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Research Article

Synthesis, characterisation, evaluation of antimicrobial & antifungal activity of novel pyrazolopyrimidine & pyrazolopyridine derivatives

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Abstract

A new series of substituted Pyrazolo Pyrimidine & Pyrazolopyridine were designed to meet the structural requirement of antimicrobial, Analgesic & antifungal drugs which has been known to possess a broad spectrum of biological activities such as analgesic, antimicrobial antifungal etc. The therapeutic importance of these rings prompted us to develop selective molecules. The starting material of 1-{1-phenyl-3-(substituted phenyl)-1H-pyrazol-4-yl}-3-phenyl-1-propen-3-one (Chalcone) (3a-c) prepared from by claisen–schmidt reaction from respective 1-phenyl-3-(substituted phenyl)-1H-pyrazoles-4-carboxaldehyde (Vilsmeier-Haack Reaction) (2a-c). 1-{1-phenyl-3-(substituted phenyl)-1H-pyrazol-4-yl}-3-phenyl-1-propen-3-one (Chalcone) (3a-c) further treated with thiourea, 3 amino-s-triazole, ethylcyanoacetate, cynothioacetamide to produced 1,2,3,4 tetrahydropyrimidine (4 a-c), triazolopyrimidine (5 a,b), cynophenylpyridine (6 a-c) & phenyl pyridine thione derivatives. The structures of newly Synthesised compounds were characterized by spectral data & studied for their antimicrobial & Antifungal activities. Compounds 4a, 4c, 5a, 6c, 7c showed high antimicrobial activity using disc diffusion method with reference to using Amoxicillin as standard drug active against *Bacillus substillus*, *staphylococcus aureus*, *Proteus vulgaris* and *pseudomonas*. Similarly compound 4c &7c showed maximum antifungal activity against fungal species *Candida albicans.*

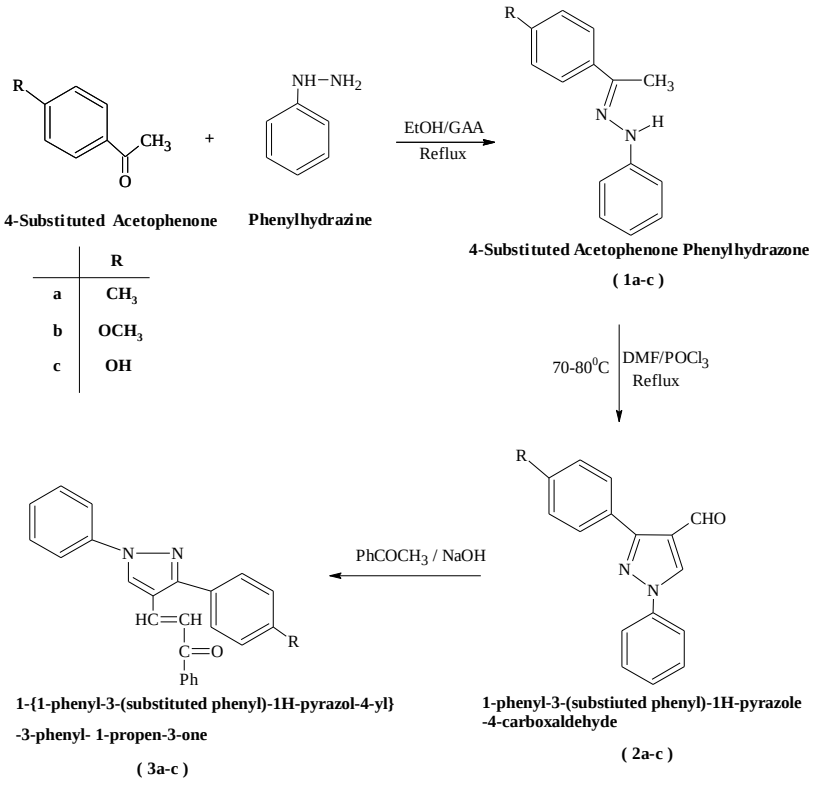
Keywords: Pyrazolo Pyrimidine, Pyrazolopyridine, Antimicrobial, Antifungal.

