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In silico studies and antimicrobial investigation of synthesised novel N-acylhydrazone derivatives of indole

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ABSTRACT

Indole is an exceptional scaffold in the discovery and design of drugs with different mechanisms and biological activity. Numerous research has been conducted on N-acylhydrazones of indole and its derivatives, however, no account of the synthesis, molecular docking and the antimicrobial activities of the eleven synthesised compounds has never been reported. The study was therefore designed to synthesize, characterize, carry out molecular docking and determine the antimicrobial activities of novel N-acylhydrazone derivatives of indole.

Aldol condensation of synthesised hydrazides with aromatic aldehydes led to the formation of the N-acylhydrazone derivatives of indole. The structure of the compounds were confirmed by proton nuclear magnetic Resonance (^{1}H NMR) and electron impact mass spectrometry (EIMS). The N-acylhydrazones were screened against ten microbes (four fungi three Gram-positive bacteria, and three Gram-negative bacteria) at concentrations of 100 to 6.26 mg/mL, using agar well diffusion and fungi carpeted antimicrobial assays. Gentamicin (5 mg/mL) was the positive control for bacteria while tioconazole (70%) was used for fungi. The molecular docking studies was done and the docking scores recorded accordingly on four antimicrobial receptors with PDB codes (3CR7, 3FHV, 6L1L and 6SPC).

The synthesised compounds displayed moderate activity against the test organisms with the zone of inhibitions range 24 ± 0.00 to 18 ± 0.70 mm at 100 mg/mL as compared to the standards (gentamicin 10 µg/mL (36 ± 1.41), tioconazole 70% (26 ± 4.24)). All synthesised N-acylhydrazone derivatives exhibited a good number of hydrophobic interactions, classical and non-classical hydrogen bonding with the docked protein enzymes. Compound 10 and 1 were predicted to exhibit highest binding energy of -9.7 and -9.2 kcal/mole respectively with the binding site of 6SPC. Other compounds were found to have higher predicted binding energies than the standard drugs. Previously, there has been no report

Abbreviations: PDB, protein data bank; R_f, retention factor; ^{1}H NMR, proton nuclear magnetic resonance; EIMS, electron impact mass spectrometry; DMSO, dimethylsulfoxide; DNA, deoxyribonucleic acid; *J*, coupling constant; SD, standard deviation; PPM, part per million; s, singlet; d, doublet; MeOH, methanol; t, triplet; m, multiplet; ds, doublet singlet; δ, chemical shift; *m/z*, mass to charge ratio; dd, double doublet.

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on the synthesis of N-acylhydrazone derivatives of Indole used in this study, their molecular docking and antimicrobial analyses is being reported for the first time. Since microbial resistance poses a major threat to human health globally, this research screened the synthesised compounds on fungi and bacteria and were found to be active against the selected microbes. These compounds if ameliorated, will reduce the uncontrollable rise in the multidrug-resistant (MDR) bacteria in Africa and in the universe.

Therefore, since the synthesised N-acylhydrazone derivatives have potential in inhibiting microbial growth and showed good binding parameters with the tested enzymes, they could be effectively developed into antimicrobial agents useful for treating a wide range of microbial infections. Further research should conducted on the drugability, toxicity, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the drug compounds.

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Introduction

Indole is a bicyclic aromatic heterocyclic ring compound that is formed by a union of benzene and pyrrole ring at carbon 2 and 3 positions. Indole is aromatic with a 4n+2 π electron system; with 10-π electrons arising from pi-bonds and lone a pair on nitrogen which are delocalised around the indole ring. The indole ring system is one of the most abundant and important heterocycles occurring in nature which are found in a diverse array of biological significance in natural compounds [1]. Indoles are a prevalent class of natural and synthetic compounds found to be in abundance in biologically active compounds such as agrochemicals, medicines, and alkaloids [2].

