Bioorganic & Medicinal Chemistry Letters 19 (2009) 1632–1635

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Identification and SAR of novel diaminopyrimidines. Part 2: The discovery of RO-51, a potent and selective, dual $P2X_3/P2X_{2/3}$ antagonist for the treatment of pain

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ARTICLE INFO

Article history:
Received 17 December 2008
Revised 28 January 2009
Accepted 29 January 2009
Available online 4 February 2009

Keywords:
RO-51
$P2X_3/P2X_{2/3}$
Purinoceptor
ATP
Diaminopyrimidine
Neuropathic pain
RO-4
$P2X_{2/3}$

ABSTRACT

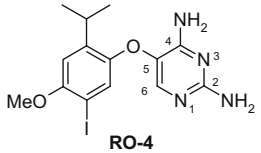
The purinoceptor subtypes $P2X_3$ and $P2X_{2/3}$ have been shown to play a pivotal role in models of various pain conditions. Identification of a potent and selective dual $P2X_3/P2X_{2/3}$ diaminopyrimidine antagonist **RO-4** prompted subsequent optimization of the template. This paper describes the SAR and optimization of the diaminopyrimidine ring and particularly the substitution of the 2-amino group. The discovery of the highly potent and drug-like dual $P2X_3/P2X_{2/3}$ antagonist **RO-51** is presented.

P2X purinoceptors are ligand gated ion channels with adenosine-5′-triphosphate (ATP) as endogenous ligand.[1,2] In the P2X family of receptors, the homomeric $P2X_3$ and the heteromeric $P2X_{2/3}$ receptors are mainly localized on sensory afferent neurons and mediate the primary sensory effects of ATP. Studies using $P2X_3$-KO mice and antagonists in the chronic constriction injury (CCI) and other models have clearly implicated a role in regulating pain behavior in rodents.[3–6]

The discovery of the first selective, non-nucleotide dual $P2X_3/P2X_{2/3}$ antagonist A-317491 and its in vivo efficacy in multiple chronic inflammatory and neuropathic pain models validated the potential use of $P2X_3/P2X_{2/3}$ antagonists as a new class of therapeutic agents for the treatment of pain.[7,8] In the preceding communication, we reported the discovery of **RO-4** (Fig. 1), a highly potent and selective, drug-like dual $P2X_3/P2X_{2/3}$ antagonist.

This compound was discovered by an extensive structure–activity relationship (SAR) investigation of the phenyl ring substituents and linkage atom of a novel 5-benzyl diaminopyrimidine screening hit identified by a high-throughput campaign of the Roche compound collection. As a continuation of our work, we extended the SAR of this series to substituents on the diaminopyrimidine ring.

Among several heterocycles investigated, the diaminopyrimidine was the only ring system that retained reasonable potency. All the attempts to replace the diaminopyrimidine ring with other heterocycles resulted in compounds with potencies > 10 μM. Also, substitution at C-6 consistently lowered the antagonistic potencies at both $P2X_3$ and $P2X_{2/3}$ receptors, limiting the exploration of this position. Although substitution on the C-4 amino group was tolerated, it generally resulted in decreased antagonist potency. These initial results led us to investigate substitution at the C-2 position.



RO-4

$rP2X_3(IC_{50})$ = 13 nM
$hP2X_{2/3}(IC_{50})$ = 25 nM

Figure 1. Dual acting $P2X_3$, $P2X_{2/3}$ receptor antagonist RO-4.

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doi:10.1016/j.bmcl.2009.01.097

Removal of the C-2 amino group, or replacement with an alkyl group, also resulted in loss of activity (Table 1, Entries 1 and 2). Next, we investigated substituted amino groups at this position. The chemistry and SAR pertaining to C-2 amino-substitutions are presented here.

Compounds **12–14**, substituted at the C-2 position of the pyrimidine ring, were prepared following the synthetic route shown in Scheme 1.

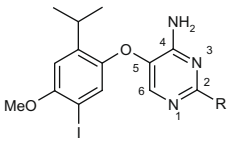
Treatment of acetophenone **1** with 2.2 equiv of MeMgCl at 5 °C gave 90% yield of the tertiary alcohol (**2**) which was hydrogenated over Pd/C in the presence of HCl, without further purification to the corresponding isopropyl group (**3**).[9] Attempts to purify the tertiary alcohol **2** consistently produced varying amounts of the methylstyrene, produced by elimination of the hydroxyl group, which was resistant to hydrogenation under various conditions.

