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Synthesis and SAR of pyrimidine-based, non-nucleotide $P2Y_{14}$ receptor antagonists

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ABSTRACT

A weak antagonist of the pyrimidinergic receptor $P2Y_{14}$ containing a dihydropyridopyrimidine core was identified through high-throughput screening. Subsequent optimization led to potent, non-UTP competitive antagonists and represent the first reported non-nucleotide antagonists of this receptor. Compound **18q** was identified as a 10 nM $P2Y_{14}$ antagonist with good oral bioavailability and provided sufficient exposure in mice to be used as a tool for future in vivo studies.

G protein-coupled receptors (GPCRs) are a large family of receptors characterized by their ability to respond to diverse extracellular stimuli such as photons, odorants, chemokines, nucleotides, lipids, amino acids and peptides. Importantly, modulation of GPCRs serves as the basis of many disease treatments.[1–3] Growing evidence shows that nucleotides not only play key intracellular functions in signaling and genetics, they also behave as potent extracellular signaling molecules.[4] They are released from most tissues and exert their effects by interacting with two classes of membrane receptors, P2X (ligand gated ion channels) and P2Y (GPCRs) which are expressed on virtually all cell types.[5] P2Y receptors are activated specifically by adenine and uridine nucleotides and nucleotide sugars. Specifically, $P2Y_{1,11,12,13}$, respond to ADP and ATP; in contrast, $P2Y_{2,4,6}$ respond to UDP or UTP and $P2Y_{14}$ to UDP-sugars such as UDP-glucose.[6] We as well as others have recently shown that UDP is also a potent agonist of $P2Y_{14}$.[7] $P2Y_{14}$ signals via Gi and the consequent inhibition of cAMP[8] and via Gq and the consequent increase in calcium flux.[9] It is expressed in a broad range of tissues such as in the stomach, intestine, placenta, spleen, adipose tissue, brain, lung, heart and several types of immune cells.[10] Several studies have demonstrated UDP-glucose-mediated signaling in neutrophils,[8] dendritic cells[9] and airway epithelium[11] thus suggesting a potential role of $P2Y_{14}$ in immune response modulation. The precise physiological role of $P2Y_{14}$ remains unknown.

To date, the only known agonists or antagonists of $P2Y_{14}$ had been nucleotide derivatives.[12,13] The identification of selective, non-nucleotide small molecule tools would prove invaluable in deciphering the cellular function of $P2Y_{14}$. Herein, we report the first series of non-nucleotide small molecule antagonists of $P2Y_{14}$. Using a $P2Y_{14}$ FLIPR (fluorometric imaging plate reader) calcium mobilization assay in HEK cells, a high-throughput screening (HTS) of the Merck sample collection identified compound **1** as a weak antagonist of $P2Y_{14}$ (Fig. 1). Although not very potent (IC_{50} = 4.9 μM) this non-nucleotide lead was attractive based on its modular structure that seemed amenable to SAR studies.

In order to explore the SAR of this series of compounds, a flexible synthesis of the core tetrahydropyrido pyrimidine was required. Initial efforts involved exploration of the SAR of the phenyl urea moiety and with this in mind, we developed a concise synthesis of the late stage intermediate **7** (Scheme 1). Condensation of Boc-protected ethyl 4-oxopiperidine-3-carboxylate **2** with pyridine-3-carboximidamide **3** gave intermediate **4** in excellent yield. Introduction of the 2-tolyl group was accomplished by Suzuki coupling of tosylate **5** with 2-methylphenyl boronic acid to give **6** in 89% yield. Removal of the Boc group gave the key intermediate **7** which was coupled with various isocyanates to afford analogs **8**.

