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Synthesis and anti hyperlipidemic activty of azetidinone derivatives in combination with nicotinic acid moiety and other Schiff's bases

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ABSTRACT

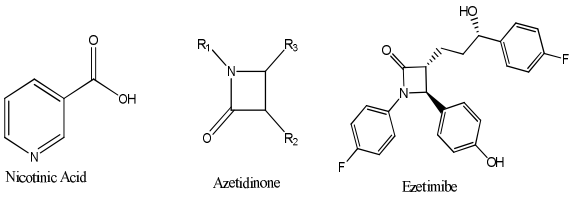
A series of novel condensed N-(3-chloro-2-oxo- 4-phenylazetidin-1-yl) nicotinamide has been synthesized by involving the cyclocondensation of the appropriate Schiff's bases with chloroacetyl chloride, followed by the addition of triethylamine in the presence of molecular sieves and evaluated for Lipid Lowering activity in high fat diet fed hyper lipidemic Sprague Dawley rats. The aim of this study was to investigate the effect of the nicotinic acid, salicylic acids and benzoic acid at the N 1 position and other substituents on C 4 positions of 2-Azetidinone. Most of the synthesized compounds significantly affected the lipid profile of the test animals. Compounds **II,V,VI** and **VII** exhibited remarkable effects in lowering the serum cholesterol, triglyceride levels and elevating the serum High Density Lipoproteins evels at high doses (100 mg/kg) & low doses(50 mg/kg) in test animal.

Keywords: ANOVA, Azetidin-2-one, Ezetimibe, lipid lowering agents, lipid profile, Tukey's Test, Sprague Dawley.

1. INTRODUCTION

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 2 recognized sources of cholesterol in the serum: biosynthesis in the liver and absorption of dietary cholesterol in the small intestine [3,4]. Statins has 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 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 the statins. The structure-activity relationships (SAR) studies revealed that 2-azetidinone is required for activity, there are references which state that amide group in the C-(3) side chain was not critical for the cholesterol absorption inhibition activity [7]. The C 4 substituted aryl residue showed good activity when 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. It is known that bio isosterism 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 [8-10]. Since 2- azetidinone nucleus containing drug Ezetimibe is known to act on Cholesterol absorption from the intestine and has no effect on the HDL level, it was thought rational to combine substituted azetidinones with Nicotinic acid which is known to reduce serum cholesterol, VLDL, LDL and TG levels in types II, III, IV, and V hyper-lipoproteinemia and also increase HDL. It is known that niacin decreases lipolysis in adipose tissue, decreases TG esterification in the liver andincrease LPL activity [11]. Nicotinic acid moiety was therefore logically introduced at N 1 position in 2 azetidinone, substituted aromatic groups like methoxy and Chloro group at C 4 position of the basic pharmacophore, electronegative group was retained at C 3 position and it's the effect was seen on the lipid profiles oftest animals. Other acids

substituted in place ofnicotinic acid were salicylic acids and benzoicacid [7].



Manisha Fig 1: Structure of Nicotinic acid, Azetidinone and ezetimibe

The traditional synthesis of 2-azetidinone reported in the literature involves cycloaddition reaction of primary amines with aromatic aldehydes and various acid chlorides in dry organic solvent (dichloromethane) using triethylamine (TEA) as a catalyst. This required long refluxing time (12–16 h). The reported reactions involved use of Deanstark apparatus for the removal of water molecules from the reaction and formation of byproducts. Tedious workup procedures were required for isolation of pure products resulting in very low yields. The green chemical route method reported in the literature involved stirring in presence of nitrogen or argon gas at very low temperatures (– 70 to – 90 °C). The synthetic approaches reported hence required specialized glassware for synthesis. Of all the methods reported for synthesis of β-lactams, the cycloaddition reaction of ketenes with imines (Staudinger reaction) for the construction of β-lactam ring has found wide acceptance. This is mainly because of its simplicity, predictability of stereo chemical outcome and proven utility of this method for the synthesis of large number of monocyclic, bicyclic, tricyclic and spirocyclic β-lactams [12] . The method used by us involved MWI of respective aldehydes with hydrazide to yield the Schiff base. This was then dissolved in suitable solvent and stirred with addition of chloroacetyl chloride and triethylamine, at low temperature (0–5 °C). The reaction was carried out in the presence of molecular sieves [MS (1–2 g, 3 Å · 1.5 mm), The product was washed with concentrated brine and sodium bicarbonate solution and recrystallized from alcohol yielding 2-azetidinones of aryl acids hydrazones in good yields.

2. Experimental

2.1. Chemistry

All reagents and chemicals used were of LR grade and standard quality. Melting points were determined on scientific melting point apparatus in open capillaries and were uncorrected. The 1 H NMR spectra were recorded in $CDCl_3$ using NMR Varian Mercury YH-300 MHz spectrometer and chemical shifts are given inunits as parts per million, downfield from TMS (tetramethylsilane) as an internal standard. Mass spectra were obtained on a Shimadzu GCMS-QP2010 spectrometer. Elemental analyses were obtained using a Flash EA 1112 Thermofinnigan instrument. The IR spectra of the synthesized compounds were recorded on Perkin Elmer (USA) Spectrum BX 2 FT-IR spectrophotometer in potassium bromide discs.

