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Synthesis and Characterization of Cobalt (II) Complexes of 2-{[(2-hydroxy-5-nitrophenyl) methylidene] amino} Nicotinic acid derived from o-phenylenediamine and 5- nitrosalicaldehyde

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ABSTRACT: The objective of this paper was to synthesize and characterize cobalt (II) complexes of 2-{[(2 hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid derived from o-phenylenediamine and 5- nitrosalicaldehyde using FTIR, 1HNMR, 13CNMR, GCMS and screened to establish their potential as antimicrobial agents, compared with standards Ampiclox for bacteria and ketoconazole for fungal infections. Antimicrobial activity screening of the Schiff base exhibited biological activity virtually against all forms of bacteria andfungi isolates with the B. subtilis and E coli having same minimum inhibition concentrations of 1.8mm each and with the exception of Candidas albicans which exhibited zero diameter zone of inhibition. In this study, it was found that the synthesized Schiff bases exhibited two digits purity range, implying that they were relatively stable. Furthermore, the in silico molecular docking analysis of the Scihff bases is incorporated to predict their binding affinity to established molecular targets in C. albicans, S. aureus, and E. coli. The findings posited that the binding affinity of the Schiff base (9.3 kJ mol–1) is better than that of both the complex (9.0 kJ mol–1) and even the standard drugs (7.6 and 7.9 kJ mol–1) respectively. This lends credence to the Schiff Base as a better pharmaceutical than the present drug in usage, hence the need to harness them for daily use by pharmacies.

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The development of antibiotics stopped millions of deaths globally and transformed the provision of healthcare. Antibiotic misuse, however, has resulted in the emergence of resistant strains and multi-drug resistance, raising implications for public health (Altamira and Barr, 2021). As a result, combating this situation requires a multifaceted approach. Researchers are now interested in schiff base compounds and their complexes due to their wide range of biological and pharmacological properties as well as their applications in numerous human undertakings. They belong to a class of substances that are created when primary amines and ketones or aldehydes condense (Daila *et al*., 2018). Because of the biological, pharmacological, anticancer, and chelating capabilities of Schiff bases and their metal complexes, their usage in medicine has significantly improved. In recent times, these compounds have

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garnered significant attention due to their diverse biological properties, which include antibacterial, antifungal, antimalarial, antiproliferative, antiviral, and antipyretic effects. Additionally, Schiff bases and their metal complexes have demonstrated efficacy as catalysts, medicine enhancers, antitumor, and antidiabetic agents. Furthermore, they possess a high capacity to form chelates and stabilize polymers (Lindgren et al., 2014; Osigbemhe et al., 2022). Furthermore, their binding affinities to known molecular targets in C. albicans, S. aureus, and E. coli were predicted using in silico models. Consequently, the objective of this paper is to synthesize and characterize cobalt (II) complexes of 2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid derived from o-phenylenediamine and 5-nitrosalicaldehyde.

MATERIALS AND METHODS

Reagents: 2-aminonicotinic acid (2-aminopyridine-3-carboxylic acid); Salicylaldehyde; 5-bromosalicylaldehyde (5-bromo-2-hydroxybenzaldehyde); 5-nitrosalicylaldehyde (2-hydroxy-5- nitrobenzaldehyde); 5-methoxysalicylaldehyde (2-hydroxy-5-methoxybenzaldehyde); 2-amino-1,3,4-thiodiazole; Furfuraldehyde; Thiophene-2-carboxaldehyde; Ethanol; Methanol; Nutrient agar. All the reagents were of analytical grade from Sigma-Aldrich, Merck, Germany.

Organisms: Bacteria: Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Enterobacter aeruginosa, and *Proteus mirabilis.*

Fungi- Candida albicans, Penicillium notatum and *Aspergillus niger:* Specimens were obtained from the Department of Medical Microbiology, University of Benin Teaching Hospital (UBTH). All were checked for purity at Pax Herbal Clinic and Research Laboratories, Ewu, Edo State and maintained at $4^{O}C$ in slants of Nutrient Agar and Sabourand Dextrose Agar (SDA) slants for both.

Equipment/Apparatus: Gas chromatography, Mass spectrometry (GCMS); Thermal Scientific DSQ II Focus Instrument model; Fourier Transform Nuclear Magnetic Resonance Spectrometer (FTNMR) – Bruker $400MH_z$ machine; Ultra-violet spectra were recorded on a Hitachi U-2000 double beam spectrophotometer; Infra-red spectra (KBr Discs) were recorded on a Hitachi Model 200-50 IR spectrophotometre. Melting points were taken on a Gellenkamp apparatus and are uncorrected. All instrumental determinations were carried out in Durham University, Chemistry Department, United Kingdom.

Syntheses of Schiff Base, 2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid: Equimolar portions of 2-aminonicotinic acid (0.01mol) with 5-nitrosalicylaldehyde (0.01mol) [Fig: 1.0], were mixed together in ethanol (30-40mL) containing a few drop of conc. H_2SO_4 at a pH between 3.5 and 4.5. The resultant mixture was then heated under reflux for 2 hours and filtered hot by suction filtration. The product of reaction was allowed to crystallize from filtrate left at room temperature of $25^{0}C$ for over two days. The crystals formed were recrystallized hot in ethanol and dried in a desiccator over $CaCl_2$ vacuum and the yield was calculated (Oviawe and Elemikkhe, 2012; Izuagbe *et al.,* 2023).

