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Research Article

Synthesis, Biological Evaluation and Molecular Docking Studies of Benzimidazole Derivatives Substitutes as New Antimicrobial Agents

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Abstract

Benzimidazole derivatives have assumed an imperative moiety in the hypothetical improvement in heterocyclic science and furthermore utilized broadly in organic synthesis. The synthesis, structure and biological activities of benzimidazole derivatives have been focal point of research enthusiasm for the field of medication because of potential exercises displayed by them. Developments of more current lead atoms are utilized to improve pharmacological action and lessen drug toxicities. After extensive literature review it was thought worthwhile to synthesize some Benzimidazole derivatives and evaluate their antibacterial and antifungal activities. A series of new 2-(1*H*-benzimidazole-2-*yl*) phenyl)-2-(substituted benzylidene) hydrazine was designed for showing the above activity. The different hydrazine derivatives (4a-4i) were synthesized by using different substituted benzaldehyde compounds. The structures of synthesized compounds were characterized by IR, ^{1}H NMR, Mass spectral data and elemental analysis. Synthesized compounds were tested *in vitro* for different kinds of pharmacological activity of this class of medications including antibacterial and antifungal action. The compounds 4d and 4e found most active against *E.coli* and *P. aeurigenosa* and 4i found to be most active against *B.subtilis* and *S.aureus.* The derivative 4c shows good activity against *C.albicans* and *A.niger.* Molecular docking analysis was performed to investigate the binding affinity of the synthesized compounds with target proteins. By means of this research it is concluded that Benzimidazole derivatives are a potent compound compressing of different pharmacological activities and this has been proved by docking studies too. Further evaluation of their properties is required before they can be adopted for widespread use.

Keywords

Benzimidazole, Antibacterial, Antifungal, Benzaldehyde, Docking Analysis

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1. Introduction

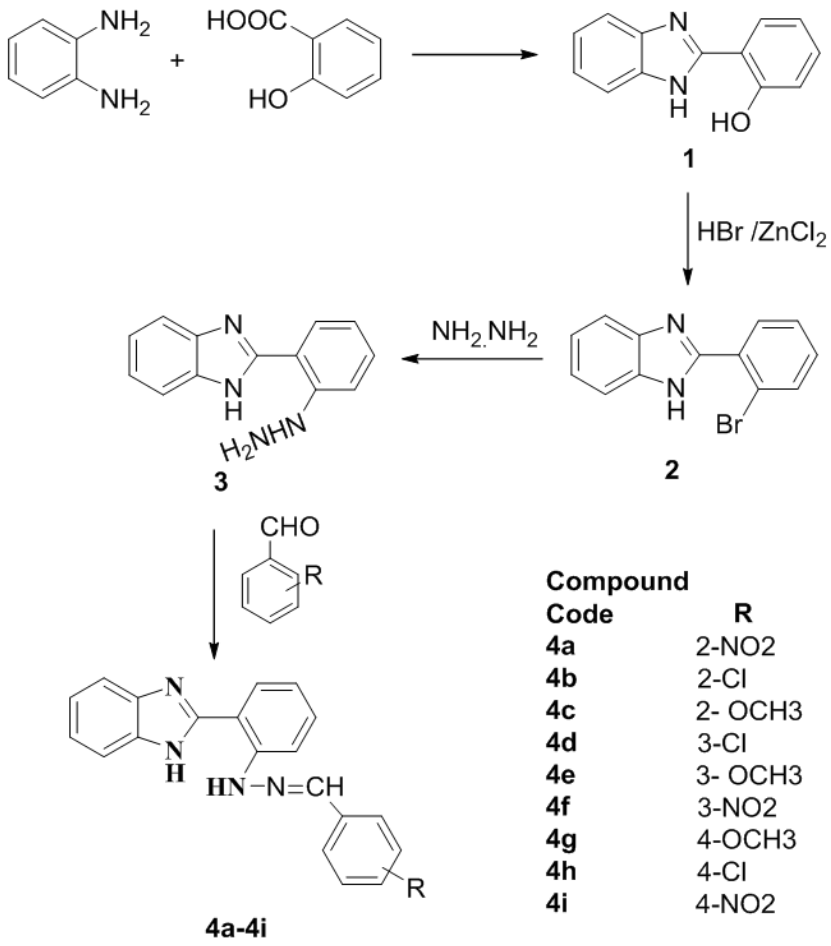
In nature, heterocyclic chemical compounds have a wide distribution and necessary for life. Heterocyclic substances have been involved in the metabolism of all living cells in an important manner. Heterocyclic compounds based on nitrogen are crucial to humankind. Particularly within the entire class of nitrogen-based heterocyclic compounds, benzimidazole has a significant impact on both biological and industrial processes. Benzimidazole is an important heterocyclic system, according to extensive study, since it has biological action against a variety of infections and physical illnesses. In therapeutic compounds including antiviral, anticancer, anthelmintics, anti-inflammatory, analgesics, antihistaminic, antiparasitic, anticonvulsants, antiulcer, antihypertensives, antifungals, proton pump inhibitors, and anticoagulants, among others, benzimidazole derivatives play an active role. Numerous researchers designed the benzimidazole-based compounds and investigated their antibacterial efficacy against various bacterial strains. A key problem nowadays is the rise of bacterial resistance to antibiotics. Due to bacterial resistance, many antibacterial medications are insignificant against germs. The World Health Organization recently released an antibiotic resistance bacterium priority list. An important heterocyclic aromatic chemical with a benzene and imidazole ring is benzimidazole. It is a collection of atoms with potential for use in a range of pharmaceutical objectives. Given its enormous therapeutic usefulness, the discovery and development of medicines containing the benzimidazole moiety is currently a significant and alluring topic of study [1]. Due to their many biological functions, benzoimidazole compounds have recently become a prominent study focus [2]. The most significant nitrogen-containing heterocycles are benzimidazole rings, which are extensively researched and used by the pharmaceutical industry for drug discovery [3]. The benzimidazole compounds were examined for their antibacterial, antiviral (anti-HIV), anticancer, antiplatelet, antiarrhythmic, anti-inflammatory, and analgesic effects [4-12]. They also contain a wide range of pharmacological characteristics. The synthesis, structure and biological activities of Benzimidazole derivatives have been focal point of research enthusiasm for the field of medication because of potential exercises displayed by them.

2. Material and Methods

The reagents were all of high commercial grade. Standard methods were used to dry and purify the solvents. TLC was used to determine the purity of all the freshly synthesized compounds on silica gel G plates. The melting points were recorded in an uncorrected open capillary tube. The 1H NMR spectra were taken on a Bruker DRX 300 MHz spectrometer in DMSO using TMS (tetramethylsilane) as an internal standard, and the mass spectra were taken on an MS-ESI (SHIMADZU-2010 AT, software class VP). The UV spectra were taken on a SHIMADZU spec-1700 spectrophotometer. Elemental analysis was conducted on Elementa, VarioEL III Carlo Erba 1108.

