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SYNTHESIS OF 9-BROMO-N-SUBSTITUTED- 6H- INDOLO [2, 3-b] QUINOXALINE-3-SULFONAMIDE DERIVATIVES CONTAINING QUINOXALINE MOIETY AS PROSPECTIVE ANTIMICROBIAL AGENTS

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ABSTRACT

O-phenylene diamine is reacted with 5-bromo Isatin and the resultant compound is reacted with various aromatic and aliphatic amines to form the 9-bromo-N-substituted- 6H- indolo [2,3-b] quinoxaline-3-sulfonamide derivatives of Quinoxaline (QXN 1 to QXN 12). All the compounds were structurally elucidated with physical and analytical methods. All the compounds were evaluated with anti-microbial activity against a variety of bacterial strains (both Gram *+ve* and Gram *–ve*) and fungal strains and some of these compounds have shown significant antibacterial and antifungal activities.

Key words: o-phenylene diamine, 5-bromo isatin, Quinoxaline derivatives, antimicrobial activity.

INTRODUCTION

Quinoxaline is an important nitrogen containing heterocyclic compound and has been considered as a wonder nucleus which posses almost all types of biological activities. This diversity in the biological response profile has attracted the attention of many researchers to explore this skeleton to its multiple potential against several activities [1]. Substituted quinoxalines are an important class of benzoheterocycles, which constitute the building blocks of wide range of pharmacologically active compounds having antibacterial [2], antifungal [3], anticancer [4], antitubercular [5], antileishmanial [6], antimalarial [7] and antidepressant activities [8] potent antithrombotic [9], anti-pain and anti-inflammatory activities [10- 11]. In addition, quinoxaline derivatives are reported for their application in dyes, efficient electroluminescent materials, organic semiconductors and DNA cleaving agents [12]. Keeping many points in view, it's worthwhile to design the synthesis of newer Quinoxaline derivative compounds, where in *o*-phenylene diamine is reacted with 5-bromo isatin and the resultant compound is reacted with various aromatic and aliphatic amines to form the title compounds (QXN 1 to QXN 12).

A walkthrough inspection of literature helped to develop innovative scheme which is so far not seen in any article which is described in detail in this manuscript. So it's our zeal to develop various quinoxaline derivatives from innovative procedure and finally evaluating them for Antimicrobial activity.

MATERIALS AND METHODS

All the melting points were taken in open capillary tube and are uncorrected. The purity of the compounds was checked routinely by TLC using silica gel coated plates and spots were visualized by exposing the dry plates in iodine vapours. IR spectra (υ_{max} in cm^{-1}) were recorded on FT-IR-Spercle Elmer

DHF1FT-IR using KBr technique. The ^{1}HNMR and ^{13}C NMR spectra of the compounds were carried out in Bruker AMX 400 MHz NMR instrument using $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal reference (chemical shifts in δ ppm). The mass spectra of the compounds were carried out in Agilent 1100 series LC-MSD.

Step 1: Synthesis of 9-bromo-6H-indolo [2, 3-b] quinoxaline (1): Solution of O-phenylenediamine (0.0015mol) in rectified spirit (12ml) is added to a warm solution of 5-Bromo Isatin (0.0015mol) in rectified spirit (12ml). The mixture is warmed for 30 mins in a water bath/ reflux the mixture for 30mins. Addition of water drop wise results slight cloudiness persistence. Resultant solution is cooled; separated product is filtered and crystallized from alcohol. The yield, melting point range, solubility, Rf value was found to be 67%, 110-115^{0}C, ethyl acetate, 0.87 respectively.

Step 2: Synthesis [13] of 9-bromo-6H-indolo [2,3-b] quinoxaline-3-sulfonyl chloride (2): To 9-bromo-6H-indolo [2,3-b] quinoxaline (0.01mol) was added chlorosulfonic acid (0.0015 mol) and the reaction mixture was refluxed for a period of 5hr. The mixture was poured slowly on ice-water mixture; white solid precipitated out was filtered, washed thoroughly with cold water to make it acid free and re-crystallized by using ethanol. .

