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Design, Synthesis, in silico and in vitro antibacterial studies and molecular docking analysis of novel Benzotriazole derivatives

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ABSTRACT

Heterocyclic Chemistry plays an important role in medicinal chemistry. Benzo-condensed compounds containing hetero atoms have been extensively studied for their biological activities. So, it was aimed to synthesize biological active compounds with high potency, lower toxicity and enhanced action mechanism. The present research work deals with the study of ADMET, synthesis, molecular docking and in-vitro antimicrobial activities. All the synthesized compounds were well characterized using various spectroscopic techniques. Further, the compounds were screened for antimicrobial activities and moreover these synthesized compounds along with standard drug Amoxicillin were docked with crystal structure of S. aureus TarS with PDB ID 5TZ8. Based on in vitro and in silico studies in comparison to amoxicillin as standard reference drugs, compound 2a has revealed efficient results towards selected bacterial and strains.

Keywords: *Benzotriazole · Docking · ADMET · Antibacterial*

1. INTRODUCTION

In past few years there is rise in the development of novel pharmaceutical lead compounds with exceptional antimicrobial activities, high potency, reduced systemic adverse effects, lower toxicity, economically viability and enhanced action mechanism with different molecular scaffold. Many recent and upgraded sophisticated tools have been accessed by the medicinal chemists to aid in the process of drug development. Molecular Docking has proven to be an important tool for helping scientists to identify structural determinants necessary for efficient ligand-receptor binding [1]. In recent studies synthesis and testing of newly synthesized compounds which are more biologically efficient against the infectious diseases are aided with molecular docking. Undesirable pharmacokinetics and toxicity of the candidate compounds are the main failure of drug development. So it is the need of the hour to evaluate the absorption, distribution, metabolism, excretion and toxicity (ADMET) of chemicals. This has been carried out to predict and assist in accelerating the drug research and development process thereby selecting promising lead compounds for further exploration [2]. Subsequently, Heterocyclic compounds have majorly contributed in development of society from both biological and industrial point of view and thus interpreting the life processes and ultimately improving the standard of life [3]. Among various active heterocyclic moieties, Benzotriazole is a miraculous heterocyclic compound with multi-skilled biological activitiesand is an area of considerable interest for research scientists in past few decades due to itsbenign properties such as antimicrobial [4], antibacterial [5], antifungal [6], antihelmintic [7], antitubercular [8] and anticancer [9] activities. The biological significances of these class of heterocycles impelled us to synthesize some novel derivatives of benzotriazole .In this study we report the docking and antibacterial screening of synthesized derivatives of benzotriazole which are N-(3-(1H-benzotriazol-1-yl) propyl) substituted aniline compounds 2a-2d on protein receptor with PDB ID 5TZ8.

2. MATERIALS AND METHODS

As a part of research planning, experimental work with reported methods and procedures were carried out leading to synthesis of compound 1. Compounds 2a,2b, 2c and 2d were synthesized using compound 1 in similar manner by modifying the substituted aniline followed by analytical studies. The reagent grade AVRA chemicals were purchased from the commercial

sources. The progress of reaction was monitored by silica gel-G coated TLC plates.The products were purified through column chromatographic using

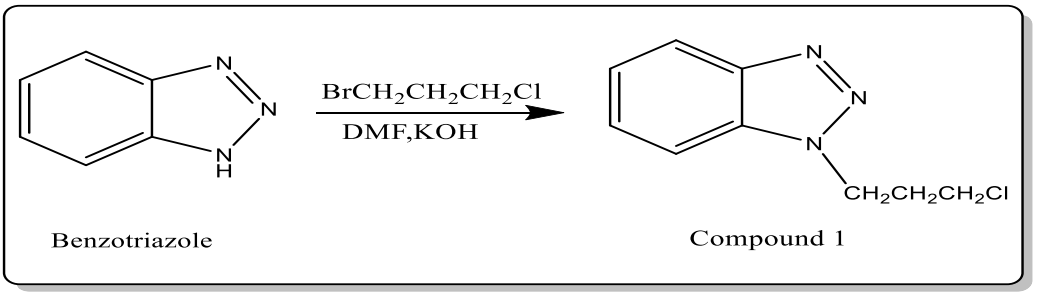
Merck silica gel 60-120 mesh. Melting points were determined using digital melting point apparatus. The spectroscopic data supported the formation of the products in end step. IR spectra were recorded on Perkin Elmer FT-IR Spectrometer with KBr pellets and ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance Neo-500 MHz NMR spectrometer in $CDCl_3$ at 500 MHz scale. The HRMS was recorded on SYNAPT-XS mass spectrometer. The analytical data for all the compounds were quite up to the mark. The reagent grade chemicals were purchased from the commercial sources. To predict and assist in accelerating the drug research and development process thereby selecting promising lead compounds for further exploration the pharmacokinetic, physiochemical and drug-likeliness properties were evaluated using a web based tool ADMETlab 2.0 **[10]**

