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Identification of 2-oxo-*N*-(phenylmethyl)-4-imidazolidinecarboxamide antagonists of the $P2X_7$ receptor

Lee Abberley, Aude Bebius, Paul J. Beswick, Andy Billinton, Katharine L. Collis, David K. Dean, Elena Fonfria, Robert J. Gleave, Stephen J. Medhurst, Anton D. Michel, Andrew P. Moses, Sadhana Patel, Shilina A. Roman, Tiziana Scoccitti, Beverley Smith, Jon G. A. Steadman, Daryl S. Walter *

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

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ABSTRACT

A backup molecule to compound **2** was sought by targeting the most likely metabolically vulnerable site in this molecule. Compound **18** was subsequently identified as a potent $P2X_7$ antagonist with very low in vivo clearance and high oral bioavailability in all species examined. Some evidence to support the role of $P2X_7$ in the etiology of pain is also presented.

The $P2X_7$ receptor is an ATP-gated ion-channel[1–3] which controls the activation and release of pro-inflammatory cytokines such as interleukin-1β (IL-1β).[4] Expression on cells in both the immune and central nervous systems,[5] together with preclinical in vivo studies[6] and reports detailing the activity of small molecule $P2X_7$ antagonists in animal models of neuropathic pain[7–10] directly implicate the involvement of the $P2X_7$ receptor in pain states.[11] Consequently the potential for $P2X_7$ antagonists to function as novel human analgesics has resulted in significant interest in the pharmaceutical sector.[12] In our earlier publications[13] we described two series of compounds (exemplified by **1** and **2**, Fig. 1) which were shown to be active in animal models of pain.

Compound **2**, in particular, was highlighted as having an excellent preclinical physicochemical profile and a pharmacokinetic profile in rats (see Table 1) which was suitable for progression to clinical studies.[13,16] However, the cross-species pharmacokinetic profile,[17] and particularly the short half-life in dog and monkey raised concerns that the human half-life might also be relatively short. Whilst the physicochemical profile of this compound suggested that a delayed release formulation would be a viable approach to addressing this potential issue we also sought to identify a backup molecule with an improved preclinical pharmacokinetic profile. One of the approaches we decided to adopt in order to mitigate the risk of a short half-life in humans was to explore modifications of compound **2** which, in the first instance, were most likely to reduce the clearance rate in our preclinical species. Initially we sought to achieve this in rats by blocking the sites most prone to metabolic processes. Route of metabolism studies suggested that hydroxylation and subsequent glucuronidation of one of the carbon atoms in the pyrrolidinone ring (see **3** in Fig. 2) was the most prevalent metabolic route and initial efforts to block this pathway focused on substitution of the carbon atom adjacent to the carbonyl group (which was predicted to be the

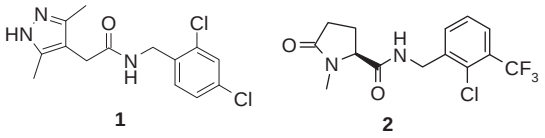

Figure 1. Compound **1**: Human $P2X_7$ pIC_{50} 8.1; rat $P2X_7$ pIC_{50} 7.1; MW = 312; LE = 0.55;[14] LLE = 5.4;[15] compound **2**: human $P2X_7$ pIC_{50} 8.5; rat $P2X_7$ pIC_{50} 6.6; rat MW = 334; LE = 0.53;[14] LLE = 6.2.[15]

Table 1
Summary of the cross-species pharmacokinetic profile ($n = 3$)[a] of compound **2**

Species	Rat	Dog	Monkey
CLb (mL/min/kg)	9	14	17
V_{ss} (L/kg)	1.0	0.9	1.1
$t^{1/2}$ (h)	1.5	0.7	0.9
F_{po} (%)	65	69	57

[a] Data generated from 1 mg/kg iv or 3 mg/kg po profiles.

* Corresponding author.
E-mail address: darylwmail-web@yahoo.co.uk (D.S. Walter).

