Synthesis, Biological Evaluation and Docking Studies of Some Novel (E)-(4-(2-(benzo[d]thiazol-2-yl)hydrazono)methyl-2,6-diphenylpiperidin-1-yl)(phenyl)methanone Derivatives

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ABSTRACT. A series of biologically active some novel (E)-(4-(2-(benzo[d] thiazol-2-yl)hydrazono)-3-methyl-2,6-diphenylpiperidin-1-yl)(phenyl)methanone derivatives (**14-19**) were synthesized and characterized by IR, ^1H NMR, ^{13}C NMR and HSQC spectral analysis. Molecular docking studies were also carried out for compounds **14-19** using the crystal structure of Ribonucleotide Reductase to disclose the binding mode and orientation of target molecule into the active site of respective receptors. The synthesized compounds were also screened for their antimicrobial activity against selected panel strains Antimicrobial studies revealed that the synthesized compound **14** exhibited noticeable activity against *B.cerus* whereas compounds **17** and **18** displayed appreciable activity against *K.pneumoniae*. Bromo substituted phenyl group at C-2 and C-6 of piperidine ring showed excellent antibacterial activity against *K.pneumoniae* and *B.cerus* at MIC of 25 $\mu\text{g/ml}$. The antifungal studies revealed that compounds **17**, **18** and **19** exposed significant antifungal activity against the pathogenic strains used in this study. In particular compound **19** are showed excellent antifungal activity against *C.albicans* and *A.niger*.

1. Introduction

Nitrogen containing heterocycles always signified a subject of great interest due to their ubiquity in nature and massive presence as part of the skeletal backbone of many therapeutic agents [1]. Piperidin-4-one nucleus are interested to study because of their broad spectrum of biological activities such as antibacterial and antifungal [2-3] local anesthetic [4] antiviral [5] antitumor [6]. The utility of substituent's at second, third and sixth positions, particularly aromatic substituent at second and/or sixth positions with regard to its biological activity have well documented by many workers [7-8]. Hydrazone derivatives have been claimed to exhibit appreciable antimicrobial [9-12] antitubercular [13] anticonvulsant [14] antiviral [15] and anti-inflammatory [16] activities.

Ribonucleotide Reductase (RNR) is a ubiquitous cytosolic enzyme in the cell, responsible for converting ribonucleotides into deoxyribonucleotides, the eventual substrates for DNA polymerase [17-19] and also repairs DNA in all living cells [20]. In mammalian cells, this enzyme contains two dissimilar protein components (R1 and R2) which are encoded by two different genes located on different chromosomes [21]. Protein R1 is a homodimeric structure, with a molecular mass of 168kDa and has substrate and allosteric effector sites that control enzyme activity and substrate specificity. Protein R2 is a homodimer with a molecular mass of 88kDa and forms two equivalent dinuclear iron centers that stabilize a tyrosyl free radical, required for the initiation of electron transformation during catalysis. R1 and R2 proteins interact at their C-terminal ends to form an active holoenzyme [19].

On the basis of literature importances, we have the impetus to synthesize a number of hydrazones of the synthetically accessible 2,6-diarylpiperidin-4-one derivatives with a prospect to incorporate diverse bioactive heterocyclic nucleus intact for evaluating their antibacterial and antifungal activity. The docking study was also performed for the crystal structure of human RNR

with (E)-(4-(2-(benzo[d]thiazol-2-yl)hydrazono)methyl-2,6-diphenylpiperidin-1-yl)(phenyl) methanone and the observed results were suitably interpreted.

2. EXPERIMENTAL SECTION

2.1. MATERIALS AND METHODS

All the reported melting points were taken in open capillaries and are uncorrected. IR spectra were recorded in AVATAR-330 FT-IR spectrophotometer (Thermo Nicolet) and only noteworthy absorption levels (reciprocal centimeters) are listed. ^1H NMR spectra were recorded at 400 MHz on Bruker AMX 400 MHz spectrometer using CDCl_3 as solvent and TMS as an internal Standard. ^{13}C NMR spectra were recorded at 100 MHz on Bruker AMX 400 MHz spectrometer in CDCl_3 . The tubes used for recording NMR spectra are of 5mm diameter. The reagents and solvents used were of high grade and purchased from Himedia and Merck and the solvents were distilled prior to use.

