ORIGINAL ARTICLE

Design, *in silico* analysis, synthesis, and evaluation of novel benzofused nitrogen-containing heterocyclic N-substituted mercaptobenzimidazole derivatives as potential antimicrobial agent

Tejaswini D. Patil[1]*, Sunil V. Amrutkar[2]

[1]Department of Pharmaceutical Chemistry, Progressive Education Society's, Modern College of Pharmacy, Nigadi, Pune, Maharashtra, India, [2]Department of Pharmaceutical Chemistry, Gokhale Education Society's Sir Dr. M. S. Gosavi College of Pharmaceutical Education and Research, Nashik, Maharashtra, India

Abstract

Background: There is an urgent need for the development of novel antimicrobial drugs due to the rapid development of antimicrobial drug resistance. Benzimidazole containing mercapto group at 2-position is attractive nucleus for modification with wider pharmacological activities. **Objective:** The aim of this study is to design benzofused nitrogen-containing heterocyclic derivatives of mercaptobenzimidazole using molecular docking, synthesis of active derivative and evaluation as potential antimicrobial agent. **Materials and Methods:** Using an effective procedure, N-substituted mercaptobenzimidazole derivatives were synthesized based on the literature review. The antimicrobial activity of all synthesized compounds was tested against four different organisms: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. Molecular docking of mercaptobenzimidazole derivatives against DNA gyrase subunit B Protein Data Bank (PDB): 5l3j and *S. aureus* tyrosyl-tRNA synthetase PDB: 1jij was performed using docking protocol. The compound binds to the active site of DNA gyrase subunit B (1KZN) in a docking study, indicating that it may have antimicrobial activity. **Conclusion:** The compounds MB3 and MB5 have antimicrobial capacity, according to the findings of this report. MB4 has the high activity against *C. albicans*.

Key words: Mercaptobenzimidazole**,** antimicrobial, molecular docking, DNA gyrase, tyrosyl-tRNA synthetase, *in silico* screening

INTRODUCTION

Mercapto group containing heterocycles are significant in organic chemistry because of their diverse biological and pharmacological properties. Derivatives of 2-meracaptobenzimidazoles, benzoxazole, benzothiazole, and quinazolinone are commercially available in some therapeutic areas.[1-3] 2-Mercaptobenzimidazole is a benzimidazole derivative with a thiol group in the second position. It is also known by the names o-phenylen thiourea and benzimidazol-2-thion, and has the formula $C_7H_6N_2S$.[4,5]

2-mercaptobenzimidazole derivatives have become extremely valuable in the therapeutic and pharmacological fields in recent years.[6,7] 2-Mercaptobenzimidazole displays various pharmacological[8] activities such as antimicrobial,[9-11] anticonvulsant,[12] analgesic, and anti-inflammatory activities.[13,14] In non-biological applications, 2-mercaptobenzimidazole is commonly used as a rubber accelerator[15] and an antioxidant for rubber and plastics.[16] It also used as a plant growth regulators[17] and as mild steel corrosion inhibitors in neutral medium.[18]

Address for correspondence:
D. Patil Tejaswini, Department of Pharmaceutical Chemistry, Progressive Education Society's, Modern College of Pharmacy, Nigadi, Pune, Maharashtra, India.
Tel/Fax: (020)-27661315/14. Mobile: +91-9881624094.
E-mail: dhanshrirmali@gmail.com

Received: 18-05-2021
Revised: 02-08-2021
Accepted: 18-08-2-21

Microbial infections cause more than 400 million years of life to be lost each year throughout the world, a greater burden than cancer or cardiovascular disease.[19] Malaria and other diseases that have long afflicted humans, such as tuberculosis, appear to take a severe impact. The past few decades have also seen the introduction of novel diseases with high virulence, such as the human immunodeficiency virus, the Ebola virus, and others West Nile virus, severe acute respiratory syndrome coronavirus, as well as the Zika virus.[20,21]

There is major issue of the development of resistance among the microorganism to available drug. Hence, this work was targeted to design mercaptobenzimidazole derivatives as an alternative to available drug to tackle with the resistant bacteria.

The present work focusses in designing, synthesis, and evaluation of novel mercaptobenzimidazole derivatives as potent antimicrobial agent.

MATERIALS AND METHODS

The starting materials and reagents were obtained from commercial sources and used without further purification. An open-ended capillary tube was used to calculate melting points. Thin-layer analysis was used to determine the purity of the synthesized compounds as well as the success of the reaction using aluminum silica gel sheet and detection in an iodine chamber.

The structure of the synthesized compounds was determined by IR spectra in υmax (cm-1) on FT-IR (Shizmadu-8400 series and Vertex 80 FTIR) using KBr disc technique and 1H-NMR spectra in δ units (ppm) relative to an internal standard of tetramethylsilane on 1H-NMR (ECZR Series 600 MHz NMR spectrometer) in deuterated chloroform or dimethyl sulfoxide (CDCl3/DMSO). Mass spectra were measured on AccuTOF GC mass spectrometer. Elemental analyses for final compounds were performed on FLASH EA 1112 series analyzer and the observed values were within the acceptable limits (±0.4%). The synthetic method is represented in Scheme 1.

Procedure

Preparation of 2-mercaptobenzimidazole (compound-I)[22-24]

The general procedure given by Wang and Liu is used for preparing 2-mercaptobenzimidazole.

