Bioorganic & Medicinal Chemistry Letters 20 (2010) 4653–4656

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Structure–activity relationships and in vivo activity of (1*H*-pyrazol-4-yl)acetamide antagonists of the P2X$_7$ receptor

Paul J. Beswick, Andy Billinton, Laura J. Chambers, David K. Dean, Elena Fonfria, Robert J. Gleave, Stephen J. Medhurst, Anton D. Michel, Andrew P. Moses, Sadhana Patel, Shilina A. Roman, Sue Roomans, Stefan Senger, Alexander J. Stevens, Daryl S. Walter *

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 14 April 2010
Revised 26 May 2010
Accepted 29 May 2010
Available online 8 June 2010

Keywords:
P2X$_7$
SAR
Pain

ABSTRACT

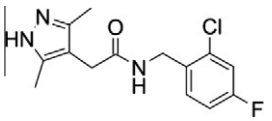
Structure–activity relationships (SAR) of analogues of lead compound **1** were investigated and compound **16** was selected for further study in animal models of pain. Compound **16** was shown to be a potent anti-hyperalgesic agent in both the rat acute complete Freund's adjuvant (CFA) model of inflammatory pain [Iadarola, M. J.; Douglass, J.; Civelli, O.; Naranjo, J. R. *rain Res.* **1988**, *455*, 205] and the knee joint model of chronic inflammatory pain [Wilson, A. W.; Medhurst, S. J.; Dixon, C. I.; Bontoft, N. C.; Winyard, L. A.; Brackenborough, K. T.; De Alba, J.; Clarke, C. J.; Gunthorpe, M. J.; Hicks, G. A.; Bountra, C.; McQueen, D. S.; Chessell, I. P. *Eur. J. Pain* **2006**, *10*, 537].

The P2X$_7$ receptor is an ATP-gated ion-channel,[1,2] which, when activated, induces a non-specific cation flux[3] as well as the activation and release of pro-inflammatory cytokines such as interleukin-1β (IL-1β).[4] The localisation of the P2X$_7$ receptor to key cells of the immune system and the emerging role of purinergic signalling in the central nervous system,[5] 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. Preclinical in vivo studies have directly implicated the P2X$_7$ receptor in pain states[6] and small molecule P2X$_7$ antagonists have been demonstrated to be efficacious in animal models of neuropathic pain.[7–10] Furthermore, two compounds (AZD-9056 and CE-224535), have progressed to early proof-of-concept clinical trials in rheumatoid arthritis patients.[11,12]

In order to explore the utility of P2X$_7$ antagonists as potential therapeutic agents for the treatment of pain, a high-throughput screen (HTS) was carried out. Of the hits identified from this exercise, a series of (1*H*-pyrazol-4-yl)acetamides emerged as amongst the most attractive, having good ligand efficiency and good potency at both human and rat orthologues. An earlier publication[13] described the preliminary optimisation of this series, which initially suffered from moderate levels of solubility and poor metabolic stability in rat microsomes, to give compound **1** (Fig. 1).

Compound **1** is relatively potent, as highlighted by the promising ligand efficiency[17] (LE) and ligand lipophilicity efficiency[18] (LLE) values, metabolically stable, has very good aqueous solubility, and has excellent oral bioavailability (~90%) and relatively low clearance (15 mL/min/kg) in rats.[13] Whilst compound **1** clearly represented an advance over the initial hits in this series further optimisation was initiated (compounds were prepared as previously described[13,14]) to identify the most suitable analogues for progression to key animal model studies.

Although some changes to the pyrazole ring were made during the earlier optimisation phase of this series,[13] some additional changes were explored at this stage to probe the possibilities for improving in vitro potency whilst maintaining the favourable properties associated with the lead compound **1**. Our earlier studies had suggested that both methyl substituents on the pyrazole ring were required for potency but different alkyl substituents had not been fully explored. The diethyl analogue **2** was slightly more potent than **1** but larger groups in one or both of the 3- and 5-positions were generally not well tolerated (e.g., **3**, **4**



1

Figure 1. Compound **1**, 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*^{21} at pH 7.4 = 1.6; solubility in water = 732 μg/mL (1 h timepoint); MW = 296; LE = 0.53;[17] LLE = 6.1.[18]

* Corresponding author.
E-mail addresses: daryl.s.walter@gsk.com, darylwmail-web@yahoo.co.uk (D.S. Walter).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.05.107

and **5**) and aryl substitution (as in **6**) led to a sharp decline in activity. Replacing a methyl group with a trifluoromethyl was tolerated and compound **7** was essentially equipotent relative to **1**. The isomeric methyl substituted (1*H*-pyrazol-5-yl)-acetamide **8** was considerably less potent than **1** and dialkylation in either the 4- or 3-position (see **9** and **10**) only improved the potency to a modest degree.