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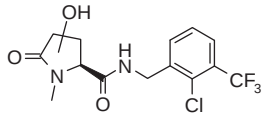

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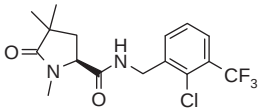
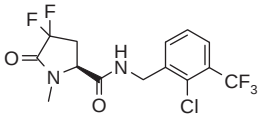
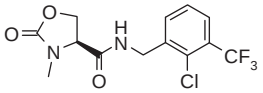
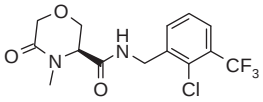
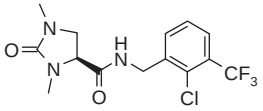
Figure 2. Proposed major metabolite of compound **2** in rats.

Table 2
Analogues of **2** in which the α-carbon of the pyroglutamide ring is substituted or replaced by a heteroatom

Compd	R	Human $P2X_7$ pIC_{50}[a]	Rat CLi[b] mL/min/g liver
4		7.6	<0.5
5		8.4	0.7
6		7.8	—
7		8.4	<0.5
8		9.2	<0.5

[a] Data generated using an ethidium bromide release assay (Ref. 19), reporting an average value of n >3.
[b] Microsomal clearance method described in Ref. 20.

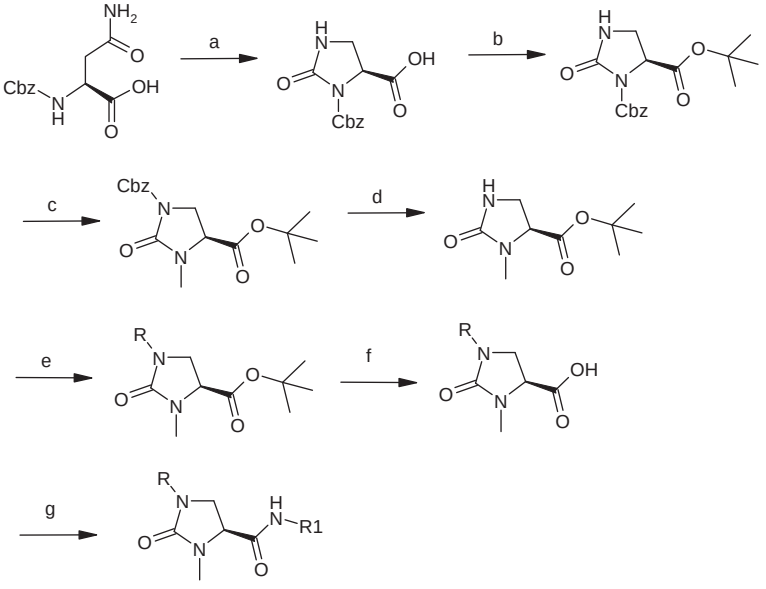
most metabolically vulnerable position) or replacement of the carbon atom with a heteroatom.[18]

The dimethylated analogue **4** (see Table 2) was 10-fold less potent whilst difluorination gave **5** which was essentially equipotent with **2**. The intrinsic clearance in rat microsomes also increased slightly, possibly indicative of a shift towards an alternative metabolic route or an increase in metabolic rate. Replacing the carbon atom with an oxygen **6** again led to a loss in activity whilst increasing the ring size as in the morpholinone **7** was better tolerated and low rat microsomal clearance was maintained. The most marked effect, however, was observed when the carbon atom in the ring was replaced with an *N*-methyl group. This led to a significant increase in potency whilst also maintaining an encouraging in vitro rat clearance profile. The remainder of this letter will focus on the work done to exploit this latter observation.

Compound **8** showed an increase in potency and maintained many of the favourable properties of the pyroglutamide **2**, including the potential for good brain penetration (compound **8**: PSA = 52, *c* log *D* @ pH7.4 = 2.9, Pgp efflux ratio = 1.5[28]), whilst eliminating the metabolically labile carbon atom. Importantly the nitrogen atom in this position provided a convenient synthetic handle. We envisaged that appropriate substitution in this position could lead to compounds with relatively low clearance and/or higher volumes of distribution hence providing a complimentary approach to increasing the in vivo half-life in rats. Compounds of this general formula could be prepared[21,22] according to the method outlined in Scheme 1.

