Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 2, 2013

Research Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL 2-MERCAPTO PYRIMIDINES

M. MUMTAZ MOHAMMED HUSSAIN[a*], K. ISHWAR BHAT[b], B.C. REVANASIDDAPPA[b], D.R. BHARATHI[c]

[a]Department of Pharmaceutical Chemistry, [c] Department of Pharmacology, SJM College of Pharmacy Chitradurga 577502, [b] Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences, Nitte University, Mangalore 575018, Karnataka, India.
Email: mumtazmhussain@gmail.com

Received: 18 Mar 2013, Revised and Accepted: 30 Apr 2013

ABSTRACT

Objective: Synthesis and antibacterial, antifungal and antitubercular evaluation of a novel series of 2-mercapto pyrimdines.

Methods: A novel series of 2-mercapto Pyrimidines (4a-l) were synthesized by reacting (E)-thienyl chalcones (3a-l) with thiourea in alcohol medium. (E)- thienyl chalcones were prepared by the reaction of thiophene-2-aldehyde(1) with various substituted acetophenones(2) in presence of NaOH. The structures of the newly synthesized compounds were established on the basis of IR, ^{1}H-NMR, Mass spectral data and elemental analysis. All the new compounds were evaluated for their *In-Vitro* antibacterial, antifungal and antitubercular activities.

Results and Conclusion: Some of the compounds have exhibited promising antibacterial, antifungal and antitubercular activities.

Keywords: Chalcones, 2-mercapto pyrimidines, Antibacterial activity, Antifungal activity, Antitubercular activity.

INTRODUCTION

Pyrimidines are the most important six membered heterocyclic compounds containing two nitrogen atoms. Pyrimidines occur in living system in the form of nucliec acids and vitamins. As pyrimidine is a basic nucleus in DNA & RNA, it has been found to be associated with diverse biological activities. Further research led to the development of antimalarial drugs like Pyrimethamine and Trimethoprim. Supplementary modifications have yielded useful Pyrimidine derivatives like oxythiamine.

The molecules containing pyrimidine nucleus possess wide range of biological activities such as antiviral[1], antileishmanial[2], antimalarial[3], antibacterial[4], antihistaminic[5], cytotoxic[6], anti-inflammatory[7] and antitubercular[8] activities. Chalcones act as intermediates in the biosynthesis of various flavonoids and also have been used as intermediates in the synthesis of various pharmacologically significant heterocyclic molecules such as Pyrimidines, Pyrazolines, Isoxazolines and Benzodiazepines.

By considering the above facts, it was contemplated to synthesize a new series of 2-mercapto Pyrimidines **(4a-l).** The final synthesized compounds were screened for their *In-Vitro* antibacterial, antifungal and antitubercular activities.

(E)-thienyl Chalcones **(3a-l)** were synthesized by Claisen-Schmidt condensation reaction of Thiophene-2-carbaldehyde **(1)** with various substituted acetophenones **(2)** in presence of NaOH in alcohol medium as per the reported procedure[9]. Chalcones **(3a-l)** undergoes selective cyclization with thiourea to yield the title compounds 2-mercapto pyrimidines **(4a-l).** The synthetic strategies adopted to obtain the target compounds are depicted in **Scheme-01.**

MATERIALS AND METHODS

The IR spectrum is recorded by using Alpha Bruker IR Spectrometer using a thin film on KBr pellets and frequencies are expressed in cm^{-1}. The ^{1}H-NMR spectra were recorded on Bruker Avance II 400 MHz NMR Spectrometer. All spectra were obtained in $CDCl_3$ and DMSO-d6 as a solvent. Chemical shift values are reported as values in ppm relative to TMS (δ=0) as internal standard. Mass spectra were recorded on ESI. Melting points were determined by open capillary method and are uncorrected. All the synthesized compounds were purified by recrystallization. Elemental analysis was performed on Carlo Elba 1108 analyzer.

