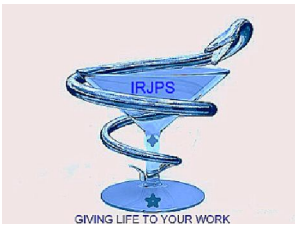


SYNTHESIS OF SOME NEW BENZIMIDAZOLE DERIVATIVES CONTAINING PTERIDINE RING SYSTEM AND THEIR ANTIMICROBIAL EVALUATION

Ankur Bakshi[*1], C.H.S. Venkataramana[2],

[1]IMS Health, DLF Cyber City, Phase II, Gurgaon.
[2]Department of Pharmaceutical Chemistry, M.S. Ramaiah College of Pharmacy Bangalore – 560054

Submitted on: 01.12.2013; Reviewed on: 11.12.2013; Accepted on: 13.12.2013

Abstract
Substituted *o*-phenylene diamines were treated with chloroacetic acid in acidic medium to obtain substituted 2-chloromethyl benzimidazoles. Different dicarbonyl compounds were condensed with 2,4,5-triamino-6-hydroxypyrimidine sulphate in aqueous medium to get substituted pteridines. Finally these substituted pteridines were fused with substituted 2-chloromethyl benzimidazoles to obtain various derivatives. The resulting compounds were screened for antimicrobial activity.

Keywords: Pteridine, benzimidazole, chloroacetic acid, antimicrobial activity, antifungal activity.

Corresponding Address: Ankur Bakshi
Tel: +918447240875
Email address: ankur.bakshi@mail.com

Introduction

Benzimidazoles constitute an important class of heterocyclic compounds possessing a wide spectrum of biological activities[1]. Of these, the 2-substituted derivatives have been found to be the most potent[2]. This ring system is present in numerous antiparasitic, fungicidal, anthelmintic and anti-inflammatory drugs. Some benzimidazole nucleosides, particularly 5,6-dichloro benzimidazole-1β-D-ribofuranoside (DRB) and its 2-substituted derivatives **show** activity against human cytomegalovirus[3]. Apart from the anti-hypertensive, anti-viral, anti-fungal, anti-tumor and anthelmintic activity substituted benzimidazoles are potent inhibitors of parietal cell proton pump, the H^+/K^+ ATPase.

Inhibitors of Dihydrofolate Reductase (DHFR), an enzyme that catalyzes NADPH-dependent reduction of 7,8-dihydrofolate to 5,6,7,8-tetrahyddrofolate have been used as antimicrobial as well as antimetabolite drugs and majority of DHFR inhibitors which are currently in use or being under investigation are derivatives of folic acid having 2,4-diamino substitution in pyrimidine ring and structurally belong to different classes viz. pyrimidines, pteridines, pyrido-pyrimidines[4]. DHFR is considered as an ideal target for rational and effective drug design for antimycobacterial agents[5].

A number of compounds with pteridine ring system have diverse pharmacological activity such as antibacterial, antitumor, cardiotonic, hepatoprotective antihypertensive, anti-bronchitis, antialergic, antimalerial, analgesic and antifungal[6].

Material and methods

Experimental

The chemicals and solvents were of reagent grade. Melting points were determined by open capillary method and are uncorrected. The IR spectra were recorded on a Fourier Transform IR spectrometer (8400S, Shimadzu) at M.S. Ramaiah college of pharmacy, Bangalore. The elemental analysis reports of compounds were obtained from Uwin Global Services, Bangalore using Flash EA 1112 series Thermo finnigan instrument.^{1}H NMR spectra were recorded on NMR spectrometer (AMX-400, Bruker) at Indian Institute of Science Bangalore using DMSO and chemical shifts (δ) are reported in parts per million downfield from internal reference Tetramethylsilane (TMS). Mass spectra were **provided by** Uwin Global Services, Bangalore, which were recorded on Mass spectrometer (LCMS-2010 A, Shimadzu).

General procedure for Condensation of substituted 1,2-Diol compounds with 2,4,5-triaminopyrimidine-6-hydroxysulfate (1)

2, 4, 5-triamino-6-hydroxy **pyrimidine sulfate** (0.01 mol) was dissolved in water (250 ml) and the solution was heated to boiling with stirring, a solution of substituted Isatin (0.01 mol) in hot water was added to it. The mixture was heated for 30 mins. Then it was neutralized with anhydrous sodium carbonate and then the solution was cooled to room temp. Solid **precipitate** appeared. The **precipitate** was re-crystallized from hot water.

