Research Article **www.ijcps.com**

International Journal of Chemical and Pharmaceutical Sciences
2015, Mar., Vol. 6 (1)

ISSN: 0976-9390
IJCPS

Synthesis of some new thienopyrimidines and triazole fused thienopyrimidines and their antimicrobial activities

[1] Naveen P. Badiger, [2] Santhosh L. Gaonkar and [2] Nitinkumar S. Shetty*.

[1] Department of Chemistry, Karnatak University, Dharwad, Karnataka, India.

[2] Department of chemistry, Manipal Institute of Technology, Manipal, Karnataka, India.

* **Corresponding Author:** E-Mail: snitinshetty@gmail.com

Received: 11 Mar 2015, Revised and Accepted: 13 Mar 2015

ABSTRACT

Some more new derivatives of thienopyrimidines and 1, 3, 4 -triazole fused thienopyrimidines were synthesized efficiently from 2-amino-thieno [2, 3-c] thiochromene-1-carbonitrile 1. Using the Gewald reaction, the precursor 1 was prepared. The structures of newly synthesized thiophene derivatives were ascertained by spectral and analytical data and they were screened for antimicrobial activity where some of them have showed promising antibacterial and antifungal activities.

Keywords: Thienopyrimidine, Triazolothienopyrimidine, Antibacterial activity, Antifungal activity.

1. INTRODUCTION

Compounds containing the thienopyrimidine nucleus have been prepared by several chemists because of their diverse potent biological properties. [1-6] For instance, a number of thienopyrimidines are known to possess antimalerial, antiallergic, hypo cholesterolemic, analgesic, anti-inflammatory, diuretic, CNS depressant and antiviral activities. [7-9] Literature survey also reveals that triazoles and N-bridged heterocycles fused with them possess diverse pharmacological activities. [10-12] In view of these reports and in continuation of our work on biologically active nitrogen and sulfur heterocycles. [13-21] we have synthesized some more novel triazolothienopyrimidines and evaluated them for 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.[22, 23]

2. MATERIALS AND METHODS

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 spectrum was recorded on Finnigan MAT (Model MAT8200) spectrometer and elemental analyses were carried out using Heraus CHN rapid analyzer.

2.1. Preparation of 2-amino-4*H*-thieno[2,3-*c*]thiochromene-1-carbonitrile (1)

To a well-stirred mixture of thiochromane-4-one **3** (0.05mol) and malononitrile (3.3g, 0.05mol) in ethanol (45mL) was added elemental sulfur (1.68g, 0.0525mol), diethylamine (5mL) with vigorous stirring during 2 mins. The reaction mixture was stirred at 40-45^{o}C for 1hr. The yellow-orange solid that separated was filtered, washed with hot ethanol and recrystallized from dioxane to yield analytically pure yellow needles. Yield 54%, m.p. 284-286^{o}C; IR (KBr) νcm^{-1}: 3360, 3224, 3033, 2918, 2191, 1629, 1579, 1534; ^{1}H NMR (300MHz, DMSO-d_6) δ: 3.94(s, 2H, S-CH_2), 5.65(s, 2H, NH_2, D_2O exchangeable), 7.01(t, *J*=7.5Hz, 1H, Ar-H), 7.12(t, *J*=7.5Hz, 1H, Ar-H), 7.43(d, *J*=8.1Hz, 1H, Ar-H), 7.68(d, *J*=8.1Hz, 1H, Ar-H).

2.2.Thiochromene[2,3-*c*]thieno[2,3-*d*]pyrimidin-4-one (2)

Microwave irradiation of mixture of **1 (**1 g**)** and formic acid (18 mL) at 80^o C for 15 minutes yield **2**. The excess of formic acid was removed under reduced pressure. The resulting residue

was crystallized from ethanol to yield pale yellow granules. Yield 78%, m.p. 161-163°C, IR (KBr) ν cm-1: 2934, 1672, 1580, 1364, 974; ^{1}H NMR (300MHz, $CDCl_3$) δ: 3.77(s, 2H, S-CH_2), 6.91(t, *J*=7.5Hz, 1H, Ar-H), 7.11(t, *J*=7.5Hz, 1H, Ar-H), 7.49(d, *J*=8.1Hz, 1H, Ar-H), 7.60(d, *J*=8.1Hz, 1H, Ar-H), 7.86 (*s*, C2-H, pyrimidine), 11.56 (*br s*, 1H, NH, D_2O exchangeable).

