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Identification and SAR of novel diaminopyrimidines. Part 1: The discovery of RO-4, a dual $P2X_3/P2X_{2/3}$ antagonist for the treatment of pain

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ABSTRACT

P2X purinoceptors are ligand-gated ion channels whose endogenous ligand is ATP. Both the $P2X_3$ and $P2X_{2/3}$ receptor subtypes have been shown to play an important role in the regulation of sensory function and dual $P2X_3/P2X_{2/3}$ antagonists offer significant potential for the treatment of pain. A high-throughput screen of the Roche compound collection resulted in the identification of a novel series of diaminopyrimidines; subsequent optimization resulted in the discovery of RO-4, a potent, selective and drug-like dual $P2X_3/P2X_{2/3}$ antagonist.

P2X purinoceptors are ligand-gated ion channels activated by adenosine 5′-triphosphate (ATP). Seven P2X receptor subunits have been identified ($P2X_{1-7}$) and each channel shown to be assembled from three subunits.[1] Understanding the pharmacology is complex since each subunit has the ability to form heteromeric channels. A total of seven heteromeric P2X family members have been identified.[2] Homomeric $P2X_3$, and the closely related heteromultimeric $P2X_{2/3}$, receptors are predominantly localized 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. $P2X_3$ receptor expression is upregulated in DRG neurons following ligation of the sciatic nerve in the chronic constriction injury (CCI) model.[3] $P2X_3$-KO mice demonstrate a reduced sensitivity to thermal stimuli and decreased pain behaviors.[4] Furthermore reduction of $P2X_3$ expression through intrathecal administration of $P2X_3$-selective antisense oligonucleotide,[5] and more recently siRNA,[6] also causes a significant decrease in pain behaviors in mice.

Despite the mounting evidence for the importance of these receptors, the field was hampered by the lack of potent and selective small molecule antagonists. 2′,3′-*O*-(2,4,6-Trinitrophenyl)adenosine 5′-triphosphate (TNP-ATP) (**1**) was the only reported dual $P2X_3/P2X_{2/3}$ antagonist though its nucleotide nature primarily limits its use to in vitro experiments.[7] In a more recent report the peptide spinorphin has been reported as a selective $P2X_3$ antagonist but no data at $P2X_{2/3}$ is described[8] (Fig. 1).

The discovery of A-317491 (**2**) marked a significant breakthrough; for the first time a truly selective, non-nucleotide, small molecule dual $P2X_3/P2X_{2/3}$ antagonist was reported.[9] The impressive in vivo efficacy profile in multiple chronic inflammatory and neuropathic pain models validated the importance of these receptors in pain signaling pathways. More recent data demonstrating the efficacy of A-317491 in a cyclophosphamide induced cystitis model also signals the potential of targeting $P2X_3$-containing receptors for the treatment of overactive bladder.[10] While displaying an impressive array of in vivo activity, the potential of A-317491 as a therapeutic agent is limited by the poly-acidic nature it shares with many of the non-selective antagonists. The presence of the three carboxylic acid groups significantly limits both the oral bioavailability and the CNS penetration. In this Letter we describe the discovery and structure–activity relationships of a novel series of diaminopyrimidines resulting in the discovery of RO-4; a potent, selective[11] and drug-like[12] dual $P2X_3/P2X_{2/3}$ receptor antagonist.

A high-throughput screening campaign of the Roche compound collection using the rat recombinant $P2X_3$ receptor was performed.

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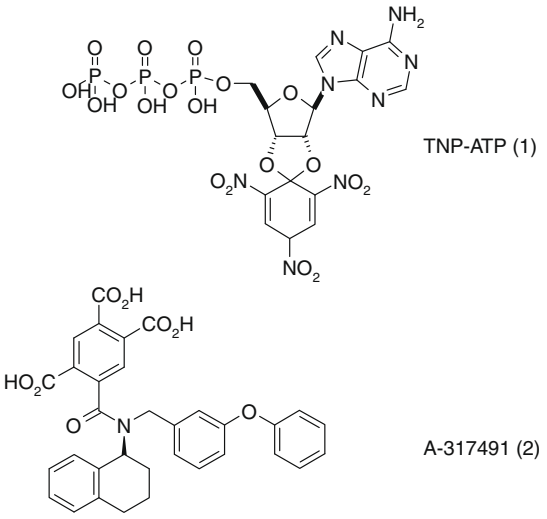


Figure 1.

In addition to a very low overall hit rate of 0.01%, many of the more potent hits contained poly-acidic functionality, similar to the known antagonists, with the implied poor drug-like properties associated with these functional groups. 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. The low molecular weight diaminopyrimidine **3** was identified with a pIC_{50} of 5.8 at $P2X_3$ but with no activity at $P2X_{2/3}$ below 10 μM ($pIC_{50} < 5$). This molecule was originally synthesized as an analog of the anti-bacterial diaminopyrimidine trimethoprim (**4**) and shares many of its structural features (Fig. 2).

The lead optimization strategy for the diaminopyrimidine series focused on three key features; the small alkyl side-chain, the arene–pyrimidine linker and the 3,4-disubstituted arene. Although necessary for activity, the nature of the diaminopyrimidine SAR will be the subject of a subsequent communication.

