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Synthesis and antimicrobial activity of some novel thienopyrimidines and triazolothienopyrimidines

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Abstract. Novel tricyclic thienopyrimidines (**3**, **5**, **6**, **9**, **11**, **12**) and triazole fused tetracyclic thienopyrimidines (**4a–c**, **10a–c**) were synthesized from precursors 2-amino-6-methyl-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonitrile **1** and 2-amino-7-oxo-4,5,6,7-tetrahydro-1-benzothio-phene-3-carbonitrile **7** respectively. The corresponding precursors were prepared by employing the Gewald reaction. The structures of newly synthesized compounds were characterized by spectral and analytical data. All the compounds were screened for their biological activities. Some of the compounds displayed promising antibacterial and antifungal activities.

Keywords. Thienopyrimidine; triazolothienopyrimidine; Gewald reaction; antibacterial activity; antifungal activity.

1. Introduction

Since resistance of pathogenic bacteria towards available antibiotics is rapidly becoming a major worldwide problem, the design of new compounds to deal with resistant bacteria has become one of the most important areas of antibacterial research today. In addition, it is known that antifungal drugs do not have selective activity because of the biochemical similarity between human cell and fungi forms. Therefore there are many studies focused on antibacterial and antifungal compounds.[1–3]

Thienopyrimidines and thiadiazolothienopyrimidines have been found to exhibit a variety of biological activities viz. anti inflammatory,[4,5] antimicrobial,[6] analgesic[7] activities, inhibition of cancer cell proliferation,[8] antagonism of α_1 adrenoceptors[9] and prevention of cartilage destruction in articular diseases.[10] Consequently, thienopyrimidines[11,12] have become a wellsought-privileged class of compounds in drug discovery programs. In view of these reports and in continuation of our work on biologically active nitrogen and sulfur heterocycles,[13–15] we report here the synthesis of some novel thienopyrimidines and thienotriazolopyrimidines for the evaluation of their antimicrobial properties. The synthesized compounds were tested against two Gram (+) Bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), two Gram (–) bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) and two yeast-like fungi *Candida albicans* and *Candida parapsilosis* using the broth microdilution method.

2. Experimental

2.1 *General*

Melting points were determined in open capillaries and are uncorrected. The IR spectra were recorded on Nicolet Impact 410 FT IR spectrophotometer using KBr pellets. ^{1}H and ^{13}C NMR were recorded on Bruker 300-MHz FT NMR spectrometer in $CDCl_3$ and DMSO-d_6 with TMS as internal standard. Mass spectra were recorded on Finnigan MAT (Model MAT8200) spectrometer and elemental analysis were carried out using Heraus CHN rapid analyser.

2.1a *2-Amino-6-methyl-4,5,6,7-tetrahydro-benzo-[b]thiophene-3-carbonitrile* (**1**)*:* To a well stirred mixture of 4-methylcyclohexanone (8 g, 71 mmole) and malononitrile (4·7 g, 71 mmole) in ethanol (45 mL) was added elemental sulfur (2·31 g, 72 mmole). To this cooled reaction mixture was added diethylamine (5 mL) with vigorous stirring

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during 1 min. Reaction mixture was stirred at 40–45°C for about 1 h. The yellow–orange solid separated was filtered, washed with hot ethanol and recrystallised from dioxane to yield analytically pure yellow needles.[16] Yield 9·7 g (71%); m.p. 138–140°C.

2.1b *N-(3-Cyano-6-methyl-4,5,6,7-tetrahydro-benzo [b]thiophene-2-yl)formimidic acid ethyl ester* (**2**)*:* A solution of **1** (1·10 g, 57 mmole) in triethylorthoformate (12 mL) was heated under reflux for 18 h, excess triethylorthoformate was removed under vaccum. The residue was treated with petroleum ether. Solid that separated was filtered and recrystallized with petroleum ether to afford light brown crystals. Yield 1·18 g (83%); m.p. 136–138°C; IR (KBr) ν cm^{-1} 3090, 2921, 2866, 2212, 1625, 1573, 1541; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1·26 (*d*, 3H, *J* = 5·3 Hz, CH_3), 1·41 (*t*, *J* = 7·1 Hz, 3H, $CH_2\underline{CH_3}$), 2·03 (*m*, 1 H, C_6–H), 2·22 (*d*, *J* = 3·2 Hz, 2H, CH_2), 2·48 (*t*, 2 H, CH_2), 2·60 (*t*, 2H, CH_2), 4·42 (*q*, *J* = 7·08 Hz, 2 H, $\underline{CH_2}CH_3$), 7·94 (*s*, 1 H, N=CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14·7, 18·9, 19·5, 37·2, 39·3, 41·0, 59·5, 112·8, 115·9, 133·4, 136·0, 144·2, 163·9; Anal. calcd. for $C_{13}H_{16}N_2OS$: C, 62·87; H, 6·49; N, 11·28. Found: C, 62·76; H, 6·57; N, 11·38.

