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Discovery, synthesis and SAR of azinyl- and azolylbenzamides antagonists of the P2X$_7$ receptor

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ABSTRACT

The discovery, of a series of 2-Cl-5-heteroaryl-benzamide antagonists of the P2X$_7$ receptor via parallel medicinal chemistry is described. Initial analogs suffered from poor metabolic stability and low Vd$_{ss}$. Multi parametric optimization led to identification of pyrazole **39** as a viable lead with excellent potency and oral bioavailability. Further attempts to improve the low Vd$_{ss}$ of **39** via introduction of amines led to analogs **40** and **41** which maintained the favorable pharmacology profile of **39** and improved Vd$_{ss}$ after iv dosing. But these analogs suffered from poor oral absorption, probably driven by poor permeability.

The P2X$_7$ receptor is a member of the purinoreceptor superfamily; this family includes both G-protein coupled P2Y receptors and ionotropic P2X receptors, a group of ligand-gated ion channels.[1] The P2X$_7$ receptor has been implicated in a number of disease states such as pain, neurodegeneration, rheumatoid and Oseteoarthritis, making it a very attractive target for therapeutic intervention.[2,3] Herein, we describe the discovery of azinyl and azolylbenzamides, which are potent antagonists of the P2X$_7$ receptor.

High throughput screening of the Pfizer compound file identified a number of hits with compound **1** being the most attractive. Medium speed analoging efforts led to the 2-chlorophenethyl derivative **2** as a potent and orally bioavailable lead. Further optimization of **2** led to the clinical candidate **CE-224,535 (3)** (Fig. 1).[4]

In spite of its excellent attributes the 6-azauracil series had an intrinsic liability characterized by low Vd$_{ss}$ in dog and monkey which resulted in a short predicted half-life in humans. To overcome these liabilities, we initiated parallel efforts to find alternate modalities for the 6-azauracil ring of **2**, which we felt was a primary contributor to the observed short half-life.[5] We envisioned that a parallel medicinal chemistry approach would offer an optimal opportunity to identify a replacement for the 6-azauracil in our lead series. Retrosynthetically we hypothesized that a readily accessible boronate such as **5** would allow for the parallel preparation of bi-aryl amides **A** via a Suzuki reaction (Scheme 1).

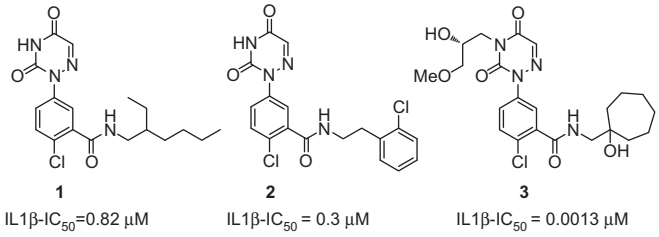
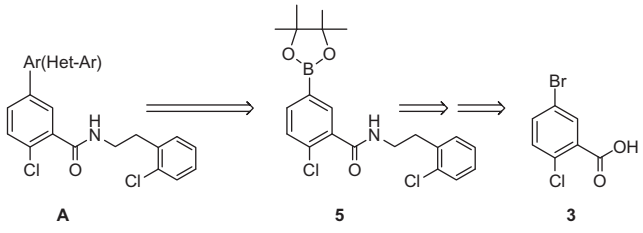


Figure 1. Lead structures and candidate CE-224535.



Scheme 1. Proposed synthesis of targets.

Thus reaction of acid **3** with *o*-Cl-phenethylamine under standard coupling conditions yielded the amide **4** in excellent yield.