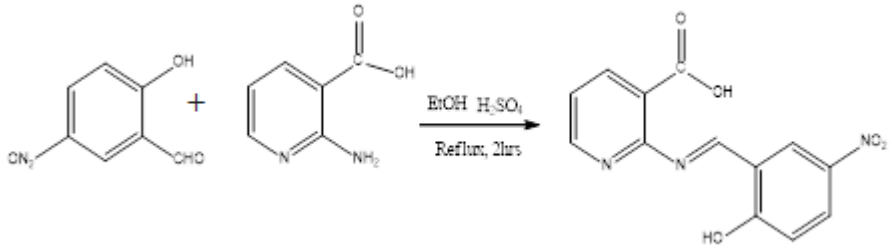

Fig. 1: Synthesis of 2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid

Synthesis of Cobalt Complex of 2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid.

The metal complexes of Cobalt (II), was prepared by reaction of equimolar (0.01mol) of each metal salt with a corresponding (0.01mol) of the Schiff base ligand. Various 0.01mol of the metal salts were each refluxed with 0.01mol of the ligand in ethanol as medium for 2hours. All were filtered and washed with ethanol after which they were allowed to stand for 24days. The resulting crystals were then dried and melting point determined. All synthesized complexes were coloured.

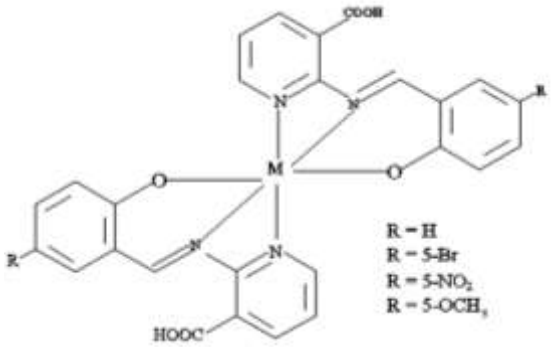

Fig 2: Synthesis of Cobalt Complex of 2-{[(2-hydroxy-5-nitrophenyl) methylidene] amino} nicotinic acid.

Antimicrobial Assay: The synthesized compound (the Schiff base/complex) was assayed for their antimicrobial activity using the disc diffusion technique by Kirby-Bauer. The Whatman filter paper (Whatman No. 1) were cut into size of 6mm diameter with office perforator and sterilized at 105^{O}C for 1 hour. The sterile discs were impregnated with 20uL of 100mg/mL of the synthesized Schiff base or complex and dried in the oven at 60^{O}C for about 15-30mins. Mueller Hinton agar plates were seeded with standardized broth culture of test organisms containing 100cfu/mL equivalent to 0.5 Mcfarland standards (NCCLS) and the prepared discs containing 2mg of the compound were placed on the plates. They were then incubated at 37^{O}C for 24 hours and observed for clear zones diameters of inhibition against the organisms. The zones diameters were measured with a transparent ruler and the result recorded in millimeters (mm). The assay was done in duplicates. Sterilized disc were soaked in 100% DMSO as negative and 2mg/mL of Ampicillin-Cloxacillin (Ampiclox) for bacterial isolates and Ketoconazole for fungi as positive control, (Khan *et al.,* 2017).

Preparation of Inoculum: A loopful of the test organism was taken from their respective agar slants and subcultured into test-tubes containing Mueller Hinton Broth for bacteria and Saboraud Dextrose liquid for fungi. The test-tubes were incubated for 18hours at 37^{0}C for bacteria and for 48 hours at 30^{O}C for the fungi. The obtained microorganisms in the broth were standardized using normal saline to obtain a population density of 100cfu/mL for the bacteria. For the fungi, fungal spores were harvested after visible notice of growth and suspension was standardized (Khan *et al.,* 2017).

Preparation of the Media: 38 g of Mueller Hinton Agar, 52g of SDA were weighed independently into different conical flask 1000mL of distilled water was added and capped with a cotton wool. The media were boiled to dissolution and then sterilized at 121^{O}C at 15mins.The media were then allowed to cool to 45^{O}C and 20mL of the sterilized medium was poured into sterile Petri-dishes and allowed to cool and solidify. The plates were labeled with the test microorganism (each plate with a test microbe). The microbes were spread evenly over the surface of the medium with the aid of a glass spreader. The plates were dried at 37^{O}C for 30mins respectively, (Khan *et al.,* 2019).

Minimum Inhibitory Concentration – Broth Dilution Method: The minimum inhibitory concentration of the compound was carried out using Macro Broth dilution technique (Boron and Fingold, 1990). Nine (9.0) mL of each Broth was dispersed into separate test-tubes and was sterilized at 121^{O}C for 15mins and the allowed to cool. Dilutions of the compound were made from the stock concentration to obtain 0.6, 0.9,1.2,1.5,1.8 and 2.1 mg/mL. The standardized inoculum (0.1mL) of the microbes were inoculated into the different concentrations of the compound in the broth. The test tubes of the broth were incubated at 37^{O}C for 24 hours and 30^{O}C for 1-7 days for bacteria and fungi respectively and observed for turbidity. The lowest concentration which showed on turbidity in the test tube was recorded as the MIC, (Akinyemi *et al.,* 2005).

