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The synthesis of 4,7-disubstituted-2*H*-benzo[*b*][1,4]-oxazin-3(4*H*)-ones using Smiles rearrangement and their in vitro evaluation as platelet aggregation inhibitors

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ABSTRACT

A series of novel benzo[*b*][1,4]oxazin-3(4*H*)-one derivatives were synthesized as platelet aggregation inhibitors for structure–activity relationships (SAR) analysis. The synthetic pattern, involved Smiles rearrangement for the preparation of benzoxazine, was proven to be more efficient than the conventional methods. Biological evaluation demonstrated that among all the synthesized compounds, compound **9u** (IC_{50} = 9.20 μM) exhibited the most potent inhibition activity compared with aspirin, the positive control (IC_{50} = 7.07 μM). Molecular docking revealed that these set of compounds could be the GPIIb/IIIa antagonist for that they could be situated in the binding site of GPIIb/IIIa receptor quite well.

Ischemic heart disease and cerebrovascular disease are the two leading death causes for adults aged 15–59 years throughout the world nowadays, which get to even bring about one fifth deaths to them.[1] Thrombosis, as a consequence of the losing balance between coagulation function and anticoagulant function in blood, turns out to be the potential fact that leads to the diseases such as ischemic stroke,[2] myocardial infarction,[3] angina pectoris,[4] and so on.

As a vital process in primary stage for the formation of haemostasis, platelet aggregation, activated by thrombin which principally transforms the soluble fibrinogen to the insoluble fibrin, may have close relationship with thrombosis.[5] Thus, in 1960s, Coller B.S. firstly reviewed that platelet aggregation inhibitors appeared to be a logical approach for antithrombtic therapy.[6] According to the mode of action the inhibitors of platelet aggregation can be classified as: (1) substances affecting metabolism of arachidonic acid; (2) agents affecting ADP depending pathway of platelet aggregation; (3) the platelet activation factor (PAF) receptor antagonists.[7] However, such mostly clinically utilized antiaggregatory agents suffer from the flaws of the insufficiency of the efficacy and selectivity in affecting platelet aggregation. Besides, these agents only inhibit one possible way of the platelets activation, while the platelet aggregation could be generated through other approaches.[8]

The final obligatory step of the platelet aggregation is that the fibrinogen binds to the activated platelet fibrinogen receptors (glycoprotein IIb/IIIa; GPIIb/IIIa) regardless of which agonist is involved in initiation of platelet aggregation (ADP, epinephrine, collagen, PAF and others).[9,10] It is suggested that blockade of GPIIb/IIIa receptors might be a desirable therapeutic strategy because GPIIb/IIIa is platelet specific and the monoclonal antibodies to GPIIb/IIIa are more potent than aspirin. Furthermore, inhibition of GPIIb/IIIa still leaves platelet adhesion largely intact and platelet adhesion may contribute to hemostasis without leading to ischemic damage.[6] Consequently, a number of nonpeptide mimics have been investigated and found to be potent inhibitors of platelet aggregation.[11] In this way, besides the conventional platelet aggregation inhibitors such as aspirin and ticlopidine, novel molecules including eptifibatide, tirofiban and lamifiban have been introduced into clinical application as therapeutically useful antiaggregatory agents (Fig. 1).[12]

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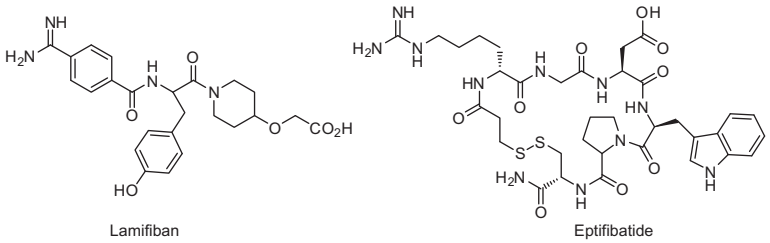
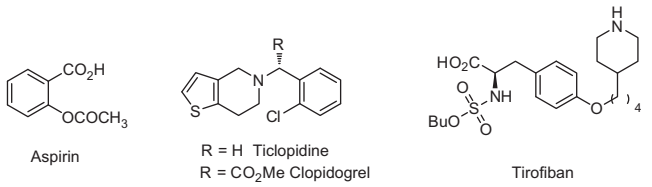


Figure 1. Chemical structures of some clinical platelet aggregation inhibitors.



