World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Design, synthesis and antimicrobial activity of tricyclic tetrahydro thieno [2,3-d] pyrimidine derivatives

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Received: 30-01-2015 / Revised: 11-02-2015 / Accepted: 19-02-2015

ABSTRACT

Novel tricyclic tetrahydro thienopyrimidines (**4a-h**) were prepared by a simple synthetic protocol from the corresponding 2-amino-3-cyanothiophenes (**1a** & **1b**). The required precursors were prepared by employing the Gewald reaction. The structures of all the newly synthesized tricyclic thienopyrimidines were established by the spectral and analytical data. The synthesized compounds were screened for their antibacterial and anti fungal activity and some of the compounds have displayed considerable antibacterial and anti fungal activities.

Keywords: Thienopyrimidine, Gewald reaction, Antibacterial activity.

INTRODUCTION

The rapid development of resistance to existing antimicrobial and antifungal drugs poses a major threat to public health. Consequently, there is a need to develop new antimicrobial and antifungal agents with potent activity. Among the broad range of templates, heterocyclic scaffolds represent the most promising molecules as leading structures for the discovery of novel synthetic drugs. In particular, the classes of multicyclic compounds bearing thienopyrimidine scaffolds have been the focus of great interest because of their remarkable biological properties including antiallergic, anti-inflammatory, analgesic, antifungal and antibacterial activities [1-12]. Consequently, thienopyrimidines have become a well sought privileged class of compounds in drug discovery programs and the interest in developing this class of bioactive compounds remains high in medicinal chemistry [13-15]. Considering all these biological activities of thienopyrimidines which were reported to be more potent and less toxic, it has been felt worthwhile to take up the present investigation in an effort to incorporate morpholine, piperidine ring system into thienopyrimidine framework to synthesize novel thieno[2,3-d] pyrimidine as antimicrobial and antifungal agents. We utilized thieno[2,3-*d*]pyrimidine scaffold as key prototype structural unit with important pharmacophoric groups like morpholine and piperidine.

MATERIALS AND METHODS

The reagents and solvents used in this study were of analytical grade and used without further purification. All the reactions were monitored on Merck aluminium thin layer chromatography (TLC, UV_{254nm}) plates. Column chromatography was carried out on silica gel (60-120 mesh). Melting points were determined by open capillary method and are uncorrected. Commercial reagents were used without purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker WM-300 or 400 using $CDCl_3$ and DMSO-d_6 as the solvent. Chemical shift are reported in parts per million (δ-value). IR spectra (KBr disc) were recorded on a Nicolet -5700 FT-IR spectrophotometer and reported in wave number (cm^{-1}). ESI mass spectra were recorded on Shimadzu LC-MS after dissolving the compounds in acetonitrile and methanol.

General procedure for preparation of 2-amino-4,5,6,7-tetrahydrobenzo [b]thiophene-3-carbonitrile (1a) and 2-amino-6-methyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (1b): To a well stirred mixture of cyclohexanone or 4-methylcyclohexanone (71 mmole) and malononitrile (4.7g, 71mmole) in ethanol (45 mL) was added elemental sulfur (2.31g, 72 mmole). To this cooled reaction mixture was added diethylamine (5 mL) with vigorous stirring during

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1 min. Reaction mixture was stirred at 40-45°C for about 1 h. The yellow-orange solid that separated was filtered, washed with hot ethanol and recrystallised from dioxane to yield analytically pure yellow needles **[**19**].**

General procedure for preparation of 2a and 2b: A mixture of **1a** or **1b (**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.

5, 6, 7, 8-tetrahydro[1]benzothieno[2,3-d] pyrimi din-4[3H]-one (2a): Yield 89%, mp 124-126 °C, IR (KBr) ν cm-1: 3158, 3070, 1664, 1580. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.89 (m, 4H, CH_2), 2.80 (t, J = 8Hz, 2H, CH_2), 2.91 (t, J = 8Hz, 2H, CH_2), 8.51 (s, 1H, CH), 11.67 (br s, 1H, NH, D_2O exchangeable); Anal. calcd. for $C_{10}H_{14}N_2OS$: C, 57.11; H, 6.71; N, 13.32; S, 15.25 Found: C, 57.51; H, 6.91; N, 13.52; S, 15.45.

