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Hypocholesterolemic activity of some novel azetidin-2-ones in diet and diabetes induced hypercholesterolemia in rats

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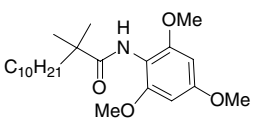
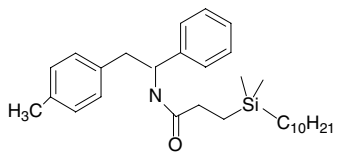
Some novel substituted azetidin-2-ones (**5**–**8**) were synthesized via {2 + 2} cycloaddition reactions of imines and ketenes and evaluated for their ability to prevent diet and diabetes induced hypercholesterolemia. The test compounds **5a** and **7a** significantly ($p < 0.01$) inhibited the rise in serum total cholesterol induced by peanut oil (5.5%), cholesterol (1.5%) and cholic acid (0.5%) diet in both acute and chronic models in a dose dependent manner. Compound **5a** also raised the high density lipoprotein-cholesterol levels in chronic diet models by peanut oil (5.5%), cholesterol (1.5%) and cholic acid (0.5%). In a diabetes induced model of hypercholesterolemia, the test compounds were evaluated for preventing diabetes-induced hypercholesterolemia (protocol 1) as well as for lowering post diabetic hypercholesterolemia (protocol 2). Test compounds **5a**–**g** and **7a**–**d** significantly ($p < 0.05$) reduced serum total cholesterol with a greater reduction in protocol 1 as compared with protocol 2. Based on SAR studies, the substituents that favor hypocholesterolemic activity around the azetidin-2-one nucleus are discussed and a possible mechanism of action is proposed on the basis of their differential effects in two protocols of diabetes-induced hypercholesterolemia.

1. Introduction

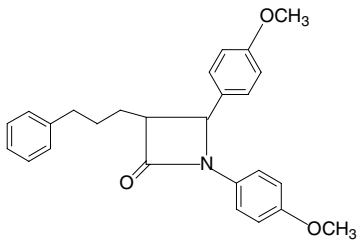
Hypercholesterolemia is an independent risk factor for the development of coronary heart disease (CHD). Pharmacological reduction of serum cholesterol levels has been linked to reduction in CHD mortality as well as to reversal of atherosclerotic disease as indicated by the degree of occlusion of coronary arteries (Brown et al. 1990). Endogenously synthesized cholesterol and dietary cholesterol are important sources for cholesterol (Sliskovic and White 1991). Thus serum cholesterol can be reduced by inhibiting the absorption of dietary and biliary cholesterol from the intestines and/or by inhibiting endogenous cholesterol biosynthesis and promoting hepatic cholesterol clearance from plasma (Krause et al. 1995). Attempts to inhibit the absorption of dietary cholesterol have been focused primarily on the inhibition of acyl co-A cholesterol acyltransferase (ACAT), a major enzyme associated with chol-



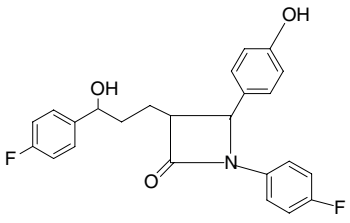
CI-976



(SA-58035)



(SCH-48461)



(SCH-58235)

esterol esterification (Sliskovic and White 1991). Several chemical classes have been investigated for ACAT inhibition viz. fatty acid anilides (Roth et al. 1992) heterocyclic amides (White et al. 1996), disubstituted ureas (Trivedi et al. 1993), *N*-chlorosulfonyl compounds (Picard et al. 1996), etc. However, none of these has shown significant clinical efficacy. In the last decade azetidin-2-ones have been reported as a new class of hypercholesterolemic agents, including conformationally restricted analogs of fatty acid anilides such as Cl-976 and SA 58035 (Burnett et al. 1994). As a result there has been a sporadic increase in the design and synthesis of novel substituted azetidin-2-ones, Such attempts have resulted in the development of SCH 48461 (Clader et al. 1996) and SCH 58235 (Rosenblum et al. 1998) and of these later (Ezitimib) has recently been approved for clinical use (Earl and Krik 2003). These reports of azetidin-2-ones as clinically effective cholesterol absorption inhibitors prompted us to synthesize suitably substituted novel azetidin-2-ones and evaluate them for their cholesterol absorption inhibitory activity.

2. Investigations and results

2.1. Synthesis of compounds

Imines, prepared by the condensation of a primary amine with the equivalent proportion of aldehyde in dichloromethane in the presence of anhydrous magnesium sulphate, were treated with ketenes, generated *in situ* from acid chlorides in the presence of triethylamine, to give the desired azetidin-2-ones (Scheme). Stereochemistry of the synthesized compounds **5a–g, 6, 7a–d** and **8** (Table 1) was assigned on the basis of the reported coupling constant for methine H-3/H-4 protons. (J = 2–4 Hz and 4–6 Hz for *trans*-azetidinones and *cis*-azetidinones respectively) (Sharma et al. 1996).

