www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.2, pp 648-654, April-June 2012

Synthesis and Antimicrobial Studies of Some Novel Thieno[2,3-*d*]pyrimidine derivatives

Yerra Rajeshwar[1], Naresh.K[1]*, Jayaveera K.N[2]

[1]S.R.College of Pharmacy, Warangal, A.P, India.

[2]Jawaharlal Nehru Technological University Ananthapur, Ananthapur, A.P, India.

Corres. Author: naresh4nani@yahoo.co.in

Abstract: Different 4, 5-substituted, 3-amino thiophene-2-Carbonitriles were prepared by using various ketones. From the 3-amino thiophene-2-carbonitriles different 4-amino thieno [2,3-*d*]pyrimidines were prepared and further these compounds were condensed with various aldehydes to afford Schiff's bases of Thieno[*2,3-d*]pyrimidines. The title compounds thus prepared were characterized by their physical (TLC, M.P) and spectral data (NMR, IR & Mass). Then the title compounds were screened for anti-microbial activity against different strains of micro-organisms. Antibacterial activity was carried out by using both Gram positive and Gram negative bacterial strains, the anti fungal activity was carried against *Candiada* and *Aspergillus* species.
Key words: Thiophene, Thienopyrimidine, Anti-microbial, *Candida*, Schiff's base.

Introduction

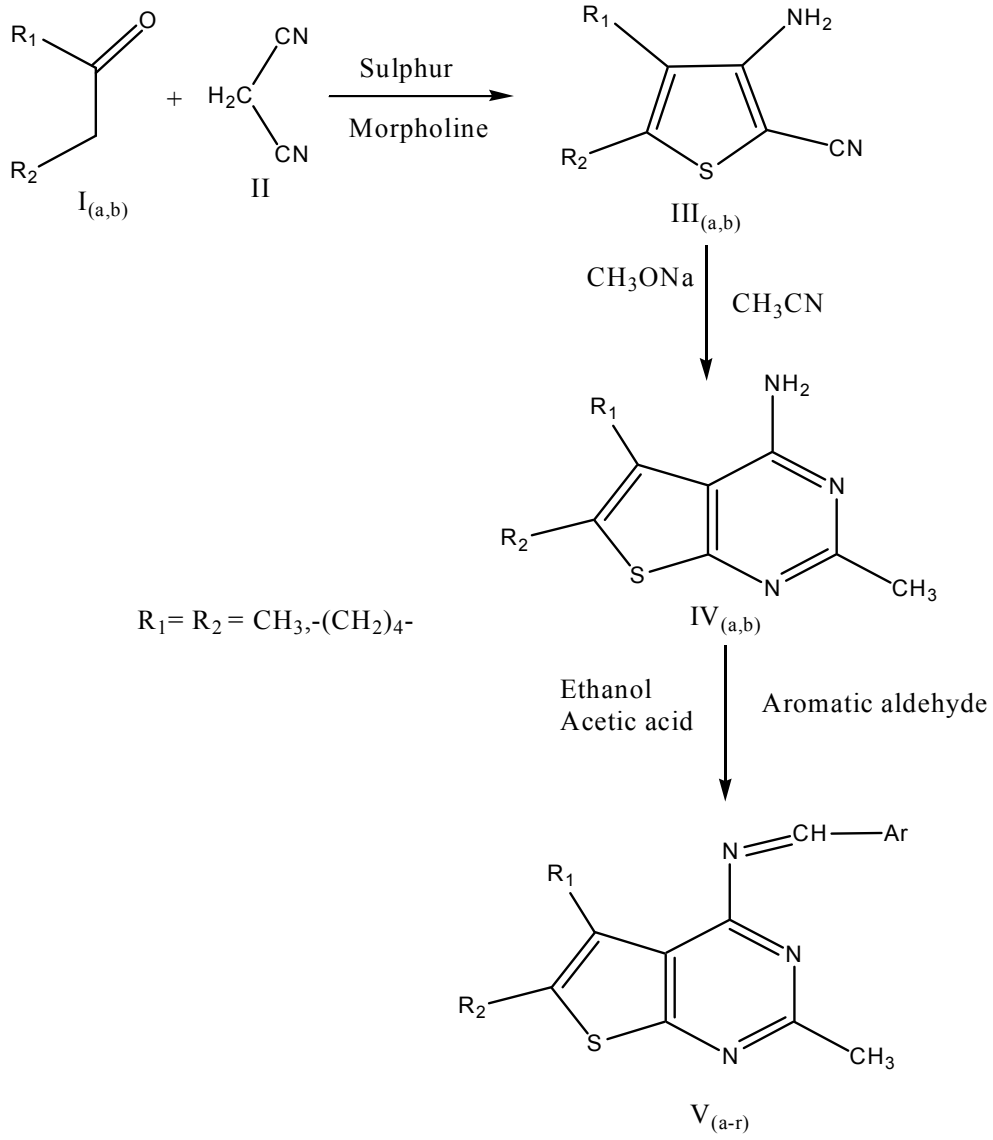
Thienopyrimidine is a biologically important heterocyclic moiety and attracting attention in medicinal chemistry research since last two decades due to its diverse range of biological activities. An intensive literature review on thienopyrimidines and its derivatives revealed that they were found to possesses different biological activities such as antitumor , antimicrobial, anti inflammatory, bronchodilatory activity[1,2,3] inhibitors of enzymes like VEGFR-2 kinase[4], tyrosine kinase and phosphodiesterases and most of them were patented[5].

