

博士論文

選択的ホスホジエステラーゼ 10A (PDE10A) 阻害剤の合成と
構造活性相関に関する研究

2016 年 4 月

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Design, synthesis, and structure-activity relationships of selective phosphodiesterase 10A (PDE10A) inhibitors

April, 2016

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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
ADDP	1,1'-(azodicarbonyl)dipiperidine
aq.	aqueous
cAMP	cyclic adenosine monophosphate
<i>n</i> Bu	normal butyl
cDNA	complementary DNA
cGMP	cyclic guanosine monophosphate
CAN	ammonium hexanitratocerate(IV)
CL _{int}	intrinsic clearance
CL _{total}	total clearance
CMBP	cyanomethylenetriethylphosphorane
CTAB	cetyltrimethylammonium bromide
CYP	cytochrome P450
DAST	<i>N,N</i> -diethylaminosulfur trifluoride
DMA	<i>N,N</i> -dimethylacetamide
DME	1,2-dimethoxyethane
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethyl sulfoxide
ED ₅₀	50% effective dose
Et	ethyl
GABA	γ -aminobutyric acid
G _{ai}	G _i alpha subunit
HLM	human liver microsomes
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
IC ₅₀	50% inhibitory concentration
i.p.	intraperitoneal
LC–MS	liquid chromatography–mass spectrometry
LiHMDS	lithium bis(trimethylsilyl)amide

MPE	mean photo effect
Me	methyl
MLM	mouse liver microsomes
NADP ⁺	nicotinamide adenine dinucleotide phosphate
NMDA	<i>N</i> -methyl-D-aspartate
NORT	novel object recognition test
NRU	neutral red uptake
PCP	phencyclidine
PCR	polymerase chain reaction
PDB	protein data bank
Pd-C	palladium on carbon
PdCl ₂ (dppf)·CH ₂ Cl ₂	[1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane complex
Pd ₂ (dba) ₃	tris(dibenzylideneacetone)dipalladium(0)
PDE	phosphodiesterase
PET	positron emission tomography
Ph	phenyl
PK	pharmacokinetics
PKA	protein kinase A
p.o.	per os
<i>i</i> Pr	isopropyl
quant	quantitative yield
ROS	reactive oxygen species
SAR	structure-activity relationship
SOR	social odor recognition
s.c.	subcutaneous injection
SUV	standardized uptake value
t _{1/2}	half-life period
THF	tetrahydrofuran
<i>p</i> -TsOH	<i>p</i> -toluenesulfonic acid
Xphos	2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl
WSC·HCl	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

序論

第一節 本研究の背景

統合失調症（schizophrenia）は、主に思春期から成人早期に発症し、陽性症状、陰性症状、認知機能障害を主な症状とする慢性・進行性の精神疾患である。陽性症状とは、本来あるべきでないものが症状として出てくるもので、幻覚、妄想などがある。陰性症状は、正常な行動が欠如ないし減少しているもので、情動反応の平板化、会話の貧困、自発性や持続性の欠如、無快楽症、社会的引きこもりなどがある。認知機能障害には、注意を保持することの困難さ、学習・記憶の障害、他者の感情や意図を読み取る能力の低下などが含まれる。

統合失調症の生涯罹患率は約 1%と報告されており、性別、人種や地域による差はほとんどなく、普遍性の高い疾患であると言える¹⁾。また、統合失調症は再発率の非常に高い疾患であり、再発した場合には治療は長期におよぶ。本邦においては精神疾患で入院している患者のうち半数以上を統合失調症の患者が占めており、入院期間は1年以上にわたることが多く、患者、家族への負担や実生活への影響が大きい²⁾。統合失調症患者一人あたりの社会的費用は生涯推計費用で1億円を超えと言われており、医療経済の面からも社会的な問題である³⁾。

ハロペリドールやリスペリドンに代表される既存の抗精神病薬の多くは、D2 受容体への拮抗作用を示す。統合失調症患者では線条体の D2 受容体の発現量が増加していることが報告されており⁴⁾、抗精神病薬の陽性症状に対する効果は線条体での D2 受容体の占有率と相関していることも知られている⁵⁾。ただし、抗精神病薬の線条体 D2 受容体への過度な拮抗作用は錐体外路症状などの副作用を誘発する⁶⁾。また、ハロペリドールなどの定型抗精神病薬は、線条体以外の部位で薬物が作用することにより、認知機能障害を増悪させてしまう可能性が指摘されているなど^{7,8)}、認知機能障害に対する既存の抗精神病薬の改善効果は乏しく、統合失調症の認知機能障害に効果のある薬剤が望まれている。

D2 受容体はドパミンの結合を受けて G タンパク質 G_{ai} と共役して、アデニル酸シクラーゼによる cAMP 産生および cAMP/protein kinase A (PKA) シグナルを抑制する。統合失調症患者の線条体ではド

パミン D2 受容体の発現量亢進により、cAMP/PKA シグナルならびに線条体からの抑制性 GABA シグナルの出力が低下していると考えられる。その結果、線条体を含む大脳基底核によって調節されている視床への抑制性 GABA シグナルの入力が低下していることが示唆される。視床は外部からの情報を適切に選別して大脳皮質に転送するというフィルターとしての機能を果たしているが、統合失調症患者では、視床への抑制性シグナルの入力が低下していることによりフィルター機能の異常を生じ、グルタミン酸シグナルを介して不必要な情報まで大脳皮質への入力が行われ、種々の症状を引き起こしていると考えられる (Figure 1)。この考えに基づくと、線条体からの出力シグナルを増強することにより、大脳皮質への過度な情報入力を抑制でき、それにより生じていると考えられる統合失調症の各種症状を改善できる可能性がある。

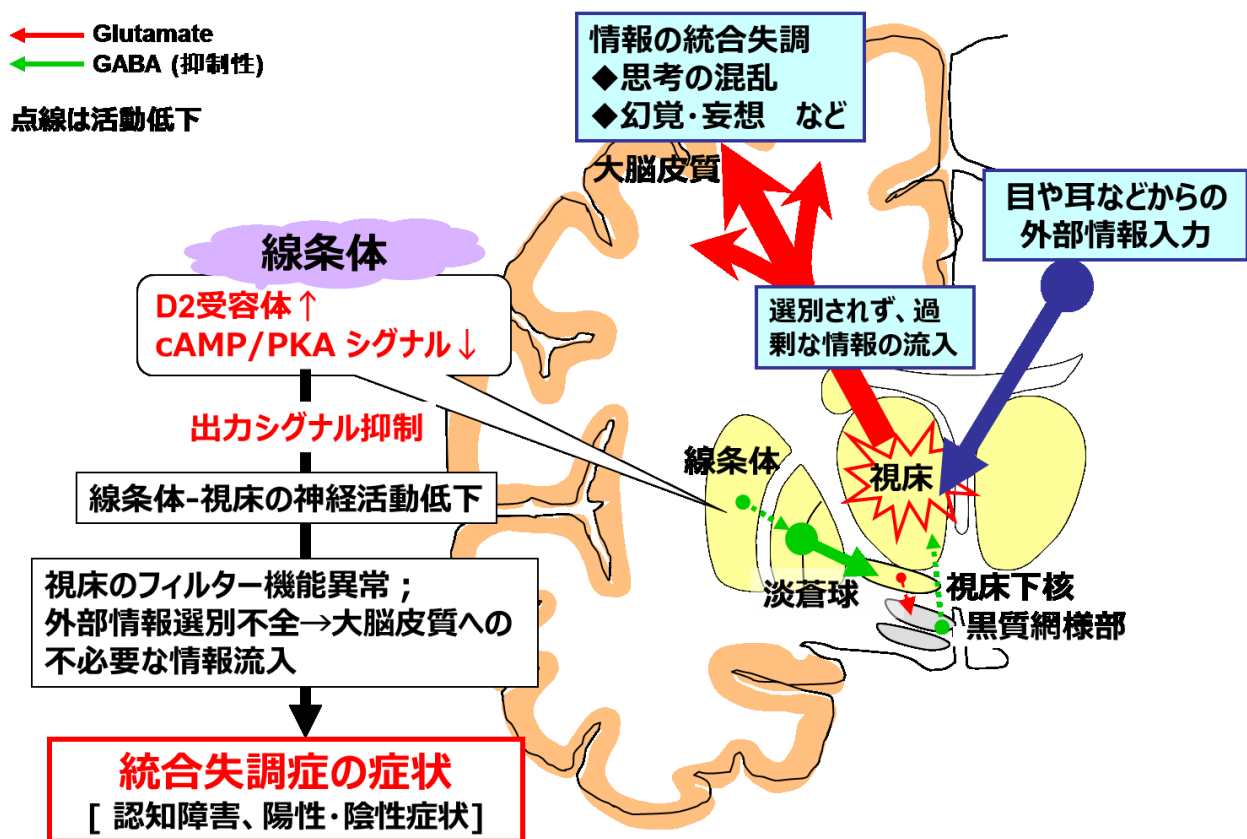


Figure 1. 統合失調症の病態仮説

統合失調症患者での線条体の機能低下を改善する手段として、線条体の cAMP 濃度を上昇させることにより cAMP/PKA シグナルを増強することを考え、cAMP の分解酵素であるホスホジエステラーゼ (PDE)

に着目した。PDE のサブタイプの中で、PDE10A は脳内の線条体に特に高発現しており^{9,10)}、cAMP に対する加水分解能がcGMP より 10 倍程度高い特徴を有する¹¹⁾。従ってPDE10A 阻害剤は、線条体のcAMP 濃度上昇による線条体からの出力シグナル増強を介して、線条体から視床に至る神経活動ならびに視床のフィルター機能を回復し、認知機能障害を含む統合失調症の各症状を改善することが期待できる (Figure 2)。線条体 cAMP の分解は PDE10A が主に担っているが、PDE1B や PDE4 も関わっているために¹¹⁾、選択的 PDE10A 阻害剤は線条体の機能を過度に抑制することではなく、錐体外路症状などの副作用を起こしにくいと考えられる。また、PDE10A は線条体以外の脳内部位での発現は高くないため、選択的 PDE10A 阻害剤は線条体にのみ効果的に作用することにより、定型抗精神病薬のような二次的な認知機能障害を引き起こしにくいと考えられる。また、PDE10A 阻害剤であるパパベリンは、マウスへ投与すると線条体の cAMP 濃度を上昇させることや、陽性症状モデルでの有効性を示すこと、NMDA 受容体アンタゴニストである MK-801 により誘発される認知機能障害を改善することも報告されている¹²⁾。一方、PDE10A ノックアウトマウスは社会性行動が多くなることも示されている¹³⁾。以上の結果から、選択的 PDE10A 阻害剤は、統合失調症の認知機能障害を含む各症状に対し効果的に作用することが期待できる。

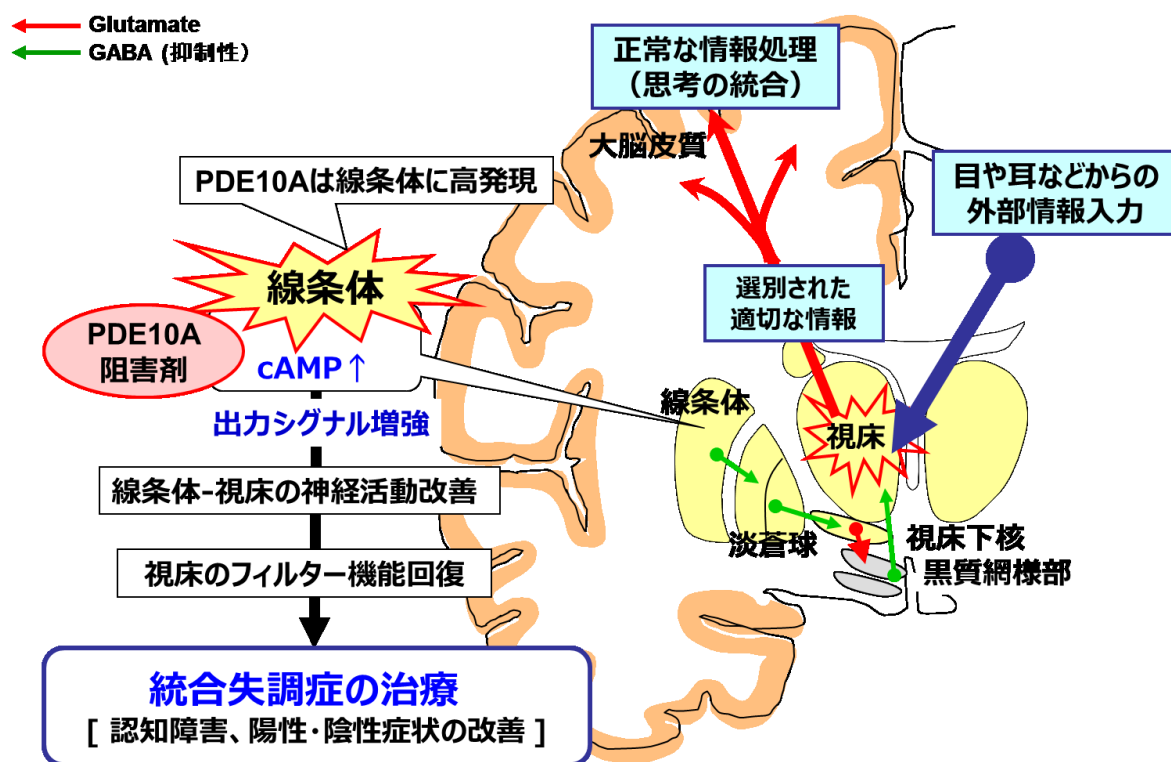


Figure 2. PDE10A 阻害剤の推定効果

現在までに構造が報告されている代表的な PDE10A 阻害剤を Figure 3 に示す。AMG-579、TAK-063、OMS-824 に関しては統合失調症を適応疾患として現在開発中との情報があり、臨床試験の結果が待たれている。

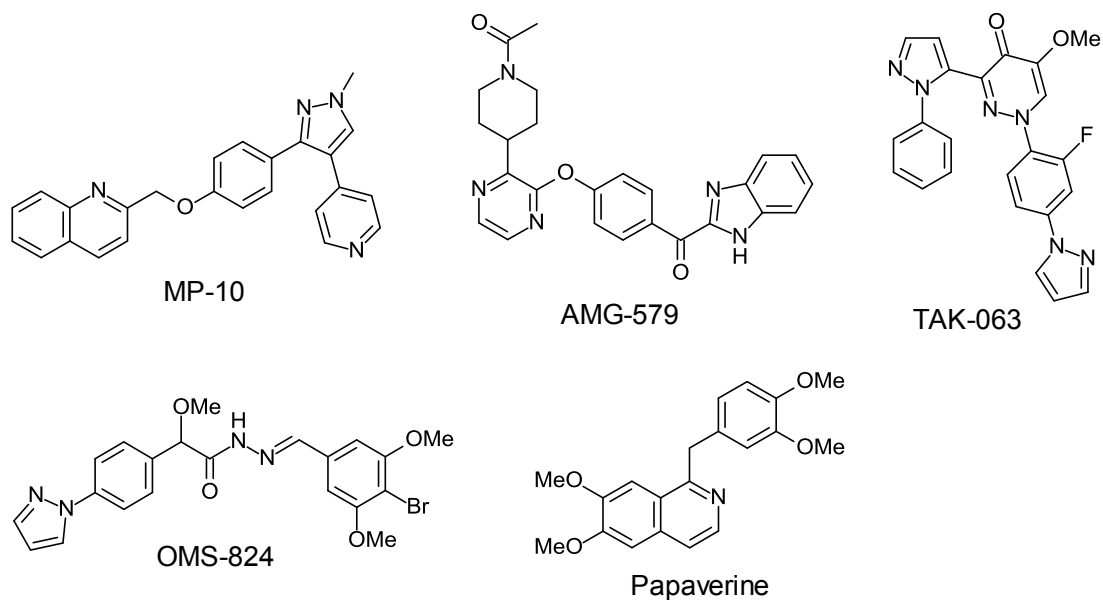


Figure 3. Representative PDE10A inhibitors.

第二節 本研究の概要

本研究では、統合失調症治療薬、特に認知機能障害改善薬として有用性の高い新規 PDE10A 阻害剤の創製を目的に研究を行った。

目標とする化合物のプロフィールとして以下の項目を設定した。

- (1) PDE10A に対する高い阻害活性、ならびに高い PDE サブタイプ選択性を示すこと
- (2) げっ歯類の認知機能障害モデルにおいて低用量で有効性を示すこと
- (3) 吸収、代謝等の薬物動態が良好であること
- (4) 薬物-薬物相互作用、光毒性などの臨床上の薬物使用を制限させる懸念が少ないこと

研究方針として、研究開始時に臨床試験段階にあったファイザー社の MP-10 から新規化合物を設計・合成し、新規 PDE10A 阻害剤の創製を目指した。MP-10 は高い *in vitro* PDE10A 阻害活性を示すものの、認知機能を評価する *in vivo* モデルである正常ラットの新奇物体認識試験 (NORT) において無効との報告があった¹⁴⁾。そこで MP-10 から合成展開を行い、認知機能障害モデルで有効性を示すリード化合物を見出すこととした。次に見出したリード化合物について CYP3A4 阻害作用、光毒性を含めた構造活性相関を明らかにし、最終的に上記目標プロフィールを満たす化合物を創出することとした。

第一章では、げっ歯類の認知機能障害モデルで有効性を示す PDE10A 阻害剤の取得を目的とした。MP-10 はげっ歯類の肝ミクロソーム中でのクリアランスが大きく、それが上記の *in vivo* モデルにおいて無効となる原因と考え、MP-10 代謝安定性の改善を目指した。MP-10 の代謝物検索から得られた情報を参考に、キノリン環とピラゾール環のリンカー部位を変換したところ、マウス肝ミクロソーム中での代謝安定性が改善し、統合失調症の認知機能障害モデルと考えられる新生児期にフェンサイクリジン (PCP) を投与したマウスの NORT において有効性を示すリード化合物 **14** を見出した (Figure 4)。

第二章では、リード化合物 **14** のさらなる PDE10A 阻害活性の増強および CYP3A4 阻害作用の減弱を目指した。**14** のピラゾール環 4 位のピリジン環が強い CYP3A4 阻害作用の主要因であると考え、ピリ

ジン環を *N*-メチルピリドン環へと変換することにより CYP3A4 阻害作用の大幅な低減に成功した。また、この過程で、ピラゾール環 4 位置換基の水素結合受容能と PDE10A 阻害活性に相関があることも見出した。次いでピラゾール環とフェニル環のリンカーを変換することにより、PDE10A 阻害活性の増強と CYP3A4 阻害作用低減を達成した化合物 **82b** を見出した。化合物 **82b** は、げっ歯類に静脈内投与後、脳の線条体に集積することが PET 試験において確認され、マウスの NORT においても有効性を示した (Figure 4)。

第三章では、リード化合物 **14** の PDE10A 阻害作用の増強および光毒性の回避を達成し、マウスの NORT においてさらに強力な薬効を示す化合物を見出すことを目指した。リード化合物 **14** の光毒性発現の主要因はビアリールユニットであるキノリニルフェニル部にあると考えて分子設計を行い、キノリン環を *N*-メチルベンズイミダゾール環へと変換することにより、化合物 **14** と同等の PDE10A 阻害活性を保持しつつ光毒性を回避することに成功した。また、この過程でビアリールユニットが平面構造になるコンフォメーションの存在確率が低いほど、光毒性発現の懸念も低いことを見出した。さらに、第二章で得た知見を適用し、薬物-薬物相互作用や光毒性の懸念が低く、リード化合物 **14** と比較して 100 倍低用量で、マウスの NORT において有効性を示す **116** を見出した (Figure 4)。この **116** のセスキリン酸塩を開発候補化合物 ASP9436 として選択した。

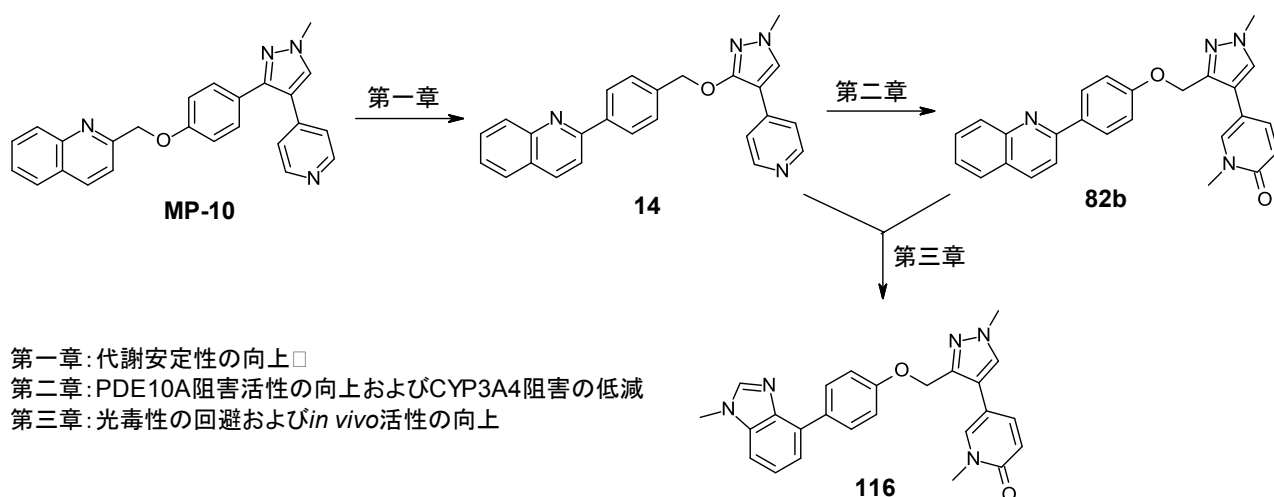


Figure 4. 本研究の概要

本論

第一章 新規キノリン誘導体の合成および *in vivo* 薬効評価

第一節 分子設計

新規選択的 PDE10A 阻害剤を取得するために、研究開始時に PDE10A 阻害剤として報告されていた MP-10 および TP-10 に着目した (Figure 5)。これらの化合物の PDE10A 阻害活性は強力であり他の PDE サブタイプへの選択性も高いものの¹⁵⁾、げっ歯類の肝ミクロソーム中での代謝安定性が悪いことがわかった (Figure 5)。また、MP-10 は認知機能障害モデルである MK-801 を投与したラットの social odor recognition (SOR) 試験において有効性を示した一方、正常ラットを用いた NORT においては無効であったとの報告がなされており、MP-10 の投与タイミングの違いが2つのモデルでの有効性の違いに影響したと示唆されている¹⁴⁾。つまり、記憶学習の訓練開始前に MP-10 を投与する NORT では無効であったのに対し、訓練後に MP-10 を投与した SOR 試験では有効性を示している。この結果から、記憶が形成されるのに重要なタイミングは訓練後であるということが示唆され、MP-10 はげっ歯類での代謝安定性が低いために、訓練前に投与する NORT においては、記憶形成時に薬効を発現するに十分な濃度が保たれていない、と考えた。そこで、MP-10 のマウス肝ミクロソーム (MLM) 中での代謝安定性を向上させ、重要なモデルと位置付けていた新生児期に PCP を投与したマウスの NORT において有効性を示すリード化合物を取得することを目的として、MP-10 の構造変換を行うこととした。

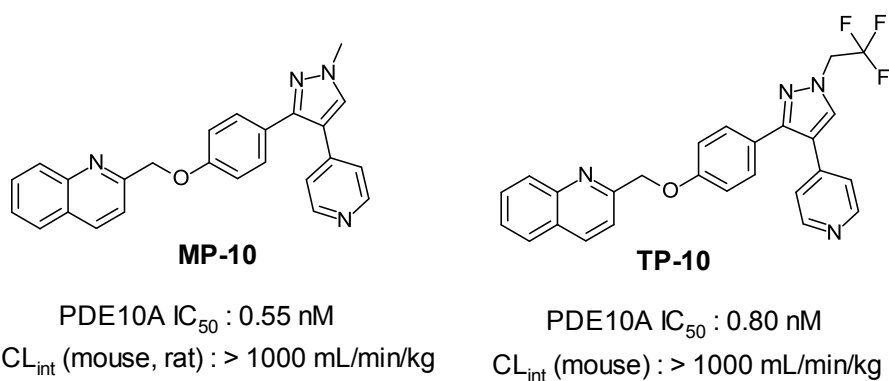


Figure 5. Structures of MP-10 and TP-10.

MP-10や TP-10の構造変換を行うに先立ち、代謝安定性を向上させる手がかりを得るために MP-10の MLM 中での代謝物検索を行った。その結果 Figure 6に示すように、主代謝経路は MP-10からキノリニルメチル部が脱離した化合物**1**へと至る経路、ならびに MP-10のキノリン環が酸化された化合物**2**へと至る経路であることがわかった。また化合物**1**からピラゾール上のメチル基が脱離した化合物**3**も検出された。Figure 7に示すように、MP-10と PDE10A の X 線共結晶構造解析の結果、キノリン環は PDE10A のいわゆる'selectivity pocket'を占め、キノリン環の窒素原子は PDE10A の Tyr693と相互作用していることが報告されており¹⁶⁾、キノリン環が他の PDE サブタイプとの選択性発現に重要である可能性があった。そこで、MP-10のキノリン環は固定し、MP-10から化合物 **1** および**3**へと至る代謝経路を抑制するために、「ピラゾール-フェノキシメチレン」部を変換し代謝安定性の向上を目指すこととした。

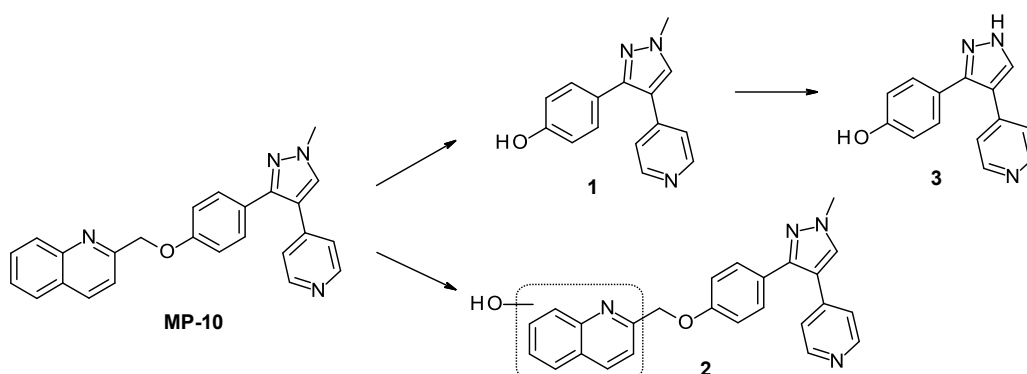


Figure 6. Proposed major *in vitro*-metabolic pathways of MP-10 in mouse liver microsomes.

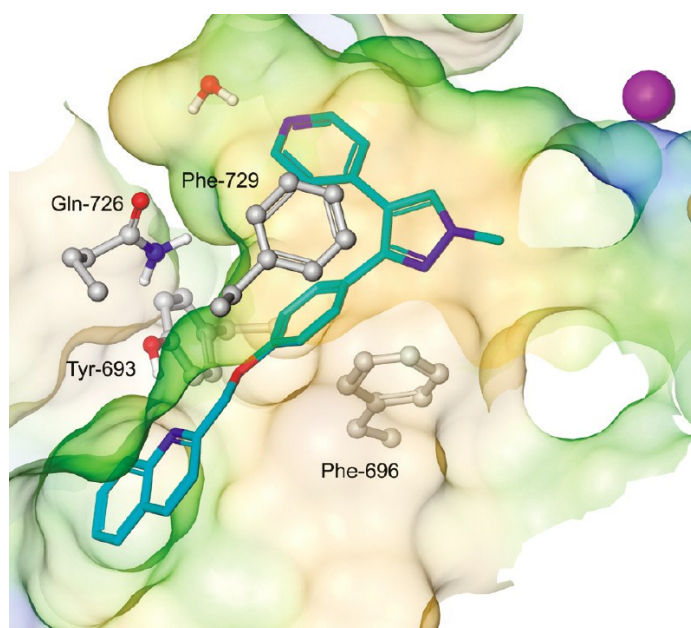
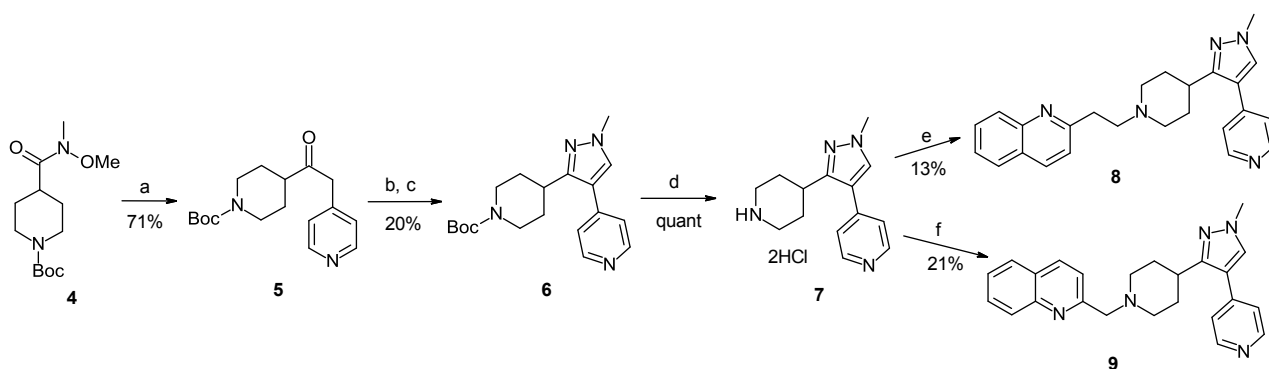


Figure 7. MP-10 bound in PDE10A pocket (PDB 3HR1)¹⁶⁾.

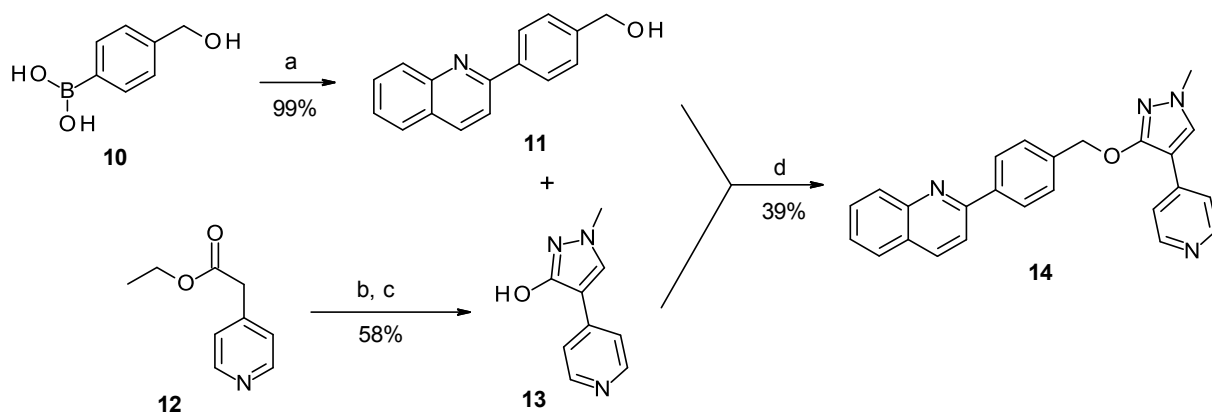
第二節 化合物の合成

一連の MP-10誘導体は以下に示す方法を用いて合成した。ピラゾール環とキノリン環のリンカーにピペリジンを有する化合物**8**および**9**の合成法を Scheme 1に示す。まず、市販の Weinreb アミド**4**をリチウムジイソプロピルアミド (LDA) で処理した4-メチルピリジンと反応させケトン体**5**を得た。化合物**5**を、*N,N*-ジメチルホルムアミドジメチルアセタール (DMF-DMA) と反応させた後、メチルヒドラジンを反応させることにより環化反応を行いピラゾール**6**とした。**6**の *tert*-ブトキシカルボニル基 (Boc 基) を除去しアミン**7**とした後、2-ビニルキノリンまたは2-(クロロメチル)キノリンと反応させることにより、目的物**8, 9**をそれぞれ合成した。

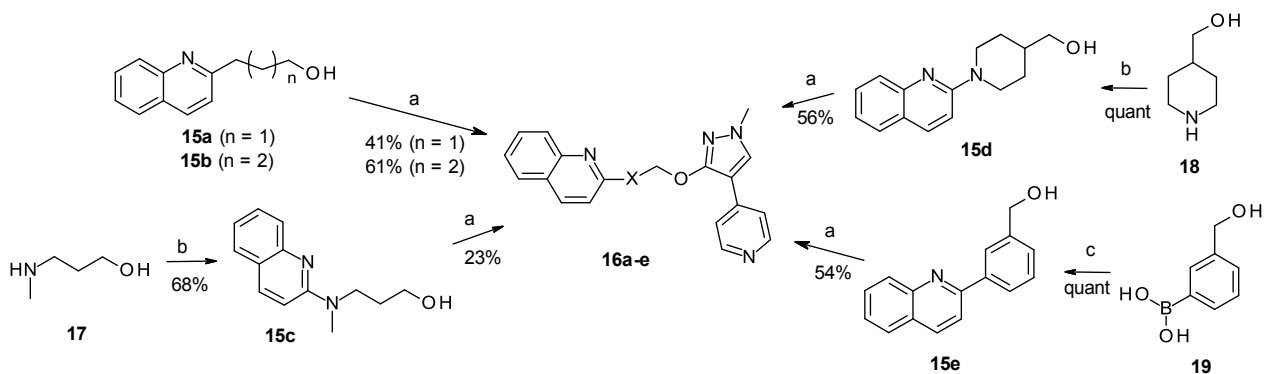


Scheme 1. Reagents and conditions: (a) 4-methylpyridine, LDA, THF; (b) DMF-DMA, DMF; (c) methylhydrazine, AcOH, EtOH; (d) HCl, dioxane, MeOH; (e) Et₃N, MeOH; then 2-vinylquinoline, AcOH, EtOH; (f) 2-(chloromethyl)quinoline, *i*Pr₂NEt, DMF.

次にリンカーにエーテル部を有する MP-10誘導体の合成法を示す。化合物**14**は Scheme 2に示す経路により合成した。市販のボロン酸**10**と2-クロロキノリンの鈴木-宮浦カップリングにより**11**を合成した。市販のエステル**12**を DMF-DMA、次いでメチルヒドラジンと連続して反応させてピラゾール体**13**を得、1,1'-(アゾジカルボニル)ジピペリジン (ADDP) とトリブチルホスフィン存在下で**11**と反応させることにより、目的物**14**を得た。Scheme 3に示すように化合物**16a-e** も、対応するアルコール**15a-e** と**13**を ADDP とトリブチルホスフィン存在下で反応させることにより合成した¹⁷⁾。なお、**15c** および**15d** は市販のアミン**17**または**18**を炭酸カリウム存在下で2-クロロキノリンと *ipso*-置換反応させることによりそれぞれ合成した。**15e** は市販のボロン酸**19**と2-クロロキノリンとの鈴木-宮浦カップリング反応により合成した。



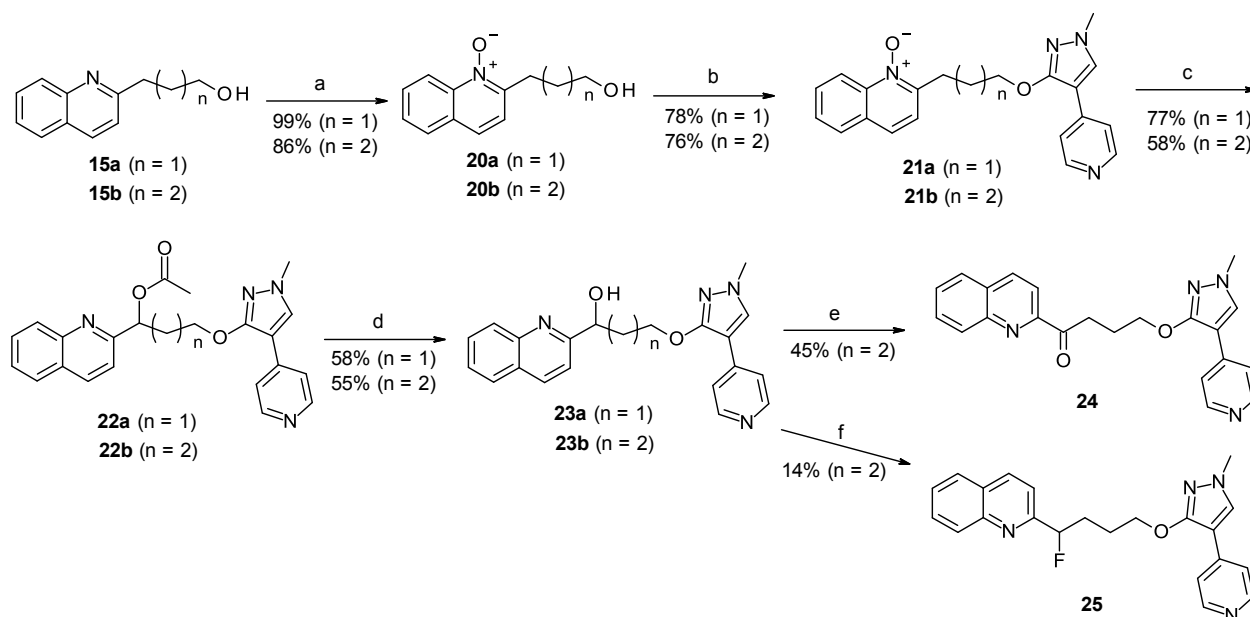
Scheme 2. Reagents and conditions: (a) 2-chloroquinoline, $\text{Pd}(\text{PPh}_3)_4$, Na_2CO_3 , DME, H_2O ; (b) DMF-DMA, DMF; (c) methylhydrazine, AcOH, EtOH; (d) ADDP, $n\text{Bu}_3\text{P}$, THF.



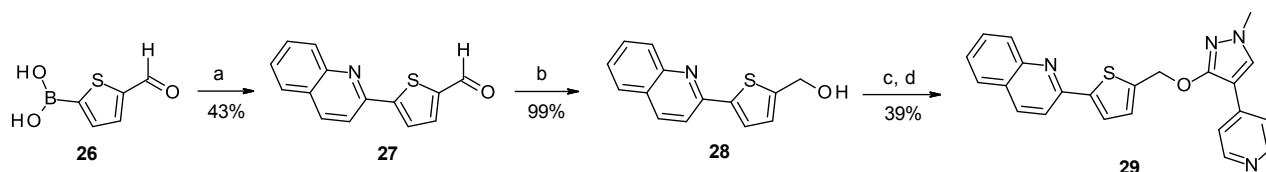
Scheme 3. Reagents and conditions: (a) **13**, ADDP, $n\text{Bu}_3\text{P}$, THF; (b) 2-chloroquinoline, K_2CO_3 , DMF; (c) 2-chloroquinoline, $\text{Pd}(\text{PPh}_3)_4$, Na_2CO_3 , DME, H_2O .

次に、化合物**23a**, **23b**, **24**, **25**の合成法を Scheme 4に示す。まず、**15a**, **15b**を3-クロロ過安息香酸で酸化しN-オキシド体**20a**, **20b**とし、次いで ADDP とトリブチルホスフィン存在下で**13**と反応させ、**21a**, **21b**とした。**21a**, **21b**を無水酢酸と反応させ**22a**, **22b**とし、次いでエステルを加水分解することによりアルコール体**23a**, **23b**を合成した。また**23b**に関しては、二酸化マンガンの酸化することによってケトン体**24**へと変換し、また三フッ化ジエチルアミノ硫黄 (DAST) によりヒドロキシル基をフルオロ基へ変換することにより**25**を得た。

リンカーにチオフェンを有する化合物**29**の合成法を Scheme 5に示す。市販のボロン酸**26**と2-クロロキノリンとの鈴木-宮浦カップリング反応により**27**を合成し、次いでアルデヒドを水素化ホウ素ナトリウムで還元し、アルコール**28**を得た。最後に**28**のヒドロキシル基を塩化チオニルでクロロ化した後、**13**と炭酸カリウム存在下で反応させることにより、目的物**29**を合成した。

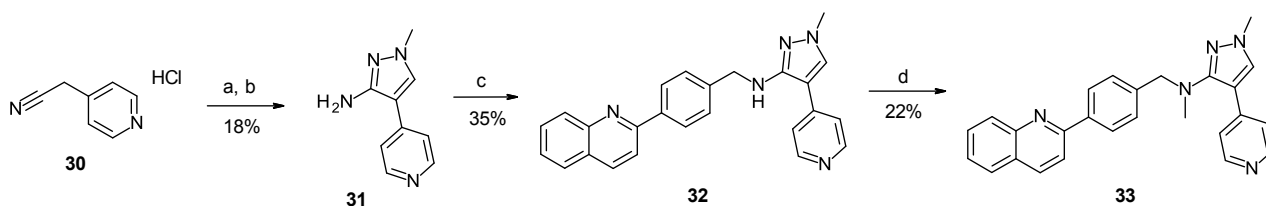


Scheme 4. Reagents and conditions: (a) 3-chloroperbenzoic acid, CH_2Cl_2 ; (b) **13**, ADDP, $n\text{Bu}_3\text{P}$, THF; (c) Ac_2O ; (d) aq NaOH, MeOH; (e) MnO_2 , CH_2Cl_2 ; (f) diethylaminosulfur trifluoride, CH_2Cl_2 .



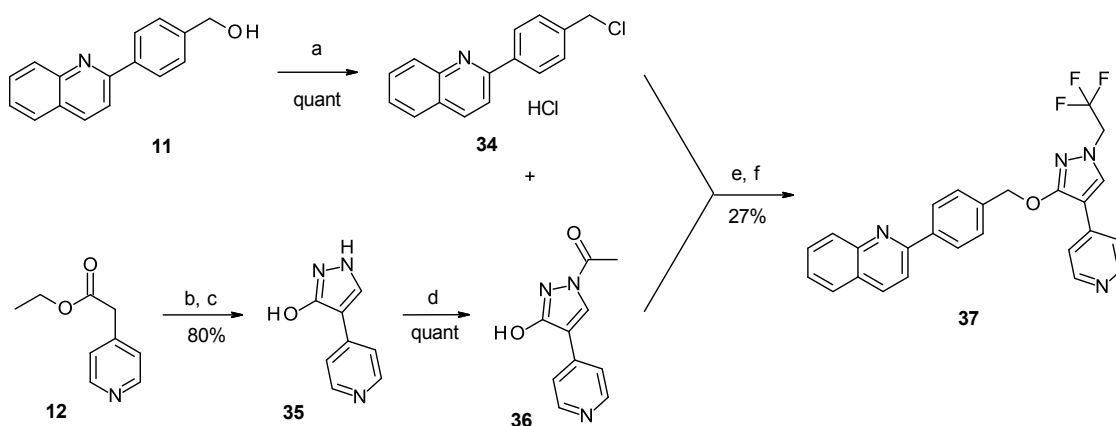
Scheme 5. Reagents and conditions: (a) 2-chloroquinoline, $\text{Pd}(\text{PPh}_3)_4$, Na_2CO_3 , DME, H_2O ; (b) NaBH_4 , EtOH; (c) SOCl_2 , CH_2Cl_2 ; (d) **13**, K_2CO_3 , DMF.

リンカー部にアミンを有する化合物**32**, **33**の合成法を Scheme 6に示す。市販のピリジン-4-イルアセトニトリル塩酸塩 (**30**) を水酸化ナトリウムでフリー体にした後、DMF-DMA 続いてメチルヒドラジンと反応させ、アミノピラゾール**31**を得た。4-(キノリン-2-イル)ベンズアルデヒドと化合物**31**の還元的アミノ化反応により**32**へと変換し、さらにホルマリンと反応させ *N*-メチル体**33**を合成した¹⁸⁾。



Scheme 6. Reagents and conditions: (a) aq NaOH, EtOH, THF; then DMF-DMA, DMF; (b) methylhydrazine, AcOH, MeOH; (c) 4-(quinolin-2-yl)benzaldehyde, $\text{Ti}(\text{O}i\text{Pr})_4$, 1,2-dichloroethane; then NaBH_4 , MeOH; (d) formalin, $\text{NaBH}(\text{OAc})_3$, AcOH, CH_2Cl_2 .

