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Synthesis, antifungal and antibacterial activities of 3-substitutedphenyl-6-substitutedphenyl-(1,2,4)-triazolo-(4,3-b)-pyridazines

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ABSTRACT

A series of 3-substituted phenyl -6-substituted phenyl (1,2,4) triazolo (4,3-b) pyridazine has been synthesized. An appropriate aromatic hydrocarbon reacts with succinic anhydride in presence of $AlCl_3$ to yield β-aroyl propionic acid. The corresponding acid was cyclised with hydrazine hydrate to give 6-(substituted aryl)-2,3,4,5-tetrahydro-3-pyridazinone, which was heated on steam bath with phosphorous oxy chloride to yield 3-chloro 6-substituted phenyl pyradazine. This intermediate after reaction with hydrazine hydrate was converted into 3-hydrazino-6-substituted phenyl pyradazine. The resulting product was converted into3-substituted phenyl 6-substituted phenyl (1,2,4) triazolo (4,3-b) pyridazine by reacting with substituted aroyl chloride. Spectral data (IR, NMR, mass spectra) confirmed the structures of the synthesized compounds. The synthesized compounds were investigated for their in vitro antifungal and antibacterial activities. The results indicated that the synthesized compounds have mild to potent activities with reference to their appropriate reference standards.

Keywords: Pyridazin**,** *In vitro* antifungal, antibacterial.

INTRODUCTION

Triazole and it's derivatives are noteworthy for their physiological and biological importance. They paved the attention of medicinal chemist due to their wide range of biological activities like anti-inflammatory (1-5), antibacterial (6-8), anticonvulsant (9), antifungal (10-14) and anticancerous (15) etc. In view of above facts and inspired by the research going on triazole and its derivatives, particularly in relation to microbial infections, 3-substituted phenyl 6-substituted phenyl (1,2,4) triazolo (4,3-b) pyridazine has been synthesized. The final compounds was synthesized as per reaction sequence is outlined in Scheme-I. Friedal-crafts acylation of appropriate hydrocarbons with succinic anhydride, in presence of anhydrous $AlCl_3$ yield β-aroyl propionic acid followed by hydrazinolysis with hydrazine hydrate to get substituted pyridazinones, which reacted with phosphorous oxy chloride to give 3-chloro 6-substituted phenyl pyradazine. This intermediate after reaction with hydrazine hydrate was converted into 3-hydrazino-6-substituted phenyl pyradazine. This product was reacted with substituted aroyl chloride to yield 3-substituted phenyl 6-substituted phenyl (1,2,4) triazolo (4,3-b) pyridazine.

All the final compounds was structurally elucidated on the basis of NMR, IR and mass spectral data. The final synthesized compounds were evaluated for antifungal and antibacterial activities. The physiochemical data of the final synthesized compounds are mentioned in Table-I.

MATERIALS AND METHODS

The melting points was determined on a X-4 microscope melting point apparatus and are uncorrected. The NMR spectra were recorded in $CDCl_3$ as solvent (using TMS as an internal standard).The NMR and mass spectra were recorded on Jeol FX-100FT-NMR and Jeol BX 102/DA-6000 mass spectrometer respectively. The infrared spectra in KBr were recorded, on Buck Scientific M-500 Infrared Spectrophotometer. Solvent system used through out the experimental work for running TLC plates Toluene, Ethyl formate, and Formic acid in the ratio of 5:4:1.