In RBF, 10.8 g (0.1 mole) of o-phenylenediamine, 5.65 g (0.1 mole) of potassium hydroxide, and 7.67 g (0.1 mole, 6.19 ml) of carbon disulfide, 100 ml of 95% ethanol and 15 ml of water were added. The reaction mixture was heated under reflux for 3 h. Then, 1–1.5 g of charcoal was added with precaution and the mixture is further heated at the reflux for 10 min. After cooling reaction mixture, the charcoal was removed by filtration. The filtrate was further heated to 60–70°C, 100 ml of warm water was added. The filtrate was then acidified with dilute acetic acid with good stirring. The 2-mercaptobenzimidazole was separated as glistening white crystals, and the mixture was placed in a refrigerator for 3 h to complete the crystallization. The product is collected on a Buckner funnel, dried, and recrystallized with ethanol. The melting point is 296–300°C, yield: 73%.

General procedure for the synthesis of 2-chloro-N-substituted acetamide (Compound- II)[25-27]

With continuous shaking, 0.01 M of substituted aromatic amine was introduced to a conical flask containing a 10% NaOH solution. In a fuming hood, the conical flask was cooled on an ice bath, and (0.015 M) chloroacetyl chloride was added drop by drop using a dropping funnel. The solution was stirred on magnetic stirrer until complete addition of chloroacetyl chloride and fumes from the reaction mixture ceased completely. The solution was then stirred overnight. After pouring the reaction mixture into ice-cold water, the desired product was separated as a precipitate. Filtered precipitate was washed with cold water and dried. 95% ethanol was used to recrystallize.

General procedure for the synthesis of 2-(1H mercaptobenzimidazole-1-yl)-N-substituted acetamide (III)[27,28]

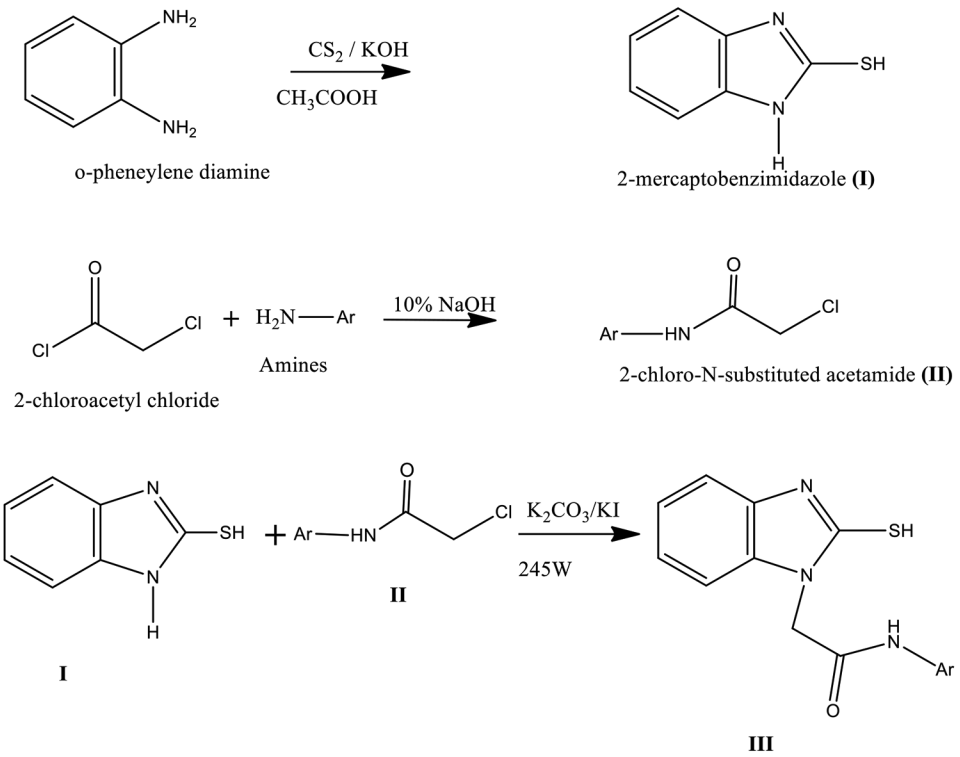
2-chloro-N-Aryl acetamide derivative (II) (0.02 mole) in DMF was introduced to 2-mercaptobenzimidazole (I) (0.02 mole) in DMF and refluxed in the presence of potassium carbonate and potassium iodide using microwave irradiation at 245 W. TLC was used to monitor the reaction's progress. After the reaction was completed, the solution was poured into ice-cold water, and the precipitate was filtered, dried, and recrystallized using ethanol.

Antimicrobial Activity

This method is built on the presumption that moisture absorbs from an antibiotic-impregnated disc placed on agar previously inoculated with the test bacterium, causing the antibiotic to diffuse radially outward through the agar medium, resulting in an antibiotic concentration gradient. If an antibiotic disc prevents bacterial development, a clear zone or ring develops around it after incubation.[29]

Method[30-33]

Mueller-Hinton agar is used for the disc diffusion process because it has high reproducibility, is low in sulfonamide, trimethoprim, and tetracycline inhibitors, and helps most bacterial pathogens to mature well.



Scheme 1: Synthesis of mercaptobenzimidazole derivatives

Agar medium was filled in the petri dishes. After solidification, the Petri plates were deposited inverted so that water could condense in the upper lid. The compounds were dissolved in dimethyl sulfoxide and added at a concentration of 8.0 mg mL^{-1}. In the disc diffusion assay, antimicrobial activity was measured using the zone of inhibition against the test organisms.

