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Synthesis and biological activity of a series of tetrasubstituted-imidazoles as $P2X_7$ antagonists

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ABSTRACT

A series of analogues of the pyrazole lead **1** were synthesized in which the heterocyclic core was replaced with an imidazole. A number of potent antagonists were identified and structure–activity relationships (SAR) were investigated both with respect to activity at the $P2X_7$ receptor and in vitro metabolic stability. Compound **10** was identified as a potent $P2X_7$ antagonist with reduced in vitro metabolism and high solubility.

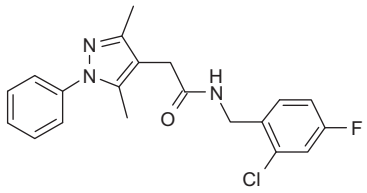
The $P2X_7$ receptor is a member of the P2X family of ligand gated ion channels. Like other members, it is activated by ATP and can act as a non-specific cation channel. It is however unique amongst this family, in that its activation can also lead to the release of biologically active interleukin-1-β (IL-1β),[1] and the up-regulation of other proinflammatory mediators.[2]

$P2X_7$ receptors are up-regulated and activated after inflammatory insults and are located within cells of the immune system.[3] This localization, together with the established role for IL-1β in inflammatory cascades, suggests a key role in a number of disease states. Recent data from experiments with knock-out mice has shown, for example, a lack of the development of a pain phenotype in models of inflammatory and neuropathic pain.[4] A second study has shown an improved score for $P2X_7$ −/− mice when compared to wild-type mice in a monoclonal antibody induced arthritis model.[5] Recently a number of novel antagonists have been disclosed, together with encouraging in vivo data in animal models of inflammatory bowel disease, rheumatoid arthritis, neuropathic and inflammatory pain.[6]

This evidence suggests the development of a potent antagonist could provide an exciting approach to the treatment of a number of inflammatory disorders. High throughput screening against the $P2X_7$ receptor lead to the discovery of a series of *N*-aryl-pyrazoles, for example compound **1** (Fig. 1).[7] This compound showed encouraging potency against both the human and rat channels, however showed moderate aqueous solubility (0.11 mg/mL) and lacked in vitro metabolic stability in rat and human liver microsomes. As part of the SAR studies around this template, it was decided to replace the central pyrazole core. If tolerated, it was hoped this would lead to an improvement in the overall developability profile of this series.

Imidazole was selected as an isostere which could retain the relative position of the ring substituents and the ability to act as a hydrogen bond acceptor which was believed to be important for $P2X_7$ antagonism.[7] This ring replacement would also lead to a lower lipophilicity[8] and solubility might also be gained through increased basicity.

2-Aryl imidazoles, as shown in Figure 2, were selected for initial investigation. A synthetic route was required which would enable variation of each of the imidazole substituents. Literature precedent identified keto-amides as imidazole precursors which would enable facile variation of the nitrogen substituent.[9] Incorporation of an ester functionality, if tolerated in the cyclisation reaction, would enable rapid optimization of the benzylic moiety.



1

Figure 1. Lead identified through screening. $P2X_7$ pIC_{50} h 7.4, r 7.0; in vitro clearance (Cli, mL/min/g liver) human >50, rat 46.

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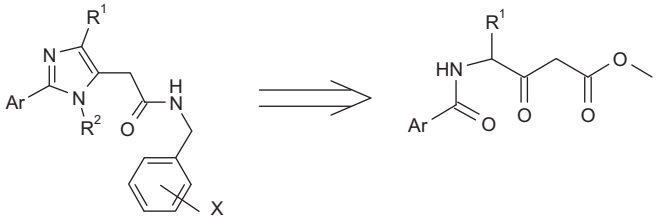


Figure 2. Proposed target molecule and key intermediate.