2.2. Molecular docking

2.2.1. Target protein structure preparation

The three dimensional structure of target protein was obtained from protein data bank (<http://www.rcsb.org/pdb>). During the docking process, water molecules of crystallization were removed from the complex and hydrogen atoms were added to the structure. The protein was optimized. Partial atomic charges were also assigned according to the force field. Minimizations were performed until the average root mean square deviation of the non-hydrogen atoms reached 5Å using OPLS-2005 force field to remove the steric hindrance using the protein preparation wizard of Schrödinger (Maestro 9.3.5 version).

2.2.2. Ligand structure preparation

The structure is imported in to the software's workspace and energy minimized using two algorithms: steepest descent and conjugate gradient of the impact module having default force field. Receptor Grid Generation program was used to prepare 4BMO Grid and all ligands were optimized by LigPrep program by using OPLS-2005 force field to generate lowest energy state of ligands.

2.3. Synthesis

2.3.1. General procedure for synthesis of N-benzoyl-2, 6-diarylpiperidin-4-one (7-12)

Piperidin-4-one (0.01 mol) and triethylamine (0.01 mol) in 30 mL of dry benzene, benzoylchloride (0.01 mol) in 20 mL of benzene was added dropwise through the separating funnel for about half an hour. Stirring was continued with (0°C) a magnetic stirrer. After the completion of the reaction, it was poured into water and extracted with ether in three 50 mL portion. The combined ether extract was then washed well with 3% sodium bicarbonate solution and dried over anhydrous sodium sulphate. This upon evaporation and subsequent recrystallization in distilled ethanol furnished the compound (7) in pure form with good yield. The compounds (8-12) were also synthesized adopting the similar procedure.

2.3.2. Synthesis of 2-hydrazinobenzothiazole (13)

Equimolar solution of 2-mercaptobenzothiazole (0.01 mol) and hydrazine hydrate (0.01 mol) in methanol (20 mL) was refluxed on a steam bath for about 10 hrs. The obtained filtrate was washed with ice water. The product was dried and recrystallized from ethanol to yield the pure compound, yield 60%, m.p. 201-205 °C.

2.3.3. General procedure for the synthesis of (Z)-(4-(2-(benzo[d]thiazol-2-yl)hydrazono)-3-methyl-2,6-diphenylpiperidin-1-yl)(phenyl)methanone (14-19)

A mixture of N-benzoyl-2,6-diarylpiperidine-4-one (0.01 mol) in methanol and 2-hydrazinobenzothiazole (HBT) (0.01 mol) was added with constant stirring and the reaction mixture was refluxed on a water bath. The completion of the reaction was monitored by TLC. The obtained product was filtered and then washed with water. The product was purified by column chromatography (Ethyl acetate & Hexane 1:4 ratio). All the synthesized compounds were obtained in good yield (Table 1).

Table 1. Analytical data of 14-19

Compounds	R	R ₁	R ₂	Yield (%)	M.F	M.W	m.p (°c)
1, 7, 14	H	H	CH ₃	68	C ₃₂ H ₂₈ N ₄ OS	516.66	168-170
2, 8, 15	CH ₃	H	CH ₃	64	C ₃₄ H ₃₂ N ₄ OS	544.71	169-172
3, 9, 16	OCH ₃	H	CH ₃	69	C ₃₄ H ₃₂ N ₄ O ₃ S	576.71	165-168
4, 10, 17	F	H	CH ₃	64	C ₃₂ H ₂₆ N ₄ OSF ₂	552.64	172-175
5, 11, 18	Cl	H	CH ₃	63	C ₃₂ H ₂₆ N ₄ OSCl ₂	585.55	174-176
6, 12, 19	Br	H	CH ₃	70	C ₃₂ H ₂₆ N ₄ OSBr ₂	674.45	167-171

2.3.4. (E)-(4-(2-(benzo[d]thiazol-2-yl)hydrazono)methyl-2,6-diphenylpiperidin-1-yl)(phenyl)methanone derivatives (14)

