博士論文

慢性炎症性貧血治療薬を指向した
経口ヘプシジン産生阻害剤の創製研究

本論文は静岡県立大学大学院薬学研究院
博士論文である。

2019 年 3 月

福田　剛

Discovery of orally active hepcidin production inhibitors as agents for anemia of chronic disease

March, 2019

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目次

略語表

本論文中における以下の用語、試薬は下記のように略記した。

Ac	Acetyl
ACD	Anemia of chronic disease
ALK2	Activin receptor type-1
ALK3	Bone morphogenetic proteic receptor type-1A
AUC	Area under the blood concentration-time curve
ATP	Adenosine triphosphate
BA	Bioavailability
BMP6	Bone morphogenetic protein 6
Boc	*tert*-Butoxycarbonyl
CDI	1,1'-Carbonyldiimidazole
CHr	Reticulocyte hemoglobin content
CL	Total clearance
Cmax	Maximum plasma concentration
DIAD	Diisopropyl azodicarboxylate
DMAP	4-Dimethylaminopyridine
DMF	*N,N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
DMP	Disintegrations per minute
DMT-MM	4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride
DPPF	1,1'-Bis(diphenylphosphino)ferrocene
DYRK	Dual specificity tyrosine-phosphorylation-regulated kinase

EPO	Erythropoietin
Hb	Hemoglobin
HTS	High throughput screening
IDA	Iron deficient anemia
IL-6	Interleukin-6
i.p.	Intraperitoneal administration
i.v.	Intravenous administration
MC	Methyl cellulose
MS	Metabolic stability
PB	Protein binding
PEG	Polyethylene glycol
p.o.	Oral administration
QoL	Quality of Life
SAR	Structure – activity relationships
STAT	Signal transduction and activator of transcription
TBAF	Tetrabutylammonium fluoride
TBDMS	*tert*-Butyldimethylsilyl
TEG	Triethylene glycol
Tf	Trifluoromethanesulfonyl
THF	Tetrahydrofuran
Tmax	Time to reach maximum plasma concentration
p-Ts	*p*-Toluenesulfonyl
UGT	UDP-glucuronosyltransferase
Vd	Volume of distribution

序論

第一節　貧血とは

血液の重要な役割の一つは、酸素を全身の各組織に運搬することである。この働きを担うのが、赤血球に含まれる血色素 (ヘモグロビン、Hb)であり、血液が酸素を運搬する能力は、Hb 量とほぼ比例する。

また貧血とは、赤血球あるいは Hb 量が正常より少なくなった状態と定義される。私たちの身体は酸素と栄養素をエネルギー源として生命を維持しているため、貧血状態に陥り酸素の運搬が十分に行われなくなると、各組織が酸素不足になり様々な症状が生じる。重症度や進行の速さによって、症状は様々である。軽度の貧血では、疲労・脱力感を覚えたり、顔色蒼白を呈する程度であるが、重度の貧血では失神・めまい、頻脈、呼吸が速くなるなどの症状が現れることがある。

世界保健機構 (WHO)は、貧血と診断する Hb の基準値を、小児及び妊婦では 11 g / dL 未満、思春期 (男性、女性)、及び成人女性では 12 g / dL 未満、成人男性では 13 g /dL 未満と定義している [1] (**Table 1**)。

Table 1. Hemoglobin levels to diagnose anemia at sea level

Population	Non-Anemia*	Anemia*		
		Mild	Moderate	Severe
6 – 59 months of age	11.0 or higher	10.0-10.9	7.0-9.9	lower than 7.0
5 – 11 years of age	11.5 or higher	11.0-11.4	8.0-10.9	lower than 8.0
12 – 14 years of age	12.0 or higher	11.0-11.9	8.0-10.9	lower than 8.0
Non-pregnant women (15 years of age and above)	12.0 or higher	11.0-11.9	8.0-10.9	lower than 8.0
Pregnant women	11.0 or higher	10.0-10.9	7.0-9.9	lower than 7.0
Men (15 years of age and above)	13.0 or higher	11.0-12.9	8.0-10.9	lower than 8.0

* Hemoglobin in grams per deciliter　　(文献 1 を参考に作成)

世界全体での貧血の推定患者数は 16 億 2000 万人に及んでおり、これは全人口の 24.8 %に相当する。 最も罹患率が高いのは就学前児 (47.4 %)である。 また患者が最も多いのは、妊娠していない女性の 4 億 6800 万人と推定されている [2] (**Table 2**)。

Table 2. Global anemia prevalence and number of individuals affected

Population group	Prevalence of Anemia Percent	Population affected Number (million)
Preschool-age children	47.4	293
School-age children	25.4	305
Pregnant women	41.8	56
Non-pregnant women	30.2	468
Men	12.7	260
Elderly	23.9	164
Total population	24.8	1620

(文献 2 を参考に作成)

また地域別にみると、就学前児と妊娠していない女性に関して、罹患率が最も高いのはアフリカで 47.5–67.6 %に上り、患者数が最も多い地域は東南アジアで約 3 億 1500 万人が罹患していると推定され、特に発展途上地域において深刻な問題である (**Table 3**)。

Table 3. Anemia prevalence and number of individuals affected in preschool-age children, pregnant women, and non-pregnant women in each WHO region

WHO region	Preschool-age children		Pregnant women		Non-pregnant women	
	Prevalence (%)	Affected (millions)	Prevalence (%)	Affected (millions)	Prevalence (%)	Affected (millions)
Africa	67.6	83.5	57.1	17.2	47.5	69.9
Americas	29.3	23.1	24.1	3.9	17.8	39.0
South-East Asia	65.5	115.3	48.2	18.1	45.7	182.0
Europe	21.7	11.1	25.1	2.6	19.0	40.8
Eastern Mediterranean	46.7	0.8	44.2	7.1	32.4	39.8
Western Pacific	23.1	27.4	30.7	7.6	21.5	97.0
Global	**47.4**	**293.1**	**41.8**	**56.4**	**30.2**	**468.4**

*Population subgroups: Preschool-age children (0.00-4.99 yrs): Pregnant women (no range defined): Non-pregnant women (15.00-49.99 yrs)

(文献 2 を参考に作成)

また日本に関して述べると、平成 29 年 3 月に厚生労働省が発表した平成 27 年国民健康・栄養調査報告によると、Hb が 13 g / dL 未満の成人男性の割合が 10.5 %であり、年齢を増すごとに上昇している。また、女性のうち Hb が 12 g / dL 未満の割合は 16.8 %、40 代女性では 20.8 %と世界的にも高頻度である[3]。栄養状態が良いと考えられる本国においても、改善すべき疾患であることが分かる。

第二節　ヘプシジンと慢性炎症性貧血 (ACD)

鉄はほぼ全ての生物に不可欠な微量元素であり、酸化的リン酸化・核酸合成、酸素運搬など重要な役割を担っている[4]。一方で過剰の鉄は Fenton 反応[5]に代表される反応を介してフリーラジカル種を産生させ細胞障害を引き起こす[6, 7]。このため体内の鉄の量は様々な状況に応じて迅速かつ厳密にコントロールされている。

生命にとって鉄は貴重な栄養素であったため能動的に排出する仕組みが発達していない。このため体内の鉄の量は、小腸からの吸収段階で制御されている[8]。つまり鉄欠乏状態では吸収率が上昇し、鉄過剰状態では低下する。また量的収支を考えると、ヒト体内の鉄の総量は 3,000～5,000 mg であるのに対し、吸収と排泄は 1 日あたり 1 mg 程度しかない。つまり赤血球合成などに必要な鉄の大部分は生体内での再利用により賄われている[9]。

鉄代謝は様々な臓器により厳密にコントロールされているが、それを調節する因子がヘプシジンというペプチドホルモンである。Park らは、内因性抗菌ペプチドのクローニングを目的にヒトの尿より単離した新規ペプチドが、肝臓(hep)由来の抗菌物質(cidin)であったことから、ヘプシジン「hepcidin」と命名した[10]。このヘプシジンは Krause らが 2000 年 LEAP-1 としてヒト血中から分離した disulfide 結合をもつ抗菌作用を有するペプチドと同じものであった[11a, b]。2002 年に、転写因子である USF-2 ノックアウトマウスが鉄過剰を呈することから、その責任遺伝子のクローニングによりヘプシジンが同定された[12a, b]。また、ヘプシジン高発現マウスが重度の鉄欠乏性貧血を呈することなどから[13]、ヘプシジンが鉄代謝の主要調節因子として認知されるようになった。

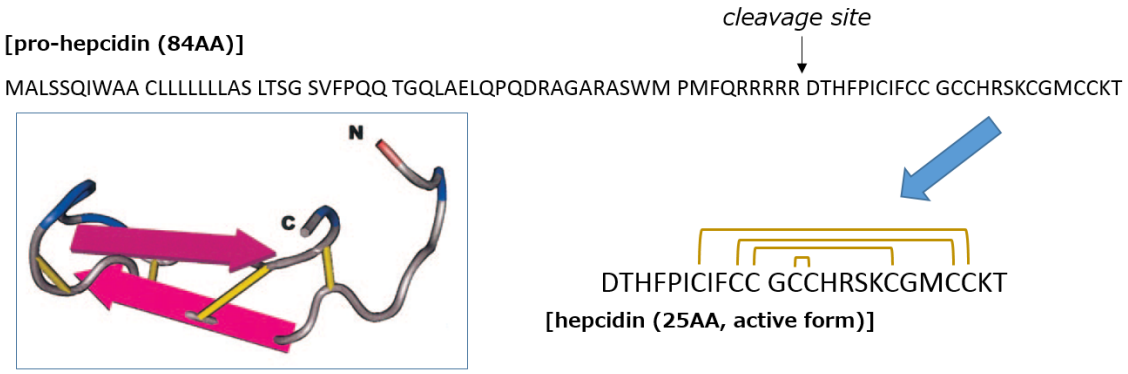


Figure 1. Amino acid sequence and a model of the major form of human hepcidin
The amino and carboxy termini are labeled as N and C, respectively. Disulfide bridges are in yellow, basic amino acids in blue, and acidic in red. The pattern of disulfide linkages between the 8 cysteines is also shown in the amino acid sequence.

ヘプシジンは 84 アミノ酸鎖のペプチドとして肝臓で産生され、25 アミノ酸鎖に分解されて活性型となる (**Figure 1**, 文献 11a, 16 より一部改変)。

ヘプシジンは小腸基底膜にある細胞から鉄をくみ出す輸送体である ferroportin と結合し、ferroportin の分解を促進することにより腸管上皮細胞から循環血への鉄移動を抑制する [14]。またヘプシジンはマクロファージに存在する ferroportin にも同様の機序で働き、マクロファージから循環血へ移動する鉄を減少させる [15]。したがって、ヘプシジンは生体における鉄利用の抑制因子であるといえる [16, 17]。

生体は細菌感染や炎症状態、体内の鉄飽和状態、造血シグナル、低酸素シグナルなど様々な要因の変化に対し、ヘプシジン発現を厳密に制御することで鉄量の調節を行っていると考えられている [18]。

貧血は鉄欠乏性貧血 (Iron deficiency anemia, IDA)と慢性炎症性貧血 (Anemia of chronic disease, ACD)に大分される。

IDA は、体内に鉄が不足することにより、充分に Hb を産生できなくなることで生じる貧血のことである。

一方、ACD は感染症や自己免疫疾患、悪性腫瘍を基礎疾患として、誘導され

たIL-6などの炎症性サイトカインの働きにより、ヘプシジン産生が亢進する[19]。炎症性サイトカインによりヘプシジン産生を亢進すると、赤血球合成のために鉄が必要であるにもかかわらず、利用可能な鉄が減少し貧血を発症する[20, 21]。

このように慢性炎症性貧血はヘプシジンの不適切な亢進により発症すると考えられている[22]。

第三節　治療法と課題

貧血の治療には、主に組み換え Erythropoietin (EPO) 製剤、及び経口・静注鉄剤が使用される。EPO は主に腎臓で生成される赤血球産生を促進するサイトカインである。骨髄中の血芽細胞前駆体細胞に作用し、赤血球への分化・増殖を促進する。組み換え EPO 製剤は分化ののちに赤血球となる器を増やす作用により、貧血の改善を促す薬剤である。一方、鉄剤は血芽細胞が赤血球へと分化する際に必要となるヘム鉄の供給を促進することにより、赤血球の増殖促進を促す薬剤である (**Figure 2**)。

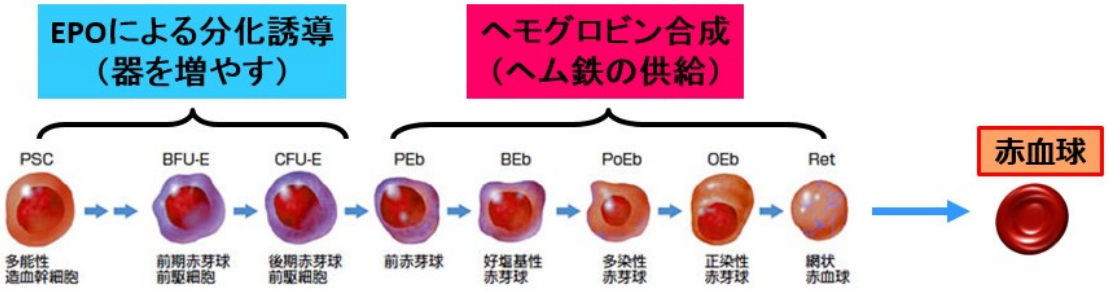


Figure 2. Differentiation of red blood cell and anemia treatment

前節で述べたが、慢性炎症性貧血では純粋に体内の鉄分が不足して発症する鉄欠乏性貧血と異なり、体内の鉄分が十分であるにも関わらず造血組織において鉄欠乏が生じている。逆説的なことに、鉄量を補うために鉄剤を負荷することで更なるヘプシジンを誘導し、効率的な血球への鉄補充を妨げている。

つまり、生体内の鉄の利用効率を上げることを目的にしたヘプシジン産生の抑制が、慢性炎症性貧血の治療戦略として望ましいと考えられる。

近年、ヘプシジンを標的として鉄利用効率の改善を指向した薬物として、NOX-H94 (抗ヘプシジンアプタマー [Spiegelmer], 第Ⅱ相臨床試験)、LY2928057

(抗 Ferroportin 抗体, 第 I 相臨床試験)、LY2787106 (抗ヘプシジン抗体, 第 I 相臨床試験)などの生物製剤の臨床試験が行われている。

しかしながら、生物製剤は投薬コンプライアンスや医療費の観点で課題も多く、患者さんの Quality of Life (QoL)向上や発展途上国をはじめより多くの患者さんへ治療の選択肢を提示できることから、経口投与可能なヘプシジン産生阻害剤の開発が期待されている。一方で、経口投与可能なヘプシジン産生阻害剤の報告は無い状況であった。

第四節　本研究の概要

本研究において著者は、生体内の鉄代謝を司る因子として近年明らかとなったペプチドホルモンであるヘプシジンに着目し、ヘプシジン産生阻害剤が慢性炎症性貧血の有効な治療薬となると考え、創薬研究を行った。

序論では、貧血の疫学、慢性炎症性貧血と鉄代謝におけるヘプシジンの役割、及び国内外の医療現場で用いられている治療法の現状と課題について述べた。

第一章では、High throughput screening (HTS) により得られたヒット化合物 **1** の誘導体展開により、アミノピリミジン誘導体 **DS42450411** を創製した経緯を述べる。キナーゼプロファイリング評価によって Dual-specificity tyrosine phosphorylation-regulated kinase 1a (DYRK1A)阻害能を発見し、DYRK1A との X 線結晶構造解析データを基にしたドラッグデザイン、及び最適化研究により強力な *in vivo* 薬効を示す **DS42450411** の創製に成功した (**Figure 3**)。

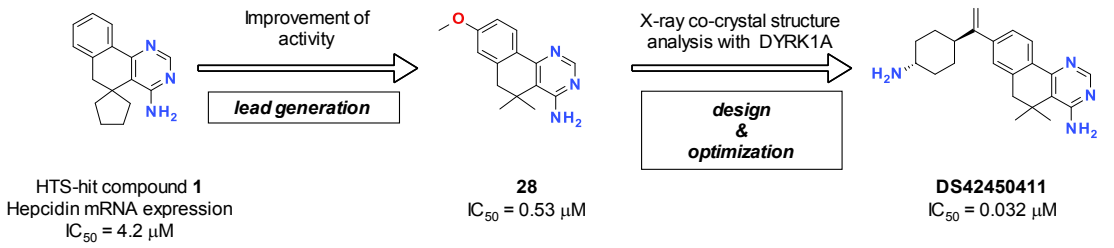


Figure 3. Discovery of **DS42450411** (chapter 1)

第二章では、もう一つのヒット化合物 **67** からの誘導体展開により経口投与で *in vivo* 薬効を示す化合物 **129** を創出した経緯を述べる。ヒット化合物の初期誘導体展開で見出したインダゾール 6 位に *para*-ヒドロキシフェニル基を有する化合物 **109** は IL-6 誘発高ヘプシジンマウスモデルを用いた *in vivo* 薬効評価において、腹腔内投与で血清ヘプシジン低下作用を示すものの経口薬として満

足のいくプロファイルではなかった。そこで化合物 **109** の 6 位フェノール性ヒドロキシ基の代替基探索により経口投与時の血中暴露量を改善し、経口投与で高い *in vivo* 薬効を示す化合物 **129** を創出した。

第三章では、化合物 **129** の誘導体展開により **DS28120313**、**DS79182026** を創製した経緯を述べる。広範なキナーゼ阻害能を有する化合物 **129** のキナーゼ阻害能低減を指向した scaffold hopping による新規母核の探索、及び最適化研究により強力な *in vivo* 薬効を示すインダゾール誘導体 **DS28120313**、及びベンゾイソキサゾール誘導体 **DS79182026** の創製に成功した (**Figure 4**)。

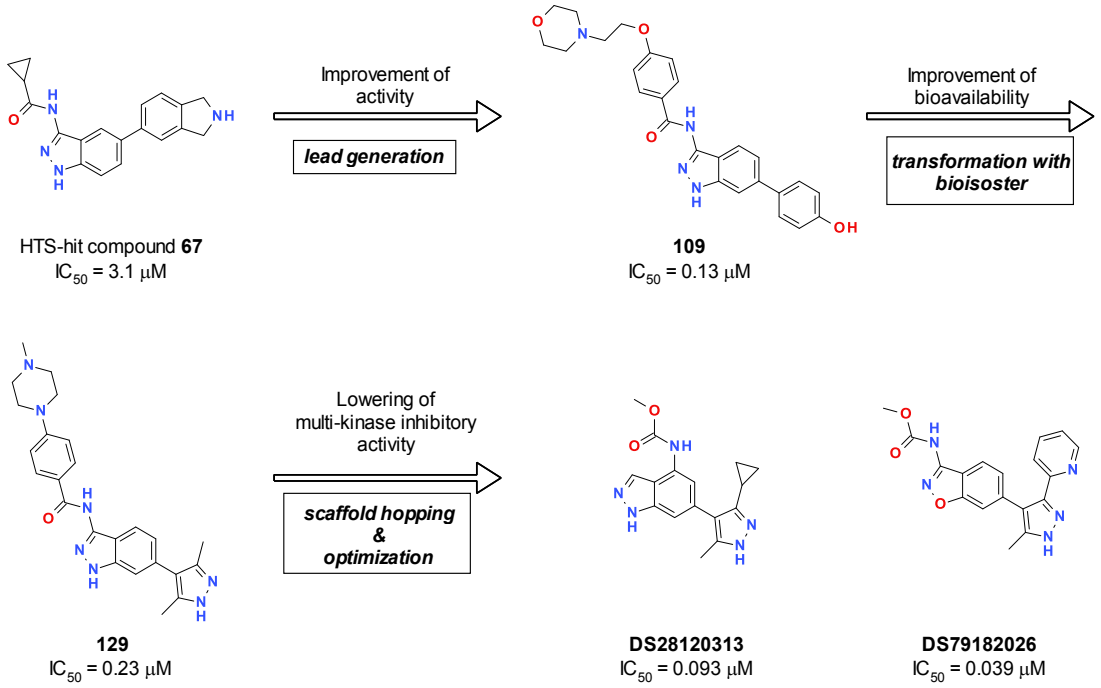


Figure 4. Discovery of **DS28120313** and **DS79182026** (chapter 2 and 3)

第四章では、**DS79182026** の薬理学的標的タンパク質の同定研究に用いる高活性プローブのデザイン・合成、及び薬理学的研究について述べる。**DS79182026** の適切な位置にリンカーを付与することで高い *in vitro* 活性を保持した化合物 **218** を得た。このリンカー体から得た化合物固定化ビーズと HepG2 セルライセートを用いて結合実験を実施し、13 種類の候補タンパク質を同定し

た。候補タンパク質の中で入手可能な 10 種類のリコンビナントタンパク質とトリチウム標識化した活性化合物による結合実験を実施し、**DS79182026** の薬理学的標的タンパク質として Activin receptor type-1 (ALK2) / Bone morphogenetic proteic receptor type-1A (ALK3)の 2 種を同定した (**Figure 5**)。

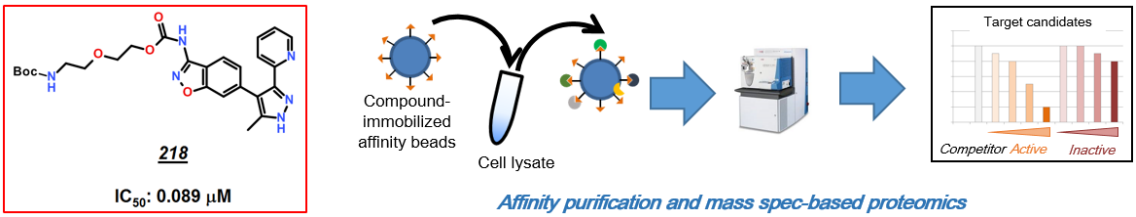


Figure 5. Overview of target identification (chapter 4)

以下、各章にて本研究内容を詳細に記述する。

第一章　アミノピリミジン母核を有する新規ヘプシジン産生阻害剤の合成と構造活性相関

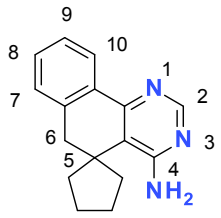
第一節　HTS ヒット化合物 1

序論第三節で述べたように、ヘプシジン産生を阻害することにより効率的な鉄吸収、及び貯蔵鉄からの造血細胞への鉄補充が期待されることから、経口貧血治療薬を指向したヘプシジン産生阻害剤創製を目的として研究に着手した。

*In vitro*評価系として、ヘプシジンは主に肝臓で産生されることからヒト肝癌細胞株HepG2を用い、培養上清中のヘプシジンmRNAを定量化する系を構築した。本評価系では炎症性サイトカインによるヘプシジン産生誘導を阻害する化合物を取得するため、ヘプシジン産生誘導刺激にBone morphogenetic protein 6 (BMP6)を選択した。

第一三共株式会社保有の化合物ライブラリーを用いたHTSにより、3つの環が縮合し、かつスピロシクロペンタン構造を有する4-アミノピリミジン誘導体**1**が見出された。しかしながら、化合物**1**のIC_{50} (50 %阻害濃度)は4.2 μMと弱く、阻害活性の大幅な向上が必要であった (**Figure 6**)。

そこで、化合物**1**からの誘導体展開を開始することとした。

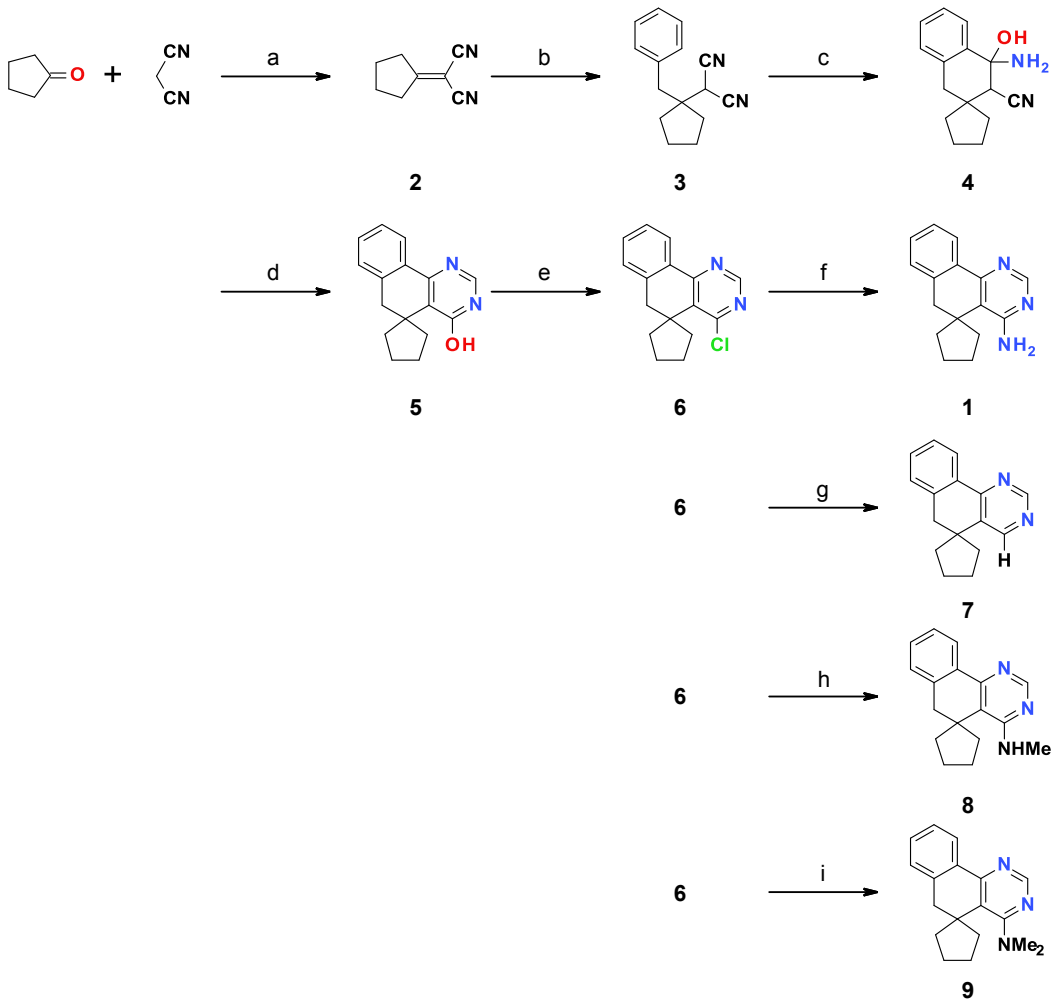


1 IC_{50} = 4.2 μM

Figure 6. Structure of HTS-hit of hepcidin production inhibitor **1**

第二節　誘導体の合成法

三環性骨格 5 位にスピロシクロペンタン環を有する誘導体の合成法を **Scheme 1** に示す。

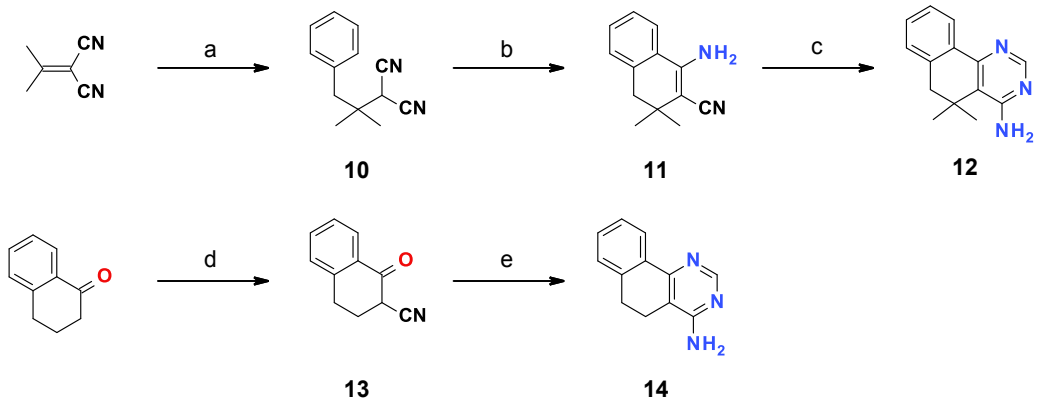


Scheme 1. Reagents and conditions: (a) NH_4OAc, AcOH, benzene, reflux, 95%; (b) Arylmethylmagnesium chloride, THF, 77%; (c) conc. H_2SO_4, 0°C~r.t., 42%; (d) $HCONH_2$, $POCl_3$, 130°C, 75%; (e) $POCl_3$, 95°C, 86%; (f) NH_3 gas, $HCONH_2$, 150°C, 79%; (g) H_2 / Pd-C, AcOEt, 4%; (h) 40% $MeNH_2$-MeOH solution, reflux, 90%; (i) 50% aqueous Me_2NH, reflux, 68%.

シクロペンチリデンマロノニトリル **2** へのベンジルグルニャール試薬のマイケル付加反応により、ジニトリル **3** を合成した。**3** に濃硫酸を作用させることにより環化反応が進行し、ヘミアミナール **4** を中程度の収率で得た。ヘミアミナ

ール **4** をホルムアミド溶媒中、オキシ塩化リンを加えて 130℃で加熱することにより 4-ヒドロキシピリミジン **5** を得た。また、化合物 **5** にオキシ塩化リンを作用させることにより、4-クロロピリミジン **6** を得た。クロロピリミジン **6** を芳香族求核置換反応によるアミノ化、もしくは接触還元による脱クロロ化を行うことにより、化合物 **1, 7-9** を得た。

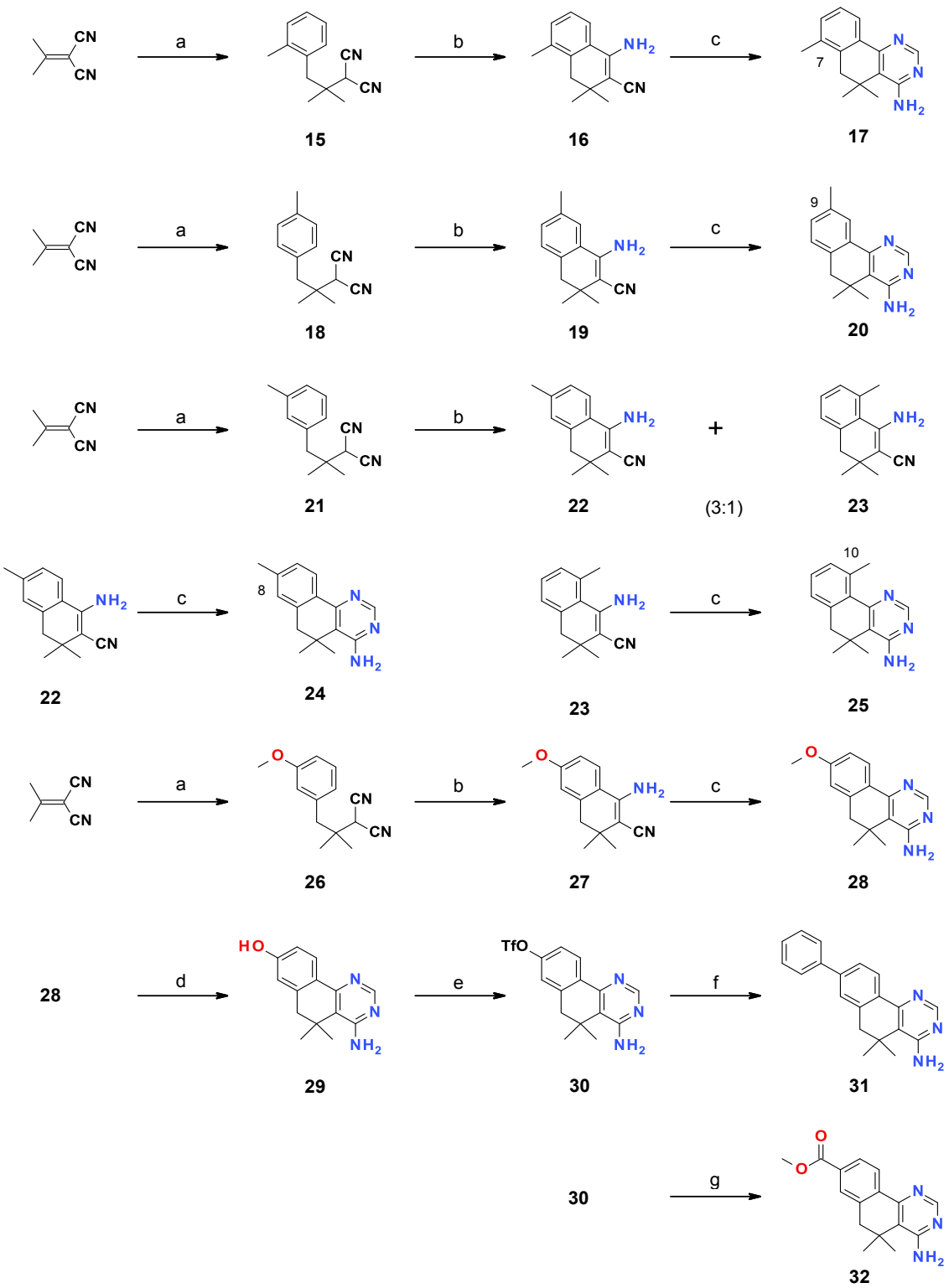
次に、5位にスピロ構造を有さない化合物の合成法を**Scheme 2**に示す。



Scheme 2. Reagents and conditions: (a) Benzylmagnesium chloride, THF, 71%; (b) conc. H_2SO_4, 0°C~r.t., 10%; (c) $HCONH_2$, 130°C, 43%; (d) Lithium diisopropylamide, Tosyl cyanide, -78~0°C, 25%; (e) $HCONH_2$, $POCl_3$, 125°C, 20%.

ジニトリル**10**に濃硫酸を作用させることにより環化反応が進行し、低収率ながらアミノニトリル**11**を得た。**11**をホルムアミド中130℃で加熱することにより、5位ジメチル誘導体**12**を得た。また、α–テトラロンをLDA/トシルシアニドを用いてカルボニル基のα位をシアノ化、続く環化反応により5位無置換体**14**を得た。

次に、左側ベンゼン環に各種置換基を有する化合物の合成法を**Scheme 3**に示す。7-10位にメチル基を導入した誘導体は、イソプロピリデンマロノニトリルと*ortho*、*meta*、及び*para*-メチルベンジルグリニャール試薬を用いて合成した。ジニトリル**21**のトリフルオロメタンスルホン酸を用いた環化反応では、メチル基の立体障害の影響で、環化体**22**と**23**の生成比は3:1であった。

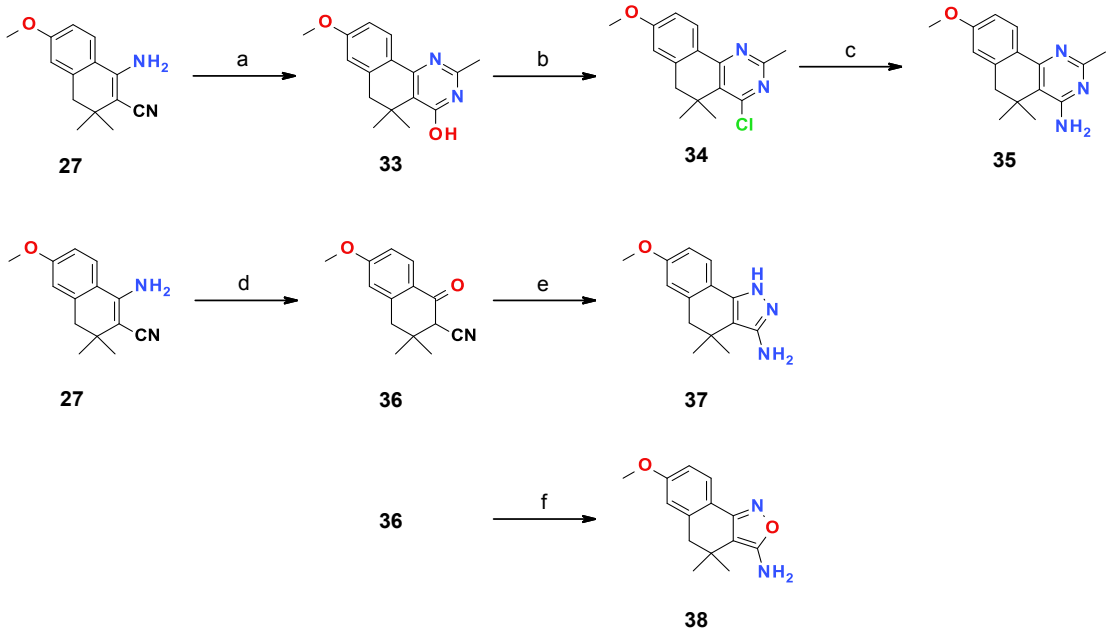


Scheme 3. Reagents and conditions: (a) Arylmethylmagnesium chloride, THF, 39-73%; (b) Trifluoromethanesulfonic acid, CH_2Cl_2, 73% - quant.; (c) Formamide, 32-72%; (d) BBr_3, THF, -40°C, 82%; (e) Tf_2O, pyridine, 50%; (f), Phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, 1,4-dioxane, 15%; (g) CO gas, MeOH, $Pd(OAc)_2$, DPPF, DMSO, 80°C, 51%.

一方、*meta*-位にメトキシ基を有するジニトリル**26**の環化反応は、メトキシ基の*para*-位で選択的に環化した**27**を単一生成物として与えた。

8位にフェニル基、及びメトキシカルボニル基を有する化合物**31, 32**は、**28**から二工程で合成したトリフラート中間体**30**を用い、鈴木カップリング反応、もしくはパラジウム触媒を用いた一酸化炭素挿入反応を用いて合成した。

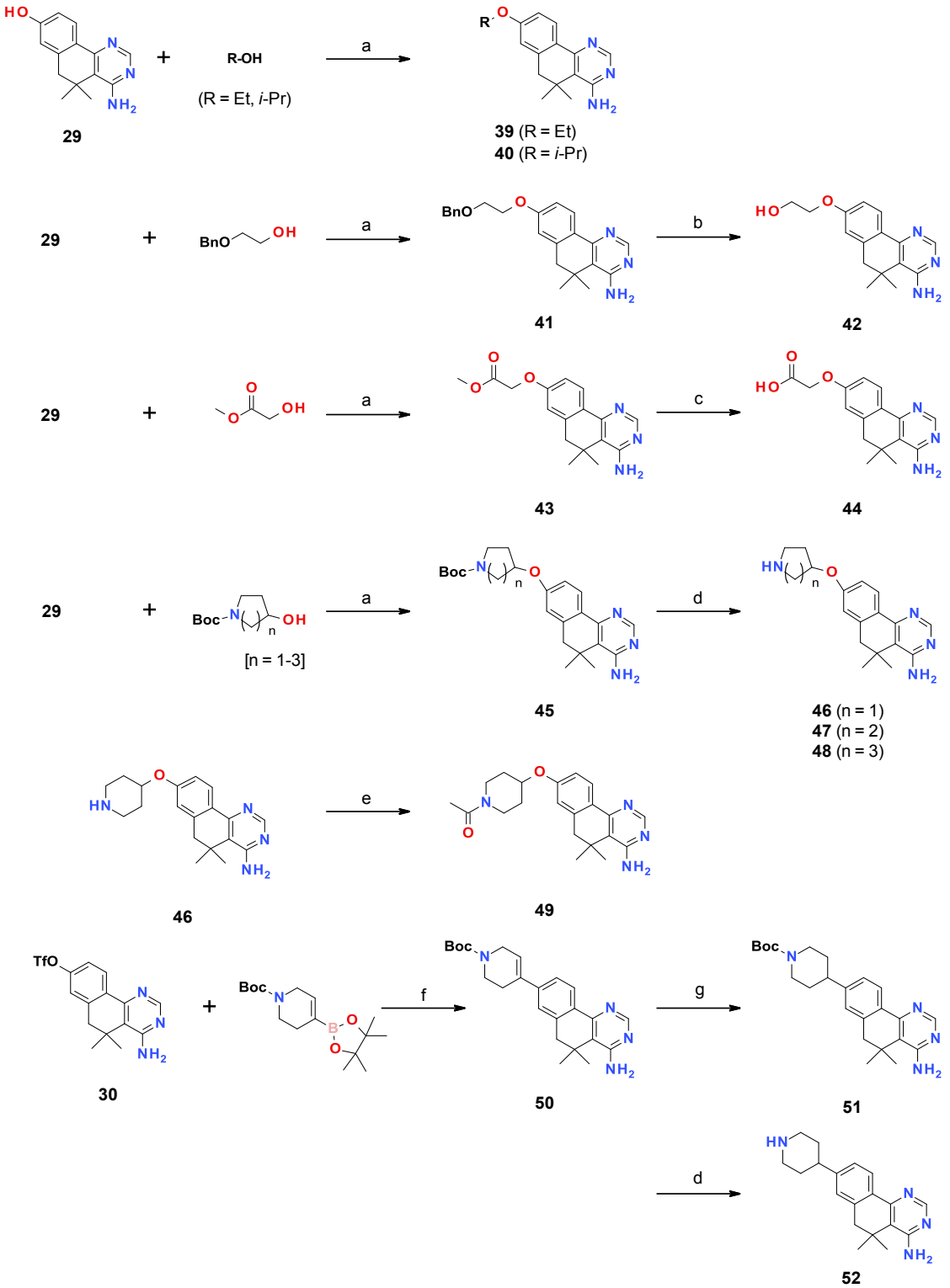
4-アミノピリミジン部位を変換した化合物の合成ルートを**Scheme 4**に示す。



Scheme 4. Reagents and conditions: (a) Acetyl chloride, Toluene, 39%; (b) $POCl_3$, 99%; (c) NH_3 gas, $HCONH_2$, 160°C, 29%; (d) H_2SO_4 aq., MeOH, 80°C, 86%; (e) Hydrazine monohydrate, AcOH, EtOH, 72%; (f) Hydroxylamine hydrochloride, AcOH, EtOH, 56%.

アミノニトリル**27**にアセチルクロリドを作用させることにより、アセチル化ー環化反応が連続的に進行し、4-ヒドロキシピリミジン**33**を与えた。クロロ化、アミノ化を経て2-メチル誘導体**35**を合成した。また、シアノテトラトン**36**に対し、ヒドラジン一水和物、もしくはヒドロキシルアミン塩酸塩を作用させることにより、アミノピラゾール誘導体**37**、及びアミノイソキサゾール誘導体**38**を合成した。

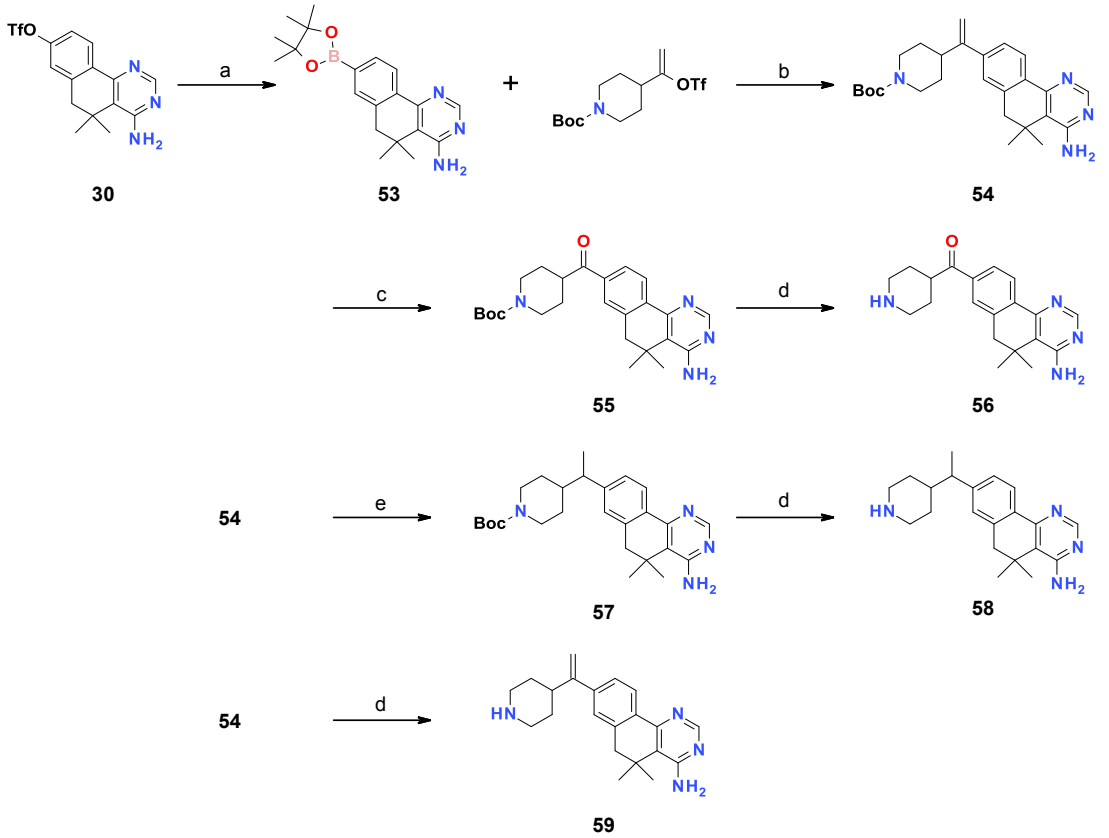
次に8位に置換アルコキシ基を有する化合物の合成法を**Scheme 5**に示す。



Scheme 5. Reagents and conditions: (a) DIAD, PPh_3, THF, 39-99%; (b) H_2 / Pd-C, EtOH/MeOH, 31%; (c) NaOH aq. MeOH, 75%; (d) HCl - dioxane, CH_2Cl_2, 25-82%; (e) Ac_2O, Et_3N, CH_2Cl_2, 83%; (f) $Pd(PPh_3)_4$, Na_2CO_3, Toluene/EtOH/H_2O, 69%; (g) H_2 / Pd-C, MeOH, 76%.

8-ヒドロキシ体**29**を原料とし、光延反応を用いて種々8-アルコキシ誘導体を合成した (化合物**39, 40, 42, 44, 46-48**)。また、化合物**48**のピペリジンNHのアセチル化反応は、無水酢酸を用いることでピリミジン4位アミノ基を保護することなく目的物**49**を得ることができた。8位にエーテルリンカーを持たない直結ピペリジン型誘導体**52** はトリフラート中間体**30**を用いて鈴木カップリング反応、オレフィンの還元、Boc基の脱保護反応により合成した。

次に、8位に種々のリンカーを介してピペリジン環を有する化合物の合成法を**Scheme 6**に示す。

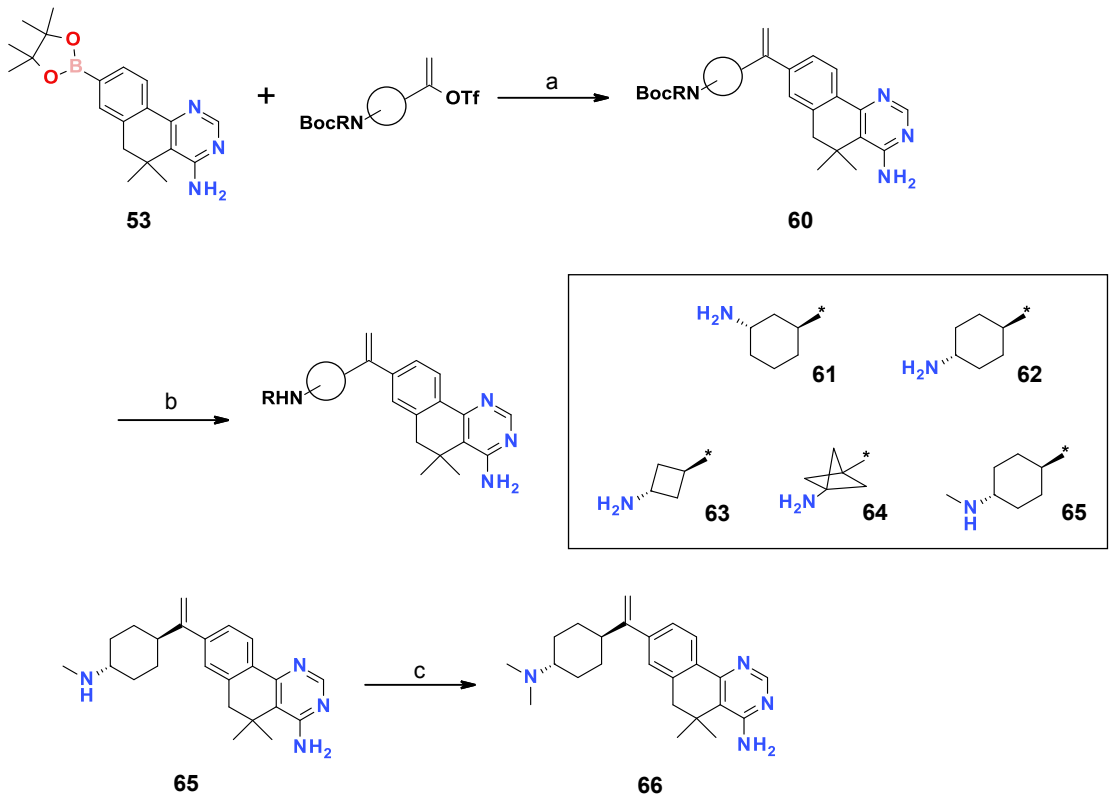


Scheme 6. Reagents and conditions: (a) Bis(pinacolato)diboron, Pd(dppf)Cl_2-CH_2Cl_2, KOAc, 1,4-dioxane, 90%; (b) $Pd(PPh_3)_4$, Na_2CO_3, 1,4-dioxane/H_2O, 78%; (c) OsO_4, *N*-Methylmorpholine-*N*-oxide, $NaIO_4$, *t*-BuOH, 87%; (d) Trifluoroacetic acid, CH_2Cl_2, 73-97%; (e) Pd-C - H_2, MeOH, 93%.

前述のトリフレート中間体**30**から誘導したピナコールエステル**53**とビニルトリフラートの鈴木カップリング反応により、*exo*-オレフィン構造を持つ化合物

54を得た。化合物**54**のオレフィン部位を四酸化オスミウムで酸化することで、ケトン体**55**を合成した。また、**54**のオレフィン部分を還元することで、メチル体**57**を合成した。ピペリジンBoc保護体**54, 55, 57**をトリフルオロ酢酸で脱保護し、目的化合物**56, 58, 59**を得た。

次に、*exo*-オレフィンの先の環状アミノ基を変換した化合物の合成法を**Scheme 7**に示す。



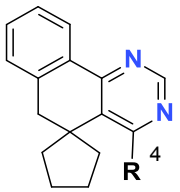
Scheme 7. Reagents and conditions: (a) Chloro(2-dicyclohexylphosphino-2',4',6'-triisopropyl-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II), K_3PO_4, 1,4-dioxane/H_2O, 69-81%; (b) Trifluoroacetic acid, CH_2Cl_2, 43-92%; (c) Formaldehyde solution, $NaBH(OAc)_3$, CH_2Cl_2, 36%.

Scheme 6で示した化合物**59**の合成法に準じて、対応するビニルトリフラートとの鈴木カップリング反応、Boc基の脱保護反応により化合物**61-65**を合成した。また化合物**65**のホルムアルデヒドを用いた還元的アミノ化反応により、化合物**66**を合成した。

第三節　リード化合物 28 の創出

はじめにピリミジン環の 4 位アミノ基の変換を実施した。しかしながら無置換体、アルコール、置換アミノ基への変換により *in vitro* 活性は完全に消失したことから、一級アミノ基は活性発現に必須であると判断した **(Table 4)**。

Table 4. SAR of 4-position of pyrimidine derivatives

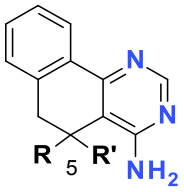


compound	R	IC_{50} (μM)
1	NH_2	4.2
5	OH	> 30
7	H	> 30
8	NHMe	> 30
9	NMe_2	> 30

次に、多面的な誘導体展開の妨げとなっている 5 位スピロペンタン環の変換を実施した **(Table 5)**。

5 位無置換体 **14** への変換は *in vitro* 活性を消失する結果であった。しかしながら、より単純なジメチル基に変換した **12** は **1** と同等の *in vitro* 活性を示し、5 位スピロシクロペンタン構造は必ずしも必須ではないことが分かった。

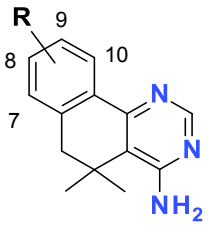
Table 5. SAR of 5-substituted pyrimidine derivatives



compound	R, R’	IC_{50} (μM)
1	cyclopentyl	4.2
12	Me, Me	4.5
14	H, H	> 30

次にベンゼン環上への置換基導入の可能性検討を実施した(**Table 6**)。

Table 6. SAR of substituted 4-aminopyrimidine derivatives

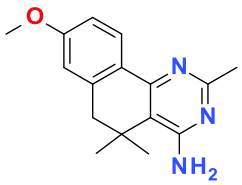


compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM)
12	H	4.5	**25**	10-Me	4.7
17	7-Me	5.0	**31**	8-Ph	1.5
24	8-Me	0.60	**32**	8-CO_2Me	11
20	9-Me	2.8	**28**	8-OMe	0.53

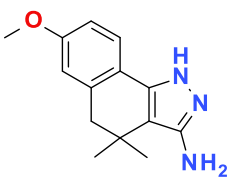
7-10 位にメチル基を導入した化合物を評価したところ、8 位への置換基導入で大幅に活性が向上することが分かった (**24**, IC_{50} = 0.60 μM)。

そこで種々の置換基を 8 位に導入したところ、フェニル基では IC_{50} = 1.5 μM と *in vitro* 活性はやや減弱し、メトキシカルボニル基の導入では大幅に活性が減弱した。しかしながら、メトキシ基を導入した **28** は高い *in vitro* 活性を示すことが明らかとなったので、**28** をリード化合物として誘導体展開を行うこととした。

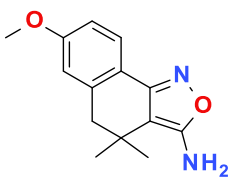
続いて、2 位無置換 4-アミノピリミジン以外の骨格について検討を行った。2 位にメチル基を導入した **35** の活性は消失した。またピリミジン環をアミノピラゾール、アミノイソキサゾール環へと変換した **37, 38** ともに活性が消失することから 2 位無置換 4-アミノピリミジン骨格は活性発現に必須であることが分かった (**Figure 7**)。



35 IC_{50} > 5.0 μM



37 IC_{50} > 30 μM



38 IC_{50} > 30 μM

Figure 7. *in vitro* activity of other scaffolds

第四節　化合物 28 のキナーゼプロファイリング評価と DYRK1A

前節で述べたように、HTS ヒット化合物 **1** の誘導体展開により、高い *in vitro* 活性を示すリード化合物 **28** を獲得することに成功した。

そこで、本系統の化合物がヘプシジン産生阻害活性を示すメカニズムを考察するために、**28** のキナーゼプロファイリング評価を実施した (216 キナーゼ)。

その結果、CMGC ファミリーに属する CLK2、DYRK1A、及び DYRK1B の 3 種のキナーゼに対し、高い阻害能を示すことが判明した (**Figure 8**)。

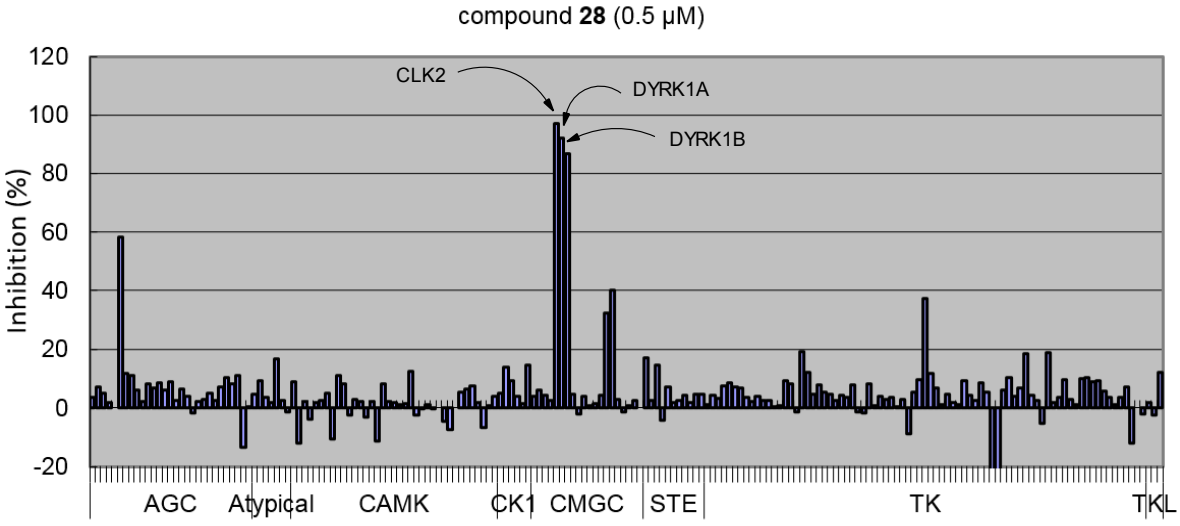


Figure 8. Kinase inhibitory profiles of compound **28**

本結果より、**28**が発現するヘプシジン産生阻害のメカニズムはCMGCファミリーに属するキナーゼ阻害に基づくと推測した。そこで*in vitro*活性向上を指向した化合物デザインの糸口を得るために、DYRK1Aと化合物**28**のX線共結晶を作製し、構造解析を実施した (**Figure 9**)。

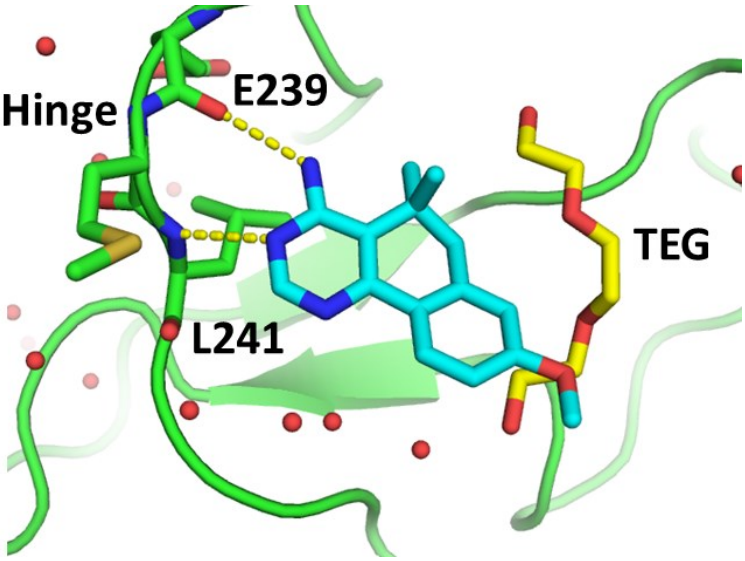


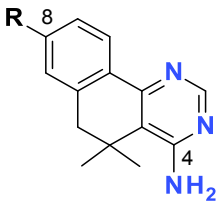
Figure 9. Co-crystal structure of DYRK1A and compound **28** determined at 1.5 Å resolution (PDB: 6A1F). Triethylene glycol (TEG) was placed to the position of unidentified density observed within the ATP-binding pocket of DYRK1A.

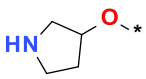
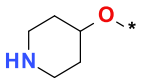
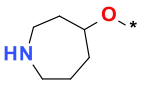
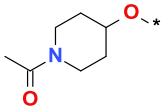
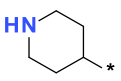
その結果、**28**はDYRK1AのATP結合ポケットに結合し、アミノピリミジン部分はヒンジ領域グルタミン酸239と相互作用していることが明らかとなった。また、8位メトキシ基の方向に空間許容性があり、置換基導入の余地があることが判明した。

第五節　X 線結晶構造解析結果を基にしたデザインと DS42450411 の創製

前節で示した通り、**28**とDYRK1AとのX線結晶構造解析の結果、8位メトキシ基の方向に空間許容性が示唆された。そこでDYRK1Aの阻害能を向上することによりヘプシジン阻害活性が向上するのではないかと考え、空間許容性が示唆された8位置換基の最適化研究を実施することとした (**Table 7**)。

Table 7. SAR of 4-amino-8-substituted pyrimidine derivatives

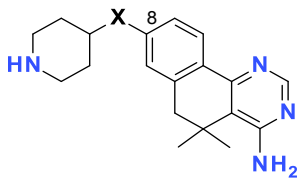


compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM)
28	MeO	0.53	**46**		0.18
39	EtO	0.42	**47**		0.23
40	*i*-PrO	0.49	**48**		0.24
42	$HOCH_2CH_2O$	1.1	**49**		0.76
44	HO_2CCH_2O	11	**52**		0.81

エトキシ基、イソプロピルオキシ基への変換においても、メトキシ基と同等の活性を維持することが明らかとなった (**39, 40**)。次に導入可能な官能基について検討を行った。ヒドロキシ基の導入により活性がやや減弱し、カルボキシ基の導入により、大幅に活性が減弱することが分かった (**42, 44**)。一方、環状アミノ基の導入で活性が向上することが明らかとなった (**46-48**)。ピペリジン環の二級アミノ基をアセトアミド基に変換すると活性が減弱することから、*in vitro*活性の向上には塩基性置換基が有効であることが示唆された (**49**)。また、8位にピペリジン環が直結した**52**の活性が減弱することから、母核と塩基性置換基を繋ぐリンカーが必要であると判断した。

そこで、リンカーの変換を実施した (**Table 8**)。

Table 8. SAR of various linkers at 8-position



compound	X	IC_{50} (μM)
47	O	0.23
56	C=O	0.30
58	*rac*-CHMe	0.073
59	$C=CH_2$	0.021

エーテルリンカーをカルボニル基に変換した**56**は、**47**と比較して*in vitro*活性の向上は見られなかった。しかし、カルボニル基をメチル基に変換した化合物**58**、更には*exo*-メチレンリンカー (**59**)への変換により、飛躍的に*in vitro*活性が

向上することが明らかとなった。

そこで、*in vitro*活性向上の原因を考察するために化合物**59**とDYRK1AとのX線結晶構造解析を行った **(Figure 10)**。

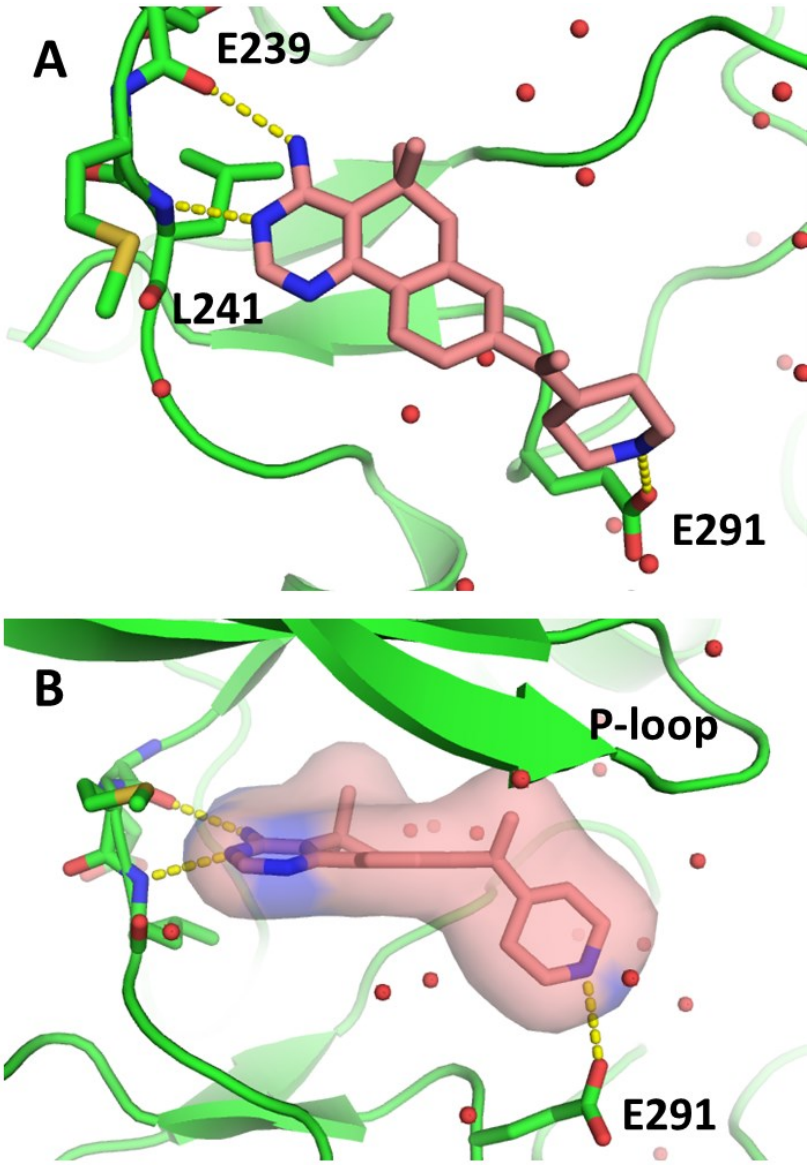


Figure 10. Co-crystal structure of DYRK1A and compound **59** determined at 2.15 Å resolution (PDB: 6A1G). **A**: Sideview, **B** Topview.

59は**28**と同様にヒンジ領域のグルタミン酸239との結合を維持しつつ、新たに導入したピペリジン環の二級アミノ基の水素原子がグルタミン酸291と強固な水素結合を形成していることが分かった。加えて、*exo*-メチレンリンカーにより*P*-ループとの空間相補性が向上していることも確認された。この新たに獲得した2つの相互作用の結果、**59**の*in vitro*活性が大幅に向上したと考えられる。

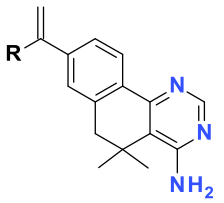
そこで代表化合物のDYRK1A阻害活性を測定した。その結果、**Table 9**に示すように非常に弱いヘプシジン産生阻害活性を示す**14**のDYRK1A阻害活性は非常に減弱している。また、リード化合物**28**と比べて高いヘプシジン阻害活性を示す**59**はDYRK1A阻害活性に関しても大幅に向上していることが確認できた。すなわち、ヘプシジン産生阻害活性とDYRK1A阻害活性には正の相関関係が見られた。このことから、本系統誘導体が示すヘプシジン阻害能はDYRK1Aに基づく可能性が示唆された。

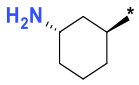
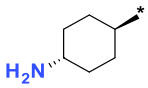
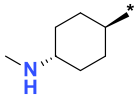
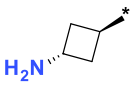
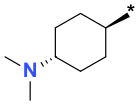
Table 9. SAR of inhibitory activity between hepcidin production and DYRK1A

compound	Hepcidin IC_{50} (μM)	DYRK1A IC_{50} (μM)
28	0.53	0.33
14	> 30 (20 %inh. @30 μM)	57
35	>5.0	2.7
47	0.23	0.56
59	0.021	0.054

アミノ基の導入、及び*exo*-メチレンリンカーへの変換が*in vitro*活性向上に有効であることが分かったので、アミノ基の最適化研究を実施することとした(**Table 10**)。

Table 11. SAR and AUC values of 8-*exo*-olefin derivatives



compound	R	IC_{50} (μM) / AUC[a] (h*μg/mL)	compound	R	IC_{50} (μM) / AUC[a] (h*μg/mL)
61		0.042 / 0.095	**64**		0.12 / 0.18
62 (DS42450411)		0.032 / 2.58	**65**		0.045 / 0.038
63		0.031 / 0.084	**66**		0.11 / 2.90

[a] Average of two values dosed at 30 mg/kg orally (p.o.) in C57BL/6J mice (0.5% Methylcellulose suspension).

シクロヘキサン、及びシクロブタン環を介して一級アミノ基を有する化合物**61-63**はいずれも強力な*in vitro*活性を示した。一方で、ベンゼン環等価体として用いられるビシクロ[1.1.1]ペンタン環[23] (**64**)はやや活性が減弱することが明らかとなった。またアミノ基を二級 (**65**)、三級 (**66**)へと変換すると、活性は減弱傾向を示した。

また、マウス30 mg/kg経口投与におけるAUC (血中濃度曲線下面積)を比較すると、一級アミノ基を有する化合物の中で、**62**が高い血中暴露を示した。

そこで8位アミノ基の最適化で創製した**62**を**DS42450411**と命名し、更なる高次評価を実施することとした。

第六節　DS42450411 の *in vivo* 薬効評価

前節では、DYRK1AとのX線結晶構造解析のデータを基にしたドラックデザイン、及び8位置換基の最適化研究を行うことにより、高い*in vitro* ヘプシジン産生阻害活性を示す**DS42450411**創製の経緯を述べた。

Table 11に**DS42450411**のマウスを用いた薬物動態試験の結果を示す。

Table 11. Physicochemical properties and pharmacokinetic parameters of **DS42450411**

LogD	PB (free %)	Cmax[a] (μg/mL)	Tmax[a] (h)	AUC[a] (h*μg/mL)
1.6	6.5	0.61	1.17	2.58

[a] Average of two values dosed at 30 mg/kg orally (p.o.) in C57BL/6J mice (0.5% Msokodeethylcellulose suspension).