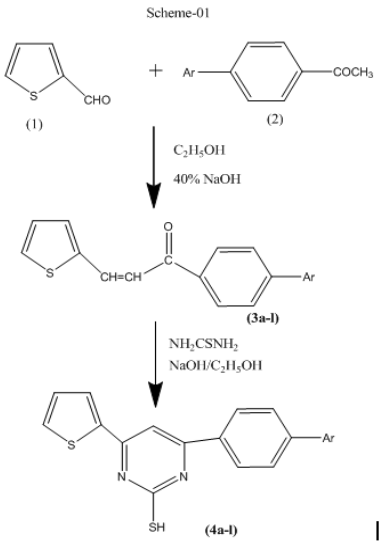

Fig. 1: Experimental Scheme for the synthesis of 2-mercapto pyrimidines (4a-l)

Synthesis of 2-mercapto pyrimidines (4a-l)

A mixture of (E)-thienyl Chalcones **(3a-l)** (0.01 mol) and thiourea (0.01 mol) was dissolved in ethanol (30 ml). 2ml of 20% NaOH was added and the reaction mixture was refluxed on a water bath for about 18-30 hrs. The progress of the reaction was monitored by TLC (benzene: chloroform 8:2).The reaction mixture was allowed to cool and poured into crushed ice. The solid which precipitates out was filtered and recrystallized from ethanol. The physical data of the compounds **(4a-l)** is given in table-1.

4a: IR (cm^{-1}): 3078 (CH), 1625 (C=N), 1593 (C=C), 1512 & 1365(NO_2), 683 (C-S).**^{1}H NMR (δ PPM) :** 6.68-8.07 (m, 8H, Ar-H), 9.42 (s, 1H, SH).**Mass:** 316 (M^{+1})

4b:IR (cm^{-1}): 3085 (CH), 1616 (C=N), 1565 (C=C), 829 (C-Cl), 700 (C-S). **^{1}H NMR (δ PPM):** 6.93-8.05(m, 8H, Ar-H), 10.37 (s, 1H, SH). **Mass:** 304 (M^+)

4c: IR (cm^{-1}): 3076 (CH), 1633 (C=N), 1568(C=C), 806 (C-Cl), 714 (C-S). **^{1}H NMR (δ PPM):** 9.86 (s, 1H, SH).6.86-7.90 (m, 8H, Ar-H), 2.37 (s, 3H, CH_3). **Mass:** 285 (M^{+1})

4d: IR (cm^{-1}): 3323 (NH_2), 3091 (CH), 1634(C=N), 1599(C=C), 681(C-S). **^{1}H NMR (δ PPM) :** 8.89 (s, 1H, SH).6.63-7.93 (m, 8H, Ar-H,), 4.92(s, 2H, NH_2). **Mass:** 285 (M^+)

Antimicrobial activity

The *In-Vitro* anti-microbial screening of the newly synthesized 2-mercapto Pyrimidines **(4a-l)** was carried out against Gram-positive organisms (*E. Fecalis and S. Aureus),*Gram-negative organisms (*K.pneumoneae and E. Coli)* and Fungi *(C. albicans and A. niger*) by conventional tube dilution method[10] and compared with that of the standard drugs Ciprofloxacin and Fluconazole respectively. MIC of each drug was defined as the lowest concentration that produces no visible turbidity after incubation time. Dilutions of each drug were prepared with brain heart infusion broth (BHI) for MIC. A stock solution of drug with concentration 1000 µg/10µl was prepared in DMF. The tubes were incubated for 24 hrs and observed for turbidity. The antimicrobial activity of the compounds **(4a-l)** is given in table-2.

The antibacterial activity results revealed that, compounds showed significant activity against *Gram Positive* organisms. The compounds **4a, 4g, 4i, 4l** showed good activity against *E. Fecalis* and most of the compounds displayed significant activity against *S.Aureus.* However the compounds showed only moderate activity against the *Gram Negative* organisms when compared to the standard drug.

In the antifungal activity, compounds showed modertae to weak activity against *C.Albicans.* The compounds **4c, 4d, 4f, 4g, 4h, 4l** showed significant activity against *A.Fumigatus* when compared to the standard drug.

