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Research article

DESIGN, INSILICO SCREENING, MOLECULAR DOCKING, SYNTHESIS AND BIOLOGICAL EVALUATION OF BENZO-FUSED FIVE MEMBERED NITROGEN CONTAINING HETEROCYCLE AGAINST DNA GYRASE SUBUNIT B AS POTENTIAL ANTIMICROBIAL AGENT

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ABSTRACT

Because of its function in DNA replication, DNA gyrase subunit B (1KZN) is a promising target for antimicrobial drug development. There is an urgent requirement for the designing and improvement of novel antimicrobial drugs due to the rapid development of antimicrobial drug resistance. The aim of this study is to use molecular docking to design, synthesise, and identify benzo-fused five-membered nitrogen containing heterocycle against DNA gyrase subunit B (1KZN). Using an effective procedure, 2-(1H-1,2,3-Benzotriazol-1-yl)-N-substituted acetamide was synthesised based on the literature review. The antimicrobial activity of all synthesised compounds was tested against four different organisms: E. coli, P. aeruginosa, S. aureus, and Candida albicans. The compound binds to the active site of DNA gyrase subunit B (1KZN) in a docking study, indicating that it may have antimicrobial activity. The compounds BT4 and BT6 have antimicrobial capacity, according to the findings of this report. BT3 has the ability to be an antibacterial agent for Staphylococcus aureus.

KEYWORDS: Benzotriazole, molecular docking, antimicrobial, DNA gyrase, in silico.

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INTRODUCTION

Due to a lack of adequate and effective antimicrobial drugs, bacterial and fungal infections have develop into a worldwide health issue, especially in immune-compromised people.[1] The spread of infection is accelerated by the growth, propagation, and proliferation of multidrug-resistant (MDR) bacteria that do not respond to conventional treatments.[2,3] Multidrug resistance and extensive drug resistance are already common in various bacteria and lead to limited treatment options.[4,5] The extension of antimicrobial drug compound space has been proposed as a remedy to the antibacterial drug discovery method's deadlock.[3]

Topoisomerases are enzymes that can modify the way DNA supercoils.[6] Another topoisomerase, DNA gyrase, catalyses the introduction of negative super helical transforms into closed duplex DNA that is initially relaxed or positively supercoiled in an ATP-dependent manner.[7,8] It is critical for bacterial survival and growth, making it a promising target for antibacterial drug development.[9]

Several findings pointed to benzo of used rings as promising moieties for developing novel antibacterial structures.[10] Medicinal chemists are especially interested in heterocyclic compounds because of their unique chemical and biological profiles.[11] The wide variety of biological activities of benzofusedheterocycles attracts researchers. To shape indole, Benzofuran, benzene can be fused to a heterocyclic ring containing one heteroatom. Benzimidazole, bentyhiazole, and benoxazole are formed when it is fused to two heterocyclic containing rings. Benzotriazole is a three heteroatom containing benzofused heterocyclic drug.[12] The 1H-benzo[d][1,2,3]triazole is a preferred structure because of its diverse pharmacological actions.[13] The synthesis of heterocycles using benzotriazole is rapidly progressing, and it can be used as a scaffold to create novel pharmacologically active molecules.[12,14]

Benzotriazoles are a type of heterocyclic organic compound with a three-nitrogen atom five membered ring system and a fused benzene ring.[15] There are two isomers of N-substituted benzotriazoles: 1H-and 2H-substituted. In solids and solutions, the 1H-substituted dominated, while in the gas phase, the quantity of the 2H-tautomer increased.[16] Because of its chemical properties, such as its ability to act as an electron donor or a precursor to radicals or carbanions, Benzotriazole is one of a kind. Condensation, addition reactions, and benzotriazolyl-alkylation are among the reactions it goes through. It is simple to add different groups and heterocycles to benzotriazole using this reaction, leading in the formation

of a new heterocycles.[12,17,18] This Benzofusedheterocycle has recently been the focus of a lot of research to learn more about its biological properties[19], which include α-amylase and α-glucosidase inhibitors[11], antioxidant, antiulcer[20], antitumor[21], anti-inflammatory[22,23], antimycobacterial[24,25], and antiviral properties along with antimicrobial[26]. Accordingly, the present work comprises of designing, molecular docking and synthesis of 2-(1H-1,2,3-Benzotriazol-1-yl)-N-substituted acetamide and evaluating its antimicrobial activity against gram-positive, gram negative bacteria and Fungi.