2.2. Pharmacological investigations

2.2.1. Experimental setup

Based on structure activity relationship and receptor binding studies of SCH 48461 and SCH 48678, to define the binding conformation for SCH 48461, which demonstrated that the nature of the C_3 substituents was critical for hypocholesterolemic activity (Dugar et al. 1995), we initiated the examination of four azetidinones with varied substituents at C_3 and tested their efficacy in acute and chronic models of PCC diet induced hypercholesterolemia. The promising results obtained with compounds **5a** and **7a** prompted us to establish the SAR in variously substituted compounds **5a** and **7a**. The studies reported to date have failed to reveal the exact mechanism of action of these compounds (Clader et al. 1996). Hence, based on the following facts, an indirect mode has been devised: i) diabetes has been established as a secondary cause for hypercholesterolemia and atherosclerosis, ii) augmentation of ACAT activity as early as three days after induction of diabetes, which may be responsible for diabetic hypercholesterolemia (Maechler et al. 1992) and iii) inhibition of hypercholesterolemia induced by diabetes by different specific ACAT inhibitors (Matsurba et al. 1988 and Sakuma et al. 1997). In order to understand the mechanism of hypocholesterolemic action of these compounds observed in diet-induced models in rats, the effect of the test compounds on serum total cholesterol levels was studied in two different protocols of dia-

Scheme

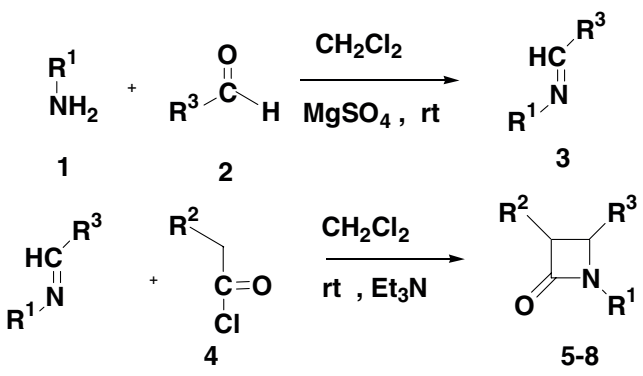
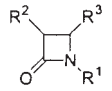
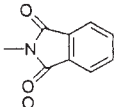
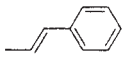
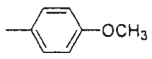
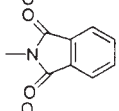
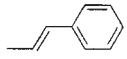
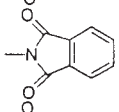
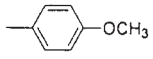
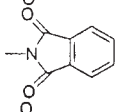
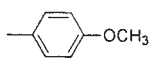
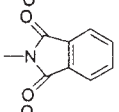
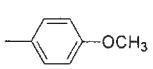
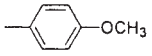
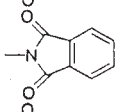
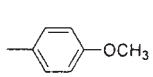
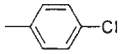
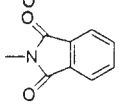
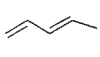
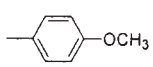
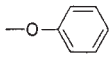
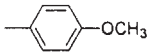
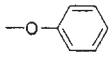
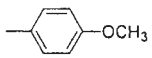
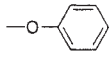
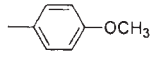
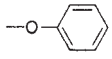
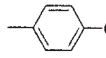
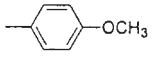


Table 1: Structure of compounds 5–8



Compound	R^1	R^2	R^3
5a			
5b			
5c			
5d			
5e			
5f			
5g			
6			
7a			
7b			
7c			
7d			
8			

betes induced hypercholesterolemia. In protocol 1 treatment with the test compounds was started from the day of alloxan treatment whereas in protocol 2 the treatment was started seven days after alloxan injection, on confirmation of diabetes.

2.2.2. Effect of test compounds on serum cholesterol profile of rats in acute and chronic PCC diet (PCCD)-induced hypercholesterolemia

