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名古屋市立大学学位論文

メラニン凝集ホルモン受容体１拮抗薬に関する合成研究およびその薬理作用

2016 年度（**2016** 年 **10** 月）

井川 英之

1. 本論文は、2016 年 10 月に名古屋市立大学大学院薬学研究科において審査されたものである。

主査　　中川秀彦　教授
副査　　樋口恒彦　教授
　　今川正良　教授
　　中村精一　教授

2. 本論文は、学術情報雑誌に収載された次の報文を基礎とするものである。

1. Hideyuki Igawa, Masashi Takahashi, Keiko Kakegawa, Asato Kina, Minoru Ikoma, Jumpei Aida, Tsuneo Yasuma, Yayoi Kawata, Shuntaro Ashina, Syunsuke Yamamoto, Mrinalkanti Kundu, Uttam Khamrai, Hideki Hirabayashi, Masaharu Nakayama, Yasutaka Nagisa, Shizuo Kasai, and Tsuyoshi Maekawa.

   Melanin-concentrating hormone receptor 1 antagonists lacking an aliphatic amine: synthesis and structure–activity relationships of novel 1-(imidazo[1,2-*a*]pyridin-6-yl)pyridin-2(1*H*)-one derivatives.

   J. Med. Chem., **59(3)**, 1116–1139 (2016).

2. Hideyuki Igawa, Masashi Takahashi, Mikio Shirasaki, Keiko Kakegawa, Asato Kina, Minoru Ikoma, Jumpei Aida, Tsuneo Yasuma, Shoki Okuda, Yayoi Kawata, Toshihiro Noguchi, Syunsuke Yamamoto, Yasushi Fujioka, Mrinalkanti Kundu, Uttam Khamrai, Masaharu Nakayama, Yasutaka Nagisa, Shizuo Kasai, Tsuyoshi Maekawa.

   Amine-free melanin-concentrating hormone receptor 1 antagonists: Discovery of novel 1-(1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one derivatives and design to avoid CYP3A4 time-dependent inhibition.

   Bioorg. Med. Chem., **24(11)**, 2486–2503 (2016).

3. Hideyuki Igawa, Masashi Takahashi, Minoru Ikoma, Hiromi Kaku, Keiko Kakegawa, Asato Kina, Jumpei Aida, Shoki Okuda, Yayoi Kawata, Toshihiro Noguchi, Natsu Hotta, Syunsuke Yamamoto, Masaharu Nakayama, Yasutaka Nagisa, Shizuo Kasai, Tsuyoshi Maekawa.

   Amine-free melanin-concentrating hormone receptor 1 antagonists: Novel non-basic 1-(2*H*-indazole-5-yl)pyridin-2(1*H*)-one derivatives and mitigation of mutagenicity in Ames test.

   Bioorg. Med. Chem., **24(11)**, 2504–2518 (2016).

3. 本論文の基礎となる研究は、武田薬品工業株式会社循環代謝創薬ユニットにおいて前川毅志博士および河西静夫博士の指導の下に行われた。

略語表

5HT	5-Hydroxytryptamine (Serotonin)	
		5-ヒドロキシトリプタミン (セロトニン)
Ac	Acetyl	アセチル
Ar	Aryl	アリール
Asn	Asparagine	アスパラギン
Asp	Aspartic acid	アスパラギン酸
AUC	Area under the curve	曲線下面積
ADDP	1,1'-(Azodicarbonyl)dipiperidine	
		1,1'-(アゾジカルボニル)ジピペリジン
BBB	Blood brain barrier	血液脳関門
Bu	Butyl	ブチル
CHO	Chinese hamster ovary	チャイニーズハムスター卵巣細胞
CYP	Cytochrome P450	シトクロム P450
DIO	Diet-induced obesity	食餌性肥満
DIPEA	*N*,*N*-Diisopropylethylamine	*N*,*N*-ジイソプロピルエチルアミン
DMA	Dimethylacetamide	ジメチルアセトアミド
DME	Dimethoxyethane	ジメトキシエタン
DMEAD	Di-(2-methoxyethyl)azodicarboxylate	
		アゾジカルボン酸ジ (2-メトキシエチル)
DMEDA	*N*,*N'*-Dimethylethylenediamine	
		N,*N'*-ジメチルエチレンジアミン
DMF	Dimethylformamide	ジメチルホルムアミド
DMSO	Dimethyl sulfoxide	ジメチルスルホキシド
ECL	Extracellular loop	細胞外ループ
Et	Ethyl	エチル
Gln	Glutamine	グルタミン
GHS	Glutathione	グルタチオン
HATU	1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo(4,5-b) pyridinium 3-oxide hexafluorophosphate	

HBA	Hydrogen-bonding acceptor	水素結合受容基
HBD	Hydrogen-bonding donor	水素結合供与基
hERG	Human ether-a-go-go related gene	ヒト遅延整流性カリウムチャネル遺伝子
HLM	Huma liver microsome	ヒト肝ミクロソーム
HOBt	1-Hydroxybenzotriazole	1-ヒドロキシベンゾトリアゾール
IPE	Diisopropyl ether	ジイソプロピルエーテル
iv	Intravenous	静脈
LHS	Left hand side	(MCHR1 拮抗薬のファーマコフォアにおける) 左側部分
LLE	Ligand-lipophilicity efficiency	脂溶性効率
Me	Methyl	メチル
HMDS	Hexamethyldisilazane	ヘキサメチルジシラザン
MCH	Melanin-concentrating hormone	メラニン凝集ホルモン
MCHR1	Melanin-concentrating hormone receptor 1	メラニン凝集ホルモン受容体 1
mp	Melting point	融点
MS	Molecular sieve	モレキュラーシーブ
MW	Molecular weight	分子量
NMR	Nuclear magnetic resonance spectroscopy	核磁気共鳴スペクトル
Ph	Phenyl	フェニル
PLsis	Phospholipidosis	ホスホリピドーシス
po	Per os	経口投与
Pr	Propyl	プロピル
^{c}Pr	Cyclopropyl	シクロプロピル
^{n}Pr	Normal propyl	ノルマルプロピル
PSA	Polar surface area	極性表面積

RHS	Right hand side	(MCHR1 拮抗薬のファーマコフォアにおける) 右側部分
SAR	Structure-activity relationship	構造活性相関
TDI	Time-dependent inhibition	時間依存的阻害
TEA	Triethylamine	トリエチルアミン
TFA	Trifluoroacetic acid	トリフルオロ酢酸
TFAA	Trifluoroacetic anhydride	トリフルオロ酢酸無水物
THF	Tetrahydrofuran	テトラヒドロフラン
THP	Tetrahydropyran	テトラヒドロピラン
Thr	Threonine	トレオニン
TPSA	Topological polar surface area	位相幾何学的極性表面積
Tyr	Tyrosine	チロシン
WSC	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride	1-エチル-3-(3-ジメチルアミノプロピル)カルボジイミド塩酸塩

理論の部

実験の部

第 1 章　緒言

第 1 節　肥満と抗肥満薬の現状

世界保健機構の 2014 年の調査では、世界における成人の 19 億人以上が過体重 [ボディマス指数 (BMI) ≥ 25] であり、そのうち 6 億人が肥満 (BMI ≥ 30) であるとされている[1]。肥満はカロリー摂取量がカロリー消費量に比べ過剰な状態により惹起され、過剰な体脂肪により産生された炎症性アディポカインが糖尿病、高血圧、脂質異常症、うつ病、冠動脈疾患およびある種の癌などの疾患の誘起に関連している[2]。そのため、肥満人口の増加は深刻な社会問題として認識されている[3]。

現在、肥満の治療方法としては、食事療法、運動療法、外科的療法 (胃バイパス術や胃バインディング術など) および薬物療法がある。食事療法および運動療法では満足な結果が得られないことが多く、外科療法には手術に伴う危険性がある。

薬物療法に関しては、欧米では抗肥満治療薬として中枢性摂食抑制薬 Phentermine や膵リパーゼ阻害薬 Orlistat が用いられているものの、Phentermine は副作用から使用期間が 3 ヶ月以内と制限されており、Orlistat は脂肪便といった副作用を有している[4] (Figure 1)。また過去には、中枢性摂食抑制薬として使用されてきたノルアドレナリン / セロトニン再吸収阻害薬 Sibutramine[5] が心臓への影響から 2010 年に欧州医薬品庁から使用中止の勧告を受け[6]、また同じく中枢性摂食抑制薬である中枢性カンナビノイド CB1 受容体インバースアゴニスト Rimonabant は、欧州での承認後まもなく重篤なうつと自殺のリスクが報告されたことから、米国では非承認、欧州でも 2008 年に販売中止となっている[7]。

2012 年から 2014 年にかけて連邦食品医薬品局 (FDA) は、新たに 3 つの低分子肥満薬 [Lorcaserin (セロトニン 2C アゴニスト)、Qsymia (Phentermine と Topiramate の合剤) および Contrave (Bupropion と Naltrexone の合剤)] を承認した。しかし、これらの薬剤の場合、BMI ≥ 30 以上もしくは BMI ≥ 27 以上かつ高血圧、2 型糖尿病、脂質異常症等の合併症を併発している患者層に投薬は限定されており、3 ヶ月の投与で 5% 以上の体重低下が無ければ使用を中止する必要がある (Qsymia については Phentermine 15 mg/Topiramate 92 mg 錠が対象)。またこれらの薬剤には心血管イベントに関する長期間の市販後調査が求められている。一方、2015 年に承認された Liraglutide (GLP-1 アナログ) は安全性の高い薬剤として期待されているが、注射剤で高価といったペプチド製剤特有の課題を抱える。このように既存の抗肥満薬には効果と安全性の面に課題があることから、抗肥満薬の unmet medical needs は高い[8]。

なお、日本においては肥満症の薬物治療は浸透しておらず、使用できる抗肥満薬は Mazindol のみである。しかし、Mazindol の適用は重度肥満症の患者層に限定されており、また中枢性の副作用のリスクから使用期間が 3 ヶ月に制限されている。

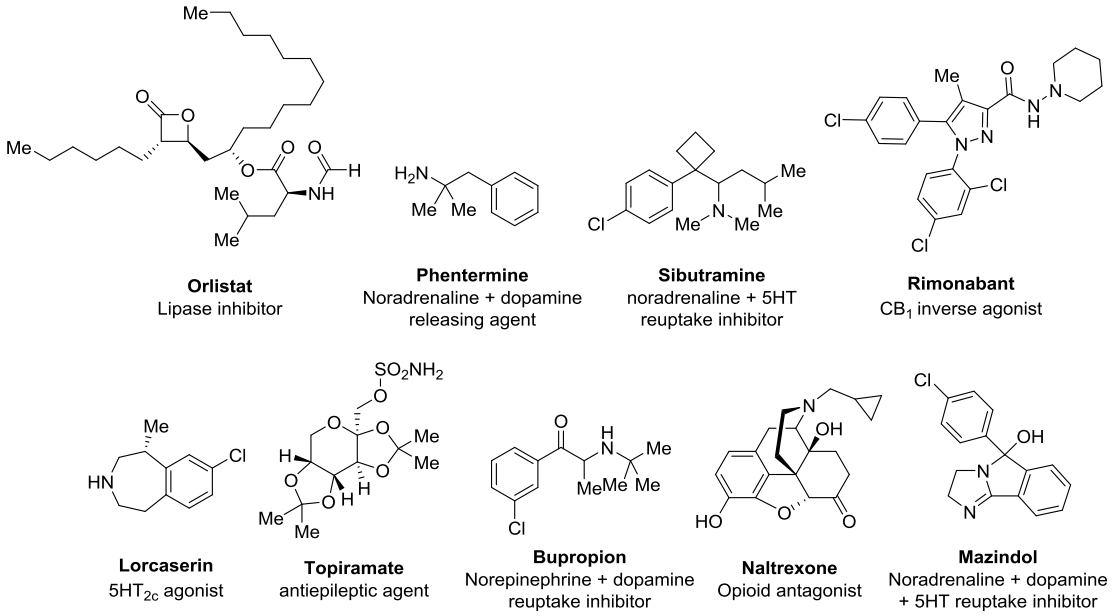


Figure 1. Chemical structures of existing antiobesity agents.

第２節　メラニン凝集ホルモンとメラニン凝集ホルモン受容体 1

メラニン凝集ホルモン (MCH) は主に視床下部外側野や不確帯において産生される 19 アミノ酸残基から成るペプチドホルモンであり (Figure 2)、その神経線維は脳内に広く投射されている [9]。MCH の受容体としては 7 回膜貫通型 G-タンパク質共役型受容体 (GPCR) である MCH 受容体 1 (MCHR1) と MCH 受容体 2 (MCHR2) が報告されている [10]。MCHR1 は大脳皮質、尾状核・被殻、海馬、視床下部など中枢の各部位に発現している一方 [11]、MCHR2 はげっ歯類において発現していない [12a–f]。最近になって、MCHR2 が食餌性肥満に対する抵抗性を示すとの報告があるものの [12g]、その機能に対する理解は未だ限定的である。また、多くの MCHR1 拮抗薬が報告されているのに対し、MCHR2 拮抗薬の報告は μM オーダーの IC_{50} 値を示す例のみである [12h]。

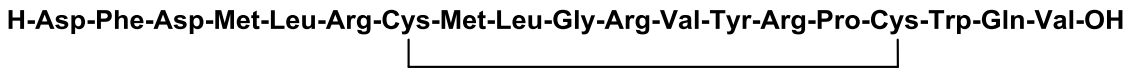


Figure 2. Amino acid sequence of MCH.

MCH/MCHR1 系は摂食行動およびエネルギー消費において重要な役割を果たしていることがこれまでの研究により明らかとなっている。すなわち、食餌性肥満ラット (DIO ラット) [13] や遺伝的な肥満を呈する *ob/ob* マウス [14] や *db/db* マウス [15]、$A^{y/a}$ (agouti) マウス [16]、Zucker (*fa/fa*) ラット [17] において MCH の mRNA レベルおよび発現レベルの上昇

が認められている。また、MCH の脳室内投与は、特に高脂肪食負荷において過食、体重増加および高インスリン血症を惹起することが報告されている [18]。さらに、視床下部外側野における MCH の過剰発現マウスは高脂肪食負荷において肥満およびインスリン抵抗性を呈することが知られている [19]。一方、MCH もしくは MCHR1 の遺伝的欠損マウスは痩せの表現型を示し、代謝亢進と食餌性肥満に対する抵抗性が認められる [20]。また、肥満患者では視床下部における MCH の産生が増加していることが明らかとなっている [21]。さらには、ヒトにおいて MCHR1 の機能欠損型変異 (R210H もしくは P377S) が体重低下を引き起こすことが確認されており、痩せの表現型と MCHR1 シグナル低下との関連性が示唆されている [22]。

これらの報告は MCH/MCHR1 系が摂食行動およびエネルギー消費に対して深く関与していることを示しており、MCHR1 拮抗薬は新しい分子機構に基づく抗肥満薬となると考えられる。これを受け多数の研究機関によって MCHR1 を創薬ターゲットとした抗肥満薬の研究が実施され、Figure 3 に示す 6 つの低分子化合物 (AMG-076、GW865464、NGD-4715、Alb-127158(a)、BMS-830216 および AZD1979) の臨床開発が行われたが、有効性と安全性の両立の困難さから未だ上市に至った候補化合物は無い。

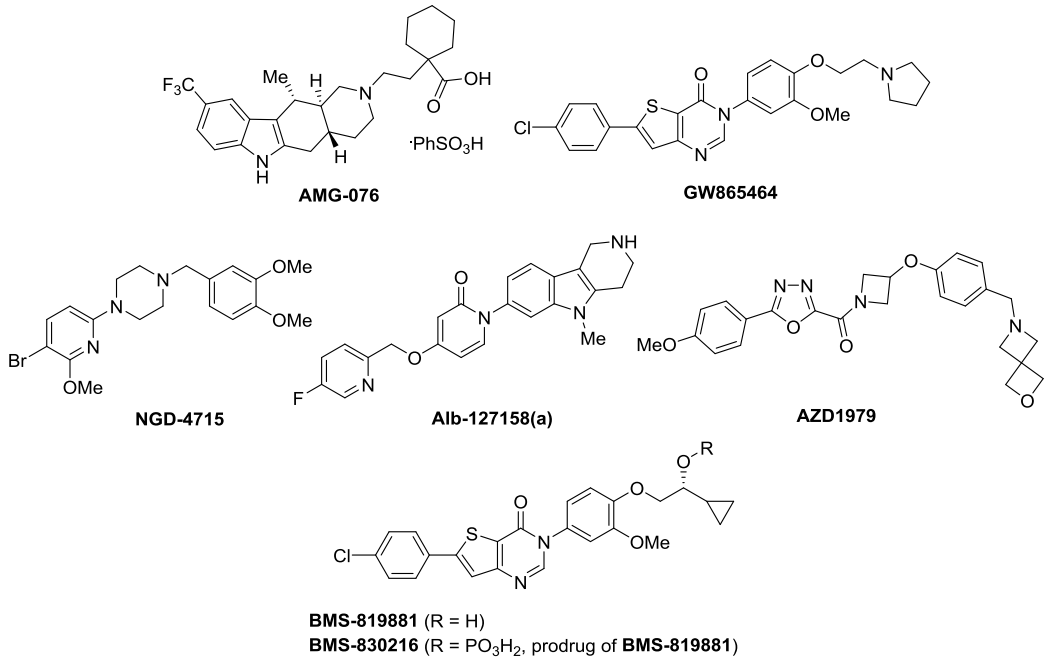


Figure 3. Clinical candidates of MCHR1 antagonists.

第３節　研究方針および論文の概要

1999 年に当グループが MCH は MCHR1 の内因性リガンドであることを見出し [23]、低分子 MCHR1 拮抗薬 T-226296 (**1a**, (−)enantiomer) を報告して以来 [24]、我々は種々の低

分子 MCHR1 拮抗薬を創製している [25] (Figure 4)。一般にこれらの化合物は、分子中央のアミド結合を中心に、脂溶性部位から成る left hand side (LHS)、二環性縮合環とアルキルアミン部位から成る right hand side (RHS) が左右に配置された構造的特性を有する。また、ウシのロドプシンに基づく MCHR1 のホモロジーモデルを用いたテトラリン誘導体 **1b** のドッキング解析の結果、中央カルボニル基と Gln127、アルキルアミン部位と Asn294 との相互作用が示唆されている [25a] (Figure 5)。これらの MCHR1 拮抗薬は、肥満モデル動物において強力な摂食抑制作用を示したが、致死的不整脈を誘発する hERG 阻害作用やリン脂質の臓器への蓄積を伴うホスホリピドーシス (PLsis) 等、安全性の懸念から、その後の開発は中止された。この様な状況の下、安全性の向上した新規 MCHR1 拮抗薬を見出すべく研究を開始した。

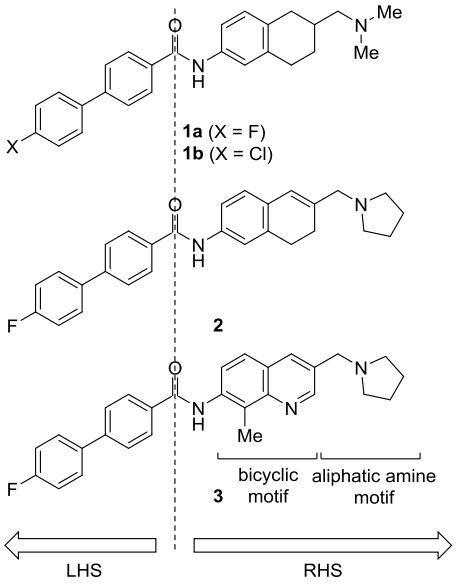


Figure 4. Chemical structures of our MCHR1 antagonists.

未だいずれの研究機関も MCHR1 拮抗薬の臨床開発に成功していない要因の一つとして、安全面での課題、特に hERG 阻害作用の問題が挙げられる。これまでに、既存の MCHR1 拮抗薬および hERG 阻害薬の網羅的解析によって両者のファーマコフォアの類似性が指摘されており、これが MCHR1 拮抗薬が hERG 阻害作用を起こしやすい原因と考えられた [26]。すなわち、既存 MCHR1 拮抗薬におけるアルキルアミン部位は、MCHR1 に結合する上での重要な部分構造である反面、カチオン–π 相互作用により hERG チャネルとの結合においても鍵構造である。これまでの検討により、本アルキルアミン部位はアミド基やカルバマート基に置換可能であり、それにより hERG 阻害作用を回避できることを報告しているが [25a]、これら初期型の非アミン性 MCHR1 拮抗薬は中枢移行性の低下により in vivo で効果を発揮するには至らなかった。

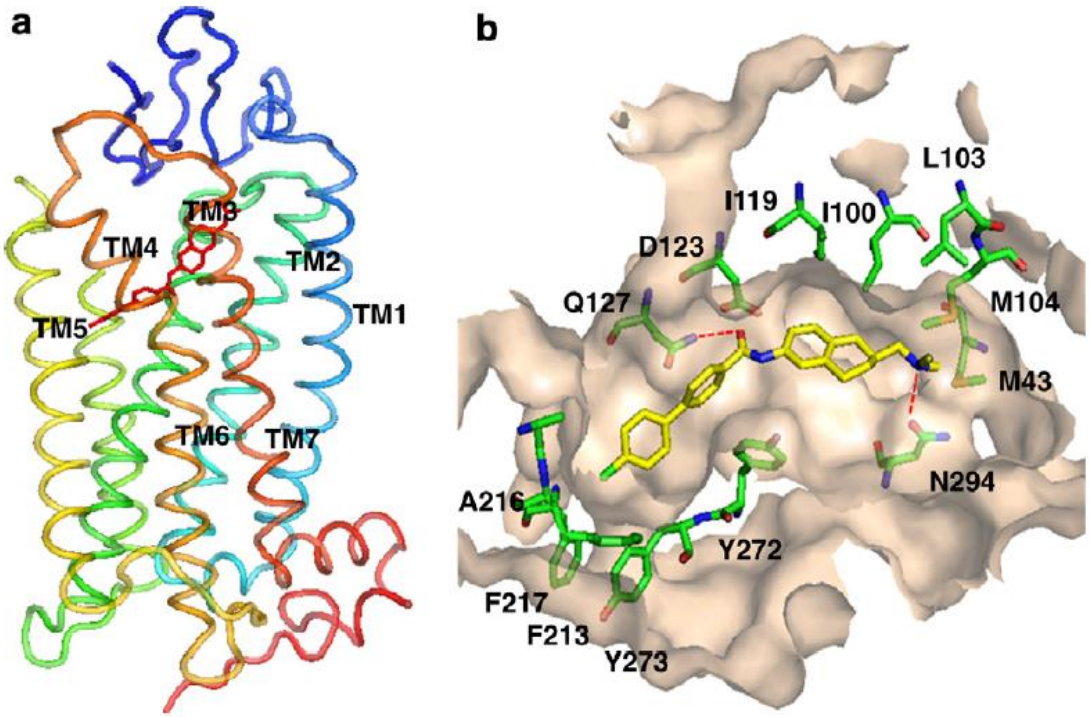


Figure 5. Docking model of **1b** with hMCHR1 generated by homology modeling. (a) overall structure (colored for helices) and (b) binding pocket of hMCHR1 with **1b** (carbon atoms in yellow for ligand and in green for receptor, nitrogen atoms in blue, oxygen atoms in red, and chloride atom in light green).

一方、Sasmal らは β2-アドレナリン受容体に基づく独自のホモロジーモデルにより、Figure 6 に示すドッキング解析を報告している [27]。すなわち、2-アミノキナゾリン誘導体 **4** のキナゾリン環上の二つの窒素原子がそれぞれ AspIII:08 および細胞外ループ (ECL) 上の Thr と相互作用し、二環性縮合環が受容体との結合に重要な役割を果たしているとしている。一方、Figure 5 で論じた我々のドッキング解析では化合物 **1b** のテトラリン環近傍に Asp123 および Tyr 272 の存在が示唆されており [25a]、水素結合を利用することで二環性縮合環部位と受容体との相互作用が可能と考えられた。そこでこの新たな相互作用獲得により、アルキルアミン部位を持たない非アミン性 MCHR1 拮抗薬の設計が可能であり、それにより hERG 阻害作用や PLsis リスクの軽減された安全性の高い薬剤の創出が可能と考えた (Figure 7)。本研究方針に基づき、アルキルアミン部位を薬物設計に用いない、非アミン性 MCHR1 拮抗薬の探索に着手した。

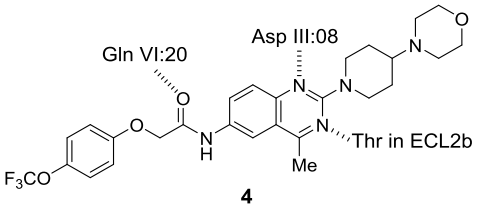


Figure 6. Docking model of the quinazoline derivative **4** reported by 7TM Pharma on the basis of the β2-X-ray structure. Dotted lines denote the hydrogen-bonding interaction with the receptor. ECL refers to the extracellular loop.

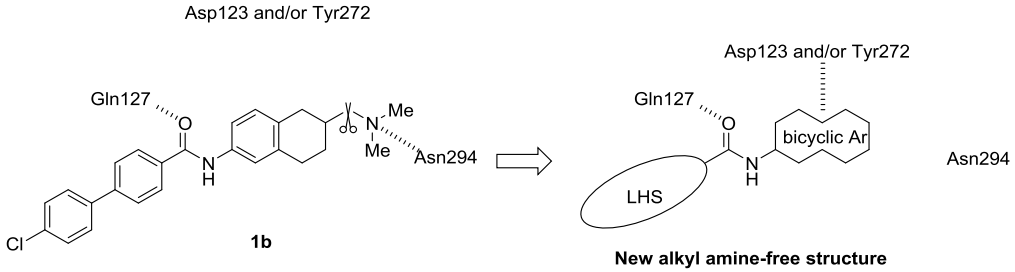


Figure 7. Design concept of alkyl amine-free MCHR1 antagonists. Dotted lines denote the hydrogen-bonding interaction with the receptor.

本論文では、筆者が武田薬品工業株式会社において実施した下記の内容について論じる。第２章では、上述の研究方針に基づいたリード化合物創出の戦略と、新規イミダゾピリジン誘導体の構造活性相関と薬理作用について論じる。第３章では、イミダゾピリジン環の更なる変換によって見出されたベンズイミダゾール誘導体の構造活性相関と薬理作用、ならびにチオフェン誘導体の CYP3A4 時間依存的阻害 (TDI) 作用回避の戦略について論じる。さらに第４章では、中性 MCHR1 拮抗薬の創製と、インダゾール誘導体の TA1537 株における遺伝毒性リスク回避の戦略について論じる。

第２章　新規イミダゾピリジン誘導体の構造活性相関および薬理作用

第１節　非アミン性 MCHR1 拮抗薬リード化合物創出の戦略

第１項　薬物設計

第１章で述べた研究方針に従い安全性と薬効に優れたリード化合物を創出すべく、アルキルアミン部位を持たない非アミン性 MCHR1 拮抗薬の設計を行うに際し、以下に論じる物理化学的指針を指標とした薬物設計を実施した。

Ploemen らは既存の PLsis 陽性化合物を解析することで、PLsis 回避のモデル [$(pK_a)^2$ + $(ClogP)^2$ < 90 もしくは pK_a < 8 もしくは ClogP < 1] を提唱している [28]。本モデルより、PLsis 回避を指向した薬物設計の指標として pK_a < 8 を選択した。また、MCHR1 は主に中枢に発現していることから、その拮抗薬は血液脳関門 (BBB) を透過する必要がある。一般に中枢薬は、末梢性の薬剤と比較し、より制限された物理化学的パラメータの範囲内で設計することが推奨される。これまでに、市販後もしくは臨床開発段階における中枢薬の解析や、薬物排出トランスポーターである P-gp の基質性評価の結果から、複数の中枢移行性に関する経験則が報告されている [29]。Hichcock らは、それらの経験則を統合した極性表面積 (PSA)、ClogP 値、分子量 (MW) および水素結合供与基 (HBD) 数で規定される中枢移行性を指向した chemical space を提唱しており、良好な中枢移行性の獲得には PSA < 70、2 < ClogP < 4、MW < 450 および HBD 数 = 0 もしくは 1 で定義される範囲内で薬物設計を行うことを推奨している [30]。この chemical space は、中枢移行性が求められる本ターゲットに対しても有効な指針となると考えた。以上の考察を踏まえ、本章で述べる薬物設計は、安全性および中枢移行性を指向した五つの物理化学的パラメータから定義される chemical space (pK_a < 8、PSA < 70、2 < ClogP < 4、MW < 450 および HBD 数 = 0 もしくは 1) を指標に行うこととした。

アルキルアミン部位を持たない非アミン性 MCHR1 拮抗薬を設計するにあたり、Figure 8 に記載した一般式に基づいて、受容体との親和性が高いことが期待される二環性縮合環を設計した。すなわち、Asp 123 もしくは Tyr 272 との相互作用獲得を狙い、水素結合受容基 (X) を環上に有する 5–5, 5–6 および 6–6 の縮合環を設計し、さらに R^1 および R^2 部分には脂溶性相互作用を指向したアルキル側鎖を配置した。本設計に基づき選択した二環性縮合環は、対応するアミン **I** を既知の LHS (一般式 A および B 参照) と縮合することによりスクリーニングし、その有効性を確かめた。

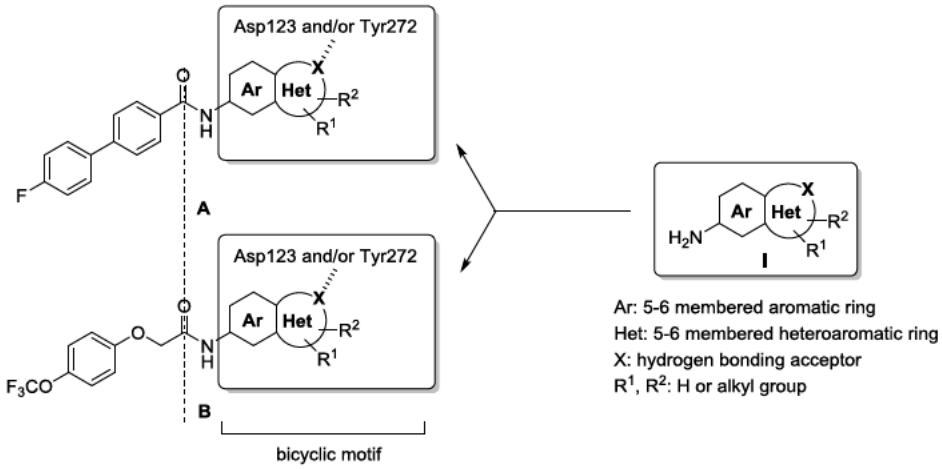
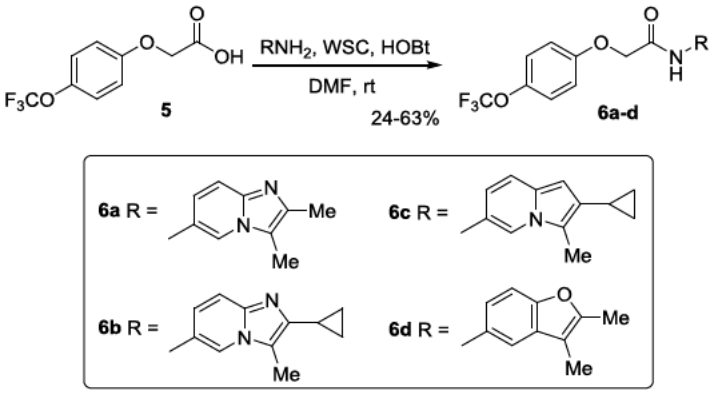


Figure 8. Lead identification strategy via RHS exploration. A number of bicyclic motifs were designed on the basis of the illustrated general structure.

第２項　合成

フェノキシアセトアミド誘導体 **6a–d** の合成を Scheme 1 に示した。カルボン酸 **5** を WSC による縮合反応に付し、目的物 **6a–d** を得た。本反応で用いた芳香族アミン試薬は常法を用いて合成した。

Scheme 1



第３項　生物活性と考察

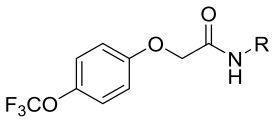
二環性縮合環のスクリーニングにより得られた結果の抜粋を Table 1 に示した。百種類を超えるアミン **I** (Figure 8) を評価した結果、イミダゾピリジン誘導体 **6a** および **6b** のみが IC_{50} 値 10^{-9} M オーダーの強力な in vitro 結合活性を示した *)。これらの化合物はアルキルアミン部位を持たないことから、イミダゾピリジン環が受容体との相互作用に寄

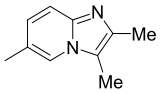
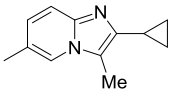
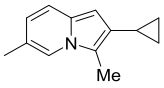
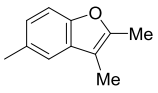
*) 化合物 **6a** の CHO 細胞における拮抗活性は IC_{50} 値 4.4 nM であった。

与していると考えられる。また両化合物の物理化学的性質は、概ね第 1 項で設定した中枢移行性を指向した chemical space (PSA < 70、2 < ClogP < 4、MW < 450 および HBD 数 = 0 もしくは 1) の範囲内であった。一方、インドリジン誘導体 **6c** およびベンゾフラン誘導体 **6d** の in vitro 活性は大きく減弱した。本結果は、イミダゾピリジン環 1 位窒素原子が活性発現に寄与していることを示唆している。

本項で論じた結果から、二環性縮合環のスクリーニングにより、非アミン性 MCHR1 拮抗薬を設計する上での鍵構造となるイミダゾピリジン環を見出すことに成功した。

Table 1. In vitro binding affinity of compounds **6a–d**



Compound	R	IC_{50} (nM)[a]
		hMCHR[b]
6a		3.8
6b		3.0
6c		>1000
6d		>1000

[a]IC_{50} values were calculated using an experiment performed in duplicate, with a standard deviation of 3-fold. [b]Binding affinity for human MCHR1.

第２節　ピリミジノン誘導体の創出

第１項　薬物設計

一般に鎖状アミドを有する化合物には化学的、酵素的に分解の懸念がある。実際、化合物 **6a** および **6b** は代謝的に不安定であった為、安定性の向上を指向し、化合物 **6b** の中央アミド部位を環化、芳香化したピリドン誘導体を設計した (Figure 9)。

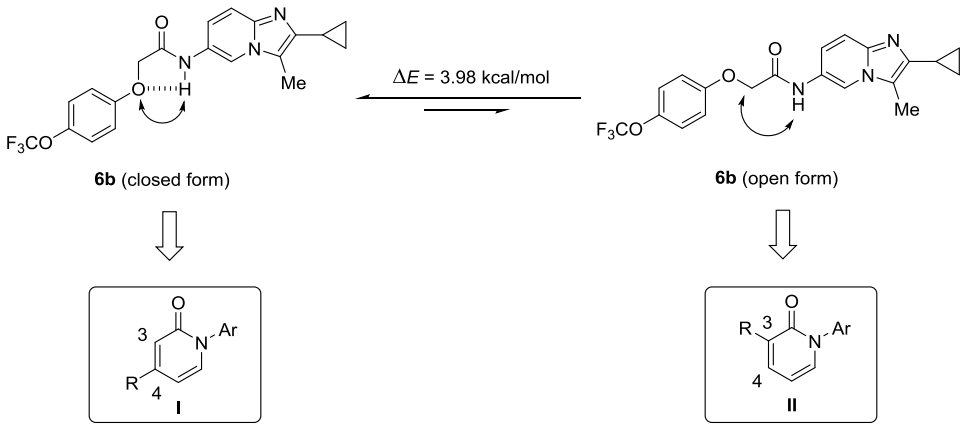


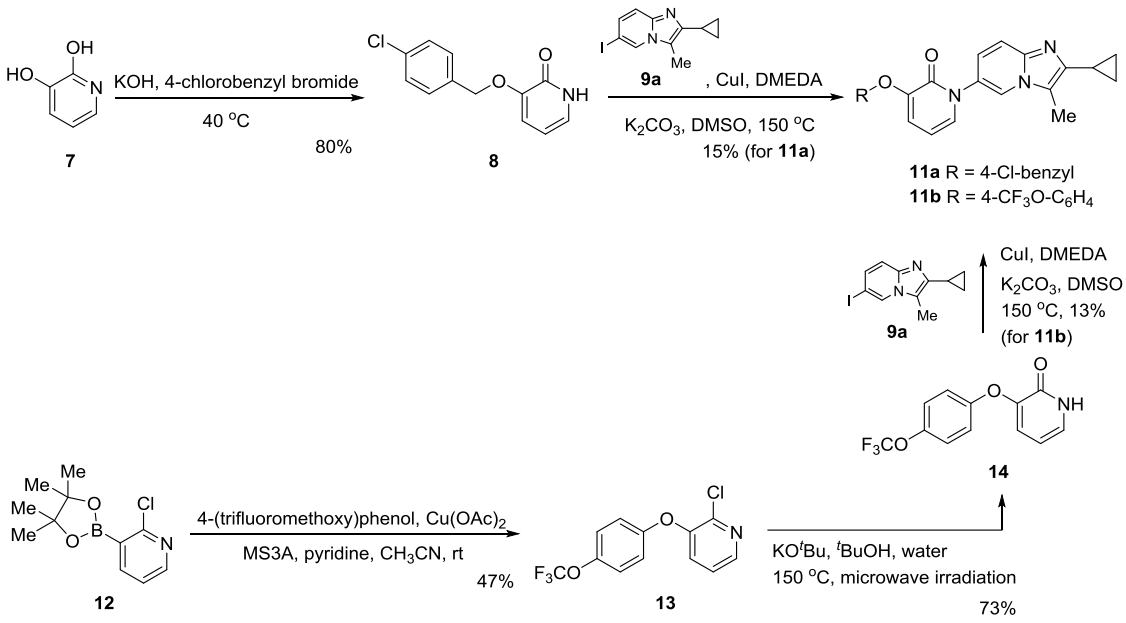
Figure 9. Design of cyclic amides on the basis of two conformers of **6b**. The energy barrier between the 2 conformers (Δ*E* value) was calculated using MOE.[31]

化合物 **6b** は closed form と open form の二つの局所安定配座を取ると考えられ、その間には 3.98 kcal/mol のエネルギー障壁が存在する (MOE [31] による計算結果に基づく)。中央アミド部分を環化構造に組み込んだピリドン誘導体として、化合物 **6b** の closed form からはピリドン 4 位置換体 (**I**)、open form からはピリドン 3 位置換体 (**II**) が設計できる。そこで筆者は、化合物 **6b** が強力な MCHR1 結合親和性を有することから、活性コンフォメーションは最安定構造である closed form と近い構造であると考え、ピリドン 4 位置換体 (**I**) を中心とした薬物設計を実施した。本節では、より安定なリード化合物の創出を指向したピリドン誘導体の創出と、ピリドン環部位の他のアジン環への構造変換について論じる。

第２項　合成

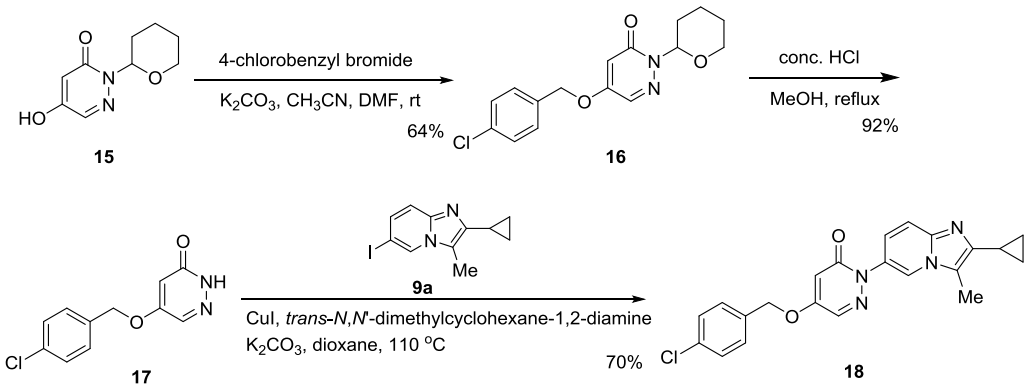
3-アルコキシピリドン誘導体 **11a** および **11b** は Scheme 2 に示した手法により合成した。すなわち、ピリジン-2,3-ジオール (**7**) の 3 位選択的アルキル化反応 [32] により 3-ベンジルオキシピリドン **8** を得、続くヨウ化銅 (I) 存在下におけるカップリング反応 [33] により目的物 **11a** へと導いた。一方、ボロン酸エステル **12** と対応するフェノールとの Chan–Lam–Evans カップリング反応 [34] により中間体 **13** を調製した後、塩素原子を置換することで、ピリドン 1 位無置換体 **14** を得、ヨウ化銅 (I) 存在下におけるカップリング反応により目的物 **11b** へと導いた。

Scheme 2

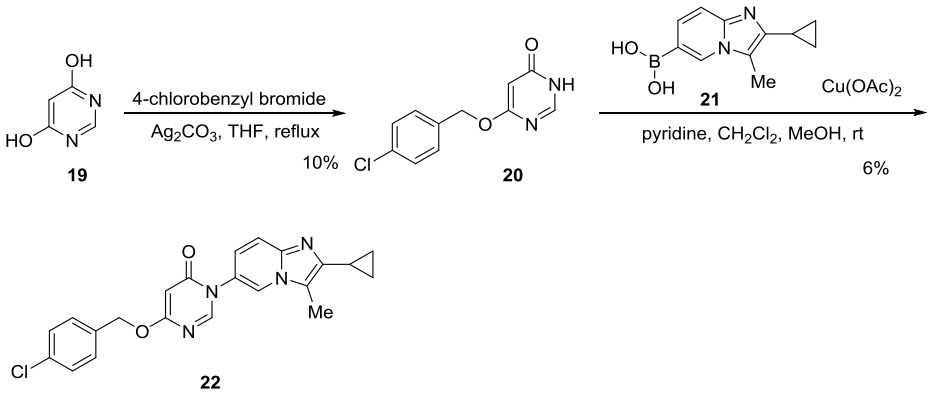


ピリダジノン誘導体 **18**、ピリミジノン誘導体 **22** および **26** の合成を Schemes 3–5 に示した。ピリダジノン誘導体 **18** は、ピリダジノン-5-オール **15** を出発原料とし、アルキル化反応に続く THP 基脱保護反応ならびにヨウ化銅 (I) によるカップリング反応を経て合成した (Scheme 3)。ピリミジノン誘導体 **22** は、4,6-ジヒドロキシピリミジン (**19**) のモノアルキル化反応により得られたピリミドン **20** を用いて合成した。ピリミドン **20** に対してヨウ化銅 (I) によるカップリング反応は進行しなかったため、Chan–Lam–Evans カップリング反応を用い目的物 **22** を得た (Scheme 4)。ピリミジノン誘導体 **26** は 2,4-ジクロルピリミジン (**23**) より合成した (Scheme 5)。一段階目の S_NAr 反応は 4 位選択的に進行し [35]、中間体 **24** を与えた。続いて中間体 **24** の 2 位塩素原子を水酸基に置換した後、得られたピリミジノン誘導体 **25** をヨウ化銅 (I) を用いたカップリング反応に付し目的物 **26** へと導いた。

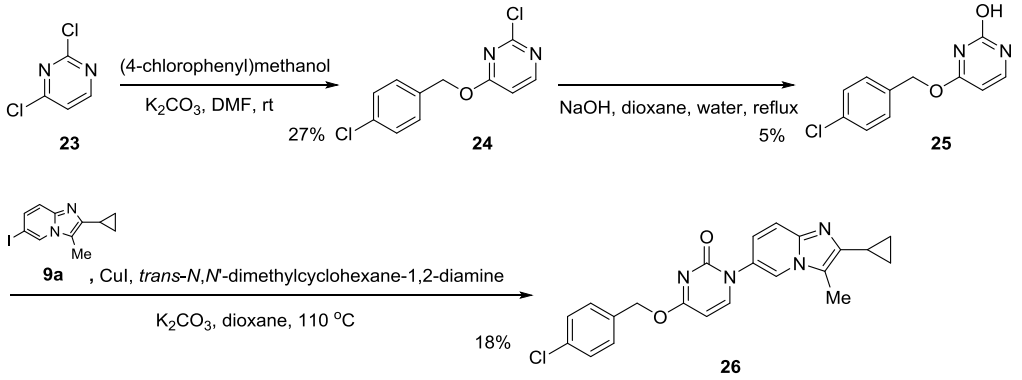
Scheme 3



Scheme 4



Scheme 5



上述の反応に用いたイミダゾピリジン-6-ボロン酸 **21** は常法に従って合成した。また、6-ヨードイミダゾピリジン **9a**、化合物 **10a** および **10b** の合成については、第４節における 4-アルコキシピリドン誘導体の一般合成法において述べた。

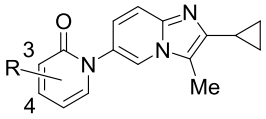
第３項　生物活性と考察

第１項で論じた化合物 **6b** の二つの局所安定配座をもとに設計したピリドン 3 位および 4 位置換体の in vitro 活性を Table 2 に示した。

ピリドン 4 位置換体 **10a** が強力な in vitro 活性を示したのに対し、ピリドン 3 位置換体 **11a** および **11b** では大幅に活性が減弱した。これは活性コンフォメーションが化合物 **6b** の closed form に類似するとする第１項の仮説を支持する結果である。一方、4-フェノキシ誘導体 **10b** では 4-ベンジルオキシ誘導体 **10a** と比較して活性が低い事が明らかとなった。本結果を考察すべく、計算の都合上構造を単純化した **10a'** および **10b'** の最安定構造を鎖状アミド **6b'** の構造と比較したところ、化合物 **10a'** の末端アリール基が、化合物 **6b'** の OCF_3 基とよく重なることが明らかとなった (Figure 10)。一方、化合物 **10b'** の末端アリール基は化合物 **6b'** の OCF_3 基と異なる配向を取っており、これが化合物

10b' の活性減弱の要因となっていることが考えられた。

Table 2. In vitro binding affinity of compounds **10a**, **10b**, **11a**, and **11b**



Compound	position	R	IC_{50} (nM)[a]	
			hMCHR[b]	rMCHR[c]
10a	4	OCH_2(4-Cl-C_6H_4)	26	20
10b	4	O(4-CF_3O-C_6H_4)	>1000	950
11a	3	OCH_2(4-Cl-C_6H_4)	990	650
11b	3	O(4-CF_3O-C_6H_4)	270	240

[a]IC_{50} values were calculated using an experiment performed in duplicate, with a standard deviation of 3-fold. [b]Binding affinity for human MCHR1. [c]Binding affinity for rat MCHR1

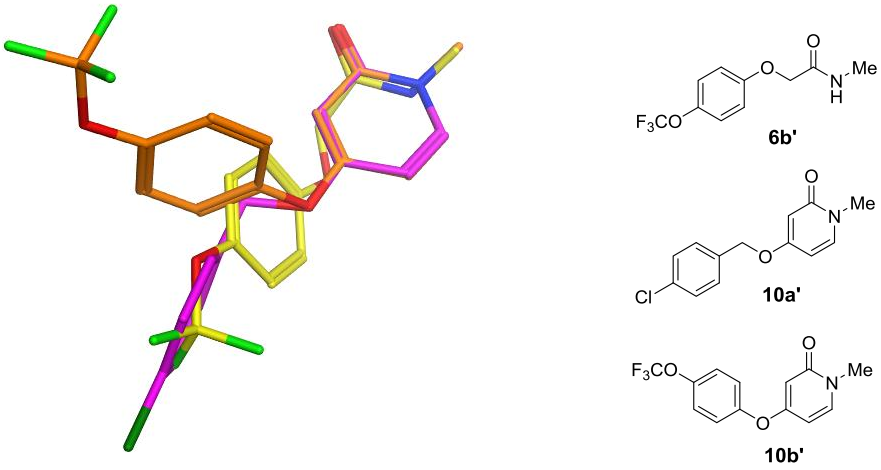


Figure 10. Superposition of the lowest energy conformers of **6b′** (yellow), **10a′** (purple), and **10b′** (orange) using MOE[31] (for the calculation cost, the imidazopyridine ring was simplified with a methyl group).

