Bioorganic & Medicinal Chemistry Letters 20 (2010) 5807–5810

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Novel pyrrole- and 1,2,3-triazole-based 2,3-oxidosqualene cyclase inhibitors

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ARTICLE INFO

Article history:
Received 20 July 2010
Revised 28 July 2010
Accepted 29 July 2010
Available online 3 August 2010

Keywords:
2,3-Oxidosqualene cyclase inhibitor
Virtual screening
Hypercholesterolemia
Structure–activity-relationship

ABSTRACT

Pyrrole- and 1,2,3-triazole-based 2,3-oxidosqualene cyclase (OSC) inhibitors **3** and **4** were discovered by conducting a virtual screening, a docking study based on the crystallographic structure of OSC, and biological assays. The hit rate of the assays was increased by establishing appropriate substructural filters in the virtual screening stage. Amide derivatives of **8** and **12** preserved the inhibitory activity of parent compound **3**, which provided a reasonable starting point for further structure–activity-relationship (SAR) studies on related compounds.

Elevated plasma cholesterol levels are a major risk factor for coronary artery disease; however, cholesterol biosynthesis inhibitors can be used for the prevention of heart disease.[1] To this end, HMG-CoA reductase inhibitors such as statins are used clinically for the treatment of hypercholesterolemia.[2] However, high doses of statins cause severe side effects, including myopathy, because the enzyme catalyzes the rate limiting step of cholesterol synthesis, depleting isoprenoids such as coenzyme Q.[3] 2,3-Oxidosqualene cyclase (OSC, lanosterol synthase)[4] which catalyzes the conversion of 2,3-oxidosqualene to lanosterol is an alternative target for the inhibition of cholesterol biosynthesis. Because the conversion of 2,3-oxidosqualene–lanosterol is downstream from HMG-CoA in the biosynthesis of cholesterol, OSC inhibitors are not expected to produce statin-derived adverse effects.

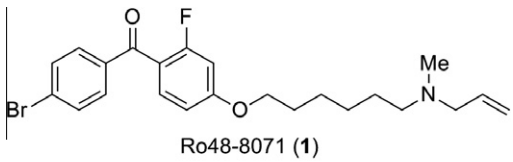
Various substances have shown inhibitory activity against OSC,[5–20] and representative compounds are listed in Figure 1. Among active compounds, Ro 48-8071 (**1**)[16] is one of the most potent inhibitors. However, OSC inhibitors have not been approved as clinical drugs for the treatment of hypercholesterolemia because of toxicological issues such as skin and epididymal changes and cataract formation. Alternatively, OSC inhibitors with unique structural scaffolds are expected to show cholesterol-lowering activity without the toxicity.

Herein, we describe the discovery of pyrrole and 1,2,3-triazole-based OSC inhibitors. By considering the crystal structure of **1** bound to OSC, a virtual screening based on the 3D structure of commercially available small molecules was performed. Among the resultant candidates, eight substances were found to display OSC inhibitory activity.

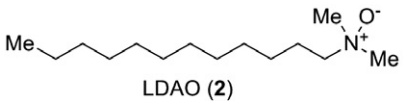
To identify OSC inhibitors, docking study was performed in which conformers in 3D small molecule database ZINC[21] were superimposed onto the crystal structure of **1** bound to human OSC[22] and optimized energetically. Thus, the results of the computational experiment were highly dependent on the quality of the virtual library.

At the time when this screening started, ZINC contains the energetically optimal structures of 3,300,000 compounds. Because performing a docking study for every compound would be time-consuming, the database was subdivided into 46 subsets based on the supplier of the compounds, and five subsets (222,000 chemicals altogether) from the 46 subsets were selected for virtual screening.

The selected five subsets were filtered according to several parameters ($-2 < x \log P < 4$, 150< molecular weight <350, hydrogen bond donor $\leqslant 3$, hydrogen bond acceptor $\leqslant 6$) to obtain a subset of 110,507 substances. A docking study was performed on the selected subsets using the CDOCKER application (Discovery Studio 2.1, Accelrys), where the virtual hits were selected based on two



Ro48-8071 (**1**)



LDAO (**2**)

Figure 1. Structure of OSC inhibitors, Ro 48-8071 (**1**) and LDAO (**2**).

