Research Article
ISSN: 0976-1209

Available online through
www.ijcas.info

Synthesis and Antimicrobial activity of Novel Pyrazolo [3,4-d] Pyrimidine derivatives

Mukesh M. Chavda, Manish J. Solanki* and Jatin Upadhyay
Chemical Engineering Department, IDS, Nirma University, Ahmedabad
Department of chemistry, Bahauddin Science college, Junagadh,
Department of chemistry, M.V.M.Science and Homescience college Rajkot-360005, India.

Received on: 20-05-2010; Revised on: 16-06-2010; Accepted on:15-07-2010

ABSTRACT

A series of new *N*-(2,5-dimethylphenyl)-7-methyl/7-methyl -3-oxo-5-phenyl-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*] pyrimidine-6-carboxamide have been synthesized by a three component (MCR) reaction involving *N*-(2,5-dimethylphenyl)-3-oxobutanamide,thioura and substituted benzaldehyde gives *N*-(2,5-dimethylphenyl)-6-methyl-4-phenyl-2-sulfanyl-1,2,3,4-tetrahydro pyrimidine-5-carboxamide which on reaction with dibromoethane /chloro acetylchloride respectively. The newly synthesized compounds were well characterized by elemental analysis, IR, 1H NMR and mass spectral studies. The newly synthesized compounds were screened for their antibacterial and antifungal activities and have exhibited moderate to excellent growth inhibition of bacteria and fungi. The results of such studies have been discussed in this paper.

Keywords: *N*-(2,5-dimethylphenyl)-3-oxobutanamide,Thiazolo[3,2-a]pyrimidinne-6-carboxamide; Antimicrobial activity.

INTRODUCTION

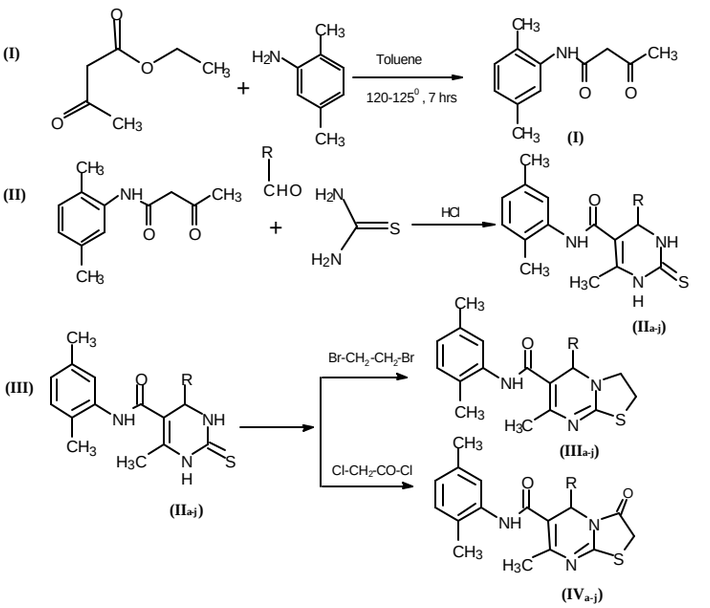
Multi-component reactions (MCRs) are theoretically useful organic reactions involving three or more starting materials which react to give a product [1]. They are one of the most important protocols in organic synthesis and medicinal chemistry [2]. They constitute a major part in the present day organic synthesis with advantages ranging from lower reaction times, increased reaction rates to higher yields and reproducibility. The diversity, efficiency and rapid access to small and highly functionalized organic molecules make this approach of central current interest in the construction of combinatorial libraries and optimization in drug discovery process [3].They constitute a superior tool for diversity-oriented and complexity- generating organic synthesis for drug discovery [4].The 3,4-dihydropyrimidin-2(1H)-ones have recently emerged as important target molecules due to their therapeutic and pharmacological properties [5] such as antiviral [6], antimitotic [7], anticarcinogenic [8], antihypertensive [9] and noteworthy, as calcium channel modulators [10]. Additionally, their particular structure has been found in natural marine alkaloid batzelladine A and B which are the first low molecular weight natural products reported in the literature to inhibit the binding of HIV gp-120 to CD4 cells, so disclosing a new field towards the development of AIDS therapy [11]. Thiazoles and their derivatives are also found to be associated with various biological activities such as antibacterial, antifungal and anti-inflammatory [12-15]. Prompted by the chemo therapeutic importance of pyrimidine derivatives and in a view to synthesize bioactive molecules [16], it was contemplated to synthesize a series of novel fused pyrimidine derivatives possessing 4-substitutedphenyl moiety and study their biological properties. Results of such studies are discussed in this paper.

