Bioorganic & Medicinal Chemistry Letters 21 (2011) 3708–3711

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Optimization of the physicochemical and pharmacokinetic attributes in a 6-azauracil series of $P2X_7$ receptor antagonists leading to the discovery of the clinical candidate CE-224,535

Allen J. Duplantier *, Mark A. Dombroski, Chakrapani Subramanyam, Aimee M. Beaulieu, Shang-Poa Chang, Christopher A. Gabel, Crystal Jordan, Amit S. Kalgutkar, Kenneth G. Kraus, Jeff M. Labasi, Christopher Mussari, David G. Perregaux, Rick Shepard, Timothy J. Taylor, Kristen A. Trevena, Carrie Whitney-Pickett, Kwansik Yoon

Pfizer Global Research and Development, Groton Laboratories, Eastern Point Road, Groton, CT 06340, USA

ARTICLE INFO

Article history:
Received 7 March 2011
Revised 15 April 2011
Accepted 19 April 2011
Available online 24 April 2011

Keywords:
$P2X_7$
Antagonist
Rheumatoid arthritis
6-Azauracil
Pharmacokinetic
Log *P*

ABSTRACT

High throughput screening (HTS) of our compound file provided an attractive lead compound with modest $P2X_7$ receptor antagonist potency and high selectivity against a panel of receptors and channels, but also with high human plasma protein binding and a predicted short half-life in humans. Multi-parameter optimization was used to address the potency, physicochemical and pharmacokinetic properties which led to potent $P2X_7R$ antagonists with good disposition properties. Compound **33** (CE-224,535) was advanced to clinical studies for the treatment of rheumatoid arthritis.

© 2011 Elsevier Ltd. All rights reserved.

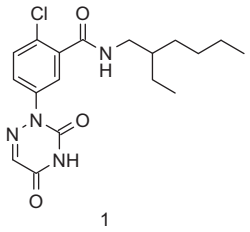
The P2X family of purinoreceptors are ligand gated ion channels of which there are seven known receptors ($P2X_1$–$P2X_7$) with 30 – 50% amino acid sequence identity.[1] The largest of the group, the $P2X_7$ receptor ($P2X_7R$), is expressed in the periphery on immune cells and in the CNS on microglia and astrocytes and has been shown to be linked to glutamate release.[2] Because of its role in interleukin-1 (IL-1) posttranslational processing,[3] this receptor has been the target of drug discovery efforts in the treatment of inflammatory diseases, pain[4] and neurodegenerative diseases such as Alzheimer's disease (AD).[5]

Activation of the $P2X_7R$ on leukocytes leads to opening of a non-selective cation channel which in turn can lead to complete depolarization of the membrane potential.[6] Within seconds of opening, the $P2X_7R$ channel transitions to a pore state that is permeable to large organic molecules, such as YoPro Yellow.[7] This feature, which is a hallmark of the $P2X_7R$, creates a state of osmotic imbalance and can cause LPS-activated monocytes and macrophages to die. Recent studies have shown that $P2X_7R$ activation is associated with activation of the inflammasome, an assembly of cytoplasmic proteins that facilitates caspase-1 activation and, in turn, proteolytic processing of proIL-1β and release of the mature cytokine to the medium. This unique type of programmed cellular death response resulting in the release of proinflammatory mediators has been termed pyroptosis[8] to distinguish it from a non-inflammatory apoptotic process. Thus, a $P2X_7R$ antagonist should block IL-1 post-translational processing and lead to a reduction in the release of IL-1β. In support of this hypothesis, studies using $P2X_7R$ knockout mice have shown that absence of the $P2X_7R$ leads to an inability of macrophages and monocytes to generate and release mature IL-1β in response to ATP challenge.[9] In vivo, $P2X_7R$-deficient mice demonstrated less severe outcomes in anticollagen-induced arthritis[9] and formalin-induced pain disease models.[4] In efforts to test the hypothesis of whether a $P2X_7R$ antagonist would improve the signs and symptoms of rheumatoid arthritis (RA) in humans, we sought to discover an orally active clinical candidate that could be safely administered to humans at doses capable of achieving free systemic concentrations above its IC_{90} for blocking IL-1β release. The high bar of achieving >90% inhibition of IL-1β in the clinic was set in efforts to improve upon the efficacy of anakinra, a natural IL-1 receptor antagonist for the treatment of RA.

