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Research Article

# SYNTHESIS, CHOLESTEROL ABSORPTION INHIBITION AND ANTI-BACTERIAL ACTIVITY OF SOME NOVEL 2-AZETIDINONE DERIVATIVES

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**ABSTRACT**

Nine new derivatives of the 2-azetidinone cholesterol absorption inhibitors have been synthesized. The structures of synthesized compounds were established by spectroscopic (FT-IR, 1H NMR, Mass) and elemental analyses. All the new compounds were evaluated for their activity to inhibit cholesterol absorption in rats and for their antimicrobial activity. Most of them showed comparable effects in lowering the levels of total cholesterol in the of serum cholesterol-fed hamsters and anti-bacterial screening reveal that all the compounds showed moderate to good anti-bacterial activity against S. aureus.

**Keywords:** 2-azetidinone, cholesterol absorption inhibitors and antibacterial activity.

## INTRODUCTION

The synthesis of hetrocyclic compound has always drawn the attention of chemist over the years mainly because of their important biological properties. One such hetrocyclic, 2-Azetidinone, a β-lactam four member compound involved in research which is aimed to evaluate new products that possess interesting biological activities. 2-Azetidinone compounds reported for their antimicrobial and antifungal activities[1] in resent past.

Most of the researches up to early 90s focused on synthesis of 2-azetidinones and study of their antibacterial property. In recent years, renewed interest has been focused on the synthesis and modification of β-lactam ring to obtain compounds with diverse pharmacological activities like cholesterol absorption inhibitory activity, human tryptase, thrombin and chymase inhibitory activity, vasopressin V1a antagonist activity, antidiabetic, anti-inflammatory, antiparkinsonian and anti-HIV activity[2-7]. They are also found to be a potent inhibitor of serine protease, human leukocyte elastase and human cytomegalovirus protease enzyme[8-11], and are effective on central nervous system; in recent past these derivatives are also found to be moderately active against several types of cancer[12]. The biological activity of the β - lactam skeleton is generally believed to be associated with the chemical reactivity of their β-lactam ring and on the substituent's especially at nitrogen of the 2-azetidinone ring. The oxo group is at 2nd position i.e. 2-azetidinone is important for the activity whereas the substituent's at the N-1, C-3 and C-4 position may be varied.

Atherosclerotic coronary heart disease (CHD) has been the major cause of death and cardiovascular morbidity in the world[13]. The prominent risk factor associated with CHD was the elevation of serum cholesterol levels[14].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[15]. 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[16]. 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[17]. Ezetimibe , 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[18]. 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[19].

The reported structure–activity relationships (SAR) studies revealed that the 2-azetidinone was required for activity, the C-(3) side chain 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[20].

In order to investigate the effect of the polarity of the C-(3) side chain on cholesterol absorption inhibition, bioisosteric interchange and amide group introduction to the C-(3) carbon chain has been done to increase the polarity of C-(3) side chain. The relative configuration at C-(3) and C-(4) of compounds 2a–e were all trans. our research was also aimed at investigating the hydrophobic requirements for cholesterol absorption inhibition ability. Because hydrophobic forces may be the most important single factor responsible for interaction between drug and receptor. Hydrophobicity could be increased by introducing another aromatic substituted hydrazine group to N-(1) Nitrogen chain. 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, compounds 1-9 (Fig. 1) were designed, synthesized and their ability to inhibit cholesterol absorption was evaluated.

