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Original article

2-Azetidinone derivatives: Design, synthesis and evaluation of cholesterol absorption inhibitors

Yubin Wang [a], Huibin Zhang [a], Wenlong Huang [a,*], Jing Kong [b], Jinpei Zhou [a], Beibei Zhang [c]

[a] New Drug Research Center, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China
[b] Department of Pharmacology, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China
[c] Jiangsu Environmental Monitoring Center, 241 Fenghuangxijie, Nanjing 210036, China

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ABSTRACT

Fourteen new derivatives of the 2-azetidinone cholesterol absorption inhibitors have been synthesized, and three of them were enantiomerically pure. All the new compounds were evaluated for their activity to inhibit cholesterol absorption in rats, and most of them showed comparable effects in lowering the levels of total cholesterol in the serum.

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–e**, increasing the polarity of C-(3) sidechain. The relative configuration at C-(3) and C-(4) of compounds **2a–e** were all *trans*. In order to acquire further insights into structure–activity relationships (SAR), enantiomerically pure 2-azetidinone **4a–c** were also synthesized by using (*S*)-(+)-4-phenyl-2-oxazolidinone. The configuration at C-(3) and C-(4) of compounds **4a–c** were all (3*R*,4*S*). On the other hand, another chemical modification of **1** in our research was aimed at investigating the hydrophobic requirements for their cholesterol absorption inhibition ability. Because

* Corresponding author. Tel./fax: +86 25 83271480.
E-mail address: hktkcpu@126.com (W. Huang).

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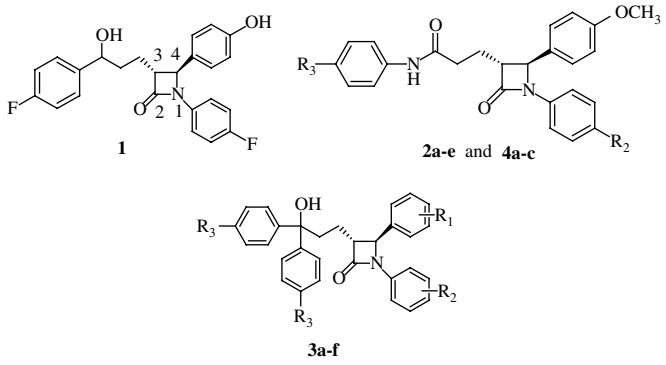


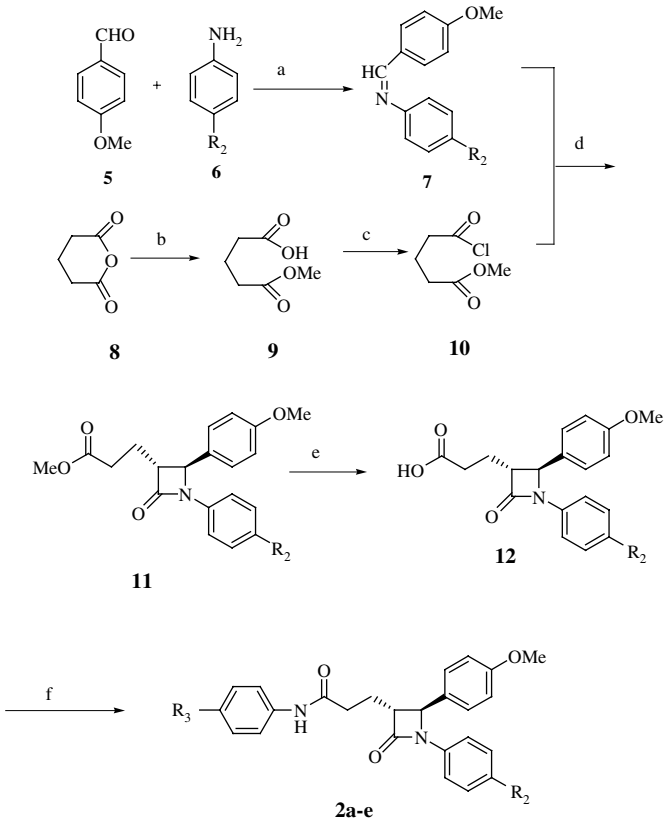
Fig. 1.

hydrophobic forces may be the most important single factor responsible for interaction between drug and receptor. Hydrophobicity could be increased by introducing another substituted phenyl group to C-(3) carbon chain in **4a–c**. In this way, the binding of CAIs to their target protein may be enhanced and may be helpful to their cholesterol absorption inhibition activity. As a result, the ezetimibe analogs **2a–e**, **3a–f** and **4a–c** (Fig. 1) were designed, synthesized and their ability to inhibit cholesterol absorption was evaluated.