A significant rise ($p < 0.001$) in serum total cholesterol was observed in the PCC diet control group as compared to the normal diet control group in both acute and chronic PCC diet models. Compounds **5a, 6, 7a** and **8** were given at three different dose levels (5, 7.5, 10 mg/kg) in order to study the optimal effect. Compounds **5a** and **7a** significantly ($p < 0.01$) inhibited the rise in serum total cholesterol (TC) induced by PCC diet in a dose dependent manner in both models. Compound **5a** also raised high density lipoprotein cholesterol (HDL-C) levels in the chronic PCC diet model. Compounds **5a** and **7a** were chosen for further studies (Table 2). Analogs of compound **5a** (compounds **5b**–**5g**) at a dose of 5 mg/kg significantly lowered the TC and non-HDL cholesterol ($p < 0.05$) and HDL-cholesterol levels were raised significantly ($p < 0.05$) as compared to normal diet group. The order of inhibitory activity was **5a** > **5f** > **5b** > **5c** > **5d** > **5g** > **5e**. Test compounds **7b**–**d** showed significant ($p < 0.05$) inhibition of the rise of TC and non-HDL cholesterol, but HDL cholesterol levels were not raised significantly. The order of inhibitory activity was **7c** > **7b** > **7a** < **7d**. (Table 3). Test compounds **5a**–**g** and 7**a**–**d** significantly ($p < 0.05$) reduced the atherogenic index.

2.2.3. Effect of test compounds on hepatic ACAT activity

The liver content of both TC and esterified cholesterol was significantly raised ($p < 0.001$) in the PCC diet-control group as compared to the normal diet group indicating higher supply and esterification of cholesterol. Percentage of esterified cholesterol was calculated for each group and it was found that the test compounds **5a**–**g** and **7a**–**d** significantly ($p < 0.05$) reduced the percentage esterified cholesterol in the liver as compared to the PCC diet group. At the same time accumulation of cholesterol in the liver was also significantly ($p < 0.05$) inhibited by the test compounds (Table 4).

Table 2: Effect of test compounds 5a, 6, 7a and 8 on serum cholesterol of rats in acute and chronic PCC diet model

Treatment Group	Dose mg/kg	Acute PCC diet model	Chronic PCC diet model			
		Total cholesterol (mg/dl)	Total cholesterol (mg/dl)	HDL-C (mg/dl)	Non HDL-C (mg/dl)	Atherogenic index
ND Control	–	72.34 ± 1.58	72.34 ± 1.58	31.04 ± 1.58	41.3 ± 2.16	1.33
PCCDcontrol	–	129.3 ± 2.09[a]	258.6 ± 3.1[a]	20.9 ± 1.42[a]	237.7 ± 3.9[a]	11.37[a]
PCCD + **5a**	5.0	78.25 ± 1.51[b]	93.50 ± 9.6[b]	41.2 ± 4.3[b]	52.4 ± 6.38[b]	1.27[b]
PCCD + **5a**	7.5	71.13 ± 6.18[b]	90.60 ± 10.5[b]	46.8 ± 4.7[b]	43.8 ± 6.66[b]	0.93[b]
PCCD + **5a**	10.0	62.25 ± 4.41[b]	85.30 ± 7.8[b]	46.6 ± 5.2[b]	38.7 ± 5.54[b]	0.83[b]
PCCD + **6**	5.0	108.2 + 9.28	220.5 ± 15.4	32.3 ± 3.4	118.2 ± 13.31[b]	2.39[b]
PCCD + **6**	7.5	105.3 ± 6.60	218.6 ± 21.3	30.8 ± 3.8	187.8 ± 15.8[b]	6.09[b]
PCCD + **6**	10.0	103.8 ± 11.4	215.4 ± 22.5	36.40 ± 6.4	179.0 ± 23.5[b]	4.91[b]
PCCD + **7a**	5.0	90.94 ± 6.75[b]	115.7 ± 9.3[b]	30.6 ± 8.3	85.1 ± 8.7[b]	2.78[b]
PCCD + **7a**	7.5	83.38 ± 8.80[b]	100.6 ± 10.5[b]	37.8 ± 4.7	62.8 ± 5.6[b]	1.66[b]
PCCD + **7a**	10.0	78.62 ± 9.40[b]	96.30 ± 8.7[b]	32.4 ± 4.6	63.9 ± 5.4[b]	1.97[b]
PCCD + **8**	5.0	136.5 ± 11.8	240.6 ± 13.6	44.6 ± 3.5	196.0 ± 10.8[b]	4.39[b]
PCCD + **8**	7.5	130.5 ± 6.39	248.3 ± 15.8	43.8 ± 4.5	204.5 ± 11.5[b]	4.66[b]
PCCD + **8**	10.0	128.4 ± 10.4	228.6 ± 16.7	41.4 ± 4.8	187.2 ± 15.6[b]	4.52[b]

a = $p < 0.001$ when compared with normal diet controls b = $p < 0.001$ when compared with control PCC diet controls
ND = Normal diet PCCD= Peanut oil cholesterol and cholic acid diet, high density lipoprotein-cholesterol (HDL-C)

Table 3: Effect of test compounds 5a–5g and 7a–7d on serum cholesterol of rats in chronic PCC diet model

