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Research Article

Synthesis, Antimicrobial Activity and Molecular Docking Study of Some Novel Isoxazole Incorporated Benzimidazole Derivatives

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ABSTRACT

A series of novel isoxazole-incorporated benzimidazole derivatives was synthesized and investigated for antimicrobial activity. The structures of all synthesized compounds were confirmed by means of elemental analysis, infrared spectroscopy (IR), proton nuclear magnetic resonance (^{1}H-NMR), and liquid chromatography-mass spectrometry (LC-MS). All compounds were evaluated for antimicrobial activity cup plate method against *Staphylococcus aureus, Bacillus anthracis, Pseudomonas aeruginosa, Escherchia coli, Candida albicans* and *Aspegillus niger.* The 4d, 4f and 4j compounds showed significant activity against gram-positive and gram-negative bacteria. On the basis of the interaction energy criterion, compound 4f showed the best docking interactions equal to 7.0 kcal/mol.

INTRODUCTION

According to the Centers for Disease Control and perevntion (CDC) more than 19 million antimicrobial-resistant infections occur every year, and more than 2.5 lakh people die as a result.[1-6] Many pathogenic microorganisms such as bacteria, fungi, and viruses are reported to cause serious issues, including local irritation, systemic toxicity, drug resistance, hypersensitivity, superinfection, and inadequate nutrition.[7-17] Hence, several alternative strategies have been discovered to combat the booming microbial infection cases and curb their resistance mechanisms toward antimicrobial agents. Several naturally derived compounds have been isolated and identified as potential antimicrobial drugs.[18-24] Multiple plant-based phenolic compounds have become promising antimicrobial agents for human pathogenic bacteria.[25-33] The issue has been identified as a major concern by multiple organizations and governments that have implemented "global action plans" and exists an urgent need for the development of novel, inexpensive antimicrobials for the treatment of drug-resistant bacterial infections.[34-40]

The isoxazole and benzimidazole are flexible lead molecules used in pharmaceutical creation and have various biological functions.[41,42] The isoxazole and benzimidazole derivatives both are of great importance in medicinal chemistry and can be used for the synthesis of numerous heterocyclic compounds with different biological activities such as anticonvulsant,[43,44] antidepressant,[45,46] antituberculosis,[47,48] anti-inflammatory,[49,50] antibacterial,[51,52] antiviral,[53,54] antifungal,[55,56] and anticancer[57,58] activities etc.

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In the present work, we planned to develop novel isoxazole-incorporated benzimidazole derivatives and screen for antibacterial and antifungal activity.

Material And Methods

All the melting points reported were determined by open capillary tube method and were not corrected. The synthesis and analytical studies of the compounds were carried out using laboratory grade and analytical grade reagents as the case may be standard procedure or reported method were followed with or without modification, appropriately as and when required. Elemental analysis (C, H and N) was undertaken with a Perkin-Elmer model 240C analyzer, and all analyses were consistent with theoretical values (within 0.4%) unless indicated. IR absorption spectra were recorded on Bruker alpha. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on the Bruker DPX-400 instrument at 400 MHz. The ^{1}H chemical shifts are reported as parts per million (ppm) downfield from TMS (Me4Si). The compounds' liquid chromatography-mass spectrometry (LC-MS) were recorded on Shimadzu 8201PC spectrometer. The homogeneity of the compounds was monitored by ascending thin-layer chromatography (TLC) on silica gel G (Merck)-coated aluminum plates, visualized by iodine vapor.

1-(1*H*-benzo[d]imidazol-2-yl)ethanol (1)

o-Phenylenediamine (0.25 mol) was mixed with lactic acid (0.36 mol) in RBF and refluxed for 3 hours. The reaction mixture was cooled and added with 10% NaOH until basicity to litmus paper. The crude pink-colored product obtained was dissolved in 400 mL of boiling water. To this add 2 gm of decolorising carbon and heated for 15 minutes. The mixture was filtered rapidly at the pump through a preheated Buchner funnel. The product obtained was further filtered, washed with 25 mL cold water, and dried at 100°C.