2.3. 4-Chloro-thiochromene[2,3-*c*]thieno[2,3-*d*]pyrimidine (3)

A solution of **2** (2.5 mmol) in dry dioxane (8 mL) was treated with phosphorus oxychloride (1.80 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. Yield 74%, m.p. 92-94°C; IR cm^{-1}: 3065, 1587, 1323, 965; ^{1}H NMR (300MHz, $CDCl_3$), δ: 3.55(s, 2H, S-CH_2), 6.97(t, *J*=7.5Hz, 1H, Ar-H), 7.23(t, *J*=7.5Hz, 1H, Ar-H), 7.54(d, *J*=8.1Hz, 1H, Ar-H), 7.77(d, *J*=8.1Hz, 1H, Ar-H), 8.01 (*s*, C2-H, pyrimidine).

2.4. 4-Hydrazino-thiochromene[2,3-*c*]thieno[2,3-*d*]pyrimidine (4)

A mixture of compound **3** (2.5 mmol) and hydrazine hydrate 80 % (2 mL) in ethanol (15 mL) was heated at 50°C for 3 h. The reaction mixture was allowed to cool to room temperature. The deposited so precipitate was filtered off and crystallized from dioxane. Yield 72%, m.p. 143-145°C; IR cm^{-1}: 3385, 3320, 3115, 1562, 980; ^{1}H NMR (300MHz, $CDCl_3$), δ: 3.72(s, 2H, S-CH_2), 4.56 (br s, 2H, NH_2, D_2O exchangeable), 5.79 (br s, 1H, NH, D_2O exchangeable), 7.04(t, *J*=7.5Hz, 1H, Ar-H), 7.20(t, *J*=7.5Hz, 1H, Ar-H), 7.50(d, *J*=8.1Hz, 1H, Ar-H), 7.68(d, *J*=8.1Hz, 1H, Ar-H), 8.08 (s, C2-H, pyrimidine).

2.5. Triazolothienopyrimidine derivative (5a)

A mixture of compound **4** (2 mmol), formic acid (2 mL), and a catalytic amount of concentrated hydrochloric acid was heated under reflux for 7 hours. The reaction mixture was allowed to cool to room temperature and poured into water (200 mL). The precipitate that was formed was collected by filteration, washed with ethanol (50 mL), dried and crystallized from dioxane:ethanol mixture(2:1). Yield 65%, m.p. 143-145°C; IR cm^{-1}: 3100, 2995, 1581, 1360, 961; ^{1}H NMR (300MHz, $CDCl_3$) δ: 3.72(s, 2H, S-CH_2), 7.02(t, *J*=7.5Hz, 1H, Ar-H), 7.18(t, *J*=7.5Hz, 1H, Ar-H), 7.48(d, *J*=8.1Hz, 1H, Ar-H), 7.67(d, *J*=8.1Hz, 1H, Ar-H), 7.91(s, C2-H, pyrimidine), 9.27 (s, 1H, triazole).

2.6. Triazolothienopyrimidine derivative (5b)

A mixture of compound **4** (2 mmol), glacial acetic acid (6 mL) was heated under reflux for 15 hours. The reaction mixture was allowed to cool to room temperature and poured into water (200 mL). The precipitate that was formed was collected by filteration, dried and crystallized from acetic acid. Yield 68%, m.p. 174-176°C; IR cm^{-1}: 3109, 3018, 1584, 1309, 970; ^{1}H NMR (300MHz, $CDCl_3$) δ: 3.72(s, 2H, S-CH_2), 7.02(t, *J*=7.5Hz, 1H, Ar-H), 7.18(t, *J*=7.5Hz, 1H, Ar-H), 7.48(d, *J*=8.1Hz, 1H, Ar-H), 7.67(d, *J*=8.1Hz, 1H, Ar-H), 7.98 (s, C2-H, pyrimidine).

