ISSN: 2277- 7695
CODEN Code: PIHNBQ
ZDB-Number: 2663038-2
IC Journal No: 7725

ISSN 2277-7695

Vol. 1 No. 9 2012
Online Available at www.thepharmajournal.com

THE PHARMA INNOVATION

Synthesis, Characterization and Evaluation For Antimicrobial Activity Of 2-Substituted Benzimidazole Derivatives

Kuldeep Kumar[1]*, Prof.(Dr). D. P. Pathak[1]

1. Department of Pharmaceutical Chemistry, Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), Sector-3, Pushp Vihar, New Delhi-110017, India

Some new 2-substituted benzimidazole derivatives were synthesized from microwave irradiation method by condensation of 2-nitroaniline with different carboxylic acids (aliphatic,aromatic and hetrocyclic).The compounds synthesized were identified by 1HNMR, and FT-IR spectroscopic techniques. All compounds studied in this work were screened for their in vitro antimicrobial activities against the standard strains: Escherichia coli, pseudomonas aeruginosa, Bacillus subtilis, bacillus pumilus, Candida albicans, Aspergillus niger.The diameter(in mm) of zone of inhibition was determined by agar well diffusion method. Compound 2-Pyridin-3-yl-1H-benzimidazole (1f) was found to be the most active antimicrobial compound amongst the series. Compounds 2-(2-Chloro-4-nitro-phenyl)-1H-benzimidazole (1a), 2-(1H-Benzimidazol-2-yl)-6-nitro-benzoic acid (1e) also showed good antimicrobial activity.

Keyword: Benzimidazoles, Microwave, Antimicrobial Activity

INTRODUCTION: Benzimidazoles are very useful intermediates/subunits for the development of molecules of pharmaceutical or biological interest.

Corresponding Author's Contact information:
Kuldeep Kumar*
Department of Pharmaceutical Chemistry, Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), Sector-3, Pushp Vihar, New Delhi, India
E-mail: Kkr036@gmail.com
Mob: +91-9911443726

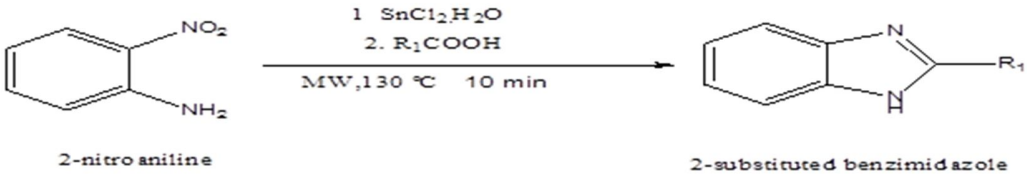
Substituted benzimidazole derivatives have found applications in diverse therapeutic areas including antimicrobial, antihelminthic, anticancer , antibacterial, anticonvulsant, anti-inflammatory, antiarrythmic, antimycobacterial, antioxidant, antiulcer, androgen receptor antagonist, antiprotozoal, antitumour, antiviral, anti hypertensives, antihistaminics, cysticidal, antifolate, antifungal, antiserotonin, nematicidal, radioprotective activity to name just a few.[1-27] The widespread interest in benzimidazole containing structures has promted extensive studies for their synthesis. There are two general methods for the synthesis of 2-substituted

benzimidazoles. One is the coupling of 2-nitroaniline/phenylenediamine with carboxylic acids[28] at high temperature conditions usually 190-195˚C using HCl, PPA (polyphosphoric acid), $SnCl_2.H_2O$ or the use of microwave irradiation[29].The other way involves a two-step procedure that includes the oxidative cyclo-dehydrogenation of aniline Schiff, bases. Partially due to the availability of a vast number of carboxylic acids, the first method has been extensively used.