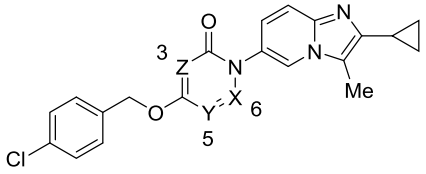
続いてピリドン誘導体 **10a** の中央の環の構造活性相関を検証すべく、アジン誘導体 **18**、**22** および **26** を in vitro 試験に供した (Table 3)。ピラジン-6-オン誘導体 **18** およびピリミジン-4-オン誘導体 **22** では in vitro 活性が減弱し、それに伴い脂溶性効率 (LLE 値) *) が低下した。一方、ピリドン環 3 位への窒素原子導入は活性に影響せず、脂溶性を低下

*) $pIC_{50} - \log D_{7.4}$ により算出した [36]。一般的に値が高いものがリード化合物として適していると考えられる。

させた [log $D_{7.4}$ [37] = 3.2 (**10a**)、log $D_{7.4}$ = 2.8 (**26**)]。結果としてピリミジン-2-オン誘導体 **26** は化合物 **10a** より良好な LLE 値を示し、リード化合物として適切であることが示唆された [*)]。

本項で論じた、より安定なリード化合物の創出を目的とした研究の結果、鎖状アミド誘導体 **6b** の最安定配座を基に化合物 **10a** を見出した。また、続くピリドン環の構造変換により化合物 **26** を非アミン性 MCHR1 拮抗薬のリード化合物として見出すことに成功した。

Table 3. In vitro binding affinity, log *D*, and LLE of compounds **10a**, **18**, **22**, and **26**



Compound	X	Y	Z	IC_{50} (nM)[a]		Log $D_{7.4}$[d]	LLE [32]
				hMCHR[b]	rMCHR[c]		
10a	CH	CH	CH	26	20	3.2	4.4
18	N	CH	CH	92	110	3.7	3.3
22	CH	N	CH	150	140	2.9	3.9
26	CH	CH	N	37	30	2.8	4.6

[a]IC_{50} values were calculated using an experiment performed in duplicate, with a standard deviation of 3-fold. [b]Binding affinity for human MCHR1. [c]Binding affinity for rat MCHR1. [d]The logD value at pH 7.4.[37]

第３節　フロピリドン誘導体の創出

第１項　薬物設計

前節においてピリドン誘導体 **10a** より設計したアジン誘導体を種々合成し、その中でピリミジン-2-オン誘導体 **26** がピリミジン-4-オン誘導体 **22** より強力な活性を示すことを示した (Table 3)。また同節における検討によって LHS の配向が活性に影響することが明らかとなっていることから、化合物 **26** および **22** においても LHS の配向が活性差に寄与していると考えた。

一般に 2-アルコキシアジン誘導体は、酸素上と窒素上の孤立電子対の反発を避ける配向

*) *) 化合物 **10a**、**18** および **26** の CHO 細胞における拮抗活性はそれぞれ IC_{50} 値 23 nM、40 nM および 140 nM であった。

を取ることが知られている。この効果により、ピリミジン-2-オン誘導体 **26** およびピリミジン-4-オン誘導体 **22** のベンジルオキシ基は異なる安定配座を取ることが予想される (Figure 11A)。すなわち、化合物 **22** ではベンジロキシ基が式中下方向に伸長した **22-B**、化合物 **26** では式中左方向に伸長した **26-A** が安定配座と考えられ、計算結果からも支持されている。本項では、優れた活性を有するピリミジン-2-オン誘導体 **26** の安定配座 **26-A** が活性コンフォメーションに類似していると考え、安定配座の固定化による in vitro 活性増強を目的とした薬物設計を行った。具体的にはピリドン環 5 位への置換基導入、もしくはベンジル位とピリドン環 3 位を環状に固定化した化合物を設計し (Figure 11B)、更なるリード化合物創出を試みた。

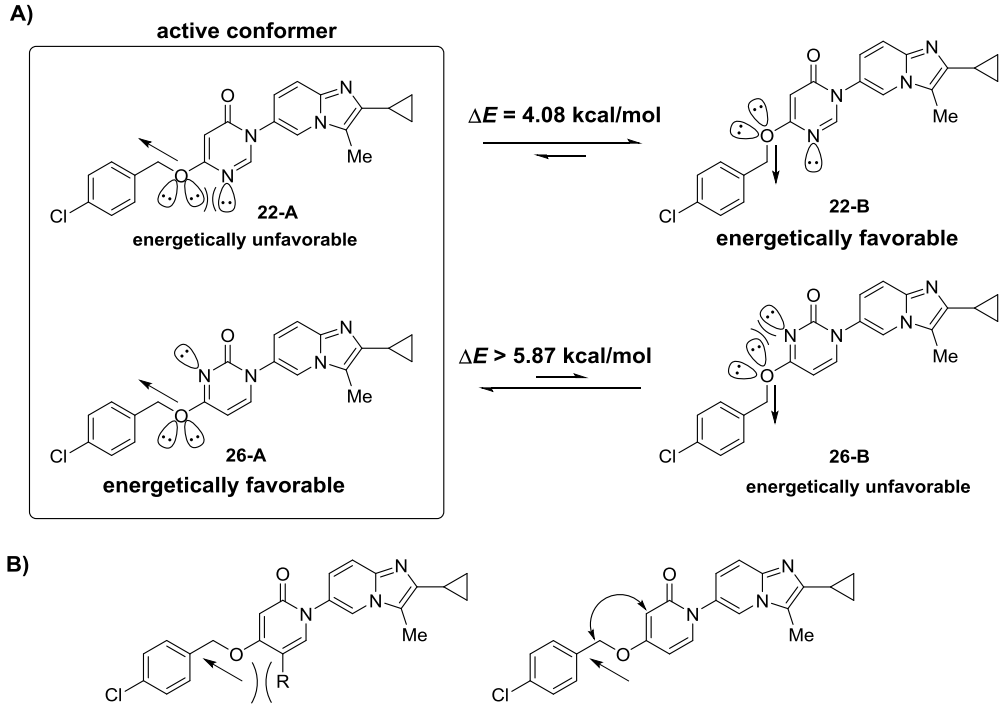
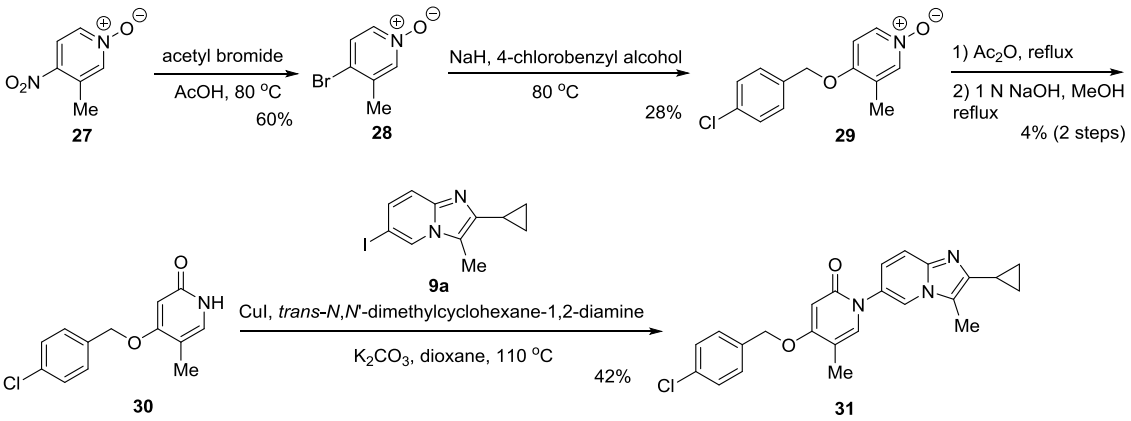


Figure 11. A) Conformational preference of compounds **22** and **26**, and prediction of the active conformer. B) Design based on the putative active conformer. The energy barrier between the 2 conformers (Δ*E* value) was calculated using MOE.[31]

第２項　合成

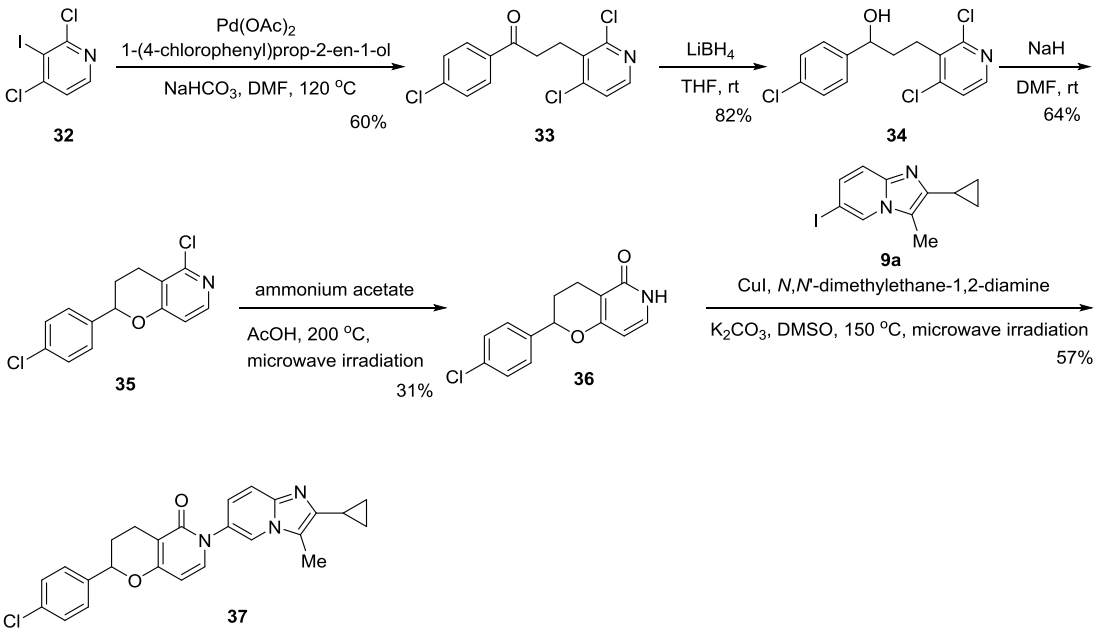
5 位にメチル基を有する 4-アルコキシピリドン誘導体 **31** は Scheme 6 に示す手法により合成した。すなわち、3-メチル-4-ニトロピリジン-*N*-オキシド (**27**) から、ニトロ基の置換反応による臭素化 [38]、S_NAr 反応によるベンジルオキシ基導入、ピリジン-*N*-オキシドへの付加－脱離反応を経る三段階で調製した 5-メチルピリドン **30** を用い、ヨウ化銅 (I) 存在下カップリング反応により合成した。

Scheme 6



テトラヒドロピラノピリドン誘導体 **37** は Scheme 7 に示した手法により合成した。ヨードピリジン **32** と 1-(4-chlorophenyl)prop-2-en-1-ol との Heck 反応によりケトン **33** を得、続く水素化ホウ素リチウムによる還元反応によりベンジルアルコール **34** を調製した。続いて環化前駆体 **34** を水素化ナトリウムで処理する事により速やかに分子内環化が進行し、中間体 **35** を得た。中間体 **35** は塩素原子の置換反応 [39]、続くヨウ化銅 (I) によるカップリング反応により目的物 **37** へと導いた。

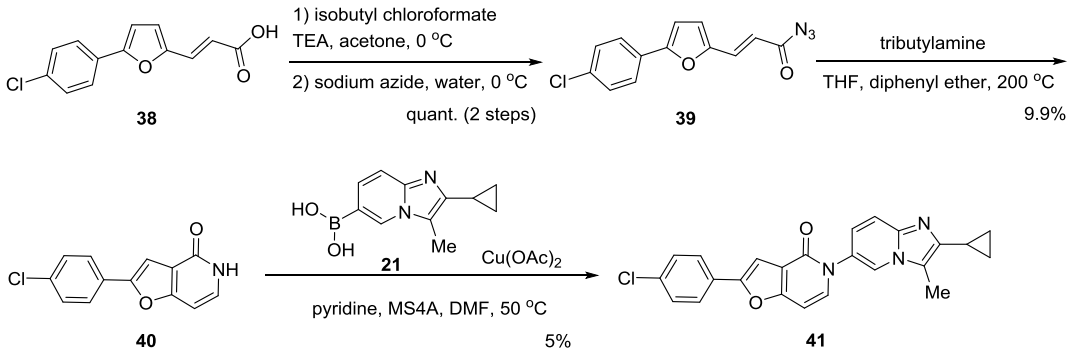
Scheme 7



フロピリドン誘導体 **41** の合成法を Scheme 8 に示した。アクリル酸誘導体 **38**[40] からアシルアジド **39** を合成した後、アジド **39** を塩基性条件下 200 度で加熱する事によりフロピリドン 1 位無置換体 **40** へと導いた。続いて Chan–Lam–Evans によるカップリン

グ反応により目的物 **41** へと導いた。

Scheme 8



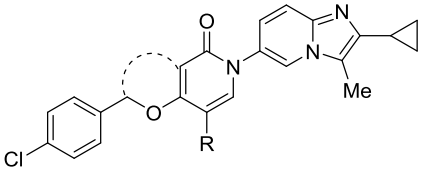
第３項　生物活性と考察

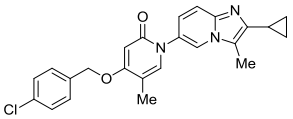
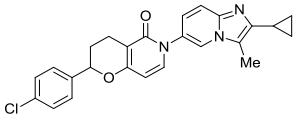
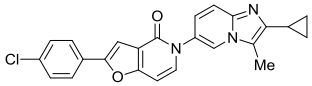
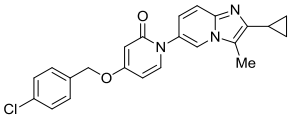
予想活性コンフォメーションの固定化により設計した化合物の in vitro 活性を Table 4 に示した。5-メチルピリドン誘導体 **31** はリード化合物 **10a** と比較して活性が低く、ピリドン 5 位の置換基許容性が低いことが明らかとなった。テトラヒドロピラノピリドン誘導体 **37** の最安定構造を計算した結果、クロロフェニル基はリード化合物 **10a** のものと計算上良好な重なりを示したが、その活性は **10a** に比べわずかに低かった。ここでの活性低下は、化合物 **10a** には無いテトラヒドロピラン環のエチレン部位と受容体の反発によるものと考え、縮合環部分をより小さいフラン環に変換したところ、フロピリドン誘導体 **41** は hMCHR1 に対して IC_{50} 値 10^{-9} M オーダーの強力な in vitro 活性を示すことが明らかとなった *)。

本項における結果により、化合物 **10a** のベンジルオキシ基をより活性コンフォメーションに近い構造に固定化することで活性向上が可能であり、本目的においてフロピリドン環が scaffold として適切なことが明らかとなった。

*) 化合物 **37** および **41** の CHO 細胞における拮抗活性はそれぞれ IC_{50} 値 330 nM および 42 nM であった。

Table 4. In vitro binding affinity of compounds **10a**, **31**, **37**, and **41**



Compound	Structure	IC_{50} (nM)[a]	
		hMCHR[b]	rMCHR[c]
31		210	150
37		94	62
41		6.8	11
10a		26	20

[a]IC_{50} values were calculated using an experiment performed in duplicate, with a standard deviation of 3-fold. [b]Binding affinity for human MCHR1. [c]Binding affinity for rat MCHR1.

第４節　ピリドン誘導体の構造活性相関

第１項　薬物設計

本章におけるここまでの検討により、RHS にイミダゾピリジン環を有する新規非アミン性 MCHR1 拮抗薬 **10a**、**26** および **41** を見出すことに成功した。これらの化合物は良好な MCHR1 結合活性を有し、さらに CHO 細胞を用いた MCH 刺激による Ca^{2+} mobilization assay において良好な MCHR1 拮抗活性を有することが明らかとなった (Figure 12)。そこで本項では、最も細胞系で強力であった化合物 **10a** の LHS 上末端アリール基、イミダゾピリジン環上 2 位および 3 位置換基の更なる最適化について論じる。

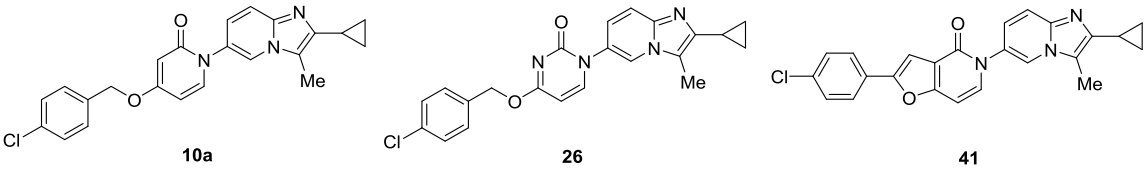


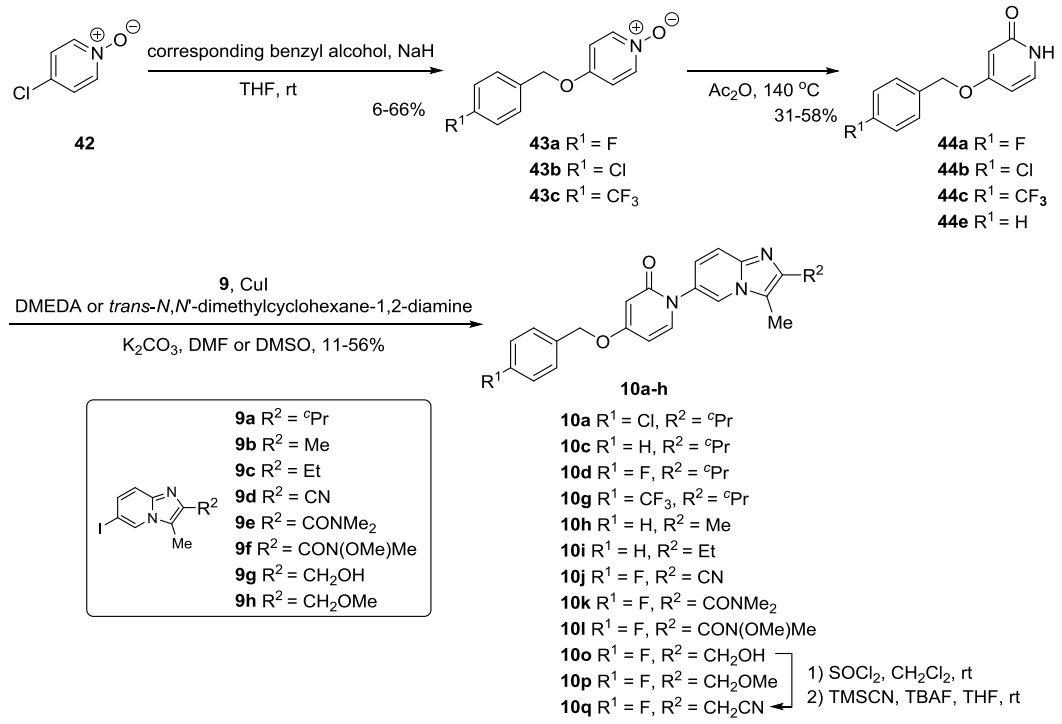
Figure 12. Binding affinities and antagonistic activities of lead compounds **10a**, **26**, and **41**.

なお、イミダゾピリジン環 3 位へのアルコキシ基や水酸基等の極性基の導入により活性は低下し、本位置の置換基としてはメチル基が最適であった。この構造活性相関 (SAR) は類似の MCHR1 拮抗薬 [41] の SAR とよく一致しており、同様の結合様式を取っていることが推察された。

第２項　合成

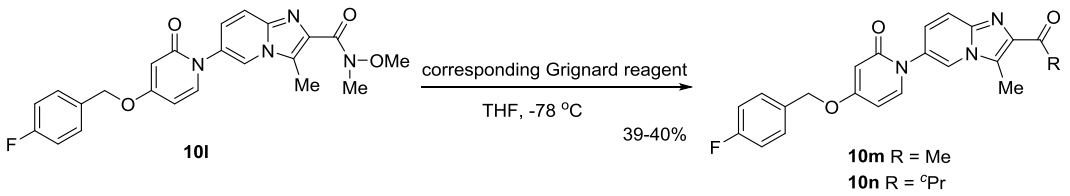
4-アルコキシピリドン誘導体 **10a, 10c, 10d, 10g–l** および **10o–q** は Scheme 9 に示した一般合成法に基づいて合成した。原料の 4-クロルピリジン-*N*-オキシド (**42**) とベンジルアルコールとの S_NAr 反応により中間体 **43a–c** を得、続く無水酢酸との反応によりピリドン 1 位無置換体 **44a–c** へと導いた。得られたピリドン 1 位無置換体は、ヨウ化銅 (I) 存在下、種々の 6-ヨードイミダゾピリジン **9a–h** とカップリングさせることにより、目的物へと導いた。なお、本カップリング反応においては、配位子として DMEDA もしくは *trans-N,N′*-dimethylcyclohexane-1,2-diamine のいずれかを使用し、原料 **44a–c** の反応性の低さから化学量論量のヨウ化銅 (I) を用いた。2-シアノメチル誘導体 **10q** は、2-ヒドロキシメチル誘導体 **10o** から塩素化、TMSCN によるシアノ化を経て合成した。また、6-ヨードイミダゾピリジン誘導体 **9a-h** の合成は実験の部に記した。

Scheme 9



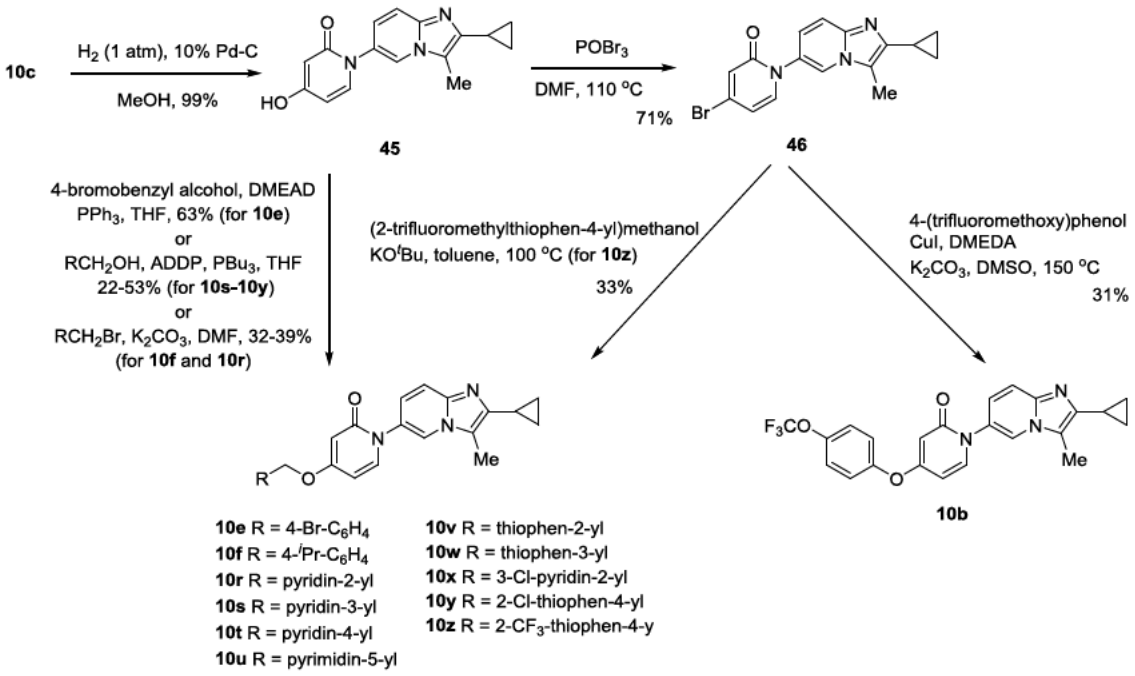
Scheme 9 で得た化合物 **10l** は、Grignard 試薬との反応によりメチルアミド誘導体 **10m** およびシクロプロピルアミド誘導体 **10n** へと導いた (Scheme 10)。

Scheme 10



ところで、ピリドン環 4 位に様々なアルコキシ基を導入した誘導体を合成することを考慮すると、4-アルコキシピリドン誘導体を、4-ヒドロキシピリドン **45** もしくは 4-ブロモピリドン **46** から一段階で合成することが望ましい。そこで、Scheme 11 に示す経路による合成を行った。原料の 4-ヒドロキシピリドン **45** は化合物 **10c** の加水素分解反応により合成でき、4-ブロモピリドン **46** は続くオキシ臭化リンを用いた臭素化反応により調製した。4-ヒドロキシピリドン **45** に対するアルキル基導入は、塩基性条件下もしくは光延反応条件下で行い、目的物 **10e**、**10f** および **10r–y** を得た。一方、4-ブロモピリドン **46** に対するアルコキシ化反応はヨウ化銅 (I) を用いたカップリング反応もしくは S_NAr 反応により進行し、目的物 **10b** および **10z** が得られた。

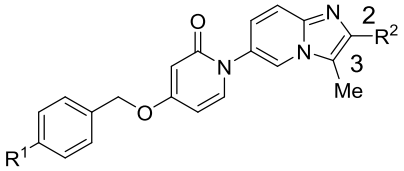
Scheme 11



第３項　生物活性と考察

末端ベンゼン環上の初期 SAR を Table 5 に示した (化合物 **10c–g**)。これまでの当グループにおけるジヒドロナフタレン誘導体 [25a] やキノリン誘導体 [25c] の検討結果から、LHS は TM5 部位の Phe213、Ala 216 および Phe217、ならびに TM 6 部位における Tyr273 から形成される脂溶性領域に結合しており (第一章第三節、Figure 5 参照)、パラ位置換ベンゼンの導入が最適なことが想定された。そこでベンゼン環上パラ位の構造変換を行ったところ、無置換ベンゼン誘導体 **10c** および *p*-フッ素体 **10d** では、*p*-クロロ体 **10a** と比較して活性が減弱した。無置換体 **10c** および *p*-フッ素体 **10d** に活性差が無いことから、ベンゼン環上の電子密度は活性に影響しないことが考えられる。*p*-クロロ体 **10a** の塩素原子を臭素原子に置換した *p*-ブロモ体 **10e** では、**10a** と比較して in vitro 活性が二倍増強した。ここまでの結果は、パラ位のハロゲン原子の van der Waals 半径と活性の正相関を示している。そこで次に、van der Waals 半径の増したトリフルオロメチル基およびイソプロピル基の導入を試みたが、化合物 **10f**、**10g** いずれも in vitro 活性が減弱した。これらの結果から、ベンゼン環上パラ位には塩素原子もしくは臭素原子程度の van der Waals 半径を有する置換基導入が最適であることが明らかとなった。

Table 5. In vitro binding affinity of compounds **10a**, **10c–k**, and **10m–q**



Compound	R^1	R^2	IC_{50} (nM)[a]	
			hMCHR[b]	rMCHR[c]
10a	Cl	^{c}Pr	26	20
10c	H	^{c}Pr	42	28
10d	F	^{c}Pr	47	28
10e	Br	^{c}Pr	12	9.3
10f	^{i}Pr	^{c}Pr	410	240
10g	CF_3	^{c}Pr	72	82
10h	H	Me	78	80
10i	H	Et	120	120
10j	F	CN	>1000	>1000
10k	F		>1000	>1000
10m	F		>1000	>1000
10n	F		>1000	>1000
10o	F	CH_2OH	>1000	>1000
10p	F	CH_2OMe	>1000	>1000
10q	F	CH_2CN	180	170

[a]IC_{50} values were calculated using an experiment performed in duplicate, with a standard deviation of 3-fold. [b]Binding affinity for human MCHR1. [c]Binding affinity for rat MCHR1.

次にイミダゾピリジン環 2 位の構造変換を行った (化合物 **10h–k** および **10m–q**)。同位置の置換基は、既存のアミン含有化合物 (**1–3**) においてアルキルアミン部位が Asn294 と相互作用する領域に向かって伸長すると考えられる。そこで、HBD もしくは HBA の導入により、Asn294 との相互作用を介した活性増強が期待できると考えた。そこで本仮説に基づき、種々の官能基を有する置換基をイミダゾピリジン 2 位に導入し、その効果を調べた。シクロプロピル基をメチル基 (**10h**)、もしくはエチル基 (**10i**) に置換したところ、in vitro 活性はそれぞれ 2–3 倍および 3–4 倍減弱した。次にシアノ基 (**10j**)、カルボ

キシアミド基 (**10k**)、ケトン等 (**10m** および **10n**) の極性基を導入したところ、いずれも活性が大きく低下した。この結果は、電子求引性の置換基導入によりイミダゾピリジン環 1 位窒素原子上の電子密度が低下し、受容体との相互作用が弱くなった為と考えられる。実際、窒素原子上の電子密度を PM3 法 (MOPAC)[42] により計算したところ、いずれの化合物においても電子密度の低下が認められた (Figure 13)。そこで、電子密度に影響しない置換基を導入した化合物 **10o–q** を評価したが (イミダゾピリジン環上 1 位窒素原子の電子密度：**10d**, −0.104; **10o**, −0.108; **10p**, −0.103; **10q**, −0.097)、シアノメチル体 **10q** のみが 10^{-7} M オーダーの弱い活性を示すに留まり、2 位への極性基導入は受容体と相互作用する上で不利なことが明らかとなった。以上の結果から、イミダゾピリジン環 2 位置換基としてはシクロプロピル基が最適であり、Asn294 との相互作用を狙った極性官能基の導入による活性向上は達成できなかった。

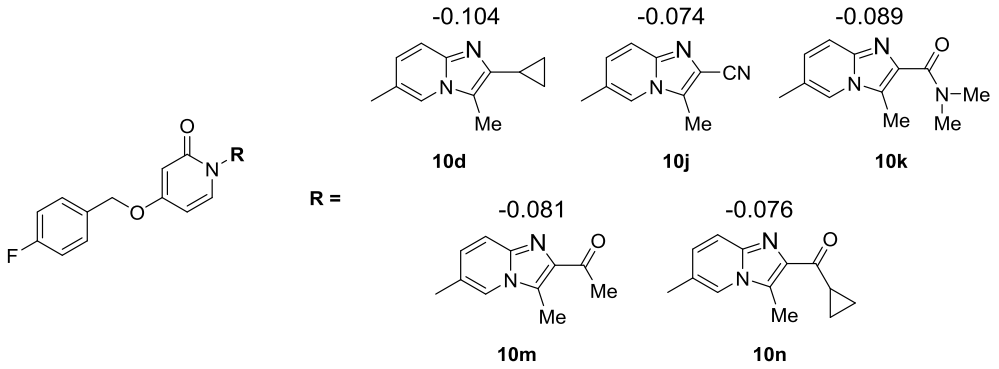
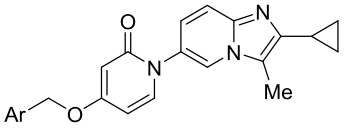
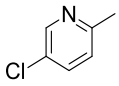
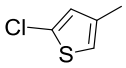
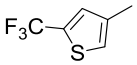
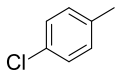


Figure 13. Calculated electron densities (PM3, MOPAC) on the nitrogen atom on the imidazopyridine ring of **10d**, **10j**, **10k**, **10m**, and **10n**.

続いて、末端アリール基の更なる最適化を目的に、種々の芳香族複素環を導入した (Table 6)。まず無置換体の導入により、芳香族複素環の活性に与える影響を評価した (化合物 **10r–w**)。2-ピリジル誘導体 **10r** は、3-ピリジル誘導体 **10s** および 4-ピリジル誘導体 **10t** と比較して良好な活性を示し、ヒトおよびラットに対してそれぞれ IC_{50} 値が 240 nM および 150 nM の値を示した。また、高極性のピリミジン誘導体 **10u** では大幅な活性低下を招いた。窒素原子の許容性は置換位置によって若干異なるが (化合物 **10r–t**)、脂溶性置換基を好む傾向は、これまでの当グループにおける塩基性 MCHR1 拮抗薬の検討結果と一致した[25a]。一方、チオフェン誘導体 **10v** および **10w** は強力な in vitro 活性を示し、特に 3-チエニル誘導体 **10w** の活性は対応するベンゼン体 **10c** より強力であった (25 vs 42 nM)。

Table 6. In vitro binding affinity of compounds **10a** and **10r–z**



Compound	Ar	IC_{50} (nM)[a]	
		hMCHR[b]	rMCHR[c]
10r		240	150
10s		>1000	>1000
10t		>1000	>1000
10u		>1000	>1000
10v		95	130
10w		25	54
10x		24	19
10y		7.7	7.5
10z		17	15
10a		26	20

[a]IC_{50} values were calculated using an experiment performed in duplicate, with a standard deviation of 3-fold. [b]Binding affinity for human MCHR1. [c]Binding affinity for rat MCHR1.

次に受容体の脂溶性領域との結合を更に強固にすべく、これらの複素芳香環上への脂溶性置換基の導入を試みた (**10x–z**)。3-クロロピリジン誘導体 **10x** は、対応する無置換体 **10r** より強力な in vitro 活性を持ち、またリード化合物 **10a** より高い LLE 値を示した [LLE = 5.2 (**10x**)、4.4 (**10a**)]。クロロチオフェン誘導体 **10y** はリード化合物 **10a** よりおよそ三倍強力な in vitro 活性を示し、ヒトおよびラットともに IC_{50} 値 10^{-9} M オーダーの強力な IC_{50} 値が認められた。一方、トリフルオロメチルチオフェン誘導体 **10z** はクロロ

チオフェン誘導体 **10y** には若干劣るものの、リード化合物 **10a** より強力な in vitro 活性を示した。

本項で論じた最適化研究により、リード化合物 **10a** より強力な MCHR1 結合活性を有するクロロチオフェン誘導体 **10y** を見出すことに成功した。また、本化合物は CHO 細胞において優れた MCHR1 拮抗活性を示すことが明らかとなった (**10y**: IC_{50} = 19 nM)。

第５節　イミダゾピリジン誘導体 **10a** の薬理作用

第１項　食餌性肥満 F344 ラットによる二日間摂食抑制確認試験

研究方針の妥当性を検証すべく、リード化合物 **10a** を各種プロファイリング試験に供した。化合物 **10a** の pK_a 値 [43] は 7.9 であり、また中枢移行性を指向した chemical space の範囲内に概ね収まる物理化学的性質を有していた (TPSA[*)] = 49、ClogP = 5.0、MW = 405、HBD 数 = 0)。また、化合物 **10a** は in vitro 評価において PLsis 陰性であり、パッチクランプ試験において hERG 阻害作用を示さなかった (IC_{50} > 10 μM)。さらに、化合物 **10a** はラットにおいて、良好な経口吸収性と血中暴露を示した (Table 7)。

Table 7. Pharmacokinetic parameters of **10a** in rats[a]

Compound	F[b] (%)	iv (0.1 mg/kg)		po (1 mg/kg)		
		CL_{total}[c] ($mL \cdot h^{-1} \cdot kg^{-1}$)	V_{ss}[d] ($mL \cdot kg^{-1}$)	C_{max}[e] ($ng \cdot mL^{-1}$)	T_{max}[f] (h)	$AUC_{0-8\ h}$[g] ($ng \cdot h \cdot mL^{-1}$)
10a	58	312	1053	313	2.0	1880

[a]n = 3; SD rats (male, 8 weeks old). [b]Bioavailability. [c]Total clearance. [d]Volume of distribution at steady state. [e]Maximal plasma concentration. [f]Time of maximal concentration. [g]Area under the plasma concentration–time curve (0–8 h).

化合物 **10a** の in vivo における効果を確認すべく、化合物 **10a** を DIO F344 ラットにおける二日間摂食抑制確認試験に供した。化合物 **10a** (3 および 10 mg/kg) を一日一回、二日間経口投与したところ、3 mg/kg 投与群で−15.1%、10 mg/kg 投与群で−29.6%の用量依存的な摂食量の低下が認められた (Figure 14)。投与後 24 時間後の血中及び脳内の薬物濃度は、3 mg/kg 投与群においてそれぞれ 351 ng/mL および 218 ng/mL、10 mg/kg 投与群において 1350 ng/mL および 841 ng/mL であった (脳/血中濃度比: 0.61 および 0.62)。化合物 **10a** の DIO F344 ラットの血漿中における非結合型分率 [**)] は 0.02 であることか

*) 位相幾何学的極性表面積。分子の三次元構造を発生させずに高速に PSA を計算するための手法。PSA と TPSA はその値が良く相関する事が報告されている [44]。
**) 血中における血漿タンパク質と結合していない薬物の割合。

ら、投与 24 時間後の血漿中フリー体濃度は 3 mg/kg および 10 mg/kg 投与群に対してそれぞれ 7.0 ng/mL および 27 ng/mL (17.2 nM および 66.5 nM) と算出され、それぞれ IC_{50} 値の 0.86 倍および 3.3 倍のフリー体が血漿中に存在すると考えられた。

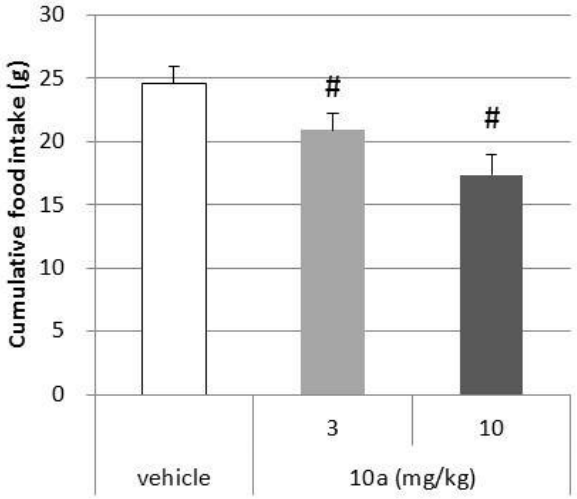


Figure 14. Effects of **10a** in a 2-day food intake study in DIO F344 rats. Inhibition of cumulative food intake for 2 days in DIO F344 rats was evaluated. The compound was administered once daily, and food intake from the initial administration to 2 days later was measured. The cumulative food intake inhibition rate was calculated by dividing the average food intake of each treatment group by that of the vehicle group. Each data represents mean ± SD (n = 6 for each group). (#) $p < 0.025$ vs. the vehicle group (Williams test).

第２項　食餌性肥満 F344 ラットにおける二週間連続投与試験

化合物 **10a** の抗肥満作用を DIO F344 ラットにおける二週間連続投与試験において評価した (Figure 15)。化合物 **10a** (3 および 10 mg/kg) を一日一回、二週間経口投与したところ、有意かつ用量依存的な体重低下作用が 3 mg/kg 投与群から確認され、vehicle 群に対して 3 mg/kg 投与群で 3.7%、10 mg/kg 投与群で 8.6% の体重低下が認められた。また、その時の摂餌量は vehicle 群と比較し、それぞれ 15.4% および 36.2% 減少していた。

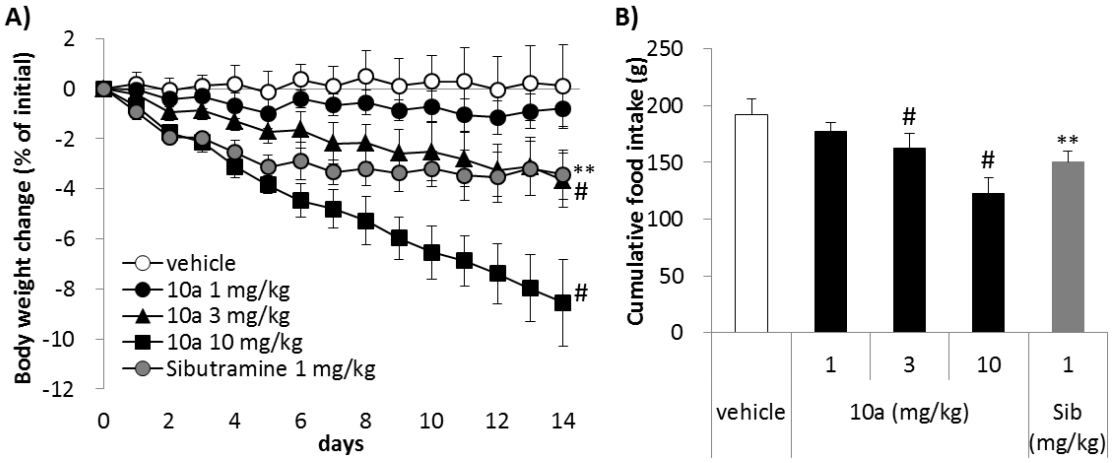


Figure 15. Effects of **10a** in a repeated-dose study in DIO F344 rats. A) Body weight change from initial during 2 weeks of dosing. B) Cumulative food intake for 2 weeks of dosing. The compounds were administered once daily for 2 weeks and body weight and food intake were measured before drug administration. Each data represents mean ± SD (n = 6 for each group). (#) $p<0.025$ vs. the vehicle group (Williams test), (**) $p<0.01$ vs. vehicle group (Student's t-test). Sib = Sibutramine.

第３項　MCHR1 欠損マウスにおける選択性確認試験

化合物 **10a** の摂食抑制作用が MCHR1 を介した作用であることを証明すべく、次に MCHR1 欠損マウスを用いて化合物 **10a** の作用を検証した (Figure 16)。化合物 **10a** (10 および 30 mg/kg) を一日一回、三日間経口投与したところ、正常マウスでは用量依存的な摂食抑制作用が認められたのに対し、MCHR1 欠損マウスにおいては作用が認められなかった。本結果は、第二項で述べた化合物 **10a** の摂食抑制作用および体重低下作用が、MCHR1 拮抗作用を介する効果であることを示している。

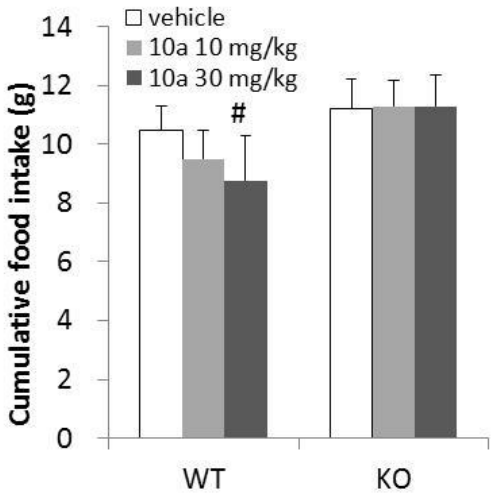
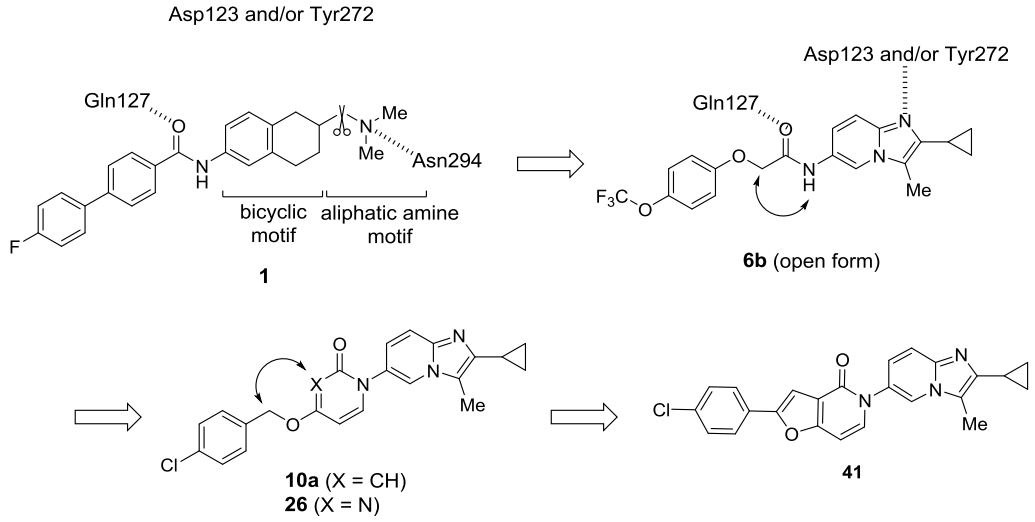


Figure 16. Effects of **10a** in a 3-day food intake study in MCHR1-deficient and wild-type mice. The mice were fed a high-fat diet. The cumulative food intake was measured for 3 days. Each data represents mean ± SD (n = 5 or 6 for each group). (#) $p < 0.025$ vs. the vehicle group (Williams test).

第 6 節　小括

第 1 章で述べた研究方針に基づき、既存化合物より安全性に対する懸念の低い MCHR1 拮抗薬を見出すべく、活性発現に重要であるが、同時に hERG 阻害作用および PLsis 惹起を誘発するアルキルアミン部位を持たない非アミン性 MCHR1 拮抗薬の設計を行った。その際、安全性および中枢移行性を指向した五つの物理化学的パラメータから定義される chemical space ($pK_a < 8$、PSA < 70、2 < ClogP < 4、MW < 450 および HBD 数 = 0 もしくは 1) を指標にリード化合物創出を試みた結果、良好な MCHR1 結合活性および細胞系での活性を有するピリドン誘導体 **10a**、ピリミジノン誘導体 **26** およびフロピリドン誘導体 **41** を見出すことに成功した。代表化合物 **10a** は in vitro 評価において PLsis 陰性であり、パッチクランプ試験において hERG 阻害作用を示さなかった。また、化合物 **10a** は食餌性肥満ラットにおいて MCHR1 拮抗作用に基づく強力な摂食抑制作用および体重低下作用を示した。これらの結果は、五つの物理化学的パラメータより規定される安全性および中枢移行性を指向した chemical space を用いた我々のリード創出戦略が有効であり、それにより in vivo で薬効を発揮し、かつ hERG 阻害作用および PLsis 惹起のリスクが低減された非アミン性 MCHR1 拮抗薬の創出が可能なことを示している。


Asp123 and/or Tyr272
Gln127
O
N
H
Me
N
Me
Asn294
bicyclic
motif
aliphatic amine
motif
F
1
Asp123 and/or Tyr272
Gln127
O
O
N
H
N
N
Me
F3C
O
6b (open form)
O
X
N
N
N
Me
O
Cl
10a (X = CH)
26 (X = N)
O
N
N
N
Me
Cl
O
41

第３章　新規ベンズイミダゾール誘導体の構造活性相関および薬理作用

第１節　低塩基性二環性縮合環化合物の探索

第１項　背景

安全性の向上を指向した非アミン性 MCHR1 拮抗薬の創出を目的に、前章では強力な in vitro および in vivo での活性を示すイミダゾピリジン誘導体 **10a** を見出した (Figure 17)。本化合物では hERG 阻害作用および PLsis 惹起リスクが低減しており、我々の期待するプロファイルを示すことが明らかとなった。しかし、更に精査試験を継続したところ、化合物 **10a** は 10 μM において CYP3A4 阻害作用を示すことが明らかとなった。本作用は、イミダゾピリジン環 1 位の塩基性窒素原子が CYP3A4 上のヘム鉄に配位することが原因で起こったものと考えられる。そこで本節では、イミダゾピリジン環に代わるより塩基性の低い二環性縮合環を見出すべく、水素結合受容基を有する他の低塩基性縮合環 (アザイミダゾピリジン環およびベンズイミダゾール環) を持つ化合物を設計し、in vitro 活性に与える影響を検証した。

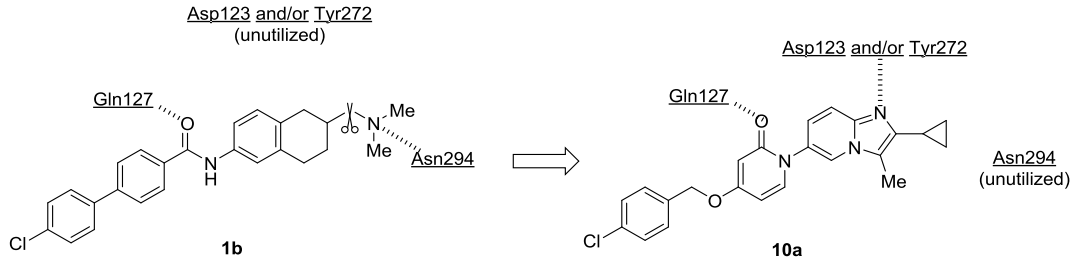
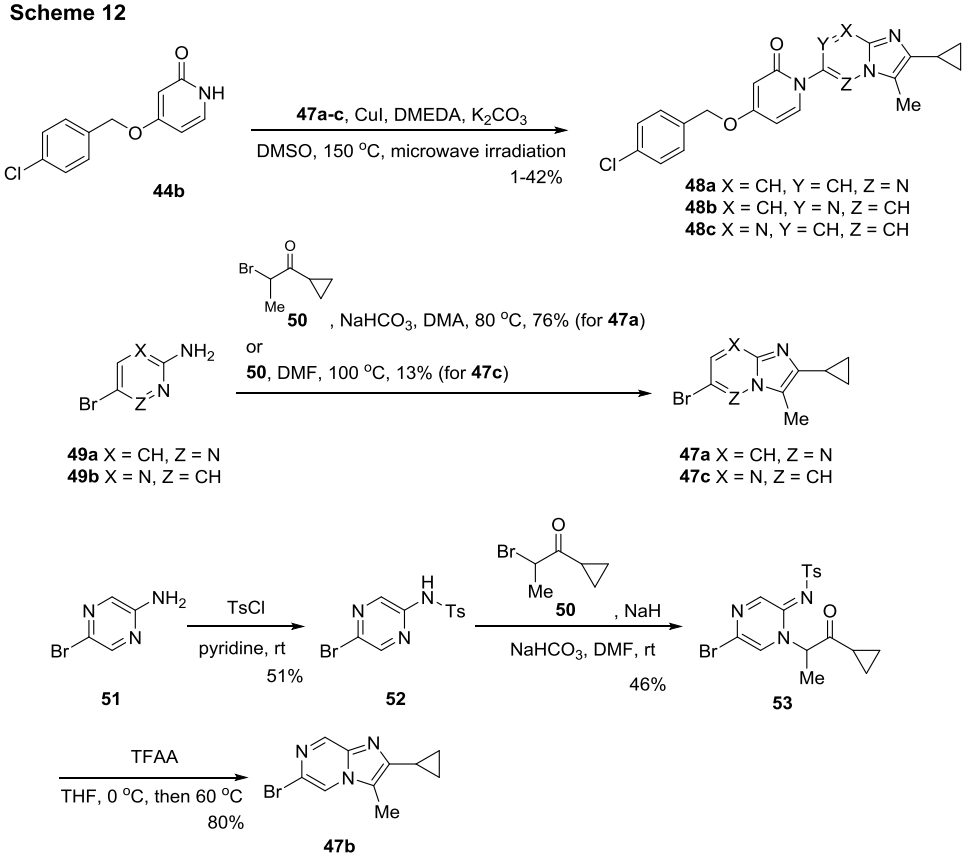


Figure 17. Chemical structures of **1b** and the amine-free MCHR1 antagonist **10a**. Dotted lines depict putative interactions with MCHR1.