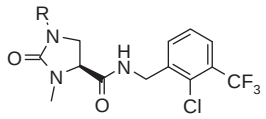
Hofmann cyclisation[23] of Cbz-protected asparagine followed by *tert*-butyl esterification gave the key *tert*-butyl 2-oxo-*N*-(Cbz)-4-imidazolidine carboxylate. The next step exploited an unusual alkylative rearrangement,[22] and then Cbz deprotection, and subsequent alkylation (or palladium-catalysed aryl halide cross-coupling reaction) provided the desired *tert*-butyl 1,3-disubstituted 2-oxo-4-imidazolidine carboxylates. Subsequent ester deprotection and amide coupling then provided the target compounds in good overall yields.

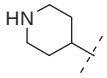
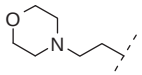
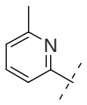
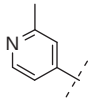
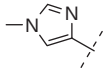
A selection of the more interesting compounds prepared to probe the effects of substitution on the ring nitrogen atom, is shown in Table 3. We explored the effects of basic groups, of varying strength, envisaging that a charged group could increase the volume of distribution.[25] At the same time we sought to ensure that the overall physical properties of the compounds remained in



Scheme 1. Synthesis of 2-oxo-*N*-(phenylmethyl)-4-imidazolidine carboxamide analogues. Reagents and conditions: (a) bromine, aq NaOH;[23] (b) *t*-BuOH, $POCl_3$, pyridine, $CHCl_3$;[24] (c) NaH, methyl iodide, DMF, 0 °C; (d) H_2, Pd–C, MeOH; (e) NaH, alkyl halide, DMF, −10 °C; or aryl bromide, $Pd_2(dba)_3$, Cs_2CO_3, Xantphos™, 1,4-dioxane; (f) TFA, DCM; (g) $R1NH_2$, EDAC, HOBT, NEM, DCM.

Table 3
Structure–activity data for N-substituted analogues of compound **8**



Compd	R	PSA Å^2	*c* log *D* @ pH 7.4[27]	Human P2X$_7$ pIC$_{50}$[a]	Rat CLi[b] mL/min/g liver	Pgp efflux ratio[28]
9	H	61	2.2	8.4	0.6	2.1
10		64	0.4	7.5	0.5	3.2
11		65	2.1	7.6	4.4	11.9
12		65	2.6	8.3	2.6	4.4
13		65	3.6	7.1	6.1	–
14		65	3.1	8.0	3.7	1.9
15		70	2.2	8.4	0.9	5.3
16		70	2.0	8.6	<0.5	–

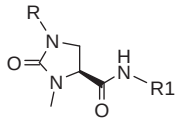
[a] Data generated using an ethidium bromide release assay (Ref. 19), reporting an average value of n >3.
[b] Microsomal clearance method described in Ref. 20.

a desirable space particularly with respect to CNS penetration.[26] In addition, to ensure maximal CNS penetration, the propensity for active efflux by P-glycoprotein (Pgp) was also monitored.[28]

Compound **9**, the des-methyl analogue of **8**, maintained a good level of potency and in vitro clearance and Pgp efflux remained at acceptable levels. The amino-substituted side-chains in **10** and **11** were less well tolerated by the P2X$_7$ receptor and significant increases in Pgp efflux ratios were also noted. The three isomeric pyridines shown, **12**, **13**, and **14**, retained a good level of potency in all but the 2-isomer, but in all cases (and in step with increasing lipophilicity) the in vitro clearance was significantly higher than in the case of **8**. The two imidazoles, **15** and **16**, maintained good levels of potency but the increased polar surface area resulted in increased levels of Pgp efflux. In general, only those analogues with hydrogen (e.g., **9**) or small alkyl (e.g., **8**) substituents in this position provided the appropriate mix of potency at the P2X$_7$ receptor, with low intrinsic clearance in liver microsomes, and relatively low Pgp efflux.

Subsequently, and to explore the effects of the right-hand benzamide substitution pattern on potency and pharmacokinetics, a two-dimensional array of NH and *N*-methyl substituted imidazolidinecarboxamides was prepared and profiled. The choice of amides prepared was largely based on the SAR previously derived from the optimization of the pyroglutamide series (as exemplified by **2**). A subset of data obtained from the compounds prepared is listed in Table 4.

