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ARTICLE

Design, *in silico* Analysis, Synthesis and Evaluation of Novel Benzofused Nitrogen Containing Heterocyclic *N*-Substituted Mercaptobenzimidazole Derivatives as Potential Antimicrobial Agent

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ABSTRACT

Benzimidazole containing mercapto group at the 2nd position is attractive nucleus for the modification with wider pharmacological activities. The aim of this study is to design benzofused nitrogen containing heterocyclic derivatives of mercapto benzimidazole using molecular docking. Using an effective procedure, *N*-substituted mercapto benzimidazole derivatives was synthesized. The antimicrobial activity of all the synthesized compounds was tested against four different organisms *viz*. *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. Molecular docking of mercapto benzimidazole derivatives against DNA gyrase subunit B PDB: 5l3j and *Staphylococcus 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. Compounds **MB3** and **MB5** have good antimicrobial capacity whereas compound **MB4** has the high activity against *Candida albicans*.

KEYWORDS

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]. 2-Mercaptobenzimidazole is a benzimidazole derivative with a thiol group in the second position. It also known by the names *o*-phenylene thiourea and benzimidazol-2-thione [2,3].

2-Mercaptobenzimidazole derivatives have become extremely valuable in the therapeutic and pharmacological fields in recent years [4]. 2-Mercaptobenzimidazole displays various pharmacological activities like antimicrobial [5,6], anticonvulsant [7], analgesic and anti-inflammatory activities [8]. In non-biological applications, 2-mercaptobenzimidazole is commonly used as a rubber accelerator [9] and an antioxidant for rubber and plastics [10]. It also used as a plant growth regulators [11] and as mild steel corrosion inhibitors in neutral

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medium [12]. The present work focusses in designing, synthesis and evaluation of novel mercaptobenzimidazole derivatives as potent antimicrobial agents.

EXPERIMENTAL

The starting materials and reagents were obtained from commercial sources and used without further purification. Thin layer analysis was used to determine the purity of the synthesized compounds as well as the success of the reaction using aluminium silica gel sheet and detection in an iodine chamber.

The structure of the synthesized compounds was analyzed by IR spectra on FT-IR (Shizmadu-8400 series and Vertex 80 FTIR) using KBr disc technique and ^{1}H NMR spectra in δ units (ppm) relative to an internal standard of tetramethylsilane on ^{1}H NMR (ECZR Series 600 MHz NMR spectrometer) in deuterated chloroform or dimethyl sulfoxide ($CDCl_3$/DMSO). Mass spectra were measured on AccuTOF GC mass spectrometer. Elemental analyses of the synthesized compounds were performed on FLASH EA 1112 series analyzer and the observed values were within the acceptable limits (± 0.4%).

Synthesis of 2-mercaptobenzimidazole (I): In round bottom flask, 10.8 g (0.1 mol) of *o*-phenylenediamine 5.65 g (0.1 mol) of KOH and 7.67 g (0.1 mol, 6.19 mL) of CS_2, 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 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 ºC, 100 mL of warm water was added. The filtrate was then acidified with dil. 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 [13-15]. The product was collected on a Büchner funnel, dried and recrystallized with ethanol (yield: 73%; m.p.: 296-300 ºC).

Synthesis of 2-chloro-*N*-substituted acetamide (II): 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) chloro acetyl chloride was added dropwise. The solution was stirred on magnetic stirrer until complete addition of chloro acetyl 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 [16-18]. Filtered precipitate was washed with cold water, dried and finally recrystallized with 95% ethanol.

Synthesis of 2-(1*H*-1,2,3-benzotriazol-1-yl)-*N*-substituted acetamide (III): 2-Chloro-*N*-aryl acetamide derivative (**II**) (0.02 mol) in DMF was introduced to 2-mercaptobenzimidazole (**I**) (0.02 mol) in DMF and refluxed in the presence of K_2CO_3 and KI 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 [18,19].

