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Discovery, synthesis and biological evaluation of 2-(4-(*N*-phenethylsulfamoyl)phenoxy)acetamides (SAPAs) as novel sphingomyelin synthase 1 inhibitors

Ya-li Li [a], Xiang-yu Qi [a], Hui Jiang [b], Xiao-dong Deng [a], Yan-ping Dong [d], Ting-bo Ding [a], Lu Zhou [a], Peng Men [a], Yong Chu [a], Ren-xiao Wang [c,*], Xian-cheng Jiang [a,b,*], De-yong Ye [a,*]

[a] Department of Medicinal Chemistry, School of Pharmacy, Fudan University, No. 826, Zhangheng Rd., Shanghai 201203, China
[b] State University of New York Downstate Medical Center, Brooklyn, NY 11203, USA
[c] State Key Lab of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China
[d] Department of Food and Pharmaceutical Engineering, Suihua University, Suihua 152061, China

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ABSTRACT

Sphingomyelin synthase (SMS) has been proved to be a potential drug target for the treatment of atherosclerosis. However, few SMS inhibitors have been reported. In this paper, structure-based virtual screening was performed on *h*SMS1. SAPA **1a** was discovered as a novel SMS1 inhibitor with an IC_{50} value of 5.2 μM in enzymatic assay. A series of 2-(4-(*N*-phenethylsulfamoyl)phenoxy)acetamides (SAPAs) were synthesized and their biological activities toward SMS1 were evaluated. Among them, SAPA **1j** was found to be the most potent SMS1 inhibitor with an IC_{50} value of 2.1 μM in in vitro assay. The molecular docking studies suggested the interaction modes of SMS1 inhibitors and PC with the active site of SMS1. Site-directed mutagenesis validated the involvement of residues Arg342 and Tyr338 in enzymatic sphingomyelin production. The discovery of SAPA derivatives as a novel class of SMS1 inhibitors would advance the development of more effective SMS1 inhibitors.

1. Introduction

Sphingomyelin (SM) is the most abundant sphingolipid in plasma membrane. It also accounts for approximately 20 percent of the phospholipids in human plasma, but 63–75 percent of plasma SM exists in atherogenic low density lipoprotein (LDL) and very low density lipoprotein (VLDL).[1,2] Epidemic investigations have revealed that patients with type II hyperlipidemia have increased plasma SM levels and increased proportions of SM in polar lipoprotein lipids.[3] Furthermore, plasma SM level has been proved as an independent risk factor for coronary artery disease in humans.[4,5]

Sphingomyelin synthase (SMS) is the last enzyme in SM biosynthetic pathway. It transfers the phosphocholine head group of phosphatidylcholine (PC) onto ceramide and thus releases SM and diacylglycerol (DAG).[6] As a SMS isoform, SMS1 was expressed ubiquitously in all the tested tissues. However, SMS1 is the major SMS in macrophages. SMS1 is primarily localized to the *trans* Golgi[7,8], and is a key factor in the control of SM level in the cells and on the cell plasma membrane.[6,9] Over-expression of SMS1 significantly increased the level of intracellular SM, atherogenic potential and subsequent atherosclerotic lesions in mice.[10,11] Contrarily, SMS1 deficiency could reduce plasma and cell membrane SM levels and attenuate atherogenesis in mice.[12,13] Thus SMS1 might serve as a potential therapeutic target of atherosclerosis. SMS1 inhibitors would be potent drugs for the treatment of atherosclerosis.[14]

There has been no report of SMS1 purified in its active form so far. However, human SMS1 has been cloned more than ten years ago.[15,16] SMS1 has an N-terminal Sterile Alpha Motif (SAM) which has been proved to have no effect on SMS activity.[7,17] Except the