1. Introduction

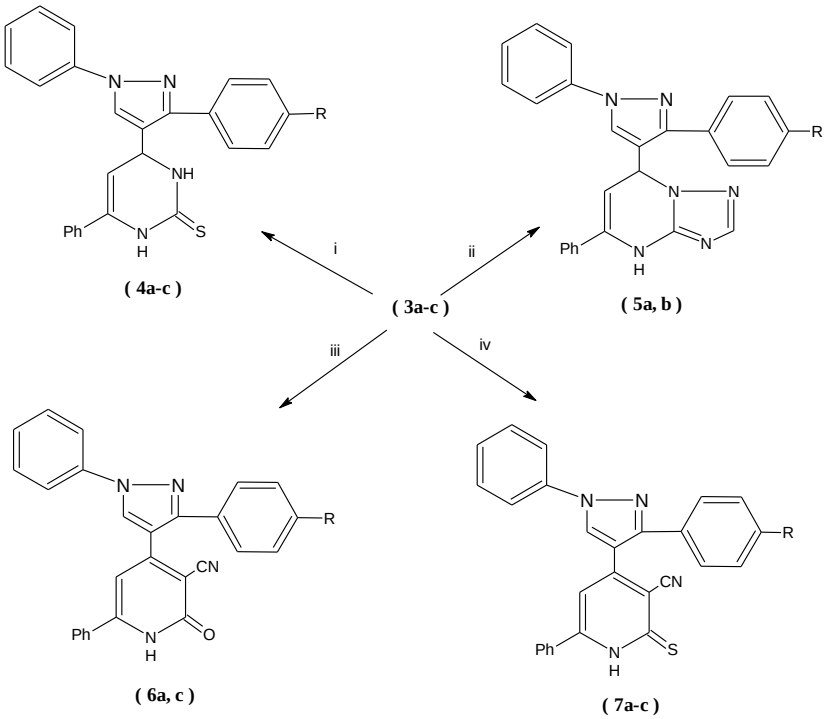
The Pyrazole nucleus in general and its chemistry has found much more attention during the last few decades [1,5] because of outstanding biological activities. It acts as antipyretic, analgesic [16], anti-inflammatory, kinase inhibitors, and as insecticides. It has good properties as antibacterial, antifungal, and an antiparasitic, as well. Considering all of these benefits and in continuation of our work to synthesize new heterocycles starting from 1, 3-diphenyl-1*H*-pyrazole-4-carboxaldehyde, we introduce herein several new pharmacophores, such as pyrimidine, pyrimidinethione, triazolopyrimidine, pyridine, pyridine-thione and thiazole moieties onto the pyrazole nucleus in an effort to obtain compounds with enhanced potency. The starting material of 1-{1-phenyl-3- (substituted phenyl) - 1H-pyrazol-4-yl}-3-phenyl-1-propen-3-one (Chalcone) (3a-c) prepared from by claisen –schmidt reaction from respective 1-phenyl-3-(substituted phenyl)-1H- pyrazoles – 4 - carboxaldehyde (Vilsmeier - Haack Reaction) (2a-c). 1-{1-phenyl-3-(substituted phenyl) - 1H-pyrazol-4-yl}-3-phenyl-1-propen-3-one (Chalcone) (3a-c) further treated with theorem, 3amino-s-triazole, ethylcyanoacetate, cynothioacetamideto produced 1,2,3,4 trtrahydropyrimidine (4 a-c), triazolopyrimidine (5 a,b), cynophenylpyridine (6 a-c) & phenyl pyridine thione derivatives. The structures of newly Synthesised compounds were characterized by spectral data & studied for their antimicrobial & antifungal activities.

1.1 Scheme for the preparation of pyrazolopyrimidine and pyrazolopyridine derivatives

Scheme I



Scheme II



i. NH_2CSNH_2
ii. 3-amino-s-triazole
iii. $CNCH_2CO_2Et$
iv. $CNCH_2CSNH_2$

2. Materials and methods

The Progress of reaction was monitored by thin layer chromatography (TLC) on silica gel & Visualized using iodine vapour melting points are determined in one end open capillary tubes and were uncorrected. All the synthesized compounds were characterized by spectral analysis (IR, NMR (H) & elemental analysis. Melting points were determined in open tube capillaries method & are corrected.

IR spectra of final compounds was recorded on Jasco FT IR spectrophotometer using KBR pellet method & 1H NMR of the final compounds was recorded in CDCl3 solution on FTNMR, varian mercury 300mHz using tetramethyl silane as internal standard to determine structure of new compounds. All the new compounds gave satisfactory analytical results.

