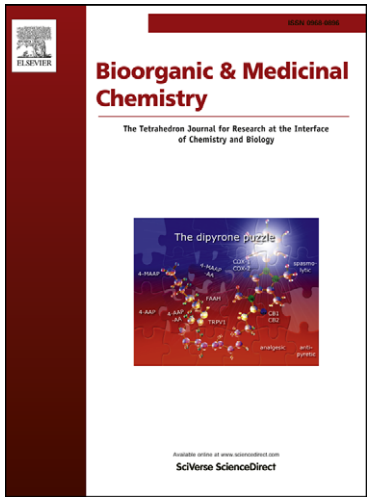

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PII: S0968-0896(13)00035-7
DOI: http://dx.doi.org/10.1016/j.bmc.2013.01.006
Reference: BMC 10526

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 9 October 2012
Revised Date: 5 January 2013
Accepted Date: 7 January 2013

Please cite this article as: Kusakabe, K-i., Tada, Y., Iso, Y., Sakagami, M., Morioka, Y., Chomei, N., Shinonome, S., Kawamoto, K., Takenaka, H., Yasui, K., Hamana, H., Hanasaki, K., Design, synthesis, and binding mode prediction of 2-pyridone-based selective CB2 receptor agonists, *Bioorganic & Medicinal Chemistry* (2013), doi: http://dx.doi.org/10.1016/j.bmc.2013.01.006

Design, synthesis, and binding mode prediction of 2-pyridone-based selective CB2 receptor agonists

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ABSTRACT. Selective CB2 agonists have the potential for treating pain without central CB1-mediated adverse effects. Screening efforts identified 1,2-dihydro-3-isoquinolone **1**; however, this compound has the drawbacks of being difficult to synthesize with two asymmetric carbons on an isoquinolone scaffold and of having a highly lipophilic physicochemical property. To address these two major problems, we designed the 2-pyridone-based lead **15a**, which showed moderate affinity for CB2. Optimization of **15a** led to identification of **39f** with high affinity for CB2 and selectivity over CB1. Prediction of the binding mode of **39f** in complex with an active-state CB2 homology model provided structural insights into its high affinity for CB2.

KEYWORDS. Cannabinoid; CB2; Agonist; Pyridone; Pyridine-2-one; Homology model.

ABBREVIATIONS. cAMP, 3',5'-cyclic adenosine monophosphate; CB, cannabinoid; CHO, Chinese hamster ovary; CNS, central nervous system; DCM, dichloromethane; DMF, *N*,*N*-dimethylformamide; DPPA, diphenylphosphonic azide; ECL, extracellular loop; ESI, electrospray ionization; LRMS, low-resolution mass spectrometry; Pd/C, palladium on carbon; SAR, structure-activity relationship; THC, tetrahydrocannabinol; TLC, thin-layer chromatography.

1. Introduction

Cannabis sativa has been widely used for hundreds of years as a medicine.[1] Δ^9-Tetrahydrocannabinol (Δ^9-THC), the predominant active ingredient of cannabis, and other cannabinoids are known to have therapeutic benefits for treating pain, emesis, anxiety, glaucoma, sleep disorders, appetite disorders, cancer, Alzheimer's disease, and epilepsy,[2] while having undesirable central nervous system (CNS) side effects such as catalepsy and dependency. Cannabinoids act on G protein-coupled receptors, CB1 and CB2. The CB1 receptor is widely expressed in CNS regions such as the brain, spinal cord, and sensory neurons,[4] while the CB2 receptor is mainly expressed in the spleen and immune cells[5] as well as in the brain.[6] Most of the CNS side effects caused by cannabinoids are induced by activation of the central CB1 receptor.[7]

Selective CB2 agonists have been shown to mediate analgesic effects in preclinical models without CNS side effects. For example, AM-1241, a selective CB2 agonist with 80-fold selectivity over CB1, was efficacious in models of inflammatory and neuropathic pain without CNS side effects.[8] GSK554418A, a highly potent CB2 agonist with >1200-fold selectivity, showed strong efficacy in models of both acute and chronic pain.[9] No significant CB1 mediated effects were observed in *in vivo* study of this compound. There have been considerable efforts to identify selective CB2 agonists, which include classical cannabinoids, indoles, pyrimidines, 4-oxo-1,4-dihydroquinolines, pyrazoles, pyridones, thiazoles, thiazines, and benzimidazolones.[8-12, 23, 26] Several of these agonists have entered clinical trials.

We initiated our efforts to identify a selective CB2 agonist with drug-like properties. 1,2-Dihydro-3-isoquinolone, compound **1** (Figure 1), was identified as a potent and selective CB2 agonist during high-

throughput screening; however, despite its good potency and selectivity, its synthetic accessibility was a concern due to its two asymmetric carbons, which makes it difficult to conduct efficient structure-activity relationship (SAR) studies. Furthermore, the isoquinolone scaffold is generally highly lipophilic and is thus less advantageous from a drug-likeness point of view. Therefore, we designed a 2-pyridone-based scaffold in order to remove the two asymmetric carbons and reduce lipophilicity in isoquinolone **1** (Figure 2). Firstly, to reduce the lipophilicity of **1**, the benzene ring in **1** was truncated as shown in **A**. Secondly, to remove the asymmetric carbons in **A**, the piperidine-2-one was aromatized to give the pyridone scaffold **B**. Finally, the thioether link in **B** was replaced by an oxygen link to give pyridone **C** because thioether-containing analogs generally have metabolic concerns.[15] To test this design, we synthesized 2-pyridone **15a** (Table 1), which showed moderate affinity for CB2 (Ki = 976 nM). Although the CB2 affinity of **15a** was decreased compared with **1**, we felt that this compound provided a good starting point for the design of selective CB2 agonists.

Herein, we report the design, synthesis, and SAR of a novel potent and selective series of 2-pyridones. This work led to compound **39f**, which showed good agonist activity and selectivity over CB1. Prediction of the binding mode of **39f** by use of a CB2 homology model provided structural insights into the high affinity of **39f** for CB2.

2. Chemistry

We synthesized 5,6-dimethylpyridone **15a** and thiopyridones **18a-e** as shown in Scheme 1 and 2. Enamine **8** was obtained by heating commercially available β-keto ester **7** with benzylamine in toluene. Acylation of enamine **8** with methoxyacetyl chloride afforded **9** that was treated with sodium in ethanol to give 4-hydroxy-2-pyridone **10**.[13] Compound **11**, obtained from the reaction of 4-hydroxy-pyridone **10** with 5-chloro-1-phenyl-1*H*-tetrazole, was reduced by hydrogenation to afford the key intermediate **12**. *N*-Alkylation of **12** with alkyl iodides in the presence of NaH gave alkyl pyridones **13a-e**. Pyridone **13a** was treated with pyridinium chloride to afford **14a**, which was reacted with 2-chlorobenzoxazole to give 5,6-dimethylpyridone **15a**. Reactions of alkyl pyridones **13a-e** with Lawesson's reagent in toluene

resulted in alkyl thiopyridones **16a-e**. Thiopyridones **18a-e** were prepared in a similar manner to that of pyridone **15a**.

The synthesis of thiopyridones **23** and **26** are presented in Scheme 3. Thiopyridone **23** was synthesized in a similar manner to thiopyridone **18a**. 3-Hydroxy-2-thiopyridone **22** was treated with *N*,*N*,*N*′,*N*′-tetramethylmethylenediamine to yield amine **24**. Compound **25** was obtained by alkylation of amine **24** followed by a reaction of triphenylphosphine then saponification. The reaction of **25** with 2-chlorobenzo[*d*]oxazole gave 4-methylthiopyridone **26**. 5-Methylthiopyridone **32** was synthesized as shown in Scheme 4. 3-Methoxypyridone **19** was reacted with *N*,*N*,*N*′,*N*′-tetramethylmethylenediamine to give amine **27**, which was converted to 5-methylthiopyridone **32** in a similar manner to **23**.

The synthesis of amide analogues is presented in Scheme 5. Ester **33** was allowed to react with *n*-butylamine to yield amide **34**, which was cyclized by 2-methyl-3-oxobutanal sodium salt to afford pyridone **35**. Saponification of **35** followed by Crutius reaction gave Cbz-protected 3-aminopyridone **37** that was deprotected by hydrogenation to afford 3-aminopyridone **38**. Acylation of **38** with various acid chlorides yielded the final compounds **39a-j**.

3. Results and discussion

3.1. Optimization of the pyridone-based lead compound 15a

Initial SAR around **15a** revealed that replacement of the carbonyl group of **15a** with thiocarbonyl group (**18a**) led to improvement in CB2 affinity (Table 1). We then investigated modification of the *N*-alkyl group attached to the thiopyridone ring. Extending or truncating the *n*-butyl group led to decreased binding affinity, as shown by compounds **18b-e**, suggesting that the lipophilicity of this region was important for the activity, but the space was rather limited. Finally, the *n*-butyl group was found to be optimal.

Next, we explored the effect of substitution on the thiopyridone ring (Table 2). Addition of a methyl group at the 4- and 5-positions led to **26** and **32**, which showed increased binding affinity for CB2 as compared with the unsubstituted analogue **23**. The optimal substituent was 5,6-dimethyl-thiopyridone **18a**. Thus, addition of the 5,6-dimethyl substituents to the pyridone ring provided a 5-fold increase in

binding potency compared with **23**. These observations indicated that lipophilicity on the pyridone ring is important for gaining CB2 affinity. Although we identified the optimized analogue *N*-*n*-butyl-5,6,-dimethylthiopyridone such as **18a**, its thiocarbonyl group was a concern due to its ability of covalent binding of proteins.[16] Therefore, we selected *N*-*n*-butyl-5,6-dimethylpyridone for further exploration.

