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Bioorganic & Medicinal Chemistry Letters 17 (2007) 101–104
Bioorganic & Medicinal Chemistry Letters

Ezetimibe analogs with a reorganized azetidinone ring: Design, synthesis, and evaluation of cholesterol absorption inhibitions

Xianxiu Xu,[a,b] Renzhong Fu,[a] Jin Chen,[a] Shengwu Chen[c] and Xu Bai[a,b,*]

[a]*The Center for Combinatorial Chemistry and Drug Discovery, Jilin University, 75 Haiwai Street, Changchun, Jilin 130012, PR China*
[b]*Changchun Discovery Sciences, Ltd, 75 Haiwai Street, Changchun, Jilin 130012, PR China*
[c]*Jilin Natural Pharmatech Co., Ltd, Changchun, Jilin 130012, PR China*

Received 31 May 2006; revised 23 September 2006; accepted 28 September 2006
Available online 30 September 2006

Abstract—The underlying principle of drug design in this paper is that the maximum retention of the functional groups that exist in the marketed drug would provide a higher probability for comparable safety while the conformational changes in the newly created analogs should not constitute a significant structural variation to adversely affect biological activity. Four individual isomers of backbone re-organized ezetimibe analogs were designed and synthesized. Their effects on the cholesterol levels in rat serum were evaluated by a high-cholesterol and high-fat diet feeding experiment. All the new analogs showed significant effect in lowering the levels of total cholesterol in serum.

Atherosclerotic coronary heart disease (CHD) remains a major concern in healthcare due to its high morbidity and mortality.[1] Lowering the level of cholesterol in blood has been shown to be an effective way to treat and prevent CHD.[2] There are two recognized sources of cholesterol in the serum: biosynthesis in the liver and absorption of dietary cholesterol in the small intestine.[3,4] Statins have been prescribed as the predominant class of cholesterol-lowering agents since 1980s.[5] They inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the catalyst of the rate-limiting step of cholesterol biosynthesis in liver.[6] Recently, ezetimibe (Fig. 1, **1**), a blocker of intestinal sources of cholesterol, has become an increasingly important choice for reducing serum cholesterol level. It inhibits the absorption of dietary or recycled cholesterol in the intestine and can be used either alone or in combination with a statin. Thus far, ezetimibe is the only marketed example of this new class of anti-cholesterolemia drugs.[7–9]

Ezetimibe was discovered through in vivo screening utilizing an animal-feeding model to identify active compounds. Recently, many reports on the potential molecular target of ezetimibe have been disclosed. Evidence suggests that the compound interacts with the Niemann Pick C1-like 1 (NPC1L1) protein which plays a critical role in cholesterol transporting through the intestine wall.[10] Despite the absence of a molecular target the SAR studies on azetidinone structures have been thoroughly investigated. Very recently, it has been disclosed that the azetidinone backbone possibly plays the role of a structural scaffold to appropriately position the required ring substituents since the mesylated azetidine analog (Fig. 1, **2**) showed comparable bioactivity by an in vitro assay.[11] This study could further encour-

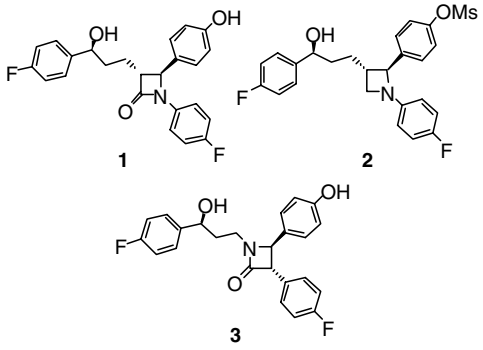

Figure 1. The structures of ezetimibe **1**, its azetidine analog **2**, and reorganized analogs **3**.