2.1. Chemistry

The sequence for the synthesis of 2-(1*H*-benzimidazole-2-yl)phenyl)-2-(substitutedbenzylidene)hydrazine (4a-4i) was shown in the scheme 1. Synthesis starts from the reaction between *o*-phenylenediamine and salicylic acid in the presence of 4N HCl to give 2-(1*H*-benzo[*d*]imidazole-2-yl) phenol (1). The intermediate compound (1) further experiences reaction in the presence of hydrogen bromide and zinc chloride to frame 2-(2-bromophenyl)-1*H*-benzo[d]imidazole (2) which on further reaction with hydrazine hydrate produces 1-(2-(1*H*-benzo[d]imidazole-2-yl)phenyl) hydrazine (3). The compound (3) further react with benzaldehyde derivatives to produce 2-(1*H*-benzimidazole-2-yl)phenyl)-2-(substitutedbenzylidene)hydrazine (4a-4i). All the synthesized compounds 4a-4i were characterized by physical parameters, chromatographic techniques and spectroscopic strategies (IR, ^{1}H NMR and Mass spectroscopy) and assessed for antimicrobial (antibacterial and antifungal activities) activity. The resultant products have consistent estimations of C, H and N contents with anticipated structure. The structures of recently synthesized benzimidazole derivatives were accomplished through IR, ^{1}HNMR and mass spectral data. In IR spectra of series, significant bands were appeared at 1192 (aromatic C-C), 1329 (aromatic C-N), 1668 (aromatic C=N), 3312 (aromatic N-H) and 3080 (aromatic C-H). In ^{1}HNMR spectra of these compounds a broad multiplet of aromatic proton were shown between 6.30 to 8.50 and a significant peak at 4.538 ppm (s, 1H, Ar. N-H) was observed. All derivatives showed M+1and M+2 peak in their mass spectra. All compounds were screened for antibacterial and antifungal activity.



Scheme 1. *Protocol for the synthesis of benzimidazole derivatives.*

2.1.1. General Method for Synthesis of 2-(1H-Benzo[d]imidazole-2-yl) Phenol (1)

Equimolar quantity of o-phenylenediamine (0.04mol) and salicylic acid (0.04mol) were dissolved in 90ml of 4N HCl in a 500ml round bottom flask. The mixture was refluxed for 18hrs. On cooling, needle shaped crystals were obtained which were washed with ice-cold water. Crude product was recrystallized from hot water to get pure white crystalline compound (1) [13].

2.1.2. Procedure for the Preparation of 2-(2-Bromophenyl)-1H-benzo[d] Imidazole (2)

In a round bottom flask, fitted with a reflux condenser (the top of which is connected to a guard tube for absorbing hydrogen chloride gas), Zinc chloride (2.09g, 0.02 mol) was dissolved in 30 ml of hydrobromic acid by shaking and 2-(1*H*-Benzo[d]imidazole-2-yl) phenol (1) (2.1g, 0.01 mol) was added to this mixture. The reaction mixture was refluxed for 15 hrs. The completion of reaction was monitored by TLC. Then reaction mixture was kept overnight in refrigerator, crude product was filtered was recrystallized from water to obtained the pale brown colored product (2) [13].

2.1.3. Procedure for the Preparation of 1-(2-(1H-Benzo[d]imidazole-2-yl)phenyl) Hydrazine (3)

The intermediate formed in previous reaction 2-(2-Bromophenyl)-1*H*-benzo[d]imidazole (2) (0.05 mol) and hydrazine hydrate (0.05 mol) in 1,4- dioxane (35 ml) was refluxed on water bath for 5 hrs. The excess of solvent was removed and the product was recrystallized with methanol to obtain the product (3) [14].

2.1.4. Procedure for the Preparation of 2-(1H-Benzimidazole-2-yl)phenyl)-2-(substitutedbenzylidene)hydrazine (4a-4i)

Various benzaldehyde derivatives (0.01 mol) was added to a solution of 1-(2-(1*H*-Benzo[d]imidazole-2-yl)phenyl) hydrazine (3) (0.01 mol) in methanol (20 ml). Reaction mixture was refluxed on water bath at 65 ^{0}C for 6 – 10 hrs. The reaction mixture was cooled and the solid obtained was filtered was recrystallized with aqueous ethanol to give compounds (4a-4i) [15].

2.2. Molecular Docking Analysis

Molecular docking analysis was done by Autodock vina and Autodock tool 1.5.6, and visualizations of ligand protein interactions was carried out by using Biovia Discovery Studio visualizer v21.1.0.20298. Five target proteins were selected binding affinity analysis. 3HSB is the crystal structure of YmaH (Hfq) from *Bacillus subtilis* in complex with an RNA aptamer. For antibacterial affinity analysis in *E. coli* PDB 1EI1 was downloaded from RCSB. Same as PDB 1JIJ (Crystal structure of *S. aureus* TyrRS in complex with SB-239629), 6TZ6 (Crystal Structure of *Candida Albicans* Calcineurin A, Calcineurin B, FKBP12 and FK506), 4ZA4 (Structure of *A. niger* Fdc1 with the prenylated-flavin cofactor in the iminium form) was selected for the docking studies. Before docking, protein was prepared for missing sidechains and atoms.

2.3. Pharmacokinetic Descriptors Calculation

Various physicochemical and pharmacokinetic descriptors of the synthesized compounds were evaluated by using the tool Molinspiration Cheminformatics server. This online server offers broad range of tools supporting molecule manipulation and processing, including SMILES and SDfile conversion, and calculation of various molecular properties needed in QSAR. The Lipinski rule of five deals four simple physicochemical parameter ranges (MWT ≤ 500, log P ≤ 5, H-bond donors ≤ 5, H-bond acceptors ≤ 10) associated with 90% of orally active drugs that have passed phase II clinical status.

3. Spectral Analysis of the Synthesised Compound

3.1. 1-(2-(1*H*-Benzo[d]imidazole-2-yl)phenyl)-2-(2-nitrobenzylidene)hydrazine (4a)

FTIR (KBr, ν, cm^{-1}): 3332(Aromatic N-H stretching (2^oamine)), 3087(Aromatic C-H str.), 2951(Aliphatic C-H str.), 1645(C=N stretching), 1514 (Aromatic C=C str.), 1529 (asymmetric N=O str.), 1423(C-N stretching), 1357 (symmetric N=O str.). ^{1}H NMR (300 MHz, DMSO, δ ppm): 6.372-7.865 ppm (m, 12H, Ar-H), 8.463 (s, 1H, Ar-H), 8.492 (s, 1H, N-H, D_2O exchangeable), 8.965(s, 1H, Ar. N-H, D_2O exchangeable). MS (ESI) m/z [% rel. abundance]: 357.15 $[M]^+$, 358.13 $[M+1]^+$ Fragments- 357.15, 193.07, 164.46, 118.53, 105.12, 90.34, 78.11.

Elemental analysis: Anal. Calcd. For $C_{20}H_{15}N_5O_2$: C, 67.62; H, 4.48; N, 19.75; O, 8.95%. Found: C, 67.69; H, 4.44; N, 19.51%

3.2. 1-(2-(1*H*-benzo[d]imidazole-2-yl)phenyl)-2-(2-chlorobenzylidene)hydrazine (4b)

FTIR (KBr) ν (cm^{-1}): 3308(Aromatic N-H str. (2^oamine)), 3076(Aromatic C-H str.), 2909(Aliphatic C-H str.), 1677(Aromatic C-C str.), 1676(C=N stretching), 1499(C-N str.), 1055(C-Cl str.). ^{1}H NMR (300 MHz, DMSO, δ ppm): 6.489-7.789 ppm (m, 12H, Ar-H), 7.324 (s, 1H, CH), 8.562 (s, 1H, N-H, D_2O exchangeable), 9.867 (s, 1H, Ar. N-H, D_2O exchangeable). MS (ESI) m/z [% rel. abundance]: 346.10 $[M]^+$, 347.52 $[M+1]^+$, 348.47 $[M+2]^+$ Fragments- 346.10, 193.77, 153.02, 117.63, 90.45, 78.44. Elemental analysis: Anal. Calcd. For $C_{20}H_{15}ClN_4$: C, 69.67; H, 4.61; N, 16.21%, Found: C, 69.47; H, 4.68; %.