White solid: m.p. 168-170°C and yield 68 %. IR (KBr) (cm⁻¹): 3063, 2964, 2920 and 2843 (C-H stretching); and 1632 (N-C=O stretching); 1594 (C=N stretching); and 3310 (NH stretching). ¹H NMR (400 MHz-CDCl₃ ppm): δ 0.92 (d, 3H, CH₃, J= 4Hz), 2.00 (t, 1H, H_{5a}, J=1.8Hz); 2.23 (s, 1H, H_{3a}), 3.52 (d, 2H, H_{2a}, H_{5e}, J=2Hz), 3.85 (d, 1H, H_{6a}, J=3.5Hz) 6.95 (s, 1H, hydrazine NH), 7.02-7.87 (m, 17H, aromatic protons). ¹³C NMR (100 MHz-CDCl₃ ppm): δ 170.49 (CO), 165.11 (C=N), 157.90 (C-4, C=N), 118.06-137.02 (aromatic and *ipso* carbon), 77.04 (C-2), 60.07 (C-6), 37.02 (C-3), 29.72 (C-5), 14.22 (CH₃).

2.3.5. (E)-(4-(2-(benzo[d]thiazol-2-yl)hydrazono)-3-methyl-2,6-di-p-tolylpiperidin-1-yl)(phenyl)methanone (15)

White solid: m.p. 169-172°C and yield 64%. IR (KBr) (cm⁻¹): 2920, 2849 (C-H stretching) and 1632 (N-C=O stretching); 1605 (C=N stretching); and 3310 (NH stretching); ¹H NMR (400 MHz-CDCl₃ ppm): δ 1.05 (d, 1H, CH₃, J=2Hz), 1.27 (m, 6H, PhCH₃), 2.19 (t, 1H, H_{5a}, J=2.6Hz), 2.06 (s, 1H, 3_a), 3.20 (m, 2H, H_{2a}, H_{5e}), 4.13 (q, 1H, H_{6a}), 6.90 (s, 1H, NH), 6.92-7.66 (m, 17H, aromatic proton). ¹³C NMR (100 MHz-CDCl₃ ppm): δ 169.08, (CO), 168.9 (C=N), 158.5 ((C-4, C=N), 121.35-137.08 (aromatic and *ipso* carbon), 77.03 (C-2), 60.42 (C-6), 29.71 (Ph-CH₃), 21.09 (C-3), 20.99 (C-5), 14.65 (CH₃).

2.3.6. (E)-(4-(2-(benzo[d]thiazol-2-yl)hydrazono)-2,6-bis(4-methoxyphenyl)-3-methylpiperidin-1-yl)(phenyl)methanone (16)

White solid: m.p. 165-168°C and yield 69%. IR (KBr) (cm⁻¹): 2964, 2920, 2849 and 2832 (C-H stretching) and 1616 (N-C=O stretching); 1556 (C=N stretching); and 3420 (NH stretching); ¹H NMR (400 MHz-CDCl₃ ppm): δ 0.91 (d, 1H, CH₃, J=2.4Hz), 2.13 (t, 1H, H_{5a}, J=2.4Hz), 2.47 (s, 1H, H_{3a}), 2.92 (d, 2H, H_{2a}, H_{5e}, J=8Hz), 3.73 (m, 6H, OCH₃), 4.14 (d, 1H, H_{6a}, J=1.8Hz), 6.62 (s, 1H, hydrazine NH), 6.75-7.56 (m, 17H, aromatic proton). ¹³C NMR (100 MHz-CDCl₃ ppm): δ 171.49 (CO), 168.16 (C=N), 159.09 (C-4, C=N), 113.37 -135.36 (aromatic and *ipso* carbon), 77.03 (C-2), 68.66 (C-6), 36.53 (C-3), 29.72 (C-5), 21.08 (CH₃).