2.7. Triazolothienopyrimidine derivative (5c)

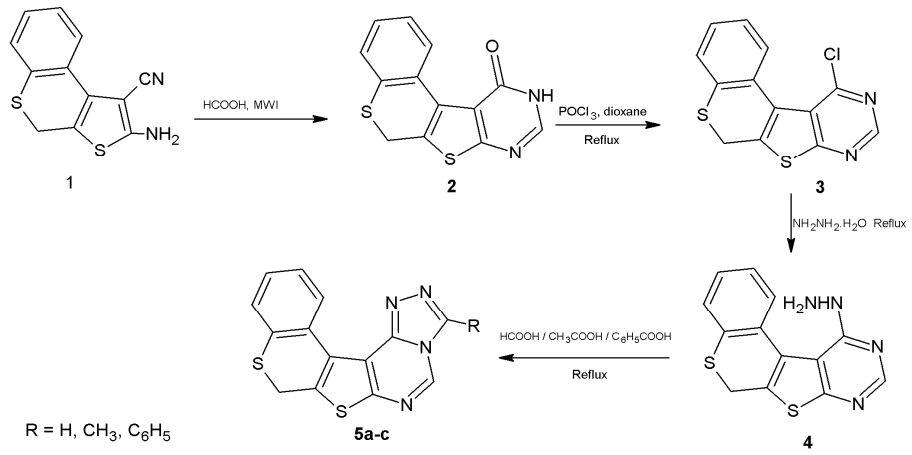
A mixture of compound **4** (2 mmol), Benzoyl chloride (6 mL) was stirred under reflux for 8 hours. The reaction mixture was allowed to cool to room temperature and poured into water (200 mL). The precipitate that was formed was collected by filteration, washed with ethanol (50 mL), dried and crystallized from dioxane:ethanol mixture (2:1). Yield 82%, m.p. 175-177°C; IR cm^{-1}: 3142, 3018, 1560, 1311, 965; ^{1}H NMR (300MHz, $CDCl_3$) δ: 3.68(s, 2H, S-CH_2), 7.11(t, *J*=7.5Hz, 1H, Ar-H), 7.19(t, *J*=7.5Hz, 1H, Ar-H), 7.52(d, *J*=8.1Hz, 1H, Ar-H), 7.68(d, *J*=8.1Hz, 1H, Ar-H), 8.02 (s, C2-H, pyrimidine).

3. RESULTS AND DISCUSSION

As shown in **scheme I**, we employed α-aminocarbonitrile[15, 16] as a precursor for the synthesis of biologically active thienopyrimidines and triazolothienopyrimidines. 2-amino-thieno [2, 3-c] thiochromene-1-carbo- nitrile **1** was prepared from thiochromane-4-one under conditions reported by K. Gewald.[17, 18] Formation of thiophene having α-aminonitrile was characterized by the presence of band at 2215 cm^{-1} due to cyano group and N-H stretching bands at 3327 and 3189 cm^{-1}. Further it is also supported by the presence of D_2O exchangeable broad singlet at δ 7.56 in ^{1}H NMR spectrum due to NH_2 group.

Thienopyrimidin-4-one **2** was prepared by the microwave irradiation of 2-amino-thieno [2, 3-c] thio- chromene-1-carbonitrile **1** in presence of formic acid. The structure of **2** was ascertained by the absence of 2212 cm^{-1} due to cyano group and the presence of $\nu_{c=o}$ in IR and a signal at δ 8.26 due to N=CH. And also a D_2O exchangeable broad singlet at δ 11.4 for NH in the ^{1}H NMR spectrum, along with the other expected signals.

Thienopyrimidin-4-one **2** on treatment with dry dioxane and phosphorous oxychloride afforded the 4-chlorothienopyrimidine derivative **3**. Formation of this product was confirmed by the absence of ν_{NH} and $\nu_{C=O}$ bands in IR. Thus obtained 4-chlorothienopyrimidine **3** on treatment with hydrazine hydrate afforded the hydrazino derivative **4.** Formation of the product



1 2 3 4 5a-c

Scheme - 1: Synthesis of thienopyrimidines and triazole fused thienopyrimidines.

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	64	256	256	16	128
4	512	32	256	256	64	64
5a	256	12	128	256	32	64
5b	512	32	256	256	16	128
5c	512	12	256	256	64	256
Ampicillin	4	8	4	-	-	-
Fluconazole	-	-	-	-	8	0.25

was confirmed by the presence of bands at 3429, 3352 and 3238 cm^{-1} in IR spectrum, due to amino functional groups. ^1H NMR spectrum shows D_2O exchangeable singlets at δ 4.86 and 4.39 due to amino groups and the C_2-H of pyrimidine resonated at δ 8.20 as a singlet along with other expected signals.

