Synthesis and Biological Evaluation of Ezetimibe Analogs as Possible Cholesterol Absorption Inhibitors

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Abstract: In order to investigate the SAR of Ezetimibe analogs for cholesterol absorption inhibitions, amide group and electron-deficient pyridine ring were introduced to the C-(3) carbon chain of Ezetimibe. Eight new derivatives of the 2-azetidinone cholesterol absorption inhibitors have been synthesized, and all of them were enantiomerically pure. All the new compounds were evaluated for their activity to inhibit cholesterol absorption in hamsters, and most of them showed comparable effects in lowering the levels of total cholesterol in the serum.

Keyword: 2-Azetidinone derivatives, Enantiomerically pure, Cholesterol absorption inhibition.

1. INTRODUCTION

Atherosclerotic coronary heart disease (CHD) has been the major cause of death and cardiovascular morbidity in the world [1]. The prominent risk factor associated with CHD was the elevation of serum cholesterol levels [2]. Well established clinical treatment for CHD has focused on life style changes and the reduction of serum cholesterol. These reductions have been shown to correlate strongly with the decrease of CHD mortality and the reversal of atherosclerosis as evidenced by the regression of occlusion of coronary arteries [3]. Pharmacologically these reductions have focused on the use of “statins” or HMG-CoA reductase inhibitors to affect both the biosynthesis of cholesterol and clearance mechanisms [4]. The other major contributor to serum cholesterol is from exogenous (dietary) or intestinal sources (enterohepatic circulation of biliary cholesterol). Blocking intestinal sources of cholesterol represents a scientifically and pharmacologically interesting mechanism for affecting serum cholesterol as it complements existing therapies in the clinic [5].

Ezetimibe (1) (Fig. 1), which was approved in late 2002 for use either alone or in combination with a statin, was the only example to date of a drug that involves inhibition of intestinal cholesterol absorption [6]. A recent report from the Schering-Plough Research Institute has described the discovery of Niemann-Pick C1 Like 1 (NPC1L1) protein as critical for the intestinal absorption of cholesterol. Knockout mice lacking the NPC1L1 gene showed markedly reduced cholesterol absorption and were no longer sensitive to further reduction of cholesterol absorption by ezetimibe. Thus NPC1L1 lies in the ezetimibe sensitive pathway for cholesterol absorption, making it a likely candidate for the target of ezetimibe [7].

The reported structure-activity relationships (SAR) studies revealed that the 2-azetidinone was required for activity, the C-(3) sidechain was optimal at three linking atoms bearing a pendent aryl group and the C-(4) aryl residue was required and was optimally substituted with a polar moiety at the para position, the N-aryl ring was also required and was tolerant of a wide variety of substitutions [8-10]. It is known that bioisosterism is an important lead modification approach that has been shown to be useful to attenuate toxicity or to modify the activity of a lead, and many have a significant role in the alteration of pharmacokinetics of a lead. In order to investigate the effect of the polarity of the C-(3) sidechain on cholesterol absorption inhibition, we used bioisosteric interchange and introduced amide group to the C-(3) carbon chain in compounds 2a-d, increasing the polarity of C-(3) sidechain. The relative configuration at C-(3) and C-(4) of compounds 2a-d were all (3R, 4S). On the other hand, another chemical modification of 1 in our research was electron-deficient pyridine ring and ester group to the C-(3) carbon chain in compounds 3a-d. As a result, the ezetimibe analogs 2a-d and 3a-d (Fig. 1) were designed, synthesized and their ability to inhibit cholesterol absorption was evaluated [11].

2. RESULTS AND DISCUSSION

2.1. Chemistry

The synthetic route to 2a-d is summarized in Scheme 1 [12]. Refluxing glutaric anhydride (4) with an equivalent amount of anhydrous MeOH afforded monomethyl glutarate (5), and treatment of 5 in refluxing SOCl2 yielded methyl 4-(chloroformyl)butyrate (6) in excellent yield 84.9% without further purification. Reactions of methyl 4-(chloroformyl)butyrate (6) with (S)-(+) 4-phenyl-2-oxazolidinone in the presence of Et3N in anhydrous CH2Cl2 at room temperature gave intermediate 7. Reaction of a substituted aromatic aldehyde 8 with a substituted aromatic amine 9 in refluxing isopropyl alcohol gave imines 10. Then, intermediate 7 was treated with TiCl4, Hüning’s base, and the corresponding imine 10 to give the intermediate β-aminoxazolidinone 11. The major diastereomer was purified to homogeneity by crystallization and then cyclized in two steps by first silylation with bistrimethylsilylacetamide.
(BSA) followed by treatment with a catalytic amount of tetrabutylammonium fluoride (TBAF) to get a single enantiomerically pure intermediate 12. Then hydrolysis and amidation of 12 led to enantiomerically pure 2-azetidinone analogs 2a-d.

The synthesis of target compounds 3a-d is summarized in Scheme 2. The intermediate 12 was used as starting material. Preparation of alcohol 14 was the key step in synthesis of target compounds because the β-lactam ring would be opened by reductive reagents. According to literature,
Scheme 2. Reagents and conditions: (a) LiOH, THF/H₂O, r.t, overnight; (b) NaBH₄, I₂, THF, reflux, 6h; (c) NaBH₄, THF, reflux, 24h; (d) substituted aromatic acid, DCC/DMAP, CH₂Cl₂, r.t, overnight.