Keywords: Backbone re-organized ezetimibe analogs; Azetidin-2-one; Cholesterol absorption inhibition.
* Corresponding author. Tel.: +86 431 5188955; fax: +86 431 5188900; e-mail: xbai@jlu.edu.cn

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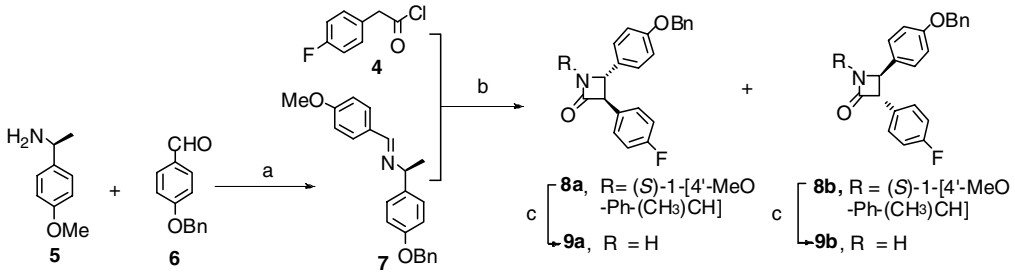
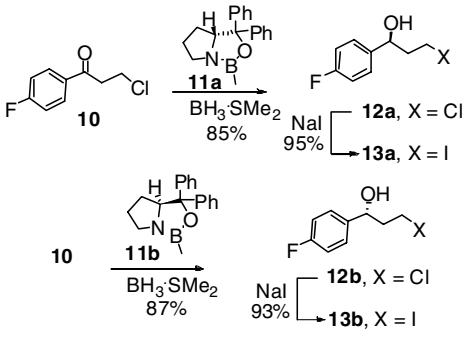
doi:10.1016/j.bmcl.2006.09.078



Scheme 1. Synthesis of *trans*-azetidinone fragments. Reagents and conditions: (a) toulene, reflux; (b) Et_3N, toluene, 47% for **8a**, 26% for **8b** in two steps; (c) CAN, 53% for **9a**, 55% for **9b**.

age the search for new cholesterol absorption inhibitors away from the traditional azetidinone structures. We have been interested in discovering compounds that inhibit cholesterol absorption by exploring ezetimibe analogs in which the substituents are regiochemically rearranged on the azetidinone scaffold (Fig. 1, **3**). This paper discloses results of the design, synthesis, and in vivo evaluation of cholesterol absorption inhibition.

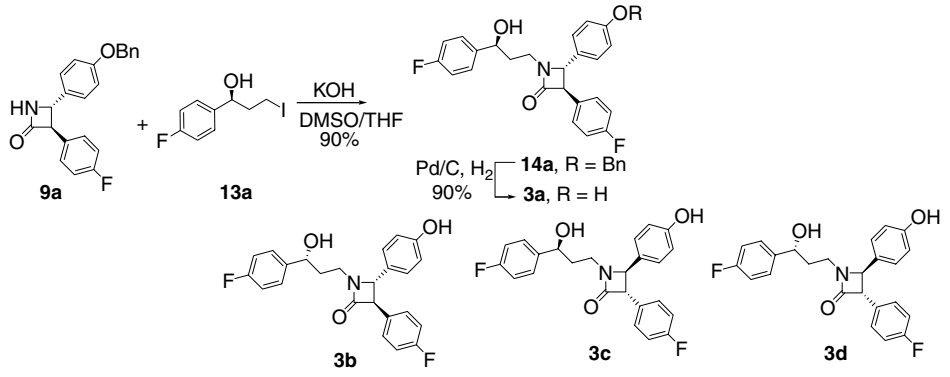
As reported in the literature, the pharmacophore of ezetimibe has the following requirements: (1) azetidinone as the required backbone; (2) *N*-phenyl is required, but could tolerate a range of substituents; (3) the phenyl tethered with three-carbon chain at 3-position is necessary for activity; (4) *p*-hydroxyl or *p*-methoxyphenyl at 4-position is a necessity; (5) unlike the chiral center at C-4 which shows a clear preference for *S* configuration, both 3*S* and 3*R* forms often demonstrate comparable activity without consistent preference. These SAR results, in particular the last point regarding the stereochemistry at 3-position prompted us to consider swapping the substituents between the *N*-position and the 3-position. The underlying principle is that the maximum retention of the functional groups that exist in the marketed drug would provide a higher probability for comparable safety while the conformational changes in the newly created backbone should not constitute a significant structural variation to adversely affect biological activity. As a result, the re-organized ezetimibe analog **3** (Fig. 1) was devised.



