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Discovery of Agonists of Cannabinoid Receptor 1 with Restricted Central Nervous System Penetration Aimed for Treatment of Gastroesophageal Reflux Disease

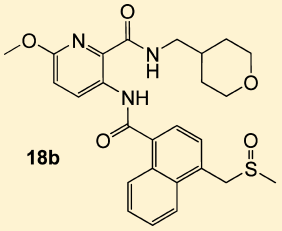
Alleyn T. Plowright,*[,†] Karolina Nilsson,[†] Madeleine Antonsson,[†] Kosrat Amin,[†] Johan Broddefalk,[†] Jörgen Jensen,[†] Anders Lehmann,[†] Shujuan Jin,[‡] Stephane St-Onge,[‡] Mirosław J. Tomaszewski,[‡] Maxime Tremblay,[‡] Christopher Walpole,[‡] Zhongyong Wei,[‡] Hua Yang,[‡] and Johan Ulander[†]

[†]AstraZeneca Research and Development, Pepparedsleden 1, Mölndal, 43183, Sweden

[‡]AstraZeneca Research and Development, 7171 Frederick-Banting, Saint-Laurent, Quebec, H4S 1Z9, Canada

Supporting Information

ABSTRACT: Agonists of the cannabinoid receptor 1 (CB1) have been suggested as possible treatments for a range of medical disorders including gastroesophageal reflux disease (GERD). While centrally acting cannabinoid agonists are known to produce psychotropic effects, it has been suggested that the CB1 receptors in the periphery could play a significant role in reducing reflux. A moderately potent and highly lipophilic series of 2-aminobenzamides was identified through focused screening of GPCR libraries. Development of this series focused on improving potency and efficacy at the CB1 receptor, reducing lipophilicity and limiting the central nervous system (CNS) exposure while maintaining good oral absorption. Improvement of the series led to compounds having excellent potency at the CB1 receptor and high levels of agonism, good physical and pharmacokinetic properties, and low penetration into the CNS. A range of compounds demonstrated a dose-dependent inhibition of transient lower esophageal sphincter relaxations in a dog model.



hCB1 IC_{50} = 15 nM

rat $C_{u,br}$ / $C_{u,pl}$ = 0.026

oral bioavailability (rat) = 21%

TLESR inhibition (0.1 μmol/kg) = 62%

INTRODUCTION

The cannabinoid receptor 1 (CB1) is a GPCR that is located both in the central nervous system (CNS) and on peripheral neurons.[1] Activation of CB1 receptors reduces calcium flux through voltage dependent calcium channels, resulting in reduced neurotransmitter release. The endogenous ligand for the CB1 receptor, anandamide (**1**), has been shown to block the N-type calcium channel (Figure 1).[2] As a consequence of these effects, agonists of the CB1 receptor have been suggested as possible medical treatments for a range of disorders including pain, inflammation, spasticity, neurodegenerative disorders, glaucoma, and gastroesophageal reflux disease (GERD).[3−9] In healthy adults, the large majority of all reflux episodes results from transient lower esophageal sphincter relaxation (TLESR) and is hence the single most important motility event as a cause of reflux in all GERD patients.[10] TLESRs are equally common in both healthy individuals and GERD patients. However, TLESRs in GERD patients are more frequently associated with acid reflux than those in healthy people. The centrally acting mixed CB1/cannabinoid receptor 2 (CB2) agonists, WIN 55,212-2 (**2**) and Δ^9-tetrahydrocannabinol (**3**) (Figure 1), have been shown to dose-dependently inhibit TLESRs in dogs.[8,9] Marinol, of which the active ingredient is **3**, has been shown to reduce TLESRs in healthy human volunteers.[9] The effect of **2** on TLESRs in dog could be reversed by the selective CB1 antagonist rimonabant[11] but not by the selective CB2 antagonist 5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-*N*-[(1*S*,2*S*,4*R*)-1,3,3-trimethylbicyclo[2.2.1]-hept-2-yl]-1*H*-pyrazole-3-carboxamide (SR144528),[12] indicating that CB1 alone mediates the action of **2**, and thereby CB1 agonists rather than CB2 agonists were desired.[8] Although the exact location of CB1 receptors involved in the inhibition of TLESRs is still uncertain, it has been suggested that the CB1 receptors in the periphery could play a significant role.[13,14] Centrally acting CB1 receptor agonists are known to produce psychotropic events that are not a viable therapeutic option for GERD patients, and therefore, targeting CB1 receptors in the periphery would be required.[15] While a number of cannabinoid ligands, particularly CB1 receptor antagonists, targeting peripheral restriction have recently been reported,[16−19] potent CB1 or CB1/CB2 agonists that combine low CNS penetration together with good physicochemical, pharmacokinetic, and in vivo efficacy characteristics are more scarce.[5,20]

Herein, a novel CB1 receptor agonist series is reported with high in vitro binding affinities and full agonism, good ligand lipophilicity efficiency (LLE)[21] and physicochemical properties, and low ratios of unbound concentration of compound in brain versus plasma ($C_{u,br}/C_{u,pl}$). Compounds in this series inhibit TLESRs in a dose dependent manner in an in vivo dog model.

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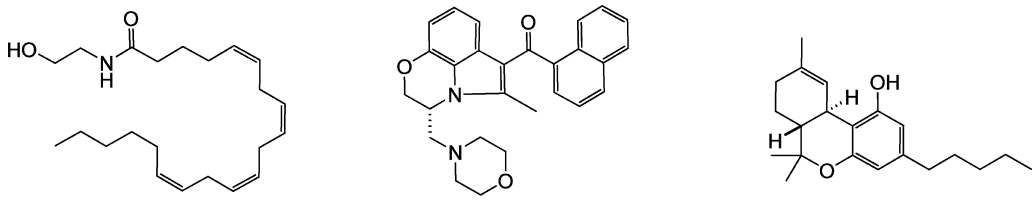

1 (anandamide) 2 (WIN 55,212-2) 3 (Δ^9-tetrahydrocannabinol)

Figure 1. Structures of CB1 receptor agonists: anandamide (**1**), WIN 55,212-2 (**2**), and Δ^9-tetrahydrocannabinol (**3**).

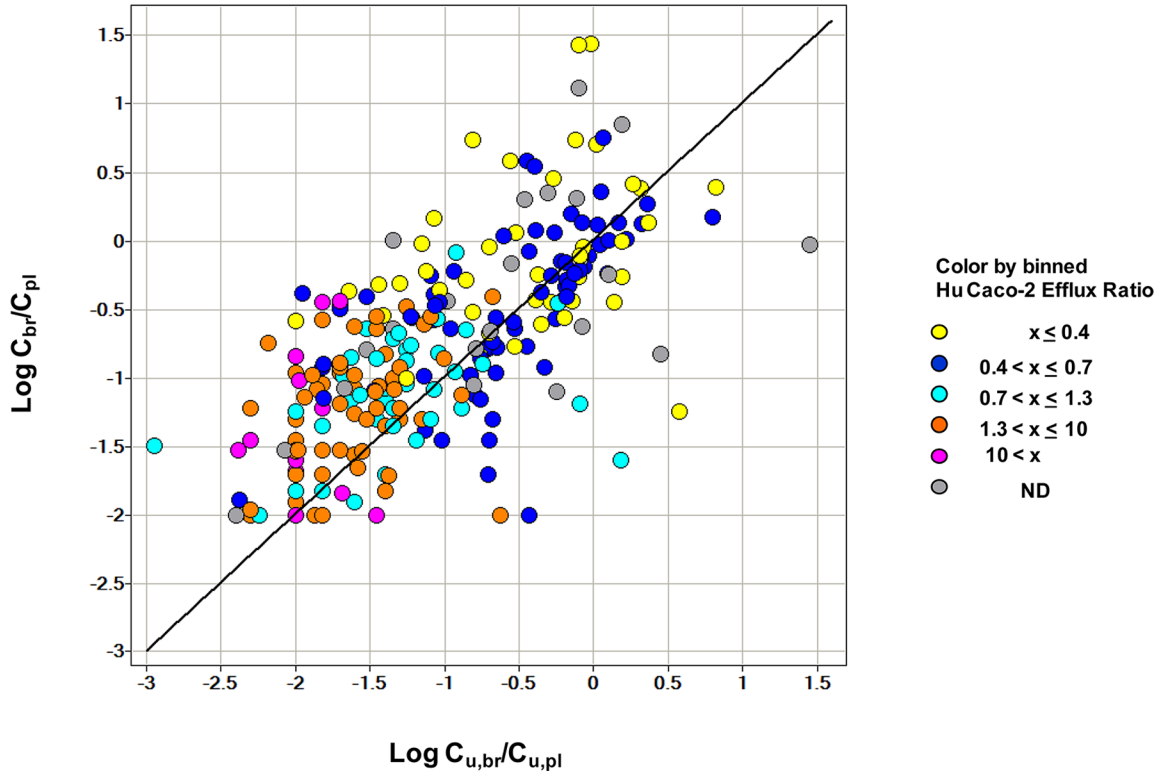

Figure 2. Experimental data showing total (C_{br}/C_{pl}) versus unbound ($C_{u,br}/C_{u,pl}$) ratios for 258 compounds from a variety of AstraZeneca projects and marketed drugs.[33] The coloring is based on experimental Caco-2 efflux data. The relevance of the efflux ratio is demonstrated by a clear shift in the plot where compounds with higher efflux generally have lower $C_{u,br}/C_{u,pl}$ ratios.

RESULTS AND DISCUSSION

Design and Identification of the Hit Series. The driver for biological response is the concentration of the drug available to interact with the target receptor in the required effect compartments.[22,23] In general, the amount of nonspecific binding differs between brain and plasma and the pharmacologically relevant ratio is the unbound brain-to-plasma concentration ratio $C_{u,br}/C_{u,pl}$.[24,25] The brain is separated from the systemic circulation by the BBB and the blood cerebrospinal fluid barrier. The CNS endothelial cells differ from their peripheral counterparts by having tighter junctions, limited pinocytic transport, and no fenestrations.[26] The net permeation across the BBB is a combination between permeability and efflux properties, and any substance with a finite permeability can maintain asymmetry of unbound concentration only by active-transport. One of the more prominent efflux transporters is P-gp.[27] P-gp transporters are expressed both in the BBB and in the gut, and it has been reported that most marketed CNS drugs are not PGP substrates.[26] The X-ray structure of apo P-gp has shed some light on putative mechanisms for P-gp mediated transport.[28] The crystal structure reveals a large flexible cavity of 6000 Å^3, making structure-based attempts to model P-gp specificity highly challenging. In addition, P-gp is just one of several potential transporters. The transporter breast cancer resistance protein (BCRP) has been shown to have, along with P-gp, the highest expression in human brain microvessels.[29] Therefore, while one strategy in the present molecular design was to achieve a high level of efflux from the CNS, it was not confined only to P-gp mediation. The extent of efflux (efflux ratio) was measured using Caco-2 cells.[30] P-gp and BCRP are both expressed in the Caco-2 cell line.[31] Brain exposure was described as the steady-state unbound brain-to-plasma concentration ratio $C_{u,br}/C_{u,pl}$ measured in rats where the compounds were administered as 4 h constant-rate intravenous infusions (both unbound and total ratios are reported on key compounds for comparison).[32] Figure 2 shows the C_{br}/C_{pl} and $C_{u,br}/C_{u,pl}$ ratios for 258 compounds from a variety of AstraZeneca projects where the coloring is based on measured Caco-2 efflux ratios.[33] The relevance of the efflux ratio is demonstrated by a clear shift in the plot where compounds with higher efflux generally have lower $C_{u,br}/C_{u,pl}$ ratios.

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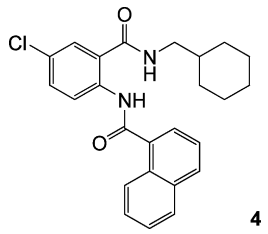
Much attention has recently been given to lipophilicity and in particular the consequences of making very lipophilic compounds in the search for highly potent compounds.[34] Lipophilicity influences permeability and is an important parameter in the design of peripheral compounds. However, lipophilicity alone is not expected to influence the asymmetry of unbound concentration directly, and the lipophilicity of non-CNS drugs is not expected to differ from that of CNS drugs by default.[35] LLE (pIC_{50} − log *D* or pIC_{50} − clogP) is one simple measure of the degree to which the potency is driven by forces other than hydrophobicity.[21] As the lead compounds in this study were highly lipophilic, LLE was a key parameter driving the design. Two human CB1 (hCB1) assays were used for estimation of CB1 potency, one competitive receptor binding assay (IC_{50})[36,37] and one assay for stimulation of [^{35}S]-guanosine 5′-*O*-(3-thiotriphosphate) (GTPγ[^{35}S]) binding assay (EC_{50}).[36,38] A corresponding competitive receptor binding assay was also utilized for the estimation of human CB2 (hCB2) potency.[36]

Screening a set of targeted GPCR libraries (75 000 compounds) led to the identification of the aminobenzamide derivative **4** (Figure 3). Compound **4** displayed reasonable in vitro potency, as measured in the competitive receptor binding assay, but was highly lipophilic[39] and displayed partial agonism (Table 1).



4

Figure 3. Initial CB1 receptor agonist lead (**4**) identified from screening a set of targeted GPCR libraries (75 000 compounds).

Chemical Synthesis. The syntheses of the pyridyl and pyrazinyl derivatives are described in general in Schemes 1−3. Introduction of the amide functional groups into the 2- and 3-position of the heterocyclic core structure was performed by two methods. Coupling 3-aminopyridine-2-carboxylic acid **5a**, 3-amino-6-methoxypyridine-2-carboxylic acid **5b** (synthesized from 3-(acetylamino)-6-methoxypyridine-2-carboxylic acid[40]), or 3-aminopyrazine-2-carboxylic acid **5c** to the required acid chloride followed by heating in the presence of *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate (HATU) gave the oxazin-4-one derivatives **6a−e** (Scheme 1). Alternatively, the required amine was first coupled to **5a−c** using HATU mediated coupling conditions to give amides **7a**,**b**. The oxazin-4-one derivatives **6a−e** then underwent ring opening upon treatment with the required amine, or the amides **7a**,**b** underwent a second acid coupling with the required substituted carboxylic acid to give the dicarboxamide derivatives **8a−o**. Some compounds were derivatized further. For example, where R^3 was a 4-substituted-1-naphthoic acid group, the acid was reduced via the mixed anhydride to the alcohol, which was subsequently converted to a mesylate. The mesylate was then displaced with a variety of nucleophiles to provide derivatives **9a−f**. A methoxy group in the 6-position of the pyridyl ring also provided a handle for further derivatization. Treatment of **8n**, where R^1 was a methoxy group, with pyridine hydrochloride at 160 °C gave the pyridone which was subsequently O-alkylated either using a variety of electrophiles in the presence of silver(I) carbonate at 130 °C or using Mitsunobu reaction conditions with the required alcohol to give the products **10a**,**b**.[41]

Further manipulation of the naphthalene ring led to the introduction of triazole and sulfur containing functionality into the 4-position. The triazole derivatives were synthesized by first coupling **5a−c** with 4-methyl-1-naphthalenecarbonyl chloride and then trapping the resultant acid with methyl iodide to give the esters **11a−c** (Scheme 2). The methyl substituted naphthalene was then brominated under radical conditions to give the bromomethyl intermediate, which then underwent a S_N2 reaction upon treatment with 1,2,3-triazole to give **12a−c**. Finally the amide was introduced at the 2-position of the heterocyclic core by treating **12a−c** with the required amine to give the triazoles **13a−e**. Treatment of **13c**, where R^1 was a methoxy group, with pyridine hydrochloride at 160 °C gave the pyridone which was subsequently O-alkylated using silver(I) carbonate and 3,3,3-trifluoropropylsulfonyl chloride to give **14**.

The sulfur containing derivatives were synthesized by two methods (Scheme 3). Treatment of **5b**,**c** with HCl in methanol gave the methyl esters **15a**,**b**, which were then reacted with 4-[(methylthio)methyl]-1-naphthoyl chloride (synthesized from 4-(bromomethyl)-1-naphthoic acid[42]) to give the amides **16a**,**b**. Treatment of **16a**,**b** with the required amines gave the thiomethylnaphthalene intermediates **17b** and **17d−e**. Alternatively, treatment of intermediates **7a**,**b** with 4-[(methylthio)-methyl]-1-naphthoyl chloride gave **17a** and **17c**. Finally, oxidation of **17a−e** using *m*-CPBA gave the sulfoxides **18a−g** and the sulfone derivative **19**. The R^1 group was varied by treating **17e**, where R^1 was a methoxy group, with pyridine hydrochloride to give the pyridone. The pyridone was then oxidized using *m*-CPBA to give the sulfoxide which was subsequently O-alkylated with silver(I) carbonate and 2-(2-chloroethoxy)ethanol to give **20**.

Biological Evaluation and Structure−Activity Relationship Study. Explorative chemistry aimed at improving the overall properties showed that the phenyl core in the lead compound **4** could be replaced with the pyridyl (**8a**) or pyrazinyl (**8b**) ring systems and lead to improved potency (Table 1). In the case of the pyridyl-2-carboxamide derivative **8a**, affinity to the hCB1 receptor was increased greater than 10-fold and full efficacy achieved. The increase in binding affinity for **8a** and **8b** is believed to be attributed to the intramolecular hydrogen bond that exists between either the pyridyl or pyrazinyl nitrogen atom and the NH moiety of the 2-carboxamide group which conformationally restricts the structure to provide a planar conformation. In contrast, moving the pyridine nitrogen atom to give the corresponding 3-carboxamide (**8c**), 4-carboxamide (**8d**), or 2-amino-3-carboxamide (**8e**) derivatives gave a significant decrease in potency compared to **4**.