2-amino-4-hydroxy pteridine (1A): Red colour solid precipitate; mp >360º C; % yield 71.82%; Rf 0.68 Petroleum ether: Ethyl acetate::(9:1);IR (KBr) ν (2873.74 cm^{-1} (Ar, C-H str), 3330.84 cm^{-1} (N-H,str),1523.66 cm^{-1} (N-H,bnd),1336.72 cm^{-1} (Ar,CN str),1112.86 cm^{-1} (OH bnd)

8-amino-3-chloro-11H-5,7,9,10,11-pentaazabenzo[b]fluoren-6-ol (1B): Red colour solid precipitate; mp >360º C; % yield 71.82%; Rf 0.68 Petroleum ether: Ethyl acetate:: (9:1);IR (KBr) ν (3346 cm^{-1} (N-H,str),2998.60 cm^{-1} (Ar, C-H str), 1573.81 cm^{-1} (N- H,bnd),1353.94 cm^{-1} (Ar,CN str),1076.21 cm^{-1} (OH bnd),752.19 cm^{-1} (C-Cl str).

8-amino-3-chloro-11H-5,7,9,10,11-pentaazabenzo[b]fluoren-6-ol (1C): Red colour solid precipitate; mp >360º C; % yield 71.82%; Rf 0.68 Petroleum ether: Ethyl acetate:: (9:1);IR (KBr) ν (3296.12 cm^{-1} (N-H,str),2997.17 cm^{-1} (Ar, C-H str), 1558.33 cm^{-1} (N-H,bnd),1332.87 cm^{-1} (Ar,CN str),1195.78 cm^{-1} (OH bnd),796.04 cm^{-1} (C-Br str).

8-amino-3-chloro-11H-5,7,9,10,11-pentaazabenzo[b]fluoren-6-ol (1D): Red colour solid precipitate; mp >360º C; % yield 71.82%; Rf 0.68 Petroleum ether: Ethyl acetate::

(9:1);IR (KBr) ν (3336.62 cm^{-1} (N-H,str),2977.89 cm^{-1} (Ar, C-H str), 1554.52 cm^{-1} (N- H,bnd),1379.01 cm^{-1} (Ar,CN str),1164.92 cm^{-1} (OH bnd),644.18 cm^{-1} (C-I str).

5-nitro-2-Chloromethyl benzimidazole (2A): Red colour solid precipitate; mp >360º C; % yield 71.82%; Rf 0.68 Petroleum ether: Ethyl acetate::(9:1);IR (KBr) ν (3139.90 cm^{-1} (N-H,str),2999.25 cm^{-1} (Ar, C-H str), 1579.99 cm^{-1} (N-H,bnd),1512.09 cm^{-1} (Ar N-O ,str)1440.73 cm^{-1} (Ar,CN str),1141.78 cm^{-1} (OH bnd)

4,6-dichloro-2-chloromethyl benzimidazole (2B): Red colour solid precipitate; mp >360º C; % yield 71.82%; Rf 0.68 Petroleum ether: Ethyl acetate:: (9:1);IR (KBr) ν (3326.98 cm^{-1} (N-H,str),3047.32 cm^{-1} (Ar, C-H str), 1506.30 cm^{-1} 1340.43 cm^{-1} (Ar,CN str),1130.36 cm^{-1} (OH bnd),931.95 cm^{-1} (C-F str).

Synthesis of 6,7-disubstituted-2-[(5-substituted-1*H*-benzo[*d*]imidazol-2-yl) methyl amino]pteridine-4-ol [3A1-3B4].

2-chloro methyl benzimidazole (1.66g, 0.01 mol) was dissolved in ethanol (30ml). Then the 8-amino-11H-5,7,9,10,11-pentaazabenzo[b]fluoren-6-ol (2.52g, 0.01 mol) was dissolved in ethanol (20ml). Both the solutions were mixed and slightly excess of anhydrous potassium carbonate was added to the reaction mixture. It was refluxed for 5 to 8 hours and poured in the crushed ice. The orange-white solid separated was filtered and recrystallized from hot ethanol.