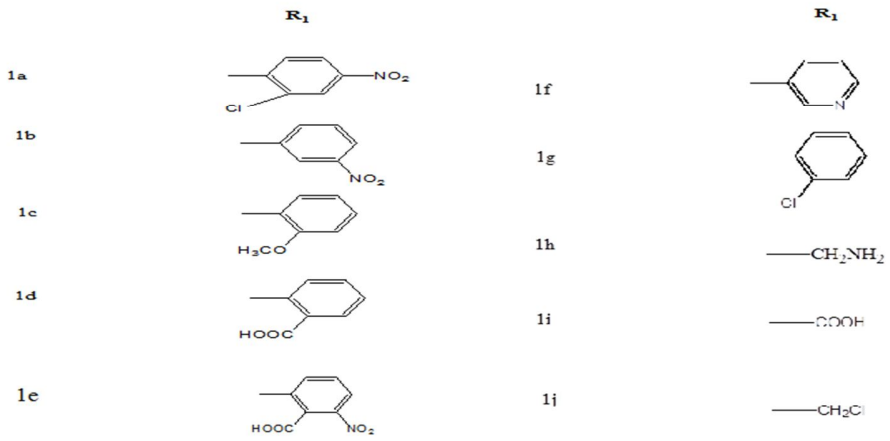
Experimental

Melting point ranges of newly synthesized compounds were determined by open glass capillary tube using Visual melting point apparatus and were uncorrected. Commercially available reagent grade chemicals were used as received. All reactions were followed by TLC, with detection by UV light and/or spots were visualised by exposure to iodine vapours.IR spectra were recorded as thin films in KBr pellets with a Nicolet spectrophotometer.1HNMR spectra were recorded on a Brucker in DMSO-d6. Chemical shift values are reported in ppm relative to $SiMe_4$ (Tetra methyl silane) as internal reference, unless otherwise stated; s (singlet), d(doublet), t(triplet), m (multiplet) ; J in hertz.

Scheme :-



2-nitro aniline — 2-substituted benzimidazole



Method of synthesis

Method : Preparation of 2- Substituted benzimidazole derivatives (Scheme):

In a CEM Microwave vial with a stir bar(optional), the 2-nitroaniline(0.36mol), carboxylic acid(0.35 mol),and $Sncl_2.2H_2O$(3.0mol)were combined and heated at130˚C for 10 minutes .After completion of reaction the solution was cooled at room temperature, then it was poured in to the crushed ice. Then aqueous sodium hydroxide sol^n (50%) was added dropwise to neutralize the reaction mixture and the resulting solid was filtered, washed with cold water, dried and recrystallized in ethyl alcohol.TLC examination was done by using solvent TEF(5:4:1) which gave single spot.

2-(2-Chloro-4-nitro-phenyl)-1H-benzimidazole ***(1a)***

The compound was obtained as orange solid; Yield 75%; M.P 310-312˚C

IR spectral data *(γ cm -1 ,KBr):* 3414 N-H Stretching *sec.* amine , 1573,1511(C=C stretching), 3048 C-H stretching (aromatic), 1511,1378 N-O stretching, 771(C-Cl group), 1640(C=N stretching), 1343(C-N amine *) 1 H NMR (DMSO d6, 300 MHz)* δ **:** 7.2-8.3(m,7H,Ar-H), 4.8(s,1H,NH)

2-(3-Nitro-phenyl)-1H-benzimidazole ***(1b)***
The compound was obtained as yellow solid; Yield 71%; M.P 260-262˚C

IR spectral data *(γ cm -1 , KBr)*: 3346(N-H Stretching sec. amine) , 1428,1530 (C=C stretching), 3048 C-H stretching (aromatic), 1530,1385(NO stretching), 1626 (C=N stretching), 1348(C-N amine) **1 H NMR** *(DMSO d6, 300 MHz) δ :* 7.2-8.4(m,8H,Ar-H), 4.7(s,1H,NH)

2-(2-Methoxy-phenyl)-1H-benzimidazole ***(1c)***
The compound was obtained as pale yellow; Yield 74%; M.P 255-257˚C
IR spectral data (γ cm $^{-1}$, KBr): 3372(N-H Stretching *sec.* amine) , 1597,1463(C=C stretching), 2947(C-H stretching aromatic), 1671(C=N stretching), 1256(C-N amine) **^{1} H NMR (DMSO d_6, 300 MHz)** δ **:** 7.0-7.8(m,8H,Ar-H), 4.9(s,1H,NH) ,3.9(s,3H,OCH_3 (methoxy group)

2-(1H-Benzimidazol-2-yl)-benzoic acid ***(1d)***
The compound was obtained as off white solid; Yield 75%; M.P 283-285˚C
IR spectral data (γ cm $^{-1}$, KBr): 3496(N-H Stretching *sec.* amine) , 1592,1455(C=C stretching), 3023(C-H stretching aromatic), 1624(C=N stretching), 1267(C-N amine), 1712(C=O stretching),2857(OH group) **^{1} H NMR (DMSO d_6, 300 MHz)** δ **:** 7.2-8.1(m,8H,Ar-H), 4.8(s,1H,NH), 11.9(s,1H,COOH)

