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Discovery of 2-chloro-*N*-((4,4-difluoro-1-hydroxycyclohexyl)methyl)-5-(5-fluoropyrimidin-2-yl)benzamide as a potent and CNS penetrable $P2X_7$ receptor antagonist

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ABSTRACT

Focused SAR studies were carried out around 5-heteroaryl and 1-amide portions of the 2-chlorobenzamide scaffold, resulting in the discovery of a potent, metabolically stable and centrally penetrable antagonist against $P2X_7$ receptor.

The $P2X_7$ receptor ($P2X_7R$), a ligand-gated ion channel, is expressed primarily on both peripheral and central immune cells including macrophages, monocytes, microglia and astrocytes,[1] and can be activated by elevated concentration of extracellular ATP (>100 μM).[2] Upon brief activation, $P2X_7R$ undergoes structural changes to form a 'typical' cation channel to allow influx of Ca^{2+} and efflux of Na^+/K^+. If activation is repeated or prolonged, a large non-selective membrane pore with increased cell permeability to molecules up to 900 Da molecular mass is gradually opened, presumably as the result of activation of pannexin-1, a membrane pore-forming protein.[3] These functional changes trigger multiple downstream effects including Caspase 1 and glial cell activations which in turn lead to release of mature and biologically active IL-1β and neurotransmitters (glutamate and GABA, etc.), implicating that $P2X_7R$ plays a potential role in pain signaling.[4] The therapeutic importance of $P2X_7R$ in mediating inflammatory and neuropathic pain is further supported by in vivo studies demonstrating that $P2X_7R$ knockout mice were resistant to inflammatory/neuropathic pain[5] and $P2X_7R$ selective antagonists exhibited dose-dependent antinociceptive effects in inflammatory/neuropathic pain animal models.[6,7] Therefore $P2X_7R$ as a therapeutic target for treatment of inflammatory and neuropathic pain has attracted considerable interest from the pharmaceutical industry.[8] However, whether to target peripheral or central $P2X_7R$ has not yet been clearly demonstrated, but the significant role of activated central glia in initiation/maintenance of chronic pain through regulating IL-1β and perhaps glutamate suggests that $P2X_7R$ may be a central player.[9] Based on such rationale, we initiated a program to identify $P2X_7R$ antagonists targeting central $P2X_7R$ for treatment of inflammation and pain.

Numerous classes of $P2X_7R$ antagonists were disclosed featuring increasing drug-like properties, but no centrally penetrable $P2X_7R$ antagonists have been specifically described so far.[10] Our approach toward developing potent $P2X_7R$ antagonists with CNS penetration was based on the potent 2-chlorobenzamide template disclosed previously.[11] Compound **1** was identified as a suitable lead for optimization. It not only possesses desirable physicochemical parameters under preferred CNS drug chemical space (HBD $\leqslant$ 2, TPSA $\leqslant$ 75, etc.),[12] but also has potent antagonism activity against $P2X_7R$ with IC_{50} of 32 nM and relatively low Pgp efflux activity assessed by MDR1 of 2.0, although metabolically unstable [human liver microsomal intrinsic clearance (HLM CL_{int}) of 80 mL/min/kg]. Here we report our SAR efforts around **1** to improve its druggability leading to the discovery of a potent and CNS penetrable $P2X_7R$ antagonist with an excellent PK profile.

The preparation of compounds shown in Table 1 is illustrated in Scheme 1. Commercially available 5-bromo-2-chlorobenzoic acid and 1-(aminomethyl)cycloheptanol were coupled to give amide **2** in the presence of HBTU and DIEA which was subsequently converted into the key intermediate, boron ester **3** according to a pub-

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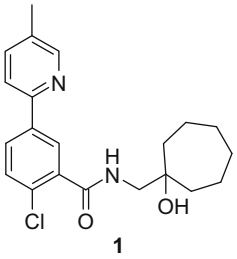
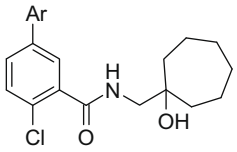
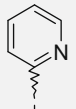
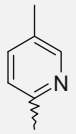
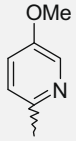
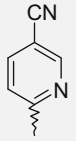
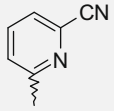
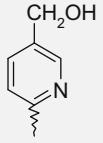
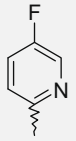
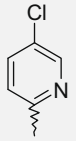
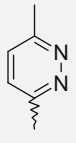
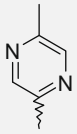
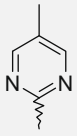
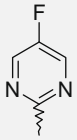


