ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2014; 5(1): 124 - 131

ORIGINAL RESEARCH ARTICLE

Synthesis and Biological Evaluation of Some New Benzimidazole Derivatives as Antimicrobials

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Received 19 Oct 2013; Revised 04 Feb 2014; Accepted 16 Feb 2014

ABSTRACT

Benzimidazole and its derivatives represent one of the most biologically active class of compounds, possessing a wide spectrum of activities and these are well documented in the literature. Some derivatives of benzimidazole were synthesized by nucleophilic substitution. The present work comprises of synthesis of new antimicrobial agent, in which 4- chloro-ortho phenylene diamine are used as a starting material to which formic acid/acetic acid reacts and form 2-substituted 6-chloro-benzo[d]imidazole, this further heated with ethylbromoacetate and forms 2-substituted Ethyl 2-(6-chloro-benzo[d]imidazol-1-yl)acetate. The resulting intermediate on treatment with hydrazine hydrate yields Ethyl 2-(6-chloro-2-substituted-1H-benzo[d]imidazol-1-yl)hydrazide which on further reaction with one equivalent of different substituted aromatic carboxylic acids in the presence of phosphoryl chloride afforded the corresponding target compounds 6-chloro-2-substituted-1-[{(5-substituted aryl)-1,3,4-oxadiazol-2-yl} methyl]-1H-benzimidazole. The compounds were synthesized in appreciable yields and the structures of the synthesized compounds were evaluated by spectral methods of analyses. All the synthesized compounds were screened for their antimicrobial activity against *Escherichia coli* representing Gram – negative bacteria, *Bacillus subtilis* and *Staphylococcus aureus* representing Gram - positive bacteria, *Saccharomyces Cerevisiae* representing Fungi. The result revealed that most of newly synthesised compounds exhibited promising antibacterial and antifungal activities. Generally the test compounds showed good activity against Gram – positive bacteria, Gram – positive bacteria, fungi. Other compounds showed moderate activity against Gram – positive bacteria, Gram – positive bacteria, fungi. The results showed that all of the compounds have exhibited antimicrobial activity.

Key words: Benzimidazole, 1,3,4-oxadiazol, antimicrobial activity, antifungal activity.

INTRODUCTION

In recent years, attention has increasingly been given to the synthesis of benzimidazole derivatives as a source of new antimicrobial agents. The synthesis of novel benzimidazole derivatives remains a main focus of medicinal research. Many of the currently available drugs are toxic, enable recurrence because they are bacteriostatic/fungistatic and not bactericidal/fungicidal or lead to the development of resistance due in part to the prolonged periods of administration. Recent observations suggest that benzimidazole molecules are effective against various strains of microorganisms [1–7]. This was confirmed by various biochemical and pharmacological studies. Benzimidazoles constitute an important class of heterocyclic compounds possessing a wide spectrum of biological activities. Specifically, this nucleus is a constituent of vitamin-B12 [8]. Benzimidazoles ring play a vital role in biological fields such as anti-inflammatory [10–12] anticonvulsant [13], antioxidant [14–16], antiparasitic [17,18], antihelmintics [19], anti-HIV [20], antihypertensive [21], antineoplastic [22,23] activities.

Literature review suggest that that oxadiazole nucleus is also gives pharmacological actions [24,25]. It shows antifungal [26], anticonvulsant [27] and antimycobacterial [28] activities. Looking at the importance of

