Bioorganic & Medicinal Chemistry Letters 22 (2012) 204–206

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one derivatives as platelet aggregation inhibitors

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ARTICLE INFO

Article history:
Received 12 August 2011
Revised 20 October 2011
Accepted 9 November 2011
Available online 16 November 2011

Keywords:
2*H*-Benzo[*b*][1,4]oxazin-3(4*H*)-one
Smiles rearrangement
Platelet aggregation inhibition
ADP
Structure–activity relationship

ABSTRACT

Novel 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-ones have been synthesized by condensation, reduction, *O*-alkylation and Smiles rearrangement using 3-bromo-4-hydroxy benzaldehyde, anilines, and chloroacetyl chloride as starting materials. All the synthesized compounds have been characterized by ^{1}H NMR, ^{13}C NMR, and HRMS, and tested for the inhibitory ability on platelet aggregation. The results have shown that the ADP (adenosine 5′-diphosphate)-induced platelet aggregation was inhibited by **7a**–**g** with the IC_{50} value at 10.14–18.83 μmol/L. Compound **7a** exhibited the most potent inhibitory effect (IC_{50} = 10.14 μmol/L) among all the compounds, but less potent than the control drug ticlopidine (3.18 μmol/L) and aspirin (6.07 μmol/L). The preliminary structure–activity relationship (SAR) was initially investigated in the study.

Arterial thromboembotic diseases, for example, ischemic heart failure, myocardial infarction, unstable angina, and stroke, which are caused by platelet aggregation, are the major causes of death in developed countries.[1] Platelets, or thrombocytes, show no interaction with the inner surface of normal blood vessel endothelium (BVE) but adhere promptly where endothelial cells are altered or extracellular matrix substrates are exposed. The final pathway in platelet aggregation induced by diverse agents, such as collagen, ADP (adenosine 5′-diphosphate), and thrombin, involves binding of the adhesive protein fibrinogen to the glycoprotein IIb/IIIa (GPIIb/IIIa) on the surface of activated platelets.[2] Since under pathological conditions platelet is a primary factor in thrombosis and heart disease the inhibition of platelet function represents a well-established approach to prevent these diseases.[3]

A number of current clinically used anti-platelet drugs are represented as aspirin, ticlopidine, dipiridamole, and tirofiban (Fig. 1). In spite of the therapeutic anti-platelet agents, improvements are needed due to the limited number of the agents and their side effects.[4] Therefore, it is of great interest to explore new agents, not only for use as drugs but also because such compounds could be used as pharmacological tools to provide important information regarding platelet function.

On the other hand, benzoxazines represent a very important heterocyclic scaffold which gives accessibility of specific compounds with various biological activities such as anti-microbial,[5] anti-inflammatory,[6] anti-tumor,[7] and act as Na/H exchange inhibitors,[8] renin inhibitors,[9] COX-2-inhibitors,[10] and nonsteroidal progesterone receptor agonists.[11]

Jakobsen et al. found that benzoxazines showed inhibitory activities on the tissue factor/factor VIIa-induced activation of factor X. The factor X is required to convert prothrombin to thrombin, which then converts fibrinogen to fibrin as a final stage in forming fibrin clot.[12] The in vitro anti-aggregatory study on 2,7-di-substituted 2*H*-1,4-benzoxazine-3(4*H*)-ones indicated that such benzoxazines exhibited high affinity towards $\alpha_{IIb}\beta_3$ integrins. The binding acts like Arg-Gly-Asp (RGD) peptides, a key recognition signal for GPIIb/IIIa, whose importance was confirmed by the inhibition of glycoprotein adhesion in the presence of small RGD-containing peptides.[13,14]

Molecular modeling revealed that 4,7-di-substituted 2*H*-1,4-benzoxazine-3(4*H*)-one analogs should have more 'cup-shaped' minimum conformations while retaining the generally accepted distance requirement between carboxylate and amidine moieties.[13] Based on these findings, we describe the design, the synthesis, and the evaluation of the anti-aggregatory activities of a series of 4,7-di-substituted 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-ones including the preliminary interpretation of their structure–activity relationships. The set consists of 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-ones containing a alkyl fragment at 4-position, as well as an aryl unit at 7-positions (Scheme 1).