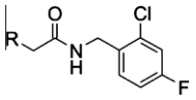
The analogous isoxazole **11** was also somewhat less potent than **1**. Increasing lipophilicity tended to be detrimental with respect to microsomal metabolic stability (see **4** and **5**) whereas the trifluoromethyl analogue **7** and the isomeric pyrazole and isoxazole analogues maintained a good degree of in vitro metabolic stability.

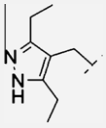
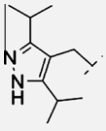
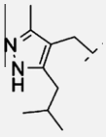
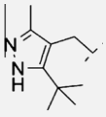
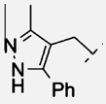
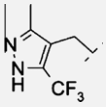
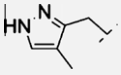
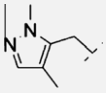
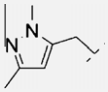
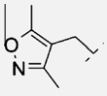
Earlier optimisation of this series[13] had demonstrated that the amide portion of the molecule was crucial to identifying compounds with good potency and lipophilic benzamides were generally best in this regard. Substitution in this position was reexamined in this more advanced subset of compounds and a similar trend was observed (see Table 2). Compound **12** highlights the requirement for a lipophilic substituent in the 2-position, losing almost 10-fold potency when chlorine was replaced with fluorine. Chlorine alone (**13**), however, is not sufficient to maintain potency either and the positional mono-Cl isomers (**14** and **15**) were similarly reduced in activity compared to compound **1**.

However, replacing the 4-F substituent with chlorine (**16**) was tolerated and gave a modest increase in potency. Introducing a CF_3 group into the 2-position (**17**) led to a small decrease in potency relative to **1** whereas compound **18**, with the 3-CF_3, 4-Cl substitution pattern had similar activity despite the lack of substitution in the 2-position. Combining this finding with a 2-Cl substituent gave compound **19** which showed a marked increase in potency.

Combining some of the more active substituted amides with the 2-(1,4-dimethyl-1*H*-pyrazol-5-yl)- and 2-(3,5-dimethyl-4-isoxazolyl)acetamide left-hand side analogues described earlier (see Table 1) also proved fruitful. The 2,4-dichloro- and 2-chloro-3-trifluoromethylbenzyl-2-(1,4-dimethyl-1*H*-pyrazol-5-yl)acetamides (**20** and **21**) and the 2,4-dichloro- and 2-chloro-3-trifluorometh-

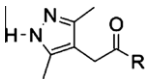
Table 1
Structure–activity data for 3,5-dialkyl-(1*H*-pyrazol-4-yl)-acetamides and alternative heterocyclic analogues of compound **1**

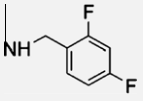
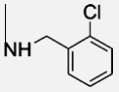
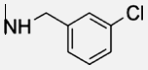
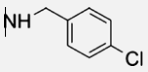
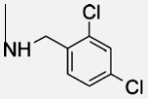
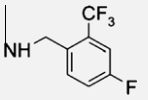
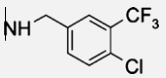
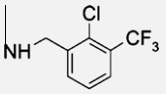


Compd	R	Human P2X$_7$ pIC$_{50}$[a]	Rat Cli[b] (mL/min/g) liver
2		8.0	–
3		6.9	–
4		7.0	12.5
5		6.8	6.7
6		<6	–
7		7.9	1.6
8		6.4	–
9		6.8	0.9
10		6.5	–
11		7.2	1.0

[a] Data generated using an ethidium bromide release assay (Ref. 15), reporting an average value of $n > 3$.
[b] Microsomal clearance method described in Ref. 16.

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



Compd	R	Human P2X$_7$ pIC$_{50}$[a]
12		6.8
13		6.6
14		6.8
15		6.2
16		8.1
17		7.4
18		7.2
19		8.6

[a] Data generated using an ethidium bromide release assay (Ref. 15), reporting an average value of $n > 3$.