Melting Point: 178–180°C; Yield: 77%; R_f value: 0.84; Solvent system: Benzene: Methanol (9:1); IR (υ_{max}, cm^{-1}): 3348, 3234 (N–H), 3065 (Ar. C–H), 1542, 1486 (Ar. C=C), 1432 (–CH_3), 3624 (O–H).

1-(1*H*-benzo[*d*]imidazol-2-yl)ethanone (2)

A solution of compound 1 (0.01 mol) in dil. H_2SO_4 (5%, 40 mL) was added dropwise to the solution of $K_2Cr_2O_7$ (0.15 mol) in H_2SO_4 (25%, 80 mL) with constant stirring at room temperature over a period of 20 minutes. Further the reaction mixture was stirred at room temperature for 2 hours. After completion of the reaction, the reaction mixture was neutralized with aqueous ammonia solution (1:1) and resultant orange solid was filtered, washed with water, dried and recrystallized from ethyl acetate.

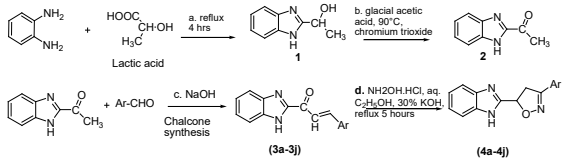

Fig. 1: Synthesis of isoxazole incorporated benzimidazole derivatives

Melting Point: 238–240°C; Yield: 87%; R_f value: 0.72; Solvent system: Benzene: Methanol (8:2); IR (υ_{max}, cm^{-1}): 3356, 3249 (N–H), 3068 (Ar. C–H), 1722 (C=O), 1549, 1521 (Ar. C=C), 1411 (–CH_3).

1-(1*H*-benzo[*d*]imidazol-2-yl)-3-phenylprop-2-en-1-one (3a)

The compound 4 (0.01 mol) and appropriately substituted aromatic aldehydes (0.012 mol) were mixed in ethanol (20 mL) containing 10% aq. KOH (8 mL) and magnetically stirred the solution constantly at room temperature for 10 hours. The whole mixture transferred into 100 mL ice cold water and acidified with dil. HCl. The solid formed was filtered, washed, dried and recrystallized from absolute ethanol.

Melting Point: 200–204°C; Yield: 71%; R_f value: 0.85; Solvent system: Benzene: Methanol (9:1); IR (υ_{max}, cm^{-1}): 3445, 3341 (N–H), 3083 (Ar. C–H), 1735 (C=O), 1587, 1491 (Ar. C=C).

1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(4-hydroxyphenyl) prop-2-en-1-one (3b)

Melting Point: 182–186°C; Yield: 68%; R_f value: 0.79; Solvent system: Benzene: Methanol (9:1); IR (υ_{max}, cm^{-1}): 3466, 3375 (N–H), 3061 (Ar. C–H), 1723 (C=O), 1469, 1488 (Ar. C=C).

1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(3-methoxyphenyl)prop-2-en-1-one (3c)

Melting Point: 188–192°C; Yield: 82%; R_f value: 0.67; Solvent system: Benzene: Methanol (9:1); IR (υ_{max}, cm^{-1}): 3364, 3264 (N–H), 3055 (Ar. C–H), 1721 (C=O), 1565, 1479 (Ar. C=C), 1264 (C–O).

1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (3d)

Melting Point: 172–176°C; Yield: 71%; R_f value: 0.86; Solvent system: Benzene: Methanol (9:1); IR (υ_{max}, cm^{-1}): 3465, 3324 (N–H), 3081 (Ar. C–H), 1733 (C=O), 1543, 1483 (Ar. C=C), 1264 (C–O).

1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3e)

Melting Point: 204–208°C; Yield: 77%; R_f value: 0.79; Solvent system: Benzene: Methanol (9:1); IR (υ_{max}, cm^{-1}): 3244, 3167 (N–H), 3059 (Ar. C–H), 1728 (C=O), 1545, 1462 (Ar. C=C), 1156 (C–O).

