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Synthesis and evaluation of 2-aryl substituted benzimidazole derivatives bearing 1,3,4-oxadiazole nucleus for antimicrobial activity

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ABSTRACT

*In the present study, a series of benzimidazole derivatives bearing 1, 3, 4-oxadiazole nucleus (**5a-5h**) were synthesized and screened for the in vitro antimicrobial activity against a panel of two Gram positive bacteria: Staphylococcus epidermidis, Bacillus subtilis, two Gram negative bacteria: Escherichia coli, Pseudomonas aeruginosa and two fungal strain: Candida albicans and Aspergillus niger respectively. Compounds **(5e-5h)** had shown good antibacterial activity. Compound **(5b)** exhibited equipotent activity against fungal strain A. niger in comparison to standard drug. The H^1NMR and IR spectra were found to be in accord with assigned structures.*

Key words: Benzimidazole, 1, 3, 4-oxadiazole, antibacterial activity, antifungal activity, minimum inhibitory concentration (MIC)

INTRODUCTION

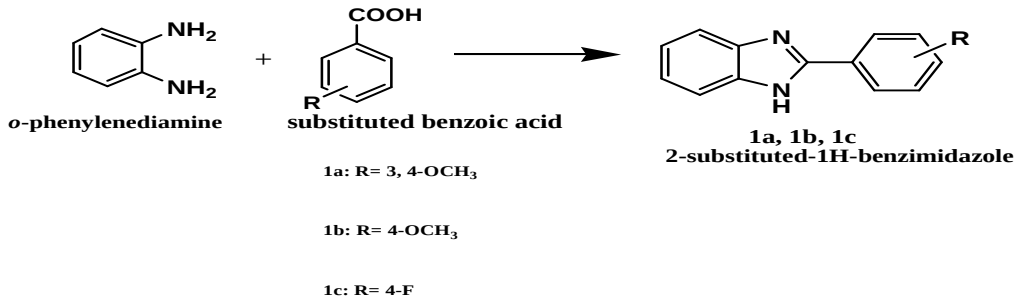
Since last decennium, heterocyclic molecules has recieved much attention due to their versatile pharmacological profile in the research and development of new pharmaceutical molecules [1]. One such pharmacophore is 'Benzimidazole', because the molecule acts as a biomimetic of nucleotide [2], a number of initiatives have been made in generating libraries of these compounds and to screen them for their potential biological activities. The ring is present as nucleus in many compounds acting as antioxidant [3], antiparasitic [4], anthelmintic [5], antiproliferative [6], anti-HIV [7], anticonvulsant activities [8], antimicrobial [9] activities. Oxadiazole nucleus is also associated with a variety of pharmacological activities like antiproliferative, anticonvulsant, antifungal. Appraising the importance of benzimidazole and oxadiazole nucleus design and synthesise of some new benzimidazole derivatives was intended bearing oxadiazole moiety and to contemplate their potential biological activities, *viz*, antibacterial and antifungal [10].

MATERIALS AND METHHODS

All the chemicals and reagents were of analytical grade and were used without further purification. Melting points were determined by capillary tube method and are uncorrected. All the reactions and purity of synthesized compounds was deduced by thin layer chromatography (TLC) using silica gel-G plate. The plates were developed by exposing to the iodine vapours. Infrared spectra were recorded by Perkin Elmer spectrophotometer using KBr pellets. Proton nuclear magnaetic resonance spectra (1H NMR) were recorded on Bruker Avance II 400 NMR spectrophotometer. Chemical shifts are expressed as δ values (ppm) downfield using tetramethylsilane as internal standard.