Procedures for the synthesis of Benzotriazole Derivatives

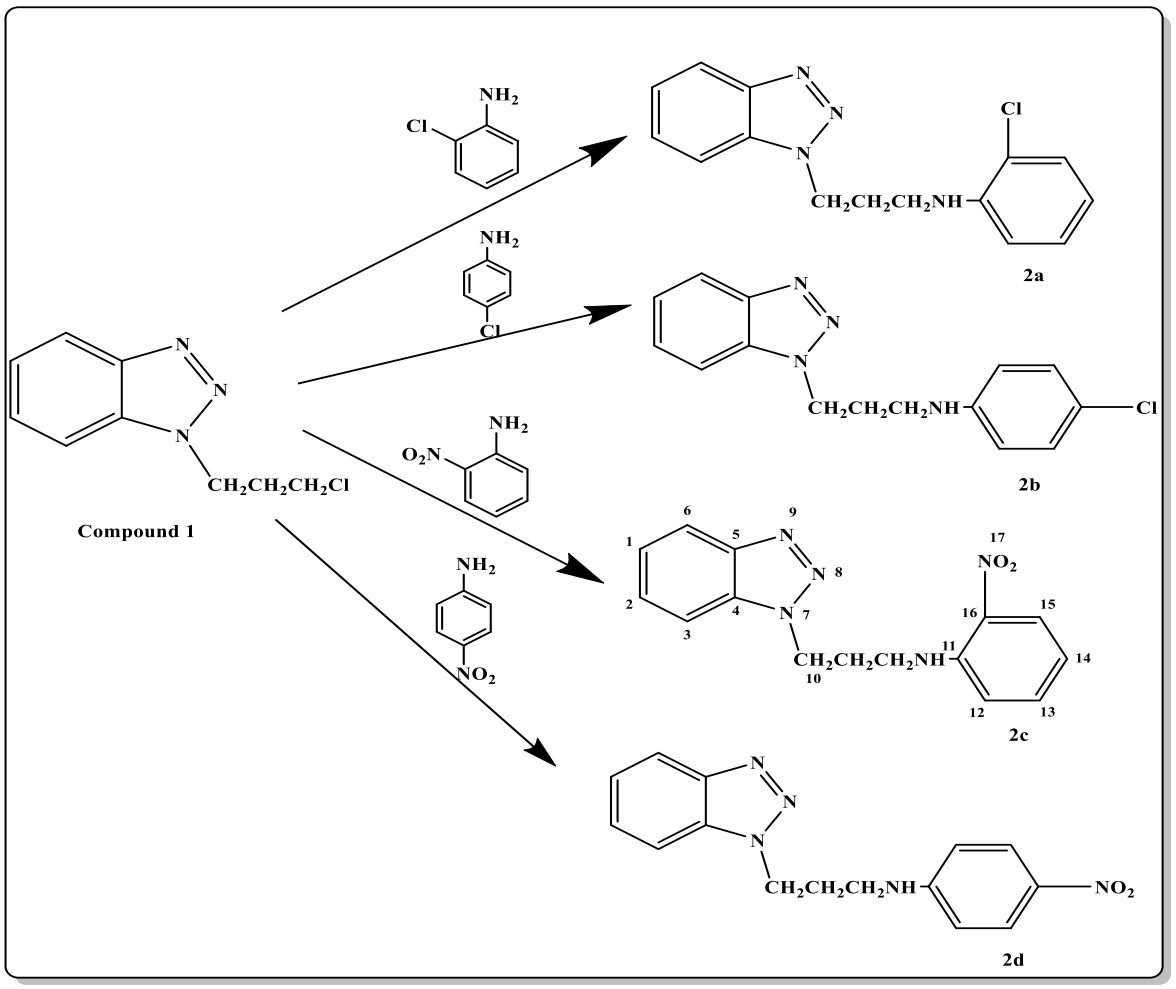
2.1.1 Synthesis of Compound 1: 1-(3-chloropropyl)-1H-benzotriazole-1. :Benzotriazole (0.21 mol) was dissolved in DMF(15 ml) and (0.21mol) of K_2CO_3 was dissolved in 1-bromo-3-chloropropane (15ml) . Both the mixture was added in a round bottom flask. The mixture was stirred and refluxed on a magnetic stirrer for about 6 hours. The completion of the reaction was monitored by TLC plates coated with silica gel-G. The crude product was readily purified by passing it through chromatographic column packed with silica gel (slurry made in hexane) using the system Hexane: Ethylacetate (7:3 v/v) as eluent to obtain pure derivative. The excess of solvent was evaporated by Rota-evaporator. The resulting purified product was re-crystallized from chloroform to yield the compound **(Scheme 1).**