TP-10誘導体**37**の合成法を Scheme 7に示す。まず、エステル**12**を DMF-DMA と反応させた後、ヒドラジン水和物で環化反応を行いピラゾール体**35**を得た。次いで、ピラゾールの窒素原子を無水酢酸でアセチル化して**36**とした。化合物**11**のヒドロキシル基を塩化チオニルでクロロ化した**34**を用いて**36**をアルキル化した後、ピラゾールのアセチル基を脱保護し、さらに炭酸セシウム存在下1,1,1-トリフルオロ-2-ヨードエタンでアルキル化することにより、目的物**37**を合成した。



Scheme 7. Reagents and conditions: (a) SOCl_2 , CH_2Cl_2 ; (b) DMF-DMA, DMF; (c) hydrazine hydrate, AcOH, EtOH; (d) Ac_2O , pyridine; (e) K_2CO_3 , DMF; then MeOH, H_2O ; (f) 1,1,1-trifluoro-2-iodoethane, Cs_2CO_3 , DMF.

第三節 PDE10A 阻害活性および代謝安定性の評価結果ならびに考察

合成した化合物の *in vitro* PDE10A 阻害活性は、ヒトリコンビナント PDE10A による cAMP 加水分解反応を50%阻害する化合物濃度を IC₅₀値として算出することにより評価した。

第一節で述べたように、MP-10の「ピラゾール-フェノキシメチレン」部を変換するにあたり、まず特許で報告されていた MP-10や TP-10のピラゾール部変換体を評価することとした (Table 1)¹⁹⁾。なお、Table 1に示した MP-10や TP-10を含む化合物の構造は特許に記載されているが、具体的な PDE10A 阻害活性ならびに代謝安定性についての記載はなかった。Table 1のピラゾール誘導体の中では、化合物**38**や**40**が強い PDE10A 阻害活性を示したが、MLM およびヒト肝ミクロソーム (HLM) 中での固有クリアランスは大きな値を示した。また、トリアゾール誘導体**41**に関しては、1000 nM までの濃度において PDE10A 阻害活性を示さなかった。MP-10や TP-10と比較すると、化合物**40**は PDE10A 阻害活性の面では同等であったが、HLM 中でのクリアランス値は MP-10や TP-10よりも大きな値を示した。これらの結果から、この部位は MP-10のピラゾール構造に固定し、MP-10のフェノキシメチレンに代わるリンカーを探索し、代謝安定性の改善を目指すこととした。

Table 2に示すように、まず MP-10のフェノキシメチレン部をピペリジンを含むリンカーに変換したが、化合物**8**および**9**は PDE10A 阻害活性を消失した。ピペリジン環の強い塩基性が PDE10A 阻害活性を消失させた可能性がある。直鎖エーテル誘導体**16a** は8.9 nM の IC₅₀値を示し、強い PDE10A 阻害活性を有することがわかった。また、一炭素増炭した**16b** も**16a** と同等の活性を示した。MP-10が PDE10A に結合している構造 (PDB code: 3HR1) に化合物**16a** および**16b** を重ね合わせたところ、Figure 8に示すように、これら3つの化合物のピリジン環とキノリン環は良い重なりを示すことがわかった。これらの結果より、MP-10のフェノキシメチレンリンカーは PDE10A 阻害活性の発現に必須ではなく、ピリジン環とキノリン環を適切な位置に配置する役割を果たしている可能性が考えられる。一方、化合物**16a** および**16b** はいずれも MLM および HLM 中で不安定であることが判明した。そこで**16a** と**16b** のキノリン環に結合している炭素原子が酸化的に代謝されている可能性を考え²⁰⁾、ヒドロキシル基でこの位置をブロックした**23a** と**23b** を評価したところ、**23a** は PDE10A 阻害活性が大幅に減弱したが、**23b** の活性は4倍程度の減

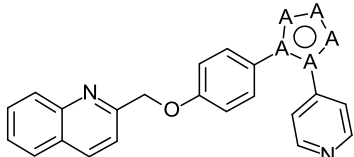
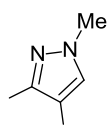
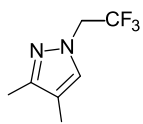
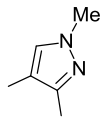
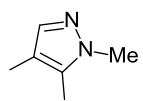
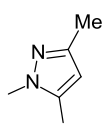
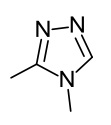
弱にとどまり中程度の PDE10A 阻害活性を示した。**23b** は MLM 中では不安定であったが、**16b** と比較して HLM 中でのクリアランスには若干の改善が見られた。そこで**23b** のヒドロキシル基をオキソおよびフルオロ基へと変換した**24**および**25**の評価を行ったが、PDE10A 阻害活性は減弱し HLM での代謝安定性も悪化する結果となった。また、**16b** のキノリン環に隣接する炭素原子を窒素原子に変換した**16c** に関しては、PDE10A 阻害活性は減弱したものの HLM 中でのクリアランスには若干の改善が見られた。

次に、**16c** のリンカーに環構造を導入することにより、代謝安定性を改善することを試みた。**16c** の直鎖アミン部を環状のピペリジンへと変換した**16d** は HLM 中での代謝安定性は向上しなかったが、中程度の PDE10A 阻害活性を示すことがわかり、別の環状構造の導入を検討した。**16d** のピペリジン環をフェニル環へと変換した**14**は**16d** と同程度の PDE10A 阻害活性を示した。また化合物**14**の MLM 中での代謝安定性は**16d** や MP-10と比較して大幅に改善しており、HLM 中での代謝安定性も**16d** や MP-10と比較して改善していることがわかった。化合物**14**を MP-10と比較すると、フェノキシメチル基とフェニル基の Hammett の置換基定数がほぼ同じことから²¹⁾、キノリン環の電子密度に大きな違いはないと考えられ、リンカー部の違いがキノリン環の酸化的代謝速度に与える影響は小さいと考えられる。したがって、**14** のオキシメチレンフェニル部そのものが MP-10のリンカーであるフェノキシメチレン部よりも代謝的に安定であることが示唆される。次いで、**14**のフェニル環を生物学的等価体であるチオフェン環へと変換した**29**を評価したところ、期待通り PDE10A 阻害活性は保持し、MLM および HLM 中での代謝安定性も MP-10と比較して良好であった。一方、化合物**14**のエーテルリンカーをアミンリンカーへと変換した**32**および**33**や、**14**のフェニル基上の置換位置をパラ位からメタ位に変えた**16e** の PDE10A 阻害活性は減弱する結果となった。

これまでに述べた化合物の中で、**14**は MLM および HLM 中で最も安定で且つ中程度の PDE10A 阻害活性を示した。そこで**14**のピラゾール環上のメチル基を TP-10と同様のトリフルオロエチル基へと変換した化合物**37**の評価を行った。しかしながら MP-10と TP-10の関係と異なり、**14**と比較して**37**の PDE10A 阻害活性は大幅に減弱する結果となった (Table 3)。化合物**37**の PDE10A 阻害活性が大幅に減弱した理由は不明であるが、化合物**37**の PDE10A への結合様式が TP-10とは異なっている可能性があり、その結果トリフルオロエチル基のかさ高さが PDE10A との相互作用に不利であった可能性がある。

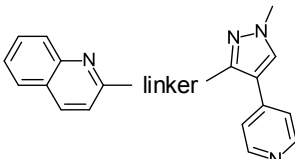
以上の検討により得られた化合物**14**について、他の PDE サブタイプとの選択性を調べた結果、Table 4 に示すように PDE1, 2, 3, 4B, 4D, 5 および 9 に対して 540 倍以上の選択性を示すことがわかった。次に、化合物**14**の *in vivo* での血漿中および脳内濃度を TP-10 と比較した。化合物**14**および TP-10 を 30 mg/kg の用量でマウスに腹腔内投与したところ、投与後 1～2 時間後において、**14**は TP-10 よりも 2～3 倍程度高い血漿中濃度を示した (Table 5)。化合物**14**は TP-10 よりも MLM 中での代謝安定性が大幅に向上したことが、**14**の高い血漿中濃度に寄与したと考えられる。また、化合物**14**は脳内濃度も高く、投与後 30 分から 2 時間までの $K_{p,brain}$ 値は 2.3～3.1 であり、TP-10 より中枢移行性が良好であることが判明した。

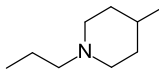
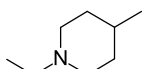
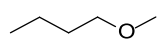
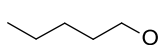
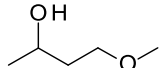
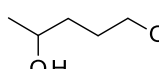
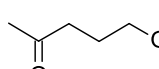
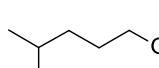
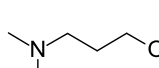
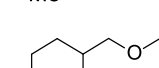
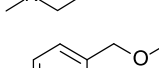
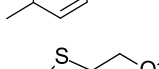
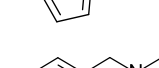
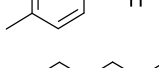
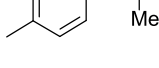
Table 1. PDE10A potency and intrinsic clearance for heterocyclic derivatives

				
comps	A	PDE10A IC ₅₀ (nM)	mouse CL _{int} (mL/min/kg)	human CL _{int} (mL/min/kg)
MP-10		0.55	>1000	186
TP-10		0.80	>1000	218
38		4.4	984	274
39		183	841	193
40		0.50	>1000	721
41		>1000	NT ^a	NT ^a

^a Not tested

Table 2. Effect of linker unit on PDE10A inhibitory activity and intrinsic clearances



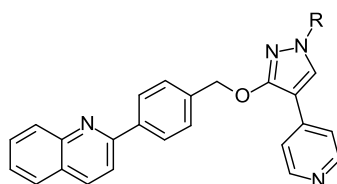
comps	linker	PDE10A IC ₅₀ (nM)	mouse CL _{int} (mL/min/kg)	human CL _{int} (mL/min/kg)
8		>1000	NT ^a	NT ^a
9		>1000	NT ^a	NT ^a
16a		8.9	>1000	>1000
16b		8.7	>1000	>1000
23a		719	NT ^a	NT ^a
23b		37	>1000	527
24		790	NT ^a	NT ^a
25		115	NT ^a	>1000
16c		102	NT ^a	660
16d		19	>1000	589
14		29	366	124
29		25	556	138
32		215	NT ^a	NT ^a
33		100	NT ^a	NT ^a
16e		>100	NT ^a	NT ^a

^a Not tested



Figure 8. Superposition of MP-10 (orange), **16a** (magenta) and **16b** (cyan).

Table 3. Substitution effect on pyrazole ring



compds	R	PDE10A IC ₅₀ (nM)	mouse CL _{int} (mL/min/kg)	human CL _{int} (mL/min/kg)
14	Me	29	366	124
37	-CH ₂ CF ₃	239	NT ^a	NT ^a

^a Not tested

Table 4. Selectivity of compound **14** toward PDEs isoforms

isoform	Selectivity
PDE1	5700
PDE2	2500
PDE3	850
PDE4B	640
PDE4D	540
PDE5	1400
PDE9	5700

Table 5. Plasma and brain concentrations of compound **14** and TP-10 after 30 mg/kg intraperitoneal dosage

time (h)		0.5	1	2	4
14	plasma conc. (ng/mL)	9040	9100	9377	NT ^a
	brain conc. (ng/g)	20383	25383	29100	NT ^a
TP-10	plasma conc. (ng/mL)	NT ^a	4097	3263	1117
	brain conc. (ng/g)	NT ^a	5807	3893	1183

^a Not tested

第四節 行動薬理試験における薬効評価

第三節で述べたように、化合物**14**は中程度の PDE10A 阻害活性を示し、マウスに腹腔内投与後の脳内濃度も良好であったことから、統合失調症の陽性症状のモデルである PCP 誘発マウス過活動の系で、化合物**14**の評価を行った。その結果、Figure 9A に示すように、化合物**14**の腹腔内投与により投与量依存的にマウスの自発運動量 (locomotor activity) を抑制し、その ED₅₀値は14 mg/kg であった。さらに、Figure 9B に示すように、化合物**14**は経口投与でもマウスの過活動を抑制し、その ED₅₀値は19 mg/kg であった。なお、この結果から、**14**はマウスにおいて良好な生物学的利用能を示すことが示唆される。

次に、統合失調症の認知機能障害のモデルと考えられる新生児期に PCP を投与したマウスの NORT を行い、化合物**14**の *in vivo* 薬効を評価した。なお、NORT は新奇性を好むというげっ歯類の特性を利用した、認知機能の評価する試験系である。Figure 10に示すように、正常マウスが65%程度の時間を新奇物体の探索に費やしたのに対し、新生児期に PCP を投与されたマウスは新奇物体の探索に50%程度の時間を費やすのみであり、視覚認識記憶が障害されていた。この PCP を投与したマウスに化合物**14**を経口投与したところ、0.1 mg/kg および0.3 mg/kg の投与量で新奇物体の探索時間が統計学的に優位に増加し、マウスの記憶障害が改善された。

化合物**14**の *in vitro* PDE10A 阻害活性は MP-10より弱いにも関わらず、上記の NORT において視覚認識記憶障害の改善作用を示しており、記憶形成時の化合物**14**の脳内濃度が薬効発現に十分であったと示唆される。MP-10と比べて化合物**14**の高い代謝安定性が、その *in vivo* 薬効発現に寄与していると考えられる。

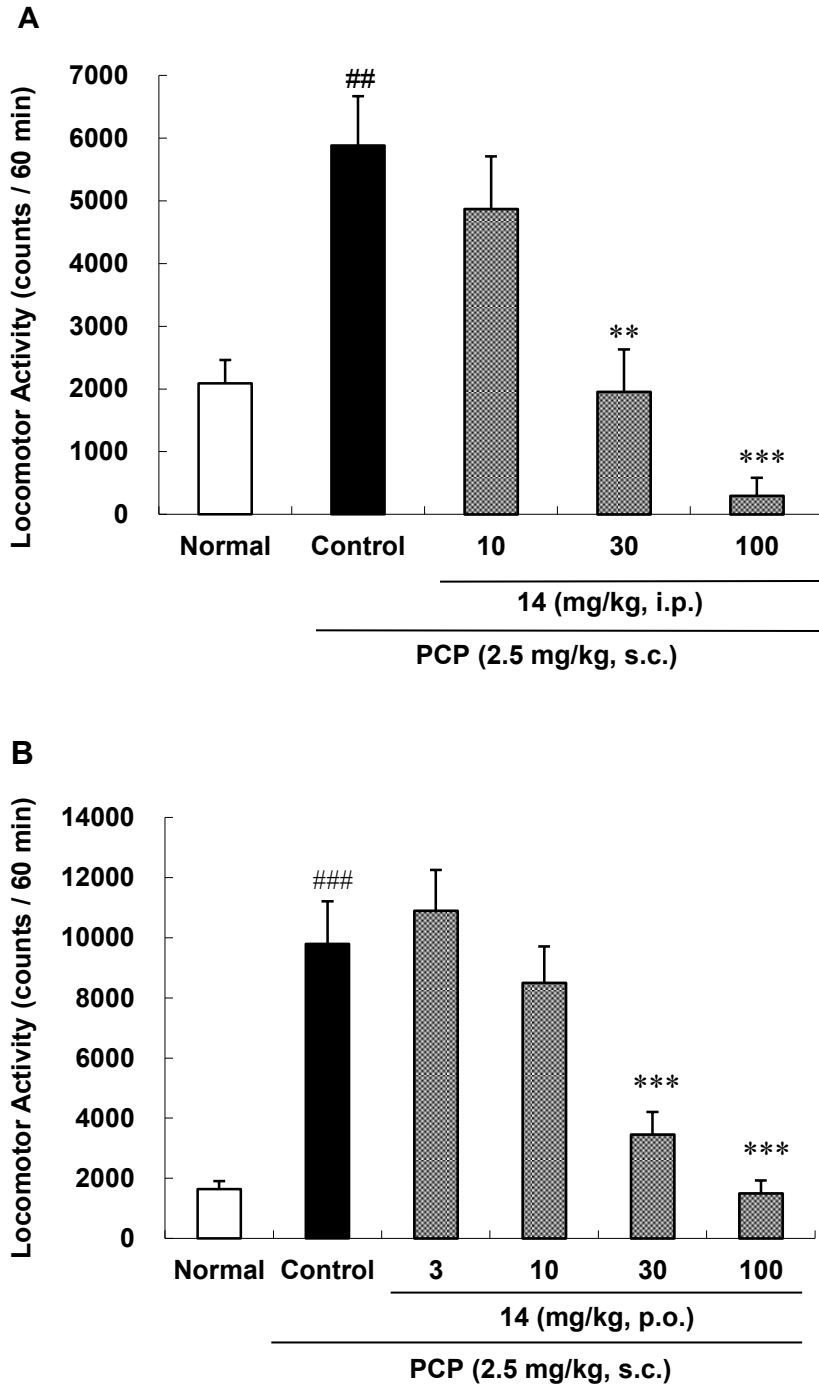


Figure 9. Effect of **14** on PCP-induced hyperlocomotion in mice. PCP was administered subcutaneously. The data represent the mean \pm SEM ($n = 6$ in each group): (##) $p < 0.01$, (###) $p < 0.001$ vs normal group (Student's t -test); (**) $p < 0.01$, (***) $p < 0.001$ vs control group (Dunnett's test). (A) **14** was administered intraperitoneally. (B) **14** was administered orally.

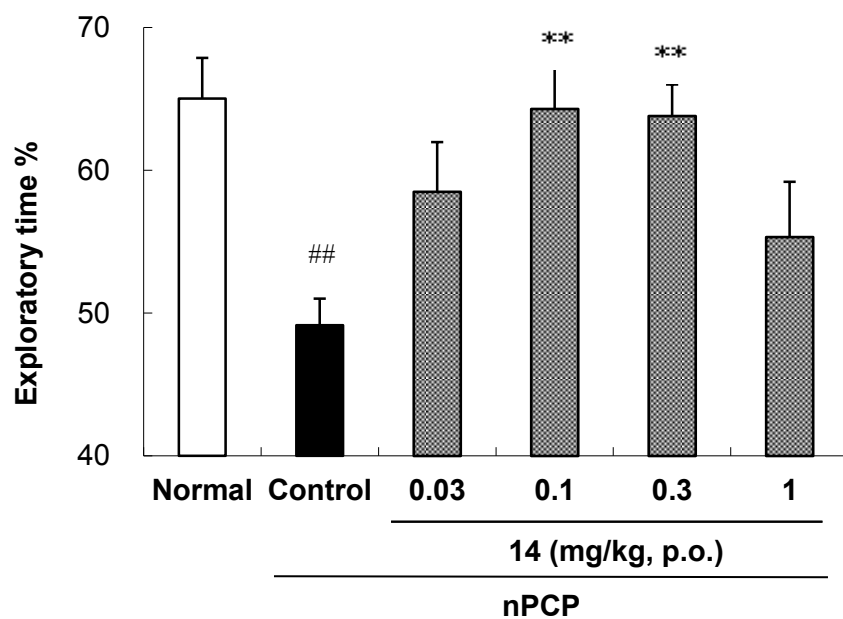


Figure 10. Effect of **14** on neonatal PCP treatment-induced learning deficit in mice during novel object recognition test. The data represent the mean \pm SEM ($n = 12$ in each group): (##) $p < 0.01$ vs normal group (Student's t -test); (**) $p < 0.01$ vs control group (Dunnett's test).

第五節 本章のまとめ

本章では、MP-10の MLM 中での代謝安定性の改善を目指し、MP-10の MLM 中での推定代謝経路を参考にして MP-10の構造変換を行った。その結果、MP-10のリンカーにあたるフェノキシメチレン部をオキシメチレンフェニルへと変換した化合物**14**が、MLM および HLM 中で高い代謝安定性を示すことを見出した。化合物**14**は他の PDE サブタイプへの選択性も良好で、マウスに腹腔内投与後の血漿中および脳内濃度も高いことがわかった。さらに、化合物**14**を腹腔内投与もしくは経口投与することにより、PCP 誘発マウス過活動の系において投与量依存的に過活動を抑制し、新生児期に PCP を投与したマウスの NORT においても、**14**は0.1, 0.3 mg/kg (p.o.) で視覚認識記憶の改善作用を示した。これらの結果から、化合物**14**は新規 PDE10A 阻害剤を見出すためのリード化合物になり得ると考えられる (Figure 11)。

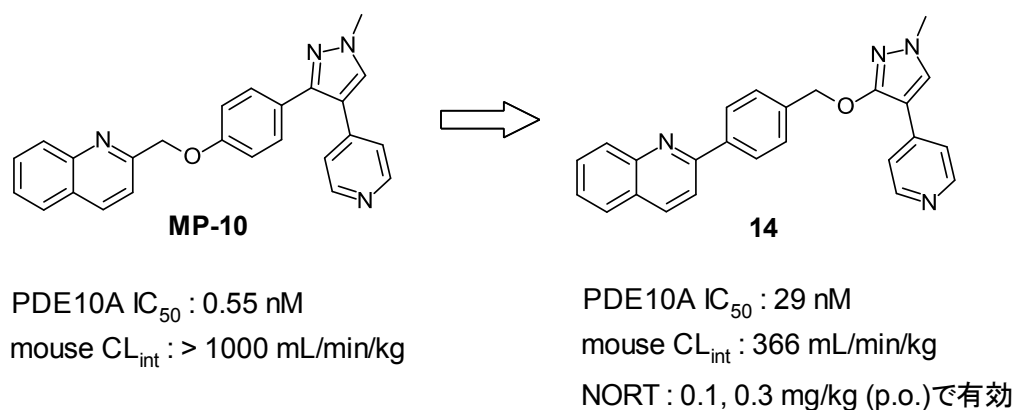


Figure 11. Summary of chapter 1.

第二章 CYP3A4阻害を減弱したキノリニルフェニル誘導体の合成および構造活性相関

第一節 分子設計

第一章で述べた通り、ファイザー社より報告されていた選択的 PDE10A 阻害剤である MP-10から合成展開を行い、そのリンカーにあたるフェノキシメチレン部を変換することにより MLM および HLM 中の代謝安定性が向上した新規リード化合物**14**を見出した (Figure 12)。化合物**14**は統合失調症の陽性症状ならびに認知機能障害のマウス行動薬理モデルで有効性を示したが、**14**の PDE10A 阻害活性は中程度にとどまった。また、**14**と MP-10は強い CYP3A4阻害作用を有することが判明した。CYP3A4は上市されている薬の半数以上の代謝に関わっているとされているため、CYP3A4の阻害は臨床現場で薬物-薬物相互作用を引き起こす懸念がある²²⁾。

化合物**14**や MP-10に含まれる4-ピリジル基のような塩基性ヘテロ芳香環は CYP のへムに配位することができ、CYP 阻害を引き起こすことが知られている²³⁾。よって、化合物**14**の4-ピリジル基の窒素原子の近傍に立体障害となる置換基導入や、ピリジン環から他のヘテロ環への変換により、化合物が CYP のへムに配位するのを妨げ CYP3A4阻害作用の減弱が達成できると考えた。一方で、MP-10と PDE10A の X 線共結晶構造から¹⁶⁾、**14**のピリジン環の窒素原子は、PDE10A のポケット内の水分子と水素結合により相互作用していると推定され、PDE10A 阻害活性に重要な可能性も考えられた。そこで、PDE10A 阻害活性の増強と CYP3A4阻害の低減を両立できる構造を見出すべく、新たな合成展開を開始した。

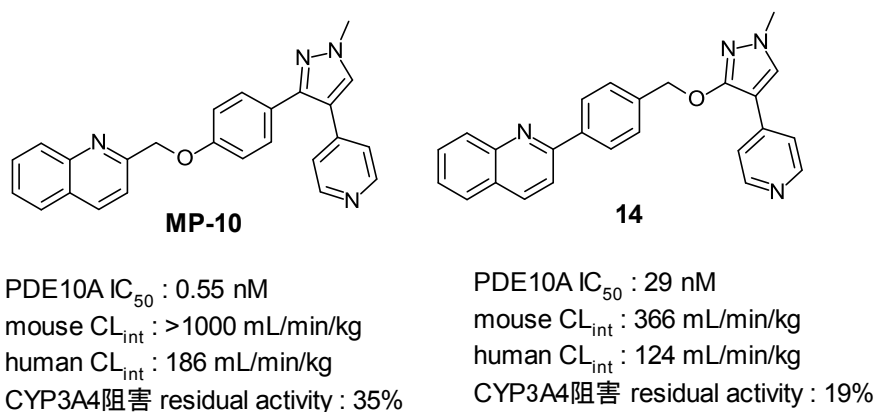
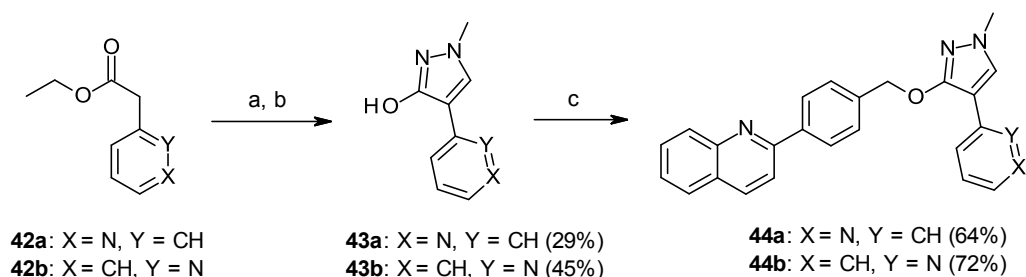


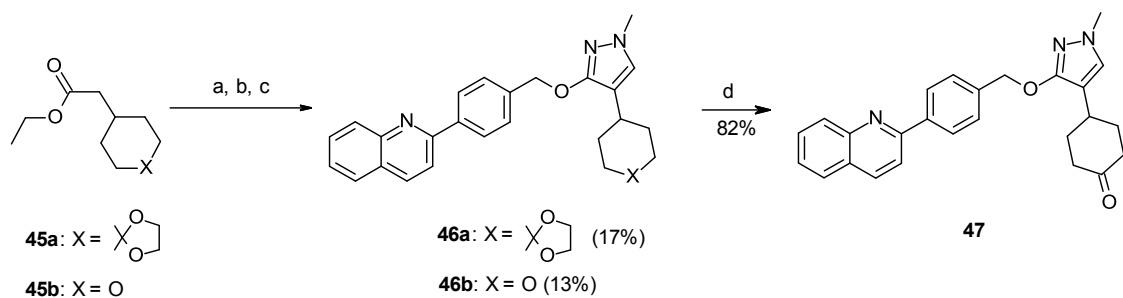
Figure 12. Structures of MP-10 and compound **14**.

第二節 化合物の合成

第二章で評価した化合物**14**の誘導体は以下に示す方法を用いて合成した。3-ピリジル体**44a** および2-ピリジル体**44b** は Scheme 8に示す方法により合成した。市販のエステル**42a**, **42b** を DMF-DMA と反応させた後、メチルヒドラジンによりピラゾール環を構築し**43a**, **43b** とした。次いで、ADDP およびトリブチルホスフィン存在下、第一章で合成法を報告済みの**11**と反応させることにより目的物**44a**, **44b** を得た。また、この Scheme 8と同様の方法により、市販試薬である**45a**, **45b** から化合物**46a**, **46b** を合成した (Scheme 9)。化合物**47**に関しては、**46a** のアセタール部を脱保護することにより合成した。



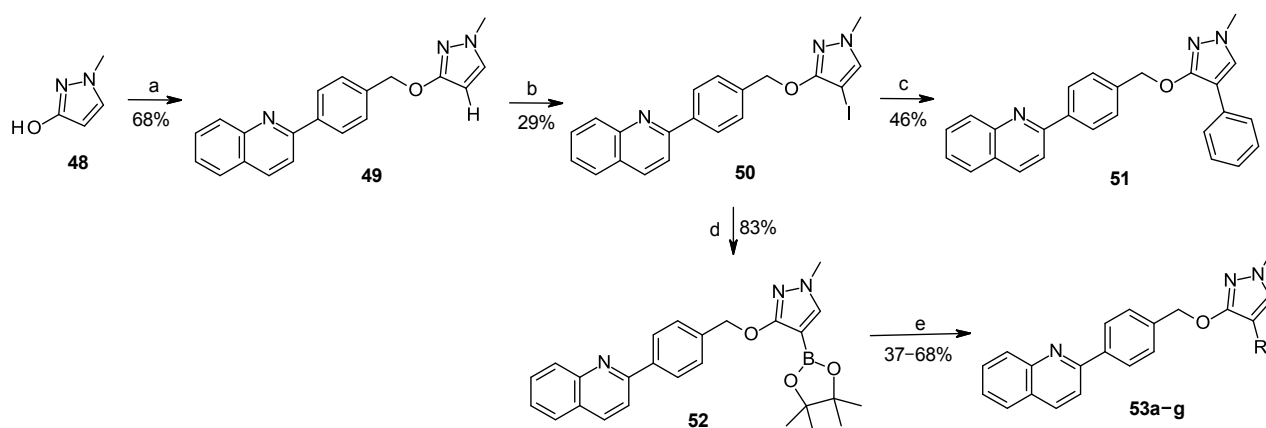
Scheme 8. Reagents and conditions: (a) DMF-DMA, DMF; (b) methylhydrazine, AcOH, EtOH; (c) **11**, ADPP, $n\text{Bu}_3\text{P}$, THF.



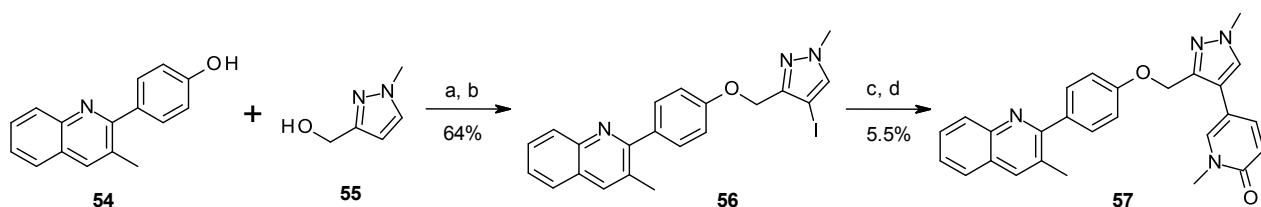
Scheme 9. Reagents and conditions: (a) LiHMDS, HCO_2Me , THF; (b) methylhydrazine, EtOH; (c) **34**, K_2CO_3 , DMF; (d) $p\text{-TsOH}\cdot\text{H}_2\text{O}$, THF, H_2O .

Scheme 10に化合物**51**および**53a-g**の合成法を示す。ヒドロキシピラゾール誘導体**48**とアルコール**11**を ADPP とトリブチルホスフィン存在下で反応させ**49**を得た。**49**のピラゾール4位を硝酸セリウム(IV)アンモニウム (CAN) 存在下ヨウ素でヨウ素化することにより中間体**50**へと変換し、続いてフェニルボ

ロン酸との鈴木-宮浦カップリングにより目的物**51**を合成した。また中間体**50**をイソプロピルマグネシウムクロライドによるハロゲン-金属交換反応後、2-イソプロポキシ-4,4,5,5-テトラメチル-1,3,2-ジオキサボロランを添加することにより、ボロン酸エステル**52**を得た²⁴⁾。この**52**と種々の置換ハライドを鈴木-宮浦カップリングにより反応させることで目的の**53a-g**を得た。3-メチルキノリン誘導体も同様の方法により合成した。Scheme 11に示すように、化合物**54**と**55**をシアノメチレントリブチルホスホラン (CMBP) 存在下で反応させた後²⁵⁾、ピラゾール4位をヨウ素化することにより、ヨードピラゾール**56**を得た²⁶⁾。化合物**56**のイソプロピルマグネシウムクロライドによるハロゲン-金属交換反応後、2-イソプロポキシ-4,4,5,5-テトラメチル-1,3,2-ジオキサボロランによりボロン酸エステルへと変換し、続いて5-ブromo-1-メチルピリジン-2(1*H*)-オンとの鈴木-宮浦カップリングにより目的物**57**を合成した。

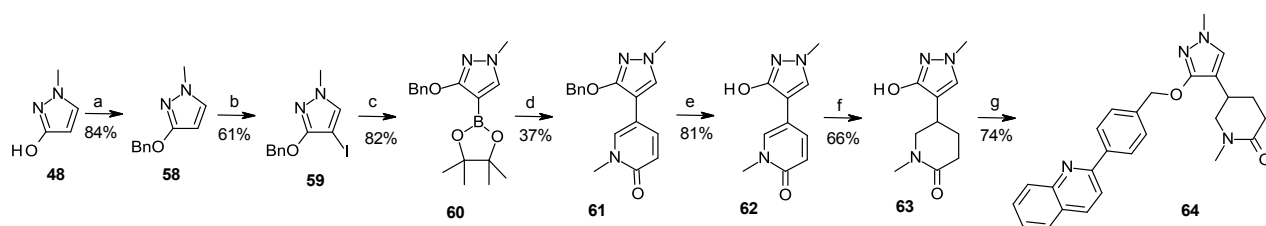


Scheme 10. Reagents and conditions: (a) **11**, ADDP, *n*Bu₃P, THF; (b) CAN, I₂, MeCN; (c) PhB(OH)₂, Pd₂(dba)₃, Xphos, K₃PO₄, *n*BuOH; (d) *i*PrMgCl, 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, THF; (e) R-Br or R-Cl, PdCl₂(dppf)·CH₂Cl₂, Na₂CO₃, DMF, H₂O.



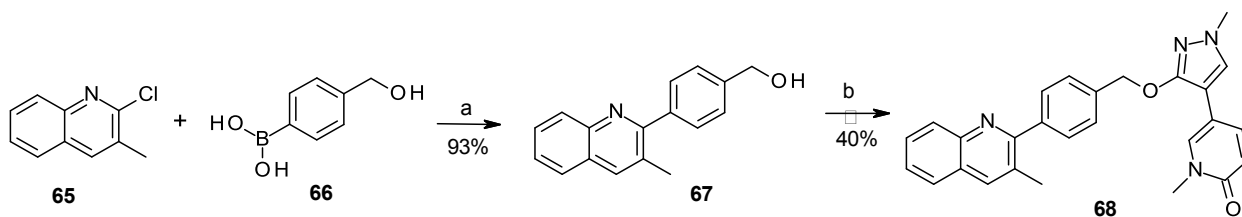
Scheme 11. Reagents and conditions: (a) CMBP, toluene; (b) CAN, I₂, MeCN; (c) *i*PrMgCl, 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, THF; (d) 5-bromo-1-methylpyridin-2(1*H*)-one, PdCl₂(dppf)·CH₂Cl₂, Na₂CO₃, DMF, H₂O.

ピラゾール4位にピペリドン有する化合物**64**の合成法を Scheme 12に示す。市販のピラゾール**48**のヒドロキシル基をベンジル基 (Bn) で保護することで**58**へと変換し、続いて CAN 存在下ヨウ素でピラゾール4位をヨード化し**59**を得た。中間体**59**のハロゲン-金属交換反応後、2-イソプロポキシ-4,4,5,5-テトラメチル-1,3,2-ジオキサボロランを添加することにより、ボロン酸エステル**60**へと変換し、続いて5-ブromo-1-メチルピリジン-2(1*H*)-オンとの鈴木-宮浦カップリングにより**61**を合成した。**61**のベンジル基を脱保護し**62**とした後、接触水素化によりピリドン環をピペリドン環に還元した**63**を得た。この**63**を炭酸カリウム存在下、**34**でアルキル化することで目的物**64**を合成した。



Scheme 12. Reagents and conditions: (a) benzyl bromide, K_2CO_3 , DMF; (b) CAN, I_2 , MeCN; (c) $iPrMgCl$, 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, THF; (d) 5-bromo-1-methylpyridin-2(1*H*)-one, $PdCl_2(dppf) \cdot CH_2Cl_2$, Na_2CO_3 , DMF, H_2O ; (e) H_2 , Pd-C, EtOH; (f) H_2 , PtO_2 , AcOH; (g) **34**, K_2CO_3 , DMF.

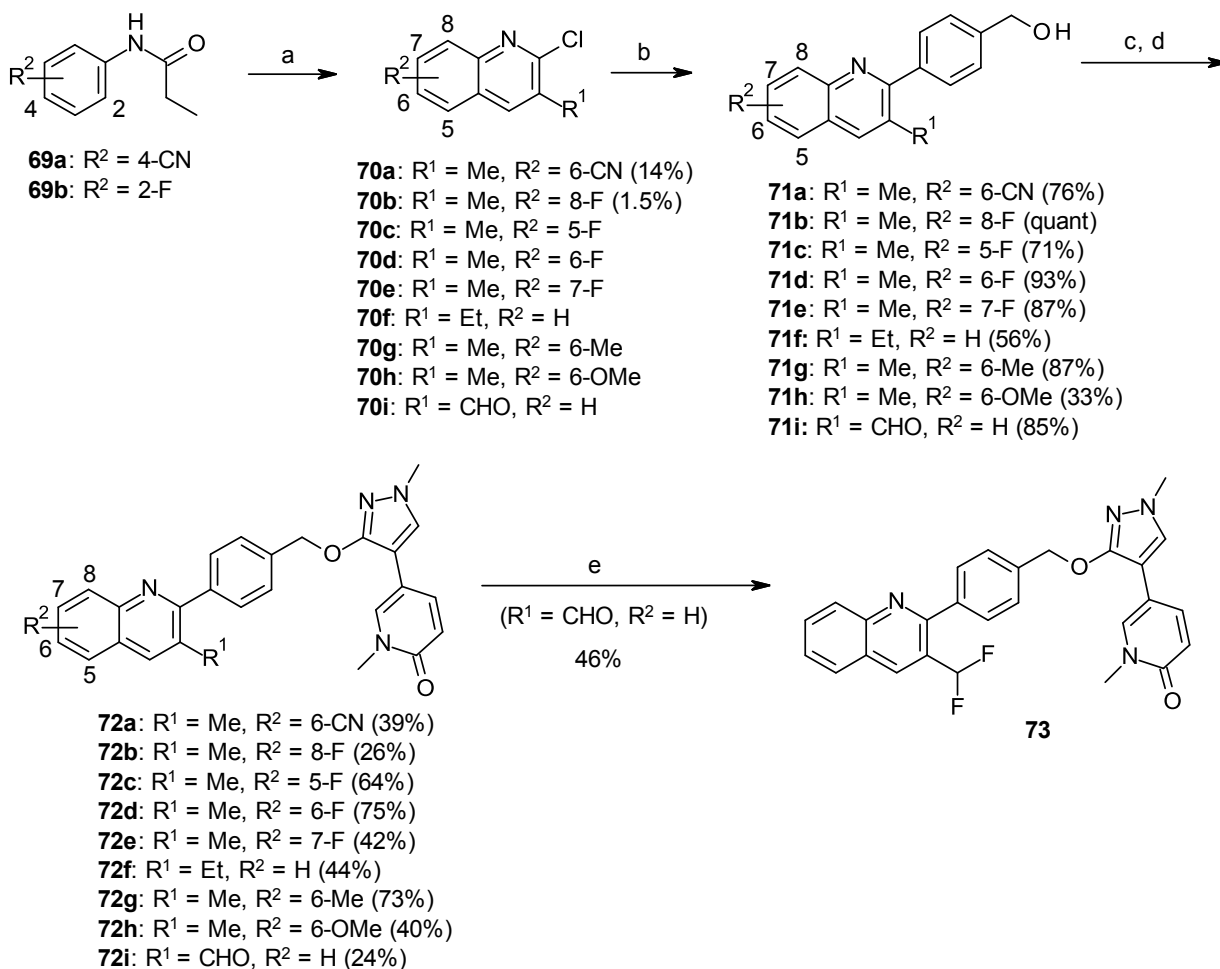
キノリン環にメチル基を導入した化合物 **68** の合成法を Scheme 13 に示す。クロロキノリン誘導体 **65** とボロン酸 **66** との鈴木-宮浦カップリングにより **67** へと変換し、続いて CMBP を用いて化合物 **62** と反応させることにより、目的物 **68** を合成した。



Scheme 13. Reagents and conditions: (a) $Pd(PPh_3)_4$, Na_2CO_3 , DME, H_2O ; (b) **62**, CMBP, toluene.

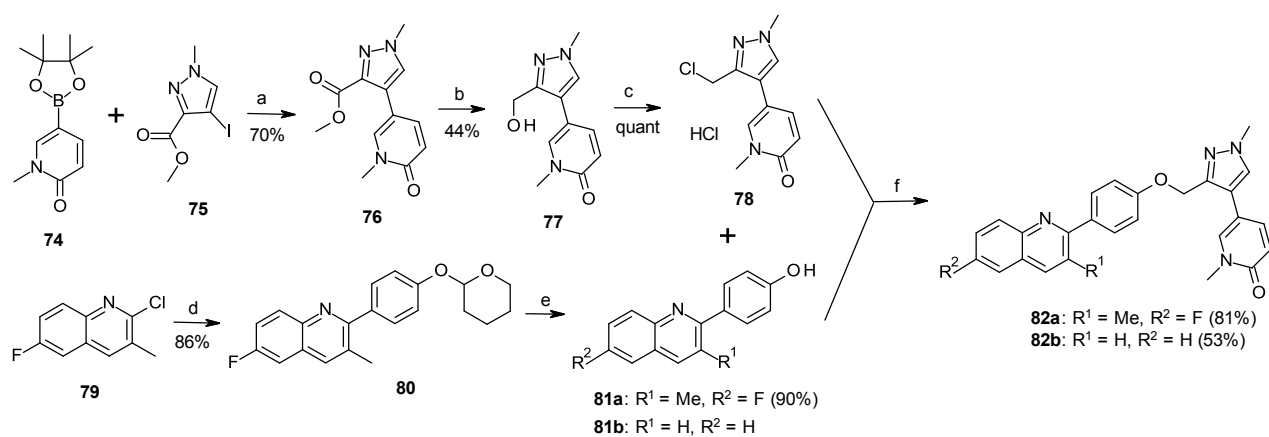
次に、キノリン環上の置換基を種々変換した化合物**72a-h**, **73**の合成は Scheme 14に記載した方法で行った。原料の**70a**, **70b**に関しては、それぞれ市販のアミド体**69a**, **69b**を Vilsmeier-Haack 反応の条件で環化反応を行い合成した²⁷⁾。なお、**70d-i**は市販試薬であり、**70c**の合成法は既に報告されている²⁸⁾。続いて、クロロキノリン誘導体**70a-i**とボロン酸**66**の鈴木-宮浦カップリングにより、**71a-i**へと変換した。

71a-i に関しては塩化チオニルによりヒドロキシル基をクロロ化した後、中間体**62**と炭酸カリウム存在下で反応させることにより**72a-i**を合成した。また**72i**のアルデヒド部を Deoxo-Fluor[®]でジフルオロメチル基へと変換し、**73**を得た。



Scheme 14. Reagents and conditions: (a) POCl₃, CTAB, DMF; (b) **66**, Pd(PPh₃)₄, Na₂CO₃, DME, H₂O; (c) SOCl₂, CH₂Cl₂; (d) **62**, K₂CO₃, DMF; (e) Deoxo-Fluor[®], CH₂Cl₂.

化合物**82a**, **82b**の合成法を Scheme 15に示す。まず、ボロン酸エステル**74**とヨードピラゾール**75**の鈴木-宮浦カップリングにより**76**を合成し、エステルを水素化ホウ素リチウムで還元することでアルコール**77**へと変換した。次いで**77**のヒドロキシル基を塩化チオニルでクロロ化することで中間体**78**を得た。また、クロロキノリン誘導体**79**とボロン酸との鈴木-宮浦カップリングにより**80**を合成し、次いでテトラヒドロピラニル基を塩酸で脱保護することで**81a**を得た。最後に**81a**および**81b**を炭酸カリウム存在下中間体**78**でアルキル化することにより、目的物**82a**および**82b**を合成した²⁹⁾。



Scheme 15. Reagents and conditions: (a) Pd(PPh₃)₄, Cs₂CO₃, DMF, H₂O; (b) LiBH₄, EtOH, THF; (c) SOCl₂, CH₂Cl₂; (d) 4-(2-tetrahydropyranyloxy)phenylboronic acid, Pd(PPh₃)₄, Na₂CO₃, DME, H₂O; (e) HCl, THF, H₂O; (f) K₂CO₃, DMF.

