



**DRUG DESIGN, SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL
STUDIES OF NOVEL BENZIMIDAZOLE DERIVATIVES AS DNA GYRASE
INHIBITOR IN *ESCHERICHIA COLI***

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ABSTRACT

Background: A novel series of N-Mannich bases of benzimidazole analogues were designed and synthesized with an effort to overcome the increasing antibiotic resistance. DNA gyrase is an essential bacterial enzyme that catalyzes the ATP-dependent negative super-coiling of double-stranded closed-circular DNA. Benzimidazole is having the ability to inhibit the bacterial DNA gyrase. In this study, we target DNA gyrase enzyme on *Escherichia coli*.

Method: In this work we aimed to design, synthesis and evaluate the N-Mannich bases of benzimidazole. The ligand molecules were subjected to molecular docking studies with enzyme DNA gyrase. Computational Autodock 4.2 tools will be employed in this study for docking of benzimidazole ligand molecules against DNAGyrase (PDB code: 1KZN). Molinspiration server was used for lead optimization.

Results and Conclusion: The molecular docking studies are supported to compare *in-vitro* antibacterial activity by the use of binding energy of the docked ligand molecules. The newly synthesized compounds were characterized by UV and various physico-chemical methods. Further, the antibacterial activity of benzimidazole compounds were assessed with zone of inhibition by agar well diffusion method using gram negative bacterial strain *Escherichia coli*.

Keywords: DNAGyrase, Benzimidazole, Molecular docking, N-Mannich base, *Escherichia coli*

INTRODUCTION

Benzimidazole [1] is a heterocyclic aromatic organic compound. This bicyclic compound consists of the fusion of benzene and imidazole. Benzimidazole moieties are very important class of heterocyclic compounds that have many applications in pharmaceutical chemistry. Mannich bases [2] of benzimidazole derivatives shows antimicrobial activities, which are designed and synthesized with an effort to overcome the increasing antibiotic resistance. Mannich reaction is the condensation of a compound having one or more active hydrogen atoms with formaldehyde and ammonia or a primary or secondary amine usually as hydrochloride, resulting in the formation of β -aminocarbonyl compound is known as Mannich reaction. Bacteria is a unicellular microorganism and that cause serious diseases. *Escherichia coli*, also known as *E. coli*, is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms. *Escherichia coli* (*E. coli*) bacteria normally live in the intestines of healthy people and animals. DNAgyrase [3] is an essential bacterial enzyme that catalyzes the ATP-dependent negative super-coiling of double-stranded closed-circular DNA. Gyrase belongs to a class of enzymes known as topoisomerases

that are involved in the control of topological transitions of DNA.

The current research is aimed to design of enzyme inhibitors as target for the drug discovery. Drug discovery [4-7] tools helps in designing the new molecular entities which are safe and effective without consuming much of the research hours. Literature review suggests that benzimidazole can inhibit the DNAgyrase that present in the *E.coli* is an essential bacterial enzyme that catalyzes the ATP-dependent negative super-coiling of double-stranded closed-circular DNA.

Drug likeness score helps to find out the bioavailability. Lipinski's Rule of Five is a rule of thumb to evaluate drug likeness, or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. By considering the above observations, an attempt is made here to design and synthesize various N-Mannich bases of benzimidazole as DNAgyrase inhibitors.

MATERIALS AND METHODOLOGY

Molecular modeling

Lipinski's rule of five [8-10] helps to find the drug likeness score by evaluating the oral absorption property of the ligand. Solubility, diffusion, log P, molecular weight etc are evaluated. Lipinski's rule of five can be found out by using

Molinspiration server. In order to find out the structural interactions of the N-mannich base of benzimidazole with the enzyme, DNAgyrase, molecular docking simulation was done by utilizing Autodock 4.2 [11-13]. The crystal structure of DNAgyrase from *Escherichia coli* (PDB entry: 1KZN) was retrieved from the Protein Data Bank. The remaining docking protocol was described from our previous studies. The final docked conformations were ranked according to their binding free energy. The pharmacophore modelling of the docked pose were generated (Figure 1-6).

Preparation of Benzimidazole [14]

Place 0.06 mol of o-Phenylene diamine in a round bottom flask. Add 0.15 mol of 90% formic acid, heat the mixture in a water bath at 100°C for 2 hours. Cool and add 10% sodium hydroxide slowly with constant stirring till the solution becomes alkaline to litmus. Filter and wash with ice water. Dissolve in boiling water and add 0.16 mol charcoal and digest for 15 minutes. Filter and cool the filtrate at 10°C.