2-(2-Mercapto-1*H*-benzo[*d*]imidazole-1-yl)-*N*-phenylacetamide (MB1): Yield: 65.42%, m.p.: 242-244 ºC; R_f: 0.58. IR (KBr, ν_{max}, cm^{-1}): 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. calcd. (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-1*H*-benzo[*d*]imidazole-1-yl)-*N*-(*p*-tolyl)acetamide (MB2): Yield: 61.30%, m.p.: 248-250 ºC; R_f: 0.60. IR (KBr, ν_{max}, cm^{-1}): 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-1*H*-benzo[*d*]imidazole-1-yl)-*N*-(4-chlorophenyl) acetamide (MB3): Yield: 64.20%, m.p.: 262-264 ºC; R_f: 0.59. IR (KBr, ν_{max}, cm^{-1}): 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. calcd. (found) % for $C_{15}H_{12}N_3OSCl$: 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-1*H*-benzo[*d*]imidazole-1-yl)-*N*-(4-nitrophenyl)acetamide (MB4): Yield: 75.25%, m.p.: 196-198 ºC; R_f: 0.62. IR (KBr, ν_{max}, cm^{-1}): 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-1*H*-benzo[*d*]imidazole-1-yl)-*N*-(4-fluorophenyl)acetamide (MB5): Yield: 56.40%, m.p.: 232-234 ºC; R_f: 0.56. IR (KBr, ν_{max}, cm^{-1}): 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 *str.*), 793.9-705.6 (aromatic ring), 1069.4 (C-F).

Antimicrobial activity: Mueller-Hinton Agar (MHA) 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. 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 DMSO 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 [20-23]. *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923) were used to determine antibacterial activity and *Candida albicans* (ATCC10231) for antifungal activity using gentamycin and nystatin as reference compound.

Molecular docking: Chemdraw Ultra programme 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) [24]. The three-dimensional structures of the molecular target (DNA

gyrase subunit B PDB: 5l3j and *Staphylococcus aureus* tyrosyl-tRNA synthetase PDB:1jij) were obtained from the Protein Data Bank (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 [25]. The grid boxes of appropriate size were used to identify and define active sites. Autodock Vina [26] was used to conduct the docking investigation and visualization with the help of Discovery studio 3.5 visualizer (DS visualizer) [27].

Two targets were chosen for study *viz. Staphylococcus 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 [28]. DNA gyrase is a key element of DNA synthesis. DNA gyrase introduces negative supercoils in DNA in front of the replication fork [29,30].

While Tyrosyl-tRNA synthetase catalyses the covalent binding of amino acids to their corresponding tRNA to form charged tRNA and plays an important role in protein synthesis [31]. As a result, it's a promising target enzyme for discovering new antibacterial agents [32,33].

***in silico* Screening:** Online chemical property calculator Molinspiration (http://www.molinspiration.com) were used to asses physico-chemical properties and other pharmacokinetic properties and toxicity were assed using the PreADMET server (http://preadmet.bmdrc.org/) and SwissADME (http://www.swissadme.ch) [34].

To predict a molecule's potential as a drug, its physio-chemical properties must be described. Lipinski's principles are a series of guidelines for predicting medication acceptability [35,36]. The pharmaceutical pharmacokinetic properties like absorption, distribution, metabolism and toxicity can be modelled using computational programmes like PreADMET and SwissADME to predict the behaviour of compounds that may be used as pharmaceuticals in the future [37,38].

RESULTS AND DISCUSSION

2-Mercaptobenzimidazole was synthesized from the reported procedure with good yield. 2-Chloro-*N*-substituted acetamide was synthesized by adding chloroacetyl chloride in aqueous amine solution containing 10% NaOH. The final desired product was obtained by condensing 2-mercaptobenzimidazole with acetamide in the presence of K_2CO_3 as base.

The novel derivatives of the synthesized mercaptobenzimidazole derivatives (**MB1-MB5**) were obtained in good yield with high purity. The structures of synthesized derivatives were confirmed using ^1H & ^{13}C NMR and FTIR. In the FTIR spectrum, a band observed in the range 1675-1652 cm^{-1} was 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 3113-2918 cm^{-1}, also the S-H stretching was observed between 2956-2829 cm^{-1}. In ^1H NMR spectra, the peak for secondary amide observed at δ 7.25 and different peak for aromatic proton of benzoimidazole ring observed at higher δ than the phenyl ring. The ^{13}C NMR spectra show the presence of amide carbon at δ 164 and mercapto carbon at 168. This confirmed the structure of synthesized compounds.