Treatment Group	Dose mg/kg	Chronic PCC diet model			
		Total cholesterol (mg/dl)	HDL-C (mg/dl)	NonHDL-C (mg/dl)	Atherogenic index
ND Control	–	72.34 ± 1.58	31.04 ± 1.58	41.3 ± 2.16	1.33
PCCDcontrol	–	258.6 ± 3.1[a]	20.9 ± 1.42[a]	237.7 ± 3.9[a]	11.37[a]
PCCD + **5a**	5	93.50 ± 9.6[b]	41.2 ± 4.3[b]	52.4 ± 6.38[b]	1.27[b]
PCCD + **5b**	5	101.4 ± 1.5[b]	39.4 ± 2.1[b]	62.0 ± 2.7b	1.57[b]
PCCD + **5c**	5	117.4 ± 1.8[b]	38.9 ± 3.1[b]	78.5 ± 2.4[b]	2.02[b]
PCCD + **5d**	5	126.9 ± 3.8[b]	34.6 ± 2.9[b]	92.3 ± 2.3[b]	2.67[b]
PCCD + **5e**	5	182.6 ± 2.3[b]	40.6 ± 1.9[b]	142.0 ± 3.1[b]	3.54[b]
PCCD + **5f**	5	100.3 ± 1.3[b]	40.3 ± 2.3[b]	60.6 ± 2.5[b]	1.49[b]
PCCD + **5g**	5	143.7 ± 1.4[b]	31.3 ± 1.4	112.4 ± 1.9[b]	3.59[b]
PCCD + **7a**	5	115.7 ± 9.3[b]	30.6 ± 8.3	85.1 ± 8.7[b]	2.78[b]
PCCD + **7b**	5	108.3 ± 8.6[b]	32.5 ± 5.4	75.8 ± 6.7[b]	2.33[b]
PCCD + **7c**	5	100.5 ± 7.5[b]	33.8 ± 4.8	66.7 ± 6.9[b]	1.97[b]
PCCD + **7d**	5	118.3 ± 6.6[b]	30.6 ± 4.5	87.7 ± 8.7[b]	2.86[b]

a = $p < 0.001$ when compared with control normal diet controls b = $p < 0.001$ when compared with PCC diet controls
ND = Normal diet PCCD = Peanut oil, cholesterol and cholic acid diet., high density lipoprotein-cholesterol (HDL-C)

Table 4: Effect of 5a–5g and 7a–7d on the hepatic cholesterol profile of rats in chronic PCC diet model

Treatment Group	Dose mg/kg	Total cholesterol (mg/g of liver tissue)	Esterified cholesterol (mg/ g of liver tissue)	% age Esterified
ND Control	–	233.3 ± 8.8	86.66 ± 3.4	37.1
PCCD Control	–	771.3 ± 6.60[a]	401.5 ± 4.6[a]	52.05[a]
PCCD + **5a**	5	282.0 ± 7.3[b]	73.05 ± 2.99[b]	25.8[b]
PCCD + **5b**	5	288.8 ± 6.60[b]	68.8 ± 5.15[b]	23.8[b]
PCCD + **5c**	5	328 ± 5.6[b]	83 ± 4.35[b]	25.3[b]
PCCD + **5d**	5	377.5 ± 5.31[b]	82.5 ± 4.2[b]	21.9[b]
PCCD + **5e**	5	428.8 ± 7.45[b]	83.8 ± 5.54[b]	19.5[b]
PCCD + **5f**	5	636 ± 21.54[b]	83.0 ± 5.82[b]	13.1[b]
PCCD + **5g**	5	343.8 ± 9.65[b]	80.0 ± 6.12[b]	23.3[b]
PCCD + **7a**	5	365.6 ± 8.92[b]	76.80 ± 7.57[b]	21.0[b]
PCCD + **7b**	5	323.0 ± 7.06[b]	72.02 ± 6.18[b]	22.7[b]
PCCD + **7c**	5	277.5 ± 7.24[b]	63.01 ± 5.53[b]	14.3[b]
PCCD + **7d**	5	324.5 ± 11.52[b]	76.25 ± 6.88[b]	23.5[b]

a is $P < 0.001$ as compared with normal diet (ND) control b is $P < 0.001$ as compared with PCC diet (PCCD) control

Table 5: Hypocholesterolemic effect of test compounds in diabetic hypercholesterolemia model

