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Potent $P2Y_1$ urea antagonists bearing various cyclic amine scaffolds

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ABSTRACT

A number of new amine scaffolds with good inhibitory activity in the ADP-induced platelet aggregation assay have been found to be potent antagonists of the $P2Y_1$ receptor. SAR optimization led to the identification of isoindoline **3c** and piperidine **4a** which showed good in vitro binding and functional activities, as well as improved aqueous solubility. Among them, the piperidine **4a** showed the best overall profile with favorable PK parameters.

Blocking the $P2Y_1$ receptor continues to be an attractive strategy to prevent clot formation[1] and research groups have recently started to describe small molecule antagonists.[2] We previously described new azole $P2Y_1$ antagonists showing potent in vitro binding but poorer activity in an ADP-induced platelet aggregation assay run in platelet-rich plasma.[3] In an effort to improve the translation from in vitro binding to the functional ADP-induced platelet aggregation assay, we pursued SAR studies which involved the addition of polar and solubilizing groups to lead structure **1**.[4] It was hoped that this approach would potentially decrease the high lipophilicity and improve the aqueous solubility which are common liabilities with our earlier $P2Y_1$ antagonists.[3,4a] Adding polar or basic amine groups to different parts of compound **1** generally resulted in significant loss of binding affinity and antiplatelet activity except for the addition of amine substituents replacing the trifluoromethoxy portion of the phenyl ring. The resulting amines showed improved solubility and similar antiplatelet activity as **1**.[4b] In addition, our continued research led to the incorporation of amines into the *t*-butylphenoxy region of the lead structure **1**. This report describes the SAR of the various cyclic amine scaffolds identified. Our strategy to tie back an amine containing ring to **1** was based on the assumption that high affinity on the receptor would be maintained while the resulting amine backbones would

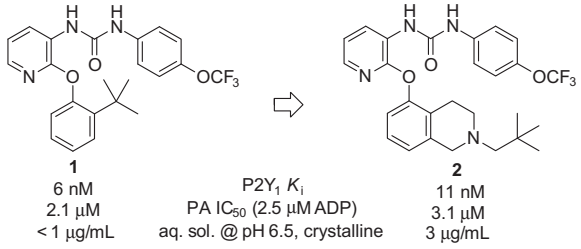

Figure 1. *t*-Butyl tetrahydroisoquinoline replacement.

be less lipophilic and potentially more soluble in aqueous media. Tetrahydroisoquinoline **2** bearing a *N*-neopentyl group was first identified as a compound having similar potency to the *tert*-butyl analog **1** both in terms of binding affinity and inhibition of platelet aggregation (Fig. 1). Platelet aggregation was measured in human platelet rich plasma and binding of the agonist [β-^{33}P]-2MeS-ADP to cloned human P2Y1 receptors stably expressed in HEK293 cells was used in the binding assay; see Ref. 4a for details). The *N*-neopentyl was found optimum for binding affinity as summarized in Table 1. Removing just one methyl group from the neopentyl group afforded the *N*-isobutyl analog **2a** and this minor change resulted in a about five-fold decrease in affinity against $P2Y_1$ (entry 2). This *N*-neopentyl to *N*-isobutyl transformation also resulted in a similar decrease in binding affinity on other amine scaffolds (vide infra). The linear *N*-propyl and *N*-ethyl analogs **2b** and **2c** along with the *N*-isopropyl analog **2d** all showed significantly reduced affinity

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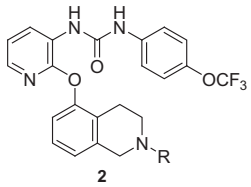
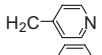
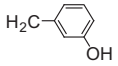
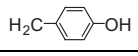
Table 1
Tetrahydroisoquinoline *N*-alkyl SAR



2

Entry	Compound	R	$P2Y_1$[a] (K_i, nM)
1	**2**	CH_2t-Bu	11
2	**2a**	CH_2i-Pr	60
3	**2b**	*n*-Pr	620
4	**2c**	Et	1700
5	**2d**	*i*-Pr	560
6	**2e**	H	3000
7	**2f**	CH_2CH_2t-Bu	190
8	**2g**	CH_2Ph	55
9	**2h**	CH_2CH_2Ph	290
10	**2i**		1100
11	**2j**		1200
12	**2k**		1040
13	**2l**		260
14	**2m**		2200
15	**2n**		3400