Minimum Bactericidal/Fungicidal Concentration – Macro Broth Dilution Method: Fresh Muller Hinton agar media were prepared, sterilized at 121^{O}C for 15mins and was poured into sterile petri-dishes and left to cool and solidify. The contents of the MIC tubes (that is the tubes that showed no growth) were then sub-cultured onto the media and incubated at 37^{O}C for 24hours and 30^{O}C for 1-3 days for bacteria and fungi respectively. It was then observed for colony growth. The MBC/MFC was the plate with the lowest extract concentration and without colony growth, (Gajendra *et al.,* 2010).

In-Silico Molecular Docking Studies: The compounds' 3D structure was obtained from PubChem

(https://pubchem.ncbi.nlm.nih.gov/), optimized using the Density Functional Theory (DFT-B3LYP/6-31G(d,p)) method, and then docked with the receptors using Auto Dock Vina integrated in PyRx software (Krishnan *et al.,* 2022; Kirubhanand *et al.,* 2023; Shil *et al.,* 2023; Anthony *et al.,* 2024). The receptor proteins, specifically dihydrofolate reductase (PDB ID: 4H95), dihydropteroate synthase (PDB ID: 5V7A), and GyrB ATPase domain (PDB ID: 5Z9N), were obtained from the protein data bank website (https://www.rcsb.org/). They were then subjected to cleaning procedures using Edupymol version 1.7.4.4 and BIOVIA Discovery Studio 2021, (Anthony *et al.,* 2024; Adepoju *et al.,* 2022; Oyebamji *et al.,* 2023). The BIOVIA Discovery Studio 2021 software was utilized to visualize the two-dimensional structure of the receptor complexed with a co-ligand to identify the active region of each receptor. Additionally, a grid box was established around the active site. The coordinates for the center and size of the grid box for the 4H95 receptor are (x = -7.0568, y = 18.3021, z = -0.5798) and (x = 24.3832, y = 32.2190, z = 25.0000). For the 5V7A receptor, the coordinates are (x = -24.5881, y = 5.7818, z = 105.0706) and (x = 32.6966, y = 36.0716, z = 25.0000). Lastly, for the 5Z9N receptor, the coordinates are (x = -33.4074, y = 2.6100, z = -24.2488) and (x = 28.4834, y = 25.8876, z = 25.0000).

RESULTS AND DISCUSSION

Chemistry of the Schiff Base 2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid: Its yield was found to be 68% as yellow powder; m.p. 140-141^{0}C; IR (KBr, Cm^{-1}): 1741.82 (OH, Carboxylic acid) 3285.85 (OH, Phenol), 1382.04 (C=O, carboxylic acid), 1631.83(HC=N), 1529.5 (C=N, pyridine); the ^{1}H NMR (DMSO-d_6δ, ppm); 7.30 (d, IH, d=7.81H_3, phenyl C_3-H), 6.90 (dd, IH, d=7.82, 5.23H_3, pyridine C_5-H), 8.32 (d, IH, d = 7.82H_z, pyridine C_4-H), 8.41 (d, IH, d=7.81, 2.33H_z, phenyl C_6-H), 8.68 (d, IH, d=2.33H_3, phenyl C_6-H), 8.75 (d,1H,d=5.23$H_z$pyridine C_6-H), 8.93 (S,IH, CH=N), 10.45 (S,IH, OH), 11.42 (S,IH, COOH).

Cobalt (II) 2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid: Its yield was found to be 72% as red crystals; m.p. (decomposed) 190 -192^{0}C; IR (KBr, Cm^{-1}): 1741.82 (OH, Carboxylic acid) 3285.85 (OH, Phenol), 1382.04 (C=O, carboxylic acid), 1631.83(HC=N), 1529.5 (C=N, pyridine); the ^{1}H NMR (DMSO-d_6δ, ppm); 7.30 (d, IH, d=7.81H_3, phenyl C_3-H), 6.90 (dd, IH, d=7.82, 5.23H_3, pyridine C_5-H), 8.32 (d, IH, d = 7.82H_z, pyridine C_4-H), 8.41 (d, IH, d=7.81, 2.33H_z, phenyl C_6-H), 8.68 (d, IH, d=2.33H_3, phenyl C_6-H), 8.75 (d,1H,d=5.23$H_z$pyridine C_6-H), 8.93 (S,IH, CH=N), 10.45 (S,IH, OH), 11.42 (S,IH, COOH), 550 (M-N), 450 (M-O).

The IR Spectra of Schiff base due to 2-aminonicotinic acid and the Salicylaldehyde: The IR spectra (as shown in Appendix 1) of these Schiff bases showed bands resulting from the OH stretching of the phenol and carboxyl function in the 3282-3286cm^{-1} and 1735-1741cm^{-1} regions respectively, whereas the carboxyl (C=O) stretching were observed in the 1382-1383cm^1 regions. The azomethine (HC=N) stretching were observed in the 1630-1635cm^{-1} region, and the pyridine (C=N) stretching appeared at 1610cm^{-1} in all the structures synthesized with this combination of amines and aldehydes.