1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(4-nitrophenyl) prop-2-en-1-one (3f)

Melting Point: 180–184°C; Yield: 81%; R_f value: 0.78; Solvent system: Benzene: Methanol (9:1); IR (υ_{max}, cm^{-1}):

3465, 3394 (N–H), 3153 (Ar. C–H), 1723 (C=O), 1526, 1478 (Ar. C=C), 1456 (–NO_2).

1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(4-chlorophenyl) prop-2-en-1-one (3g)

Melting Point: 224–228°C; Yield: 74%; R_f value: 0.66; Solvent system: Benzene: Methanol (9:1); IR (υ_{max}, cm^{-1}): 3346, 3248 (N–H), 3089 (Ar. C–H), 1735 (C=O), 1526, 1483 (Ar. C=C) 762 (C–Cl).

1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(4-fluorophenyl) prop-2-en-1-one (3h)

Melting Point: 176–180°C; Yield: 68%; R_f value: 0.81; Solvent system: Benzene: Methanol (9:1); IR (υ_{max}, cm^{-1}): 3465, 3349 (N–H), 3083 (Ar. C–H), 1737 (C=O), 1539, 1481 (Ar. C=C).

1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(4-bromophenyl) prop-2-en-1-one (3i)

Melting Point: 170–172°C; Yield: 73%; R_f value: 0.76; Solvent system: Benzene: Methanol (9:1); IR (υ_{max}, cm^{-1}): 3464, 3369 (N–H), 3126, 3079 (Ar. C–H), 1731 (C=O), 1676 (Ar. C=N) 1564 (Ar. C=C).

1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (3j)

Melting Point: 164–168°C; Yield: 77%; R_f value: 0.68; Solvent system: Benzene: Methanol (9:1); IR (υ_{max}, cm^{-1}): 3346, 3249 (N–H), 3081 (Ar. C–H), 1665 (C=O), 1546, 1494 (Ar. C=C), 2146 (C–C).

2-(3-phenyl-4,5-dihydroisoxazol-5-yl)-1*H*-benzo[*d*] imidazole (4a)

A mixture of chalcone (3a) (0.01 mol) and hydroxylamine hydrochloride (0.01 mol) along with 30% KOH in 50 mL ethanol was stirred and refluxed for 5 to 6 hours. TLC monitored the progress of the reaction. The reaction mixture was cooled and poured into ice cold water, filtered and dried to get the final product recrystallized from aqueous ethanol.

MeltingPoint: 212–214°C; Yield: 84%; $R_f$value:0.81; Solventsystem:Benzene: Ethylacetate: Methanol (9: 0.5: 0.5); Anal. Calcd. for $C_{16}H_{13}N_3O$ (363.29): C, 72.99; H, 4.98; N, 15.96.

Found: C, 72.54; H, 4.59; N, 15.95; IR (υ_{max}, cm^{-1}): 3412, 3379 (N–H), 3062, (Ar. C–H), 1655 (C=N), 1591, 1448 (Ar. C=C), 1262 (C–O); ^{1}H-NMR (400 MHz, DMSO-d_6); δ: 3.22 (m, 1H, CH_2), 3.42 (m, 1H, CH_2), 5.91 (t, 1H, CH), 6.91 (s, 1H, NH), 7.01-7.52 (m, 9H, Ar-H); LCMS (m/z): $[M]^+$; 263.11

A similar procedure was adopted to synthesize derivatives (4b-4j).

4-(5-(1*H*-benzo[*d*]imidazol-2-yl)-4,5-dihydroisoxazol-3-yl)phenol (4b)

Melting Point: 228–230°C; Yield: 88%; R_f value: 0.76; Solvent system: Benzene: Ethylacetate: Methanol (9:0.5:0.5); Anal. Calcd. for $C_{16}H_{13}N_3O_2$ (279.29): C, 68.81; H, 4.69; N, 15.05.