MATERIAL AND METHOD

All reagents and solvents were bought from commercial sources and used as provided. The reactions were monitored using thin layer chromatography (TLC) on 0.2 mm silica gel plates. IR, NMR (1H) and HRMS analysis were used to determine the chemical structures of the final products. IR spectra were recorded on Vertex 80 FTIR. 1H and 13C-NMR spectra were recorded by ECZR Series 600 MHz NMR spectrometer in deuterated chloroform or dimethyl sulfoxide (CDCl3/DMSO). Mass spectra were recorded on AccuTOF GCV mass spectrometer. CHNS analysis was carried out using FLASH EA 1112 series analyser.

General method for the Synthesis of 1H-1,2,3 Benzotriazole (I)

Using procedure given in literature benzotriazole was synthesized. o-phenylenediamine (0.1mole) was dissolved in beaker containing a mixture of glacial acetic acid (0.2mole) and 30ml water, which was slightly warmed to dissolve o-phenylenediamine. The solution was chilled to 15°c, then magnetically stirred and a solution of sodium nitrate (0.11mole) in 15 mL of water was added to above solution at once. The reaction mixture starts to warm shortly after addition, reaching a temperature of about 85°C in around 2-3 minutes before cooling and changing color from deep red to pale brown. Stirring was continued for another 15 minutes, during which the temperature had fallen to 35-40°C, and the mixture was completely chilled in an ice-water bath for 30 minutes. Vacuum filtration was used to filter the precipitated material, which was then washed three times with ice-cold water. Recrystallization from boiling water with activated charcoal further purified the compound.[27] 67.90 %, 96-99°C.

General method for the Synthesis of 2-chloro-N-substituted acetamide (II)

With continuous shaking, 0.01 M of substituted aromatic amine was introduced to a 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 drop by drop using a dropping funnel. 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. Filtered precipitate was washed with cold water and dried. 95% ethanol was used to re-crystallize it.[28–30]

General method for the Synthesis of 2-(1H-1,2,3-Benzotriazol-1-yl)-N-substituted acetamide (III)

2-chloro-N-Aryl acetamide derivative (II) (0.02mole) in DMF was introduced to Benzotriazole (I) (0.02mole) in DMF and potassium carbonate and potassium iodide was refluxed using microwave irradiation at 245W. 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.[30,31]

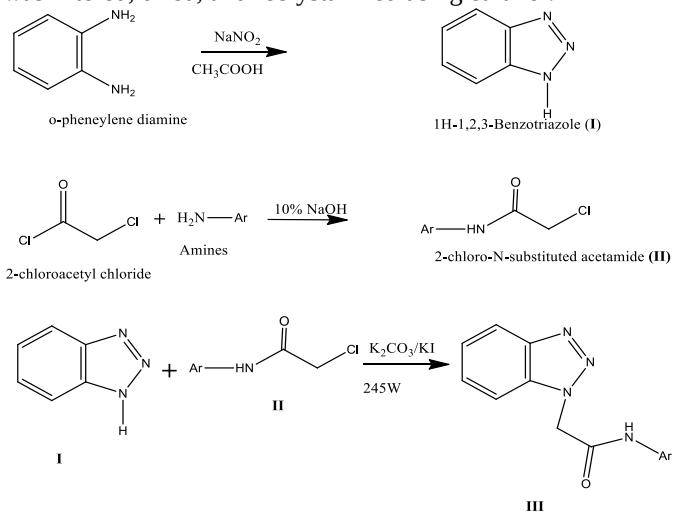


Figure 1: Scheme for synthesis of 2-(1H-1,2,3-Benzotriazol-1-yl)-N-substituted acetamide

