Journal of Young Pharmacists, 2026; 18(2):347-356.
https://www.jyoungpharm.org

Original Article

Design, Molecular Docking and ADMET Analysis of Mannich-Base Substituted Benzimidazole Derivatives as Potential Antimicrobial Agents

Nivetha Jasmine Edwardraj[1,*], Hemalatha Kuppan[2], Santhiya Gopalsamy[3], Manimegalai Karnan[4]

[1]Department of Pharmaceutical Chemistry, School of Pharmacy, SBV Karaikal Campus, Sri Balaji Vidyapeeth (Deemed to be University), Karaikal, Puducherry, INDIA.
[2]Department of Pharmaceutical Chemistry, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences (A Government of Puducherry Institution, Affiliated to Pondicherry University), Puducherry, INDIA.
[3]Department of Pharmacy Practice, School of Pharmacy, SBV Karaikal Campus, Sri Balaji Vidyapeeth (Deemed to be University), Karaikal, Puducherry, INDIA.
[4]Department of Pharmaceutics, School of Pharmacy, SBV Karaikal Campus, Sri Balaji Vidyapeeth (Deemed to be University), Karaikal, Puducherry, INDIA.

ABSTRACT

Background: The growing incidence of antimicrobial resistance necessitates the development of new therapeutic agents with improved safety and efficacy. Benzimidazole derivatives are recognized as promising pharmacophores because of their broad biological activities, particularly their potential to inhibit DNA gyrase. This study aims to design and computationally evaluate novel Mannich base-substituted benzimidazole derivatives as potential antimicrobial agents targeting DNA gyrase. **Materials and Methods:** Ten benzimidazole derivatives are designed and optimized using various *in silico* approaches. Molecular docking is performed to analyze their binding affinity toward DNA gyrase. Physicochemical properties, bioactivity scores, toxicity risks, and ADMET parameters are predicted using Molinspiration, OSIRIS, and SwissADME tools. **Results:** Docking results demonstrate favorable interactions of the designed molecules within the active site of DNA gyrase. Among them, compound 2ASA exhibits the highest binding affinity (-9.06 kcal/mol), surpassing the reference drug ciprofloxacin. All compounds satisfy Lipinski's rule of five, indicating good oral drug-likeness. Bioactivity predictions suggest enzyme inhibitory potential, and most derivatives display high gastrointestinal absorption with minimal predicted toxicity. **Conclusion:** These findings identify compound 2ASA as a promising lead molecule and support further experimental validation of Mannich base-substituted benzimidazole derivatives for antimicrobial drug development.

Keywords: ADMET, Antimicrobial, Benzimidazole, Mannich base, Molecular Docking, OSIRIS, Swiss ADME.

Correspondence:
Ms. E. Nivetha Jasmine
Assistant Professor, Department of Pharmaceutical Chemistry, School of Pharmacy, SBV Karaikal Campus, Sri Balaji Vidyapeeth (Deemed to be University), Karaikal-609609, Puducherry, INDIA.
Email: nivejasmine2000@gmail.com

Received: 12-01-2026;
Revised: 24-02-2026;
Accepted: 08-05-2026.

INTRODUCTION

The problem of Antimicrobial Resistance (AMR) has become a sharp public health issue on the global agenda and has already created an emergency need to continue searching and developing new, safer, and more effective antimicrobial agents to address it on a long-term basis (Salam *et al*., 2023). The growing rate of resistance in microbial strains has seriously reduced the clinical efficiencies of most of the remaining antibiotics, hence the need to adopt other forms of therapeutic interventions. Benzimidazole-based scaffolds have garnered significant attention in medicinal chemistry because of their pleiotropic and well-documented biological activities, such as antimicrobial, anthelmintic, antiviral, and anticancer activity (Monga *et al*., 2024; Chakrabarti, 2023). The structural versatility of the benzimidazole nucleus that allows extensive chemical modification is the main reason why this pharmacological profile is extensive. This type of structural flexibility allows adjustment of physicochemical properties, selectivity on targets, and pharmacokinetic behaviour, which eventually results in increased antimicrobial potency (Chakrabarti, 2023). Due to the heteroatoms and aromatic rings, benzimidazole analogs may successfully interact with the microbial target via hydrogen bonding, pi-pi stacking, and hydrophobic interactions. In parallel, another group of biologically active organic compounds is represented by Mannich bases. They are typified by the existence of a β amino carbonyl group and have been recorded to have varying pharmacological