第２項　合成

アザイミダゾピリジン誘導体 **48a–c** は、アザイミダゾピリジン **47a–c** を用いて、前章第４節で示したヨウ化銅 (I) を用いたカップリング反応により合成した (Scheme 12)。ここで用いたイミダゾピリダジン **47a** および **47c** は、原料となる芳香族アミン **49a** もしくは **49b** に対する α-ブロモケトン **50** によるアルキル化、続く環化反応をワンポットで実施する事により調製した。一方、イミダゾピラジン **47b** はアルキル化反応および環化工程を段階的に実施することで合成した。すなわち、アミン **51** のトシル保護体 **52** を α-ブロモケトン **50** によるアルキル化反応に付すことで環化前駆体 **53** とし、その後無水トリフルオロ酢酸による環化反応を経て良好な収率で目的物 **47b** を得ることができた。

Scheme 12

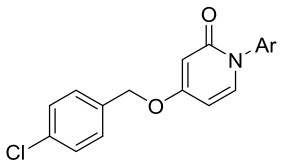


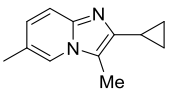
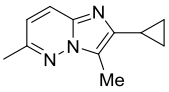
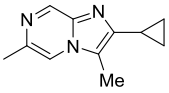
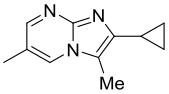
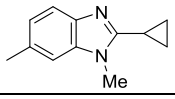
ベンズイミダゾール誘導体 **54a** の合成法は、第２節におけるベンズイミダゾール誘導体の一般合成法において述べる。

第３項　生物活性と考察

イミダゾピリジン誘導体 **10a** の二環性縮合環部分の SAR 結果を Table 8 に示した。イミダゾピリジン環の 6 員環部分に窒素原子を導入したアザイミダゾピリジン誘導体 **48a–c** の共役酸の pK_a[43] 値を計算したところ、いずれも化合物 **10a** より塩基性が低いことが示された。これらの化合物を in vitro 評価に供したところ、イミダゾピラジン誘導体 **48b** では化合物 **10a** と比較して in vitro 活性がおよそ 2 倍弱く、イミダゾピラジン誘導体 **48a** およびイミダゾピリミジン誘導体 **48c** では、それぞれ 10 倍および 50 倍活性が低くなることが明らかとなった (**48a**: IC_{50} = 380 nM、**48c**: IC_{50} > 1000 nM)。続いて、医薬品の部分構造として用いられることが多く、安全面での懸念が低いと考えられるベンズイミダゾール環 [45] を導入した結果、化合物 **54a** はリード化合物 **10a** と同等の強力な in vitro 活性を示すことが明らかとなった。化合物 **54a** におけるベンズイミダゾール環 1 位窒素原子上の共役酸の pK_a 値は 5.71 であったことから、塩基性の低減した新規リード化合物として、活性向上を目指した更なる最適化研究を実施することとした。

Table 8. In vitro binding affinities and pK_a values of compounds **10a**, **48a–c**, and **54a**



Compound	Ar	IC_{50} (nM)[a] hMCHR1[b]	pK_a[c]
10a		26	7.85
48a		380	6.35
48b		44	5.35
48c		>1000	6.35
54a		35	5.71

[a] IC_{50} values were calculated using an experiment performed in duplicate with a three-fold standard deviation. [b] Binding affinity for human MCHR1. [c] pK_a values of conjugate acids were calculated using ACD Labs ver. 12.0.[43]

第２節　ベンズイミダゾール誘導体の構造活性相関

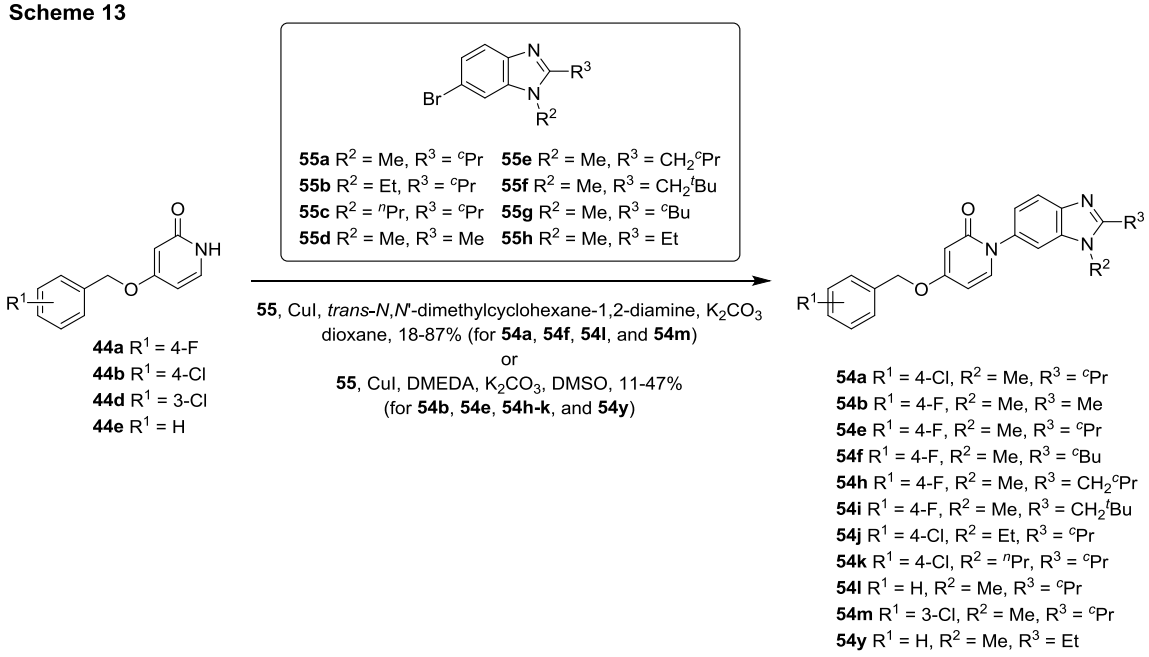
第１項　薬物設計

前章におけるイミダゾピリジン誘導体の SAR では、イミダゾピリジン環 2 位への極性基の導入は活性を減弱させた。この傾向は、ベンズイミダゾール環 2 位においても同様であり、アルコキシ基、水酸基、ケトンもしくはアミド基の導入により in vitro 活性が減弱した。そこで本節では、種々のアルキル鎖導入による脂溶性相互作用の最大化を目的としたベンズイミダゾール 2 位置換基の最適化についてのみ論じる。また、LHS におけるアリール基の構造変換では、既報のジヒドロナフタレン誘導体およびキノリン誘導体の SAR も参考に、まずはハロゲン置換ベンゼン、ピリジンおよびピリミジンの効果を検証し、その後、前章において活性増強に効果のあったチオフェン環の導入を試みた。以下の項において詳細を論じる。

第２項　合成

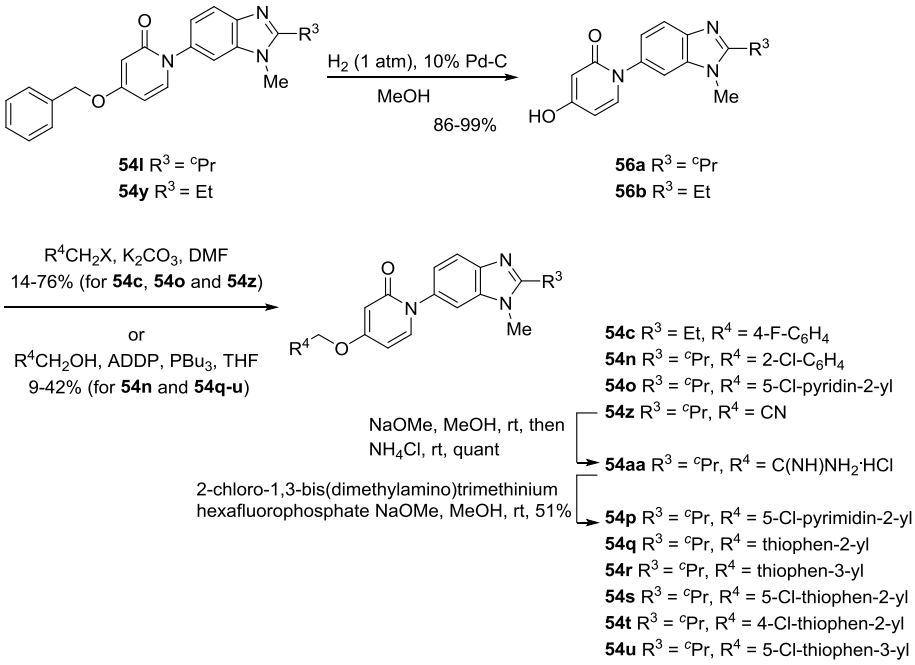
4-アルコキシピリドン誘導体 **54** の一般合成法を Scheme 13 に示した。共通中間体 **44a–d** の合成、およびヨウ化銅 (I) を用いたカップリング反応は前章第４節に示した手法に倣い実施し、目的物を得た。

Scheme 13



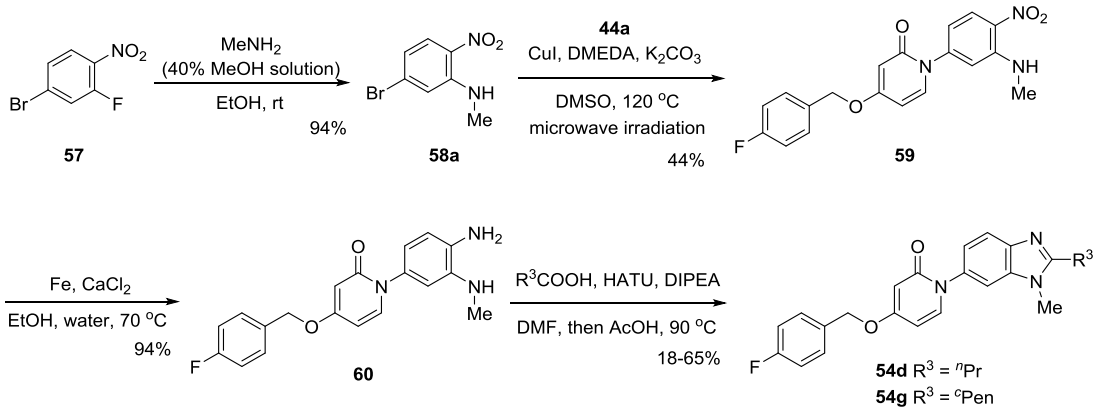
４位の効率的変換を目的とした 4-アルコキシピリドン **54** の別途合成法を Scheme 14 に示した。Scheme 13 において合成した化合物 **54l** もしくは **54y** の加水素分解反応により合成した鍵中間体 **56a** および **56b** に対し、塩基性条件下もしくは光延反応条件下でアルキル化することにより目的物を得た。末端アリール部位にピリミジン環を有する化合物 **54p** は、ニトリル誘導体 **54z** をアミジン **54aa** に変換した後、ジホルミル等価体との環化反応に付す事により合成した。

Scheme 14



ベンズイミダゾール環 2 位の効率的構造変換を目的とした合成法による 4-アルコキシピリドン **54d** および **54g** の合成を Scheme 15 に示した。原料 **57** に対するメチルアミンの S_NAr 反応により *p*-ニトロブロモベンゼン **58a** を得、続くヨウ化銅 (I) によるカップリング反応、ニトロ基の還元反応 [46] を経て中間体 **60** を調製した。続いて、中間体 **60** に対し 1 当量のカルボン酸を HATU によって縮合させた後、得られたアミドを酢酸中加熱する事でベンズイミダゾール環を構築し、目的とする化合物 **54d** および **54g** を得た。

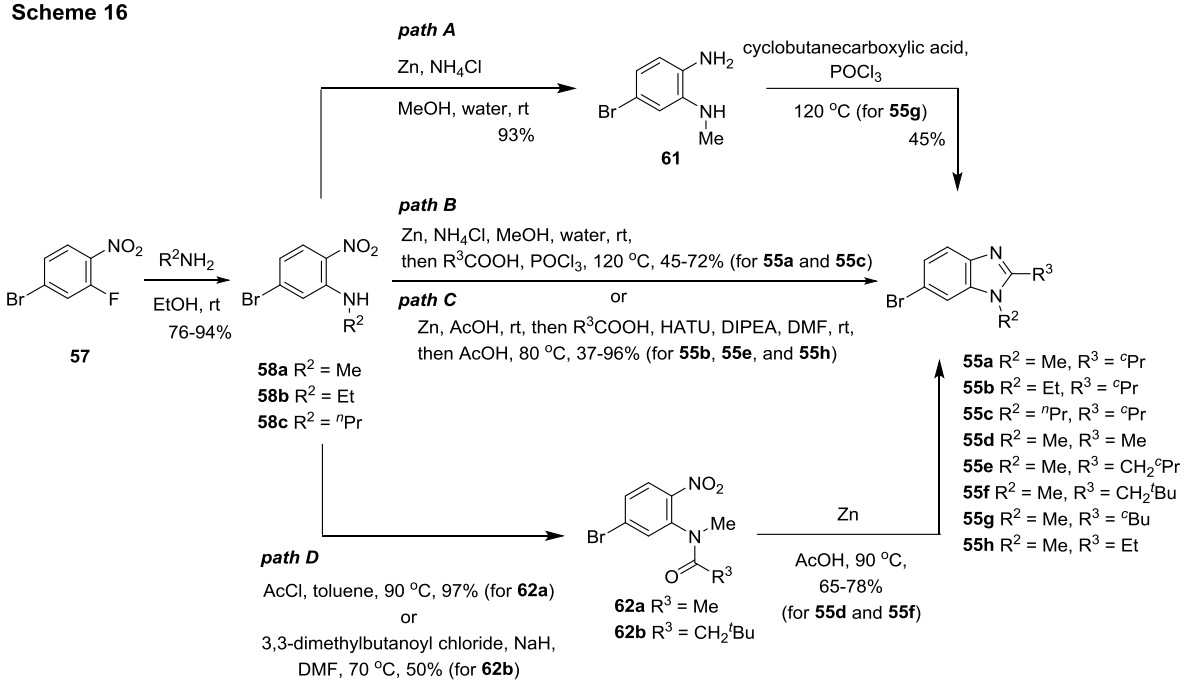
Scheme 15



6-ブロモベンズイミダゾール **55a–h** は *p*-ニトロブロモベンゼン **58a–c** を用い、Scheme 16 に示した手法により合成した。*p*-ニトロブロモベンゼン **58a–c** の還元反応によりジアミン中間体を得、続いてオキシ塩化リン溶媒中、対応するカルボン酸と反応させることでイミダゾール環を構築し、目的物とした (path A および B)。また、*p*-ニトロブロモ

ベンゼン **58a** もしくは **58b** の還元の後、得られたジアミン中間体へのアミド化反応、酢酸中加熱条件下での環化反応により目的物を得た (path C)。さらに、*p*-ニトロブロモベンゼン **58a** をアミド化反応に付し、得られたアミドを亜鉛存在下において酢酸中加熱することで、ニトロ基の還元反応およびイミダゾール環構築が効率よく進行し、目的物へと導くことも可能であった (path D)。

Scheme 16



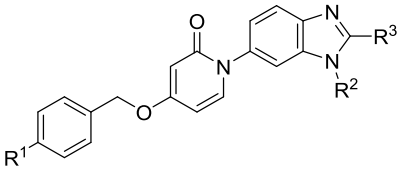
第３項　生物活性と考察

ベンズイミダゾール環 2 位の SAR を Table 9 に示した。初めにアルキル鎖長の検討を行ったところ (**54b–d**)、メチル誘導体 **54b** の活性が中程度に留まったのに対し (hMCHR1: IC_{50} = 77 nM)、エチル誘導体 **54c** および *n*-プロピル誘導体 **54d** では 2 倍程度活性が高かった。次にシクロアルキル基の検討を行ったところ (**54e–g**)、シクロプロピル誘導体 **54e** では同アルキル鎖長のエチル誘導体 **54c** と比較して若干活性が向上した。しかし、他のシクロアルキル基は環サイズ依存的な活性減弱を招くことが明らかとなった (**54e** > **54f** > **54g**)。また、シクロプロピルメチル誘導体 **54h** は同アルキル鎖長の *n*-プロピル誘導体 **54d** と比較して約 3 倍活性が減弱し、ネオペンチル誘導体 **54i** においては測定濃度範囲内で活性が認められなかった。これらの結果から、ベンズイミダゾール 2 位方向は狭い脂溶性領域に位置しており、立体的に小さな 2 ないし 3 アルキル鎖長から成る脂溶性置換基、中でもシクロプロピル基が最適な置換基であることが明らかとなった。

続いてベンズイミダゾール 1 位の SAR を確認したところ、エチル基の導入で rMCHR1 に対する in vitro 活性が若干減弱し (**54a** vs **54j**)、*n*-プロピル基の導入で活性が

大幅に減弱した (**54a** vs **54k**)。これは、1 位の置換基許容性が低く、メチル基が最適であることを示している。

Table 9. In vitro binding affinities of compounds **54a–k**

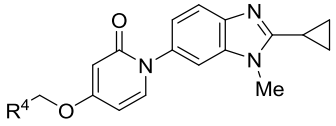


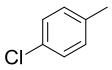
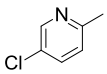
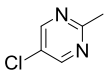
Compound	R^1	R^2	R^3	IC_{50} (nM)[a]	
				hMCHR1[b]	rMCHR1[c]
54b	F	Me	Me	77	65
54c	F	Me	Et	48	38
54d	F	Me	^{n}Pr	34	39
54e	F	Me	^{c}Pr	40	28
54f	F	Me	cyclobutyl	240	210
54g	F	Me	cyclopentyl	600	460
54h	F	Me	$CH_2{}^c$Pr	90	140
54i	F	Me	$CH_2{}^t$Bu	>1000	>1000
54j	Cl	Et	^{c}Pr	37	34
54k	Cl	^{n}Pr	^{c}Pr	85	110
54a	Cl	Me	^{c}Pr	35	21

[a] IC_{50} values were calculated using an experiment performed in duplicate with a three-fold standard deviation. [b] Binding affinity for human MCHR1. [c] Binding affinity for rat MCHR1.

次に、LHS における末端アリール基の効果を検証した (Table 10)。無置換体 **54l** の IC_{50} 値がヒトおよびラットにおいて、それぞれ 45 nM および 43 nM であったのに対し、パラ位へのフッ素原子 (**54e**) および塩素原子 (**54a**) の導入により活性が向上した。一方、メタ位 (**54m**) およびオルト位 (**54n**) への塩素原子の導入により活性は大きく減弱した。末端アリール基として芳香族複素環を導入したところ、活性は脂溶性が低下するのに従って減弱した (**54o**: ClogP = 3.56、**54p**: ClogP = 2.56) [43]。これらの結果は、LHS が狭い疎水的な環境下にあり、ベンゼン環上への置換基導入はパラ位が最適であるという既報の塩基性 MCHR1 拮抗薬の結果とよく一致した [25]。

Table 10. In vitro binding affinities of compounds **54a**, **54e**, and **54l–p**

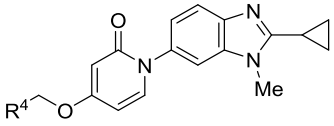


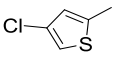
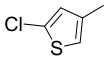
Compound	R^4	IC_{50} (nM)[a]	
		hMCHR1[b]	rMCHR1[c]
54l		45	43
54e		40	28
54a		35	21
54m		100	72
54n		160	140
54o		73	47
54p		>1000	900

[a] IC_{50} values were calculated using an experiment performed in duplicate with a three-fold standard deviation. [b] Binding affinity for human MCHR1. [c] Binding affinity for rat MCHR1.

続いて末端アリール基としてチオフェン環を導入し、その活性に与える影響を評価した (Table 11)。2-チエニル誘導体 **54q** の活性が中程度であったのに対し、3-チエニル誘導体 **54r** はベンゼン誘導体 **54l** と比較して強力な活性を示した。続いてこれらのチオフェン環上に塩素原子を導入したところ (**54s–u**)、いずれの化合物においても活性向上が認められ、中でも 5-クロロ-3-チエニル誘導体 **54u** は最も強力な活性を示した。

Table 11. In vitro binding affinities of compounds **54q–u**



Compound	R^4	IC_{50} (nM)[a]	
		hMCHR1[b]	rMCHR1[c]
54q		76	120
54r		28	29
54s		19	11
54t		17	18
54u		14	9.3

[a] IC_{50} values were calculated using an experiment performed in duplicate with a three-fold standard deviation. [b] Binding affinity for human MCHR1. [c] Binding affinity for rat MCHR1.

本節における最適化研究の結果、リード化合物 **54a** の末端アリール基を種々のクロロ置換チオフェンとすることにより、強力な MCHR1 結合活性を示す一連の化合物群を見出すことに成功した。

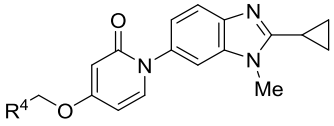
第３節　チオフェン置換体の CYP3A4 時間依存的阻害回避の戦略

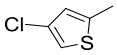
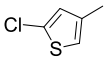
第１項　背景

強力な in vitro 活性を示した前節のチオフェン誘導体を精査した結果、化合物 **54r**、**54t** および **54u** において CYP3A4 時間依存的阻害作用 (TDI 作用)[*)] が認められることが明らかとなった (Table 12)。

*) 代謝物に由来する不可逆的な CYP3A4 阻害作用。臨床での重篤な薬物間相互作用に繋がる。

Table 12. CYP3A4 TDI risk of compounds **54q–u**



Compound	R^4	CYP3A4 TDI[a] (% remaining)
54q		NT[b]
54r		74
54s		83
54t		25
54u		44

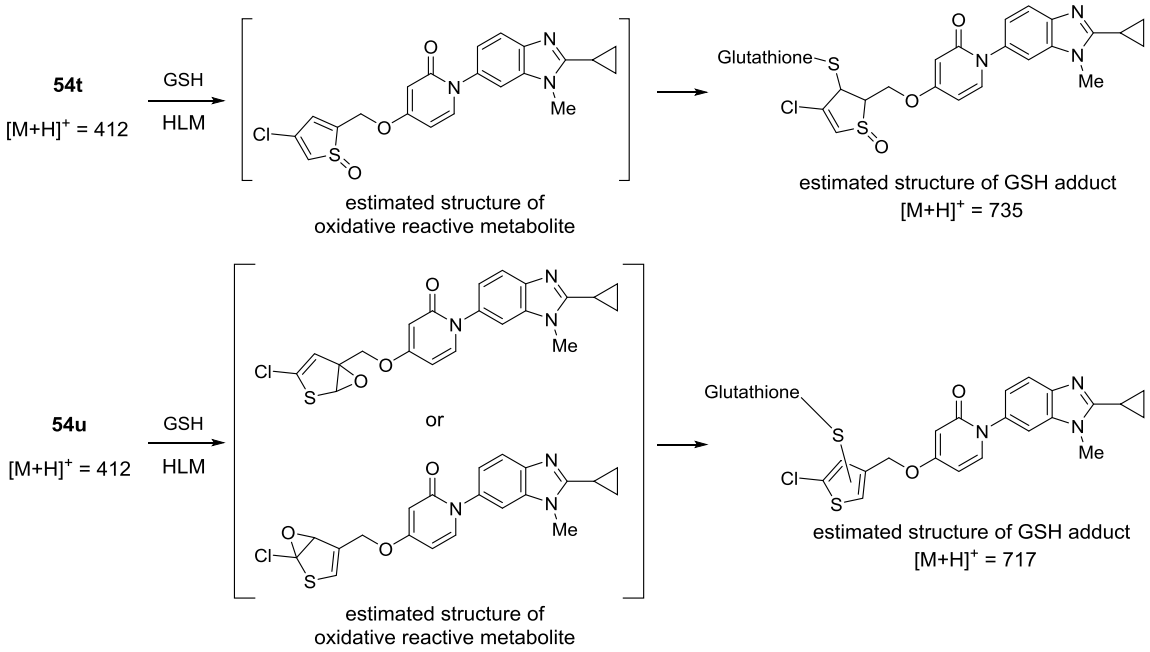
[a] CYP3A4 time-dependent inhibition assay (n = 2). The remaining activity of CYP3A4 after pre-incubation with a test compound was determined. [b] Not tested.

CYP3A4 は生体内に最も豊富に存在する CYP アイソフォームであり、様々な薬物の酸化代謝に関与していることから、CYP3A4 に対する TDI リスクを有する薬剤には臨床での薬物間相互作用が懸念され、開発が困難となる。ベンゼン誘導体 **54e** およびピリジン誘導体 **54o** は TDI 作用を示さなかったことから、筆者はチオフェン環が TDI 作用の原因構造であると考えた。チオフェン環が酸化代謝により反応性代謝物を生じやすく、これが生体分子と結合することによる毒性発現や、CYP3A4 の不可逆的阻害による薬物間相互作用を引き起こす懸念があるというこれまでの報告からも、チオフェン環の TDI 作用への関与が疑われた [47]。

そこで化合物 **54t** および **54u** の CYP3A4 TDI 作用の機構を考察すべく、グルタチオントラップ試験を実施した。化合物 **54t** および **54u** をヒト肝ミクロソーム存在下、グルタチオンと反応させた結果、化合物 **54t** からは "**54t** + GSH + O" の分子量に相当する GSH 付加体が、化合物 **54u** からは "**54u** + GSH – H_2" の分子量に相当する GSH 付加体が確認された (Scheme 17)。さらに、GSH 付加体のイオンスペクトルより、GSH はチオフェン環に付加していると考えられた。これらの結果から、化合物 **54t** からは酸化代謝によりスルホキシド種が生じ、続く GSH の付加 [*)] によって GSH がチオフェン環上 3

[*)] チオフェン環の開裂、再環化を経る多段階機構が報告されている [48]。

位に結合していると考えられた。また、化合物 **54u** からは酸化代謝によりエポキシド種が生じ、続く GSH の付加、脱水反応を経て GSH 付加体が生じていると考えられた。これらの結果から、Table 4 で述べた CYP3A4 TDI 作用は、チオフェンの酸化代謝により反応性のスルホキシド種もしくはエポキシド種が発生し、これが CYP3A4 と共有結合性の複合体を形成することにより酵素活性を阻害することに起因すると推察された。



Scheme 17. Plausible mechanisms of glutathione adduct formation by incubation of test compounds **54t** and **54u** with GSH and human liver microsomes (HLM). The test compounds (30 μM) were incubated with HLM (1.0 mg/mL) in the presence of GSH (1 mM) at 37 ℃ for 60 min. The structures of the GSH adducts were estimated by LC/MS/MS analysis.

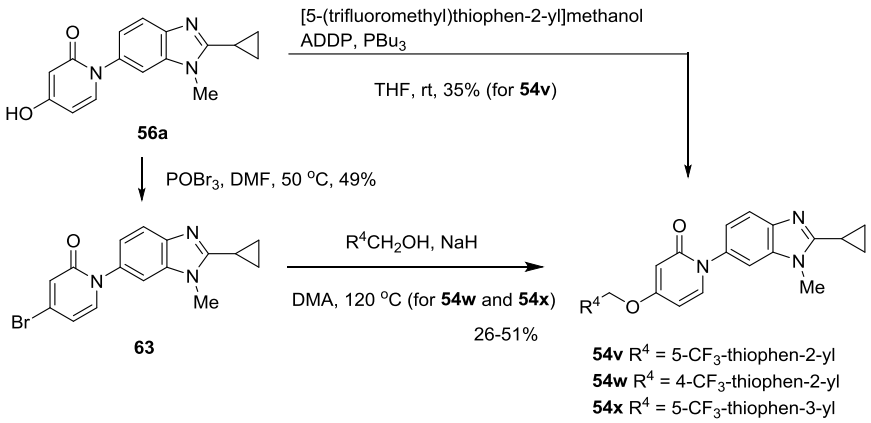
第２項　薬物設計

前項の CYP3A4 TDI 作用の機構解析に基づき、(1) チオフェン環上への嵩高い置換基導入、もしくは (2) チオフェン環の電子密度の低減により、チオフェン誘導体の CYP3A4 TDI 作用が回避可能と考えた。事実、前項の Table 12 に示した化合物 **54s** の TDI リスクは他の化合物と比較して低い。これはチオフェンの 2, 5 位が置換されると同時に、比較的嵩高い塩素原子がチオフェン環への代謝酵素の接近を妨げている為であると考えられ、上記の (1) の有効性を支持する結果である。本項では上記 (2) の有効性を検証すべく、強力な電子求引基であるトリフルオロメチル基を導入したチオフェン誘導体を設計し、その CYP3A4 TDI 阻害作用回避に対する効果について検証した。

第３項　合成

トリフルオロメチル置換チオフェン誘導体 **54v–x** は、前項において合成した 4-ヒドロキシピリドン誘導体 **56a** に対する光延反応、もしくは **56a** より調製した 4-ブロモピリドン誘導体 **63** に対するアルコキシ化反応により合成した (Scheme 18)。

Scheme 18

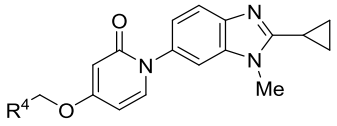


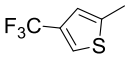
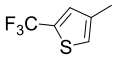
第４項　生物活性および考察

トリフルオロメチル置換チオフェン誘導体 **54v–x** の in vitro 活性および CYP3A4 TDI 作用を Table 13 に示した。5-トリフルオロメチル-2-チエニル誘導体 **54v** は対応するクロロ置換体 **54s** と同等の in vitro 活性を示したが、4-トリフルオロメチル-2-チエニル誘導体 **54w** はクロロ置換体 **54t** と比較して活性が低かった。また、5-トリフルオロメチル-3-チエニル誘導体 **54x** ではクロロ置換体 **54u** の比較して、およそ 3 倍低活性であった。続いて、TDI 作用を評価した結果、2, 5 位が置換された化合物 **54v** のみならず、2 位あるいは 5 位の一方が無置換であり、よりチオフェン環の酸化反応が進行しやすいと考えられる化合物 **54w** および **54x** においても 電子求引性のトリフルオロメチル基の導入により TDI 作用が大幅に軽減されることが明らかとなった。化合物 **54w** および **54x** に対応するクロロ置換体 **54t** および **54u** が TDI 陽性であることからも (Table 12)、トリフルオロメチル基の TDI 作用回避における有効性は明らかといえる。

チオフェンは、その反応性代謝物生成の懸念より、しばしば薬物の部分構造として導入することが避けられる傾向がある。本項での検討により、トリフルオロメチル置換チオフェンが反応性代謝物を生じる懸念の少ない安全性の高い building block として、創薬研究に使用できる可能性が示された。

Table 13. In vitro binding affinities and CYP3A4 TDI risk of compounds **54s** and **54v–x**



Compound	R^4	IC_{50} (nM)[a]		CYP3A4 TDI[d] (% remaining)
		hMCHR1[b]	rMCHR1[c]	
54v		16	13	97
54w		34	17	87
54x		41	29	96
54s		19	11	83

[a] IC_{50} values were calculated using an experiment performed in duplicate with a three-fold standard deviation. [b] Binding affinity for human MCHR1. [c] Binding affinity for rat MCHR1. [d] CYP3A4 time-dependent inhibition assay (n = 2). The remaining activity of CYP3A4 after pre-incubation with a test compound was determined.

第４節　ベンズイミダゾール誘導体 **54s** の薬理作用

第１項　食餌性肥満 F344 ラットにおける二日間摂食抑制確認試験

上記の検討で見出された、強力な MCHR1 結合活性を示し、かつ CYP3A4 TDI 作用を回避した化合物 **54s** および **54v** を更なるプロファイリング試験に供した。これらは CHO 細胞を用いた細胞系試験において良好な MCHR1 拮抗活性を示し (**54s**: IC_{50} = 24 nM、**54v** IC_{50} = 18 nM)、またラットにおいて良好な経口吸収性と血中暴露を示した (Table 14)。

化合物 **54s** および **54v** の in vivo における効果を確認すべく、両化合物を DIO F344 ラットにおける二日間摂食抑制確認試験に供した。化合物 **54s** (3 および 10 mg/kg) を一日一回、二日間経口投与したところ、3 mg/kg 投与群で −11.6%、10 mg/kg 投与群で −36.5% の用量依存的な摂食量の低下が認められた (Figure 18)。一方、化合物 **54v** の 3 mg/kg 投与群は、化合物 **54s** とほぼ同等 (−12.6%) の摂食抑制作用を示したが、10 mg/kg 投与群の作用は化合物 **54s** の 10 mg/kg 投与群と比べて軽微であった (−20.3%)。これは、化合物 **54v** の溶解性 (0.25 μg/mL at pH 6.8) が、化合物 **54s** (2.3 μg/mL at pH 6.8) より低いことによる非線形動態により、化合物 **54v** の 10 mg/kg 投与群の血中濃度が不十分である

為と考えられた。これらの結果を受け、化合物 **54s** を続く連続投与試験用化合物として選択した。

Table 14. Pharmacokinetic parameters of **54s** and **54v** in rats[a]

Compound	F[b]	iv (0.1 mg/kg)		po (1 mg/kg)		
		CL_{total}[c]	V_{ss}[d]	C_{max}[e]	T_{max}[f]	$AUC_{0-8\ h}$[g]
	(%)	($mL \cdot h^{-1} \cdot kg^{-1}$)	($mL \cdot kg^{-1}$)	($ng \cdot mL^{-1}$)	(h)	($ng \cdot h \cdot mL^{-1}$)
54s	23	450	920	164.7	1.0	514.9
54v	57	285	979	297.8	2.7	2015.6

[a] n = 3; SD rats (male, eight weeks old). [b] Bioavailability. [c] Total clearance. [d] Volume of distribution at steady state. [e] Maximal plasma concentration. [f] Time of maximal concentration. [g] Area under the plasma concentration–time curve (0–8 h).

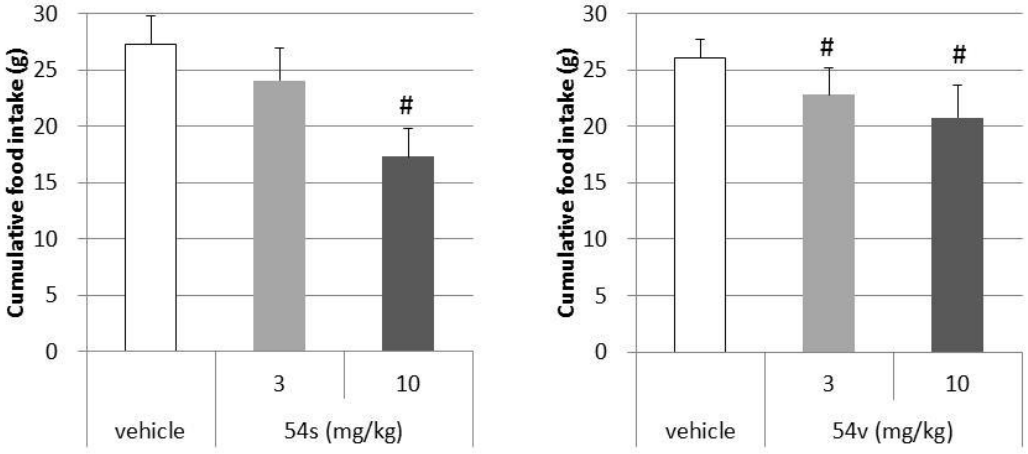


Figure 18. Effects of **54s** and **54v** in a two-day food intake study in DIO F344 rats. Inhibition of cumulative food intake over two days in DIO F344 rats. The compounds were administered once daily, and food intake from the initial administration to two days later was measured. The cumulative food intake inhibition rate was calculated by dividing the average food intake of each treatment group by that of the vehicle group (n = 6 for each group). (#) $p < 0.025$ vs. the vehicle group (Williams test).

第２項　食餌性肥満 F344 ラットにおける二週間連続投与試験

化合物 **54s** の抗肥満作用を DIO F344 ラットにおける二週間連続投与試験において評価した (Figure 19)。化合物 **54s** (1、3 および 10 mg/kg) を一日一回、二週間経口投与したところ、有意かつ用量依存的な体重低下作用が 3 mg/kg 投与群から確認され、vehicle 群に対して 3 mg/kg 投与群で 2.2%、10 mg/kg 投与群で 4.1% の体重低下が認められた。また、その時の摂餌量は vehicle 群と比較し、それぞれ 11.6% および 20.9% 低かった。

化合物 **54s** 10 mg/kg 投与群の血漿中薬物濃度のトラフ値 *) は 0.79 μM であり、化合物 **54s** の非結合型分率が 0.02 であることから、フリー体換算で 15.8 nM と算出される。この結果は、化合物 **54s** の 10 mg/kg 投与群では IC_{50} 値の 1.44 倍の血漿中フリー体濃度が試験を通じて担保されていることを示しており、これが 4.7% の体重低下に必要な薬物濃度と考えられた。また、化合物 **54s** の脳/血中濃度比は 0.5 であることから、十分な BBB 透過性を有することが明らかとなった。

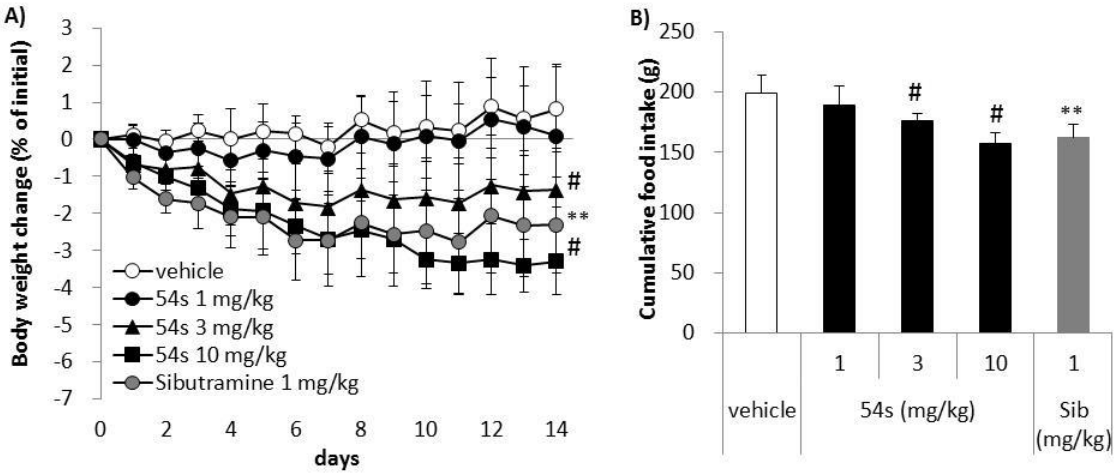


Figure 19. Effects of **54s** in a repeated-dose study in DIO F344 rats. A) Body weight change from initial value during two weeks of dosing. B) Cumulative food intake for two weeks of dosing. The compounds were administered once daily for two weeks and the body weight and food intake were measured before drug administration. Each data point represents mean ± SD (n = 5 or 6 for each group). (#) $p < 0.025$ vs. the vehicle group (Williams test), (**) $p < 0.01$ vs. the vehicle group (Student's t-test). Sib = Sibutramine.

第３項　MCHR1 欠損マウスにおける選択性確認試験

前項における化合物 **54s** の摂食抑制作用および体重低下作用が MCHR1 を介した作用であることを証明すべく、次に MCHR1 欠損マウスを用いて化合物 **54s** の作用を検証した (Figure 20)。化合物 **54s** (10 および 30 mg/kg) を一日一回、三日間経口投与したところ、正常マウスでは用量依存的な抗肥満作用が認められたのに対し、MCHR1 欠損マウスにおいては作用が認められなかった。また、正常マウスでは統計的に有意ではないが用量依存的な摂食抑制傾向が認められたのに対し、MCHR1 欠損マウスにおいては摂食抑制効果が認められなかった。本結果は、化合物 **54s** の抗肥満作用が MCHR1 拮抗作用を介する効果であることを示している。

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*) 薬物を反復投与したときの定常状態における最低血中薬物濃度。

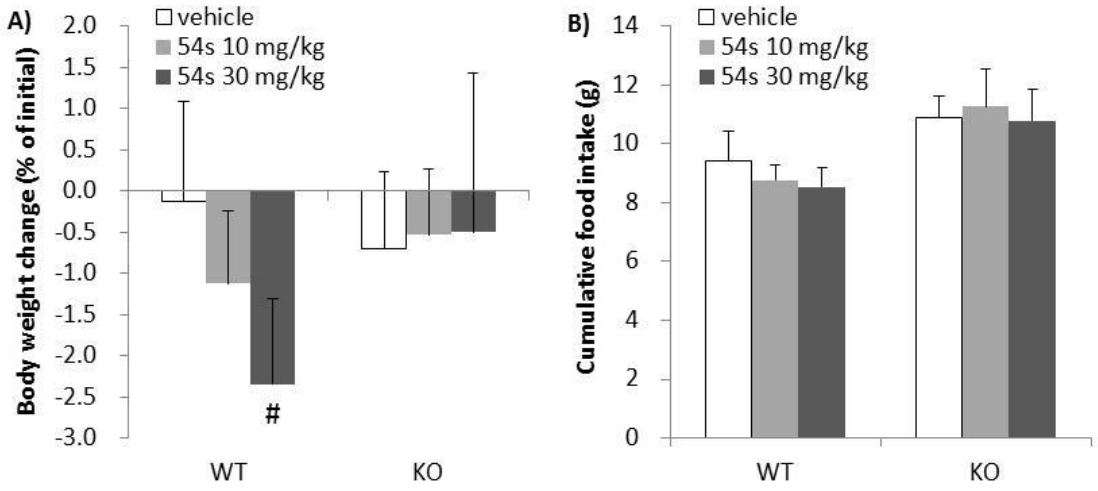


Figure 20. Effects of **54s** in a three-day study in MCHR1-deficient (KO) and wild-type (WT) mice. The mice were fed a high-fat diet. The body weight change (A) and cumulative food intake (B) were measured for three days. Each data point represents mean + SD (n = 6 for each group). (#) $p < 0.025$ vs. the vehicle group (Williams test).

第５節　小括

イミダゾピリジン環に代わる低塩基性の二環性縮合環を見出すべく縮合環を探索した結果、良好な in vitro 活性を有するベンズイミダゾール誘導体を見出した。また、続く最適化において、末端アリール基にチオフェン環を有する化合物群が強力な in vitro 活性を示すことが明らかとなった。これらのチオフェン誘導体は CYP3A4 TDI 作用を示したが、チオフェン環 5 位への置換基導入、もしくは立体的に嵩高く、電子求引性のトリフルオロメチル基を導入することにより TDI 作用を回避できることを示した。チオフェンは反応性代謝物生成の懸念より、しばしば薬物の部分構造として導入することが避けられる傾向があるが、本章で見出したトリフルオロメチル置換チオフェンは反応性代謝物を生じる懸念の少ない有用な building block となると考えられる。

強力な in vitro 活性を有し、TDI 作用が回避された化合物 **54s** は前章のイミダゾピリジン誘導体で認められた 10 μM における可逆的 CYP3A4 阻害作用を示さず、また、in vitro において PLsis 惹起リスクおよび hERG 阻害作用を示さなかった。化合物 **54s** は DIO rat において強力な体重低下作用を示したことから、有望な薬剤候補化合物となり得ると考えられる。

第４章　新規インダゾール誘導体の構造活性相関および薬理作用

第１節　新規インダゾール誘導体の発見

第１項　背景

安全性の高い新規抗肥満薬の創製を目的とした、非アミン性 MCHR1 拮抗薬の創薬研究の結果、第２章においてはイミダゾピリジン環を、第３章においてはベンズイミダゾール環を RHS に有するピリドン誘導体について報告した (Figure 21)。インドール誘導体 **64** の活性が大きく低下していることからも、当初の狙い通り化合物 **10a** および **54s** は、縮合環上窒素原子が受容体上の Asp123 もしくは Tyr272 と相互作用することにより活性を示していると考えられた。一方、塩基性の異なる化合物 **10a** および **54s** が同等の in vitro 結合活性を示していることから、本相互作用は縮合環上窒素原子の塩基性に影響を受けないことが推測された。これらの非アミン性 MCHR1 拮抗薬では既存のアミン性 MCHR1 拮抗薬と異なり hERG 阻害作用および PLsis 惹起リスクが軽減しており、また食餌性肥満ラットにおいて強力な摂食抑制作用と体重低下作用を示すことをこれまでに論じた。

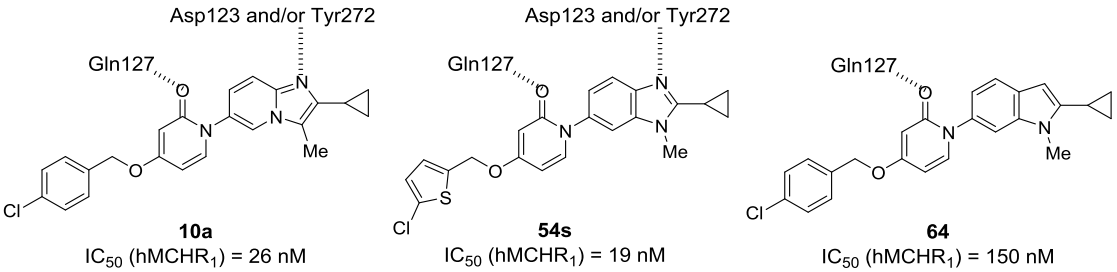


Figure 21. Chemical structures of amine-free MCHR1 antagonists (**10a** and **54s**) and indole derivative **64**. Dotted lines depict putative interactions with MCHR1.

一方、最近になって Washburn らは、hERG チャネル等に対しターゲット選択性が高い中性 MCHR1 拮抗薬を報告している [49] (第一章 Figure 3 における BMS-819881)。既報の MCHR1 拮抗薬において中性分子は非常に稀であるが、選択性および安全性の面において中性分子は塩基性化合物に比べ有利と考えられた。そこで本章では、我々の非アミン性 MCHR1 拮抗薬を更に中性 MCHR1 拮抗薬へと発展させるべく化合物設計を行った。

第２項　薬物設計

中性 MCHR1 拮抗薬を創出する上では、これまでのイミダゾピリジン環およびベンズイミダゾール環と同等の受容体親和性を保持した非塩基性縮合環の設計が重要である。受容体との親和性に対する縮合環上窒素原子の塩基性の関与が大きくないとの考えの下、式 I 中の X^1 部分に受容体との相互作用が可能な窒素原子を有し、かつその共役酸の pK_a 値の低いピラゾロピリジン環 (pK_a [43] = 4.3) およびインダゾール環 (pK_a = 2.9) を有する化合物 **65** および **66a** を設計した (Figure 22)。なお、インダゾール誘導体研究の初期段階では、インダゾール環 2 位へのシクロプロピル基導入法を構築するに至っていなかったため、対応するメチル誘導体 **66a** を用いてインダゾール誘導体の効果を評価することとした。

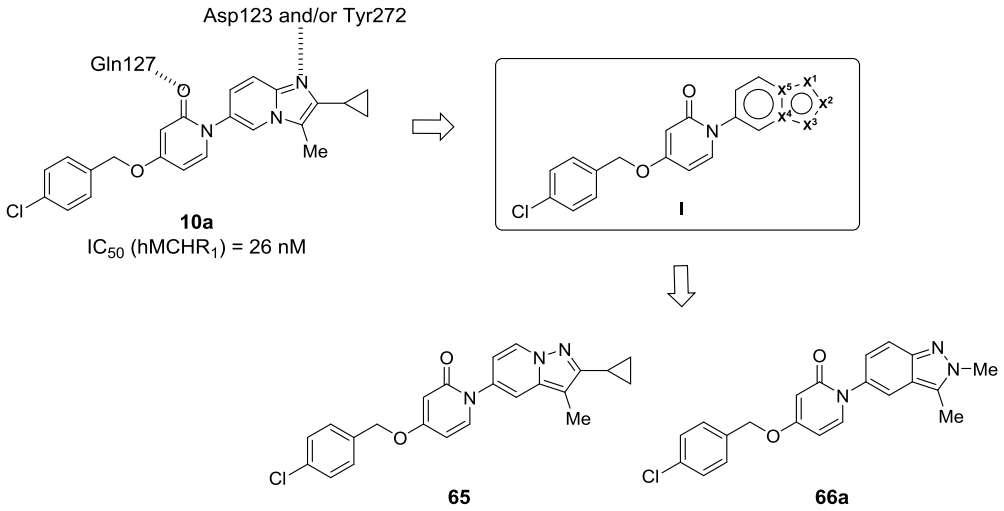
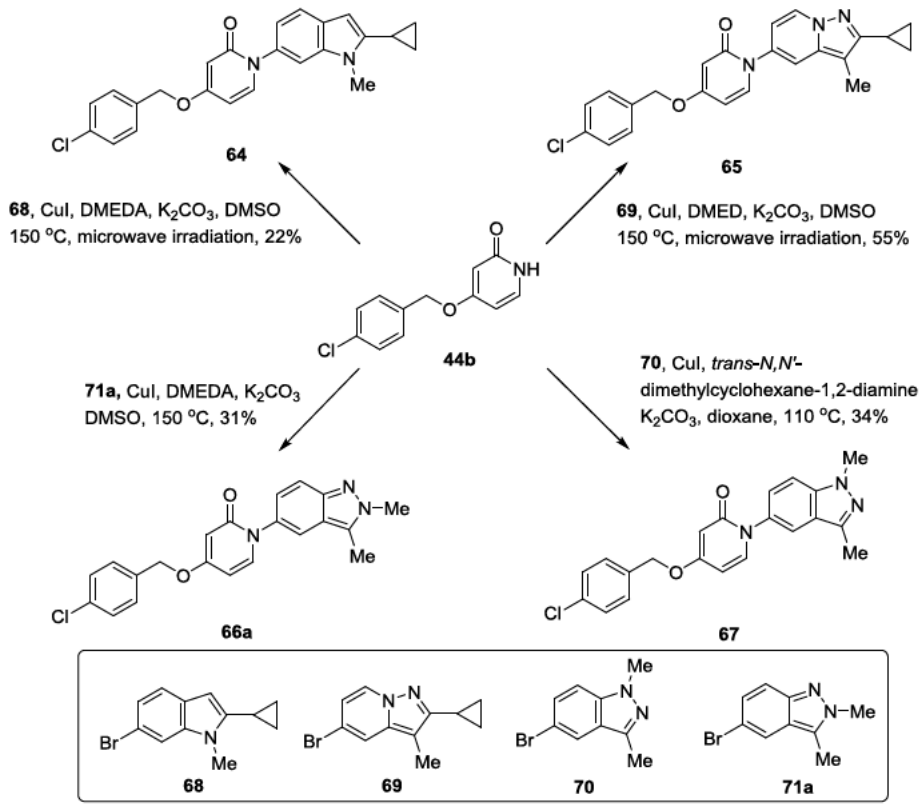


Figure 22. Design of pyrazolo[1,5-*a*]pyridine derivative **65** and 2*H*-indazole derivative **66a**.

