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Discovery and structure–activity relationships of a series of pyroglutamic acid amide antagonists of the $P2X_7$ receptor

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ABSTRACT

A computational lead-hopping exercise identified compound **4** as a structurally distinct $P2X_7$ receptor antagonist. Structure–activity relationships (SAR) of a series of pyroglutamic acid amide analogues of **4** were investigated and compound **31** was identified as a potent $P2X_7$ antagonist with excellent in vivo activity in animal models of pain, and a profile suitable for progression to clinical studies.

$P2X_7$, an ATP-gated ion-channel,[1–3] controls the activation and release of pro-inflammatory cytokines such as interleukin-1β (IL-1β).[4] Antagonists of the $P2X_7$ receptor can modulate such responses in $P2X_7$-expressing cells in both the immune and central nervous systems,[5] suggesting a potential role for $P2X_7$ receptor antagonists in the treatment of a wide range of conditions. In particular, 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] Two compounds (AZD-9056 and CE-224535), have progressed to early proof-of-concept clinical trials in rheumatoid arthritis patients[11,12] and whilst both of these compounds have now been discontinued the question of whether a $P2X_7$ antagonist, with the appropriate physicochemical and pharmacokinetic profile, could be therapeutically efficacious in treating human pain states remains unanswered.

In two earlier publications[13] we described the optimization of a series of (1*H*-pyrazol-4-yl)acetamides, derived from hit compound **1** (Fig. 1) which had been identified via high-throughput screening. From this series, compound **2**, *N*-[(2,4-dichlorophenyl)methyl]-2-(3,5-dimethyl-1*H*-pyrazol-4-yl)acetamide, was selected for evaluation in in vivo models of pain and shown to be a potent antihyperalgesic agent in both the rat acute complete Freund's adjuvant (CFA) model of inflammatory pain[19] and the knee joint model of chronic inflammatory pain.[20] However, compounds of this class were subsequently found to be prone to time-dependently inhibit the CYP3A4[21] isozyme and routes of metabolism studies indicated oxidation of the methyl substituents as the most likely cause.

Whilst this issue could be addressed (unpublished data) by replacing the methyl groups with alternative substituents (e.g.,

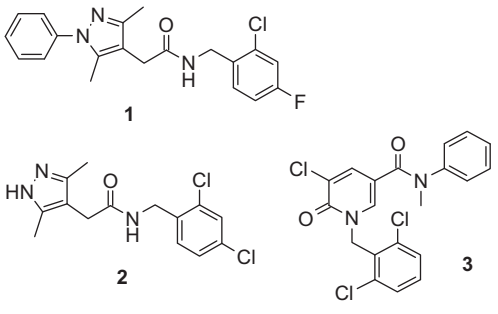

Figure 1. Compound **1**: human $P2X_7$ pIC_{50}[14] 7.4; rat $P2X_7$ pIC_{50} 7.0; rat in vitro clearance[15] = 46 mL/min/g liver; *c* log *D*[16] at pH 7.4 = 3.4; M_W = 372; LE = 0.39[17]; LLE[18] = 4.0; compound **2**: human $P2X_7$ pIC_{50} 8.1; Rat $P2X_7$ pIC_{50} 7.1; rat in vitro clearance 1.3 mL/min/g; *c* log *D* at pH 7.4 = 2.7; M_W = 312; LE = 0.55; LLE = 5.4; compound **3**: human $P2X_7$ pIC_{50} 6.9; rat $P2X_7$ pIC_{50} 6.5; rat in vitro clearance >50 mL/min/g; *c* log *D* at pH 7.4 = 4.4; M_W = 421; LE = 0.35; LLE = 2.5.

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CF_3) this usually resulted in a significant loss of potency. In order to address the potential developability risk presented by this observation we sought to identify alternative classes of $P2X_7$ antagonists which were free of this issue. Compound **3** (see Fig. 1) was also identified as a $P2X_7$ antagonist following our initial high-throughput screening of the GSK compound collection and, whilst this template was less attractive in general (higher molecular weight, lower ligand efficiency (LE)), it presented the opportunity to identify common pharmacophoric features that are shared by different $P2X_7$ antagonist chemotypes. In order to identify these common pharmacophoric features a simple overlay[22] of compounds **1** and **3** was performed. As can be seen in Figure 2 (circled) the resulting superposition of the two molecules indicated that the carbonyl oxygens of the two (acyclic) amide groups as well as the pyrazole nitrogen in **1** and the oxygen atom of the carbonyl in the pyridone ring of **3**, respectively, can interact with the same putative hydrogen-bond donor feature of the $P2X_7$ receptor in each case.

This observation led us to adopt the hypothesis that two suitably positioned hydrogen-bond donor features are required (or at least beneficial) when trying to achieve activity at the $P2X_7$ receptor in a low molecular weight template. Since only limited SAR was available (particularly around compound **3**) and we felt that we did not have sufficient information to build a pharmacophore that would be fit for virtual screening we decided to use fingerprint-based similarity searching in order to identify additional chemotypes of interest.[23] After applying property filters (e.g., hydrogen-bond acceptor count $\geqslant 2$, based on the overlay shown in Fig. 2) hits of interest were selected by stepping through the framework clusters and visually inspecting the members of the individual clusters.[24] A preference was given to molecules where the bond count between the two hydrogen-bond acceptor atoms was similar to that found in the query molecules (i.e., 5 in **1** and 6 in **3**).