The triazole derivatives was synthesized as per Scheme-I. The overall reaction involves five steps for synthesis and characterization of compound It is illustrated with the synthesis of compound **1e.**

Synthesis of β-Benzoyl propionic acid (1a)
After suspending anhydrous aluminum chloride (0.15 mol) in dry benzene (50ml) under anhydrous conditions, the contents was refluxed on a water bath. Succinic anhydride (0.10 mol) was then added to the reaction mixture in small portions with continuous stirring. Stirring and heating was continued for 6 h and the contents after leaving overnight at room temperature, ice cold solution of concentrated hydrochloric acid (2.5% v/v) was then added to the reaction mixture and the contents was concentrated to a small volume by heating on a water bath. The solid compound which separated out, was filtered. It was purified by dissolving in 5% w/v sodium bicarbonate solution, followed by extraction with ether. The aqueous layer on acidification with dilute hydrochloric acid gave benzoyl propionic acid, crystallized from aqueous ethanol to give a colourless compound.m.p.125^oC; R_f 0.25; %yield 73; ^{1}H-NMR(δ) 2.59 (t, 2H, CH_2), 3.23 (t, 2H, CH_2), 7.53-7.62 (m, 3H, H-3'-H-5'), 7.97 (d, 2H, H-2', H-6'), 12.17 (s, 1H, COOH).

All the remaining acids was synthesized by analogous procedure with minor modification in temperature of reaction and use of nitrobenzene as solvent.

Synthesis of 6-Phenyl 2,3,4,5-Tetrahydro pyridazin-3-one (1b)
To a solution of β-benzoyl propionic acid **(1a)** (0.1 mol) in methanol (30ml), hydrazine hydrate (1ml) and sodium acetate (0.5g) was added and the contents refluxed for 6 h. After completion of the reaction, methanol was distilled off and the contents was poured into cold water. The solid that separated out, was filtered and crystallized from methanol, m.p.250 ^oC; R_f 0.45; % yield 72;IR (cm^{-1}) 3306(NH), 1678(C=O); ^{1}H-NMR (δ) 2.45 (t, 2H, CH_2), 2.93 (t, 2H, CH_2), 7.41(m, 3H, H-3'-H-5'), 7.74 (d, 2H, H-2', H-6'), 10.94 (s, 1H, CONH); Ms (m/z) 174,159, 147, 130, 115, 109.

Synthesis of 3-chloro 6- phenyl pyradazine (1c)
A mixture of 6-Phenyl 2,3,4,5-Tetrahydropyradazin-3-one (0.01 mole)and phosphorous oxy chloride ($POCl_3$) was heated on a steam bath for 6 h. After heating, the mixture was carefully poured on crushed ice. The water was rendered alkaline by sodium bicarbonate. Crude 3-chloro pyradazine was collected by filtration. m.p.135^oC, R_f 0.70, %yield 76; IR(cm^{-1}) 1630(C=N), 1590(C=C), 940, 702 (C-Cl);^{1}H-NMR(δ) 7.42−7.51 ((m,7H,Ar-H).

Synthesis of 3-hydrazino-6- phenyl pyradazine (1d)
To an ethanolic solution of 3-chloro pyradazine (0.01 mole) in hydrazine hydrate (99 %) was added. The resulting reaction mixture was refluxed on steam bath for 16 h. The contents were concentrated, cooled and poured into crushed ice. The resulting solid which separated out is filtered, washed with water, dried and recrystallized from alcohol. m.p.168 ^oC, R_f 0.75, %yield 79; IR(cm^{-1}) 3440(NH), 961(CH); 1638(C=N), 1582 (C=C), 938, 680; ^{1}H-NMR (δ) 2.5 (s, 2H ,NH_2), 7.17-8.05 (m, 7H, Ar-H), 8.15 (m,1H, Ar-NH);

Synthesis of 3,6-di-phenyl (1,2,4) triazolo (4,3-b) pyridazine (1e)
Compound 1d dissolve in xylene (20 ml) were added triethylamine (0.67 gm, 6.7 mol) and benzoyl chloride (0.58 gm, 4.4 m mol). The mixture was stirred at room temp. for 1 hr. and then heated at reflux for 16 hrs. The solution was cooled to room temp., the solvent evaporated under reduced pressure and the residue partitioned between CH_2Cl_2 (150 ml) and water (30 ml). The aqueous phase was separated and extracted further with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and evaporated and the residue was chromatographed on silica gel, eluted with ethyl acetoacetate to get the final compound, recrystallised with alcohol; m.p.219 ^oC, R_f 0..76, % yield 95. IR

(cm^{-1}) 3004 (CH), 1686 (C=C), 1291, 907 , 931, 805, ^{1}H-NMR (δ) 7.22-8.45 (m, 12H, Ar-H); Ms (m/z) 272 (M^+), 257, 242, 230, 212, 168,104;

The remaining compounds was synthesized with analogous procedure and their m.p., %ge yield and molecular formula are mentioned in table I.