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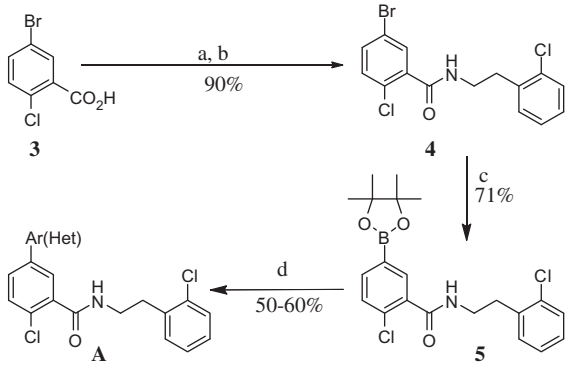


Scheme 2. Synthesis of 2-Cl-5-aryl benzamides. Reagents: (a) $SOCl_2/\Delta$; (b) *o*-Cl-phenythylamine, DIEA, DCE; (c) bis-pinacolatodiboron, KOAc, $PdCl_2(DPPF)$, DMF, Δ; (d) ArX, $PdCl_2(DPPF)$, 2.0 M Na_2CO_3, DMF.

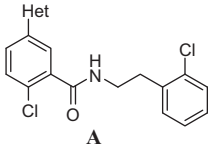
Conversion of **4** to the boronate **5** was accomplished by reaction with bis-pinacolatodiboron in presence of $PdCl_2(DPPF)$.[6] Finally Suzuki–Miyaura coupling[6] of **5** with a variety of aryl and heteroaryl halides led to the desired targets in modest isolated yield after high throughput purification via reverse phase HPLC (Scheme 2).[7]

Analogs from this array were evaluated for the ability to inhibit the release of lL-1β from monocytes stimulated by ATP,[8] The activity spanned a range from 500 nM to double digit μM. The 2-pyridyl analog **6** was the most potent, with activity similar to that of the lead **2**. Removal of the methyl group from **6** (compound **7**) led to a 10-fold drop in potency. The 3-pyridyl and 4-pyridyl analogs (**8** and **9**) were much less potent. Replacement of the methyl group in **6** with CF_3 (compound **10**) led to considerable loss of activity. Walking the methyl group around the pyridyl ring in **6** (compounds **11**–**13**) also led to a substantial drop in potency. Replacing the methyl group of **6** with either chloro or primary amide (**14** and **15**) led to 5- to 7-fold loss in activity. Replacing the primary amide with other electron withdrawing groups such as cyano or methyl ketone (**16** and **17**), however, led to a considerable loss in activity. Reduction of the ketone to the secondary alcohol (**18**) restored most of the activity. The pyridazine **19** and **20** were 2- to 3-fold less potent than **6** while the C-linked 6-azauracil analog **21** was 10-fold less potent (Table 1).

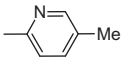
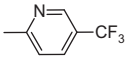
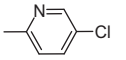
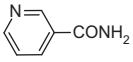
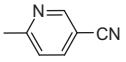
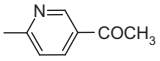
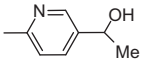
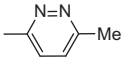
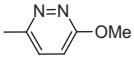
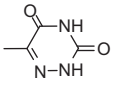
Although we were able to find some alternatives for the 6-azauracil portion of **2**, these analogs all suffered from relatively high lipophilicity. This was reflected in their high in vitro microsomal clearance.[9] To address these issues, we embarked on an analoging effort focused on reducing the lipophilicity of these analogs and at the same time improving the activity. To lower lipophilicity, we borrowed from the work in the 6-azauracil series, and replaced the *o*-Cl-phenethyl side chain of the potent analogs described in Table 1 with 1-hydroxycycloheptane.[3] Simultaneously we also explored varying the heterocycle portion of compound **6**. The synthesis of these analogs is depicted in Schemes 3 and 4. Thus the readily accessible boronate **25** was converted to the desired targets by a sequence that involved Suzuki reaction with the heteroaryl halide followed by saponification of the ester and amide coupling with the cycloheptanol amine (Scheme 3).

