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Synthesis and SAR development of novel $P2X_7$ receptor antagonists for the treatment of pain: Part 2

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ABSTRACT

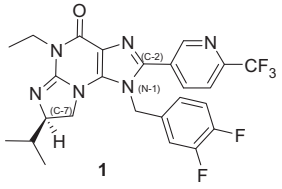
Novel $P2X_7$ antagonists were developed using a purine scaffold. These compounds were potent and selective at the $P2X_7$ receptor in human and rodent as well as efficacious in rodent pain models. Compound **15a** was identified to have oral potency in several pain models in rodent similar to naproxen, gabapentin and pregabalin. Structure–activity relationship (SAR) development and results of pain models are presented.

Located primarily in lymphocytes, macrophages, and mast cells,[1] the $P2X_7$ ligand-gated ion channel belongs to the super family of adenosine 5′-triphosphate (ATP)-sensitive (P2) receptors.[2] The P2 receptor family has been shown to play a role in the modulation and transmission of nociceptive signals altered by various forms of injury.[3] Of the seven ionotropic P2X subtypes, $P2X_4$ and $P2X_7$ have been associated with the release of proinflammatory cytokines, such as Il-1β.[4] This has been shown to facilitate pain transmission.[4,5] While ATP has been shown to be a non selective agonist for the P2X family,[2] it has been demonstrated in the literature, due to its unusual phenotypic characteristics[6] that it is possible to synthesize small molecules which are selective $P2X_7$ antagonists.[7] It is also important to note that $P2X_7$ receptor knockout mice did not have inflammatory or neuropathic hypersensitivity, however normal nociceptive processing was persevered.[8] For these reasons we believe that $P2X_7$ is an attractive target in the treatment of pain.

Our recently published work[9] described the discovery of compound **1** (Fig. 1) as a prototype $P2X_7$ antagonist. This compound exhibited good affinity for $P2X_7$ in human and rat with a moderate pharmacokinetic (PK) profile the in rapid rat PK model.[10] It showed oral efficacy similar to that of naproxen in rat mono-iodacetate (MIA)-induced hyperalgesia model but had only marginal efficacy in chronic constriction injury (CCI) rat model.[11] In our quest to develop a compound that would treat patients across the broad spectrum pains, we continued SAR efforts in this area. Our thoughts were that a compound with good whole blood potency and sustainable oral exposure would achieve our goal.

Our previous work has indicated that bulkier substitutions were tolerated at the C-2 position of the core. To evaluate this in detail, the 2-trifluoromethylpyridine substitution at the C-2 position was replaced with a biphenyl and we investigated various fluorinated benzyl substituents at N-1 position (Fig. 2). These initial set of compounds were prepared using Scheme 1 and results are presented in Table 1.

Alkylation of commercially available **3** with 3,4-difluorobenzyl chloride provided compound **4** as a mixture of regio-isomers. The



1

h $P2X_7$ IC_{50} = 50 nM
r $P2X_7$ IC_{50} = 32 nM
h WB (IL-1β) IC_{50} = 82 nM
RR AUC = 2685 nM. hr
$Conc._{6h}$ = 227

Figure 1. Previously disclosed $P2X_7$ antagonist.[9]