DOI: 10.5530/jyp.20260044

actions especially strong antimicrobial effects (Raju *et al.*, 2023; Raoof and Sadiq, 2022; Yamali *et al.*, 2023). The addition of the functionality of Mannich base has been reported to enhance the molecular lipophilicity, solubility, and membrane permeability, which are important factors of antimicrobial activity. Thus, the conjugation of the benzimidazole core with the moiety of a Mannich base seems to be a reasonable and promising solution to the design of the new antimicrobial agents. This hybridisation will likely be able to combine the favourable attributes of the two pharmacophores, which may result in synergy, increased activity on resistant microbial strains of S. aureus, and enhanced safety in combination with improved safety profiles (Wang *et al.*, 2023; Zalaru *et al.*, 2022). Past research studies on N-Mannich base derivatives of benzimidazole have shown great antibacterial, antifungal, and antiprotozoal effects, thus supporting the therapeutic promise of such hybrid molecules (Ablo *et al.*, 2023). Computational methodologies have now become essential tools to the accelerated identification and optimisation of potential drug candidates in modern drug discovery, significantly saving time and cost compared to traditional experimental methods, which is what is referred to as the first and third way of drug discovery (Chakrabarti, 2023; Salam *et al.*, 2023). Molecular docking is one of the most popular *in silico* methods, which allows predicting the binding orientation, interaction patterns, and binding affinities of small molecules in the open sites of biological targets, including essential microbial enzymes (Monga *et al.*, 2024; Raju *et al.*, 2023; Pattapulavar *et al.*, 2025). Molecular docking is useful to understand the interaction between ligands and targets and help in the prioritisation of useful compounds that should be validated further through experiments. Besides docking studies, early ADMET property assessment is also of paramount importance in drug development. Exhibition of poor pharmacokinetic behaviour or toxicity profile are significant reasons of drug attrition in clinical trials (Salam *et al.*, 2023; Monga *et al.*, 2024; Chakrabarti, 2023). As a result, prediction of ADMET during drug design has become obligatory in the early stages of drug design as a method of determining drug-like candidates with favourable safety and pharmacokinetic profiles. In the current study, the computer-aided drug design technique was used to design and screen Mannich base-substituted benzimidazole analogs as potential antimicrobial agents. The interaction between the designed compounds and critical microbial target proteins were evaluated by molecular docking, and the pharmacokinetic characteristics as well as toxicity properties of these compounds were predicted by full-scale ADMET analysis simultaneously. The overall goal of the work is to find prospective lead candidates, which can be further developed into effective antimicrobial agents to deal with the rising challenge of antimicrobial resistance.

MATERIALS AND METHODS

Molecular Docking Studies

Virtual screening software was used to analyse the expected optimal binding conformation of projected ligands into the enzyme-binding pocket. The docking protocol was applied to testing of the binding affinities with the scoring functions accounting for both steric and electrostatic interactions allowing thereby screening a range of compounds for future lead optimisation. All the molecular modelling studies were performed by using Auto Dock 4.2 (Hryhoriv *et al.*, 2021; Kingsley and Abraham, 2022; Edache *et al.*, 2022; Srivastava, 2021). Lamarckian Genetic Algorithm (LGA) was utilised to examine the ligand conformational space and minimise the Binding affinity between the ligands and the target receptor. Auto Dock Tools was used to prepare the receptor and ligand structure and grid maps of the active site were created to define the docking search space. The docking simulations were carried out, and the results analysed according to the binding energy and the predicted interaction patterns.

Xray Crystal Structure

X-ray crystal structure of the target enzyme DNA gyrase (1KZN) Figure 1 are obtained from Protein Data Bank. For the docking study a crystal structure with 2.00 Å resolution was used.

Protein Preparation

The protein configuration were prepared by voiding water molecules, co-crystallisation ligands and any other non-protein constituents (Pattapulavar *et al.*, 2025; Hryhoriv *et al.*, 2021; Kingsley and Abraham, 2022). Polar hydrogens were added and Gasteiger partial charges were attributed to all atoms with use of Auto Dock Tools (Kingsley and Abraham, 2022; Edache *et al.*, 2022). Afterwards, processed protein structures were retained in PDBQT format for extended molecular docking (Chakrabarti, 2023).

Ligand Preparation

ChemSketch was employed to rendered the two-dimensional configuration of the ten modeled benzimidazole derivatives. Figure 2 ligands with one substituent (Mannich base) and the reference drug ciprofloxacin. The 2D structures were then transformed to 3D structures and stored in PDB format through Open Babel. The 3D structures were then energy-minimised with the help of appropriate force fields to get the low-energy conformers which were stable (Chand *et al.*, 2025; Edache *et al.*, 2022). For molecular docking simulations, polar hydrogen was added and Gasteiger partial charge was assigned to all of the ligand molecules (Kingsley and Abraham, 2022; Edache *et al.*, 2022). Finally, the ligands were optimized and retained in PDBQT format to perform docking simulations with AutoDock (Chakrabarti, 2023).

Figure 2: General Structure of designed Benzimidazole Compound.

Molecular Docking

The simulations of molecular docking were conducted with AutoDock 4.2 (Salam *et al.*, 2023; Chakrabarti, 2023). The target protein active sites were defined as a grid box that defines the search space of ligand binding. The size and position of the grid box was established either using co-crystallised ligands or using standard active site residues that have been reported in the previous studies (Pattapulavar *et al.*, 2025; Chand *et al.*, 2025; Kingsley and Abraham, 2022). The docking simulations were used to forecast the manner in which the ligands oriented themselves in the protein active site and calculate the respective binding energies. All the posed docked structures and binding energies were analysed and main interaction, such as hydrogen bonds, hydrophobic contacts, and pi-pi stacking Interactions were observed and visually represented (Edache *et al.*, 2022).

ADMET Property Prediction

Computational *in silico* tools were used to evaluate the pharmacokinetic behaviour and toxicological risks of the designed ligands were:

Swiss ADME

This online platform was employed to forecast physicochemical characteristics, such as molecular weight, log P, Topological Polar Surface Area (TPSA), and water solubility. It also gave data on the pharmacokinetic properties including gastrointestinal absorption and penetration of the blood-brain barrier Figure 3. Also, the Lipinski, Ghose, Veber, Egan, and Muegge filters were used to determine drug-likeness, and the medicinal chemistry suitability of each drug was determined (Chakrabarti, 2023; Chanda and Katari, 2025; Tahiroğlu *et al.*, 2025).

OSIRIS Property Explorer

The software was employed to compute the drug-likeness, drug scores and predict possible toxicity risks, such as mutagenicity, tumorigenicity, irritancy and reproductive toxicity. The findings were presented in the form of colour codes; green was low or no risk, orange medium risk and red high risk (Bhat *et al.*, 2025; Salihu and Salleh, 2023; Shehab *et al.*, 2024; Srivastava, 2021).