第三節 PDE10A 阻害活性および CYP3A4阻害活性の評価結果ならびに考察

第二節で合成した化合物の PDE10A 阻害活性は、第一章で述べた方法により評価した。化合物の CYP3A4阻害活性は、化合物 (5 μ M) と HLM を30分間ブレインキュベーションした後、CYP3A4の基質であるミダゾラムを用いて CYP3A4の残存活性を測定することにより評価した。

初めにリード化合物**14**のピリジン環の窒素原子の位置が活性に与える影響を検討したところ、**14**の4-ピリジルが最も良く、**44a**の3-ピリジル、**44b**の2-ピリジルという順に PDE10A 阻害活性が減弱した (Table 6)。リード化合物**14**のピリジン環にメチル基を導入した**53a**は PDE10A 阻害活性を保持したが、さらにメチル基を導入した**53b**は若干活性が減弱した。CYP3A4阻害活性に関してはメチル基を導入するごとに阻害活性の減弱が認められ、**53a**や**53b**のメチル基によりピリジン環の窒素原子が CYP3A4のヘムに配位するのを妨げていることが示唆された。**53a**のメチル基をメトキシ基に変換した**53c**では PDE10A 阻害活性が減弱することがわかった。次に、**14**のピリジン環をフェニル基へと変換したところ、化合物**51**は CYP3A4阻害作用をほとんど示さないことがわかった。また**51**は PDE10A 阻害活性も失う結果となった。この結果から、ピリジン環の窒素原子が CYP3A4阻害の主要因であると同時に PDE10A 阻害活性にも大きな寄与をしていることが示された。水素結合受容能を有するが塩基性を示さない環であれば、PDE10A 阻害活性を保持しながら、CYP3A4阻害を低減できると考え検討を行った。水素結合受容能は pK_{BHX} という値で定量化でき、*N*-メチルピリドン環は高い pK_{BHX} を有することが報告されていた ($pK_{BHX} = 2.50$)³⁰⁾。そこで、リード化合物**14**のピリジン環を *N*-メチルピリドン環へと変換したところ、化合物**53e**は**14**と同等の PDE10A 阻害活性を示す一方、CYP3A4阻害は低減された。しかし、**53e**の位置異性体である**53d**は CYP3A4阻害が低減されたものの、PDE10A 阻害活性も大幅に減弱した。*N*-メチルピリドン環内に窒素原子をもう一つ導入した**53f**や**53g**では、PDE10A 阻害活性が減弱した。ピリドン環を飽和化しピペリドン環へと変換した化合物**64**は、**53e**と比較して PDE10A 阻害活性は同等であったが、CYP3A4阻害活性は若干悪化した。この CYP3A4阻害の悪化は、**53e**の脂溶性 (ACD/logP = 3.2) と比べて、化合物**64**の脂溶性が高い (ACD/logP = 4.9) ことに起因している可能性がある^{24,31)}。シクロヘキサノンやテトラヒドロピラン環を有する**47**や**46b**の PDE10A 阻害活性は、**53e**と比べて減弱する結果となっ

た。

Table 7に示す化合物について pK_{BHX} 値と PDE10A 阻害活性の pIC_{50} 値との関係を調べたところ^{30,32)}、 pK_{BHX} と pIC_{50} 値との間に正の相関関係 ($R^2 = 0.779$) があり、水素結合受容能が高い程、PDE10A 阻害活性も強くなることがわかった (Figure 13)。MP-10のピリジン窒素原子が PDE10A の活性ポケット内の水分子と相互作用していることも考慮に入れると、Figure 12の結果は、本誘導体と PDE10A 活性ポケット内の水分子との水素結合の強さが、PDE10A 阻害活性に重要であることを示唆している。


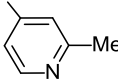
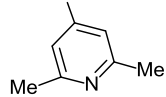
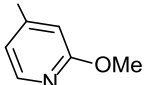
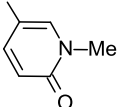
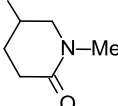
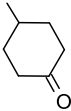

53e のキノリン環上の置換基効果についても検討を行った (Table 8)。**53e** のキノリン環の3位にメチル基を導入 (**68**) することにより、PDE10A 阻害活性が向上した。メチル基の電子供与性がキノリン窒素原子の水素結合受容能を上昇させ、化合物**68**の PDE10A 阻害活性が向上した可能性が考えられる。CYP3A4阻害に関しては、キノリン3位のメチル基により悪化する結果となった。**68**, **53e** の ACD/logP 値はそれぞれ3.7, 3.2であり、**68**の高い脂溶性が強い CYP3A4阻害を示す原因となった可能性がある。**68**のメチル基をエチル基へと変換した**72f** は、**68**よりも活性が減弱した。また、電子求引性のジフルオロメチル基 (**73**) は CYP3A4阻害を改善したが、PDE10A 阻害活性は減弱する結果となった。次いで、キノリン3位以外の置換基効果を検討した。フルオロ基により CYP3A4阻害が改善する事例が報告されていたので³³⁾、**68**のキノリン環にフルオロ基を導入したところ、7位および8位のフルオロ基 (**72e**, **72b**) は CYP3A4阻害活性にほとんど影響を与えなかったが、5位および6位のフルオロ基 (**72c**, **72d**) は CYP3A4阻害を改善することがわかった。PDE10A 阻害活性に関しては6位にフルオロ基を導入した**72d** が最も強く、 $IC_{50} = 15 \text{ nM}$ の活性を示した。そこで、**72d** のフルオロ基をメトキシ基、メチル基、シアノ基へとそれぞれ変換したところ、メチル基 (**72g**) およびシアノ基 (**72a**) を有する場合、PDE10A 阻害活性が減弱したが、メトキシ基を有する**72h** では**72d** と同等の PDE10A 阻害活性を示した。ただし、**72h** の CYP3A4阻害活性は、アッセイバッファーへの低溶解性のために評価することが出来なかった。

Table 6. PDE10A inhibitory activity and CYP3A4 inhibition

comps	R	PDE10A IC ₅₀ (nM)	CYP3A4 residual activity (%) ^b
14		29	19
44a		274	7.5
44b		>1000	NT ^a
53a		25	31
53b		54	48
53c		135	NT ^a
51		>100000	96
53e		24	59
53d		151	65
53f		73	46
53g		289	78
64		27	49
47		138	NT ^a
46b		155	92

^a Not tested. ^b Residual activities of HLM for metabolism of midazolam in the presence of test compounds were determined following preincubation for 30 min.

Table 7. pIC₅₀ of PDE10A inhibitory activity and p*K*_{BHX} values

compds	R	pIC ₅₀	p <i>K</i> _{BHX}
14		7.54	1.86
53a		7.60	2.03
53b		7.27	2.14
53c		6.87	0.99
53e		7.59	2.50
64		7.57	2.60
47		6.86	1.39
46b		6.81	1.23

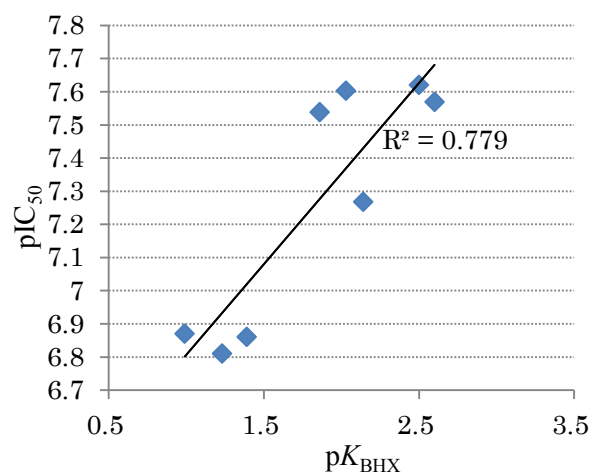
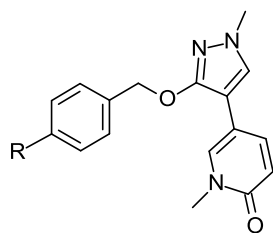


Figure 13. Scatter plot of pIC₅₀ against p*K*_{BHX} values.

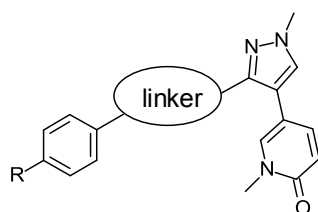
Table 8. Substituent effect on PDE10A potency and CYP3A4 inhibition

comps	R	PDE10A IC ₅₀ (nM)	CYP3A4 residual activity (%) ^c
53e		24	59
68		1.6	30
72f		22	insoluble ^b
73		100	86
72c		43	53
72d		15	66
72e		52	27
72b		37	35
72h		21	insoluble ^b
72g		157	70
72a		91	NT ^a

^a Not tested; ^b Insoluble in assay buffer; ^c Residual activities of HLM for metabolism of midazolam in the presence of test compounds were determined following preincubation for 30 min.

リード化合物**14**よりも強い PDE10A 阻害活性を有し、CYP3A4阻害が改善した化合物として**53e**, **68**および**72d**を得ることが出来た。最後にこれらの化合物の「オキシメチレン」リンカーを変換することにした。上述の通り、本章のキノリン誘導体は、その脂溶性が低い程、CYP3A4阻害活性も低いことが示唆されており、**53e**, **68**および**72d**の CYP3A4阻害をさらに低減させるために ACD/logP 値を低下させることを目指した。Table 9に示すように、**53e**, **68**および**72d**の「オキシメチレン」リンカーを「メチレンオキシ」リンカーに置き換えることにより ACD/logP 値は1以上低下し、期待通り CYP3A4阻害が低減された (**82b**, **57**, **82a**)。また、この変換により PDE10A 阻害活性も向上した。**57**は最も強い PDE10A 阻害活性を示したが、HLM 中での代謝安定性が悪かった。化合物**68**と**82a**も強い PDE10A 阻害活性を示したが、HLM 中でのクリアランス値は**53e** や**82b** に比べて大きい値を示した。これらの結果は、キノリン3位のメチル基は PDE10A 阻害活性の向上に寄与しているが、それと同時に化合物を代謝的に不安定にしていることを示唆するものである。

化合物**82b**は強い PDE10A 阻害活性を示し、CYP3A4阻害作用も改善され、かつ HLM 中での代謝安定性も良好であったため、**82b**の PDE サブタイプ選択性を評価したところ、PDE1, 3, 4, 5, 6, 7, 8, 9および11に対して1200倍以上の選択性を示した。次に、**82b**と PDE10A の共結晶を取得し、複合体の X 線結晶構造解析によって、結合様式の確認を行った。Figure 14に示すように、**82b**のキノリン窒素原子が PDE10A の活性ポケットの Tyr693と相互作用しており、かつキノリン環は PDE10A の Gly725および Met713と CH- π 相互作用していることがわかった。Figure 13の共結晶構造中に水分子は低解像度 (3.1 Å) のために観測されていないが、**82b**のピリドン酸素原子は MP-10のピリジン窒素原子とほぼ同じ場所に位置しており、**82b**のピリドン酸素原子も PDE10A 活性ポケット内の水分子と水素結合しているものと考えられる (Figure 15)。

Table 9. Effect of linker unit on PDE10A potency, CYP3A4 inhibition and intrinsic clearance

Compds	R	linker	PDE10A IC ₅₀ (nM)	CYP3A4 residual activity (%) ^a	human CL _{int} (mL/min/kg)	ACD/logP
53e		-CH ₂ O-	24	59	168	3.2
68		-CH ₂ O-	1.6	30	239	3.7
72d		-CH ₂ O-	15	66	263	4.4
82b		-OCH ₂ -	5.1	83	120	2.0
57		-OCH ₂ -	1.6	67	576	2.4
82a		-OCH ₂ -	2.8	82	445	3.2

^a Residual activities of HLM for metabolism of midazolam in the presence of test compounds were determined following preincubation for 30 min.

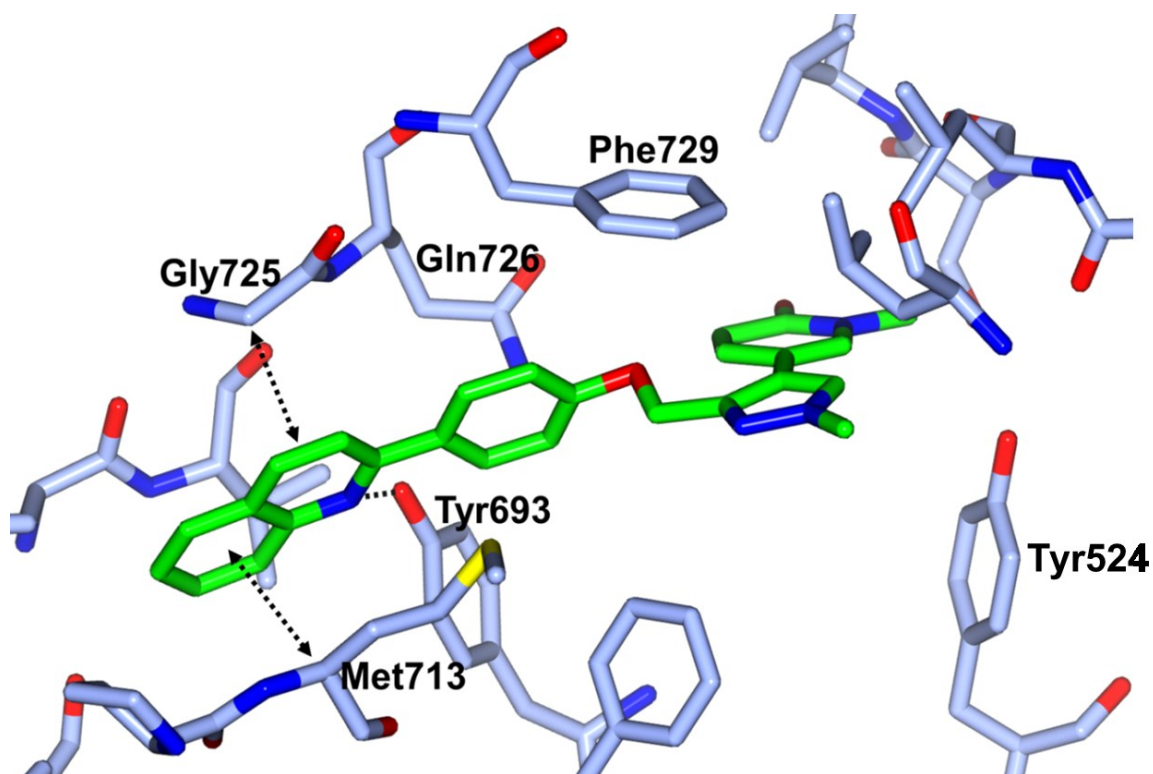


Figure 14. Crystal structure of **82b** (lime green) bound to the PDE10A (PDB code : 4WN1).

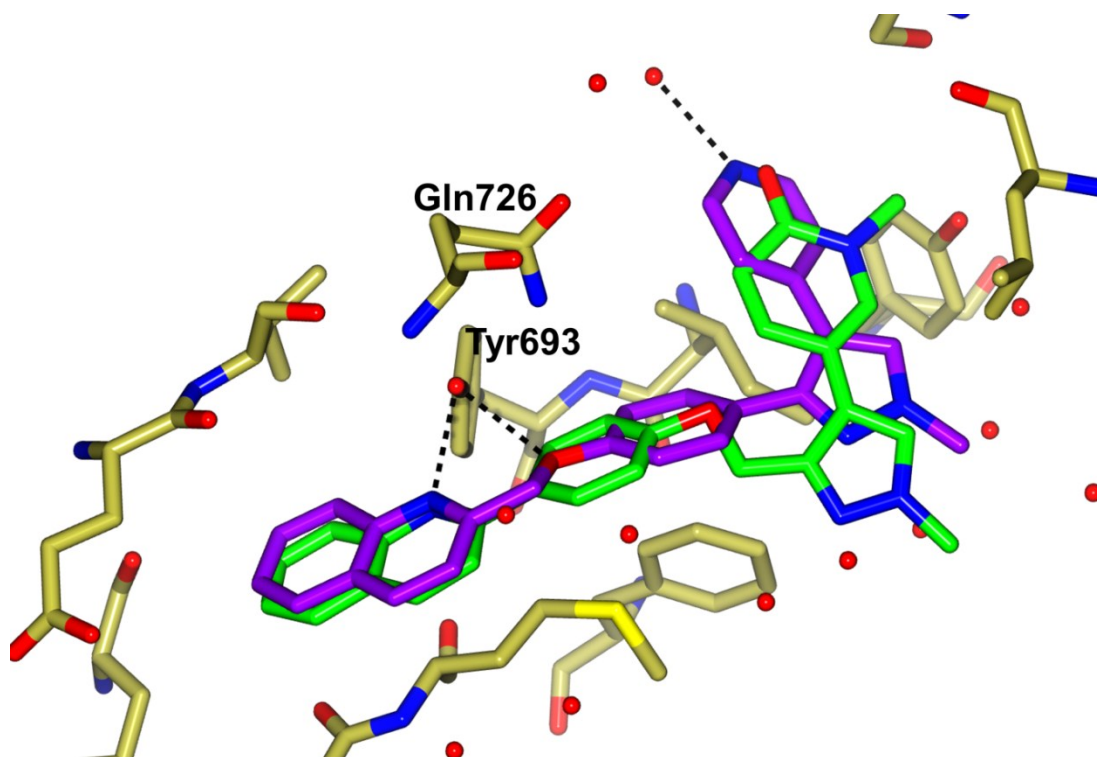
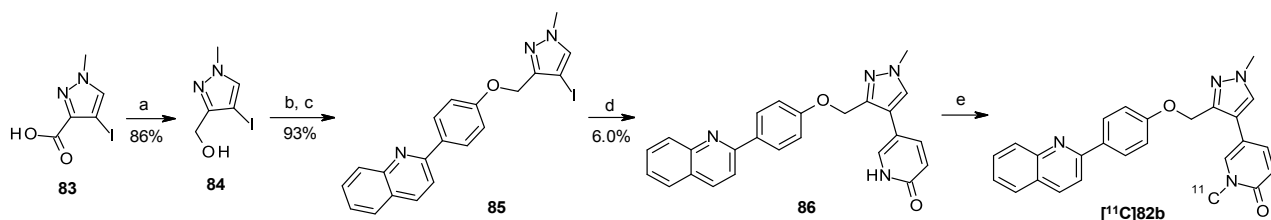


Figure 15. Superimposition of **82b** (lime green) on the crystal structure of MP-10 (purple) bound PDE10A (PDB code: 3HR1). Red spheres indicate water molecules and dashed lines indicate hydrogen bonds.

第四節 陽電子放射断層撮影法および *in vivo* 薬効評価

まず、**82b** の線条体への集積を確認するために、 ^{11}C で標識した**82b** ($[^{11}\text{C}]\mathbf{82b}$) を用いた陽電子放射断層撮影法 (PET) を検討した。

$[^{11}\text{C}]\mathbf{82b}$ は Scheme 16に示す方法で合成した。カルボン酸**83**を1,1'-カルボニルジイミダゾール (CDI) で活性化後、水素化ホウ素ナトリウムで還元することによりアルコール**84**を得た。**84**のヒドロキシル基を塩化チオニルでクロロ化し、炭酸カリウム存在下**81b** と反応させ、**85**へと変換した。**85**とボロン酸エステルとの鈴木-宮浦カップリングにより $[^{11}\text{C}]\mathbf{82b}$ の前駆体**86**を合成した。最後に、 ^{11}C で標識したトリフルオロメチルメタンスルホン酸メチルと**86**との反応により $[^{11}\text{C}]\mathbf{82b}$ を合成した。なお、最終工程の合成時間は EOB (end of bombardment; 照射終了) から36分であった。合成終了時から計算した減衰補正後の放射化学的収率は24–27%で、3–3.8GBq の収量、95%の放射化学的純度、29–60GBq の比放射能であった。



Scheme 16. Reagents and conditions: (a) CDI, THF; then NaBH_4 , H_2O ; (b) SOCl_2 , CH_2Cl_2 ; (c) **81b**, K_2CO_3 , DMF; (d) 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine-2-ol, $\text{PdCl}_2(\text{dppf})\cdot\text{CH}_2\text{Cl}_2$, Na_2CO_3 , MeCN, H_2O ; (e) $[^{11}\text{C}]\text{CH}_3\text{OTf}$, NaH, THF, DMSO.

合成した $[^{11}\text{C}]\mathbf{82b}$ をマウスに静脈内投与し、60分後の線条体と小脳の放射能を測定した結果、PDE10A が高発現している線条体においてより高い standardized uptake value (SUV) が認められた (Figure 16)。ラットにおける PET イメージング試験の結果 (Figure 17) も、 $[^{11}\text{C}]\mathbf{82b}$ は静脈内投与60分後においても線条体に集積していることを示している。さらに、MP-10 (10 mg/kg) を共投与することにより $[^{11}\text{C}]\mathbf{82b}$ の線条体への集積が阻害されることもわかった。これらの結果は、げっ歯類において**82b** は良好な中枢移行性を示し、線条体の PDE10A に特異的に結合していることを示唆している。

次に、第一章で報告したマウスの NORT の系を用いて、**82b** の *in vivo* 薬効を検証した。その結果、Figure

18に示すように、**82b** は、0.1および0.3 mg/kg の経口投与量で統計学的に有意に視覚認識記憶障害を改善することがわかった。

82b の更なるプロファイルを Figure 19に示した。**82b** はMP-10よりも CYP3A4阻害が弱く CYP1A2, 2C9, 2C19, 2D6阻害作用も弱いことが判明した。ラット、イヌ、サルの肝ミクロソーム中でも比較的安定であり、ラット、イヌ、サルの PK プロファイルも良好であった。これらの結果から、化合物**82b** は MP-10 よりも薬物-薬物相互作用の懸念が低い統合失調症の治療薬としての可能性を有していると言える。

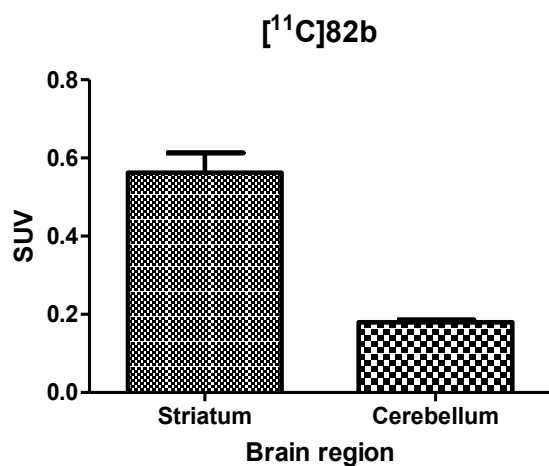


Figure 16. SUV after intravenous injection of [¹¹C]82b in mice striatum and cerebellum.

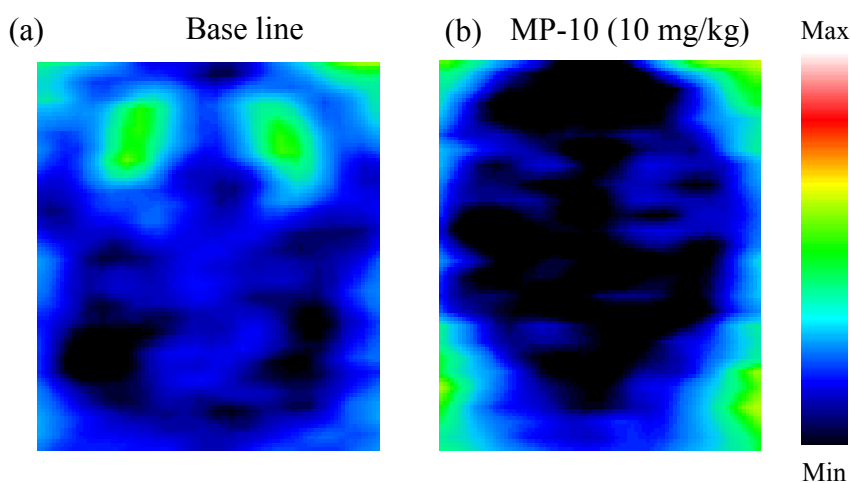


Figure 17. PET images of [¹¹C]82b in rat brain. (a) PET image after intravenous administration of [¹¹C]82b. (b) PET image after intravenous administration of [¹¹C]82b in the presence of MP-10.

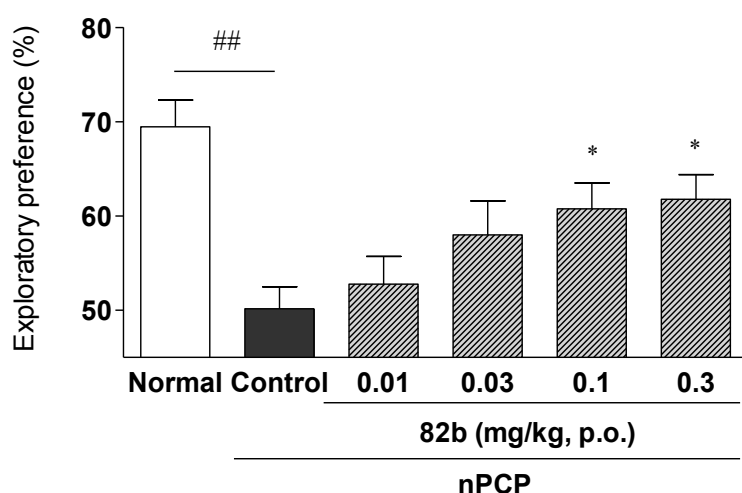
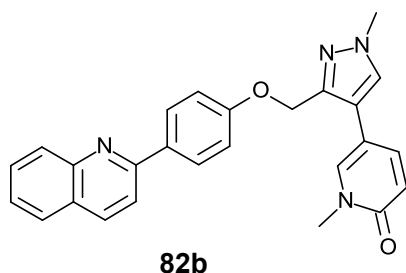


Figure 18. Effect of **82b** on neonatal PCP treatment-induced learning deficit in mice during novel object recognition test. The data represent the mean \pm SEM (n = 13 or 14 in each group): (##) $p < 0.01$ vs normal group (Student's t-test); (*) $p < 0.05$ vs control group (Dunnett's test).



in vitro properties

PDE10A IC₅₀: 5.1 nM
other PDEs: >1200 fold

in vivo properties

mice PCP-HL ED₅₀: 2.0 mg/kg, p.o.
mice NORT MED: 0.1 mg/kg, p.o.

Pharmacokinetics

CYP inhibition (1A2, 2C9, 2C19, 2D6); IC₅₀: >10uM
CYP3A4 inhibition; residual activity: 83%
CL_{int} (mL/min/kg) (human, mouse, rat, dog, monkey): 120, 285, 484, 148, 299
F (1.0 mg/kg; rat, dog, monkey): 45, 112, 105 %
CL_{total} (mL/min/kg) (i.v.; rat, dog, monkey): 12, 1.1, 1.7 (mL/min/kg)
t_{1/2} (h) (i.v.; rat, dog, monkey) : 1.3, 4.5, 2.5

Figure 19. Profiles of compound **82b**.

第五節 本章のまとめ

本章では、CYP3A4阻害に関連する薬物-薬物相互作用の懸念が少なく、強力な PDE10A 阻害活性を示す化合物を取得するために、リード化合物**14**誘導体の PDE10A 阻害活性ならびに CYP3A4阻害活性の SAR 探索を行った。その結果、化合物**14**のピリジン環を塩基性を有さない *N*-メチルピリドン環へ変換した**53e** が、PDE10A 阻害活性を保ちつつ CYP3A4阻害を低減できることがわかった。その過程で、PDE10A 阻害活性の pIC_{50} 値とピラゾール環の4位置換基の pK_{BHX} 値の間に正の相関関係があることを見出した。**53e** の「オキシメチレン」リンカーを「メチレンオキシ」リンカーへと変換した**82b** は、強い PDE10A 阻害活性を示し、CYP3A4阻害も改善していることがわかった。PET 試験により、化合物**82b** はげっ歯類において良好な中枢移行性を示し、線条体に特異的に集積していることも明らかになった。また、化合物**82b** を経口投与することにより、NORT において0.1, 0.3 mg/kg で視覚認識記憶障害を改善し、**82b** は優れた *in vivo* 薬効を示すことがわかった (Figure 20)。

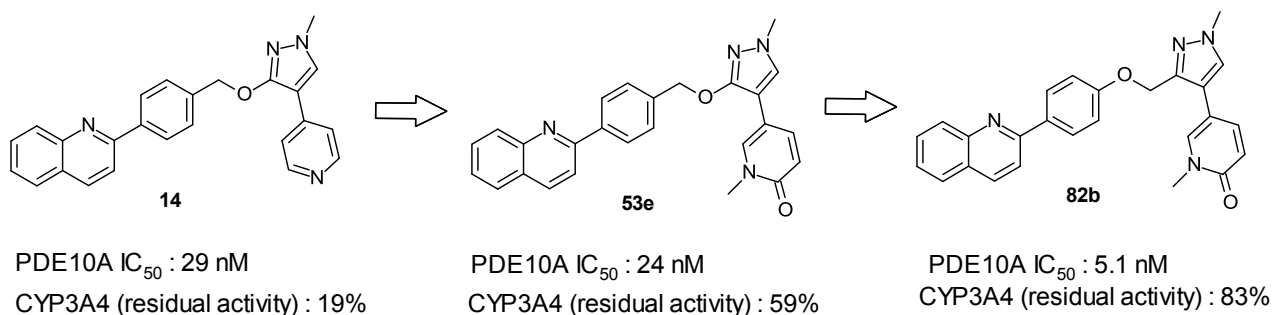


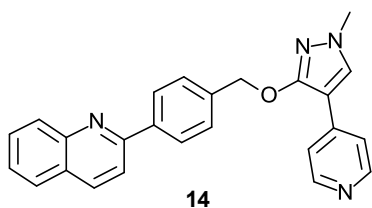
Figure 20. Summary of chapter 2.

第三章 光毒性回避した新規ビアリール誘導体の合成および構造活性相関

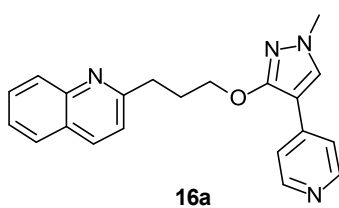
第一節 分子設計

第一章で見出したリード化合物**14** (Figure 20) は中程度の PDE10A 阻害活性を示し、HLM および MLM 中での代謝安定性が改善された。しかし、**14**は第二章で述べた CYP3A4阻害活性が強いという問題に加えて、*in vitro* 3T3ニュートラルレッド取り込み光毒性試験 (3T3 NRU test) において、光毒性を示すことが判明した³⁴⁾。光毒性は薬剤性光線過敏症の一つであり、化合物を全身投与後、日光に含まれる紫外線を浴びることにより浮腫や紅斑などの症状を引き起こす毒性所見である。そのため、光毒性を有する化合物は臨床上で使用が制限される可能性があり、光毒性を示さない化合物が求められる。

第一章で報告した化合物**16a** (Figure 21) は代謝的に不安定という問題点はあったが、リンカー部にフェニル基を有さず、3T3 NRU test において光毒性作用を示さないことがわかった。この結果は、化合物**14**のビアリール部であるキノリニルフェニル部の長い π 共役系が光毒性の主な原因であることを示唆しており³⁵⁾、このビアリール部の変換により光毒性を回避した PDE10A 阻害剤が創出できると考えた。化合物**14**において、フェニル基は代謝安定性に寄与していると考えられたため、フェニル基は維持しつつ、化合物**14**のキノリン環の変換ならびにキノリニルフェニル部への置換基導入により、光毒性を回避した PDE10A 阻害剤の創出を目指す方針を立案した。



PDE10A IC₅₀: 29 nM
mouse CL_{int}: 366 mL/min/kg
光毒性: 陽性

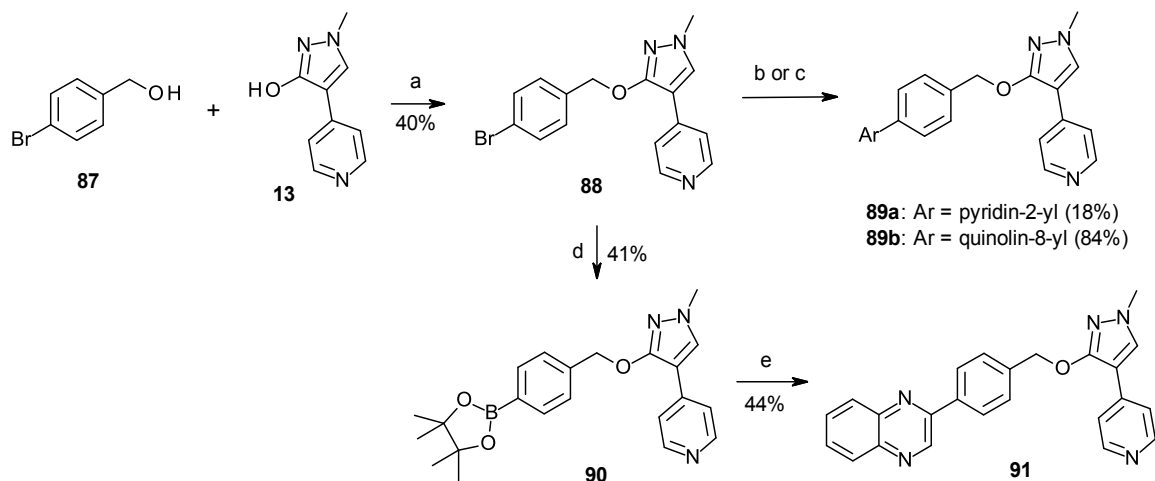


PDE10A IC₅₀: 8.9 nM
mouse CL_{int}: >1000 mL/min/kg
光毒性: 陰性

Figure 21. Structures of **14** and **16a**.

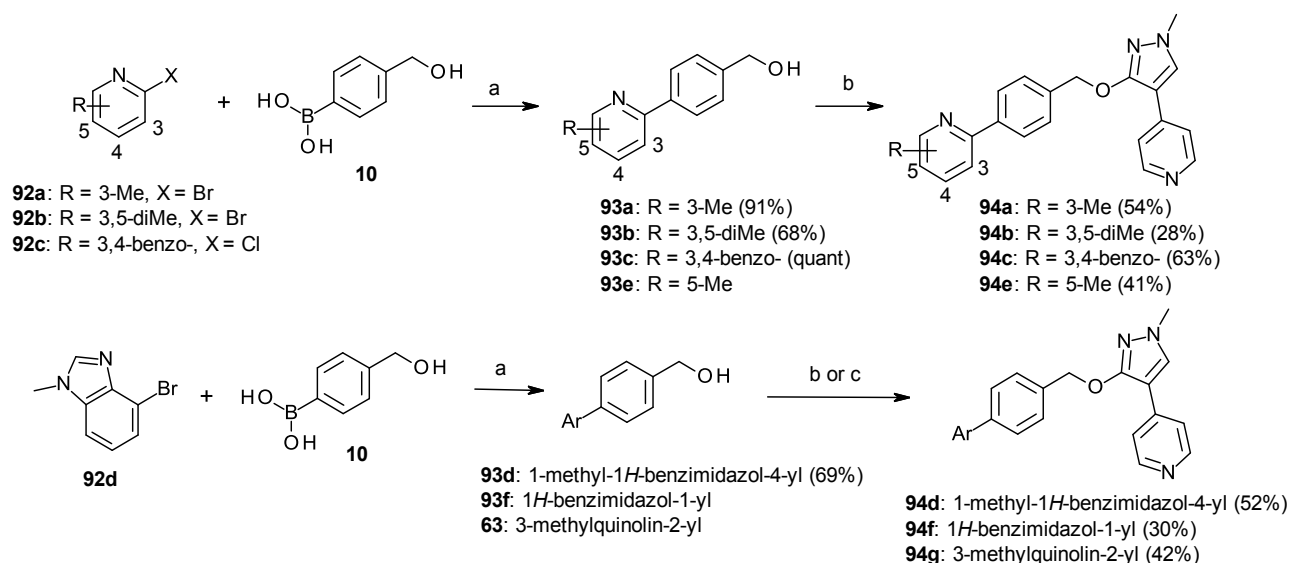
第二節 化合物の合成

種々のビアリアル誘導体は以下に示す方法で合成した。まず化合物**89a**, **89b** および**91**の合成法を Scheme 17に示す。4-ブロモベンジルアルコール (**87**) とヒドロキシピラゾール**13**を CMBP 存在下で反応させることにより中間体**88**を合成した。中間体**88**と臭化2-ピリジル亜鉛との根岸カップリングにより目的物**89a**を、**88**と8-キノリンボロン酸との鈴木-宮浦カップリングにより目的物**89b**を合成した。また、中間体**88**はボロン酸エステル**90**に変換後、2-クロロキノキサリンとの鈴木-宮浦カップリングにより目的物**91**に誘導した。

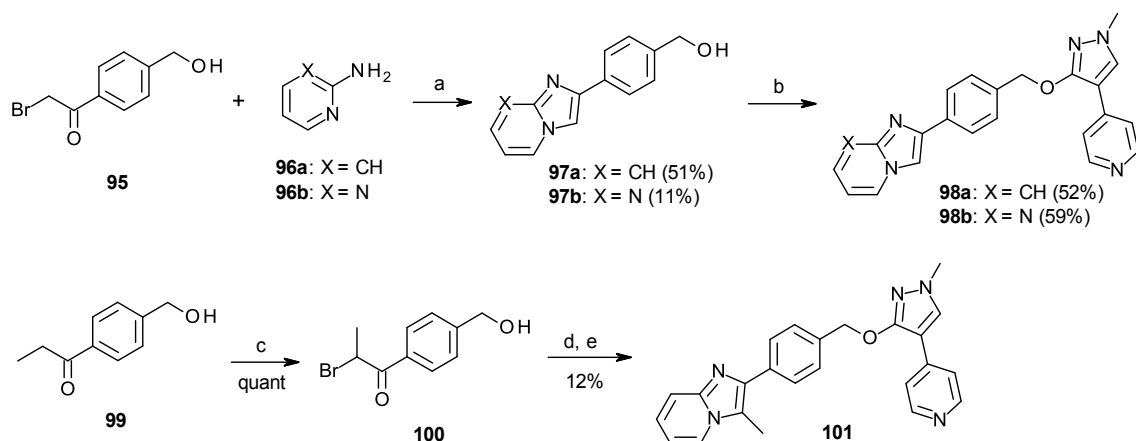


Scheme 17. Reagents and conditions: (a) CMBP, toluene; (b) 2-pyridylzinc bromide, $\text{Pd}(\text{PPh}_3)_4$, THF; (c) 8-quinolineboronic acid, $\text{Pd}(\text{PPh}_3)_4$, K_2CO_3 , dioxane, H_2O ; (d) bis(pinacolato)diboron, $\text{PdCl}_2(\text{dppf}) \cdot \text{CH}_2\text{Cl}_2$, KOAc, dioxane; (e) 2-chloroquinoline, $\text{Pd}(\text{PPh}_3)_4$, K_2CO_3 , dioxane, H_2O .

種々のビアリアル誘導体**94a-g**の合成法を Scheme 18に示す。まず市販試薬**92a-d**とボロン酸**10**との鈴木-宮浦カップリングにより、アルコール体**93a-d**を合成した。**93a-f**, **63**と化合物**13**を CMBP もしくは ADDP とトリブチルホスフィンを用いて反応させることにより、目的物**94a-g**を合成した。また、Scheme 19に示すように、化合物**98a**, **98b**もベンジルアルコール**97a**, **97b**と化合物**13**を ADDP とトリブチルホスフィンを用いて反応させることにより得ることができた。なお、**97a**, **97b**はブロモケトン**95**とアリールアミン**96a**, **96b**との反応により合成した³⁶⁾。また、イミダゾピリジン誘導体**101**は、市販試薬**99**をブロモ化することにより調製した**100**とアミノピリジン**96a**から、上述の方法と同様にして合成した。

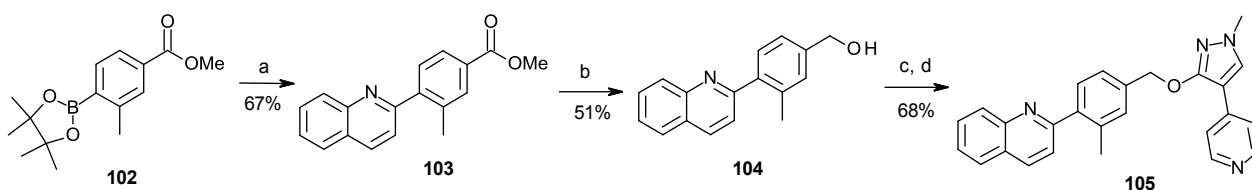


Scheme 18. Reagents and conditions: (a) Pd(PPh₃)₄, Na₂CO₃, DME, H₂O; (b) **13**, CMBP, toluene; (c) **13**, ADDP, *n*Bu₃P, THF.



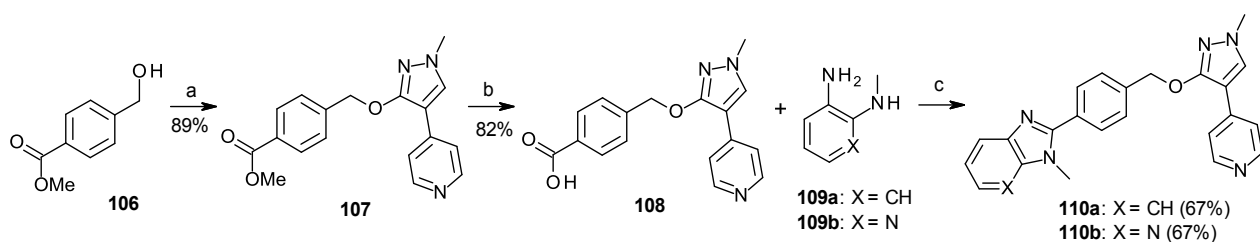
Scheme 19. Reagents and conditions: (a) EtOH; (b) **13**, ADDP, *n*Bu₃P, THF; (c) pyridinium tribromide, THF; (d) **96a**, NaHCO₃, EtOH; (e) **13**, ADDP, *n*Bu₃P, THF.

フェニル環にメチル基を導入した化合物**105**の合成法を Scheme 20に示す。市販のボロン酸エステル**102**と2-クロロキノリンの鈴木-宮浦カップリングによりエステル**103**を合成し、続いて水素化ホウ素リチウムによりエステルを還元し、ベンジルアルコール誘導体**104**へと変換した。この**104**のヒドロキシル基を塩化チオニルでクロロ化した後、ヒドロキシピラゾール**13**と炭酸カリウム存在下で反応させることにより、目的物**105**を合成した。



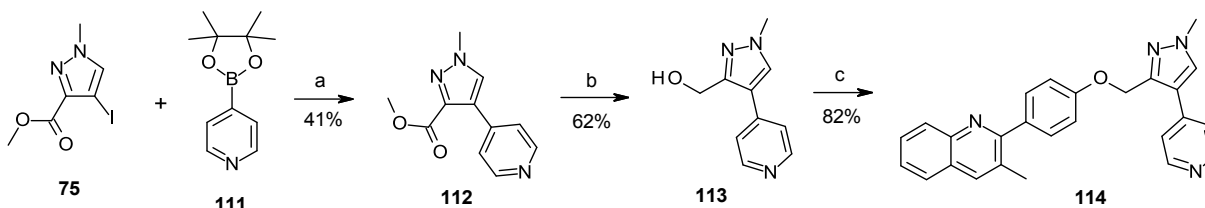
Scheme 20. Reagents and conditions: (a) 2-chloroquinoline, $\text{Pd}(\text{PPh}_3)_4$, Na_2CO_3 , DME, H_2O ; (b) LiBH_4 , EtOH, THF; (c) SOCl_2 , CH_2Cl_2 ; (d) **13**, K_2CO_3 , DMF.

