



## Discovery and optimization of RO-85, a novel drug-like, potent, and selective P2X<sub>3</sub> receptor antagonist

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### ABSTRACT

Despite the extensive literature describing the role of the ATP-gated P2X<sub>3</sub> receptors in a variety of physiological processes the potential of antagonists as therapeutic agents has been limited by the lack of drug-like selective molecules. In this paper we report the discovery and optimization of RO-85, a novel drug-like, potent and selective P2X<sub>3</sub> antagonist. High-throughput screening of the Roche compound collection identified a small hit series of heterocyclic amides from a large parallel synthesis library. Rapid optimization, facilitated by high-throughput synthesis, focusing on increasing potency and improving drug-likeness resulted in the discovery of RO-85.

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P2X receptors are ligand gated ion channels activated by adenosine 5'-triphosphate (ATP) and related di- and tri-phosphate nucleotides. Seven P2X receptor subunits have been identified P2X<sub>1-7</sub> and each channel shown to be assembled from three subunits.<sup>1</sup> Understanding the pharmacology of the channels is complex in that, with the exception of P2X<sub>6</sub>, in addition to homomeric channels, each subunit also has the ability to form heteromeric channels and a total of seven heteromeric P2X family members have been identified.<sup>2</sup> Homomeric P2X<sub>3</sub>, and the closely related heteromultimeric P2X<sub>2/3</sub>, receptors are predominantly colocalized on small to medium diameter sensory afferent neurons and have become increasingly recognized as playing a major role in mediating the primary sensory effects of ATP. P2X<sub>3</sub>-KO mice demonstrate a reduced sensitivity to thermal stimuli and decreased pain behaviors.<sup>3</sup> Furthermore reduction of P2X<sub>3</sub> expression through intrathecal administration of P2X<sub>3</sub>-selective antisense,<sup>4</sup> and more recently siRNA,<sup>5</sup> also causes a significant decrease in pain behaviors in mice. By eliminating the P2X<sub>3</sub> subunit these gene deletion and knockdown techniques also eliminate the P2X<sub>2/3</sub> receptor so a clear understanding of the receptor responsible for these effects is hard to ascertain.

Despite significant advances in elucidating the pharmacology of P2X<sub>3</sub> receptors, the field has suffered from a lack of drug-like, potent and selective ligands. Many of the reported selective P2X<sub>3</sub> antagonists (Fig. 1), including the most recently reported highly potent peptide antagonist Spinorphin (**1**),<sup>6</sup> either do not report selectivity over P2X<sub>2/3</sub> or are dual P2X<sub>3</sub>/P2X<sub>2/3</sub> antagonists like TNP-ATP (**2**)<sup>7</sup> and A-317491 (**3**),<sup>8</sup> as well as our recently published diaminopyrimidines, (**4a**) and (**4b**).<sup>9,10</sup>

In addition to the unknown, or lack of selectivity over P2X<sub>2/3</sub> making their pharmacology hard to understand, these ligands also show poor drug-like properties. TNP-ATP and A-317491, similar to many of the non-selective P2X<sub>3</sub> receptor antagonists, contain polyacidic functional groups that limit their bioavailability and overall potential as drug candidates. Despite this A-317491 has been shown to be active in a number of in vivo models of chronic inflammatory pain, neuropathic pain<sup>8</sup> and overactive bladder.<sup>11</sup> No data has been published on the drug-like properties of spinorphin which has also been shown to be effective in the tail pinch assay following intracerebroventricular (ICV) administration.<sup>12</sup> In this Letter we report the discovery and optimization of a series of drug-like potent and selective P2X<sub>3</sub> antagonists.

A high-throughput screening campaign of the Roche compound collection using the rat recombinant P2X<sub>3</sub> receptor was performed. Disappointingly, in addition to a very overall low hit rate of 0.01%, many of the more potent sub-micromolar hits contained similar

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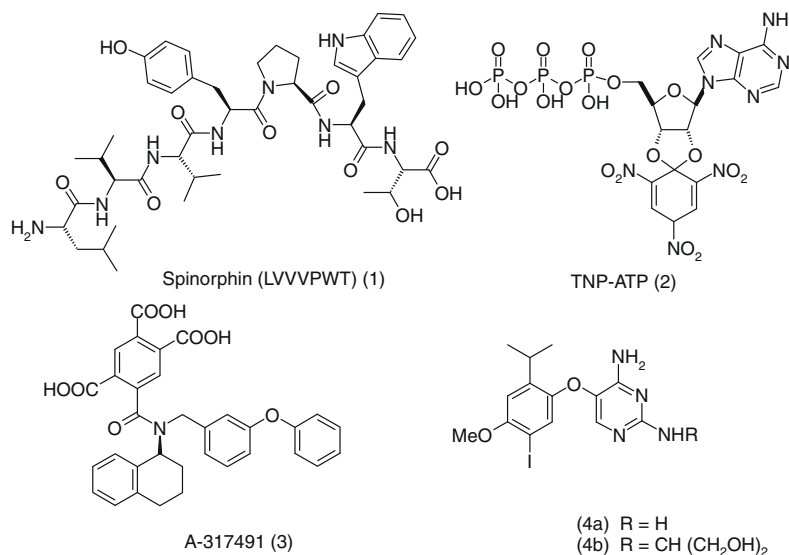


Figure 1. Structures of known P2X<sub>3</sub> antagonists.

poly-acidic functionality to the known antagonists with their implied poor drug-like properties. These were not deemed suitable starting points for a medicinal chemistry program aimed at producing potential therapeutic agents so a careful analysis of the less potent hits was undertaken. Four compounds, initially synthesized as part of a ~4500 member parallel synthesis library, were identified as hits. The library was based on a 2,4-disubstituted-oxazole-5-carboxylic acid amide core structure. The hits were characterized by a 2-phenyl substituent on the oxazole core and, more specifically, the presence of a 1-methyl-2-(4-pyrimidin-2-yl-piperazin-1-yl)-ethylamine amide (Fig. 2).

