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Research Article

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Molecular Docking of Some Monoazaphenothiazine Derivatives as Antimicrobial Agents

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Abstract

Purpose: *This study was carried out to determine the antibacterial activities and molecular docking interactions of some aniline derivatives of monoazaphenothiazine earlier reported.*

Methods: *The antimicrobial activities were determined by agar well diffusion method on Bacillus SPP, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli. The 3D crystal structures of cryptogein complexed with cholesterol molecule, (PDB Code: 1LRI) and glucosamine 6-phosphate synthase (2VF5) complexed with glucosamine 6 phosphate (PDB Code: 2VF5) used for the present molecular docking studies were retrieved from the Protein Data Bank (PDB).*

Results: *Compound 21 was most sensitive to Staphylococcus aureus with an MIC of 0.0625 mg/ml while compound 19 was most sensitive to Bacillus spp (MIC = 0.0625 mg/ml). Compound 22 gave the highest binding affinity with 2VF5 (11.51 kcal/mol). Compounds 21 and 23 showed significant binding affinity for 1LRI comparable to the standard drug fluconazole.*

Conclusion: *The aniline derivatives of monoazaphenothiazines were found to possess interesting antimicrobial activities. The in silico studies showed that the compounds had strong binding interactions with the drug receptors.*

Keywords: Antibacterial Activity, Monoazaphenothiazines, Molecular Docking

Introduction

Monoazaphenothiazines possess pharmaceutical properties due to the presence of phenothiazine an active component in antimicrobial, antifungal and antibacterial agents [1-3]. Moreover, phenothiazines and its derivatives have prompted a lot of research due to its vast application as drugs, dyes, pesticides, industrial antioxidants. Consequently, there have been variations in their structures which have enabled the synthesis of both linear and non-linear derivatives [4]. It is worthy to note that the basic nitrogen of phenothiazine rings is responsible for its pharmacological activities because of its tendency to donate electrons to biological receptors by a charge transfer mechanism. Consequently, synthesis of aza-analogues of phenothiazines have received a great attention by chemists [5]. Phenothiazines make up one of the largest classes of organic compounds in official compendia. More than four thousand compounds have been presently synthesized and about 100 have been used in clinical practice [6]. Linear phenothiazines derivatives are very useful drugs which are widely used as tranquilizers in psychiatric treatment [7]. The inventions and use of phenothiazine derivatives into the treatment of mental diseases has changed the modern psychiatry. This innovation has improved the life style of patients and allowed rapid development of ambulatory system of treatment for such sickness [8]. The common use of phenothiazine has prompted the need for quick and reliable methods for quality control of phenothiazine pharmacenticals and monitoring them in clinical

samples [8,9]. Phenothiazines, especially its linear derivatives are interesting from analytical perspective due to their peculiar structure which involves the presence of chemically active sulphur and nitrogen atoms in positions 5 and 10 and substituents in position 2 and alkyl amine side chains at N10 atom [10]. Phenothiazines are antipsychotic drugs which work on the positive symptoms of psychosis such as delusions, hallucinations, and looseness of association, disorganized speech and bizarre behaviour [3]. On the other hand, *in silico* studies which simply mean performed on computer or through computer simulation was used to characterize biological experiments carried out mainly in a computer [11]. It helps to predict how drugs interact with the body and with pathogens. *In silico* analysis involves variety of techniques such as bacterial sequencing techniques, molecular modeling and whole cell simulations [12]. Additionally, the subjection of monoazaphenothiazines to *in vitro* studies, which means studies conducted with components of an organism that have been isolated from their normal biological environment such as microorganisms, cells or biological molecules has also helped in the characterization of specific adsorption, metabolism, distribution and excretion of a drug and even the chemicals inside a particular living organism [1,13].

Two druggable targets have been chosen to study the antimicrobial activity of the compounds *in silico*. They are cryptogein (PDB ID: 1LRI) and glucosamine 6phosphate (PDB ID: 2VF5) representing fungal and bacterial drug targets respectively.