In order to explore replacements of the tolyl group, the steps of the synthesis were rearranged. 4-Oxopiperidine **9** was reacted with

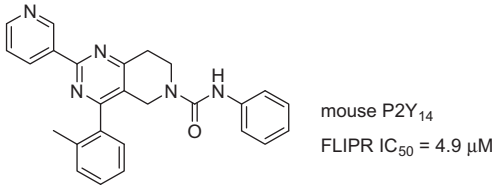

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Figure 1. Hit from high-throughput screening.

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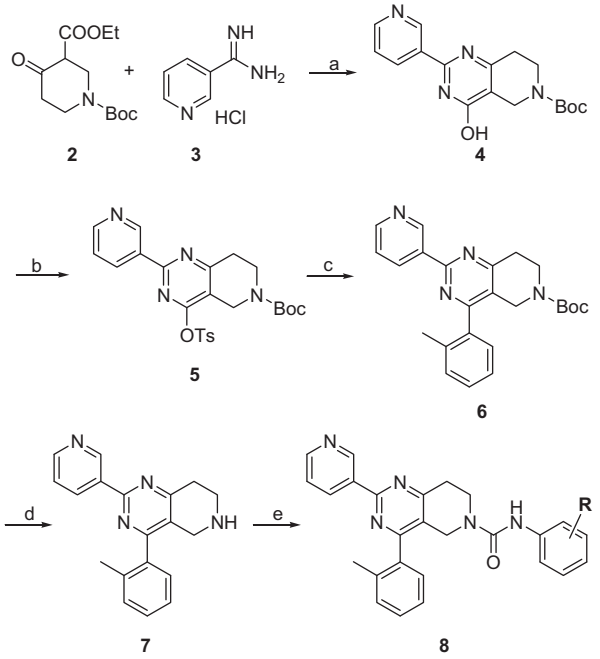
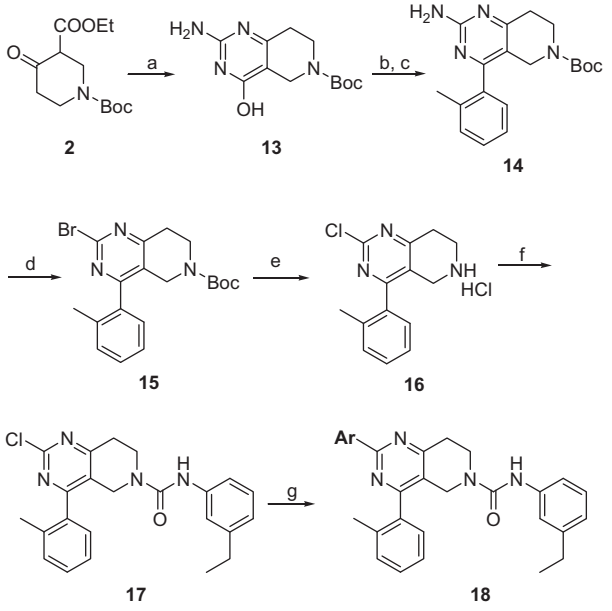
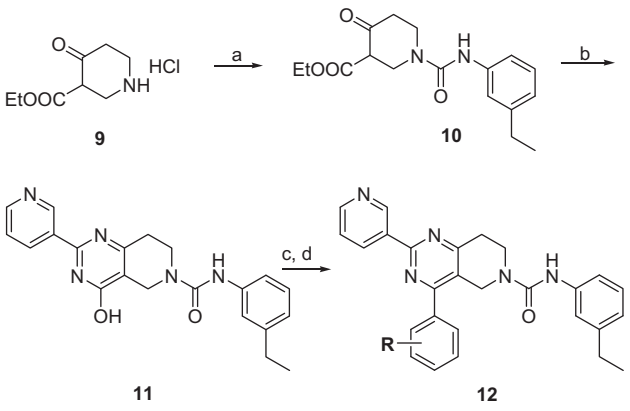