The ^{1}H-NMR spectra due to 2-amino nicotinic acid and their Salicylaldehydes: In the Schiff bases of 5-bromo, 5-nitro and 5-methoxysalicylaldehyde, the ^{1}H-NMR spectra exhibited the OH protons of the phenol at δ 10.21 – 10.45 and the carboxyl OH protons at δ 11.31 – 11.42 as three separate singlets. The azomethine (HC=N) protons of all the Schiff bases appeared as singlets at δ 8.66 – 8.93. The ^{1}H-NMR spectrum of 5-bromo, 5-nitro, and 5-methoxySalicylaldehyde displayed phenyl C_3-H as a doublet at δ 7.15 and δ 7.16, respectively. The phenyl C_6-H, experiencing a de-shielding effect due to the inductive effect of HC=N function, resonated as a doublet at δ 7.89 and δ 7.86 respectively. The phenyl C_4-H appeared as a double doublet at δ 7.25 and δ 7.21, respectively.

The ^{13}C-NMR spectra of Schiff base ligands nicotinic acid and the Salicylaldehyde: The ^{13}C-NMR spectra of 5-bromo-, 5-nitro- and 5-methoxy displayed peaks at δ 165,δ 158,δ 147,δ 143,δ 110, and δ 108. The carbonyl in carboxylic group experiencing a de-shielding effect occurs at the downfield of δ 165. The imine group was found at δ 158 while the benzene carbon occurred at δ 108 - δ 143.

The GC-MS fragmentation of2-{[(2-hydroxy-5-nitrophenyl)-methylidene] amino} nicotinic acid: The GC-MS showed the mass ion at 287.2 and major fragment at 167, 166, 139, 120, 105, 93, 79, 57(base peaks) 44, 43.

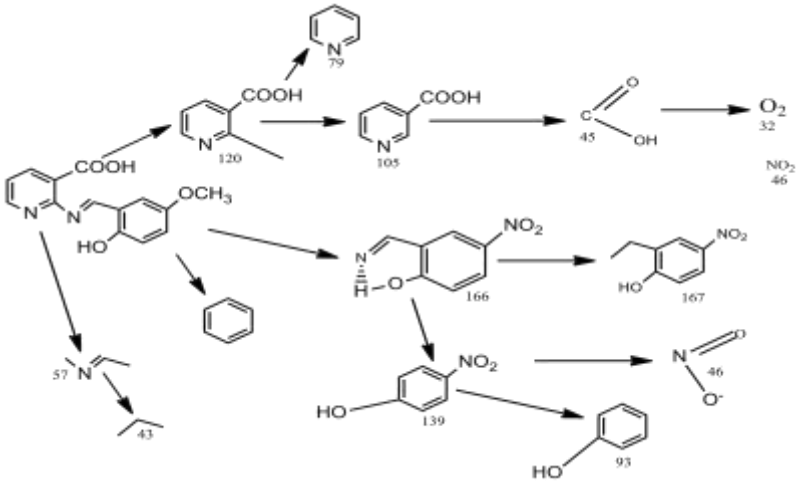

Fig 3: The GC-MS fragmentation of 2-{[(2-hydroxy-5-nitrophenyl)-methylidene]amino} nicotinic acid

Preliminary Screening

Table 1: Result of *in vitro* anti-bacteria activities of Schiff bases

Compounds	Diameter zone of inhibition (mm)						
	B. Subtilis	*E. coli*	*E. aerogenes*	*K. pneumonia*	*P. Aeruginosa*	*S. aureus*	*P. mirabilis*
2-{[(2-hydroxy-5-nitrophenyl)methylidene] amino} nicotinic acid	24	24	25	14	12	30	12
Ampiclox	19	0	0	0	17	19	0
DMSO	0	0	0	0	0	0	0

Table 2: Result of i*n vitro*anti-fungiactivitiesof Schiff base

Compounds	Diameter zone of inhibition (mm)		
	Aspergillus niger	*Candida albicans*	*Penicillium notatum*
2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid	29	0	35
Ampiclox	0	0	0
Ketonazole	0	0	9
DMSO	0	0	0

Table 3: Results of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal (MBC) (mg/ml) of Schiff bases

Compounds	Minimum Inhibitory concentration (MIC) and Minimum Bactericidal (MBC mg/mL)													
	B. Subtilis		*E. coli*		*E. aerogenes*		*K. pneumonia*		*P. Aeruginosa*		*S. aureus*		*P. mirabilis*	
	MIC	*MBC*	*MI C*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*
2-{[(2-hydroxy-5-nitrophenyl)methylidene] amino} nicotinic acid	1.2	1.5	0.9	1.2	1.2	1.5	1.5	1.8	1.5	1.8	1.5	1.8	1.5	1.8

The MIC/MBC values were determined as mg/mL of active compound in medium.

Biological Activities of Ligands and Its metal Complexes

Biological Activity of Schiff Bases: This study examined many Schiff bases and the associated metal complexes on isolates of seven bacteria and three fungi, respectively. Tables 1-4 displayed the in vitro antibacterial activity data. The diameter zone of inhibition for bacteria and fungi were measured 24 hours after incubation at a constant temperature of 37 ^{O}C, and 2–5 days after incubation at 30 ^{O}C. When compared to standard treatment (ketoconazole for fungal infections and ampicillin-cloxacillin for bacteria), the diameter zone of inhibition obtained shows that the majority of Schiff bases were even more active against the isolates of bacteria and fungi.

The Schiff base 2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid demonstrated antimicrobial action almost against every bacterial and fungal isolate, with the exception

of C. albicans, a fungal isolate that recorded a zero diameter zone of inhibition, according to the results of the antimicrobial activity screening. The fungus P. notatum and the bacterium isolate S. aureus showed the greatest susceptibility to it, with recorded zones of inhibition measuring 30 and 35 mm, respectively.