General procedure

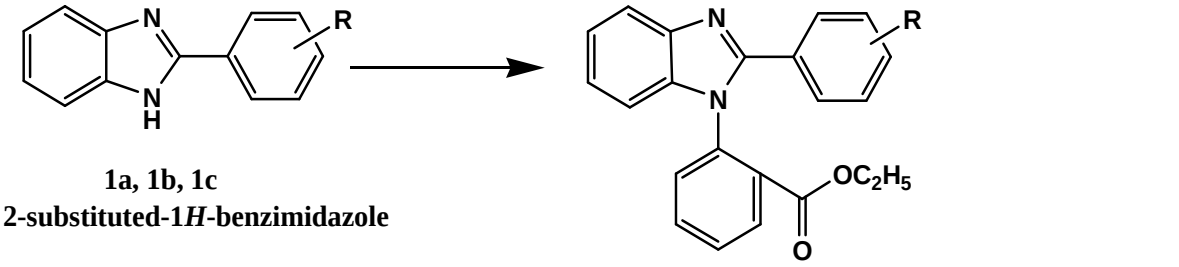
a) Synthesis of 2-substituted-1H-benzimdazole (1a, 1b, 1c) [11]



**Reagents and conditions: o-phosphoric acid, refluxed for 6 hrs.*

In a round bottom flask, a mixture of *o*-phenylenediamne (0.1 mol, 10.8g) and substituted benzoic acid (0.1 mol) was taken and refluxed in *o*-phosphoric acid for 6 hrs. After completion of reaction, the reaction mixture was cooled and was poured onto crushed ice. Concentrated ammonia solution was added to the cooled mixture dropwise, until got neutralized, and the resulting solid was filtered, washed with cold water, dried and recrystallized from ethanol.

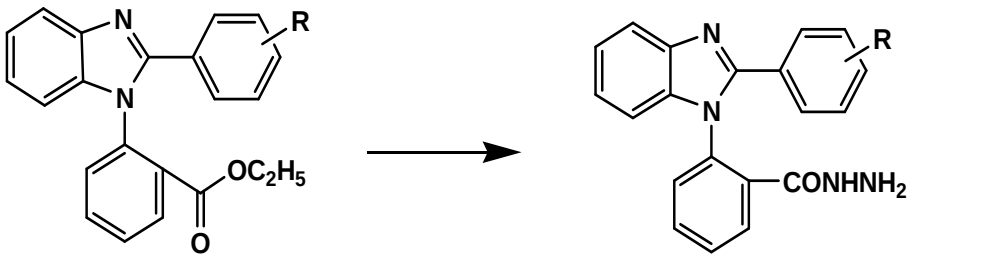
b) Synthesis of ethyl-(2-substituted 1H-benzimidzol-1-yl) benzoate (2a, 2b, 2c) [12]



**Reagents and conditions: C_6H_5COOH, anhydrous K_2CO_3, acetone, stirred for 10-12 hrs.*

To a suspension of 2-substituted-1*H*-benzimidazole (0.01 mol) **1a, 1b, 1c,** anhydrous potassium carbonate (0.01mol, 1.5g) in dry acetone was added. To the reaction mixture added ethyl chloro benzoate (0.01 mol, 1.8 ml) dropwise at room temperature for 20-30 mins. The reaction mixture was stirred at room temperature for 10-12 hrs. The inorganic solid was filtered off and the filtrate was concentrated under reduced pressure using rotary evaporator.

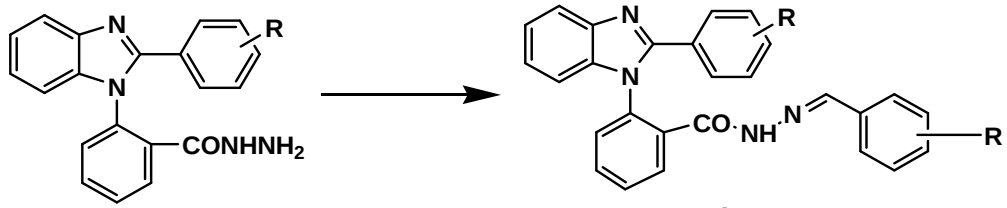
c) Synthesis of 2-(2-substituted 1*H*-benzimidazol-1-yl) benzohydrazide 3a, 3b, 3c [12]



**Reagents and conditions: C_2H_5OH, hydrazine hydrate (99%), refluxed for 5 hrs.*

To an ethanolic solution of Ethyl-(2-substituted-1*H*-benzimizol-1-yl) benzoate **2a, 2b, 2c,** (0.01 mol) in a round bottom flask, hydrazine hydrate (99%) (0.01 mol, 0.50 ml) was added and the mixture was refluxed for 5 hrs. After completion of reaction, the reaction mixture was cooled and the solid so obtained was filtered and washed with cold water, dried and recrystallized from ethanol.