Scheme 1. Reagents and conditions: (a) aq K_2CO_3, MeOH, reflux, 16 h, 94%; (b) TsCl, Et_3N, DMAP cat., CH_2Cl_2, rt, 4 h, 92%; (c) *o*-tolyl boronic acid, K_3PO_4, $Pd(OAc)_2$, 2-(dicyclohexylphosphino)biphenyl, DMF, H_2O, 90 °C, 2 h, 89%; (d) HCl/dioxane or TFA, CH_2Cl_2, rt, 4 h, 97%; (e) RC_6H_4NCO, Et_3N, CH_2Cl_2, rt, 2 h, or $RC_6H_4NH_2$, $(Cl_3CO)_2CO$, CH_2Cl_2, aq $NaHCO_3$, 0 °C to rt, 16 h, 77–94%.



Scheme 3. Reagents and conditions: (a) guanidine HCl, aq K_2CO_3, MeOH, reflux, 16 h, 92%; (b) TsCl, Et_3N, DMAP cat., CH_2Cl_2, rt, 16 h, 85%; (c) *o*-tolyl boronic acid, K_3PO_4, $Pd(OAc)_2$, 2-(dicyclohexyl-phosphino)biphenyl, DMF, H_2O, 80 °C, 16 h, 62%; (d) *t*-BuONO, $SbBr_3$, CH_2Cl_2, 0 °C, 16 h, 23%; (e) 4 M HCl/dioxane, rt, 3 h, 100%; (f) 3-EtC_6H_4NCO, Et_3N, CH_2Cl_2, rt, 2 h, 92%; (g) $ArB(OH)_2$, $PdCl_2(dppf)$, Na_2CO_3 aq, DMF, 90 °C, 16 h, 45–85%.



Scheme 2. Reagents and conditions: (a) 3-EtC_6H_4NCO, Et_3N, CH_2Cl_2, rt, 2 h, 99%; (b) **3**, aq K_2CO_3, MeOH, reflux, 7 h, 94%; (c) TsCl, Et_3N, DMAP, CH_2Cl_2, rt, 16 h, 82%; (d) $RC_6H_4B(OH)_2$, K_3PO_4, $Pd(OAc)_2$, 2-(dicyclohexylphosphino)biphenyl or DavePhos, DMF, H_2O, 90 °C, 2–12 h, 50–92%

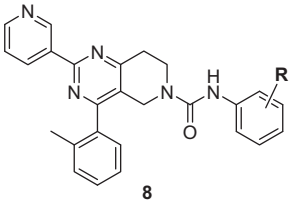
3-ethylphenyl isocyanate to give urea **10** (Scheme 2). Condensation of **10** with the carboximidamide **3** gave intermediate **11** in high yield. Formation of the corresponding tosylate provided the key intermediate for SAR of the bottom phenyl moiety. Suzuki coupling with various phenyl boronic acids was typically carried out using palladium acetate, potassium carbonate and 2-(dicyclohexylphosphino)biphenyl to afford the desired compounds **12**. However, for sterically hindered examples such as 2,6-disubstituted phenyl boronic acids, this coupling required Davephos® as the ligand.[14]

Finally, replacement of the 3-pyridyl group with other aromatic moieties could be carried out according to chemistry described above and replacing **3** with different aryl amidines. However, in order to be more convergent, we decided to slightly modify the approach so we could access a key intermediate such as **17** and introduce diverse aryl moieties at the final step (Scheme 3). Condensation of **2** with guanidine hydrochloride under basic conditions gave **13** in very good yield. Selective formation of the *O*-tosylate followed by the usual Suzuki coupling afforded intermediate **14**. Conversion of this 2-aminopyrimidine into the 2-bromopyrimidine **15** was accomplished using modified Sandmeyer conditions.[15] Removal of the Boc group with dry hydrochloric acid also effected a halogen exchange of the heterocyclic template to give **16** quantitatively. Reaction of **16** with 3-ethylphenyl isocyanate secured intermediate **17** which could be coupled with a variety of phenyl, aryl and heteroaryl boronic acids to give the desired analogs **18**.