2-[(5-nitro-1*H*-benzo[*d*]imidazol-2-yl)methylamino]pteridine-4-ol [3A1]:

Reddish-brown powder; mp. >360º C; % yield 35.60 %; Rf 0.43Ammonium hydroxide (10%); IR (KBr) ν 3074.32 (Ar C-H, str),1539.09 (N-H, bnd),1340.43 (Ar N-O, str),
1220.86 (Ar C-N, str), 1070.42 (O-H, bnd). 1H NMR (400 MHz, DMSO) δ 4.7 (2H, CH2), 7.4 (1H, NH)8.3-8.8 (5H, Ar)11.9 (1H, OH)12.4 (1H, NH). Anal Cal C,(49.71); H (2.98); N (33.12) ; Found C,(49.75); H (3.02); N (33.16) MS (APCI +) m/z 339 (M) +.

6,7-dimethyl-2-[(5-nitro-1*H*-benzo[*d*]imidazol-2-yl)methylamino] pteridine-4-ol [3A2]:

Dark red powder; mp. >360º C; % yield 52.52 %; Rf 0.41 Petroleum ether: ethyl acetate:: (9: 1); IR (KBr) ν 3249.83 cm^{-1} (O-H,str), 3063.11 (Ar C-H, str),
3024.18 (alkane C-H, str),1517.37 (N-H, bnd),1340.43 (Ar N-O, str). ^{1}H NMR (400 MHz, DMSO) δ 2.8 (6H, CH_3), 4.8 (2H, CH_2), 7.1 (1H, NH), 7.9-8.3(3H, Ar), 11.7(1H, OH), 12.5(1H, NH). Anal Cal C,(52.46); H (3.85); N (30.59) ; Found C,(52.49); H (3.89); N (30.61) MS (APCI +) *m/z* 367 $(M)^+$.

2-[(5-nitro-1*H*-benzo-[*d*]-imidazol-2yl)methylamino]pteridine-4,6,7-triol [3A3]:

Yellow solid powder; mp. >360º C; % yield 43.95 %; Rf 0.55 Petroleum ether: Ethyl acetate :: (9 : 1);IR (KBr) ν (3223.26 cm^{-1} (N-H,str),2837.09 cm^{-1} (Ar, C-H str),
1541.02 cm^{-1} (N- H,bnd),1399.72 cm^{-1} (Ar,CN str),1016.42 cm^{-1} (OH bnd),742.54 cm^{-1} (C-Br, str) .1H NMR (400 MHz,DMSO) δ 4.3(2H, CH2),7.0-7.9(7H, Ar),
8.1(1H, NH attached to methyl group), 11.1-11.2(1H, OH), 11.9-12.0(1H, NH),
12.1-12.2(1H, NH). Anal Cal C,(52.08); H (2.84); N (24.29) ; Found C,(
52.13); H (2.87); N (24.33) MS (APCI +) *m/z* 463 $(M+2)^+$.

6,7-diphenyl-2-[(5-nitro-1*H*-benzo[*d*]imidazol-2-yl)methylamino] pteridine-4-ol [3A4]:

Yellow solid powder; mp. >360º C; % yield 45.12 %; Rf 0.53 Petroleum ether: Ethyl acetate :: (9 : 1); IR (KBr) ν (3120.61 cm^{-1} (N-H,str),3058.89 cm^{-1} (Ar, C-H str),
1517.37 cm^{-1} (N-H,bnd),1450.94 cm^{-1} (Ar,CN str),1010.63 cm^{-1} (OH bnd),744.47 cm^{-1} (C-I str) . 1H NMR (400 MHz, DMSO) δ 4.3-4.4(2H, CH2), 6.9-7.6(7H, Ar),
7.8(1H, NH attached to methyl group), 11.2-11.4(1H, OH), 11.9-12.2-12.3(1H,
NH), 12.4-12.5(1H, NH). Anal Cal C,(47.26); H (2.58); N (22.05) ; Found C, (47.30); H (2.62); N (22.09) MS (APCI +) *m/z* 508 $(M)^+$.

2-[(4,6-dichloro-1*H*-benzo[*d*]imidazol-2-yl)methylamino]pteridine-4-ol [3B1]:

Yellow solid powder; mp. >360º C; % yield 44.57 %; Rf 0.46 Petroleum ether: Ethyl acetate:: (9: 1); IR (KBr) ν (3096.96 cm^{-1} (N-H, str), 3016.46 cm^{-1} (Ar, C-H str), 1517.87 cm^{-1} (N- H,bnd), 1502.44 cm^{-1} (Ar,N-O,str)1450.37 cm^{-1} (Ar,CN str),1076.21 cm^{-1} (OH bnd) cm^{-1}. 1H NMR (400 MHz, DMSO) δ 4.2-4.3(2H, CH2), 7.1-8.4(7H, Ar), 9.1(1H,

NH attached to methyl group), 11.2-11.4(1H, OH), 11.9-12.1-12.2(1H, NH), 12.6-12.8(1H, NH). Anal Cal C,(56.21); H (3.07); N (29.50) ; Found C,(56.25); H (3.11); N (29.53) MS (APCI +) *m/z* 427 $(M)^+$.

