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# Synthesis, Antimicrobial and Antihyperlipidemic Activities of Some 4-Substituted-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidines

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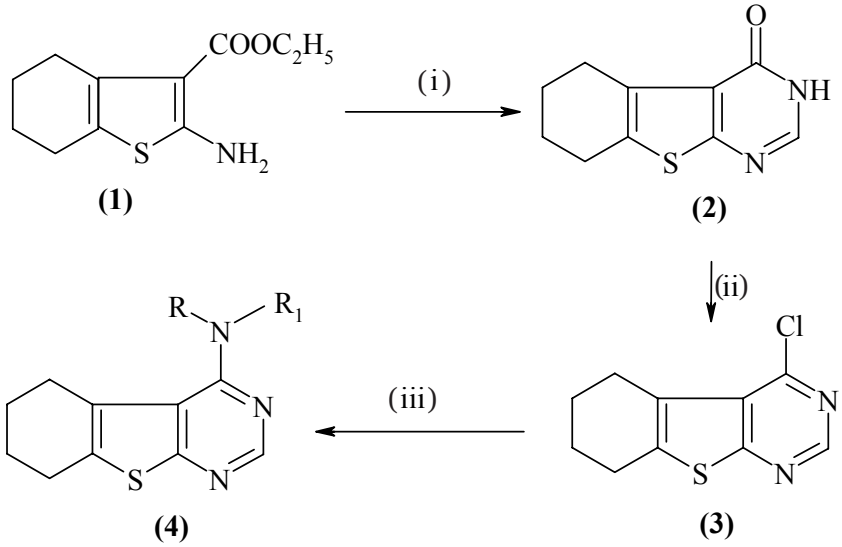
A series of 4-substituted-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine derivatives (**4a-f**) were prepared by the displacement reaction between various amines and 4-chloro-5,6,7,8-tetrahydro[1]-benzothieno[2,3-d]pyrimidine (**3**). The structure of the compounds has been established on their analytical and spectral data. All the compounds have been screened for antihyperlipidemic and antimicrobial activities. The compounds **4c** and **4d** showed good antihyperlipidemic activity in reducing serum cholesterol level when compared with standard, gemfibrozil. Compounds **4a** and **4d** were found to have excellent antimicrobial activity against *Staphylococcus aureus* and *Streptococcus pneumoniae* when compared with standard used (amoxicillin-clavulanic acid).

**Key Words: Thienopyrimidine, Antihyperlipidemic activity, Antimicrobial activity.**

## INTRODUCTION

The biological activities of condensed pyrimidines as sedatives, antibacterials and antimalarials are well documented[1-3]. In practice, many thienopyrimidines have been evaluated pharmacologically for their anticancer[4], antiviral[5,6], antihyperlipidemic[7], antimicrobial[8,9] and gastric antisecretory activities[10]. We report herein the synthesis of some new 4-substituted-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine (**4a-f**) and result of their antihyperlipidemic and antimicrobial activities. 4-Substituted-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine derivatives (**4a-f**) were prepared by the displacement reaction between various amines and 4-chloro-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine (**3**), which was obtained by refluxing 5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine-4(3H)-one (**2**) with phosphorous oxychloride. Compound **2** was obtained by cyclization of ethyl 2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (**1**) with formamide. Compound **1** was prepared by Gewald thiophene ring synthesis[11]. The structures of the synthesized compounds

have been established based on their analytical and spectral data. All the compounds have been screened for antihyperlipidemic and antimicrobial activities.




(i) formamide, reflux, 160-180ºC 6 h
(ii) $POCl_3$, triethylamine, reflux, 140ºC, 1.5 h
(iii) methanol, corresp[onding amine, reflux 2 h

**Scheme-I**

## EXPERIMENTAL

The melting points of the compounds were determined in open capillaries and are uncorrected. Purity of the compounds was checked by micro TLC using silica gel G coated glass plates using benzene-methanol (9:1; v/v) as irritant and iodine vapour as detecting agent. The IR (KBr) spectra were recorded on Jasco FT/IR-5300 spectrophotometer. $^1$H NMR spectra ($CDCl_3$) were recorded on Bruker DPX-400 MHz NMR spectrophotometer; chemical shifts (δ) are reported in ppm, with TMS as internal standard. GC Mass spectra were recorded on a Shimadzu QP 50000. Elemental analysis for C, H and N were performed on a Perkin Elmer 240 C elemental analyzer and were with in ± 0.4 % of the theoretical values. Physical data of the compounds and percentage yield of various reactions are given in Table-1.