2.1.2 General Method of Synthesis of N-(3-(1H-benzotriazol-1-yl)propyl)substituted aniline Compound (2a-2d):

1-(3-chloropropyl)-1H-benzotriazole Compound 1 (0.006 mol) was dissolved in 10 ml N,N-Dimethylformamide (DMF) in a round bottom flask. Substituted aniline (0.018mol) and powdered K_2CO_3 (0.006 mol) was added to the solution. The mixture was stirred and refluxed on a magnetic stirrer for about 5 hours 30 minutes maintaining the temperature between 80 °C. The completion of the reaction was monitored by TLC plates coated with silica gel-G. The product obtained was cooled at room temperature and 10 % HCl was added to it in order to remove excess of aniline. Further compound was extracted by adding 30ml of $CHCl_3$ using separating funnel. The excess of $CHCl_3$ was removed by subjecting the compound to rotary-evaporator. Finally it was readily purified by passing it through chromatographic column packed with silica gel(slurry made in hexane) using the system CH_3COCH_3 : CH_3OH (6:4 v/v) as eluent to obtain pure derivative. The resulting purified product was re-crystallized from ethanol to yield the compounds. Other compounds 2b-2d was synthesized in the similar manner by treating compound 1 with selected substituted aniline **(Scheme 2).**



Scheme 1: Synthesis of 1-(3-chloropropyl)-1H-benzotriazole



Scheme 2: Synthesis of (N-(3-(1H-benzotriazol-1-yl) propyl) substituted aniline (Compound 2a-2d)

Table 1: Yield Analysis of synthesized compounds 1,2(a-d)

Compound	Molecular Formula	Recrystallizing solvent	M.P.(˚C)	Yield(%)	R_f Value
1	$C_9H_{10}N_3Cl$	Ethanol	72-74	85	0.5
2a	$C_{15}H_{15}N_4Cl$	Ethanol	162-164	75	0.21
2b	$C_{15}H_{15}N_4Cl$	Ethanol	166-168	70	0.17
2c	$C_{15}H_{15}N_5O_2$	Ethanol	97-99	80	0.25
2d	$C_{15}H_{15}N_5O_2$	Ethanol	127-129	82	0.13

2.2.3 Spectral Data of synthesized Compounds:

1-(3-chloropropyl)-1H-benzotriazole(1)**: ^{1}H NMR:** (500 MHz, $CDCl_3$, TMS), δ: 7.92-7.34 (m, 4H, ArH), 4.85 (t, 2H), 3.53 (s, 2H), 2.49(m, 2H), **IR: ν_{max} (KBr cm^{-1}):** 3068.92 (Ar C-H), 2857.36 (aliphatic C-H), 1453.89 (-CH_2-), 1271.98 (C-

N), 746.11(C–Cl), **^{13}C NMR:**(125 MHz, $CDCl_3$) δ:138.01,127.97, 127.5, 124.35,119.81,115.73,45.47, 41.40, 32.29, **HR-MS(m/z);** 196.0671$(M)^+$, 160.0871

N-(3-(1H-benzotriazol-1-yl) propyl)-2-chloroaniline(2a): ^{1}H NMR: (500 MHz, $CDCl_3$, TMS), δ: 9.40(brs N-H), 7.94-7.49(m, 8H, ArH), 4.39 (t, 2H), 2.89 (t, 2H), 1.38(t, 2H),

IR: ν_{max} (KBr cm^{-1}): 3416.71(N-H), 3090.10(Ar C-H), 2852.87 (aliphatic C-H), 1620.11(N-H), 1466.02(-CH_2-), 748.86 (C-Cl) **^{13}C NMR spectrum:** (125 MHz, $CDCl_3$), δ: 141.10, 134.47, 131.13, 130.64, 129.97, 128.21, 125.69, 124.23, 122.26, 120.28, 119.08, 114.92, 39.67, 39.33, 39.00, **HR-MS (m/z):** 251.1283$(M)^+$, 196.0643, 160.0865

N-(3-(1H-benzotriazol-1-yl)propyl)-4-chloroaniline(2b): ^{1}H NMR: (500 MHz, $CDCl_3$, TMS), δ: 9.29(brs N-H), 7.90-7.47(m, 8H, ArH), 4.39 (t, 2H), 2.75(t, 2H), 1.38(m, 2H), **IR: ν_{max} (KBr cm^{-1}):** 3432.08(N-H), 3050.54 (Ar C-H), 2852.66 (aliphatic C-H), 1633.94(N-H), 1466.73 (-CH_2-), 749.91 (C-Cl), **^{13}C NMR spectrum** (125 MHz, $CDCl_3$), δ: 167.72, 152.51, 141.11, 140.82, 132.25, 130.97, 129.52, 128.87, 126.26, 126.01, 114.98, 113.40, 61.67, 35.10, 14.16, **HR-MS(m/z):** 251.1283$(M)^+$, 160.0866