Step 3: Synthesis [13] of 9-bromo-6H-indolo [2,3-b] quinoxaline-3-sulfonamide (3): Dissolve 0.05 mol of aromatic amine derivative in a mixture of 40ml anhydrous acetone and 6ml of dry pyridine. Add 0.05 mole of step1 product and reaction mixture is set aside overnight. Add water next day after which filtration should be done, recrystallized from acetone. The characterization data of the resultant title compounds is shown in **Table No.1**.

SPECTRAL DATA OF THE SYNTHESIZED DERIVATIVES:

QXN 01: IR(υ, in **KBr, cm^{-1}):**594.68(str, Ar-Br), 643.92(str, C-S), 1118.12(Sym, $S(=O)_2$), 1334.85(Asym, $S(=O)_2$), 1474.79-1603.38(str, C-N, C=N), (str1514.71(str, Ar, C=C), 1701(str, Ar, C=N), 3112.25(str, Ar, C-H), 3311.99 (str, NH).

^{1}H NMR(δ in $CDCl_3$, ppm): 3.508(s, 1H, NH), 7.256(d, 1H,Ar-H), 8.35(d, 1H, Ar-H), 8.556(d, 1H, Ar-H), 11.1(s, 1H, NH).

QXN 02: IR(υ, in **KBr, cm^{-1}):** 588.76(str, Ar-Br), 614.66(str, C-S), 1276.16(str, C-N), 1118.59(Sym, $S(=O)_2$), 1333.72(Asym, $S(=O)_2$), 1514.38(str, Ar, C=C), 1593.53 (str, Ar,C=N), 1706.28(C=O), 3308.73 (str, N-H).

.**^{1}H NMR(δ in $CDCl_3$, ppm):** 2.506(s, 3H, CH_3), 4.108(s, 1H, NH), 6.635(d, 1H, Ar-H), 6.693(d, 1H, Ar-H), 7.504(d, 1H, Ar-H), 7.798(s, 1H, Ar-H), 9.524(s, 1H, NH).

QXN 03: R(υ, in **KBr, cm^{-1}):** 591.62(str, C-Br), 644.85(str, C-S), 1279.54(str, C-N), 1118.58(Sym, $S(=O)_2$), 1323.10 (Asym, $S(=O)_2$), 1515.58(str, Ar, C=C), 1657.92 (str, Ar, C=N), 1708.02(str, C=O), 2926.35(str, CH_3), 3036.55(str, Ar-H), 3366.33(str, NH).

^{1}H NMR(δ in $CDCl_3$, ppm): 2.559(s, 3H, CH_3), 3.1812(s, 1H, NH), 6.855(d, 1H, Ar-H), 6.81(d, 1H, Ar-H), 7.317(d, 1H, Ar-H), 8.905(s, 1H, NH).

QXN 04: IR (υ, in **KBr, cm^{-1}):** 591.61(C-Br), 643.57(str, C-S), 1204.17(str, C-N),1117.51(Sym, S $(=O)_2$) 1382.08(Asym, $S(=O)_2$), 1513.73(str, C=C), 1651.65(str, C=N), 3383.55(str, N-H).

^{1}H NMR(δ in $CDCl_3$, ppm): 4.167(s, 1H, NH), 7.834(d, 1H, Ar-H), 8.300(d, 1H, Ar-H), 8.605(s, 1H, Ar-H), 10.737(s, 1H, N-H)

^{13}C NMR(δ in $CDCl_3$, ppm): 115.54(^{16}C), 121.96(^{28}C), 143.50(^{143}C), 151.42(^{6}C).