2.1c *4-Imino-7-methyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-3[4H]-amine* (**3**)*:* A mixture of **2** (1·38 g, 55 mmole) and hydrazine hydrate (7 mL) was stirred at room temperature for 2 h and then diluted with ethanol (20 mL). The resulting fine solid suspension was filtered, washed with ethanol and purified by recrystallisation from ethanol-dioxane mixture to afford analytically pure pale yellow granules. Yield 1 g (77%); m.p. 152–154°C; IR (KBr) ν cm^{-1} 3382, 3271, 3180, 3037, 2929, 1614, 1548; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1·12 (*d*, *J* = 5·2 Hz, 3 H, CH_3), 2·15 (*m*, C_7–H, 1H), 2·32 (*d*, 2 H, *J* = 3·2 Hz, CH_2), 2·38 (*t*, 2H, CH_2), 2·40 (*t*, 2H, CH_2), 4·72 (*br s*, 2 H, NH_2, D_2O exchangeable), 5·54 (*br s*, 1 H, NH, D_2O exchangeable), 7·96 (*s*, 1 H, C_2–H, pyrimidine); ^{13}C NMR (75 MHz, DMSO-d_6) δ 18·2, 20·1, 32·5, 38·0, 43·9, 119·8, 128·2, 135·0, 142·4, 162·5, 163·9; Anal. calcd. For $C_{11}H_{14}N_4S$: C, 56·38; H, 6·02; N, 23·91. Found: C, 56·23; H, 6·12; N, 23·99.

2.1d *7-Methyl-5,6,7,8-tetrahydro[1]benzothieno [2,3-d]pyrimidin-4[3H]-one* (**5**): A mixture of 2-amino-6-methyl-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carbonitrile **1** (1 g, 52 mmole) and formic acid (15 mL) was heated under reflux for 5 h. The excess of formic acid was removed under reduced pressure. The resulting residue was crystallized from ethanol. Yield 0·92 g (80%); m.p. 154–156°C; IR (KBr) ν cm^{-1} 3158, 3070, 1664, 1580, 1364, 974; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1·35 (*d*, *J* = 5·3 Hz, 3H, CH_3), 2·09 (*m*, 1H, C_7–H), 2.20 (*d*, *J* = 3·2 Hz, 2H, CH_2), 2·45 (*t*, 2H, CH_2), 2·76 (*t*, 2H, CH_2), 7·86 (*s*, 1H, C_2–H, pyrimidine), 11·80 (*br s*, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16·4, 18·1, 31·9, 38·2, 42·1, 130·9, 136·0, 142·5, 143·1, 161·9, 169·9; Anal. calcd. for $C_{11}H_{12}N_2OS$: C, 59·97; H, 5·49; N, 12·72. Found: C, 59·89; H, 5·69, N, 12·37.