3.3. 1-(2-(1*H*-Benzo[d]imidazole-2-yl)phenyl)-2-(2-methoxybenzylidene)hydrazine (4c)

FTIR (KBr) ν (cm^{-1}): 3280 (Aromatic N-H str. (2^oamine)), 3048 (Aromatic C-H str.), 2872 (Aliphatic C-H str.), 1657(C=N stretching), 1588 (Aromatic C-C str.), 1517 (C-N str.), 1348 (C-O-C, asymmetric). ^{1}H NMR (300 MHz, DMSO, δ ppm): 3.73(s, 3H, CH_3), 6.976-7.7992 ppm (m, 12H, Ar-H), 8.234 (s, 1H,-CH), 8.922 (s, 1H, N-H, D_2O exchangeable), 9.185 (s, 1H, Ar. N-H, D_2O exchangeable). MS (ESI) m/z [% rel. abundance]: 342.52 $[M]^+$, 343.46 $[M+1]^+$, 3044.89 $[M+2]^+$ Fragments- 342.52, 193.73, 149. 63, 118.56, 90.57, 78.66. Elemental analysis: Anal. Calcd. For $C_{21}H_{18}N_4O$: C, 73.64; H, 5.51; N, 16.17; O, 4.70%, Found: C, 73.67; H, 5.35; N, 16.32%

3.4. 1-(2-(1*H*-Benzo[d]imidazole-2-yl)phenyl)-2-(3-chlorobenzylidene)hydrazine (4d)

FTIR (KBr) ν (cm-1): 3322(Aromatic N-H str. (2^oamine)), 3096(Aromatic C-H str.), 2907(Aliphatic C-H str.), 1664(C=N stretching), 1612(Aromatic C-C str.), 1482(C-N str.), 1048(C-Cl str.). ^{1}H NMR (300 MHz, DMSO, δ ppm): 6.489(s, 1H, Ar-H), 7.105-7.854 ppm (m, 11H, Ar-H), 7.987 (s, 1H,-CH), 8.235 (s, 1H, N-H, D_2O exchangeable), 9.546 (s, 1H, Ar. N-H, D_2O exchangeable), MS (ESI) m/z [% rel. abundance]: 346.25 $[M]^+$, 347.68 $[M+1]^+$, 348.56 $[M+2]^+$. Elemental analysis: Anal. Calcd. For $C_{20}H_{15}ClN_4$: C, 69.67; H, 4.31; N, 16.21; Cl, 10.34%, Found: C, 69.45; H, 4.37; Cl, 10.16%

3.5. 1-(2-(1*H*-Benzo[d]imidazole-2-yl)phenyl)-2-(3-methoxybenzylidene)hydrazine (4e)

FTIR (KBr) ν (cm^{-1}): 3298 (Aromatic N-H str. (2^oamine)), 3075 (Aromatic C-H str.), 2898 (Aliphatic C-H str.), 1649(C=N stretching), 1568 (Aromatic C-C str.), 1510 (C-N str.), 1308 (C-O-C, asymmetric). ^{1}H NMR (300 MHz, DMSO, δ ppm): 3.657 (s, 3H, CH_3), 6.524-7.829 ppm (m, 11H, Ar-H), 7.145(s, 1H, Ar-H), 8.231 (s, 1H, -CH), 8.587 (s, 1H, Ar. N-H,

D_2O exchangeable), 8.489 (s, 1H, N-H, D_2O exchangeable). MS (ESI) m/z [% rel. abundance]: 342.67 $[M]^+$, 343.50 $[M+1]^+$, 344.94 $[M+2]^+$. Elemental analysis: Anal. Calcd. For $C_{21}H_{18}N_4O_2$: C, 73.04; H, 4.51; N, 16.47; O: 4.78%, Found: C, 73.47; H, 4.59; O: 4.53%

3.6. 1-(2-(1*H*-Benzo[d]imidazole-2-yl)phenyl)-2-(3-nitrobenzylidene)hydrazine (4f)

FTIR (KBr) ν (cm-1): 3326(Aromatic N-H stretching (2°amine)), 3089(Aromatic C-H str.), 2931(Aliphatic C-H str.), 1685(C=N stretching), 1529 (Aromatic C-C str.), 1513 (asymmetric N=O str.), 1358 (symmetric N=O str.), 1424(C-N stretching), ^{1}H NMR (300 MHz, DMSO, δ ppm): 6.548 -8.692 ppm (m, 11H, Ar-H), 8.549 (s, 1H,-CH), 8.682 (s, 1H, Ar-CH), 8.693 (s, 1H, N-H, D_2O exchangeable), 9.548 (s, 1H, Ar. N-H, D_2O exchangeable). MS (ESI) m/z [% rel. abundance]: 357.48 $[M]^+$, 358.62$[M+1]^+$. Elemental analysis: Anal. Calcd. For $C_{20}H_{15}N_4O_2$: C, 67.66; H, 4.28; N, 19.55; O, 8.85%. Found: C, 67.33; H, 4.28; O, 8.79%

3.7. 1-(2-(1*H*-Benzo[d]imidazole-2-yl)phenyl)-2-(4-methoxybenzylidene)hydrazine (4g)

FTIR (KBr) ν (cm-1): 3289 (Aromatic N-H str. (2°amine)), 3074 (Aromatic C-H str.), 2888 (Aliphatic C-H str.), 1674(C=N stretching), 1543 (Aromatic C-C str.), 1542 (C-N str.), 1303 (C-O-C, asymmetric). ^{1}H NMR (300 MHz, DMSO, δ ppm): 3.467 (s, 3H,-CH_3), 6.821- 7.547(dd, 4H, Ar-CH), 6.984 -7.798 ppm (m, 8H, Ar-H), 8.397 (s, 1H, -CH), 8.745 (s, 1H, Ar. N-H, D_2O exchangeable), 9.643 (s, 1H, N-H, D_2O exchangeable). MS (ESI) m/z [% rel. abundance]: 342.74 $[M]^+$, 343.58 $[M+1]^+$, 344.62 $[M+2]^+$. Elemental analysis: Anal. Calcd. For $C_{21}H_{18}N_4O$: C, 73.64; H, 5.31; N, 16.47; O, 4.85%, Found: C, 73.82; H, 5.52; N, 16.25%,

3.8. 1-(2-(1*H*-Benzo[d]imidazole-2-yl)phenyl)-2-(4-chlorobenzylidene)hydrazine (4h)