6,7-dimethyl-2-[(5-nitro-1*H*-benzo[*d*]imidazol-2-yl)methylamino] pteridine-4-ol [3B2]:
Yellow solid powder; mp. >360º C; % yield 61.28 %; Rf 0.48 Petroleum ether: Ethyl acetate:: (9: 1); IR (KBr) ν (3253.69 cm^{-1} (N-H, str), 3103.25 cm^{-1} (Ar, C-H str), 1517.87cm^{-1} (N- H,bnd), 1379.01 cm^{-1} (Ar,CN str),1186.14 cm^{-1} (OH bnd),854.41 cm^{-1} (C-F str) cm^{-1}. 1H NMR (400 MHz, DMSO) δ 4.7(2H, CH2), 7.2-8.7(7H, Ar and 1H, NH attached to methyl group), 11.5(1H, OH), 11.8-11.9(1H, NH), 13.0-13.2(1H, NH). Anal Cal C,(60.00); H (3.27); N (27.99) ; Found C,(60.04); H (3.29); N (28.03) MS (APCI +) *m/z* 401$(M+1)^+$.

2-[(4,6-dichloro-1*H*-benzo-[*d*]-imidazol-2-yl)methylamino] pteridine-4,6,7-triol [3B3]:
Yellow solid powder; mp. >360º C; % yield 63.14 %; Rf 0.43 Petroleum ether: Ethyl acetate:: (9 : 1); IR (KBr) ν (3097.41 cm^{-1} (N-H, str), 2896.80 cm^{-1} (Ar, C-H str), 1510.16 cm^{-1} (N- H,bnd), 1442.66 cm^{-1} (Ar,CN str),1217 cm^{-1} (OH bnd),846.69 cm^{-1} (C-F str) cm^{-1}. 1H NMR (400 MHz, DMSO) δ 4.4(2H, CH2), 7.1-7.9(7H, Ar), 8.1(1H, NH attached to methyl group), 11.5(1H,OH),11.6-11.7(1H, NH),12.8-12.9(1H,NH) Anal Cal C,(62.82); H (3.69); N (29.30) ; Found C,(62.86); H (3.72); N (29.34). Anal Cal C,(60.00); H (3.27); N (27.99) ; Found C,(60.03); H (3.30); N (28.02) MS (APCI +) *m/z* 401 $(M+1)^+$.

6,7-diphenyl-2-[(4,6-dichloro-1*H*-benzo[*d*]imidazol-2- yl)methylamino] pteridine-4-ol [3B4]:
Yellow solid powder; mp. >360º C; % yield 7.86 %; Rf 0.50 Petroleum ether: Ethyl acetate:: (9 : 1); IR (KBr) ν (3099.39 cm^{-1} (N-H,str),3016.46 cm^{-1} (Ar, C-H str), 2827.45 cm^{-1} (alkane C-H, str)1510.16 cm^{-1} (N-H,bnd),1402.15 cm^{-1} (Ar,CN str),1026.06 cm^{-1} (OH bnd)cm^{-1}. 1H NMR (400 MHz, DMSO) δ 2.3(3H, CH3), 4.2(2H, CH2), 7.2-7.8(7H, Ar and 1H, NH attached to methyl group), 11.3-11.5(1H, OH), 11.9-12.1(1H, NH), 12.5-12.6(1H, NH).,Anal Cal C,(63.63); H (4.07); N (28.27) ; Found C,(64.05); H (4.05); N (28.35) MS (APCI +) *m/z* 396 $(M)^+$.

RESULTS AND DISCUSSION

Antibacterial activity

The antibacterial activity of newly synthesized Benzimidazole derivatives containing pteridine ring system was carried out by agar diffusion method against *Staphylococcus aureus and Bacillus Subtilis (*gram-positive) and *Klebsiella and Proteus Vulgaris (gram-negative)* using **:** Amoxicillin and Ciprofloxacin as standard reference drugs. The results are presented in Table-IA.