Synthesis of Synthesis of 3-(4'-tolyl)-6-phenyl (1,2,4) triazolo (4,3-b) pyridazine (1f)
IR (cm^{-1}) 3002(CH), 1682(C=C), 1632(C=N), 980 , 932, 810; ^{1}H-NMR (δ) 3.26(t, 3H, CH_3), 7.27-8.38 (m, 11H, Ar-H);

Synthesis of 3-phenyl 6-(4'-tolyl) (1,2,4) triazolo (4,3-b) pyridazine (2a)
IR (cm^{-1}) 1023, 3066(CH), 1686(C=C), 1451, 1422, 1023 , 917; ^{1}H-NMR (δ) 2.34(s, 3H, CH_3), 7.19-8.16 (m, 6H, Ar-H); Ms (m/z) 288(M^++2), 265, 233, 146, 115,88;

Synthesis of 3,6-di(4'-tolyl) (1,2,4) triazolo (4,3-b) pyridazine (2b)
IR (cm^{-1}) 2992(CH), 1631(C=N), 1602(C=C), 936, 798; ^{1}H-NMR (δ) 2.3(s, 6H, 2xCH_3), 6.98-8.01 (m, 10H, Ar-H);

Synthesis of 3-phenyl 6-(2',4'-dimethyl phenyl) (1,2,4) triazolo (4,3-b) pyridazine (3a)
IR (cm^{-1}) 2963(CH), 1687(C=C), 1492, 1290, 1023, 930; ^{1}H-NMR (δ) 2.5(s, 6H, 2xCH_3), 7.29-8.15 (m, 10H, Ar-H);

Synthesis of 3-(4'-tolyl)-6-(2',4'-dimethyl phenyl) (1,2,4) triazolo (4,3-b) pyridazine (3b)
IR (cm^{-1}) 2971(CH), 1674(C=C), 1283, 752; ^{1}H-NMR (δ) 2.4(s, 9H, 3xCH_3), 7.19 (m, 9H, Ar-H); Ms (m/z) 314(M^++2), 289, 273, 146, 115,106;

Synthesis of 3-phenyl 6-(4'-phenoxy phenyl) (1,2,4) triazolo (4,3-b) pyridazine (4a)
IR (cm^{-1}) 2962(CH), 1632(C=N), 1584(C=C), 932, 672; ^{1}H-NMR (δ) 7.24-8.14 (m, 16H, Ar-H);

Synthesis of 3-(4'-tolyl)-6-(4'-phenoxy phenyl) (1,2,4) triazolo (4,3-b) pyridazine (4b)
IR (cm^{-1}) 2965(CH), 1630(C=N), 1584(C=C), 930, 671; ^{1}H-NMR (δ) 2.38(s, 3H, CH_3), 6.97-8.01 (m, 18H, Ar-H);

Synthesis of 3-phenyl 6-(4'-chloro phenyl) (1,2,4) triazolo (4,3-b) pyridazine (5a)
IR (cm^{-1}) 2980(CH), 1636(C=N), 1608(C=C), 1220, 1173, 1036, 754; ^{1}H-NMR (δ) 7.16-8.33 (m, 11H, Ar-H);