2.1.1. General synthesis procedure of Schiff's bases [13]

Hydrazide of acid (0.03 M) and substituted benzaldehyde (0.03 M) in water were irradiated under microwave using a microwave synthesizer (Make-Raga's Scientific) at power level 3 (240 W, 35 % irradiation) until the completion of the reaction. The reaction was monitored by TLC (Toluene: Methanol-4:1). The reaction mixture was filtered. The residue obtained was washed with water, followed by sodium thiosulphate ($Na_2S_2O_3$)solution, and then dried. The crude product was obtained upon recrystallization from alcohol. The product (85–90 % yield) having m.p. above 200 °C were obtained for Schiff's bases.

2.1.2. General synthesis procedure of 2-azetidinones using Schiff's bases

The 2-azetidinones were synthesized as per reported method of stirring (Thomas et al., 2011). The appropriate Schiff 's base was (0.0025 M) dissolved in DMF, chloroacetyl chloride (0.0037 M). Triethylamine (0.0075 M) was added drop wise with constant stirring at low temperature (0–5°C). The reaction was carried out in thepresence of molecular sieves [MS (1–2 g, 3 Å · 1.5 mm)]. The reaction mixture was further stirred at room temperature until the completion of reaction. [TLC (Toluene/Methanol = 4.5:0.5)]. The reaction mixture was added into crushed ice and stirred to obtain the crude product. The product obtained was washed with concentrated brine and sodium bicarbonate solution and then dried. The crude product on recrystallization from alcohol yielded the pure 2-azetidinones of aryl acids hydrazones. The synthesized compounds were characterized on the basis of their spectral and analytical data (IR, 1H NMR, MASS).[13]

2.2. Lipid lowering activity

The experiments were carried on Sprague Dawley rats (150– 200 g) of either sex. The animals were housed at a temperature of 20 ± 2 °C with relative humidity of 50 ± 10 % with 12 h light and dark cycles. The activity was divided in 2 major groups of compounds first major group was of compounds **I–V** and second group was of compounds **VI–X.** Ezetimibe (1 mg/kg and 3 mg/kg) was used as standard for the comparison

Lipid Lowering activity. Normal diet was made available for 7 days to Group I (normal control) and vehicle (2 % acacia solution. *p.o.*) was administered for 7 days. Hyperlipidemia was induced to Group II (High fed suspension control) by orally administering a suspension of cholesterol (500 mg/kg) and cholic acid (250 mg/kg) in groundnut oil (10 ml/kg) daily for 7 days. Standard treatment groups (Group III & Group IV) were orally administered Ezetimibe at dose 1 mg/kg and 3 mg/kg*p.o.* respectively for 7 days daily in High fed animals. Test groups (Groups V–XIV) of each major group were orally administered 10 synthesized compounds each respective major group at dose 50 mg/kg and 100 mg/kg, respectively for 7 days daily in high fat diet fed animals (Figure 1). The blood was collected on 8th day; animals were kept on fasting for 14 h before the blood withdrawal. The blood was withdrawn by retro orbital method under light ether anesthesia and serum was separated by centrifugation at 3000 rpm for 10 min. and evaluated for serum total cholesterol, triglyceride and HDL level using commercial diagnostic kits (Biolabs Pvt. Ltd., Mumbai, India) where asatherogenic index, coronary risk index, serum LDL, VLDL levels were calculated by the reported formulae [14-16].

3. RESULTS AND DISCUSSION

3.1. Chemistry

The present protocol describes a simple and efficient method for the synthesis of 2-azetidinones by different Schiff bases of hydrazides of nicotinic acid, salicylic acid and benzoic acid[17]. It has been demonstrated that cyclo-condensation of Schiff bases with chloroacetyl chloride in triethylamine gives fairly high yields in a relatively short reaction time and easy work-up procedures. This enables this method to be applicable for the synthesis of 2-azetidinone based heterocyclic compounds.

The completion of the synthesis of target compounds were confirmed by TLC. IR absorption band at 1 550 cm $^{-1}$ to 1 560 cm $^{-1}$ for stretching vibration of -CH = N- and Mass spectra m/e: 225.1(M+), 105.3, 121.3 confirms the condensation of reactants to form Schiff -base. Similarly IR, 1HNMR and mass spectral data obtained are in correlation with synthesized azetidinone. Target Synthesis was done by reported procedure from literature survey [13] (Table 1and Figure 2).