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doi:10.1016/j.bmcl.2010.07.131

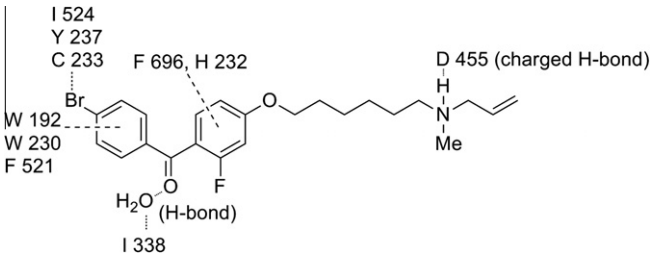


Figure 2. Interactions between **1** and human OSC.[23]

scoring functions. Specifically, hits were chosen for biological assays if they displayed a LigScore2 >7 and a Consensus >2 calculated with Score Ligand Poses and Consensus Score applications (Discovery Studio 2.1, Accelrys). The virtual assay yielded 157 hits and 40 compounds were purchased for the examination of OSC inhibitory activity according to Ebizuka's procedure.[17] However, the results indicated that the samples obtained from the virtual assay did not inhibit OSC in vitro. Thus, by selecting substructures that are expected to bind to the active site of OSC, we constructed a more focused subset to increase the probability of hits.

A co-crystallographic structure of **1** and human OSC (Fig. 2) clearly demonstrates that the carboxyl group of Asp 455 forms a charged hydrogen bond with the allylmethylamino group, and the amide NH of Ile 338 interacts with the carbonyl oxygen of **1** by a hydrogen bond via adventitious water molecule. Two of the benzene rings of **1** interact with several of the aromatic amino acid residues of the enzyme: Trp 192, Trp 230, His 232, Phe 521, and Phe 696. The bromine atom also interacts with Ile 524, Tyr 237,

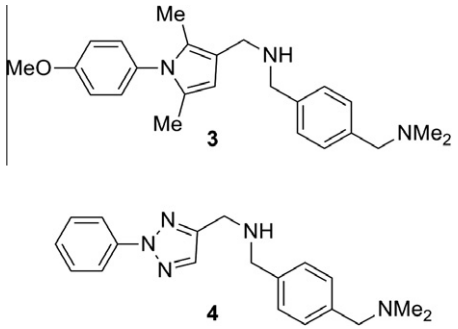
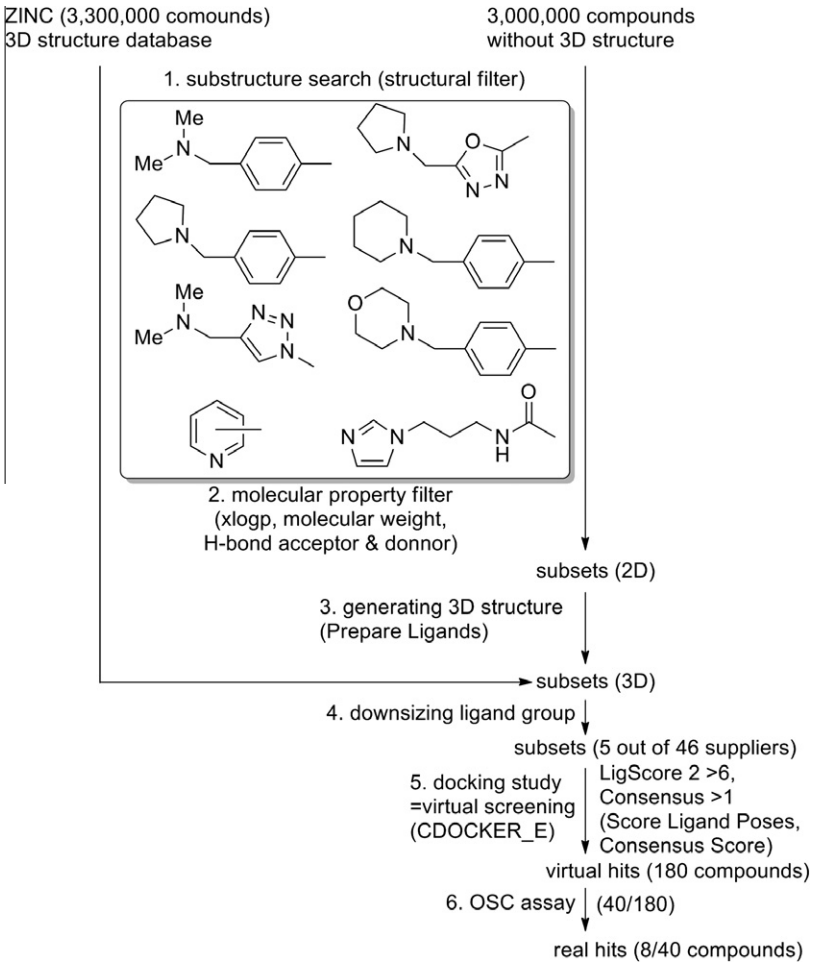


