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Applying the pro-drug approach to afford highly bioavailable antagonists of $P2Y_{14}$

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ABSTRACT

Our series of competitive antagonists against the G-protein coupled receptor $P2Y_{14}$ were found to be highly shifted in the presence of serum (>99% protein bound). A binding assay using 2% human serum albumin (HSA) was developed to guide further SAR studies and led to the identification of the zwitterion **2**, which is substantially less shifted (18-fold) than our previous lead compound **1** (323-fold). However, as the bioavailability of **2** was low, a library of ester pro-drugs was prepared (**7a–7j**) and assessed in vitro. The most interesting candidates were then profiled in vivo and led to the identification of the pro-drug **7j**, which possesses a substantially improved pharmacokinetic profile.

The engineering of biologically active molecules is a complex, multi-faceted process that encompasses multiple levels of fine-tuning. Too often this endeavor is shunted in one of the last crucial steps, which involves delivery of the active compound to the targeted system via oral absorption.

While many of the strategies that have been devised to circumvent this issue revolve around the formulation of the active ingredient, another approach that has gained considerable attention in recent times involves the preparation of pro-drugs.[1] These modified active compounds possess favorable physicochemical properties that can significantly improve oral absorption and then undergo efficient in vivo conversion to the active drug by various metabolic processes.

During our investigations of the biological role of $P2Y_{14}$,[2]a widely expressed G-protein-coupled receptor that binds UTP-glucose,[3,4] we generated several series of potent non-nucleotide antagonists.[5,6] Many of these antagonists displayed both high affinity for the $P2Y_{14}$ receptor and good pharmacokinetic profiles in mice. For example, compound **1** (see Fig. 1) has a K_i of 4.0 nM in the chimpanzee $P2Y_{14}$ binding assay,[7] and is orally bioavailable in mice (75 mg/kg, BID, 0.25% methocel, $F = 67\%$, $T_{1/2} = 3.0$ h, Cl = 1.6 mL/min/kg).

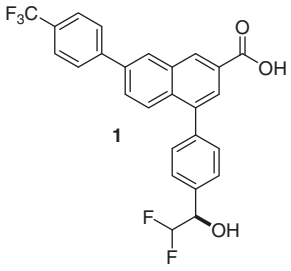

Figure 1. Previously disclosed 4,7-disubsituted naphthoic acid antagonist of $P2Y_{14}$.

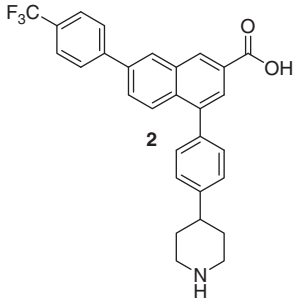

Figure 2. Less shifted antagonist of $P2Y_{14}$ identified using the binding assay utilizing 2% HSA.

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However, further studies revealed that compound **1** had a very high affinity for serum proteins and was >99% bound in the presence of 4.5% human serum albumin (HSA). Such high levels of protein binding often negatively impact in vivo activity due to the low level of free drug in circulation.

A binding assay using 2% HSA was thus developed to rapidly assess the extent of protein binding for various antagonists. Under these newly developed assay conditions, compound **1** displayed a K_i of 1292 nM, which represented a 323-fold shift from the binding assay in the absence of HSA. Armed with this new tool, an SAR campaign was initiated to identify less shifted compounds. These efforts ultimately led to the identification of the zwitterion **2** (see Fig. 2) which showed good affinity for the $P2Y_{14}$ receptor (K_i = 1.9 nM in the chimpanzee $P2Y_{14}$ binding assay), and was only 18-fold shifted in the presence of 2% HSA (K_i = 35 nM).

However, as is often the case, the improvement of one molecular property inadvertently leads to the demise of other crucial elements. In this case, the oral bioavailability of compound **2** (mice, 50 mg/kg, 0.25% methocel) suffered considerably (F = 5%, C_{24h} = 0.68 μM). Aware that the polar zwitterionic properties of compound **2** might be responsible for the low bioavailability, we reasoned that using an ester pro-drug to cap the acid moiety might improve oral absorption.

This strategy was thus applied to antagonist **2** with the aim of delivering a highly bioavailable pro-drug that would be efficiently converted to high circulating levels of the active compound. We decided that the most efficient manner to identify the best pro-drug would be to prepared a library of esters whose potential would first be assessed in vitro, followed by in vivo profiling of the most promising candidates.