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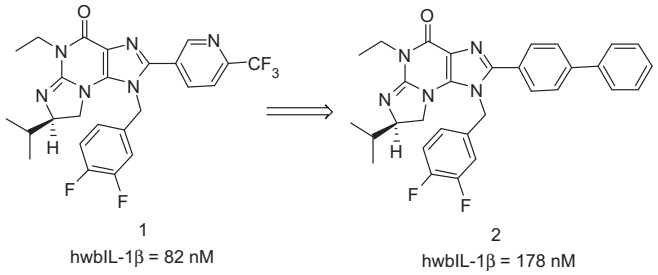
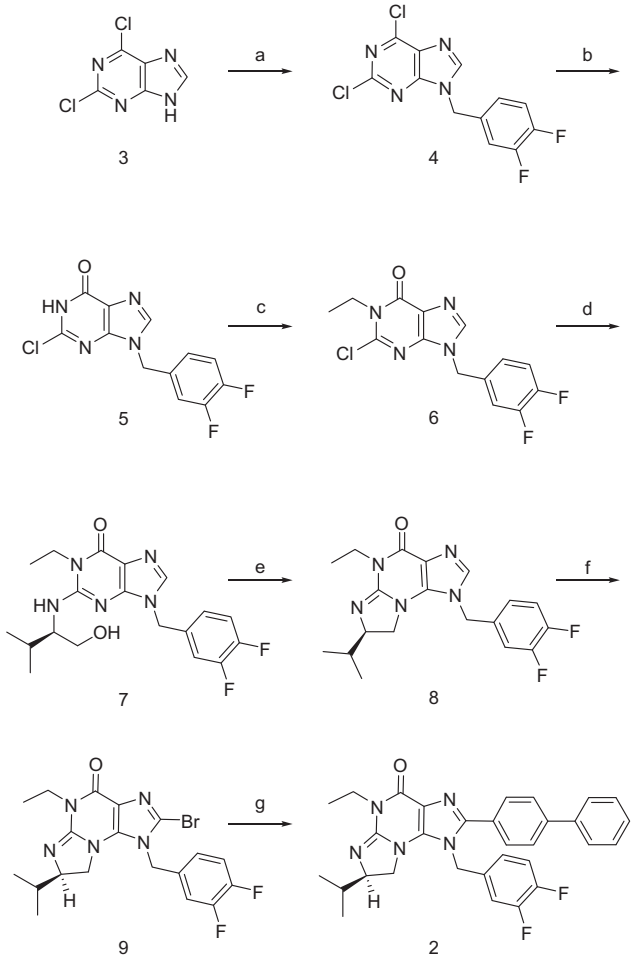


Figure 2. SAR of selected C-2 substitutions.

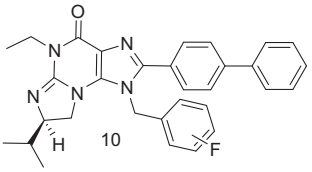


Scheme 1. Reagents and conditions: (a) 3,4-Difluorobenzyl chloride, K_2CO_3, DMF, rt 24 h; (b) 0.2 N NaOH reflux, 24 h; (c) ethyl iodide, K_2CO_3, DMF, rt 24 h; (d) (*R*)-(−)-2-amino-3-methyl-1-butanol, TEA, NMP 150 °C 24 h; (e) thionyl chloride, DCM, rt 1 h; (f) NBS, DCM rt 1 h; (g) 4-biphenyl boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, CH_3CN/H_2O (3:1), μλ 130 °C, 30 min.

hydrolysis of the major regio-isomer of **4** in aq sodium hydroxide afforded compound **5**. Alkylation of **5** using ethyl iodide produced **6**, which on displacement with (*R*)-(−)-2-amino-3-methyl-1-butanol gave intermediate **7**. Cyclization of **7** using thionyl chloride, produced compound **8**. Compound **8** was treated with NBS to give compound **9**. The synthesis of the desired targets was completed by performing a Suzuki coupling to give compound **2**. Other analogs were prepared using this synthetic route.

As described in Table 1, substitution of fluorine at the *ortho* position (Entry **10a**) was least tolerated and a fourfold loss in whole blood activity was observed compared to *meta* and *para* positions

Table 1
Initial SAR modifications



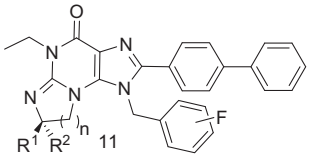
Entry	Fluorine substitution pattern on benzyl ring	hwb IL-1β[12] IC_{50} (nM)
2	3,4	178
10a	2	937
10b	3	269
10c	4	237
10d	2,3	870
10e	2,4	845
10f	2,5	1036
10g	3,5	107

The IC_{50} and K_i data are an average of at least three measurements.

