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ABSTRACT

The synthesis, SAR and preclinical characterization of a series of 6-chloro-N-(2-(4,4-difluoropiperidin-1-yl)-2-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)quinoline-5-carboxamide based P2X7 antagonists is described herein. The lead compounds are potent inhibitors in Ca^{2+} flux and whole blood IL-1β P2X7 release assays at both human and mouse isoforms. Compound **1e** showed a robust reduction of IL-1β release in a mouse ex vivo model with a 50 mg/kg oral dose. Evaluation of compound **1e** in the mouse SNI tactile allodynia, carrageenan-induced paw edema or CIA models resulted in no analgesic or anti-inflammatory effects.

The P2X7 receptor is an ATP-gated non-selective ion channel belonging to the P2X superfamily (P2X1-7) of purinoreceptors. It is abundantly expressed on monocytes, macrophages and lymphocytes in the periphery and on astrocytes and microglia in the CNS. *In vitro* activation of the P2X7 receptor by elevated levels of ATP, or benzoylbenzoyl-ATP (BzATP), results in cation flux and the release of proinflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18) in cells primed with lipopolysaccharide (LPS).[1,2]

The P2X7 receptor has been implicated in a number of disease states including chronic pain, inflammation, rheumatoid arthritis, Crohn's disease, mood disorders, multiple sclerosis, Alzheimer's, and Huntington's disease.[3] As a result many groups in both industry and academia have dedicated significant research efforts toward the development of P2X7 antagonists. Most of the early work focused on the discovery of P2X7 antagonists for treating diseases related to pain and/or inflammation. These efforts resulted in at least four compounds, GSK1482160[4,5], AZD9056[6], CE-224535[7,8], and EVT 401[9] (structure not disclosed), that progressed into clinical trials (Figure 1). To our knowledge these compounds showed no or limited efficacy in the clinic despite robust target engagement and were not advanced into further development.[6,7] A detailed review of P2X7 clinical candidate outcomes has been reviewed elsewhere.[10]

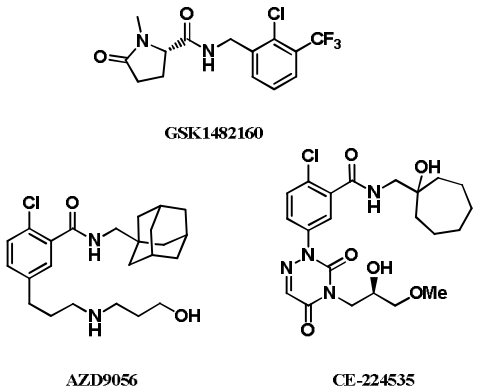

Figure 1: P2X7 clinical antagonists

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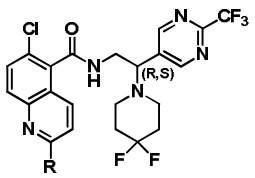
Despite recent setbacks in the clinic, efforts to unlock the therapeutic potential of the P2X7 receptor are ongoing with new antagonists continuing to appear in the literature. Described herein is the discovery and profiling of a series of peripherally restricted P2X7 antagonists with potency at both human and mouse isoforms and robust *in vitro* and *ex vivo* reduction of IL-1β release. A general structure of our antagonist series is shown in Figure 2. Rodent P2X7 pharmacology was an important criterion for our program as at least two compounds that failed in the clinic had weak rodent activity and as such were most likely never evaluated in rodent models of pain or inflammation.[6,7]

The series of P2X7 antagonists described in the following text was identified through an HTS screen followed by a brief medicinal chemistry effort. For convenience these compounds were generated and tested as racemic mixtures as the identity of the stereocenter in Figure 2 has no bearing on the physical properties or potency. Our work on this series led us to explore all possible points of diversity. The most fruitful changes were shown to occur at the 2-position of the quinoline moiety (Figure 2). Compounds generated as part this effort are the focus of this article. A complete list of the compounds generated in the course of our work on this series can be found in our recent disclosure.[11]