2.1 Synthesis of 4-substituted Acetophenone phenyl-hydrazone (1a-c).

Concentrated acetic acid (1ml) and phenylhydrazine hydrochloride (3g, 20 mmol) were added to solution of substituted acetophenone (24 mmol) in 90 ml of ethanol. Then, the reaction mixture was refluxed for 1 hour. The precipitate was filtered and washed with ethanol. After drying in vacuum, product **(1a-c)** was obtained.

2.2 Synthesis of 1-phenyl-3-(substituted phenyl)-1H-pyrazoles-4-carboxaldehyde (Vilsmeier- Haack Reaction) (2a-c).

Vilsmeier-Haack reagent was prepared from previously from separately cooled Dimethyl formamide (2.58g, 35.3 mmol) and $POCl_3$ (5.4g, 35.5 mmol) at 0^0C and stirred at 0^0C. A solution of **1** (3g, 11.76 mmol) in DMF (3ml) was added drop wise to the Vilsmeier- Haack reagent, which was then warmed at room temperature and refluxed at 70-80^{0}C for 4-5 hours. After cooling at room temperature the mixture was basified with a cool saturated K_2CO_3 solution. The precipitate was filtered, strongly washed with water and recrystallized from ethanol.

2.3 Synthesis of 1-{1-phenyl-3-(substituted phenyl)-1H-pyrazol-4-yl}-3-phenyl-1-propen-3-one (Chalcone) (3a-c):

0.01 mole of ethanolic solution of acetophenone and 0.01 mole of 1,3– diphenylpyrazoles-4-carboxaldehyde were mixed together and stirred. 10ml of 40% sodium hydroxide solution was added to it. The mixture was kept overnight at room temperature. The content was then poured over crushed ice and acidified with dilute hydrochloric acid. The solid obtained was filtered, dried and recrystallised with ethanol.

2.4 Synthesis of 4-{1-phenyl-3-(substituted phenyl)-1H-pyrazol-4-yl}-6-phenyl-1,2,3,4-tetrahydropyrimidine-2-thione (4a-c):

A mixture of chalcone 3 (3.5gm, 0.01mol) and thiourea (0.76g, 0.01 mol) in ethanolic C_2H_5ONa solution (0.25g, Na in 30 ml abs C_2H_5OH) was heated under reflux for 2-4 hours, cooled and neutralised with diluted CH_3COOH. The precipated solid was collected and recrystallised from benzene as fine white needles.

2.5 Synthesis of 4,7-Dihydro-7-{1-phenyl-3-(substituted phenyl)-1H-pyrazol-4-yl}-5-phenyl-s-triazolo[1,5-a]pyrimidine (5a,b)

A mixture of chalcone 3 (3.5g, 0.01 mol) and 3-amino-s-triazole (0.84g, 0.01 mol) in CH_3COOH (40ml) was heated under reflux for 2-4 hours and allowed to cool. The product thus separated was collected and recrystallised from C_2H_5OH to give white crystals,

2.6 Synthesis of 3-Cyano-4-{1-phenyl-3-(substituted phenyl)-1H-pyrazol-4-yl}-6-phenylpyridine-2(1H)-one (6a,c)

A mixture of chalcone 3 (3.5g, 0.01 mol), ethyl cyanoacetate (2.26, 0.02mol) and CH_3COONH_4 (7.7g, 0.1 mol) was heated at 150^{0}C in an oil bath for 5-7hour . The solid which precipated after cooling and dilution with water was collected and recrystallized from CH_3COOH as white crystals

2.7 Synthesis of 3-Cyano-4-(1,3-Diphenyl-1H-pyrazol-4-yl)-6-phenylpyridine-2(1H)-thione(7a-c):

To a suspension of chalcone 3 (7.0g, 0.02 mol) and cynothioacetamide (2.0g, 0.02 mol) in absolute C_2H_5OH (150ml), triethylamine (1.0 ml) was added. The reaction mixture was heated under reflux for 6-8 hour, concentrated and left to cool. The product thus formed was collected and recrystallised form CH_3COOH as yellow fine needles,

2.8 Antimicrobial Sensitivity test [4,5]

Antimicrobial activity of newly synthesized compounds (4a,4c,5a,6c,7c) was determined by disc diffusion method in nutrient agar for bacterial & saborsuds agar for fungi. In this work *Bacillus substitis, Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa* are used to investigate the Antimicrobial activities & *Candida albicans* were used to investigate the antifungal activities. Minimum inhibitory concentration (MIC) of all compounds was determined which is defined as the lowest concentration of inhibitor at which bacterial growth was not visually apparent.