According to our previous work,[15] Smiles rearrangement, an intramolecular nucleophilic reaction, is employed in the preparation

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doi:10.1016/j.bmcl.2011.11.027

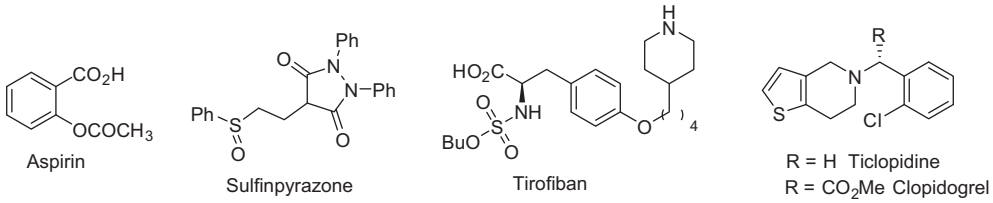


Figure 1. Structures of the most prominent anti-platelet agents currently.

of this set of compounds. 2*H*-Benzo[*b*][1,4] oxazin-3(4*H*)-ones (**7a**–**g**) were synthesized in satisfactory yields as shown in Scheme 1. The Schiff's bases **4** were synthesized by applying the condensation reaction of anilines **3** and 3-bromo-4-hydroxybenzaldehyde in anhydrous ethanol. The reduction of compounds **4** by $NaBH_4$ provided compound **5**, which then reacted with 2-chloro-*N*-alkylacetamides **2** in K_2CO_3/MeCN system to afford the O-alkylated compounds **6**. The following Smiles rearrangement of compound **6** in refluxing DMF with Cs_2CO_3 as base gave 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-ones **7** as desired compounds.

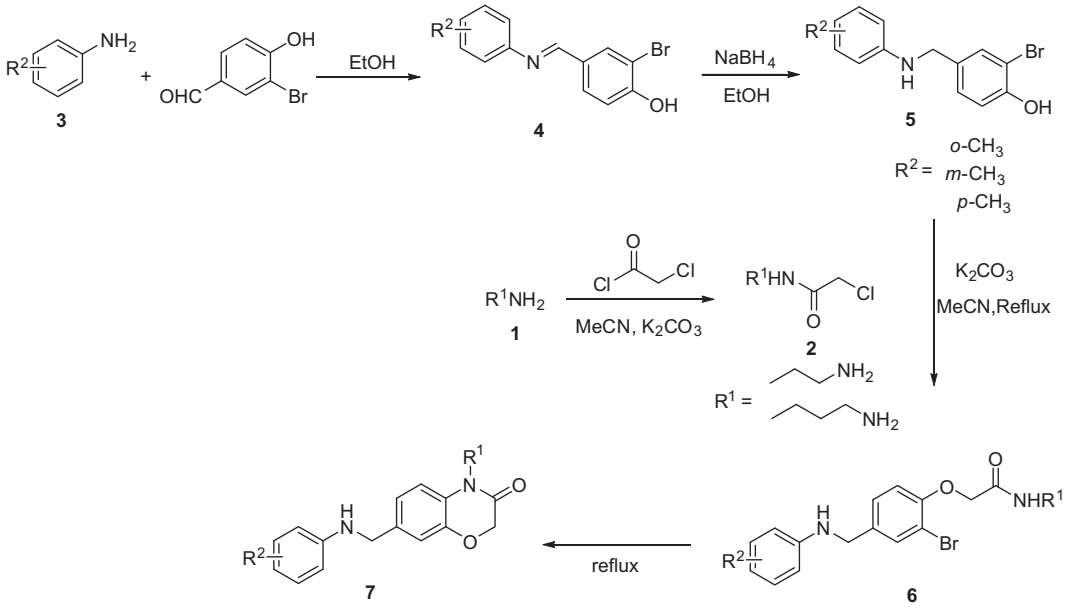
As we mentioned above, benzoxazines are associated with various pharmacological functions. Based on these surveys, we studied the anti-platelet aggregation activity of compounds **7a**–**g**. In the platelet aggregation assays, ADP was employed as the inducer. The ability of the tested compounds to inhibit platelet aggregation was evaluated by IC_{50}, calculated from the inhibition ratio linearity regression equation which was fitted based on the inhibition ratio. The result is presented in Table 1.