[a] K_i values are reported as the mean of at least three individual determinations (for assay conditions see Ref. 4a). Variability around the mean was <50%.

for the $P2Y_1$ receptor (entries 3–5). These small alkyl substituents had nevertheless a positive effect on receptor affinity since completely removing the lipophilic substituent from the tetrahydroisoquinoline nitrogen resulted in **2e** which showed an even greater loss in binding. Comparison between *N*-isopropyl analog **2d** and *N*-isobutyl analog **2a** suggests that the methylene spacer in the latter is beneficial (entries 2 and 5). Adding one methylene in the side-chain of compound **2** resulted in **2f** which showed about 20-fold reduction in binding affinity against $P2Y_1$ (entries 1 and 7). The same trend was observed with the pair **2g** and **2h** where the benzyl analog was more potent than the phenethyl analog.

Adding heteroatoms or polar groups to the phenyl ring of **2g** resulted in decreased $P2Y_1$ binding affinity (entries 10–15). While all pyridine regioisomers **2i**–**k** had similar binding affinity, the 2-hydroxyphenyl derivative **2l** was about 10-fold more active than the corresponding 3- and 4-hydroxyisomers **2m** and **2n**. The SAR described in Table 1 therefore suggests that the more active compounds are tertiary amines bearing a side-chain capped by a large lipophilic substituent. Furthermore, this large alkyl or aryl group is ideally separated from the ring nitrogen by a methylene unit. In the ADP-induced platelet aggregation assay (at 2.5 μM ADP), compound **2** had an IC_{50} comparable to **1** (3.1 and 2.1 μM, respectively). Tetrahydroisoquinoline **2** and compound **1** showed similarly low aqueous solubility (Fig. 1), so we therefore explored other amine scaffolds.[5]

Figure 2 shows various analogs (**3a**–**d** and **4a**–**c**) resulting from the modification of the tetrahydroisoquinoline ring system. Although these compounds were not more potent than **2** as above that bore the bulkier *t*-butyl group. In the indoline and piperidine series, the binding affinity against $P2Y_1$ was indeed higher for the

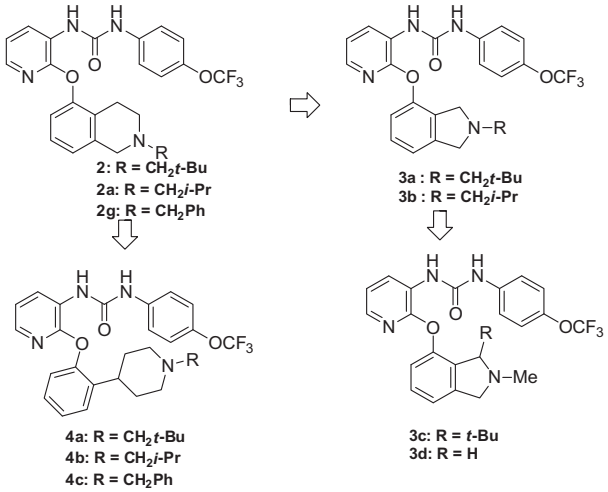


Figure 2. Analogs of tetrahydroisoquinoline **2**.

N-neopentyl analogs **3a** and **4a** by comparison to *N*-isobutyl analogs **3b** and **4b**, respectively (Table 2, entries 1–2 and 5–6). As was the case also with the tetrahydroisoquinoline series, the binding affinities of benzyl and isobutyl analogs, **4c** and **4b**, respectively, were in the same range but the latter was more potent in the functional assay (PA IC_{50} = 7.6 μM). The inhibition of ADP-induced platelet aggregation was weak however for the isoindoline pairs **3a** and **3b** (PA IC_{50} >10 μM). Moving the *t*-butyl group from the nitrogen atom to the adjacent benzylic position provided **3c** which, showed significantly improved functional activity (PA IC_{50} = 1.2 μM) in spite of showing similar binding affinity to *N*-neopentyl analog **3a**. The piperidine **4a** also showed comparable IC_{50} in the ADP-induced platelet aggregation assay (PA IC_{50} = 2.4 μM), a significant improvement over our previously reported azoles,[3] and both compounds **3c** and **4a** showed improved aqueous solubility (270 and 105 μg/mL, amorphous and partially crystalline, respectively, Table 3).