2.3.7. (E)-(4-(2-(benzo[d]thiazol-2-yl)hydrazono) -2,6-bis (4-fluorophenyl) -3-methyl piperidin-1-yl)(phenyl)methanone (17)

White solid: m.p. 172-175°C and yield 64%. IR (KBr) (cm⁻¹): 3020, 2949, 2836, 2815 and 2854 (C-H stretching); and 1710 (N-C=O stretching); 1585 (C=N stretching) and 3200 (NH stretching); ¹H NMR (400 MHz-CDCl₃ ppm): δ 1.13 (d, 1H, CH₃, J= 2Hz), 1.90 (s, 1H, H_{5a}), 2.89 (t, 1H, H_{3a}, J=1.5Hz), 3.23 (t, 1H, H_{5e}, J=2Hz), 3.71 (d, 1H, H_{2a}, J=1.2Hz), 3.8 (d, 1H, H_{6a}, J=3Hz), 6.7 (s, 1H, hydrazine NH), 6.79-7.64 (m, 17H, aromatic protons). ¹³C NMR (100 MHz-CDCl₃ ppm): δ 173.49 (ph-CO), 169.11 (C=N), 162.90 (C-4, C=N), 114.79-136.75 (aromatic and *ipso* carbon), 77.30 (C-2), 77.05 (C-6), 76.79 (C-3), 53.45 (C-5), 29.88 (CH₃).

2.3.8. (E)-(4-(2-(benzo[d]thiazol-2-yl)hydrazono)-2,6-bis(4-chlorophenyl)-3-methylpiperidin-1-yl)(phenyl)methanone (**18**)

White solid: m.p. 174-176°C and yield 63%. IR (KBr) (cm⁻¹): 3020, 2949, 2836, 2815 and 2854 (C-H stretching) and 1710 (N-C=O stretching); 1585 (C=N stretching); and 3210 (NH stretching); ¹H NMR (400 MHz-CDCl₃ ppm): δ 0.84 (d, 1H, CH₃, J=0.8Hz), 2.06 (t, 1H, H_{5a}, J=0.4Hz), 2.61 (m, 1H, H_{3a}), 3.52 (d, 2H, H_{2a}, H_{5e}, J=1.2Hz), 3.77 (d, 1H, H_{6a}, J=1.6Hz), 6.85 (s, 1H, hydrazine NH), 7.12-7.62 (m, 17H, aromatic proton). ¹³C NMR (100 MHz-CDCl₃ ppm): δ 170.28 (CO), 155.10 (C=N), 149.62 (C-4, C=N), 118.00-141.33 (aromatic and *ipso* carbon), 68.34 (C-2) 59.83 (C-6), 45.01 (C-3), 36.73 (C-5), 12.07 CH₃).

2.3.9. (E)-(4-(2-(benzo[d]thiazol-2-yl)hydrazono)-2,6-bis(4-bromophenyl)-3-methylpiperidin-1-yl)(phenyl)methanone (**19**)

White solid: m.p. 167-171°C and yield 70%. IR (KBr) (cm⁻¹): 3052 2969, 2936, 2915 and 2854 (C-H stretching) and 1720 (N-C=O stretching); 1605 (C=N stretching) and 3200 (NH stretching); ¹H NMR (400 MHz-CDCl₃ ppm): δ 0.83 (d, 1H, CH₃, J=1.4Hz), 1.30 (t, 1H, H_{5a}, J=2Hz), 2.61 (m, 1H, H_{3a}), 3.59 (d, 2H, H_{2a}, H_{5e}, J=2Hz), 4.04 ((d, 1H, H_{6a}, J=4Hz), 6.85 (s, 1H, hydrazine NH), 7.26-8.12 (m, 17H, aromatic proton). ¹³C NMR (100 MHz-CDCl₃ ppm): δ ¹³C NMR (100 MHz-CDCl₃ ppm): δ 172.00 (CO), 169.80 (C=N), 158.5 (C-4, C=N), 113.00-150.51 (aromatic and *ipso* carbon), 61.50 (C-2) 58.3 (C-6), 45.01 (C-3), 37.20 (C-5) 12.09 (CH₃).

2.4. Biological Screening

2.4.1. Microbiology

All the bacterial strains namely *Klebsiella pneumonia*, *Bacillus cerus*, *Salmonella typhi*, *Escherichia coli*, *Vibriae cholera* and fungal strains namely *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* and *Cryptococcus neoformans* were obtained from the Department of Microbiology, Annamalai University, Annamalaiagar, Tamilnadu, India.