DS42450411は適度な脂溶性と中程度のタンパク結合率を示し、30 mg/kg 経口投与において高い血中濃度を示すことが明らかとなった。

次に、マウス*in vivo*モデルにおける**DS42450411**の薬効評価を検討した。序章第二節で述べたようにヘプシジンは慢性炎症状態に応答して産生される急性期タンパク質として知られている。

そこで**DS42450411**の薬効評価に先立って、炎症反応に伴う血清ヘプシジンの濃度推移を調べた。血清ヘプシジン濃度は、炎症性サイトカインであるInterleukin-6 (IL-6)の静脈内投与後1時間ですばやく増加し、6時間まで一定であることが明らかとなった。

したがって、マウスにIL-6を静脈内注射した血清中のヘプシジンの急性期誘導に対する**DS42450411**の効果を評価した。

C57BL/6Jマウス (9週齢、雄)に**DS42450411**を30 mg/kg経口投与し、30分後にIL-

6を静脈内投与し、IL-6投与4時間後の血清ヘプシジン濃度を測定した (**Figure 11**)。その結果、0.5％メチルセルロース投与群において有意に血清ヘプシジンが上昇しているのに対し、**DS42450411**投与群では有意に血清ヘプシジン濃度を低下させることが明らかとなった。

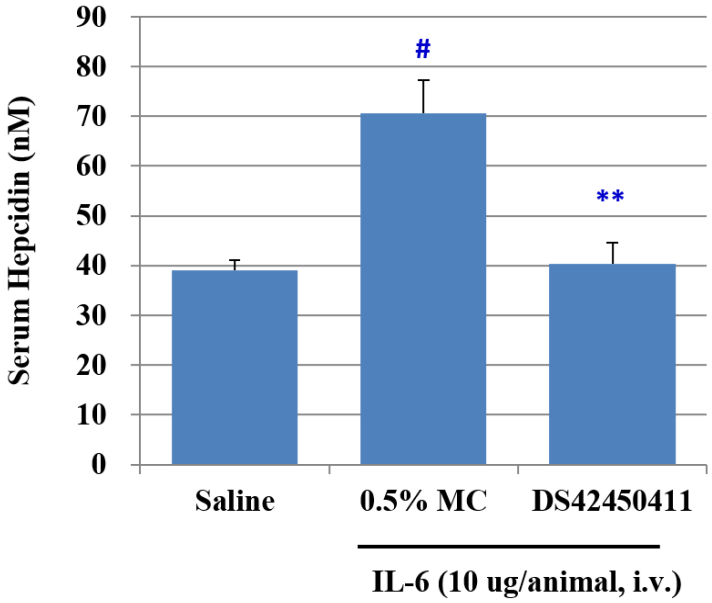


Figure 11. Effect of **DS42450411**. The compound was administered to mice at dose of 30 mg/kg (p.o., 0.5% Methylcellulose, suspension, $n = 4$) before IL-6 treatment. #, $p < 0.05$ vs Saline treated group (t-test), **, $p < 0.01$ vs 0.5% MC treated group (t-test).

第七節　小括

本章では、第一三共株式会社保有の化合物ライブラリーを用いたHTSにより見出したヒット化合物**1**の誘導体展開により、アミノピリミジン誘導体**DS42450411**創製の経緯を述べた。

ヒット化合物**1**の初期誘導体展開により見出した中程度の*in vitro*活性を示すリード化合物**28**のキナーゼプロファイリング評価を実施したところ、CMGCファミリーに属するキナーゼDYRK1Aに高い阻害能を示すことが明らかとなった。

そこで**28**とDYRK1AとのX線結晶構造解析を実施し、空間許容性やアミノ酸残基のデータを基に、更なる*in vitro*活性向上を指向し8位置換基のデザイン・合成を実施した。

その結果、8位置換基として*exo*-メチレンをリンカーに有し、環状アミノ基を配置した誘導体で飛躍的に*in vitro*活性が向上することが明らかとなった。

環状アミノ基の最適化検討の結果、経口薬として優れたPKプロファイルを有し、IL-6誘発高ヘプシジンマウスモデルを用いた*in vivo*薬効評価において経口投与で強力な血清ヘプシジン低下作用を示す**DS42450411**の創製に成功した。

第二章　3-アミノインダゾール母核を有する新規ヘプシジン産生阻害剤の合成と構造活性相関

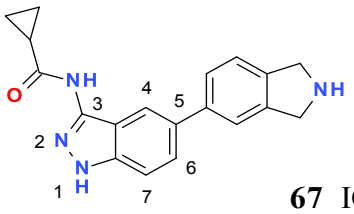
第一節　HTS ヒット化合物 67

前章では、経口薬として優れたPKプロファイルを有し、IL-6誘発高ヘプシジンマウスモデルを用いた*in vivo*薬効評価において経口投与で強力な血清ヘプシジン低下作用を示す4-アミノピリミジン誘導体**DS42450411**創製の経緯を述べた。

しかしながら一連の4-アミノピリミジン誘導体は、マウスに高用量を投与した安全性評価において急性毒性を呈する化合物が散見された。患者さんへの長期投与が可能なより安全性の高い薬剤を開発するために、異なる骨格を持つヘプシジン産生阻害剤の創製を目指すこととした。

そこで、先述のHTSにより見出されたもう一つのヒット化合物である3,5-二置換インダゾール誘導体**67**からの誘導体展開を開始した。

化合物**67**のIC_{50}は3.1 μMと弱く、阻害活性の大幅な向上が必要であった (**Figure 12**)。



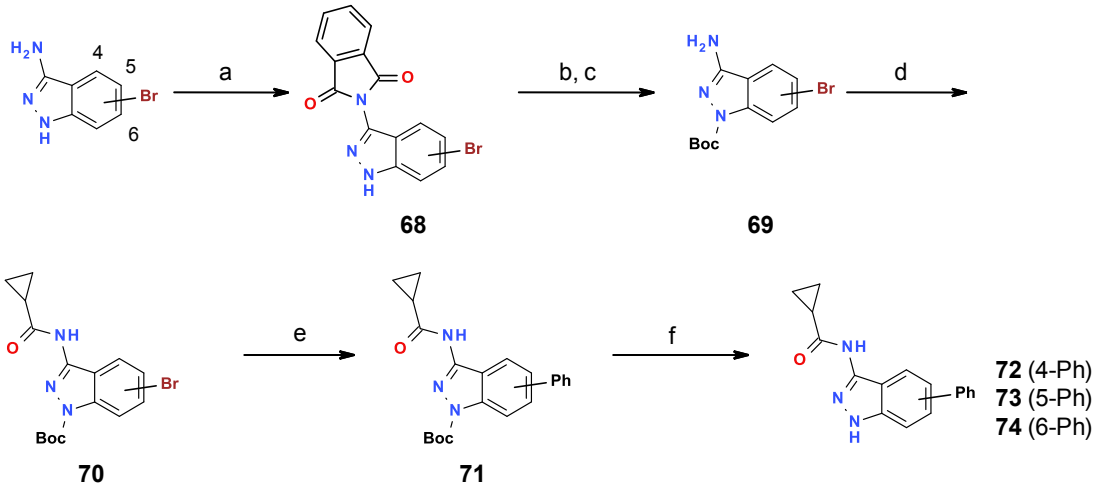
67 IC_{50} = 3.1 μM

Figure 12. Structure of HTS-hit of indazole-based hepcidin production inhibitor **67**

第二節　誘導体の合成法

3-置換インダゾール誘導体

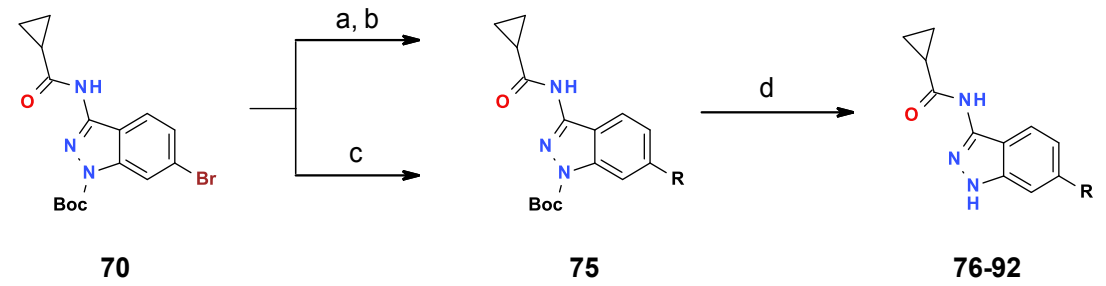
インダゾール4-6位にフェニル基を有する誘導体の合成法を **Scheme 8** に示す。



Scheme 8. Reagents and conditions: (a) Phthalic anhydride, 1,4-dioxane, 57-100%; (b) Boc_2O, Et_3N, DMAP, CH_3CN, 98-99%; (c) Hydrazine monohydrate, EtOH/CH_2Cl_2, 81-82%; (d) Cyclopropanecarbonyl chloride, pyridine, CH_2Cl_2, 49-97%; (e) Phenylboronic acid, Pd(dppf)Cl_2-CH_2Cl_2, K_3PO_4-nH_2O, 1,2-dimethoxyethane/H_2O, 3%-84%; (f) 4N-HCl/1,4-dioxane, 86-95%.

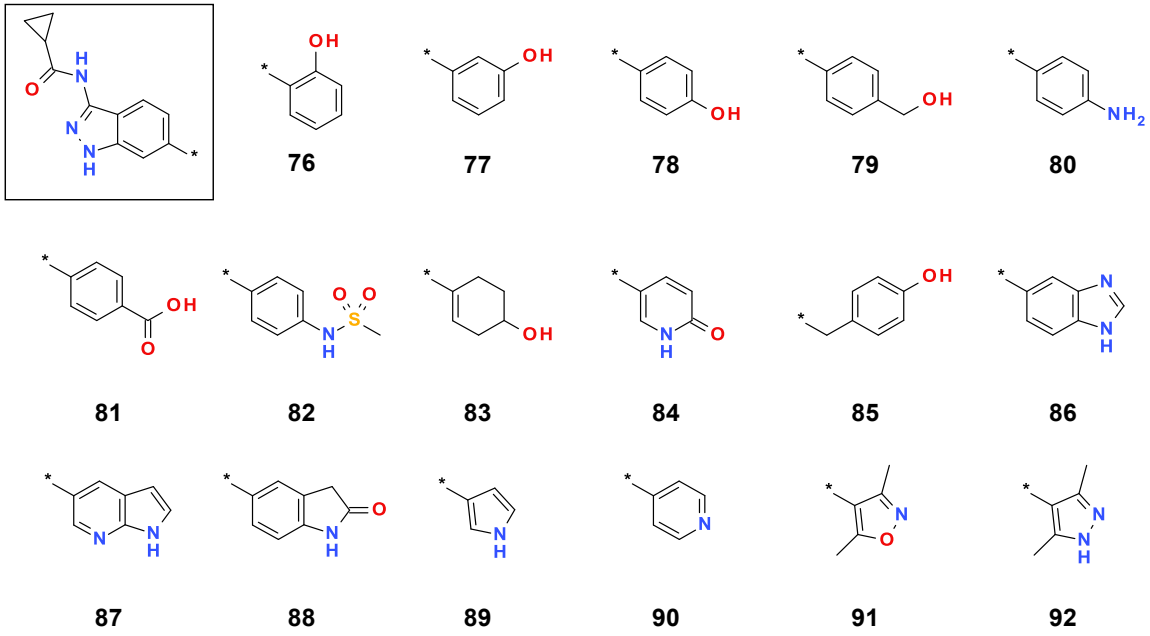
4-6 位にブロモ基をもつ 3-アミノインダゾールの保護基の掛け替えを行い、1 位を Boc 基で保護した中間体 **69** を合成した。中間体 **69** の 3 位アミド化、フェニルボロン酸との鈴木カップリング反応、続く酸性条件での Boc 基の脱保護により目的化合物 **72-74** を合成した。

次に 3 位にシクロプロパンカルボキサミドを有し、6 位に様々な置換基を有するインダゾール誘導体の合成法を **Scheme 9** に示す。



70 **75** **76-92**

Scheme 9. Reagents and conditions: (a) Bis(pinacolato)diboron, Pd(dppf)Cl_2-CH_2Cl_2, KOAc, dioxane, quant.; (b) R-halide or R-OTf, Pd(dppf)Cl_2-CH_2Cl_2, K_3PO_4-nH_2O, 1,2-dimethoxyethane/H_2O, 23-65%; (c) R-boronic acid or R-boronic acid ester, Pd(dppf)Cl_2-CH_2Cl_2, K_3PO_4-nH_2O, 1,2-dimethoxyethane/H_2O, 37-96%; (d) 4N-HCl/1,4-dioxane, 73-95%.



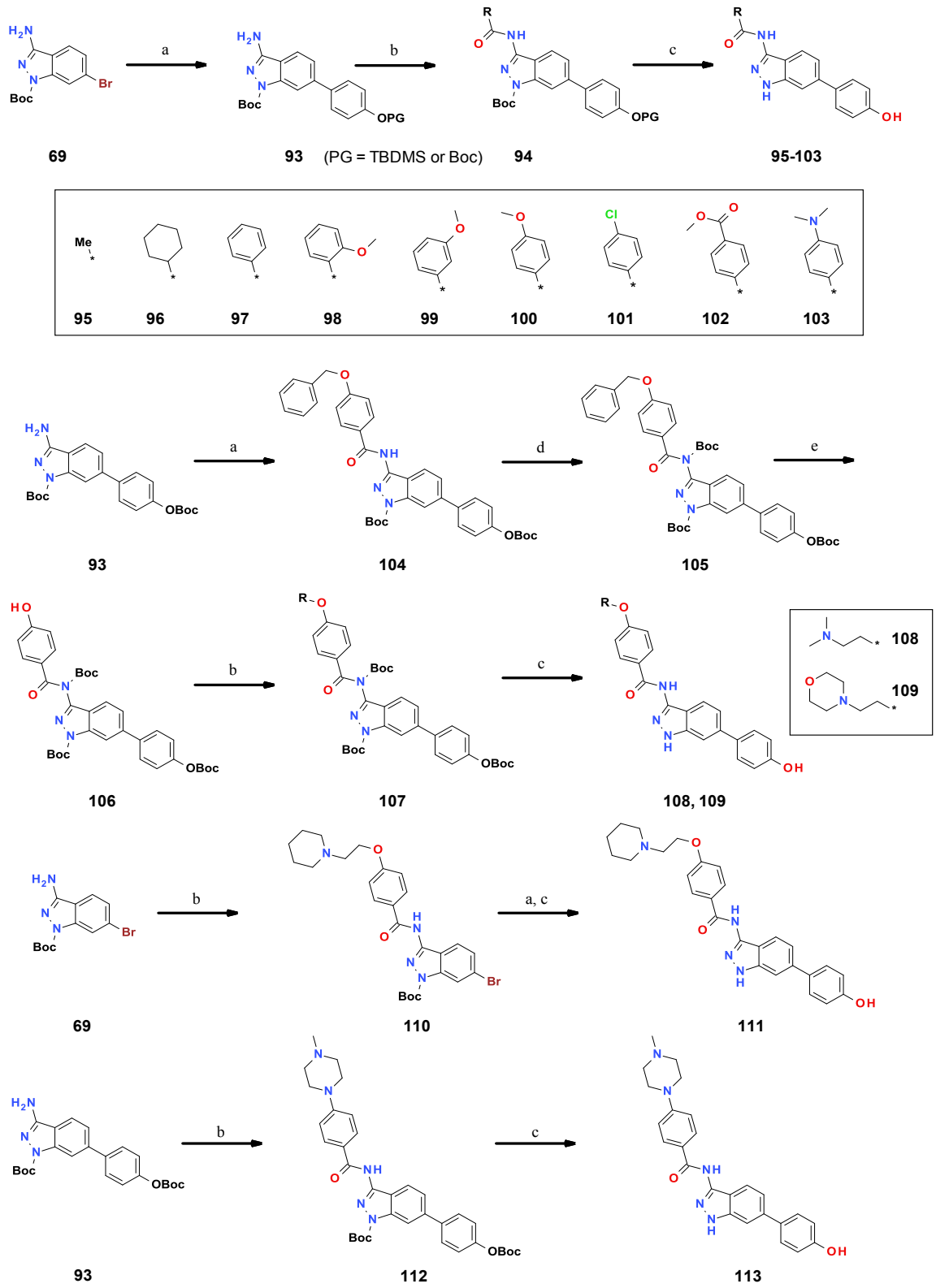
76 **77** **78** **79** **80**

81 **82** **83** **84** **85** **86**

87 **88** **89** **90** **91** **92**

6-ブロモインダゾール中間体 **70**、もしくは **70** より一工程で導くことができるボロン酸ピナコールエステル中間体を用いた鈴木カップリング、続く酸性条件での脱保護反応により、上記目的化合物 **76-92** を得た。

次に 6 位に *p*-ヒドロキシフェニル基を有し、3 位に様々なアミド置換基を有するインダゾール誘導体の合成法を **Scheme 10** に示す。

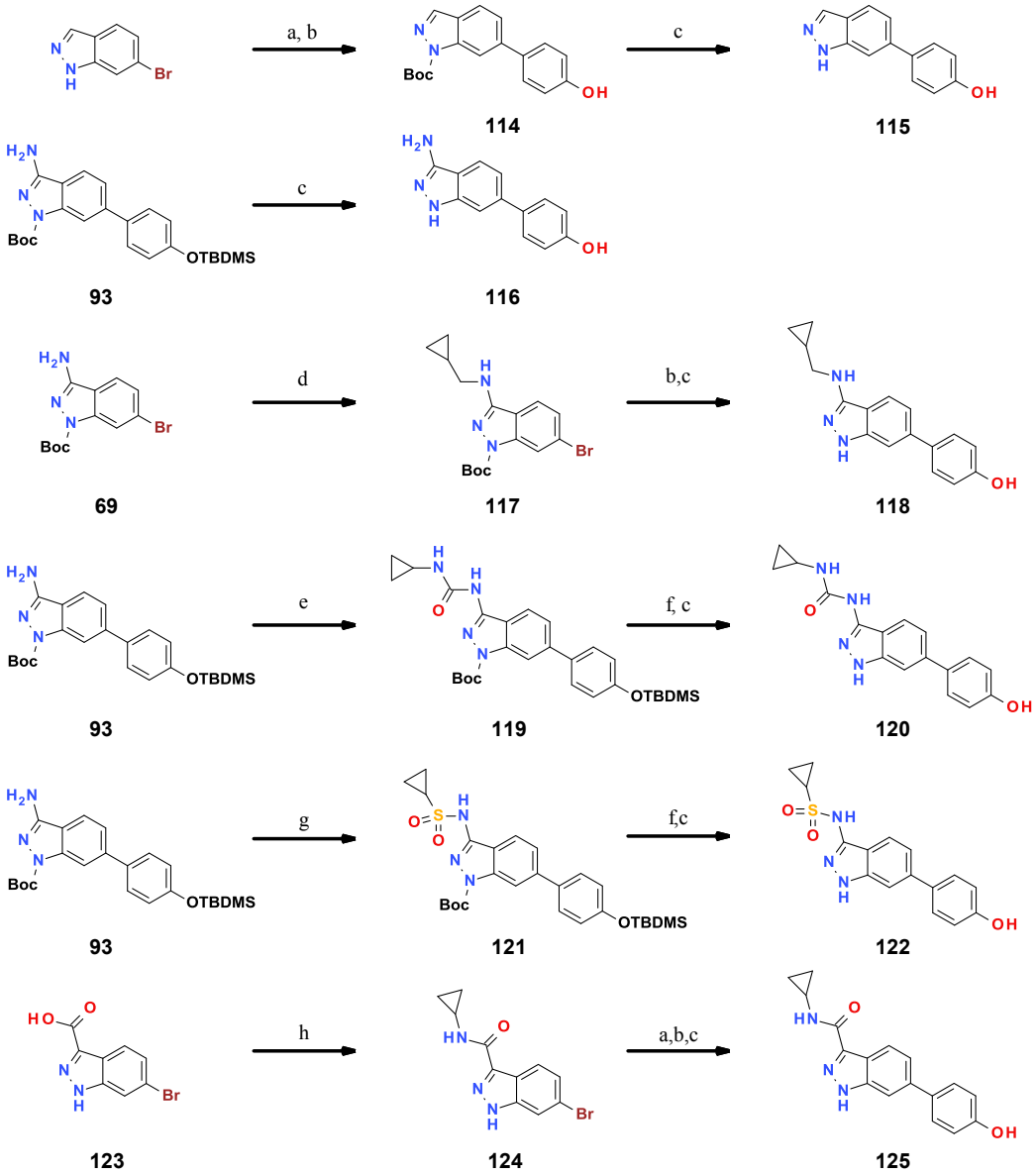


Scheme 10. Reagents and conditions: (a) 4-(*tert*-Butyldimethylsiloxy)phenylboronic acid pinacol ester or 4-(*tert*-Butoxycarbonyloxy)phenylboronic acid pinacol ester, Pd(dppf)Cl_2-CH_2Cl_2, K_3PO_4-nH_2O, 1,2-dimethoxyethane/H_2O, 56-99%; (b) RCOCl, pyridine, CH_2Cl_2, 93-95%; (c) 4N-HCl/1,4-dioxane, 90-94%; (d) Boc_2O, Et_3N, DMAP, CH_3CN, 50-59%; (e) Pd-C / H_2, EtOH/CH_2Cl_2, 83%; (f) R-OH, 1,1'-(Azodicarbonyl)dipiperidine, *n*-Bu_3P, THF, 66-92%.

中間体 **69** とヒドロキシ基を保護した *p*-ヒドロキシフェニルボロン酸の鈴木カップリング反応により、3 位アミノインダゾール中間体 **93** を合成した。中間体 **93** に対して、種々の酸クロリドを反応させ、続く酸性条件下での脱保護反応により、上記目的化合物 **95-103** を得た。また、中間体 **93** に *p*-ベンジルオキシ安息香酸クロリドを作用し、インダゾール 3 位アミド基の NH を Boc 基で保護した後、ベンジル基を脱保護することにより、*p*-ヒドロキシフェニル中間体 **106** を合成した。中間体 **106** を 1,1'-(アゾジカルボニル)ジピペリジン/トリブチルホスフィンを用いた光延反応、続く酸性条件下での三箇所の脱 Boc 化により化合物 **108, 109** を合成した。また、化合物 **111**、**113** は *para*-位に置換基を有する安息香酸クロリドを用いたアミド化により数工程で合成した。

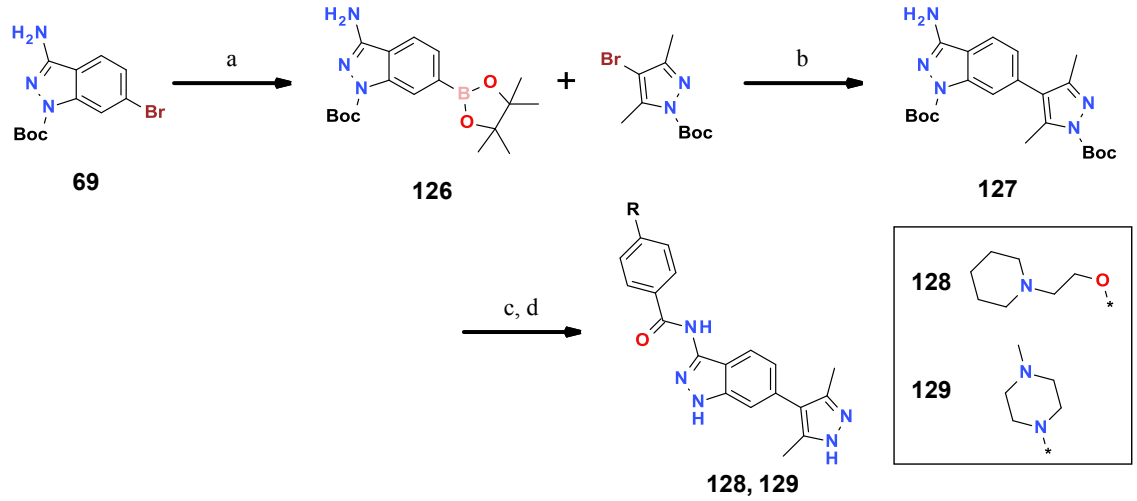
次に 6 位に *p*-ヒドロキシフェニル基を有し、3 位にアミド基以外の様々な置換基を有するインダゾール誘導体の合成法を **Scheme 11** に示す。

市販の 6-ブロモインダゾールの 1 位を Boc 基で保護し、*p*-ヒドロキシフェニルボロン酸の鈴木カップリング反応、続く脱保護により、3-無置換誘導体 **115** を合成した。中間体 **93** を酸性条件下で TBDMS 基の脱保護を行い、3-アミノ誘導体 **116** を合成した。中間体 **69** にシクロプロパンカルボキシアルデヒドを用いた還元的アミノ化反応により 3-シクロプロピルメチルアミノ誘導体 **118** を合成した。前述の中間体 **93** にシクロプロピルイソシアネート、もしくはスルホニルクロリドを反応させ、続く脱保護を行うことによりウレア誘導体 **120** 及びスルホンアミド誘導体 **122** を合成した。6-ブロモインダゾール-3-カルボン酸を出発原料に、CDI を用いたシクロプロピルアミンとのアミド化反応により、リバースアミド誘導体 **125** を合成した。



Scheme 11. Reagents and conditions: (a) Boc_2O, Et_3N, DMAP, CH_3CN, 50-59%; (b) 4-Hydroxyphenylboronic acid pinacol ester, Pd(dppf)Cl_2-CH_2Cl_2, K_3PO_4-nH_2O, 1,2-dimethoxyethane/H_2O, 56-97%; (c) 4N-HCl/1,4-dioxane, 90-94%; (d) Cyclopropanecarboxaldehyde, Sodium triacetoxyborohydride, CH_2Cl_2, 62%; (e) Cyclopropyl isocyanate, *N,N*-Diisopropylethylamine, THF, 39%; (f) TBAF, THF, 76-97%; (g) Cyclopropanesulfonyl chloride, pyridine, 39%; (h) Cyclopropylamine, CDI, Et_3N, *N,N*-Dimethylformamide, 64%.

次に、インダゾール 6 位にジメチルピラゾール基を有し、3 位にアミド置換基を有する化合物の合成ルートを **Scheme 12** に示す。



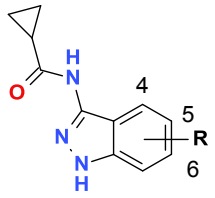
Scheme 12. Reagents and conditions: (a) Bis(pinacolato)diboron, Pd(dppf)Cl_2-CH_2Cl_2, KOAc, dioxane, quant.; (b) Pd(dppf)Cl_2-CH_2Cl_2, K_3PO_4-nH_2O, 1,2-dimethoxyethane/H_2O, 56-97%; (c) R-COCl, pyridine, CH_2Cl_2, 78-84%; (d) 4N-HCl/1,4-dioxane, 86-93%.

中間体 **69** を 6-ボロン酸ピナコールエステル中間体 **126** に導いた後、市販の Boc 保護されたブロモジメチルピラゾールを鈴木カップリング反応させることにより、3-アミノ中間体 **127** を合成した。中間体 **127** に酸クロリドを反応し、酸性条件下で脱保護することにより、目的化合物 **128, 129** を合成した。

第三節　腹腔内投与で *in vivo* 薬効を示すリード化合物 109 の創出

はじめに、ヒット化合物 **67** のインダゾール母核の最適な置換位置を検証するために、単純化したベンゼン環を 4 位、5 位、及び 6 位に置換した誘導体(**72-74**)を評価した (**Table 12**)。

Table 12. SAR of substituted indazole derivatives



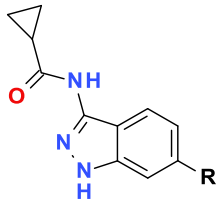
compound	R	inhibition (%) at 30 μM
72	4-Ph	8
73	5-Ph	-4
74	6-Ph	23

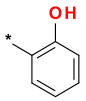
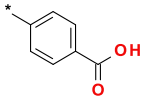
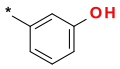
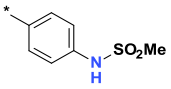
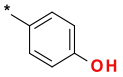
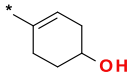
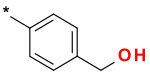
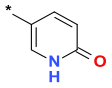
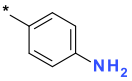
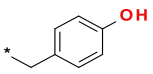
その結果、置換位置として 6 位が好ましいことが明らかとなった。

次に、インダゾール 6 位に置換ベンゼンを中心に、様々な置換基の導入を検討した結果を **Table 13** に示す。

meta-ヒドロキシフェニル誘導体 **77** は中程度の活性を示した一方で、*para*-ヒドロキシフェニル誘導体 **78** は非常に高い *in vitro* 活性を示すことが明らかとなった。そこで *para*-位に様々な官能基を有するベンゼン環の検討を行った。ベンジルアルコール (**79**)、及びアニリン誘導体 (**80**)は **78** に比べて活性が減弱する結果であった。またフェノールと同様に酸性プロトンを有する安息香酸 (**81**)、スルホンアミド誘導体 (**82**)は活性が完全に消失した。更に、脂肪族アルコール (**83**)、ピリドン (**84**)、及びメチレンリンカーを介した *para*-ヒドロキシフェニル誘導体 (**85**)はいずれも活性が消失する結果であった。

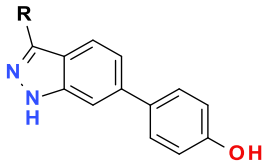
Table 13. SAR of 6-substituted indazole derivatives

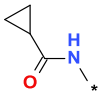
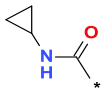
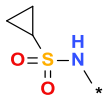
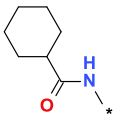
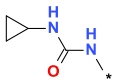
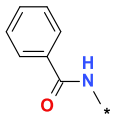


compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM)
76		>30	**81**		>10
77		4.9	**82**		>30
78		0.40	**83**		>30
79		1.4	**84**		>30
80		4.5	**85**		>30

インダゾール環の 6 位置換基として *para*-ヒドロキシフェニル基が好ましいことが明らかとなったので、続いてインダゾール 3 位の変換を実施することとした (**Table 14**)。

Table 14. SAR of 3-substituted indazole derivatives



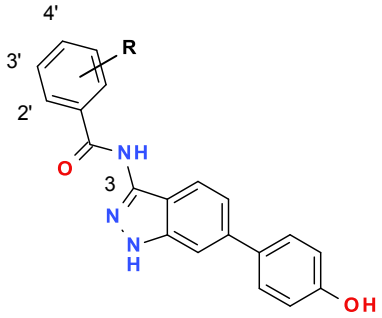
compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM)
78		0.40	**125**		4.2
115		>3.0	**122**		>10
116		1.0	**95**		1.1
118		1.6	**96**		0.93
120		0.94	**97**		0.47

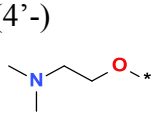
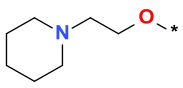
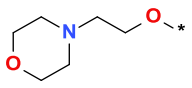
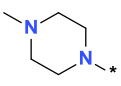
インダゾール 3 位無置換体 **115** は *in vitro* 活性が低下した。一級、及び二級アミン誘導体 **(116, 118)**、シクロプロピルウレア誘導体 **120**、及びリバースアミド型誘導体 **125** は活性がやや減弱した。一方で、スルホンアミド誘導体 **122** の活性は完全に消失する結果であった。また 3 位アミド基としてアセトアミド、シクロヘキシルアミド、及びベンズアミド誘導体 **(95-97)**を比較した結果、空間許容性が確認できた。**78** と **97** がともに高い *in vitro* 活性を示したことから、sp^2 性

炭素を有するアミド置換基が好適であることが示唆された。

次に、更なる活性向上を目指してベンズアミド誘導体のベンゼン環上の置換基の最適化を実施した (**Table 15**)。

Table 15. SAR and metabolic stability of 3-benzamide indazole derivatives



compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM) / MS (%)[a]
97	H	0.47	**103**	4'-NMe_2	0.33
98	2'-OMe	>30	**108**	(4'-) 	0.16
99	3'-OMe	0.70	**111**	(4'-) 	0.12 / 66
100	4'-OMe	0.42	**109**	(4'-) 	0.13 / 89
101	4'-Cl	0.98	**113**	(4'-) 	0.085 / 25
102	4'-CO_2Me	1.1			

[a] Remaining (%) of the tested compound after 0.5 h incubation with mouse liver microsome (0.5 mg/mL).

ortho、*meta*、及び*para*-位にメトキシ基を導入したところ、*para*-位が好適である

ことが分かった (**98-100**)。また*para*-位にクロロ基、メトキシカルボニル基、及びジメチルアミノ基を導入した誘導体 (**101-103**)を比較したところ、電子供与性が好ましいことが明らかとなった。

さらにベンゼン環*para*-位の電子供与性置換基の最適化を実施した結果、エトキシリンカーを介して環状アミンを有する誘導体**109, 111**、及び*N*-メチルピペラジン環を持つ**113**が非常に高い*in vitro*活性を示すことを見出した。

高い*in vitro*活性とCYP代謝安定性を両立した**109**のPKパラメータを**Table 16**に示す。化合物**109**はマウスに30 mg/kg 腹腔内投与 (i.p.)することにより、高い血中薬物濃度を示した。

Table 16. Physicochemical property and PK parameters of **109**

LogD	MS[a] (%)	Cmax[b] (μg/mL)	Tmax[b] (h)	AUC[b] (h*μg/mL)
3.4	89	2.64	1.33	6.93

[a] Remaining (%) of the tested compound after 0.5 h incubation with mouse liver microsome (0.5 mg/mL).

[b] Average of two values administered at 30 mg/kg i.p. to C57BL/6J mice (0.5% Msokodeethylcellulose suspension).

そこで、前述のIL-6誘発高ヘプシジンマウスモデルを用いて評価したところ、化合物**109**は30 mg/kg腹腔内投与において、強力な*in vivo*ヘプシジン産生抑制作用を示すことが明らかとなった (**Figure 13**)。

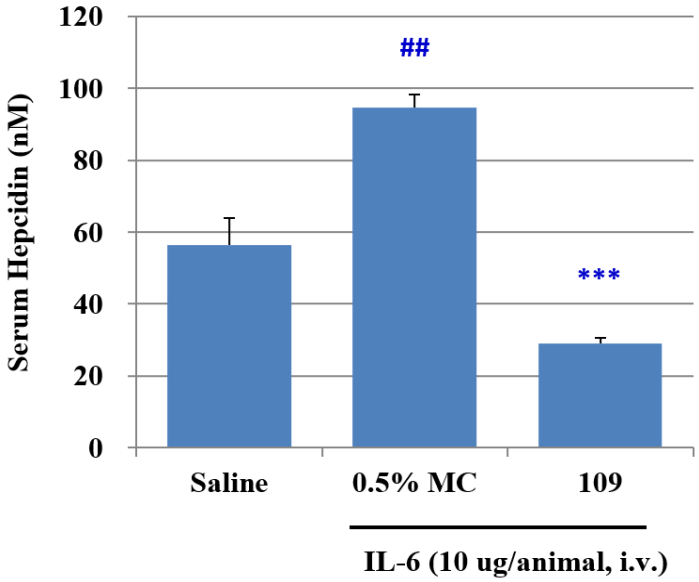


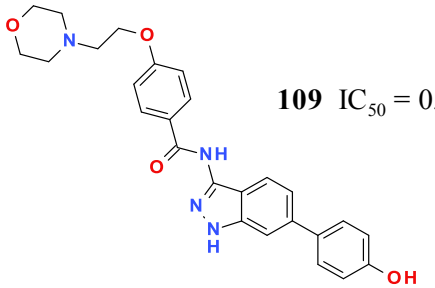
Figure 13. Effect of compound **109.** The compound was administered to an IL-6 pretreated mouset at doses of 30 mg/kg (i.p., 0.5% Methylcellulose, suspension, $n = 4$) . ##, $p < 0.01$ vs saline treated group (t-test), ***, $p < 0.001$ vs 0.5% MC treated group (t-test).

第四節　経口投与で *in vivo* 薬効を示す化合物 **129** の創出

前節では、HTS ヒット化合物 **67** の誘導体展開により、IL-6 誘発高ヘプシジンマウスモデルにおいて腹腔内投与で *in vivo* 薬効を示す **109** 創出の経緯を述べた。

しかしながら化合物 **109** は、30 mg/kg 経口投与時の血中暴露量が腹腔内投与の約 1/8 と低く、経口薬として満足できるプロファイルを有していないことが明らかとなった (**Table 17**)。

Table 17. IC_{50} value and physicochemical properties and PK parameters of **109**



109 IC_{50} = 0.13 μM

LogD	MS[a] (%)	UGT[b] (%)	Cmax (μg/mL)	Tmax (h)	AUC (h*μg/mL)
3.4	89	76	2.64[c]	1.33[c]	6.93[c]
			0.32[d]	1.50[d]	0.89[d]

[a] Remaining (%) of the tested compound after 0.5 h incubation with mouse liver microsomes (0.5 mg/mL).

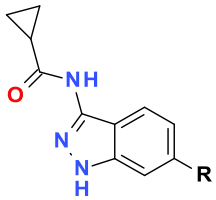
[b] Remaining (%) of the tested compound after 0.5 h incubation with mouse liver microsomes (0.5 mg/mL) and UGT reactionmix solution.

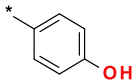
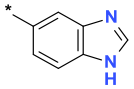
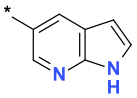
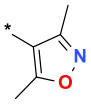
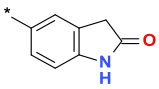
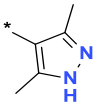
[c] Average of two values dosed at 30 mg/kg i.p. with C57BL/6J mice (0.5% Methylcellulose, suspension).

[d] Average of two values dosed at 30 mg/kg p.o. with C57BL/6J mice (0.5% Methylcellulose, suspension).

化合物 **109** の示す経口投与時の低血中暴露量の原因は、高い *in vitro* 活性の発現に必須であるインダゾール母核 6 位の *para*-ヒドロキシフェニル基が UDP-glucuronosyltransferase (UGT)により抱合代謝を受けることであると考え、*para*-ヒドロキシフェニル基の代替置換基の探索を行うこととした (**Table 18**)。

Table 18. Alternatives of *para*-hydroxyphenyl group



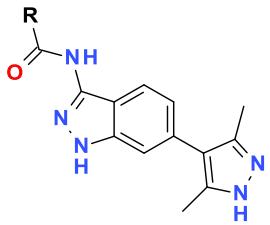
compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM)
78		0.40	**89**		>30
86		>3	**90**		7.2
87		11	**91**		1.0
88		>30	**92**		0.33

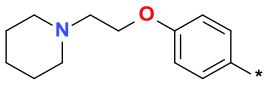
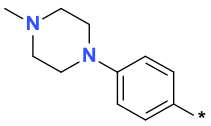
始めにフェノール性ヒドロキシ基の生物学的等価体 (bioisostere)として知られている二環式複素環への変換を行った[24]。フェノール性ヒドロキシ基と類似の酸性度をもちH-bond donorとして機能するようにデザインされたベンゾイミダゾール (**86**)、ピロロピリジン (**87**)、及びオキシインドール誘導体 (**88**)はいずれも大幅な*in vitro*活性の低下を示した。

次に単環複素環への変換を検討した。ピロール環 (**89**)、及び4-ピリジン環へ変換した誘導体 (**90**)は大幅に活性が減弱した。しかしながら、イソキサゾール誘導体**91**は中程度の活性を示した。さらに3,5-ジメチルピラゾール誘導体**92**は化合物**109**と同等の*in vitro*活性を示すことが明らかとなった。

そこで、インダゾール6位に*para*-ヒドロキシフェニル基をもつ誘導体において*in vitro*活性向上に寄与した3位置換基を**92**に導入した誘導体**128, 129**を検討したところ、これらは高い*in vitro*活性を示すことが明らかとなった (**Table 19**)。

Table 19. SAR and metabolic stability of 6-dimethylpyrazole derivatives



compound	R	IC_{50} (μM) / MS (%)[a]
92		0.33 / 7
128		0.26 / 70
129		0.23 / 80

[a] Remaining (%) of the tested compound after 0.5 h incubation with mouse liver microsome (0.5 mg/mL).

新たに見出した二置換ピラゾール誘導体**129**のPKパラメータを**Table 20**に示す。化合物**109**と比較しUGT代謝に対する安定性が大幅に改善し、30 mg/kg経口投与時の血中薬物濃度は大幅に向上することが明らかとなった。

Table 20. Physicochemical properties and PK parameters of **129**

LogD	MS[a] (%)	UGT[b] (%)	Cmax[c] (μg/mL)	Tmax[c] (h)	AUC[c] (h*μg/mL)
2.4	80	94	0.92	3.25	2.55

[a] Remaining (%) of the tested compound after 0.5 h incubation with mouse liver microsomes (0.5 mg/mL).

[b] Remaining (%) of the tested compound after 0.5 h incubation with mouse liver microsomes (0.5 mg/mL) and UGT reactionmix solution.

[c] Average of two values dosed at 30 mg/kg p.o. with C57BL/6J mice (0.5% Methylcellulose, suspension).

そこで、前述のIL-6誘発高ヘプシジンマウスモデルを用いて評価したところ、化合物**129**は30 mg/kg経口投与において、強力な*in vivo*ヘプシジン産生抑制作用を示すことが明らかとなった (**Figure 14**)。

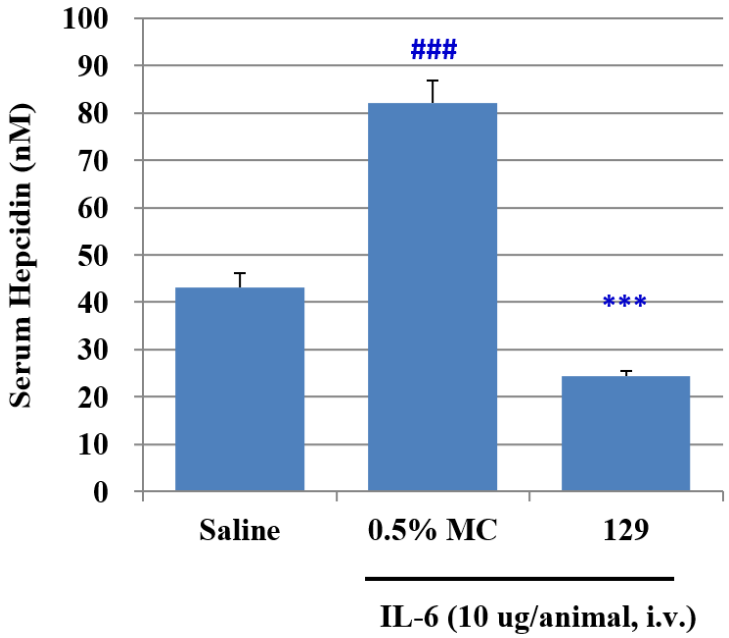


Figure 14. Effect of compound **129.** The compound was administered to mice at dose of 30 mg/kg (p.o., 0.5% Methylcellulose, suspension, n = 4) before IL-6 treatment. ###, $p < 0.001$ vs Saline treated group (t-test), ***, $p < 0.001$ vs 0.5% MC treated group (t-test).

第五節　小括

本章では、HTSヒット化合物**67**の誘導体展開により、経口投与で*in vivo*薬効を示す化合物**129**を創出した経緯を述べた。

化合物 **67** の初期誘導体展開で見出したインダゾール 6 位に *para*-ヒドロキシフェニル基を有する化合物 **109** は IL-6 誘発高ヘプシジンマウスモデルを用いた *in vivo* 薬効評価において、腹腔内投与で血清ヘプシジン低下作用を示すものの経口薬として満足のいくプロファイルを有していなかった。そこで、化合物 **109** の 6 位フェノール性ヒドロキシ基の代替基探索により経口吸収性を改善し、経口投与で高い *in vivo* 薬効を示す化合物 **129** を創出した。

第三章　3-アミノベンゾイソキサゾール母核を有する新規ヘプシジン産生阻害剤の合成と構造活性相関

第一節　マルチキナーゼ阻害能回避を指向した化合物デザイン

前章では、化合物 **109** の *para*-ヒドロキシフェニル基の代替基探索により、経口投与時の血中暴露量を改善し、IL-6 誘発高ヘプシジンマウスモデルにおいて経口投与で *in vivo* 薬効を示す **129** を創出した経緯を述べた。

そこで、第一章で述べた 4-アミノピリミジン誘導体が示す DYRK1A をはじめとする CMGC ファミリーに属するキナーゼ阻害能の有無を確認するために化合物 **129** のキナーゼプロファイリング評価を実施した (216 キナーゼ)。その結果、**129** は DYRK1A への阻害能は低かった(6.3 % inhibition @ 0.45 μM)。このことから前章の 4-アミノピリミジン誘導体とは異なるメカニズムでヘプシジン阻害活性を発現していると考えられる。一方で多くのキナーゼに対し阻害能を持つことが明らかとなった (**Figure 15**)。

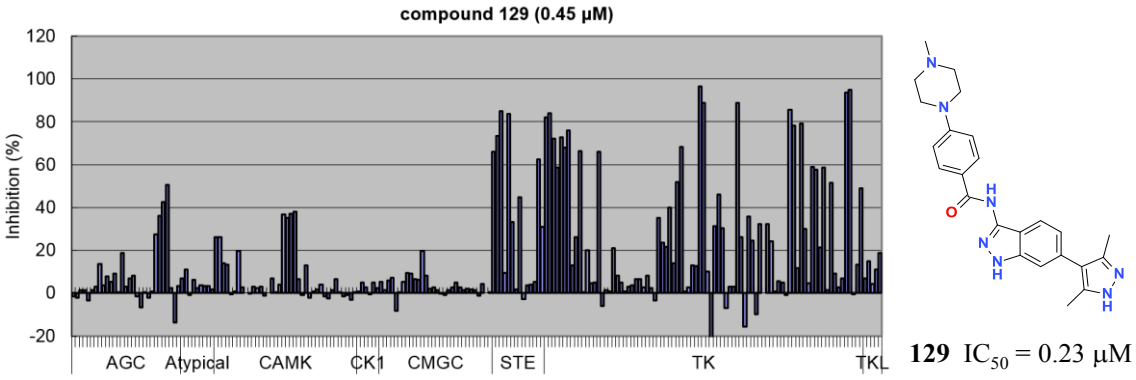


Figure 15. IC_{50} value and kinase inhibitory profiles of compound **129**

マルチキナーゼ阻害剤は慢性疾患の治療薬としては適していないと考えられているため[25]、キナーゼ阻害活性を低下させることが必須と考えた。

一般的に生物学的認識プロセスにおいて、水素結合は最も重要な特異的相互作用の 1 つであると考えられている。特にキナーゼ阻害剤の多くは Adenosine triphosphate (ATP) との拮抗作用に基づくものが多く、ATP とキナーゼの結合に重要と考えられるのがヒンジ結合部位である。キナーゼに対する強力な阻害能の発現にはヒンジ結合部位の主鎖のアミド結合との強固な水素結合が不可欠であると考えられている [26]。

我々は、化合物 **129** の 3-アミノインダゾール母核がキナーゼ阻害能を発現する主因と考えた (**Figure 16**)。

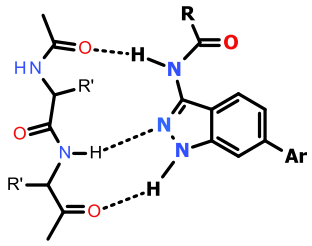


Figure 16. Estimated interaction between hihge binding site and 3-aminindazole moiety

そこで、ヒンジ結合部位と強固な水素結合を形成していると考えられる 3 つの部位を変換した 3 種類の scaffold をデザインした (**Figure 17**)。

一つ目は、インダゾール 3 位にアリール基を導入することで NH 基を除去した 3-アリールインダゾール誘導体、二つ目は、3 位置換基を 4 位に移動させた 4-アミノインダゾール誘導体、そして三つ目は、インダゾール 1 位の窒素原子を酸素原子に置き換えた 3-アミノベンゾイソキサゾール誘導体である。

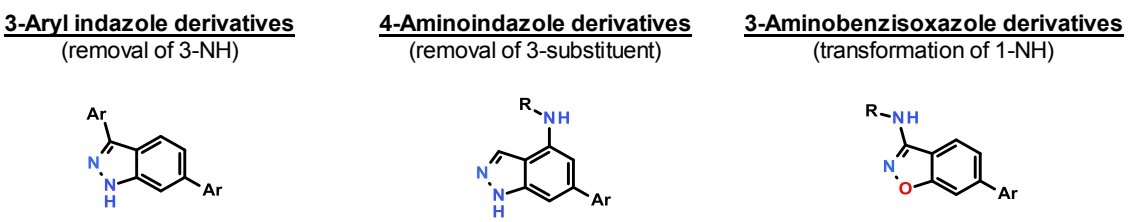


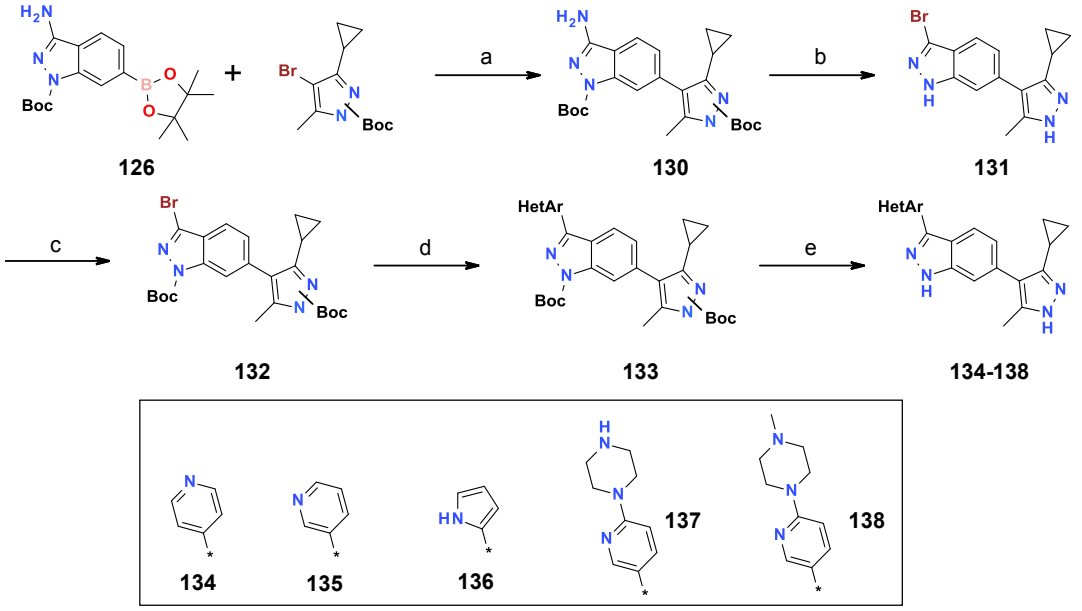
Figure 17. Newly designed scaffolds

第二節　誘導体の合成法

3 位に芳香族複素環を有するインダゾール誘導体

インダゾール 3 位に芳香族複素環を有する化合物の合成ルートを **Scheme 13** に示す。

第二章第二節に記載の中間体 **126** より合成した 3-アミノインダゾール中間体 **130** を臭化銅-亜硝酸ナトリウム-臭化水素酸水溶液を用いた Sandmeyer 反応により、3-ブロモインダゾール中間体 **131** を合成した。インダゾール 1 位及び 6 位ピラゾリル基を Boc 基で再保護し、種々のアリールボロン酸試薬との鈴木カップリング反応、続く脱保護反応を行うことにより、目的化合物 **134-138** を合成した。

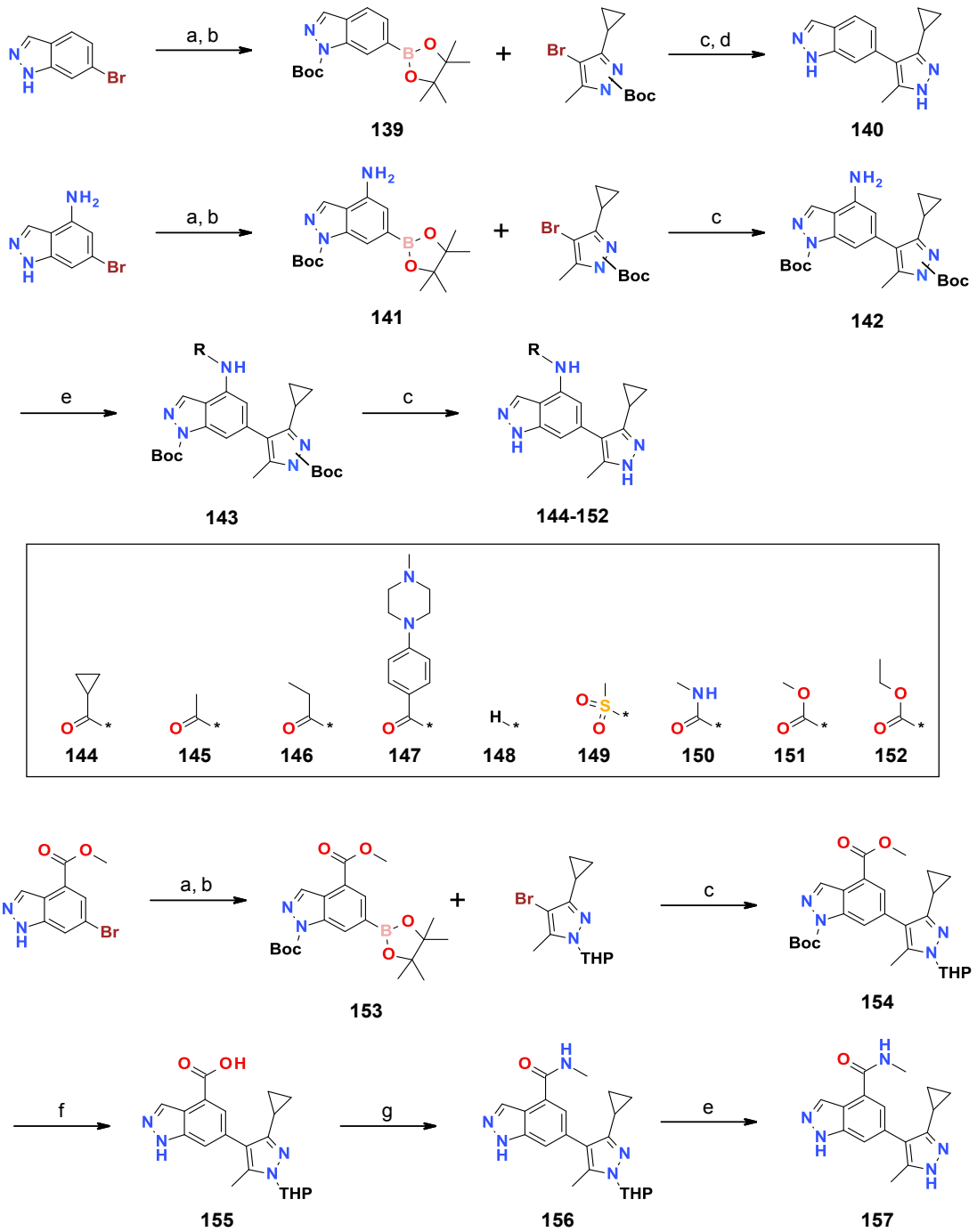


Scheme 13. Reagents and conditions: (a) $Pd(dppf)Cl_2$-CH_2Cl_2, K_3PO_4-nH_2O, 1,2-dimethoxyethane/H_2O, 91%; (b) CuBr, $NaNO_2$, HBr aq., AcOH/H_2O, 29%; (c) Boc_2O, Et_3N, DMAP, CH_3CN, 89%; (d) Heteroarylboronic acid, $Pd(dppf)Cl_2$-CH_2Cl_2, K_3PO_4-nH_2O, 1,2-dimethoxyethane/H_2O, 46-83%; (4) 4N-HCl/1,4-dioxane, 71-97%. (The Boc protected compounds were isolated as a single regioisomer, but the position of Boc groups were not determined.)

4 位に置換基を有するインダゾール誘導体

6 位にシクロプロピルメチルピラゾリル基を有し、4 位に様々な置換基を有す

るインダゾール誘導体の合成法を **Scheme 14** に示す。

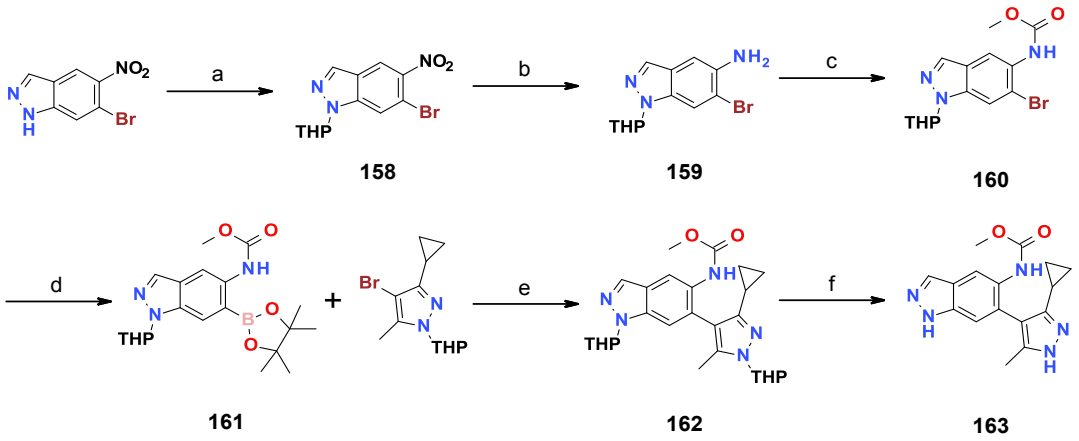


Scheme 14. Reagents and conditions: (a) Boc_2O, Et_3N, DMAP, CH_3CN, 55%; (b) Bis(pinacolato) diboron, Pd(dppf)Cl_2-CH_2Cl_2, KOAc, 1,4-dioxane, 99%; (c) Pd(dppf)Cl_2-CH_2Cl_2, K_3PO_4-nH_2O, 1,2-dimethoxyethane/H_2O, 72%; (d) 4N-HCl/1,4-dioxane, 16-90%; (e) Acyl chloride, pyridine, CH_2Cl_2 or Alkyl chloroformate, pyridine, CH_2Cl_2 or *p*-Nitrophenyl chloroformate, pyridine, then $MeNH_2$ hydrocholide - Et_3N, THF or Methanesulfonyl chloride, pyridine, CH_2Cl_2, 27-61%; (f) aqueous NaOH, THF-MeOH, 99%; (g) $MeNH_2$ hydrochoride, DMT-MM, *N*-Methylmorpholine, THF-MeOH, 68%. (The Boc protected compounds were isolated as a single regioisomer, but the position of Boc groups were not determined.)

市販の 6-ブロモインダゾールを出発原料に 1 位を Boc 基で保護した 6-ボロン酸ピナコールエステル中間体 **139** を合成し、ブロモピラゾールとの鈴木カップリング反応、続く脱保護反応により 4 位無置換体 **140** を合成した。また、市販の 4-アミノ-6-ブロモインダゾールを出発原料に、鈴木カップリング反応により 4-アミノインダゾール中間体 **142** を合成した。中間体 **142** をアミド化、スルホニル化、ウレア化、もしくはカーバメート化した後に脱保護することにより、目的物 **144-152** を合成した。市販の 6-ブロモインダゾール-4-カルボン酸メチルを出発原料に、同様の反応により 4-カルボン酸中間体 **155** を合成し、続くアミド化、脱保護反応によりリバースアミド誘導体 **157** を合成した。

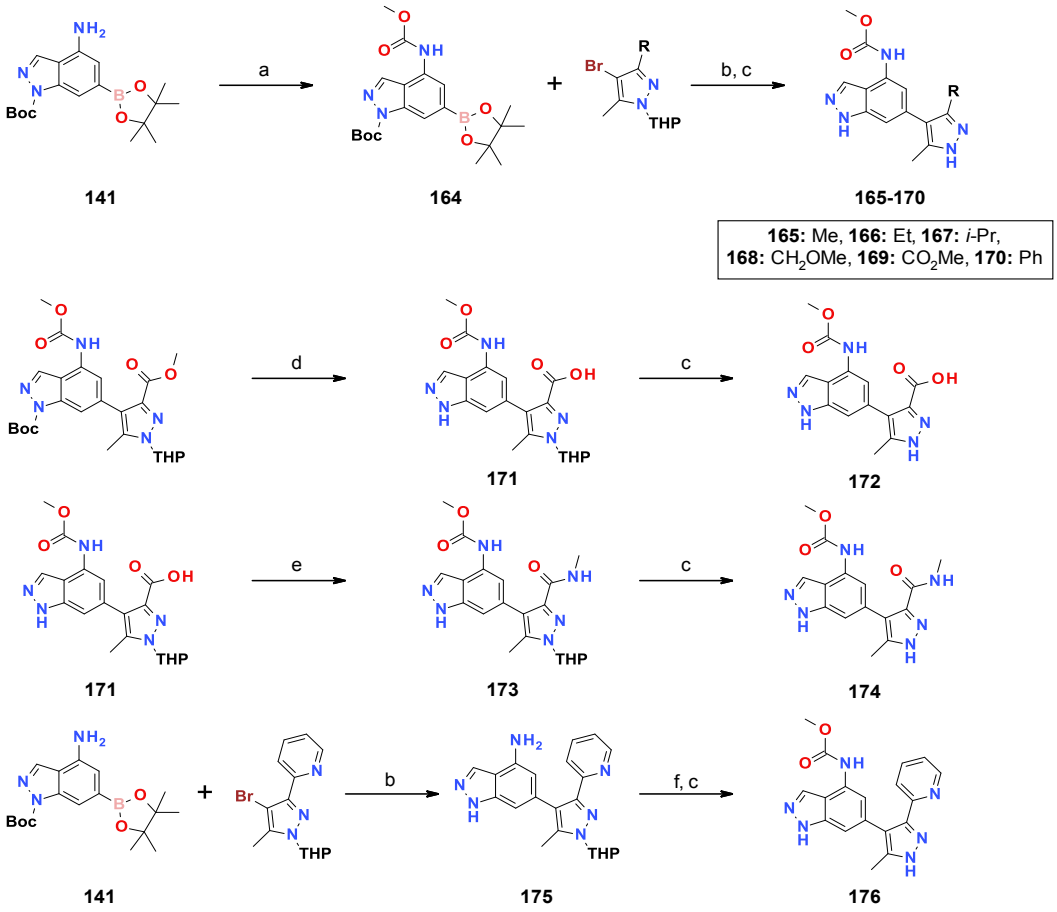
次に、6 位にシクロプロピルメチルピラゾリル基を有し、5 位に置換基を有するインダゾール誘導体の合成法を **Scheme 15** に示す。

6-ブロモ-5-ニトロインダゾールの 1 位を THP で保護した後、鉄を用いた還元反応により 5-アミノインダゾール中間体 **159** を合成した。中間体 **159** をメチルカーバメート化、6 位ピラゾール環の導入、続く酸性条件下での THP 基の脱保護を行い、目的化合物 **163** を合成した。



Scheme 15. Reagents and conditions: (a) Dihydropyran, *p*-TsOH monohydrate, THF, 87%; (b) Fe, NH_4Cl, $EtOH/H_2O$, 85%; (c) Methyl chloroformate, pyridine, CH_2Cl_2, 83%; (d) Bis(pinacolato)diboron, Pd(dppf)Cl_2-CH_2Cl_2, KOAc, 1,4-dioxane, 64%; (e) Chloro(2-dicyclohexylphosphino-2',4',6'-triisopropyl-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II), K_3PO_4, 1,4-dioxane/H_2O, 54%; (f) 4N-HCl/1,4-dioxane, 72%.

次に 4 位にメトキシカルボニルアミノ基を有し、6 位に様々な置換基を有するインダゾール誘導体の合成法を **Scheme 16** に示す。



Scheme 16. Reagents and conditions: (a) Methyl chloroformate, pyridine, CH_2Cl_2, 92%; (b) Chloro(2-dicyclohexylphosphino-2',4',6'-triisopropyl-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II), K_3PO_4, 1,4-dioxane/H_2O, 54%; (c) 4N-HCl/1,4-dioxane, 31-90%; (d) aqueous NaOH, 1,4-dioxane, 68%; (e) $MeNH_2$ hydrochoride, DMT-MM, *N*-Methylmorpholine, THF/MeOH, 77%; (f) Methyl chloroformate, pyridine, CH_2Cl_2, 71%.

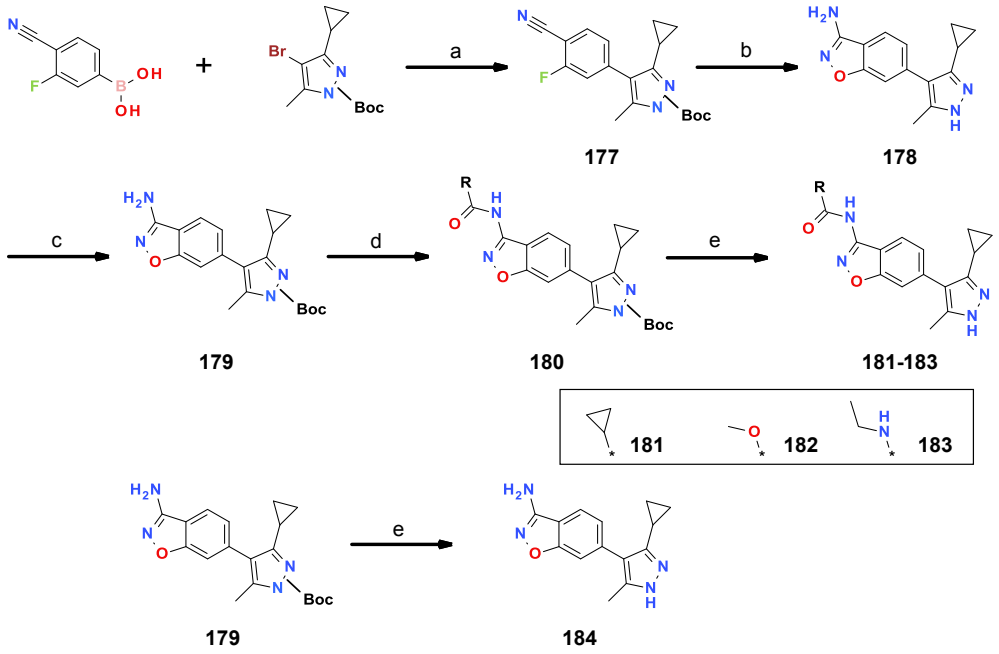
前述の中間体 **141** とクロロギ酸メチルとの反応によって得られた **164** に対し、THP で保護した種々のブロモピラゾール [27] との鈴木カップリング反応と続く酸性条件下での Boc 基と THP 基の脱保護により、目的物 **165-170** を合成した。また、メチルエステル中間体のアルカリ加水分解反応により合成したカルボン酸中間体 **171** を脱保護することでカルボン酸誘導体 **172** を、またカルボン酸中間体 **171** に対し縮合剤に DMT-MM を用いてメチルアミンと縮合後、脱保護することによりメチルカルバモイル誘導体 **174** を合成した。化合物 **176** はア

ミノピナコールエステル中間体 **141** の鈴木カップリング反応、メチルカーバメート化、続く THP 基の脱保護により合成した。

ベンゾイソキサゾール誘導体

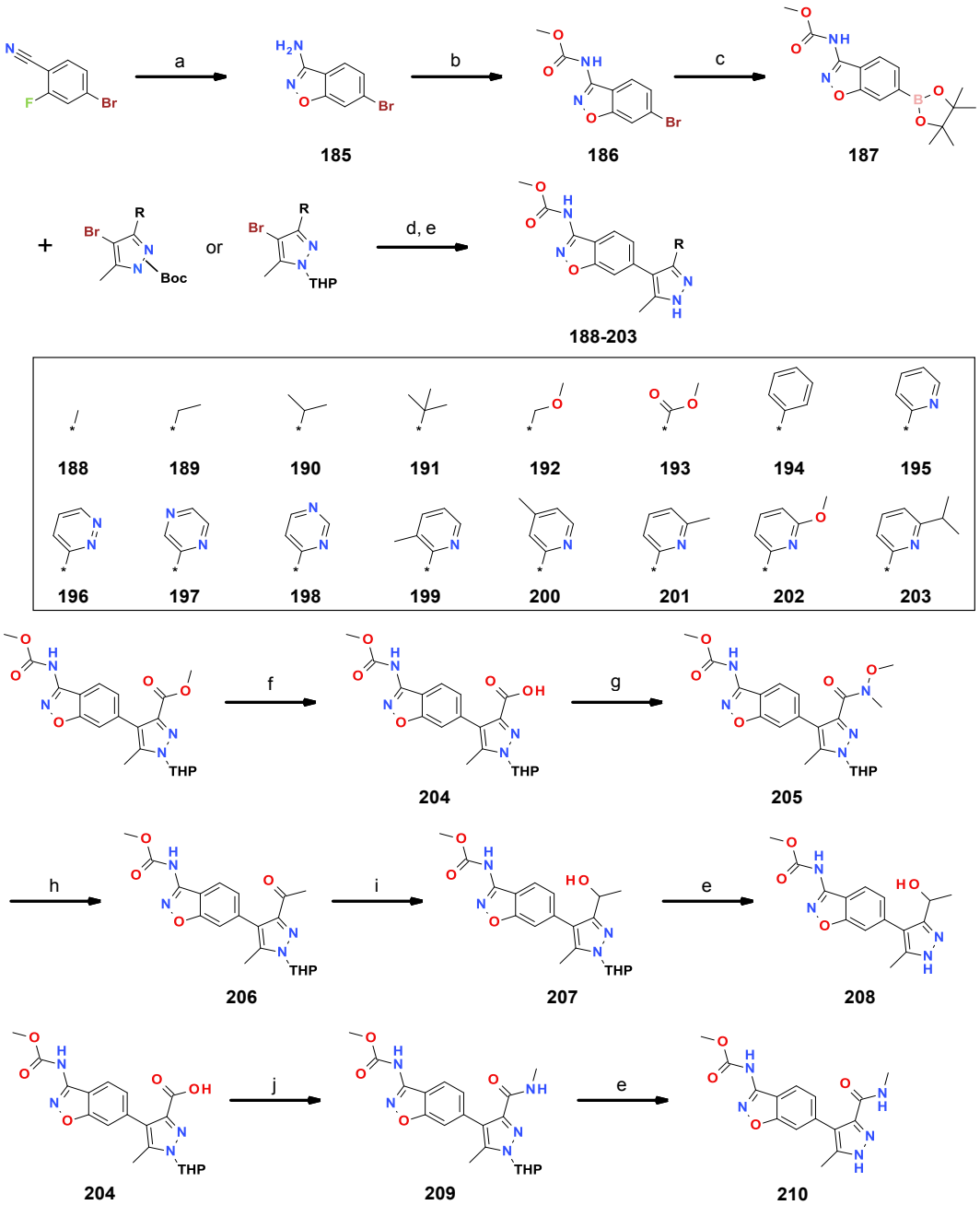
6 位にシクロプロピルメチルピラゾリル基を有し、3 位に置換基を有するベンゾイソキサゾール誘導体の合成法を **Scheme 17** に示す。

市販の 4-シアノ-3-フルオロフェニルボロン酸とブロモピラゾールの鈴木カップリング反応により合成した化合物 **177** に対し、アセトヒドロキサム酸を作用させることにより環化反応が進行し、3-アミノベンゾイソキサゾール **178** を得た。6 位ピラゾールを Boc 基で再保護の後、3 位アミノ基をアミド化、カーバメート化、もしくはウレア化することにより、目的化合物 **181-183** を得た。中間体 **179** を酸性条件下で脱保護することにより、3 位アミノ誘導体 **184** を合成した。



Scheme 17. Reagents and conditions: (a) $Pd(dppf)Cl_2$-CH_2Cl_2, K_3PO_4-nH_2O, 1,2-dimethoxyethane/H_2O, 58%; (b) Acetohydroxamic acid, Potassium *tert*-butoxide, DMF, 99%; (c) Boc_2O, Et_3N, DMAP, THF, 74%; (d) Cyclopropanecarbonyl chloride, pyridine, CH_2Cl_2 or Methyl chloroformate, pyridine, CH_2Cl_2 or Ethyl isocyanate, Et_3N, THF, 20-95%; (e) 4N-HCl/1,4-dioxane, 71-97%.
(The Boc protected compounds were as a single regioisomer, but the position of Boc groups were not determined.)