Molinspiration Cheminformatics

This programme was applicable to compute molecular properties including Log P, TPSA and the rotatable number of bonds, and to assess the adherence to Lipinski Rule of Five, giving an idea into the oral bioavailability and drug-likeness (Bhat *et al.*, 2025; Salihu and Salleh, 2023; Panigrahi and Sahu, 2025). The molecules with a molecular weight less than 500 Dalton, less than 10 H-bond acceptors, less than 5 H-bond donors, and Log P less than 5 were deemed prone to have good oral bioavailability (Chand *et al.*, 2025; Sampat *et al.*, 2022).

Analysis and Visualization of Data

All the computational data such as docking data, docking results, ligand-protein interactions data and ADMET predictions were collected, arranged and reviewed. The correct statistical procedures were used, where required, to compare the characteristics of the designed derivatives. BIOVIA Discovery Studio Visualiser was used to create molecular visualisation and protein-ligand binding orientations that allowed easy visualisation of the ligand-protein interactions and identification of the important binding site residues (Yavuz, 2024).

RESULTS

Drug-Likeness and Bioactivity

Molinspiration analysis revealed that all the designed Mannich bases substituted benzimidazole derivatives complied with Lipinski's Rule of Five Table 1. The molecular weight was between 281.31 and 436.49 g/mol. The donor and acceptors of hydrogen bonds were acceptable. The TPSA ranged from 67.15 to 127.32Å. The prediction of bioactivity showed that all derivatives had high scores on enzyme inhibition Table 2. The compounds were moderately active to GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, and protease inhibitors.

Toxicity

OSIRIS Property Explorer analysis indicated that there was no significant risk of mutagenic, tumorigenic, irritant, and reproductive toxicity Table 3.

ADMET and Pharmacokinetic Properties

The outcomes of Swiss ADME revealed positive gastrointestinal absorption of most of the compounds except 2PA Table 4. Compounds 1AA and 1BA had the lowest TPSA (67.15 Å) and were predicted to penetrate the Blood Brain Barrier. It was estimated that the rest of the derivatives would possess low BBB permeability. Profiling of CYP inhibition indicated possible CYP2D6 inhibition of some of the derivatives, but lacked expected CYP inhibition of 1PA, 1ASA, and 2ASA. The values of skin permeability (Log Kp) were between -5.31 and -6.82 cm/s. The bioavailability scores were 0.55-0.56.

Lipophilicity and Medicinal Chemistry Evaluation

The lipophilicity parameters (iLOGP, XLOGP, WLOGP, MLOGP, consensus LogP) were acceptable drug-like, Table 5. No PAINS notifications were identified. Brenk alerts were minimal. The score of synthetic accessibility was 2.39 to 3.17, Table 6.

Molecular Docking

The derivatives were successfully docked into the active site of DNA gyrase (PDB ID: 1KZN). The binding energies ranged from -6.0 to -9.24 kcal/mol Table 7. The binding affinities of 2BA and

2ASA were the highest (-9.24 and -9.06 kcal/mol, respectively) and were greater than that of ciprofloxacin (-7.33 kcal/mol). Hydrogen bond interactions were found with Val120, Asn46, Thr165, and Gly119. 3.93 and 3.71 Å were also observed to have a π-sigma interaction. Other π-alkyl contacts with Ile78, Val167, and Ala47 and a π-anion interaction with Gln50 were also found Figures 4-6.

DISCUSSION

The compliance with Lipinski's criteria indicates that the designed derivatives possess favorable oral drug-likeness characteristics. The observed TPSA range suggests an optimal balance between polarity and membrane permeability, which is essential for intestinal absorption. The strong enzyme inhibition scores support the rational design strategy targeting DNA gyrase. Minimal toxicity risks and acceptable drug scores suggest a favorable safety profile. High predicted gastrointestinal absorption and moderate bioavailability scores further support oral administration potential. Limited BBB permeability for most compounds may reduce central nervous system-related adverse effects. The absence of predicted CYP inhibition in selected derivatives (1PA, 1ASA, 2ASA) suggests reduced risk of drug-drug interactions.

DNA gyrase was selected as the primary molecular target in the present study because it plays a crucial role in bacterial DNA replication, transcription, and chromosome segregation, making it an essential enzyme for bacterial survival. Inhibition of DNA gyrase has been widely exploited in antibacterial therapy, as demonstrated by the success of fluoroquinolone antibiotics. Moreover, several studies have reported that benzimidazole derivatives exhibit inhibitory activity against bacterial DNA gyrase, which provided a strong rationale for selecting this enzyme for molecular docking analysis. Although other microbial targets such as topoisomerase and Dihydrofolate Reductase (DHFR) are also important in antimicrobial drug discovery, the current study focused on DNA gyrase to specifically evaluate the interaction potential of the designed Mannich base-substituted benzimidazole derivatives with this validated target. Future studies may include multi-target docking and experimental validation against additional enzymes such as topoisomerase and

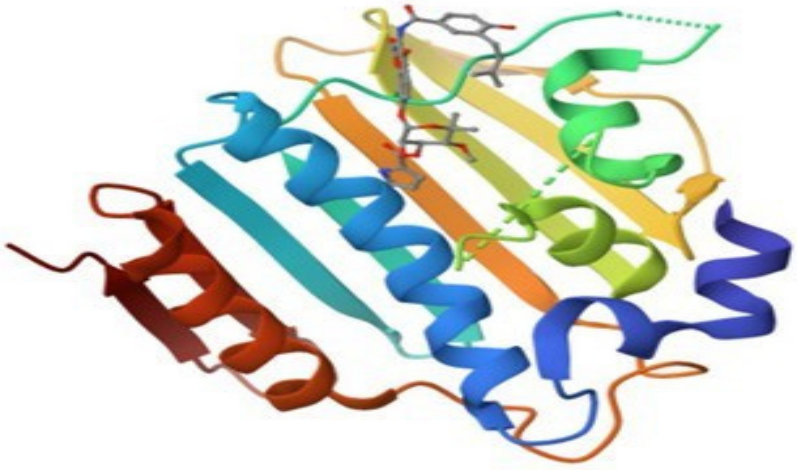
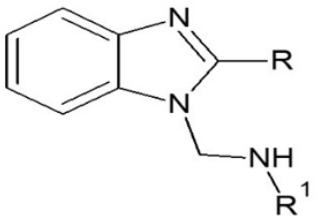


Figure 1: Crystal structure of DNA Gyrase (PDB ID - 1KZN).