Preparation of N-Mannich bases of benzimidazole [15]

To a solution of synthesized benzimidazole (0.01 mol) in DMF, formaldehyde (0.02 mol) was added under stirring. The reaction mixture was stirred at room temperature for 30 minute to complete the reaction of formaldehyde and to yield methylol derivative. To this solution, 0.02 mol

primary or secondary amine was added drop wise and refluxed for 2hrs. The reaction mixture was poured into ice cold water and filtered and washed with hot water. Finally it was dried & purified by recrystallisation from ethanol.

N-(1*H*-benzimidazol-1-ylmethyl)aniline

Solid, %yield: 67.16, m.p.: 108-110, Rf: 0.92

N-(1*H*-benzimidazol-1-ylmethyl)-*N*-phenylaniline

Solid, %yield: 62.87, m.p.: 130-132, Rf: 0.88.

4-[(1*H*-benzimidazol-1-yl)methyl]amino}benzoic acid

Solid,%yield: 70.37, m.p.: 56-58, Rf: 0.91

N-(1*H*-benzimidazol-1-ylmethyl)benzamide

Solid,%yield: 77.29, m.p.: 120-122, Rf: 0.87

N-(1*H*-benzimidazol-1-ylmethyl)benzene-1,2-diamine

Solid,%yield: 68.48, m.p.: 110-112, Rf: 0.88

Antibacterial screening [16]

The antibacterial activity of synthesized compounds (M₁-M₅) was assessed against *Escherichia coli* agar disc diffusion method. The sterilized (autoclave at 120°C for 30 minutes) nutrient agar medium (40-50°C) was inoculated with the suspension of microorganism and mixture was transferred to sterile petridishes and allowed to solidify. In each plate 10mm

disc was placed. Ciprofloxacin disc of conc. 1µg placed kept as standard and 20µl synthesized compounds (200µg/µl) solutions in DMF is poured. The plates were kept in refrigerator for 30 minutes to allow the diffusion of sample to the surrounding agar medium. The plates were incubated at 37±2°C for 24 hours and observed for antibacterial activity. The diameter of zone of inhibition were measured and compared with that of standard.

RESULTS

For the present study, we have selected DNAgyrase as target enzyme based on literature review [17-29]. Safety and efficacy of the lead compounds were evaluated by computation of drug like properties. Five compounds (**M₁₋₅**) were subjected to *in-silico* lead optimization (**Table 1**).

For the docking study, DNAgyrase (1KZN.pdb) proteins were used. Docking of the ligands was done by the use of AutoDock 4.2 software and the interactions with the active site of enzyme were studied. The derivatives were subjected to interactions with DNAgyrsae (inhibited by chlorobiocin). Most of the derivatives were interacting with the key active site of the DNAgyrase with superior binding energy (**Table 2**). In N-Mannich base of bezimidazole series all the compound shown excellent binding interaction. Among

these diphenyl amine derivative of benzimidazole (**M₂**) shown highest binding energy – 7.81kcal/mol compared to standard ciprofloxacin (-6.75 kcal/mol). Other derivatives such as **M₁** (**acetanilide derivative**) and **M₅** (**o-pheneylne diamine derivative**) also showed very good similar binding energy -7.01 kcal/mol. But compound **M₃** (**p-amino benzoic acid derivative**) and **M₄** (**benzamide derivative**) showed least binding energy -5.51 kcal/mol and -6.56 kcal/mol respectively.

In this scheme (**Figure 7**), the N-Mannich base of secondary and primary amine is prepared by two step process. At the first step we have prepared benzimidazole from ortho phenylene diamine and formic acid. Then benzimidazole from the step 1 is treated with formaldehyde and secondary or primary amine under reflux for 2-5 hrs gives the product. Melting point of all the newly synthesized compounds was determined. R_f values were determined to confirm the completion of the reaction. Antibacterial screening of the synthesized compounds were done by Agar well plate method in nutrient agar medium and ciprofloxacin was used as the standard. All newly synthesized compounds **M₁₋₅** showed antifungal activity towards the tested clinical strain of *Escherichia coli*. The values were tabulated in **Table 3**. Among these, N-Mannich bases compound **M₅** (**o-phenylene diamine derivative**) and **M₂** (**diphenyl amine derivative**) showed good zone of inhibition of 3mm and 2mm respectively. Compound **M₁**

showed moderate zone of inhibition of 1mm. concentration.

M₃ and M₄ has no antibacterial activity at this

Table 1: Drug likeness scores of M₁₋₅ using molinspiration server

Sl.No	Compound code	mLogP	MW	No. of H acceptors	No. of H donors	No. of violations
01	M ₁	2.57	265.32	4	0	0
02	M ₂	4.91	299.38	3	0	0
03	M ₃	2.88	267.29	5	2	0
04	M ₄	2.30	251.29	4	1	0
05	M ₅	2.40	238.29	4	3	0

The ligands were subjected to find any violation of Lipinski's rule of Five.