A detailed pharmacological and drug-likeness characterization of (5a) was undertaken. (5a) was shown to be a selective antagonist of rP2X<sub>3</sub> (pIC<sub>50</sub> 6.6) with no antagonist activity at P2X<sub>2/3</sub> or other family members tested (pIC<sub>50</sub> <5). In a standard CEREP profile of 80 other enzymes, receptors and ion channels only five other activities were identified in the 5–10 μM range.<sup>13</sup> In an assessment of drug-likeness; with positive attributes of moderate molecular weight (434) and calculated log *P* (2.7), and high permeability without efflux it was disappointing to find high in vitro clearance in rat and human microsomes and hepatocytes, and >99% protein binding in rat plasma. In addition (5a) was a moderate inhibitor of the CYP450 isozymes 3A4 and 2D6 with IC<sub>50</sub>'s <10 μM. A parallel optimization approach exploring replacement of the oxazole core and its substituents, and the amide portion of the molecule was undertaken.

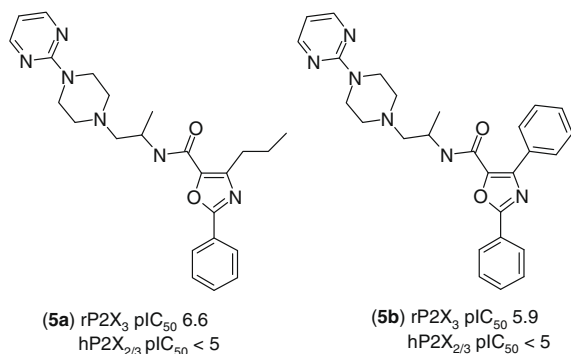


Figure 2. Hits structures identified by high-throughput screening and their pIC<sub>50</sub>'s at the rat P2X<sub>3</sub> receptor.

The role of the oxazole scaffold was investigated using both a general collection of acids fitting a similar motif and by a traditional iterative SAR approach. A representative set of similar amides is shown in Table 1. For five-membered scaffolds, pyrazole amides (6a) and (6b) and furans (6c) and (6d) had similar activity to one of the other initial hits (5b). Substitutions that altered the geometry of the amide bond, (6g) and (6h) were not tolerated. Substitution of the butyl or phenyl group for a methyl gave a significant drop in activity (6i) versus (5a) and (5b). Interestingly, substitution of the phenyl groups in a 3,4 relationship as in (6j) and (6f) led to no loss in activity.

A six-membered ring scaffold was also accepted, as shown in examples (8a–8f), even with only one aryl substituent. An interesting 5-5-bicyclic core, 1-methyl-3-phenyl-1*H*-thieno[2,3-*c*]pyrazole-5-carboxylic amide (7), discovered using parallel synthesis was found to show promising opportunities by allowing truncation of the propyl group to a methyl group.

Exploration of the piperazinyl-ethylamine linker of the molecule showed little opportunity for modification (see Fig. 3) with the exception of a preference for the *R*-stereochemistry (Table 2). The pyrimidine group could, however be modified with other heterocycles, without losing activity at the P2X<sub>3</sub> receptor (9a–9f) as long as a nitrogen was present in the 2-position to act as an elector acceptor (see Table 2). The *m*-pyridine and *p*-pyridine were inactive (9g and 9h). Interestingly, a simple carbonyl group was shown to be sufficient for good affinity as long as the group remained planar. For example, acetyl amide (10a), thioacetyl amide (10c) and urea (10d) were all active, whereas the cyclopropylcarbonylamide (10f) and other amides not shown here, the substituted urea (10e), sulfonamide (10g) and ethyl carbamate (10h) were inactive. Of particular significance was the promising activity of (10a) with a concurrent improvement in molecular weight and lipophilicity. These analogs were synthesized easily starting from Boc-D-alaninal as shown in Scheme 1.

Reaction of Boc-D-alaninal with *N*-acetylpiperazine in the presence of NaBH(OAc)<sub>3</sub> afforded intermediate (15) in 85% yield. Removal of the Boc group with TFA, followed by coupling with commercially-available 1-methyl-3-phenyl-1*H*-thieno[2,3-*c*]pyrazole-5-carboxylic acid using a HBTU coupling afforded the amide, which was converted the oxalate salt (16).<sup>14</sup> Similar reductive amination conditions, followed by deprotection were used to prepare *N*-arylpiperidines (14a–14h), which were coupled to 2-(4-fluorophenyl)-4-propyl-oxazole-5-carboxylic acid<sup>15</sup> using DCC and HOBT

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