Bioorganic & Medicinal Chemistry Letters 20 (2010) 3161–3164

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and structure–activity relationships of a series of (1*H*-pyrazol-4-yl)acetamide antagonists of the P2X$_7$ receptor

Laura J. Chambers *, Alexander J. Stevens, Andrew P. Moses, Anton D. Michel, Daryl S. Walter, David J. Davies, David G. Livermore, Elena Fonfria, Emmanuel H. Demont, Mythily Vimal, Pam J. Theobald, Paul J. Beswick, Robert J. Gleave, Shilina A. Roman, Stefan Senger

Neurosciences Centre of Excellence for Drug Discovery, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, United Kingdom

ARTICLE INFO

Article history:
Received 22 February 2010
Revised 25 March 2010
Accepted 25 March 2010
Available online 30 March 2010

Keywords:
P2X7
P2X7 antagonist
Lead optimisation

ABSTRACT

High-throughput screening identified compound **1** as a potent P2X$_7$ receptor antagonist suitable for lead optimisation. Structure–activity relationships (SAR) of a series of (1*H*-pyrazol-4-yl)acetamides were investigated and compound **32** was identified as a potent P2X$_7$ antagonist with enhanced potency and favourable physicochemical and pharmacokinetic properties.

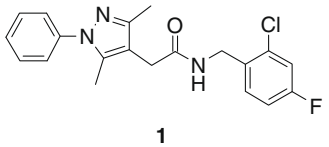
© 2010 Elsevier Ltd. All rights reserved.

The P2X$_7$ receptor is an ATP-gated ion-channel which is expressed in cells of the hematopoietic lineage,[1,2] (e.g., macrophages, microglia, mast cells, and lymphocytes) and has been the subject of considerable interest in the recent scientific and patent literature as a target for potential therapeutic intervention.[2–8] Activation of P2X$_7$ receptors has numerous effects including non-specific cation flux;[9] subsequent formation of a membrane pore, which can facilitate the transit of relatively large molecules;[10] and activation and release of pro-inflammatory cytokines such as interleukin-1β (IL-1β).[11] Furthermore, the P2X$_7$ receptor is also expressed in the central nervous system[12] and has been shown to mediate glutamate release in glial cells.[13]

The localisation of the P2X$_7$ receptor to key cells of the immune system, coupled with its ability to release important inflammatory mediators from these cells and the emerging role of purinergic signaling in the central nervous system,[14] suggests a potential role for P2X$_7$ receptor antagonists in the treatment of a wide range of conditions including inflammatory disease, pain and neurodegenerative disorders. Recent preclinical in vivo studies have directly implicated the P2X$_7$ receptor in both inflammatory and neuropathic pain[15] and small molecule P2X$_7$ antagonists have been demonstrated to be efficacious in animal models of neuropathic pain.[16–19] Consequently, P2X$_7$ antagonists are of interest as novel therapeutic agents in a number of disease states. Two compounds of undisclosed structure, AZD-9056 and CE-224535, have progressed to early proof-of-concept clinical trials in rheumatoid arthritis patients.[20,21]

In order to identify novel P2X$_7$ chemotypes, a high-throughput screen (HTS) of the GSK compound collection was carried out. This led to the discovery of compound **1** (Fig. 1), which exhibited a good level of in vitro potency against both the human and rat orthologues of P2X$_7$ but displayed poor in vitro stability in microsomal preparations.

The synthesis of this compound was achieved in four synthetic steps as shown in Scheme 1. The first step involved alkylation of acetylacetone to produce a functionalized 1,3-diketone. This was followed by pyrazole formation with phenylhydrazine, ester hydrolysis and amide coupling. Variation of the hydrazine or amine components allowed rapid preparation of analogues with two main points of diversity. Compound **1** demonstrated encouraging levels of potency and low molecular weight; highlighted by the promising ligand efficiency[22] (LE) and ligand lipophilicity effi-

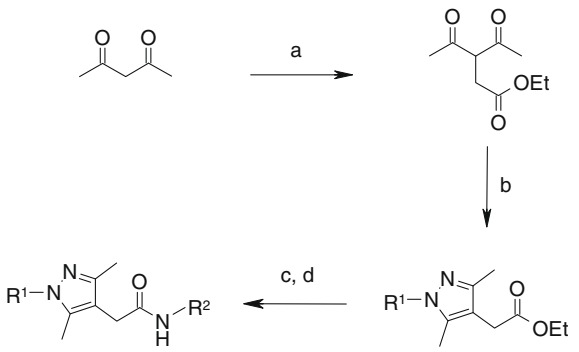


1

Figure 1. Compound **1**, a P2X$_7$ antagonist identified from HTS. Relevant data: Human P2X$_7$ pIC$_{50}$ 7.4; Rat P2X$_7$ pIC$_{50}$ 7.0; Rat in vitro clearance = 46 mL/min/g liver; cLog *D* @ pH 7.4 = 3.4; Solubility in water = 110 μg/mL (1 h timepoint); MW = 372; LE = 0.39; LLE = 4.0.