Bioisosteric replacements of the benzoxazole group of **15a** with amide groups were investigated (Table 3). Introduction of the acetamide group (**39a**) resulted in a significant decrease in affinity, while phenyacetamide **39b** was equipotent with the corresponding benzoxazole analogue **15a**. We were pleased to observe that benzamide **39c** showed a 15-fold increase in CB2 affinity compared with **39b**, with a Ki value of 89 nM. Encouraged by this result, the effect of substitution on the phenyl ring was investigated. 2-Fluoro analogue **39d** was equipotent with unsubstituted **39c**, while introduction of a 2-chloro group (**39e**) led to an increase in affinity with a Ki value of 16 nM. Also, the 2-methyl analogue **39f** retained high affinity for CB2 with a 29-fold selectivity for CB1. Moving the methyl group to the *meta*- and *para*-position resulted in compounds **39g** and **39h** with a reduction in activity (2-fold and 5-fold, respectively), indicating that introduction of lipophilic substituents at the *ortho*-position was important for gaining CB2 affinity. 1-Naphthyl analog **39i** was equipotent with **39f**, but showed lower selectivity for CB2 over CB1 (1.6-fold). There was a marked decrease in CB2 potency as the substitution changed from 1-naphthyl **39i** to 2-naphthyl **39j**. Having identified the potent and selective analogue **39f**, its functional activity was assessed by measuring the maximal effects in reversing forkolin-evoked accumulation of cAMP in CHO cells expressing human CB2, **39f** was found to be a highly potent CB2 agonist with a cAMP IC_{50} value of 13 nM. Taken together, we identified **39f** as a potent and selective CB2 agonist, which had good lead profiles[17] with low molecular weight (MW = 312), low polar surface area (tPSA = 49Å^2),[18] moderate lipophilicity (CLog P = 3.6),[18] and high ligand efficiency (LE = 0.47).[19]

3.2. Putative binding mode of 39f in an active-state CB2 homology model

To gain insight into the molecular interactions of **39f** in CB2 receptor, we constructed an active-state CB2 homology model on MOE[25] using the crystal structure of the β_2 adrenergic receptor-Gs protein

complex.[20] Compound **39f** was docked into the CB2 model using ASEDock on MOE (Figure 3).[21] The model of **39f** bound to the CB2 receptor reveals some of the important binding interactions between the ligand and the receptor. The amide oxygen atom in **39f** forms a hydrogen bond with S193$^{5.42}$ (superscripts indicate Ballesteros-Weinstein numbers[27]). The tolyl ring displays a hydrophobic interaction with I110$^{3.29}$, T114$^{3.33}$, Y190$^{5.39}$, and F281$^{7.35}$. The pyridone ring lies in a hydrophobic pocket defined by F117$^{3.36}$, W194$^{5.43}$, and F197$^{5.46}$. The *N*-1 *n*-butyl chain fits well in a pocket formed by W258$^{6.48}$, V261$^{6.51}$, F281$^{7.35}$, and C288$^{7.42}$.

Replacement of the benzoxazole ring of **15a** with the amide group (**39f**) led to a 70-fold increase in CB2 affinity. This observation is explained by the hydrogen bond of the amide carbonyl of **39f** with S193$^{5.42}$, because the hydrogen bond basicity of the amide group is rather strong compared with the oxazole ring.[22] The *ortho*-methyl group on the amide phenyl ring played an important role in conferring the CB2 affinity. This methyl group is located in the lipophilic pocket defined by Y190$^{5.39}$ and F281$^{7.35}$, thus explaining the increase in activity (Figure 3 (b), (c)). On the other hand, transposition of the methyl group of **39f** from the *ortho*-position to the *meta*- or *para*-position (**39g** or **39h**) caused ~5-fold loss in affinity. Introduction of the *para*-methyl group on **39f** in our homology model results in a steric crash with residues in the second extracellular loop (ECL2) (Figure 3 (c)), which explains its loss of activity. This observation also explains why the 2-naphthyl derivative **39j** is significantly less potent.

As for the intracellular side of CB2, the 5,6-dimethyl groups on the pyridone ring of **39f** were directed into a hydrophobic pocket involving the hydrophobic residues F197$^{5.46}$ and I198$^{5.47}$ (Figure 3 (b), (c)). Installation of hydrophobic dimethyl groups on the pyridone ring led to a 4-fold increase of CB2 affinity (**18a** and **23**), supporting our proposed docking model. The pyridone ring itself displays a van der Waals interaction with hydrophobic residues W194$^{5.43}$ and F117$^{3.36}$ (Figure 3 (a), (b); F117$^{3.36}$ is not shown). This observation may explain why the more hydrophobic thiopyridone ring shows better affinity for CB2 (**15a** and **18a**). The *n*-butyl group on the pyridone ring fits well in the intracellular pocket, and the terminal carbon of the *n*-butyl group makes contact with W258$^{6.48}$ (Figure 3 (b), (c)), which explains why the *n*-butyl group is optimal.

4. Conclusion

A series of 2-pyridone-based potent and selective CB2 agonists was identified. Replacement of the 1,2-dihydroisoquinoline scaffold of **1** with 2-pyridone was performed to remove two asymmetric carbons and its lipophilic benzene ring. Introduction of various amide groups at the 3-position on the pyridone ring resulted in improved CB2 affinity, leading to the discovery of **39f**, which has good lead-like profiles: MW (312), tPSA (49Å^2), CLog P (3.6), and LE (0.47). Our active state CB2 homology model in complex with the optimized **39f** explained the SAR around this compound. The results of continued optimization and *in vivo* studies of its promising compounds will be presented in due course.

5. Experimental section

5.1. General chemistry information.

All commercial reagents and solvents were used as received unless otherwise noted. Thin layer chromatography (TLC) analysis was performed using Merck silica gel 60 F254 thin layer plates (250 μm thickness). Flash column chromatography was carried out using an automated purification system with Yamazen prepacked silica gel columns. 1H NMR spectra were recorded on a Varian Gemini 300 MHz. Spectral data are reported as follows: chemical shift (as ppm referenced to tetramethylsilane), multiplicity (s = singlet, d = doublet, dd = double doublet, dt = double triplet, t = triplet, q = quartet, m = multiplet, br = broad peak), coupling constant, and integration value. Analytical LC/MS was performed on a Waters X-Bridge (C18, 5 μm, 4.6 mm × 50 mm, a linear gradient from 10% to 100% B over 3 min and then 100% B for 1 min (A = H_2O + 0.1% formic acid, B = MeCN + 0.1% formic acid), flow rate 3.0 mL/min) using a Waters system equipped with a ZQ2000 mass spectrometer, 2525 binary gradient module, 2996 photodiode array detector (detection at 254 nm), and 2777 sample manager.

5.1.1. Ethyl 3-(benzylamino)-2-methylbut-2-enoate (8)

A flask with a Dean-Stark apparatus was charged with ethyl 2-methylacetoacetate (49.1 mL, 347 mmol), benzylamine (39.8 mL, 364 mmol, 1.05 equiv.), and toluene (250 mL). The mixture was heated to reflux for 10 h. After evaporation of toluene, the crude product was dried in vacuo to give compound

8 (84.2 g, 104%) as a yellow oil. ^{1}H NMR (300 MHz, CDCl3) δ 1.28 (t, *J* = 7.2 Hz, 3H), 1.80 (s, 3H), 1.93 (s, 3H), 4.13 (q, *J* = 7.2 Hz, 2H), 4.43 (d, *J* = 6.3 Hz, 2H), 7.20-7.40 (m, 5H), 9.65 (br s, 1H).

5.1.2. Ethyl 3-(*N*-benzyl-2-methoxyacetamido)-2-methylbut-2-enoate (9)

To a solution of **8** (83.2 g, 357 mmol) and 2-methoxyacetyl chloride (34.2 mL, 374 mmol, 1.05 equiv.) in ethyl ether (500 mL) was added pyridine (30.2 mL, 374 mmol, 1.05 equiv.) dropwise at such a rate that the internal temperature was did not rise above 5 °C. After completion of the addition, the reaction mixture was allowed to warm to room temperature, stirred for 3 h, and poured onto ice water. The aqueous layer was separated and extracted with ethyl ether. The combined organic extracts were washed with H_2O, saturated aqueous solution of $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and concentrated to give **9** (102 g, 94%) as an orange oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21 (t, *J* = 7.2 Hz, 3H), 1.74 (s, 3H), 1.89 (s, 3H), 3.44 (s, 3H), 3.97 (d, *J* = 14.7 Hz, 1H), 3.98 (q, *J* = 7.2 Hz, 2H), 4.12 (d, *J* = 14.7 Hz, 1H), 4.31 (d, *J* = 14.4 Hz, 1H), 4.95 (d, *J* = 14.4 Hz, 1H), 7.20-7.40 (m, 5H). LRMS (ESI) m/z calcd for $C_{17}H_{24}NO_4$ $[M+H]^+$ 306.2, found 306.2.

5.1.3. Benzyl-4-hydroxy-3-methoxy-5,6-dimethylpyridin-2(1*H*)-one (10)

To a solution of ethanol (19.8 mL, 340 mmol) in toluene (400 mL) was added sodium (7.82 g, 340 mmol) at room temperature and heated to reflux. After being refluxed, **9** (100.8 g, 330 mmol) in toluene (100 mL) was added dropwise to the refluxed reaction mixture over 60 min. The mixture was stirred at reflux for 2 h and allowed to cool to room temperature. The reaction was quenched by dropwise addition of 4M HCl (85 mL), and the resulting precipitate was collected by filtration and washed with toluene. The solid was diluted with $CHCl_3$ and 2 M HCl. The aqueous layer was separated and extracted with $CHCl_3$. The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was diluted with EtOAc and heated to reflux, and the resulting solid was collected on a glass filter to give **10** (47.3 g, 55%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.03 (s, 3H), 2.19 (s, 3H), 3.99 (s, 3H), 5.38 (br s, 2H), 6.41 (br s, 1H), 7.11-7.33 (m, 5H). LRMS (ESI) m/z calcd for $C_{15}H_{17}NO_3$ $[M+H]^+$ 260.1, found 260.1.