R=CH3, C_6H_5, HOC_6H_4, C_6H_4COOH, $CH_3COOC_6H_4$

R^1=C_6H_4COOH, $C_6H_4SO_2NH_2$

Figure 2: General Structure of designed Benzimidazole Compound.

Table 1: Drug likeness score of designed ligands using Molinspiraton software.

Code	miLogP	TPSA	nAtoms	MW (gm)	nON	nOHNH	Nviolation	Nrotb	Vol
1AA	2.96	67.15	21	281.31	5	2	0	4	253.42
1BA	4.99	67.15	26	343.39	5	2	0	5	308.27
1SA	4.73	87.38	27	359.38	6	3	0	5	316.29
1PA	4.52	104.45	29	387.39	7	3	0	6	335.27
1ASA	4.50	93.46	30	401.42	7	2	0	7	352.80
2AA	1.75	90.02	22	316.39	6	3	0	4	269.14
2BA	3.78	90.02	27	378.46	6	3	0	5	323.99
2SA	3.51	110.25	28	394.46	7	4	0	5	332.00
2PA	3.30	127.32	30	422.47	8	4	0	6	350.99
2ASA	3.28	116.32	31	436.49	8	3	0	7	368.52

Table 2: Bioactivity scores of designed Ligands using Molinspiration software.

Code	Bioactivity score for various targets					
	GPCR	ICM	KI	NRL	PI	EI
1AA	-0.20	-0.22	-0.25	-0.46	-0.52	-0.01
1BA	-0.12	-0.10	-0.05	-0.17	-0.30	0.10
1SA	-0.12	-0.14	-0.06	-0.13	-0.32	0.11
1PA	-0.04	-0.09	-0.04	-0.11	-0.24	0.11
1ASA	-0.17	-0.17	-0.17	-0.11	-0.31	0.10
2AA	-0.29	-0.31	-0.18	-0.84	-0.31	0.07
2BA	-0.21	-0.18	-0.02	-0.52	-0.17	0.16
2SA	-0.20	-0.20	-0.02	-0.45	-0.17	0.18
2PA	-0.11	-0.16	-0.05	-0.31	-0.09	0.21
2ASA	-0.26	-0.24	-0.15	-0.42	-0.19	0.15

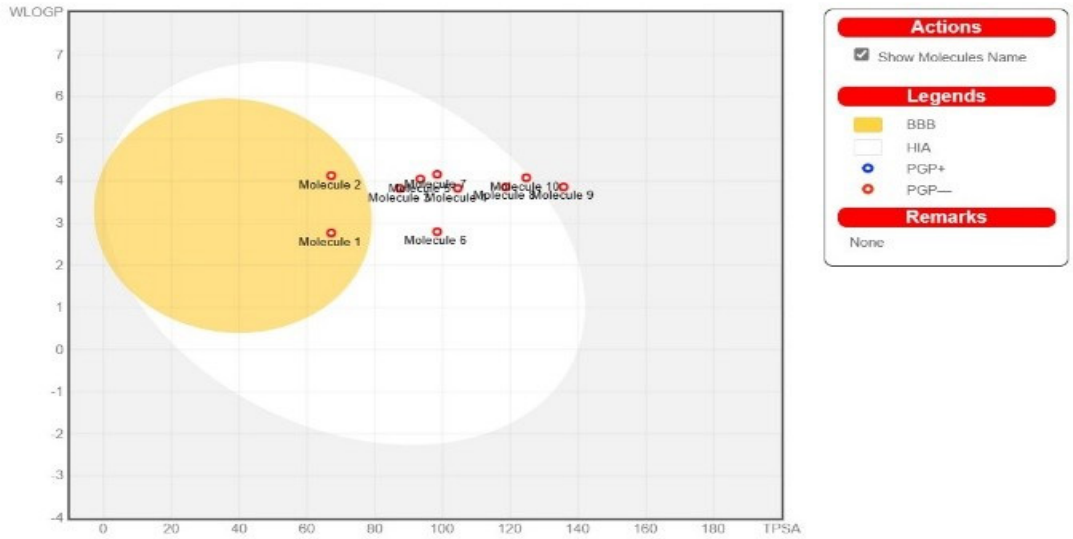


Figure 3: BOILED-Egg concept of the designed ligands.

Table 3: **Toxicity Profile of designed Ligands using OSIRIS Property Explorer.**

Code	C LogP	Solubility	Drug likeness	Drug score	Mutagenic Effect	Tumorigenic Effect	Irritant Effect	Reproductive Effect
1AA	2.53	-2.66	1.29	0.79				
1BA	3.94	-5.17	2.19	0.56				
1SA	3.59	-4.88	1.27	0.57				
1PA	3.43	-5.19	-1.4	0.36				
1ASA	3.93	-5.47	1.82	0.5				
2AA	1.98	-2.54	3.93	0.88				
2BA	3.39	-5.06	4.78	0.61				
2SA	3.04	-4.76	3.94	0.64				
2ASA	3.38	-5.36	4.35	0.54				
Ciprofloxacin (Standard)	-1.53	-3.32	2.07	0.82				

