1,4-Benzoxazine-3(4H)-ones as Potent Inhibitors of Platelet Aggregation: Design, Synthesis and Structure–Activity Relations

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A series of novel potentially platelet aggregation-inhibiting 1,4-benzoxazine-3(4H)-one derivatives was designed and synthesized through Smiles rearrangement, reduction and acetylation reactions. The antiaggregatory activities of the target molecules on arterial blood samples from rabbits, expressed by IC_{50} values (μM), were then evaluated in vitro against ADP induced platelet aggregation. The favorable IC_{50} values of compound 8c (IC_{50}=8.99 μM) and 8d (IC_{50}=8.94 μM) indicated that these two compounds were the most potent molecules among all the synthesized compounds. A detailed molecular docking study to explore the interaction of compounds 8c and 8d with GP Ib/IIa receptor showed that they these two compounds were docked into the active site of GPIIb/IIIa receptor. These results suggest that the 1,4-benzoxazine-3(4H)-one derivatives are promising lead compounds to develop new platelet aggregation inhibitors.

Key words benzazinone; platelet aggregation inhibitor; ADP; molecular docking; Smiles rearrangement; structure–activity relationship

Thrombotic disease represents one of the most important causes of morbidity and mortality. Anti-thrombotic therapy agents, mainly aiming at treating thrombin platelet and thrombosis, are divided into 1) anti-platelet drugs, to prevent the coronary and cerebrovascular thrombosis from forming, such as aspirin and ticlopidine; 2) drugs against thrombin; 3) thrombolytic drugs such as fibrinolytic drugs. Currently, the most widely clinically applicable anti-platelet drugs are cyclooxygenase inhibitor, ADP receptor antagonists, phosphodiesterase inhibitor and GPIIb/IIIa receptor antagonist. It has been found that there are a large number of GPIIb/IIIa receptors on the surface of platelets. After activation, GPIIb/IIIa receptor is exposed, accelerating the Arg-Gly-Asp (RGD) sequence of fibrinogen to combine with platelet receptors. A fibrinogen molecule could bind to several platelets and one platelet might also be combined with a plurality of fibrinogen, so by “bridging effect” platelet aggregation occurs. Ultimately, the burden of thrombus overwhelms the luminal area of the vessel, potentially resulting in myocardial ischaemia and necrosis. Activation of the platelet surface receptor GPIIb/IIIa is the final common pathway of platelet aggregation, regardless of the initiating stimulus, thus blocking the platelet GPIIb/IIIa receptor could effectively suppress platelet aggregation.

Most GPIIb/IIIa inhibitors are available in the intravenous form and are only limited in hospital applications. Moreover, several compounds designed as oral GPIIb/IIIa antagonists have been discontinued because of a lack of efficacy and increased mortality. Therefore, the low efficacy and high side effects of these existing drugs allowed us to rapidly search for more novel ones. Considering that low molecular weight inhibitors to be a major goal in scientists’ research these years, the GPIIb/IIIa inhibitors with low molecular weight remain an important target in the discovery of novel anti thrombotic compounds. On the other hand, it has been reported that 1,4-benzoxazine-3(4H)-one derivatives owned a wide range of biological activities. As shown in Fig. 1, compound 1 was designed as a small molecule rennin inhibitor and compound 2 (PHRL0010) was considered as a cardiotonic agent. Besides, 1,4-benzoxazine-3(4H)-one derivatives have also been discovered to possess a good affinity with GPIIb/IIIa receptor, which in turn met with the mechanism as platelet membrane GPIIb/IIIa receptor antagonists. In our laboratory, a set of 1,4-benzoxazine-3(4H)-one molecules 3 have been synthesized to be the inhibitors of platelet aggregation.