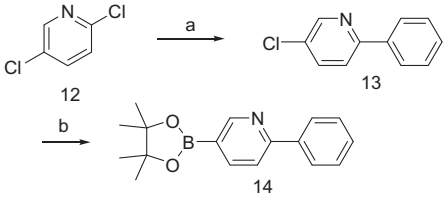
(**10b** and **10c**). A similar drop in whole blood activity was also observed with all disubstituted analogs (**10b–f**) which had fluorine at the *ortho* position. However, 3,4 and 3,5-difluorobenzyl derivatives (e.g., **2** and **10g**) exhibited h-whole-blood potency similar to our prototype compound **1**. Both compounds (**2** and **10g**) were dosed (10 mg/kg) in the rapid rat PK model[10] to determine oral exposures. Compound **10g** exhibited an AUC of 1113 nM h with a 93 nM concentration at 6 h, while **2** exhibited an AUC (3763 nM h) with a concentration of 392 nM at 6 h. Compared to compound **1**, neither compound displayed superior oral exposure. To this end we went back and explored the substitution around the C-7 position with the biphenyl at C-2. All compounds were prepared using methods described in Scheme 1 and the data is presented in Table 2.

From previous work[9] we knew that small substitutions were tolerated at C-7. Gem-dimethyl was the first substitution evaluated. It showed an IC_{50} of 85 nM in h-whole-blood assay. When this substitution was expanded to include a spirocycle (**11b**) a twofold reduction in h-whole blood potency was observed. When the ring was expanded by one carbon (**11c**) it provided a compound equipotent to that of compound **2**. We then began to explore the substitution pattern around the benzyl ring to ensure that the 3,4-difluoro substitution was optimal. We found that **11d** was equipotent to that of its isopropyl counterpart **10c** (Table 1). However, when fluorine was added to the *ortho* position (**11e**) an increase in whole-blood potency was observed. Compound **11f** was equipotent to that of its isopropyl counterpart (**10g**). Two compounds (**11a** and **11f**) with good h-whole blood potency were examined for oral exposure in the rapid rat PK model[10] at 10 mg/kg dose. Compound **11a** exhibited very good exposure with an AUC of 6863 nM h and a plasma concentration of 1369 nM at 6 h. Based on its good exposure, compound **11a** was evaluated in rodent whole blood assay and showed a rodent whole-blood potency of 121 nM. Based this, compound **11a** was evaluated in variety of rodent pain models encompassing both inflammatory and neuropathic pain. Gabapentin (50 mg/kg) and naproxen (10 mg/kg) were used as positive controls. All efficacy measurement was made at 4 h post dose. In the Streptozocin (STZ)-induced diabetic neuropathy model[13] in rat, **11a** exhibited efficacy comparable to gabapentin at an oral dose of 10 mg/kg. Similarly, compound **11a** produced efficacy similar to gabapentin when dosed orally (at 60 mg/kg) in chronic constriction injury (CCI) model of neuroinflammatory pain. Compound **11a** also produced a naproxen like effect when dosed at 50 mpk in the rat mono-iodacetate (MIA)-induced hyperalgesia model.[14] Compound **11a** was the first

Table 2
Core SAR modifications



Entry	R^1	R^2	*n*	Substitution of fluorines on benzyl ring	hwb IL-1β[12] IC_{50} (nM)
2	Isopropyl	H	0	3,4	178
11a	Me	Me	0	3,4	85
11b	Cyclohexyl		0	3,4	388
11c	Me	Me	1	3,4	108
11d	Me	Me	0	4	276
11e	Me	Me	0	2,4	700
11f	Me	Me	0	3,5	91



Scheme 2. Reagents and conditions: (a) Phenyl boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene/ethanol/water (2.4:1:1.4), 80 °C, 24 h; (b) bispinacolediborane, Pd(dba), Xphos, KOAc, dioxane, 80 °C, 24 h.

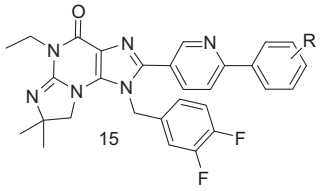
compound in this series which showed efficacy similar to control in all three pain models. This compound was screened against a panel of ion channels and was found to have greater than 80% inhibition at 10 μM for several different ion channels (Na^+ Channel (site 2), Ca^+ Channel (L, diltiazem), Cl^- Channel and Nor epinephrine transporter). In addition, **11a** exhibited 55% inhibition of $P2X_4$ at 10 μM.