Found: C, 69.15; H, 4.58; N, 15.27; IR (υ_{max}, cm^{-1}): 3674 (O–H), 397, 3309 (N–H), 2923 (Ar. C–H), 1646 (C=N), 1575, 1506 (Ar. C=C), 1159 (C–O); ^{1}H -NMR (400 MHz, DMSO-d_6); δ: 3.25–3.28 (m, 1H, CH_2), 3.42-3.47 (m, 1H, CH_2), 3.98 (s, 1H, OH), 5.95 (t, 1H, CH), 6.98 (s, 1H, NH), 7.01-7.60 (m, 8H, Ar-H); LCMS (m/z): $[M]^+$; 279.10.

2-(3-(3-methoxyphenyl)-4,5-dihydroisoxazol-5-yl)-1*H*-benzo[*d*]imidazole (4c)

Melting Point: 222–224°C; Yield: 85%; R_f value: 0.78; Solvent system: Benzene: Ethylacetate: Methanol (9:0.5:0.5); Anal. Calcd. for $C_{17}H_{15}N_3O_2$ (293.32): C, 69.61; H, 5.15; N, 14.33.

Found: C, 69.54; H, 5.02; N, 14.21; IR (υ_{max}, cm^{-1}): 3432, 3364 (N–H), 3075 (Ar. C–H), 1675 (C=N), 1546, 1486 (Ar. C=C), 1284 (C–O); ^{1}H-NMR (400 MHz, DMSO-d_6); δ: 3.22 (m, 1H, CH_2), 3.34 (m, 1H, CH_2), 3.76 (s, 3H, OCH_3), 5.69 (t, 1H, CH), 6.94 (s, 1H, NH), 7.03–7.59 (m, 8H, Ar-H); LCMS (m/z): $[M]^+$; 293.12

2-(3-(3,4-dimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)-1*H*-benzo[*d*]imidazole (4d)

Melting Point: 236–238°C; Yield:87%; $R_f$value:0.79; Solvent system: Benzene: Ethylacetate: Methanol (9: 0.5: 0.5); Anal. Calcd. for $C_{18}H_{17}N_3O_3$ (323.35): C, 66.86; H, 5.30; N, 13.00. Found: C, 67.10; H, 5.19; N, 12.68; IR (υ_{max}, cm^{-1}): 3463 (N–H), 3068 (Ar. C–H), 1674 (C=N), 1567, 1499 (Ar. C=C), 1255 (C–O); ^{1}H-NMR (400 MHz, DMSO-d_6); δ: 3.26 (m, 1H, CH_2), 3.38 (m, 1H, CH_2), 3.78 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 5.74 (t, 1H, CH), 6.87 (s, 1H, NH), 6.97–7.45 (m, 7H, Ar-H); LC-MS (m/z): $[M]^+$; 323.13.

2-(3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)-1*H*-benzo[*d*]imidazole (4e)

Melting Point: 246–248°C; Yield:86%; R_f value:0.83; Solvent system: Benzene: Ethylacetate: Methanol (9: 0.5: 0.5); Anal. Calcd. for $C_{19}H_{19}N_3O_4$ (353.37): C, 64.58; H, 5.42; N, 11.89.

Found: C, 64.78; H, 5.54; N, 12.02; IR (υ_{max}, cm^{-1}): 3345, 3264 (N–H), 3076 (Ar. C–H), 1657 (C=N), 1524, 1451 (Ar. C=C), 1178 (C-O); ^{1}H-NMR (400 MHz, DMSO-d_6); δ: 3.29 (m, 1H, CH_2), 3.42 (m, 1H, CH_2), 3.81 (s, 3H, OCH_3), 3.82 (s, 6H, OCH_3), 5.69 (t, 1H, CH), 6.92 (s, 1H, NH), 7.06-7.59 (m, 6H, Ar-H); LCMS (m/z): $[M]^+$; 353.14

2-(3-(4-nitrophenyl)-4,5-dihydroisoxazol-5-yl)-1*H*-benzo[*d*]imidazole (4f)

Melting Point: 238–240°C; Yield: 89%; R_f value:0.72; Solvent system: Benzene: Ethylacetate: Methanol (9: 0.5: 0.5); Anal. Calcd. for $C_{16}H_{12}N_4O_3$ (308.29): C, 62.33; H, 3.92; N, 18.17.