N-(3-(1H-benzotriazol-1-yl) propyl)-2-nitroaniline (2c): ^{1}H NMR: (500 MHz, $CDCl_3$, TMS), δ: 8.12(brs N-H), 7.37-6.72(m, 8H, ArH), 5.95 (t, 2H), 2.76(t, 2H), 2.17(m, 2H), **HR-MS (m/z)**: 297.12 $(M)^+$, 251.1299, 196.065, 160.0863

N-(3-(1H-benzotriazol-1-yl) propyl)-4-nitroaniline (2d) ^{1}H NMR: (500 MHz, $CDCl_3$, TMS), δ: 9.35 (brs N-H), 8.08-6.61 (m, 8H, ArH) 4.39 (t, 2H), 2.73(t, 2H), 1.38(m, 2H),

IR: ν_{max} (KBr cm^{-1}): 3362.22 (N-H), 3081.57(Ar C-H), 2923.53 (aliphatic C-H), 1630.68 (N-H) 1598.27(N-O) 1468.72(-CH_2-), **^{13}C NMR spectrum** (125 MHz, $CDCl_3$), δ: 167.74, 152.63, 139.07, 132.23, 131.00, 128.86, 126.37, 126.04, 120.03, 118.01, 113.38, 61.69, 35.06, 14.10, **HR- MS (m/z)**: 251.1283$(M)^+$ 196.0643,160.0865

Biological, ADMET and Molecular Docking Studies:

Biological studies:

Antibacterial Activity: All the synthesized compounds were screened for antibacterial activities, against gram-positive organisms like *Staphylococcus aureus*&*Bacillus licheniformis*& gram-negative organisms like *Escherichia Coli* & *Pseudomonas aeruginosa* by Well diffusion method using nutrient agar as cultural medium**(10)** (Fig 1). The nutrient agar and were prepared as per the composition, autoclaved for 121 ^{0}C for 15 lbs and cooled and poured on sterilized petri plates and allowed for solidification. DMSO is used as a solvent. Amoxycillin was used as standard for antibacterial screening. The plates were incubated for 37^{0}C for 24 hours for bacteria and results were recorded in terms of zone of inhibition in mm as shown in **Table 2.**

Table 2. Antibacterial activities of compounds 2a-2d (Zone of inhibition in mm)

		Gram(+ve) bacteria		**Gram(-ve)bacteria**	
Compound	**Conc. (MIC)**	***S. aureus***	***B. licheniformis***	***Pseudomonas sp***	***E. coli***
		Zone of inhibition in mm			
2a	25µg/ml	2	1	-	-
	50 µg/ml	5	2	-	-
	75 µg/ml	10	7	7	-
	100 µg/ml	11	9	10	2
2b	25µg/ml	-	1	-	-
	50 µg/ml	-	1	-	-
	75 µg/ml	-	1	-	1
	100 µg/ml	-	2	-	2

2c	25µg/ml	-	2	1	-
	50 µg/ml	-	3	2	-
	75 µg/ml	-	5	4	3
	100 µg/ml	3	9	7	5
2d	25µg/ml	-	-	-	2
	50 µg/ml	5	-	3	6
	75 µg/ml	8	1	5	7
	100 µg/ml	9	2	7	9
Amoxycillin	100 µg/ml	2	2	4	4

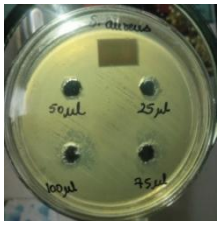
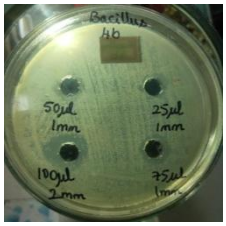
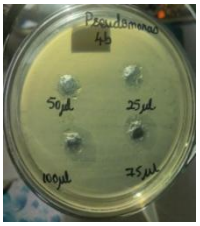
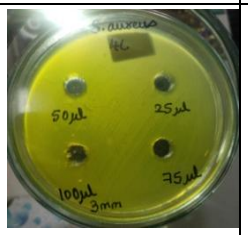
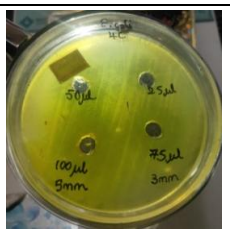
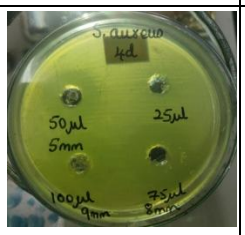
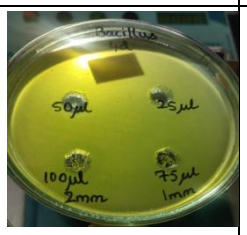
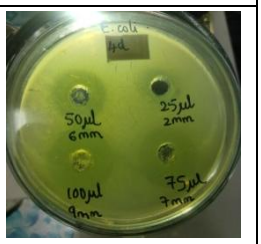
S.no	Compound	*S. aureus*	*B. licheniformis*	*P. aeruginosa*	*E. coli*
1	2a				
2	2b				
3	2c				
4	2d				