**Synthesis of 5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine-4(3H)-one (2):** A mixture of ethyl 2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (**1**) (2.25 g, 0.01 mol) and formamide (15

mL) was refluxed at 160-180ºC for 6 h and then allowed to cool at room temperature. The solid separated was filtered, washed with water, dried and recrystallized from DMF-water (2:1) to get white crystals. Yield 1.6 g, 78 %.

TABLE-1
PHYSICAL DATA OF 4-SUBSTITUTED-5,6,7,8-TETRAHYDRO[1]BENZOTHIENO[2,3-d]PYRIMIDINES

| Compd. | R | $R_1$ | Recrystallization solvent | Yield (%) / m.p. (ºC) | m.f. / (m.w.) | $R_f$ value* |
|---|---|---|---|---|---|---|
| **4a** | Me | Me | DMF | 69 (63) | $C_{12}H_{15}N_3S$ (233) | (0.56) |
| **4b** | Et | Et | DMF | 71 (60) | $C_{14}H_{19}N_3S$ (261) | (0.63) |
| **4c** | Ph | Ph | DMF | 71 (40) | $C_{22}H_{19}N_3S$ (357) | (0.58) |
| **4d** | $-(CH_2)_5-$ | | DMF | 74 (115) | $C_{15}H_{19}N_3S$ (273) | (0.64) |
| **4e** | $-CH_2CH_2NHCH_2CH_2-$ | | 1,4-Dioxane | 62 (69) | $C_{14}H_{18}N_4S$ (274) | (0.62) |
| **4f** | $-CH_2CH_2OCH_2CH_2-$ | | 1,4-Dioxane | 68 (110) | $C_{14}H_{17}N_3OS$ (275) | (0.56) |

*$R_f$ value was determined in benzene: methanol (9: 1). DMF–Dimethyl formamide.

**5,6,7,8-Tetrahydro[1]benzothieno[2,3-d]pyrimidine-4(3H)-one (2):** IR (KBr, $cm^{-1}$) 3155 ν(N-H), 1655 ν(C=O), 1587 ν(C=N) and 1282 ν(C-N); The $^1H$ NMR ($CDCl_3$, δ ppm): 11.61 (broad, 1H, CONH), 7.94 (s, 1H, 2-pyrimidinyl-H), 3.02-3.05 (t, 2H, $-CH_2-$), 2.79-2.80 (t, 2H, $-CH_2-$) and 1.85-1.90 (m, 4H, $-(CH_2)_2-$); m/z: 206 ($M^+$); Anal. ($C_{10}H_{10}N_2OS$) Found (%) C, 58.09; H, 4.62; N, 13.33. Calculated (%): C, 58.23; H, 4.89; N, 13.58.

**Synthesis of 4-chloro-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine (3):** 5,6,7,8-Tetrahydro[1]benzothieno[2,3-d]pyrimidine-4(3H)-one (**2**) (2.06 g, 0.01 mol) was dissolved in phosphorus oxychloride ($POCl_3$) (10 mL). Triethylamine (1.5 mL) was added and the mixture was refluxed for 1.5 h in an oil bath at 140ºC. The excess of $POCl_3$ was removed under reduced pressure and the suspension was poured into cold water and neutralized with 10 % NaOH solution. The residue was collected, washed with water, dried and recrystallized from DMF-water (2:1). Yield 1.7g, 75 %.

**4-Chloro-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine (3):** IR (KBr, $cm^{-1}$) 2935 ν(aliphatic C-H str), 1558 ν(C=N) and 729 ν(C-Cl); The $^1H$ NMR ($CDCl_3$, δ ppm): 7.94 (s, 1H, 2-pyrimidinyl-H), 3.02-3.05 (t,

2H, -$CH_2$-), 2.79-2.80 (t, 2H, -$CH_2$-) and 1.85-1.90 (m, 4H, -$(CH_2)_2$-); m/z: 224 (M+); Anal. ($C_{10}H_9N_2SCl$) Found (%): C, 53.57; H, 4.25; N, 12.28. Calculated (%): C, 53.45; H, 4.04; N, 12.47.