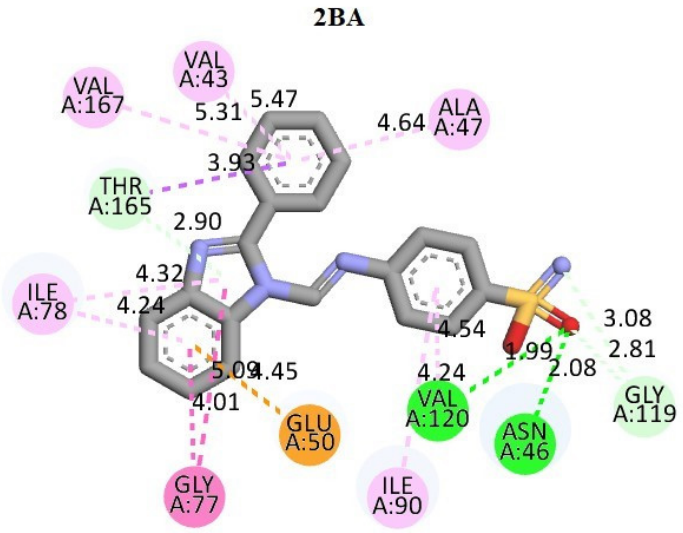


Figure 4: Interaction of Compound 2BA with the active site residues of DNA Gyrase.

DHFR to further broaden the antimicrobial evaluation of these compounds.

Substituents at the R position play a crucial role in modulating activity. Derivatives containing aromatic or electron-withdrawing substituents, such as Carboxyphenyl (C_6H_4COOH), Hydroxy Phenyl (C_6H_4OH), and Acetoxyphenyl ($CH_3COOC_6H_4$) groups, exhibited improved docking scores compared with simple electron releasing substituents like alkyl/ aryl substituents [methyl (CH_3) and Phenyl (C_6H_5)]. These aromatic substituents increase hydrophobic interactions and π-stacking interactions within the enzyme binding pocket. In particular, the presence of polar functional groups such as -COOH and -OH enhances hydrogen bonding with key residues including Val120, Asn46, Thr165, and Gly119, thereby stabilizing the ligand-protein complex.

The R_1 substituent also significantly affects the binding interactions. Compounds containing Sulfonamide ($C_6H_4SO_2NH_2$) groups

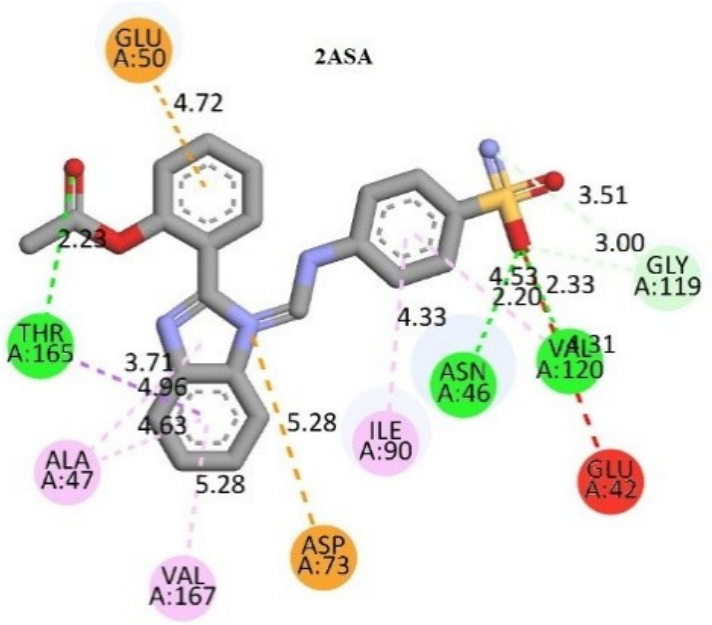


Figure 5: Interaction of Compound 2ASA with the active site residues of DNA Gyrase.

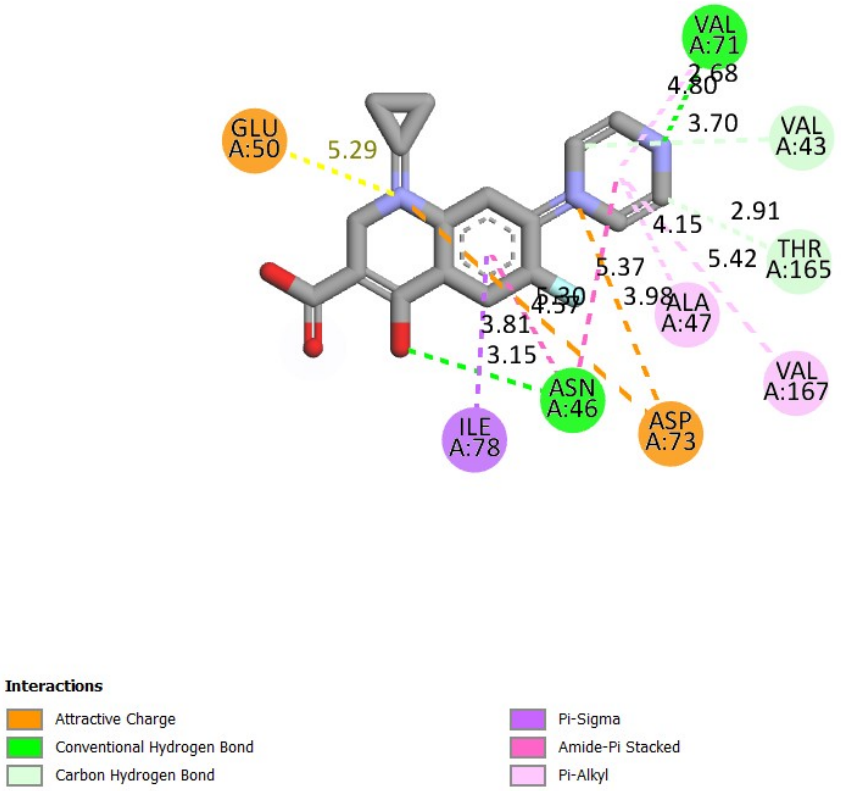


Figure 6: Interaction of standard ciprofloxacin with the active site residues of DNA Gyrase.