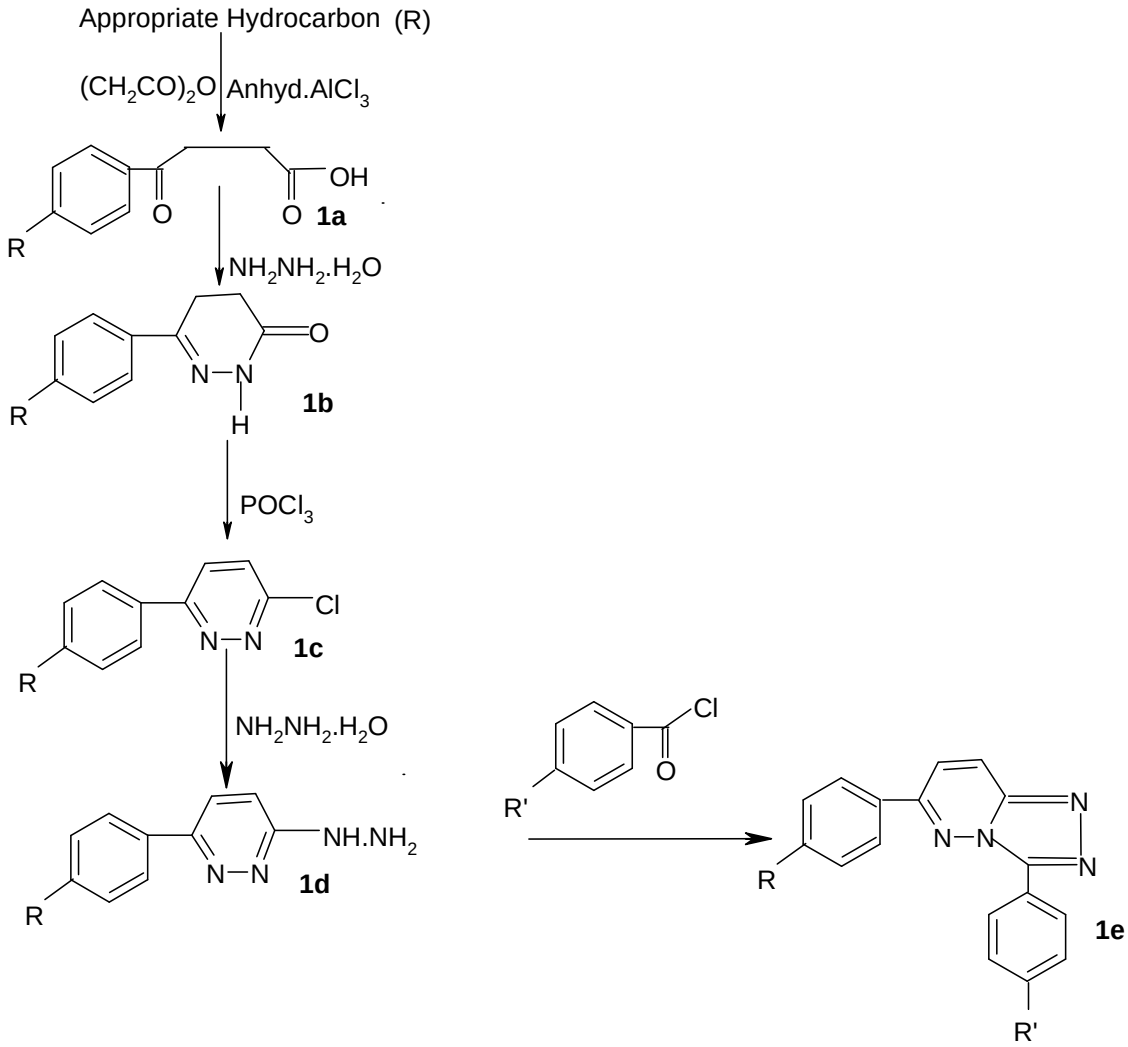
Synthesis of 3-(4'-tolyl)-6-(4'-chloro phenyl) (1,2,4) triazolo (4,3-b) pyridazine (5b)
IR (cm^{-1}) 2916(CH), 1605(C=C), 1223, 1170, 1038, 749; ^{1}H-NMR (δ) 2.4(s, 3H, CH_3), 7.25-8.0 (dd, 10H, Ar-H); Ms (m/z) 321\320.5(M^+), 221, 211;