Fig. 1 Pictorial representation of Zone of inhibition of Compounds N-(3-(1*H*- benzotriazol-1-yl)propyl)-substituted aniline 2(a-d) against selected gram +ve and –ve bacteria strains.

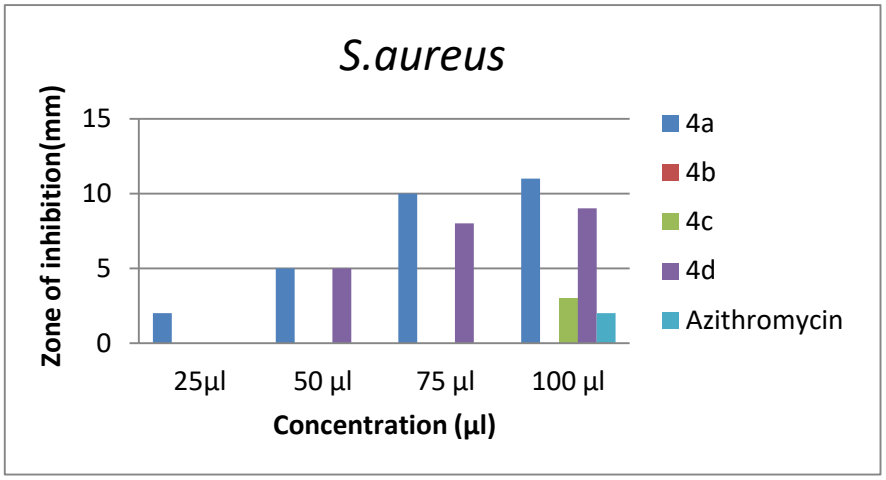


Fig. 2 Graphical representation of Antibacterial activity

Compounds 2a and 2d have shown significant results towards *S. aureus* and *Pseudomonas Sp (Table 2 and Fig 2).*

Molecular Docking Studies

As a part of research planning, in-silico study was carried out for all the compounds (2a-2d) of scheme 1 and the with respect to standard antibiotic Amoxycillin using ArgusLab 4.0.1 software with PDB ID 5TZ8: S. aureus TarS. The structure of S. aureus TarS with PDB ID 5TZ8 was obtained from https://www.rcsb.org. All the reported compounds were used as ligands to bind the pockets of the PDB ID 5TZ8 receptor bacterial agent respectively. This exercise was carried out to predict the tentative binding parameters of ligand-receptor complex beforehand. For the determination of structural interactions Biovia Discovery Studio v21.1.0.20298 was taken.

Ligand-receptor binding presentation of compounds

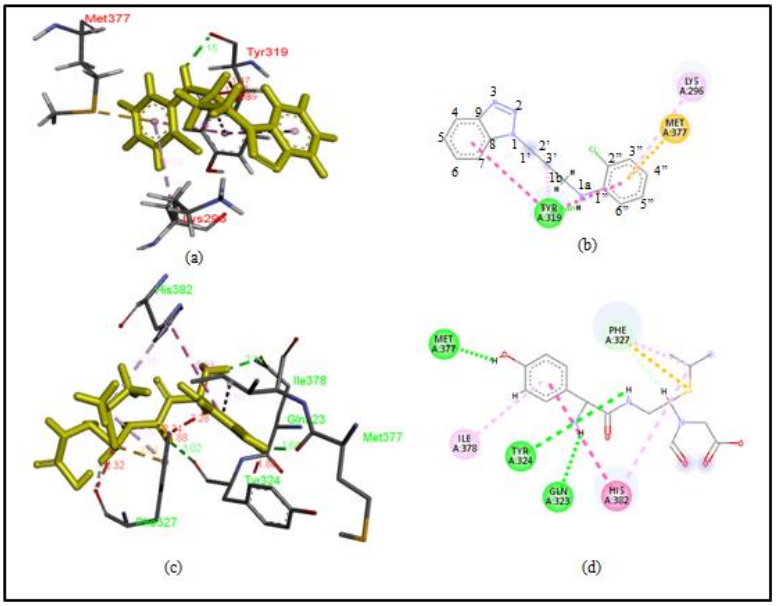


Fig. 3 (a) 3D structure of 2a (b) 2D structure of 2a (c) 3D structure of Amoxicillin (d) 2D structure of amoxicillin

