Full Paper

Design, Synthesis, and Antihyperlipidemic Evaluation of Novel 2-[1-(Substitutedphenyl)-4-oxo-azetidin-2-yl]-5,6-disubstituted-thieno[2,3-*d*]pyrimidin-4(3*H*)-ones

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Novel thienopyrimidine derivatives of azetidinone possessing the combined features of the cholesterol absorption inhibitor drug ezetimibe and potential antihyperlipidemic 2-substitutedthienopyrimidin-4-ones were synthesized and characterized by spectroscopic data and elemental analysis. These compounds were evaluated for their lipid-lowering activity in Wistar albino rats. Some of them showed significant lipid-lowering effects comparable to those of the standard drug, gemfibrozil, at the same dose levels.

Keywords: Antihyperlipidemic / Azetidinone / Gewald / Thienopyrimidines

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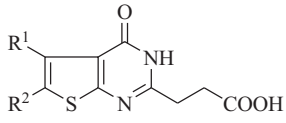
Introduction

Congestive heart disease (CHD) and stroke remain the leading causes of death and disability across the world. In both cases, the risk factor is directly related to atherosclerosis [1], which in turn is linked to increased levels of lipids, mainly the cholesterol and triglycerides (Tg) in the blood.

Though many drug therapies are available in the form of the fibrates, lipid-lowering agents (LLA), including the statins (HMG CoA reductase inhibitors), as well as the recently introduced cholesterol absorption inhibitors drugs like ezetimibe, a few limitations and drawbacks with these drugs still continue to plague the current therapy for hyperlipidemia, with one or more of the problems; presently, no good drugs are available for covering the hitherto untreatable cases of type II hyperlipidemia, wherein drugs like clofibrate, nicotinic acid, D-thyroxin, etc. are used without much success. Also no drugs capable of blocking the stimuli that lead to the formation of an atherosclerotic lesion are available. New drugs needed to be developed, to bring about regression of the already existing atherosclerotic lesions and the most widely used "statins" also suffer from limitations like cost, intolerance, and adverse effects, ineffective, or only partially effective in lowering of cholesterol levels and thus capable of reducing only up to 40% risk of CHD.

Development of newer LLA with mechanism of action distinct from statins are now required to achieve target cholesterol and Tg levels in many individuals [2]. Antihyperlipidemic activity has been reported in some thieno[2,3-*d*]-pyrimidine derivatives, **1**–**4** [3–6]. The detail pharmacokinetics and pharmacodynamics studies on compound **4** suggested the gastrointestinal tract to be the primary site of action for its antihyperlipidemic activity [7].



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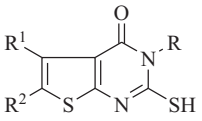

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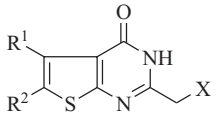

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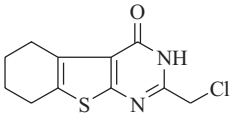

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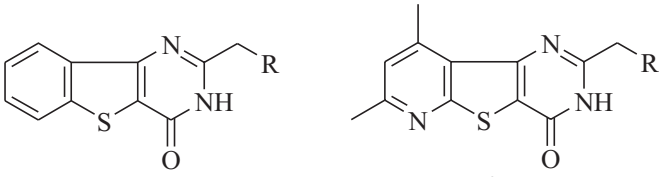
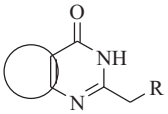
Further, a detailed QSAR study, of isomeric 2-substituted-thieno[3,2-*d*]pyrimidin-4(3*H*)-ones **5** and **6**, indicated the direct positive influence of the electronic nature of the 2-substituents of these compounds on their antihyperlipidemic activity [8]. Thus, the mechanism of action of these compounds appears to be through the inhibition of cholesterol absorption through the gastrointestinal tract.



5 **6**

R = H, Cl, OCH_3, OC_2H_5, OH

The condensed 2-substituted alkylpyrimidine nucleus, **7**, appears to be a potential pharmacophore for antihyperlipidemic activity.



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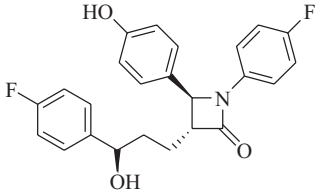
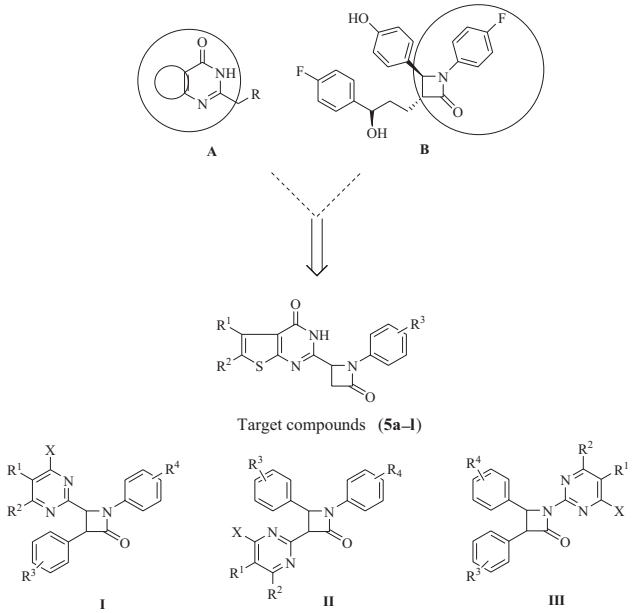
Recently, more potent analogs of **4** have been prepared through subtle manipulation of the substituent at the 2- and 4-positions of the condensed 2-substituted pyrimidine pharmacophore, **7**, and evaluated in hyperlipidemic rats to give results comparable to gemfibrozil and ezetimibe [9, 10].