Medicine and biochemistry fields of study are also interested in many parts of Indole chemistry. Therefore, it is not surprising that Indoles act as main compounds and are vital building blocks in several drug design [2,20]. Schiff bases are chief precursors for the synthesis of numerous bioactive compounds. Likewise, they are reported to display diverse biological actions including antifungal, antibacterial, anti-cancer, and herbicidal activities [2,4]. Recently, the bioactive N-acylhydrazone moiety has been one of the most popular functional groups in drug discovery, and it has been identified in a huge number of hit and lead compounds that affects numerous kind of molecular target [21–27]. A myriad of N-acylhydrazone compounds that show good antimicrobial activity had earlier been synthesised [3–7]; compounds with good to moderate antimicrobial activity as compared to the well-known broad-spectrum antifungal and antibacterial agent ciclopirox [4]. The synthesis of antibacterial diketopiperazine Indole alkaloids was also carried out [8]. Developments in the field of structural biology have provided numerous opportunities for synthetic chemists to design small-molecular drug with better biological action and minimal side effects for a syndrome-specific target [9,32,33]. Molecular docking is an interesting area to understand as it explains drug biomolecular interaction for the rational of the design and discovery of drug. It is a systematic study done by placing a ligand into the preferred binding location of the target region of the DNA of the protein (the receptor) mainly in a non-covalent manner to produce a stable complex of prospective efficacy and more specificity [10,11]. The information obtained from the docking procedure can be used to predict how stable a complex species is, also its binding energy and free energy [10,11]. Communicable diseases remain the principal cause of death in the universe and bacteria have become more resilient to orthodox antibiotics in recent years. The introduction of antibiotics has played a vital role in reducing morbidity due to contagious diseases. The extensive use of antimicrobial medicines is thought to further strengthen the advent of antimicrobial-resistant strains. This condition of increased drug resistance, coupled with the variety of side effects by present market antimicrobial agents and the advent of diseases with no discovered treatment yet, makes the search for improved antimicrobial drugs a highly relevant and important subject which is the aim of this research. The uncontrollable rise in the multidrug-resistant (MDR) bacteria to the present antibacterial drugs is a very important issue as it represents the major cause of treatment failure and has increased the percentage of death rate globally [12,13].

Previously, microwave-assisted synthesis of some novel Indole containing compounds was carried out, the antifungal and antibacterial assays of the new products were also determined [34].

This study was therefore designed to synthesize, characterize, and dock the synthetic analogues for ligand-receptor interaction on the enzyme on four antibacterial receptors, PDB code: 3CR7, 3FHV, 6L1L and 6SPC, also, to screen the N-acylhydrazone derivatives against selected microbes in order to evaluate their antimicrobial potentials. Thus, it becomes a need to develop novel antibacterial agents that will not only prevent the process of drug resistance but also improve on the treatment of infectious diseases.