Synthesis of 3-phenyl 6-(4'-bromo phenyl) (1,2,4) triazolo (4,3-b) pyridazine (6a)
IR (cm^{-1}) 2989(CH), 1632(C=N), 1605(C=C),1180, 976, 758; ^{1}H-NMR (δ) 7.37-8.14 (m, 11H, Ar-H); Ms (m/z) 352(M-1), 331, 287, 221,211, 167, 91;

Synthesis of 3-(4'-tolyl)-6-(4'-bromo phenyl) (1,2,4) triazolo (4,3-b) pyridazine (6b)
IR (cm^{-1}) 2987(CH), 1631(C=N), 1607(C=C),1175, 968, 678; ^{1}H-NMR (δ) 3.2(s, 3H, CH_3), 7.32-8.09 (m, 10H, Ar-H);

Synthesis of 3-phenyl 6-(4'-bi phenyl) (1,2,4) triazolo (4,3-b) pyridazine (7a)
IR (cm^{-1}) 2985(CH), 1627(C=N), 1603(C=C), 1085, 958, 718; ^{1}H-NMR (δ) 7.23-8.13 (m, 16H, Ar-H);

Synthesis of 3-(4'-tolyl)-6-(4'-bi phenyl) (1,2,4) triazolo (4,3-b) pyridazine (7b)
IR (cm^{-1}) 2989(CH), 1631(C=N), 1609(C=C); ^{1}H-NMR (δ) 2.38(s, 3H, CH_3), 6.99-8.01 (m, 15 H, Ar-H);



Scheme-I Synthesis of 3-substituted phenyl 6-substituted phenyl (1,2,4) triazolo (4,3-b) pyridazine

Biological Evaluation

Antibacterial activity (16)

The antibacterial activity of synthesized compounds from all the three series have been performed by adopting cup plate method. This method depends on the diffusion of a sample solution from a vertical cylinder or a cavity through the solidified agar layer of a petridish, such that growth of the added microorganism is prevented entirely in a circular area or a zone around the cylinder or cavity containing a solution of the sample if the added sample posses antibacterial activity.

Freshly prepared liquid agar medium (35ml/ Petridish) was poured in to the petridishes (8 Petridishes/sample) and kept for solidification. Then the 200 µl-standardized culture (99ml Nutrient broth media + 1ml culture) of organism was spread on each petridishes by L-shaped spreader. With the help of the borer (5mm), three bores were made on each plate. The synthetic compounds were made dilutions with Dimethyl Sulphoxide (DMSO). The different concentrations (50 µg, 100 µg, and 200 µg/ml) of the sample solutions was added in each well separately. The petridishes were kept aseptically for 4 to 5 hr for diffusion of the sample. After the completion of diffusion period, all petridishes were kept for incubation at 37°C for 24 h. After 24 h the activity of sample in the way of zone of inhibition was observed for each compound against four (2 gram positive & 2 gram negative) microorganisms namely *Staphylococcus aureus, Micrococcus luteus*, *Escherichia coli and Klebsiella pneumoniae*. The antibacterial activity of synthesized compounds from all the series are depicted in Table 2.

Antifungal Activity (17)

The sabouraud agar media (Dextrose 4%, Peptone 1%, & agar 1.5%) was used for anti-fungal activity. The media was prepared and sterilized in autoclaves at 15Psi for 15 mts. Then it was poured in sterilized petriplates, aseptically. The fungal strains *candida albicans* & *cryptococcus neoformans* were inoculated on the surface of petriplates separately after 2 h. of pouring the agar media, when the media sets on petriplates the cups (diameter 6 mm) was made in the sabouraud agar media using sterilized cup borer in aseptic condition. The 0.1 ml of each standard and test (10 mg/ml) prepared by dissolving it in DMSO was added in cups. The petriplates were incubated at 28 ± 2^0C for 48 h growth and zone of inhibition (in mm) was recorded. The antifungal activity of synthesized compounds from all the series are depicted in Table 3.