Amoxicillin & clotrimazole were used as standard drugs for bacterial & fungi respectively. Initial screening of the test compounds & standard drugs were performed at fixed concentration of (500µg/ml). Inhibition was recorded by measuring the diameter of the inhibition zone at the end of 24hrs for Bacteria & 72 hrs for fungi. Each Experiment was repeated twice.

3. Results

3.1 Spectral data of some pyrazolopyrimidine pyrazolopyridine derivatives:

4a:-4{1-phenyl-3-(substituted phenyl)-1H-pyrazol-4-yl}-6-phenyl-1,2,3,4-tetrahydropyrimidine-2-thione

-yield (70%), lemon yellow crystal, M.P. 210^{0}C IR (cm^{-1}) 3116.40 (Ar-CH), 1448 (Ar-C=C), 1648.84 (-C=N), 1315.21(Ar-C-N), 2915.84(-CH3)-3170.40 (N-H), 1220.72 (C=S)

1H NMR (CDCl3) δppm 8.308 (s,1H,H5 of pyrazole) 5.44 (S,H4 of Pyrimidine), 5.19 (S,H5 of Pyrimidine), 9.496 (S,1H,-NH1 of Pyrimidine), 10.001 (S,1H,NH3 of Pyrimidine, 7.256 (m,5H of 1 Phenyl of Pyrazole), 7.431-7.94 (d,4H of 3 Phenyl of Pyrazole), 2.415 (S,3H of CH3 of Phenylm ring)

4c:4-{1-phenyl-3-(4-hydroxyphenyl)-1H-pyrazol-4-yl}-6-phenyl-1,2,3,4 tetrahydropyrimidine-2-thione

Yield (43.82%), faint brownish yellow, M.P.(135^{0}C)

IR (cm-1)3056 (Ar-CH), 1502.28 (Ar-C=C), 1677.77 (-C=N),1357.64 (Ar-C-N), 3660.23 (OH)3168.47 (N-H) 1218.79 (C=S), NMR 1H ($CDCl_3$) , δppm 8.345 (s,1H,H5 of Pyrazole), 9.897, (s,1H,NH of pyrimidine), 9.970 (s,1H, NH3 of Pyrimidine), 5.554 (s,1H,5 CH of pyrimidine) 6.799- 6.814(m, H2,3 of 1 phenyl of pyrazole) 7.204-7.247 (m,5H 4,5,6 of 1 phenyl of pyrazole) 7.851-7.877 (d,2H H 3,5 of 3 phenyl of pyrazole) 7.751-7.788 (s,H 2,6 of 3 Phenyl pyrazole), 7.332-7.513 (m.5H of phenyl ring of pyrimidine),8.012 (s,1h of OH of 3 Phenyl of Pyrazole).

5a:- 4,7-Dihydro-7-{1-phenyl-3-(4-methylphenyl)-1H-pyrazol-4-yl}-5-phenyl-s-triazolo[1,5-a]pyrimidine

Dark yellow crystals, M.P. (195^{0}C) yield (86.79%),

IR(cm-1) 3116.40 (Ar-CH), 1502.28 (Ar-C=C), 1646.91 (-C=N), 1315.21 (Ar-C-N), 2915.84(CH3), 3166.54(NH),

NMR 1H($CDCl_3$) δppm 8.315 (S,1H,H5 of pyrazole) 8.155 (S,CH of Triazole), 9.487 (S,1H of Pyrimidine), 2.419 (S,3H,-CH3 of phenyl ring)4.955 (S,1H of Pyrimidine),6.879 (S,1H, of Pyrimidine), 7.260-7.339 (m,5H of 1 Phenyl of Pyrazole),7.449-7.488 (m,5H of 1 Phenyl of pyrimidine),7.752-7.776 (d,4H of 1 phenyl pyrazole)

6c:- 3-Cyano-4-{1-phenyl-3-(Hydroxy phenyl)-1H-pyrazol-4-yl}-6-phenylpyridine-2(1H)-one

Yield 57.78, M.P. (165^{0}C), dark brown.

