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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SUBSTITUTED 4-[(1*H*-BENZO[D]IMIDAZOL-2YL)METHYL]PHENOL DERIVATIVES

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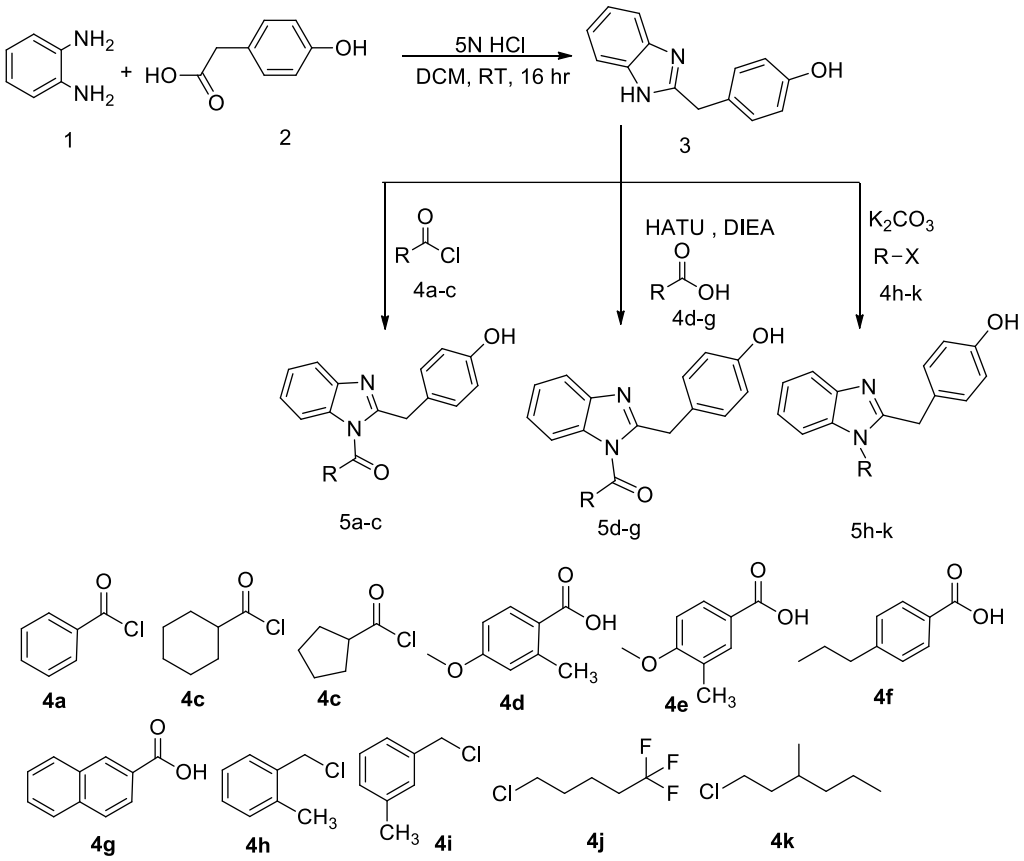
ABSTRACT: A new set of *N*-substituted benzimidazole analogues were prepared by the reaction of respective benzimidazoles with different acids, acid chlorides and alky alides under appropriate conditions. All the compounds' structure was analysed by FT-IR, ^{1}H-NMR, and Mass spectral data. The synthesized compounds were evaluated for their *in vitro* antibacterial and antifungal activity and also studied their molecular docking.

KEYWORDS: Benzimidazole, benzenediamine, 2-(4-hydroxyphenyl)acetic acid, Antimicrobial and Antifungal Activity, Docking studies.

INTRODUCTION: Benzimidazole derivatives are privileged heterocyclic compounds in medicinal chemistry and drug development due to their wide variety of applications pharmaceutical industry. Benzimidazole derivatives are possess multiple biological activities such as antitumor[i], antibacterial[ii], antifungal[iii], antiviral[iv], anticonvulsant[V], antidepressant[vi], analgesic[vii], anti-inflammatory[viii] and antidiabetic[ix] properties. Benzimidazole derivatives are also found in progesterone receptor antagonist and luteinizing hormone-releasing hormone antagonists. Moreover, thiabendazole, cambendazole, parbendazole, mebendazole, albendazole and flubendazole are widely-used anti-helminth drugs[x], used to treat people and animals with gastrointestinal worm infections. In addition, pyrethroid pesticides is a benzimidazole derivatives are widely used in agriculture to protect crops and in households to control insect pests because of their high efficiency, low toxicity and low residue. Bezimidazole scaffold have offered high biological activities that have proven useful for the development of new medicinal agents having improved potency and lesser toxicity. Consequently, developing environmentally benign approaches using cheap and unharmful starting materials is highly desirable. It was considered worthwhile that to synthesize convinced new chemical entities include two active pharmacophores such as benzimidazoles and phenol nucleus single molecular frame work and to get them evaluated for their antimicrobial activity.