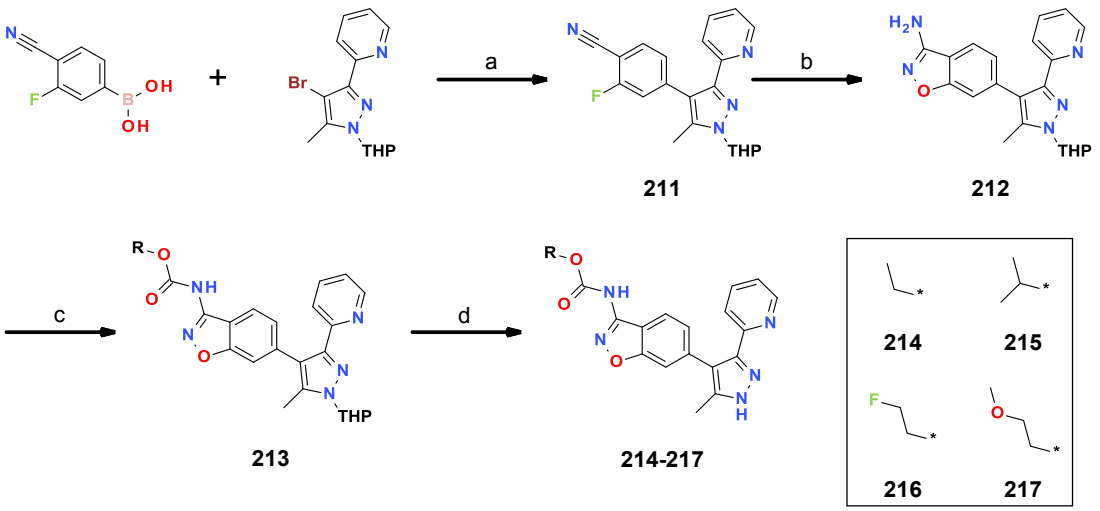
次に 3 位にメトキシカルボニルアミノ基を有し、6 位に様々な置換基を有するベンゾイソキサゾール誘導体の合成法を **Scheme 18** に示す。



Scheme 18. Reagents and conditions: (a) Acetohydroxamic acid, Potassium *tert*-butoxide, DMF, 94%; (b) Methyl chloroformate, pyridine, CH_2Cl_2, 94%; (c) Bis(pinacolato)diboron, Pd(dppf)Cl_2-CH_2Cl_2, KOAc, 1,4-dioxane, 66%; (d) Chloro(2-dicyclohexylphosphino-2',4',6'-triisopropyl-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II), K_3PO_4, 1,4-dioxane/H_2O,79-99%; (e) 4N-HCl/1,4-dioxane, 71-97%; (f) aqueous NaOH, 1,4-dioxane, 99%; (g) *N,O*-Dimethylhydroxylamine hydrochloride, DMT-MM, *N*-Methylmorpholine, THF/MeOH, 83%; (h) Methylmagnesium chloride, THF, 35%; (i) $NaBH_4$, MeOH, 99%; (j) *N,O*-Dimethylhydroxylamine hydrochloride, DMT-MM, *N*-Methylmorpholine, THF/MeOH, 99%.
(The Boc protected compounds were as a single regioisomer, but the position of Boc groups were not determined.)

市販の 4-ブロモ-2-フルオロベンゾニトリルを出発原料に **Scheme 17** と同様の反応により合成した 3-アミノ-6-ブロモベンゾイソキサゾール **185** への 3 位メチルカーバメート化、6 位へのピラゾール環の導入により、目的化合物 **188-203** を合成した。メチルエステル中間体のアルカリ加水分解により合成した中間体 **204** を用いて、Weinreb アミド **205** への変換と続くメチル基の導入によりメチルケトン **206** へと誘導後、水素化ホウ素ナトリウムによる還元反応により、二級アルコール誘導体 **208** を合成した。中間体 **204** とメチルアミンとの縮合、及び脱保護により化合物 **210** を合成した。

次に、6 位にメチルピリジルピラゾリル基を有し、3 位に様々なアルコキシカルボニルアミノ基を有する誘導体の合成法を **Scheme 19** に示す。



Scheme 19. Reagents and conditions: (a) $Pd(dppf)Cl_2$-CH_2Cl_2, K_3PO_4-nH_2O, 1,2-dimethoxyethane/H_2O, 89%; (b) Acetohydroxamic acid, Potassium *tert*-butoxide, DMF, 64%; (c) Alkyl chloroformate, pyridine, CH_2Cl_2 98-100%; (d) 4N-HCl/1,4-dioxane, 52-82%.

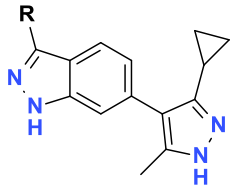
Scheme17 と同様の反応により合成した 3-アミノベンゾイソキサゾール中間体 **212** に種々のクロロギ酸アルキルを作用させた後、脱保護することにより目的物 **214-217** を合成した。

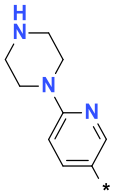
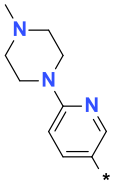
第三節　3-アリールインダゾール誘導体の展開

本節では、キナーゼ阻害能低減を指向した一つ目のデザインである3位アミノ基を芳香族複素環に変換した3-アリールインダゾール誘導体の誘導体展開について述べる。

まず始めに、3位アミノ基をアリール基に変換した誘導体の*in vitro*評価結果を**Table 21**に示す。

Table 21. SAR of 3-heteroaryl-substituted indazole derivatives



compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM)
134		2.6	**137**		0.17
135		2.3	**138**		0.12
136		3.1			

単環複素環のピリジン環、及びピロール環に変換した誘導体 **(134-136)**は、大幅に *in vitro* 活性が減弱する結果であった。しかしながら、ピリジン環の先にピ

ペラジン環を導入した化合物 **137, 138** は高い *in vitro* 活性を示すことが分かった。

そこで、高い *in vitro* 活性を示した **138** のキナーゼプロファイリング評価を実施したところ、改善傾向を示したものの依然として多くのキナーゼに対する阻害能を有することが明らかとなった (**Figure 18**)。

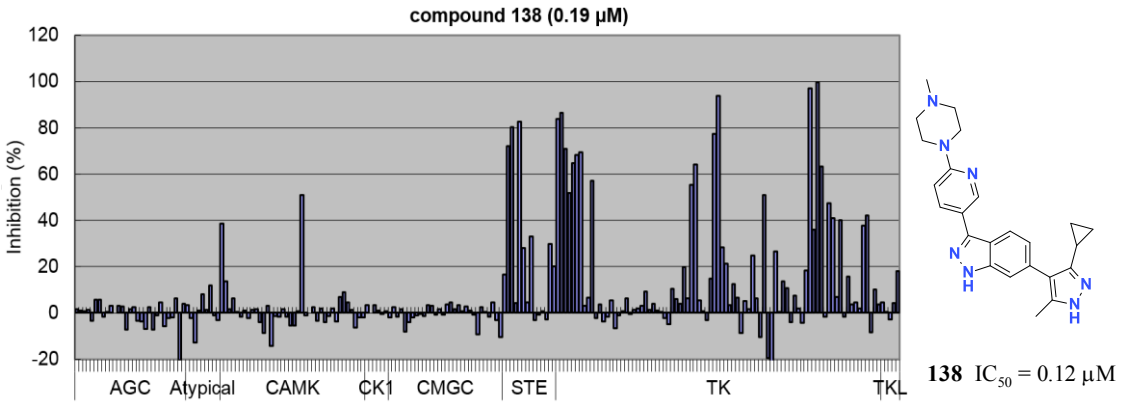


Figure 18. IC_{50} value and kinase inhibitory profiles of compound **138**

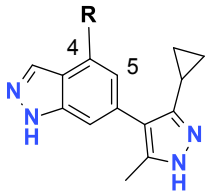
キナーゼ阻害能が低下しなかった原因は、3 位に新たに導入したピリジン環上の水素原子が新たな H-bond donor として機能したためと推測している [28]。

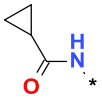
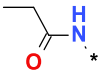
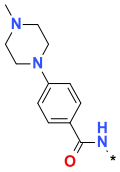
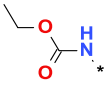
第四節　4-アミノインダゾール誘導体の展開と DS28120313 の創製

本節では、二つ目のデザインである3位アミノ基を4位へ移動した4-アミノインダゾール誘導体の誘導体展開について述べる。

まず始めに、4位に様々な置換基を有する3位無置換インダゾール誘導体の*in vitro*評価結果を**Table 22**に示す。

Table 22. SAR of 4-substituted indazole derivatives



compound	R	IC_{50} (μM)	Compound	R	IC_{50} (μM)
140	H	4.1	**157**		2.2
144		1.0	**149**		1.2
145		0.19	**150**		0.34
146		0.45	**151 (DS28120313)**		0.093
147		1.9	**152**		0.66
148		2.7	**153** (5-)		>10

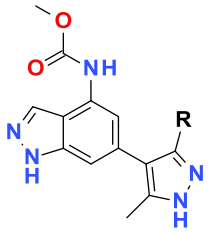
無置換誘導体**140**は大幅に*in vitro*活性が減弱した。一方で4位にシクロプロピルアミド基をもつ**144**は中程度の活性を示すことが分かった。4位アミド置換基は立体的に嵩が低いほど*in vitro*活性が高く、インダゾール3位への導入で非常に高い活性を示した*para*-ピペラジニルベンズアミド基は中程度の活性を示すにとどまり、前節で述べたインダゾール3位誘導体とは異なるSARを示した (**145-147**)。

次に4位へのアミド基以外の置換基の導入を検討した。一級アミノ基 (**148**)、リバースアミド (**157**)、及びスルホンアミド誘導体 (**149**)の活性は中程度であった。一方、メチルウレア誘導体 (**150**)、及びメチルカーバメート誘導体 (**151**)は非常に高い*in vitro*活性を示すことが分かった。4位置換基の空間許容性は非常に少なく、エチルカーバメートへの変換で*in vitro*活性が6倍程度減弱する。また、5位にメチルカーバメート基を有する化合物**153**の*in vitro*活性は完全に消失した。

インダゾール4位の置換基としてメチルカーバメートが好適であることが明らかとなったので、6位置換基である二置換ピラゾール環上の置換基の最適化を実施した (**Table 23**)。

種々のアルキル基(**151, 165-167**)はいずれも高い*in vitro*活性を示したが、エーテル置換基 (**168**)は活性が減弱することが明らかとなった。またメチルエステル誘導体 (**169**)は良好な活性を保持するものの、カルボン酸誘導体 (**172**)、及びメチルカルバモイル誘導体 (**174**)の活性は低下する結果であった。シクロプロピル基やエステル基と同様のsp^2性の炭素を持つ2-ピリジル誘導体 (**176**)は高い*in vitro*活性を示した。

Table 23. SAR of 6-substituted indazole derivatives



compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM)
151 (DS28120313)	*c*-Pr	0.093	**169**	CO_2Me	0.13
165	Me	0.15	**172**	CO_2H	>10
166	Et	0.18	**174**	CONHMe	2.0
167	*i*-Pr	0.19	**170**	Ph	1.1
168	CH_2OMe	3.9	**176**		0.32

そこで、高い *in vitro* 活性を示した **151** のキナーゼプロファイリング評価を実施したところ、期待通り評価した 216 種のキナーゼに対して 30 %以上阻害を示さないことを確認した (**Figure 19**)。

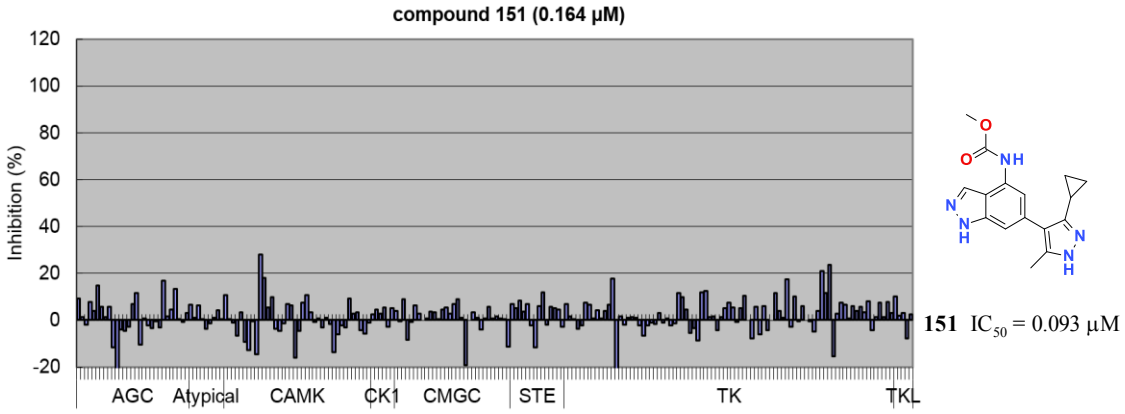


Figure 19. IC_{50} value and kinase inhibitory profiles of compound **151**

強力な*in vitro*活性を有し、かつ広範なキナーゼに対する阻害能を低減させることに成功した**151**を**DS28120313**と命名し、*in vivo*薬効評価を行うにあたり、マウスを用いた薬物動態試験を実施した (**Table 24**)。

Table 24. Physicochemical properties and PK parameters of **DS28120313**

LogD	MS[a] (%)	PB (free %)	Cmax[b] (μg/mL)	Tmax[b] (h)	AUC[b] (h*μg/mL)
3.1	84	24	4.94	0.67	14.7

[a] Remaining (%) test compound after 0.5 h incubation with mouse liver microsomes (0.5 mg/mL).

[b] Average of two values dosed at 30 mg/kg orally (p.o.) in C57BL/6J mice (0.5% Methylcellulose suspension).

DS28120313は適度な脂溶性と低タンパク結合率を示し、30 mg/kg 経口投与において良好なPKプロファイルを示した。そこで、IL-6誘発高ヘプシジンマウスモデルを用いて評価したところ、**DS28120313**は30 mg/kg経口投与において、強力な*in vivo*ヘプシジン産生抑制作用を示すことが明らかとなった (**Figure 20**)。

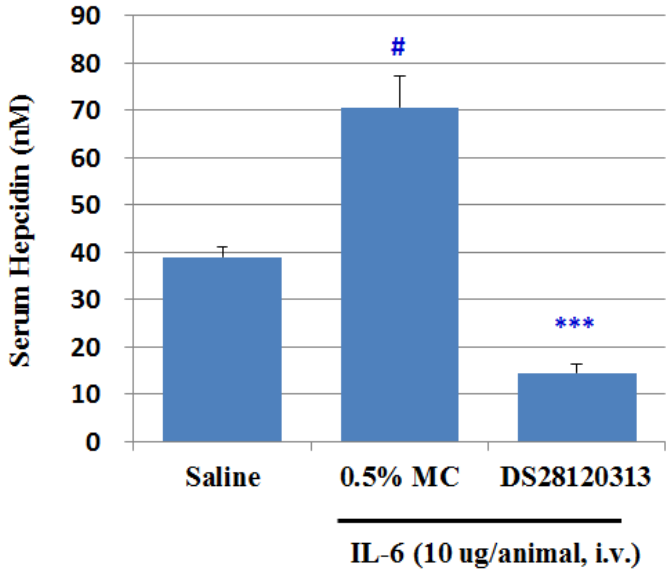
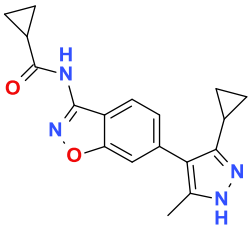


Figure 20. Effect of compound **DS28120313**. The compound was administered to mice at dose of 30 mg/kg (p.o., 0.5% Methylcellulose, suspension, $n = 4$) before IL-6 treatment.
#, $p < 0.05$ vs Saline treated group (t-test), ***, $p < 0.001$ vs 0.5% MC treated group (t-test).

第五節　ベンゾイソキサゾール誘導体の展開と DS79182026 の創製

本節では、三つ目のデザインであるベンゾイソキサゾール誘導体の誘導体展開について述べる。

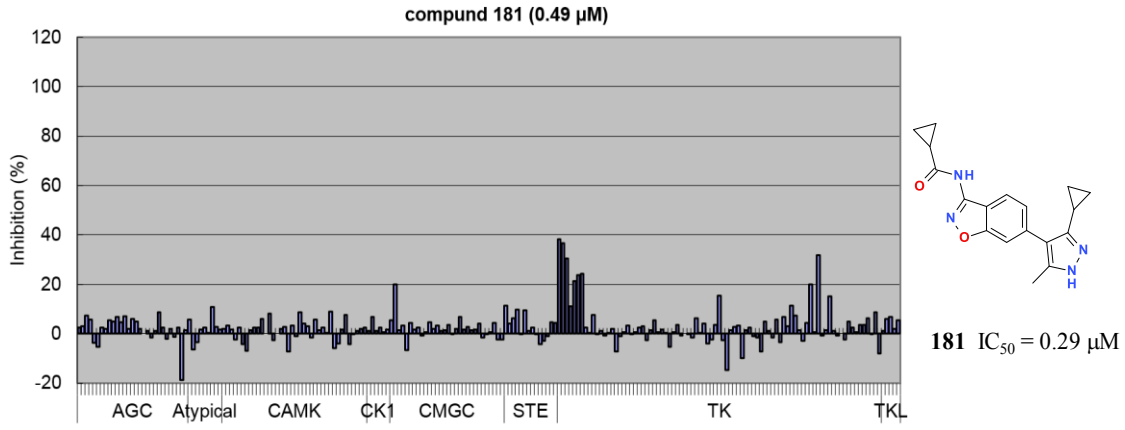
インダゾール1位の窒素原子を酸素原子に置換することにより、ヒンジ結合部位との水素結合を形成しているH-donorを減らしたベンゾイソキサゾール誘導体**181**は中程度のヘプシジン産生阻害活性を示すことが分かった **(Figure 21)**。



181 IC_{50} = 0.29 μM

Figure 21. *In vitro* activity of benzisoxazole scaffold **181**

そこで化合物 **181** のキナーゼプロファイリング評価を実施したところ、ヘプシジン産生阻害の IC_{50} の 2 倍の濃度において、期待通り広範なキナーゼに対する阻害能の低減に成功したことを確認した **(Figure 22)**。



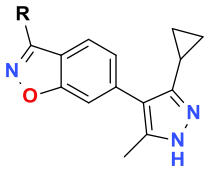
181 IC_{50} = 0.29 μM

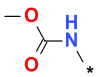
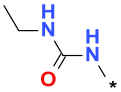
Figure 22. IC_{50} value and kinase inhibitory profiles of compound **181**

ベンゾイソキサゾール母核への変換が、キナーゼ阻害能の大幅な低減に効果的であることが確認できたので、ベンゾイソキサゾール誘導体の合成展開を実施することとした。

始めに、ベンゾイソキサゾール 3 位に様々な置換基を導入した結果を **Table 25** に示す。

Table 25. SAR of 3-substituted benzisoxazole

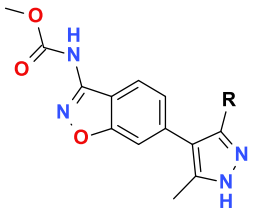


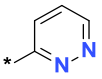
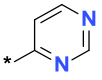
compound	R	IC_{50} (μM)
181		0.29
184		1.4
182		0.21
183		0.72

一級アミノ基を有する**184**の活性は減弱したが、メチルカーバメート誘導体**182**は高い*in vitro*活性を示した。一方、エチルウレア誘導体**183**はやや活性が減弱する結果であった。

次に、ベンゾイソキサゾール6位の二置換ピラゾール上に様々な置換基を導入した化合物の結果を**Table 26**に示す。

Table 26. SAR of 6-substituted benzisoxazole derivatives



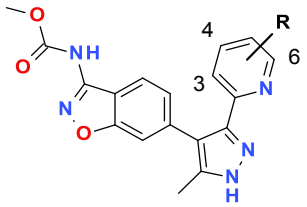
compound	R	IC_{50} (μM)	Compound	R	IC_{50} (μM)
182	*c*-Pr	0.21	**193**	CO_2Me	2.9
188	Me	0.58	**210**	CONHMe	4.4
189	Et	0.46	**194**	Ph	0.54
190	*i*-Pr	0.91	**195 (DS79182026)**		0.039
191	*t*-Bu	2.6	**196**		0.35
192	CH_2OMe	1.1	**197**		0.21
208	CH(OH)Me	1.2	**198**		2.9

立体的に嵩高いアルキル基を導入すると*in vitro*活性が減弱していくことが明らかとなった (**190, 191**)。またエーテル基 (**192**)、ヒドロキシ基 (**208**)、エステル基 (**193**)、及びアミド基 (**210**)のような極性官能基の導入により、いずれも*in vitro*活性は減弱することが明らかとなった。アルキル置換基の検討で、立体的に最も小さいメチル基よりもシクロプロピル基が高い*in vitro*活性を示した。このことから、sp^2性の炭素を持つ置換基が好適ではないかと考え合成したフェニル誘導体 (**194**)は比較的高い*in vitro*活性を示した。そこで、様々な含窒素複素環を導入した

ところ、2-ピリジル基を導入した化合物**195**が非常に高い*in vitro*活性を示した。この結果より、sp^2炭素を有することに加えて、窒素原子の位置も活性向上に重要であることがわかった。

次に、2-ピリジル基上への置換基導入を検討した結果を**Table 27**に示す。

Table 27. SAR of 2-pyridyl pyrazole derivatives

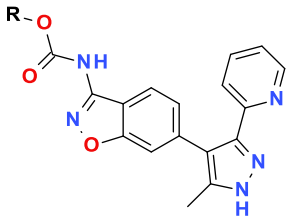


compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM)
195 (DS79182026)	H	0.039	**201**	6-Me	0.042
199	3-Me	4.8	**202**	6-OMe	0.40
200	4-Me	2.6	**203**	6-*i*-Pr	4.8

2-ピリジル基の3位 (**199**)、及び4位 (**200**)へのメチル基導入により*in vitro*活性は100倍程度減弱することが明らかとなった。対照的に、6位にメチル基を導入した**201**は非常に高い*in vitro*活性を維持することが分かった。しかしながら、6位にメトキシ基 (**202**)、及び嵩高いイソプロピル基 (**203**)を導入すると*in vitro*活性が減弱することから、置換基導入の許容性は低いと判断した。

二置換ピラゾール上の置換基として、2-ピリジル基が最適であることが明らかとなったので、ベンゾイソキサゾール3位のアルキルカーバメート基の最適化を実施した結果を**Table 28**に示す。

Table 28. SAR of substituted carbamate derivatives



compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM)
195 (DS79182026)	Me	0.039	**216**	FCH_2CH_2-	0.047
214	Et	0.040	**217**	$MeOCH_2CH_2$-	0.069
215	*i*-Pr	0.16			

分岐したイソプロピルカーバメート誘導体**215**は*in vitro*活性の減弱傾向を示したが、直鎖アルキルカーバメート誘導体**214, 216, 217**は非常に高い*in vitro*活性を保持することから、ベンゾイソキサゾール3位置換基として母核近傍を除いて空間許容性が高いことが確認できた。最適化研究で創出した化合物**195**のキナーゼプロファイリング評価 (48キナーゼ)により、広範なキナーゼに対する阻害能を持たないことを確認した (**Figure 23**)。

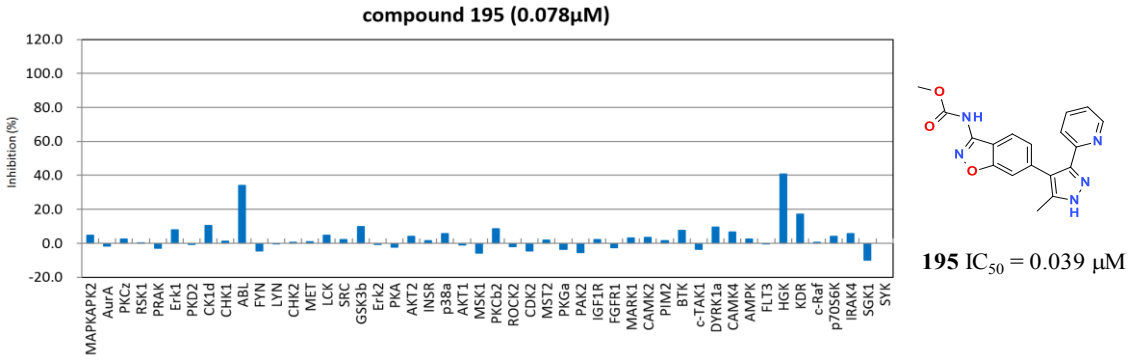


Figure 23. IC_{50} values and kinase inhibitory profiles of compound **195**

創出した化合物**195**を**DS79182026**と命名し、*in vivo*薬効評価を行うにあたり、マウスを用いた薬物動態試験を実施した (**Table 29**)。

適度な脂溶性を有し、高い代謝安定性と血漿中のフリー体濃度を示す**DS79182026**は、30 mg/kg 経口投与において良好な血中暴露を示し、経口薬として望ましいプロファイルを有することが明らかとなった。

Table 30. Physicochemical properties and PK parameters of **DS79182026**

LogD	MS[a] (%)	PB (free %)	Cmax[b] (μg/mL)	Tmax[b] (h)
2.5	87	17	9.17	1.0

AUC[b] (h*μg/mL)	Vd[c] (L/kg)	CL[c] (mL/min/kg)	BA[b] (%)
36.2	0.91	14.5	100

[a] Remaining (%) of the tested compound after 0.5 h incubation with mouse liver microsomes (0.5 mg/mL).

[b] Average of two values dosed at 30 mg/kg p.o. with C57BL/6J mice (0.5% Methylcellulose, suspension).

[c] Average of two values dosed at 3 mg/kg i.v. with C57BL/6J mice (92 mM SBE7-β-CyD, solution).

そこで前述のIL-6誘発高ヘプシジンマウスモデルを用いて評価したところ、**DS79182026**は30 mg/kg経口投与において、強力な*in vivo*ヘプシジン産生抑制作用を示すことが明らかとなった (**Figure 24**)。

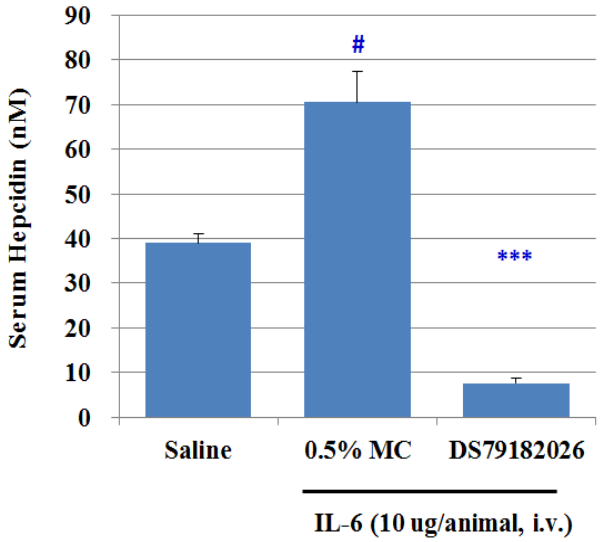


Figure 24. Effect of **DS79182026**. The compound was administered to mice at dose of 30 mg/kg (p.o., 0.5% Methylcellulose, suspension, $n = 4$) before IL-6 treatment. #, $p < 0.05$ vs Saline treated group (t-test), ***, $p < 0.001$ vs 0.5% MC treated group (t-test).

DS79182026はIL-6誘発高ヘプシジンマウスモデルでより強力な活性を示したので、更なる高次評価を実施した。ヘプシジン産生過剰により生じる機能的鉄欠乏性貧血に対する治療効果を確認するため、鉄剤誘導貧血モデルマウスを用いた評価を実施した。鉄剤を静脈内投与することにより高ヘプシジン状態を維持したマウスに対して、エリスロポエチン製剤を投与し造血を促すと、十分な鉄の供給が起きず網状赤血球中ヘモグロビン量 (CHr)は低下する。しかしながら**DS79182026**の1日2回経口投与を5日間継続するとCHrを回復させることが明らかとなった。このことにより、ヘプシジン産生阻害によって機能性鉄欠乏状態を改善することが示唆された (**Figure 25**)。

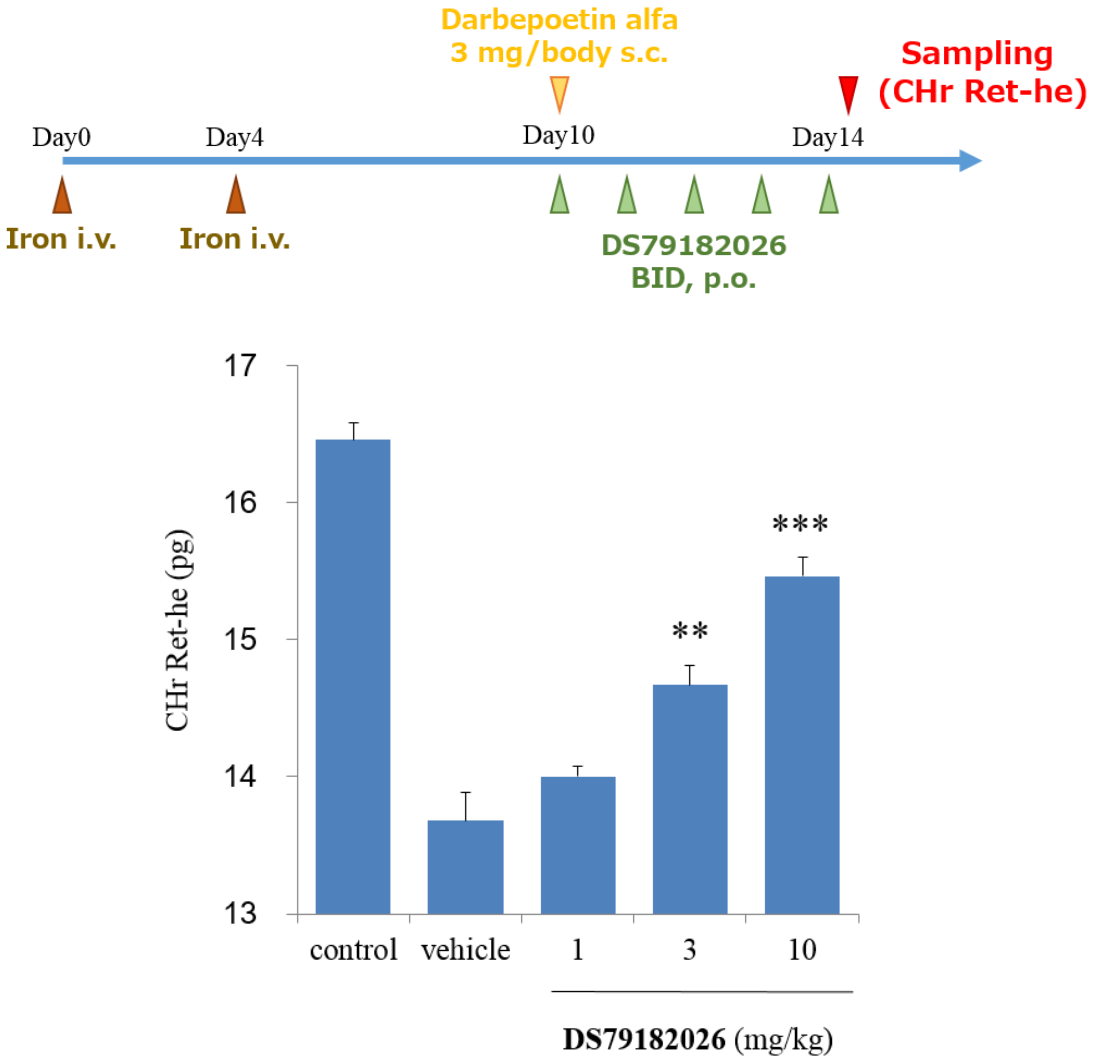


Figure 25. Effect of **DS79182026** on reticulocyte hemoglobin content (CHr) in iron-induced anemia mouse model. *, $p < 0.05$ vs vehicle treated group, **, $p < 0.01$ vs Vehicle treated group, ***, $p < 0.001$ vs vehicle treated group (Dunnett multiple comparison test).

第六節　小括

本章では、経口投与で*in vivo*薬効を示す化合物**129**からマルチキナーゼ阻害能の低減を意図した誘導体展開により、**DS79182026**を創製した経緯を述べた。

化合物**129**は3-アミノインダゾール部位がキナーゼのヒンジ結合部位と強固な水素結合を形成しているために広範なキナーゼに対して阻害能を示すと考えた。

そこでヒンジ結合部位と考えられる部位を変換した3-アリールインダゾール、4-アミノインダゾール、及び3-アミノベンゾイソキサゾール誘導体の3種類のscaffoldをデザインし、合成展開を実施した。その結果、4-アミノインダゾール、及び3-アミノベンゾイソキサゾール誘導体へのscaffold hoppingにより高いヘプシジン産生阻害活性を保持し、キナーゼ阻害能を大幅に低減できることを見出した。

更に3-ベンゾイソキサゾール誘導体の最適化検討の結果、経口薬として優れたPKプロファイルを有し、IL-6誘発高ヘプシジンマウスモデルを用いた*in vivo*薬効評価において経口投与で強力な血清ヘプシジン低下作用を示す**DS79182026**の創製に成功した。さらに**DS79182026**は鉄剤誘導貧血モデルマウスを用いた*in vivo*薬効評価において血球パラメータ (CHr Ret-he) の有意な改善を示し、ヘプシジン産生阻害剤が機能性鉄欠乏性貧血治療薬として有効であることが示唆された。

第四章　DS79182026 の薬理学的標的タンパク質の探索研究

第一節　標的タンパク質探索研究の概要

前章では、マルチキナーゼ阻害能が低減し、鉄剤誘導貧血モデルマウスにおいて高い *in vivo* 薬効を示す有望化合物 **DS79182026** 創製の経緯を述べた。

DS79182026 はヘプシジン産生量を指標とした cell-based アッセイを用いた最適化研究によって創製した化合物であり、ヘプシジン産生阻害を示す薬理学的標的分子は不明であったため、その同定研究に着手することとした。

一般的に、創薬研究ならびに医薬品臨床開発において、最適な対象疾患の決定、対象患者の選定、及び副作用の可能性予測を行うために、薬理学的標的分子の同定は大変重要な項目と考えられている [29]。

その方法論は様々あるが、間接的アプローチ [30-35]、もしくは分子生物学や生化学技術を駆使して薬物の作用する分子を同定する直接的アプローチ [36-42] の二つに大分される。

近年の定量的プロテオミクスの著しい進歩により、1000 を超えるタンパク質が同時に定量可能となった。そこで定量的プロテオミクス技術と化学プローブと組み合わせた「chemical proteomics」の手法で、様々な薬理学的標的分子同定が実施されてきた。これらの「chemical proteomics」の手法の中で、化合物固定化ビーズを用いたアフィニティー精製が最も成功確率が高い方法であると考えられている [43]。

我々は、アフィニティー精製を用いた「chemical proteomics」と放射性化合物を用いた結合評価を組み合わせることにより、**DS79182026** の薬理学的分子標的タンパク質の同定研究を行うこととした (**Figure 26**)。

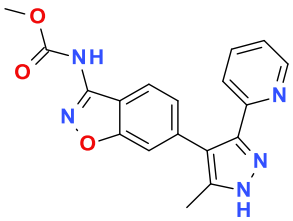
Figure 26. Schematic representation of chemical proteomics approach to identify candidates of molecular targets of DS79182026, a hepcidin production inhibitor

Cell lysate was subjected to affinity purification using compound-immobilized affinity beads to enrich proteins which bound to the compound with or without active/inactive competitors. Enriched proteins were identified and quantified by liquid chromatography - tandem mass spectrometry.

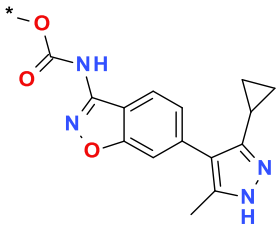
第二節　ツール化合物のデザイン及び合成

前節では、**DS79182026** の薬理学的標的探索研究の概要について述べた。

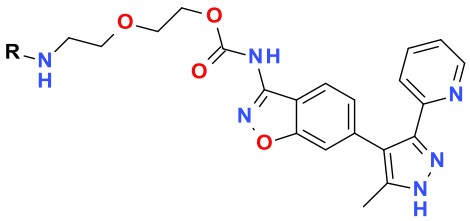
アフィニティー精製法を行うためには、ビーズへ固定化しても標的タンパク質との結合親和性を損なうことのないリンカー化合物を設計・合成することが必要ある。そこで著者は、アフィニティー精製に用いるビーズに固定化するための化学プローブを設計、合成した。一方で、より直接的な標的分子への結合を評価するためのツール化合物として、放射性同位体で置換したプローブも併せて合成した (**Figure 27**)。



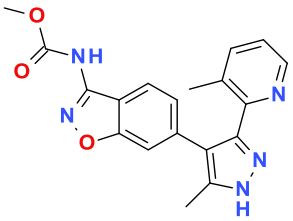
DS79182026
(IC_{50} = 0.039 μM)



[^{3}H]-**182**
(IC_{50} = 0.21 μM)



R = Boc: **218** (IC_{50} = 0.089 μM)
R = H: **219**



199
(IC_{50} = 4.8 μM)

Figure 27. Compound structures

DS79182026 and **182**: Hepcidin production inhibitors obtained from derivatization of the hit compound from phenotypic screening. *Hydrogen atom on this carbon was tritiated in [^{3}H]-**182**.

218 and **219**: Tool compounds for target identification by affinity purification using compound-immobilized beads.

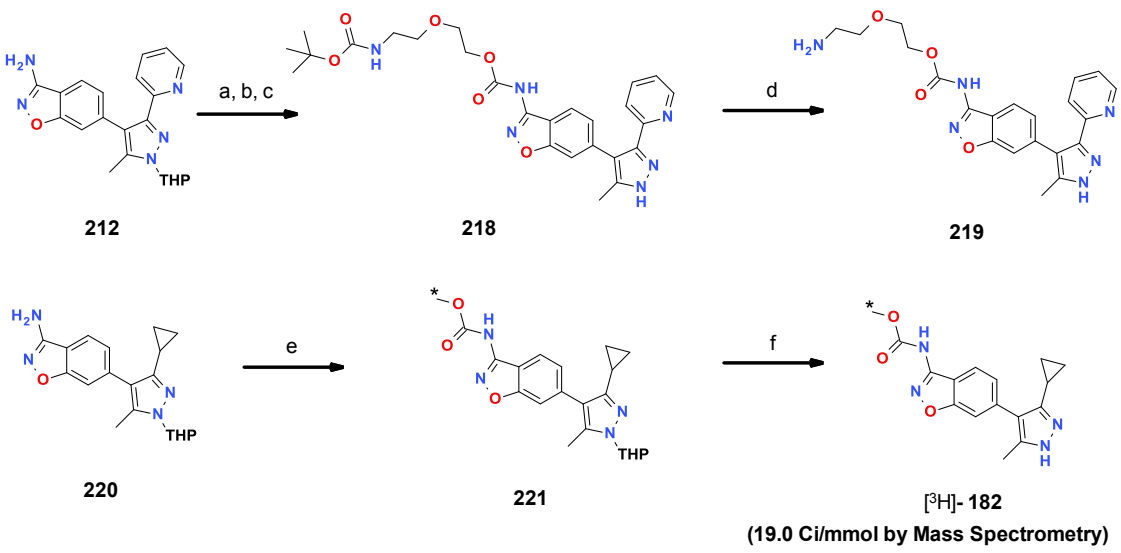
199: Negative competitor for affinity purification.

Hepcidin production inhibitory activity of each compound is shown as IC_{50} values.

前章第八節において、ベンゾイソキサゾール 3 位は母核近傍を除いて空間許容性が高いことが確認されており、標的分子との結合ポケットの外側にあることも示唆されている。そこで、ベンゾイソキサゾール 3 位カーバメート基にリンカーを導入した化合物をデザインした。

Boc アミノ基を持つ polyethylene glycol (PEG)リンカー体 **218** の *in vitro* 活性は IC_{50} = 0.089 μM であり、**DS79182026** に近い高活性を示した。PEG リンカーの先に大きな置換基の導入が可能であることが確認でき、**218** の Boc 基を除去したアミン **219** を用いて作製した化合物固定化ビーズは薬理学的標的分子と高い親和性を持つことが期待された。

Boc アミノ基、及びアミノ基を持つリンカー体 **218, 219** の合成法を **Scheme 20** で示す。



Scheme 20. Reagents and conditions: (a) 2-[2-(*tert*-Butoxycarbonylamino)ethoxy]ethyl carbonochloridate, pyridine, CH_2Cl_2, crude; (b) 4N-HCl/1,4-dioxane, crude; (c) Boc_2O, Et_3N, CH_2Cl_2 - THF, 37%; (d) Trifluoroacetic acid, CH_2Cl_2, 77%; (e) [^{3}H]-Methyl chloroformate, pyridine; (f) 4N-HCl/1,4-dioxane.

第三節　標的候補タンパク質の同定

前節では、薬理学的標的タンパク質の同定研究に用いるツール化合物のデザイン、及び合成について述べた。

本節ではツール化合物を用いて共同研究者により検討された標的タンパク質の同定研究について述べる。

ヘプシジンは主に肝臓で産生されることから、評価試料として *in vitro* 評価と同じヒト肝癌細胞株 HepG2 を用いた。まず始めに 1 %の界面活性剤 NP-40 を用いて可溶化したセルライセート、及びその残渣である不溶性成分のどちらに対してヘプシジン産生阻害剤と特異的な結合を示すタンパク質が含まれているかを、トリチウムで標識した[^{3}H]-**182** を用いて調べることとした。HepG2 セルライセートと[^{3}H]-**182** との結合量は、用いたタンパク質濃度依存的に上昇し、かつトリチウムで標識していない化合物 **182** の添加濃度依存的に減少した **(Figure 28A)**。一方、不溶性成分と[^{3}H]-**182** との結合量は、タンパク質濃度、標識していない化合物 **182** の添加濃度いずれにも相関性が見られなかった **(Figure 28B)**。

この結果より、ヘプシジン産生阻害剤と特異的な結合を示すタンパク質は可溶化した HepG2 セルライセートに存在すると結論づけた。

次に HepG2 セルライセートに存在する特異的な結合を示すタンパク質を同定するため、アミノリンカーをもつ化合物 **219** を固定したセファロースビーズを用いた実験を実施した。

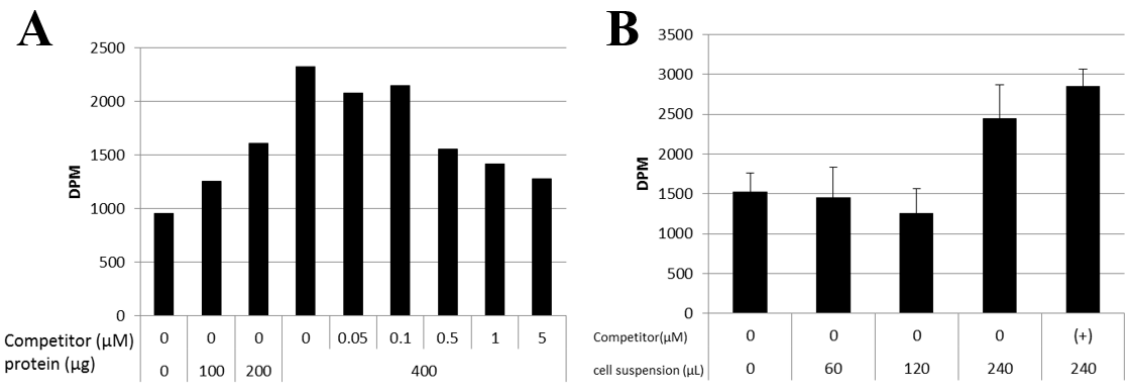


Figure 28. Confirmation of the specific binding of [^{3}H]-182 to HepG2 whole-cell lysate
A: HepG2 whole-cell lysate was incubated with [^{3}H]**-182** (50 nM) with or without unlabeled **182** as a competitor. Protein-bound radioactive compounds were separated by a gel filtration column and measured by scintillation counting as disintegrations per minute (DPM).
B: HepG2 suspension was incubated with [^{3}H]**-182** (50 nM) with or without unlabeled **182** as a competitor. Protein-bound radioactive compounds were separated by glass filter filtration and measured by scintillation counting as DPM.

219 を固定化したビーズ、もしくは化合物を固定化していないコントロールビーズを用い、競合化合物として **DS79182026** 存在/非存在下で HepG2 セルライセートの精製を行った。また、ヘプシジン阻害活性と関連しないものの化合物に対して非特異的な結合を示すタンパク質を除外するために、**DS79182026** と構造類似性が高く、ヘプシジン阻害活性が著しく低下した陰性対照化合物 **199** を用いることとした (IC_{50} = 4.8 μM, **Figure 27**)。

溶出成分内のタンパク質の同定は、LC-MS/MS を用いて行った。

同定されたすべてのタンパク質から化合物への特異性、及びヘプシジン阻害活性との関連性を示すタンパク質か否かを判断するため、以下 4 つのクライテリアを設定した。

① **DS79182026** 非存在下 **219** を固定化したビーズを用いて精製した時、コントロールビーズに比べてタンパク量が 2 倍以上に増加する

② 10 μM の **DS79182026** にて競合阻害を行った条件下で **219** を固定化したビーズを用いて精製した時、**DS79182026** 非存在下に比べて、タンパク量が減少する

③ 添加する **DS79182026** の濃度依存的にタンパク量が減少する

④ 10 μM の陰性対照化合物 **199** 存在下で **219** を固定化したビーズを用いて精製した時、化合物 **199** 非存在下に比べて、タンパク量が変化しない

上記の 4 つのクライテリアを満たす 13 種のタンパク質が、ヘプシジン産生阻害剤の薬理学的標的タンパク質として候補に挙げられた **(Table 30)**。

Official gene symbol (common name)	Protein name	Uniprot ID	Total peptide number	Unique peptide number
ACVR1 (ALK2)	Activin receptor type-1	Q04771	20	20
BMPR1A (ALK3)	Bone morphogenetic protein receptor type-1A	P36894	16	16
CIT	Citron Rho-interacting kinase	Q2M5E1	99	25
CSNK1D	Casein kinase 1, delta	P48730	24	10
CTNNB1	Catenin beta-1	E7ERS9	18	15
GAPVD1	GTPase-activating protein and VPS9 domain-containing protein 1	Q14C86-2	59	59
GSK3B	Glycogen synthase kinase-3 beta	P49841-2	24	18
HMMR	Hyaluronan-mediated motility receptor	O75330-2	17	17
PRKD2	Serine/threonine-protein kinase D2	Q9BZL6	20	17
RIPK2	Receptor-interacting serine/threonine-protein kinase 2	O43353	35	35
SNX24	Sorting nexin-24	Q9Y343-2	10	10
TEX264	Testis-expressed sequence 264 protein	C9JXQ7	2	2
TGFBR2	TGF-beta receptor type-2	P37173	35	1

Table 30. Candidate proteins identified by affinity purification

HepG2 cell lysates were subjected to **219**-immobilized beads followed by quantitative proteomics. Reproducible proteins that passed those criteria in biological replicate experiments (n = 4) were regarded as candidate proteins of the molecular target of the compound.

第四節　トリチウム標識体を用いた候補タンパク質のバリデーション

前節では、化合物固定化ビーズを用いて実施した 13 種の薬理学的標的候補タンパク質の同定について述べた。

本節では 13 種の候補タンパク質とヘプシジン産生阻害能の関連性を詳細に検討するために、共同研究者によって実施された入手可能な 10 種のリコンビナントタンパク質とトリチウムで標識した[^{3}H]-**182** を用いた競合実験について述べる。

用いるタンパク量に依存して[^{3}H]-**182** との結合量が増加し、かつトリチウムで標識していない化合物 **182** の過剰量の添加により、結合量が減少するタンパク質として、ALK2 (official gene symbol: ACVR1) と ALK3 (official gene symbol: BMPR1A) が同定された (**Figure 29**)。

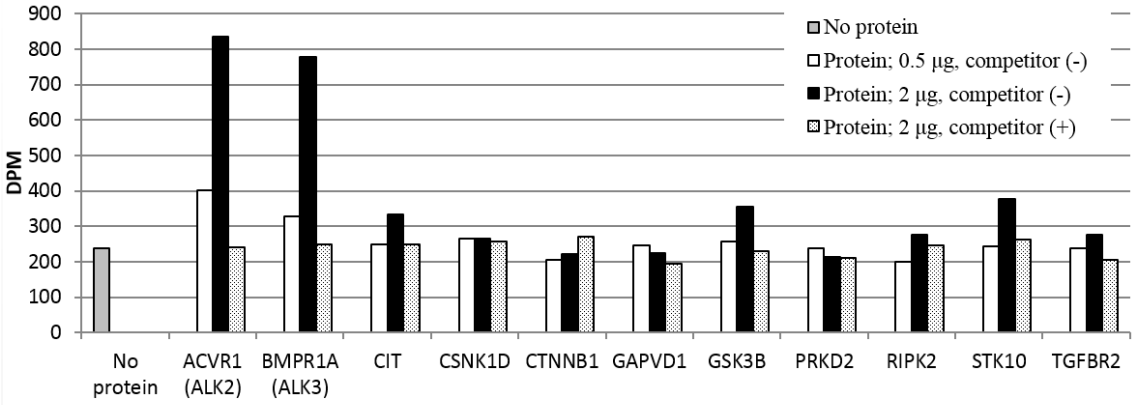


Figure 29. Validation of candidate proteins
Binding assay of [^{3}H]-**182** with recombinant proteins. Recombinant proteins were incubated with [^{3}H]-**182** (50 nM) after 2 h of pre-incubation with the unlabeled **182** as a competitor (5 mM). Protein-bound radioactive compounds were separated by a gel filtration column and measured by scintillation counting as disintegrations per minute (DPM).

ALK2 と ALK3 は I 型骨形成タンパク質 (Bone morphogenetic protein, BMP)受容体として知られており、ヘプシジン発現に関連しているため、我々はそれらをヘプシジン産生阻害剤の分子標的の非常に有望な候補と考えた[44]。また近年、ヘプシジンの転写活性化に ALK2 が重要な役割を果たしていることが報告されているため[45]、ALK2 についてより詳細にヘプシジン産生阻害能の関連性を検討することとした。

HepG2 細胞を用いて、siRNA により ALK2 の発現をノックダウンすると STAT シグナル伝達または BMP シグナル伝達経路のリガンドである IL-6 および BMP6 による処理の有無にかかわらず、ヘプシジン mRNA 発現が阻害されることを見出した (**Figure 30**)。

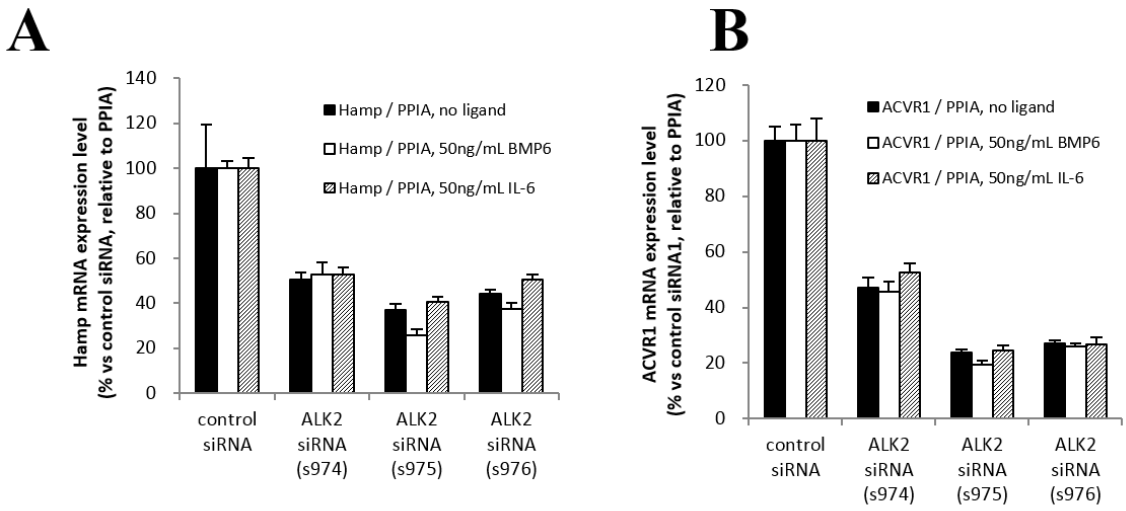
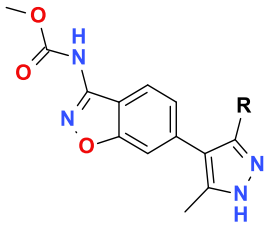
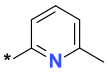
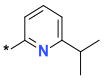


Figure 30. Validation of a candidate protein
A and **B**: Effect of ALK2 gene targeting on hepcidin mRNA expression. Hepcidin (Hamp) mRNA expression was downregulated by each ALK2 gene targeting siRNA. This downregulation was observed in the same way in BMP6, IL-6, or untreated conditions (**A**). ALK2 mRNA gene silencing levels were consistent with hepcidin mRNA expression levels (**B**).

前章第八節で詳細に述べたが、ベンゾイソキサゾール 6 位の二置換ピラゾール上の置換基はヘプシジン産生阻害活性に大きな影響を与えることが分かっている。そこで ALK2 阻害とヘプシジン産生阻害能の関連性を検討するために、種々のベンゾイソキサゾール誘導体を用いて、ヘプシジン産生阻害能と ALK2 に対する結合能との相関性を評価した **(Figure 31A)**。

A



	R	IC_{50} of hepcidin (μM)	Binding to ALK2 (% of control)		R	IC_{50} of hepcidin (μM)	Binding to ALK2 (% of control)
195		0.039	8.58	**188**		0.58	34.1
201		0.042	20.4	**192**		1.1	59.3
182		0.21	40.7	**191**		2.6	76.6
197		0.21	32.1	**198**		2.9	132
196		0.35	32.3	**203**		4.8	128
194		0.54	19.2	**199**		4.9	110

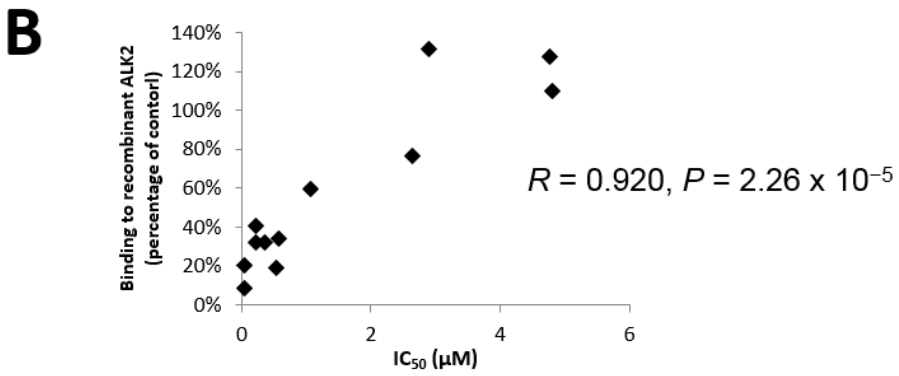


Figure 31. Correlation between pharmacological activity and binding affinity to purified recombinant ALK2

A: The structure, hepcidin production inhibitory activity (shown as IC_{50}), and competition efficiency of the binding of [^{3}H]-**182** to recombinant ALK2 of competitors. **B**: Correlation between IC_{50} of hepcidin production inhibitory activity and competition efficiency of selected compounds to the binding of [^{3}H]-**182** to recombinant ALK2. Recombinant proteins were incubated with [^{3}H]-**182** (50 nM) after 2 h of pre-incubation of the competitors (150 nM). Protein-bound radioactive compounds were separated by a gel filtration column and measured. Competition efficiency is shown as a percentage based on the ratio of the decreased radioactive count by the competitors to the radioactive count without a competitor.

その結果、ALK2 への結合とヘプシジン産生阻害活性の IC_{50} との間のピアソン相関係数は、有意な相関 ($R = 0.920$、$P = 2.26\times10^{-5}$)を示すと共に (**Figure 31B**)、**DS79182026**、及び化合物 **182** の ALK2 阻害活性を測定したところ、IC_{50} はそれぞれ 0.54, 2.29 μM であった。

以上の二つの結果より、ヘプシジン産生阻害剤 **DS79182026** の薬理学的標的タンパク質は ALK2 であることが強く示唆された。

第五節　小括

本章では、**DS79182026** の薬理学的標的タンパク質の同定研究により、候補タンパク質として ALK2 を同定した経緯について述べた。

ベンゾイソキサゾール 3 位置換基は空間許容性が高いことから、**DS79182026** の 3 位メチルカーバメート基の先にアミノ基を持つ PEG リンカーを導入した **218** は **DS79182026** と同程度の高い *in vitro* 活性を保持していた。

トリチウムで標識した[^{3}H]-**182** を用いた評価により、HepG2 セルライセートにヘプシジン産生阻害剤と特異的な結合を示すタンパク質が含まれることが明らかとなったため、アミノリンカー体 **219** を固定化したセファロースビーズを用いた候補タンパク質のアフィニティー精製を行った。

同定実験より候補に挙げられた 10 種のリコンビナントタンパク質を用いて、トリチウムで標識した[^{3}H]-**182** と標識していない **182** を用いた競合実験を行ったところ、ALK2 と ALK3 が標的候補タンパク質として同定された。

HepG2 細胞において siRNA を用いて ALK2 の発現をノックダウンすると、ヘプシジン mRNA 発現が阻害された。また種々のベンゾイソキサゾール誘導体のヘプシジン産生阻害能と ALK2 に対する結合能の間に高い相関性が確認された。以上の結果から、ALK2 がヘプシジン産生阻害剤 **DS79182026** の薬理学的標的タンパク質であることが強く示唆された。

総括

著者は、生体内の鉄代謝を司る因子として近年明らかとなったペプチドホルモンであるヘプシジンに着目し、ヘプシジン産生阻害剤が慢性炎症性貧血の有効な治療薬となると考え、創薬研究を行った。

第一章では、第一三共株式会社保有の化合物ライブラリーを用いたHTSにより見出したヒット化合物**1**の誘導体展開により、アミノピリミジン誘導体**DS42450411**を創製した経緯を述べた。

HTSヒット化合物**1**の初期誘導体展開により見出した中程度の*in vitro*活性を示すリード化合物**28**のキナーゼプロファイリング評価を実施したところ、DYRK1Aに高い阻害能を示すことを発見した。

そこで**28**とDYRK1AとのX線結晶構造解析を実施し、空間許容性やアミノ酸残基のデータを基に、更なる*in vitro*活性向上に向け8位置換基のデザイン・合成を実施した。

その結果、8位置換基として*exo*-メチレンをリンカーとして環状アミノ基を配置した誘導体で飛躍的に*in vitro*活性が向上することが明らかとなった。

環状アミノ基の最適化検討の結果、経口薬として優れたPKプロファイルを有し、IL-6誘発高ヘプシジンマウスモデルを用いた*in vivo*薬効評価において経口投与で強力な血清ヘプシジン低下作用を示す**DS42450411**の創製に成功した。

第二章では、もう一つのHTSヒット化合物**67**の誘導体展開により、経口投与で*in vivo*薬効を示す化合物**129**を創出した経緯を述べた。

化合物 **67** の初期誘導体展開で見出したインダゾール 6 位に *para*-ヒドロキシフェニル基を有する化合物 **109** は IL-6 誘発高ヘプシジンマウスモデルを用いた *in vivo* 薬効評価において、腹腔内投与で血清ヘプシジン低下作用を示すものの経口薬として満足のいくプロファイルではなかった。そこで化合物 **109** の 6 位

フェノール性ヒドロキシ基の代替基探索により経口吸収性を改善し、化合物 **129** を創出した。この化合物は、経口投与で高い *in vivo* 薬効を示した。

第三章では、経口投与で*in vivo*薬効を示す化合物**129**からの誘導体展開により、**DS79182026**を創製した経緯を述べた。

化合物**129**は3-アミノインダゾール部位がキナーゼのヒンジ結合部位と強固な水素結合を形成しているために広範なキナーゼに対して阻害能を示すと考えた。

そこでヒンジ結合部位を変換した3-アリールインダゾール、4-アミノインダゾール、及び3-アミノベンゾイソキサゾール誘導体の3種類のscaffoldをデザインし、合成展開を実施した。その結果、4-アミノインダゾール、及び3-アミノベンゾイソキサゾール誘導体へのscaffold hoppingによりヘプシジン産生阻害活性を保ちつつキナーゼ阻害能を大幅に低減できることを見出した。

3-ベンゾイソキサゾール誘導体の最適化検討の結果、経口薬として優れたPKプロファイルを有し、IL-6誘発高ヘプシジンマウスモデルを用いた*in vivo*薬効評価において経口投与で強力な血清ヘプシジン低下作用を示す**DS79182026**の創製に成功した。さらに**DS79182026**は鉄剤誘導貧血モデルマウスを用いた*in vivo*薬効評価において血球パラメータ (CHr Ret-he) の有意な改善を示したことから、ヘプシジン産生阻害剤が機能性鉄欠乏性貧血治療薬として有効であることが示唆された。

第四章では、**DS79182026**の薬理学的標的タンパク質の同定研究に向けたプローブ化合物の設計、合成、及びそれらを用いて候補タンパク質としてALK2を同定した経緯について述べた。

ベンゾイソキサゾール 3 位置換基は空間許容性が高いことから、**DS79182026** の 3 位メチルカーバメート基の先にアミノ基を持つ PEG リンカーを導入した化合物をデザインした。合成した Boc アミノリンカー体 **218** は **DS79182026** と同

程度の高い *in vitro* 活性を保持していた。

トリチウムで標識した[^{3}H]-**182** を用いた評価により、HepG2 セルライセートにヘプシジン産生阻害剤と特異的な結合を示すタンパク質が含まれることが明らかとなったため、化合物 **219** を固定化したセファロースビーズを用いた候補タンパク質の同定実験を行った。　同定実験により候補に挙がった 10 種のリコンビナントタンパク質を用いて、トリチウムで標識した[^{3}H]-**182** と標識していない **182** を用いた競合実験を行ったところ、ALK2 と ALK3 が同定された。

HepG2 細胞において siRNA を用いて ALK2 の発現をノックダウンすると、ヘプシジン mRNA 発現が阻害された。また種々のベンゾイソキサゾール誘導体のヘプシジン産生阻害能と ALK2 に対する結合能の間に高い相関性が確認されたことにより、ヘプシジン産生阻害剤 **DS79182026** の薬理学的標的タンパク質は ALK2 であることが強く示唆された。

以上、著者は HTS により見出された二つのヒット化合物の構造活性相関研究を実施し、新規経口ヘプシジン産生阻害剤として **DS79182026** を創製した。**DS79182026** は経口投与において強力な *in vivo* ヘプシジン産生阻害作用を示すとともに、鉄剤誘導貧血モデルマウスにおいて血球パラメータの有意な改善を示す化合物である。

本研究における X 線結晶構造解析を用いた化合物デザインや標的探索研究に用いるプローブ化合物のデザイン・合成等の知見は、他の薬物の探索合成研究に応用可能であり、本研究で得られた知見が広く活用されることを期待する。

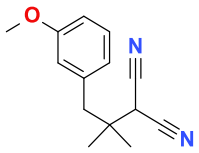
実験の部

Compound Preparation.

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. NMR spectra were recorded on a Varian Mercury 400 or 500 spectrometer with tetramethylsilane as an internal reference. Data for ^{1}H NMR are reported with chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, dt = double triplet, brs = broad singlet), coupling constant (Hz), and integration. Mass spectra were recorded on an Agilent Technologies Agilent 1100 series LC/MS. Unless otherwise noted, all LCMS ions listed are [M + H]. TLC analysis was performed on 60F254 plates (Merck). Flash column chromatography was performed on Shoko scientific cartridge series (SI- 60). All temperatures are degrees Celsius unless otherwise noted.

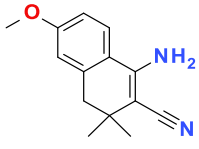
8-methoxy-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (28)

[1-(3-methoxyphenyl)-2-methylpropan-2-yl]propanedinitrile (26)



To a solution of (propan-2-ylidene)propanedinitrile (1.04 g, 9.80 mmol) in THF (15 mL) in a round-bottom flask was added 3-methoxybenzylmagnesium chloride (0.25 mol/L, 50 mL, 13.0 mmol) at 0 ºC. The resulting mixture was stirred at room temperature for 4.5 hours. The reaction mixture was quenched by 1 mol/L hydrogen chloride at 0 ºC, then concentrated under reduced pressure. The residue was added to water and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 60% AcOEt/*n*-hexane linear gradient) provided the title compound. (900 mg, 3.94 mmol, 40% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (dt, *J* = 8.0, 3.0 Hz, 1H), 6.81 (d, *J* = 3.0 Hz, 1H), 6.74 (d, *J* = 3.0 Hz, 1H), 6.71-6.68 (m, 1H), 3.78 (s, 3H), 3.40 (s, 1H), 2.77 (s, 2H), 1.24 (s, 6H).