Table 2: Binding energies of M₁₋₅ with DNAGyrase (1KZN.pdb)

Sl. No	Compound Code	Binding energy ($\Delta G = \text{kcal/mol}$)	Binding Interaction
01	M ₁	-7.01	PRO 79, ILE78, THR165, ASP73 and ASN46
02	M ₂	-7.81	ASN 46, THR 165, ASP 73, PRO 79 and Ile 78
03	M ₃	-5.51	ILE78, THR165, ASP73 and ASN46
04	M ₄	-6.56	PRO 79, ILE78, THR165, ASP73 and ASN46
05	M ₅	-7.01	ILE78, THR165, ASP73 and ASN46
06	Ciprofloxacin	-6.75	ILE78, THR165, ASP73 and ASN46

Tabulation of dock score using autodock4.2. Binding interaction towards the aminoacid also tabulated

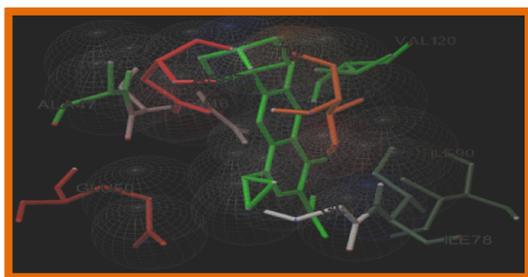


Figure 1: Snapshot of Ciprofloxacin with DNAGyrase

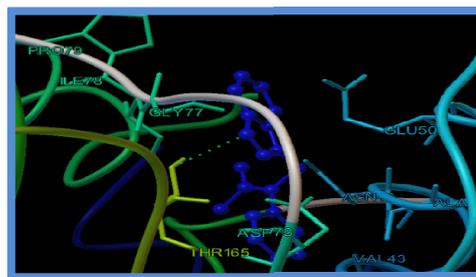


Figure 2: Snapshot of M₁

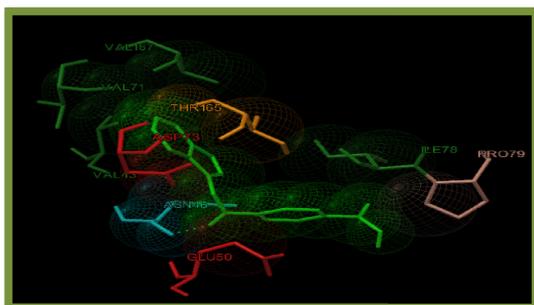


Figure 3: Snapshot of M₂

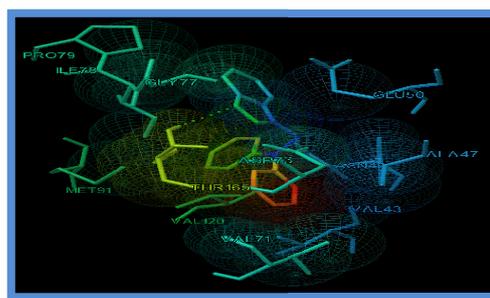


Figure 4: Snapshot of M₃

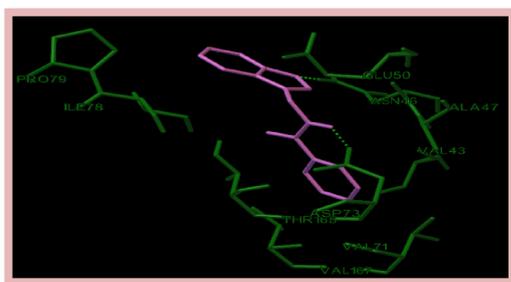


Figure 5: Snapshot of M₄

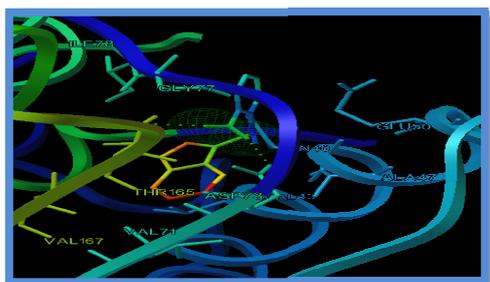


Figure 6: Snapshot of M₅

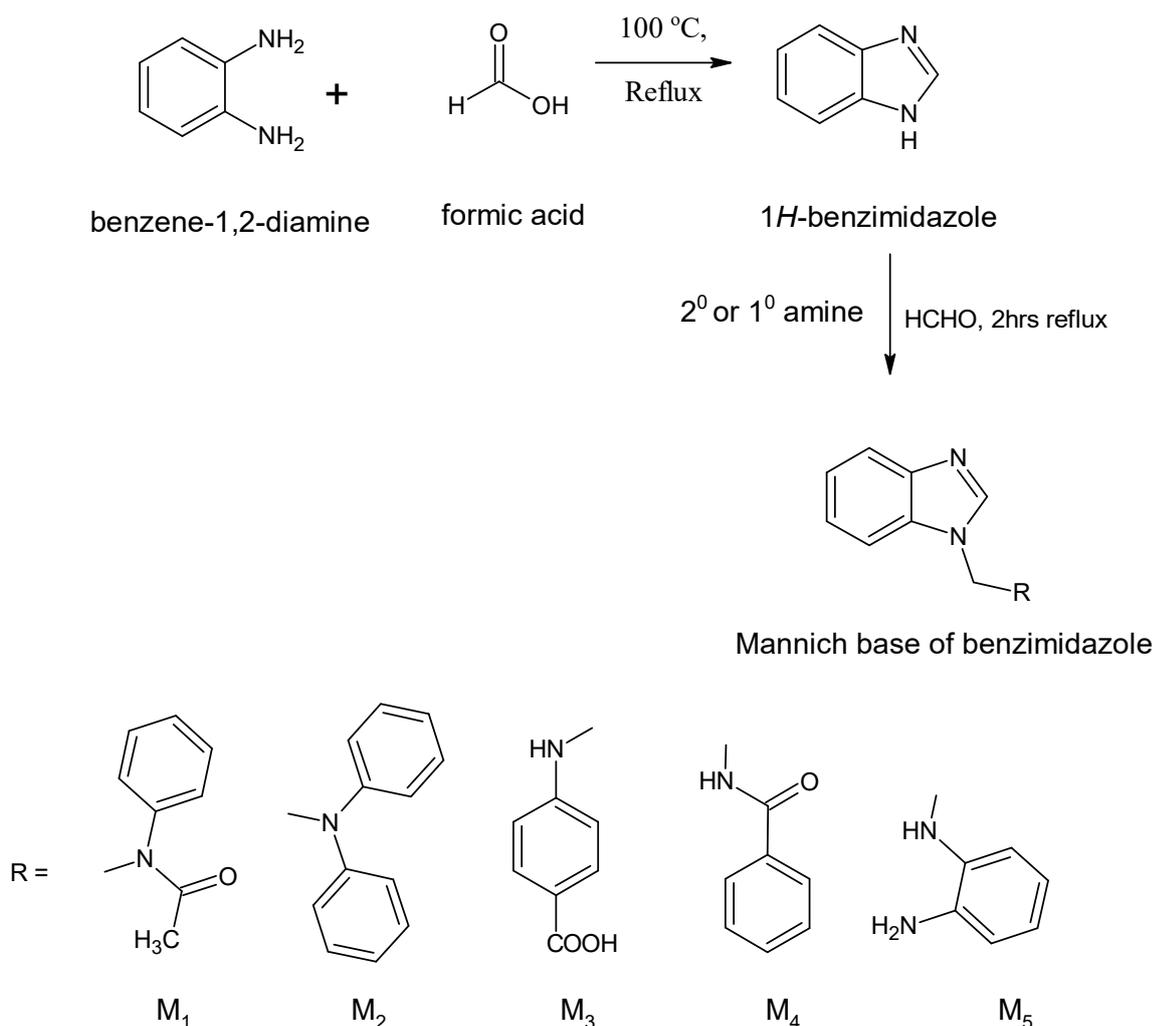


Figure 7: Synthetic Scheme for the preparation of derivatives of N-Mannich bases of Benzimidazole

Table 3: Antibacterial Screening of test compounds *Escherichia coli*

Compound Code	Zone of Inhibition (mm)	
	<i>Escherichia coli</i>	
M ₁	1	
M ₂	2	
M ₃	-	
M ₄	-	
M ₅	3	
Ciprofloxacin	10	

Zone of inhibition of the synthesized compound with *Escherichia coli* by agar disc diffusion method

DISCUSSION

Computer aided drug design helps to minimise the tedious drug discovery process over the traditional method. Drug likeness score of the ligands observed helped to predict a better pharmacokinetic

activity and oral bioavailability of the designed leads. The binding energies obtained from docking study of DNAGyrase confirms that the lead compound inhibit the enzymes present in *Escherichia coli*. Designed schemes were

utilised in the preparation of novel benzimidazole incorporated N-mannich bases of secondary and primary amine. The screening of synthesized compounds for antibacterial study revealed that o-phenylene diamine substituted N-mannich base of benzimidazole, diphenyl amine substituted N-mannich base of benzimidazole showed excellent activity. Thus the present study depicts that the utilization of computer aided drug design is an efficient tool in predicting the effectiveness of a series of compounds under study and thus can result in the design of potent antibacterial agent.

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