7-Methyl-5, 6, 7, 8-tetrahydro[1] benzothieno [2,3-d]pyrimidin-4[3H]-one (2b): Yield 80%, mp 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 = 5.3Hz, 3H, CH_3), 2.09 (m, 1H, C_7-H), 2.20 (d, J = 3.2Hz, 2H, CH_2), 2.45 (t, 2H, CH2), 2.76 (t, 2H, CH2), 7.86 (s, 1H, C2-H, pyrimidine), 11.80 (br s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, CDCl3) 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.

General procedure for preparation of 3a and 3b: A solution of **2a** or **2b** (2.5 mmol) in dry dioxane (7.5 mL) was treated with phosphorus oxychloride (1.75 mL) and the mixture was stirred under reflux for 3 h. The reaction mixture was allowed to cool to room temperature and poured into ice-water (200 g); the solid that separated was filtered off and crystallized from petroleum ether.

4-Chloro-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine (3a): Yield 80%, mp. 110-112 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.89 (m, 4H, CH_2), 2.80 (t, J = 8Hz, 2H, CH_2), 2.91 (t, J =8Hz, 2H, CH_2), 8.3 (s, C2-H, pyrimidine); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.6, 33.4, 37.5, 42.8, 123.9, 128.9, 134.6, 141.8, 158.2, 158.9;

4-Chloro-7-methyl-5,6,7,8-tetrahydro[1]benzo thieno[2,3-d]pyrimidine (3b): Yield 82%, mp. 88-90 °C; ^{1}H NMR (300 MHz, $CDCl_3$), δ: 1.40 (d, 3H, CH_3), 2.09 (m, 1H, C9-H), 2.24 (d, 2H, CH_2), 2.49 (t, 2H, CH2), 2.78 (t, 2H, CH_2), 8.21 (s, C_2-H, pyrimidine); ^{13}C NMR (75 MHz, CDCl3) δ: 19.9, 21.6, 33.4, 37.5, 42.8, 123.9, 128.9, 134.6, 141.8, 158.2, 158.9; Anal. calcd. for $C_{11}H_{11}ClN_2S$: C, 55.34; H, 4.64; N, 11.73. Found: C, 55.39; H, 4.70; N, 11.80%.

General procedure for preparation of 4a-h: The Compound **3a** or **3b** (19.4 mmol) was dissolved in n-butanol (20 mL) and then added 1^0 or 2^0 amine (7.04 mmol) followed by triethyl amine (19.2 mmol). The reaction mixture was heated to 100 °C for 12 h. The reaction mixture was concentrated in vacuo and the residue was treated with ethanol (25 mL), the seperated solid was filtered, washed with excess of ethanol and recrystalised form chloroform to obtain analytically pure target compounds **(4a-h)** in good yields.

3,5-dimethyl-4-(5,6,7,8-tetrahydrobenzo [4,5] thieno[2,3-d]pyrimidin-4-yl)morpholine(4a): Yield 72%, mp. 165-168 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.3(d,6H), 1.89 (m, 4H, CH_2), 2.80 (t, J = 8Hz, 2H, CH_2), 2.93 (t, J = 8Hz, 2H, CH_2), 3.3-3.7(m,6H) 8.51 (s, 1H, CH), ^{13}C NMR (75 MHz, $CDCl_3$); 15.1, 23, 24.3, 25, 56, 74.6,113.7, 127.6, 137.5, 145.7, 156.3, 167.7 . Anal. calcd for: $C_{16}H_{21}N_3OS$ C, 63.33; H, 6.98; N, 13.85; O, 5.27; S, 10.57 Found:C, 63.52; H, 6.66; N, 13.70; O, 5.22; S, 10.5 m/z: 303

1-(5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d] pyrimidin-4-yl)piperidine-4-carbonitrile(4b): Yield 85%, mp. 180-182 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.87-1.90 (m, 4H, CH_2), 2.60 (t, J = 8Hz, 2H), 2.71 (t, J = 8Hz, 2H,)1.70-1.78 (m, 4H), 1.97-2.03 (m, 4H), 2.75 -2.81(m, 1H), 2.90(s,2H),2.94(s,2H),7.84(s,1H,pyrimidineH)^{13}CNMR(75MHz,$CDCl_3$):23,23.5,24,24.8,25.1,49.5,113.7,119.2,127.6,137.5,145.7,156.3,167.7 m/z: 298 Anal. calcd for: $C_{16}H_{18}N_4S$ C, 64.40; H, 6.08; N, 18.78; S, 10.75 Found: C, 64.20; H, 6.34; N,18.58; S, 10.94

