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Towards dual antithrombotic compounds – Balancing thrombin inhibitory and fibrinogen GPIIb/IIIa binding inhibitory activities of 2,3-dihydro-1,4-benzodioxine derivatives through regio- and stereoisomerism

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ABSTRACT

Enantiomers of 2,3-dihydro-1,4-benzodioxine derivatives possessing both thrombin and fibrinogen GPIIb/IIIa binding inhibitory activities were prepared from (*R*)- and (*S*)-glycidol as potential dual antithrombotic compounds. The influence of chirality and substitution pattern on thrombin inhibition and on inhibition of fibrinogen binding to GPIIb/IIIa was analyzed. Docking studies were used in an attempt to rationalize the results. The (*S*)-isomers of both 2,3-dihydro-1,4-benzodioxine regioisomers at positions 6 and 7 were found to be better thrombin inhibitors than the corresponding (*R*)-enantiomers, whereas we observed that stereochemistry does not display a consistent influence on fibrinogen GPIIb/IIIa binding inhibitory activity. Compound **11b**, the (*S*)-isomer of the 6-substituted regioisomer, possessed the best balanced dual activity, with $K_{i(thrombin)}$ = 1.67 ± 0.27 μM and $IC_{50(GPIIb/IIIa)}$ = 0.665 ± 0.26 μM, raising the hope that merging anticoagulant and platelet antiaggregatory activities in the same molecule could lead to successful multitarget antithrombotic agents.

1. Introduction

Cardiovascular diseases are the main cause of death in developed countries [1]. Existing anticoagulant and antiplatelet therapy, despite its importance and effectiveness in the treatment of cardiovascular diseases, has numerous limitations such as bleeding, metabolism that differs in different individuals due to genetic polymorphism, and interaction with other drugs and food [2,3].

During the last decade much effort have been made to design novel anticoagulant drugs that target the coagulation enzymes thrombin, factor Xa, factor VII and factor IX, with the aim of obtaining a simple and satisfactory, orally applicable replacement for the existing warfarin treatment [4–11]. Ximelagatran, a prodrug of melagatran, was the first oral direct thrombin inhibitor introduced to the market, however it was withdrawn from the market in 2006 due to expressed hepatotoxicity [12,13]. Dabigatran etexilate is a new oral prodrug for the direct thrombin inhibitor dabigatran (Fig. 1) that entered the market in 2008 for the prevention of blood clotting following hip or knee surgery, and for the prevention of stroke in patients with non-valvular atrial fibrillation [14]. Good progress has also been made in the field of direct and indirect factor Xa inhibitors. Rivaroxaban, a recently introduced, highly selective oral factor Xa inhibitor, promised effectiveness and safety in preventing venous thromboembolism [15]. Other novel factor Xa inhibitors include the recently approved apixaban, and idraparinux that is in the final stage of clinical investigation [16].

Platelets play an essential role in the maintenance of hemostasis; they are responsible for clot formation when damage of endothelium of blood vessels occurs [17]. The platelet glycoprotein IIb/IIIa antagonists abciximab, eptifibatide and tirofiban are potent antiplatelet agents, and are used to prevent pathological platelet

Abbreviations: GPIIb/IIIa, glycoprotein IIb/IIIa; PDB, Protein Data Bank; DMAP, dimethylaminopyridine; DCM, dichloromethane; DMF, *N*,*N*-dimethylformamide; DIAD, diisopropylazodicarboxylate; TFA, trifluoroacetic acid.

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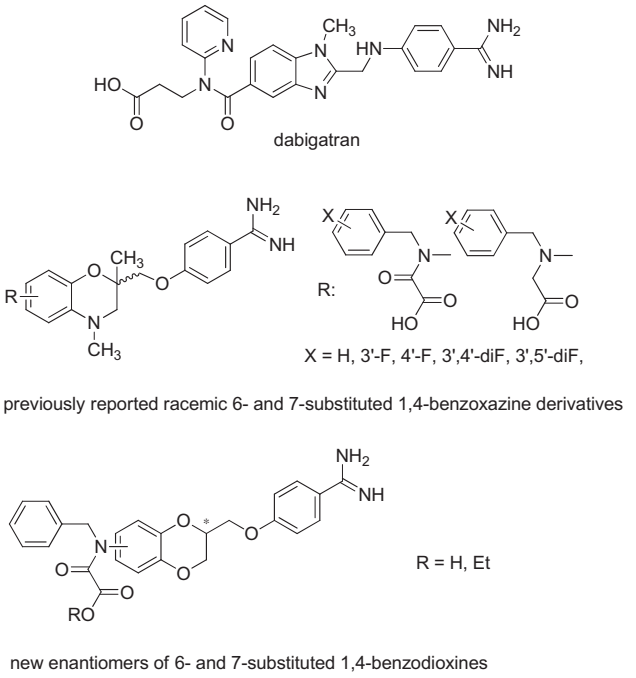


Fig. 1. Structures of the clinically used thrombin inhibitor dabigatran, the racemic 1,4-benzoxazine derivatives [25,30] and the enantiomers of 1,4-benzodioxine derivatives reported in this paper.

aggregation in patients with acute coronary syndrome, heart attack and those undergoing invasive heart procedures [18].