Cryptogein produced by the phytoparasitic oomycete *Phytophthora ryptogea*, is a small 10 kDa elicitor which has a sterol carrier activity [14]. It has a large inner non-specific hydrophobic binding cavity where a number of 3β-hydroxy sterols can be fitted. Lascombe, et al. has proposed that this protein acts as a sterol shuttle helping the pathogen to grow and complete its life cycle [14]. Cryptogein has become an important drug target as is inhibition will cause the death of the pathogen. The 3D crystal structure of cryptogein with its co-crystallized ligand is shown in Figure 1a.

Glucosamine-6-phosphate synthase (GlcN-6-P) is an important drug target in antimicrobial chemotherapy. Its biochemical functions ultimately leads to the formation of uridine 5′diphospho-N-acetyl-d-glucosamine (UDP-GlcNAc). UDP-GlcNAc is an important component of peptido glycan layer mostly found in the bacterial and fungal cell walls [15]. Disruption of this process will be detrimental to the organism. Ezeokonkwo, et al. have detailed the roles and mechanism of actions of glucosamine6-phosphate synthase [16]. The 3D crystal structure of glucosamine-6-phosphate synthase with its co-crystallized ligand is shown in Figure 1b.

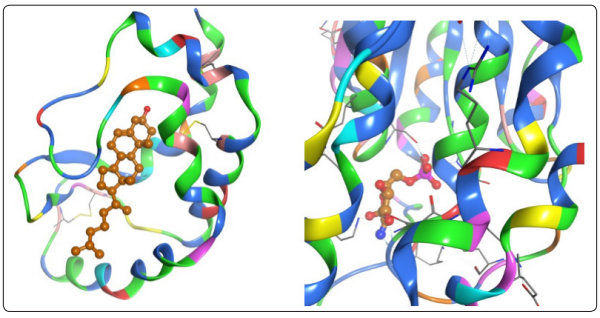


Figure 1: 3D crystal structures of (a) cryptogein (1LRI) complexed with cholesterol molecule and (b) glucosamine 6-phosphate synthase (2VF5) complexed with glucosamine 6-phosphate

Experimental

All starting reagents were obtained from Sigma-Aldrich and were used without further purification. Melting points of the compounds synthesized were determined using electro thermal melting point apparatus in open capillaries and are uncorrected. Ultraviolet-visible spectra were recorded on a UNICO-UV2102 PC spectrophotometer (Pure and Industrial Chemistry Department, UNN) using matched 1 cm quarts cells. The solvent was ethanol and absorption maxima are given in nanometers (nm); the figures in parentheses are the log E values. Infrared spectra data was obtained on a Magna–IR system 750 spectrophotometer (NARICT, Zaria, and Kaduna State) using KBr discs and absorptions were given in per-centimeter (cm^{-1}). Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) were determined using varian NMR mercury 200BB spectrophotometer (Obafemi Awolowo University, Ile Ife). Chemical shifts are reported in δ scale (neat). Elemental analysis was carried out to determine the percentage abundance of the elements present.

Procedure for the synthesis of 3-chloro-10H-pyrido [3,2-*b*][1,4] benzothiazine (19)

The compounds were synthesized and reported by Egbujor, et al. [17]. 2-aminothiophenol (2 g, 18 mmol) was placed in the reaction flask containing (1.79 g, 44 mmol) of potassium hydroxide in 50ml of water. The mixture was warmed until the material dissolved at a temperature of about 85°C. 2,3,5-Trichloropyridine (2.97 g, 20 mmol) in 50ml of DMF was added in drops during a period of 15min. The entire mixture was refluxed with stirring for 4hrs. It was later poured into a beaker, diluted with water to the 500ml mark and cooled, filtered and the residue recrystallized from ethanol. Greenish yellow crystals of 3-chloro-10*H*-pyrido [3,2-b][1,4]benzothiazine (4.81g, 52% yield).