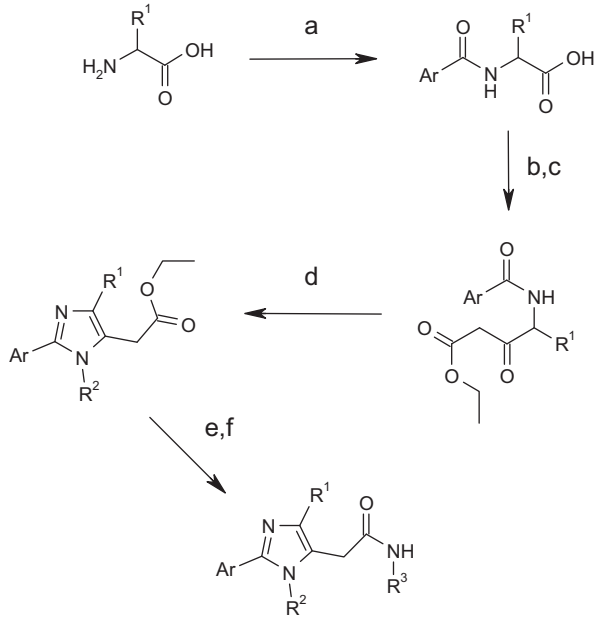
The desired intermediates were prepared by a two step procedure (Scheme 1). Coupling of glycine or alanine with an acid chloride was carried out in good yield with transient protection of the acid terminus using chlorotrimethylsilane (TMSCl). The intermediate carboxylic acids were converted using 1,1′-carbonyldiimidazole (CDI) to the imidazolides, to which was added the magnesium enolate of mono-ethyl malonate. Upon addition, a decarboxylation led to the desired cyclisation precursors in good to excellent yield.

Imidazole formation was successfully achieved by heating the keto-amide, amine and acetic acid in xylenes using a Dean–Stark apparatus in a typically moderate to good yield. Subsequent attempts to replicate this reaction using microwave irradiation proved unsuccessful. Ester cleavage using lithium hydroxide and amide coupling led to formation of the desired analogues.

Initially the aryl and R^1 substituents were held constant as phenyl and methyl, respectively. Imidazole nitrogen substituents (R^2) were evaluated in combination with a small set of benzylic groups which had been identified during SAR analysis around the original pyrazole template and had shown to be well tolerated by the $P2X_7$ receptor[7] (Table 1).

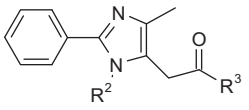
Encouragingly, the imidazole analogue **2** of the lead pyrazole **1** was shown to be equipotent against the human receptor with a small drop-off observed in the rat assay. An increase in metabolic stability was also observed with a reduction in human and rat in vitro clearance upon replacement of the pyrazole core.

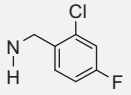
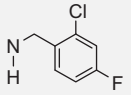
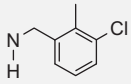
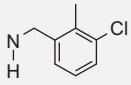
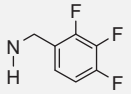
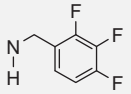
Each of the selected benzylic substituents showed similar human potency. A greater variation was observed with the rat ortho-



Scheme 1. (a) Reagents and conditions: (i) TMSCl, NEt_3, DCM, 0 °C to rt, 1 h; (ii) ArCOCl, 0 °C to rt, 18 h, 25–80%; (b) CDI, THF, rt 5 h; (c) $EtO_2CCH_2CO_2H$, $Mg(OEt)_2$, rt 18 h, 89–98%; (d) R^2NH_2, AcOH, xylenes, reflux, 2.5 h, 16–78%; (e) LiOH, THF, H_2O, 0–5 °C, 4 h; (f) R^3NH_2, EDCI, HOBT, *N*-ethyl morpholine, DMF, rt, 18 h.