In our continuous study on the synthesis of 1,4-benzoxazine-3(4H)-ones and their biological activities, we developed a variety of new benzoxazinone derivatives, through the attach-

Fig. 1. Bioactive 1,4-Benzoxazinone Derivatives

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Results and Discussion

Chemistry The synthetic route for the synthesis of 1,4-benzoxazine-3(4H)-one is shown in Chart 1. Firstly, the starting material alkylamine 4 was converted to the intermediate 2-chloro-N-substituted acetamide 5 through acetylation reaction with chloroacetyl chloride at 0–5°C in dichloromethane. Then the obtained 2-chloro-N-substituted acetamide 5 was reacted with 2-chloro-4-nitrophenol to construct the benzoxazinone skeleton 6 via Smiles rearrangement, in the system of NaH/N,N-dimethylformamide (DMF). The reduction of the nitro group on the benzene ring gave 7-amino-4-substituted-2H-benzo[b][1,4]oxazin-3(4H)-one 7 through the use of hydrogen catalyzed by Pd/C, followed by the amidation reaction with substituted acetyl chloride at room temperature, to afford the final product 8.

Notably, considering the stability of the intermediate and the total yield of the reactions, at first we tried to attach the substituted acyl group to the amine moiety at the 7-position, which was obtained by the reduction of the nitro group of the O-alkylated product of 2-chloro-4-nitrophenol by the compound 5, and then cyclized to construct the benzoxazinone core through Smiles rearrangement in the final step. However, due to the multi byproducts and extremely low yield of the reactions, we then turned to beginning with the reaction between 2-chloro-4-nitrophenol and 2-chloro-N-substituted acetamide 5 to give benzoxazinone, which was then reduced and acylated to afford the target molecules. In addition, the previous method for the synthesis of benzoxazinone via two steps, Chart 2, at first the reaction of 2-chloro-4-nitrophenol with 2-chloro-N-substituted acetamide to get an O-alkylated intermediate in K2CO3/CH3CN system, followed by initiating Smiles rearrangement after separation and purification, was difficultly handled and time consuming. In contrast, the one-pot reaction in refluxing NaH/DMF system, which was screened from NaOH, Cs2CO3 and K2CO3 in CH3Cl2, MeCN and DMF, respectively, greatly improved the total yield of the reactions and dramatically shortened the reaction time, shown in Chart 3. The structures of the target molecules were characterized by 1H-NMR, 13C-NMR, high resolution-mass spectrum (HR-MS) spectra and compound 8g was further confirmed by X-ray single crystal diffraction (Fig. 2).

Biological Activity The antiaggregatory activities of the target molecules 8 on the artery blood sample from rabbits, were assayed against ADP-induced platelet aggregation. Aspirin and ticlopidine were used as the positive control and the inhibitory activities of all the compounds, expressed by IC50 values (concentration required to inhibit platelet aggregation by 50%), were illustrated in Table 1.

As depicted in Table 1, the IC50 values of all the synthesized compounds ranged from 8.94 to 18.96 µM, among which the preferable values 8.99 and 8.94 µM belonged to compound 8c and 8d, respectively. These compounds exhibited stronger inhibition activities against platelet aggregation than those without containing an amide group,17) illustrated by the generally lower IC50 values. This trend suggested that the amide moiety formed additional interactions with the surface of the GP IIb/IIIa receptor. The shorter alkyl group ethyl on the 4-position of the oxazine ring, contributed to the stronger inhibition activity, which is consistent with our previous results. On the other hand, the compounds containing substituent phenyl and chloroethyl group in R2, behaved better biological activities than those with a methyl and ethyl moiety, namely longer branched substituent or ring structure on C-7 was more conducive to bind the receptor.

Molecular Docking Besides, to further study the specific binding mode between the synthesized molecules and the GP IIb/IIIa receptor, the two molecules performing the most...
potent activities in the anti-platelet aggregation experiments were docked into the binding site of the GPIIb/IIIa receptor, as depicted in Fig. 3. As we could see, the two molecules $8c$ and $8d$ were both docked into the same cavity which was enclosed by the two chains of the GPIIb/IIIa. The two carbonyl groups in molecule $8c$ might have two hydrogen bonds with the amino acid residues ALA-217 and TYR-166 respectively in the blue chain and the green chain of GPIIb/IIIa receptor in Fig. 3a, and the derivative $8d$ might have hydrophobic bond with TYR-190 which was positioned green chain in Fig. 3b. Furthermore, the molecular configuration of compound $8c$ was more twisted and folded, compared to the compound $8d$.