Melting point is 161-161.6oC. IR(KBr), Vmax 3439cm^{-1} (N-H stret), 3050cm^{-1} (Ar-C-H), 1616 cm^{-1} (C=C of aromatic rings), 1483-1418cm^{-1} (C=N stret), 1350cm^{-1} -1306cm^{1}(monosubstituted-Cl), 757cm^{-1}(C-S-C). UV-Visible, λmax (ethanol), 30932nm(logE=2.491), 292nm(2.465), 361nm(2.557). ^{1}H-NMR ($CDCl_3$), 6.30-8.06δ (6H,m,Ar-H), 4.1δ (^{1}H,s,N-H). ^{13}C-NMR(CDC_{13}), 115.4-147.5δ (11C,m,Ar-C). Analysis calculated for $C_{11}H_7N_2ClS$; C, 56.31, H,3.01, N,11.91, Cl,15.15, S,13.66. Analysis found: C, 56.41, H, 3.02, N,11.79, Cl,15.21, S,13.62.

Coupling procedure for the synthesis of aniline derivatives of 3-chloro-10*H*-pyrido[3,2-*b*][1,4]benzothiazine

Palladium acetate was pre-activated in a 100ml three neck flasks for 2min at 80°C, 3-chloro-10H-pyrido[3,2-b][1,4]benzothiazine, potassium carbonate, amines (aniline, 3-nitroaniline, 4nitroaniline, 4-hydroxyaniline) and 2ml of tertiary butanol were added. On stirring for 2 hours at 110°C and recrystallized from ethyl acetate, the following compounds (20-23) were obtained via Buchwald-Hartwig cross-coupling reaction.

N-phenyl-10*H*-pyrido[3,2-*b*][1,4]benzothiazin-3-amine (20)

It was obtained as a dark tan solid in (0.26 g, 95 %) yield, melting at 117-119°C . IR (KBr), Vmax 3440-3061cm^{-1} (N-H stret), 3210-2951cm^{-1} (C=C-H), 1611cm^{-1} (C=N), 1361cm^{-1} (CN), 746cm^{-1} (C-S-C). UV-Visible, λmax (ethanol), 238nm(logE=2.376), 261.9nm(2.419), 308nm(2.488), 409.9nm(2.64). ^{1}HNMR($CDCl_3$),6.30-7.78δ, (9H,m,Ar-H), 4.1δ(2H,s,N-H). ^{13}C-NMR ($CDCl_3$),112.7-147.07 (16C,m,Ar-C). Analysis calculated for $C_{17}H_{13}N_3S$: C, 70.11, H, 4.48, N, 14.44, S,11.01. Analysis found: C,69.90, H,4.51, N,14.49, S,11.14.

N-(4-nitrophenyl)-10H-pyrido[3,2-b][1,4]benzothiazin-3-amine (21)

It was obtained as a grey solid in (2.60g, 97%) yield, melting at 97-99°C. IR (KBr), Vmax 3071cm^{-1}(N-H), 3230-2941cm^{-1} (C=C-H, C-H stret), 1621cm^{-1}(C=N) 1311cm^{-1} (C-N), 1308cm^{-1} (-NO_2), 748cm-1(C-S-C). UV-Visible, λmax (ethanol), 210.5nm (log E=2.324), 247nm(2.392), 311nm(2.4912), 370.5nm(2.569), 497.9nm (2.70). ^{1}HNMR ($CDCl_3$), 6.30-7.95δ (8H, m, Ar-H), 4.1δ (2H, s, N-H). ^{13}C-NMR ($CDCl_3$), 112.8-152.9δ (15C, m, Ar-C). Analysis calculated for $C_{17}H_{12}N_4SO_2$: C, 60.72, H, 3.58, N, 16.68, S, 9.53. Analysis found: C, 60.81, H, 3.62, N, 16.61, S, 9.49.