**Synthesis of 4-substituted-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidines (4a-f):** A mixture of 4-chloro-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine (**3**) (2.24 g, 0.01 mol) and appropriate amine (10 mL) in methanol (20 mL) was refluxed for 2 h. The reaction mixture was concentrated to 1/3 of the initial volume and cooled to room temperature. The crystals formed were filtered off and recrystallized from appropriate solvent.

**N,N-Dimethyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine-4-amine (4a):** IR (KBr, $cm^{-1}$) 2854 ν(aliphatic C-H str), 1568 ν(C=N) and 1365 ν(C-N); The $^1$H NMR ($CDCl_3$, δ ppm): δ: 8.04 (s, 1H, 2-pyrimidinyl-H), 3.21(s, 6H, -$NCH_3$), 3.02-3.05 (t, 2H, -$CH_2$-), 2.79-2.80 (t, 2H, -$CH_2$-) and 1.85-1.90 (m, 4H, -$(CH_2)_2$-); m/z: 233 ($M^+$); Anal. ($C_{12}H_{15}N_3S$) Found (%): C, 61.53; H, 6.80; N, 17.79. Calculated (%): C, 61.77; H, 6.48; N, 18.01.

**N,N-Diethyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine-4-amine (4b):** IR (KBr, $cm^{-1}$) 2937 ν(aliphatic C-H str), 1558 ν(C=N) and 1319 ν(C-N); The $^1$H NMR ($CDCl_3$, δ ppm): δ: 8.71 (s, 1H, 2-pyrimidinyl-H), 3.10(q, 4H, -$CH_2CH_3$), 2.89 (m, 4H, -$(CH_2)_2$-), 1.92-1.93 (t, 6H, -$CH_3$) and 1.65 (m, 4H, -$(CH_2)_2$-); m/z: 261 ($M^+$); Anal. ($C_{14}H_{19}N_3S$) Found (%): C, 64.67; H, 7.64; N, 16.32. Calculated (%): C, 64.33; H, 7.33; N, 16.08.

**N,N-Diphenyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine-4-amine (4c):** IR (KBr, $cm^{-1}$) 3042 ν(aromatic C-H str), 1593 ν(C=N) and 1315 ν(C-N); The $^1$H NMR ($CDCl_3$, δ ppm): δ: 8.71 (s, 1H, 2-pyrimidinyl-H), 7.06-7.08 (m, 10H, Ar-H), 3.09-3.10 (t, 2H, -$CH_2$- ), 2.88-2.89 (t, 2H, -$CH_2$-) and 1.91-1.93 (m, 4H, -$(CH_2)_2$-); m/z: 357 ($M^+$); Anal. ($C_{22}H_{19}N_3S$) Found (%): C, 73.76; H, 5.10; N, 11.54. Calculated (%): C, 73.92; H, 5.36; N, 11.75.

**4-Piperidin-1-yl-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine (4d):** IR (KBr, $cm^{-1}$) 2930 ν(aliphatic C-H str), 1556 ν(C=N) and 1365 ν(C-N); The $^1$H NMR ($CDCl_3$, δ ppm): δ: 8.50 (s, 1H, 2-pyrimidinyl-H), 3.31-3.33 (m, 4H, -$N(CH_2)_2$-), 2.87-2.93 (m, 4H, -$(CH_2)_2$-), 1.92-1.94 (m, 4H, -$(CH_2)_2$-) and 1.73-1.82 (m, 6H, -$(CH_2)_3$-); m/z: 273 (M+); Anal. ($C_{15}H_{19}N_3S$) Found (%): C, 66.02; H, 7.23; N, 15.71. Calculated (%): C, 65.90; H, 7.00; N, 15.37.

**4-Piperazin-1-yl-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine (4e):** IR (KBr, $cm^{-1}$) 3503 ν(NH), 2932 ν(aliphatic C-H str), 1537 ν(C=N) and 1369 (C-N); The $^1$H NMR ($CDCl_3$, δ ppm): δ: 8.52 (s, 1H, 2-pyrimidinyl-H), 4.94 (broad, 1H, NH), 3.35-3.36 (m, 4H, -$N(CH_2)_2$-), 3.03-3.05 (m, 4H, -$N(CH_2)_2$-), 2.88-2.92 (m, 4H, -$(CH_2)_2$-) and 1.79-1.83

(m, 4H, -$(CH_2)_2$-); m/z: 274 ($M^+$); Anal. ($C_{14}H_{18}N_4S$) Found (%): C, 61.49; H, 6.29; N, 20.09. Calculated (%): C, 61.28; H, 6.61; N, 20.42.