Preparation of the pyrazole targets **36**–**38** involved intermediacy of the methyl ketone **34**. Thus Sonogashira reaction[10] of bromide **23** with trimethylsilyl acetylene followed by acid catalyzed hydration with formic acid[11] led to the methyl ketone **34** which was converted to pyrazoles **36** and **39** via the enamine **35**. Finally alkylation of pyrazole NH led to the N-methyl analogs **37** and **38** (Scheme 4).

Table 1
SAR for Aryl analogs of compound **2**

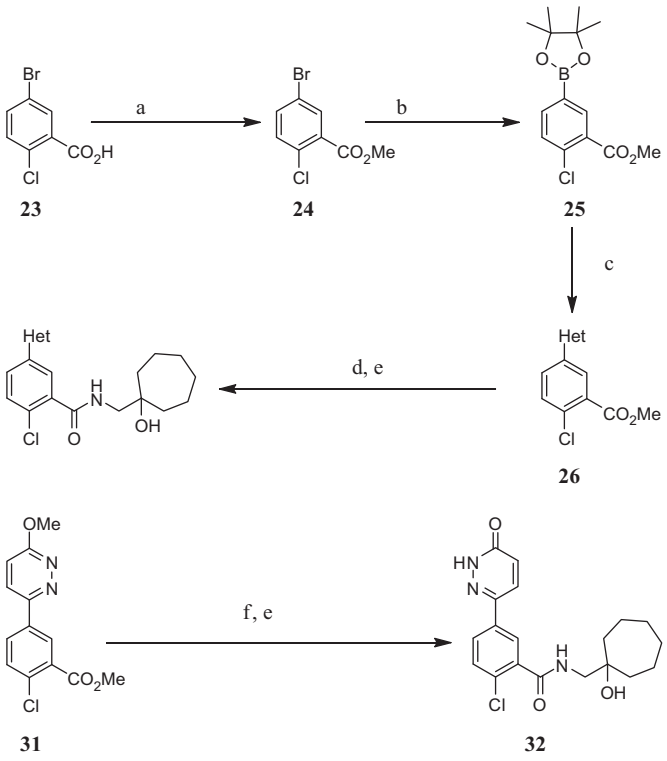


A

Compound	Ar=	*c* log *P*	Monocyte IC_{50}[a] (μM)
6		4.42	0.11
7		4.42	1.60
8		4.21	4.8
9		4.21	5.2
10		5.44	4.6
11		4.92	0%inh@1.0 μM
12		4.62	0%inh@1.0 μM
13		4.92	0%inh@1.0 μM
14		5.21	0.46
15		3.57	0.72
16		4.04	0%inh@1.0 μM
17		4.25	0%inh@1.0 μM
18		3.69	0.668
19		3.73	0.25
20		3.89	0.49
21		2.51	1.05

[a] Inhibition of the stimulation of the release of lL-1β from monocytes by ATP
[b] Data is for $n = 2$.

As predicted, a number of these analogs either maintained or improved the activity of the corresponding *o*-Cl phenethyl amides and also improved human microsomal clearance. The pyridyl analog **27** was equipotent to the analog with a 6-azauracil head group.[3] Pyridazinone **32**, which mimics the 6-azauracil in compound **2** was threefold less potent. The five-membered analogs (**28–30**, **36–39**) in general were more potent than the six-membered heterocyclic analogs probed. *N*-Methylation of pyrazole **36** led to a drop in activity with the *N*-2 isomer **37** more potent than the *N*-1 analog **38**. The regioisomeric analog where the C5-position has been methylated (compound **39**) provided an unexpected