3.2. Spectral data of representative compounds

3.2.1. N-(3-chloro-2-oxo-4-phenylazetidin-1-yl) benzamide (I)

IR (KBr): 1657(γ CONH); 3071,3040 (γ ArCH) 2985 (γ CH), 834(γ Cl) 3296(γ NH), . 1H NMR (300 MHz, CDCl 3): 4.212 (d, 2H, -CO-N-*CH*Ar of azetidinone), (d, 1H, -N-CH- ***CH*** - Cl of azetidinone), 7.241– 7.908 (m, 10H, Ar- *H* & -N *H*) MS (m/e): 299.7 (M +), 104, 222, 250. Anal. Calcd. for C 16 H 13 ClN 2 O 2 .

3.2.2. N-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-2-hydroxy benzamide (III)

IR (KBr): 1629(γ CONH); 3032(γ ArCH); 3232 (γ NH); 3611(γ OH); 749,639 (γ Cl) 1H NMR (300 MHz, CDCl 3): 4.225 (d, 2H, -CO-N*CH*-Ar of azetidinone), 4.406 (s, 1H, -N-CH- *CH*- Cl of azetidinone), 4.346 (s, 1H, Ar-OH), 6.979–7.789 (m, 9H, Ar- *H* & -N *H*).

3.2.3. N-(3-chloro-2-(3-methoxyphenyl)-4-oxoazetidin-1-yl) benzamide (V)

IR (KBr): 2922(γ CH); 1651(γ -CONH-, -CON-); 1449(γ Ar C-C); 3002 (γ Ar-H);1266 (γ Ar-O-R); 792(γ R-Cl) 1 H NMR (300 MHz, CDCl 3): 4.459 (d, 2H, -CO-N- *CH*- Ar of azetidinone), 5.138 (d, 1H, -N-CH*CH*- Cl of azetidinone), 6.958–7.908 (m, 9H, Ar- *H* & -N *H*).

3.2.4. N-(3-Chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-yl)-2- hydroxybenzamide. (VIII)

IR (KBr): 3356.21(γ O-H), 1735.22(γ C = O), 1478.42(γ C-N), 2980.37 (γ Ar C-H), 763.76(γ Ar), 703.35(γ C-Cl), 3 421.55(γ N-H). MS (m/e): 350.29 (M +).

3.2.5. N-(3-chloro-2-(3-methoxyphenyl)-4-oxoazetidin-1-yl)-2- hydroxybenzamide (IX)

IR (KBr): 3350.38(γ O-H), 3557.74(γ N-H), 1788.81(γ C = O), 1680.94(γ C-N), 3002.01(γ ArC-H), 774.24(γ Ar), 1241.97 (γ C-O-C) 1 H NMR (300 MHz, CDCl 3): 6.984–7.955 (m, 9H,Ar-*H* &N *H*), 4.35(m,1H,-N-CH- *CH*- Cl of azetidinone, 3.81(m,2H,Ar- *OH* &-CON *CH*- Ar of azetidinone), 1.56 (s, 3H, Ar- *OCH3*) MS (m/e): 346(M +).

3.3. Lipid lowering activity

The test compounds **I–X** were evaluated for antihyperlipidemic activity in high fat diet fed Sprague Dawley rats for 1 week. The test animals were divided broadly into 14 groups. Compounds were administered at dose 50 mg/kg and 100 mg/kg according to acute toxicity studies, *p.o.,* whereas, ezetimibe[18]a well-known cholesterol absorption inhibitor was administered as the standard drug at dose 1 mg/kg and 3 mg/kg *p.o.* Further, the proposed mechanism of action for compounds is closely related to the title compound which acts by interfering with cholesterol reabsorption and is effective by oral route. These points led to the selection of ezetimibe as the standard drug during the biological evaluation of the title compounds.

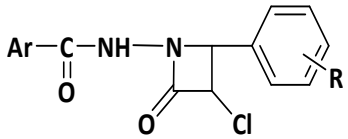
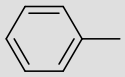
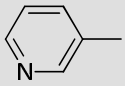
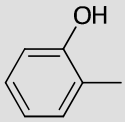
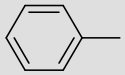
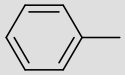
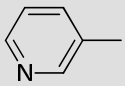
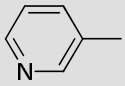
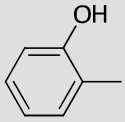
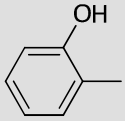
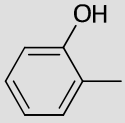


Table - 1: Physical data for the N-(2-oxo-4-phenylazetidin-1-yl) aromatic amide (I–V).