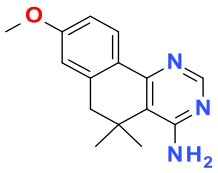
1-amino-6-methoxy-3,3-dimethyl-3,4-dihydronaphthalene-2-carbonitrile (27)



To a solution of [1-(3-methoxyphenyl)-2-methylpropan-2-yl]propanedinitrile (**26**, 3.28 g,

14.4 mmol) in CH_2Cl_2 (100 mL) in a round-bottom flask was added trifluoromethanesulfonic acid (6.30 mL, 71.8 mmol) at 0 °C. The resulting mixture was stirred at 0 °C for 1 hour. The reaction mixture was quenched by saturated aqueous sodium hydrogen carbonate solution, and extracted with CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude title compound was used next reaction without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, *J* = 8.6 Hz, 1H), 6.80 (dd, *J* =8.6, 2.4 Hz, 1H), 6.73 (d, *J* = 2.4 Hz, 1H), 4.50 (brs, 2H), 3.84 (s, 3H), 2.68 (s, 2H), 1.16 (s, 6H); LCMS m/z 229 $[M + H]^+$.

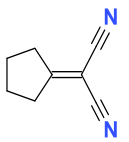
8-methoxy-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (28)



1-Amino-6-methoxy-3,3-dimethyl-3,4-dihydronaphthalene-2-carbonitrile (crude of **27**, 14.4 mmol) was dissolved in formamide (100 mL). The resulting mixture was stirred at 180 °C for 4 hours. The reaction mixture was added to water and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 40% AcOEt/*n*-hexane linear gradient) provided the title compound. (2.64 g, 10.3 mmol, 72% yield in 2 steps): ^{1}H NMR (500 MHz, DMSO-*d*6) δ 8.26 (s, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 6.88 (dd, *J* =8.4, 2.6 Hz, 1H), 6.80 (d, *J* = 2.6 Hz, 1H), 6.37 (brs, 2H), 3.80 (s, 3H), 2.76 (s, 2H), 1.29 (s, 6H); ^{13}C NMR (125 MHz, DMSO-*d*6) δ 161.02, 160.60, 155.45, 155.31, 138.28, 126.77, 125.25, 115.65, 112.73, 112.17, 55.08, 45.10, 32.46, 25.18; LCMS m/z 256 $[M + H]^+$; HRMS (Positive ESI) m/z 256.1436 (256.1372 calcd for $C_{15}H_{17}N_3O$ + H).

6*H*-spiro[benzo[*h*]quinazoline-5,1'-cyclopentan]-4-ol (5)

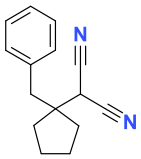
cyclopentylidenepropanedinitrile (2)



To a solution of cyclopentanone (10.0 g, 119 mmol) and malononitrile (8.05 g, 121 mmol) in toluene (100 mL) in a round-bottom flask were added ammonium acetate (1.96 g, 25.4 mmol) and acetic acid (5.5 mL, 96.0 mmol) at room temperature. The resulting mixture was stirred at 105 °C for 4 hours. The reaction mixture was added to water and extracted with Toluene. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated

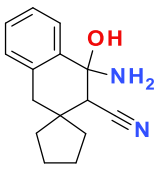
under reduced pressure. Purification by flash chromatography on silica gel (10% AcOEt/*n*-hexane) provided the title compound (15.0 g, 113 mmol, 95% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.84-2.78 (m, 4H), 1.96-1.91 (m, 4H); LCMS m/z 131 $[M - H]^+$.

(1-benzylcyclopentyl)propanedinitrile (3)



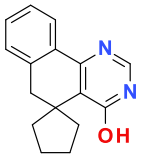
To a solution of cyclopentylidenepropanedinitrile (**2**, 3.03 g, 22.9 mmol) in THF (60 mL) in a round-bottom flask was added 0.96 mol/L benzylmagnesium chloride THF solution (35 ml, 34.0 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 1.75 hours. The reaction mixture was cooled to 0 °C and quenched by 1 mol/L aqueous hydrogen chloride solution (34 mL, 34 mmol). The residual solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (8% AcOEt/*n*-hexane) provided the title compound. (3.94 g, 17.6 mmol, 77% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.25 (m, 3H), 7.20-7.15 (m, 2H), 3.57 (s, 1H), 2.87 (s, 2H), 1.88 (m, 8H).

4'-amino-4'-hydroxy-3',4'-dihydro-1'*H*-spiro[cyclopentane-1,2'-naphthalene]-3'-carbonitrile (4)



(1-Benzylcyclopentyl)propanedinitrile (**3**, 3.89 g, 17.3 mmol) in a round-bottom flask was added to concentrated sulfuric acid (9.0 mL, 173 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 2 hours. The reaction mixture was cooled to 0 °C and quenched by 28% aqueous ammonia solution. The residual solution was extracted with CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (5% MeOH/CH_2Cl_2) provided the title compound. (1.76 g, 7.26 mmol, 42% yield) ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.71 (brs, 1H), 8.15-8.11 (m, 1H), 8.09 (s, 1H), 7.39-7.30 (m, 2H), 7.20-7.15 (m, 1H), 2.86 (s, 2H), 2.38-2.29 (m, 2H), 1.97-1.86 (m, 2H), 1.81-1.73 (m, 2H), 1.58-1.50 (m, 4H); HRMS (Positive ESI) m/z 243.1497 (243.1496 calcd for $C_{15}H_{19}N_2O$ + H).

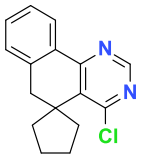
6*H*-spiro[benzo[*h*]quinazoline-5,1'-cyclopentan]-4-ol (5)



To a solution of 4'-amino-4'-hydroxy-3',4'-dihydro-1'*H*-spiro[cyclopentane-1,2'-naphthalene]-3'-carbonitrile (**4**, 1.10 g, 4.54 mmol) in formamide (30 mL) in a round-bottom flask was added phosphoric trichloride (1.70 mL, 18.2 mmol) at 0 °C. The resulting mixture was stirred at 130 °C for 19 hours. The reaction mixture was cooled to 0 °C and quenched by saturated aqueous sodium hydrogen carbonate solution. The residual solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (2% MeOH/CH_2Cl_2) provided the title compound. (859 mg, 3.41 mmol, 75% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 8.16 (d, *J* = 7.2 Hz, 1H), 7.33 (dt, *J* = 7.2, 6.8 Hz, 1H), 7.29 (dt, *J* = 7.2, 6.8 Hz, 1H), 7.21 (dt, *J* = 6.8 Hz, 1H), 4.13 (brs, 1H), 2.88 (s, 2H), 2.09-2.01 (m, 2H), 1.93-1.84 (m, 2H), 1.77-1.64 (m, 4H); HRMS (Positive ESI) m/z 253.1347 (253.1341 calcd for $C_{16}H_{17}N_2O$ + H).

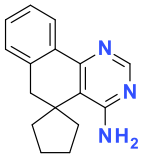
6*H*-spiro[benzo[*h*]quinazoline-5,1'-cyclopentan]-4-amine (1)

4-chloro-6*H*-spiro[benzo[*h*]quinazoline-5,1'-cyclopentane] (6)



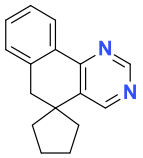
To a solution of 6*H*-spiro[benzo[*h*]quinazoline-5,1'-cyclopentan]-4-ol (**5**, 855 mg, 3.39 mmol) in CH_2Cl_2 (5.0 mL) in a round-bottom flask was added phosphoric trichloride (3.10 mL, 33.9 mmol) at room temperature. The resulting mixture was stirred at 95 °C for 2 hours. The reaction mixture was cooled to 0 °C and quenched by saturated aqueous sodium hydrogen carbonate solution. The residual solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (4% MeOH/CH_2Cl_2) provided the title compound. (789 mg, 2.91 mmol, 86% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.21 (d, *J* = 7.5 Hz, 1H), 7.40 (dt, *J* = 7.5, 7.2 Hz, 1H), 7.37 (dt, *J* = 7.5, 7.2 Hz, 1H), 7.19 (dt, *J* = 7.2 Hz, 1H), 2.94 (s, 2H), 2.41-2.32 (m, 2H), 2.02-1.91 (m, 2H), 1.90-1.78 (m, 2H), 1.74-1.65 (m, 2H); LCMS m/z 271 $[M + H]^+$.

6*H*-spiro[benzo[*h*]quinazoline-5,1'-cyclopentan]-4-amine (1)



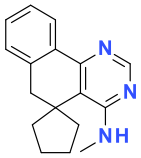
To a solution of 4-chloro-6*H*-spiro[benzo[*h*]quinazoline-5,1'-cyclopentane] (**6**, 695 mg, 2.57 mmol) in formamide (10 mL) in a round-bottom flask was stirred bubbling with ammonia gas at 150 °C for 6.5 hours. The reaction mixture was cooled to room temperature and added to water. The residual solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (3% MeOH/CH_2Cl_2) provided the title compound. (508 mg, 2.02 mmol, 79% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 8.16 (d, *J* = 7.2 Hz, 1H), 7.33 (dt, *J* = 7.2, 6.8 Hz, 1H), 7.29 (dt, *J* = 7.2, 6.8 Hz, 1H), 7.21 (dt, *J* = 6.8 Hz, 1H), 4.91 (brs, 2H), 2.88 (s, 2H), 2.09-2.01 (m, 2H), 1.93-1.84 (m, 2H), 1.77-1.64 (m, 4H); ^{13}C NMR (125 MHz, DMSO-*d*6) δ 160.14, 157.39, 155.93, 136.53, 132.98, 130.08, 127.79, 127.19, 125.72, 118.38, 43.51, 43.40, 35.07, 26.41; LCMS m/z 252 $[M + H]^+$; HRMS (Positive ESI) m/z 252.1499 (252.1501 calcd for $C_{16}H_{18}N_3$ + H); Anal. Calcd for $C_{16}H_{17}N_3$: C, 76.46; H, 6.82; N, 16.72. Found: C, 76.41; H, 6.84; N, 16.54.

6*H*-spiro[benzo[*h*]quinazoline-5,1'-cyclopentane] (7)



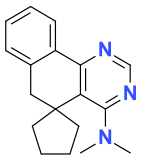
To a solution of 4-chloro-6*H*-spiro[benzo[*h*]quinazoline-5,1'-cyclopentane] (**6**, 58.5 mg, 0.217 mmol) in AcOEt (2 mL) in a round-bottom flask was added 10% palladium on carbon (49.0 mg, 0.039 mmol) at room temperature. The resulting mixture was stirred at room temperature in hydrogen atmosphere for 5 hours. The palladium on carbon was filtrated off with Celite pad and washed with AcOEt. The filtrate was concentrated under reduced pressure. Purification by thin layer chromatography on silica gel (8% AcOEt/CH_2Cl_2) provided the title compound. (2.3 mg, 0.0096 mmol, 4.4% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 8.16 (d, *J* = 7.2 Hz, 1H), 7.33 (dt, *J* = 7.2, 6.8 Hz, 1H), 7.32 (s, 1H), 7.29 (dt, *J* = 7.2, 6.8 Hz, 1H), 7.21 (dt, *J* = 6.8 Hz, 1H), 2.88 (s, 2H), 2.09-2.01 (m, 2H), 1.93-1.84 (m, 2H), 1.77-1.64 (m, 4H); LCMS m/z 237 $[M + H]^+$.

_N_-methyl-6_H_-spiro[benzo[_h_]quinazoline-5,1'-cyclopentan]-4-amine (8)



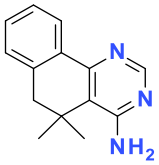
4-Chloro-6*H*-spiro[benzo[*h*]quinazoline-5,1'-cyclopentane] (**6**, 88.0 mg, 0.325 mmol) was dissolved in 40% methylamine MeOH solution (10 mL) at room temperature. The resulting mixture was stirred at 80 ^{o}C for 1 hour. The reaction mixture was cooled to 0 ^{o}C and quenched by saturated aqueous sodium hydrogen carbonate solution. The residual solution was extracted with CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by thin layer chromatography on silica gel (3% MeOH/CH_2Cl_2) provided the title compound. (77.5 mg, 0.292 mmol, 90% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H), 8.13 (d, *J* = 7.2 Hz, 1H), 7.30 (dt, *J* = 7.2, 6.8 Hz, 1H), 7.28 (dt, *J* = 7.2, 6.8 Hz, 1H), 7.11 (dt, *J* = 6.8 Hz, 1H), 4.69 (brs, 1H), 3.05 (d, *J* = 4.7 Hz, 3H), 2.86 (s, 2H), 2.04-1.94 (m, 2H), 1.93-1.82 (m, 2H), 1.75-1.64 (m, 4H); LCMS m/z 266 $[M + H]^{+}$.

_N,N_-dimethyl-6_H_-spiro[benzo[_h_]quinazoline-5,1'-cyclopentan]-4-amine (9)



To a solution of 4-chloro-6*H*-spiro[benzo[*h*]quinazoline-5,1'-cyclopentane] (**6**, 80.9 mg, 0.299 mmol) in isopropyl alcohol (2 mL) in a round-bottom flask was added 50% aqueous dimethylamine solution (8.0 mL) at room temperature. The resulting mixture was stirred at 80 ^{o}C for 1.25 hours. The reaction mixture was cooled to 0 ^{o}C and quenched by saturated aqueous sodium hydrogen carbonate solution. The residual solution was extracted with CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by thin layer chromatography on silica gel (3% MeOH/CH_2Cl_2) provided the title compound. (56.6 mg, 0.203 mmol, 68% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.14 (d, *J* = 6.8 Hz, 1H), 7.32 (dt, *J* = 6.8, 6.4 Hz, 1H), 7.29 (dt, *J* = 6.8, 6.4 Hz, 1H), 7.14 (dt, *J* = 6.4 Hz, 1H), 2.81 (s, 6H), 2.78 (s, 2H), 2.38-2.28 (m, 2H), 1.82-1.74(m, 4H), 1.71-1.63 (m, 2H); LCMS m/z 280 $[M + H]^{+}$; HRMS (Positive ESI) m/z 280.1810 (280.1814 calcd for $C_{18}H_{22}N_3$ + H).

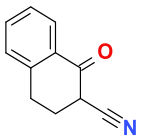
5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (12)



12 was prepared in a similar manner described for **28**. 43% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 8.17 (d, *J* = 7.2 Hz, 1H), 7.33 (dt, *J* = 7.2, 6.8 Hz, 1H), 7.31 (dt, *J* = 7.2, 6.8 Hz, 1H), 7.21 (dt, *J* = 6.8 Hz, 1H), 5.05 (brs, 2H), 2.83 (s, 2H), 1.35 (s, 6H); LCMS m/z 226 $[M + H]^+$; HRMS (Positive ESI) m/z 226.1347 (226.1344 calcd for $C_{14}H_{16}N_3$ + H).

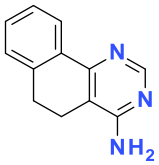
5,6-dihydrobenzo[*h*]quinazolin-4-amine (14)

1-oxo-1,2,3,4-tetrahydronaphthalene-2-carbonitrile (13)



To a solution of α-tetralone (1.66 g, 11.3 mmol) in THF (7 mL) in a round-bottom flask was added 0.41 mol/L lithium diisopropylamide (28.8 mL, 11.7 mmol) at -78 ºC. The resulting mixture was stirred at -78 ºC for 15 minutes. The reaction mixture was added to tosyl cyanide (3.35 g, 18.4 mmol) in THF (35 mL). The resulting mixture was stirred at -78 ºC for 3 hours. The reaction mixture was allowed to 0 ºC and quenched by 28% aqueous ammonia solution. The residual solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by thin layer chromatography on silica gel (2% MeOH/CH_2Cl_2) provided the title compound. (395 mg, 2.31 mmol, 20% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 7.8 Hz, 1H), 7.45 (dt, *J* = 7.8, 1.6 Hz, 1H), 7.34 (dt, *J* = 7.8, 1.6 Hz, 1H), 7.31 (dt, *J* = 7.8 Hz, 1H), 3.87 (t, *J* = 7.0 Hz, 1H), 3.09 (t, *J* = 7.8 Hz, 2H), 2.45 (dt, *J* = 7.8, 7.0 Hz, 2H).

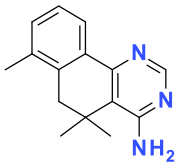
5,6-dihydrobenzo[*h*]quinazolin-4-amine (14)



To a solution of 1-oxo-1,2,3,4-tetrahydronaphthalene-2-carbonitrile (**13**, 435 mg, 2.30 mmol) in formamide (10 mL) in a round-bottom flask was added phosphoric trichloride (0.841 mL, 9.19 mmol) at room temperature. The resulting mixture was stirred at 125 ºC for 5 hours. The reaction mixture was cooled to 0 ºC and quenched by saturated aqueous sodium hydrogen

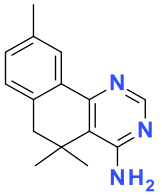
carbonate solution. The residual solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (3% MeOH/CH_2Cl_2) provided the title compound. (89.1 mg, 0.452 mmol, 20% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 8.28 (s, 1H), 8.11-8.02 (m, 1H), 7.36-7.20 (m, 3H), 2.94-2.84 (m, 2H), 2.77-2.63 (m, 2H); LCMS m/z 198 $[M + H]^+$.

5,5,7-trimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (17)



17 was prepared in a similar manner described for **28**. 50% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.24 (s, 1H), 7.90 (d, *J* = 7.4 Hz, 1H), 7.18 (dd, *J* = 7.4, 2.4 Hz, 1H), 7.15 (d, *J* = 7.4 Hz, 1H), 6.44 (brs, 2H), 3.30 (s, 2H), 2.27 (s, 3H), 1.26 (s, 6H); LCMS m/z 240 $[M + H]^+$; HRMS (Positive ESI) m/z 240.1501 (240.1501 calcd for $C_{15}H_{18}N_3$ + H).

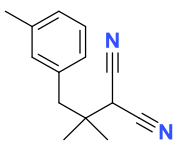
5,5,9-trimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (20)



20 was prepared in a similar manner described for **28**. 47% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.24 (s, 1H), 7.85 (s, 1H), 7.13 (d, *J* = 5.1 Hz, 1H), 7.08 (d, *J* = 5.1 Hz, 1H), 6.44 (brs, 2H), 3.30 (s, 2H), 2.29 (s, 3H), 1.25 (s, 6H); LCMS m/z 240 $[M + H]^+$; HRMS (Positive ESI) m/z 240.1501 (240.1501 calcd for $C_{15}H_{18}N_3$ + H).

5,5,8-trimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (24) and 5,5,10-trimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (25)

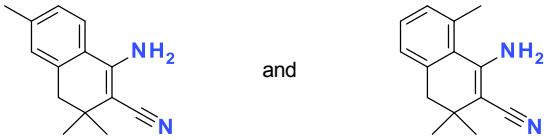
[2-methyl-1-(3-methylphenyl)propan-2-yl]propanedinitrile



To a solution of (propan-2-ylidene)propanedinitrile (2.01 g, 19.0 mmol) in THF (50 mL) in a

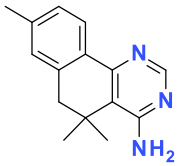
round-bottom flask was added 3-methylbenzylmagnesium chloride (0.50 M, 50 mL, 25.0 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 3 hours. The reaction mixture was quenched by 1 mol/L hydrogen chloride at 0 °C, then concentrated under reduced pressure. The residue was added to water and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 15% AcOEt/*n*-hexane linear gradient) provided the title compound. (2.81g, 3.94 mmol, 70% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (t, *J* = 7.8, 1H), 7.08 (d, *J* = 7.8 Hz, 1H), 6.96 (d, *J* = 7.8 Hz, 1H), 6.95 (d, *J* = 7.8 Hz, 1H), 3.38 (s, 1H), 2.75 (s, 2H), 2.32 (s, 3H), 1.24 (s, 6H).

1-amino-3,3,6-trimethyl-1,2,3,4-tetrahydronaphthalene-2-carbonitrile and 1-amino-3,3,8-trimethyl-1,2,3,4-tetrahydronaphthalene-2-carbonitrile



To a solution of [2-methyl-1-(3-methylphenyl)propan-2-yl]propanedinitrile (1.05 g, 4.95 mmol) in CH_2Cl_2 (30 mL) in a round-bottom flask was added trifluoromethanesulfonic acid (2.18 mL, 24.6 mmol) at 0 °C. The resulting mixture was stirred at 0 °C for 1 hour. The reaction mixture was quenched by saturated aqueous sodium hydrogen carbonate solution, and extracted with CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 65% CH_2Cl_2/*n*-hexane linear gradient) provided the title compound. **1-amino-3,3,6-trimethyl-1,2,3,4-tetrahydronaphthalene-2-carbonitrile** [more polar] (574 mg, 2.38 mmol, 48% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, *J* = 7.8 Hz, 1H), 6.80 (d, *J* = 7.8 Hz, 1H), 6.73 (s, 1H), 4.50 (brs, 2H), 2.64 (s, 2H), 2.33 (s, 3H), 1.12 (s, 6H); LCMS m/z 213 $[M + H]^+$. **1-amino-3,3,8-trimethyl-1,2,3,4-tetrahydronaphthalene-2-carbonitrile** [less polar] (192 mg, 0.888 mmol, 18% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (dd, *J* = 7.4, 7.4 Hz, 1H), 7.06 (d, *J* = 7.4 Hz, 1H), 7.00 (d, *J* = 7.4 Hz, 1H), 4.66 (brs, 2H), 2.64 (s, 2H), 2.59 (s, 3H), 1.09 (s, 6H); LCMS m/z 213 $[M + H]^+$.

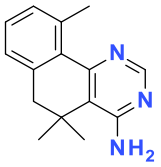
5,5,8-trimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (24)



1-Amino-3,3,6-trimethyl-1,2,3,4-tetrahydronaphthalene-2-carbonitrile (568 mg, 2.37 mmol) was dissolved in formamide (17 mL). The resulting mixture was stirred at 180 °C for 4.5

hours. The reaction mixture was added to water and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 3% MeOH/CH_2Cl_2 linear gradient) provided the title compound. (383 mg, 1.60 mmol, 68% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.23 (s, 1H), 7.91 (d, *J* = 7.8 Hz, 1H), 7.09 (d, *J* =7.8 Hz, 1H), 7.01 (s, 1H), 6.40 (brs, 2H), 2.70 (s, 2H), 2.29 (s, 3H), 1.24 (s, 6H); LCMS m/z 240 $[M + H]^+$; HRMS (Positive ESI) m/z 240.1503 (240.1501 calcd for $C_{15}H_{18}N_3$ + H).

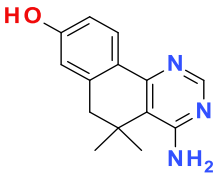
5,5,10-trimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (25)



1-Amino-3,3,8-trimethyl-1,2,3,4-tetrahydronaphthalene-2-carbonitrile (188 mg, 0.886 mmol) was dissolved in formamide (5.6 mL). The resulting mixture was stirred at 180 ºC for 5 hours. The reaction mixture was added to water and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 40% AcOEt/*n*-hexane linear gradient) provided the title compound. (67.9 mg, 0.284 mmol, 32% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.24 (s, 1H), 7.17 (dd, *J* = 7.4, 7.4 Hz, 1H), 7.08 (d, *J* = 7.4 Hz, 1H), 7.02 (d, *J* = 7.4 Hz, 1H), 6.36 (brs, 2H), 2.66 (s, 2H), 2.57 (s, 3H), 1.20 (s, 6H); LCMS m/z 240 $[M + H]^+$; HRMS (Positive ESI) m/z 240.1503 (240.1501 calcd for $C_{15}H_{18}N_3$ + H).

5,5-dimethyl-8-phenyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (31)

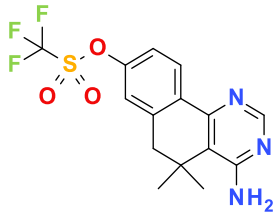
4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-ol (29)



To a solution of 8-methoxy-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (**28**, 3.25 g, 12.7 mmol) in THF (90 mL) in a round-bottom flask was added boron tribromide (1 mol/L CH_2Cl_2 solution, 38 mL, 38.0 mmol) at -40 ºC. The resulting mixture was stirred at room temperature for 1 hour and at 80 ºC for 7 hours. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 60 °C. The title compound was obtained as a pale green solid. (2.52 g, 10.4 mmol, 82% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 9.72 (s, 1H), 8.18 (s, 1H), 7.84 (d, *J* = 8.6 Hz, 1H), 6.65 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.56

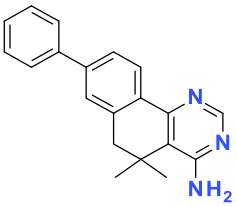
(d, J = 2.4 Hz, 1H), 6.30 (brs, 2H), 2.64 (s, 2H), 1.23 (s, 6H); LCMS m/z 242 $[M + H]^+$.

4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl trifluoromethanesulfonate (30)



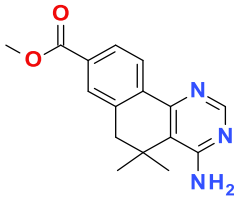
To a solution of 4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-ol (**29**, 1.50 g, 6.22 mmol) in THF (50 mL) in a round-bottom flask were added *N*-phenylbis(trifluoromethanesulfonimide) (2.66 g, 7.45 mmol) and potassium carbonate (2.58 g, 18.6 mmol). The resulting mixture was stirred for 15 hours at room temperature and the reaction mixture was quenched by saturated aqueous sodium hydrogen carbonate solution. The resulting solution was extracted with CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 100% AcOEt/ CH_2Cl_2 linear gradient) provided the title compound. (1.66 g, 4.45 mmol, 72% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s,1H), 8.33 (d, J = 8.6 Hz, 1H), 7.24 (dd, J =8.6, 2.4 Hz, 1H), 7.13 (d, J = 2.4 Hz, 1H), 5.14 (brs, 2H), 2.90 (s, 2H), 1.40 (s, 6H); LCMS m/z 374 $[M + H]^+$.

5,5-dimethyl-8-phenyl-5,6-dihydrobenzo[h]quinazolin-4-amine (31)



To a solution of 4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl trifluoromethanesulfonate (**30**, 80.0 mg, 0.21 mmol) and phenylboronic acid (52.0 mg, 0.43 mmol) in 1,4-dioxane (2 mL) were added tetrakis(triphenylphosphine)palladium(0) (12.0 mg, 0.11 mmol) and sodium carbonate (68.0 mg, 0.64 mmol) at room temperature. The resulting mixture was stirred at 130 °C for 1 hour with microwave reactor. The reaction mixture was added to water and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 40% AcOEt/hexane linear gradient) provided the title compound. (10.0 mg, 0.033 mmol, 15% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.23 (s, 1H), 7.91 (d, J = 7.8 Hz, 1H), 7.55-7.50 (m, 2H), 7.42-7.37 (m, 3H), 7.09 (d, J =7.8 Hz, 1H), 7.01 (s, 1H), 6.40 (brs, 2H), 2.70 (s, 2H), 2.29 (s, 3H), 1.24 (s, 6H); LCMS m/z 302 $[M + H]^+$.

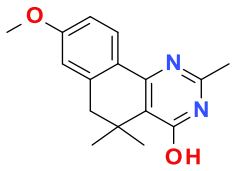
methyl 4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazoline-8-carboxylate (32)



To a solution of 4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl trifluoromethanesulfonate (**30**, 3.00 g, 8.03 mmol) in MeOH (50 mL) and DMF (50 mL) in a round-bottom flask were added [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (1.31 g, 1.61 mmol), triethylamine (4.46 mL, 32.1 mmol) at room temperature. The resulting mixture was stirred at 85 ºC for 6 hours. The reaction mixture was added to water and extracted with toluene. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (50% AcOEt/*n*-hexane) provided the title compound. (2.10 g, 7.40 mmol, 92% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 1H), 8.25 (d, *J* = 7.8 Hz, 1H), 7.98 (d, *J* =7.8 Hz, 1H), 7.85 (s, 1H), 5.09 (brs, 2H), 3.91 (s, 3H), 2.86 (s, 2H), 1.35 (s, 6H); LCMS m/z 284 $[M + H]^+$; HRMS (Positive ESI) m/z 284.1397 (284.1399 calcd for $C_{16}H_{18}N_3O_2$ + H).

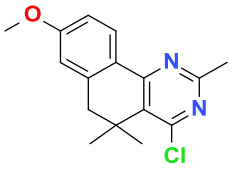
8-methoxy-2,5,5-trimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (35)

8-methoxy-2,5,5-trimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-ol (33)



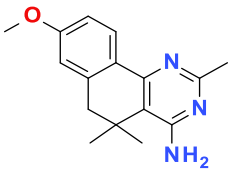
To a solution of 1-amino-6-methoxy-3,3-dimethyl-3,4-dihydronaphthalene-2-carbonitrile (**27**, 402 mg, 1.76 mmol) in toluene (12 mL) in a round-bottom flask was added acetyl chloride (0.188 mL, 2.65 mmol) at room temperature. The resulting mixture was stirred at 65 ºC for 4.5 hours. The reaction mixture was quenched by saturated aqueous sodium hydrogen carbonate solution and extracted with CH_2Cl_2/MeOH (v/v = 20/1). The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting solid was collected and dried under reduced pressure at room temperature. The title compound was obtained as a white solid. (184 mg, 0.681 mmol, 39% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.95 (d, *J* = 8.6 Hz, 1H), 6.86 (d, *J* =8.6 Hz, 1H), 6.81 (s, 1H), 4.13 (brs, 1H), 3.75 (s, 3H), 2.69 (s, 3H), 2.32 (s, 2H), 1.24 (s, 6H).

4-chloro-8-methoxy-2,5,5-trimethyl-5,6-dihydrobenzo[*h*]quinazoline (34)



8-Methoxy-2,5,5-trimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-ol (**33**, 73.4 mg, 0.272 mmol) was added to phosphoric trichloride (0.600 mL, 6.55 mmol) at room temperature. The resulting mixture was stirred at 90 °C for 1 hour. The reaction mixture was added to iced water, saturated aqueous sodium hydrogen carbonate solution and extracted with CH_2Cl_2/MeOH (20/1, v/v). The resulting solid was collected and dried under reduced pressure at room temperature. The title compound was obtained as a pale yellow solid. (77.9 mg, 0.270 mmol, 99% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 9.0 Hz, 1H), 6.88 (d, *J* = 9.0 Hz, 1H), 6.71 (s, 1H), 3.88 (s, 3H), 2.84 (s, 2H), 2.68 (s, 3H), 1.48 (s, 6H).

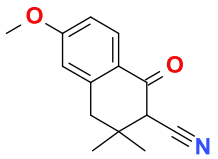
8-methoxy-2,5,5-trimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (35)



To a solution of 4-chloro-8-methoxy-2,5,5-trimethyl-5,6-dihydrobenzo[*h*]quinazoline (**34**, 77.9 mg, 0.270 mmol) in formamide (3 mL) in a round-bottom flask was stirred bubbling with ammonia gas at 160 °C for 2.5 hours. The reaction mixture was quenched by saturated aqueous sodium hydrogen carbonate solution and extracted with CH_2Cl_2/MeOH (20/1, v/v). The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by thin layer chromatography on silica gel (3% MeOH/CH_2Cl_2) provided the title compound. (21.0 mg, 0.078 mmol, 29% yield): ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.95 (d, *J* = 8.6 Hz, 1H), 6.88 (d, *J* =8.6 Hz, 1H), 6.75 (s, 1H), 6.26 (brs, 2H), 3.76 (s, 3H), 3.31 (s, 3H), 2.69 (s, 2H), 1.22 (s, 6H); LCMS m/z 270 $[M + H]^+$; HRMS (Positive ESI) m/z 270.1607 (270.1606 calcd for $C_{16}H_{20}N_3O$ + H).

<u>7-methoxy-4,4-dimethyl-4,5-dihydro-1*H*-benzo[*g*]indazol-3-amine (37)</u>

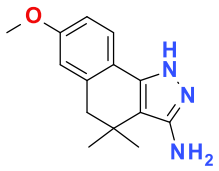
6-methoxy-3,3-dimethyl-1-oxo-1,2,3,4-tetrahydronaphthalene-2-carbonitrile (36)



To a solution of 1-amino-6-methoxy-3,3-dimethyl-3,4-dihydronaphthalene-2-carbonitrile (**27**,

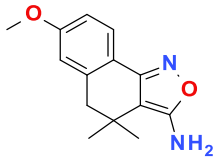
1.12 g, 4.90 mmol) in MeOH (30 mL) in a round-bottom flask were added water (0.88 mL) and concentrated sulfuric acid (0.26 mL, 4.89 mmol) at room temperature. The resulting mixture was stirred at 80 ºC for 12 hours. The reaction mixture was quenched by saturated aqueous sodium hydrogen carbonate solution and extracted with CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0.5% AcOEt/ CH_2Cl_2) provided the title compound. (970 mg, 4.23 mmol, 86% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 7.4 Hz, 1H), 6.83 (d, *J* =7.4 Hz, 1H), 6.65 (s, 1H), 3.85 (s, 3H), 3.52 (s, 1H), 2.91 (q, *J* =11.8 Hz, 2H), 1.31 (s, 3H), 1.12 (s, 3H).

7-methoxy-4,4-dimethyl-4,5-dihydro-1*H*-benzo[*g*]indazol-3-amine (37)



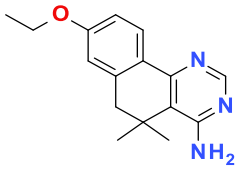
To a solution of 6-methoxy-3,3-dimethyl-1-oxo-1,2,3,4-tetrahydronaphthalene-2-carbonitrile (**36**, 340 mg, 1.48 mmol) in EtOH (15 mL) in a round-bottom flask were added hydrazine monohydrate (0.14 mL, 2.97 mmol) and acetic acid (0.22 mL, 3.71 mmol) at room temperature. The resulting mixture was stirred at reflux for 22 hours. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (70% to 100% AcOEt/*n*-hexane linear gradient) provided the title compound. (260 mg, 1.07 mmol, 72% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, *J* = 8.0 Hz, 1H), 6.79 (s, 1H), 6.78 (brs, 1H), 6.76 (d, *J* = 8.0 Hz, 1H), 3.83 (s, 3H), 3.64 (brs, 2H), 2.76 (s, 2H), 1.30 (s, 6H); LCMS m/z 244 $[M + H]^+$.

7-methoxy-4,4-dimethyl-4,5-dihydronaphtho[1,2-*c*][1,2]oxazol-3-amine (38)



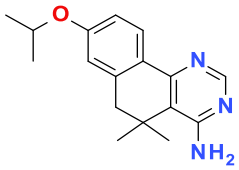
38 was prepared in a similar manner described for **37**. 56% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.6 Hz, 1H), 6.79 (d, *J* = 8.6 Hz, 1H), 6.72 (s, 1H), 4.22 (brs, 2H), 3.80 (s, 3H), 2.69 (s, 2H), 1.22 (s, 6H); LCMS m/z 245 $[M + H]^+$; HRMS (Positive ESI) m/z 245.1282 (245.1290 calcd for $C_{14}H_{17}N_2O_2$ + H).

8-ethoxy-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (39)



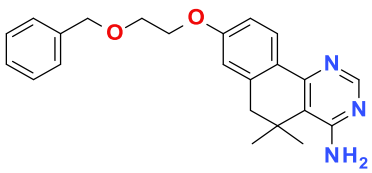
To a solution of 4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-ol (**29**, 49.4 mg, 0.205 mmol) and triphenylphosphine (68.0 mg, 0.259 mmol) in THF (2.5 mL) in a round-bottom flask were added EtOH (0.030 mL, 0.514 mmol) and diisopropyl azodicarboxylate (0.0483 mL, 0.245 mmol) at room temperature. The resulting mixture was stirred at room temperature for 3 hours. The reaction mixture was concentrated under reduced pressure. Purification by thin layer chromatography on silica gel (40% AcOEt/CH_2Cl_2) provided the title compound. (36.9 mg, 0.137 mmol, 67% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 8.10 (d, *J* = 8.6 Hz, 1H), 6.83 (dd, *J* =8.6, 1.8 Hz, 1H), 6.80 (d, *J* = 1.8 Hz, 1H), 4.99 (brs, 2H), 4.04 (q, *J* = 7.1 Hz, 2H), 2.77 (s, 2H), 1.40 (t, *J* = 7.1 Hz, 3H), 1.33 (s, 6H); LCMS m/z 270 $[M + H]^+$; HRMS (Positive ESI) m/z 270.1606 (270.1606 calcd for $C_{16}H_{20}N_3O$ + H).

5,5-dimethyl-8-[(propan-2-yl)oxy]-5,6-dihydrobenzo[*h*]quinazolin-4-amine (40)



40 was prepared in a similar manner described for **39**. 65% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 8.09 (d, *J* = 8.6 Hz, 1H), 6.81 (dd, *J* =8.6, 1.8 Hz, 1H), 6.64 (d, *J* = 1.8 Hz, 1H), 4.99 (brs, 2H), 4.59 (quintet, *J* = 6.3 Hz, 1H), 2.76 (s, 2H), 1.34 (s, 6H), 1.33 (d, *J* = 6.3 Hz, 6H); LCMS m/z 284 $[M + H]^+$; HRMS (Positive ESI) m/z 284.1755 (284.1763 calcd for $C_{17}H_{22}N_3O$ + H).

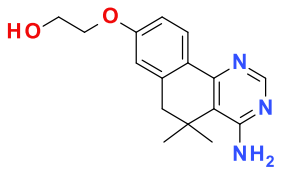
2-[(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)oxy]ethan-1-ol (42)
8-[2-(benzyloxy)ethoxy]-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (41)



41 was prepared in a similar manner described for **39**. 70% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 1H), 8.17 (d, *J* = 8.6 Hz, 1H), 7.45-7.25 (m, 5H), 6.92 (dd, *J* =8.6, 2.2 Hz, 1H), 6.76 (d, *J* = 2.2 Hz, 1H), 5.06 (brs, 2H), 4.68 (s, 2H), 4.27-4.21 (m, 2H), 3.92-3.86 (m, 2H),

2.84 (s, 2H), 1.40 (s, 6H); LCMS m/z 376 $[M + H]^+$; HRMS (Positive ESI) m/z 376.2041 (376.2025 calcd for $C_{23}H_{26}N_3O_2$ + H).

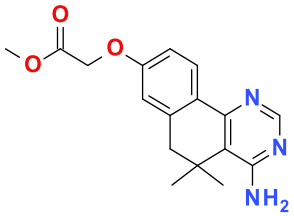
2-[(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)oxy]ethan-1-ol (42)



To a solution of 8-[2-(benzyloxy)ethoxy]-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (**41**, 50.4 mg, 0.134 mmol) in EtOH (1 mL) and MeOH (1 mL) in a round-bottom flask was added 10% palladium on carbon (49.4 mg, 2.17 mmol) at room temperature. The resulting mixture was stirred at room temperature in hydrogen atmosphere for 7.5 hours. The palladium on carbon was filtrated off with Celite pad and washed with AcOEt. The filtrate was concentrated under reduced pressure. Purification by thin layer chromatography on silica gel (5% AcOEt/CH_2Cl_2) provided the title compound. (12.0 mg, 0.0421 mmol, 31% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.20 (s, 1H), 7.94 (d, *J* = 9.0 Hz, 1H), 6.83 (dd, *J* = 9.0, 2.3 Hz, 1H), 6.76 (d, *J* = 2.3 Hz, 1H), 6.35 (brs, 2H), 4.85 (t, *J* = 5.6 Hz, 1H), 4.02-3.96 (m, 2H), 3.72-3.65 (m, 2H), 2.71 (s, 2H), 1.24 (s, 6H); LCMS m/z 286 $[M + H]^+$; HRMS (Positive ESI) m/z 286.1555 (286.1556 calcd for $C_{16}H_{20}N_3O_2$ + H).

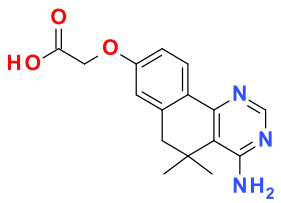
[(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)oxy]acetic acid (44)

methyl [(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)oxy]acetate (43)



43 was prepared in a similar manner described for **39**. 39% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (s, 1H), 8.13 (d, *J* = 8.6 Hz, 1H), 6.82 (dd, *J* =8.6, 1.9 Hz, 1H), 6.70 (d, *J* = 1.9 Hz, 1H), 4.98 (brs, 2H), 4.65 (s, 2H), 3.79 (s, 3H), 2.78 (s, 2H), 1.33 (s, 6H); LCMS m/z 314 $[M + H]^+$; HRMS (Positive ESI) m/z 314.1508 (314.1505 calcd for $C_{17}H_{20}N_3O_3$ + H).

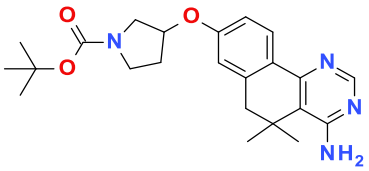
[(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)oxy]acetic acid (44)



To a solution of methyl [(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)oxy]acetate (**43**, 43.9 mg, 0.140 mmol) in MeOH (1.5 mL) in a round-bottom flask was added 1 mol/L aqueous sodium hydroxide solution (0.420 mL, 0.420 mmol) at room temperature. The resulting mixture was stirred at room temperature for 2 hours. The reaction mixture was quenched by 1 mol/L aqueous hydrochloric acid solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a pale green solid. (31.3 mg, 0.105 mmol, 75% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 13.04 (s, 1H), 8.21 (s, 1H), 7.94 (d, *J* = 8.6 Hz, 1H), 6.80 (dd, *J* =8.6, 2.8 Hz, 1H), 6.74 (d, *J* = 2.8 Hz, 1H), 6.37 (brs, 2H), 4.68 (s, 2H), 2.71 (s, 2H), 1.24 (s, 6H); LCMS m/z 300 $[M + H]^+$; HRMS (Positive ESI) m/z 300.1353 (300.1348 calcd for $C_{16}H_{18}N_3O_3$ + H).

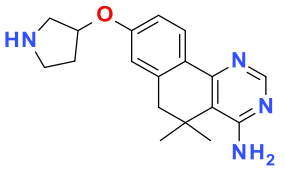
5,5-dimethyl-8-[(pyrrolidin-3-yl)oxy]-5,6-dihydrobenzo[*h*]quinazolin-4-amine (46)

***tert*-butyl 3-[(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)oxy]pyrrolidine-1-carboxylate (45)**



45 was prepared in a similar manner described for **39**. quantative yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.18 (s, 1H), 7.93 (d, *J* = 8.2 Hz, 1H), 6.78 (dd, *J* =8.2, 2.0 Hz, 1H), 6.68 (d, *J* = 2.0 Hz, 1H), 6.25 (brs, 2H), 4.88-4.81 (m,1H), 3.09-3.01 (m, 1H), 2.92-2.79 (m, 2H), 2.75-2.71 (m, 1H), 2.72 (s, 2H), 2.04-1.92 (m, 1H), 1.77-1.68 (m, 1H), 1.42 (s, 9H), 1.23 (s, 6H).

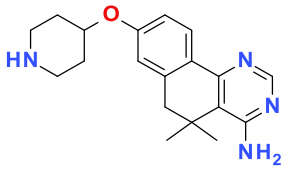
5,5-dimethyl-8-[(pyrrolidin-3-yl)oxy]-5,6-dihydrobenzo[*h*]quinazolin-4-amine (46)



To a solution of *tert*-butyl 3-[(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)oxy]pyrrolidine-1-carboxylate (**45**, 356 mg, 0.870 mmol) in CH_2Cl_2 (7 mL) in a round-bottom flask was added 4 mol/L hydrogen chloride in 1,4-dioxane (7 mL, 28.0 mmol) at room temperature. The resulting mixture was stirred at room temperature for 2 hours. The reaction mixture was quenched by saturated aqueous sodium hydrogen carbonate solution and extracted with CH_2Cl_2/MeOH (20/1, v/v). The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting solid was

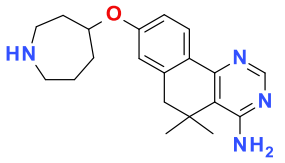
washed with CH_2Cl_2/MeOH-hexane and dried under reduced pressure at room temperature. The title compound was obtained as a white solid. (194 mg, 0.625 mmol, 72% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.20 (s, 1H), 7.93 (d, *J* = 8.6 Hz, 1H), 6.79 (dd, *J* =8.6, 2.4 Hz, 1H), 6.70 (d, *J* = 2.4 Hz, 1H), 6.35 (brs, 2H), 4.88-4.81 (m,1H), 3.06-3.00 (m, 1H), 2.90-2.77 (m, 2H), 2.75-2.71 (m, 1H), 2.70 (s, 2H), 2.04-1.92 (m, 1H), 1.77-1.68 (m, 1H), 1.24 (s, 6H); LCMS m/z 311 $[M + H]^+$; HRMS (Positive ESI) m/z 311.1869 (311.1872 calcd for $C_{18}H_{23}N_4O$ + H).

5,5-dimethyl-8-[(piperidin-4-yl)oxy]-5,6-dihydrobenzo[*h*]quinazolin-4-amine (47)



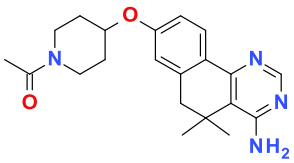
47 was prepared in a similar manner described for **46**. 89% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.20 (s, 1H), 7.92 (d, *J* = 8.6 Hz, 1H), 6.82 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.70 (d, *J* = 2.3 Hz, 1H), 6.33 (brs, 2H), 4.45-4.37 (m, 1H), 4.10-4.04 (m, 1H), 2.94-2.86 (m, 2H), 2.59-2.47 (m, 2H), 2.70 (s, 2H), 1.91-1.85 (m, 2H), 1.44-1.31 (m, 2H), 1.24 (s, 6H); LCMS m/z 325 $[M + H]^+$; HRMS (Positive ESI) m/z 325.2035 (325.2028 calcd for $C_{19}H_{25}N_4O$ + H).

8-[(azepan-4-yl)oxy]-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (48)



48 was prepared in a similar manner described for **46**. 80% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.20 (s, 1H), 7.93 (d, *J* = 9.0 Hz, 1H), 6.78 (dd, *J* = 9.0, 2.3 Hz, 1H), 6.76 (d, *J* = 2.3 Hz, 1H), 6.33 (brs, 2H), 4.45-4.37 (m, 1H), 4.10-4.04 (m, 1H), 2.85-2.60 (m, 4H), 2.69 (s, 2H), 2.07-1.88 (m, 2H), 1.80-1.60 (m, 3H), 1.57-1.40 (m, 1H), 1.23 (s, 6H); LCMS m/z 339 $[M + H]^+$; HRMS (Positive ESI) m/z 339.2185 (339.2185 calcd for $C_{20}H_{27}N_4O$ + H).

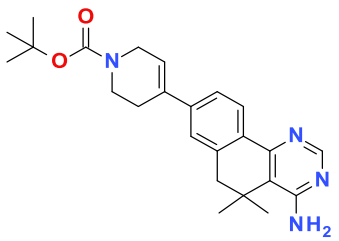
1-{4-[(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)oxy]piperidin-1-yl}ethan-1-one (49)



To a solution of 5,5-dimethyl-8-[(piperidin-4-yl)oxy]-5,6-dihydrobenzo[*h*]quinazolin-4-amine (**47**, 40.6 mg, 0.125 mmol) and triethylamine (0.0348 mmol, 0.250 mmol) in CH_2Cl_2 (1.0 mL) in a round-bottom flask was added acetic anhydride (0.100 mL, 0.137 mmol) at room temperature. The resulting mixture was stirred at room temperature for 2 hours. The reaction mixture was added to water and extracted with CH_2Cl_2/MeOH (v/v = 20/1). The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by thin layer chromatography on silica gel (7% 7 mol/L ammonia MeOH solution/CH_2Cl_2) provided the title compound. (38.0 mg, 0.104 mmol, 83% yield): ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.21 (s, 1H), 7.94 (d, *J* = 8.6 Hz, 1H), 6.88 (dd, *J* = 8.6, 1.6 Hz, 1H), 6.81 (d, *J* = 1.6 Hz, 1H), 6.36 (brs, 2H), 4.69-4.60 (m, 1H), 3.89-3.81 (m, 1H), 3.70-3.62 (m, 1H), 3.35-3.27 (m, 1H), 3.21-3.13 (m, 1H), 2.71 (s, 2H), 1.98 (s, 3H), 2.00-1.82 (m, 2H), 1.64-1.51 (m, 1H), 1.50-1.42 (m, 1H), 1.23 (s, 6H); LCMS m/z 367 $[M + H]^+$; HRMS (Positive ESI) m/z 367.2133 (367.2134 calcd for $C_{21}H_{27}N_4O_2$ + H).

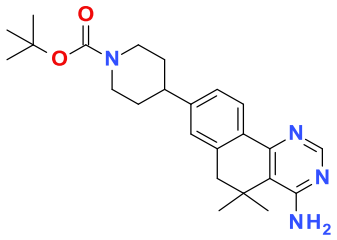
5,5-dimethyl-8-(piperidin-4-yl)-5,6-dihydrobenzo[*h*]quinazolin-4-amine (52)

***tert*-butyl 4-(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (50)**



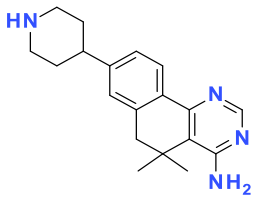
To a solution of 4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl trifluoromethanesulfonate (**30**, 4.80 g, 12.9 mmol) and *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (6.76 g, 21.9 mmol) in toluene (200 mL) and EtOH (25 mL) were added tetrakis(triphenylphosphine)palladium(0) (1.49 g, 1.29 mmol), sodium carbonate (5.45 g, 51.4 mmol) and water (50 mL) at room temperature. The resulting mixture was stirred at 100 °C for 40 minutes. The reaction mixture was added to water and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 100% AcOEt/CH_2Cl_2 linear gradient) provided the title compound. (3.60 g, 8.89 mmol, 69% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 8.13 (d, *J* = 8.2 Hz, 1H), 7.34 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.15 (d, *J* = 1.6 Hz, 1H), 6.13 (brs, 2H), 5.02 (brs, 1H), 4.08 (d, *J* = 9.4 Hz, 2H), 3.62 (t, *J* = 5.5 Hz, 2H), 2.82 (s, 2H), 2.53 (t, *J* = 8.2 Hz, 2H), 1.42 (s, 9H), 1.23 (s, 6H).

***tert*-butyl 4-(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)piperidine-1-carboxylate (51)**



To a solution of *tert*-butyl 4-(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (**50**, 60.4 mg, 0.149 mmol) in EtOH (1.5 mL) in a round-bottom flask was added 10% palladium on carbon (42.0 mg, 0.037 mmol) at room temperature. The resulting mixture was stirred at room temperature in hydrogen atmosphere for 5 hours and at 40 °C for 3.5 hours. The palladium on carbon was filtrated off with Celite pad and washed with AcOEt. The filtrate was concentrated under reduced pressure. Purification by thin layer chromatography on silica gel (6% AcOEt/CH_2Cl_2) provided the title compound. (33.0 mg, 0.0808 mmol, 54% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 8.10 (d, *J* = 7.8 Hz, 1H), 7.15 (dd, *J* = 7.8, 2.5 Hz, 1H), 6.96 (d, *J* = 2.5 Hz, 1H), 5.00 (brs, 1H), 4.35-4.12 (m, 1H), 3.60-3.45 (m, 1H), 2.85-2.70 (m, 2H), 2.79 (s, 2H), 2.70-2.55 (m, 1H), 1.85-1.75 (m, 1H), 1.69-1.60 (m, 2H), 1.45 (s, 9H), 1.34 (s, 6H); LCMS m/z 409 [M + H]$^+$; HRMS (Positive ESI) m/z 409.2605 (409.2604 calcd for $C_{24}H_{33}N_4O_2$ + H).

5,5-dimethyl-8-(piperidin-4-yl)-5,6-dihydrobenzo[*h*]quinazolin-4-amine (52)

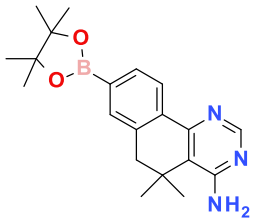


To a solution of *tert*-butyl 4-(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)piperidine-1-carboxylate (**51**, 31.3 mg, 0.0766 mmol) in CH_2Cl_2 (1.0 mL) in a round-bottom flask was added 4 mol/L hydrogen chloride in 1,4-dioxane (1.0 mL, 4.0 mmol) at room temperature. The resulting mixture was stirred at room temperature for 2 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and extracted with CH_2Cl_2/MeOH (v/v = 15/1). The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting solid was collected. The solid was washed with hexane/CH_2Cl_2 and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (17.6 mg, 0.0571 mmol, 75% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.27 (s, 1H), 7.98 (d,

J = 8.8 Hz, 1H), 7.16 (dd, *J* = 8.8, 1.6 Hz, 1H), 7.07 (d, *J* = 1.6 Hz, 1H), 6.44 (brs, 2H), 3.47-3.28 (m, 2H), 3.08-3.01 (m, 2H), 2.76 (s, 2H), 2.64-2.55 (m, 2H), 1.75-1.67 (m, 2H), 1.59-1.46 (m, 2H), 1.28 (s, 6H); LCMS m/z 309 $[M + H]^+$; HRMS (Positive ESI) m/z 309.2077 (309.2079 calcd for $C_{19}H_{25}N_4$ + H).

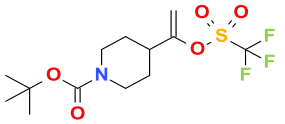
5,5-dimethyl-8-[1-(piperidin-4-yl)ethenyl]-5,6-dihydrobenzo[*h*]quinazolin-4-amine (59)

5,5-dimethyl-8-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydrobenzo[*h*]quinazolin-4-amine (53)



To a solution of 4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl trifluoromethanesulfonate (**30**, 10.0 g, 26.8 mmol) in 1,4-dioxane (100 mL) in a round-bottom flask were added bis(pinacolato)diboron (8.84 g, 34.8 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (1.09 g, 1.34 mmol) and potassium acetate (7.89 g, 80.3 mmol). The resulting mixture was stirred for 6 hours at 90 °C and the reaction mixture was added to water. The resulting solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (10% AcOEt/*n*-hexane) provided the title compound. (8.50 g, 24.0 mmol, 90% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s,1H), 8.20 (d, *J* = 7.4 Hz, 1H), 7.80 (d, *J* =7.4 Hz, 1H), 7.63 (s, 1H), 5.19 (brs, 2H), 2.87 (s, 2H), 1.37 (s, 12H), 1.36 (s, 6H); LCMS m/z 352 $[M + H]^+$.

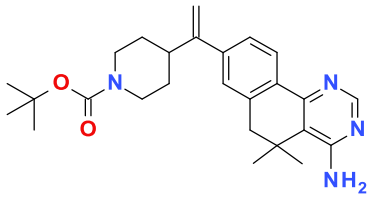
***tert*-butyl 4-{1-[(trifluoromethanesulfonyl)oxy]ethenyl}piperidine-1-carboxylate**



To a solution of *tert*-butyl 4-acetylpiperidine-1-carboxylate (2.74 g, 12.1 mmol) in THF (50 mL) in a round-bottom flask were added 1 mol/L sodium bis(trimethylsilyl)amide tetrahydrofuran solution (13.3 mL, 13.3 mmol) at -78 °C. The resulting mixture was stirred at -78 °C for 1 hour, then added *N*-phenylbis(trifluoromethanesulfonimide) (4.74 g, 13.3 mmol) at 0 °C. The resulting mixture was stirred at 0 °C for 3 hours, and the reaction mixture was quenched by saturated ammonium chloride solution. The resulting solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and

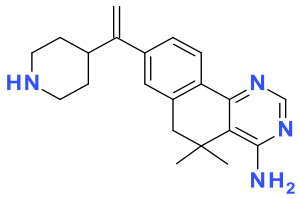
concentrated under reduced pressure. Purification by flash chromatography on silica gel (20% AcOEt/*n*-hexane) provided the title compound. (3.92 g, 10.9 mmol, 91% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.10 (d, *J* = 3.9 Hz,1H), 4.90 (d, *J* = 3.9 Hz,1H), 4.25-4.05 (m, 2H), 2.76-2.59 (m, 2H), 2.38-2.28 (m, 1H), 1.91-1.83 (m, 2H), 1.42 (s, 9H), 1.42-1.31 (m, 2H).

tert-butyl 4-[1-(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)ethenyl]piperidine-1-carboxylate (54)



To a solution of 5,5-dimethyl-8-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydrobenzo[*h*]quinazolin-4-amine (**53**, 880 mg, 2.51 mmol) in 1,4-dioxane (15 mL) in a round-bottom flask were added *tert*-butyl 4-{1-[(trifluoromethanesulfonyl)oxy]ethenyl}piperidine-1-carboxylate (1.35 g, 3.76 mmol), tetrakis(triphenylphosphine)palladium(0) (145 mg, 0.125 mmol), sodium carbonate (531 mg, 5.01 mmol) and water (1.5 mL). The resulting mixture was stirred for 3 hours at 100 ºC and the reaction mixture was added to water. The resulting solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (10% AcOEt/*n*-hexane) provided the title compound. (853 mg, 1.96 mmol, 78% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 8.18 (dd, *J* = 5.1, 1.2 Hz, 1H), 7.32 (dd, *J* = 4.2, 1.2 Hz, 1H), 7.14 (dd, *J* = 5.1, 4.2 Hz, 1H), 5.26 (s, 1H), 5.05 (brs, 2H), 5.04 (s, 1H), 4.25-3.95 (m, 2H), 3.75-3.68 (m, 1H), 3.28-3.19 (m, 1H), 2.85-2.72 (m, 1H), 1.90-1.78 (m, 1H), 1.41 (s, 6H), 1.43-1.37 (m, 2H), 1.36 (s, 9H); LCMS m/z 435 $[M + H]^+$.

5,5-dimethyl-8-[1-(piperidin-4-yl)ethenyl]-5,6-dihydrobenzo[*h*]quinazolin-4-amine (59)

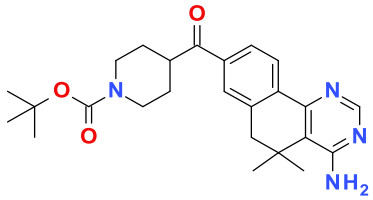


To a solution of *tert*-butyl 4-[1-(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)ethenyl]piperidine-1-carboxylate (**54**, 1.20 g, 2.80 mmol) in CH_2Cl_2 (5 mL) in a round-bottom flask was added trifluoroacetic acid (5 mL). The resulting mixture was stirred for 3 hours at room temperature and the reaction mixture was concentrated. Purification by flash chromatography on NH-silica gel (10% MeOH/AcOEt) provided the title compound. (782

mg, 2.34 mmol, 85% yield): ^{1}H NMR (500 MHz, DMSO-*d*6) δ 8.29 (s, 1H), 8.02 (d, *J* = 8.2 Hz, 1H), 7.33 (d, *J* = 8.2 Hz, 1H), 7.24 (s, 1H), 6.46 (brs,2H), 5.27 (s, 1H), 5.04 (s, 1H), 3.45-3.09 (m, 2H), 3.01-2.92 (m, 2H), 2.80 (s, 2H), 2.60-2.51 (m, 4H), 1.70-1.64 (m, 2H), 1.29 (s, 6H), 1.29-1.20 (m, 2H); ^{13}C NMR (125 MHz, DMSO-*d*6) δ 161.16, 155.53, 154.97, 153.08, 142.86, 136.33, 131.53, 125.38, 125.00, 124.45, 116.84, 110.89, 46.41, 44.88, 32.66, 32.46, 25.10; LCMS m/z 335 $[M + H]^{+}$; HRMS (Positive ESI) m/z 335.2254 (335.2157 calcd for $C_{21}H_{26}N_4$ + H).

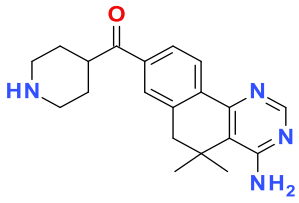
(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)(piperidin-4-yl)methanone (56)

***tert*-butyl 4-(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazoline-8-carbonyl)piperidine-1-carboxylate (55)**



To a solution of *tert*-butyl 4-[1-(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)ethenyl]piperidine-1-carboxylate (**54**, 457 mg, 1.05 mmol) in 1,4-dioxane (10 mL) in a round-bottom flask were added osmium tetroxide solution, 2.5 wt. % in *tert*-butanol (668 mg, 1.05 mmol) and 4-methylmorpholine *N*-oxide (185 mg, 1.58 mmol) at room temperature. The resulting mixture was stirred at 105 °C for overnight. The reaction mixture was added sodium periodate (338 mg, 1.58 mmol) and stirred at room temperature for 1 hour. The reaction mixture was quenched by saturated aqueous sodium thiosulfate solution and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (20% AcOEt/*n*-hexane) provided the title compound. (398 mg, 0.912 mmol, 87% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 8.09 (d, *J* = 7.8 Hz, 1H), 7.32 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.14 (dd, *J* = 1.2 Hz, 1H), 5.00 (brs, 2H), 4.35-4.25 (m, 1H), 3.35-3.20 (m, 1H), 2.81 (s, 2H), 2.47-2.35 (m, 1H), 1.95-1.82 (m, 2H), 1.40 (s, 9H), 1.36 (s, 6H), 1.10-0.98 (m, 2H), 0.96-0.89 (m, 2H); LCMS m/z 437 $[M + H]^{+}$.

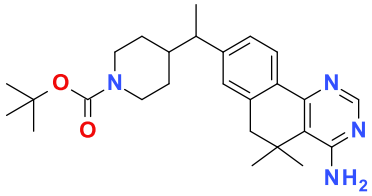
(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)(piperidin-4-yl)methanone (56)



56 was prepared in a similar manner described for **59**. 73% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 1H), 8.27 (d, *J* = 7.8 Hz, 1H), 7.85 (dd, *J* = 7.8, 2.0 Hz, 1H), 7.74 (d, *J* = 2.0 Hz, 1H), 5.09 (brs,2H), 3.42-3.33 (m, 1H), 3.20-3.15 (m, 2H), 2.89 (s, 2H), 2.80-2.70 (m, 2H), 1.86-1.80 (m, 2H), 1.72-1.60 (m, 2H), 1.36 (s, 6H); LCMS m/z 337 $[M + H]^+$.

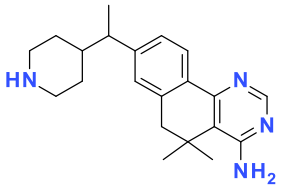
5,5-dimethyl-8-[1-(piperidin-4-yl)ethyl]-5,6-dihydrobenzo[*h*]quinazolin-4-amine (58)

***tert*-butyl 4-[1-(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)ethyl]piperidine-1-carboxylate (57)**



To a solution of *tert*-butyl 4-[1-(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)ethenyl]piperidine-1-carboxylate (**54**, 95.0 mg, 0.219 mmol) in MeOH (5 mL) in a round-bottom flask was added 10% palladium on carbon (120 mg, 0.112 mmol) at room temperature. The resulting mixture was stirred at room temperature in hydrogen atmosphere for 5 hours. The palladium on carbon was filtrated off with Celite pad and washed with MeOH. The filtrate was concentrated under reduced pressure. Purification by flash chromatography on silica gel (20% AcOEt/*n*-hexane) provided the title compound. (89.0 mg, 0.204 mmol, 95% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 8.18 (dd, *J* = 5.1, 1.2 Hz, 1H), 7.32 (dd, *J* = 4.2, 1.2 Hz, 1H), 7.14 (dd, *J* = 5.1, 4.2 Hz, 1H), 5.05 (brs, 2H), 4.25-3.95 (m, 2H), 3.75-3.68 (m, 1H), 3.40-3.30 (m, 1H), 3.28-3.19 (m, 1H), 2.85-2.72 (m, 1H), 1.90-1.78 (m, 1H), 1.41 (s, 6H), 1.43-1.37 (m, 2H), 1.36 (s, 9H), 1.24 (d, *J* = 7.4 Hz, 1H).

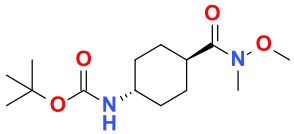
5,5-dimethyl-8-[1-(piperidin-4-yl)ethyl]-5,6-dihydrobenzo[*h*]quinazolin-4-amine (58)



58 was prepared in a similar manner described for **59**. 78% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.27 (s, 1H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 7.01 (s, 1H), 6.44 (brs, 2H), 3.40-3.30 (m, 1H), 2.97-2.80 (m, 2H), 2.76 (s, 2H), 2.60-2.51 (m, 4H), 1.70-1.64 (m, 2H), 1.28 (s, 6H), 1.24 (d, *J* = 7.4 Hz, 3H), 1.29-1.20 (m, 2H); LCMS m/z 337 [M + H]$^+$.

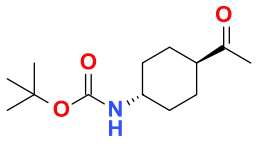
8-{1-[(1*r*,4*r*)-4-aminocyclohexyl]ethenyl}-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (62, DS42450411)

***tert*-butyl {(1*r*,4*r*)-4-[methoxy(methyl)carbamoyl]cyclohexyl}carbamate**



To a solution of (1*r*,4*r*)-4-[(*tert*-butoxycarbonyl)amino]cyclohexane-1-carboxylic acid (1.22 g, 5.00 mmol) in THF (10 mL) and MeOH (10 mL) in a round-bottom flask were added *N,O*-dimethylhydroxylamine hydrochloride (540 mg, 5.50 mmol), 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (1.52 g, 5.50 mmol) and *N*-methylmorpholine (1.00 g, 10.0 mmol). The resulting mixture was stirred at room temperature for 2 hours, then concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 100% AcOEt/CH_2Cl_2 linear gradient) provided the title compound. (1.42 g, 4.96 mmol, 99% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.37 (brs, 1H), 3.88-3.67 (m, 1H), 3.70 (s, 3H), 3.51-3.36 (m, 1H), 3.17 (s, 3H), 2.67-2.52 (m, 1H), 2.15-2.02 (m, 2H), 1.88-1.78 (m, 2H), 1.69-1.54 (m, 2H), 1.45 (s, 9H), 1.21-1.08 (m, 2H).

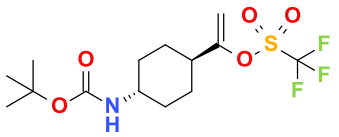
***tert*-butyl [(1*r*,4*r*)-4-acetylcyclohexyl]carbamate**



To a solution of *tert*-butyl {(1*r*,4*r*)-4-[methoxy(methyl)carbamoyl]cyclohexyl}carbamate (1.42 g, 4.96 mmol) in THF (50 mL) in a round-bottom flask was added 3 mol/L

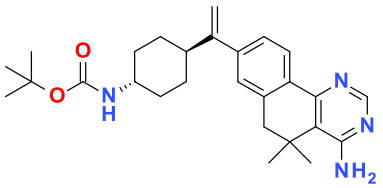
methylmagnesium bromide in 2-methyltetrahydrofuran (4.96 mL, 14.9 mmol) at 0 °C. The resulting mixture was stirred for 3.5 hours at 0 °C, and the reaction mixture was quenched by saturated aqueous ammonium chloride solution. The resulting solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (20% AcOEt/*n*-hexane) provided the title compound. (340 mg, 1.41 mmol, 28% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.45-4.36 (brs, 1H), 4.03 (s, 3H), 3.48-3.22 (m, 1H), 2.32-2.21 (m, 1H), 2.14 (s, 3H), 2.13-2.04 (m, 2H), 1.99-1.91 (m, 2H), 1.62-1.57 (m, 1H), 1.49-1.36 (m, 1H), 1.44 (s, 9H), 1.18-1.06 (m, 2H).

1-{(1*r*,4*r*)-4-[(*tert*-butoxycarbonyl)amino]cyclohexyl}ethenyl trifluoromethanesulfonate



To a solution of *tert*-butyl [(1*r*,4*r*)-4-acetylcyclohexyl]carbamate (340 mg, 1.41 mmol) in THF (15 mL) in a round-bottom flask was added 1 mol/L sodium bis(trimethylsilyl)amide tetrahydrofuran solution (1.55 mL, 1.55 mmol) at -78°C. The resulting mixture was stirred at -78 °C for 30 minutes, then added *N*-phenylbis(trifluoromethanesulfonimide) (554 mg, 1.55 mmol) at 0 °C. The resulting mixture was stirred at 0 °C for 3 hours, and the reaction mixture was quenched by saturated aqueous ammonium chloride solution. The resulting solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (20% AcOEt/*n*-hexane) provided the title compound. (330 mg, 0.884 mmol, 63% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.08 (d, J = 3.7 Hz,1H), 4.92 (d, J = 3.7 Hz,1H), 4.38 (brs, 1H), 3.47-3.37 (m, 1H), 2.22-2.14 (m, 1H), 2.14-2.07 (m, 2H), 2.07-1.99 (m, 2H), 1.44 (s, 9H), 1.39-1.24 (m, 2H), 1.22-1.09 (m, 2H).