Abbreviations: SM, sphingomyelin; SMS, sphingomyelin synthase; PC, phosphatidylcholine; DAG, diacylglycerol; TM, transmembrane; *h*SMS1, human sphingomyelin synthase 1; His, histidine; Asp, aspartic acid; SAPA, 2-(4-(*N*-phenethylsulfamoyl)phenoxy)acetamide; SAR, structure–activity relationships; Phe, phenylalanine; Leu, leucine; Ala, alanine; Arg, arginine; Trp, tryptophane; Tyr, tyrosine; C6-NBD-Cer, 6-((*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)hexanoyl)-sphingosine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; C6-NBD-SM, *N*-(*N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)-epsilon-aminohexanoyl)sphingosyl phosphoryl choline; HPLC, high performance liquid chromatography; IC_{50}, 50% inhibitory concentration.

* Corresponding authors. Tel.: +86 21 54925128 (R.W.); tel.: +1 718 270 6701 (X.J.); tel./fax: +86 21 51980125 (D.Y.).

E-mail addresses: wangrx@mail.sioc.ac.cn (R.-x. Wang), xjiang@downstate.edu (X.-c. Jiang), dyye@shmu.edu.cn (D.-y. Ye).

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SAM domain, the six transmembrane (TM1–TM6) and four highly conserved motifs (D1–D4) of *h*SMS1 reside on the sequence of M130-Q353 amino acids.[15,16] Two conserved motifs D3 (C277-T286 aa) and D4 (H328-H348 aa) localize at TM4 and TM6, respectively. Especially, motif D4 (H328-H348 aa) at TM6 is critical for the catalytic function of SMS1.[18] For the discovery of SMS1 inhibitor, a three-dimensional structure of human sphingomyelin synthase 1 (*h*SMS1, M130-Q353 aa) has been built by homology modeling, optimized through molecular dynamics and proved the rationality.[19] Moreover, a domain locating on motifs D3 and D4 of *h*SMS1 was discovered to be the active site for SM production.[15] Three residues His285, His328 and Asp332 in motifs D3 and D4, forming a HHD triad, have been proved to be key amino acids involved in SM production by site-directed mutagenesis.[17] However, the roles of other amino acids in the active site as well as the catalytic mechanism involved in SM production were still under investigation.

Up to now, very few SMS inhibitors have been reported in the literatures. Potassium tricyclo[5.2.1.0(2,6)]-decan-8-yl-dithiocarbonate **D609** (Fig. 1), which was firstly found as a cytotoxic antivirus and anti-tumor reagent,[20,21] was reported with a weak SMS inhibitory effect in numerous enzyme or cell types in vitro (IC_{50} = 177–600 μM).[6,22–24] But the highly instable structure of **D609** ($t_{1/2}$ = 19.5 min in saline solution at 24 °C) hindered its further development to become an effective SMS inhibitor. Although the prodrug modification of **D609** could improve its chemical stability,[23] the inhibitory activity toward SMS should be enhanced. Furthermore, α-aminonitrile derivatives have been identified as the first series of SMS inhibitors discovered through rational design. Among them, the representative compound **D2** (Fig. 1) exhibited more potent SMS inhibitory activity than **D609**.[25,26] However, the molecule of compound **D2** contains α-aminonitrile group, which is regarded as a potential toxigenic structure. Therefore, more effective SMS1 inhibitors should be discovered to understand the role of SMS1 in cell functions and animal phenotypes as well as to meet the further study of SMS1 inhibitors as potent therapeutical drugs for the treatment of atherosclerosis.

In this study, structure-based virtual screening was performed to discover novel SMS1 inhibitors. A series of SAPA derivatives were designed and synthesized through structure sectionalized modification for in vitro enzymatic assay and SAR studies. Fortunately, six of them showed potent SMS1 inhibitory activities with IC_{50} values lower than 10 μM in enzymatic assay. The binding modes of SMS1 inhibitors and substrate PC with *h*SMS1 were investigated. The involvement of residues Arg342 and Tyr338 at the active site of *h*SMS1 in SM production were evaluated by site-directed mutagenesis.