4-(10H-pyrido[3,2-b][1,4]benzothiazin-3-ylamino)phenol (22)

It was obtained as a resin in (0.27g, 96 %) yield, melting p at 117-119°C. IR (KBr), Vmax, 3440-3071cm^{-1} (N-H, O-H stret), 3070-3941cm^{-1} (C=C-H. C-H stret), 2391cm^{-1} (C=C, C=N) 1361cm^{-1} (C-N), 747cm^{-1} (C-S-C).UV-Visible λmax (ethanol), 237.5nm (log E =2.376), 307.5nm (2.489), 379nm (2.578), 496.3nm (2.693). ^{1}HNMR ($CDCl_3$), 6.29-7.79δ, (8H, m, Ar– H), 4.1δ (2H, s,N-H), 5.1δ(1H,s,O-H). ^{13}C-NMR ($CDCl_3$), 112.7-147.5δ (15C, m, Ar-C). Analysis calculated for $C_{17}H_{12}N_3SO$: C, 66.68, H, 3.93, N, 13.74, S, 10.47. Analysis found: C, 66.71, H, 3.81, N, 13.82, S, 10.51.

N-(3-nitrophenyl)-10H-pyrido[3,2-b][1,4]benzothiazin-3-amine (23)

It was obtained as a grey solid in (2.76 g, 96%) yield, melting point 87-89oC. IR (KBr), Vmax 3770-3085cm^{-1}(N-H), 3210-2951cm^{-1}(C-H stret), 1620-1511cm^{-1} (C=C, C=N stret), 1341cm^{-1}(C-N), 1338cm^{-1}(-NO_2), 743cm^{-1}(C-S-C). UV-Visible λmax (ethanol), 2I3nm(log E =2.327), 248.3nm (2.396), 308nm (2.488), 361nm (2.557). ^{1}HNMR ($CDCl_3$), 6.30-7.78δ(10H,m,Ar-H), 4.0δ(2H,s,N-H). ^{13}C-NMR ($CDCl_3$), 110.2-149.3δ (17C,m,Ar-C). Analysis calculated for $C_{17}H_{12}N_4SO_2$: C, 60.72, H, 3.58, N, 16.68, S, 9.53. Analysis found: C, 60.80, H,3.49, N,16.65, S,9.49.

Preparation of the Inoculums

The isolated organisms of Bacillus spp, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli were obtained and the analysis was carried out at Department of Microbiology Laboratory Renaissance University, Enugu, Nigeria. The strains of the organisms involved were propagated on nutrient agar plates the temperature was maintained at 4°C. The isolates were sub-cultured in nutrient broth at a temperature of 37°C for about 8hrs before antibacterial testing.

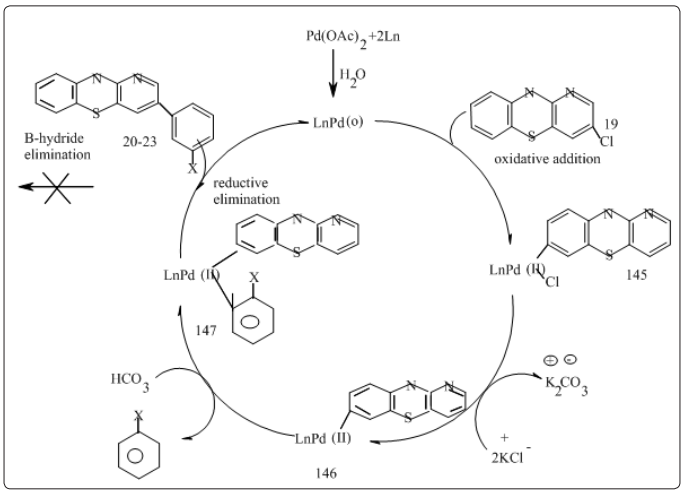
Antibacterial Sensitivity Testing of Compounds