Ezetimibe, chemically an azetidinone, is a selective cholesterol absorption inhibitor, blocking Niemann-Pick C1-Like1 (NPC1L1) protein involved in cholesterol absorption in the proximal part of small intestine [11, 12]. It reduces LDL-C between 10–19% in monotherapy. Interestingly, the reduction of LDL-C in combination of ezetimibe with a statin (simvastatin or atorvastatin) is additive. It has opened the doors for the design and synthesis of new potential cholesterol absorption inhibiting drugs, in this class of compounds.

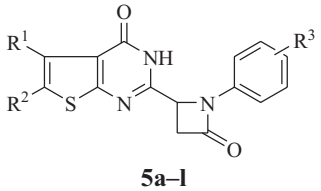


Ezetimibe

Rational designing approaches in which structural features from selected ligands are combined into one single entity have produced ligands that act on a variety of targets [13]. The so called dual acting drugs can be designed by overlapping the pharmacophores of two drugs into a single chemical entity by taking advantage of common structural features of two or more classes of drugs. There are numerous examples of hybrid molecules wherein dual bioactivities have been packed into a single chemical entity [13]. So it was thought to logically hybridize these two potential pharmacophores, i.e., 2-substituted methylthienopyrimidin-4(3*H*)-one (**A**) and ezetimibe (**B**), and evaluate the new hybrid structures in the series for their lipid-lowering activities. Further, a systematic probe into the synthesis and evaluation in the series **I–III** for their lipid-lowering properties and detailed QSAR studies was planned.

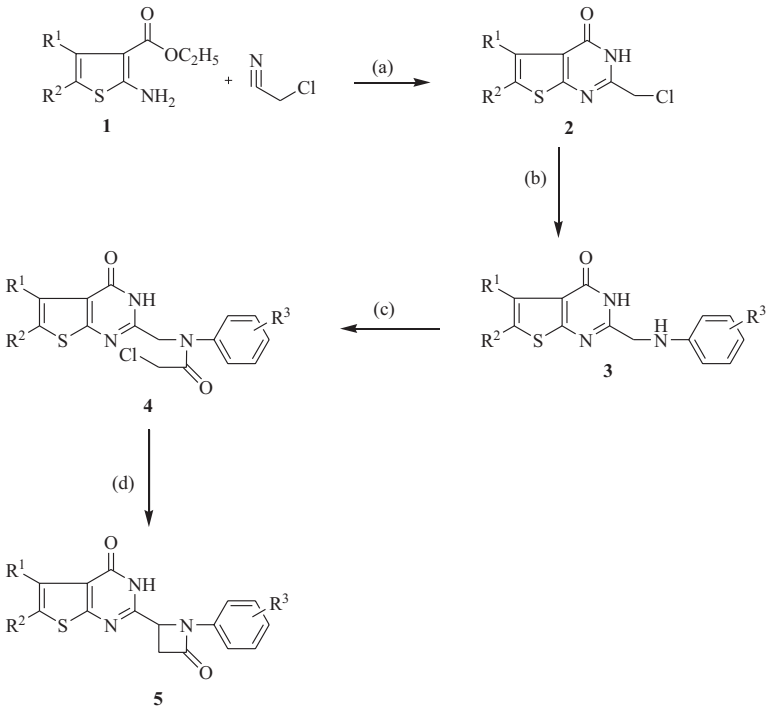


Thus, with this broad goal in mind initially, we have completed the work on series of 2-[1-(substitutedphenyl)-4-azetidin-2-yl]-5,6-disubstitutedthieno[2,3-*d*]pyrimidin-4-ones (**5a–l**), which were synthesized and their ability to inhibit cholesterol absorption was evaluated.



5a–l

where R^1, R^2 = aryl, cycloalkyl, H, etc.; R^3 = H, alkyl, halo, etc.



Where, R^1, R^2 = -$(CH_2)_4$-, -$(CH_2)_5$-, 4-ClC_6H_5;
R^3 = H, 2-CH_3, 3-CH_3, 4-CH_3, 4-OCH_3, 4-F, 4-Br.

Scheme 1. Reagents and conditions: (a) conc. HCl/MWI; (b) Ar–NH_2, DMF/reflux; (c) $ClCH_2COCl$, K_2CO_3, CH_2Cl_2/RT; (d) NaH/DMSO 60–65°C, CH_2Cl_2/RT.