RESULTS AND DISCUSSION

All the final synthesized compounds were evaluated for Antitubercular activity. Stock solutions of test compounds is prepared in DMSO. MIC of rifampin is calculated by established procedures . All the synthesized compounds screened at 6.25 µ/\ml show the percentage inhibition ranging from 30 to 72 %.. The final synthesized compounds were evaluated for their antibacterial activity against *E.Coli*, *S.aureus*, *Micrococcus luteus* and *Klebsiella pneumonia* by using cup plate technique in the nutrient agar at 100µg/ml concentration and the zone of inhibition (mm) of each compound is mentioned in Table 2. DMSO is used as a control, The results of antibacterial exhibit that all compounds having comparable activity against the bacterial strain. Compounds **2b, 3b** and **4b** are the most active derivatives, which shows significant activity against these bacteria comparable to standard drug, ampicillin and chloramphenicol. All the final compounds were evaluated for antifungal activity against *C.albicans* and *C.neoformans* by using cup-plae method in the sabouraud agar media (Dextrose 4%, Peptone 1%, & agar 1.5 %).The zone of inhibition (mm) of each compound is determined and compared with standard drug fluconazole. The result is depicted in Table 3 The compounds **3a, 3b** and **5b** were found to be active derivatives of this series against the microorganism**.**

Table 1 Physicochemical data of 3-substituted phenyl 6-substituted phenyl (1,2,4) triazolo (4,3-b) pyridazine

Compd no.	R	R'	M.P.(^{0}C)	Mol.Formula	%ge yield
1e	H	H	219	$C_{17}H_{12}N_4$	76
1f	H	$-CH_3$	232	$C_{18}H_{14}N_4$	97
2a	$-CH_3$	H	243	$C_{18}H_{14}N_4$	99
2b	$-CH_3$	$-CH_3$	236	$C_{19}H_{16}N_4$	97
3a	2,4-$(CH_3)_2$-	H	276	$C_{19}H_{16}N_4$	93
3b	2,4-$(CH_3)_2$-	$-CH_3$	246	$C_{20}H_{18}N_4$	98
4a	$-OC_6H_5$	H	296	$C_{23}H_{16}N_4O$	93
4b	$-OC_6H_5$	$-CH_3$	278	$C_{24}H_{18}N_4O$	98
5a	4-Cl	H	274	$C_{17}H_{11}N_4Cl$	90
5b	4-Cl	$-CH_3$	298	$C_{18}H_{13}N_4Cl$	79
6a	4-Br	H	310	$C_{17}H_{11}N_4Br$	79
6b	4-Br	$-CH_3$	298	$C_{18}H_{13}N_4Br$	81
7a	$-C_6H_5$	H	246	$C_{23}H_{16}N_4$	83
7b	$-C_6H_5$	$-CH_3$	276	$C_{24}H_{18}N_4$	78

Table 2 *Invitro* Antibacterial activity of 3-substituted phenyl 6-substituted phenyl (1,2,4) triazolo (4,3-b) pyridazine

Compound	Concentration (µg/ml)	Zone of Inhibition (in mm)			
		Gram positive		Gram negative	
		Staph. aureus	*M. luteus*	*E. coli*	*K.pneumoniae*
1e	50	-	-	-	-
	100	-	-	-	-
	200	10	-	9	-
1f	50	-	-	-	-
	100	7	5	6	8
	200	14	12	13	16
2a	50	-	-	-	-
	100	-	6	8	11
	200	-	14	13	15
2b	50	-	-	-	-
	100	11	9	13	9
	200	20	19	21	19
3a	50	-	-	-	-
	100	-	-	8	9
	200	19	-	18	18
3b	50	-	-	-	-
	100	8	10	9	11
	200	21	17	19	20
4a	50	-	-	-	-
	100	7	5	-	11
	200	13	12	-	17
4b	50	-	-	-	-
	100	8	7	10	6
	200	16	18	20	14
5a	50	-	-	-	-
	100	6	5	8	9
	200	13	12	18	17
5b	50	-	-	-	-
	100	9	-	-	7
	200	16	-	-	14
6a	50	-	-	-	-
	100	-	5	-	6
	200	10	9	-	14
6b	50	-	-	-	-
	100	5	4	7	5
	200	14	11	18	13
7a	50	-	-	-	-
	100	5	3	5	6
	200	11	9	10	13
7b	50	-	-	-	-
	100	6	7	8	-
	200	16	14	12	-
Ampicillin	50	23	21	25	21
Chloramphenicol	50	22	25	23	20