Antimicrobial Activity

This method is based on the concept that moisture permeates through an antibiotic-impregnated disc mounted 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. The antibiotic concentration is highest near the disk's edge and gradually declines as the distance between the disc and the organism grows, until it no longer inhibits the organism's expansion. After incubation, if an antibiotic disc stops bacterial development, a clear zone forms around it.[32]

Method [33–35]

Mueller-Hinton Agar (MHA) is used for the disc diffusion allows most bacterial pathogens to develop well. The petri plates were filled with agar medium. The petri plates were stored inverted after solidifications so that water could condense in the upper lid. The compounds were dissolved in dimethyl process because it is the best medium for routine susceptibility tests because it has good reproducibility, is low in sulfonamide, trimethoprim, and tetracycline inhibitors and sulfoxide and added at an 8.0 mg mL-1 final concentration. The zone of inhibition against the test species was used to assess antimicrobial activity in the disc diffusion assay.

Strains used

E. coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 25923,Candida albicans ATCC10231

Incubation period : 35 to 37°C for 24 hours

Reference compound : Gentamycin and Nystatin

Molecular Docking

Structures of synthesised molecule was drawn using Chemdraw Ultra software V.12.0.2 and optimized and converted to 3D structures that are compatible for docking using Open Babel (version 3.0.0)[36]. The molecular target's (DNA gyrase subunit b Center (DNAG) PDB: 1KZN) three-dimensional structures were obtained from Protein Data Bank (PDB) (www.rcsb.org). The receptor protein is prepared for docking by removing heteroatom, water and adding polar hydrogen and charges (Kollman and Gasteiger). The active site of receptor is visualised in PyMol.[37]

The active sites are defined using grid box of suitable size. The study of docking was carried out with the help of Autodockvina[38] and visualization with the help of Discovery studio 3.5 visualizer (DS visualizer)[39].

In silico screening

Physicochemical properties was analysed using online chemical property calculator Molinspiration (http://www.molinspiration.com) and other pharmacokinetic properties and toxicity was evaluated using the PreADMET server (http://preadmet.bmdrc.org/) and SwissADME (http://www.swissadme.ch).[40]

RESULT

Chemistry

As a benzofused nitrogen containing heterocycle, novel benzotrizole derivatives were synthesised. The procedure described in the literature yielded good yields of benzotriazole. Then, in the presence of 10% NaOH, various 2-chloro-N-substituted acetamides were produced by slowly adding chloroacetyl chloride to a solution of various amines. The desired derivatives were then obtained by condensing this acetamide with benzotriazole using $K_2CO=$ as a base, as shown in scheme-I.

Table 1 summarises the synthesis result in terms of yield and physical properties. TLC was used to track the synthesis reactions (Scheme 1) of these compounds, which used a 70:30 n-hexane and ethyl acetate mixture as a solvent phase.

Elemental analysis was used to validate the structure of solid compounds. MS, 1H NMR, 13C NMR, and FTIR spectroscopy were used to characterise the derivatives.

Table 1: Physical properties of synthesised Benzotriazole derivatives

Compound	Ar	mp (℃)	Yield (%)	Rf value
BT1	Phenyl	210-214	77.58	0.62
BT2	4-Methylphenyl	220-222	62.36	0.63
BT3	4-Chlorophenyl	186-188	65.50	0.56
BT4	4- Nitro phenyl	242-246	75.25	0.59
BT5	2- Nitro phenyl	232-238	72.40	0.57
BT6	4- fluro Phenyl	292-294	64.20	0.61
BT7	4-methoxy Phenyl	172-176	70.12	0.66

2-(1H-1,2,3-Benzotriazol-1-yl)-N-phenylacetamide (BT1)

IR (KBr)γ_{max} (cm^{-}): 3281.02 (N-H str.), 3074.63 (=C-H str.)1681.98 (C=O str.), 1562.75 (N-H bend) ,1249.91 (C-N stre) , 752.26 (Aromatic ring).