d) Synthesis of 2-[2-substituted-1*H*-benzimidazol-1-yl] *N*'-[substituted-phenyl methylidene]benzohydrazide (4a, 4b, 4c) [12]



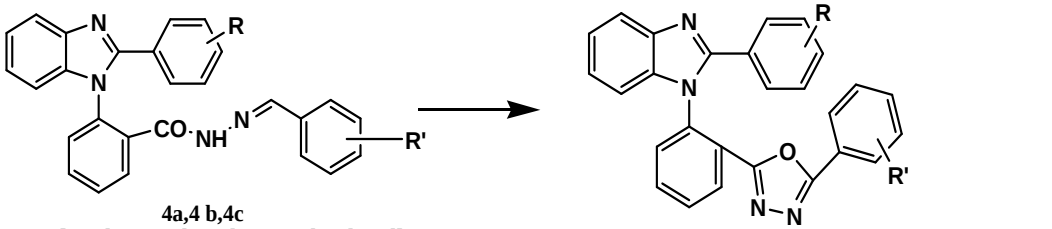
3a, 3b, 3c
2-(2-substituted-1*H*-benzimidazol-1-yl) benzohydrazide

4a ,4 b,4c
2-[2-substituted 1*H*-benzimidazol-1-yl] *N'*-[substituted-phenylmethylidene] benzohydrazide

**Reagents and Conditions: R'= 4-NO_2benzaldehyde, 4-OCH_3benzaldehyde, 3,4dimethyaminobenzaldehyde, C_2H_5OH, glacial acetic acid, refluxed for 7 hrs.*

A mixture of benzyl (2-substituted-1*H*-benzimidazol-1-yl) benzohydrazide, **3a, 3b, 3c,** (0.0025 mol) and substituted benzaldehyde (0.0025) in ethyl alcohol (10 ml) and few drops of glacial acetic acid was added, and refluxed for 7 hrs. The reaction mixture was poured in ice cold water, filtered, dried and recrystallized from ethanol.

e) Synthesis of 2-substituted-1-(2-(5-substituted phenyl-1, 3, 4 oxadiazol-2yl)-phenyl)-*1H*-benzimidazole [12]



4a,4 b,4c
2-[2-substituted-1*H*-benzimidazol-1-yl] *N'*-[substituted-phenylmethylidene] benzohydrazide

5a,5b,5c
2-substituted-1-(2-(5-substituted phenyl-1,3,4 oxadiazol-2yl)-phenyl)1*H*-benzimidazole

**Reagents and Conditions: C_2H_5OH, chloramine T, refluxed for 5 hrs.*

To an ethanolic solution of 2-[2-substituted-1*H*-benzimidazol-1-yl] *N*- [substituted-phenylmethylidene] benzohydrazide **4a, 4b, 4c** (0.0025 mol), chloramine T (0.0025) was added. The reaction mixture was refluxed for 5 hrs, filtered off the sodium chloride separated out during the reaction. Excess of ethanol was completely removed from the reaction mixture by distillation under reduced pressure using rotary evaporator after completion of reaction, leaving behind a solid mass which was recrystallized from ethanol.

Antimicrobial activity

The evaluation of antimicrobial activity was conducted *via* serial dilution method. The method determines the minimum inhibitory concentration (MIC). MIC is the lowest concentration of antimicrobial agent required to inhibit the microbial growth *in-vitro*, preventing appearance of turbidity. The synthesised compounds were screened against the following strains of micro-organisms:

Bacteria		**Fungus**
Gram positive	***Gram negative***	*Aspergillus niger* (MTCC-8189) *Candida albicans* (MTCC- 227)
Bacillus subtilis (MTCC-2063) *S. epidermidis* (MTCC-435)	*Pesudomonas aeruginosa* (MTCC-4215) *Escherichia coli* (MTCC-40)	

Preparation of standard and stock solution

Stock solution of test compounds and that of standard drugs, ciprofloxacin and fluconazole for antibacterial and antifungal activity was prepared in DMSO to give a concentration of 1000µg/ml. 1 ml of the above solution was taken and made upto 10 ml by dissolving it in 9 ml of DMSO to give a concentration of 100µg/ml.