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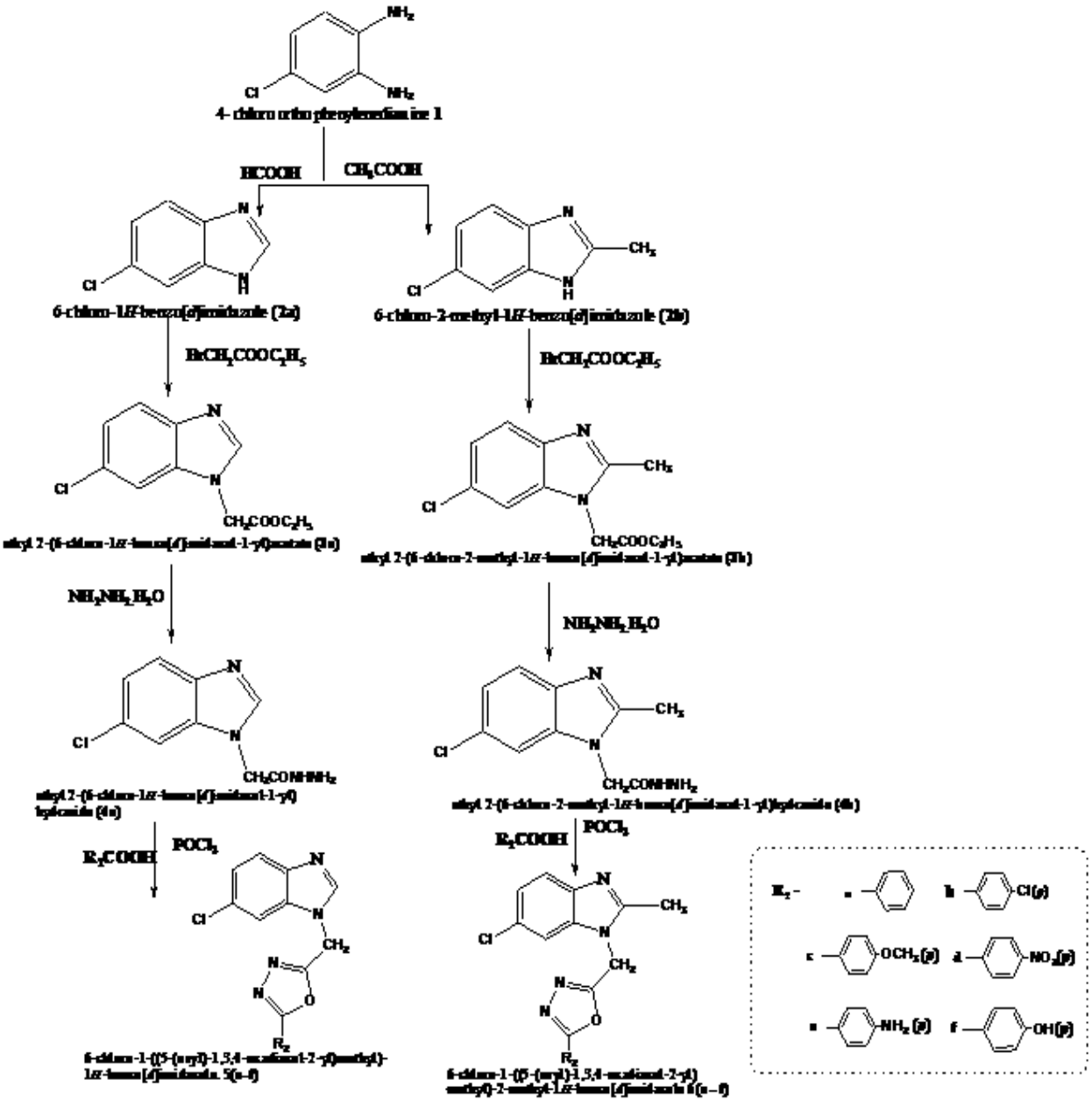
benzimidazole and oxadiazole nucleus, it was thought that it would be worthwhile to design and synthesize some new benzimidazole derivatives having oxadiazole moiety and screen them for potential biological activities. The present work comprises of synthesis of new antimicrobial agent, in which 4- chloro-ortho phenylene diamine are used as a starting material. 12 novel benzimidazole derivatives were synthesized from 2 series as mentioned in scheme.

EXPERIMENTAL

Melting point was determined by thiel's tube method using liquid paraffin and was uncorrected. Infrared (IR) spectra were recorded on a Shimadzu (Japan) 8400 S FT-IR spectrophotometer model using nujol and potassium bromide pellets (υ_{max} in cm^{-1}). ^{1}HNMR spectra were recorded on Brucker multinuclear FT NMR spectrometer model AV-400, 400 MHz using deuterated-chloroform or deuterated dimethylsulfoxide-containing tetramethylsilane (Me_4Si) as internal standard (chemical shifts in δ, ppm). The spin multiplicities are indicated by symbols, s (singlet), d (doublet), t (triplet), m (multiplet), and q (quartet). The purity of compounds was established by thin layer chromatography (TLC). Precoated silica gel aluminium plate 60F-254 (20 cm X 20 cm with 250 μm thickness were used for TLC (E. Merck). Iodine was used to develop the TLC plates. All the solvents were distilled prior to use according to standard procedures. Anhydrous potassium carbonate was used as drying agent.