Results and discussion

Chemistry

The title compounds, **5a–l**, were prepared through a 4-step synthetic protocol as depicted in Scheme 1. The starting materials, thiophene-*o*-aminoesters (**1a**, **1b**, and **1c**, Scheme 1, Table 1) were chosen as the substrates and are often referred to as "Gewalds" after the reported method given by Gewald et al. [14]. The first step involved the cyclocondensation of **1**, with chloroacetonitrile under acidic conditions and MWI, to yield the corresponding condensed 2-chloromethylpyrimidin-4-ones (**2a**, **2b**, and **2c**, Table 2) in good yields by a procedure reported by us [15]. The second step involved the nucleophilic displacement of the chlorine of the 2-chloromethyl group with various substituted anilines in refluxing DMF in presence of three to four drops of conc. HCl as a catalyst,

Table 1. Physical data of synthesized compounds **1a–c**.

Compound	R^1	R^2	Molecular formula	Molecular weight	Melting point (°C)
1a	$-(CH_2)_4-$		$C_{11}H_{15}NO_2S$	225.31	110–112
1b	4-ClC_6H_4	–H	$C_{13}H_{12}ClNO_2S$	281.76	102–104
1c	$-(CH_2)_5-$		$C_{12}H_{17}NO_2S$	239.33	115–118

Table 2. Physical data of synthesized compounds **2a–c**.

Compound	R^1	R^2	Molecular formula	Molecular weight	Melting point (°C)
2a	$-(CH_2)_4-$		$C_{11}H_{11}ClN_2OS$	254.74	275–277
2b	4-ClC_6H_4	–H	$C_{13}H_8Cl_2N_2OS$	311.19	233–234
2c	$-(CH_2)_5-$		$C_{12}H_{13}ClN_2OS$	268.76	261–265

Table 3. Physical data of synthesized compounds **3a–l**.

Compound	R^1	R^2	R^3	Molecular formula	Molecular weight	Melting point (°C)
3a	$-(CH_2)_4-$		−H	$C_{17}H_{17}N_3OS$	311.4	186–190
3b	$-(CH_2)_4-$		2-CH_3	$C_{18}H_{19}N_3OS$	325.43	220–223
3c	$-(CH_2)_4-$		4-CH_3	$C_{18}H_{19}N_3OS$	325.43	160–162
3d	$-(CH_2)_4-$		4-F	$C_{17}H_{16}FN_3OS$	329.39	150–152
3e	4-ClC_6H_4	−H	−H	$C_{19}H_{14}ClN_3OS$	367.85	75–77
3f	4-ClC_6H_4	−H	2-CH_3	$C_{20}H_{16}ClN_3OS$	381.88	111–115
3g	4-ClC_6H_4	−H	4-CH_3	$C_{20}H_{16}ClN_3OS$	381.88	170–172
3h	4-ClC_6H_4	−H	4-F	$C_{19}H_{13}ClFN_3OS$	385.84	85–88
3i	$-(CH_2)_4-$		3-CH_3	$C_{18}H_{19}N_3OS$	325.43	152–155
3j	$-(CH_2)_4-$		4-OCH_3	$C_{18}H_{19}N_3O_2S$	341.43	171–175
3k	$-(CH_2)_4-$		4-Br	$C_{17}H_{16}BrN_3OS$	390.3	140–143
3l	$-(CH_2)_5-$		4-F	$C_{18}H_{18}FN_3OS$	343.42	180–183

Table 4. Physical data of synthesized compounds **4a–l**.

Compound	R^1	R^2	R^3	Molecular formula	Molecular weight	Melting point (°C)
4a	$-(CH_2)_4-$		−H	$C_{19}H_{18}ClN_3O_2S$	387.88	96–100
4b	$-(CH_2)_4-$		2-CH_3	$C_{20}H_{20}ClN_3O_2S$	401.91	203–205
4c	$-(CH_2)_4-$		4-CH_3	$C_{20}H_{20}ClN_3O_2S$	401.91	170–172
4d	$-(CH_2)_4-$		4-F	$C_{19}H_{17}ClFN_3O_2S$	405.87	125–127
4e	4-ClC_6H_4	−H	−H	$C_{21}H_{15}Cl_2N_3O_2S$	444.33	125–130
4f	4-ClC_6H_4	−H	2-CH_3	$C_{22}H_{17}Cl_2N_3O_2S$	458.36	80–82
4g	4-ClC_6H_4	−H	4-CH_3	$C_{22}H_{17}Cl_2N_3O_2S$	458.36	85–87
4h	4-ClC_6H_4	−H	4-F	$C_{21}H_{14}Cl_2FN_3O_2S$	462.32	92–94
4i	$-(CH_2)_4-$		3-CH_3	$C_{20}H_{20}ClN_3O_2S$	401.91	150–154
4j	$-(CH_2)_4-$		4-OCH_3	$C_{20}H_{20}ClN_3O_3S$	417.91	110–113
4k	$-(CH_2)_4-$		4-Br	$C_{19}H_{17}BrClN_3O_2S$	466.78	132–135
4l	$-(CH_2)_5-$		4-F	$C_{20}H_{19}ClFN_3O_2S$	419.9	108–111

to afford the requisite 2-substitutedphenylaminomethyl-5,6-disubstitutedthieno[2,3-*d*]pyrimidin-4-ones (**3a–l**, Table 3) in good yields. These intermediates were further *N*-acylated using chloroacetyl chloride under anhydrous conditions to yield **4a–l** (Scheme 1, Table 4), which on further cyclization under basic conditions afforded the target molecules (**5a–l**, Scheme 1, Table 5). The structural assignments of the intermediates **2–4** and target compounds **5a–l** are based on the correct spectral data and elemental analysis of these compounds.