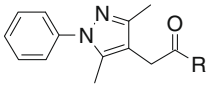
* Corresponding author. Tel.: +44 1279643070; fax: +44 1279643210.
E-mail address: laura.j.chambers@gsk.com (L.J. Chambers).

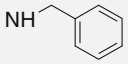
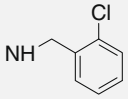
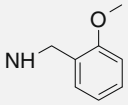
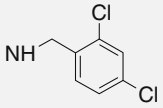
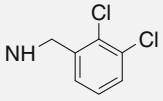
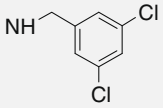
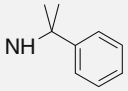
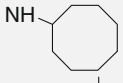
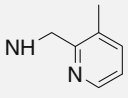
0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.03.096



Scheme 1. Synthesis of pyrazolacetamide analogues. Reagents and conditions: (a) ethyl bromoacetate, KOH, 1,4-dioxane, water, rt, ca. 16 h; (b) (i) R^1NHNH_2, AcOH, EtOH, reflux, 2 h; or (ii) R^1NHNH_2·HCl, NaOAc, EtOH, reflux, 2 h; (c) LiOH, H_2O, THF, 50 °C, 2 h; (d) R^2NH_2, EDAC, HOBT, 4-ethylmorpholine, DCM, DMF, rt, ca 16 h.

Table 1
Structure–activity data for a range of 2-(3,5-dimethyl-1-phenyl-1*H*-pyrazol-4-yl)-acetamides



Compd	R	Human P2X$_7$ pIC$_{50}$[a]
2		<6.0
3		6.0
4		<6.0
5		7.1
6		7.6
7		⩽6.0[b]
8		6.9
9		6.8
10		<6.0

[a] Data generated using an ethidium bromide release assay (see Ref. 24), reporting an average value of n >3.
[b] This value is an average of four individual values: <6.0; 6.2; <6.0; 6.0.

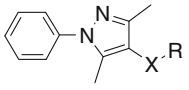
ciency[23] (LLE) values. This compound was therefore selected as a promising starting point for further optimisation.

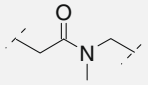
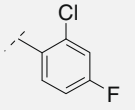
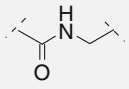
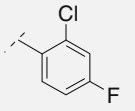
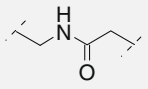
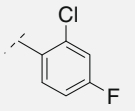
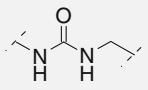
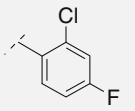
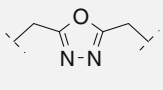
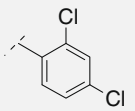
Modifications of the amide substituent (R^2 in Scheme 1) were explored first and are summarized in Table 1. Although compounds **2**–**7** in Table 1 are benzamides, the moderate inhibition of compound **9** illustrated that an aromatic ring was not necessarily required for activity. Overall, the changes made in this part of the molecule suggested that the amide substituent was binding into a relatively large pocket and that a moderate level of antagonism could be obtained if this was filled appropriately. For instance, the α,α′-dimethyl benzamide analogue (**8**) retained a good level of inhibition. Regarding the benzylic group, the di-halo substitution patterns of compounds **5** and **6** appeared optimal, however neither of these modifications offered a significant advantage when compared to compound **1**.

In order to determine the importance of the amide group to receptor binding, a number of alternative linkers were prepared[25] (exemplified by compounds shown in Table 2). Methylation of the amide nitrogen (compound **11**) led to a marked loss of inhibition, which suggested that either the N–H was participating in an important interaction, or that the secondary amide conformation was preferred. Interestingly, the urea analogue (compound **14**) was tolerated, although potency was reduced somewhat compared to compound **1**. A range of heterocyclic amide isosteres (exemplified by **15**) also maintained a degree of activity, which suggested that a hydrogen bond donor in this region of space is not an absolute requirement. The H-bond acceptor and appropriate placement of the amide group appeared to be the important factors in retaining inhibitory activity in this series.