FTIR (KBr) ν (cm-1): 3312(Aromatic N-H str. (2°amine)), 3089(Aromatic C-H str.), 2905(Aliphatic C-H str.), 1688(C=N stretching), 1610(Aromatic C-C str.), 1482(C-N str.), 1046(C-Cl str.). ^{1}H NMR (300 MHz, DMSO, δ ppm): 6.464- 7.895ppm (m, 8H, Ar-H), 7.458 – 7.642 (dd, 4H, Ar-CH), 8.563 (s, 1H, -CH), 8.684 (s, 1H, Ar. N-H, D_2O exchangeable), 9.432 (s, 1H, N-H, D_2O exchangeable). MS (ESI) m/z [% rel. abundance]: 346.38 $[M]^+$, 347.70 $[M+1]^+$, 348.69 $[M+2]^+$. Elemental analysis: Anal. Calcd. For $C_{20}H_{15}ClN_4$: C, 69.35; H, 4.31; N, 16.27; Cl, 10.34%, Found: C, 69.57; H, 4.17; N, 16.59%

3.9. 1-(2-(1*H*-Benzo[d]imidazole-2-yl)phenyl)-2-(4-nitrobenzylidene)hydrazine (4i)

FTIR (KBr) ν (cm-1): 3313(Aromatic N-H stretching (2°amine)), 3089(Aromatic C-H str.), 2927(Aliphatic C-H str.), 1678(C=N stretching), 1539 (Aromatic C-C str.), 1516 (asymmetric N=O str.), 1449(C-N stretching), 1356 (symmetric N=O str.). ^{1}H NMR (300 MHz, DMSO, δ ppm): 6.548-7.702 ppm (m, 8H, Ar-H), 7.947 – 8.292 (dd, 4H, Ar-CH), 8.428 (s, 1H, -CH), 8.621 (s, 1H, Ar. N-H, D_2O exchangeable), 9.642 (s, 1H, N-H, D_2O exchangeable). MS (ESI) m/z [% rel. abundance]: 357.65 $[M]^+$, 358.86$[M+1]^+$. Elemental analysis: Anal. Calcd. For $C_{20}H_{15}N_5O_2$: C, 67.66; H, 4.48; N, 19.55; O, 8.72%. Found: C, 67.86; H, 4.61; N, 19.70; O, 8.72%

4. Biological Evaluation

4.1. Antibacterial Activity

Gram-positive and gram-negative bacteria was examined for antibacterial activity using Muller Hinton agar (Hi-media) plates at 37 ℃ for 24 hours using the agar diffusion cup plate technique. At a concentration of 50 g/ml, compounds were tested for antibacterial activity against the bacterial strains *S. aureus*, *P. aeureginosa*, *B. subtilis*, and *E. coli*. The benchmark medication for comparing antibacterial activity was ciprofloxacin. The results, which are displayed in Tables 1 and 2, showed that 4d and 4e, which were created compounds, were most effective against *E. coli* and *B. subtilis*, respectively, while 4i was most effective against *P. aeurigenosa* and *S. aureus*. The consequences of antibacterial investigations are available in Tables 1 and 2 and in (Figure 1) [16].

Table 1. *Antibacterial activity of the synthesized compounds.*

No of compound	*E. coli*			*B. subtilis*		
	25μgml^{-1}	50μgml^{-1}	100μgml^{-1}	25μgml^{-1}	50 μg ml^{-1}	100 μg ml^{-1}
4a	8.22 ±0.38	11.16 ±0.19	15.87 ±0.16	8.42 ±0.21	11.28 ±0.64	15.28 ±0.17
4b	9.54 ±0.29	14.07 ±0.41	16.67 ±0.24	9.94 ±0.34	10.57 ±0.17	16.65 ±0.15
4c	7.54 ±0.23	10.97 ±0.40	14.82 ±0.37	8.46 ±0.19	11.69 ±0.91	15.53 ±0.34
4d	10.24 ±0.28	14.21 ±0.27	15.18 ±0.43	7.43 ±0.16	11.51 ±0.26	14.72 ±0.27

No of compound	*E. coli*			*B. subtilis*		
	25μgml^{-1}	50μgml^{-1}	100μgml^{-1}	25μgml^{-1}	50 μg ml^{-1}	100 μg ml^{-1}
4e	9.54 ±0.19	11.57 ±0.64	15.02 ±0.42	10.28 ±0.23	14.64 ±0.46	17.22 ±0.41
4f	9.21 ± 0.37	13.94 ±0.19	15.94 ±0.84	8.87 ±0.12	11.35 ±0.94	15.57 ±0.47
4g	7.56 ±0.51	10.14 ±0.72	14.54 ±0.74	9.29 ±0.57	11.69 ±0.16	16.32 ±0.51
4h	8.11 ±0.14	11.56 ±0.72	14.57 ±0.41	10.95 ±0.78	14.39 ±0.42	16.97 ±0.24
4i	7.79 ±0.64	11.34 ±0.43	15.24 ±0.87	9.42 ±0.57	10.64 ±0.72	15.27 ±0.43
Ciprofloxacin	-	14.38 ±0.13	-	-	14.82 ±0.21	-

Table 2. *Antibacterial activity of the synthesized compounds.*

No of compound	*P. aeruginosa*			*S. aureus*		
	25 μg ml^{-1}	50 μg ml^{-1}	100 μg ml^{-1}	25 μg ml^{-1}	50μgml^{-1}	100μgml^{-1}
4a	10.57 ±0.35	11.36 ±0.54	14.54 ±0.17	10.52 ±0.11	14.08 ±0.49	17.06 ±0.35
4b	9.54 ±0.12	12.04 ±0.31	15.28 ±0.35	9.14 ±0.17	11.61 ±0.35	14.35 ±0.29
4c	10.24 ±0.18	14.38 ±0.46	17.5 ±1 0.17	8.87 ±0.42	12.26 ±0.14	15.37 ±0.64
4d	8.18 ±0.21	11.64 ±0.54	15.21 ±0.13	7.12 ±0.11	10.84 ±0.49	15.87 ±0.27
4e	10.64 ±0.17	14.24 ±0.12	17.69 ± 0.37	8.57 ±0.53	11.64 ±0.35	15.49 ±0.17
4f	9.42 ±0.72	11.03 ±0.31	15.68 ± 0.68	7.28 ±0.33	12.54 ±0.15	16.65 ±0.24
4g	8.27 ±0.34	11.26 ±0.19	14.34 ± 0.54	10.84 ±0.29	13.97 ±0.46	17.28 ±0.15
4h	8.86 ±0.47	12.35 ±0.42	15.69 ±0.36	7.39 ±0.74	11.31 ±0.79	14.10 ±0.48
4i	10.86 ±0.24	14.38 ±0.68	16.51 ±0.74	10.27 ±0.31	14.38 ±0.42	15.84 ±0.28
Ciprofloxacin	-	14.49 ±0.16	-	-	14.79 ±0.12	-