The antibacterial activity of the synthesized compounds was determined using well diffusion method on Mueller-Hinton agar (MHA) described by Mueller, et al. [18]. Sensitivity test agar were inoculated with 0.1ml of overnight culture of the bacteria strain involved. The inoculated agar plates were properly labelled after they got dried. Uniformed wells were bored in the inoculated nutrient agar with the help of 6mmplastic cork borer. 100µl of 10 mg/ml each of the test compound solution was introduced into each well by the help of micropipette. The positive standard ciprofloxacin was tested and the plates were allowed on the bench for 30min so that the compound can diffuse into the agar. Then the plates were incubated at 37°C for 24hrs after which the monitored for possible inhibition zones around the wells and the zones diameters were measured to the nearest whole number millimeter using meter rule.

Results and Discussion

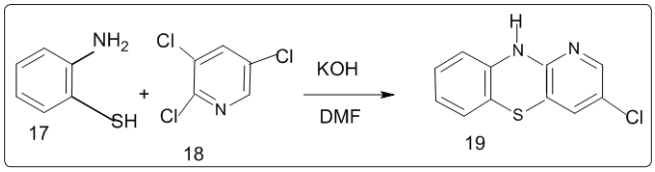
Chemistry

The palladium catalyzed syntheses of 3-chloro-10*H*-pyrido[3,2-*b*][1,4]benzothiazine (19) as an aryl chloride intermediate by reaction of 2-aminophenol with 2,3,5-trichloropyridine and its derivatives by condensation with substituted aniline derivatives via Buchwald-Hartwig amination reaction are described. Reaction steps involved in the syntheses are: (1) oxidative addition of Pd$^{(0)}$ to the aryl halide to form Pd$^{(II)}$ aryl halide. (2) Pd$^{(II)}$-amine formation in which the Pd$^{(II)}$ aryl amine is formed by direct displacement of the halide by the amine via a Pd$^{(II)}$- alkoxide intermediate. (3) Reductive elimination that results in the formation of the desired C-N bond and the Pd$^{(o)}$ catalyst is regenerated. The first step in the reaction mechanism is the oxidative addition of palladium (0) to 3-chloro-10*H*-pyrido [3,2-*b*][1,4] benzothiazine (142) to for compound 145. In the second step, the palladium (II)-aryl amide was formed by direct displacement of the chloride group. Compound 146 reacts with aniline to give Palladium (II)-aryl amine(147) which results in the formation of the various anilino derivatives of 3chloro-monophenothiazine(20-23) and the catalyst was regenerated as shown below in scheme 1.



Scheme 1: Reaction mechanism for the synthesis of anilino derivatives of 3-chloro-10*H*-pyrido [3,2-*b*] [1,4] benzothiazine represented in a catalytic cycle

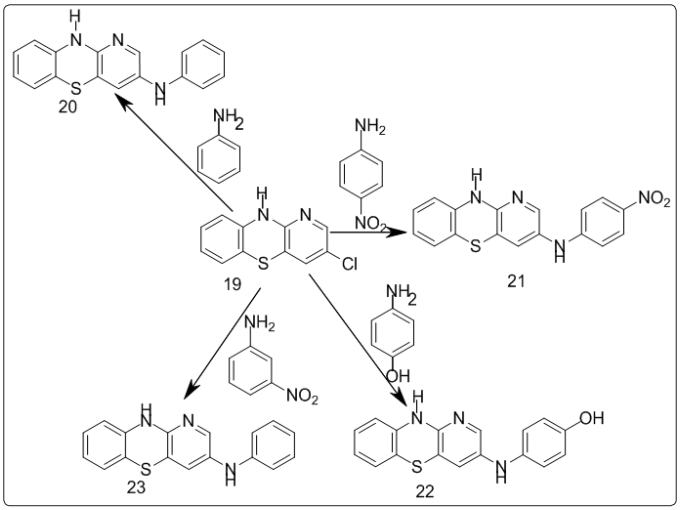
3-chloro-10*H*-pyrido[3,2-*b*][1,4]benzothiazine (19) served as an important aryl chloride intermediate in the synthesis of aniline derivatives of linear monoazaphenothiazine. The synthesis of the intermediate compound (19) was achieved by the reaction of 2-aminothiophenol (17) and 2,3,5-trichlorophyridine (18) in the presence of potassium hydroxide and DMF as shown below in scheme 2.