2-(1H-1,2,3-Benzotriazol-1-yl)-N-(p-tolyl)acetamide (BT2)

IR (KBr)γ_{max} (cm^{-}): 3276.18 (N-H str.), 3082.35(=C-H str.), 1680.05 (C=O str.), 1537.32 (N-H bend), 1251.84 (C-N str.), 746.48 (Aromatic ring); ^{1}H NMR (600 MHz, δ ppm):1.60(s, CH_3), 3.66 (s, CH_2), 7.26(s, NH), 7.91(d,=CH), 7.90 (d,=CH), 6.67-6.64(m, CH_2Ar); ^{13}C NMR(600 MHz, δ ppm): 25.01 (CH_3), 39.63 (CH_2), 172.31(C=O), 115.07-119.12(CH Ar.), 130-134 (=CH Benzotriazole); m/z: 266(M^{+}), Anal. Cal. (Found) for $C_{15}H_{14}N_4O$: C 67.65 (67.91), H 5.30(5.18), N21.04 (20.93), O 6.01 (5.92).

2-(1H-1,2,3-Benzotriazol-1-yl)-N-(4-chloro-phenyl)-acetamide (BT3)

IR (KBr)γ_{max} (cm^{-}): 3266.59 (N-H str.), 3055.36(=C-H str.), 1668.48 (C=O str.), 1541.18 (N-H bend), 1280.78 (C-N str.), 758.05 (Aromatic ring), 678.97 (C-Cl).

2-(1H-1,2,3-Benzotriazol-1-yl)-N-(4-nitrophenyl)acetamide (BT4)

IR (KBr)γ_{max} (cm^{-}): 3363.77(N-H str.), 3052.39(=C-H str.), 1665.59 (C=O str.), 1573.19 (N-H bend),1624.42 and 1422.51 (NO_2 str.), 1291.52 (C-N str.), 770.95 (Aromatic Ring), ^{1}H NMR (600 MHz, δ ppm): 5.30 (s, CH_2), 7.25(s, NH), 7.88(d,=CH), 7.87 (d,=CH), 6.99-6.75(m, CH_2Ar); ^{13}C NMR(600 MHz, δ ppm): 39.63 (CH_2), 167.50(C=O), 113.67-118.38(CH Ar.), 130-134 (=CH Benzotriazole); m/z: 297(M^{+}), Anal. Cal. (Found) for $C_{15}H_{14}N_4O$: C 56.56 (54.94), H 3.73(3.62), N 23.56 (23.49), O 16.15 (15.92).

2-(1H-1,2,3-Benzotriazol-1-yl)-N-(2-nitrophenyl)acetamide (BT5)

IR (KBr)γ_{max} (cm^{-}): 3275.24 (N-H str.), 3059.20 (=C-H str.), 1662.69(C=O str.), 1543.10 (N-H bend), 1570.11 and 1460.52(NO_2 str.), 1257.63 (C-N str.), 763.84 (Aromatic Ring).

2-(1H-1,2,3-Benzotriazol-1-yl)-N-(4-fluorophenyl) acetamide (BT6)

IR (KBr)γ_{max} (cm^{-}): 3290.67 (N-H str.), 3063.06 (=C-H str.), 1647.26 (C=O str.), 1550.82 (N-H bend), 1244.13 (C-N stre) , 746.48 (Aromatic ring), 1072 (C- F)

2-(1H-1,2,3-Benzotriazol-1-yl)-N-(4-methoxyphenyl) acetamide (BT7)

IR (KBr)γ_{max} (cm^{-}): 3279.10 (N-H str.), 3090.07 (=C-H str.), 1670.41 (C=O str.), 1535.39 (N-H bend), 1257.63 (C-N stre) , 1030.02(O-C str.), 744.55 (Aromatic ring).

Antimicrobial Study

The antimicrobial activity of the synthesised compound was assessed using the well-known well diffusion method, with gentamycin as the antibacterial standard and nystatin as the antifungal standard. The antimicrobial activity of the synthesised compound is evaluated by measuring the zone of inhibition in millimetres and comparing it to the standard drug as a parameter. The antimicrobial activity of the synthesised molecule was assessed using the well-known well diffusion method, using gentamycin as the antibacterial standard and nystatin as the antifungal standard. The antibacterial activity of the synthesised molecule is evaluated by measuring the zone of inhibition in millimetres and comparing it to the reference drug as a parameter.