In silico studies of all the reported compounds 2(a-d) mentioned in scheme 2 along with reference standard Amoxycillin were carried out using ArgusLab 4.0.1 software with 5TZ8: *S. aureus* TarS . Table 3 represented the details of docking studies of reported compounds 2(a-d) and the standard reference drug Amoxycillin including number of poses, docking score/binding energy, docking run time, binding amino acid, interacting atom of the ligand and H-bond distance. Fig 3 indicates various interactions of 5TZ8 receptor site with reported compounds. Fig 3 represents the interactions of 5TZ8 receptor site with standard antibiotic Amoxycillin. It has been observed that N-H at position 1a (linker part) of the compound 2a, 2c and 2d described conventional and carbon hydrogen bonding respectively. But N-H of compound 2a exhibited CHB (Conventional hydrogen bond) interaction with TYR 319 (2.15367 Å). This type of interaction was also found in Amoxycillin that displayed N-H (linker part) interaction also with TYR 324 (3.01696 Å). Compounds 2c and 2d have also displayed N-H interaction (linker part) intramolecular in 2c and Carbon Hydrogen bonding (2d) with ASP 349 (2.28986 A°). Such type of linker interactions were not found in compound 2b. Therefore it can be proposed that compound 2a has shown good docking scene due to the presence of N-H interaction with TYR as that of Amoxicillin.

Table 3 Molecular Docking Studies with 5TZ8: *S. aureus* TarS.

Compound	**Re-clustering the final poses**	**Docking score/ Binding energy:**	**Dock-ing run time in sec**	**Binding amino acid**	**H-bondin g (A°)**	**Fig.**
2a	139	-10.16 kcal/mol	196	640 GLY:Coil 640 GLY:Coil 641 GLY:Coil	2.64 2.25 2.54	2.4.3
2b	134	-10.12 kcal/mol	181	640 GLY:Coil 640 GLY:Coil 641 GLY:Coil	2.80 2.41 2.42	2.4.4
2c	74	-9.85 kcal/mol	233	647 ASN:Coil 640 GLY:Coil 640 GLY:Coil 641 GLY:Coil 650 TYR:Coil	2.36 2.81 2.40 2.53 2.90	2.4.5
2d	62	-9.11 kcal/mol	220	640 GLY:Coil 640 GLY:Coil 641 GLY:Coil 723 SER:Coil 954 THR:Coil 966 THR:Coil	2.52 2.38 2.70 2.97 2.36 2.90	2.4.6
Amoxicillin	53	-8.73 kcal/mol	146	891 ASP: Coil 997 THR:Coil 1444 ARG:β Strand 1441 GLN:Coil	2.90 2.79 2.55	2.4.7

				1440 GLY: Coil 1498 HIS:Coil 1494 ILE:Coil 1553 ILE: β Strand 1445 PHE: β Strand 1442 TYR:Coil 1443 TYR:Coil		

ADMET Studies:

Prediction of the physicochemical, pharmacokinetic, and drug-likeness properties of compound was achieved using the web-based tool ADMETlab 2.0 which offers a straightforward approach to the comprehensive, accurate, and efficient prediction of ADMET properties **[11].** The ADMET properties were predicted like absorption, plasma protein binding, fraction unbound in plasma, volume of distribution, clearance, environmental toxicity) and other properties and toxicological endpoints were predicted based on classification models (P-glycoprotein inhibitor and substrate, blood–brain barrier penetration, inhibitor/substrate of human cytochromes involved in the metabolism of chemicals, skin sensitization, hepatotoxicity, etc.)

Table 4 Predictions regarding the absorption, distribution, and excretion of the reported compounds of scheme 1: HIA—human intestinal absorption, P-gp—permeability glycoprotein, BBB—blood–brain barrier, CL—clearance

Compound	**HIA < 30%**	**Pgp Substrate**	**Pgp Inhibitor**	**BBB Permeation**	**CL (mL/min/kg)**
2a	0.005	0.005	0.292	0.812	8.283
2b	0.007	0.004	0.717	0.74	7.911
2c	0.007	0.001	0.017	0.649	7.726
2d	0.008	0.001	0.095	0.571	7.602

HIA

● Result interpretation: A molecule with an absorbance of less than 30% is considered to be poorly absorbed. Accordingly, molecules with a HIA >30% were classified as HIA- (Category 0), while molecules with a HIA < 30% were classified as HIA+ (Category 1). The output value is the probability of being HIA+, within the range of 0 to 1.

● Empirical decision: 0-0.3: excellent; 0.3-0.7: medium; 0.7-1.0: poor

Hence almost all the compounds are have revealed high intestinal absorption

Pgp-substrate

● Results interpretation: Category 0: Non-substrate; Category 1: substrate. The output value is the probability of being Pgp-substrate, within the range of 0 to 1.