Regioselective iodination *ortho* to the methoxy group was achieved following the protection of the phenolic OH as the tosylate (**4**) and subsequent treatment with I_2 in the presence of *m*-CPBA with acetic acid as solvent (**5**).[10] Hydrolysis of the tosylate using aqueous NaOH in ^{t}BuOH produced phenol **6** which was alkylated with $TsOCH_2CN$ in THF to give **7** in 90% yield. Using either bromo or iodoacetonitrile instead of $TsOCH_2CN$ consistently gave lower yields. Phenoxy acetonitrile **7** was heated with Bredereck's reagent (*tert*-butoxy-bis(dimethyl-amino)methane) in DMF at 100 °C for 18 h. This reaction generally produced a mixture of two adducts (**8** and **9**), both of which were transformed to enamine **10** on heating at 100 °C with aniline HCl in DMF.[10] Finally, heating intermediate **10** with the appropriate guanidine/amidine (**11**) in the presence of NaOMe in EtOH produced the desired 2-amino-substituted-pyrimidines (**12–14**) in satisfactory yields. Compounds **12a** and **12b** (Entries 1 and 2, Table 1), in which the C-2 position of the pyrimidine ring was substituted with H and Me, were prepared by condensing the enamine **10** with the corresponding amidines (**11**: R = H, Me).

For synthesis of diaminopyrimidines (**13**), in which R′ is a hydroxyalkyl group, the alcohol(s) on the corresponding guanidine precursors (**19**) had to be protected prior to condensation with the enamine **10**. The general route for preparation of the protected guanidines (**11**, R = NHR′) and hydroxyalkyl-2-aminopyrimidine analogs is outlined in Scheme 2 and is exemplified by the synthesis of **13t** (**RO-51**).

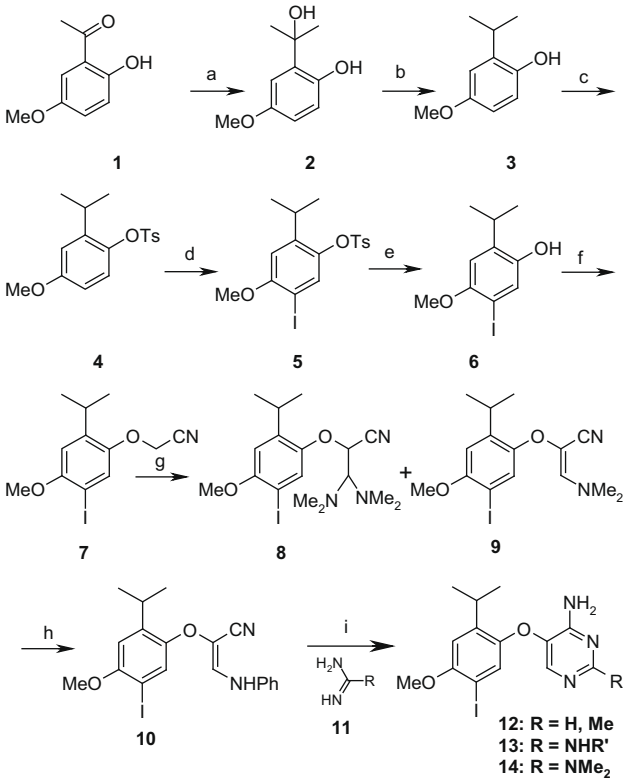
Stirring the commercially available *N,N*-bis(benzyloxycarbonyl)-1*H*-pyrazole-1-carboxamidine (**15**) with 2-amino-1,3-propanediol (**16**) for 7 h at ambient temperature produced guanidine (**17**) in excellent yield.[11] It was essential to protect the hydroxyls before condensing the substituted guanidine fragment with the intermediate **11**. In this case, TBS protection of both hydroxyl groups of **17** was achieved using standard conditions (TBDMSOTf/lutidine) to give **18**.[12] The desired guanidine intermediate