Comp. No.	Ar	R	Mol. Formula (Solvent of recrystallization)*	M.P. (°C) (Mol. wt.)	Yield (%)
I		H	$C_{16}H_{13}ClN_2O_2$(E)	85-90 (300.74)	91
II		H	$C_{15}H_{12}ClN_3O_2$ (E)	105-110 (301.73)	71
III		H	$C_{16}H_{13}ClN_2O_3$ (E)	95-100 (316.74)	84
IV		2-Cl	$C_{16}H_{12}Cl_2N_2O$ (E)	120-125 (335.18)	80
V		3-OCH_3	$C_{17}H_{15}ClN_2O_3$ (E)	85-90 (330.77)	86
VI		2-Cl	$C_{15}H_{11}Cl_2N_3O_2$ (E)	226-229(336.17)	78
VII		3-OCH_3	$C_{16}H_{14}ClN_3O_3$ (E)	238-242(331.75)	72
VIII		2-Cl	$C_{16}H_{12}Cl_2N_2O_3$ (E)	242-245(351.18)	84
IX		3-OCH_3	$C_{17}H_{15}ClN_2O_4$ (E)	232-235(346.76)	80
X		3-OH	$C_{16}H_{13}ClN_2O_4$ (E)	238-240(332.74)	86

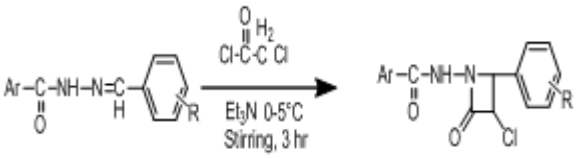


Figure - 2: Scheme for synthesis of compunds

One week after the treatment, all the animal groups were fasted for 14 h and blood was withdrawn from retro-orbital plexus of the test animals for the evaluation of serum level of total cholesterol, triglyceride and HDL [19] . The obtained results revealed that feeding rats with high fat diet for 7 days, significantly elevated the serum levels of total cholesterol and triglycerides, as compared to the normal control rats. Moreover, induction of hyperlipidemia significantly decreased serum HDL levels of these animals as compared to the normal control ones. It was found that the test compounds showed significant changes in lipid profile,*i. e.*, decrease in serum level of total cholesterol, triglycerides and increase in HDL at a dose of 50 mg/kg (Low dose) & 100 mg/kg (High dose) body weight *p.o.* as compared with the high fed diet group. A perusal of (Table 2,3 and Figure 4) reveals that :-

- At 50 mg/ kg and 100 mg/kg body weight *p.o.* dose levels, of compounds **II, VI** in Low dose and **II, IV, V, VI, VII** in High dose **,** caused significant reduction in serum total cholesterol levels, comparable to that caused by ezetimibe at a dose level of 1 mg/kg and 3 mg/kg body weight *p.o*. compounds, while compounds **IV, VII, IX** in low dose and **IX** in high dose remained moderately effective.
- The test compounds **II, VI** in low dose and **II, V, VI, VII** in high dose caused significant reduction in serum triglycerides levels (Table 2,3 and Figure 5,6), comparable to that caused by ezetimibe at a dose level of 1 mg/kg and 3 mg/kg body weight *p.o* . compounds, while compounds **V, VII** in low dose and **IX** in high dose remained moderately effective.
- The compounds **VI, VII** in low dose and **II, VII, IX** in high dose also exhibited good HDL levels enhancing activity in the test animals (Table 2,3 and Figure 7,8), while other compounds **II, V**, **V I, VII** in low dose and **V, VII, IX** in high dose remained moderately effective in this activity.
- The compounds **II, VI, VII** in low dose and **II, IV, V, VI, VII, IX** in high dose exhibit good VLDL and LDL reducing activity, comparable to that caused by ezetimibe at a dose level of 1 mg/kg and 3 mg/kg body weight *p .o*. (Table 2 , 3 and Figure 9-12). While compounds **V, VII** and **IV, IX** remained moderately effective.
- All compounds exhibit good atherogenic index (Normal value of AI = < 3.5). Total 4 test compounds **II, V, VI** and **VII** of the series significantly affecting the lipid profile (decreasing total cholesterol & triglycerides & increasing the HDL levels) of the test animals and can be looked upon as potential leads for further development and investigations.

3.4. Statistical analysis

All the values were expressed as the mean ± SEM and were subjected to 1-Way Analysis of Variance (ANOVA) followed by Tukey's test, where P < 0.001 was considered as statistical significant. Each value mean presents the ± S.E.M. of 6 observations by ANOVA followed by Tukey's test, ### P< 0.001 statistically significant as compared to Normal control group. *P < 0.05, **P < 0.01, ***P < 0.001 statistical significance as compared to cholesterol fed diet group. ns = not significant **.**

3.5. Figures and illustration

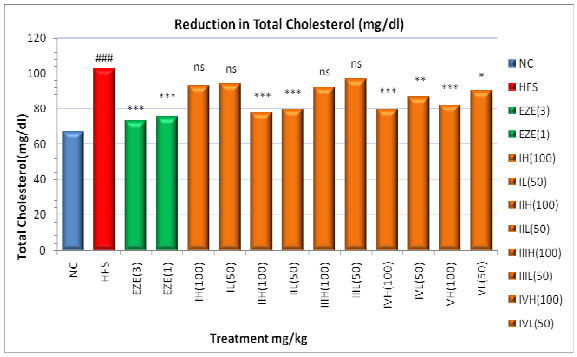


Figure – 3: Effect of the synthesized test compounds (I–V) on serum total cholesterol levels of test animals.