CHEMISTRY

N-(2,5-dimethylphenyl)-6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide 1 was synthe- sized in a one pot Biginelli reaction involving Substituted benzaldehyde, *N*-(2,5-dimethylphenyl)-3-oxo- butanamide and thiourea in presence of SnCl2$2H2O catalyst according to the procedure reported in the literature [17].These compound were used further in order to synthesize a series of novel N-bridged heterocycles. The title compounds i.e *N*-(2,5-dimethylphenyl)-7-methyl/7-methyl -3-oxo-5-phenyl-2,3-dihydro-5*H*-[1,3] thiazolo[3,2-*a*]pyrimidine-6-carboxamidehave been synthesized in a one pot reaction involving 1, and dibromo- ethane/chloroacetylchloride/ in presence of anhydrous sodium acetate as catalytic amount (Scheme 1). The structures of some of the newly synthesized compounds have been established on the basis of elemental analysis, IR, 1H NMR and mass spectral studies. The physical properties of these compounds have been discussed in (Table I) and then all the compounds were screened for their *in vitro* biological activity, such as antimicrobial activity towards gram positive and gram negative bacterial strains and antifungal activity at different concentrations. The biological activities of the synthesized components are compared with standard drugs, such as ampicillin, chloramphenicol, ciprofloxacin, norfloxacin and griseofulvin.

***Corresponding author.**
Manish J. Solanki
Chemical Engineering Department, IDS,
Nirma University, Ahmedabad,India.
Tel.: + 91-
E-mail:manish_oraganic@rediffmail.com

Reaction Scheme



EXPERIMENTAL

Melting pointswere determined routinely in an open capillary tube and are uncorrected. Formation of compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and the spots were located by iodine.Ascientific The PMR spectra were recorded in CDCl3 on a Brucker DRX-300 at 300 MHz. The IR spectra were recorded on a Shimadzu-8400 FT-IR spectrometer in KBr (λ in cm-1). Elemental analyses of thenewly synthesized compounds were carried out on a Carlo Erba 1108 analyzer and results within the range of the theoretical value were found. Mass spectra were scanned on a GCMS-QP200 instrument.

General procedure for synthesis of the title compounds (IIIa–IIIj and IVa-IVj)

Preparation of *N*-(2, 5-dimethylphenyl)-3-oxobutanamide (1):
A mixture of ethyl acetatoacetate (0.01 mol) and 2,5-dimethyl aniline (0.01 mol) in 20 ml of ethanol containing 0.3 g of sodium hydroxide was refluxed for 5 hrs.The reaction mixture was concentrated and the solid product obtained was filtered off and recrystallized with ethyl acetate. Yield, 76%; m.p., 124_C. IR (KBr, cm-1): 3315, 3070, 1672, 1329. 1H NMR (DMSO-d6): 1.76 (s, 6H, 2CH3), 2.15 (s, 3H, CH3), 3.78 (s, 2H, CH2), 7.12–7.83 (m, 4H, ArH), 8.52 (bs,1H, NH, D2O exchangeable).

Preparation of *N*-(2,5-dimethylphenyl)-6-methyl-4-(substituted phenyl)-2-thioxo-1,2,3,4-tetrahydro- pyrimidine -5-carboxamide (IIa-j)

A mixture of N-(2,5-dimethylphenyl)-3-oxo-butyramide (1), substituted aryl aldehydes (0.01 mol), and thiourea (0.015 mol) in 20 ml of ethanol was refluxed for 7–9 h in the presence of a catalytic amount of concentrated hydrochloric acid. The reaction mixture was kept overnight and the precipitate obtained was filtered and recrystallized with ethanol.The physical data are given in table I.