MS:*m/z 579.4(M+1)*

QXN 05: IR(υ, in **KBr, cm^{-1}):**595.17(Ar-Br), 644.77(C-S), 1053.94(Ar-Cl), 119.17(Ar-F), 1278.73(str, Ar, C-N),1119.17(Sym, $S(=O)_2$), 1383.30(Asym, $S(=O)_2$), 1516.61(str, Ar, C=C), 1655.30(str, Ar, C=N),3043.97(str, Ar-H) 3209.09(str, N-H),

^{1}H NMR (δ in $CDCl_3$, ppm):3.490(s, 1H, NH), 6.666(d, 1H, Ar-H), 7.260(d, 1H, Ar-H), 7.727(d, 1H, Ar-H), 7.820(d, 1H, Ar-H), 8.598(s, 1H, Ar-H), 11.221(s, 1H, N-H),)

QXN 06: IR(υ, in **KBr, cm^{-1}):**593(str, Ar-Br), 643(str, Ar, C-S), 1203.43(str, Ar, C-N), 1117.28(Sym, S(=O)2), 1383.70(Asym, $S(=O)_2$), 1514.69(str, Ar, C=C), 1652.66(str, C=N), 1704.67(str, C=O), 3013.46(str, Ar-H), 3212.16(str, NH)

^{1}H NMR(δ in $CDCl_3$, ppm): 3.463(s, 1H, NH), 6.673(d, 2H, Ar-H), 7.260(d, 1H, Ar-H), , 7.814(d, 1H, Ar-H), 8.112(s, 1H, Ar-H), 11.022(s, 1H, COOH).

QXN 07: IR(υ, in **KBr, cm^{-1}):** 641.65(str, Ar-Br), 695.63(str, C-S), 1052.92(str, Ar-Cl), 1205.65 (Str, Ar, C-N), 1144.03(sym, $S(=O)_2$), 1316.11(Asym, $S(=O)_2$), 1460.75(str, Ar-C=C), 1655.28(str, Ar-C=N), 1702.19(str, C=O), 3305.69(str, NH).

^{1}H NMR(δ in $CDCl_3$, ppm: 3.531(s, 1H, NH), 7.218(d, 1H, Ar-H), 7.239(s, 1H, Ar-H), 7.0405(m, 1H, Ar-H), 7.455(m, 2H, Ar-H), 7.472(s, 1H, Ar-H), 7.531(m, 1H, Ar-H), 7.620(s, 1H, Ar-H), 9.959(s, 1H, NH).

QXN 08: IR(υ, in **KBr, cm^{-1}):** 595.33(Ar-Br), 643.19(str, C-S), 1205.45(str, Ar, C-N),

1118.33(sym, $S(=O)_{2)}$, 1389.16(Asym, $S(=O)_2$), 1518.65(str, Ar, C=C), 1657.24(str, Ar, C=N), 3116.93(str, Ar-H), 3350.23(str, NH), 3452.32(str, NH).

^{1}H NMR(δ in $CDCl_3$, ppm): 3.494(s, 1H, NH), 7.339(d, 1H, Ar-H, 7.508(d, 1H, Ar-H), 7.837(d, 1H, Ar-H), 8.319(s, 1H, Ar-H), 10.807(s, 1H, NH),

QXN 09: IR(υ, in **KBr, cm^{-1}):**595.17(Ar-Br), 643.56(str, C-S), 1207.27(str, Ar, C-N),1119.94(sym, $S(=O)_{2)}$, 1333.19(Asym, $S(=O)_2$), 1516.35(str, Ar, C=C), 1657.35 (str, Ar, C=N), 3018.73(str, Ar-H), 3368.50(str, NH).

^{1}H NMR(δ in $CDCl_3$, ppm):3.493(s, 1H, NH), 6.692(t, 1H, Ar-H), 7.260(m, 2H, Ar-H), 7.316(t, 2H, Ar-H), 7.816(d, 1H, Ar-H), 8.577(s, 1H, Ar-H), 10.884(s, 1H, NH).