2.1e *7-Methyl-5,6,7,8-tetrahydro[1]benzothieno [2,3-d]pyrimidin-4-amine* (**6**)*:* Imidoformate **2** (1·38 g, 55 mmole) was treated with anhydrous ethanolic ammonia (35 mL) at 0°C. The bright yellow solid that separated within 10 min was further stirred overnight. Solvent was removed *in vaccuo* and the residue was dissolved in DMF (35 mL) to which sodium ethoxide (0·8 g) was added and the stirred solution was heated to 100–112°C for 1 h. The solution was concentrated under reduced pressure. The residue was treated with warm water and the product was filtered and recrystallized with aq. ethanol to afford pale yellow granules. Yield 0·70 g (80%); m.p. 134–136°C; IR (KBr) ν cm^{-1} 3360, 3313, 3113, 1645, 1571; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2·01 (*d*, *J* = 5·2 Hz, 3H, CH_3), 2·23 (*m*, 1H, C_7-H), 2·50 (*d*, *J* = 3·1 Hz, 2H, CH_2), 2·85 (*t*, 2H, CH_2), 2·96 (*t*, 2H, CH_2), 6·74 (*br s*, 2H, NH_2, D_2O exchangeable), 8·16 (*s*, 1H, C2–H), pyrimidine; ^{13}C NMR (75 MHz, $CDCl_3$) δ 20·1, 20·9, 30·5, 38·1, 44·1, 121·2, 129·2, 133·3, 142·0, 157·4, 167·7; Anal. calcd. for $C_{11}H_{13}N_3S$: C, 60·24; H, 5·97; N, 19·16. Found: C, 60·39; H, 5·81, N, 19·28.

2.1f *9-Methyl-8,9,10,11-tetrahydro[1]benzothieno [3,2-e][1,2,4]triazolo[1,5-c] pyrimidine* (**4a**)*:* A mixture of **3** (0·125 g, 0·053 mmole), triethylorthoformate (0·6 mL) and dimethylformamide (0·6 mL) was heated on water bath (90°C) for 4 h. The reaction mixture was cooled to room temperature and diluted with petroleum ether (2 mL). The solid that separated was washed with benzene and petroleum ether and dried. The crude product was purified by column chromatography (EtOAc : benzene :: 6 : 4) to furnish yellow granules. Yield 0·09 g (70%); m.p. 122–124°C; IR (KBr) ν cm^{-1} 3076, 2949, 2921,

1620, 1485; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1·16 (*d*, J = 5·4 Hz, 3H, CH_3), 2·09 (*d*, J = 3·2 Hz, 2H, CH_2), 2·12 (*m*, C_9–H, 1H), 2·58 (*t*, 2H, CH_2), 2·99 (*t*, 2H, CH_2), 8·42 (*s*, 1H, C_2–H, pyrimidine), 9·20 (*s*, 1H, triazole); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18·1, 19·8, 22·2, 35·6, 41·9, 122·4, 129·3, 133·6, 142·7, 145·9, 149·1, 157·2; Anal. calcd. for $C_{12}H_{12}N_4S$: C, 58·99; H, 4·95; N, 22·93. Found: C, 59·08; H, 4.88; N, 23·03.

2.1g *2-Methyl-9-methyl-8,9,10,11-tetrahydro[1] benzothieno[3,2-e][1,2,4]triazolo[1,5-c] pyrimidine* (**4b**)*:* A mixture of **3** (0·125 g, 0·053 mmole), triethylorthoacetate (0·6 mL) and dimethylformamide (0·6 mL) was heated on water bath (90°C) for 4 h. The reaction mixture was cooled to room temperature and diluted with petroleum ether (2 mL). The solid that separated was washed with benzene and petroleum ether and dried. The crude product was purified by column chromatography (EtOAc : benzene :: 6 : 4) to furnish yellow granules. Yield 0·09 (66%); m.p. 149–151°C; IR (KBr) ν cm^{-1} 3044, 2953, 2927, 1616, 1555; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1·25 (*d*, J = 5·2 Hz, 3H, CH_3), 2·05 (*d*, J = 3·1 Hz, 2H, CH_2), 2·20 (*m*, 1H, C_9–H), 2·43 (*t*, 2H, CH_2), 2·81 (*t*, 2H, CH_2), 3·35 (*s*, 3H, CH_3), 8·98 (*s*, 1H, C_2–H, pyrimidine); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15·4, 19·9, 22·4, 34·5, 38·0, 43·1, 121·1, 127·6, 133·0, 139·9, 148·0, 156·7, 159·0; Anal. calcd. for $C_{13}H_{14}N_4S$: C, 60·44; H, 5·46; N, 21·69. Found: C, 60·59; H, 5·38; N, 21·75.