Found: C, 62.68; H, 4.16; N, 18.26; IR (υ_{max}, cm^{-1}): 3421 (N–H), 3071 (Ar. C–H), 1635 (C=N), 1564, 1474 (Ar. C=C) 1486 (–NO_2); ^{1}H-NMR (400 MHz, DMSO-d_6); δ: 3.33 (m, 1H,

CH_2), 3.49 (m, 1H, CH_2), 5.78 (t, 1H, CH), 6.96 (s, 1H, NH), 7.22-7.86 (m, 8H, Ar-H); LCMS (m/z): $[M]^+$; 308.09

2-(3-(4-chlorophenyl)-4,5-dihydroisoxazol-5-yl)-1*H*-benzo[*d*]imidazole (4g)

Melting Point: 216–218°C; Yield: 81%; R_f value: 0.75; Solvent system: Benzene: Ethylacetate: Methanol (9: 0.5: 0.5); Anal. Calcd. for $C_{16}H_{12}ClN_3O$ (297.74): C, 64.54; H, 4.06; N, 14.11.

Found: C, 64.78; H, 4.23; N, 15.95; IR (υ_{max}, cm^{-1}): 3431 (N–H), 3081 (Ar. C–H), 1654 (C=N), 1539, 1451 (Ar. C=C), 744 (C–Cl); ^{1}H -NMR (400 MHz, DMSO-d_6); δ: 3.27 (m, 1H, CH_2), 3.43 (m, 1H, CH_2), 5.73 (t, 1H, CH), 6.92 (s, 1H, NH), 7.16-7.58 (m, 8H, Ar-H); LCMS (m/z): $[M]^+$; 297.07

2-(3-(4-fluorophenyl)-4,5-dihydroisoxazol-5-yl)-1*H*-benzo[*d*]imidazole (4h)

Melting Point: 206–208°C; Yield: 78%; R_f value: 0.81; Solvent system: Benzene: Ethylacetate: Methanol (9: 0.5: 0.5); Anal. Calcd. for $C_{16}H_{12}FN_3O$ (281.28): C, 68.32; H, 4.30; N, 14.94.

Found: C, 68.61; H, 4.18; N, 14.71; IR (υ_{max}, cm^{-1}): 3321, 3246 (N–H), 3061 (Ar. C–H), 1674 (C=N), 1535, 1474 (Ar. C=C); ^{1}HNMR (400 MHz, DMSO-d_6); δ: 3.25 (m, 1H, CH_2), 3.39 (m, 1H, CH_2), 5.65 (t, 1H, CH), 6.88 (s, 1H, NH), 7.13-7.64 (m, 8H, Ar-H); LCMS (m/z): $[M]^+$; 281.10

2-(3-(4-bromophenyl)-4,5-dihydroisoxazol-5-yl)-1*H*-benzo[*d*]imidazole (4i)

Melting Point: 226–228°C; Yield: 68%; R_f value: 0.74; Solvent system: Benzene: Ethylacetate: Methanol (9: 0.5: 0.5); Anal. Calcd. for $C_{16}H_{12}BrN_3O$ (342.19): C, 56.16; H, 3.53; N, 12.28.

Found: C, 56.43; H, 3.71; N, 12.54; IR (υ_{max}, cm^{-1}): 3343, 3264 (N–H), 3080 (Ar. C–H), 1659 (C=N), 1532, 1492 (Ar. C=C); ^{1}H-NMR (400 MHz, DMSO-d_6); δ: 3.21 (m, 1H, CH_2), 3.36 (m, 1H, CH_2), 5.71 (t, 1H, CH), 6.94 (s, 1H, NH), 7.08-7.63 (m, 8H, Ar-H); LCMS (m/z): $[M]^+$; 341.02

2-(3-(4-(trifluoromethyl)phenyl)-4,5-dihydroisoxazol-5-yl)-1*H*-benzo[*d*]imidazole (4j)

Melting Point: 214–216°C; Yield: 73%; R_f value: 0.81; Solvent system: Benzene: Ethylacetate: Methanol (9: 0.5: 0.5); Anal. Calcd. for $C_{17}H_{12}F_3N_3O$ (331.29): C, 61.63; H, 3.65; N, 12.68.