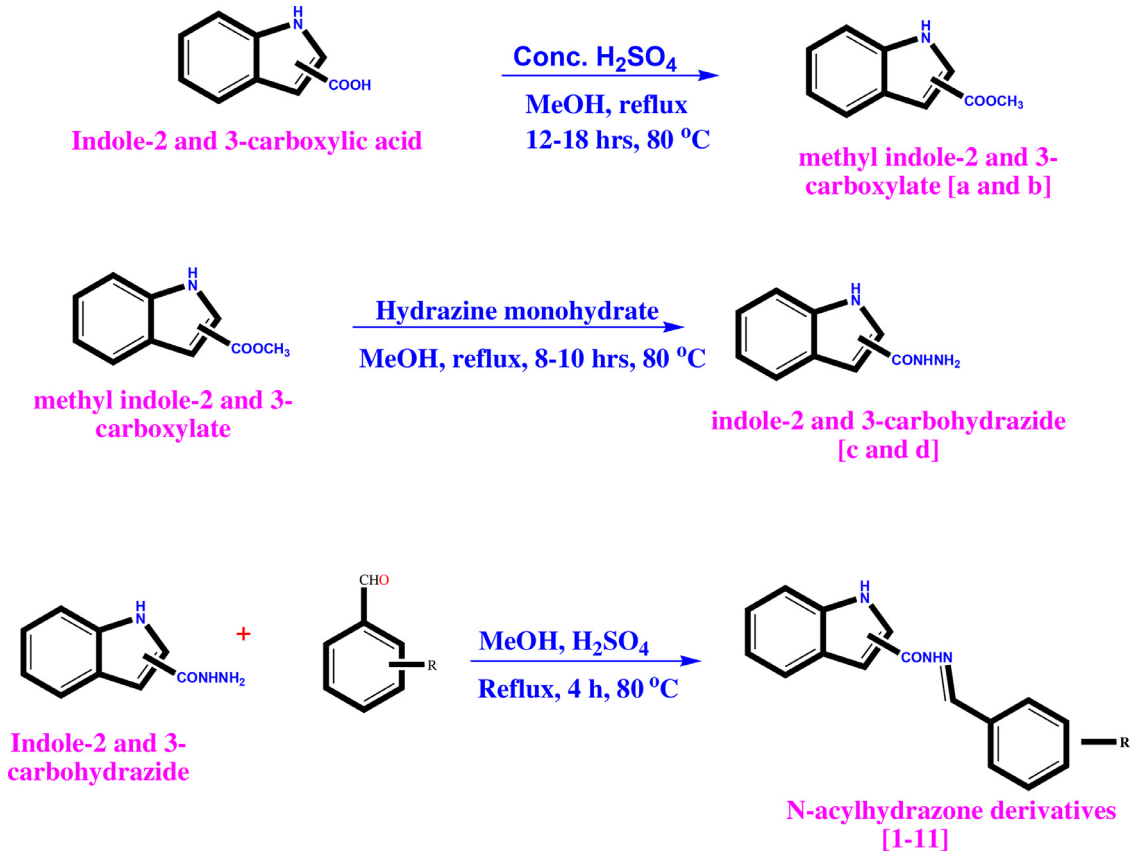


Fig. 1. Schematic illustrations of indole esters, hydrazides and N-acylhydrazone preparation.

Materials and methods

General

Melting point was determined with a Buchi-535 melting point apparatus. The thin-layer chromatography (TLC) was carried out on pre-coated silica gel GF-254 aluminum plates. Reagents and solvents were obtained from Sigma-Aldrich (USA) and Merck (Germany). Spots on TLC plate was visualised under an ultraviolet lamp at 254 nm and 366 nm. Electron impact mass spectra (EI-MS) was recorded on a Finnigan MAT 311 mass spectrometer. The ^{1}H NMR spectra were recorded in deuterated DMSO-d_6 on Avance Bruker AM-300, and AMX-400 MHz machines. Chemical shifts (δ) were shown on ppm scale and coupling constants or J values were expressed in Hz.

Synthesis

Synthesis of Methyl 1H-indole-(2) and (3)-carboxylate (a and b)

Methyl 1H-indole-(2) and (3)-carboxylate was synthesised using the esterification process, by dissolving 1.61 g (10 mmol) of 1H-indole-2-carboxylic acid and 1H-indole-3-carboxylic acid in 20 mL methanol (solvent), a catalytic amount of concentrated sulphuric acid (0.5 mL) was added. The mixture was refluxed for 10–18 h and the progress of the reaction was monitored by TLC (Fig. 1). Upon consumption of the starting material, indicating the completion of the reaction, which lasted for 12–18 h, the reaction mixture was poured in ice flakes and precipitation occurred. The precipitate was filtered, dried, and recrystallised in ethanol.

Synthesis of 1H-indole-(2) and (3)-carbohydrazide (c and d)

Exactly 0.875 g (≈5mmole) each of 1H-indole-2- and 1H-indole-3 carboxylates were further subjected to hydrazide formation by dissolving the esters in 15 mL of methanol respectively and refluxed for 12 h. The progress of the reaction was monitored using TLC. Upon consumption of the starting material, which lasted for 8–10 h, the reaction mixture was poured into crushed ice, precipitation occurred and the precipitate was filtered, dried to give the intermediate benzohydrazide compounds (Fig. 1).