Table 4
P2X$_7$ activity data for selected 2-oxo-*N*-(phenylmethyl)-4-imidazolidinecarboxamides



Compd	R	R1	Human P2X$_7$ pIC$_{50}$[a]	Rat CLi[b] mL/min/g liver	Rat CLb[c] mL/min/kg	Rat $t^{1/2c}$ (h)
8	Me	2-Cl-3-CF$_3$-Phenylmethyl	9.2	<0.5	37	0.7
9	H	2-Cl-3-CF$_3$-Phenylmethyl	8.4	0.6	14	2.3
17	Me	2,4-DiCl-phenylmethyl	8.7	<0.5	30	0.6
18	H	2,4-DiCl-phenylmethyl	8.0	<0.5	6	2.8
19	Me	2,3-DiCl-phenylmethyl	8.0	<0.5	49	0.4
20	H	2,3-DiCl-phenylmethyl	7.6	–	–	–

[a] Data generated using an ethidium bromide release assay (Ref. 19), reporting an average value of n >3.
[b] Microsomal clearance method described in Ref. 20.
[c] As determined following a single iv infusion (1 mg/kg).

A trend that emerged from this data was that whilst intrinsic clearance in liver microsomes was low in all cases, the in vivo rat clearance was generally higher in the *N*-Me examples (compare **8** with **9**, and **17** with **18**). This may hint at N-demethylation being a route of metabolism in rats but no studies were conducted to confirm this. Although the NH analogues were generally slightly less potent, the improved pharmacokinetic profile (and low clearance in particular) in rats singled these compounds out for further study. Compound **18**, a compound with high ligand efficiency (Fig. 3), had very low in vivo clearance and displayed an oral bioavailability of ~100% in a rat iv/po crossover study ($n = 3$), and was selected for progression to in vivo studies in animal models of pain.[17]

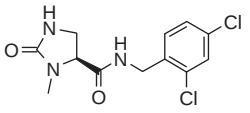
Compound **18** is a selective antagonist of the $P2X_7$ receptor, showing no appreciable off-target activities (both in in-house and CEREP[29] selectivity panels). The rat receptor affinity of **18** is relatively modest but we were confident that the selectivity for the $P2X_7$ receptor; and the high exposures obtained on oral dosing in rats, together with the excellent solubility (0.78 mg/mL in FeSSIF[30]), and low protein binding (65% in rat plasma) would allow us to reliably evaluate the in vivo efficacy of this compound.

Indeed, compound **18** produced (Fig. 4) a highly significant reversal of FCA-induced hypersensitivity in the knee joint model of chronic inflammatory pain.[31] An oral dose of 20 mg/kg produced a maximal reversal of hypersensitivity which was statistically indistinguishable from the effect obtained with the standard, celecoxib. Blood concentrations of **18** at 20 mg/kg, 1.0 h post-dose, were 56.4 μM and the terminal brain to blood ratio was measured as 0.3:1.

We were interested in exploring the role of centrally expressed $P2X_7$ receptors in the etiology of pain and decided to also profile a compound with low CNS penetration in our animal models. In contrast to the result shown above for compound **18**, a compound with a higher Pgp efflux ratio (see compound **21**, Fig. 5) and poor brain penetration (following oral dosing in rats) showed little or no efficacy in the same rat pain model (see Fig. 6) even after 5 days of dosing. Average blood concentrations of **21** at 20 mg/kg, 1.0 h post-dose on day 1, were 16.8 μM and the terminal brain to blood ratio was measured as <0.1:1. Protein binding of this compound was relatively low (77% in rat plasma) so whilst the free concentrations of **21** in the periphery are lower than those shown for **18** in a similar study, the increased rat $P2X_7$ activity of **21** relative to **18**, along with the data reported for compound **2** in our earlier paper, would suggest that this compound should have been active in this model if the efficacy was driven by peripheral concentrations alone. A potential hypothesis to explain the differential activity displayed by compounds **18** and **21** in the joint pain model could be that the efficacy is driven by the free CNS concentrations in each case and would lend support to the postulated role of $P2X_7$ in the release of IL-1β and/or glutamate in activated glia in the CNS.[32]

Finally, compound **18** was also profiled in dog and monkey and pleasingly it was found that the modest improvements in half-life in rats, relative to compound **2**, translated to more substantial improvements in the pharmacokinetics in these two additional species (Table 5). In light of this data, compound **18** was selected for further development as a potential analgesic agent.