Found: C, 61.78; H, 3.84; N, 12.47; IR (υ_{max}, cm^{-1}): 3465, 3346 (N–H), 3065 (Ar. C–H), 2244 (C=N), 1508, 1472 (Ar. C=C); ^{1}H-NMR (400 MHz, DMSO-d_6); δ: 3.26 (m, 1H, CH_2), 3.41 (m, 1H, CH_2), 5.73 (t, 1H, CH), 6.98 (s, 1H, NH), 7.15-7.78 (m, 8H, Ar-H); LCMS (m/z): $[M]^+$; 331.09

In-silico Prediction of Absorption and Drug-likeness

The calculation of molecular properties like drug likeliness and bioactivity were predicted by the molinspiration property engine v2009.01 program.

The molinspiration home page was opened online, in which free online cheminformatics services link option was opened. The molecule to be analyzed was pasted, whose structure was already saved in smile format (through any chemistry software) then with the help of calculating properties or predict bioactivity options, calculations were obtained and saved.[59-61]

"Lipinski rule or rule of five is like that to be drug-like, a candidate should have less than five hydrogen bond donors (HBD), less than 10 hydrogen bond acceptors (HBA), a molecular weight of less than 500 Da, and a partition coefficient log P of less than 5. The aim of the rule of five is to highlight possible bioavailability problems if two or more properties are violated."[59-61]

"Absorption (%ABS) was calculated by %ABS = 109-(0.345 X TPSA)."[59-61]

Molecular Docking Study

Hardware and software

Windows 10 (64-bit) operating systems with 4 GB RAM and 2.50 GHz Intel(R) Core$^{(TM)}$ i5-7200U processor was used for executing the docking process. PyRx version 0.8, available at https://pyrx.sourceforge.io/ was used to perform the docking in Autodock Vina Wizard.[62] Autodock Tools 4.2.6 which is made accessible by the Scripps Research Institute at https://autodock.scripps.edu/, was used for preparing the proteins and for grid generation, ligands were processed using Open babel[63] and PyRx 0.8 and interaction poses of ligands were visualized and analysed using Discovery Studio Visualizer.

Selection of target proteins

The molecular docking studies were conducted on two microbial proteins to assess antimicrobial potential. PDB ID 2EG7-*E. coli* dihydrorootase in complex with HDDP and PDB ID 5D6P-ATP Binding domain of GyrB of *S. aureus* in complex with 57U were chosen.[64]

Protein and Ligand Processing for Docking

Protein preparation

The crystal structures of target protein PDB id: 2EG7-*E. coli* dihydrorootase in complex with HDDP[64] were downloaded from the RCSB-Protein Data Bank. The proteins were prepared using Autodock Tools 4.2.6. In this step, attached water molecules and bound heteroatoms/ligand were removed, polar hydrogens and Kollman charges were added, the charge was spread equally over all atoms and residues were checked for missing atoms if any. The prepared PDB files were then converted to the PDBQT format for executing the next step.

Ligand processing

Ligands in smiles format were converted to sdf files and 3D coordinates for all ligands were generated using Open Babel using command line. The 3D structure data files

were processed in PyRx using UFF energy minimization and then converted to PDBQT format (autodock detectable format).

Grid generation

The grid box was first set over attached ligands using AutoDock Tools and then manually adjusted to desired dimensions in PyRx. The grid dimensions were set as 30.329 x 40.334 x 80.415 $Å^{3,}$ keeping number of points as 25 in X, Y, Z direction for PDB ID: 2EG7.

Docking and visualization of results

The docking was implemented in Vina Wizard of PyRx Tool, using exhaustiveness of 8 and the resultant out files were split into individual pose files. These files and the protein structure were then taken for visualization of interactions using Discovery Studio Visualizer.