With the knowledge that good affinity and full agonism could be achieved, the focus shifted toward improving ADMET properties to meet the required peripheral candidate drug profile. The different positions on the core structure were modified to build up knowledge of how different substitution patterns and groups would affect these properties. The 2-carboxamide side chain of the pyridine ring was varied showing that both lipophilicity and molecular weight could be reduced without significant loss of potency (Table 2). For example, replacement of the cyclohexylmethyl moiety with tetrahydro-

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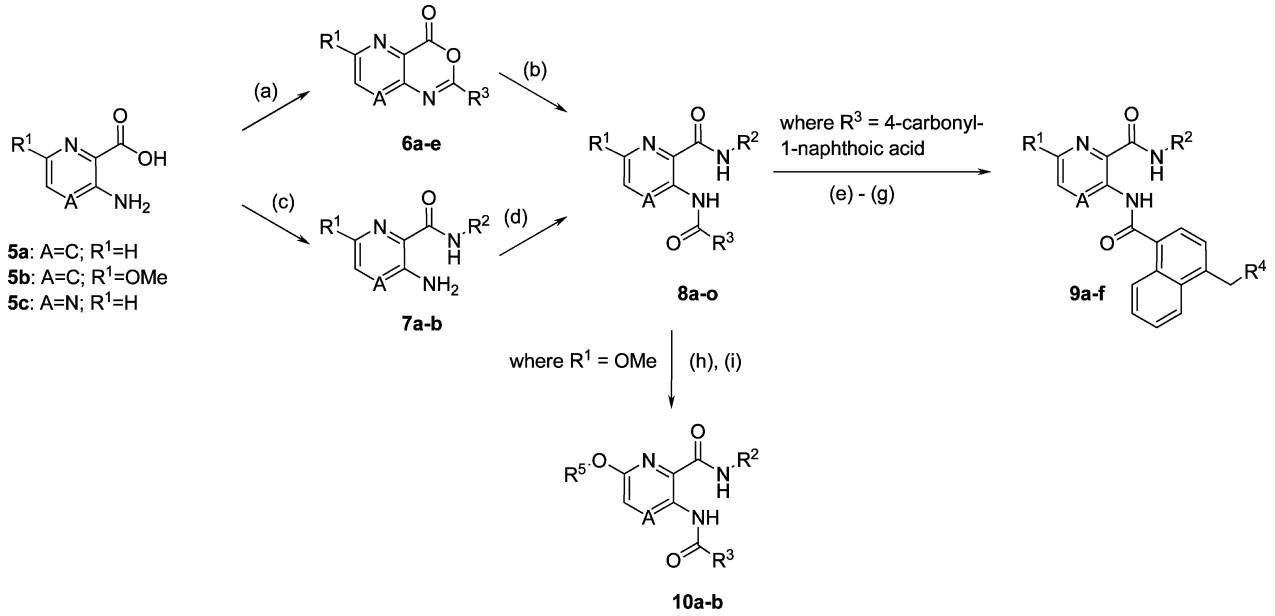
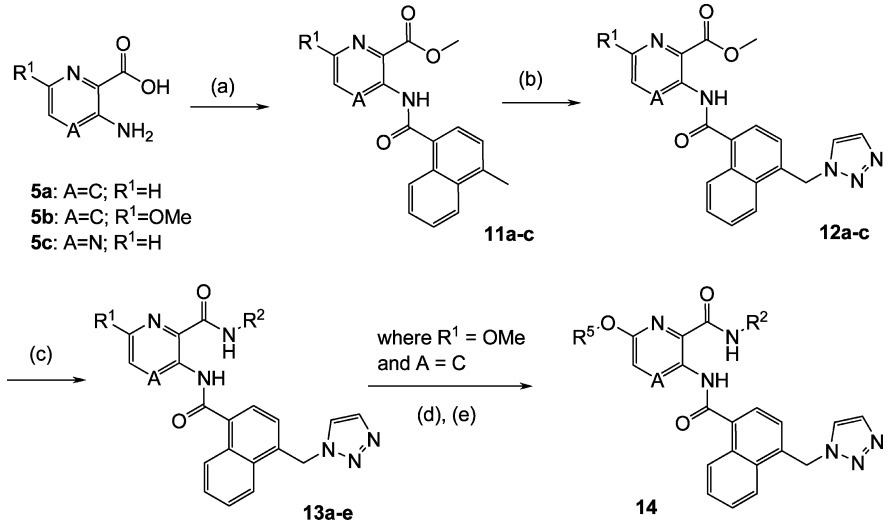
Scheme 1. Synthesis of Substituted Pyridine and Pyrazine Derivatives[a]



[a]Reagents and conditions: (a) (i) $R^3C(O)Cl$, *N,N*-diisopropylethylamine (DIPEA), DMF, CH_2Cl_2, 0 °C; (ii) DIPEA, HATU, 50 °C; (b) R^2NH_2, DIPEA, DMF; (c) R^2NH_2, HATU, DIPEA, DMF; (d) R^3COOH, HATU, DIPEA, DMF; (e) (i) oxalyl chloride, DCE, 70 °C; (ii) $NaBH_4$, I_2; (f) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0 °C; (g) nucleophile (R^4H) (e.g., pyrrole for **9c**), KI, DMF, 80 °C; (h) pyridine hydrochloride, 160 °C; (i) Ag_2CO_3, R^5Cl, DMF, 130 °C or R^5OH, diisopropyl azodicarboxylate, Ph_3P, THF, 50 °C. The structures of R^1–R^5 and the molecule cores are presented in Tables 1–3.

Scheme 2. Synthesis of Triazole Derivatives[a]



[a]Reagents and conditions: (a) (i) 4-methyl-1-naphthalenecarbonyl chloride, DIPEA, DMF, 50 °C; (ii) MeI, K_2CO_3; (b) (i) NBS, benzoyl peroxide, CCl_4; (ii) 1,2,3-triazole, DMF; (c) R^2NH_2, DMF, 80 °C; (d) pyridine hydrochloride, 160 °C; (e) Ag_2CO_3, R^5Cl, DMF, 130 °C. The structures of R^1, R^2, and R^5 are presented in Tables 3 and 4.

pyranylmethyl (**8f**) gave a 3-fold reduction in binding affinity with a significant reduction in lipophilicity while replacement with cyclobutylmethyl (**8g**) gave similar potency compared to **8a**. Compound **8f** showed significant CNS exposure (C_{br}/C_{pl} = 3), which was comparable to that of **2** (C_{br}/C_{pl} = 2.5), highlighting that significant work was required to achieve peripheral compounds in this series.

A large contributing factor to the overall lipophilicity of these compounds was the naphthalene in the 3-position of the pyridine ring. Replacement of the naphthalene ring with alternative bicyclic systems, such as benzodioxane (**8h**), benzothiazole (**8i**), or quinoxaline (**8j**) led to a drop in potency compared with the naphthalene derivative **8f**. Introduction of a nitrogen atom to give regioisomeric quinoline or isoquinoline derivatives was also explored. The 5-substituted isoquinoline (**8k**) and 8-substituted quinoline (**8l**) both showed reduced binding affinity compared with **8g**. However, the 4-substituted quinoline (**8m**) displayed greater potency than the other regioisomers and also maintained full efficacy and had no selectivity versus hCB2. Further substitution in the 6-position of the pyridine ring of **8m** with a methoxy group gave **8n** which was also well tolerated (Table 3).

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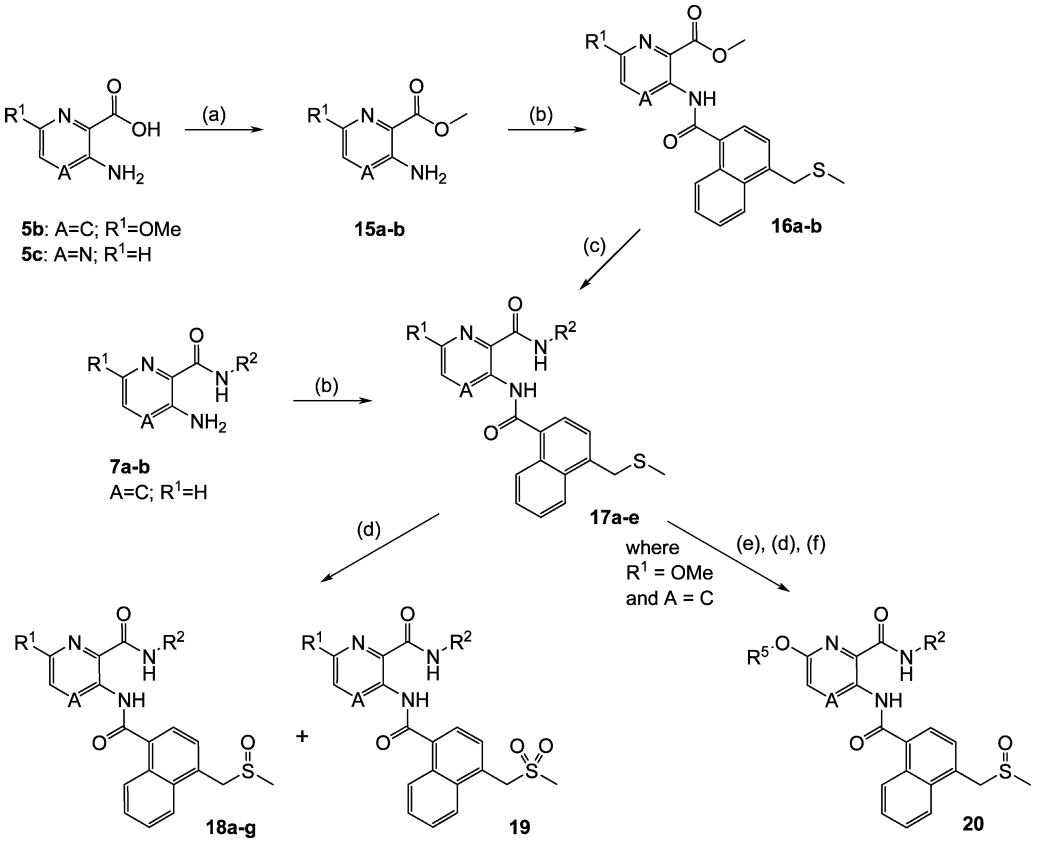
Scheme 3. Synthesis of Sulfoxide Derivatives[a]



[a]Reagents and conditions: (a) HCl, MeOH; (b) 4-[(methylthio)methyl]-1-naphthoyl chloride, pyridine, DMAP, $CHCl_3$, 50 °C; (c) R^2NH_2, DMF, 80 °C; (d) *m*-CPBA, CH_2Cl_2, 0 °C; (e) pyridine hydrochloride, 160 °C; (f) Ag_2CO_3, R^5Cl, DMF, 130 °C. The structures of R^1, R^2, and R^5 are presented in Tables 3 and 4.

As a nitrogen atom could be incorporated into the 4-position of the naphthalene ring, this led to an investigation into alternative substitution at this position in an attempt to further enhance potency while reducing lipophilicity. This was also an opportunity to introduce additional polarity, including hydrogen bond donors and acceptors, in an attempt to pick up recognition elements with CNS efflux transporters such as P-gp and also modify molecular properties to restrict brain penetration.[17,43] While acidic side chains such as in **8o** were not tolerated, it was found that a wide variety of polar substituents could successfully be incorporated into this position to achieve high levels of hCB1 inhibition, including both neutral and basic side chains (Table 3). This included substituents such as methoxymethyl (**9a**) and dimethylaminomethyl (**9b**). The pyrrole derivative (**9c**) lost potency compared to the unsubstituted naphthalene (**8g**). The imidazole (**9d**) and pyrazole (**9e**) derivatives had comparable potency, while the triazole (**9f**) had excellent subnanomolar binding affinity to hCB1 and full agonism. As expected, **9f** had reduced lipophilicity relative to **8a** and hence achieved an improved LLE and also good selectivity over hCB2. This is most likely due to the triazole ring picking up an additional binding interaction with the hCB1 receptor. Alternatively the methylsulfoxide (**18a**) and methylsulfone (**19**) were also tolerated with the sulfoxide displaying significantly greater binding affinity and LLE compared with the sulfone **19**. As a methoxy group was well tolerated in the 6-position of the pyridine ring as shown with **8n**, additional modifications were made in this position. This is exemplified with the hydroxy ethers (**10a** and **10b**) (Table 3). The derivative containing the hydroxyethoxyethoxy side chain (**10b**) gave the highest affinity for hCB1 and highest LLE. Substitution at the 6-position also had a large effect on the selectivity over hCB2, where increasing the size and length of the substituent from a hydrogen atom to the hydroxyethoxyethoxy side chain in **10b** increased the selectivity significantly.

With the SAR of the three side chains established, the best substituents were combined to address the overall design criteria including achieving compounds with a very low $C_{u,br}/C_{u,pl}$ ratio (Table 4). The series of 1,4-naphthylmethyltriazoles (**13a–d**) achieved excellent hCB1 potency and showed a reduction in log *D* of 3 log units across the series, whereas the drop in potency is less than 1 log unit. Thus, an increase in LLE is achieved where the LLE for **13d** is the greatest. Other properties including permeability, as measured in Caco-2 cells, and solubility are also improved as log *D* decreases across this series. The $C_{u,br}/C_{u,pl}$ ratio also decreases from **13a** to **13d**. The polar surface area (PSA) is similar between **13a–d** (PSA = 112 for **13a** and PSA = 111 for **13d**), and interestingly the efflux ratio decreases as the $C_{u,br}/C_{u,pl}$ decreases. This may be caused by several reasons, including that other transporter mechanisms are involved. The triazole derivatives **13a**, **13c**, and **13d** maintained good selectivity over hCB2, while **13d** also displayed full efficacy. At the beginning of this investigation it was shown that a pyrazine ring could replace the pyridine core structure. The preferred side chains from the SAR exploration

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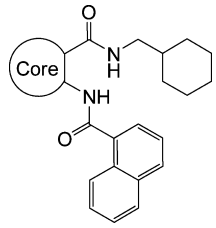
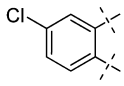
Table 1. In Vitro Binding, Efficacy, and Lipophilicity Data for the Core Variations



Compound	Core	hCB1 IC_{50} (μM)[a]	hCB2 IC_{50} (μM)[a]	hCB1 EC_{50} (μM)[b]	hCB1 Emax (%)[c]	logD[d]	LLE[e]
2		0.059	0.0034	0.11	100	5.2	2.0
4		0.16	1.8	0.26	77	7.8	-1.0
8a		0.010	0.11	0.024	110	8.1	-0.11
8b		0.091	1.4	0.48	120	ND[f]	ND
8c		1.4	9.0	ND	ND	6.1	-0.24
8d		3.6	>8.9	ND	ND	5.5	-0.05
8e		>10	>10	ND	ND	4.7	<0.30

[a] IC_{50} values against hCB1 and hCB2 were determined using membranes from HEK293S cells expressing the cloned hCB1 receptor or Sf9 cells expressing the cloned hCB2 receptor and ^{3}H-CP55,940 as the radioligand.[36,37] Curve fitting and IC_{50} calculations were performed using Xlfit4 (IDBS, Inc.). IC_{50} values were obtained from three independent experiments and are expressed as the mean. [b] EC_{50} values from GTPγ[^{35}S] binding assay were measured on cloned hCB1 receptors in membranes of HEK 293S cells.[36,38] Curve fitting and EC_{50} calculations were performed using Xlfit4 (IDBS, Inc.). EC_{50} values were obtained from three independent experiments and are expressed as the mean. [c] E_{max} is the maximal effect of the test compounds and expressed as a percentage of that value obtained with **2**. [d] Chromatographic log *D* measurements performed in a similar fashion to that described previously.[39] The log *D* was calculated from retention time versus standard curve. [e] LLE defined as pIC_{50}(hCB1 IC_{50}) − log *D*.[21] [f] ND means not determined.

with the pyridine derivatives were applied to the pyrazine core which gave the triazole derivative **13e**, a potent hCB1 receptor agonist. Further exploration of the 6-position of the pyridine ring led to the discovery of the 1,1,1-trifluoropropylsulfonate containing derivative **14**,[44] which was a highly potent and full agonist of hCB1 with excellent selectivity over hCB2. Compound **14** had an even lower $C_{u,br}/C_{u,pl}$ ratio compared to **13d**, most likely reflecting the increased PSA (158) as well as being an efflux substrate as shown in the Caco-2 assay. A set of 1,4-naphthylmethylsulfoxides with different substituents in the 2- and 6-position of the pyridyl ring were also prepared. This set of compounds, including **18b,c**, again showed that good binding affinity against hCB1 can be achieved with compounds with significantly reduced lipophilicity and hence improved LLEs. Compounds **18b,c** have good permeability and are efflux substrates in Caco-2 cells, which leads to low $C_{u,br}/C_{u,pl}$ ratios. The enantiomers of the sulfoxide moiety show a significant difference in binding affinity to hCB1 as exemplified by the enantiomers of **18c**, compounds **18d** and **18e**. The more potent enantiomer **18d** has a high efflux ratio and is

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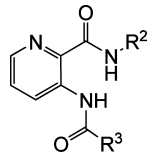
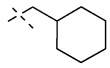
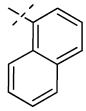
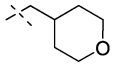
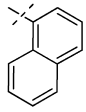
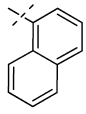
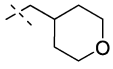
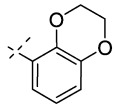
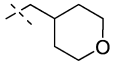
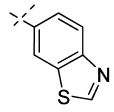
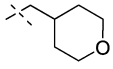
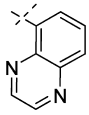
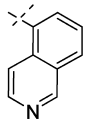
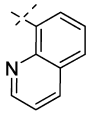
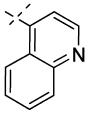
Table 2. In Vitro Binding, Efficacy, Lipophilicity, and LLE Data for R^2 and R^3 Variations



Compound	R^2	R^3	hCB1 IC_{50} (μM)[a]	hCB2 IC_{50} (μM)[a]	hCB1 EC_{50} (μM)[b]	hCB1 Emax (%)[c]	logD[d]	LLE[e]
8a			0.010	0.11	0.024	110	8.1	-0.11
8f			0.031	0.26	0.081	110	5.1	2.4
8g			0.0061	0.026	0.023	110	ND[f]	ND
8h			1.2	5.7	ND	ND	3.8	2.1
8i			>6.7	>10	ND	ND	3.5	<1.7
8j			>9.9	>10	ND	ND	2.5	<2.5
8k			0.22	0.038	ND	ND	5.1	1.5
8l			0.32	0.36	ND	ND	5.5	1
8m			0.028	0.082	0.079	94	ND	ND

[a] IC_{50} values against hCB1 and hCB2 were determined using membranes from HEK293S cells expressing the cloned hCB1 receptor or Sf9 cells expressing the cloned hCB2 receptor and ^{3}H-CP55,940 as the radioligand.[36,37] Curve fitting and IC_{50} calculations were performed using Xlfit4 (IDBS, Inc.). IC_{50} values were obtained from three independent experiments and are expressed as the mean. [b] EC_{50} values from GTPγ[^{35}S] binding assay were measured on cloned human CB1 receptors in membranes of HEK 293S cells.[36,38] Curve fitting and EC_{50} calculations were performed using Xlfit4 (IDBS, Inc.). EC_{50} values were obtained from three independent experiments and are expressed as the mean. [c] E_{max} is the maximal effect of the test compounds and expressed as a percentage of that value obtained with **2**. [d] Chromatographic log *D* measurements performed in a similar fashion to that described previously.[39] The log *D* was calculated from retention time versus standard curve. [e] LLE defined as pIC_{50}(hCB1 IC_{50}) − log *D*.[21] [f] ND means not determined.