Escherichia coli (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 25923) were used to determine antibacterial activity and *Candida albicans* (ATCC 10231) for antifungal activity using gentamycin and nystatin as reference compound.

Molecular Docking

The compound which selected for docking will have electron withdrawing group and also some lipophilic group which will help in binding to the selected target proteins. The derivatives were designed accordingly which will contain electron-withdrawing group such as chloro, fluoro, and nitro substitution on phenyl ring.

ChemDraw Ultra program V.12.0.2 was used to draw the configurations of the synthesized molecules, which were then refined and transformed to 3D structures that could be docked using Open Babel (version 3.0.0).[34]

The three-dimensional structures of the molecular target (DNA gyrase subunit B Protein Data Bank (PDB): 5l3j and *S. aureus* tyrosyl-tRNA synthetase PDB: 1jij) were obtained from the PDB (www.rcsb.org). The receptor protein was prepared for docking by eliminating heteroatoms and water and replacing them with polar hydrogen and charges (Kollman and Gasteiger). In PyMol, the active site of the receptor is visualized.[35]

Grid boxes of appropriate size were used to identify and define active sites. Autodock Vina[36] was used to conduct the docking investigation and visualization with the help of Discovery studio 3.5 visualizer (DS visualizer).[37]

In Silico Screening

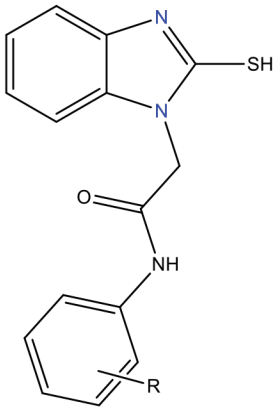
Online chemical property calculator Molinspiration (http://www.molinspiration.com) was used to asses physicochemical properties, and other pharmacokinetic properties and toxicity were assed using the PreADMET server (http://preadmet.bmdrc.org/) and SwissADME (http://www.swissadme.ch).[38]

RESULTS

Chemistry

Mercaptobenzimidazole was synthesized from the procedure given in the literature with good yield. Chloroacetamide was synthesized by adding chloroacetyl chloride in aqueous amine solution containing 10% NaOH. The final desired

product was obtained by condensing mercaptobenzimidazole with acetamide in the presence of potassium carbonate as base (Scheme 1). Elemental analysis was used to validate the structure of solid compounds. MS, 1H-NMR, 13C-NMR, and FTIR spectroscopy were used to characterize the derivatives.



General structure of mercaptobenzimidazole derivatives

2-(2-mercapto-1H-benzo[d]imidazole-1-yl)-N-phenylacetamide (MB1)

IR (KBr)γ_{max} (cm^{-}): 3211.7 (N-H str.), 2977.6 (=C-H str.), 2929.2 (-SH str.), 1671.6 (C=O str.), 1594.0 (N-H bend), 1251.3 (C-N str.), 798.6–698.0 (Aromatic ring); ^{1}H-NMR (600 MHz, δ ppm): 4.36 (s, CH_2), 8.38 (s, NH), 7.90 (d,=CH), 7.87 (d,=CH), 7.74–7.05 (m, CH_2 Ar), 12.4 (s, SH); ^{13}C-NMR (600 MHz, δ ppm): 39.63 (CH_2), 164.47 (C=O), 168.11 (C-SH), 119.85–109.54 (CH Ar.), 133.90–122.37 (CH- Benzimidazole); m/z: 283(M^+), Anal. Cal. (Found) for $C_{15}H_{13}N_3OS$: C 63.58 (62.91), H 4.62(4.81), N 14.84 (14.93), O 5.65 (5.92), S 11.32 (10.65).

2-(2-mercapto-1H-benzo[d]imidazole-1-yl)-N-(p-tolyl)acetamide (MB2)

IR (KBr)γ_{max} (cm^{-}): 3335.17 (N-H str.), 3113.80 (=C-H str.), 2985.92 (-SH str.), 1652.91 (C=O str.), 1514.15 (N-H bend), 1260.78 (C-N str.), 789.72–718.17 (Aromatic ring).

2-(2-mercapto-1H-benzo[d]imidazole-1-yl)-N-(4-chlorophenyl)acetamide (MB3)

IR (KBr)γ_{max} (cm^{-}): 3211.4 (N-H str.), 2976.3 (=C-H str.), 2928.3 (-SH str.), 1673.2 (C=O str.), 1533.9 (N-H bend), 1251.7 (C-N str.), 798.8–717.8 (Aromatic ring), 698.2 (C-Cl), ^{1}H-NMR (600 MHz, δ ppm): 4.75 (s, CH_2), 7.25 (s, NH), 7.87 (d,=CH), 7.73 (d,=CH), 7.71–7.09 (m, CH_2 Ar), 12.54 (s, SH); ^{13}C-NMR (600 MHz, δ ppm): 39.63 (CH_2), 164.47 (C=O), 168.07 (C-SH), 113.38–109.54 (CH Ar.), 122.36–119.70 (=CH Benzoimidazole), 134.46 (C-Cl); m/z: 317(M^+), Anal. Cal. (Found) for $C_{15}H_{12}ClN_3OS$: C 56.69 (55.94), H 3.81(3.62), N 13.22 (12.53), O 5.03 (5.20)S 10.09 (9.63).