RESULTS AND DISCUSSIONS:

CHEMISTRY: For the synthesis of 4-((1H-benzo[d]imidazol-2-yl) methyl) phenol (**3**), a condensation reaction was employed using 1,2-diaminobenzene **(1)**, 4-hydroxyphenylaceticacid **(2)** and hydrochloric acid used as catalyst in DCM medium to afford.4-((1H-benzo[d]imidazol-2-yl) methyl) phenol in good yield. A several number of targeted substituted benzimidazole analogues (**5a-l**) were synthesized with excellent yields by the reaction between 4-((1H-benzo[d]imidazol-2-yl) methyl) phenol (**3**) with different substituted aromatic acyl halides (**4a-c**), carboxylic acids (**4d--g**) and aryl & alkyl halides (**4h-f**). HATU is a reagent used in peptide coupling chemistry to generate an active amide from a carboxylic acid. HATU is used along with Hünig's base (N, N-diisopropylethylamine, DIPEA), or triethylamine to form amide bonds. Typically DMF is used as solvent, although other polar aprotic solvents can also be used. The structures of the newly synthesized derivatives **(5a-k)** were confirmed based on their FT-IR, ^{1}H-NMR, 13C-NMR and Mass spectrometry.



ANTIMICROBIAL ACTIVITY: The *in vitro* antibacterial activity was performed against a series of Gram-positive bacteria and Gram-negative bacteria such as *Mycobacterium tuberculosis (M. tub), Micrococcus luteus (M. lut), Methicillin-resistant Staphylococcus aureus (MRSA), Bacillus subtilis (B. sub) and Bacillus cereus (B. cer),* in such as *Pseudomonas aeruginosa (P. aer), Klebsiella pneumonia (K. pne), Escherichia coli (E. col), Proteus vulgaris (P. vul), Salmonella typhi (S. typ).* Ciprofloxacin used as standard reference drug and the activity screened using agar well diffusion technique. Among all synthesised derivatives, the compounds **5c, 5d** and **5e** have shown good inhibition activity against almost all stains. All compounds were also screened for their antifungal activity against four pathogenic fungi such as *Aspergillus Niger Candida albicansusing, Fusarium Oxysporum,* and *Fusarium solan.* Nystatin used as standard reference drug and the activity screened using agar well diffusion technique. All the compounds showed moderate antifungal activity against the tested fungal

organism. The derivatives **5c, 5e, 5g** and **5k** have shown good inhibition activity against fungal organism.

MOLECULAR DOCKING STUDIES: The in-silico screening of all newly synthesized molecules was carried out by using Auto dock tools against the Wild-type Bacillus Subtilis lipase A of *Bacillus Subtilis* bacteria and Secreted aspartic proteinase of Candida albicans fungus. The Auto dock 4.2 suite uses Lamarckian genetic algorithm to find the best conformer and its binding interactions. Almost all the new ligands have shown interactions with the target molecules of *Bacillus Subtilis* and *Candida albicans*.

Table-1: Docking results of antibacterial activity of compounds **5a-k** against the Bacillus subtilis lipase A (PDB ID: 5CRI) of Bacillus subtilis.

S. No	Ligand	Binding Energy	No. of Interactions	Binding Site
1	5a	-8.0	2	ILE87 & LEU108
2	5b	-8.12	2	ASN48 & LYS88
3	5c	-7.93	1	GLY116
4	5d	-7.95	1	GLY116
5	5e	-7.63	1	GLY93
6	5f	-8.41	1	THR117
7	5g	-7.83	1	LEU90
8	5h	-8.31	1	LEU90
9	5i	-8.16	1	LYS88
10	5j	-8.26	3	ASN48, GLY116 & THR117
11	5k	-8.4	2	ASN48 & LYS88
12	Std	-7.58	2	ASN48 & LYS88

Compound **5e** has shown the highest docking score of -8.41 with one interaction at the binding site THR117 of Bacillus subtilis lipase A and Compound **5k** showed a lower score of -7.58 with two interactions at binding sites ASN48 and LYS88.