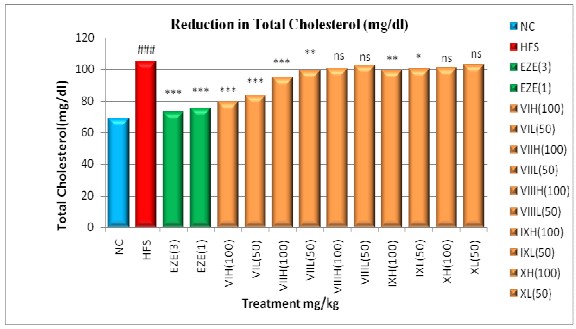


Figure - 4: Effect of the synthesized test compounds (VI–X) on serum total cholesterol levels of test animals.

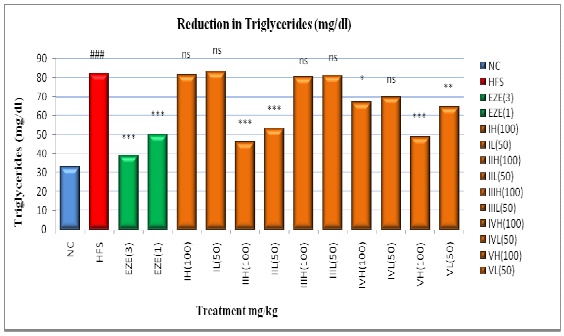


Figure - 5: Effect of the synthesized test compounds (I–V) on serum triglyceride levels of test animals.

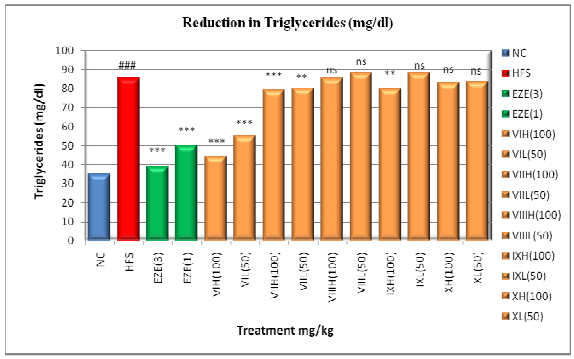


Figure - 6: Effect of the synthesized test compounds (VI-X) on serum triglyceride levels of test animals.

Table - 2: Effect of title compounds (I–V) on lipid profile in high fat diet fed hyperlipidemic Sprague Dawley rats (First Major group of compounds).

Groups	TC	TG	HDL	VLDL	LDL	AI	CRI
NC	67.27 ±3.47	33.07±2.29	47.34±5.32	6.61±0.45	13.31±7.78	0.34±0.19	1.49±0.20
HFS	102.8 ±1.62### (↑52.81 ±1.62)	82.26±2.35### (↑148.74±2.35)	28.86±1.24### (↓39.04 ±1.24)	16.46±0.47### (↑149.00±0.47)	57.51±2.12### (↑332.00±2.12)	2.00±0.13###	3.58±0.16###
EZE 3	73.23±2.17*** (↓28.76 ±2.17)	39.11±0.73*** (↓52.45 ±0.73)	45.07±0.52*** (↑56.16 ±0.52)	7.82±0.14*** (↓52.49±0.14)	20.34±2.59*** (↓64.63±2.59)	0.45±0.06***	1.62±0.06***
EZE 1	75.44±1.97*** (↓26.61±1.97)	50.26±0.97*** (↓38.90 ±0.97)	43.04±0.57*** (↑49.13 ±0.57)	10.05±0.19*** (↓38.94±0.19)	22.34±1.88*** (↓61.15±1.88)	0.52±0.04***	1.75±0.04***
IH	92.98 ±2.54ns (↓9.55 ±2.54)	81.53±2.31ns (↓0.88 ±2.31)	30.71±2.67ns (↑6.41 ±2.67)	16.31±0.46ns (↓0.91±0.46)	45.97±3.25ns (↓20.06±3.25)	1.55±0.21ns	3.09±0.25ns
IL	94.12 ± 1.58ns (↓8.44± 1.58)	83.18±2.15ns (↓1.11 ±2.15)	29.89±0.97ns (↑3.56 ±0.97)	16.64±0.43ns (↓1.09±0.43)	47.59±2.29ns (↓17.24±2.29)	1.6±0.11ns	3.16±0.13ns
IIH	77.91±1.56*** (↓24.21±1.56)	46.49±2.34*** (↓43.48 ±2.34)	41.62±1.022** (↑44.21±1.022)	9.29±0.46*** (↓43.56±0.46)	26.99±2.43*** (↓53.06±2.43)	0.65±0.07***	1.87±0.07***
IIL	79.82±1.39*** (↓22.35±1.39)	53.46±2.12*** (↓35.01 ±2.12)	39.56±0.22* (↑37.07 ±0.22)	10.69±0.42*** (↓35.05±0.42)	29.58±1.50*** (↓48.56±1.50)	0.74±0.03***	2.02±0.03***
IIIH	91.97±1.08ns (↓10.53±1.08)	80.45±3.24ns (↓2.18 ±3.24)	29.45±1.98ns (↑2.04 ±1.98)	16.09±0.65ns (↓2.24±0.65)	46.44±2.58ns (↓19.24±2.58)	1.61±0.17ns	3.16±0.22ns
IIIL	96.58±3.15ns (↓6.05±3.15)	81.14±3.39ns (↓1.36 ±3.39)	29.80±1.61ns (↑3.25 ±1.61)	16.23±0.67ns (↓1.39±0.67)	50.55±3.11ns (↓12.10±3.11)	1.70±0.10ns	3.25±0.10ns
IVH	79.77±1.78*** (↓22.40±1.78)	67.08±3.49* (↓18.45 ±3.49)	35.72±1.90ns (↑23.76 ±1.90)	13.42±0.69* (↓18.46±0.69)	30.64±0.40*** (↓46.72±0.40)	0.86±0.03***	2.24±0.06***
IVL	86.77±1.20** (↓15.59±1.20)	69.94±1.35ns (↓14.97 ±1.35)	31.34±1.85ns (↑8.59 ±1.85)	13.99±0.27ns (↓15.00±0.27)	41.44±2.56ns (↓27.94±2.56)	1.35±0.16*	2.80±0.19*
VH	81.99±2.24*** (↓22.24±2.24)	48.87±0.54*** (↓41.07 ±0.54)	40.37±0.91* (↑39.88 ±0.91)	9.77±0.10*** (↓40.64±0.10)	31.85±2.91*** (↓44.61±2.91)	0.79±0.08***	2.03±0.09***
VL	90.34±4.24* (↓12.12±4.24)	64.58±7.08** (↓21.49 ±7.08)	39.19±1.59* (↑35.79 ±1.59)	12.92±1.41*** (↓21.50±1.41)	38.24±5.35* (↓33.50±5.35)	0.98±0.16***	2.31±0.16***