2. Results and discussion

2.1. Chemistry

The synthetic route to **2a–e** is summarized in Scheme 1 [8]. Reaction of 4-methoxybenzaldehyde (**5**) with a substituted aromatic amine **6** in refluxing isopropyl alcohol gave imines **7**. Refluxing glutaric anhydride (**8**) with an equivalent amount of anhydrous MeOH afforded monomethyl glutarate (**9**), and treatment of **9** in refluxing $SOCl_2$ yielded methyl 4-(chloroformyl)butyrate (**10**) in excellent yield 84.9% without further purification. A preferred *trans* configuration of 2-azetidinone intermediate **11** was established by modification of the ketene–imine reaction. Compound **10** was added to a refluxing solution of imine **7** in anhydrous toluene in the presence of tri(*n*-butyl)amine. Maintaining the mixture refluxing overnight, gave 2-azetidinone intermediate **11**. Hydrolysis of **11** with LiOH solution affords acid **12** in almost quantitative yield. Finally the reaction of **12** with substituted aromatic amine in the presence of DCC/DMAP in anhydrous CH_2Cl_2 at room temperature gave 2-azetidinone derivatives **2a–e** in good yields (60.8–66.4%). In the ^1H NMR spectra the coupling constants between H-C(3) and H-C(4) of compounds **2a–e** were about 2 Hz, indicating that the relative configuration at C-(3) and C-(4) of compounds **2a–e** were all *trans* ($J = \sim 2$ Hz is characteristic for *trans*-coupling between H-C(3) and H-C(4), whereas $J = 5$–6 Hz corresponds with the *cis*-configuration [11]).



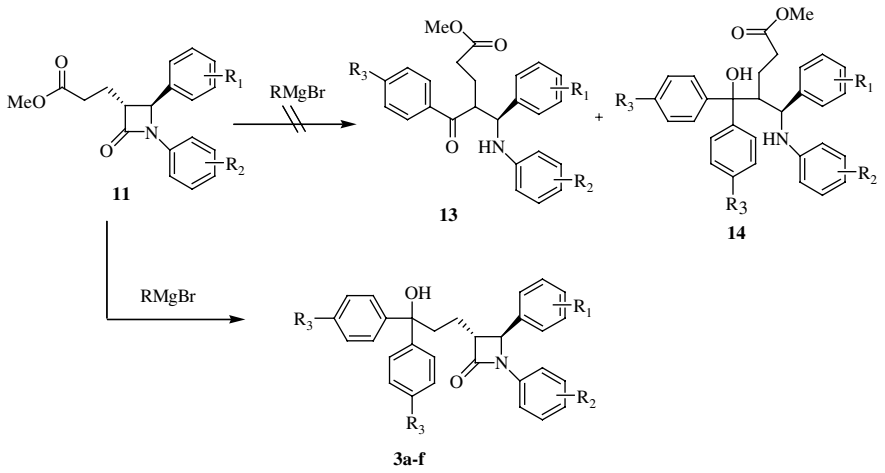
Scheme 1. Reagents and conditions: (a) *i*PrOH, reflux; (b) CH_3OH, reflux; (c) $SOCl_2$, reflux; (d) *n*-Bu_3N, toluene, reflux; (e) LiOH, THF/H_2O, r.t.; (f) substituted aromatic amine, DCC/DMAP, CH_2Cl_2, r.t.

Compounds **3a–f** were obtained by reaction intermediate **11** with various Grignard reagents (Scheme 2). According to literature [12], upon treatment of the β-lactam compound with aryl Grignard reagents in ether as solvent at −40 °C, the β-lactam would be opened by Grignard reagents (Fig. 2). But the expected open product β-amino ketones **13** and β-amino carbibols **14** were not obtained in our research, even upon treatment with 4-fold excess of Grignard reagents. The reaction invariably led to the corresponding tertiary alcohols **3** and the starting β-lactam **11**. We attributed this to the electronic features of substituents on the β-lactam nitrogen atom. When electron withdrawing groups were connected to nitrogen atom in 1-position of the β-lactam ring, such as carbonyl in Ref. [12], the β-lactam ring could be easily opened by Grignard reagents because of large ring strain in the four-membered ring. While the substituent on the β-lactam nitrogen was electron donating, such as an aromatic ring, the β-lactam ring was stable to Grignard reagents, and the ester group in the 3-C sidechain was able to react with Grignard reagents smoothly. Curiously, when the same reaction was carried out in tetrahydrofuran as solvent, neither the ring-opened product nor the ester-addition product was obtained and the starting material was recovered unchanged. It was unexpected that the solvent has such a dramatic effect on this reaction.

In order to acquire further insights into structure–activity relationships (SAR), enantiomerically pure 2-azetidinone **4a–c** were also synthesized by using (*S*)-(+)-4-phenyl-2-oxazolidinone. Our synthesis of the enantiomerically pure 2-azetidinone analogs **4a–c** were based on an asymmetric synthesis of ezetimibe as depicted in Scheme 3 [13]. Reactions of methyl 4-(chloroformyl)butyrate (**10**) with (*S*)-(+)-4-phenyl-2-oxazolidinone (**15**) in the presence of Et_3N in anhydrous CH_2Cl_2 at room temperature gave intermediate **16**. Then, **16** was treated with $TiCl_4$, Hünig's base, and the corresponding imine **7** to give the intermediate β-aminoxazolidinone **17**. 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 **18**. Then hydrolysis and amidation of **18** led to enantiomerically pure 2-azetidinone analogs **4a–c**.