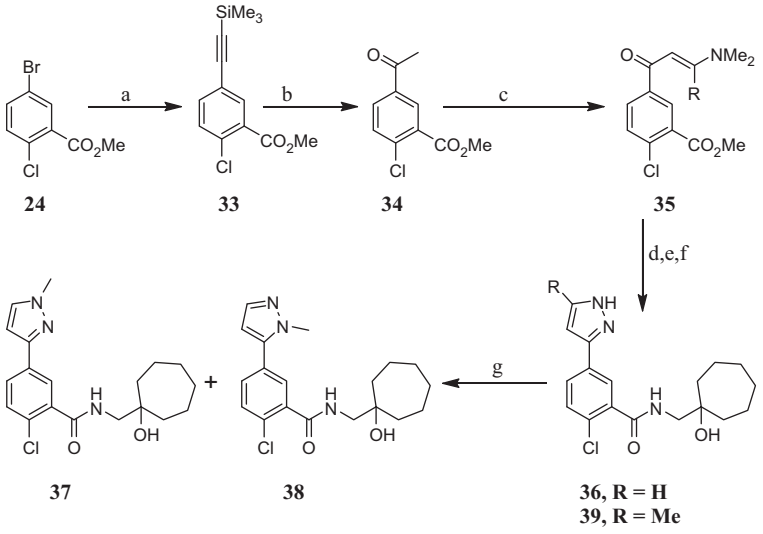


Scheme 3. Preparation of cycloheptanol analogs. Reagents and conditions: (a) HCl, MeOH, 98%; (b) bis-pinacalotodiboron, KOAc, $PdCl_2$(DPPF), DMF, Δ, 60%; (c) Het-X, $Pd(PPh_3)_4$, Cs_2CO_3, 4 Å sieves, dioxane, 80 °C, 50–60%; (d) NaOH, MeOH, 100%; (e) 1-(aminomethyl)cycloheptanol, HOBT, EDCI, TEA, DMF, 60–70%; (f) HBr, Δ.

20-fold boost in activity while maintaining a favorable $t_{1/2}$ in the HLM assay (Table 2). Selected compounds were also tested for their ability to inhibit uptake of YOPRO-1 by ATP-treated $P2X_7R$ over-expressing HEK293 cells to provide direct evidence of $P2X_7R$ impact.[12] A number of these analogs were evaluated for their ability to inhibit LPS/ATP-induced IL-1β release in diluted human blood.[8] As shown in Table 2, the activity in this assay was 2- to 15-fold less potent than that observed in low serum monocyte assay.

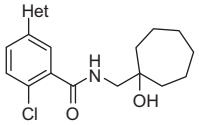
Compound **39** emerged as a viable lead from the non 6-azauracil series and was profiled further (Fig. 2). It showed good oral bioavailability in rats. The exquisite selectivity of compound **39** for human receptor versus murine/rat precluded its evaluation in standard rodent models of inflammation. However, an in vivo model was developed that allowed a pharmacodynamic assessment to be conducted. In this model, LPS-activated human monocytes are implanted into the peritoneal cavity of Balbc mice. Subsequently, these animals are dosed orally with test compound, followed 60-min later by an ip injection of ATP to promote IL-1β posttranslational processing. IL-1β released extracellularly is measured using a human-specific ELISA. In this model, **39** is a very effective inhibitor of ATP-induced IL-1β output (Fig. 3) after an oral dosing of 4 and 20 mpk. Unfortunately, compound **39** suffered from the same low Vd_{ss} and short $t_{1/2}$ observed for the 6-azauracil analogs **2**. We believed that these shortcomings could be addressed by improving overall polarity of the molecule. Specifically we hypothesized that introduction of strategically placed basic amino substituent could overcome the low volume of distribution liability of **39**.[13] In silico tools predicted such amino substituents would provide a 4- to 6-fold increase in Vd_{ss} while maintaining the favorable clearance profiles. Synthesis of the amino substituted analogs is shown in Scheme 5. Compound **39** was alkylated with chiral epoxy nosylate using a phosphazene base[14] followed by opening of the intermediate epoxide with ammonia and provided the desired amines **40**, **41** (Scheme 5).