Table.I. Physical constants of (II_{a-j}).

Comp No.	R	Molecular Formula	M.W	Yield %	M.P. ^{0}C	R_f Value R_{f1}/R_{f2}	% of Nitrogen Calcd.	Found
III	4-NO_2-C_6H_4	$C_{22}H_{22}N_4O_3S$	422	61	216	0.56	13.25	13.30
III_a	4-OCH_3-C_6H_4	$C_{23}H_{25}N_3O_2S$	407	60	206	0.58	10.30	10.34
III_b	4-OH-C_6H_4	$C_{22}H_{23}N_3O_2S$	393	58	201	0.53	10.67	10.71
III_c	4-F-C_6H_4	$C_{22}H_{22}FN_3OS$	395	58	187	0.51	10.61	10.66
III_d	4-Cl-C_6H_4	$C_{22}H_{22}ClN_3OS$	411	56	198	0.54	10.19	10.23
III_e	3-NO_2-C_6H_4	$C_{22}H_{22}N_4O_3S$	422	53	220	0.50	13.25	13.30
III_f	3-Cl-C_6H_4	$C_{22}H_{22}ClN_3OS$	411	54	214	0.61	10.19	10.23
III_g	2-NO_2-C_6H_4	$C_{22}H_{22}N_4O_3S$	422	63	209	0.52	13.25	13.30
III_h	2-Cl-C_6H_4	$C_{22}H_{22}ClN_3OS$	411	52	216	0.56	10.19	10.23
III_i	2-OH-C_6H_4	$C_{22}H_{23}N_3O_2S$	393	50	218	0.48	10.67	10.71

Preparation of 5-(4-chlorophenyl)-*N*-(2,5-dimethylphenyl)-7-methyl-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*] pyrimidine-6-carboxamide (IIIc):

A mixture of compounds IIc (0.01 mol), dibromoethane (0.01 mol) and sodium acetate (0.5 g) in 15 ml DMF was refluxed for 8–10 hrs. The reaction mixture was poured into cold water and neutralized the solid thus obtained was filtered and recrystallized with DMF to get the titled compounds.The other compounds **II1a-j** were prepared in a similar manner. The physical data are given in table II..

Table .II. Physical constants of (III_{a-j}).

Comp No.	R	Molecular Formula	M.W	Yield %	M.P. ^{0}C	R_f Value R_{f1}/R_{f2}	% of Nitrogen Calcd.	Found
IV	4-NO_2-C_6H_4	$C_{22}H_{20}N_4O_4S$	436	61	219	0.61	12.82	12.88
IV_a	4-OCH_3-C_6H_4	$C_{23}H_{23}N_3O_3S$	421	60	211	0.60	09.97	10.03
IV_b	4-OH-C_6H_4	$C_{22}H_{21}N_3O_3S$	407	58	176	0.59	10.30	10.36
IV_c	4-F-C_6H_4	$C_{22}H_{20}FN_3O_2S$	409	58	180	0.57	10.26	10.31
IV_d	4-Cl-C_6H_4	$C_{22}H_{20}ClN_3O_2S$	425	56	204	0.59	09.87	09.92
IV_e	3-NO_2-C_6H_4	$C_{22}H_{20}N_4O_4S$	436	53	216	0.54	12.82	12.88
IV_f	3-Cl-C_6H_4	$C_{22}H_{20}ClN_3O_2S$	425	54	224	0.63	09.87	09.92
IV_g	2-NO_2-C_6H_4	$C_{22}H_{20}N_4O_4S$	436	63	219	0.55	12.82	12.88
IV_i	2-Cl-C_6H_4	$C_{22}H_{20}ClN_3O_2S$	425	52	202	0.51	09.87	09.92
IV_j	2-OH-C_6H_4	$C_{22}H_{21}N_3O_3S$	407	50	226	0.49	10.30	10.35

Preparation of Preparation of 5-(4-chlorophenyl)-*N*-(2, 5-dimethyl phenyl)-7-methyl-2,3-dihydro-5*H*-[1,3] thiazolo[3,2-*a*] pyrimidine-6-carboxamide (IVc):

A mixture of compounds IIc (0.01 mol), chloroacetylchloride (0.015 mol) and sodium acetate (0.5 g) in 15 ml DMF was refluxed for 6–8 hrs. The reaction mixture was poured into cold water and neutralized the solid thus obtained was filtered and recrystallized with DMF to get the titled compounds. The other compounds **IIIa-j** were prepared in a similar manner. The physical data are given in table III.