2-(1H-Benzimidazol-2-yl)-6-nitro-benzoic acid ***(1e)***
The compound was obtained as off white solid; Yield 70%; M.P 302-304˚C

IR spectral data (γ cm $^{-1}$,KBr): 3593(N-H Stretching *sec.* amine) , 1586,1535(C=C stretching), 3087(C-H stretching aromatic), 1535,1348 NO stretching, 1586(C=N stretching), 1348(C-N amine),1686(C=O stretching),3087(OH group) **^{1} H NMR (DMSO d_6, 300 MHz)** δ **:** 7.2-8.3(m,7H,Ar-H), 3.9(s,1H,NH), 8.4(s,1H,COOH)

2-Pyridin-3-yl-1H-benzimidazole **(1f)**
The compound was obtained as yellow solid; Yield 79%; M.P 215-217˚C
IR spectral data (γ cm $^{-1}$,KBr): 3384(N-H Stretching *sec.* amine) , 1597,1457(C=C stretching), 3030(C-H stretching aromatic), 1632(C=N stretching), 1271(C-N amine) **^{1} H NMR (DMSO d_6, 300 MHz)** δ **:** 7.2-9.1(m,7H,Ar-H), 4.2(s,1H,NH),

2-(2-Chloro-phenyl)-1H-benzimidazole ***(1g)***
The compound was obtained as yellow solid; Yield 80%; M.P 208-210˚C
IR spectral data (γ cm $^{-1}$,KBr): 3385(N-H Stretching *sec.* amine),1497,1402(C=C stretching), 3288(C-H stretching aromatic),1587(C=N stretching), 1272(C-N amine),746(C-Cl stretching) **^{1} H NMR (DMSO d_6, 300 MHz)** δ **:** 7.2-7.9(m,8H,Ar-H), 4.3(s,1H,NH),

C-(1H-Benzimidazol-2-yl)-methylamine ***(1h)***
The compound was obtained as yellow solid; Yield 68%; M.P 222-225˚C
IR spectral data (γ cm $^{-1}$,KBr): 3386(N-H Stretching *sec.* amine) , 1591,1502(C=C stretching), 3189(C-H stretching aromatic), 1633(C=N stretching), 1273(C-N amine), **^{1} H NMR (DMSO d_6, 300 MHz)** δ **:** 7.2-7.7(m,4H,Ar-H), 4.6(s,1H,NH), 3.8(2H,CH_2 methylene), 2.5(2H,NH_2)

1H-Benzimidazole-2-carboxylic acid ***(1i)***
The compound was obtained as white solid; Yield 72%; M.P 162-165˚C
IR spectral data (γ cm $^{-1}$,KBr): 3446(N-H Stretching *sec.* amine) , 1501,1419(C=C stretching), 3048(C-H stretching aromatic),

1614(C=N stretching), 1248(C-N amine),1681(C=O stretching),2881(OH group) **^{1} H NMR (DMSO d_6, 300 MHz)** δ **:** 7.2-7.5(m,4H,Ar-H), 4.9(s,1H,NH), 10.7(s,1H,COOH)

2-Chloromethyl-1H-benzimidazole ***(1j)***
The compound was obtained as white solid; Yield 70%; M.P 188-190◦C
IR spectral data (γ cm $^{-1}$,KBr): 2945(N-H Stretching *sec*. amine) , 1586,1440(C=C stretching), 2845(C-H stretching aromatic), 1640(C=N stretching), 1313(C-N amine),740(C-Cl group) **^{1} H NMR (DMSO d_6, 300 MHz)** δ **:** 7.2-7.6(m,4H,Ar-H), 4.9(s,1H,NH), 4.8(s, 2H CH_2)