Treatment Group	Dose mg/kg	Total cholesterol (mg/dl) Protocol 1	Totalcholesterol (mg/dl) Protocol 2
Diabetic Control	–	146.37 ± 4.02	146.37 ± 4.02
Gliclazide	8	47.29 ± 2.50[a]	75.9 ± 2.56[a]
	5	76.64 ± 7.59[a]	103.1 ± 6.7[a]
	5	85.63 ± 7.03[a]	112.5 ± 8.5[a]
	5	96.5 ± 7.56[a]	117.3 ± 9.5[a]
	5	100.3 ± 8.9[a]	124.4 ± 10.3[a]
	5	113.4 ± 9.5[a]	128.7 ± 10.5[a]
	5	83.6 ± 7.4[a]	107.1 ± 6.9[a]
	5	101.6 ± 9.4[a]	126.6 ± 7.8[a]
	5	123.4 ± 10.5[a]	137.7 ± 11.3[a]
	5	120.3 ± 10.3[a]	130.7 ± 11.5[a]
	5	111.6 ± 9.5[a]	118.8 ± 9.4[a]
	5	126.3 ± 10.4[a]	136.6 ± 11.4[a]

a = $P < 0.001$ as compared with diabetic control

2.2.4. Effect of test compounds on diabetes induced hypercholesterolemia

The effect of the test compounds was evaluated in alloxan-induced diabetic rats. The diabetic group developed significant ($p < 0.05$) hypercholesterolemia over 21 days compared with the normal control group. Test compounds **5a**–**g** and **7a**–**d** significantly ($p < 0.05$) reduced serum TC in protocols 1 and 2. The effect of the test compounds was much better in protocol 1 as compared to protocol 2 (Table 5).

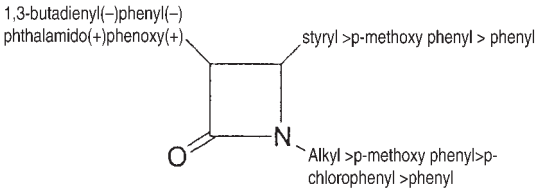
3. Discussion

Azetidin-2-ones such as SCH 48461 and SCH58235 have been reported as effective cholesterol absorption inhibitors (CAI). The substituent at C_3 has been reported to interact with the receptor and is critical for inhibition of cholesterol absorption. However, few novel azetidin-2-ones have been synthesized with varied substituents at the N_1, C_3 and C_4 positions. Accordingly four compounds viz. **5a**, **6**, **7a**, **8** were synthesized and the pharmacological results indicated that the presence of simple aryl/alkyl substituents at C_3 was unfavorable while aryl substituents having a suitable spacer atom at C_3 were favorable. These observations are in accordance with those reported in the literature.

On the basis of the hypocholesterolemic effects of compounds **5a**–**g** observed in the CPCC diet model the order of favorable substituents for 3-phthalamido azetiden-2-one was found to be: Alkyl > *p*-methoxy phenyl > *p*-chlorophenyl > phenyl at N_1 and styryl > *p*-methoxy phenyl at C_4. Similarly for 3-phenoxy azetidin-2-ones (**7a**–**d**) the order of favorable substitution at N_1 was *p*-methoxy phenyl > phenyl and at C_4 *p*-methoxyphenyl > phenyl.

In diabetes-induced hypercholesterolemia the test compounds showed a significantly better effect in protocol-1 than in protocol 2. This indicated that the test compounds inhibited the induction of intestinal ACAT in Protocol-1. The decreased inhibitory effect in protocol 2 may be due to partial reduction of ACAT activity. Gliclazide used as a reference drug also showed similar results but these test compounds were observed to be less effective than gliclazide in controlling serum cholesterol levels. The lowering of the percentage esterified cholesterol by all the test compounds is indicative of hepatic ACAT inhibition. The reduction of TC in liver further suggests that the supply of cholesterol to the liver is also reduced. These observations indicated that both intestinal absorption and hepatic ACAT activity might have been reduced. The following conclusions may be drawn on the basis of the above discussion:

- Favorable substitutions at N_1, C_3, C_4 are as shown below
  (+) Favorable, (−) Favorable



- Mechanism of action of these compounds is not well understood. and might be due to the intestinal ACAT inhibition.
- These compounds might reduce the risk of atherosclerosis as these are hepatic ACAT inhibitors and significantly reduced the atherogenic index in diet-induced as well as in diabetes-induced hypercholesterolemia

4. Experimental

4.1. Chemistry

Melting points were determined using open glass capillaries and are uncorrected. NMR spectra were recorded on Bruker 200 MHz and 300 MHz spectrometers. The ^{1}H-chemical shifts are expressed in ppm relative to tetra methyl silane (Me_4Si). IR spectra were obtained on a Shimadzu FT-IR. Microanalysis for C, H, and N were performed on a Perkin Elmer CHN analyzer. All the results were within an acceptable error range. Mass spectra were obtained on a Shimadzu GCMS-QP-2000 mass spectrometer.

4.1.1. General procedure

To a stirred solution of imine (10 m. mol) and triethylamine (15 m. mol) in dry dichloromethane (20 ml) at room temperature was added dropwise a solution of acid chloride (12 mmol) in dry dichloromethane (10 ml). After completion of the reaction (TLC), the resulting mixture was washed with saturated sodium bicarbonate solution (2×20 ml) and then with water (2×30 ml) and dried over anh. sodium sulphate. Solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (eluent: ethyl acetate/hexane in 1 : 9 ratio).