Molecular docking: The docking findings revealed that all of the compounds in the binding pocket had major bonding interactions. The binding affinity of the synthesized mercaptobenzimidazole derivative to the target protein with amino acid interaction are shown in Table-1. Figs. 1 and 2 shows the 2D and

TABLE-1
BINDING AFFINITY AND INTERACTION OF SYNTHESIZED MERCAPTOBENZIMIDAZOLE DERIVATIVES (MB1-MB5) AND REFERENCE WITH TARGET PROTEIN (1jij AND 5l3j)

Compd. code	Binding affinity (kcal/mol)	Type of interaction	Interacting amino acid	Bond length
			Protein 1jij	
MB1	-7.3	van der Waals, Carbon hydrogen Bond, Pi-Donar 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)
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)
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)
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)
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)
Ligand	-7.9	van der Waals, Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Donar 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)

			Protein 5l3j	
MB1	-6.6	van der waals, Amide-pi-stackedPi-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	-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	-7.5	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	-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)
MB5	-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	-7.8	Conventional hydroge 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)

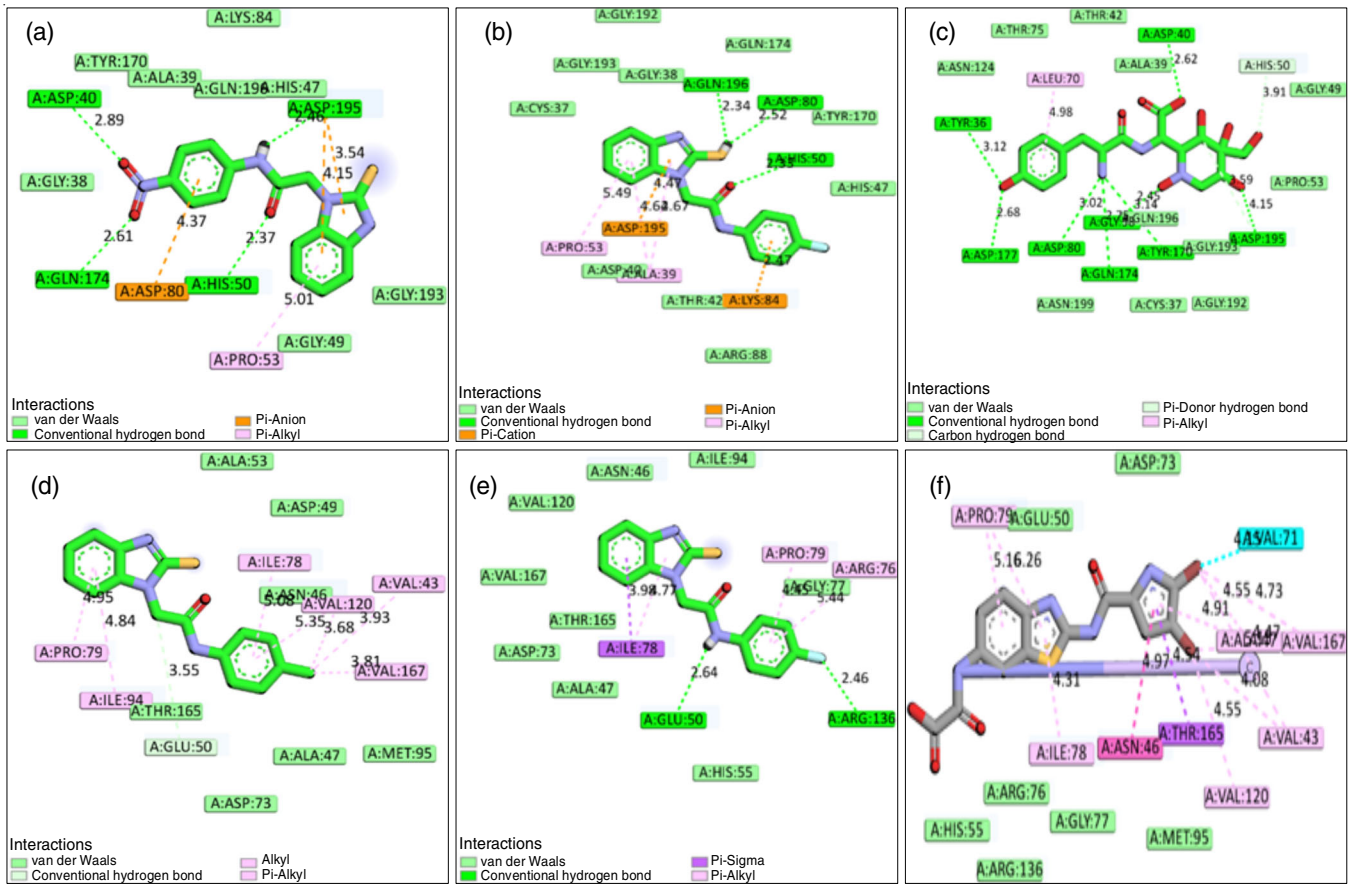

Fig. 1. (a) 2D interaction of compound **MB4** with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB: 1jij); (b) 2D interaction of compound **MB5** with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB: 1jij); (c) 2D interaction of ligand with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB: 1jij); (d) 2D interaction of compound **MB2** with DNA gyrase subunit B (PDB: 5l3j); (e) 2D interaction of compound **MB5** with DNA gyrase subunit B (PDB: 5l3j); (f) 2D interaction of Ligand with DNA gyrase subunit B (PDB: 5l3j)

3D interaction of the synthesized mercapto benzoimidazole derivatives respectively with the target protein.

The binding affinity of mercaptobenzoimidazole against *Staphylococcus aureus* tyrosyl-tRNA synthetase was -7.3 to -8.3 kcal/mol where as against DNA gyrase subunit B it was -6.6 to -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 presence of amide in structure all the derivatives have good binding affinity towards t-RNA synthetase. MB4 has lowest docking score of -8.3 kcal/mol against t-RNA synthetase and compound **MB2** has lowest binding score of -8.1 kcal/mol against DNA gyrase subunit B.

***in silico* Screening:** Table-2 shows Lipinski's rule, as well as hydrogen bond acceptor and donor, log P and TPSA. Both synthesized derivatives obey the Lipinski's law and have a high log P value. The ADME and toxicity of the synthesized

