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Original article

Synthesis, structure–property relationships and pharmacokinetic evaluation of ethyl 6-aminonicotinate sulfonylureas as antagonists of the $P2Y_{12}$ receptor

Peter Bach [a,*], Jonas Boström [a], Kay Brickmann [a], J.J.J. van Giezen [b], Robert D. Groneberg [c], Darren M. Harvey [c], Michael O'Sullivan [c], Fredrik Zetterberg [a,**]

[a] Department of Medicinal Chemistry, AstraZeneca R&D Mölndal, Pepparedsleden 1, S-43183 Mölndal, Sweden
[b] Department of Bioscience, AstraZeneca R&D Mölndal, Pepparedsleden 1, S-43183 Mölndal, Sweden
[c] Array Biopharma, 3200 Walnut Street, Boulder, CO 80301, USA

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ABSTRACT

The present paper describes the development of a new series of $P2Y_{12}$ receptor antagonists based on our previously reported piperazinyl urea series **1** (IC_{50} binding affinity = 0.33 μM, aq solubility <0.1 μM, microsomal CLint (HLM) ≥300 μM/min/mg). By replacement of the urea functionality with a sulfonylurea group we observed increased affinity along with improved stability and solubility as exemplified by **47** (IC_{50} binding affinity = 0.042 μM, aq solubility = 90 μM, microsomal CLint (HLM) = 70 μM/min/mg). Further improvements in affinity and metabolic stability were achieved by replacing the central piperazine ring with a 3-aminoazetidine as exemplified by **3** (IC_{50} binding affinity = 0.0062 μM, aq solubility = 83 μM, microsomal CLint (HLM) = 28 μM/min/mg). The improved affinity observed in the in vitro binding assay also translated to the potency observed in the WPA aggregation assay (**47**: 19 nM and **3**: 9.5 nM) and the observed in vitro ADME properties translates to the in vivo PK properties observed in rat. In addition, we found that the chemical stability of the sulfonylureas during prolonged storage in solution was related to the sulfonyl urea linker and depended on the type of solvent and the substitution pattern of the sulfonyl urea functionality.

1. Introduction

The platelet $P2Y_{12}$ receptor, also known as the ADP receptor, plays an important role in the amplification phase of platelet aggregation. $P2Y_{12}$ is a 7-transmembrane, G-protein-coupled receptor that is activated by adenosine diphosphate (ADP) [1]. When platelets are stimulated, they release ADP from their dense-granules. ADP interacts with the $P2Y_{12}$ receptor and causes a down-regulation of intracellular adenylyl–cyclase activity [2]. This results in decreased cyclic-AMP levels and prolonged calcium signaling which both stabilize the formed aggregates. The important role of the $P2Y_{12}$ receptor in platelet function makes it an attractive target for the development of novel anti-platelet therapies [3].

The first class of compounds to show benefit in clinical studies were thienopyridine pro-drugs like ticlopidine and clopidogrel, whose active metabolites bind irreversibly to the receptor [4]. However, preclinical data suggested that reversible binding would not only lead to a faster off-set of effect, but also improved separation between the anti-thrombotic effect and bleeding risk [5,6]. The clinical benefits of ticagrelor, the first reversibly binding, direct-acting $P2Y_{12}$ antagonist, were demonstrated in the phase III PLATelet inhibition and patient Outcomes (PLATO) trial; in comparison with clopidogrel, ticagrelor significantly reduced the rate of the primary composite endpoint of myocardial infarction, stroke, and death from vascular causes [7]. Ticagrelor was developed via a