- shows no antibacterial activity.

Table 3 *Invitro* Antifungal activity of 3-substituted phenyl 6-substituted phenyl (1,2,4) triazolo (4,3-b) pyridazine

Compound	Concentration (µg/ml)	Zone of Inhibition (in mm)	
		C.albicans.	*C.neoformans*
1e	100	-	-
	250	-	8
	500	11	10
1f	100	-	-
	250	7	8
	500	16	17
2a	100	-	-
	250	8	9
	500	17	16
2b	100	-	-
	250	11	14
	500	19	18
3a	100	6	5
	250	10	9
	500	18	18
3b	100	3	4
	250	8	9
	500	18	19
4a	100	-	-
	250	8	5
	500	11	10
4b	100	3	-
	250	8	11
	500	13	17
5a	100	-	-
	250	12	13
	500	15	16
5b	100	8	8
	250	11	10
	500	21	20
6a	100	-	4
	250	11	9
	500	14	13
6b	100	-	-
	250	13	14
	500	19	20
7a	100	-	3
	250	6	7
	500	12	14
7b	100	-	-
	250	8	7
	500	16	14
Fluconazole	100	24	28
Grisieofulvin	100	23	26

- shows no antifungal activity.

CONCLUSION

From the above result, it is concluded that compound **2b, 3b** and **4b** are active against gram positive and gram negative bacteria. Compound **3a, 3b** and **5b** are potent antifungal drugs.

REFERENCES

[1] M Amir ., S Kumar ., *Ind J Het Chem*., **2004,**14., 51
[2] H, D, Boschelli.,D T Connor., A, D, Bornemeier., *J. Med. Chem*.**, 1802**,36., 993
[3]R Mehboob., T Ramalingam., P, B Satur., *Ind J Chem*., 1989,28B., 695
[4] M Tandon, J P, Berthwal, T , N, Bhalla., *Ind J Chem*.**, 1981**, 20B., 1017
[5] R, S Desai, S C Benner, *Ind J Chem*., **1998,** 37B., 61

[6] K Mogilaiah., Srinivasa., D Chowdary., R, B Rao., *Ind. J. Chem*.**, 2001,** 40B., 43
[7] B Talawar., R Desai ., S Somannavar., *Ind J Het Chem*., **1996,** 5., 215
[8] M Bhat ., S, A Khan., N Siddiqui., *Ind J Het Chem* ., **2005,** 14., 271
[9] M, S, Y Khan., S Drabu ., *Ind J Het Chem*., **2001,** 11., 119
[10] A, D Kumar., N Sangwan ., *Ind J Chem*, **1994,** 33B., 1043
[11] Nizamuddin., M Mishra ., M K Srivastva., *Ind. J Chem* **2001,** 40B., 49
[12] P S Upadhya., R N Vansdadia.,A J Baxi., *Ind. J Chem.*, **1990,** 29B., 793
[13] R, K Khare. , M, K Srivastava., H Sing ., *Ind J Chem*., **1995,** 34B., 828
[14] K Singh , N Tiwari ; Nizamuddin., *Ind J Chem*.**, 1993,** 32B., 1080
[15] S Jantova , S Letasiova ;.R Ovadekova and M Muckova *Neoplasm*; **2006,** 53(4); 291
[16] D Greenwood.,R C B Slack.,J F Peutherer., Microbiology, **1992,** 14th ed., p.1, Churchill and Livingstone, Spencerswood