Pharmacological activity

Hyperlipidemia is a condition characterized by increased concentration of lipids (triglyceride, cholesterol) and

Table 5. Physical data of synthesized compounds **5a–l**.

Compound	R^1	R^2	R^3	Molecular formula[a)]	Molecular weight	Melting point (°C)
5a	$-(CH_2)_4-$		−H	$C_{19}H_{17}N_3O_2S$	351.42	70–72
5b	$-(CH_2)_4-$		2-CH_3	$C_{20}H_{19}N_3O_2S$	365.45	210–212
5c	$-(CH_2)_4-$		4-CH_3	$C_{20}H_{19}N_3O_2S$	365.45	182–185
5d	$-(CH_2)_4-$		4-F	$C_{19}H_{16}FN_3O_2S$	369.41	206–208
5e	4-ClC_6H_4	−H	−H	$C_{21}H_{14}ClN_3O_2S$	407.87	190–192
5f	4-ClC_6H_4	−H	2-CH_3	$C_{22}H_{16}ClN_3O_2S$	421.9	180–182
5g	4-ClC_6H_4	−H	4-CH_3	$C_{22}H_{16}ClN_3O_2S$	421.9	186–188
5h	4-ClC_6H_4	−H	4-F	$C_{21}H_{13}ClFN_3O_2S$	425.86	179–182
5i	$-(CH_2)_4-$		3-CH_3	$C_{20}H_{19}N_3O_2S$	365.45	140–143
5j	$-(CH_2)_4-$		4-OCH_3	$C_{20}H_{19}N_3O_3S$	381.45	135–138
5k	$-(CH_2)_4-$		4-Br	$C_{19}H_{16}BrN_3O_2S$	430.32	126–130
5l	$-(CH_2)_5-$		4-F	$C_{20}H_{18}FN_3O_2S$	383.44	130–133

[a)] Satisfactory elemental analysis (±0.4 of the calculated values of % C, % H, and % N obtained).

Table 6. Biological data profile of synthesized compounds (**5a–h**).

Compound	R^1	R^2	R^3	% Reduction in total cholesterol (mg/dL)	% Reduction in total triglyceride (mg/dL)	% Change in total HDL (mg/dL)
5a	$-(CH_2)_4-$		–H	33.50 ± 3.35	22.97 ± 3.09	31.87 ± 2.36
5b	$-(CH_2)_4-$		2-CH_3	46.83 ± 3.01	67.42 ± 2.70	27.49 ± 2.40
5c	$-(CH_2)_4-$		4-CH_3	7.15 ± 1.84	54.77 ± 3.25	33.17 ± 2.98
5d	$-(CH_2)_4-$		4-F	24.83 ± 2.21	41.47 ± 2.55	19.14 ± 2.24
5e	4-ClC_6H_4	–H	–H	38.16 ± 2.13	48.22 ± 2.82	19.90 ± 3.12
5f	4-ClC_6H_4	–H	2-CH_3	19.77 ± 3.01	72.62 ± 2.84	28.87 ± 1.86
5g	4-ClC_6H_4	–H	4-CH_3	26.37 ± 1.99	44.81 ± 2.66	27.62 ± 2.72
5h	4-ClC_6H_4	–H	4-F	22.74 ± 1.63	23.30 ± 2.55	24.21 ± 2.73
Std	Gemfibrozil			42.42 ± 1.2	37.57 ± 1.68	35.1 ± 1.3

Results are expressed as mean ± standard error, statistically significant ($p < 0.05$, *t*-test, $n = 6$). The results of the eight compounds selected for screening were correlated statistically. Triton WR 1339 model with regression $r = 0.9$, i.e., showed good correlation.

lipoprotein (LDL and VLDL) in the blood. Triton WR 1339 induced hyperlipidemia in Wistar albino rat [16] was the model used for the biological evaluation of title compounds. This model is less time consuming, rapid, robust, and requires smaller quantity of test compounds for evaluation. The lipid profile (cholesterol, triglycerides, and HDL) for the hyperlipidemic and control Wistar albino rats were studied with oral administration of selected test compounds (**5a–h**, Table 6). Total eight test compounds were evaluated and, thus, eight test groups were involved in the study. Besides this, gemfibrozil was administered to the standard group. Further, two more animal groups were taken, the control group and the cholesterol control group, to assess and compare the effects of cholesterol and the test compounds as well as standard drug, on the lipid levels of test animals in the study.