2.1h *2-Ethyl-9-methyl-8,9,10,11-tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine* (**4c**)*:* Prepared as per the procedure mentioned for compound **4a** and **4b**. In this case triethylorthopropionate was used as cyclizing agent. yield 0·11 g (76%); pale yellow granules; m.p. 136–138°C; IR (KBr) ν cm^{-1} 3051, 2967, 2911, 1616, 1573; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1·13 (*d*, J = 5·3 Hz, 3H, CH_3), 1·44 (*t*, J = 6·6 Hz, 3H, $CH_2\underline{CH_3}$), 2·07 (*m*, 1H, C_9–H, 1H), 2·11 (*d*, J = 3·2 Hz, 2H, CH_2), 2·62 (*t*, 2H, CH_2), 2·85 (*t*, 2H, CH_2), 4·48 (*q*, J = 7·02 Hz, 2H, $\underline{CH_2}CH_3$), 8·90 (*s*, 1H, C_2–H, pyrimidine); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15·9, 19·3, 22·6, 24·3, 33·1, 36·5, 41·9, 122·0, 127·3, 135·1, 140·6, 145·9, 157·2, 160·0; Anal. calcd. for $C_{14}H_{16}N_4S$: C, 61·74; H, 5·92; N, 20·57. Found: C, 61·62; H, 5·85; N, 20·64.

2.1i 2-*Amino-7-oxo-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonitrile* (**7**)*:* To a well stirred mixture of 1,3-cyclohexanedione (8·2 g, 73 mmole) and malononitrile (4·8 g, 73 mmole) in ethanol (45 mL) was added elemental sulfur (2·3 g, 72 mmole). To this cooled reaction mixture was added diethylamine (5 mL) with vigorous stirring during 1 min. Reaction mixture was stirred at 40–45°C for about 1 h. The yellow–orange solid separated was filtered, washed with hot ethanol and recrystallised from dioxane to yield analytically pure yellow needles. Yield 9·8 g (70%); m.p. 215–217°C; IR (KBr) ν cm^{-1} 3339, 3190, 2962, 2901, 2213, 1645, 1623; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2·27 (*t*, 2H, CH_2), 2·50 (*m*, 2H, CH_2), 2·72 (*t*, 2H, CH_2), 8·30 (*s*, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ 19·1, 23·5, 39·8, 115·3, 116·2, 142·0, 144·8, 148·1, 188·2; Anal. calcd. for $C_9H_8N_20S$: C, 56·23; H, 4·19; N, 14·57. Found: C, 56·36; H, 4·05; N, 14·63.

2.1j *N-(3-Cyano-7-oxo-4,5,6,7-tetrahydro-benzo[b] thiophene-2-yl)-formimidic acid ethyl ester* (**8**)*:* A solution of **7** (1·10 g, 57 mmole) in triethylorthoformate (12 mL) was heated under reflux for 18 h, excess triethylorthoformate was removed under vaccum. The residue was treated with petroleum ether. Solid that separated was filtered and recrystallized with petroleum ether to afford light brown crystals. Yield 0·92 g (65%); m.p. 97–99°C; IR (KBr) ν cm^{-1} 2989, 2932, 2882, 2219, 1663, 1573, 1541; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1·43 (*t*, J = 6·9 Hz, 3H, $CH_2\underline{CH_3}$), 2·60 (*t*, 2H, CH_2), 2·89 (*m*, 2H, CH_2), 2·93 (*t*, 2H, CH_2), 4·49 (*q*, J = 7·09 Hz, 2H, $\underline{CH_2}CH_3$), 8·04 (*s*, 1H, N=CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14·1, 22·9, 25·1, 39·8, 58·3, 114·6, 116·4, 143·2, 146·0, 148·5, 164·1, 188·6; Anal. calcd. for $C_{12}H_{12}N_2O_2S$: C, 58·05; H, 4·87; N, 11·28. Found: C, 57·93; H, 4·97; N, 11·39.