Abbreviations: 3-aze, 3-amino-azetidinyl; CDI, *N,N′*-carbonyl diimidazole; CLint, intrinsic clearance; cy-Pr, cyclopropyl; Caco-2, adenocarcinoma cells from human colon; DIPEA, *N,N*-diisopropylethylamine; DMA, dimethylacetamide; DMAP, 4-dimethylamino-pyridine; DSC, *N,N′*-disuccinimide carbonate; EDCI, *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride; GTPγS, guanosine 5′-*O*-[γ-thio]triphosphate; HLM, human liver microsomes; HOBT, 1-hydroxybenzotriazole; LC, liquid chromatography; MES, 2-morpholinoethanesulphonic acid monohydrate; MW, single node heating in a microwave oven; paz, piperazinyl; 4-pip, 4-aminopiperidinyl; PS, polymer-supported; RLM, rat liver microsomes; rt, room temperature; TEA, triethylamine; TEER, transepithelial electrical resistance; TEG, triethyleneglycol; Troc-Cl, 2,2,2-trichloroethyl chloroformate; WPA, washed platelet assay.

* Corresponding author. Tel.: +46 738 20 86 12; fax: +46 317 72 13 94.
** Corresponding author. Tel.: +46 317 76 13 37; fax: +46 317 76 37 10.
E-mail addresses: bach@chalmers.se (P. Bach), fredrik.zetterberg@astrazeneca.com (F. Zetterberg).

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medicinal chemistry program starting from adenosine triphosphate (ATP), the natural antagonist of the $P2Y_{12}$ receptor [8]. Other series of $P2Y_{12}$ antagonists are for example piperazinyl-glutamate-pyridines [9], thienopyrimidines [10], anthraquinones [11], adenosine analogs [12], and dinucleoside polyphosphates and nucleotides [13].

The present paper describes the discovery of a novel series of ethyl 6-aminonicotinate sulfonylureas as potent antagonists of the $P2Y_{12}$ receptor. The sulfonylureas were developed from our recently reported series of piperazinyl-pyridine ureas [14], exemplified by compound **1** (Fig. 1). Many compounds in the urea series had potencies in the submicromolar range, but the urea compounds generally suffered from low solubility and low metabolic stability in liver microsomes. Compounds to be tested in vivo by oral administration must be dissolved in order to be absorbed from the intestinal fluid, thus low solubility often results in low systemic exposure and poor in vivo activity [15]. Issues with low solubility can be addressed at different stages in the drug discovery process. In the early stage, one strategy is molecular design, in the later stage formulation can be an option, however neither of these have any guarantee of success. Our strategy to use molecular design to increase the solubility ultimately led to the discovery of the series of sulfonylureas presented here. Likewise, our attempts to increase the microsomal stability of the novel series by replacement of the ethyl ester functionality in structures like **2** and **3** will be described.

Compounds in the sulfonylurea series are composed of a pyridine moiety (A), that is substituted in the 6-position with a cyclic amine (B ring), exemplified by a piperazine **2** (Fig. 1) and an azetidine **3**, but also other mono- and bicyclic B-rings have been incorporated into the structure. A sulfonyl urea linker connects the A–B system to an aryl group (C). From a synthetic perspective, compounds like **2** and **3** were attractive target molecules since building blocks A, B and C could be coupled by efficient parallel synthesis procedures.

In the present study, SAR (structure–activity relationships) arising from variations of the different parts of the molecules is presented. Firstly, the variations of the linker that led to the discovery of the sulfonyl urea linker will be described. To study the sulfonylureas further, three different A-rings (all ethyl nicotinates) with variations of the pyridine 2- and 5-substituents have been employed. Variations of the B-ring, based on shape matching to the azetidinyl and piperazinyl compounds, were made to test the effects of changing ring type, size, rigidity, and substitution pattern, while substituted phenyls, benzyls and 5-chloro-2-thienyl were introduced as C-rings. Then follows an investigation of the chemical stability in solution of the sulfonylureas. Finally, in vivo PK data in rat on six selected compounds are presented.

2. Chemistry

Four different 6-chloronicotinic acid or ester building blocks (A-rings) were used as starting materials (Fig. 2). The 2-CF_3/5-CN building block (**4**) was described previously [14], the 2-H/5-Cl building blocks (**5** and **6**) were commercially available, and the 2-CH_3/5-CN building block (**7**) was made by a modified literature procedure [16,17] via the corresponding pyridone [18]. The 6-chloro nicotinates **4**, **5**, and **7** were reacted with an amine (B-ring) to give 6-amino nicotinates (A–B). Subsequently, these were treated with (derivatives of) sulfonyl isocyanates to form sulfonylureas. Alternatively, a more convergent route was used, in which a B-ring and a C-ring were joined via a sulfonyl urea linker before being coupled with an A-ring.

