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# The identification of 4,7-disubstituted naphthoic acid derivatives as UDP-competitive antagonists of $P2Y_{14}$

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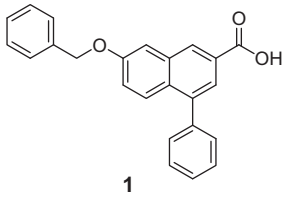
ABSTRACT

A weak, UDP-competitive antagonist of the pyrimidinergic receptor $P2RY_{14}$ with a naphthoic acid core was identified through high-throughput screening. Optimization provided compounds with improved potency but poor pharmacokinetics. Acylglucuronidation was determined to be the major route of metabolism. Increasing the electron-withdrawing nature of the substituents markedly reduced glucuronidation and improved the pharmacokinetic profile. Additional optimization led to the identification of compound **38** which is an 8 nM UDP-competitive antagonist of $P2Y_{14}$ with a good pharmacokinetic profile.



$P2Y_{14}$ is a G-protein-coupled receptor that binds UDP-glucose and is widely expressed in a broad range of tissues.[1,2] The physiological role of $P2Y_{14}$ is currently unknown and mechanistic studies would benefit from the identification of small-molecule tools. In the previous Letter in this journal, we have described the identification of the first non-nucleotide antagonist of $P2Y_{14}$.[3] This non-competitive antagonist was derived from a hit in an HTS campaign using the FLIPR-based calcium flux assay in HEK cells over-expressing either mouse or chimpanzee $P2Y_{14}$.

Using the same assay, additional hits were identified, including the 4,7-disubstituted naphthoic acid derivative **1** (Fig. 1). This compound had FLIPR $IC_{50}$'s of 3.5 μM on both the mouse and chimpanzee receptors, was fully reversible and inhibited UDP-glucose mediated signaling. In addition, **1** was competitive with $^3$H-UDP in a recombinant simian $P2Y_{14}$ binding assay with a $K_i$ of 0.16 μM, indicating that it was an active site antagonist. Since we felt that the successful identification of a potent, competitive antagonist would be advantageous to the elucidation of the pharmacological role of $P2Y_{14}$, we decided to pursue the optimization of **1**.




**1**

**Figure 1.** A UDP competitive naphthoic acid antagonist of $P2Y_{14}$.

To explore the SAR of the naphthoic acid lead **1** we utilized both single compound and library syntheses. For both, ethyl 4-hydroxy-7-bromo-2-naphthoate **2** was the ideal building block (Scheme 1). It was prepared from 3-bromobenzaldehyde and 4-*tert*-butyl-1-ethyl-2-(diethoxyphosphoryl) succinate via Stobbe condensation and Friedel–Crafts cyclization as described by Boger et al.[4] Alternatively, derivatives bearing an aryl group at C-4 and an oxygen-linked substituent at C-7 were prepared from ethyl 4-hydroxy-7-benzyloxy-2-naphthoate **3** (obtained using the same method). Derivatization of the substituents at the 4- or the 7- positions was accomplished by appropriate sequencing of Pd-catalyzed cross-couplings of activated derivatives (the bromide **2**, the triflate **6** or the pinacolato arylboronic ester **9**). Mitsunobu and/or base-catalysed