Methods of synthesis



Scheme

1. Synthesis of 6-chloro-1H-benzo[d]imidazole (2a)

Take 0.24 mol of 4-chloro-o-phenylenediamine in round-bottomed flask and add 0.48 mol of 90% formic acid. Heat the mixture on water bath at 100 C for 2 h. Cool, add 10% NaOH solution slowly with constant rotation of the flask until the mixture is just alkaline to litmus. Filter out the crude benzimidazole at the pump, wash with ice-cold water. Dissolve the crude product in 400 mL of boiling water, add 2 g of decolourizing carbon and digest for 15 min, filter, cool the filtrate to about 10 C, filter off the benzimidazole, wash with 25 mL of cold water and dry at 100 C.Mp: 220 °C. % Yield : 60.08. Molecular Weight: 152.01

FTIRν_{max} (KBr): 2987.9 C-H (stretching aromatic) 1558.8, 1460.0 C = C (stretching aromatic) 3427.27 N-H stretching (Hetrocyclic) 790.9 C - Cl aromatic cm^{-1}.

^{1}H NMR ($CDCl_3$): δ 7.98 (s, 1H), 7.44 (s, 1H), 7.20 (d, *J* = 16.3 Hz, 2H), 6.83 (s, 1H).

2. Synthesis of 6-chloro-2-methyl-1H-benzo[d]imidazole (2b)

Heat together the mixture of 0.03 mol of of 4-chloro-o-phenylenediamine dihydrochloride, 20 mL of water and 0.09 mol of acetic acid under reflux for 45 min, make the cooled reaction mixture distinctly basic by gradual addition of conc. ammonia solution, collect the precipitate product and recrystallized it from 10% aqueous ethanol. Mp: 132 °C. % Yield : 68.38 Molecular Weight: 166.64

FTIRν_{max} (KBr): 3043.4 C-H (stretching aromatic) 1515.9, 1400.0 C = C (stretching aromatic) 3362.06 N-H stretching (Hetrocyclic) 740.4 C - Cl aromatic 2752 C-H stretching (Hetrocyclic) cm^{-1}.

^{1}H NMR ($CDCl_3$): ^{1}H NMR (500 MHz, Chloroform) δ 7.87 – 7.24 (m, 1H), 7.24 – 7.13 (m, 2H), 6.74 (s, 1H), 2.51 (s, 3H).

3. Synthesis of Ethyl 2-(6-chloro-1H-benzo[d]imidazol-1-yl)acetate (3a)

A mixture of equimolar alkaline solution (0.5 mL, 4 N NaOH) of 4-chloro benzimidazole 2a (0.01 mol, 1.18 g) in MeOH (50 mL) and ethylbromoacetate (0.01 mol, 1 mL) in MeOH (30 mL) was heated gently on boiling water bath for 0.5 h. The solid thus obtained on cooling was recrystallized from chloroform to give 3a. Mp: 210 °C. % Yield : 57.07 Molecular Weight: 238.67

FTIRν_{max} (KBr): 2821.06 C-H (stretching aromatic) 1454 C = C (stretching aromatic) 3500.6 N-H stretching (Hetrocyclic) 782.4 C - Cl aromatic 1693.32 C=O stretching for ester 1012.30 C - O stretching for ester cm^{-1}.

^{1}H NMR ($CDCl_3$): δ 9.87 (s, 1H), 7.85 (s, 1H), 7.43 (s, 1H), 7.22 (s, 1H), 7.09 (s, 1H), 5.11 (s, 1H), 4.96 (s, 1H), 3.92 – 3.88 (m, 2H), 3.09 – 3.05 (m, 2H).