ベンズイミダゾール誘導体**110a** およびイミダゾピリジン誘導体**110b** の合成法を Scheme 21に示す。まず市販のアルコール**106**とヒドロキシピラゾール**13**を ADDP、トリブチルホスフィン存在下で反応させ**107**を合成し、次いでエステルを加水分解することによりカルボン酸**108**へと変換した。この**108**をジアミン**109a** または**109b** を用いてそれぞれアミド化し、続いて酢酸溶媒下、加熱することにより環化させ、**110a**, **110b** をそれぞれ合成した。



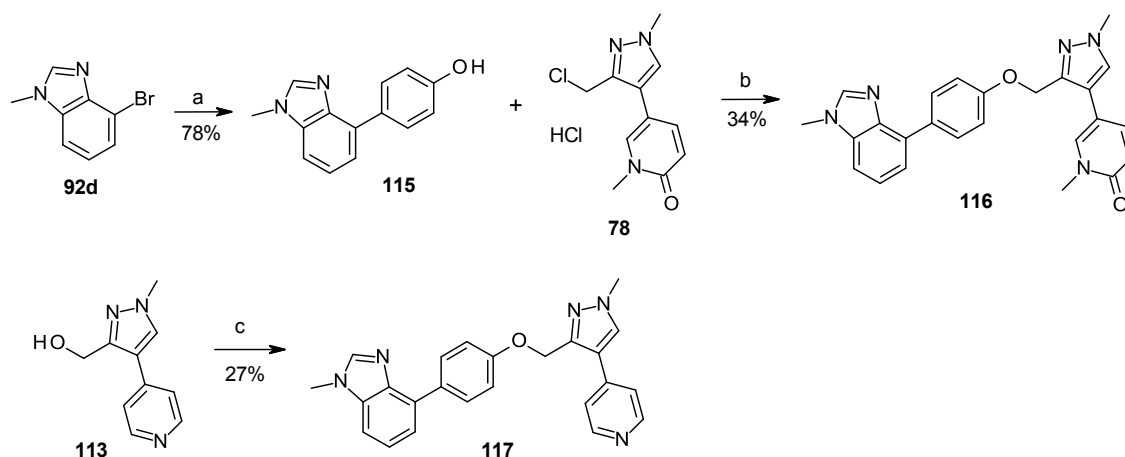
Scheme 21. Reagents and conditions: (a) **13**, ADDP, $n\text{Bu}_3\text{P}$, THF; (b) NaOH , H_2O , THF, MeOH; (c) WSC·HCl, HOBt, Et_3N , DMF, then AcOH.

Scheme 22に3-メチルキノリン誘導体**114**の合成法を示す。市販のヨードピラゾール誘導体**75**とボロン酸エステル**111**の鈴木-宮浦カップリングにより**112**を得た後、エステルを LiAlH_4 で還元しアルコール体**113**へと変換した。この**113**をフェノール誘導体**54**と CMBP 存在下で反応させ、目的物**114**を合成した。



Scheme 22. Reagents and conditions: (a) $\text{Pd}(\text{PPh}_3)_4$, Cs_2CO_3 , DMF, H_2O ; (b) LiAlH_4 , THF; (c) **54**, CMBP, toluene.

ベンズイミダゾール誘導体**116**および**117**の合成法を Scheme 23に示す。まず市販の**92d** と4-ヒドロキシフェニルボロン酸との鈴木-宮浦カップリングにより**115**を合成し、続いて第二章で合成法を報告済みの中間体**78**でアルキル化反応を行い、目的の**116**を得た。目的物**117**に関しては、**113**のヒドロキシル基を塩化チオニルでクロロ化した中間体を、炭酸カリウム存在下**115**と反応させることにより合成した。

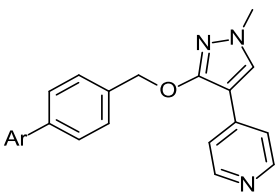


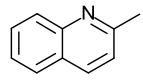
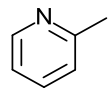
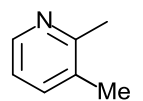
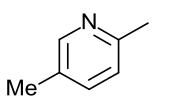
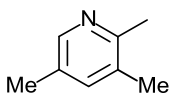
Scheme 23. Reagents and conditions: (a) 4-hydroxyphenylboronic acid, $\text{Pd(PPh}_3)_4$, Na_2CO_3 , DME, H_2O ; (b) K_2CO_3 , DMF; (c) SOCl_2 , CH_2Cl_2 ; then **115**, K_2CO_3 , DMF.

第三節 PDE10A 阻害活性および光毒性の評価結果ならびに考察

第二節で合成した化合物の PDE10A 阻害活性は、第一章で述べた方法により評価した。また、化合物の光毒性のポテンシャルは *in vitro* 3T3 NRU 光毒性試験により評価した。この評価系は光照射の有無で化合物が細胞の生存率に与える影響の違いに基づいて、化合物の光細胞毒性を評価する系である³⁴⁾。なお、ガイドラインに基づき、3T3 NRU test において0.15以上の Mean Photo Effect (MPE) 値を示す化合物を光毒性陽性として定義した³⁴⁾。

リード化合物**14**は中程度の PDE10A 阻害活性 ($IC_{50} = 29 \text{ nM}$) を示したが、3T3 NRU 試験で0.21の MPE 値を示し、光毒性を示す化合物であった。**14**の光毒性を回避するために、キノリン環をピリジン環へと変換し π 共役系を短くすることを試みた (Table 10)。その結果、化合物**89a** の PDE10A 阻害活性はリード化合物**14**より30倍程度弱かったが、その MPE 値は0.02であり、3T3 NRU 試験において光毒性を示さないことがわかった。次にピリジン環への置換基導入で PDE10A 阻害活性が向上するかを検討した。ピリジン環の3位または5位にメチル基を導入することにより、PDE10A 阻害活性は向上した (**94a**, **94e**)。ピリジン環3,5-ジメチル体**94b** とすることにより PDE10A 阻害活性がさらに向上したが、キノリン体**14**と比較すると活性はまだ3倍程度弱いことがわかった。光毒性に関しては、Table 10に示した単環のピリジン誘導体はすべて陰性であったが、PDE10A 阻害活性に関しては、**14**と比較して向上が見られなかった。

Table 10. *In vitro* activity and phototoxicity of pyridylpyrazole derivatives


compds	Ar	PDE10A IC ₅₀ (nM)	MPE
14		29	0.21
89a		861	0.02
94a		500	0.04
94e		147	0.00
94b		78	0.00

化合物の HOMO–LUMO エネルギー差 (HL-gap) は光毒性を予測できる因子として報告されており、大きな HL-gap は光による化合物の励起を防ぐことに貢献すると考えられる³⁵⁾。そこで、化合物**14**よりも大きな HL-gap となる二環性芳香環を検討することにした。なお、HL-gap の算出には、化合物の最安定コンフォメーションの HOMO および LUMO の軌道エネルギー値を用いた。Table 11に示すように、化合物**14**よりも大きな HL-gap を有する**94f**は、3T3 NRU 試験において光毒性を示さなかった。しかし、**94f**と同程度の HL-gap を有する**98a**は0.32という大きな MPE 値を示し光毒性陽性であった。また、化合物**14**に近い HL-gap を有するイミダゾピリミジン誘導体**98b**は、MPE 値は若干低下したものの光毒性陽性であり、化合物**14**よりも小さい HL-gap 値を有するキノキサリン誘導体**91**は大きな MPE 値を示し光毒性陽性であった。これらの結果から、化合物**14**の誘導体の光毒性を HL-gap のみで予測することは困難

であると考えた。HL-gap の算出には、化合物の最安定コンフォメーションのみを考慮に入れているので、そのことが光毒性の予測に不十分であった可能性がある。

次に、芳香環の平面性がその π 電子の非局在化に繋がり、そのことが光毒性を誘発する光吸収を容易にするという考えのもと、化合物**14**のビアリール部であるキノリニルフェニル部が同一面になるコンフォメーションが光毒性を誘発しており、ビアリール部の同一平面性を乱すことが光毒性の回避に繋がると仮定した。まず、最安定コンフォメーションにおけるフェニル環と二環性ヘテロ芳香環との二面角を計算したところ、光毒性陰性の**94f**の二面角は 26° であった。光毒性陽性の化合物に関しては、**98a**, **98b**の二面角は**94f**より小さかったのに対し、**14**と**91**の二面角は光毒性陰性の**94f**よりも大きかった。これらの結果は最安定構造における二面角が大きいことが、必ずしも光毒性回避に直結しない事を示唆している。

次いで、平面構造の存在確率の指標として、最安定コンフォメーションとビアリール部が平面構造となるコンフォメーションとのエネルギー差を計算することとした (Figure 22)。このエネルギー差を以下、平面化エネルギー (Flattening Energy) と称する。リード化合物**14**の平面化エネルギーは 0.49 kcal/mol であった。この化合物**14**を含め、Table 11に示した光毒性陽性化合物はすべて平面化エネルギーが 0.5 kcal/mol 未満であったのに対し、光毒性陰性である**94f**の平面化エネルギーは 1.6 kcal/mol と比較的大きい値を示した。この結果は、化合物**14**誘導体の光毒性を予測するのに平面化エネルギーの値が有用であることを示唆している。

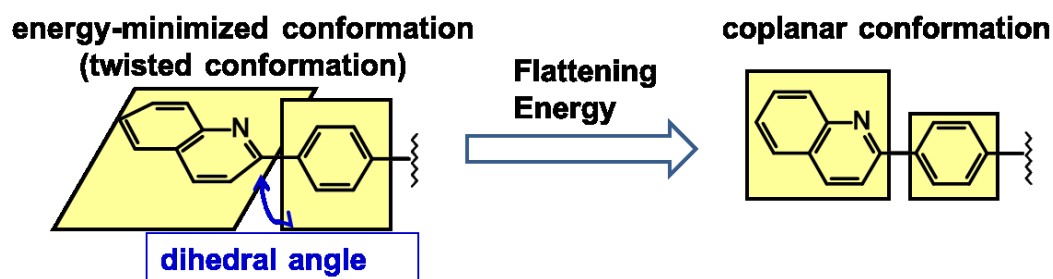


Figure 22. Energy difference (Flattening Energy) between the energy-minimized conformation and coplanar conformation.

Table 11. *In vitro* activity and phototoxicity of biaryl-substituted derivatives

compds	Ar	PDE10A IC ₅₀ (nM)	MPE	HL-gap (eV)	dihedral angle (°)	Flattening Energy (kcal/mol)
14		29	0.21	7.85	34	0.49
94f		262	-0.02	8.16	26	1.6
98a		4.8	0.32	8.05	9.7	<0.1
98b		111	0.17	7.91	9.1	<0.1
91		203	0.73	7.52	28	0.29

Figure 23に示す MP-10や第二章で述べた化合物**82b** のキノリン環は PDE10A の'selectivity pocket'を占めており、キノリン環が他の PDE に対する選択性発現に重要である可能性も考えられた。そこで、Table 11で最も高い PDE10A 阻害活性を示すイミダゾピリジン環を有する**98a** の PDE1, 2, 3, 4D, 5および9に対する選択性を調べたところ、PDE4D に対しては130倍程度の選択性に留まることがわかった (Table 12)。**98a** のイミダゾピリジン環の6員環部は PDE4D との選択性を発現するのにそれほど適した位置を占めていない可能性がある。

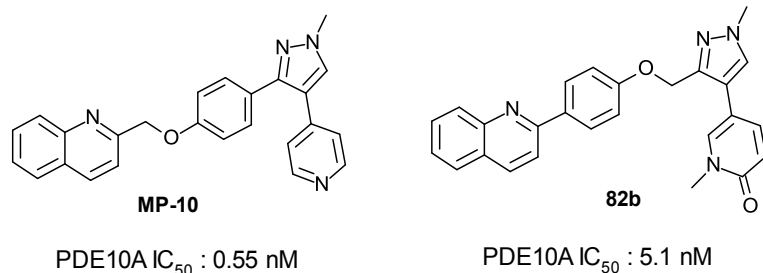
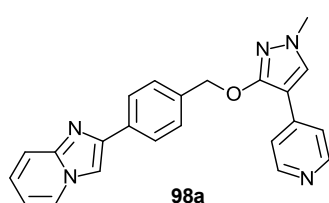


Figure 23. Structures of MP-10 and compound **82b**.

Table 12. Selectivity of compound **98a** toward PDEs isoforms



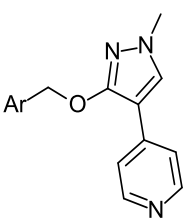
Isoform	selectivity
PDE1	>42000
PDE2	>4200
PDE3	>4200
PDE4D	130
PDE5	310
PDE9	>42000

強力な PDE10A 阻害活性と高い PDE 選択性を示し、かつ光毒性陰性の化合物を得るべく、光毒性陰性である**94f**が示す平面化エネルギー1.6 kcal/mol 以上を示す化合物を検討した (Table 13)。化合物**14**のフェニル基上にメチル基を導入した化合物**105**は、5.4 kcal/mol と大きな平面化エネルギーを有し、光毒性も陰性であった。同様に、複素芳香環部にメチル基を導入した**94g**, **101**, **110a** および**110b** は大きな平面化エネルギーを有し、光毒性陰性であることがわかった。PDE10A 阻害活性に関しては、フェニル環にメチル基を導入した**105**は活性を消失した。本誘導体が PDE10A に結合する際に、フェニル環の周囲にはメチル基が収まる空間がない可能性が考えられる。一方、3-メチルキノリン誘導体**94g** はリード化合物**14**よりも5倍程度高い活性を示した。これは、**94g**のメチル基の電子供与性がキノリン環の窒素原子の水素結合受容能を上昇させ、PDE10A 阻害活性が向上したと考えられる。次いで、**89b**, **94c**, **94d** のようなビアリール環が曲がった方向となる化合物 (bent-type) を検討した。これら化合物の平面化エネルギーは光毒性陰性である**94f**の1.6 kcal/mol よりも大きく、光毒性を示さないことがわかった。PDE10A

阻害活性に関しては、**94d** のみリード化合物**14**に匹敵する活性を示した。この結果は、**94d** のベンズイミダゾール環の窒素原子がPDE10AのTyr693との水素結合に適した場所に位置していることを示唆している。なお、各化合物のHL-gap 値も Table 13に示した。光毒性陽性であった**98a** のHL-gap 値8.05 eV よりも小さいHL-gap 値を示す**105, 94g, 89b, 94c** が光毒性陰性であったことを考えると、本誘導体の光毒性の予測にはやはり HL-gap よりも平面化エネルギーがより適していると考えられる。

続いて、**94d** と**94g** の「オキシメチレン」リンカーとピリジン環を変換することとした。第二章で、ピラゾール環とフェニル環の間の「オキシメチレン」部を「メチレンオキシ」リンカーに変換することで、PDE10A 阻害活性が向上することを明らかにしている。またピリジン環を *N*-メチルピリドン環に変換することで、CYP3A4阻害が改善し PDE10A 阻害活性も保持することを見出している。これらの知見を**94d, 94g** に適応したところ、Table 14に示すように、ベンズイミダゾール誘導体**117**および**116**は強いPDE10A 阻害活性を示すことが判明し、メチルキノリン誘導体**114**および第二章で報告済みの化合物**57**はさらに強いPDE10A 阻害活性を示した。光毒性に関しては、**94d, 94g, 117, 114**および**116**は光毒性を示さなかったが、化合物**57**は平面化エネルギーが13 kcal/mol と大きいにもかかわらず光毒性陽性であり、別の要因が光毒性を誘発していることが示唆された。活性酸素（ROS）が関与する機構がいくつかのピリドン誘導体の光毒性に関与しているとの報告がある³⁸⁾。したがって、ピリドン誘導体である**57**の光毒性もROS が関与する機構で発現している可能性がある。HL-gap はROS 産生を反映する因子であるという報告もあることを考慮に入れ³⁵⁾、ピリドン誘導体**116**と**57**のHL-gap 値を計算することとした。その結果、**116**と**57**のHL-gap はそれぞれ7.95 eV, 7.53 eV であり、**57**のHL-gap は**116**よりもかなり小さいことがわかった。この**57**の小さいHL-gap 値が、**57**が光毒性陽性である結果を説明可能と考えられる。また、Table 14に示すように**94d, 117**および**114**は強い CYP2C19阻害作用を示したが、幸運なことに**116**のCYP2C19阻害作用は弱いことがわかった。

Table 13. *In vitro* activity and phototoxicity of biaryl-substituted derivatives



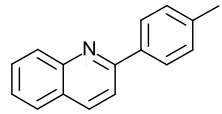
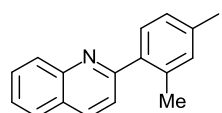
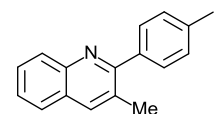
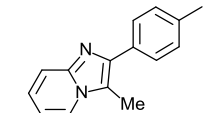
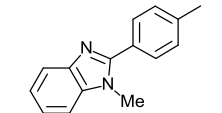
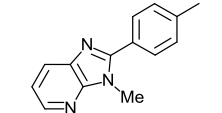
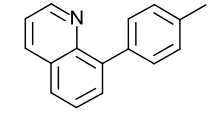
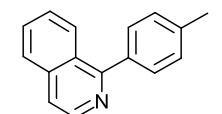
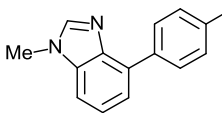
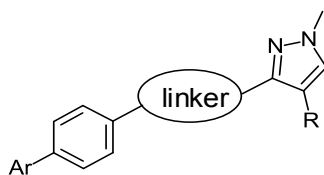
comps	Ar	PDE10A IC ₅₀ (nM)	MPE	HL-gap (eV)	Flattening Energy (kcal/mol)
14		29	0.21	7.85	0.49
105		657	0.06	7.85	5.4
94g		5.3	0.02	7.96	13
101		22	0.02	8.15	3.2
110a		58	0.06	8.34	8.2
110b		65	0.07	8.31	6.3
89b		90	0.00	7.71	7.9
94c		510	0.01	7.87	19
94d		21	0.03	8.06	3.1

Table 14. *In vitro* activity, phototoxicity and CYP2C19 inhibition of methylquinoline and benzimidazole analogues



comps	Ar	linker	R	PDE10A IC ₅₀ (nM)	MPE	HL-gap (eV)	Flattening Energy (kcal/mol)	CYP2C19 IC ₅₀ (μM)
94d				21	0.03	8.06	3.1	<0.31
94g				5.3	0.02	7.96	13	insoluble ^a
117				4.4	0.02	7.91	2.1	1.3
114				1.1	0.13	7.89	13	<0.16
116				8.0	0.08	7.95	2.5	>20
57				1.6	0.36	7.53	13	>10

^a Insoluble in assay buffer

以上の結果から化合物**116**が最も良好な化合物と判断し、さらなる評価を行った。X 線共結晶構造解析の結果、**116**は予想通りベンズイミダゾール環の窒素原子が Tyr693と水素結合していることが判明した (Figure 24)。さらに、**116**のピラゾール環と PDE10A の Ile692との間に CH- π 相互作用が認められ、これが**116**の強力な PDE10A 阻害作用に貢献している可能性がある。また興味深いことに、**116**の PDE10A への結合様式は、MP-10や**82b**とは異なっていることもわかった。つまり、Figure 25に示すように、MP-10や**82b**では、ピリジン環の窒素原子やピリドン環の酸素原子がそれぞれ PDE10A のポケット内の水分子と水素結合していることが強く示唆されているのに対し、**116**ではそのピリドン環は**82b**のピラゾール環とほぼ同じ部位に位置し、ピラゾール環上のメチル基が PDE10A の Tyr524近傍の空間を占めていることがわかった。**116**の結合様式が、ベンズイミダゾール環の窒素原子と PDE10A の Tyr693間の強力な水素結合形成に好ましいという可能性が考えられる。

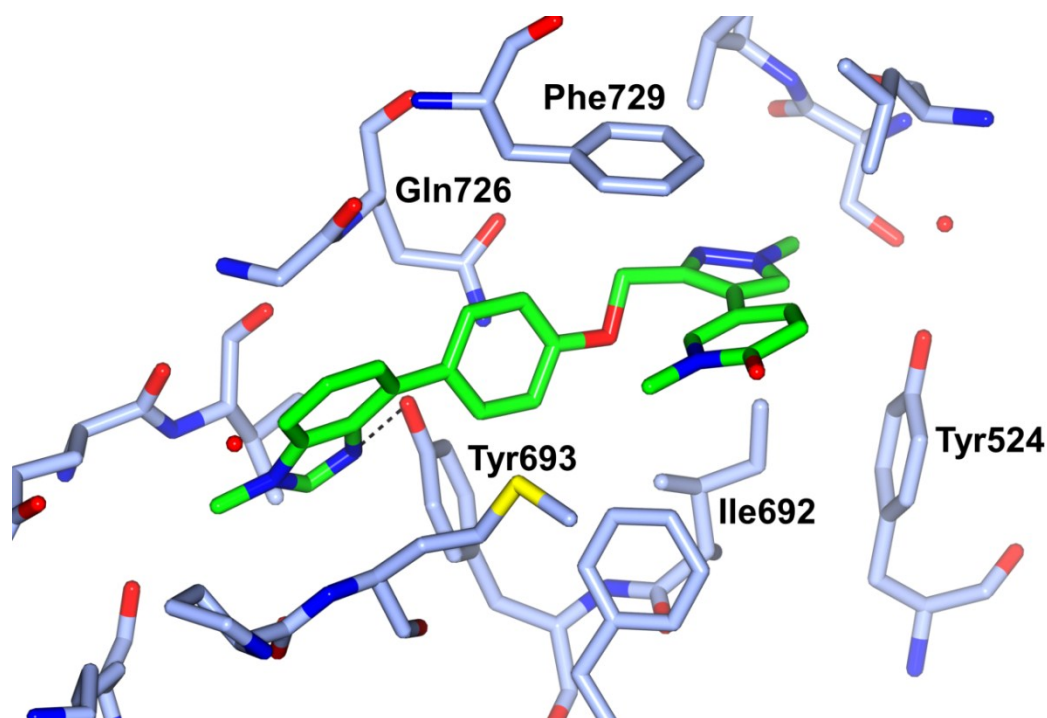


Figure 24. Crystal structure of PDE10A complexed with **116** (PDB code: 4XY2). Dashed lines indicate hydrogen bonds.

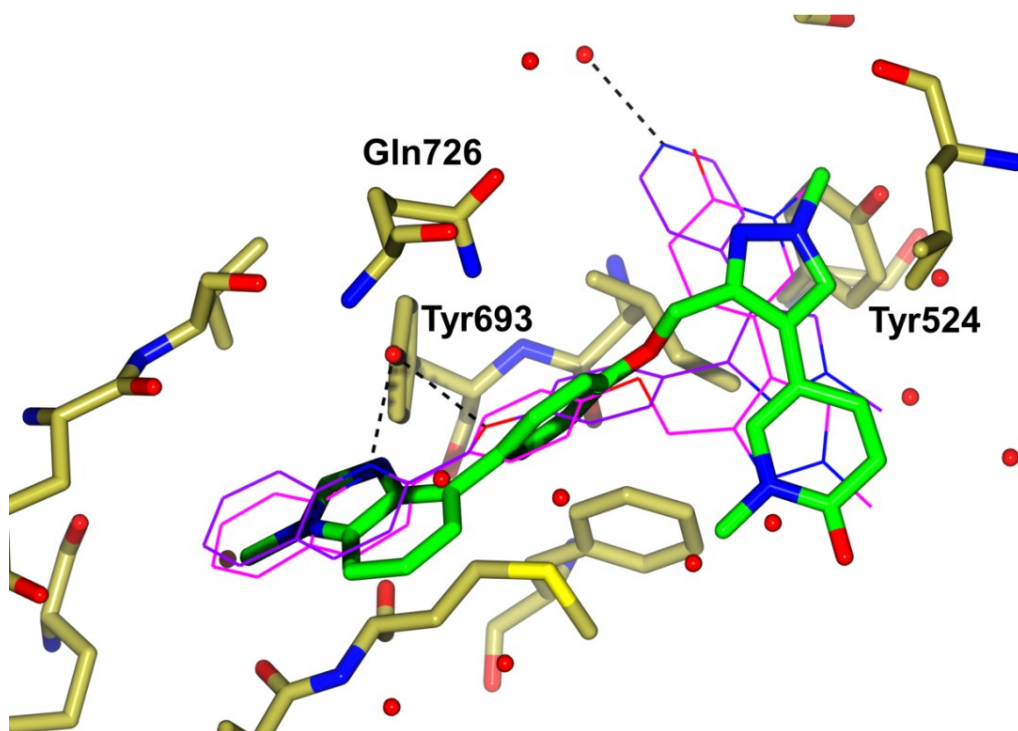


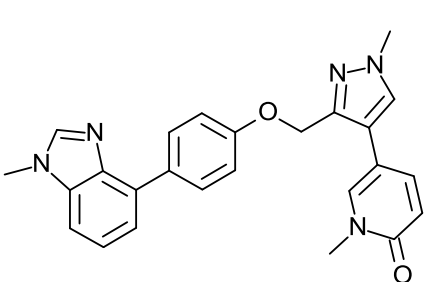
Figure 25. Superimposition of **116** (lime green) and **82b** (pink) on the crystal structure of MP-10 (purple) bound PDE10A (PDB code: 3HR1). Red spheres indicate water molecules and dashed lines indicate hydrogen bonds.

第四節 マウス行動薬理モデルにおける *in vivo* 薬効評価および PK 評価

化合物**116**の他の PDE サブタイプに対する選択性を評価した結果、Table 15に示すように PDE1, 2, 3, 4D, 5, 6, 7, 8, 9および11に対して、少なくとも420倍以上の選択性を示すことがわかった。そこで、統合失調症の陽性症状のモデルである PCP 誘発マウス過活動の系を用いて、**116**の *in vivo* 薬効を検証した (Figure 26)。その結果、**116**を経口投与することにより投与量依存的にマウスの過活動が抑制され、その ED₅₀ 値は7.0 mg/kg であった。また、新生児期に PCP を投与したマウスを用い NORT を行った結果、**116**は経口投与で0.001 mg/kg および0.003 mg/kg で視覚認識記憶障害を統計学的に有意に改善することがわかった (Figure 27)。つまり**116**の NORT における最少有効投与量 (MED) は0.001 mg/kg であり、知る限りでは**116**は NORT において最も低い投与量で薬効を示す PDE10A 阻害剤である。次に、**116**をマウスに経口投与後の脳内濃度を調べた結果、Table 16に示すように K_{p,brain} 値は投与量によって大きく異なることがわかった。NORT の MED である0.001 mg/kg という低用量では15というかなり高い K_{p,brain} 値を示すのに対し、10 mg/kg の高用量では0.021という低い K_{p,brain} 値を示した。この0.001 mg/kg における中枢移行性の高さが、**116**が NORT において低用量で薬効発現した要因である可能性がある。また、PCP 誘発過活動の系における MED である10 mg/kg をマウスに経口投与した1時間後の**116**の脳内フリー体濃度は12 nmol/kg (4.9 ng/g) であり、この値は**116**の *in vitro* PDE10A 阻害活性の IC₅₀値である8.0 nM に近い値であった (Table 16)。PDE10A 酵素への化合物の結合率が50%程度で、げっ歯類の薬物誘発過活動が抑制されるという報告がある³⁷⁾。この報告は脳内フリー体濃度が *in vitro* PDE10A 阻害の IC₅₀値に近い10 mg/kg の投与量で、化合物**116**がマウス過活動を抑制したという本節の結果と矛盾しないものと言える。

化合物**116**のさらなるプロファイルを Figure 28に示した。**116**は CYP1A2, 2C9, 2C19, 2D6, 3A4に対する阻害作用が低く、マウス、ラット、イヌおよびサルにおいて代謝的に安定で良好な PK プロファイルを示した。本章で述べた結果は、**116**は光毒性を示さず、かつ統合失調症の認知機能障害治療薬としてのポテンシャルを有していることを示しており、**116**のセスキリン酸塩である ASP9436を臨床試験の候補化合物として選択した。ASP9436は、統合失調症の認知機能障害の治療薬としての試験に向けて、さらなる検討を行うに値する化合物であると言える。

Table 15. Selectivity of compound **116** toward PDEs isoforms

 116	isoform	selectivity
	PDE1	>4200
	PDE2	>4200
	PDE3	>42000
	PDE4D	>4200
	PDE5	>42000
	PDE6	>420
	PDE7	>420
	PDE8	>420
	PDE9	>420
	PDE11	>420

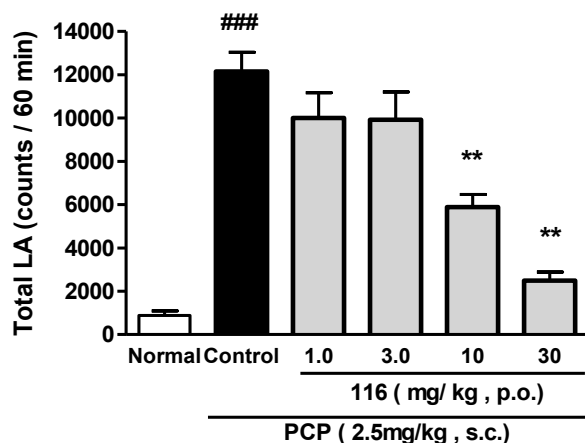


Figure 26. Effect of oral administration of **116** on PCP-induced hyperlocomotion in mice. PCP was administered subcutaneously (s.c). The data represent the mean \pm SEM: (###) $p < 0.001$ vs normal group (Student's t-test); (**) $p < 0.01$ vs control group (Dunnett's test).

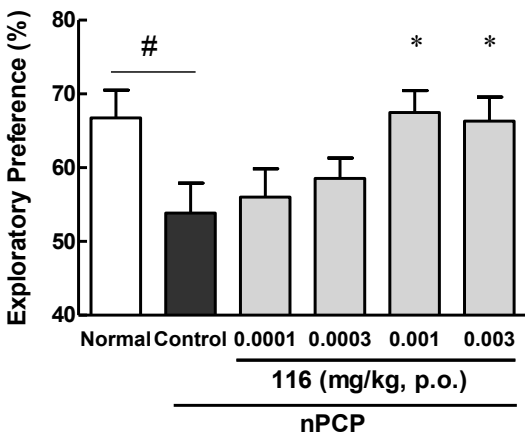


Figure 27. Effect of **116** on neonatal PCP treatment-induced learning deficit in mice during novel object recognition test. The data represent the mean \pm SEM: (#) $p < 0.05$ vs normal group (Student's t-test); (*) $p < 0.05$ vs control group (Dunnett's test).

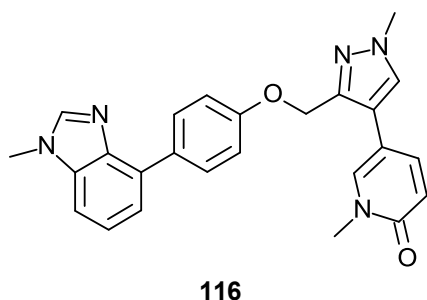
Table 16. Plasma and brain concentrations of **116** at 1 h after oral administration to mice

dose (mg/kg) ^a	plasma (ng/mL)	brain (ng/g)	Kp,brain ^b	free brain conc. (ng/g) ^c
0.001	0.03	0.4	15	0.02
10	4658	98	0.021	4.9

^a ddY mice and ICR mice were used for 0.001 mg/kg and 10 mg/kg dosage, respectively.

^b Average value of individual Kp (brain/plasma) (n = 3).

^c Free brain conc. was calculated by multiplying brain conc. by fu,brain (0.05).



in vitro properties

PDE10A IC₅₀: 8.0 nM
other PDEs: >420 fold

in vivo properties

mice PCP-HL ED₅₀: 7.0 mg/kg, p.o.
mice NORT MED: 0.001 mg/kg, p.o.

Pharmacokinetics

CYP inhibition (1A2, 2C9, 2C19, 2D6) IC₅₀: >10 μ M

CYP3A4 inhibition (residual activity) : 80%

CL_{int} (mL/min/kg) (human, mouse, rat, dog, monkey): 97, 100, 121, 153, 61

F (rat, dog, monkey): 49, 97, 144 %

t_{1/2} (h) (i.v.; rat (1.0 mg/kg), dog (0.3 mg/kg), monkey (0.3 mg/kg)) : 2.2, 5.5, 7.4

CL_{total} (mL/min/kg) (i.v.; rat (1.0 mg/kg), dog (0.3 mg/kg), monkey (0.3 mg/kg)) : 7.4, 0.8, 3.8

Figure 28. Profiles of compound **116**.

第五節 本章のまとめ

本章では、PDE10A 阻害剤である化合物**14**のようなビアリール誘導体の光毒性を回避するために SAR 探索を行った。その結果、ビアリール部が平面構造となるコンフォメーションの存在確率の指標となる平面化エネルギーが、ビアリール誘導体の光毒性を予測・説明するのに有用であることを見出した。リード化合物**14**のキノリン環の *N*-メチルベンズイミダゾール環への変換が平面化エネルギーを増大させ、その結果化合物**94d** は3T3 NRU 試験における光毒性を回避することが出来た。第二章で得た知見を用い、化合物**94d** のオキシメチレン部をメチレンオキシリンカーに変換し、さらにピリジン環を *N*-メチルピリドン環へと変換することにより得た化合物**116**は光毒性の懸念がなく、強力な PDE10A 阻害活性を示すことがわかった。この化合物**116**は他の PDE サブタイプに対しても良好な選択性を示し、また**116**を経口投与することにより、NORT の系において0.001, 0.003 mg/kg の投与量で視覚認識記憶を改善できることがわかった。これらの結果より、**116**のセスキリン酸塩である ASP9436を治験候補化合物として選択することができた (Figure 29)。

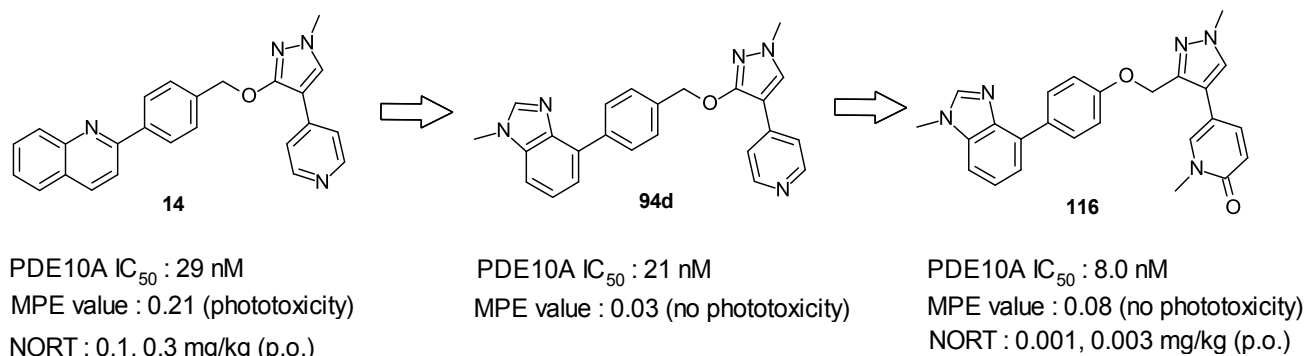


Figure 29. Summary of chapter 3.

結論

著者は、統合失調症治療薬、特に統合失調症の認知機能障害改善薬として有用性の高い新規 PDE10A 阻害剤の創製を目指し、ファイザー社から報告されていた選択的 PDE10A 阻害剤である MP-10 から合成展開を行った結果、以下の知見を得た。

第一章では、MP-10のマウスおよびヒト肝ミクロソーム中での代謝安定性の改善を目指し、MP-10の MLM 中での推定代謝経路を参考にして、MP-10のリンカーにあたるフェノキシメチレン部の構造変換を行った。その結果、化合物**14**が MLM および HLM 中で高い代謝安定性を示し中程度の PDE10A 阻害活性を示すことを見出した。化合物**14**は他の PDE サブタイプへの選択性も良好で、マウスに腹腔内投与後の血漿中および脳内濃度も高いことがわかった。さらに、化合物**14**を腹腔内投与もしくは経口投与することにより、PCP 誘発マウス過活動の系において用量依存的に過活動を抑制し、新生児期に PCP を投与したマウス NORT の系においても経口投与で0.1, 0.3 mg/kg の用量で視覚認識記憶の改善作用を示すことを見出し、**14**をリード化合物として選択した。

第二章では、リード化合物**14**の CYP3A4阻害作用に基づく薬物-薬物相互作用の懸念が少なく、強い PDE10A 阻害活性を示す化合物を得るために、**14**誘導体の PDE10A 阻害活性ならびに CYP3A4阻害活性の SAR 探索を行った。その結果、化合物**14**のピリジン環の塩基性が CYP3A4阻害の主要因であることを確認し、このピリジン環を塩基性のない N-メチルピリドン環へ変換した**53e** が、PDE10A 阻害活性を保ちつつ CYP3A4阻害を低減できることを見出した。その過程で、PDE10A 阻害活性の pIC_{50} 値とピラゾール環の4位置換基の pK_{BHX} 値の間に正の相関関係があることを見出した。**53e** の「オキシメチレン」リンカーを「メチレンオキシ」リンカーへと変換した**82b** は、強い PDE10A 阻害活性を示し、CYP3A4阻害もさらに改善していることがわかった。PET 試験により、化合物**82b** はげっ歯類において良好な中枢移行性を示し、線条体に特異的に集積していることが明らかになった。さらに化合物**82b** を経口投与することにより、NORT において0.1, 0.3 mg/kg で視覚認識記憶障害を改善し、**82b** は優れた *in vivo* 薬効を有

することを明らかにした。

第三章では、リード化合物**14**のようなビアリール誘導体の光毒性を回避するために SAR 探索を行った。その結果、ビアリール部が平面構造となるコンフォメーションの存在確率の指標となる平面化エネルギーが、ビアリール誘導体の光毒性を予測・説明するのに有用であることを見出した。すなわち、平面化エネルギーが大きいビアリール誘導体は、光毒性を回避できる傾向にあることを明らかにし、リード化合物**14**のキノリン環の *N*-メチルベンズイミダゾール環への変換により得られた化合物**94d** は3T3 NRU 試験における光毒性を回避することが出来た。さらに第二章で得た知見を用い、化合物**94d** のオキシメチレン部をメチレンオキシリンカーに変換し、ピリジン環を *N*-メチルピリドン環へと変換することにより得た化合物**116**は光毒性の懸念がなく、強力な PDE10A 阻害活性を示すことを見出した。この化合物**116**は他の PDE サブタイプに対しても良好な選択性を示し、また**116**を経口投与することにより、NORT の系において0.001, 0.003 mg/kg という低用量で視覚認識記憶を改善できることがわかった。これらの結果より、**116**のセスキリン酸塩である ASP9436を統合失調症の認知機能障害治療薬の治験候補化合物として選択した。

以上、本研究により、著者は強力で選択的な PDE10A 阻害活性を有する化合物 **116** を見出した。**116** は CYP3A4 阻害や光毒性といった、臨床上薬剤の使用を制限することに繋がる問題点を改善した PDE10A 阻害剤であるのみならず、優れた PK プロファイルを有し、且つ統合失調症の認知機能障害のモデルと考えられる新生児期に PCP を投与したマウスの NORT において、0.001 mg/kg (p.o.) という低用量で視覚認識記憶障害を改善したことは、意義深いものであると考えられる。また、CYP3A4 阻害の改善や、光毒性の回避を目指した中で得られた知見は、PDE10A 阻害剤の研究のみならず、創薬研究全般に応用できるものであると思われる。最後に、ASP9436 のような優れたプロファイルを示す PDE10A 阻害剤が統合失調症の認知機能障害の新たな治療薬となることを期待する。

実験の部

合成の部

¹H NMR spectra were recorded on a Varian VNS-400, JEOL JNM-LA400, or JEOL JNM-AL400 and the chemical shifts were expressed in δ (ppm) values with tetramethylsilane as an internal reference (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, td = triplet of doublets, tt = triplet of triplets, and br = broad peak). Mass spectra (MS) were recorded on Thermo Electron LCQ Advantage, Thermo Electron TRACE DSQ-2, Waters UPLC/ZQ, Waters UPLC/SQD, or Agilent 6140. Elemental analyses were performed using Yanaco MT-6 (C, H, N), Elementar Vario EL III (C, H, X), and Dionex ICS-3000 (S, halogene) and were within $\pm 0.4\%$ of theoretical values. Electrospray ionization positive high resolution mass spectrum (HRMS) was obtained using Waters LCT Premier.

第一章に関する実験

***tert*-Butyl 4-(pyridin-4-ylacetyl)piperidine-1-carboxylate (5)** Under argon gas atmosphere, to a mixture of 4-methylpyridine (2.63 g, 28.3 mmol) in THF (50 mL) cooled with ice-water bath was added lithium diisopropylamide (2.0 M solution in THF/heptane/ethylbenzene, 17.0 mL, 34.0 mmol), and the mixture was stirred at the same temperature for 30 min. To the resultant mixture cooled with dryice-acetone bath was slowly added a solution of *tert*-butyl 4-(*N*-methoxy-*N*-methylcarbamoyl)-1-piperidinecarboxylate (**4**, 5.12 g, 18.8 mmol) in THF (100 mL), and the mixture was stirred at the same temperature for 1 h. The mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10 to 50% EtOAc in CHCl₃) to give **5** (4.05 g, 71%) as a yellow oil. ¹H NMR (CDCl₃) δ 1.45 (s, 9H), 1.50–1.63 (m, 2H), 1.82 (brd, 2H, J = 12.2 Hz), 2.58 (tt, 1H, J = 11.2, 3.9 Hz), 2.78 (brt, 2H, J = 11.2 Hz), 3.76 (s, 2H), 4.03–4.21 (m, 2H), 7.11–7.14 (m, 2H), 8.55–8.58 (m, 2H); MS (ESI) m/z 305 [M+H]⁺.

1-Methyl-4-(pyridin-4-yl)-1*H*-pyrazol-3-ol (13) To a solution of ethyl pyridine-4-ylacetate (**12**, 25.0 g, 151 mmol) in DMF (100 mL) was added DMF-DMA (50.4 mL, 378 mmol), and the mixture was stirred at 80 °C for 2 h. The reaction was cooled at room temperature and concentrated *in vacuo*. To the residue in EtOH (250 mL) cooled with ice-water bath were added methylhydrazine (15.9 mL, 302 mmol) and AcOH (60 mL), and the mixture was stirred at room temperature for 16 h. The mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (0 to 10% MeOH in CHCl₃) to give the crude product, which was washed with EtOAc–hexane to give an orange solid. The orange solid was purified again by silica gel column chromatography (0 to 10% MeOH in CHCl₃) to give **13** (15.3 g, 58%) as a pale yellow solid. ¹H NMR

(DMSO- d_6) δ 3.67 (s, 3H), 7.55–7.59 (m, 2H), 8.09 (s, 1H), 8.39–8.44 (m, 2H), 10.71 (brs, 1H); MS (ESI) m/z 176 $[M+H]^+$.

tert-Butyl 4-[1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]piperidine-1-carboxylate (6) Compound **6** was prepared from **5** in a manner similar to that described for compound **13**, with a yield of 20% as a colorless solid. ^1H NMR (CDCl_3) δ 1.46 (s, 9H), 1.72–1.89 (m, 4H), 2.66–2.90 (m, 2H), 2.93–3.04 (m, 1H), 3.90 (s, 3H), 4.19 (brs, 2H), 7.22–7.25 (m, 2H), 7.47 (s, 1H), 8.56–8.61 (m, 2H); MS (ESI) m/z 343 $[M+H]^+$.

