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Synthesis, Biological Evaluation, and Molecular Docking Studies of Some New 2-(2-(Substituted piperazin-1-yl)-phenyl)-1*H*-benzo[d]imidazoles as Potential Antibacterial, Anticancer, and Antifungal Agents

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ABSTARCT Some new 2-(2-(substituted piperazin-1-yl)-phenyl)-1*H*-benzo[d]imidazoles **(3a-3g)** were designed, synthesized, and evaluated by the docking studies using glide tool for their antimicrobial and anticancer activities. The structures of these compounds were characterized by infrared, proton nuclear magnetic resonance, mass spectral data, and elemental analysis. Each analog was tested *in vitro* for various types of pharmacological activities, including antibacterial, antifungal, and anticancer activity. The compound **3c** was found to be most active against *Escherichia coli* and *Pseudomonas aeruginosa* and compound **3e** against *Bacillus subtilis* and *Staphylococcus aureus*. The derivative **3g** showed good activity against *Candida albicans* and *Aspergillus niger*. Among all the tested compounds, **3a** and **3b** were found with significant anticancer activity in comparison to Adriamycin standard drug. The obtained results revealed that most of the synthesized compounds exhibited significant antifungal, antibacterial, and anticancer activity. It can be deduced that these synthesized compounds can be regarded as a promising starting point for developing a single molecule with multiple targets.

KEYWORDS: Benzimidazole, Antibacterial, Antifungal, Anticancer, Molecular docking, Pharmacokinetic parameters calculation.

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INTRODUCTION

Benzimidazole is an organic aromatic heterocyclic compound that has an imidazole ring fused with a benzene ring. Imidazole ring is a part of numerous natural products, including purine, histamine, histidine, and nucleic acid.[1] Due to its broad range of pharmacological properties, benzimidazole is one of the privileged structures in medicinal chemistry and has been screened for its antimicrobial,[2-6] anticancer,[7-12] antiviral (anti-HIV),[13] antimalarial,[14] anthelmintic,[15] antiplatelet,[16] anti-inflammatory,[17] and analgesic[18] activities.

Benzimidazole is well known antibacterial agent and its derivatives have also been investigated for the development of anticancer drugs.[19] The design of new compounds to cope with resistant bacteria and fungi has been one of today's most critical fields of antibacterial and antifungal science, as pathogenic bacteria and fungi resistance to existing antimicrobial drugs are increasingly becoming a major problem worldwide. For chemists and pharmacists today, the development of novel and potent antibacterial as well as an antifungal agent is a challenging issue.[20]

Significant attempts were made in the drug development of possible antitumor molecules to synthesize novel heterocyclic motifs as the primary structural model. These heterocyclic scaffolds (Benzimidazole) were known as master scaffolds, taking their wide spectrum of biological profiles and target-related affinities into account. The molecules based on benzimidazole derivatives have been

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reported to show potential anticancer activities against different types of cancer.[8]

Benzimidazole is used as a precursor for the development of newer biological molecules.[21] Various techniques have been reported for the synthesis of benzimidazole and their derivatives.[22-24]

Work focused on piperazine has gained significant interest in recent years. Because of its proper alkalinity, water solubility, capacity to shape hydrogen bonds, piperazine integration is an outstanding technique in product design.[25]

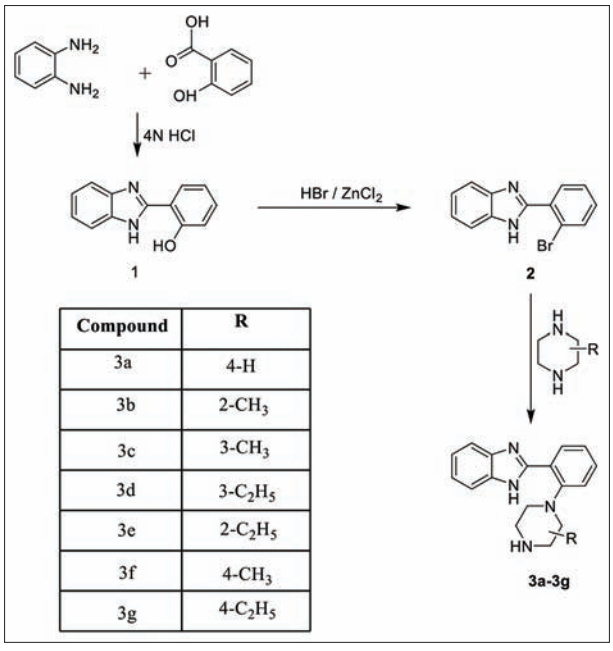
In view of these facts, we wish to report herein the synthesis of some new 2-(2-(substituted piperazin-1-yl)-phenyl)-1*H*-benzo[d]imidazole derivatives as potential antibacterial, anticancer, and antifungal agents.