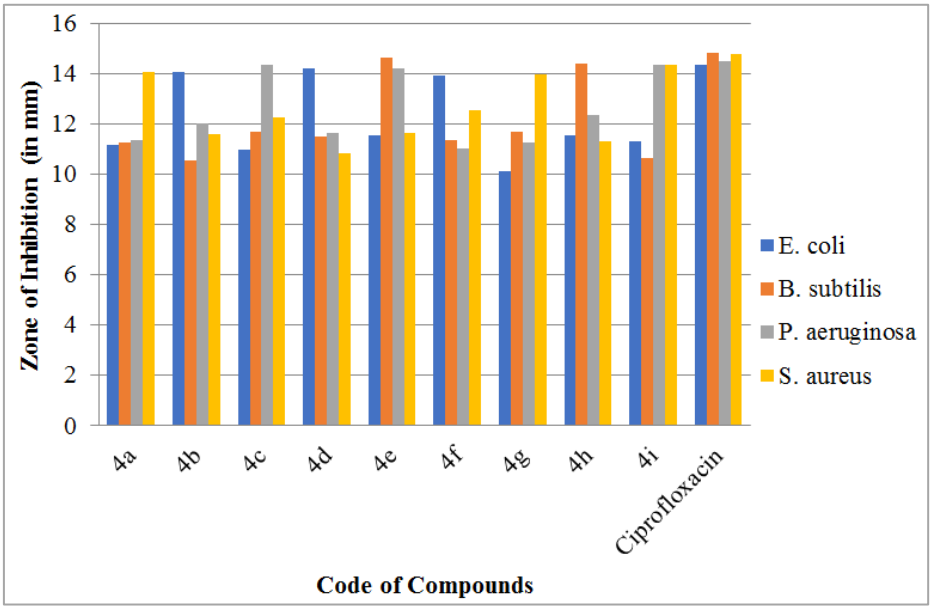


Figure 1. *Antibacterial activity of the synthesized compounds.*

4.2. Antifungal Activity

On Sabouraud's dextrose agar (Himedia) plates, antifungal activity was evaluated using the agar diffusion cup plate technique against the dimorphic fungus *C. albicans* and *A. niger*. At a concentration of 50 g/ml, antifungal activity was tested for. The benchmark medicine for comparing antifungal activity was fluconazole. As a solvent control for antifungal activity, DMSO was employed. The results shown in Table 3 and in (Figure 2) revealed that Compound 4c shows good activity against *C.albicans* and *A.niger* [16].

Table 3. *Antifungal activity of the synthesized compounds.*

Compounds	*C. albicana*			*A. niger*		
	25μgml^{-1}	**50μgml^{-1}**	**100μgml^{-1}**	**25μgml^{-1}**	**50μgml^{-1}**	**100μgml^{-1}**
4a	6.39 ±0.5	8.55 ±0.3	11.6 ±0.42	6.47 ±0.31	8.65 ±0.59	10.9 ±0.6
4b	7.48 ±0.2	8 ±0.9	10.8 ± 0.8	8.37 ±0.31	9.8 ±0.5	10.42 ±0.21
4c	8.19 ±0.17	11.47 ±0.5	13.56 ±0.40	8.75 ±0.37	11.15 ±0.9	13.26 ± 0.7
4d	7.60 ±0.4	10.3 ±0.9	12.55 ±0.5	8.04±0.71	10.4 ±0.8	12.5 ±0.31
4e	6.3 ±0.9	9.9 ±0.2	11.3 ±0.15	6.3 ±0.6	9.63 ±0.7	12.70 ±0.29
4f	7.77 ±0.3	10.9 ±0.7	13.20 ±0.6	8.6 ±0.3	10.6 ±0.5	12.7 ±0.12
4g	7.89 ±0.60	11.6 ±0.9	13.3 ±0.41	7.52 ±0.7	11.10 ±0.4	13.51 ±0.4
4h	8.13 ±0.11	11.09 ±0.97	13.02 ±0.11	7.65 ±0.9	11 ±0.2	13.6 ±0.46
4i	7.9 ±0.14	10.16 ±0.3	12.53 ±0.46	6.63 ±0.5	9.86 ±0.1	12.4 ±0.8
Fluconazole		12.4 ±0.5			11.2 ±0.8	

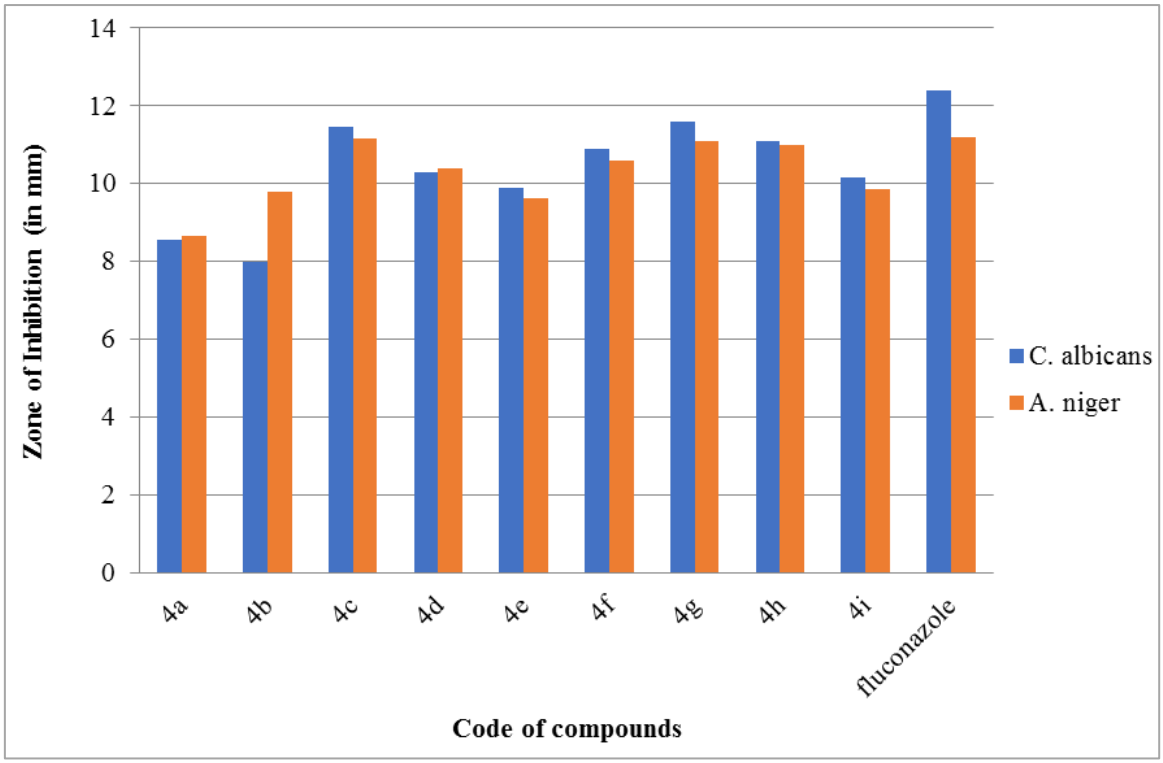


Figure 2. *Antifungal activity of the synthesized compounds.*

The outcome revealed that the compounds with nitro and methoxy groups were found to be most active against gram +ve, gram –ve and fungal strains.

5. Molecular Docking Evaluation

Docking analysis was performed to explore the binding interactions with the target proteins. The results of the docking analysis are given in Table 4. Compound 4f shows excellent binding with the 1EI1 protein with -9.9 docking score (Figure 3). Same as for binding with *S. aureus*, compound 4a exhibit highest docking score -10.4 with two hydrogen bonds with the residues Thr75 and Gly38 (Figure 4). Compound 4b and 4f shows excellent binding with *B. subtilis* protein (3HSB). Figures 5 and 6 denotes ligand interaction diagram of compound 4b and 4f. Compounds 4a and 4f shows excellent binding with the target protein 6TZ6 (Figures 7 and 8). Compound 4d exhibit docking score -9.1 for the target protein 4ZA4 (Figure 9).