2.2. Biological studies

Cholesterol absorption inhibition was assessed in orally dosed, cholesterol-fed hamsters as reported in literature [14]. The result is presented in the Table 1. As can be seen from the data, most of the



Scheme 2. Reagents and conditions: R_3MgBr, Et_2O, reflux.

new compounds demonstrated moderate effect in lowering the total cholesterol in serum, especially compound **4a** and **4c**, although their potency was still somewhat below that of ezetimibe. It was also found that **3c**, **3d**, **3e** and **3f** could raise high-density lipoprotein cholesterol (HDL-C) levels markedly. This activity may be good for prevention and treatment of CHD. The current work suggests that the amide group in the C-(3) sidechain was not critical for the cholesterol absorption inhibition activity and an increase of compunds hydrophobicity may lead to the elevation of HDL-C. 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, fourteen 2-azetidinone derivatives were synthesized and 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.

4. Experimental

4.1. Chemistry

4.1.1. General

All reagents were purchased from Shanghai Chemical Reagent Company. Column chromatography (CC): silica gel 60 (200–300 mesh). Thin-layer chromatography (TLC): silica gel 60 F254 plates (250 mm; Qingdao Ocean Chemical Company, China). M.p.: capillary tube; uncorrected. IR spectra: Shimadzu FTIR-8400S spectrophotometer; in cm^{-1}. ^1H NMR spectra: Bruker ACF-300Q apparatus at 300 MHz, in $CDCl_3$ unless otherwise indicated; δ in ppm rel. to Me_4Si, J in Hz. Mass spectrometry (MS): Hewlett–Packard 1100 LC/MSD spectrometer; in m/z. Elemental analyses: CHN-O-Rapid instrument.

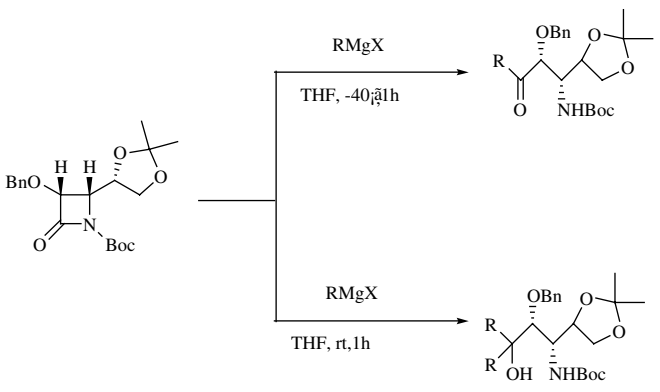
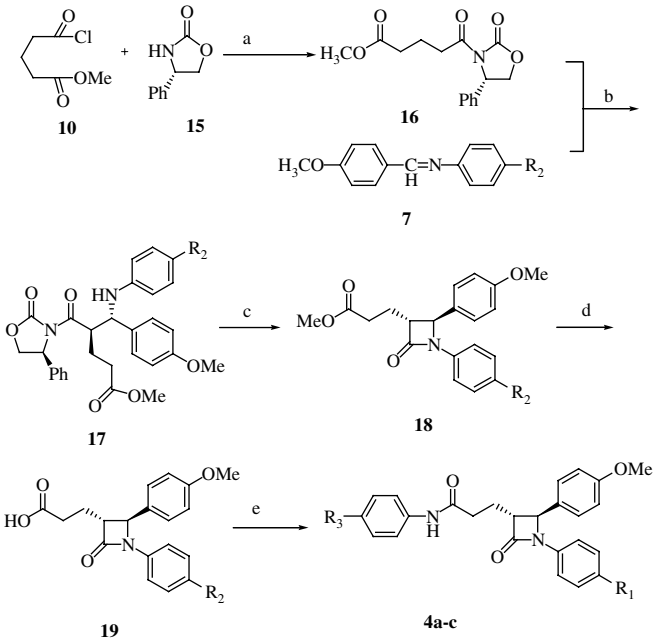


Fig. 2.