All compounds have shown antibacterial activity against the gram-positive and gram-negative bacteria tested. The order of the antibacterial activity for the synthesized compounds is as follows.

a) Against *Staphylococcus aureus*
3B4 > 3A1, 3B2, 3B3 >3B1 > 3A2, 3A4 > 3A3
b) Against *Bacillus Subtilis*
3A1 >3B1 >3B3 >3A2, 3B2 >3A3, 3B4 >3A4
c) Against *Klebsiella*
3A4>3A1>3A2, 3B1, 3B4 >3A3, 3B2 >3B3
d) Against *Proteus Vulgaris*
3B2> 3A4> 3A2, 3B3> 3B4 >3A3, 3B1> 3A1

Antifungal activity

The antifungal activity was evaluated against *Aspergillus niger* and *Candida Albicans* by agar diffusion method. The standards used are Fluconazole and Amphotericin B. The results are presented in **Table-1**. All compounds have shown antifungal activity and the order of activity is as follows.

a)* Against *Aspergillus niger
3B2> 3A2> 3B1> 3A4> 3A1, 3A3, 3B3>3B4
b)* Against *Candida Albicans
3B3> 3A2, 3B2, 3B4> 3A1> 3A3, 3A4> 3B1

Conclusion
The objective of the present work was to synthesize, purify, characterize and evaluate the antimicrobial of the newly synthesized benzimidazole derivatives. The yield of the products ranged from 30-53%. The purity was checked by TLC and elemental analysis. The structures of the newly synthesized compounds **[3A1-3B4]** are characterized and confirmed by spectral data viz. IR, 1H NMR and Mass spectra and all the synthesized compounds **[3A1-3B4]** were screened for antimicrobial activity. Some of these derivatives, specifically 3A2 and 3B2, have shown reasonable antimicrobial activity. It appears and unsubstituted and substitution with bulky group like Phenyl is detrimental to anti-microbial activity for the synthesized ring system.

Acknowledgement
The work was carried out in the Research Laboratory of Department of Pharmaceutical Chemistry, M.S. Ramaiah College of Pharmacy Bangalore-560054. The authors thank Gokula Education Foundation (Medical) for providing the facilities and also thank faculties of Dept. of Pharmaceutical Chemistry – M. Narayana Babu, B.V. Suma, Judy Jays for their valuable insights. The authors thank the M.pharm 2008-10 batch research students who were carrying out their research work at that time, - for extending valuable opinions and making the research work enjoying.

Author's contribution

A B : performed the synthesis, antimicrobial activity and drafting the manuscript.
C.H.S. V: chalked out the synthetic scheme and participated in interpretation of spectral data interpretation.

REFERENCES
1. Dubey PK, Kumar R, Cameron TS, Grossert JS, Hooper DL. Studies on bromination of 2-styrylbenzimidazoles. *Indian J Chem* 1999 Aug;38B:p 914-9.
2. Ramaiah K, Dubey PK, Rao DE, Ramanatham J, Kumar R, Grossert JS, Hooper DL. Synthesis and spectral properties of a variety of 2-styrylbenzimidazoles. *Indian J Chem* 2000 Dec;39B:p 904-14.
3. Kazimierczuk Z, Upcroft JA, Upcroft P, Górska A, **Starósciak** B, Laudy A. Synthesis, antiprotozoal and antibacterial activity of nitro and halogen substituted benzimidazole derivatives. *Acta biochimica polonica* 2002 Jan;49(1):p 185-95.
4. Banjanac M, Tatic I, Ivezic Z, Tomic S, Dumic J. Pyrimido-pyrimidines: A novel class of dihydrofolate reductase inhibitors. *Food technol Biotechnol* 2009;47(3):p 236-45.
5. Suiling WJ, Seitz LE, Pathak V, Westbrook L, Barrow EW, Zywno-Van-Ginkel S etal. Antimicrobial activities of 2,4-diamino-5-deazapteridine derivatives and effects on mycobacterial dihydrofolate reductase. *Antimicrob.ag Chemotherapy* 2000 Oct;44(10):p 2784-93.
6. Deb ML, Bhuyan PJ. Synthesis of novel classes of pyrido[2,3-*d*]-pyrimidines, pyra -no[2,3-*d*]-pyrimidines and pteridines.*Syn comm* 2006;36:p 3085-90.