The compound **4** was further converted into triazolothienopyrimidine derivatives **(5a-c)** by treatment with aliphatic acids such as formic or acetic acid or acid chlorides such as benzoyl chloride. The formation of triazole ring involving both amino groups was evident by the absence of absorption bands due to either of these groups in the IR spectrum of **4**. Further ^1H NMR spectrum also exhibited the presence of two characteristic protons each as singlet at δ 8.46 and δ 9.25 due to pyrimidine and triazole proton respectively.

3.1. Antimicrobial activity

Minimum inhibitory concentration (MIC) values for the synthesized compounds were determined by using the broth microdilution method [24, 25]. 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. Twofold dilutions of the synthesized compounds were prepared (1024, 512… …2 µg/mL), and twofold 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 was prepared in Mueller-Hinton Broth (Merck) which had been kept at 36^0C overnight and was diluted with broth to give a final concentration of 5 x10^5 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 36^0C 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 2.5 x 10^3 cfu/mL. The microplates were incubated at 36^0C 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 microorganism's growth. The minimum inhibitory concentrations (MIC) were recorded and as shown in table 1, none of the title compounds had activity against *S. aureus*, *P. aeruginosa* and *E. coli,* but, generally, the title compounds were found to be active against *B. subtilis* and the *fungi*. The antibacterial activity of compounds **5a** and **5c** was 66% of that of ampicillin against *B. subtilis*. Therefore it can be suggested that these compounds show promising antibacterial activity. The antifungal activity of compound **3** and **5b** was 50% of that of fluconazole against *C. albicans*.

4. CONCLUSION

Herein we report the efficient synthesis of some tricyclic and triazole fused tetracyclic thienopyrimidines. All the compounds were characterised by spectral and analytical data and evaluated for their antimicrobial properties. The investigation of antibacterial screening reveals that the compounds **5a** and **5c** have exhibited good antibacterial activity against *B. subtilis* comparable to the standard ampicillin, while **3** and **5b** displayed better antifungal activity against *candida albicans* comparable to the standard fluconazole.

Acknowledgment

We are very thankful to MIT, Manipal for providing us facilities and motivation for completion of the research work.

5. REFERENCES

1. a) Rosowsky A, Chaykovsky M, Chen KKN, Lin M and Modest EJ. 2,4-Diaminothieno[2,3-d]pyrimidines as antifolates and antimalarials: Synthesis of 2,4-diamino-5,6,4,8-tetrahydrothianaphtheno[2,3-d]pyrimidines and related compounds**. J. Med. Chem,**. 1973; 16: 185-188.
2. Rosowsky A, Chen KKN and Lin M. 2,4-Diaminothieno[2,3-d]pyrimidines as antifolates and antimalarials: Synthesis of 5,6-disubstituted derivatives and related tetracyclic analogs**. J. Med. Chem.,** 1973; 16: 191-194.
3. Shishoo CJ, Devani MB, Karvekar MD, Ullas GV, Anathan S, Bhadti VS, Patel RB and Gandhi TP. Synthesis and Biological Activity of Tetrazolothienopyrimidines. **Indian J. Chem**., 1982; 21B: 666-668.
4. Cannito A, Perrissin M, Luu-Duc C, Huguet F, Gaultier C and Nareisse G. Synthesis and pharmacological properties of thieno[2,3-d]-4-one 2-thiones. **Eur. J. Med Chem**., 1990; 25: 635-639.
5. Russo F, Romeo G, Santagati NA, Venturini R and Spampinato S. Synthesis and pharmacological activity of 2-alkylthio substituted thieno[2,3-d]pyrimidine-4-one and 5H-pyrimido [5,4-b]indol-4-one. **Pharmazie**. 1990; 45: 493-500.
6. Munchhof MJ, Beebe JS, Casavant JM, Cooper BA, Doty JL, Higdon RC, Hillerman SM, Soderstrom CI, Knauth EA, Marx MA, Rossi AMK, Sobolov SB and Sun J. Design and SAR of thienopyrimidine and thienopyridine inhibitors. **BioOrg. Med. Chem. Lett**., 2004; 14: 21-27.
7. Kulshreshtha MJ, Bhatt S, Pardasani M and Khanna NM. **J. Indian Chem. Soc**., 1981; LVIII**:** 982.
8. Shishoo CJ, Devani MB, Anathan S, Jain KS, Bhadti VS, Mohan S and Patel LJ. Synthesis of some 2-Substituted Quinazolin-4-ones and Thienopyrimidin-4-ones of Biological Interest and Isolation of o-functionalized Amidine Intermediates. **Indian J. Chem**., 1989; 28B: 1039-1045.
9. Shishoo CJ and Jain KS. 2,4-Diaminopyridines and condensed 2,4-diaminpyrimidines have exhibited promising antifolate activities**. J. Het. Chem**., 1992; 29: 883-887.
10. Bradbury RH and Revett JE. 1,2,4-Triazolo[4,3-a]pyrazine derivatives with human renin inhibitory activity: Synthesis and biological properties of aminodeoxystatine and difluorostatone derivatives. **J. Med. Chem**., 1991; 34: 151-157.
11. Holla BS, Kalluraya, Sridhar KR, Drake E, Thomas LM, Bhandary KK and Levine MJ. **Eur. J. Med. Chem**., 1994; 29: 301-304.
12. Burni SSF, Castanz A, Guerrini G, Ajello PM, Lavstrone G and Martini C. Synthesis and preliminary evaluation of pyrazolo[1,5-c]pyrido[3,4-c]pyrimidine-6-one. **Eur. J. Med. Chem**., 1992; 27: 985-900.
13. Kolavi G, Hegde V and Khazi I. Intramolecular amidation: Synthesis of