Table 1
Effect of C-2 substituents on $P2X_3/P2X_{2/3}$ receptor affinity

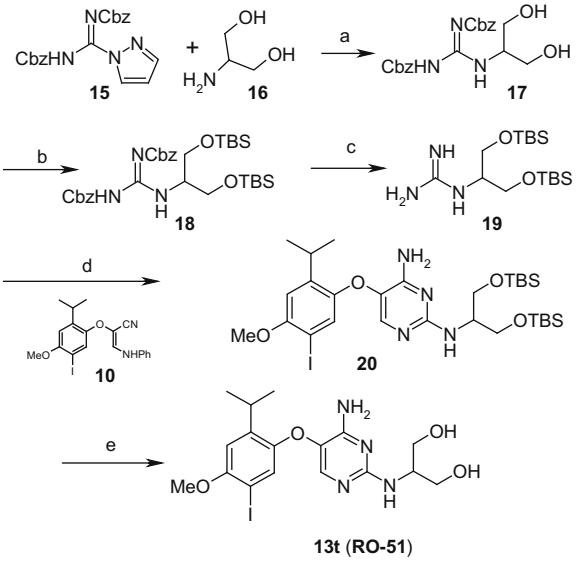


Entry	Compound	R	$P2X_3$[a,c]	$P2X_{2/3}$[b,c]
1	**12a**	H	6.4	5.8
2	**12b**	Me	5.6	5.6
3	**RO-4**	NH_2	8.0	7.4
4	**13a**	NHMe	7.3	6.7
5	**13b**	$NHCH_2CF_3$	7.1	6.7
6	**14**	NMe_2	<5.0	<5.0

[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 1. Preparation of C-2 substituted compounds. Reagents and conditions: (a) MeMgCl, THF, 5 °C to rt, 90%; (b) H_2, Pd/C, EtOH–HCl; (c) TsCl, NaOH, THF–H_2O, 95%; (d) I_2, *m*-CPBA, AcOH, 90–95%; (e) NaOH, ^{t}BuOH, H_2O, 85–90%; (f) $TsOCH_2CN$, KO^{t}Bu, THF, 85–90%; (g) ^{t}BuOCH(NMe)$_2$, DMF 100 °C; (h) $PhNH_3Cl$, DMF, 100 °C, 90–95% from **7**; (i) **11**, NaOMe, EtOH, microwave 160 °C, 15–30 min.



Scheme 2. Preparation of C-2 hydroxyalkylamino compounds. Reagents and conditions: (a) THF, 25 °C, 7 h, 95%; (b) TBDMSOTf (3.2 equiv), lutidine, CH_2Cl_2, 1.5 h, 70%; (c) Pd/C, H_2 (3 atm), EtOH, 2 h, 80%; (d) **10**, microwave, NaOMe, EtOH, 160 °C 15–30 min; 86% (e) TBAF, (3.2 equiv), THF, 30 min, 84%.

19, ready for cyclization, was then generated by the removal of the Cbz groups with H_2 over Pd/C. The required diaminopyrimidine ring was constructed by condensing **19** with **10** under microwave heating at 160 °C to produce **20** in excellent yield. Finally, the deprotection of TBS groups was accomplished with TBAF under standard conditions producing **13t** (Scheme 2).

Initial SAR results (Table 1) clearly indicated that the C-2 position of the pyrimidine ring must retain a single N–H group. Replacement with either a non-amino moiety, such as H (**12a**, Entry 1) or Me (**12b**, Entry 2) or a tertiary amino group, such as NMe_2 (**14**, Entry 6) was detrimental to antagonist activity at both $P2X_3$, and $P2X_{2/3}$ receptors. On the contrary, both monoalkyl amino analogs **13a** and **13b** (Entries 4 and 5, Table 1) retained moderate inhibitory potencies for both $P2X_3$ and $P2X_{2/3}$ receptor compared to **RO-4**.

The essential requirement of NH at C-2 for $P2X_3$ and $P2X_{2/3}$ receptors activity prompted us to investigate the SAR of mono substituted amines (R = NHR′) (Table 2). All compounds (**13a–13w**) in Table 2 were prepared by condensing enamine (**10**) with the appropriately substituted guanidines **11** (R = NHR′, Scheme 1).

In general, compounds containing basic amines (**13c, 13f–13j**, Entry 1, 4–8; Table 2) exhibited only moderate antagonistic potencies at $rP2X_3$ and $hP2X_{2/3}$ receptors. Interestingly, acylation of the terminal C-2 NH_2 of **13c**, compound **13d** (Entry 2, Table 2), resulted in a 14-fold increase in $rP2X_3$ and a 15-fold increase in $hP2X_{2/3}$ antagonist potency.

With the exception of **13v** (Entry 20), all the analogs containing one or more OH groups were consistently highly potent. It is possible that the steric hindrance due to the *gem*-dimethyl group in **13v** created the unfavorable conformation of the R group and therefore had a negative impact on potencies relative to **13p** and **13q** (Entry 14 and 15). Conversion of the hydroxyl group of **13m** to the corresponding methyl ether **13n** (Entry 12, Table 2) resulted in substantial loss in potency at both targeted receptors. SAR of the C-2 hydroxyalkylamino analogs also revealed a modest preference for the two carbon alkyl chain over the three carbon one. Pyrimidines **13m** and **13t** (Entries 11 and 18, Table 2) containing hydroxyethyl moieties were more potent than their homologated analogs **13o** and **13u** (Entries 13 and 19, Table 2).