PK data in rats was obtained for compounds **2**, **3c** and **4a**. Clearance was moderate for piperidine **4a**, similar to that of compound **1**, but higher in compounds **2** and **3c**. Clearance values correlated well with microsomal stability data. All compounds exhibited high volume of distribution with half-life of 14 and 11 h for compounds **2** and **4a**, respectively, while isoindoline **3c** half-life was only 1.7 h. Compound **4a** had superior oral bioavailability compared to **2** and **3c**. In addition, compared with the *t*-butyl lead compound **1**, piperidine **4a** had significantly higher bioavailability (89%) and improved aqueous solubility, providing **4a** as a potential tool molecule to assess its pharmacology as a $P2Y_1$ antagonist. Despite improved pharmacokinetic profile, compound **4a** however showed less desirable hERG profile (hERG flux assay IC_{50} = 4.6 μM, Table 2). The more potent analogs in Table 2 also showed undesirable hERG activity. Furthermore, the GPCR selectivity profile of **4a** showed greater than 50% inhibition against 5-HT2A, D2, and NET at 10 μM concentration. Similar issues were also observed with other amphiphilic amine analogs.[4b] Consequently, **4a** was not advanced to in vivo efficacy/bleeding studies.

A typical synthesis of isoindolines shown in Scheme 1 starts with commercially available 3-methoxy benzoyl chloride (**5**). Amide formation upon treatment with methylamine afforded **6** quantitatively. *ortho*-Directed lithiation followed by the addition of pivaloyl chloride gave the benzyl alcohol intermediate **7** which was directly reduced using a mixture of triethylsilane and trifluoroacetic acid. The resulting lactam **8** was reduced to the isoindoline ring using lithium aluminum hydride and the methyl ether was

Table 2
K_i, and PA IC_{50} of amine scaffolds **3**–**4**

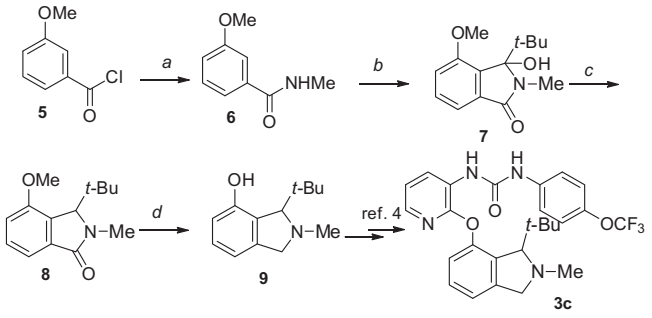
Entry	Compound	$P2Y_1$[a] (K_i, nM)	PA IC_{50} (μM) @2.5 μM ADP[b]	hERG flux IC_{50} (μM)
1	**3a**	29	>10	nd[c]
2	**3b**	68	>10	nd[c]
3	**3c**	57	1.2	7.2
4	**3d**	3700	nd[c]	nd[c]
5	**4a**	16	2.4	4.6
6	**4b**	150	7.6	6.0
7	**4c**	98	12.7	nd[c]

[a] K_i values are reported as the mean of at least three individual determinations (for assay conditions see Ref. 4a). Variability around the mean was <50%.
[b] Platelet aggregation (PA) was tested with 2.5 μM of ADP, $n \geqslant 3$.
[c] Not determined.

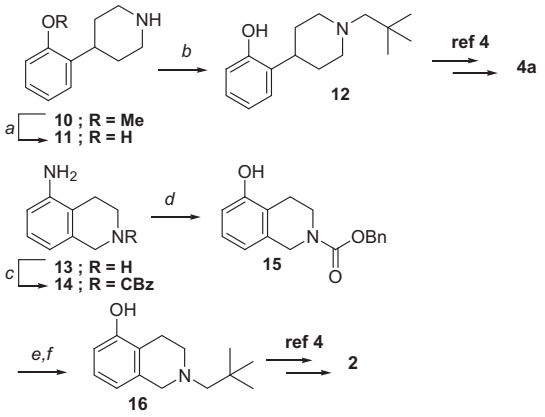
Table 3
In vitro and rat PK profile of diaryl ureas **1**, **2**, **3c**, and **4a**