2. Results and discussion

2.1. Structure-based virtual screening of the SPECS library

A multi-step structure-based virtual screening was employed to discover potential small molecule SMS1 inhibitors. The SPECS library (http://www.specs.net) was updated and contained over 220,000 compounds which were to be screened through molecular docking. The reported structural model of *h*SMS1[19] was energy optimized before being employed in the virtual screening. The molecular docking was conducted by targeting the validated active site of *h*SMS1 and performed on the GOLD software and the GLIDE software sequentially. A total of 95 compounds were finally selected among the top-ranked candidates after visual examination. The screening procedure was fully detailed in Section 4.

Samples of these 95 compounds were purchased from SPECS Inc and tested for their biological activities without further purification. The SMS1 inhibitory activities of these compounds were evaluated in an in vitro enzymatic assay. Among them, 2-(4-(*N*-phenethylsulfamoyl)phenoxy)acetamide (SAPA) derivative **1a** (Fig. 2), the only active compound, exhibited dose-dependent inhibition toward SMS1 overexpressed Hela cell lysate with an IC_{50} value of 5.2 μM (Fig. 3**a**). It was approximately forty fold more potent than **D609** which showed modest SMS1 inhibitory activity with an IC_{50} value of 219 μM (Fig. 3b). Therefore SAPA **1a**, as a novel scaffold of SMS1 inhibitors, was considered to be the lead compound for further structural modification.

2.2. Chemistry

SAPA derivatives were designed and synthesized following the general route described in Scheme 1. The *N*-substituted-2-chloroacetamides **3a**–**3r** were synthesized by the nucleophilic substitution reaction of various substituted aniline or benzylamine **2a**–**2r** with chloracetyl chloride in the presence of pyridine. Besides the key intermediate, 4-hydroxybenzene-1-sulfonyl chloride **5** was obtained from chloroformylation of compound **4** by thionyl chloride with catalytic amount of DMF. Then compounds **7a**–**7d** were prepared by the reaction of the intermediate **5** with different amines **6a**–**6d** in moderate yields after recrystallization. Finally the target compounds SAPA **1a** and **1d**–**1w** were achieved through the general electrophilic substitution of compounds **7a**–**7d** by 2-chloroacetamides **3a**–**3r**. The yields were in the range of 30–80% and the unfavorable attacking of nitrogen atom on sulfonamide group could be avoided by controlling temperature and ratio of substrates.

In order to investigate the role of sulfonyl group on **1a**, SAPA analogues **1b** and **1c** were designed and synthesized following the route described in Scheme 2. 4-Hydroxybenzoic acid **8** was treated with thionyl chloride in methanol under reflux condition to afford the corresponding methyl ester **9**. Then the intermediate **10** was afforded by the reaction of compound **9** with *N*-substituted-2-chloroacetamides **3a**. Subsequently the hydrolysis of compound **10** was carried out to give compound **11**, which was treated respectively with amines **6a** and **6e** to afford the corresponding SAPA analogues **1b** and **1c**.

The structures of all new compounds were elucidated from their analytical and spectroscopic data which were collected in the Section 4.

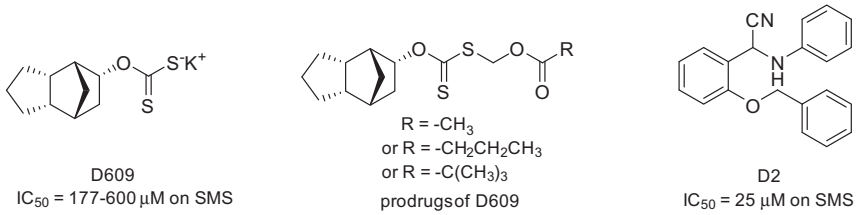

Figure 1. Chemical structures of reported sphingomyelin synthase inhibitors.