RESULTS AND DISCUSSION

Chemistry

The sequence for the synthesis of 2-(2-(substituted piperazin-1-yl)-phenyl)-1*H*-benzo[d]imidazoles (**3a-3g**) is shown in **Scheme 1**. First, the reaction between *o*-phenylenediamine and salicylic acid in the presence of 4N HCl provided 2-(1*H*-benzo[d]imidazole-2-yl)phenol (**1**). The intermediate compound **1** underwent reaction with HBr and $ZnCl_2$ at high temperature to generate 2-(2-bromophenyl)-1*H* benzo[*d*]imidazole (**2**) which on reaction with substituted piperazines afforded target compounds **3a-3g.**[26]

All the newly synthesized compounds **3a-3g** were characterized by physical parameters [**Table 1**], chromatographic and spectroscopic methods (infrared [IR], proton nuclear magnetic resonance [^{1}H NMR], and mass spectrometry), and elemental analysis.



Compound	R
3a	4-H
3b	2-CH_3
3c	3-CH_3
3d	3-C_2H_5
3e	2-C_2H_5
3f	4-CH_3
3g	4-C_2H_5

Scheme 1: Synthetic protocol of compounds **(3a-3g)**

Antibacterial activity

All the synthesized benzimidazole derivatives **3a-g** were screened for their *in-vitro* antibacterial activity against *Staphylococcus aureus, Bacillus subtilis* (Gram-positive) and *Escherichia coli, Pseudomonas aeruginosa* (Gram-negative) bacterial strain. Ciprofloxacin was used as a standard drug. Prelude screening for all the synthesized derivatives was performed at concentrations of 50 μgml^{-1}. Dimethyl sulfoxide (DMSO) was used as a solvent control for antibacterial activity. The antibacterial screening exposed that all the tested compounds **3a-3g** possess moderate to good inhibition toward all the bacterial strains (both Gram-positive and Gram-negative).

Antifungal activity

All the derivatives **3a-g** were evaluated for antifungal activity against *Aspergillus niger* and *Candida albicans* fungal strains. Fluconazole was used as a standard drug for comparative study. The fungal strains were grown on agar medium. Prelude screening for all the synthesized derivatives was performed at concentrations of 50 μgml^{-1}. The Petri plates were incubated at 26°C for 72 h and zones of inhibition formed were measured. The antifungal screening exposed that all the tested compounds **3a-3g** possess moderate to good inhibition toward all the fungal strains.

Anticancer activity

All the newly synthesized benzimidazole derivatives were screened for anticancer activity against Human breast cancer (MCF 7) cell line by Sulforhodamine B (SRB) assay. The SRB assay possesses a colorimetric endpoint and is nondestructive and indefinitely stable. These practical advances make the SRB assay an appropriate and sensitive assay to measure percent growth inhibition. The results indicated that the compound **3a** has minimum GI_{50} value among all the tested compounds, that is, <10 μgml^{-1}.

Molecular docking analysis

Molecular docking and pharmacokinetic descriptor calculations were performed to probe the ligand-binding interaction and strengthen the scope of the investigation. The results are shown in **Table 2**.

The integration process of experimental and computational methods is an extremely attractive strategy for the design and optimization of drug candidates. For anticancer activity, the binding mode of the active novel derivative **3a** is quite consistent with the mode of binding of camptothecin [**Figure 1**].

For antifungal evaluation, all synthesized compounds, including fluconazole as reference drugs, were successfully docked with a target protein. Compound **3g** shows excellent binding with target protein with the highest Glide Gscore −7.748 which indicates **3g** binds with protein more selectively in comparison with reference drug fluconazole which has Glide Gscore −6.423. The binding interactions are shown in **Figure 2**. As depicted in **Table 3**.