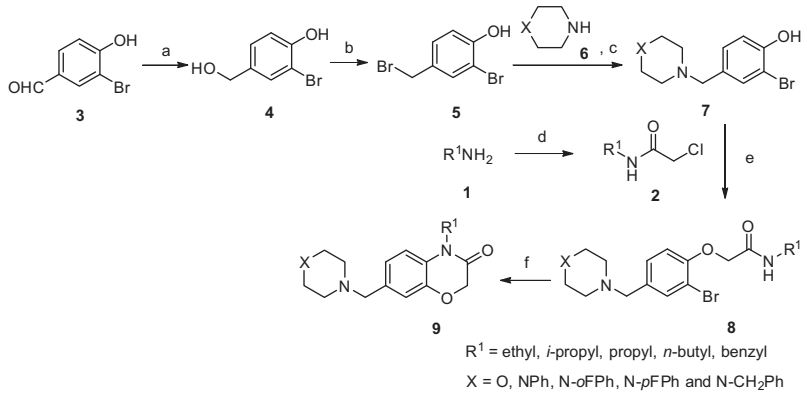
In spite of the clinically useful antiaggregatory agents, exploration for effective and safe platelet aggregation inhibitors as GPIIb/IIIa receptor antagonists are, still remains challenging not only in terms of identifying the clinical indications for which these agents will be efficacious with acceptable safety margins, but also in terms of moving this novel approach from short-term-use paradigms to potential long-term indications without damaging activity.[13] Additionally, from accumulated data in the various clinical trials with this class of drugs, low doses of antagonists may activate the receptor, resulting in increased aggregation.[14] Therefore, it is of tremendous interest to seek for new ideal GPIIb/IIIa antagonist in antithrombotic research these years.[15–17]

Benzoxazines, with nitrogen atom and oxygen atom contained in the heterocyclic ring, manifest diverse pharmaceutical functions, for example, antimicrobial,[18–20] anti-tumor,[21] antifungal activity,[22,23] renin inhibition[24] and puromycin-sensitive aminopeptidase inhibition activity,[25] and as antagonist of nonsteroidal progesterone receptor.[26] Besides, benzoxazine analogues were also found to show desirable activity as platelet aggregation inhibitors. In 2000, Dudley et al. discovered an approach to 2-phenyl-2*H*-1,4-benzoxazin-3(4*H*)-one derivatives and identified them as considerable inhibitors of a factor Xa.[27] Jakobsen et al. reported that 2-aryl substituted-4*H*-3,1-benzoxazin-4-ones could be the inhibitors of the tissue factor/factor VIIa-induced coagulation.[28,29] As for the GPIIb/IIIa receptor antagonist, Anderluh et al.[30] and Ilaš et al.[31] demonstrated that substituted-2*H*-1,4-benzoxazine derivatives could perform desire glycoprotein IIb/IIIa receptor antagonistic activity. Furthermore, Anderluh and co-workers even reported a novel class of 1,4-benzoxazin-3(4*H*)-one derivatives possessing both thrombin inhibitory and fibrinogen receptor antagonistic activity in the same molecule.[32]