第３項　合成

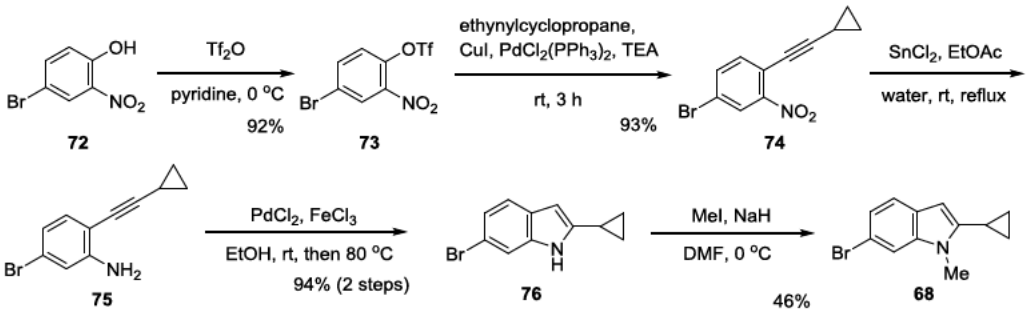
インドール誘導体 **64**、ピラゾロピリジン誘導体 **65**、インダゾール誘導体 **66a** および **67** は、第２章第４節に示したヨウ化銅 (I) を用いたカップリング反応により、ピリドン **44b** から対応する臭化物 **68**、**69**、**71a** および **70** を用いて合成した (Scheme 19)。

Scheme 19



上記のインドール 6 位臭素体 **68** は Scheme 20 に示した手法に基づいて合成した。まず、出発原料 **72** のトリフラート化、薗頭カップリング反応に続く、ニトロ基の還元の三工程を経て環化前駆体である *o*-アルキニルアニリン誘導体 **75** を得た。環化前駆体 **75** を用いたインドール環構築は、触媒量の塩化パラジウムに、0 価パラジウムから 2 価パラジウムへ再酸化する目的で塩化鉄 (III) を共存させて行い [50]、N-メチル化の後に目的とするインドール誘導体 **68** へと導いた。

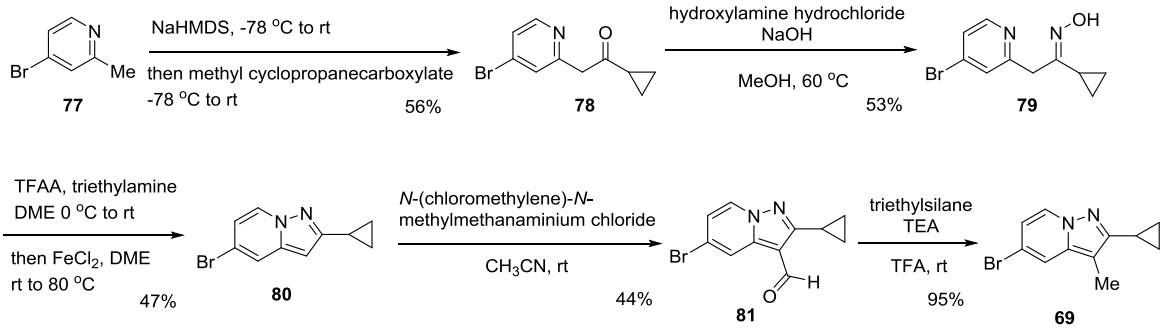
Scheme 20



ピラゾロピリジン 5 位臭素体 **69** は、Scheme 21 に示したオキシム **79** を鍵中間体とする手法により合成した。原料の 2-メチルピリジン **77** のメチル基を NaHMDS により

脱プロトン化した後、シクロプロパンカルボン酸メチルエステルと反応させることでケトン **78** とし、続くオキシム化反応を経て鍵中間体 **79** を得た。続いて鍵中間体 **79** を TFAA で処理することで反応性のアジリンを系中に発生させ、アジリンの熱的環拡大反応を経てピラゾロピリジン環を構築した [51]。その後、Vilsmeier 試薬を用いたピラゾロピリジン環 3 位のホルミル化反応、続くホルミル **81** のシラン還元を経て目的物 **69** を得た。

Scheme 21

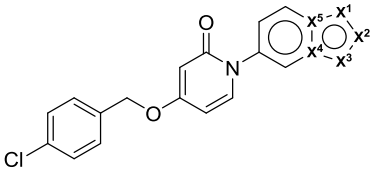


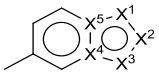
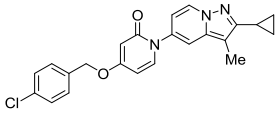
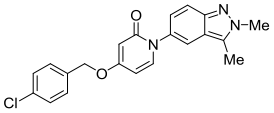
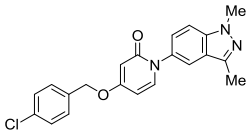
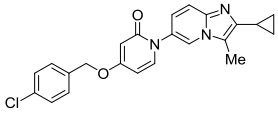
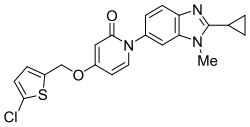
なお、インダゾール 5 位臭素置換体 **70** および **71a** の合成法は、第２節において示すインダゾール環の一般合成法において述べた。

第４項　生物活性と考察

二環性縮合環部の構造変換結果を Table 15 に示した。ピラゾロピリジン誘導体 **65** では化合物 **10a** と比較して活性が 2–3 倍低くなった。一方、興味深いことに中性の 2*H*-インダゾール誘導体 **66a** は化合物 **10a** とほぼ同等の活性を示した。縮合環の共役酸の pK_a 値と in vitro 活性に相関関係が認められず、また中性分子 **66a** でも活性を示していることから、活性発現には受容体と相互作用可能な窒素原子上の孤立電子対を適切な配向に配置できる二環性縮合環が重要であると考えられる。一方、1*H*-インダゾール誘導体 **67** では、2*H*-インダゾール誘導体 **66a** と比較して 3 倍程度活性が低かった。これは、化合物 **67** の 1 位窒素原子上の孤立電子対と受容体との相互作用が失われたためと考えられる。本項における結果から、イミダゾピリジン環およびベンズイミダゾール環と同等の受容体親和性を保持した非塩基性縮合環として、2*H*-インダゾール環を見出すことに成功した。

Table 15. In vitro binding affinities and pK_a values of compounds **10a**, **54s**, **65**, **66a**, and **67**



Compound		IC_{50} (nM)[a] hMCHR1[b]	pK_a value on X^{1}[c]
65		69	4.3
66a		35	2.9
67		99	-
10a		26	7.9
54s		19	5.7

[a] IC_{50} values were calculated using an experiment performed in duplicate, with a standard deviation of three-fold. [b] Binding affinity for human MCHR1. [c] pK_a values of conjugate acids on $\mathbf{X^1}$ were calculated by ACD Labs ver. 12.0.[43]

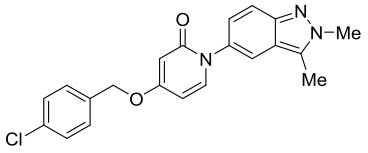
第２節　TA1537 株における遺伝毒性リスク回避の戦略

第１項　背景

インダゾール誘導体 **66a** を更なるプロファイリング試験に供したところ、TA1537 株を用いた Ames 試験 *) において陽性であり、発がん性や催奇形性につながる遺伝毒性のリ

*) 復帰突然変異試験。ヒスチジン要求性突然変異を有するネズミチフス菌を用い、被験物質の変異原性を評価するための試験法。

スクを有することが明らかとなった (Figure 23)。

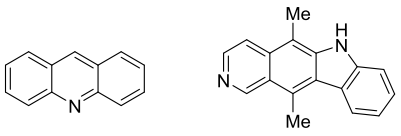


66a

Ames test
TA1537, S9-: positive
TA1537, S9+: negative

Figure 23. Result of the Ames test of compound **66a** using TA1537.

すなわち、S9 *) 非存在下、化合物 **66a** を TA1537 株に作用させた結果、250 μg/plate から 2 倍以上の変異復帰コロニーの増加が確認された。一方、S9 存在下、すなわち代謝活性化条件下において化合物 **66a** は変異原性を示さなかったことから、化合物 **66a** 自体が変異原性を誘発していると考えられた。一般に TA1537 株は、DNAの塩基対間に入り込みその複製を阻害する DNA インターカレーターを検出し易い菌株として知られており、平面性の高い多環性芳香環である acridine や ellipticine が TA1537 株に対して DNA インターカレーションによるフレームシフト型変異を起こすことが知られている [52] (Figure 24)。また、化合物 **66a** は反応性の部分構造を持たず、DNA との共有結合形成反応を経てその複製を阻害する可能性は低いことから、化合物 **66a** で認められた変異原性は DNA インターカレーション作用に基づくものであると推察された。



acridine　　ellipticine

Figure 24. Chemical structures of polycyclic aromatic DNA intercalators: acridine and ellipticine

一方、化合物 **66a** の推定代謝部位は LHS 上のベンジル位であり、主代謝物はヒドロキシピリジン誘導体 **82a** と考えられる (Figure 25)。化合物 **82a** は TA1537 株を用いた Ames 試験にて評価した結果、陰性であった。よって、S9 存在下において化合物 **66a** が陰性であった理由として、代謝により系中で生じた末端アリール基を持たない化合物 **82a** が変異原性を誘起しないからであると考えられた。

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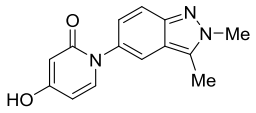
*) 肝ミクロソーム S9 画分

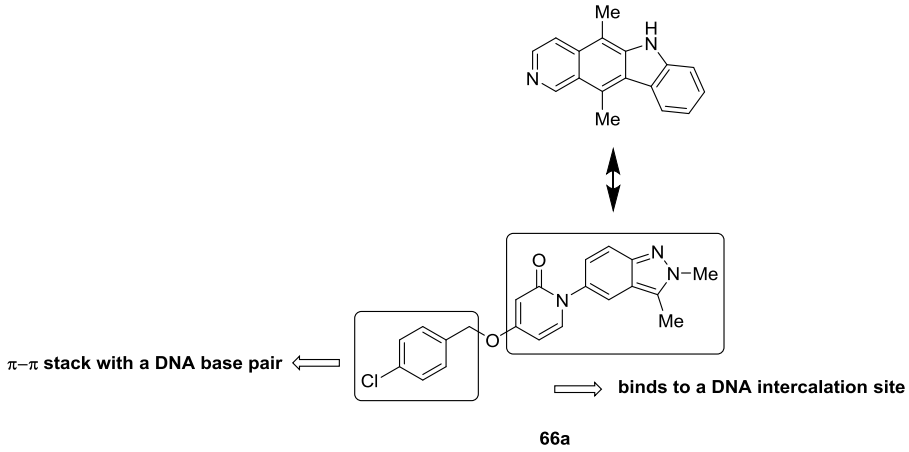


82a

Figure 25. Chemical structure of a possible degradation product of compound **66a**.

第２項　薬物設計

前項で論じた末端アリール基の変異原性への関与、および化合物 **66a** と既存 DNA インターカレーターである ellipticine との重ね合わせより、変異原性は以下の結合様式に基づく DNA インターカレーション作用により惹起されると考えた。すなわち、(1) インダゾール環から中央ピリドン環にわたる平面性の高い部分構造と、DNA の塩基対との π–π 相互作用、ならびに (2) LHS 上末端アリール基と DNA との付加的な π–π 相互作用である (Figure 26)。



66a

Figure 26. Hypothesized binding mode of compound **66a** to DNA.

ところで、Albertini らは $5HT_{2c}$ アゴニストの創薬研究において、インデノピロール誘導体およびインデノピラゾール誘導体の母核のジェミナル位へジメチル基を導入して平面構造を回避すると、TA1537 株における変異原性リスク回避に効果的であることを報告している [52b]。

上述の化合物 **66a** と DNA との予想結合様式、および平面構造回避による TA1537 株における変異原性リスク回避の報告例を踏まえ、下記に示す DNA インターカレーション回避の戦略を立てた。

(1)　末端アリール部位の電子密度の低下。

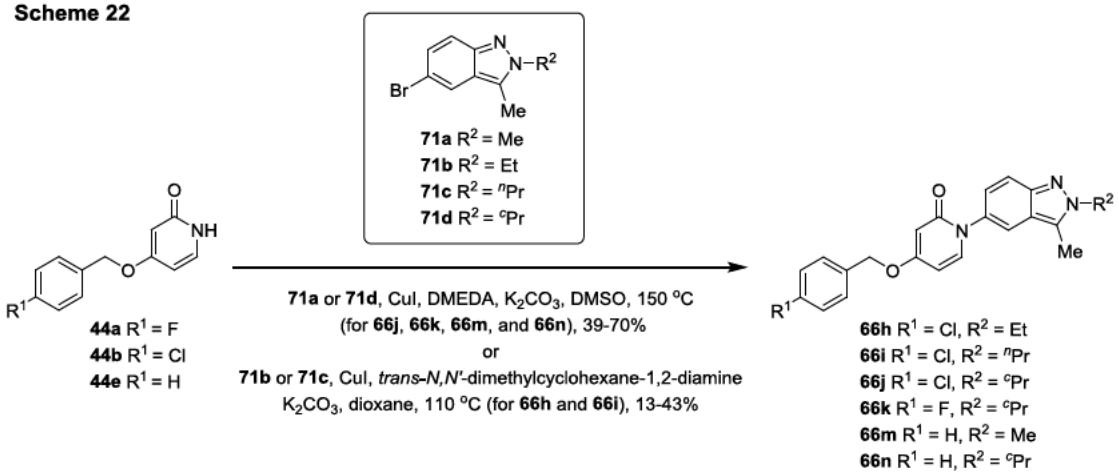
(2)　インダゾール環への立体的に嵩高い置換基導入による平面性構造の回避。

本節では、上述の TA1537 株における遺伝毒性リスク回避の戦略の有効性について論じる。

第３項　合成

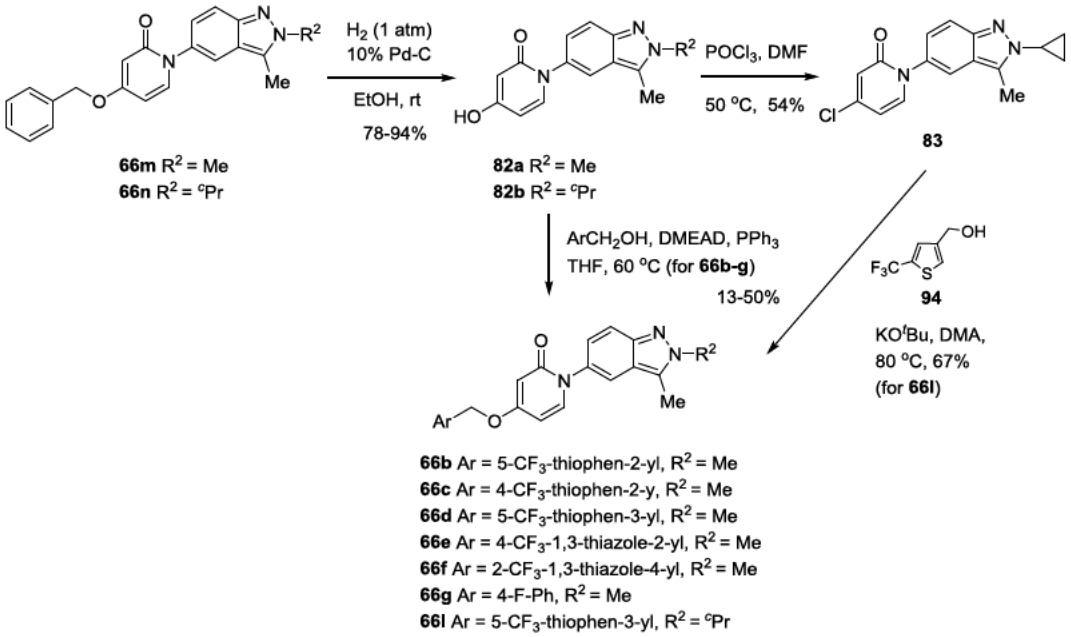
4-アルコキシピリドン誘導体 **66** は、第２章・第４節で示したヨウ化銅 (I) を用いたカップリング反応により、共通中間体 **44a**、**44b** および **44e** より合成した (Scheme 22)。

Scheme 22



4 位の効率的構造変換を目的とした 4-アルコキシピリドン誘導体の別途合成は、Scheme 23 に示す前章第３節と同様の手法により実施した。

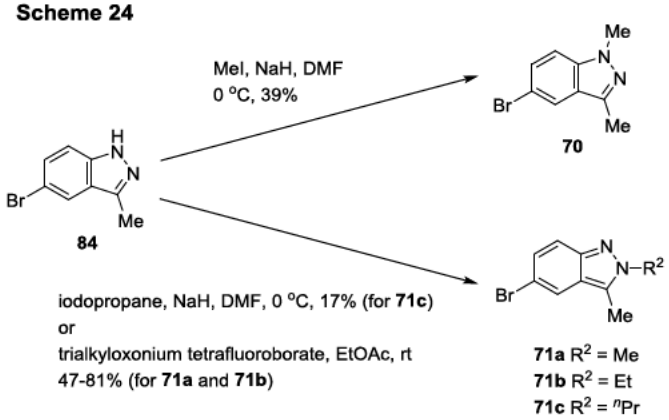
Scheme 23



インダゾール 5 位臭素置換体 **70** および **71a–c** の合成法を Scheme 24 に示した。市販のインダゾール **84** への塩基性条件下でのアルキル化反応は概ね 1 位選択的に進行し

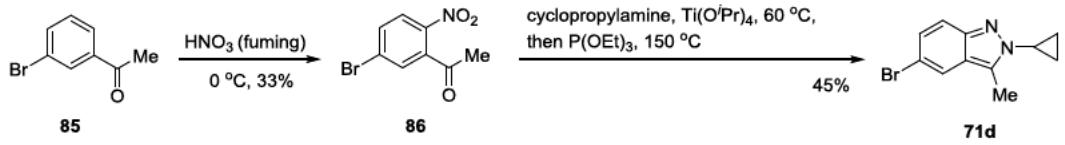
(化合物 **70**)、2 位アルキル化体の合成は低収率に留まった (化合物 **71c**)。そこで 2 位選択的なアルキル化反応を検討した結果、Meerwein 試薬を用いることでアルキル化が 2 位選択的に進行することを見出し、良好な収率で目的物 **71a** および **71b** を得ることができた。しかし、本手法で導入できる置換基は入手可能な Meerwein 試薬に依存し、限定される為、引き続き、インダゾール環 2 位への選択的な置換基導入法の検討を継続した。

Scheme 24



インダゾール環 2 位への置換基導入を可能とする Cadogan 反応 [53] の改良法を Scheme 25 に示した。鍵となる環化前駆体 **86** は市販の原料 **85** より合成した。アセトフェノン **86** に対しチタンテトライソプロポキシドを用いエナミン形成を行った後、亜リン酸トリエチル中 150 度で加熱することにより、系中で生じたナイトレン種の NH 基への挿入反応を経て目的とする 2-シクロプロピルインダゾール **71d** を得た。シクロプロピル基等の嵩高い置換基を 2,3-置換-2*H*-インダゾール環の 2 位へ導入する手法、およびエナミンに対する Cadogan 反応はこれまでに報告が無い。今回筆者の見出した、エナミン形成ーナイトレン種の挿入反応を経るワンポット Cadogan 反応改良法は、インダゾール 2 位置換体の新規合成法として有用と考えられる。

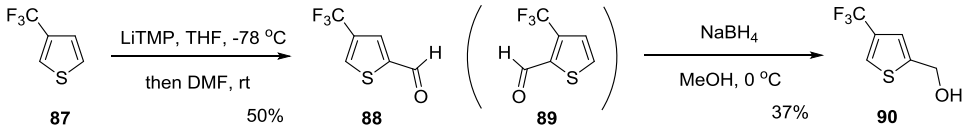
Scheme 25



チオフェンメタノール CF_3 置換体 **90** および **94** は Scheme 26 ならびに 27 に示した手法により合成した。3-トリフルオロメチルチオフェン (**87**) に対するホルミル化は非選択的に進行し、ホルミル体 **88** および **89** が 3:2 の比率で生成した (Scheme 26)。続く水素化ホウ素ナトリウムによる還元、シリカゲルクロマトグラフィーによる精製を経て目的物 **90** を得た。また、ヨードチオフェン **92** に対してトリフルオロメチル基を導入する事も可能である (Scheme 27)。すなわち、ヨウ化銅 (I) と methyl 2,2-difluoro-2-(fluorosulfonyl)acetate から発生させた CF_3–Cu 種をヨードチオフェン **92** と

反応させることで化合物 **93** を得 [54]、続く還元反応により目的物 **94** を得た。

Scheme 26



Scheme 27

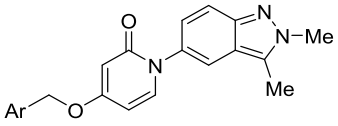
第４項　生物活性と考察

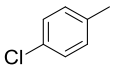
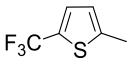
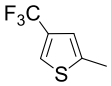
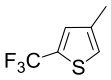
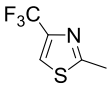
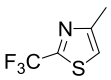
本項での検討では、これまでの SAR 情報に基づき、強力な in vitro 活性が期待できる置換基に検討範囲を限定した。まず、第２項で論じた DNA インターカレーション作用回避を指向した第一の戦略に基づき、末端アリール基をトリフルオロメチル基もしくはフッ素原子が置換した電子不足系芳香環へと置換した (Table 16)。

前章における最適化研究で見出したチオフェン環を導入した結果 (**66b–d**)、2,4-置換チオフェン誘導体 **66c** および **66d** が IC_{50} 値 10^{-8} M オーダーの良好な活性を示したのに対して、2,5-置換チオフェン誘導体 **66b** は活性が低かった。次に、電子密度が低下したチアゾール誘導体 **66e** および **66f** を評価したところ、いずれも対応するチオフェン誘導体と比較して活性が弱かった (**66c** vs **66e**、**66d** vs **66f**)。ここで化合物 **66d** を選択し、TA1537 株を用いた Ames 試験に供したところ、313 μg/plate から 2 倍の変異復帰コロニーの増加が認められ、陽性であることが確認された。以上の結果から、第一の戦略に基づく末端アリール基の電子密度低減は、活性の低下を招き、また化合物 **66d** も依然 Ames 陽性反応を示したことから、変異原性リスク回避の有効な手段ではないと判断した。

興味深いことに、4-フッ素置換ベンゼン誘導体 **66g** は 5000 μg/plate の濃度まで変異原性リスクを示さず、Ames 陰性であった。詳細な理由は不明であるが、フッ素原子の強力な電子求引性誘起効果によるベンゼン環の電子密度低下の他、Ellis らの報告にある様に、分子長が短くなることによる末端アリール部分と DNA 主鎖との相互作用減弱が要因と推測される [56]。

Table 16. Biological activities of compounds **66a–g**



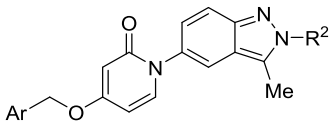
Compound	Ar	IC_{50} (nM)[a]		Ames (TA1537, S9−)[55]
		hMCHR1[b]	rMCHR1[c]	
66a		35	36	positive[d]
66b		170	90	NT[e]
66c		74	38	NT
66d		90	70	positive
66e		130	76	NT
66f		230	130	NT
66g		110	76	negative

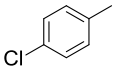
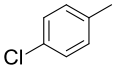
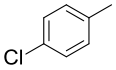
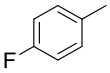
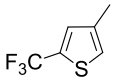
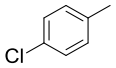
[a] IC_{50} values were calculated using an experiment performed in duplicate, with a standard deviation of three-fold. [b] Binding affinity for human MCHR1. [c] Binding affinity for rat MCHR1. [d] >Two-fold increase of the revertant colony compared with vehicle control. [e] Not tested.

続いて第二の戦略に基づき、インダゾール環 2 位の置換基変換を実施した (Table 17)。化合物 **66a** のメチル基を、エチル基、*n*-プロピル基と変換するに従い、鎖長依存的な活性低下が認めらた (**66h** および **66i**)。次に、2-シクロプロピルインダゾール誘導体 (**66j–l**) を設計し、その活性および変異原性に与える効果を検証した。計算上シクロプロピル基は、インダゾール環の平面より 42–68º 立ち上がっていることが示されており、分子の平面性低下に効果的と考えられた (Figure 27)。さらに、第二章、第三章における検討からも、シクロプロピル基の導入は活性向上にも効果的なことが期待された。そこで、前項で論じた 2*H*-インダゾール環の新規合成経路に従い、化合物 **66j–l** を合成、評価に供した。2-シクロプロピル誘導体 **66j–l** は対応するメチル体と比較して強力な MCHR1 結合活性を示し (**66a** vs **66j**、**66g** vs **66k**、**66d** vs **66l**)、第２章のイミダゾピリジン誘導体、第３章のベンズイミダゾール誘導体に続きシクロプロピル基の有効性が示された。さらに、これらの化合

物は TA1537 株を用いた Ames 試験において、5000 μg/plate まで変異原性リスクを示さず、陰性であった。

Table 17. In vitro binding affinities and results of the Ames test of compounds **66a** and **66h–l**



Compound	Ar	R^2	IC_{50} (nM)[a]		Ames (TA1537, S9−)[55]
			hMCHR1[b]	rMCHR1[c]	
66h		Et	140	65	NT[d]
66i		^{n}Pr	210	130	NT
66j		^{c}Pr	31	23	negative
66k		^{c}Pr	43	35	negative
66l		^{c}Pr	38	26	negative
66a		Me	35	36	positive[e]

[a] IC_{50} values were calculated using an experiment performed in duplicate with a three-fold standard deviation. [b] Binding affinity for human MCHR1. [c] Binding affinity for rat MCHR1. [d] Not tested. [e] >Two-fold increase of the revertant colony compared with vehicle control.

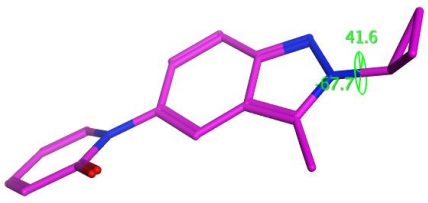


Figure 27. The lowest energy conformers of **66l′** calculated using MOE[31] (for the calculation cost, the structure was simplified).

本項で論じた結果により、分子の平面性を低下させ、DNA インターカレーションを回避することが、TA1537 株における変異原性リスク軽減に有効なことが証明された。また、

同目的ではインダゾール環 2 位へのシクロプロピル基導入が効果的であることを発見した。

第３節　インダゾール誘導体 **66l** の薬理作用

上記の検討から、強力な MCHR1 結合活性を示し、かつ TA1537 株における変異原性リスクを回避した化合物 **66l** および **66j** を in vivo における薬効試験に供した。これらは CHO 細胞を用いた細胞系試験において良好な MCHR1 拮抗活性を示し (**66j**: IC_{50} = 33 nM、**66l**: IC_{50} = 79 nM) *)、ラットにおいて良好な経口吸収性と血中暴露を示した (Table 18)。これら 2 化合物のうち、DIO F344 ラット における二日間摂食抑制確認試験においてより強力な摂食抑制作用 (**66j**: 7.8% at 3 mg/kg, 5.6% at 10 mg/kg, **66l**: 21.5% at 3 mg/kg, 30.6% at 10 mg/kg) を示した化合物 **66l** を続く連続投与試験用化合物として選択した。

Table 18. Pharmacokinetic parameters of **66j** and **66l** in rats[a]

Compound		iv (0.1 mg/kg)		po (1 mg/kg)		
	F[b] (%)	CL_{total}[c] ($mL \cdot h^{-1} \cdot kg^{-1}$)	V_{ss}[d] ($mL \cdot kg^{-1}$)	C_{max}[e] ($ng \cdot mL^{-1}$)	T_{max}[f] (h)	$AUC_{0-8\ h}$[g] ($ng \cdot h \cdot mL^{-1}$)
66j	37	207	676	291	4.0	1813
66l	78	296	949	426	2.7	2879

[a] n = 3; SD rats (male, eight weeks old). [b] Bioavailability. [c] Total clearance. [d] Volume of distribution at steady state. [e] Maximal plasma concentration. [f] Time of maximal concentration. [g] Area under the plasma concentration–time curve (0–8 h).

化合物 **66l** の抗肥満作用を DIO F344 ラットにおける二週間連続投与試験において評価した (Figure 28)。化合物 **66l** (5 および 10 mg/kg) を一日一回、二週間経口投与したところ、有意かつ用量依存的な体重低下作用が 5 mg/kg 投与群から確認され、vehicle 群に対して 5 mg/kg 投与群で 3.9%、10 mg/kg 投与群で 7.6% の体重低下が認められた。また、その時の摂餌量は vehicle 群と比較し、それぞれ 12.2% および 23.2% 減少していた。化合物 **66l** の 10 mg/kg 投与群の血漿中薬物濃度のトラフ値は 3.36 μM であり、DIO F344 ラットにおける化合物 **66l** の非結合型分率は 0.022 であることから、フリー体換算で 73.9 nM と算出される。この結果は、化合物 **66l** の 10 mg/kg 投与群では IC_{50} 値の 2.8 倍の血漿中フリー体濃度が試験を通じて担保されており、これが 7.6% の体重低下に必要な薬物濃度と考えられる。また、化合物 **66l** の脳/血中濃度比は 0.66 であることから、十分な BBB 透過性を有することが明らかとなった。さらに、化合物 **66l** は正常マウスでは

*) 化合物 **66j** の MCHR2 に対する拮抗活性は IC_{50} > 10 μM であり、MCHR1 選択的であった。

摂食抑制作用を示したのに対し、MCHR1 欠損マウスにおいては作用が認められなかった。よって、上述の摂食抑制作用および体重低下作用は MCHR1 拮抗作用を介する効果と考えられる。

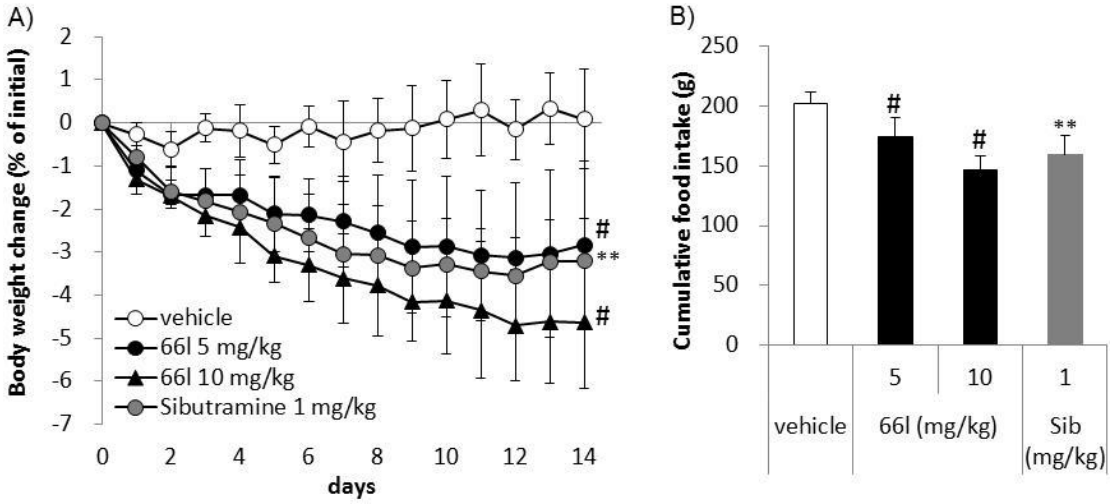


Figure 28. Effects of **66l** in a repeated-dose study in DIO F344 rats. A) Body weight change from the initial value during two weeks of dosing. B) Cumulative food intake for two weeks of dosing. The compounds were administered once daily for two weeks and the body weight and food intake were measured before drug administration. Each data point represents mean ± SD (n = 6 for each group). (#) $p < 0.025$ vs. the vehicle group (Williams test), (**) $p < 0.01$ vs. the vehicle group (Student's t-test). Sib = Sibutramine.

第４節　小括

ターゲット選択性および安全性の向上が期待できる中性 MCHR1 拮抗薬の探索を行った結果、良好な in vitro 活性を有するインダゾール誘導体を見出した。初期のリード化合物 **66a** は TA1537 株を用いた Ames 試験において陽性であったが、DNA との予想結合様式に基づき、変異原性リスクの回避を試みた結果、インダゾール環へシクロプロピル基を導入することによる平面性の低下により、TA1537 株における変異原性を回避できることが明らかとなった。これらの結果は、既知の DNA インターカレーターとしては多環性の塩基性化合物が多数を占める中、2 環性骨格から成る中性分子も DNA と相互作用し得ることを示しており、今後の創薬における重要な注意喚起となると考えられる。

強力な in vitro 活性を有し、Ames 試験における変異原性リスクが回避された化合物 **66l** は 10 μM において主要 CYP アイソフォーム (1A2/2C8/2C9/2D6/3A4) に対する可逆的阻害作用を示さず、また CYP3A4 TDI 作用、PLsis 惹起リスクおよび hERG 阻害作用を示さなかった。さらに、化合物 **66l** は DIO F344 ラットにおいて強力な体重低下作用を

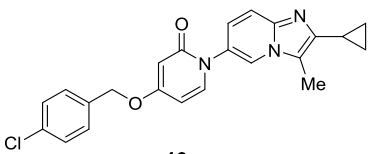
示したことから、中性 MCHR1 拮抗薬として独自性の高い薬剤候補化合物となり得ると考えられる。

第５章　結語

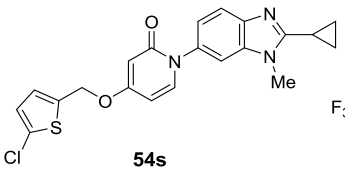
抗肥満薬の創製を指向した MCHR1 拮抗薬の分子設計と合成を実施した。既報の MCHR1 拮抗薬と比較し、より安全性の高い化合物の創出を目的とし、強塩基性のアルキルアミン部位を有さない新規非アミン性 MCHR1 拮抗薬を設計した。リード化合物創出の戦略として、アルキルアミンを用いない構造的制約に加え、塩基性の低減 ($pK_a < 8$) と中枢薬を指向した物性パラメータ (PSA < 70、2 < ClogP < 4、MW < 450 および HBD 数 = 0 もしくは 1) を指標として薬物設計することで、良好な MCHR1 結合活性を有し、in vitro 評価において hERG 阻害作用および PLsis 惹起リスクを示さない新規イミダゾピリジン誘導体を見出す事に成功した。また、続く二環性縮合環の構造変換によって、塩基性が低減されたベンズイミダゾール誘導体、さらには中性分子であるインダゾール誘導体を設計し、それぞれより in vitro における十分な安全性と肥満モデルラットにおける強力な抗肥満作用を有する薬剤候補化合物 **54s** および **66l** を見出した。

また、ベンズイミダゾール誘導体の合成研究においては、チオフェン環の代謝活性化の機構を解析することで、チオフェン環 2, 5 位への置換基導入、もしくは立体的に嵩高く電子求引性のトリフルオロメチル基の導入により CYP3A4 TDI 作用の回避に成功した。さらに、インダゾール誘導体の合成研究では、Ames 試験において TA1537 株で認められた遺伝毒性リスクが、化合物の平面性に基づく DNA インターカレーションに起因すると考え、平面性を低下させる部分構造を導入する事で遺伝毒性リスクを回避できることを見出した。

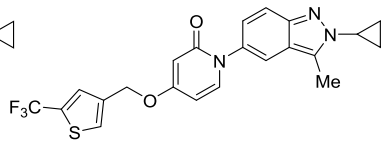
以上、MCHR1 拮抗薬の研究により、有力な抗肥満薬となり得る複数の化合物を見いだした。本論文で示したリード化合物創出に関する方法論や、チオフェン環の CYP3A4 TDI 作用回避の戦略、および TA1537 株における遺伝毒性リスク回避の戦略等の新たな知見は、今後の創薬研究において非常に有用である。



10a



54s



66l

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Experimental section

General. Melting points were determined on a Yanaco melting point apparatus Mp-500D and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III (300 MHz) or a Bruker Advance III plus (400 MHz) spectrometer. Chemical shifts are given in parts per million (ppm) downfield from tetramethysilane (δ) as the internal standard in deuterated solvent, and coupling constants (J) are in Hertz (Hz). Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, dd = doublet of doublets, td = triplet of doublets, and bs = broad signal), and coupling constants. Protons of the methyl group on the 3 position of the imidazopyridine ring were frequently merged with solvent peak of DMSO, and not observed. Reagents and solvents were obtained from commercial sources and used without further purification. Reaction progress was determined by thin layer chromatography (TLC) analysis on Merck Kieselgel 60 F254 plates or Fuji Silysia NH plates. Chromatographic purification was performed on silica gel columns [(Merck Kieselgel 60, 70–230 mesh size or 230–400 mesh size, Merck) or (Chromatorex NH-DM 1020, 100–200 mesh size)] or on Purif-Pack (SI or NH, particle size: 60 μm, Fuji Silysia Chemical, Ltd.). LC−MS analysis was performed on a Shimadzu Liquid Chromatography–Mass Spectrometer System, operating in APCI (+ or −) or ESI (+ or −) ionization mode. Analytes were eluted using a linear gradient of 0.05% TFA containing water/acetonitrile or 5 mM ammonium acetate containing water/acetonitrile mobile phase and detected at 220 nm. Analytical HPLC was performed with Corona CAD (Charged Aerosol Detector) or photo diode array detector. The column was a Capcell Pak C18AQ (50 mm × 3.0 mm I.D., Shiseido, Japan) or L-column 2 ODS (30 mm × 2.0 mm I.D., CERI, Japan) with a temperature of 50 °C and a flow rate of 0.5 mL/min. Mobile phase A and B under a neutral condition were a mixture of 50 mmol/L Ammonium acetate, water and acetonitrile (1:8:1, v/v/v) and a mixture of 50 mmol/L ammonium acetate and acetonitrile (1:9, v/v), respectively. The ratio of mobile phase B was increased linearly from 5% to 95% over 3 min, 95% over the next 1 min. Mobile phase A and B under an acidic condition were a mixture of 0.2% formic acid in 10 mmol/L ammonium formate and 0.2% formic acid in acetonitrile, respectively. The ratio of mobile phase B was increased linearly from 14% to 86% over 3 min, 86% over the next 1 min. The purities of compounds submitted for biological evaluation were >95% as determined by elemental analyses within ±0.4% of the calculated values or analytical HPLC. Yields are not optimized.

Experiments concerning Chapter 2

2,3-Dimethylimidazo[1,2-*a*]pyridin-6-amine. To a solution of **9b** (1.05 g, 3.85 mmol), cesium carbonate (2.51 g, 7.70 mmol), Xantphos (156 mg, 0.27 mmol) and $Pd_2(dba)_3$ (106 mg, 0.12 mmol) in DMF (12 mL) was added benzophenone imine (0.71 mL, 4.24 mmol) at ambient temperature. The mixture was stirred at 80 °C under Ar atmosphere overnight. The mixture was poured into water and extracted with EtOAc–THF. The organic layer was separated, washed with brine twice, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 80/20 to 20/80) to give the title compound (448 mg, 72%) as a dark yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.25 (3H, s), 4.76 (2H, br s), 6.73 (1H, dd, *J* = 9.5, 1.9 Hz), 7.17 (1H, d, *J* = 9.5 Hz), 7.29 (1H, d, *J* = 1.9 Hz). MS (ESI/APCI) *m/z* 162.0 $[M + H]^+$.

2-Cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-amine. A mixture of **9a** (900 mg, 3.02 mmol), diphenylmethanimine (602 mg, 3.32 mmol), cesium carbonate (1967 mg, 6.04 mmol), $Pd_2(dba)_3$ (41.5 mg, 0.05 mmol), Xantphos (52.4 mg, 0.09 mmol), and DMF (10 mL) was heated at 80 °C under Ar atmosphere for 5 h. The mixture was poured into water, and extracted with EtOAc. The mixture was washed with brine, dried over $MgSO_4$ and concentrated. The residue was dissolved in THF (5 mL), and treated with 3 N HCl (5 mL) at rt for 10 min. Then the mixture was poured into sat. $NaHCO_3$ solution, and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, concentrated, and purified by column chromatography (NH silica gel, hexane/EtOAc = 80/20 to 20/80) to give the title compound (360 mg, 64%) as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.72–0.90 (4H, m), 1.90–2.02 (1H, m), 2.33 (3H, s), 4.76 (2H, s), 6.71 (1H, dd, *J* = 9.3, 2.1 Hz), 7.15 (1H, d, *J* = 9.4 Hz), 7.28 (1H, d, *J* = 1.5 Hz). MS (ESI/APCI) *m/z* 188.2 $[M + H]^+$.

2-Cyclopropyl-3-methylindolizin-6-amine hydrochloride. 6-Methylpyridin-3-amine (3.2 g, 29.6 mmol) was dissolved in THF (50 mL) and a solution of sodium hexamethyldisilazane (1.9 M THF solution, 34.3 mL, 65.10 mmol) was added dropwise at rt with stirring over 15 min. After stirring for 10 min, a solution of di-*tert*-butyl dicarbonate (9.62 mL, 41.4 mmol) in THF (15 mL) was added dropwise over 10 min. After stirring for 3 h, the reaction solution was extracted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 95/5 to 70/30) to give di-*tert*-butyl (6-methylpyridin-3-yl)imidodicarbonate (4.0 g, 44%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (18H, s), 2.57 (3H, s), 7.15 (1H, d, *J* = 7.9 Hz), 7.36 (1H, dd, *J* = 7.9, 2.6 Hz), 8.29 (1H, d, *J* = 2.6 Hz). MS (ESI/APCI) *m/z* 309.2 $[M + H]^+$.

A mixture of di-*tert*-butyl (6-methylpyridin-3-yl)imidodicarbonate (1.3 g, 4.22 mmol), 2-bromo-1-cyclopropylpropan-1-one (**50,** 1.12 g, 6.32 mmol) and sodium bicarbonate (0.850 g,

10.1 mmol) in 2-butanone (14 mL) was stirred under reflux overnight. The mixture was quenched with water at rt and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 90/10) to give di-*tert*-butyl (2-cyclopropyl-3-methylindolizin-6-yl)imidodicarbonate (812 mg, 50%) as a green solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.58–0.69 (2H, m), 0.91–0.98 (2H, m), 1.45 (18H, s), 1.82–1.96 (1H, m), 2.45 (3H, s), 6.02 (1H, s), 6.42 (1H, dd, *J* = 9.4, 1.5 Hz), 7.21 (1H, d, *J* = 9.4 Hz), 7.51 (1H, s). MS (ESI/APCI) *m/z* 387.2 $[M + H]^+$.

A mixture of di-*tert*-butyl (2-cyclopropyl-3-methylindolizin-6-yl)imidodicarbonate (400 mg, 1.03 mmol) and 4 N HCl in EtOAc (2.6 mL, 10.4 mmol) in EtOAc (1 mL) was stirred at rt for 2 h. The insoluble material was collected and washed with EtOAc to give the title compound (110 mg, 48%) as a brown solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.52–0.66 (2H, m), 0.87–1.00 (2H, m), 1.87–1.97 (1H, m), 2.44 (3H, s), 6.12 (1H, s), 6.59 (1H, dd, *J* = 9.4, 1.5 Hz), 7.39 (1H, d, *J* = 9.4 Hz), 8.02 (1H, s), 10.14 (3H, br s). MS (ESI/APCI) *m/z* 187.1 $[M + H]^+$.

6-Iodo-3-methylimidazo[1,2-*a*]pyridine-2-carboxylic acid. A mixture of 2-oxo-butyric acid (10.0 g, 98.0 mmol), EtOH (110 mL), benzene (50 mL) and *p*-toluenesulfonic acid (200 mg) was heated under reflux for 4 h. The reaction mixture was then cooled to rt and concentrated. The residue was diluted with a mixture of DCM (500 mL) and water (200 mL), and DCM layer was separated. DCM layer was washed with brine, dried over Na_2SO_4, and concentrated to give ethyl 2-oxobutanoate (10 g, 77 %) as colorless liquid that was used for the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.97 (3H, t, *J* = 7.2 Hz), 1.26 (3H, t, *J* = 7.2 Hz), 2.82 (2H, q, *J* = 7.2 Hz), 4.21 (2H, q, *J* = 7.2 Hz).

To a stirred solution of ethyl 2-oxobutanoate (10.0 g, 77 mmol) in a mixture of EtOAc (100 mL) and $CHCl_3$ (50 mL) was added $CuBr_2$ (54 g, 231 mmol), and then the mixture was heated under reflux for 16 h. The reaction mixture was then cooled to rt, filtered through the Celite® pad, and the filtrate was concentrated to afford ethyl 3-bromo-2-oxobutanoate (15 g, 94%) as green oil that was used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (3H, t, *J* = 7.2 Hz), 1.79 (3H, d, *J* = 7.2 Hz), 4.38 (2H, m), 5.15 (1H, q, *J* = 7.2 Hz).

To ethyl 3-bromo-2-oxobutanoate (16 g, 76.5 mmol) and 5-iodo-pyridin-2-ylamine (8.42 g, 38.25 mmol) was added EtOH (100 mL), and the mixture was heated under reflux for 16 h. The reaction mixture was cooled to rt and concentrated. The resulting residue was diluted with DCM (300 mL), and the DCM layer was washed with water and brine, successively. The DCM layer was dried over Na_2SO_4, concentrated, purified by column chromatography (silica gel, hexane/EtOAc = 75/25) to afford ethyl 6-iodo-3-methylimidazo[1,2-*a*]pyridine-2-carboxylate (4.0 g, 32%) as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (3H, t, *J* = 7.2 Hz), 2.77 (3H, s), 4.45 (2H, q, *J* = 7.2 Hz), 7.37

(1H, dd, *J* = 9.6, 1.2 Hz), 7.44 (1H, d, *J* = 9.2 Hz), 8.16 (1H, s). MS (ESI/APCI) *m/z* 331.0 $[M + H]^+$.

To a stirred solution of ethyl 6-iodo-3-methylimidazo[1,2-*a*]pyridine-2-carboxylate (1.5 g, 4.54 mmol) in a mixture of THF (10 mL) and H_2O (10 mL) was added $LiOH \cdot H_2O$ (381 mg, 9.0 mmol), and the resulting mixture was stirred at rt for 16 h. The mixture was concentrated, and the residue was washed with EtOAc (10 mL) and then diluted with H_2O (10 mL). The aqueous layer was neutralized with 2 N HCl solution. The mixture was extracted with EtOAc, and the organic layer was dried over Na_2SO_4 and concentrated to give the title compound (1.0 g, 73%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.77 (3H, s), 7.60 (1H, d, *J* = 9.4 Hz), 7.99 (1H, d, *J* = 9.3 Hz), 9.02 (1H, s). MS (ESI/APCI) *m/z* 302.9 $[M + H]^+$.

***N*-(2,3-Dimethylimidazo[1,2-*a*]pyridin-6-yl)-2-[4-(trifluoromethoxy)phenoxy]acetamide (6a).** A mixture of **5** (146 mg, 0.62 mmol), 2-methyl-3-methylimidazo[1,2-*a*]pyridin-6-amine (100 mg, 0.62 mmol), WSC (238 mg, 12.4 mmol), HOBt (16.8 mg, 0.12 mmol) and DMF (4 mL) was stirred at rt overnight. The mixture was poured into water, and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, concentrated, and purified by column chromatography (NH silica gel, hexane/EtOAc = 90/10 to 50/50) followed by recrystallization from EtOAc–hexane to give the title compound **6a** (89.2 mg, 38%) as an off-white solid; mp 202–204 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (3H, s), 2.34 (3H, s), 4.79 (2H, s), 7.08–7.16 (2H, m), 7.22 (1H, dd, *J* = 9.4, 1.9 Hz), 7.34 (2H, d, *J* = 8.3 Hz), 7.38–7.49 (1H, m), 8.72 (1H, d, *J* = 1.1 Hz), 10.23 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.9, 13.1, 67.3, 114.0, 115.6, 116.0, 116.1, 118.5, 120.1 (q, *J* = 256.5 Hz), 122.4, 124.9, 139.3, 140.7, 142.2, 156.6, 166.4. MS (ESI/APCI) *m/z* 380.1 $[M + H]^+$. Anal. Calcd. for $C_{18}H_{16}F_3N_3O_3$: C, 56.99; H, 4.25; N, 11.08. Found: C, 56.92; H, 4.26; N, 11.03.

***N*-(2-Cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)-2-[4-(trifluoromethoxy)phenoxy]acetamide (6b).** The title compound was prepared in 63% yield using 2-cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-amine in an analogous manner to **6a**. White solid; mp 158 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.82–0.92 (4H, m), 1.97–2.11 (1H, m), 2.42 (3H, s), 4.78 (2H, s), 7.12 (2H, d, *J* = 9.4 Hz), 7.20 (1H, dd, *J* = 9.4, 1.9 Hz), 7.31–7.43 (3H, m), 8.72 (1H, s), 10.22 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 7.9, 8.0, 67.2, 113.7, 115.6, 115.9, 116.0, 118.4, 120.6 (q, *J* = 253.5 Hz), 122.5, 124.9, 140.8, 142.2, 144.4, 156.6, 166.4. MS (ESI/APCI) *m/z* 406.1 $[M + H]^+$. Anal. Calcd. for $C_{20}H_{18}F_3N_3O_3$: C, 59.26; H, 4.48; N, 10.37. Found: C, 59.02; H, 4.49; N, 10.29.

***N*-(2-Cyclopropyl-3-methylindolizin-6-yl)-2-[4-(trifluoromethoxy)phenoxy]acetamide (6c)**. The title compound was prepared in 24% yield using 2-cyclopropyl-3-methylindolizin-6-amine hydrochloride in an analogous manner to **6a**. Off-white solid; mp 168–170 °C (EtOAc–hexane). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.45–0.62 (2H, m), 0.83–0.95 (2H, m), 1.84–1.97 (1H, m), 2.39 (3H, s), 4.75 (2H, s), 5.97 (1H, s), 6.69 (1H, dd, *J* = 9.5, 1.9 Hz), 7.04–7.16 (2H, m), 7.26 (1H, d, *J*

= 9.5 Hz), 7.34 (2H, d, *J* = 8.3 Hz), 8.50 (1H, s), 9.97 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.4, 8.3, 9.2, 67.3, 94.7, 111.7, 112.4, 116.0, 117.6, 117.8, 120.1 (q, *J* = 253.5 Hz), 122.5, 123.4, 128.57, 128.61, 142.1, 142.2, 156.7, 166.1. MS (ESI/APCI) *m/z* 405.2 $[M + H]^+$. Anal. Calcd. for $C_{21}H_{19}F_3N_2O_3$: C, 62.37; H, 4.74; N, 6.93. Found: C, 62.24; H, 4.71; N, 6.92.

***N*-(2,3-Dimethyl-1-benzofuran-5-yl)-2-[4-(trifluoromethoxy)phenoxy]acetamide (6d).** The title compound was prepared in 62% yield using 2,3-dimethyl-1-benzofuran-5-amine in an analogous manner to **6a**; mp 156–159 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.09 (3H, s), 2.35 (3H, s), 4.74 (2H, s), 7.01 (2H, d, *J* = 9.0 Hz), 7.32–7.39 (4H, m), 7.81 (1H, s), 10.08 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.5, 11.6, 67.4, 109.6, 109.9, 110.1, 116.0, 116.1, 120.1 (q, *J* = 253.5 Hz), 122.5, 129.9, 133.2, 142.11, 142.13, 149.8, 151.3, 156.7, 165.9. MS (ESI/APCI) *m/z* 380.2 $[M + H]^+$. Anal. Calcd. for $C_{19}H_{16}F_3NO_4$: C, 60.16; H, 4.25; N, 3.69. Found: C, 59.89; H, 4.26; N, 3.69.

3-[(4-Chlorobenzyl)oxy]pyridin-2-ol (8). To a stirred solution of KOH (0.360 g, 9.00 mmol) in MeOH (10 mL) was added **7** (1.00 g, 9.00 mmol) portionwise. To the resulting red solution was added 4-chlorobenzyl bromide (1.85 g, 9.00 mmol), and the reaction mixture was stirred at rt for 30 min, then at 40 °C for 1.5 h. The mixture was concentrated, and partitioned between EtOAc, THF and water. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The precipitate was collected by filtration, and washed with IPE to give the title compound (1.69 g, 80%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.12 (2H, s), 6.14 (1H, d, *J* = 7.0 Hz), 6.74 (1H, d, *J* = 7.3 Hz), 7.02 (1H, d, *J* = 6.3 Hz), 7.31–7.40 (4H, m), 12.36–12.56 (1H, m). MS (ESI/APCI) *m/z* 236.0 $[M + H]^+$.

2-Cyclopropyl-6-iodo-3-methylimidazo[1,2-*a*]pyridine (9a). A mixture of 2-bromo-1-cyclopropylpropan-1-one (**50**, 1.00 g, 5.65 mmol), 5-iodopyridin-2-amine (1.24 g, 5.65 mmol) and EtOH (10 mL) was heated at 70 °C for 48 h. The mixture was poured into sat. $NaHCO_3$ solution, and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, concentrated, and purified by column chromatography (silica gel, hexane/EtOAc = 95/5 to 65/45) to give the title compound (520 mg, 31%) as a pale orange solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.81–0.94 (4H, m), 1.96–2.11 (1H, m), 2.46 (3H, s), 7.21–7.32 (2H, m), 8.44 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.3, 8.4, 8.5, 75.3, 116.3, 117.5, 128.6, 130.6, 142.1, 144.9. MS (ESI/APCI) *m/z* 299.0 $[M + H]^+$.