Scheme 2: synthesis of 3-chloro-10*H*-pyrido[3,2-*b*][1,4] benzothiazine intermediate

When a mixture 3-chloro-10*H*-pyrido[3,2-*b*][1,4]benzothiazine (19), aniline derivatives and K_2CO_3 reacted with an activated palladium catalyst solution, the aniline derivatives of 3-chloro-

10*H*-pyrido[3,2-*b*][1,4]benzothiazine (20-23) were obtained in excellent yields (90-96%) as shown below in scheme 3.



Scheme 3: Aniline derivatives of 3-chloro-10*H*-pyrido[3,2-*b*][1,4]benzothiazine (20-23)

The assigned structure is supported by spectral analysis. The ^{1}HNMR and ^{13}CNMR, IR and UV agreed with the structure of the compounds.

Antibacterial Activity

The new derivatives of 3-chloro-10*H*-pyrido[3,2-*b*][1,4]benzothiazine were screened for their antibacterial activities against some pathogenic bacteria viz Bacillus spp, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli using agar cup diffusion method. The antibacterial investigation shows that the monoazaphenothiazine derivatives has antibacterial activity (Table 1).

Table 1: Minimum Inhibitory Concentrations (mg/ml) of Samples

		MIC OF SAMPLES (mg/ml)				
S/N	**Microorganism**	**19**	**20**	**21**	**22**	**23**
1.	*Bacillus cereus (gram +ve)*	0.0625	0.125	0.125	-	-
2.	*Staphylococcus aureus (gram +ve)*	0.125	-	0.0625	0.125	0.125
3.	*Escherichia coli (gram -ve)*	0.0625	0.0625	0.125	0.250	0.0625
4.	*Pseudomonas aeruginosa (gram -ve)*	0.125	0.0625	0.250	0.125	-

Key: resistant (no inhibition)

Escherichia coli was found to be sensitive to all the synthesized derivatives of monoazaphenothiazine (19-23). However, monoazaphenothiazine derivatives exhibited different MICs against different strains. Notably, compounds 19 and 21 showed significant anti-bacterial activity against the tested microorganisms. Compound 21 was most sensitive to *Staphylococcus aureus* with an MIC of 0.0625 mg/ml while compound 19 was most sensitive to *Bacillus spp* (MIC = 0.0625 mg/ml). It can be inferred, therefore, that compounds 19 and 21 have broad spectra of activities against gram +ve and gram -ve organisms.

In silico Studies

Molecular Modeling Studies

The 3D crystal structures of cryptogein complexed with cholesterol molecule, (PDB Code: 1LRI) and glucosamine 6-phosphate synthase (2VF5) complexed with glucosamine 6 phosphate (PDB Code: 2VF5) used for the present molecular docking studies were retrieved from the Protein Data Bank (PDB), (http://www.pdb.org) database. The structures of the ligand's ciprofloxacin (CID: 2764) and fluconazole (CID: 3365) were retrieved from Pubchem database (https://pubchem.ncbi.nlm.nih.gov). MMFF94 force field was used for energy minimization of the ligand molecules. The prepared compounds were then subjected to interact with each of the 2 receptors through molecular docking. The protocol facilitates flexible compound docking for various compound conformers within the rigid receptor. The Best conformation for each compound was chosen and the interaction was visualized in Discovery studio.