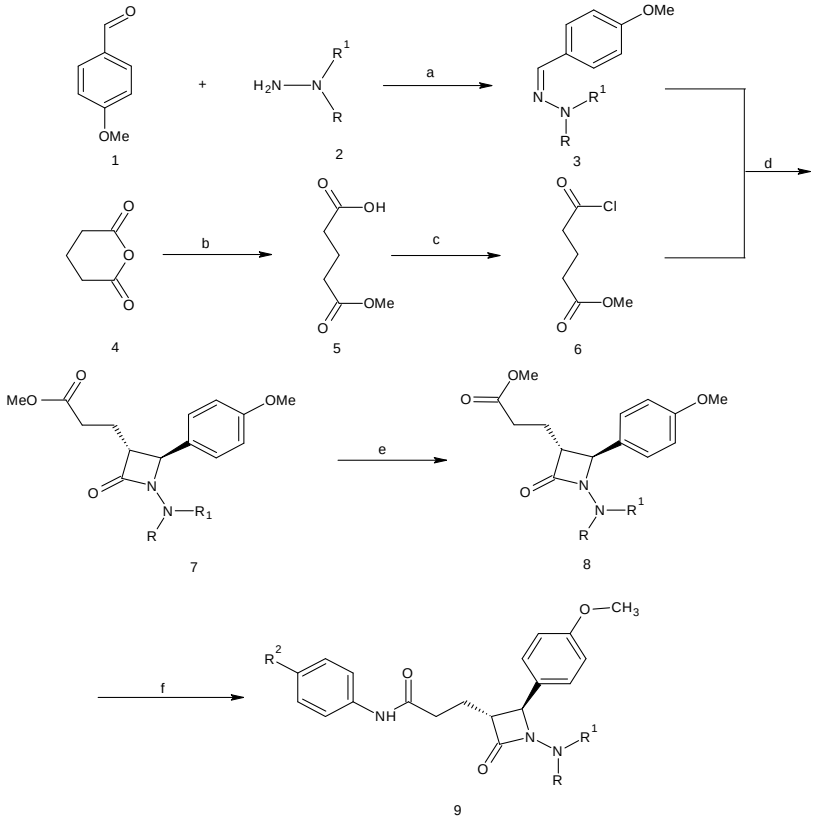
Since the discovery of hydrazides strong antituberculotic action[21], a lot of compounds, which contain the mono- or diacylhydrazine moiety, have been synthesized and tested for anti-inflammatory[22], antibacterial[23], tuberculostatic[24] and antimicrobial activity[25]. Such compounds can be used also as HIV integrase inhibitors[26] or as prostaglandin E receptor antagonists[27]. 1,3,4-Oxadiazole and 1,3,4-thiadiazole derivatives, which can be readily produced using diacylhydrazines as starting materials[28], have been also shown to display antifungal[29], antimicrobial[30] and antiserotonin activity[31]. Although to the best of our knowledge there are no examples of the substitutions of hydrazine derivatives at the nitrogen of Azetidinone ring.

Compounds with 2-azetidinone and hydrazines functions shows mainly biological activities, but, so far, no molecules containing both hydrazine and 2-azetidinone functions, have been recognized for their potential biological properties. Hence in present work the azitidinone analogs with hydrazine substitutions on nitrogen position were designed, synthesized and their ability to inhibit cholesterol absorption and antibacterial properties was evaluated.

## RESULTS AND DISCUSSION

The synthetic route to 1–9 is summarized in Scheme 1. Reaction of 4-methoxybenzaldehyde (1) with a substituted hydrazine (2) in refluxing isopropyl alcohol gave imines (3). Refluxing glutaric anhydride (4) with an equivalent amount of anhydrous MeOH afforded monomethyl glutarate (5), and treatment of (5) in refluxing $SOCl_2$ yielded methyl 4-(chloroformyl) butyrate (6) in excellent yield 84.9% without further purification. Compound (6) was added to a refluxing solution of imine (3) in anhydrous toluene in the presence of tri(n-butyl)amine. Maintaining the mixture refluxing overnight, gave 2-azetidinone intermediate 7. Hydrolysis of 7 with LiOH solution affords acid 8 in

almost quantitative yield. Finally the reaction of 8 with substituted aromatic amine in the presence of DCC/DMAP in anhydrous CH2Cl2 at room temperature gave 2-azetidinone derivatives 1-9 in good yields (60.8–66.4%).



Reagents and conditions: (a) iPrOH, reflux; (b) $CH_3OH$, reflux; (c) $SOCl_2$,reflux; (d) n-Bu3N, toluene, reflux; (e) LiOH, $THF/H_2O$ ; (f) substituted aromatic amine, DCC/DMAP, $CH_2Cl_2$.