A whole cell Ca^{2+} mobilization assay was used to assess compound potencies. Specifically, we tested the ability of compounds to inhibit UDP-glucose-stimulated Ca^{2+} release in human embryonic kidney (HEK) cells co-expressing $P2Y_{14}$ and $G\alpha_{qi5}$. All compounds were tested against both the mouse and chimpanzee receptors. The selection of chimpanzee $P2Y_{14}$ (99% identical to human) was driven by the larger window (signal/background) with this receptor over the human $P2Y_{14}$, while the mouse receptor (83% identical to human) was used to enable future evaluation of compounds in mouse models of disease.

The effect of having different substituents on the phenyl urea was studied first and results are summarized in Table 1. Addition of a chloride at the *ortho*-, *meta*- or *para*-position revealed that substitution at the *ortho*-position was detrimental to potency whereas substitution at the *meta*-position was beneficial (**8a**–**c**). Replacing the 3-chloro for a 3-methoxy group (**8d**) did not affect the potency on the chimpanzee receptor but reduced 10-fold its activity on the mouse receptor. On the other hand, opting for a more lipophilic 3-methylthio substituent had no effect on the potency for the mouse receptor but increased the potency on the chimpanzee receptor by 25-fold (**8f** vs **8b**). Switching to alkyl groups, a similar trend was found where small non-polar substituents in the 3-position provided the most potent $P2Y_{14}$ receptor antagonists. A simple methyl group as in **8g** only marginally improved the potency compared to the parent **1** but one carbon homologation to the ethyl analog **8h** led to a 60-fold potency increase on the mouse receptor and over

Table 1
Potency of antagonists **8** on the mouse and the chimpanzee $P2Y_{14}$ receptors



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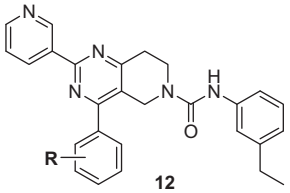
Compd	*R*	Mouse $P2Y_{14}$ IC_{50}[a] (μM)	Chimpanzee $P2Y_{14}$ IC_{50}[a] (μM)
1	H	4.9	10
8a	2-Cl	>20[b]	>20[b]
8b	3-Cl	0.58	2.1
8c	4-Cl	5.8	6.7
8d	3-MeO	5.1	2.3
8e	3-EtO	0.42	0.86
8f	3-MeS	0.63	0.081
8g	3-Me	1.9	1.0
8h	3-Et	0.081	0.025
8i	3-Pr	0.15	0.024
8j	3-(CH=CH_2)	1.2	0.098
8k	3-CH(CH_2)$_2$	1.2	0.040
8m	3-$COCH_3$	6.5	6.9
8n	3-$CH(OH)CH_3$	8.2	6.0
8p	3-$CHFCH_3$	0.029	0.033
8q	3-CF_2CH_3	0.62	0.062
8r	3-Et, 5-Cl	0.16	0.017

[a] Values are means of at least two experiments where values were typically within 3-fold of each other.
[b] Less than 50% inhibition at a maximum concentration of 20 μM.

Table 2
Potency of antagonists **12** on the mouse and the chimpanzee $P2Y_{14}$ receptors



12

Compd	*R*	Mouse $P2Y_{14}$ IC_{50} (μM)[a]	Chimpanzee $P2Y_{14}$ IC_{50}[a] (μM)
8h	2-Me	0.081	0.025
12a	2-Cl	0.44	0.014
12b	2-F	3.7	0.93
12c	3-Cl	12	>20[b]
12d	2-Me, 4-Cl	7.0	6.3
12e	2-Et	0.39	2.5
12f	2-OMe	3.3	18
12g	2-OH	6.5	0.56
12h	2-CH_2OH	1.1	3.2
12i	2-Me, 6-Me	0.009	0.032
12j	2-Me, 6-Cl	0.050	0.016
12k	2-Cl, 6-Cl	0.051	0.034

[a] Values are means of at least two experiments where values were typically within 3-fold of each other.
[b] Less than 50% inhibition at a maximum concentration of 20 μM.