5.1.4. Benzyl-3-methoxy-5,6-dimethyl-4-(1-phenyl-1H-tetrazol-5-yloxy)pyridin-2(1*H*)-one (11)

To a suspension of **10** (25 g, 96 mmol) and K_2CO_3 (26.6 g 193 mmol, 2 equiv.) in DMF (160 mL) was added 5-chloro-1-phenyl-1*H*-tetrazole (20.9 g, 116 mmol, 1.2 equiv.) and stirred at room temperature for 24 h. The reaction mixture was diluted with H_2O and EtOAc, and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated. The resulting solid was washed with ethyl ether and hexane to give **11** (23.9 g, 61%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.07 (s, 3H), 2.28 (s, 3H), 3.79 (s, 3H), 5.41 (br s, 2H), 7.15-7.84 (m, 10H). LRMS (ESI) m/z calcd for $C_{22}H_{21}N_5O_3$ $[M+H]^+$ 404.2, found 404.2. mp 178°C.

5.1.5. 3-Methoxy-5,6-dimethylpyridin-2(1*H*)-one (12)

A mixture of **11** (27.2 g, 67.4 mmol) and Pd/C (10wt. % on carbon, 5.4 g) in DMF (272 mL) was stirred under 5 kg/cm^2 of H_2 for 48 h. The mixture was filtered on celite, and the filtrate was diluted with H_2O (160 mL) and heated to 85 °C. The resulting precipitate was removed by filtration and washed with hot water, and the filtrate was evaporated. The residue was diluted with acetone, and the resulting solid was collected on a glass filter to give compound **12** (8.23 g, 80%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.05 (s, 3H), 2.28 (s, 3H), 3.81 (s, 3H), 6.59 (s, 1H), 12.74 (s, 1H). mp 215–219°C.

5.1.6. Butyl-3-methoxy-5,6-dimethylpyridin-2(1*H*)-one (13a)

To a mixture of **12** (306 mg, 2.0 mmol) and potassium hydroxide (157 mg, 2.8 mmol, 1.4 equiv.) in 1-butanol (13 mL) was added 1-butyl iodide (0.44 mL, 3.86 mmol, 1.9 equiv.). The reaction mixture was heated to 85 °C for 24h and then evaporated. The residue was diluted with EtOAc and H_2O, and the separated aqueous layer was extracted with EtOAc. The combined organic layers were washed with H_2O, dried over $MgSO_4$, filtered, and evaporated. The crude product was purified by flash column chromatography (silica gel, toluene/acetone = 3/1) to give **13a** (124 mg, 30%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, J = 7.2 Hz, 3H), 1.36-1.48 (m, 2H), 1.60-1.70 (m, 2H), 2.09 (s, 3H), 2.26 (s, 3H), 3.78 (s, 3H), 4.08 (t, J = 7.8 Hz, 2H), 6.44 (s, 1H).

5.1.7. Butyl-3-hydroxy-5,6-dimethylpyridin-2(1*H*)-one (14a)

A mixture of **13a** (124 mg, 0.591 mmol) and pyridine hydrochloride (293 mg, 2.54 mmol, 4.3 equiv.) was heated to 200 °C and stirred for 30 min then cooled to room temperature. The reaction mixture was

diluted with ethyl ether and H_2O. The aqueous layer was separated, extracted with EtOAc, washed with H_2O, dried over $MgSO_4$, filtered, and concentrated to give **14a** (94 mg, 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, *J* = 7.2 Hz, 3H), 1.37-1.50 (m, 2H), 1.61-1.72 (m, 2H), 2.08 (s, 3H), 2.26 (s, 3H), 4.10 (t, *J* = 7.8 Hz, 2H), 6.66 (br s, 2H). mp 112–116°C.

5.1.8. 3-(Benzo[*d*]oxazol-2-yloxy)-1-butyl-5,6-dimethylpyridin-2(1*H*)-one (15a)

To a solution of **14a** (94 mg, 0.481 mmol) in DMF (4.1 mL) was added sodium hydride (60%; 24 mg, 0.75 mmol, 1.25 equiv.) at 0 °C. The suspension was allowed to warm to room temperature and stirred for 10min. To this suspension was added 2-chlorobenzo[*d*]oxazoles (125 mg, 1.42 mmol, 1.7 equiv.) at room temperature and stirred for an additional 2 h. The reaction was quenched with methanol and diluted with EtOAc and H_2O. The aqueous layer was separated, extracted with EtOAc, washed with H_2O, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (silica gel, toluene/acetone = 4/1) to give **15a** (100 mg, 67%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, *J* = 7.5 Hz, 3H), 1.35-1.48 (m, 2H), 1.62-1.72 (m, 2H), 2.15 (s, 3H), 2.35 (s, 3H), 4.10 (t, *J* = 7.8 Hz, 2H), 7.19-7.25 (m, 2H), 7.33 (s, 1H), 7.38-7.42 (m, 1H), 7.46-7.49 (m, 1H). mp 106–108°C. Anal. Calcd for $C_{18}H_{20}N_2O_3$: C, 69.21; H, 6.45; N, 8.97. Found C, 69.27; H, 6.61; N, 8.97.

5.1.9. Butyl-3-methoxy-5,6-dimethylpyridine-2(1*H*)-thione (16a)

A mixture of **13a** (222 mg, 1.06 mmol) and Lawesson's reagent (502 mg, 1.24 mmol, 1.2 equiv.) in toluene (8 mL) was heated to reflux and stirred for 7 h. The mixture was allowed to cool to room temperature, diluted with methanol (25 mL), and stirred for additional 1 h at room temperature. The reaction mixture was evaporated and purified by flash chromatography (silica gel, toluene/acetone = 4/1) to give **16a** (177 mg, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (t, *J* = 7.2 Hz, 3H), 1.43-1.55 (m, 2H), 1.70-1.95 (br s, 2H), 2.22 (s, 3H), 2.46 (s, 3H), 3.89 (s, 3H), 4.90 (br s, 2H), 6.54 (s, 1H). mp 111–112°C.

5.1.10. Butyl-3-hydroxy-5,6-dimethylpyridine-2(1*H*)-thione (17a)

Compound **17a** was prepared in a similar manner as **14a** after substituting **16a** for **13a**. The desired product **17a** was isolated in 74% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (t, *J* = 7.2 Hz, 3H), 1.45-

1.57 (m, 2H), 1.70-1.90 (m, 2H), 2.21 (s, 3H), 2.45 (s, 3H), 4.72 (br s, 2H), 6.87 (s, 1H), 8.44 (br s, 1H). mp 81–88°C.

5.1.11. 3-(Benzo[*d*]oxazol-2-yloxy)-1-butyl-5,6-dimethylpyridine-2(1*H*)-thione (18a)

Compound **18a** was prepared in a similar manner as **15a** after substituting **17a** for **14a**. The desired product **18a** was isolated in 46% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (t, J = 7.2 Hz, 3H), 1.42-1.54 (m, 2H), 1.83 (br s, 2H), 2.25 (s, 3H), 2.53 (s, 3H), 4.80 (br s, 2H), 7.18-7.26 (m, 2H), 7.36 (s, 1H), 7.42-7.49 (m, 2H).mp 185–187°C. Anal. Calcd for $C_{18}H_{20}N_2O_2S$: C, 65.83; H, 6.14; N, 8.53; S, 9.76. Found C, 65.53; H, 6.07; N, 8.45; S, 9.50.

5.1.12. Ethyl-3-methoxy-5,6-dimethylpyridin-2(1*H*)-one (13b)

Compound **13b** was prepared in a similar manner as **13a** after substituting ethyl iodide for 1-butyl iodide. The desired product **13b** was isolated in 42% yield. LRMS (ESI) m/z calcd for $C_{10}H_{16}NO_2$ $[M+H]^+$ 182, found 182.

5.1.13. Ethyl-3-methoxy-5,6-dimethylpyridine-2(1*H*)-thione (16b)

Compound **16b** was prepared in a similar manner as **16a** after substituting **13b** for **13a**. The desired product **16b** was isolated in 71% yield. LRMS (ESI) m/z calcd for $C_{10}H_{16}NOS$ $[M+H]^+$ 198, found 198. mp 111–113°C.

5.1.14. Ethyl-3-hydroxy-5,6-dimethylpyridine-2(1*H*)-thione (17b)

Compound **17b** was prepared in a similar manner as **14a** after substituting **16b** for **13a**. The desired product **17b** was isolated in 90% yield. LRMS (ESI) m/z calcd for $C_9H_{14}NOS$ $[M+H]^+$ 184, found 184. mp 42–70°C.

5.1.15. 3-(Benzo[*d*]oxazol-2-yloxy)-1-ethyl-5,6-dimethylpyridine-2(1*H*)-thione (18b)

Compound **18b** was prepared in a similar manner as **15a** after substituting **17b** for **14a**. The desired product **18b** was isolated in 50% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (t, J = 7.2 Hz, 3H), 2.25 (s, 3H), 2.55 (s, 3H), 4.92 (br s, 2H), 7.18-7.24 (m, 2H), 7.37 (s, 1H), 7.42-7.49 (m, 2H). mp 190–191°C. Anal calcd for $C_{16}H_{16}N_2O_2S$: C, 63.98; H, 5.37; N, 9.33; S, 10.67. Found: C, 63.57; H, 5.33; N, 9.22; S, 10.53.

5.1.16. 3-(Benzo[*d*]oxazol-2-yloxy)-5,6-dimethyl-1-propylpyridine-2(1*H*)-thione (18c)

Compound **18c** was prepared in a similar manner as **15a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04 (t, *J* = 7.2 Hz, 3H), 1.89 (br s, 2H), 2.25 (s, 3H), 2.52 (s, 3H), 4.71 (br s, 2H), 7.19-7.26 (m, 2H), 7.36 (s, 1H), 7.42-7.49 (m, 2H). LRMS (ESI) calcd for $C_{17}H_{19}N_2O_2S$ $[M+H]^+$ 314. Found: 314.