To investigate the contribution of the pyrazole ring to receptor binding, a number of heterocyclic replacements were prepared[26] (Table 3). The results obtained for compounds **16** and **17** demonstrated that removal of one of the methyl groups from the pyrazole resulted in a reduction of potency so, where possible, methyl substituents were retained in analogous heterocycles. Most alternative heterocycles were poorly tolerated (compounds **18–21**), with compound **22** being a notable exception.[27] Although interpretation was complicated by missing methyl groups in some instances, there was a suggestion that one of the pyrazole/imidazole ring nitrogen atoms participates in a binding interaction with the receptor, one that the other ring systems are unable to access.

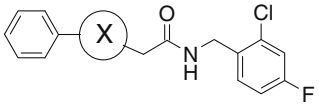
Table 2
Structure–activity data for a range of amide linker variations

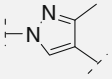
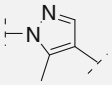
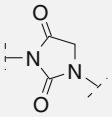
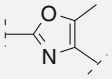
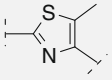
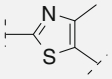
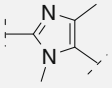


Compd	X	R	Human P2X$_7$ pIC$_{50}$[a]
11			<6.0
12			<6.0
13			<6.0
14			6.6
15			5.9

[a] Data generated using an ethidium bromide release assay (see Ref. 24), reporting an average value of n >3.

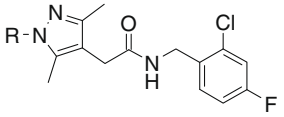
Table 3
Structure–activity data for a range of heterocycle variations

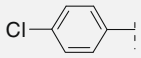
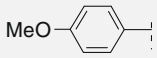
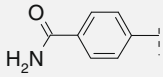
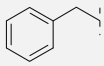
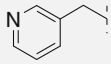
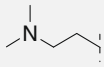
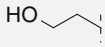


Compd	X	Human $P2X_7$ pIC_{50} [a]
16		6.8
17		6.8
18		<6.0
19		<6.0
20		<6.0
21		<6.0
22		7.3

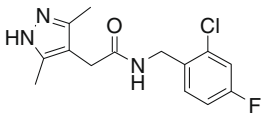
[a] Data generated using an ethidium bromide release assay (see Ref. 24), reporting an average value of n >3.

Table 4
Structure–activity data for a range of N-substituents



Compd	R	Human $P2X_7$ pIC_{50}[a]	Rat Cli[b] mL/min/g liver
23		7.2	12.0
24		7.0	–
25		8.0	3.2
26		7.3	1.8
27		6.5	>50
28		6.9	20.0
29		6.5	5.7
30		6.7	2.4
31	Me	6.8	3.5
32	H	7.7	0.58

[a] Data generated using an ethidium bromide release assay (see Ref. 24), reporting an average value of n >3.
[b] Microsomal clearance method described in Ref. 29.



32

Figure 2. Lead compound from the optimisation of compound **1**. Relevant data: Human $P2X_7$ pIC_{50} 7.7; Rat $P2X_7$ pIC_{50} 6.8; Rat in vitro clearance 0.58 mL/min/g; *c*Log *D* @ pH 7.4 = 1.6; Solubility in water = 732 μg/mL (1 h timepoint); MW = 296; LE = 0.53; LLE = 6.1.

Table 5
Summary of the pharmacokinetic profile of compound **32**

Route of administration	Dose	Property	Measured value
IV[a]	1 mg/kg	CLb (mL/min/kg)	15
		$T_{1/2}$ (h)	1.7
		VD_{ss} (L/kg)	1.6
PO[b]	3 mg/kg	T_{max} (h)	1.0
		C_{max} (μM)	2.92 ± 0.33
		AUC/dose (min·kg/L)	58 ± 3.6
		F_{po}	90%

[a] Compound **32** was dissolved in 0.9% (w/v) saline containing 10% (w/v) HPB (hydroxypropyl-β-cyclodextrin) and 2% (v/v) DMSO at a target concentration of 0.2 mg/mL. It was administered as a 1 h intravenous infusion to 1 rat to achieve a target dose of 1 mg/kg.
[b] Compound **32** was dissolved in 1% (w/v) methylcellulose in water at a concentration of 0.6 mg/mL and dosed by oral gavage to 3 rats at a target dose of 3 mg/kg.

