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Research paper

New highly active antiplatelet agents with dual specificity for platelet $P2Y_1$ and $P2Y_{12}$ adenosine diphosphate receptors

Ivan B. Yanachkov [a, *], Hung Chang [c, d], Milka I. Yanachkova [a], Edward J. Dix [a], Michelle A. Berny-Lang [b], Thomas Gremmel [b], Alan D. Michelson [b, c], George E. Wright [a], Andrew L. Frelinger III [b, c]

[a] GLSynthesis, Inc. Worcester, MA, USA
[b] Center for Platelet Research Studies, Division of Hematology/Oncology, Boston Children's Hospital, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA
[c] Center for Platelet Function Studies, Department of Pediatrics, University of Massachusetts Medical School, Worcester, MA, USA
[d] Hematology Division, Chang Gung Memorial Hospital, Chang Gung University, Taipei, Taiwan

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ABSTRACT

Currently approved platelet adenosine diphosphate (ADP) receptor antagonists target only the platelet $P2Y_{12}$ receptor. Moreover, especially in patients with acute coronary syndromes, there is a strong need for rapidly acting and reversible antiplatelet agents in order to minimize the risk of thrombotic events and bleeding complications. In this study, a series of new P^1,P^4-di(adenosine-5′) tetraphosphate (Ap_4A) derivatives with modifications in the base and in the tetraphosphate chain were synthesized and evaluated with respect to their effects on platelet aggregation and function of the platelet $P2Y_1$, $P2Y_{12}$, and P2X1 receptors. The resulting structure–activity relationships were used to design Ap_4A analogs which inhibit human platelet aggregation by simultaneously antagonizing both $P2Y_1$ and $P2Y_{12}$ platelet receptors. Unlike Ap_4A, the analogs do not activate platelet P2X1 receptors. Furthermore, the new compounds exhibit fast onset and offset of action and are significantly more stable than Ap_4A to degradation in plasma, thus presenting a new promising class of antiplatelet agents.

1. Introduction

Platelets play critical roles in hemostasis and its pathophysiology [1]. Undesired platelet activation can be a result of many common pathologies or interventions e.g. atherosclerotic plaque rupture, surgeries, percutaneous interventions, or major traumas, and may lead to excessive platelet aggregation and generation of occlusive thrombi. The ischemic events that follow, such as acute myocardial infarction and stroke, are leading causes of death and incapacitation in the developed world and are major contributors to health care costs. It is also increasingly recognized that platelets are an essential and integral part of the immune system [2–4], and abnormal platelet activation can be a contributing factor to vascular inflammation and associated vascular injury and atherosclerosis [5]. As a result, therapeutics that control platelet reactivity have achieved significant use in clinical practice, and the development of new drugs of the class has been a major focus of the research community and the pharmaceutical industry [6,7].

ADP plays a major role in the process of platelet activation [8]. It

Abbreviations used: Ap_4A, P^1,P^4-di(adenosine-5′) tetraphosphate; bs, broad singlet; dd, doublet of doublets; MeCN, acetonitrile; 2-MeSADP, 2-methylthioadenosine 5′-diphosphate; MeSAMP, 2-methylthioadenosine 5′-monophosphate; MeSAMP(S), 2-methylthioadenosine 5′-monothiophosphate; PRI, Platelet Reactivity Index; PRP, platelet-rich plasma; PPP, platelet-poor plasma; VASP, vasodilator-stimulated phosphoprotein.

* Corresponding author. GLSynthesis, Inc., One Innovation Drive, Worcester, MA 01605, USA.
E-mail address: ivan.yanachkov@glsynthesis.com (I.B. Yanachkov).

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is released by activated platelets [9], and is an agonist at two platelet purinergic G-protein coupled transmembrane receptors – the G_q coupled $P2Y_1$ and the G_i coupled $P2Y_{12}$. A third platelet P2 receptor, P2X1, is an ATP-activated ion channel. $P2Y_1$ activation initiates ADP-induced platelet aggregation, and is responsible for platelet shape change [10]. However, without $P2Y_{12}$ activation, the result is a small and reversible platelet aggregation. $P2Y_{12}$ activation results in amplification and stabilization of the aggregation response. There is a complex interplay between $P2Y_1$ and $P2Y_{12}$ receptors [11], and co-activation of both is necessary for full platelet aggregation [12]. The role of P2X1 may be associated with platelet shape change in response to ATP, and may contribute to the activation of platelets by low collagen concentrations and high shear stress, thus playing a role in localized thrombus formation in small arteries [13].

The $P2Y_{12}$ receptor is the most important platelet drug target. It is irreversibly inhibited by the major class of antiplatelet agents – the thienopyridines [14,15]. It is also the target of the newer reversible antiplatelet drugs ticagrelor [16], cangrelor [17], elinogrel [18], and other drug candidates [19] in various stages of development. $P2Y_1$-selective antagonists have been identified [20,21], but the lack of clinical candidates contrasts with the essential role of this receptor in platelet aggregation [22]. By using selective inhibitors, Nylander et al. [23] found that simultaneous targeting of $P2Y_1$ and $P2Y_{12}$ is highly synergistic. We [24,25] have reported that Ap_4A and its phosphonate analogs inhibit both human platelet $P2Y_1$ and $P2Y_{12}$ receptors and that the IC_{50}s for inhibition of ADP-induced human platelet aggregation were lower than the IC_{50}s for each of the receptors.

Both ADP and ATP scaffolds have been heavily modified in search of new P2 receptor agonists and antagonists (for reviews, see Refs. [22,26–29]), and the efforts resulted in the discovery of the highly potent $P2Y_{12}$ antagonists cangrelor [17] and ticagrelor [16], and highly potent $P2Y_1$ agonists and antagonists [30,31].