Procedure to determine MIC

Suspension of organism were prepared by dissolving 0.1 ml of freshly prepared inoculum in 10 ml of sterile isotonic solution of sodium chloride (0.9% w\v). 1 mL of sterilized media was poured into sterilized test tubes. 1 mL of 100 µg/mL test solution was transferred in one tube and serially diluted to give a concentration of 50 µg/mL, 25 µg/ml, 12.5 µg/mL, 6.25 µg/mL, 3.12 µg/mL, 1.56 µg/mL respectively. In the same way five different concentrations of stock solution (100 µg/mL) of standard drug were prepared. To the all tubes 0.1 mL of suspension of microbe in saline was added and tubes were incubated for the prescribed incubation time. Incubation time - 37±1ºC for 24 hr *(B.subtilis, S.epidermidis, P.aeruginosa, E.coli*), 37±1ºC for 48 hr *(Candida albicans)*, 25±1ºC for 7 days *(Aspergilus niger)* respectively. The concentration for drug that completely diminish the growth of microorganism was taken as MIC by observing the growth in the tubes in form of turbidity and the inhibition was determined by absence of growth.

RESULTS AND DISCUSSION

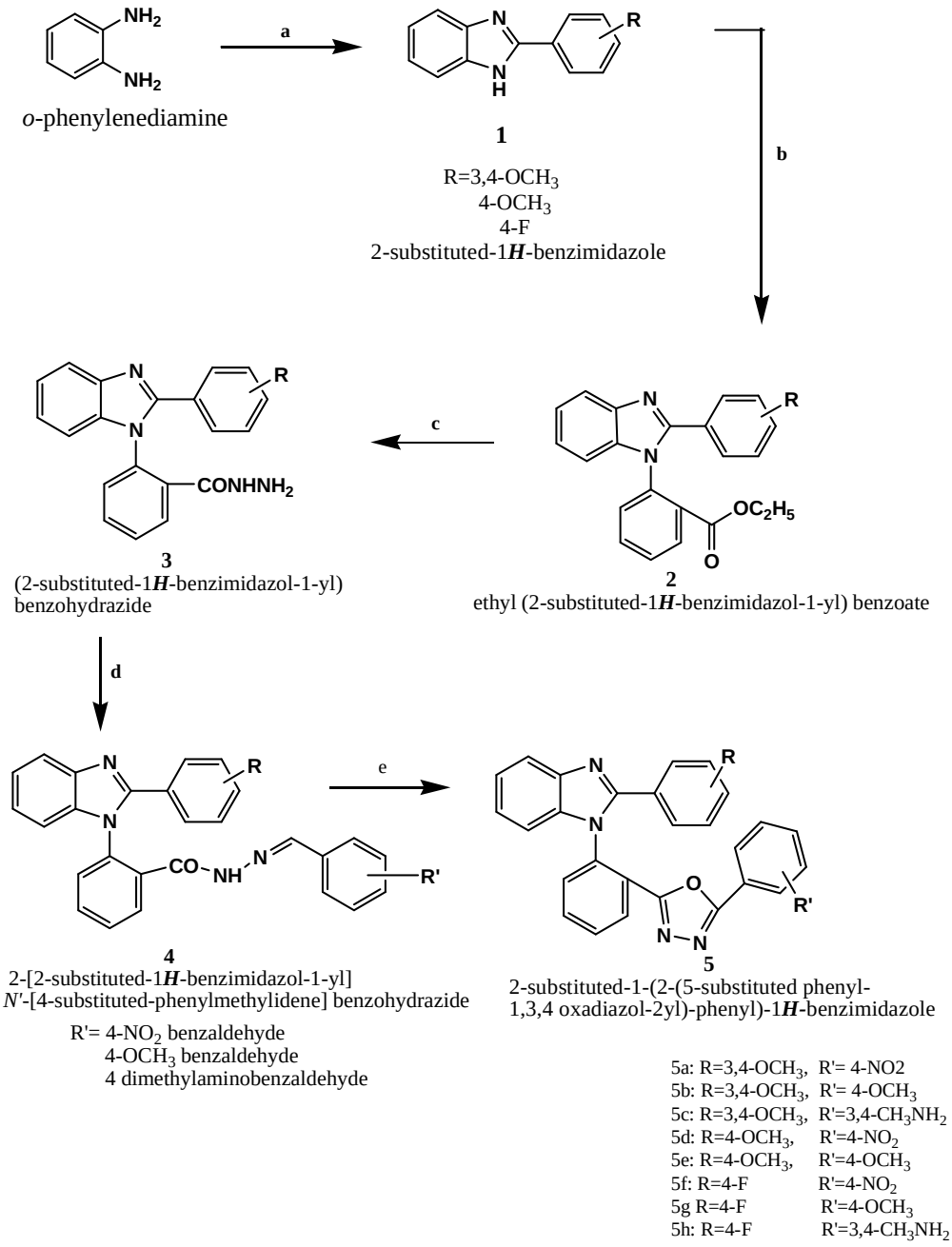
Chemistry

o-phenylenediamine upon reaction with aromatic acids in presence of *o*-phosphoric acid gives 2-aryl benzimidazole **(1)** which reacted with ethyl chloro benzoate yielded 2-substituted-1*H*-benzimidazol-1-yl benzoate **(2)**. This on further reaction with hydrazine hydrate yielded benzohydrazide **(3)**. The benzohydrazide then reacts with different aromatic aldehydes to yield Schiff's bases **(4)**. The synthesised Schiff's bases undergone cyclization in presence of chloramine-T synthesising different compounds **(5)** shown in the general scheme (fig 1). The derivatives were obtained in appreciable yield which were purified by recrystallization from ethanol. The purity of derivatives was confirmed by single spot on TLC (silica gel) plates and spot was detected by exposing the silica gel plate to iodine vapours. The physicochemical analysis of the synthesized compounds is presented in table 1.