ANTIMICROBIAL ACTIVITY

The antimicrobial activities of the compounds were investigated against bacterial strains i.e, Escherichia coli, Pseudomonas aeruginosa , Bacillus pumilus, Bacillus subtilis, and fungal strains i.e, Aspergillus niger, Candida albicans using agar well diffusion method.(Table 1) Ciprofloxacin and Fluconazole were used as standard drug for antibacterial and antifungal studies respectively. Nutrient broth (20g of the powder was dissolved in 1000ml of distilled water) was employed as culture media for antibacterial studies. For antifungal studies Sabouraud dextrose agar medium (Hi Media) was used as culture media. The sterilization of the culture medias , petridishes and other glassware was done by autoclaving at 15 lbs (121^{O}C) for 15 min for antibacterial studies, incubation was carried out at 37^{O}C for 24 hours. For antifungal studies incubation was carried out at at 35^{O}C for 48 hours. The cell density of each inoculums was adjusted in a concentration of approximately10^5 CFU ml^{-1}.During antimicrobial evaluation the medium after sterilization was poured into sterile petridishes under asceptic conditions in a laminar flow chamber. When the medium in the plate solidified, 0.1 ml of inoculums (of 104 to 106 CFU / ml population prepared from standardized culture, adjusted with peptone water) was spread on the agar plate by spread plate technique. Using flamed sterile borer the medium was bored . Accurately measured (0.1 ml) solution of each test sample and standard samples were added to the cups with a micropipette. The test solutions of synthesized compounds were prepared in DMSO at concentrations of 2.5, 5 and 10mg /ml. Ciprofloxacin and Fluconazole was used as standard and dissolved in DMSO to get a final concentration of 5µg /ml. DMSO(0.1ml) was used as solvent control. Inhibition zones were measured and the diameter was calculated in millimetres.

RESULTS AND DISCUSSION

The structures of the compounds were elucidated on the basis of FT-IR, ^{1}HNMR.All the final compounds have strong absorption between 3000-3100 cm^{-1} which is evidence for the presence of aromatic C-H bonds. Presence of aromatic C=C bonds was confirmed by the presence of medium weak multiple absorption band between 1400-1600 cm^{-1}.IR data also confirms the presence of specific functional groups present in the final synthesized compounds. The chemical shift of all other carbons of final compounds was seen as expected.

Out of all ten compounds evaluated for antimicrobial studies, compound no. **1f (*2-Pyridin-3-yl-1H-benzimidazole)*** showed appreciable antimicrobial activity against all six microbial strains used (Zone of inhibition in agar well diffusion method- 22 mm against Escherichia coli, 20 mm against pseudomonas aeruginosa, 20 mm against Bacillus subtilis, 19 mm against bacillus pumilus, 22 mm against Candida albicans, 20 mm against Aspergillus niger).

Table1. Antimicrobial activity of the synthesized compounds using agar well diffusion method
Zone of inhibition (in mm)

Compound		*E.coli*	*P.aeruginosa*	*B.subtilis*	*B.pumilus*	*C.albicans*	*A.niger*
	C1	11	NZ	NZ	12	18	12
	C2	17	18	19	18	20	21
1a	C3	NZ	18	21	18	18	17
	C1	NZ	NZ	10	NZ	16	12
	C2	23	12	13	12	18	16
1b	C3	20	12	10	10	13	14
	C1	10	11	NZ	NZ	15	12
	C2	20	16	15	12	20	15
1c	C3	14	12	21	NZ	18	11
	C1	NZ	10	NZ	NZ	18	14
	C2	14	16	20	18	20	18
1d	C3	NZ	12	16	NZ	20	18
	C1	11	14	10	NZ	16	17
	C2	18	16	16	18	19	18
1e	C3	18	NZ	12	12	17	16
	C1	NZ	NZ	NZ	NZ	15	NZ
	C2	22	20	20	19	22	20
1f	C3	12	NZ	14	16	NZ	12
	C1	10	11	NZ	NZ	12	10
	C2	15	17	20	14	15	16
1g	C3	12	16	22	12	11	12
	C1	14	12	NZ	NZ	18	12
	C2	21	18	20	12	10	18
1h	C3	14	14	12	16	15	18
	C1	NZ	12	NZ	NZ	16	NZ
	C2	20	21	12	11	16	14
1i	C3	NZ	12	16	18	11	10
	C1	14	NZ	12	12	14	NZ
	C2	20	18	20	16	12	12
1j	C3	14	16	12	14	17	11
Std.		23	20	22	21	23	22
DMSO		NZ	NZ	NZ	NZ	NZ	NZ

CONCLUSION

A series of 2-substituted benzimidazole derivatives were synthesized and evaluated for their antimicrobial activity against ciprofloxacin (for antibacterial activity) and fluconazole (for anti fungal activity).In the present studies ,Compound *2-Pyridin-3-yl-1H-benzimidazole* **(1f)** was found to be the most active antimicrobial compound amongst other in the series. These new data might be helpful in the future development of benzimidazole analogues as novel antimicrobial agent.