**Scheme** 1

## BIOLOGICAL STUDIES

### Cholesterol absorption inhibition

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 new compounds demonstrated moderate to good 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) side chain was not critical for the cholesterol absorption inhibition activity and an increase of compounds hydrophobicity may lead to the elevation of HDL-C. These SAR trends may provide insights into the further design of novel cholesterol absorption inhibitors.

2.2.2 .Anti-bacterial activity of newly synthesized compounds 1-9 was evaluated against various pathogenic bacterial strains viz., Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), The anti-bacterial activities were evaluated by Filter paper disc method at concentrations of 200 lg/ml and 1 mg/ml as per the guidelines of the National Committee for Clinical Laboratory.

## CONCLUSIONS

In an effort to understand the SAR around cholesterol absorption inhibition, nine 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. However the compounds with the phthalazine and 1,3-dinitrobenzene substituted hydrazine showed comparable cholesterol absorption inhibition. This information could be valuable for further investigation of SAR and will be useful in later research of cholesterol absorption inhibitors.

The results of anti-bacterial screening reveal that among all the compounds screened, compounds 1,2,5 and 6 showed moderate anti-bacterial activity while compound 6 9,8,7, and 3 displayed good anti-bacterial activity when compared with lincomycin against S. aureus. None of the test compounds could exhibit comparable activity to that of the standard ceftazidime against E. coli. Particularly, compound 8 and 9 which is carrying propylbenzene substituent hydrazine appears to exhibit the highest anti-bacterial activity (zone of inhibition up to 15– 24 mm at concentration of 200 lg/ml and 1 mg/ml.) against S. aureus. Also compound with benzaldehyde substituted hydrazine shows comparable activity.

In antibacterial activity a further test can be done using a higher concentration of more than 1 mg/ml if diffusion is not the barrier. In future, some more test organisms of gram positive and gram negative types can be used. In conclusion we suggest that the future studies on these azetidinone derivatives could be useful for the management of bacterial infections and inflammatory diseases.

## EXPERIMENTAL

### Chemistry

### 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 CDCl3 unless otherwise indicated; d in ppm rel. to Me4Si, J in Hz. Mass spectrometry (MS): Hewlett–Packard 1100 LC/ MSD spectrometer; in m/z. Elemental analyses: CHN-O-Rapid instrument.

**General procedure for the preparation of 1-9**

A mixture of the appropriate 4-methoxybenzaldehyde (**1**) (10 mmol) and substituted Hydrazine **2** (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 **3.** Refluxing glutaric anhydride (**4**) (0.44 mol) with equivalent anhydrous methanol (30 mL) affords monomethyl glutarate (**5**) and then treatment of **5** in refluxing SOCl2 (100 mL) gave methyl 4-(chloroformyl) butyrate (**6**) (0.37 mol) in good yield (84.9%) without further purification. Compound **6** (77 mmol) was added to refluxing solution of imine **3** (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, NaHCO3(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 **7**. 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 **7** (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 **8** of sufficient purity to be used without further purification. To a stirring solution of acid **8** (5 mmol), 4- dimethylaminopyridine (0.25 mmol) and substituted aromatic amine (5.5 mmol) in dry CH2Cl2 (40 mL) under N2 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.

***4.1***

*1.* ***3-[2-(4-methoxyphenyl)-1-[methyl(phenyl) amino]-4-oxoazetidin-3-yl]-N-(4-methyl phenyl)propanamide*** Yield: 46.9%. White crystals, m.p. 102–103 _C. IR (KBr): 3380, 1731, 1443, 1245, 1037, 833, 752. $^1$H NMR (CDCl3): 1.91-2.23(m, 2H,–CH2CH2–), 2.01-2.29(d, 2H, –NH-NH-),2.35 (s, 3H, –CH3), 2.47 (s, 3H, –CH3), 4.7 (d, J ¼2.1 Hz, 1H, –CHN–), 6.72–7.01 (m, 4H, Ar-H), 7.04–7.52 (m, 4H, Ar-H), 8.0 (1H, s, –NH–CO–); MS: 366.14 ([M+ H]$^+$); Anal. calc. for: $C_{21}H_{25}N_3O_3$ (367.44): C 68.64 H 6.86 N 11.44 found 67.52 H 7.93 N 10.71