Scheme 2. Synthesis of alcohol fragments.

Four individual isomers of **3** with *trans* configuration of the azetidinone backbone were prepared according to Schemes 1–3. Scheme 1 depicts the synthesis of *trans*-azetidinone fragments in optically active forms. Commercially available *S*-1-(4-methoxyphenyl) ethanamine **5** was treated with 4-(benzyloxy)benzaldehyde **6** in refluxing toluene to give imine **7**. Reactions of acid chloride **4** and imine **7** using Et_3N as the base in refluxing toluene led to diastereoisomers **8a** and **8b** with a ratio of 2 to 1 in 73% combined yield over two steps. Diastereoisomers **8a** and **8b** were readily separated by flash chromatography. The oxidative removal of the chiral benzylamine group by ceric ammonium nitrate (CAN) gave more than 50% of the desired products **9**.

The optically active alcohol fragments **13a** and **13b** were prepared by a modified literature procedure[12] as depicted in Scheme 2. Both optically pure enantiomers of CBS catalyst **11** were obtained by Corey's procedure.[13]



Scheme 3. Synthesis of final targets.

Table 1. Physical properties of backbone re-organized ezetimibe analogs

	3a	3b	3c	3d
^{1}H NMR (300 MHz, $CDCl_3$)	1.85–1.92 (m, 2H), 2.93–3.02 (m, 1H), 3.70–3.80 (m, 1H), 4.14 (d, 1H, J = 2.1 Hz), 4.41 (d, 1H, J = 2.1 Hz), 4.69–4.73 (m, 1H), 6.89 (d, 2H, J = 8.4 Hz), 6.97–7.07 (m, 4H), 7.18–7.29 (m, 6H)	1.83–1.92 (m, 2H), 2.76 (d, 1H, OH, J = 3.9 Hz), 3.16–3.25 (m, 1H), 3.45–3.54 (m, 1H), 4.14 (d, 1H, J = 2.1 Hz), 4.43 (d, 1H, J = 2.1 Hz), 4.74–4.79 (m, 1H), 5.43 (s, 1H, OH), 6.88 (d, 2H, J = 8.4 Hz), 6.98–7.07 (m, 4H), 7.21–7.31 (m, 6H)	1.83–1.91 (m, 2H), 3.18–3.25 (m, 1H), 3.46–3.53 (m, 1H), 4.14 (d, 1H, J = 2.1 Hz), 4.43 (d, 1H, J = 2.1 Hz), 4.75–4.79 (m, 1H), 6.87 (d, 2 H, J = 8.1 Hz), 6.97–7.07 (m, 4H), 7.20–7.33 (m, 6H)	1.86–1.93 (m, 2H), 2.94–3.02 (m, 1H), 3.09 (d, 1H, OH, J = 3.6 Hz), 3.71–3.80 (m, 1H), 4.15 (d, 1H, J = 2.1 Hz), 4.41 (d, 1H, J = 2.1 Hz), 4.69–4.75 (m, 1H), 5.69 (s, 1H, OH), 6.90 (d, 2H, J = 8.7 Hz), 6.98–7.08 (m, 4H), 7.19–7.30 (m, 6H)
^{13}C NMR (75 MHz, DMSO-d^6)	37.78, 37.86, 62.85, 63.72, 70.09, 115.41 (d, J = 21.23 Hz), 116.22 (d, J = 20.03 Hz), 116.38, 128.21 (d, J = 8.03 Hz), 128.22, 128.70, 130.08 (d, J = 8.03 Hz), 132.53 (d, J = 2.85 Hz), 142.49 (d, J = 2.33 Hz), 158.34, 161.80 (d, J = 240.98 Hz), 162.11 (d, J = 241.58 Hz), 168.13	37.86, 38.10, 63.18, 63.59, 70.14, 115.39 (d, J = 20.63 Hz), 116.21 (d, J = 18.3 Hz), 116.33, 128.25 (d, J = 8.03 Hz), 128.30, 128.74, 130.08 (d, J = 8.18 Hz), 132.51 (d, J = 2.25 Hz), 142.40 (d, J = 2.25 Hz), 158.34, 161.79 (d, J = 241.5 Hz), 162.10 (d, J = 241.5 Hz), 168.18.	37.87, 38.10, 63.18, 63.59, 70.14, 115.40 (d, J = 20.63 Hz), 116.24 (d, J = 21.23 Hz), 116.33, 128.25 (d, J = 7.5 Hz), 128.30, 128.75, 130.09 (d, J = 8.03 Hz), 132.55 (d, J = 2.85 Hz), 142.39 (d, J = 2.33 Hz), 158.34, 161.80 (d, J = 240.98 Hz), 162.10 (d, J = 242.10 Hz), 168.17	37.78, 37.85, 62.84, 63.71, 70.07, 115.40 (d, J = 20.55 Hz), 116.20 (d, J = 19.5 Hz), 116.33, 128.21 (d, J = 6.9 Hz), 128.25, 128.68, 130.08 (d, J = 8.03 Hz), 132.51, 142.48, 158.32, 161.79 (d, J = 240.38 Hz), 162.10 (d, J = 241.5 Hz), 168.10
Mp (°C)	65.8–67.3	150–151.3	149–150.8	67.6–68.5
MS $[M+H-18]^+$	392.2	392.2	392.2	392.2
RT (min)	3.042	3.021	3.028	3.037
$[\alpha]_D^{20}$ (c = 1, MeOH)	+90.2°	+128.2°	−127.4°	−90.2°