While several small molecule $P2X_7R$ antagonists have been reported in the literature,[10] our efforts began with high throughput screening of our compound file against the $P2X_7R$ using a YOPRO-1

* Corresponding author.
E-mail address: allen.j.duplantier@pfizer.com (A.J. Duplantier).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.04.077



$P2X_7R$ Yo-Pro IC_{50} = 1.1 μM
IL-1β IC_{50} = 0.8 μM
MW = 379
cLog P = 4.45

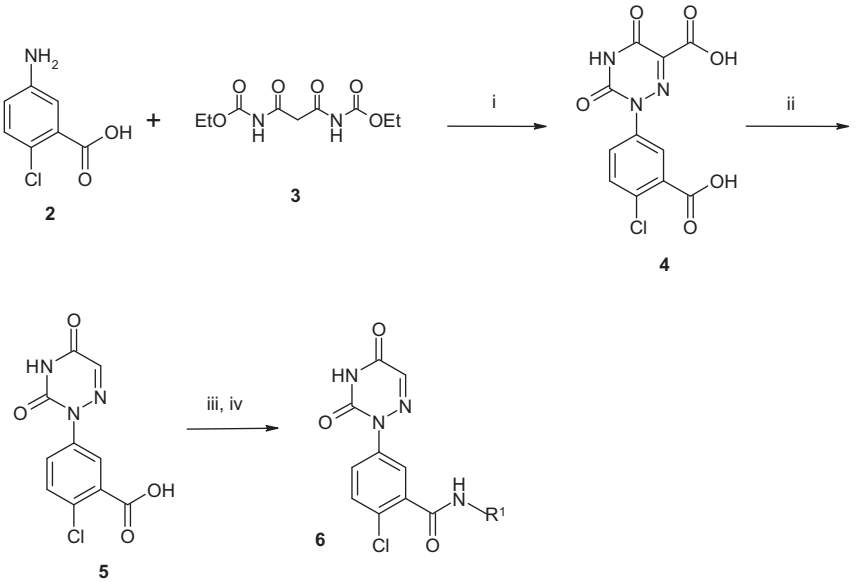
Figure 1. Properties of compound **1**.

fluorescence assay[11] and uncovered compound **1** as a novel hit. Although compound **1** had moderate $P2X_7R$ antagonist activity, and relatively low MW it did have moderately high *c* Log *P* (Fig. 1), and thus our first goal was to improve potency and lower *c* Log *P* simultaneously. Initial efforts towards this goal were to explore the amide region via parallel chemistry. Thus acid **5** was coupled with a variety of readily available amines (Scheme 1) and biological testing[11] of the resulting amides (**6**) provided a wealth of SAR information.

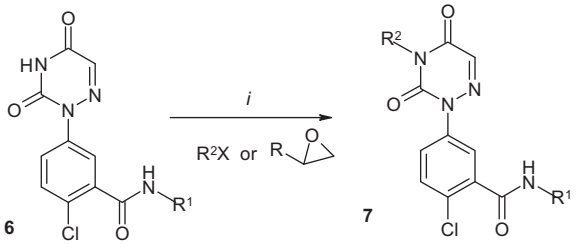
The synthesis of 1-phenyl-6-azauracils has been reported, and using appropriate modifications of the literature procedures,[12] the preparation of compound **1** and its analogs began with diazotization of 2-chloro-5-aminobenzoic acid (**2**) (Scheme 1). Addition of the diethylcarbamate of malonic acid (**3**) to the diazonium salt of **2** at room temperature followed by heating sequentially in the presence of sodium acetate and then sulfuric acid provided the 1-aryl-6-azauracil-5-carboxylate **4** in moderate yield (46%). Subsequent decarboxylation with sodium hydroxide in mercapto-acetic acid provided intermediate **5**. The carboxylic acid moiety of **5** was readily esterified or converted to a variety of amides (**6**) via acylation, and the 3-position of the 6-azauracil ring could then be alkylated with either alkyl halides in the presence of base, or by epoxides by heating in DMF without the addition of base to provide analogs **7** (Scheme 2).