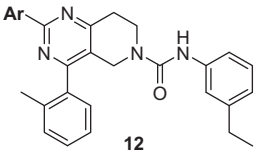
400-fold increase of potency on the chimpanzee ortholog. The propyl-substituted derivative **8i** was similar in potency while the vinyl and cyclopropyl derivatives (**8j**, **k**) were significantly less potent on the mouse receptor but retained most of their activity on the chimpanzee receptor. Polar substituents were not well tolerated (e.g., **8m**, **n**) and electron-withdrawing groups such as the 1,1-difluoroethyl compound **8q** maintained good potency on the chimpanzee receptor but suffered 8-fold potency loss on the mouse receptor. Although the monofluoroethyl analog **8p** was found to be the most potent in both assays, the electrophilic nature of this moiety precluded its further evaluation. Eventually, we found that 3,4- and 3,5-disubstituted phenyl ureas were also potent antagonists as long as the 3-ethyl is present as in analog **8r**. Overall, it appeared that the 3-ethylphenyl urea was the best motif for these $P2Y_{14}$ antagonists and that the chimpanzee receptor was more tolerant of the nature of substituents on the phenyl ring.

Next, we examined the SAR of the *o*-tolyl group appended at the 4-position of the core heterocyclic template while keeping other portions of the molecule constant. Changing the 2-methyl substituent of **8h** for a 2-chloro led to a slight increase of potency on the chimpanzee receptor but also resulted in a 5-fold loss of potency on the mouse receptor (**12a**, Table 2). The smaller and more electronegative fluorine atom resulted in a very significant loss of potency (**12b**). Moving the chloride atom to the *meta*- (**12c**) or to the *para*-positions (**12d**) led to a dramatic loss in potency compared to the starting compound. These results seemed to indicate little tolerance for substituents at the 3- and 4-positions of this phenyl ring. Increasing the size of the 2-methyl to a 2-ethyl group (**12e**) proved to be much more detrimental for potency on the chimpanzee receptor with a 100-fold loss compared to the mouse receptor (5-fold). Introduction of a polar group also led to significant reduction of activity in the assays (e.g., **12f**– **h**). Finally we found that both *ortho*-positions could have a methyl or a chloro substituent to provide $P2Y_{14}$ antagonists with very good potency on both receptors (**12i**–**k**).

The third moiety we wanted to explore in terms of SAR modification was the 3-pyridyl appended at the 2-position of the pyrimidine core template. For this, we elected to keep constant the 3-ethylphenyl urea and the *o*-tolyl moieties and results are summarized in Table 3. Oxidation of the pyridine nitrogen led to a large loss of potency (**18a**). While the 2-pyridyl isomer **18b** was totally inactive, the 4-pyridyl analog **18c** was found to be 17-fold less potent on the mouse receptor but at least 3-fold more potent on the chimpanzee ortholog. Changing the 3-pyridyl for a phenyl had no effect on the potency on the mouse receptor and only reduced its activity 3-fold on the chimpanzee receptor (**18d**). Addition of alkyl substituents was not well tolerated (e.g., **18e–h**) but small electron-withdrawing groups such as fluorine and nitrile provided very potent antagonists on both receptors (e.g., **18i–m**). Replacing the phenyl with a 2-naphthyl (**18n**) was somewhat detrimental but a 3-quinolyl group gave a potent antagonist on the mouse receptor (**18p**). The 3,4-methylenedioxyphenyl derivative **18q** was found to be potent with IC_{50}'s of 10 nM on the mouse receptor and of 81 nM on the chimp receptor. Finally, we found that small nitrogen containing heterocycles bearing electron-withdrawing groups provided the most active $P2Y_{14}$ receptor antagonists (e.g., **18r–u**).