Figure 2: P2X7 antagonist general structure



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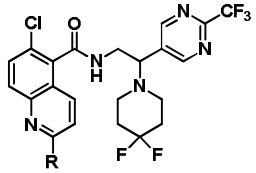
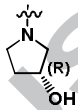
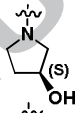
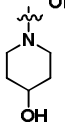
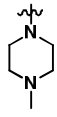
The 2-position of the quinoline moiety was shown to be highly flexible allowing for the incorporation of a variety of functional groups (Figure 2). Appending small alkyl, alkoxy, ether, or amine moieties via an amine linkage ultimately provided the most favorable combination of potency and drug-like properties. A subset of compounds generated for this series is shown in Table 1 along with their potency in a P2X7 Ca^{2+} flux assay.

Compounds with (*R*)-pyrrolidin-3-ol (**1e**) and morpholine (**1h**) on the 2-position of the quinoline moiety provided the most favorable combination of potency at both the human and mouse isoforms of the P2X7 receptor. Interestingly, (*S*)-pyrroldin-3-ol **1f** showed a 4 fold reduction in potency when compared to **1e**, indicating the absolute stereochemistry of the pyrrolidin-3-ol influences antagonist potency. Cyclopropylamine **1c** was also shown to inhibit Ca^{2+} flux at both human and mouse isoforms. The 2-aminoquinoline **1a** was a highly potent antagonist of human P2X7 but was significantly less potent at the mouse receptor. Aside from (*R*)-pyrrolidin-3-ol **1e**, incorporating other aminoalcohols (**1d** and **1g**) and an N-methylpiperazine, **1i**, resulted in antagonists with modest potency.

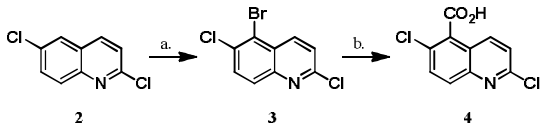
Table 1: IC_{50} data for compounds **1a-i**



Compound	R =	Human P2X7 IC_{50} (nM)[a]	Mouse P2X7 IC_{50} (nM)[a]
1a		9±7	1091±31
1b		82 (n=1)	305±21 (n=2)
1c		64±43	123±32
1d		78 (n=1)	ND
1e		35±29	80±29
1f		151 (n=1)	ND
1g		120 (n=1)	ND
1h		25±17	103±59
1i		85±80 (n=2)	486±375 (n=2)

[a]IC_{50}s were determined using a FLIPR Ca^{+2} flux assay. The values are the average of at least three experiments, unless otherwise noted. h denotes human and m denotes mouse.

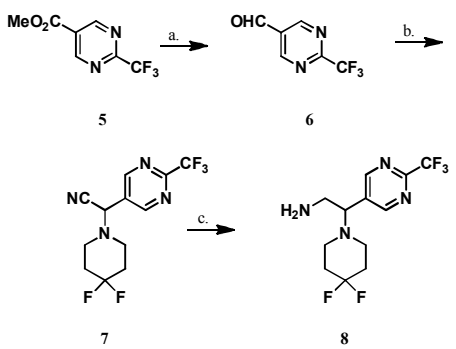
The compounds detailed in Table 1 were prepared using the reaction sequences shown in Schemes 1-3. The synthesis of these antagonists was highly convergent, scalable and allowed for diversification in the ultimate transformation of the synthesis. The requisite 2,6-dichloroquinoline-5-carboxylic acid (**4**, Scheme 1) was prepared by the treatment of commercially available 2,6-dichloroquinoline (**2**) with $AlCl_3$ and bromine to provide 2,6-dichloro-5-bromoquinoline (**3**). Treatment of resulting 2,6-dichloro-5-bromoquinoline (**3**) with isopropyl magnesium chloride followed by carbon dioxide completed the synthesis of 2,6-dichloroquinoline-5-carboxylic acid **4**.[12]



2 **3** **4**

Scheme 1: Synthesis of compounds **1a-1i**: a. $AlCl_3$, Br_2, 83%; b. *i*PrMgCl, CO_2, THF, 0 °C → rt, 74%.