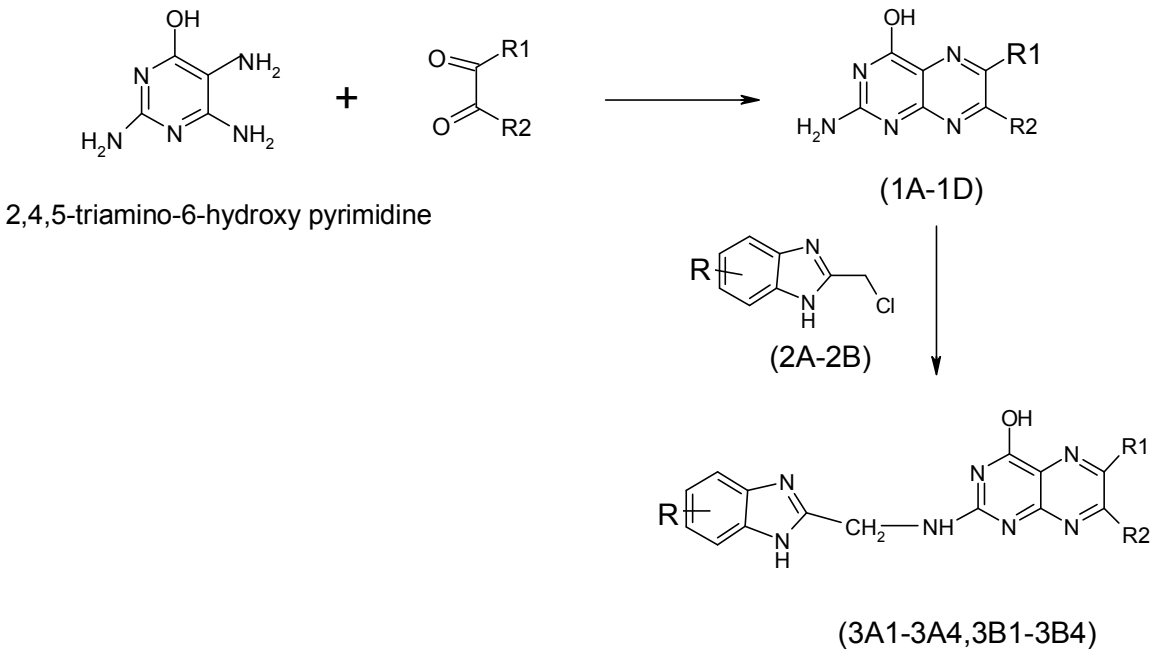
BIOLOGICAL ACTIVITY

TABLE-1: RESULTS OF ANTIMICROBIAL ACTIVITY

Comp.	R	R^1	R^2	ANTIBACTERIAL ACTIVITY Zone of Inhibition (mm)				ANTIFUNGAL ACTIVITY Zone of Inhibition (mm)	
				S.aureus	*B.Subtilis (Gram +ve)*	*Klebsiella (Gram -ve)*	*Proteus Vulgaris (Gram -ve)*	*Aspergillus niger*	*Candida Albicans*
3A1	5-NO_2	H	H	12	13	11	6	8	7
3A2	5-NO_2	CH_3	CH_3	10	10	10	10	11	8
3A3	5-NO_2	OH	OH	9	8	8	8	8	6
3A4	5-NO_2	Ph	Ph	10	6	12	11	9	6
3B1	4,6-Di Cl	H	H	11	12	10	8	10	4
3B2	4,6-Di Cl	CH_3	CH_3	12	10	8	12	12	8
3B3	4,6-Di Cl	OH	OH	12	11	7	10	8	9
3B4	4,6-Di Cl	Ph	Ph	13	8	10	9	6	8
Ciprofloxacin	……	……	……	34	26	24	30	-	-
Amoxycillin	……	……	……	42	33	25	35	-	-
Fluconazole	……	……	……	-	-	-	-	35	24
Amphotericin B	……	……	……	-	-	-	-	38	30
Control (DMF)	……	……	……	NI	NI	NI	NI	NI	NI

NOTE: - Average zone diameter of triplicates in mm.
NI : - No inhibition

SCHEME FOR SYNTHETIC METHODLOGY:



(3A1-3A4,3B1-3B4)

R- 5-NO_2, 4,6-Di Cl

R_1, R_2- H, CH_3, OH, Ph

S.No.	COMPOUND	R	R_1	R_2
1	3A1	5-NO_2	H	H
2	3A2	5-NO_2	CH_3	CH_3
3	3A3	5-NO_2	OH	OH
4	3A4	5-NO_2	Ph	Ph
5	3B1	4,6-Di Cl	H	H
6	3B2	4,6-Di Cl	CH_3	CH_3
7	3B3	4,6-Di Cl	OH	OH
8	3B4	4,6-Di Cl	Ph	Ph

Table 2: Synthesized compounds - Substitutions