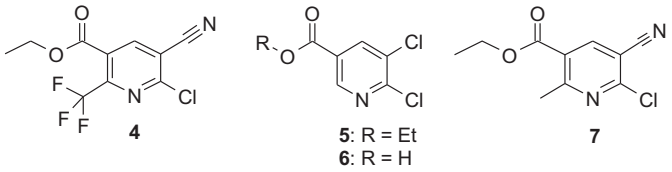
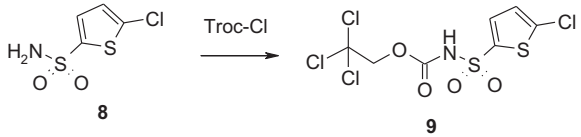


Fig. 2. 6-Chloronicotinic acid and ethyl 6-chloro nicotinate building blocks.

A synthetically efficient method to introduce the 5-chloro-2-thienyl C-ring was needed. The 5-chlorothiophene-2-sulfonamide (**8**, Scheme 1) was commercially available, however its corresponding sulfonyl isocyanate was not. Consequently, chemistry procedures were developed to convert **8** into the corresponding sulfonyl isocyanate or synthetic equivalents thereof. Activation of **8** with *N*,*N*′-disuccinimide carbonate (DSC) [19], *N*,*N*′-carbonyldiimidazole (CDI), or *n*-butyl isocyanate/phosgene [20] generated intermediates that were reacted in situ. Alternatively, reaction of **8** with Troc-Cl (2,2,2-trichloroethyl chloroformate) [21] gave a stable intermediate **9** that could be isolated and stored. Compound **9** became the reagent-of-choice due to its easy preparation, handling, and the reasonably high yields obtained in reactions of **9** with A–B systems.

Ethyl 2-CF_3/5-CN/6-piperazinyl nicotinates were synthesized from the 6-chloro-nicotinate **4** as outlined in Scheme 2 [22]. Compound **4** was treated with two different piperazines to form piperazinyl-pyridines **10** and (±)-**11**, respectively. Treatment of **10** with the CDI-derivative of **8** gave **12**, while treatment with commercially available sulfonyl isocyanates produced sulfonylureas **13**–**18** in 18–84% yield. Treatment of (±)-**11** with the CDI-derivative of **8** gave (±)-**19** after *tert*-butyl deprotection.

Ethyl 2-H/5-Cl/6-piperazinyl nicotinates were synthesized as outlined in Scheme 3. Urea compound (±)-**22** was prepared in three steps, starting from commercially available (±)-**20**. Compounds **2**, **24**–**26**, and **28**–**32** were synthesized by treating the piperazinyl-pyridine **23** with different iso(thio)cyanates. Compound **27** was



Scheme 1. Activation of 5-chlorothiophene-2-sulfonamide (**8**) with Troc-Cl (2,2,2-trichloroethyl chloroformate).

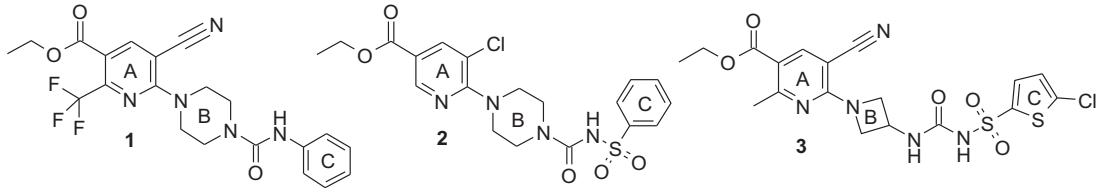


Fig. 1. Replacement of a urea linker (like in **1**) with a sulfonyl urea linker (like in **2** and **3**).