Table. III: Physical constants of (IV_{a-j}).

Comp No.	R	Molecular Formula	M.W	Yield %	M.P. ^{0}C	R_f Value R_{f1}/R_{f2}	% of Nitrogen Calcd.	Found
IV_a	4-NO_2-C_6H_4	$C_{22}H_{20}N_4O_4S$	436	61	219	0.61	12.82	12.88
IV_b	4-OCH_3-C_6H_4	$C_{23}H_{23}N_3O_3S$	421	60	211	0.60	09.97	10.03
IV_c	4-OH-C_6H_4	$C_{22}H_{21}N_3O_3S$	407	58	176	0.59	10.30	10.36
IV_d	4-F-C_6H_4	$C_{22}H_{20}FN_3O_2S$	409	58	180	0.57	10.26	10.31
IV_e	4-Cl-C_6H_4	$C_{22}H_{20}ClN_3O_2S$	425	56	204	0.59	09.87	09.92
IV_f	3-NO_2-C_6H_4	$C_{22}H_{20}N_4O_4S^2$	436	53	216	0.54	12.82	12.88
IV_g	3-Cl-C_6H_4	$C_{22}H_{20}ClN_3O_2S$	425	54	224	0.63	09.87	09.92
IV_h	2-NO_2-C_6H_4	$C_{22}H_{20}N_4O_4S^2$	436	63	219	0.55	12.82	12.88
IV_i	2-Cl-C_6H_4	$C_{22}H_{20}ClN_3O_2S$	425	52	202	0.51	09.87	09.92
IV_j	2-OH-C_6H_4	$C_{22}H_{21}N_3O_3S^2$	407	50	226	0.49	10.30	10.35

Antimicrobial activity

Antimicrobial activity testing was carried out using the cup-plate method,7 which is described below.

Antibacterial activity:

Streptococcus pyogenes MTCC-442, *Streptococcus aureus* MTCC-96 and *Bacillus subtilis* MTCC-441 (Gram positive bacteria) were grown in nutrient broth and *E. coli* MTCC-443 (Gram negative bacterium) in Peptone water (PW, 1%bacteriological peptone and 0.5%NaCl) for 24 h; this gave the optimum growth of the test bacteria. Each purified compound was dissolved in dimethylformamide (DMF), which had been sterilized by filtration through a sintered glass filter, and stored at 4 °C. Each agent was then added tomolten nutrient agar in the following concentrations (λg/ml): 0 (control), 5, 10, 25, 50, 100, 200,500 and poured into a sterile petri dish. The pH of the media was maintained at 7.2 to 7.4. The inoculum consisted of an overnight growth broth culture of a bacterium diluted in such a manner that a 2 mm (internal diameter) loopful of the culture contained 105 colony- forming units (CFU). These were then spot inoculated onto nutrient agar plates containing increasing amounts of a compound, incubated at 37 °C for up to 24 h to determine the minimum inhibitory concentration (MIC), [18,19] which were recorded as zones of inhibition in mm for the bacteria.