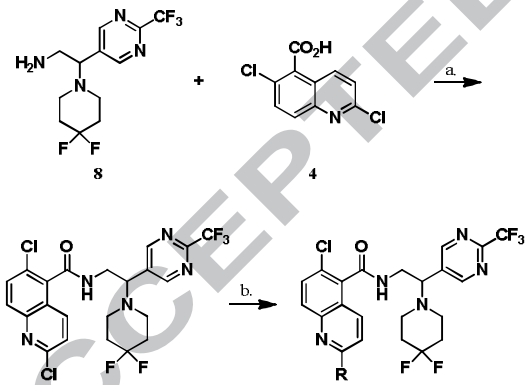
Synthesis of 2-(4,4-difluoropiperidin-1-yl)-2-(2-(trifluoromethyl)pyrimidin-5-yl)ethanamine (**8**) commenced from commercially available methyl-2-(trifluoromethyl)pyrimidine-5-carboxylate (**5**) which was reduced to the aldehyde (**6**) by treatment with DIBAL. The aldehyde was subjected to Strecker reaction conditions to provide nitrile **7** in good yield. The reduction of nitrile **7** to the requisite 2-(4,4-difluoropiperidin-1-yl)-2-(2-(trifluoromethyl)pyrimidin-5-yl)ethanamine (**8**) was accomplished in good yield by treatment with Raney Nickel under an H_2 atmosphere.



5 6 7 8

Scheme 2: Synthesis of compounds **1a-1i**: a. DIBAL, -78 °C, DCM; b. 4,4-difluoropiperidine hydrochloride, Me_3SiCN, acetic acid, 45% over 2 steps; c. Raney Nickel, 7N NH_3 in methanol, H_2 (45 psi), MeOH, 98%.

Coupling of the 2,6-dichloroquinoline-5-carboxylic acid (**4**) and 2-(4,4-difluoropiperidin-1-yl)-2-(2-(trifluoromethyl)pyrimidin-5-yl)ethanamine (**8**) occurred in the presence of (benzotriazol-1-yloxy)tris(dimethylamino)-phosphonium hexafluorophosphate (BOP) to provide compound **9.** The completion of the synthesis was accomplished through a SNAr on the 2-chloro moiety under standard SNAr conditions employing the desired amine.



8 4 9 1a-1

Scheme 3: Synthesis of Compounds **1a-1i**: a. BOP, NEt_3, DCM, 85%; b. amine, NEt_3, MeCN, 90 °C, 15-95%.

Compounds showing the most favorable potency in the Ca^{2+} flux assay were further profiled in mouse and human P2X7 whole blood assays. The whole blood assays are conducted in undiluted whole blood to best mimic an *in vivo* environment and measure the attenuation of P2X7 mediated IL-1β release initiated by LPS challenge and subsequent stimulation by BzATP.[13] The whole blood assay results are shown in Table 2.

A rightward shift in potency was observed for all compounds tested in the whole blood P2X7 assays with respect to the Ca^{2+} flux which can be attributed to the inclusion of plasma protein inherent in the undiluted whole blood. Compound **1e** was most effective at attenuating the release of IL-1β in both human and mouse isoforms (Table 2). Both compounds **1c** and **1h** were shown to have moderate human and mouse whole blood potencies.

Table 2: P2X7 whole blood assay data‡

Compound	Human $P2X_7$ WB IC_{50} (nM)†	Mouse $P2X_7$ WB IC_{50} (nM)†
1c	141	631
1e	87	316*
1h	376	631

† The values are the mean of at least two experiments, unless otherwise noted. * Single data point. ‡ Mouse blood obtained for male C57/bl6 mice from Charles River Laboratories

The reasonable mouse whole blood potency of compound **1e** prompted additional profiling to determine its utility as a tool compound to explore mouse *ex vivo* pharmacodynamic and *in vivo* efficacy models. A mouse tissue distribution study showed compound **1e** is orally bioavailable and restricted to the periphery (Table 3). A 50 mg/kg oral dose resulted in high plasma concentrations (6415 ng/mL) and negligible compound concentrations (21 ng/mL) in the brain. The high plasma concentrations and lack of CNS exposure upon oral dosing coupled with its reasonable mouse whole blood potency suggested compound **1e** could serve as an adequate tool compound to explore the role of peripherally localized P2X7 receptors in pain and inflammation.