Table 1: Characteristic data of the synthesized compounds 3a-3g

Compound code	R	Molecular formula	Molecular weight (g/mol)	Melting point (°C)	R_f value*	Yield (%)
3a	4-H	$C_{17}H_8N_4$	278.35	212–214	0.72	84
3b	2-CH_3	$C_{18}H_{20}N_4$	292.38	223–224	0.61	81
3c	3-CH_3	$C_{18}H_0N_4$	292.38	220–221	0.78	88
3d	3-C_2H_5	$C_{19}H_{22}N_4$	306.4	192–193	0.83	76
3e	2-C_2H_5	$C_{19}H_{22}N_4$	306.4	195–196	0.62	79
3f	4-CH_3	$C_{18}H_{20}N_4$	292.38	184–185	0.64	88
3g	4-C_2H_5	$C_{19}H_{22}N_4$	306.4	204–205	0.68	72

*Solvent used: Diethyl ether: *n*-Hexane: Acetic acid (7: 2.6: 0.4)

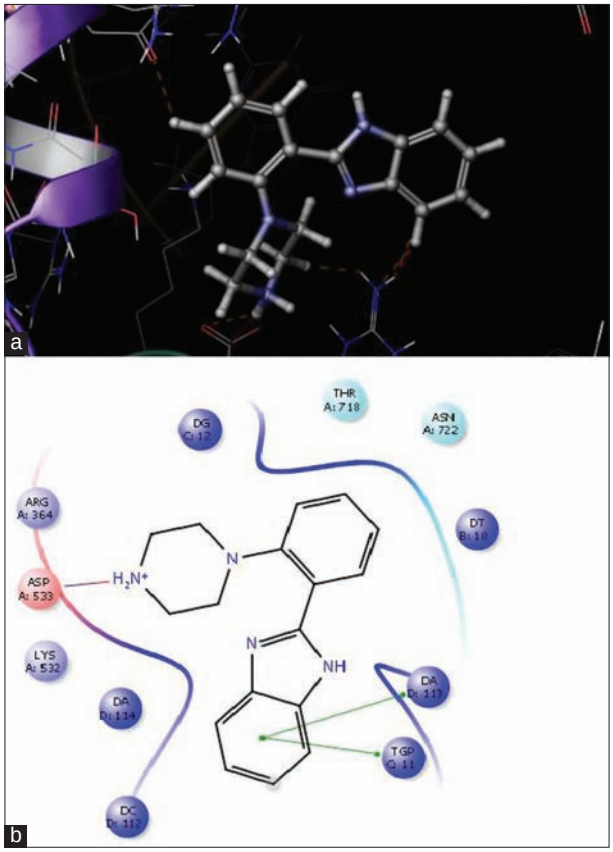


Figure 1: (a and b) Compound **3a** in the binding pocket of **1T8I**

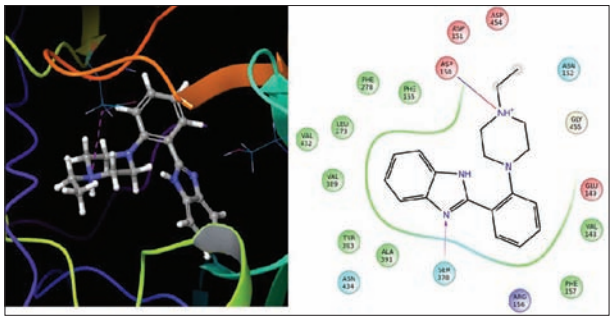


Figure 2: Compound **3g** in the binding pocket of **4CAW**

Compound **3c** shows better binding with target protein **1FJG** in comparison with ciprofloxacin as shown in **Figure 3**. This indicates compound **3c** could be potential antibacterial agents.