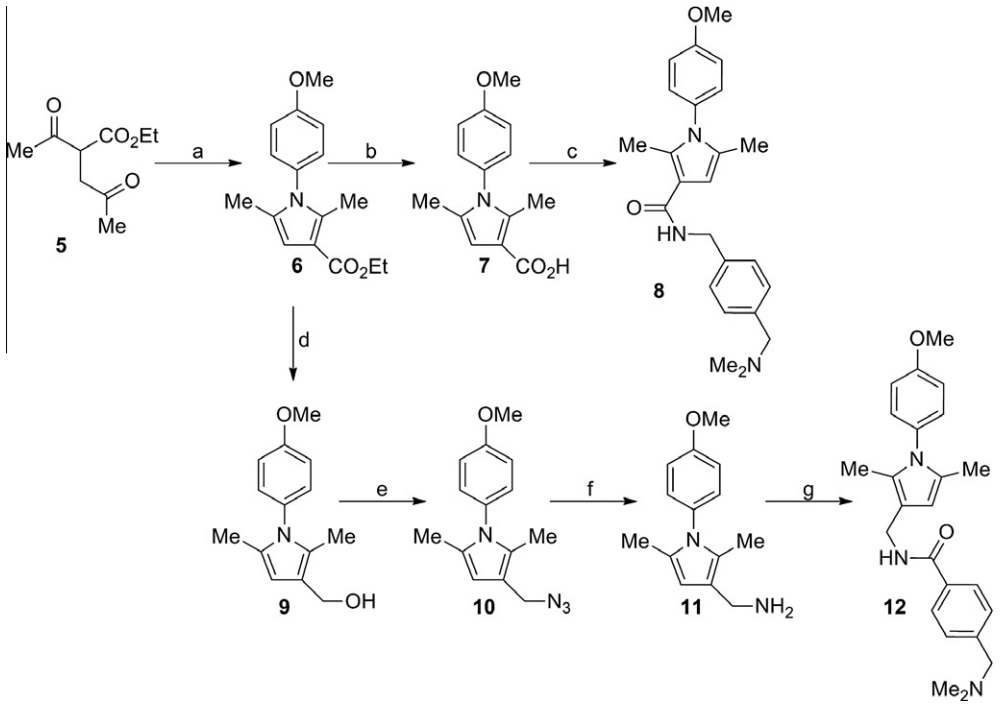
Figure 3. OSC inhibitors identified during the virtual screening.

Table 1
LigScore 2 and OSC inhibitory activity (IC_{50}: μM) of virtual hits and amide analogs

Compounds	LigScore 2	OSC inhibitory activity
3	7.58	12.7
4	6.99	1.41
8	7.36	35.4
12	7.38	58.8
LDAO (2)	nd	2.15



Scheme 1. Virtual screening of OSC inhibitors.



Scheme 2. Reagents and conditions: (a) 4-methoxybenzylaniline, toluene, rt, 72 h, 98%; (b) KOH, aqueous EtOH, 70 °C, 7 h, 65%; (c) 4-dimethylaminomethylbenzylamine, WSC·HCl, HOBt, TEA, DMF, rt, 16 h, 26%. (d) DIBAL, THF, −78 °C to 0 °C, 3 h, 16%; (e) HN_3, DEAD, PPh_3, THF, rt, 2 h, 61%; (f) H_2, 10% Pd/C, AcOEt, rt, 16 h, 85%; (g) 4-dimethylaminomethylbenzoic acid, WSC·HCl, HOBt, TEA, DMF, rt, 16 h, 26%.

and Cys 233. Based on the crystallographic information, a structural filter was established to construct a new subset for virtual screening, as shown in Scheme 1. Each substructure possessed the basic characteristic which was expected to make a charged hydrogen bond with Asp 455 of OSC.

In the second virtual screening, 3D structures of the compounds that were not included in ZINC were generated using Prepare Ligands application (Discovery Studio 2.1, Accelrys), and used in the docking study. The molecular property filter was utilized as described previously. In the second virtual screening, the threshold for hits was changed to a LigScore2 >6 and a Consensus >1 because only a few virtual hits were obtained when more stringent thresholds were applied. The second virtual screening yielded 180 hits, of which the 40 most readily available substances were purchased and tested for OSC inhibitory activity.