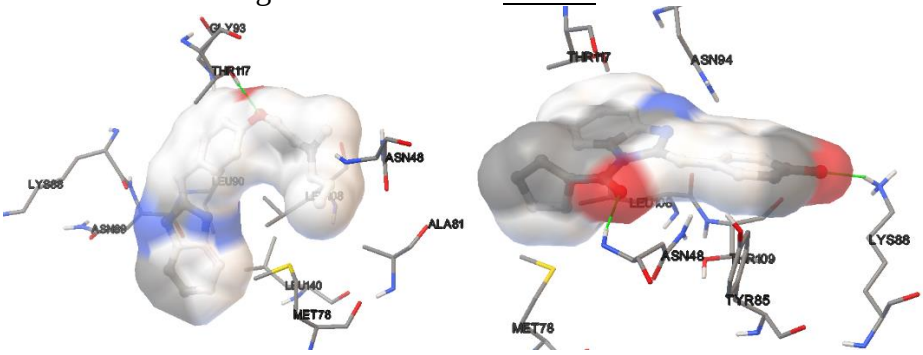


Figure-1: Docking pose of compound s **5e** & **5k** with Bacillus subtilis lipase A.

Table-2: Docking results of antifungal activity of compounds **5a-k** against Secreted aspartic proteinase (PDB ID: 2QZW) of Candida albicans.

S.No	**Ligand**	**Binding Energy**	**No. of interactions**	**Binding Site**
1	5a	-8.15	1	ARG192
2	5b	-8.18	1	SER336
3	5c	-8.15	1	ASN131
4	5d	-8.15	1	ASN 132
5	5e	-6.86	0	--

6	5f	-7.57	0	--
7	5g	-7.89	1	SER336
8	5h	-10.84	1	SER36
9	5i	-8.88	1	SER36
10	5j	-8.75	1	ARG192
11	5k	-9.89	1	SER36
12	Std	-8.94	1	SER36

The compound **5h** has shown highest docking score of -10.84 with an interaction at binding site SER36 of aspartic proteinase of Candida albicans and Compounds **5e** and **5f** have not shown any interaction with least docking score of -6.86 & -7.57 respectively.

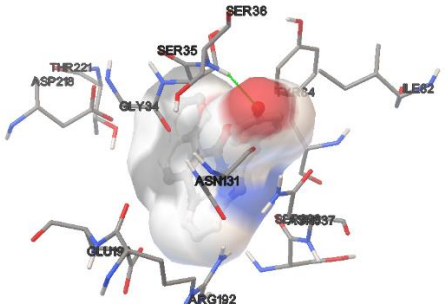


Figure-3: Docking pose of Compound 7 with aspartic proteinase (PDB ID:2QZW)

EXPERIMENTAL: Melting points are uncorrected and were find out in open capillary tubes in sulphuric acid bath. TLC was carrying out on silica gel-G, and spotting was done using UV light. IR spectra were recorded using Perkin-Elmer 1000 instrument in KBr phase, The 1 H NMR spectra were record on a Varian as 400 MHz instrument in $CDCl_3$, chemical shifts are given in ppm relative to TMS, and coupling constants (J) are expressed in hertz (Hz). Combinations of the following abbreviations are used to describe NMR spectra: s-singlet; d-doublet; t-triplet; m-multiplet. 13C NMR spectra were recorded with a Bruker Advance 400 (100 MHz) spectrometer. Mass spectra on Agilent LCMS instrument giving only (M+H) values.

General procedure for preparation of compounds 5a & 5b: A solution of acid halide (**4a-b**) (10 mmol) in DCM (10 vol) was added to amine (**3**) (10 mmol) the reaction mixture was stirred at RT for 16 h and monitored by TLC. After complete conversion of starting materials, the mixture was poured in to ice cold water (10 ml) extracted with DCM (3 X 20 mL) the organic layer was washed with brine (2 x 10 ml) the organic layer was dried over anhydrous sodium sulphate, filtered and concentrated in vacuo. The crude product was purified by column chromatography using silica gel (100-200 mesh) eluted at 10% ethyl acetate in pet ether to afford pure products **5a & 5b**.

(2-(4-hydroxybenzyle)-1H-benzo[d]imidazole-1-yl)(phenyl)methanone (5a): Yield 93%; MP 156-159°C; IR spectrum, ν, cm^{-1}: 3360, 3078, 2740, 1680; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.07-8.10 (d, 2H), 7.21-7.69 (m, 11H), 6.96-6.98 (d, 2H), 4.56 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 183.5, 152.4, 151.0, 141.7, 138.5, 130.1, 127.9, 124.3, 122.7, 119.5, 114.9, 35.0 ppm; MS (ESI+): m/z = 328.