peripherally restricted with a low $C_{u,br}/C_{u,pl}$ ratio. Replacement of the pyridine core in **18a** with a pyrazine ring provided the sulfoxide derivative **18f**, which had good potency against the hCB1 receptor. The enantiomers of **18f** were separated, and as with the pyridine core, one sulfoxide enantiomer (**18g**) showed much greater potency versus hCB1. Analysis of the matched

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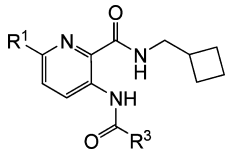
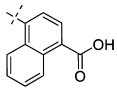
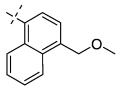
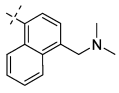
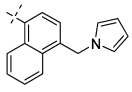
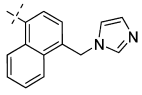
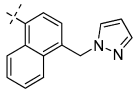
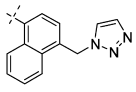
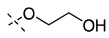
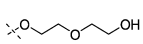
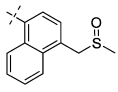
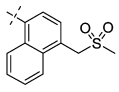
Table 3. In Vitro Binding, Efficacy, Selectivity, and LLE Data for R^1 and R^3 Variations



Compound	R^1	R^3	hCB1 IC_{50} (nM)[a]	hCB2 IC_{50} (nM)[a]	hCB1 EC_{50} (nM)[b]	hCB1 Emax (%)[c]	logD[d]	LLE[e]
8n	OMe		15	100	16	51	5.9	1.9
8o[f]	H		>10000	>10000	ND[g]	ND	ND	ND
9a	H		2.0	19	5.9	110	ND	ND
9b	H		41	190	180	100	ND	ND
9c	H		89	340	170	82	ND	ND
9d	H		16	330	40	100	ND	ND
9e	H		13	69	23	93	6.2	1.7
9f	H		0.71	65	1.4	110	5.3	3.8
10a			73	2500	120	51	4	3.1
10b			10	1200	14	130	4.1	3.9
18a	H		7.0	ND	5.9	110	4.6	3.6
19	H		240	ND	260	77	5.2	1.4

[a] IC_{50} values against hCB1 and hCB2 were determined using membranes from HEK293S cells expressing the cloned hCB1 receptor or Sf9 cells expressing the cloned hCB2 receptor and ^{3}H-CP55,940 as the radioligand.[36,37] Curve fitting and IC_{50} calculations were performed using Xlfit4

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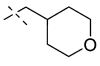
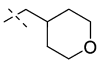
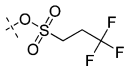
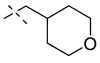
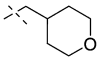
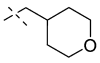
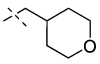
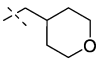
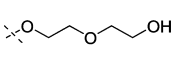
Table 3. continued

(IDBS, Inc.). IC_{50} values were obtained from three independent experiments and are expressed as the mean. [b]EC_{50} values from GTPγ[^{35}S] binding assay were measured on cloned human CB1 receptors in membranes of HEK 293S cells.[36,38] Curve fitting and EC_{50} calculations were performed using Xlfit4 (IDBS, Inc.). EC_{50} values were obtained from three independent experiments and are expressed as the mean. [c]E_{max} is the maximal effect of the test compounds and expressed as a percentage of that value obtained with **2**. [d]Chromatographic log *D* measurements performed in a similar fashion to that described previously.[39] The log *D* was calculated from retention time versus standard curve. [e]LLE defined as pIC_{50}(hCB1 IC_{50}) − log *D*.[21] [f]Compound **8o** contains tetrahydropyranylmethyl instead of cyclobutylmethyl in the R^2 position. [g]ND means not determined.

Table 4. In Vitro Binding, Lipophilicity, and Caco-2 Data and $C_{u,br}/C_{u,pl}$ Data for the Best R^1, R^2, and R^4 Combinations

Compound	R^1	R^2	R^4	hCB1 IC_{50} (nM)[a]	hCB2 IC_{50} (nM)[a]	hCB1 EC_{50} (nM)[b]	hCB1 Emax (%)[c]	logD[d]	LLE[e]	Hu Caco-2 P_{app} A to B ($1E^{-6}$cm/s)[f]	Hu Caco-2 Efflux Ratio[g]	$C_{u,br}/C_{u,pl}$[h]	C_{br}/C_{pl}[i]
13a	OMe			0.85	110	1.7	57	6.7	2.4	0.19	ND[j]	0.18	0.18
13b	OMe			0.97	ND	1.3	54	5.7	3.3	0.96	2.6	0.093	0.31
13c	OMe			2.4	470	2.9	60	4.2	4.5	47	1.5	0.044	0.10
13d	H			5.0	1200	8.0	110	3.6	4.7	43	0.55	0.019	0.093
13e[k]	H			4.3	ND	8.1	110	3.4	5.0	50	0.9	0.054	0.078
14				14	>10000	11	110	4.8	3.0	21	3.3	0.015	0.016
18b	OMe			15	>690	13	79	3.4	4.4	35	3.2	0.026	0.031
18c	H			49	2500	87	110	2.8	4.5	23	2.4	0.010	0.018
18d[l]	H			21	ND	31	100	2.9	4.8	26	17	0.030	0.031
18e[l]	H			600	ND	ND	ND	2.9	3.3	0.61	ND	ND	ND
18f[k]	H			47	300	120	120	2.7	4.6	20	6.3	0.030	0.022
18g[k,m]	H			37	ND	32	110	2.7	4.7	9	5.4	0.011	0.020
20				7.2	500	5.1	120	3.2	4.9	26	11	0.023	0.020

[a]IC_{50} values against hCB1 and hCB2 were determined using membranes from HEK293S cells expressing the cloned hCB1 receptor or Sf9 cells expressing the cloned hCB2 receptor and ^{3}H-CP55,940 as the radioligand.[36,37] Curve fitting and IC_{50} calculations were performed using Xlfit4 (IDBS, Inc.). IC_{50} values were obtained from three independent experiments and are expressed as the mean. [b]EC_{50} values from GTPγ[^{35}S] binding assay were measured on cloned human CB1 receptors in membranes of HEK 293S cells.[36,38] Curve fitting and EC_{50} calculations were performed using Xlfit4 (IDBS, Inc.). EC_{50} values were obtained from three independent experiments and are expressed as the mean. [c]E_{max} is the maximal effect of the test compounds and expressed as a percentage of that value obtained with **2**. [d]Chromatographic log *D* measurements performed in a similar fashion to that described previously.[39] The log *D* was calculated from retention time versus standard curve. [e]LLE defined as pIC_{50}(hCB1 IC_{50}) − log *D*.[21] [f]Apparent permeability measurements carried out in Caco-2 cells at pH 7.4 for apical (A) to basolateral (B) transport. [g]Efflux ratio defined as P_{app}(B to A)/P_{app}(A to B) where both P_{app}(A to B) and P_{app}(B to A) are measured in Caco-2 cells. [h]Calculated by combining the total brain to plasma ratio determined from in vivo samples from Sprague−Dawley rats with estimates of the unbound fraction in the brain from rat brain slices[45] and unbound fraction in plasma by equilibrium dialysis.[32,46] [i]The total brain to plasma ratio determined from in vivo samples from Sprague−Dawley rats. [j]ND means not determined. [k]Compounds contain pyrazine core. [l]The two enantiomers of **18c**. [m]Most active enantiomer of **18f**.

pairs of the pyridine and pyrazine containing compounds showed that the pyrazine compounds were in general 5- to 10-fold less potent versus hCB1 with similar efficacy.[47] The pyrazines were also less lipophilic and had higher predicted PSA, similar selectivity versus hCB2, and generally improved solubility (3- to 100-fold). Compounds **18f** and **18g** were

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peripherally restricted with low $C_{u,br}/C_{u,pl}$ ratios. Finally, increasing the size of the 6-substituent on the pyridine core, exemplified with compound **20**, maintained excellent potency and led to excellent selectivity against hCB2.

Now that compounds displaying good potency and efficacy versus hCB1 and low $C_{u,br}/C_{u,pl}$ ratios had been identified, the concept of inhibition of TLESRs through CB1 receptor agonism was tested in an in vivo dog model.[9,48] Prior to testing in vivo, it was confirmed that compounds in this series, for example, **13c**, **18b**, and **20**, showed excellent potency at the dog CB1 receptor with EC_{50} values that were similar to that shown at the hCB1 receptor (Table 5). The compounds tested

Table 5. In Vitro Dog CB1 EC_{50} Values (GTPγ[^{35}S] Binding Assay) and Mean Inhibition of Transient Lower Esophageal Sphincter Relaxations (TLESRs) in the Dog in Vivo Model at Different Doses

compd	dog CB1 EC_{50} (nM)[a]	mean inhibition of TLESRs (%)[b]	±SEM	dose (μmol/kg)[c]	n[d]
2	47	14.1	12.7	0.006	6
		58	10.4	0.019	6
		81.7	1.2	0.06	4
13a	1.5	43.2	16.2	0.25	4
		81.5		0.5	1
13c	4.1	28.9	6.9	0.05	4
		57.2	6.8	0.1	4
		90.4	9.6	0.2	2
13d	ND[e]	13.5	9.6	0.2	2
		50	10.7	0.4	4
13e	31	21.5	7.5	1.5	4
		38.1	16.3	3	4
14	19	28.6	4.7	0.1	4
		33.5	12.8	0.2	4
		58.4	13	0.4	2
18b	16	38.6	2.6	0.05	2
		62.2	23.4	0.1	2
20	3.0	5.9	2	1	2
		31.7	16.8	2	2

[a] EC_{50} values from GTPγ[^{35}S] binding assay were measured on cloned dog CB1 receptors in membranes of HEK 293S cells.[38] Curve fitting and EC_{50} calculations were performed using Xlfit4 (IDBS, Inc.). EC_{50} values were obtained from three independent experiments and are expressed as the mean. [b] Inhibition of TLESRs through CB1 receptor agonism tested in an in vivo dog model.[9,48] The compounds tested demonstrated a dose-dependent inhibition of TLESRs. [c] Administered intragastrically except **2**, **14**, and **18b**, which were administered by an iv bolus. [d] n = number of experiments for each dose. [e] ND means not determined.

in vivo demonstrate a dose-dependent inhibition of TLESRs in the dog, delivering greater than 50% inhibition for most of the compounds tested (Table 5). Figure 4 shows the dose response plots for **13c** and **14**. The level of inhibition achieved is comparable in magnitude to what has been shown previously for the more centrally acting CB1 agonists, **2** and **3**, in the same animal model.[8,9] In dogs, centrally acting cannabinoids induce a behavioral pattern known as static ataxia, including sedation, prancing, and motor incoordination.[49] Single behavioral experiments with the CB1 receptor agonists with restricted CNS penetration demonstrated an increased margin (e.g., approximately 5 and 10 times for **13c** and **14**, respectively) between plasma concentrations producing 50% inhibition of TLESRs and the plasma concentrations that induce these CNS side effects in comparison with **2**. Compound **2** showed no margin between the plasma concentrations required to reach 50% inhibition of TLESRs and CNS side effects. The present data demonstrate the potential for achieving a robust inhibition of TLESRs with CB1 agonists with restricted CNS penetration, reducing the risk for CNS mediated side effects.

Further profiling of this series revealed that a number of compounds were strong inhibitors of the cytochrome P450 (CYP) isoforms 3A4 and 2C9, in some cases with IC_{50} values of around 100 nM (Table 6). These interactions are highly undesirable because of potential drug−drug interactions in the clinic.[50] The potency versus CYP 2C9 was particularly sensitive to changes in R^1. Changing a H atom to a methoxy group increased potency at CYP 2C9; for example, compare **13d** with **13c** or **18c** with **18b**. This effect of the methoxy group on the interactions with CYP 2C9 could be decreased by increasing the size and flexibility of the R^1 substituent, as shown by comparing **18b** with **20**. The potency at CYP 3A4 was impacted to some extent by the R^1 substituent. However, it was particularly sensitive to variations on the naphthalene ring (R^4). For example, while the methyltriazole substituted naphthalene in compound **13c** significantly increased the hCB1 potency and LLE, it also led to strong inhibition of CYP 3A4. It has been reported that nitrogen atoms in heterocyclic ring systems can bind strongly to the heme moiety within the active site of the CYP enzymes.[51] Hence, attempts were made to substitute the triazole ring to increase the steric bulk around the triazole nitrogen atoms to reduce the affinity. However, in this case this approach had minimal impact on reducing the interactions. Alternatively, replacement of the triazole ring with a sulfoxide group led to significantly reduced potency at the CYP enzymes; for example, compare **13c** and **18b**. Applying the learning from R^1 and R^4 in combination led to significantly reduced CYP interactions and provided compounds with reduced inhibition of both CYP 2C9 and 3A4 isoforms (e.g., **18c**). Applying the same learning with the pyrazine core also provided compounds, such as **18f**, with significantly reduced inhibition of the CYP enzymes.

The most promising sulfoxide derivatives **18b**, **18c**, and **18f** were profiled further (Table 7). As well as showing good permeability in Caco-2 cells and having low $C_{u,br}/C_{u,pl}$ ratios, these compounds display good solubility and low inhibition of the hERG encoded potassium channel.[52] These compounds were also tested in in vivo rat PK studies and show moderate clearance and moderate to good oral bioavailability. For example, despite efflux properties contributing to very low concentrations in the CNS, **18b** showed acceptable oral bioavailability and clearance while the pyrazine derivative **18f** had similar clearance but good oral bioavailability.

CONCLUSION

The design of efficient orally bioavailable yet peripherally restricted compounds involves optimizing properties that are often in conflict with each other. Sufficient bioavailability is required; thus, permeability across the intestinal membrane is needed, while permeation across the BBB needs to be minimized. This can be achieved, as the concentration of the compound is much higher in the intestine, potentially leading to saturation of efflux transporters.[54] A moderately potent and highly lipophilic series of 2-aminobenzamides was identified through focused screening of GPCR libraries. Opportunities were found to vary the core structure to pyridine or pyrazine to increase potency at the hCB1 receptor by restricting the

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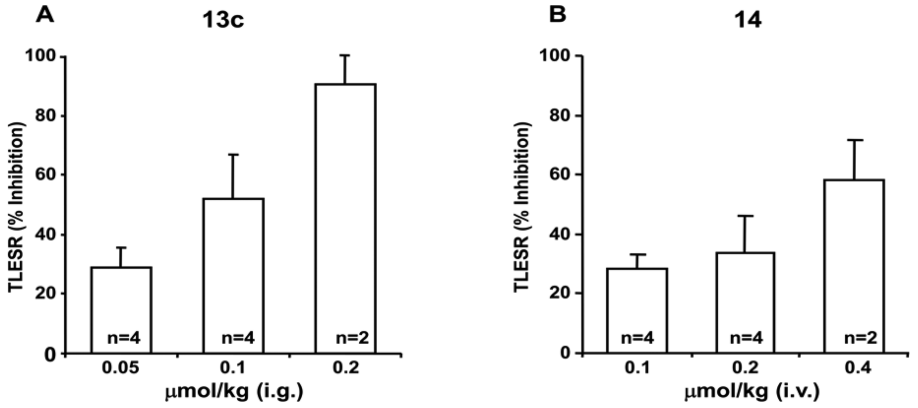

Figure 4. Compounds **13c** and **14** produced dose-dependent inhibition of transient lower esophageal sphincter relaxations (TLESRs) in the dog in vivo.[9,48] The graphs show mean percentage inhibition and SEM (n = number of experiments for each dose).

Table 6. CYP 3A4 and 2C9 Inhibition Data

	IC_{50} (μM)[a]	
compd	CYP 3A4	CYP 2C9
13c	0.36	0.11
13d	0.61	0.66
18b	8.8	1.6
20	5.5	6.7
18c	>20	19
18f	>20	>20

[a]Inhibition values show the inhibition of metabolic degradation of the corresponding substrate by human recombinant CYP 3A4 and 2C9 at 37 °C (the percent of inhibition is determined at five different concentrations and is reported as IC_{50}). Data were analyzed using nonlinear regression analysis with ActivityBase software.

Table 7. $C_{u,br}/C_{u,pl}$, Rat PK, hERG, and Solubility Data for Leading Derivatives

compd	$C_{u,br}/C_{u,pl}$[a]	C_{br}/C_{pl}[b]	rat F[c] (%)	rat CL[c] (mL min^{-1} kg^{-1})	hERG IC_{50} (μM)[d]	solubility (μM)[e]
13c	0.044	0.10	5.8	22	11	3.7
18b	0.026	0.031	21	17	>32	93
18c	0.010	0.018	31	14	>33	79
18f	0.030	0.022	52	26	17	87

[a]Calculated by combining the total brain to plasma ratio determined from in vivo samples from Sprague–Dawley rats with estimates of the unbound fraction in the brain from rat brain slices[45] and unbound fraction in plasma by equilibrium dialysis.[32,46] [b]The total brain to plasma ratio determined from in vivo samples from Sprague–Dawley rats. [c]Compounds **18b**, **18c**, and **18f** were dosed at 2 μmol/kg (iv) and 10 μmol/kg (po), and **13c** was dosed at 1 μmol/kg (iv) and 15 μmol/kg (po) to Sprague–Dawley rats. [d]Patch clamp assay using IonWorks technology in hERG-expressing CHO cells.[53] [e]Solubility determined by adding Hank's balanced saline solution (pH 7.4) to a solution of the compound in DMSO and shaking for 24 h. The mixtures were then filtered and analyzed to give an estimate of the solubility.

conformation through an intramolecular hydrogen bond. The series was developed further focusing on improving potency and efficacy at the hCB1 receptor and reducing lipophilicity to improve compound properties. Design strategies were applied to limit CNS exposure, including increasing PSA and introducing polar functionality in an attempt to increase recognition by CNS efflux transporters while simultaneously maintaining properties for good oral absorption. A rewarding strategy to achieve the required criteria was to introduce polar functionality, particularly triazole or sulfoxide groups, into the 4-position of the naphthalene ring located in the 3-position of the heterocyclic scaffold. This led to compounds with excellent potency at the hCB1 receptor, lipophilicity values in the preferred range for oral drug-like compounds, and hence, significantly improved LLEs. These modifications also gave compounds with low $C_{u,br}/C_{u,pl}$ ratios. Changes were tolerated in the 6-position of the core heterocyclic ring structure, allowing further modification of compound properties as well as increasing selectivity versus hCB2. In general, the sulfoxide derivatives such as **18b** and **18f** gave higher efflux ratios than the triazoles and also better properties including less inhibition of the CYP enzymes and good in vivo rat PK profiles.