**Conclusion**

In summary, as our continuous exploration for the potent biologically active compounds, we have successfully designed and synthesized a series of 1,4-benzoxazine-3(4H)-one derivatives, whose activities as inhibitors of platelet aggregation were further investigated. The experimental results displayed that this series of compounds possessed potent anti-platelet aggregation abilities, among which compounds $8c$ and $8d$.

### Table 1. The Anti-platelet Aggregation Activities of the Novel 1,4-Benzoxazine-3(4H)-ones

| Compound | $R^1$     | $R^2$     | Yield (%) | IC$_{50}$ (μM) $^b$ |
|----------|-----------|-----------|-----------|---------------------|
| $8a$     | CH$_3$CH$_2$ | CH$_3$   | 61        | 15.11               |
| $8b$     | CH$_3$CH$_2$ | CH$_3$CH$_2$ | 56        | 12.16               |
| $8c$     | CH$_3$CH$_2$ | Ph       | 82        | 8.99                |
| $8d$     | CH$_3$CH$_2$ | CH$_3$CH$_2$ | 52        | 8.94                |
| $8e$     | CH$_3$CH$_2$ | CH$_3$   | 62        | 17.09               |
| $8f$     | CH$_3$CH$_2$ | CH$_3$CH$_2$ | 78        | 9.76                |
| $8g$     | CH$_3$CH$_2$ | Ph       | 50        | 10.06               |
| $8h$     | CH$_3$CH$_2$ | CH$_3$CH$_2$ | 61        | 18.96               |
| $8i$     | CH$_3$CH$_2$ | CH$_3$   | 54        | 13.71               |
| $8j$     | CH$_3$CH$_2$ | Ph       | 71        | 14.49               |
| $8k$     | CH$_3$CH$_2$ | CH$_3$CH$_2$ | 48        | 10.56               |
| Aspirin  |           |          | 6.81      |                     |
| Ticlopidine |         |          | 3.33      |                     |

$^a$ Isolated yield of the final step. $^b$ Inhibitory activity was assayed by testing the changes in optical density of ADP-induced platelet rich plasma.
showed the most potent activities. It was inferred that the benzoxazinone with an amide bond at C-7 position, which was linked to a long branched chain or a cyclic structure, exhibited stronger platelet aggregation inhibition activity, after the SAR analysis. The shorter alkyl chain linked to the nitrogen atom of the oxazinone ring, for less steric effect reason, contributed to stronger inhibition activity. Furthermore, a docking study was performed to confirm the specific binding mode between the compounds 8c and 8d and the GPIIb/IIIa receptor and it was shown that they were both docked into the binding site of the GPIIb/IIIa receptor. These results suggested that the 1,4-benzoxazine-3(4H)-one derivatives are promising lead compounds to develop a new class of platelet aggregation inhibitors.

Experimental

Chemistry All of the reagents were obtained from commercial sources. Solvents were dried and purified with known conventional methods. Melting points (uncorrected) were determined on a micro melting point apparatus (Shanghai Shengguang Instrument Co., Ltd., China). 1H- and 13C- nuclear magnetic resonance (NMR) spectra (at 400 MHz and 100 MHz, respectively) were recorded in CDCl3 with tetramethylsilane as internal reference on a Bruker Advance 500 FT spectrometer. Chemical shifts were reported in parts per million. Mass spectra (MS) were measured by the ESI method on an Agilent 6150 Q-TOF mass spectrometer. CDCl3 was used as delivered from Adamas Co., Ltd. (Shanghai, China). Silica gel (70–230 mesh) was used for flash column chromatography. All reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm silica gel plates with UV indicator (Shanghai Jingpeng Technology Co., Ltd., China). Unless otherwise noted, other reagents were obtained from commercial suppliers and used without further purification.