**4-Morpholin-1-yl-5,6,7,8-tetrahydro[1]benzothieno[2,3-d] pyrimidine (4f):** IR (KBr, $cm^{-1}$) 1556 ν(C=N), 1363 ν(C-N) and 1113 ν(-C-O-C-); The $^1H$ NMR ($CDCl_3$, δ ppm): δ: 8.54 (s, 1H, 2-pyrimidinyl-H), 3.86-3.88 (m, 4H, -$O(CH_2)_2$-), 3.39-3.42 (m, 4H, -$N(CH_2)_2$-), 2.89-2.92 (m, 4H, -$(CH_2)_2$-) and 1.80-1.95 (m, 4H, -$(CH_2)_2$-); m/z: 275 (M+); Anal. ($C_{14}H_{17}N_3OS$) Found (%): C, 61.84; H, 6.41; N, 15.52. Calculated (%): C, 61.66; H, 6.22; N, 15.26.

**Acute toxicity studies:** The acute toxicity studies were carried out by the Miller and Tainter method[12]. Overnight fasted mice were weighed and divided into various groups of six in each. The test compounds **4a-f** was given in various doses (1000-2000 mg/kg) by oral route. After administration of the test compounds, the animals were observed continuously for the first 2 h for the death due to acute toxicity. The percentage mortality values were converted to probit values by reading the corresponding probit unit from probit value. The values were plotted against log doses and $LD_{50}$ values were read as the dose that corresponds to probit 5. The $LD_{50}$ values are given in Table-3.

**Antihyperlipidemic activity:** The synthesized compounds were assessed for the antihyperlipidemic activity using wistar rats. The hyperlipidemia in rats was produced by triton (surfactant). Animals were divided into nine groups of 6 rats each. The synthesized compounds (**4a-f**) were reconstituted in distilled water using microcrystalline cellulose as the suspending agent. Male albino rats (120-200 g) of wistar strain were used for the study. The animals were kept in polypropylene cages, at optimum temperature conditions (21-25ºC) and humidity of 45 ± 5 % RH. The animals received Hindustan Lever pellet diet and water *ad libitium*. Hyperlipidemia was induced in groups I to VIII by subcutaneous injection of triton (200 mg/kg b.w.). The rats in control group received vehicle (0.25 % CMC solution), while groups II to VII and received test compounds **4a-f** and gemfibrozil by per oral route in doses of 160, 140, 140, 145, 145, 140 and 108 mg/kg body weight, respectively 1 h prior to triton injection. The second dose of test compounds **4a-f** was given 20 h later. At the end of 24 h after triton injection, animals were sacrificed and blood was collected by cardiac puncture. The animals were kept fasted through the experimental period, but were provided water *ad libitium*. Serum from blood was analyzed for total cholesterol using stangen cholesterol kit and triglyceride using span triglyceride kit, respectively. The data obtained was subjected to statistical analysis using student's t-test and $p < 0.05$ was considered statistically significant.

TABLE-2
ANTIHYPERLIPIDEMIC ACTIVITY OF THE TITLE COMPOUNDS

| Design of treatment | Dose (mg/kg) | Total cholesterol (mg %) ± SEM | Triglycerides (mg %) ± SEM |
|---|---|---|---|
| Control (vehicle) | 1 mL of 0.25 % CMC | 69.13 ± 2.10 | 84.49 ± 4.75 |
| Triton | 200 | 140.86 ± 3.45[X] | 120.89 ± 6.31[X] |
| Gemfibrozil | 108 | 74.00 ± 4.21[a] | 88.44 ± 6.04[b] |
| Compound **4a** | 160 | 110.88 ± 4.61[a] | 112.10 ± 5.86[d] |
| Compound **4b** | 140 | 120.67 ± 6.84[b] | 116.9 ± 7.15[d] |
| Compound **4c** | 140 | 84.24 ± 6.77[a] | 111.08 ± 6.37[d] |
| Compound **4d** | 145 | 87.91 ± 3.12[a] | 113.86 ± 7.24[d] |
| Compound **4e** | 145 | 112.52 ± 7.01[b] | 97.04 ± 7.48[c] |
| Compound **4f** | 140 | 93.81 ± 3.76[a] | 107.38 ± 9.00[d] |

Values are expressed as mean ± SEM for six animals.
Data were analyzed by using student's t-test.
[x]$p < 0.001$ compared to respective control group.
[a]$p < 0.001$ compared to respective triton induced group.
[b]$p < 0.01$ compared to respective triton induced group.
[c]$p < 0.001$ compared to respective triton induced group.
[d]$p < 0.001$ compared to respective triton induced group.