Trimethoprim (**4**) is prepared via the on the metric ton scale.[13] This synthesis is based on an aldol condensation of an aldehyde with cyanomethoxyethane to build in the precursor of the diaminopyrimidine ring. Because this method failed to give appreciable amounts of aldol adduct with hindered aromatic aldehydes, we chose to develop a new route based on lithiated 2,4-dimethoxypyrimidine.[14] This five step synthesis is outlined in Scheme 1.

Orthollithiation of 2,4-dimethoxypyrimidine was accomplished with lithium 2,2,6,6-tetramethylpiperidine (LTMP) in THF at 0 °C. Addition of a substituted aromatic aldehyde[15] gave a secondary alcohol which was oxidized with MnO_2 to a ketone. Treatment of the ketone with NH_3/MeOH at 80 °C gave a 5-acyl-2,4-diaminopyrimidine. Reduction of the ketone via $LiAlH_4$ followed by treatment with TFA/Et_3SiH gave the target diaminopyrimidines (typically 15% for 5 steps).

Initial optimization efforts focused on preparation of side-chain analogs (Table 1). Screening hit **3**, which contained an ethyl side-chain, was moderately active at $P2X_3$. Methyl (**5**) and *n*-propyl

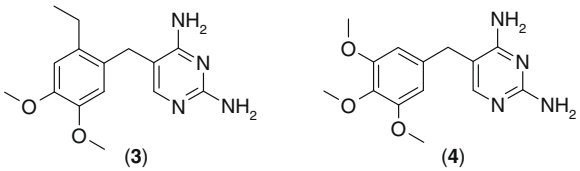
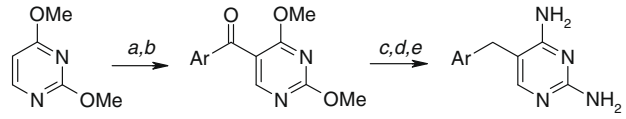
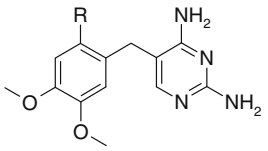


Figure 2.



Scheme 1. Preparation of C-linked diaminopyrimidines. Reagents and conditions: (a) LiTMP, THF, RCHO, 0 °C; (b) MnO_2, toluene, reflux; (c) 7 M NH_3, MeOH, 80 °C, sealed tube; (d) $LiAlH_4$, THF, reflux; (e) TFA, CH_2Cl_2, Et_3SiH.

Table 1
Optimization of the arene side-chain



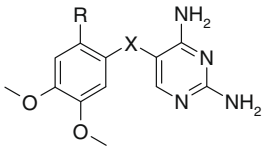
Entry	Compound	R	$P2X_3$[a,c]	$P2X_{2/3}$[b,c]
1	**5**	Me	< 5.0	<5.0
2	**3**	Et	5.8	<5.0
3	**6**	*n*-Pr	<5.0	<5.0
4	**7 (RO-3)**	***i*-Pr**	**6.9**	**5.7**
5	**8**	*c*-Pr	5.9	<5.0
6	**9**	*i*-Bu	<5.0	5.0
7	**10**	*t*-Bu	<5.0	<5.0
8	**11**	*c*-Bu	<5.0	<5.0

[a] FLIPR: mean pIC_{50}, $rP2X_3$ CHO cell.
[b] FLIPR: mean pIC_{50}, $hP2X_{2/3}$ 1321N1 (astrocytoma) cells.
[c] pIC_{50} values are the mean of at least three experiments performed in triplicates, standard deviation ±20%.

(**6**) analogs were devoid of all $P2X_3$ activity. However, isopropyl arene **7** (RO-3) was *10-fold* more potent than ethyl arene **3**. Furthermore, incorporation of one additional methyl group, either at the benzylic carbon (*tert*-butyl **10**) or the terminal carbon (*iso*-butyl **9**) resulted in complete loss of activity. Also, the *c*-butyl analog was not active. Together these results suggest that although there seems to be a requirement for a small side-chain, a major contributing force for $P2X_3$ binding must be the torsional angle of the side-chain. Specifically, analogs such as *i*-propyl arene **7** can adopt a low-energy conformation which places the side-chain C–H in plane (toward the diaminopyrimidine). This orients a methyl group into a region of space inaccessible to inactive analogs such as *tert*-butyl arene **10**.

Next the role of arene–diaminopyrimidine linker was examined (Table 2). Both ketone **13** and alcohol **12** were completely inactive. Extended straight-chain aliphatic analogs such as phenethyl **14**

Table 2
Ether containing linker leads to an impressive boost in potency



Entry	Compound	Structure	$P2X_3$[a,c]	$P2X_{2/3}$[b,c]
1	**12**	R = *i*-Pr, X = CHOH	<5.0	<5.0
2	**13**	R = *i*-Pr, X = C = O	<5.0	<5.0
3	**14**	R = Et, X = CH_2CH_2	<5.0	<5.0
4	**15**	R = Et, X = O	6.8	5.7
5	**16**	**R = *i*-Pr, X = O**	**7.6**	**6.3**

[a] FLIPR: mean pIC_{50}, $rP2X_3$ CHO cell.
[b] FLIPR: mean pIC_{50}, $hP2X_{2/3}$ 1321n1c cell.
[c] pIC_{50} values are the mean of at least three experiments performed in triplicates, standard deviation ±20%.