4.1.2. Cis/trans-3-phthalamido-1-isopropyl-4-styrylazetidin-2-one (**5a**)

Yield: 77%, mp: 158–159 °C, IR (KBr): 1730 cm^{-1} (C = O). ^{1}H NMR: (200 MHz, $CDCl_3$): δ: 1.28 (d, J = 6.8 Hz, 3 H, C*H*$_3$), 1.39 (d, J = 6.7, 3 H, C*H*$_3$), 3.85–4.02 (m, 1 H, CH<) 4.59 (dd, J = 2.4 Hz and 9.1 Hz, 1 H, H-4), 5.05 (d, J = 2.4 Hz, 1 H, *H*-3 cis isomer), 5.41 (d, J = 5.2 Hz, 1H, *H*-3 trans isomer), 6.21 (dd, J = 15.8 Hz and 9.1 Hz, 1 H, *H*-1′), 6.65 (d, 1 H, J = 15.8 *H*-2′), 7.15–7.36 (m, 5 H, Ar*H*), 7.65–7.85 (m, 4 H, Ar*H*), mass (m/z) M^+ 360.
$C_{22}H_{120}N_2O_3$

4.1.3. Trans-3-phthalamido-1-(4-methoxyphenyl)-4-styrylazetidin-2-one (**5b**)

Yield: 60.0%, m.p.: 66–67 °C, IR (KBr): 1720 cm^{-1} (C = O). ^1H NMR: (200 MHz, $CDCl_3$): δ: 3.80 (s, 3 H, OCH_3), 5.07 (dd, 1 H, J = 5.5 and 8.6 Hz, *H*-4), 5.69 (d, J = 5.5 Hz, 1 H, *H*-3), 6.31 (dd, J = 8.6 Hz and 14.1 Hz, 1 H, *H*-1′), 6.82 (d, 1 H, J = 14.1 *H*-2′) 6.88 (d, 2 H, J = 8.6 Ar*H*), 7.23–7.28 (m, 5 H, Ar*H*), 7.48 (d, 2 H, J = 8.9 Hz Ar*H*), 7.86–7.90 (m, 4 H, Ar*H*), mass (m/z) M^+ 424.
$C_{26}H_{20}N_2O_4$

4.1.4. Cis/trans-3-phthalamido-1-isopropyl-4-(4′-methoxyphenyl) azetidin-2-one (**5c**)

Yield: 80%, m.p.: 100–101 °C, IR (KBr): 1720 cm^{-1} (C = O). ^1H NMR: (200 MHz, $CDCl_3$): δ: 1.23 (d, J = 6.6 Hz, 3 H, CH_3), 1.38 (d, J = 6.6 Hz, 3 H, CH_3), 3.81 (s, 3 H, OCH_3), 3.85–4.02 (m, 1 H, C*H*-<) 4.88 (d, J = 2.4 Hz, 1 H, H-4), 5.07 (d, J = 2.4 Hz, 1 H, H-3 trans isomer), 5.40 (d, J = 5.2 Hz, 1 H, H-3 cis isomer), 6.72 (d, 1 H, J = 8.8 Hz, Ar*H*), 6.82 (d, 1 H, J = 8.8 Hz, Ar*H*), 7.22–7.31 (m, 2 H, Ar*H*), 7.64–7.87 (m, 4 H, Ar*H*), mass (m/z) M^+ 364.
$C_{21}H_{20}N_2O_4$

4.1.5. Trans-3-phthalamido-1-cyclohexyl-4-(4-methoxyphenyl) azetidin-2-one (**5d**)

Yield: 80% m.p.: 100–101 °C, IR (KBr): 1720 cm^{-1} (C = O). ^1H NMR: (300 MHz, $CDCl_3$): δ: 1.09–2.12 (m, 10 H, cyclohexyl), 3.44–3.67 (m, 1 H), 3.82 (s, 3 H, OCH_3) 4.92 (d, J = 2.4 Hz, 1 H, *H*-4), 5.07 (d, J = 2.4 Hz, 1 H, *H*-3), 6.90 (d, 2 H, J = 8.6 Hz Ar*H*), 7.26–7.32 (m, 2 H, Ar*H*), 7.72–7.8 (m, 4 H, Ar*H*), mass (m/z) M^+ 404.
$C_{24}H_{24}N_2O$

4.1.6. Trans-3-phthalamido-1-phenyl-4-(4-methoxyphenyl) azetidin-2-one (**5e**)

Yield: 58% m.p.: 118–119 °C, IR (KBr): 1730 cm^{-1} (C = O). ^1H NMR: (200 MHz, CDCl3): δ: 3.73 (s, 3 H, OC*H*3), 5.13 (d, J = 2.5 Hz, 1 H, *H*-4), 5.22 (d, J = 2.5 Hz, 1 H, *H*-3), 6.80–7.83 (m, 13 H, Ar*H*), mass (m/z) M^+ 398.
$C_{24}H_{18}N_2O_4$