Table 1
P2X7 inhibition at human and rat receptors together with metabolic stability for compounds **2–9**



	R^2	R^3	Human (rat) $P2X_7$ pIC_{50}[a]	Human (Rat) Cli[b] (mL/min/g liver)	*c* log *P*[c]
2	Me		7.4 (6.6)	9.5 (5.1)	3.7
3	Et		7.0 (6.8)	16 (13)	4.3
4	Me		7.3 (5.8)	16 (21)	4.0
5	Et		7.3 (6.2)	>50 (43)	4.6
6	Me		7.3 (5.5)	22 (43)	4.0
7	Et		7.3 (6.3)	>50 (>50)	4.6
8	Me		7.0 (5.3)	4.7 (2.0)	3.2
9	Et		6.8 (5.4)	—	3.7

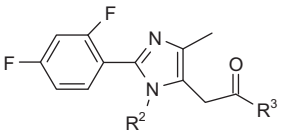
[a] Compounds tested in an ethidium bromide release assay.[10] Values represent a mean with $n \geqslant 2$.
[b] In vitro microsomal stability.[11]
[c] Daylight *c* log *P*.

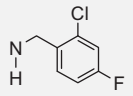
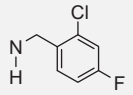
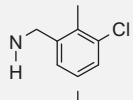
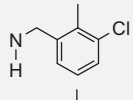
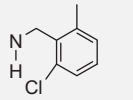
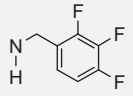
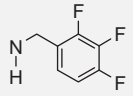
logue, for example, 2,3,4-trifluorobenzyl (**8**, **9**) was poorly tolerated by the rat receptor, whereas 2-chloro-4-fluorobenzyl analogues (**2**, **3**) had a much smaller drop-off in potency between species. Increasing the size of the N-substituent had minimal effect on potency and led to a decrease in metabolic stability. An emerging correlation between lipophilicity and metabolic stability was observed with the least lipophilic analogue **8** showing the greatest stability in the microsomal preparation.

A potential metabolic liability within this series was thought to be the unsubstituted phenyl ring and it was proposed that introduction of a halogen substituent, such as fluorine, could block oxidative metabolism. A series of compounds were synthesized, using a method analogous to that shown in Scheme 1, with a 2,4-difluorophenyl moiety (Table 2). The addition of fluorine yielded little change in the affinity of analogues to $P2X_7$ and generally showed little benefit in terms of metabolic stability. An exception was compound **10** which showed a good combination of potency at each species and low to moderate metabolic turnover in both human and rat.

A third set of analogues were prepared and evaluated in which the potentially metabolically vulnerable R^1 substituent at C4 was replaced by hydrogen (Table 3). Removal of a methyl at this position was detrimental to human potency in some cases, although

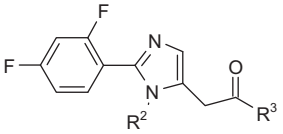
Table 2
$P2X_7$ inhibition at human and rat receptors together with metabolic stability for compounds **10**–**16**

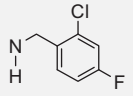
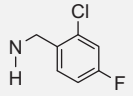
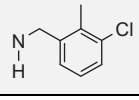


	R^2	R^3	Human (rat) $P2X_7$ pIC_{50}[a]	Human (rat) Cli[b] (mL/min/g liver)	*c* log *P*[c]
10	Me		7.4 (6.5)	3.0 (3.4)	4.0
11	Et		7.0 (6.8)	20 (13)	4.6
12	Me		7.5 (6.2)	16 (16)	4.3
13	Et		7.4 (6.4)	—	4.9
14	Et		7.3 (6.2)	>50 (>50)	4.9
15	Me		7.2 (<5)	—	3.5
16	Et		6.9 (6.0)	18 (3.6)	4.0

[a–c] See footnotes to Table 1 for details.