QXN 10: IR(υ, in **KBr, cm^{-1}):**643.56(str, C-S), 1204.08(str, Ar, C-N),1113.93(sym, $S(=O)_{2)}$, 1331.77(Asym, $S(=O)_2$), 1512.45(str, Ar, C=C), 1552.33(str, C=S), 1652.15 (str, Ar, C=N), 3345.23(str, NH), 3412.58(str, NH).

^{1}H NMR(δ in $CDCl_3$, ppm):2.017(d, 4H, NH), 3.491(s, 1H, NH), 7.243(d, 1H, Ar-H), 7.311(s, 1H, C-H), 7.339(d, 1H, C-H), 8.553(s, 2H,NH), 8.598(S, 1H, C-H), 10.015(s, NH).

QXN 11: IR(υ, in **KBr, cm^{-1}):** 593.39(Ar-Br), 642.79(str, C-S), 1204.43(Str, Ar, C-N), 1118.23(sym, S(=O)2), 1384.22(Asym, $S(=O)_2$), 1513.31(str, Ar, C=C), 1551.90(str, C=S) 1651.03(str, Ar, C=N), 3014.12(str, Ar-H),3295.65(str, NH), 3416.54(str, NH).

^{1}H NMR(δ in $CDCl_3$, ppm):3.493(s, 1H, NH), 5.793(s, 1H, NH), 6.694(d, 1H, NH), 7.429(d, 1H, Ar-H), 7.817(s, 1H, C-H), 8.090(d, 1H, C-H), 8.302(s, 2H, NH), 8.468(d, 1H, Ar-H), 10.689(s, 1H, NH).

QXN 12: IR(υ, in **KBr, cm^{-1}):** 578.65(Ar-Br), 690.40(str, C-S), 1258.99(str, Ar, C-N), 1114.71(sym, S(=O)2), 1335.56(Asym, S(=O)2), 1509.93(str, Ar, C=C), 1606.07(str, Imine, C=N), 3013.16(str, Ar-H), 3101.25(str, N-H), 3409.64(str, N-H).

^{1}H NMR (δ in $CDCl_3$, ppm):3.493(s, 1H, NH), 6.692(d, 1H, Ar-H), 7.314(d, 1H, Ar-H), 7.406(d, 1H, Ar-H), 7.503(d, 2H, Ar-H), 8.321(s, 2H, NH), 10.061 (s, 1H, NH)

ANTIBACTERIAL ACTIVITY

The *invitro* antibacterial screening of all the Quinoxaline derivatives were evaluated against Gram- positive organisms *Staphylococcus aureus* (NCIM 2079), *Bacillus pimilis* (NCIM 2063) and Gram- negative organism *Escherichia coli* (MTCC 443) by cup and plate method [14]. The culture was maintained on nutrient agar slants. Twenty milliliters of sterilized nutrient agar medium was inoculated with the bacteria and spread in a petridish and allowed to set for 30 min. Four bores (10 mm in diameter) were made at equal distance in the petridish and filled with a single standard concentration (50 µg/ml) of standard drug Streptomycin and different concentrations (50 and 100 µg/ml) of Quinoxaline derivatives were introduced. Chloroform was used as control. After introduction of standard drug and derivatives, the plates were placed in a refrigerator at 8-10^{0}C for proper diffusion into media. After two hrs of cold incubation, the petri plates are transferred to incubator and maintained at 37^0 for 24 hrs. After the incubation period, the petri plates were observed for zone of inhibition by using vernier scale. The results evaluated by comparing the zones of inhibition shown by the derivatives with standard drug.

ANTIFUNGAL ACTIVITY

Aspergillus niger (MTCC 277) and *Candida albicans* (MTCC 227) were employed for testing antifungal activity using the cup-plate method [14]. Miconazole nitrate is used as standard and chloroform is used as control.