It was found from the results that the test compounds showed significant changes in lipid profile, i.e., decrease in total cholesterol, triglycerides and increase in HDL at dose of 400 mg/kg body weight p.o. as compared to the hyperlipidemic group (Figs. 1–3).

The compounds **5b**, **5e**, and **5a** have shown good reduction in % cholesterol level showing 46.83 ± 3.01%, 38.16 ± 2.13%, and 33.50 ± 3.35%, respectively, which is comparable to the standard 42.42 ± 1.2% (Fig. 1). The test compounds **5f**, **5b**, **5c**, **5e**, **5g**, and **5d** have shown good ability in reducing the serum triglyceride levels in the test animals; i.e., % reduction 72.62 ± 2.84, 67.42 ± 2.70, 54.77 ± 3.25, 48.22 ± 2.82, 44.81 ± 2.66, 41.47 ± 2.55, respectively, as compared to standard 37.57 ± 1.68% (Fig. 2).

The test compounds **5c** and **5a** have elevated the serum HDL levels (31.87 ± 2.36% and 33.17 ± 2.98%, respectively) though slightly less than the standard (35.1 ± 1.3%) (Fig. 3).

Conclusion

Some novel 2-(*N*-aryl-4-oxo)azetidinyl-5,6-disubstitutedthieno-[2,3-*d*]pyrimidin-4(3*H*)-one derivatives (**5a–l**) were synthesized and were screened in Triton WR 1339-induced hyperlipidemic rats, for antihyperlipidemic activity. The present investigation showed significant antihyperlipidemic activity to some of these compounds, comparable to the standard drug, gemfibrozil. Compound **5b** was found to be the most active of all the compounds, exhibiting significant lipid-lowering effects like reducing serum levels of cholesterol and triglycerides in test animals. Another derivative, compound **5c** significantly elevated the serum HDL levels in test animals.

Hence the present series could be developed and evaluated to obtain potential antihyperlipidemic agents. However, further structural modifications are planned towards lead optimization.

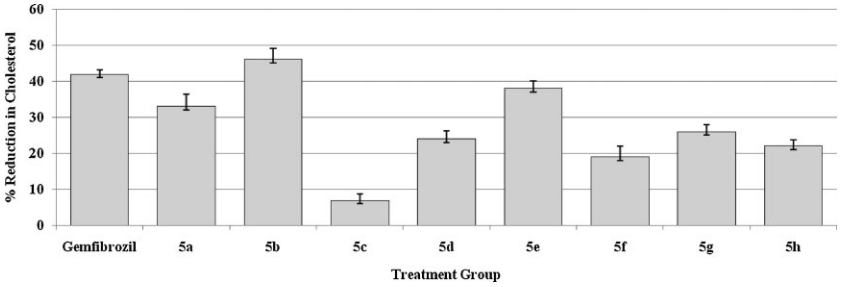


Figure 1. % Reduction in total serum cholesterol levels.

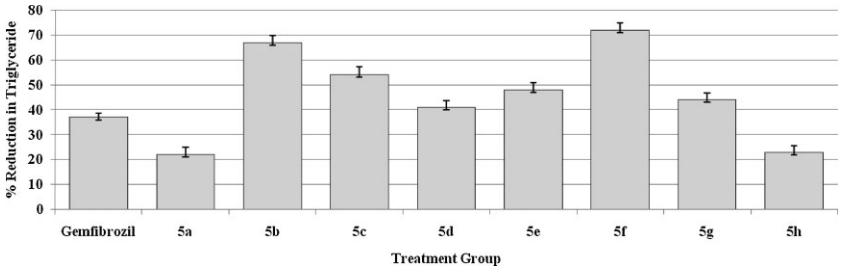


Figure 2. % Reduction in serum triglyceride levels.

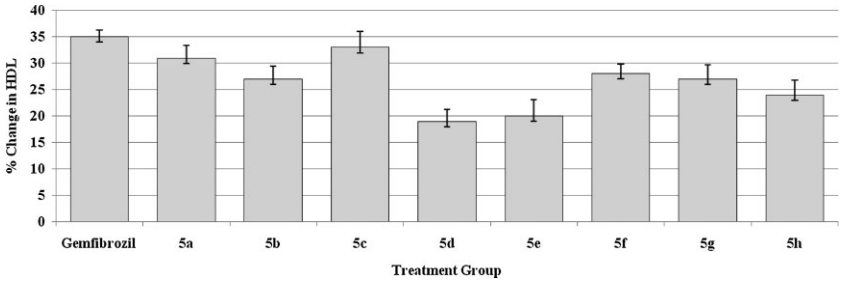


Figure 3. % Change in serum HDL level.