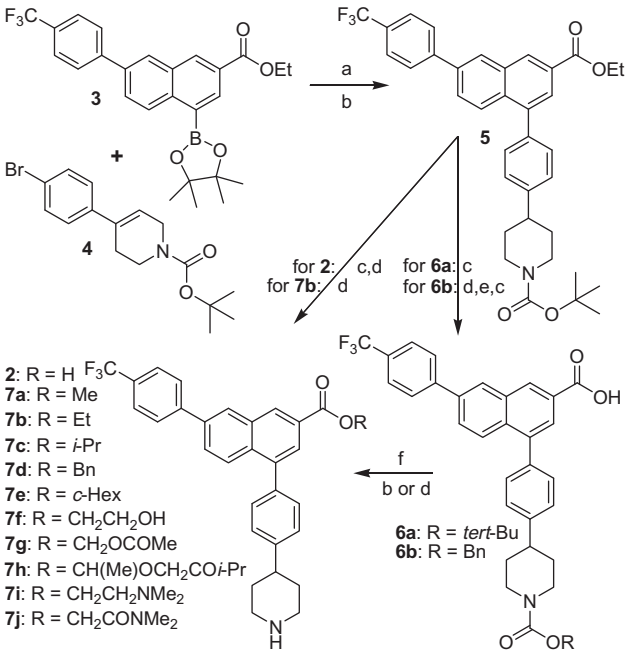
The synthetic route devised (see Scheme 1) began from the previously described boronic ester **3**,[4] which was cross-coupled with the aryl bromide **4** to afford the desired biaryl which was converted to compound **5** by hydrogenation of the double bond. The zwitterionic compound **2** could be prepared by hydrolysis of the ethyl ester using lithium hydroxide (LiOH) in a mixture of tetrahydrofuran and methanol (THF/MeOH) followed by benzyloxycarbonyl (BOC) deprotection using trifluoroacetic acid (TFA) in dichloromethane (DCM). The ethyl ester pro-drug **7b** was prepared by simple BOC deprotection using similar conditions.

While the preparation of the BOC-protected piperidine **6a** required a simple hydrolysis of the ethyl ester using LiOH in THF/H_2O at 45 °C, the synthesis of the CBZ-protected piperidine **6b** required a three-step sequence. First, the removal of the BOC protecting group was again accomplished using TFA in DCM, the piperidine was then treated with benzyl chloroformate (CBZ-Cl) and sodium carbonate (Na_2CO_3) in THF/H_2O, and the ethyl ester was finally hydrolyzed as described above to afford compound **6b**.

While the BOC-protected piperidine **6a** was utilized in the alkylation of the free carboxylic acid using benzyl chloride, the CBZ-protected piperidine **6b** was utilized in the alkylation reaction with all other electrophiles. The BOC and CBZ protecting groups of this library of compounds were then removed respectively using TFA in DCM and using hydrogen with palladium on charcoal, to afford the desired pro-drug compounds **7a–7j**.[7]

This library of pro-drugs was first evaluated for metabolic stability in vitro by parallel incubation of compounds **7a–7j** in whole blood or liver microsomes + NADPH (see Fig. 3). These experiments demonstrated that pro-drugs **7g, 7h, 7i and 7j** were among the most labile and gave high conversion to the parent drug **2** in the presence of whole blood.[8]

In vivo profiling of pro-drug **7h** revealed inefficient hydrolysis of the ester functionality and afforded low levels of the parent drug



Scheme 1. Reagents and conditions: (a) $PdCl_2(dppf)\cdot CH_2Cl_2$, aq Na_2CO_3, DMF, 85 °C; (b) H_2, Pd/C*, EtOAc; (c) LiOH, THF/MeOH, 45 °C; (d) TFA, DCM; (e) $ClCO_2Bn$, Na_2CO_3, THF/H_2O; (f), R–Cl, K_2CO_3, DMF, rt → 45 °C.