***2. 3-[2-(4-methoxyphenyl)-4-oxo-1-(phenyl amino)azetidin-3-yl]-N-(4-methylphenyl) propanamide***

Yield: 46.9%. White crystals, m.p. 102–103 _C. IR (KBr): 3328, , 1635, 1624, 1467, 1378, 1108, 1243, $^1$H NMR (CDCl3): 1.91-2.23(m, 2H,–CH2CH2–), 2.01-2.29(d, 2H, –NH-NH-),2.35 (s, 3H, –CH3), 3.73 (3H, s, –OCH3), 4.7 (d, J ¼2.1 Hz, 1H, –CHN–),6.66–7.18 (m, 5H, Ar-H), 6.72–7.01 (m, 4H, Ar-H), 7.0–7.52 (m, 4H, Ar-H), 8.0 (1H, s, –NH–CO–);MS: 476.21 ([M+ H]$^+$); Anal. calc. for: $C_{26}H_{27}N_3$28 (429.51): C 72.71 H 6.34 N 9.78 found 71.52 H 7.83 N 10.71

***3. N-(3-{2-[(4-chlorophenyl)carbamoyl]ethyl}-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl) benzamide***

Yield: 46.9%. White crystals, m.p. 102–103 _C. IR (KBr): 3429, 1750, 1609, 1489, 1250, 1071, 745.

$^1$H NMR (CDCl3): 1.91-2.23(m, 2H,–CH2CH2–), 2.01-2.29(d, 2H, –NH-NH-),3.45 (s, 1H, –CH–$CH_2$–),3.73 (3H, s, –OCH3), 4.7 (d, J ¼2.1 Hz, 1H, –CHN–),6.72–7.01 (m, 4H, Ar-H), 7.25–7.58 (m, 4H, Ar-H), 7.44–7.95 (m, 5H, Ar-H), 8.0 (1H, s, –NH–CO–);

MS: 476.8 ([M+ H]$^+$); Anal. calc. for: $C_{26}H_{24}ClN_3O_4$ (477.93): C 65.34 H 5.06 N 8.79 found 64.52 H 7.93 N 9.81

**4.** ***3-{1-[(2,4-dinitrophenyl)amino]-2-(4-meth oxyphenyl)-4-oxoazetidin-3-yl}-N-(4-methyl phenyl)propanamide***

Yield: 46.9%. White crystals, m.p. 102–103 _C. IR (KBr): 3501, 2923, 1731, 1666, , 1510, 1375, 1278, , 1173, 1089, 1029.$^1$H NMR (CDCl3): 1.91-2.23(m, 2H,–CH2CH2–), 2.01-2.29(d, 2H, –NH-NH-),2.35 (s, 3H, –CH3), 3.45 (s, 1H, –CH–$CH_2$–),3.73 (3H, s, –OCH3), 4.7 (d, J ¼2.1 Hz, 1H, –CHN–), 6.72–7.01 (m, 4H, Ar-H), 7.04–7.52 (m, 4H, Ar-H), 7.18–9.04 (m, 3H, Ar-H), 8.0 (1H, s, –NH–CO–); MS: 520.27 ([M+ H]$^+$); Anal. calc. for: $C_{26}H_{27}N_5O_7$ (521.52): C 59.88 H 5.22 N 13.43 found 58.02 H 6.83 N 14.93

***5. 3-{1-[(2,4-dinitrophenyl)amino]-2-(4-meth oxyphenyl)-4-oxoazetidin-3-yl}-N-phenyl propanamide***

Yield: 46.9%. White crystals, m.p. 102–103 _C. IR (KBr): 3501, 2923, 1731, 1666, 1613, 1512, 1395, 1298, 1246, 1173, 1089, 1029. $^1$H NMR (CDCl3): 1.91-2.23(m, 2H,–CH2CH2–), 2.01-2.29(d, 2H, –NH-NH-), 3.45 (s, 1H, –CH–$CH_2$–), 3.73 (3H, s, –OCH3), 4.7 (d, J ¼2.1 Hz, 1H, –CHN–), 6.72–7.01 (m, 4H, Ar-H), 7.0–7.64 (m, 5H, Ar-H), 7.16–9.04 (m, 3H, Ar-H), 8.0 (1H, s, –NH–CO–); MS: 504.27 ([M+ H]$^+$); Anal. calc. for: $C_{25}H_{23}N_5O_7$ (505.47): C 59.40 H 4.59 N 13.85 found 58.12 H 5.83 N 14.73