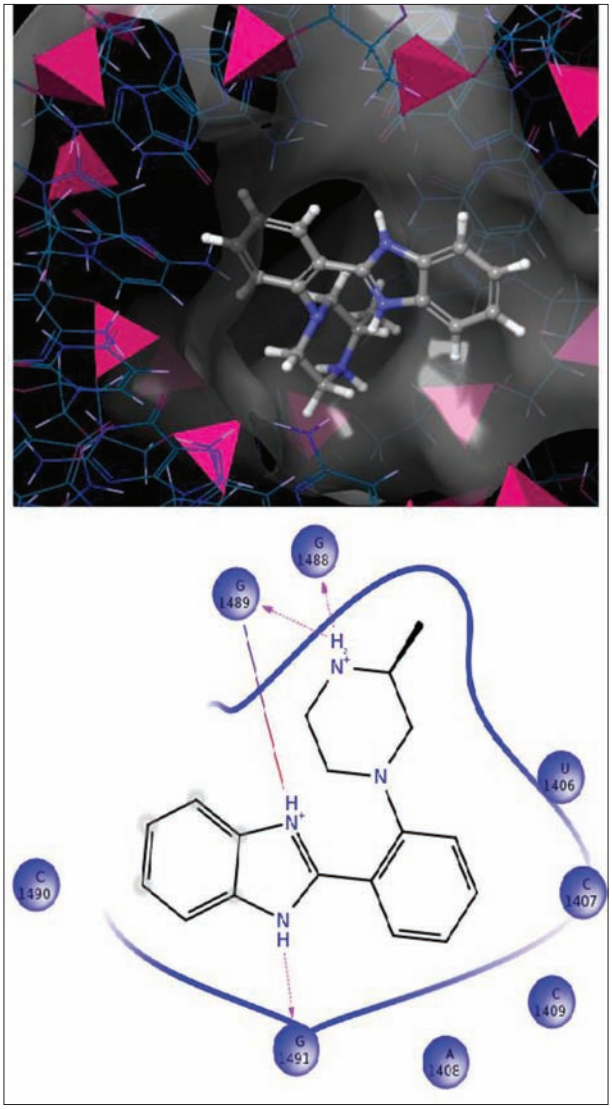


Figure 3: Compound **3c** in the binding pocket of **1FJG**

Pharmacokinetic parameters calculation results suggest that none of the synthesized compound shows a violation for druglikeness.[26]

EXPERIMENTAL

All the reagents were of commercial quality. Solvents were dried and purified using standard techniques. The

Table 2: Principle pharmacokinetic descriptors of the compounds

Compound	CNS	Volume	Donor hb	Accpt HB	QPlogPo/w	QPlogS	QPlogBB	Rule of five
3a	1	935.413	2	4	2.795	−3.256	0.397	0
3b	1	969.908	2	4	3.015	−3.293	0.436	0
3c	2	991.753	2	4	3.2	−3.69	0.473	0
3d	2	1049.525	2	4	3.602	−4.057	0.465	0
3e	1	1026.547	2	4	3.37	−3.638	0.373	0
3f	2	997.434	1	4.5	3.316	−3.722	0.62	0
3g	2	1054.849	1	4.5	3.699	−4.101	0.588	0

Table 3: Glide docking score and Prime/MMGBSA binding energy of compounds and reference molecules

Compound	Glide Gscore			dG_Bind	dG_Bind_Coulomb	dG_Bind_Hbond	dG_Bind_Lipo
	1T8I	1FJG	4CAW				
3a	−9.113	−6.017	−7.422	−24.815	−98.395	−0.887	−15.442
3b	−7.927	−6.287	−7.629	−30.119	−98.5200	−0.316	−16.540
3c	−8.642	−7.317	−5.663	−64.139	−500.046	−0.192	−17.697
3d	−8.773	−6.369	−6.240	−25.143	−100.357	−1.197	−19.019
3e	−6.248	−6.132	−5.825	−31.540	−78.666	−0.212	−10.193
3f	−7.045	−6.932	−6.259	−42.795	−98.673	−0.473	−17.207
3g	−8.028	−5.475	−7.748	−76.426	−499.894	−0.482	−18.394
Camptothecin	−7.948	-	-	−74.102	−13.485	−1.425	−16.300
Ciprofloxacin	-	−4.542	-	−70.452	−42.295	−0.952	−15.872
Fluconazole	-	-	−6.423	−71.580	−105.740	−1.120	−17.426

purity of all the newly synthesized compounds was monitored by thin-layer chromatography (TLC) on silica gel G plates. Melting points were taken in an open capillary tube and are uncorrected. The ultraviolet spectra were recorded on a SHIMADZU spec-1700 spectrophotometer, IR spectra were taken on a SHIMADZU 8400S spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker DRX 300 MHz spectrometer in DMSO using tetramethylsilane as an internal standard. Mass spectra were recorded on an MS-ESI (SHIMADZU-2010 AT, software class VP). Elemental analysis was carried out on elemental Vario EL III Carlo Erba 1108.

Synthesis of 2-(1H-benzo[d]imidazole-2-yl) phenol (1)

Compound **1** was synthesized from o-phenylenediamine by procedure reported by Singh *et al*.[26]

Synthesis of 2-(2-bromophenyl)-1H-benzo [d] imidazole (2)

Compound **2** was synthesized from compound **1** by procedure reported by Singh *et al*.[26]

General procedure for the synthesis of 2-(2-(substituedpiperazin-1-yl)-phenyl)-1H-benzo[d] imidazoles (3a-3g)