Table 2 shows the antimicrobial activity effects, which are also shown as bar diagrams in Figure 2.

Table 2: Antimicrobial evaluation of benzotriazole derivatives

Sample Code.	E. coli (ATCC 25922)	Pseudomonas aeruginosa (ATCC27853)	Staphylococcus aureus (ATCC 25923)	Candida sp. (ATCC10231)
BT1	No zone	No zone	No zone	12 mm.
BT2	No zone	No zone	No zone	No zone
BT3	No zone	10 mm.	21 mm.	15 mm.
BT4	15 mm.	09 mm.	11 mm.	12 mm.
BT5	No zone	No zone	No zone	No zone
BT6	11 mm.	07 mm.	14 mm.	16 mm.
BT7	10mm	08mm	09mm	10mm
SD1	23 mm.	26 mm.	24 mm.	--
SD2	--	--	--	29 mm.

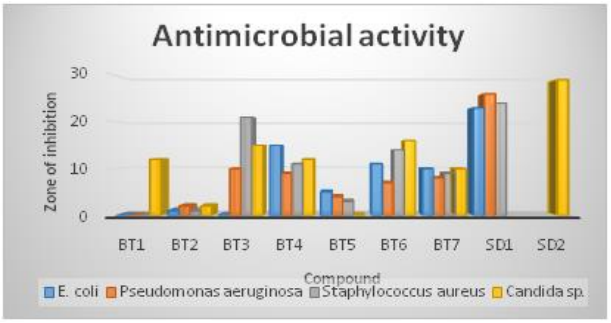


Figure 2: Antimicrobial activity of benzotriazole derivatives

Molecular docking

The binding affinities of newly synthesised compounds and the target protein 1KZN were investigated using molecular docking. The docking results showed that all of the compounds had significant bonding interactions in 1KZN's binding pocket, with docking scores ranging from –7.4 to –7.8 kcal/mol. Table 3 presents the binding affinity of the benzotriazole derivative with the target protein with amino acid interaction. Figure 3 to 6 shows the 2D and 3D interaction of benzotriazole derivative with the target protein

Table 3: Binding affinity and interaction of Benzotriazole derivatives and reference with target protein 1KZN