IR (KBR) cm-1 3058.55 (Ar-CH), 1502.28,(Ar-C=C),1598.70 (C=N),1338.36 (Ar-C-N) ,3664.09 (OH), 3195.47(N-H),1691.21 (C=O),2213.88 (C-N)

NMR 1H (CDCl3)) δppm 8.193 (s. 1H,H5 of Pyrazole),6.761 (s,1h CH of Pyridine),9.918 (s,1h NH of pyridine),7.229 (m,5 H, of 1 phenyl of pyridine, 7.624-7.687 (d,4H of 3 phenyl of pyrazole).8.023 (s,1H,OH of 3 Phenyl ring.)

7c: 3-Cyano-4-{1 phenyl-3-(4-hydroxyphenyl)-1H-pyrazol-4-yl}-6-phenyl pyridine-2(1H)-thione

Yield (75%) M.P (155^{0}C) Brown

IR (KBR) cm-1 3060.48 (Ar-CH) 1502.28 (Ar-C=C), 1594.84 (C=N), 1353.78 (Ar-C-N), 3642.87 (OH) 3180.04 (N-H), 2206.17 (C=N)

NMR 1H ($CDCl_3$)) δppm8.337 (s,1H,H5 of Pyrazole) 6.838 (s,1H of CH of Pyridine) 7.987 (s,1H of OH of 3 phenyl , 7.247 (m, 5H of 1 phenyl of pyridine), 7.763 (d,2H H3,5 of 3 Phenyl of pyrazole) 7.481 (m, 5H of 1 phenyl of pyrazole.

Table 1: Various Substitution of the synthesized Pyrazolo Pyrimidine & Pyrazolopyridine

Sr. No	Compounds	Substitution
1	4a	CH_3
2	4c	OH
3	5a	CH_3
4	6c	OH
5	7c	OH

Table 2: Physicochemical data of Pyrazolo Pyrimidine & Pyrazolopyridine acid derivatives

Sr. No.	Compound	Molecular Formula	Molecular weight	Melting point (^{0}C)	Yield (%)	Rf Value
1.	4a	$C_{26}H_{22}N_4S$	422	210	77.80	0.90
2.	4c	$C_{25}H_{20}N_4SO$	424	135	43.82	0.62
3.	5a	$C_{27}H_{22}N_6$	430	195	86.79	0.76
4.	6c	$C_{27}H_{18}N_4O_2$	430	165	57.78	0.95
5.	7c	$C_{27}H_{18}N_4SO$	446	155	75	0.7

3.2 Antimicrobial Sensitivity test

Based on the result of zone of inhibition, The Minimum inhibitory concentration (MIC) of potent compounds (4a,4c,5a,6c,7c) against all bacterial & fungal growth was determined by stock solutions of tested compound were prepared with DMSO as Inoculums of the bacterial and fungal cultures were also prepared. To a series of tubes containing 1.0ml each of test compound solution

with different concentrations and 0.2mL of the test compound solution with different concentrations and 0.2 mL of the inoculums was added. Further 3.8ml of the sterile water was added to each of the tubes. These test tubes were incubated for 24hours at 37oc and observed for the presence of turbidity. This method was repeated by changing test compound with standard drugs Amixicillin & Clotriamazole for comparision. The Minimum Inhibitory concentration at which no growth was observed was taken as the MIC values. The Comparison of the MICs (in µg/mL) of potent compounds and standard drug Amoxicillin 10 mg/Disc concentrations and DMSO as the vehicle against tested strains are presented in table. Similarly the MIC for Antifungal Activity was determined using 72hours old Saborauds cultures. The results were compared with Clotriamazole (10 mg/Ml of DMSO as the vehicle and summarized in Table

Table 3: Antibacterial activity

Name of organism	4a	4b	4c	5a	5b	6a	6c	7a	7b	7c	Control (DMSO)	Standard
Bacillus substilis	+	+	-	-	+	+	-	+	++	++	-	+++
Staphylococcus aureus	++	+	+++	-	-	-	-	-	++	-	-	+++
Proteus vulgaris	++	++	-	-	-	-	-	-	-	-	-	++
Pseudomonas aeruginosa	++	++	++	+++	-	++	+++	-	++	++	-	++

Standard: Amoxicillin (10mg/1ml of DMSO.)