According to our previous work, 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one analogues proved to be potent inhibitors of platelet aggregation.[33] Smiles rearrangement, an intramolecular nucleophilic aromatic substitution reaction discovered in 1931 by Samuel Smiles through the conversion of iso-β-naphthol sulfide into 2-napthol-1-sulfide,[34] was the prominent reaction for the synthesis of them. Compared with the conventional methods featuring harsh conditions and expensive catalysts[35] for example, palladium catalyzed coupling cyclocondensation reaction,[36] *p*-ClPIFA or PIFA catalyzed intramolecular electrophilic substitution reaction to form the benzoxazin skeleton,[37] Smiles rearrangement method is characterized with high selectivity, mild reaction conditions and good to excellent yield.[38]

Herein, we designed and synthesized a set of new benzoxazine scaffold, aiming at enhancing the activity as platelet aggregation inhibitors compared to the molecules prepared before[33] in ADP-induced platelet rich plasma or exploring the specific structure-activity relationships. Also, we performed the docking computation to discover the plausible mechanism of platelet aggregation inhibition of these synthesized compounds.

Encouraged by our previous work,[33] with 3-bromo-4-hydroxybenzaldehyde (**3**) as starting material, through the introduction of pharmacologically active heterocyclic moiety morpholyl or piperizyl ring to the benzoxazine core, the protocol for the synthesis of 4,7-disubstituted-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one **9a–u** was quite straightforward and efficient involving the reduction of aldehydes, halogenation of alcohols, N-acylation, Williamson ether synthesis, followed by Smiles rearrangement of the intermediate **8** (Scheme 1). Herein, the reduction of 3-bromo-4-hydroxybenzaldehyde (**3**) by $NaBH_4$ in ethanol gave

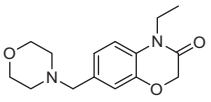
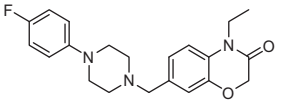
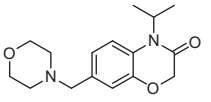
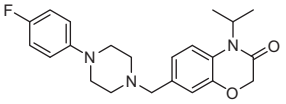
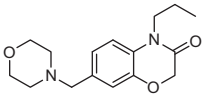
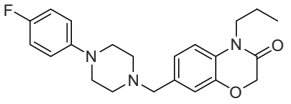
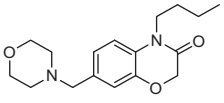
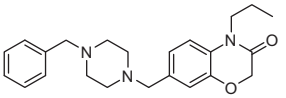
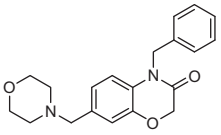
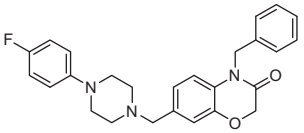
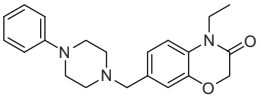
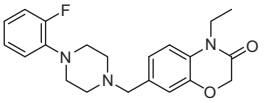
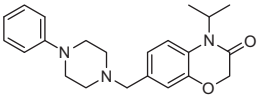
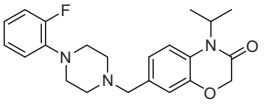
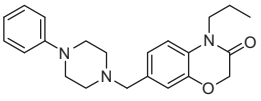
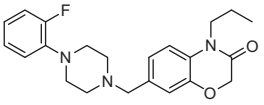
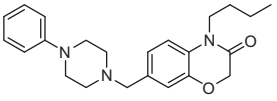
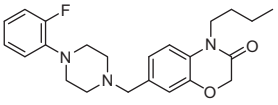
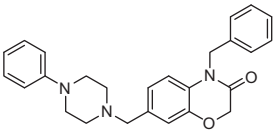
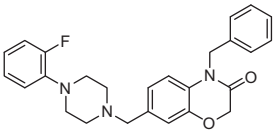
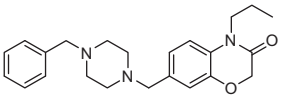


Scheme 1. Synthesis of 4,7-disubstituted-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one derivatives (**9a–u**). Reagents and conditions: (a) $NaBH_4$, EtOH, rt; (b) phosphorus tribromide, CH_2Cl_2, rt; (c) K_2CO_3, CH_3CN, rt; (d) chloroacetyl chloride, K_2CO_3, 0–5 °C; (e) K_2CO_3, CH_3CN, reflux; (f) Cs_2CO_3, DMF, 120 °C.