4.1.7. Trans-3-phthalamido-1-(4-methoxyphenyl)-4-(4-methoxyphenyl)azetidin-2-one (**5f**)

Yield: 69%, m.p.: 190–191 °C, IR (KBr): 1720 cm^{-1} (C = O). ^1H NMR: (200 MHz, $CDCl_3$): δ: 3.76 (s, 3 H, OCH_3), 3.82 (s, 3 H, OCH_3), 4.95 (d, J = 2.5 Hz, 1 H, *H*-4), 5.05 (d, J = 2.5 Hz, 1 H, *H*-3), 6.54 (d, J = 8.8 Hz, 2 H, Ar*H*), 6.66 (d, J = 8.8 Hz, 2 H, Ar*H*), 6.96–7.24 (m, 4 H, Ar*H*), 7.57–7.63 (m, 4 H, Ar*H*), mass (m/z) M^+ 428.
$C_{25}H_{20}N_2O_5$

4.1.8. Trans-3-phthalamido-1-(4-chlorophenyl)-4-(4-methoxyphenyl) azetidin-2-one (**5g**)

Yield: 68%, m.p.: 114–115 °C, IR (KBr): 1722 cm^{-1} (C = O). ^1H NMR: (200 MHz, $CDCl_3$): δ: 3.78 (s, 3 H, OCH_3), 5.19 (d, J = 2.5 Hz, 1 H, *H*-4), 5.28 (d, J = 2.5 Hz, 1 H, *H*-3), 6.87 (d, J = 8.6 Hz, 2 H, Ar*H*) 7.03–7.07 (m, 1 H, ArH), 7.19–7.31 (m, 5 H, Ar*H*), 7.70–7.87 (m, 4 H.Ar*H*), mass (m/z) M^+ 434.
$C_{21}H_{20}N_2O_4$

4.1.9. Trans-3-(1,3-butadienyl)-1-phenyl-4-(4-methoxyphenyl)azetidin-2-one (**6**)

Yield: 70% m.p.: 104–105 °C, IR (KBr): 1747 cm^{-1} (C = O). ^1H NMR: (300 MHz, $CDCl_3$): δ: 3.74 (dd, J = 2.4 Hz and 8.1 Hz, 1 H, *H*-3), 3.80 (s, 3 H, OCH_3), 4.75 (d, J = 2.4 Hz, 1 H, *H*-4) 5.17 (dd, J = 9.6 Hz and 15.6 Hz, 1 H, Ha), 5.24 (dd, J = 9.6 Hz and 15.6 Hz, 1 H, Hb), 5.82–5.90 (m, 1 H, *Hc*), 6.26–6.41 (m, 2 H, Hd, He), 6.90 (d, 2 H, J = 8.8 Hz Ar*H*), 6.99–7.06 (m, 1 H, Ar*H*), 7.21–7.31 (m, 6 H, ArH), mass (m/z) M^+ 305.
$C_{20}H_{19}NO_2$

4.1.10. Cis-3-phenoxy-1-phenyl-4-phenylazetidin-2-one (**7a**)

Yield: 43% m.p.: 172–173 °C IR (KBr): 1716 cm^{-1} (C = O), ^1H NMR ($CDCl_3$, 200 MHz): δ, 5.39 (d, 1 H, J = 5.0 Hz H-4), 5.56 (d, 1 H, J = 5.0 Hz, H-3), 6.76–7.41 (m, 15 H, ArH), mass (m/z) M^+ 345.
$C_{21}H_{17}NO_2$

4.1.11. Cis-3-phenoxy-1-phenyl-4-(4-methoxyphenyl) azetidin-2-one (**7b**)

Yield: 43%, m.p.: 77–78 °C IR (KBr): 1716 cm^{-1} (C = O), ^1H NMR ($CDCl_3$, 200 MHz): δ 3.71 (s, 3 H, OCH_3), 5.32 (d, J = 4.7 Hz , 1 H, H-4), 5.50 (d, J = 4.7 Hz, 1 H, H-3), 6.75–6.79 (m, 4 H, ArH), 7.09–7.14 (m, 5 H, ArH), 7.22–7.36 (m, 5 H, ArH), mass (m/z) M^+ 375.
$C_{22}H_{19}NO_3$