4.1.2. General procedure for the preparation of ***2a–e***

A mixture of the appropriate 4-methoxybenzaldehyde (**5**) (10 mmol) and substituted aromatic amine **6** (10 mmol) in isopropanol (40 mL) was heated to reflux, cooled to room temperature, and diluted with hexanes and allowed to stand overnight. The resulting precipitate was collected via vacuum filtration, washed with cold hexanes and dried under vacuum to give imines **7**. Refluxing glutaric anhydride (**8**) (0.44 mol) with equivalent anhydrous methanol (30 mL) affords monomethyl glutarate (**9**) and then treatment of **9** in refluxing $SOCl_2$ (100 mL) gave methyl 4-(chloroformyl)butyrate (**10**) (0.37 mol) in good yield (84.9%) without further purification. Compound **10** (77 mmol) was added to refluxing solution of imine **7** (38 mmol) in anhydrous toluene (150 mL) in the presence of *n*-tributylamine (114 mmol). The mixture was heated to reflux overnight, cooled to room temperature, 1 M HCl was added, the resulting mixture was stirred for 15 min, transferred to a separatory funnel, diluted with ethyl acetate, washed with 1 M HCl, $NaHCO_3$(satd), water and brine, dried over anhydrous sodium sulfate, and concentrated to an oil. The resulting oil was loaded onto a chromatography column prepacked with silica gel and 20% ethyl acetate/hexane. Elution with the same solvent provided *trans* 2-azetidinone intermediate **11**. Lithium hydroxide (0.82 g, 19.6 mmol) was dissolved in water (20 mL) and added to a room temperature solution of *trans* 2-azetidinone intermediate **11** (16.3 mmol) in THF (60 mL). After 6 h, the reaction was quenched with HCl (1 M), transferred to a separatory funnel, diluted with ethyl acetate, washed with HCl (1 M), water and brine, dried over anhydrous sodium sulfate, and concentrated to give acid **12** of sufficient purity to be used without further purification. To a stirring solution of acid **17** (5 mmol), 4-dimethylaminopyridine (0.25 mmol) and substituted aromatic amine (5.5 mmol) in dry CH_2Cl_2 (40 mL) under N_2 was added dicylohexylcarbodimide (5.5 mmol). The mixture was stirred at room temperature for 36 h and the solids were filtered. The filtrate was concentrated in vacuum and the residue was chromatographed (15% ethyl acetate/hexane). Initially target compounds **2a–e** were obtained.



Scheme 3. Reagents and conditions: (a) Et_3N, CH_2Cl_2, r.t.; (b) $TiCl_4$, DIPEA, CH_2Cl_2, −30 to −40 °C;(c) BSA, TBAF, toluene, 40–50 °C; (d) LiOH, THF/H_2O, r.t.; (e) 4-methylaniline, DCC/DMAP, CH_2Cl_2, r.t.

*4.1.3. General procedure for the preparation of **3a–f***

Compound **11** (5 mmol) resolved in 150 ml ether was added to refluxing Grignard reagents R_3MgBr (12.5 mmol). The mixture was refluxing for 12 h, cooled to room temperature, 1 M HCl was added, transferred to a separatory, diluted with ethyl acetate, washed with $NaHCO_3$ (satd), water and brine, dried over anhydrous sodium sulfate, and concentrated to oil. The resulting oil was loaded onto a chromatography column prepacked with silica gel and 10% ethyl acetate/hexane. Initially target compounds **3a–f** were obtained.

*4.1.4. General procedure for the preparation of **4a–c***

(*S*)-4-phenyl-2-oxazolidinone (**15**) (20 g, 0.12 mol) in CH_2Cl_2 (20 ml) was added 4-dimethylaminopyridine (1.2 g, 0.01 mol) and Et_3N (42.3 ml, 0.31 mol) and cooled the reaction to 0 °C. Then, a solution of methyl 4-(chloroformyl)butyrate (**10**; 20 g, 0.12 mol) in CH_2Cl_2 (150 ml) was added dropwise over 1 h and the mixture was heated to reflux. After 12 h, H_2O and H_2SO_4 (2 N, 75 ml) were added, separated the layers and washed the organic layer sequentially with NaOH (10%), NaCl (satd) and water. Dried the organic layer over $MgSO_4$ and concentrated to obtain solid product **16**. To a solution of $TiCl_4$ (3 ml, 27.5 mmol) in CH_2Cl_2 (100 ml) at −10 °C added compound **16** (8.2 g, 28.3 mmol) as a solution in CH_2Cl_2 (50 ml). After 5 min. added diisopropylethylamine (DIPEA) (11 ml, 61 mmol) and stirred at −20 °C. for 1 h. Cooled the reaction mixture to −35 °C, and added imine **7** (61 mmol) as a solid. Stirred the reaction vigorously for 4 h. at −30 °C. Added acetic acid as a soln. in CH_2Cl_2 dropwise over 15 min., allowed the reaction to warm to 0 °C and added H_2SO_4 (2 N). Stirred the reaction an additional 1 h, separated the layers, washed with water, separated and dried the organic layer. Crystallized the crude from ethanol to obtain the pure intermediate **17**. To a solution of **17** (7.5 mmol) in toluene (50 mL) at 60 °C added *N*,*O*-bis(trimethylsilyl)acetamide (BSA) (3.6 ml, 15.2 mmol) and stirred the reaction at 60 °C. for an additional 3 h. Cooled the reaction mixture to room temperature, added CH_3OH (5 ml), washed the reaction mixture with HCl (1 N), $NaHCO_3$(1 N) and NaCl (satd), dried the organic layer over $MgSO_4$, and concentrated to an oil. The resulting oil was loaded onto a chromatography column prepacked with silica gel and 20% ethyl acetate/hexane. Elution with the same solvent provided a single enantiomerically pure intermediate **18**. Lithium hydroxide (0.82 g, 19.6 mmol) was dissolved in water (20 ml) and added to a room temperature solution of enantiomerically pure intermediate **18** (16.3 mmol) in THF (60 ml). After 6 h, the reaction was quenched with HCl (1 M), transferred to a separatory funnel, diluted with ethyl acetate, washed with HCl (1 M), water and brine, dried over anhydrous sodium sulfate, and concentrated to give acid **18** of sufficient purity to be used without further purification. To a stirring solution of acid **18** (5 mmol), 4-dimethylaminopyridine (0.25 mmol) and substituted aromatic amine (5.5 mmol) in dry CH_2Cl_2 (40 mL) under N_2 was added dicyclohexylcarbodimide (5.5 mmol). The mixture was stirred at room temperature for 36 h and the solids were filtered. The filtrate was concentrated in vacuum and the residue was chromatographed (15% ethyl acetate/hexane). Initially target compounds **4a–c** were obtained.