2-bromo-4-(hydroxymethyl)phenol (**4**) in a yield higher than 90%, which was then brominated to afford 2-bromo-4-(bromomethyl)phenol (**5**). The morpholyl or piperizyl ring was then conveniently introduced to the 7-position methylene of the intermediate **5** by the reaction of it with the heterocyclic compound **6**, which gave the compound **7** as a crucial substrate for the construction of the target molecules. The O-alkylated product **8** was cyclized to 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one **9** using Smiles rearrangement, as the desired compound for antiaggregatory assay.

During the exploration of the target molecules, we found that the aldehyde group in 3-bromo-4-hydroxybenzaldehyde (**3**) was extremely unstable in the presence of the base under Smiles rearrangement condition, so we firstly converted the aldehyde moiety into hydroxyl and then obtained the benzoxazin skeleton in the last step of the reactions. Whereas, in an alternative method involving firstly the formation of benzoxazine ring followed by the introduction of heterocyclic ring to the aldehyde moiety, the by-products formed in the process greatly decreased the yields of the reactions. The Smiles rearrangement reaction was performed under Cs_2CO_3/DMF condition, for its moderate basicity, good solubility and the high yield it brought, compared with other bases like NaH, K_2CO_3, NaOH and EtONa in various solvents. Further experiments revealed that 120 °C was the best reaction temperature inducing the rearrangement. For the amines containing sterically hindered R^1 group in general, the conversion to the target molecules led to lower yields in the rearrangement reaction (the

Table 1
In vitro anti-platelet aggregation of the benzoxazinone derivatives in platelet rich plasma

Compd	Structure	IC_{50}[a] (μM)	Compd	Structure	IC_{50}[a] (μM)
9a		10.45 ± 0.17	**9k**		11.11 ± 0.13
9b		11.02 ± 0.09	**9l**		13.35 ± 0.18
9c		9.63 ± 0.19	**9m**		11.43 ± 0.13
9d		14.40 ± 0.11	**9n**		15.24 ± 0.09
9e		12.15 ± 0.15	**9o**		19.45 ± 0.21
9f		10.11 ± 0.09	**9p**		12.66 ± 0.11
9g		10.82 ± 0.17	**9q**		13.58 ± 0.13
9h		12.82 ± 0.20	**9r**		13.79 ± 0.17
9i		15.46 ± 0.14	**9s**		17.12 ± 0.10
9j		13.87 ± 0.11	**9t**		15.34 ± 0.16
			9u		9.20 ± 0.11
	Aspirin	7.07 ± 0.14			
	Ticlopidine	3.72 ± 0.08			

[a] Inhibitory activity was assayed by testing the changes in optical density of ADP-induced platelet rich plasma. Data were presented as mean values ± SDs of the three independent experiments.

compounds **9a**, **9f**, **9k** and **9p** with smaller R^1 had higher yields 75% than their analogues, 55–65%). All the synthesized 21 molecules (**9a**–**u**) were confirmed by spectra data (^{1}H NMR, ^{13}C NMR, and HRMS) and possessed perfect stability.

Then the synthesized compounds **9a**–**u** were evaluated for their inhibitory activity in ADP-induced rich platelet plasma of New Zealand rabbit[39] and the anti-platelet aggregation drugs aspirin and ticlopidine were used as positive controls (Table 1).