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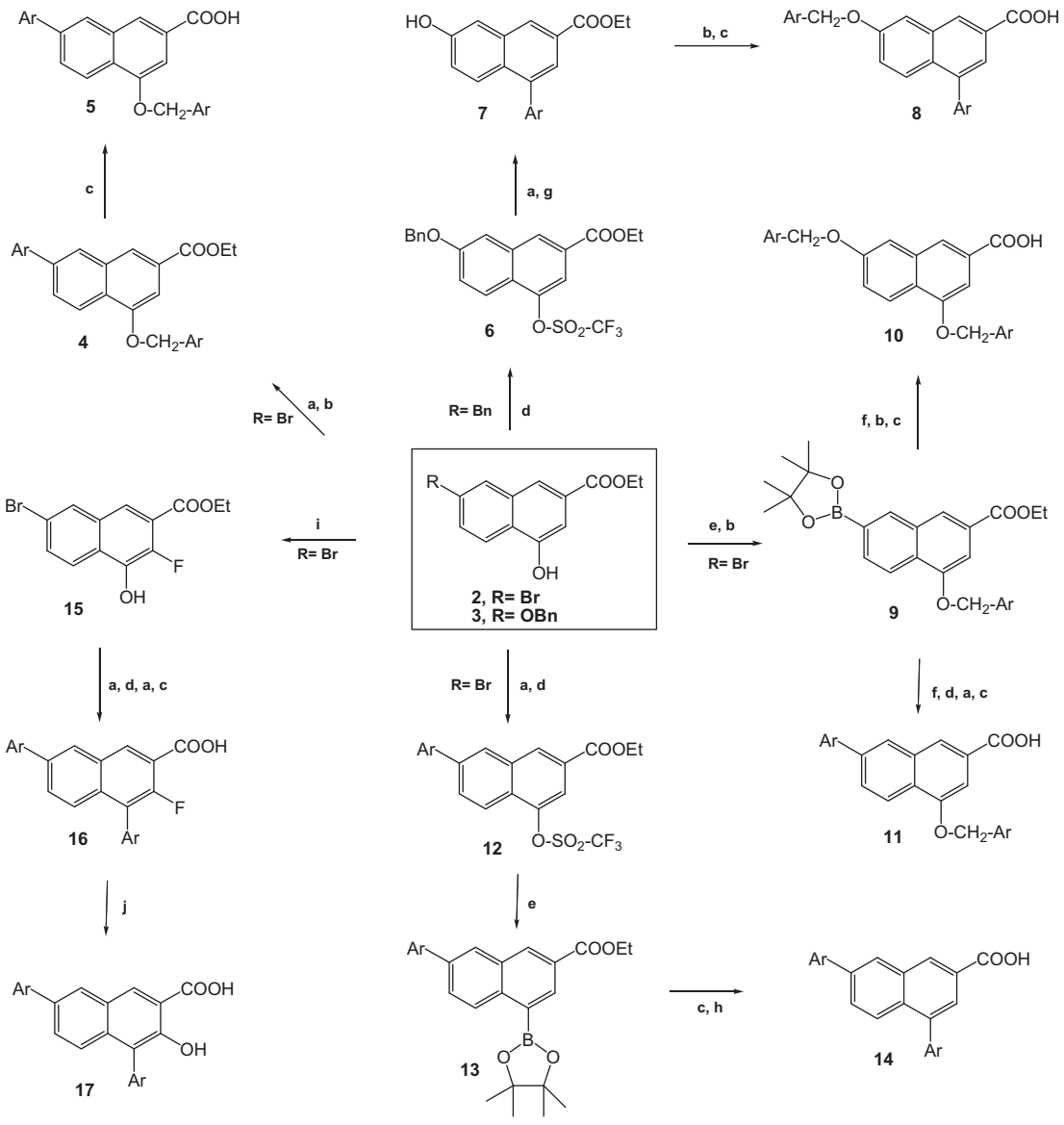



**Scheme 1.** Reagents and conditions: (a) Ar-B(OH)$_2$, PdCl$_2$dppf, 2 M Na$_2$CO$_3$, DMF, 85 °C; (b) ArCH$_2$X (X = Cl, Br, I), K$_2$CO$_3$, DMF; (c) NaOH, EtOH, THF; (d) (CF$_3$SO$_2$)$_2$O, pyridine, CH$_2$Cl$_2$; (e) bis(pinacolato)diboron, KOAc, PdCl$_2$dppf, dioxane, 85 °C; (f) H$_2$O$_2$ MeOH; (g) H$_2$, Pd/C, EtOAc; (h) Ar-Br, PdCl$_2$dppf, 2 M Na$_2$CO$_3$, DMF, 85 °C; (i) *N*-fluoropyridinium triflate, 1,2-dichloroethane; (j) LiOH, DMSO, 150 °C.

alkylations, followed by ester hydrolysis generated the carboxylic acids as shown in Scheme 1.

The 3-fluoro derivative was prepared from **2** using *N*-fluoropyridinium triflate[5] to produce ethyl 3-fluoro-4-hydroxy-7-bromo-2-naphthoate **15** and then elaborated as described above to furnish **16**. The fluorine atom could then be displaced with hydroxide to produce the 3-hydroxy derivative **17**.

Our initial evaluation of **1** focused on replacement of the carboxylic acid by a methyl ester, acylsulfonamide, acetic acid, or phosphate, but all of these derivatives were found to be markedly less active than **1** (data not shown).

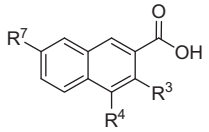
Replacement of the C-7 benzyloxy moiety by either a methoxy group (**18**) or a phenoxy group (**19**) led to a significant loss of potency (Table 1). An acidic substituent on the benzyl ring (**20**) was not tolerated.

Conversely, the addition of lipophilic substituents to the benzyloxy moiety increased functional activity and improved binding. The addition of chlorine atoms or methyl groups as shown in **21** and **22** led to potent antagonists. The importance of the linker itself was evidenced by the loss of activity observed upon reversal of the benzyloxy to a phenoxymethyl as in **23**.