Table 4: Physicochemical and Pharmacokinetics properties prediction of designed Ligands.

Code	Physicochemical Properties				Pharmacokinetics Properties					
	MW	RB	TPSA (Å)	ESOL Class	GIA	BBB P	Pgp S	CYP¹	CYP²	Log Kp (cm/s)
1AA	281.31	4	67.15	Sol	High	Yes	No	Yes	Yes	-5.83
1BA	343.39	5	67.15	M Sol	High	Yes	No	Yes	Yes	-5.31
1SA	359.38	5	87.38	M Sol	High	No	No	Yes	Yes	-5.67
1PA	387.39	6	104.45	M Sol	High	No	No	No	No	-5.92
1ASA	401.42	7	93.46	Sol	High	No	No	No	No	-5.84
2AA	316.39	4	90.02	M Sol	High	No	No	Yes	Yes	-6.73
2BA	378.46	5	90.02	M Sol	High	No	No	Yes	Yes	-6.21
2SA	394.46	5	110.25	M Sol	High	No	No	Yes	Yes	-6.56
2PA	422.47	6	127.32	M Sol	Low	No	No	Yes	No	-6.82
2ASA	436.49	7	116.32	M Sol	High	No	No	No	No	-6.74

Table 5 : Lipophilicity and Drug likeness properties of designed Ligands.

Code			Lipophilicity			Drug Likeness					BAS
							No. of Violations				
	I Log P	X Log P	W Log P	M Log P	Cons. Log P	L	G	V	E	M	
1AA	1.91	3.08	2.77	1.32	2.21	0	0	0	0	0	0.85
1BA	2.43	4.34	4.13	2.27	3.24	0	0	0	0	0	0.85
1SA	2.47	3.98	3.83	1.72	2.91	0	0	0	0	0	0.56
1PA	2.00	3.86	3.83	1.88	2.81	0	0	0	0	0	0.56
1ASA	2.76	4.09	4.05	2.10	3.20	0	0	0	0	0	0.56
2AA	1.61	2.11	2.80	1.29	1.69	0	0	0	0	0	0.55
2BA	2.20	3.38	4.16	2.27	2.74	0	0	0	0	0	0.55
2SA	1.66	3.02	3.86	1.74	2.29	0	0	0	0	0	0.55
2PA	1.42	2.90	3.86	2.18	2.30	0	0	0	1	0	0.56
2ASA	2.60	3.13	4.08	2.40	2.78	0	0	0	0	0	0.55

Table 6: Medicinal Chemistry Assessment of the Designed Ligands.

Code	PAINS #alerts	Brenk #alerts	Leadlikeness #alerts	Synthetic Accessibility
1AA	0	0	Yes	2.39
1BA	0	0	No	2.76
1SA	0	0	No	2.80
1PA	0	0	No	2.97
1ASA	0	1	No	3.04
2AA	0	0	Yes	2.68
2BA	0	0	No	2.96
2SA	0	0	No	2.97
2PA	0	0	No	3.08
2ASA	0	1	No	3.17

Table 7: Energy minimization of DNA Gyrase (PDB ID - 1KZN).

Code	Binding Energy (Kcal/mol)	Ligand Efficiency	Inhibitory Constant	Vander waals Desolvation Energy
1AA	-6.0	-0.29	40.09µM	-7.09
1BA	-7.44	-0.29	3.53µM	-9.24
1SA	-7.40	-0.27	3.79µM	-9.02
1PA	-6.76	-0.23	11.16µM	-9.02
1ASA	-6.94	-0.23	8.13µM	-9.26
2AA	-7.22	-0.33	5.08µM	-8.13
2BA	-9.24	-0.34	169.77nM	-10.78
2SA	-8.75	-0.31	384.59nM	-10.25
2PA	-8.20	-0.27	981.53nM	-10.1
2ASA	-9.06	-0.29	228.36nM	-11.21
Ciprofloxacin (Standard)	-7.33	-0.31	4.27µM	-7.76

showed stronger binding affinity due to their ability to act as hydrogen bond donors and acceptors. This facilitates stronger hydrogen bonding with amino acid residues in the active site and contributes to improved ligand stabilization.

Among the designed compounds, 2BA and 2ASA showed the highest binding affinities (-9.24 and -9.06 kcal/mol, respectively). The enhanced activity of these compounds can be attributed to the combined effect of aromatic substituents, hydrogen bonding capability, and favorable hydrophobic interactions. Docking analysis indicated that these derivatives form multiple stabilizing interactions including hydrogen bonds, π-sigma interactions, π-alkyl contacts with residues such as Ile78, Val167, and Ala47, and π-anion interactions with Gln50.

Overall, the SAR analysis suggests that increasing aromaticity and introducing polar functional groups capable of hydrogen bonding significantly enhance the inhibitory potential of benzimidazole-Mannich derivatives against DNA gyrase. These findings highlight compounds 2BA and 2ASA as promising lead molecules for further optimization and antimicrobial development.

CONCLUSION

In this study, a series of Mannich base-substituted benzimidazole derivatives were designed and evaluated using computational approaches to explore their potential as antimicrobial agents targeting DNA gyrase. Drug-likeness analysis indicated that all the designed compounds complied with Lipinski's Rule of Five, suggesting favorable physicochemical properties and good oral bioavailability. Bioactivity prediction further supported their potential as enzyme inhibitors.