2-Methyl-6-iodo-3-methylimidazo[1,2-*a*]pyridine (9b). To a solution of 5-iodopyridin-2-amine (2.50 g, 11.4 mmol) in DMF (20 mL) was added 3-bromo-2-butanone (1.19 mL, 11.4 mmol), and the mixture was stirred at 90 °C overnight. The mixture was neutralized with sat. $NaHCO_3$ solution, and extracted with EtOAc/THF (1:1). The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 75/25 to 0/100) to give the title compound (980 mg, 32%) as a dark yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (3H, s), 2.38 (3H, s), 7.20–

7.39 (2H, m), 8.46 (1H, s). MS (ESI/APCI) *m/z* 272.9 $[M + H]^+$.

2-Ethyl-6-iodo-3-methylimidazo[1,2-*a*]pyridine (9c). The title compound was prepared in 31% yield using 2-bromopentan-3-one in an analogous manner to **9a**. Yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.19 (3H, t, *J* = 7.2 Hz), 2.38 (3H, s), 2.65 (2H, q, *J* = 7.2 Hz), 7.27–7.33 (2H, m), 8.47 (1H, s). MS (ESI/APCI) *m/z* 286.8 $[M + H]^+$.

6-Iodo-3-methylimidazo[1,2-*a*]pyridine-2-carbonitrile (9d). To a stirred solution of 6-iodo-3-methylimidazo[1,2-*a*]pyridine-2-carboxylic acid (1.0 g, 3.3 mmol) in DMF (15 mL) were added HATU (1.88 g, 4.92 mmol) and Et_3N (1.38 mL, 9.9 mmol) at 0 °C. The mixture was allowed to warm to rt for 30 min, and then NH_4Cl (725 mg, 13.5 mmol) was added. The resultant mixture was stirred at the same temperature for 18 h. The mixture was then concentrated, and the residue was diluted with DCM (100 mL), and washed with sat. NH_4Cl solution (40 mL), sat. $NaHCO_3$ solution (20 mL), water (30 mL) and brine (50 mL) successively. DCM layer was then dried over Na_2SO_4, and concentrated to give 6-iodo-3-methylimidazo[1,2-*a*]pyridine-2-carboxamide (600 mg, 60%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.73 (3H, s), 7.33 (1H, br s), 7.39 (1H, d, *J* = 9.3 Hz), 7.49 (1H, d, *J* = 9.4 Hz), 7.63 (1H, br s), 8.63 (1H, s). MS (ESI/APCI) *m/z* 301.9 $[M + H]^+$.

To a stirred solution of 6-iodo-3-methylimidazo[1,2-*a*]pyridine-2-carboxamide (600 mg, 1.99 mmol) was added $POCl_3$ (40 mL), and the mixture was heated at reflux for 3 h. The mixture was then cooled to rt, and concentrated. The residue was poured into ice-cold sat. $NaHCO_3$ solution (100 mL). The mixture was extracted with EtOAc (150 mL) twice, and the combined EtOAc layers were washed with water (100 mL) and brine (100 mL), dried over Na_2SO_4 and concentrated to give the title compound (500 mg, 88%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.60 (3H, s), 7.46 (1H, d, *J* = 9.5 Hz), 7.60 (1H, d, *J* = 9.3 Hz), 8.74 (1H, s). MS (ESI/APCI) *m/z* 283.9 $[M + H]^+$.

6-Iodo-*N*,*N*,3-trimethylimidazo[1,2-*a*]pyridine-2-carboxamide (9e). To a stirred solution of 6-iodo-3-methylimidazo[1,2-*a*]pyridine-2-carboxylic acid (150 mg, 0.49 mmol) in DMF (5 mL) were added HATU (283 mg, 0.74 mmol), and DIPEA (0.17 mL, 0.99 mmol) at 0 °C. The mixture was allowed to warm to rt for 30 min, and dimethylamine (2 N THF solution, 0.25 mL, 0.49 mmol) was added. The resultant mixture was stirred at rt for 18 h. The mixture was concentrated, and the residue was diluted with DCM (100 mL), washed with sat. NH_4Cl solution (40 mL), sat. $NaHCO_3$ solution (20 mL), water (30 mL) and brine (50 mL). DCM layer was then dried over Na_2SO_4, and concentrated to give the title compound (150 mg, 93%) as brown oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.52 (3H, s), 2.99 (3H, s), 3.18 (3H, s), 7.41 (1H, d, *J* = 9.3 Hz), 7.46 (1H, dd, *J* = 9.3, 1.2 Hz), 8.62 (1H, s). MS (ESI/APCI) *m/z* 329.8 $[M + H]^+$.

6-Iodo-*N*-methoxy-*N*,3-dimethylimidazo[1,2-*a*]pyridine-2-carboxamide (9f). To a stirred solution of 6-iodo-3-methylimidazo[1,2-*a*]pyridine-2-carboxylic acid (600 mg, 1.98 mmol) in

DMF (10 mL) were added HATU (1.50 g, 3.97 mmol), DIEPA (1.30 mL, 7.94 mmol) and *N*,*O*-dimethylhydroxylamine hydrochloride (385 mg, 3.97 mmol) at 0 °C. The resultant mixture was stirred at rt for 16 h. The mixture was then diluted with water (60 mL), and extracted with EtOAc (100 mL) twice. The organic layers were successively washed with sat. $NaHCO_3$ solution (50 mL), water (50 mL), and brine (50 mL). The EtOAc layer was then dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (silica gel, hexane/EtOAc = 80/20) to give the title compound (600 mg, 88%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.56 (3H, s), 3.39 (3H, s), 3.73 (3H, s), 7.42 (1H, d, *J* = 9.4 Hz), 7.48 (1H, d, *J* = 9.4 Hz), 8.64 (1H, s). MS (ESI/APCI) *m/z* 345.6 $[M + H]^+$.

(6-Iodo-3-methylimidazo[1,2-*a*]pyridin-2-yl)methanol (9g). To a stirred solution of ethyl 6-iodo-3-methylimidazo[1,2-*a*]pyridine-2-carboxylate (2.0 g, 6.06 mmol) in DCM (20 mL) was added DIBAL-H (1.76 M toluene solution, 7.72 mL, 13.6 mmol) at −19 °C . The resultant mixture was stirred at the same temperature for 3 h, and then at rt for 4 h. The reaction mixture was quenched with MeOH and water (2 mL) at −40 °C. The mixture was acidified with a few drops of 5 N HCl solution, and pouted into sat. $NaHCO_3$ (20 mL) solution. The mixture was extracted with EtOAc (150 mL) three times, and the combined EtOAc layers were washed with brine (50 mL), dried over Na_2SO_4, and concentrated. The crude residue was purified by column chromatography (silica gel, DCM/MeOH = 98/2) to give the title compound (1.1 g, 62%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.45 (3H, s), 4.55 (2H, s), 5.03 (1H, br s), 7.31–7.39 (2H, m), 8.53 (1H, s). MS (ESI/APCI) *m/z* 288.9 $[M + H]^+$.

6-Iodo-2-(methoxymethyl)-3-methylimidazo[1,2-*a*]pyridine (9h). To a stirred solution of **9g** (200 mg, 0.69 mmol) in DCM (2 mL) was added $SOCl_2$ (56 μL, 0.78 mmol) at rt, the mixture was stirred at the same temperature for 4 h. $SOCl_2$ (125 μL, 0.78 mmol) was added, and stirred at rt for further 3 h. The reaction mixture was diluted with DCM (100 mL), washed with sat. $NaHCO_3$ (20 mL), dried over Na_2SO_4, and concentrated to give 2-(chloromethyl)-6-iodo-3-methylimidazo[1,2-*a*]pyridine (150 mg, 24%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.48 (3H, s), 4.88 (2H, s), 7.36 (1H, d, *J* = 9.3 Hz), 7.42 (1H, dd, *J* = 9.3, 1.3 Hz), 8.56 (1H, s). MS (ESI/APCI) *m/z* 306.6 $[M + H]^+$.

To a stirred solution of 2-(chloromethyl)-6-iodo-3-methylimidazo[1,2-*a*]pyridine (150 mg, 0.49 mmol) and MeOH (2.5 mL) was added NaOMe (65 mg, 1.06 mmol), and the resulting mixture was heated at reflux for 3 h. The reaction mixture was then cooled to rt and concentrated. The mixture was poured into water (50 mL), and extracted with EtOAc (50 mL) twice. Combined EtOAc layers were washed with brine (30 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (silica gel, DCM/MeOH = 97/3) to give the title compound (100 mg, 67%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.50 (3H, s), 3.30 (3H, s), 4.49 (2H, s), 7.33–7.38 (2H, m), 8.55 (1H, s). MS (ESI/APCI) *m/z* 303.0 $[M + H]^+$.

4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)pyridin-2(1*H*)-one (10a). Two batches of a mixture of **44b** (1.00 g, 4.24 mmol), **9a** (1.27 g, 4.24 mmol), DMEDA (0.48 mL, 4.24 mmol), CuI (0.81 g, 4.24 mmol), K_2CO_3 (1.76 g, 12.7 mmol), and DMSO (15 mL) were heated at 150 °C for 3 h. The two batches were combined, and poured into 14% NH_3 solution. The mixture was extracted with EtOAc/THF (1:1). The extract was washed with brine, dried over $MgSO_4$, concentrated, and purified by column chromatography (silica gel, hexane/EtOAc = 90/10 to 0/100, then EtOAc/MeOH = 100/0 to 85/15) followed by column chromatography (NH silica gel, hexane/EtOAc = 90/10 to 0/100) to give a crude product (350 mg).

A mixture of **44b** (200 mg, 0.85 mmol), **9a** (253 mg, 0.85 mmol), DMEDA (95.0 μL, 0.85 mmol), CuI (162 mg, 0.85 mmol), K_2CO_3 (352 mg, 2.55 mmol), and DMSO (3 mL) was heated at 110 °C under microwave irradiation for 1 h. Another two batches of a mixture of **44b** (1.00 g, 4.24 mmol), **9a** (1.27 g, 4.24 mmol), DMEDA (0.48 mL, 4.24 mmol), CuI (0.81 g, 4.24 mmol), K_2CO_3 (1.76 mg, 12.7 mmol), and DMSO (13 mL) were heated at 110 °C under microwave irradiation for 1 h. The reaction mixtures were combined, and poured into 14% NH_3 solution. The mixture was extracted with EtOAc/THF (1:1). The extract was washed with brine, dried over $MgSO_4$, concentrated, and purified by column chromatography (silica gel, hexane/EtOAc = 90/10 to 0/100, then EtOAc/MeOH = 100/0 to 85/15) followed by column chromatography (NH silica gel, hexane/EtOAc = 90/10 to 0/100) to give a crude product (950 mg).

These two crude lots were combined, and recrystallized from EtOH–water to give the title compound (1.25 g, 17% in total) as a white solid; mp 230–232 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.84–0.96 (4H, m), 2.01–2.13 (1H, m), 2.47 (3H, s), 5.16 (2H, s), 6.00 (1H, d, *J* = 2.6 Hz), 6.14 (1H, dd, *J* = 7.6, 2.6 Hz), 7.11 (1H, dd, *J* = 9.4, 1.9 Hz), 7.43 (1H, d, *J* = 9.4 Hz), 7.50 (4H, s), 7.66 (1H, d, *J* = 7.6 Hz), 8.36 (1H, d, *J* = 1.1 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 8.0, 8.1, 68.8, 97.7, 100.2, 114.9, 116.6, 121.9, 123.2, 127.2, 128.5, 129.7, 132.8, 134.9, 139.7, 141.9, 145.0, 162.8, 167.0. MS (ESI/APCI) *m/z* 406.1 $[M + H]^+$. Anal. Calcd. for $C_{23}H_{20}ClN_3O_2 \cdot H_2O$: C, 65.17; H, 5.23; N, 9.91. Found: C, 65.09; H, 5.24; N, 9.87.

1-(2-Cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)-4-[4-(trifluoromethoxy)phenoxy]pyridin-2(1*H*)-one (10b). A mixture of **46** (100 mg, 0.29 mmol), 4-(trifluoromethoxy)phenol (103 mg, 0.58 mmol), DMEDA (31.0 μL, 0.29 mmol), K_2CO_3 (120 mg, 0.87 mmol), CuI (55.3 mg, 0.29 mmol), and DMSO (3 mL) was heated at 150 °C for 3 h. The mixture was purified by column chromatography (NH silica gel, hexane/EtOAc = 90/10 to 0/100) to give the title compound (39.6 mg, 31%) as a white solid; mp 227–228 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.85–0.96 (4H, m), 2.08 (1H, br s), 5.58 (1H, d, *J* = 2.4 Hz), 6.26 (1H, dd, *J* = 7.4, 2.6 Hz), 7.13 (1H, d, *J* = 9.5 Hz), 7.35–7.48 (3H, m), 7.53 (2H, d, *J* = 8.7 Hz), 7.82 (1H, d, *J* = 7.5 Hz), 8.41 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 7.97, 8.05, 99.5, 100.6, 115.0, 116.6, 120.0 (q, *J* = 254.3 Hz),122.0, 122.8, 123.0, 123.2, 127.0, 141.2, 141.9, 145.2, 145.6, 151.7, 162.4, 167.1. MS (ESI/APCI) *m/z* 442.3 [M

+ H]$^+$. Anal. Calcd. for $C_{23}H_{18}F_3N_3O_3$: C, 62.58; H, 4.11; N, 9.52. Found: C, 62.46; H, 4.10; N, 9.48.

4-(Benzyloxy)-1-(2-cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)pyridin-2(1*H*)-one (10c). A mixture of **9a** (1.48 g, 4.97 mmol), **44e** (1.00 g, 4.97 mmol), CuI (946 mg, 4.97 mmol), DMEDA (0.534 ml, 4.97 mmol), K_2CO_3 (2.06 g, 14.9 mmol), and DMSO (13 mL) was heated at 110 °C for 1 h under microwave irradiation. The mixture was quenched with 28% NH_3 solution, and extracted with EtOAc/THF (1:1). The organic layer was separated, washed with water and brine, dried over $MgSO_4$, passed through silica gel pad (EtOAc), and concentrated in vacuo. The residue was purified by silica gel column chromatography (silica gel, EtOAc/MeOH = 100/0 to 85/15) and recrystallized from EtOH (80 mL) to give the title compound (1.03 g, 56%) as an off-white solid; mp 233–235 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.83–0.96 (4H, m), 2.02–2.12 (1H, m), 2.47 (3H, s), 5.16 (2H, s), 6.01 (1H, d, *J* = 2.6 Hz), 6.09–6.18 (1H, m), 7.11 (1H, dd, *J* = 9.4, 1.9 Hz), 7.32–7.53 (6H, m), 7.66 (1H, d, *J* = 7.5 Hz), 8.37 (1H, d, *J* = 1.1 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 7.9, 8.1, 69.7, 97.7, 100.3, 114.8, 116.6, 121.9, 123.2, 127.2, 127.9, 128.2, 128.5, 135.8, 139.7, 141.8, 145.1, 162.8, 167.2. MS (ESI/APCI) *m/z* 372.2 [M + H]$^+$. Anal. Calcd. for $C_{23}H_{21}N_3O_2$: C, 74.37; H, 5.70; N, 11.31. Found: C, 74.21; H, 5.65; N, 11.28.

1-(2-Cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)-4-((4-fluorobenzyl)oxy)pyridin-2(1*H*)-one (10d). To a stirred mixture of **44a** (200 mg, 0.912 mmol), **9a** (285 mg, 0.956 mmol), and K_2CO_3 (378 mg, 2.73 mmol) in DMF (6 mL) was added CuI (34.7 mg, 0.182 mmol) and *trans*-*N*,*N*′-dimethylcyclohexane-1,2-diamine (26 mg, 0.182 mmol). The reaction vessel was sealed and heated at 130 °C for 16 h. DMF was removed in vacuo, and then the residue was partitioned between EtOAc (100 mL) and water (20 mL). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Two batches were combined and purified by preparative HPLC to give the title compound (80 mg, 11%) as an off-white solid; mp 233–235 °C. ^{1}H NMR (300 MHz, $CDOD_3$) δ 0.95–0.99 (4H, m), 2.06 (1H, m), 2.51 (3H, s), 5.14 (2H, s), 6.11 (1H, d, *J* = 2.6 Hz), 6.28 (1H, dd, *J* = 7.6, 2.6 Hz), 7.14 (2H, t, *J* = 8.7 Hz), 7.21 (1H, dd, *J* = 9.4, 1.9 Hz), 7.46–7.51 (3H, m), 7.60 (1H, d, *J* = 7.6 Hz), 8.33 (1H, d, *J* = 1.3 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.8, 7.9, 8.0, 68.9, 97.6, 100.2, 115.3 (d, *J* = 21.2 Hz), 114.8, 116.5, 121.9, 123.1, 127.2, 130.2 (d, *J* = 9.1 Hz), 132.0 (d, *J* = 3.0 Hz), 139.7, 141.9, 145.0, 161.9 (d, *J* = 245.4 Hz), 162.7, 167.1. MS (ESI/APCI) *m/z* 389.8 [M + H]$^+$. Purity > 99.9% (HPLC).

4-[(4-Bromobenzyl)oxy]-1-(2-cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)pyridin-2(1*H*)-one (10e). To a solution of **45** (500 mg, 1.78 mmol) in THF (5 mL) was added 4-bromobenzyl alcohol (332 mg, 1.78 mmol), (E)-bis(2-methoxyethyl) diazene-1,2-dicarboxylate (541 mg, 2.31 mmol), and PPh_3 (606 mg, 2.31 mmol) at rt, and the mixture was stirred for 16 h at rt. The mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by

column chromatography (silica gel, EtOAc/MeOH = 90/10), and the product was crystalized from EtOAc to give the title compound (502 mg, 63 %) as white crystals; mp 228–229 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.82–0.96 (4H, m), 2.07 (1H, br s), 2.47 (3H, s), 5.15 (2H, s), 5.99 (1H, d, *J* = 2.4 Hz), 6.14 (1H, dd, *J* = 7.9, 2.5 Hz), 7.06–7.15 (1H, m), 7.43 (3H, d, *J* = 8.8 Hz), 7.59–7.69 (3H, m), 8.37 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 8.0, 8.1, 68.8, 97.7, 100.2, 114.9, 116.6, 121.3, 121.9, 123.2, 127.2, 130.0, 131.4, 131.5, 135.3, 139.8, 141.9, 145.1. MS (ESI/APCI) *m/z* 450.3 $[M + H]^+$. Anal. Calcd. for $C_{23}H_{20}BrN_3O_2$: C, 61.34; H, 4.48; N, 9.33. Found: C, 61.14; H, 4.40; N, 9.25.

1-(2-Cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)-4-{[4-(propan-2-yl)benzyl]oxy}pyridin-2(1*H*)-one (10f). To a solution of **45** (200 mg, 0.71 mmol) in DMF (3 mL) was added 1-(bromomethyl)-4-(*tert*-butyl)benzene (0.131 mL, 0.78 mmol) and K_2CO_3 (197 mg, 1.42 mmol), and the mixture was stirred at rt for 16 h. The mixture was poured into water, and the resultant solid was collected by filtration. The solid was recrystallized from acetone–MeOH to give the title compound (114 mg, 39%) as white crystals; mp 244–246 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.84–0.99 (4H, m), 1.21 (6H, d, *J* = 6.9 Hz), 2.08 (1H, d, *J* = 5.5 Hz), 2.47 (3H, br s), 2.91 (1H, dt, *J* = 13.7, 6.8 Hz), 5.11 (2H, s), 6.00 (1H, d, *J* = 2.5 Hz), 6.12 (1H, dd, *J* = 7.5, 2.4 Hz), 7.05–7.16 (1H, m), 7.24–7.33 (2H, m), 7.35–7.40 (2H, m), 7.43 (1H, d, *J* = 9.5 Hz), 7.65 (1H, d, *J* = 7.7 Hz), 8.37 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.8, 7.9, 8.0, 23.8, 33.1, 69.6, 97.6, 100.2, 114.8, 116.5, 121.9, 123.2, 126.4, 127.2, 128.0, 133.1, 139.6, 141.9, 145.0, 148.5, 162.8, 167.2. MS (ESI/APCI) *m/z* 414.4 $[M + H]^+$. Anal. Calcd. for $C_{26}H_{27}N_3O_2 \cdot 0.1H_2O$: C, 75.19; H, 6.60; N, 10.12. Found: C, 75.19; H, 6.54; N, 10.10.

1-(2-Cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)-4-{[4-(trifluoromethyl)benzy]oxy}pyridin-2(1*H*)-one (10g). To a stirred mixture of **44c** (110 mg, 0.409 mmol), **9a** (121 mg, 0.409 mmol), K_2CO_3 (168 mg, 1.22 mmol), and dioxane (10 mL) was added CuI (15 mg, 0.079 mmol) and *trans-N,N'*-dimethylcyclohexane-1,2-diamine (11 mg, 0.079 mmol). The reaction vessel was sealed and heated at 110 °C for 16 h. The mixture was filtered through Celite®, and the filtrate was concentrated in vacuo. The residue was diluted with water, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (silica gel, DCM/MeOH = 98/2) to give the title compound (40 mg, 22%) as an off-white solid; mp 240–242 °C. ^{1}H NMR (400 MHz, $CDOD_3$) δ 0.95–1.01 (4H, m), 2.06 (1H, m), 2.51 (3H, s), 5.28 (2H, s), 6.10 (1H, d, *J* = 2.4 Hz), 6.33 (1H, dd, *J* = 7.6, 2.4 Hz), 7.21 (1H, dd, *J* = 9.2, 2.0 Hz), 7.47 (1H, d, *J* = 9.6 Hz), 7.62–7.67 (3H, m), 7.72 (2H, d, *J* = 8.4 Hz), 8.33 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 8.0, 8.1, 68.7, 97.8, 100.1, 114.9, 116.6, 121.9, 123.2, 124.2 (q, *J* = 270.8 Hz), 125.4 (q, *J* = 3.8 Hz), 127.2, 128.2, 128.6 (q, *J* = 31.5 Hz), 139.8, 140.7, 141.9, 145.1, 162.8, 166.9. MS (ESI/APCI) *m/z* 440.2 $[M + H]^+$. Anal. Calcd. for $C_{24}H_{20}F_3N_3O_2$: C, 65.50; H, 4.59; N, 9.56. Found: C, 65.45; H, 4.65; N, 9.53.

4-(Benzyloxy)-1-(2,3-dimethylimidazo[1,2-*a*]pyridin-6-yl)pyridin-2(1*H*)-one (10h). The title compound was prepared in 50% yield using **9b** and **44e** in an analogous manner to **10g**. Off-white solid; mp 220–221 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.33 (3H, s), 2.39 (3H, s), 5.17 (2H, s), 5.99 (1H, d, *J* = 2.4 Hz), 6.14 (1H, dd, *J* = 7.6, 2.3 Hz), 7.12–7.14 (1H, m), 7.45–7.49 (5H, m), 7.67 (1H, d, *J* = 7.6 Hz), 8.38 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.9, 13.1, 68.8, 97.7, 100.2, 114.9, 117.0, 122.2, 123.1, 127.2, 128.5, 129.6, 132.7, 134.8, 139.7, 140.0, 141.7, 162.7, 167.0. MS (ESI/APCI) *m/z* 379.8 $[M + H]^+$. Anal. Calcd. for $C_{21}H_{18}ClN_3O_2 \cdot H_2O$: C, 63.40; H, 5.07; N, 10.56. Found: C, 63.36; H, 5.07; N, 10.50.

4-(Benzyloxy)-1-(2-ethyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)pyridin-2(1*H*)-one (10i). The title compound was prepared in 25% using **9c** and **44e** in an analogous manner to **10g**. Off-white solid; mp 218–220 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.22 (3H, t, *J* = 7.5 Hz), 2.39 (3H, s), 2.69 (2H, dd, *J* = 14.9, 7.3 Hz), 5.16 (2H, s), 5.99 (1H, d, *J* = 2.6 Hz), 6.14 (1H, dd, *J* = 7.6, 2.6 Hz), 7.13 (1H, dd, *J* = 9.4, 1.9 Hz), 7.48–7.49 (5H, m), 7.67 (1H, d, *J* = 7.6 Hz), 8.38 (1H, d, *J* = 1.3 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.8, 14.1, 20.3, 68.8, 97.7, 100.2, 115.1, 116.2, 122.3, 123.1, 127.2, 128.5, 129.6, 132.7, 134.8, 139.7, 141.8, 145.5, 162.7, 167.0. MS (ESI/APCI) *m/z* 394.0 $[M + H]^+$. Anal. Calcd. for $C_{22}H_{20}ClN_3O_2 \cdot 0.25H_2O$: C, 66.33; H, 5.19; N, 10.55. Found: C, 66.22; H, 5.10; N, 10.54.

6-{4-[(4-Fluorobenzyl)oxy]-2-oxopyridin-1(2*H*)-yl}-3-methylimidazo[1,2-*a*]pyridine-2-carbonitrile (10j). The title compound was prepared in 34% yield using **9d** and **44a** in an analogous manner to **10g**. Off-white solid; mp 230–232 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.63 (3H, s), 5.15 (2H, s), 6.04 (1H, d, *J* =2.4 Hz), 6.18 (1H, dd, *J* =2.5, 1.6 Hz), 7.23–7.28 (2H, m), 7.47 (1H, dd, *J* = 9.7, 1.7 Hz), 7.51–7.54 (2H, m), 7.67–7.69 (2H, m), 8.69 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 8.7, 69.0, 97.6, 100.6, 115.1, 115.2, 115.3 (d, *J* = 21.2 Hz), 116.5, 123.7, 128.0, 129.6, 130.2 (d, *J* = 9.1 Hz), 131.2, 132.0 (d, *J* = 3.0 Hz), 139.3, 143.1, 161.9 (d, *J* = 245.4 Hz), 162.6, 167.3. MS (ESI/APCI) *m/z* 375.0 $[M + H]^+$. Anal. Calcd. for $C_{21}H_{15}FN_4O_2 \cdot 0.25H_2O$: C, 66.57; H, 4.12; N, 14.79. Found: C, 66.74; H, 4.09; N, 14.78.

6-{4-[(4-Fluorobenzyl)oxy]-2-oxopyridin-1(2*H*)-yl}-*N*,*N*,3-trimethylimidazo[1,2-*a*]pyridine-2-carboxamide (10k). The title compound was prepared in 34% yield using **9e** and **44a** in an analogous manner to **10g**. White solid; mp 252–254 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.54 (3H, s), 3.01 (3H, s), 3.22 (3H, s), 5.15 (2H, s), 6.03 (1H, d, *J* = 2.4 Hz), 6.16 (1H, dd, *J* = 7.5, 2.4 Hz), 7.24–7.31 (3H, m), 7.51–7.54 (2H, m), 7.62 (1H, d, *J* = 9.6 Hz), 7.69 (1H, d, *J* = 7.6 Hz), 8.56 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) 8.8, 35.0, 38.3, 69.0, 97.6, 100.4, 115.3 (d, *J* = 21.2 Hz), 116.4, 123.0, 123.5, 125.5, 128.4, 130.2 (d, *J* = 9.1 Hz), 132.0 (d, *J* = 4.0 Hz), 137.2, 139.5, 141.0, 161.9 (d, *J* = 245.4 Hz), 162.7, 164.7, 167.2. MS (ESI/APCI) *m/z* 421.4 $[M + H]^+$. Anal. Calcd. for $C_{23}H_{21}FN_4O_3 \cdot 0.15H_2O$: C, 65.29; H, 5.07; N, 13.24. Found: C, 65.29; H, 5.02; N, 13.21.

6-{4-[(4-Fluorobenzyl)oxy]-2-oxopyridin-1(2*H*)-yl}-*N*-methoxy-*N*,3-dimethylimidazo[1,2-*a*]pyridine-2-carboxamide (10l). To a stirred mixture of **44b** (500 mg, 2.28 mmol), **9f** (945 mg, 2.73 mmol), K_2CO_3 (945 mg, 6.84 mmol), and dioxane (20 mL) were added CuI (174 mg, 0.91 mmol) and *trans-N,N'*-dimethyl-cyclohexane-1,2-diamine (130 mg, 0.91 mmol). The reaction vessel was sealed, and heated at 110 °C for 16 h. The reaction mixture was then cooled to rt, filtered through Celite®, and the concentrated. The residue was diluted with EtOAc (150 mL), washed with brine (50 mL), dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (silica gel, DCM/MeOH = 98/2) to give the title compound (320 mg, 32%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.57 (3H, s), 3.41 (3H, s), 3.75 (3H, s), 5.15 (2H, s), 6.03 (1H, d, *J* = 2.6 Hz), 6.16 (1H, dd, *J* = 7.6, 2.6 Hz), 7.23–7.32 (3H, m), 7.51–7.54 (2H, m), 7.62 (1H, d, *J* = 9.6 Hz), 7.70 (1H, d, *J* = 7.5 Hz), 8.58 (1H, s). MS (ESI/APCI) *m/z* 437.4 $[M + H]^+$.

1-(2-Acetyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (10m). To a stirred solution of **10l** (150 mg, 0.34 mmol) in THF (10 mL) was added a solution of MeMgBr (3 M in ether, 344 μL, 1.03 mmol) at −78 °C, and the resulting mixture was stirred at same temperature for 2 h. The reaction mixture was then quenched with sat. NH_4Cl solution (40 mL), and allowed to warm to rt. The reaction mixture was concentrate, and extracted with EtOAc (100 mL). The organic layer was washed with brine (40 mL), dried over Na_2SO_4, and concentrated. The resulting residue was purified by column chromatography (silica gel, DCM/MeOH = 97/3) to give the title compound (55 mg, 40%) as a white solid; mp 249–251 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.61 (3H, s), 2.73 (3H, s), 5.15 (2H, s), 6.04 (1H, d, *J* = 2.2 Hz), 6.16 (1H, dd, *J* = 7.7, 2.6 Hz), 7.24–7.28 (2H, m), 7.35 (1H, dd, *J* = 9.7, 1.7 Hz), 7.51–7.54 (2H, m), 7.68–7.71 (2H, m), 8.63 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 9.2, 27.7, 69.0, 97.6, 100.5, 115.3 (d, *J* = 21.2 Hz), 117.1, 123.3, 125.6, 126.7, 126.6, 129.2, 130.2 (d, *J* = 8.1 Hz), 132.0 (d, *J* = 3.0 Hz), 139.1, 139.4, 141.4, 161.9 (d, *J* = 245.4 Hz), 162.6, 167.2, 196.8. MS (ESI/APCI) *m/z* 392 $[M + H]^+$. Purity 98.9% (HPLC).

1-[2-(Cyclopropylcarbonyl)-3-methylimidazo[1,2-*a*]pyridin-6-yl]-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (10n). The title compound was prepared in 39% yield using ^{c}PrMgBr in an analogous manner to **10m**. White solid; mp 233–234 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.04 (4H, m), 2.73 (3H, s), 3.34 (1H, m), 5.15 (2H, s), 6.04 (1H, d, *J* = 2.5 Hz), 6.16 (1H, dd, *J* = 7.5, 2.4 Hz), 7.24–7.28 (2H, m), 7.37 (1H, dd, *J* = 9.5, 1.5 Hz), 7.51–7.54 (2H, m), 7.69 (1H, d, *J* = 3.9 Hz), 7.72 (1H, d, *J* = 1.6 Hz), 8.64 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 9.2, 11.0, 17.4, 69.0, 97.6, 100.5, 115.3 (d, *J* = 21.2 Hz), 117.2, 123.3, 125.4, 126.6, 129.2, 130.2 (d, *J* = 8.1 Hz), 132.0 (d, *J* = 3.0 Hz), 139.1, 139.4, 141.6, 161.9 (d, *J* = 245.4 Hz), 162.6, 167.2, 198.0. MS (ESI/APCI) *m/z* 418 $[M + H]^+$. Purity 98.3% (HPLC).

4-[(4-Fluorobenzyl)oxy]-1-[2-(hydroxymethyl)-3-methylimidazo[1,2-*a*]pyridin-6-yl]pyridin-2(

1*H*)-one (10o). The title compound was prepared in 43% yield using **9g** and **44a** in an analogous manner to **10g**. White solid; mp 270–273 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.46 (3H, s), 4.58 (2H, d, *J* = 5.3 Hz), 5.04 (1H, m), 5.14 (2H, s), 6.02 (1H, d, *J* = 2.5 Hz), 6.13 (1H, dd, *J* = 7.6, 2.6 Hz), 7.18 (1H, dd, *J* = 9.5, 1.6 Hz), 7.24–7.28 (2H, m), 7.51–7.54 (3H, m), 7.68 (1H, d, *J* = 7.6 Hz), 8.44 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 8.1, 57.0, 68.9, 97.6, 100.3, 115.3 (d, *J* = 22.2 Hz), 115.6, 122.6, 123.7, 127.6, 130.2 (d, *J* = 9.1 Hz), 132.0 (d, *J* = 3.0 Hz), 139.6, 141.6, 143.7, 161.9 (d, *J* = 245.4 Hz), 162.7, 167.1. MS (ESI/APCI) *m/z* 380.0 $[M + H]^+$. Anal. Calcd. for $C_{21}H_{18}FN_3O_3 \cdot 0.5H_2O$: C, 64.94; H, 4.93; N, 10.82. Found: C, 64.89; H, 4.74; N, 10.87.

4-[(4-Fluorobenzyl)oxy]-1-[2-(methoxymethyl)-3-methylimidazo[1,2-*a*]pyridin-6-yl]pyridin-2(1*H*)-one (10p). The title compound was prepared in 26% yield using **9h** and **44a** in an analogous manner to **10g**. White solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.49 (3H, s), 3.42 (3H, s), 4.65 (2H, s), 5.01 (2H, s), 6.04 (1H, d, *J* = 2.5 Hz), 6.08 (1H, dd, *J* = 7.5, 2.5 Hz), 7.08–7.14 (3H, m), 7.26 (1H, m), 7.39 (2H, dd, *J* = 8.4, 5.4 Hz), 7.63 (1H, d, *J* = 9.4 Hz), 7.98 (1H, s). MS (ESI/APCI) *m/z* 394.0 $[M + H]^+$.

(6-{4-[(4-Fluorobenzyl)oxy]-2-oxopyridin-1(2*H*)-yl}-3-methylimidazo[1,2-*a*]pyridin-2-yl)acetonitrile (10q). To a stirred solution of compound **10o** (120 mg, 0.31 mmol) in DCM (1 mL) was added $SOCl_2$ (1 mL), and the mixture was stirred at rt for 18 h. The mixture was concentrated, diluted with DCM (100 mL), and quenched with sat. $NaHCO_3$ solution (50 mL). The organic layer was separated, washed successively with water (50 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated to give 1-[2-(chloromethyl)-3-methylimidazo[1,2-*a*]pyridin-6-yl]-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (120 mg, 96%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.91 (2H, s), 5.14 (2H, s), 6.02 (1H, d, *J* = 2.4 Hz), 6.15 (1H, dd, *J* = 7.6, 2.5 Hz), 7.23–7.28 (3H, m), 7.51–7.57 (3H, m), 7.68 (1H, d, *J* = 7.6 Hz), 8.48 (1H, s). MS (ESI/APCI) *m/z* 398.2 $[M + H]^+$.

To a stirred solution of 1-[2-(chloromethyl)-3-methylimidazo[1,2-*a*]pyridin-6-yl]-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (150 mg, 0.32 mmol) in THF (5 mL) were added TMSCN (115 μL, 1.13 mmol) and TBAF (1 M solution in THF, 1.13 mL, 1.13 mmol) at rt, and the resulting mixture was stirred for 4 h. The reaction mixture was then quenched with sat. $FeSO_4$ solution (10 mL), and extracted with DCM (100 mL) twice. The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, and concentrated. The crude material was purified by column chromatography (silica gel, DCM/MeOH = 97/3) to afford the title compound (60 mg, 48%) as a white solid; mp 220–222 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.46 (3H, s), 4.15 (2H, s), 5.14 (2H, s), 6.03 (1H, s), 6.15 (1H, d, *J* = 7.2 Hz), 7.24–7.28 (3H, m), 7.51–7.59 (3H, m), 7.67 (1H, d, *J* = 7.6 Hz), 8.48 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.7, 16.4, 69.0, 97.6, 100.4, 115.4 (d, *J* = 21.0 Hz), 115.6, 118.3, 118.5, 122.9, 124.8, 128.0, 130.0 (d, *J* = 9.0 Hz), 132.0 (d, *J* = 3.8 Hz), 133.6, 139.6, 142.2, 162.0

(d, *J* = 243.0 Hz), 162.7, 167.2. MS (ESI/APCI) *m/z* 389.4 $[M + H]^+$. Anal. Calcd. for $C_{22}H_{17}FN_4O_2 \cdot 0.25H_2O$: C, 67.25; H, 4.49; N, 14.26. Found: C, 67.30; H, 4.46; N, 14.18.

1-(2-Cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)-4-(pyridin-2-ylmethoxy)pyridin-2(1*H*)-one (10r). The title compound was prepared in 32% yield using 2-(bromomethyl)pyridine hydrobromide in an analogous manner to **10f**. White crystals; mp 217–220 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.05 (2H, d, *J* = 3.8 Hz), 1.16 (2H, d, *J* = 8.3 Hz), 2.23–2.32 (1H, m), 2.58 (3H, s), 5.29 (2H, s), 6.07 (1H, d, *J* = 2.3 Hz), 6.30 (1H, dd, *J* = 7.7, 2.4 Hz), 7.41–7.53 (1H, m), 7.62 (1H, d, *J* = 7.9 Hz), 7.72 (1H, d, *J* = 7.8 Hz), 7.85–7.92 (1H, m), 7.93–8.01 (2H, m), 8.65 (1H, d, *J* = 4.5 Hz), 9.00 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 5.9, 7.7, 8.1, 68.6, 97.8, 100.9, 110.5, 120.1, 123.8, 124.5, 124.8, 131.2, 132.1, 135.5, 137.0, 139.3, 141.0, 146.4, 152.9, 162.4, 167.2. MS (ESI/APCI) *m/z* 373.2 $[M + H]^+$. Anal. Calcd. for $C_{22}H_{20}N_4O_2 \cdot 2HCl \cdot H_2O$: C, 57.03; H, 5.22; N, 12.09. Found: C, 57.13; H, 5.19; N, 12.09.

1-(2-Cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)-4-(pyridin-3-ylmethoxy)pyridin-2(1*H*)-one (10s). To a solution of **45** (150 mg, 0.53 mmol), pyridin-3-yl-methanol (116 mg, 1.06 mmol), and tributyl phosphine (322 mg, 1.59 mmol) in THF (15 mL) was added ADDP (401 mg, 1.59 mmol). The mixture was stirred under sonication at 60 °C for 4 h. The reaction mixture was then cooled to rt and concentrated. The residue was diluted with DCM (60 mL), washed with water (30 mL) twice and brine (30 mL), dried over Na_2SO_4, and concentrated. The resulting residue was purified by column chromatography (silica gel, DCM/MeOH = 96/4) to give the title compound (61 mg, 31%) as an off-white solid; mp 210–212 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88–0.92 (4H, m), 2.07 (1H, m), 5.21 (2H, s), 6.05 (1H, d, *J* = 2.5 Hz), 6.14 (1H, dd, *J* = 7.5, 2.6 Hz), 7.11 (1H, dd, *J* = 9.4, 1.7 Hz), 7.42–7.48 (2H, m), 7.67 (1H, d, *J* = 7.6 Hz), 7.89–7.91 (1H, m), 8.37 (1H, s), 8.61 (1H, br s), 8.70 (1H, br s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 8.0, 8.1, 67.4, 97.7, 100.2, 114.9, 116.6, 122.0, 123.2, 123.6, 127.2, 131.5, 135.9, 139.8, 141.9, 145.1, 149.2, 149.5, 162.8, 167.0. MS (ESI/APCI) *m/z* 373.4 $[M + H]^+$. Anal. Calcd. for $C_{22}H_{20}N_4O_2 \cdot 0.1H_2O$: C, 70.61; H, 5.44; N, 14.97. Found: C, 70.75; H, 5.38; N, 14.89.

1-(2-Cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)-4-(pyridin-4-ylmethoxy)pyridin-2(1*H*)-one (10t). The title compound was prepared in 28% yield using pyridin-4-yl-methanol in an analogous manner to **10s**. White solid; mp 251–253 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.98–1.08 (4H, m), 1.98–2.02 (1H, m), 2.50 (3H, s), 5.11 (2H, s), 6.02 (1H, d, *J* = 2.6 Hz), 6.15 (1H, dd, *J* = 7.6, 2.7 Hz), 7.06 (1H, dd, *J* = 9.4, 1.8 Hz), 7.31 (1H, d, *J* = 7.6 Hz), 7.36 (2H, d, *J* = 5.6 Hz), 7.57 (1H, d, *J* = 9.4 Hz), 7.94 (1H, s), 8.68 (2H, d, *J* = 5.8 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 8.0, 8.1, 67.8, 97.8, 100.1, 114.9, 116.6, 121.8, 122.0, 123.2, 127.1, 139.9, 141.9, 144.9, 145.1, 149.8, 162.7, 166.8. MS (ESI/APCI) *m/z* 373.3 $[M + H]^+$. Anal. Calcd. for $C_{22}H_{20}N_4O_2 \cdot 0.5H_2O$: C, 69.28; H, 5.55; N, 14.69. Found: C, 69.33; H, 5.50; N, 14.57.

1-(2-Cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)-4-(pyrimidin-5-ylmethoxy)pyridin-2(1

***H*)-one (10u).** The title compound was prepared in 33% yield using pyrimidin-5-yl-methanol in an analogous manner to **10s**. White solid; mp 236–238 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88–0.92 (4H, m), 2.05–2.09 (1H, m), 2.48 (3H, s), 5.26 (2H, s), 6.08 (1H, d, *J* = 2.5 Hz), 6.16 (1H, dd, *J* = 7.5, 2.6 Hz), 7.11 (1H, dd, *J* = 9.5, 1.8 Hz), 7.43 (1H, d, *J* = 9.4 Hz), 7.68 (1H, d, *J* = 7.8 Hz), 8.38 (1H, s), 8.95 (2H, s), 9.22 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 8.0, 8.1, 65.1, 97.7, 100.1, 114.9, 116.6, 122.0, 123.2, 127.1, 129.7, 139.9, 141.9, 145.1, 156.5, 158.3, 162.8, 166.8. MS (ESI/APCI) *m/z* 374.2 $[M + H]^+$. Anal. Calcd. for $C_{21}H_{19}N_5O_2.0.75H_2O$: C, 65.19; H, 5.34; N, 18.10. Found: C, 65.32; H, 5.22; N, 18.11.

1-(2-Cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)-4-(thiophen-2-ylmethoxy)pyridin-2(1*H*)-one (10v). The title compound was prepared in 22% yield using thiophen-2-yl-methanol in an analogous manner to **10s**. Off-white solid; mp 225 °C (decomposition). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88–0.92 (4H, m), 2.06–2.08 (1H, m), 5.35 (2H, s), 6.07–6.12 (2H, m), 7.07 (1H, dd, *J* = 4.8, 3.5 Hz), 7.09–7.13 (1H, m), 7.27 (1H, d, *J* = 3.1 Hz), 7.43 (1H, d, *J* = 9.4 Hz), 7.61 (1H, d, *J* = 4.9 Hz), 7.64 (1H, d, *J* = 7.5 Hz), 8.37 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.8, 7.9, 8.0, 64.5, 97.7, 100.1, 114.8, 116.5, 121.9, 123.2, 126.9, 127.1, 127.3, 128.3, 137.7, 139.7, 141.9, 145.0, 162.7, 166.7. MS (ESI/APCI) *m/z* 378.3 $[M + H]^+$. Anal. Calcd. for $C_{21}H_{19}N_3O_2S{\cdot}0.4H_2O$: C, 65.57; H, 5.19; N, 10.92. Found: C, 65.57; H, 5.01; N, 10.86.

1-(2-Cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)-4-(thiophen-3-ylmethoxy)pyridin-2(1*H*)-one (10w). The title compound was prepared in 40% yield using thiophen-3-yl-methanol in an analogous manner to **10s**. White solid; mp 234 °C (decomposition). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88–0.94 (4H, m), 2.04–2.10 (1H, m), 5.15 (2H, s), 6.02 (1H, d, *J* = 2.6 Hz), 6.10 (1H, dd, *J* = 7.6, 2.6 Hz), 7.07 (1H, dd, *J* = 9.5, 1.8 Hz), 7.19 (1H, d, *J* = 4.9 Hz), 7.43 (1H, d, *J* = 9.4 Hz), 7.59 (1H, dd, *J* = 4.8, 2.9 Hz), 7.64–7.66 (2H, m), 8.37 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.8, 7.9, 8.0, 65.2, 97.5, 100.2, 114.8, 116.5, 121.9, 123.2, 124.7, 126.8, 127.2, 127.5, 136.6, 139.6, 141.9, 145.0, 162.8, 167.1. MS (ESI/APCI) *m/z* 378.3 $[M + H]^+$. Anal. Calcd. for $C_{21}H_{19}N_3O_2S{\cdot}0.6H_2O$: C, 64.96; H, 5.24; N, 10.82. Found: C, 64.84; H, 5.01; N, 10.71.

4-[(5-Chloropyridin-2-yl)methoxy]-1-(2-cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)pyridin-2(1*H*)-one (10x). The title compound was prepared in 53% yield using (5-chloropyridin-2-yl)methanol in an analogous manner to **10s**. White solid; mp 200–201 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.84–0.96 (4H, m), 2.01–2.12 (1H, m), 2.47 (3H, s), 5.24 (2H, s), 5.99 (1H, d, *J* = 2.6 Hz), 6.18 (1H, dd, *J* = 7.6, 2.7 Hz), 7.11 (1H, dd, *J* = 9.5, 1.8 Hz), 7.43 (1H, d, *J* = 9.5 Hz), 7.61 (1H, d, *J* = 8.3 Hz), 7.68 (1H, d, *J* = 7.7 Hz), 8.03 (1H, dd, *J* = 8.3, 2.4 Hz), 8.37 (1H, s), 8.67 (1H, d, *J* = 2.4 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 8.0, 8.1, 69.8, 97.8, 100.1, 114.9, 116.6, 121.9, 123.2, 123.5, 127.1, 130.5, 136.9, 139.9, 141.9, 145.1, 147.8, 154.0, 162.7, 166.9. MS (ESI/APCI) *m/z* 407.1 $[M + H]^+$. Anal. Calcd. for $C_{22}H_{19}ClN_4O_2$: C, 64.94; H, 4.71; N, 13.77. Found: C, 64.80; H, 4.72; N, 13.69.

4-[(5-Chlorothiophen-3-yl)methoxy]-1-(2-cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)pyridin-2(1*H*)-one (10y). The title compound was prepared in 41% yield using (5-chloro-thiophen-3-yl)-methanol in an analogous manner to **10s**. White solid; mp 233–235 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88–0.92 (4H, m), 2.05–2.09 (1H, m), 2.47 (3H, s), 5.06 (2H, s), 6.01 (1H, d, *J* = 2.6 Hz), 6.10 (1H, dd, *J* = 7.6, 2.7 Hz), 7.11 (1H, dd, *J* = 9.5, 1.9 Hz), 7.20 (1H, d, *J* = 1.7 Hz), 7.43 (1H, d, *J* = 9.4 Hz), 7.56 (1H, d, *J* = 1.2 Hz), 7.65 (1H, d, *J* = 7.7 Hz), 8.36 (1H, d, *J* = 1.3 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 8.0, 8.1 65.1, 97.5, 100.2, 114.9, 116.6, 121.9, 123.2, 124.3, 127.0, 127.2, 128.9, 136.4, 139.7, 141.9, 145.1, 162.8, 166.9. MS (ESI/APCI) *m/z* 412.0 $[M + H]^+$. Anal. Calcd. for $C_{21}H_{18}ClN_3O_2S \cdot 0.4H_2O$: C, 60.18; H, 4.52; N, 10.03. Found: C, 60.01; H, 4.47; N, 10.08.

1-(2-Cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)-4-{[5-(trifluoromethyl)thiophen-3-yl]methoxy}pyridin-2(1*H*)-one (10z). A mixture of **46** (111 mg, 0.32 mmol), 2-(trifluoromethylthiophen-4-yl)methanol (88 mg, 0.48 mmol) and KO^{t}Bu (109 mg, 0.97 mmol) in toluene (3 mL) was heated at 100 °C for 1 h. The mixture was poured into water, and extracted with EtOAc/THF (1:1). The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 97/3 to 0/100). The solid was recrystallized from IPA–IPE to give the title compound (47.5 mg, 33 %) as a white solid; mp 209–210 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83–0.99 (4H, m), 2.07 (1H, br s), 5.17 (2H, s), 6.05 (1H, s), 6.13 (1H, d, *J* = 5.0 Hz), 7.12 (1H, d, *J* = 9.9 Hz), 7.44 (1H, d, *J* = 9.3 Hz), 7.67 (1H, d, *J* = 7.5 Hz), 7.81 (1H, s), 8.07 (1H, s), 8.37 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.8, 7.9, 8.0, 64.6, 97.5, 100.1, 114.8, 116.5, 121.9, 122.4 (q, *J* = 269.7 Hz), 123.1, 127.1, 129.6, 129.8 (q, *J* = 38.4 Hz), 130.3 (q, *J* = 4.0 Hz), 137.1, 139.7, 141.9, 145.1, 162.7, 166.9. MS (ESI/APCI) *m/z* 446.4 $[M + H]^+$. Anal. Calcd. for $C_{22}H_{18}F_3N_3O_2S$: C, 59.32; H, 4.07; N, 9.43. Found: C, 59.46; H, 4.20; N, 9.35.

3-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)pyridin-2(1*H*)-one (11a). The title compound was prepared in 15% yield using **8** and **9a** in an analogous manner to **10a**. White solid. 180 °C (decomposition). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94–1.11 (4H, m), 1.94–2.04 (1H, m), 2.48 (3H, s), 5.13 (2H, s), 6.15 (1H, t, *J* = 7.2 Hz), 6.73 (1H, d, *J* = 7.3 Hz), 7.03 (1H, d, *J* = 6.8 Hz), 7.08 (1H, d, *J* = 9.4 Hz), 7.31–7.44 (4H, m), 7.56 (1H, d, *J* = 9.4 Hz), 7.99 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 8.0, 8.1, 69.0, 104.2, 115.1, 116.0, 116.7, 122.0, 122.9, 127.4, 128.4, 129.6, 131.0, 132.5, 135.5, 141.9, 145.2, 148.1, 157.3. MS (ESI/APCI) *m/z* 406.1 $[M + H]^+$. Anal. Calcd. for $C_{23}H_{20}ClN_3O_2 \cdot 0.5H_2O$: C, 66.58; H, 5.10; N, 10.13. Found: C, 66.30; H, 5.22; N, 10.21.