Compound number	$P2Y_1$ K_i (nM)	PA IC_{50} (μM)	Solubility[d] (μg/ml)	RLM[e]	Caco-2 Pc (A–B) (nm/s)	PAMPA (nm/s)	CL (mL/min/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	*F*%	*h* PB % bound
1[a]	6	2.1	<1*	86	21	ND[g]	16	5.6	15	7	99.3
2[b]	11	2.5	3*	27	—[f]	—[f]	48	22	14	16	100
3c[b]	57	1.2	270***	6	<15	784	84	10	1.7	13	99.7
4a[c]	16	2.4	105**	96	ND[g]	286	18	14	11	89	99.9

[a] Discrete PK. Dose: 1 mg/kg (IV/PO), vehicle: 10% DMAC/10% EtOH/10% cremophor/70% H_2O.
[b] Compounds **2** and **3c** were part of a cassette dosing (five compounds). Dose: 0.5 mg/kg (IV/PO), vehicle: 10% DMAC/10% EtOH/10% cremophor/70% H_2O.
[c] Discrete PK. Dose: 2 mg/kg (IV), 5 mg/kg (PO), vehicle: 10% EtOH/10% cremophor/80% H_2O.
[d] Aqueous solubility was tested at pH 6.5 using 25 μM phosphate buffer with either crystalline (*), partially crystalline (**), or amorphous materials (***).
[e] Percentage remaining after 10 min in rat liver microsomal (RLM) incubation.
[f] Compounds were undetected in initial stock solution or after assay incubation.
[g] Not determined.



Scheme 1. Representative synthesis of isoindolines. Reagents and conditions: (a) $MeNH_2$, quantitative yield; (b) (i) *n*-BuLi, THF; (ii) *t*-BuCOCl; (c) Et_3SiH, TFA; (d) (i) LAH; (ii) BBr_3; 20% yield from **6**.



Scheme 2. Representative piperidine and tetrahydroisoquinoline syntheses. Reagents and conditions: (a) BBr_3, quantitative yield; (b) (i) trimethylacetaldehyde, $CH(OMe)_3$, AcOH, NMP; (ii) $NaBH_4$, MeOH, 70%; (c) BnOCOCl, Hunig's base, DCM, 65%; (d) $NaNO_2$, H_2O–H_2SO_4, 62%; (e) H_2, Pd/C, quantitative yield; (f) as in (b) 55%.

deprotected with boron tribromide to afford **9** in 20% overall yield from **6**. The phenol obtained was then transformed into **3c** using the previously described sequence:[4a] (i) addition to 2-chloro-3-nitropyridine, (ii) reduction with zinc and (iii) addition to *p*-trifluoromethoxyphenyl isocyanate.

Scheme 2 describes the preparation of the phenol building blocks used to prepare piperidines **4a** and tetrahydroisoquinolines **2**. The commercially available 4-(2-methoxyphenyl)piperidine (**10**) was cleaved quantitatively to phenol **11** which was then submitted to a reductive amination protocol with trimethylacetaldehyde to provide **12** in 70% yield. In the tetrahydroisoquinoline series, phenol **16** was prepared by first protecting the aniline **13** as the CBz carbamate **14**. Treatment of the latter with sodium nitrite in sulfuric acid provided **15** in 62% yield. Transformation to the *N*-neopentyl derivative **16** then simply involved the removal of CBz group followed by a reductive amination as above. Both phenols **12** and **16** were then transformed to the final compounds **4a** and **2**, respectively, following our previously described sequence.[4a]

In summary, various cyclic amine scaffolds (**2**–**4**) were identified as replacements of the *t*-butyl group in lead structure **1**. Several compounds reported herein showed improved functional activity in the ADP-induced platelet aggregation assay in comparison to previously described azole antagonists.[3] Piperidine **4a** showed the best overall profile with functional activity comparable to that of the corresponding *tert*-butyl analog **1**. Furthermore, piperidine **4a** showed significantly improved solubility and oral bioavailability compared to other compounds in this series and compared to *tert*-butyl compound **1**.

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