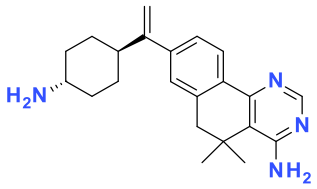
***tert*-butyl {(1*r*,4*r*)-4-[1-(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)ethenyl]cyclohexyl}carbamate**



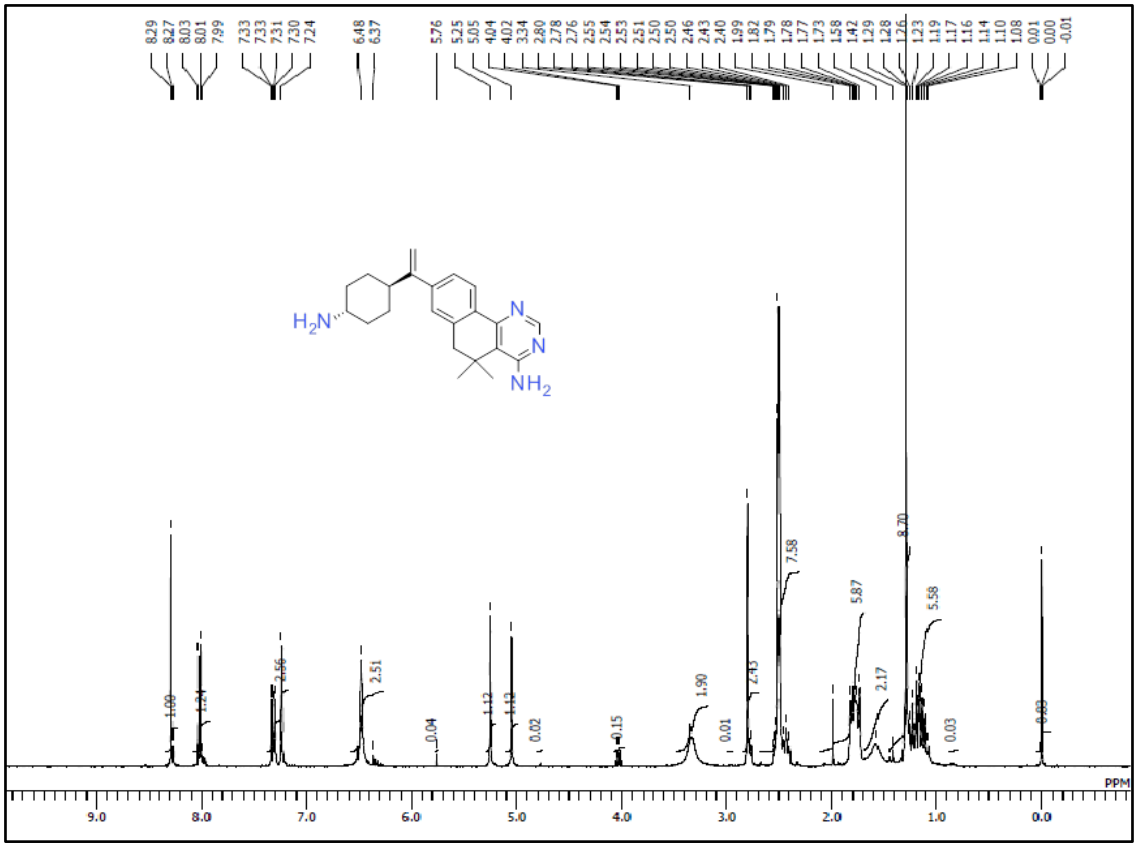
To a solution of 5,5-dimethyl-8-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydrobenzo[*h*]quinazolin-4-amine (176 mg, 0.500 mmol) in 1,4-dioxane (6 mL) in a round-bottom flask were added 1-{(1*r*,4*r*)-4-[(*tert*-butoxycarbonyl)amino]cyclohexyl}ethenyl

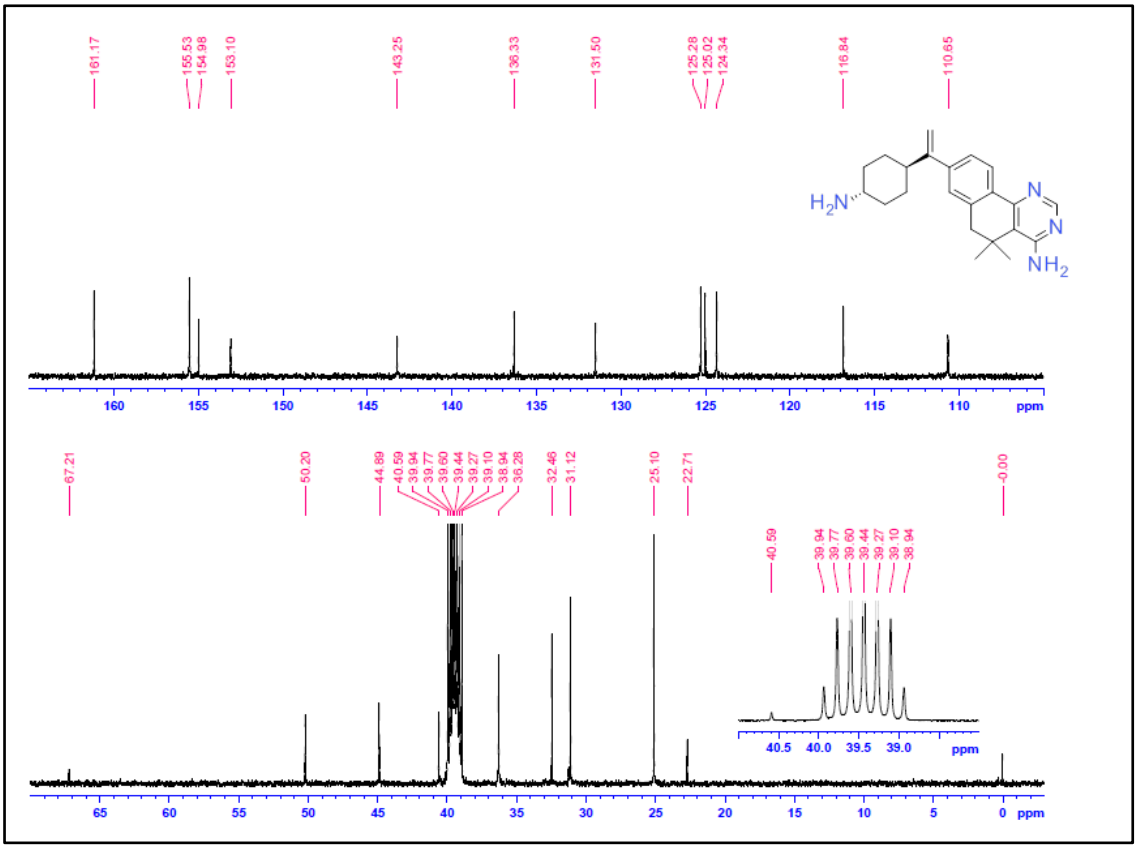
trifluoromethanesulfonate (373 mg, 1.00 mmol), chloro(2-dicyclohexylphosphino-2',4',6'-triisopropyl-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II) (39.3 mg, 0.0500 mmol), tripotassium phosphate hydrate (212 mg, 1.00 mmol) and water (2 mL). The resulting mixture was stirred for 2 hours at 100 °C and the reaction mixture was added to water. The resulting solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (10% AcOEt/*n*-hexane) provided the title compound. (155 mg, 0.346 mmol, 69% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 1H), 8.15 (d, *J* = 8.0 Hz, 1H), 7.30 (dd, *J* = 8.0, 1.8 Hz, 1H), 7.14 (dd, *J* = 1.8 Hz, 1H), 5.24 (s, 1H), 5.06 (s, 1H), 5.06 (brs, 2H), 4.45-4.35 (m, 1H), 3.50-3.37 (m, 1H), 2.86 (s, 2H), 2.86-2.79 (m, 1H), 2.47-2.37 (m, 1H), 2.12-2.02 (m, 1H), 1.95-1.86 (m, 2H), 1.45 (s, 9H), 1.39 (s, 6H), 1.39-1.29 (m, 2H), 1.27-1.14 (m, 2H); LCMS m/z 449 $[M + H]^+$.

8-{1-[(1*r*,4*r*)-4-aminocyclohexyl]ethenyl}-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (62, DS42450411)

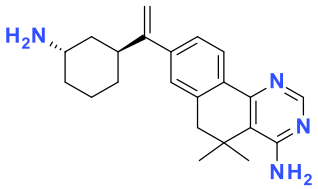


To a solution of *tert*-butyl {(1*r*,4*r*)-4-[1-(4-amino-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-8-yl)ethenyl]cyclohexyl}carbamate (482 mg, 1.07 mmol) in CH_2Cl_2 (10 mL) in a round-bottom flask was added trifluoroacetic acid (10 mL). The resulting mixture was stirred for 30 minutes at room temperature and the reaction mixture was concentrated. Purification by flash chromatography on NH-silica gel (15% MeOH/AcOEt) provided the title compound. (281 mg, 0.805 mmol, 75% yield): ^{1}H NMR (500 MHz, DMSO-*d*6) δ 8.29 (s, 1H), 8.02 (d, *J* = 8.2 Hz, 1H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.23 (s, 1H), 6.46 (brs,2H), 5.25 (s, 1H), 5.05 (s, 1H), 3.34 (brs, 2H), 2.80 (s, 2H), 2.60-2.50 (m, 1H), 2.48-2.40 (m, 1H), 1.85-1.71 (m, 4H), 1.30 (s, 6H), 1.29-1.10 (m, 4H); ^{13}C NMR (125 MHz, DMSO-*d*6) δ 161.17, 155.53, 154.98, 153.10, 143.25, 136.33, 131.50, 125.28, 125.02, 124.34, 116.84, 110.65, 67.21, 50.20, 44.89, 40.59, 36.28, 32.46, 31.12, 25.10, 22.71; LCMS m/z 349 $[M + H]^+$; HRMS (Positive ESI) m/z 349.2405 (349.2314 calcd for $C_{22}H_{28}N_4$ + H).



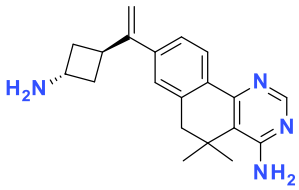


8-{1-[(1*S*,3*S*)-3-aminocyclohexyl]ethenyl}-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (61)



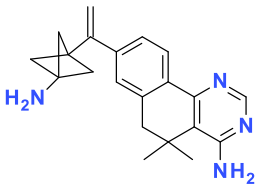
61 was prepared in a similar manner described for **59**. 43% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (s, 1H), 8.11 (d, *J* = 8.2 Hz, 1H), 7.30 (dd, *J* =8.2, 1.6 Hz, 1H), 7.12 (d, *J* = 1.6 Hz, 1H), 5.23 (s, 1H), 5.09 (brs, 2H), 5.04 (s, 1H), 3.30-3.23 (m, 1H), 2.98-2.88 (m, 1H), 2.82 (s, 2H), 1.81-1.73 (m, 1H), 1.72-1.60 (m, 2H), 1.58-1.40 (m, 2H), 1.35 (s, 6H), 1.30-1.22 (m, 2H); LCMS m/z 349 $[M + H]^+$.

8-{1-[(1*r*,3*r*)-3-aminocyclobutyl]ethenyl}-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (63)



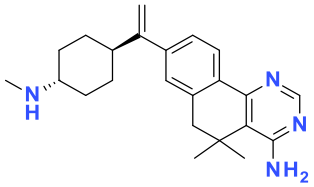
63 was prepared in a similar manner described for **59**. 78% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 8.11 (d, *J* = 8.2 Hz, 1H), 7.31 (dd, *J* =8.2, 2.0 Hz, 1H), 7.15 (d, *J* = 2.0 Hz, 1H), 5.47 (s, 1H), 5.15 (brs, 2H), 5.03 (s, 1H), 3.60-3.43 (m, 2H), 2.82 (s, 2H), 2.30-2.20 (m, 2H), 2.05-1.93 (m, 2H), 1.35 (s, 6H); LCMS m/z 321 $[M + H]^+$.

8-[1-(3-aminobicyclo[1.1.1]pentan-1-yl)ethenyl]-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (64)



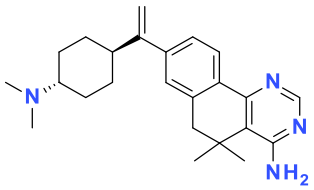
64 was prepared in a similar manner described for **59**. 71% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 1H), 8.17 (d, *J* = 8.2 Hz, 1H), 7.37 (dd, *J* =8.2, 1.5 Hz, 1H), 7.15 (d, *J* = 1.5 Hz, 1H), 5.30 (d, *J* = 2.2 Hz, 1H), 5.20 (brs, 2H), 5.16 (d, *J* = 2.0 Hz, 1H), 2.88 (s, 2H), 2.05 (s, 6H), 1.42 (s, 6H); LCMS m/z 333 $[M + H]^+$.

5,5-dimethyl-8-{1-[(1*r*,4*r*)-4-(methylamino)cyclohexyl]ethenyl}-5,6-dihydrobenzo[*h*]quinazolin-4-amine (65)



65 was prepared in a similar manner described for **59**. 92% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 8.14 (d, *J* = 8.2 Hz, 1H), 7.30 (dd, *J* =8.2, 1.6 Hz, 1H), 7.12 (d, *J* = 1.6 Hz, 1H), 5.22 (s, 1H), 5.04 (s, 1H), 5.02 (brs, 2H), 2.84 (s, 2H), 2.50-2.47 (m, 1H), 2.39-2.25 (m, 1H), 2.05-1.95 (m, 2H), 1.93-1.82 (m, 2H), 2.43 (s, 3H), 1.37 (s, 6H), 1.31-1.08 (m, 4H); LCMS m/z 363 $[M + H]^+$.

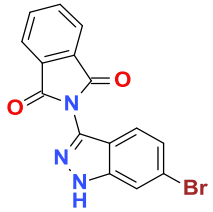
8-{1-[(1*r*,4*r*)-4-(dimethylamino)cyclohexyl]ethenyl}-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (66)



To a solution of 5,5-dimethyl-8-{1-[(1*r*,4*r*)-4-(methylamino)cyclohexyl]ethenyl}-5,6-dihydrobenzo[*h*]quinazolin-4-amine (**65**, 59.2 mg, 0.163 mmol) in CH_2Cl_2 (3 mL) in a round-bottom flask was added 37% formaldehyde solution (0.024 mL, 0.327 mmol) and sodium triacetoxyborohydride (51.9 mg, 0.245 mmol) at room temperature. The resulting mixture was stirred at room temperature for 3 hours. The reaction mixture was quenched by saturated aqueous sodium hydrogen carbonate solution and extracted with CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (20% AcOEt/*n*-hexane) provided the title compound. (22.0 mg, 0.0584 mmol, 36% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 8.12 (d, *J* = 8.2 Hz, 1H), 7.29 (dd, *J* =8.2, 1.6 Hz, 1H), 7.10 (d, *J* = 1.6 Hz, 1H), 5.20 (s, 1H), 5.02 (s, 1H), 5.00 (brs, 2H), 2.83 (s, 2H), 2.50-2.47 (m, 1H), 2.39-2.25 (m, 1H), 2.05-1.95 (m, 2H), 1.93-1.82 (m, 2H), 2.26 (s, 6H), 1.36 (s, 6H), 1.31-1.08 (m, 4H); LCMS m/z 377 $[M + H]^+$.

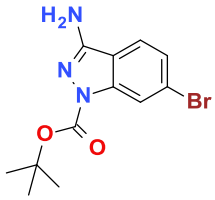
***N*-(6-phenyl-1*H*-indazol-3-yl)cyclopropanecarboxamide (74)**

2-(6-bromo-1*H*-indazol-3-yl)-1*H*-isoindole-1,3(2*H*)-dione (68)



To a solution of 6-bromo-1*H*-indazol-3-amine (5.01 g, 23.6 mmol) in 1,4-dioxane (150 mL) in a round-bottom flask was added 2-benzofuran-1,3-dione (4.20 g, 28.4 mmol) at room temperature. The resulting mixture was stirred at reflux for 4 hours. The reaction mixture was concentrated under reduced pressure. The resulting solid was collected. The solid was washed with diethylether and dried under reduced pressure at 60 °C. The title compound was obtained as a pale green solid. (8.08 g, 23.6 mmol, 99% yield): ^{1}H NMR (500 MHz, DMSO-*d*6) δ 13.56 (s, 1H), 8.03-8.00 (m, 2H), 7.95-7.92 (m, 2H), 7.83 (s, 1H), 7.71-7.66 (d, *J* = 7.8 Hz, 1H), 7.31-7.26 (d, *J* = 7.8 Hz, 1H); LCMS m/z 342 $[M + H]^+$.

***tert*-butyl 3-amino-6-bromo-1*H*-indazole-1-carboxylate (69)**

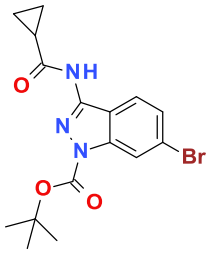


To a solution of 2-(6-bromo-1*H*-indazol-3-yl)-1*H*-isoindole-1,3(2*H*)-dione (**68**, 6.84 g, 20.0 mmol) in acetonitrile (200 mL) in a round-bottom flask were added di-*tert*-butyl dicarbonate (4.80 g, 22.0 mmol), triethylamine (4.05 g, 40.0 mmol) and 4-dimethylaminopyridine (244 mg, 2.00 mmol) at room temperature. The resulting mixture was stirred at room temperature for 1.5 hours. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 35% AcOEt/*n*-hexane linear gradient) provided *tert*-butyl 6-bromo-3-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-1*H*-indazole-1-carboxylate (6.30 g, 14.2 mmol, 71% yield).

To a solution of *tert*-butyl 6-bromo-3-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-1*H*-indazole-1-carboxylate (6.30 g, 14.2 mmol) in EtOH (150 mL) and CH_2Cl_2 (100 mL) in a round-bottom flask was added hydrazine monohydrate (3.45 mL, 71.2 mmol) at room temperature. The resulting mixture was stirred at room temperature for 15 hours. The insoluble materials were filtrated off and the filtrate was concentrated under reduced pressure. Purification by flash chromatography on silica gel (25% to 50% AcOEt/*n*-hexane linear gradient) provided the title compound. (4.06 g, 13.0 mmol, 91% yield): ^{1}H NMR (500 MHz,

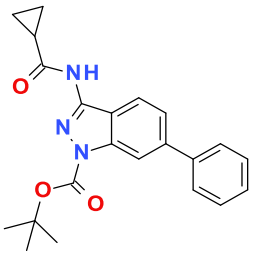
$CDCl_3$) δ 7.35 (d, *J* = 1.2 Hz, 1H), 7.23 (s, 1H), 7.22 (d, *J* = 1.2 Hz, 1H), 4.39 (brs, 2H), 1.66 (s, 9H).

***tert*-butyl 6-bromo-3-[(cyclopropanecarbonyl)amino]-1*H*-indazole-1-carboxylate (70)**



To a solution of *tert*-butyl 3-amino-6-bromo-1*H*-indazole-1-carboxylate (**69**, 800 mg, 2.56 mmol) and pyridine (0.32 mL, 3.84 mmol) in CH_2Cl_2 (30 mL) in a round-bottom flask was added cyclopropanecarbonyl chloride (0.322 mL, 3.08 mmol) at 0 ºC. The resulting mixture was stirred at 0 ºC for 30 minutes. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (50% to 100% AcOEt/*n*-hexane linear gradient) provided the title compound. (930 mg, 2.45 mmol, 95% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.05-8.96 (brs, 1H), 8.40-8.35 (m, 1H), 8.16-8.09 (m, 1H), 7.45-7.40 (m, 1H), 1.73 (s, 9H), 1.68-1.58 (m, 1H), 1.21-1.15(m, 2H), 1.00-0.95 (m, 2H).

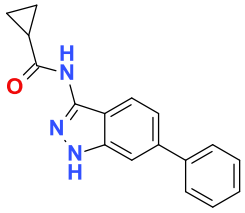
***tert*-butyl 3-[(cyclopropanecarbonyl)amino]-6-phenyl-1*H*-indazole-1-carboxylate (71)**



To a solution of *tert*-butyl 6-bromo-3-[(cyclopropanecarbonyl)amino]-1*H*-indazole-1-carboxylate (**70**, 100 mg, 0.263 mmol) and 4,4,5,5-tetramethyl-2-phenyl-1,3,2-dioxaborolane (59.1 mg, 0.289 mmol) in 1,2-dimethoxyethane (3 mL) in a round-bottom flask were added [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (10.7 mg, 0.0132 mmol), tripotassium phosphate hydrate (167 mg, 0.789 mmol) and water (0.75 mL). The resulting mixture was stirred at reflux for 1 hour. The reaction mixture was added to water and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 35% AcOEt/*n*-hexane linear gradient) provided the title compound. (77.0 mg, 0.204 mmol, 78% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 8.33-8.15 (m, 2H), 7.70-7.65(m, 2H), 7.54 (d, *J* = 8.6 Hz, 1H), 7.50-7.43 (m, 1H), 7.46 (d, *J* = 8.6 Hz, 1H), 7.41-7.37 (m, 1H), 1.57 (s, 9H), 1.56-1.51 (m, 1H), 1.19-1.15 (m, 2H), 0.98-

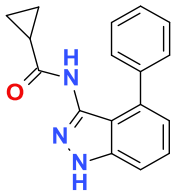
0.93 (m, 2H).

***N*-(6-phenyl-1*H*-indazol-3-yl)cyclopropanecarboxamide (74)**



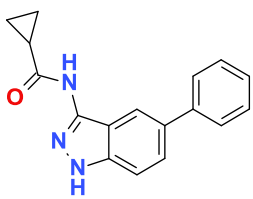
tert-Butyl 3-[(cyclopropanecarbonyl)amino]-6-phenyl-1*H*-indazole-1-carboxylate (**71**, 77.0 mg, 0.204 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (5.0 mL, 20.0 mmol) at room temperature. The resulting mixture was stirred at room temperature for 16 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (53.7 mg, 0.194 mmol, 95% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.70 (brs, 1H), 7.86 (d, *J* = 8.6 Hz, 1H), 7.75-7.68 (m, 2H), 7.60 (s, 1H), 7.51-7.45 (m, 2H), 7.41-7.15 (m, 1H), 7.33 (d, *J* = 8.6 Hz, 1H), 1.98-1.90 (m, 1H), 0.88-0.80 (m, 4H); LCMS m/z 278 $[M + H]^+$.

<u>*N*-(4-phenyl-1H-indazol-3-yl)cyclopropanecarboxamide (72)</u>



72 was prepared in a similar manner described for **74**. 86% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.99 (brs, 1H), 9.77 (s, 1H), 7.48 (d, *J* = 8.2 Hz, 1H), 7.43-7.35 (m, 6H), 6.99 (d, *J* = 2.6 Hz, 1H), 1.48-1.39 (m, 1H), 0.55-0.45 (m, 2H), 0.30-0.25 (m, 2H); LCMS m/z 278 $[M + H]^+$.

<u>*N*-(5-phenyl-1*H*-indazol-3-yl)cyclopropanecarboxamide (73)</u>

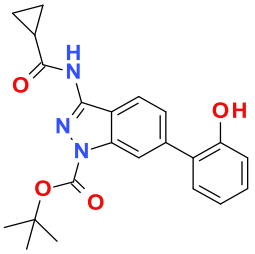


73 was prepared in a similar manner described for **74**. 89% yield: ^{1}H NMR (400 MHz,

DMSO-*d*6) δ 10.72 (brs, 1H), 8.03 (s, 1H), 7.65-7.59 (m, 3H), 7.58-7.48 (m, 1H), 7.48-7.42 (m, 2H), 7.36-7.30 (m, 2H), 1.99-1.90 (m, 1H), 0.88-0.80 (m, 4H); LCMS m/z 278 $[M + H]^+$.

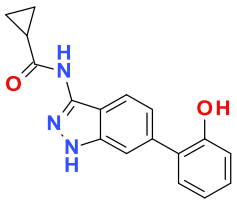
<u>*N*-[6-(2-hydroxyphenyl)-1*H*-indazol-3-yl]cyclopropanecarboxamide (76)</u>

***tert*-butyl 3-[(cyclopropanecarbonyl)amino]-6-(2-hydroxyphenyl)-1*H*-indazole-1-carboxylate (75)**



To a solution of *tert*-butyl 6-bromo-3-[(cyclopropanecarbonyl)amino]-1*H*-indazole-1-carboxylate (**70**, 122 mg, 0.301 mmol) and 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (83.4 mg, 0.379 mmol) in 1,2-dimethoxyethane (3 mL) in a round-bottom flask were added [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (12.9 mg, 0.0158 mmol), tripotassium phosphate hydrate (201 mg, 0.947 mmol) and water (0.75 mL). The resulting mixture was stirred at reflux for 20 minutes. The reaction mixture was added to water and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (10% to 80% AcOEt/*n*-hexane linear gradient) provided the title compound. (122 mg, 0.310 mmol, 98% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 8.33-8.15 (m, 1H), 7.70-7.65(m, 2H), 7.54 (d, *J* = 8.6 Hz, 1H), 7.50-7.43 (m, 1H), 7.46 (d, *J* = 8.6 Hz, 1H), 7.41-7.37 (m, 1H), 5.40 (brs, 1H), 1.65 (s, 9H), 1.56-1.51 (m, 1H), 1.19-1.15 (m, 2H), 0.98-0.93 (m, 2H).

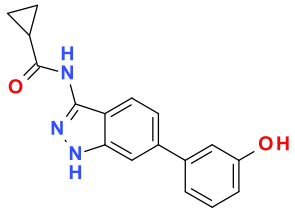
***N*-[6-(2-hydroxyphenyl)-1*H*-indazol-3-yl]cyclopropanecarboxamide (76)**



tert-Butyl 3-[(cyclopropanecarbonyl)amino]-6-(2-hydroxyphenyl)-1*H*-indazole-1-carboxylate (**75**, 122 mg, 0.310 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (5.0 mL, 20.0 mmol) at room temperature. The resulting mixture was stirred at room temperature for 16 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid

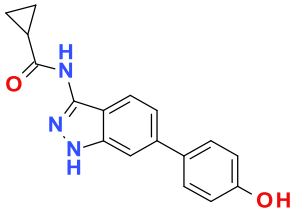
was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (67.7 mg, 0.230 mmol, 74% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.56 (brs, 1H), 10.60 (brs, 1H), 7.87 (d, *J* = 8.6 Hz, 1H), 7.52 (s, 1H), 7.27 (d, *J* = 7.4 Hz, 1H), 7.19-7.10 (m, 2H), 6.92 (d, *J* = 7.4 Hz, 1H), 6.84 (t, *J* = 7.4 Hz, 1H), 1.94-1.85 (m, 1H), 0.82-0.74 (m, 4H); LCMS m/z 294 $[M + H]^+$.

<u>*N*-[6-(3-hydroxyphenyl)-1*H*-indazol-3-yl]cyclopropanecarboxamide (77)</u>



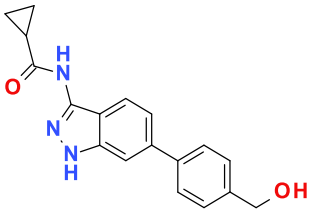
77 was prepared in a similar manner described for **76**. 66% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.67 (brs, 1H), 7.80 (d, *J* = 8.4 Hz, 1H), 7.50 (s, 1H), 7.27-7.20 (m, 2H), 7.07 (d, *J* = 7.4 Hz, 1H), 7.03 (s, 1H), 6.75 (d, *J* = 8.4 Hz, 1H), 6.84 (t, *J* = 7.4 Hz, 1H), 1.94-1.87 (m, 1H), 0.84-0.74 (m, 4H); LCMS m/z 294 $[M + H]^+$.

<u>*N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]cyclopropanecarboxamide (78)</u>



78 was prepared in a similar manner described for **76**. 85% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.64 (brs, 1H), 7.76 (d, *J* = 8.6 Hz, 1H), 7.50 (d, *J* = 8.5 Hz, 2H), 7.45 (s, 1H), 7.22 (d, *J* = 8.6 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 2H), 1.94-1.87 (m, 1H), 0.84-0.74 (m, 4H); LCMS m/z 294 $[M + H]^+$.

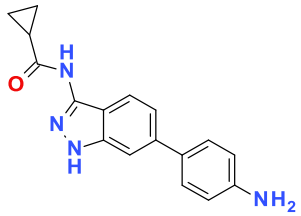
<u>*N*-{6-[4-(hydroxymethyl)phenyl]-1*H*-indazol-3-yl}cyclopropanecarboxamide (79)</u>



79 was prepared in a similar manner described for **76**. 98% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.67 (brs, 1H), 7.81 (d, *J* = 8.6 Hz, 1H), 7.64 (d, *J* = 8.3 Hz, 2H), 7.56 (s, 1H),

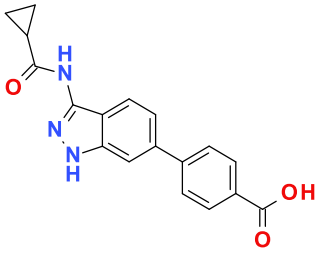
7.39 (d, *J* = 8.3 Hz, 2H), 7.30 (d, *J* = 8.6 Hz, 1H), 4.51 (s, 2H), 1.93-1.83 (m, 1H), 0.84-0.74 (m, 4H); LCMS m/z 308 $[M + H]^+$.

<u>*N*-[6-(4-aminophenyl)-1*H*-indazol-3-yl]cyclopropanecarboxamide (80)</u>



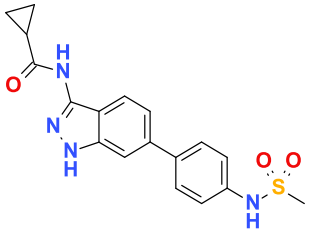
80 was prepared in a similar manner described for **76**. 73% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.48 (brs, 1H), 10.60 (brs, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.40 (s, 1H), 7.35 (d, *J* = 8.3 Hz, 2H), 7.21 (d, *J* = 8.2 Hz, 1H), 6.62 (d, *J* = 8.3 Hz, 2H), 5.23 (brs, 2H), 1.92-1.85 (m, 1H), 0.82-0.75 (m, 4H); LCMS m/z 293 $[M + H]^+$.

<u>4-{3-[(cyclopropanecarbonyl)amino]-1*H*-indazol-6-yl}benzoic acid (81)</u>



81 was prepared in a similar manner described for **76**. 96% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.98 (brs, 1H), 12.76 (brs, 1H), 10.71 (brs, 1H), 8.01 (d, *J* = 8.6 Hz, 2H), 7.86 (d, *J* = 8.6 Hz, 1H), 7.82 (d, *J* = 8.2 Hz, 2H), 7.66 (s, 1H), 7.36 (d, *J* = 8.6 Hz, 1H), 1.94-1.87 (m, 1H), 0.85-0.77 (m, 4H); LCMS m/z 322 $[M + H]^+$.

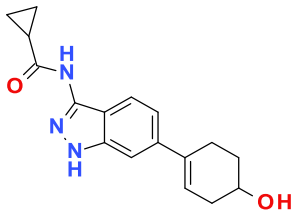
<u>*N*-(6-{4-[(methanesulfonyl)amino]phenyl}-1*H*-indazol-3-yl)cyclopropanecarboxamide (82)</u>



82 was prepared in a similar manner described for **76**. 86% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.62 (brs, 1H), 10.64 (brs, 1H), 9.85 (brs, 1H), 7.79 (d, *J* = 8.6 Hz, 1H), 7.64

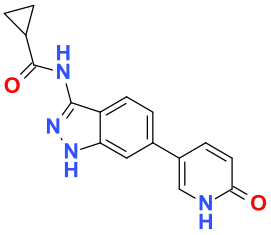
(d, *J* = 8.5 Hz, 2H), 7.53 (s, 1H), 7.27 (d, *J* = 8.6 Hz, 1H), 7.24 (d, *J* = 8.5 Hz, 2H), 2.94 (s, 3H), 1.94-1.87 (m, 1H), 0.83-0.75 (m, 4H); LCMS m/z 371 $[M + H]^+$.

<u>*N*-[6-(4-hydroxycyclohex-1-en-1-yl)-1*H*-indazol-3-yl]cyclopropanecarboxamide (83)</u>



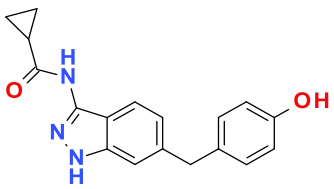
83 was prepared in a similar manner described for **76**. 55% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.62 (brs, 1H), 7.65 (d, *J* = 8.6 Hz, 1H), 7.26 (s, 1H), 7.12 (d, *J* = 8.6 Hz, 1H), 6.08 (s, 1H), 3.78 (brs, 1H), 2.45-2.36 (m, 1H), 2.08-1.99 (m, 2H), 1.93-1.84 (m, 4H), 1.65-1.52 (m, 1H), 0.81-0.74 (m, 4H); LCMS m/z 298 $[M + H]^+$.

<u>*N*-[6-(6-oxo-1,6-dihydropyridin-3-yl)-1*H*-indazol-3-yl]cyclopropanecarboxamide (84)</u>



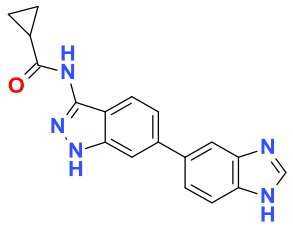
84 was prepared in a similar manner described for **76**. 88% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.61 (brs, 1H), 11.83 (brs, 1H), 10.65 (brs, 1H), 7.86 (d, *J* = 9.4 Hz, 1H), 7.77 (d, *J* = 8.6 Hz, 1H), 7.71 (s, 1H), 7.46 (s, 1H), 7.19 (d, *J* = 8.6 Hz, 1H), 6.42 (d, *J* = 9.4 Hz, 1H), 1.95-1.85 (m, 1H), 0.85-0.75 (m, 4H); LCMS m/z 295 $[M + H]^+$.

<u>*N*-{6-[(4-hydroxyphenyl)methyl]-1*H*-indazol-3-yl}cyclopropanecarboxamide (85)</u>



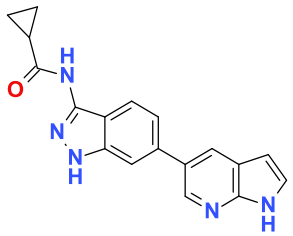
85 was prepared in a similar manner described for **76**. 49% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.40 (brs, 1H), 10.55 (brs, 1H), 9.17 (s, 1H), 7.60 (d, *J* = 8.2 Hz, 1H), 7.10 (s, 1H), 6.99 (d, *J* = 8.6 Hz, 2H), 6.83 (d, *J* = 8.2 Hz, 1H), 6.63 (d, *J* = 8.6 Hz, 1H), 3.87 (s, 2H), 1.89-1.82 (m, 1H), 0.79-0.73 (m, 4H); LCMS m/z 308 $[M + H]^+$.

***N*-[6-(1*H*-benzimidazol-5-yl)-1*H*-indazol-3-yl]cyclopropanecarboxamide (86)**



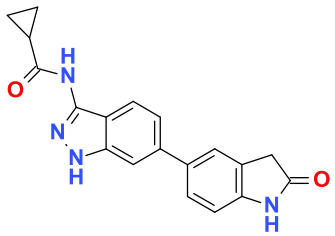
86 was prepared in a similar manner described for **76**. 78% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.67 (brs, 1H), 8.23 (s, 1H), 7.84 (s, 1H), 7.80 (d, *J* = 8.6 Hz, 1H), 7.65 (d, *J* = 8.2 Hz, 1H), 7.59 (s, 1H), 7.52 (d, *J* = 8.2 Hz, 1H), 7.35 (d, *J* = 8.6 Hz, 1H), 1.95-1.88 (m, 1H), 0.85-0.76 (m, 4H); LCMS m/z 318 $[M + H]^+$.

***N*-[6-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)-1*H*-indazol-3-yl]cyclopropanecarboxamide (87)**



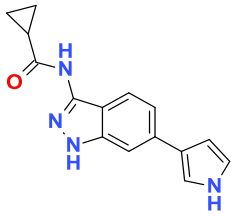
87 was prepared in a similar manner described for **76**. 85% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.68 (brs, 1H), 8.52 (d, *J* = 2.0 Hz, 1H), 8.22 (d, *J* = 2.0 Hz, 1H), 7.84 (d, *J* = 8.2 Hz, 1H), 7.61 (s, 1H), 7.49 (d, *J* = 3.5 Hz, 1H), 7.35 (d, *J* = 8.2 Hz, 1H), 6.49 (d, *J* = 3.5 Hz, 1H), 1.95-1.88 (m, 1H), 0.85-0.76 (m, 4H); LCMS m/z 318 $[M + H]^+$.

***N*-[6-(2-oxo-2,3-dihydro-1*H*-indol-5-yl)-1*H*-indazol-3-yl]cyclopropanecarboxamide (88)**



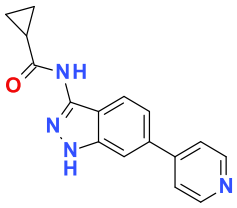
88 was prepared in a similar manner described for **76**. 45% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.60 (brs, 1H), 10.64 (brs, 1H), 10.44 (brs, 1H), 7.79 (d, *J* = 8.6 Hz, 1H), 7.54 (s, 1H), 7.50 (d, *J* = 8.2 Hz, 1H), 7.49 (s, 1H), 7.24 (d, *J* = 8.6 Hz, 1H), 6.88 (d, *J* = 8.2 Hz, 1H), 3.52 (s, 2H), 1.93-1.88 (m, 1H), 0.84-0.75 (m, 4H); LCMS m/z 333 $[M + H]^+$.

***N*-[6-(1*H*-pyrrol-3-yl)-1*H*-indazol-3-yl]cyclopropanecarboxamide (89)**



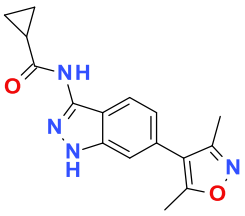
89 was prepared in a similar manner described for **76**. 58% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.36 (brs, 1H), 10.94 (brs, 1H), 10.55 (brs, 1H), 7.65 (d, *J* = 8.6 Hz, 1H), 7.40 (s, 1H), 7.26 (s, 1H), 7.12 (d, *J* = 8.6 Hz, 1H), 6.81-6.75 (m, 1H), 6.48-6.42 (m, 1H), 1.93-1.88 (m, 1H), 0.84-0.75 (m, 4H); LCMS m/z 267 $[M + H]^+$.

***N*-[6-(pyridin-4-yl)-1*H*-indazol-3-yl]cyclopropanecarboxamide (90)**



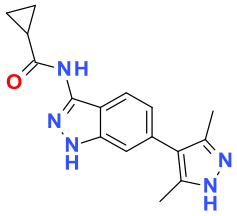
90 was prepared in a similar manner described for **76**. 98% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.97 (brs, 1H), 10.78 (brs, 1H), 8.79 (d, *J* = 7.2 Hz, 2H), 8.09 (d, *J* = 7.2 Hz, 2H), 7.94 (d, *J* = 8.2 Hz, 1H), 7.89 (s, 1H), 7.50 (d, *J* = 8.2 Hz, 1H), 1.95-1.87 (m, 1H), 0.85-0.76 (m, 4H); LCMS m/z 279 $[M + H]^+$.

***N*-[6-(3,5-dimethyl-1,2-oxazol-4-yl)-1*H*-indazol-3-yl]cyclopropanecarboxamide (91)**



91 was prepared in a similar manner described for **76**. 74% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.69 (brs, 1H), 10.69 (brs, 1H), 7.80 (d, *J* = 8.2 Hz, 1H), 7.34 (s, 1H), 6.99 (d, *J* = 8.2 Hz, 1H), 2.29 (s, 3H), 2.21 (s, 3H), 1.94-1.86 (m, 1H), 0.82-0.76 (m, 4H); LCMS m/z 297 $[M + H]^+$.

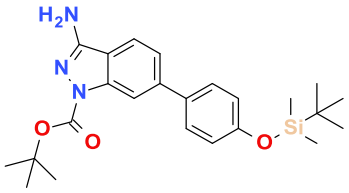
***N*-[6-(3,5-dimethyl-1*H*-pyrazol-4-yl)-1*H*-indazol-3-yl]cyclopropanecarboxamide (92)**



92 was prepared in a similar manner described for **76**. 70% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.50 (brs, 1H), 10.61 (brs, 1H), 7.72 (d, *J* = 8.6 Hz, 1H), 7.19 (s, 1H), 6.93 (d, *J* = 8.6 Hz, 1H), 2.18 (s, 6H), 1.92-1.86 (m, 1H), 0.82-0.75 (m, 4H); LCMS m/z 296 $[M + H]^+$.

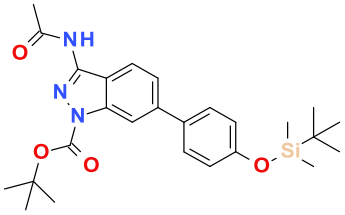
***N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]acetamide (95)**

***tert*-butyl 3-amino-6-(4-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-1*H*-indazole-1-carboxylate (93)**



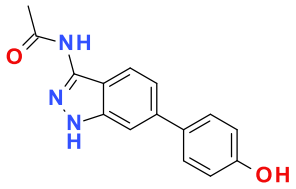
To a solution of *tert*-butyl 3-amino-6-bromo-1*H*-indazole-1-carboxylate (**69**, 624 mg, 2.00 mmol) and (4-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)boronic acid (756 mg, 3.00 mmol) in 1,2-dimethoxyethane (8 mL) in a round-bottom flask were added [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (81.7 mg, 0.100 mmol), tripotassium phosphate hydrate (1.27 g, 6.00 mmol) and water (2 mL). The resulting mixture was stirred at reflux for 50 minutes. The reaction mixture was added to water and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 100% AcOEt/*n*-hexane linear gradient) provided the title compound. (490 mg, 1.12 mmol, 56% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, *J* = 8.6 Hz, 2H), 7.35 (d, *J* = 1.2 Hz, 1H), 7.23 (s, 1H), 7.22 (d, *J* = 1.2 Hz, 1H), 6.89 (d, *J* =8.6 Hz, 2H), 4.38 (brs, 2H), 1.68 (s, 9H), 0.97 (s, 9H), 0.21 (s, 6H).

***tert*-butyl 3-acetamido-6-(4-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-1*H*-indazole-1-carboxylate (94)**



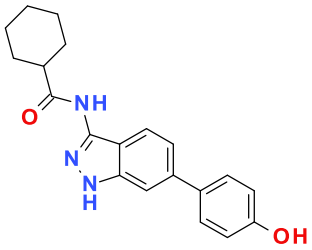
To a solution of *tert*-butyl 3-amino-6-(4-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-1*H*-indazole-1-carboxylate (**93**, 245 mg, 0.557 mmol) and pyridine (0.0673 mL, 0.836 mmol) in CH_2Cl_2 (10 mL) in a round-bottom flask wa added acetyl chloride (0.0436 mL, 0.613 mmol) at 0 ºC. The resulting mixture was stirred at 0 ºC for 1.25 hours. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (30% to 100% AcOEt/*n*-hexane linear gradient) provided the title compound. (261 mg, 0.542 mmol, 97% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (brs, 1H), 7.53 (d, *J* = 8.6 Hz, 2H), 7.50 (d, *J* = 1.2 Hz, 1H), 7.22 (s, 1H), 7.20 (d, *J* = 1.2 Hz, 1H), 6.89 (d, *J* =8.6 Hz, 2H), 2.25 (s, 3H), 1.68 (s, 9H), 0.97 (s, 9H), 0.21 (s, 6H).

***N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]acetamide (95)**



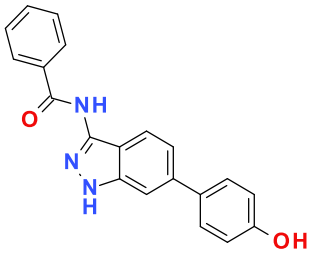
tert-Butyl 3-acetamido-6-(4-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-1*H*-indazole-1-carboxylate (**94**, 261 mg, 0.542 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (10 mL, 40.0 mmol) at room temperature. The resulting mixture was stirred at room temperature for 21 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (130 mg, 0.486 mmol, 90% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.56 (brs, 1H), 10.34 (brs, 1H), 9.60 (brs, 1H), 7.77 (d, *J* = 8.6 Hz, 1H), 7.50 (d, *J* = 8.5 Hz, 2H), 7.45 (s, 1H), 7.22 (d, *J* = 8.6 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 2H), 3.30 (s, 3H); LCMS m/z 268 $[M + H]^+$.

***N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]cyclohexanecarboxamide (96)**



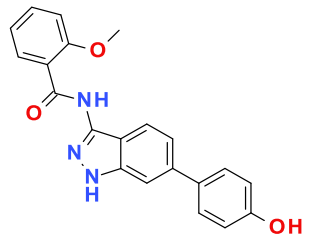
96 was prepared in a similar manner described for **95**. 94% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.18 (brs, 1H), 7.71 (d, *J* = 8.6 Hz, 1H), 7.50 (d, *J* = 8.5 Hz, 2H), 7.45 (s, 1H), 7.23 (d, *J* = 8.6 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 2H), 2.46-2.38 (m, 1H), 1.87-1.77 (m, 2H), 1.76-1.69 (m, 2H), 1.66-1.57 (m, 1H), 1.50-1.38 (m, 2H), 1.37-1.10 (m, 3H); LCMS m/z 336 $[M + H]^+$.

***N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]benzamide (97)**



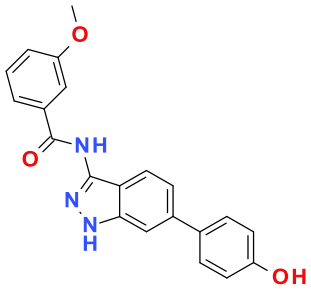
97 was prepared in a similar manner described for **95**. 94% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.05 (d, *J* = 8.2 Hz, 2H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.65-7.48 (m, 6H), 7.27 (d, *J* = 8.2 Hz, 1H), 6.83 (d, *J* = 8.2 Hz, 2H); LCMS m/z 330 $[M + H]^+$.

***N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]-2-methoxybenzamide (98)**



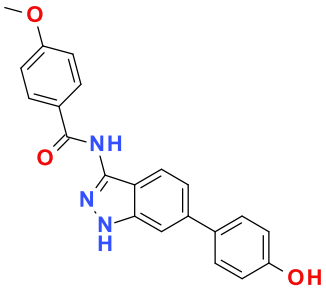
98 was prepared in a similar manner described for **95**. 95% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.35 (brs, 1H), 7.87 (d, *J* = 8.6 Hz, 1H), 7.80 (d, *J* = 7.5 Hz, 1H), 7.53 (d, *J* = 8.6 Hz, 1H), 7.52 (d, *J* = 6.7 Hz, 2H), 7.52 (s, 1H), 7.30-7.05 (m, 3H), 6.84 (d, *J* = 6.7 Hz, 2H), 3.94 (s, 3H); LCMS m/z 360 $[M + H]^+$.

***N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]-3-methoxybenzamide (99)**



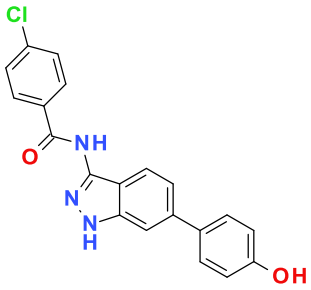
99 was prepared in a similar manner described for **95**. 99% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.71 (d, *J* = 8.6 Hz, 1H), 7.64 (d, *J* = 7.9 Hz, 1H), 7.60 (s, 1H), 7.53 (s, 1H), 7.52 (d, *J* = 7.8 Hz, 2H), 7.41 (t, *J* = 8.2 Hz, 1H), 7.28 (d, *J* = 9.0 Hz, 1H), 7.14 (d, *J* = 8.2 Hz, 1H), 6.84 (d, *J* = 7.8 Hz, 2H), 3.82 (s, 3H); LCMS m/z 360 $[M + H]^{+}$.

***N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]-4-methoxybenzamide (100)**



100 was prepared in a similar manner described for **95**. 89% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.05 (d, *J* = 9.0 Hz, 2H), 7.70 (d, *J* = 8.6 Hz, 1H), 7.52 (s, 1H), 7.51 (d, *J* = 8.6 Hz, 2H), 7.27 (d, *J* = 8.6 Hz, 1H), 7.03 (d, *J* = 9.0 Hz, 2H), 6.83 (d, *J* = 8.6 Hz, 2H), 3.82 (s, 3H); LCMS m/z 360 $[M + H]^{+}$.

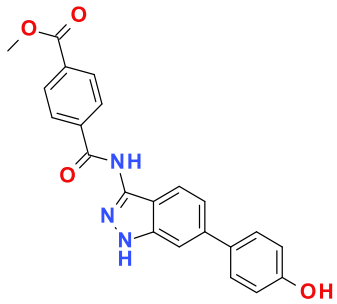
4-chloro-*N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]benzamide (101)



101 was prepared in a similar manner described for **95**. 88% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.06 (d, *J* = 8.6 Hz, 2H), 7.72 (d, *J* = 8.6 Hz, 1H), 7.59 (d, *J* = 8.6 Hz, 2H),

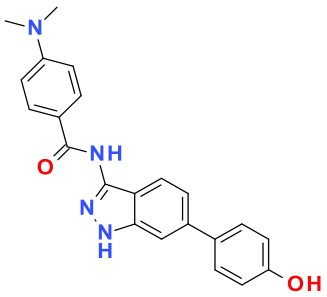
7.53 (s, 1H), 7.51 (d, *J* = 8.6 Hz, 1H), 7.27 (d, *J* = 9.0 Hz, 1H), 6.83 (d, *J* = 8.6 Hz, 2H); LCMS m/z 364 $[M + H]^+$.

methyl 4-{[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]carbamoyl}benzoate (102)



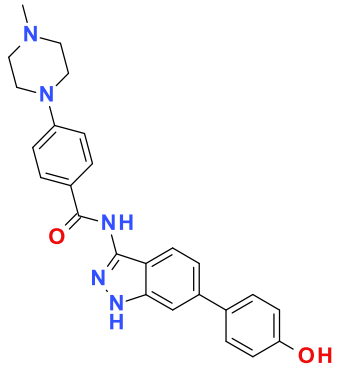
102 was prepared in a similar manner described for **95**. 90% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.16 (d, *J* = 7.8 Hz, 2H), 8.05 (d, *J* = 7.8 Hz, 2H), 7.74 (d, *J* = 8.6 Hz, 2H), 7.54 (s, 1H), 7.50 (d, *J* = 7.0 Hz, 1H), 7.28 (d, *J* = 7.0 Hz, 1H), 6.84 (d, *J* = 8.6 Hz, 2H), 3.87 (s, 3H); LCMS m/z 388 $[M + H]^+$.

4-(dimethylamino)-*N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]benzamide (103)



103 was prepared in a similar manner described for **95**. 99% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.64 (s, 1H), 10.38 (s, 1H), 9.55 (s, 1H), 7.94 (d, *J* = 9.0 Hz, 2H), 7.69 (d, *J* = 8.2 Hz, 1H), 7.51 (d, *J* = 9.0 Hz, 2H), 7.49 (s, 1H), 7.26 (d, *J* = 8.2 Hz, 1H), 6.83 (d, *J* = 8.6 Hz, 1H), 6.72 (d, *J* = 8.6 Hz, 2H), 3.30 (s, 6H); LCMS m/z 373 $[M + H]^+$.

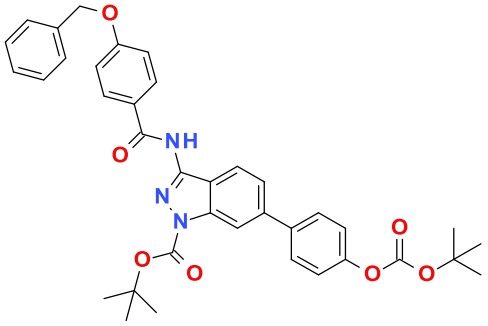
***N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]-4-(4-methylpiperazin-1-yl)benzamide (113)**



113 was prepared in a similar manner described for **95**. 93% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.51 (s, 1H), 8.00 (d, *J* = 9.0 Hz, 2H), 7.75 (d, *J* = 8.2 Hz, 1H), 7.57 (d, *J* = 9.0 Hz, 2H), 7.56 (s, 1H), 7.32 (d, *J* = 8.2 Hz, 1H), 7.05 (d, *J* = 9.0 Hz, 2H), 6.89 (d, *J* = 9.0 Hz, 2H), 3.35-3.29 (m, 4H), 2.50-2.42 (m, 4H), 2.26 (s, 6H); LCMS m/z 428 $[M + H]^+$.

***N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]-4-[2-(morpholin-4-yl)ethoxy]benzamide (109)**

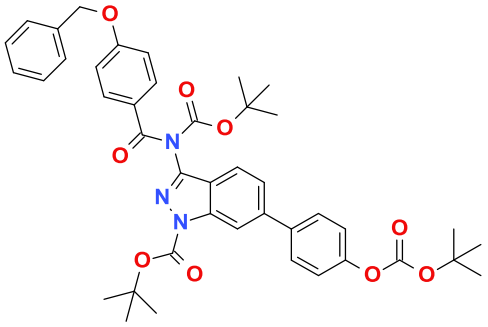
***tert*-butyl 3-[4-(benzyloxy)benzamido]-6-{4-[(*tert*-butoxycarbonyl)oxy]phenyl}-1*H*-indazole-1-carboxylate (104)**



To a solution of *tert*-butyl 3-amino-6-{4-[(*tert*-butoxycarbonyl)oxy]phenyl}-1*H*-indazole-1-carboxylate (**93**, 440 mg, 1.03 mmol) and pyridine (0.167 mL, 2.07 mmol) in CH_2Cl_2 (10 mL) in a round-bottom flask was added 4-(benzyloxy)benzoyl chloride (383 mg, 1.55 mmol) at 0 ºC. The resulting mixture was stirred at 0 ºC for 1 hour. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 50% AcOEt/*n*-hexane linear gradient) provided the title compound. (655 mg, 1.03 mmol, 99% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (brs, 1H), 8.32 (brs, 1H), 8.30-8.25 (m, 2H), 7.91 (d, *J* = 9.0 Hz, 2H), 7.56 (d, *J* = 9.0 Hz, 2H), 7.51 (d, *J* = 8.6 Hz, 2H), 7.35-7.29 (m, 3H), 7.25 (d, *J* = 8.6 Hz, 2H), 7.04 (d, *J* = 8.6 Hz, 2H), 5.13 (s, 2H), 1.69 (s, 9H), 1.55 (s,

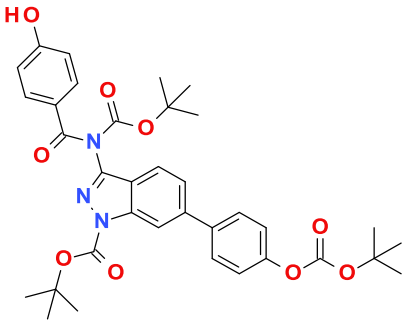
9H); LCMS m/z 636 $[M + H]^+$.

***tert*-butyl 3-{[4-(benzyloxy)benzoyl](*tert*-butoxycarbonyl)amino}-6-{4-[(*tert*-butoxycarbonyl)oxy]phenyl}-1*H*-indazole-1-carboxylate (105)**



To a solution of *tert*-butyl 3-[4-(benzyloxy)benzamido]-6-{4-[(*tert*-butoxycarbonyl)oxy]phenyl}-1*H*-indazole-1-carboxylate (**104**, 620 mg, 0.978 mmol) in acetonitrile (50 mL) in a round-bottom flask were added di-*tert*-butyl dicarbonate (319 mg, 1.46 mmol), triethylamine (0.270 mL, 1.95 mmol) and 4-dimethylaminopyridine (11.9 mg, 0.0975 mmol) at room temperature. The resulting mixture was stirred at room temperature for 14 hours. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 50% AcOEt/*n*-hexane linear gradient) provided the title compound. (620 mg, 0.843 mmol, 86% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (brs, 1H), 7.82 (d, *J* = 8.6 Hz, 2H), 7.62 (d, *J* = 8.6 Hz, 2H), 7.56 (d, *J* = 8.6 Hz, 1H), 7.49 (d, *J* = 8.6 Hz, 1H), 7.45-7.29 (m, 5H), 7.25 (d, *J* = 8.6 Hz, 2H), 6.97 (d, *J* = 8.6 Hz, 2H), 5.11 (s, 2H), 1.67 (s, 9H), 1.55 (s, 9H), 1.29 (s, 9H).

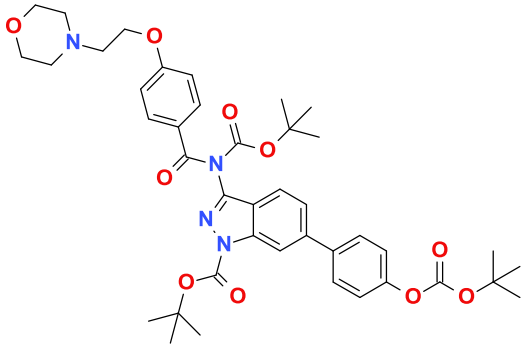
***tert*-butyl 3-[(*tert*-butoxycarbonyl)(4-hydroxybenzoyl)amino]-6-{4-[(*tert*-butoxycarbonyl)oxy]phenyl}-1*H*-indazole-1-carboxylate (106)**



To a solution of *tert*-butyl 3-{[4-(benzyloxy)benzoyl](*tert*-butoxycarbonyl)amino}-6-{4-[(*tert*-butoxycarbonyl)oxy]phenyl}-1*H*-indazole-1-carboxylate (**105**, 620 mg, 0.843 mmol) in EtOH (20 mL) in a round-bottom flask was added 10% palladium on carbon (120 mg, 0.094 mmol) at room temperature. The resulting mixture was stirred at room temperature in hydrogen atmosphere for 1.5 hours. The palladium on carbon was filtrated off with Celite pad

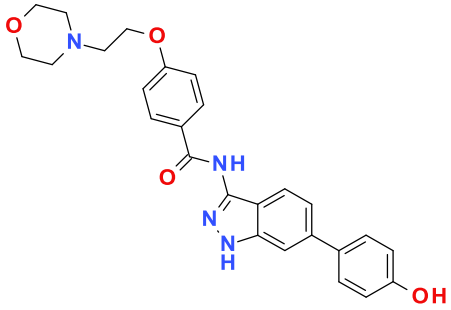
and washed with EtOH. The filtrate was concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 50% AcOEt/*n*-hexane linear gradient) provided the title compound. (470 mg, 0.728 mmol, 86% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (brs, 1H), 7.69 (d, *J* = 8.6 Hz, 2H), 7.63 (d, *J* = 8.6 Hz, 2H), 7.57 (d, *J* = 8.2 Hz, 1H), 7.51 (d, *J* = 8.2 Hz, 1H), 7.25 (d, *J* = 8.6 Hz, 2H), 6.78 (d, *J* = 8.6 Hz, 2H), 6.26 (brs, 2H), 1.67 (s, 9H), 1.55 (s, 9H), 1.30 (s, 9H); LCMS m/z 646 $[M + H]^+$.

***tert*-butyl 3-[(*tert*-butoxycarbonyl){4-[2-(morpholin-4-yl)ethoxy]benzoyl}amino]-6-{4-[(*tert*-butoxycarbonyl)oxy]phenyl}-1*H*-indazole-1-carboxylate (107)**



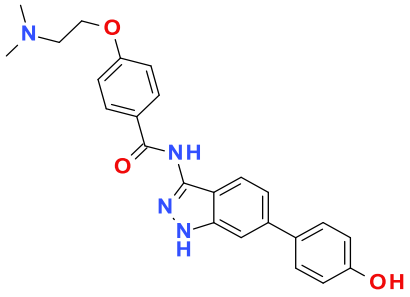
To a solution of *tert*-butyl 3-[(*tert*-butoxycarbonyl)(4-hydroxybenzoyl)amino]-6-{4-[(*tert*-butoxycarbonyl)oxy]phenyl}-1*H*-indazole-1-carboxylate (**106**, 100 mg, 0.155 mmol) and 2-(morpholin-4-yl)ethan-1-ol (40.6 mg, 0.310 mmol) in THF (10 mL) in a round-bottom flask were added tributylphosphine (62.7 mg, 0.310 mmol) and 1,1'-(azodicarbonyl)dipiperidine (78.2 mg, 0.310 mmol) at room temperature. The resulting mixture was stirred at room temperature for 13 hours. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (50% to 100% AcOEt/*n*-hexane linear gradient) provided the title compound. (108 mg, 0.142 mmol, 92% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (brs, 1H), 7.82 (d, *J* = 8.9 Hz, 2H), 7.62 (d, *J* = 8.9 Hz, 2H), 7.56 (d, *J* = 8.2 Hz, 1H), 7.50 (d, *J* = 8.2 Hz, 1H), 7.25 (d, *J* = 8.6 Hz, 2H), 6.90 (d, *J* = 8.6 Hz, 2H), 4.13 (t, *J* = 5.6 Hz, 2H), 3.75-3.68 (m, 4H), 2.79 (t, *J* = 5.6 Hz, 2H), 2.59-2.51 (m, 4H), 1.67 (s, 9H), 1.55 (s, 9H), 1.31 (s, 9H); LCMS m/z 759 $[M + H]^+$.

***N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]-4-[2-(morpholin-4-yl)ethoxy]benzamide (109)**



tert-Butyl 3-[(*tert*-butoxycarbonyl){4-[2-(morpholin-4-yl)ethoxy]benzoyl}amino]-6-{4-[(*tert*-butoxycarbonyl)oxy]phenyl}-1*H*-indazole-1-carboxylate (**107**, 110 mg, 0.145 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (3 mL, 12.0 mmol) at room temperature. The resulting mixture was stirred at 50 ºC for 45 minutes, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (65.5 mg, 0.143 mmol, 99% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.63 (brs, 1H), 8.03 (d, *J* = 8.6 Hz, 2H), 7.70 (d, *J* = 8.2 Hz, 1H), 7.53 (s, 1H), 7.52 (d, *J* = 8.6 Hz, 2H), 7.27 (d, *J* = 8.2 Hz, 1H), 7.04 (d, *J* = 8.6 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 4.16 (t, *J* = 5.6 Hz, 2H), 3.59-3.52 (m, 4H), 2.69 (t, *J* = 5.6 Hz, 2H), 2.47-2.40 (m, 4H); LCMS m/z 459 $[M + H]^+$.

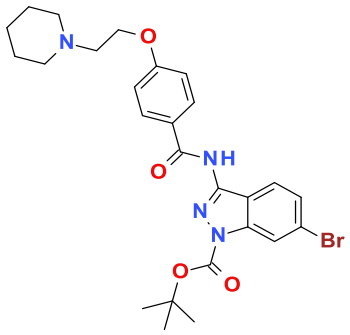
4-[2-(dimethylamino)ethoxy]-*N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]benzamide (108)



108 was prepared in a similar manner described for **109**. 75% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.70 (s, 1H), 10.62 (s, 1H), 9.55 (s, 1H), 8.03 (d, *J* = 8.6 Hz, 2H), 7.70 (d, *J* = 8.2 Hz, 1H), 7.52 (d, *J* = 8.6 Hz, 2H), 7.51 (s, 1H), 7.27 (d, *J* = 8.2 Hz, 1H), 7.04 (d, *J* = 8.6 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 4.16 (t, *J* = 5.9 Hz, 2H), 3.30 (s, 6H), 2.69 (t, *J* = 5.9 Hz, 2H); LCMS m/z 417 $[M + H]^+$.

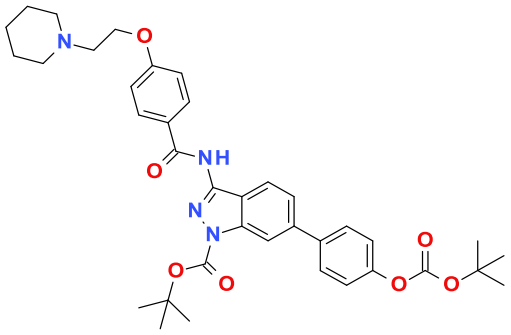
<u>*N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]-4-[2-(piperidin-1-yl)ethoxy]benzamide (111)

***tert*-butyl 6-bromo-3-{4-[2-(piperidin-1-yl)ethoxy]benzamido}-1*H*-indazole-1-carboxylate (110)**



To a solution of *tert*-butyl 3-amino-6-bromo-1*H*-indazole-1-carboxylate (**69**, 312 mg, 1.00 mmol) and pyridine (0.322 mL, 4.00 mmol) in CH_2Cl_2 (30 mL) in a round-bottom flask was added 4-[2-(piperidin-1-yl)ethoxy]benzoyl chloride hydrogen chloride (912 mg, 3.00 mmol) at room temperature. The resulting mixture was stirred at room temperature for 2 hours. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (0% to 50% AcOEt/*n*-hexane linear gradient) provided the title compound. (173 mg, 0.318 mmol, 32% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (brs, 1H), 8.34 (brs, 1H), 8.12 (d, *J* = 8.6 Hz, 1H), 7.86 (d, *J* = 8.6 Hz, 2H), 7.40 (d, *J* = 8.6 Hz, 1H), 6.97 (d, *J* = 8.6 Hz, 2H), 4.15 (t, *J* = 5.8 Hz, 2H), 2.77 (t, *J* = 5.8 Hz, 2H), 2.52-2.45 (m, 4H), 1.68 (s, 9H), 1.61-1.53 (m, 4H), 1.48-1.38 (m, 2H); LCMS m/z 543 $[M + H]^+$.

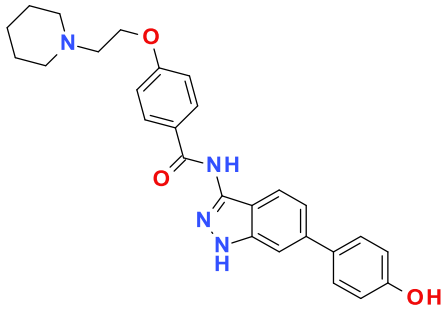
***tert*-butyl 6-{4-[(*tert*-butoxycarbonyl)oxy]phenyl}-3-{4-[2-(piperidin-1-yl)ethoxy]benzamido}-1*H*-indazole-1-carboxylate**



To a solution of *tert*-butyl 6-bromo-3-{4-[2-(piperidin-1-yl)ethoxy]benzamido}-1*H*-indazole-1-carboxylate (**110**, 85.0 mg, 0.156 mmol) and *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl carbonate (60.1 mg, 0.188 mmol) in 1,2-dimethoxyethane (3 mL) in a round-bottom flask were added [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (6.39 mg,

0.00782 mmol), tripotassium phosphate hydrate (99.6 mg, 0.469 mmol) and water (1 mL). The resulting mixture was stirred at reflux for 40 minutes. The reaction mixture was added to water and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 100% AcOEt/*n*-hexane linear gradient) provided the title compound. (101 mg, 0.154 mmol, 98% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (brs, 1H), 8.32 (brs, 1H), 8.26 (d, *J* = 9.0 Hz, 1H), 7.89 (d, *J* = 8.6 Hz, 2H), 7.65 (d, *J* = 8.6 Hz, 2H), 7.50 (d, *J* = 9.0 Hz, 1H), 7.26 (d, *J* = 8.6 Hz, 2H), 6.97 (d, *J* = 8.6 Hz, 2H), 4.16 (t, *J* = 5.8 Hz, 2H), 2.78 (t, *J* = 5.8 Hz, 2H), 2.52-2.45 (m, 4H), 1.68 (s, 9H), 1.61-1.53 (m, 4H), 1.55 (s, 9H), 1.48-1.38 (m, 2H).

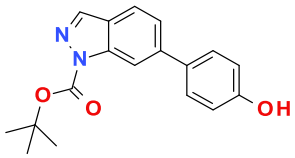
***N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]-4-[2-(piperidin-1-yl)ethoxy]benzamide (111)**



tert-Butyl 6-{4-[(*tert*-butoxycarbonyl)oxy]phenyl}-3-{4-[2-(piperidin-1-yl)ethoxy]benzamido}-1*H*-indazole-1-carboxylate (101 mg, 0.154 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (5 mL, 20.0 mmol) at room temperature. The resulting mixture was stirred at room temperature for 15 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (66.0 mg, 0.145 mmol, 94% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.62 (brs, 1H), 8.03 (d, *J* = 9.0 Hz, 2H), 7.69 (d, *J* = 8.6 Hz, 1H), 7.51 (d, *J* = 8.6 Hz, 2H), 7.49 (s, 1H), 7.27 (d, *J* = 8.6 Hz, 1H), 7.02 (d, *J* = 9.0 Hz, 2H), 6.83 (d, *J* = 8.6 Hz, 2H), 4.13 (t, *J* = 6.3 Hz, 2H), 2.65 (t, *J* = 6.3 Hz, 2H), 2.45-2.35 (m, 4H), 1.50-1.42 (m, 4H), 1.40-1.30 (m, 2H); LCMS m/z 457 $[M + H]^+$.