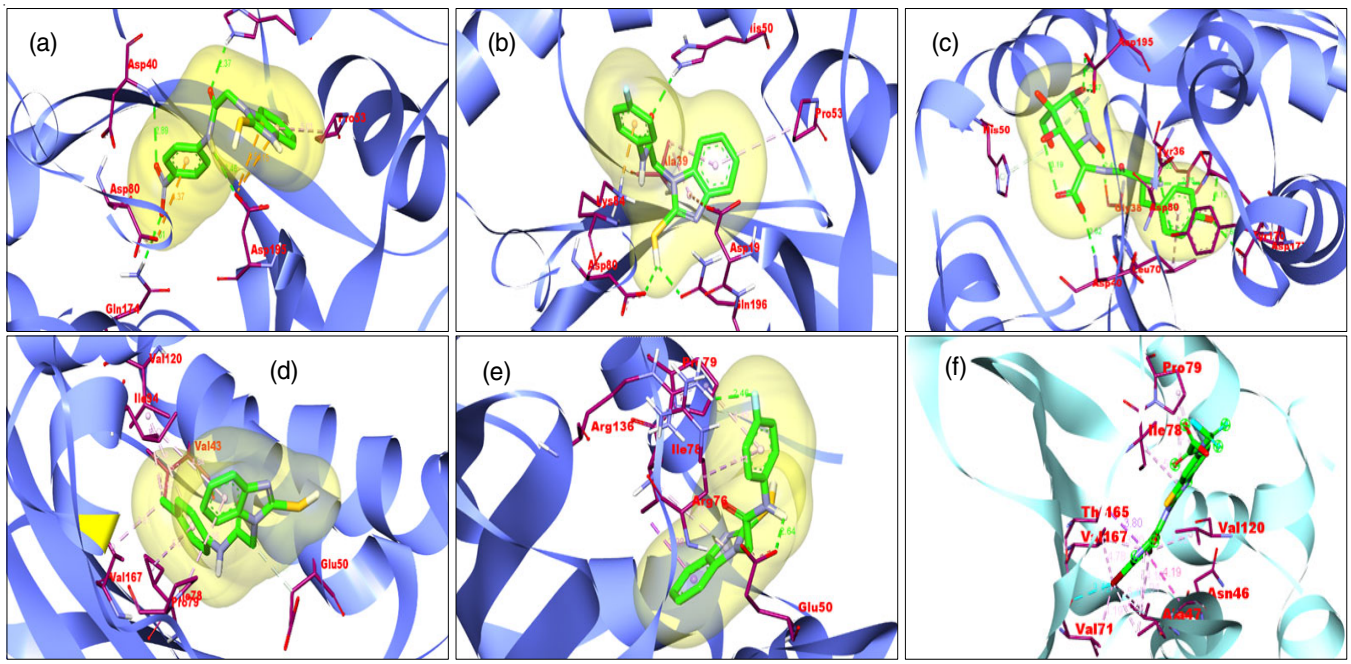

Fig. 2. (a) 3D interaction of compound **MB4** with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB: 1jij); (b) 3D interaction of compound **MB5** with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB: 1jij); (c) 3D interaction of Ligand with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB: 1jij); (d) 3D interaction of compound **MB2** with DNA gyrase subunit B (PDB: 5l3j); (e) 3D interaction of compound **MB5** with DNA gyrase subunit B (PDB: 5l3j); (f) 3D interaction of Ligand with DNA gyrase subunit B (PDB: 5l3j)

TABLE-2
PHYSICO-CHEMICAL PARAMETER AND DRUGLIKENESS OF THE SYNTHESIZED MERCAPTOBENZIMIDAZOLE DERIVATIVES (**MB1-MB5**)

Compd. code	m.f.	m.w.	Rotatable bonds	H-bond acceptors	H-bond donors	TPSA	log P	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	$C_{15}H_{12}N_3OSCl$	317.79	4	2	1	85.72	2.71	YES	0
MB4	$C_{15}H_{12}N_4O_3S$	328.35	5	4	1	131.54	1.16	YES	0
MB5	$C_{15}H_{12}N_3OSF$	301.34	4	3	1	85.72	2.59	YES	0

compound are shown in Table-3. All the newly synthesized mercaptobenzimidazoles have the significant pharmacological profiles with the modest toxicity. The *in silico* screening data show that mercaptobenzimidazole derivatives have drug-like properties and also obey the Lipinski's 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. In terms of toxicity, the derivatives have a low toxicity and safe to use.