2-(2-mercapto-1H-benzo[d]imidazole-1-yl)-N-(4-nitrophenyl)acetamide (MB4)

IR (KBr)γ_{max} (cm^{-}): 3258.94(N-H str.), 3093.39(=C-H str.), 2951.71(-SH str.),1666.08 (C=O str.), 1593.47 (N-H bend),1626.01 and 1405.52 (NO_2 str.), 1291.57 (C-N str.), 743.94 (Aromatic Ring),

2-(2-mercapto-1H-benzo[d]imidazole-1-yl)-N-(4-fluorophenyl)acetamide (MB5)

IR (KBr)γ_{max} (cm^{-}): 3369.4 (N-H str.), 2918.7 (=C-H str.), 2850.2 (-SH str.), 1675.7 (C=O str.), 1553.3 (N-H bend), 1269.3 (C-N stre), 793.9–705.6 (Aromatic ring), 1069.4 (C-F).

Antimicrobial Study

The biological evaluation of synthesized derivative for the antimicrobial activity was carried out by the well-known well-diffusion method,[39,40] with gentamycin as the antibacterial standard and nystatin as the antifungal standard. Antimicrobial activity was assessed by measuring zone of inhibition in mm and compared with the standard drug as shown in Table 2 and diagrammatically presented as bar diagrams in Figure 1.

Molecular Docking

Two targets were chosen for study: *S. aureus* tyrosyl-tRNA synthetase (PDB: 1jij) and DNA gyrase subunit B (PDB: 5l3j). Bacterial DNA gyrase is a topoisomerase type II enzyme that has attracted interest since its discovery in 1976, when it was first isolated from *E. coli* and identified as a target of the well-known aminocoumarin family of antibacterial compounds.[41] DNA gyrase is a key element of DNA synthesis. DNA gyrase introduces negative supercoils in DNA in front of the replication fork.[42,43]

Tyrosyl-tRNA synthetase, catalyzes the covalent binding of amino acids to their corresponding tRNA to form charged tRNA and hence, plays an important role in protein synthesis.[44] As a result, it is a promising target enzyme for discovering new antibacterial agents.[45,46]

Molecular docking was used to examine the binding affinities of newly synthesized compounds[47] and the target proteins (1jij and 5l3j). The docking findings revealed that all of the compounds in the binding pocket had major bonding interactions. The binding affinity of the mercaptobenzimidazole derivative to the target protein with amino acid interaction is shown in Table 3.

Figures 2-7 show the 2D and 3D interaction of mercaptobenzimidazole derivative with the target protein.

In Silico Screening

To predict a molecule's potential as a drug, its physiochemical properties must be described.[48] Lipinski's principles are a

series of guidelines for predicting medication acceptability.[49] Table 4 shows Lipinski's theorem, as well as hydrogen bond acceptor and donor, LogP, and TPSA. Both synthesized derivatives obey the Lipinski law, and they have a high LogP value.

Pharmaceutical pharmacokinetic properties such as absorption, distribution, metabolism, and toxicity can be modeled using computational programs such as PreADMET and SwissADME to predict the behavior of compounds that may be used as pharmaceuticals in the future.[50,51] The ADME and toxicity of the synthesized compound are shown in Table 5. All newly synthesized mercaptobenzimidazoles have the significant pharmacological profile with modest toxicity.

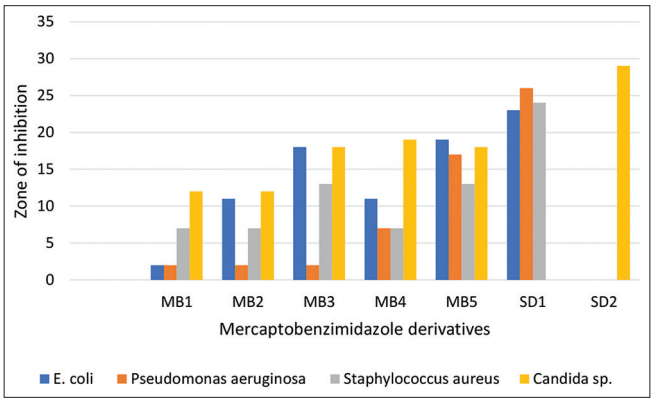


Figure 1: Antimicrobial activity of mercaptobenzimidazole derivatives

Table 1: Physical properties of synthesized mercaptobenzimidazole derivatives

Compound	Ar	mp (°C)	Yield (%)	Rf value
MB1	Phenyl	242–246	65.42	0.58
MB2	4-Methylphenyl	248–252	61.30	0.60
MB3	4-Chlorophenyl	262–264	64.20	0.59
MB4	4-Nitrophenyl	196–198	75.25	0.62
MB5	4-Flurophenyl	232–238	56.40	0.56

DISCUSSION

Chemistry

Using Scheme 1, novel derivatives of mercaptobenzimidazole were synthesized. All synthesized compounds were obtained in good yield with high purity. The physicochemical properties of synthesised derivatives are summarised in Table 1. The structures of synthesized derivatives were confirmed using MS, ^{1}H-NMR, ^{13}C-NMR, and FTIR. In the FTIR spectrum, a band observed in the range of 1675–1652 cm^{-1} attributed to the stretching of C = O group of amides, a band 3369–3211 cm^{-1} for the N-H stretching band for secondary amide, and C- H stretching between 3113 and 2918 cm^{-1}. Furthermore, S-H stretching was observed between 2829 and 2956 cm^{-1}. In ^{1}H spectra, the peak for secondary amide observed at δ, 7.25 and different peak for aromatic proton of benzimidazole ring observed at higher δ than phenyl ring.The ^{13}C NMR spectra display the presence of amide carbon at δ, 164 and mercapto carbon at 168. This finding confirms the structure of synthesized compound.