Antitubercular activity

The antitubercular activity of the newly synthesized 2-mercapto Pyrimidines **(4a-l)** were carried out by using Middle brook 7H9 agar medium against *M. tuberculosis* H_{37}Rv strain by Microplate Alamar Blue Assay (MABA) [11] method. Microplate Alamar Blue Assay (MABA) is an non-toxic rapid, inexpensive and high throughput assay for antitubercular drug screening. The principle behind Microplate Alamar Blue Assay is that, in the presence of cellular metabolism resazurin (oxidized form of Alamar blue,) which is non fluorescent blue in colour is converted to rezorufin (reduced form of Alamar blue) which is fluorescent pink in colour, by the action of variety of enzymes. The Middle brook 7H9 agar medium containing different derivatives, standard drug as well as control was inoculated with *M. tuberculosis* H_{37}Rv strain. The inoculated plates were incubated at 37^0 C for four weeks. At the end of four weeks they were checked for growth. The new compounds **4a, 4b, 4c, 4h, 4i, 4l** have showed significant antitubercular activity with MIC ranging from 0.8 to 6.25 µg. INH was used as standard drug for comparison purpose. The antitubercular data of the compounds **(4a-l)** is given in table-3.

RESULTS AND DISCUSSION

A novel series of 2-mercapto Pyrimidine **(4a-l)** derivatives have been synthesized and screened for their *In-Vitro* antibacterial, antifungal and antitubercular activities. The physical data of the final synthesized compounds is given in table-01.

Table 1: Physical data of 2-mercapto pyrimidine derivatives (4a-l)

Comp	Ar-$COCH_3$	M.W.	M.P (^{0}C)	Elemental analysis Calculated (Found)			Yield (%)
				C (%)	H (%)	N (%)	
4a	4-NO_2	315	174-176	53.32 (53.17)	2.88 (2.72)	13.32 (13.23)	50
4b	4-Cl	304	94-96	55.16 (54.98))	2.98 (2.83)	11.63 (11.48)	60
4c	4-CH_3	284	124-126	63.35 (63.24)	4.25 (4.21)	9.85 (9.69)	70
4d	4-NH_2	285	103-105	58.92 (58.81)	3.89 (3.79)	14.72 (14.58)	50
4e	H	270	60-62	62.19 (62.10)	3.73 (3.66)	10.36 (10.19)	54
4f	4-F	288	69-71	58.31 (58.15)	3.15 (2.97)	9.71 (9.62)	53
4g	3-NO_2	315	205-207	53.32 (53.04)	2.88 (2.69)	13.32 (13.18)	58
4h	4-Br	349	180-182	48.14 (47.96)	2.60 (2.49)	8.02 (7.91)	65
4i	4-OCH_3	300	153-155	59.97 (59.85)	4.03 (3.91)	9.33 (9.21)	70
4j	4-OH	286	140-142	58.72 (58.59)	3.52 (3.38)	9.78 (9.62)	75
4k	1-Naphthyl	320	73-75	67.47 (67.36)	3.77 (3.65)	8.74 (8.61)	60
4l	2-Thiophene	276	187-189	52.14 (52.02)	2.92 (2.78)	10.14 (9.94)	57

M.W: Molecular Weight; M.P: Melting point in ˚C

Table 2: Antimicrobial activity of 2-mercapto pyrimidine derivatives (4a-l) by tube dilution method

Comp	Minimum Inhibitory Concentration in (µg)					
	E.Fecalis	*S. Aureus*	*K. Pneumoniae*	*E. coli*	*C. albicans*	*A. fumigatus*
4a	0.4	0.4	12.5	R	R	3.12
4b	0.8	0.2	6.25	25	100	6.25
4c	1.6	0.2	12.5	50	100	0.2
4d	1.6	0.2	50	100	100	0.2
4e	0.8	0.2	50	R	R	3.12
4f	0.8	0.2	25	R	R	0.2
4g	0.2	0.2	25	100	50	0.2
4h	0.8	0.2	50	100	50	0.2
4i	0.2	0.2	25	100	50	3.12
4j	0.8	0.2	1.6	6.25	50	3.12
4k	R	0.2	12.5	100	100	0.4
4l	0.2	12.5	25	100	50	0.2
Ciprofloxacin[a]	**1µg**	**2µg**	**1µg**	**2µg**	**-**	**-**
Fluconazole [a]	**-**	**-**	**-**	**-**	**16.6µg**	**8.3 µg**

* Resistant, [a]Standard drugs

Table 3: Antitubercular activity of 2-mercaptopyrimidines (4a-l) by Microplate Alamar Blue Assay (MABA)

Comp	MIC in µg
4a	6.25
4b	6.25
4c	6.25
4d	12.5
4e	12.5
4f	12.5
4g	R
4h	6.25
4i	0.8
4j	12.5
4k	12.5
4l	6.25
INH	**0.2**

The structures of the newly synthesized compounds were established on the basis of spectral data and elemental analysis. The compounds were purified by recrystallization from appropriate solvents. The completion of the reaction was monitored by TLC.