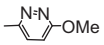
Amino alcohols **40** and **41** were very potent in inhibiting the release of IL1-β from monocytes both in the presence and absence of human blood (IC_{50} = <0.005 μm). In addition, introduction of the polarity also improved their microsomal clearance (HLM $t_{1/2}$ >120 min). Unfortunately, these modifications also led to poorly permeable analogs as evidenced by their poor flux in Caco-2 cells and this was reflected in poor oral bioavailability of **40** in rats (Fig. 4). We did, however, establish that introduction of the amino groups improved Vd_{ss} and $t_{1/2}$ of these analogs upon iv dosing. Modulating the basicity of the terminal amine and reducing the number of H-bond donors[15] in **40** may provide a path forward to improve the oral bioavailability of these analogs.

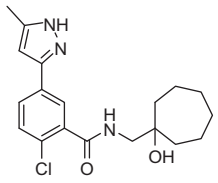


Scheme 4. Synthesis of pyrazoles. Reagents: (a) Trimethylsilylacetylene, $PdCl_2(PPh_3)_2$, PPh_3, CuI, TEA, 98%; (b) 98% formic acid, Δ, 75%; (c) *N*,*N*-dimethylformamide dimethyl acetal (or) *N*,*N*-dimethylacetamide dimethyl acetal, Δ, 90%; (d) hydrazine hydrate, THF, EtOH; (e) KOH, MeO; (f) 1-(aminomethyl)cycloheptanol, HOBT, EDCI, TEA, DMF, 60–70 % over three steps; (g) NaH, MeI (for R = H in **36**).

Table 2
Activity of cycloheptanol analogs



Compound	Ar=	*c* Log *P*	Monocyte IC_{50} (μM)	$P2X_7R$ Yo-Pro IC_{50} (μM)	Whole blood IC_{50} (μM)	HLM $t_{1/2}$ (min)
27		4.13	0.095	0.031	0.514	34.7
28		2.96	0.51		NT	85
29		3.96	0.28	0.006	NT	NT
30		3.39	0.24		NT	22.3
31		3.10	0.28	0.028	0.51	77
32		1.71	0.93	0.18	NT	NT
36		3.26	0.18	0.012	0.61	12
37		3.22	0.238		0.44	3.7
38		3.22	>1.0	0.266	NT	NT
39		3.53	0.007	0.003	0.121	75



39

Monocyte IC_{50} = 0.007 μM
Yo-Pro IC50 = 0.003 μM
Whole Blood IC_{50} = 0.121 μM
HLM $t_{1/2}$ = 75 min
Papp($X10^{-6}$/s) = 26
Rat in-vivo PK:
VD_{ss} = 0.7 L/kg
$t_{1/2}$ = 0.7 h
%F = 59

Figure 2. In vitro and PK profile of **39**.

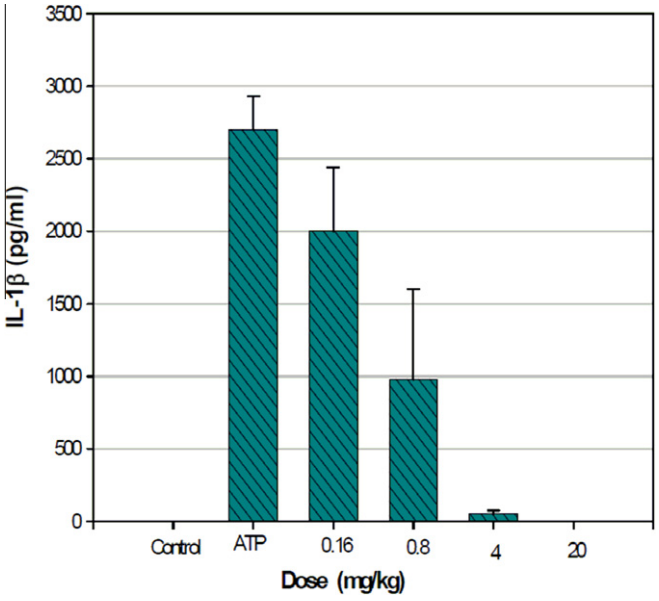
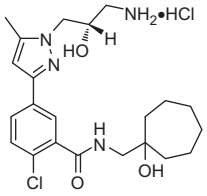
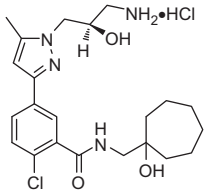


Figure 3. In vivo activity of **39** in murine implant model.