The necessity of the amide NH moiety of compound **1** was confirmed by the dramatic loss in activity when it was either methylated (**8**) or replaced with oxygen (**9**) (Table 1). Likewise, the *ortho*-chloro substituent of **1** was also shown to be important as replacement with hydrogen (**10**) greatly diminished the blocking of IL-1β release. The 2-ethyl-1-hexyl substituent of **1** could be reduced in size to 2-ethyl-1-butyl (**11**), *n*-hexyl (**12**) or *n*-pentyl (**13**) without loss of activity, but the *n*-butyl group (**14**) and other 1-4 carbon alkyl groups (data not shown) did not appear large enough to adequately fill the lipophilic pocket (IL-1β IC_{50} >5 μM) (Table 2). Tying the alkyl side chain of **11** into a cyclohexyl ring (**15**) did not affect potency, but surprisingly the analogous cyclopentyl analog (**16**) was much less active, and direct attachment of carbocycles to the amide nitrogen led to inactivity (data not shown). In efforts to reduce lipophilicity, an oxygen atom was walked around the aforementioned active analogs (**17**–**19**), but this change led to loss of activity. Next, the amide side chain was replaced with benzyl (**20**) and then homologated out to phenethyl (**21**) and phenylpropyl (**22**). Of these, only compound **21** had an IL-1β IC_{50} under 5 μM. A variety of subtituents were substituted around the phenethyl moiety (data not shown) and 2-chlorophenethyl (**23**) emerged as a viable lead (IL-1β IC_{50} = 0.29 μM). Compound **23** was equipotent in the YOPRO assay (IC_{50} = 0.3 μM), but was inactive in the presence of human blood (IL-1β IC_{50} >10 μM), likely due to high human plasma protein binding of > 99.5%. However, the in vitro ADME profile of **23** was favorable [little to no turnover in human liver microsomes and human hepatocytes, excellent absorptive permeability (CACO P_{app} AB = 10×10^{-6} cm/sec, BA = 21×10^{-6} cm/sec)], and an in vivo rat pharmacokinetic (PK) study showed decent oral bioavailability (*F* = 44%), consistent with the observed low plasma clearance (*CLp* = 9.5 mL/min/kg).

Our follow-up strategy for compound **23** was to further improve potency through amide side chain manipulation while reducing plasma protein binding through further reduction of *c* Log *P*. In efforts toward that end, the systematic insertion of nitrogen atoms around the phenethyl moiety of **21** lowered lipophilicity, but unfortunately led to a substantial loss in activity (compounds **24–26**). However, sytematically inserting polar groups, such as hydroxyl and cyano around the cyclohexyl ring of **15** gave some promising results. While hydroxylation at the 2-, 3- and 4-position of the cyclohexyl ring led to a significant loss in activity (data not shown), quaternary substitution at the 1-position provided an increase in activity and a decrease in *c* Log *P* (e.g., compound **27**,

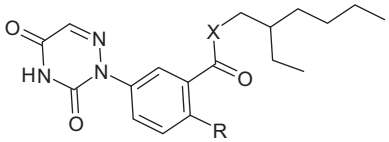


Scheme 1. Reagents and conditions: (i) AcOH, 12 N HCl (3.3 equiv), $NaNO_2$, 10 ~ 15 °C, 30 min; then NaOAc (1 equiv), addition of compound **3**, 10 °C–rt, 80 min; then NaOAc (2 equiv), reflux, 6 h; then 50% H_2SO_4, reflux, 2 h (one pot, isolated by filtration, purified by ether trituration, 46% yield); (ii) mercaptoacetic acid (1.1 equiv), NaOH (2.2 equiv), water, 175 °C, isolated by filtration, recrystallized from methanol/water, 75%; (iii) oxalyl chloride, DCM, 92%; (iv) amine, 1 N NaOH (2 equiv).