Results and Discussion

as per scheme (see Fig. 1), isoxazole-incorporated benzimidazole derivatives were synthesized in 4 steps. In the first step, 1-(1*H*-benzo[*d*]imidazol-2-yl)ethanol (1) was synthesized by condensation of *o*-Phenylenediamine with lactic acid. In the second step, 1-(1*H*-benzo[*d*]imidazol-2-yl)ethanone (2) was prepared by oxidation of compound 1. In the third step, the chalcone derivatives (3a-3j) were synthesized by condensation of 1-(1*H*-benzo[*d*]imidazol-2-yl)ethanone (2) with arylaldehydes. In the final step, isoxazole-incorporated benzimidazole derivatives (4a-4j) were synthesized by cyclization of chalcone derivatives (3a-3j) by using hydroxylamine.

The structures of title compounds (4a-4j) were also confirmed by infrared spectroscopy with the absence of C=O peak and by ^{1}H-NMR and mass spectrometry. The purity of compounds was also ascertained by elemental analysis (C, H and N) (Table 1).

All the title compounds synthesized were tested against two gram-positive bacterial strains *S. aureus*, *Bacillus anthracis*, two gram-negative bacterial strains *Pseudomonas aeruginosa*, *E. coli* and two fungal strain (*C. albicans*, *A. niger*) by cup-plate method for antimicrobial activity.

The solutions of 1000 µg/mL concentration of test compounds were prepared in dimethylsulphoxide (solvent) for the study. Streptomycin and fluconazole were used as standard for antibacterial and antifungal activity, respectively. Standard drug and solvent control were maintained for the study.[65]

In case of antibacterial activity, the zone of inhibition ranged from 08 to 29 mm and 09 to 27 mm for gram-positive bacterial and gram-negative bacterial strains, respectively.

At the same time, it was noted that compounds 4d, 4f and 4j showed significant activity against gram-positive and gram-negative bacteria.

The calculation of molecular properties like drug likeliness and bioactivity were predicted by Molinspiration property engine v2009.01 program. It was observed that all the compounds (4a-4j) exhibited a great %absorbance ranging from 75.84 to 91.65% and none of the compounds showed any violation of Lipinski rules (Table 2).

The molecular docking studies were carried out for assessing antimicrobial potential. PDB ID 2EG7-*E. coli* dihydrorootase in complex with was chosen.

Streptomycin as the reference drug and compounds (4a-4j) studied for molecular docking analysis on each of these ligands at two different binding sites, (PDB ID 2EG7- *E. coli* dihydrorootase in complex with HDDP.

On the basis of the interaction energy criterion, compound 4f showed the best docking interactions equal to -7.0 kcal/mol and the residues of binding site regions are following. Compound 4f showed five hydrogen bond interactions

Table 1: Antimicrobial activity of title compounds

Compound (1000 µg/mL)	*Zone of Inhibition (mm)*					
	S. aureus	*B. anthracis*	*P. aeruginosa*	*E. coli*	*C. albicans*	*A. niger*
Streptomycin	34	35	30	31	-	-
Fluconazole	-	-	-	-	27	29
4a	15	8	20	17	18	17
4b	22	18	10	23	16	15
4c	10	8	12	17	11	10
4d	27	27	26	25	22	24
4e	24	17	9	16	13	14
4f	28	26	25	24	20	19
4g	16	8	18	20	12	15
4h	22	19	18	18	16	17
4i	14	21	16	10	12	13
4j	29	27	27	25	21	18

Table 2: Drug likeliness and *in-silico* prediction of % absorption

Compounds	*Log P*	*TPSA*	*MW*	*nON*	*nOHNH*	*nviolation*	*nrotb*	*Vol*	*%abs*
4a	3.11	50.28	263.30	4	1	0	2	235.31	91.65
4b	2.63	70.51	279.30	5	2	0	2	243.32	84.67
4c	3.15	59.52	293.33	5	1	0	3	260.85	88.47
4d	2.76	68.75	323.35	6	1	0	4	286.40	85.28
4e	2.75	77.98	353.38	7	1	0	5	311.94	82.10
4f	3.07	96.11	308.30	7	1	0	3	258.64	75.84
4g	3.79	50.28	297.75	4	1	0	2	248.84	91.65
4h	3.28	50.28	281.29	4	1	0	2	240.24	91.65
4i	3.92	50.28	342.20	4	1	0	2	253.19	91.65
4j	4.01	50.28	331.30	4	1	0	3	266.60	91.65