Table 3
$P2X_7$ inhibition at human and rat receptors together with metabolic stability for compounds **17**–**19**



	R^2	R^3	Human (rat) $P2X_7$ pIC_{50}[a]	Human (rat) Cli[b] (mL/min/g liver)	*c* log *P*[c]
17	Me		6.7 (6.6)	2.7 (6.7)	3.8
18	Et		6.3 (6.4)	—	4.3
19	Me		7.2 (6.2)	10 (18)	4.1

[a–c] See footnotes to Table 1 for details.

when 2-methyl-3-chlorobenzyl is introduced (**19**) potency is maintained, indicating further evaluation of SAR may be warranted. The R^1 substituent seems unlikely to be a site of metabolism as its removal has little effect on microsomal stability.

Table 4
Solubility profile of compounds **1** and **10** (μg/mL, 1 h)

Aqueous media	pH	Solubility	
		1	**10**
Water		110	920
Fed state simulated intestinal fluid (FeSSIF)	5	100	900
Fasted state simulated intestinal fluid (FaSSIF)	6.5	120	370
Simulated gastric fluid	1.2	410	>1000

The hydrochloride salt of compound **10** was selected for further evaluation of its developability profile since it showed the best overall combination of potency and metabolic stability in both species. The compound showed a moderate level of protein binding (HSA 91% bound) and was not a potent competitive inhibitor of CYP1A2, 2C9, 2C19, 2D6 and 3A4 with an IC_{50} of 5.5 μM at 3A4 and >10 μM at each of the other isoforms.[12] Solubility was assessed in water and a number of simulated physiological fluids (Table 4).[13] The data showed an improved profile compared with the pyrazole lead with high solubility in water, gastric fluid and simulated fed state fluid. The compound showed moderate solubility in the fasted state fluid, which may be a reflection of the fluid having a pH value close to the measured pK_a of compound **10** (6.47).

Compound **10** was also tested against 35 other ion channels, receptors and enzymes. No significant off-target activity was observed. Following on from this positive data the in vivo pharmacokinetics of **10** were assessed in the rat. The compound was administered orally at 3 mg/kg.[14] Promisingly, oral exposure was observed with a C_{max} of 0.43 μM (±0.04), AUC (0–6 h)/dose 6.8 min kg/L (±0.5) and a T_{max} of 0.5 h indicating rapid absorption.

In summary, a combination of homologation and cyclisation synthetic steps proved to be a versatile route to a number of tetra-substituted imidazoles. This enabled a range of analogues to be prepared for SAR studies. Replacement of the central core of the pyrazole screening hit **1** with an imidazole was well tolerated. Potency was successfully retained across a range of analogues at both the human and rat receptor, whilst aqueous solubility was increased. Several strategies were employed to block potential sites of metabolism and to increase the in vitro metabolic stability. Compound **10** was identified with an encouraging combination of $P2X_7$ inhibition and rat oral exposure and therefore with a suitable profile to take forward into in vivo PD studies.

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8. *c* log $D_{7.4}$ (ACD) compound **1** = 3.4, compound **2** = 2.2.
9. Gordon, T. D.; Singh, J.; Hansen, P. E.; Morgan, B. A. *Tetrahedron Lett.* **1993**, *34*, 1901.
10. Ethidium bromide assay format described in patent application WO 2007 141267 A1. Briefly, the assay is carried out in HEK293 cells expressing recombinant $P2X_7$ receptors. ATP activation of the channel leads to an opening of in cell wall and accumulation of ethidium bromide within the cell, levels of which are determined using measurement of fluorescence. Inhibition of the ATP response by a novel compound may be measured across a range of concentrations to generate an IC_{50}.
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13. Galia, E.; Nicolaides, E.; Horter, D.; Lobenberg, R.; Reppas, C.; Dressman, J. B. *Pharm. Res.* **1998**, *15*, 698.
14. Compound **10** was dosed to three rats orally by gavage at a target dose of 3 mg/kg. The compound was prepared, on the day of dosing, in 1% (w/v) methylcellulose aq at a concentration of 0.6 mg/mL and administered at 5 mL/kg. Serial blood samples were taken from each rat up to 6 h after dose administration. Diluted blood samples were analysed for parent compound by LC/MS/MS. 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.