N-(2-(piperidin-1-yl)ethyl)-5,6,7,8-tetrahydro benzo[4,5]thieno[2,3-d]pyrimidin-4-amine (4c): Yield 75%, mp. 170-172 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.80-2.30 (m, 10 H), 2.21 (t, 4H, -N-CH_2), 2.50-3.10 (m, 4H, CH_2) 2.80 (t, J = 8Hz, 2H, CH_2), 3.16 (t, 2H, -NH-CH_2), 4.01 (br s, 1H, NH, D_2O exchangeable), 7.86 (s, 1H, C2-H, pyrimidine); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.50, 25.10, 30.10, 47.81, 54.12, 55.21, 113.51, 127.37, 137.51, 145.71, 156.41, 160.12; Anal. calcd. for $C_{17}H_{24}N_4S$: C 64.52, H 7.64, N 17.70. Found: C 64.00, H 7.74, N 17.60.

N-(morpholinomethyl)-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-d]pyrimidin-4-amine (4d): Yield 65%, mp. 168-172 °C; ^{1}H NMR (300 MHz, CDCl3) δ: 1.81-2.10 (m, 8 H), 2.21 (t, 4H, -N-

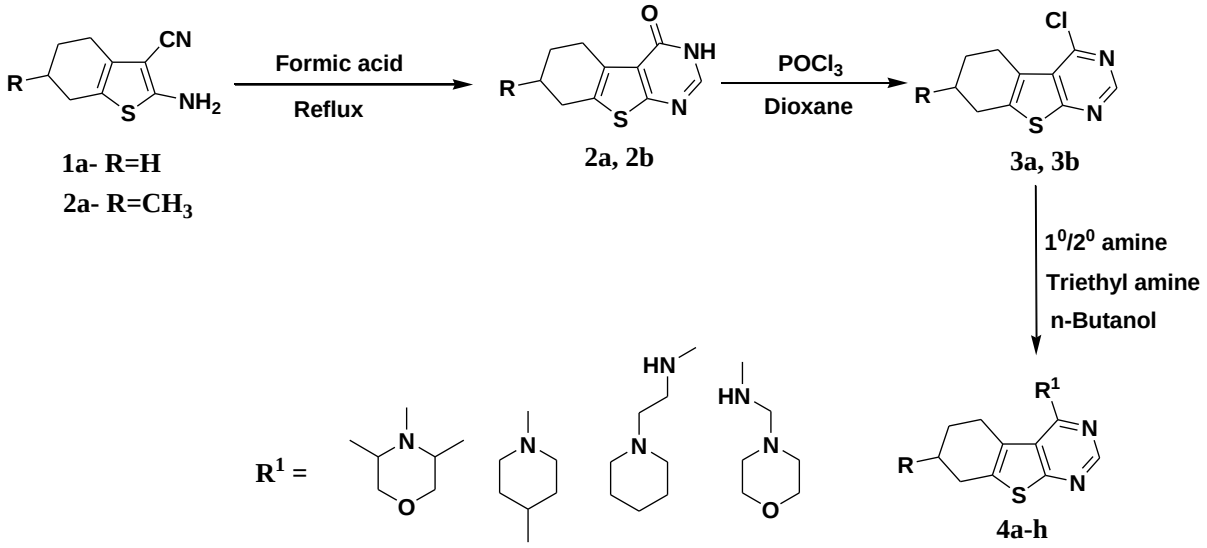
CH_2), 2.80 (t, J = 8Hz, 2H, CH_2), 2.91 (t, J = 8Hz, 2H, CH_2), 3.16 (t, 2H, -NH-CH_2), 4.01 (br s, 1H, NH, D_2O exchangeable), 7.86 (s, 1H, C2-H, pyrimidine) ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.51, 24.31, 25.01, 50.12, 67.29, 72.81, 113.81, 127.62, 137.51, 145.71, 156.31, 157.21; Anal. calcd. for $C_{15}H_{20}N_4OS$: C 59.18, H 6.62, N 18.41. Found: C 59.25, H 6.42, N 18.30.