As an orally bioavailable therapeutic agent was ultimately required, it was important to understand and address the high in vitro clearance observed with early representatives of this series. In silico profiling of compound (**1**) using the METASITE[28] program postulated the phenyl ring as a highly likely site of metabolism. To test this hypothesis and to probe the SAR in this region of space, ring substitutions and ring replacements were explored. As shown in Table 4, most of these changes resulted in a good level of inhibition, and the binding site appeared to be tolerant of both lipophilic and hydrophilic groups in this position. 4-Substitution of the phenyl ring (compounds **23** and **25**), or replacement by pyrimidine (**26**) also successfully reduced the in vitro clearance. Incorporating a hydrophilic group seemed to successfully lower the clearance; however compound **23** is a notable exception, which may be explained by the 4-substituent blocking a potential site of oxidation. Homologation to a benzyl group (**27**) led to a reduction of activity, although the analogous pyridyl methyl group (**28**) did show a slight reduction to the in vitro clearance.

Small alkylated derivatives (compounds **29**–**31**) retained a degree of activity, and offered some significant metabolic advantages, but removal of the N-substituent (compound **32**) provided the most significant improvements to both inhibition and metabolic stability. Removing the pyrazole nitrogen substituent (R^1 in Scheme 1), which does not appear to make a significant interaction with the human receptor, significantly lowered both the molecular weight and the *c*Log *D*[30] of the molecule (see Fig. 2). This can be demonstrated by the improved ligand efficiency and high ligand lipophilicity efficiency of compound **32**. This modification also resulted in the lowest level of in vitro metabolism observed in the series to this point, as well as a marked improvement in solubility when compared to **1**. Compound **32** was selected for further progression to determine whether the improved in vitro properties translated to a desirable in vivo profile[31] (Table 5).

Following intravenous administration (1 mg/kg), compound **32** had a low blood clearance (15 mL/min/kg, approximately 17% of liver blood flow). The steady-state volume of distribution (VD_{ss}) was

1.6 L/kg, indicative of good distribution into tissues, and a half-life of 1.7 h was recorded. Compound **32** also exhibited good oral absorption following a 3 mg/kg dose. Comparison of the intravenous and oral studies resulted in an estimated oral bioavailability of 90%.

In conclusion, it was demonstrated that the pyrazole N-substituent of the initial hit **1** offered the greatest opportunity to improve the overall profile of this series. Compound **32** was subsequently identified and shown to be a potent $P2X_7$ antagonist at both human and rat orthologues; with good oral absorption, low in vivo clearance and an excellent physicochemical profile.