Antimicrobial Study

All synthesized compounds MB1-5 were tested for *in vitro* antibacterial activity against two different strains of Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive (*S. aureus*) bacteria using gentamicin as a standard medicine. Nystatin is a standard medication that was used to evaluate the antifungal efficacy of all newly synthesized compounds against *C. albicans*.

Antimicrobial tests revealed that MB5 has the greatest antibacterial activity against *E. coli*, while MB4 has the highest antibacterial activity against *S. aureus* and *C. albicans*. MB5 is also the most powerful antifungal against *C. albicans*. MB3 has a modest level of activity against all of the bacteria tested.

MB4 contains very strong electron withdrawing group which may be responsible for highest antibacterial activity. MB5 also has electron withdrawing fluoro group which may enhances antimicrobial activity by binding with the enzyme. Compound

Table 2: Antimicrobial evaluation of mercaptobenzimidazole derivatives

Sample code	*Escherichia coli* (ATCC 25922)	*Pseudomonas aeruginosa* (ATCC 27853)	*Staphylococcus aureus* (ATCC 25923)	*Candida* sp. (ATCC 10231)
MB1	No zone	No zone	7 mm	12 mm
MB2	11 mm	No zone	7 mm	12 mm
MB3	18 mm	No zone	13 mm	18 mm
MB4	11 mm	7 mm	7 mm	19 mm
MB5	19 mm	17 mm	13 mm	18 mm
SD1	23 mm	26 mm	24 mm	-
SD2	-	-	-	29 mm

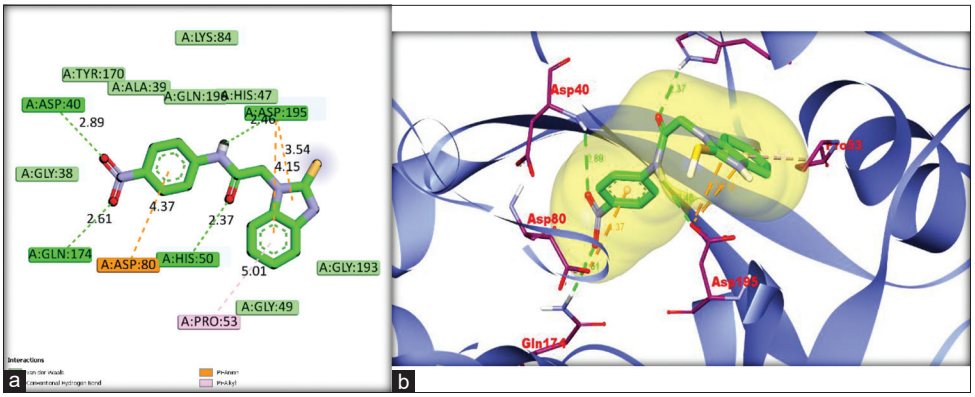


Figure 2: (a) 2D interaction of MB4 with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB: 1jij). (b) 3D interaction of MB4 with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB: 1jij)

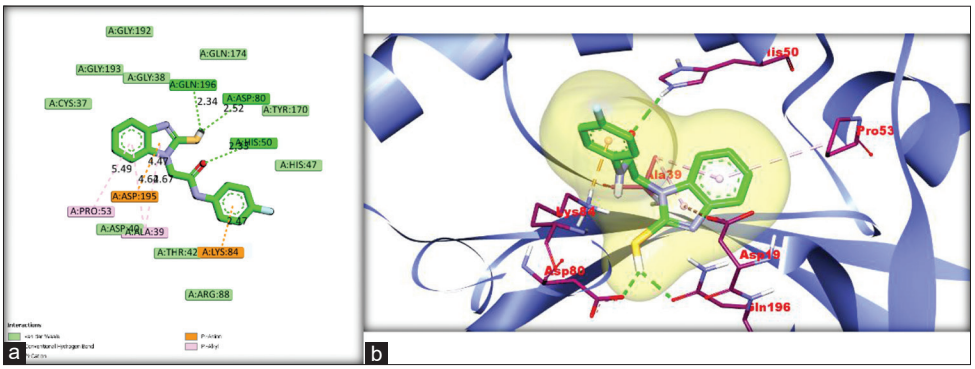


Figure 3: (a) 2D interaction of MB5 with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB: 1jij). (b) 3D interaction of MB5 with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB: 1jij)

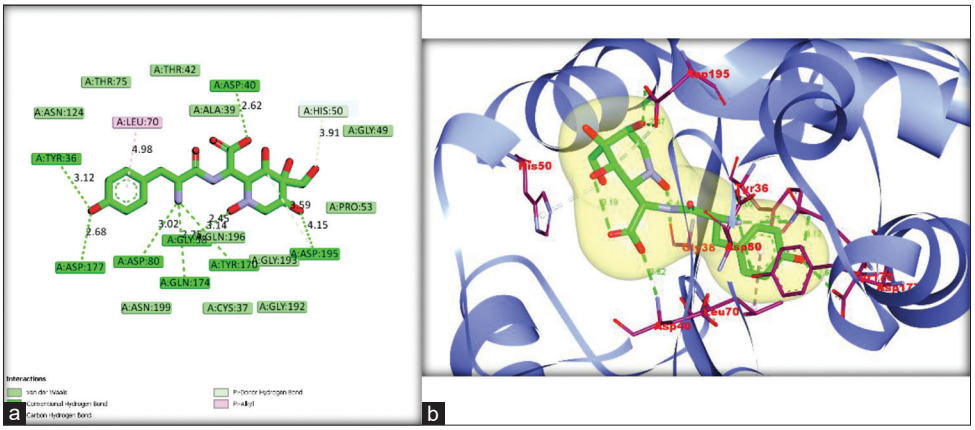


Figure 4: (a) 2D interaction of ligand with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB: 1jij). (b) 3D interaction of ligand with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB: 1jij)