Cyclohexyl (2-(4-hydroxy benzyl)-1H-benzo[d] imidazol-1-yl)methanone (5b): Yield 91%: MP 151-155°C; IR spectrum, ν, cm^{-1}: 3365, 3045, 2870, 1670 cm^{-1}; ^{1}H-NMR (400MHz, $CDCl_3$): 7.18-7.77 (m, 8H), 6.84-6.85 (d, 2H), 4.50 (s, 2H), 2.45-2.48 (m, 5H), 1.35-1.99 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 184.0, 152.5, 151.5, 141.9, 139.2, 131.4, 122.0, 111.0, 49.1, 30.5, 29.5, 27.9, 24.7, 20.7, 12.1 ppm; MS (ESI+): m/z = 309.

Cyclopentyl (2-(4-hydroxybenzyl)-1H-benzo[d]imidazol-1-yl)methanone (5c): Yield: 94%; MP: 153–157°C; IR spectrum, ν, cm^{-1}: 3365, 3060, 1660; 1H-NMR (400MHz, $CDCl_3$): δ 7.33-7.49 (m, 4H), 7.52-7.65 (m, 4H), 6.90-6.92 (d, 2H), 4.50 (s, 2H), 2.65-2.75 (m, 3H), 1.55-2.00 (m, 6H),; ^{13}C-NMR (100MHz, $CDCl_3$): δ 183.7, 148.3, 141.5, 138.9, 133.0, 130.7, 129.4, 123.0, 121.5, 115.2, 114.0, 32.0, 12.2, 12.3, 11.4; MS (ESI+): m/z =361.

General procedure for preparation of compounds 5d-g: A solution acid **(4d-g)** (15 mmol) in DMF (10 vol) was added to HATU (15 mmol) then add amine **(3)** (10 mmol) followed by addition of DIPEA (25 mmol) the reaction mixture was stirred at RT for 16 hrs and monitored by TLC for complete conversion of starting materials. After complete, the mixture was poured in to ice cold water (10 ml) extract with ethyl acetate (3 X 20ml) the organic layer was washed with brine (2x10 ml) the organic layer was dried over anhydrous sodium sulphate and concentrated in vacuo. The crude product was purified by silica gel eluted at 10% ethyl acetate in pet ether to afford desired products **5d-g.**

(2-(4-hydroxybenzyl)-1H-benzo[d]imidazole-1-yl)(4-methoxy-2-methylphenyl) methanone (5d) : Yield: 97%; MP 142-147°C; IR spectrum, ν ,cm^{-1}: 3365, 3040, 2950, 1680, 1240; ^{1}H-NMR (400MHz, $CDCl_3$): 7.77-7.22 (m, 9H), 6.83-6.81 (d, 2H), 4.31 (s, 2H), 3.88 (s, 3H), 2.65 (s, 3H); ^{13}C-NMR (100MHz, $CDCl_3$): 163.0, 150.2, 144.5, 133.7, 130.2, 122.6, 120.2, 117.2, 111.2, 55.3, 38.8, 22.5 ppm; MS (ESI+): m/z =373.11 ([M+H]+); and MS (ESI+): m/z = 373.50

(2-(4-hydroxybenzyl)-1H-benzo[d]imidazole-1-yl)(4-methoxy-3-methylphenyl)methanone (5e): Yield 88%; MP 156-159°C; IR spectrum, ν, cm^{-1}: 3360, 2926, 2870, 2855, 1660; ^{1}H-NMR; (400MHz, $CDCl_3$): 7.72-7.18 (m, 9H), 6.92-6.90 (d, 2H) 4.34 (s, 2H) 3.90 (s, 3H), 2.28 (s, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): 163.0, 150.2, 144.5, 132.5, 130.3, 130.2, 122.5, 109.4, 55.5, 35.4, 22.7 ppm ; MS (ESI+): m/z = 373.50

(2-(4-Hydroxybenzyl)-1H-benzo[d]imidazole-1-yl)(4-propylphenyl)methanone (2i): Yield: 97%; MP: 156–159°C; IR spectrum, ν, cm^{-1}: 3350, 2950, 2860, 1680; ^{1}H-NMR (400MHz, $CDCl_3$): 7.58-7.22 (m, 8H) 7.16-6.92 (m, 5H), 4.28 (s, 2H), 0.83-088 (m, 6H); ^{13}C-NMR (100MHz, $CDCl_3$) 165.6, 150.1, 149.4, 133.8, 130.6, 128.8, 126.6, 122.4, 38.1, 31.9, 24.22, 14.09 ppm; MS (ESI+): m/z = 371.00.