As shown in Table 1, all the synthesized compounds **9a**–**u** displayed platelet aggregation inhibition activity, with the IC_{50} values ranging from 9.20 to 19.45 μM, as well as the positive controls. Especially, the IC_{50} values of compounds **9c** and **9u** were lower than 10 μM, very close to that of aspirin. Among all the synthesized compounds, the compounds with sterically less hindered group in R^1 (ethyl, isopropyl and propyl) displayed higher activity compared with the compounds containing the groups with stereospecific blockade (benzyl). Generally, the compounds with a morpholyl ring at 7-position of the 2*H*-benzo[*b*][1,4]-oxazin-3(4*H*)-one, **9a**–**e**, gave a higher pharmacological potency than those with than those with phenylpiperizyl, 4-fluorophenylpiperizyl and 2-fluorophenyl piperizyl substituents. Furthermore, the polarity of the target molecules had a remarkable effect on their activity as platelet aggregation inhibitors. Compound **9u**, the most polar molecule, had the lowest IC_{50} value among all the synthesized compounds. To our disappointment, when the drug-active fluorine-substituted phenylpiperizyl rings were introduced to the benzoxazin scaffolds (**9k**–**t**), these derivatives performed relatively less potent against the ADP-induced platelet rich plasma.

Then a docking study of the most effective compound **9u** was performed using the surflex-dock module of Sybyl-X 1.1 software,[40] in order to explore its binding mode against the active site of GPIIb/IIIa receptor. The X-ray structure of GPIIb/IIIa receptor (PDB: 2VDM) was selected for the docking study since it has been demonstrated that GPIIb/IIIa is a good model for the development of antiaggregatory agent.[41] The top 20 ranked docking poses of compound **9u** were generated and the best superimposition was shown in Figure 2. The compound **9u** was docked into a restricted box around the active site and the benzoxazine moiety was situated in the binding pocket. To point out, a hydrogen bond formed between the carbonyl oxygen of the benzoxazine moiety and the amino acid residue SER123 of the receptor, which could explain the compounds with sterically less hindered group in R^1 performed more potent anti-platelet aggregation activity. In addition, there might be hydrophobic interactions between the benzene ring of the benzoxazin skeleton and SER225, SER226 and SER161.

In conclusion, a number of 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one derivatives have been synthesized with the assistance of Smiles rearrangement as platelet aggregation inhibitors for structure-activity relationships analysis. Among all the synthesized compounds, the compound **9u** with the most polarity exhibited the most potent inhibition activity against ADP-induced platelet aggregation. The structure–activity relationship study indicated that the compounds with sterically less hindered group at 4-position of benzoxazine ring displayed higher activity compared with the compounds containing the groups with stereospecific blockade. Furthermore, docking calculation revealed that the compound **9u** could be docked in the binding site of GPIIb/IIIa receptor quite well and a hydrogen bond formed between the carbonyl oxygen of the benzoxazine moiety and the amino acid residue SER123 of the receptor. Aiming at improve their levels of anti-aggregation activity, further structural optimization of 2*H*-benzo[*b*][1,4] oxazin-3(4*H*)-one derivatives is well under way, along with more detailed biological testing of the most active compound.

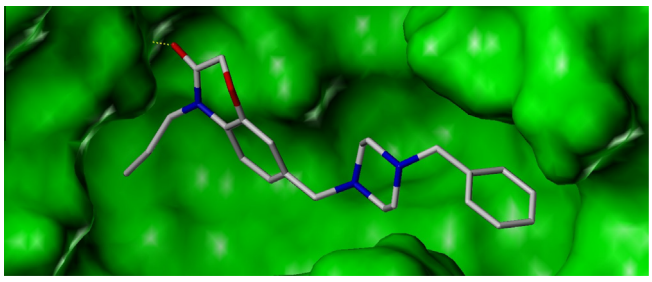


Figure 2. Compound **9u** was docked in the binding site of GPIIb/IIIa receptor (PDB code: 2VDM).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.02.014.