REFERENCE:

1. Tuncbilek M., Kiper T. and Altanlar N. 'Syntheis and in vitro antimicrobial activity of some novel substituted benzimidazole derivatives having potent activity against MRSA', *Eur. J. Med. Chem.*,**2009** Vol. 44, pp. 1024-1033.

2. Himaja M., Rajiv and Ramana M.V. 'synthesis of 6-Nitrobenzimidazol-1-acetyl amino acids and peptides as potent anthelmintic agents', *Indian J. Heterocycl. Chem*., **2002**, Vol. 12, pp. 121-124.
3. Chassaing C. And Berger M. ' Highly water soluble prodrug of Anthelmintic Benzimidazole Carbamates : Synthesis, Pharmacodynamic and Pharmacokinetics', *J. Med. Chem*., **2008** ,Vol. 51, pp. 1111-1114
4. Gellis A., Kovacic H., Boufatah N., and Vanelle P. 'Synthesis and cytotoxicity evaluation of some Benzimidazole-47-diones as bio reductive anticancer agents', *Eur. J. Med. Chem*., **2008**, Vol.43, pp. 1858-1864.
5. Andrzejewska M., Mulia L.Y., Rivera C.R., Tapia A., Vilpo L., Vilpo J. and Kazimierczuk Z. ' Synthesis, antiprotozoal and anticancer activity of substituted 2-trifluoromethyl and 2-pentafluoroethyl benzimidazoles', *Eur. J. Med. Chem*., **2002** ,Vol. 37, pp. 973-978.
6. Gupta S. K., Imran M., Rehman Z. U. and Khan S. A. 'Synthesis and biological evaluation of some 2-substituted benzimidazoles' *Asian J. Chem*., **2005**, Vol. 17 , No. 4 , pp. 2741-2747
7. Rajanarendar E., Ramu K., Reddy A. S. R. and Shaik F. P. 'A series of 2/3 (1*H*-benzimidazole-2-yl)-N-(5-methyl-3-isooxazolyl)-benzamide, acrylamide and propianomides' *Indian J. Chem*,.**2008**, Vol. 47B, pp. 1284-1290.
8. Sharma V.P 'Synthesis of 3-(2-benzimidazolyl) chromones' *Indian J. Heterocycl. Chem*., **2003**, Vol. 13, pp. 171-172.
9. Anisimova V.A., Spasov A.A., Stepanv A. V., Arkova N. V. and Jakovlev D.S. 'Synthesis and pharmacological activity of N-aryloxethyl derivatives of 9-*H*-2,3-Dihydroimidazo and 10-*H*-2,310-tetrahydropyrimido[1,2-a] benzimidazoles' *Pharm. Chem. J*., **2006**, Vol. 40 , No. 9, pp. 485-488
10. Kazimeirczuk V., Koci J. and Pour N. 'Synthesis and preliminary evaluation of benzimidazole derivatives as antimycobacterial agents', *Eur. J. Med. Chem*., **2005**, Vol. 37, pp. 409-418.
11. Dianov V.M. 'Synthesis and antioxidant properties of 3-methylsubstituted thiazolo [3,2-a] benzimidazole', *Pharm. Chem. J*.,**2007**, Vol. 41, pp. 308-309.
12. Anisimova V.A., Spasov A.A., Kosolapov V.A., Chernikov M.V., Stukovina A.Y., Larionov N.P., Libinzon R.E., and Vatolkina O.E. 'synthesis and biological activity of 9-dialkylaminoethyl-2-oxy(dioxy)-phenylimidazo[1.2a]benzimidazoles', *Pharm. Chem. J*.,**2006**, Vol. 40, No. 5,pp. 261-267.
13. Bariwal J.B. and Shah A.K., 'Synthesis and antiulcer activity of novel pyrimidylthiomethyl- and pyrimidylsulfinylmethyl benzimidazoles as potential reversible proton pump inhibitors', *Indian J. Pharm. Educ. Res*., **2008**, Vol. 42, pp. 225-231.
14. Dubey P.K., Naidu A., Reddy P.V.V.P., Kumar N.D.M and Vineel V.G. 'Studies on synthesis of unsymmetrical 2,2'-Bisbenzimidazole sulphides of pharmacological interest', *Indian J. Chem*,**2008**, Vol. 47B, pp.1443-1446.
15. Ng. R.A., Guan J., Alford V.C., Lundeen S. G. and Sui Z. 'Synthesis and SAR of potent and selective androgen receptor antagonists: 5, 6-dichlorobenzimidazole derivatives', *Bioorg. Med. Chem. Lett*.**, 2007**, Vol. 17, pp. 784-788.
16. Gomez H.T., Nunez E.H., Rivera I.L., Alverez J.G., Rivera R.C., Puc R.M., and Ramos R.A. 'Design, synthesis and in vitro anti protozoal activity of benzimidazole-pentamidine hybrids', *Bioorg. Med. Chem. Lett*., **2008**, Vol. 18, pp. 3147-3151.