4-[1-Methyl-3-(piperidin-4-yl)-1H-pyrazol-4-yl]pyridine dihydrochloride (7) To a mixture of **6** (342 mg, 1.00 mmol) in MeOH (7.3 mL) was added 4 M HCl/dioxane (3.6 mL, 14.4 mmol), and the mixture was stirred at 50 °C for 1 h. After cooling at room temperature, the mixture was concentrated *in vacuo* to give **7** (315 mg, quant) as a pale yellow solid. ^1H NMR (DMSO- d_6) δ 1.88–2.06 (m, 4H), 3.00–3.12 (m, 2H), 3.26–3.50 (m, 3H), 3.90 (s, 3H), 8.07 (d, 2H, $J = 6.7$ Hz), 8.58 (s, 1H), 8.78 (d, 2H, $J = 6.7$ Hz), 8.97 (brs, 1H), 9.24 (brs, 1H); MS (ESI) m/z 398 $[M+H]^+$.

2-(2-{4-[1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]piperidin-1-yl}ethyl)quinoline trihydrochloride (8) To a solution of **7** (100 mg, 0.32 mmol) in MeOH (30 mL) was added Et_3N (90 μL , 0.65 mmol), and the mixture was concentrated *in vacuo*. To the residue in EtOH (2.0 mL) were added 2-vinylquinoline (100 mg, 0.65 mmol) and AcOH (30 μL , 0.53 mmol), and the mixture was stirred at 80 °C for 12 h. After cooling at room temperature, the mixture was partitioned between EtOAc and saturated NaHCO_3 aqueous solution. The organic layer was washed with brine, dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (1 to 10% MeOH in CHCl_3) to give a pale brown solid, which was dissolved in MeOH (5.0 mL) and 4 M HCl/dioxane (0.25 mL, 1.00 mmol) was added to the mixture. The mixture was concentrated *in vacuo*, and the residue was washed with diisopropyl ether to give **8** (21 mg, 13%) as a dark green solid. ^1H NMR (DMSO- d_6) δ 2.04–2.26 (m, 4H), 3.27 (brs, 2H), 3.45 (brs, 1H), 3.69 (brs, 6H), 3.91 (s, 3H), 7.71–7.80 (m, 2H), 7.90–7.97 (m, 1H), 8.07 (d, 2H, $J = 6.6$ Hz), 8.13 (d, 1H, $J = 8.0$ Hz), 8.21 (d, 1H, $J = 8.3$ Hz), 8.58 (s, 1H), 8.68 (d, 1H, $J = 8.0$ Hz), 8.79 (d, 2H, $J = 6.6$ Hz); MS (ESI) m/z 398 $[M+H]^+$. Anal. Calcd for $\text{C}_{25}\text{H}_{27}\text{N}_5 \cdot 3\text{HCl} \cdot 4.3\text{H}_2\text{O} \cdot 0.3\text{C}_6\text{H}_{14}\text{O}$: C, 52.34; H, 7.01; N, 11.39; Cl, 17.29. Found: C, 52.44; H, 6.81; N, 11.09; Cl, 16.98.

2-({4-[1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]piperidin-1-yl}methyl)quinoline trihydrochloride (9) To a mixture of **7** (144 mg, 0.46 mmol) and 2-(chloromethyl)quinoline (100 mg, 0.47 mmol) in DMF (8.0 mL) was added *N*-ethyl-*N*-isopropylpropan-2-amine (0.33 mL, 1.90 mmol), and the mixture was stirred at 60 °C for 4 h.

After cooling at room temperature, the mixture was diluted with EtOAc and washed with saturated NaHCO₃ aqueous solution and brine, dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (1 to 9% MeOH in CHCl₃) to give a pale yellow solid, which was dissolved in MeOH (5 mL) and 4 M HCl/EtOAc (0.35 mL) was added to the mixture. The mixture was concentrated *in vacuo* to give **9** (47 mg, 21%) as a beige solid. ¹H NMR (DMSO-*d*₆) δ 2.04–2.29 (m, 4H), 3.28–3.47 (m, 3H), 3.53–3.63 (m, 2H), 3.99 (s, 3H), 4.67 (s, 2H), 7.70 (dd, 1H, *J* = 7.2, 7.2 Hz), 7.82–7.89 (m, 1H), 7.94 (d, 1H, *J* = 8.5 Hz), 8.02–8.13 (m, 4H), 8.54 (d, 1H, *J* = 8.5 Hz), 8.58 (s, 1H), 8.77 (d, 2H, *J* = 6.7 Hz); MS (ESI) *m/z* 384 [M+H]⁺. Anal. Calcd for C₂₄H₂₅N₅·3HCl·4.1H₂O: C, 50.86; H, 6.44; N, 12.36; Cl, 18.77. Found: C, 51.01; H, 6.41; N, 12.24; Cl, 18.56.

[4-(Quinolin-2-yl)phenyl]methanol (11) Under argon gas atmosphere, to a mixture of 2-chloroquinoline (1.05 g, 6.42 mmol), [4-(hydroxymethyl)phenyl]boronic acid (**10**, 976 mg, 6.42 mmol) and Pd(PPh₃)₄ (384 mg, 0.33 mmol) in DME (15 mL) and water (5 mL) was added Na₂CO₃ (1.65 g, 15.6 mmol), and the mixture was stirred at 100 °C for 14 h. After cooling at room temperature, the mixture was partitioned between EtOAc and water. The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10 to 100% EtOAc in hexane) to give **11** (1.50 g, 99%) as an off-white solid. ¹H NMR (DMSO-*d*₆) δ 4.60 (d, 2H, *J* = 5.7 Hz), 5.28 (t, 1H, *J* = 5.7 Hz), 7.50 (d, 2H, *J* = 8.2 Hz), 7.56–7.62 (m, 1H), 7.75–7.81 (m, 1H), 7.99 (d, 1H, *J* = 8.1 Hz), 8.07 (d, 1H, *J* = 8.5 Hz), 8.14 (d, 1H, *J* = 8.7 Hz), 8.25 (d, 2H, *J* = 8.2 Hz), 8.44 (d, 1H, *J* = 8.7 Hz); MS (ESI) *m/z* 236 [M+H]⁺.

[3-(Quinolin-2-yl)phenyl]methanol (15e) Compound **15e** was prepared from [3-(hydroxymethyl)phenyl]boronic acid (**19**) and 2-chloroquinoline in a manner similar to that described for compound **11**, with a quantitative yield as a yellow oil. ¹H NMR (CDCl₃) δ 2.13 (brs, 1H), 4.81 (brs, 2H), 7.43–7.56 (m, 3H), 7.71–7.76 (m, 1H), 7.83 (d, 1H, *J* = 8.1 Hz), 7.87 (d, 1H, *J* = 8.6 Hz), 8.03–8.07 (m, 1H), 8.16–8.24 (m, 3H); MS (ESI) *m/z* 236 [M+H]⁺.

2-[3-([1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)methyl]phenyl]quinoline dihydrochloride (16e) To a mixture of **13** (200 mg, 1.14 mmol), **15e** (295 mg, 1.26 mmol) and tributylphosphine (346 mg, 1.71 mmol) in THF (20 mL) was added 1,1'-(azodicarbonyl)dipiperidine (ADDP, 432 mg, 1.71 mmol), and the mixture was stirred at room temperature for 14 h before the mixture was concentrated *in vacuo*. The residue was suspended in EtOAc, and the insoluble material was removed by filtration. The filtrate was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (0 to 3% MeOH in CHCl₃) to give a colorless oil, which was dissolved in EtOAc (20 mL). To the mixture was added 4 M HCl/EtOAc (1.1 mL) and the precipitate

was collected by filtration give **16e** (289 mg, 54%) as an off-white solid. ^1H NMR (DMSO- d_6) δ 3.85 (s, 3H), 5.55 (s, 2H), 7.63–7.75 (m, 3H), 7.86–7.92 (m, 1H), 8.11 (d, 1H, J = 8.1 Hz), 8.15 (d, 2H, J = 7.0 Hz), 8.23–8.31 (m, 3H), 8.45 (s, 1H), 8.67 (d, 1H, J = 8.7 Hz), 8.70–8.74 (m, 3H); MS (ESI) m/z 393 $[\text{M}+\text{H}]^+$. Anal. Calcd for $\text{C}_{25}\text{H}_{20}\text{N}_4\text{O}\cdot 2\text{HCl}\cdot 0.9\text{H}_2\text{O}$: C, 62.35; H, 4.98; N, 11.63; Cl, 14.72. Found: C, 62.70; H, 5.31; N, 11.45; Cl, 14.83.

2-[4-([1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)methyl]phenyl]quinoline dihydrochloride (14)

Compound **14** was prepared from **11** and **13** in a manner similar to that described for compound **16e**, with a yield of 39% as an off-white solid. ^1H NMR (DMSO- d_6) δ 3.84 (s, 3H), 5.52 (s, 2H), 7.66–7.72 (m, 1H), 7.74 (d, 2H, J = 8.3 Hz), 7.85–7.91 (m, 1H), 8.10 (d, 1H, J = 8.1 Hz), 8.15 (d, 2H, J = 7.0 Hz), 8.20–8.27 (m, 2H), 8.34 (d, 2H, J = 8.3 Hz), 8.64 (d, 1H, J = 8.7 Hz), 8.71 (s, 1H), 8.74 (d, 2H, J = 7.0 Hz); MS (ESI) m/z 393 $[\text{M}+\text{H}]^+$. Anal. Calcd for $\text{C}_{25}\text{H}_{20}\text{N}_4\text{O}\cdot 2\text{HCl}\cdot 3.1\text{H}_2\text{O}$: C, 57.61; H, 5.45; N, 10.75; Cl, 13.60. Found: C, 57.76; H, 5.51; N, 10.76; Cl, 13.54.

[1-(Quinolin-2-yl)piperidin-4-yl]methanol (15d) To a mixture of 2-chloroquinoline (2.50 g, 15.3 mmol) and piperidin-4-ylmethanol (**18**, 5.28 g, 45.8 mmol) in DMF (20 mL) was added K_2CO_3 (3.17 g, 22.9 mmol), and the mixture was stirred at 110 °C for 1 day. After cooling at room temperature, the mixture was concentrated *in vacuo*. The residue was partitioned between EtOAc and water, and the organic layer was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (20 to 50% EtOAc in hexane) to give **15d** (3.69 g, quant) as a pale yellow solid. ^1H NMR (DMSO- d_6) δ 1.08–1.20 (m, 2H), 1.62–1.80 (m, 3H), 2.84–2.92 (m, 2H), 3.28 (t, 2H, J = 5.8 Hz), 4.47 (t, 1H, J = 5.3 Hz), 4.51–4.58 (m, 2H), 7.15–7.21 (m, 1H), 7.23 (d, 1H, J = 9.2 Hz), 7.46–7.56 (m, 2H), 7.64–7.68 (m, 1H), 7.98 (d, 1H, J = 9.2 Hz); MS (ESI) m/z 243 $[\text{M}+\text{H}]^+$.

3-[Methyl(quinolin-2-yl)amino]propan-1-ol (15c) Compound **15c** was prepared from **17** and 2-chloroquinoline in a manner similar to that described for compound **15d**, with a yield of 68% as a colorless oil. ^1H NMR (DMSO- d_6) δ 1.70–1.78 (m, 2H), 3.13 (s, 3H), 3.42–3.48 (m, 2H), 3.67 (t, 2H, J = 7.0 Hz), 4.69 (t, 1H, J = 5.3 Hz), 7.07 (d, 1H, J = 9.2 Hz), 7.13–7.18 (m, 1H), 7.46–7.52 (m, 2H), 7.64–7.68 (m, 1H), 7.99 (d, 1H, J = 9.1 Hz); MS (ESI) m/z 217 $[\text{M}+\text{H}]^+$.

2-(3-[1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)propyl]quinoline dihydrochloride (16a) Compound **16a** was prepared from **13** and **15a** in a manner similar to that described for compound **16e**, with a yield of 41% as a yellow oil. ^1H NMR (DMSO- d_6) δ 2.40–2.50 (m, 2H), 3.47–3.55 (m, 2H), 3.79 (s, 3H), 4.46 (t, 2H, J = 5.8 Hz), 7.82 (t, 1H, J = 7.6 Hz), 7.94–8.09 (m, 4H), 8.19 (d, 1H, J = 8.3 Hz), 8.46 (brd, 1H, J = 8.3 Hz), 8.59 (s, 1H), 8.64 (d, 2H, J = 7.0 Hz), 8.92 (brd, 1H, J = 8.3 Hz); MS (ESI) m/z 345 $[\text{M}+\text{H}]^+$. Anal. Calcd for

C₂₁H₂₀N₄O·2HCl·3H₂O: C, 53.51; H, 5.99; N, 11.89; Cl, 15.04. Found: C, 53.56; H, 6.03; N, 11.82; Cl, 15.22.

2-(4-([1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)butyl)quinoline dihydrochloride (16b) Compound **16b** was prepared from **13** and **15b**¹⁷⁾ in a manner similar to that described for compound **16e**, with a yield of 61% as a pale pink solid. ¹H NMR (DMSO-*d*₆) δ 1.87–1.98 (m, 2H), 2.03–2.14 (m, 2H), 3.35 (t, 2H, *J* = 7.5 Hz), 3.76 (s, 3H), 4.35 (t, 2H, *J* = 6.2 Hz), 7.86 (dd, 1H, *J* = 7.5, 7.5 Hz), 7.98 (d, 1H, *J* = 8.3 Hz), 8.06 (t, 1H, *J* = 7.8 Hz), 8.11 (d, 2H, *J* = 7.0 Hz), 8.25 (d, 1H, *J* = 8.1 Hz), 8.44 (d, 1H, *J* = 8.1 Hz), 8.61 (s, 1H), 8.73 (d, 2H, *J* = 7.0 Hz), 8.96 (brd, 1H); MS (ESI) *m/z* 359 [M+H]⁺. Anal. Calcd for C₂₂H₂₂N₄O·2HCl·3H₂O: C, 54.44; H, 6.23; N, 11.54; Cl, 14.61. Found: C, 54.30; H, 5.97; N, 11.51; Cl, 14.71.

N-Methyl-N-(3-([1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)propyl)quinolin-2-amine dihydrochloride (16c) Compound **16c** was prepared from **13** and **15c** in a manner similar to that described for compound **16e**, with a yield of 23% as an off-white solid. ¹H NMR (DMSO-*d*₆) δ 2.22–2.32 (m, 2H), 3.44 (s, 3H), 3.73 (s, 3H), 4.02–4.14 (m, 2H), 4.46 (t, 2H, *J* = 5.9 Hz), 7.47–7.52 (m, 2H), 7.73–7.79 (m, 1H), 7.88–7.92 (m, 1H), 8.01 (d, 2H, *J* = 6.6 Hz), 8.16 (d, 1H, *J* = 8.3 Hz), 8.35 (d, 1H, *J* = 9.8 Hz), 8.55 (s, 1H), 8.65 (d, 2H, *J* = 6.6 Hz); MS (ESI) *m/z* 374 [M+H]⁺. Anal. Calcd for C₂₂H₂₃N₅O·2HCl·0.8H₂O: C, 57.34; H, 5.82; N, 15.20; Cl, 15.39. Found: C, 57.44; H, 5.76; N, 15.16; Cl, 15.63.

2-[4-([1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)methyl]piperidin-1-yl]quinoline dihydrochloride (16d) Compound **16d** was prepared from **13** and **15d** in a manner similar to that described for compound **16e**, with a yield of 56% as a colorless solid. ¹H NMR (DMSO-*d*₆) δ 1.49–1.61 (m, 2H), 2.00–2.08 (m, 2H), 2.31–2.43 (m, 1H), 3.40–3.50 (m, 2H), 3.80 (s, 3H), 4.24 (d, 2H, *J* = 6.6 Hz), 4.76 (brd, 2H, *J* = 12.4 Hz), 7.47–7.54 (m, 1H), 7.65 (d, 1H, *J* = 9.8 Hz), 7.75–7.81 (m, 1H), 7.93 (dd, 1H, *J* = 8.0, 1.2 Hz), 8.11 (d, 2H, *J* = 7.0 Hz), 8.41–8.50 (m, 2H), 8.69 (s, 1H), 8.73 (d, 2H, *J* = 7.0 Hz); MS (ESI) *m/z* 400 [M+H]⁺. Anal. Calcd for C₂₄H₂₅N₅O·2.2HCl·3.1H₂O: C, 53.82; H, 6.29; N, 13.08; Cl, 14.56. Found: C, 53.87; H, 6.28; N, 12.96; Cl, 14.78.

4-(1-Oxidoquinolin-2-yl)butan-1-ol (20b) To a mixture of **15b** (1.49 g, 7.38 mmol) in CH₂Cl₂ (30 mL) was added 3-chloroperbenzoic acid (75% purity; 2.21 g, 9.59 mmol), and the mixture was stirred at room temperature for 4 h. The mixture was partitioned between CHCl₃ and 1 M NaOH aqueous solution, and the organic layer was dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 3% MeOH in CHCl₃) to give **20b** (1.38 g, 86%) as a pale yellow solid. ¹H NMR (DMSO-*d*₆) δ 1.47–1.56 (m, 2H), 1.71–1.82 (m, 2H), 3.02 (t, 2H, *J* = 7.7 Hz), 3.42–3.48 (m, 2H), 4.42 (t, 1H, *J* = 5.2 Hz), 7.54 (d, 1H, *J* = 8.6 Hz), 7.65–7.72 (m, 1H), 7.77–7.83 (m, 1H), 7.87 (d, 1H, *J* = 8.6 Hz), 8.05 (d, 1H, *J* =

8.1 Hz), 8.58 (d, 1H, $J = 8.7$ Hz); MS (ESI) m/z 218 $[M+H]^+$.

3-(1-Oxidoquinolin-2-yl)propan-1-ol (20a) Compound **20a** was prepared from **15a** in a manner similar to that described for compound **20b**, with a yield of 99% as a brown solid. ^1H NMR (DMSO- d_6) δ 1.84–1.95 (m, 2H), 3.06 (t, 2H, $J = 7.5$ Hz), 3.45–3.52 (m, 2H), 4.65 (t, 1H, $J = 5.3$ Hz), 7.54 (d, 1H, $J = 8.6$ Hz), 7.66–7.72 (m, 1H), 7.78–7.84 (m, 1H), 7.88 (d, 1H, $J = 8.6$ Hz), 8.05 (d, 1H, $J = 8.1$ Hz), 8.58 (d, 1H, $J = 8.6$ Hz); MS (ESI) m/z 204 $[M+H]^+$.

2-(4-{[1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy}butyl)quinoline 1-oxide (21b) To a mixture of **13** (930 mg, 5.31 mmol), **20b** (1.27 g, 5.84 mmol) and tributylphosphine (2.15 g, 10.6 mmol) in THF (93 mL) was added ADDP (2.68 g, 10.6 mmol), and the mixture was stirred at room temperature for 2 h before the mixture was concentrated *in vacuo*. The residue was suspended in toluene and the precipitate was removed by filtration. The filtrate was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (0 to 50% EtOAc in CHCl_3) to give **21b** (1.50 g, 76%) as a pale yellow solid. ^1H NMR (DMSO- d_6) δ 1.82–1.98 (m, 4H), 3.11 (t, 2H, $J = 7.2$ Hz), 3.72 (s, 3H), 4.29 (t, 2H, $J = 6.0$ Hz), 7.52–7.60 (m, 3H), 7.65–7.71 (m, 1H), 7.77–7.84 (m, 1H), 7.87 (d, 1H, $J = 8.6$ Hz), 8.05 (d, 1H, $J = 8.1$ Hz), 8.20 (s, 1H), 8.40–8.45 (m, 2H), 8.57 (d, 1H, $J = 8.6$ Hz); MS (ESI) m/z 375 $[M+H]^+$.

2-(3-{[1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy}propyl)quinoline 1-oxide (21a) Compound **21a** was prepared from **13** and **20a** in a manner similar to that described for compound **21b**, with a yield of 78% as a pale yellow solid. ^1H NMR (DMSO- d_6) δ 2.25–2.34 (m, 2H), 3.21 (t, 2H, $J = 7.4$ Hz), 3.72 (s, 3H), 4.33 (t, 2H, $J = 6.2$ Hz), 7.45–7.50 (m, 2H), 7.57 (d, 1H, $J = 8.6$ Hz), 7.65–7.71 (m, 1H), 7.78–7.84 (m, 1H), 7.85 (d, 1H, $J = 8.6$ Hz), 8.00–8.05 (m, 1H), 8.20 (s, 1H), 8.34 (d, 2H, $J = 5.6$ Hz), 8.58 (d, 1H, $J = 8.8$ Hz); MS (ESI) m/z 361 $[M+H]^+$.

4-{[1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy}-1-(quinolin-2-yl)butyl acetate (22b) A mixture of **21b** (1.40 g, 3.74 mmol) and Ac_2O (14.2 mL, 150 mmol) was stirred at 80 °C for 4 h. After cooling at room temperature, the mixture was concentrated *in vacuo*. The residue was purified by NH silica gel column chromatography (0 to 50% EtOAc in CHCl_3) to give **22b** (902 mg, 58%) as a yellow oil. ^1H NMR (DMSO- d_6) δ 2.15 (s, 3H), 3.26–3.32 (m, 4H), 3.70 (s, 3H), 4.26 (t, 2H, $J = 6.3$ Hz), 5.86–5.92 (m, 1H), 7.52–7.64 (m, 4H), 7.75–7.80 (m, 1H), 7.97–8.01 (m, 2H), 8.19 (s, 1H), 8.38–8.44 (m, 3H); MS (ESI) m/z 417 $[M+H]^+$.

3-{[1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy}-1-(quinolin-2-yl)propyl acetate (22a) Compound **22a** was prepared from **21a** in a manner similar to that described for compound **22b**, with a yield of 77% as a yellow

oil. ^1H NMR ($\text{DMSO-}d_6$) δ 2.13 (s, 3H), 3.27–3.35 (m, 2H), 3.71 (s, 3H), 4.34–4.40 (m, 2H), 6.04–6.10 (m, 1H), 7.42–7.46 (m, 2H), 7.56–7.63 (m, 2H), 7.74–7.80 (m, 1H), 7.94–8.03 (m, 2H), 8.19 (s, 1H), 8.31–8.35 (m, 2H), 8.38 (d, 1H, $J = 8.5$ Hz); MS (ESI) m/z 403 $[\text{M}+\text{H}]^+$.

4-[[1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy]-1-(quinolin-2-yl)butan-1-ol (23b) To a mixture of **22b** (895 mg, 2.15 mmol) in MeOH (13 mL) was added 1 M NaOH aqueous solution (6.45 mL, 6.45 mmol), and the mixture was stirred at room temperature for 3 h. The mixture was partitioned between CHCl_3 and brine, and the organic layer was dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (1 to 5% MeOH in EtOAc) to give **23b** (444 mg, 55%) as a pale yellow solid. ^1H NMR ($\text{DMSO-}d_6$) δ 1.80–2.07 (m, 4H), 3.71 (s, 3H), 4.25 (t, 2H, $J = 6.0$ Hz), 4.80–4.85 (m, 1H), 5.63 (d, 1H, $J = 4.8$ Hz), 7.53–7.60 (m, 3H), 7.70 (d, 1H, $J = 8.5$ Hz), 7.71–7.76 (m, 1H), 7.94–7.98 (m, 2H), 8.19 (s, 1H), 8.36 (d, 1H, $J = 8.5$ Hz), 8.41–8.44 (m, 2H); MS (ESI) m/z 375 $[\text{M}+\text{H}]^+$. Anal. Calcd for $\text{C}_{22}\text{H}_{22}\text{N}_4\text{O}_2 \cdot 0.1\text{H}_2\text{O}$: C, 70.23; H, 5.95; N, 14.89. Found: C, 70.12; H, 5.90; N, 14.79.

3-[[1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy]-1-(quinolin-2-yl)propan-1-ol (23a) Compound **23a** was prepared from **22a** in a manner similar to that described for compound **23b**, with a yield of 58% as a pale yellow solid. ^1H NMR ($\text{DMSO-}d_6$) δ 2.17–2.28 (m, 1H), 2.35–2.46 (m, 1H), 3.72 (s, 3H), 4.35–4.45 (m, 2H), 4.96–5.02 (m, 1H), 5.76 (d, 1H, $J = 4.9$ Hz), 7.44–7.48 (m, 2H), 7.54–7.60 (m, 1H), 7.71–7.77 (m, 2H), 7.92–8.00 (m, 2H), 8.19 (s, 1H), 8.33–8.38 (m, 3H); MS (ESI) m/z 361 $[\text{M}+\text{H}]^+$. Anal. Calcd for $\text{C}_{21}\text{H}_{20}\text{N}_4\text{O}_2$: C, 69.98; H, 5.59; N, 15.55. Found: C, 69.99; H, 5.61; N, 15.48.

4-[[1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy]-1-(quinolin-2-yl)butan-1-one (24) To a solution of **23b** (312 mg, 0.83 mmol) in CH_2Cl_2 (31 mL) was added MnO_2 (290 mg, 3.33 mmol), and the mixture was stirred at room temperature for 1 day. The mixture was filtered through Celite pad and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 5% MeOH in EtOAc) to give a solid, which was triturated with EtOAc to give **24** (138 mg, 45%) as a colorless solid. ^1H NMR ($\text{DMSO-}d_6$) δ 2.20–2.28 (m, 2H), 3.56 (t, 2H, $J = 7.1$ Hz), 3.70 (s, 3H), 4.36 (t, 2H, $J = 6.3$ Hz), 7.52–7.55 (m, 2H), 7.73–7.78 (m, 1H), 7.85–7.90 (m, 1H), 8.04–8.11 (m, 2H), 8.13 (d, 1H, $J = 8.5$ Hz), 8.19 (s, 1H), 8.37–8.41 (m, 2H), 8.54 (d, 1H, $J = 8.5$ Hz); MS (ESI) m/z 373 $[\text{M}+\text{H}]^+$. Anal. Calcd for $\text{C}_{22}\text{H}_{20}\text{N}_4\text{O}_2 \cdot 0.35\text{H}_2\text{O}$: C, 69.77; H, 5.51; N, 14.79. Found: C, 69.85; H, 5.38; N, 14.68.

2-(1-Fluoro-4-[[1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy]butyl)quinoline dihydrochloride (25)

Under argon gas atmosphere, to a mixture of **23b** (128 mg, 0.34 mmol) in CH_2Cl_2 (5.1 mL) cooled with

dry-ice-acetone bath was added diethylaminosulfur trifluoride (72 mg, 0.44 mmol), and the mixture was stirred at the same temperature for 2 h and at 0 °C for another 6 h. The reaction was quenched with water and extracted with CHCl₃. The organic layer was washed with saturated NaHCO₃ aqueous solution and brine, dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 5% MeOH in EtOAc) to give a colorless oil (28 mg), which was dissolved in EtOAc (3.8 mL) and 4 M HCl/EtOAc (85 µL, 0.34 mmol) was added to the mixture. The mixture was stirred at room temperature for 2 h, and the precipitate was collected by filtration to give **25** (21 mg, 14%) as a pale yellow solid. ¹H NMR (DMSO-*d*₆) δ 1.95–2.06 (m, 2H), 2.19–2.35 (m, 2H), 3.77 (s, 3H), 4.38 (t, 2H, *J* = 6.5 Hz), 5.79–5.97 (m, 1H), 7.63–7.69 (m, 1H), 7.72 (d, 1H, *J* = 8.4 Hz), 7.79–7.84 (m, 1H), 8.03 (dd, 2H, *J* = 8.7, 8.7 Hz), 8.09 (d, 2H, *J* = 7.0 Hz), 8.52 (d, 1H, *J* = 8.6 Hz), 8.62 (s, 1H), 8.70 (d, 2H, *J* = 7.0 Hz); MS (ESI) *m/z* 377 [M+H]⁺; HRMS Calcd for C₂₂H₂₁FN₄O [M+H]⁺ 377.1778. Found 377.1780.

5-(Quinolin-2-yl)thiophene-2-carbaldehyde (27) Compound **27** was prepared from 5-formyl-2-thienylboronic acid (**26**) and 2-chloroquinoline in a manner similar to that described for compound **11**, with a yield of 43% as a beige solid. ¹H NMR (DMSO-*d*₆) δ 7.61–7.67 (m, 1H), 7.78–7.84 (m, 1H), 8.00–8.06 (m, 2H), 8.12 (d, 1H, *J* = 4.0 Hz), 8.20 (d, 1H, *J* = 4.0 Hz), 8.26 (d, 1H, *J* = 8.6 Hz), 8.51 (d, 1H, *J* = 8.6 Hz), 9.99 (s, 1H); MS (EI) *m/z* 239 [M]⁺.

[5-(Quinolin-2-yl)-2-thienyl]methanol (28) To a mixture of **27** (172 mg, 0.72 mmol) in EtOH (5.2 mL) cooled with ice-water bath was added NaBH₄ (27 mg, 0.72 mmol), and the mixture was stirred at the same temperature for 2 h. The mixture was partitioned between EtOAc and a 1:1 mixture of brine and water, and the organic layer was dried over MgSO₄, filtered and concentrated *in vacuo* to give **28** (171 mg, 99%) as a pale yellow solid. ¹H NMR (DMSO-*d*₆) δ 4.68 (dd, 2H, *J* = 5.7, 0.8 Hz), 5.57 (t, 1H, *J* = 5.7 Hz), 7.03–7.06 (m, 1H), 7.52–7.58 (m, 1H), 7.71–7.77 (m, 1H), 7.83 (d, 1H, *J* = 3.7 Hz), 7.91–7.96 (m, 2H), 8.06 (d, 1H, *J* = 8.7 Hz), 8.37 (d, 1H, *J* = 8.5 Hz); MS (ESI) *m/z* 242 [M+H]⁺.

2-[5-([1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)methyl]-2-thienyl]quinoline hemisuccinate (29) To a solution of **28** (320 mg, 1.33 mmol) in CH₂Cl₂ (6.4 mL) was added SOCl₂ (0.29 mL, 3.97 mmol), and the mixture was stirred at room temperature for 3 h before the addition of toluene. The precipitate was collected by filtration to give a beige solid (302 mg). To a mixture of above-obtained beige solid and **13** (179 mg, 1.02 mmol) in DMF (6.0 mL) was added K₂CO₃ (352 mg, 2.55 mmol), and the mixture was stirred at 60 °C for 7 h. After cooling at room temperature, the mixture was partitioned between EtOAc and a 1:1 mixture of brine and water, and the aqueous layer was extracted with EtOAc. The combined organic layer was dried over MgSO₄, filtered and

concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 10% MeOH in CHCl₃) to give a beige oil (213 mg), which was dissolved in EtOAc (9.0 mL). To the solution was added succinic acid (35 mg), and the mixture was stirred at room temperature for 1 h. The precipitate was collected by filtration to give **29** (205 mg, 39%) as a beige solid. ¹H NMR (DMSO-*d*₆) δ 2.42 (s, 2H), 3.80 (s, 3H), 5.55 (s, 2H), 7.34 (d, 1H, *J* = 3.7 Hz), 7.53–7.61 (m, 3H), 7.72–7.79 (m, 1H), 7.92 (d, 1H, *J* = 3.7 Hz), 7.93–7.99 (m, 2H), 8.11 (d, 1H, *J* = 8.7 Hz), 8.29 (s, 1H), 8.40 (d, 1H, *J* = 8.6 Hz), 8.44–8.50 (m, 2H), 12.16 (brs, 1H); MS (ESI) *m/z* 399 [M+H]⁺. Anal. Calcd for C₂₃H₁₈N₄OS·0.5C₄H₆O₄: C, 65.63; H, 4.63; N, 12.25; S, 7.01. Found: C, 65.37; H, 4.75; N, 12.16; S, 6.98.

1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-amine (31) To a mixture of pyridin-4-ylacetonitrile hydrochloride (**30**, 2.01 g, 13.0 mmol) in THF (30 mL) and EtOH (30 mL) was added 1 M NaOH aqueous solution (13.0 mL, 13.0 mmol), and the mixture was filtered and the filtrate was concentrated *in vacuo*. To the residue dissolved in DMF (19 mL) was added DMF-DMA (3.48 mL, 26.0 mmol), and the mixture was stirred at 80 °C for 1 h. After cooling at room temperature, the mixture was concentrated *in vacuo*. To the residue in MeOH (20 mL) were added AcOH (780 mg, 13.0 mmol) and methylhydrazine (826 μ L, 15.6 mmol), and the mixture was stirred at 60 °C for 12 h. After cooling at room temperature, the mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (1 to 10% MeOH in CHCl₃) to give **31** (400 mg, 18%) as an orange solid. ¹H NMR (DMSO-*d*₆) δ 3.64 (s, 3H), 4.90 (s, 2H), 7.44–7.47 (m, 2H), 7.96 (s, 1H), 8.40–8.43 (m, 2H); MS (ESI) *m/z* 175 [M+H]⁺.

1-Methyl-4-(pyridin-4-yl)-N-[4-(quinolin-2-yl)benzyl]-1H-pyrazol-3-amine (32) To a mixture of 4-(quinolin-2-yl)benzaldehyde¹⁸⁾ (284 mg, 1.22 mmol) and **31** (177 mg, 1.02 mmol) in 1,2-dichloroethane (1.0 mL) was added titanium(IV) isopropoxide (0.45 mL, 1.53 mmol), and the mixture was stirred at 85 °C for 2 h. To the resultant mixture cooled with ice-water bath were added MeOH (5.0 mL) and NaBH₄ (270 mg, 7.14 mmol), and the mixture was stirred at room temperature for 5 h. The reaction was quenched with saturated NaHCO₃ aqueous solution and diluted with CHCl₃. The mixture was filtered through Celite pad and the organic layer of the filtrate was washed with brine, dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (1 to 7% MeOH in CHCl₃) to give **32** (138 mg, 35%) as a pale yellow solid. ¹H NMR (DMSO-*d*₆) δ 3.67 (s, 3H), 4.46 (d, 2H, *J* = 6.0 Hz), 5.95 (t, 1H, *J* = 6.0 Hz), 7.50–7.53 (m, 2H), 7.55–7.61 (m, 3H), 7.75–7.80 (m, 1H), 7.97–8.01 (m, 2H), 8.06 (d, 1H, *J* = 8.4 Hz), 8.13 (d, 1H, *J* = 8.7 Hz), 8.20–8.24 (m, 2H), 8.42–8.47 (m, 3H); MS (ESI) *m/z* 392 [M+H]⁺. Anal. Calcd for C₂₅H₂₁N₅·1.3H₂O·0.1CHCl₃: C, 70.63; H, 5.60; N, 16.41. Found: C, 70.90; H, 5.37; N, 16.17.

***N*,1-Dimethyl-4-(pyridin-4-yl)-*N*-[4-(quinolin-2-yl)benzyl]-1*H*-pyrazol-3-amine dihydrochloride (33)** To a solution of **32** (77 mg, 0.20 mmol) and AcOH (0.80 mL) in CH₂Cl₂ (4.0 mL) was added 36% formaldehyde aqueous solution (150 µL, 1.96 mmol), and the mixture was stirred at room temperature for 10 min. To the resultant mixture was added sodium triacetoxyborohydride (167 mg, 0.79 mmol), and the mixture was stirred at room temperature for 2 h. To the resultant mixture were added 36% formaldehyde aqueous solution (150 µL, 1.96 mmol) and sodium triacetoxyborohydride (167 mg, 0.79 mmol), and the mixture was stirred at room temperature overnight. To the resultant mixture were added 36% formaldehyde aqueous solution (150 µL, 1.96 mmol) and sodium triacetoxyborohydride (167 mg, 0.79 mmol) again, and the mixture was stirred at room temperature for 12 h. The reaction was quenched with saturated NaHCO₃ aqueous solution and extracted with CHCl₃. The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (1 to 7% MeOH in CHCl₃) to give a pale yellow amorphous, which was dissolved in MeOH (5 mL) and 4 M HCl/EtOAc (0.15 mL, 0.60 mmol) was added to the mixture. The mixture was concentrated *in vacuo*, and the residue was washed with diisopropyl ether/2-propanol to give **33** (21 mg, 22%) as a yellow solid. ¹H NMR (DMSO-*d*₆) δ 2.68 (s, 3H), 3.82 (s, 3H), 4.32 (s, 2H), 7.54 (d, 2H, *J* = 8.3 Hz), 7.65–7.70 (m, 1H), 7.83–7.89 (m, 1H), 8.08 (d, 1H, *J* = 8.0 Hz), 8.17–8.27 (m, 6H), 8.58–8.63 (m, 2H), 8.78 (d, 2H, *J* = 7.0 Hz); MS (ESI) *m/z* 406 [M+H]⁺. Anal. Calcd for C₂₆H₂₃N₅·2HCl·2.5H₂O·0.75C₃H₈O: C, 59.68; H, 6.38; N, 12.32; Cl, 12.47. Found: C, 59.91; H, 6.42; N, 12.18; Cl, 12.24.

2-[4-(Chloromethyl)phenyl]quinoline hydrochloride (34) To a mixture of **11** (500 mg, 2.13 mmol) in CH₂Cl₂ (7.5 mL) was added SOCl₂ (758 mg, 6.37 mmol), and the mixture was stirred at room temperature for 2 h. To the reaction mixture was added toluene and the precipitate was collected by filtration to give **34** (616 mg, quant) as a colorless solid. ¹H NMR (DMSO-*d*₆) δ 4.88 (s, 2H), 7.64–7.71 (m, 3H), 7.85–7.90 (m, 1H), 8.07–8.11 (m, 1H), 8.19 (d, 1H, *J* = 8.5 Hz), 8.23 (d, 1H, *J* = 8.7 Hz), 8.27–8.31 (m, 2H), 8.63 (d, 1H, *J* = 8.7 Hz); MS (ESI) *m/z* 254, 256 [M+H]⁺.

4-(Pyridin-4-yl)-1*H*-pyrazol-3-ol (35) Compound **35** was prepared from **12** and hydrazine hydrate in a manner similar to that described for compound **13**, with a yield of 80% as a pink solid. ¹H NMR (DMSO-*d*₆) δ 7.65 (s, 2H), 8.12 (s, 1H), 8.38 (s, 2H), 10.6 (brs, 1H), 12.1 (brs, 1H); MS (ESI) *m/z* 162 [M+H]⁺.

1-[3-Hydroxy-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl]ethanone (36) To a mixture of **35** (7.84 g, 48.6 mmol) in pyridine (78 mL) was added Ac₂O (4.81 mL, 50.9 mmol), and the mixture was stirred at 100 °C for 2 h before the mixture was concentrated *in vacuo*. The residue was washed with water and hexane to give **36** (9.89 g, quant) as a yellow solid. ¹H NMR (DMSO-*d*₆) δ 2.55 (s, 3H), 7.80–7.84 (m, 2H), 8.53–8.56 (m, 2H), 8.92 (s, 1H), 12.0 (brs,

1H); MS (ESI) m/z 204 $[M+H]^+$.

2-[4-({[4-(Pyridin-4-yl)-1-(2,2,2-trifluoroethyl)-1H-pyrazol-3-yl]oxy}methyl)phenyl]quinoline

dihydrochloride (37) To a mixture of **36** (2.03 g, 10.0 mmol) and **34** (3.19 g, 11.0 mmol) in DMF (77 mL) was added K_2CO_3 (4.15 g, 30.0 mmol), and the mixture was stirred at 60 °C for 3 h before the mixture was concentrated *in vacuo*. To the residue were added MeOH (80 mL) and water (20 mL), and the mixture was stirred at 60 °C for 3 h before the mixture was concentrated *in vacuo*. The residue was washed with water and purified by silica gel column chromatography (0 to 5% MeOH in $CHCl_3$) to give a yellow solid (1.52 g). To a mixture of the yellow solid obtained above (568 mg) and 1,1,1-trifluoro-2-iodoethane (630 mg, 3.00 mmol) in DMF (20 mL) was added Cs_2CO_3 (1.47 g, 4.50 mmol), and the mixture was stirred at 60 °C for 5 h before the mixture was concentrated *in vacuo*. The residue was partitioned between EtOAc and water, and the organic layer was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (1 to 7% MeOH in $CHCl_3$) to give free form of the title compound, which was dissolved in EtOAc (20 mL). To the mixture was added 4 M HCl/EtOAc (1.5 mL) and the mixture was stirred at room temperature for 30 min. The precipitate was collected by filtration and washed with EtOAc to give **37** (650 mg, 27%) as a pale yellow solid. 1H NMR ($DMSO-d_6$) δ 5.19 (q, 2H, $J = 8.9$ Hz), 5.54 (s, 2H), 7.69–7.75 (m, 1H), 7.78 (d, 2H, $J = 8.4$ Hz), 7.89–7.94 (m, 1H), 8.13 (d, 1H, $J = 7.8$ Hz), 8.23–8.38 (m, 6H), 8.71 (d, 1H, $J = 8.7$ Hz), 8.79–8.84 (m, 2H), 8.89 (s, 1H); MS (ESI) m/z 461 $[M+H]^+$. Anal. Calcd for $C_{26}H_{19}F_3N_4O \cdot 2.3HCl \cdot 0.1C_4H_8O_2 \cdot 2.8H_2O$: C, 52.53; H, 4.63; N, 9.28; Cl, 13.51; F, 9.44. Found: C, 52.58; H, 4.71; N, 9.40; Cl, 13.75; F, 9.43.

第二章に関する実験

1-Methyl-4-(pyridin-3-yl)-1H-pyrazol-3-ol (43a) A mixture of ethyl pyridin-3-ylacetate (**42a**, 2.00 g, 12.1 mmol) and DMF-DMA (3.61 g, 30.3 mmol) in DMF (10 mL) was stirred at 110 °C for 2 h. After cooling at room temperature, the mixture was concentrated *in vacuo* to give a reddish brown oil. To this reddish brown oil in EtOH (20 mL) were added AcOH (5.0 mL) and methylhydrazine (1.12 g, 24.2 mmol), and the mixture was stirred at room temperature for 16 h before the mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 10% MeOH in $CHCl_3$) to give **43a** (620 mg, 29%) as a beige solid. 1H NMR ($DMSO-d_6$) δ 3.67 (s, 3H), 7.29–7.34 (m, 1H), 7.94–7.99 (m, 2H), 8.30 (dd, 1H, $J = 4.7, 1.6$ Hz), 8.83 (d, 1H, $J = 1.6$ Hz), 10.43 (brs, 1H); MS (ESI) m/z 176 $[M+H]^+$.

1-Methyl-4-(pyridin-2-yl)-1H-pyrazol-3-ol (43b) Compound **43b** was prepared from **42b** in a manner similar to that described for compound **43a**, with a yield of 45% as a brown solid. 1H NMR ($DMSO-d_6$) δ 3.69 (s, 3H), 7.10–7.15 (m, 1H), 7.65–7.69 (m, 1H), 7.75–7.80 (m, 1H), 8.07 (s, 1H), 8.44–8.46 (m, 1H), 10.94 (brs, 1H); MS

(ESI) m/z 176 $[M+H]^+$.

2-[4-([1-Methyl-4-(pyridin-3-yl)-1H-pyrazol-3-yl]oxy)methyl]phenyl]quinoline dihydrochloride (44a) To a stirred mixture of **43a** (263 mg, 1.50 mmol) and **11** (388 mg, 1.65 mmol) in THF (20 mL) were added ADDP (568 mg, 2.25 mmol) and tributylphosphine (455 mg, 2.25 mmol), and the mixture was stirred at room temperature for 12 h before the mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel; 0 to 5% MeOH in $CHCl_3$, then NH silica gel; 20 to 50% EtOAc in hexane) to give a free form of the title compound, which was diluted with EtOH (20 mL) and treated with 4 M HCl/EtOAc (1.5 mL). After the mixture was stirred at room temperature for 30 min, the mixture was concentrated *in vacuo* and recrystallized from EtOH/EtOAc to give **44a** (446 mg, 64%) as a cream-colored solid. 1H NMR (DMSO- d_6) δ 3.81 (s, 3H), 5.49 (s, 2H), 7.66–7.77 (m, 3H), 7.85–7.93 (m, 1H), 8.05 (dd, 1H, J = 8.2, 5.7 Hz), 8.11 (d, 1H, J = 7.9 Hz), 8.25 (d, 2H, J = 8.7 Hz), 8.33 (d, 2H, J = 8.4 Hz), 8.45 (s, 1H), 8.62–8.70 (m, 3H), 8.71–8.75 (m, 1H); MS (ESI) m/z 393 $[M+H]^+$; Anal. Calcd for $C_{25}H_{20}N_4O \cdot 2HCl \cdot 3.3H_2O$: C, 57.21; H, 5.49; N, 10.68; Cl, 13.51. Found: C, 57.09; H, 5.77; N, 10.31; Cl, 13.89.