5.1.17. 3-Methoxy-5,6-dimethyl-1-pentylpyridin-2(1*H*)-one (13d)

Compound **13d** was prepared in a similar manner as **13a** after substituting 1-pentyl iodide for 1-butyl iodide. The desired product **13d** was isolated in 36% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88-0.93 (m, 3H), 1.35-1.40 (m, 4H), 1.64-1.66 (m, 2H), 2.09 (s, 3H), 2.25 (s, 3H), 3.78 (s, 3H), 4.07 (t, *J* = 7.9 Hz, 2H), 6.44 (s, 1H).

5.1.18. 3-Methoxy-5,6-dimethyl-1-pentylpyridine-2(1*H*)-thione (16d)

Compound **16d** was prepared in a similar manner as **16a** after substituting **13d** for **13a**. The desired product **16d** was isolated in 97% yield. LRMS (ESI) m/z calcd for $C_{13}H_{22}NOS$ $[M+H]^+$ 240, found 240. mp 82–83°C.

5.1.19. 3-Hydroxy-5,6-dimethyl-1-pentylpyridine-2(1*H*)-thione (17d)

Compound **17d** was prepared in a similar manner as **14a** after substituting **16d** for **13a**. The desired product **17d** was isolated in 79% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93-0.97 (m, 3H), 1.42-1.47 (m, 4H), 1.86 (br s, 2H), 2.21 (s, 3H), 2.45 (s, 3H), 4.71 (br s, 2H), 6.87 (s, 1H), 8.44 (s, 1H). mp 62–68°C.

5.1.20. 3-(Benzo[*d*]oxazol-2-yloxy)-5,6-dimethyl-1-pentylpyridine-2(1*H*)-thione (18d)

Compound **18d** was prepared in a similar manner as **15a** after substituting **17d** for **14a**. The desired product **18d** was isolated in 64% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, *J* = 7.2 Hz, 3H), 1.36-1.48 (m, 4H), 1.85 (br s, 2H), 2.25 (s, 3H), 2.53 (s, 3H), 2.53 (s, 3H), 4.76 (br s, 2H), 7.18-7.26 (m, 2H), 7.36 (s, 1H), 7.42-7.49 (m, 2H). mp 149–150°C. Anal. Calcd for $C_{19}H_{22}N_2O_2S$: C, 66.64; H, 6.48; N, 8.18;S, 9.36. Found C, 66.65; H, 6.58; N, 8.18; S, 9.33.

5.1.21. Hexyl-3-methoxy-5,6-dimethylpyridin-2(1*H*)-one (13e)

Compound **13e** was prepared in a similar manner as **13a** after substituting 1-hexyl iodide for 1-butyl iodide. The desired product **13e** was isolated in 37% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, *J* =

7.0 Hz, 2H), 1.31-1.44 (m, 6H), 1.63-1.73 (m, 2H), 2.12 (s, 3H), 2.28 (s, 3H), 3.81 (s, 3H), 4.10 (t, *J* = 7.9 Hz, 2H), 6.46 (s, 1H)..

5.1.22. Hexyl-3-methoxy-5,6-dimethylpyridine-2(1*H*)-thione (16e)

Compound **16e** was prepared in a similar manner as **16a** after substituting **13e** for **13a**. The desired product **16e** was isolated in 70% yield. LRMS (ESI) m/z calcd for $C_{14}H_{24}NOS$ $[M+H]^+$ 254, found 254. mp 76–77°C.

5.1.23. Hexyl-3-hydroxy-5,6-dimethylpyridine-2(1*H*)-thione (17e)

Compound **17e** was prepared in a similar manner as **14a** after substituting **16e** for **13a**. The desired product **17e** was isolated in 91% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, *J* = 7.0 Hz, 3H), 1.38-1.52 (m, 6H), 1.88 (br s, 2H), 2.23 (s, 3H), 2.48 (s, 3H), 4.73 (br s, 2H), 6.90 (s, 1H). mp 43–48°C.

5.1.24. 3-(Benzo[*d*]oxazol-2-yloxy)-1-hexyl-5,6-dimethylpyridine-2(1*H*)-thione (18e)

Compound **18e** was prepared in a similar manner as **15a** after substituting **17e** for **14a**. The desired product **18e** was isolated in 60% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, *J* = 7.2 Hz, 3H), 1.30-1.50 (m, 6H), 1.84 (br s, 2H), 2.25 (s, 3H), 2.52 (s, 3H), 4.79 (br s, 2H), 7.17-7.26 (m, 2H), 7.35 (s, 1H), 7.42-7.49 (m, 2H). mp 116–117°C. Anal. calcd for $C_{20}H_{24}N_2O_2S$: C, 67.38; H, 6.79; N, 7.86; S, 8.99. Found: C, 67.16; H, 6.70; N, 7.88; S, 8.91.

5.1.25. Butyl-3-methoxypyridin-2(1*H*)-one (20)

To a solution of 3-methoxypyridin-2(1*H*)-one **19** (5.0 g, 40 mmol) in DMF (40 mL) was added sodium hydride (60%, 2.24 g, 56 mmol, 1.4 equiv.) at 0 °C. The reaction mixture was stirred at room temperature, and 1-butyl iodide (9.6 mL, 84 mmol, 2.1 equiv.) was added to the reaction mixture. The mixture was stirred for an additional 40 min. The mixture was poured onto ice water and extracted with CHCl3, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (silica gel, $CHCl_3$/MeOH = 50/1) to give **20** (6.29 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 (t, *J* = 7.2 Hz, 2H), 1.30-1.42 (m, 2H), 1.68-1.78 (m, 2H), 3.81 (s, 3H), 3.97 (t, *J* = 7.2 Hz, 2H), 6.09 (t, *J* = 7.2 Hz, 1H), 6.59 (dd, *J* = 7.2, 1.5 Hz, 1H), 6.88 (dd, *J* = 7.2, 1.5 Hz, 1H).

5.1.26. Butyl-3-methoxypyridine-2(1*H*)-thione (21)

A mixture of **20** (6.44 g, 35.5 mmol) and Lawesson's reagent (16.8 g, 41.6 mmol, 1.2 equiv.) in toluene (180 mL) was heated to reflux for 3 h. The reaction mixture was allowed to cool to room temperature, added methanol (100 mL), and concentrated. The residue was purified by flash column chromatography (silica gel, toluene/acetone = 4/1) to give **21** (5.6 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, *J* = 7.3 Hz, 3H), 1.36-1.48 (m, 2H), 1.84-1.94 (m, 2H), 3.92 (s, 3H), 4.62 (t, *J* = 7.6 Hz, 2H), 6.61 (dd, *J* = 7.9, 6.2 Hz, 1H), 6.69 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.38 (dd, *J* = 6.2, 1.2 Hz, 1H).

5.1.27. Butyl-3-hydroxypyridine-2(1*H*)-thione (22)

Compound **22** was prepared in a similar manner as **14a** after substituting **21** for **13a**. The desired product **22** was isolated in 78% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (t, *J* = 7.3 Hz, 3H), 1.43 (m, 2H), 1.91 (m, 2H), 4.53 (t, *J* = 7.6 Hz, 2H), 6.66 (dd, *J* = 7.6, 6.7 Hz, 1H), 6.97 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.34 (dd, *J* = 6.7, 1.2 Hz, 1H), 8.61 (br s, 1H).

5.1.28. 3-(Benzo[*d*]oxazol-2-yloxy)-1-butylpyridine-2(1*H*)-thione (23)

Compound **23** was prepared in a similar manner as **15a** after substituting **22** for **14a**. The desired product **23** was isolated in 96% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, *J* = 7.3 Hz, 3H), 1.37-1.49 (m, 2H), 1.83-1.94 (m, 2H), 4.57 (t, *J* = 7.6 Hz, 2H), 6.65-6.70 (m, 1H), 7.22-7.27 (m, 2H), 7.43-7.51 (m, 3H), 7.68 (dd, *J* = 6.4, 1.5 Hz, 1H). Anal calcd for $C_{16}H_{16}N_2O_2S$: C, 63.98; H, 5.37; N, 9.33, S, 10.67. Found: C, 63.83; H, 5.31; N, 9.32; S, 10.57. IR (KBr): 2966, 1620, 1574, 1537, 1319, 1207, 1138, 1041.

5.1.29. Butyl-4-((dimethylamino)methyl)-3-hydroxypyridine-2(1*H*)-thione (24)

A mixture of **22** (1.0 g, 5.51 mmol) and *N*,*N*,*N*',*N*'-tetramethylmethylenediamine (1.70 g, 16.6 mmol, 3 equiv.) in ethanol/H_2O (20 mL/2 mL) was heated to 75 °C for 24 h. The reaction mixture was concentrated to give **24** (1.26 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (t, *J* = 7.3 Hz, 3H), 1.39-1.47 (m, 2H), 1.86-1.93 (m, 2H), 2.29 (s, 6H), 3.48 (s, 2H), 4.51 (t, *J* = 7.3Hz, 2H), 6.87 (d, *J* = 6.7 Hz, 1H), 7.32 (d, *J* = 6.7 Hz, 1H).

5.1.30. Butyl-3-hydroxy-4-methylpyridine-2(1*H*)-thione (25)

To a solution of **24** (1.0 g, 4.16 mmol) in DCM (17 mL) was added methyl iodide (0.91 mL, 14.5 mmol, 3.5 equiv.) and stirred at room temperature for 1h. The reaction mixture was evaporated and diluted with ethanol (10 mL). To this solution was added triphenyl phosphine (1.6 g, 6.1 mmol. 1.5 equiv.) and heated to 75 °C overnight. The mixture was evaporated, diluted with methanol (10 mL) and 1M NaOH (8 mL), and heated to 60 °C for 2 h. The reaction mixture was evaporated, and the residue was purified by flash chromatography (silica gel, toluene/acetone = 4/1) to give **25** (0.57 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, *J* = 7.3 Hz, 3H), 1.36-1.48 (m, 2H), 1.84-1.94 (m, 2H), 2.25 (s, 3H), 4.50 (t, *J* = 7.6 Hz, 2H), 6.55 (d, *J* = 6.7 Hz, 1H), 7.25 (d, *J* = 6.7 Hz, 1H), 8.67 (s, 1H).