novel imidazo[2, 1-b][1, 3, 4] thiadiazole and imidazo[2, 1-b][1, 3] thiazole fused diazepinones. **Tetrahedron Letters**. 2006; 47: 2811-2814.

14. Kolavi G, Hegde V, Khazi I and Gadad P. Synthesis and evaluation of antitubercular activity of imidazo[2, 1-b][1, 3, 4]thiadiazole derivatives. **Bioorganic & Medicinal Chemistry**. 2006; 14: 3069- 3080.
15. Hegde VS, Kolavi G.D.; Khazi IM. Chemoselectivity of thiophene dicarboxylate towards hydrazine hydrate: synthesis of some bis heterocycles from thiophene monocarbohydrazide. **J. Sulf. Chem.**. 2006; 27: 307-314.
16. Shetty NS, Lamani RS, Khazi IM. Synthesis and antimicrobial activity of some novel thienopyrimidines and triazolothinopyrimidines. **J. Chem. Sci**., 2009; 121:301-307.
17. Shetty NS, Gaonkar SL, Pai VV. Synthesis and Antimicrobial Activity of Tricyclic Thienopyrimidines and 1, 3, 4-Triazole Fused Thienopyrimidines. **Journal of Chemical science and Technology**. 2013; 2:93-96.
18. Dave CG, Shah RD. Synthesis of 7H-tetrazolo[1,5-c]pyrrolo[3,2-e] pyrimidines and their reductive ring cleavage to 4-aminopyrrolo[2,3-d] pyrimidines. **J. Het. Chem**., 1998; 35:1295-1300.
19. Review: Sebnis RW, Ragnekar DW, Sonawane ND. 2-aminothiophenes by the Gewald reaction. **J. Het. Chem.,** 1999; 36:333-345.
20. Gewald K, Schinke E, Boettcher H. Aminothiophene synthesis. **Chem Ber**, 1966; 99: 94-100.
21. Rosowsky A, Chaykovsky M, Chen KKN, Lin M, Modest EJ. 2,4-diaminothieno[2, 3-d]pyrimidines as antifolates and antimalarials. **J. Med. Chem.,** 1973;16:185-188.
22. National Committee for Clinical Laboratory Standards. Method for broth dilution antifungal susceptibility testing yeast; Approved standard, M27-A, 15, 10, NCCLS, VA Medical Center, Tuscon, 1996.
23. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Third ed. Approved standard. NCCLS document M100-S12, NCCLS, Wayne, PA, USA, 2002.