In view of the biological importance and the past research of the thienopyrimidines and its derivatives, it is worthwhile to synthesize some novel thieno[*2,3-d*]Pyrimidine schiff's bases. The synthesis of title compounds was achieved by a systematic approach is outlined in the **Scheme-I.**

Experimental

Materials and methods:

Melting points of all the compounds were determined in open capillaries using Toshniwal and Cintex melting point apparatus and are uncorrected. IR spectra of the compounds were recorded on SCHIMADZU FT-IR Spectrophotometer by using KBr discs, ^{1}H NMR spectra were recorded on Gemini 300 MHz and Mass spectra were recorded on Aglient 1100 series. Progress of the each reaction in the present investigation was monitored by TLC using E-Merck 0.25 mm silica gel plates. The elemental analysis of title compounds were determined by using Carlo Erba 1108 elemental analyzer.



SCHEME-I

Synthesis of 2-amino 3-cyano 4, 5-substituted thiophene derivatives (IIIa& IIIb):

Equimolar amount (0.01mole) of Sulphur, Melanonitrile and a ketone (2-butanone/cyclohexanone) were taken in round bottom flask containing 20 ml of ethanol. The mixture was stirred for five minutes then morpholine(0.012mole) was slowly added to the reaction mixture at 50^{o}C with constant stirring for 10-15 minutes. Later, the reaction mixture was allowed to stir for five hours at room temperature and left in refrigerator over night. The crystals thus formed were collected by filtration under reduced pressure and washed with cold ethanol. The compound was further purified by recrystallization from ethanol. (Melting point: compound **IIIa** 145-147^{0}C and compound **IIIb** 142-144^{0}C)

Synthesis of 2-methyl 5, 6-substituted thieno [2, 3-*d*] pyrimidin-4-amine (IVa& IVb): 2-0.001moles of Amino-3-cyano thiophene III, 0.001moles of Methyl nitrile along with sodium methoxide (0.002moles) were taken in dry methanol. The reaction mixture was heated under reflux for eight hours. The excess solvent was removed under vacuum and added to crushed ice. The aqueous mixture was neutralized with dilute hydrochloric acid to liberate the compound out of the solution. The compound thus precipitated was collected by filtration under vacuum, which was purified by recrystallization from alcohol. (Melting point: compound **IVa** 137-139^{0}C and compound **IVb** 133-135^{0}C)

Synthesis of 2-methyl-4{[(Aryl)methylene] amino}-5,6-Substituted-thieno[2,3-*d*]pyrimidine derivatives(Va-r)

0.001 mole of 2-methyl 5, 6-substituted thieno [2, 3-*d*] pyrimidin-4-amine (IV) and aromatic aldehyde were dissolved in absolute ethanol, and then catalytic amount of glacial acetic acid was added dropwise and refluxed for 17h. The reaction mixture was then cooled in ice bath and the crude product thus obtained was collected by filtration. The crude compound was further purified by recrystallization from ethanol. The physical data and elemental analysis of compounds Va-r are presented in Table 1 and Table 2 respectively.

V_a: IR(KBr): 2938.3 and 2867cm^{-1}(-CH), 1615 cm^{-1} (C=N) :^{1}H NMR ($CDCl_3$): 1.98(s, 3H,-CH_3), 2.37(s, 3H, -CH_3), 2.46(s, 3H, -CH_3), 7.50(d, 3H, -ArH), 7.73(t, 2H, -ArH), 8.40(s, 1H, =CH): MS: *m/z* 282(M^{+1}).