● Empirical decision: 0-0.3: excellent; 0.3-0.7: medium; 0.7-1.0: poor

Pgp-inhibitor

. ● Results interpretation: Category 0: Non-inhibitor; Category 1: Inhibitor. The output value is the probability of being Pgp-inhibitor, within the range of 0 to 1.

● Empirical decision: 0-0.3: excellent; 0.3-0.7: medium; 0.7-1.0: poor

Hence all the compounds were not considered substrates and inhibitors of permeability glycoprotein.

BBB Penetration

● Result interpretation: The unit of BBB penetration is cm/s. Molecules with logBB> -1 were classified as BBB+ (Category 1), while molecules with logBB ≤ -1 were classified as BBB- (Category 0). The output value is the probability of being BBB+, within the range of 0 to 1.

● Empirical decision: 0-0.3: excellent; 0.3-0.7: medium; 0.7-1.0: poor

Compound 2a was not considered to be able to penetrate the blood-brain barrier where as compound 2c, 2d exposed a reasonable probability to penetrate the blood-brain barrier

Clearance of Drug

● Result interpretation: The unit of predicted CL penetration is ml/min/kg. >15 ml/min/kg: high clearance; 5- 15 ml/min/kg: moderate clearance; < 5: poor.

All the compounds have revealed optimal distribution and high clearance.

Table 5: Predictions regarding the metabolism of the reported compounds of scheme 1: CYP— cytochrome, s—substrate, i—inhibitor

Compound	CYP1A2-inh	CYP1A2-sub	CYP2C19-inh	CYP2C19-sub	CYP2C9-inh	CYP2C9-sub	CYP2D6-inh	CYP2D6-sub	CYP3A4-inh	CYP3A4-sub
2a	0.972	0.772	0.948	0.178	0.864	0.803	0.554	0.858	0.723	0.244
2b	0.979	0.539	0.959	0.155	0.849	0.739	0.652	0.761	0.496	0.253
2c	0.96	0.239	0.937	0.281	0.861	0.908	0.539	0.796	0.766	0.146
2d	0.929	0.112	0.919	0.138	0.78	0.899	0.566	0.854	0.542	0.192

CYP 1A2 / 2C19 / 2C9 / 2D6 / 3A4 inhibitor

CYP 1A2 / 2C19 /2C9 / 2D6 / 3A4 substrate

● Result interpretation: Category 0: Non-substrate / Non-inhibitor; Category 1: substrate / inhibitor. The output value is the probability of being substrate / inhibitor, within the range of 0 to 1

All the compounds illustrated reasonable to high probabilities to inhibit CYP isozymes and similarly reasonable to high probabilities to be substrates for aforesaid CYP isozymes.

Table 6 Predicted toxicological endpoints of the reported compounds of scheme 1: hERG— cardiotoxicity, HT—hepatotoxicity

Compd	hERG	HT	Skin Sensitization	Eye Irritation
2a	0.015	0.195	0.498	0.62
2b	0.028	0.129	0.717	0.592
2c	0.025	0.265	0.742	0.728
2d	0.047	0.21	0.847	0.864

hERG

● Result interpretation: Molecules with IC50 more than 10 μM or less than 50% inhibition at 10 μM were classified as hERG - (Category 0), while molecules with IC50 less than 10 μM or more than 50% inhibition at 10 μM were classified as hERG+ (Category 1). The output value is the probability of being hERG+, within the range of 0 to 1.

● Empirical decision: 0-0.3: excellent; 0.3-0.7: medium; 0.7-1.0: poor

None of the compound revealed high probabilities of producing cardiotoxicity by the blockage of the h-ERG potassium

channel.

HT

● Result interpretation: Category 0: H-HT negative (-); Category 1: H-HT positive (+). The output value is the probability of being toxic, within the range of 0 to 1.

● Empirical decision: 0-0.3: excellent; 0.3-0.7: medium; 0.7-1.0: poor

As predicted compounds are not considered for showing hepatotoxicity.

Skin Sensitization

. ● Result interpretation: Category 1: Sensitizer; Category 0: Non-sensitizer. The output value is the probability of being toxic, within the range of 0 to 1.

● Empirical decision: 0-0.3: excellent; 0.3-0.7: medium; 0.7-1.0: poor

Furthermore, compounds 2a, 2b, 2c and 2d revealed a reasonable probability of producing skin sensitization

Eye Irritation

● Result interpretation: Category 1: corrosives / irritants chemicals; Category 0: non-corrosives / non-irritants chemicals. The output value is the probability of being toxic, within the range of 0 to 1.

● Empirical decision: 0-0.3: excellent; 0.3-0.7: medium; 0.7-1.0: poor

Compounds 2b and 2d revealed a reasonable probability of producing Eye irritation.