4. Synthesis of Ethyl 2-(6-chloro-2-methyl-1H-benzo[d]imidazol-1-yl)acetate (3b)

Ethylchloroacetate (0.01 mol, 1.06 mL) was added to a solution of 2- methyl -4-chloro - benzimidazole (0.01 mol, 1.32 g) in dry acetone (20 mL). To that mixture, anhydrous K2CO3 (1 g) was added and the reaction mixture was refluxed for 10 h. Acetone was removed after completion of reaction and the residue crystallized from ethanol to give compound 3b. Mp: 155 °C. % Yield : 66.50 Molecular Weight: 252.07

FTIRν_{max} (KBr): 3000.9 C-H (stretching aromatic) 1478.8 C = C (stretching aromatic) 3604.3 N-H stretching (Hetrocyclic) 761.0 C - Cl aromatic 1670.2 C=O stretching for ester 1080.8 C - O stretching for ester cm^{-1}.

^{1}H NMR ($CDCl_3$): δ 9.98 (s, 1H), 7.43 (s, 1H), 7.22 (s, 1H), 7.09 (s, 1H), 5.20 (s, 1H), 4.94 (s, 1H), 3.91 – 3.87 (m, 2H), 3.10 – 3.06 (m, 2H), 2.52 – 2.48 (m, 3H).

5. Synthesis of Ethyl 2-(6-chloro-1H-benzo[d]imidazol-1-yl)hydrazide (4a)

To a solution of compounds 3a (0.01 mol) dissolved in dry methanol (50 mL) 99% hydrazine hydrate (1 mL) was added and the mixture was refluxed for 4–5 h. The reaction mixture was cooled and the solid obtained was filtered, washed with small quantity of cold methanol to give 4a.Mp: 130 °C. % Yield : 33.26Molecular Weight: 240.65

FTIRν_{max} (KBr): 3051.1 C-H (stretching aromatic) 1402.1 C = C (stretching aromatic) 3500.9 N-H (stretching Amide) 1672.4 C=O (stretching amide) 1487.0 C-N (stretching amide) cm^{-1}.

^{1}H NMR ($CDCl_3$): δ 7.92 (s, 1H), 7.52 (s, 1H), 7.19 (s, 1H), 7.15 (s, 1H), 6.86 (s, 1H), 5.02 (d, *J* = 1.3 Hz, 2H), 3.62 (s, 1H), 2.58 (s, 1H).

6. Synthesis of Ethyl 2-(6-chloro-2-methyl-1H-benzo[d]imidazol-1-yl)hydrazide (4b).

To a solution of compounds 3b (0.01 mol) dissolved in dry methanol (50 mL) 99% hydrazine hydrate (1 mL) was added and the mixture was

refluxed for 4–5 h. The reaction mixture was cooled and the solid obtained was filtered, washed with small quantity of cold methanol to give 4b.Mp: 120 °C. % Yield : 25.07 Molecular Weight: 254.67.

FTIRν_{max} (KBr): 3141.3 C-H (stretching aromatic) 1390.3 C = C (stretching aromatic)
3444.8 N-H (stretching Amide) 1564.1 C=O (stretching amide) 1471.0 C-N (stretching amide) cm^{-1}.
^{1}H NMR ($CDCl_3$): δ 7.51 (s, 1H), 7.22 (d, *J* = 10.4 Hz, 2H), 6.64 (s, 1H), 5.14 (s, 1H), 4.88 (s, 1H), 3.21 (s, 1H), 2.51 – 2.47 (m, 3H), 2.19 (s, 1H).

7. Synthesis of 6-chloro-2-substituted-1-[{(5-substituted aryl)-1,3,4-oxadiazol-2-yl} methyl]-1H-benzimidazole. (5a-f, 6a-f)

General method:. An equimolar mixture of compound 4 (0.001 mol) and substituted carboxylic acid in phosphoryl chloride was refluxed for 10–16 h. Then reaction mixture was cooled, poured into ice-cold water and neutralized with 20% NaHCO3 solution. The resultant solid was filtered, washed with water and recrystallized from ethanol to give the title compounds.