The antimicrobial activity of the compounds showed good activity against *Gram Positive* organisms. Most of the synthesized compounds showed significant activity against *S.aureus*.

The compounds also displayed good activity against the fungal organism *A.fumigatus* and significant antitubercular activity against *M. tuberculosis* $H_{37}Rv$ strain by Microplate Alamar Blue Assay method. The results of antimicrobial and antitubercular studies are depicted in table 2 and table 3 respectively.

ACKNOWLEDGEMENTS

The authors of thankful to authorities of Department of Pharmaceutical Chemistry, SJM College of Pharmacy, Chitradurga and NITTE university, Mangalore for providing necessary facilities. Authors are also thankful to authorities of Department of Microbiology, Maratha Mandal's NRGH Dental College, Belgaum for antimicrobial and antitubercular studies. The authors are thankful to SAIF, Panjab University, Chandigarh for ^{1}H NMR spectral data and Mass spectral data.

REFERENCES

1. Stuart AL, Ayisi NK, Tourigny G, Gupta VS. Antiviral activity, antimetabolic cactivity and cytotoxicity of 3-substituted deoxy pyrimidine nucleosides. J Pharm Sci 1985; 74(3): 246-49.
2. Agarwal A, Ramesh, Ashutosh, Goyal N, Chauhan PM, Gupta S. Dihydropyrido[2,3- d]pyrimidines as a new class of antileishmanial agents. Bio-Org Med Chem 2005; 13(24): 6678-84.
3. Agarwal A, Srivastava K, Puri SK, Chauhan PM. Synthesis of 4-pyrido-6- aryl-2- substituted amino pyrimidines as a new class of antimalarial agents. Bio-Org Med Chem 2005; 13(22): 6226-32.
4. Ajitha M, Rajnarayana K. Synthesis and Evaluation of new-3-substituted-[3,4-dihyropyrimidinones-indolin-2-ones for cytotoxic activity. Int J Pharm Pharm Sci 2011; 3(4): 89-93.
5. Rahaman SA, Prasad YR, Kumar P, Kumar B. Synthesis and anti-histaminic activity of some novel pyrimidines. Saudi Phar J 2009; 17(3): 259-63.
6. Shilpa B, Alka NC, Preeti K Microwave assisted synthesis antibacterial activity of pyrimidine derivatives. Int J Pharm PHarm Sci 2013; 5(1): 346-348.
7. Sushma D, Archana, Sukhjinder S, Munirajam S, Nitin K. Synthesis and anti-inflammatory activity of some 2-amino pyridines. Ind J Het Chem 2008; 17: 411-12.
8. Kiran S. Nimavat, Popat KH, Joshi H. Synthesis, anticancer, antitubercular and antimicrobial activity of some new pyrimidine derivatives. Ind J Het Chem 2003; 12: 217-20.
9. Mumtaz MH, Ishwar BK, Revanasiddappa BC, Nataraj GR. Synthesis, antitubercular, antibacterial and antifungal evaluation of 2-amino pyrimidines derivatives. Ind J Het Chem 2012; 22: 159-64.
10. Sadaf Q Macro- and Microdilution Methods of Antimicrobial Susceptibility Testing. In: Schwalbe R, Moore LS, Goodwin AC, Editors. Antimicrobial Susceptibility Testing Protocols. CRC Press, Taylor and Francis Group, 2007; pp. 75-79.
11. Lisa AC, Scott GF. Microplate Alamar Blue Assay versus BACTEC 460 System for High-Throughput Screening of Compounds against Mycobacterium tuberculosis and Mycobacterium avium. Amtimicrobial Agents and Chemotherapy 1997; 41: 1004-1009.