References and notes

1. Collo, G.; Neidhart, S.; Kawashima, E.; Kosco-Vibois, M.; North, R. A.; Buell, G. *Neuropharmacology* **1997**, *36*, 1277.
2. DiVirgilio, F.; Vishwanath, V.; Ferrari, D. *Handb. Exp. Pharmacol.* **2001**, *151*, 355.
3. North, R. A. *Physiol. Rev.* **2002**, *82*, 1013.
4. Jacobsen, K. A.; Jarvis, M. F.; Williams, M. *J. Med. Chem.* **2002**, *45*, 4057.
5. Gever, J. R.; Cockayne, D. A.; Dillon, M. P.; Burnstock, G.; Ford, A. P. D. W. *Eur. J. Physiol.* **2006**, *452*, 513.
6. Donnelly-Roberts, D. L.; Jarvis, M. F. *Br. J. Pharmacol.* **2007**, *151*, 571.
7. Romagnoli, R.; Baraldi, P. G.; Cruz-Lopez, O.; Lopez-Cara, C.; Preti, D.; Borea, P. A.; Gessi, S. *Expert Opin. Ther. Targets* **2008**, *12*, 647.
8. 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.
9. Buisman, H. P.; Steinberg, T. H.; Fischbarg, J.; Silverstein, S. C.; Vogelzang, S. A.; Ince, C.; Ypey, D. L.; Leijh, P. C. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7988.
10. Pelgrin, P.; Suprenant, A. *EMBO J.* **2006**, *25*, 5071.
11. Ferrari, D.; Pizzirani, C.; Adinolfi, E.; Lemoli, R. M.; Curti, A.; Idzko, M.; Panther, E.; Di Virgilio, F. *J. Immunol.* **2006**, *176*, 3877.
12. Sperlágh, B.; Vizi, E. S.; Wirkner, K.; Illes, P. *Prog. Neurobiol.* **2006**, *78*, 327.
13. Duan, S.; Anderson, C. M.; Keung, E. C.; Chen, Y.; Chen, Y.; Swanson, R. A. *J. Neurosci.* **2003**, *23*, 1320.
14. Fields, D.; Burnstock, G. *Nat. Rev. Neurosci.* **2006**, *7*, 423.
15. Chessel, 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. A.; Casula, M. A.; Yiangou, Y.; Birch, R.; Anand, P.; Buell, G. N. *Pain* **2005**, *114*, 386.
16. Honore, P. M.; Donnelly-Roberts, D.; Namovic, M.; Hsieh, G.; Zhu, C.; Mikusa, J.; Hernandez, G.; Zhong, C.; Gauvin, D. M.; Chandran, P.; Harris, R.; Medrano, A. P.; Carroll, W.; Marsh, K.; Sullivan, J. P.; Faltynek, C. R.; Jarvis, M. F. *J. Pharmacol. Exp. Ther.* **2006**, *319*, 1376.
17. Broom, D. C.; Matson, D. J.; Bradshaw, E.; Buck, M. E.; Meade, R.; Coombs, S.; Matchett, M.; Ford, K. K.; Yu, W.; Yuan, J.; Sun, S. H.; Ochoa, R.; Krause, J. E.; Wustrow, D. J.; Cortright, D. N. *J. Pharmacol. Exp. Ther.* **2008**, *327*, 620.
18. Perez-Medrano, A.; Donnelly-Roberts, D. L.; Honore, P.; Hsieh, G. C.; Namovic, M. T.; Peddi, S.; Shuai, Q.; Wang, Y.; Faltynek, C. R.; Jarvis, M. F.; Carroll, W. A. *J. Med. Chem.* **2009**, *52*, 3366.
19. Nelson, D. W.; Gregg, R. J.; Kort, M. E.; Perez-Medrano, A.; Voight, E. A.; Wang, Y.; Grayson, G.; Namovic, M. T.; Donnelly-Roberts, D. L.; Niforatos, W.; Honore, P.; Jarvis, M. F.; Faltynek, C. R.; Carroll, W. A. *J. Med. Chem.* **2006**, *49*, 3659.
20. McInnes, I. B.; Snell, N. J.; Perrett, J. H.; Parmar, H.; Wang, M. M.; Astbury, C. *Arthritis Rheum [71st Annu Sci Meet Am Coll Rheumatol (Nov 6–11, Boston) 2007]*, **2007**, *56* (9, Suppl.): Abst 2085.
21. *ClinicalTrial.gov Identifier*: NCT00628095.
22. Abad-Zapatero, C. *Expert Opin. Drug Disc.* **2007**, *2*, 469–488.
23. (a) Leeson, P. D.; Springthorpe, B. *Nat. Rev. Drug Disc.* **2007**, *6*, 881; The same concept was independently proposed by researchers at Pfizer and termed lipE: (b) Ryckmans, T.; Edwards, M. P.; Horne, V. A.; Monica Correia, A.; Owen, D. R.; Thompson, L. R.; Tran, I.; Tutt, M. F.; Young, T. *Bioorg. Med. Chem. Lett.* **2009**, *15*, 4406.
24. Potencies are measured using a competition experiment using the natural ligand ATP. ATP activation leads to formation of a large opening in the cell wall and accumulation of ethidium bromide dye within the cell, levels of which are measured using fluorescence. All test compounds had I_{max} values of 100%. Full details of the assay protocol can be found in patent application WO 2007 141267 A1.
25. Compound **12** was prepared from commercial 3,5-dimethyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid. Compound **13** was prepared from commercial [(3,5-dimethyl-1-phenyl-1*H*-pyrazol-4-yl)methyl]amine. Compound **14** was prepared from commercial 3,5-dimethyl-1-phenyl-1*H*-pyrazol-4-amine. Compound **15** was prepared via cyclodehydration of an intermediate hydrazide.
26. The preparation of compounds **16** and **17** is described in patent application WO 2008 138876 A1. Compound **18** was prepared via alkylation of commercial 3-phenyl-2,4-imidazolidinedione. Compounds **19**–**21** were prepared from commercially available acetic acids.
27. The synthesis and optimisation of compound **22** will be described in a forthcoming publication.
28. METASITE v3.0, developed by Molecular Discovery, predicts metabolic transformations related to cytochrome-mediated reactions in phase I metabolism.
29. Clarke, S. E.; Jeffrey, P. *Xenobiotica* **2001**, *31*, 591.
30. Calculated log *D* values were obtained using ACD Labs software v8.0.
31. All experiments were performed in accordance with the United Kingdom Animals (Scientific Procedures) Act, 1986 under Project Licence as well as under the review and approval of the GlaxoSmithKline Procedures Review Panel. GlaxoSmithKline safety regulations were adhered to at all times.