β-lactam compound 15 and 17 could undergo reductive opening in the presence of sodium borohydride in methanol or isopropanol (Fig. 2) [13-14]. At beginning, methyl ester 12 was reduced by NaBH₄ in THF as solvent at room temperature for 5h. Unfortunately neither the ester-reducing product nor ring-opening product was obtained, even when the reaction was warmed to reflux. After that the solvent was changed to methanol according to literature [12]. It was observed that methyl ester 12 was transformed to alcohol 14 in yield of 12% along with the starting material in majority. The key intermediate 14 was not acquired from methyl ester 12 in good yield, but these results were also encouraging to us, since it was found that the β-lactam ring in our compounds were more stable comparatively, at least upon treatment with NaBH₄. It was known that reduction of carboxylic acids to alcohols was an important transformation in synthetic organic chemistry and several methods were available for this purpose such as NaBH₄-I₂ in THF. So methyl ester 12 was first hydrolyzed by LiOH-H₂O to give acid 13 in almost quantitative yield, and then reduced to obtain alcohol 13. In a typical procedure [15], the carboxylic acid 13 was added slowly to the suspension of NaBH₄ in THF and the mixture stirred until gas evolution ceased. Iodine in THF was then added slowly at room temperature and then the contents were warmed to reflux for 1.5-2 h. The reaction was terminated at once the starting material disappeared basically. After the usual workup, the alcohol 14 was obtained in ideal yield.

Finally the reaction of 14 with substituted aromatic acid in the presence of DCC/DMAP in anhydrous CH₂Cl₂ at room temperature gave 2-azetidinone derivatives 3a–d in good yields (60.8–66.4%).

![Scheme 2](image_url)

**Fig. (2).** Reductive opening of β-lactam ring.
Table 1. Structures, Yields, Melting Points and of the Target Compounds

| Compd.* | R₁ | R₂ | R₃ | Yield, % | mp, °C |
|---------|----|----|----|---------|--------|
| 2a      | 3,4-dioxolmethylene | 4-Me |  | 53.6 | 115−117 |
| 2b      | 3,4-dioxolmethylene | 4-Me | n-C₃H₇ | 41.8 | 235-237 |
| 2c      | 3,4-dioxolmethylene | 4-Me |  | 62.4 | 155-157 |
| 2d      | 4-OMe | 4-OMe |  | 65.0 | oil |
| 3a      | 3,4-dioxolmethylene | 4-Me |  | 52.1 | 98-101 |
| 3b      | 3,4-dioxolmethylene-6-bromo- | 4-Me |  | 42.6 | oil |
| 3c      | 3,4-dioxolmethylene | 4-Me |  | 52.3 | 120-122 |
| 3d      | 3,4-dioxolmethylene | 4-Me |  | 47.3 | 118-120 |