Equimolar quantity (0.01 mole) of compound **2** and piperazine derivatives in 45 ml of 1, 4-dioxane was refluxed for 14 h with continuous stirring at 80°C. The completion of the reaction was checked by TLC. The reaction mixture was kept overnight in a refrigerator. The solvent was evaporated and a gummy solid mass was obtained. The gummy solid was then washed with cold water and recrystallized from ethanol and water (4:6) to obtain the compounds **3**a-**3**g. Molecular weight, R_f value, melting point, and percentage yield for the synthesized derivatives are given in **Table 1.**

2-(2-(Piperazine-1-yl)phenyl)-1H-benzo(d)imidazole (3a)

FTIR (KBr) ν (cm^{-1}): 3405 (Aliphatic N-H str.), 3330 (Aromatic N-H stretching (2°amine)), 3080 (Aromatic C-H str.), 2921 (Aliphatic C-H str.), 1575 (Aromatic C-C str.), 1429 (Aliphatic C-N stretching), 1342 ((Aromatic C-N stretching); ^{1}H NMR (300 MHz, DMSO, δ ppm): 2.02 (s, 1H, -NH (piperazine ring) D_2O exhangeable), 2.78–2.81 (t, 4H, -CH_2, Aliphatic), 3.47–3.51 (t, 4H, -CH_2, Aliphatic), 5.01 (s, 1H, -NH, benzimidazole, D_2O exhangeable), 6.65–7.70 (m, 8H, Ar-H, Aromatic); MS (ESI) m/z [% rel. abundance]: 278.35 (100) [M]$^+$, 279.34 (92) [M+1]$^+$, Fragments: 194.23 (28), 118.14 (43), 78.11 (78); Elemental analysis: Calcd. For $C_{17}H_{18}N_4$: C, 73.35; H, 6.52; N, 20.13%; Found: C, 73.32; H, 6.56; N, 20.11%.

2-(2-(2-Methyppiperazin-1-yl) phenyl)-1H-benzo[d] imidazole (3b)

FTIR (KBr) ν (cm^{-1}): 3390 (Aliphatic N-H str.), 3315 (Aromatic N-H stretching (2°amine)), 3090 (Aromatic C-H str.), 2912 (Aliphatic C-H str.), 1560 (Aromatic C-C str.), 1432 (Aliphatic C-N stretching), 1332 ((Aromatic C-N stretching); ^{1}H NMR (300 MHz, DMSO, δ ppm): 1.19–1.22 (d, 3H, -CH_3, Aliphatic), 2.12 (s, 1H, -NH (piperazine ring) D_2O exhangeable), 2.58–2.60 (d, 2H, -CH_2, Aliphatic),

2.76–2.79 (t, 2H, -CH_2, Aliphatic), 3.01–3.12 (m, 1H, -CH, Aliphatic) 3.48–3.52 (t, 2H, -CH_2, Aliphatic), 5.04 (s, 1H, -NH, benzimidazole, D_2O exhangeable), 6.61–7.68 (m, 8H, Ar-H, Aromatic); MS (ESI) m/z [% rel. abundance]: 292.38 (100) $[M]^+$, 293.37 (92) $[M+1]^+$, Fragments: 278.38 (40), 194.25 (31), 118.16 (41), 78.10 (74); Elemental analysis: Calcd. For $C_{18}H_{20}N_4$: C, 73.94; H, 6.89; N, 19.16%; Found: C, 73.90; H, 6.86; N, 19.13%.

2-(2-(3-Methylpiperazin-1-yl) phenyl)-1H-benzo[d] imidazole (3c)

FTIR (KBr) ν (cm^{-1}): 3310 (Aliphatic N-H str.), 3220 (Aromatic N-H stretching (2°amine)), 2970 (Aromatic C-H str.), 2262 (Aliphatic C-H str.), 1470 (Aromatic C-C str.), 1420 (Aliphatic C-N stretching), 1330 ((Aromatic C-N stretching); ^{1}H NMR (300 MHz, DMSO, δ ppm): 1.26–1.29 (d, 3H, -CH_3, Aliphatic), 2.04 (s, 1H, -NH (piperazine ring) D_2O exhangeable), 2.60–2.67 (d, 2H, -CH_2, Aliphatic), 2.59–2.88 (t, 2H, -CH_2, Aliphatic), 3.06–3.70 (m, 1H, -CH, Aliphatic) 3.42–3.66 (t, 2H, -CH_2, Aliphatic), 5.41 (s, 1H, -NH, benzimidazole, D_2O exhangeable), 6.55–7.80 (m, 8H, Ar-H, Aromatic); MS (ESI) m/z [% rel. abundance]: 292.38 (100) $[M]^+$, 293.37 (92) $[M+1]^+$, Fragments: 278.38 (40), 194.25 (31), 118.16 (41), 78.10 (74); Elemental analysis: Calcd. For $C_{18}H_{20}N_4$: C, 73.94; H, 6.89; N, 19.16%; Found: C, 73.90; H, 6.86; N, 19.13%.