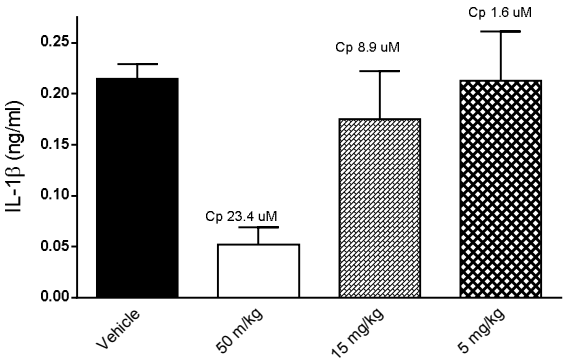
Table 3: Mouse tissue distribution for **1e**

Compound	PO Dose	Plasma[b] (ng/mL)	Brain[b] (ng/mL)
1e	50.0 mg/kg[a]	6416	21

[a] vehicle = 30% SBECD 5 mL/kg, p.o., n = 5, [b]data collected at 4 hours

Compound **1e** was taken into a mouse P2X7 *ex vivo* IL-1β reduction assay where it was shown to attenuate the release of IL-1β dose dependently (5, 15 and 50 mg/kg, oral dose) with a robust reduction observed at the 50 mg/kg dose (Figure 2). Two hours post dose blood was collected and IL-1β release was measured in response to LPS/BzATP treatment. In addition to the robust attenuation of IL-1β at the 50 mg/kg dose, a modest reduction was also observed at the 15 mg/kg dose. No significant reduction in IL-1β was observed at the 2 mg/kg dose.

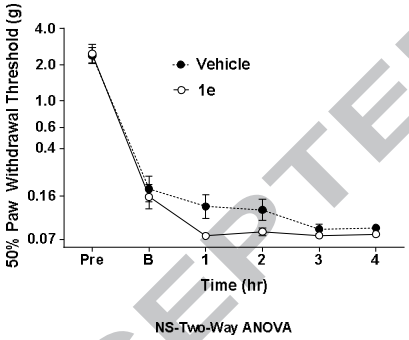
Figure 2: Mouse ex vivo IL-1β Reduction



Male C57/bl6 mice obtained from Charles River Laboratories were employed in this study. vehicle = 30% SBECD 5 mL/kg, p.o., n = 8, data collected at 2 hours post dose

The robust reduction in IL-1β observed in the mouse *ex vivo* model at the 2 hour time point with a 50 mg/kg dose prompted the progression of compound **1e** to a mouse spared nerve injury (SNI) model of tactile allodynia (Figure 3).[14] The time frame of the SNI model was designed to correspond to the *ex vivo* IL-1β reduction model to ensure target coverage for a majority of the experimental paradigm. Paw withdrawal thresholds were measured before and after induction of nerve injury and again after compound **1e** was administered orally. Measurements were recorded every hour for 4 hours and at no point were significant analgesic effects observed. Similarly compound **1e** produced no anti-inflammatory or analgesic effects in the carrageenan-induced model of paw edema at the same oral dose of 50 mg/kg (data not shown).

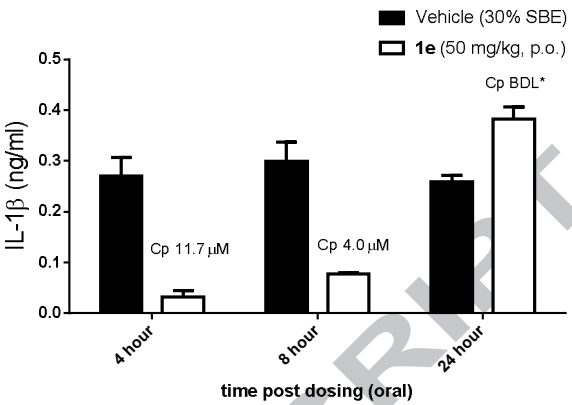
Figure 3: Mouse SNI tactile allodynia



vehicle = 30% SBECD 5 mL/kg, p.o., n = 8; **1e** 50 mg/kg, p.o, n = 8. Male C57/bl6 mice obtained from Charles River Laboratories were employed in this study.