comp code	Binding affinity (kcal/mol)	Type of interaction	Amino acid in Hydrogen bond	Interacting Amino acid	Distance
BT1	-7.6	Carbon hydrogen Bond, Pi-alkyl, van der waals		Val43, Asn46, Ala47, Glu50, Val71, Ile78, Pro79, Ile90, Val120, Thr165, Val167	Val43(5.45), Ala47 (4.62), Ile78(5.32, 4.53), Ile90 (4.80), Thr165(2.90), Val167(5.19)
BT2	-7.4	Carbon hydrogen Bond, Pi- cation, Pi-anion, alkyl Pi-alkyl, van der waals		Val43, Asn46, Ala47, Glu50, Arg76, Asp73, Gly77, Ile78, Pro79, Ile90, Met91, Val120, Arg136, Thr165, Val167	Ala47 (2.78,5.32)Glu50 (3.94), Arg76 (3.88), Ile78(3.370), Pro79 (4.29,4.86)
BT3	-7.4	Carbon hydrogen Bond, Pi-cation, Pi-anion, alkyl Pi-alkyl, van der waals		Val43, Asn46, Ala47, Glu50, Arg76, Asp73, Gly77, Ile78, Pro79, Ile90, Met91, Val120, Arg136, Thr165, Val167	Ala47 (2.77,5.34)Glu50 (3.80), Arg76 (3.88), Ile78(5.36), Pro79 (4.37,4.88)
BT4	-7.6	Conventional Hydrogen Bond, Carbon hydrogen Bond, Pi-cation, Pi-anion, alkyl Pi-alkyl, van der waals	Arg136	Val43, Asn46, Ala47, Glu50, Arg76, Asp73, Gly77, Ile78, Pro79, Ile90, Met91, Val120, Arg136, Thr165, Val167	Ala47 (2.82,5.39), Glu50 (3.93), Arg76 (2.72,3.93), Ile78(5.33), Pro79 (4.89), Arg136 (2.01)
BT5	-7.6	Conventional Hydrogen Bond,Carbon hydrogen Bond, Pi-alkyl, van der waals	Asn46, Arg76	Val43, Asn46, Ala47, Glu50, Arg76, Asp73, Ile78, Pro79, Ile90, Met91, Val120, Arg136, Thr165, Val167	Asn46(2.31,5.23, Ala47(3.54), Arg76(2.78), Ile78(5.26,5.47), Pro79(4.27), Ile90(5.47), Val120(5.40)
BT6	-7.8	Conventional Hydrogen Bond, Carbon hydrogen Bond, Halogen, Pi-alkyl, van der waals	Val167	Val43, Asn46, Ala47, Glu50, Val71, Asp73, Ile78, Pro79, Ile90, Val120, Thr165, Met166, Val167	Val43(5.45), Ala47 (4.66), Val71 (2.89), Ile78 (5.44, 4.71), Ile90 (4.67), Thr165(3.01), Val167 (5.30,2.67)
BT7	-7.4	Conventional Hydrogen Bond, Carbon hydrogen Bond, Pi-cation, Pi-anion, alkyl, Pi-alkyl, van der waals	Arg136	Val43, Asn46, Ala47, Glu50, Arg76, Asp73, Gly77, Ile78, Pro79, Ile90, Met91, Val120, Arg136, Thr165, Val167	Ala47 (2.74, 5.29, 5.48), Glu50 (3.90), Arg76 (3.93), Ile78(5.37), Pro79 (4.64, 4.82), Arg136 (2.37), Val120(5.45)
Ref	-6.4	Conventional Hydrogen Bond, Attractive Charges, Carbon hydrogen Bond, unfavorale Acceptor-Acceptor, van der waals	Glu42, Asn46, Asp49	Glu42, Asp45, Asn46, Asp49, Glu50, Arg76, Gly77, Ile78, pro79, Ile90, Gly119, Gly117, Arg136	Val43(5.45), Ala47 (4.66), Val71 (2.89), Ile78 (5.44, 4.71), Ile90 (4.67), Thr165(3.01), Val167 (5.30,2.67)

In silico screening

Physiochemical properties must be defined in order to predict the molecule's potential as a drug. Lipinski's rules are a set of rules that predict drug likeness. The lipinski's law, as well as hydrogen bond acceptor and donor, log p, and tpsa, were shown in table 4. The lippinski rule is followed by all synthesized derivatives, and they have a high oral bioavailability.

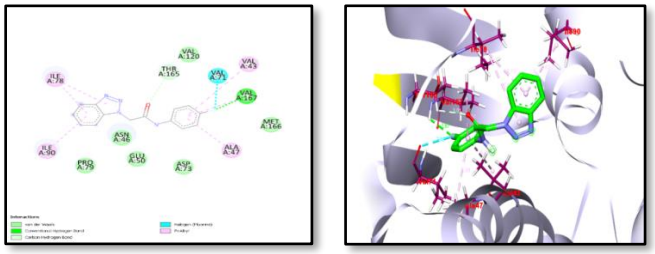
Table 4: physicochemical parameter and druglikeness of Benzotriazole Derivatives

Comp Code	Formula	MW	Rotatable bonds	H-bond acceptors	H-bond donors	TPSA	LOGP	Follow lipinski	Lipinski violations
BT1	$C_{14}H_{12}N_4O$	252.27	4	3	1	59.81	2.16	YES	0
BT2	$C_{15}H_{14}N_4O$	266.3	4	3	1	59.81	2.48	YES	0
BT3	$C_{14}H_{11}ClN_4O$	286.72	4	3	1	59.81	2.61	YES	0
BT4	$C_{14}H_{11}N_5O_3$	297.27	5	5	1	105.63	2.12	YES	0
BT5	$C_{14}H_{11}N_5O_3$	297.27	5	5	1	105.63	2.07	YES	0
BT6	$C_{14}H_{11}FN_4O$	270.26	4	4	1	59.81	2.32	YES	0
BT7	$C_{15}H_{14}N_4O_2$	282.3	5	4	1	69.04	2.22	YES	0

Pharmaceutical pharmacokinetic properties such as absorption, delivery, metabolism, and toxicity can be predicted using computational programmes such as pre-admet and swiss adme to forecast the behavior of compounds that may be used as pharmaceuticals in the future. Table 5 shows the synthesized compound's adme and toxicity. The pharmacological profile of all newly synthesized benzotriazole derivatives was significant, with moderate toxicity.