*4.1.5. Trans-*N*-(4-Methylphenyl)-3-(3-[2-oxo-4-(4-methoxypheyl)-1-(4-methoxyphenyl)-azetidinyl])propanamide (**2a**)*

Yield: 43.2%. Yellow crystals, m.p. 56–57 °C. IR (KBr): 3494, 2930, 1731, 1715, 1512, 1295, 1247, 1173, 1030. ^{1}H NMR ($CDCl_3$): 2.39 (s, 3H, $-CH_3$), 2.26–2.45 (m, 2H, $-CH_2-CH_2-$), 2.62 (t, $J = 7.2$ Hz, 2H,

Table 1
Cholesterol absorption inhibition of new analogs and reference compounds in orally dosed seven-day cholesterol-fed hamsters.

Compound[a]	R_1	R_2	R_3	TC[b] (% reduction)	HDL-C[c] (% increase)
2a	–	MeO	Me	20.9	12.4
2b	–	H	CH_3	NE[d]	13.9
2c	–	CH_3	Cl	26.3*	17.8
2d	–	CH_3	CH_3	18.7	23.9
2e	–	MeO	Cl	16.3	20.1
3a	3,4-Dioxolmethylene	4-Cl	H	28.7*	11.4
3b	4-OMe	6-Me	H	34.8**	14.5
3c	4-OMe	4-F	H	16.3	35.0**
3d	3,4-Dioxolmethylene	6-Me	F	10.4	24.8
3e	4-OMe	4-Br	F	11.2	32.9**
3f	3,4-Dioxolmethylene-6-bromo-	4-OMe	F	19.8	31.8**
4a	–	MeO	Me	28.1*	21.7
4b	–	H	CH_3	NE	22.1
4c	–	CH_3	Cl	31.6*	13.7
Ezetimibe	–	–	–	48.7**	37.3**

**$P < 0.01$; *$P < 0.05$.

[a] 6–8 Hamsters per group; dose: 50 mg/kg.

[b] Reduction of total cholesterol comparing to the one in animals fed by high-cholesterol diets.

[c] Increase of HDL-C comparing to the one in animals fed by high-cholesterol diets.

[d] NE: no effect.

–CH_2–CH_2–), 3.09–3.11 (m, 1H, –CH–CH–), 3.74 (s, 6H, –OCH_3), 4.66 (d, $J = 2.3$ Hz, 1H, –CHN–), 6.80 (dd, $J = 8.4$, 17.9 Hz, 4H, Ar-H), 7.21–7.27 (m, 4H, Ar-H), 7.32–7.36 (m, 4H, Ar-H), 7.79 (s, 1H, –NH–CO–); MS: 445.2 ($[M + H]^+$); Anal. calc. for $C_{27}H_{28}N_2O_4$ (444.21): C 72.95, H 6.35, N 6.30; found: C 72.70, H 6.60, N 5.92.

*4.1.6. Trans-*N*-(4-Methylphenyl)-3-(3-[2-oxo-4-(4-methoxyphenyl)-1-phenyl-azetidinyl])propanamide (**2b**)*

Yield: 46.9%. White crystals, m.p. 102–103 °C. IR (KBr): 3328, 2916, 2848, 2635, 1624, 1467, 1378, 1308, 1243, 1088; ^{1}H NMR ($CDCl_3$): 2.31 (s, 3H, –CH_3), 2.39–2.46 (m, 2H, –CH_2–CH_2–), 2.60–2.65 (m, 2H, –CH_2–CH_2–), 3.07–3.13 (m, 1H, –CH–CH–), 3.78 (3H, s, –OCH_3), 4.69 (d, $J = 2.1$ Hz, 1H, –CHN–), 7.01–7.05 (m, 4H, Ar-H), 7.09–7.17 (m, 5H, Ar-H), 7.28–7.45 (m, 4H, Ar-H), 7.83 (1H, s, –NH–CO–); MS: 453.1 ($[M + K]^+$); Anal. calc. for: $C_{26}H_{26}N_2O_3$ (414.13): C 75.34, H 6.32, N 6.76; found: C 74.89, H 6.48, N 6.74.