RESULTS AND DISCUSSION

The *in vitro* antibacterial activity data of the synthesized Quinoxaline derivatives against tested organisms displayed significant activity. The compounds QXN 5 has shown significant activity against *Gram +ve* organisms *S. aureus* and *B. pimilis.* The compound QXN 10 has shown good activity on *Gram +ve S. aureus*. The compounds QXN 6 and QXN 12 have shown good activity on *Gram –ve E. coli.* The compounds QXN 4 and QXN 11 have shown remarkable activity on both *Gram +ve* and *Gram –ve* organisms which are taken. Remaining compounds have shown moderate or less activity. All the compounds were compared to the standard Streptomycin. Chloroform was taken as control. The zones of inhibition of the compounds on the bacteria were shown in **Table No.2.**

A total of 12 synthesized Quinoxaline derivatives were evaluated for Antifungal activity with cup plate method at concentrations of 50 and 100 µg/ml. The compounds QXN 5 and QXN 11 have shown moderate activity against *Aspergillus niger.* QXN 4 and QXN 10 are effective against both *Aspergillus Niger* and *Pencillum notatum*. Remaining compounds were found to show minimal or no activity against fungi. Miconazole nitrate was taken as standard. Chloroform was taken as control. The zones of inhibition of the compounds on the bacteria were shown in **Table No.3.**

CONCLUSION

9-bromo-N-substituted- 6H- indolo [2, 3-b] quinoxaline-3-sulfonamide derivatives containing Quinoxaline moiety were synthesized and characterized for their structure elucidation. Antibacterial and antifungal studies of these compounds indicated that the compounds were found to be showing comparable activity against some bacteria compared to standard antibiotic drugs.

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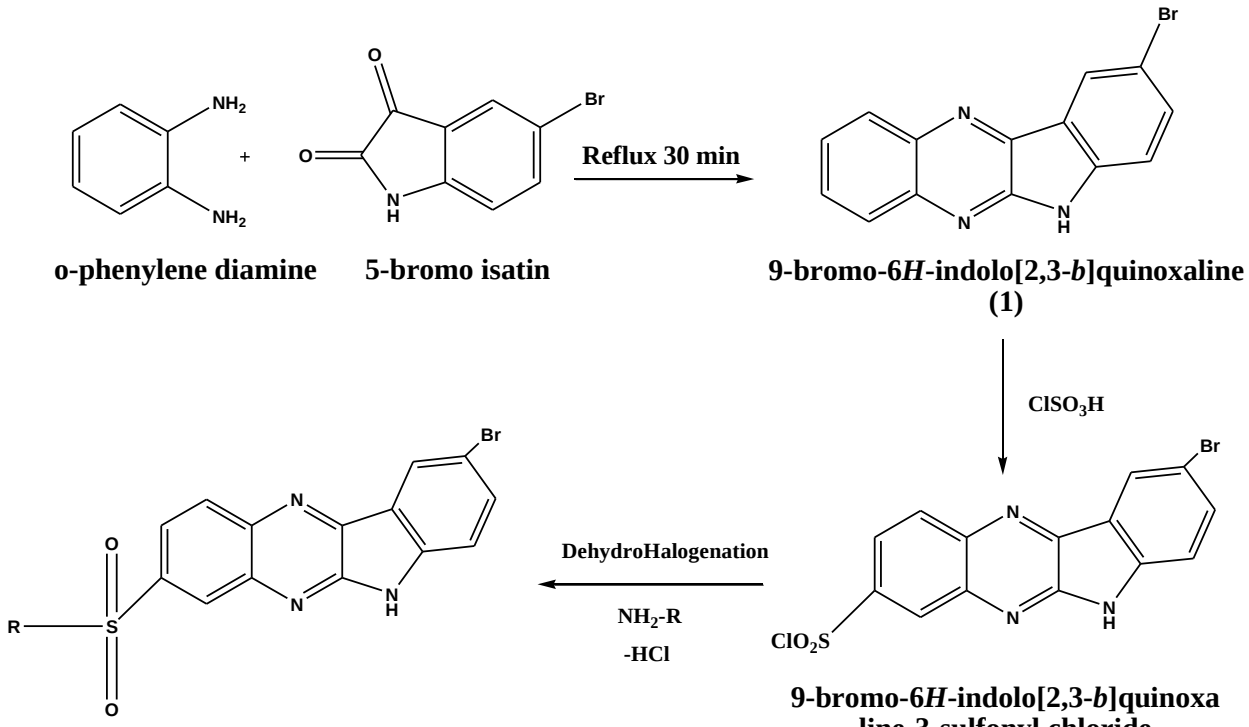
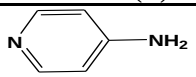
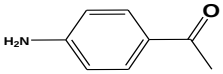
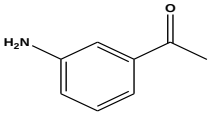
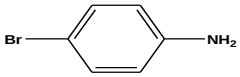