Table 4. *Docking results of the synthesized compounds.*

Code	3HSB for affinity (kcal/mol)	1EI1 for affinity (kcal/mol)	1JIJ for affinity (kcal/mol)	4ZA4 for affinity (kcal/mol)	6TZ6 for affinity (kcal/mol)
4a	-8.4	-9.4	-10.4	-8.8	-10.6
4b	-9.0	-9.2	-8.6	-8.7	-10.1
4c	-8.5	-8.9	-9.2	-8.4	-10.1
4d	-8.6	-9.1	-9.7	-9.1	-9.6
4e	-8.2	-9.0	-9.2	-8.5	-9.4
4f	-9.0	-9.9	-10.0	-8.8	-10.6
4g	-8.2	-9.1	-8.8	-8.8	-9.6
4h	-8.2	-9.2	-9.5	-8.6	-9.6
4i	-7.6	-9.6	-8.1	-9.0	-9.8

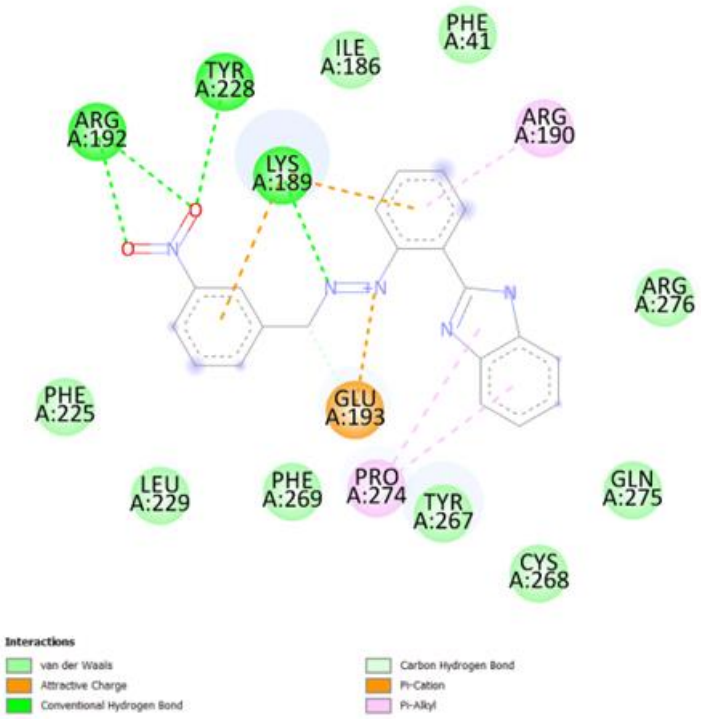


Figure 3. *2D ligand interaction diagram of compound 4f with 1EI1.*

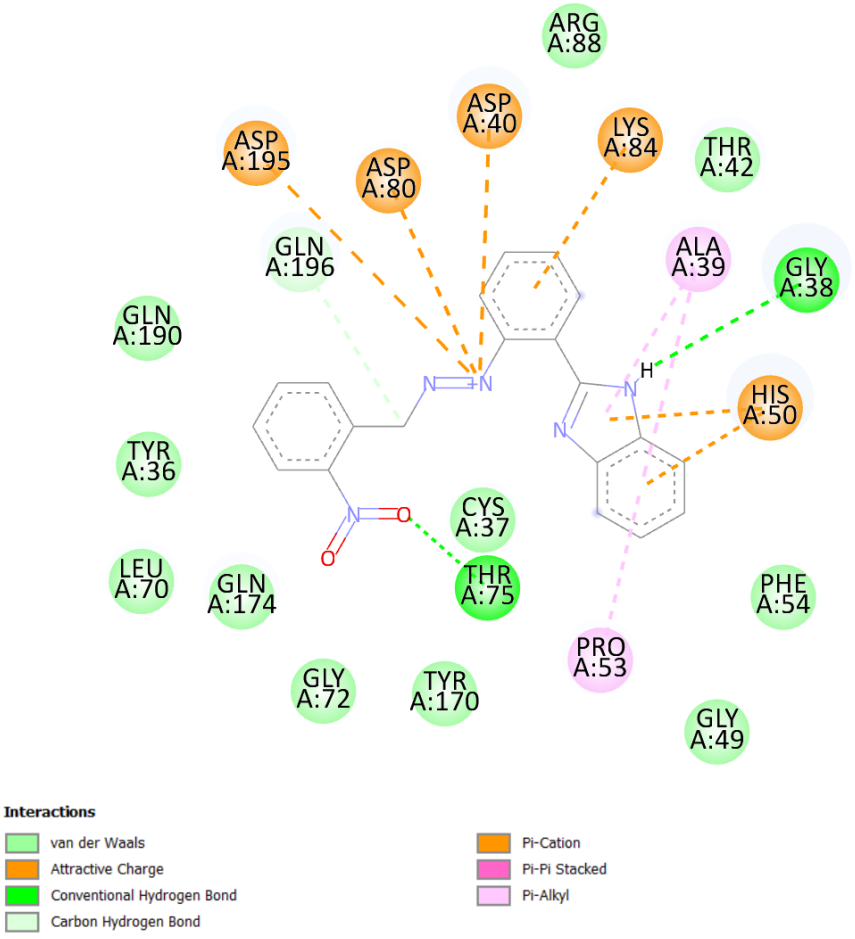


Figure 4. *2D ligand interaction diagram of compound 4a with 1JIJ.*

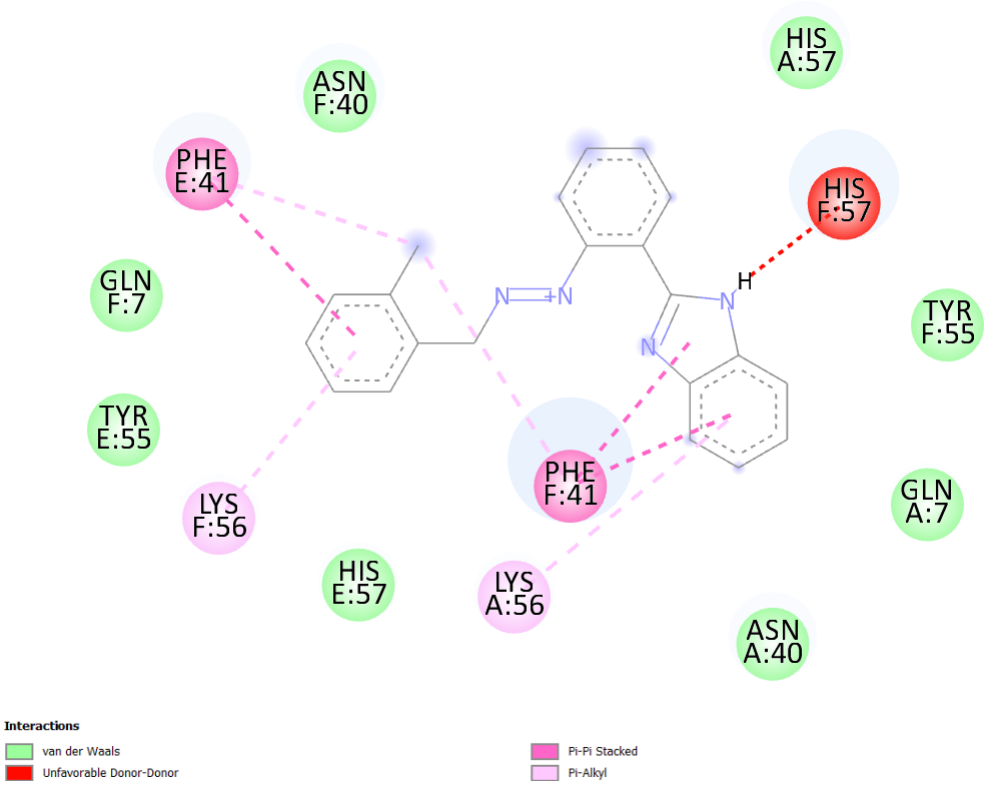


Figure 5. *2D ligand interaction diagram of compound 4b with 3HSB.*

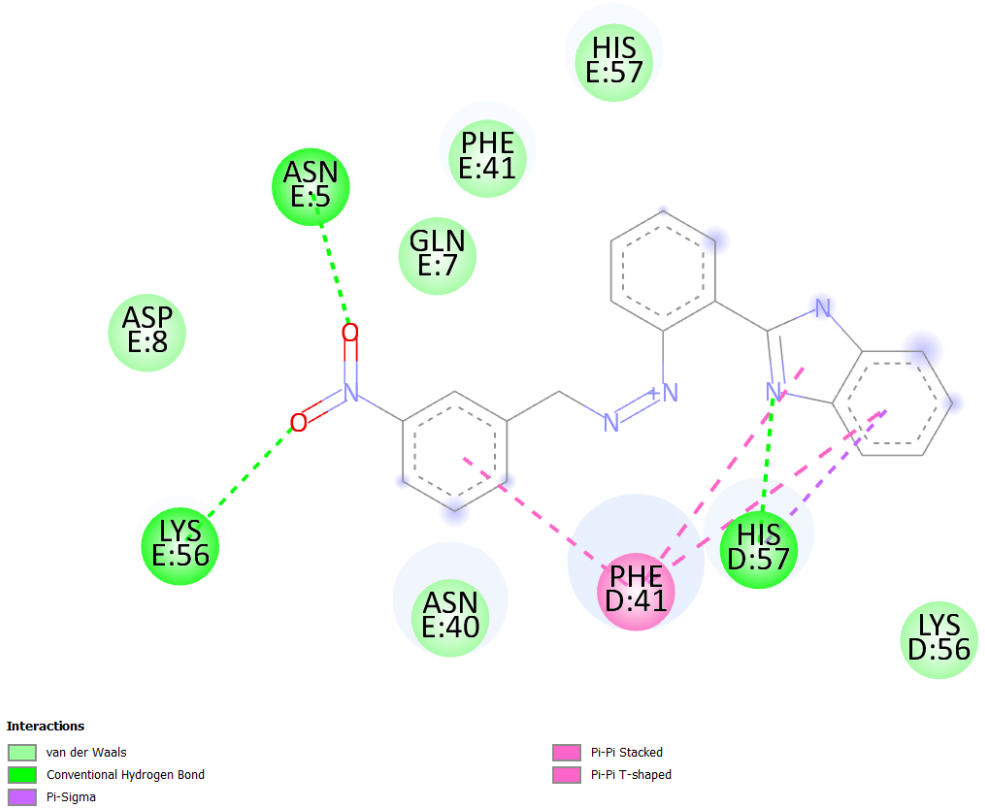


Figure 6. *2D ligand interaction diagram of compound 4f with 3HSB.*

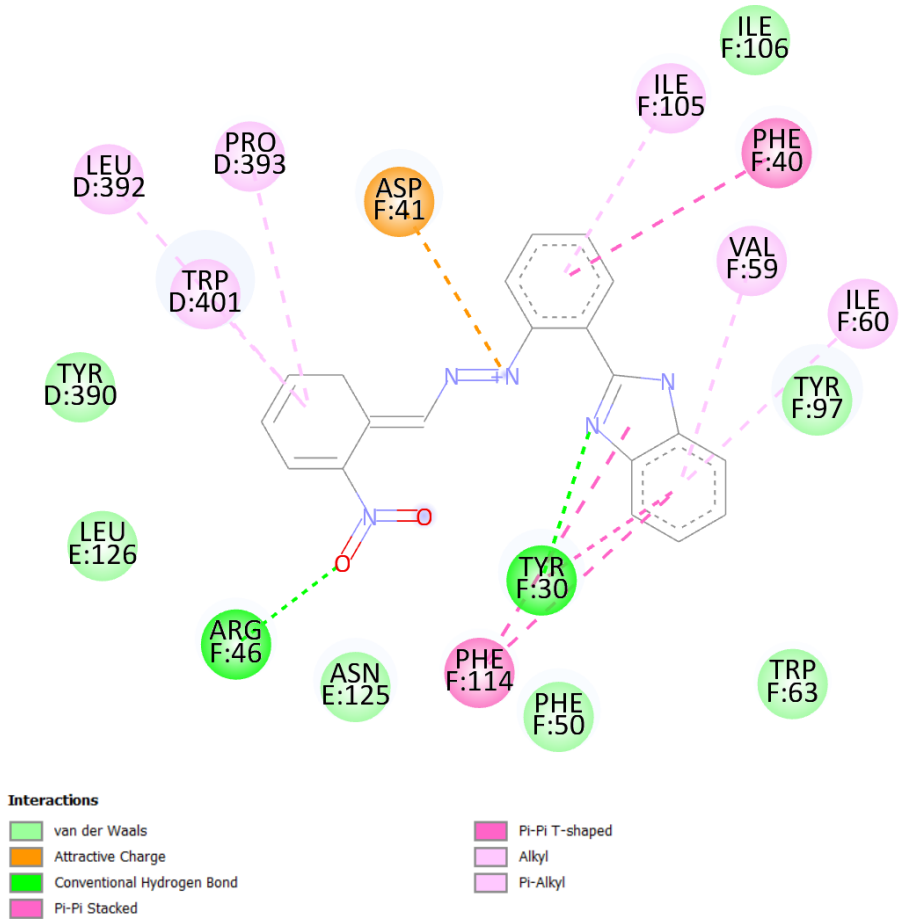


Figure 7. *2D ligand interaction diagram of compound 4a with 6TZ6.*

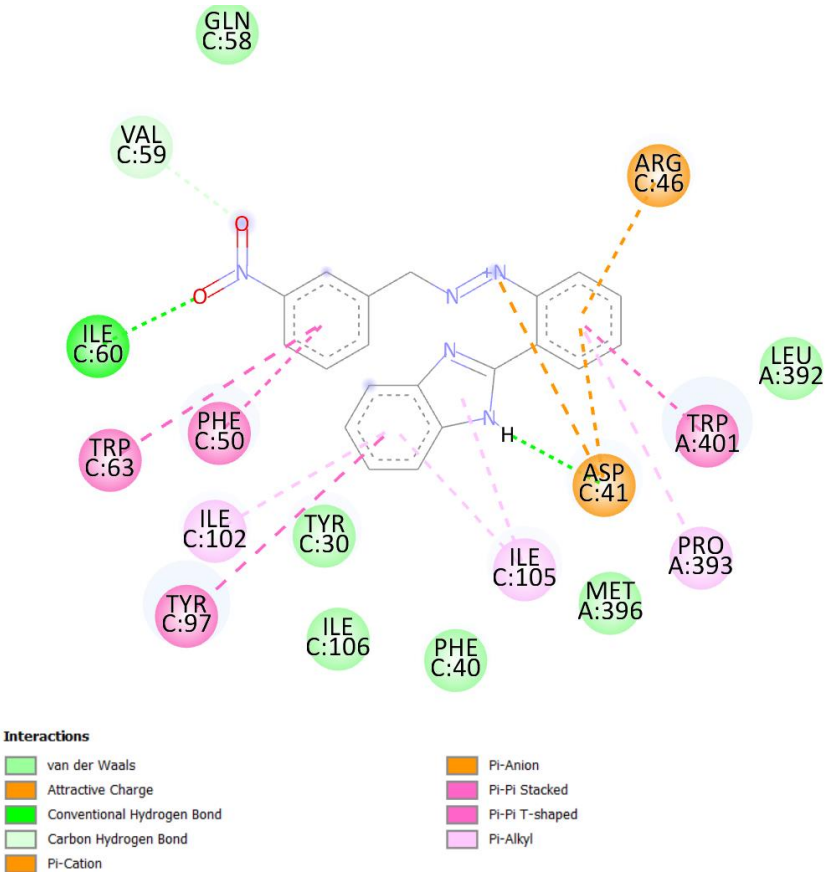


Figure 8. *2D ligand interaction diagram of compound 4f with 6TZ6.*

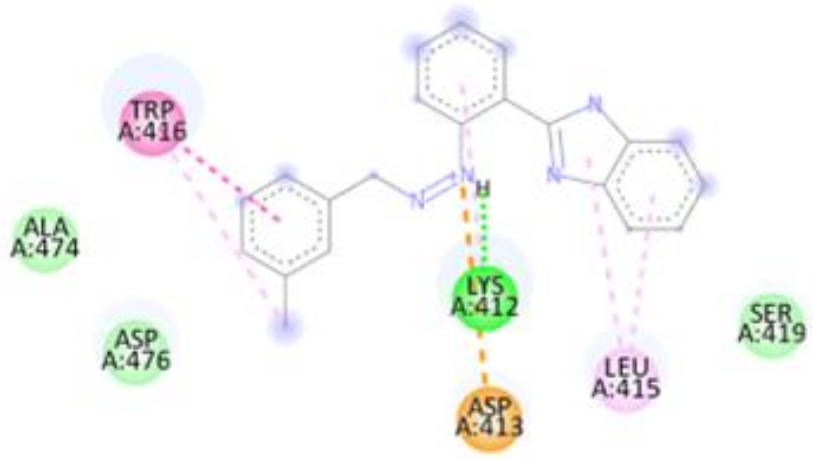


Figure 9. *2D ligand interaction diagram of compound 4d with 4ZA4.*

6. Pharmacokinetic Parameters Calculation

All synthesized compounds were analyzed for pharmacokinetic and physicochemical properties. The results are given in Table 5. All compounds have molecular weight in the range (Mol.Wt. ≤ 500). Compounds having low molecular weight are easily absorbed, diffused and transported as compared to high molecular weight compounds. With increase in molecular weight except certain limit, the bulkiness of the compounds is also increasing comparably. The LogP (octanol / water partition co efficient) of all compounds were calculated and shown violation for all compounds. The LogP value is used to calculate the lipophilic efficiency that measures the potency of drug. All compounds show one violation for Lipinski's rule of five of drug likeness.