**Antimicrobial activity:** The antimicrobial activity of the title compounds was evaluated by zone of inhibition method[13] against six bacterial strains *viz.*, *Streptococcus pneumoniae* (ATCC 49619) (gram positive), *Staphylococcus aureus* (ATCC 25923) (gram positive), *Streptococcus pyogenes* (ATCC 23162) (gram positive), *Escherichia coli* (ATCC 25922) (gram negative), *Pseudomonas aeruginosa* (ATCC 27853) (gram negative), *Shigella dysenteriae* (ATCC 49247) (gram negative) and two fungal strains namely *Candida albicans* and *Aspergillus fumigatus*. the compounds were tested at a concentration of 1 mg/mL in DMSO solution using amoxicillin-clavulanic acid (1 mg/mL) (for gram positive bacteria), cefixime (1 mg/mL) (for gram negative bacteria) for antibacterial and ketoconazole (1 mg/mL) for antifungal activity as standard for comparison of antibacterial and antifungal activity respectively. Inhibition was recorded by measuring the diameter of the inhibition zone at the end of 24 h for bacteria and 48 h for fungi. Each experiment was repeated thrice and average of three independent determinations was recorded.

## RESULTS AND DISCUSSION

All the compounds have been screened for antihyperlipidemic activity and antimicrobial activity against six bacterial strains *viz.*, *Streptococcus pneumoniae* (ATCC 49619) (gram positive), *Staphylococcus aureus* (ATCC

25923) (gram positive), *Streptococcus pyogenes* (ATCC 23162) (gram positive), *Escherichia coli* (ATCC 25922) (gram negative), *Pseudomonas aeruginosa* (ATCC 27853) (gram negative), *Shigella dysenteriae* (ATCC 49247) (gram negative) and two fungal strains namely *Candida albicans* and *Aspergillus fumigatus*. From the Table-2, the reduction of total serum cholesterol by compounds **4c** and **4d** is comparable to the standard, gemfibrozil. From the Table-3, it is clear that compounds **4a** and **4d** have excellent antimicrobial activity against *Staphylococcus aureus* and *Streptococcus pneumoniae* when compared with standard. Compound **4b** have good activity against *Streptococcus pneumoniae* and *Escherichia coli* when compared with standard. Compound **4e** has excellent activity against *Staphylococcus* aureus when compared with standard.

TABLE-3
ANTIBACTERIAL, ANTIFUNGAL ACTIVITY and $LD_{50}$ VALUES OF THE TILE COMPOUNDS

| Micro-organism | Diameter of zone (mm) Compounds (1 mg/mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 4a | 4b | 4c | 4d | 4e | 4f | Std |
| | Antibacterial activity | | | | | | |
| *S. pyogenes* | 4 | 2 | NA | NA | NA | NA | 14* |
| *S. aureus* | 12 | 6 | 2 | 18 | 12 | 6 | 8* |
| *S. pneumoniae* | 16 | 10 | NA | 10 | 8 | NA | 10* |
| *S. dysenteriae* | 8 | 6 | NA | 10 | NA | NA | 12* |
| *E. coli* | 14 | 12 | NA | 4 | NA | NA | 13* |
| *P. aeruginosa* | NA | NA | NA | 6 | 5 | 4 | 15* |
| | Antifungal activity | | | | | | |
| *C. albicans* | 6 | 4 | 2 | 2 | 5 | NA | 18** |
| *A. fumigatus* | 10 | NA | NA | 6 | 3 | 8 | 16** |
| | $LD_{50}$ values | | | | | | |
| $LD_{50}$ (mg/kg) | 1585 | 1380 | 1380 | 1445 | 1445 | 1380 | – |

NA: No activity; *Standard drugs: Amoxicillin-clavulanic acid (for gram positive bacteria); Cefixime (for gram negative bacteria); **Standard drug: Ketoconazole

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