o-phenylene diamine **5-bromo isatin** **9-bromo-6*H*-indolo[2,3-*b*]quinoxaline (1)**

9-bromo-6*H*-indolo[2,3-*b*]quinoxaline-3-sulfonyl chloride

9-bromo-*N*-substituted-6*H*-indolo[2,3-*b*] quinoxaline-3-sulfonamide (QXN 01- QXN 12)

SCHEME 1: Synthetic route to the title compounds

S. No	Comp. code	Attachment (R)
1	QXN 01	
2	QXN 02	
3	QXN 03	
4	QXN 04	

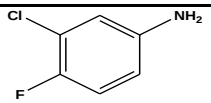
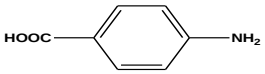
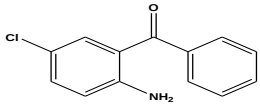
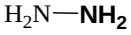
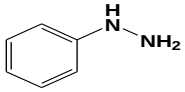
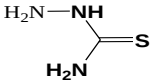
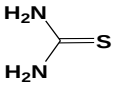
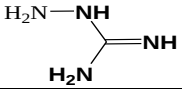
5	QXN 05	
6	QXN 06	
7	QXN 07	
8	QXN 08	
9	QXN 09	
10	QXN 10	
11	QXN 11	
12	QXN 12	

Table No. 1: Characterization data of synthesized derivatives compounds

Comp. Code	Molecular Formula	Mol. Wt	Melting Point ^{O}C	% Yield (w/w)	Rf Value*
QXN 01	$C_{19}H_{12}BrN_5O_2S$	454.30	220	39	0.86
QXN 02	$C_{22}H_{15}BrN_4O_3S$	495.35	180	37	0.8
QXN 03	$C_{22}H_{15}BrN_4O_3S$	495.35	180	31	0.8
QXN 04	$C_20H_{12}Br_2N_4O_2S$	532.21	200	20	0.82
QXN 05	$C_{20}H_{11}BrClFN_4O_2S$	505.75	225	47.5	0.86
QXN 06	$C_{21}H_{13}BrN_4O_4S$	497.32	225	38	0.87
QXN 07	$C_{27}H_{16}BrClN_4O_3S$	591.86	110	40	0.84
QXN 08	$C_{14}H_{10}BrN_5O_2S$	392.23	260	36.4	0.85
QXN 09	$C_{20}H_{14}BrN_5O_2S$	468.33	235	42.7	0.81
QXN 10	$C_{15}H_{11}BrN_6O_2S_2$	451.32	165	47	0.84
QXN 11	$C_{15}H_{10}BrN_5O_2S_2$	436.31	235	32	0.78
QXN 12	$C_{15}H_{12}BrN_7O_2S$	434.27	240	32	0.82