4.1.12. Cis-3-phenoxy-1-(4-methoxyphenyl)-4-(4-methoxyphenyl) azetidin-2-one (**7c**)

Yield: 68%, m.p.: 83–85 °C IR (KBr): 1716 cm^{-1} (C = O), ^1H NMR ($CDCl_3$, 200 MHz): δ 3.75 (s, 3 H, OCH_3), 3.79 (s, 3 H, OCH_3), 5.27 (d, J = 4.6 Hz, 1 H, H-4), 5.47 (d, J = 4.6 Hz, 1 H, H-3), 6.74–6.82 (m, 4 H, ArH), 6.87–7.03 (m, 5 H, ArH), 7.06–7.48 (m, 4 H, ArH), mass (m/z) M^+ 375.
$C_{23}H_{21}NO_4$

4.1.13. Cis-3-phenoxy-1-phenyl-4-(4-chlorophenyl) azetidin-2-one (**7d**)

Yield: 66%, m.p.: 130–132 °C IR (KBr): 1720 cm^{-1} (C = O), ^1H NMR ($CDCl_3$, 200 MHz): δ 5.41 (d, J = 4.8 Hz, 1 H, H-4), 5.60 (d, J = 4.8 Hz, 1 H, *H*-3), 6.82 (d, J = 7.62 Hz, 2 H, Ar*H*), 6.99 (d, J = 7.62 Hz, 2 H, Ar*H*), 7.13–7.22 (m, 3 H, Ar*H*), 7.26–7.40 (m, 7 H, Ar*H*), mass (m/z) M^+ 349.
$C_{21}H_{16}NO_2Cl$

4.1.14. Trans-3-phenyl-1-(4-methoxyphenyl)-4-(4-methoxyphenyl)azetidin-2-one (**8**)

Yield: 66%, m.p.: 110–112 °C IR (KBr): 1720 cm^{-1} (C = O), ^1H NMR (CDCl3, 300 MHz): δ 3.73 (s, 3 H, OC*H*3), 3.80 (s, 3 H, OC*H*3), 4.23 (d, 1 H, J = 2.4 Hz *H*-4), 4.83 (d, 1 H, J = 2.4 Hz, *H*-3), 6.80 (d, 2 H, J = 6.8 Hz, Ar*H*), 6.91 (d, 2 H, J = 11.4 Hz, Ar*H*), 7.24–7.39 (m, 9 H, Ar*H*), mass (m/z) M^+ 359.
$C_{23}H_{21}NO_3$

4.2. Pharmacology

4.2.1. Animals

Wistar rats of either sex housed in the Central Animal House facility of Guru Nanak Dev University and weighing between 150–200 g, were used. The animals were housed under standard laboratory conditions, maintained on a natural light and dark cycle and given free access to food and water. Animals were acclimatized to laboratory conditions before the experimentation. All experiments were carried out between 0900 and 1500 h. The experimental protocols were approved by the Institutional Ethics Committee and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

4.2.2. Diet induced hypercholesterolemia

In the acute PCC (APCC) diet model hypercholesterolemia was induced by keeping on a peanut oil (5.5%), cholesterol (1.5%) and cholic acid (0.5%) PCC diet for 24 h. In the test groups the test compounds (β-lactams) were administered between 9.00–10.00 am once a day and blood samples were taken 24 h after administration of the test compounds for serum total cholesterol estimations. In the chronic PCC Diet Model (CPCC) the rats were kept on the PCC diet for seven days to establish hypercholesterolemia and in the test groups the animals received the test compounds once a day along with the PCC diet for the next seven days (White et al. 1996). The test compounds (β-lactams) were suspended in water using carboxy methyl cellulose (1.5%) and Tween-20 (0.2%) for oral administration. On the 15th day the blood samples were taken by cardiac puncture under light ether anesthesia and were analyzed for total cholesterol (TC), high-density lipoprotein (HDL)-cholesterol and non-HDL-cholesterol. Atherogenic index was calculated by taking the ratio of non-HDL cholesterol to HDL-cholesterol (Dixit et al. 1998). Animals were sacrificed on the last day to obtain liver tissue. Total lipids were extracted from liver tissue (Folch et al. 1957) and the estimation of total and esterified cholesterol was carried out and results expressed as mg/g of liver tissue (Sperry and Webb 1950).

4.2.3. Diabetes induced hypercholesterolemia

In this model diabetes was produced in animals by injecting alloxan (150 mg/kg ip). Drug treatment was given in two different protocols: in protocol 1 it was started from the first day of induction of diabetes for 21 days and in protocol 2 it was started one week after induction of diabetes on confirmation of diabetes for the next two weeks. Blood samples were collected after drug treatment on the 22nd day for estimation for serum cholesterol.

4.2.4. Statistical analysis

One specific group of rats was assigned to one specific drug treatment condition and each group comprised of 6 rats (n = 6). The data were expressed as mean ± standard error mean (SEM). Data were analyzed by one way analysis of variance (ANOVA) followed by Dunnet's t test. In all the tests the criterion for statistical significance was $p < 0.05$.

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