***6. 3-[2-(4-methoxyphenyl)-4-oxo-1-[(phthal azin-1-yl)amino]azetidin-3-yl]-N-(4-methyl phenyl)propanamide***

Yield: 46.9%. White crystals, m.p. 102–103 _C. IR (KBr): 3501, 2923, 1731, 1666, 1613, 1512, 1395, 1298, 1246, 1173, 1089, 1029. $^1$H NMR (CDCl3): 1.91-2.23(m, 2H,–CH2CH2–), 2.01-2.29(d, 2H, –NH-NH-),2.35 (s, 3H, –CH3), 3.45 (s, 1H, –CH–$CH_2$–),3.73 (3H, s, –OCH3), 4.7 (d, J ¼2.1 Hz, 1H, –CHN–), 6.72–7.01 (m, 4H, Ar-H), 7.0–7.52 (m, 4H, Ar-H), 7.93–8.66 (m, 5H, Ar-H), 8.0 (1H, s, –NH–CO–);MS: 480.4 ([M+ H]$^+$); Anal. calc. for: $C_{28}H_{27}N_5O_3$ (481.54568) C 69.84 H 5.65 N 14.54, found C 68.7 H 6. 85 N 15.41

***7. 3-[2-(4-methoxyphenyl)-4-oxo-1-[(phthal azin-1-yl)amino]azetidin-3-yl]-N-phenyl propanamide***

Yield: 46.9%. White crystals, m.p. 102–103 _C. IR (KBr): 3461, 2823, 1742, 1492, 1447, 1390, 1246, 1040, 1176, 1089.$^1$H NMR (CDCl3): 1.91-2.23(m, 2H,–CH2CH2–), 2.01-2.29(d, 2H, –NH-NH-),3.45 (s, 1H, –CH–$CH_2$–),3.73 (3H, s, –OCH3), 4.7 (d, J ¼2.1 Hz, 1H, –CHN–),6.72–7.01 (m, 4H, Ar-H), 7.00–7.64 (m, 5H, Ar-H), 7.93–8.66 (m, 5H, Ar-H), 8.0 (1H, s, –NH–CO–);

MS: 466.21 ([M+ H]$^+$); Anal. calc. for: $C_{27}H_{25}N_5O_3$ (467.5191) C 69.36 H 5.39 N 14.98; found C 68.42 H 6.48 N 15.01

**8.** ***3-[2-(4-methoxyphenyl)-4-oxo-1-[(2-phenyl ethyl)amino]azetidin-3-yl]-N-(4-Methyl phenyl)propanamide***

Yield: 46.9%. White crystals, m.p. 102–103 _C. IR (KBr): 3518, 3331, 2901, 2548, 1888, 1755, 1613, 1537, 1392, 1249,1176, 1089, 1033 $^1$H NMR (CDCl3): 1.91-2.23(m, 2H,–CH2CH2–)

,2.01-2-29(d, 2H, –NH-NH-), 2.35 (s, 3H, –CH3), 2.98–2.81 (m, 2H, –CH2–CH2–), 3.45 (s, 1H, –CH–$CH_2$–),3.73 (3H, s, –OCH3), 4.7 (d, J ¼2.1 Hz, 1H, –CHN–), 6.72–7.05 (m, 4H, Ar-H), 7.08–7.21 (m, 5H, Ar-H), 7.04–7.52 (m, 4H, Ar-H), 8.0 (1H, s, –NH–CO–); MS:456.42 ([M+ H]$^+$); Anal. calc. for: $C_{28}H_{31}N_3O_3$ (457.56): C 73.50 H 6.83 N 9.18 ; found C 72.89, H 6.93, N 9.16