Antimicrobial study: The biological evaluation of synthesized derivatives (**MB1-MB5**) for the antimicrobial activity was carried out by the well-known well diffusion method.The antimicrobial activity was assessed by measuring zone of inhibition in mm and compared with the standard drug as shown in Table-4. The antimicrobial tests revealed that compound **MB5** has the greatest antibacterial activity against *E. coli*, while compound **MB4** has the highest antibacterial activity against *S. aureus* and *Candida albicans*. Compound **MB5** also acted the most powerful antifungal against *Candida albicans*, whereas compound **MB3** has a modest level of activity against all of the bacteria tested.

Conclusion

The novel benzofused heterocylic compounds were synthesized and characterized as derivatives of *N*- substituted mercaptobenzimidazole. When compared to standard drugs,

TABLE-3
ADME AND TOXICITY DATA OF THE SYNTHESIZED MERCAPTOBENZIMIDAZOLE DERIVATIVES (**MB1-MB5**)

Compd. code	BBB	Caco-2 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

TABLE-4
ANTIMICROBIAL EVALUATION OF THE SYNTHESIZED MERCAPTOBENZIMIDAZOLE DERIVATIVES (**MB1-MB5**)

Compd. code	*E. coli* (ATCC 25922)	*Pseudomonas aeruginosa* (ATCC27853)	*Staphylococcus aureus (ATCC 25923)*	*Candida* sp. (ATCC10231)
MB1	No zone	No zone	07 mm	12 mm
MB2	11 mm	No zone	07 mm	12 mm
MB3	18 mm	No zone	13 mm	18 mm
MB4	11 mm	07 mm	07 mm	19 mm
MB5	19 mm	17 mm	13 mm	18 mm
Gentamycin	23 mm	26 mm	24 mm	–
Nystatin	–	–	–	29 mm

the compounds showed moderate the 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. Compounds **MB3** and **MB5** possesses the good the antimicrobial activity.

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