1-(2-Cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)-3-[4-(trifluoromethoxy)phenoxy]pyridin-2(1*H*)-one (11b). The title compound was prepared in 13% yield using **9a** and **14** in an analogous manner to **10a**. Pale yellow crystals; mp 188–190 °C (EtOAc–hexane). ^{1}H NMR (400

MHz, DMSO-d_6) δ 0.83–0.96 (4H, m), 2.03–2.14 (1H, m), 6.39 (1H, t, *J* = 7.1 Hz), 7.09 (2H, d, *J* = 9.0 Hz), 7.16–7.24 (1H, m), 7.34 (2H, d, *J* = 8.7 Hz), 7.46 (2H, t, *J* = 9.5 Hz), 7.70 (1H, d, *J* = 5.5 Hz), 8.49 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.8, 7.9, 8.0, 104.2, 115.0, 116.7, 117.7, 120.1 (q, *J* = 256.5 Hz), 122.0, 122.5, 122.7, 127.0, 128.2, 136.2, 141.9, 143.1 (q, *J* = 2.0 Hz), 144.1, 145.3, 155.6, 157.3. MS (ESI/APCI) *m/z* 442.3 $[M + H]^+$. Anal. Calcd. for $C_{23}H_{18}F_3N_3O_3$: C, 62.58; H, 4.11; N, 9.52. Found: C, 62.43; H, 4.30; N, 9.35.

2-Chloro-3-[4-(trifluoromethoxy)phenoxy]pyridine (13). To a solution of **12** (2.00 g, 8.35 mmol) and 4-(trifluoromethoxy)phenol (1.19 mL, 9.19 mmol) in CH_3CN (50 mL) was added $Cu(OAc)_2$ (3.03 g, 16.7 mmol), MS3A (2.00 g) and pyridine (3.38 mL, 41.8 mmol) at rt, and the mixture was vigorously stirred at rt for 10 days. The insoluble material was removed by filtration, and the filtrate was concentrate in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 0/100) to give the title compound (1.15 g, 47%) as an orange oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.82 (1H, d, *J* = 8.9 Hz), 7.16 (3H, d, *J* = 9.0 Hz), 7.42 (2H, d, *J* = 8.8 Hz), 7.46–7.54 (1H, m), 7.65 (1H, dd, *J* = 8.0, 1.3 Hz), 8.29 (1H, dd, *J* = 4.5, 1.3 Hz). MS (ESI/APCI) *m/z* 289.9 $[M + H]^+$.

3-[4-(Trifluoromethoxy)phenoxy]pyridin-2(1*H*)-one (14). A mixture of **13** (1.02 g, 3.52 mmol), KO^{t}Bu (1.19 g, 10.1 mmol), water (0.19 mL, 10.1 mmol) and ^{t}BuOH (15 mL) was heated at 150 °C for 1 h under microwave irradiation. The mixture was poured into water, and extracted with EtOAc. The organic layer was separated, washed with water and brine successively, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 75/25) to give the title compound (0.702 g, 73%) as white crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.21 (1H, d, *J* = 6.8 Hz), 6.97 (2H, d, *J* = 9.0 Hz), 7.26–7.36 (4H, m), 12.02 (1H, br s). MS (ESI/APCI) *m/z* 272.9 $[M + H]^+$.

5-Hydroxy-2-(tetrahydro-2*H*-pyran-2-yl)pyridazin-3(2*H*)-one (15). To a stirred solution of 4,5-dichloropyridazin-3(2*H*)-one (2.0 g, 12.1 mmol) in THF (12 mL) was added 3,4-dihydro-2*H*-pyran (1.4 mL, 15.4 mmol) and *p*-toluenesulfonic acid (185 mg, 0.97 mmol), and the reaction mixture was heated under reflux for 16 h. Additional 3,4-dihydro-2*H*-pyran (1.4 mL, 15.4 mmol) was added to the reaction mixture, and the reaction mixture was heated under reflux for further 24 h. The mixture was then cooled to rt, concentrated under reduced pressure, and poured into sat. $NaHCO_3$ solution (100 mL). The mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, concentrated, and purified by column chromatography (silica gel, hexane/EtOAc = 90/10) to give 4,5-dichloro-2-(tetrahydro-2*H*-pyran-2-yl)pyridazin-3(2*H*)-one (2.3 g, 76%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.43–1.52 (2H, m), 1.65–1.70 (2H, m), 1.92–1.95 (1H, m), 2.01–2.05 (1H, m), 3.58–3.64 (1H, m), 3.94–3.97 (1H, m), 5.82 (1H, dd, *J* = 10.3, 1.8 Hz), 8.23 (1H, s). MS (ESI/APCI) *m/z* 249.0 $[M + H]^+$.

To a stirred solution of 4,5-dichloro-2-(tetrahydro-2*H*-pyran-2-yl)pyridazin-3(2*H*)-one (2.3 g, 9.27 mmol) in MeOH (26 mL) was added KOH (519 mg, 9.27 mmol) at 0 °C. The reaction mixture was heated at 50 °C for 30 min and then stirred at rt for 19 h. The mixture was concentrated, diluted with water (50 mL), and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, concentrated, and triturated with 10% ether–hexane to give 4-chloro-5-methoxy-2-(tetrahydro-2*H*-pyran-2-yl)pyridazin-3(2*H*)-one (2.1 g, 92%) as a light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.50–1.52 (2H, m), 1.63–1.66 (2H, m), 1.93–2.05 (2H, m), 3.59–3.60 (1H, m), 3.93–3.96 (1H, m), 4.09 (3H, s), 5.87 (1H, dd, *J* = 10.8, 2.0 Hz), 8.29 (1H, s). MS (ESI/APCI) *m/z* 245.0 $[M + H]^+$.

To a stirred suspension of 4-chloro-5-methoxy-2-(tetrahydro-2*H*-pyran-2-yl)pyridazin-3(2*H*)-one (2.1 g, 8.6 mmol) in water (20 mL) was added KOH (578 mg, 10.3 mmol) at rt and the reaction mixture was heated under reflux for 3 h. The mixture was cooled to rt and acidified with 1 N HCl solution to pH = 5. The mixture was extracted with EtOAc, and the organic layer was washed with water and brine, dried over Na_2SO_4, concentrated, and triturated with ether to give 4-chloro-5-hydroxy-2-(tetrahydro-2*H*-pyran-2-yl)pyridazin-3(2*H*)-one (1.9 g, 96%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44–1.49 (2H, m), 1.59–1.70 (2H, m), 1.90–2.09 (2H, m), 3.53–3.62 (1H, m), 3.91–3.94 (1H, m), 5.82 (1H, d, *J* = 10.2 Hz), 7.78 (1H, s). MS (ESI/APCI) *m/z* 231.0 $[M + H]^+$.

To a stirred solution of 4-chloro-5-hydroxy-2-(tetrahydro-2*H*-pyran-2-yl)pyridazin-3(2*H*)-one (1.0 g, 4.34 mmol) in MeOH (20 mL) and Et_3N (606 μL, 4.34 mmol) was added Pd-C (10%, 25 mg), and the mixture was stirred under hydrogen atmosphere at rt for 16 h. The insoluble material was filtered through the Celite® pad, and the filtrate was concentrated and purified by column chromatography (silica gel, DCM/MeOH = 96/4) to give the title compound (600 mg, 70%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44–1.49 (2H, m), 1.54–1.57 (1H, m), 1.61–1.69 (1H, m), 1.90–1.93 (1H, m), 1.99–2.08 (1H, m), 3.51–3.57 (1H, m), 3.90–3.94 (1H, m), 5.81 (1H, dd, *J* = 9.6, 1.8 Hz), 5.94 (1H, d, *J* = 2.5 Hz), 7.68 (1H, d, *J* = 2.5 Hz), 11.57 (1H, br s). MS (ESI/APCI) *m/z* 197.2 $[M + H]^+$.

5-(4-Chlorobenzyloxy)-2-(tetrahydro-pyran-2-yl)-2*H*-pyridazin-3-one (16). To a stirred solution of **15** (200 mg, 1.02 mmol) and 4-chlorobenzyl bromide (209 mg, 1.02 mmol) in MeCN (8 mL) and DMF (1 mL) was added K_2CO_3 (282 mg, 2.04 mmol), and the resulting mixture was stirred at rt for 16 h. The reaction mixture was then concentrated, diluted with water (30 mL), and extracted with EtOAc (75 mL) three times. The organic layers were washed with water (50 mL) and brine (50 mL) successively, dried over Na_2SO_4, and concentrated to give the title compound (210 mg, 64%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44–1.49 (2H, m), 1.56–1.59 (1H, m), 1.62–1.66 (1H, m), 1.90–1.93 (1H, m), 2.00–2.10 (1H, m), 3.52–3.59 (1H, m), 3.91–3.94 (1H, m), 5.14 (2H, s), 5.81 (1H, dd, *J* = 10.5, 2.0 Hz), 6.38 (1H, d, *J* = 2.7 Hz), 7.48 (4H, s), 7.83

(1H, d, J = 2.7 Hz). MS (ESI/APCI) m/z 321.2 $[M + H]^+$.

5-(4-Chlorobenzyloxy)-2*H*-pyridazin-3-one (17). To a stirred suspension of **16** (210 mg, 0.65 mmol) in MeOH (5 mL) was added conc. HCl (0.5 mL) at rt, and then the reaction mixture was heated at reflux for 3 h. The mixture was cooled to rt, concentrated, and neutralized with sat. $NaHCO_3$ (20 mL). The resulting precipitate was collected by filtration, washed with water, and dried to give the title compound (140 mg, 91%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.12 (2H, s), 6.27 (1H, s), 7.48 (4H, s), 7.72 (1H, d, J = 2.5 Hz), 12.6 (1H, br s). MS (ESI/APCI) m/z 237.2 $[M + H]^+$.

5-[(4-Chlorobenzyl)oxy]-2-(2-cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)pyridazin-3(2*H*)-one (18). The title compound was prepared in 70% yield using **9a** and **17** in an analogous manner to **10g**. White solid; mp 190–192 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88–0.92 (4H, m), 2.06–2.09 (1H, m), 2.47 (3H, s), 5.23 (2H, s), 6.54 (1H, d, J = 2.6 Hz), 7.23 (1H, dd, J = 9.3, 1.7 Hz), 7.46 (1H, d, J = 9.4 Hz), 7.50–7.55 (4H, m), 8.01 (1H, d, J = 2.7 Hz), 8.46 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.7, 7.9, 8.0, 69.5, 104.7, 114.8, 116.5, 121.3, 121.8, 127.9, 128.6, 130.0, 133.1, 133.6, 134.0, 141.8, 145.2, 159.0, 160.7. MS (ESI/APCI) m/z 407.2 $[M + H]^+$. Anal. Calcd. for $C_{22}H_{19}ClN_4O_2$: C, 64.94; H, 4.71; N, 13.77. Found: C, 64.88; H, 4.82; N, 13.55.

6-[(4-Chlorobenzyl)oxy]pyrimidin-4(3*H*)-one (20). To a stirred solution of **19** (2.5 g, 22.3 mmol) in THF (25 mL) was added Ag_2CO_3 (15.33 g, 55.75 mmol) and 4-chlorobenzyl bromide (4.58 g, 22.3 mmol). The resultant mixture was heated at reflux for 2 h. The reaction mixture was then cooled to rt, filtered through Celite®, and concentrated. The crude material was purified by column chromatography (silica gel, DCM/MeOH = 95/5 to 90/10) to afford the title compound (500 mg, 10%) as a white solid; mp 217–219 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.23 (2H, s), 5.60 (1H, s), 7.44 (4H, m), 8.11 (1H, s), 12.39 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 67.4, 91.7, 128.4, 129.6, 132.6, 135.3, 150.4, 163.1, 169.2. MS (ESI/APCI) m/z 237.2 $[M + H]^+$. Anal. Calcd. for $C_{11}H_9ClN_2O_2 \cdot 0.6H_2O$: C, 55.49; H, 3.88; N, 11.77. Found: C, 55.53; H, 3.89; N, 11.70.

(2-Cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)boronic acid (21). To a solution of **9a** (10 g, 33.5 mmol) in THF (335 mL) was added *n*-BuLi (1.6 M hexane solution, 62.9 mL, 101 mmol) at −78 °C. The mixture was stirred at the same temperature under N_2 atmosphere for 30 min. Boric acid triisopropyl ester (11.5 mL, 50.3 mmol) was added, and the mixture was stirred at −78 °C for 30 min and then at rt for 3 h. The mixture was neutralized with 6 N HCl solution and then concentrated in vacuo. The residue was dissolved with MeOH (30 mL) and then 3 N HCl solution (30 mL) was added to the mixture. The mixture was stirred at 60 °C overnight. The mixture was neutralized with 8 N NaOH solution at 0 °C and MeOH was evaporated. The mixture was basified with 8 N NaOH solution and washed with ether. The aqueous layer was neutralized with 6 N HCl solution at 0 °C. The precipitate was collected, and washed with water and ether to give the title compound (6.12 g, 84%) as a brown solid; mp 215 °C (decomposition). ^{1}H NMR (300 MHz,

CD_3OD) δ 0.93–1.00 (2H, m), 1.19 (2H, dd, *J* = 8.9, 2.1 Hz), 2.11–2.24 (1H, m), 2.61 (3H, s), 7.54 (1H, s), 7.87–8.01 (1H, m), 8.23 (1H,s). MS (ESI/APCI) *m/z* 217.3 $[M + H]^+$.

6-[(4-Chlorobenzyl)oxy]-3-(2-cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)pyrimidin-4(3*H*)-one (22). To a mixture of **20** (150 mg, 0.63 mmol) and **21** (275 mg, 1.26 mmol) in a mixture of DCM (15 mL) and MeOH (15 mL) were added $Cu(OAc)_2$ (346 mg, 1.89 mmol) and pyridine (0.5 mL, 6.3 mmol). The resulting reaction mixture was stirred at rt for 16 h. The insoluble material was then filtered through Celite® and the filtrate was poured into 1 N HCl solution. The mixture was extracted with DCM (100 mL) twice, and the combined DCM layers were washed with sat. $NaHCO_3$ solution (50 mL) and brine (50 mL) successively, dried over Na_2SO_4, and concentrated. The resulting residue was purified by preparative HPLC to afford the title compound (15 mg, 6%) as an off-white solid; mp 225–226 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 0.95–1.02 (4H, m), 2.05 (1H, m), 2.52 (3H, s), 5.37 (2H, s), 5.88 (1H, s), 7.24 (1H, dd, *J* = 9.5, 1.8 Hz), 7.38–7.49 (5H, m), 8.42 (2H, d, *J* = 6.2 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 8.00, 8.04, 67.8, 91.0, 114.9, 116.6, 122.5, 123.0, 123.7, 128.5, 129.7, 132.7, 135.2, 142.1, 145.3, 152.4, 161.9, 168.6. MS (ESI/APCI) *m/z* 407.0 $[M + H]^+$. Anal. Calcd. for $C_{22}H_{19}ClN_4O_2$: C, 64.94; H, 4.71; N, 13.77. Found: C, 64.71; H, 4.74; N, 13.62.

2-Chloro-4-[(4-chlorobenzyl)oxy]pyrimidine (24). To a stirred solution of **23** (10.4 g, 70.1 mmol) and (4-chlorophenyl)methanol (10 g, 70.0 mmol) in DMF (50 mL) was added K_2CO_3 (14.5 g, 105.2 mmol), and the resulting mixture was stirred at rt for 16 h. The mixture was then diluted with water (100 mL), and the resulting precipitate was collected by filtration and dried to give the title compound (4.8 g, 27%) as an off-white solid; mp 112–120 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.40 (2H, s), 7.06 (1H, d, *J* = 5.6 Hz), 7.46–7.52 (4H, m), 8.49 (1H, d, *J* = 5.7 Hz). MS (ESI/APCI) *m/z* 255.2 $[M + H]^+$.

4-[(4-Chlorobenzyl)oxy]pyrimidin-2-ol (25). To a stirred mixture of **24** (2.8 g, 11.0 mmol) in dioxane and water (1:4, 30 mL) was added NaOH (440 mg, 11 mmol), and the mixture was heated at reflux for 3 h. The reaction mixture was then cooled to 0 °C, and the precipitate was collected by filtration, washed with cold water and dried under vacuum to give the title compound (130 mg, 5%) as an off-white solid; mp 206–208 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 5.29 (2H, s), 5.92 (1H, d, *J* = 7.0 Hz), 7.45 (4H, s), 7.72 (1H, d, *J* = 6.8 Hz), 11.36 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 66.3, 93.5, 128.4, 130.0, 132.6, 135.2, 146.1, 156.1, 171.1. MS (ESI/APCI) *m/z* 237.2 $[M + H]^+$. Anal. Calcd. for $C_{11}H_9ClN_2O_2 \cdot 0.14H_2O$: C, 55.24; H, 3.91; N, 11.71. Found: C, 55.23; H, 3.84; N, 11.92.

4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)pyrimidin-2(1*H*)-one (26). The title compound was prepared in 18% using **9a** and **25** in an analogous manner to **10g**. Off-white solid; mp 205–207 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.85–0.92 (4H, m), 2.05–2.09 (1H, m), 2.47 (3H, s), 5.40 (2H, s), 6.21 (1H, d, *J* = 7.2 Hz), 7.19 (1H, dd, *J* = 9.5, 1.6 Hz),

7.44–7.52 (5H, m), 8.11 (1H, d, *J* = 7.2 Hz), 8.47 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 8.0, 8.1, 66.9, 94.7, 115.0, 116.6, 122.1, 122.8, 127.1, 128.5, 130.1, 132.8, 134.9, 141.9, 145.2, 150.1, 154.9, 171.1. MS (ESI/APCI) *m/z* 407.2 $[M + H]^+$. Anal. Calcd. for $C_{22}H_{19}ClN_4O_2 \cdot 0.18H_2O$: C, 64.43; H, 4.76; N, 13.66. Found: C, 64.42; H, 4.70; N, 13.57.

4-Bromo-3-methyl-pyridine 1-oxide (28). To a stirred solution of **27** (3.00 g, 19.4 mmol) in acetic acid (36 mL) was added dropwise acetyl bromide (23 mL) at 0 °C. After complete addition, the reaction mixture was heated at 80 °C for 3 h. The reaction mixture was cooled to rt and poured over crushed ice (200 g). The resulting mixture was neutralized with 1 N NaOH solution, and extracted with DCM (100 mL) twice. The organic layer was washed with water (100 mL) and brine (100 mL), dried over Na_2SO_4, and concentrated to give the title compound (2.2 g, 60%) as a light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.24 (3H, s), 7.62 (1H, d, *J* = 6.8 Hz), 7.97 (1H, dd, *J* = 6.8, 1.7 Hz), 8.28 (1H, s). MS (ESI/APCI) *m/z* 188.1 $[M + H]^+$.

4-[(4-Chlorobenzyl)oxy]-3-methylpyridine 1-oxide (29). The title compound was prepared in 28% yield using **28** and 4-chlorobenzyl alcohol in an analogous manner to **43b**. Off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.10 (3H, s), 5.19 (2H, s), 7.04 (1H, d, *J* = 7.1 Hz), 7.47 (4H, s), 8.01 (1H, d, *J* = 7.0 Hz), 8.07 (1H, s). MS (ESI/APCI) *m/z* 250.2 $[M + H]^+$.

4-[(4-Chlorobenzyl)oxy]-5-methylpyridin-2(1*H*)-one (30). To **29** (500 mg, 1.9 mmol) was added acetic anhydride (5 mL), and the solution was heated at reflux for 4 h. The reaction mixture was then cooled to rt, and concentrated. The residue was diluted with a mixture of MeOH (20 mL) and 1 N NaOH solution (10 mL), and the resulting solution was heated at reflux for 1 h. The mixture was cooled to rt, and concentrated. The residue was diluted with water (30 mL), and extracted with DCM (75 mL) three times. The combined DCM layers were concentrated, and purified by preparative HPLC to afford the title compound (20 mg, 4%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.89 (3H, s), 5.08 (2H, s), 5.76 (1H, s), 7.09 (1H, s), 7.47 (4H, s), 10.95 (1H, s). MS (ESI/APCI) *m/z* 250.2 $[M + H]^+$.

4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)-5-methylpyridin-2(1*H*)-one (31). The title compound was prepared in 42% yield using **9a** and **30** in an analogous manner to **10g**. Off-white solid; mp 238–239 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88–0.92 (4H, m), 1.97 (3H, s), 2.06 (1H, m), 2.47 (3H, s), 5.19 (2H, s), 5.98 (1H, s), 7.10 (1H, dd, *J* = 9.4, 1.9 Hz), 7.42 (1H, d, *J* = 9.4 Hz), 7.50 (4H, s), 7.54 (1H, s), 8.35 (1H, d, *J* = 1.3 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 8.0, 8.1, 112.2, 68.7, 97.2, 108.3, 114.8, 116.5, 121.9, 123.3, 127.3, 128.6, 129.3, 132.6, 135.1, 136.9, 141.9, 145.0, 162.5, 166.1. MS (ESI/APCI) *m/z* 420.2 $[M + H]^+$. Purity 99.8% (HPLC).

1-(4-Chlorophenyl)-3-(2,4-dichloropyridin-3-yl)propan-1-one (33). To a solution of **32** (1.12 g, 4.09 mmol) in DMF (40 mL) were added 1-(4-chlorophenyl)prop-2-en-1-ol (1.03 g, 6.13 mmol), $NaHCO_3$ (0.687 g, 8.18 mmol), and $Pd(OAc)_2$ (0.092 g, 0.41 mmol), and the mixture was heated at

120 °C for 15 h. The mixture was poured into water, and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 85/15) to give the title compound (0.772 g, 60%) as a light brown amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.20–3.26 (2H, m), 3.32–3.39 (2H, m), 7.29 (1H, d, *J* = 5.1 Hz), 7.45 (2H, d, *J* = 8.5 Hz), 7.92 (2H, d, *J* = 8.5 Hz), 8.19 (1H, d, *J* = 5.1 Hz).

1-(4-Chlorophenyl)-3-(2,4-dichloropyridin-3-yl)propan-1-ol (34). To a solution of **33** (100 mg, 0.32 mmol) in THF (3 mL) was added $LiBH_4$ (6.92 mg, 0.32 mmol) at rt. After 1 h, the mixture was quenched with sat. $NaHCO_3$ solution, and extracted with EtOAc. The organic layer was separated, washed with sat. $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 70/30) to give the title compound (83 mg, 82%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.93–2.10 (3H, m), 2.85–2.98 (1H, m), 3.01–3.14 (1H, m), 4.81 (1H, t, *J* = 7.8 Hz), 7.24 (1H, d, *J* = 5.1 Hz), 7.34 (4H, s), 8.13 (1H, d, *J* = 5.1 Hz).

5-Chloro-2-(4-chlorophenyl)-3,4-dihydro-2*H*-pyrano[3,2-*c*]pyridine (35). To a solution of **34** (678 mg, 2.14 mmol) in DMF (5 mL) was added NaH (60% oil dispersion, 86 mg, 2.14 mmol) at 0 °C, and the mixture was allowed to warm to rt with vigorous stirring. After 17 h, the mixture was quenched with water and extracted with EtOAc. The organic layer was separated, washed with 0.1 N NaOH solution and brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 70/30) to give the title compound (386 mg, 64%) as a pale yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95–2.13 (1H, m), 2.26–2.37 (1H, m), 2.79–3.02 (2H, m), 5.21 (1H, d, *J* = 9.9 Hz), 6.99 (1H, d, *J* = 5.4 Hz), 7.29–7.47 (4H, m), 7.97–8.11 (1H, m).

2-(4-Chlorophenyl)-2,3,4,6-tetrahydro-5*H*-pyrano[3,2-*c*]pyridin-5-one (36). The mixture of **35** (384 mg, 1.37 mmol), ammonium acetate (528 mg, 6.85 mmol) and AcOH (5 mL) was heated at 200 °C for 1 h under microwave irradiation. The solvent was evaporated and the residue was dissolved to EtOAc. The mixture was poured into sat. $NaHCO_3$ solution, and extracted with EtOAc. The organic layer was separated, washed with sat. $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (silica gel, EtOAc/MeOH = 100/0 to 90/20) to give the title compound (112 mg, 31%) as a pale orange amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.86–2.00 (1H, m), 2.25 (1H, dt, *J* = 13.9, 2.7 Hz), 2.48–2.80 (2H, m), 4.99–5.06 (1H, m), 6.01 (1H, d, *J* = 7.3 Hz), 7.15–7.19 (1H, m), 7.27 (1H, s), 7.29–7.35 (2H, m), 7.35–7.40 (2H, m). MS (ESI/APCI) *m/z* 279.8 $[M + H]^+$.

2-(4-Chlorophenyl)-6-(2-cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)-2,3,4,6-tetrahydro-5*H*-pyrano[3,2-*c*]pyridin-5-one (37). A mixture of **36** (30 mg, 0.11 mmol), **9a** (68.3 mg, 0.23 mmol), DMEDA (0.018 mL, 0.17 mmol), CuI (24.0 mg, 0.13 mmol), K_2CO_3 (47.5 mg, 0.34 mmol)

and DMSO (2 mL) was heated at 150 °C for 1 h under microwave irradiation. The reaction mixture was filtered through NH silica gel (EtOAc/MeOH = 90/10). The filtrate was poured into 1 N NaOH solution, and extracted with EtOAc. The organic layer was separated, washed with 0.1 N NaOH solution and brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (NH silica gel, EtOAc/MeOH = 100/0 to 90/10) to give the title compound (28.4 mg, 57%) as white crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.81–0.97 (4H, m), 1.88–2.02 (1H, m), 2.02–2.15 (1H, m), 2.22 (1H, d, *J* = 14.6 Hz), 2.48 (3H, s), 2.53–2.60 (2H , m), 5.23 (1H, d, *J* = 8.3 Hz), 6.12 (1H, d, *J* = 7.5 Hz), 7.13 (1H, d, *J* = 9.4 Hz), 7.40–7.54 (5H, m), 7.59 (1H, d, *J* = 7.7 Hz), 8.37 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 8.0, 8.1, 19.2, 27.9, 76.7, 99.9, 106.7, 114.9, 116.5, 122.0, 123.3, 127.5, 128.0, 128.5, 132.5, 137.2, 139.4, 141.9, 145.1, 161.8, 162.1. MS (ESI/APCI) *m/z* 432.4 $[M + H]^+$. Anal. Calcd. for $C_{25}H_{22}ClN_3O_2 \cdot 0.55H_2O$: C, 67.91; H, 5.25; N, 9.47. Found: C, 67.96; H, 5.27; N, 9.51.

3-[5-(4-Chlorophenyl)furan-2-yl]prop-2-enoyl azide (39). To a solution of **38** (1 g, 4.02 mmol) and triethylamine (0.729 mL, 5.23 mmol) in acetone (20 mL) at 0 °C was added dropwise isobutyl carbonochloridate (0.684 mL, 5.23 mmol). After stirring for 1 h at 0 °C, sodium azide (0.340 g, 5.23 mmol) in water (4 mL) was added, and the resultant mixture was stirred for further 30 min at 0 °C and at rt for 30 min. Water (40 mL) was added, and the resulting precipitate was collected by filtration, washed with water and dried to give the title compound (1.10 g, quant.) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.37 (1H, d, *J* = 15.5 Hz), 6.71–6.82 (2H, m), 7.35–7.42 (1H, m), 7.49 (1H, d, *J* = 15.5 Hz), 7.60–7.71 (2H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 109.9, 115.5, 120.8, 126.2, 127.9, 129.1, 132.2, 133.4, 149.8, 155.3, 171.1.

2-(4-Chlorophenyl)furo[3,2-*c*]pyridin-4(5*H*)-one (40). To a stirred mixture of diphenylether (40 mL) and tributylamine (10.0 mL, 41.7 mmol) at 200 °C was added dropwise a solution of **39** (3.83 g, 14.0 mmol) in diphenylether (60 mL) and THF (20 mL). After addition, the resulting brown mixture was stirred for 30 min before cooling to rt. Hexane (200 mL) was added, and the resulting suspension was filtered. The precipitate was washed with EtOH, filtered and dried to give the title compound (340 mg, 9.9%) as a light brown amorphous solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.70 (1H, d, *J* = 7.9 Hz), 7.34 (1H, d, *J* = 7.2 Hz), 7.49–7.59 (3H, m), 7.87 (2H, d, *J* = 8.7 Hz), 11.51 (1H, br s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 94.4, 103.0, 117.3, 125.8, 128.0, 129.0, 132.1, 133.0, 152.2, 159.1, 159.8. MS (ESI/APCI) *m/z* = 246.0 $[M + H]^+$.

2-(4-Chlorophenyl)-5-(2-cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)furo[3,2-*c*]pyridin-4(5*H*)-one (41). A mixture of **40** (100 mg, 0.41 mmol), **21** (106 mmol, 0.49 mmol), $Cu(OAc)_2$ (4.99 mg, 0.04 mmol), pyridine (66.0 μL, 0.81 mmol), MS4A (48.9 mg), and DMF (5 mL) was stirred at rt for 4 h and at 50 °C overnight. After filtration of the reaction mixture through Celite®, the filtrate was poured into 1 N HCl at rt and extracted with EtOAc. the organic layer was separated, washed with sat. $NaHCO_3$ solution and brine successively, dried over $MgSO_4$ and concentrated in vacuo.

The residue was purified by column chromatography (silica gel, hexane/EtOAc = 20/80 to 0/100). The solid was collected and washed with EtOH to give the title compound (10 mg, 5%) as an off-white solid; mp 278–279 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.84–0.99 (4H, m), 2.04–2.14 (1H, m), 6.94 (1H, dd, *J* = 7.6, 0.8 Hz), 7.21 (1H, dd, *J* = 9.4, 1.9 Hz), 7.49 (1H, d, *J* = 9.4 Hz), 7.53–7.59 (2H, m), 7.66 (1H, s), 7.75 (1H, d, *J* = 7.6 Hz), 7.88–7.96 (2H, m), 8.48 (1H, d, *J* = 1.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 8.0, 8.1, 95.3, 103.4, 115.0, 116.6, 117.1, 122.4, 123.3, 125.9, 127.3, 128.0, 129.1, 133.0, 137.1, 142.0, 145.2, 153.2, 158.4, 159.2. MS (ESI/APCI) *m/z* = 416.1 $[M + H]^+$. Anal. Calcd. for $C_{24}H_{18}ClN_3O_2$: C, 69.31; H, 4.36; N, 10.10. Found: C, 69.11; H, 4.48; N, 10.09.

4-[(4-Fluorobenzyl)oxy]pyridine 1-oxide (43a). The title compound was prepared in 6% yield using (4-fluorophenyl)methanol in an analogous manner to **43b**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.15 (2H, s), 7.04–7.13 (2H, m), 7.19–7.30 (2H, m), 7.46–7.57 (2H, m), 8.07–8.14 (2H, m). MS (ESI/APCI) *m/z* 220.1 $[M + H]^+$.

4-[(4-Chlorobenzyl)oxy]pyridine 1-oxide (43b). A solution of (4-chloropheny)methanol (49.5 g, 347 mmol) in THF (200 mL) was added dropwise to a suspension of NaH (60% oil dispersion, 16.7 g, 419 mmol) in THF (200 mL) at 0 °C. After the mixture was stirred at 0 °C for 30 min, **7b** (45.0 g, 347 mmol) was added portionwise to the reaction mixture. After completion of the addition, the mixture was stirred at rt for 5 h. The mixture was quenched with water (400 mL) at 0 °C, and extracted with EtOAc/THF (1:1) four times. The organic layers were combined, passed through NH-silica gel pad (EtOAc/MeOH) and concentrated. The filtrate was concentrated, and the residual solid was washed with IPE and dried to give the title compound (54.3 g, 66%) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.17 (2H, s), 7.08 (2H, d, *J* = 6.9 Hz), 7.48 (4H, s), 8.10 (2H, d, *J* = 7.0 Hz). MS (ESI/APCI) *m/z* 236.0 $[M + H]^+$.

4-{[4-(Trifluoromethyl)benzyl]oxy}pyridine 1-oxide (43c). The title compound was prepared in 29% yield using [4-(trifluoromethyl)phenyl]methanol in an analogous manner to **43b**. Yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.29 (2H, s), 7.10 (2H, d, *J* = 7.6 Hz), 7.67 (2H, d, *J* = 8.0 Hz), 7.78 (2H, d, *J* = 8.4 Hz), 8.11 (2H, d, *J* = 7.6 Hz). MS (ESI/APCI) *m/z* = 270.2 $[M + H]^+$.

4-[(4-Fluorobenzyl)oxy]pyridine-2(1*H*)-one (44a). The title compound was prepared in 58% yield using **43a** in an analogous manner to **44b**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.04 (2H, s), 5.78 (1H, d, *J* = 2.3 Hz), 5.90 (1H, dd, *J* = 7.2, 2.7 Hz), 7.17–7.28 (3H, m), 7.43–7.53 (2H, m), 11.10 (1H, br s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 68.5, 97.9, 99.1, 115.3 (d, *J* = 21 Hz), 130.2 (d, *J* = 8.3 Hz), 132.2 (d, *J* = 3 Hz), 135.4, 161.9 (d, *J* = 242.3 Hz), 164.0, 167.3. MS (ESI/APCI) *m/z* = 220.1 $[M + H]^+$.

4-[(4-Chlorobenzyl)oxy]pyridine-2(1*H*)-one (44b). A mixture of **43b** (54.3 g, 230 mmol), and acetic anhydride (540 mL, 5.71 mol) was stirred at 140 °C for 2 h. After concentration of the mixture, the residue was dissolved in MeOH (300 mL). Water (450 mL) was added to the mixture,

followed by stirring at rt for 1 h. The resulting precipitate was collected by filtration, washed with IPA, and dried to give the title compound (29.3 g, 54%) as a gray solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.99 (2H, s), 5.93 (1H, d, *J* = 2.3 Hz), 6.03 (1H, dd, *J* = 7.4, 2.5 Hz), 7.23 (1H, d, *J* = 7.2 Hz), 7.29–7.44 (4H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 68.4, 98.0, 99.1, 128.5, 129.7, 132.7, 135.0, 135.5, 163.9, 167.2. MS (ESI/APCI) *m/z* 236.0 $[M + H]^+$.

4-{[4-(Trifluoromethyl)benzyl]oxy}pyridin-2(1*H*)-one (44c). The title compound was prepared in 31% yield using **43c** in an analogous manner to **44b**. Off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.19 (2H, s), 5.77 (1H, d, *J* = 2.4 Hz), 5.93 (1H, dd, *J* = 7.2, 2.4 Hz), 7.26 (1H, d, *J* = 7.6 Hz), 7.64 (2H, d, *J* = 8.0 Hz), 7.77 (2H, d, *J* = 8.4 Hz), 11.12 (1H, s). MS (ESI/APCI) *m/z* 270.0 $[M + H]^+$.

1-(2-Cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)-4-hydroxypyridin-2(1*H*)-one (45). A mixture of **10c** (2.2 g, 5.92 mmol), 10% Pd-C (0.22 g, 2.07 mmol) and MeOH (40 mL) was stirred under H_2 atmosphere at ambient temperature for 1 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to give the title compound (1.65 g, 99%) as off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.82–0.98 (4H, m), 2.02–2.12 (1H, m), 2.47 (3H, s), 5.64 (1H, s), 5.97 (1H, d, *J* = 5.6 Hz), 7.10 (1H, d, *J* = 9.3 Hz), 7.41 (1H, d, *J* = 9.4 Hz), 7.57 (1H, d, *J* = 7.5 Hz), 8.33 (1H, s), 11.07 (1H, br s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 8.0, 8.1, 98.1, 101.2, 114.7, 116.5, 121.8, 123.5, 127.6, 139.6, 141.9, 144.9, 163.1, 168.7. MS (ESI/APCI) *m/z* 345.0 $[M + H]^+$.

4-Bromo-1-(2-cyclopropyl-3-methylimidazo[1,2-*a*]pyridin-6-yl)pyridin-2(1*H*)-one (46). To a solution of **45** (1.95 g, 6.93 mmol) in DMF (20 mL) was added phosphorus (V) tribromide oxide (1.77 mL, 17.4 mmol) at rt, and the mixture was heated at 110 °C for 1 h. The mixture was poured into sat. $NaHCO_3$ solution, and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 90/10 to 0/100) to give the title compound (1.70 g, 71%) as an off-white solid; mp 198–200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83–0.98 (4H, m), 2.04–2.12 (1H, m), 2.47 (3H, s), 6.60 (1H, d, *J* = 7.3 Hz), 6.88 (1H, s), 7.16 (1H, d, *J* = 9.5 Hz), 7.46 (1H, d, *J* = 9.3 Hz), 7.74 (1H, d, *J* = 7.3 Hz), 8.47 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.8, 7.99, 8.04, 109.3, 115.0, 116.7, 121.9, 122.1, 122.7, 126.7, 136.0, 140.3, 142.0, 145.3, 160.4. MS (ESI/APCI) *m/z* 345.0 $[M + H]^+$.

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4-[(3-Chlorobenzyl)oxy]pyridine-2(1*H*)-one (44d). The title compound was prepared in 49% yield using (3-chloropheny)methanol in an analogous manner to **44b**. Off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.07 (2H, s), 5.76 (1H, d, *J* = 2.4 Hz), 5.92 (1H, dd, *J* = 7.2 2.4 Hz), 7.25 (1H, d, *J* = 7.3 Hz), 7.34–7.45 (3H, m), 7.50 (1H, br s), 11.10 (1H, br s). MS (ESI/APCI) *m/z* = 236.0 $[M + H]^+$.

6-Bromo-2-cyclopropyl-3-methylimidazo[1,2-*b*]pyridazine (47a). To a solution of **49a** (1.0 g, 5.75 mmol) in DMA (10 mL) was added **50** (1.40 mL, 11.5 mmol) and $NaHCO_3$ (0.97 g, 11.5 mmol) at rt, and the mixture was stirred at 80 °C for 16 h. The mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 70/30) to give the title compound (1.10 g, 76%) as yellow crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.87–1.01 (4H, m), 2.14 (1H, br s), 2.51–2.53 (3H, s), 7.28 (1H, d, *J* = 9.3 Hz), 7.90 (1H, d, *J* = 9.3 Hz). MS (ESI/APCI) *m/z* = 252.0 $[M + H]^+$.

6-Bromo-2-cyclopropyl-3-methylimidazo[1,2-*a*]pyrazine (47b). To a solution of **53** (1.24 g, 2.92 mmol) in THF (10 mL) was added TFAA (0.826 mL, 5.84 mmol) at 0 °C, and the mixture was heated at 60 °C for 3 h. The mixture was poured into sat. $NaHCO_3$ solution at rt and extracted with EtOAc. The organic layer was separated, washed with 1 N NaOH solution and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 50/50) to give the title compound (0.59 g, 80%) as pale yellow crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.81–1.07 (4H, m), 2.15 (1H, t, *J* = 4.8 Hz), 2.53 (3H, s), 8.61 (1H, s), 8.68 (1H, s). MS (ESI/APCI) *m/z* = 252.2 $[M + H]^+$.

6-Bromo-2-cyclopropyl-3-methylimidazo[1,2-*a*]pyrimidine (47c). To a solution of **49b** (500 mg, 2.87 mmol) in DMF (10 mL) was added **50** (0.70 mL, 5.75 mmol) at rt, and the mixture was stirred at 100 °C for 24 h. The mixture was poured into 1 N NaOH solution and extracted with EtOAc. The organic layer was separated, washed with 1 N NaOH solution and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 100/0 to 50/50) to give the title compound (89 mg, 12%) as pale yellow crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.84–1.02 (4H, m), 2.04–2.15 (1H, m), 2.50 (3H, br s), 8.41 (1H, s), 8.98 (1H, s).

4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-3-methylimidazo[1,2-*b*]pyridazin-6-yl)pyridin-2(1*H*)-one (48a). The title compound was prepared in 28% yield using **44b** and **47a** in an analogous manner to **48c**. Pale yellow crystals; mp 234–236 °C (EtOAc–hexane). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.87–1.08 (4H, m), 2.17 (1H, br s), 2.52 (3H, br s), 5.19 (2H, s), 6.04 (1H, s), 6.23 (1H, d, *J* = 7.8 Hz), 7.30 (1H, d, *J* = 9.3 Hz), 7.50 (4H, s), 7.82 (1H, d, *J* = 7.8 Hz), 8.02 (1H, d, *J* =

9.4 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.6, 8.29, 8.31, 69.0, 97.7, 101.1, 115.6, 120.9, 124.0, 128.6, 129.8, 132.8, 134.7, 136.5, 137.8, 146.2, 148.1, 162.5, 167.5. MS (ESI/APCI) *m/z* = 407.3 $[M + H]^+$. Anal. Calcd for $C_{22}H_{19}ClN_4O_2$: C, 64.94; H, 4.71; N, 13.77. Found: C, 64.86; H, 4.63; N, 13.70.

4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-3-methylimidazo[1,2-*a*]pyrazin-6-yl)pyridin-2(1*H*)-one (48b). The title compound was prepared in 42% yield using **44b** and **47b** in an analogous manner to **48c**. Pale yellow crystals; mp 221–222 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92–1.04 (4H, m), 2.14–2.24 (1H, m), 2.54 (3H, s), 5.18 (2H, s), 6.01 (1H, d, *J* = 2.4 Hz), 6.17 (1H, dd, *J* = 7.7, 2.6 Hz), 7.50 (4H, s), 7.78 (1H, d, *J* = 7.7 Hz), 8.68 (1H, s), 8.83 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.5, 8.1, 8.7, 68.8, 97.7, 100.4, 115.1, 119.4, 128.5, 129.7, 132.8, 134.8, 135.8, 138.0, 138.3, 138.9, 149.2, 162.5, 166.9. MS (ESI/APCI) *m/z* = 407.4 $[M + H]^+$. Purity 97.8% (HPLC).

4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-3-methylimidazo[1,2-*a*]pyrimidin-6-yl)pyridin-2(1*H*)-one (48c). A mixture of **44b** (83 mg, 0.35 mmol), **47c** (89 mg, 0.35 mmol), DMEDA (0.075 mL, 0.71 mmol), CuI (66.6 mg, 0.35 mmol), K_2CO_3 (146 mg, 1.06 mmol), and DMSO (3 mL) was heated at 150 °C for 1 h under microwave irradiation. The mixture was poured into 28% NH_3 solution at rt and extracted with EtOAc. The organic layer was separated, washed with 0.1 N NaOH solution and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 50/50) to give the title compound (1.1 mg, 0.77 %) as white crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.95 (4H, d, *J* = 2.0 Hz), 2.06–2.17 (1H, m), 2.45 (3H, s), 5.18 (2H, s), 6.01–6.06 (1H, m), 6.16–6.24 (1H, m), 7.50 (4H, s), 7.68–7.75 (1H, m), 8.34–8.41 (1H, m), 8.87–8.94 (1H, m). MS (ESI/APCI) *m/z* = 407.4 $[M + H]^+$. Purity 99.2% (HPLC).

***N*-(5-Bromopyrazin-2-yl)-4-methylbenzenesulfonamide (52).** To a solution of **51** (2.0 g, 11.5 mmol) in pyridine (40 mL) was added TsCl (3.29 g, 17.2 mmol) at rt, and the mixture was stirred at rt overnight. The solvent was evaporated, and the residue was poured into sat. NH_4Cl solution, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 50/50) to give the title compound (1.92 g, 51%) as white crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.36 (3H, s), 7.40 (2H, d, *J* = 8.2 Hz), 7.82 (2H, d, *J* = 8.3 Hz), 8.18 (1H, s), 8.44 (1H, s), 11.67 (1 H, br s). MS (ESI/APCI) *m/z* = 328.0 $[M - H]^-$.

***N*-[(2*E*)-5-Bromo-1-(1-cyclopropyl-1-oxopropan-2-yl)pyrazin-2(1*H*)-ylidene]-4-methylbenzenesulfonamide (53).** To a solution of **52** (1.73 g, 5.27 mmol) in DMF (20 mL) was added NaH (60% oil dispersion, 0.32 g, 7.9 mmol) at 0 °C, and the mixture was stirred at rt for 30 min. To the mixture was added **50** (1.87 g, 10.5 mmol) at rt and the resulting mixture was stirred overnight. The mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was

purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 50/50) to give the title compound (1.02 g, 46%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.74–1.00 (4H, m), 1.71 (3H, t, *J* = 7.3 Hz), 2.21–2.29 (1H, m), 2.36 (3H, s), 5.63 (1H, d, *J* = 7.3 Hz), 7.34 (2H, d, *J* = 8.0 Hz), 7.69 (2H, d, *J* = 8.2 Hz), 8.31 (1H, s), 8.76 (1H, s).

4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (54a). To a stirred degassed mixture of **55a** (502 mg, 2.0 mmol), **44a** (470 mg, 2.0 mmol), and K_2CO_3 (552 mg, 4.0 mmol) in dioxane (15 mL) were added CuI (76 mg, 0.4 mmol) and *trans*-*N*,*N*′-dimethyl-cyclohexane-1,2-diamine (56 mg, 0.4 mmol). The reaction vessel was sealed and heated at 110 °C for 16 h. The reaction mixture was cooled to rt and concentrated. The resulting residue was diluted with DCM (250 mL), washed with brine (100 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (silica gel, DCM/MeOH = 97/3 to 96/4) to give the title compound (150 mg, 18%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 1.14–1.20 (4H, m), 2.24 (1H, m), 3.90 (3H, s), 5.16 (2H, s), 6.09 (1H, d, *J* = 2.6 Hz), 6.27 (1H, dd, *J* = 7.6, 2.7 Hz), 7.15 (1H, dd, *J* = 8.5, 2.0 Hz), 7.41–7.50 (5H, m), 7.69 (2H, t, *J* = 8.3 Hz). MS (ESI/APCI) *m/z* = 406.0 $[M + H]^+$. Anal. Calcd for $C_{23}H_{20}N_3O_2Cl$: C, 68.06; H, 4.97; N, 10.35. Found: C, 67.96; H, 5.01; N, 10.30.

1-(1,2-Dimethyl-1*H*-benzimidazol-6-yl)-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (54b). The title compound was prepared in 25% yield using **44c** and **55d** in an analogous manner to **54e**. White solid; mp 256–258 °C (EtOAc–hexane). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.55 (3H, s), 3.73 (3H, s), 5.14 (2H, s), 5.99 (1H, s), 6.09 (1H, d, *J* = 6.8 Hz), 7.06 (1H, d, *J* = 8.5 Hz), 7.26 (2H, t, *J* = 8.7Hz), 7.45–7.64 (5H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 13.4, 29.7, 68.8, 97.8, 99.8, 108.6, 115.4 (d, *J* = 22.2 Hz), 117.8, 120.2, 130.2 (d, *J* = 8.1 Hz), 132.1 (d, *J* = 3.0 Hz), 134.6, 135.6, 139.7, 141.5, 141.5, 153.6, 161.9 (d, *J* = 245.4 Hz), 162.7, 166.6. MS (ESI/APCI) *m/z* = 364.3 $[M+H]^+$. Anal. Calcd for $C_{21}H_{18}N_3O_2F$: C, 69.41; H, 4.99; N, 11.56. Found: C, 69.29; H, 5.04; N, 11.45.

1-(2-Ethyl-1-methyl-1*H*-benzimidazol-6-yl)-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (54c). A suspension of **56b** (5.00 g, 18.6 mmol), 1-(chloromethyl)-4-fluorobenzene (5.37 g, 37.1 mmol), K_2CO_3 (7.70 g, 55.7 mmol), and DMF (50 mL) was stirred at rt for 19 h. The resulting precipitate was collected by filtration, and the solid was washed with IPE and water successively to give a crude product (3.89 g). Other two bathes using 35 g and 50 g of **9b** gave 33.2 g and 50.0 g of crude product, respectively. Three lots were combined and recrystallized from MeOH–water to give the title compound (71.4 g, 57%, three bathes) as a white solid; mp 228–229 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (3H, t, *J* = 7.6 Hz), 2.93 (2H, q, *J* = 7.5 Hz), 3.73 (3H, s), 5.02 (2H, s), 6.01–6.11 (2H, m), 7.05–7.16 (3H, m), 7.24–7.45 (6H, m), 7.77 (1H, d, *J* = 8.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 11.3, 20.1, 29.5, 68.9, 97.8, 99.8, 108.8, 115.4 (d, *J* = 21.0 Hz), 118.1, 120.3, 130.3 (d, *J* = 8.3 Hz), 132.2 (d, *J* = 3.0 Hz), 134.7, 135.7, 139.7, 141.5, 157.8, 162.0 (d, *J* = 243.0 Hz), 162.8,

166.7. MS (ESI/APCI) *m/z* = 378.3 $[M + H]^+$. Anal. Calcd for $C_{22}H_{20}FN_3O_2$: C, 70.01; H, 5.34; N, 11.13. Found: C, 69.90; H, 5.26; N, 11.12.

4-[(4-Fluorobenzyl)oxy]-1-(1-methyl-2-propyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (54d). The mixture of **61** (90 mg, 0.27 mmol), HATU (106 mg, 0.28 mmol), *n*-butyric acid (0.024 mL, 0.27 mmol), DIPEA (0.136 mL, 0.80 mmol), and DMF (2 mL) was stirred at ambient temperature for 1 h. The mixture was quenched with water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved with AcOH (2.0 mL) and stirred at 90 °C for 1 h. After evaporating, the residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 90/10 to 0/100). The residual solid was recrystallized from EtOAc–MeOH to give the title compound (48.7 mg, 47%) as an off-white solid; mp 217–219 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.00 (3H, t, *J* = 7.4 Hz), 1.74–1.88 (2H, m), 2.86 (2H, t, *J* = 7.6 Hz), 3.74 (3H, s), 5.13 (2H, s), 5.98 (1H, s), 6.09 (1H, dd, *J* = 7.6, 3.0 Hz), 7.06 (1H, dd, *J* = 8.5, 2.1 Hz), 7.21–7.31 (2H, m), 7.50–7.56 (3H, m), 7.56–7.61 (2H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 13.7, 20.1, 28.4, 29.6, 68.8, 97.8, 99.8, 108.7, 115.3 (d, *J* = 21.2 Hz),118.0, 120.3, 130.2 (d, *J* = 8.1 Hz), 132.1 (d, *J* = 3.0 Hz), 134.6, 135.6, 135.6, 139.7, 141.5, 156.7, 161.9 (d, *J* = 245.4 Hz), 162.7, 162.8, 166.6. MS (ESI/APCI) *m/z* = 392.2 $[M + H]^+$. Anal. Calcd for $C_{23}H_{22}FN_3O_2 \cdot 0.1H_2O$: C, 70.25; H, 5.69; N, 10.69. Found: C, 70.28; H, 5.57; N, 10.71.