**9. *3-[2-(4-methoxyphenyl)-4-oxo-1-[(2-phenyl ethyl)amino]azetidin-3-yl]-N-Phenyl propanamide***

Yield: 46.9%. White crystals, m.p. 102–103 _C. IR (KBr): 3384, 3381, 2901, 1848, 1755, 1627, 1514, 1252, 1029.$^1$H NMR (CDCl3): 1.91-2.23(m, 2H,–CH2CH2–), 2.01-2.29(d, 2H, –NH-NH-), 2.81-2.98 (m, 2H,–CH2CH2–), 3.45 (s, 1H, –CH–$CH_2$–),3.73 (3H, s, –OCH3), 4.7 (d, J ¼2.1 Hz, 1H, –CHN–),6.72–7.12 (m, 4H, Ar-H), 6.72–7.21 (m, 5H, Ar-H), 7.00–7.64 (m, 5H, Ar-H), 8.0 (1H, s, –NH–CO–); MS:442.3742 ([M+ H]$^+$); Anal. calc. for: $C_{27}H_{29}N_3O_3$ (443.53746): C 73.11 H 6.59 N 9.47 ; found C 72.91 H 6.83 N 9.43

**Evaluation of hypocholesterolemic effects**

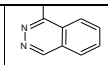
Cholesterol absorption inhibition activities of new analogs were assessed in orally dosed, cholesterol-fed hamsters as reported in literature [14]. Hypocholesterolemic hamsters were used to test the efficacy of the compounds. Male hamsters, weighing between 200 and 250 g, were maintained on rodent chow 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 gavages 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.

**Antibacterial activity**

The results of the antibacterial screening of the test compounds are presented in table no 2. Antibacterial activity of the test compounds in DMF was determined by Filter paper disc method at concentrations of 200 µg/ml and 1 mg/ml. All the compounds showed comparable activity as that of the standard lincomycin against S. aureus (MTCC 96) (see Figs. 5–8). None of the test compounds could exhibit comparable activity to that of the standard ceftazidime against E. coli (MTCC 722). The test compounds showed a better activity at the tested higher concentration (1 mg/ml) than at the lower concentration (200 lg/ml) against S. aureus. The compounds 2,4,8 and 9 are promising ones against S. aureus.

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

| Compound[a] | R1 | R2 | R3 | TC[b] (% Reduction) | HDL-C[c] (% increase) |
|---|---|---|---|---|---|
| 1 | H | $CH_3$ | Me | NE[d] | 11.2 |
| 2 | $C_6H_5$ | H | Me | 18.2 | 21.2 |
| 3 |  | H | Cl | 25.3 | 31.2 |
| 4 |  | H | Me | 23.4 | 29.3 |
| 5 |  | H | H | 15.3 | 14.1 |
| 6 |  | H | Me | 29.3 | 33.2 |

| | | | | | |
|---|---|---|---|---|---|
| 7 |  | H | H | 21.2 | 29.1 |
| 8 |  | H | Me | 26.8 | 29.2 |
| 9 |  | H | H | 22.4 | 28.2 |
| Ezetimibe | | | | 48.4 | 37.2 |

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.

**Table 2: Antibacterial activity of test compounds against S. aureus and E. coli.**

| Test compounds | Zone of inhibition in mm against S. aureus | | Zone of inhibition in mm against E. coli | |
|---|---|---|---|---|
| | 200 µg/ml | 1 mg/ml | 200 µg/ml | 1 mg/ml |
| 1 | 14 | 19 | 7 | 12 |
| 2 | 12 | 16 | 8 | 12 |
| 3 | 16 | 22 | 6 | 11 |
| 4 | 15 | 21 | 7 | 11 |
| 5 | 12 | 18 | 8 | 15 |
| 6 | 12 | 17 | 6 | 15 |
| 7 | 14 | 20 | 7 | 14 |
| 8 | 15 | 22 | 10 | 13 |
| 9 | 15 | 24 | 7 | 11 |
| Standard | 18 | 24 | 17 | 27 |

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