2-(2-(3-Ethylpiperazin-1-yl) phenyl)-1H-benzo[d]imidazole (3d)

FTIR (KBr) ν (cm^{-1}): 3400 (Aliphatic N-H str.), 3320 (Aromatic N-H stretching (2°amine)), 3070 (Aromatic C-H str.), 2977 (Aliphatic C-H str.), 1775 (Aromatic C-C str.), 1529 (Aliphatic C-N stretching), 1382 ((Aromatic C-N stretching); ^{1}H NMR (300 MHz, DMSO, δ ppm): 1.03–1.07 (t, 3H, -CH_3, Aliphatic), 1.40–1.45 (m, 2H, -CH_2, Aliphatic), 2.42 (s, 1H, -NH (piperazine ring) D_2O exhangeable), 2.66–2.69 (d, 2H, -CH_2, Aliphatic), 2.75–2.91 (t, 2H, -CH_2, Aliphatic), 3.01–3.08 (m, 1H, -CH, Aliphatic) 3.53–3.59 (t, 2H, -CH_2, Aliphatic), 5.05 (s, 1H, -NH, benzimidazole, D_2O exhangeable), 6.68–7.75 (m, 8H, Ar-H, Aromatic); MS (ESI) m/z [% rel. abundance]: 306.4 (100) $[M]^+$, 307.3 (92) $[M+1]^+$, Fragments: 278.38 (40), 194.25 (31), 118.16 (41), 78.10 (74); Elemental analysis: Calcd. For $C_{19}H_{22}N_4$: C, 74.48; H, 7.24; N, 18.29%; Found: C, 74.45; H, 7.26; N, 18.27%.

2-(2-(2-Ethylpiperzin-1-yl) phenyl)-1H-benzo[d]imidazole (3e)

FTIR (KBr) ν (cm^{-1}): 3415 (Aliphatic N-H str.), 3338 (Aromatic N-H stretching (2°amine)), 3083 (Aromatic C-H str.), 2961 (Aliphatic C-H str.), 1576 (Aromatic C-C str.), 1459 (Aliphatic C-N stretching), 1348 ((Aromatic C-N stretching); ^{1}H NMR (300 MHz, DMSO, δ ppm): 1.04–1.08 (t, 3H, -CH_3, Aliphatic), 1.40–1.45 (m, 2H, -CH_2, Aliphatic), 2.12 (s, 1H, -NH (piperazine ring) D_2O exhangeable), 2.60–2.65 (d, 2H, -CH_2, Aliphatic), 2.73–2.88 (t, 2H, -CH_2, Aliphatic), 3.09–3.50 (m, 1H, -CH, Aliphatic) 3.52–3.57 (t, 2H, -CH_2, Aliphatic), 5.09 (s, 1H, -NH, benzimidazole, D_2O exhangeable), 6.67–7.78 (m, 8H, Ar-H, Aromatic); MS (ESI) m/z [% rel. abundance]: 306.4 (100) $[M]^+$, 307.3 (92) $[M+1]^+$, Fragments: 278.38 (40), 194.25 (31), 118.16 (41), 78.10 (74); Elemental analysis: Calcd. For $C_{19}H_{22}N_4$: C, 74.48; H, 7.24; N, 18.29%; Found: C, 74.45; H, 7.26; N, 18.27%.

2-(2-(4-Methylpiperzin-1-yl) phenyl)-1H-benzo[d] imidazole (3f)

FTIR (KBr) ν (cm^{-1}): 3409 (Aliphatic N-H str.), 3370 (Aromatic N-H stretching (2°amine)), 3050 (Aromatic C-H str.), 2972 (Aliphatic C-H str.), 1579 (Aromatic C-C str.), 1469 (Aliphatic C-N stretching), 1349 ((Aromatic C-N stretching); ^{1}H NMR (300 MHz, DMSO, δ ppm): 2.97 (s, 3H, -N-CH_3, 2.70–2.83 (t, 4H, -CH_2, Aliphatic), 3.46–3.59 (t, 4H, -CH_2, Aliphatic), 5.08 (s, 1H, -NH, benzimidazole, D_2O exhangeable), 6.35–7.50 (m, 8H, Ar-H, Aromatic); MS (ESI) m/z [% rel. abundance]: 292.38 (100) $[M]^+$, 293.37 (92) $[M+1]^+$, Fragments: 278.35 (67), 194.23 (28), 118.14 (43), 78.11 (78); Elemental analysis: Calcd. For $C_{18}H_{20}N_4$: C, 73.94; H, 6.89; N, 19.16%; Found: C, 73.91; H, 6.86; N, 19.14%.