8. 6-chloro-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-1H-benzo[d] imidazole (5a)
FTIRν_{max} (KBr): 3120.61 C-H (stretching aromatic) 1460.3 C = C (stretching aromatic)
3164.9 C - H Stertching hetroaromatic 1174.5 ring strectching due to C =C & C = N
3425.3 N- H strecthing for azole. cm^{-1}.

^{1}H NMR (DMSO) δ 7.79 (s, 1H), 7.69 (s, 1H), 7.58 – 7.52 (m, 3H), 7.44 – 7.39 (m, 2H), 7.34 (s, 1H), 7.25 (s, 1H), 5.09 (s, 2H). Molecular Weight: 310.74

9. 6-chloro-1-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-benzo[d]imidazole (5b)
FTIRν_{max} (KBr): 2880 C-H (stretching aromatic) 1460.3 C = C (stretching aromatic)
3025 C - H Stertching hetroaromatic 1174.5 ring strectching due to C =C & C = N
3340 N - H strecthing for azole. 796 C - Cl aromatic cm^{-1}.

^{1}H NMR (DMSO) δ 7.95 (s, 1H), 7.85 (s, 1H), 7.62 (s, 1H), 7.53 – 7.48 (m, 2H), 7.46 – 7.41 (m, 2H), 7.27 (s, 1H), 5.79 (s, 2H). Molecular Weight: 345.18

10. 6-chloro-1-((5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-benzo[d]imidazole (5c)
FTIRν_{max} (KBr): 3168 C-H (stretching aromatic) 1440 C = C (stretching aromatic)
3130 C - H Stertching hetroaromatic 1124 ring strectching due to C =C & C = N
3342 N- H strecthing for azole. 1151 C - O -C Stretching cm^{-1}.

^{1}H NMR (DMSO) : δ 7.96 (s, 1H), 7.86 (s, 1H), 7.62 (s, 1H), 7.57 – 7.52 (m, 2H), 7.27 (s, 1H), 7.06 – 7.01 (m, 2H), 5.80 (s, 2H), 3.75 (s, 3H). Molecular Weight: 340.76

11. 6-chloro-1-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-benzo[d]imidazole (5d)
FTIRν_{max} (KBr): 2910 C-H (stretching aromatic) 1450 C = C (stretching aromatic)
1122 ring strectching due to C =C & C = N 1500 N- O Asymmetric strecthing
1391 N- O symmetric strecthing 825 C - N strectching for amine cm^{-1}.

^{1}H NMR (DMSO): δ 8.38 – 8.33 (m, 2H), 7.95 (s, 1H), 7.89 – 7.81 (m, 3H), 7.62 (s, 1H), 7.28 (s, 1H), 5.75 (s, 2H). Molecular Weight: 355.74

12. 6-chloro-1-((5-(4-amino phenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-benzo[d] imidazole (5e)
FTIRν_{max} (KBr): 3064 C-H (stretching aromatic) 1450 C = C (stretching aromatic)
1176 ring strectching due to C =C & C = N 3577 N- H strecthing for amine.
1027 C - N strectching for amine cm^{-1}.

^{1}H NMR (DMSO): δ 7.95 (s, 1H), 7.86 (s, 1H), 7.62 (s, 1H), 7.44 – 7.39 (m, 2H), 7.27 (s, 1H), 6.80 – 6.75 (m, 2H), 5.81 (s, 1H), 5.43 (s, 2H). Molecular Weight: 325.75

13. 6-chloro-1-((5-(4-hydroxy phenyl)-1,3,4-oxadiazol-2-yl)methyl) -1H-benzo[d]imidazole (5f)
FTIRν_{max} (KBr): 3133 C-H (stretching aromatic) 1450 C = C (stretching aromatic)
1244 ring strectching due to C =C & C = N 3200 O- H strecthing for alcohol
1141 C - O strectching for alcohol cm^{-1}.