Scheme 2. Reagents and conditions: (i) alkyl bromide, Cs_2CO_3, acetone; or substituted epoxide, DMF, 80 °C.

Table 1
Importance of NH and Cl groups in compound **1**



Compd	R	X	IL-1β IC_{50}, μM or % inh @ 5 μM[a,b]
1	Cl	NH	0.8
8	Cl	NMe	0% inh
9	Cl	O	19% inh
10	H	NH	0% inh

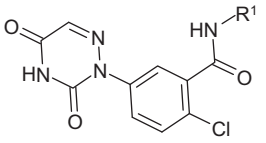
[a] Inhibition of the release of lL-1β from monocytes stimulated by ATP.
[b] IC_{50} value is an average of $n = 2$.

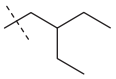
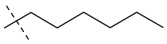
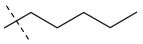
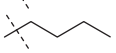
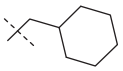
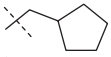
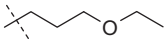
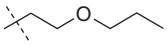
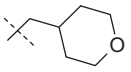
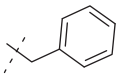
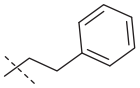
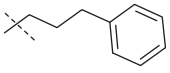
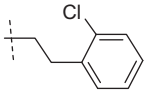
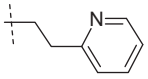
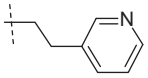
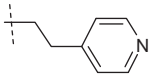
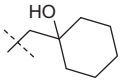
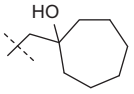
IC_{50} = 0.4 μM, *c* Log *P* = 2.3). Furthermore, the 1-hydroxycycloheptyl homolog **28** was 4-fold more potent than **27** and was significantly less protein bound compared to **23** (human plasma Fu = 0.18 vs 0.005), had sub-micromolar potency in the presence of human blood (IC_{50} = 564 nM, IC_{90} = 1000 nM), low lipophilicity (*c* Log *P* = 2.8) and low predicted clearance from human liver microsomal and hepatocyte studies.

Attempts to improve potency by replacement of the *ortho*-Cl group of **28** with methyl (IC_{50} = 0.7 μM), fluoro (20% inhibition at 1 μM) or cyano (0% inhibition at 1 μM) were unsuccessful. Subsequently, we held the 1-hydroxycycloheptyl group of compound **28** constant and explored substitution off of the 3-position of the 6-azauracil ring (Table 3). Methyl substitution (**29**) revealed that the N3 proton was not necessary for activity. Furthermore, although the ethyl analog (**30**) was equipotent with **28** in the IL-1β assay, it did not lose significant potency in the presence of human blood (IC_{50} = 0.185 μM). Many close-in analogs were prepared varying the substituent at the 3-position (e.g., **31**–**33**), and of these, the (*R*)-1-hydroxy-2-methoxyethyl group (**33**) proved optimal as its low potency in the IL-1β assay (IC_{50} = 0.001 μM) coupled with lower plasma protein binding (Fu_p = 0.27) translated into exceptional potency in the presence of human blood (IC_{50} = 0.001 μM, IC_{90} = 0.0047 μM).

The pharmacology and PK profile of compounds **23**, **28** and **33** is shown in Table 4. In rats, compound **33** had low CL_p (11 mL/min/kg) and a large V_{dss} of 7.6 L/kg, which resulted in a half-life of 2.4 h. Upon oral administration to rats at 5 mg/kg, compound **33** provided maximal plasma exposure (C_{max}) that was ~90 fold over its IC_{90} in human blood (C_{max} = 0.21 μg/mL or 0.44 μM). The oral bioavailability of **33** was low in rats (*F* = 2.6%), but this was believed to be a rat specific phenomenon since corresponding oral bioavailability in both dog (59%) and monkey (22%) was adequate. At 10 μM compound **33** had no effect on a CEREP panel of 107 receptors, channels and transporters, nor did it have any interaction with the major human CYP isozymes (CYP-1A2, 2C19, 2C9, 2D6 or 3A4, IC_{50}'s >30 μM). From a safety point of view, **33** was negative in our biolumAmes and micronucleus assays, had no effect on hERG at 1 μM, and did not show toxicity in rats when dosed daily at 500 mg/kg, p.o. over a 4 day period. Unfortunately, we were unable to obtain meaningful data for **33** in a rodent disease model because of its weak potency against the mouse $P2X_7R$. For example, 10 μM concentrations of **33** yielded minimal inhibitions of ATP-induced IL-1β posttranslational processing when using LPS-activated murine peritoneal macrophages.

