

KAPLAT Achievement Report

Synthesis and evaluation of some piperazine derivatives as potential candidates for SREBP Inhibitors

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1. Introduction

SREBP is a transcription factor that controls lipid biosynthesis and is often overactivated in cancer and metabolic diseases. Therefore, inhibition of SREBP pathway might be a potential approach to treating these disease conditions. Fatostatin, a diarylthiazole derivative, was originally discovered from a chemical library to inhibit insulin-induced adipogenesis [1], and animal studies demonstrated that it decreases the amounts of fatty acid, triglyceride, and low-density lipoprotein and thereby reduces body weight in obese mice with low cytotoxicity [2]. However, fatostatin showed only moderate potency in mice, and its utility was limited by low aqueous solubility. Another compound, named FGH10019, a methanesulfonamide derivative of fatostatin, exhibited the more potent activity in a cell-based assay and exhibited better *in vitro* and *in vivo* physicochemical properties than fatostatin [3]. FGH10019 is a novel sterol regulatory element-binding protein (SREBP) inhibitor with IC_{50} of 0.7 μ M. Both Fatostatin and FGH10019 have been serving as research tools for investigation of biological roles of SREBP. In this study, a series sulfonamide derivative containing piperazine moiety were synthesized, and their effects on SREBP were evaluated through luciferase reporter assays with the goal of discovering superior SREBP inhibitors for further *in vivo* evaluation in a variety of disease models.

2. Experimental

General procedure for the synthesis of sulfonamide derivative was described in Scheme 1 in which compound (1) was synthesized from the reaction of 4-Toluenesulfonyl chloride and p-amino benzoic acid at room temperature for more than 1 day [4]. In the next step, a mixture of (1) (1equiv) and N,N,N,N-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (1equiv) in DMF was stirred at room temperature for 1 h. Then, a solution of the appropriate amine derivative (1 equiv) in TEA (2.5 equiv) was added dropwise. The reaction mixture was stirred overnight, then quenched with water and extracted with EtOAc. The organic phase was dried with Na_2SO_4 and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (DCM/MeOH 10:1) affording the target compounds which were recrystallized in ethanol (if needed).

Luciferase reporter assay. On day 0, CHO-K1 cells were plated out onto a 96-well plate (8×10^4 cells/ml, 100 μ L/well). On day 1 (transfection), added reporter plasmid and β -gal plasmid into Opti-MEM followed by FuGENE®HD. To the cells were added 5 μ L of complex (Opti-MEM: 5 μ L/well, reporter plasmid: 0.1 μ g/well, β -gal: 0.005 μ g/well, FuGENE®HD: 0.3151 μ L/well) then incubation for 20h at 37°C. On day 2 (lipid treatment): mevalonate lithium salt (50 μ M) and compactin (50 μ M) were added into the mixture of DMEM/F-12 and 5% Lipid depleted serum (+1% P/S). To this medium was added the tested compounds (5 μ M in DMSO) and 90 μ L of medium containing compounds was added to the well. After incubation for 24h at 37°C, the cells in each well were lysed, and aliquots were used to measure luciferase and β -galactosidase activities. Luciferase activity was normalized using the activity of β -galactosidase and the result was shown in Figure 1.