2.4.2. In Vitro Antibacterial and Antifungal Activity

The *in vitro* activities of compounds (**14-19**) were tested in Sabourauds dextrose broth (SDB) (Himedia, Mumbai) for fungi and nutrient broth (NB) (Himedia, Mumbai) for bacteria by the disc diffusion method. MIC values were determined by twofold serial dilution method (Dhar et al., 1968). The test compounds were dissolved in dimethylsulfoxide (DMSO) to obtain 1mg ml⁻¹ solution. Seeded broth (broth containing microbial spores) was prepared in nutrient broth from 24h-old bacterial cultures on nutrient agar (Himedia, Mumbai) at 37± 1°C, while fungal spores from 24 h to 7 days old Sabouraud's agar slant cultures were suspended in SDB. The petriplates are incubated in BOD incubator (Sigma Instruments, Chennai, India) at 37°C for bacteria and 28°C for fungi. The zone of inhibition is recorded by visual observation after 24 hrs of inhibition for bacteria and after 72-96 hrs of inhibition for fungi. Moreover, the zone of inhibition is measured by excluding the diameter of the paper disc. Streptomycin was used as standard for bacteria and Amphotericin B as standard for fungi under analogous conditions.

Scheme 1

3. RESULTS AND DISCUSSION

3.1. Analytical Results

Schematic representation of the synthesized compounds **14-19** depicted in scheme 1. In this series, the structural interpretation of the representative compound **16** was discussed as follows. In FT-IR spectrum of compound **16**, the disappearance of NH stretching frequency around 3300 cm^{-1} confirms the complete conversion of NH into N-benzoylpiperidone and also the N-CO bond will have partial double bond character. Hence, two rotamers are possible. However, in no case separate NMR signals corresponding to the two rotamers were seen. Obviously, the rotation about the N-CO bond seems to be fast on NMR time scale. The disappearance of $\text{C}=\text{O}$ stretching frequency around 1710 cm^{-1} and the presence of $\text{C}=\text{N}$ stretching frequency confirm the formation of hydrazone derivatives (**14-19**). The weak band appeared at 3420 cm^{-1} corresponds to the $-\text{NH}$ stretching mode of the thiazole moiety. The aromatic and aliphatic C-H stretching vibrations are observed in the range of $2854\text{--}3052\text{ cm}^{-1}$. In ^1H NMR spectrum of compound **16**, there was a multiplet in the region of $6.85\text{--}7.56\text{ ppm}$ corresponding to 17 protons and it is due to the aromatic protons. The NH proton was assigned as singlet at 6.80 ppm and the methoxy protons were appeared at 3.73 ppm as a doublet and the broadening of benzylic C-6 proton was appeared as doublet in the region of 3.43 ppm . The H-2a proton coupled with H-5e proton and their signal was appeared as doublet in the region of 2.92 ppm . The signals appeared at 2.47 ppm and 2.13 ppm was assigned to H-3a and H-5a protons. In ^{13}C NMR spectrum of compound **16**, the signals at 77.03 , 68.66 , 36.53 and 29.72 ppm in the aliphatic region are assigned to C-2, C-6, C-3 and C-5 carbons of the piperidone ring, respectively. The hydrazone carbon (C-4) signal appeared at 159.09 ppm and amide carbonyl carbon signal appeared at 171.49 ppm . The aromatic and ipso carbon signals appeared in the region of $113.37\text{--}135.36\text{ ppm}$. From the HSQC spectrum of compound **16**, it is evident that benzylic proton at C-6 has cross peak with low intensity signal at 64.50 ppm . Thus, it is due to C-6 benzylic carbon. Similarly, the signal at 45.05 ppm shows cross peak with C-2 and C-5 protons confirmed the C-2

and C-5 protons. The signal at 46.00 ppm shows cross peak with methelinic proton at C-3, it is due to the benzylic carbon at C-3. Further the methoxy protons signal appeared at 3.73 ppm shows cross peaks with corresponding *ipso* carbon. ^1H and ^{13}C NMR signal and it's splitting patterns of compound **14-19** are observed as almost same as of compound **14**.