*4.1.7. Trans-*N*-(4-Chlorophenyl)-3-(3-[2-oxo-4-(4-methoxyphenyl)-1-(4-methylphenyl)-azetidinyl])propanamide (**2c**)*

Yield: 35.8%. Yellow oil. IR (KBr): 3518, 3331, 2901, 2548, 1888, 1755, 1613, 1537, 1392, 1249, 1176, 1089, 1033; ^{1}H NMR ($CDCl_3$): 2.24 (s, 3H, –CH_3), 2.26–2.43 (m, 2H, –CH_2–CH_2–), 2.61–2.67 (m, 2H, –CH_2–CH_2–), 3.08–3.15 (m, 1H, –CH–CH–), 3.77 (s, 3H, –OCH_3), 4.88 (d, 1H, $J = 2.2$ Hz, –CHN–), 7.08–7.16 (m, 4H, Ar-H), 7.18–7.29 (m, 4H, Ar-H), 7.40–7.49 (m, 4H, Ar-H), 9.60 (1H, br, –NH–CO–); MS: 449.3 ($[M + H]^+$); Anal. calc. for $C_{26}H_{25}ClN_2O_3$ (448.16): C 69.18, H 5.94, N 6.30; found: C 69.56, H 5.61, N 6.24.

*4.1.8. Trans-*N*-(4-Methylphenyl)-3-(3-[2-oxo-4-(4-methoxyphenyl)-1-(4-methylphenyl)-azetidinyl])propanamide (**2d**)*

Yield: 36.6%. Yellow oil. IR (KBr): 3848, 2900, 1742, 1600, 1513, 1442, 1392, 1249, 1065; ^{1}H NMR ($CDCl_3$): 2.29 (s, 6H, –CH_3), 2.27–2.44 (m, 2H, –CH_2–CH_2–), 2.62–2.67 (m, 2H, –CH_2–CH_2–), 3.06–3.12 (m, 1H, –CH–CH–), 3.77 (s, 3H, –OCH_3), 4.66 (d, $J = 2.3$ Hz, 1H, –CHN–), 7.11–7.16 (m, 4H, Ar-H), 7.15–7.27 (m, 4H, Ar-H), 7.35–7.46 (m, 4H, Ar-H), 7.98 (s, 1H, –NH–CO–); MS: 429.3 ($[M + H]^+$); Anal. calc. for $C_{27}H_{28}N_2O_3$ (428.21): C 75.93, H 6.27, N 6.49; found: C 75.68, H 6.59, N 6.54.

*4.1.9. Trans-*N*-(4-Chlorophenyl)-3-(3-[2-oxo-4-(4-methoxyphenyl)-1-(4-methoxyphenyl)-azetidinyl])propanamide (**2e**)*

Yield: 43.7%. Yellow crystals, m.p. 58–59 °C. IR (KBr): 3501, 2923, 1731, 1666, 1613, 1512, 1395, 1298, 1246, 1173, 1089, 1029; ^{1}H NMR ($CDCl_3$, 300 M): 2.19–2.25 (m, 2H, –CH_2–CH_2–), 2.61–2.68 (m, 2H, –CH_2–CH_2–), 3.05–3.11 (m, 1H, –CH–CH–), 3.70 (s, 6H, –OCH_3), 4.65 (d, $J = 2.3$ Hz, 1H, –CHN–), 6.84–7.05 (m, 4H, Ar-H), 7.09–7.17 (m, 4H, Ar-H), 7.30–7.42 (m, 4H, Ar-H), 8.16 (s, 1H, –NH–CO–); MS: 465.3 ($[M + H]^+$); Anal. calc. for: $C_{26}H_{25}ClN_2O_4$ (464.15): C 67.17, H 5.42, N 6.03; found: C 66.95, H 5.38, N 5.96.

*4.1.10. Trans-1-(4-Chlorophenyl)-3-(3-hydroxy-3,3-diphenylpropyl)-4-(benzo[*d*][1,3]dioxol-5-yl)-2-azetidinone (**3a**)*

Yield: 33.7%. White crystals. m.p.148–150 °C. IR (KBr): 3461, 1742, 1492, 1447, 1390, 1246, 1040, 702; ^{1}H NMR ($CDCl_3$): 1.85–1.98 (m, 2H, –CH_2CH_2–), 2.35–2.70 (m, 2H, –CH_2CH_2–), 3.04–3.18 (m, 1H, –CHCH–), 4.46 (d, $J = 2.1$ Hz, 1H, –CHN–), 5.97 (s, 2H, –OCH_2O–), 6.75–6.80 (m, 3H, Ar-H), 7.19–7.29 (m, 5H, Ar-H), 7.30–7.35 (m, 5H, Ar-H), 7.39–7.42 (m, 4H, Ar-H); MS: 534.3 ($[M + Na]^{\bullet+}$); Anal. calc. for: $C_{31}H_{26}ClNO_4$ (511.16): C 72.51, H 5.39, N 2.96; found: C 72.72, H 5.12, N 2.74.

