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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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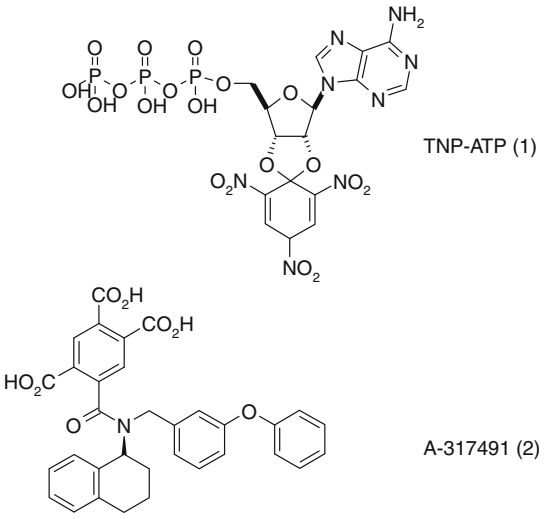


Figure 1.

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

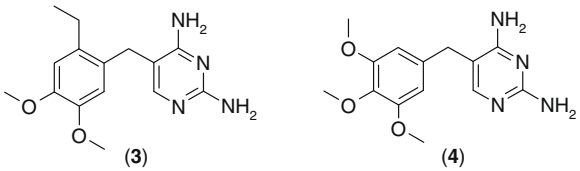
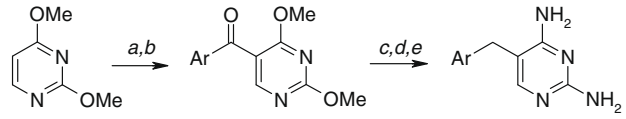
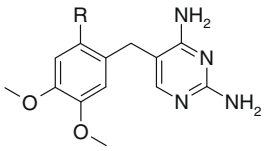


Figure 2.



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



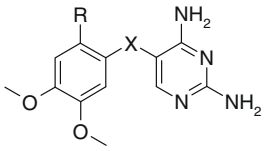
Entry	Compound	R	$P2X_3$[a,c]	$P2X_{2/3}$[b,c]
1	**5**	Me	< 5.0	<5.0
2	**3**	Et	5.8	<5.0
3	**6**	*n*-Pr	<5.0	<5.0
4	**7 (RO-3)**	***i*-Pr**	**6.9**	**5.7**
5	**8**	*c*-Pr	5.9	<5.0
6	**9**	*i*-Bu	<5.0	5.0
7	**10**	*t*-Bu	<5.0	<5.0
8	**11**	*c*-Bu	<5.0	<5.0

[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**.

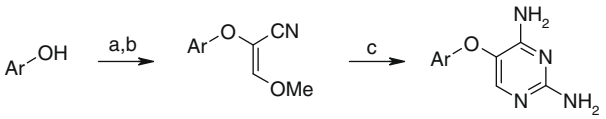
Next the role of arene–diaminopyrimidine linker was examined (Table 2). Both ketone **13** and alcohol **12** were completely inactive. Extended straight-chain aliphatic analogs such as phenethyl **14**

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%.



Scheme 2. Preparation of O-linked diaminopyrimidines. Reagents and conditions: (a) iodoacetonitrile, K_2CO_3, DMF, 50 °C; (b) NaH, DME, Ethyl formate, 80 °C, MeI; (c) quanidine carbonate, NaOMe, DMSO, 110 °C.

were also inactive. However, replacing the methylene linker of ethyl arene **3** with oxygen gave a *10-fold boost* in $P2X_3$ activity. Additionally, the cooperative effect of the oxygen linker with the isopropyl side-chain of ether **16** led to an impressive increase in $P2X_3$ potency (*18-fold* increase). Molecular modeling studies suggest that replacement of the bridging methylene by oxygen restricts the rotation of the diaminopyrimidine by the formation of an intramolecular hydrogen bond between it and the NH_2 group of the diaminopyrimidine.

The preparation of oxygen-linked analogs began with an alkylation of a 2-alkyl phenol (Scheme 2). This transformation was accomplished in DMF with iodoacetonitrile in the presence of K_2CO_3 at 50 °C. The resulting cyanomethylphenyl ether was treated with NaH in DME and refluxed for 3 h in the presence of excess ethyl formate. The ensuing unstable enol was alkylated with methyl iodide to furnish an enol ether as a mixture of *E*/*Z* isomers (typically 10:1). The final diaminopyrimidine was prepared by condensing the enol ether with guanidine carbonate in DMSO at 110 °C.

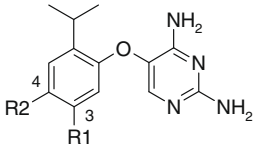
Having determined the optimal side-chain and linker we shifted our attention to modifying the methoxy groups (Table 3). To determine the individual binding contributions of each methoxy group, we prepared three deletion analogs: unsubstituted benzene **17**, 3-methoxy benzene **18** and 4-methoxy benzene **19** (entries 1–3). Unsubstituted benzene **17** was almost completely inactive ($P2X_3$ $pIC_{50} = 5.4$). But 3-methoxy arene **18** was *10-fold* more potent while 4-methoxy arene **19** was only *fourfold* more potent than unsubstituted analog (**17**). Because of the differing contributions on binding, we suspect that the two methoxy groups occupy separate hydrophobic pockets.[16]

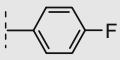
Because demethylation of the 4-methoxy group was the primary metabolite observed in this series, we attempted to find a suitable methoxy group replacement (R^1; Table 3, entries 4–7). Although slightly less active, bromobenzene **20** and iodobenzene **21** were found to be suitable methoxy replacements. However, fluoro-phenylbenzene **6** and other small heterocyclic groups resulted in a significant loss of activity. Furthermore, the highly electron-withdrawing cyano benzene **23** was completely inactive at $P2X_3$. Thus the hydrophobic pocket occupied by substituents in the 4-position (R^2) is small and tolerates groups that are lipophilic in nature.