40

logP = 2.6 logD = 1.6
IL-1β IC50 (μM) = 0.002
Yo-Pro IC50 (μM) = 0.0005
Whole Blood IC50 (μM) = 0.003
HLM = >120min
Caco-2 A→B = $2.98x10^{-6}$ cm/sec
MDR A→B = $0.26x10^{-6}$ cm/sec
MDCK B→A = $2.39x10^{-6}$ cm/sec
MDCK/MDR1: 11.5
Rat in-vivo PK:
VD_{ss} = 11.7, $t_{1/2}$ = 8.36 h, %F = 9

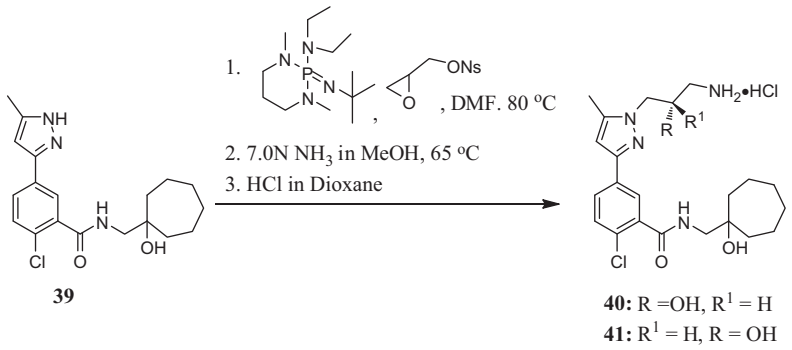


41

logP = 2.6 logD = 1.6
IL-1β IC50 (μM) = 0.007
Yo-Pro IC50 (μM) = 0.003
Whole Blood IC50 (μM) = 0.01
HLM = >120 min
Caco-2 A→B = $10.5x10^{-6}$ cm/sec
MDR A→B = $0.26x10^{-6}$ cm/sec
MDCK B→A = $2.2x10^{-6}$ cm/sec
MDCK/MDR1: 10.5

Figure 4. Profile of amino alcohols **40** and **41**.

In summary, a new series of potent $P2X_7$ receptor antagonists has been discovered. The neutral analogs in this series possessed good properties and oral bioavailability, but were hampered by a low volume of distribution coupled with a moderate clearance which led to a short half-life in vivo. Attempts to overcome these issues by introduction of amino substituents improved these attributes. Unfortunately this change led to analogs with less than optimal oral absorption, presumably due to the negative changes in permeability. Future endeavors will need to address the balance of these properties for successful identification of a development candidate.[16]



Scheme 5. Preparation of amino alcohols.

Acknowledgments

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5. We hypothesized that the weakly acidic nature of compound **2** may be the primary contributor to the observed low Vd_{ss}.
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8. Analogs were evaluated as antagonists of the $P2X_7$ R by assessing whether they inhibited the release of IL-1β from LPS activated human monocytes maintained in the presence of low serum. Promising compounds were re-evaluated in an analogous assay, employing human whole blood samples sequentially treated with LPS and ATP. For assay protocols, see: Duplantier, A. J.; Subramanyam, C. WO 2,003,042,191, 2003.
9. Most of these analogs had in vitro clearance of >100 μl/min/mg.
10. Chinchilla, R.; Najera, C. *Chem. Rev.* **2007**, *107*, 874.
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