Experimental

General

All the synthesized compounds were characterized by spectral data (UV, IR, mass, and ^{1}H NMR). The ultraviolet (UV) absorption spectra were determined in methanol on JASCO V-530, UV–Visible double beam spectrophotometer (Easton, USA). The IR spectra of the synthesized compounds were recorded on Perkin Elmer BX-FTIR spectrophotometer (Massachusetts, USA) in potassium bromide discs. The ^{1}H NMR were taken on a NMR Varian Mercury YH-300 MHz spectrometer (California, USA) using $CDCl_3$ as solvent and TMS as an internal standard (chemical shift in δ ppm). Mass spectra were obtained on a Shimadzu GCMS-QP2010 spectrometer (Kyoto, Japan). The purity of the compounds was monitored by elemental analysis and thin-layer chromatography. Elemental analyses for compounds were obtained using a Flash EA 1112 Thermofinnigan instrument (San Diego, USA). TLC silica gel 60 F_{254} plates (200 mm; Merck, Darmstadt, Germany) were used to assess preliminary purity of compounds. All melting points were taken in open capillaries using a Veego electronic melting point apparatus model MP-D (Mumbai, India) containing silicon oil bath with stirrer and are uncorrected.

Triton WR 1339 (Sigma, Bangalore, India) were procured commercially. The total lipid profile was determined by using infinite liquid cholesterol solution ready to use diagnostic kits (Accurex India Biomedicals, Mumbai, India). All the biological activity carried out in this study was approved by the Institutional Animal Ethics Committee (SCOP/IAEC/Approval/2008-09/24).

Chemistry

The starting materials, thiophene-*o*-aminoesters (**1a**, **1b**, and **1c**) and the condensed 2-chloro-methylpyrimidin-4-ones (**2a**, **2b**, and **2c**) were prepared as per reported methods [14, 15]. The synthetic steps a, b, and c are based on literature reported procedures [5, 17, 18].

*General procedure for the preparation of compounds **3a–l***

The reaction mixture of the condensed 2-chloromethylpyrimidin-4-one (**2**) (0.01 mol), aryl amine (0.015 mol), and three to four drops of conc. HCl in DMF (20 mL) was refluxed for 8–10 h. On completion of reaction (TLC), the reaction mixture was cooled and poured onto ice-water mixture (50 mL). The solid separated was washed with dilute HCl followed by water and filtered under vacuum, dried, and recrystallized to give the desired compound **3**.

*General procedure for the preparation of compounds **4a–l***

To a vigorously stirred mixture of **3** (0.01 mol) and activated potassium carbonate (0.01 mol) in anhydrous DCM (50 mL) at RT was added chloroacetyl chloride (0.015 mol) dropwise over 30 min. On completion of the reaction (TLC), the reaction mixture was poured onto ice-water (100 mL), the organic layer separated and was washed successively with aqueous sodium bicarbonate solution (10% w/v), brine solution, and water. The dried organic layer (Na_2SO_4) was distilled (vacuum) and the product was recrystallized to give the desired compound **4**.

*General procedure for the preparation of compounds **5a–l***

Sodium hydride (50% w/w, 0.6 g, 0.02 mol) in DMSO (10 mL) was warmed on oil bath at 60–65°C under vigorous stirring until the color of the solution turned light brown and then cooled to RT. To the above suspension, the solution of **4** (0.01 mol) in DCM (30 mL) was added dropwise with stirring. The reaction mixture was further stirred till the completion of reaction (TLC) and thereafter quenched with water. The organic layer was washed with dilute HCl. The dried organic layer (Na_2SO_4) was distilled (vacuum) and the product was recrystallized to give the desired compound **5**.

*2-[1-Phenyl-4-oxo-azetidin-2-yl]-5,6,7,8-tetrahydrobenzo-(b)thieno[2,3-d]pyrimidin-4(3H)-one (**5a**)*

IR (KBr): 3310, 2926, 2854, 1754, 1682, 1450, 1240, 1034 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.04 (s, 1H, *NH*), 7.11–7.62 (m, 5H,

ArH), 4.24 (m, 1H, *CH* at azetidinyl-2), 3.65 (d, 2H, CH_2 at azetinidinyl-3), 2.66–2.75 (t, 4H, CH_2 at 5 and 8), 2.34–2.41 (m, 4H, CH_2 at 6 and 7); MS (TOF): $m/z = 352$ (M+1); $C_{19}H_{17}N_3O_2S$ (351.42); requires (Found): C, 64.94 (64.57); H, 4.88 (4.66); N, 11.96 (11.68).

*2-[1-(2-Methylphenyl)-4-oxo-azetidin-2-yl]-5,6,7,8-tetrahydrobenzo(b)thieno[2,3-d]pyrimidin-4(3H)-one (**5b**)*

IR (KBr): 3248, 2932, 2856, 1748, 1686, 1532, 1492, 1237, 1194, 1155, 1036, 966 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.2–7.8$ (m, 5H, *ArH* and *NH*), 3.72–3.73 (m, 1H, *CH* at azetidinyl-2), 3.54–3.59 (d, 2H, CH_2 at azetinidinyl-3), 2.88–3.02 (m, 4H, CH_2 at 5 and 8), 2.68–2.72 (s, 3H, CH_3), 2.2–2.34 (m, 4H, CH_2 at 6 and 7); MS (TOF): $m/z = 366$ (M+1); $C_{20}H_{19}N_3O_2S$ (365.45); requires (Found): C, 65.73 (66.02); H, 5.24 (5.61); N, 11.50 (11.37).