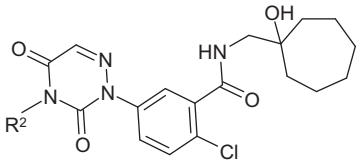
Table 2
SAR around the amide moiety of compound **1**

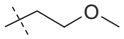
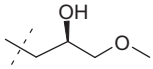


Compd	R1	IL-1β IC_{50}, μM or % inh @ 5 μM[a,b]	*c* Log *p*
11		1.1	3.4
12		3.6	3.5
13		2.8	3.0
14		0% inh	2.5
15		1.3	3.5
16		0% inh	3.0
17		2% inh	1.8
18		>5	2.0
19		>5	1.1
20		15% inh	2.8
21		0.94	3.0
22		0% inh	3.3
23		0.29	3.7
24		15% inh	1.5
25		22% inh	1.5
26		22% inh	1.5
27		0.40	2.3
28		0.083 ($n = 67$)	2.9

[a] Inhibition of the release of lL-1β from monocytes stimulated by ATP.
[b] IC_{50} values are an average of $n = 2$ unless otherwise noted.

Table 3
Substitution on N3 of 6-azauracil moiety and its effect on $P2X_7R$ potency in the presence of human blood



Compd	R^2	IL-1β IC_{50}[a,b] (μM)	IL-1β (human blood) IC_{50}[a,b] (μM)
28	H	0.083 (n = 67)	0.447 (n = 7)
29	Me	0.042	NT[c]
30	Et	0.086	0.185 (n = 1)
31		0.034	0.200 (n = 1
32		0.019	0.071
33		0.0014 (n = 5)	0.0008 (n = 13)

[a] Inhibition of the release of IL-1β from monocytes stimulated by ATP.
[b] IC_{50} values are an average of n = 2 unless otherwise noted.
[c] NT = not tested.

Table 4
Pharmacology and pharmacokinetics of key $P2X_7R$ antagonists.

		Compd **23**	Compd **28**	Compd **33**
	Yo-Pro IC_{50} (nM)	300	15	4
	IL-1β IC_{50} (nM)	285	83	1
	Human blood IC_{50} (nM)	>10,000	447	1
	Human blood IC_{90} (nM)		1000	4.7
	Human plasma Fu	0.005	0.18	0.27
	c Log *P*	3.7	2.9	2.6
Rat PK:	CL_p (mL/min/kg)	9.5	20	11
	V_{dss} (L/kg)	0.3	8.5	7.6
	*T*1/2 (h)	3	3.2	2.4
	C_{max} (μg/mL)	2	1.3	0.21
	F (%)	44	75	2.6
Dog PK:	CL_p (mL/min/kg)		3.8	12
	V_{dss} (L/kg)		0.6	0.61
	*T*1/2 (h)		3.8	0.46
	C_{max} (μg/mL)		7.4	3.5
	F (%)		84	59
Monkey PK:	CL_p (mL/min/kg)		21	16
	V_{dss} (L/kg)		1.5	0.68
	*T*1/2 (h)		3.4	0.77
	C_{max} (μg/mL)		0.6	0.55
	F (%)		72	22

Compound **33**, also referred to as CE-224,535, was recently evaluated in the clinic for the treatment of rheumatoid arthritis (RA).[13] Although CE-224,535 (500 mg, BID) was generally well tolerated over a 12 week study where median trough plasma concentrations reached ~250 ng/mL (519 nM), it was not effective in treating RA in patients with an inadequate response to methotrexate.[13]