The most promising hit with a ligand efficiency = 0.43[17] was pyroglutamic amide **4** (see Fig. 3), one of only nine compounds selected for screening, and ranked 595th amongst the 2000 top-ranked hits from the 3-point pharmacophoric fingerprint similarity search.[26] Whilst the human potency was good, the potency at the rat receptor was lower than preferred, but the low molecular weight and good ligand efficiency of **4** were particularly appealing. Synthesis of the (*S*)-enantiomer of **4** (i.e., **4a**) confirmed the activity of the initial sample and we resolved to prepare additional analogues to profile this template more thoroughly.

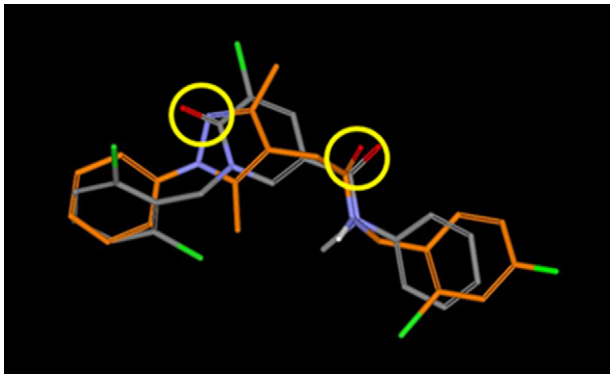
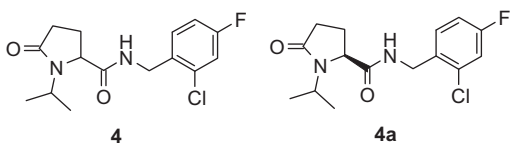
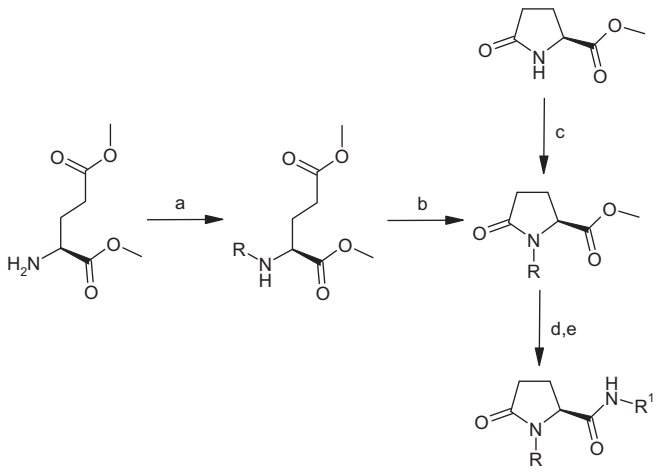


Figure 2. Overlay of compounds **1** (in orange) and **3**.



4 **4a**

Figure 3. Compound **4**: human $P2X_7$ pIC_{50} 6.5; rat $P2X_7$ pIC_{50} <5; *c* log *D* at pH 7.4 = 2.6; M_W = 312; LE = 0.43[17]; LLE = 3.9.[18] Compound **4a**: human $P2X_7$ pIC_{50} 7.0; rat $P2X_7$ pIC_{50} 5.8; rat in vivo clearance 31 mL/min/kg.



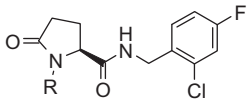
Scheme 1. Synthesis of pyroglutamic acid amide analogues. Reagents and conditions: (a) aldehyde or ketone, AcOH, $NaBH_4$, MeOH, 0 °C, ca. 1 h; (b) toluene, reflux, Dean–Stark, ca. 16 h; (c) NaHMDS, alkyl halide, THF, −78 °C to room temperature, ca. 1 h; or aryl bromide, $Pd_2(dba)_3$, Cs_2CO_3, Xantphos™, toluene, reflux, ca. 18 h (d) 2M NaOH (aq), MeOH, 0 °C to room temperature, ca. 4 h (e) R^1NH_2, EDAC, HOBT, DCM/DMF (~3:1), rt, ca. 16 h.

Analogues were, in the first instance, prepared using one of two methods as shown in Scheme 1.[27]

Reductive alkylation of the dimethyl ester of glutamic acid followed by thermal cyclisation gave the required N-substituted methyl pyroglutamates. Alternatively these key intermediates could be accessed by alkylation of methyl pyroglutamate with an alkyl halide or arylation using Buchwald coupling conditions. Subsequent saponification and amide coupling then provided the N-substituted pyroglutamic acid amides in good overall yields.

Using the above methods we first prepared a number of analogues which retained the 2-Cl, 4-*F*-benzylamide group and explored the structure–activity relationships (SAR) around the *N*-alkyl substituent in the five-membered ring (Table 1). Removing the isopropyl group in **4a** resulted in a complete loss of activity (**5**) whereas replacement by methyl, ethyl and propyl gave

Table 1
Structure–activity data for pyroglutamic acid amide analogues of compound **4**



Compd	R	Human $P2X_7$ pIC_{50}[a]	Rat CLi[b] (mL/min/g) liver	Ligand efficiency[17]
5	H	<6	—	—
6	Me	7.0	—	0.50
7	Et	7.5	<0.5	0.52
8	*n*-Pr	6.8	—	0.45
9	2-Me-propyl	6.4	—	0.40
10	Benzyl	<6	—	—
11	Cyclobutyl	7.6	2.3	0.47
12	Cyclopentyl	6.3	—	0.38
13	Phenyl	6.1	—	0.35

[a] Data generated using an ethidium bromide release assay (Ref. 14), reporting an average value of $n > 3$.
[b] Microsomal clearance method described in Ref. 15.