Table 4: Results of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of Schiff bases

Compounds	Diameter zone of inhibition (mm)					
	A. niger		*C. albicans*		*P. notatum*	
	MIC	MBC	MIC	MBC	MIC	MBC
2-{[(2-hydroxy-5-nitrophenyl)methylidene] amino}nicotinic acid	1.2	1.5	0	0	0.9	0.2

The MIC/MBC values were determined as mg/mL of active compound in medium.

Table 5: Result of *in vitro* anti-bacteria activities of metal complexes

Compounds	Diameter zone of inhibition (mm)						
	B. subtilis	*E. coli*	*E. Aerogenes*	*K. pneumonia*	*P. Aeruginosa*	*S. aureus*	*P. mirabilis*
Cobalt (II)2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid	9	12	16	14	14	21	12
Ampiclox	19	0	0	0	17	19	0
DMSO	0	0	0	0	0	0	0

Table 6: Result of i*n vitro* anti-fungi activities of metal complexes

Compounds	Diameter zone of inhibition (mm)		
	Aspergillus niger	*Candida albicans*	*Penicillium notatum*
Cobalt (II)2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid	15	10	18
Ampiclox	0	0	0
Ketonazole	0	0	9
DMSO	0	0	0

Table 7: Results of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal (MBC) (mg/mL) of metal complexes

Compounds	Minimum Inhibitory concentration (MIC) and Minimum Bactericidal (MBC mg/mL)													
	B. subtilis		*E. coli*		*E. aerogenes*		*K. pneumonia*		*P. Aeruginosa*		*S. aureus*		*P. mirabilis*	
	MIC	*MBC*	*MI C*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*
Cobalt (II)2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid	1.8	2.1	1.8	2.1	1.5	1.8	1.5	1.8	1.5	1.8	0.9	1.2	1.8	2.1

The MIC/MBC values were determined as mg/ml of active compound in medium.

Table 8: Results of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of metal complexes

Compounds	Diameter zone of inhibition (mm)					
	A. niger		*C. albicans*		*P. notatum*	
	MIC	MBC	MIC	MBC	MIC	MBC
Cobalt (II) 2-{[(5-Bromo-2- hydroxyphenyl) methylidene] amino} nicotinic acid	1.5	1.8	0	0	1.8	2.1

The MIC/MBC values were determined as mg/ml of active compound in medium.

Table 9: Binding affinities of active Compounds with the receptors (ID: 4H95), (ID: 5V9A), and (ID: 5Z9N).

S/N	LIGANDS	CODE	5Z9N	5V7A	4H95
			Binding affinity (kJ mol^{-1})	Binding affinity (kJ mol^{-1})	Binding affinity (kJ mol^{-1})
1	2-{[(2-hydroxy-5-nitrophenyl) methylidene]amino} nicotinic acid	M1	-6.7	-7.3	-7.6
2	Ampiclox	M2	-7.1	-8.0	-7.9
3	Ketonazole	M3	-7.6	-8.1	-9.0
4	Cobalt (II)2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid	M4	--7.9	-7.4	-9.3