3,5-dimethyl-4-(7-methyl-5,6,7,8-tetrahydro benzo[4,5]thieno[2,3-d]pyrimidiyl) morpholine (4e): Yield 81 %, mp. 171-172 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.31-1.36 (m, 9H, CH_3) , 2.09 (m, 1H, C7-H), 2.20 (d, J = 3.2Hz, 2H, CH_2), 2.45 (t, 2H, CH_2), 2.76 (t, 2H, CH_2),3.3-3.7(m,6H) 7.81 (s, 1H, C2-H, pyrimidine); ^{13}C NMR(75MHz,$CDCl_3$); 15.3, 20.1, 21.8, 29.3, 30.2, 33.4, 56.2,74.6,113.7,127.6,137.5, 156.3, 145.7,167.7. m/z: 317 Anal. calcd for: $C_{17}H_{23}N_3OS$: C, 64.32; H, 7.30; N, 13.24; O, 5.04; S, 10.10 Found:C, 64.00; H, 7.40; N, 13.14; O, 5.04; S, 10.15

1-(7-methyl-5,6,7,8-tetrahydrobenzo [4,5]thieno [2,3-d]pyrimidin-4-yl)piperidine carbonitrile (4f): Yield 78%, mp. 210-212 °C; ^{1}H NMR (300 MHz, $CDCl_3$); δ: 1.35 (*d*, J = 5.3Hz, 3H, CH_3), 2.09 (*m*, 1H, C_7-H), 2.20 (d, J = 3.2Hz, 2H,), 2.45 (*t*, 2H,), 2.7 (*t*, 2H,), 1.70-1.78 (m, 2H), 1.95-1.99 (m, 2H), 2.78 -2.83(m, 1H), 2.90 (s, 2H), 2.94 (s, 2H) ,7.86 (*s*, 1H, pyrimidine H) m/z: 312.1 , ^{13}CNMR (75MHz,$CDCl_3$) ;20.1, 21.5, 23,25.1,29.1,30.2,49.5,113.7,127,136.5,145.7 ,156.3,167.7. Anal. calcd for $C_{17}H_{20}N_4S$;C, 65.35; H, 6.45; N, 17.93; S, 10.26 found: C, 65.23; H, 6.15; N, 17.83; S, 10.56

7-methyl-N-(2-(piperidin-1-yl)ethyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-amine(4g): Yield 82%, mp. 186-188 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.35 (d, J = 5.3Hz, 3H, CH_3),1.81-2.10 (m, 10 H), 2.09 (m, 1H, C7-H), 2.20 (d, J = 3.2Hz, 2H, CH_2), 2.45 (t, 2H, CH2), 2.76 (t, 2H, CH_2),2.80 (t, 2H, N-CH_2), 3.16 (t, 2H, -NH-CH_2), 4.01 (br s, 1H, NH, D2O exchangeable), 7.86 (s, 1H, C2-H, pyrimidine); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.3, 26.1, 29.2, 30.2, 33.5, 47.7, 53.4, 54.1, 111.5, 121.6, 137.7, 145.8, 156.7, 160.1; Anal. calcd. for $C_{18}H_{26}N_4S$: C 65.42; H 7.93, N 16.95. Found: C 65.38; H 7.85, N 17.01.

7-methyl-N-(morpholinomethyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-amine (4h): Yield 75%, mp. 160-162 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.35 (d, J = 5.3Hz, 3H, CH_3), 2.09 (m, 1H, C7-H), 2.20 (d, J = 3.2Hz, 2H, CH2), 2.45 (t, 2H, CH2), 2.76 (t, 2H, CH2), 3.16 (t, 2H, -NH-CH_2), 3.61-4.10 (m, 8 H), 4.01 (br s, 1H, NH, D_2O exchangeable), 7.86 (s, 1H, C2-H, pyrimidine); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.30, 30.20, 33.50, 51.15, 66.52, 72.81, 116.54, 127.20, 137.50, 145.7, 156.30, 157.31; Anal. calcd. for $C_{16}H_{22}N_4OS$: C, 60.35 H 6.96, N 17.59. Found: C 60.05, H 6.75, N, 17.59.



Scheme 1: Synthetic protocol for the target compounds (**4a-h**).