1

MW = 373
HBD = 2
TPSA = 62
ClogD = 3.5
IC_{50} = 32 nM
MDR1 = 2.0
HLM CL_{int} = 80 mL/min/kg

Table 1
In vitro profiles of $P2X_7R$ antagonists with six-membered heteroaryl variation



Entry	Ar	TPSA	ClogD @ pH 7.4	IC_{50}[a] (nM)	HLM CL_{int}[b] (mL/min/kg)	MDR1[c]
4a		62	3.1	40	53	nm[d]
1		62	3.5	32	80	2.0
4b		71	3.3	213	122	1.2
4c		86	2.7	134	37	nm[d]
4d		86	3.5	232	47	nm[d]
4e		82	1.9	17	20	2.8
4f		62	3.0	32	29	1.2
4g		62	3.9	56	52	nm[d]
4h		75	2.0	18	23	1.5
4i		75	2.7	22	36	1.4
4j		75	3.7	8	53	nm[d]
4k		75	3.3	16	21	1.2

[a] IC_{50} of the $P2X_7R$ antagonism was determined by ATP-induced YO-PRO-1 uptake inhibition assay.[19]
[b] Human liver microsomal intrinsic clearance was measured according to the standard human liver microsomal stability assay protocol.
[c] MDR1 was expressed as the ratio of permeability$^{B\rightarrow A}$/permeability$^{A\rightarrow B}$ using the MDCK cell line transfected with the human MDR-1 gene to assess the P-glycoprotein (Pgp) efflux activity.
[d] Not measured.

Table 2
In vivo pharmacokinetic profiles of $P2X_7R$ antagonists

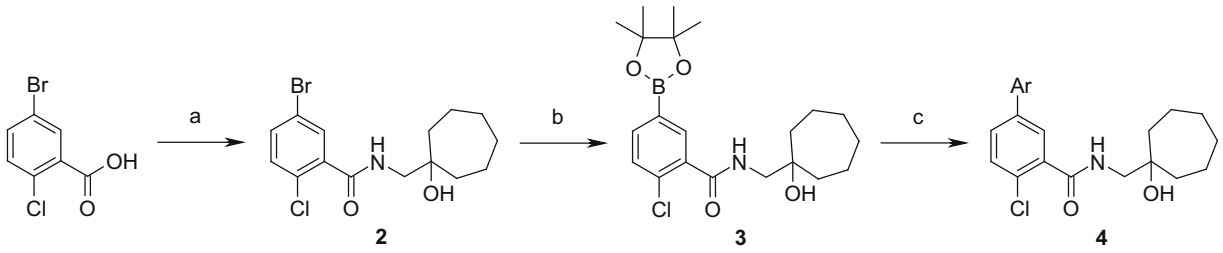
Compound	Rat IV/PO crossover PK[a]				Rat b/p[c]
	CL (mL/min/kg)	V_{dss} (L/kg)	Effective $T_{1/2}$[b] (h)	Bioavailability (%)	
4k	17	0.9	0.6	35	0.7
7f	3.3	1.3	4.6	84	1.3

[a] Dosed at 1 mpk in male Sprague Dawley rats.
[b] Effective $T_{1/2}$ was calculated as $V_{dss}/CL * \ln 2$.
[c] b/p was measured as the ratio of compound concentration in brain over that in plasma following a steady-state IV infusion in male Sprague Dawley rats.

lished procedure.[13] Palladium-catalyzed Suzuki cross-coupling of **3** with a variety of six-membered heteroaryl halides afforded **4** in good overall yields.

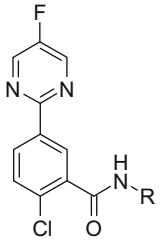
Compounds shown in Table 3 were synthesized via a slightly different sequence (Scheme 2). Palladium-catalyzed Suzuki cross-coupling of commercially available boronic acid **5** with 2-chloro-5-fluoropyrimidine gave the acid intermediate **6** which was then converted into the desired amide **7** under typical amide coupling conditions.