5.1.31. 3-(Benzo[*d*]oxazol-2-yloxy)-1-butyl-4-methylpyridine-2(1*H*)-thione (26)

Compound **26** was prepared in a similar manner as **15a** after substituting **25** for **14a**. The desired product **26** was isolated in 29% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, *J* = 7.3 Hz, 3H), 1.34-1.46 (m, 2H), 1.79-1.90 (m, 2H), 2.29 (s, 3H), 4.51 (t, *J* = 7.4 Hz, 2H), 6.55 (d, *J* = 6.6 Hz, 1H), 7.20-7.28 (m, 2H), 7.43-7.48 (m, 2H), 7.59 (d, *J* = 6.6 Hz, 1H). Anal. calcd for $C_{17}H_{18}N_2$: C, 64.94; H, 5.77; N, 8.91. Found: C, 64.47; H, 5.58; N, 8.85.

5.1.32. 5-((Dimethylamino)methyl)-3-methoxypyridin-2(1*H*)-one (27)

Compound **27** was prepared in a similar manner as **24** after substituting **19** for **22**. The desired product **27** was isolated in 53% yield after flash column chromatography (silica gel, $CHCl_3$/methanol/H_2O = 6/4/1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.21 (s, 6H), 3.17 (s, 2H), 3.87 (s, 3H), 6.86 (d, *J* = 1.8 Hz, 1H), 6.90 (d, *J* = 1.8 Hz, 1H).

5.1.33. 3-Methoxy-5-methylpyridin-2(1*H*)-one (28)

Compound **28** was prepared in a similar manner as **25** after substituting **27** for **24**. The desired product **28** was isolated in 71% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.21 (d, *J* = 1.2 Hz, 3H), 3.84 (s, 3H), 6.62 (d, *J* = 2.1 Hz, 1H), 6.80 (dd, *J* = 2.1, 1.2 Hz, 1H).

5.1.34. Butyl-3-methoxy-5-methylpyridin-2(1*H*)-one (29)

Compound **29** was prepared in a similar manner as **20** after substituting **28** for **19**. The desired product **29** was isolated in 63% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, *J* = 7.3 Hz, 3H), 1.29-1.42 (m, 2H),

1.66-1.76 (m, 2H), 2.08 (d, *J* = 1.2 Hz, 3H), 3.80 (s, 3H), 3.92 (t, *J* = 7.3 Hz, 2H), 6.45 (d, *J* = 1.2 Hz, 1H), 6.65 (dd, *J* = 2.1, 1.2 Hz, 1H).

5.1.35. Butyl-3-methoxy-5-methylpyridine-2(1*H*)-thione (30)

Compound **30** was prepared in a similar manner as **16a** after substituting **29** for **13a**. The desired product **30** was isolated in 100% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, *J* = 7.4 Hz, 3H), 1.35-1.48 (m, 2H), 1.83-1.93 (m, 2H), 2.21 (s, 3H), 3.91 (s, 3H), 4.59 (t, *J* = 7.7 Hz, 2H), 6.55 (s, 1H), 7.21 (s, 1H).

5.1.36. Butyl-3-hydroxy-5-methylpyridine-2(1*H*)-thione (31)

Compound **31** was prepared in a similar manner as **14a** after substituting **30** for **13a**. The reaction was performed at 160°C rather than 200°C. The desired product **31** was isolated in 76% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (t, *J* = 7.3 Hz, 3H), 1.37-1.50 (m, 2H), 1.85-1.95 (m, 2H), 2.19 (d, *J* = 0.9 Hz, 3H), 4.49 (t, *J* = 7.6 Hz, 2H), 6.86 (d, *J* = 1.2 Hz, 1H), 7.16 (dd, *J* = 1.9, 0.9 Hz, 1H), 8.55 (s, 1H).

5.1.37. 3-(Benzo[*d*]oxazol-2-yloxy)-1-butyl-5-methylpyridine-2(1*H*)-thione (32)

Compound **32** was prepared in a similar manner as **15a** after substituting **31** for **14a**. The desired product **32** was isolated in 78% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, *J* = 7.3 Hz, 3H), 1.36-1.46 (m, 2H), 1.82-1.92 (m, 2H), 4.54 (t, *J* = 7.6 Hz, 2H), 7.19-7.27 (m, 2H), 7.40-7.52 (m, 4H). mp 146.0–146.5°C. Anal. calcd for $C_{17}H_{18}N_2O_2S$: C, 64.94; H, 5.77; N, 8.91; S, 10.20. Found: C, 65.06; H, 5.77; N, 8.90; S, 10.23. IR (KBr): 3053, 2954, 1624, 1576, 1530, 1321, 1240. 1172, 1093.

5.1.38. *N*-Butyl-2-cyanoacetamide (34)

n-Butyl amine (43.7 mL, 442 mmol) was added dropwise to ethyl 2-cyanoacetate **33** (47 mL, 442 mmol) over 10 min at room temperature. The reaction mixture was stirred for 30 min and allowed to cool to 0 °C. The resulting suspension was diluted with hexane and ethyl ether, and resulting solid was collected on a glass filter to give **34** (46.6 g, 75%) as a white solid. mp 71–72°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, *J* = 7.3 Hz, 3H), 1.31-1.43 (m, 2H), 1.49-1.59 (m, 2H), 3.31 (dd, *J* = 13.0, 7.0 Hz, 2H), 3.37 (s, 2H), 6.14 (br s, 1H).

5.1.39. Butyl-5,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (35)

To a solution of **34** (2.8 g, 20 mmol), piperidine (396 μL, 4.0 mmol, 0.2 equiv.), and acetic acid (1.49 mL, 26 mmol, 1.3 equiv.) in DMF (28 mL) was added 2-methyl-3-oxobutanal sodium salt (3.2 g, 26 mmol, 1.3 equiv.). The reaction mixture was heated to 135 °C for 9 h, allowed to cool to room temperature, and diluted with EtOAc and H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (silica gel, EtOAc/hexane 10-100% gradient) to give **35** (3.74 g, 92%) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, J = 7.3 Hz, 3H), 1.37-1.47 (m, 2H), 1.60-1.71 (m, 2H), 2.12 (s, 3H), 2.41 (s, 3H), 4.09 (t, J = 8.0 Hz, 2H), 7.57 (s, 1H). LRMS (ESI) m/z calcd for $C_{12}H_{17}N_2O$ $[M+H]^+$ 205.1, found 205.1.

5.1.40. Butyl-5,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carboxylic acid (36)

A mixture of **35** (3.39 g, 16.6 mmol) and potassium hydroxide (85%; 8.76 g, 133 mmol, 8 equiv.) in ethanol/H_2O (40 mL/10 mL) was heated to reflux and stirred for 8 h. The mixture was allowed to cool to room temperature and diluted with ethyl ether and H_2O. The aqueous layer was washed with ethy ether and acidified with concentrated HCl. The resulting solid was collected on a glass filter and dried to give **36** (2.55 g, 69%) as a white solid. mp 105–106°C ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.93 (t, J = 7.2 Hz, 3H), 1.34-1.44 (m, 2H), 1.55-1.65 (m, 2H), 2.19 (s, 3H), 2.49 (s, 3H), 4.16 (t, J = 7.9 Hz, 2H), 8.19 (s, 1H). LRMS (ESI) m/z calcd for $C_{12}H_{18}NO_3$ $[M+H]^+$ 223.1. Found 223.1.

5.1.41. Benzyl 1-butyl-5,6-dimethyl-2-oxo-1,2-dihydropyridin-3-ylcarbamate (37)

To a solution of **36** (2.0 g, 8.96 mmol) and triethylamine (3.73 mL, 26.9 mmol, 3 equiv.) in dioxane (20 mL) was added diphenylphosphoryl azide (2.12 mL, 9.85 mmol, 1.1 equiv.). The reaction mixture was heated to reflux for 2 h and then allowed to cool to room temperature. To this solution was added benzyl alcohol (1.02 mL, 9.85 mmol, 1.1 equiv.) and heated to reflux for additional 2 h. The mixture was cooled and diluted with EtOAc and H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (silica gel, EtOAc/hexane 10-80% gradient) to give **37** (2.77 g, 94%) as a yellow oil. mp 65–66°C. ^{1}H

NMR (300 MHz, $CDCl_3$) δ 0.97 (t, *J* = 7.2 Hz, 3H), 1.39-1.44 (m, 2H), 1.58-1.69 (m, 2H), 2.10 (s, 3H), 2.28 (s, 3H), 4.05-4.13 (m, 2H), 5.19 (s, 2H), 7.28-7.39 (m, 5H), 7.83 (s, 1H), 7.85 (s, 1H). LRMS (ESI) m/z calcd for $C_{19}H_{25}N_2O_3$ $[M+H]^+$ 329.2. Found 329.2.

5.1.42. 3-Amino-1-butyl-5,6-dimethylpyridin-2(1*H*)-one (38)

To a solution of **37** (2.77 g, 8.43 mol) in methanol (200 mL) was added 10% Pd/C (359 mg). The mixture was degassed and then charged with hydrogen. The suspension was stirred under hydrogen for 3 h and then filtered through celite, washed with methanol, and concentrated. The residue was purified by flash column chromatography (silica gel, EtOAc/hexane 20-100% gradient) to give **38** (1.37 mg, 84%) as a yellow solid. mp 94–97°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, *J* = 7.3 Hz, 3H), 1.43 (td, *J* = 14.9, 7.4 Hz, 2H), 1.63-1.68 (m, 2H), 2.03 (s, 3H), 2.24 (s, 3H), 3.79 (s, 2H), 4.07 (t, *J* = 7.9 Hz, 2H), 6.42 (s, 1H).