RESULTS AND DISCUSSION

The reaction sequences employed for the synthesis of title compounds is presented in scheme 1. We have explored the fact that α-amino carbonitriles [16,17] are general precursors for the synthesis of broad range of biologically active thienopyrimidines. The amino nitriles **(1a)** and **(1b)** were prepared by the reactions of cyclohexanone and 4-methylcyclohexanone respectively under conditions reported by K. Gewald [18]. Formation of thiophene having α-aminonitrile was confirmed by the IR spectrum which shows the intense stretching band at 2210 cm^{-1} due to cyano group and N-H stretching bands at 3339 and 3190 cm^{-1}. Further it was also confirmed by ^1H NMR spectrum, which shows a D_2O exchangeable broad singlet at δ 7.48 ppm due to NH_2 group. Thienopyrimidin-4-ones **(2a** and **2b)** were prepared by refluxing 2-amino-3-cyanothiophenes **(1a and 1b)** with formic acid, which on treating with phosphorous oxychloride in dioxane afforded the 4-chlorothienopyrimidines **(3a** and **3b)**. Formation of these intermediate products were confirmed by the absence of ν_{NH} and $\nu_{C=O}$ bands in IR spectrum. Thus obtained 4-chlorothienopyrimidines **(3a** and **3b)** on treating with primary and secondary amines afforded the title compounds **(4a-h)** in good yields. The structral integreity of all novel compounds was established by specctral analysis.

Antibacterial Assay: All the novel compounds **4a-h** were evaluated for their antibacterial activities against two Gram-positive bacteria (*S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633) and two Gram-negative bacteria (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853). The bacterial suspension was adjusted with sterile saline to a concentration of 1×10^{-5} CFU. The tested compounds were dissolved in DMSO to prepare the stock solutions.

The tested compounds and reference drugs were prepared in Mueller- Hinton broth by two-fold serial dilution to obtain the required concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5 µg/mL. These dilutions were inoculated and incubated at 37 ^{0}C for 24 h. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment.

Antifungal assays: All the newly synthesized compounds were evaluated for their antifungal activity against Candida albicans (ATCC 10231) and Candida parapsilosi (ATCC 90018). A spore suspension in sterile distilled water was prepared from 1-day old culture of the fungi growing on Sabouraud agar (SA) media. The final spore concentration was $1 - 5 \times 10^3$ spore mL^{-1}. From the stock solutions of the tested compounds and reference antifungal Fluconazole, dilutions in sterile medium were made resulting in eleven desired concentrations (0.5 - 512 mg/mL) of each tested compound. These dilutions were inoculated and incubated at 37 ^{0}C for 24 h. The minimum inhibitory concentration values (MIC) (in µg/mL) are summarized in Table 1.

Table-1: Antibacterial and antifungal activities of the compounds as MIC values

Compounds	**MIC_{50} (µg / mL)**					
	C. albicans	***C. parapsilosis***	***S. aureus***	***B. subtilis***	***E. coli***	***P. aeruginosa***
4a	16	8	**8**	16	32	16
4b	32	32	16	**8**	128	128
4c	**4**	4	16	16	128	>512
4d	16	16	**8**	**8**	64	16
4e	64	64	32	**8**	256	128
4f	128	32	32	128	64	32
4g	8	16	16	128	32	128
4h	**4**	32	**8**	16	8	16
A	-	-	8	8	2	4
B	4	2	-	-	-	-

A: Ampicillin; B: Fluconazole.

MIC values 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 had activity against *P. aeruginosa*, E. *coli* and *C. parapsilosi* but, generally, the title compounds were found to be active against *B. subtilis, S aureus* and C. *albicans*. The compounds **4a, 4b, 4d and 4e** have

exhibited good antibacterial activity comparable to the standard ampicilin, while compounds **4c** displayed better antifungal activity against *Candida albicans* comparable to the standard fluconazole. Compound **4h** exhibited considerable antibacterial as well as antifungal activity.

CONCLUSION

As part of our continuous search for the potential antimicrobial heterocyclic compounds, a series of novel tricyclic thienopyrimidine derivatives **4a-h** were synthesized and evaluated for their antibacterial and antifungal activity. The synthesis involves the cyclisation leading to the formation benzothieno[2,3-d]pyrimidin-4[3H]-one, which is derivatized by reaction with various 1^0 and 2^0 amines. The products were obtained in high purity with good yields, which have been, unambiguously, characterized by ^{1}H NMR, ^{13}C NMR, and LC-MS analysis. All the newly synthesized compounds were screened for antibacterial and antifungal activity and some of the compounds showed promising antibacterial as well as antifungal activities. In general, antibacterial activity was found to be more prominent than antifungal activity. The SAR profile suggests that attachment of amine group to thienopyrimidine provides valuable inputs for further leads in the development of molecules towards inhibiting existing drug resistant forms of bacterial pathogens.

ACKNOWLEDGEMENT

We greatly acknowledge University Science Instrument Centre (USIC), Karnatak University, Dharwad for spectral analysis.

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