MB3 has chloro group which also enhanced binding with enzyme and ultimately potentiate activity. Hence, overall study shows that the mercaptobenzimidazole derivatives with electron-withdrawing group on phenyl ring enhance activity.

Docking

The compound was subjected to docking study so as to observe the non-bonding interaction as well as the binding affinity. The binding affinity of mercaptobenzimidazole against *S. aureus* tyrosyl-tRNA synthetase was –7.3—8.3 kcal/mol whereas against DNA gyrase subunit B, it was –6.6—8.1 kcal/mol as compare ligand having binding affinity to –7.9 kcal/mol and -7.8 kcal/mol, respectively. This amino acid interacts with the ligand through carbon hydrogen bonding, Pi-cation, Pi-anion, alkyl Pi-alkyl, van der Waals, and traditional hydrogen bonding. Due to the presence of amide in structure, all the derivatives have good binding affinity toward t-RNA synthetase. MB4 has lowest docking score of –8.3 kcal/mol against t-RNA synthetase and MB2 has lowest binding score of -8.1 kcal/mol against DNA gyrase subunit B. The docking results reveal that electron-withdrawing group has strong affinity for binding with the enzyme. They may form extra binding interaction with the

Table 3: Binding affinity and interaction of mercaptobenzimidazole derivatives and reference with target protein (1jij and 5l3j)

Comp code	Protein 1jij				Protein 5l3j			
	Binding affinity (kcal/mol)	Type of interaction	Interacting amino acid	Bond length	Binding affinity (kcal/mol)	Type of interaction	Interacting amino acid	Bond length
MB1	–7.3	Van der Waals, Carbon hydrogen Bond, Pi-Donor hydrogen bond, Pi-Alkyl	Tyr36, Cys37, Gly38, Ala39, Asp40, Thr42, Phe54, Pro53, His50, Asp80, Thr75, Lys84, Arg88,Tyr170, Gln174, Asp177, Gln190, Val 191, Gly192, Gly193, Asp195,Gln196, Ile200	Cys 37 (5.08), Ala39 (2.06, 4.82), Asp40 (3.05), Asp195 (3.47)	–6.6	van der Waals, Amide-pi-stacked Pi- sigma Pi-alkyl,	Val43, Ala47,Asn46, Glu50, Ala53, Asp73, Gly77, Ile78, Pro79, Ile 94, Arg136, Val120, Thr165, Val167	Asn46 (4.61,4.39), Ala47 (4.32), Ile78 (4.70,5.36), Val120 (5.24), Thr165 (3.91), Val167 (5.30)
MB2	–7.4	Van der Waals, Pi- Cation, Pi-anion, Pi-sigma, Pi-Pi -T-Shaped, Amide-Pi- Stacked, Alkyl	Val 4, Glu7, Trp11, Asp8, Arg12, His6, Phe273, Agr59, Glu62, Leu274, Gly275, Lys276.	Glu7 (4.57), Asp8 (4.99), Arg12 (3.72), Arg59 (4.09), Glu62 (4.70), Phe273 (5.57)	–8.1	van der Waals, Carbon Hydrogen Bond, Alkyl, Pi-alkyl,	Val43, Ala47, Asn46, Asp49, Glu50, Ala53, Asp73, Ile78, Pro79, Ile 94, Met95, Val120, Thr165, Val167	Val43 (3.93), Ile78 (5.08), Pro79 (4.95), Ile94 (4.84), Val120 (5.35, 3.68), Thr165 (3.55), Val167 (3.81)
MB3	–7.4	Van der Waals, Pi- Cation, Pi-anion, Pi-sigma, Pi-Pi -T-Shaped, Amide-Pi- Stacked, Alkyl	Tyr36, Gly38, Ala39, Asp40, Thr42, Pro53, His50, Asp80, Leu70, Lys84, Tyr170, Gln174, Asp177, Gln190, Gly192,Gly193, Asp195, Gln196,	Tyr36 (5.04), Ala39 (2.84, 3.20, 4.08), Asp40 (2.46, 4.08), His50 (4.59,5.45), Pro53 (5.35), Leu70 (3.81), Asp195 (4.93,4.62)	–7.5	van der Waals, Conventional Hydrogen Bond, Pi- sigma, Alkyl, Pi-alkyl,	Ala47,Asn46, Glu50, His55, Asp73,Arg76, Gly77, Ile78, Pro79, Ile 94, Arg136, Val120, Thr165, Val167	Glu50 (2.60), Ile78 (3.94,4.66), Pro79 (4.46,4.16)
MB4	–8.3	Van der Waals, Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-anion, Pi-Pi -T- Shaped, Alkyl, Pi- Alkyl	Gly38, Ala39, Asp40, His47, Gly49, Pro53, His50, Asp80, Lys84, Tyr170, Gln174, Gly192,Gly193, Asp195, Gln196,	Asp40 (2.89), His50 (2.37), Pro53 (5.01), Asp80 (4.37), Gln174 (2.621), Asp195 (2.46, 3.54)	–7	van der Waals, Conventional Hydrogen Bond, Pi- sigma, Amide-pi-stacked, Pi-alkyl,	Val43, Ala47, Asn46, Asp49, Glu50, Ala53, Asp73, Gly75, Arg76, Gly77, Ile78, Ile 94, Val120, Thr165, Val167	Asn46 (4.60,4.32), Asp73 (2.14), Ile78 (4.81,5.46), Val120 (5.24), Thr165 (3.86), Val167 (5.23)