17. Suthakaran R., Nagarajan G., Balasubramanium V., Suganthi K., Kannan M., and Valrajan G. 'Synthesis and antitumour activity of 4'-aryl/alkyl-2"-D-aldosugar disubstituted bis-benzimidazoles', *Indian J. Heterocycl. Chem*., **2005**, Vol. 15, pp. 173-176.
18. Zou R., Ayres K.R., Drach J.C. and Townsend L.B.'Synthesis and antiviral evaluation of certain di-substituted benzimidazoles ribonucleosides', *J.Med. Chem*., **1996**, Vol. 39, pp. 3477-3482.
19. Gudmundsson S.K. and Freeman G.A., 'Synthesis of flurosugar analogues of 2, 5, 6-trichloro-1-(β-*D*-ribofuranosyl) benzimidazole as antiviral with potential increased glycosidic bond stability', *J. Med. Chem*.,**2000**, Vol. 37, pp. 2473-2478.
20. Kohara Y., Kubo K., Imamiya E., wada T., Inada Y., and Naka T. 'synthesis and angiotensin II receptor antagonistic activities of benzimidazole derivatives bearind acidic heterocycles as novel tetrazole bioisosteres', *J. Med. Chem*.,**1996**, Vol. 39, pp. 5228-5235.
21. Aslanian R., Zhu X., Vaccaro H.A., Shih N.Y. and West R.E. 'Benzimidazole-substituted (3-phenoxypropyl)amines as histamine H_3 receptor ligands', *Bioorg. Med. Chem. Lett*., **2008**, Vol. 18, pp. 5032-5036.
22. Alonso F.P., Cook H.J., Villanueva J.P., Piliado J.C., Morales S.R., Hernandez G.P., Balbiaux N.L., Campos A.H., Castillo R. and Luis F.H. 'Synthesis and in vitro cysticidal activity of new benzimidazole derivatives', *Eur. J. Med. Chem*., **2009**, Vol. 44, pp. 1794-1800.
23. Dolzhenko A.V. and Chui W.K. 'Synthesis and biological activity of 1, 3, 5-triazino [1,2-a]benzimidazole-2-amines', *Pharm. Chem. J*., **2007**, Vol. 41., No. 9, pp. 470-473.
24. Singh D.N., Verma N., Pathak L.P., Raghuwanshi S., Shukla P.K. and Kulshreshtha D.K.'Synthesis and antifungal activity of novel benzimidazole incorporated anthraquinones', *Indian J. Heterocycl. Chem*.,**2007**, Vol. 17, pp. 161-164.
25. Spasov A.A., Chernikov M.V., Yakovlev D.S. and Anisimova V.A. 'Antiserotonin properties of tricyclic benzimidazole derivatives', *Pharm. Chem. J*., **2006**, Vol. 40, No. 11, pp.603-606.
26. Srinivasan N., Balaji A., Nagaranjan G., Suthakarant R., Kumar Y. and Jagadesh D. 'Synthesis and biological screening of some new 3-[2-methyl-1,2-dihydropyrimio(1,2-c)benzimidazole-1-thionyl]-6,8-dibromo-2-substituted-3*H*-quinazolin-4-one', *Asian J. Chem*.,**2008**, Vol. 21, No.6, pp. 4934-4936.
27. Hosseinimehr S.J. 'Trends in the development of Radioprotective agents', *Drug Dis. Today*, **2007**, Vol. 12, No.19/20, pp. 794-805.
28. Bhatnagar I and George MV,*Tetrahedron*,**1968**, 24,1293.
29. Beaulieu P L, Hache B and Von Moos E, *Synthesis*,**2003,**11, 1683-1692.
30. Pratap R et al, 'Antimicrobial activity by agar well diffusion method' *Int. J. of ChemTech Research;***2011** Vol. 3, No.3, pp 1172-1178.