*Each value mean presents the ± S.E.M. of six observations by ANOVA followed by Tukey's test, ###P<0.001 statistically significance as compared to Normal control group. *P<0.05, **P<0.01, ***P<0.001 statistical significance as compared to cholesterol fed diet group Values in Parenthesis indicates, ↓- % Reduction and ↑- % Rise.*

H - High Dose (100mg/kg), **L -** Low Dose (50mg/kg), **CFD**-Cholesterol Fed Diet Group, **EZ**-Ezetimibe, **TC**-Total Cholesterol, **TG-**Triglyceride, **HDL-**High Density Lipoprotein, **LDL-**Low Density Lipoprotein, **VLDL**-Very Low nsity Lipoprotein, **AI**-Atherogenic Index, **CRI**-Coronary Risk Index, **NC**-Normal control, **HFS-** High fat substance, **ns-** non significant.

Table - 3: Effect of title compounds (VI–X) on lipid profile in high fat diet fed hyperlipidemic Sprague Dawley rats (second Major group of compounds).

Group (mg/kg)	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	AI (mg/dl)	CRI (mg/dl)
NC	69.21 ±1.58	34.96 ±0.38	45.84 ±1.55	6.99±0.07	16.38±2.38	0.36±0.064	1.51±0.06
HFS	105.1 ±1.41### (↑51.85 ±1.41)	85.42 ±1.51### (↑144.33±1.51)	30.23 ±0.09### (↓34.06 ±0.09)	17.09±0.30### (↑144.34±0.30)	57.81±1.48### (↑252.93±1.4)	1.91±0.045###	3.47±0.039###
EZE 3	73.23 ±2.17*** (↓30.32 ±2.17)	39.11 ±0.73*** (↓54.21 ± 0.73)	45.07 ±0.52*** (↑49.09 ±0.52)	7.82±0.14*** (↓54.21±0.14)	20.34±2.59*** (↓64.81±2.59)	0.45±0.063***	1.62±0.06***
EZE 1	75.44 ±0.52*** (↓28.22 ±0.52)	50.26±0.38*** (↓41.16 ±0.38)	43.04 ±0.57*** (↑42.37 ±0.57)	10.05±0.07*** (↓41.15±0.07)	22.34±0.92*** (↓61.35±0.92)	0.51±0.027***	1.75±0.03***
VIH	79.56 ±0.72*** (↓24.30 ±0.72)	44.31 ±0.21*** (↓48.13 ±0.21)	37.50 ±0.31*** (↑24.40 ±0.31)	8.86±0.04*** (↓48.15±0.04)	33.20±0.55*** (↓42.57±0.55)	0.88±0.013***	2.12±0.01***
VIL	83.76 ± 0.47*** (↓20.30± 0.47)	55.05 ±0.45*** (↓35.55 ±0.45)	34.86 ±0.16** (↑15.31 ±0.16)	11.01±0.09*** (↓35.53±0.09)	37.90±0.42*** (↓34.44±0.42)	1.088±0.013***	2.40±0.01***
VIIH	94.74 ±0.48*** (↓9.85 ±0.48)	79.36 ±0.26*** (↓7.09 ±0.26)	34.73 ±0.08** (↑14.88 ±0.08)	15.87±0.05*** (↓7.08±0.05)	44.13±0.58*** (↓23.66±0.58)	1.27±0.019***	2.73±0.01***