(Contd...)

Table 3: *(Continued)*

Comp code	Protein 1jij				Protein 5l3j			
	Binding affinity (kcal/mol)	Type of interaction	Interacting amino acid	Bond length	Binding affinity (kcal/mol)	Type of interaction	Interacting amino acid	Bond length
MB5	–8.1	Van der Waals, Conventional Hydrogen Bond, Pi- cation, Pi-anion, Pi- Alkyl	Cys37, Gly38, Ala39, Asp40, Thr42, His47, Gly49, Pro53, His50, Asp80, Lys84, Arg88, Tyr170, Gln174, Gly192,Gly193, Asp195, Gln196,	Ala39 (4.67, 4.64), His50 (2.33), Pro53 (5.49), Asp80 (2.52), Lys84 (2.47), Asp195 (4.47), Gln196 (2.34).	–7.8	van der Waals, Conventional Hydrogen Bond, Pi- sigma, Pi-alkyl,	Ala47, Asn46, Glu50, His55, Asp73, Gly75, Arg76, Gly77, Ile78, Ile 94, Val120, Arg136, Thr165, Val167	Glu50 (4.23), Arg76 (7.20), Ile78 (5.50, 5.10), Pro79 (5.73)
Ligand	–7.9	Van der Waals, Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Donor Hydrogen Bond, Pi- Alkyl	Tyr36, Cys37, Ala39, Asp40, Thr42, Pro53, Gly49, His50, Gly58 Asp80, Leu70, Thr75, Lys84, Asn124, Tyr170, Gln174, Asp177, Gln190, Gly192, Gly193, Asp195, Gln196,	Tyr36 (3.12), Gly38 (2.45), Asp40 (2.62), His50 (3.91), Leu70 (4.98), Asp80 (3.02), Gln174 (2.75), Asp177 (2.68) Asp195 (4.15)	–7.8	Conventional hydrogen bond, Pi- sigma Pi-alkyl, van der Waals	Ala47, Asn46, Asp49, Glu50, His55, Asp73, Gly77, Arg76, Ile78, Ile 94, Val120, Arg136, Thr165, Val167	Asn46 (3.71), Glu50 (3.55,3.26 ,3.54, 3.42), Gly77 (3.17), Ile78 (5.38), Thr165 (3.09)

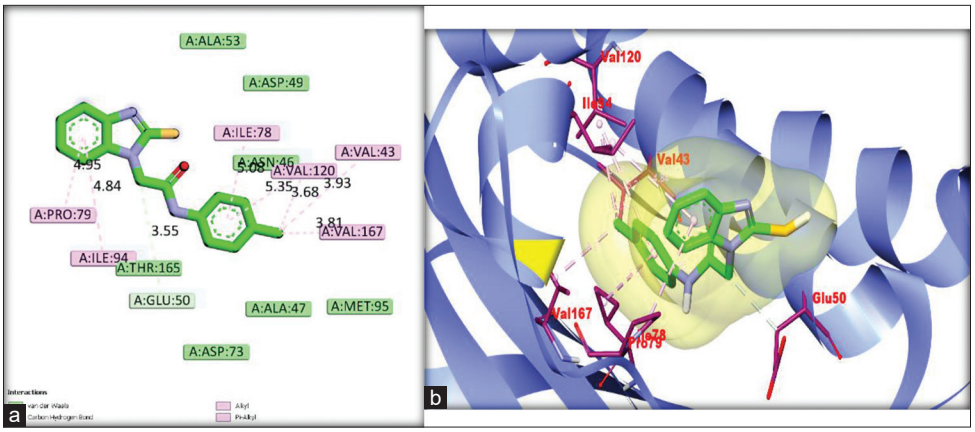


Figure 5: (a) 2D interaction of MB2 with DNA gyrase subunit B (PDB: 5l3j). (b) 3D interaction of MB2 with DNA gyrase subunit B (PDB: 5l3j)

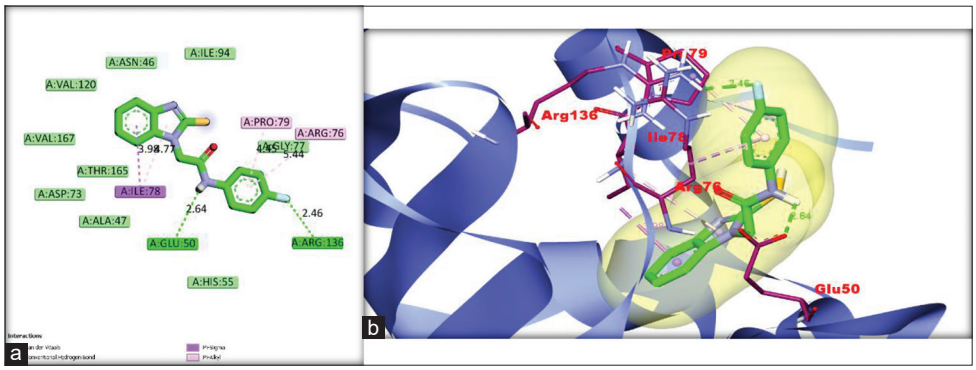


Figure 6: (a) 2D interaction of MB5 with DNA gyrase subunit B (PDB: 5l3j). (b) 3D interaction of MB5 with DNA gyrase subunit B (PDB: 5l3j)