The ultimate goal for this series was to demonstrate that CB1 agonists with restricted CNS penetration are efficacious in inhibition of TLESRs. The in vivo data presented demonstrate that compounds from this series, such as **18b**, dose dependently inhibit TLESRs in dogs in a way comparable to what has previously been reported for the centrally acting compounds **2** and **3**. This suggests that the peripheral concept of CB1 receptor agonism can deliver high efficacy while minimizing CNS-related side effects and have a potential benefit for the treatment of GERD.

EXPERIMENTAL SECTION

Chemistry. All solvents and reagents were obtained from commercially available sources and used without further purification. Reactions were carried out under nitrogen atmosphere unless otherwise stated. Reactions carried out using a microwave reactor were performed using a Biotage initiator or Personal Chemistry [Biotage] Emrys optimizer. Flash chromatography was carried out on prepacked silica gel columns supplied by Biotage and using Horizon/Biotage systems. Analytical HPLC/MS was conducted on a Waters Zevo QTof or Waters LCT Premiere mass spectrometer using an Acquity PDA (Waters) UV detector monitoring either at (a) 210 nm with an Acquity BEH C18 column (2.1 mm × 100 mm, 1.7 μm, 0.7 mL/min flow rate), using a gradient of 2% v/v CH_3CN in H_2O (ammonium carbonate buffer, pH 10) to 98% v/v CH_3CN in H_2O or (b) 230 nm with an Acquity HSS C18 column (2.1 mm × 100 mm, 1.8 μm, 0.7 mL/min flow rate), using a gradient of 2% v/v CH_3CN in H_2O (ammonium formate buffer, pH 3) to 98% v/v CH_3CN in H_2O. Preparative HPLC was conducted using a Waters Fraction Lynx purification system using either (i) Xbridge Prep C18 5 μm OBD, 19 mm × 150 mm columns. The mobile phase used was varying gradients of CH_3CN and 0.2% NH_3 buffer at flow rate of 30 mL/min or (ii) Kromasil, C8, 10 μm, 50.8 mm × 300 mm columns. The mobile phase used was varying gradients of CH_3CN and 0.1 M NH_4OAc buffer at

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flow rate of 30 mL/min or (iii) Kromasil, C8, 10 μm, 20 mm × 50 mm columns. The mobile phase used was varying gradients of CH_3CN and 0.1 M HCO_2H buffer at flow rate of 30 mL/min. MS-triggered fraction collection was used for (a)−(c). All tested compounds were determined to be >95% pure using the analytical method (a) or (b) described above based on the peak area percentage. ^{1}H NMR spectra were generated on a Varian 300 MHz, Varian 400 MHz, Varian 500 MHz, or Varian 600 MHz instrument as indicated. Chemical shifts (δ) are given in parts per million (ppm), with the residual solvent signal used as a reference. Coupling constants (J) are reported as Hz. NMR abbreviations are used as follows: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

***N*-[4-Chloro-2-[[(cyclohexylmethyl)amino]carbonyl]phenyl]-1-naphthalene-carboxamide (4).** 1-Cyclohexylmethanamine (6.8 g, 60 mmol) was added to a solution of 6-chloro-2*H*-3,1-benzoxazine-2,4(1*H*)-dione (6.0 g, 30 mmol) and *N*,*N*-diisopropylethylamine (DIPEA, 3.8 g, 30 mmol) in DMF (50 mL) at room temperature. After 2 h, the reaction mixture was quenched with H_2O (100 mL) and Et_2O (50 mL). The precipitate was collected to provide 2- amino-5-chloro-*N*-(cyclohexylmethyl)benzamide (7.0 g, 87%).

A solution of 1-naphthoyl chloride (296 mg, l.5 mmol) in CH_2Cl_2 (1 mL) was added to a mixture of DIPEA (190 mg, 1.5 mmol) and 2-amino-5-chloro-*N*-(cyclohexylmethyl)benzamide (270 mg, 1.0 mmol) in DMF (10 mL) at 0 °C. The reaction mixture was then stirred for 2 h at room temperature and then quenched with H_2O (20 mL). The precipitate was collected to provide the title compound (178 mg, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (m, 2H), 1.22 (m, 3H), 1.56 (m, 1H), 1.75 (m, 5H), 3.23 (d, J = 6.4, 2H), 6.21 (brs, 1H), 7.46 (m, 1H), 7.53 (m, 4H), 7.84 (dd, J = 7.2, 1.2, 1H), 7.89 (m, 1H), 7.97 (d, J = 8.0, 1H), 8.52 (m, 1H), 8.88 (d, J = 9.2, 1H), 11.53 (brs, 1H); HRMS (ESI) m/z calcd for $C_{25}H_{25}ClN_2O_2$ $[M + H]^+$ 421.1683; found 421.168.

3-Amino-6-methoxypyridine-2-carboxylic Acid (5b). A mixture of 3-(acetylamino)-6-methoxypyridine-2-carboxylic acid[40] (8.0 g, 37.9 mmol) and a 2.5 M aqueous solution of NaOH (80 mL) was refluxed for 80 min. The solution was cooled to 0 °C and 4 M aqueous HCl was added until the solution was pH 4. The precipitate was collected and washed with cold H_2O to give the title compound as a solid (5.7 g, 89%). ^{1}H NMR (300 MHz, CD_3OD) δ 3.87 (s, 3H), 6.86 (d, J = 9.0, 1H), 7.25 (d, J = 9.0 Hz, 1H).

3-Amino-*N*-(tetrahydro-2*H*-pyran-4-ylmethyl)pyridine-2-carboxamide (7a). HATU (2.63 g, 6.93 mmol) and 1-(tetrahydro-2*H*-pyran-4-yl)methanamine (0.80 g, 6.94 mmol) were added to a solution of 3-amino-2-pyridinecarboxylic acid (**5a**, 0.91 g, 6.60 mmol) and DIPEA (1.26 mL, 7.26 mmol) in DMF (120 mL) at 0 °C. The reaction mixture was warmed to room temperature and heated to 50 °C for 3 h. The solvent was concentrated in vacuo, and the residue was partitioned between EtOAc and water. The organic phase was washed with saturated aqueous $NaHCO_3$ solution, saturated aqueous NaCl solution, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by flash column chromatography (Et_3N 0.1%, MeOH 3%, and acetone 5% in CH_2Cl_2) to give the title compound as a white solid (1.40 g, 90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.34−1.48 (m, 2H), 1.66−1.77 (m, 2H), 1.82−1.96 (m, 1H), 3.34 (t, J = 6.6, 2H), 3.37−3.45 (m, 2H), 4.01 (dd, J = 11.2, 4.0, 2H), 5.96 (s, 2H), 7.03 (d, J = 11.2, 1H), 7.17 (dd, J = 8.3, 4.3, 1H), 7.81−7.91 (m, 1H), 8.25 (s, 1H).

3-Amino-*N*-(cyclobutylmethyl)pyridine-2-carboxamide (7b). *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (2.32 g, 7.24 mmol) and DIPEA (2.02 mL, 11.6 mmol) were added to a solution of **5a** (1.00 g, 7.24 mmol) in DMF (5 mL), and after 15 min 1-cyclobutylmethanamine (0.62 g, 7.24 mmol) was added. The reaction mixture was stirred at room temperature for 1 h and was then partitioned between EtOAc and H_2O. The organic phase was washed with H_2O, saturated aqueous solution of $NaHCO_3$, and saturated solution of NaCl. The combined aqueous phases were extracted with EtOAc. The combined organic phases was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by flash column chromatography (heptane/EtOAc 1:1) to give the title compound as a clear oil (1.05 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68−1.81 (m, 2H), 1.83−1.97 (m, 2H), 2.01−2.15 (m, 2H), 2.46−2.65 (m, 1H), 3.34−3.5 (m, 2H), 5.93 (s, 2H), 6.96 (dd, J = 8.3, 1.3, 1H), 7.12 (dd, J = 8.4, 4.3, 1H), 7.82 (dd, J = 4.3, 1.3, 1H), 8.07 (s, 1H).

***N*-(Cyclohexylmethyl)-3-[(1-naphthalenylcarbonyl)amino]-2-pyridinecarboxamide (8a).** 1-Naphthalenecarbonyl chloride (400 mg, 2.1 mmol) in CH_2Cl_2 (2 mL) was added to a solution of 3-aminopyridine-2-carboxylic acid (**5a**, 280 mg, 2.0 mmol) and DIPEA (280 mg, 2.2 mmol) in DMF (10 mL) at 0 °C. The reaction mixture was stirred for 16 h at room temperature, and then DIPEA (280 mg, 2.2 mmol) and HATU (0.84 g, 2.2 mmol) were added. After being stirred for 1 h at room temperature, the reaction mixture was heated at 50 °C to provide 2-(1-naphthalenyl)-*H*-pyrido[3,2-*d*][1,3]oxazin-4-one (**6a**) which was used in the next step with no further purification.

A solution of 1-cyclohexylmethanamine (0.261 g, 2.3 mmol) in MeOH (0.5 mL) was added to a solution of **6a** (130 mg, 0.47 mmol) in DMF (2 mL) at 0 °C. The reaction mixture was stirred for 18 h at room temperature and then concentrated in vacuo to leave a residue, which was purified by flash chromatography (hexane/EtOAc 9:1) to provide the title compound as a solid (172 mg, 95%). ^{1}H NMR (400 MHz, CD_3OD) δ 0.90−1.00 (m, 2H), 1.13−1.28 (m, 3H), 1.52−1.75 (m, 6H), 3.16 (d, J = 6.83, 2H), 7.55−7.61 (m, 4H), 7.88−7.90 (m, 1H), 7.94−7.96 (m, 1H), 8.05−8.07 (m, 1H), 8.36 (dd, J = 4.5, 1.6, 1H), 8.41−8.43 (m, 1H), 9.29 (dd, J = 8.6, 1.4, 1H); HRMS (ESI) m/z calcd for $C_{24}H_{25}N_3O_2$ $[M + H]^+$ 388.2025; found 388.2014.

***N*-(Cyclohexylmethyl)-3-(1-naphthoylamino)pyrazine-2-carboxamide (8b).** Following the procedure for **8a**, using 3-aminopyrazine-2-carboxylic acid (**5c**, 139 mg, 1.0 mmol) in place of **5a** gave 2-(naphthalen-1-yl)-4*H*-pyrazino[2,3-*d*][1,3]oxazin-4-one (**6b**). Treatment of **6b** (69 mg, 0.25 mmol) with 1-cyclohexylmethanamine (113 mg, 1.0 mmol) provided the title compound as its TFA salt after purification by reversed-phase HPLC (6 mg, 5%). ^{1}H NMR (400 MHz, CD_3OD) δ 0.96 (m, 2H), 1.22 (m, 3H), 1.72 (m, 6H), 3.19 (m, 2H), 7.55 (m, 3H), 7.95 (m, 2H), 8.06 (m, 1H), 8.48 (m, 3H); MS (ESI) m/z $C_{23}H_{24}N_4O_2$ $[M + H]^+$ found 389.0.

***N*-(Cyclohexylmethyl)-4-[(naphthalen-1-ylcarbonyl)amino]-pyridine-3-carboxamide (8c).** Following the procedure for **8a**, using 4-aminopyridine-3-carboxylic acid (138 mg, 1.0 mmol) in place of **5a** gave 2-(naphthalen-1-yl)-4*H*-pyrido[4,3-*d*][1,3]oxazin-4-one (**6c**). Treatment of **6c** (0.137 mg, 0.5 mmol) with 1-cyclohexylmethanamine (226 mg, 2.0 mmol) provided the title compound as its TFA salt after purification by reversed-phase HPLC (39 mg, 16%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (m, 2H), 1.23 (m, 3H), 1.63 (m, 1H), 1.76 (m, 5H), 3.22 (d, J = 6.8, 2H), 7.61 (m, 3H), 7.98 (m, 2H), 8.14 (d, J = 8.4, 1H), 8.53 (m, 1H), 8.72 (m, 1H), 9.05 (s, 1H), 9.22 (d, J = 6.8, 1H); HRMS (ESI) m/z calcd for $C_{24}H_{25}N_3O_2$ $[M + H]^+$ 388.2025; found 388.2064.

***N*-(Cyclohexylmethyl)-3-[(naphthalen-1-ylcarbonyl)amino]-pyridine-4-carboxamide (8d).** Following the procedure for **8a**, using 3-aminopyridine-4-carboxylic acid (138 mg, 1.0 mmol) in place of **5a** gave 2-(naphthalen-1-yl)-4*H*-pyrido[3,4-*d*][1,3]oxazin-4-one (**6d**). Treatment of **6d** (137 mg, 0.5 mmol) with 1-cyclohexylmethanamine (226 mg, 2.0 mmol) provided the title compound as its TFA salt after purification by reversed-phase HPLC (55 mg, 22%). ^{1}H NMR (400 MHz, CD_3OD) δ 0.99 (m, 2H), 1.22 (m, 3H), 1.70 (m, 6H), 3.22 (d, J = 7.2, 2H), 7.59 (m, 3H), 7.90 (dd, J = 7.2, 1.2, 1H), 7.96 (m, 1H), 7.99 (brs, 1H), 8.08 (d, J = 8.4, 1H), 8.47 (m, 1H), 8.64 (brs, 1H), 10.1 (brs, 1H); HRMS (ESI) m/z calcd for $C_{24}H_{25}N_3O_2$ $[M + H]^+$ 388.2025; found 388.2064.

***N*-(Cyclohexylmethyl)-2-[(naphthalen-1-ylcarbonyl)amino]-pyridine-3-carboxamide (8e).** Following the procedure for **8a**, using 2-aminopyridine-3-carboxylic acid in place of **5a** gave 2-(naphthalen-1-yl)-4*H*-pyrido[2,3-*d*][1,3]oxazin-4-one (**6e**). Treatment of **6e** (100 mg, 0.36 mmol) with 1-cyclohexylmethanamine (226 mg, 2.0 mmol) provided the title compound as its TFA salt after purification by reversed-phase HPLC (89 mg, 62%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.76−0.93 (m, 2H), 0.99−1.14 (m, 3H), 1.39−1.79 (m, 6H), 3.02 (t, J = 6.3, 2H), 7.30 (dd, J = 7.6, 4.9, 1H), 7.49−7.64 (m, 3H), 7.80 (dd, J = 7.2, 1.0, 1H), 7.94−8.10 (m, 3H), 8.33−8.39 (m, 1H), 8.48 (dd, J = 4.9, 1.8, 1H), 8.60 (brs, 1H), 11.24 (brs,

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1H); HRMS (ESI) m/z calcd for $C_{24}H_{25}N_3O_2$ $[M + H]^+$ 388.2025; found 388.2051.

3-[(1-Naphthalenylcarbonyl)amino]-*N*-[(tetrahydro-2*H*-pyran-4-yl)methyl]-2-pyridinecarboxamide (8f). Following the procedure for **8a**, treating **6a** (122 mg, 0.446 mmol) with 1-(tetrahydro-2*H*-pyran-4-yl)methanamine (62 mg, 0.535 mmol) in place of 1-cyclohexylmethanamine provided the title compound as a solid (139 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (m, 2H), 1.23 (m, 3H), 1.56 (m, 1H), 1.76 (m, 5H), 3.25 (t, J = 6.4, 2H), 7.54 (m, 4H), 7.90 (m, 2H), 7.98 (d, J = 8.0, 1H), 8.28 (dd, J = 8.4, 1.6, 1H), 8.53 (m, 2H), 9.41 (dd, J = 8.4, 0.8, 1H), 12.87 (s, 1H); HRMS (ESI) m/z calcd for $C_{23}H_{23}N_3O_3$ $[M + H]^+$ 390.1818; found 390.1812.

***N*-(Cyclobutylmethyl)-3-[(1-naphthalenylcarbonyl)amino]-2-pyridinecarboxamide (8g).** Following the procedure for **8a**, treating **6a** (100 mg, 0.37 mmol) with 1-cyclobutylmethanamine (45 mg, 0.53 mmol) in place of 1-cyclohexylmethanamine provided the title compound as a solid (160 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.69−1.78 (m, 2H), 1.81−1.91 (m, 2H), 1.99−2.07 (m, 2H), 2.51−2.62 (m, 1H), 3.34 (d, J = 7.0, 2H), 7.52−7.59 (m, 4H), 7.87−7.89 (m, 1H), 7.92−7.96 (m, 1H), 8.03−8.05 (m, 1H), 8.30−8.35 (m, 1H), 8.42−8.45 (m, 1H), 9.27 (dd, J = 8.6, 1.2, 1H). MS (ESI) m/z $C_{22}H_{21}N_3O_2$ $[M + H]^+$ found 360.0.

3-[(2,3-Dihydro-1,4-benzodioxin-5-ylcarbonyl)amino]-*N*-(tetrahydro-2*H*-pyran-4-ylmethyl)pyridine-2-carboxamide (8h). A mixture of **7a** (125 mg, 0.53 mmol), crude 1,4-benzodioxan-5-carboxylic acid chloride (0.88 mmol, prepared from 1,4-benzodioxan-5-carboxylic acid and oxalyl chloride), and DIPEA (0.15 mL, 0.88 mmol) in CH_2Cl_2 (10 mL) was stirred for 16 h. A saturated aqueous solution of $NaHCO_3$ was added, and the phases were separated. The organic phase was dried (phase separator) and concentrated in vacuo. The residue was purified by preparative reversed phase HPLC to give the title compound as a solid (150 mg, 71%). ^{1}H NMR (400 MHz, CD_3OD) δ 1.34−1.47 (m, 2H), 1.68−1.81 (m, 2H), 1.89−2.01 (m, 1H), 3.31−3.40 (m, 2H), 3.41−3.51 (m, 2H), 3.95−4.04 (m, 2H), 4.37−4.44 (m, 2H), 4.52−4.57 (m, 2H), 6.98 (t, J = 7.9, 1H), 7.09 (dd, J = 1.6, 8.0, 1H), 7.5−7.62 (m, 2H), 8.36 (dd, J = 1.3, 4.4, 1H), 9.26 (dd, J = 1.3, 8.6, 1H); HRMS (ESI) m/z calcd for $C_{21}H_{24}N_3O_5$ $[M + H]^+$ 398.1716; found 398.1725.

***N*-{2-[(Tetrahydro-2*H*-pyran-4-ylmethyl)carbamoyl]pyridin-3-yl}-1,3-benzothiazole-6-carboxamide (8i).** Following the procedure for **8h**, treating **7a** (71 mg, 0.30 mmol) with 1,3-benzothiazole-6-carboxylic acid chloride (1.0 mmol, prepared from 1,3-benzothiazole-6-carboxylic acid and oxalyl chloride) in place of 1,4-benzodioxan-5-carboxylic acid chloride provided the title compound as a solid (54 mg, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35−1.49 (m, 2H), 1.71 (brd, 2H), 1.84−1.98 (m, 1H), 3.34−3.44 (m, 4H), 3.99 (dd, 2H), 7.50 (dd, 1H), 8.17−8.28 (m, 3H), 8.67 (brt, 1H), 8.71 (s, 1H), 9.14 (s, 1H), 9.34 (d, 1H), 13.30 (s, 1H); HRMS (ESI) m/z calcd for $C_{20}H_{20}N_4O_3S$ $[M + H]^+$ 397.1334; found 397.1313.