General procedure for the preparation of N-acylhydrazone derivatives (1–11)

Equal amount (1 mmole) of appropriate aromatic aldehyde was added to a solution of hydrazide compound in 20 mL of methanol, in presence of catalytic amount of acetic acid (0.2 mL). Reaction mixture was heated at 80 °C under reflux with continuous stirring for about 2–6 h. The progress of the reaction was monitored by TLC. Upon consumption of the starting materials, the reaction mixture was poured into ice flakes. Precipitation was observed, the precipitate was filtered and washed with cold water. Scheme of the reaction is as shown in Fig. 1. The reaction was carried out using the standard method [14–16].

The work up of most of the N-acylhydrazone compounds, were carried out in water; for those insoluble in water. For those soluble in water, the solvents were allowed to dry, the resulting mixture neutralised with sodium bicarbonate solution, the resulting solution was partitioned with ratio (1:1) dichloromethane and water, the organic phase was dried and recrystallised in ethanol to obtain a pure product. This afforded the respective N-acylhydrazone derivatives of indole (compound 2, 6 and 9). The synthetic analogues, were characterised using different spectroscopic techniques which include ^{1}H NMR (Proton Nuclear Magnetic Resonance) and EI-MS (Electron Impact Mass Spectrometry).

Further purification of synthetic compounds was done by crystallization in ethanol solvent (Fig. 1). Spectroscopic techniques EI-MS and ^{1}H NMR were used to elucidate the structures of synthetic analogues (Table 1).

Antimicrobial inhibition analysis

Preparation of samples for antimicrobial assay

About 1.0 g each of the sample was weighed and dissolved into 5.0 mL of Dimethyl sulfoxide (DMSO), for proper dissolution to give 200 mg/mL. Serial dilution was carried out to obtain a lower concentrations of 100, 50, 25, 12.5 and 6.26 mg/mL solutions respectively. Gentamycin (5 mg/mL) was used as a positive control for bacteria while Tioconazole (70%) for fungi and the negative control contained the solvent that was used in dissolving the compounds [17,18].

Agar diffusion for bacteria (Pour plate method)

Cultures of the organisms used were prepared overnight. The organisms used include three Gram-positive bacteria (*Staphylococcus aureus* (ATCC 29,213), *Bacillus subtilis* (ATCC 9372), *Stregitococcus viridians*) and three Gram-negative bacteria [*Escherichia coli* (ATCC 25,922), *Pseudomonas aeruginosa* (ATCC 27,853), *Salmonella typhi* (ATCC 50,013)]. A sterile cork-borer was used to make a hole (well) in the set plates. The test solutions of the synthesised compounds were introduced into each of the cups on each plate ensuring no spillage take place. Same amount of the standard antimicrobial control and solvent were introduced using syringes into the remaining cups on each plate to serve as positive and negative controls respectively. The plates were then left to stand at room temperature for about 2 h, allowed to diffuse into the medium, turned upside-down and thereafter incubated at 37 °C for 24 h in an incubator. Clear zones of inhibition were observed. Activity or inactivity of each compound were observed. Activity of the compounds were recorded in triplicate, inhibition were measured in millimeter (mm) with a transparent well-calibrated ruler. The positive control for bacteria is gentamycin at the concentration at 5 mg/mL. The analysis was done in triplicates in accordance to the reported method [17,18].

Agar diffusion plate method for fungi

Sterile Sabouraud Dextrose Agar (SDA) was poured aseptically into the sterile plates and allowed to cool down for 45 min. The test organisms; *Candida albicans (ATCC 90,028), Rhizopus stolonifer, Aspergillus niger* (ATCC 11,394) and *Penicillum* notatum; clinical isolates, were spread on the surface using a sterile spreader. Then, a sterile cork-borer was used to create wells inside the plates. The same procedure described for antibacterial activity above was followed from this stage. The positive control for the fungi tioconazole 70%. All the plates for the fungi were incubated at 28 °C for 48 h. The clear zones of inhibition were observed and recorded by measuring in millimeter (mm) using a well graduated ruler [17,18].