Ketone **10** was reduced by BH_3SMe_2 in presence of CBS catalyst **11a** or **11b** to give the corresponding *S*-3-chloro-1-(4-fluorophenyl)propan-1-ol **12a** and **12b** in good yields, respectively. Iodine exchange of **12a** and **12b** provided *S*-3-iodo analog **13a** and *R*-isomer **13b** in high yields.

Alkylation of precursors **9** with **13** in the presence of KOH in DMSO/THF at room temperature provided the final four target compounds **14a–d**, respectively, in high yields, which were debenzylated with 1 atm H_2 and Pd/C to provide **3a–d** (Scheme 3). The physical data of compounds **3a–d** are listed in Table 1.

The absolute configurations of final compounds **3a–d** were elucidated by combination of X-ray diffraction analysis and chemical correlations. The stereochemical structures of **3a** and intermediate **8b** were determined by X-ray analysis.[14,15] Since the chiral center in the side chain is *S* (known from starting amine **5**) the rest of the chiral centers in **8b** were easily deduced based on its X-ray structure. Therefore the chiral centers in **8b** are 3*R*, 4*S*, and 1′*S* as shown in Figure 2. Consequently the azetidinone moiety in **3a** must be 3*S*, 4*R* (The opposite to those of **8b**), and the stereochemistry of **3a** are (3*S*, 4*R*, 3′*S*) as shown by its X-ray structure in Figure 3.

The new analogs **3a–d** were subjected to a cholesterol-feeding experiment in rats. The results are summarized in Table 2. All the new compounds demonstrated signif-

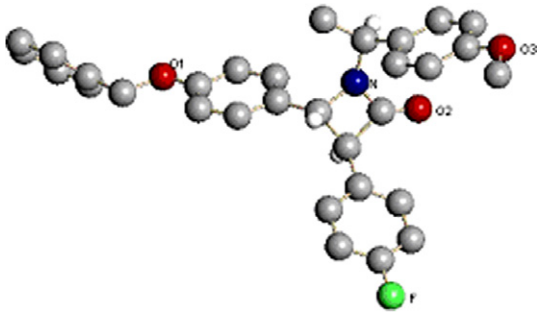


Figure 2. The X-ray structure of **8b** (3*R*, 4*S*, 1′*S*).