The physicochemical properties to evaluate the drug-likeness of the synthesized compounds are shown in Table 2. Lipinski's rule of five (Ro5) is vital in assessing the drug-likeness of a molecule [19]. A molecule must have molecular weight value of ≤ 500, hydrogen bond donor ≤ 5, hydrogen bond acceptor ≤ 10, and partition coefficient (Log P) value ≤ 5. Violation of more than one parameter may pose a challenge to the bioavailability of the molecule in case of oral formulation. From the results in Table 2, the synthesized compounds are in agreement with (Ro5). The TPSA, a reflection of the ligand hydrophilicity, is very vital in protein-ligand interaction. NoRB ≤ 10 and TPSA ≤ 140 Å2 would have a high probability of good oral bioavailability in rat [20].

Drug-likeness evaluation

Table 2: Drug-Likeness Parameters of Synthesized Compounds

Comp	HBA	HBD	NoRB	logP(o/w)	logS	TPSA	MW	LNV
20	1	2	2	3.93	-4.28	36.95	291.38	0
21	1	2	3	3.87	-5.07	82.77	336.38	0
22	2	3	2	3.63	-3.92	57.18	307.38	0
23	1	2	3	3.91	-5.07	82.77	336.38	0
19	1	1	0	3.14	-3.40	24.92	234.71	0
Fluco	5	1	3	4.63	-3.77	75.69	304.26	0
Cipro	3	2	3	1.04	-2.71	77.46	332.35	0

MW: molecular weight; HBA: hydrogen bond acceptor; HBD: hydrogen bond donor; TPSA: total polar surface area; NoRB: number of rotatable bond; LNV: Lipinski's number of violations; Fluco: Fluconazole; Cipro: Ciprofloxacin.

Molecular docking

The calculated free binding energy after molecular docking is given in Table 3. Our compounds showed a strong binding affinity with the receptors. Compound **22** gave the lowest binding energy (the highest binding affinity) with **2VF5** (11.51 kcal/mol) compounds **21** and **23** showed significant binding affinity for **1LRI** compared to fluconazole. The binding poses of these compounds (**21** and **22**) in the binding cavities of the drug receptors are shown in Figures 2, 3, 4.

Table 3: Free binding energy of synthesized compounds

		ΔG (kcal/mol) for:	
S/N	Comp	1LRI	2 VF 5
1	20	-10.23	-10.30
2	21	-11.22	-10.58
3	22	-10.62	-11.51
4	23	-11.85	-10.57
5	19	-9.18	-8.68
6	fluconazole	-9.98	ND
7	ciprofloxacin	ND	-15.25

ND = Not determined

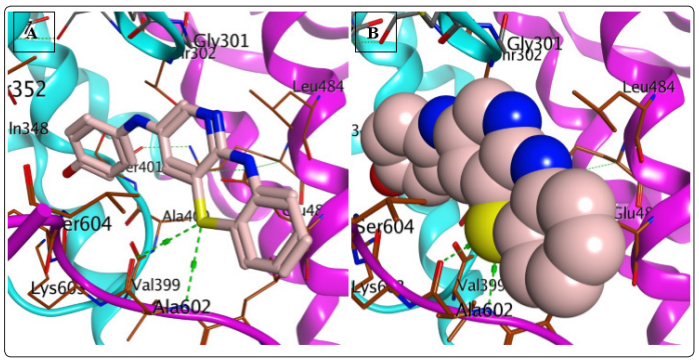


Figure 2: Binding pose of compound 22 in the binding cavity of 2VF5 (A) stick representation (B) CPK representation (green dotted lines represent hydrogen bonds)

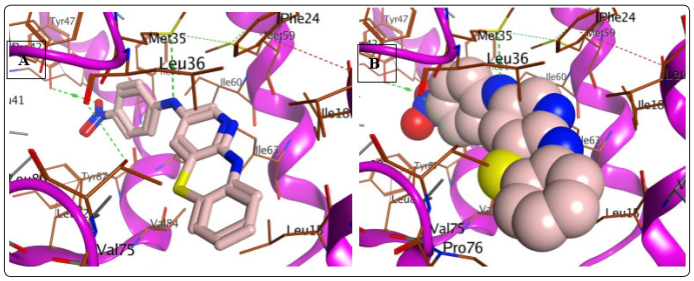


Figure 3: Binding pose of compound 21 in the binding cavity of 1LRI (A) stick representation (B) CPK representation (green dotted lines represent hydrogen bonds)