4-(1*H*-indazol-6-yl)phenol (115)

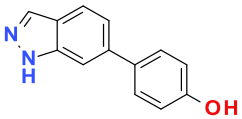
***tert*-butyl 6-(4-hydroxyphenyl)-1*H*-indazole-1-carboxylate (114)**



To a solution of 6-bromo-1*H*-indazole (1.01 g, 5.13 mmol) in acetonitrile (50 mL) in a round-bottom flask were added di-*tert*-butyl dicarbonate (1.23 g, 5.64 mmol), triethylamine (1.42 mL, 10.3 mmol) and 4-dimethylaminopyridine (62.6 mg, 0.513 mmol) at room temperature. The resulting mixture was stirred at room temperature for 30 minutes. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 20% AcOEt/*n*-hexane linear gradient) provided *tert*-butyl 6-bromo-1*H*-indazole-1-carboxylate (1.05 g, 3.53 mmol, 69% yield).

To a solution of *tert*-butyl 6-bromo-1*H*-indazole-1-carboxylate (1.05 g, 3.53 mmol) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (933 mg, 4.24 mmol) in 1,2-dimethoxyethane (30 mL) in a round-bottom flask were added [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (289 mg, 0.353 mmol), tripotassium phosphate hydrate (2.25 g, 10.6 mmol) and water (7.5 mL). The resulting mixture was stirred at reflux for 1 hour. The reaction mixture was added to water and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 40% AcOEt/*n*-hexane linear gradient) provided the title compound. (620 mg, 2.00 mmol, 57% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 8.20 (s, 1H), 7.78 (d, *J* = 8.3 Hz, 1H), 7.60 (d, *J* = 8.6 Hz, 2H), 7.55 (d, *J* = 8.3 Hz, 1H), 6.96 (d, *J* = 8.6 Hz, 2H), 5.11 (brs, 1H), 1.61 (s, 9H); LCMS m/z 311 $[M + H]^+$.

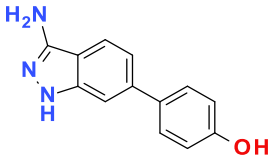
4-(1*H*-indazol-6-yl)phenol (115)



tert-Butyl 6-(4-hydroxyphenyl)-1*H*-indazole-1-carboxylate (**114**, 75.0 mg, 0.242 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (5 mL, 20.0 mmol) at room temperature. The resulting mixture was stirred at room temperature for 17 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (42.3 mg, 0.201 mmol, 83% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.00 (s, 1H),

7.73 (d, *J* = 8.2 Hz, 1H), 7.57 (s, 1H), 7.49 (d, *J* = 8.6 Hz, 2H), 7.30 (d, *J* = 8.2 Hz, 1H), 6.82 (d, *J* = 8.6 Hz, 2H); LCMS m/z 211 $[M + H]^+$.

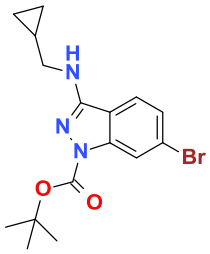
4-(3-amino-1*H*-indazol-6-yl)phenol (116)



tert-Butyl 3-amino-6-(4-hydroxyphenyl)-1*H*-indazole-1-carboxylate (77.0 mg, 0.237 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (5 mL, 20.0 mmol) at room temperature. The resulting mixture was stirred at room temperature for 17 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (52.8 mg, 0.234 mmol, 99% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 11.32 (brs, 1H), 7.65 (d, *J* = 8.2 Hz, 1H), 7.45 (d, *J* = 8.6 Hz, 2H), 7.27 (s, 1H), 7.09 (d, *J* = 8.2 Hz, 1H), 6.80 (d, *J* = 8.6 Hz, 2H), 5.29 (brs, 2H); LCMS m/z 226 $[M + H]^+$.

4-{3-[(cyclopropylmethyl)amino]-1*H*-indazol-6-yl}phenol (118)

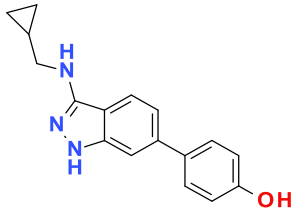
***tert*-butyl 6-bromo-3-[(cyclopropylmethyl)amino]-1*H*-indazole-1-carboxylate (117)**



To a solution of *tert*-butyl 3-amino-6-bromo-1*H*-indazole-1-carboxylate (**69**, 470 mg, 1.50 mmol) and cyclopropanecarbaldehyde (110 mg, 1.50 mmol) in CH_2Cl_2 (30 mL) in a round-bottom flask was added sodium triacetoxyborohydride (380 mg, 1.80 mmol) at room temperature. The resulting mixture was stirred at room temperature for 46 hours. The reaction mixture was quenched by saturated aqueous sodium hydrogen carbonate solution and extracted with CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 30% AcOEt/*n*-hexane linear gradient) provided the title compound. (340 mg, 0.929 mmol, 62% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, *J* = 8.6 Hz, 1H), 7.35 (s, 1H), 7.31 (d, *J* = 8.6 Hz, 1H), 4.31-4.27 (m, 1H), 3.35-3.28 (m, 2H), 1.18-1.06 (m, 1H), 0.59-

0.50 (m, 2H), 0.29-0.24 (m, 2H); LCMS m/z 366 $[M + H]^+$.

4-{3-[(cyclopropylmethyl)amino]-1*H*-indazol-6-yl}phenol (118)

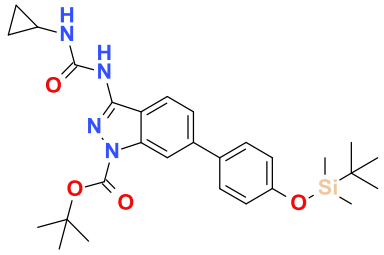


To a solution of *tert*-butyl 6-bromo-3-[(cyclopropylmethyl)amino]-1*H*-indazole-1-carboxylate (**117**, 320 mg, 0.874 mmol) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (211 mg, 0.962 mmol) in 1,2-dimethoxyethane (10 mL) in a round-bottom flask were added [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (35.7 mg, 0.0436 mmol), tripotassium phosphate hydrate (556 mg, 2.62 mmol) and water (2.5 mL). The resulting mixture was stirred at reflux for 40 minutes. The reaction mixture was added to water and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 50% AcOEt/*n*-hexane linear gradient) provided *tert*-butyl 3-[(cyclopropylmethyl)amino]-6-(4-hydroxyphenyl)-1*H*-indazole-1-carboxylate (322 mg, 0.849 mmol, 97% yield).

tert-Butyl 3-[(cyclopropylmethyl)amino]-6-(4-hydroxyphenyl)-1*H*-indazole-1-carboxylate (322 mg, 0.849 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (10 mL, 40.0 mmol) at room temperature. The resulting mixture was stirred at room temperature for 21 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (218 mg, 0.781 mmol, 92% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 11.32 (brs, 1H), 7.71 (d, *J* = 8.6 Hz, 1H), 7.46 (d, *J* = 8.6 Hz, 2H), 7.27 (s, 1H), 7.08 (d, *J* = 8.6 Hz, 1H), 6.79 (d, *J* = 8.6 Hz, 2H), 5.94 (t, *J* = 5.8 Hz, 1H), 3.10 (t, *J* = 5.8 Hz, 2H), 1.17-1.07 (m, 1H), 0.45-0.35 (m, 2H), 0.24-0.17 (m, 2H); LCMS m/z 280 $[M + H]^+$.

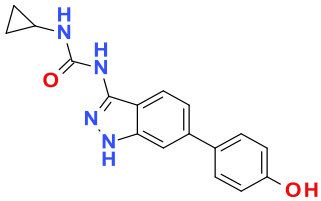
***N*-cyclopropyl-*N*'-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]urea (120)**

***tert*-butyl 6-(4-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-3-[(cyclopropylcarbamoyl)amino]-1*H*-indazole-1-carboxylate (119)**



To a solution of *tert*-butyl 6-(4-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-3-[(cyclopropylcarbamoyl)amino]-1*H*-indazole-1-carboxylate (**119**, 81.0 mg, 0.155 mmol) in THF (5 mL) in a round-bottom flask was added isocyanatocyclopropane (166 mg, 2.00 mmol) at 0 °C. The resulting mixture was stirred at 70 °C for 5 hours. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 50% AcOEt/*n*-hexane linear gradient) provided the title compound. (81.0 mg, 0.155 mmol, 39% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (brs, 1H), 7.98 (d, *J* = 9.0 Hz, 1H), 7.53 (d, *J* = 8.6 Hz, 2H), 7.49 (d, *J* = 9.0 Hz, 1H), 6.88 (d, *J* = 8.6 Hz, 2H), 2.88-2.82 (m, 1H), 1.68 (s, 9H), 0.96 (s, 9H), 0.88-0.82 (m, 2H), 0.70-0.65 (m, 2H), 0.20 (s, 6H); LCMS m/z 523 $[M + H]^+$.

***N*-cyclopropyl-*N*'-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]urea (120)**



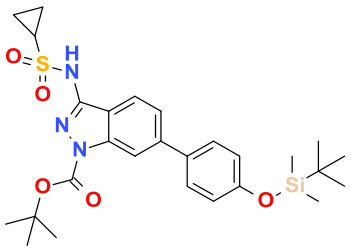
To a solution of *tert*-butyl 3-amino-6-(4-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-1*H*-indazole-1-carboxylate (**93**, 176 mg, 0.400 mmol) and triethylamine (0.278 mL, 2.00 mmol) in THF (10 mL) in a round-bottom flask was added 1 mol/L tetrabutylammonium fluoride in THF (2.5 mL, 2.50 mmol) at room temperature. The resulting mixture was stirred at room temperature for 15 minutes. The reaction mixture was added to water and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 50% AcOEt/*n*-hexane linear gradient) provided *tert*-butyl 3-[(cyclopropylcarbamoyl)amino]-6-(4-hydroxyphenyl)-1*H*-indazole-1-carboxylate (48.0 mg, 0.118 mmol, 76 % yield).

tert-Butyl 3-[(cyclopropylcarbamoyl)amino]-6-(4-hydroxyphenyl)-1*H*-indazole-1-

carboxylate (48.0 mg, 0.118 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (5 mL, 20.0 mmol) at room temperature. The resulting mixture was stirred at room temperature for 21 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (34.9 mg, 0.113 mmol, 96% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.29 (brs, 1H), 9.42 (brs, 1H), 7.97 (d, *J* = 8.2 Hz, 1H), 7.92 (brs, 1H), 7.50 (d, *J* = 8.6 Hz, 2H), 7.42 (s, 1H), 7.20 (d, *J* = 8.2 Hz, 1H), 6.83 (d, *J* = 8.6 Hz, 2H), 2.68-2.60 (m, 1H), 0.70-0.63 (m, 2H), 0.48-0.32 (m, 2H); LCMS m/z 309 $[M + H]^+$.

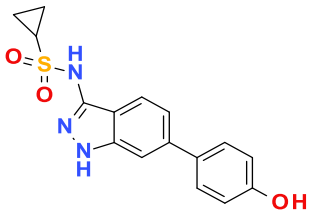
<u>*N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]cyclopropanesulfonamide (122)</u>

***tert*-butyl 6-(4-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-3-[(cyclopropanesulfonyl)amino]-1*H*-indazole-1-carboxylate (121)**



To a solution of *tert*-butyl 6-(4-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-3-[(cyclopropylcarbamoyl)amino]-1*H*-indazole-1-carboxylate (**119**, 170 mg, 0.387 mmol) in pyridine (5 mL) in a round-bottom flask was added cyclopropanesulfonyl chloride (65.2 mg, 0.464 mmol) at room temperature. The resulting mixture was stirred at 70 °C for 4.5 days. The reaction mixture was concentrated under reduced pressure. The residue mixture was added to AcOEt and washed with saturated aqueous ammonium chloride solution. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 70% AcOEt/*n*-hexane linear gradient) provided the title compound. (81.3 mg, 0.150 mmol, 39% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (brs, 1H), 8.32 (brs, 1H), 8.03 (d, *J* = 8.6 Hz, 1H), 7.60 (d, *J* = 8.6 Hz, 1H), 7.57 (d, *J* = 8.5 Hz, 2H), 6.97 (d, *J* = 8.5 Hz, 2H), 2.80-2.75 (m, 1H), 1.76 (s, 9H), 1.33-1.26 (m, 2H), 1.12-1.05 (m, 2H), 1.05 (s, 9H), 0.24 (s, 6H); LCMS m/z 543 $[M + H]^+$.

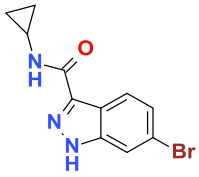
***N*-[6-(4-hydroxyphenyl)-1*H*-indazol-3-yl]cyclopropanesulfonamide (122)**



122 was prepared in a similar manner described for **120**. 99% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.67 (d, *J* = 8.6 Hz, 1H), 7.49 (d, *J* = 8.2 Hz, 2H), 7.40 (s, 1H), 7.19 (d, *J* = 8.6 Hz, 1H), 6.82 (d, *J* = 8.2 Hz, 2H), 2.78-2.68 (m, 1H), 0.89-0.73 (m, 4H); LCMS m/z 330 [M + H]$^+$.

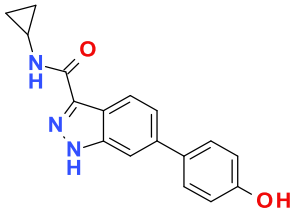
<u>*N*-cyclopropyl-6-(4-hydroxyphenyl)-1*H*-indazole-3-carboxamide (125)</u>

6-bromo-*N*-cyclopropyl-1*H*-indazole-3-carboxamide (124)



To a solution of 6-bromo-1*H*-indazole-3-carboxylic acid (1.00 g, 4.15 mmol) in DMF (30 mL) in a round-bottom flask was added 1,1'-carbonyldiimidazole (807 mg, 4.98 mmol) at room temperature. The resulting mixture was stirred at 70 °C for 30 minutes. The reaction mixture was allowed to room temperature and added cyclopropanamine (355 mg, 6.22 mmol) and triethylamine (1.15 mL, 8.30 mmol). The resulting mixture was stirred at room temperature for 1.5 hours. The reaction mixture was added to AcOEt and washed with water. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure and the resulting solid was collected. The solid was washed with CH_2Cl_2 and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (739 mg, 2.64 mmol, 64% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.70 (s, 1H), 8.55 (brs, 1H), 8.13 (d, *J* = 8.7 Hz, 1H), 7.87 (s, 1H), 7.41 (d, *J* = 8.7 Hz, 1H), 2.95-2.79 (m, 1H), 0.73-0.65 (m, 4H).

***N*-cyclopropyl-6-(4-hydroxyphenyl)-1*H*-indazole-3-carboxamide (125)**



To a solution of 6-bromo-*N*-cyclopropyl-1*H*-indazole-3-carboxamide (**124**, 739 mg, 2.64 mmol) in acetonitrile (50 mL) in a round-bottom flask were added di-*tert*-butyl dicarbonate

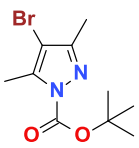
(633 mg, 2.90 mmol), trimethylamine (0.731 mL, 5.28 mmol) and *N,N*-dimethylaminopyridine (32.2 mg, 0.264 mmol) at room temperature. The resulting mixture was stirred at room temperature for 1 hour. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 30% AcOEt/*n*-hexane linear gradient) provided *tert*-butyl 6-bromo-3-(cyclopropylcarbamoyl)-1*H*-indazole-1-carboxylate (501 mg, 1.32 mmol, 50% yield).

To a solution of *tert*-butyl 6-bromo-3-(cyclopropylcarbamoyl)-1*H*-indazole-1-carboxylate (250 mg, 0.658 mmol) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (173 mg, 0.789 mmol) in 1,2-dimethoxyethane (3 mL) in a round-bottom flask were added [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (26.9 mg, 0.0328 mmol), tripotassium phosphate hydrate (419 mg, 10.6 mmol) and water (0.75 mL). The resulting mixture was stirred at reflux for 30 minutes. The reaction mixture was added to water and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 70% AcOEt/*n*-hexane linear gradient) provided *tert*-butyl 3-(cyclopropylcarbamoyl)-6-(4-hydroxyphenyl)-1*H*-indazole-1-carboxylate (132 mg, 0.336 mmol, 51% yield).

tert-Butyl 3-(cyclopropylcarbamoyl)-6-(4-hydroxyphenyl)-1*H*-indazole-1-carboxylate (132 mg, 0.336 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (5 mL, 20.0 mmol) at room temperature. The resulting mixture was stirred at room temperature for 17.5 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (72.0 mg, 0.245 mmol, 73% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.34 (brs, 1H), 8.11 (d, *J* = 8.2 Hz, 1H), 7.63 (s, 1H), 7.52 (d, *J* = 8.6 Hz, 2H), 7.42 (d, *J* = 8.2 Hz, 1H), 6.83 (d, *J* = 8.6 Hz, 2H), 2.90-2.82 (m, 1H), 0.69-0.60 (m, 4H); LCMS m/z 294 $[M + H]^+$.

<u>*N*-[6-(3,5-dimethyl-1*H*-pyrazol-4-yl)-1*H*-indazol-3-yl]-4-(4-methylpiperazin-1-yl)benzamide (129)</u>

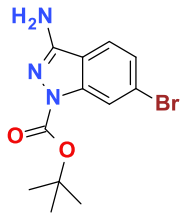
***tert*-butyl 4-bromo-3,5-dimethyl-1*H*-pyrazole-1-carboxylate**



To a solution of 4-bromo-3,5-dimethyl-1*H*-pyrazole (5.30 g, 30.3 mmol) in THF (200 mL) in

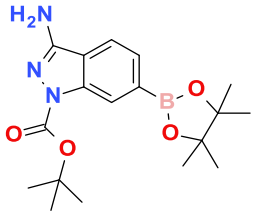
a round-bottom flask were added di-*tert*-butyl dicarbonate (7.93 g, 36.3 mmol), triethylamine (12.6 mL, 90.9 mmol) and 4-dimethylaminopyridine (370 mg, 3.03 mmol). The resulting mixture was stirred at room temperature for 1 hour, then concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 20% AcOEt/*n*-hexane linear gradient) provided the title compound. (8.10 g, 29.0 mmol, 97% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.47 (s, 3 H), 2.25 (s, 3H), 1.59 (s, 9H).

***tert*-butyl 3-amino-6-bromo-1*H*-indazole-1-carboxylate**



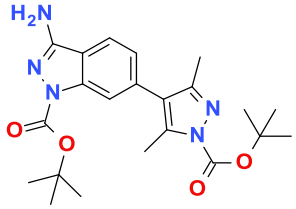
To a solution of 6-bromo-1*H*-indazole-3-amine (20.3 g, 95.7 mmol) in acetonitrile (200 mL) in a round-bottom flask were added di-*tert*-butyl dicarbonate (31.3 g, 144 mmol), triethylamine (19.9 mL, 144 mmol) and 4-dimethylaminopyridine (1.17 g, 9.57 mmol). The resulting mixture was stirred at room temperature for 7 hours, then concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 50% AcOEt/*n*-hexane linear gradient) provided the title compound. (14.9 g, 47.8 mmol, 50% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.31 (brs,1 H), 7.38 (s, 2H), 4.44 (brs, 2H), 1.67 (s, 9H).

***tert*-butyl 3-amino-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole-1-carboxylate (126)**



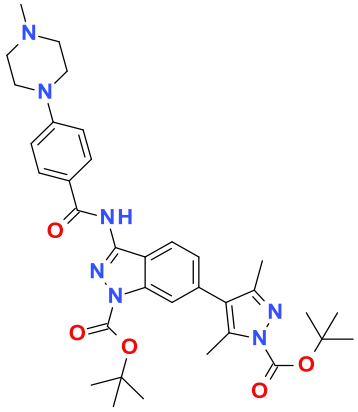
To a solution of *tert*-butyl 3-amino-6-bromo-1*H*-indazole-1-carboxylate (1.56 g, 5.00 mmol) in 1,4-dioxane (50 mL) in a round-bottom flask were added bis(pinacolato)diboron (1.52 g, 6.00 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with dichloromethane (408 mg, 0.500 mmol) and potassium acetate (1.47 g, 15.0 mmol). The resulting mixture was stirred for 1.5 hours at reflux. The insoluble materials were filtrated off with Celite pad and the filtrate was concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 70% AcOEt/*n*-hexane linear gradient) provided the title compound. (1.80 g, 5.00 mmol, 100% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56 (brs,1 H), 7.63 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 4.09 (brs, 2H), 1.67 (s, 9H), 1.32 (s, 6H), 1.21 (s, 6H); LCMS m/z 360 $[M + H]^+$.

***tert*-butyl 3-amino-6-[1-(*tert*-butoxycarbonyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]-1*H*-indazole-1-carboxylate (127)**



To a solution of *tert*-butyl 3-amino-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole-1-carboxylate (**126**, 1.80 g, 5.00 mmol) in 1,2-dimethoxyethane (50 mL) in a round-bottom flask were added *tert*-butyl 4-bromo-3,5-dimethyl-1*H*-pyrazole-1-carboxylate (1.65 g, 6.01 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (409 mg, 0.500 mmol), tripotassium phosphate hydrate (3.46 g, 15.0 mmol) and water (15 mL). The resulting mixture was stirred for 45 minutes at reflux and the reaction mixture was added to water. The resulting solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 70% AcOEt/*n*-hexane linear gradient) provided the title compound. (880 mg, 2.01 mmol, 41% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (brs,1H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.08 (d, *J* = 7.8 Hz, 1H), 4.42 (brs, 2H), 2.46 (s, 3H), 2.25 (s, 3H), 1.65 (s, 18H); LCMS m/z 428 $[M + H]^+$.

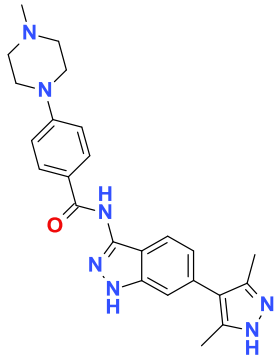
***tert*-butyl 6-[1-(*tert*-butoxycarbonyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]-3-{[4-(4-methylpiperazin-1-yl)benzoyl]amino}-1*H*-indazole-1-carboxylate**



To a solution of *tert*-butyl 3-amino-6-[1-(*tert*-butoxycarbonyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]-1*H*-indazole-1-carboxylate (427 mg, 1.00 mmol) in CH_2Cl_2 (30 mL) in a round-bottom flask were added 4-(4-methylpiperazin-1-yl)benzoyl chloride dihydrochloride (934 mg, 3.00 mmol) and pyridine (0.402 mL, 5.00 mmol). The resulting mixture was stirred at room temperature for 3 hours, then concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (0% to 100% AcOEt/*n*-hexane linear gradient) provided

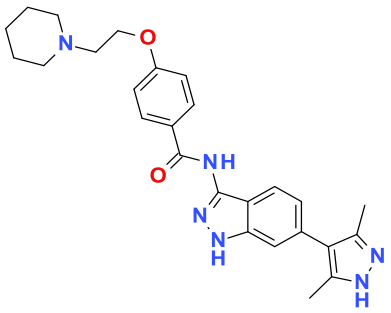
the title compound. (190 mg, 0.301 mmol, 30% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (m, 1H), 8.28 (d, *J* = 10.3 Hz, 1H), 7.96 (brs,1H), 7.84 (d, *J* = 8.6 Hz, 2H), 7.14 (d, *J* = 10.3 Hz, 1H), 6.93 (d, *J* = 8.6 Hz, 2H), 3.39-3.32 (m, 4H), 2.58-2.51 (m, 4H), 2.47 (s, 3H), 2.34 (s, 3H), 2.26 (s, 3H), 1.67 (s, 9H), 1.65 (s, 9H); LCMS m/z 630 $[M + H]^+$.

_N_-[6-(3,5-dimethyl-1_H_-pyrazol-4-yl)-1_H_-indazol-3-yl]-4-(4-methylpiperazin-1-yl)benzamide (129)



tert-Butyl 6-[1-(*tert*-butoxycarbonyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]-3-{[4-(4-methylpiperazin-1-yl)benzoyl]amino}-1*H*-indazole-1-carboxylate (85.0 mg, 0.135 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (5 mL, 20.0 mmol). The resulting mixture was stirred at 50 ºC for 1.5 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (46.7 mg, 0.109 mmol, 81% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.61 (s, 1H), 12.31 (brs, 1H), 10.47 (s, 1H), 7.93 (d, *J* = 10.3 Hz, 2H), 7.65 (d, *J* = 8.6 Hz, 1H), 7.23 (s, 1H), 6.99 (d, *J* = 10.3 Hz, 2H), 6.95 (d, *J* = 8.6 Hz, 1H), 3.31 (s, 3H), 3.29-3.24 (m, 4H), 2.45-2.40 (m, 4H), 2.26 (s, 6H); LCMS m/z 430 $[M + H]^+$; HRMS (Positive ESI) m/z 430.2378 (430.2277 calcd for $C_{24}H_{27}N_7O$ + H).

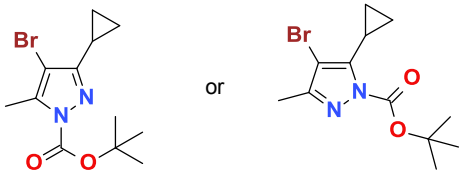
_N_-[6-(3,5-dimethyl-1_H_-pyrazol-4-yl)-1_H_-indazol-3-yl]-4-[2-(piperidin-1-yl)ethoxy]benzamide (128)



128 was prepared in a similar manner described for **129**. 93% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.66 (brs, 1H), 10.62 (brs, 1H), 8.03 (d, *J* = 9.0 Hz, 2H), 7.66 (d, *J* = 8.6 Hz, 1H), 7.25 (s, 1H), 7.27 (d, *J* = 8.6 Hz, 1H), 7.05 (d, *J* = 9.0 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 1H), 4.13 (t, *J* = 6.3 Hz, 2H), 2.65 (t, *J* = 6.3 Hz, 2H), 2.30-2.22 (m, 4H), 1.55-1.42 (m, 4H), 1.43-1.37 (m, 2H); LCMS m/z 459 $[M + H]^+$.

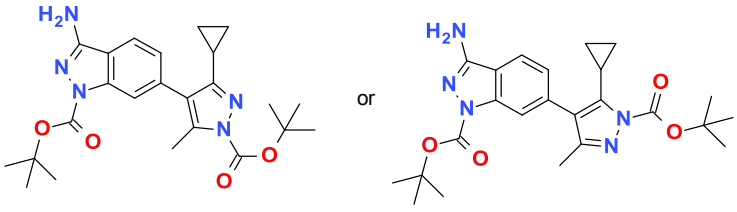
6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-3-[6-(4-methylpiperazin-1-yl)pyridin-3-yl]-1*H*-indazole (138)

***tert*-butyl 4-bromo-3-cyclopropyl-5-methyl-1*H*-pyrazole-1-carboxylate (or *tert*-butyl 4-bromo-5-cyclopropyl-3-methyl-1*H*-pyrazole-1-carboxylate)** [The Boc protected compounds were as a single regioisomer, but the position of Boc groups were not determined]



To a solution of 4-bromo-5-cyclopropyl-3-methyl-1*H*-pyrazole hydrochloride (14.4 g, 60.6 mmol) in THF (300 mL) in a round-bottom flask were added di-*tert*-butyl dicarbonate (15.9 g, 72.8 mmol), triethylamine (25.2 mL, 182 mmol) and 4-dimethylaminopyridine (741 mg, 6.06 mmol). The resulting mixture was stirred at room temperature for 17 hours, then concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 50% AcOEt/*n*-hexane linear gradient) provided the title compound. (16.6 g, 55.1 mmol, 91% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44 (s, 3 H), 1.89-1.81 (m, 1H), 1.59 (s, 9H), 1.03-0.97 (m, 2H), 0.93-0.84 (m, 2H); LCMS m/z 201 $[M – Boc + H]^+$.

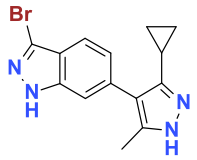
***tert*-butyl 3-amino-6-[1-(*tert*-butoxycarbonyl)-3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl]-1*H*-indazole-1-carboxylate (or *tert*-butyl 3-amino-6-[1-(*tert*-butoxycarbonyl)-5-cyclopropyl-3-methyl-1*H*-pyrazol-4-yl]-1*H*-indazole-1-carboxylate) (130)**



To a solution of *tert*-butyl 3-amino-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole-1-carboxylate (**126**, 15.0 g, 33.0 mmol) in 1,2-dimethoxyethane (200 mL) in a round-bottom flask were added *tert*-butyl 4-bromo-3-cyclopropyl-5-methyl-1*H*-pyrazole-1-

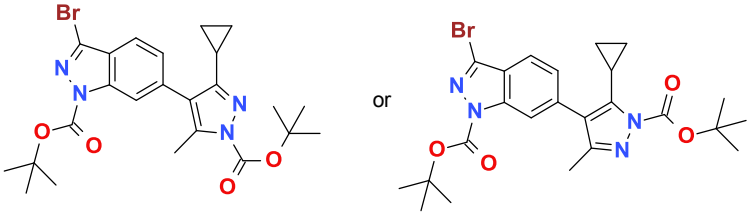
carboxylate (or *tert*-butyl 4-bromo-5-cyclopropyl-3-methyl-1*H*-pyrazole-1-carboxylate) (10.9 g, 36.3 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (539 mg, 0.660 mmol), tripotassium phosphate hydrate (22.8 g, 99.0 mmol) and water (80 mL). The resulting mixture was stirred for 2 hours at reflux and the reaction mixture was added to water. The resulting solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 70% AcOEt/*n*-hexane linear gradient) provided the title compound. (13.6 g, 30.0 mmol, 91% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (brs,1H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.22 (d, *J* = 7.8 Hz, 1H), 4.43 (brs, 2H), 2.44 (s, 3H), 1.78-1.68 (m, 1H), 1.66 (s, 9H), 1.62 (s, 9H), 1.05-0.99 (m, 2H), 0.85-0.80 (m, 2H); LCMS m/z 354 $[M - Boc + H]^+$.

3-bromo-6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazole (131)



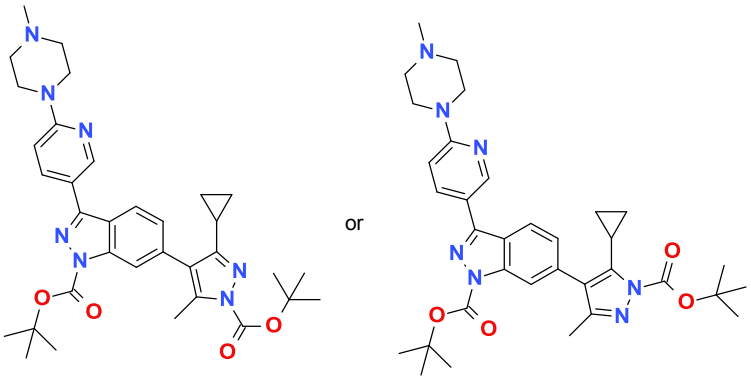
To a solution of a *tert*-butyl 3-amino-6-[1-(*tert*-butoxycarbonyl)-3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl]-1*H*-indazole-1-carboxylate (or *tert*-butyl 3-amino-6-[1-(*tert*-butoxycarbonyl)-5-cyclopropyl-3-methyl-1*H*-pyrazol-4-yl]-1*H*-indazole-1-carboxylate) (**130**, 3.00 g, 6.62 mmol) in 48% hydrobromic acid (5 mL) and acetic acid (5 mL) in a round-bottom flask was added a solution of sodium nitrite (548 mg, 7.94 mmol) in water (2 mL) at 0ºC. The resulting mixture was stirred at 0 ºC for 30 minutes and added copper bromide (1.90 g, 13.2 mmol) at 0 ºC. The resulting mixture was stirred at room temperature overnight and the reaction mixture was quenched by saturated aqueous sodium hydrogen carbonate solution. The resulting solution was extracted with AcOEt and CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (0% to 10% MeOH/CH_2Cl_2 linear gradient) provided the title compound. (610 mg, 1.90 mmol, 29% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.14 (brs, 1H), 7.64 (d, *J* = 8.8 Hz, 1H), 7.41 (s, 1H), 7.26 (d, *J* = 8.8 Hz, 1H), 2.29 (s, 3H), 1.87-1.78 (m, 1H), 0.91-0.82 (m, 2H), 0.81-0.75 (m, 2H) ; LCMS m/z 317 $[M + H]^+$.

***tert*-butyl 3-bromo-6-[1-(*tert*-butoxycarbonyl)-3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl]-1*H*-indazole-1-carboxylate (or *tert*-butyl 3-bromo-6-[1-(*tert*-butoxycarbonyl)-5-cyclopropyl-3-methyl-1*H*-pyrazol-4-yl]-1*H*-indazole-1-carboxylate) (132)**



To a solution of 3-bromo-6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazole (**131**, 190 mg, 0.599 mmol) in acetonitrile (20 mL) in a round-bottom flask were added di-*tert*-butyl dicarbonate (288 mg, 1.32 mmol), triethylamine (0.332 mL, 2.40 mmol) and 4-dimethylaminopyridine (7.3 mg, 0.0599 mmol). The resulting mixture was stirred at room temperature for 1 hour, then concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 20% AcOEt/*n*-hexane linear gradient) provided the title compound. (275 mg, 0.532 mmol, 89% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (brs,1H), 7.66 (d, *J* = 8.3 Hz, 1H), 7.36 (d, *J* = 8.3 Hz, 1H), 2.44 (s, 3H), 1.73-1.65 (m, 1H), 1.68 (s, 9H), 1.63 (s, 9H), 1.07-1.02 (m, 2H), 0.86-0.80 (m, 2H); LCMS m/z 417 $[M - Boc + H]^+$.

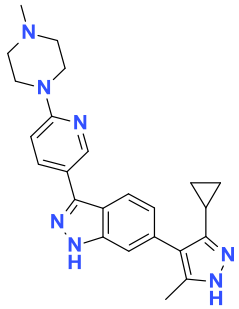
***tert*-butyl 6-[1-(*tert*-butoxycarbonyl)-3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl]-3-[6-(4-methylpiperazin-1-yl)pyridin-3-yl]-1*H*-indazole-1-carboxylate (or *tert*-butyl 6-[1-(*tert*-butoxycarbonyl)-5-cyclopropyl-3-methyl-1*H*-pyrazol-4-yl]-3-[6-(4-methylpiperazin-1-yl)pyridin-3-yl]-1*H*-indazole-1-carboxylate) (133)**



To a solution of *tert*-butyl 3-bromo-6-[1-(*tert*-butoxycarbonyl)-3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl]-1*H*-indazole-1-carboxylate (or *tert*-butyl 3-bromo-6-[1-(*tert*-butoxycarbonyl)-5-cyclopropyl-3-methyl-1*H*-pyrazol-4-yl]-1*H*-indazole-1-carboxylate) (**132**, 130 mg, 0.251 mmol) in 1,2-dimethoxyethane (5 mL) in a round-bottom flask were added 1-methyl-4-[5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl]piperazine (152 mg, 0.503 mmol),

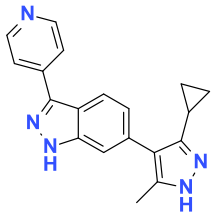
[1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (20.5 mg, 0.0251 mmol), tripotassium phosphate hydrate (174 mg, 0.754 mmol) and water (2 mL). The resulting mixture was stirred for 1 hour at 100 ºC and the reaction mixture was added to water. The resulting solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (0% to 67% AcOEt/*n*-hexane linear gradient) provided the title compound. (128 mg, 0.209 mmol, 83% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.83 (s, 1H), 8.17 (d, *J* = 8.4 Hz, 1H), 8.12 (brs,1H), 7.98 (d, *J* = 8.8 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 6.77 (d, *J* = 8.8 Hz, 1H), 3.69-3.65 (m, 4H), 2.56-2.51 (m, 4H), 2.46 (s, 3H), 2.34 (s, 3H), 1.78-1.71 (m, 1H), 1.70 (s, 9H), 1.63 (s, 9H), 1.07-1.03 (m, 2H), 0.87-0.82 (m, 2H); LCMS m/z 614 $[M + H]^+$.; HRMS (Positive ESI) m/z 614.3452 (614.3377 calcd for $C_{34}H_{43}N_7O_4$ + H).

6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-3-[6-(4-methylpiperazin-1-yl)pyridin-3-yl]-1*H*-indazole (138)



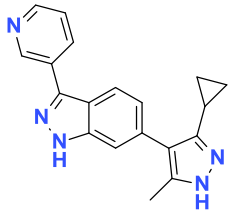
tert-Butyl 6-[1-(*tert*-butoxycarbonyl)-3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl]-3-[6-(4-methylpiperazin-1-yl)pyridin-3-yl]-1*H*-indazole-1-carboxylate (or *tert*-butyl 6-[1-(*tert*-butoxycarbonyl)-5-cyclopropyl-3-methyl-1*H*-pyrazol-4-yl]-3-[6-(4-methylpiperazin-1-yl)pyridin-3-yl]-1*H*-indazole-1-carboxylate) (**133**, 128 mg, 0.209 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (9.58 mL, 313 mmol). The resulting mixture was stirred at 60 ºC for 1 hour, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (58.0 mg, 0.140 mmol, 67% yield): ^{1}H NMR (500 MHz, DMSO-*d*6) δ 13.00 (s, 1H), 12.23 (brs, 1H), 8.72 (s, 1H), 8.09 (d, *J* = 8.7 Hz, 1H), 7.99 (d, *J* = 8.3 Hz, 1H), 7.45 (brs,1H), 7.19 (d, *J* = 8.7 Hz, 1H), 6.96 (d, *J* = 8.3 Hz, 1H), 3.59-3.51 (m, 4H), 2.42-2.37 (m, 4H), 2.23 (brs, 3H), 2.21 (s, 3H), 1.83-1.76 (m, 1H), 0.80-0.74 (m, 4H); LCMS m/z 414 $[M + H]^+$; HRMS (Positive ESI) m/z 414.2411 (414.2328 calcd for $C_{24}H_{27}N_7$ + H).

6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-3-(pyridin-4-yl)-1*H*-indazole (134)



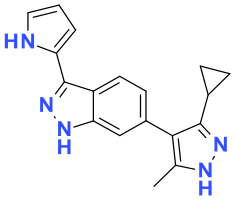
134 was prepared in a similar manner described for **138**. 10% yield: ^{1}H NMR (500 MHz, DMSO-*d*6) δ 13.49 (s, 1H), 12.22 (brs, 1H), 8.66 (d, *J* = 5.8 Hz, 2H), 8.18 (d, *J* = 8.2 Hz, 1H), 7.99 (d, *J* = 5.8 Hz, 2H), 7.54 (s,1H), 7.30 (d, *J* = 8.3 Hz, 1H), 2.23 (brs, 3H), 1.24-1.19 (m, 1H), 0.80-0.74 (m, 4H); LCMS m/z 316 $[M + H]^+$.

6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-3-(pyridin-3-yl)-1*H*-indazole (135)



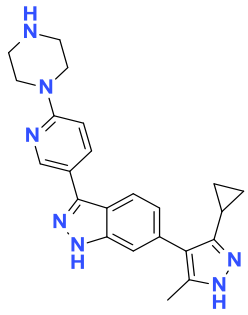
135 was prepared in a similar manner described for **138**. 32% yield: ^{1}H NMR (500 MHz, DMSO-*d*6) δ 13.32 (s, 1H), 12.26 (brs, 1H), 9.19 (s, 1H), 8.60-8.57 (m, 1H), 8.38-8.33 (m, 1H), 8.09 (d, *J* = 8.3 Hz, 1H), 7.56-7.50 (m, 2H), 7.25 (d, *J* = 8.3 Hz, 1H), 2.25 (brs, 3H), 1.24-1.19 (m, 1H), 0.80-0.74 (m, 4H); LCMS m/z 316 $[M + H]^+$.

6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-3-(1*H*-pyrrol-2-yl)-1*H*-indazole (136)



136 was prepared in a similar manner described for **138**. 74% yield: ^{1}H NMR (500 MHz, DMSO-*d*6) δ 12.83 (s, 1H), 12.23 (brs, 1H), 11.32 (brs, 1H), 7.99 (d, *J* = 8.3 Hz, 1H), 7.40 (s, 1H), 7.17 (d, *J* = 8.3 Hz, 1H), 6.83-6.80 (m, 1H), 6.70-6.66 (m, 1H), 6.18-6.14 (m, 1H), 2.23 (brs, 3H), 1.24-1.19 (m, 1H), 0.80-0.74 (m, 4H); LCMS m/z 304 $[M + H]^+$.

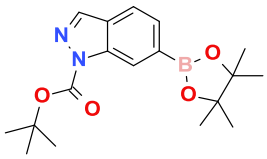
6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-3-[6-(piperazin-1-yl)pyridin-3-yl]-1*H*-indazole (137)



137 was prepared in a similar manner described for **138**. 74% yield: ^{1}H NMR (500 MHz, DMSO-*d*6) δ 12.99 (s, 1H), 12.24 (brs, 1H), 8.71 (s, 1H), 8.08 (d, *J* = 8.7 Hz, 1H), 7.99 (d, *J* = 8.3 Hz, 1H), 7.45 (brs,1H), 7.18 (d, *J* = 8.7 Hz, 1H), 6.92 (d, *J* = 8.3 Hz, 1H), 3.59-3.51 (m, 4H), 2.42-2.37 (m, 4H), 2.23 (brs, 3H), 1.83-1.76 (m, 1H), 0.80-0.74 (m, 4H); LCMS m/z 400 $[M + H]^+$.

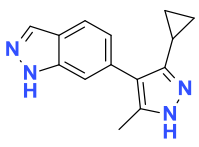
6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazole (140)

***tert*-butyl 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole-1-carboxylate (139)**



To a solution of *tert*-butyl 6-bromo-1*H*-indazole-1-carboxylate (1.06 g, 3.56 mmol) in 1,4-dioxane (20 mL) in a round-bottom flask were added bis(pinacolato)diboron (1.08 g, 4.27 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (145 mg, 0.178 mmol) and potassium acetate (1.05 g, 10.7 mmol) at room temperature. The resulting mixture was stirred for 1 hour at reflux. The insoluble materials were filtrated off with Celite pad and the filtrate was concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 25% AcOEt/*n*-hexane linear gradient) provided the title compound (1.22 g, 3.54 mmol, 100% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.18 (s, 1H), 7.73 (s, 2H), 1.74 (s, 9H), 1.37 (s, 12H); LCMS m/z 245 $[M + H - Boc]^+$.

6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazole (140)

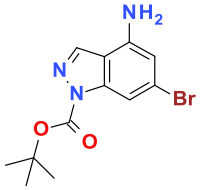


To a solution of *tert*-butyl 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole-1-carboxylate (**139**, 200 mg, 0.581 mmol) and *tert*-butyl 4-bromo-3-cyclopropyl-5-methyl-1*H*-pyrazole-1-carboxylate (or *tert*-butyl 4-bromo-5-cyclopropyl-3-methyl-1*H*-pyrazole-1-carboxylate) (263 mg, 0.872 mmol) in 1,2-dimethoxyethane (20 mL) in a round-bottom flask were added [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (47.4 mg, 0.0581 mmol), tripotassium phosphate hydrate (401 mg, 1.74 mmol) and water (8 mL) at room temperature. The resulting mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (20% to 90% AcOEt/*n*-hexane linear gradient) provided *tert*-butyl 6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazole-1-carboxylate (62.0 mg, 0.180 mmol, 32% yield).

tert-Butyl 6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazole-1-carboxylate (62.0 mg, 0.180 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (8.4 mL, 3.36 mmol) at room temperature. The resulting mixture was stirred at 60 °C for 2 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution. The resulting solution was extracted with AcOEt and CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (0% to 5% MeOH/CH_2Cl_2 linear gradient) provided the title compound. (10.0 mg, 0.042 mmol, 23% yield): ^{1}H NMR (500 MHz, DMSO-*d*6) δ 12.95 (s, 1H), 12.22 (brs, 1H), 8.02 (s, 1H), 7.74 (d, *J* = 8.3 Hz, 1H), 7.42 (s, 1H), 7.12 (d, *J* = 8.3 Hz, 1H), 2.23 (brs, 3H), 1.83-1.76 (m, 1H), 0.80-0.74 (m, 4H); LCMS m/z 239 $[M + H]^+$.

methyl [6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazol-4-yl]carbamate (151, DS28120313)

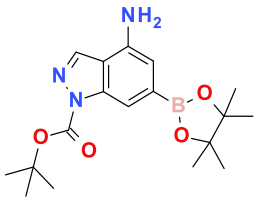
***tert*-butyl 4-amino-6-bromo-1*H*-indazole-1-carboxylate**



To a solution of 6-bromo-1*H*-indazol-4-amine (25.0 g, 118 mmol) in acetonitrile (500 mL) in a round-bottom flask were added di-*tert*-butyl dicarbonate (28.3 g, 130 mmol), triethylamine (18.0 mL, 130 mmol) and 4-dimethylaminopyridine (1.44 g, 11.8 mmol). The resulting mixture was stirred at room temperature for 7 hours, then concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 100% AcOEt/CH_2Cl_2 linear gradient) provided the crude compound. The crude solid was washed with *n*-hexane

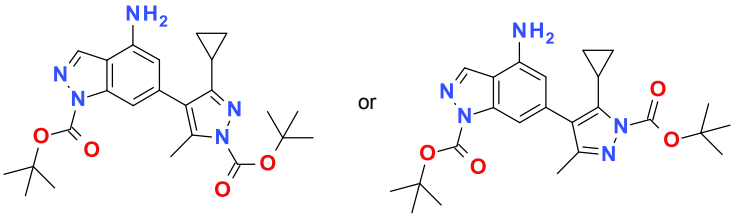
/AcOEt (v/v = 3/1) and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (20.4 g, 65.4 mmol, 55% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 8.01 (s, 1H), 6.63 (s, 1H), 4.21 (brs, 2 H), 1.68 (s, 9H); LCMS m/z 212 [M – Boc + H]$^+$.

***tert*-butyl 4-amino-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole-1-carboxylate (141)**



To a solution of *tert*-butyl 4-amino-6-bromo-1*H*-indazole-1-carboxylate (11.0 g, 35.2 mmol) in 1,4-dioxane (200 mL) in a round-bottom flask were added bis(pinacolato)diboron (9.84 g, 38.8 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (1.44 g, 1.76 mmol) and potassium acetate (10.4 g, 106 mmol). The resulting mixture was stirred for 2 hours at reflux. The insoluble materials were filtrated off with Celite pad and the filtrate was concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 30% AcOEt/CH_2Cl_2 linear gradient) provided the title compound. (12.6 g, 35.1 mmol, 99% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 8.03 (s, 1H), 6.90 (s, 1H), 4.11 (brs, 2 H), 1.69 (s, 9H), 1.32 (s, 12H); LCMS m/z 260 [M – Boc + H]$^+$.

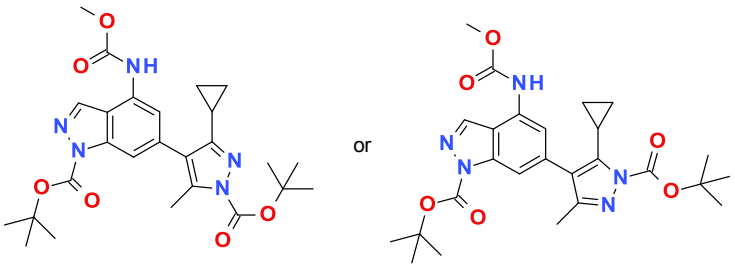
***tert*-butyl 4-amino-6-[1-(*tert*-butoxycarbonyl)-3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl]-1*H*-indazole-1-carboxylate (or *tert*-butyl 4-amino-6-[1-(*tert*-butoxycarbonyl)-5-cyclopropyl-3-methyl-1*H*-pyrazol-4-yl]-1*H*-indazole-1-carboxylate) (142)**



To a solution of *tert*-butyl 4-amino-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole-1-carboxylate (**141**, 3.39 g, 9.44 mmol) in 1,2-dimethoxyethane (100 mL) in a round-bottom flask were added *tert*-butyl 4-bromo-3-cyclopropyl-5-methyl-1*H*-pyrazole-1-carboxylate (or *tert*-butyl 4-bromo-5-cyclopropyl-3-methyl-1*H*-pyrazole-1-carboxylate) (2.84 g, 9.44 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (385 mg, 0.472 mmol), tripotassium phosphate hydrate (6.52 g, 28.3 mmol) and water (40 mL). The resulting mixture was stirred for 1 hour at reflux. The resulting solution

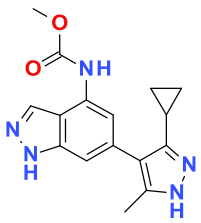
was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (50% to 70% AcOEt/*n*-hexane linear gradient) provided the title compound. (3.10 g, 6.80 mmol, 72% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.48 (s, 1H), 6.45 (s, 1H), 4.21 (brs, 2H), 2.45 (s, 3H), 1.79-1.71 (m, 1H), 1.68 (s, 9H), 1.62 (s, 9H), 1.06-1.10 (m, 2H), 0.88-0.81 (m, 2H); LCMS m/z 354 $[M - Boc + H]^+$.

tert-butyl 6-[1-(_tert_-butoxycarbonyl)-3-cyclopropyl-5-methyl-1_H_-pyrazol-4-yl]-4-[(methoxycarbonyl)amino]-1_H_-indazole-1-carboxylate (or _tert_-butyl 6-[1-(_tert_-butoxycarbonyl)-5-cyclopropyl-3-methyl-1_H_-pyrazol-4-yl]-4-[(methoxycarbonyl)amino]-1_H_-indazole-1-carboxylate) (143)



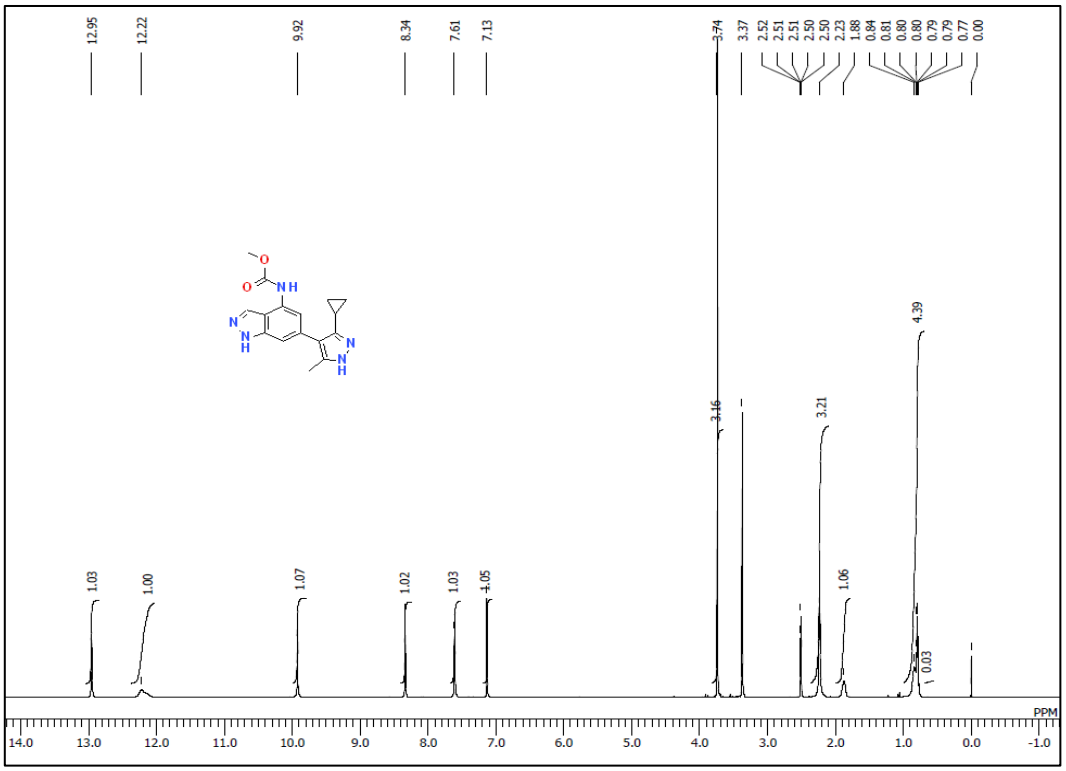
To a solution of *tert*-butyl 4-amino-6-[1-(*tert*-butoxycarbonyl)-3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl]-1*H*-indazole-1-carboxylate (or *tert*-butyl 4-amino-6-[1-(*tert*-butoxycarbonyl)-5-cyclopropyl-3-methyl-1*H*-pyrazol-4-yl]-1*H*-indazole-1-carboxylate) (**142**, 2.10 g, 4.63 mmol) in CH_2Cl_2 (40 mL) in a round-bottom flask were added methyl chloroformate (0.394 mL, 5.09 mmol) and pyridine (0.410 mL, 5.09 mmol). The resulting mixture was stirred at room temperature for 30 minutes, then concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (0% to 30% AcOEt/CH_2Cl_2 linear gradient) provided the title compound. (1.44 g, 2.81 mmol, 61% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.86 (s, 1H), 7.65 (brs,1H), 6.94 (s, 1H), 3.83 (s, 3H), 2.46 (s, 3H), 1.79-1.71 (m, 1H), 1.68 (s, 9H), 1.62 (s, 9H), 1.06-1.10 (m, 2H), 0.88-0.81 (m, 2H); LCMS m/z 512 $[M + H]^+$; HRMS (Positive ESI) m/z 512.2550 (512.2431 calcd for $C_{26}H_{33}N_5O_6$ + H).

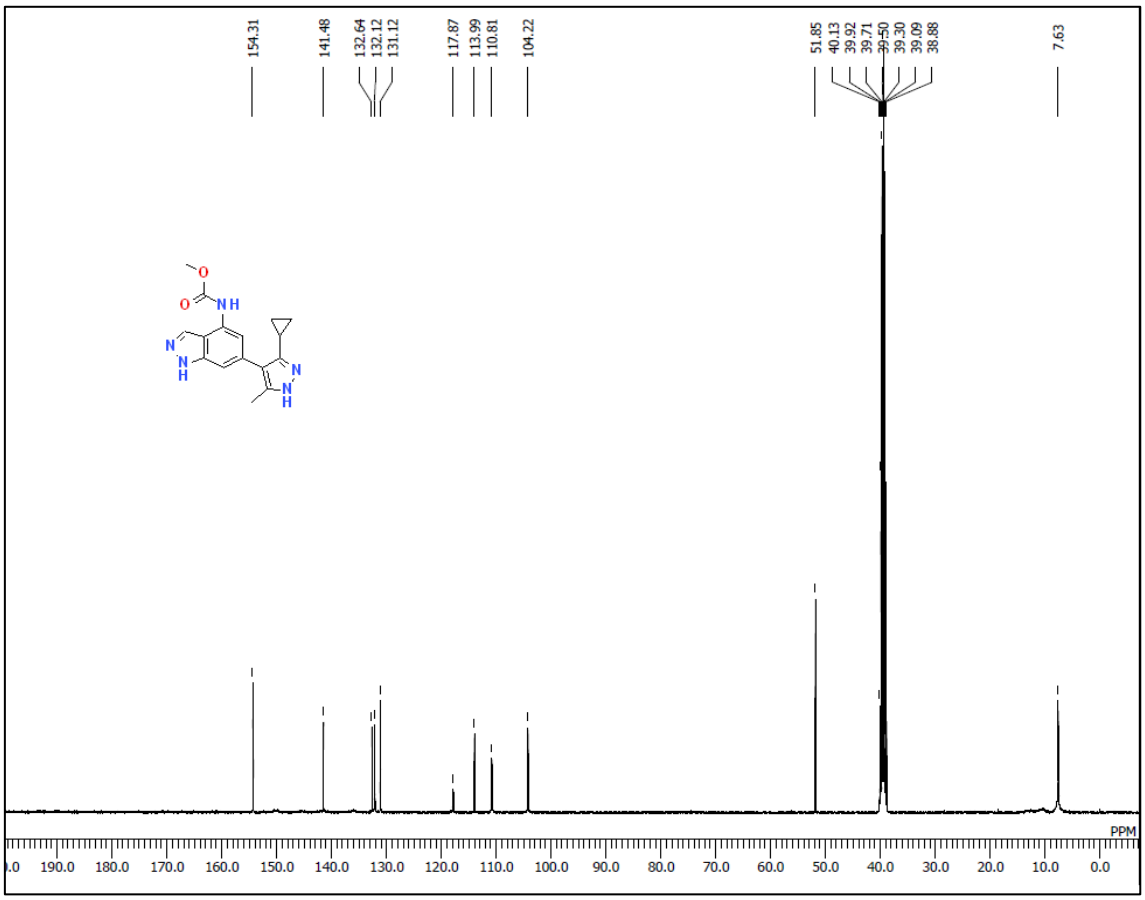
methyl [6-(3-cyclopropyl-5-methyl-1_H_-pyrazol-4-yl)-1_H_-indazol-4-yl]carbamate (151, DS28120313)



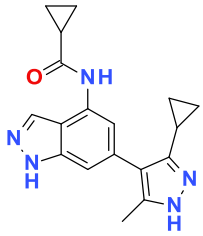
tert-Butyl 6-[1-(*tert*-butoxycarbonyl)-3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl]-4-[(methoxycarbonyl)amino]-1*H*-indazole-1-carboxylate (or *tert*-butyl 6-[1-(*tert*-

butoxycarbonyl)-5-cyclopropyl-3-methyl-1*H*-pyrazol-4-yl]-4-[(methoxycarbonyl)amino]-1*H*-indazole-1-carboxylate) (**143**, 160 mg, 0.313 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (14.4 mL, 57.6 mmol). The resulting mixture was stirred at 60 ºC for 4 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as an offwhite solid. (88.0 mg, 0.280 mmol, 90% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.95 (s, 1H), 12.23-12.03 (brs, 1H), 9.92 (s, 1H), 8.34 (s, 1H), 7.61 (s, 1H), 7.13 (s, 1H), 3.74 (s, 3H), 2.23 (brs, 3H), 1.90-1.84 (m, 1H), 0.87-0.74 (m, 4H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 154.31, 141.48, 132.64, 132.12, 131.12, 117.87, 113.99, 110.81, 104.22, 51.85, 7.63; LCMS m/z 312 $[M + H]^{+}$; HRMS (Positive ESI) m/z 312.1467 (312.1382 calcd for $C_{16}H_{17}N_5O_2$ + H).



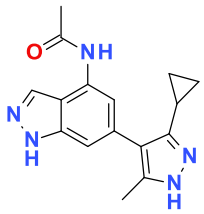


<u>*N*-[6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazol-4-yl]cyclopropanecarboxamide (144)</u>



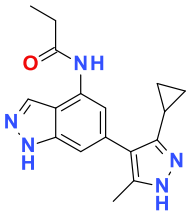
144 was prepared in a similar manner described for **151**. 49% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.94 (s, 1H), 12.18 (brs, 1H), 10.20 (s, 1H), 8.26 (s, 1H), 7.75 (s, 1H), 7.12 (s, 1H), 2.20 (brs, 3H), 2.05-1.98 (m, 1H), 1.90-1.84 (m, 1H), 0.87-0.70 (m, 8H); LCMS m/z 322 $[M + H]^{+}$.

***N*-[6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazol-4-yl]acetamide (145)**



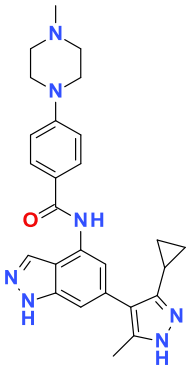
145 was prepared in a similar manner described for **151**. 52% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.93 (s, 1H), 12.19 (brs, 1H), 9.97 (s, 1H), 8.24 (s, 1H), 7.76 (s, 1H), 7.11 (s, 1H), 2.21 (brs, 3H), 2.14 (s, 3H), 1.90-1.84 (m, 1H), 0.87-0.74 (m, 4H); LCMS m/z 296 $[M + H]^+$.

***N*-[6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazol-4-yl]propanamide (146)**



146 was prepared in a similar manner described for **151**. 27% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.93 (s, 1H), 9.89 (s, 1H), 8.24 (s, 1H), 7.77 (s, 1H), 7.12 (s, 1H), 2.44 (q, *J* = 7.8 Hz, 2H), 2.19 (brs, 3H), 1.90-1.84 (m, 1H), 1.10 (t, *J* = 7.8 Hz, 3H), 0.87-0.74 (m, 4H); LCMS m/z 310 $[M + H]^+$.

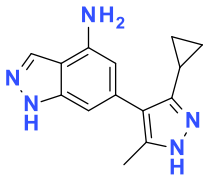
***N*-[6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazol-4-yl]-4-(4-methylpiperazin-1-yl)benzamide (147)**



147 was prepared in a similar manner described for **151**. 28% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.90 (s, 1H), 12.21 (brs, 1H), 10.09 (s, 1H), 8.15 (s, 1H), 7.89 (d, *J* = 9.3 Hz, 1H), 7.52 (s, 1H), 7.19 (s, 1H), 7.01 (d, *J* = 9.3 Hz, 2H), 3.29-3.25 (m, 4H), 2.45-2.40 (m,

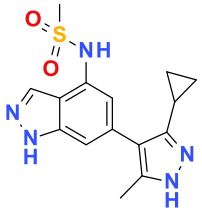
4H), 2.20 (s, 3H), 2.23 (brs, 3H), 1.90-1.84 (m, 1H), 0.87-0.74 (m, 4H); LCMS m/z 456 [M + H]$^+$.

6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazol-4-amine (148)



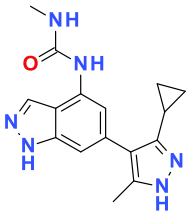
148 was prepared in a similar manner described for **151**. 22% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.51 (s, 1H), 12.07 (brs, 1H), 8.02 (s, 1H), 6.53 (s, 1H), 6.15 (s, 1H), 5.67 (brs, 2H), 2.17 (brs, 3H), 1.90-1.84 (m, 1H), 0.87-0.74 (m, 4H); LCMS m/z 254 [M + H]$^+$.

***N*-[6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazol-4-yl]methanesulfonamide (149)**



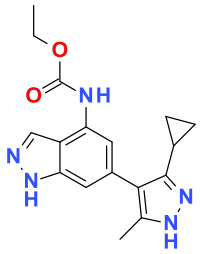
149 was prepared in a similar manner described for **151**. 84% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 13.02 (s, 1H), 12.24 (brs, 1H), 10.04 (s, 1H), 8.27 (s, 1H), 7.19 (s, 1H), 7.10 (s, 1H), 3.03 (s, 3H), 2.23 (brs, 3H), 1.90-1.84 (m, 1H), 0.87-0.74 (m, 4H); LCMS m/z 322 [M + H]$^+$.

***N*-[6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazol-4-yl]-*N*'-methylurea (150)**



150 was prepared in a similar manner described for **151**. 16% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.86 (s, 1H), 12.15 (brs, 1H), 10.04 (s, 1H), 8.67 (brs, 1H), 8.03 (brs, 1H), 7.68 (s, 1H), 6.96 (brs, 1H), 6.19 (q, d, *J* =5.8 Hz, 1H), 2.65 (d, *J* =5.8 Hz, 3H), 2.23 (brs, 3H), 1.90-1.84 (m, 1H), 0.87-0.74 (m, 4H); LCMS m/z 311 [M + H]$^+$.

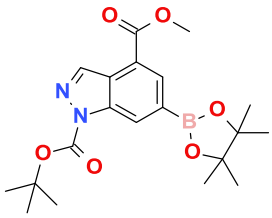
ethyl [6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazol-4-yl]carbamate (152)



152 was prepared in a similar manner described for **151**. 21% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.95 (s, 1H), 12.23-12.03 (brs, 1H), 9.90 (s, 1H), 8.36 (s, 1H), 7.62 (s, 1H), 7.14 (s, 1H), 4.22 (q, *J* = 7.0 Hz, 2H), 2.23 (brs, 3H), 1.90-1.84 (m, 1H), 1.32 (t, *J* = 7.0 Hz, 3H), 0.87-0.74 (m, 4H); LCMS m/z 326 $[M + H]^+$.

6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-*N*-methyl-1*H*-indazole-4-carboxamide (157)

1-*tert*-butyl 4-methyl 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole-1,4-dicarboxylate (153)

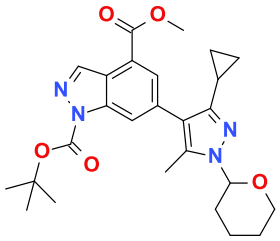


To a solution of methyl 6-bromo-1*H*-indazole-4-carboxylate (3.53 g, 13.8 mmol) in acetonitrile (80 mL) in a round-bottom flask were added di-*tert*-butyl dicarbonate (3.62 g, 16.6 mmol), triethylamine (2.30 mL, 16.6 mmol) and 4-dimethylaminopyridine (169 mg, 1.38 mmol). The resulting mixture was stirred at room temperature for 22 hours, then concentrated under reduced pressure. Purification by flash chromatography on silica gel (2% AcOEt/CH_2Cl_2) provided 1-*tert*-butyl 4-methyl 6-bromo-1*H*-indazole-1,4-dicarboxylate (3.65 g, 10.3 mmol, 74% yield).

To a solution of 1-*tert*-butyl 4-methyl 6-bromo-1*H*-indazole-1,4-dicarboxylate (3.65 g, 10.3 mmol) in 1,4-dioxane (70 mL) in a round-bottom flask were added bis(pinacolato)diboron (3.15 g, 12.4 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (421 mg, 0.516 mmol) and potassium acetate (3.17 g, 32.3 mmol). The resulting mixture was stirred for 1 hour at reflux. The insoluble materials were filtrated off with Celite pad and the filtrate was concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 30% AcOEt/CH_2Cl_2 linear gradient) provided the title compound as a pale yellow solid. (1.96 g, 4.88 mmol, 48% yield): ^{1}H NMR (400 MHz,

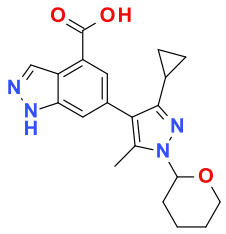
$CDCl_3$) δ 8.89 (s, 1H), 8.72 (s, 1H), 8.39 (s, 1H), 3.98 (s, 3H), 1.71 (s, 9H), 1.34 (s, 12H).

1-*tert*-butyl 4-methyl 6-[3-cyclopropyl-5-methyl-1-(oxan-2-yl)-1*H*-pyrazol-4-yl]-1*H*-indazole-1,4-dicarboxylate (154)



To a solution of 1-*tert*-butyl 4-methyl 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole-1,4-dicarboxylate (**153**, 1.03 g, 2.57 mmol) in 1,4-dioxane (30 mL) in a round-bottom flask were added 4-bromo-3-cyclopropyl-5-methyl-1-(oxan-2-yl)-1*H*-pyrazole (807 mg, 2.83 mmol), chloro(2-dicyclohexylphosphino-2′,4′,6′-triisopropyl-1,1′-biphenyl)[2-(2′-amino-1,1′-biphenyl)]palladium(II) (205 mg, 0.261 mmol), tripotassium phosphate hydrate (1.77 g, 7.69 mmol) and water (6 mL). The resulting mixture was stirred at 85 °C for 1 hour and the reaction mixture was added to water. The resulting solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (25% AcOEt/*n*-hexane) provided the title compound. (881 mg, 1.83 mmol, 69% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.39 (s, 1H), 8.08 (s, 1H), 5.28-5.22 (m, 1H), 4.07-4.02 (m, 1H), 3.68-3.58 (m, 1H), 3.99 (s, 3H), 2.52-2.40 (m, 1H), 2.33 (s, 3H), 2.14-2.05 (m, 1H), 1.95-1.88 (m, 1H), 1.79-1.70 (m, 1H), 1.69 (s, 9H), 1.68-1.50 (m, 3H), 0.98-0.84 (m, 2H), 0.82-0.75 (m, 2H).

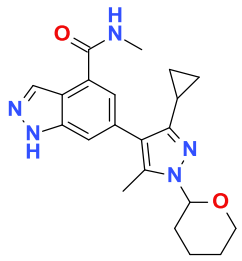
6-[3-cyclopropyl-5-methyl-1-(oxan-2-yl)-1*H*-pyrazol-4-yl]-1*H*-indazole-4-carboxylic acid (155)



To a solution of 1-*tert*-butyl 4-methyl 6-[3-cyclopropyl-5-methyl-1-(oxan-2-yl)-1*H*-pyrazol-4-yl]-1*H*-indazole-1,4-dicarboxylate (**154**, 872 mg, 1.81 mmol) in THF (25 mL) and MeOH (9 mL) in a round-bottom flask was added 1 mol/L aqueous sodium hydroxide solution (5.4 mL, 5.40 mmol) at room temperature. The resulting mixture was stirred at room temperature for 1.5 hours and 45 °C for 1.5 hours. The reaction mixture was quenched by 1 mol/L aqueous

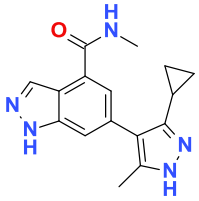
hydrochloric acid solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a pale yellow solid. (660 mg, 1.80 mmol, 99% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 13.40 (brs, 1H), 8.35 (s, 1H), 7.79 (s, 1H), 7.68 (s, 1H), 5.35-5.29 (m, 1H), 3.90-3.82 (m, 1H), 3.65-3.55 (m, 1H), 2.30-2.18 (m, 1H), 2.27 (s, 3H), 1.98-1.90 (m, 1H), 1.82-1.75 (m, 1H), 1.74-1.55 (m, 2H), 1.52-1.42 (m, 2H), 0.80-0.72 (m, 4H); HRMS (nega ESI) m/z 365.1614 (365.1614 calcd for $C_{20}H_{21}N_4O_3$ - H).