Antifungal activity:

Candida albicans MTCC-227 and *Aspergillus niger* MTCC-282 were employed for the testing of the antifungal activity using the cup-plate method. The culture was maintained on Sabouraud's agar for 72 h; this gave the optimum growth of the test fungal spores. Each purified compound was dissolved in dimethylformamide, sterilized by filtration using a sintered glass filter and stored. Each agent was then added to Sabouraud's agar in the following concentrations (λg/ml): 0 (control), 5, 10, 25, 50, 100, 200, 500 and poured into a sterile petri dish. The inoculum consisted of an overnight-grown broth culture of a fungus diluted in such a manner that a 2 mm (internal diameter) loopful of the culture contain 105 colony-forming units (CFU).These were then spot inoculated onto Sabouraud's agar plates containing increasing amounts of the compound and then incubated at 37 °C for up to 48 h to determine the minimum inhibitory concentration (MIC). [18,19]

RESULTS AND DISCUSSION

The physical data for compound **IIIc** are as follows: Yield 65 %, m.p. 121 °C. Anal. Calcd. for $C_{22}H_{22}ClN_3OS$; Required: C, 64.08; H, 5.34; N, 10.19 % Found: C, 64.12; H, 5.37; N, 10.23 %. IR (KBr) _max cm–1: 3400 (N–H str.), 3040 (C–H str.), 2920 (C–H str., asym.), 2858 (C–H str., sym.), 1620 (N–N def.), 1596 (N–H def.), 1550 (C=N), 1455 (C–H def., asym.), 1434 (–CH2 bending), 1380 (–CH3 bending), 1365 (C–H def., sym.), 1323 (C–N str.), 1170 (C–H i.p. def.), 1130 (C–N str.), 692 (C–H o.o.p.def.). PMR _ / ppm (TFA): 0.901 (*t*, 3H, – CH3), 1.364 (*q*, 2H, –CH2), 1.673 (*m*, 2H, –CH2), 2.676 (*t*, 2H, –CH2), 3.802 (*s*, 3H, –OCH3), 3.841 (*s*, 3H, –OCH3), 6.526–7.356 (*m*, 13H, Ar-H + – NH + –SH). Mass spectrum of the compound exhibited a molecular ion peak at *m/z* 410 (M+). The physical data of the other prepared compounds(III_{a-j}, IV_{a-j}) are given in Table II and Table III

Antimicrobial activity

TheMIC values of the test solutions are recorded in Tables IV and V which are given in zones of inhibition in mm for the bacteria and fungi.

Table V: comparative antimicrobial activity of (IV_{a-j}). (Different Inhibition Concentration in µg/ml).

Comp. No.	R	Antibactrial activity (Zones of inhibition in m.m.) *S.pyogens* MTCC-442					*S.aureus* MTCC-96				
		5	25	50	100	250	5	25	50	100	250
IV	4-NO_2-C_6H_4	-	11	16	18	20	-	13	16	18	19
IV_a	4-OCH_3-C_6H_4	-	11	15	16	19	-	11	18	15	17
IV_b	4-OH-C_6H_4	-	12	13	15	19	-	12	13	14	16
IV_c	4-F-C_6H_4	-	13	16	18	21	-	15	19	20	21
IV_d	4-Cl-C_6H_4	-	11	15	16	18	-	11	15	16	18
IV_e	3-NO_2-C_6H_4	-	13	16	17	20	-	13	15	16	19
IV_f	3-Cl-C_6H_4	-	12	14	18	18	-	12	14	19	20
IV_g	2-NO_2-C_6H_4	-	13	14	19	21	-	12	17	18	20
IV_h	2-Cl-C_6H_4	-	11	16	16	20	-	12	15	15	17
IV_i	2-OH-C_6H_4	-	11	14	16	17	-	12	15	14	15
IV_j											
Comparative activity of (I_{a-j}) with known chosen standard drugs											
Standard drug		**Antibacterial activity**									
Amplicilline		**11**	**14**	**16**	**18**	**19**	**10**	**13**	**14**	**16**	**18**
Chloramphenicol		**10**	**13**	**19**	**20**	**20**	**12**	**14**	**19**	**20**	**21**
Ciprofloxacin		**16**	**19**	**21**	**21**	**22**	**17**	**19**	**21**	**22**	**21**
Norfloxacin		**18**	**19**	**20**	**21**	**21**	**19**	**22**	**25**	**26**	**28**

ACKNOWLEDGEMENTS:

Authors are thankful to Dr. R.P.Bhatt, Principal, Bahauddin Science College Janagadh, for providing research facility and for his valuable encouragement during my research work. Authors are also thanking to Punjab University, Chandigadh for providing NMR spectra and Saurashtra University, Rajkot for providing IR spectral and mass spectral data.