In conclusion, through a combination of parallel chemistry and property focused medicinal chemistry, we optimized an HTS hit $P2X_7R$ antagonist (**1**) to identify a clinical candidate **33** (CE-224,535). The excellent PK and safety profile of compound **33** allowed for us to adequately test the effect of the $P2X_7R$ antagonist mechanism in the clinic as a potential treatment for RA. Although ineffective for the treatment of RA at adequate drug exposures, compound **33** may still hold promise for the treatment of other diseases such as pain and AD.

References and notes

1. Koles, L.; Furst, S.; Illes, P. *Drug News Perspect.* **2005**, *18*, 85.
2. Papp, L.; Vizi, E. S.; Sperlagh, B. *NeuroReport* **2004**, *15*, 2387.
3. Solle, M.; Labasi, J.; Perregaux, D. G.; Stam, E.; Petrushova, N.; Koller, B. H.; Griffiths, R. J.; Gabel, C. A. *J. Biol. Chem.* **2001**, *276*, 125.
4. Chessell, I. P.; Hatcher, J. P.; Bountra, C.; Michel, A. D.; Hughes, J. P.; Green, P.; Egerton, J.; Murfin, M.; Richardson, J.; Peck, W. L.; Grahames, C. B.; Casula, M. A.; Yiangou, Y.; Birch, R.; Anand, P.; Buell, G. N. *Pain* **2005**, *114*, 386.
5. Parvathenani, L. K.; Tertyshnikova, S.; Greco, C. R.; Roberts, S. B.; Robertson, B.; Posmantur, R. *J. Biol. Chem.* **2003**, *278*, 13309.
6. Perregaux, D. G.; McNiff, P.; Laliberte, R.; Conklyn, M.; Gabel, C. A. *J. Immunol.* **2000**, *165*, 4615.
7. Gunosewoyo, H.; Coster, M. J.; Bennett, M. R.; Kassiou, M. *Bioorg. Med. Chem.* **2009**, *17*, 4861.
8. Bergsbaken, T.; Fink, S. L.; Cookson, B. T. *Nat. Rev. Microbiol.* **2009**, *7*, 99.
9. Labasi, J. M.; Petrushova, N.; Donovan, C.; McCurdy, S.; Lira, P.; Payette, M. M.; Brissette, W.; Wicks, J. R.; Audoly, L.; Gabel, C. A. *J. Immunol.* **2002**, *168*, 6436.
10. (a) Guile, S. D.; Alcaraz, L.; Birkinshaw, T. N.; Bowers, K. C.; Ebden, M. R.; Furber, M.; Stocks, M. J. *J. Med. Chem.* **2009**, *52*, 3123; (b) Carroll, W. A.; Donnelly-Roberts, D.; Jarvis, M. F. *Purinergic Signalling* **2009**, *5*, 63; (c) Donnelly-Roberts, D. L.; Jarvis, M. F. *Br. J. Pharmacol.* **2007**, *151*, 571; (d) Romagnoli, R.; Baraldi, P. G.; Cruz-Lopez, O.; Lopez-Cara, C.; Preti, D.; Borea, P. A.; Gessi, S. *Exp. Opin. Ther. Targets* **2008**, *12*, 647.
11. Analogs of compound **1** were evaluated as antagonists of the $P2X_7R$ 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. The final concentration of ATP was 6 mM. 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. For assay protocols, see: Duplantier, A. J. and Subramanyam, C. WO 2003042191, 2003.
12. Miller, M. W.; Mylari, B. L.; Howes, H. L., Jr.; Lynch, J. E.; Lynch, M. J.; Koch, R. C. *J. Med. Chem.* **1979**, *22*, 1483.
13. Stock, T.; Bloom, B. J.; Wang, X.; Gupta, P. and Mebus, C. *Eur. League Against Rheumatism*, Rome, Italy, June 16–19, 2010; THU0217.