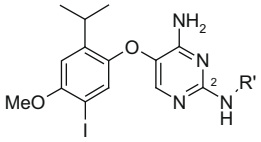
As evident from the pIC_{50} data in Table 2, compounds with dihydroxyalkyl groups (**13s**, Entry 17) were somewhat more potent than their mono-hydroxyalkyl analogs (**13o**, Entry 13). Compound **RO-51** (**13t**, Entry 18) was the most potent analog in the series. Compared to **RO-4** (Entry 3, Table 1), **RO-51** is seven- and fivefold more potent as an antagonist at $rP2X_3$ (IC_{50} 2 nM) and $hP2X_{2/3}$ (IC_{50} 5 nM), respectively.

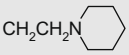
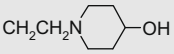
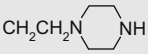
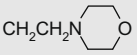
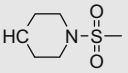
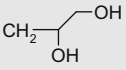
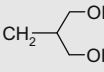
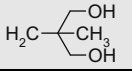
Encouraged by its high potency, **RO-51** was chosen for further pharmacological evaluation. This ligand proved to be highly selective for $P2X_3$ and $P2X_{2/3}$ exhibiting no antagonistic activity at other P2X receptor family members tested ($P2X_1$, $P2X_2$, $P2X_4$, $P2X_5$, and $P2X_7$) at concentrations up to 10 μM. CEREP profiling against the standard panel of 50 receptors, ion channels, enzymes, and transporters, showed no significant activity (IC_{50} >10 μM).[13] In vitro safety assessment (CYP_{450} inhibition, hERG blockade, Ames test, phototoxicity) of **RO-51** also revealed an attractive profile for further evaluation. In permeability measurements using Caco-2 cells, **RO-51** proved highly permeable with P_{app} (AB) of 9.43×10^{-6} cm/s and a lack of any efflux tendencies.

Single dose rat pharmacokinetics (SDPK) of **RO-51** were also evaluated. The results are summarized in Table 3 and are comparable to those of **RO-4**.

In rat both compounds suffered rapid clearance, short half-lives, and high protein binding. However, in a dog SDPK assessment, **RO-51** (CL: 4.65; $T_{1/2}$: 3.88 h; %*F*: 79) was comparatively superior to **RO-4** (CL: 13; $T_{1/2}$: 1.5 h; %*F*: 72).

Table 2
Structures and activities of mono substituted amines at C-2



Entry	Compound	R′	rP2X3[a,c]	hP2X2/3[b,c]
1	**13c**	$CH_2CH_2NH_2$	6.6	5.9
2	**13d**	$CH_2CH_2NHCOMe$	8.0	7.4
3	**13e**	$CH_2CH_2SO_2Me$	7.8	7.4
4	**13f**	$CH_2CH_2NMe_2$	6.8	6.6
5	**13g**		6.6	6.2
6	**13h**		6.4	6.0
7	**13i**		7.2	6.6
8	**13j**		6.8	6.4
9	**13k**		6.9	6.4
10	**13l**		7.0	6.5
11	**13m**	CH_2CH_2OH	8.0	7.8
12	**13n**	CH_2CH_2OMe	7.2	7.2
13	**13o**	$CH_2CH_2CH_2OH$	7.8	7.2
14	**13p**		8.2	7.8
15	**13q**		8.0	7.5
16	**13r**		7.9	7.6
17	**13s**		8.1	7.5
18	**13t** (**RO-51**)		8.7	8.3
19	**13u**		8.2	7.5
20	**13v**		7.0	6.1
21	**13w**		8.0	7.3

[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%.

In summary, further optimization of the first selective, drug-like $P2X_3/P2X_{2/3}$ antagonist **RO-4** resulted in the discovery of the more potent **RO-51**. Selectivity testing, in vitro safety assessment and

Table 3
Rat SDPK data[a] of **RO-4** and **RO-51**

Compound	T_{max} (h)	C_{max} (ng/ml)	AUC (ng/h/ml)	$T_{1/2}$ (h)	CL (mg/min/kg)	%F
RO-4 (iv)	0.083	589	628	0.94	52.8	
RO-51 (iv)	0.167	554	495	1.07	71.9	
RO-4 (po)	1.33	64.3	240	2.09	—	39
RO-51 (po)	0.833	104	237	1.52	—	48

[a] Calculated from 2 mg/kg iv and oral (po) dosing.

SDPK evaluation indicated that **RO-51** can serve as a valuable tool in determining the in vivo pharmacological effects of dual $P2X_3$/$P2X_{2/3}$ antagonists.

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