***N*-{2-[(Tetrahydro-2*H*-pyran-4-ylmethyl)carbamoyl]pyridin-3-yl}quinoxaline-5-carboxamide (8j).** Following the procedure for **8h**, treating **7a** (125 mg, 0.53 mmol) with quinoxaline-5-carboxylic acid chloride (0.883 mmol, prepared from quinoxaline-5-carboxylic acid and oxalyl chloride) in place of 4-benzodioxan-5-carboxylic acid chloride provided the title compound as a solid (63 mg, 30%). ^{1}H NMR (400 MHz, CD_3OD) δ 1.33−1.48 (m, 2H), 1.68−1.80 (m, 2H), 1.87−2.01 (m, 1H), 3.33−3.38 (m, 2H), 3.39−3.49 (m, 2H), 3.93−4.04 (m, 2H), 7.56−7.68 (m, 1H), 7.98−8.09 (m, 1H), 8.3−8.46 (m, 2H), 8.65 (dd, J = 1.3, 7.4, 1H), 9.06 (d, J = 1.6, 1H), 9.13 (d, J = 1.6, 1H), 9.29 (dd, J = 1.3, 8.6, 1H); HRMS (ESI) m/z calcd for $C_{21}H_{21}N_5O_3$ $[M + H]^+$ 392.1722; found 392.1708.

***N*-[2-[[(Cyclobutylmethyl)amino]carbonyl]-3-pyridinyl]-5-isoquinolinecarboxamide (8k).** DIPEA (0.17 mL, 0.97 mmol) was added to a solution of **7b** (100 mg, 0.49 mmol) and isoquinoline-5-carboxylic acid (168 mg, 0.97 mmol) in DMF (20 mL) at 0 °C. The mixture was stirred for 20 min, and then HATU (369 mg, 0.97 mmol) was added. The reaction mixture was stirred for 24 h at room temperature and was then quenched with water and extracted with EtOAc. The combined organic phases were combined and concentrated in vacuo to leave a residue which was purified by reversed-phase HPLC to provide the title compound as its TFA salt (97 mg, 42%). ^{1}H NMR (400 MHz, CD_3OD) δ 1.47−1.96 (m, 4H), 2.02−2.10 (m, 2H), 2.58−2.65 (m, 1H), 3.39 (d, J = 7.2, 2H), 7.62 (dd, J = 8.6, 4.6, 1H), 8.10 (dd, J = 8.3, 7.3, 1H), 8.39 (dd, J = 4.6, 1.4, 1H), 8.59−8.64 (m, 3H), 8.98−8.90 (m, 1H), 9.26 (dd, J = 8.6, 1.4, 1H), 9.73−9.80 (brs, 1H); HRMS (ESI) m/z calcd for $C_{21}H_{20}N_4O_2$ $[M + H]^+$ 361.1664; found 361.1643.

***N*-{2-[(Cyclobutylmethyl)carbamoyl]pyridin-3-yl}quinoline-8-carboxamide (8l).** A solution of methyl 3-aminopyridine-2-carboxylate (0.62 g, 4.06 mmol), pyridine (0.7 mL, 8.1 mmol), and DMAP (15 mg, 0.12 mmol) in $CHCl_3$ (6 mL) was added to a suspension of quinoline-8-carbonyl chloride (1.16 g, 6.08 mmol) in $CHCl_3$ (10 mL), and the reaction mixture was heated at reflux for 14 h. The reaction mixture was then diluted with CH_2Cl_2 and was washed with saturated aqueous solution of Cu(II)SO_4, 1 M aqueous NaOH, and a saturated aqueous solution of NaCl. The organic phase was dried (Na_2SO_4) and concentrated in vacuo to leave a residue which was purified by flash column chromatography (toluene/EtOH 15:1) to give methyl 3-[(quinolin-8-ylcarbonyl)amino]pyridine-2-carboxylate as a solid (0.50 g, 40%).

Methyl 3-[(quinolin-8-ylcarbonyl)amino]pyridine-2-carboxylate (50 mg, 0.16 mmol), dissolved in DMF (1.5 mL), was added to 1-cyclobutylmethanamine (36 mg, 0.42 mmol). The reaction mixture was stirred at 80 °C for 16 h. Additional 1-cyclobutylmethanamine (28 mg) was added, and the reaction mixture was stirred for 5 h at 80 °C. The reaction mixture was concentrated in vacuo and then purified by HPLC to give the title compound (8 mg, 14%) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.74−1.86 (m, 2H), 1.88−1.99 (m, 2H), 2.07−2.20 (m, 2H), 2.55−2.68 (m, 1H), 3.43−3.55 (m, 2H), 7.47 (dd, J = 4.4, 8.6, 1H), 7.54 (dd, J = 4.2, 8.3, 1H), 7.68 (t, J = 7.7, 1H), 7.97−8.04 (m, 1H), 8.23−8.34 (m, 3H), 8.66 (dd, J = 1.4, 7.3, 1H), 9.18 (dd, J = 1.8, 4.2, 1H), 9.42 (dd, J = 1.4, 8.6, 1H), 14.34 (s, 1H); HRMS (ESI) m/z calcd for $C_{21}H_{20}N_4O_2$ $[M + H]^+$ 361.1659; found 361.1658.

***N*-[2-[[(Cyclobutylmethyl)amino]carbonyl]-3-pyridinyl]-4-quinolinecarboxamide (8m).** Following the procedure for **8k**, treating **7b** (50 mg, 0.24 mmol) with quinoline-4-carboxylic acid (50 mg, 0.29 mmol) in place of isoquinoline-5-carboxylic acid provided the title compound as its TFA salt (9 mg, 8%). ^{1}H NMR (400 MHz, CD_3OD) δ 1.71−1.93 (m, 4H), 2.02−2.10 (m, 2H), 2.57−2.64 (m, 1H), 3.38 (d, J = 7.2, 2H), 7.64 (m, 1H), 7.76−7.78 (m, 1H), 7.82−7.96 (m, 2H), 8.17−8.19 (d, 1H), 8.41 (dd, J = 4.6, 1.5, 1H), 8.50−8.52 (m, 1H), 9.10 (d, J = 4.7, 1H), 9.27 (dd, J = 8.6, 1.5, 1H); MS (ESI) m/z $C_{21}H_{20}N_4O_2$ $[M + H]^+$ found 361.2.

***N*-{2-[(Cyclobutylmethyl)carbamoyl]-6-methoxypyridin-3-yl}quinoline-4-carboxamide (8n).** A solution of quinoline-4-carbonyl chloride (16.5 mmol, prepared from quinoline-4-carboxylic acid and oxalyl chloride) and pyridine (7.8 mL, 97 mmol) in $CHCl_3$ (20 mL) was added to a solution of **15a** (2.00 g, 11.0 mmol), DMAP (0.302 g, 2.47 mmol), and pyridine (1.0 mL, 12 mmol) in $CHCl_3$ (10 mL) at 70 °C, and the reaction mixture was stirred at 70 °C for 1 h. The reaction mixture was then partitioned between CH_2Cl_2 and saturated aqueous solution of $NaHCO_3$. The organic phase was washed with saturated aqueous solution of $NaHCO_3$, dried (phase separator), and concentrated in vacuo to give crude methyl 6-methoxy-3-[(quinolin-4-ylcarbonyl)amino]pyridine-2-carboxylate (2.19 g, 59%).

A mixture of crude methyl 6-methoxy-3-[(quinolin-4-ylcarbonyl)-amino]pyridine-2-carboxylate (56 mg, 0.17 mmol) and crude 1-cyclobutylmethanamine (50 mg, 0.50 mmol) in DMF (2 mL) was heated at 80 °C for 5 h and at room temperature for 16 h. The reaction mixture was concentrated in vacuo and the residue was purified by flash column chromatography (heptane/EtOAc 4:1 → 1:1) to give the title compound as a white solid (52 mg, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.69−1.81 (m, 2H), 1.85−1.98 (m, 2H), 2.05−2.15 (m, 2H), 2.52−2.65 (m, 1H), 3.39−3.45 (m, 2H), 3.95 (s, 3H), 7.04 (d, J = 9.1, 1H), 7.59−7.65 (m, 1H), 7.71 (d, J = 4.3, 1H), 7.74−7.80 (m, 1H), 8.12−8.20 (m, 2H), 8.47 (d, J = 8.0, 1H), 9.04 (d, J = 4.3, 1H), 9.30 (d, J = 9.1, 1H), 12.82 (bs, 1H); HRMS (ESI) m/z calcd for $C_{22}H_{22}N_4O_3$ $[M + H]^+$ 391.177; found 391.1762.

4-{[(2-{[(Tetrahydro-2*H*-pyran-4-ylmethyl)amino]carbonyl}-pyridin-3-yl)amino]carbonyl}-1-naphthoic Acid (8o). A solution of **7a** (67 mg, 0.28 mmol) and DIPEA (1 mL, 5.74 mmol) in DCE (2 mL) was added to a solution of naphthalene-1,4-dicarbonyl dichloride (1.15 mmol, prepared from naphthalene 1,4-dicarboxylic acid and thionyl chloride) in DCE (20 mL). The reaction mixture was stirred for 3 h at room temperature and then quenched with H_2O. The organic phase was separated and dried (Na_2SO_4). The solvent was concentrated in vacuo and the residue was purified by preparative HPLC to provide the title compound as its TFA salt (20 mg, 16%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.49 (dd, J = 12.9, 2.2, 2H), 2.07 (d, J = 3.9, 2H), 3.12 (m, 2H), 3.19 (m, 2H), 3.32 (s, 2H), 3.78 (dd, J = 10.7, 3.3, 2H), 3.89 (s, 1H), 7.67 (t, J = 7.7, 1H), 7.73 (dd, J = 8.6, 4.7, 2H), 7.92 (d, J = 7.4, 1H), 8.19 (d, J = 7.6, 1H), 8.35 (d, J = 8.2, 1H), 8.42 (dd, J = 4.5, 1.4, 1H), 8.85 (d, J = 8.4, 1H), 9.20 (dd, J = 8.5, 1.3, 1H), 13.0 (s, 1H); MS (ESI) m/z for $C_{24}H_{23}N_3O_5$ $(M + H)^+$ 434.0

***N*-(Cyclobutylmethyl)-3-{[4-(methoxymethyl)-1-naphthoyl]-amino}pyridine-2-carboxamide (9a).** A solution of **7b** (3.0 g, 14.6 mmol) and Et_3N (2.6 mL, 14.6 mmol) in MeCN (50 mL) was added to a solution of naphthalene-1,4-dicarbonyl dichloride (4.7 g, 18.5 mmol, prepared from naphthalene 1,4-dicarboxylic acid and thionyl chloride) in MeCN (700 mL) at 0 °C, and the reaction mixture was stirred for 2 h. NaOH (0.1 M aqueous solution, 0.44 mL) was then added. The reaction mixture was stirred for 1 h, and then an excess of NaOH (0.1 M aqueous solution) was added. The reaction mixture was concentrated in vacuo to leave a residue which was taken up in H_2O. The precipitate was filtered, and the filtrate was acidified with concentrated HCl. The resulting precipitate was then filtered and taken up in CH_2Cl_2 and dried (Na_2SO_4). The solvent was concentrated in vacuo to provide the naphthoic acid product as a solid (5.43 g, 92%).

Oxalyl chloride (0.015 mL, 0.18 mmol) was added to a mixture of 4-{[(2-{[(cyclobutylmethyl)amino]carbonyl}pyridin-3-yl)amino]-carbonyl}-1-naphthoic acid (50 mg, 0.11 mmol) and DCE (20 mL) at 0 °C. The reaction mixture was warmed to room temperature, heated to 70 °C for 1 h, and then cooled to 0 °C. Sodium borohydride (22 mg, 0.57 mmol) and iodine (one crystal) were added, and the reaction mixture was stirred for 1 h at 0 °C. Then EtOAc was added. The mixture was washed with H_2O and then concentrated in vacuo to leave the alcohol as an oil (41 mg, 67%).

Methanesulfonyl chloride (0.24 mL, 3.11 mmol) was added to a solution of *N*-(cyclobutylmethyl)-3-{[4-(hydroxymethyl)-1-naphthoyl]amino}pyridine-2-carboxamide (1.01 g, 2.59 mmol) and Et_3N (0.45 mL, 3.23 mmol) in CH_2Cl_2 (150 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 3 h. The reaction mixture was washed with a saturated aqueous solution of $NaHCO_3$, H_2O, and then brine and then dried (Na_2SO_4). The mixture was concentrated in vacuo to leave a residue which was purified using flash column chromatography to provide the mesylate as a colorless oil (340 mg, 28%).

A mixture of (4-{[(2-{[(cyclobutylmethyl)amino]carbonyl}pyridin-3-yl)amino]carbonyl}-1-naphthyl)methyl methanesulfonate (60 mg, 0.13 mmol) in NaOMe (20% in MeOH, 15 mL) was heated at 70 °C for 1 h. The reaction mixture was concentrated in vacuo to leave a residue which was taken up in EtOAc. The solution was washed with a saturated aqueous solution of $NaHCO_3$, H_2O, and then brine and then dried (Na_2SO_4). The solution was concentrated in vacuo to leave a residue which was purified by preparative HPLC to provide the title compound as its TFA salt (30 mg, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68−1.81 (m, 2H), 1.83−1.99 (m, 2H), 2.03−2.16 (m, 2H), 2.52−2.64 (m, 1H), 3.42 (t, J = 6.1, 2H), 3.47 (s, 3H), 4.92−4.99 (m, 2H), 7.52 (dd, J = 3.1, 1.4, 1H), 7.59 (dd, J = 6.6, 2.7, 3H), 7.87 (d, J = 7.2, 1H), 8.14 (dd, J = 6.6, 2.9, 1H), 8.28 (s, 1H), 8.43 (s, 1H), 8.56 (dd, J = 6.6, 2.9, 1H), 9.40 (d, J = 8.2, 1H), 12.9 (s, 1H); MS (ESI) m/z for $C_{24}H_{25}N_3O_3$ $[M + H]^+$ 404.0.

***N*-(Cyclobutylmethyl)-3-({4-[(dimethylamino)methyl]-1-naphthoyl}amino)pyridine-2-carboxamide (9b).** Intermediate (4-{[(2-{[(cyclobutylmethyl)amino]carbonyl}pyridin-3-yl)amino]-carbonyl}-1-naphthyl)methyl methanesulfonate (prepared in **9a**) (60 mg, 0.13 mmol), dimethylamine hydrochloride (0.20 g, 2.45 mmol), potassium iodide (138 mg, 0.84 mmol), and DMF (2 mL) were mixed and heated at 70 °C for 1 h. The reaction mixture was concentrated in vacuo to leave a residue which was taken up in EtOAc. The solution was washed with a saturated aqueous solution of $NaHCO_3$, H_2O, and then brine and then dried (Na_2SO_4). The solution was concentrated in vacuo to leave a residue which was purified by preparative HPLC to provide the title compound as its TFA salt (30 mg, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.69−1.80 (m, 2H), 1.85−1.98 (m, 2H), 2.05−2.16 (m, 2H), 2.53−2.64 (m, 1H), 2.84 (s, 6H), 3.38−3.45 (m, 2H), 4.73−4.79 (m, 2H), 7.55 (dd, J = 8.5, 4.6, 1H), 7.63−7.74 (m, 2H), 7.85 (dd, J = 6.4, 7.3, 2H), 8.17 (d, J = 7.8, 1H), 8.3 (dd, J = 4.5, 1.4, 1H), 8.46 (t, J = 5.7, 1H), 8.56 (dd, J = 8.4, 1.2, 1H), 9.38 (dd, J = 8.6, 1.6, 1H), 12.99 (s, 1H); MS (ESI) m/z for $C_{25}H_{29}N_4O_2$ $(M + H)^+$ 417.3.

***N*-(Cyclobutylmethyl)-3-{[4-(1*H*-pyrrol-1-ylmethyl)-1-naphthoyl]amino}pyridine-2-carboxamide (9c).** Intermediate (4-{[(2-{[(cyclobutylmethyl)amino]carbonyl}pyridin-3-yl)amino]-carbonyl}-1-naphthyl)methyl methanesulfonate (prepared in **9a**) (85 mg, 0.18 mmol), pyrrole (624 mg, 9.30 mmol), potassium iodide (33 mg, 0.20 mmol), and DMF (2 mL) were mixed and heated at 80 °C for 1 h. The reaction mixture was concentrated in vacuo to leave a residue which was taken up in EtOAc. The solution was washed with a saturated aqueous solution of $NaHCO_3$, H_2O, and then brine and then dried (Na_2SO_4). The solution was concentrated in vacuo to leave a residue which was purified by preparative HPLC to provide the title compound as its TFA salt (29 mg, 28%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67−1.84 (m, 3H), 1.85−1.97 (m, 2H), 2.04−2.17 (m, 2H), 2.52−2.64 (m, 1H), 3.42 (dd, J = 7.1, 6.2, 2H), 4.45−4.50 (m, 2H), 6.06−6.11 (m, 1H), 6.18 (q, J = 2.7, 1H), 6.62−6.68 (m, 1H), 7.38 (d, J = 7.4, 1H), 7.48−7.61 (m, 3H), 7.84 (d, J = 7.2, 1H), 8.09−8.15 (m, 1H), 8.28 (dd, J = 4.5, 1.6, 1H), 8.45 (t, J = 5.8, 1H), 8.54−8.59 (m, 1H), 9.40 (dd, J = 8.6, 1.6, 1H), 12.9 (s, 1H); MS (ESI) $C_{24}H_{23}N_7O_2$ $[M + H]^+$ found 439.0.

***N*-(Cyclobutylmethyl)-3-{[4-(1*H*-imidazol-1-ylmethyl)-1-naphthoyl]amino}pyridine-2-carboxamide (9d).** Following the procedure for **9c**, using imidazole in place of pyrrole provided the title compound as its TFA salt (50 mg, 18%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68−1.81 (m, 2H), 1.83−1.99 (m, 2H), 2.04−2.16 (m, 2H), 2.52−2.64 (m, 1H), 3.37−3.45 (m, 2H), 5.83 (s, 2H), 7.04 (s, 1H), 7.36 (s, 1H), 7.46 (d, J = 7.4, 1H), 7.54 (dd, J = 8.6, 4.5, 1H), 7.60−7.69 (m, 2H), 7.82−7.92 (m, 2H), 8.31 (dd, J = 4.5, 1.4, 1H), 8.47 (t, J = 6.0, 1H), 8.55−8.62 (m, 1H), 8.98 (s, 1H), 9.38 (dd, J = 8.6, 1.4, 1H), 13.00 (s, 1H); MS (ESI) m/z for $C_{26}H_{25}N_5O_2$ $[M + H]^+$ 440.2066.