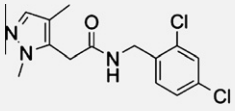
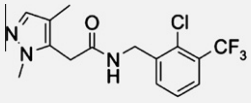
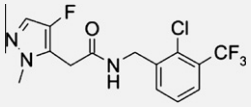
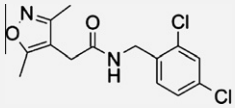
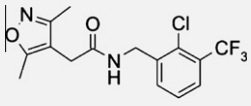
ylbenzyl-2-(3,5-dimethyl-4-isoxazolyl)acetamides (**23** and **24**) were essentially equipotent to the corresponding 2-(3,5-dimethyl-1*H*-pyrazol-4-yl)-acetamides. This fits with the earlier suggestion[13] that one of the pyrazole ring nitrogen atoms in **1** participates in a binding interaction with the receptor. However, there appears to be some flexibility in how this interaction is made given that the H-bond acceptor in question does not occupy an exactly analogous position in all cases. Interestingly, the 2-chloro-3-trifluoromethylbenzyl substitution also allowed for substitution of one of the ring methyl groups by fluorine (**22**).

Whilst the above optimisation process identified a number of compounds with very good activity in the primary assay and excellent cross-target selectivity (CEREP), not all of these compounds had favourable metabolic stability profiles in rat microsomal preparations (e.g., compound **19**, rat CLi = 3.5 mL/min/g liver. Also see values shown in Table 3). Generally it was found that for compounds within this series to have reasonable rat oral pharmacokinetics the microsomal CLi needed to be lower than 2 mL/min/g liver.

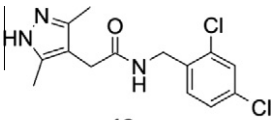
Of those that demonstrated low levels of in vitro metabolism compound **16** (see Fig. 2 below) offered the best balance of properties. In some ways this was disappointing as this compound differs only very slightly from the starting compound **1**. However, based on the slightly better rat receptor activity compound **16** was preferred for progression to in vivo studies[22] as it offered the possibility of examining the efficacy of compounds in this series in our rat pain models thus providing additional target validation for the application of $P2X_7$ antagonists as potential analgesics and confidence to progress work on this series further.

Following intravenous administration (1 mg/kg), compound **16** had low blood clearance (9 mL/min/kg); a steady-state volume of distribution of 1.0 L/kg; and an excretion halflife of 1.7 h. Compound **16** also exhibited good oral absorption following a 3 mg/kg dose and an estimated oral bioavailability of ~100%. On oral dosing in the rat acute complete Freund's adjuvant (CFA) model of inflammatory pain,[19] **16** is a potent antihyperalgesic agent

Table 3
2-(1,4-Dimethyl-1*H*-pyrazol-5-yl)acetamide and 2-(3,5-dimethyl-4-isoxazolyl)acetamide containing analogues of **1** incorporating optimised amide substituents

Compd	R	Human $P2X_7$ pIC_{50}[a]	Rat Cli[b] (mL/min/g) liver
20		8.0	3.2
21		8.5	2.9
22		8.0	1.6
23		7.8	2.1
24		8.6	2.4

[a] Data generated using an ethidium bromide release assay (Ref. 15), reporting an average value of $n > 3$.
[b] Microsomal clearance method described in Ref. 16.



16

Figure 2. Human $P2X_7$ pIC_{50} 8.1; rat $P2X_7$ pIC_{50} 7.1; rat in vitro clearance 1.3 mL/min/g; *c* log *D* at pH 7.4 = 2.7; MW = 312; LE = 0.55;[17] LLE = 5.4.[18]

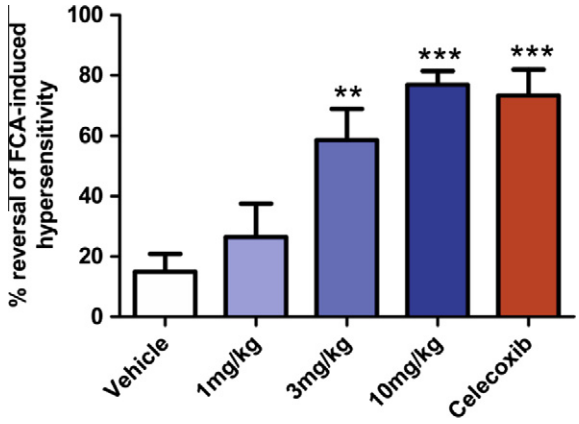


Figure 3. Effect of oral dose of **16** on FCA-induced hypersensitivity in rats. Effect of 10 mg/kg oral dose of celecoxib is included for comparison.

which showed a dose-related reversal of induced hypersensitivity with a calculated ED_{50} of 2.5 mg/kg at 1.5 h post challenge (Fig. 3). The maximum effect at 10 mg/kg compared favourably to that seen with a comparable dose of the gold standard, celecoxib. Average blood and brain concentrations of **16** at 10 mg/kg were 20.9 and 4.9 μM, respectively.