**Mobile phase: Ethyl acetate: Benzene (7:3)* *Recrystallization solvent: Ethyl acetate*

Table No.2: Zone of inhibition obtained on bacteria

Comp. Code	Gram +*ive*				Gram -*ive*	
	S.aureus		*B. Pimilis*		*E.Coli*	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
QXN 01	10	11	_	_	10	13
QXN 02	_	_	_	10	_	_
QXN 03	_	_	11	12	_	_
QXN 04	14*	16*	14*	16*	16*	18*
QXN 05	15*	17*	17*	18*	11	12
QXN 06	_	11	_	10	14*	16*
QXN 07	10	12	_	_	_	_
QXN 08	11	12	_	10	_	10
QXN 09	_	12	_	_	_	_
QXN 10	14*	16*	10	11	_	_
QXN 11	13	15*	14*	15*	15*	17*
QXN 12	10	11	_	12	16*	18*
Chloroform	_		_		_	
Streptomycin (100 µg/ml)	18		20		20	

Note: (-) not active and (*) significant zone of inhibition Bore size: 10mm

Table No. 3: Zone of inhibition on fungi

Compound Code	*Aspergillus niger*		*Penicillum notatum*	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
QXN 01	_	_	15*	17*

QXN 02	10	11	11	13
QXN 03	10	11	_	11
QXN 04	17*	21*	17*	19*
QXN 05	14*	15*	11	13
QXN 06	_	10	_	10
QXN 07	10	11	12	13
QXN 08	_	10	_	11
QXN 09	_	10	_	11
QXN 10	17*	18*	16*	19*
QXN 11	12	14*	_	10
QXN 12	10	12	10	11
Chloroform	_	_	_	_
Miconazole Nitrate(50 µg/ml)	23		20	

Note: (-) not active and (*) significant zone of inhibition Bore size: 10mm

REFERENCE

1. Ashutosh kumar Patidar, International Journal of PharmTech Research CODEN (USA): IJPRIF ISSN: 0974-4304 Vol. 3, No.1, 386-392, Jan-Mar 2011.
2. Badran M.M., Abouzid K.A.M. and Hussein M.H.M., Part II. Arch. Pharm. Res., 2003, 26, 107–113
3. Tandon V.K., Yadav D.B., Maurya H.K., Chaturvedi A.K. and Shukla P.K., Bioorg. Med. Chem., 2006,14,6120-1626
4. Sanna P., Carta A., Loriga M., Zanetti S., and Sechi L. Part 2: II ,Farmaco., 1999,54,161-168
5. Jaso A., Zarranz B., Aldana I., and Monge A., Eur. J. Med. Chem., 2003,38,791–800
6. Guillon J., Forfar I., Matsuda M.M., Desplat V., Saliege M. and Thiolat D., Bioorg. Med. Chem. Lett., 2007,15,194-210
7. Rangisetty J.B., Gupta C.N.V.H.B., Prasad A.L., Srinivas P., Sridhar N., Parimoo P. and Veeranjaneyulu A., J. Pharmacy and Pharmacology.,2001,53,1409–1413
8. Sarges R., Howard H.R., Browne R.G., Lebel L.A., Seymour P.A. and Koe B.K, J. Med. Chem.,1990,33,2240- 2254
9. Ries U.J., Priekpe H.W., Havel N.H., Handschuh S., Mihm G., Stassen J.M., Wienen W., and Nar H., Bioorg. Med. Chem. Lett., 2003,13,2297-2302
10. Wendt G.R. and Ledig K.W., U.S. Patent, 3,431,262, Chem. Abstr.,1969,70,106512
11. Su D.S. and Bock M.G., US Patent. Appl., 2005, (20050020591)
12. Srinivas C., Kumar C.N.S.P., Rao V.J. and Palaniappan S., Catal. Lett., 2008, 121,291- 296
13. Pratap Y. Pawar, , Asian Journal of Chemisttry vol.19, No.2 (2007), 1473-1481
14. Dr.Dushyanth "Text Book of Pharmaceutical Microbiology" B.S Shah Prakashan Publications, Ahamedabad, India, 2004, 106-132