***N*-(Cyclobutylmethyl)-3-{[4-(1*H*-pyrazol-1-ylmethyl)-1-naphthoyl]amino}pyridine-2-carboxamide (9e).** Following the procedure for **9c**, using pyrazole in place of pyrrole provided the title compound as its TFA salt (33 mg, 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67−1.81 (m, 2H), 1.84−1.98 (m, 2H), 2.04−2.16 (m, 2H), 2.52−2.64 (m, 1H), 3.42 (dd, J = 7.2, 6.3 Hz, 2H), 5.85 (s, 2H), 6.30 (s, 1H), 7.22−7.28 (m, 1H), 7.33 (s, 1H), 7.52 (dd, J = 8.6, 4.5, 1H), 7.56−7.61 (m, 2H), 7.61−7.65 (m, 1H), 7.85 (d, J = 7.4, 1H), 7.98−8.06 (m, 1H), 8.28 (dd, J = 4.5, 1.6, 1H), 8.44 (t, J = 5.8, 1H), 8.53−8.61 (m, 1H), 9.39 (dd, J = 8.6, 1.4, 1H), 12.90 (s, 1H); HRMS (ESI) m/z calcd for $C_{26}H_{25}N_5O_2$ $[M + H]^+$ 440.2086; found 440.2066.

***N*-(Cyclobutylmethyl)-3-{[4-(1*H*-1,2,3-triazol-1-ylmethyl)-1-naphthoyl]amino}pyridine-2-carboxamide (9f).** Following the procedure for **9c**, using 1,2,3-triazole in place of pyrrole provided the title compound as its TFA salt (63 mg, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68−1.81 (m, 2H), 1.85−1.98 (m, 2H), 2.05−2.16 (m, 2H), 2.52−2.65 (m, 1H), 3.42 (dd, J = 7.1, 6.2, 2H), 6.08 (s, 2H), 7.43 (s, 1H), 7.48 (d, J = 7.2, 1H), 7.54 (dd, J = 8.6, 4.5, 1H), 7.57−7.66 (m, 2H), 7.76 (s, 1H), 7.88 (d, J = 7.4, 1H), 7.95−8.02 (m, 1H), 8.30 (dd, J = 4.5, 1.4, 1H), 8.48 (t, J = 5.8, 1H), 8.52−8.59 (m, 1H), 9.39 (dd, J = 8.6, 1.6, 1H), 12.95 (s, 1H); HRMS (ESI) m/z calcd for $C_{25}H_{24}N_6O_2$ $[M + H]^+$ 441.2039; found 441.2044.

***N*-{2-[(Cyclobutylmethyl)carbamoyl]-6-(2-hydroxyethoxy)-pyridin-3-yl}quinoline-4-carboxamide (10a).** Pyridine hydrochloride (31.4 g, 0.27 mol) was added to compound **8n** (1.06 g, 2.72 mmol), and the mixture was heated at 180 °C for 25 min. The

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mixture was then cooled to room temperature, and a saturated aqueous solution of $NaHCO_3$ was added. The pH of the mixture was made basic by addition of solid $NaHCO_3$. The precipitated material was filtered and washed with H_2O. The solid material was then suspended in CH_2Cl_2 and a mixture of saturated aqueous $NaHCO_3$ and saturated aqueous NaCl. The CH_2Cl_2 phase was discarded and the solid material filtered, washed with H_2O and Et_2O, and dried to give *N*-{2-[(cyclobutylmethyl)carbamoyl]-6-hydroxypyridin-3-yl}-quinoline-4-carboxamide (0.71 g, 69%).

Silver(I) carbonate (0.220 g, 0.798 mmol) was added to a solution of the hydroxypyridine (112 mg, 0.266 mmol) in DMF (4 mL), and the mixture was stirred at 90 °C for 5 min. 3-Bromo-1-propanol (0.145 mL, 1.60 mmol) was then added, and the reaction mixture was stirred at 90 °C for 45 min. The reaction mixture was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The organic phase was dried (phase separator) and concentrated in vacuo to leave a residue. The residue was purified by preparative HPLC to give the title compound as a solid (49 mg, 44%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.70−1.80 (m, 2H), 1.84−2.00 (m, 2H), 2.05−2.15 (m, 2H), 2.52−2.69 (m, 1H), 3.38−3.45 (m, 2H), 3.99−4.06 (m, 2H), 4.39−4.45 (m, 2H), 7.06 (dd, J = 2.0, 9.1, 1H), 7.60−7.66 (m, 1H), 7.70−7.74 (m, 1H), 7.75−7.81 (m, 1H), 8.06−8.15 (m, 1H), 8.18 (d, J = 8.5, 1H), 8.48 (d, J = 8.5, 1H), 9.01−9.09 (m, 1H), 9.31 (dd, J = 2.0, 9.1, 1H), 12.83 (s, 1H). HRMS (ESI) m/z calcd for $C_{23}H_{24}N_4O_4$ $[M + H]^+$ 421.1876; found 421.1881.

***N*-{2-[(Cyclobutylmethyl)carbamoyl]-6-[2-(2-hydroxyethoxy)ethoxy]pyridin-3-yl}quinoline-4-carboxamide (10b).** Intermediate *N*-{2-[(cyclobutylmethyl)carbamoyl]-6-hydroxypyridin-3-yl}quinoline-4-carboxamide (prepared in **10a**) (115 mg, 0.31 mmol), diethylene glycol (162 mg, 1.528 mmol), and triphenylphosphine (480 mg, 1.83 mmol) were dissolved in THF (10 mL), and diisopropyl azodicarboxylate (370 mg, 1.83 mmol) was added. The reaction mixture was heated at 50 °C for 30 min. CH_2Cl_2 and a saturated aqueous solution of $NaHCO_3$ were added, and the phases were separated. The organic layer was dried (phase separator) and concentrated in vacuo to leave a residue which was purified by flash column chromatography (EtOAc in heptane 10% → 100%) to give the title compound as a solid (73 mg, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.66−1.80 (m, 2H), 1.82−1.96 (m, 2H), 2.03−2.14 (m, 2H), 2.44 (s, 1H), 2.49−2.64 (m, 1H), 3.40 (t, J = 6.6, 2H), 3.63−3.71 (m, 2H), 3.74−3.81 (m, 2H), 3.84−3.92 (m, 2H), 4.38−4.48 (m, 2H), 7.05 (d, J = 9.2, 1H), 7.58−7.64 (m, 1H), 7.69 (d, J = 4.4, 1H), 7.72−7.79 (m, 1H), 8.08 (t, J = 5.6, 1H), 8.16 (d, J = 8.4, 1H), 8.46 (d, J = 7.9, 1H), 9.02 (d, J = 4.4, 1H), 9.28 (d, J = 9.2, 1H), 12.80 (s, 1H); HRMS (ESI) m/z calcd for $C_{25}H_{28}N_4O_5$ $[M + H]^+$ 465.2138; found 465.2152.

Methyl 3-{[(4-Methylnaphthalen-1-yl)carbonyl]amino}-pyridine-2-carboxylate (11a). A solution of 4-methyl-1-naphthalenecarbonyl chloride (0.65 g, 3.16 mmol) in $CHCl_3$ (2 mL) was added to a solution of **5a** (0.321 g, 2.11 mmol), DMAP (8 mg, 0.06 mmol), and pyridine (0.34 mL, 4.2 mmol) in $CHCl_3$ (4 mL), and the reaction mixture was heated at reflux for 5 h. The reaction mixture was cooled to room temperature, diluted with CH_2Cl_2, and washed with a saturated aqueous solution of copper(II) sulfate, a 1 M aqueous solution of NaOH, and a saturated aqueous solution of NaCl. The organic phase was dried (Na_2SO_4) and concentrated in vacuo to leave a residue, which was purified by flash column chromatography (toluene/EtOAc 20:1) to give the title compound (0.65 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.77 (s, 3H), 4.00 (s, 3H), 7.42 (d, J = 7.2, 1H), 7.55−7.66 (m, 3H), 7.80 (d, J = 7.2, 1H), 8.03−8.14 (m, 1H), 8.49 (dd, J = 4.4, 1.3, 1H), 8.53−8.6 (m, 1H), 9.42 (dd, J = 8.6, 1.2, 1H), 11.59 (s, 1H).

Methyl 3-{[(4-Methylnaphthalen-1-yl)carbonyl]amino}-pyrazine-2-carboxylate (11c). A solution of 4-methyl-1-naphthalenecarbonyl chloride (1.50 g, 7.31 mmol) in $CHCl_3$ (3 mL) was added to a suspension of methyl 3-aminopyrazine-2-carboxylate[55] (**15b**, 0.224 g, 1.46 mmol) and DMAP (18 mg, 0.15 mmol) in pyridine (5 mL), and the reaction mixture was stirred at 50 °C for 20 h. The reaction mixture was cooled to room temperature. $NaHCO_3$ (614 mg, 7.31 mmol) was carefully added, and the mixture was then concentrated in vacuo. The residue was partitioned between CH_2Cl_2 and H_2O, and the organic phase was washed with a saturated aqueous solution of $NaHCO_3$, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by flash column chromatography (toluene/EtOH 30:1; toluene/EtOH 10:1) to give methyl 3-{bis[(4-methylnaphthalen-1-yl)carbonyl]amino}pyrazine-2-carboxylate (0.69 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.47 (s, 6H), 3.99 (s, 3H), 6.98 (d, J = 7.4, 2H), 7.41−7.52 (m, 4H), 7.76−7.84 (m, 2H), 7.98 (d, J = 7.3, 2H), 8.26−8.33 (m, 2H), 8.52 (d, J = 2.3, 1H), 8.57 (d, J = 2.3, 1H).

2-Propanol (5 mL) and hydrazine monohydate (0.067 mL, 1.39 mmol) were added to a solution of methyl 3-{bis[(4-methylnaphthalen-1-yl)carbonyl]amino}pyrazine-2-carboxylate (0.680 g, 1.38 mmol) in dioxane (5 mL) at 100 °C. The reaction mixture was heated at reflux for 30 min and concentrated in vacuo. The residue was purified by flash column chromatography (toluene/EtOH 15:1) to give the title compound (0.44 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.75 (s, 3H), 4.01 (s, 3H), 7.40 (d, J = 7.3, 1H), 7.54−7.63 (m, 2H), 7.81 (d, J = 7.3, 1H), 8.02−8.09 (m, 1H), 8.44 (d, J = 2.3 Hz, 1H), 8.58−8.66 (m, 1H), 8.72 (d, J = 2.3, 1H), 11.22 (s, 1H).

Methyl 3-({[4-(1*H*-1,2,3-Triazol-1-ylmethyl)naphthalen-1-yl]-carbonyl}amino)pyridine-2-carboxylate (12a). NBS (0.418 g, 2.35 mmol) and benzoyl peroxide (0.54 g, 0.22 mmol) were added to a mixture of **11a** (1.80 g, 5.14 mmol) in carbon tetrachloride (50 mL), and the reaction mixture was heated at reflux for 80 min. 1,2,3-Triazole (1.30 mL, 22.4 mmol) was added, and the reaction mixture was heated at reflux for 16 h. The reaction mixture was concentrated in vacuo to leave a residue which was purified by preparative HPLC to provide the title compound (0.20 g, 23%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.83 (s, 3H), 6.21 (s, 2H), 7.44 (d, J = 7.4, 1H), 7.66−7.74 (m, 3H), 7.77 (s, 1H), 7.85 (d, J = 7.3, 1H), 8.23 (s, 1H), 8.32 (dd, J = 6.8, 2.6, 1H), 8.38 (dd, J = 6.9, 2.8, 1H), 8.46−8.52 (m, 2H), 11.06 (s, 1H).

***N*-(Cyclohexylmethyl)-6-methoxy-3-{[4-(1*H*-1,2,3-triazol-1-ylmethyl)-1-naphthoyl]amino}pyridine-2-carboxamide (13a).** DIPEA (11.1 mL, 63.6 mmol) and 4-methyl-1-naphthalenecarbonyl chloride (2.65 g, 13.0 mmol) were added to a solution of **5b** (1.78 g, 10.6 mmol) in DMF (30 mL). The reaction mixture was stirred for 1 h at room temperature and then for 1 h at 50 °C. The reaction mixture was cooled to room temperature, and K_2CO_3 (2.2 g, 15.9 mmol) was added followed by the addition of MeI (3.3 mL, 53 mmol). The reaction mixture was stirred for 16 h and then concentrated in vacuo to leave a residue, which was taken up in H_2O. The mixture was filtered to leave a solid that was washed with H_2O and then EtOH. The solid was suspended in a mixture of EtOAc and MeOH and then filtered, washed with MeOH and Et_2O to give methyl 6-methoxy-3-[(4-methyl-1-naphthoyl)amino]pyridine-2-carboxylate (**11b**, 2.0 g, 54%), which was used directly in the next step.

NBS (0.96 g, 5.39 mmol) and benzoyl peroxide (0.13 g, 0.51 mmol) were added to a mixture of **11b** (1.8 g, 5.14 mmol) in carbon tetrachloride (100 mL), and the reaction mixture was refluxed for 1.5 h. DMF (2.5 mL) and 1,2,3-triazole (3.0 mL, 51.4 mmol) were added, and the reaction mixture was refluxed for 16 h. The reaction mixture was concentrated in vacuo to leave a residue which was taken up in cold H_2O to leave a precipitate. The mixture was filtered to leave a solid, which was washed with H_2O and then purified by flash chromatography (CH_2Cl_2/MeOH 100:0 → 100:1) to give methyl 6-methoxy-3-{[4-(*1H*-1,2,3-triazol-1-ylmethyl)-1-naphthoyl]amino}-pyridine-2-carboxylate (**12b**, 1.55 g, 72%) as a solid.

A solution of **12b** (0.5 g, 1.2 mmol) and 1-cyclohexylmethanamine (0.41 g, 3.6 mmol) in DMF (3 mL) was heated at 80 °C for 40 min. The reaction mixture was then concentrated in vacuo to leave a residue, which was dissolved in CH_2Cl_2. H_2O (50 mL) and 2 M aqueous HCl (13 mL) were added, and the organic phase was separated, washed with a saturated aqueous solution of $NaHCO_3$ and then saturated aqueous solution of NaCl, and then dried ($MgSO_4$). The organic phase was concentrated in vacuo to leave a residue, which was purified by preparative HPLC to give the title compound (520 mg, 86%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.93−1.02 (m, 2H), 1.09−1.27 (m, 3H), 1.50−1.58 (m, 1H), 1.62−1.78 (m, 5H), 3.22 (t, J = 6.7, 2H), 3.94 (s, 3H), 6.04 (s, 2H), 7.01 (d, J = 9.1, 1H), 7.36 (s, 1H), 7.41 (d, J = 7.2, 1H), 7.53−7.60 (m, 2H), 7.66 (s, 1H),

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7.83 (d, J = 7.2, 1H), 7.98 (d, J = 7.8, 1H), 8.23 (t, J = 6.5, 1H), 8.53 (d, J = 8.5, 1H), 9.31 (d, J = 9.1, 1H), 12.62 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.6, 166.8, 157.7, 136.2, 134.2, 133.1, 132.8, 131.5, 130.9, 129.7, 127.8, 127.6, 126.7, 126.6, 124.8, 123.4, 123.0, 115.7, 53.5, 52.1, 45.3, 38.0, 30.8, 26.3, 25.8; HRMS (ESI) m/z calcd for $C_{28}H_{30}N_6O_3$ $[M + H]^+$ 499.2458; found 499.2468.

6-Methoxy-*N*-(cyclobutylmethyl)-3-{[4-(1*H*-1,2,3-triazol-1-ylmethyl)-1-naphthoyl]-amino}pyridine-2-carboxamide (13b). **13b** was prepared using essentially the same procedure as described for **13a**, using 1-cyclobutylmethanamine in place of 1-cyclohexylmethanamine to give the product (980 mg, 87%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.64−1.82 (m, 2H), 1.83−2.00 (m, 2H), 2.02−2.19 (m, 2H), 2.46−2.67 (m, 1H), 3.29−3.53 (m, 2H), 3.94 (s, 3H), 6.05 (s, 2H), 7.02 (d, J = 9.2, 1H), 7.35−7.46 (m, 2H), 7.51−7.64 (m, 2H), 7.67 (s, 1H), 7.85 (d, J = 7.3, 1H), 7.94−8.07 (m, 1H), 8.1−8.25 (m, 1H), 8.44−8.69 (m, 1H), 9.32 (d, J = 9.2, 1H), 12.63 (s, 1H); MS (ESI) $(M + H)^+$ m/z $C_{26}H_{26}N_6O_3$ $[M + H]^+$ found 471.04.

6-Methoxy-*N*-(tetrahydro-2*H*-pyran-4-ylmethyl)-3-{[4-(1*H*-1,2,3-triazol-1-ylmethyl)-1-naphthoyl]amino}pyridine-2-carboxamide (13c). **13c** was prepared using essentially the same procedure as described for **13a** using 1-(tetrahydro-2*H*-pyran-4-yl)methanamine in place of 1-cyclohexylmethanamine to give the product (470 mg, 79%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30−1.41 (m, 2H), 1.60−1.70 (m, 2H), 1.80−1.94 (m, 1H), 3.26−3.43 (m, 4H), 3.96 (s, 3H), 3.96−4.02 (m, 2H), 6.06 (s, 2H), 7.04 (d, J = 9.2, 1H), 7.39 (d, J = 0.8, 1H), 7.43 (d, J = 7.2, 1H), 7.54−7.64 (m, 2H), 7.69 (d, J = 0.8, 1H), 7.85 (d, J = 7.2, 1H), 7.96−8.04 (m, 1H), 8.27 (t, J = 6.2, 1H), 8.51−8.59 (m, 1H), 9.33 (d, J = 9.1, 1H), 12.6 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.6, 167.0, 157.8, 136.2, 134.2, 133.2, 133.2, 132.9, 131.5, 130.9, 129.4, 127.8, 127.6, 126.6, 126.6, 124.8, 123.5, 123.0, 115.9, 67.5, 53.5, 52.1, 44.8, 35.4, 30.6; HRMS (ESI) m/z calcd for $C_{27}H_{28}N_6O_4$ $[M + H]^+$ 501.225; found 501.2228.