Toxicity assessment using OSIRIS Property Explorer revealed minimal risks of mutagenicity, tumorigenicity, irritancy, and reproductive toxicity for most of the derivatives. In addition, SwissADME analysis demonstrated acceptable pharmacokinetic properties, including high gastrointestinal absorption for most compounds and suitable lipophilicity values. These findings suggest that the designed molecules possess promising drug-like characteristics.

Molecular docking studies showed that all the derivatives interacted effectively with the active site of DNA gyrase (PDB ID: 1KZN). The binding energies ranged from -6.0 to -9.24 kcal/mol, indicating favorable ligand-protein interactions. Among the compounds, 2BA and 2ASA exhibited the strongest binding affinities, which were higher than that of the reference drug ciprofloxacin. The enhanced activity of these compounds can be attributed to the presence of aromatic substituents and functional groups capable of forming hydrogen bonds and hydrophobic interactions with key amino acid residues within the enzyme binding pocket.

Overall, the results suggest that benzimidazole-Mannich base derivatives represent promising scaffolds for the development of new antimicrobial agents targeting DNA gyrase. Among the designed compounds, 2ASA and 2BA emerged as potential lead molecules with favorable binding affinity and pharmacokinetic properties. However, further *in vitro* and *in vivo* studies are necessary to validate their antimicrobial activity and therapeutic potential.

ACKNOWLEDGEMENT

The authors are thankful to DR. S. Govindarajan, Dean and Prof. DR. K. Girija, Principal, College of Pharmacy, MTPG&RIHS (A Govt. of Puducherry Institution), Affiliated to Pondicherry University for their kind support.

ABBREVIATIONS

AMR: Antimicrobial Resistance; **ADMET:** Absorption, Distribution, Metabolism, Excretion and Toxicity; **TPSA:** Topological Polar Surface Area; **BBB:** Blood-Brain Barrier; **GI:** Gastrointestinal; **GPCR:** G-Protein Coupled Receptor; **ICM:** Ion Channel Modulator; **KI:** Kinase Inhibitor; **NRL:** Nuclear Receptor Ligand; **PI:** Protease Inhibitor; **EI:** Enzyme Inhibitor; **CYP:** Cytochrome P450; **PDB:** Protein Data Bank; **LGA:** Lamarckian Genetic Algorithm; **PAINS:** Pan-Assay Interference Compounds; **MW:** Molecular Weight; **Log P:** Partition Coefficient; **HBA:** Hydrogen Bond Acceptor; **HBD:** Hydrogen Bond Donor.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Ablo, E., Dosso, O., Coulibaly, B., Adingra, K. F., Coulibaly, P. M. A., Achi, A. P., ... & Coulibali, S. (2023). Novel Mannich bases of benzimidazole derivatives: an antibacterial study of environmental bacterial strains. *Adv. Biol. Chem.*, *13*, 182-191. https://doi.org/10.4236/abc.2023.135013

Bhat, N., Revanasiddappa, B. C., Daksha, S., Chouta, D., Bidye, D. P., Dixit, S. R., & Awasthi, A. (2025). Exploring the therapeutic potential of some alkaloidal phytoconstituents for diabetes management: a thorough in-silico approachment. *Future Journal of Pharmaceutical Sciences*, *11*(1), 1-15. https://doi.org/10.1186/s43094-025-00901-8

Chand, J., Jupudi, S., Ahmad, S. F., Emran, T. B., & Subramanian, G. (2025). Therapeutic mechanistic study of novel indole derivatives as SIRTUIN3 modulators in Parkinson's disease with *in vitro* evaluation. *Scientific reports*, *15*(1), 15196. https://doi.org/10.1038/s41598-025-99534-3

Chanda, H. M. K. C., & Katari, S. K. (2025). *In silico* Discovery of Natural and Synthetic Inhibitors Targeting AKT1 in Prostate Cancer. https://doi.org/10.21203/rs.3.rs-7627265/v1

Edache, E. I., Uzairu, A., Mamza, P. A., & Shallangwa, G. A. (2022). QSAR, homology modeling, and docking simulation on SARS-CoV-2 and pseudomonas aeruginosa inhibitors, ADMET, and molecular dynamic simulations to find a possible oral lead candidate. *Journal of Genetic Engineering and Biotechnology*, *20*(1), 88. https://doi.org/10.1186/s43141-022-00362-zhttps://doi.org/10.1186/s43141-022-00362-z

Feng, J., Zheng, Y., Ma, W., Weng, D., Peng, D., Xu, Y., ... & Wang, X. (2024). A synthetic antibiotic class with a deeply-optimized design for overcoming bacterial resistance. *Nature Communications*, *15*(1), 6040. https://doi.org/10.1038/s41467-024-50453-3

Hryhoriv, H., Mariutsa, I., Kovalenko, S. M., Sidorenko, L., Perekhoda, L., Filimonova, N., ... & Georgiyants, V. (2021). Structural modification of ciprofloxacin and norfloxacin for searching new antibiotics to combat drug-resistant bacteria. *ScienceRise: Pharmaceutical Science*, (5 (33)), 4-11.http://doi.org/10.15587/2519-4852.2021.242997

Kingsley, D., & Abraham, J. (2022). *In vitro* analysis of antimicrobial compounds from Euphorbia milli. *Current Trends in Biotechnology and Pharmacy*, *16*(Supplement 1), 15-27.https://doi.org/10.5530/ctbp.2022.2s.27

Monga, J., Ghosh, N. S., Rani, I., Singh, R., Deswal, G., Dhingra, A. K., & Grewal, A. S. (2024). Unlocking the pharmacological potential of benzimidazole derivatives: a pathway to drug development. *Current Topics in Medicinal Chemistry*, *24*(5), 437-485. https://doi.org/10.2174/0115680266283641240109080047