VIIL	99.15 ±0.34** (↓5.66 ±0.34)	80.26 ±0.32** (↓6.04 ±0.32)	33.90 ±0.12* (↑12.14 ±0.12)	16.05±0.06** (↓6.03±0.06)	49.20±0.36** (↓14.89±0.36)	1.45±0.012***	2.92±0.01***
VIIIH	100.5 ±0.42ns (↓4.37 ±0.42)	85.37 ±0.43ns (↓0.05 ±0.43)	29.08 ±0.29ns (↓ 3.80 ±0.29)	17.08±0.08 ns (↓0.00±0.08)	54.38±0.65 ns (↓5.93±0.65)	1.87±0.04 ns	3.46±0.05 ns
VIIIL	102.5 ±0.70ns (↓2.47 ±0.70)	88.26 ±0.46 ns (↑3.32 ±0.46)	29.76 ±0.32ns (↓1.55 ±0.32)	17.65±0.09 ns (↑3.33±0.09)	55.12±0.88 ns (↓4.65±0.88)	1.85±0.045 ns	3.44±0.04 ns
IXH	99.18 ±0.40** (↓5.63 ±0.40)	79.77 ±0.63** (↓6.61 ±0.63)	33.83 ±0.22* (↑11.90 ±0.22)	15.96±0.12** (↓6.55±0.12)	49.40±0.15** (↓14.54±0.15)	1.45±0.006***	2.93±0.01***
IXL	99.98 ±0.38* (↓4.87 ±0.38)	88.41 ±1.67ns (↑3.50 ±1.67)	28.67 ±0.52ns (↓5.16 ±0.52)	17.68±0.33 ns (↑3.51±0.33)	53.64±0.74 ns (↓7.21±0.74)	1.87±0.058 ns	3.49±0.06 ns
XH	101.0 ±1.05ns (↓3.90 ±1.05)	82.87 ±0.45ns (↓2.98 ±0.45)	31.12 ±0.49 ns (↑2.94 ±0.49)	16.58±0.09 ns (↓2.92±0.09)	53.28±0.90 ns (↓7.83±0.90)	1.71±0.037 ns	3.24±0.04 ns
XL	102.9 ±0.45ns (↓2.09 ±0.45)	83.7 ±1.65ns (↓2.01 ±1.65)	27.76 ±1.48 ns (↑8.17 ±1.48)	16.75±0.33 ns (↓1.93±0.33)	58.44±1.93 ns (↑1.08±1.93)	2.13±0.19 ns	3.74±0.21 ns

*Each value mean presents the ± S.E.M. of six observations by ANOVA followed by Tukey's test, ###P<0.001 statistically significance as compared to Normal control group. *P<0.05, **P<0.01, ***P<0.001 statistical significance as compared to cholesterol fed diet group Values in Parenthesis indicates, ↓- % Reduction and ↑- % Rise.*

H - High Dose (100mg/kg), **L -** Low Dose (50mg/kg), **CFD**-Cholesterol Fed Diet Group, **EZ**-Ezetimibe, **TC**-Total Cholesterol, **TG-**Triglyceride, **HDL-**High Density Lipoprotein, **LDL-**Low Density Lipoprotein, **VLDL**-Very Low Density Lipoprotein, **AI**-Atherogenic Index, **CRI**-Coronary Risk Index, **NC**-Normal control, **HFS**- High fat substance, **ns-** non significant.

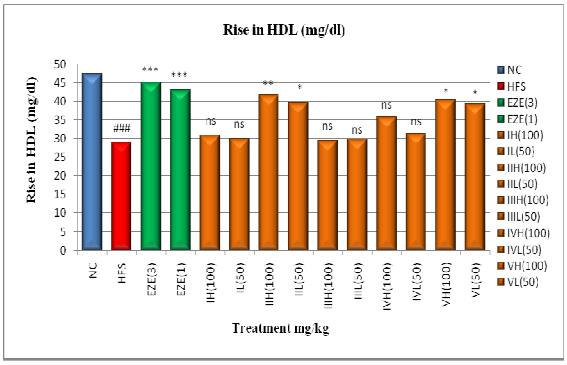


Figure - 7: Effect of the synthesized test compounds (I–V) on serum HDL levels of test animals.

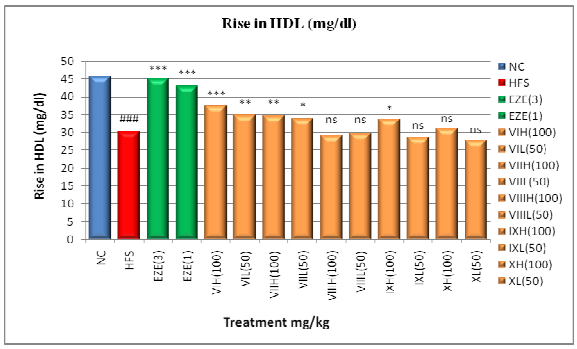


Figure - 8: Effect of the synthesized test compounds (VI-X) on serum HDL levels of test animals.