References and notes

1. Lopez, A. D.; Mathers, C. D.; Ezzati, M.; Jamison, D. T.; Murray, C. J. L. *Lancet* **2006**, *367*, 1747.
2. Donnan, G. A.; Fisher, M.; Macleod, M.; Davis, S. M. *Lancet* **2008**, *371*, 1612.
3. Ridker, P. M.; Hennekens, C. H.; Lindpainter, K.; Stampfer, M. J.; Eisenberg, P. R.; Miletich, J. P. N. *Engl. J. Med.* **1995**, *332*, 912.
4. Tanne, D.; Shotan, A.; Goldbourt, U.; Haim, M.; Boyko, V.; Adler, Y.; Mandelzweig, L.; Behar, S. *Stroke* **2002**, *33*, 245.
5. Bode, W. *J. Thromb. Haemost.* **2005**, *3*, 2379.
6. Coller, B. S. *Circulation* **1995**, *92*, 2373.
7. Urmey, W. F.; Rowlingson, J. *Reg. Anesth. Pain Med.* **1998**, *23*, 146.
8. Andronati, S. A.; Karaseva, T. L.; Krysko, A. A. *Curr. Med. Chem.* **2004**, *11*, 1183.
9. Van de Graaff, E.; Steinhubl, S. R. *Ann. Med.* **2000**, *32*, 561.
10. Phillips, D. R.; Charo, I. F.; Parise, L. V.; Fitzgerald, L. A. *Blood* **1988**, *71*, 831.
11. Zablocki, J. A.; Miyano, M.; Garland, R. B.; Pireh, D.; Schretzman, L.; Rao, S. N.; Lindmark, R. J.; Toth, M. V.; Zupec, M. E.; McMackins, D. E.; Adams, S. P.; Miyano, M.; Markos, C. S.; Milton, M. N.; Paulson, S.; Herin, M.; Jacqmin, P.; Nicholson, N. S.; Panzer-Knodle, S. G.; Haas, N. F.; Page, J. D.; Szalony, J. A.; Taite, B. B.; Salyers, A. K.; King, L. W.; Campion, J. G.; Feigen, L. P. *J. Med. Chem.* **1993**, *36*, 1811.
12. Kong, D. F.; Califf, R. M.; Miller, D. P.; Moliterno, D. J.; White, H. D.; Harrington, R. A.; Tcheng, J. E.; Lincoff, A. M.; Hasselblad, V.; Topol, E. J. *Circulation* **1998**, *98*, 2829.
13. Scarborough, R. M.; Kleiman, N. S.; Phillips, D. R. *Circulation* **1999**, *100*, 437.
14. Peter, K.; Schwarz, M.; Nordt, T.; Bode, C. *Thromb. Res.* **2001**, *103*, S21.
15. Ojima, I.; Chakravarty, S.; Dong, Q. *Bioorg. Med. Chem.* **1995**, *3*, 337.
16. Topol, E. J.; Moliterno, D. J.; Herrmann, H. C.; Powers, E. R.; Grines, C. L.; Cohen, D. J.; Cohen, E. A.; Bertrand, M.; Neumann, F.-J.; Stone, G. W.; DiBattiste, P. M.; Demopoulos, L. N. *Engl. J. Med.* **2001**, *344*, 1888.
17. Adler, M.; Lazarus, R. A.; Dennis, M. S.; Wagner, G. *Science* **1991**, *253*, 445.
18. Pathak, S.; Debnath, K.; Hossain, S. T.; Mukherjee, S. K.; Pramanik, A. *Tetrahedron Lett.* **2013**, *54*, 3137.
19. Mathew, B. P.; Kumar, A.; Sharma, S.; Shukla, P. K.; Nath, M. *Eur. J. Med. Chem.* **2010**, *45*, 1502.
20. Besson, T.; Rees, C. W.; Cottenceau, G.; Pons, A.-M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2343.
21. Das, B. C.; Madhukumar, A. V.; Anguiano, J.; Mani, S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4204.
22. Macías, F. A.; Marín, D.; Oliveros-Bastidas, A.; Molinillo, J. M. G. *Nat. Prod. Rep.* **2009**, *26*, 478.
23. Borate, H. B.; Maujan, S. R.; Sawargave, S. P.; Chandavarkar, M. A.; Vaiude, S. R.; Joshi, V. A.; Wakharkar, R. D.; Iyer, R.; Kelkar, R. G.; Chavan, S. P.; Kunte, S. S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 722.
24. 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, I.; Zhang, E.; Ryan, M. J.; Bryant, J.; Collard, W.; Ferreira, S.; Gu, C.; Collins, R.; Edmunds, J. J. *Bioorg. Med. Chem.* **2007**, *15*, 5912.
25. Kakuta, H.; Koiso, Y.; Takahashi, H.; Nagasawa, K.; Hashimoto, Y. *Heterocycles* **2001**, *55*, 1433.
26. Zhang, P.; Terefenko, E. A.; Fensome, A.; Zhang, Z.; Zhu, Y.; Cohen, J.; Winneker, R.; Wrobel, J.; Yardley, J. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 787.