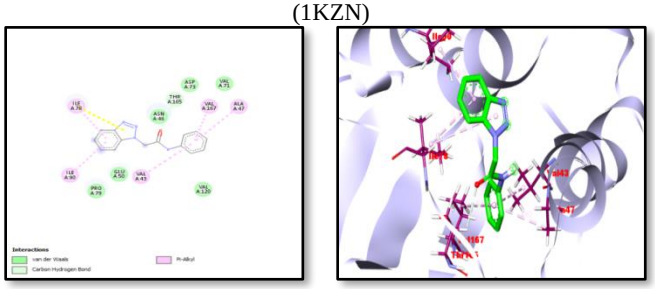
Table 5: ADME and toxicity of Benzotriazole derivatives

Comp Code	BBB	Caco2++ Cell permeability	HIA	MDCK	PPB	Skin Permeability	Algaeat	Ames test	Carcino Rat	hERG inhibition
BT1	1.14254	20.5038	95.71427	22.4985	100	-3.48279	0.0850593	mutagen	negative	Medium risk
BT2	0.741288	20.8373	95.75007	24.7306	100	-3.41304	0.0526432	mutagen	negative	Medium risk
BT3	0.43395	21.672	95.98776	14.8835	99.55019	-3.52879	0.0378092	mutagen	negative	Medium risk
BT4	0.0278888	20.9278	93.94095	5.64767	98.71858	-3.54964	0.0804334	mutagen	positive	Medium risk
BT5	0.161515	19.3246	93.94078	26.1458	97.45237	-3.52878	0.0896459	mutagen	positive	Medium risk
BT6	0.327233	22.5743	95.71651	7.22047	91.96729	-3.75733	0.0736706	mutagen	positive	Medium risk
BT7	0.542045	23.8975	96.11326	14.1237	90.16409	-3.69014	0.0674 75	mutagen	negative	Medium risk



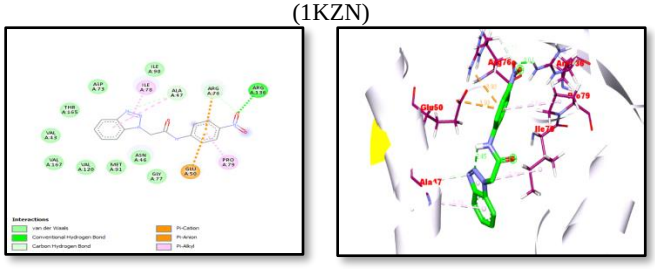
(a) (b)

Figure 3: (a) 2D interaction of BT6 with DNA gyrase subunit B (1KZN), (b) 3D interaction of BT6 with DNA gyrase subunit B (1KZN)



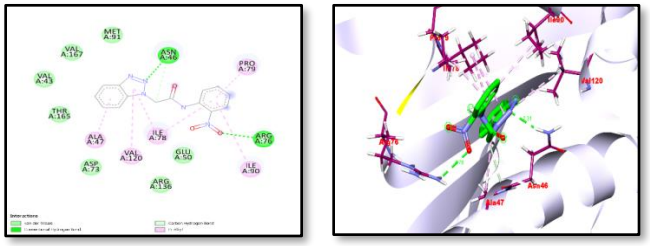
(a) (b)

Figure 4: (a) 2D interaction of BT1 with DNA gyrase subunit B (1KZN), (b) 3D interaction of BT1 with DNA gyrase subunit B (1KZN)



(a) (b)

Figure 5: (a) 2D interaction of BT4 with DNA gyrase subunit B (1KZN), (b) 3D interaction of BT4 with DNA gyrase subunit B (1KZN)



(a) (b)

Figure 6: (a) 2D interaction of BT5 with DNA gyrase subunit B (1KZN), (b) 3D interaction of BT5 with DNA gyrase subunit B (1KZN)

Figure legends

Docking images of ligand with molecular target protein (E. Coli DNA gyrase PDB: 1KZN). The 2D (2 Dimentional) interaction (a) with Amino acid in the active site of target along with type of interaction. The 3D interaction (b) depicts the ligand's binding in the target protein's pocket, as well as the bonding distance.