^{1}H NMR (DMSO): δ 8.62 (s, 1H), 7.95 (s, 1H), 7.86 (s, 1H), 7.62 (s, 1H), 7.44 – 7.39 (m, 2H), 7.27 (s, 1H), 6.93 – 6.88 (m, 2H), 5.80 (s, 2H), 4.99 (s, 1H). Molecular Weight: 326.74

14. 6-chloro-2-methyl-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-1H-benzo[d]imidazole (6a)

FTIRν_{max} (KBr): 2883 C-H (stretching aromatic) 1408 C = C (stretching aromatic)
2900 C - H Stertching hetroaromatic 1135 ring strectching due to C =C & C = N
3505 N- H strecthing for azole. cm^{-1}.

^{1}H NMR (DMSO): δ 7.86 (s, 1H), 7.63 – 7.54 (m, 3H), 7.44 – 7.39 (m, 2H), 7.35 (s, 1H), 7.26 (s, 1H), 5.81 (s, 1H), 5.68 (s, 1H), 2.57 (s, 3H). Molecular Weight: 324.76

15. 6-chloro-1-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-methyl-1H benzo[d]imidazole. (6b)

FTIRν_{max} (KBr): 3118 C-H (stretching aromatic) 1460.3 C = C (stretching aromatic)
3391 C - H Stertching hetroaromatic 1174.5 ring strectching due to C =C & C = N
3340 N - H strecthing for azole. 742 C - Cl aromatic cm^{-1}.

^{1}H NMR (DMSO): δ 7.86 (s, 1H), 7.60 (s, 1H), 7.54 – 7.49 (m, 2H), 7.46 – 7.41 (m, 2H), 7.26 (s, 1H), 5.68 (s, 2H), 2.53 (s, 3H). Molecular Weight: 359.21

16. 6-chloro-1-((5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-methyl-1H benzo[d]imidazole. (6c)

FTIRν_{max} (KBr): 3070 C-H (stretching aromatic) 1407 C = C (stretching aromatic)
3101 C - H Stertching hetroaromatic 1108 ring strectching due to C =C & C = N
3380 N- H strecthing for azole. 1151 C - O -C Stretching cm^{-1}.

^{1}H NMR (DMSO): δ 7.86 (s, 1H), 7.60 (s, 1H), 7.55 (s, 2H), 7.26 (s, 1H), 7.03 (s, 2H), 5.81 (s, 2H), 3.78 (s, 3H), 2.54 (s, 3H). Molecular Weight: 354.79

17.6-chloro-2-methyl-1-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-benzo[d]imidazole. (6d)

FTIRν_{max} (KBr): 3072 C-H (stretching aromatic) 1460 C = C (stretching aromatic)
1164 ring strectching due to C =C & C = N 1510 N- O Asymmetric strecthing
1324 N- O symmetric strecthing 891 C - N stretching for amine cm^{-1}.

^{1}H NMR (DMSO): δ 8.38 – 8.33 (m, 2H), 7.89 – 7.80 (m, 3H), 7.61 (s, 1H), 7.27 (s, 1H), 5.98 (s, 2H), 2.56 (s, 3H). Molecular Weight: 369.74

18. 6-chloro-2-methyl-1-((5-(4-aminophenyl)-1,3,4-oxadiazol-2-yl) methyl)-1H-benzo[d]imidazole (6e)

FTIRν_{max} (KBr): 3089 C-H (stretching aromatic) 1415 C = C (stretching aromatic)
1141 ring strectching due to C =C & C = N 3607 N- H strecthing for amine.
1052 C - N stretching for amine cm^{-1}.

^{1}H NMR (DMSO): δ 7.86 (s, 1H), 7.60 (s, 1H), 7.44 – 7.39 (m, 2H), 7.26 (s, 1H), 6.81 – 6.76 (m, 2H), 5.81 (s, 2H), 5.44 (s, 2H), 2.57 (s, 3H). Molecular Weight: 339.78

19. 4-(5-((6-chloro-2-methyl-1H-benzo[d]imidazol-1-yl)methyl)-1,3,4-oxadiazol-2-yl)phenol. (6f)

FTIRν_{max} (KBr): 2921 C-H (stretching aromatic) 1450 C = C (stretching aromatic)
1263 ring strectching due to C = C & C = N 3436 O- H strecthing for alcohol
1104 C - O stretching for alcohol cm^{-1}.