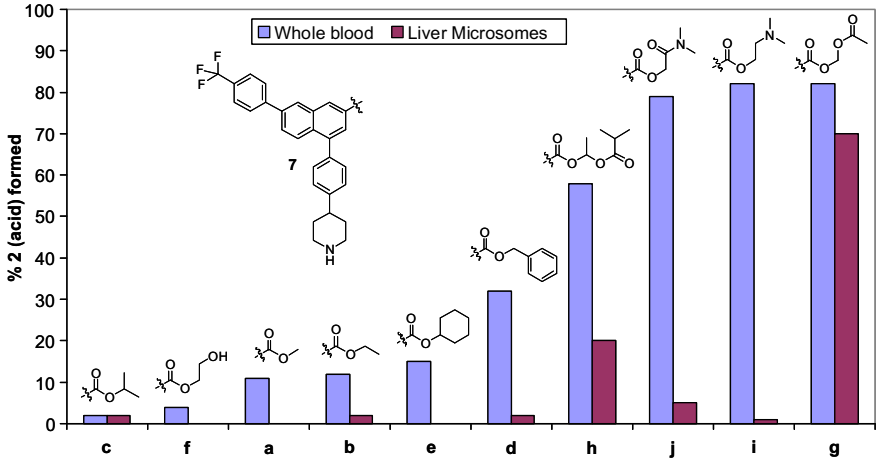


Figure 3. In vitro screening of the pro-drug library in whole blood and in liver microsomes (1 h incubation, % conversion to zwitterion **2**).

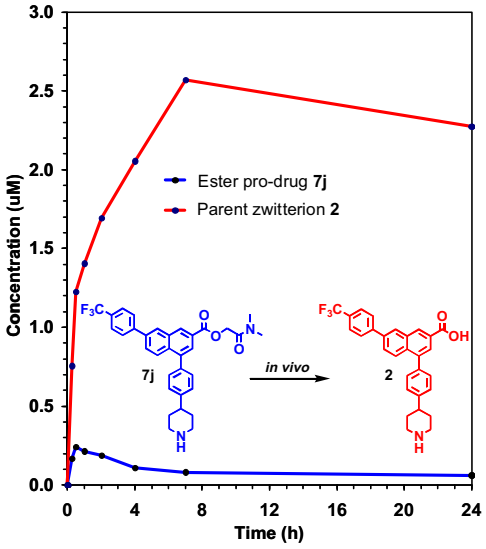


Figure 4. Pharmacokinetic profile of ester pro-drug **7j** (blue line) and its conversion to zwitterion **2** (red line).

2. On the other hand, pro-drugs **7i** and **7j** were efficiently converted in vivo to the parent zwitterion **2**, with pro-drug **7j** displaying the highest levels of compound **2** and was identified as the most promising candidate.

The pharmacokinetic profile of pro-drug **7j** (mice, 50 mg/kg, 0.25% methocel) afforded plasma levels ($F_{virtual}$ = 67%, C_{24h} = 2.28 μM, AUC = 54 μM h, Cl = 1.6 mL/min/kg) of the parent drug **2** that were substantially higher than when compound **2** was administered directly as a zwitterion (F = 5%, C_{24h} = 0.68 μM, AUC = 23 μM h). Furthermore, in vivo conversion of pro-drug **7j** to parent compound **2** was found to be very efficient as the measured plasma levels of the pro-drug **7j** represented less than 5% of the AUC of the parent compound **2** (AUC = 2.2 vs 54.4 μM h), with circulating levels of the pro-drug **7j** at 24 h of less than 0.1 μM (see Fig. 4).

In summary, our newly developed binding assay for the G-protein coupled receptor $P2Y_{14}$ utilizing 2% HSA has enabled us to identify zwitterion **2** (K_i = 35 nM, 18-fold shifted), which is significantly less shifted in the presence of protein. This advance was limited by the erosion of bioavailability when compared with our initial lead **1**. Solving this issue required the preparation of a small library of ester pro-drugs (**7a–7j**) that were first evaluated in vitro in the presence of whole blood and liver microsomes, followed by in vivo profiling of the three most promising candidates. This strategy led to the discovery of pro-drug **7j** which possesses a substantially enhanced pharmacokinetic prolife over its parent compound **2**, and which opens the path for further in vivo studies aimed at the elucidation of the biological role of the $P2Y_{14}$ receptor.

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7. The mesylate salts of all compounds (**2** and **7a–7j**) were prepared for the purpose of in vivo evaluation.
8. Pro-drug **7g** was not further profiled in vivo as it was found to be chemically unstable.