***N*-[(Tetrahydro-2*H*-pyran-4-yl)methyl]-3-[[[4-(1*H*-1,2,3-triazol-1-ylmethyl)-1-naphthalenyl]carbonyl]amino]-2-pyridinecarboxamide (13d).** A solution of **12a** (0.20 g, 0.51 mmol) and 1-(tetrahydro-2*H*-pyran-4-yl)methanamine (0.153 g, 1.33 mmol) in DMF (3 mL) was heated at 80 °C for 4 h. The reaction mixture was then concentrated in vacuo to leave a residue which was purified by preparative HPLC to provide the title compound as a solid (0.19 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37−1.51 (m, 2H), 1.66−1.70 (m, J = 12.7, 2H), 1.81−1.92 (m, 1H), 3.31 (t, J = 6.6, 2H), 3.36−3.42 (m, 2H), 3.98−4.02 (m, 2H), 6.09 (s, 2H), 7.44−7.46 (m, 1H), 7.48 (d, J = 7.2, 1H), 7.56 (dd, J = 8.6, 4.5, 1H), 7.61−7.65 (m, 2H), 7.79 (s, 1H), 7.88 (d, J = 7.2, 1H), 7.98−7.80 (m, 1H), 8.31 (dd, J = 4.5, 1.4, 1H), 8.54−8.56 (m, 1H), 8.60−8.64 (m, 1H), 9.40 (dd, J = 8.6, 1.6, 1H), 12.86 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.1, 167.2, 142.4, 138.3, 135.9, 134.3, 133.3, 133.1, 131.5, 130.8, 128.8, 127.9, 127.7, 127.6, 126.6, 126.6, 124.9, 123.5, 123.1, 67.5, 52.1, 44.9, 35.3, 30.6; HRMS (ESI) m/z calcd for $C_{26}H_{26}N_6O_3$ $[M + H]^+$ 471.2144; found 471.2135.

***N*-(Cyclobutylmethyl)-3-{[4-(1*H*-1,2,3-triazol-1-ylmethyl)-1-naphthoyl]amino}pyrazine-2-carboxamide (13e).** Following the procedure for **13a**, using **11c** (139 mg, 1.0 mmol) in place of **11b** gave methyl 3-({[4-(1*H*-1,2,3-triazol-1-ylmethyl)naphthalen-1-yl]-carbonyl}amino)pyrazine-2-carboxylate (**12c**, 1.44 g, 58%). Treatment of **12c** (0.620 g, 1.60 mmol) with 1-cyclobutylmethanamine (316 mg, 3.71 mmol) in place of 1-cyclohexylmethanamine provided the title compound as a solid (0.47 mg, 66%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.62−1.85 (m, 4H), 1.86−2.02 (m, 2H), 2.48−2.58 (m, 1H), 3.23−3.39 (m, 2H), 6.21 (s, 2H), 7.40 (d, J = 7.3, 1H), 7.61−7.73 (m, 2H), 7.78 (s, 1H), 7.87 (d, J = 7.3, 1H), 8.23 (s, 1H), 8.30 (d, J = 8.0, 1H), 8.41 (d, J = 8.4, 1H), 8.47 (d, J = 2.1, 1H), 8.66 (d, J = 2.1, 1H), 9.15 (t, J = 5.6, 1H), 12.21 (brs, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.9, 165.3, 147.9, 145.8, 138.3, 135.2, 134.8, 133.8, 133.0, 131.0, 130.1, 127.5, 127.4, 126.1, 126.0, 125.6, 125.4, 124.0, 50.7, 44.2, 34.6, 25.3, 17.9; HRMS (ESI) m/z calcd for $C_{24}H_{23}N_7O_2$ $[M + H]^+$ 442.1991; found 442.1997.

6-{[(Tetrahydro-2*H*-pyran-4-ylmethyl)amino]carbonyl}-5-{[4-(l*H*-1,2,3-triazolylmethyl)-1-naphthoyl]amino}pyridin-2-yl-3,3,3-trifluoropropane-1-sulfonate (14). A mixture of **13c** (145 mg, 0.3 mmol) and pyridine hydrochloride (3.8 g, 32.9 mmol) was heated at 150 °C for 30 min. Water was added at room temperature and the formed precipitate was collected, washed with water, dried, and purified by preparative HPLC to give 6-hydroxy-*N*-(tetrahydro-2*H*-pyran-4-ylmethyl)-3-{[4-(*1H*-1,2,3-triazol-1-ylmethyl)-1-naphthoyl]amino}pyridine-2-carboxamide (113 mg, 80%).

Silver(I) carbonate (0.40 g, 1.45 mmol) was added to a solution of the hydroxypyridine (50 mg, 0.10 mmol) and 3,3,3-trifluoropropylsulfonyl chloride (40 mg, 0.21 mmol) in CH_3CN (20 mL), and the mixture was heated at reflux for 2.5 h. A mixture of CH_2Cl_2/MeOH was then added, and the mixture was filtered. The filtrate was concentrated in vacuo to leave a residue which was partitioned between CH_2Cl_2 and saturated aqueous solution of $NaHCO_3$. The organic phase was washed with a saturated aqueous solution of NaCl, dried (Na_2SO_4), and concentrated in vacuo to leave a residue. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH 100:1.5) to give the title compound as a solid (260 mg, 78%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.34−1.40 (m, 2H), 1.60−1.66 (m, 2H), 1.78−1.88 (m, 1H), 2.88−2.98 (m, 2H), 3.28−3.40 (m, 4H), 3.65−3.71 (m, 2H), 3.95−4.01 (m, 2H), 6.08 (s, 2H), 7.40−7.45 (m, 3H), 7.59−7.66 (m, 2H), 7.71 (s, 1H), 7.86 (d, J = 7.3, 1H), 7.94−7.99 (m, 1H), 8.02−8.08 (m, 1H), 8.52−8.57 (m, 1H), 9.64 (d, J = 9.0, 1H), 12.81 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.0, 165.8, 148.7, 138.1, 135.2, 134.3, 134.2, 133.7, 131.6, 131.1, 130.8, 128.0, 127.9, 126.4, 125.2 (q, J = 276), 125.1, 123.6, 123.2, 120.4, 67.5, 52.0, 45.4 (q, J = 3), 45.1, 35.2, 30.5, 29.2 (q, J = 32); HRMS (ESI) m/z calcd for $C_{29}H_{29}F_3N_6O_6S$ $[M + H]^+$ 647.1899; found 647.1904.

Methyl 3-Amino-6-methoxypyridine-2-carboxylate (15a). Compound **4b** (15.1g, 89.8 mmol) was treated with saturated methanolic solution of HCl (300 mL) for 60 h at room temperature. The reaction mixture was concentrated in vacuo to leave a residue, and the residue was suspended in Et_2O. The solid material was filtered, washed with Et_2O, and dried (Na_2SO_4) to give the title compound as the corresponding HCl salt (19.5g, 99%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.74 (s, 3H), 3.78 (s, 3H), 6.87 (d, J = 8.9, 1H), 7.28 (d, J = 8.9, 1H); MS (ESI) m/z for $C_8H_{11}N_2O_3$ $(M + H)^+$ 183.0.

Methyl 6-Methoxy-3-[({4-[(methylsulfanyl)methyl]-naphthalen-1-yl}carbonyl)amino]pyridine-2-carboxylate (16a). Sodium methanethiolate (5.6 g, 80.5 mmol) was added in portions to a solution of 4-(bromomethyl)-1-naphthoic acid[42] (19.9 g, 75.2 mmol) in THF (140 mL) at 0 °C, and the reaction mixture was stirred at room temperature for 2 h. The mixture was cooled to 0 °C, H_2O was added, and the pH was adjusted to ∼4 with the aid of a 4 M aqueous solution of HCl to give a precipitate. The precipitate was filtered and washed with cold H_2O to give 4-[(methylthio)methyl]-1-naphthoic acid (16.1g, 92%).

Oxalyl chloride (10.5 mL, 124 mmol) was added slowly to a suspension of 4-[(methylthio)methyl]-1-naphthoic acid (2.89 g, 12.4 mmol) in CH_2Cl_2 (100 mL), and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was then concentrated in vacuo to leave a residue, which was taken up in toluene and concentrated in vacuo to leave 4-[(methylthio)methyl]-1-naphthoyl chloride (3.06 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.06 (s, 3H), 4.15 (s, 2H), 7.48 (d, J = 7.6, 1H), 7.61−7.73 (m, 2H), 8.21 (d, J = 8.2, 1H), 8.48 (d, J = 7.7, 1H), 8.79 (d, J = 8.7, 1H).

A solution of 4-[(methylthio)methyl]-1-naphthoyl chloride (690 mg, 2.74 mmol) in $CHCl_3$ (4 mL) was added to a solution of **15a** (400 mg, 1.83 mmol), pyridine (0.59 mL, 7.3 mmol), and DMAP (7.0 mg, 0.06 mmol) in $CHCl_3$ (6 mL), and the reaction mixture was stirred at 50 °C for 16 h. The mixture was then diluted with CH_2Cl_2 and washed with a saturated aqueous solution of copper(II) sulfate, a 1 M aqueous solution of NaOH, and a saturated aqueous solution of NaCl. The organic phase was dried (Na_2SO_4) and concentrated in vacuo to leave a residue, which was purified by flash column chromatography (toluene/EtOAc 20:1) to give the title compound (550 mg, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.09 (s, 3H), 3.95 (s, 3H), 4.00 (s, 3H), 4.17 (s, 2H), 7.08 (d, J = 9.2, 1H), 7.46 (d, J = 7.3, 1H), 7.56−7.7 (m, 2H), 7.78 (d, J = 7.3, 1H), 8.13−8.27 (m, 1H), 8.46−8.62 (m, 1H), 9.28 (d, J = 9.2, 1H), 11.36 (s, 1H); MS (ESI) m/z $C_{21}H_{21}N_2O_4S$ $(M + H)^+$ 397.1.

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N-(Cyclobutylmethyl)-3-({4-[(methylthio)methyl]-1-naphthoyl}amino)pyridine-2-carboxamide. (17a). Compound **7b** (80 mg, 0.39 mmol) was added to a solution of 4-[(methylthio)-methyl]-1-naphthoyl chloride (prepared in **16a**) (143 mg, 0.57 mmol), pyridine (0.32 mL, 3.9 mmol), and DMAP (0.5 mg, 0.004 mmol) in $CHCl_3$ (1 mL). The reaction mixture was heated at reflux for 2 h. H_2O and CH_2Cl_2 were added and the organic phase was filtered through a phase separator and concentrated in vacuo to leave a residue. The residue was purified by flash column chromatography (EtOAc in heptane 0% → 40%) to give the title compound (140 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.66−1.79 (m, 2H), 1.82−1.98 (m, 2H), 2.05 (s, 3H), 2.03−2.13 (m, 2H), 2.49−2.65 (m, 1H), 3.36−3.44 (m, 2H), 4.14 (s, 2H), 7.43 (d, J = 7.3, 1H), 7.49 (dd, J = 8.6, 4.5, 1H), 7.53−7.63 (m, 2H), 7.80 (d, J = 7.2, 1H), 8.14−8.21 (m, 1H), 8.25 (dd, J = 4.5, 1.3, 4.5, 1H), 8.36−8.47 (m, 1H), 8.51−8.59 (m, 1H), 9.38 (dd, J = 8.6, 1.3, 1H), 12.82 (s, 1H); MS (ESI) m/z for $C_{24}H_{26}N_3O_2S$ $(M + H)^+$ 420.1.

6-Methoxy-3-({4-[(methylthio)methyl]-1-naphthoyl}amino)-N-(tetrahydro-2H-pyran-4-ylmethyl)pyridine-2-carboxamide (17b). 1-(Tetrahydro-2*H*-pyran-4-yl)methanamine (8.72 g, 75.7 mmol) was added to a solution of **16a** (5.0 g, 12.6 mmol) in DMF (50 mL). The reaction mixture was stirred at 80 °C for 3 h and then cooled to room temperature. H_2O and CH_2Cl_2 were added, and the phases were separated. The organic phase was washed with H_2O and a saturated aqueous solution of NaCl and then dried (Na_2SO_4) and concentrated in vacuo to leave a residue. The residue was recrystallized using CH_2Cl_2/EtOH to give the title compound (5.5 g, 91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30−1.51 (m, 2H), 1.54−1.73 (m, 2H), 1.75−1.95 (m, 1H), 2.08 (s, 3H), 3.24−3.45 (m, 4H), 3.95 (s, 3H), 3.96−4.02 (m, 2H), 4.16 (s, 2H), 7.03 (d, J = 9.2, 1H), 7.44 (d, J = 7.3, 1H), 7.52−7.68 (m, 2H), 7.79 (d, J = 7.3, 1H), 8.14−8.35 (m, 2H), 8.49−8.62 (m, 1H), 9.34 (d, J = 9.2, 1H), 12.47 (s, 1H); MS (ESI) m/z $C_{26}H_{30}N_3O_4S$ $(M + H)^+$ 480.0.

N-(Cyclobutylmethyl)-3-({4-[(methylsulfinyl)methyl]-1-naphthoyl}amino)pyridine-2-carboxamide (18a). *m*-CPBA (82 mg, ∼70%, 0.33 mmol) was added to a solution of **17a** (140 mg, 0.33 mmol) in CH_2Cl_2 (20 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 10 min. A saturated aqueous solution of $NaHCO_3$ was then added, and the organic phase was dried (Na_2SO_4) and concentrated in vacuo to leave a residue. The residue was purified by flash column chromatography (EtOAc in toluene 20% → 100%) to give the title compound (51 mg, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67−1.78 (m, 2H), 1.82−1.95 (m, 2H), 2.02−2.13 (m, 2H), 2.50 (s, 3H), 2.51−2.53 (m, 1H), 3.34−3.43 (m, 2H), 4.33 (d, J = 12.9, 1H), 4.69 (d, J = 12.9, 1H), 7.10−7.17 (m, 1H), 7.47−7.54 (m, 1H), 7.58−7.66 (m, 2H), 7.85 (d, J = 7.3, 1H), 8.09−8.15 (m, 1H), 8.24−8.28 (m, 1H), 8.39−8.46 (m, 1H), 8.51−8.58 (m, 1H), 9.33−9.39 (m, 1H), 12.89 (s, 1H); HRMS (ESI) m/z calcd for $C_{24}H_{25}N_3O_3S$ $[M + H]^+$ 436.1695; found 436.1701.

6-Methoxy-3-({4-[(methylsulfinyl)methyl]-1-naphthoyl}-amino)-N-(tetrahydro-2H-pyran-4-ylmethyl)pyridine-2-carboxamide (18b). A solution of *m*-CPBA (120 mg, ∼70%, 0.49 mmol) in CH_2Cl_2 (3 mL) was added to a solution of **17b** (220 mg, 0.46 mmol) in CH_2Cl_2 (7 mL) at 0 °C, and the reaction mixture was stirred at 0 °C for 2.5 h. The reaction mixture was then diluted with CH_2Cl_2, washed with a saturated aqueous solution of $NaHCO_3$ and a saturated aqueous solution of NaCl, dried (Na_2SO_4), and concentrated in vacuo to leave a residue. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH 50:1 → 25:1) to give the title compound (166 mg, 73%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29−1.45 (m, 2H), 1.60−1.69 (m, 2H), 1.78−1.91 (m, 1H), 2.54 (s, 3H), 3.27−3.42 (m, 4H), 3.92−4.01 (m, 2H), 3.95 (s, 3H), 4.38 (d, J = 12.9, 1H), 4.71 (d, J = 12.9, 1H), 7.03 (d, J = 9.2, 1H), 7.54 (d, J = 7.3, 1H), 7.57−7.67 (m, 2H), 7.84 (d, J = 7.3, 1H), 8.09−8.15 (m, 1H), 8.23−8.29 (m, 1H), 8.52−8.58 (m, 1H), 9.32 (d, J = 9.2, 1H), 12.54 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.7, 167.0, 157.7, 135.5, 133.2, 133.2, 132.2, 130.9, 129.7, 129.4, 128.1, 127.5, 127.4, 126.6, 125.0, 123.8, 115.8, 67.5, 59.0, 53.5, 44.8, 38.2, 35.4, 30.6; HRMS (ESI) m/z calcd for $C_{26}H_{29}N_3O_5S$ $[M + H]^+$ 496.1906; found 496.1925.

3-({4-[(Methylsulfinyl)methyl]-1-naphthoyl}amino)-N-(tetrahydro-2H-pyran-4-ylmethyl)pyridine-2-carboxamide (18c). 4-Methyl-1-naphthalenecarbonyl chloride (6.14 g, 30.0 mmol) was added to a solution of **7a**, (2.90 g, 12.3 mmol), pyridine (3.0 mL, 37 mmol), and DMAP (45 mg, 0.37 mmol) in $CHCl_3$ (160 mL), and the reaction mixture was heated at reflux for 1.5 h. The reaction mixture was then partitioned between CH_2Cl_2 and H_2O. The organic phase was dried (phase separator) and concentrated in vacuo to leave a residue. The residue was purified by flash column chromatography (heptane/EtOAc 9:1) to give 3-{[(4-methylnaphthalen-1-yl)-carbonyl]amino}-*N*-(tetrahydro-2*H*-pyran-4-ylmethyl)pyridine-2-carboxamide (4.24 g, 85%). ^{1}H NMR (400 MHz, $CDCL_3$) δ 1.29−1.42 (m, 2H), 1.61−1.68 (m, 2H), 1.77−1.91 (m, 1H), 2.72 (s, 3H), 3.29 (t, J = 6.6, 2H), 3.31−3.39 (m, 2H), 3.95 (dd, J = 11,2, 3.4, 2H), 7.38 (d, J = 7.2, 1H), 7.49 (dd, J = 8.6, 4.5, 1H), 7.53−7.59 (m, 2H), 7.79 (d, J = 7.2, 1H), 8.02−8.09 (m, 1H), 8.24 (dd, J = 4.5, 1.4, 1H), 8.5−8.61 (m, 2H), 9.38 (dd, J = 8.6, 1.4, 1H), 12.72 (s, 1H).