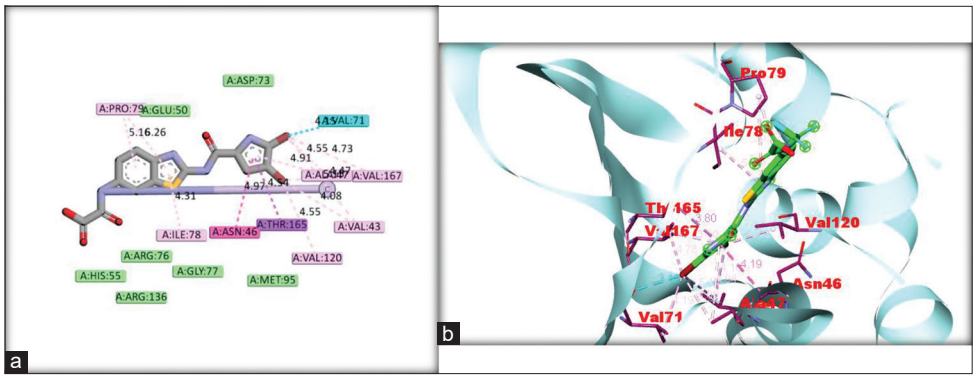


Figure 7: (a) 2D interaction of ligand with DNA gyrase subunit B (PDB: 5l3j). (b) 3D interaction of ligand with DNA gyrase subunit B (PDB: 5l3j)

Table 4: Physicochemical parameter and druglikeness of mercaptobenzimidazole derivatives

Comp Code	Formula	MW	Rotatable bonds	H-bond acceptors	H-bond donors	TPSA	LOGP	Follow Lipinski	Lipinski violations
MB1	$C_{15}H_{13}N_3OS$	283.35	4	2	1	85.72	2.2	YES	0
MB2	$C_{16}H_{15}N_3OS$	297.37	4	2	1	85.72	2.45	YES	0
MB3	$C_{15}H_{12}ClN_3OS$	317.79	4	2	1	85.72	2.71	YES	0
MB4	$C_{15}H_{12}N4O_3S$	328.35	5	4	1	131.54	1.16	YES	0
MB5	$C_{15}H_{12}FN_3OS$	301.34	4	3	1	85.72	2.59	YES	0

Table 5: ADME and toxicity of mercaptobenzimidazole derivatives

Comp code	BBB	Caco2++ Cell permeability	HIA	MDCK	PPB	Skin permeability	Algae at	Ames test	Carcino rat	hERG inhibition
MB1	1.31045	28.3659	96.74649	239.064	100	–3.29433	0.0548876	Mutagen	Positive	Medium risk
MB2	1.01121	29.6858	96.67333	113.784	100	–3.23134	0.0268641	Mutagen	Positive	Medium risk
MB3	0.958442	38.2864	96.53377	95.1014	100	–3.34072	0.0166982	Mutagen	Positive	Medium risk
MB4	0.0199087	19.1873	94.25417	0.119231	100	–3.3518	0.0405241	Mutagen	Positive	Medium risk
MB5	0.731757	30.6775	96.74066	37.8621	100	–3.5876	0.0405621	Mutagen	Positive	Medium risk

amino acid present in active site of enzyme which will result into significant inhibition as reveal by docking score.

In silico Screening

The findings of *in silico* screening show that mercaptobenzimidazole derivatives have drug-like properties and that they all obey the Lipinski rule. Four rotatable bonds with 3–5 hydrogen acceptor atoms are found in the majority of derivatives. According to the ADME profile, these derivatives have marginal to detectable concentrations in the CNS. The oral bioavailability of derivatives is demonstrated by CaCO2++ cell permeability, HIA, and MDCK. In terms of toxicity, the derivatives have a low toxicity and are safe to use.

CONCLUSION

The novel benzofused heterocyclic compounds were synthesized and characterized as derivatives of N-substitutes mercaptobenzimidazole. When compared to standard drugs, the compounds showed moderate antimicrobial activity. Increased dosages of the respective compounds resulted in increased activity. The findings also showed that synthesized compounds could be more effective antibacterial agents. According to a docking analysis, this compound has antimicrobial activity. Compounds MB3 and MB5 possess the best antimicrobial activity.

ACKNOWLEDGMENTS

The author is especially grateful to the Management, Principal, and Faculty of Progressive Education Society's, Modern College of Pharmacy. The author is also thankful to the Management, Principal, and Faculty of Gokhale Education Society's Sir Dr. M. S. Gosavi College of Pharmaceutical Education and Research for providing facility and support to carry out the study. The author is thankful to SAIF, IIT, Bombay, for providing instrumental analysis of compound.

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Source of Support: Nil. **Conflicts of Interest:** None declared.