Spectral analysis

IR and ^{1}H-NMR spectra of selected compounds were recorded and interpreted thoroughly and are consistent with the assigned structure of the derivatives. The IR spectra of compound **1b**, **1c** appeared in the range of 3188-3057cm^{-1}(C-H strech aromatic), 1490-1600 cm^{-1} (C=C aromatic), 1685-1645cm^{-1} (C=N), 1334-111cm^{-1} (C-O) revealing completion of cyclization and preparation of 2-substituted benzimidazole pharmacophore. The IR peaks coming in the range of 1683-1653cm^{-1}(C=O), confirmed the presence of ester group substitution. Further (C-N stretch) in the range 1237-1018cm^{-1}, 1653-1597cm^{-1} (N-H bend) indicated the formation of benzohydrazide. Further derivatives (5a-5h) were prepared and we witnessed the appearance of peaks at 1685-1645cm^{-1} (C=N), 1543-1680cm^{-1}(N-H stretch),1334-1111cm^{-1} (CO),1334-1564cm^{-1}(N=O), 1018-1417cm^{-1} indicating the formation of 2-substituted-1-(2-(5-substituted phenyl-1,3,4-oxadiazol-2yl)-phenyl)-1*H*-benzimidazole.

The ^{1}H NMR spectrum of synthesised compounds revealed the appearance of chemical shift value for aromatic protons in the range of 7.69-7.24 δ(ppm) as doublet (d) confirming the presence of aromatic ring of benzimidazole in **1a**. The presence of N-H proton was observed at 9.83δ (ppm), appeared as a singlet (s) in **1a** and disappeared in further synthesised compounds. Peaks appeared in the range 3.90-3.88 δ(ppm) as singlet (s) confirmed the presence of protons for -OCH_3 groups at 2-aryl benzimidazole. Singlet (s) appeared in the range 2.30-2.12 δ(ppm) and at 3.87 δ(ppm) confirmed that -CH_3 and -OCH_3 groups substituted in 2-phenyl ring of 1,3,4-oxadiazole.