To improve selectivity of **11a** against the ion channels, we decided to investigate the incorporation of pyridine in the interior phenyl ring of the C-2 biphenyl. The rational behind this was prior knowledge that pyridine substitutions at C-2[9] provided compounds that were potent in the whole-blood assay. Scheme 2 provided the synthetic route to achieve this substitution pattern. Compound **14** was synthesized via a Suzuki coupling of **12** and commercially available boronic acids. Compound **13** was then converted to the boronic ester and taken through the final Suzuki coupling as described in Scheme 1. This combined schemes provided access to the compounds presented in Table 3.

Replacement of the interior phenyl ring of **11a** with a pyridine ring gave **15a**. This compound had very good potency in human whole blood assay. A variety of substituents were investigated at the distal phenyl moiety. Substitution around the distal phenyl ring was also well tolerated with most compounds exhibiting potencies under 100 nM in the whole-blood assay. However, once trifluoromethyl and trifluoromethoxy (**15e, 15g, 15i** and **15l**) groups were substituted, a drop in whole-blood potencies was observed. This is most dramatically demonstrated by **15l**. Further differentiation of these compounds came when their oral exposures were measured in the rapid rat PK model. Methyl substitution (**15d** and **15h**) produced compounds with extremely low exposures. Although compounds **15b**, **15f**, and **15j** showed significant exposures, compound **15a** had best sustained levels in plasma.

The in vivo potency of this compound was measured in several inflammatory and neuropathic pain models as described for

Table 3
Distal phenyl SAR modifications



Entry	R	hwb IL-1β[12] IC_{50} (nM)	RR AUC[10] nM h	$Concn_{6\ h}$ nM
15a	H	24	13670	2638
15b	2-Fluoro	52	2714	537
15c	2-Methoxy	101	1621	224
15d	3-Methyl	25	231	0
15e	3-Trifluoromethyl	104	1836	395
15f	3-Fluoro	28	3559	897
15g	3-Trifluoromethoxy	110	ND	ND
15h	4-Methyl	39	0	0
15i	4-Trifluoromethyl	137	5677	1569
15j	4-Fluoro	45	3559	897
15k	4-Methoxy	50	0	0
15l	4-Trifluoromethoxy	1944	ND	ND
15m	2,4-Difluoro	95	4137	537
15n	3,5-Difluoro	55	7538	1263

compound **11a**. The potency of compound **15a** (10 mg/kg dose) was similar to that of **11a** in STZ-induced diabetic neuropathy model, but was much more potent (1–3 mg/kg dose) in both MIA and CCI models in rat. In addition, potency of **15a** was also measured in the spinal nerve ligation (SNL) model[15] for neuropathic pain in rat. Pregabalin (10 mg/kg) was used as a positive control. It exhibited efficacy comparable pregabalin at a dose between 10 and 30 mg/kg given via the oral route. All efficacy measurements were carried out at 4 h post dose. Compared to **11a**, compound **15a** was also found to be significantly more selective when screened for its inhibitory activity against other ion channels.

Excited with these results, we further studied compound **15a** for oral exposures in higher species. To our disappointment the presence of glutathione adducts was observed in dog urine. These adducts were determined to form through enzymatic epoxidation of the distal phenyl ring. The glutathione formation was mitigated by the addition of electron withdrawing groups on the distal phenyl ring. Unfortunately these substitutions resulted in significant

drop in potency in the whole blood assay. Work is currently in progress to identify a compound with oral efficacy similar to that of **15a** without this glutathione issue.

In conclusion, current efforts identified compound **15a** with sustained oral exposures and very good potency in the human whole blood assay. This compound was very efficacious in inflammatory (MIA), inflammatory/neuropathic (CCI) and neuropathic (STZ and SNL) pain models in rat. Its efficacy was similar or better than positive controls (naproxen, gabapentin and pregabalin) used in these studies. Unfortunately, it also formed glutathione adducts in higher species. Our current SAR development is focused on resolving this issue and results will be discussed in further publications.

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