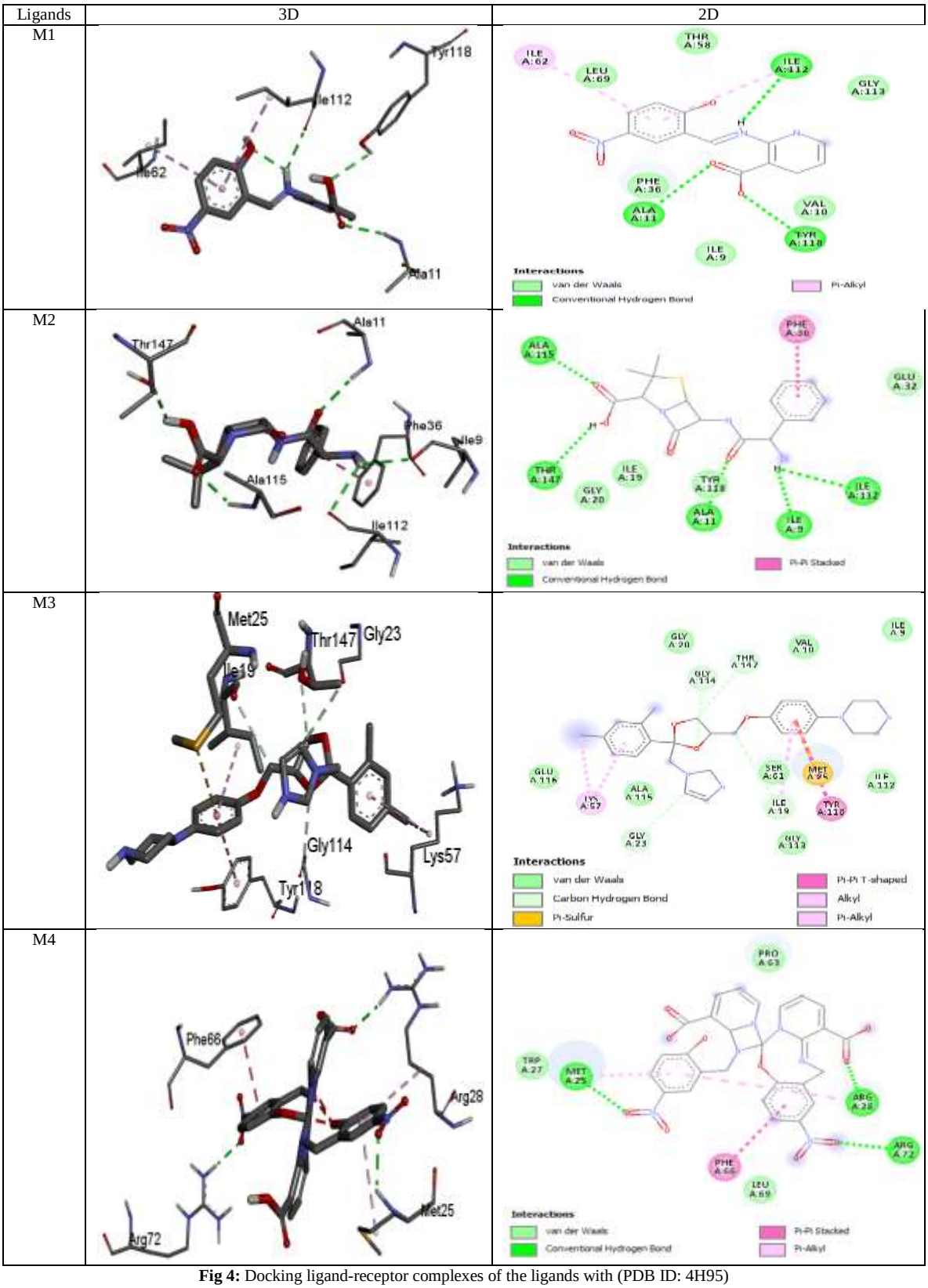

Fig 4: Docking ligand-receptor complexes of the ligands with (PDB ID: 4H95)

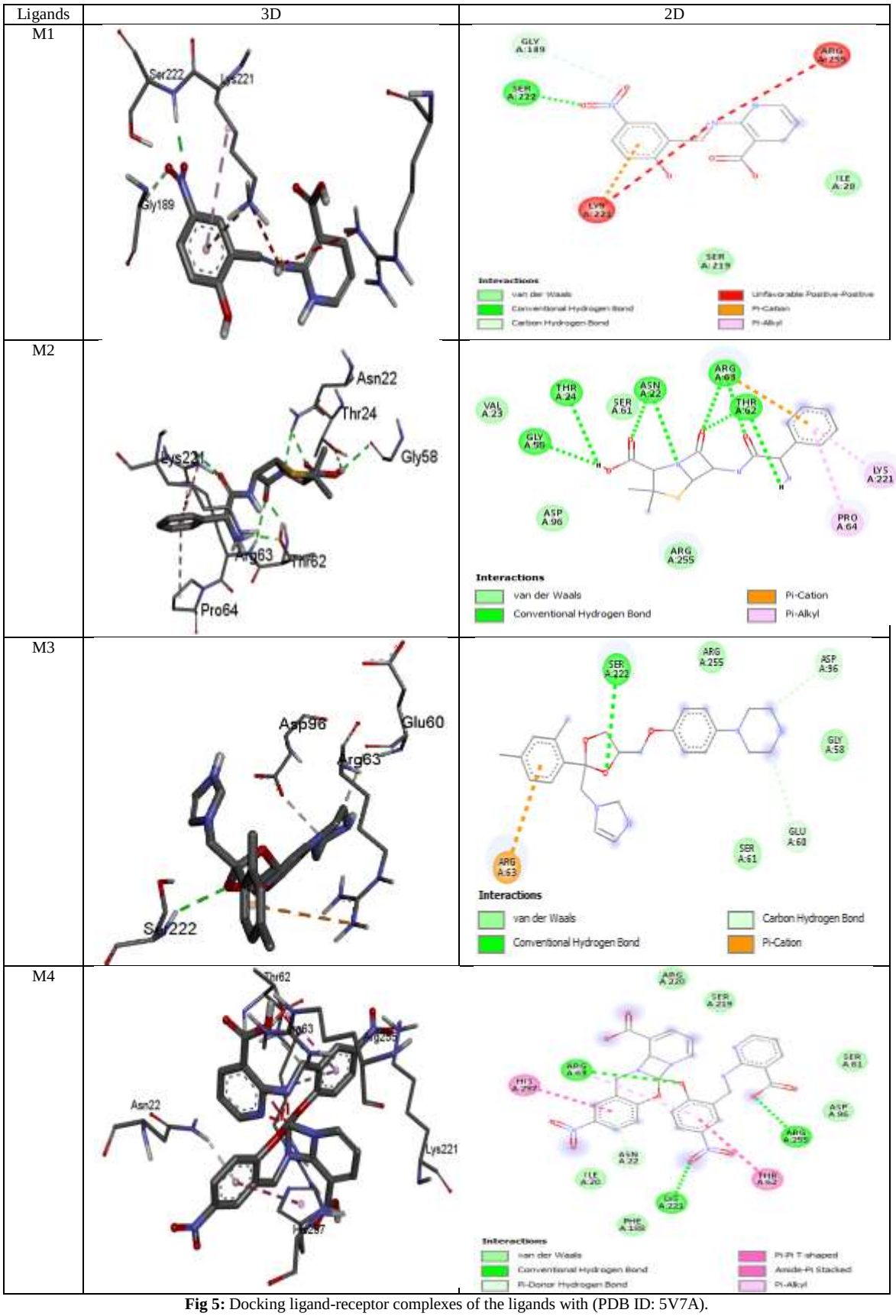

Fig 5: Docking ligand-receptor complexes of the ligands with (PDB ID: 5V7A).