*4.1.11. Trans-1-(6-Methylphenyl)-3-(3-hydroxy-3,3-diphenylpropyl)-4-(4-methoxyphenyl)-2-azetidinone (**3b**)*

Yield: 35.1%. White oil. IR (KBr): 3384, 1727, 1514, 1252, 1029, 749, 701; ^{1}H NMR ($CDCl_3$): 1.92–1.96 (m, 2H, –CH_2CH_2–), 2.44 (s, 3H, –CH_3), 2.64–2.69 (m, 2H, –CH_2CH_2–), 3.15–3.21 (m, 1H, –CHCH–), 3.77 (s, 3H, –OCH_3), 4.69 (d, $J = 2.4$ Hz, 1H, –CHN–), 6.83–7.07 (m, 4H, Ar-H), 7.20–7.27 (m, 5H, Ar-H), 7.30–7.34 (m, 5H, Ar-H), 7.39–7.46 (m, 4H, Ar-H); MS: 500.3 ($[M + Na]^{\bullet+}$); Anal. calc. for: $C_{32}H_{31}NO_3$ (477.23): C 80.23, H 6.69, N 2.81; found: C 80.47, H 6.54, N 2.93.

*4.1.12. Trans-1-(4-Fluorophenyl)-3-(3-hydroxy-3,3-diphenylpropyl)-4-(4-methoxyphenyl)-2-azetidinone (**3c**)*

Yield: 36.4%. White crystals, m.p.157–159 °C. IR (KBr): 3436, 1720, 1510, 1389, 1249, 827, 704; ^{1}H NMR ($CDCl_3$): 1.85–1.94 (m, 2H, –CH_2CH_2–), 2.56–2.62 (m, 2H, –CH_2CH_2–), 3.07–3.13 (m, 1H, –CHCH–), 3.81 (s, 3H, –OCH_3), 4.50 (d, $J = 2.1$ Hz, 1H, –CHN–), 6.88–6.91 (m, 4H, Ar-H), 7.19–7.28 (m, 5H, Ar-H), 7.30–7.33 (m, 5H, Ar-H), 7.39–7.43 (m, 4H, Ar-H); MS: 504.1 ($[M + Na]^{\bullet+}$); Anal. calc. for: $C_{32}H_{28}FNO_3$ (481.21): C 77.61, H 5.63, N 2.74; found: C 77.32, H 5.86, N 2.91.

*4.1.13. Trans-1-(6-Methylphenyl)-3-(3,3-bis(4-fluorophenyl)-3-hydroxypropyl)-4-(benzo[*d*][1,3]dioxol-5-yl)-2-azetidinone (**3d**)*

Yield: 21.4%. White crystals, m.p. 89–91 °C. IR (KBr): 3449, 1732, 1446, 1245, 1037, 833, 752; ^{1}H NMR ($CDCl_3$): 1.87–1.92 (m, 2H, –CH_2CH_2–), 2.37 (s, 3H, –CH_3), 2.59–2.68 (m, 2H, –CH_2CH_2–), 2.72 (s, 1H, -OH), 3.15–3.16 (m, 1H, –CHCH–), 4.65 (d, $J = 2.1$ Hz, 1H, –CHN–), 5.93 (s, 2H, –OCH_2O–), 6.74–6.76 (m, 3H, Ar-H), 6.97–7.03 (m, 4H, Ar-H), 7.07–7.15 (m, 4H, Ar-H), 7.35–7.40 (m, 4H, Ar-H); MS: 550.2 ($[M + Na]^{\bullet+}$); Anal.calc. for: $C_{32}H_{27}F_2NO_4$ (527.19): C 72.64, H 5.47, N 2.71; found: C 72.85, H 5.16, N 2.66.

*4.1.14. Trans-1-(4-Bromophenyl)-3-(3,3-bis(4-fluorophenyl)-3-hydroxypropyl)-4-(4-methoxyphenyl)-2-azetidinone (**3e**)*

Yield: 19.9%. White crystals, m.p. 122–124 °C. IR (KBr): 3429, 1750, 1609, 1489, 1250, 1071, 827; ^{1}H NMR ($CDCl_3$): 1.85–1.87 (m, 2H, –CH_2CH_2–), 2.47 (s, 1H, –OH), 2.51–2.59 (m, 2H, –CH_2CH_2–), 3.09–3.10 (m, 1H, –CHCH–), 3.80 (s, 3H, –OCH_3), 4.50 (d, $J = 2.4$ Hz, 1H, –CHN–), 6.88–6.91 (m, 4H, Ar-H), 6.99–7.14 (m, 4H, Ar-H), 7.21–7.33 (m, 4H, Ar-H), 7.35–7.38 (m, 4H, Ar-H); MS: 601.0 ($[M + Na]^{\bullet+}$); Anal. calc. for: $C_{31}H_{26}BrF_2NO_3$ (577.11): C 67.9, H 4.27, N 2.49; found: C 64.37, H 4.53, N 2.42.