V_d: IR(KBr): 2938 and 2867 cm^{-1}(-CH), 1615 cm^{-1} (C=N): ^{1}H NMR ($CDCl_3$): 1.96(s, 3H,-CH_3), 2.37(s, 3H, -CH_3), 2.46(s, 3H, -CH_3), 3.05(s, 6H, -CH_3), 6.9(d, 2H, -ArH), 7.50(s, 2H, -ArH), 8.36(s, 1H, =CH): MS: *m/z* 324(M^+).

V_f : IR(KBr): 2938 and 2867 cm^{-1}(-CH), 1615 cm^{-1} (C=N): ^{1}H NMR ($CDCl_3$): 1.97(s, 3H,-CH_3), 2.41(s, 3H, -CH_3), 2.46(s, 3H, -CH_3), 3.83(s, 6H, -OCH_3), 6.9(d, 1H, -ArH), 7.4(d, 1H, -ArH), 7.58(s, 1H, -ArH), 8.59(s, 1H, =CH).

V_h : IR(KBr): 2938.3 and 2867 cm^{-1}(-CH), 1615.1 cm^{-1} (C=N): ^{1}H NMR ($CDCl_3$): 1.9(s, 3H,-CH_3), 2.36(s, 3H, -CH_3), 2.41(s, 3H, -CH_3), 7.01(s, 1H, -ArH), 7.2(s, 1H, -ArH), 7.55(s, 1H, -ArH), 7.55(s, 1H, -ArH), 8.20(s, 1H, -ArH) 8.78(s, 1H, =CH).

V_n : IR(KBr): 2935.3 and 2864.2 cm^{-1}(-CH), 1615.1 cm^{-1} (C=N): ^{1}H NMR ($CDCl_3$): 1.20(t, 6H,-CH_3), 1.81(m, 4H,-CH_2), 2.43(s, 3H, -CH_3), 2.7(t, 2H, -CH_2), 2.9(t, 2H, -CH_2), 3.4(m, 4H, -CH_2), 7.01(s, 1H, -ArH), 6.9(d, 2H, -ArH), 7.68(d, 2H, -ArH), 8.20(s, 1H, -ArH) 8.45(s, 1H, =CH): MS: *m/z* 379(M+1).

Table: 1 Physical data table

Compound	Molecular formula	Melting point	Yield (%)	Molecular weight
V_a	$C_{16}H_{15}N_3S$	124-128	88	281
V_b	$C_{16}H_{14}ClN_3S$	117-120	87	315
V_c	$C_{16}H_{14}BrN_3S$	128-130	64	359
V_d	$C_{18}H_{20}N_4S$	164-168	72	324
V_e	$C_{20}H_{24}N_4S$	158-160	75	352
V_f	$C_{18}H_{19}N_3O_2S$	122-124	67	341
V_g	$C_{17}H_{17}N_3OS$	120-122	62	311
V_h	$C_{18}H_{16}N_4S$	152-154	74	320
V_i	$C_{14}H_{13}N_3S_2$	88-91	57	287
V_j	$C_{18}H_{17}N_3S$	118-121	79	307
V_k	$C_{18}H_{16}ClN_3S$	124-128	82	341
V_l	$C_{18}H_{16}BrN_3S$	205-207	72	386
V_m	$C_{20}H_{22}N_4S$	190-195	78	350
V_n	$C_{22}H_{26}N_4S$	119-123	69	378
V_o	$C_{20}H_{21}N_3O_2S$	154-158	62	367
V_p	$C_{19}H_{19}N_3OS$	173-176	73	337
V_q	$C_{20}H_{18}N_4S$	170-172	70	346
V_r	$C_{16}H_{15}N_3S_2$	100-105	68	313