^{1}H NMR (DMSO): δ 8.62 (s, 1H), 7.86 (s, 1H), 7.60 (s, 1H), 7.44 – 7.39 (m, 2H), 7.26 (s, 1H), 6.88 (s, 2H), 5.80 (s,2H), 2.57 (s, 3H). Molecular Weight: 340.76

Antimicrobial activity test

All the synthesized compounds were screened for their antimicrobial activity against *Escherichia coli* representing Gram – negative bacteria, *Bacillus subtilis* and *Staphylococcus aureus* representing Gram - positive bacteria, *Saccharomyces Cerevisiae* representing Fungi.

Antimicrobial activity of the synthesized compounds was tested by the disc diffusion method under standard conditions using Mueller–Hinton agar medium as described by NCCLS [29]. Sterile filter paper discs (6 mm diameter) containing specific amounts of an antimicrobial agent (300 mg for the synthesized compounds) were placed on the surface of an agar plate inoculated with a standardized suspension of the microorganisms tested. The plates were incubated at 37^{o}C for 48 hrs for evaluating antimicrobial activity. The diameters of inhibition zones (in mm) of triplicate sets were measured and the results of Minimum inhibitory concentration (MIC) by tube dilution are reported in (**Table 2 & 3**) respectively. Paper discs with dimethyl sulfoxide (DMSO) only were utilized as negative controls.

Table 1: List of Synthesized Compounds

S. No	COMPOUND	M.P. (°C)	% YIELD	MOL. WT.	MOL. FORMULA
1	6-chloro-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-1H-benzo[d]imidazole (**5a**)	185	32.82	310.74	$C_{16}H_{11}ClN_4O$
2	6-chloro-1-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-benzo[d]imidazole (**5b)**	160	35.56	345.18	$C_{16}H_{10}Cl_2N_4O$
3	6-chloro-1-((5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-benzo[d]imidazole **(5c)**	175	38.76	340.76	$C_{17}H_{13}ClN_4O_2$
4	6-chloro-1-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-benzo[d]imidazole (**5d)**	145	31.45	355.74	$C_{16}H_{10}ClN_5O_3$
5	6-chloro-1-((5-(4-amino phenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-benzo[d]imidazole (**5e)**	170	34.92	325.75	$C_{16}H_{11}ClN_5O$
6	6-chloro-1-((5-(4-hydroxy phenyl)-1,3,4-oxadiazol-2-yl)methyl) -1H-benzo[d]imidazole (**5f)**	175	37.85	326.74	$C_{16}H_{11}ClN_4O_2$
7	6-chloro-2-methyl-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-1H-benzo[d]imidazole **(6a)**	165	57.85	324.76	$C_{17}H_{13}ClN_4O$
8	6-chloro-1-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-methyl-1H-benzo[d]imidazole. **(6b)**	115	46.85	359.21	$C_{17}H_{12}Cl_2N_4O$
9	6-chloro-1-((5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-methyl-1H-benzo[d]imidazole. **(6c)**	185	28.39	354.79	$C_{18}H_{15}ClN_4O_2$
10	6-chloro-2-methyl-1-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-benzo[d]imidazole. **(6d)**	195	52.16	369.74	$C_{17}H_{12}ClN_5O_3$
11	6-chloro-2-methyl-1-((5-(4-aminophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-benzo[d]imidazole **(6e)**	210	28.89	339.78	$C_{17}H_{14}ClN_5O$
12	4-(5-((6-chloro-2-methyl-1H-benzo[d]imidazol-1-yl)methyl)-1,3,4-oxadiazol-2-yl)phenol. **(6f)**	205	66.34	340.76	$C_{17}H_{13}ClN_4O_2$

RESULTS AND DISCUSSION

The present work comprises of synthesis of new antimicrobial agent, in which 4- chloro-ortho phenylene diamine are used as a starting material to which formic acid/acetic acid reacts and form 2-substituted 6-chloro-benzo[d]imidazole (2a,2b), this further heated with ethylbromoacetate and forms 2-substituted Ethyl 2-(6-chloro-benzo[d]imidazol-1-yl)acetate (3a,3b). The resulting intermediate on treatment with hydrazine hydrate yields Ethyl 2-(6-chloro-2-substituted-1H-benzo[d]imidazol-1-yl)hydrazide (4a,4b) which on further reaction with one equivalent of different substituted aromatic carboxylic acids in the presence of phosphoryl chloride afforded the corresponding target compounds 6-chloro-2-substituted-1-[{(5-substituted aryl)-1,3,4-oxadiazol-2-yl} methyl]-1H-benzimidazole (5a-f, 6a-f). All the spectral data of compounds 2a, 2b, 3a, 3b, 4a, 4b and 5(a-f), 6(a-f) were in accordance with assumed structures.