Table 5. Physicochemical and pharmacokinetic parameters result of the synthesized compounds.

Code	Log P	TPSA	Mol. Wt.	nON	nOHNH	No. of violations
4a	6.26	98.90	357.37	7	2	1
4b	6.98	53.07	346.82	4	2	1
4c	6.36	62.31	342.40	5	2	1
4d	6.95	53.07	346.82	4	2	1
4e	6.33	62.31	342.40	5	2	1
4f	6.23	98.90	357.37	7	2	1
4g	6.38	62.31	342.40	5	2	1
4h	7.00	53.07	346.82	4	2	1
4i	6.28	98.90	357.37	7	2	1

7. Results and Discussion

All of the recently synthesized compounds 4a–4i were evaluated for their antimicrobial (antibacterial and antifungal activities) activity using physical characteristics, chromatographic methods, and spectroscopic techniques (IR, ^{1}H NMR, and mass spectroscopy). The final products contain predictable structures and reliable estimates of the concentrations of C, H, and N. Using IR, ^{1}HNMR, and mass spectral data, the structures of freshly synthesized benzimidazole derivatives were determined. Significant bands were seen at 1192 (aromatic C-C), 1329 (aromatic C-N), 1668 (aromatic C=N), 3312 (aromatic N-H), and 3080 (aromatic C-H) in the IR spectra of the series. These compounds ^{1}HNMR spectra revealed a broad multiplet of aromatic proton between 6.30 and 8.50 and a notable peak at 4.538 ppm (s, 1H, Ar. N-H). All derivatives mass spectra have M+1 and M+2 peaks. All substances underwent testing for antibacterial and antifungal properties.

Gram positive and Gram negative bacteria were tested for antibacterial activity using Muller Hinton agar (Hi-media) plates at 37 ℃ for 24 hours using the agar diffusion cup plate method. At a concentration of 50 g/ml, compounds were tested for antibacterial activity against the bacterial strains *S. aureus*, *P. aeureginosa*, *B. subtilis*, and *E. coli*. The benchmark drug for comparing antibacterial activity was ciprofloxacin. Tables 1 and 2 results demonstrate that 4d and 4e, two freshly synthesized compounds, were most effective against *E. coli* and *B. subtilis*, respectively, while 4i was most effective against *P. aeurigenosa* and S. aureus. On Sabouraud's dextrose agar (Himedia) plates, antifungal