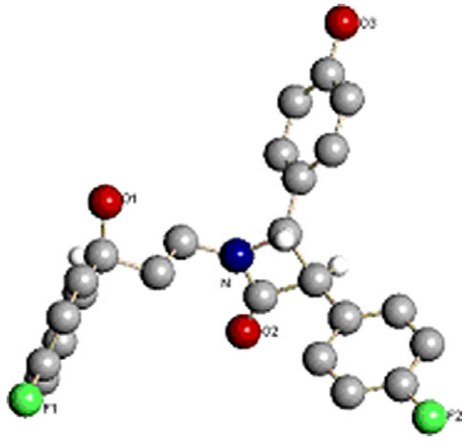


Figure 3. The X-ray structure of **3a** (3*S*, 4*R*, 3′*S*).

Table 2. Results of in vivo experiments[16]

Entry	Animal group[a]	TC or Changes (%)[b]	LDL-cho or Changes (%)[b]
1	High-cholesterol diet	5.40 ± 2.25	2.93 ± 1.54
2	Normal diet	$-62^{***,c}$	-59^{***}
3	ezetimibe	-51^{***}	-47^{**}
4	**3a**	-26^{*}	−22
5	**3b**	−17	−5
6	**3c**	-25^{*}	-29^{*}
7	**3d**	−18	−35

$^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$.

[a] 8–18 rats per group.

[b] Percentile was calculated by comparing to the one in animals fed by high-cholesterol diets.

[c] Compared to high-cholesterol diet.

icant effect in lowering the total cholesterol and LDL (**3b** is the exception) in serum. These SAR trends may provide insights into the further design of novel cholesterol absorption inhibitors.

In conclusion, four individual isomers of backbone reorganized ezetimibe analogs were designed and synthesized. Their effects on the cholesterol levels in rat serum were evaluated by a high-cholesterol and high-fat diet-feeding experiment. All of the new compounds showed significant effects in lowering the levels of total cholesterol in serum. This information could be valuable in designing new cholesterol absorption inhibitors.

Acknowledgments

The authors wish to express gratitude to Mr. Guangdi Yang, Ms. Ling Ye, and Dr. Mao Li for conducting X-ray analysis of products **3a** and **8b**. This work was partially supported by a Grant (No. 2005132) from the Bureau of Science and Technology of Changchun City.

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14. CCDC 620888 contains the supplementary crystallographic data for **8b**. These data can be obtained free of charge from The Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/data_request/cif.
15. CCDC 620889 contains the supplementary crystallographic data for **3a**. These data can be obtained free of charge from The Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/data_request/cif.
16. Cholesterol-feeding experiment: Male Wistar rats with weight of 150–180 g were purchased from the Center of Research Animals, the College of Basic Medicines, Jilin University. The high-fat and high-cholesterol diet was made from 87.8% regular feeds, 2% cholesterol, 10% hog fat, and 0.2% propylthiouracil. The animals were raised for 1 week, divided into individual groups according to their levels of TC and LDL-cholesterol in serum, and then fed for 2 weeks on regular diet, high-fat and high-cholesterol diet, high-fat and high-cholesterol diet with ezetimibe or individual drugs, respectively. The drugs were made to a mixture with 0.5% sodium carboxy methyl cellulose(40 mg/100 ml), administered at a single dosage of 4 mg/kg/day by intragastric perfusion. After fasted for 16 h, blood samples were collected by cutting the tails, and the serum was isolated and measured for the level of total cholesterol and LDL-cholesterol. The data were calculated by statistical methods.