5.1.43. *N*-(1-Butyl-5,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)acetamide (39a)

To a solution of **38** (98 mg, 0.504 mmol) in pyridine (1 mL) was added acetyl chloride (40 μL, 0.555 mmol, 1.1 equiv.) at 0 °C, and stirred for 30 min. The mixture was diluted with saturated aqueous $NaHCO_3$ and EtOAc. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with 1M HCl, H_2O and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (silica gel, EtOAc/hexane 10-70% gradient) to give **39a** (108 mg, 91%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, *J* =7.5 Hz, 3H), 1.37-1.50 (m, 2H), 1.60-1.70 (m, 2H), 2.12 (s, 3H), 2.17 (s, 3H), 2.30 (s, 3H), 4.10 (t, *J* = 7.8 Hz, 2H), 8.20 (s, 1H), 8.35 (br s, 1H). Anal. calcd for $C_{13}H_{20}N_2O_2$: C, 66.07; H, 8.53; N, 11.85; O, 13.54. Found: C, 65.93; H, 8.55; N, 11.78.

5.1.44. *N*-(1-Butyl-5,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)-2-phenylacetamide (39b)

Compound **39b** was prepared in a similar manner as **39a** after substituting phenylacetyl chloride for acetyl chloride. The desired product **39b** was isolated in 40% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, *J* = 7.2 Hz, 3H), 1.35-1.47 (m, 2H), 1.57-1.67 (m, 2H), 2.09 (s, 3H), 2.28 (s, 3H), 3.72

(s, 2H), 4.05 (t, *J* = 7.8 Hz, 2H), 7.28-7.40 (m, 5H), 8.22 (s, 1H), 8.40 (br s, 1H). Anal. calcd for $C_{19}H_{24}N_2O_2$: C, 73.05; H, 7.74; N, 8.97; Found: C, 72.88; H, 8.84; N, 9.05.

5.1.45. *N*-(1-Butyl-5,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)benzamide (39c)

Compound **39c** was prepared in a similar manner as **39a** after substituting benzoyl chloride for acetyl chloride. The desired product **39c** was isolated in 92% yield as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (t, *J* = 7.2 Hz, 3H), 1.40-1.52 (m, 2H), 1.64-1.74 (m, 2H), 2.17 (s, 3H), 2.34 (s, 3H), 4.14 (t, *J* = 7.8 Hz, 2H), 7.44-7.57 (m, 3H), 7.92-7.95 (m, 2H), 8.41 (s, 1H), 9.22 (br s, 1H). Anal. calcd for $C_{18}H_{22}N_2O_2$: C, 72.46; H, 7.43; N, 9.39; Found C, 72.74; H, 7.61; 9.60.

5.1.46. *N*-(1-Butyl-5,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)-2-fluorobenzamide (39d)

Compound **39d** was prepared in a similar manner as **39a** after substituting 2-fluorobenzoyl chloride for acetyl chloride. The desired product **39d** was isolated in 93% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (t, *J* = 7.2 Hz, 3H), 1.39-1.52 (m, 2H), 1.64-1.74 (m, 2H), 2.16 (s, 3H), 2.34 (s, 3H), 4.13 (t, *J* = 7.8 Hz, 2H), 7.15-7.24 (m, 1H), 7.30 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.47-7.54 (m, 1H), 8.12 (dt, 7.8, 1.8 Hz, 1H), 8.42 (s, 1H), 9.75 (br s, 1H). Anal. calcd for $C_{18}H_{21}FN_2O_2$: C, 68.34; H, 6.69; N, 8.85; F, 6.01. Found: C, 68.33; H, 6.71; N, 8.87; F, 5.96. LRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{18}H_{22}FN_2O_2$, 317.2; Found, 317.2.

5.1.47. *N*-(1-Butyl-5,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)-2-chlorobenzamide (39e)

Compound **39e** was prepared in a similar manner as **39a** after substituting 2-chlorobenzoyl chloride for acetyl chloride. The desired product **39e** was isolated in 79% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, *J* = 7.2 Hz, 3H), 1.38-1.50 (m, 2H), 1.64-1.72 (m, 2H), 2.17 (s, 3H), 2.34 (s, 3H), 4.11 (t, *J* = 7.8 Hz, 2H), 7.31-7.47 (m, 3H), 7.73 (dd, *J* = 7.2, 2.1 Hz, 1H), 8.41 (s, 1H), 9.13 (br s, 1H). Anal. calcd for $C_{18}H_{21}ClN_2O_2$: C, 64.96; H, 6.36; N, 8.42; Cl, 10.65. Found: C, 65.01; H, 6.42; N, 8.51; Cl, 10.47.

5.1.48. *N*-(1-Butyl-5,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)-2-methylbenzamide (39f)

Compound **39f** was prepared in a similar manner as **39a** after substituting 2-methylbenzoyl chloride for acetyl chloride. The desired product **39f** was isolated in 88% yield as a white solid. ^{1}H NMR (300

MHz, $CDCl_3$) δ 0.98 (t, *J* = 7.2 Hz, 3H), 1.38-1.50 (m, 2H), 1.61-1.72 (m, 2H), 2.17 (s, 3H), 2.34 (s, 3H), 2.53 (s, 3H), 4.11 (t, *J* = 7.8 Hz, 2H), 7.20-7.26 (m ,2H), 7.32-7.37 (m, 1H), 7.54 (d, *J* = 7.8 Hz, 1H), 8.39 (s, 1H), 8.74 (br s, 1H). Anal. calcd for $C_{19}H_{24}N_2O_2$: C, 73.05; H, 7.74; N, 8.97. Found: C, 72.43; H, 7.76; N, 8.96. LRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{19}H_{25}N_2O_2$, 313.2; Found, 313.2.

5.1.49. *N*-(1-Butyl-5,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)-3-methylbenzamide (39g)

Compound **39g** was prepared in a similar manner as **39a** after substituting 3-methylbenzoyl chloride as acetyl chloride. The desired product **39g** was isolated in 95% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (t, *J* = 7.2 Hz, 3H), 1.40-1.52 (m, 2H), 1.64-1.74 (m, 2H), 2.17 (s, 3H), 2.34 (s, 3H), 2.42 (s, 3H), 4.13 (t, *J* = 7.8 Hz, 2H), 7.35 (m, 2H), 7.74 (m, 2H), 8.41 (s, 1H), 9.21 (br s, 1H). Anal. calcd for $C_{19}H_{24}N_2O_2$: C, 73.05; H, 7.74; N, 8.97. Found: C, 72.86; H, 7.87; N, 9.06.

5.1.50. *N*-(1-Butyl-5,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)-4-methylbenzamide (39h)

Compound **39h** was prepared in a similar manner as **39a** after substituting 4-methylbenzoyl chloride for acetyl chloride. The desired product **39h** was isolated in 90% yield as a white solid ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (t, *J* = 7.2 Hz, 3H), 1.40-1.52 (m, 2H), 1.64-1.74 (m, 2H), 2.16 (s, 3H), 2.34 (s, 3H), 2.41 (s, 3H), 4.13 (t, *J* = 7.8 Hz, 2H), 7.27 (d, *J* = 8.1 Hz, 2H), 7.84 (d, *J* = 8.1 Hz, 2H), 8.40 (s, 1H), 9.20 (br s, 1H). Anal. calcd for $C_{19}H_{24}N_2O_2$: C, 73.05; H, 7.74; N, 8.97. Found: C, 72.70; H, 7.81; N, 9.07.

5.1.51. *N*-(1-Butyl-5,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)-1-naphthamide (39i)

Compound **39i** was prepared in a similar manner as **39a** after substituting 1-naphthoyl chloride for acetyl chloride. The desired product **39i** was isolated in 89% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, *J* = 7.2 Hz, 3H), 1.38-1.50 (m, 2H), 1.62-1.73 (m, 2H), 2.20 (s, 3H), 2.36 (s, 3H), 4.12 (t, *J* = 7.8 Hz, 2H), 7.46-7.59 (m, 3H), 7.79 (dd, *J* = 7.2, 1.2 Hz, 1H), 7.88 (dd, *J* = 7.2, 1.5 Hz, 1H), 7.95 (d, *J* = 8.1 Hz, 1H), 8.45 (dd, *J* = 7.5, 1.5 Hz, 1H), 8.50 (s, 1H), 8.95 (br s, 1H). Anal. calcd for $C_{22}H_{24}N_2O_2$: C, 75.83; H, 6.94; N, 8.04. Found: C, 75.64; H, 7.07; N, 7.98.

5.1.52. *N*-(1-Butyl-5,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)-2-naphthamide (39j)

Compound **39j** was prepared in a similar manner as **39a** after substituting 2-naphthoyl chloride for acetyl chloride. The desired product **39j** was isolated in 89% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (t, J = 7.2 Hz, 3H), 1.42-1.54 (m, 2H), 1.66-1.76 (m, 2H), 2.19 (s, 3H), 2.36 (s, 3H), 4.16 (t, *J* = 7.8 Hz, 2H), 7.53-7.62 (m, 2H), 7.88-8.03 (m, 4H), 8.47 (s, 2H), 9.41 (br s, 1H). Anal. calcd for $C_{22}H_{24}N_2O_2$: C, 75.83; H, 6.94; N, 8.04. Found: C, 75.56; H, 6.99; N, 8.03.