AIBN (15 mg, 0.06 mmol) was added to a solution of 3-{[(4-methylnaphthalen-1-yl)carbonyl]amino}-*N*-(tetrahydro-2*H*-pyran-4-ylmethyl)pyridine-2-carboxamide (400 mg, 0.99 mmol) and NBS (360 mg, 2 mmol) in DCE (20 mL) at room temperature. The solution was heated at 80 °C for 2.5 h, then cooled to room temperature and concentrated in vacuo to leave the crude bromomethylnaphthalene derivative, which was used directly with no further purification. Sodium methanethiolate (1.22 g, 17.4 mmol) was added to a solution of 3-{[4-(bromomethyl)-1-naphthoyl]amino}-*N*-(tetrahydro-2*H*-pyran-4-ylmethyl)pyridine-2-carboxamide (4.20 g, 8.71 mmol) in DMF (50 mL) at 0 °C. The reaction mixture was stirred at room temperature for 3 h, and then H_2O and EtOAc were added and the phases were separated. The organic phase was washed with H_2O, dried (Na_2SO_4), and concentrated in vacuo to leave a residue. The residue was purified by flash column chromatography (EtOAc in heptane 25% → 50%) to give the crude 3-({4-[(methylthio)methyl]-1-naphthoyl}-amino)-*N*-(tetrahydro-2*H*-pyran-4-ylmethyl)pyridine-2-carboxamide (**17c**, 1.81 g, 46%) that was used directly in the next step.

m-CPBA (0.99 g, ∼70%, 4.03 mmol) was added to a solution of the crude methylthionaphthyl derivative (**17c**, 1.81 g, 4.03 mmol) in CH_2Cl_2 (100 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 10 min. A saturated aqueous solution of $NaHCO_3$ was then added, and the organic phase was dried (Na_2SO_4) and concentrated in vacuo to leave a residue. The residue was purified by flash column chromatography (EtOAc in toluene 20% → 60%) to give the title compound as a solid (0.82 g, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29−1.41 (m, 2H), 1.59−1.68 (m, 2H), 1.78−1.88 (m, 1H), 2.51 (s, 3H), 3.25−3.39 (m, 4H), 3.91−3.99 (m, 2H), 4.36 (d, J = 12.9, 1H), 4.69 (d, J = 12.9, 1H), 7.49−7.55 (m, 2H), 7.58−7.66 (m, 2H), 7.84 (d, J = 7.3, 1H), 8.09−8.15 (m, 1H), 8.24−8.28 (m, 1H), 8.51−8.58 (m, 2H), 9.34−9.39 (m, 1H), 12.81 (s, 1H); HRMS (ESI) m/z calcd for $C_{25}H_{27}N_3O_4S$ $[M + H]^+$ 466.1801; found 466.1779.

3-[(4-{[(R)-Methylsulfinyl]methyl}-1-naphthoyl)amino]-N-(tetrahydro-2H-pyran-4-ylmethyl)pyridine-2-carboxamide and 3-[(4-{[(S)-Methylsulfinyl]methyl}-1-naphthoyl)amino]-N-(tetrahydro-2H-pyran-4-yl-methyl)pyridine-2-carboxamide (18d and 18e). **18d** and **18e** were obtained by separating the enantiomers of **18c** using chiral chromatography at 40 °C on a ReproSil HPLC column (250 mm × 20 mm, 8 μm) and eluting with MeOH at a flow rate of 18 mL/min and detected at 265 nm. The first eluted compound was collected and evaporated to yield **18d** (320 mg, 99.6% ee) $[\alpha]^{20}_D$ +101 (c 1.0, CH_3CN) as a solid. The second eluted compound was collected and evaporated to yield **18e** (340 mg, 99.7% ee) $[\alpha]^{20}_D$ −99.7 (c 1.0, CH_3CN) as a solid.

N-(Cyclobutylmethyl)-3-({4-[(methylsulfinyl)methyl]-1-naphthoyl}amino)pyrazine-2-carboxamide (18f). NBS (88 mg, 0.49 mmol) and AIBN (∼2 mg, 0.01 mmol) were added to a solution of **11c** (155 mg, 0.482 mmol) in CCl_4 (15 mL) at 77 °C, and the reaction mixture was heated at reflux for 2 h. The reaction mixture was then concentrated in vacuo to leave a residue, and the residue was partitioned between EtOAc and H_2O. The organic phase was washed with a saturated aqueous solution of NaCl, dried (Na_2SO_4) and concentrated in vacuo to give crude methyl 3-{[4-(bromomethyl)-1-

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naphthoyl]amino}pyrazine-2-carboxylate (193 mg). This crude product was taken to the next synthetic step without further purification.

Sodium methanethiolate (29 mg, 0.41 mmol) was added to a solution of crude methyl 3-{[4-(bromomethyl)-1-naphthoyl]amino}-pyrazine-2-carboxylate (190 mg) in DMF (3 mL) at 0 °C. The reaction mixture was stirred at room temperature for 4 h and then partitioned between EtOAc and H_2O. The aqueous phase was extracted with EtOAc and the combined organic phases were dried (Na_2SO_4) and concentrated in vacuo to leave a residue, which was purified using flash chromatography (EtOAc in heptane 50% → 100%) to give crude methyl 3-({4-[(methylthio)methyl]-1-naphthoyl}-amino)pyrazine-2-carboxylate (**16b**, 102 mg).

The final two steps were performed by an analogous method to **16a** → **17b** → **18b** using 1-cyclobutylmethanamine in place of 1-(tetrahydro-2*H*-pyran-4-yl)methanamine to give crude *N*-(cyclobutylmethyl)-3-[({4-[(methylsulfanyl)methyl]naphthalen-1-yl}-carbonyl)amino]pyrazine-2-carboxamide (**17d**) and then performing the last oxidation step with *m*-CPBA to give the title compound as a solid (33 mg, 16% overall yield from **11c**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.73−1.81 (m, 2H), 1.89−1.99 (m, 2H), 2.10−2.17 (m, 2H), 2.55 (s, 3H), 2.57−2.64 (m, 1H), 3.45−3.48 (m, 2H), 4.39 (d, J = 12.9, 1H), 4.74 (d, J = 12.9, 1H), 7.58 (d, J = 7.3, 1H), 7.63−7.70 (m, 2H), 7.94 (d, J = 7.3, 1H), 8.15−8.20 (m, 2H), 8.31 (d, J = 2.3, 1H), 8.67−8.70 (m, 1H), 8.74 (d, J = 2.3, 1H), 12.75 (s, 1H); HRMS (ESI) m/z calcd for $C_{23}H_{24}N_4O_3S$ $[M + H]^+$ 437.1647; found 437.1664.

***N*-(Cyclobutylmethyl)-3-[(4-{[(*R*)-methylsulfinyl]methyl}-1-naphthoyl)amino]pyrazine-2-carbox-amide or *N*-(Cyclobutylmethyl)-3-[(4-{[(*S*)-methylsulfinyl]methyl}-1-naphthoyl)-amino]pyrazine-2-carboxamide (18g).** **18g** was obtained by separating the enantiomers of **18f** using chiral chromatography at 40 °C on a Chiralpak IA HPLC column (205 mm × 20 mm, 5 μm), eluting with heptane/EtOH 30:70 at a flow rate of 12 mL/min and detected at 265 nm. The first eluted compound was collected and evaporated to yield 18g (310 mg, 99.9% ee) $[\alpha]^{20}_D$ +100 (c 1.0, CH_3CN) as a solid. The second eluted compound was collected and evaporated to yield the second enantiomer (265 mg, 99.9% ee) $[\alpha]^{20}_D$ −131 (c 1.0, CH_3CN) as a solid.

***N*-(Cyclobutylmethyl)-3-({4-[(methylsulfonyl)methyl]-1-naphthoyl}amino)pyridine-2-carboxamide (19).** In the synthesis of **18a**, the title compound (**19**, 51 mg, 34%) could also be isolated as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.78−1.66 (m, 2H), 1.94−1.83 (m, 2H), 2.12−2.03 (m, 2H), 2.50−2.62 (m, 1H), 2.77 (s, 3H), 3.35−3.43 (m, 2H), 4.79 (s, 2H), 7.17−7.12 (m, 1H), 7.53−7.48 (m, 1H), 7.68−7.58 (m, 2H), 7.88 (d, J = 7.3, 1H), 8.15 (d, J = 8.0, 1H), 8.28−8.26 (m, 1H), 8.46−8.40 (m, 1H), 8.52−8.59 (m, 1H), 9.38−9.34 (m, 1H), 12.9 (s, 1H); HRMS (ESI) m/z calcd for $C_{24}H_{25}N_3O_4S$ $[M + H]^+$ 452.1644; found 452.1649.

***N*-(Cyclobutylmethyl)-6-[2-(2-hydroxyethoxy)ethoxy]-3-({4-[(methylsulfinyl)methyl]-1-naphthoyl}amino)pyridine-2-carboxamide (20).** Following the procedure for **17b**, using **16a** (1.7 g, 4.19 mmol) and 1-cyclobutylmethanamine (1.0 g, 11.7 mmol) in place of 1-(tetrahydro-2*H*-pyran-4-yl)methanamine) gave *N*-(cyclobutylmethyl)-6-methoxy-3-({4-[(methylthio)methyl]-1-naphthoyl}amino)-pyridine-2-carboxamide (17e, 1.68 g, 89%).

Compound **17e** (810 mg, 1.80 mmol) was added to melted pyridine hydrochloride (21 g, 0.11 mol) at 160 °C, and the mixture was stirred at 160 °C for 30 min. The reaction mixture was cooled to room temperature, and H_2O was added. The precipitated material was filtered, washed with H_2O, and dried to give *N*-(cyclobutylmethyl)-3-({4-[(methylthio)methyl]-1-naphthoyl}amino)-6-oxo-1,6-dihydropyridine-2-carboxamide (680 mg, 87%).

A solution of *m*-CPBA (2.70 g, ∼70%, 11.0 mmol) in CH_2Cl_2 (200 mL) was added slowly to a solution of the thiomethyl intermediate (4.64 g, 10.7 mmol) in $CHCl_3$ (200 mL) at 0 °C, and the reaction mixture was stirred at 0 °C for 1 h. A second portion of *m*-CPBA (105 mg, ∼70%, 0.43 mmol) was added, and the reaction mixture was stirred at 0 °C for an additional 10 min. H_2O was added. The phases were separated, and the aqueous phase was extracted with CH_2Cl_2. The organic phases were washed with a saturated aqueous solution of NaCl, dried (Na_2SO_4), and concentrated in vacuo to leave a residue, which was purified by flash column chromatography (CH_2Cl_2/MeOH 40:1 → 25:1) to give the hydroxypyridine (4.50 g, 94%).

Silver(I) carbonate (0.74 g, 2.67 mmol) was added to a solution of the hydroxypyridine (400 mg, 0.89 mmol) and 2-(2-chloroethoxy)-ethanol (3.30 g, 26.5 mmol) in DMF (8 mL), and the mixture was heated in a microwave at 130 °C for 8 h. A mixture of CH_2Cl_2/MeOH was then added, and the mixture was filtered. The filtrate was concentrated in vacuo to leave a residue which was partitioned between EtOAc and H_2O. The organic phase was washed with a saturated aqueous solution of $NaHCO_3$ and a saturated aqueous solution of NaCl, dried (Na_2SO_4), and concentrated in vacuo to leave a residue. The residue was purified by preparative HPLC to give the title compound as a solid (128 mg, 27%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.73−1.82 (m, 2H), 1.84 (s, 1H), 1.89−1.99 (m, 2H), 2.08−2.15 (m, 2H), 2.56 (s, 3H), 2.57−2.64 (m, 1H), 3.42−3.45 (m, 2H), 3.71−3.74 (m, 2H), 3.81−3.83 (m, 2H), 3.92−3.95 (m, 2H), 4.40 (d, J = 12.9, 1H), 4.47−4.49 (m, 2H), 4.77 (d, J = 12.9, 1H), 7.10 (d, J = 9.1, 1H), 7.56 (d, J = 7.3, 1H), 7.62−7.70 (m, 2H), 7.87 (d, J = 7.3, 1H), 8.09−8.12 (m, 1H), 8.15−8.17 (m, 1H), 8.57−8.60 (m, 1H), 9.37 (d, J = 9.1, 1H), 12.64 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.7, 166.9, 157.0, 135.5, 133.4, 133.3, 132.2, 130.9, 129.7, 129.6, 128.1, 127.5, 127.4, 126.6, 125.0, 123.8, 116.0, 72.6, 69.4, 65.4, 61.8, 59.0, 44.2, 38.2, 34.9, 25.6, 18.3; HRMS (ESI) m/z calcd for $C_{28}H_{33}N_3O_6S$ $[M + H]^+$ 540.2168; found 540.2157.

Biological Evaluation. hCB_1 and hCB_2 Receptor Binding Assay.[36] Membranes are produced from either HEK 293S cells expressing the cloned hCBl receptor (clone no. 24) or Sf9 cells, using the baculovirus system, expressing the cloned hCB2 receptor. The membranes are thawed at 37 °C, passed 3 times through a 23 gauge blunt-end needle, and diluted in the cannabinoid binding buffer (50 mM Tris, 2.5 mM EDTA, 5 mM $MgCl_2$, and 0.5 mg/mL BSA fatty acid free, pH 7.4), and 80 μL aliquots containing the appropriate amount of protein are distributed in 96-well plates. The IC_{50} values of the compounds (150 μL) at hCBl and hCB2 are evaluated from 10-point dose−response curves performed with ^{3}H-CP55,940 (70 μL) at 20000−25000 dpm per well (0.17−0.21 nM) in a final volume of 300 μL. The total and nonspecific binding are determined in the absence and presence of 0.2 μM of HU210 (150 μL). The plates are vortexed, incubated for 60 min at room temperature, and filtered through Unifilters GF/B (presoaked in 0.1% polyethyleneimine) with the Packard harvester using 3 mL of wash buffer (50 mM Tris, 5 mM $MgCl_2$, 0.05% BSA, pH 7.0). The filters are dried for 1 h at 55 °C. The specific binding (SB) is calculated as TB-NS, and the SB in the presence of various ligands is expressed as percentage of control SB. Values of IC_{50} and Hill coefficient (n_H) for ligands in displacing specifically bound radioligand are calculated in ActivityBase with Xlfit4 (IDBS, Inc.). The concentration of compounds to use and dilutions are also calculated with ActivityBase. The radioactivity (cpm) is counted in a TopCount (Packard) after adding 65 μL/well Microscint 20 (Packard Biosciences) scintillation fluid.

GTPγ[^{35}S] Binding Assay.[36] GTPγ[^{35}S] binding was measured on cloned human CB1 receptors in membranes of HEK 293S cells or cloned human CB2 receptors in membranes of Sf9 cells. The membranes are thawed at 37 °C, passed 3 times through a 23 gauge blunt-end needle, and diluted in the GTPγ[^{35}S] binding buffer (50 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (Hepes), 20 mM NaOH, 100 mM NaCl, 1 mM EDTA, 5 mM $MgCl_2$, pH 7.4, 0.1% BSA, and 15 μM GDP). The EC_{50} and E_{max} of the compounds at hCBl and hCB2 are evaluated from 10-point dose−response curves. The assay, performed in 96-well plates, consisted of 300 μL, containing 150 μL of buffer alone or compound at varying concentrations, 80 μL of membranes (5 μg of protein/well) mixed with 56 μM GDP (15 μM final). Finally, 70 μL of the tracer GTPγ[^{35}S] (Dupont/NEN, Mandel Scientific, St-Laurent) (100000−130000 dpm/well) is added to start the reaction. Eight wells were used to define basal (negative control) binding and eight for positive control (maximal binding) using 10 μM **2**. The plates were then mixed by hand on an orbital mixer and incubated for 1 h at room temperature, filtered on Unifilters GF/B

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(presoaked in deionized water) with the Packard harvester using 3 mL of wash buffer (50 mM Tris, 5 mM $MgCl_2$, 50 mM NaCl, pH 7.4). The filters are dried for 1 h at 55 °C before adding 50 μL of Microscint 20 (Packard Biosciences) scintillation fluid. The radioactivity (cpm) on the filter plates was counted in a TopCount (Packard). The cpm values of GTPγ[^{35}S] binding in the eight wells containing GTPγ[^{35}S] and membranes were averaged to define basal binding, and the values of the eight wells containing 10 μM **2** were averaged to define GTPγ[^{35}S] maximal binding. The stimulation of GTPγ[^{35}S] binding observed for each concentration of compound was expressed as a percentage of maximal effect elicited by 10 μM **2**. GTPγ[^{35}S] specific binding is calculated by subtracting the basal binding. Curve fitting and EC_{50} calculations were performed using Xlfit4 (IDBS, Inc.).

Dog CB1 GTPγ[^{35}S] binding assays were conducted using cloned dog CB1 receptors stably expressed in HEK 293S cells using the same procedures described for the human CB1 receptors stably expressed in the same cell line.

TLESR Measurements in Vivo in Dogs. The method applied for the in vivo studies of TLESR in dogs and the definitions of motility parameters have been described previously.[9,48] In brief, a water-perfused Dentsleeve multilumen assembly was introduced to dogs through the esophagostomy for the measurement of gastric, lower esophageal sphincter (LES) and esophageal pressures. A pH electrode above the LES was used to measure acid reflux episodes, and a catheter was placed in the hypopharynx to measure swallows. TLESRs were stimulated by gastric infusion of an acidified liquid nutrient (10% peptone, 5% D-glucose, 5% Intralipid, pH 3.0; 30 mL/kg; 100 mL/min) followed by air insufflation (500 mL/min) to maintain gastric pressure at 10 ± 1 mmHg. TLESRs were defined as a rapid decrease in LES pressure (>1 mmHg/s) to a pressure of <2 mmHg above gastric pressure and a duration of >1s without any swallowing <2 s before onset. The compounds were administered intragastrically or intravenously 30 or 10 min before start of the experiment, respectively, and the number of TLESRs was measured for 45 min. Each dog served as its own control, and inhibition of TLESRs was calculated with regard to five preceding control experiments for each dog. Data are presented as the mean ± SEM. All procedures were approved by the Ethical Committee for Animal Experiments of the Gothenburg Region.

■ ASSOCIATED CONTENT

Supporting Information

Experimental methods used to determine dog behavior, solubility, Caco-2 transport and efflux ratios, CYP enzyme inhibition, measurement of unbound brain exposure in the rat, $C_{u,br}/C_{u,pl}$, and hERG inhibition. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +46 31 7761572. Fax: +44 31 7763868. E-mail: alleyn.plowright@astrazeneca.com.

Notes

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■ ABBREVIATIONS USED

BCRP, breast cancer resistance protein; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; $C_{u,br}/C_{u,pl}$, unbound brain-to-plasma concentration ratio; DIPEA, *N*,*N*-diisopropylethylamine; GERD, gastroesophageal reflux disease; GTPγ[^{35}S], [^{35}S]guanosine 5′-*O*-(3-thiotriphosphate); HATU, *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate; HBSS, Hank's balanced saline solution; Hepes, *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid; hu Caco-2 A−B, human Caco-2 cells apical to basolateral; hCB1, human cannabinoid receptor 1; hCB2, human cannabinoid receptor 2; LLE, ligand lipophilicity efficiency; LES, lower esophageal sphincter; P_{app}, apparent permeability; TLESR, transient lower esophageal sphincter relaxation

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