Table 3: Molecular docking of compounds (4a-4j) with PDB 2EG7

S. No.	*Compound*	*Binding energy (Kcal/mol)*	*Hydrogen bonding interactions (Conventional)*	*Receptor ligand interactions*
1.	4a	-6.2	-	ALA A:252, ALA A:266, LEU A:222, ASN A:44
2.	4b	-6.6	HIS A:177, ASP A:250, ALA A:266	ALA A:46, HIS A:18, ALA A:252
3.	4c	-6.6	HIS A:18, ARG A:20, HIS A:254	HIS A:177, HIS A:16, ASP A:250, LEU A:222
4.	4d	-6.8	ASN A:44	ALA A:252, ALA A:266, HIS A:254, MET A:24, ARG A:20, LEU A:45
5.	4e	-6.9	ASN A:44	ALA A:252, ALA A:266, HIS A:254, ARG A:20, LEU A:45
6.	4f	-7.0	ALA A:266, HIS A:16, HIS A:18, HIS A:139, HIS A:177	ALA A:252, ALA A:46, HIS A:254
7.	4g	-6.5	ASN A:44	ARG A:20, GLY A:267, ALA A:252, ALA A:266, CYS A:221, LEU A:222
8.	4h	-6.4	ASN A:44	ALA A:252, ALA A:266, HIS A:254, ARG A:20
9.	4i	-6.1	ALA A:266	LEU A:222, ALA A:252, ALA A:46, AGRG A:20, HIS A:254
10.	4j	-6.9	ASN A:44, LEU A:222	ARG A:20, ALA A:266, ASP A:250, ALA A:252, HIS A:18, HIS A:254
11.	Streptomycin	-6.0	ASP A:21, HIS A:254, ALA A:266	ARG A:258

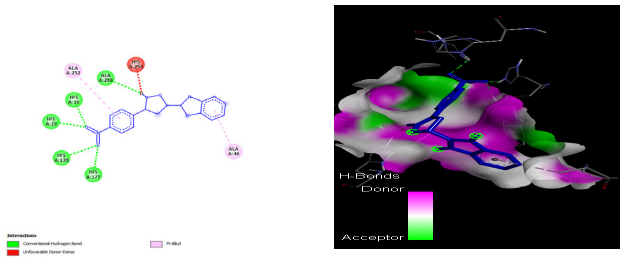

Fig. 2: 2D and 3D binding conformation of Compound **4f** at HDDP binding site of *E. coli* dihydrorootase (PDB ID: 2EG7)

with ALA:266, HIS:16, HIS:18, HIS:139 and HIS:177. It formed two π-alkyl interactions with ALA:46 and ALA;252 (Fig. 2, Table 3). These interactions are generally spherical with a radius of 4Å and cover most of the ligand. So, for the displacement of the ligand in the enzyme binding site, the first level interactions are used first, and the presence of hydrogen bond interaction explains that we have a good interaction between the two molecules and the studied protein.

Conclusion

Benzimidazole and Isoxazole derivatives constitute an important class of heterocycles with analgesic-antiinflammatory, antimicrobial, anticonvulsant, antimalarial, anticancer, antioxidant, antidepressant, anti-leishmanial, neuroprotective and other pharmacological activities. Isoxazole incorporated benzimidazoles (4a-4j) were tested against 2 gram-positive bacterial strains *S. aureus, B. anthracis*, 2 gram -ve bacterial strains *P. aeruginosa, E. coli*, and 2 fungal strains (*A. niger and C. albicans*) by cup-plate method for antimicrobial activity. The 4d, 4f and 4j compounds showed significant activity against gram-positive and gram-negative bacteria. On

the basis of the interaction energy criterion, compound 4f showed the best docking interactions equal to -7.0 kcal/mol.

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