Table: 2 spectral data of title compounds

Compound	Substituent's			Elemental analysis Found(calculated)		
	R_1	R_2	Ar	%C	%H	%N
V_a	CH_3	CH_3	$-C_6H_5$	73.48 (73.44)	4.94 (4.99)	12.25 (12.23)
V_b	CH_3	CH_3	p-Cl-C_6H_4	66.67 (66.75)	4.25 (4.27)	11.15 (11.12)
V_c	CH_3	CH_3	p-Br-C_6H_4	59.72 (59.72)	3.87 (3.82)	9.50 (9.95)
V_d	CH_3	CH_3	p-$N(CH_3)_2$-C_6H_4	66.68 (66.63)	6.22 (6.21)	17.27 (17.27)
V_e	CH_3	CH_3	p-$N(C_2H_5)_2$-C_6H_4	68.15 (68.15)	6.84 (6.86)	15.85 (15.89)
V_f	CH_3	CH_3	p,m-$(OCH_3)_2$-C_6H_3	63.30 (63.32)	5.60 (5.61)	12.30 (12.31)
V_g	CH_3	CH_3	p-(OCH_3)-C_6H_4	65.55 (65.57)	5.53 (5.50)	13.52 (13.49)
V_h	CH_3	CH_3	3-indolyl	72.26 (72.22)	4.75 (4.74)	14.67 (14.65)
V_i	CH_3	CH_3	3-thienyl	58.53 (58.51)	4.53 (4.56)	14.65 (14.62)
V_j	$-(CH_2)_4-$		$-C_6H_5$	70.35 (70.33)	5.58 (5.57)	13.69 (13.67)
V_k	$-(CH_2)_4-$		p-Cl-C_6H_4	63.30 (63.24)	4.74 (4.72)	12.30 (12.29)
V_l	$-(CH_2)_4-$		p-Br-C_6H_4	55.94 (55.96)	4.25 (4.17)	10.87 (10.88)
V_m	$-(CH_2)_4-$		p-$N(CH_3)_2$-C_6H_4	68.55 (68.54)	6.35 (6.33)	15.95 (15.99)
V_n	$-(CH_2)_4-$		p-$N(C_2H_5)_2$-C_6H_4	69.85 (69.80)	6.94 (6.92)	14.82 (14.80)
V_o	$-(CH_2)_4-$		p,m-$(OCH_3)_2$-C_6H_3	65.39 (65.37)	5.79 (5.76)	11.48 (11.44)
V_p	$-(CH_2)_4-$		p-(OCH_3)-C_6H_4	67.54 (67.63)	5.54 (5.68)	12.40 (12.45)
V_q	$-(CH_2)_4-$		3-indolyl	69.30 (69.34)	5.20 (5.24)	16.20 (16.17)
V_r	$-(CH_2)_4-$		3-thienyl	61.30 (61.31)	4.79 (4.82)	13.44 (13.41)

Biological activity

Antibacterial activity:

All the newly synthesized compounds (V_{a-r}) were screened for their possible antibacterial activity by disc diffusion technique[6-8] against *Bacillus subtilis, Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escheresia coli* bacterial strains. The test compounds and standard were dissolved in DMSO, solvent and growth controls were kept for comparison. The zone of inhibition (mm) were measured after incubating the Petri plates for 24h at 37^0C and the results are given in Table 3.

Antifungal activity:

The newly synthesized compounds were also screened for antifungal activity[6-8] against fungal strains *Candida albicans (NCIM), Candida krusei (NCIM), Aspergilus flavus (NCIM) and Aspergilus fumigatus (NCIM)*. The diameter of zone of inhibition was noted. Clotrimazole was used as standard to compare the activity of title compounds. The results are given in Table 4.

Table:3 Antibacterial data of title compounds(Va-r)

Compound		Diameter of zone of inhibition(mm)			
		S. aureus	***B. subtilis***	***P. aeruginosa***	***E. coli***
V_a	500µg	14	13	14	14
	1000 µg	17	15	16	15
V_b	500µg	17	16	16	14
	1000 µg	19	18	16	16
V_c	500µg	13	13	14	13
	1000 µg	15	14	17	17
V_d	500µg	15	14	15	13
	1000 µg	18	16	17	15
V_e	500µg	17	16	14	14
	1000 µg	17	17	18	19
V_f	500µg	14	13	13	14
	1000 µg	17	15	16	16
V_g	500µg	12	13	13	13
	1000 µg	14	14	15	16
V_h	500µg	15	15	15	15
	1000 µg	17	15	18	19
V_i	500µg	16	16	14	14
	1000 µg	18	18	18	19
V_j	500µg	14	14	14	14
	1000 µg	15	14	16	17
V_k	500µg	16	16	13	14
	1000 µg	18	18	14	14
V_l	500µg	12	11	13	13
	1000 µg	13	12	14	15
V_m	500µg	12	12	13	13
	1000 µg	13	12	15	15
V_n	500µg	14	13	13	15
	1000 µg	16	15	16	17
V_o	500µg	13	14	13	14
	1000 µg	14	14	17	18
V_p	500µg	13	13	14	14
	1000 µg	14	15	17	19
V_q	500µg	16	17	15	15
	1000 µg	18	18	19	20
V_r	500µg	15	16	15	16
	1000 µg	17	18	19	20
S	100 µg	23	24	21	22