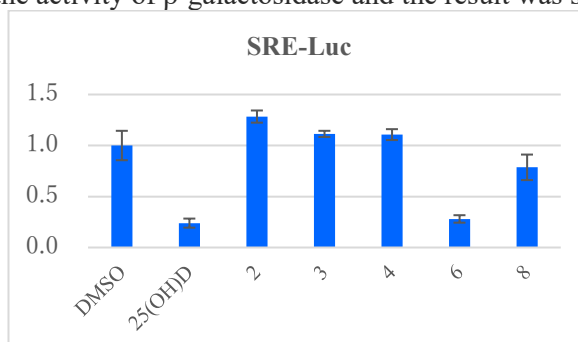
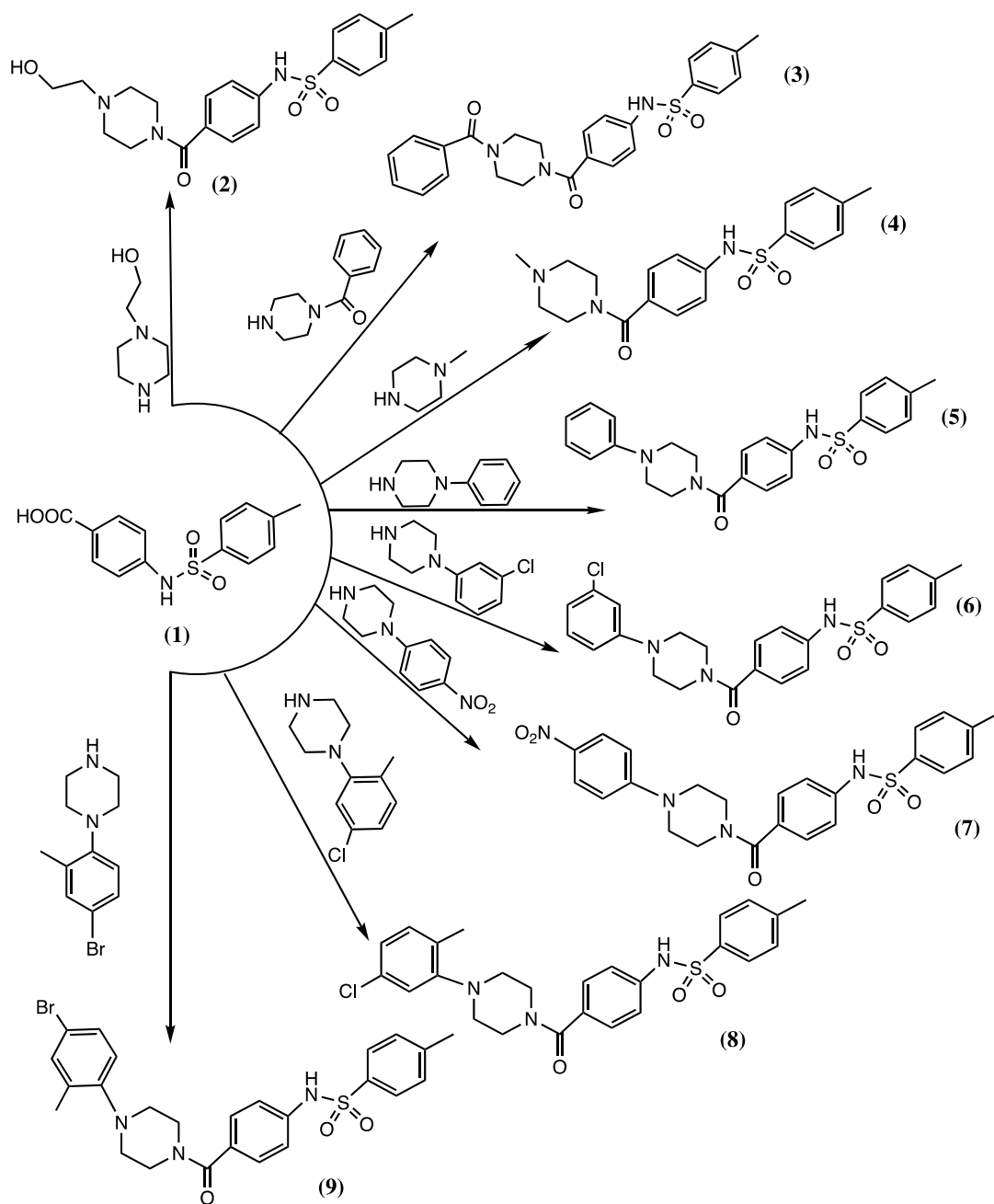


Figure 1. Effects of piperazine derivatives on the activation of SREBP

Scheme 1. Synthesis of piperazine derivatives



Reference

1. Choi Y, Kawazoe Y, Murakami K, Misawa H, Uesugi M. Identification of bioactive molecules by adipogenesis profiling of organic compounds. *J Biol Chem* **2003**; 278:7320-4.
2. Kamisuki S, Mao Q, Abu-Elheiga L, Gu Z, Kugimiya A, Kwon Y, et al. A small molecule that blocks fat synthesis by inhibiting the activation of SREBP. *Chem Biol* **2009**;16:882-92.
3. Shinji Kamisuki, Takashi Shirakawa, Akira Kugimiya, Lutfi Abu-Elheiga, Hea-Young Park Choo, Kohei Yamada, Hiroki Shimogawa, Salih J. Wakil and Motonari Uesugi, Synthesis and Evaluation of Diarylthiazole Derivatives That Inhibit Activation of Sterol Regulatory Element-Binding Proteins, *J. Med. Chem.* **2011**, 54, 4923-4927.
4. Jagrut, V. B.; Netankar, P. D.; Jawale, D. V.; Mane, R. A.; Jadhav, W. N., An Efficient Synthetic Route for New 1,3,4-Oxadiazoles Having Sulphonamido Pharmacophore, *Bull. Korean Chem. Soc.* **2009**, 30, 2812-2814.