DISCUSSION

Chemistry

The novel N- substituted benzimidazole was synthesised using scheme I. All derivatives were obtained in good yield and with high purity. The structures of synthesised derivatives were confirmed using MS, ^{1}H NMR, ^{13}C NMR, and FTIR. In the FTIR spectrum, a band observed in the range 1681 to 1647 cm^{-1} attributed to the stretching of C = O group of amides. The N-H stretching band for secondary amide was observed in the range of 3363- 3263 cm^{-1}and C- H stretching between 3090-3052 cm^{-1}.

In ^{1}H spectra the peak for secondary amide observed at δ, 7.25 and different peak for aromatic proton of benzotriazole ring observed at higher δ, 7.87-7.91 than phenyl ring proton at δ,6.99-6.64. In ^{13}C spectra shows the presence of amide carbon at δ, 167. This finding confirms the structure of synthesised compound.

Antimicrobial study

Using Gentamicin as a standard drug, all of the synthesized compounds BT1-7 were tested for in vitro antibacterial activity against two separate strains of Gram-negative (Escherichia coli and Pseudomonas aeruginosa) and Gram-positive (Staphylococcus aureus) bacteria. Nystatin is used as a standard medication to test antifungal activity of all synthesized compound against Candida albicans. After antimicrobial testing, it was discovered that BT4 has the highest activity against E. Coli, while BT3 has the highest activity against S. aureus. BT6 also has the best antifungal activity against Candida albicans. BT4 and BT6 has moderate activity against all the bacteria under investigation.

Docking

The compound was subjected to docking study so as to observe the non-bonding interaction as well as the binding affinity. Molecular docking result reveals that Val43, Asn46, Ala47, Glu50, Val71, Ile78, Pro79, Ile90, Val120, Thr165, Val167 are the amino acids found in the active site of DNA gyrase subunit B Center (DNAG) PDB: 1KZN and are involved in ligand binding. 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, resulting in a docking score of –7.4 to –7.8 kcal/mol. BT6 bind with the target protein molecule with lowest docking score of –7.8 kcal/mol. It forms a traditional hydrogen bond with Val167 amino acid residue and various interactions with other amino acids such as Carbon hydrogen Bond, Halogen, Pi-alkyl, and van der Waals. BT1, BT4, and BT5 are other benzotriazole derivatives with a moderate binding affinity and a docking score of -7.6 kcal/mol.

In silico screening

In silico screening results indicate that benzotriazole derivatives have drug-like properties and all follow the Lipinski law without exception. The majority of derivatives have four rotatable bonds with 3-5 hydrogen acceptor atoms. These derivatives have marginal to measurable concentrations in the CNS, according to the ADME profile.

The result of in silico screening suggests that benzotriazole derivatives have the drug likeness properties and all obey Lipinski rule with no violation. Most of derivatives has 4 rotatable bonds with 3-5 atom as hydrogen acceptor. ADME profile suggests that these derivatives have negligible to detectable concentration in CNS. The oral bioavailability of derivatives is demonstrated by CaCO2++ cell permeability, HIA, and MDCK. In terms of toxicity, the compound has a low toxicity and is safe to use.

CONCLUSION

The novel benzofused heterocylic compounds were synthesised and characterised as derivatives of 2-(1H-1,2,3-Benzotriazol-1-yl)-N-substituted acetamide. 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 synthesised compounds could be more effective antifungal agents than antibacterial agents. According to a docking analysis, this compound has antimicrobial activity. Compound BT6 possesses the best antimicrobial activity.

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