Panigrahi, D., & Sahu, S. K. (2025). Computational approaches: atom-based 3D-QSAR, molecular docking, ADME-Tox, MD simulation and DFT to find novel multi-targeted anti-tubercular agents. *BMC chemistry*, *19*(1), 39. https://doi.org/10.1186/s13065-024-01357-2

Pattapulavar, V., Ramanujam, S., Shah, M., Thirunavukkarasu, M. K., Arumugam, S., Karuppasamy, R., ... & Christopher, J. G. (2025). Streptomyces sp. VITGV156 secondary metabolite binds pathogenic protein PBP2a and Beta-lactamase. *Frontiers in Bioinformatics*, *5*, 1544800. https://doi.org/10.3389/fbinf.2025.1544800https://doi.org/10.3389/fbinf.2025.1544800

Raju, S. K., Vengadhajalaphathy, P., Sundaram, R., Periyasamy, S., Chinnaraj, T., & Sekar, P. (2023). Recent advances in biological applications of Mannich bases—An overview. *Int. J. Pharm. Chem. Anal*, *10*(1), 15-27. https://doi.org/10.18231/j.ijpca.2023.004https://doi.org/10.18231/j.ijpca.2023.004

Raoof, S. S., & Sadiq, A. S. (2022). Mannich bases: Synthesis, pharmacological activity, and applications: A review. *Iraqi Journal of Science*, 5086-5105.https://doi.org/10.24996/ijs.2022.63.12.1

Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., & Alqumber, M. A. (2023). Antimicrobial resistance: a growing serious threat for global public health. In *Healthcare* (Vol. 11, No. 13, p. 1946). MDPI. https://doi.org/10.3390/healthcare11131946

Salihu, A. S., & Salleh, W. M. N. H. W. (2023). Exploring the potential of furofuran lignans isolated from beilschmiedia pulverulenta for drug development: a computational approach. *Yuzuncu Yıl University Journal of Agricultural Sciences*, *33*(3), 491-502.https://doi.org/10.29133/yyutbd.1294240

Sampat, G., Suryawanshi, S. S., Palled, M. S., Patil, A. S., Khanal, P., & Salokhe, A. S. (2022). Drug likeness screening and evaluation of physicochemical properties of selected medicinal agents by computer aided drug design tools. *Adv. Pharmacol. Pharm*, *10*(4), 234-246.https://doi.org/10.13189/app.2022.100402

Shehab, W. S., Elsayed, D. A., Abdel Hamid, A. M., Assy, M. G., Mouneir, S. M., Hamed, E. O., ... & El-Bassyouni, G. T. (2024). CuO nanoparticles for green synthesis of significant anti-Helicobacter pylori compounds with *in silico* studies. *Scientific reports*, *14*(1), 1608.https://doi.org/10.1038/s41598-024-51708-1

Srivastava, R. (2021). Theoretical studies on the molecular properties, toxicity, and biological efficacy of 21 new chemical entities. *ACS omega*, *6*(38), 24891-24901. https://doi.org/10.1021/acsomega.1c03736

Tahiroğlu, V., Gören, K., Kotan, G., & Yüksek, H. (2025). *In silico* drug evaluation by molecular docking, ADME studies and synthesis, characterization, biological activities, DFT, SAR analysis of the novel Mannich bases. *BMC chemistry*, *19*(1), 249.https://doi.org/10.1186/s13065-025-01615-x

TI, M. C. (2023). Revolutionizing antimicrobial drug discovery: computational design and ADMET studies of emerging potent anti-microbial agents. https://dx.doi.org/10.22159/ijpps.2023v15i8.48526

Tokalı, F. S., Taslimi, P., Demircioğlu, İ. H., Şendil, K., Tuzun, B., & Gülçin, İ. (2022). Novel phenolic Mannich base derivatives: synthesis, bioactivity, molecular docking, and ADME-Tox Studies. *Journal of the Iranian Chemical Society*, *19*(2), 563-577.. https://doi.org/10.1007/s13738-021-02331-8

Yamali, C., Gul, M., & Gul, H. I. (2023). Current pharmaceutical research on the significant pharmacophore mannich bases in drug design. *Current Topics in Medicinal Chemistry*, *23*(27),2590-2608. https://doi.org/10.2174/0115680266256102230922101939

Yavuz, S. C. (2024). Synthesis of new two 1, 2-disubstituted benzimidazole compounds: their *in vitro* anticancer and *in silico* molecular docking studies. *BMC chemistry*, *18*(1), 146.https://doi.org/10.1186/s13065-024-01241-z

Yeşilçayır, E., Çelik, İ., Şen, H., Rızvanoğlu, S., Eryılmaz, M., & Kilcigil, G. (2022). Novel benzimidazole-based compounds as antimicrobials: Synthesis, molecular docking, molecular dynamics and *in silico* ADME profile studies. *Acta Chimica Slovenica*, *69*(2). http://doi.org/10.17344/acsi.2022.7314

Zalaru, C., Dumitrascu, F., Draghici, C., Tarcomnicu, I., Marinescu, M., Nitulescu, G. M., ... & Chifiriuc, M. C. (2022). New pyrazolo-benzimidazole Mannich bases with antimicrobial and antibiofilm activities. *Antibiotics*, *11*(8), 1094.https://doi.org/10.3390/antibiotics11081094

Cite this article: Nivetha Jasmine, Hemalatha, Santhiya, Manimegalai. Design, Molecular Docking and ADMET Analysis of Mannich-Base Substituted Benzimidazole Derivatives as Potential Antimicrobial Agents. J Young Pharm. 2026;18(2):347-56.