Molecular docking protocol

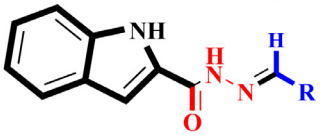
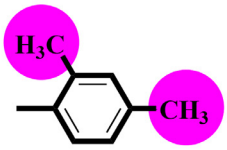
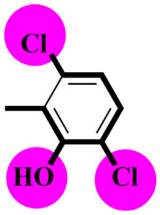
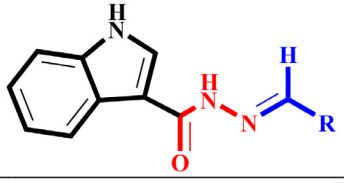
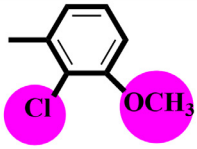
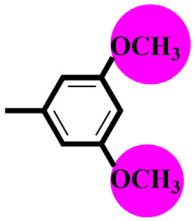
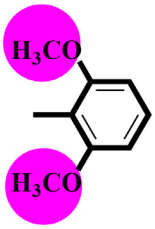
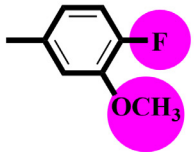
Information about the active sites of each of the four receptors used was obtained from their respective associating literature that accompanied the X-ray crystallography structure on the RCSB webserver (http//:www.rcsb.org) and BIOVIA software [19].

All ligands were modeled using the ChemDraw® Ultra software package [28]. Ligands optimization was done using the MMFF (Molecular mechanics Force Field) at steepest descent for the 4 receptors via Avogadro® software package [29]. The 13 ligands were further re-optimised using the Open babel's plugin in the Pyrex® software [30] and the compounds were then converted into AutoDock's ligand format, which registers each ligand using a new extension called .pdbqt.

The PDB codes of the four receptors used for the docking studies are 3CR7, 3FHV, 6L1L and 6SPC. The receptors were downloaded from the RCSB webserver (http//:www.rcsb.org) and saved in PDB format. The downloaded protein were then prepared by removing associated water of crystallization, polar hydrogens computed while non-polar hydrogens were merged together. Kollman's charges were added and resultant structure were stored in AutoDock' PDBQT format using AutoDock® tools software package [31] (Tables 2a, 2b, 2c, 2d).

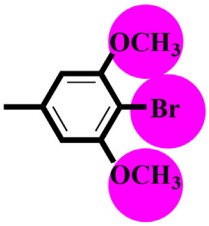
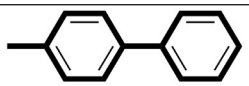
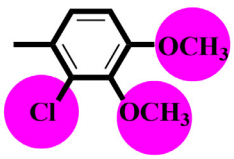
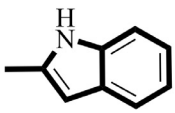
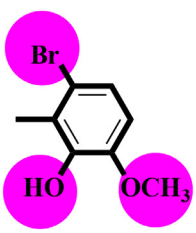
Molecular Docking of the ligands and receptors was done using the Pyrx® [30] software package and docked score recorded accordingly Table 6.

Table 1
Summary of the synthesised compounds.

Compounds	R
Group "A" (Schiff Bases of 1*H*-indole-2-carbohydrazide) 	
1	
2	
Group "B" (1H-indole-3-carbohydrazide Derivatives) 	
3	
4	
5	
6	

(*continued on next page*)

Table 1 (*continued*)

7	
8	
9	
10	
11	

Statistical analysis

All data are expressed as mean ± SD of triplicate analyses. Statistical significance was analyzed by the one-way ANOVA and post-hoc tests by using the statistical software package. Differences with $P < 0.05$ were considered as statistically significant.