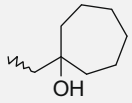
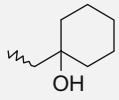
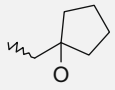
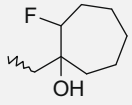
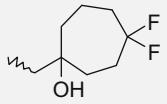
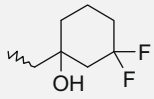
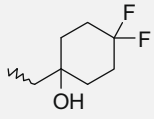
The preparation of the fluorinated amines is shown in Scheme 3. Treatment of commercial cycloheptanone with Selectfluor in methanol at room temperature overnight yielded 2-fluoro-cycloheptanone **8**. Ketone **8** was readily converted into a cyanohydrin ether intermediate via addition of trimethylsilyl cyanide catalyzed by zinc iodide in methylene chloride.[14] The crude cyanohydrin ether was subsequently reduced with borane dimethyl sulfide complex in THF to an ethanolamine which was further converted into the Boc-protected intermediate **9** for the purpose of easy isolation. Deprotection of Boc under the acidic conditions gave the desired amine **10** as an HCl salt. Ring expansion of commercial 4,4-difluorocyclohexanone with trimethylsilyl diazomethane catalyzed by boron trifluoride diethyl etherate afforded 4,4-difluorocycloheptanone **11** in low yield.[15] Following similar procedures as for **10**, both **11** and 4,4-difluorocyclohexanone were converted into the corresponding amine HCl salts, **13** and **15**, respectively. Cyanation of commercially available 2-cyclohexen-1-one with KCN gave 3-cyano-cyclohexanone **16**,[16] where the ketone



Scheme 1. Reagents and conditions: (a) HBTU, DIEA, 1-(aminomethyl)cycloheptanol, DMF, room temperature, 1 h, 80%; (b) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, DMSO, 80 °C, 2 h, 85%; (c) aryl halide, $Pd(PPh_3)_4$, Cs_2CO_3, DME/H_2O (5:1 v/v), 80 °C, overnight, 84%.

Table 3
In vitro profiles of $P2X_7R$ antagonists with amide variation



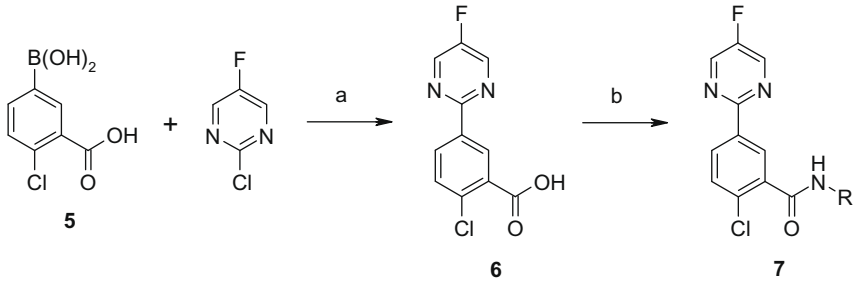
Entry	R	ClogD @ pH 7.4	IC_{50} (nM)	HLM CL_{int} (mL/min/kg)	MDR1
4k		3.3	16	21	1.2
7a		2.7	32	12	1.1
7b		2.1	449	12	1.3
7c		3.0	17	15	1.2
7d		2.1	46	<8	nm
7e		2.0	51	10	1.8
7f		1.8	27	<8.8	1.1

functionality was further transformed into difluoro with Deoxofluor to yield **17**. Compound **17** was sequentially treated with LDA, oxygen and acetyl chloride to afford a peracetoxy-nitrile intermediate **18**.[17] Without further purification, **18** was reduced with borane dimethyl sulfide complex in THF to an ethanolamine, followed by protection of the amine with Boc_2O to yield the intermediate **19**. Final deprotection of Boc gave the desired amine HCl salt **20**.