*4.1.15. Trans-1-(4-Methoxyphenyl)3-(3,3-bis(4-fluorophenyl)-3-hydroxypropyl)-4-(6-bromobenzo[*d*][1,3]dioxol-5-yl)-2-azetidinone (**3f**)*

Yield: 21.5%. Yellow crystals, m.p. 104–106 °C. IR (KBr): 3487, 1720, 1510, 1240, 1034, 930, 830; ^{1}H NMR ($CDCl_3$): 1.93–1.99 (m, 2H, –CH_2CH_2–), 2.47–2.54 (m, 2H, –CH_2CH_2–), 3.02 (d, $J = 2.1$ Hz, 1H, –CHCH–), 3.76 (s, 3H, –OCH_3), 5.00 (d, $J = 2.1$ Hz, 1H, –CHN–), 5.95 (s, 2H, –OCH_2O–), 6.80–6.83 (m, 2H, Ar-H), 6.96–7.04 (m, 4H, Ar-H), 7.15–7.18 (m, 4H, Ar-H), 7.35–7.39 (m, 4H, Ar-H); MS: 644.1/646.2 ($[M + Na]^{\bullet+}$); Anal. calc. for: $C_{32}H_{26}BrF_2NO_5$ (622.45): C 72.64, H 5.47, N 2.71; found: C 61.75, H 4.21, N 2.25.

*4.1.16. (3*R*,4*S*)-N-(4-Methylphenyl)-(3-[2-oxo-4-(4-methoxyphenyl)-1-(4-methoxyphenyl)-azetidinyl]) propanamide (**4a**)*

Yield: 22.7%. Yellow oil. IR (KBr): 3316, 2923, 1728, 1599, 1537, 1511, 1249, 1110, 834; ^{1}H NMR ($CDCl_3$): 2.31 (s, 3H, –CH_3), 2.24–2.30 (m, 2H, –CH_2–CH_2–), 2.59–2.64 (m, 2H, –CH_2–CH_2–), 3.09–3.14 (m, 1H, –CH–CH–), 3.78 (s, 6H, –OCH_3), 4.66 (d, $J = 2.0$ Hz, 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, –NH–CO–); MS: 445.4 ($[M + H]^+$); Anal. calc. for $C_{27}H_{28}N_2O_4$ (444.20): C 72.73, H 6.49, N 6.41; found: C 72.95, H 6.35, N 6.30.

*4.1.17. (3*R*,4*S*)-N-(4-Methylphenyl)-3-(3-[2-oxo-4-(4-methoxyphenyl)-1-phenyl-azetidinyl])propanamide (**4b**)*

Yield: 19.1%. White crystals, m.p. 115–117 °C. IR (KBr): 3332, 2927, 1737, 1669, 1598, 1513, 1386, 1249, 1178, 1033, 818, 754, 691; ^{1}H NMR ($CDCl_3$): 2.31 (s, 3H, –CH_3), 2.22–2.27 (m, 2H, –CH_2–CH_2–),

2.60–2.65 (m, 2H, –CH_2–CH_2–), 3.11–3.16 (m, 1H, –CH–CH–), 3.78 (s, 3H, –OCH_3), 4.69 (d, $J = 2.1$ Hz, 1H, –CHN–), 6.83–7.05 (m, 4H, Ar-H), 7.09–7.17 (m, 5H, Ar-H), 7.28–7.45 (m, 4H, Ar-H), 7.69 (s, 1H, –NH–CO–); MS: 453.3 ($[M + K]^+$); Anal. calc. for: $C_{26}H_{26}N_2O_3$ (414.19): C 75.39, H 6.31, N 6.79; found: C 74.89, H 6.48, N 6.74.

*4.1.18. (3R,4S)-N-(4-Chlorophenyl)-3-(3-[2-oxo-4-(4-methoxyphenyl)-1-(4-methylphenyl)-azetidinyl])propanamide (**4c**)*

Yield: 23.6%. Yellow oil. IR (KBr): 2924, 2868, 1728, 1598, 1537, 1512, 1246, 1236, 1108, 823. ^{1}H NMR ($CDCl_3$): 2.23 (s, 3H, Me), 2.25–2.39 (m, 2H, –CH_2–CH_2–), 2.60–2.65 (m, 2H, –CH_2–CH_2–), 3.08–3.13 (m, 1H, –CH–CH–), 3.76 (s, 3H, MeO), 4.63 (d, $J = 2.1$, 1H, –CHN–), 6.82–6.89 (m, 4H, Ar-H), 7.02–7.14 (m, 4H, Ar-H), 7.21–7.27 (m, 4H, Ar-H), 8.35 (s, 1H, –NH–CO–); MS: 471.3 ($[M + Na]^+$). Anal. calcd for $C_{26}H_{25}N_2O_3Cl$ (448.16): C 69.85, H 5.43, N 6.57; found: C 69.56, H 5.61, N 6.24.

4.2. Evaluation of hypocholesterolemic effects

Cholesterol absorption inhibition activities of new analogs were assessed in orally dosed, cholesterol-fed hamsters as reported in literature [14]. Hypocholesterolemic hamsters were used to test the efficacy of the compounds. Male hamsters, weighing between 200 and 250 g, were maintained on rodent chow and provided with water. In order to induce a hypercholesterolemia in hamsters their chow diet must be supplemented with 1% cholesterol and 0.5% cholic acid. Treatment protocols consisted of feeding this diet for 7 days. Test compounds, dissolved in 0.5 ml corn oil, were administered to the animals by oral gavage daily (mid-light cycle) during this time period. On the last day the animals were sacrificed and blood sample was taken for lipid analyses. Plasma cholesterol levels and HDL-C levels were determined by a commercial modification of the cholesterol oxidase method which was available in a kit form.

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