2-[4-([1-Methyl-4-(pyridin-2-yl)-1H-pyrazol-3-yl]oxy)methyl]phenyl]quinoline dihydrochloride (44b) Compound **44b** was prepared from **43b** in a manner similar to that described for compound **44a**, with a yield of 72% as a cream-colored solid. 1H NMR (DMSO- d_6) δ 3.86 (s, 3H), 5.53 (s, 2H), 7.67–7.78 (m, 4H), 7.89–7.95 (m, 1H), 8.14 (d, 1H, J = 7.9 Hz), 8.23 (d, 1H, J = 8.3 Hz), 8.26–8.36 (m, 4H), 8.44–8.50 (m, 1H), 8.64–8.67 (m, 1H), 8.72 (d, 1H, J = 8.6 Hz), 8.88 (s, 1H); MS (ESI) m/z 393 $[M+H]^+$; Anal. Calcd for $C_{25}H_{20}N_4O \cdot 2HCl \cdot 0.4C_2H_6O \cdot 1.9H_2O$: C, 59.82; H, 5.49; N, 10.82; Cl, 13.69. Found: C, 59.51; H, 5.81; N, 10.42; Cl, 14.06.

2-[4-([4-(1,4-Dioxaspiro[4.5]dec-8-yl)-1-methyl-1H-pyrazol-3-yl]oxy)methyl]phenyl]quinoline (46a) To a stirred mixture of lithium bis(trimethylsilyl)amide (LiHMDS, 1 M solution in THF, 30.1 mL, 30.1 mmol) in THF (45 mL) cooled with dry ice-acetone bath were dropwisely added a solution of ethyl 1,4-dioxaspiro[4.5]dec-8-ylacetate (**45a**, 6.55 g, 28.7 mmol) with keeping the temperature below $-65^\circ C$, and the mixture was stirred at the same temperature for 50 min. To the resultant mixture was dropwisely added methyl formate (3.45 g, 57.4 mmol) with keeping the temperature below $-65^\circ C$, and the mixture was stirred at the same temperature for 10 min. The resultant mixture was allowed to warm up to room temperature and stirred for 3 h. The reaction was cooled with ice-water bath and quenched with ca. 40 mL of 1 M HCl aqueous solution. The mixture was diluted with brine and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to give an orange oil. To this orange oil in EtOH (59 mL) was added methylhydrazine (2.64

g, 57.4 mmol), and the mixture was stirred at 100 °C for 3 h before cooling at room temperature. The mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (0 to 15% MeOH in CHCl₃) to give a white solid (2.91 g). To this white solid (1.48 g) and **34** (1.8 g, 6.20 mmol) in DMF (18 mL) was added K₂CO₃ (2.14 g, 15.5 mmol), and the mixture was stirred at 60 °C for 1 h before cooling at room temperature. The mixture was diluted with water and brine, and the mixture was extracted with CHCl₃/MeOH (5:1) for 3 times. The combined organic layer was dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 3% MeOH in CHCl₃) to give **46a** (1.10 g, 17%) as a pale yellow oil. ¹H NMR (DMSO-*d*₆) δ 1.46–1.72 (m, 8H), 2.35–2.44 (m, 1H), 3.56 (s, 3H), 3.83–3.86 (m, 4H), 5.14 (s, 2H), 7.15 (s, 1H), 7.60–7.65 (m, 3H), 7.77–7.83 (m, 1H), 8.02 (d, 1H, *J* = 8.2 Hz), 8.09 (d, 1H, *J* = 8.4 Hz), 8.19 (d, 1H, *J* = 8.7 Hz), 8.33 (d, 2H, *J* = 8.4 Hz), 8.48 (d, 1H, *J* = 8.7 Hz); MS (ESI) *m/z* 456 [M+H]⁺.

2-[4-({[1-Methyl-4-(tetrahydro-2*H*-pyran-4-yl)-1*H*-pyrazol-3-yl]oxy}methyl)phenyl]quinoline

hydrochloride (46b) Free form of title compound was prepared from **45b** in a manner similar to that described for compound **46a**, with a yield of 25% as a colorless oil. To this colorless oil (139 mg, 0.35 mmol) in EtOAc (9.7 mL) was added 4 M HCl/EtOAc (0.174 mL), and the mixture was stirred at room temperature for 1 h. The precipitate was collected by filtration to give **46b** (76 mg, 13%) as a beige solid. ¹H NMR (DMSO-*d*₆) δ 1.46–1.66 (m, 4H), 2.53–2.63 (m, 1H), 3.33 (td, 2H, *J* = 11.5, 2.5 Hz), 3.58 (s, 3H), 3.79–3.88 (m, 2H), 5.19 (s, 2H), 7.27 (s, 1H), 7.68 (d, 2H, *J* = 8.4 Hz), 7.72 (d, 1H, *J* = 7.9 Hz), 7.86–7.95 (m, 1H), 8.08–8.17 (m, 1H), 8.20–8.39 (m, 4H), 8.69 (d, 1H, *J* = 8.4 Hz); MS (ESI) *m/z* 400 [M+H]⁺; Anal. Calcd for C₂₅H₂₅N₃O₂·1.55HCl·1.2H₂O: C, 62.87; H, 6.11; N, 8.80; Cl, 11.51. Found: C, 62.96; H, 6.23; N, 8.90; Cl, 11.45.

4-(1-Methyl-3-{[4-(quinolin-2-yl)benzyl]oxy}-1*H*-pyrazol-4-yl)cyclohexanone dihydrochloride (47) To a stirred solution of **46a** (1.10 g, 2.40 mmol) in THF (11 mL) and water (11 mL) was added 4-methylbenzenesulfonic acid hydrate (*p*-TsOH·H₂O, 229 mg, 1.20 mmol), and the mixture was stirred at room temperature for 3 days. The mixture was diluted with NaHCO₃ aqueous solution and brine, and extracted with CHCl₃ for 2 times. The combined organic layer was dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 3% MeOH in CHCl₃) to give a free form of the title compound (989 mg, quant) as a pale yellow oil. To this pale yellow oil (117 mg, 0.28 mmol) in EtOAc (3.5 mL) cooled with ice-water bath was added 4 M HCl/EtOAc (0.21 mL, 0.84 mmol), and the mixture was stirred at the same temperature for 15 min. The precipitate was collected by filtration to give **47** (105 mg, 82%) as a white solid. ¹H NMR (DMSO-*d*₆) δ 1.65–1.79 (m, 2H), 2.09–2.28 (m, 4H), 2.43–2.57 (m, 2H), 2.83–2.93 (m, 1H), 3.32 (s, 3H), 5.15 (s, 2H), 7.45 (d, 2H, *J* = 8.3 Hz), 7.71 (t, 1H, *J* = 7.3 Hz), 7.90 (t, 1H, *J* = 7.3 Hz), 8.00 (s, 1H), 8.12 (t, 1H, *J* = 8.0 Hz), 8.20–8.29 (m, 4H), 8.69 (s, 1H); MS (ESI) *m/z* 412 [M+H]⁺; Anal. Calcd for

$\text{C}_{26}\text{H}_{25}\text{N}_3\text{O}_2 \cdot 1.7\text{HCl} \cdot 1.75\text{H}_2\text{O}$: C, 61.95; H, 6.02; N, 8.34; Cl, 11.96. Found: C, 62.10; H, 6.35; N, 8.36; Cl, 12.08.

2-(4-((1-Methyl-1*H*-pyrazol-3-yl)oxy)methyl)phenyl)quinoline (49) To a solution of 1-methyl-1*H*-pyrazol-3-ol (**48**, 1.01 g, 10.3 mmol), **11** (2.43 g, 10.3 mmol) and ADDP (3.91 g, 15.5 mmol) in THF (122 mL) was added tributylphosphine (3.14 g, 15.5 mmol), and the mixture was stirred at room temperature for 6 h. The precipitate was filtered off and the filtrate was concentrated *in vacuo*. The residue was suspended in EtOAc, and the insoluble material was removed by filtration and the soluble extract concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 50% EtOAc in hexane) to give **49** (2.20 g, 68%) as a pale yellow solid. ^1H NMR ($\text{DMSO}-d_6$) δ 3.69 (s, 3H), 5.21 (s, 2H), 5.71 (d, 1H, $J = 2.3$ Hz), 7.49 (d, 1H, $J = 2.3$ Hz), 7.58–7.63 (m, 3H), 7.77–7.81 (m, 1H), 8.01 (dd, 1H, $J = 8.2, 1.1$ Hz), 8.08 (d, 1H, $J = 8.2$ Hz), 8.16 (d, 1H, $J = 8.7$ Hz), 8.28–8.31 (m, 2H), 8.47 (d, 1H, $J = 8.4$ Hz); MS (ESI) m/z 316 $[\text{M}+\text{H}]^+$.

2-(4-((4-Iodo-1-methyl-1*H*-pyrazol-3-yl)oxy)methyl)phenyl)quinoline (50) To a solution of **49** (2.20 g, 6.97 mmol) in MeCN (44 mL) were added ammonium hexanitratocerate(IV) (CAN, 2.29 g, 4.18 mmol) and iodine (1.06 g, 4.18 mmol), and the mixture was stirred at room temperature for 1 h. The mixture was concentrated *in vacuo*, and to the residue were added CHCl_3 and 5% NaHSO_3 aqueous solution during ice-water cooling. The organic layer was washed with brine, dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 30% EtOAc in hexane) to give **50** (893 mg, 29%) as a colorless solid. ^1H NMR (CDCl_3) δ 3.77 (s, 3H), 5.34 (s, 2H), 7.20 (s, 1H), 7.50–7.55 (m, 1H), 7.62 (d, 2H, $J = 8.1$ Hz), 7.70–7.75 (m, 1H), 7.83 (d, 1H, $J = 8.1$ Hz), 7.89 (d, 1H, $J = 8.6$ Hz), 8.15–8.20 (m, 3H), 8.23 (d, 1H, $J = 8.6$ Hz); MS (ESI) m/z 442 $[\text{M}+\text{H}]^+$.

2-(4-((1-Methyl-4-phenyl-1*H*-pyrazol-3-yl)oxy)methyl)phenyl)quinoline hydrochloride (51) To a mixture of **50** (300 mg, 0.68 mmol), phenylboronic acid (124 mg, 1.02 mmol), 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (Xphos, 65 mg, 0.14 mmol), $\text{Pd}_2(\text{dba})_3$ (62 mg, 0.068 mmol), and K_3PO_4 (289 mg, 1.36 mmol) was added *n*-butanol (2 mL) under argon gas atmosphere, and the mixture was stirred at 80 °C for 90 min. The mixture was concentrated *in vacuo*, and the residue was partitioned between EtOAc and water. The organic layer was washed with brine, dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 30% EtOAc in hexane) to give a free form of the title compound, which was dissolved in a mixture of EtOH and EtOAc. The solution was treated with 4 M HCl/EtOAc and concentrated *in vacuo*. The residue was washed with a mixture of EtOH and Et_2O to give **51** (135 mg, 46%) as a yellow solid. ^1H NMR ($\text{DMSO}-d_6$) δ 3.75 (s, 3H), 5.41 (s, 2H), 7.13–7.17 (m, 1H), 7.31–7.36 (m, 2H), 7.65–7.71 (m, 5H), 7.83–7.89 (m, 1H), 8.03 (s, 1H), 8.08 (d, 1H, $J = 7.8$ Hz), 8.16 (d, 1H, $J =$

8.4 Hz), 8.23 (d, 1H, $J = 8.7$ Hz), 8.31 (d, 2H, $J = 8.4$ Hz), 8.61 (d, 1H, $J = 8.7$ Hz); MS (ESI) m/z 392 $[M+H]^+$; Anal. Calcd for $C_{26}H_{21}N_3O \cdot 0.6HCl \cdot 0.6H_2O$: C, 73.62; H, 5.42; N, 9.91; Cl, 5.02. Found: C, 73.81; H, 5.42; N, 10.03; Cl, 4.80.

2-[4-([1-Methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazol-3-yl]oxy)methyl]phenyl]quinoline (52) To a mixture of **50** (2.0 g, 4.53 mmol) in THF (40 mL) cooled with MeOH-ice bath was dropwisely added isopropylmagnesium chloride (2 M in THF solution, 2.60 mL, 5.20 mmol) with keeping the temperature below -10 °C, and the mixture was stirred at temperature between -18 to 10 °C for 45 min. To the resultant mixture cooled with MeOH-ice bath was added 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1.39 mL, 6.80 mmol), and the mixture was allowed to stir at room temperature for 90 min. To the resultant mixture was cooled at -15 °C were added isopropylmagnesium chloride (2 M in THF solution, 1.00 mL, 2.00 mmol) and 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (422 mg, 2.27 mmol), and the mixture was stirred at room temperature for 1 h. The reaction was diluted with EtOAc and washed with saturated NH_4Cl aqueous solution and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 40% EtOAc in hexane) to give **52** (1.67 g, 83%) as a colorless solid. 1H NMR ($CDCl_3$) δ 1.33 (s, 12H), 3.73 (s, 3H), 5.40 (s, 2H), 7.45 (s, 1H), 7.49–7.55 (m, 1H), 7.65 (d, 2H, $J = 8.4$ Hz), 7.70–7.75 (m, 1H), 7.83 (d, 1H, $J = 8.1$ Hz), 7.89 (d, 1H, $J = 8.6$ Hz), 8.13–8.18 (m, 3H), 8.22 (d, 1H, $J = 8.6$ Hz); MS (ESI) m/z 442 $[M+H]^+$.

2-[4-([1-Methyl-4-(2-methylpyridin-4-yl)-1H-pyrazol-3-yl]oxy)methyl]phenyl]quinoline dihydrochloride (53a) Under argon gas atmosphere, to the mixture of **52** (200 mg, 0.45 mmol), 4-bromo-2-methylpyridine (156 mg, 0.91 mmol), and Na_2CO_3 (144 mg, 1.36 mmol) in DMF (2.0 mL) and water (0.87 mL) was added $PdCl_2(dppf) \cdot CH_2Cl_2$ (22 mg, 0.027 mmol), and the mixture was stirred at 100 °C for 1 h. After cooling at room temperature, the mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (0 to 5% MeOH in $CHCl_3$) to give a free from of the title compound, which was dissolved in MeOH and the mixture was treated with 4 M HCl/EtOAc. The precipitate formed was collected by filtration and washed with Et_2O to give **53a** (91 mg, 42%) as a colorless solid. 1H NMR ($DMSO-d_6$) δ 2.67 (s, 3H), 3.83 (s, 3H), 5.51 (s, 2H), 7.68 (t, 1H, $J = 7.1$ Hz), 7.73 (d, 2H, $J = 8.4$ Hz), 7.84–7.89 (m, 1H), 7.98 (dd, 1H, $J = 6.5, 1.8$ Hz), 8.03 (brd, 1H, $J = 1.8$ Hz), 8.08 (d, 1H, $J = 7.8$ Hz), 8.20 (d, 1H, $J = 8.4$ Hz), 8.24 (d, 1H, $J = 8.7$ Hz), 8.34 (d, 2H, $J = 8.4$ Hz), 8.58–8.65 (m, 3H); MS (ESI) m/z 407 $[M+H]^+$; Anal. Calcd for $C_{26}H_{22}N_4O \cdot 2.3HCl \cdot 3H_2O$: C, 57.36; H, 5.61; N, 10.29; Cl, 14.98. Found: C, 57.28; H, 5.67; N, 10.15; Cl, 15.22.

2-[4-([4-(2,6-Dimethylpyridin-4-yl)-1-methyl-1H-pyrazol-3-yl]oxy)methyl]phenyl]quinoline

dihydrochloride (53b) Compound **53b** was prepared from **52** and 4-bromo-2,6-dimethylpyridine hydrobromide in a manner similar to that described for compound **53a**, with a yield of 62% as a colorless solid. ¹H NMR (DMSO-*d*₆) δ 2.65 (s, 6H), 3.82 (s, 3H), 5.52 (s, 2H), 7.67 (t, 1H, *J* = 7.5 Hz), 7.72 (d, 2H, *J* = 8.4 Hz), 7.85–7.88 (m, 3H), 8.08 (d, 1H, *J* = 7.9 Hz), 8.19 (d, 1H, *J* = 8.4 Hz), 8.23 (d, 1H, *J* = 8.7 Hz), 8.34 (d, 2H, *J* = 8.3 Hz), 8.57 (s, 1H), 8.61 (d, 1H, *J* = 8.7 Hz); MS (ESI) *m/z* 421 [M+H]⁺; Anal. Calcd for C₂₇H₂₄N₄O·2.2HCl·3.5H₂O: C, 57.52; H, 5.94; N, 9.94; Cl, 13.83. Found: C, 57.87; H, 5.83; N, 9.81; Cl, 13.64.

2-[4-([4-(2-Methoxypyridin-4-yl)-1-methyl-1H-pyrazol-3-yl]oxy)methyl]phenyl]quinoline dihydrochloride (53c) Compound **53c** was prepared from **52** and 4-bromo-2-methoxypyridine in a manner similar to that described for compound **53a**, with a yield of 51% as a colorless solid. ¹H NMR (DMSO-*d*₆) δ 3.79 (s, 3H), 3.95 (s, 3H), 5.47 (s, 2H), 7.29 (s, 1H), 7.41 (dd, 1H, *J* = 5.9, 1.3 Hz), 7.71–7.76 (m, 3H), 7.89–7.95 (m, 1H), 8.13 (d, 1H, *J* = 8.2 Hz), 8.17 (d, 1H, *J* = 5.9 Hz), 8.28 (d, 2H, *J* = 8.7 Hz), 8.33 (d, 2H, *J* = 8.4 Hz), 8.45 (s, 1H), 8.72 (d, 1H, *J* = 8.6 Hz); MS (ESI) *m/z* 423 [M+H]⁺; Anal. Calcd for C₂₆H₂₂N₄O₂·2.2HCl·3.5H₂O: C, 56.10; H, 5.58; N, 10.06; Cl, 12.74. Found: C, 56.33; H, 5.68; N, 9.88; Cl, 12.52.

1-Methyl-4-(1-methyl-3-[4-(quinolin-2-yl)benzyl]oxy)-1H-pyrazol-4-yl]pyridin-2(1H)-one dihydrochloride (53d) Compound **53d** was prepared from **52** and 4-bromo-1-methylpyridin-2(1H)-one in a manner similar to that described for compound **53a**, with a yield of 68% as a colorless solid. ¹H NMR (DMSO-*d*₆) δ 3.39 (s, 3H), 3.75 (s, 3H), 5.42 (s, 2H), 6.54 (dd, 1H, *J* = 7.1, 2.0 Hz), 6.74 (d, 1H, *J* = 1.9 Hz), 7.65 (d, 1H, *J* = 7.1 Hz), 7.68–7.73 (m, 3H), 7.87–7.93 (m, 1H), 8.11 (d, 1H, *J* = 8.0 Hz), 8.19–8.23 (m, 2H), 8.27 (d, 1H, *J* = 8.7 Hz), 8.31 (d, 2H, *J* = 8.4 Hz), 8.68 (brd, 1H, *J* = 8.7 Hz); MS (ESI) *m/z* 423 [M+H]⁺; Anal. Calcd for C₂₆H₂₂N₄O₂·2HCl·4H₂O: C, 55.03; H, 5.68; N, 9.87; Cl, 12.50. Found: C, 55.15; H, 5.84; N, 9.58; Cl, 12.46.

1-Methyl-5-(1-methyl-3-[4-(quinolin-2-yl)benzyl]oxy)-1H-pyrazol-4-yl]pyridin-2(1H)-one dihydrochloride (53e) Compound **53e** was prepared from **52** and 5-bromo-1-methylpyridin-2(1H)-one in a manner similar to that described for compound **53a**, with a yield of 39% as a pale yellow solid. ¹H NMR (DMSO-*d*₆) δ 3.46 (s, 3H), 3.72 (s, 3H), 5.41 (s, 2H), 6.47 (d, 1H, *J* = 9.4 Hz), 7.67–7.78 (m, 4H), 7.87 (s, 1H), 7.92–7.97 (m, 2H), 8.16 (d, 1H, *J* = 7.9 Hz), 8.28–8.34 (m, 4H), 8.77 (d, 1H, *J* = 8.7 Hz); MS (ESI) *m/z* 423 [M+H]⁺; Anal. Calcd for C₂₆H₂₂N₄O₂·2.2HCl·2.7H₂O: C, 56.64; H, 5.41; N, 10.16; Cl, 14.15. Found: C, 56.97; H, 5.78; N, 9.93; Cl, 14.25.

1-Methyl-5-(1-methyl-3-[4-(quinolin-2-yl)benzyl]oxy)-1H-pyrazol-4-yl]pyrimidin-2(1H)-one dihydrochloride (53f) Compound **53f** was prepared from **52** and 5-bromo-1-methylpyrimidin-2(1H)-one in a manner similar to that described for compound **53a**, with a yield of 37% as a pale yellow solid. ¹H NMR

(DMSO- d_6) δ 3.55 (s, 3H), 3.75 (s, 3H), 5.40 (s, 2H), 7.64–7.71 (m, 3H), 7.83–7.88 (m, 1H), 7.96 (s, 1H), 8.07 (d, 1H, J = 8.1 Hz), 8.16 (d, 1H, J = 8.6 Hz), 8.22 (d, 1H, J = 8.7 Hz), 8.30 (d, 2H, J = 8.4 Hz), 8.60 (d, 1H, J = 8.7 Hz), 8.67 (d, 1H, J = 3.2 Hz), 8.86 (d, 1H, J = 3.2 Hz); MS (ESI) m/z 424 $[M+H]^+$; Anal. Calcd for $C_{25}H_{21}N_5O_2 \cdot 2HCl \cdot 3.3H_2O$: C, 54.02; H, 5.37; N, 12.60; Cl, 12.76. Found: C, 54.03; H, 5.66; N, 12.46; Cl, 12.44.

2-Methyl-6-(1-methyl-3-{[4-(quinolin-2-yl)benzyl]oxy}-1H-pyrazol-4-yl)pyridazin-3(2H)-one hydrochloride (53g) Compound **53g** was prepared from **52** and 6-chloro-2-methylpyridazin-3(2H)-one in a manner similar to that described for compound **53a**, with a yield of 49% as a pale yellow solid. 1H NMR (DMSO- d_6) δ 3.67 (s, 3H), 3.76 (s, 3H), 5.43 (s, 2H), 6.98 (d, 1H, J = 9.6 Hz), 7.73–7.81 (m, 4H), 7.95–8.01 (m, 1H), 8.04 (s, 1H), 8.19 (d, 1H, J = 8.0 Hz), 8.30–8.34 (m, 3H), 8.41 (d, 1H, J = 8.5 Hz), 8.85 (d, 1H, J = 8.5 Hz); MS (ESI) m/z 424 $[M+H]^+$; Anal. Calcd for $C_{25}H_{21}N_5O_2 \cdot 1.5HCl \cdot 1.7H_2O$: C, 59.02; H, 5.13; N, 13.76; Cl, 10.45. Found: C, 59.24; H, 5.53; N, 13.79; Cl, 10.33.

2-{4-[(4-Iodo-1-methyl-1H-pyrazol-3-yl)methoxy]phenyl}-3-methylquinoline (56) To a solution of (1-methyl-1H-pyrazol-3-yl)methanol (**55**, 4.00 g, 35.7 mmol) and 4-(3-methylquinolin-2-yl)phenol (**54**, 8.53 g, 36.3 mmol) in toluene (120 mL) was added CMBP (13.1 g, 54.4 mmol), and the mixture was stirred at 100 °C for 8 h. The mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 5% MeOH in $CHCl_3$) to give a brown syrup, which was dissolved in MeCN (250 mL). To this solution were added CAN (16.0 g, 29.3 mmol) and iodine (7.43 g, 29.3 mmol), and the mixture was stirred at room temperature for 15 min. To the mixture was added iodine (7.43 g, 29.3 mmol), and the mixture was stirred at room temperature for further 30 min. The reaction was concentrated *in vacuo*, and the residue was diluted with $CHCl_3$ and washed with saturated sodium thiosulfate aqueous solution. The organic layer was dried over Na_2SO_4 , filtered and concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (20 to 50% EtOAc in hexane) to give **56** (10.6 g, 64%) as a brown solid. 1H NMR (DMSO- d_6) δ 2.48 (s, 3H), 3.87 (s, 3H), 5.01 (s, 2H), 7.14–7.18 (m, 2H), 7.54–7.64 (m, 3H), 7.67–7.72 (m, 1H), 7.89–7.93 (m, 2H), 7.97 (d, 1H, J = 8.6 Hz), 8.23 (s, 1H); MS (ESI) m/z 456 $[M+H]^+$.

1-Methyl-5-(1-methyl-3-{[4-(3-methylquinolin-2-yl)phenoxy]methyl}-1H-pyrazol-4-yl)pyridin-2(1H)-one dihydrochloride (57) To a solution of **56** (6.00 g, 13.2 mmol) in THF (120 mL) cooled with MeOH-ice bath was dropwisely added isopropylmagnesium chloride (2.0 M solution in THF, 8.24 mL, 16.5 mmol), and the mixture was stirred at the same temperature for 45 min. To the resultant mixture was added 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (4.30 mL, 21.1 mmol), and the mixture was allowed to stir at room temperature for 2 h. The reaction was quenched with NH_4Cl aqueous solution and extracted with EtOAc.

The organic layer was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (10 to 60% EtOAc in hexane, then 30 to 60% EtOAc in hexane) to give 3-methyl-2-(4-{{[1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazol-3-yl]methoxy}phenyl})quinoline (2.32 g, 39%) as a brown solid. To a mixture of this brown solid (500 mg, 1.10 mmol) and 5-bromo-1-methylpyridin-2(1*H*)-one (413 mg, 2.20 mmol) in DMF (5 mL) and water (1 mL) were added PdCl₂(dppf)·CH₂Cl₂ (54 mg, 0.066 mmol) and Na₂CO₃ (349 mg, 3.29 mmol), and the mixture was stirred at 100 °C for 1 h. The reaction was diluted with water and extracted with EtOAc. The organic layer was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (0 to 5% MeOH in CHCl₃) to give a free form of the title compound, which was diluted with EtOH (5 mL). This mixture was treated with 4 M HCl/EtOAc (1.1 mL) and stirred at room temperature for 30 min. The mixture was concentrated *in vacuo*, and the residue was washed with EtOAc to give **57** (80 mg, 14%) as a beige solid. ¹H NMR (DMSO-*d*₆) δ 2.55 (s, 3H), 3.40 (s, 3H), 3.88 (s, 3H), 5.21 (s, 2H), 6.44 (d, 1H, *J* = 9.3 Hz), 7.35 (d, 2H, *J* = 8.8 Hz), 7.58 (dd, 1H, *J* = 9.3, 2.6 Hz), 7.76–7.82 (m, 3H), 7.88 (t, 1H, *J* = 7.5 Hz), 7.94 (s, 1H), 8.04 (t, 1H, *J* = 7.6 Hz), 8.23 (d, 1H, *J* = 8.1 Hz), 8.30 (d, 1H, *J* = 8.5 Hz), 8.98 (s, 1H); MS (ESI) *m/z* 437 [M+H]⁺; Anal. Calcd for C₂₇H₂₄N₄O₂·1.95HCl·0.1C₄H₈O₂·1.7H₂O: C, 60.16; H, 5.56; N, 10.24; Cl, 12.64. Found: C, 60.28; H, 5.89; N, 9.92; Cl, 12.59.

3-(Benzyloxy)-1-methyl-1*H*-pyrazole (58) To a suspension of **48** (8.09 g, 82.5 mmol) and K₂CO₃ (13.7 g, 99.0 mmol) in DMF (100 mL) cooled with ice-water bath was added benzyl bromide (16.9 g, 98.6 mmol), and the mixture was allowed to stir at room temperature for 1.5 h. The mixture was stirred at 50 °C for another 4 h. The resultant mixture was partitioned between EtOAc and water, and the organic layer was washed with NaCl aqueous solution, dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10 to 30% EtOAc in hexane) to give **58** (13.1 g, 84%) as a colorless oil. ¹H NMR (CDCl₃) δ 3.74 (s, 3H), 5.18 (s, 2H), 5.64 (d, 1H, *J* = 2.3 Hz), 7.12 (d, 1H, *J* = 2.3 Hz), 7.28–7.33 (m, 1H), 7.34–7.39 (m, 2H), 7.43–7.46 (m, 2H); MS (ESI) *m/z* 189 [M+H]⁺.

3-(Benzyloxy)-4-iodo-1-methyl-1*H*-pyrazole (59) Compound **59** was prepared from **58** in a manner similar to that described for compound **50** with a yield of 61% as a brown oil. ¹H NMR (CDCl₃) δ 3.77 (s, 3H), 5.25 (s, 2H), 7.19 (s, 1H), 7.29–7.33 (m, 1H), 7.35–7.40 (m, 2H), 7.45–7.48 (m, 2H); MS (ESI) *m/z* 315 [M+H]⁺.

3-(Benzyloxy)-1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (60) Compound **60** was prepared from **59** in a manner similar to that described for compound **52** with a yield of 82% as a gum. ¹H NMR (CDCl₃) δ 1.31 (s, 12H), 3.72 (s, 3H), 5.32 (s, 2H), 7.24–7.30 (m, 1H), 7.32–7.37 (m, 2H), 7.43 (s, 1H),

7.49 (d, 2H, $J = 7.5$ Hz); MS (ESI) m/z 315 $[M+H]^+$.

5-[3-(Benzyloxy)-1-methyl-1H-pyrazol-4-yl]-1-methylpyridin-2(1H)-one (61) Under argon gas atmosphere, to the mixture of **60** (3.5 g, 11.1 mmol), 5-bromo-1-methylpyridin-2(1H)-one (2.53 g, 13.5 mmol), and Na_2CO_3 (3.54 g, 33.4 mmol) in DMF (33 mL) and water (15 mL) was added $\text{PdCl}_2(\text{dppf})\cdot\text{CH}_2\text{Cl}_2$ (543 mg, 0.67 mmol), and the mixture was stirred at 100 °C for 2 h. After cooling at room temperature, the mixture was diluted with EtOAc and water, and filtered through Celite pad. The organic layer of the filtrate was washed with NaCl aqueous solution, dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 5% MeOH in CHCl_3) to give **61** (1.23 g, 37%) as a pale green solid. ^1H NMR (CDCl_3) δ 3.51 (s, 3H), 3.78 (s, 3H), 5.32 (s, 2H), 6.58 (d, 1H, $J = 9.4$ Hz), 7.30 (s, 1H), 7.32–7.50 (m, 6H), 7.70 (d, 1H, $J = 2.5$ Hz); MS (ESI) m/z 296 $[M+H]^+$.

5-(3-Hydroxy-1-methyl-1H-pyrazol-4-yl)-1-methylpyridin-2(1H)-one (62) The mixture of **61** (1.2 g, 4.06 mmol) and 10% Pd–C (150mg) in EtOH (20 mL) was stirred at room temperature for 3 days under 3 atm of H_2 atmosphere. The mixture was filtered through Hyflo pad and washed with MeOH/EtOH/ CHCl_3 , and the filtrate was concentrated *in vacuo* to give **62** (678 mg, 81%) as a colorless solid. ^1H NMR ($\text{DMSO}-d_6$) δ 3.43 (s, 3H), 3.62 (s, 3H), 6.40 (d, 1H, $J = 9.4$ Hz), 7.64 (dd, 1H, $J = 9.4, 2.6$ Hz), 7.69 (s, 1H), 7.83 (d, 1H, $J = 2.6$ Hz), 10.26 (brs, 1H); MS (ESI) m/z 206 $[M+H]^+$.

5-(3-Hydroxy-1-methyl-1H-pyrazol-4-yl)-1-methylpiperidin-2-one (63) The suspension of **62** (285 mg, 1.39 mmol) and PtO_2 (50 mg, 0.22 mmol) in AcOH (10 mL) was stirred at room temperature for 15 h under 3 atm of H_2 atmosphere. The mixture was filtered through Hyflo pad and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 10% MeOH in CHCl_3) to give **63** (193 mg, 66%) as a colorless solid. ^1H NMR ($\text{DMSO}-d_6$) δ 1.72–1.92 (m, 2H), 2.19–2.35 (m, 2H), 2.80 (s, 3H), 2.82–2.90 (m, 1H), 3.15–3.22 (m, 1H), 3.33–3.39 (m, 1H), 3.56 (s, 3H), 7.23 (s, 1H), 9.56 (s, 1H); MS (ESI) m/z 210 $[M+H]^+$.

1-Methyl-5-(1-methyl-3-{[4-(quinolin-2-yl)benzyl]oxy}-1H-pyrazol-4-yl)piperidin-2-one dihydrochloride (64) A mixture of **63** (150 mg, 0.72 mmol), **34** (208 mg, 0.72 mmol), and K_2CO_3 (248 mg, 1.79 mmol) in DMF (4.2 mL) was stirred at 60 °C for 10 h. After cooling at room temperature, the mixture was partitioned between EtOAc and water. The organic extracts were washed with NaCl aqueous solution, dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 5% MeOH in CHCl_3) to give a free form of the title compound, which was dissolved in a mixture of EtOH and EtOAc. To the solution was added 4 M HCl/EtOAc. The resulting precipitate was collected by filtration to give **64** (266 mg, 74%) as a

pale yellow solid. ^1H NMR (DMSO- d_6) δ 1.80–2.00 (m, 2H), 2.22–2.40 (m, 2H), 2.82 (s, 3H), 2.95–3.03 (m, 1H), 3.24–3.30 (m, 1H), 3.39–3.44 (m, 1H), 3.66 (s, 3H), 5.33 (s, 2H), 7.43 (s, 1H), 7.71 (d, 2H, J = 8.4 Hz), 7.81–7.86 (m, 1H), 8.01–8.06 (m, 1H), 8.25 (d, 1H, J = 7.8 Hz), 8.32 (d, 2H, J = 8.4 Hz), 8.38 (d, 1H, J = 8.7 Hz), 8.56 (d, 1H, J = 8.5 Hz), 8.97 (d, 1H, J = 8.7 Hz); MS (ESI) m/z 427 $[\text{M}+\text{H}]^+$; Anal. Calcd for $\text{C}_{26}\text{H}_{26}\text{N}_4\text{O}_2 \cdot 2.3\text{HCl} \cdot 1.9\text{H}_2\text{O}$: C, 57.34; H, 5.94; N, 10.29; Cl, 14.97. Found: C, 57.36; H, 6.04; N, 10.33; Cl, 15.18.

[4-(3-Methylquinolin-2-yl)phenyl]methanol (67) To a suspension of 2-chloro-3-methylquinoline (**65**, 533 mg, 3.00 mmol) and [4-(hydroxymethyl)phenyl]boronic acid (**66**, 501 mg, 3.30 mmol) in DME (20 mL) were added $\text{Pd}(\text{PPh}_3)_4$ (173 mg, 0.15 mmol) and 1 M Na_2CO_3 aqueous solution (7.5 mL), and the mixture was stirred at 90 °C for 19 h under argon gas atmosphere. After cooling at room temperature, the mixture was partitioned between EtOAc and water. The organic layer was dried over Na_2SO_4 , filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 5% MeOH in CHCl_3) to give **67** (696 mg, 93%) as a pale yellow oil. ^1H NMR (DMSO- d_6) δ 2.46 (s, 3H), 4.60 (d, 2H, J = 5.7 Hz), 5.27 (t, 1H, J = 5.7 Hz), 7.45 (d, 2H, J = 8.3 Hz), 7.56–7.65 (m, 3H), 7.67–7.73 (m, 1H), 7.93 (d, 1H, J = 8.1 Hz), 7.98 (d, 1H, J = 8.2 Hz), 8.25 (s, 1H); MS (ESI) m/z 250 $[\text{M}+\text{H}]^+$.

1-Methyl-5-(1-methyl-3-{[4-(3-methylquinolin-2-yl)benzyl]oxy}-1H-pyrazol-4-yl)pyridin-2(1H)-one dihydrochloride (68) To a suspension of **62** (200 mg, 0.98 mmol) and **67** (292 mg, 1.17 mmol) in toluene (15 mL) was added CMBP (353 mg, 1.46 mmol) and the mixture was stirred at 100 °C for 10 h before the mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel; 0 to 5% MeOH in CHCl_3 , then NH silica gel; 50 to 80% EtOAc in hexane) to give a free form of the title compound, which was dissolved in a mixture of EtOAc and EtOH, and the mixture was treated with 4 M HCl/EtOAc (0.97 mL). The precipitate was collected by filtration and washed with Et_2O to give **68** (182 mg, 40%) as a pale blue solid. ^1H NMR (DMSO- d_6) δ 2.52 (s, 3H), 3.46 (s, 3H), 3.73 (s, 3H), 5.44 (s, 2H), 6.47 (d, 1H, J = 9.4 Hz), 7.71 (dd, 1H, J = 9.4, 2.6 Hz), 7.75 (d, 2H, J = 8.3 Hz), 7.84 (d, 2H, J = 8.3 Hz), 7.88–7.94 (m, 3H), 8.04 (t, 1H, J = 7.7 Hz), 8.25 (d, 1H, J = 8.1 Hz), 8.35 (d, 1H, J = 8.6 Hz), 9.00 (s, 1H); MS (ESI) m/z 437 $[\text{M}+\text{H}]^+$; Anal. Calcd for $\text{C}_{27}\text{H}_{24}\text{N}_4\text{O}_2 \cdot 1.9\text{HCl} \cdot 3\text{H}_2\text{O}$: C, 57.87; H, 5.74; N, 9.85; Cl, 12.47. Found: C, 58.08; H, 5.77; N, 9.88; Cl, 12.19.

2-Chloro-3-methylquinoline-6-carbonitrile (70a) Under argon atmosphere, to a mixture of *N*-(4-cyanophenyl)propanamide (**69a**, 3.61 g, 20.7 mmol) and CTAB (754 mg, 2.07 mmol) cooled with ice-water bath were added POCl_3 (9.5 mL, 104 mmol) and DMF (3.2 mL, 41.5 mmol), and the mixture was stirred at 120 °C for 12 h. The mixture was concentrated *in vacuo*, and the residue was poured into ice and diluted with

CHCl₃. To the mixture was added silica gel and filtered. The filtrate was extracted with CHCl₃ for 2 times. The combined organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 20% EtOAc in hexane) to give **70a** (595 mg, 14%) as a beige solid. ¹H NMR (CDCl₃) δ 2.59 (s, 3H), 7.82 (dd, 1H, *J* = 8.7, 1.7 Hz), 8.03 (s, 1H), 8.08 (d, 1H, *J* = 8.5 Hz), 8.16 (d, 1H, *J* = 1.7 Hz); MS (ESI) *m/z* 203 [M+H]⁺.

2-Chloro-8-fluoro-3-methylquinoline (70b) Compound **70b** was prepared from **69b** in a manner similar to that described for compound **70a** with a yield of 1.5%. ¹H NMR (CDCl₃) δ 2.56 (d, 3H, *J* = 1.0 Hz), 7.34–7.39 (m, 1H), 7.44–7.49 (m, 1H), 7.54 (d, 1H, *J* = 8.1 Hz), 8.01 (s, 1H); MS (ESI) *m/z* 196 [M+H]⁺.

[4-(5-Fluoro-3-methylquinolin-2-yl)phenyl]methanol (71c) Under argon atmosphere, a mixture of 2-chloro-5-fluoro-3-methylquinoline (**70c**, 443 mg, 2.27 mmol) and **66** (379 mg, 2.49 mmol), and Pd(PPh₃)₄ (130 mg, 0.11 mmol) in 1 M Na₂CO₃ aqueous solution (5.7 mL) and DME (15 mL) was stirred at 90 °C for 12 h. The mixture was filtered through Celite pad and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 10% MeOH in CHCl₃) to give **71c** (432 mg, 71%) as a white solid. ¹H NMR (CDCl₃) δ 2.50 (s, 3H), 4.61 (d, 2H, *J* = 5.7 Hz), 5.29 (t, 1H, *J* = 5.7 Hz), 7.38–7.49 (m, 3H), 7.59–7.63 (m, 2H), 7.66–7.73 (m, 1H), 7.85 (d, 1H, *J* = 8.5 Hz), 8.38 (s, 1H); MS (ESI) *m/z* 268 [M+H]⁺.

2-[4-(Hydroxymethyl)phenyl]-3-methylquinoline-6-carbonitrile (71a) Compound **71a** was prepared from **70a** in a manner similar to that described for compound **71c**, with a yield of 76% as a beige solid. ¹H NMR (CDCl₃) δ 1.93 (t, 1H, *J* = 5.9 Hz), 2.52 (d, 3H, *J* = 0.8 Hz), 4.80 (d, 2H, *J* = 5.9 Hz), 7.49–7.53 (m, 2H), 7.59–7.63 (m, 2H), 7.80 (dd, 1H, *J* = 8.8 Hz), 8.07 (s, 1H), 8.18–8.21 (m, 2H); MS (ESI) *m/z* 275 [M+H]⁺.

[4-(8-Fluoro-3-methylquinolin-2-yl)phenyl]methanol (71b) Compound **71b** was prepared from **70b** in a manner similar to that described for compound **71c** with a quantitative yield as a yellow amorphous solid. ¹H NMR (CDCl₃) δ 2.04 (brs, 1H), 2.49 (s, 3H), 4.75 (s, 2H), 7.31–7.37 (m, 1H), 7.41–7.48 (m, 3H), 7.56 (d, 1H, *J* = 8.2 Hz), 7.61 (d, 2H, *J* = 8.1 Hz), 8.03 (s, 1H); MS (ESI) *m/z* 268 [M+H]⁺.

[4-(6-Fluoro-3-methylquinolin-2-yl)phenyl]methanol (71d) Compound **71d** was prepared from 2-chloro-6-fluoro-3-methylquinoline (**70d**) in a manner similar to that described for compound **71c**, with a yield of 93% as a beige solid. ¹H NMR (CDCl₃) δ 2.13 (t, 1H, *J* = 6.0 Hz), 2.46 (s, 3H), 4.77 (d, 2H, *J* = 6.0 Hz), 7.37–7.49 (m, 4H), 7.54–7.58 (m, 2H), 7.97 (s, 1H), 8.12 (dd, 1H, *J* = 9.2, 5.4 Hz); MS (ESI) *m/z* 268 [M+H]⁺.

[4-(7-Fluoro-3-methylquinolin-2-yl)phenyl]methanol (71e) Compound **71e** was prepared from 2-chloro-7-fluoro-3-methylquinoline (**70e**) in a manner similar to that described for compound **71c**, with a yield of 87% as a beige solid. ¹H NMR (CDCl₃) δ 1.95 (brs, 1H), 2.46 (s, 3H), 4.78 (s, 2H), 7.28–7.35 (m, 1H), 7.48 (d, 2H, *J* = 8.1 Hz), 7.58 (d, 2H, *J* = 8.1 Hz), 7.64–7.70 (m, 1H), 7.73–7.79 (m, 1H), 8.02 (s, 1H); MS (ESI) *m/z* 268 [M+H]⁺.

[4-(3-Ethylquinolin-2-yl)phenyl]methanol (71f) Compound **71f** was prepared from 2-chloro-3-ethylquinoline (**70f**) in a manner similar to that described for compound **71c**, with a yield of 56% as a beige solid. ¹H NMR (CDCl₃) δ 1.20 (t, 3H, *J* = 7.5 Hz), 2.12 (t, 1H, *J* = 6.1 Hz), 2.76–2.84 (m, 2H), 4.77 (d, 2H, *J* = 6.1 Hz), 7.46 (d, 2H, *J* = 8.3 Hz), 7.51–7.56 (m, 3H), 7.63–7.70 (m, 1H), 7.81 (dd, 1H, *J* = 8.1, 1.2 Hz), 8.06 (s, 1H), 8.13 (d, 1H, *J* = 8.4 Hz); MS (ESI) *m/z* 264 [M+H]⁺.