3.2. Molecular Docking protocol

The entire docking calculations were performed using the induced fit docking module of the schrodinger. It performs flexible protein-ligand docking and searches for favorable interactions between one typically small ligand molecule and a typically larger protein molecule. Docking process is divided into three steps. Primary glide docking, wherein protein preparation inhibited refinement is carried out with a maximum of 20 poses. Prime induced fit, wherein the side chains are optimized and refinement of residues takes place, if the ligand poses are within 5.0Å. Step 3 consists of the glide redocking step by means of standard precision mode. The best docked structure was chosen by three criterias: glide score function, glide energy and the number of amino acids matches (hydrogen bonds) with original drug complex. Binding interaction of compound with active site residues of RNR is shown in Fig. 1. Molecular analysis of compound **19** indicated that hydrogen bond, hydrophobic and mild polar interactions are three major interactions incorporating the attachment of this ligand to Ribonucleotide Reductase acceptor. Hydrophilic and hydrophobic interactions are shown in Fig. 2. The two-dimensional interaction diagram of docked compound **19** (**Fig.2**) revealed that the ligand is surrounded by hydrophobic residues such as TRP74, TYR41, LEU99, PHE45, PHE78, MET77, ALA80 and MET8. This observation concluded that hydrophobic interactions and desolvation effects involved in the binding process. Further the hydrophilic residues like THR42, THR43, ASN71, ASN73, ASN11 and SER69 are situated around the surface of the target ligand **19**. Hence binding affinities also enhanced by these hydrophilic residues. In compound **19**, the binding interactions are further surprisingly enhanced by π - π stacking interactions between benzoyl and benzothiazole moieties with TRY74 binding site residue. Among the synthesized compounds **14-19**, the *para*-bromo substituted compound **19** showed moderate G score -4.95 and glide energy -41.95kcal/mol. It is indicated that compound **19** has better ligand-protein interactions and reflected that compound **19** will have better antimicrobial character.

Table 2. G core, glide energy, hydrophilic and hydrophobic interaction of 14-19

Compounds	G score	Glide energy (kcal/mol)	Hydrophobic interaction residues	Hydrophilic interaction residues
14	-3.82	-28.4	TRP74, PHE45, ALA80, MET77, PHE78.	THR43, ASN47, ASN71, THR42.
15	-3.66	-37.2	TRP74, PHE78, MET77, PHE45, MET 8.	THR45, ASN71, THR42, ASN73, THR9.
16	-3.48	-25.9	TRP74, MET77, MET8, PHE45.	ASN71, THR42, ASN47, THR43, THR9.
17	-3.76	-35.7	PHE45, TRP74, MET77, TYR41, MET8, LEU99.	THR43, THR42, SER69, ASN71, ASN73.
18	-4.31	-28.1	TRP74, PHE78, MET77, LEU99, MET8, PHE45.	ASN71, ASN73, THR42, THR43, THR9.
19	-4.95	-41.9	TRP74, TYR41, LEU99, PHE45, PHE78, MET77, ALA80, MET8.	THR42, THR43, ASN71, ASN73, ASN11, SER69,

The root-mean-square deviation (RMSD) is the measure of the average distance between the atoms (usually the backbone atoms) of superimposed proteins. Rmsd data calculated for structure pairs of different sizes cannot be compared, because the rmsd value obviously depends on the number atoms included in the structural alignment. The rmsd value of the native ligand is 0.02Å and the rmsd value of synthesized compound (**19**) is 0.023Å from this evident the compound **19** is approximately same size compared to reference ligand. To conclude this observation, the synthesized compounds subjected for antimicrobial studies and the results are interpreted. The experimentally observed microbial results indicated that the compound **19** exhibited excellent antibacterial and antifungal activities. The docking score, glide energy, hydrophilic and hydrophobic interactions of amino acids of compounds **14-19** are given in Table 2.

Fig. 1 Binding interaction of 14-19 with active site residues of 4bmo receptor

The synthesized compounds (**14-19**) were tested for their *in vitro* antibacterial activity against selected Gram positive and Gram negative pathogens. (E)-(4-(2-(benzo[d]thiazol-2-yl)hydrazono)-3-methyl-2,6-diphenylpiperidin-1-yl)(phenyl)methanone (**14-19**) derivatives exhibited a wide spectrum of antibacterial profile in *In vitro* against all the tested organisms as shown in Table 3.