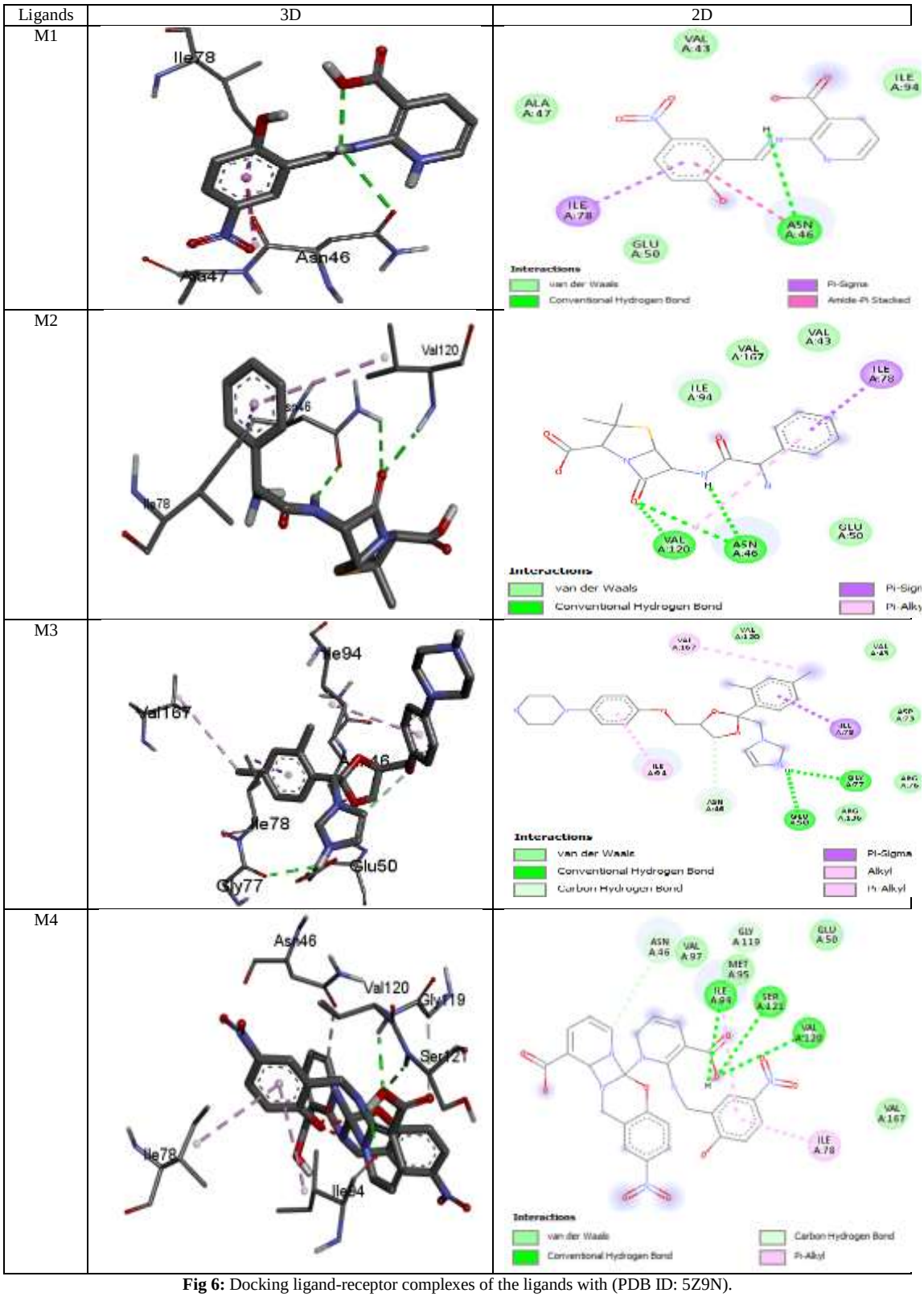

Fig 6: Docking ligand-receptor complexes of the ligands with (PDB ID: 5Z9N).

Biological Activity of Metal Complexes: In this study, the antibacterial properties of the metal complexes were also contrasted with those of the common medications ketoconazole and ampicilin-loxacillin (Ampiclox). The diameter zone of inhibition data show that most metal complexes were, on average,

more active than the usual medications utilized against the isolated bacteria and fungi. This result was consistent with the findings of several transition metal complexes of Schiff base that were reported to have antibacterial action and were generated from 5-nitrosalicaldehyde and o-phenylenediamine (Fasina and Ogundele, 2014). In their research, they found that the Schiff bases had greater activity than the metal complexes against all types of bacteria, with the complexes' activity being related to the presence of different metal ions. It appears that the shape of the metal complex influences the activity of the complexes that are produced. The microbial cells' ribosome variations or the impermeability of the bacteria' cells determine how differently various metal complexes behave against various germs (Gajendra *et al.*, 2010; Sengupta *et al.*, 1998).

MIC and MBC/MFC of Schiff bases: The antimicrobial properties of the Schiff bases were further investigated by macro-dilution to determine their minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration for Bacterial isolates and Minimum Fungicidal Concentration (MFC) applicable to fungi isolates. The MIC of Schiff bases: Schiff base 2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid notably exhibited high but good MIC and MBC results, fairly MIC/MFC result on *P. notatum*.