2-(2-(4-Ethylpiperzin-1-yl) phenyl)-1H-benzo[d]imidazole (3g)

FTIR (KBr) ν (cm^{-1}): 3427 (Aliphatic N-H str.), 3337 (Aromatic N-H stretching (2°amine)), 3040 (Aromatic C-H str.), 2928 (Aliphatic C-H str.), 1535 (Aromatic C-C str.), 1427 (Aliphatic C-N stretching), 1362 ((Aromatic C-N stretching); ^{1}H NMR (300 MHz, DMSO, δ ppm): 1.06–1.11 (t,3H, CH_3 Aliphatic), 2.32–2.40 (q, 2H, -N-CH_2, 2.68- 2.71 (t, 4H, -CH_2, Aliphatic), 3.46–3.58 (t, 4H, -CH_2, Aliphatic), 5.51 (s, 1H, -NH, benzimidazole, D_2O exchangeable), 6.35–7.77 (m, 8H, Ar-H, Aromatic); MS (ESI) m/z [% rel. abundance]: 306.4 (100) $[M]^+$, 307.39 (92) $[M+1]^+$, Fragments: 278.35 (67), 194.23 (28), 118.14 (43), 78.11 (78); Elemental analysis: Calcd. For $C_{19}H_{22}N_4$: C, 74.48; H, 7.24; N, 18.29%; Found: C, 74.48; H, 7.24; N, 18.29%.

Antibacterial activity

The antibacterial activity of newly synthesized compounds was performed by Mueller–Hinton agar (Hi-media) plates (37°C, 24 h) by agar diffusion cup plate method against following microorganism: *S. aureus*, *B. subtilis* (Gram-positive) and *E. coli*, *P. aeruginosa* (Gram-negative).[26] All the compounds were screened for antibacterial activity at the concentration level of 50 μgml^{-1}

The zone of inhibitions (mm) produced by synthesized compounds are summarized in **Table 4**.

Antifungal activity

Antifungal activity was tested on Sabouraud's dextrose agar (Hi-media) plates (26°C, 48–72 h) by agar diffusion cup plate method against fungi *C. albicans* and *A. niger*.[26] All the compounds were screened for antifungal activity at the concentration level of 50 μgml^{-1}.

Table 4 shows the result of antifungal activity.

Anticancer activity

All the newly synthesized benzimidazole derivatives were screened for anticancer activity against MCF 7 cell line

Table 4: Zone of inhibition of newly synthesized compounds (3a-3g)

Compound	Zone of inhibition (mm^2)					
	Gram-positive bacteria		Gram-negative bacteria		Fungal strain	
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Candida albicans*
3a	12.55	14.17	16.58	16.45	8.54	8.62
3b	9.42	12.60	12.74	15.83	9.06	9.90
3c	11.81	10.79	19.43	21.06	11.38	10.04
3d	12.60	12.13	11.96	13.82	10.24	10.87
3e	16.37	15.50	13.34	13.64	9.84	9.63
3f	11.75	12.80	12.21	14.43	10.76	10.70
3g	12.83	10.73	12.16	12.26	11.09	10.38
Standard	20.40[a]	16.70[a]	22.09[a]	25.65[a]	12.93[b]	12.88[b]
Control	0.00	0.00	0.00	0.00	0.00	0.00

[a]Ciprofloxacin, [b]Fluconazole

by SRB assay.[27] The SRB assay possesses a colorimetric endpoint and is non-destructive and indefinitely stable. These practical advances make the SRB assay an appropriate and sensitive assay to measure percent growth inhibition. The results are shown in **Table 5**.

Percentage growth inhibition was calculated using the formula:

% Growth Inhibition (GI_{50})= ([Ti-Tz]/[C-Tz])×100

Concentrations for which Ti>Tz, (Ti−Tz) is positive or zero.

% Growth Inhibition (GI_{50})= ([Ti−Tz[/Tz)×100

Concentrations for which Ti<Tz, (Ti−Tz) is negative.

Where,

GI_{50} = Drug concentration resulting in 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation.