6-[3-cyclopropyl-5-methyl-1-(oxan-2-yl)-1*H*-pyrazol-4-yl]-*N*-methyl-1*H*-indazole-4-carboxamide (156)



To a solution of 6-[3-cyclopropyl-5-methyl-1-(oxan-2-yl)-1*H*-pyrazol-4-yl]-1*H*-indazole-4-carboxylic acid (**155**, 153 mg, 0.450 mmol) and methylamine hydrochloride (60.8 mg, 0.901 mmol) in THF (5 mL) and MeOH (5 mL) in a round-bottom flask were added 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (190 mg, 0.687 mmol) and *N*-methylmorpholine (0.170 mL, 1.50 mmol) at room temperature. The resulting mixture was stirred at room temperature for 1.5 hours. The reaction mixture was added to water and extracted with CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (4% MeOH/ CH_2Cl_2) provided the title compound (108 mg, 0.306 mmol, 68% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.18 (brs, 1H), 8.44 (q, *J* = 4.7 Hz, 1H), 8.34 (s, 1H), 7.55 (s, 1H), 7.50 (s, 1H), 5.35-5.30 (m, 1H), 3.89-3.82 (m, 1H), 3.65-3.56 (m, 1H), 2.79 (d, *J* = 4.7 Hz, 3H), 2.28-2.20 (m, 1H), 2.24 (s, 3H), 1.99-1.92 (m, 1H), 1.82-1.60 (m, 2H), 1.68-1.59 (m, 1H), 1.52-1.44 (m, 2H), 0.78-0.70 (m, 4H).

6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-*N*-methyl-1*H*-indazole-4-carboxamide (157)

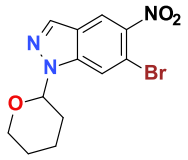


To a solution of 6-[3-cyclopropyl-5-methyl-1-(oxan-2-yl)-1*H*-pyrazol-4-yl]-*N*-methyl-1*H*-

indazole-4-carboxamide (**156**, 104 mg, 0.274 mmol) in 1,2-dichloroethane (3 mL) in a round-bottom flask was added 4 mol/L hydrogen chloride in 1,4-dioxane (3 mL, 12.0 mmol). The resulting mixture was stirred at 60 °C for 2 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (48.0 mg, 0.163 mmol, 59% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 13.18 (s, 1H), 12.32 (brs, 1H), 8.46 (q, *J* = 4.7 Hz, 1H), 8.38 (s, 1H), 7.62 (s, 1H), 7.59 (s, 1H), 2.86 (d, *J* = 4.7 Hz, 1H), 2.27 (brs, 3H), 1.92-1.83 (m, 1H), 0.87-0.74 (m, 4H); HRMS (positive ESI) m/z 296.1518 (296.1511 calcd for $C_{16}H_{18}N_5O$ + H).

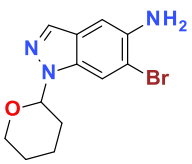
methyl [6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazol-5-yl]carbamate (163)

6-bromo-5-nitro-1-(oxan-2-yl)-1*H*-indazole (158)



To a solution of 6-bromo-5-nitro-1*H*-indazole (1.00 g, 4.14 mmol) in THF (20 mL) in a round-bottom flask were added 3,4-dihydro-2H-pyran (0.945 mL, 10.3 mmol) and *p*-toluenesulfonic acid monohydrate (171 mg, 0.899 mmol). The resulting mixture was stirred at 70 °C for 4 hours and quenched by saturated aqueous sodium hydrogen carbonate solution. The resulting solution was extracted with CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (20% AcOEt/*n*-hexane) provided the title compound. (1.28 g, 3.91 mmol, 95% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 8.10 (s,1H), 7.96 (s, 1H), 5.73-5.68 (m, 1H), 4.01-3.93 (m, 1H), 3.86-3.78 (m, 1H), 2.48-2.38 (m, 1H), 2.18-2.00 (m, 2H), 1.81-1.60 (m, 3H).

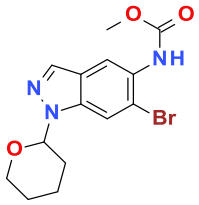
6-bromo-1-(oxan-2-yl)-1*H*-indazol-5-amine (159)



To a solution of 6-bromo-5-nitro-1-(oxan-2-yl)-1*H*-indazole (**158**, 584 mg, 1.79 mmol) in EtOH (10 mL) and water (10 mL) in a round-bottom flask were added ammonium chloride (107 mg, 2.00 mmol) and iron powder (422 mg, 7.56 mmol) at room temperature. The resulting mixture was stirred at 80 °C for 1 hour. The insoluble materials were filtrated off

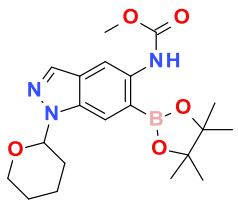
with Celite pad and the filtrate was extracted with CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (8% AcOEt/CH_2Cl_2) provided the title compound. (450 mg, 1.52 mmol, 85% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.74 (s,1H), 7.00 (s, 1H), 5.59-5.54 (m, 1H), 4.02-3.93 (m, 1H), 3.94 (brs, 2H), 3.73-3.64 (m, 1H), 2.40-2.30 (m, 1H), 2.15-2.00 (m, 2H), 1.79-1.59 (m, 3H).

methyl [6-bromo-1-(oxan-2-yl)-1*H*-indazol-5-yl]carbamate (160)



To a solution of 6-bromo-1-(oxan-2-yl)-1*H*-indazol-5-amine (**159**, 450 mg, 1.52 mmol) and pyridine (0.134 mL, 1.67 mmol) in CH_2Cl_2 (13.5 mL) in a round-bottom flask was added methyl chloroformate (0.129 mL, 1.67 mmol) at room temperature. The resulting mixture was stirred at room temperature for 1 hour. The reaction mixture was added to water and extracted with CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (6% AcOEt/CH_2Cl_2) provided the title compound. (447 mg, 1.26 mmol, 83% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (brs, 1H), 7.92 (s, 1H), 7.82 (s,1H), 7.06 (brs, 1H), 5.64-5.58 (m, 1H), 4.01-3.93 (m, 1H), 3.78 (s, 3H), 3.75-3.67 (m, 1H), 2.51-2.38 (m, 1H), 2.15-1.99 (m, 2H), 1.75-1.60 (m, 3H).

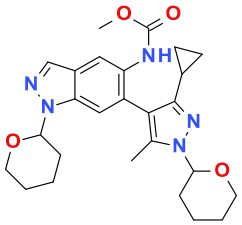
methyl [1-(oxan-2-yl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazol-5-yl]carbamate (161)



To a solution of methyl [6-bromo-1-(oxan-2-yl)-1*H*-indazol-5-yl]carbamate (**160**, 447 mg, 1.26 mmol) in 1,4-dioxane (15 mL) in a round-bottom flask were added bis(pinacolato)diboron (395 mg, 1.56 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (51.4 mg, 0.0629 mmol) and potassium acetate (390 mg, 3.97 mmol). The resulting mixture was stirred at 90 ^{o}C for 2 hours. The insoluble materials were filtrated off with Celite pad and the filtrate was concentrated under reduced pressure. Purification by flash chromatography on silica gel

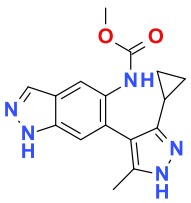
(6% AcOEt/CH_2Cl_2) provided the title compound. (323 mg, 0.805 mmol, 64% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 (brs, 1H), 8.46 (brs, 1H), 7.94 (s, 1H), 7.92 (s,1H), 5.74-5.69 (m, 1H), 4.02-3.95 (m, 1H), 3.79-3.71 (m, 1H), 3.75 (s, 3H), 2.63-2.51 (m, 1H), 2.18-2.08 (m, 1H), 2.04-1.96 (m, 1H), 1.78-1.68 (m, 2H), 1.65-1.56 (m, 1H), 1.37 (s, 12H).

methyl {6-[3-cyclopropyl-5-methyl-1-(oxan-2-yl)-1*H*-pyrazol-4-yl]-1-(oxan-2-yl)-1*H*-indazol-5-yl}carbamate (162)



To a solution of methyl [1-(oxan-2-yl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazol-5-yl]carbamate (**161**, 157 mg, 0.390 mmol) in 1,4-dioxane (7.5 mL) in a round-bottom flask were added 4-bromo-3-cyclopropyl-5-methyl-1-(oxan-2-yl)-1*H*-pyrazole (77.9 mg, 0.273 mmol), chloro(2-dicyclohexylphosphino-2',4',6'-triisopropyl-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II) (31.2 mg, 0.0397 mmol), tripotassium phosphate hydrate (266 mg, 1.16 mmol) and water (1.5 mL). The resulting mixture was stirred at 90 °C for 35 minutes and the reaction mixture was added water. The resulting solution was extracted with CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (50% to 66% AcOEt/ *n*-hexane linear gradient) provided the title compound (101 mg, 0.211 mmol, 54% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (brs, 1H), 7.97 (brs, 1H), 7.39 (dd, *J* = 20.7, 3.9 Hz, 1H), 6.64 (d, *J* = 20.7 Hz, 1H), 6.68-6.60 (m, 1H), 5.65-5.57 (m, 1H), 5.24-5.20 (m, 1H), 4.10-3.95 (m, 2H), 3.70 (d, *J* = 8.6 Hz, 3H), 3.68-3.60 (m, 1H),2.50-2.40 (m, 1H), 2.18-1.90 (m, 7H), 2.10 (brs, 3H), 1.75-1.50 (m, 4H), 1.68-1.57 (m, 1H), 0.88-0.82 (m, 2H), 0.72-0.65 (m, 2H).

methyl [6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazol-5-yl]carbamate (163)

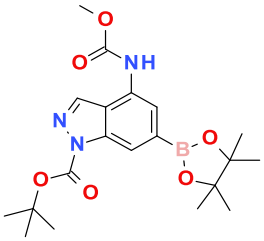


To a solution of methyl {6-[3-cyclopropyl-5-methyl-1-(oxan-2-yl)-1*H*-pyrazol-4-yl]-1-(oxan-2-yl)-1*H*-indazol-5-yl}carbamate (**162**, 92.4 mg, 0.193 mmol) in 1,2-dichloroethane (2.5 mL) in a round-bottom flask were added 4 mol/L hydrogen chloride in 1,4-dioxane (2.5 mL, 10.0 mmol). The resulting mixture was stirred at 60 °C for 2.5 hours, then concentrated

under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and extracted with CH_2Cl_2/MeOH (v/v = 20/1). The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by thin layer chromatography on silica gel (4% AcOEt/CH_2Cl_2) provided the title compound as a pale yellow solid. (43.3 mg, 0.139 mmol, 72% yield):^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.47 (brs, 1H), 8.05 (s, 1H), 7.31 (s, 1H), 6.59 (brs, 1H), 3.72 (s, 3H), 2.15 (s, 3H), 1.61-1.50 (m, 1H), 0.82-0.65 (m, 4H); HRMS (Positive ESI) m/z 312.1461 (312.1460 calcd for $C_{16}H_{18}N_5O_2$ + H).

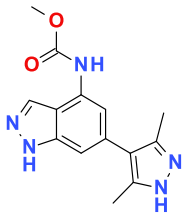
methyl [6-(3,5-dimethyl-1*H*-pyrazol-4-yl)-1*H*-indazol-4-yl]carbamate (165)

***tert*-butyl 4-[(methoxycarbonyl)amino]-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole-1-carboxylate (164)**



To a solution of *tert*-butyl 4-amino-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole-1-carboxylate (**141**, 1.00 g, 2.78 mmol) and pyridine (0.246 mL, 3.06 mmol) in CH_2Cl_2 (10 mL) in a round-bottom flask was added methyl chloroformate (0.237 mL, 3.06 mmol) at room temperature. The resulting mixture was stirred at room temperature for 30 minutes. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 20% AcOEt/CH_2Cl_2 linear gradient) provided the title compound. (980 mg, 2.30 mmol, 84% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (brs, 1H), 8.23 (s, 1H), 7.85 (brs, 1H), 6.87 (s, 1H), 3.84 (s, 3H), 1.73 (s, 9H), 1.35 (s, 12H).

methyl [6-(3,5-dimethyl-1*H*-pyrazol-4-yl)-1*H*-indazol-4-yl]carbamate (165)

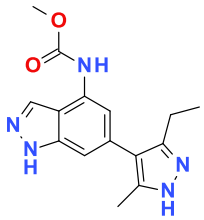


To a solution of *tert*-butyl 4-[(methoxycarbonyl)amino]-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole-1-carboxylate (**164**, 200 mg, 0.479 mmol) in 1,4-dioxane (5 mL) in a round-bottom flask were added *tert*-butyl 4-bromo-3,5-dimethyl-1*H*-pyrazole-1-carboxylate (158 mg, 0.575 mmol), chloro(2-dicyclohexylphosphino-2',4',6'-triisopropyl-

1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II) (37.7 mg, 0.0479 mmol), tripotassium phosphate hydrate (221 mg, 0.959 mmol) and water (1 mL). The resulting mixture was stirred for 30 minutes at 100 °C and the reaction mixture was added water. The resulting solution was concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 20% AcOEt/CH_2Cl_2 linear gradient) provided *tert*-butyl 6-[1-(*tert*-butoxycarbonyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]-4-[(methoxycarbonyl)amino]-1*H*-indazole-1-carboxylate (83.0 mg, 0.170 mmol, 36% yield).

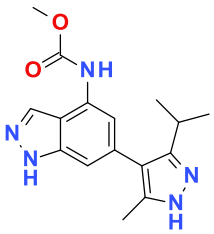
tert-butyl 6-[1-(*tert*-butoxycarbonyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]-4-[(methoxycarbonyl)amino]-1*H*-indazole-1-carboxylate (83.0 mg, 0.170 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (5.2 mL, 20.8 mmol). The resulting mixture was stirred at 60 °C for 2 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as an offwhite solid. (48.0 mg, 0.170 mmol, 98% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.90 (brs, 1H), 12.29 (brs, 1H), 9.89 (s, 1H), 8.29 (s, 1H), 7.45 (s, 1H), 6.97 (s, 1H), 3.69 (s, 3H), 2.19 (d, *J* = 23.1 Hz, 6H); LCMS m/z 286 $[M + H]^+$.

methyl [6-(3-ethyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazol-4-yl]carbamate (166)



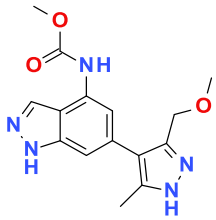
166 was prepared in a similar manner described for **165**. 55% yield : ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.90 (brs, 1H), 12.29 (brs, 1H), 9.89 (s, 1H), 8.29 (s, 1H), 7.43 (s, 1H), 6.94 (s, 1H), 3.69 (s, 3H), 2.65-2.52 (m, 2H), 2.17 (d, *J* = 22.7 Hz, 3H), 1.15-1.01 (m, 3H); LCMS m/z 300 $[M + H]^+$.

methyl {6-[5-methyl-3-(propan-2-yl)-1*H*-pyrazol-4-yl]-1*H*-indazol-4-yl}carbamate (167)



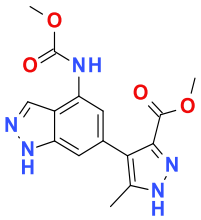
167 was prepared in a similar manner described for **165**. 98% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.95 (brs, 1H), 9.90 (s, 1H), 8.31 (s, 1H), 7.42 (s, 1H), 6.95 (s, 1H), 3.69 (s, 3H), 3.03 (quintet, *J* = 7.0 Hz, 1H), 2.14 (s, 3H), 1.15 (d, *J* = 7.0 Hz, 6H); LCMS m/z 314 $[M + H]^+$.

methyl {6-[3-(methoxymethyl)-5-methyl-1*H*-pyrazol-4-yl]-1*H*-indazol-4-yl}carbamate (168)



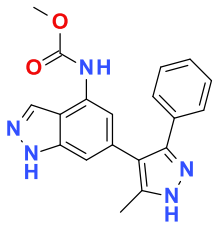
168 was prepared in a similar manner described for **165**. 20% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 13.03 (brs, 1H), 12.69 (brs, 1H), 9.94 (s, 1H), 8.35 (s, 1H), 7.55 (s, 1H), 7.22 (brs, 1H), 4.35 (brs, 2H), 3.76 (s, 3H), 3.28 (s, 3H), 2.33 (brs, 3H); LCMS m/z 316 $[M + H]^+$.

methyl 4-{4-[(methoxycarbonyl)amino]-1*H*-indazol-6-yl}-5-methyl-1*H*-pyrazole-3-carboxylate (169)



169 was prepared in a similar manner described for **165**. 34% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 13.02 (brs, 1H), 9.94 (s, 1H), 8.37 (s, 1H), 7.42 (s, 1H), 7.09 (s, 1H), 3.75 (s, 3H), 3.70 (brs, 3H), 2.24 (brs, 3H); LCMS m/z 330 $[M + H]^+$.

methyl [6-(5-methyl-3-phenyl-1*H*-pyrazol-4-yl)-1*H*-indazol-4-yl]carbamate (170)

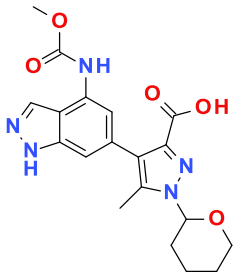


170 was prepared in a similar manner described for **165**. 69% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.93 (brs, 1H), 9.93 (s, 1H), 8.36 (s, 1H), 7.47-7.30 (m, 2H), 7.40 (s, 1H),

7.29-7.19 (m, 2H), 6.92 (s, 1H), 3.71 (s, 3H), 2.25 (s, 3H); LCMS m/z 348 $[M + H]^+$.

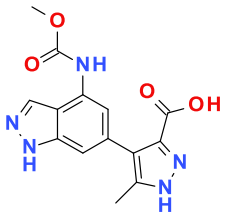
4-{4-[(methoxycarbonyl)amino]-1*H*-indazol-6-yl}-5-methyl-1*H*-pyrazole-3-carboxylic acid (172)

4-{4-[(methoxycarbonyl)amino]-1*H*-indazol-6-yl}-5-methyl-1-(oxan-2-yl)-1*H*-pyrazole-3-carboxylic acid (171)



To a solution of methyl 4-{4-[(methoxycarbonyl)amino]-1-(oxan-2-yl)-1*H*-indazol-6-yl}-5-methyl-1-(oxan-2-yl)-1*H*-pyrazole-3-carboxylate (720 mg, 1.40 mmol) in 1,4-dioxane (14 mL) in a round-bottom flask was added 1 mol/L aqueous sodium hydroxide solution (14 mL, 14.0 mmol) at room temperature. The resulting mixture was stirred at room temperature for 1 hour. The reaction mixture was quenched by 1 mol/L aqueous hydrochloric acid solution and concentrated under reduced pressure. Purification by flash chromatography on silica gel (5% to 10% MeOH/CH_2Cl_2 linear gradient) provided the title compound. (380 mg, 0.950 mmol, 68% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.91 (brs, 1H), 9.82 (s, 1H), 8.26 (s, 1H), 7.37 (s, 1H), 5.35-5.30 (m, 1H), 3.89-3.82 (m, 1H), 3.65-3.56 (m, 1H), 3.74 (s, 3H), 2.28-2.20 (m, 1H), 2.27 (s, 3H), 1.99-1.92 (m, 1H), 1.82-1.60 (m, 4H); LCMS m/z 400 $[M + H]^+$.

4-{4-[(methoxycarbonyl)amino]-1*H*-indazol-6-yl}-5-methyl-1*H*-pyrazole-3-carboxylic acid (172)

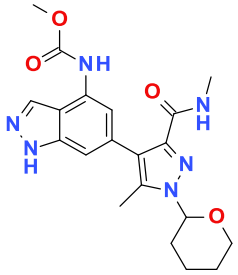


4-{4-[(methoxycarbonyl)amino]-1*H*-indazol-6-yl}-5-methyl-1-(oxan-2-yl)-1*H*-pyrazole-3-carboxylic acid (**171**, 100 mg, 0.250 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (7.7 mL, 30.8 mmol). The resulting mixture was stirred at 60 °C for 5 hours. The reaction mixture was quenched by 1 mol/L aqueous hydrochloric acid solution and concentrated under reduced pressure. Purification by flash chromatography on ODS-silica gel (50% acetonitrile/H_2O) provided the title compound. (27.0 mg, 0.0860 mmol, 34% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.82 (brs, 1H), 9.73 (s, 1H), 8.21 (s, 1H), 7.36 (s, 1H),

7.17 (s, 1H), 3.67 (s, 3H), 2.08 (brs, 3H); LCMS m/z 316 $[M + H]^+$.

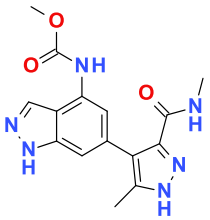
methyl {6-[5-methyl-3-(methylcarbamoyl)-1*H*-pyrazol-4-yl]-1*H*-indazol-4-yl}carbamate (174)

methyl {6-[5-methyl-3-(methylcarbamoyl)-1-(oxan-2-yl)-1*H*-pyrazol-4-yl]-1*H*-indazol-4-yl}carbamate (173)



To a solution of 4-{4-[(methoxycarbonyl)amino]-1*H*-indazol-6-yl}-5-methyl-1-(oxan-2-yl)-1*H*-pyrazole-3-carboxylic acid (**171**, 100 mg, 0.250 mmol) and methylamine hydrochloride (33.8 mg, 0.501 mmol) in THF (5 mL) and MeOH (5 mL) in a round-bottom flask were added 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (104 mg, 0.376 mmol) and *N*-methylmorpholine (0.083 mL, 0.751 mmol) at room temperature. The resulting mixture was stirred at room temperature for 3 hours. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 5% $MeOH/CH_2Cl_2$ linear gradient) provided the title compound. (80.0 mg, 0.190 mmol, 77% yield): ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.48 (brs, 1H), 7.01 (s, 1H), 6.97 (q, J = 5.1 Hz, 1H), 5.31-5.27 (m, 1H), 4.08-4.01 (m, 1H), 3.70-3.61 (m, 1H), 3.64 (s, 3H), 2.92 (d, J = 5.1 Hz, 3H), 2.52-2.42 (m, 1H), 2.19 (s, 3H), 2.16-2.07 (m, 1H), 1.99-1.92 (m, 1H), 1.72-1.65 (m, 1H), 1.63-1.55 (m, 2H); LCMS m/z 413 $[M + H]^+$.

methyl {6-[5-methyl-3-(methylcarbamoyl)-1*H*-pyrazol-4-yl]-1*H*-indazol-4-yl}carbamate (174)

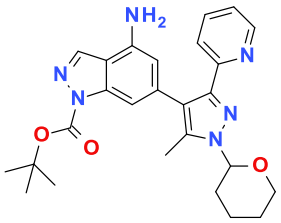


methyl {6-[5-methyl-3-(methylcarbamoyl)-1-(oxan-2-yl)-1*H*-pyrazol-4-yl]-1*H*-indazol-4-yl}carbamate (**173**, 80.0 mg, 0.190 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (5.9 mL, 23.6 mmol). The resulting mixture was stirred at 60 °C for 5 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with

water and dried under reduced pressure at 50 °C. The title compound was obtained as an offwhite solid. (44.0 mg, 0.130 mmol, 69% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.98 (brs, 1H), 9.81 (s, 1H), 8.26 (s, 1H), 7.96 (q, *J* = 4.7 Hz, 1H), 7.35 (s, 1H), 7.07 (s, 1H), 3.68 (s, 3H), 2.63 (d, *J* = 4.7 Hz, 3H), 2.19 (brs, 3H); LCMS m/z 329 $[M + H]^+$.

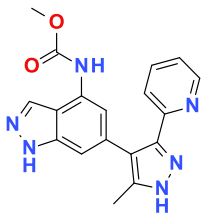
methyl {6-[5-methyl-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1*H*-indazol-4-yl}carbamate (176)

***tert*-butyl 4-amino-6-[5-methyl-1-(oxan-2-yl)-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1*H*-indazole-1-carboxylate (175)**



To a solution of *tert*-butyl 4-[(methoxycarbonyl)amino]-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole-1-carboxylate (**164**, 2.00 g, 4.14 mmol) in 1,4-dioxane (20 mL) in a round-bottom flask were added 2-[4-bromo-5-methyl-1-(oxan-2-yl)-1*H*-pyrazol-3-yl]pyridine (1.60 g, 4.97 mmol), chloro(2-dicyclohexylphosphino-2',4',6'-triisopropyl-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II) (326 mg, 0.414 mmol), tripotassium phosphate hydrate (1.91 g, 8.28 mmol) and water (4 mL). The resulting mixture was stirred at 100 °C for 1 hour and the reaction mixture was added to water. The resulting solution was concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (0% to 100% AcOEt/CH_2Cl_2 linear gradient) provided the title compound. (2.17 g, 4.57 mmol, 95% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57-8.54 (m, 1H), 8.09 (s, 1H), 7.51-7.45 (m, 1H), 7.37 (brs, 1H), 7.35-7.31 (m, 1H), 7.10-7.07 (m, 1H), 5.45-5.40 (m, 1H), 4.11-4.05 (m, 1H), 4.06 (brs, 2H), 3.70-3.61 (m, 1H), 2.52-2.42 (m, 1H), 2.38 (s, 3H), 2.16-2.07 (m, 1H), 1.99-1.92 (m, 1H), 1.72-1.65 (m, 1H), 1.63-1.55 (m, 2H), 1.60 (s, 9H); LCMS m/z 475 $[M + H]^+$.

methyl {6-[5-methyl-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1*H*-indazol-4-yl}carbamate (176)



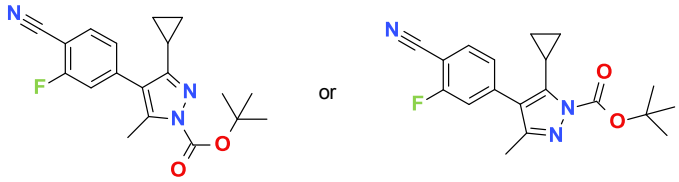
To a solution of *tert*-butyl 4-amino-6-[5-methyl-1-(oxan-2-yl)-3-(pyridin-2-yl)-1*H*-pyrazol-

4-yl]-1*H*-indazole-1-carboxylate (**175**, 400 mg, 0.759 mmol) and pyridine (0.0733 mL, 0.910 mmol) in CH_2Cl_2 (10 mL) in a round-bottom flask was added methyl chloroformate (0.0703 mL, 0.910 mmol) at room temperature. The resulting mixture was stirred at room temperature for 1 hour. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (0% to 30% AcOEt/CH_2Cl_2 linear gradient) provided *tert*-butyl 4-[(methoxycarbonyl)amino]-6-[5-methyl-1-(oxan-2-yl)-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1*H*-indazole-1-carboxylate (288 mg, 0.541 mmol, 71% yield).

tert-Butyl 4-[(methoxycarbonyl)amino]-6-[5-methyl-1-(oxan-2-yl)-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1*H*-indazole-1-carboxylate (288 mg, 0.541 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (14.9 mL, 59.6 mmol). The resulting mixture was stirred at 60 °C for 2 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as an offwhite solid. (132 mg, 0.379 mmol, 70% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 13.11 (brs, 1H), 12.82 (s, 1H), 9.93 (s, 1H), 8.57-8.54 (m, 1H), 8.09 (s, 1H), 7.51-7.45 (m, 1H), 7.37 (brs, 1H), 7.35-7.31 (m, 1H), 7.10-7.07 (m, 1H), 3.65 (s, 3H), 2.23 (brs, 3H); LCMS m/z 349 $[M + H]^+$.

<u>*N*-[6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1,2-benzoxazol-3-yl]cyclopropanecarboxamide (181)</u>

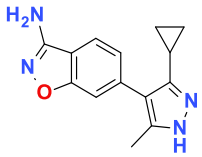
***tert*-butyl 4-(4-cyano-3-fluorophenyl)-3-cyclopropyl-5-methyl-1*H*-pyrazole-1-carboxylate (or *tert*-butyl 4-(4-cyano-3-fluorophenyl)-5-cyclopropyl-3-methyl-1*H*-pyrazole-1-carboxylate) (177)**



To a solution of *tert*-butyl 4-bromo-3-cyclopropyl-5-methyl-1*H*-pyrazole-1-carboxylate (or *tert*-butyl 4-bromo-5-cyclopropyl-3-methyl-1*H*-pyrazole-1-carboxylate) (16.6 g, 55.0 mmol) in 1,2-dimethoxyethane (200 mL) in a round-bottom flask were added 4-cyano-3-fluorophenylboronic acid (8.25 g, 50.0 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (2.04 g, 2.50 mmol), tripotassium phosphate hydrate (31.8 g, 150 mmol) and water (50 mL). The resulting mixture was stirred for 3 hours at reflux and the reaction mixture was added to water. The resulting solution was extracted with AcOEt. The organic extracts were combined, dried over

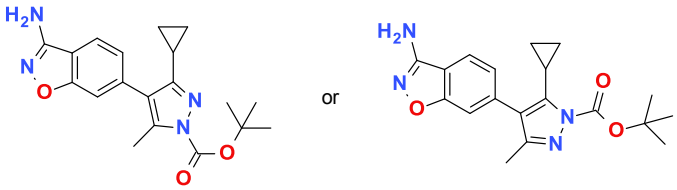
anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 70% AcOEt/*n*-hexane linear gradient) provided the title compound. (8.53 g, 25.0 mmol, 50% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70-7.62 (m, 1H), 7.27-7.17 (m, 2H), 2.43 (s, 3 H), 1.66-1.62 (m, 1H), 1.62 (s, 9H), 1.06-0.99 (m, 2H), 0.89-0.82 (m, 2H).

6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1,2-benzisoxazol-3-amine (178)



To a solution of acetohydroxamic acid (516 mg, 6.88 mmol) in DMF (10 mL) in a round-bottom flask was added potassium *tert*-butoxide (772 mg, 6.88 mmol). The resulting mixture was stirred at room temperature for 30 minutes and added to a solution of *tert*-butyl 4-(4-cyano-3-fluorophenyl)-3-cyclopropyl-5-methyl-1*H*-pyrazole-1-carboxylate (or *tert*-butyl 4-(4-cyano-3-fluorophenyl)-5-cyclopropyl-3-methyl-1*H*-pyrazole-1-carboxylate) (**177**, 940 mg, 2.75 mmol) in DMF (5 mL) at room temperature. The resulting mixture was stirred at 50 °C for 3 hours and the reaction mixture was added to water. The resulting solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (50% to 100% AcOEt/*n*-hexane linear gradient) provided the title compound. (421 mg, 1.66 mmol, 60% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, J = 8.2 Hz, 1H), 7.45 (s, 1H), 7.32 (d, J = 8.2 Hz, 1H), 4.41 (brs, 2H), 2.34 (s, 3 H), 1.93-1.84 (m, 1H), 0.96-0.89 (m, 2H), 0.88-0.82 (m, 2H) ; LCMS m/z 255 $[M + H]^+$.

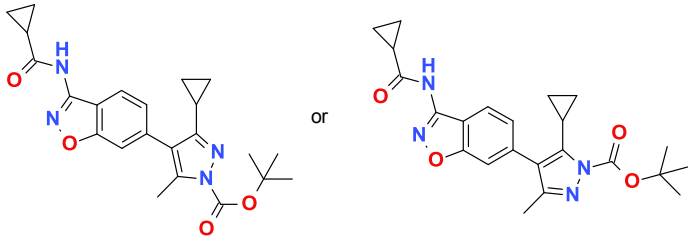
***tert*-butyl 4-(3-amino-1,2-benzisoxazol-6-yl)-3-cyclopropyl-5-methyl-1*H*-pyrazole-1-carboxylate (or *tert*-butyl 4-(3-amino-1,2-benzisoxazol-6-yl)-5-cyclopropyl-3-methyl-1*H*-pyrazole-1-carboxylate) (179)**



To a solution of 6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1,2-benzisoxazol-3-amine (**178**, 3.23 g, 12.7 mmol) in THF (100 mL) in a round-bottom flask were added di-*tert*-butyl dicarbonate (3.05 g, 14.0 mmol), triethylamine (5.28 mL, 38.1 mmol) and 4-dimethylaminopyridine (155 mg, 1.27 mmol). The resulting mixture was stirred at room temperature for 17 hours, then concentrated under reduced pressure. Purification by flash

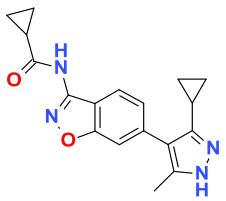
chromatography on silica gel (0% to 50% AcOEt/*n*-hexane linear gradient) provided the title compound. (2.40 g, 6.77 mmol, 53% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 8.5 Hz, 1H), 7.40 (s, 1H), 7.24 (d, *J* = 8.5 Hz, 1H), 4.41 (brs, 2H), 2.45 (s, 3 H), 1.76-1.68 (m, 1H), 1.65 (s, 9H), 1.07-1.03 (m, 2H), 0.90-0.82 (m, 2H) ; LCMS m/z 355 $[M + H]^+$.

tert-butyl 3-cyclopropyl-4-{3-[(cyclopropylcarbonyl)amino]-1,2-benzisoxazol-6-yl}-5-methyl-1*H*-pyrazole-1-carboxylate (or _tert_-butyl 5-cyclopropyl-4-{3-[(cyclopropylcarbonyl)amino]-1,2-benzisoxazol-6-yl}-3-methyl-1*H*-pyrazole-1-carboxylate) (180)



To a solution of *tert*-butyl 4-(3-amino-1,2-benzisoxazol-6-yl)-3-cyclopropyl-5-methyl-1*H*-pyrazole-1-carboxylate (or *tert*-butyl 4-(3-amino-1,2-benzisoxazol-6-yl)-5-cyclopropyl-3-methyl-1*H*-pyrazole-1-carboxylate) (**179**, 177 mg, 0.500 mmol) in CH_2Cl_2 (15 mL) in a round-bottom flask were added cyclopropanecarbonyl chloride (57.5 mg, 0.550 mmol) and pyridine (0.121 mL, 1.50 mmol). The resulting mixture was stirred at room temperature for 2.5 hours, then concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 50% AcOEt/*n*-hexane linear gradient) provided the title compound. (140 mg, 0.331 mmol, 66% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (brs, 1H), 8.24 (d, *J* = 8.6 Hz, 1H), 7.47 (s, 1H), 7.27 (d, *J* = 8.6 Hz, 1H), 2.46 (s, 3 H), 1.83-1.73 (m, 1H), 1.73-1.68 (m, 1H), 1.65 (s, 9H), 1.25-1.19 (m, 2H), 1.09-0.98 (m, 4H), 0.89-0.82 (m, 2H).

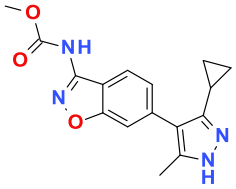
_N_-[6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1,2-benzisoxazol-3-yl]cyclopropanecarboxamide (181)



tert-Butyl 3-cyclopropyl-4-{3-[(cyclopropylcarbonyl)amino]-1,2-benzisoxazol-6-yl}-5-methyl-1*H*-pyrazole-1-carboxylate (or *tert*-butyl 5-cyclopropyl-4-{3-[(cyclopropylcarbonyl)amino]-1,2-benzisoxazol-6-yl}-3-methyl-1*H*-pyrazole-1-carboxylate) (**180**, 140 mg, 0.331 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (5 mL, 20.0 mmol). The resulting mixture was stirred at 50 °C for 17 hours, then

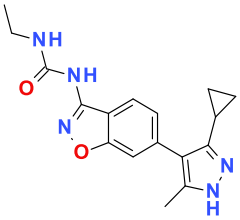
concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (103 mg, 0.320 mmol, 96% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 11.42 (s, 1H), 8.07 (d, *J* = 7.9 Hz, 1H), 7.64 (s, 1H), 7.40 (d, *J* = 7.9 Hz, 1H), 2.27 (s, 3H), 2.08-2.00 (m, 1H), 1.94-1.86 (m, 1H), 0.94-0.78 (m, 8H); LCMS m/z 323 $[M + H]^+$; HRMS (Positive ESI) m/z 323.1512 (323.1430 calcd for $C_{18}H_{18}N_4O_2$ + H).

methyl [6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1,2-benzoxazol-3-yl]carbamate (182)



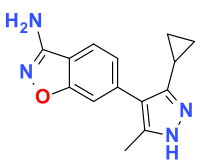
182 was prepared in a similar manner described for **181**. 81% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.31 (brs, 1H), 8.02 (d, *J* = 7.9 Hz, 1H), 7.57 (s, 1H), 7.38 (d, *J* = 7.9 Hz, 1H), 3.79 (s, 3H), 2.24 (s, 3H), 1.90-1.80 (m, 1H), 0.88-0.80 (m, 2H), 0.78-0.73 (m, 2H); LCMS m/z 313 $[M + H]^+$.

***N*-[6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1,2-benzoxazol-3-yl]-*N*'-ethylurea (183)**



183 was prepared in a similar manner described for **181**. 92% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.38 (brs, 1H), 10.11 (s, 1H), 8.17 (d, *J* = 8.6 Hz, 1H), 7.57 (s, 1H), 7.40 (d, *J* = 8.6 Hz, 1H), 7.36 (t, *J* = 5.5 Hz, 1H), 3.00-2.92 (m, 2H), 2.25 (brs, 3H), 1.90-1.80 (m, 1H), 1.13 (t, *J* = 7.3 Hz, 3H), 0.89-0.80 (m, 2H), 0.79-0.73 (m, 2H); LCMS m/z 326 $[M + H]^+$.

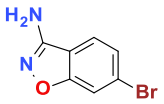
6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1,2-benzoxazol-3-amine (184)



tert-Butyl 4-(3-amino-1,2-benzisoxazol-6-yl)-3-cyclopropyl-5-methyl-1*H*-pyrazole-1-carboxylate (or *tert*-butyl 4-(3-amino-1,2-benzisoxazol-6-yl)-5-cyclopropyl-3-methyl-1*H*-pyrazole-1-carboxylate) (**179**, 230 mg, 0.649 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (5 mL, 20.0 mmol). The resulting mixture was stirred at 50 ºC for 4.5 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (136 mg, 0.535 mmol, 82% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.83 (d, *J* = 8.5 Hz, 1H), 7.39 (s, 1H), 7.28 (d, *J* = 8.5 Hz, 1H), 6.39 (brs, 2H), 2.23 (s, 3H), 1.90-1.80 (m, 1H), 0.88-0.80 (m, 2H), 0.78-0.73 (m, 2H); LCMS m/z 255 $[M + H]^+$.

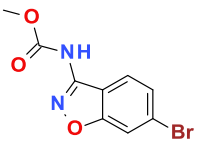
<u>methyl [6-(3,5-dimethyl-1*H*-pyrazol-4-yl)-1,2-benzoxazol-3-yl]carbamate (188)</u>

6-bromo-1,2-benzoxazol-3-amine (185)



To a solution of acetohydroxamic acid (11.3 g, 150 mmol) in DMF (150 mL) in a round-bottom flask was added potassium *tert*-butoxide (16.8 g, 150 mmol) at room temperature. The resulting mixture was stirred at room temperature for 40 minutes, then added 4-bromo-2-fluorobenzonitrile (15.0 g, 75.0 mmol) and stirred at room temperature for 3.5 hours. The reaction mixture was added to water and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting solid was collected. The solid was washed with hexane/CH_2Cl_2 (v/v = 20/1) and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (12.3 g, 57.7 mmol, 77% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 1.2 Hz, 1H), 7.39 (s, 1H), 7.26 (d, *J* = 1.2 Hz, 1H), 4.38 (brs, 2H); LCMS m/z 213 $[M + H]^+$.

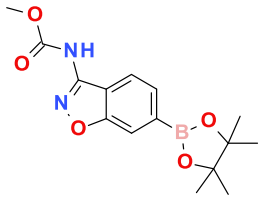
methyl (6-bromo-1,2-benzoxazol-3-yl)carbamate (186)



To a solution of 6-bromo-1,2-benzoxazol-3-amine (**185**, 4.06 g, 19.1 mmol) and pyridine (7.67 mL, 95.3 mmol) in CH_2Cl_2 (100 mL) in a round-bottom flask was added methyl chloroformate (3.09 mL, 40.0 mmol) at room temperature. The resulting mixture was stirred at room temperature for 18 hours. The reaction mixture was washed with 0.5 mol/L aqueous hydrogen chloride solution and brine. The organic layer was dried over anhydrous sodium

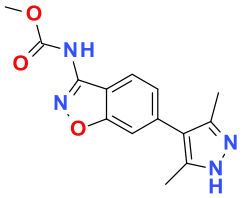
sulfate and concentrated under reduced pressure. The resulting solid was collected. The solid was washed with ether and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (4.86 g, 17.9 mmol, 94% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J* = 8.6 Hz, 1H), 7.82 (brs, 1H), 7.71 (s, 1H), 7.43 (d, *J* = 8.6 Hz, 1H), 3.89 (s, 3H).

methyl [6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-benzoxazol-3-yl]carbamate (187)



To a solution of methyl (6-bromo-1,2-benzoxazol-3-yl)carbamate (**186**, 4.86 g, 17.9 mmol) in 1,4-dioxane (60 mL) in a round-bottom flask were added bis(pinacolato)diboron (5.03 g, 19.8 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (790 mg, 0.967 mmol) and potassium acetate (5.29 g, 53.9 mmol). The resulting mixture was stirred at 100 °C for 4 hours. The insoluble materials were filtrated off with Celite pad and the filtrate was concentrated under reduced pressure. Purification by flash chromatography on silica gel (15% to 50% AcOEt/*n*-hexane linear gradient) provided the title compound. (3.61 g, 11.3 mmol, 63% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, *J* = 7.9 Hz, 1H), 7.95 (s, 1H), 7.70 (d, *J* = 7.9 Hz, 1H), 7.49 (brs, 1H), 3.88 (s, 3H), 1.38 (s, 12H).

methyl [6-(3,5-dimethyl-1*H*-pyrazol-4-yl)-1,2-benzoxazol-3-yl]carbamate (188)

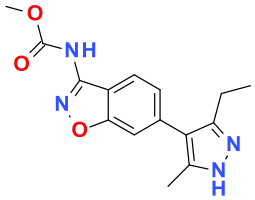


To a solution of methyl [6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-benzoxazol-3-yl]carbamate (**187**, 190 mg, 0.597 mmol) in 1,2-dimethoxyethane (15 mL) in a round-bottom flask were added *tert*-butyl 4-bromo-3,5-dimethyl-1*H*-pyrazole-1-carboxylate (246 mg, 0.896 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (24.4 mg, 0.0299 mmol), tripotassium phosphate hydrate (380 mg, 1.78 mmol) and water (4 mL). The resulting mixture was stirred for 2 hours at reflux. The resulting solution was added to AcOEt and washed with saturated aqueous ammonium chloride solution. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 10% AcOEt/*n*-hexane linear gradient) provided *tert*-butyl 4-{3-[(methoxycarbonyl)amino]-1,2-benzoxazol-6-yl}-

3,5-dimethyl-1*H*-pyrazole-1-carboxylate (20.0 mg, 0.0518 mmol, 8.7% yield).

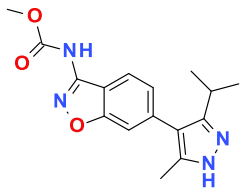
tert-Butyl 4-{3-[(methoxycarbonyl)amino]-1,2-benzoxazol-6-yl}-3,5-dimethyl-1*H*-pyrazole-1-carboxylate (20.0 mg, 0.0518 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (5 mL, 20.0 mmol). The resulting mixture was stirred at 50 ºC for 2.5 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (11.9 mg, 0.0416 mmol, 80% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.87 (s, 1H), 8.07 (d, *J* = 8.5 Hz, 1H), 7.60 (s, 1H), 7.33 (d, *J* = 8.5 Hz, 1H), 3.77 (s, 3H), 2.29 (s, 6H); LCMS m/z 267 $[M + H]^+$.

methyl [6-(3-ethyl-5-methyl-1*H*-pyrazol-4-yl)-1,2-benzoxazol-3-yl]carbamate (189)



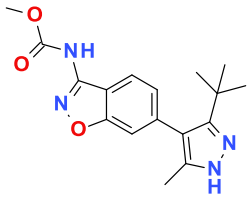
189 was prepared in a similar manner described for **188**. 93% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.44 (brs, 1H), 10.83 (s, 1H), 7.99 (d, *J* = 5.6 Hz, 1H), 7.46 (s, 1H), 7.24 (d, *J* = 5.6 Hz, 1H), 3.73 (s, 3H), 2.61 (q, *J* = 7.8 Hz, 1H), 2.19 (brs, 3H), 1.07 (t, *J* = 7.8 Hz, 3H); LCMS m/z 301 $[M + H]^+$.

methyl {6-[5-methyl-3-(propan-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (190)



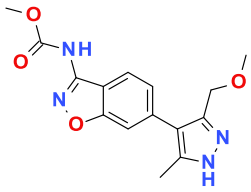
190 was prepared in a similar manner described for **188**. 99% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.84 (s, 1H), 8.04 (d, *J* = 8.5 Hz, 1H), 7.49 (s, 1H), 7.25 (d, *J* = 8.5 Hz, 1H), 3.77 (s, 3H), 3.10-2.98 (m, 1H), 2.18 (s, 3H), 1.17 (d, *J* = 7.4 Hz, 6H); LCMS m/z 315 $[M + H]^+$.

methyl [6-(3-*tert*-butyl-5-methyl-1*H*-pyrazol-4-yl)-1,2-benzoxazol-3-yl]carbamate (191)



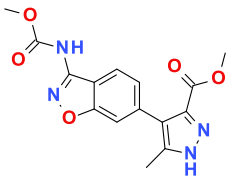
191 was prepared in a similar manner described for **188**. 79% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.26 (brs, 1H), 10.88 (brs, 1H), 7.99 (d, *J* = 7.9 Hz, 1H), 7.45 (s, 1H), 7.18 (d, *J* = 7.9 Hz, 1H), 3.75 (s, 3H), 1.92 (brs, 3H), 1.13 (s, 9H); LCMS m/z 329 $[M + H]^+$.

methyl {6-[3-(methoxymethyl)-5-methyl-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (192)



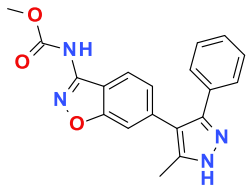
192 was prepared in a similar manner described for **188**. 85% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.82 (brs, 1H), 10.85 (brs, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.60 (s, 1H), 7.37 (d, *J* = 8.0 Hz, 1H), 4.34 (s, 2H), 3.74 (s, 3H), 3.26 (s, 3H), 2.32 (s, 3H); LCMS m/z 317 $[M + H]^+$.

methyl 4-{3-[(methoxycarbonyl)amino]-1,2-benzoxazol-6-yl}-5-methyl-1*H*-pyrazole-3-carboxylate (193)



193 was prepared in a similar manner described for **188**. 98% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.96 (d, *J* = 7.9 Hz, 1H), 7.53 (s, 1H), 7.23 (d, *J* = 7.9 Hz, 1H), 3.72 (s, 3H), 3.68 (s, 3H), 2.27 (s, 3H); LCMS m/z 331 $[M + H]^+$.

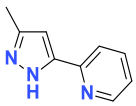
methyl [6-(5-methyl-3-phenyl-1*H*-pyrazol-4-yl)-1,2-benzoxazol-3-yl]carbamate (194)



194 was prepared in a similar manner described for **188**. 97% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.85 (s, 1H), 7.96 (d, *J* = 8.5 Hz, 1H), 7.47 (s, 1H), 7.33-7.26 (m, 5H), 7.10 (d, *J* = 8.5 Hz, 1H), 3.76 (s, 3H), 2.26 (brs, 3H); LCMS m/z 349 $[M + H]^+$.

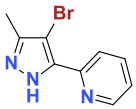
methyl {6-[5-methyl-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (195, DS79182026)

2-(5-methyl-1*H*-pyrazol-3-yl)pyridine



To a solution of methyl pyridine-2-carboxylate (21.4 g, 182 mmol) and acetone (26.8 mL, 365 mmol) in THF (250 mL) in a round-bottom flask was added 28% sodium methoxide methanol solution (71.8 mL, 365 mmol). The resulting mixture was stirred at room temperature for 3.5 hours and the reaction mixture was quenched by addition of equimolar 5 mol/L hydrochloric acid. The resulting was solution was extracted with CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was dissolved in EtOH (260 mL) and added hydrazine hydrate (10.6 mL, 219 mmol). The resulting mixture stirred at reflux for 19 hours, then concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (30% to 100% AcOEt/*n*-hexane linear gradient) provided the title compound. (27.9 g, 175 mmol, 96% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.75 (brs, 1H), 8.60 (d, *J* = 4.8 Hz, 1H), 7.77-7.67 (m, 2H), 7.21 (d, *J* = 6.8 Hz, 1H), 6.57 (s, 1H), 2.37 (s, 3H) ; LCMS m/z 160 $[M + H]^+$.

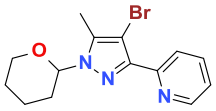
2-(4-bromo-5-methyl-1*H*-pyrazol-3-yl)pyridine



To a solution of 2-(5-methyl-1*H*-pyrazol-3-yl)pyridine (27.9 g, 175 mmol) in carbon tetrachloride (300 mL) and CH_2Cl_2 (300 mL) in a round-bottom flask was added *N*-bromosuccinimide (31.2 g, 175 mmol). The resulting mixture was stirred at room temperature for 21 hours and the reaction mixture was added to water. The resulting solution was extracted

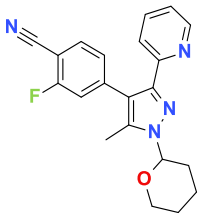
with CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting solid was washed with hexane/AcOEt (v/v =10/1) and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (37.5 g, 158 mmol, 90% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 3.1 Hz, 1H), 8.28 (d, *J* = 7.8 Hz, 1H), 7.80 (t, *J* = 7.8 Hz, 1H), 7.28 (t, *J* = 7.8 Hz, 1H), 2.34 (s, 3H).

2-[4-bromo-5-methyl-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-3-yl]pyridine



To a solution of 2-(4-bromo-5-methyl-1*H*-pyrazol-3-yl)pyridine (17.9 g, 75.0 mmol) in THF (500 mL) in a round-bottom flask were added 3,4-dihydro-2H-pyran (17.1 mL, 188 mmol) and *p*-toluenesulfonic acid monohydrate (2.85 g, 15.0 mmol). The resulting mixture was stirred at 60 °C for 19 hours and quenched by saturated aqueous sodium hydrogen carbonate solution. The resulting solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 50% AcOEt/*n*-hexane linear gradient) provided the title compound. (16.2 g, 50.3 mmol, 67% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (d, *J* = 4.8 Hz, 1H), 7.96 (d, *J* = 7.9 Hz, 1H), 7.73 (dt, *J* = 1.9, 7.9 Hz, 1H), 7.24 (dt, J = 1.9, 4.8 Hz, 1H), 5.43-5.38 (m, 1H), 4.09-4.03 (m, 1H), 3.76-3.63 (m, 1H), 2.58-2.43 (m, 1H), 2.42 (s, 3H), 2.17-2.10 (m, 1H), 2.08-1.98 (m, 1H), 1.78-1.66 (m, 2H), 1.66-1.61 (m, 1H) ; LCMS m/z 322 $[M + H]^+$.

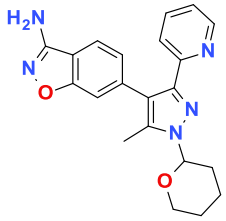
2-fluoro-4-[5-methyl-3-(pyridin-2-yl)-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-4-yl]benzonitrile (211)



To a solution of 2-[4-bromo-5-methyl-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-3-yl]pyridine (6.44 g, 20.0 mmol) in 1,2-dimethoxyethane (150 mL) in a round-bottom flask were added 4-cyano-3-fluorophenylboronic acid (3.63 g, 22.0 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (817 mg, 1.00 mmol), tripotassium phosphate hydrate (12.7 g, 60.0 mmol), and water (60 mL). The resulting mixture was stirred for 3 hours at reflux and the reaction mixture was added to water. The resulting solution was extracted with AcOEt. The organic extracts were combined, dried over

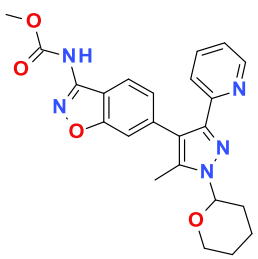
anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 50% AcOEt/*n*-hexane linear gradient) provided the title compound. (7.01 g, 19.3 mmol, 97% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, *J* = 4.3 Hz, 1H), 7.70-7.64 (m, 3H), 7.55 (t, *J* = 7.3 Hz, 1H), 7.17 (t, *J* = 7.3 Hz, 1H), 7.10 (d, *J* = 9.2Hz, 1H), 5.45-5.40 (m, 1H), 4.13-4.03 (m, 1H), 3.76-3.63 (m, 1H), 2.66-2.50 (m, 1H), 2.37 (s, 3H), 2.23-2.13 (m, 1H), 2.12-1.98 (m, 1H), 1.81-1.68 (m, 2H), 1.67-1.60 (m, 1H) ; LCMS m/z 363 $[M + H]^+$.

6-[5-methyl-3-(pyridin-2-yl)-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzisoxazol-3-amine (212)



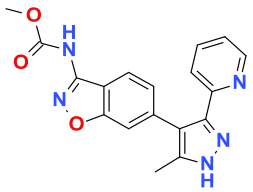
To a solution of acetohydroxamic acid (7.26 g, 96.7 mmol) in DMF (150 mL) in a round-bottom flask was added potassium *tert*-butoxide (10.9 g, 96.7 mmol). The resulting mixture was stirred at room temperature for 1 hour and a solution of 2-fluoro-4-[5-methyl-3-(pyridin-2-yl)-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-4-yl]benzonitrile (**211**, 7.01 g, 19.3 mmol) in DMF (50 mL) was added at room temperature. The resulting mixture was stirred at 50 °C for 16 hours and the reaction mixture was added water. The resulting solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (30% to 100% AcOEt/*n*-hexane linear gradient) provided the title compound. (2.65 g, 7.06 mmol, 37% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (dd, *J* = 1.2, 4.3 Hz, 1H), 7.55 (dt, *J* = 1.8, 7.3 Hz, 1H), 7.45 (dd, *J* = 1.8, 7.3 Hz, 1H), 7.44 (d, *J* = 8.6 Hz, 1H), 7.30 (s, 1H), 7.12 (dt, *J* = 1.2, 7.3 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 1H), 5.48-5.43 (m, 1H), 4.37 (brs, 2H), 4.14-4.09 (m, 1H), 3.76-3.67 (m, 1H), 2.70-2.57 (m, 1H), 2.38 (s, 3H), 2.21-2.07 (m, 2H), 1.80-1.69 (m, 2H), 1.67-1.59 (m, 1H) ; LCMS m/z 376 $[M + H]^+$.

methyl *N*-{6-[5-methyl-3-(pyridin-2-yl)-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzisoxazol-3-yl}carbamate (213)

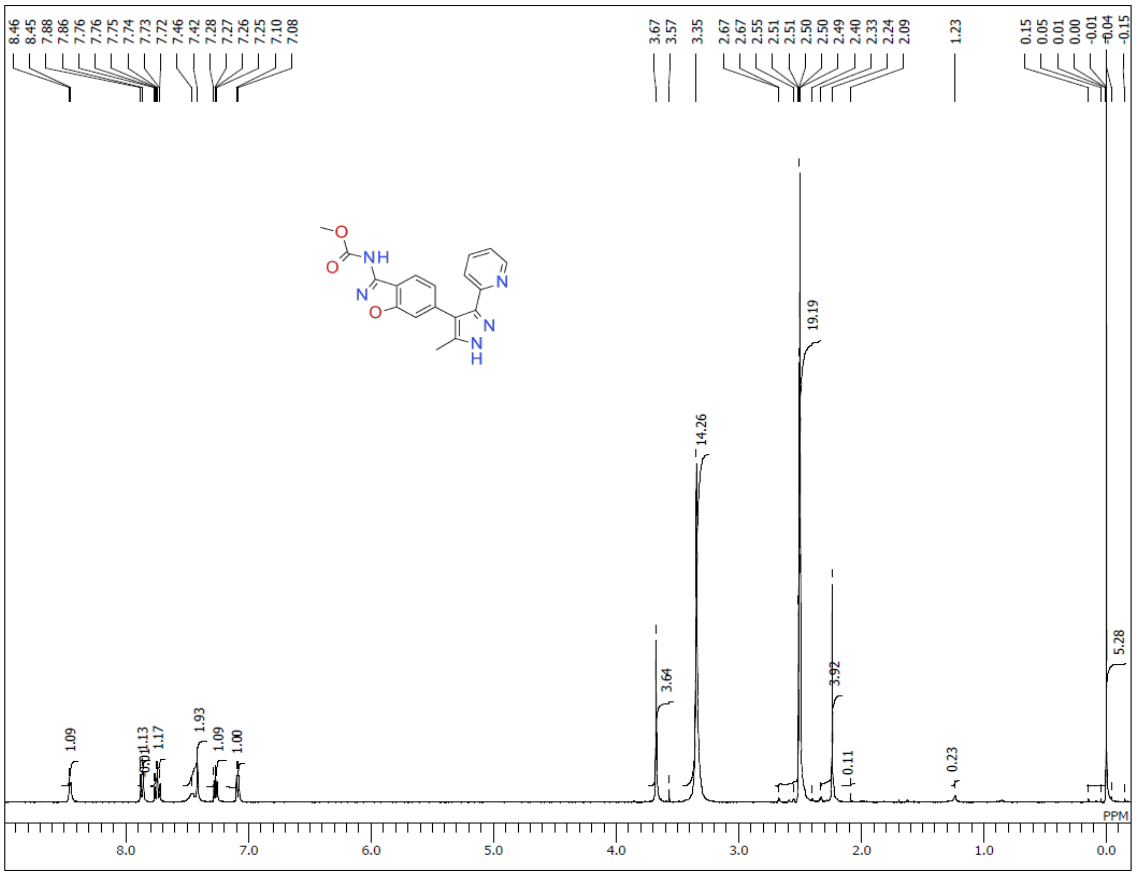


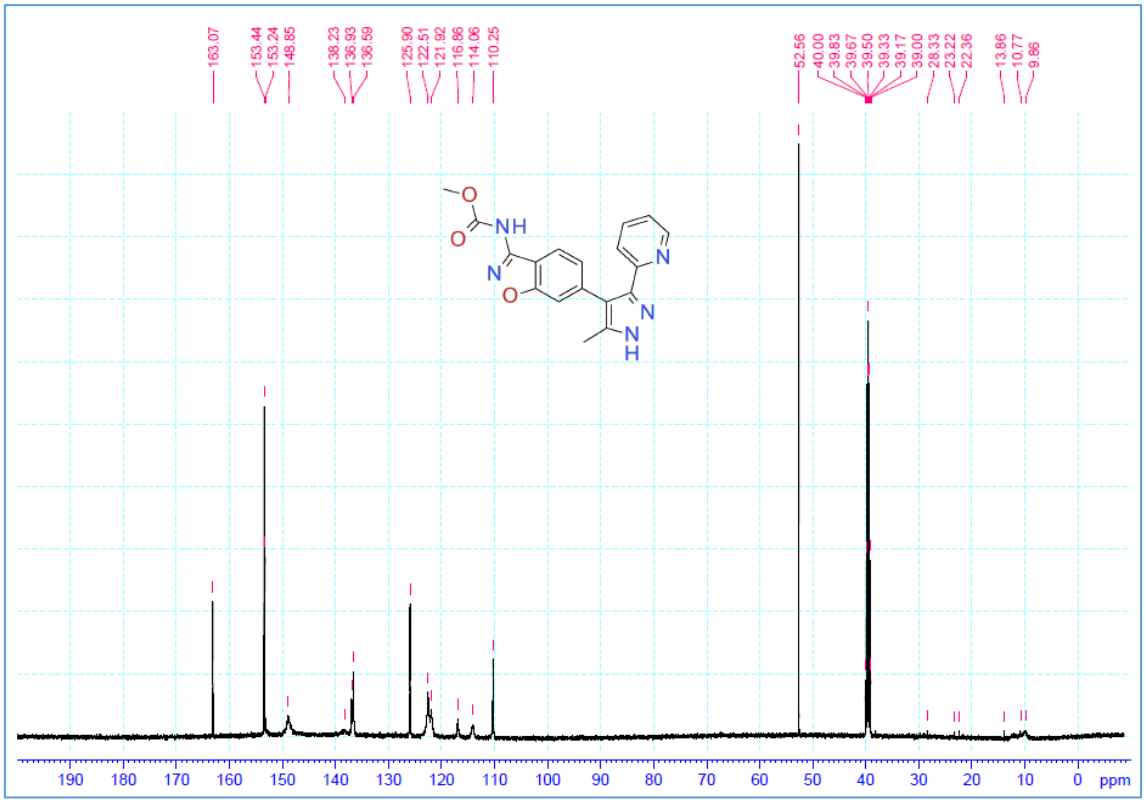
To a solution of 6-[5-methyl-3-(pyridin-2-yl)-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzisoxazol-3-amine (**212**, 2.63 g, 7.00 mmol) in CH_2Cl_2 (150 mL) in a round-bottom flask was added methyl chloroformate (1.08 mL, 14.0 mmol) and pyridine (1.69 mL, 21.0 mmol). The resulting mixture was stirred at room temperature for 1 hour, then concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (0% to 100% AcOEt/*n*-hexane linear gradient) provided the title compound. (2.95 g, 6.81 mmol, 97% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, *J* = 3.2 Hz, 1H), 8.06 (d, *J* = 8.6 Hz, 1H), 7.56 (dt, *J* = 8.0, 1.8 Hz, 1H), 7.51 (d, *J* = 7.5 Hz, 1H), 7.49 (d, *J* = 7.9 Hz, 1H), 7.36 (s, 1H), 7.14 (d, *J* = 6.8 Hz, 1H), 7.12 (d, *J* = 7.5 Hz, 1H), 5.48-5.43 (m, 1H), 4.16-4.08 (m, 1H), 3.87 (s, 3H), 3.75-3.67 (m, 1H), 2.70-2.55 (m, 1H), 2.39 (s, 3H), 2.22-2.10 (m, 2H), 1.83-1.68 (m, 2H), 1.67-1.58 (m, 1H); LCMS m/z 434 $[M + H]^+$.

methyl *N*-{6-[5-methyl-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzisoxazol-3-yl}carbamate (195, DS79182026)

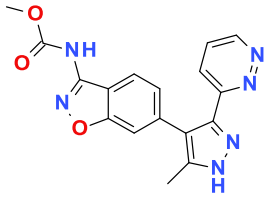


Methyl *N*-{6-[5-methyl-3-(pyridin-2-yl)-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzisoxazol-3-yl}carbamate (213, 2.95 g, 6.81 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (50 mL, 200 mmol). The resulting mixture was stirred at 50 °C for 1 hour, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The crude solid was washed with diisopropyl ether/EtOH(v/v =20/1) and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (2.06 g, 5.90 mmol, 87% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.45 (d, *J* = 4.3 Hz, 1H), 7.87 (d, *J* = 8.6 Hz, 1H), 7.74 (dt, *J* = 1.8, 7.3 Hz, 1H), 7.46 (brs, 1H), 7.42 (s, 1H), 7.26 (dt, *J* = 1.8, 6.7 Hz, 1H), 7.09 (d, *J* = 7.9 Hz, 1H), 3.67 (s, 3H), 2.09 (s, 3H); ^{13}C NMR (125 MHz, DMSO-*d*6) δ 163.07, 153.44, 153.24, 148.85, 138.23, 136.93, 136.59, 125.90, 122.51, 121.92, 116.86, 114.06, 110.25, 52.56, 9.86; LCMS m/z 350 $[M + H]^+$; HRMS (Positive ESI) m/z 350.1258 (350.1175 calcd for $C_{18}H_{15}N_5O_3$ + H).


8.46
8.45
7.88
7.86
7.76
7.76
7.75
7.74
7.73
7.72
7.46
7.42
7.28
7.27
7.26
7.25
7.10
7.08
3.67
3.57
3.35
2.67
2.67
2.55
2.51
2.51
2.50
2.50
2.49
2.40
2.33
2.24
2.09
1.23
0.15
0.05
0.01
0.00
-0.01
-0.04
-0.15
19.19
14.26
5.28
3.64
3.92
1.09
1.13
1.17
1.93
1.09
1.00
0.11
0.23
PPM
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

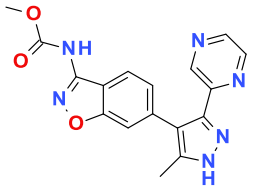

163.07
153.44
153.24
148.85
138.23
136.93
136.59
125.90
122.51
121.92
116.86
114.06
110.25
52.56
40.00
39.83
39.67
39.50
39.33
39.17
39.00
28.33
23.22
22.36
13.86
10.77
9.86
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

methyl {6-[5-methyl-3-(pyridazin-3-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (196)



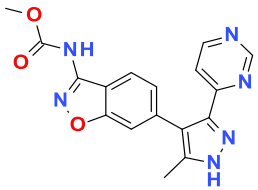
196 was prepared in a similar manner described for **195**. 68% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 9.11 (dd, *J* = 4.9, 1.8 Hz, 1H), 7.93 (d, *J* = 7.9 Hz, 1H), 7.88 (d, *J* = 8.3 Hz, 1H), 7.69 (dd, *J* = 8.3, 4.9 Hz, 1H), 7.53 (s, 1H), 7.16 (d, *J* = 7.9 Hz, 1H), 3.74 (s, 3H), 2.30 (s, 3H); LCMS m/z 351 $[M + H]^+$.

methyl {6-[5-methyl-3-(pyrazin-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (197)



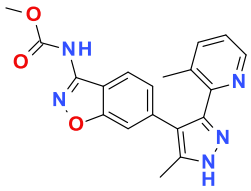
197 was prepared in a similar manner described for **195**. 64% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 13.36 (brs, 1H), 10.88 (brs, 1H), 8.89 (brs, 1H), 8.52 (d, *J* = 2.4 Hz, 1H), 8.47 (brs, 1H), 7.94 (d, *J* = 8.6 Hz, 1H), 7.54 (s, 1H), 7.17 (d, *J* = 8.6 Hz, 1H), 3.75 (s, 3H), 2.29 (s, 3H); LCMS m/z 351 $[M + H]^+$.

methyl {6-[5-methyl-3-(pyrimidin-4-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (198)



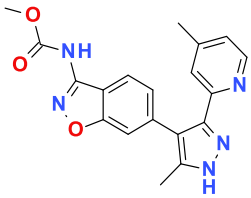
198 was prepared in a similar manner described for **195**. 70% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.96 (brs, 1H), 8.74 (d, *J* = 4.8 Hz, 1H), 7.95 (d, *J* = 9.2 Hz, 1H), 7.71 (brs, 1H), 7.54 (s, 1H), 7.19 (d, *J* = 9.2 Hz, 1H), 3.74 (s, 3H), 2.25 (s, 3H); LCMS m/z 351 $[M + H]^+$.

methyl {6-[5-methyl-3-(3-methylpyridin-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (199)



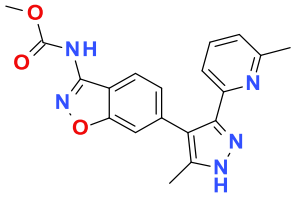
199 was prepared in a similar manner described for **195**. 84% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.89 (s, 1H), 8.75 (d, *J* = 5.5 Hz, 1H), 8.36 (d, *J* = 7.8 Hz, 1H), 7.97 (d, *J* = 8.5 Hz, 1H), 7.93 (dd, *J* = 7.8, 5.5 Hz, 1H), 7.46 (s, 1H), 7.05 (d, *J* = 8.5 Hz, 1H), 3.75 (s, 3H), 2.46 (s, 3H), 2.00 (s, 3H); LCMS m/z 364 $[M + H]^+$.

methyl {6-[5-methyl-3-(4-methylpyridin-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (200)



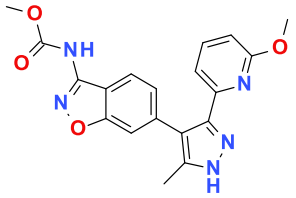
200 was prepared in a similar manner described for **195**. 84% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.15 (brs, 1H), 8.45 (d, *J* = 4.6 Hz, 1H), 8.23 (d, *J* = 7.9 Hz, 1H), 7.75 (brs, 1H), 7.49 (s, 1H), 7.28 (d, *J* = 6.1 Hz, 1H), 7.01 (d, *J* = 6.1 Hz, 1H), 3.91 (s, 3H), 2.28 (s, 3H), 2.14 (s, 3H); LCMS m/z 364 $[M + H]^+$.

methyl {6-[5-methyl-3-(6-methylpyridin-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (201)



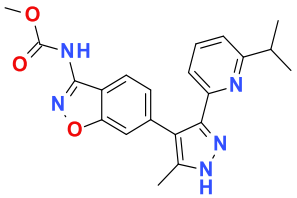
201 was prepared in a similar manner described for **195**. 84% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.91 (d, *J* = 8.6 Hz, 1H), 7.60 (t, *J* = 7.3 Hz, 1H), 7.52 (s, 1H), 7.21 (brs, 1H), 7.16 (d, *J* = 8.6 Hz, 1H), 7.13 (d, *J* = 8.6 Hz, 1H), 3.72 (s, 3H), 2.34 (s, 3H), 2.19 (s, 3H); LCMS m/z 364 $[M + H]^+$.

methyl {6-[3-(6-methoxypyridin-2-yl)-5-methyl-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (202)



202 was prepared in a similar manner described for **195**. 91% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.90 (d, *J* = 7.9 Hz, 1H), 7.66 (t, *J* = 7.9 Hz, 1H), 7.46 (s, 1H), 7.22 (brs, 1H), 7.13 (d, *J* = 8.9 Hz, 1H), 6.64 (d, *J* = 8.9 Hz, 1H), 3.68 (s, 3H), 3.31 (brs, 3H), 2.21 (s, 3H); LCMS m/z 380 $[M + H]^+$.