*2-[1-(4-Methylphenyl)-4-oxo-azetidin-2-yl]-5,6,7,8-tetrahydrobenzo(b)thieno[2,3-d]pyrimidin-4(3H)-one (**5c**)*

IR (KBr): 3275, 2929, 1676, 1608, 1452, 1210, 1035, 975 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 6.93–7.67$ (m, 5H, *ArH* and *NH*), 3.24–3.30 (m, 1H, *CH* at azetidinyl-2), 2.8–2.92 (d, 2H, CH_2 at azetinidinyl-3), 2.67–2.70 (s, 3H, CH_3), 2.55–2.63 (m, 4H, CH_2 at 5 and 8), 1.83–1.94 (m, 4H, CH_2 at 6 and 7); $C_{20}H_{19}N_3O_2S$ (365.45); requires (Found): C, 65.73 (65.52); H, 5.24 (5.43); N, 11.50 (11.64).

*2-[1-(4-Fluorophenyl)-4-oxo-azetidin-2-yl]-5,6,7,8-tetrahydrobenzo(b)thieno[2,3-d]pyrimidin-4(3H)-one (**5d**)*

IR (KBr): 2937, 2856, 1752, 1683, 1509, 1448, 1231, 1155, 1034, 966, 840 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.14–7.77$ (m, 5H, *ArH* and *NH*), 3.19–3.33 (m, 1H, *CH* at azetidinyl-2), 2.73–2.77 (d, 2H, CH_2 at azetinidinyl-3), 2.15–2.26 (m, 4H, CH_2 at 5 and 8), 1.7–2.0 (m, 4H, CH_2 at 6 and 7); MS (TOF): $m/z = 370$ (M+1); $C_{19}H_{16}FN_3O_2S$ (369.41); requires (Found): C, 61.78 (62.07); H, 4.37 (4.70); N, 11.37 (11.75).

*5-(4-Chlorophenyl)-2-[1-phenyl-4-oxo-azetidin-2-yl]-thieno[2,3-d]pyrimidin-4(3H)-one (**5e**)*

IR (KBr): 3362, 2921, 1670, 1596, 1394, 958, 720 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 8.12$ (s, 1H, *NH*), 6.9–7.83 (m, 10H, *ArH* and *H* at 6), 4.02 (m, 1H, *CH* at azetidinyl-2), 3.25 (d, 2H, CH_2 at azetinidinyl-3); $C_{21}H_{14}ClN_3O_2S$ (407.87); requires (Found): C, 61.84 (62.09); H, 3.46 (3.56); N, 10.30 (10.52).

*5-(4-Chlorophenyl)-2-[1-(2-methylphenyl)-4-oxo-azetidin-2-yl]-thieno[2,3-d]pyrimidin-4(3H)-one (**5f**)*

IR (KBr): 3239, 2949, 1686, 1614, 1552, 1410, 1092, 979, 772 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.99$ (s, 1H, *NH*), 6.84–7.65 (m, 9H, *ArH* and *H* at 6), 3.94–4.11 (m, 1H, *CH* at azetidinyl-2), 3.34 (d, 2H, CH_2 at azetinidinyl-3), 2.51 (s. 3H, *Ar–*CH_3); $C_{22}H_{16}ClN_3O_2S$ (421.9); requires (Found): C, 62.63 (62.25); 3.82 (3.93); N, 9.96 (10.11).

*5-(4-Chlorophenyl)-2-[1-(4-methylphenyl)-4-oxo-azetidin-2-yl]-thieno[2,3-d]pyrimidin-4(3H)-one (**5g**)*

IR (KBr): 3340, 2937, 1689, 1555, 1446, 1120, 1035, 686 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 8.14$ (s, 1H, *NH*), 6.80–7.71 (m, 9H, *ArH* and *H* at 6), 3.48–3.69 (m, 1H, *CH* at azetidinyl-2), 3.34 (m, 2H, CH_2 at azetidinyl-3), 2.51 (s. 3H, *Ar–*CH_3); $C_{22}H_{16}ClN_3O_2S$ (421.9); requires (Found): C, 62.63 (62.41); 3.82 (3.68). N, 9.96 (10.09).

*5-(4-Chlorophenyl)-2-[1-(4-fluorophenyl)-4-oxo-azetidin-2-yl]thieno[2,3-d]pyrimidin-4(3H)-one (**5h**)*

IR (KBr): 3270, 2937, 1689, 1536, 1278, 814 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 8.0$ (s, 1H, *NH*), 7.1–7.73 (m, 9H, *ArH* and *H* at 6), 4.15 (m, 1H, *CH* at azetidinyl-2), 3.45 (m, 2H, CH_2 at azetidinyl-3); $C_{21}H_{13}ClFN_3O_2S$ (425.86); requires (Found): C, 59.23 (59.61); H, 3.08 (2.97); N, 9.87 (9.82).