[4-(3,6-Dimethylquinolin-2-yl)phenyl]methanol (71g) Compound **71g** was prepared from **70g** in a manner similar to that described for compound **71c**, with a yield of 87% as a white solid. ¹H NMR (CDCl₃) δ 1.94 (t, 1H, *J* = 5.7 Hz), 2.45 (s, 3H), 2.54 (s, 3H), 4.77 (d, 2H, *J* = 5.7 Hz), 7.45–7.51 (m, 3H), 7.54 (s, 1H), 7.56–7.60 (m, 2H), 7.92 (s, 1H), 8.01 (d, 1H, *J* = 8.6 Hz); MS (ESI) *m/z* 264 [M+H]⁺.

[4-(6-Methoxy-3-methylquinolin-2-yl)phenyl]methanol (71h) Compound **71h** was prepared from 2-chloro-6-methoxy-3-methylquinoline (**70h**) in a manner similar to that described for compound **71c**, with a yield of 33% as a beige solid. ¹H NMR (CDCl₃) δ 2.04 (t, 1H, *J* = 5.7 Hz), 2.45 (s, 3H), 3.94 (s, 3H), 4.76 (d, 2H, *J* = 5.7 Hz), 7.04 (d, 1H, *J* = 2.8 Hz), 7.32 (dd, 1H, *J* = 9.2, 2.8 Hz), 7.46 (d, 2H, *J* = 8.0 Hz), 7.57 (d, 2H, *J* = 8.0 Hz), 7.92 (s, 1H), 8.02 (d, 1H, *J* = 9.2 Hz); MS (ESI) *m/z* 280 [M+H]⁺.

2-[4-(Hydroxymethyl)phenyl]quinoline-3-carbaldehyde (71i) Compound **71i** was prepared from 2-chloroquinoline-3-carboxaldehyde (**70i**) in a manner similar to that described for compound **71c**, with a yield of 85% as a yellow solid. ¹H NMR (DMSO-*d*₆) δ 4.64 (d, 2H, *J* = 5.8 Hz), 5.34 (t, 1H, *J* = 5.8 Hz), 7.53 (d, 2H, *J* = 8.3 Hz), 7.67–7.75 (m, 3H), 7.94–7.99 (m, 1H), 8.13 (d, 1H, *J* = 8.4 Hz), 8.28 (d, 1H, *J* = 8.1 Hz), 8.97 (s, 1H), 10.09 (s, 1H); MS (ESI) *m/z* 264 [M+H]⁺.

3-Methyl-2-[4-({[1-methyl-4-(1-methyl-6-oxo-1,6-dihydropyridin-3-yl)-1H-pyrazol-3-yl]oxy}methyl)phenyl]quinoline-6-carbonitrile (72a) To a stirred mixture of **71a** (591 mg, 2.15 mmol) in CH₂Cl₂ (12 mL) was added SOCl₂ (0.47 mL, 6.44 mmol), and the mixture was stirred at room temperature for 2 h. The reaction was diluted with EtOAc, and the precipitate was collected by filtration to give a

2-[4-(chloromethyl)phenyl]-3-methylquinoline-6-carbonitrile hydrochloride (547 mg, 77%) as a beige solid. A mixture of this beige solid (300 mg, 0.91 mmol), **62** (206 mg, 1.00 mmol) and K₂CO₃ (378 mg, 2.74 mmol) in DMF (6 mL) was stirred at 60 °C for 12 h. After cooling at room temperature, the mixture was diluted with water and stirred for a while. The precipitate was collected by filtration and purified by silica gel column chromatography (0 to 10% MeOH in CHCl₃) to give a yellow solid, which was washed with EtOAc, then EtOH to give **72a** (216 mg, 51%) as a yellow solid. ¹H NMR (DMSO-*d*₆) δ 2.50 (s, 3H), 3.44 (s, 3H), 3.72 (s, 3H), 5.38 (s, 2H), 6.44 (d, 1H, *J* = 9.4 Hz), 7.62 (d, 2H, *J* = 8.2 Hz), 7.66–7.72 (m, 3H), 7.85 (s, 1H), 7.89 (d, 1H, *J* = 2.5 Hz), 7.99 (dd, 1H, *J* = 8.7, 1.8 Hz), 8.13 (d, 1H, *J* = 8.7 Hz), 8.39 (s, 1H), 8.60 (d, 1H, *J* = 1.7 Hz); MS (ESI) *m/z* 462 [M+H]⁺; Anal. Calcd for C₂₈H₂₃N₅O₂·0.2H₂O: C, 72.30; H, 5.07; N, 15.06. Found: C, 72.36; H, 4.92; N, 15.00.

5-(3-{[4-(3,6-Dimethylquinolin-2-yl)benzyl]oxy}-1-methyl-1*H*-pyrazol-4-yl)-1-methylpyridin-2(1*H*)-one dihydrochloride (72g) To a stirred mixture of **71g** (252 mg, 0.96 mmol) in CH₂Cl₂ (5 mL) was added SOCl₂ (0.21 mL, 2.88 mmol), and the mixture was stirred at room temperature for 2 h. The reaction was diluted with EtOAc, and the precipitate was collected by filtration to give 2-[4-(chloromethyl)phenyl]-3,6-dimethylquinoline hydrochloride (245 mg, 81%) as a beige solid. To this beige solid (244 mg, 0.77 mmol) and **62** (173 mg, 0.84 mmol) in DMF was added K₂CO₃ (318 mg, 2.30 mmol), and the mixture was stirred at 60 °C for 12 h. After cooling at room temperature, the mixture was diluted with water and stirred for a while. The precipitate was collected by filtration and purified by silica gel column chromatography (0 to 10% MeOH in CHCl₃) to give a white amorphous solid, which was dissolved in EtOH (5 mL). To the solution was added 4 M HCl/EtOAc (2 mL), and the precipitate was collected by filtration to give **72g** (360 mg, 90%) as a beige solid. ¹H NMR (DMSO-*d*₆) δ 2.51 (s, 3H), 2.59 (s, 3H), 3.45 (s, 3H), 3.72 (s, 3H), 5.44 (s, 2H), 6.46 (d, 1H, *J* = 9.4 Hz), 7.70 (dd, 1H, *J* = 9.4, 2.6 Hz), 7.75 (d, 2H, *J* = 8.2 Hz), 7.82 (d, 2H, *J* = 8.2 Hz), 7.87 (s, 1H), 7.88–7.93 (m, 2H), 8.01 (s, 1H), 8.21 (d, 1H, *J* = 8.8 Hz), 8.89 (brs, 1H); MS (ESI) *m/z* 451 [M+H]⁺.

5-(3-{[4-(8-Fluoro-3-methylquinolin-2-yl)benzyl]oxy}-1-methyl-1*H*-pyrazol-4-yl)-1-methylpyridin-2(1*H*)-one trihydrochloride (72b) Compound **72b** was prepared from **71b** in a manner similar to that described for compound **72g**, with a yield of 26% as a beige solid. ¹H NMR (DMSO-*d*₆) δ 2.48 (s, 3H), 3.46 (s, 3H), 3.73 (s, 3H), 5.39 (s, 2H), 6.48 (d, 1H, *J* = 9.4 Hz), 7.50–7.80 (m, 8H), 7.87 (s, 1H), 7.93 (d, 1H, *J* = 2.5 Hz), 8.36 (brs, 1H); MS (ESI) *m/z* 455 [M+H]⁺; Anal. Calcd for C₂₇H₂₃FN₄O₂·2.9HCl·3.4H₂O: C, 52.18; H, 5.30; N, 9.01; Cl, 16.54; F, 3.06. Found: C, 52.19; H, 5.06; N, 9.04; Cl, 16.76; F, 3.31.

5-(3-{[4-(5-Fluoro-3-methylquinolin-2-yl)benzyl]oxy}-1-methyl-1*H*-pyrazol-4-yl)-1-methylpyridin-2(1*H*)-one dihydrochloride (72c) Compound **72c** was prepared from **71c** in a manner similar to that described for

compound **72g**, with a yield of 64% as a beige solid. ^1H NMR ($\text{DMSO-}d_6$) δ 2.52 (d, 3H, J = 0.7 Hz), 3.45 (s, 3H), 3.73 (s, 3H), 5.40 (s, 2H), 6.46 (d, 1H, J = 9.4 Hz), 7.49 (dd, 1H, J = 9.6, 7.8 Hz), 7.65 (d, 2H, J = 8.3 Hz), 7.68–7.81 (m, 4H), 7.86 (s, 1H), 7.89–7.94 (m, 2H), 8.55 (brs, 1H); MS (ESI) m/z 455 $[\text{M}+\text{H}]^+$; Anal. Calcd for $\text{C}_{27}\text{H}_{23}\text{FN}_4\text{O}_2 \cdot 2\text{HCl} \cdot 2\text{H}_2\text{O}$: C, 57.55; H, 5.19; N, 9.94; Cl, 12.58; F, 3.37. Found: C, 57.59; H, 5.32; N, 9.94; Cl, 12.61; F, 3.22.

5-(3-{[4-(6-Fluoro-3-methylquinolin-2-yl)benzyl]oxy}-1-methyl-1H-pyrazol-4-yl)-1-methylpyridin-2(1H)-one dihydrochloride (72d) Compound **72d** was prepared from **71d** in a manner similar to that described for

compound **72g**, with a yield of 75% as a beige solid. ^1H NMR ($\text{DMSO-}d_6$) δ 2.53 (s, 3H), 3.50 (s, 3H), 3.74 (s, 3H), 5.45 (s, 2H), 6.54 (d, 1H, J = 9.4 Hz), 7.73–7.84 (m, 5H), 7.89 (s, 1H), 7.91–8.00 (m, 2H), 8.06 (dd, 1H, J = 9.0, 2.8 Hz), 8.35 (dd, 1H, J = 9.4, 5.0 Hz), 8.89 (s, 1H); MS (ESI) m/z 455 $[\text{M}+\text{H}]^+$; Anal. Calcd for $\text{C}_{27}\text{H}_{23}\text{FN}_4\text{O}_2 \cdot 2\text{HCl} \cdot 4\text{H}_2\text{O}$: C, 54.10; H, 5.55; N, 9.35; Cl, 11.83; F, 3.17. Found: C, 54.28; H, 5.55; N, 9.30; Cl, 11.78; F, 3.16.

5-(3-{[4-(7-Fluoro-3-methylquinolin-2-yl)benzyl]oxy}-1-methyl-1H-pyrazol-4-yl)-1-methylpyridin-2(1H)-one dihydrochloride (72e) Compound **72e** was prepared from **71e** in a manner similar to that described for

compound **72g**, with a yield of 42% as a beige solid. ^1H NMR ($\text{DMSO-}d_6$) δ 2.47 (s, 3H), 3.44 (s, 3H), 3.72 (s, 3H), 5.40 (s, 2H), 6.45 (d, 1H, J = 9.3 Hz), 7.59–7.74 (m, 6H), 7.81 (dd, 1H, J = 9.3, 2.4 Hz), 7.86 (s, 1H), 7.91 (d, 1H, J = 2.4 Hz), 8.14 (dd, 1H, J = 9.1, 6.2 Hz), 8.54 (brs, 1H); MS (ESI) m/z 455 $[\text{M}+\text{H}]^+$; Anal. Calcd for $\text{C}_{27}\text{H}_{23}\text{FN}_4\text{O}_2 \cdot 1.9\text{HCl} \cdot 3.8\text{H}_2\text{O}$: C, 54.76; H, 5.53; N, 9.46; Cl, 11.37; F, 3.21. Found: C, 54.98; H, 5.57; N, 9.29; Cl, 11.08; F, 3.16.

5-(3-{[4-(3-Ethylquinolin-2-yl)benzyl]oxy}-1-methyl-1H-pyrazol-4-yl)-1-methylpyridin-2(1H)-one dihydrochloride (72f) Compound **72f** was prepared from **71f** in a manner similar to that described for

compound **72g**, with a yield of 44% as a beige solid. ^1H NMR ($\text{DMSO-}d_6$) δ 1.20 (t, 3H, J = 7.5 Hz), 2.83 (q, 2H, J = 7.5 Hz), 3.46 (s, 3H), 3.73 (s, 3H), 5.44 (s, 2H), 6.47 (d, 1H, J = 9.4 Hz), 7.71 (dd, 1H, J = 9.4, 2.6 Hz), 7.75 (d, 2H, J = 8.3 Hz), 7.79 (d, 2H, J = 8.3 Hz), 7.87–7.95 (m, 3H), 8.06 (t, 1H, J = 7.8 Hz), 8.27–8.36 (m, 2H), 9.06 (brs, 1H); MS (ESI) m/z 451 $[\text{M}+\text{H}]^+$; Anal. Calcd for $\text{C}_{28}\text{H}_{26}\text{N}_4\text{O}_2 \cdot 2.3\text{HCl} \cdot 2\text{H}_2\text{O}$: C, 58.96; H, 5.71; N, 9.82; Cl, 14.30. Found: C, 58.83; H, 5.87; N, 9.77; Cl, 14.38.

5-(3-{[4-(6-Methoxy-3-methylquinolin-2-yl)benzyl]oxy}-1-methyl-1H-pyrazol-4-yl)-1-methylpyridin-2(1H)-one dihydrochloride (72h) Compound **72h** was prepared from **71h** in a manner similar to that described for

compound **72g**, with a yield of 40% as a beige solid. ^1H NMR ($\text{DMSO-}d_6$) δ 2.50 (s, 3H), 3.45 (s, 3H), 3.72 (s,

3H), 3.98 (s, 3H), 5.43 (s, 2H), 6.46 (d, 1H, $J = 9.4$ Hz), 7.63 (d, 1H, $J = 2.5$ Hz), 7.66–7.77 (m, 4H), 7.80 (d, 2H, $J = 8.2$ Hz), 7.87 (s, 1H), 7.92 (d, 1H, $J = 2.5$ Hz), 8.24 (d, 1H, $J = 9.4$ Hz), 8.83 (brs, 1H); MS (ESI) m/z 467 $[M+H]^+$; Anal. Calcd for $C_{28}H_{26}N_4O_3 \cdot 2HCl \cdot 2.6H_2O$: C, 57.36; H, 5.71; N, 9.56; Cl, 12.09. Found: C, 57.72; H, 5.87; N, 9.35; Cl, 11.79.

2-[4-([1-Methyl-4-(1-methyl-6-oxo-1,6-dihydropyridin-3-yl)-1H-pyrazol-3-yl]oxy)methyl]phenyl]quinoline-3-carbaldehyde (72i) Compound **72i** was prepared from **71i** in a manner similar to that described for compound **72a**, with a yield of 24% as a dark yellow solid. 1H NMR ($CDCl_3$) δ 3.56 (s, 3H), 3.79 (s, 3H), 5.44 (s, 2H), 6.61 (d, 1H, $J = 9.4$ Hz), 7.31 (s, 1H), 7.49–7.53 (m, 1H), 7.63–7.68 (m, 3H), 7.71–7.74 (m, 3H), 7.86–7.92 (m, 1H), 8.02 (dd, 1H, $J = 8.2, 1.3$ Hz), 8.19–8.23 (m, 1H), 8.86 (s, 1H), 10.21 (s, 1H); MS (ESI) m/z 451 $[M+H]^+$.

5-[3-([4-[3-(Difluoromethyl)quinolin-2-yl]benzyl]oxy)-1-methyl-1H-pyrazol-4-yl]-1-methylpyridin-2(1H)-one dihydrochloride (73) To a solution of **72i** (150 mg, 0.33 mmol) in CH_2Cl_2 (3 mL) was added bis(2-methoxyethyl)aminosulfur trifluoride (Deoxo-Fluor[®], 125 mg, 0.57 mmol), and the mixture was stirred at room temperature overnight. The reaction was quenched with water and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (NH silica gel; 38 to 70% $CHCl_3$ in hexane) to give a free form of the title compound, which was dissolved in EtOAc. The solution was treated with 4 M HCl/EtOAc, and the precipitate was collected by filtration to give **73** (83 mg, 46%) as a pale yellow solid. 1H NMR ($DMSO-d_6$) δ 3.47 (s, 3H), 3.73 (s, 3H), 5.40 (s, 2H), 6.50 (d, 1H, $J = 9.4$ Hz), 7.20 (t, 1H, $J = 54$ Hz), 7.64–7.79 (m, 6H), 7.88 (s, 1H), 7.93–7.98 (m, 2H), 8.14 (d, 1H, $J = 8.5$ Hz), 8.26 (d, 1H, $J = 7.9$ Hz), 8.93 (s, 1H); MS (ESI) m/z 473 $[M+H]^+$; Anal. Calcd for $C_{27}H_{22}F_2N_4O_2 \cdot 2.3HCl \cdot 2H_2O$: C, 54.74; H, 4.82; N, 9.46; Cl, 13.77; F, 6.41. Found: C, 54.83; H, 4.93; N, 9.53; Cl, 13.73; F, 6.50.

Methyl 1-methyl-4-(1-methyl-6-oxo-1,6-dihydropyridin-3-yl)-1H-pyrazole-3-carboxylate (76) To a mixture of 1-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2(1H)-one (**74**, 2.3 g, 9.78 mmol) and methyl 4-iodo-1-methyl-1H-pyrazole-3-carboxylate (**75**, 1.25 g, 4.70 mmol) in DMF (40 mL) and water (10 mL) were added $Pd(PPh_3)_4$ (814 mg, 0.71 mmol) and CS_2CO_3 (3.06 g, 9.40 mmol), and the mixture was stirred at 80 °C for 12 h. The mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (0 to 5% MeOH in $CHCl_3$) to give **76** (812 mg, 70%) as a colorless solid. 1H NMR ($DMSO-d_6$) δ 3.44 (s, 3H), 3.74 (s, 3H), 3.91 (s, 3H), 6.39 (d, 1H, $J = 9.3$ Hz), 7.51 (dd, 1H, $J = 9.4, 2.6$ Hz), 7.86 (d, 1H, $J = 2.6$ Hz), 7.94 (s, 1H); MS (ESI) m/z 248 $[M+H]^+$.

5-[3-(Hydroxymethyl)-1-methyl-1*H*-pyrazol-4-yl]-1-methylpyridin-2(1*H*)-one (77) To a mixture of **76** (8.24 g, 33.3 mmol) in THF (200 mL) were added LiBH₄ (1.45 g, 66.7 mmol) and EtOH (5.83 mL), and the mixture was stirred under reflux condition for 2 h. After cooling at room temperature, to the reaction mixture was added 1 M HCl aqueous solution. The mixture was neutralized with NaHCO₃ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 10% MeOH in CHCl₃) to give **77** (3.23 g, 44%) as a colorless solid. ¹H NMR (DMSO-*d*₆) δ 3.45 (s, 3H), 3.80 (s, 3H), 4.41 (d, 2H, *J* = 5.1 Hz), 5.17 (t, 1H, *J* = 5.1 Hz), 6.43 (d, 1H, *J* = 9.4 Hz), 7.65 (dd, 1H, *J* = 9.4, 2.6 Hz), 7.83 (s, 1H), 7.88 (d, 1H, *J* = 2.6 Hz); MS (ESI) *m/z* 220 [M+H]⁺.

5-[3-(Chloromethyl)-1-methyl-1*H*-pyrazol-4-yl]-1-methylpyridin-2(1*H*)-one hydrochloride (78) To a mixture of **77** (395 mg, 1.80 mmol) in CH₂Cl₂ (20 mL) was added SOCl₂ (0.39 mL, 5.40 mmol), and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo* to give **78** (493 mg, quant) as a colorless solid. ¹H NMR (DMSO-*d*₆) δ 3.47 (s, 3H), 3.83 (s, 3H), 4.80 (s, 2H), 6.47 (d, 1H, *J* = 9.3 Hz), 7.58 (dd, 1H, *J* = 9.3, 2.6 Hz), 7.79 (d, 1H, *J* = 2.6 Hz), 7.87 (s, 1H); MS (ESI) *m/z* 238, 240 [M+H]⁺.

6-Fluoro-3-methyl-2-[4-(tetrahydro-2*H*-pyran-2-yloxy)phenyl]quinoline (80) To a mixture of 2-chloro-6-fluoro-3-methylquinoline (**79**, 4.23 g, 21.6 mmol), 4-(2-tetrahydropyranyloxy)phenylboronic acid (5.04 g, 22.7 mmol), and Pd(PPh₃)₄ (1.44 g, 1.25 mmol) was added Na₂CO₃ (5.64 g, 53.2 mmol) in DME (80 mL) and water (25 mL), and the mixture was stirred at 100 °C for 16 h. After cooling at room temperature, the mixture was diluted with EtOAc and washed with water and brine, dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 10% EtOAc in CHCl₃) to give a pale brown solid, which was washed with EtOAc to give **80** (6.29 g, 86%) as a pale yellow solid. ¹H NMR (CDCl₃) δ 1.58–1.77 (m, 3H), 1.87–1.93 (m, 2H), 1.99–2.11 (m, 1H), 2.49 (d, 3H, *J* = 0.8 Hz), 3.60–3.66 (m, 1H), 3.90–3.97 (m, 1H), 5.50 (t, 1H, *J* = 3.2 Hz), 7.15–7.19 (m, 2H), 7.35–7.43 (m, 2H), 7.51–7.55 (m, 2H), 7.94 (s, 1H), 8.09 (dd, 1H, *J* = 9.1, 5.4 Hz); MS (ESI) *m/z* 338 [M+H]⁺.

4-(6-Fluoro-3-methylquinolin-2-yl)phenol (81a) To a mixture of **80** (6.29 g, 18.6 mmol) in THF (90 mL) was added 1 M HCl aqueous solution (40 mL, 40 mmol), and the mixture was stirred at room temperature for 1 h. The reaction was quenched with 1 M NaOH aqueous solution, and the precipitate was collected by filtration to give **81a** (4.72 g, 90%) as a pale yellow solid. ¹H NMR (CDCl₃) δ 2.46 (d, 3H, *J* = 0.6 Hz), 6.85–6.91 (m, 2H), 7.46–7.51 (m, 2H), 7.54–7.61 (m, 1H), 7.69 (dd, 1H, *J* = 9.5, 2.9 Hz), 8.01 (dd, 1H, *J* = 9.2, 5.5 Hz), 8.19 (s, 1H); MS (ESI) *m/z* 254 [M+H]⁺.

5-(3-{[4-(6-Fluoro-3-methylquinolin-2-yl)phenoxy]methyl}-1-methyl-1*H*-pyrazol-4-yl)-1-methylpyridin-2(1

H)-one (82a) To a stirred mixture of **81a** (270 mg, 1.07 mmol) and **78** (351 mg, 1.28 mmol) in DMF (7.0 mL) was added K₂CO₃ (369 mg, 2.67 mmol), and the mixture was stirred at 70 °C for 12 h. After cooling at room temperature, the mixture was diluted with EtOAc, washed with water. The aqueous layer was extracted with EtOAc. The combined organic layer was dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (NH silica gel; 0 to 20% EtOAc in CHCl₃) to give an off-white solid, which was washed with EtOAc to give **82a** (390 mg, 81%) as an off-white solid. ¹H NMR (DMSO-*d*₆) δ 2.47 (d, 3H, *J* = 0.6 Hz), 3.38 (s, 3H), 3.88 (s, 3H), 5.13 (s, 2H), 6.44 (d, 1H, *J* = 9.2 Hz), 7.16–7.20 (m, 2H), 7.56–7.63 (m, 4H), 7.71 (dd, 1H, *J* = 9.5, 2.9 Hz), 7.77 (d, 1H, *J* = 2.5 Hz), 7.83 (s, 1H), 8.03 (dd, 1H, *J* = 9.2, 5.5 Hz), 8.22 (s, 1H); MS (ESI) *m/z* 455 [M+H]⁺; Anal. Calcd for C₂₇H₂₃FN₄O₂·0.2H₂O: C, 70.79; H, 5.15; N, 12.23; F, 4.15. Found: C, 70.59; H, 5.11; N, 12.24; F, 4.21.

1-Methyl-5-(1-methyl-3-{[4-(quinolin-2-yl)phenoxy]methyl}-1H-pyrazol-4-yl)pyridin-2(1H)-one (82b)

Compound **82b** was prepared from 4-(quinolin-2-yl)phenol (**81b**) in a manner similar to that described for compound **82a**, with a yield of 53% as a colorless solid. ¹H NMR (DMSO-*d*₆) δ 3.37 (s, 3H), 3.87 (s, 3H), 5.15 (s, 2H), 6.41–6.45 (d, 1H, *J* = 9.3 Hz), 7.20–7.25 (m, 2H), 7.54–7.60 (m, 2H), 7.73–7.79 (m, 2H), 7.93 (s, 1H), 7.95–7.99 (m, 1H), 8.03 (d, 1H, *J* = 8.4 Hz), 8.11 (d, 1H, *J* = 8.7 Hz), 8.23–8.28 (m, 2H), 8.41 (d, 1H, *J* = 8.4 Hz); MS (ESI) *m/z* 423 [M+H]⁺; Anal. Calcd for C₂₆H₂₂N₄O₂: C, 73.92; H, 5.25; N, 13.26. Found: C, 73.89; H, 5.23; N, 13.27.

(4-Iodo-1-methyl-1H-pyrazol-3-yl)methanol (84) To a solution of 4-iodo-1-methyl-1H-pyrazole-3-carboxylic acid (**83**, 719 mg, 2.82 mmol) in THF (7 mL) was added 1,1'-carbonyldiimidazole (CDI, 685 mg, 4.23 mmol), and the mixture was stirred at room temperature for 1 h. To the resultant mixture cooled with ice-water bath was added a mixture of NaBH₄ (320 mg, 8.46 mmol) in water (7 mL), and the mixture was stirred at room temperature for 3 h. The reaction was diluted with water and extracted with EtOAc. The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 5% MeOH in CHCl₃) to give **84** (577 mg, 86%) as a white solid. ¹H NMR (DMSO-*d*₆) δ 3.80 (s, 3H), 4.30 (d, 2H, *J* = 5.3 Hz), 4.93 (t, 1H, *J* = 5.3 Hz), 7.78 (s, 1H); MS (ESI) *m/z* 239 [M+H]⁺.

2-{4-[(4-Iodo-1-methyl-1H-pyrazol-3-yl)methoxy]phenyl}quinoline (85) To a mixture of **84** (690 mg, 2.90 mmol) in CH₂Cl₂ (7 mL) cooled with ice bath was added SOCl₂ (0.32 mL, 4.39 mmol), and the mixture was stirred at room temperature for 2 h. The reaction was concentrated *in vacuo*. To the residue in DMF (10 mL) were added **81b** (488 mg, 2.21 mmol) and K₂CO₃ (800 mg, 5.79 mmol), and the mixture was stirred at 70 °C for 8 h. After cooling at room temperature, the residue was partitioned between EtOAc and water. The organic layer was

washed with brine, dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 5% MeOH in CHCl₃) to give **85** (908 mg, 93%) as a pale yellow solid. ¹H NMR (DMSO-*d*₆) δ 3.87 (s, 3H), 5.02 (s, 2H), 7.17–7.22 (m, 2H), 7.54–7.69 (m, 1H), 7.73–7.79 (m, 1H), 7.91 (s, 1H), 7.97 (brd, 1H), 8.04 (brd, 1H), 8.11 (d, 1H, *J* = 8.7 Hz), 8.23–8.28 (m, 2H), 8.41 (brd, 1H); MS (ESI) *m/z* 442 [M+H]⁺.

5-(1-Methyl-3-{{4-(quinolin-2-yl)phenoxy}methyl}-1H-pyrazol-4-yl)pyridin-2-ol (86) Compound **86** was prepared from **85** and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine-2-ol in a manner similar to that described for compound **53a**, with a yield of 6.0% as a brown solid. ¹H NMR (CDCl₃) δ 3.94 (s, 3H), 5.12 (s, 2H), 6.60 (d, 1H, *J* = 9.4 Hz), 7.17 (d, 2H, *J* = 8.9 Hz), 7.41 (s, 1H), 7.47–7.52 (m, 2H), 7.57 (dd, 1H, *J* = 9.4, 2.7 Hz), 7.68–7.73 (m, 1H), 7.78–7.85 (m, 2H), 8.11–8.20 (m, 4H), 11.61 (brs, 1H); MS (ESI) *m/z* 409 [M+H]⁺.

1-(¹¹C)Methyl-5-(1-methyl-3-{{4-(quinolin-2-yl)phenoxy}methyl}-1H-pyrazol-4-yl)pyridin-2(1H)-one ([¹¹C]82b**)** [¹¹C]CO₂ was produced by the ¹⁴N(p, α)¹¹C nuclear reaction using a Cyclone 18/9 cyclotron (IBA) and [¹¹C]CH₃I was produced from [¹¹C]CO₂ by its reduction with LiAlH₄ and subsequent reaction with HI. Generated [¹¹C]CH₃I was passed through a heated column (200 °C) of silver triflate (AgOTf), and converted to [¹¹C]methyl triflate (CH₃OTf). [¹¹C]CH₃OTf was introduced into a reaction vessel containing **86** (1.2 mg) and 2 mg of NaH (60% oil dispersion, pre-washed with hexane) in a mixed solution of THF (300 μ L) and DMSO (25 μ L) at -20 °C. The solution was heated at 60 °C for 3 min. After cooling at room temperature, the mixture was transferred to a semi-preparative reverse phase HPLC system (YMC-Pack Pro C₁₈ 10 \times 250 mm column (YMC Co., Ltd.), eluted with 55:45 [0.05 M NH₄OAc–0.1% AcOH]/MeCN, eluted at 5.0 mL), which included a solution wash of the reaction vessel with two portions of 70:30 [0.05 M NH₄OAc–0.1% AcOH]/MeCN (800, 1000 μ L). The fraction containing [¹¹C]**82b** was collected in a rotary evaporator including 25% ascorbic acid in distilled water (50 μ L) and EtOH (0.5 mL). The solvent was removed under reduced pressure. The residue was dissolved in 10% DMF, 10% PEG400-saline (3.0 mL) and sterile filtered (Millipore GS) to furnish an injectable solution of [¹¹C]**82b**. Analytical chromatography was performed with an Agilent 1200 HPLC system (Agilent Technologies Japan) and an Aloka positron detector RLC-700 (Aloka). Reverse phase chromatography was performed using a YMC-Pack C₁₈ Pro column (5 μ m, 4.6 \times 150 mm; YMC, Kyoto, Japan), eluted with 55:45 [0.05 M NH₄OAc–0.1% AcOH]/MeCN at 1 mL/min (R_t = 8 min). [¹¹C]**82b** was identified by comparison with authentic **82b**.

第三章に関する実験

4-{3-[(4-Bromobenzyl)oxy]-1-methyl-1H-pyrazol-4-yl}pyridine (88) To a mixture of

(4-bromophenyl)methanol (**87**, 3.07 g, 16.4 mmol) and **13** (2.40 g, 13.7 mmol) in toluene (100 mL) was added 95% CMBP (4.00 g, 15.7 mmol), and the mixture was stirred at 100 °C for 12 h. After cooling at room temperature, the mixture was concentrated *in vacuo*, and the residue was purified by flash column chromatography (silica gel; 0 to 5% MeOH in CHCl₃ then NH silica gel; 20 to 50% EtOAc in hexane) and recrystallized from EtOAc/hexane to give **88** (1.89 g, 40%) as a colorless solid. ¹H NMR (DMSO-*d*₆) δ 3.75 (s, 3H), 5.30 (s, 2H), 7.46 (d, 2H, *J* = 8.5 Hz), 7.56 (dd, 2H, *J* = 4.6, 1.6 Hz), 7.59–7.63 (m, 2H), 8.26 (s, 1H), 8.45 (dd, 2H, *J* = 4.6, 1.6 Hz); MS (ESI) *m/z* 344, 346 [M+H]⁺.

2-[4-([1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)methyl]phenyl]pyridine dihydrochloride (89a)

Under argon gas atmosphere, to a mixture of **88** (137 mg, 0.40 mmol) and 2-pyridylzinc bromide (0.5 M solution in THF, 1.6 mL, 0.80 mmol) in THF (1.0 mL) was added Pd(PPh₃)₄ (92 mg, 0.08 mmol), and the mixture was stirred at 120 °C for 1 h. After cooling at room temperature, the reaction was quenched with water and diluted with EtOAc, and the mixture was filtered through Celite pad. The filtrate was washed with saturated NH₄Cl aqueous solution and brine, dried over Na₂SO₄, and concentrated *in vacuo*. The residue was purified by NH silica gel column chromatography (25 to 50% CHCl₃ in hexane) to give a free form of the title compound, which was dissolved in EtOH (2 mL) and treated with 4 M HCl/EtOAc (0.2 mL). The mixture was diluted with Et₂O (5 mL) and stirred at room temperature for 1 h. The precipitate was collected by filtration and washed with Et₂O to give **89a** (30 mg, 18%) as a colorless solid. ¹H NMR (DMSO-*d*₆) δ 3.83 (s, 3H), 5.48 (s, 2H), 7.54 (brs, 1H), 7.68 (d, 2H, *J* = 8.4 Hz), 8.07–8.16 (m, 6H), 8.69 (s, 1H), 8.71–8.75 (m, 3H); MS (ESI) *m/z* 343 [M+H]⁺; Anal. Calcd for C₂₁H₁₈N₄O·2.2HCl·3.1H₂O: C, 52.72; H, 5.56; N, 11.71; Cl, 16.30. Found: C, 52.57; H, 5.60; N, 11.69; Cl, 16.30.

4-(1-Methyl-3-{[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl]oxy}-1H-pyrazol-4-yl)pyridine (90)

Under argon gas atmosphere, to a mixture of **88** (292 mg, 0.85 mmol) and bis(pinacolato)diboron (259 mg, 1.02 mmol) and potassium acetate (250 mg, 2.55 mmol) in dioxane (3.0 mL) was added PdCl₂(dppf)·CH₂Cl₂ (69 mg, 0.085 mmol), and the mixture was stirred at 100 °C for 3 h. After cooling at room temperature, the insoluble material was removed by filtration through Celite pad and washed with CHCl₃/EtOAc, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 3% MeOH in CHCl₃) to give **90** (135 mg, 41%) as a pale brown oil. ¹H NMR (CDCl₃) δ 1.35 (s, 12H), 3.81 (s, 3H), 5.37 (s, 2H), 7.47–7.53 (m, 4H), 7.58 (s, 1H), 7.85 (d, 2H, *J* = 8.1 Hz), 8.47 (brd, 2H, *J* = 4.4 Hz); MS (ESI) *m/z* 392 [M+H]⁺.

2-[4-([1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)methyl]phenyl]quinoxaline dihydrochloride (91)

Under argon gas atmosphere, to a mixture of **90** (130 mg, 0.33 mmol), 2-chloroquinoxaline (109 mg, 0.66 mmol) and K₂CO₃ (115 mg, 0.83 mmol) in dioxane (3.0 mL) and water (0.60 mL) was added Pd(PPh₃)₄ (77 mg, 0.067

mmol), and the mixture was stirred at 100 °C for 2 h. After cooling at room temperature, the insoluble material was removed by filtration through Celite pad and washed with EtOAc, and the filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel; 0 to 5% MeOH in CHCl₃, then NH silica gel; 50 to 100% EtOAc in hexane) to give a colorless solid. To this solid in EtOH (2 mL) and CHCl₃ (1 mL) was added 4 M HCl/EtOAc (0.33 mL), and the mixture was diluted with Et₂O (5 mL). After the mixture was stirred at room temperature for 2 h, the precipitate was collected by filtration and washed with Et₂O to give **91** (68 mg, 44%) as a yellow solid. ¹H NMR (DMSO-*d*₆) δ 3.84 (s, 3H), 5.52 (s, 2H), 7.76 (d, 2H, *J* = 8.3 Hz), 7.84–7.93 (m, 2H), 8.12–8.18 (m, 4H), 8.41 (d, 2H, *J* = 8.3 Hz), 8.70 (s, 1H), 8.74 (d, 2H, *J* = 7.0 Hz), 9.62 (s, 1H); MS (ESI) *m/z* 394 [M+H]⁺; Anal. Calcd for C₂₄H₁₉N₅O·1.9HCl·2.2H₂O: C, 57.38; H, 5.08; N, 13.94; Cl, 13.41. Found: C, 57.59; H, 4.99; N, 13.86; Cl, 13.17.

8-[4-({[1-Methyl-4-(pyridin-4-yl)-1*H*-pyrazol-3-yl]oxy}methyl)phenyl]quinoline dihydrochloride (89b)

Compound **89b** was prepared from **88** and quinolin-8-ylboronic acid in a manner similar to that described for compound **91**, with a yield of 84% as a beige solid. ¹H NMR (DMSO-*d*₆) δ 3.85 (s, 3H), 5.50 (s, 2H), 7.59–7.75 (m, 6H), 7.81 (dd, 1H, *J* = 7.2, 1.4 Hz), 8.06 (dd, 1H, *J* = 8.1, 1.4 Hz), 8.16 (d, 2H, *J* = 7.0 Hz), 8.50–8.55 (m, 1H), 8.71 (s, 1H), 8.74 (d, 2H, *J* = 7.0 Hz), 8.92 (dd, 1H, *J* = 4.3, 1.8 Hz); MS (ESI) *m/z* 393 [M+H]⁺; Anal. Calcd for C₂₅H₂₀N₄O·2.05HCl·0.25C₄H₈O₂·3.5H₂O: C, 56.54; H, 5.67; N, 10.14; Cl, 13.16. Found: C, 56.75; H, 5.55; N, 9.88; Cl, 13.04.

[4-(3-Methylpyridin-2-yl)phenyl]methanol (93a) Under argon gas atmosphere, to a mixture of 2-bromo-3-methylpyridine (**92a**, 860 mg, 5.00 mmol) and **10** (836 mg, 5.50 mmol) in DME (30 mL) were added Pd(PPh₃)₄ (289 mg, 0.25 mmol) and 1 M Na₂CO₃ aqueous solution (12.5 mL, 12.5 mmol), and the mixture was stirred at 90 °C for 8 h. The mixture was diluted with water and extracted with EtOAc. The organic layer was concentrated *in vacuo* and purified by silica gel column chromatography (0 to 5% MeOH in CHCl₃) to give **93a** (906 mg, 91%) as a pale yellow solid. ¹H NMR (DMSO-*d*₆) δ 2.32 (s, 3H), 4.56 (d, 2H, *J* = 5.7 Hz), 5.23 (t, 1H, *J* = 5.7 Hz), 7.28 (dd, 1H, *J* = 7.6, 4.7 Hz), 7.40 (d, 2H, *J* = 8.3 Hz), 7.50 (d, 2H, *J* = 8.3 Hz), 7.69–7.73 (m, 1H), 8.45–8.48 (m, 1H); MS (ESI) *m/z* 200 [M+H]⁺.

[4-(3,5-Dimethylpyridin-2-yl)phenyl]methanol (93b) Compound **93b** was prepared from 2-bromo-3,5-dimethylpyridine (**92b**) and **10** in a manner similar to that described for compound **93a**, with a yield of 68% as a brown syrup. ¹H NMR (DMSO-*d*₆) δ 2.29 (s, 3H), 2.30 (s, 3H), 4.55 (d, 2H, *J* = 5.7 Hz), 5.21 (t, 1H, *J* = 5.7 Hz), 7.38 (d, 2H, *J* = 8.3 Hz), 7.47 (d, 2H, *J* = 8.3 Hz), 7.52 (brs, 1H), 8.30 (brs, 1H); MS (ESI) *m/z* 214 [M+H]⁺.

[4-(Isoquinolin-1-yl)phenyl]methanol (93c) Compound **93c** was prepared from 1-chloroisoquinoline (**92c**) and **10** in a manner similar to that described for compound **93a**, with a quantitative yield as a yellow solid. ¹H NMR (DMSO-*d*₆) δ 4.63 (d, 2H, *J* = 5.8 Hz), 5.31 (t, 1H, *J* = 5.8 Hz), 7.51 (d, 2H, *J* = 8.3 Hz), 7.62–7.68 (m, 3H), 7.77–7.82 (m, 1H), 7.84 (d, 1H, *J* = 5.6 Hz), 8.02–8.07 (m, 2H), 8.58 (d, 1H, *J* = 5.6 Hz); MS (ESI) *m/z* 236 [M+H]⁺.

[4-(1-Methyl-1*H*-benzimidazol-4-yl)phenyl]methanol (93d) Compound **93d** was prepared from 4-bromo-1-methyl-1*H*-benzimidazole (**92d**) and **10** in a manner similar to that described for compound **93a**, with a yield of 69% as a colorless solid. ¹H NMR (DMSO-*d*₆) δ 3.88 (s, 3H), 4.56 (d, 2H, *J* = 5.7 Hz), 5.20 (t, 1H, *J* = 5.7 Hz), 7.35 (dd, 1H, *J* = 7.8, 7.8 Hz), 7.39–7.47 (m, 3H), 7.54 (dd, 1H, *J* = 8.0, 1.0 Hz), 8.05–8.08 (m, 2H), 8.24 (s, 1H); MS (ESI) *m/z* 239 [M+H]⁺.

3-Methyl-2-[4-([1-methyl-4-(pyridin-4-yl)-1*H*-pyrazol-3-yl]oxy)methyl]phenyl]pyridine dihydrochloride (94a) To a mixture of **13** (350 mg, 2.00 mmol) and **93a** (478 mg, 2.40 mmol) in toluene was added CMBP (724 mg, 3.00 mmol), and the mixture was stirred at 90 °C for 8 h. The mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (0 to 5% MeOH in CHCl₃) to give a free form of the title compound, which was diluted with EtOH (25 mL) and treated with 4 M HCl/EtOAc (2.0 mL). After the mixture was stirred at room temperature for 30 min, the mixture was concentrated *in vacuo* and washed with EtOAc to give **94a** (464 mg, 54%) as a beige solid. ¹H NMR (DMSO-*d*₆) δ 2.44 (s, 3H), 3.84 (s, 3H), 5.55 (s, 2H), 7.75 (s, 4H), 7.90–7.95 (m, 1H), 8.16 (d, 2H, *J* = 7.0 Hz), 8.48 (d, 1H, *J* = 7.8 Hz), 8.73–8.77 (m, 4H); MS (ESI) *m/z* 357 [M+H]⁺; Anal. Calcd for C₂₂H₂₀N₄O·2.2HCl·2.9H₂O: C, 54.05; H, 5.77; N, 11.46; Cl, 15.95. Found: C, 54.23; H, 5.93; N, 11.34; Cl, 16.26.

3,5-Dimethyl-2-[4-([1-methyl-4-(pyridin-4-yl)-1*H*-pyrazol-3-yl]oxy)methyl]phenyl]pyridine dihydrochloride (94b) Compound **94b** was prepared from **13** and **93b** in a manner similar to that described for compound **94a**, with a yield of 28% as a colorless solid. ¹H NMR (DMSO-*d*₆) δ 2.37 (s, 3H), 2.43 (s, 3H), 3.83 (s, 3H), 5.51 (s, 2H), 7.63–7.72 (m, 4H), 8.07 (brs, 1H), 8.12 (d, 2H, *J* = 7.0 Hz), 8.53 (brs, 1H), 8.68 (s, 1H), 8.73 (d, 2H, *J* = 7.0 Hz); MS (ESI) *m/z* 371 [M+H]⁺; Anal. Calcd for C₂₃H₂₂N₄O·2.3HCl·3.5H₂O: C, 53.40; H, 6.10; N, 10.83; Cl, 15.76. Found: C, 53.54; H, 6.08; N, 10.61; Cl, 15.99.

1-[4-([1-Methyl-4-(pyridin-4-yl)-1*H*-pyrazol-3-yl]oxy)methyl]isoquinoline dihydrochloride (94c) Compound **94c** was prepared from **13** and **93c** in a manner similar to that described for compound **94a**, with a yield of 63% as a cream-colored solid. ¹H NMR (DMSO-*d*₆) δ 3.85 (s, 3H), 5.60 (s, 2H), 7.80–7.93 (m, 5H),

8.08–8.19 (m, 4H), 8.31–8.37 (m, 2H), 8.67 (d, 1H, $J = 6.2$ Hz), 8.73 (s, 1H), 8.76 (d, 2H, $J = 7.0$ Hz); MS (ESI) m/z 393 $[M+H]^+$; Anal. Calcd for $C_{25}H_{20}N_4O \cdot 2.4HCl \cdot 0.2C_4H_8O_2 \cdot 2.6H_2O$: C, 56.92; H, 5.41; N, 10.29; Cl, 15.63. Found: C, 57.00; H, 5.60; N, 10.38; Cl, 15.88.