C = Control growth.

Ti = test growth in the presence of drug at the four concentration levels.

Tz = Cell population at the time of drug addiction.

Molecular docking and binding free energy calculations

To evaluate the binding pattern of synthesized compound as anticancer, antibacterial, and antifungal agents, docking analysis has been done by Glide.[28] For this, the crystal structures of proteins were retrieved from RCSB portal. Ligands were prepared using LigPrep application (Schrödinger). In this process, OPLS_2005 force field was used for energy optimization for all ligands until 0.01 Å RMSD cutoff not obtained. Tautomers and all possible ionization states were generated using Epic at target pH of 7 ± 2 and low energy ring conformation for each Ligand was also generated. Before docking, the crystal structure of the Topoisomerase-I enzyme (PDB ID: 1T8I), structure of the *Thermus thermophilus* 30 s ribosomal subunit in complex with the antibiotics (PDB ID: 1FJG) and crystal structure of *Aspergillus fumigatus* n-myristoyltransferase in complex with myristoyl CoA and a pyrazole sulfonamide ligand (PDB ID: 4CAW) was retrieved from Protein Data Bank. The protein was pre-processed using a Protein Preparation Wizard by assigning bond orders, adding missing hydrogen atoms, and adding missing atoms and loops. Following the generation of ionization and tautomeric states of het groups, finally restrained minimization till RMSD constraint value of 0.3 Å was done using OPLS_2005 as force field after optimization of hydrogen bonds, to refine the structure. To perform docking, a rectangular receptor grid box was generated in 20 Å size using grid generation module of Glide. All synthesized compounds, including reference drug camptothecin, fluconazole, and ciprofloxacin, were docked into the respective binding site of proteins followed by the grid generation process using the Glide panel in extra precision (XP) mode.

Table 5: Anticancer data of synthesized 3a-3g derivatives

Human breast cancer cell line MCF 7 % control growth drug concentrations (μg ml^{-1})					
Compound no.	10	20	40	80	IC_{50} value (μM)
3a	35.4	2.6	−15.6	−30.4	9.6
3b	40.4	45.3	38.2	30.8	9.9
3c	52.4	40.9	34.7	29.2	30.3
3d	58.6	25.6	10.0	2.8	22.3
3e	59.6	41.5	30.2	22.6	37.9
3f	53.4	38.7	27.2	13.3	29.7
3g	61.6	50.2	41.9	36.0	51.4
ADR (Std.)	0.3	−10.7	−33.6	−59.7	8.2

To explore the scope of investigation, we also performed binding energy and pharmacokinetic parameter calculations.[29]

The results of antibacterial activity, as shown in **Table 4,** revealed that among the newly synthesized compound **3c** with 3-methyl substitution was found to be most active against *E. coli* and *P. aeruginosa* and **3e** with 2-ethyl substitution found to be most active against *B. subtilis* and *S. aureus*. Beyond them, the valuable antibacterial activity was found in derivatives with the methyl group as

a substituent. The results of antifungal activity shown in **Table 4** revealed that compound **3g** with 4-ethyl substitution shows good activity against *C. albicans* and *A. niger*. Away from all the derivatives, the compounds with *p*-ethyl substitution show potent antifungal activity. Results of anticancer activity state that compound **3a** with piperazine ring substitution were found to be more potent with 35.2% control growth. Compound **3b** with 2-methyl substitution was also be active with 40.5% control growth <10 μgml^{-1} GI_{50} value. Molecular docking results are in correlation with biological activity results. The binding pattern of synthesized compounds was evaluated for all selected target proteins. Pharmacokinetic parameters calculations results suggest that synthesized compounds could be a potent drug for antibacterial, antifungal, and anticancer activity. Furthermore, the screened derivatives could be potent antibacterial, antifungal, and anticancer agents with a diverse mechanism of action. Based on the findings of these preclinical results, further studies need to be carried out to investigate the other specifications, such as *in vivo* assays and toxicological studies.

CONCLUSION

By the help of this research work, we have developed topoisomerase-I inhibitors by the combination of heterocycles, namely, piperazine with benzimidazole moiety. Hydrogen attached with nitrogen atom was found to be involved in the formation of a hydrogen bond with the active site of the target. The presence of an electron releasing group on the piperazine ring also increases the potency. Newly synthesized compounds were also found with significant antimicrobial activity. The study emphasizes that benzimidazole, in combination with other heterocycles, might be used as a lead for the finding of the potent anticancer and antimicrobial agents.

CONFLICTS OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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