2.1k *3-Amino-4-imino-5,6,7,8-tetrahydro-5H-benzo [4,5]thieno[2,3-d]pyrimidin-8-one* (**9**)*:* Prepared from compound **8** as per the procedure mentioned for preparation of **3**. Yield 0·83 g (64%); m.p. 176–178°C; light brown crystals; IR (KBr) ν cm^{-1} 3406, 3348, 3235, 3037, 2935, 1664, 1582; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2·39 (*t*, 2H, CH_2), 2·50 (*m*, 2H, CH_2), 2·90 (*t*, 2H, CH_2), 4·54 (*br s*, 2H, NH_2, D_2O exchangeable), 5·31 (*br s*, 1H, NH, D_2O exchangeable), 7·46 (*s*, 1H, C_2–H, pyrimidine); ^{13}C NMR (75 MHz, DMSO-d_6) δ 19·2, 24·0, 38·9, 130·1, 133·7, 140·5, 145·7, 164·1, 165·2, 190·8; Anal. calcd. for $C_{10}H_{10}N_4OS$: C, 51·27; H, 4·30; N, 23·91. Found: C, 51·36; H, 4·39; N, 23·82.

2.1l *6,7-Dihydro-[1]benzothieno[2,3-d]pyrimidin-4,8(3H,5H)-dione* (**11**)*:* Prepared from compound **7** as per the procedure mentioned for preparation of **5**. Yield 0·92g (80%); pale yellow granules; m.p. 154–156°C; IR (KBr) ν cm^{-1} 3087, 2955, 1672, 1585, 1375, 990; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2·45 (*t*, 2H, CH_2), 2·72 (*m*, 2H, CH_2), 2·93 (*t*, 2H, CH_2), 7·51 (*s*, 1H, C_2–H, pyrimidine), 11·98 (*br s*, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18·2, 23·8, 42·3, 136·0, 139·2, 145·1, 147·4, 164·3, 173·8, 189·0; Anal. calcd. for $C_{10}H_8N_2O_2S$: C, 54·53; H, 3·66; N, 12·72. Found: C, 54·41; H, 3·55; N, 12·86.

2.1m *4-Amino-6,7-dihydro[1]benzothieno[2,3-d]pyrimidin-8(5H)-one* (**12**)*:* Prepared from compound **8** as per the procedure mentioned for preparation of **6**. Yield 0·91 g (75%); pale yellow granules; m.p. 143–145°C; IR (KBr) ν cm^{-1} 3360, 3313, 3113, 1645, 1571; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2·50 (*t*, 2H, CH_2), 2·85 (*m*, 2H, CH_2), 2·96 (*t*, 2H, CH_2), 6·74 (*br s*, 2H, NH_2, D_2O exchangeable), 8·16 (*s*, 1H, C_2–H, pyrimidine); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24·2, 25·0, 38·9, 132·0, 135·1, 140·2, 142·1, 156·3, 166·8, 190·1; Anal. calcd. for $C_{10}H_9N_3OS$: C, 54·68; H, 4·06; N, 19·23. Found: C, 54·54; H, 3·96; N, 19·32.

2.1n *8,9,10,11-Tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine-8-one* (**10a**): Prepared from compound **9** as per the procedure mentioned for preparation of **4**. Yield 0·09 g (70%); intense yellow granules; m.p. 182–184°C; IR (KBr) ν cm^{-1} 3045, 2949, 1658, 1617, 1522, 1485, 1432; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2·79 (*t*, 2H, CH_2), 2·83 (*m*, 2H, CH_2), 2·97 (*t*, 2H, CH_2), 8·48 (*s*, 1H, C_2–H, pyrimidine), 9·33 (*s*, 1H, triazole); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22·4, 24·8, 39·6, 127·2, 133·9, 140·7, 142·3, 146·0, 147·8, 157·3, 190·1; Anal. calcd. for $C_{11}H_8N_4OS$: C, 54·09; H, 3·30; N, 22·94. Found: C, 53·91; H, 3·40; N, 23·02.