MIC and MBC/MFC of Metal complexes: Tables7 and 8 indicate that Metal complexes Cobalt(II)2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino}nicotinic acid, showed good minimum bactericidal and fungicidal activity against some selected bacteria; *K. pneumonia, B. subtilis, E.aerogenes, S.aureus* were most susceptible at minimum concentration of 1.2mg/mL, 1.8mg/mL, 1.5mg/mL and 0.9mg/mLCobalt (II) 2-{[(2-hydroxy-5-nitrophenyl) methylidene]amino}nicotinic acid. Further, the antifungal activity of metal complex cobalt (II) 2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino}nicotinic acid was found to be significant against *A. niger* and *P. notatum*.

Molecular Docking Study: To find the molecules that have a high binding affinity to the receptor pocket, the molecular docking approach was utilized. The compound showed a high degree of receptor affinity, as did the traditional medications. Through their interaction with the amino acid residue located within the receptor's active region, they perfectly conform to the receptor's active site. Protein residues, types of interaction, and binding affinities for each complex formed by the standard and the investigated chemical are shown in Table 9. The compounds are aligned with dihydrofolate reductase (PDB ID: 4H95), dihydropteroate synthase (PDB ID: 5V7A), and GyrB ATPase domain (PDB ID: 5Z9N). Figures 4–6 illustrate the strong affinities between the compounds through hydrogen bonding, carbon-hydrogen bonding, and Van der Waal interactions.

The ligand and the experimental pharmaceuticals show increased binding affinities, according to the molecular docking analysis of dihydrofolate reductase (PDB ID: 4H95) with the chemicals. However, Cobalt (II)2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid (M4) has superior binding performance compared to all other ligands. As calculated, the binding affinities for the ligand and experimental medications are as follows: M1 (-7.6 kcal/mol), M2 (-7.9 kcal/mol), M3 (-9.0 kcal/mol) and M4 (-9.3 kcal/mol) (Tables 9). These values indicate that all of these compounds can inhibit dihydrofolate reductase. However, Cobalt (II)2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid(M4) exhibits a stronger binding affinity compared to M1, M2 and M3. The compound's drug-likeness is enhanced when its binding affinity decreases, hence boosting its capacity to effectively inhibit Oyebamji *et al.*, 2023). In the 4H95–ligand complex, M1 is hydrogen bonded with ILE 112, ALa 18, and TYR 118 also interacted through π–alkyl with ILE 62; M2 interacted with Ala 115, Ala 11, THR 147, ILE 9 and ILE 112 through hydrogen bond also interacted through π- π stacked with PHE 36,M3 formed carbon-hydrogen bond to Gly 23, Gly 114, Thr 147; π–alkyl with ILE 19; alkyl with LYS 57; π- π T-shape with THR 116 also interacted through π-sulfur with MET 25, whereas M4 formed conventional hydrogen bond with MET 25, ARG 28, ARG 72 also interacted through π- π stacked with PHE 66 (Fig. 4).

Similarly, the docking results of dihydropteroate synthase (ID: 5V7A) with the ligands indicate that the ligand and the experimental medicines exhibit greater inhibitory actions (Table 9) compared to M1 (−7.3 kcal/mol), M2 (–8.0 kcal/mol), M3 (–8.1 kcal/mol) and M4 (–7.4 kcal/mol). Thus, the ligand can also function as good drug candidates with the experimental medications. The complex formed by the 5V7A ligand demonstrated that M1, M2, M3 and M4 all engaged in hydrogen bonding interactions with 5V7A. However, M2 created a π-alkyl link with PRO 64 and LYS 221, M2 and M3 formed π-cation contacts with 5V7A whereas M1 engaged through a carbon-hydrogen bond with Gly 189 whereas M4 formed conventional hydrogen bond with LYS 221, ARG 63, ARG 255 also interacted through π- π stacked with THR 62 and HIS 257 (Fig. 5).

Furthermore, for GyrB ATPase domain (ID: 5Z9N), the docking results given in Table 9 demonstrated that M1 (−6.7 kcal/mol), M2 (–7.1 kcal/mol), M3 (–7.6 kcal/mol) and M4 (–7.9 kcal/mol) displayed a comparably closed binding affinity against GyrB ATPase. In 5Z9N ligand complex demonstrated that M4 has three hydrogen bonds, M2 and M3 have two hydrogen bonds, while M1 has one hydrogen bond with amino acid residues in the active site of the 5Z9N.

Therefore, both experimental and in silico results supported 2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid, Cobalt (II)2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid, Ampiclox and Ketonazole for the treatment of bacteria and fungi diseases, and that hydrogen bonding and Van der Waal interactions (Figs. 4–6) play crucial roles in inhibitory activities of these ligands.

Conclusion: The high affinity of the Schiff bases for chelation towards transition metals has been taken advantage of in synthesizing the complexes earlier mentioned. The antifungal activity of metal complex cobalt (II) 2-{[(2-hydroxy-5-nitrophenyl) methylidene] amino} nicotinic acid was found to be significant against *A. niger* and *P. notatum*. The molecular docking results indicated that M1, M2, and M3 had higher binding affinities to the 4H95, 5V9A, and 5Z9N receptors. The ligand exhibits promising properties as a potential therapeutic candidate in conjunction with the experimental medicines, despite molecule M3 having the highest binding affinity.

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