S -Standard (Streptomycin)

Table: 4 Antifungal data of title compounds (V_a-V_r)

Compound		Diameter of zone of inhibition(mm)			
		C. albicans	***C. krusei***	***A. fumigatus***	***A. flavus***
V_a	500µg	13	14	13	-
	1000 µg	14	15	13	12
V_b	500µg	13	-	12	-
	1000 µg	16	14	13	13
V_c	500µg	13	13	12	-
	1000 µg	13	15	13	14

V_d	500µg	14	13	14	13
	1000 µg	15	13	15	15
V_e	500µg	13	13	14	13
	1000 µg	12	17	16	16
V_f	500µg	12	12	13	12
	1000 µg	13	14	16	17
V_g	500µg	12	13	14	12
	1000 µg	14	15	17	15
V_h	500µg	13	12	13	13
	1000 µg	15	16	16	13
V_i	500µg	13	13	14	13
	1000 µg	16	17	18	16
V_j	500µg	13	12	12	13
	1000 µg	14	16	16	15
V_k	500µg	14	13	13	12
	1000 µg	14	16	16	16
V_l	500µg	14	14	13	13
	1000 µg	15	17	17	14
V_m	500µg	13	14	14	14
	1000 µg	16	18	18	14
V_n	500µg	13	13	14	15
	1000 µg	17	18	17	16
V_o	500µg	13	12	14	14
	1000 µg	16	14	16	17
V_p	500µg	12	13	14	-
	1000 µg	15	17	17	13
V_q	500µg	13	14	14	14
	1000 µg	17	18	19	17
V_r	500µg	14	14	13	14
	1000 µg	16	18	19	16
S	100 µg	22	23	24	18

S -Standard (Clotrimazole)

Results and Discussion

The results of *in vitro* antibacterial activity of title compounds have shown that all the tested possesses moderate to good antibacterial activity against the strains used in the present study. Although the antibacterial activities of tested compounds are not comparable with the standard drug Streptomycin but they could exhibit moderate to potent activity at high concentrations. The antibacterial data showed that compounds V_b, V_e, V_i, V_q and V_r were showed good to potent antibacterial activity against tested strains of organism.

The antifungal data of title compounds revealed that no compound is as good as standard drug Cotrimazole against tested fungal strains. However compounds V_i, V_m, V_n, V_q and V_r showed potent antifungal activity against both Candida and Aspergillus species.

The results of the present investigation revealed that V_b, V_e, V_i, V_q and V_r exhibited promising antibacterial activity, where as compounds V_i, V_m, V_n, V_q and V_r exhibited good antifungal activity. Therefore these compounds can be recommended for further biological studies.

Acknowledgements

The authors are thankful to the Director, St Marys's College of Pharmacy, Secunderabad for laboratory facilities and also thankful to IICT Hyderabad, CDRI Lucknow for Elemental analysis and spectral data.

References:

1. Guo Z, Chen Y, Wu D, Zhu YF, Struthers RS, Saunders J, Xie Q, Chen C, Bioorg.Med.Chem.lett, 2003, 13(20), 3617-3622.
2. V. P. Litvinov, Russ. Chem. Bull, Int.Ed, 2004 Vol. 53, No. 3, 481-516.
3. Ed.A. R. Katritzky and C. W. Rees *Comprehensive Heterocyclic Chemistry*, Vol. 3, Pergamon Press, Oxford, 1984, 443.
4. Abdel fattah, M.M. Kandeel, Maha. A. Hakeem, J.Chin.Chem.Soc, 2006, vol 53,No 3,402-412.
5. Abdel-Rahman B. A, El-Gazzar, Hoda A. R, Hussein, Hend N, Hafez., *Acta Pharm*. 2007,57, 395–411.
6. Duguid J. P, Cruikshank R, Marmion B. P and Swain R. H. A, Medicinal Microbiology, 12th edition, Vol I, Churchill livingstone, London, 1975.
7. Arthrington-Skaggs B. A, Motley M, Warnock D. W and Morrison C. J, J Clin Microbiology, 2000,38, 2254.
8. Collins A. H, Microbiological Methods, 2nd edition, Butterworth, London,1976.

*****