General Procedure for the Synthesis of 8a–l

To a magnetically stirred solution of substituted amine 4 (50.0 mmol, 1.0 equiv) and K2CO3 (75.0 mmol, 1.5 equiv) in CH2Cl2 (30 mL), cooled in an ice bath, the chloroacetyl chloride (6.0 mmol, 1.2 equiv) was added dropwise slowly. The reaction mixture was stirred at room temperature and monitored by TLC (iodine as streak reagent). After the reaction was completed, the solvent was removed under vacuum and water (40 mL) was added into the residue. The mixture was then extracted with ethyl acetate (3×30 mL). The organic layers were combined, dried over anhydrous MgSO4, and evaporated under vacuum to give the crude product 8 without further purification.

N-(4-Ethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)acetamide (8a)

White solid. mp 164.2–165.2°C. 1H-NMR (CDCl3) δ: 7.77 (1H, s), 7.31–7.18 (2H, m), 6.93 (1H, d, J = 8.6 Hz), 4.58 (2H, s), 3.97 (2H, q, J = 7.1 Hz), 2.18 (3H, s), 1.27 (3H, t, J = 7.1 Hz). 13C-NMR (CDCl3) δ: 12.5, 24.5, 36.2, 67.6, 109.4, 114.3, 114.8, 124.6, 134.1, 145.5, 163.6, 168.6. Electrospay ionization (ESI)-HR-MS m/z: 235.1225 (Calcd for C12H14N2O3: 235.1004 [M+H]+).

N-(4-Ethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)propionamide (8b)

Milky white solid, mp 152.1–152.4°C. 1H-NMR (CDCl3) δ: 7.65 (1H, s), 7.29–7.21 (2H, m), 6.92 (1H, d, J = 8.6 Hz), 4.58 (2H, s), 3.97 (2H, q, J = 7.2 Hz), 2.40 (2H, q, J = 7.6 Hz), 1.29–1.23 (6H, m). 13C-NMR (CDCl3) δ: 9.7, 12.5, 30.6, 36.2, 67.7, 109.2, 114.1, 114.8, 124.4, 134.2, 145.6, 163.5, 172.3. ESI-HR-MS m/z: 249.1227 (Calcd for C13H16N2O4: 249.1161 [M+H]+).

N-(4-Ethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)benzamide (8c)

White solid, mp 118.8–119.2°C. 1H-NMR (CDCl3) δ: 7.79 (1H, s), 7.89–7.87 (2H, m), 7.59–7.55 (1H, m), 7.51–7.47 (2H, m), 7.41–7.27 (2H, m), 6.98 (1H, d, J = 8.8 Hz), 4.60 (2H, s), 4.00 (2H, q, J = 7.2 Hz), 1.30 (3H, t, J = 7.2 Hz). 13C-NMR (CDCl3) δ: 12.5, 36.2, 67.7, 109.6, 114.6, 114.9, 124.9, 127.0, 128.8, 132.0, 134.0, 134.7, 145.6, 163.5, 165.8. ESI-HR-MS m/z: 297.1233 (Calcd for C14H15N2O: 297.1161 [M+H]+).

3-Chloro-N-(4-ethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)propionamide (8d)

White solid, mp 96.8–97.5°C. 1H-NMR (CDCl3) δ: 7.32 (1H, s), 7.29–7.22 (2H, m), 7.30–7.27 (2H, m), 7.51–7.45 (1H, m), 7.41–7.37 (2H, m), 7.31–7.24 (2H, m), 6.98 (1H, d, J = 8.8 Hz), 4.61 (2H, s), 4.00 (2H, q, J = 7.1 Hz), 3.91 (2H, t, J = 6.3 Hz), 2.83 (2H, t, J = 6.2 Hz), 2.19 (3H, s). 13C-NMR (DMSO) δ: 12.5, 36.2, 39.8, 40.5, 67.7, 109.4, 114.3, 114.8, 125.0, 133.4, 145.6, 163.5, 167.6. ESI-HR-MS m/z: 283.0847 (Calcd for C14H13N2O3Cl: 283.0771 [M+H]+).