The Compounds **5(a-f), 6(a-f)** were evaluated for their antimicrobial activity against *Escherichia coli* representing Gram – negative bacteria, *Bacillus subtilis* and *Staphylococcus aureus* representing Gram - positive bacteria, *Saccharomyces Cerevisiae* representing Fungi.

The result of antimicrobial effect of all tested compounds were reported as zone of inhibition in mm and are shown in Table No. 2 and 3. The result revealed that most of newly synthesised compounds exhibited promising antibacterial and antifungal activities. Generally the test compounds **5(e, f), 6(e, f)** showed good activity against Gram – positive bacteria as compared to ciprofloxacin. Other compounds showed moderate activity against gram – positive bacteria.

Compounds **5(b-f)** showed good activity agianst Gram – negative bacteria as compared to ciprofloxacin. Compound 6(**f**), exhibited excellent activity agianst Gram – negative bacteria as compared to ciprofloxacin. Other compounds showed moderate activity against gram – negative bacteria.

Compunds 6e showed good activity agianst *Saccharomyces Cerevisiae*. Other compounds showed moderate anti fungal activity.

Table 2: Antimicrobial activity data at 100µg/ml (after 48 hr)

S. No	Compounds	Zone of Inhibition (mm) (after 48 hrs)			
		Antibacterial			Antifungal
		S. aureus	*B.subtilis*	*E.coli*	*S. Cerevisiae*
1	**5 (a)**	16	18	15	20
2	**5 (b)**	17	18	20	20
3	**5 (c)**	17	19	20	19

4	**5 (d)**	18	17	21	20
5	**5 (e)**	21	18	18	19
6	**5 (f)**	20	21	21	19
7	**6 (a)**	18	20	20	18
8	**6 (b)**	19	18	19	18
9	**6 (c)**	17	19	19	17
10	**6 (d)**	21	17	18	18
11	**6 (e)**	22	17	21	21
12	**6 (f)**	20	18	22	20
13	**Ciprofloxacin**	30	29	30	27

Table 3: MIC of compounds in µg/ml (*B. subtilis*)

Compound	MIC µg/ml
5a	45
5b	25
5c	30
5d	25
5e	15
5f	35
6a	20
6b	15
6c	45
6d	10
6e	15
6f	15

CONCLUSION

In conclusion, several substituted 6-chloro-2-substituted-1-[{(5-substituted aryl)-1,3,4-oxadiazol-2-yl} methyl]-1H-benzimidazole 5(a-f), 6(a-f) were synthesized. The pharmacological study was undertaken to evaluate the effects of substituents on the antibacterial and antifungal activities. All the synthesized compounds exhibited good antibacterial activity towards Gram-positive bacteria and some of the synthesized compounds showed good to moderate antifungal activity. These compounds however did not show any promising activity towards Gram-negative bacteria. The compounds were synthesized in appreciable yields and the structures of the synthesized compounds were evaluated by spectral methods of analyses. All the synthesized compounds were screened for their antimicrobial activity against *Escherichia coli* representing Gram – negative bacteria, *Bacillus subtilis* and *Staphylococcus aureus* representing Gram - positive bacteria, *Saccharomyces Cerevisiae* representing Fungi. The result revealed that most of newly synthesised compounds exhibited promising antibacterial and antifungal activities. Generally the test compounds showed good activity against Gram – positive bacteria, Gram – positive bacteria, fungi. Other compounds showed moderate activity against Gram – positive bacteria, Gram – positive bacteria, fungi. The results showed that all of the compounds have exhibited antimicrobial activity.

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