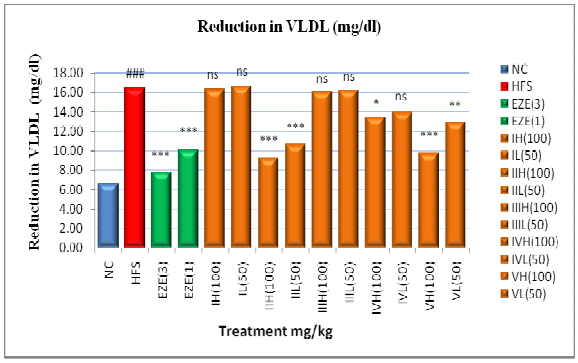


Figure - 9: Effect of the synthesized test compounds (I–V) on serum VLDL levels of test animals.

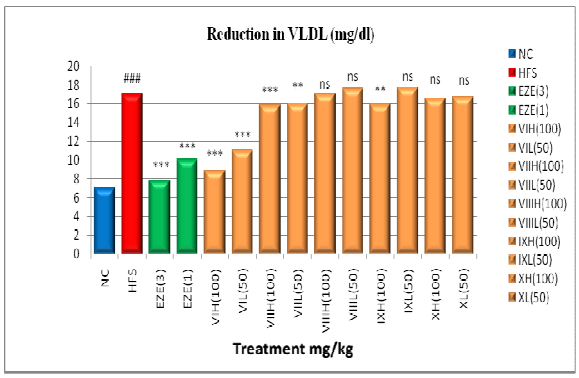


Figure - 10: Effect of the synthesized test compounds (VI-X) on serum VLDL levels of test animals.

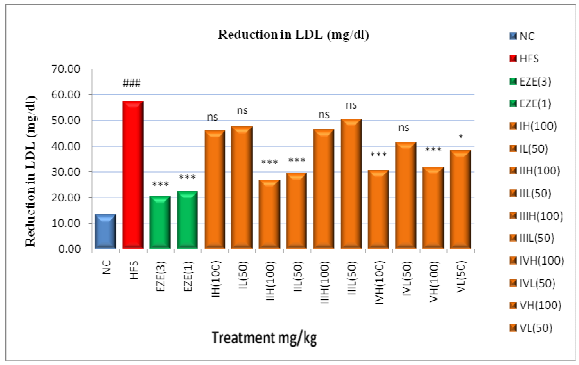


Figure - 11: Effect of the synthesized test compounds (I–V) on serum LDL levels of test animals.

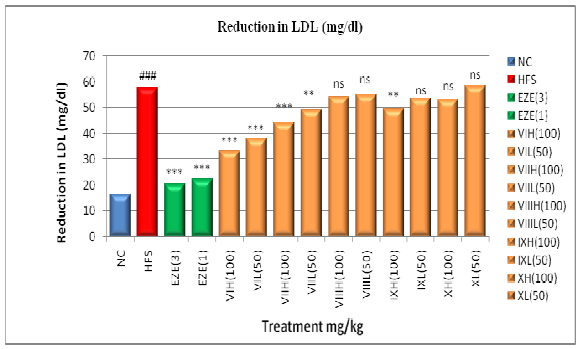


Figure - 12: Effect of the synthesized test compounds (VI–X) on serum LDL levels of test animals.

3.6. Structure activity relationship

- The synthesized compound showed good Lipid Lowering activity by significantly affecting the lipid profile (decreasing total cholesterol, triglycerides and increasing the HDL levels) of the test animals.
- The Nicotinic acid hydrazide substitution at N 1 position of basic scaffold (2-Azetidinone) showed decrease in total cholesterol, triglycerides as well as increase in the HDL levels.
- The steric bulky substitution at C_4 position in Azetidinone ring such as phenyl group bearing an electronegative atom like chlorine and methoxy group at *meta* position led to significant increase in HDL levels and also decrease in total cholesterol and triglycerides levels.

4. CONCLUSION

In summary, the newly synthesized 2-Azetidinones **(I–X)** were evaluated for Lipid Lowering activity. Though most of the compounds showed good lipid lowering activity by significantly affecting the lipid profile of the test animals, compounds **II, V, VI** and **VII** showed the best activity in reducing serum cholesterol and triglyceride levels. Compound **II** showed most significant HDL enhancing effects. The Chloro

substitution on 3-position of basic scaffold has resulted in decrease in total cholesterol, triglycerides as well as increasing the HDL levels due to its high electronegativity. N-N linkage has been used as a key structural motif in present structures as bioactive agents. Aniline might be less active if we use it in the place of acid hydrazide for schiff 's base synthesis. Overall, the title compounds **II, V, VI** and **VII** can be looked upon as potential leads for further development& investigations.

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