1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (54e). To a solution of **44c** (2.44 g, 11.2 mmol), **55a** (2.8 g, 11.15 mmol), K_2CO_3 (4.62 g, 33.5 mmol), and DMEDA (1.20 mL, 11.15 mmol) in DMSO (56 mL) was added CuI (2.12 g, 11.2 mmol), and the mixture was stirred at 150 °C under Ar atmosphere for 2 h. After cooling to 0 °C, 28% NH_3 solution (56.0 mL) was added, and the mixture was allowed to warm to rt for 2 h. The precipitate was collected by filtration, washed with water and IPE, dissolved in THF (500 mL), and filtered through a short NH silica-gel column (EtOAc). The filtrate was concentrated and the residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 90/10 to 0/100), followed by recrystallized from EtOH–water to give the title compound (1.60 g, 37%) as an off-white solid; mp 221–223 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99–1.15 (4H, m), 2.20–2.33 (1H, m), 3.85 (3H, s), 5.13 (2H, s), 5.98 (1H, d, *J* = 2.6 Hz), 6.09 (1H, dd, *J* = 7.7, 2.8 Hz), 7.00–7.09 (1H, m), 7.21–7.32 (2H, m), 7.43–7.67 (5H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.0, 8.3, 29.6, 68.8, 97.8, 99.8, 108.6, 115.3 (d, *J* = 22.2 Hz), 117.6, 120.5, 130.2 (d, *J* = 9.1 Hz), 132.1 (d, *J* = 3.0 Hz), 134.5, 135.7, 139.6, 141.0, 158.2, 161.9 (d, *J* = 245.4 Hz), 162.7, 166.6. MS (ESI/APCI) *m/z* = 390.2 $[M + H]^+$. Anal. Calcd for $C_{23}H_{20}N_3O_2F \cdot 0.1H_2O$: C, 70.61; H, 5.20; N, 10.74. Found: C, 70.53; H, 5.19; N, 10.69.

1-(2-Cyclobutyl-1-methyl-1*H*-benzimidazol-6-yl)-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (54f). The title compound was prepared in 22% yield using **44c** and **55g** in an analogous manner to **54a**. White solid; mp 246–249 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91–1.94 (1H, m), 2.05–2.12

(1H, m), 2.39–2.46 (4H, m), 3.66 (3H, s), 3.89 (1H, m), 5.13 (2H, s), 5.98 (1H, d, *J* = 2.8 Hz), 6.08 (1H, dd, *J* = 7.6, 2.8 Hz), 7.07 (1H, dd, *J* = 8.4, 2.0 Hz), 7.26 (2H, t, *J* = 8.8 Hz), 7.51–7.55 (3H, m), 7.57–7.63 (2H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 18.1, 26.4, 29.4, 31.5, 68.8, 97.8, 99.8, 108.7, 115.3 (d, *J* = 21.2 Hz), 118.2, 120.3, 130.2 (d, *J* = 9.1 Hz), 32.1 (d, *J* = 3.0 Hz), 134.7, 135.9, 139.6, 141.4, 159.2, 161.9 (d, *J* = 245.4 Hz), 162.7, 166.6. MS (ESI/APCI) *m/z* = 404.0 $[M + H]^+$. Anal. Calcd for $C_{24}H_{22}FN_3O_2 \cdot 0.11H_2O$: C, 71.10; H, 5.52; N, 10.36. Found: C, 71.14; H, 5.42; N, 10.32.

1-(2-Cyclopentyl-1-methyl-1*H*-benzimidazol-6-yl)-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (54g). The title compound was prepared in 22% yield using cyclopentanecarboxylic acid in an analogous manner to **54d**. White solid; mp 262–263 °C (EtOAc–hexane). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.59–1.85 (4H, m), 1.88–1.99 (2H, m), 2.07 (2H, br s), 3.45 (1H, t, *J* = 7.7 Hz), 3.76 (3H, s), 5.13 (2H, s), 5.99 (1H, d, *J* = 2.6 Hz), 6.09 (1H, dd, *J* = 7.5, 2.6 Hz), 7.06 (1H, dd, *J* = 8.5, 2.1 Hz), 7.26 (2H, t, *J* = 8.9 Hz), 7.46–7.64 (5H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.3, 29.6, 31.1, 36.2, 68.8, 97.8, 99.8, 108.7, 115.3 (d, *J* = 21.2 Hz), 118.1, 120.2, 130.2 (d, *J* = 8.1 Hz), 132.1 (d, *J* = 3.0 Hz), 134.6, 135.9, 139.7, 141.3, 160.3, 161.9 (d, *J* = 245.4 Hz), 162.7, 166.6. MS (ESI/APCI) *m/z* = 418.1 $[M + H]^+$. Anal. Calcd for $C_{25}H_{24}FN_3O_2 \cdot 0.14H_2O$: C, 71.49; H, 5.83; N, 10.00. Found: C, 71.48; H, 5.67; N, 10.04.

1-[2-(Cyclopropylmethyl)-1-methyl-1*H*-benzimidazol-6-yl]-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (54h). The title compound was prepared in 37% yield using **44c** and **55e** in an analogous manner to **54j**. Pale yellow solid; mp 206–214 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.27–0.37 (2H, m), 0.57–0.68 (2H, m), 1.12–1.25 (1H, m), 2.87 (2H, d, *J* = 6.4 Hz), 3.75 (3 H, s), 5.02 (2 H, s), 5.96–6.13 (2H, m), 7.04–7.16 (3H, m), 7.31 (1 H, d, *J* = 7.2 Hz), 7.35–7.45 (3 H, m), 7.79 (1 H, d, *J* = 8.3 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 4.5, 8.9, 29.8, 31.1, 68.8, 97.8, 99.8, 108.8, 115.3 (d, *J* = 21.2 Hz), 118.1, 120.3, 130.2 (d, *J* = 8.1 Hz), 132.1 (d, *J* = 3.0 Hz), 134.7, 135.6, 139.7, 141.6, 156.4, 161.9 (d, *J* = 245.4 Hz), 162.7, 166.6. MS (ESI/APCI) *m/z* = 404.2 $[M + H]^+$. Anal. Calcd for $C_{24}H_{22}N_3O_2F$: C, 71.45; H, 5.50; N, 10.42. Found: C, 70.88; H, 5.57; N, 10.13.

1-[2-(2,2-Dimethylpropyl)-1-methyl-1*H*-benzimidazol-6-yl]-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (54i). The title compound was prepared in 34% yield using **44c** and **55f** in an analogous manner to **54e**. White solid; mp 238–239 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.04 (9H, s), 2.81 (2H, s), 3.77 (3H, s), 5.14 (2H, s), 5.99 (1H, d, *J* = 2.6 Hz), 6.09 (1H, dd, *J* = 7.6, 2.6 Hz), 7.08 (1H, dd, *J* = 8.5, 2.1 Hz), 7.20–7.32 (2H, m), 7.48–7.56 (3H, m), 7.61 (2H, dd, *J* = 7.9, 5.3 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 29.4, 30.4, 32.4, 39.0, 68.8, 97.8, 99.8, 109.0, 115.3 (d, *J* = 21.2 Hz), 118.1, 120.4, 130.2 (d, *J* = 8.1 Hz), 132.1 (d, *J* = 3.0 Hz), 134.6, 135.3, 139.7, 141.6, 155.0, 161.9 (d, *J* = 245.4 Hz), 162.8, 166.7. MS (ESI/APCI) *m/z* = 420.2 $[M + H]^+$. Anal. Calcd for $C_{25}H_{26}FN_3O_2$: C, 71.58; H, 6.25; N, 10.02. Found: C, 71.46; H, 61.7; N, 9.97.

4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-1-ethyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one

(54j). A mixture of **44a** (100 mg, 0.42 mmol), **55b** (124 mg, 0.47 mmol), CuI (81 mg, 0.42 mmol), DMEDA (0.048 mL, 0.42 mmol), K_2CO_3 (147 mg, 1.06 mmol), and DMSO (2.5 mL) was heated 120 °C for 1 h under microwave irradiation. The mixture was quenched with 28% ammonia solution at rt and extracted with EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 75/25 to 0/100). The solid was crystallized from IPA–hexane to give the title compound (85 mg, 48%) as a light pink solid; mp 203–204 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08–1.19 (2H, m), 1.20–1.31 (2H, m), 1.46 (3H, t, *J* = 7.4 Hz), 1.93–2.05 (1H, m), 4.29 (2H, q, *J* = 7.2 Hz), 5.02 (2H, s), 6.01–6.08 (2H, m), 7.10 (1H, dd, *J* = 8.7, 1.9 Hz), 7.28–7.42 (6H, m), 7.70 (1H, d, *J* = 8.3 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.0, 7.1, 8.4, 15.0, 37.7, 68.7, 97.9, 99.8, 108.4, 117.8, 120.4, 128.5, 129.7, 132.7, 134.5, 134.8, 135.0, 139.8, 141.5, 157.6, 162.8, 166.6. MS (ESI/APCI) *m/z* = 420.1 $[M + H]^+$. Anal. Calcd for $C_{24}H_{22}ClN_3O_2$: C, 68.65; H, 5.28; N, 10.01. Found: C, 68.53; H, 5.29; N, 9.73.

4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-1-propyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (54k). The title compound was prepared in 11% yield using **44a** and **55c** in an analogous manner to **54j**. Off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (3H, t, *J* = 7.5 Hz), 1.08–1.20 (2H, m), 1.23–1.33 (2H, m), 1.85–1.96 (2H, m), 1.96–2.04 (1H, m), 4.20 (2H, t, *J* = 7.3 Hz), 5.02 (2H, s), 6.00–6.13 (2H, m), 7.01–7.16 (3H, m), 7.28–7.36 (2H, m), 7.37–7.45 (2H, m), 7.70 (1H, d, *J* = 8.3 Hz). MS (ESI/APCI) *m/z* = 434.2 $[M + H]^+$.

4-(Benzyloxy)-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (54l). The title compound was prepared in 87% yield using **44e** and **55a** in an analogous manner to **54a**. White solid; mp 210–211 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95–1.19 (4H, m), 2.18–2.34 (1H, m), 3.85 (3H, s), 5.15 (2H, s), 5.98 (1H, d, *J* = 3.0 Hz), 6.10 (1H, dd, *J* = 7.6, 2.6 Hz), 6.96–7.11 (1H, m), 7.29–7.66 (8H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.1, 8.3, 29.5, 69.6, 97.8, 99.8, 108.5, 117.7, 120.3, 127.8, 128.1, 128.5, 134.5, 135.8, 135.9, 139.6, 141.4, 158.3, 162.8, 166.7. MS (ESI/APCI) *m/z* = 372.0 $[M + H]^+$. Anal. Calcd for $C_{23}H_{21}N_3O_2$: C, 74.37; H, 5.70; N, 11.31. Found: C, 74.19; H, 5.76; N, 11.16.

4-[(3-Chlorobenzyl)oxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (54m). The title compound was prepared in 29% yield using **44b** and **55a** in an analogous manner to **54a**. White solid; mp 220–222 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02–1.11 (4H, m), 2.25–2.29 (1H, m), 3.85 (3H, s), 5.18 (2H, s), 5.97 (1H, d, *J* = 2.4 Hz), 6.12 (1H, dd, *J* = 7.5, 2.5 Hz), 7.04 (1H, dd, *J* = 8.5, 1.6 Hz), 7.43–7.48 (3H, m), 7.49–7.55 (3H, m), 7.59 (1H, d, *J* = 7.6 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.1, 8.4, 29.6, 68.6, 97.9, 99.8, 108.6, 117.8, 120.4, 126.4, 127.5, 128.1, 130.5, 133.1, 134.4, 135.8, 138.5, 139.8, 141.4, 158.3, 162.8, 166.5. MS (ESI/APCI) *m/z* = 405.8 $[M + H]^+$. Anal. Calcd for $C_{23}H_{20}ClN_3O_2$: C, 68.06; H, 4.97; N, 10.35. Found: C, 67.94; H, 4.91; N, 10.31.

4-[(2-Chlorobenzyl)oxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (54n). To a suspension of **56a** (100 mg, 0.36 mmol) and (2-chlorophenyl)methanol (101 mg, 0.71 mmol) in THF (2 mL) were added tributylphosphine (0.266 mL, 1.07 mmol) and ADDP (269 mg, 1.07 mmol) at 60 °C, and the mixture was stirred at the same temperature for 3 h. After solvent was removed by evaporation, the residue was purified by column chromatography (silica gel, hexane/EtOAc = 90/10 to 0/100, then EtOAc/MeOH = 100/0 to 85/15), followed by column chromatography (NH silica gel, hexane/EtOAc = 90/10 to 0/100, then EtOAc/MeOH = 100/0 to 85/15). The residual solid was recrystallized by EtOH–hexane to give the title compound (60.0 mg, 42%) as an off-white solid; mp 199–201 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.01–1.13 (4H, m), 2.23–2.31 (1H, m), 3.85 (3H, s), 5.20 (2H, s), 6.02 (1H, d, *J* = 2.6 Hz), 6.10 (1H, dd, *J* = 7.5, 2.5 Hz), 7.06 (1H, dd, *J* = 8.6, 1.6 Hz), 7.40–7.48 (2H, m), 7.50–7.57 (3H, m), 7.59 (1H, d, *J* = 7.5 Hz), 7.61–7.66 (1H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.1, 8.4, 29.6, 67.3, 97.7, 99.6, 108.6, 117.8, 120.4, 127.5, 129.5, 130.4, 130.7, 133.0, 133.1, 134.4, 135.8, 139.8, 141.4, 158.3, 162.8, 166.7. MS (ESI/APCI) *m/z* = 406.1 $[M + H]^+$. Anal. Calcd for $C_{23}H_{20}N_3O_2Cl$: C, 68.06; H, 4.97; N, 10.35. Found: C, 68.11; H, 4.88; N, 10.31.

4-[(5-Chloropyridin-2-yl)methoxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-pyridin-2(1*H*)-one (54o). The title compound was prepared in 14% yield using **56a** and (5-chloropyridin-2-yl)methanol in an analogous manner to **54c**. White solid; mp 234–236 °C (EtOH–H_2O). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.04–1.09 (4H, m), 2.26–2.32 (1H, m), 3.84 (3H, s), 5.23 (2H, s), 5.96 (1H, s), 6.12 (1H, dd, *J* = 7.7, 2.6 Hz), 7.04 (1H, m), 7.50–7.52 (2H, m), 7.59–7.61 (2H, m), 8.02–8.04 (1H, m), 8.67 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.1, 8.4, 29.6, 69.8, 98.0, 99.7, 108.5, 117.8, 120.4, 123.5, 130.5, 130.5, 134.4, 135.8, 136.9, 139.9, 141.4, 147.8, 154.1, 158.3, 162.7, 166.5. MS (ESI/APCI) *m/z* = 407.4 $[M + H]^+$. Anal. Calcd for $C_{22}H_{19}ClN_4O_2$: C, 64.94; H, 4.71; N, 13.77. Found: C, 64.77; H, 4.83; N, 13.50.

4-[(5-Chloropyrimidin-2-yl)methoxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (54p). A mixture of **54aa** (100 mg, 0.27 mmol), 2-chloro-1,3-bis(dimentylamino)trimethinium hexafluorophosphate (98 mg, 0.32 mmol), sodium methoxide (43.4 mg, 0.80 mmol), and MeOH (3 mL) was stirred at rt for 1 h. The mixture was concentrated and the residue was diluted with water. The aqueous phase was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 90/10 to 0/100) to give the title compound (56.0 mg, 51%) as a white solid; mp 217–219 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94–1.17 (4H, m), 2.26 (1H, br s), 3.85 (3H, s), 5.36 (2H, s), 5.87 (1H, br s), 6.13 (1H, d, *J* = 9.0 Hz), 7.04 (1H, d, *J* = 9.0 Hz), 7.51 (2H, br s), 7.59 (1H, d, J = 6.9 Hz), 9.02 (2H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.0, 8.3, 29.5, 69.5, 97.9, 99.6, 108.5, 117.7, 120.3, 129.8, 134.4, 135.8, 139.8, 141.4, 156.1, 158.3, 162.5, 162.6, 166.6. MS

(ESI/APCI) *m/z* = 408.3 $[M + H]^+$.

1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-4-(thiophen-2-ylmethoxy)pyridin-2(1*H*)-one (54q). The title compound was prepared in 30% yield using **56a** and thiophen-2-ylmethanol in an analogous manner to **54n**. White solid; mp 222–223 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.04–1.11 (4H, m), 2.24–2.28 (1H, m), 3.85 (3H, s), 5.34 (2H, s), 6.05–6.07 (2H, m), 7.03–7.08 (2H, m), 7.27 (1H, d, *J* = 2.8 Hz), 7.50–7.52 (2H, m), 7.57 (1H, d, *J* = 7.2 Hz), 7.61 (1H, d, *J* = 4.9 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.1, 8.3, 29.5, 64.4, 97.8, 99.7, 108.5, 117.8, 120.4, 126.9, 127.3, 128.3, 134.4, 135.8, 137.8, 139.7, 141.4, 158.3, 162.7, 166.3. MS (ESI/APCI) *m/z* = 377.8 $[M + H]^+$. Purity 99.4% (HPLC).

1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-4-(thiophen-3-ylmethoxy)pyridin-2(1*H*)-one (54r). The title compound was prepared in 37% yield using **9a** and thiophen-3-ylmethanol in an analogous manner to **54n**. Off-white solid; mp 223–225 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02–1.09 (4H, m), 2.24–2.28 (1H, m), 3.85 (3H, s), 5.13 (2H, s), 5.99 (1H, d, *J* = 2.6 Hz), 6.06 (1H, dd, *J* = 7.5, 2.6 Hz), 7.04 (1H, dd, *J* = 8.4, 1.7 Hz), 7.19 (1H, d, *J* = 4.2 Hz), 7.50–7.52 (2H, m), 7.56–7.60 (2H, m), 7.64 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.1, 8.3, 29.5, 65.1, 97.6, 99.8, 108.5, 117.8, 120.4, 124.7, 126.8, 127.6, 134.5, 135.8, 136.7, 139.6, 141.4, 158.3, 162.8. MS (ESI/APCI) *m/z* = 378.2 $[M + H]^+$. Anal. Calcd for $C_{21}H_{19}N_3O_2S \cdot 0.12H_2O$: C, 66.44; H, 5.11; N, 11.07. Found: C, 66.49; H, 5.09; N, 11.08.

4-[(5-Chlorothiophen-2-yl)methoxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (54s). The title compound was prepared in 33% yield using **56a** and (5-chlorothiophen-2-yl)methanol in an analogous manner to **54n**. White solid; mp 218–220 °C (EtOH–H_2O). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02–1.11 (4H, m), 2.23–2.28 (1H, m), 3.85 (3H, s), 5.29 (2H, s), 6.03–6.07 (2H, m), 7.02 (1H, dd, *J* = 8.5, 1.8 Hz), 7.08 (1H, d, *J* = 3.7 Hz), 7.16 (1H, d, *J* = 3.8 Hz), 7.51 (2H, dd, *J* = 5.4, 3.5 Hz), 7.57 (1H, d, *J* = 7.4 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.1, 8.4, 29.6, 64.4, 97.9, 99.6, 108.5, 117.8, 120.4, 126.6, 128.3, 129.1, 134.4, 135.8, 137.3, 139.8, 141.4, 158.3, 162.7, 166.1. MS (ESI/APCI) *m/z* = 412.3 $[M + H]^+$. Anal. Calcd for $C_{21}H_{18}ClN_3O_2S$: C, 61.23; H, 4.40; N, 10.20. Found: C, 61.34; H, 4.43; N, 10.21.

4-[(4-Chlorothiophen-2-yl)methoxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (54t). The title compound was prepared in 24% yield using **56a** and (4-chlorothiophen-2-yl)methanol in an analogous manner to **54n**. Off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.04–1.09 (4H, m), 2.26 (1H, m), 3.85 (3H, s), 5.32 (2H, s), 6.03–6.08 (2H, m), 7.04 (1H, dd, *J* = 8.5, 1.8 Hz), 7.28 (1H, s), 7.50–7.52 (2H, m), 7.58 (1H, d, *J* = 7.5 Hz), 7.64 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.1, 8.3, 29.5, 64.0, 97.9, 99.6, 108.5, 117.8, 120.3, 122.1, 123.0, 127.8, 134.4, 135.8, 139.4, 139.8, 141.4, 158.3, 162.7, 166.1. MS (ESI/APCI) *m/z* = 412.2 $[M + H]^+$. Purity >99.9% (HPLC).

4-[(5-Chlorothiophen-3-yl)methoxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (54u). The title compound was prepared in 9% yield using **56a** and (5-chlorothiophen-3-yl)methanol in an analogous manner to **54n**. White solid; mp 236–237 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02–1.11 (4H, m), 2.24–2.32 (1H, m), 3.85 (3H, s), 5.05 (2H, s), 5.98 (1H, d, *J* = 2.6 Hz), 6.06 (1H, dd, *J* = 7.6, 2.7 Hz), 7.03 (1H, dd, *J* = 8.4, 1.8 Hz), 7.20 (1H, d, *J* = 1.4 Hz), 7.51 (2H, dd, *J* = 5.4, 3.5 Hz), 7.56–7.58 (2H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.1, 8.3, 29.5, 65.0, 97.7, 99.7, 108.5, 117.7, 120.3, 124.2, 127.0, 128.8, 134.4, 135.8, 136.5, 139.7, 141.4, 158.3, 162.7, 166.5. MS (ESI/APCI) *m/z* = 412.0 $[M + H]^+$. Anal. Calcd for $C_{21}H_{18}ClN_3O_2S$: C, 61.23; H, 4.40; N, 10.20. Found: C, 61.19; H, 4.39; N, 10.17.

1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-4-{[5-(trifluoromethyl)thiophen-2-yl]methoxy}pyridin-2(1*H*)-one (54v). The title compound was prepared in 35% yield using **56a** and [5-(trifluoromethyl)thiophen-2-yl]methanol in an analogous manner to **54n**. White solid; mp 256–257 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.99–1.14 (4H, m), 2.22–2.31 (1H, m), 3.85 (3H, s), 5.45 (2H, s), 6.06 (1H, d, *J* = 2.5 Hz), 6.10 (1H, dd, *J* = 7.5, 2.7 Hz), 7.05 (1H, dd, *J* = 8.5, 1.8 Hz), 7.38 (1H, d, *J* = 2.8 Hz), 7.49–7.54 (2H, m), 7.60 (1H, d, *J* = 7.5 Hz), 7.69 (1H, d, *J* = 2.8 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.0, 8.3, 29.5, 64.0, 98.0, 99.5, 108.5, 117.8, 120.3, 125.0 (q, *J* = 269.7 Hz), 128.1, 129.4 (q, *J* = 37.4 Hz), 130.0 (q, *J* = 4.0 Hz), 134.4, 135.8, 139.9, 141.4, 143.4 (d, *J* = 2.0 Hz), 158.3, 162.6, 166.0. MS (ESI/APCI) *m/z* = 446.1 $[M + H]^+$. Anal. Calcd for $C_{22}H_{18}F_3N_3O_2S$: C, 59.32; H, 4.07; N, 9.43. Found: C, 59.43; H, 4.10; N, 9.42.

1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-4-{[4-(trifluoromethyl)thiophen-2-yl]methoxy}pyridin-2(1*H*)-one (54w). NaH (60% oil dispersion, 87 mg, 2.18 mmol) was added to a solution of [4-(trifluoromethyl)thiophen-2-yl]methanol (**90**, 397 mg, 2.18 mmol) in DMA at 0 °C. After being stirred at the same temperature for 30 min, **57** (500 mg, 1.45 mmol) was added to the reaction mixture. The mixture was stirred at 120 °C for 10 min. The mixture was quenched with water at 0 °C and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 100/0 to 0/100), followed by preparative HPLC (L-Column 2 ODS, eluted with H_2O in acetonitrile containing 0.1% TFA). The desired fraction was neutralized with sat. $NaHCO_3$ solution and extracted with EtOAc. The organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo to give the title compound (330 mg, 51%) as a pale yellow solid; mp 218–219 °C (IPA–IPE). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.95–1.14 (4H, m), 2.21–2.33 (1H, m), 3.85 (3H, s), 5.39 (2H, s), 6.05–6.10 (2H, m), 7.05 (1H, dd, *J* = 8.5, 1.2 Hz), 7.52 (2H, dd, *J* = 5.0, 3.2 Hz), 7.56–7.67 (2H, m), 8.33 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.0, 8.3, 29.5, 63.9, 97.9, 99.6, 108.5, 117.8, 120.3, 122.0 (q, *J* = 270.7 Hz),124.57, 124.59, 129.3 (q, *J* = 35.4 Hz), 129.6 (q, *J* = 4.0 Hz), 134.4, 135.8, 139.8, 141.4, 158.3, 162.7, 166.1. MS (ESI/APCI) *m/z* = 446.1 $[M + H]^+$. Anal. Calcd for $C_{22}H_{18}F_3N_3O_2S \cdot 0.25H_2O$: C, 58.72;

H, 4.14; N, 9.34. Found: C, 58.89; H, 4.21; N, 9.29.

1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-4-{[5-(trifluoromethyl)thiophen-3-yl]methoxy}pyridin-2(1*H*)-one (54x). The title compound was prepared in 26% yield using **57** and [5-(trifluoromethyl)thiophen-3-yl]methanol (**94**) in an analogous manner to **54x**. White solid; mp 236–237 °C (EtOH–H_2O). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02–1.12 (4H, m), 2.22–2.31 (1H, m), 3.85 (3H, s), 5.16 (2H, s), 6.01 (1H, d, *J* = 2.5 Hz), 6.09 (1H, dd, *J* = 7.6, 2.4 Hz), 7.04 (1H, dd, *J* = 8.5, 1.3 Hz), 7.48–7.55 (2H, m), 7.59 (1H, d, *J* = 7.5 Hz), 7.81 (1H, s), 8.06 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.0, 8.3, 29.5, 64.5, 97.7, 99.7, 108.5, 117.8, 120.3, 122.4 (q, *J* = 270.0 Hz), 129.6, 129.8 (q, *J* = 37.4 Hz), 130.2 (q, *J* = 3.0 Hz), 134.4, 135.8, 137.2, 139.7, 141.4, 158.3, 162.7, 166.4. MS (ESI/APCI) *m/z* = 446.3 $[M + H]^+$. Anal. Calcd for $C_{22}H_{18}F_3N_3O_2S$: C, 59.32; H, 4.07; N, 9.43. Found: C, 59.36; H, 4.25; N, 9.34.

4-(Benzyloxy)-1-(2-ethyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (54y). The title compound was prepared in 47% yield using **44e**and **55h** in an analogous manner to **54e**. White solid; mp 214–216 °C (MeOH–H_2O). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (3H, t, *J* = 7.5 Hz), 2.93 (2H, q, *J* = 7.6 Hz), 3.73 (3H, s), 5.06 (2H, s), 6.03–6.13 (2H, m), 7.13 (1H, dd, *J* = 8.4, 1.9 Hz), 7.28–7.48 (7H, m), 7.77 (1H, d, *J* = 8.4 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 11.3, 20.1, 29.5, 69.6, 97.8, 99.9, 108.8, 118.1, 120.3, 127.9, 128.2, 128.5, 134.7, 135.7, 135.9, 139.7, 141.5, 157.8, 162.8, 166.8. MS (ESI/APCI) *m/z* = 360.3 $[M + H]^+$. Anal. Calcd for $C_{22}H_{21}N_3O_2 \cdot 0.92H_2O$: C, 70.28; H, 6.12; N, 11.18. Found: C, 70.18; H, 5.72; N, 11.13.

{[1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-2-oxo-1,2-dihydropyridin-4-yl]oxy}acetonitrile (54z). A mixture of **56a** (1.00 g, 3.55 mmol), bromoacetonitrile (0.27 mL, 3.91 mmol), K_2CO_3 (1.47 g, 10.7 mmol), and DMF (10 mL) was stirred at 80 °C for 2 h. The mixture was poured into water and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 97/3 to 0/100) to give the title compound (0.87 g, 76%) as a white solid; mp 184–186 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.97–1.20 (4H, m), 2.27 (1H, br s), 3.85 (3H, s), 5.25 (2H, s), 6.08 (1H, br s), 6.14 (1H, d, *J* = 7.65 Hz), 7.07 (1H, d, *J* = 8.5 Hz), 7.49–7.59 (2H, m), 7.67 (1H, d, *J* = 7.4 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.1, 8.4, 29.5, 53.3, 98.2, 98.9, 108.6, 115.7, 117.8, 120.3, 134.2, 135.8, 140.4, 141.5, 158.4, 162.4, 164.9. MS (ESI/APCI) *m/z* = 321.3 $[M + H]^+$.

2-{[1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-2-oxo-1,2-dihydropyridin-4-yl]oxy}ethanimidamide hydrochloride (54aa). Sodium methoxide (2.53 mg, 0.050 mmol) was added to a solution of **54z** (300 mg, 0.94 mmol) in MeOH (4 mL) and the mixture was stirred at rt for 4 h. To the solution was added ammonium chloride (52.6 mg, 0.98 mmol) and the mixture was stirred at rt overnight. The solvent was evaporated to give the title compound (368 mg, quant.) as a light brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.19 (4H, m), 2.27 (1H, br s), 3.86 (3H, s), 4.99 (2H, s),

5.87 (1H, s), 6.16 (1H, d, *J* = 5.3 Hz), 7.04 (1H, d, *J* = 7.8 Hz), 7.48–7.57 (2H, m), 7.68 (1H, d, *J* = 7.5 Hz), 9.04 (3H, br s). MS (ESI/APCI) *m/z* = 321.3 $[M + H]^+$.

6-Bromo-2-cyclopropyl-1-methyl-1*H*-benzimidazole (55a). A mixture of **59a** (4.20 g, 18.2 mmol), zinc (5.94 g, 90.9 mmol), NH_4Cl (9.7 g, 182 mmol), MeOH (50 mL), and water (25 mL) was stirred at rt for 3 h. After MeOH was removed by evaporation, the mixture was neutralized with sat. $NaHCO_3$ solution and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Then the residue was dissolved in $POCl_3$ (1.68 mL, 18.0 mmol) and cyclopropanecarboxylic acid (2.86 mL, 36.0 mmol) was added to the mixture at rt. The mixture was stirred at 120 °C for 3 h. After cooling to 0 °C, ice water and sat. $NaHCO_3$ solution were carefully added, and the mixture was extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, concentrated to give a brown solid. This solid was dissolved in 1 N HCl solution and washed with EtOAc. The aqueous layer was basified with 4 N NaOH solution and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to give the title compound (3.3 g, 72%) as a brown solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.95–1.14 (4H, m), 2.23 (1H, tt, *J* = 7.9, 5.1 Hz), 3.83 (3H, s), 7.24 (1H, dd, *J* = 8.5, 2.1 Hz), 7.41 (1H, d, *J* = 8.7 Hz), 7.75 (1H, d, *J* = 1.9 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.0, 8.4, 29.5, 112.4, 113.4, 119.6, 123.9, 137.2, 141.1, 157.9. Anal. Calcd for $C_{11}H_{11}BrN_2$: C, 52.61; H, 4.42; N, 11.16. Found: C, 52.37; H, 4.31; N, 11.14.

6-Bromo-2-cyclopropyl-1-ethyl-1*H*-benzimidazole (55b). Zinc (8.0 g, 122 mmol) was added to a solution of **59b** (3.0 g, 12.2 mmol) in AcOH (60 mL) at rt. The mixture was stirred at ambient temperature for 30 min. The insoluble material was removed by filtration and the filtrate was concentrated in vacuo. The mixture was neutralized with sat. $NaHCO_3$ solution and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo to give an intermediate 4-bromo-N^2-ethylbenzene-1,2-diamine.

HATU (4.89 g, 12.9 mmol) was added to a solution of the intermediate 4-bromo-N^2-ethylbenzene-1,2-diamine, DIPEA (6.40 mL, 36.7 mmol), and cyclopropanecarboxylic acid (0.98 mL, 12.2 mmol) in DMF (40 mL), and the mixture was stirred at rt for 1 h. The mixture was quenched with water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in AcOH (40 mL) and the mixture was stirred at 80 °C for 1 h. After concentration of the mixture, the residue was neutralized with sat. $NaHCO_3$ solution and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 0/100) to give the title compound (1.2 g, 37%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03–1.18 (2H, m), 1.19–1.30 (2H, m), 1.46 (3H, t, *J* = 7.2 Hz), 1.96 (1H, tt, *J* = 8.2, 4.9 Hz), 4.25 (2H, q, *J* = 7.3 Hz), 7.29 (1H, dd, *J* = 8.3, 1.9 Hz), 7.43 (1H, d, *J* =

1.5 Hz), 7.50 (1H, d, *J* = 8.3 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.0, 8.4, 14.9, 37.6, 112.3, 113.5, 119.7, 123.9, 136.1, 141.2, 157.3. MS (ESI/APCI) *m/z* = 265.1 $[M + H]^+$. Anal. Calcd for $C_{12}H_{13}BrN_2$: C, 54.36; H, 4.94; N, 10.57. Found: C, 54.29; H, 4.87; N, 10.58.

6-Bromo-2-cyclopropyl-1-propyl-1*H*-benzimidazole (55c). The title compound was prepared in 56% yield using **59c** in an analogous manner to **55a**. Pink solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (4H, t, *J* = 7.4 Hz), 1.07–1.16 (2H, m), 1.21–1.30 (2H, m), 1.80–2.01 (3H, m), 4.13–4.21 (2H, m), 7.26–7.32 (1H, m), 7.42 (1H, d, *J* = 1.9 Hz), 7.50 (1H, d, *J* = 8.7 Hz). MS (ESI/APCI) *m/z* = 279.1 $[M + H]^+$.

6-Bromo-1,2-dimethyl-1*H*-benzimidazole (55d). To a solution of **63a** (30.0 g, 109 mmol) in AcOH (300 mL) was added zinc powder (35.9 g, 549 mmol) at rt. After being stirred over 90 °C for 4 h, the reaction mixture was allowed to cool to rt, and the zinc dust was removed by filtration with Celite pad and washed with EtOAc. The filtrate was concentrated and partitioned between EtOAc and sat. $NaHCO_3$ solution. The resulting precipitate was removed by filtration with Celite pad, and the filtrate was extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified by chromatography (NH silica gel, hexane/EtOAc = 100/0 to 0/100) to give the title compound (16.0 g, 65%) as a purple solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.56–2.61 (3H, m), 3.69 (3H, s), 7.32 (1H, dd, *J* = 8.5, 1.3 Hz), 7.43 (1H, s), 7.53 (1H, d, *J* = 8.4 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 13.4, 29.8, 112.6, 113.6, 119.6, 123.8, 137.1, 141.3, 153.3. MS (ESI/APCI) *m/z* = 225.1 $[M + H]^+$. Anal. Calcd for $C_9H_9BrN_2$: C, 48.02; H, 4.03; N, 12.45. Found: C, 48.12; H, 3.99; N, 12.52.

6-Bromo-2-(cyclopropylmethyl)-1-methyl-1*H*-benzimidazole (55e). The title compound was prepared in 96% yield using **59a** and cyclopropylacetic acid in an analogous manner to **55b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.31 (2H, d, *J* = 6.0 Hz), 0.63 (2H, dd, *J* = 7.9, 1.1 Hz), 1.08–1.27 (1H, m), 2.83 (2H, d, *J* = 6.4 Hz), 3.72 (3H, s), 7.34 (1H, d, *J* = 1.9 Hz), 7.45 (1H, d, *J* = 1.9 Hz), 7.58 (1H, d, *J* = 8.7 Hz). MS (ESI/APCI) *m/z* = 265.1 $[M + H]^+$.

6-Bromo-2-(2,2-dimethylpropyl)-1-methyl-1*H*-benzimidazole (55f). The title compound was prepared in 78% yield using **63b** in an analogous manner to **55d**. White solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (9H, s), 2.77 (2H, s), 3.75 (3H, s), 7.28 (1H, dd, *J* = 8.7, 1.9 Hz), 7.51 (1H, d, *J* = 8.3 Hz), 7.76 (1H, d, *J* = 1.9 Hz). MS (ESI/APCI) *m/z* = 281.0 $[M + H]^+$.

6-Bromo-2-cyclobutyl-1-methyl-1*H*-benzimidazole (55g). To a mixture of cyclobutanecarboxylic acid (352 μL, 3.73 mmol) and **62** (500 mg, 2.48 mmol) was added $POCl_3$ (10 mL) and the mixture was heated under reflux for 4 h. The mixture was cooled to rt and poured into ice-cold sat. $NaHCO_3$ solution (100 mL). The mixture was extracted with EtOAc, and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The reside was purified by column chromatography (silica gel, hexane/EtOAc = 70/30) to afford the title compound (300 mg, 45%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.89–

1.95 (1H, m), 2.01–2.12 (1H, m), 2.37–2.46 (4H, m), 3.64 (3H, s), 3.80–3.88 (1H, m), 7.27 (1H, dd, *J* = 8.4, 1.6 Hz), 7.51 (1H, d, *J* = 8.4 Hz), 7.75 (1H, d, *J* = 1.6 Hz). MS (ESI/APCI) *m/z* = 266.8 $[M + H]^+$.

6-Bromo-2-ethyl-1-methyl-1*H*-benzimidazole (55h). The title compound was prepared in 57% yield using **59a** and propanoic acid in an analogous manner to **55e**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (3H, t, *J* = 7.5 Hz), 2.89 (2H, q, *J* = 7.5 Hz), 3.69 (3H, s), 7.32 (1H, dd, *J* = 8.5, 1.8 Hz), 7.44 (1H, d, *J* = 1.5 Hz), 7.57 (1H, d, *J* = 8.5 Hz). MS (ESI/APCI) *m/z* = 239.0 $[M + H]^+$.

1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-4-hydroxypyridin-2(1*H*)-one (56a). A mixture of **54l** (2.4 g, 6.46 mmol), 10% Pd-C (1.2 g), and MeOH (60 mL) was hydrogenated under H_2 atmosphere (1 atm) at rt for 3 h. The inorganic material was removed by filtration and the filtrate was concentrated in vacuo to give the title compound (1.57 g, 86%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.99–1.13 (4H, m), 2.21–2.30 (1H, m), 3.85 (3H, s), 5.65 (1H, br s), 5.95 (1H, d, *J* = 7.4 Hz), 7.02 (1H, dd, *J* = 8.4, 2.0 Hz), 7.40–7.58 (3H, m), 10.78 (1H, br s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.1, 8.4, 29.6, 108.4, 109.0, 117.9, 120.0, 121.9, 133.8, 135.3, 135.8, 140.2, 141.7, 158.6, 160.4. MS (ESI/APCI) *m/z* = 282.1 $[M + H]^+$. Anal. Calcd for $C_{16}H_{15}N_3O_2 \cdot 0.11H_2O$: C, 67.84; H, 5.42; N, 14.83. Found: C, 67.80; H, 5.42; N, 14.81.

1-(2-Ethyl-1-methyl-1*H*-benzimidazol-6-yl)-4-hydroxypyridin-2(1*H*)-one (56b). The title compound was prepared in 99% yield using **54y** in an analogous manner to **56a**. Pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (3H, t, *J* = 7.5 Hz), 2.90 (2H, q, *J* = 7.5 Hz), 3.74 (3H, s), 5.65 (1H, d, *J* = 2.5 Hz), 5.96 (1H, dd, *J* = 7.5, 2.5 Hz), 7.04 (1H, dd, *J* = 8.4, 2.0 Hz), 7.49–7.62 (3H, m), 10.88 (1H, br s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 11.3, 20.0, 29.5, 98.4, 100.2, 108.7, 117.9, 120.3, 135.0, 135.7, 139.9, 141.4, 157.7, 163.0, 167.2. MS (ESI/APCI) *m/z* = 470.2 $[M + H]^+$. Anal. Calcd for $C_{15}H_{15}N_3O_2 \cdot 1.35H_2O$: C, 61.36; H, 6.08; N, 14.31. Found: C, 61.49; H, 5.73; N, 14.39.

5-Bromo-*N*-methyl-2-nitroaniline (58a). To a solution of **57** (25.0 g, 114 mmol) in EtOH (100 mL) was added methylamine (40% in MeOH, 34.8 mL, 341 mmol) at rt. The mixture was stirred at rt for 1 h and then cooled to 0 °C. The precipitate was collected by filtration, and washed with EtOH and IPE successively to give the title compound (24.8 g, 94%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.95 (3 H, d, *J* = 4.9 Hz), 6.83 (1 H, dd, *J* = 9.1, 1.9 Hz), 7.17 (1 H, d, *J* = 1.9 Hz), 7.98 (1 H, d, *J* = 9.1 Hz), 8.23 (1H, br s).

5-Bromo-*N*-ethyl-2-nitroaniline (58b). The title compound was prepared in 80% yield using ethylamine in an analogous manner to **58a**. Pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (3H, t, *J* = 7.2 Hz), 3.33 (2H, qd, *J* = 7.2, 5.1 Hz), 6.75 (1H, dd, *J* = 9.1, 1.9 Hz), 7.01 (1H, d, *J* = 1.9 Hz), 7.98 (1H, br s), 8.03 (1H, d, *J* = 9.1 Hz).

5-Bromo-2-nitro-*N*-propylaniline (58c). The title compound was prepared in 76% yield using *n*-propylamine in an analogous manner to **58a**. Orange solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.06

(3H, t, *J* = 7.5 Hz), 1.77 (2H, m), 3.25 (2H, td, *J* = 7.1, 5.1 Hz), 6.74 (1H, dd, *J* = 9.0, 1.9 Hz), 7.01 (1H, d, *J* =1.9 Hz), 7.92–8.11 (2H, m).

4-[(4-Fluorobenzyl)oxy]-1-[3-(methylamino)-4-nitrophenyl]pyridin-2(1*H*)-one (59). The title compound was prepared in 44% yield using **44a** and **58a** in an analogous manner to **54a**. Yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.96 (3H, d, *J* = 4.9 Hz), 5.13 (2H, s), 6.00 (1H, d, *J* = 2.3 Hz), 6.14 (1H, dd, *J* = 7.7, 2.8 Hz), 6.69 (1H, dd, *J* = 9.0, 1.9 Hz), 6.96 (1H, d, *J* = 1.9 Hz), 7.25 (2H, t, *J* = 8.9 Hz), 7.52 (2H, dd, *J* = 8.3, 5.7 Hz), 7.64 (1H, d, *J* = 7.5 Hz), 8.13 (1H, d, *J* = 9.0 Hz), 8.27 (1H, d, *J* = 4.9 Hz). MS (ESI/APCI) *m/z* = 370.1 $[M + H]^+$.

1-[4-Amino-3-(methylamino)phenyl]-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (60). A mixture of **59** (90 mg, 0.24 mmol), iron (54.4 mg, 0.97 mmol), calcium chloride (54.1 mg, 0.49 mmol), EtOH (1.5 mL), and water (1.5 mL) was heated at 70 °C for 3 h. The inorganic material was removed by filtration, and the filtrate was concentrated. The residue was neutralized with sat. $NaHCO_3$ solution and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated to give the title compound (78 mg, 94%) as an brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.85 (3 H, s), 3.29–3.41 (2 H, m), 4.99 (2 H, s), 5.90–6.08 (2 H, m), 6.52–6.65 (2 H, m), 6.74 (1 H, d, *J* = 7.9 Hz), 7.09 (2 H, t, *J* = 8.7 Hz), 7.23 (1 H, s), 7.39 (2 H, dd, *J* = 8.7, 5.3 Hz). MS (ESI/APCI) *m/z* = 340.1 $[M + H]^+$.

4-Bromo-*N*^{2}-methylbenzene-1,2-diamine (61). A solution of **58a** (350 mg, 1.51 mmol), zinc (495 mg, 7.57 mmol), and NH_4Cl (810 mg, 15.15 mmol) in MeOH (4 mL)/water (2 mL) was stirred at rt for 1 h. The insoluble material was removed by filtration and neutralized sat. $NaHCO_3$ solution. The mixture was concentrated and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo to give the title compound as a brown solid (282 mg, 93%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.68 (3H, d, *J* = 4.9 Hz), 4.60 (2H, s), 4.87 (1H, d, *J* = 4.9 Hz), 6.32–6.58 (3H, m). MS (ESI/APCI) *m/z* = 202.09 $[M + H]^+$.

***N*-(5-Bromo-2-nitrophenyl)-*N*-methylacetamide (62a).** To a solution of **58a** (50.0 g, 216 mmol) in toluene (500 mL) was added acetyl chloride (30.8 mL, 432 mmol) at rt. After being stirred at 90 °C for 15 h, acetyl chloride (7.69 mL, 108 mmol) was added and the mixture was stirred at 90 °C for further 5 h. The reaction mixture was cooled to rt, poured into EtOAc, washed with sat. $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated. The residual solid was recrystallized from EtOAc–hexane to give the title compound (57 g, 97%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.65–2.26 (3H, m), 3.00–3.52 (3H, m), 7.67–8.20 (3H, m). MS (ESI/APCI) *m/z* = 272.9 $[M + H]^+$.

***N*-(5-Bromo-2-nitrophenyl)-*N*,3,3-trimethylbutanamide (62b).** To a mixture of **58a** (300 mg, 1.30 mmol), 3,3-dimethylbutanoyl chloride (0.45 mL, 3.25 mmol), and DMF (5 mL) was added NaH (60% oil dispersion, 57.1 mg, 1.43 mmol), and the mixture was heated at 70 °C overnight. The mixture was poured into water and extracted with EtOAc. The extract was washed with brine,

dried over $MgSO_4$, concentrated, and purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 75/25) to give the title compound (214 mg, 51%) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.86–1.06 (9H, m), 1.67–2.39 (2H, m), 3.02–3.45 (3H, m), 7.68–8.13 (3H, m). MS (ESI/APCI) *m/z* = 329.0 $[M + H]^+$.

4-Bromo-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (63). To a solution of **56a** (1.0 g, 3.55 mmol) in DMF (15 mL) was added phosphoryl tribromide (1.22 g, 4.27 mmol) at ambient temperature. The mixture was stirred at 50 °C for 9 h. The mixture was poured into sat. $NaHCO_3$ solution and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residual solid was recrystallized from EtOH–hexane to give the title compound (600 mg, 49%) as a brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.99–1.16 (4H, m), 2.22–2.32 (1H, m), 3.85 (3H, s), 6.54 (1H, dd, *J* = 7.3, 2.0 Hz), 6.84 (1H, d, *J* = 2.0 Hz), 7.09 (1H, dd, *J* = 8.4, 1.9 Hz), 7.50–7.61 (2H, m), 7.67 (1H, d, *J* = 7.3 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.1, 8.3, 29.5, 98.5, 99.9, 108.5, 117.7, 120.4, 134.7, 135.8, 140.0, 141.3, 158.2, 162.9, 166.8. MS (ESI/APCI) *m/z* = 345.0 $[M + H]^+$. Anal. Calcd for $C_{16}H_{14}BrN_3O$: C, 53.78; H, 4.36; N, 11.76. Found: C, 53.85; H, 4.10; N, 11.94.

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4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-1-methyl-1*H*-indol-6-yl)pyridin-2(1*H*)-one (64). A mixture of **44b** (75 mg, 0.32 mmol), **68** (80 mg, 0.32 mmol), DMEDA (0.034 mL, 0.32 mmol), CuI (60.9 mg, 0.32 mmol), K_2CO_3 (133 mg, 0.96 mmol), and DMSO (2 mL) was heated at 150 °C for 1 h under microwave irradiation. The mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 0/100) to give the title compound (28.0 mg, 22%) as off-white crystals; mp 210–211 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.70 (2H, d, *J* = 3.6 Hz), 1.00 (2H, d, *J* = 7.4 Hz), 2.03 (1H, br s), 3.74–3.80 (3H, m), 5.15 (2H, s), 5.96 (1H, br s), 6.08 (1H, d, *J* = 6.8 Hz), 6.14 (1H, s), 6.87 (1H, d, *J* = 8.2 Hz), 7.37 (1H, s), 7.41–7.53 (5H, m), 7.57 (1H, d, *J* = 7.7 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 6.6, 7.2, 29.6, 68.7, 96.5, 97.9, 99.5, 107.9, 118.0, 119.2, 126.6, 128.5, 129.7, 132.7, 133.9, 135.0, 136.7, 139.9, 145.0, 162.8, 166.5. MS (ESI/APCI) *m/z* = 405.3 $[M + H]^+$. Anal. Calcd for $C_{24}H_{21}ClN_2O_2$: C, 71.19; H, 5.23; N, 6.92. Found: C, 71.32; H, 5.19; N, 6.93.

4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-3-methylpyrazolo[1,5-*a*]pyridin-5-yl)pyridin-2(1*H*)-one (65). The title compound was prepared in 55% yield using **44b** and **69** in an analogous manner to **64**. White crystals; mp 229–231 °C (EtOAc). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.81–1.03 (4H, m), 1.95–2.11 (1H, m), 2.27 (3H, s), 5.16 (2H, s), 5.99 (1H, d, *J* = 2.3 Hz), 6.14 (1H, dd, *J* = 7.6, 2.4 Hz), 6.65–6.74 (1H, m), 7.50 (4H, s), 7.56 (1H, s), 7.66 (1H, d, *J* = 7.5 Hz), 8.48 (1H, d, *J* = 7.3 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 6.9, 7.2, 8.1, 68.8, 97.8, 100.4, 104.5, 110.4, 113.2, 128.1, 128.5, 129.7, 132.8, 134.8, 135.2, 138.2, 138.9, 155.2, 162.3, 166.9. MS (ESI/APCI) *m/z* = 406.3 $[M + H]^+$. Anal. Calcd for $C_{23}H_{20}ClN_3O_2$: C, 68.06; H, 4.97; N, 10.35. Found: C, 67.78; H, 5.08; N, 10.24.

4-[(4-Chlorobenzyl)oxy]-1-(2,3-dimethyl-2*H*-indazol-5-yl)pyridin-2(1*H*)-one (66a). A mixture of **44b** (300 mg, 1.27 mmol), **71a** (287 mg, 1.27 mmol), DMEDA (0.137 mL, 1.27 mmol), K_2CO_3 (528 mg, 3.82 mmol), CuI (242 mg, 1.27 mmol), and DMSO (10 mL) was heated at 150 °C for 3 h. The mixture was poured into 28% NH_3 solution and extracted with EtOAc–THF. The extract was washed with brine, dried over $MgSO_4$, concentrated, and purified by column chromatography (silica gel, EtOAc/MeOH = 100/0 to 85/15) followed by recrystallization from EtOH to give the title compound (150 mg, 31%) as a white solid; mp 242–244 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.61 (3H, s), 4.07 (3H, s), 5.15 (2H, s), 5.97 (1H, d, *J* = 2.3 Hz), 6.09 (1H, dd, *J* = 7.6, 2.3 Hz), 7.10 (1H, d, *J* = 9.2 Hz), 7.47–7.55 (5H, m), 7.60 (1H, d, *J* = 7.5 Hz), 7.65 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 9.4, 37.4, 68.7, 97.9, 99.8, 116.6, 117.8, 120.1, 125.3, 128.5, 129.7, 132.7, 133.3, 134.9, 139.7, 145.6, 162.8, 166.6. MS (ESI/APCI) *m/z* = 406.3 $[M + H]^+$. Anal. Calcd for

$C_{21}H_{18}ClN_3O_2 \cdot 0.27H_2O$: C, 65.56; H, 4.86; N, 10.92. Found: C, 65.90; H, 4.75; N, 10.97.