All the compounds generally showed inhibitory effects on platelet aggregation induced by ADP in a concentration-dependent manner, similar to the reference drugs, aspirin, and ticlopidine. The tested compounds showed lower anti-aggregatory activity with IC_{50} 10.14–18.83 μmol/L, than aspirin and ticlopidine (Table 1).

The presence of propyl group at 4-position of 7-((phenyl amino)-methyl)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one gave rise to the highest activity of the series, in the product **7a**. The introduction of methyl group on the benzene ring at the 7-position of the substituted 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one produced a decrease of the anti-platelet effect, with the IC_{50} value at 12.68–18.83 μmol/L (**7c**–**g**), and the presence of prolonged alkyl chain at 4-position of nitrogen atom reduced the activity as well (**7b**), compared with compound **7a**. These results indicated that the stereospecific blockade around the nitrogen atom of the substituent aniline at 7-position of the 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one and the nitrogen atom at the 4-position can give the derivatives with a significantly lower pharmacological potency than compound **7a**. This is the case for compound **7a**, which does not contain substituent near the nitrogen atom in the aniline fragment. Therefore, the platelet receptors may easily bond to the compound, which contributed to the highest inhibitory activity of product **7a**. The highest steric blockade *o*-CH_3 around nitrogen atom on the aniline hindered the binding of the compound **7c** to the platelet receptor, and decreased the anti-platelet effect. Thus, the compound **7c** showed the highest IC_{50} value among all the synthesized compounds. Similarly, it was observed that compound **7b** exhibited weaker anti-platelet aggregation activity than compound **7a**, due to the presence of *n*-butyl group attached to the nitrogen atom of the oxazine ring.

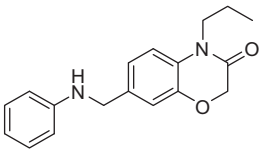
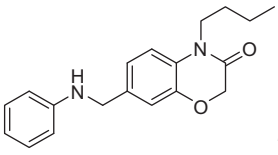
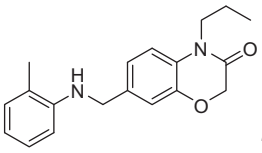
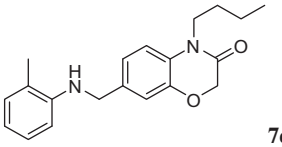
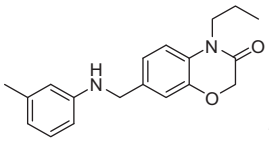
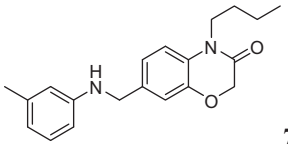
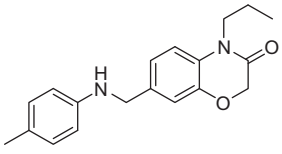
Unfortunately, the detailed structure–activity relationship (SAR) could not be established at present due to the limited variety of compounds. The further SAR of the 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-ones in order to determine in detail both the mechanism of action and the structural requirements will be studied in our laboratory.

In conclusion, set of 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one derivatives were synthesized with the assistance of Smiles rearrangement. According to the pharmacological experiments, all the compounds exhibited inhibitory activity against platelet aggregation, weaker than the control drugs aspirin and ticlopidine. The preliminary study of structure–activity relationship indicated that reducing the steric blockade at 4- and 7-position increased the inhibitory ability of the compounds bonded to platelet receptors. For presenting more detailed relationship between the structures and the anti-platelet aggregation, more compounds need to be approached.



Scheme 1. Synthesis of 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-ones.

Table 1
Anti-platelet aggregation activity of compounds **7a–g**

Compounds	IC_{50} (μmol/L)
 **7a**	10.14
 **7b**	15.17
 **7c**	18.83
 **7d**	18.78
 **7e**	13.92
 **7f**	12.68
 **7g**	14.24
Ticlopidine	3.18
Aspirin	6.07

Acknowledgments

We would like to thank the National Natural Science Foundation of China (21002081) and Technology Innovation Foundation for Graduate Students (ky2010011) of Southwest University, China to Li-Ying Wang, for financial support.

Supplementary data

Supplementary data (General procedures and spectral data.) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.027.

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