***Reagents and conditions: (a)** RC_6H_5COOH, PPA refluxed for 6 hrs, **(b)** $C_6H_5COOC_2H_5Cl$, K_2CO_3, acetone stiring for 10-12 hrs, **(c)** hydrazine hydrate,C_2H_5OH refluxed for 5 hrs, **(d)** C_2H_5OH, R'CHO, glacial acetic acid refluxed for 7 hrs, **(e)** C_2H_5OH, chloramine T refluxed for 5 hrs.

Fig 1. General synthetic scheme for the synthesis of compounds 5a-5h

Table 1. Physicochemical characterization of the synthesised compounds

S.NO	COMPOUND	MOLECULAR FORMULA	MOLECULAR WEIGHT	MELTING POINT	R_f VALUE	% YEILD
1.	5a	$C_{30}H_{21}N_5O_6$	519.52	341-349	0.74	60.93
2.	5b	$C_{31}H_{24}N_4O_5$	504.55	335-337	0.76	64.18
3.	5c	$C_{32}H_{27}N_5O_4$	517.59	342-350	0.82	65.93
4.	5d	$C_{29}H_{19}N_5O_5$	489.49	336-341	0.75	63.22
5.	5e	$C_{30}H_{22}N_4O_4$	474.52	319-326	0.81	64.90
6.	5f	$C_{28}H_{16}FN_5O_4$	477.76	321-329	0.72	71.80
7.	5g	$C_{29}H_{19}FN_4O_3$	462.49	309-314	0.78	68.71
8.	5h	$C_{30}H_{22}FN_5O_2$	477.52	328-336	0.84	68.59

TLC moblie phase: Hexane: Ethyl Acetate (5:5)

2-(4-methoxyphenyl)-1*H*-benzimidazole (1b)
IR (KBr, cm^{-1}): 3080.32(C-H str., aromatic), 2980.02 (C-H str., aliphatic), 1685.79(C=N), 1653.00 (N-H bend), 1440.83(C=C str., aromatic), 1365.60 (C-N str.,), 1325.10 (C-O str.,). ^{1}H NMR DMSO, δ (ppm): 7.69-7.24 (4H, d, Ar-H, benzimidazole), 7.50-7.49 (2H, m, 2-aryl Ar-H), 3.88-3.90 (6H, s, OCH_3).

2-(4-flourophenyl)-1*H*-benzimidazole (1c)
IR (KBr, cm^{-1}): 3061.03(C-H str., aromatic), 1683.86(C=O), 1642.86(C=N str.,), 1597.06 (C=C str., aromatic), 1018.41(C-O).

2-[2-(3,4-dimethoxyphenyl)-1*H*-benzimidazol-1-yl]benzohydrazide (3b)
IR (KBr, cm^{-1}): 3059.10 (C-H str., aromatic), 1683.86 (C=O str.,), 1600.92(C=C aromatic), 1589.92 (N-H bend), 1321.24 (C-N), 1392.61(C-F).

2-(3,4-dimethoxyphenyl)-1-{2-[5-(2-nitrophenyl-1, 3, 4-oxadiazole-2-yl]phenyl}-1*H*-benzimidazole (5a)
IR (KBr, cm^{-1}): 3057.17 (C-H str., aromatic), 2949.16 (C-H str., aliphatic), 1645.28(C=N), 1598.99 (C=C str., aromatic), 1016.49(C-O), 1490.27 (N=O) 1016.49(C-N),. ^{1}H NMR DMSO, δ (ppm): 8.37-8.22 (2H, d, 2-phenyl-1, 3, 4-oxadiazole), 7.90-7.89 (4H, m, Ar-H,benzimidazole), 7.78-7.46 (7H, m, Ar-H), 3.82 (6H, s, OCH_3).

2-(3, 4-dimethoxyphenyl)-1-{2-[5-(2-methoxyphenyl-1, 3, 4-oxadiazole-2-yl]phenyl}-1*H*-benzimidazole (5b)
IR (KBr, cm^{-1}): 3132.40(C-H str., aromatic), 2924.09 (C-H str., aliphatic), 1600.9 (C=C str., aromatic), 1645.28 (C=N), 1172.40 (C-O), 937.40(C-N),. ^{1}H NMR DMSO, δ (ppm): 7.78-7.30(4H, m, Ar-H, benzimidazole), 7.77-7.32 (6H, m, AR-H), 3.87 (2H, s, 2-phenyl-1,3,4-oxadiazole), 3.88 (6H, s, OCH_3).