5.2. Homology model of active CB2 receptor and docking of 39f

The homology model of an active CB2 receptor was constructed using the β_2 adrenergic receptor-Gs protein complex (PDB ID: 3SN6) as a template. The human CB2 receptor sequence and the β_2 adrenergic receptor were aligned based on existing information on conserved residues within class A G protein-coupled receptors.[28] Amino acids 30-315 of CB2 were used for the modeling, and the residues of T4L, Gαs, Gb, Gg, and NB35 in the β_2 adrenergic receptor-Gs protein complex were omitted. The alignment and model construction were carried out using MOE[25] according to its manual. The docking of **39f** with the CB2 homology model was performed using ASEDock[21] on MOE. The default parameter setting was used for the docking according to its own manual.[21(b)]

5.3. Biological assays

The binding assay of the compounds reported in this paper was evaluated at recombinant human CB1 and CB2 receptors stably expressed in Chinese hamster ovary (CHO) cell lines through competition binding against [H^3]-CP-55,940.[14a,b] The functional activities of selected compounds were assessed by measuring their maximal effects in reversing the forkolin-evoked accumulation of cAMP in CHO cells expressing human CB2.[14c] The CB1/CB2 Binding assay and cell-based cAMP assay were performed as described in ref. 26 (a).

Acknowledgements

We thank Naoki Yoshikawa for early input into this work and useful discussions and Rikio Ikenishi and Yumiko Takagi for the elemental analysis. K.K. also acknowledges Robert Hall for his assistance in preparing this manuscript.

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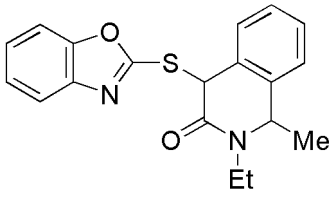
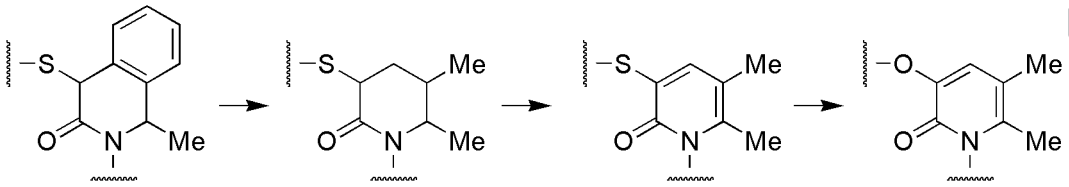


1

CB2 Ki = 42 nM
CB1 Ki = >5000 nM

Figure 1. Screening hit **1**.



A **B** **C**

Figure 2. Structural design of 2-pyridone scaffold from 1,2-dihydro-3-isoquinolone

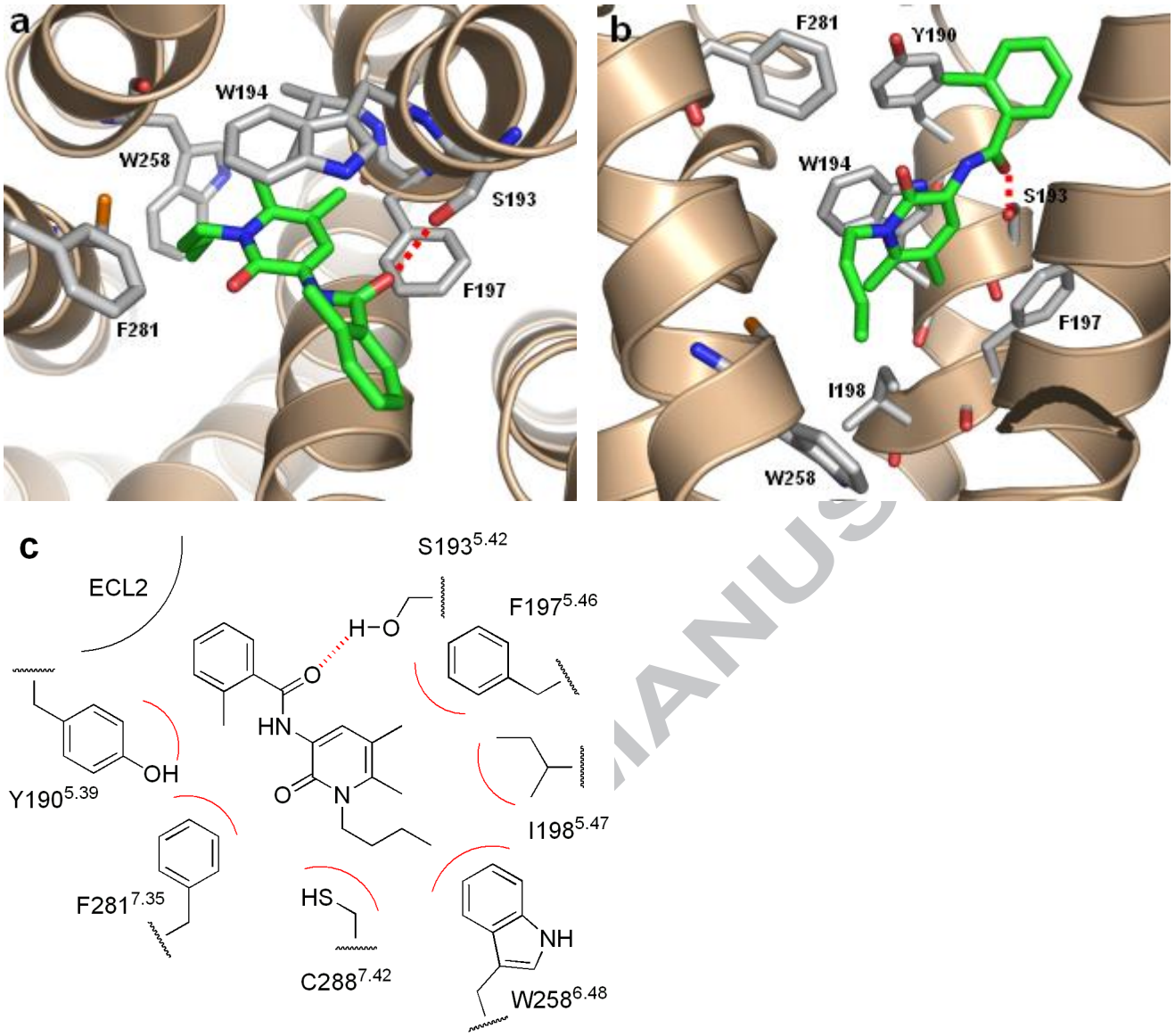
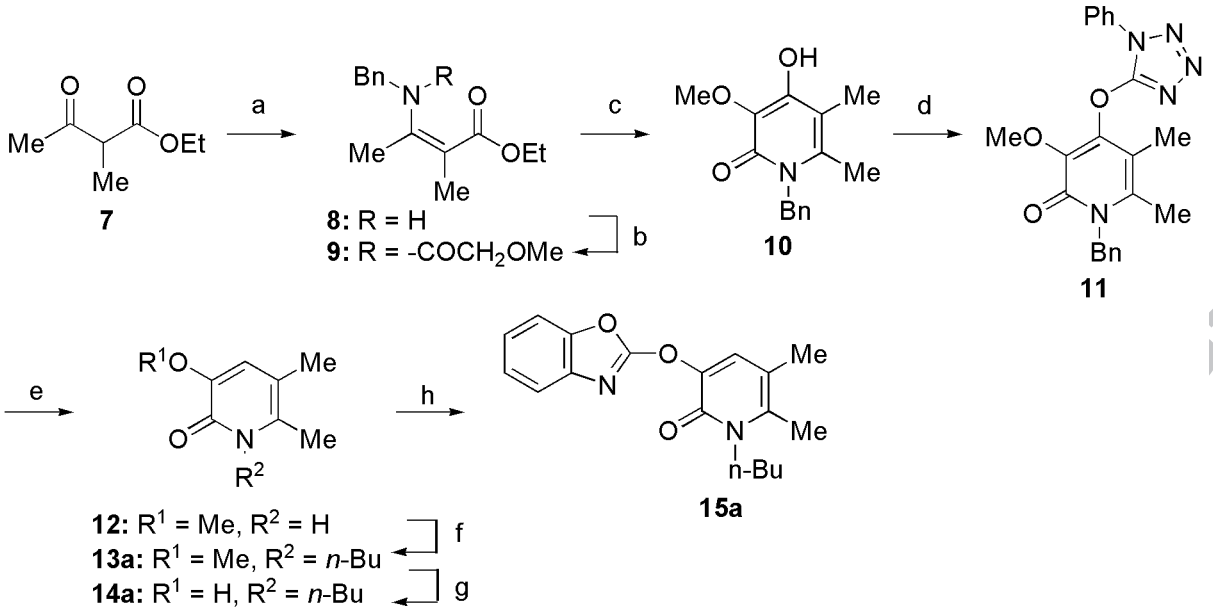
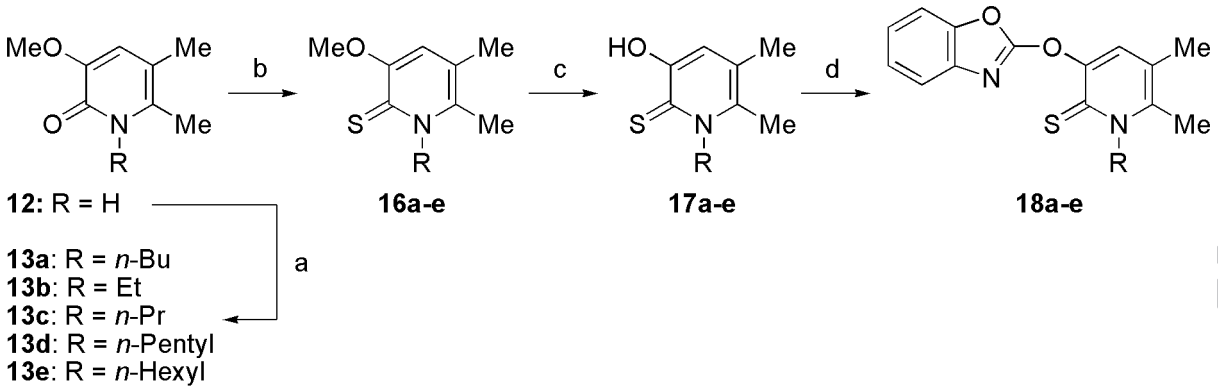