Furthermore, **16** produced a highly significant and dose-related reversal of FCA-induced hypersensitivity in the knee joint model of chronic inflammatory pain.[20] Commencing on day 13 and continuing for 4 days, **16** produced a statistically significant effect by day 14 and maximal reversal of hypersensitivity was achieved by day 15 (see Fig. 4).

In summary, we have discovered a novel series of drug-like $P2X_7$ antagonists with good in vitro potency, selectivity and pharmacokinetic properties. We have also demonstrated that a representative example has high efficacy in both the acute FCA and chronic joint pain models of inflammatory pain. Compound **16** and analogues thereof have clear utility in exploring the utility of

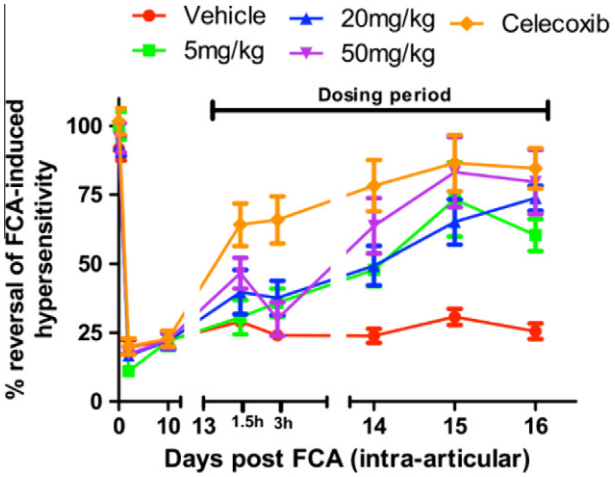


Figure 4. Effect of oral dose of **16** in the chronic joint pain model at oral doses of 5, 20, and 50 mg/kg b.i.d. for 4 days. The effect of 4 days oral dosing of 30 mg/kg b.i.d. celecoxib is included for comparison.

$P2X_7$ antagonists as potential therapeutic agents for the treatment of pain.

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.. In *Handbook of Experimental Pharmacology*; Abbraccio, M. P., Williams, M., Eds.; Springer, 2001; Vol. 151, pp 355–373.
3. 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.
4. Ferrari, D.; Pizzirani, C.; Adinolfi, E.; Lemoli, R. M.; Curti, A.; Idzko, M.; Panther, E.; Di Virgilio, F. *J. Immunol.* **2006**, *176*, 3877.
5. Fields, D.; Burnstock, G. *Nat. Rev. Neurosci.* **2006**, *7*, 423.
6. 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.
7. 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.
8. 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.
9. 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.
10. 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.
11. 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.
12. ClinicalTrial.gov Identifier: NCT00628095.
13. Chambers, L. J.; Stevens, A. J.; Moses, A. P.; Michel, A. D.; Slingsby, B.; Walter, D. S.; Davies, D. J.; Livermore, D. J.; Fonfria, E.; Demont, E. H.; Vimal, M.; Theobald, P. J.; Beswick, P. J.; Gleave, R. J.; Roman, S. A.; Senger, S.; Roomans, S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3161.
14. WO 2007/141267A1; WO 2007/141269A1; WO 2008/125600A1.
15. 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.
16. Clarke, S. E.; Jeffrey, P. *Xenobiotica* **2001**, *31*, 591.
17. Abad-Zapatero, C. *Expert Opin. Drug Discov.* **2007**, *2*, 469–488.
18. (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.
19. Iadarola, M. J.; Douglass, J.; Civelli, O.; Naranjo, J. R. *Brain Res.* **1988**, *455*, 205; Hay, C. H.; Trevethick, M. A.; Wheeldon, A.; Browers, J. S.; De Belleroche, J. S. *Neuroscience* **1997**, *78*, 843.
20. Wilson, A. W.; Medhurst, S. J.; Dixon, C. I.; Bontoft, N. C.; Winyard, L. A.; Brackenborough, K. T.; De Alba, J.; Clarke, C. J.; Gunthorpe, M. J.; Hicks, G. A.; Bountra, C.; McQueen, D. S.; Chessell, I. P. *Eur. J. Pain* **2006**, *10*, 537–549.
21. Calculated log *D* values were obtained using ACD Labs software v8.0.
22. 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.