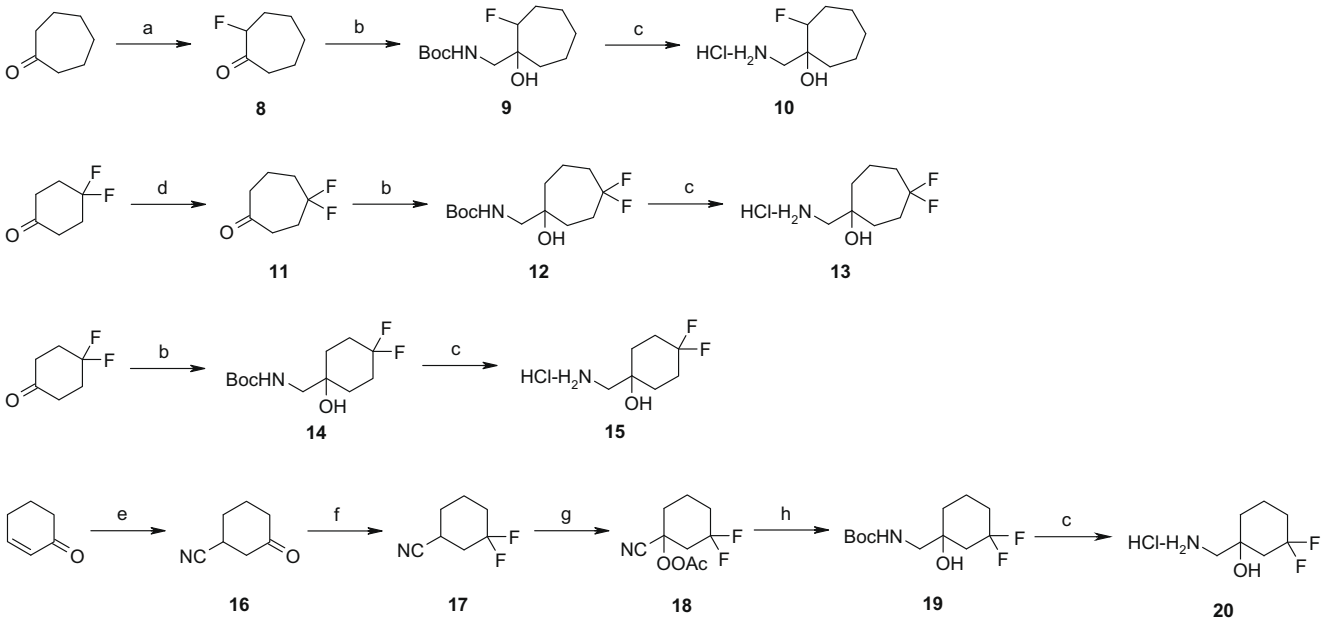
In order to improve the metabolic stability of **1**, replacement of the metabolically liable 5-methylpyridyl as revealed in Met ID studies was first investigated while keeping the rest of molecule fixed (Table 1). Removal of the 5-methyl group (**4a**) slightly improved HLM stability which was further enhanced by re-introducing a 5-fluoro group (**4f**) with retained potency. Other slightly larger 5-substitutions, whether polar or non-polar, caused some degrees of potency loss (**4b**, **4c**, **4g**). The 5-hydroxymethyl analog, **4e**, however, considerably improved both potency and HLM stability, but raised efflux as well due to an additional HBD. Potency was also weakened by moving substitution from 5 to 6 position (**4c** vs **4d**). Insertion of a nitrogen into the 5-methylpyridyl ring led to more active derivatives, **4h**, **4i** and **4j**. Among them, **4j** was the most potent but least stable analog because of its highest ClogD value. Further replacement of the 5-methylpyrimidyl group with a 5-fluoropyrimidyl group gave **4k** with substantially enhanced HLM stability while retaining $P2X_7R$ antagonism. Such transformation was not suitable for both pyridazinyl and pyrazinyl analogs as the resulting fluoro-derivatives post a structural alert.[18] Overall, good correlation between ClogD and HLM stability was observed and low efflux was achieved if HBD $\leqslant$ 2 and TPSA $\leqslant$ 75. Therefore, it is important to properly balance all physicochemical properties of new synthetic targets in the multi-parameter lead optimization. With good overall in vitro properties, **4k** was advanced for in vivo profiling.

The in vivo rat PK profile of compound **4k** is summarized in Table 2. Compound **4k** had medium clearance of 17 mL/min/kg, relatively short effective half-life of 0.6 h and good CNS exposure with a brain/plasma ratio of 0.7. The in vitro assessment of HLM stability and efflux correlated with in vivo properties reasonably well. However, the moderate PK properties of **4k** were not good enough to lead to adequately low predicted human dose. As low human dose is desirable for a CNS drug to minimize potential adverse CNS effects, it was necessary to further improve PK properties, particularly clearance. With an optimal 5-fluoropyrimidyl group, the focus was then shifted to the amide portion of the molecule.