Based on the data listed in Table 3, we believed that optimization of the 3-position (R^1) could have a dramatic impact on potency. We began with the preparation of simple alkyl analogs. Small chain analogs such as toluene **24** and ethyl benzene **25**, which are similar in size to a methoxy group, were nearly equipotent to dimethoxy benzene **16**. However, incorporation of a small electron-rich substituent in alkynyl benzene **28** afforded an impressive boost in potency ($P2X_3$ $pIC_{50} = 8.2$). In contrast, larger alkyl replacements, such as isopropyl benzene **26** and biphenyl **27**, were *10-fold* less potent at $P2X_3$.

Since the lipophilic isopropyl group is a potential site of metabolic oxidation, we decided to search for (R^1) replacements that would lower log*P* and thereby improve metabolic stability. We began by preparing a series of four halogenated analogs (Table 3: entries 13–16). Fluoroanisole **29** was nearly inactive ($P2X_3$ $pIC_{50} = 5.5$). In fact, this compound, which lacks the key 3-methoxy group, was found to be *fivefold* less potent than 4-methoxy benzene **19** ($P2X_3$ $pIC_{50} = 5.8$), suggesting that size alone is not influencing activity. Both chloroanisole **30** and bromoanisole **31** were similarly potent to dimethoxy benzene **16**. Interestingly, iodoanisole **32** (RO-4), was *fourfold* more potent than dimethoxy benzene **16** ($P2X_3$ $pIC_{50} = 8.0$). These results together suggest that the substituent at the 3-position is not only occupying a hydrophobic pocket but may be fulfilling a key hydrogen bond interaction. Since hydrogen bond strength is dominated by distance and basicity, fluorine would be expected to be a very weak acceptor (inactive),[17]

Table 3
3-Methoxy group replacements lead to potent drug-like analogs



Entry	Compound	R^1	R^2	$P2X_3$[a,c]	$P2X_{2/3}$[b,c]
1	**17**	H	H	5.4	5.7
2	**18**	OMe	H	6.4	6.8
3	**19**	H	OMe	5.8	6.4
4	**20**	OMe	Br	7.1	6.5
5	**21**	OMe	I	7.4	6.4
6	**22**	OMe		5.6	5.1
7	**23**	OMe		<5.0	<5.0
8	**24**	Me	OMe	7.0	6.4
9	**25**	Et	OMe	7.3	6.8
10	**26**	*i*-Pr	OMe	6.3	5.7
11	**27**	Ph	OMe	6.0	5.6
12	**28**		OMe	8.2	7.9
13	**29**	F	OMe	5.5	5.8
14	**30**	Cl	OMe	7.6	7.0
15	**31**	Br	OMe	7.7	7.1
16	**32 (RO-4)**	I	OMe	**8.0**	**7.1**
17	**33**		OMe	6.2	5.6
18	**34**		OMe	7.0	6.0
19	**35**		OMe	7.0	5.8
20	**36**		OMe	6.8	6.5
21	**37**		OMe	6.8	6.2
22	**38**		OMe	<5.0	<5.0
23	**39**		OMe	7.4	6.5

[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%.

while the other larger halogens have a much greater propensity to act as a hydrogen bond acceptor (more active).[18] This is an important finding because functional groups that have the ability to act as hydrogen bond acceptors can also help to improve the PK properties by lowering log*P* and decreasing polar surface area.[19]

With results suggesting a possible hydrogen bond interaction with the groups occupying the 3-position (R^1), analogs that act as hydrogen bond donors or acceptors were prepared (Table 3; entries 18–23). These analogs contained neutral, basic and acidic functional groups. Analogs that contained neutral polar functional groups such as cyano benzene **33**, sulfone **34** and carboxamide **35** were moderately active. The acidic functionality of carboxylic acid **36** was moderately active while bioisosteric tetrazole **39** was equipotent with dimethoxy benzene **16**. Finally, moderately basic imidazole **37** was active ($P2X_3$ $pIC_{50} = 6.8$) while the much more basic dihydroimidazole **38** was completely inactive.

In conclusion, a high-throughput screening campaign identified a diaminopyrimidine-based series of mixed $P2X_3/P2X_{2/3}$ antagonists. Optimization of this series resulted in an impressive boost in potency due to the cooperative effect of an isopropyl side-chain and an ether linker. Replacements for the potentially labile 4-methoxy group were explored. Substitution of the 3-methoxy group with various hydrogen bond acceptors yielded active analogs with reduced lipophilicity. Finally, **RO-4** (**32**) a potent drug-like dual $P2X_3/P2X_{2/3}$ antagonist was selected for further evaluation.

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