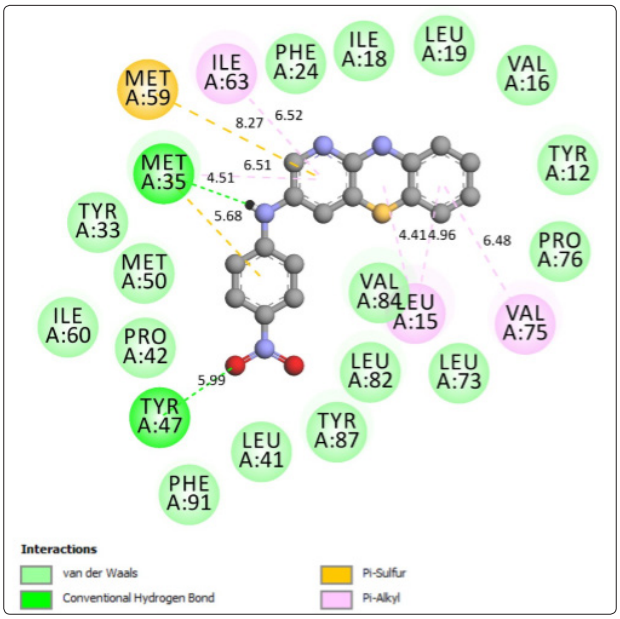


Figure 4: Binding interactions of compound 21 with amino acid residues of 1LRI

The phenolic OH group of Tyr47 is involved in a strong hydrogen bond with the O-atom of the nitro group of compound 22 (d = 5.99 Å) (Figures 3 & 4, Table 4). This finding collaborated with an in-depth research on the structure of the cryptogein-cholesterol complex: a close-up view of a sterol carrier protein (SCP) active site conducted by Lascombe, et al. [14]. They also observed that when

Tyr47 is mutated, the protein loses most of its ability to capture sterols. This shows that Tyr47 is very essential for inhibitors to bind to the receptor. The cavity is highly hydrophobic, mostly lined by leucines (Leu15, Leu18, Leu19, Leu41, Leu73, Leu82) isoleucines (Ile60 and Ile63) and other similar residues (Tyr12, Val16, Val84, Ala38, Phe24, Met50, Pro76, Phe91, Ala88).

Table 4: Binding interactions of compound 21 with amino acid residues of 1LRI

Ligand atom	Receptor	Interaction	Distance of interaction (Å)
O 24	TYR 47	H-bonding H-	5.99
6-ring (aromatic)	MET 35	bonding π-	4.51
	MET 35	sulphur π-	5.68
	MET 35	alkyl π-	6.51
	MET 59	sulphur π-	8.27
	ILE 63	alkyl π-alkyl	6.52
	LEU 15	π-alkyl	4.96
6-ring (non-aromatic)	VAL 79	π-alkyl	6.48
			4.41

Conclusion

The aniline derivatives of monoazaphenothiazines (20-23) were screened against some gram +ve and gram –ve bacteria and were found to possess interesting antimicrobial activities. Compound 21 was most sensitive to *Staphylococcus aureus* with an MIC of 0.0625 mg/ml while compound 19 was most sensitive to Bacillus spp (MIC = 0.0625 mg/ml). Compound 22 gave the lowest highest binding affinity with **2VF5** (11.51 kcal/mol). Compounds **21** and **23** showed significant binding affinity for **1LRI** comparable to the standard drug fluconazole. The *in silico* studies showed that all the compounds complied with Lipinski's rule and therefore are druggable. They also had a strong binding interaction with the drug receptors. The compounds were found to be potential antimicrobial agents.

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