1-(2,3-Dimethyl-2*H*-indazol-5-yl)-4-{[5-(trifluoromethyl)thiophen-2-yl]methoxy}pyridin-2(1*H*)-one (66b). To a suspension of **82a** (100 mg, 0.39 mmol), triphenylphosphine (308 mg, 1.18 mmol), and [5-(trifluoromethyl)thiophen-2-yl]methanol (143 mg, 0.78 mmol) in THF (5 mL) at 60 °C was added DMEAD (275 mg, 1.18 mmol). After stirring 3 h, the mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (silica gel, EtOAc/MeOH = 100/0 to 90/10) to give the title compound (71.2 mg, 43%) as a white solid; mp 191–193 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.61 (3H, s), 4.07 (3H, s), 5.44 (2H, s), 6.03–6.14 (2H, m), 7.11 (1H, dd, *J* = 9.0, 2.0 Hz), 7.36–7.41 (1H, m), 7.54 (1H, d, *J* = 8.9 Hz), 7.59–7.71 (3H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 9.4, 37.4, 64.0, 98.0, 99.6, 116.7, 117.9, 120.1, 122.3 (q, *J* = 269.7 Hz), 125.3, 128.1, 129.5 (q, *J* = 37.4 Hz), 130.0 (q, *J* = 4.0 Hz), 132.7, 133.2, 139.9, 143.4 (d, *J* = 1.5 Hz), 145.6, 162.7, 166.1. MS (ESI/APCI) *m/z* = 420.3 $[M + H]^+$. Anal. Calcd for $C_{20}H_{16}F_3N_3O_2S$: C, 57.27; H, 3.85; N, 10.02. Found: C, 57.09; H, 3.84; N, 10.00.

1-(2,3-Dimethyl-2*H*-indazol-5-yl)-4-{[4-(trifluoromethyl)thiophen-2-yl]methoxy}pyridin-2(1*H*)-one (66c). The title compound was prepared in 13% yield using **82a** and **90** in an analogous manner to **66b**. White crystals; mp 199–200 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.61 (3H, s), 4.07 (3H, s), 5.38 (2H, s), 6.04–6.11 (2H, m), 7.11 (1H, dd, *J* = 9.1, 2.0 Hz), 7.50–7.63 (3H, m), 7.63–7.68 (1H, m), 8.29–8.37 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 9.4, 37.4, 63.9, 97.9, 99.6, 116.7, 117.9, 120.1, 122.0 (q, *J* = 270.7 Hz), 124.6 (d, *J* = 2.0 Hz), 125.3, 129.3 (q, *J* = 35.4 Hz), 129.6 (q, *J* = 5.1 Hz), 132.7, 133.2, 139.8, 141.4, 145.6, 162.7, 166.2. MS (ESI/APCI) *m/z* = 420.3 $[M + H]^+$. Anal. Calcd for $C_{20}H_{16}F_3N_3O_2S \cdot 0.15H_2O$: C, 56.91; H, 3.89; N, 9.95. Found: C, 56.86; H, 3.87; N, 9.90.

1-(2,3-Dimethyl-2*H*-indazol-5-yl)-4-{[5-(trifluoromethyl)thiophen-3-yl]methoxy}pyridin-2(1*H*)-one (66d). The title compound was prepared in 43% yield using **82a** and **94** in an analogous manner to **66b**. White solid; mp 217–218 °C (EtOH–hexane). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.61 (3H, s), 4.07 (3H, s), 5.16 (2H, s), 6.01 (1H, s), 6.09 (1H, d, *J* = 7.7 Hz) 7.11 (1H, d, *J* = 9.3 Hz), 7.54 (1H, d, *J* = 8.9 Hz), 7.60 (1H, d, *J* = 7.5 Hz), 7.65 (1H, s), 7.81 (1H, s), 8.06 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 9.4, 37.4, 64.5, 97.7, 99.7, 116.6, 117.9, 120.1, 122.4 (q, *J* = 269.7 Hz),125.3, 129.6, 129.8 (q, *J* = 36.4 Hz), 130.3 (q, *J* = 4.0 Hz), 132.7, 133.3, 137.2, 139.7, 145.6, 162.8, 166.5. MS (ESI/APCI) *m/z* = 420.3 $[M + H]^+$. Anal. Calcd for $C_{20}H_{16}F_3N_3O_2S$: C, 57.27; H, 3.85; N, 10.02. Found: C, 57.34; H, 3.89; N, 10.09.

1-(2,3-Dimethyl-2*H*-indazol-5-yl)-4-{[4-(trifluoromethyl)-1,3-thiazol-2-yl]methoxy}pyridin-2(1*H*)-one (66e). The title compound was prepared in 50% yield using **82a** and [4-(trifluoromethyl)-1,3-thiazol-2-yl]methanol in an analogous manner to **66b**. White solid; mp 213–215 °C (IPA–IPE). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.61 (3H, s), 4.07 (3H, s), 5.56 (2H, s),

6.08 (1H, s), 6.16 (1H, d, *J* = 9.8 Hz), 7.11 (1H, d, *J* = 8.9 Hz), 7.54 (1H, d, *J* = 8.9 Hz), 7.66 (2H, d, *J* = 11.7 Hz), 8.61 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 9.4, 37.4, 66.3, 98.3, 99.4, 116.7, 117.9, 120.1, 120.4 (q, *J* = 270.7 Hz), 125.0 (q, *J* = 2.0 Hz), 125.2, 132.8, 133.2, 140.0, 142.4 (q, *J* = 36.4 Hz), 145.7, 162.6, 165.9, 168.2. MS (ESI/APCI) *m/z* = 421.3 $[M + H]^+$. Anal. Calcd for $C_{19}H_{15}F_3N_4O_2S$: C, 54.28; H, 3.60; N, 13.33. Found: C, 54.20; H, 3.59; N, 13.27.

1-(2,3-Dimethyl-2*H*-indazol-5-yl)-4-{[2-(trifluoromethyl)-1,3-thiazol-4-yl]methoxy}pyridin-2(1*H*)-one (66f). The title compound was prepared in 24% yield using **82a** and [2-(trifluoromethyl)-1,3-thiazol-4-yl]methanol in an analogous manner to **66b**. White solid; mp 185–188 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.61 (3H, s), 4.07 (3H, s), 5.32 (2H, s), 6.05–6.14 (2H, m), 7.11 (1H, d, *J* = 8.9 Hz), 7.54 (1H, d, *J* = 9.2 Hz), 7.61 (1H, d, *J* = 7.4 Hz), 7.66 (1H, s), 8.33 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 9.4, 37.4, 64.7, 97.8, 99.6, 116.6, 117.9, 119.7 (q, *J* = 273.7 Hz), 120.1, 124.6, 125.3, 132.7, 133.3, 139.8, 145.6, 152.3, 154.5 (q, *J* = 40.4 Hz), 162.8, 166.4. MS (ESI/APCI) *m/z* = 421.3 $[M + H]^+$. Anal. Calcd for $C_{19}H_{15}F_3N_4O_2S \cdot 1.78H_2O$: C, 50.43; H, 4.13; N, 12.38. Found: C, 50.46; H, 4.03; N, 12.38.

1-(2,3-Dimethyl-2*H*-indazol-5-yl)-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (66g). The title compound was prepared in 37% yield using **82a** and (4-fluorophenyl)methanol in an analogous manner to **66b**. White solid; mp 247–248 °C (EtOH–H_2O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.62 (3H, s), 4.14 (3H, s), 5.03 (2H, s), 6.06 (1H, dd, *J* = 7.5, 2.6 Hz), 6.09 (1H, d, *J* = 2.5 Hz), 7.12 (2H, t, *J* = 8.6 Hz), 7.21(1H, dd, *J* = 9.1, 1.7 Hz), 7.32 (1H, d, *J* = 7.5 Hz), 7.43 (2H, dd, *J* = 8.2, 5.5 Hz), 7.54 (1H, d, *J* = 1.0 Hz), 7.70 (1H, d, *J* = 9.0 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 9.4, 37.4, 68.9, 97.8, 99.8, 115.3 (d, *J* = 21.2 Hz), 116.6, 117.8, 120.1, 125.3, 130.2 (d, *J* = 9.1 Hz), 132.1 (d, *J* = 4.0 Hz), 132.7, 133.3, 139.6, 145.6, 161.9 (d, *J* = 245.4 Hz), 162.8, 166.7. MS (ESI/APCI) *m/z* = 364.1 $[M + H]^+$.

4-[(4-Chlorobenzyl)oxy]-1-(2-ethyl-3-methyl-2*H*-indazol-5-yl)pyridin-2(1*H*)-one (66h). The title compound was prepared in 13% yield using **44b** and **71b** in an analogous manner to **67**. Off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83–0.87 (3H, m), 1.84–1.91 (2H, m), 2.59 (3H, s), 4.29 (2H, t, *J* = 7.0 Hz), 7.25 (1H, dd, *J* = 9.0, 1.8 Hz), 7.48 (1H, d, *J* = 9.0 Hz), 7.94 (1H, d, *J* = 1.6 Hz). MS (ESI/APCI) *m/z* = 394.2 $[M + H]^+$. Purity 99.4% (HPLC).

4-[(4-Chlorobenzyl)oxy]-1-(3-methyl-2-propyl-2*H*-indazol-5-yl)pyridin-2(1*H*)-one (66i). The title compound was prepared in 43% yield using **44b** and **71c** in an analogous manner to **67**. Off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.86 (3H, t, *J* = 7.4 Hz), 1.86–1.92 (2H, m), 2.62 (3H, s), 4.32 (2H, t, *J* = 6.9 Hz), 5.15 (2H, s), 5.96 (1H, d, *J* = 2.6 Hz), 6.08 (1H, dd, *J* = 7.6, 2.7 Hz), 7.09 (1H, dd, *J* = 9.0, 1.8 Hz), 7.50 (4H, s), 7.55 (1H, d, *J* = 9.1 Hz), 7.60 (1H, d, *J* = 7.6 Hz), 7.65 (1H, d, *J* = 1.4 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 9.3, 10.9, 23.0, 51.1, 68.7, 97.8, 99.8, 116.8, 118.0, 120.0, 125.4, 128.5, 129.7, 132.3, 132.7, 133.3, 135.0, 139.7, 145.8, 162.8, 166.6. MS (ESI/APCI) *m/z* = 408.0 $[M + H]^+$. Purity 99.8% (HPLC).

4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-3-methyl-2*H*-indazol-5-yl)pyridin-2(1*H*)-one (66j). The title compound was prepared in 39% yield using **44b** and **71d** in an analogous manner to **64**. White crystals; mp 222–224 °C (EtOAc). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.09–1.18 (2H, m), 1.22–1.30 (2H, m), 2.70 (3H, s), 3.96 (1H, dt, *J* = 7.4, 3.6 Hz), 5.15 (2H, s), 5.97 (1H, d, *J* = 2.6 Hz), 6.09 (1H, dd, *J* = 7.6, 2.7 Hz), 7.09 (1H, dd, *J* = 9.1, 2.0 Hz), 7.49–7.55 (5H, m), 7.58 (1H, d, *J* = 7.6 Hz), 7.65 (1H, dd, *J* = 2.0, 0.7 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 6.9, 9.5, 31.4, 68.7, 97.8, 99.8, 116.9, 117.7, 120.3, 125.5, 128.5, 129.7, 132.7, 133.4, 134.1, 134.9, 139.6, 145.1, 162.7, 166.6. MS (ESI/APCI) *m/z* = 406.3 $[M + H]^+$. Anal. Calcd for $C_{23}H_{20}ClN_3O_2 \cdot 0.1H_2O$: C, 67.76; H, 4.99; N, 10.31. Found: C, 67.76; H, 4.99; N, 10.31.

1-(2-Cyclopropyl-3-methyl-2*H*-indazol-5-yl)-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (66k). The title compound was prepared in 70% yield using **44a** and **71d** in an analogous manner to **66a**. White crystals; mp 195–196 °C (EtOAc). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.14 (2H, d, *J* = 5.3 Hz), 1.27 (2H, br s), 2.70 (3H, s), 3.95 (1H, d, *J* = 3.5 Hz), 5.13 (2H, s), 5.98 (1H, br s), 6.08 (1H, d, *J* = 7.2 Hz), 7.09 (1H, d, *J* = 8.9 Hz), 7.26 (2H, t, *J* = 8.6 Hz), 7.49–7.61 (4H, m), 7.65 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 6.9, 9.5, 31.4, 68.9, 97.8, 99.8, 115.3 (d, *J* = 21.2 Hz), 116.9, 117.7, 120.3, 125.5, 130.2 (d, *J* = 9.1 Hz), 132.1 (d, *J* = 3.0 Hz), 133.5, 134.1, 139.6, 145.1, 161.9 (d, *J* = 244.4 Hz), 162.8, 166.7. MS (ESI/APCI) *m/z* = 390.3 $[M + H]^+$. Anal. Calcd for $C_{23}H_{20}FN_3O_2$: C, 70.94; H, 5.18; N, 10.79. Found: C, 70.92; H, 5.18; N, 10.78.

1-(2-Cyclopropyl-3-methyl-2*H*-indazol-5-yl)-4-{[5-(trifluoromethyl)thiophen-3-yl]methoxy}pyridin-2(1*H*)-one (66l). To a solution of **94** (1.82 g, 10.0mmol) in DMA (25 mL) was added potassium *tert*-butoxide (1.12 g, 10.0 mmol) at 0 °C and the suspension was stirred at same temperature for 15 min. To the mixture was added **83** (2.5 g, 8.34 mmol) and the mixture was heated at 80 °C for 1.5 h. The mixture was cooled to rt and water was added. After being stirred at rt overnight, the precipitate was collected by filtration and washed with water, EtOH, and IPE to give a solid. The resulting solid was recrystallized from DMSO–EtOH–water to give the title compound (2.5 g, 67%) as a solid; mp 215–216 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.09–1.19 (2H, m), 1.21–1.31 (2H, m), 2.70 (3H, s), 3.92–4.01 (1H, m), 5.16 (2H, s), 6.01 (1H, d, *J* = 2.6 Hz), 6.09 (1H, dd, *J* = 7.6, 2.7 Hz), 7.10 (1H, dd, *J* = 9.2, 2.0 Hz), 7.53 (1H, d, *J* = 9.2 Hz), 7.59 (1H, d, *J* = 7.6 Hz), 7.65 (1H, d, *J* = 1.3 Hz), 7.81 (1H, s), 8.06 (1H, d, *J* = 1.4 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 6.9, 9.5, 31.4, 64.5, 97.7, 99.7, 116.9, 117.7, 120.3, 122.4 (q, *J* = 269.7 Hz), 125.5, 129.6, 129.8 (q, *J* = 36.4 Hz), 130.3 (q, *J* = 4.0 Hz), 133.4, 134.1, 137.2, 139.6, 145.2, 162.8, 166.5. MS (ESI/APCI) *m/z* = 446.2 $[M + H]^+$. Anal. Calcd for $C_{22}H_{18}N_3O_2SF_3$: C, 59.32; H, 4.07; N, 9.43. Found: C, 59.30; H, 4.17; N, 9.37.

4-(Benzyloxy)-1-(2,3-dimethyl-2*H*-indazol-5-yl)pyridin-2(1*H*)-one (66m). The title compound was prepared in 65% yield using **44e** and **71b** in an analogous manner to **66a**. Pale yellow solid; mp 215–217 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.61 (3H, s), 4.07 (3H, s), 5.15 (2H, s), 5.98

(1H, d, *J* = 2.3 Hz), 6.09 (1H, dd, *J* = 7.5, 2.2 Hz), 7.11 (1H, d, *J* = 9.0 Hz), 7.32–7.50 (5H, m), 7.53 (1H, d, *J* = 9.2 Hz), 7.59 (1H, d, *J* = 7.5 Hz), 7.65 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 9.4, 37.4, 69.6, 97.8, 99.8, 116.6, 117.8, 120.1, 125.3, 127.8, 128.1, 128.5, 132.7, 133.3, 135.9, 139.6, 145.6, 162.8, 166.8. MS (ESI/APCI) *m/z* = 346.3 $[M + H]^+$. Purity 99.9% (HPLC).

4-(Benzyloxy)-1-(2-cyclopropyl-3-methyl-2*H*-indazol-5-yl)pyridin-2(1*H*)-one (66n). The title compound was prepared in 67% yield using **44e** and **71d** in an analogous manner to **64**. White crystals; mp 194–195 °C (EtOAc). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11–1.19 (2H, m), 1.26 (2H, d, *J* = 3.0 Hz), 2.70 (3H, s), 3.96 (1H, dt, *J* = 7.3, 3.6 Hz), 5.15 (2H, s), 5.97 (1H, d, *J* = 2.5 Hz), 6.09 (1H, dd, *J* = 7.5, 2.5 Hz), 7.10 (1H, dd, *J* = 9.1, 1.8 Hz), 7.34–7.50 (5H, m), 7.52 (1H, d, *J* = 9.2 Hz), 7.58 (1H, d, *J* = 7.5 Hz), 7.65 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 6.9, 9.5, 31.4, 69.6, 97.8, 99.8, 116.9, 117.7, 120.3, 125.5, 127.8, 128.1, 128.5, 133.5, 134.1, 135.9, 139.6, 145.1, 162.8, 166.8. MS (ESI/APCI) *m/z* = 372.3 $[M + H]^+$. Purity >99.9% (HPLC).

4-[(4-Chlorobenzyl)oxy]-1-(1,3-dimethyl-1*H*-indazol-5-yl)pyridin-2(1*H*)-one (67). To a mixture of **70** (200 mg, 0.88 mmol), **44b** (168 mg, 0.77 mmol), and K_2CO_3 (368 mg, 2.66 mmol) in dioxane (10 mL) were added CuI (51 mg, 0.36 mmol) and *trans-N,N'*-dimethyl-cyclohexane-1,2-diamine (68 mg, 0.36 mmol). The mixture was heated at 110 °C for 16 h. The mixture was cooled to rt and concentrated. The residue was diluted with DCM, washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (silica gel, DCM/MeOH = 97/3) to give the title compound (100 mg, 34%) as a off-white solid; mp 172–173 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.47 (3H, s), 3.99 (3H, s),. 5.16 (2H, s), 5.97 (1H, d, *J* = 2.6 Hz), 6.10 (1H, dd, J = 7.6, 2.6 Hz), 7.30 (1H, dd, *J* = 8.8, 1.7 Hz), 7.49 (4H, s), 7.60 (1H, d, *J* = 2.1 Hz), 7.62 (1H, d, *J* = 3.5 Hz), 7.68 (1H, d, *J* = 1.4 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 11.4, 35.1, 68.7, 97.8, 99.9, 109.6, 118.2, 122.4, 125.5, 128.5, 129.7, 132.7, 133.0, 134.9, 139.4, 139.7, 140.6, 162.8, 166.7. MS (ESI/APCI) *m/z* = 380.0 $[M + H]^+$. Anal. Calcd for $C_{21}H_{18}ClN_3O_2$: C, 66.40; H, 4.78; N, 11.06. Found: C, 66.35; H, 4.84; N, 11.09.

6-Bromo-2-cyclopropyl-1-methyl-1*H*-indole (68). To a solution of **76** (1.0 g, 4.24 mmol) in DMF (5 mL) was added methyl iodide (0.79 mL, 12.7 mmol) and NaH (40% oil dispersion, 0.17 g, 4.24 mmol) at 0 °C. After 3 h, the mixture was poured into sat. NH_4Cl solution and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 90/10) to give the title compound (0.48 g, 46%) as pale yellow crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.68 (2H, d, *J* = 3.8 Hz), 0.98 (2H, d, *J* = 6.5 Hz), 1.99 (1H, br s), 3.30 (1H, s), 3.72–3.79 (3H, m), 6.09 (1H, s), 7.07 (1H, d, *J* = 8.0 Hz), 7.35 (1H, d, *J* = 8.3 Hz), 7.62 (1H, s).

5-Bromo-2-cyclopropyl-3-methylpyrazolo[1,5-*a*]pyridine (69). To a solution of **81** (50 mg, 0.19 mmol) in TFA (3 mL) was added triethylsilane (0.60 mL, 3.77 mmol) at rt and the mixture was stirred vigorously for 16 h. The mixture was poured into 1 N NaOH solution and extracted with

EtOAc. The organic layer was separated, washed with sat. $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 85/15) to give the title compound (45.2 mg, 95%) as white crystals. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92–1.06 (4H, m), 1.91–2.01 (1H, m), 2.25 (3H, s), 6.63 (1H, dd, *J* = 7.3, 1.9 Hz), 7.46 (1H, d, *J* = 1.6 Hz), 8.09 (1H, d, *J* = 7.4 Hz). MS (ESI/APCI) *m/z* = 252.8 $[M + H]^+$.

5-Bromo-1,3-dimethyl-1*H*-indazole (70). To a stirred suspension of NaH (60% oil dispersion, 136 mg, 2.83 mmol) in DMF (10 mL) was added a solution of **84** (400 mg, 1.89 mmol) in DMF (2 mL) at 0 °C, and the mixture was stirred at the same temperature for 30 min. MeI (400 μL, 2.83 mmol) was added and the resulting mixture was stirred at 0 °C for 4 h. The reaction mixture was quenched with water and extracted with EtOAc. The combined EtOAc layers were washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 80/20) to give the title compound (200 mg, 39%) as a yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.45 (3H, s), 3.95 (3H, s), 7.46 (1H, dd, *J* = 8.9, 1.8 Hz), 7.54 (1H, d, *J* = 8.9 Hz), 7.94 (1H, d, *J* = 1.5 Hz). MS (ESI/APCI) *m/z* = 225.0 $[M + H]^+$.

5-Bromo-2,3-dimethyl-2*H*-indazole (71a). To a solution of **84** (4.25 g, 20.1 mmol) in EtOAc (100 mL) was added trimethyloxonium tetrafluoroborate (4.47 g, 30.2 mmol) and the mixture was stirred at rt for 5h. The mixture was poured into 1 N NaOH solution and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated to give the title compound (3.65 g, 81%) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.58 (3H, s), 4.04 (3H, s), 7.25 (1H, d, *J* = 9.0 Hz), 7.47 (1H, d, *J* = 9.0 Hz), 7.94 (1H, s).

5-Bromo-2-ethyl-3-methyl-2*H*-indazole (71b). The title compound was prepared in 47% yield using triethyloxonium tetrafluoroborate in an analogous manner to **71a**. Off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.43 (3H, t, *J* = 7.2 Hz), 2.62 (3H, s), 4.39 (2H, q, *J* = 7.2 Hz), 5.15 (2H, s), 5.96 (1H, d, *J* = 2.6 Hz), 6.08 (1H, dd, *J* = 7.6, 2.7 Hz), 7.09 (1H, dd, *J* = 9.1, 1.9 Hz), 7.50 (4H, s), 7.55 (1H, d, *J* = 9.1 Hz), 7.55 (1H, d, *J* = 7.6 Hz), 7.65 (1H, d, *J* = 1.6 Hz). MS (ESI/APCI) *m/z* = 240.0 $[M + H]^+$.

5-Bromo-3-methyl-2-propyl-2*H*-indazole (71c). The title compound was prepared in 17% yield using iodopropane in an analogous manner to **70**. Yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83–0.87 (3H, m), 1.84–1.91 (2H, m), 2.59 (3H, s), 4.29 (2H, t, *J* = 7.0 Hz), 7.25 (1H, dd, *J* = 9.0, 1.8 Hz), 7.48 (1H, d, *J* = 9.0 Hz), 7.94 (1H, d, *J* = 1.6 Hz). MS (ESI/APCI) *m/z* = 253.0 $[M + H]^+$.

5-Bromo-2-cyclopropyl-3-methyl-2*H*-indazole (71d). To a solution of **86** (43.0 g, 176 mmol) in toluene (250 mL) were added cyclopropylamine (24.4 mL, 352 mmol) and titanium isopropoxide (105 mL, 352 mmol) at rt, and the mixture was stirred at 60 °C for 16 h. After removal of solvent, the residue was dissolved in triethyl phosphite (91 mL, 529 mmol), and the mixture was heated to 150 °C for 2 h. The mixture was treated with NH silica gel (500 g) in EtOAc (500 mL) with stirring

overnight. The silica gel was removed by filtration, washed with EtOAc, concentrated, and filtered through a silica gel (500 g, hexane/EtOAc = 85/15). The filtrate was evaporated and the residue was recrystallized from EtOAc–hexane to give the title compound (19.8 g, 45%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.13 (2H, d, J = 5.3 Hz), 1.25 (2H, br s), 2.67 (3H, s), 3.87–3.97 (1H, m), 7.25 (1H, d, J = 9.0 Hz), 7.46 (1H, d, J = 9.0 Hz), 7.94 (1H, s). MS (ESI/APCI) *m/z* = 251.2 $[M + H]^+$.

4-Bromo-2-nitrophenyl trifluoromethanesulfonate (73). To a solution of **72** (9.43 g, 43.3 mmol) in pyridine (200 mL) was added Tf_2O (8.0 mL, 47.6 mmol) at 0 °C and the mixture was stirred for 1 h. The mixture was quenched with sat. $NaHCO_3$ solution and extracted with EtOAc. The organic layer was separated, washed with sat. $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 80/20) to give the title compound (14.0 g, 92%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.78 (1H, d, J = 8.9 Hz), 8.20 (1H, dd, J = 8.8, 2.5 Hz), 8.56 (1H, d, J = 2.5 Hz).

4-Bromo-1-(cyclopropylethynyl)-2-nitrobenzene (74). To a solution of **73** (2.0 g, 5.71 mmol) in THF (20 mL) were added ethynylcyclopropane (0.58 mL, 6.86 mmol), CuI (0.054 g, 0.29 mmol), $PdCl_2(PPh_3)_2$ (0.20 g, 0.29 mmol), and TEA (2.4 mL, 17.1 mmol) at rt under N_2 atmosphere, and the mixture was stirred at rt for 3 h. The mixture was concentrated and purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 80/20) to give the title compound (1.42 g, 93%) as a yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.79 (2H, br s), 0.90–1.00 (2H, m), 1.61 (1H, d, J = 4.0 Hz), 7.58 (1H, d, J = 8.3 Hz), 7.89 (1H, d, J = 8.3 Hz), 8.22–8.30 (1H, m).

5-Bromo-2-(cyclopropylethynyl)aniline (75). To a solution of **74** (1.42 g, 5.34 mmol) in EtOAc (20 mL) was added $SnCl_2$ (1.3 mL, 26.7 mmol) and water (0.96 mL, 53.4 mmol) at rt, and the mixture was heated at reflux for 4 h. The mixture was quenched with sat. $NaHCO_3$ solution. The insoluble material was removed by filtration and the filtrate was diluted with EtOAc. The organic layer was separated, washed with sat. $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was used for next reaction without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.77 (2H, br s), 0.81–0.90 (2H, m), 1.50–1.60 (1H, m), 5.47 (2H, br s), 6.59 (1H, d, J = 8.2 Hz), 6.85 (1H, s), 6.98 (1H, d, J = 8.3 Hz).

6-Bromo-2-cyclopropyl-1*H*-indole (76). To a solution of **75** (1.09 g, 4.62 mmol) in EtOH (20 mL) was added $PdCl_3$ (41 mg, 0.23 mmol) and $FeCl_2$ (37 mg, 0.23 mmol) at rt, and the mixture was heated at 80 °C for 2 h. The solvent was evaporated, and the residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 100/0 to 70/30) to give the title compound (1.02 g, 94 %) as yellow crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.77 (2H, d, J = 4.8 Hz), 0.97 (2H, d, J = 8.0 Hz), 1.17 (1H, t, J = 7.1 Hz), 1.95–2.05 (1H, m), 6.09 (1H, s), 7.02 (1H, d, J = 8.3 Hz), 7.31 (1H, d, J = 8.3 Hz), 7.38 (1H, s), 11.02 (1H, br s). MS (ESI/APCI) *m/z* = 236.0 $[M + H]^+$.

2-(4-Bromopyridin-2-yl)-1-cyclopropylethanone (78). To a solution of **77** (5.5 g, 32.0 mmol) in

THF (40 mL) was added NaHMDS (1.9 M THF solution, 20.2 mL, 38.4 mmol) at −78 °C, and the mixture was stirred at rt for 1 h. Methyl cyclopropanecarboxylate (3.27 mL, 32.0 mmol) was added at −78 °C, and the mixture was allowed to warm to rt for 16 h. The mixture was quenched with sat. $NaHCO_3$ solution and extracted with EtOAc. The organic layer was separated, washed with sat. $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 90/10 to 50/50) to give the title compound (4.3 g, 56%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (2H, dd, *J* = 7.5, 3.3 Hz), 1.04–1.14 (2H, m), 2.00–2.10 (1H, m), 4.03 (2H, s), 7.37 (1H, d, *J* = 5.1 Hz), 7.44 (1H, s), 8.38 (1H, d, *J* = 5.3 Hz).

2-(4-Bromopyridin-2-yl)-1-cyclopropyl-*N*-hydroxyethanimine (79). To a solution of **78** (5.86 g, 24.4 mmol) in MeOH (50 mL) was added hydroxylamine hydrochloride (8.48 g, 122 mmol) and NaOH (4.88 g, 122 mmol) at rt, and the mixture was heated at 60 °C for 14 h. The mixture was poured into sat. $NaHCO_3$ solution and extracted with EtOAc. The organic layer was separated, washed with 1 N NaOH solution and brine, dried over $MgSO_4$, and concentrated. The resulting solid was recrystallized from EtOAc–hexane to give the title compound (3.3 g, 53%) as white crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.55–0.67 (4H, m), 1.46–1.56 (1H, m), 3.66 (2H, s), 7.47–7.55 (2H, m), 8.36 (1H, d, *J* = 5.9 Hz), 10.55 (1H, s).

5-Bromo-2-cyclopropylpyrazolo[1,5-*a*]pyridine (80). To a suspension of **79** (395 mg, 1.55 mmol) in DME (4 mL) was added triethylamine (1.08 mL, 7.74 mmol) at 0 °C. TFAA (0.26 mL, 1.86 mmol) was added to the mixture portionwise at the same temperature. After 30 min, the mixture was turned to clear yellow solution. The mixture was quenched with sat. $NaHCO_3$ solution and extracted with EtOAc. The organic layer was separated, washed with sat. $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated. The residue was diluted with DME (4 mL) and $FeCl_2$ (19.6 mg, 0.15 mmol) was added to the mixture. The mixture was heated to 80 °C for 2 h. Then the mixture was poured into 1 N NaOH solution and extracted with EtOAc. The organic layer was separated, washed with 1 N NaOH solution and brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 85/15) to give the title compound (173 mg, 47%) as pale yellow crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.73–0.85 (2H, m), 0.91–1.05 (2H, m), 1.97–2.13 (1H, m), 6.30 (1H, s), 6.87 (1H, dd, *J* = 7.3, 1.9 Hz), 7.83 (1H, d, *J* = 1.8 Hz), 8.48 (1H, d, *J* = 7.4 Hz). MS (ESI/APCI) *m/z* = 238.8 $[M + H]^+$.

5-Bromo-2-cyclopropylpyrazolo[1,5-*a*]pyridine-3-carbaldehyde (81). To a solution of **80** (500 mg, 2.11 mmol) in CH_3CN (5 mL) was added *N*-(chloromethylene)-*N*-methylmethanaminium chloride (324 mg, 2.53 mmol) at 0 °C, and the mixture was stirred at rt for 1 h. The mixture was quenched with sat. NH_4Cl solution and the suspension was stirred at rt for 30 min. The mixture was poured into sat. NH_4Cl solution and extracted with EtOAc. The organic layer was separated, washed with sat. $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated. The residue

was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 75/25) to give the title compound (245 mg, 44%) as white crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.99–1.16 (4H, m), 2.58–2.70 (1H, m), 7.32 (1H, dd, *J* = 7.1, 1.7 Hz), 8.36 (1H, s), 8.73 (1H, d, *J* = 7.3 Hz), 10.17 (1H, s).

1-(2,3-Dimethyl-2*H*-indazol-5-yl)-4-hydroxypyridin-2(1*H*)-one (82a). A mixture of **66m** (1.8 g, 5.21 mmol), palladium on carbon (0.56 g, 5.21 mmol), and EtOH (40 mL) was vigorously stirred under H_2 atmosphere at rt for 3 h. The inorganic material was removed by filtration and the filtrate was concentrated to give the title compound (1.04 g, 78%) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.60 (3H, s), 4.07 (3H, s), 5.64 (1H, d, *J* = 1.9 Hz), 5.94 (1H, dd, *J* = 7.5, 2.1 Hz), 7.09 (1H, dd, *J* = 9.0, 1.5 Hz), 7.52 (2H, d, *J* = 8.3 Hz), 7.62 (1H, s), 10.8 (1H, br s).

1-(2-Cyclopropyl-3-methyl-2*H*-indazol-5-yl)-4-hydroxypyridin-2(1*H*)-one (82b). The title compound was prepared in 94% yield using **66n** in an analogous manner to **82a**. Yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.06–1.19 (2H, m), 1.26 (2H, d, *J* = 3.3 Hz), 2.69 (3H, s), 3.95 (1H, dt, *J* = 7.2, 3.5 Hz), 5.60 (1H, d, *J* = 2.1 Hz), 5.92 (1H, dd, *J* = 7.5, 2.3 Hz), 7.08 (1H, dd, *J* = 9.0, 1.8 Hz), 7.49 (2H, t, *J* = 7.9 Hz), 7.61 (1H, s), 11.05 (1H, br s). MS (ESI/APCI) *m/z* = 282.3 $[M + H]^+$.

4-Chloro-1-(2-cyclopropyl-3-methyl-2*H*-indazol-5-yl)pyridin-2(1*H*)-one (83) To a suspension of **82b** (8.0 g, 28.4 mmol) in DMF (140 mL) was added phosphorus oxychloride (3.18 mL, 34.1 mmol) at rt and the mixture was stirred at 50 °C for 21 h. The mixture was poured into EtOAc and washed with sat. $NaHCO_3$ solution. The aqueous layer was extracted with EtOAc, and the extracts were washed with water and brine, dried over Na_2SO_4, and concentrated. The resulting residue was dissolved in EtOAc–THF at 50 °C and NH-silica gel was added to the mixture. After 1 h, a mixture was purified by column chromatography (NH silica gel, EtOAc only) to give the crude product. The crude solid was triturated with IPE and collected by filtration to give the title compound (4.59 g, 54%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.11–1.20 (2H, m), 1.22–1.31 (2H, m), 2.70 (3H, s), 3.97 (1H, tt, *J* = 7.4, 3.7 Hz), 6.44 (1H, dd, *J* = 7.4, 2.5 Hz), 6.65 (1H, d, *J* = 1.9 Hz), 7.13 (1H, dd, *J* = 9.3, 2.1 Hz), 7.56 (1H, d, *J* = 9.1 Hz), 7.71–7.75 (1H, m), 7.77 (1H, d, *J* = 7.2 Hz). MS (ESI/APCI) *m/z* = 300.2 $[M + H]^+$.

1-(5-Bromo-2-nitrophenyl)ethanone (86). To nitric acid (fuming, 2 mL, 25.1 mmol) was slowly added sulfuric acid (2.5 mL, 25.1 mmol) at 0 °C and the mixture was stirred at same temperature for 15 min. To the solution was slowly added **85** (3.34 mL, 25.1 mmol) at 0 °C with vigorous stirring and the mixture was stirred at same temperature for 1 h. The mixture was poured into ice–water and extracted with EtOAc. The organic layer was washed with sat. $NaHCO_3$ solution and brine, dried over $MgSO_4$, concentrated, and purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 90/10) to give the title compound (2.04 g, 33%) as pale yellow crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.58 (3H, s), 7.92–8.00 (1H, m), 8.02–8.08 (2H, m).

4-(Trifluoromethyl)thiophene-2-carbaldehyde (88). To a solution of norpempidine (9.3 mL, 55.1 mmol) in THF (150 mL) was added n-BuLi (34.5 mL, 55.1 mmol) at −78 °C and the mixture was stirred at 0 °C for 10 min. Then the mixture was cooled to −78 °C and a solution of 3-(trifluoromethyl)thiophene (6.99 g, 46.0 mmol) in THF (5 mL) was added dropwise over 30 min. Then the mixture was stirred for 1 h at the same temperature. DMF (10.7 mL, 138 mmol) was added, and stirred at rt overnight. The mixture was quenched with 1 N HCl solution and extracted with EtOAc. The organic layer was separated, washed with 1 N HCl solution and brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 85/15) to give the title compound (4.1 g, 50%) as a light brown oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.63 (1H, d, J = 5.1 Hz), 8.31 (1H, d, J = 5.1 Hz), 8.37 (1H, s), 8.80 (1H, s), 9.97 (1H, s), 10.09 (1H, s).

[4-(Trifluoromethyl)thiophen-2-yl]methanol (90). To a solution of **88** (7.06 g, 39.2 mmol) in MeOH (50 mL) was added $NaBH_4$ (1.48 g, 39.2 mmol) at 0 °C and the mixture was stirred for 10 min. The mixture was quenched with sat. NH_4Cl solution and extracted with EtOAc. The organic layer was separated, washed with sat. NH_4Cl solution and brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 85/15) to give the title compound (2.63 g, 37%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.84–1.93 (1H, m), 4.81–4.87 (2H, m), 7.14 (1H, s), 7.66 (1H, s).

Methyl 5-iodothiophene-3-carboxylate (92). A mixture of **91** (3.48 g, 13.7 mmol), methyl iodide (1.29 mL, 20.6 mmol), K_2CO_3 (2.84 g, 20.6 mmol), and DMF (35 mL) was stirred at rt for 1 day. The mixture was poured into water and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 98/2 to 85/15) to give the title compound (3.62 g, 99%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.86 (3H, s) 7.66 (1H, s) 8.08 (1H, s).

Methyl 5-(trifluoromethyl)thiophene-3-carboxylate (93). To a solution of **92** (896 mg, 3.34 mmol), CuI (1.91 g, 10.0 mmol), and hexamethylphosphoramide (3.49 mL, 20.1 mmol) in DMF (15 mL) was added methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (2.53 mL, 20.1 mmol) at rt. The mixture was stirred at 80 °C under N_2 atmosphere for 5 h. The mixture was neutralized with sat. $NaHCO_3$ solution and extracted with EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 99/1 to 85/15) to give the title compound (526 mg, 75%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89 (3H, s) 7.86 (1H, s) 8.23 (1H, d, J = 1.0 Hz).

[5-(Trifluoromethyl)thiophen-3-yl]methanol (94). To a solution of **93** (523 mg, 2.49 mmol) in THF (10 mL) and MeOH (1 mL) was added $NaBH_4$ (2.20 g, 58.0 mmol) at rt. The mixture was stirred at 60 °C for 3 h. The mixture was quenched with water and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by

column chromatography (silica gel, hexane/EtOAc = 90/10 to 50/50) to give the title compound (449 mg, 99%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.71 (1H, t, *J* = 5.7 Hz), 4.70 (2H, d, *J* = 5.6 Hz), 7.39 (1H, s), 7.43 (1H, s).

Experiments concerning biological activities

Determination of hMCHR1 competitive inhibitory activity of test compound using binding assay. *1. Preparation of membrane fraction.* Using hMCHR1-expressing CHO cell clone 57,[57] MCHR1-expressing CHO cellular membrane fractions were prepared by the following method. In phosphate buffered saline (pH 7.4) supplemented with 5 mM EDTA (ethylenediaminetetraacetic acid) were respectively suspended human MCHR1-expressing CHO cells (1×10^8 cells) and centrifuged. Homogenate buffer [10 mL, 10 mM $NaHCO_3$, 5 mM EDTA, pH 7.5, 0.5 mM PMSF (phenylmethylsulfonyl fluoride), 20 mg/L leupeptin, 4 mg/L E-64, 1 mg/L pepstatin A] was added to the pellets of the cells and, using Polytron Homogenizer, the mixture was homogenated. The supernatant obtained after centrifugation at 400 × g for 10 min was further centrifuged at 100,000 × g for 1 h to give precipitate of the membrane fraction. The precipitate were suspended in 2 mL of assay buffer [20 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.5 mM PMSF, 20 mg/L leupeptin, 4 mg/L E-64, 1 mg/L pepstatin A]. The membrane fractions were suspended in assay buffer to a protein concentration of 2 mg/mL, and after dispensing, preserved at −80 °C and used upon thawing each time when in use.

2. Binding assay. An MCHR1-expressing CHO cellular membrane fraction (173 μL) diluted with an assay buffer was dispensed to a 96 well polypropylene plate (3363, Corning). DMSO solution (2 μL), 33 μM cold MCH (1–19) diluted with DMSO solution (2 μL), or a test compound solution diluted with DMSO solution to various concentrations (2 μL) was added, and lastly, [^{125}I]-MCH(4–19) diluted with assay buffer (hereinafter, sometimes to be referred to as "hot MCH", 25 μL) was added to each well. The mixture was reacted with stirring at rt for 1 h, and the plate was set on FilterMate Harvester (PerkinElmer). Using a polyethyleneimine-treated glass filter plate (GF/C, PerkinElmer), which had been previously set, the plate was suction-filtered and washed three times with washing buffer (50 mM Tris-HCl buffer pH 7.5). The glass filter plate was dried, MicroScint 0 (PerkinElmer) was added at 25 μL/well, and the resulting radioactivity was measured by TopCount liquid scintillation counter (PerkinElmer). The binding inhibition rate of the test compound was calculated by the following formula.

Binding inhibition (%) = 100 − (radioactivity upon addition of test compound and hot MCH − radioactivity upon addition of cold MCH and hot MCH solution)/(radioactivity upon addition of DMSO solution and hot MCH − radioactivity upon addition of cold MCH and hot MCH solution) × 100

Measurement of MCH receptor 1 antagonistic activity of test compound using Ca^{2+} mobilization assay. Using an expression vector plasmid introduced with human MCHR1 gene for expression in animal cells, human MCHR1 gene was introduced into CHO cells (CHO dhfr$^-$) by

Lipofectamine LTX (Invitrogen). The cells were cultured in selection MEMα medium [445 mL of MEMα medium without nucleic acid and added with 5 mL of Penicillin-Streptomycin (Invitrogen) and 50 mL of dialyzed fetal bovine serum]. Colony 24 clones grown in the selection medium, which were human MCHR1 gene-expressing CHO cell candidates, were selected. From these clones, clone #4 which showed the highest response to the change of Ca^{2+} concentration on stimulation by the addition of 25 nM ligand MCH (4–19) was selected by Ca^{2+} mobilization assay. In the following test, this human MCHR1-expressing CHO cell (clone #4) was used. An integrated dispensing function fluorometer (CellLux, PerkinElmer) was used for Ca^{2+} mobilization assay. The CHO cells were sown in a 96 well plate (type 3904, Corning) with a black wall and clear well bottom at a density of 20000 cells/well, and cultured in an incubator for about 24 h at 5% CO_2, 37 °C. The medium was removed, and the cells were washed with phosphate buffered saline (PBS). A Ca^{2+} indicator dye reagent (DOJINDO LABORATORIES, Ca screening no-wash kit Fluo4) was added at 100 μL/well, and the dye was allowed to penetrate into the cell for 30 min in an incubator at 5% CO_2, 37 °C. The plate was set on a plate reader. First, a test compound solution diluted with an assay buffer [10 mM HEPES (pH 7.4): 1 × Assay Buffer (DOJINDO LABORATORIES, attached to Ca screening no-wash kit Fluo4) containing 0.1% BSA] or DMSO solution was added at 50 μL/well, and then ligand MCH (4–19) peptide (final concentration 2 nM) diluted with assay buffer or DMSO was added at 50 μL/well, during which changes in intracellular fluorescence were measured at 2 seconds intervals. The antagonistic activity of the test compound was calculated by the following formula and shown as an inhibition rate (%) wherein the intracellular fluorescence activity resulting from the stimulation by the addition of ligand MCH (4–19) peptide was 100% and that of the well added with DMSO solution alone was 0%.

Inhibitory rate (%) = 100 − [fluorescence activity upon addition of test compound and MCH(4–19) peptide solution − fluorescence activity upon addition of DMSO solution only]/[fluorescence activity upon addition of DMSO solution and MCH(4–19) peptide solution − fluorescence activity upon addition of DMSO solution only] × 100

Evaluation of PLsis inducing potential.[58] DMEM medium, L-glutamine, penicillin-streptomycin, pyruvic acid, and *N*-(7-nitrobenz-2-oxa-l,3-diazol-4-yl)-l,2-hexadecanoyl-sn-glycero-3-phosphoethanolamine triethylammonium salt (NBD-PE) were purchased from Invitrogen Corporation. As bovine serum albumin (BSA), a product of Thermo Trace Ltd. (Melbourne, Australia) was used, and as Amiodarone, a product of ICN (Costa Mesa, CA) was used. A test compound was used in the form of a 10 mM DMSO solution.

FBS was added at a final concentration of 5 vol% to DMEM medium supplemented with L-glutamine, pyruvic acid and penicillin-streptomycin and subjected to the experiment. Culture was performed using 5% carbon dioxide gas–95% air as a gas phase in a CO_2 incubator at 37 °C.

HepG2 cells were suspended in a culture medium at 50×10^4 cells/mL, plated in a 96 well plate at 50 μL/well and precultured for 24 hr. After preculture, the culture medium was removed, a culture medium containing 60 μM NBD-PE was added at 50 μL/well, and a culture medium containing 0.6 μM or 20 μM test compounds were each added at 50 μL/well to HepG2 cells, and the cells were cultured for 24 hr. As a positive control, Amiodarone was used at a final concentration of 10 μM.

After exposure to the test compound for 24 hr, the fluorescence intensity (Ex. 485 nm, Em. 538 nm) of NBD-PE uptaken by the cells was measured by a fluorometer. The measurement value with addition of 0 μM test compound solution was subtracted as a blank, a relative value to the measurement value with addition of 10 μM Amiodarone was calculated, and the maximum value per unit concentration of the test compound was obtained as a phospholipidosis inducing potential.

Evaluation of time-dependent inhibition (TDI) of CYP3A4 (single-point assay). Human liver microsomes were purchased from Xenotech, LLC (Lenexa, KS). A mixture of a test compound (30 μM) and microsomes in phosphate buffer (pH 7.4) was preincubated at 37 °C in the presence of an NADPH-generating system containing $MgCl_2$, glucose-6-phosphate, β-$NADP^+$, and glucose-6-phosphate dehydrogenase. After preincubation, enzymatic activity of CYP3A4 in the incubation mixture was determined by measuring 6β-hydroxytestosterone in the reaction with testosterone by UPLC. The activity (% of control) for each preincubation time was calculated to the following: {(activity with test compound)/(activity with DMSO)} × 100. The remaining activity (% remaining) after preincubation was calculated to the following: {activity with preincubation (% of control)}/{activity without preincubation (% of control)} × 100.

GSH trapping experiment. *1. Instrument*. LC/MS system consisted of UPLC system (Waters, Milford, MA) and SYNAPT Q-TOF mass spectrometer (Waters) equipped with an electrospray ionization source.

2. Microsomal incubation with GSH. For the GSH trapping experiments each test compound (30 μM) was incubated with human liver microsomes (final protein concentration 1.0 mg/mL; XenoTech, LLC. Lenexa, KS) in the presence of an NADPH-regenerating system and GSH (1 mM) in phosphate buffer (pH 7.4) at 37 °C. The reaction was terminated after 60 min by the addition of an equal volume of acetonitrile. After centrifugation at 15000 rpm for 10 min, 5 μL of supernatant was injected into LC/MS system.

3. LC/MS/MS analysis. Microsomal incubation mixtures were separated on a BEH C_{18} column (1.7 μm, 2.1 × 100 mm; Waters) using solvent A (5% acetonitrile in 5 mM aqueous ammonium acetate) and solvent B (90% acetonitrile in 50 mM aqueous ammonium acetate). At a flow rate of 0.5 mL/min, the initial elution gradient was 98% solvent A and 2% solvent B with a linear gradient to

70% solvent B over 10 min and returned to initial condition. The column was allowed to equilibrate at 2% solvent B for 5 min before the next injection. The column temperature was 40 °C and the eluents were monitored with a PDA detector. The mass spectrometry was run in positive ion mode. The source settings were 1.20 kV capillary voltage, 35 V sampling corn voltage, 120 °C source temperature, 350 °C desolvation temperature. GSH adducts were analyzed based on their product ion spectra of the protonated molecules upon CID ramped from 15 V to 40 V.

Pharmacokinetic analysis in rat cassette dosing. Test compounds were administered intravenously (0.1 mg/kg) or orally (1 mg/kg, suspended in 0.5% methylcellulose aqueous solution) by cassette dosing to nonfasted rats. After administration, blood samples were collected and centrifuged to obtain the plasma fraction. The plasma samples were deproteinized by mixing with acetonitrile followed by centrifugation. The compound concentrations in the supernatant were measured by LC/MS/MS.

Evaluation of anorectic effect using male DIO F344/Jcl rats. Male DIO F344/Jcl rats (50 weeks old) fed with a high-fat diet (D12451: Research Diets) from 5 weeks old were used. From before the start of experiment, the rats were singly housed, given a powder high-fat diet (D12451M: Research Diets), and habituated to oral administration with tap water. The rats were grouped based on both the food intake and the body weight of day−1. The rats were orally administered vehicle (0.5% methylcellulose solution) or compounds suspended in vehicle at 2 mL/kg 1h before the onset of dark period for 2 days (6 rats per group). The food intake for 2 days from the initial administration was measured. The food intake inhibition rate of each compound administration group to the vehicle group was calculated.

Evaluation of anti-obesity effect using male DIO F344/Jcl rats. DIO F344 rats (45 weeks old) were habituated and grouped prior to treatment as described above. The rats were orally administered vehicle (0.5% methylcellulose solution) or compounds suspended in vehicle at 2 mL/kg for 2 weeks (6 rats per group). Sibutramine was used as a positive control in this study. The compounds were administered after measurement of body weight at 1–3 h before the onset of dark period and food intake was measured every 2 or 3 days. The change in body weight was presented as percentage from initial body weight.

In vivo selectivity of anorectic effect by using MCHR1-deficient mice. Male MCHR1-deficient mice and wild-type litter mate mice (45 weeks old) loaded with a high-fat diet (D12451) from 5 weeks of age were used. Before the start of the experiment, the mice were independently raised, a high-fat diet (D12451) was given, and tap water (0.5 mL) was administered for acclimation. The

mice were grouped on the basis of food intake from day−3 to day−1 and body weight of day−1 as indices. Each group was orally administered vehicle (0.5% methylcellulose solution) or compounds suspended in vehicle at 10 mL/kg for 3 days (6 mice per group). Food intake for 3 days was measured.

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