N-(3-Oxo-4-propyl-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)acetamide (8e)

White solid, mp 96.8–97.5°C. 1H-NMR (CDCl3) δ: 7.73 (1H, s), 7.31–7.16 (2H, m), 6.91 (1H, d, J = 8.7 Hz), 4.59 (2H, s), 3.88 (2H, t, J = 7.6 Hz), 2.18 (3H, s), 1.77–1.62 (2H, m), 0.97 (3H, t, J = 7.4 Hz). 13C-NMR (CDCl3) δ: 11.2, 20.4, 24.5, 42.6, 67.6, 109.3, 114.2, 115.0, 124.8, 134.0, 145.6, 163.8, 168.6. ESI-HR-MS m/z: 249.1234 (Calcd for...
N-(3-Oxy-4-propyl-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)propionamide (8f) Light yellow solid, mp 86.8–87.5°C. 1H-NMR (CDCl3): δ: 7.73 (1H, s), 7.30–7.20 (2H, m), 6.90 (1H, d, J = 8.7Hz), 4.57 (2H, q, J = 7.6Hz), 3.91–3.83 (2H, m), 2.40 (2H, q, J = 7.6Hz), 1.75–1.59 (2H, m), 1.24 (3H, t, J = 7.6Hz), 0.97 (3H, t, J = 7.4Hz). 13C-NMR (CDCl3): δ: 9.7, 11.2, 20.4, 30.6, 42.6, 67.6, 109.2, 114.0, 115.0, 124.2, 134.5, 163.8, 167.2. ESI-HR-MS m/z: 263.1388 (Calcd for C15H18N2O3: 263.1317 [M + H]+).

N-(3-Oxy-4-propyl-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-y1)benzamide (8g) White solid, mp 193.0–193.7°C. 1H-NMR (CDCl3): δ: 8.07 (1H, s), 7.91–7.84 (2H, m), 7.58–7.54 (1H, m), 7.48 (2H, t, J = 7.5Hz), 7.39–7.28 (2H, m), 6.95 (1H, d, J = 8.7Hz), 4.59 (2H, s), 3.94–3.82 (2H, m), 1.80–1.61 (2H, m), 0.99 (3H, t, J = 7.4Hz). 13C-NMR (CDCl3): δ: 11.2, 20.4, 42.6, 67.7, 109.7, 114.6, 115.1, 125.0, 127.1, 128.8, 132.0, 134.0, 134.7, 145.7, 163.8, 165.8. ESI-HR-MS m/z: 311.1380 (Calcd for C15H16N2O3Cl: 311.1317 [M + H]+).

3-Chloro-N-(3-oxy-4-propyl-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-y1)acetamide (8i) Light yellow solid, mp 129.8–130.9°C. 1H-NMR (CDCl3): δ: 7.94 (1H, s), 7.31–7.16 (2H, m), 6.92 (1H, d, J = 8.7Hz), 4.60 (2H, s), 3.95–3.84 (4H, m), 2.83 (2H, t, J = 6.4Hz), 1.77–1.61 (2H, m), 0.98 (3H, t, J = 7.4Hz). 13C-NMR (CDCl3): δ: 11.2, 20.4, 24.4, 29.1, 40.9, 46.7, 107.6, 109.3, 114.2, 115.3, 125.1, 133.5, 145.6, 163.9, 167.9. ESI-HR-MS m/z: 297.1002 (Calcd for C14H17N2O3Cl: 297.0928 [M + H]+).