Table: 7: Predicted Medicinal Chemistry/Drug likeliness aspects of reported compounds of scheme 1

Compound	Lipinski rule
2a	Accepted
2b	Accepted
2c	Accepted
2d	Accepted

Lipinski Rule

● Content: MW≤500; logP≤5; Hacc≤10; Hdon≤5

● Results interpretation: If two properties are out of range, a poor absorption or permeability is possible, one is acceptable.

● Empirical decision: < 2 violations : excellent ; ≥2 violations: poor

All the compounds are following the Lipinski's rule of druglikeliness.

3. RESULT AND DISCUSSION:

6.1. Biological evaluation:

As per the data shown it is exhibited that compound 2a has shown antibacterial activity towards Gram +ve bacteria *S. aureus* and *B.licheniformis*with MIC 50µg/ml and 75µg/ml respectively. Similarly compound 2b has shown antibacterial activity against *B.licheniformis* with MIC 25µg/ml. Compound 2c has shown the activity towards*S. aureus* and *B.licheniformis* with MIC100µg/ml and 75µg/ml respectively whereas Compound 2d has shown activity towards *S.aureus* and *B.licheniformis* with MIC 50µg/mland 75µg/ml respectively. In case of Gram -ve bacteria there is no significant activity by 4a and 4b however compound 4c with MIC 50µg/mland 75µg/ml and 4d has shown better activity with MIC 50µg/mltowards Gram –ve bacteria i.e *E. coli* and *P. aeruginosa* respectively. The following conclusion was derived:

All the compounds have shown significant results as compared to Standard reference drug.

Compounds 2a which is an ortho-chloro substituted compound has shown better activity towards Gram +ve bacteria as compared to Gram –ve strains.

Moreover electron withdrawing groups like –Cl, -NO_2 have shown promising activity against the selected bacteria. Like in

case of ortho chloro substituted compound 2a and ortho and p-nitro substituted compound 2c and 2d respectively has shown good activity towards both Gram +ve and Gram –ve strains**.**

6.2. ADMET Studies: Similarly these compounds have revealed high intestinal absorption and were not considered substrates and inhibitors of permeability glycoprotein. Major compounds were not considered to be able to penetrate the blood-brain barrier disclosed optimal to good clearance as per the computational studies by ADMETlab2.0 web software. All the compounds illustrated reasonable to high probabilities to inhibit CYP isozymes and similarly reasonable to high probabilities to be substrates for aforesaid CYP isozymes. None of the compound revealed high probabilities of producing cardiotoxicity by the blockage of the h-ERG potassium channel. Also as predicted they are not considered for showing hepatotoxicity Furthermore, compounds revealed a reasonable probability of producing skin sensitization and eye irritation. It is also observed that all the compounds are following the Lipinski's rule of drug likeliness

Correlation of Molecular Docking Studies and Antimicrobial Studies

The results of the molecular docking study of the synthesized compounds with protein target S. aureus (PDB ID: 5TZ8) reveal that their binding affinity was in the range of – 10.16 to – 9.11 kcal/mol. Compound 2a(N-(3-(1H-benzotriazol-1-yl) propyl)-2-chloroaniline)shows the best binding affinity of – 10.16 kcal/mol with protein target S. aureus (PDB ID: 5TZ8) as shown in Table 2.4.1. Among the synthesized compounds, compound 2a was found to show good inhibiting activities against *S. aureus*with the minimum inhibitory concentration of 11 mm as shown in Table 2.3.1. Compound 2d (N-(3-(1H-benzotriazol-1-yl) propyl)-4-nitroaniline)on the other hand gave a good binding affinity of – 9.11 kcal/ mol. So, it can be concluded that compound 2a has shown significant results in docking analysis as well as in biological activities. Therefore,it is recommended that further studies should be carried out in this context to see the future perspective.

4. CONCLUSIONS

Here, we have synthesize novel derivatives of benzotriazole N-(3-(1*H*-benzotriazol-1-yl) propyl) substituted aniline 2(a-d). The antibacterial activities of the synthesized compounds were tested against two Gram-positive and two Gram-negative bacterial strains. Most of the newly synthesized compounds have displayed significant results towards biological activities and docking analysis But Compound 2a was depicted as the most efficient compound based on biological and docking studies. The N-H at position 1a of compound 2a has shown interaction with the same amino acid as that of the reference. In correspondence with biological activities of the reported compounds the ADMET studies are quite relatable. Thus it can be interoperated that the compound 2a is showing broad spectrum biological activities comparable to the reference drug Amoxicillin, shows high binding energies and greater ligand receptor interactions and have shown more favorable ADMET profiles, hence are suitable for synthesis.

5. DECLARATIONS

Conflict of interest: No competing interest.

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