In addition the robust attenuation of IL-1β observed at the 2 hour time point, a 50 mg/kg oral dose of compound **1e** was also shown to sustain IL-1β reduction over an 8 hour period (Figure 4). At the 24 hour time point compound **1e** was no longer detectable and hence no attenuation of IL-1β release was observed. Therefore b.i.d. dosing of compound **1e** was employed to maintain sustained levels of IL-1β reduction over 24 hours making compound **1e** amenable to evaluation in the mouse collagen-induced arthritis (CIA) model.[15] We postulated the sustained attenuation of IL-1β release under disease state conditions may be required to see the therapeutic effect of a P2X7 antagonist.

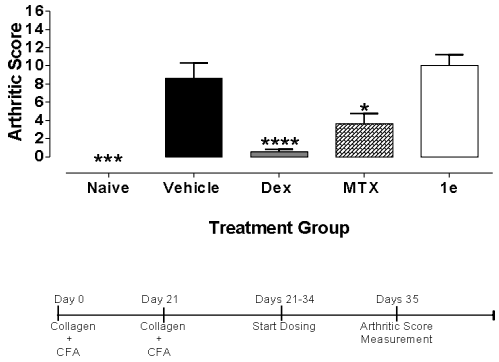
Figure 4: Duration of action for compound **1e**



Male C57/bl6 mice obtained from Charles River Laboratories were employed in this study. *BLQ = below detectable levels

Compound **1e** was evaluated in the mouse CIA at a 50 mg/kg b.i.d. oral dose. The results of this study are shown in Figure 5. Animals treated with collagen and CFA for 21 days developed a significant and pronounced arthritic like condition which was reversed with dexamethasone and methotrexate. Administration of compound **1e** commenced on day 21 and continued through day 34. Arthritic scores were measured on day 35. The arthritic scores of animals treated with compound **1e** did not differentiate from those of vehicle.

Figure 5: Mouse collagen-induced arthritis model*



One-Way ANOVA overall, ****p<0.0001; Bonferonni post-tests: * = p <0.05, *** = p<0.001, ****p<0.0001; naïve n = 5, veh. n = 11, Dex n = 11, MTX n = 12, **2e** n = 12; dosing vehicle is 30 % SBE 5 mL/kg, p.o. b.i.d.; Dex = dexamethasone 0.3 mg/kg, p.o. q.d.; MTX = methotrexate 30 mg/kg, i.p. 2x per week; **2e** 50 mg/kg, p.o. b.i.d. *Female DBA mice obtained from Charles River laboratories were employed in this study.

We have identified a novel series of P2X7 antagonists with potency in both calcium flux and whole blood assays at both human and mouse isoforms. The lead compound of this series, **1e**, is a good *in vivo* tool compound to address the role of peripheral P2X7 in animal models of inflammation and pain; as such it was shown to significantly attenuate the release of IL-1β with a 50 mg/kg dose for up to 8 hours. Surprisingly, even though **1e** suppressed IL-1β release, it was ineffective in providing an analgesic or anti-inflammatory response in the mouse SNI model and in the carrageena-induced model of paw edema after a single oral 50 mg/kg dose. In addition, compound **1e** did not reduce the arthritic scores in the mouse CIA model after 14 day continuous 50 mg/kg b.i.d. dosing. Combined these

results indicate that blockade of peripheral P2X7 mediated IL-1β release is not sufficient for efficacy in the animal models of pain and inflammation.

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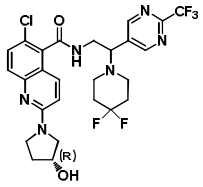

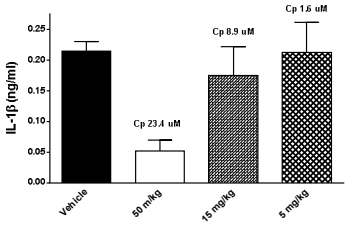

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