In summary, our backup strategy sought to address the risk of observing sub-optimal human pharmacokinetics with our first candidate **2** by targeting the primary route of metabolism of this molecule. This was achieved by replacing a metabolically labile carbon atom with a nitrogen atom and compound **18** emerged as the front runner from this new series. Compound **18** has a preclinical pharmacokinetic profile which addresses the risks associated with compound **2** and shows good efficacy in one of our key in vivo rat pain models. In addition we have also described some evidence which seems to support the role of central $P2X_7$ in the



18

Figure 3. Compound **18**: human $P2X_7$ pIC_{50} 8.0; rat $P2X_7$ pIC_{50} 6.4; MW = 302; PSA = 61 Å^2; LE = 0.58;[14] LLE = 5.1;[15] Pgp efflux ratio = 2.3; CYP_{450} inhibition pIC_{50} >25 μM at all isoforms tested (3A4, 2D6, 2C9, 1A2, 2C19); Log *D* @ pH 7.4 = 1.8.

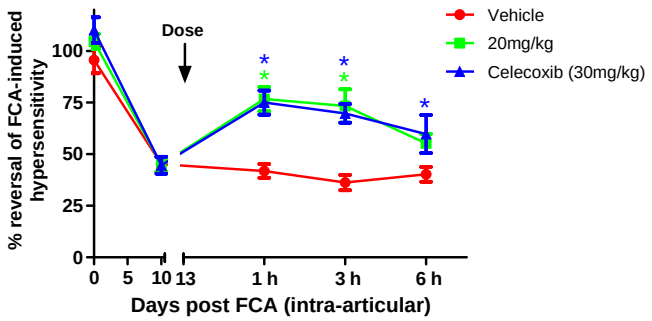
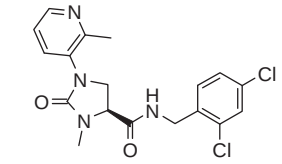


Figure 4. Effect of a 20 mg/kg oral dose of **18** in the chronic joint pain model in the rat at 1, 3, and 6 h post-dose. The effect of a 30 mg/kg oral dose of celecoxib is included for comparison.



21

Figure 5. Compound **21**: human $P2X_7$ pIC_{50} 8.0; rat $P2X_7$ pIC_{50} 7.2; MW = 393; PSA = 65 Å^2; Pgp efflux ratio = 4.9; Log *D* @ pH 7.4 = 2.2; rat in vivo clearance 17 mL/min/kg; rat $t^{1/2}$ = 2.3 h; rat C_{max} (1 h) = 1073 ng/mL @ 3 mg/kg po.

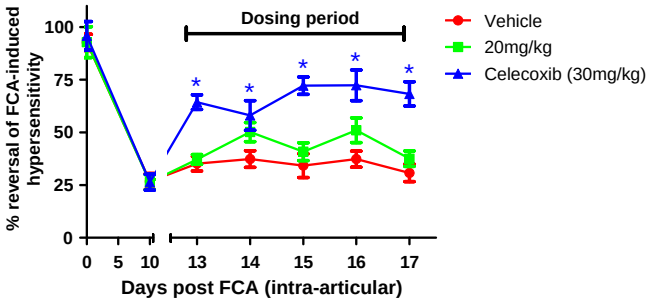


Figure 6. Effect of 5 days of dosing a 20 mg/kg bid oral dose of **21** in the chronic joint pain model in the rat. The effect of a 30 mg/kg bid oral dose of celecoxib is included for comparison.

Table 5
Summary of the cross-species pharmacokinetic profile ($n = 3$)[a] of compound **18**

Species	Rat	Dog	Monkey
CLb (mL/min/kg)	6	2	7
V_{ss} (L/kg)	1.3	0.8	1.2
$t^{1/2}$ (h)	2.8	5.7	2.9
F_{po} (%)	100	100	99

[a] Data generated from 1 mg/kg iv or 3 mg/kg po profiles.

processing of pain signals. Further details of related studies will be published in due course.

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18. Compounds shown in Table 2 were prepared using the methods described in WO2008 003697 A1, WO 2008 119685 A1, and WO 2008 119825 A1.
19. 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. All reported pIC50s deviate from the mean value by <0.3.
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