27. Dudley, D. A.; Bunker, A. M.; Chi, L. G.; Cody, W. L.; Holland, D. R.; Ignasiak, D. P.; Janiczek-Dolphin, N.; McClanahan, T. B.; Mertz, T. E.; Narasimhan, L. S.; Rapundalo, S. T.; Trautschold, J. A.; Van Huis, C. A.; Edmunds, J. J. *J. Med. Chem.* **2000**, *43*, 4063.
28. Jakobsen, P.; Pedersen, B. R.; Persson, E. *Bioorg. Med. Chem.* **2000**, *8*, 2095.
29. Jakobsen, P.; Horneman, A. M.; Persson, E. *Bioorg. Med. Chem.* **2000**, *8*, 2803.
30. Anderluh, M.; Cesar, J.; Stefanic, P.; Kikelj, D.; Janes, D.; Murn, J.; Nadrah, K.; Tominc, M.; Addicks, E.; Giannis, A.; Stegnar, M.; Dolenc, M. S. *Eur. J. Med. Chem.* **2005**, *40*, 25.
31. Ilaš, J.; Jakopin, Ž.; Borštnar, T.; Stegnar, M.; Kikelj, D. *J. Med. Chem.* **2008**, *51*, 5617.
32. Anderluh, P. Š.; Anderluh, M.; Ilaš, J.; Mravljak, J.; Dolenc, M. S.; Stegnar, M.; Kikelj, D. *J. Med. Chem.* **2005**, *48*, 3110.
33. Tian, X.; Wang, L.-Y.; Xia, S.; Li, Z.-B.; Liu, X.-H.; Yuan, Y.; Fang, L.; Zuo, H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 204.
34. Warren, L. A.; Smiles, S. *J. Chem. Soc.* **1931**, 914.
35. Ilaš, J.; Anderluh, P. Š.; Dolenc, M. S.; Kikelj, D. *Tetrahedron* **2005**, *61*, 7325.
36. Rybczynski, P. J.; Zeck, R. E.; Combs, D. W.; Turchi, I.; Burris, T. P.; Xu, J. Z.; Yang, M.; Demarest, K. T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2359.
37. Kikugawa, Y.; Nagashima, A.; Sakamoto, T.; Miyazawa, E.; Shiiya, M. *J. Org. Chem.* **2003**, *68*, 6739.
38. Zuo, H.; Meng, L.; Ghate, M.; Hwang, K.-H.; Cho, Y. K.; Chandrasekhar, S.; Reddy, C. R.; Shin, D.-S. *Tetrahedron Lett.* **2008**, *49*, 3827.
39. Born, G. V. R. *Nature* **1962**, *194*, 927.
40. Sybyl Molecular Modeling Software Packages. Tripos Inc., St. Louis, MO, 2001.
41. Xiao, T.; Takagi, J.; Coller, B. S.; Wang, J.-H.; Springer, T. A. *Nature* **2004**, *432*, 59.