Table 2. Spectral Data of the Target Compounds

| Compound | Spectral Data |
|----------|---------------|
| 2a       | ¹HNMR (CDCl₃): 1.43-1.96 (m, 10H, -(CH₂)-), 2.03-2.23 (m, 2H, -(CH₂-CH₂-), 2.32 (s, 3H, Me), 2.52-2.65 (m, 2H, -(CH₂-CH₂-)), 3.06-3.11 (m, 1H, -(CH-CH-)), 4.60 (s, 1H, -(CHN)-), 3.66-3.68 (m, 1H, -(CH₃)), 5.94 (2H, s, -(OCH₂O)-), 6.77-6.87 (m, 3H, Ar-H), 7.03-7.17 (dd, 4H, Ar-H, J = 8.1Hz); MS (70eV) m/z: 435 ([M+H]+). Anal. calc. for: C₂₇H₃₂N₂O₄ (454.22): C 71.53 H 7.14 N 6.49; found: C 71.87 H 6.96 N 6.45; [α] = +11.4 (C=0.33, D:589nm, T=20°C, solv:MeOH) |
| 2b       | ¹HNMR (CDCl₃): 0.98-1.03 (m, 3H, -(CH₃)), 1.43-1.64 (m, 2H, -(CH₂CH₂-CH₂-), 2.35 (s, 3H, Me), 2.48-2.69 (m, 2H, -(CH₂CH₂), 2.53-2.59 (m, 2H, -(CH₂-CH₂-)), 3.24-3.43 (m, 1H, -(CH-CH-)), 4.87 (s, 1H, -(CHN)-), 5.94 (2H, s, -(OCH₂O)-), 6.57-6.67 (m, 3H, Ar-H), 6.95-7.18 (dd, 4H, Ar-H, J = 7.8Hz); MS (70eV) m/z: 417 ([M+H]+). Anal. calc. for: C₂₇H₃₂N₂O₄ (394.19): C 70.29 H 6.52 N 7.97; found: C 70.03 H 6.44 N 7.10; [α] = +11.4 (C=0.055, D:589nm, T=20°C, solv:MeOH) |
| 3a       | ¹HNMR (CDCl₃): 1.59−1.73 (m, 3H, -(CH₃)), 1.92-2.08 (m, 2H, -(CH₂CH₂-)), 2.29 (s, 3H, Ar-Me), 2.39-2.49 (m, 2H, -(CH₂-CH₂-)), 2.86-2.89 (m, 1H, -(CH₂CH₂), 3.48-3.51 (m, 1H, -(CH₃)), 5.95 (2H, s, -(OCH₂O)-), 6.56 (s, 1H, Ar-H), 7.01 (s, 1H, Ar-H), 7.14-7.30 (m, 9H, Ar-H); MS (70eV) m/z: 557, 559 ([M+H]+); Anal. calc. for: C₂₇H₃₂N₂O₄ (456.20): C 73.54 H 6.33 N 6.27; found: C 73.66 H 6.18 N 6.14; [α] = -19.8 (C=0.185, D:589nm, T=20°C, solv:MeOH) |
| 3b       | ¹HNMR (CDCl₃): 2.31 (s, 3H, -(CH₃)), 2.24-2.30 (m, 2H, -(CH₂-CH₂-)), 2.59-2.64 (m, 2H, -(CH₂-CH₂-)), 3.09-3.14 (m, 1H, -(CH₂)), 3.78 (s, 6H, -(OCH₂O)-), 4.66 (d, J = 2.0Hz, 1H, -(CHN)-), 6.76-6.85 (m, 4H, Ar-H), 7.19-7.27 (m, 4H, Ar-H), 7.28-7.40 (m, 4H, Ar-H), 7.79 (s, 1H, -(CH₃), -(CH₂CH₂-CH₂-)); MS (70eV) m/z: 445.4 ([M+H]+); Anal. calc. for: C₂₇H₃₂N₂O₄ (444.20): C 72.73 H 6.49 N 6.41; found: C 72.93 H 6.53 N 6.30; [α] = +11.3 (C=0.34, D:589nm, T=20°C, solv:MeOH) |
| 3c       | ¹HNMR (CDCl₃): 3.00 (m, 4H, -(CH₂)), 3.36-3.41 (m, 4H, -(CH₂)), 3.77-3.82 (m, 4H, -(CH₂)), 5.63-5.68 (m, 4H, Ar-H), 7.03-7.17 (4H, dd, Ar-H, J = 8.4Hz); MS (70eV) m/z: 435 ([M+H]+). Anal. calc. for: C₂₇H₃₂N₂O₄ (454.22): C 71.53 H 7.14 N 6.49; found: C 71.87 H 6.96 N 6.45; [α] = +11.4 (C=0.33, D:589nm, T=20°C, solv:MeOH) |
| 3d       | ¹HNMR (CDCl₃): 1.96-2.17 (4H, m, -(CH₂)), 2.26 (s, 3H, -(CH₃)), 3.11 (1H, m, -(CH₂CH₂)), 3.48-4.40 (2H, t, -(CH₂O)-), J = 5.4Hz); 4.57 (1H, d, -(CHN)-, J = 2.0Hz), 5.95 (2H, s, -(OCH₂O)-), 6.77-6.85 (3H, m, Ar-H), 7.03-7.19 (4H, dd, Ar-H, J = 8.4Hz); 7.35-7.42 (1H, m, Py -H); 8.27-8.30 (1H, d, Py -H', J = 8.4Hz); 8.70-8.83 (1H, m, Py -H); 9.21 (1H, s, Py -H'); MS (70eV) m/z: [M+H+] 445, [M+Na+] 467, [M+K+] 483; Anal. calc. for: C₂₇H₃₂N₂O₄ (444.17): C 70.53 H 5.27 N 6.19; found: C 70.26 H 5.44 N 6.30; [α] = +15.6 (C=0.215, D:589nm, T=20°C, solv:MeOH) |
The structures and spectral characteristics of the target compounds 2a-d and 3a-d were mentioned in Table 1 and Table 2.

2.2. Biological Studies

Cholesterol absorption inhibition was assessed in orally dosed, cholesterol-fed hamsters as reported in literature [16]. The result is presented in the Table 3. As can be seen from the data, most of the new compounds demonstrated moderate effect in lowering the total cholesterol in serum, especially compound 2c 2d 3a and 3b, although their potency was still somewhat below that of ezetimibe. Compound 2a and 2b have no effect in lowering the total cholesterol in serum. It was also found that 3a and 3b could raise high-density lipoprotein cholesterol (HDL-C) levels markedly. This activity may be good for prevention and treatment of CHD, as the increased HDL-C may be crucial for raising high-density lipoprotein cholesterol. These SAR trends may provide insights into the further design of novel cholesterol absorption inhibitors.

3. CONCLUSIONS

In an effort to understand the SAR around cholesterol absorption inhibition, eight 2-azetidinone derivatives were synthesized and all of them were enantiomERICALLY pure. Their cholesterol absorption inhibition activities were evaluated.

Most of them showed comparable effects in lowering the levels of total cholesterol in the serum. These information could be valuable for further investigation of SAR and will be useful in later research of cholesterol absorption inhibitors.

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