1-Methyl-4-[4-([1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)methyl]phenyl]-1H-benzimidazole

dihydrochloride (94d) Compound **94d** was prepared from **13** and **93d** in a manner similar to that described for compound **94a**, with a yield of 52% as a colorless solid. 1H NMR (DMSO- d_6) δ 3.84 (s, 3H), 4.07 (s, 3H), 5.51 (s, 2H), 7.65–7.76 (m, 4H), 7.86 (d, 2H, $J = 8.1$ Hz), 7.88–7.94 (m, 1H), 8.15 (d, 2H, $J = 7.0$ Hz), 8.72 (s, 1H), 8.75 (d, 2H, $J = 7.0$ Hz), 9.42 (brs, 1H); MS (ESI) m/z 396 $[M+H]^+$; Anal. Calcd for $C_{24}H_{21}N_5O \cdot 2.1HCl \cdot 1.7H_2O$: C, 57.35; H, 5.31; N, 13.93; Cl, 14.81. Found: C, 57.61; H, 5.62; N, 13.94; Cl, 14.90.

5-Methyl-2-[4-([1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)methyl]phenyl]pyridine dihydrochloride

(94e) Compound **94e** was prepared from **13** and [4-(5-methylpyridin-2-yl)phenyl]methanol (**93e**) in a manner similar to that described for compound **94a**, with a yield of 41% as a beige solid. 1H NMR (DMSO- d_6) δ 2.50 (s, 3H), 3.83 (s, 3H), 5.51 (s, 2H), 7.74 (d, 2H, $J = 8.4$ Hz), 8.14 (d, 2H, $J = 7.0$ Hz), 8.19 (d, 2H, $J = 8.4$ Hz), 8.29 (d, 1H, $J = 8.3$ Hz), 8.35 (d, 1H, $J = 8.3$ Hz), 8.72–8.76 (m, 4H); MS (ESI) m/z 357 $[M+H]^+$; Anal. Calcd for $C_{22}H_{20}N_4O \cdot 2.3HCl \cdot 2.6H_2O$: C, 54.24; H, 5.69; N, 11.50; Cl, 16.74. Found: C, 54.50; H, 5.89; N, 11.37; Cl, 16.44.

1-[4-([1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)methyl]phenyl]-1H-benzimidazole dihydrochloride

(94f) To a mixture of **13** (100 mg, 0.57 mmol), [4-(1H-benzimidazol-1-yl)phenyl]methanol (**93f**, 140 mg, 0.62 mmol) and ADDP (245 mg, 0.97 mmol) in THF (7.0 mL) was added tributylphosphine (196 mg, 0.97 mmol), and the mixture was stirred at room temperature for 2 h. The mixture was concentrated *in vacuo* and purified by flash column chromatography (silica gel; 0 to 5% MeOH in $CHCl_3$, then NH silica gel; 30 to 100% $CHCl_3$ in hexane) to give a free form of the title compound, which was dissolved in EtOH (2.0 mL) and treated with 4 M HCl/EtOAc (0.29 mL). The mixture was diluted with Et₂O (5.0 mL) and stirred at room temperature for 30 min. The precipitate was collected by filtration to give **94f** (77 mg, 30%) as a colorless solid. 1H NMR (DMSO- d_6) δ 3.84 (s, 3H), 5.54 (s, 2H), 7.45–7.52 (m, 2H), 7.70–7.75 (m, 1H), 7.79–7.84 (m, 4H), 7.86–7.90 (m, 1H), 8.14 (d, 2H, $J = 7.0$ Hz), 8.70 (s, 1H), 8.74 (d, 2H, $J = 7.0$ Hz), 9.18 (brs, 1H); MS (ESI) m/z 382 $[M+H]^+$; Anal. Calcd for $C_{23}H_{19}N_5O \cdot 2.4HCl \cdot 2.3H_2O$: C, 54.13; H, 5.13; N, 13.72; Cl, 16.67. Found: C, 54.08; H, 5.24; N, 13.75; Cl, 17.00.

3-Methyl-2-[4-([1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)methyl]phenyl]quinoline dihydrochloride

(94g) Compound **94g** was prepared from **13** and **63** in a manner similar to that described for compound **94a**, with a yield of 42% as a colorless solid. 1H NMR (DMSO- d_6) δ 2.51 (s, 3H), 3.85 (s, 3H), 5.55 (s, 2H), 7.71–7.82

(m, 5H), 7.87–7.94 (m, 1H), 8.09–8.23 (m, 4H), 8.65–8.75 (brs, 1H), 8.72 (s, 1H), 8.74–8.77 (m, 2H); MS (ESI) m/z 407 $[M+H]^+$; Anal. Calcd for $C_{26}H_{22}N_4O \cdot 2.15HCl \cdot 2.6H_2O$: C, 58.73; H, 5.56; N, 10.54; Cl, 14.34. Found: C, 58.86; H, 5.47; N, 10.55; Cl, 14.30.

[4-(Imidazo[1,2-*a*]pyridin-2-yl)phenyl]methanol (97a) A solution of 2-bromo-1-[4-(hydroxymethyl)phenyl]ethanone (**95**, 1.25 g, 5.46 mmol) and 2-aminopyridine (**96a**, 770 mg, 8.19 mmol) in EtOH (12.5 mL) was stirred under reflux condition for 2 h. After the mixture was cooled at room temperature, the mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 5% $CHCl_3$ in MeOH) to give **97a** (628 mg, 51%) as a pale yellow solid. 1H NMR ($CDCl_3$) δ 4.74 (s, 2H), 6.76–6.81 (m, 1H), 7.15–7.21 (m, 1H), 7.44 (d, 2H, $J = 8.1$ Hz), 7.64 (d, 1H, $J = 8.1$ Hz), 7.87 (s, 1H), 7.96 (d, 2H, $J = 8.1$ Hz), 8.10–8.15 (m, 1H); MS (ESI) m/z 225 $[M+H]^+$.

[4-(Imidazo[1,2-*a*]pyrimidin-2-yl)phenyl]methanol (97b) Compound **97b** was prepared from **95** and 2-aminopyrimidine (**96b**) in a manner similar to that described for compound **97a**, with a yield of 11% as a dark yellow solid. 1H NMR ($DMSO-d_6$) δ 4.54 (d, 2H, $J = 5.7$ Hz), 5.22 (t, 1H, $J = 5.7$ Hz), 7.05 (dd, 1H, $J = 6.7, 4.1$ Hz), 7.41 (d, 2H, $J = 8.4$ Hz), 7.96 (d, 2H, $J = 8.4$ Hz), 8.35 (s, 1H), 8.52 (dd, 1H, $J = 4.1, 2.0$ Hz), 8.95 (dd, 1H, $J = 6.7, 2.0$ Hz); MS (ESI) m/z 226 $[M+H]^+$.

2-[4-([1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)methyl]phenyl]imidazo[1,2-*a*]pyridine dihydrochloride (98a) Compound **98a** was prepared from **13** and **97a** in a manner similar to that described for compound **94f**, with a yield of 52% as a colorless solid. 1H NMR ($DMSO-d_6$) δ 3.83 (s, 3H), 5.49 (s, 2H), 7.45 (dd, 1H, $J = 6.8, 6.8$ Hz), 7.73 (d, 2H, $J = 8.3$ Hz), 7.86–7.92 (m, 1H), 7.97 (d, 1H, $J = 9.0$ Hz), 8.09–8.14 (m, 4H), 8.70 (s, 1H), 8.73 (d, 2H, $J = 7.0$ Hz), 8.82 (s, 1H), 8.87 (d, 1H, $J = 6.8$ Hz); MS (ESI) m/z 382 $[M+H]^+$; Anal. Calcd for $C_{23}H_{19}N_5O \cdot 2HCl \cdot 2.1H_2O$: C, 56.13; H, 5.16; N, 14.23; Cl, 14.41. Found: C, 56.10; H, 5.15; N, 14.18; Cl, 14.25.

2-[4-([1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)methyl]phenyl]imidazo[1,2-*a*]pyrimidine dihydrochloride (98b) Compound **98b** was prepared from **13** and **97b** in a manner similar to that described for compound **94f**, with a yield of 59% as a colorless solid. 1H NMR ($DMSO-d_6$) δ 3.83 (s, 3H), 5.48 (s, 2H), 7.49–7.54 (m, 1H), 7.72 (d, 2H, $J = 8.4$ Hz), 8.09–8.16 (m, 4H), 8.71–8.76 (m, 4H), 8.91 (dd, 1H, $J = 4.3, 1.8$ Hz), 9.26 (dd, 1H, $J = 6.7, 1.8$ Hz); MS (ESI) m/z 383 $[M+H]^+$; Anal. Calcd for $C_{22}H_{18}N_6O \cdot 2.3HCl \cdot H_2O$: C, 52.42; H, 4.90; N, 16.67; Cl, 16.18. Found: C, 52.67; H, 5.16; N, 16.71; Cl, 16.47.

2-Bromo-1-[4-(hydroxymethyl)phenyl]propan-1-one (100) To a solution of 1-[4-(hydroxymethyl)phenyl]propan-1-one (**99**, 710 mg, 4.32 mmol) in THF (10 mL) was added pyridinium tribromide (1.45 g, 4.54 mmol), and the mixture was stirred at room temperature for 90 min. The insoluble material was removed by filtration and the filtrate was concentrated *in vacuo* to give **100** (1.05 g, quant) as a yellow oil. ¹H NMR (CDCl₃) δ 1.91 (d, 3H, *J* = 6.6 Hz), 4.80 (s, 2H), 5.29 (q, 1H, *J* = 6.6 Hz), 7.49 (d, 2H, *J* = 8.5 Hz), 8.02 (d, 2H, *J* = 8.5 Hz); MS (CI) *m/z* 243, 245 [M+H]⁺.

3-Methyl-2-[4-({1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl}oxy)methyl]phenyl]imidazo[1,2-*a*]pyridine dihydrochloride (101) A suspension of **100** (2.02 g, 4.32 mmol), **96a** (488 mg, 5.19 mmol) and NaHCO₃ (726 mg, 8.65 mmol) in EtOH (15 mL) was stirred under reflux condition for 16 h. After the mixture was cooled at room temperature, the insoluble material was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 5% MeOH in CHCl₃) to give [4-(3-methylimidazo[1,2-*a*]pyridin-2-yl)phenyl]methanol (439 mg, 43%) as a dark yellow oil. Compound **101** was prepared from this dark yellow oil and **13** in a manner similar to that described for compound **94f**, with a yield of 28% as a pale yellow solid. ¹H NMR (DMSO-*d*₆) δ 2.73 (s, 3H), 3.84 (s, 3H), 5.52 (s, 2H), 7.54–7.60 (m, 1H), 7.78 (d, 2H, *J* = 8.2 Hz), 7.89 (d, 2H, *J* = 8.2 Hz), 7.93–8.04 (m, 2H), 8.14 (d, 2H, *J* = 6.8 Hz), 8.71 (s, 1H), 8.74 (d, 2H, *J* = 6.8 Hz), 8.86 (d, 1H, *J* = 6.9 Hz); MS (ESI) *m/z* 396 [M+H]⁺; Anal. Calcd for C₂₄H₂₁N₅O·2.3HCl·3.8H₂O: C, 52.62; H, 5.69; N, 12.79; Cl, 14.89. Found: C, 52.46; H, 5.82; N, 13.10; Cl, 14.75.

Methyl 3-methyl-4-(quinolin-2-yl)benzoate (103) Compound **103** was prepared from 2-chloroquinoline and methyl 3-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (**102**) in a manner similar to that described for compound **93a**, with a yield of 67% as a beige solid. ¹H NMR (DMSO-*d*₆) δ 2.45 (s, 3H), 3.90 (s, 3H), 7.63–7.69 (m, 2H), 7.75 (d, 1H, *J* = 8.5 Hz), 7.79–7.84 (m, 1H), 7.91–7.98 (m, 2H), 8.04–8.08 (m, 2H), 8.49 (d, 1H, *J* = 8.3 Hz); MS (ESI) *m/z* 278 [M+H]⁺.

[3-Methyl-4-(quinolin-2-yl)phenyl]methanol (104) To a suspension of LiBH₄ (597 mg, 27.4 mmol) in THF (10 mL) was added a mixture of **103** (1.52 g, 5.49 mmol) in THF (5.5 mL). To the resulting mixture was dropwisely added EtOH (1.6 mL), and the mixture was stirred at room temperature for 30 min. The reaction was cooled with ice bath and quenched with brine. The mixture was extracted with CHCl₃ for 2 times. The combined organic layer was dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 3% CHCl₃ in MeOH) to give **104** (700 mg, 51%) as a white solid. ¹H NMR (DMSO-*d*₆) δ 2.40 (s, 3H), 4.56 (d, 2H, *J* = 5.7 Hz), 5.24 (d, 1H, *J* = 5.7 Hz), 7.27–7.31 (m, 2H), 7.48 (d, 1H, *J* = 7.6 Hz), 7.60–7.65 (m, 1H), 7.68 (d, 1H, *J* = 8.4 Hz), 7.76–7.81 (m, 1H), 8.01–8.05 (m, 2H), 8.43 (d, 1H, *J* = 8.4

Hz); MS (ESI) m/z 250 $[M+H]^+$.

2-[2-Methyl-4-([1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)methyl]phenyl]quinoline dihydrochloride (105)

To a suspension of **104** (486 mg, 1.95 mmol) in CH_2Cl_2 (15 mL) was added $SOCl_2$ (0.36 mL, 4.88 mmol), and the mixture was stirred at room temperature for 2.5 h. After about 10 mL of CH_2Cl_2 was removed under reduced pressure, to the mixture was added toluene. The insoluble material was collected by filtration to give 2-[4-(chloromethyl)-2-methylphenyl]quinoline hydrochloride (508 mg, 86%) as a beige solid. To this beige solid (250 mg) and **13** (144 mg, 0.82 mmol) in DMF was added K_2CO_3 (284 mg, 2.05 mmol), and the mixture was stirred at 60 °C for 2 h. After cooling at room temperature, the reaction was quenched with water and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 3% MeOH in $CHCl_3$) to give a pale yellow oil, which was diluted with EtOAc (20 mL) and treated with 4 M HCl/EtOAc (0.62 mL). The mixture was stirred at room temperature for 1 h, and the insoluble material was collected by filtration to give **105** (313 mg, 79%) as a beige solid. 1H NMR ($DMSO-d_6$) δ 2.45 (s, 3H), 3.84 (s, 3H), 5.50 (s, 2H), 7.56–7.61 (m, 2H), 7.69 (d, 1H, J = 7.9 Hz), 7.88 (dd, 1H, J = 7.5, 7.5 Hz), 8.02–8.09 (m, 2H), 8.15 (d, 2H, J = 7.0 Hz), 8.31 (d, 1H, J = 8.1 Hz), 8.44 (d, 1H, J = 8.4 Hz), 8.73–8.78 (m, 3H), 8.98 (brd, 1H, J = 7.8 Hz); MS (ESI) m/z 407 $[M+H]^+$; Anal. Calcd for $C_{26}H_{22}N_4O \cdot 2.5HCl \cdot 2.3H_2O$: C, 57.93; H, 5.44; N, 10.39; Cl, 16.44. Found: C, 58.03; H, 5.27; N, 10.32; Cl, 16.30.

Methyl 4-([1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)methylbenzoate (107) To a mixture of **13** (2.00 g, 11.4 mmol), methyl 4-(hydroxymethyl)benzoate (**106**, 2.85 g, 17.1 mmol) and ADDP (5.76 g, 22.8 mmol) in THF (200 mL) was added tributylphosphine (4.62 g, 22.8 mmol), and the mixture was stirred at room temperature for 20 min. After the removal of the solvent *in vacuo*, the residue was dissolved in EtOAc, and the insoluble material was removed by filtration and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel; 0 to 10% MeOH in $CHCl_3$, then NH silica gel; 25 to 100% EtOAc in hexane) to give **107** (3.30 g, 89%) as a colorless solid. 1H NMR ($CDCl_3$) δ 3.81 (s, 3H), 3.93 (s, 3H), 5.42 (s, 2H), 7.49–7.57 (m, 4H), 7.59 (s, 1H), 8.07 (d, 2H, J = 8.3 Hz), 8.50 (brd, 2H); MS (ESI) m/z 324 $[M+H]^+$.

4-([1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy)methylbenzoic acid (108) To a solution of **107** (1.50 g, 4.64 mmol) in THF (5.0 mL) and MeOH (5.0 mL) was added 1 M NaOH aqueous solution (5.0 mL, 5.0 mmol), and the mixture was stirred at room temperature for 1 h. The precipitate was collected by filtration and washed with water and Et_2O to give **108** (1.18 g, 82%) as a colorless solid. 1H NMR ($DMSO-d_6$) δ 3.75 (s, 3H), 5.41 (s, 2H), 7.58–7.63 (m, 4H), 7.98 (d, 2H, J = 8.1 Hz), 8.27 (s, 1H), 8.45–8.49 (m, 2H); MS (ESI) m/z 310 $[M+H]^+$.

1-Methyl-2-[4-({[1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy}methyl)phenyl]-1H-benzimidazole

dihydrochloride (110a) To a mixture of **108** (345 mg, 1.12 mmol), *N*-methylbenzene-1,2-diamine (**109a**, 164 mg, 1.34 mmol), 1-hydroxybenzotriazole (HOBt, 181 mg, 1.34 mmol) and Et₃N (169 mg, 1.67 mmol) in DMF (5.0 mL) was added *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride (WSC·HCl, 256 mg, 1.33 mmol), and the mixture was stirred at room temperature for 1 h. The mixture was diluted with EtOAc and washed with water and NaCl aqueous solution. The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo* to give a yellow oil. This yellow oil was dissolved in AcOH (5.0 mL), and the mixture was stirred at 90 °C for 19 h. After removal of AcOH, the residue was diluted with saturated NaHCO₃ aqueous solution and NaCl aqueous solution, and extracted with CHCl₃ for 3 times. The combined organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by NH silica gel column chromatography (30 to 100% CHCl₃ in hexane) to give a brown solid, which was diluted with EtOH (3.0 mL) and treated with 4 M HCl/EtOAc (1.1 mL). The mixture was stirred at room temperature for 1 h, and the precipitate was collected by filtration and washed with Et₂O to give **110a** (352 mg, 67%) as a pale brown solid. ¹H NMR (DMSO-*d*₆) δ 3.83 (s, 3H), 4.03 (s, 3H), 5.58 (s, 2H), 7.55–7.65 (m, 2H), 7.83–7.88 (m, 3H), 7.97–8.03 (m, 3H), 8.15 (d, 2H, *J* = 7.0 Hz), 8.70 (s, 1H), 8.75 (d, 2H, *J* = 7.0 Hz); MS (ESI) *m/z* 396 [M+H]⁺; Anal. Calcd for C₂₄H₂₁N₅O·2HCl·0.5H₂O: C, 60.38; H, 5.07; N, 14.67; Cl, 14.85. Found: C, 60.64; H, 5.18; N, 14.43; Cl, 15.02.

1-Methyl-2-[4-({[1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]oxy}methyl)phenyl]-1H-benzimidazole

dihydrochloride (110b) Compound **110b** was prepared from **108** and **109b** in a manner similar to that described for compound **110a**, with a yield of 67% as a beige solid. ¹H NMR (DMSO-*d*₆) δ 3.84 (s, 3H), 3.99 (s, 3H), 5.55 (s, 2H), 7.43 (dd, 1H, *J* = 8.0, 4.7 Hz), 7.78 (d, 2H, *J* = 8.4 Hz), 8.00–8.04 (m, 2H), 8.13–8.20 (m, 3H), 8.49 (dd, 1H, *J* = 4.8, 1.4 Hz), 8.71 (s, 1H), 8.73–8.76 (d, 2H, *J* = 7.0 Hz); MS (ESI) *m/z* 397 [M+H]⁺; Anal. Calcd for C₂₃H₂₀N₆O·1.7HCl·3.3H₂O: C, 53.34; H, 5.51; N, 16.23; Cl, 11.64. Found: C, 53.21; H, 5.61; N, 16.26; Cl, 11.68.

Methyl 1-methyl-4-(pyridin-4-yl)-1H-pyrazole-3-carboxylate (112) To a mixture of **75** (263 mg, 0.99 mmol) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (**111**, 1.01 g, 4.94 mmol) in DMF (3.4 mL) and H₂O (1.0 mL) were added Cs₂CO₃ (644 mg, 1.98 mmol) and Pd(PPh₃)₄ (571 mg, 0.49 mmol), and the mixture was stirred at 80 °C for 3 h. After the mixture was cooled at room temperature, the mixture was partitioned between EtOAc and water. The organic layer was washed with brine, dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 100% EtOAc in CHCl₃) to give **112** (87 mg, 41%) a pale yellow oil. ¹H NMR (DMSO-*d*₆) δ 3.77 (s, 3H), 3.95 (s, 3H), 7.49 (d, 2H, *J* = 5.3 Hz), 8.22 (s, 1H), 8.55 (d, 2H, *J* = 5.3 Hz); MS (ESI) *m/z* 218 [M+H]⁺.

[1-Methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]methanol (113) To a mixture of LiAlH₄ (22.3 mg, 0.59 mmol) in THF (3 mL) cooled with ice-water bath was dropwisely added a solution of **112** (85 mg, 0.39 mmol) in THF (2 mL), and the mixture was stirred at the same temperature for 1 h. The reaction was quenched with ammonium hydroxide and diluted with THF. After the resulting mixture was stirred at room temperature for 2 h, to the mixture was added Celite and MgSO₄. The mixture was filtered through Celite pad and washed with CHCl₃/MeOH, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 5% MeOH in CHCl₃) to give **113** (46 mg, 62%) as an off-white solid. ¹H NMR (DMSO-*d*₆) δ 3.85 (s, 3H), 4.51 (d, 2H, *J* = 5.3 Hz), 5.27 (t, 1H, *J* = 5.3 Hz), 7.61 (dd, 2H, *J* = 4.5, 1.6 Hz), 8.22 (s, 1H), 8.51 (dd, 2H, *J* = 4.5, 1.6 Hz); MS (ESI) *m/z* 190 [M+H]⁺.

3-Methyl-2-(4-{[1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]methoxy}phenyl)quinoline dihydrochloride (114) Compound **114** was prepared from **113** and **54** in a manner similar to that described for compound **94a**, with a yield of 82% as a pale yellow solid. ¹H NMR (DMSO-*d*₆) δ 2.55 (s, 3H), 3.98 (s, 3H), 5.47 (s, 2H), 7.34 (d, 2H, *J* = 8.8 Hz), 7.81 (d, 2H, *J* = 8.8 Hz), 7.86 (dd, 1H, *J* = 7.6, 7.6 Hz), 8.02 (dd, 1H, *J* = 7.6, 7.6 Hz), 8.14 (d, 2H, *J* = 6.9 Hz), 8.21 (d, 1H, *J* = 8.1 Hz), 8.39 (d, 1H, *J* = 8.5 Hz), 8.78 (s, 1H), 8.87 (d, 2H, *J* = 6.9 Hz), 8.94 (s, 1H); MS (ESI) *m/z* 407 [M+H]⁺; Anal. Calcd for C₂₆H₂₂N₄O·2.4HCl·3H₂O: C, 56.98; H, 5.59; N, 10.22; Cl, 15.53. Found: C, 56.96; H, 5.58; N, 10.32; Cl, 15.82.

4-(1-Methyl-1H-benzimidazol-4-yl)phenol (115) Compound **115** was prepared from **92d** and 4-hydroxyphenylboronic acid in a manner similar to that described for compound **93a**, with a yield of 78% as a beige solid. ¹H NMR (DMSO-*d*₆) δ 3.86 (s, 3H), 6.84–6.89 (m, 2H), 7.30 (t, 1H, *J* = 7.7 Hz), 7.37 (dd, 1H, *J* = 7.5, 1.2 Hz), 7.46 (dd, 1H, *J* = 7.9, 1.2 Hz), 7.94–7.98 (m, 2H), 8.20 (s, 1H), 9.48 (s, 1H); MS (ESI) *m/z* 225 [M+H]⁺.

Methyl-5-(1-methyl-3-{[4-(1-methyl-1H-benzimidazol-4-yl)phenoxy]methyl}-1H-pyrazol-4-yl)pyridin-2(1H)-one dihydrochloride (116) Compound **116** was prepared from **78** and **115** in a manner similar to that described for compound **64**, with a yield of 34% as a beige solid. ¹H NMR (DMSO-*d*₆) δ 3.40 (s, 3H), 3.88 (s, 3H), 4.11 (s, 3H), 5.15 (s, 2H), 6.45 (d, 1H, *J* = 9.3 Hz), 7.25–7.29 (m, 2H), 7.59 (dd, 1H, *J* = 9.3, 2.6 Hz), 7.63–7.73 (m, 4H), 7.80 (d, 1H, *J* = 2.5 Hz), 7.92–7.95 (m, 2H), 9.72 (s, 1H); MS (ESI) *m/z* 426 [M+H]⁺; Anal. Calcd for C₂₅H₂₃N₅O₂·2HCl·0.7H₂O: C, 58.76; H, 5.21; N, 13.70; Cl, 13.88. Found: C, 58.75; H, 5.25; N, 13.70; Cl, 13.67.

1-Methyl-4-(4-{[1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl]methoxy}phenyl)-1H-benzimidazole (117) To a mixture of **113** (1.51 g, 7.96 mmol) in CH₂Cl₂ (70 mL) was added SOCl₂ (2.84 g, 23.9 mmol), and the mixture

was stirred at room temperature for 2 h. The mixture was concentrated *in vacuo* to give a yellow solid. To a mixture of this yellow solid in DMF (40 mL) were added **115** (1.37 g, 6.10 mmol) and K₂CO₃ (2.11 g, 15.2 mmol), and the mixture was stirred at 70 °C for 8 h. The mixture was concentrated *in vacuo*. The residue was partitioned between EtOAc and water, and the organic layer was concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel; 0 to 5% MeOH in CHCl₃, then NH silica gel; 50 to 100% EtOAc in hexane) to give a solid, which was washed with EtOAc to give **117** (656 mg, 27%) as a colorless solid. ¹H NMR (DMSO-*d*₆) δ 3.87 (s, 3H), 3.92 (s, 3H), 5.20 (s, 2H), 7.15–7.19 (m, 2H), 7.34 (t, 1H, *J* = 7.8 Hz), 7.43 (dd, 1H, *J* = 7.5, 1.1 Hz), 7.49–7.55 (m, 3H), 8.07–8.10 (m, 2H), 8.23 (s, 1H), 8.33 (s, 1H), 8.51–8.55 (m, 2H); MS (ESI) *m/z* 396 [M+H]⁺. Anal. Calcd for C₂₄H₂₁N₅O·0.2H₂O: C, 72.23; H, 5.41; N, 17.55. Found: C, 72.20; H, 5.43; N, 17.36.

In vitro 評価

PDE10A enzyme assay protocol

Cloning and vector construction of PDE10A2. The full-length human *PDE10A2* was amplified by PCR using the 1st strand cDNA synthesized from the total RNA isolated from human neuroblastoma TGW cell line. The PCR products were cloned into a pCR2.1-TOPO vector (Invitrogen. Corp.) to confirm sequences. The confirmed plasmid was digested with restricted enzymes, BamHI/HindIII, and this digested product was inserted into a pFastBac1 vector (Invitrogen. Corp.).

Preparation of human PDE10A2 enzyme. Human PDE10A2 enzyme protein was expressed in a *Spodoptera frugiperda* Sf9 insect cell using the Bac-to-Bac Baculovirus Expression System (Invitrogen. Corp.). The infected Sf9 cells were collected by the centrifuge and removed medium. The collected cells were lysed by sonication in the lysis buffer (50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 3 mM dithiothreitol, 0.1% NP-40, 20% Glycerol with protease inhibitors), The lysate was centrifuged and supernatant was collected to obtain the PDE10A2 enzyme solution. We confirmed the PDE10A2 expression by Western blot analysis.

PDE10A2 inhibition assay. Inhibition of compounds on human PDE10A enzyme activity was assessed by measuring the amount of cAMP by the Homogeneous Time-Resolved Fluorescence (HTRF) detection method. The assay was performed in 12 μL samples containing a optimal amount of the PDE10A enzyme, a buffer (40 mM Tris-HCl pH 7.5; 5 mM MgCl₂), 0.1 μM cAMP and various concentrations of compounds (0.1 nM to 10 μM). After compounds were preincubated for 30 min with the enzyme, the reaction was initiated by adding the substrate cAMP and the mixture was incubated for 60 min at room temperature with agitation. The reaction was terminated by the addition of the fluorescence acceptor (cAMP labeled with the dye d2) and the fluorescence donor (anti-cAMP antibody labeled with Cryptate, Cisbio). After 60 min, the fluorescence transfer corresponding

to the amount of residual cAMP was measured at λ_{exc} 320 nm, λ_{em} 620 nm and λ_{em} 665 nm using an Envision plate reader (PerkinElmer) and signal ratio (665/620) was calculated. The ratio determined in the absence of enzyme was subtracted from all data. The obtained results were converted to activity relative to an uninhibited control (100%) and IC_{50} values were calculated using Prism software (GraphPad Software, Inc.).

***In vitro* enzyme assays for profiling PDE selectivity**

Phosphodiesterases 1A, 4D, and 10A were generated from full-length human recombinant clones. PDE2A was isolated from rat, while PDE3 and 5 were isolated from rabbit. PDE activity was measured with the preferred substrates using a scintillation proximity assay. For PDE1A, 2A, 3, 4D, 10A, cAMP was used, and for PDE5, cGMP was used. Inhibitory activities for PDE6AB, 7B, 8A, 9A, and 11A generated from human recombinant clones were measured by Sekisui Medical Inc. (Tokyo, Japan) using proprietary assay formats.

***In vitro* metabolic pathway in mouse liver microsomes** The incubation mixture consisted of the following: 10 μM MP-10, 100 mM phosphate buffer (pH 7.4), liver microsomes (0.2 mg protein), and an NADPH-generating system (15 mM glucose 6-phosphate, 3 mM NADP^+ , 5 mM magnesium chloride, and 0.3 U glucose 6-phosphate dehydrogenase) in a total volume of 500 μL . The mixture was preincubated for 5 min at 37 °C. A reaction was started by the addition of MP-10 (added in MeCN/water, 50:50, v/v; final solvent concentration not exceeding 0.5% [v/v]). The samples were incubated at 37 °C for 15 and 60 min, and the reaction was stopped by the addition of 1 mL cold MeCN. The reaction mixture was centrifuged at $16000 \times g$ for 5 min, and the supernatant was evaporated under a stream of nitrogen gas at 40 °C. The dried extracts containing the parent drug and metabolites were dissolved in 200 μL of MeCN/water (50:50, v/v), and 800 μL of MeCN was added. Centrifugation and evaporation of the supernatant was repeated once again. The dried extracts were then dissolved in 200 μL of MeCN/water (50:50, v/v), and filtered through a membrane filter, and 5 μL of the filtrate was analyzed using LC-MS/MS with Waters Ultra Performance Liquid Chromatography (UPLC) system and Thermo Scientific LTQ-Orbitrap mass spectrometer.

Mouse and human liver microsomal assays Pooled mouse or human liver microsomes (Xenotech LLC.) were diluted in 100 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ buffer (pH 7.4) containing 0.1 mM ethylenediaminetetraacetic acid. The incubation mixtures (270 μL total volume), which contained 0.2 mg/mL of microsomal proteins, and 1 mM NADPH (30 μL) were pre-incubated for 5 min at 37 °C. Reactions were initiated by the addition of 0.2 μM of substrates. After the appropriate incubation time (0, 15, 30, and 45 min), 50 μL of incubation mixture was transferred into 80% MeCN containing internal standard (50 ng/mL diazepam, 250 μL) and centrifuged for 10 min at 2800 rpm. The supernatant (200 μL) was prepared and analyzed via LC-MS/MS with a Surveyor HPLC system

(Thermo Fisher Scientific Inc.) and TSQ Quantum ultra tandem triple quadrupole mass spectrometer (Thermo Fisher Scientific Inc.). The *in vitro* intrinsic clearance ($CL_{\text{int, vitro}}$) was calculated using Equation 1, which is based on the time course of the residual ratio of the compounds.³⁹⁾

$$CL_{\text{int, vitro}} (\text{mL/min/kg}) = \frac{Ke(1/\text{min}) \times MS_{\text{content}}(\text{mg/kg})}{MS_{\text{Protein Conc.}}(\text{mg/mL})} \quad (1)$$

where Ke is the disappearance rate constant.

CYP3A4 inhibition

Time-dependent inhibition assay for CYP3A4 was performed in two steps, a pre-incubation step where the test compound was incubated with human liver microsomes and the secondary incubation period where CYP3A4 substrate, midazolam, was added to the preincubate to measure residual CYP3A4 activity. Midazolam 1'-hydroxylation was used to monitor the CYP3A4 activity.

Each test compound (5 μM) was pre-incubated with human liver microsomes (0.1 mg/mL) and NADPH (1.5 mM) at 37 °C. The pre-incubation times used were 0 and 30 min. Following the pre-incubation step, each compound was co-incubated with midazolam (2 μM) at 37 °C for 20 min. At the end of the incubation, the reaction was terminated by the addition of aqueous solution containing 80% MeCN. The concentration of 1'-hydroxymidazolam was determined by LC–MS analysis. The inhibition of CYP3A4 activity was assessed by comparing the amount of 1'-hydroxymidazolam formed in the presence of varying concentrations of inhibitor to the amount of 1'-hydroxymidazolam formed in the solvent control. In each study, a CYP3A4 potent and specific inhibitor, verapamil was used as positive control.

$$\% \text{ Residual Activity} = 100 \times (\text{Activity NME, 30 min} / \text{Activity vehicle, 30 min})$$

where Activity NME, 30 min is the activity in the presence of test compound and with pre-incubation, and Activity vehicle, 30 min is the activity in the absence of test compound and with pre-incubation.

CYP2C19 inhibition

Using a 96-well or 384-well plate, 3-cyano-7-ethoxycoumarin (25 μM), each test compound (from 0.16 to 20 μM), and the enzyme (0.24 pmol) were incubated at 37 °C for 20 min in 100 mM phosphate buffer (pH 7.4) containing 8.2 μM NADP⁺, 0.41 mM glucose-6-phosphate, 0.41 mM MgCl₂ and 0.4 Units/mL glucose-6-phosphate dehydrogenase. Thereafter, the reaction was stopped by adding 0.5 M 2-amino-2-hydroxymethyl-1,3-propanediol aqueous solution containing 80% MeCN, and the fluorescence intensity was measured using a fluorescence plate reader. The residual activity was calculated based on the following formula, and concentration of each test compound by which the residual activity becomes 50% (IC_{50}) was obtained.

The residual activity (%) = $(C_1 - B_1) / (C_0 - B_1) \times 100$

C₁: Fluorescence intensity in the presence of test compound having known concentration, enzyme, and substrate.

C₀: Fluorescence intensity in the absence of test compound and in the presence of enzyme and substrate.

B₁: Fluorescence intensity of blank well

***In vitro* 3T3 NRU phototoxicity test**

A 100 µL BALB/3T3 cell suspension was dispensed in culture medium in 96-well plate and incubated for 1 to 2 days. To the culture medium was added 100 µL aliquot of the Earle's Balanced Salt Solution (EBSS) containing the test compound. Following incubation in the dark for 60 min, the solution was exposed to UV (1200 µW/cm²) at room temperature for 70 min. The solution was then decanted, and 100 µL of the culture medium was added to each well, and the plate was incubated overnight. Culture medium was decanted, 100 µL of neutral red (NR) medium (50 µg/mL) was added to each well, after which the plate was incubated for 3 h. NR medium was removed, and each well was washed with 150 µL EBSS. To each well was added 150 µL aliquot of NR desorb solution, and the plate was shaken for 10 min and the absorbance at 540 nm was measured.

***In vivo* 評価**

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc. Further, Astellas Pharma Inc. Tsukuba Research Center was awarded Accreditation Status by the AAALAC International. All efforts were made to minimize the number of animals used and to avoid suffering and distress.

***In vivo* behavioral assay in mice**

Phencyclidine-induced hyperlocomotion: ICR mice aged 5–6 weeks were used to evaluate the effect of PDE10A inhibitor on hyper-locomotion induced by the NMDA antagonist phencyclidine (PCP). Immediately after oral administration of either vehicle or agent as pre-treatment, mice were placed into individual plastic test cages (30 × 35 × 17.5 cm) of a SUPERMEX system (PAT.P; Muromachi Kikai Co., Ltd.), and measurement of locomotor activity was started. After 1 h, the mice were injected with a post-treatment of saline or PCP (2.5 mg/10 mL/kg, s.c.), and locomotor activity was measured for a further 60 min. Total locomotor activity for 60 min post-treatment was calculated.

NORT in neonatally PCP-treated mice: Three-day-old male ddY mice were housed 10–12 per cage with a stepmother. Saline or PCP (15 mg/kg) was administered subcutaneously once daily on days 7, 9, and 11 after birth. The mice were separated from their mother at 3 weeks of age, and used for NORT at 8–9 weeks old. Neonatal

mice were treated with PCP, and the NORT was conducted as previously described.⁴⁰⁾

Mouse pharmacokinetic study

(第一章の評価)

Compound **14** or TP-10 was administered to ICR mice as a 30 mg/kg solution of saline containing 5% DMSO and 5% Cremophor. Blood samples were collected using syringes containing heparin sodium at 0.5, 1, 2, and 4 h after intraperitoneal administration. Blood samples were kept on ice and centrifuged within 0.5 h of collection at $16,000 \times g$, for 2 min at 4 °C to obtain plasma, which was then stored at -20 °C prior to analysis. Whole brain samples were also removed from the same animals as blood samples just after the collection of blood samples, and stored at -20 °C and homogenized in 4-fold volume of phosphate buffered saline (pH 7.4) before extraction processing. Extraction and analysis of compound concentrations were performed via LC-MS/MS with a Prominence HPLC (SHIMADZU Corporation) and API4000 Triple Quadrupole Mass Spectrometer (AB SCIEX).

(第二章の評価)

The mice were treated orally with compound **82b** suspended in 0.5% methylcellulose aqueous solution. Blood samples were collected using syringes containing heparin sodium at 1 h after oral administration. Blood samples were kept on ice and centrifuged within 0.5 h of collection at $16000 \times g$, for 2 min at 4 °C to obtain plasma, which was then stored at -20 °C prior to analysis. Whole brain samples were collected at 1 h after administration, and stored at -20 °C and homogenized in 4-fold volume of phosphate buffered saline (pH 7.4) before extraction processing. Extraction and analysis of compound concentrations were performed via LC-MS/MS with a ACQUITY UPLC (Waters) and Xevo TQ (Waters).

(第三章の評価)

The ICR mice were treated orally with compound **116** or MP-10 (3.0 mg/kg) suspended in 0.5% methylcellulose aqueous solution. Whole brain samples were collected at 1 h after administration, and stored at -20°C and homogenized in 4-fold volume of phosphate buffered saline (pH 7.4) before extraction processing. Extraction and analysis of compound concentrations were performed via LC-MS/MS with a ACQUITY UPLC (Waters) and Xevo TQ (Waters).

[¹¹C]82b distribution study

Mice brain distribution study: 8 weeks old ddY mice received [¹¹C]**82b** injection via the tail vein (approximately 7 MBq in each mice) at 60 min prior to sacrifice. Striatum and cerebellum were isolated immediately following sacrifice. Brain samples were weighed and measured for radioactivity using a γ -counter (Wallac 2480, Perkin Elmer, Waltham, MA, USA). The data obtained from the brain in units of Bq/g were converted to SUV using the following equation:

$$\text{SUV} = \frac{\text{Brain region radioactivity (Bq/g)}}{\text{Injected radioactivity per body weight (Bq/g)}}$$

Rat PET imaging study: PET scans were conducted using an Inveon Multimodality system (Siemens, Knoxville, TN, USA). PET emission scans were performed with 8 weeks old SD rats under anesthesia induced by 2.5% isoflurane. [^{11}C]**82b** (approximately 50 MBq) was bolus intravenously injected to rats treated with MP-10 (10 mg/kg, i.v.) or saline at 10 min before radioligand administration. And then, dynamic PET emission data were collected for 60 min. Data from PET acquisition was reconstructed using ASIproVM software (Siemens).

In silico 評価

(第一章の評価)

Molecular modeling The geometry of MP-10 binding to PDE10A enzyme was derived from PDB (code: 3HR1). Structure alignment of MP-10, **16a**, and **16b** was performed with the Flexible Alignment tool in the MOE program²⁰⁾ with the geometry of MP-10 as a template and the MMFF94x force field.

(第三章の評価)

Calculation of HL-gap, dihedral angel, and Flattening Energy MOE was used to build the ligand structures of test compounds. Conformational searches of ligands were conducted with Conformation Import module in MOE with MMFF94x force field. Potential energy of each conformation was calculated with the PM6 method implemented in MOPAC2012. The conformation with the lowest energy was selected as the energy-minimized conformation to calculate HL-gap and dihedral angle. HL-gap was calculated as energy difference between HOMO and LUMO energy with the PM7 method implemented in the MOPAC2012. Flattening Energy was calculated as total energy difference between energy-minimized conformation and coplanar conformation with the PM7 method implemented in the MOPAC2012.

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謝辞

本論文を提出するにあたり、懇切丁寧な御指導と御鞭撻を賜りました、静岡県立大学薬食生命科学総合学府薬学研究院教授 菅敏幸先生に深く感謝の意を表します。

また、本論文に関して審査および御指導と御助言を賜りました静岡県立大学薬食生命科学総合学府薬学研究院教授 眞鍋敬先生、同研究院教授 濱島義隆先生、同研究院教授 橋本博先生、ならびに同研究院准教授 江木正浩先生に心より御礼申し上げます。

本研究を行う機会を与えていただき、御指導、御鞭撻をいただきましたアステラス製薬株式会社 上席執行役員 竹内誠氏、アステラスリサーチテクノロジー株式会社代表取締役社長 渡辺俊博博士、名古屋市立大学特任教授 服部浩二博士、元アステラス製薬株式会社創薬化学研究所室長 富島昌紀博士に深く感謝いたします。

本研究の遂行にあたり、直接懇切なるご指導を頂きました神戸大学大学院科学技術イノベーション研究科特命准教授 増田直之博士に心より感謝いたします。また、本研究の論文発表にあたり、貴重な御指導、御助言をいただきました、創薬化学研究所機能分子第1研究室室長 四月朔日晋博士に心より感謝いたします。

本研究の合成研究に御協力いただいた、宮本聡博士、菊池重俊博士、椎名康裕博士、三水清寛博士、檜崎史恵氏、瀬尾竜志氏に深く感謝いたします。また、化合物の薬理評価、体内動態評価を行っていただきました、三原拓真博士、山崎真也子博士、嶋田健博士、鏑水順子氏、鈴木淳博士、青田正樹氏、森口博行氏、高間香織氏に深く感謝いたします。X線共結晶構造解析を実施していただきました天野靖士氏、本坊和也博士、ならびに PET 試験を行っていただきました村上佳裕博士、伏木洋司博士に深く感謝いたします。化合物のエネルギー計算について御助言をいただきました森友綾子博士に感謝いたします。また、*in vitro* 代謝安定性評価、3T3 NRU test などの各種スクリーニングならびに各種スペクトル測定を行っていただきました、川崎匡史氏ならびにアステラスリサーチテクノロジー株式会社の諸氏に御礼申し上げます。

最後に、本論文をまとめるにあたり、物心両面で支えてくれた妻 道代、長男 翔太郎、次男 航太郎に感謝いたします。