*2-[1-(3-Methylphenyl)-4-oxo-azetidin-2-yl]-5,6,7,8-tetrahydrobenzo(b)thieno[2,3-d]pyrimidin-4(3H)-one (**5i**)*

IR (KBr): 3250, 2936, 2363, 1776, 1654, 1598, 1232, 1119, 965 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.2–7.9$ (m, 5H, *ArH* and *NH*), 3.46–3.53 (m, 1H, *CH* at azetidinyl-2), 2.90–3.04 (m, 2H, CH_2 at azetidinyl-3), 2.51–2.62 (m, 4H, CH_2 at 5 and 8), 2.28 (s, 3H), 1.85–2.01 (m, 4H, CH_2 at 6 & 7); MS (TOF): $m/z = 366$ (M+1); $C_{20}H_{19}N_3O_2S$ (365.45); requires (Found): C, 65.73 (65.63); H, 5.24 (5.42); N, 11.50 (11.27).

*2-[1-(4-Methoxyphenyl)-4-oxo-azetidin-2-yl]-5,6,7,8-tetrahydrobenzo(b)thieno[2,3-d]pyrimidin-4(3H)-one (**5j**)*

IR (KBr): 3275, 2948, 2878, 2348, 1651, 1455, 1237, 1002 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.2–7.9$ (m, 5H, *ArH* and *NH*), 3.30–3.38 (m, 1H, *CH* at azetidinyl-2), 3.19 (d, 2H, CH_2 at azetidinyl-3), 2.88 (s, 3H, $ArOCH_3$), 2.37–2.51 (m, 4H, CH_2 at 5 and 8), 1.94–2.10 (m, 4H, CH_2 at 6 and 7); MS (TOF): $m/z = 382$ (M+1); $C_{20}H_{19}N_3O_3S$ (381.45); requires (Found): C, 62.98 (63.11); H, 5.02 (5.31); N, 11.02 (11.13).

*2-[1-(4-Bromophenyl)-4-oxo-azetidin-2-yl]-5,6,7,8-tetrahydrobenzo(b)thieno[2,3-d]pyrimidin-4(3H)-one (**5k**)*

IR (KBr): 3111, 2942, 2875, 1775, 1654, 1507, 1425, 1203, 1058, 963 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.26–7.97$ (m, 5H, *ArH* and *NH*), 3.10–3.24 (m, 1H, *CH* at azetidinyl-2), 2.70–2.81 (m, 2H, CH_2 at azetidinyl-3), 2.25–2.44 (m, 4H, CH_2 at 5 and 8), 1.9–2.12 (m, 4H, CH_2 at 6 and 7); MS (TOF): $m/z = 430$, 432 (M+2); $C_{19}H_{16}BrN_3O_2S$ (430.32); requires (Found): C, 53.03 (53.27); H, 3.75 (3.99); N, 9.76 (10.12).

*2-[1-(4-Fluorophenyl)-4-oxo-azetidin-2-yl]-3,5,6,7,8,9-hexahydro-10-thia-1,3-diazabenzoazulen-4-one (**5l**)*

IR (KBr): 3107, 2919, 1889, 1665, 1596, 1506, 1204, 1044, 922 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.92$ (s, 1H, *NH*), 7.02–7.70 (m, 4H, *ArH*), 4.44 (m, 1H, *CH* at azetidinyl-2), 3.58 (m, 2H, CH_2 at azetidinyl-3), 2.60–2.65 (m, 4H, CH_2 at 5 and 9), 2.23–2.30 (m, 4H, CH_2 at 6 and 8), 1.92–2.0 (m, 2H, CH_2 at 7); MS (TOF): $m/z = 384$ (M+1); $C_{20}H_{18}FN_3O_2S$ (383.44); requires (Found): C, 62.65 (62.69); 4.73 (5.02); N, 10.96 (10.66).

Pharmacological activity

The experiments were carried out with Wistar Albino rats (170–200 g). The animals were housed at a temperature of 30 ± 5°C and humidity of 40–50 ± 5% with 12 h light and 12 h dark cycles. The animals were given food and water *ad libitum*, unless specified otherwise. For all studies animals of either sex were selected at random.

Triton WR 1339, a surfactant, chemically isooctyl-polyoxyethylene phenol (tyloxapol) was used to induce hyperlipidemia.

The animals were divided into four groups of six animals each:

Group I – control group: the control group received only vehicle (2% w/v acacia gum aqueous solution p.o.).

Group II – cholesterol-control group: the cholesterol-control group received Triton WR 1339 in vehicle solution (200 mg/kg/day i.p.).
Group III – test group: The test drug treated group received Triton WR 1339 (200 mg/kg i.p.) as well as test drug suspension (**5a–h**; 400 mg/kg/day p.o.) in 2% acacia gum solution.
Group IV – the standard group received Triton WR 1339 (200 mg/kg i.p.) as well as gemfibrozil as standard drug (400 mg/kg/day p.o.) in 2% acacia gum solution.

As such eight test groups for eight test compounds were formed of six animals of either sex per group. Blood samples were collected from the retro-orbital plexus of the eyes of the animals, initially and after 24 h. The samples were analyzed for serum cholesterol (total) levels, serum triglyceride (total) levels, and serum HDL (total) levels.

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