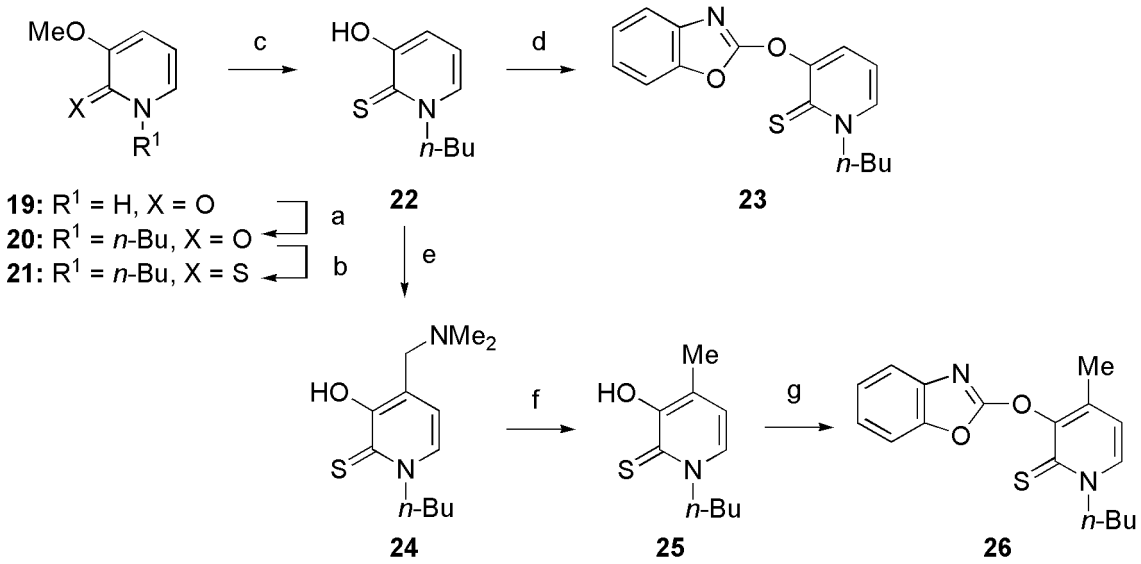
Figure 3. Docking of **39f** into the CB2 homology model. Key residues are depicted. (a) Binding pocket viewed from the extracellular surface. The hydrogen bond is shown as dotted lines. (b) Side view with extracellular side at the top. (c) Diagram of **39f** in the binding pocket. Hydrophobic interactions are shown as red lines and the hydrogen bond is shown as dotted lines.



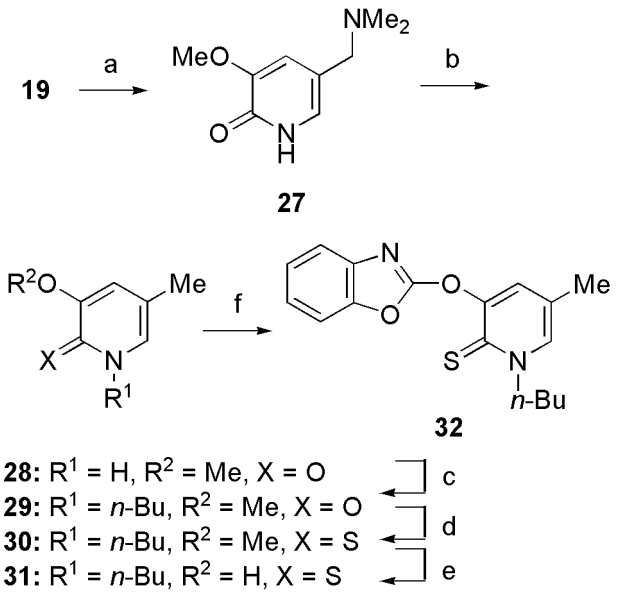
Scheme 1. Reagents and conditions: a) $BnNH_2$, toluene, reflux; b) 2-methoxyacetyl chloride, pyridine, Et_2O, 0 °C–rt; c) Na, EtOH-toluene, reflux; d) 5-chloro-1-phenyl-1*H*-tetrazole, K_2CO_3, DMF, rt; e) 10% Pd/C, H_2 (5 kg/cm^2), DMF, rt; f) *n*-BuI, NaOH, 1-butanol, 85 °C; g) pyridinium chloride, 200 °C; h) 2-chlorobenzo[*d*]oxazole, NaH, DMF, rt.



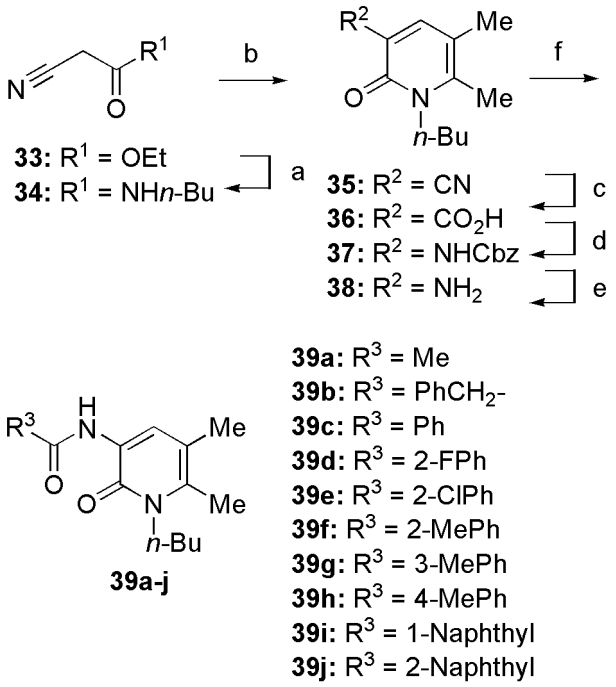
Scheme 2. Reagents and conditions: a) R-I, NaOH, 1-butanol, 85 °C; b) Lawesson's reagent, toluene, reflux; c) pyridinium chloride, 200 °C; d) 2-chlorobenzo[*d*]oxazole, NaH, DMF, rt.



Scheme 3. Reagents and conditions: a) *n*-BuI, NaH, DMF; b) Lawesson's reagent, toluene, reflux; c) pyridinium chloride, 200 °C; d) 2-chlorobenzo[*d*]oxazole, NaH, DMF, rt. e) *N*,*N*,*N*',*N*'-tetramethylmethylenediamine, EtOH-H_2O, 75 °C; f) i) MeI, DCM, rt, ii)PPh_3, EtOH, 75 °C, iii) aq. NaOH, MeOH, 60 °C; g) 2-chlorobenzo[*d*]oxazole, NaH, DMF, rt.

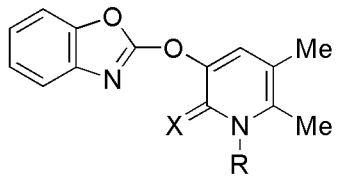


Scheme 4. Reagents and conditions: a) *N*,*N*,*N*',*N*'-tetramethylmethylenediamine, EtOH-H_2O, 75 °C; b) i) MeI, DCM rt, ii)PPh_3, EtOH, 75 °C, iii) aq. NaOH, MeOH, 60°C; c) *n*-BuI, NaH, DMF, rt; d) Lawesson's reagent, toluene, reflux; e) pyridinium chloride, 165°C; f) 2-chlorobenzo[*d*]oxazole, NaH, DMF, rt.



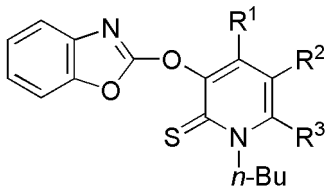
Scheme 5. Reagents and conditions: a) *n*-BuNH_2, rt; b) 2-methyl-3-oxobutanal sodium salt, piperidine, AcOH, DMF, 135 °C; c) KOH, 80% aq. EtOH, reflux, 85%; d) DPPA, Et_3N, BnOH, dioxane, 110 °C; e) 10% Pd/C, MeOH, rt; f) R^3COCl, pyridine, THF, rt.

Table 1. Binding affinity of pyridone **15a** and thiopyridones **18a-e**



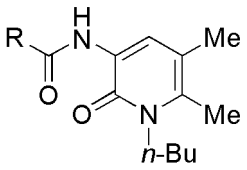
compd	X	R	CB2 Ki (nM)
15a	O	*n*-Bu	976
18a	S	*n*-Bu	101
18b	S	Et	3855
18c	S	*n*-Pr	276
18d	S	*n*-Pentyl	214
18e	S	*n*-Hexyl	609

Table 2. Binding affinity of thiopyridones **18a**, **23**, **26**, and **32**



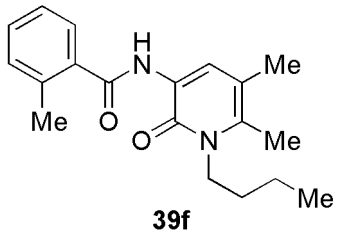
compd	R^1	R^2	R^3	CB2 Ki (nM)
18a	H	Me	Me	101
23	H	H	H	490
26	Me	H	H	235
32	H	Me	H	236

Table 3. Binding affinity of pyridones **39a-j**



compd	R	hCB2 Ki (nM)	hCB1 Ki (nM)
39a	Me	>5000	>5000
39b	$PhCH_2$-	1310	>5000
39c	Ph	89	1376
39d	2-FPh	88	617
39e	2-ClPh	16	391
39f	2-MePh	14	390
39g	3-MePh	42	343
39h	4-MePh	72	854
39i	1-Naphthyl	12	19
39j	2-Naphthyl	768	>5000

Graphical abstract:



39f

CB2 Ki = 14 nM
CB1 Ki = 390 nM