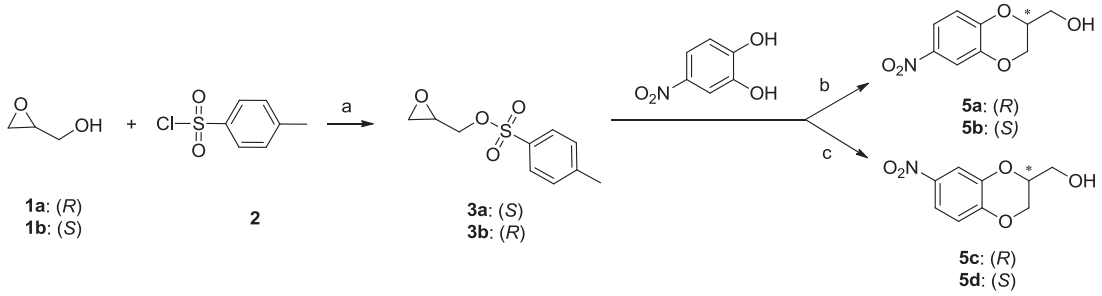
In clinical practice, an effective prophylaxis and treatment of thromboembolic diseases, such as deep vein thrombosis, thromboembolic stroke, pulmonary embolism and myocardial infarction, is achieved by using a combination of various antiaggregatory and anticoagulant drugs [19]. Since the combined use of thrombin inhibitors and glycoprotein IIb/IIIa antagonists in the prevention of cardiovascular diseases has shown additional benefits over treatment directed against thrombin or against platelets alone [20], we envisaged merging anticoagulant and platelet antiaggregatory activity in the same molecule as a promising approach towards novel multitarget antithrombotic agents [21–25]. Recently, other groups have reported the preparation of dual anticoagulant/antiplatelet agents featuring high molecular weight, polysulfated conjugates [26], in contrast to our dual acting compounds with strongly overlapping pharmacophores and low molecular weight.

In our previous work on potential dual antithrombotic compounds, combining in one molecule both thrombin inhibitory and fibrinogen receptor antagonistic activities [24,25], 3,4-dihydro-2*H*-1,4-benzoxazine [27] was selected as a suitable scaffold, on the basis of docking experiments, enabling convenient attachment of substituents in positions 2, 4, 6 and 7 [25]. The benzamidine moiety, a typical P1 group of thrombin inhibitors and an arginine mimetic of the RGD motif [28], was attached at position 2 of the benzoxazine core, while P3 benzyl and carboxylic acid moieties, required for thrombin inhibition and GPIIb/IIIa binding [29], were bound at positions 6 or 7 of the 1,4-benzoxazine skeleton (Fig. 1). Molecular modeling studies predicted 7-isomers to be better thrombin inhibitors while 6-isomers were expected to possess better inhibition of fibrinogen GPIIb/IIIa binding, as was later confirmed in biological assays [25]. Focusing on optimization of the P3 part, with a combination of different aromatic and carboxylate group moieties, we succeeded in preparing well-balanced dual compounds with thrombin K_i values and IC_{50} values for inhibition of fibrinogen binding to platelet GPIIb/IIIa in the high nanomolar/low micromolar ranges [30]. Replacement of the highly basic benzamidine moiety with the less basic [1,2,4] triazolo[4,3-*b*]pyridazine-6-yl group resulted in loss of both thrombin inhibitory and fibrinogen receptor antagonistic activities [31], showing the importance of the benzamidine group for binding to both targets. The failure to achieve better affinities of the designed compounds for thrombin and fibrinogen receptor demonstrates the difficulty in reaching a compromise that would meet the constraints imposed by both binding sites.

When designing multiple ligands with highly overlapping pharmacophores, balancing activities for both targets is demanding, so all structural information is of the utmost value [32–35]. In the present work we report a 1,4-benzodioxine series of enantiomeric compounds that combine, in the same molecule, highly overlapping pharmacophores of thrombin inhibitors and fibrinogen receptor antagonists (Fig. 1). They resulted from replacement of the 1,4-benzoxazine core by a 1,4-benzodioxine scaffold [36], a privileged heterocyclic skeleton applied in the design of selective α_1 adrenoceptor antagonists [37], antioxidants [38,39], radical scavenging compounds [40–42], hypolipidemic [43] and anti-inflammatory agents [44,45]. In order to explore the significance of chirality and regioisomerism in balancing the activity of the new 1,4-benzodioxine compounds at both targets, and thanks to the commercial availability of both (*R*)- and (*S*)-glycidol (required for enantioselective synthesis of the parent 2-substituted benzodioxine cores), we prepared both enantiomers of the benzodioxine 6- and 7-regioisomers and studied the effects of chirality and regioisomerism on binding the resulting potential dual antithrombotic compounds to thrombin and platelet fibrinogen receptor.

2. Chemistry

The synthesis of target 1,4-benzodioxine enantiomers **11a–d** from 4-nitrocatechol is presented in Schemes 1 and 2. The strongly electronegative nitro group in 4-nitrocatechol enabled effective control of the regioselectivity in construction of the 1,4-



Reagents and conditions: a) Et_3N, DMAP, DCM, 0 °C → rt, 1h; b) NaH, DMF, 80 °C, 2h; c) Na_2CO_3, DMF, 60 °C, 2h.

Scheme 1. Synthesis of compounds **5a–d**.