Table 3. Antibacterial activity of 14 -19

Compound	Minimum inhibitory concentrations. ($\mu\text{g mL}^{-1}$)				
	<i>K.pneumonia</i>	<i>B.cerus</i>	<i>S.typhi</i>	<i>E.coli</i>	<i>V.cholerae</i>
14	200	50	200	150	100
15	100	-	200	100	150
16	100	100	200	150	150
17	75	100	150	-	100
18	75	100	100	-	150
19	25	25	-	50	-
Streptomycin	25	25	25	50	25

The substitution of methyl group at C-3 position of the six-membered heterocyclic ring generally increased their antibacterial activity. Compound **14** without any substituent at *para* position of aryl groups present at C-2 and C-6 of piperidone ring exerted moderate antibacterial activity at 50-200 $\mu\text{g mL}^{-1}$ against the bacterial strains. The methyl substituent on **14** expressed an appreciable activity against *K. pneumoniae*. The replacement of methyl group at the *para* position of the aryl group through methoxy functionalities in compound **16**, did not show any appreciable antibacterial activity. The fluoro substituted compound **17** exhibited moderate antibacterial activities against *B.cerus* and *V.cholerae*. The chloro substituted compound **18** showed strong bacterial activities against *C. K.pneumoniae*. The substitution of bromo atom has excellent activity against *K. pneumonia* and *K.pneumoniae* *B.cerus* and *E.coli*.

3.4. Antifungal Activity

The *in vitro* antifungal activity of compounds (**14-19**) were examined against the fungal strains viz., *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus*, *Cryptococcus neoformans* and *Fusarium oxysporum*. Amphotericin B was used as standard drug whose minimum inhibitory concentration values are reproduced in Table 4.

Table 4. Antifungal activity of 14-19

Compound	Minimum inhibitory concentrations. ($\mu\text{g mL}^{-1}$)				
	<i>C.albicans</i>	<i>A.niger</i>	<i>A.flavus</i>	<i>C.neoformans</i>	<i>F.oxysporum</i>
14	150	250	-	50	250
15	100	50	100	250	200
16	150	50	150	250	-
17	-	100	50	150	150
18	150	100	100	50	100
19	25	25	50	50	50
Amphotericin B	25	50	25	25	50

Compound **14**, without any substituents at the *para* position of aryl groups at C-2 and C-6 of the six-membered heterocyclic ring has more antifungal activity against *C. neoformans* compared to other fungal strains. The introduction of methyl group at the *para* position of aryl group at C-2 and C-6 of piperidone ring (**compound 15**) was found to be better in enhancing the antifungal activity against *A.niger* compared to other fungal strains. Further, compound **16** has decrease in fungal activity against all the fungal strains. The substitution of fluoro atom at the *para* position of aryl group (**compound 17**) exerted remarkable activity against *A.flavus* compared to others bacterial

strains. The chloro substituted compound **18** exhibited excellent activities against *C. neoformans* compared to other fungal strains. From Table 4, it is obvious that compounds **17**, **18** and **19** were recorded excellent antifungal activity.

4. CONCLUSION

All the synthesized compounds of (E)-(4-(2-(benzo[d]thiazol-2-yl)hydrazono)-3-methyl-2,6-diphenylpiperidin-1-yl)(phenyl)methanone derivatives (**14-19**) are characterized by IR and NMR spectral data. The chemical shifts and coupling constant values indicated that all the synthesized hydrazone derivatives adopt the chair conformation in which all the alkyl and aryl substituents in the piperidone occupying equatorial orientations. Molecular docking studies revealed that compound **19** has appreciable binding interaction with the active site of the selected protein 4BMO. Compound **19** oriented in the binding site in such a manner that it favour the possibility of π - π stacking interaction on benzothiazole and benzoyl ring with TRY74. Existence of hydrophilic, hydrophobic and π - π stacking interaction on target ligand **19** with selected protein 4BMO is the reason for obtaining low docking score and glide energy. Antimicrobial studies revealed that compound **19** showed excellent antibacterial and antifungal activities as per the results obtained from the docking studies.

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