(2-(aminomethyl)-5-(5-(2-(2-(3,4-dimethoxyphenyl)-1*H*-benzimidazol-1-yl)phenyl)-1,3,4-oxadiazol-2-yl) phenyl) methanamine (5c)
IR (KBr, cm^{-1}): 3062.96 (C-H str., aromatic), 2870.08 (C-H str., aliphatic), 1675.14 (C=O str.,), 1649.14 (N-H),1600.92 (C=C aromatic), 1020.24(C-N). ^{1}H NMR DMSO, δ (ppm): 7.88-7.31(4H, m, benzimidazole), 7.77-6.61 (6H, m, Ar-H), 2.30-2.12 (4H, s, 2-phenyl-1,3,4-oxadiazole), 3.90-3.88 (6H, s, OCH_3).

2-(4-dimethoxyphenyl)-1-{2-[5-(2-nitrophenyl-1,3,4-oxadiazole-2-yl]phenyl}-1*H*-benzimidazole (5d)
IR (KBr, cm^{-1}): 3188.33 (C-H str., aromatic), 2918.16 (C-H str., aliphatic), 1653.00 (C=N str.,), 1492.70 (C=C), 1334.74 (C-N), 1018.41 (C-O).

2-(4-dimethoxyphenyl)-1-{2-[5-(2-methoxyphenyl-1,3,4-oxadiazole-2-yl]phenyl}-1*H*-benzimidazole (5e)
IR (KBr, cm^{-1}): 3088.33 (C-H str., aromatic), 2958.16 (C-H str., aliphatic), 1650.00 (C=N), 1497.70 (C=C str., aromatic), 1650.00 (C=N), 1345.74 (C-N), 1018.41 (C-O).

2-(4-fluorophenyl)-1-{2-[5-(2-nitrophenyl-1,3,4-oxadiazole-2-yl]phenyl}-1*H*-benzimidazole (5f)
IR (KBr, cm^{-1}): 3030.17 (C-H str., aromatic), 2922.16 (C-H str., aliphatic), 1664.21(C=N), 1496.76 (C=C str., aromatic), 1020.34 (C-O).

2-(4-fluorophenyl)-1-{2-[5-(2-methoxyphenyl-1,3,4-oxadiazole-2-yl]phenyl}-1*H*-benzimidazole (5g)
IR (KBr, cm^{-1}): 3057.17(C-H str., aromatic, 2981.66(C-H str., aliphatic), 1674.21(C=N), 1591.27 (C=C str., aromatic), 1346.31(C-N), 1417.68(C-F), 1012.63(C-O).

(2-(aminomethyl)-5-(5-(2-(2-(4-fluorophenyl)-1H-benzo[d]imidazol-1-yl)phenyl)-1,3,4-oxadiazol-2-yl) phenyl)methanamine (5h)
IR (KBr, cm^{-1}): 3057.17(C-H str., aromatic), 2922.16 (C-H str., aliphatic), 1681.93 (C=N), 1598.99 (C=C str., aromatic), 1305.81 (C-N), 1396.46 (C-F), 1091.71 (C- O).

Evaluation of antimicrobial activity
The evaluation of antimicrobial activity of synthesised compounds was conducted *via* serial dilution method. The standard drugs used were Ciprofloxacin and Fluconazole for antibacterial and antifungal activity respectively. The pMIC of the synthesised compounds are given in table 2.