As shown in Table 3, reducing ClogD by shrinking the amide-carbocyclic ring size from a seven- to six- and five-membered rings continued to lower HLM intrinsic clearance. However, the potency deteriorated accordingly. The seven-membered carbocyclic ring appeared to be optimal for potency while having reasonable HLM stability. Further Met ID studies of **4k** using both HLM and RLM revealed that metabolism took place predominantly on the carbocyclic ring with oxidative hydroxylation by cyps as the main clearance mechanism, suggesting that the metabolic stability could be improved if metabolically soft spots on the carbocyclic ring were blocked. As well documented in the literature that fluorination can effectively block metabolism, four fluorinated analogs were prepared and evaluated. Mono-fluorination of the seven-membered carbocyclic ring (**7c**) maintained potency but only slightly improved HLM stability. Presumably mono-fluorination at the α-position could not effectively block cyps' oxidation at positions away from the hydroxyl group while bis-fluorination at β- and γ-position indeed efficiently reduced HLM clearance although causing some drops in potency. More than 1 unit decrease in ClogD



Scheme 2. Reagents and conditions: (a) $Pd(PPh_3)_4$, Cs_2CO_3, DMF/H_2O (5:1 v/v), 80 °C, overnight, 83%; (b) HBTU, DIEA, RNH_2, DMF, room temperature, 1 h, 90%.



Scheme 3. Reagents and conditions: (a) Selectfluor, MeOH, overnight, 53%; (b) (i) TMS–CN, ZnI_2, CH_2Cl_2, 0 °C to room temperature, 2 h; (ii) BH_3·DMS, THF, room temperature, overnight; (iii) Boc_2O, NEt_3, DMAP, CH_2Cl_2, room temperature, overnight; 22–68% for three steps; (c) 4 N HCl in dioxane, room temperature, 1 h, 85–96%; (d) TMS–CH_2N_2, BF_3·OEt_2, CH_2Cl_2, −10 °C, 2 h, 20%; (e) KCN, Et_3N·HCl, MeOH–H_2O (2:1 v/v), 60 °C, 4 h, 45%; (f) Deoxofluor, CH_2Cl_2, room temperature, overnight, 50%; (g) (i) LDA, O_2, AcCl, THF, −78 °C; (ii) BH_3·DMS, THF, room temperature, overnight; (iii) Boc_2O, NEt_3, DMAP, CH_2Cl_2, room temperature, overnight; 10% for steps (g) and (h).

by bis-fluorination certainly added additional beneficial effect on HLM stability.[20] Good potency, low HLM clearance and efflux, along with achirality, made **7f** an attractive candidate for further evaluation. The in vivo rat PK assessment of **7f** showed that it had much improved PK properties over **4j**, with low clearance of 3.3 mL/min/kg, long effective half-life of 4.6 h and high oral bioavailability of 84% (Table 2). Coupled with its excellent CNS exposure (b/p of 1.3), **7f** was endorsed for further development.

In conclusion, focused SAR studies were carried out to optimize the drug properties of **1** following CNS drug design guidelines. By keeping the ClogD low, fluorinating the carbocyclic ring and constraining the number of hydrogen bond donors to two, the metabolic stability was significantly improved while maintaining potent antagonism and CNS penetration. Such effort led to the discovery of **7f** as a potent $P2X_7R$ antagonist with excellent PK properties and CNS exposure. Further assessment of **7f** could provide more insights into the role that central $P2X_7R$ plays in mediating pain signaling. This could in turn lead to development of therapeutic $P2X_7R$ antagonists for treatment of inflammation and pain.

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19. ATP-induced YO-PRO-1 uptake assay to assess potency of $P2X_7R$ antagonists by measuring inhibition of dye uptake. Briefly, HEK-293 cells stably expressing human $P2X_7R$ harvested with 0.05% trypsin were suspended at 2×10^6 cells/mL in the assay media (15 mM HEPES, 135 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgCl_2$, 5 mM glucose, 1% dialyzed fetal bovine serum, pH 7.4). Ten microliters of the cell suspension and 10 μL of 10 μM YO-PRO-1 were added to 384-well non-treated black plates containing 20 μL of antagonist in the assay media at various concentrations. The plates were incubated in dark at room temperature for 15 min, followed by addition of 10 μL of 10 mM ATP in 20 mM HEPES at pH 7.4. After incubation for additional 2 h, fluorescence derived from dye uptake was measured using a Tecan Safire fluorescence plate reader with excitation at 450 nm and emission at 535 nm.
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