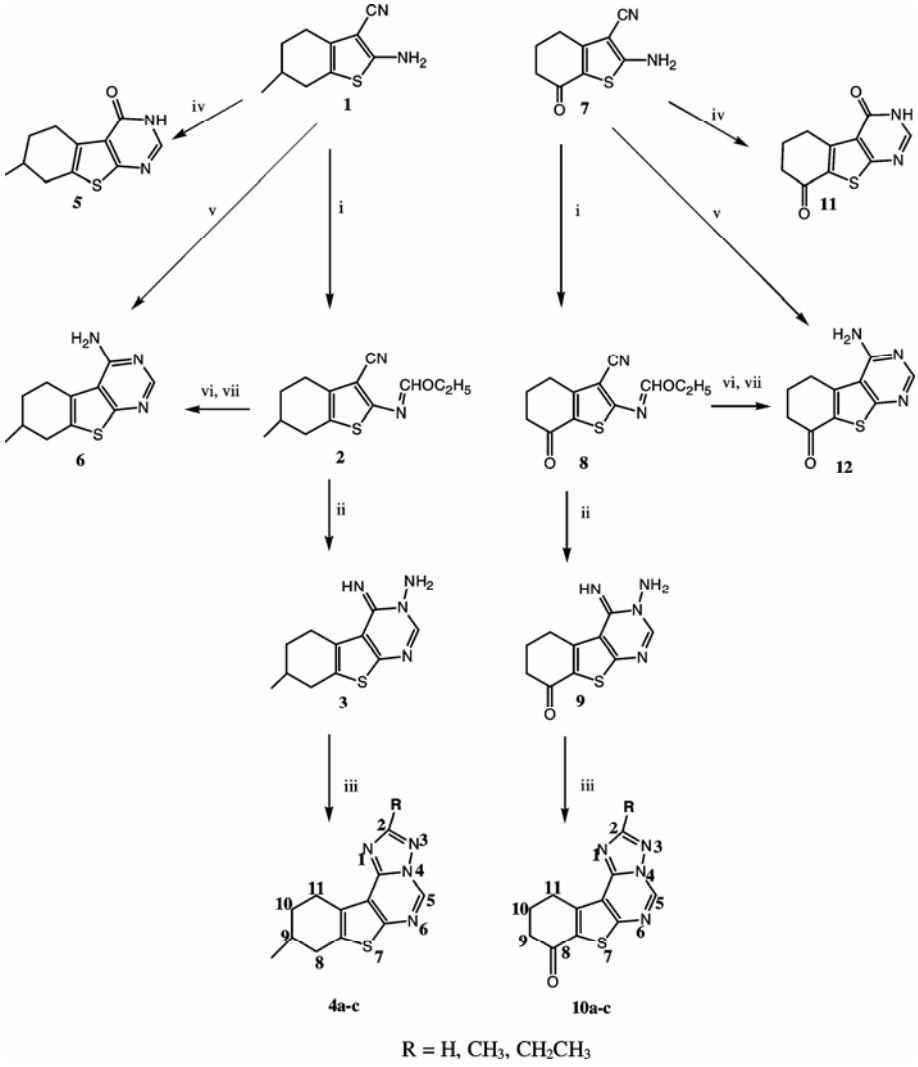
2.1o *2-Methyl-8,9,10,11-tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine-8-one* (**10b**): Prepared from compound **9** as per the procedure mentioned for preparation of **4**. Yield 0·1 g (73%); intense yellow granules; m.p. 175–177°C; IR (KBr) ν cm^{-1} 2999, 2927, 1672, 1618, 1543; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2·72 (*t*, 2H, CH_2), 2·76 (*m*, 2H, CH_2), 3·04 (*t*, 2H, CH_2), 3·77 (*s*, 3H, CH_3), 8·00 (*s*, 1H, C_2–H, pyrimidine); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15·2, 22·8, 25·3, 39·8, 131·6, 134·9, 141·2, 142·9, 146·4, 157·2, 162·2, 190·6; Anal. calcd. for $C_{12}H_{10}N_4OS$: C, 55·80; H, 3·90; N, 21·69. Found: C, 55·71; H, 3·96; N, 21·78.

2.1p *2-Ethyl-8,9,10,11-tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine-8-one* (**10c**)*:* Prepared from compound **9** as per the procedure mentioned for preparation of **4**. Yield 0·1 g (69%); intense yellow granules; m.p. 179–181°C; IR (KBr) ν cm^{-1} 2985, 2943, 2881, 1663, 1617, 1542; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1·35 (*t*, J = 6·6 Hz, 3H, $CH_2\underline{CH_3}$), 2·72 (*t*, 2H, CH_2), 2·79 (*m*, 2H, CH_2), 2·89 (*t*, 2H, CH_2), 4·23 (*q*, J = 7·02 Hz, 2H, $\underline{CH_2}CH_3$), 8·02 (*s*, 1H, C_2–H, pyrimidine); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15·9, 22·4, 24·9, 26·3, 39·9, 128·8, 134·0, 139·4, 144·2, 148·0, 157·1, 160·6, 191·1; Anal. calcd. for $C_{13}H_{12}N_4OS$: C, 57·34; H, 4·44; N, 20·57. Found: C, 57·25; H, 4·49; N, 20·62.