activity was evaluated using the agar diffusion cup plate method against the dimorphic fungus *C. albicans* and *A. niger*. At a concentration of 50 g/ml, antifungal activity was screened. The standard drug for comparing antifungal activity was fluconazole. As a solvent control for antifungal activity, DMSO was employed. According to the findings in table 3, compound 4c has good effectiveness against *C. albicans* and *A. niger*.

Against gram +ve, gram -ve, and fungal strains, the results showed that drugs having nitro and methoxy groups were more effective.

According to tables 4 and 5, Compounds 4a and 4f shows excellent binding with the target protein 6TZ6. Compound 4d exhibit docking score -9.1 for the target protein 4ZA4. All compounds show one violation for Lipinski's rule of five of drug likeness.

8. Conclusion

In conclusion, a series of novel benzimidazole derivatives was synthesized. The title compounds 4a–4i were evaluated for their antimicrobial (antibacterial and antifungal activities) activity. Preliminary antibacterial studies indicated that 4d and 4e, two freshly synthesized compounds, were most effective against *E. coli* and *B. subtilis*, respectively, while 4i was most effective against *P. aeurigenosa* and S. aureus. Specifically, compounds 4c has good effectiveness against *C. albicans* and *A. niger*. The results of this antimicrobial evaluation indicated that these compounds are a promising type of potential antibacterial agents which was shown through docking studies also. Further evaluation of their properties is required before they can be adopted for widespread use.

Abbreviations

S. aureus	*Staphylococcus aureus*
P. aeureginosa	*Pseudomonas aeureginosa*
B. subtilis	*Bacillus subtilis*
E. coli	*Escherichia coli*

C. albicans	*Candida albicans*
A. niger	*Aspergillus niger*
Ppm	parts Per Million
DMSO	Dimethyl Sulfoxide

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Conflicts of Interest

The authors declare no conflicts of interest.

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