N-(4-Butyl-oxy-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-y1)benzamide (8k) White solid, mp 142.8–144.6°C. 1H-NMR (CDCl3): δ: 7.52 (1H, s), 7.30–7.20 (2H, m), 6.91 (1H, d, J = 8.7Hz), 4.58 (2H, s), 3.91 (2H, m), 6.48 (2H, s), 4.58 (2H, s), 3.91 (2H, m), 1.68–1.58 (2H, m), 1.46–1.33 (2H, m), 0.96 (3H, t, J = 7.6Hz). 13C-NMR (CDCl3): δ: 13.9, 20.1, 29.1, 30.6, 40.9, 46.7, 109.2, 114.0, 115.0, 124.6, 134.1, 145.6, 163.8, 168.7. ESI-HR-MS m/z: 263.1389 (Calcd for C14H18N2O3: 263.1317 [M + H]+).

N-(4-Butyl-oxy-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-y1)propionamide (8l) White solid, mp 146.8–151°C. 1H-NMR (CDCl3): δ: 7.52 (1H, s), 7.30–7.20 (2H, m), 6.96 (1H, d, J = 8.7Hz), 4.58 (2H, s), 3.91 (2H, m), 2.83 (2H, t, J = 6.0Hz), 2.40 (2H, q, J = 7.6Hz), 1.68–1.60 (2H, m), 1.45–1.33 (2H, m), 1.25 (3H, t, J = 7.6Hz), 0.96 (3H, t, J = 7.3Hz). 13C-NMR (CDCl3): δ: 9.7, 13.8, 20.3, 29.1, 30.6, 40.9, 67.7, 109.6, 114.6, 115.1, 125.0, 127.1, 128.8, 132.0, 134.0, 134.7, 145.7, 163.8, 165.8. ESI-HR-MS m/z: 277.1547 (Calcd for C15H16N2O3Cl: 277.1474 [M + H]+).
6) Andronati S. A., Karaseva T. L., Krysko A. A., *Curr. Med. Chem.*, **11**, 1183–1211 (2004).
7) Wagner C. L., Mascelli M. A., Neblock D. S., Weisman H. F., Coller B. S., Jordan R. E., *Blood*, **88**, 907–914 (1996).
8) Zablocki J. A., Rico J. G., Garland R. B., Rogers T. E., Williams K., Schretzman L. A., Rao S. A., Bovy P. R., Tjoeng F. S., *J. Med. Chem.*, **38**, 2378–2394 (1995).
9) Lefkowitz J., Plow E. F., Topol E. J., *N. Engl. J. Med.*, **332**, 1553–1559 (1995).
10) Leclere J. R., *Crit. Care Med.*, **30** (Suppl.), S332–S340 (2002).
11) Quinn M. J., Byzova T. V., Qin J., Topol E. J., Plow E. F., *Arterioscler. Thromb. Vasc. Biol.*, **23**, 945–952 (2003).
12) Strong S. H., Halperin J. L., *Geriatrics*, **62**, 22–27 (2007).
13) Ruef J., Katus H. A., *Expert Opin. Investig. Drugs*, **12**, 781–797 (2003).
14) Ilić M., Ilaš J., Dunkel P., Mátyus P., Boháč A., Liekens S., Kikelj D., *Eur. J. Med. Chem.*, **58**, 160–170 (2012).
15) Powell N. A., Ciske F. L., Cai C., Holsworth D. D., Mennen K., Van Huis C. A., Jalaie M., Day J., Mastronardi M., McConnell P., Mochalkin L., Zhang E., Ryan M. J., Bryant J., Collard W., Ferreira S., Gu C., Collins R., Edmunds J. J., *Bioorg. Med. Chem.*, **15**, 5912–5949 (2007).
16) Yang K., Sun L.-P., Liu J.-Y., Cui X., Piao H.-R., *Bioorg. Med. Chem. Lett.*, **20**, 4464–4467 (2010).
17) Tian X., Wang L.-Y., Xia S., Li Z.-B., Liu X.-H., Yuan Y., Fang L., Zhao H., *Bioorg. Med. Chem. Lett.*, **22**, 204–206 (2012).
18) Born G. V. R., *Nature* (London), **194**, 927–929 (1962).
19) Xiao T., Takagi J., Coller B. S., Wang J.-H., Springer T. A., *Nature* (London), **432**, 59–67 (2004).