3. Results and discussion

In the present investigation we have synthesized novel fused tricyclic and tetracyclic thienopyrimidines and thienotriazolopyrimidines from precursors 2-amino-6-methyl-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonitrile and 2-amino-7-oxo-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonitrile respectively. The reaction sequences employed for the synthesis of title compounds is shown in scheme 1. We envisage α-aminocarbonitriles[17,18] as general precursors for the synthesis of broad range of biologically active thienopyrimidines and triazolothienopyrimidines. 2-Amino-6-methyl-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonitrile **1** was prepared by Gewald reaction as reported in the literature.[18] Similarly, 2-amino-7-oxo-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonitrile **7** was prepared by the reaction of 1,3-cyclohexanedione under conditions reported by K. Gewald.[19] Formation of thiophene having α-aminonitrile was characterized by the presence of band at 2210 cm^{-1} due to cyano group and N–H stretching bands at 3339 and 3190 cm^{-1}. Further it was also supported by the presence of D_2O exchangeable broad singlet at δ 7·48 in ^{1}H NMR spectrum due to NH_2 group.

Imidoformates (**2**, **8**) were prepared in excellent yield by treating (**1**, **7**) with triethylorthoformate in refluxing temperature. The structure of (**2**, **8**) was assigned by the absence of ν_{N-H} in IR and the presence of a triplet at δ 1·3 and a quartet at 3·99 corre-



Reagents and conditions: i, Triethylorthoformate, reflux; ii, $NH_2NH_2 \cdot H_2O$, room temp; iii, $RC(OEt)_3$, DMF, 90°C; iv, HCOOH, reflux; v, $HCONH_2$, reflux; vi, anhydrous ethanolic NH_3, rt; vii, NaOEt in DMF, 100–112°C.

Scheme 1.

sponding to protons of the ethoxy group and peak around δ 8·01 due to N=CH in the ^{1}H-NMR spectrum, along with the other expected signals.

Reactions of imidoformates (**2**, **8**) with hydrazine hydrate afforded the thienopyrimidine (**3**, **9**). Formation of the products was confirmed by the presence of bands at 3420, 3349 and 3246 cm^{-1} in IR spectrum, due to amino and imino functional groups. ^{1}H-NMR spectrum shows D_2O exchangeable singlets at δ 4·72 and 4·35 due to amino and imino groups respectively and the C_2–H of pyrimidine resonated at δ 8·15 as a singlet along with other expected signals.

Similarly the reaction of imidoformates (**2**, **8**) with ethanolic ammonia followed by the cyclisation of intermediate with sodium ethoxide in dimethyl formamide resulted in the formation of aminothienopyrimidine (**6**, **12**). Formation of the product was established by the presence of characteristic band at 3324, 3231 cm^{-1} due to amino group in IR. The ^{1}H NMR spectrum showed the presence of D_2O exchangeable broad singlet at δ 5·6 due to NH_2 group and the C_2–H of pyrimidine at δ 8·5 as a singlet. Alternatively aminothienopyrimidines (**6**, **12**) were also prepared directly from the corresponding 2-amino-3-cyanothiophenes (1, 7) precursors by their reaction with formamide. The compounds (**5**, **11**) were prepared by refluxing 2-amino-3-cyanothiophenes (**1**, **7**) with formic acid.

The compounds (**3**, **9**) were further converted into triazolopyrimidine derivatives (**4**, **10**) by treatment

Table 1. Antibacterial and antifungal activities of the compounds as MIC values (μg/mL).