The compounds **5f, 5g, 5h** (pMIC- 1.88,1.86,1.88) had shown remarkable activity against bacterial strains *E.coli*, *P.aeruginosa*, *S.epidermidis* and are moderately active against *B.subtilis.* All compounds had shown considerable activity gainst the *C.albicans* and compounds (**5e, 5f, 5g, 5h)** (pMIC- 1.88,1.88, 1.86, 1.88) are significantly active against *A.niger*. Compound (**5b)** had shown equipotent activity compared to the corresponding standard drug

Fluconazole with pMIC-1.91 against *A.niger*. Compounds with *para*-substituted electron withdrawing groups at benzene ring at C-2 of benzimidazole and benzylidene were found to exhibit moderate to good degree of activity against the gram negative bacteria, and fungal strain. Compound **(5b)** and **(5e)** with electron donating groups at the same position had shown potent activity against *A.niger* and *S.epidermidis* respectively. The lipophilic character of benzylidene derivatives **(4a, 4b, 4c)** and of synthesised compounds (**5a-5h)** was investigated. Lipophilic character is expressed as log p (table 3) and was calculated using Marvin sktech 14.8.4 ChemAxon software. The analysis of log p revealed that, log p of intermediates **(4a-4h)** were slightly lower than the synthesised title compounds (**5a-5h),** suggesting that incorporation of 1,3,4-oxadiazole moiety is not contributing as much to the antimicrobial activity of the benzimidazole derivatives which is similar to the results of *Ansari and Lal et* al (2011).

Table 2. pMIC (µmol/ml) of synthesised compounds

S. no	Sample Code	Gram Negative		Gram Positive		Fungal	
		E.coli	***P.aeruginosa***	***B.subtilis***	***S.epidermidis***	***C.albicans***	***A.niger***
1.	5a	1.61	1.61	1.61	1.61	1.61	1.61
2.	5b	1.60	1.60	1.60	1.60	1.60	1.91
3.	5c	1.61	1.61	1.61	1.61	1.61	1.61
4.	5d	1.30	1.59	1.59	1.59	1.59	1.59
5.	5e	1.58	1.88	1.58	1.88	1.58	1.88
6.	5f	1.88	1.58	1.58	1.58	1.58	1.88
7.	5g	1.56	1.86	1.56	1.56	1.56	1.86
8.	5h	1.88	1.88	1.58	1.58	1.58	1.88
9	STD	2.33^{x}	2.33^{x}	2.33^{x}	2.33^{x}	1.99^{y}	1.99^{y}

X= ciprofloxacin, y= fluconazole

Table 3. Calculated log p value of compounds (4a-4h) and (5a-5h), calculated from Marvin sketch 14.8.4 (ChemAxon softwares)

Sample Code	Log p	Sample Code	Log p
4a	5.78	**5a**	5.82
4b	5.68	**5b**	5.72
4c	6.04	**5c**	6.08
4d	5.94	**5d**	5.98
4e	5.84	**5e**	5.88
4f	6.24	**5f**	6.28
4g	6.14	**5g**	6.18
4h	6.50	**5h**	6.54

From the above discussion structure activity relationship can be deduced as follows:
1. The derivative of 2-substituted-1*H*-benzimidazole, **(5b)** had shown equipotent activity to the corresponding standard drug Fluconazole followed by compound **(5e).** This may be due to the presence of electron donating methoxy group present at the para position of benzene ring of benzylidene.
2. Antifungal activity disclosed by the compounds **(5f-5h)** is also superior suggesting that electron withdrawing group fluoro present at para position of benzene ring substituted at C-2 of benzimidazole had contributed towards the antimicrobial activity.
3. In case of *E.coli* **5f** and **5h** emerged as active candidate, perhaps due to the presence of electron withdrawing group fluoro present at para position of benzene ring substituted at of C-2 benzimidazole benzene ring.
4. For *P.aeruginosa* and *S.epidermidis* compounds **5e, 5g, 5h** were active respectively, which may again be attributed to the presence of *para*-fluoro at 2-subtituted benzimidazole benzene ring.

CONCLUSION

The *in-vitro* antimicobial study of different 2-substituted-1-(5-substituted phenyl-1,3,4 oxadiazol-2yl-phenyl)-*1H*-benzimidazole was undertaken in order to determine the effects of different substituents upon bacterial and fungal strains. Compounds **5e** and **5h** exhibited good activity against *E.coli*, compounds **5e, 5g, 5h** were active against *P. aeruginosa* and compound **5e** was found active against *S. epidermidis.* Compound **5b** and compounds **5e-5h** were potent in case in *A. niger* fungal strain. From the results, it can be concluded that substituting benzylidene benzene ring with electron donating groups are contributing substantialy towards antimicrobial activity.

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