We then explored the substituent at C-4 of the naphthalene ring. Removing the phenyl group entirely (**24**) resulted in complete loss of activity; however the benzyloxy replacement **25** showed comparable potency to **22**. The C-4 phenyl group could also be replaced by a thienyl group such as in **26**, which provided a 10-fold increase in potency in the chimpanzee functional assay. Combining these observations and adding a fluorine atom to the C-7 substituent to enhance metabolic stability led to compound **27** with a 2,6-dimethyl-4-fluoro-benzyloxy group at C-7. **27** is 250-fold and nearly 1000-fold more potent than **1** in the mouse and chimpanzee functional assays respectively with a $K_i$ of 14 nM in the chimpanzee binding assay.

To evaluate its pharmacokinetic properties, **27** was dosed in C57BL/6 mice at 50 mg/kg P.O. and 2 mg/kg I.V. An oral bioavailability of 12% was observed with a half-life of 2.7 h. To further understand the metabolic fate of this compound, we carried out a bile duct cannulation study using five wild-type and five Mrp2

**Table 1**
In vitro activity of selected analogs of the lead naphthoic acid antagonist **1**




| Compound | $R^7$ | $R^4$ | $R^3$ | Mouse $P2Y_{14}$ $IC_{50}$ (μM)[a] | Chimpanzee $P2Y_{14}$ $IC_{50}$ (μM)[a] | Chimpanzee $P2Y_{14}$ binding $K_i$ (μM)[a] |
|---|---|---|---|---|---|---|
| **1** | $-OCH_2Ph$ | Ph | H | 3.5 | 3.5 | 0.16 |
| **18** | $-OCH_3$ | Ph | H | | | 4.0 |
| **19** | –OPh | Ph | H | 4.9 | 4.1 | 1.5 |
| **20** | 4-Carboxy-benzyloxy– | Ph | H | >13 | >40 | |
| **21** | 2,6-$Cl_2$-benzyloxy– | Ph | H | 0.19 | 0.091 | 0.029 |
| **22** | 2,6-Dimethyl-benzyloxy– | Ph | H | 0.11 | 0.041 | 0.042 |
| **23** | 2,6-Dimethylphenoxymethyl– | Ph | H | 2.1 | 1.5 | 0.34 |
| **24** | 2,6-Dimethyl-benzyloxy– | H | H | 13 | 6.5 | |
| **25** | 2,6-Dimethyl-benzyloxy– | $-OCH_2$-Ph | H | 0.11 | 0.089 | 0.021 |
| **26** | 2,6-Dimethyl-benzyloxy– | 3-Thienyl | H | 0.026 | 0.006 | 0.016 |
| **27** | 2,6-Dimethyl-4-fluoro-benzyloxy– | 3-Thienyl | H | 0.014 | 0.004 | 0.014 |
| **28** | 4-$CF_3O$-phenyl– | 3-Thienyl | H | 0.054 | 0.014 | 0.029 |
| **29** | 4-$CF_3O$-phenyl– | 3-Thienyl | F | 0.38 | 0.11 | 0.11 |
| **30** | 4-$CF_3O$-phenyl– | 3-Thienyl | OH | 1.3 | 0.49 | 0.17 |
| **31** | 4-$CF_3O$-phenyl– | 4-(3-$((CF_3)_2COH)$-thienyl- | H | 0.35 | 0.091 | 0.054 |
| **32** | 4-$CF_3O$-phenyl– | 4-(3-$CH_3SO_2$)-thienyl- | H | 0.33 | 0.086 | 0.032 |
| **33** | 4-$CF_3$-phenyl– | 4-(3-$CH_3SO_2$)-thienyl- | H | 0.19 | 0.048 | 0.013 |
| **34** | 4-$CF_3$-phenyl– | 4-(4-$CH_3SO_2$)-phenyl- | H | 0.020 | 0.007 | 0.007 |
| **35** | 4-$CF_3$-phenyl– | 4-(4-$CF_3CHOH$)-phenyl- | H | 0.013 | 0.005 | 0.014 |
| **36** | 4-$CF_3$-phenyl– | 4-(*R*-4-$CF_3CHOH$)-phenyl- | H | 0.004 | 0.001 | 0.010 |
| **37** | 4-$CF_3$-phenyl– | 4-(S-4-$CF_3CHOH$)-phenyl | H | 0.051 | 0.011 | 0.021 |
| **38** | 4-$CF_3$-phenyl– | 4-(*R*-4-$CHF_2CHOH$)phenyl- | H | 0.008 | 0.001 | 0.004 |

[a] $IC_{50}$ and $K_i$ values represent an average of at least three independent titrations where values were typically within 3-fold of each other. See Ref. 1 for assay conditions.

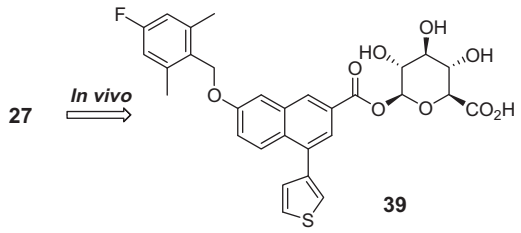



**Figure 2.** In vivo studies indicated that **27** is excreted in the bile as the acylglucuronide metabolite **39** (>99%).