Table 4: Antifungal activity

Name of organism	4a	4b	4c	5a	5b	6a	6c	7a	7b	7c	Control (DMSO)	Standard
Candida albicans	++	+	-	-	+	-	++	-	++	-	-	+++

Standard: Clotrimazole (10mg/1ml of DMSO.)

4. Conclusion

All the synthesized compounds were screened for antimicrobial, antitubercular, and analgesic activity. From the results obtained in pharmacological screening and structures of the compound we can correlate in general that compound 4c and 7c showed high antibacterial activity against both of gram + ve species, (*Bacillus substilis* and *Staphylococcus aureus*). Compound 4a, 6c and 7b Showed moderate antifungal activity against *Candida albicans.*

References

[1]. Kost A. N., Grandberg I. I.: Progress in pyrazole chemistry. *Adv. Heterocycl. Chem*. 1966; 6: 347-429.

[2]. Sammes M. P., Katritzki A. R.: The 3H-Pyrazoles. *Adv. Heterocycl. Chem*. 1983; 34: 1-52.

[3]. Elguero J., In: Katritzki A. R.; Rees C. W. (eds.): Comprehensive Heterocyclic Chemistry, Pergamon Press, Oxford 1984; Vol. 4, Chapter 4.4.

[4]. Elnagdi MH, Elgemeie GEH, Abdelaal FAE. Recent developments in the synthesis of pyrazole derivatives. *Heterocycles* 1985; 23(12):3121–53.

[5]. Mullins S. T., In: Coffey S., Ansell M. F. (eds.): Rodd's Chemistry of Carbon Compounds, Suppl. Of Vol IV$^{c/d}$, p. 1, Elsevier Science Publishers, Amsterdam (1994).

[6]. Kira M. A., Abdel-Rehman M. O., Gadalla K. Z.: The vilsmeier-haack reaction - III Cyclization of hydrazones to pyrazoles. *Tetrahedron Lett.* 1969; 2: 109-110.

[7]. Rathelot Pascal, *et al*.: 1,3-Diphenylpyrazoles: synthesis and antiparasitic activities of azomethine derivatives. *European Journal of Medicinal Chemistry* 2002; 37: 671-679.

[8]. Jagan Mohan Chary T., Narsimha Reddy Ch V., Murthy Krishna: A facile one step synthesis of fused isoxazolo-[4,5-b]pyridine-N-oxides. *Indian Journal of Chemistry* 1992; 31B: 495-498.

[9]. Pawar R. A., Patil A. A.: Studies on Vilsmeier–Hack reaction: a versatile new synthesis of 5-chloro-3-methylpyrazole-4-carboxaldehyde, 5-chloro-3-methyl - 1-phenylpyrazole-4-carboxaldehyde and related fused heterocyclic compounds and heterocyclic Schiff bases, *Indian J Chem*, 1994; 33B: 156-158.

[10]. El-Emary, T. I. and Bakhite, E. A., Synthesis and biological screening of new 1,3-diphenylpyrazoles with different heterocyclic moieties at position-4. *Pharmazie, 1999; 54*: 106-111.

[11]. Pelczar, M. J., chan, E. C. S. and Krieg, N. R., Microbiology, TATA McGRAW-HILL, 5th edition, 3-17 (1993)

[12]. Canetti G., *et al*: Advances in techniques of testing mycobacterial drug sensitivity and use of sensitivity tests in tuberculosis control programmes, *Bulletin of the WHO*, 1969; 41: 21-43.

[13]. Bauer A. W., Kirby W. W. M., Sherris, J. C., Turck, M.: Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*. 1966; 45: 493.

[14]. Kalyoncuoglu N., Rollas S., Sur-Altiner D., Yegenoglu Y., Ang O.: 1-[p-(Benzoylamino) benzoyl]-4-substituted thiosemicarbazides: synthesis and antibacterial and antifungal activities. *Pharmazie*. 1992; 47: 769.

[15]. Kulkarni, S.K., Hand Book of Experimental Pharmacology, 3rd edition 127-128 (2005)

[16]. Ghosh M. N., Fundamentals of Experimental Pharmacology, scientific Book Agency Calcutta, 153 (1981).