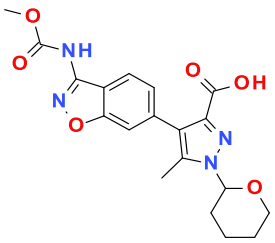
methyl (6-{5-methyl-3-[6-(propan-2-yl)pyridin-2-yl]-1*H*-pyrazol-4-yl}-1,2-benzoxazol-3-yl)carbamate (203)



203 was prepared in a similar manner described for **195**. 78% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.88 (d, *J* = 8.5 Hz, 1H), 7.66 (t, *J* = 7.9 Hz, 1H), 7.45 (s, 1H), 7.42 (brs, 1H), 7.14 (d, *J* = 8.0 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 3.57 (s, 3H), 2.85-2.76 (m, 1H), 2.24 (s, 3H), 0.94 (d, *J* = 6.7 Hz, 6H); LCMS m/z 392 $[M + H]^+$.

methyl {6-[3-(1-hydroxyethyl)-5-methyl-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (208)

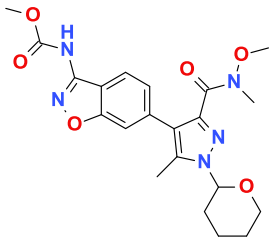
4-{3-[(methoxycarbonyl)amino]-1,2-benzoxazol-6-yl}-5-methyl-1-(oxan-2-yl)-1*H*-pyrazole-3-carboxylic acid (204)



To a solution of methyl 4-{3-[(methoxycarbonyl)amino]-1,2-benzoxazol-6-yl}-5-methyl-1-(oxan-2-yl)-1*H*-pyrazole-3-carboxylate (123 mg, 0.296 mmol) in THF (2 mL) and MeOH (3

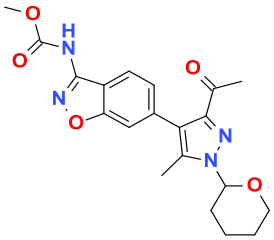
mL) in a round-bottom flask was added 1 mol/L aqueous sodium hydroxide solution (0.80 mL, 0.80 mmol) at room temperature. The resulting mixture was stirred at room temperature for 4.5 hours and 50 ºC for 1.5 hours. The reaction mixture was added to 0.1 mol/L aqueous hydrochloric acid solution and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting solid was collected and dried under reduced pressure at 50 °C. The title compound was obtained as a pale yellow solid. (116 mg, 0.290 mmol, 98% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (brs, 1H), 8.10 (d, *J* = 6.7 Hz, 1H), 7.42 (s, 1H), 7.28 (d, *J* = 6.7 Hz, 1H), 5.48-5.43 (m, 1H), 4.16-4.05 (m, 1H), 3.90 (s, 3H), 3.76-3.66 (m, 1H), 2.59-2.48 (m, 1H), 2.33 (s, 3H), 2.22-2.10 (m, 2H), 1.83-1.68 (m, 2H), 1.67-1.58 (m, 1H); LCMS m/z 401 $[M + H]^+$.

methyl (6-{3-[methoxy(methyl)carbamoyl]-5-methyl-1-(oxan-2-yl)-1*H*-pyrazol-4-yl}-1,2-benzoxazol-3-yl)carbamate (205)



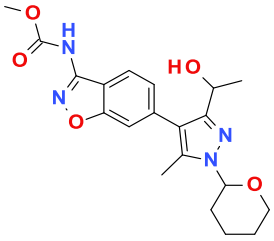
To a solution of 4-{3-[(methoxycarbonyl)amino]-1,2-benzoxazol-6-yl}-5-methyl-1-(oxan-2-yl)-1*H*-pyrazole-3-carboxylic acid (**204**, 395 mg, 0.987 mmol) and *N,O*-dimethylhydroxylamine hydrochloride (192 mg, 1.97 mmol) in THF (15 mL) and MeOH (15 mL) in a round-bottom flask were added 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (410 mg, 1.48 mmol) and *N*-methylmorpholine (299 mg, 2.96 mmol) at room temperature. The resulting mixture was stirred at room temperature for 1.25 hours. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (0% to 100% AcOEt/*n*-hexane linear gradient) provided the title compound. (365 mg, 0.823 mmol, 83% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, *J* = 8.5 Hz, 1H), 7.44 (s, 1H), 7.25 (d, *J* = 8.5 Hz, 1H), 5.42-5.46 (m, 1H), 4.09-4.03 (m, 1H), 3.89 (s, 3H), 3.74-3.68 (m, 1H), 3.66 (brs, 3H), 3.25 (s, 3H), 2.54-2.48 (m, 1H), 2.40 (s, 3H), 2.19-2.10 (m, 2H), 1.83-1.68 (m, 2H), 1.67-1.58 (m, 1H).

methyl {6-[3-acetyl-5-methyl-1-(oxan-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (206)



To a solution of methyl (6-{3-[methoxy(methyl)carbamoyl]-5-methyl-1-(oxan-2-yl)-1*H*-pyrazol-4-yl}-1,2-benzoxazol-3-yl)carbamate (**205**, 160 mg, 0.361 mmol) in THF (5 mL) in a round-bottom flask was added 3 mol/L methylmagnesium bromide diethyl ether solution (0.361 mL, 1.08 mmol) at 0 ºC. The resulting mixture was stirred at 0 ºC for 1 hour. The reaction mixture was quenched by saturated aqueous ammonium chloride solution and extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (0% to 70% AcOEt/*n*-hexane linear gradient) provided the title compound. (50.6 mg, 0.127 mmol, 35% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J* = 8.6 Hz, 1H), 7.44 (brs, 1H), 7.39 (s, 1H), 7.21 (d, *J* = 8.6 Hz, 1H), 5.45-5.40 (m, 1H), 4.12-4.05 (m, 1H), 3.89 (s, 3H), 3.74-3.68 (m, 1H), 2.58 (s, 3H), 2.54-2.48 (m, 1H), 2.29 (s, 3H), 2.25-2.15 (m, 2H), 1.80-1.63 (m, 2H), 1.67-1.58 (m, 1H); LCMS m/z 399 $[M + H]^+$.

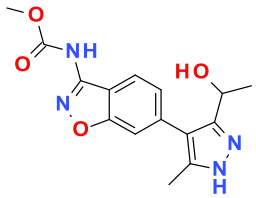
methyl {6-[3-(1-hydroxyethyl)-5-methyl-1-(oxan-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (207)



To a solution of methyl {6-[3-acetyl-5-methyl-1-(oxan-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (**206**, 212 mg, 0.532 mmol) in MeOH (10 mL) in a round-bottom flask was added sodium borohydride (22.1 mg, 0.585 mmol) at room temperature. The resulting mixture was stirred at room temperature for 20 minutes. The reaction mixture was added water and extracted with CH_2Cl_2. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (0% to 80% AcOEt/*n*-hexane linear gradient) provided the title compound. (212 mg, 0.529 mmol, 99% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, *J*

= 8.9 Hz, 1H), 7.45 (brs, 1H), 7.44 (s, 1H), 7.26 (d, *J* = 8.9 Hz, 1H), 5.35-5.29 (m, 1H), 5.01-4.93 (m, 1H), 4.12-4.05 (m, 1H), 3.90 (s, 3H), 3.74-3.68 (m, 1H), 2.59-2.50 (m, 2H), 2.31 (s, 3H), 2.25-2.15 (m, 2H), 1.80-1.63 (m, 2H), 1.67-1.58 (m, 1H), 1.37 (dd, *J* = 6.7, 5.5 Hz, 3H); LCMS m/z 401 $[M + H]^+$.

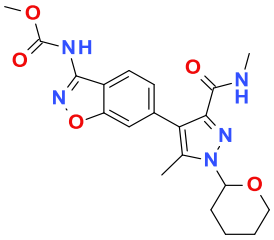
methyl {6-[3-(1-hydroxyethyl)-5-methyl-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (208)



Methyl {6-[3-(1-hydroxyethyl)-5-methyl-1-(oxan-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (**207**, 212 mg, 0.529 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (3 mL, 12.0 mmol). The resulting mixture was stirred at 50 ºC for 1.25 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (124 mg, 0.392 mmol, 74% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.00 (d, *J* = 7.8 Hz, 1H), 7.64 (s, 1H), 7.37 (d, *J* = 7.8 Hz, 1H), 5.23 (brs, 1H), 4.75 (q, *J* = 6.8 Hz, 1H), 3.75 (s, 3H), 2.23 (s, 3H), 1.35 (d, *J* = 6.8 Hz, 1H); LCMS m/z 317 $[M + H]^+$.

<u>methyl {6-[5-methyl-3-(methylcarbamoyl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (210)</u>

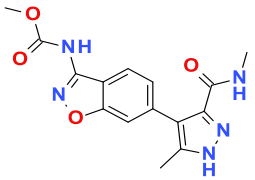
methyl {6-[5-methyl-3-(methylcarbamoyl)-1-(oxan-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (209)



To a solution of 4-{3-[(methoxycarbonyl)amino]-1,2-benzoxazol-6-yl}-5-methyl-1-(oxan-2-yl)-1*H*-pyrazole-3-carboxylic acid (**204**, 80.1 mg, 0.200 mmol) and methylamine hydrochloride (27.0 mg, 0.400 mmol) in THF (5 mL) and MeOH (5 mL) in a round-bottom flask were added 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (83.0 mg, 1.48 mmol) and *N*-methylmorpholine (60.7 mg, 0.600 mmol) at room temperature. The

resulting mixture was stirred at room temperature for 2.5 hours. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (0% to 100% AcOEt/*n*-hexane linear gradient) provided the title compound. (80.0 mg, 194 mmol, 97% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 8.5 Hz, 1H), 7.50 (brs, 1H), 7.46 (s, 1H), 7.31 (d, *J* = 8.5 Hz, 1H), 6.94 (q, *J* = 4.5 Hz, 1H), 5.39-5.34 (m, 1H), 4.12-4.05 (m, 1H), 3.87 (s, 3H), 3.74-3.68 (m, 1H), 2.90 (d, *J* = 4.5 Hz, 3H), 2.56-2.49 (m, 2H), 2.29 (s, 3H), 2.25-2.15 (m, 2H), 1.80-1.63 (m, 2H), 1.67-1.58 (m, 1H); LCMS m/z 414 $[M + H]^+$.

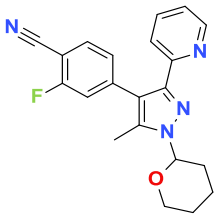
methyl {6-[5-methyl-3-(methylcarbamoyl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (210)



Methyl {6-[5-methyl-3-(methylcarbamoyl)-1-(oxan-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (**209**, 80.0 mg, 0.194 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (5 mL, 20.0 mmol). The resulting mixture was stirred at 50 ºC for 1 hour, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (48.5 mg, 0.147 mmol, 76% yield): δ 8.08 (q, *J* = 4.3 Hz, 1H), 7.92 (d, *J* = 8.6 Hz, 1H), 7.53 (s, 1H), 7.24 (d, *J* = 8.6 Hz, 1H), 3.73 (s, 3H), 2.68 (d, *J* = 4.3 Hz, 3H), 2.25 (s, 3H); LCMS m/z 330 $[M + H]^+$.

ethyl {6-[5-methyl-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (214)

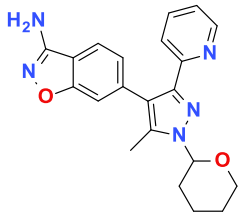
2-fluoro-4-[5-methyl-1-(oxan-2-yl)-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]benzonitrile (211)



To a solution of 2-[4-bromo-5-methyl-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-3-yl]pyridine (6.44 g, 20.0 mmol) in 1,2-dimethoxyethane (150 mL) in a round-bottom flask were added 4-cyano-3-fluorophenylboronic acid (3.63 g, 22.0 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) complex with CH_2Cl_2 (817 mg, 1.00

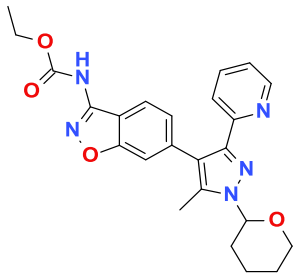
mmol), tripotassium phosphate hydrate (12.7 g, 60.0 mmol) and water (60 mL). The resulting mixture was stirred at reflux for 3 hours and the reaction mixture was added to water. The resulting solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (0% to 50% AcOEt/*n*-hexane linear gradient) provided the title compound. (7.01 g, 19.3 mmol, 97% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, *J* = 4.3 Hz, 1H), 7.70-7.64 (m, 3H), 7.55 (t, *J* = 7.3 Hz, 1H), 7.17 (t, *J* = 7.3 Hz, 1H), 7.10 (d, *J* = 9.2Hz, 1H), 5.45-5.40 (m, 1H), 4.13-4.03 (m, 1H), 3.76-3.63 (m, 1H), 2.66-2.50 (m, 1H), 2.37 (s, 3H), 2.23-2.13 (m, 1H), 2.12-1.98 (m, 1H), 1.81-1.68 (m, 2H), 1.67-1.60 (m, 1H) ; LCMS m/z 363 $[M + H]^+$.

6-[5-methyl-1-(oxan-2-yl)-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-amine (212)



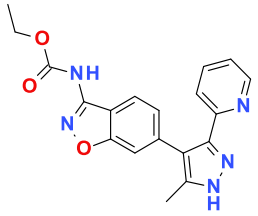
To a solution of acetohydroxamic acid (7.26 g, 96.7 mmol) in DMF (150 mL) in a round-bottom flask was added potassium *tert*-butoxide (10.9 g, 96.7 mmol). The resulting mixture was stirred at room temperature for 1 hour and a solution of 2-fluoro-4-[5-methyl-3-(pyridin-2-yl)-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-4-yl]benzonitrile (7.01 g, 19.3 mmol) in DMF (50 mL) was added at room temperature. The resulting mixture was stirred at 50 ºC for 16 hours and the reaction mixture was added water. The resulting solution was extracted with AcOEt. The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel (30% to 100% AcOEt/*n*-hexane linear gradient) provided the title compound. (2.65 g, 7.06 mmol, 37% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (dd, *J* = 1.2, 4.3 Hz, 1H), 7.55 (dt, *J* = 1.8, 7.3 Hz, 1H), 7.45 (dd, *J* = 1.8, 7.3 Hz, 1H), 7.44 (d, *J* = 8.6 Hz, 1H), 7.30 (s, 1H), 7.12 (dt, *J* = 1.2, 7.3 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 1H), 5.48-5.43 (m, 1H), 4.37 (brs, 2H), 4.14-4.09 (m, 1H), 3.76-3.67 (m, 1H), 2.70-2.57 (m, 1H), 2.38 (s, 3H), 2.21-2.07 (m, 2H), 1.80-1.69 (m, 2H), 1.67-1.59 (m, 1H) ; LCMS m/z 376 $[M + H]^+$.

ethyl {6-[5-methyl-1-(oxan-2-yl)-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (213)



To a solution of 6-[5-methyl-3-(pyridin-2-yl)-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzisoxazol-3-amine (**212**, 113 mg, 0.300 mmol) in CH_2Cl_2 (15 mL) in a round-bottom flask were added ethyl chloroformate (0.0574 mL, 0.600 mmol) and pyridine (0.0724 mL, 0.900 mmol). The resulting mixture was stirred at room temperature for 40 minutes, then concentrated under reduced pressure. Purification by flash chromatography on NH-silica gel (0% to 70% AcOEt/*n*-hexane linear gradient) provided the title compound. (134 mg, 0.299 mmol, 99% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, *J* = 4.8 Hz, 1H), 8.07 (d, *J* = 8.6 Hz, 1H), 7.56 (dt, *J* = 1.8. 8.0 Hz, 1H), 7.51 (d, *J* = 7.5 Hz, 1H), 7.49 (d, *J* = 7.9 Hz, 1H), 7.36 (s, 1H), 7.14 (d, *J* = 6.8 Hz, 1H), 7.12 (d, *J* = 7.5 Hz, 1H), 5.48-5.43 (m, 1H), 4.16-4.08 (m, 1H), 4.32 (q, *J* = 7.4 Hz, 2H), 3.75-3.67 (m, 1H), 2.70-2.55 (m, 1H), 2.39 (s, 3H), 2.22-2.10 (m, 2H), 1.83-1.68 (m, 2H), 1.67-1.58 (m, 1H), 1.36 (t, *J* = 7.4 Hz, 3H).

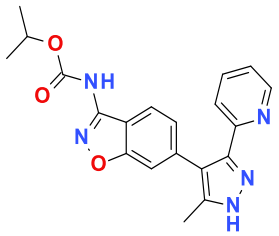
ethyl {6-[5-methyl-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (214)



Ethyl {6-[5-methyl-1-(oxan-2-yl)-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (**213**, 134 mg, 0.299 mmol) was dissolved in 4 mol/L hydrogen chloride in 1,4-dioxane (5 mL, 20.0 mmol). The resulting mixture was stirred at 50 °C for 4 hours, then concentrated under reduced pressure. The residue was quenched by saturated aqueous sodium hydrogen carbonate solution and the resulting solid was collected. The solid was washed with water and dried under reduced pressure at 50 °C. The title compound was obtained as a white solid. (89.0 mg, 0.245 mmol, 82% yield): ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.79 (brs, 1H), 8.45 (d, *J* = 4.3 Hz, 1H), 7.87 (d, *J* = 8.6 Hz, 1H), 7.74 (dt, *J* = 1.8, 7.3 Hz, 1H), 7.70 (brs, 1H), 7.52 (s, 1H), 7.29 (dt, *J* = 1.8, 6.7 Hz, 1H), 7.16 (d, *J* = 7.9 Hz, 1H), 4.22 (q, *J* = 7.3 Hz,

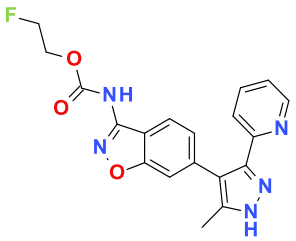
2H), 2.25 (s, 3H), 1.29 (t, *J* = 7.3 Hz, 3H); LCMS m/z 364 $[M + H]^+$.

propan-2-yl {6-[5-methyl-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (215)



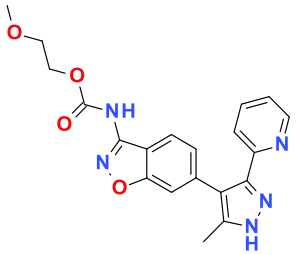
215 was prepared in a similar manner described for **214**. 55% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.71 (brs, 1H), 8.35 (d, *J* = 4.3 Hz, 1H), 7.87 (d, *J* = 8.6 Hz, 1H), 7.74 (dt, *J* = 1.8, 7.3 Hz, 1H), 7.70 (brs, 1H), 7.52 (s, 1H), 7.29 (dt, *J* = 1.8, 6.7 Hz, 1H), 7.16 (d, *J* = 7.9 Hz, 1H), 5.00-4.94 (m, 1H), 2.25 (s, 3H), 1.30 (d, *J* = 6.1 Hz, 6H); LCMS m/z 378 $[M + H]^+$.

2-fluoroethyl {6-[5-methyl-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (216)



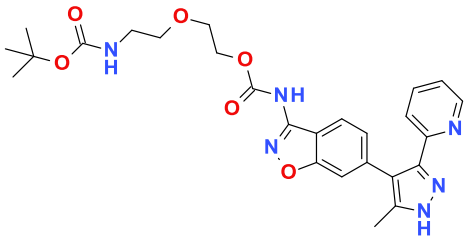
216 was prepared in a similar manner described for **214**. 82% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 13.15 (brs, 1H), 10.99 (brs, 1H), 8.44 (brs, 1H), 7.94 (d, *J* = 8.5 Hz, 1H), 7.75 (t, *J* = 6.7 Hz, 1H), 7.51 (s, 1H), 7.27 (dt, *J* = 1.9, 5.5 Hz, 1H), 7.15 (d, *J* = 8.5 Hz, 1H), 4.78-4.75 (m, 1H), 4.66-4.62 (m, 1H), 4.49-4.43 (m, 1H), 4.40-4.35 (m, 1H), 2.25 (s, 3H); LCMS m/z 382 $[M + H]^+$.

2-methoxyethyl {6-[5-methyl-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (217)



217 was prepared in a similar manner described for **214**. 52% yield: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 13.12 (brs, 1H), 10.90 (brs, 1H), 8.45 (d, *J* = 3.0 Hz, 1H), 7.93 (d, *J* = 8.6 Hz, 1H), 7.75 (dt, *J* = 1.2, 7.3 Hz, 1H), 7.51 (brs, 1H), 7.49 (s, 1H), 7.25 (dt, *J* = 1.2, 6.1 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 4.30-4.23 (m, 2H), 3.61-3.55 (m, 2H), 3.29 (s, 3H), 2.25 (s, 3H); LCMS m/z 394 $[M + H]^+$.

2-{2-[(*tert*-butoxycarbonyl)amino]ethoxy}ethyl {6-[5-methyl-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (218)



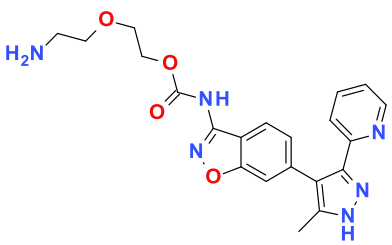
To a solution of 6-[5-methyl-3-(2-pyridyl)-1-tetrahydropyran-2-yl-pyrazol-4-yl]-1,2-benzisoxazol-3-amine (**212**, 600 mg, 1.60 mmol) in CH_2Cl_2 (40 mL) were added 2-[2-(*tert*-butoxycarbonylamino)ethoxy]ethyl carbonochloridate (1.71 g, 6.39 mmol) and pyridine (0.772 mL, 9.59 mmol). The mixture was stirred at room temperature under a nitrogen atmosphere. After 100 min, the pale ocher solution was poured into saturated aqueous ammonium chloride solution. The mixture was extracted with CH_2Cl_2. The organic layer was washed successively with saturated aqueous sodium hydrogen carbonate solution and brine and dried over anhydrous sodium sulfate. After the filtration, the filtrate was evaporated and the residue was purified by flash chromatography on NH-silica gel (15%, AcOEt/*n*-hexane) to provide 802 mg of crude 2-[2-(*tert*-butoxycarbonylamino)ethoxy]ethyl *N*-[6-[5-methyl-3-(2-pyridyl)-1-tetrahydropyran-2-yl-pyrazol-4-yl]-1,2-benzisoxazol-3-yl]carbamate as a colorless foam. LCMS *m/z* 607 $[M+H]^+$.

To a solution of 390 mg of this crude compound in CH_2Cl_2 (10 mL) was added 4 mol/L hydrochloric acid in 1,4-dioxane (5 mL). The white suspension was stirred at room

temperature for 1.5 hours under a nitrogen atmosphere. After the evaporation of solvents, the residue was purified by flash chromatography on NH-silica gel (8%, MeOH/AcOEt) to give 207 mg of crude 2-(2-aminoethoxy)ethyl *N*-[6-[5-methyl-3-(2-pyridyl)-1*H*-pyrazol-4-yl]-1,2-benzisoxazol-3-yl]carbamate as a colorless foam. LCMS *m/z* 423.4 $[M+H]^+$.

To a suspension of 100 mg of this crude compound in CH_2Cl_2/THF (v/v = 9/1) were added di-*tert*-butyl dicarbonate (65.2 mg, 0.284 mmol) and triethylamine (0.0660 mL, 0.473 mmol). The white suspension was gradually changed to a colorless solution while stirring at room temperature for 4.5 hours under a nitrogen atmosphere. The mixture was poured into saturated aqueous ammonium chloride solution and extracted with AcOEt. The organic layer was washed successively with saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by preparative HPLC (H_2O: acetonitrile+0.1% HCO_2H). The fractions that contained the title compound with high purity was evaporated to remove acetonitrile. The remaining aqueous solution was extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography on NH-silica gel (hexane/AcOEt/MeOH = 70/27/3) to give 72.7 mg (0.139 mmol, 37% total) of **5** as a colorless foam. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 11.35-10.35 (brs, 1H), 8.59 (d, *J* = 4.6 Hz, 1H), 8.22 (d, *J* = 7.6 Hz, 1H), 7.86 (brs, 1H), 7.48 (s, 1H), 7.48-7.45 (m, 1H), 7.27 (d, *J* = 8.4 Hz, 1H), 7.17 (dd, *J* = 7.6, 4.6 Hz, 1H), 7.09 (d, *J* = 8.4 Hz, 1H), 5.21-4.87 (m, 1H), 4.45-4.42 (m, 2H), 3.80-3.76 (m, 2H), 3.61 (t, *J* = 5.0 Hz, 1H), 3.40-3.29 (m, 2H), 2.27 (s, 3H), 1.44 (s, 9H); HRMS (ESI) calcd for $C_{26}H_{31}N_6O_6$ ($[M+H]^+$) 523.2305, found 523.2308; HPLC 99.9% (UV integration at 254 nm) retention time 4.993 min (H_2O: acetonitrile = 70:30–20:80 + 0.1% HCO_2H, 17 min); LCMS *m/z* 523 $[M+H]^+$ (RT 1.97 min) (ESI, H_2O: acetonitrile = 100:0–0:100, 0.1% HCO_2H, 5 min).

2-(2-aminoethoxy)ethyl {6-[5-methyl-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzoxazol-3-yl}carbamate (219)



To a solution of 2-[2-(*tert*-butoxycarbonylamino)ethoxy]ethyl *N*-[6-[5-methyl-3-(2-pyridyl)-1*H*-pyrazol-4-yl]-1,2-benzisoxazol-3-yl]carbamate (**218**, 59.0 mg, 0.113 mmol) in CH_2Cl_2 (2 mL) was added trifluoroacetic acid (0.100 mL, 1.35 mmol) at room temperature under a

nitrogen atmosphere. After the reaction mixture had been stirred for 30 min, trifluoroacetic acid (0.403 mL, 5.42 mmol) was added. The mixture was stirred for 15 min. An excess amount of potassium carbonate powder and MeOH (0.3 mL) were added to the colorless solution. The mixture was then evaporated and the residue was purified by flash chromatography on NH-silica gel (5%, MeOH/AcOEt) to give 36.7 mg (0.0869 mmol, 77%) of **6** as a colorless foam. ^{1}H-NMR (400 MHz, CD_3OD) δ 8.50 (d, *J* = 4.6 Hz, 1H), 7.97 (d, *J* = 8.4 Hz, 1H), 7.76-7.68 (m, 1H), 7.43-7.30 (m, 3H), 7.15 (d, *J* = 8.4 Hz, 1H), 4.42-4.39 (m, 2H), 3.78-3.75 (m, 2H), 3.59-3.55 (m, 2H), 2.83-2.79 (m, 2H), 2.34 (s, 3H); HRMS (ESI) calcd for $C_{21}H_{23}N_6O_4$ ($[M+H]^+$) 423.1781, found 423.1774; HPLC 98.9% (UV integration at 254 nm) retention time 10.212 min (H_2O: acetonitrile = 95:5–60:40 +0.1% HCO_2H, 17 min); LCMS *m/z* 423 $[M+H]^+$ (RT 1.26 min) (ESI, H_2O: acetonitrile = 100:0–0:100, 0.1% HCO_2H, 5 min).

Hepcidin mRNA expression inhibitory assay.

A Hep G2 human hepatocellular carcinoma cell line was purchased from American Type Culture Collection. Hep G2 cells were cultured in DMEM (Gibco, #11995) supplemented with 10% fetal bovine serum (Hyclone, #SH30071) and 1% Penicillin-Streptomycin (Gibco, #15140), and maintained under the supplier's recommended conditions.

Hep G2 cells were seeded in a 96-well plate (sumilon, #MS-8096F) at 90000 cells / well, and cultured overnight at 37°C and 5% CO_2. Solutions of test compounds in Dimethyl sulfoxide (DMSO) were prepared and diluted to the final concentration in incubation medium (DMEM + 10% FBS + 1% penicillin/streptomycin). In addition, human recombinant BMP6 (R&D systems, #507-BP) was diluted in incubation medium to a final concentration of 50 ng/mL. The culture medium was discarded and Hep G2 cells were incubated with the medium containing BMP6 and the reference or test compounds for 6 hours at 37°C and 5% CO_2. DMSO-treated cell cultures stimulated with BMP6 or left unstimulated were served as controls. All measurements were performed in triplicate.

Hep G2 cultures were washed with PBS, and plate was sealed for storage at -80°C until RNA extraction. The cellular total RNA extraction and cDNA synthesis were performed using TaqMan Gene Expression Cells-to-Ct Kit (Ambion, #4399002) or using SuperPrep Cell Lysis & RT Kit for qPCR (Toyobo, #SCQ-101) following the provider's instruction. The cDNA was stored at -20°C until analyzed by quantitative RT-PCR.

The amount of Hepcidin mRNA was determined by quantitative RT-PCR using the TaqMan technology and a Real-Time PCR System (ViiA 7 Real-Time PCR or 7900HT Fast Real-time PCR from Applied Biosystems). Cyclophilin A (PPIA) served as an endogenous control. The measurement of each sample was done in quadruplicate for ViiA 7 Real-Time PCR or singly

for 7900HT Fast Real-time PCR. The ID of TaqMan probe was as follow:
Hepcidin: Hs01057160_g1
PPIA: 4326316E
The mRNA level was calculated by ΔΔCt method, or by standard quantification method.
Where: ΔCt = "Ct of target gene (Hepcidin)" – "Ct of endogenous control gene (PPIA)".
ΔΔCt = "ΔCt of experimental sample" – "average ΔCt of DMSO-treated BMP6-induced controls".
RQ = (2^(− "ΔΔCt")) × 100
The mRNA level of test and reference compound-treated cells was expressed as % of DMSO-treated controls. The RQ of the unstimulated control were subtracted before % expression.
Where: % of DMSO-treated control = ((RQ of test sample – RQ of unstimulated control) / (RQ of DMSO treated control – RQ of unstimulated control)) × 100
Data fit for IC_{50} was determined using GraphPad Prism's nonlinear regression equation, where the bottom was fixed to 0% and the top was fixed to 100%.
The PPIA mRNA level was checked to avoid nonspecific gene expression modulation using the following calculation.
Where: PPIAΔ = "PPIA Ct of experimental sample" – "average of PPIA Ct of DMSO treated BMP6 induced controls".
PPIA RQ = − (1− (2^(PPIAΔ))) × 100
The experimental sample dosage with PPIA gene variation over or fewer than 30% of DMSO controls was excluded from IC_{50} calculation.

Kinase Profiling Assay.

Inhibitory profile on several kinases was evaluated by DAIICHI SANKYO RD NOVARE CO., LTD. using Profiler Pro Plate kit (PerkinElmer, plate 1 to 8), following the provider's instruction (http://www.perkinelmer.co.jp/reader/tabid/164/Default.aspx).

Compound 28 (0.5 μM)					
	Inhibition (%)		Inhibition (%)		Inhibition (%)
MAPKAPK2	8.0	Abl(H396P)	4.3	DYRK3	4.7
AurA	4.7	PDGFR_alpha	3.7	DYRK4	-2.0
PKCz	2.3	FGFR2(N549H)	3.0	CLK2	97.0
RSK1	2.7	Hck	1.7	MST1R	-5.3
PRAK	1.7	Flt3(D835Y)	37.3	HIPK1	32.3
Erk1	4.0	Fer	-1.3	HIPK2	40.3

PKD2	6.3	AKT3	5.0	RSK4	8.3
CK1d	14.0	CaMKII_gamma	-2.3	PDGFR-alpha (V561D)	3.0
CHK1	3.0	MSK2	0.0	EPHA8	4.7
ABL	1.0	p38-gamma	0.0	CDK5/p25	2.7
FYN	4.7	PKD1	5.3	BLK	2.3
LYN	4.0	MARK2	1.0	ALK	3.7
CHK2	2.0	BMX	2.3	PYK2	1.0
MET	4.3	CSNK1A1	14.7	DAPK1	-3.3
LCK	6.0	PKD3	7.3	Casein kinase 1g2	5.0
SRC	5.7	BRSK1	2.0	FRK	1.0
GSK3b	4.3	NEK2	-1.3	JAK2	8.7
Erk2	0.7	PIM1	-4.7	ROS (ROS1)	9.3
PKA	11.7	SGK2	-13.3	RET	10.0
AKT2	7.0	SGK3	0.3	EPHB1	2.3
INSR	4.3	Arg	2.0	FGFR3 [K650E]	0.3
p38a	3.0	DCAMKL2	-11.3	EGFR (ErbB1) T790M, L858R	8.0
AKT1	3.7	RSK2	7.0	RET Y791F	9.0
MSK1	1.7	RSK3	10.3	TXK	-12.0
PKCb2	6.0	BRSK2	-4.0	ITK	2.7
ROCK2	5.0	PKC-alpha	11.0	TYRO3	0.0
CDK2	6.0	PKC-beta1	2.0	CaMK2a	-10.7
MST2	1.7	PKC-gamma	8.3	KIT	-25.0
PKGa	4.0	PKC-delta	6.7	TRKC (NTRK3)	7.0
PAK2	4.3	PKC-epsilon	8.7	Mer	18.3
IGF1R	9.3	PKC-eta	6.0	CK1g3 (CSNK1G3)	4.0
FGFR1	8.0	PKC-theta	9.0	MET M1250T	2.7
MARK1	1.7	EPHA1	19.3	AXL	4.0
CAMK2	5.0	EPHA2	12.0	MARK4	1.3
PIM2	-7.3	EPHA3	4.7	MELK	12.3
BTK	0.3	EPHA4	7.7	CDK1/Cycline B1	4.0

c-TAK1	-2.3	EPHA5	5.3	KIT[T670I]	-34.3
DYRK1a	92.0	EPHB2	4.3	AMPK alpha2/beta1/gamma1	-12.0
CAMK4	11.0	EPHB3	3.7	PRKCI (PKC-iota)	-1.7
AMPK	9.0	EPHB4	7.7	ZIPK (DAPK3)	4.0
FLT3	9.7	DYRK1B	86.7	Ret (V804L)	10.3
HGK	2.7	LYNB	6.7	SRM (SRMS)	3.7
KDR	5.3	GCK	17.0	PRKX	2.0
c-Raf	1.7	MINK	-4.3	GSK3-alpha	1.3
p70S6K	58.3	ABL1(E255K)	8.7	FGFR1 (V561M)	0.7
IRAK4	12.0	FGR	-9.0	NTRK2 (TRKB)	19.0
SGK1	11.0	MST1	7.0	p38alpha/SAPK2a (T106M)	-1.3
SYK	1.0	FLT1	5.3	DDR2	0.7
AurB	9.3	ABL1(Y253F)	6.7	CK1-epsilon	9.3
FGFR2	4.0	FES	-1.7	MST3 (STK24)	2.3
FGFR3	3.7	FLT4	11.7	PDGFRA (D842V)	9.7
Abl(Q252H)	3.3	TEC	3.7	NuaK1	-0.3
AurC	3.7	ABL1(G250E)	7.0	CK1-gamma1	1.3
FGFR4	3.0	LTK	10.3	NEK1	2.3
EGFR	9.3	FMS	6.7	PDGFR beta	1.7
Abl(T315I)	7.3	HER4	1.0	MNK1 (MKNK1)	-2.3
IKK-beta	16.7	ROCK1	3.0	PAK4	4.7
MAPKAPK3	2.0	PASK	1.0	CaMK1a	1.7
p38-beta2	0.7	PhKg1	-0.3	PAK3	1.7
TSSK1	-6.7	Yes	-2.0	IKBKE (IKK epsilon)	1.7
PKG1-beta	6.3	PIM3	0.0	PAK5 (PAK7)	4.7
CaMKII_beta	8.3	PhKg2	0.0	CamK1d	2.3

p38-delta	2.3	DCAMKL1	2.0	LOK	14.7
TSSK2	0.7	EGFR(T790M)	-1.3	CDK3	4.3

Compound 139 (0.45 μM)					
	Inhibition (%)		Inhibition (%)		Inhibition (%)
AKT1	-1.7	PRAK	-1.7	EPHA1	21.0
AKT2	-2.0	TSSK1	-1.0	EPHA2	8.0
AKT3	1.3	TSSK2	-3.0	EPHA3	5.0
MSK1	1.3	ZIPK (DAPK3)	0.3	EPHA4	0.7
MSK2	-3.3	Casein kinase 1g2	0.7	EPHA5	3.0
p70S6K	1.3	CK1d	5.0	EPHA8	3.7
PKA	3.0	CK1-epsilon	2.7	EPHB1	6.7
PKC-alpha	13.7	CK1g3 (CSNK1G3)	-0.7	EPHB2	6.7
PKCb2	3.7	CK1-gamma1	5.0	EPHB3	2.7
PKC-beta1	7.7	CSNK1A1	2.3	EPHB4	8.0
PKC-delta	5.3	CDK1_CyclinB	5.3	Fer	2.3
PKC-epsilon	9.0	CDK1/Cycline B1	1.3	FES	-3.3
PKC-eta	0.3	CDK2	6.0	FGFR1	35.0
PKC-gamma	18.7	CDK3	7.3	FGFR1 (V561M)	23.7
PKC-theta	3.0	CDK4_CyclinD3	-8.3	FGFR2	21.7
PKCz	7.0	CDK5/p25	0.3	FGFR2 (N549H)	40.0
PKG1-beta	8.0	CDK6_CyclinD3	5.3	FGFR3	14.0
PKGa	-1.7	CDK9	9.3	FGFR3 (K650E)	52.0
PRKCI (PKC-iota)	-6.7	CLK2	9.0	FGFR3 (K650M)	68.3
PRKX	0.3	CLK3	6.7	FGFR4	0.7
ROCK1	-2.0	DYRK1a	6.3	FGFR4(V550L)	2.7
ROCK2	0.7	DYRK1B	19.7	FGR	13.0
RSK1	27.3	DYRK2	8.0	FLT1	12.7

RSK2	36.0	DYRK3	2.0	FLT3	96.7
RSK3	42.7	DYRK4	2.7	Flt3(D835Y)	89.0
RSK4	50.7	Erk1	1.3	FLT4	10.0
SGK1	2.3	Erk2	-0.3	FMS	-22.7
SGK2	-13.7	GSK3-alpha	-1.0	FRK	31.3
SGK3	3.3	GSK3b	1.3	FYN	46.0
AurA	7.0	HIPK1	2.7	Hck	30.3
AurB	11.0	HIPK2	5.0	HER4	-7.0
AurC	-1.0	HIPK3	2.7	IGF1R	3.0
IKBKE (IKK epsilon)	6.3	HIPK4	1.3	INSR	3.0
IKK-beta	2.3	MSSK1	2.0	ITK	89.0
NEK1	3.7	p38a	1.7	JAK2	26.0
NEK2	3.3	p38alpha/SAPK2a (T106M)	1.3	JAK3	-15.7
NEK6	3.3	p38-beta2	-1.3	KDR	35.7
NEK9	1.7	p38-delta	4.3	KIT	24.7
AMPK	26.0	p38-gamma	0.0	KIT(T670I)	-10.0
AMPK-alpha2/beta1/gamma1	26.0	SRPK2	0.3	LCK	32.3
BRSK1	14.0	GCK	66.0	LTK	0.0
BRSK2	13.3	HGK	73.3	LYN	32.3
CaMK1a	-0.7	KHS1	85.0	LYNB	24.3
CamK1d	0.7	LOK	9.3	Mer	0.7
CAMK2	19.7	MINK	83.7	MET	5.7
CaMK2a	2.7	MST1	33.3	MET M1250T	5.0
CAMK4	0.0	MST2	1.7	MST1R	-1.0
CaMKII_beta	-0.3	MST3 (STK24)	44.7	NTRK2 (TRKB)	85.7
CaMKII_gamma	3.0	PAK2	-2.7	PDGFR beta	78.3
CHK1	2.3	PAK3	3.7	PDGFR_alpha	11.7
CHK2	3.0	PAK4	4.0	PDGFRA	79.3

				(D842V)	
DAPK1	-1.3	PAK5 (PAK7)	5.3	PDGFR-alpha (V561D)	30.0
DCAMKL1	0.0	SLK	62.3	PYK2	4.7
DCAMKL2	7.0	YSK	31.0	RET	59.0
MAPKAPK2	0.3	ABL	82.0	RET(M918T)	57.7
MAPKAPK3	4.0	Abl(H396P)	84.0	Ret (V804L)	21.3
MARK1	36.7	Abl(Q252H)	72.0	RET Y791F	58.7
MARK2	35.0	Abl(T315I)	58.7	ROS (ROS1)	1.3
MARK4	37.0	ABL1(E255K)	72.7	SRC	51.7
MELK	38.0	ABL1(G250E)	68.0	SRM (SRMS)	9.0
MNK1 (MKNK1)	6.7	ABL1(Y253F)	76.0	SYK	2.7
MNK2	-1.0	ACK	13.0	TEC	7.0
NuaK1	13.0	ALK	26.0	TRKA	93.7
PASK	-2.0	Arg	66.3	TRKC (NTRK3)	95.0
PhKg1	0.7	AXL	0.3	TXK	-0.7
PhKg2	1.7	BLK	20.0	TYRO3	13.3
PIM1	4.0	BMX	4.7	Yes	49.0
PIM2	-1.7	BTK	5.0	c-Raf	7.0
PIM3	-2.3	DDR2	66.0	c-TAK1	15.0
PKD1	1.3	EGFR	-6.0	IRAK1	4.3
PKD2	6.7	EGFR (ErbB1) T790M L858R	1.3	IRAK4	11.0
PKD3	0.3	EGFR(T790M)	0.7	LRRK2 (G2019S)	18.7

Compound 148 (0.19 μM)					
	Inhibition (%)		Inhibition (%)		Inhibition (%)
MAPKAPK2	1.7	MSK2	-3.3	JAK2	6.3

AurA	3.3	p38-gamma	-3.0	ROS (ROS1)	-1.7
PKCz	2.3	PKD1	7.0	RET	47.3
RSK1	4.7	MARK2	-5.3	EPHB1	1.3
PRAK	1.3	BMX	-3.7	FGFR3 [K650E]	55.3
Erk1	3.7	CSNK1A1	0.7	EGFR (ErbB1) T790M L858R	-1.0
PKD2	9.0	PKD3	4.7	RET Y791F	40.0
CK1d	0.0	BRSK1	1.7	TXK	-8.3
CHK1	-8.7	NEK2	12.0	ITK	24.7
ABL	83.7	PIM1	-1.3	TYRO3	10.0
FYN	12.3	SGK2	-20.3	CaMK2a	-2.3
LYN	13.7	SGK3	4.0	KIT	-19.7
CHK2	3.0	Arg	57.0	TRKC (NTRK3)	42.0
MET	7.3	DCAMKL2	-1.7	Mer	-4.0
LCK	26.7	RSK2	-5.7	CK1g3 (CSNK1G3)	1.0
SRC	15.7	RSK3	-2.3	MET M1250T	2.0
GSK3b	3.3	BRSK2	6.3	AXL	-2.3
Erk2	4.7	PKC-alpha	-1.7	MARK4	0.7
PKA	5.7	PKC-beta1	3.0	MELK	51.0
AKT2	1.0	PKC-gamma	-7.3	CDK1/Cycline B1	2.3
INSR	1.7	PKC-delta	0.0	KIT[T670I]	-27.7
p38a	2.3	PKC-epsilon	3.0	AMPK-alpha2/beta1/gamma1	13.7
AKT1	1.7	PKC-eta	2.7	PRKCI (PKC-iota)	-7.0
MSK1	1.3	PKC-theta	1.7	ZIPK (DAPK3)	-2.0
PKCb2	0.3	EPHA1	6.3	Ret (V804L)	7.0
ROCK2	-1.0	EPHA2	-0.3	SRM (SRMS)	3.7
CDK2	-1.7	EPHA3	1.3	PRKX	2.3
MST2	4.7	EPHA4	2.0	GSK3-alpha	1.7
PKGa	-3.7	EPHA5	3.0	FGFR1 (V561M)	6.0
PAK2	-3.0	EPHB2	4.0	NTRK2 (TRKB)	18.3
IGF1R	5.0	EPHB3	1.0	p38alpha/SAPK2a (T106M)	0.3
FGFR1	10.3	EPHB4	0.3	DDR2	5.3
MARK1	-5.3	DYRK1B	3.0	CK1-epsilon	3.3

CAMK2	1.0	LYNB	10.7	MST3 (STK24)	33.0
PIM2	2.0	GCK	16.7	PDGFRA (D842V)	99.7
BTK	-1.7	MINK	82.7	NuaK1	2.3
c-TAK1	0.3	ABL1(E255K)	64.7	CK1-gamma1	-0.3
DYRK1a	3.3	FGR	-3.0	NEK1	1.3
CAMK4	1.3	MST1	28.0	PDGFR beta	97.0
AMPK	38.7	FLT1	14.7	MNK1 (MKNK1)	-1.0
FLT3	77.3	ABL1(Y253F)	69.3	PAK4	0.7
HGK	72.0	FES	-5.0	CaMK1a	0.3
KDR	51.0	FLT4	28.3	PAK3	-0.7
c-Raf	4.7	TEC	2.0	IKBKE (IKK epsilon)	1.0
p70S6K	5.7	ABL1(G250E)	68.3	PAK5 (PAK7)	-2.7
IRAK4	4.3	LTK	0.3	CamK1d	-1.7
SGK1	6.3	FMS	21.3	LOK	4.3
SYK	4.7	HER4	-8.7	CDK3	1.7
AurB	-2.3	ROCK1	-7.3	NEK6	-1.0
FGFR2	4.0	PASK	-3.3	ACK	3.0
FGFR3	6.3	PhKg1	2.0	LRRK2(G2019S)	18.0
Abl(Q252H)	71.0	Yes	3.7	KHS1	80.3
AurC	-12.7	PIM3	-3.7	NEK9	-3.0
FGFR4	5.3	PhKg2	-4.0	TRKA	37.7
EGFR	-6.7	DCAMKL1	-1.3	SLK	29.7
Abl(T315I)	51.7	EGFR(T790M)	0.7	YSK	20.0
IKK-beta	8.0	DYRK3	1.7	CDK1_CyclinB	-2.0
MAPKAPK3	-1.7	DYRK4	-0.7	CDK4_CyclinD3	-8.0
p38-beta2	-1.7	CLK2	-0.3	CDK6_CyclinD3	-2.0
TSSK1	-6.3	MST1R	-4.3	CDK9	-1.0
PKG1-beta	-3.3	HIPK1	0.7	CLK3	-1.3
CaMKII	1.7	HIPK2	2.7	DYRK2	-0.7

_beta					
p38-delta	4.7	RSK4	-2.0	HIPK3	1.0
TSSK2	-2.0	PDGFR-alpha (V561D)	63.3	HIPK4	-0.7
Abl(H396P)	86.3	EPHA8	9.3	MSSK1	-9.3
PDGFR_alpha	36.0	CDK5/p25	-4.0	IRAK1	-2.7
FGFR2 (N549H)	19.7	BLK	3.7	JAK3	-10.3
Hck	6.7	ALK	6.7	FGFR4(V550L)	0.7
Flt3(D835Y)	93.7	PYK2	-1.7	SRPK2	-10.3
Fer	-2.3	DAPK1	-14.3	MNK2	0.0
AKT3	0.7	Casein kinase 1g2	3.3	FGFR3(K650M)	64.0
CaMKII_gamma	-4.0	FRK	3.3	RET(M918T)	41.0

Compound 161 (DS28120313) (0.164 μM)					
	Inhibition (%)		Inhibition (%)		Inhibition (%)
MAPKAPK2	7.0	MSK2	4.0	JAK2	-4.3
AurA	6.7	p38-gamma	0.3	ROS (ROS1)	4.0
PKCz	11.7	PKD1	-3.0	RET	7.3
RSK1	17.0	MARK2	-4.7	EPHB1	3.0
PRAK	3.3	BMX	17.7	FGFR3 [K650E]	12.0
Erk1	7.0	CSNK1A1	5.0	EGFR (ErbB1) T790M L858R	1.0
PKD2	9.3	PKD3	2.7	RET Y791F	6.0
CK1d	4.7	BRSK1	-1.0	TXK	1.3
CHK1	5.3	NEK2	1.0	ITK	6.0
ABL	7.0	PIM1	-13.7	TYRO3	7.7
FYN	10.3	SGK2	-0.7	CaMK2a	-0.3

LYN	10.0	SGK3	3.0	KIT	4.0
CHK2	9.7	Arg	0.7	TRKC (NTRK3)	7.3
MET	0.0	DCAMKL2	-1.3	Mer	6.0
LCK	17.3	RSK2	1.7	CK1g3 (CSNK1G3)	5.3
SRC	5.7	RSK3	4.7	MET M1250T	-0.3
GSK3b	-19.3	BRSK2	-6.7	AXL	4.0
Erk2	9.0	PKC-alpha	1.3	MARK4	7.3
PKA	5.7	PKC-beta1	-11.7	MELK	10.7
AKT2	1.3	PKC-gamma	-2.7	CDK1/Cycline B1	-0.3
INSR	-6.0	PKC-delta	-22.7	KIT[T670I]	1.0
p38a	5.7	PKC-epsilon	-4.0	AMPK-alpha2/beta1/gamma1	0.3
AKT1	9.3	PKC-eta	-4.7	PRKCI (PKC-iota)	-2.3
MSK1	7.7	PKC-theta	7.0	ZIPK (DAPK3)	-1.0
PKCb2	5.7	EPHA1	1.0	Ret (V804L)	0.7
ROCK2	-3.0	EPHA2	-2.3	SRM (SRMS)	3.3
CDK2	9.0	EPHA3	-6.7	PRKX	-3.3
MST2	-11.7	EPHA4	-2.3	GSK3-alpha	1.0
PKGa	0.7	EPHA5	-1.0	FGFR1 (V561M)	4.7
PAK2	12.0	EPHB2	-1.0	NTRK2 (TRKB)	4.0
IGF1R	5.7	EPHB3	0.7	p38alpha/SAPK2a (T106M)	0.7
FGFR1	9.7	EPHB4	-2.3	DDR2	1.7
MARK1	-16.0	DYRK1B	0.3	CK1-epsilon	2.7
CAMK2	-12.7	LYNB	-0.3	MST3 (STK24)	6.0
PIM2	-6.0	GCK	7.0	PDGFRA (D842V)	23.7
BTK	-29.3	MINK	7.0	NuaK1	0.7
c-TAK1	2.0	ABL1(E255K)	-2.3	CK1-gamma1	-2.7
DYRK1a	3.3	FGR	-4.3	NEK1	-1.3
CAMK4	-14.7	MST1	-2.3	PDGFR beta	21.0
AMPK	10.7	FLT1	1.3	MNK1 (MKNK1)	3.3
FLT3	5.0	ABL1(Y253F)	6.7	PAK4	5.7
HGK	5.0	FES	11.7	CaMK1a	3.3
KDR	11.7	FLT4	5.3	PAK3	-2.0

c-Raf	10.0	TEC	-4.3	IKBKE (IKK epsilon)	0.3
p70S6K	14.7	ABL1(G250E)	7.3	PAK5 (PAK7)	5.0
IRAK4	-7.7	LTK	-2.7	CamK1d	-9.3
SGK1	0.3	FMS	-0.7	LOK	3.7
SYK	8.0	HER4	-7.7	CDK3	-8.3
AurB	1.0	ROCK1	-0.3	NEK6	4.3
FGFR2	-5.3	PASK	-3.0	ACK	0.7
FGFR3	-8.7	PhKg1	1.0	LRRK2(G2019S)	2.3
Abl(Q252H)	0.3	Yes	3.0	KHS1	8.3
AurC	6.3	PIM3	-2.3	NEK9	0.3
FGFR4	1.3	PhKg2	-1.7	TRKA	1.3
EGFR	-2.0	DCAMKL1	-4.7	SLK	4.7
Abl(T315I)	-3.7	EGFR(T790M)	1.3	YSK	-2.7
IKK-beta	-3.7	DYRK3	5.3	CDK1_CyclinB	4.0
MAPKAPK3	6.3	DYRK4	2.7	CDK4_CyclinD3	-0.7
p38-beta2	1.7	CLK2	0.7	CDK6_CyclinD3	2.7
TSSK1	-4.3	MST1R	-5.0	CDK9	0.0
PKG1-beta	-10.3	HIPK1	0.0	CLK3	3.7
CaMKII_beta	28.0	HIPK2	3.3	DYRK2	4.7
p38-delta	0.7	RSK4	13.3	HIPK3	1.0
TSSK2	-5.7	PDGFR-alpha (V561D)	-15.3	HIPK4	-4.0
Abl(H396P)	1.7	EPHA8	-1.7	MSSK1	0.7
PDGFR_alpha	11.7	CDK5/p25	6.3	IRAK1	3.0
FGFR2 (N549H)	-3.3	BLK	6.7	JAK3	0.0
Hck	0.0	ALK	4.3	FGFR4(V550L)	1.7

Flt3(D835Y)	7.3	PYK2	2.7	SRPK2	-11.3
Fer	-1.3	DAPK1	-3.7	MNK2	-0.7
AKT3	-2.0	Casein kinase 1g2	2.3	FGFR3(K650M)	12.3
CaMKII_gamma	18.0	FRK	5.0	RET(M918T)	6.7

Compound 191 (0.49 μM)					
	Inhibition (%)		Inhibition (%)		Inhibition (%)
AKT1	2.3	PRAK	-0.3	EPHA1	3.3
AKT2	3.0	TSSK1	1.0	EPHA2	-0.3
AKT3	7.3	TSSK2	2.0	EPHA3	0.7
MSK1	5.7	ZIPK (DAPK3)	2.3	EPHA4	2.3
MSK2	-3.7	Casein kinase 1g2	1.0	EPHA5	3.0
p70S6K	-5.3	CK1d	6.7	EPHA8	-2.7
PKA	2.3	CK1-epsilon	1.0	EPHB1	1.3
PKC-alpha	2.0	CK1g3 (CSNK1G3)	2.3	EPHB2	5.3
PKCb2	5.3	CK1-gamma1	0.7	EPHB3	0.7
PKC-beta1	5.0	CSNK1A1	1.7	EPHB4	1.7
PKC-delta	6.7	CDK1_CyclinB	5.3	Fer	0.3
PKC-epsilon	4.7	CDK1/Cycline B1	20.0	FES	-5.3
PKC-eta	7.0	CDK2	1.3	FGFR1	0.7
PKC-gamma	2.0	CDK3	3.3	FGFR1 (V561M)	3.7
PKC-theta	6.0	CDK4_CyclinD3	-6.7	FGFR2	-0.7
PKCz	5.0	CDK5/p25	4.3	FGFR2 (N549H)	0.0
PKG1-beta	2.0	CDK6_CyclinD3	1.7	FGFR3	-0.3
PKGa	0.0	CDK9	2.3	FGFR3 (K650E)	-1.7
PRKCI (PKC-iota)	1.0	CLK2	-0.7	FGFR3 (K650M)	6.3

PRKX	-1.7	CLK3	0.7	FGFR4	0.3
ROCK1	1.0	DYRK1a	4.7	FGFR4(V550L)	4.0
ROCK2	8.7	DYRK1B	2.0	FGR	-4.0
RSK1	2.3	DYRK2	3.3	FLT1	-2.3
RSK2	-2.0	DYRK3	1.0	FLT3	3.7
RSK3	2.0	DYRK4	1.3	Flt3(D835Y)	15.3
RSK4	-1.3	Erk1	3.7	FLT4	-2.7
SGK1	2.3	Erk2	-0.3	FMS	-14.7
SGK2	-18.7	GSK3-alpha	2.0	FRK	1.3
SGK3	1.3	GSK3b	6.7	FYN	2.7
AurA	5.7	HIPK1	1.7	Hck	3.3
AurB	-6.3	HIPK2	2.7	HER4	-10.0
AurC	-3.3	HIPK3	1.3	IGF1R	1.3
IKBKE (IKK epsilon)	1.7	HIPK4	1.7	INSR	2.3
IKK-beta	2.3	MSSK1	4.0	ITK	-1.0
NEK1	0.3	p38a	-1.7	JAK2	-1.7
NEK2	10.7	p38alpha/SAPK2a (T106M)	-0.3	JAK3	-7.3
NEK6	2.7	p38-beta2	0.7	KDR	5.0
NEK9	1.7	p38-delta	4.3	KIT	1.0
AMPK	2.0	p38-gamma	-2.3	KIT(T670I)	-1.7
AMPK-alpha2/beta1/gamma1	3.3	SRPK2	-2.3	LCK	5.7
BRSK1	1.7	GCK	11.3	LTK	-3.3
BRSK2	-2.3	HGK	4.0	LYN	6.7
CaMK1a	2.3	KHS1	6.3	LYNB	3.0
CamK1d	-4.3	LOK	9.7	Mer	11.3
CAMK2	-7.0	MINK	-0.3	MET	7.3
CaMK2a	1.3	MST1	9.3	MET M1250T	1.3
CAMK4	2.3	MST2	1.0	MST1R	-3.0
CaMKII_beta	2.3	MST3 (STK24)	2.3	NTRK2	4.3

				(TRKB)	
CaMKII_gamma	6.0	PAK2	-0.3	PDGFR beta	20.0
CHK1	0.0	PAK3	-4.3	PDGFR_alpha	0.7
CHK2	8.0	PAK4	-3.0	PDGFRA (D842V)	31.7
DAPK1	-2.7	PAK5 (PAK7)	-1.0	PDGFR-alpha (V561D)	-0.7
DCAMKL1	0.0	SLK	4.7	PYK2	1.3
DCAMKL2	2.0	YSK	4.3	RET	15.0
MAPKAPK2	2.7	ABL	38.3	RET(M918T)	1.0
MAPKAPK3	-7.3	Abl(H396P)	36.7	Ret (V804L)	-0.7
MARK1	3.3	Abl(Q252H)	30.3	RET Y791F	0.0
MARK2	-1.0	Abl(T315I)	11.0	ROS (ROS1)	-2.3
MARK4	8.7	ABL1(E255K)	21.3	SRC	5.0
MELK	4.0	ABL1(G250E)	23.7	SRM (SRMS)	2.3
MNK1 (MKNK1)	3.0	ABL1(Y253F)	24.3	SYK	0.7
MNK2	-1.7	ACK	2.3	TEC	3.7
NuaK1	5.7	ALK	0.3	TRKA	3.7
PASK	1.3	Arg	7.7	TRKC (NTRK3)	6.3
PhKg1	2.3	AXL	-0.3	TXK	-0.3
PhKg2	0.3	BLK	1.0	TYRO3	8.7
PIM1	9.0	BMX	-0.7	Yes	-8.0
PIM2	-6.0	BTK	0.3	c-Raf	1.0
PIM3	-4.0	DDR2	2.0	c-TAK1	6.0
PKD1	1.7	EGFR	-7.3	IRAK1	6.7
PKD2	7.7	EGFR (ErbB1) T790M / L858R	-1.0	IRAK4	2.0
PKD3	-4.3	EGFR(T790M)	0.7	LRRK2 (G2019S)	5.3

Compound 205 (DS79182026) (0.078μM)					
	Inhibition (%)		Inhibition (%)		Inhibition (%)
AKT1	-1.0	CHK2	0.7	ABL	34.0
AKT2	4.0	MAPKAPK2	4.7	BTK	7.7
MSK1	-5.7	MARK1	3.3	FGFR1	-2.7
p70S6K	4.0	PIM2	1.7	FLT3	-0.3
PKA	-2.3	PKD2	-0.7	FYN	-4.3
PKCb2	8.7	PRAK	-3.0	IGF1R	2.3
PKCz	2.7	CK1d	10.7	INSR	1.7
PKGa	-3.3	CDK2	-4.3	KDR	17.3
ROCK2	-2.0	DYRK1a	9.7	LCK	4.7
RSK1	0.3	Erk1	8.0	LYN	-0.3
SGK1	-10.0	Erk2	-0.7	MET	1.0
AurA	-1.7	GSK3b	10.0	SRC	2.3
AMPK	2.7	p38a	5.7	SYK	0.0
CAMK2	3.7	HGK	40.7	c-Raf	0.7
CAMK4	6.7	MST2	2.0	c-TAK1	-3.3
CHK1	1.3	PAK2	-5.3	IRAK4	5.7

Microsome Metabolic Stability Assay (MS & UGT).

Pooled human liver microsomes and pooled CD1 mouse liver microsomes were purchased from Xenotech, LLC. β-nicotinamide adenine dinucleotide phosphate (β-NADP), D-glucose-6-phosphate (G-6-P) and glucose-6-phosphate dehydrogenase (G-6-PDH) were purchased from Oriental Yeast Co., Ltd. A 100-μL of microsomes (final concentration: 0.5 mg protein/mL), 30 mM of G-6-P, 3 units/mL of G-6-PDH and the substrate (final concentration: 1 μM) were mixed to prepare the reaction mixture. The metabolic reaction was initiated by the addition of the 3 mM of β-NADP to the reaction mixture. After 0 and 30-min incubation at 37°C, a 90-μL aliquot of the reaction was drawn and the reaction was terminated by the addition to a 410 μL of mixture (acetonitrile/methanol =75/25, v/v) containing 15 ng/mL of Niflumic acid (as internal standard). Each of the incubation samples was centrifuged at 2,400 x g for 12 min at 4°C. The supernatant was subjected to LC-MS/MS analysis. MS stability (%) was calculated using the peak area ratio (PAR) of the test substance to the internal standard by the equation 1 described below.

$$\text{MS stability (\%)} = \frac{PAR\ at\ 30\ min}{PAR\ at\ 0\ min} \times 100 \qquad \text{(equation 1)}$$

Pooled human liver microsomes and pooled mouse liver microsomes were purchased from Xenotech, LLC. A 15-μL of microsomes (final concentration: 0.5 mg protein/mL), 25 mM of UDPGA (16 μL) and 5X assay buffer (40 μL) and 137 μL of distilled water were mixed to prepare the reaction mixture. The metabolic reaction was initiated by the addition of the substrate (final concentration: 1 μM) to the reaction mixture. After 0 and 30-min incubation at 37°C, a 40-μL aliquot of the reaction was drawn and the reaction was terminated by the addition to a mixture of acetonitrile (40 μL) and methanol containing IS (40 μL). Each of the incubation samples was centrifuged at 2,400 x g for 12 min at 4°C. The supernatant was subjected to LC-MS/MS analysis. UGT stability (%) was calculated using the peak area ratio (PAR) of the test substance to the internal standard by the equation 2 described below.

$$\text{UGT stability (\%)} = \frac{PAR\ at\ 30\ min}{PAR\ at\ 0\ min} \times 100 \qquad \text{(equation 2)}$$

Pharmacokinetic evaluation in mice.

Male C57BL/6N mice were purchased at 5 weeks old from Charles River Laboratories Japan, Inc (Kanagawa, Japan) and acclimated in stainless steel cages for 7–11 days in the controlled animal area. The tested compounds were suspended in a 0.5 (w/v) % methyl cellulose 400 solution (Wako pure chemical industries) for oral and intraperitoneal administration. Dosing formulations (3 mg/mL) were administered to male C57BL/6N mice at 10 mL/kg after overnight fasting. A blood sample of approximately 0.2 mL was collected from the jugular vein with a heparinized syringe. The blood was centrifuged at 14,000 rpm for 3 min at 4 ^{o}C (himac CR15D, Hitachi Koki Co., Ltd rotor: RT15A2) to obtain the plasma. The plasma was stored frozen at 20 ^{o}C until use for measurement of plasma concentration. The determination of the plasma concentration was performed by LC–MS/MS method using API 4000QTRAP (Applied Biosystems/MDS SCIEX). PK parameters were calculated by a non-compartmental model using Winnonlin (version 4.0.1, Pharsight Corp.).

Protein expression, purification, crystallization and structure determination.

The coding sequence of the human DYRK1A kinase domain was synthesized (Thermo Fisher Scientific K.K., Japan) and cloned into pET24b expression vector. Two 6x His-tags were introduced at the N-terminus of the DYRK1A kinase domain with a HRV3c protease cleavage site. Protein was expressed in an E.coli BL21(DE3). Expressed DYRK1A protein was purified through sequential Ni^{2+}-affinity chromatography, tag removal with HRV3c protease, and size-exclusion chromatography steps. The protein was concentrated to 20 mg/ml in a

final buffer of 20 mM Tris pH 8.0, 500 mM NaCl, 1 mM TCEP. A compound in DMSO solution was added to the protein to a final concentration of 6.5 mM, store at 4°C for several hours, then applied to crystallization. The DYRK1A - Compound **28** co-crystal was grown from a reservoir solution containing 2.0 M Ammonium sulfate. The DYRK1A - Compound **59** co-crystal was grown from a reservoir solution containing 20% w/v Polyethylene glycol monomethyl ether 2,000. Crystals were soaked in a solution containing the reservoir condition supplemented by 25% ethylene glycol and were flash-frozen in liquid nitrogen. Diffraction data of the soaked crystal were collected at the beamline BL15a and BL17 of Photon Factory, Japan.

The diffraction data were processed using HKL2000[46a] and the structure was solved by molecular replacement with PHASER[46b] using the DYRK1A structure (PDB code: 2VK3) as a search. The model was refined and built using Refmac[46c] and Coot[46d]. The final model and structure factors were deposited to PDB with accession codes of 6A1F for Compound **28** and 6A1G for Compound **69** complex. All figures of the crystal structures were prepared with the program PyMOL (The PyMOL Molecular Graphics System, Schrödinger, LLC).

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謝辞

本論文の提出及び発表の機会を与えて頂き、暖かいご指導、ご鞭撻を賜りました静岡県立大学薬学部教授　眞鍋敬先生に心より深く感謝の意を表します。

本論文に対し、有益な御助言を賜りました静岡県立大学薬学部教授　浅井章良先生、菅敏幸先生、濱島義隆先生に厚く御礼を申し上げます。

本研究を行う機会を与えていただき、貴重なご指導を賜りました、第一三共RDノバーレ株式会社取締役　西剛秀博士、第一三共株式会社研究開発企画部参事　長谷川雅司博士に深く感謝いたします。

本研究の遂行、及び論文執筆にあたり、貴重なご指導、ご助言をいただきました、第一三共株式会社オンコロジーラボラトリーグループ長　田中直樹博士、第一三共株式会社疼痛・神経ラボラトリーグループ長　町永信雄博士に深く感謝いたします。

本合成研究に多大なるご協力を頂いた、後藤力氏、石山崇博士、片桐隆廣博士、木方俊宏博士をはじめとする多くの共同研究者の方々に感謝いたします。

また、貴重なご助言とご協力を頂くとともに生物評価の労をとっていただきました上田健二郎博士、村松すみえ氏、橋本昌実博士、安藝杏梨氏、薬物動態評価の労をとっていただきました渡邊健悟博士、X 線結晶構造解析の労をとっていただいた馬場大地博士、標的探索研究の労をとっていただいた、佐々木真聡博士、清水弘樹博士、久保田一石博士に感謝いたします。

さらには、本論文執筆にあたり鼓舞激励いただきました、青木一真博士、武藤毅博士、金谷直明博士、永田勉博士、古川詔大博士、松藤哲義博士、菅野修博士、北澤隆之博士、辻貴司博士、松井智博士、後藤泰治博士に心より感謝いたします。

最後に、本論文の作成に際し、蔭ながら心温かく支援し、深い理解を示してくれた最愛の家族に感謝いたします。