(-/-) mice dosed with unlabeled **27**. Bile, feces and urine were collected over 24 h in the presence of 1% formic acid (to stabilize any acyl glucuronide that may be present) and analyzed by LC-MS/MS. Little parent or glucuronide were detected in the urine or feces, suggesting that **27** is well-absorbed. More than 99% of the administered dose was recovered in the bile and identified as the acyl glucuronide **39** (Fig. 2). Only traces of oxidative metabolites were observed in the bile and were not characterized. A total recovery of nearly 100% was determined based on the initial dose and authentic standards for both **27** and **39**. In addition, there were no significant differences in drug excretion between the wild-type and Mrp2 (-/-) mice suggesting that this transporter is not a major contributor to the excretion of **27**.

Based on these data, we attempted to reduce the extent of glucuronidation. It has been reported that acyl glucuronidation can be modulated by varying the electronic density of the carboxylic acid, in particular by introducing electron-withdrawing substituents.[6] To monitor the effect of such modifications on the extent of metabolism, we used an in vitro mouse hepatocyte assay[7] and tried to establish a correlation with pharmacokinetic properties (Table 2).

We first replaced the electron-donating benzyloxy group of **27** with the electron-withdrawing 4-trifluoromethoxyphenyl group obtaining **28**. This modification led to a dramatic increase in metabolic stability in vitro, as measured by the amount of parent drug remaining following a 2 h incubation with mouse hepatocytes (62% vs 8%). Moreover, the modification did not significantly affect functional activity or binding to the receptor. In an attempt to maximize electronic effects we introduced a fluorine (**29**) or a hydroxyl group (**30**) at C-3 of the naphthyl ring. Although these modifications improved both hepatocyte stability and pharmacokinetic parameters, the resulting compounds were less potent than **27**. An alternative way of improving the stability of these compounds was found by introducing a polar group on the thienyl ring. The bis-(trifluoromethyl)carbinol **31** showed good hepatocyte stability (91% parent remaining) and had improved bioavailability, clearance and half-life relative to **27**. The methylsulfone-substituted thiophene **32** had similar potency, but was not as effective at reducing metabolism.

Additional work in the C-7 position revealed that the trifluorophenyl substituent of **33** was somewhat superior to the trifluoromethoxyphenyl of **32**. Holding this group constant and optimizing the C-4 position led to the observation that the phenyl sulfone of **34** was markedly superior to the thienylsulfone, with a $K_i$ of 7 nM. As observed previously, introducing fluorinated alcohol moieties on the C-4 aryl group provided compounds with improved

**Table 2**
In vitro–in vivo correlation of metabolism in mouse hepatocytes with PK profile in mice

| Compound | % Parent remaining[a] | %F[b] | Cl (mL/min/kg)[c] | $T_{1/2}$ (h) | Cmax (μM)[b] |
|---|---|---|---|---|---|
| **27** | 8 | 12 | 7.1 | 2.7 | 23 |
| **28** | 62 | 92 | 18 | 1.1 | 18 |
| **29** | 85 | 129 | 3.3 | 4.3 | 89 |
| **30** | 93 | 240 | 7.6 | 13 | 34 |
| **31** | 91 | 45 | 1.9 | 4.1 | 37 |
| **32** | 16 | 4 | 3.7 | 3.2 | 16 |
| **38** | 39 | 67 | 1.6 | 3.0 | 113 |

[a] Following 2 h incubation with freshly isolated mouse hepatocytes ($2 \times 10^6$ cells/mL) with 20 μM of the test compound.
[b] Following an oral dose of 50 mg/kg in 5% Tween-80 except for compound **28**, dosed at 25 mg/kg.
[c] Following an IV dose of 2 mg/kg in 60% PEG-200.

potency in the functional assays and good metabolic stability (compounds **35**-**38**). The most promising derivative was **38** ($K_i$ = 4 nM), containing a chiral difluoromethylcarbinol. This compound was orally bioavailable in mice (67%) with low intrinsic clearance (1.6 mL/min/kg).

In summary, a highly potent, competitive and bioavailable antagonist of $P2Y_{14}$ was identified starting from a 3.5 μM hit from high throughput screening. The key structural features combine a 2-naphthoic acid core substituted by an electron-withdrawing 4-trifluoromethylphenyl group directly attached to C-7 and a chiral 4-difluoromethylphenethyl alcohol attached at C-4. This combination resulted in a metabolic profile where glucuronidation of the acid group was minimized, providing a compound with high oral exposure and low clearance. Compound **38** is an excellent candidate for delineating the biological role of $P2Y_{14}$ in in vivo models.

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