P^1,P^4-Di(adenosine-5′) tetraphosphate (Ap_4A) is the most important member of the group of dinucleoside polyphosphates. It is found in a variety of cells, is secreted extracellularly, and is involved in the regulation of variety of intra- and extracellular physiological functions [32]. In platelets Ap_4A is stored in dense granules and is therefore released along with ADP and ATP upon platelet activation [33]. It is well known that Ap_4A inhibits ADP-induced platelet activation [34], and modifications of Ap_4A's tetraphosphate chain have been shown to improve on this effect and to increase the biological stability [35–37]. We recently reported that Ap_4A and its tetraphosphate chain modified (P^1- and/or P^4-thio, and P^2,P^3-chloromethylene) analogs inhibit platelet aggregation by targeting both $P2Y_1$ and $P2Y_{12}$ receptors [24,25].

While the modifications of the tetraphosphate chain of the Ap_4A scaffold have been extensively explored [24,25,35–37], and some modifications of the ribose moiety, namely, the 2′,3′-*O*-benzylidene derivative have been made [38], no exploration of the base modifications has been attempted. This is due, in part, to the lack of a chemical method allowing rapid synthesis in good yield of new Ap_4A analogs. We have recently reported synthesis and properties of new reagents, diimidazolyl derivatives of diphosphoric and (methylene)bisphosphonic acids, which allow rapid synthesis of dinucleoside tetraphosphates in high yields [39]. We explored this method to prepare a number of new Ap_4A analogs with modifications in the adenosine base and the tetraphosphate moiety, and now report on their synthesis, their properties as platelet aggregation inhibitors and their activities toward platelet purinergic (P2) receptors. This SAR effort resulted in the discovery of highly potent platelet aggregation inhibitors which selectively target both platelet ADP receptors – $P2Y_1$ and $P2Y_{12}$, and have potential as novel antiplatelet drugs distinct from current antiplatelet agents, including the ATP analog cangrelor, which target only $P2Y_{12}$.

2. Results and discussion

2.1. Selection of modifications

Introduction of certain substituents at position 2 of the adenine greatly enhances the agonist potency of ADP, and the antagonist potency of ATP toward platelet ADP receptors [19]. For instance, the EC_{50} of ADP and 2-MeSADP toward $P2Y_1$ in a functional assay are 8000 nM and 6 nM, respectively, and toward $P2Y_{12}$ are 69 nM and 0.3 nM, respectively. In development of ATP type inhibitors of $P2Y_{12}$, Ingall et al. [40] synthesized a homologous series of 2-substituted ATP analogs. According to the authors, any substitution at this position increases receptor affinity, but the effect is most pronounced when the substituent is a non-polar group attached through a sulfur atom. For instance, going from adenosine 5′-(P^2,P^3-dichloromethylenetriphosphate) to its 2-ethylthio analog decreased the IC_{50} 1000 fold. Replacement of EtS group with *n*-PrS increased the inhibitory potency another 100 fold. Further increase of the chain length or introduction of additional substitution did not provide additional advantage [40].

In development of $P2Y_1$ antagonists, the group of Jacobson prepared a series of C-2, and N^6 substituted carbocyclic adenosine-3′,5′-diphosphate analogs (the so called "methanocarba" analogs) [41]. The effect of C-2 substitution at the adenine base on the antagonist potency was in the order I > Br > Me > Cl > H > F. In contrast to $P2Y_{12}$ (see above), increasing the size of a C-2 alkyl group or attaching it through a sulfur atom decreased the potency.

Substitutions at the N^6 amino group of the adenine moiety also proved to be beneficial in development of $P2Y_1$ and $P2Y_{12}$ agonist or antagonists. In Ingall's SAR study [40] mono-alkylation of N^6 improved the antagonist potency of the ATP analogs toward $P2Y_{12}$, although the effect was less pronounced than that of the substitution at C-2 position. The optimal length of the alkyl chain was 3–4 carbon atoms, and dialkylation or acylation of N^6 significantly reduced the activity. The effect of the N^6 alkylation proved to be additive with the effect of the C-2 substitution, and led to the discovery of the ATP based antiplatelet drug candidate cangrelor. In contrast to $P2Y_{12}$, the $P2Y_1$ receptor is less tolerant to bulky substituents at N^6, and the optimal modification appears to be monomethylation [42]. Both $P2Y_1$ and $P2Y_{12}$ poorly tolerate substitutions at C-8 of adenine [43].

Modifications in the polyphosphate groups of ADP and ATP have also been extensively explored. Replacement of a phosphate oxygen by sulfur is well tolerated by both $P2Y_1$ and $P2Y_{12}$, and leads in many cases to more potent analogs [26]. Actually, P^2-thioADP is a better agonist of $P2Y_1$ than ADP [30], and almost as effective as ADP toward $P2Y_{12}$ receptor [26]. An additional benefit of this modification is the improved stability of the thiophosphates toward enzymatic degradation. Replacement of the oxygen atom between P^2 and P^3 by halomethylene groups improves the inhibitory potency of the ATP scaffold in respect to $P2Y_{12}$, as well as the degradation stability [40]. All these observations are summarized in Fig. 1.

Assuming a related mode of binding of Ap_4A and ADP/ATP to $P2Y_{12}$/$P2Y_1$ receptors we chose to investigate the effects of substitutions at C2 and N^6 of the adenine base, with or without modifications in the tetraphosphate chain of the Ap_4A scaffold, on platelet aggregation and on platelet $P1Y_1$, $P2Y_{12}$, and P2X1 receptors. To this end the new Ap_4A analogs **2**–**16** shown in Fig. 2 have been synthesized and studied. The known, base unsubstituted compound **1** has been prepared and studied for reference purposes, too [24].