Compounds **18r** and **t** were eliminated from consideration due to potent binding to the hERG channel (<200 nM) following screening in a binding assay with ^{35}S-labelled MK-0499.[16] Compounds **18q**, **s** and **u** all were >5 μM in this assay (data not shown). The pharmacokinetic profiles of these promising antagonists were determined in C57B6 mice (Table 4). The compounds were dosed as oral suspensions in 0.5% methocel at 50 mg/kg. Intravenous dosing was at 5 mg/kg in 80% aqueous PEG-200. Serial tail bleeds from two animals for each dosing route were used to generate the reported data. The compounds were characterized by high volumes of distribution and long half-lives. The exposure achieved with **18q** which gave exposures well above the cell-based IC_{50}s through 24 h ($C_{24\ h}$ = 0.4 μM) suggesting that this compound would be suit-

Table 3
Potency of antagonists **18** on the mouse and the chimpanzee $P2Y_{14}$ receptors



12

Compd	Ar	Mouse $P2Y_{14}IC_{50}$[a] (μM)	Chimpanzee $P2Y_{14}$ IC_{50}[a] (μM)
8h	3-Pyr	0.081	0.025
18a	3-Pyr N-oxide	2.8	3.8
18b	2-Pyr	>20[b]	>20[b]
18c	4-Pyr	1.4	0.007
18d	Ph	0.087	0.093
18e	4-(Me)Ph	0.062	0.86
18f	3-(Me)Ph	1.3	0.73
18g	4-(*i*-Pr)Ph	>20[b]	>20[b]
18h	4-(CF_3)Ph	7.5	12
18i	3-(CN)Ph	0.14	0.13
18j	4-(CN)Ph	0.016	0.031
18k	4-(F)Ph	0.025	0.082
18m	3-(F)Ph	0.030	0.089
18n	2-Naphthyl	0.88	7.1
18p	3-Quinolyl	0.013	0.23
18q	3,4-(OCH_2O)-Ph	0.010	0.081
18r	4-(CN)-3-Pyr	0.003	0.004
18s	5-Pyrimidyl	0.012	0.008
18t	2-(CN)-5-Pyrimidyl	0.001	0.001
18u	Me_2-4-isoxazolyl	0.004	0.002

[a] Values are means of at least two experiments where values were typically within 3-fold of each other.
[b] Less than 50% inhibition at a maximum concentration of 20 μM.

Table 4
Pharmacokinetic parameters for select compounds in mouse following dosing at 50 mpk po and 5 mpk IV

Compd	*F* (%)	C_{max} (μM)	Cl (mL/min/kg)	V_{dss} (L/kg)	$T_{1/2}$ (h)
18q	134[a]	2.4	48	19	11
18s	40	6.4	38	11	12
18u	31	2.0	43	9.3	5.6

[a] This calculated value may be attributable to non-linear absorption at the high oral dose used.

able for in vivo experiments to elucidate the pharmacological role of $P2Y_{14}$. Compound **18s**, while being 10-fold more potent than **18q**, gave exposures ~10-fold lower at 8 h and 24 h, thus negating much of its utility. Compound **18u** was found to be poorly tolerated in mice following single dose administration.

Compound **18q** was further characterized in a ^{3}H-UDP filtration binding assay in membranes prepared from HEK cells overexpressing chimpanzee $P2Y_{14}$. Compound **18q** was unable to displace ^{3}H-UDP in the binding assay suggesting the compound is non-competitive with UDP.

We have succeeded in identifying a series of non-nucleotide P2Y14 antagonists. A 4.9 μM hit from high-throughput screening was optimized to give non-UDP competitive antagonists with IC_{50}s of <10 nM. These compounds were orally bioavailable and provide sufficient exposure in mice to serve as useful tools for the delineation of the biological role of $P2Y_{14}$.

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