To evaluate the inhibitory activity of the compounds, LDAO (**2**) was selected as a positive control.[23] LDAO is an inhibitor that displays an IC_{50} value for OSC inhibition that is approximately 130-times higher than that of **1**. Considering experimental error, compounds with a potency greater than 60% of the inhibitory activity of **2** were defined to have significant biological activity. OSC inhibitors that were structurally related to **1** and displayed an IC_{50} comparable to that of **2** were obtained during a SAR study for the development **1**; thus the aforementioned threshold is reasonable for a primary screening assay.[24] OSC reactions were monitored with radio-labeled 2,3-oxidosqualene according to the previously reported procedure.[17] In the assays, the sample concentration was set to 20 μM and 84% of the OSC reactions were suppressed by **2**.

The results indicated that eight compounds, including **3** and **4**, (Fig. 3) inhibited OSC in vitro with an efficacy that was comparable to that of **2**; the inhibitory activity of **3** and **4** were 86% and 128% of the activity of **2**. According to a reported protocol,[17] the IC_{50} (μM) of **2**, **3**, and **4** were determined to be 2.15, 12.7, and 1.41, respectively, as shown in Table 1. Encouraged by the results described above, we conducted a preliminary structure–activity-relationship (SAR) study on **3**.

Compound **3** contains a pyrrole and tertiary amino moieties connected by a dimethyleneamino spacer. If the linker can be surrogated by an amide, a variety of analogs can be synthesized easily by coupling corresponding primary amines to carboxylic acids. As shown in Scheme 2, the synthesis of the amide derivatives of **3** was achieved by coupling pyrrolecarboxylic acid (**6**) or aminomethylpyrrole (**11**) with 4-dimethylaminomethylbenzylamine or 4-dimethylaminomethylbenzoic acid to afford analogs **8** and **12**.[25] Subsequently, a docking study on the crystal structure of OSC was performed with analogs **8** and **12**, and LigScore2 of 7.36 and 7.38, respectively, was obtained. Indeed, compounds **8** and **12** displayed an IC_{50} for OSC that was comparable to that of parent compound **3** (**8**: 35.4 μM, **12**: 58.8 μM), and further SAR studies on amide derivatives are expected to provide more potent OSC inhibitors. A synthetic study and further evaluation of the biological activity of the remaining six hits of the in vitro assay will be reported in due course.

Acknowledgments

The authors thank Professor Dr. Yutaka Ebizuka (currently at the National Institute of Health Science) and Dr. Masaaki Shibuya (currently at Nigata University of Pharmacy and Applied Life Sciences) at the University of Tokyo for providing *Saccharomyces cervisiae* expressing human OSC and [^{14}C] (3*S*)-2,3-oxidosqualene.

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25. All the new compounds showed satisfactory analytical data. The representatives are listed below: Compound **8**: a colorless amorphous; IR (KBr) ν_{max} 3336, 2939, 1631, 1512, 1250, 1169, 1034, 840 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz); δ 7.31 (2H, d, J = 8.3 Hz), 7.27 (2H, d, J = 8.3 Hz), 7.09 (2H, m), 6.98 (2H, m), 6.04 (1H, s), 6.00 (1H, t, J = 5.5 Hz), 4.59 (1H, d, J = 5.5 Hz), 3.86 (3H, s), 3.42 (2H, s), 2.336 (3H, s), 2.24 (6H, s), 1.96 (3H, s); ^{13}C NMR ($CDCl_3$, 150 MHz); δ 165.8, 159.4, 138.0, 137.7, 134.5, 130.4, 129.4, 129.2, 128.9, 127.7, 114.4, 113.6, 104.0, 64.0, 55.5, 45.3, 42.9, 12.6, 12.2; HRMS (ESI-Orbitrap) m/z calcd for $C_{24}H_{29}N_3NaO_2^+$ [$(M+Na)^+$] 414.2152, found: 414.2141. Compound **12**: a colorless amorphous; IR (KBr) ν_{max} 3309, 2939, 1639, 1516, 1292, 1250, 1169, 1034 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz); δ 7.74 (2H, d, J = 8.2 Hz), 7.36 (2H, d, J = 8.2 Hz), 7.12 (2H, m), 6.97 (2H, m), 6.14 (1H, br), 5.94 (1H, s), 4.46 (1H, d, J = 4.8 Hz), 3.86 (3H, s), 3.46 (2H, s), 2.25 (6H, s), 2.00 (3H, s), 2.00 (3H, s); ^{13}C NMR ($CDCl_3$, 150 MHz); δ 167.0, 159.0, 142.2, 133.6, 131.4, 129.2, 129.1, 128.7, 126.93, 126.90, 114.7, 114.3, 106.2, 63.9, 55.5, 45.3, 36.8, 12.7, 10.6; HRMS (ESI-Orbitrap) m/z calcd for $C_{24}H_{29}N_3NaO_2^+$ [$(M+Na)^+$] 414.2152, found: 414.2141.