Compounds	*Staphylococcus aureus* ATCC 25923	*Bacillus subtilis* ATCC 6633	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Candida albicans* ATCC 10231	*Candida parapsilosis* ATCC 90018
3	512	128	256	256	32	128
5	512	128	256	256	64	64
6	256	11	128	256	128	64
9	512	256	256	256	16	128
11	512	11	256	256	128	256
12	256	128	256	256	128	64
4a	256	64	256	256	32	64
4b	128	128	256	256	64	128
4c	128	128	256	128	128	128
10a	128	11	128	256	128	128
10b	256	11	128	256	256	64
10c	128	11	256	128	128	64
Ampicillin	4	8	4	–	–	–
Fluconazole	–	–	–	–	8	0·25

with triethylorthoesters in dimethylformamide. The formation of triazole ring involving both amino and imino groups was evident by the absence of absorption bands due to either of these groups in the IR spectrum of (**4**, **10**). Further ^{1}H NMR spectrum also exhibited the presence of two characteristic protons each as singlet at δ 8·4 and δ 9·2 due to pyrimidine and triazole proton respectively.

All the compounds were evaluated for their antimicrobial properties. MIC's were recorded as the minimum concentration of compound, which inhibits the growth of tested microorganisms. As shown in table 1, none of the title compounds exhibited any activity against *S. aureus*, *P. aeruginosa* and *E. coli*, but, some of the synthesised compounds were found to be active against *B. subtilis*. The antibacterial activity of compounds **6**, **11**, **10a**, **10b** and **10c** was comparable with that of ampicillin against *B. subtilis*. Hence from the results it can be concluded that the tricyclic aminothienopyrimidines exhibited promising antibacterial activity, while the tetracyclic triazole fused thienopyrimidines also displayed good antibacterial activity against *B. subtilis*. The antifungal activity of compound **9** was 50% of that of fluconazole against *C. albicans*. Whereas compounds **3** and **4a** displayed moderate antifungal activity.

4. Biological activity

4.1 *Antimicrobial studies*

Minimum inhibitory concentration (MIC) values for the synthesized compounds were determined by using the broth microdilution method.[20,21] Two Gram-positive (*S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633) and two Gram-negative (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853) bacteria were used as quality control strains. For determining anti-yeast activities of the compounds, the following reference strains were tested: *Candida albicans* ATCC 10231 and *Candida parapsilosis* ATCC 90018. Ampicillin trihydrate and fluconazole were used as standard antibacterial and antifungal agents, respectively. Fluconazole was dissolved in sterile distilled water, ampicillin trihydrate in phosphate buffer (pH 8) and the stock solution of the synthesized compounds was dissolved in dimethyl sulfoxide (DMSO) and distilled water (50%) at a concentration of 2048 μg/mL. Two fold dilutions of the synthesized compounds were prepared (1024, 512… … 2 μg/mL), and two fold dilutions of the reference compounds were prepared at 64–0·125 μg/mL. All bacteria were cultivated in Mueller–Hinton Agar (Merck). The bacteria inoculums were prepared in Mueller–Hinton Broth (Merck) which had been kept at 36°C overnight and was diluted with broth to give a final concentration of 5×10^5 cfu/mL. All fungi were cultivated in sabouraud Dextrose Agar (Merck). The fungi inculums were prepared in sabouraud liquid medium (oxoid) which had been kept at 36°C overnight and was diluted with RPMI-1640 medium with L-glutamine buffered with 3-[N-morpholino]-propane sulfonic acid (MOPS) at pH 7 to give a final concentration of $2{\cdot}5 \times 10^3$ cfu/mL. The microplates were incubated at 36°C and read visually after 24 h, except for candida species when

it was at 48 h. The incubation chamber was kept humid. At the end of the incubation period, MIC values were recorded as the lowest concentrations of the substances that gave no visible turbidity. The DMSO diluents at a maximum final concentration of 12·5% had no effect on the microorganisms growth. The minimum inhibitory concentrations (MIC) were noted (table 1).

5. Conclusion

The present study reports the synthesis of novel fused tricyclic and tetracyclic thienopyrimidines and thienotriazolopyrimidines from the corresponding precursors 2-amino-6-methyl-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonitrile and 2-amino-7-oxo-4,5,6,7-tetrahydro-1-benzothio-phene-3-carbonitrile respectively. The investigation of antibacterial screening reveals that the compounds **6**, **11**, **10a**, **10b** and **10c** have exhibited good antibacterial activity against *B. subtilis* comparable to the standard ampicilin, while compounds **3**, **9** and **4a** displayed better antifungal activity against *Candida albicans* comparable to the standard fluconazole.

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