(2-(4-hydroxybenzyl)-1H-benzo[d]imidazole-1-yl)(naphthalene-1-yl)methnone (5g): Yield: 96%; MP 156–159°C; IR spectrum, ν, cm^{-1}: 3355, 3055, 2870, 1660; ^{1}H-NMR (400MHz, $CDCl_3$): 7.96-7.67 (m, 9H), 7.64-7.38 (m, 6H), 7.27-7.23 (m, 2H), 4.35 (s, 2H); ^{13}C-NMR (100MHz, $CDCl_3$): 167.7, 155.5, 141.5, 138.9, 134.5, 134.3, 133.6, 132.2, 131.9, 130.7, 130.4, 130.1, 129.3, 128.8 ppm; MS (ESI+): m/z = 379.16

General procedure for preparation of compounds 5h-k: A solution alkyl halide (1.06 mmol) in DMF (10 vol) was added to amine (0.89 mmol) than add K_2CO_3 salt (1.77m.moles) the reaction mixture was stirred at RT for 16 hr and monitored by TLC for complete conversion of starting materials the mixture was poured in to ice cold water (10ml) extract with ethyl acetate(3 X 20ml) the organic layer were washed with brine (2 X 10 ml) the organic layer was dried over anhydrous sodium sulphate filtered and concentrated in vacuo. The crude product was purified by silica gel eluted at 10% ethyl acetate in pet ether to afford desired compounds **5h-k.**

4-((1-(2-Methylbenzyl)-1H-benzo[d]imidazol-2-yl)methyl)phenol (5h): Yield 88%; MP 146–150°C; IR spectrum, ν, cm^{-1}: 3370, 3064, 2870, 2860; ^{1}H NMR (400MHz, $CDCl_3$): 7.93-7.12 (m, 10H), 6.92-6.90 (d, 2H), 5.02 (s, 2H), 4.25 (s, 2H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 158.1, 136.5, 134.5, 130.5, 128.6, 128.2, 126.2, 126.0, 122.1, 115.3, 58.5, 35.3, 19.4 ppm; MS (ESI+): m/z = 329.51.

4-(1-(3-methylbenzyl)-1H-benzo[d]imidazole-2-yl) phenol (5i): Yield 92%, MP 145–149°C. IR spectrum, ν, cm^{-1}= 3370, 3064, 2870,2860 ;1H NMR;(400MHz; $CDCl_3$): 7.91-7.17

(m, 10H), 6.92-6.90 (d, 2H), 5.02 (s.2H), 4.25 (s, 2H), 2.37 (s, 3H); ^{13}CNMR; (100 MHz, $CDCl_3$): 158.1, 154.0, 138.2, 136.4, 130.3,128.5, 128.2, 124.4, 122.4, 115.5, 58.7, 34.9, 30.7, 21.4 ppm; MS (ESI+): m/z = 329.51 ([M+H] +); MS (ESI+): m/z = 329.51.

2-(4-((5,5,5-trifluoropentyl) oxy) benzyl)-1H-benzo[d]imidazole (5j): Yield: 89%; MP: 156–160; IR spectrum, ν, cm^{-1}: 3350, 3040, 1260, 2850; ^{1}H-NMR (400MHz, $CDCl_3$): 7.84-7.20 (m, 6H), 6.94-6.92 (d, 2H), 4.27 (s, 2H), 3.91 (t, 2H), 2.22-2.01 (m, 2H), 1.82-1.21 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): 158.0, 153.6, 130.0, 128.3, 122.0, 114.7, 67.3, 58.4, 35.1, 28.2, 18.9, 18.8, 18.4 ppm; MS (ESI+): m/z = 349.48.

2-(4-methylpentyl)oxy)benzyl)-1H-benzo[d]imidazole (5k): Yield: 92%; MP: 145–150°C; IR spectrum, ν, cm^{-1}: 3350, 3340, 2860, 1250; ^{1}HNMR (400MHz, $CDCl_3$): 7.80-7.21 (m, 6H), 6.94-6.92 (d, 2H), 4.24 (s, 2H), 3.96-4.00 (t, 1H), 2.91-1.40 (m,10H); ^{13}C NMR (100 MHz, $CDCl_3$): 158.3, 130.1, 127.5, 122.1, 115.3, 66.3, 39.5, 36.2, 34.9, 29.5, 19.8, 19.6, 14.4 ppm; MS (ESI+): m/z = 323.53

CONCLUSION: To the prominence of our knowledge, this is the best report on the synthesis of substitution on benzimidazole bearing phenol ring. Among all the derivatives, the compounds **5c** and **5e** were potential to antimicrobial activity and that also evidenced by molecular docking studies.

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