REFERENCES:

1. A.Domling, Curr. Opin. *Chem. Biol*. 4 (2000) 318
2. L. Weber, Drug Discov. Today 7 (2002) 143 A. Domling, Curr. Opin. *Chem. Biol*. 6 (2002) 306
3. R.E. Dolle, K.H. Nelson, *J. Comb. Chem*. 1 (1999) 235;L.A. Thompson, J.A. Ellman, Chem. Rev. 96 (1996) 555;E.M. Gordon, M.A. Gallop, D.V. Patel, Acc. Chem. Res. 29 (1996) 144
4. D. Lee, J.K. Sello, S.L. Schreiber, *Org. Lett*. 2 (2000) 709
5. C.O. Kappe, *Eur. J. Med. Chem*. 35 (2000) 1043
6. E.W. Hurst, R. Hull, *J. Med. Pharm. Chem*. 3 (1961) 215
7. T.U. Mayer, T.M. Kapoor, S.J. Haggarty, R.W. King, S.I. Schreiber,T.J. Mitchison, Science 286 (1999) 971
8. T. Kato, Japanese Kokai Tokkyo Koho 59 190, 1984, 974
9. K.S. Atwal, B.N. Swanson, S.E. Unger, D.M. Floyd, S. Moreland, A. Hedberg, B.C. O'Reilly, *J. Med. Chem*. 34 (1991) 806; G.C. Rovnyak, K.S. Atwal, A. Hedberg, S.D. Kimball, S. Moreland, J.Z. Gougoutas, B.C. O'Reilly, J. Schwartz, M.F. Malley, J. Med.Chem. 35 (1992) 3254.
10. C.O. Kappe, Molecules 3 (1998) 1; B. Jauk, T. Pernat, C.O. Kappe, *Molecules* 5 (2000) 227.
11. A.D. Patil, N.V. Kumar, W.C. Kokke, M.F. Bean, A.J. Freyer, C. Debrossi, S. Mai, A. Trunch, D.J. Faulkner, B. Carte, A.L. Breen,R.P. Hertzberg, R.K. Johnson, J.W. Westley, B.C.M. Potts, *J.Org.Chem*. 60 (1995) 1182
12. N. Hans, Swiss Patent 592103, 1997; Chem. Abstr. [88 (1978) 22886]
13. K.J. Wilson, C.R. Utig, N. Subhasinghe, J.B. Hoffmann, N.J. Rudolph, R. Soll, C.J. Molloy, R. Bone, D. Green, *Bioorg. Med. Chem. Lett*. 11 (2001) 915
14. K.D. Berlin, M.D. Herd, Proc. Okla. Acad. Sci. 71 (1991) 29
15. B.S. Holla, K.V. Malini, B.S. Rao, B.K. Sarojini, N.S. Kumari

16. B.S. Holla, M. Mahalinga, M. Ashok, P. Karegoudar, *Phosphorus, Sulfur Silicon Relat. Elem*. 181 (2006) 1427
17. D. Russowsky, F.A. Lopes, V.S.S. da Silva, K.F.S. Canto, M.G.M. D'Oca, M.N. Godoi, *J. Braz. Chem. Soc*. 15 (2004) 165
18. R. Cruickshank, J.P. Duguid, B.P. Marion, R.H.A. Swain, *Medicinal Microbiology*, twelfth ed., vol. 11, Churchill Livingstone, London, 1975, 196
19. M.Kawase, B. Varu,A. Shah, N. Motohasbhi, S. Tani, S. Saito, S. Debnath, S. Mahapatra, S. G. Dastidar, A. N. Chakrabarty, *Arzneim. Forsch./ Drug Res.* **61** (2001) 67
20. S. G. Dastidar, A. Chaudhury, S. Annadurai, S. Roy, M. Mookerjee, A. N. Chakrabarty, *J. Chemother.* **7** (1995) 201.

Source of support: Nil, Conflict of interest: None Declared