Results and discussion

Characterization of N'-(2, 4-dimethylbenzylidene)-1H-indole-2-carbohydrazide (Compd. 1)

A white solid compound which was obtained in a yield of 70.8% (0.150 g) with a melting point of 167–168 °C and a 0.60 (ethyl acetate/MeOH, 9.5: 0.5) R_f value.

The resonances, δ (ppm) from ^{1}H NMR spectrum (400 MHz, DMSO-d_6) display a total of thirteen peaks. They are 11.78 (s, 1H, NH $_{\text{ring}}$), the most deshielded peak belongs to N-H of the Indole moiety. 8.69 (s, 1H, NH-CO), 7.77 (d, $J_{4,5} = 8.0$ Hz, 1H, H-4), 7.67 (d, $J_{7,6} = 8.0$ Hz, 1H, H-7), 7.46 (d, $J_{6',5'} = 8.5$ Hz, 1H, H- 6 '), 7.28 (s, 1H, N=CH$_{(\text{imine proton})}$), 7.21 (s, 1H, H-3), 7.08 (m, 3H, H-6, H-5 ' and H-6), 2.42 (s, 3H, CH_3–2′) and 2.30 (s, 3H, CH_3–4′). The two singlet peaks 2.42 and 2.30, observed in the upfield region were assigned to the methyl groups at 2′ and 4′ positions, respectively [Supplementary data, Fig. 1a].

The EI-MS spectrum showed the following peaks: *m/z* (relative abundance%), 292 ($M^+ + 1$, 9), 291 (M^+, 42), 160 (41), 161 (30), 145 (10), 144 (100), 142 (71), 116 (18), 88 (22) [Supplementary data, Fig. 1b].

Table 2a
Antimicrobial Result of N-acylhydrazone Derivatives in millimeter (mm).

Conc. mg/ml	S. aureus	E. coli	B. subtilis	P. aeruginosa	S. typhi	S. viridians	C. albicans	A. niger	P. notatum.	R. studonite	Compound
100	24 ± 0.00	26 ± 0.00	24 ± 0.00	22 ± 0.00	22 ± 0.00	22 ± 0.00	20 ± 0.70	18 ± 0.00	18 ± 0.00	18 ± 0.70	1
50	21 ± 0.70	22 ± 0.70	21 ± 0.00	18 ± 1.41	18 ± 0.00	18 ± 0.70	18 ± 0.00	16 ± 0.70	16 ± 0.00	16 ± 0.00	
25	17 ± 0.70	18 ± 0.00	18 ± 0.70	16 ± 0.00	16 ± 0.00	16 ± 0.70	16 ± 0.00	14 ± 0.70	14 ± 0.70	14 ± 0.00	
12.5	15 ± 0.00	14 ± 0.70	16 ± 1.41	14 ± 0.00	14 ± 0.00	14 ± 0.70	14 ± 0.70	12 ± 0.00	12 ± 0.00	11 ± 0.70	
6.25	12 ± 0.00	12 ± 0.00	14 ± 0.00	10 ± 1.41	10 ± 0.00	10 ± 0.00	10 ± 0.70	10 ± 0.00	10 ± 0.70	10 ± 0.00	
100	26 ± 1.41	24 ± 0.00	25 ± 1.41	24 ± 1.41	24 ± 0.00	20 ± 0.00	20 ± 0.00	18 ± 1.41	18 ± 1.41	20 ± 1.41	2
50	22 ± 0.00	22 ± 0.00	22 ± 0.00	22 ± 0.00	21 ± 0.70	18 ± 0.00	18 ± 0.00	16 ± 1.41	16 ± 0.00	18 ± 0.00	
25	18 ± 0.00	18 ± 0.70	19 ± 0.70	18 ± 0.70	18 ± 0.70	16 ± 0.70	16 ± 0.70	14 ± 0.00	14 ± 0.00	16 ± 0.70	
12.5	14 ± 0.70	14 ± 0.00	17 ± 1.41	14 ± 0.00	15 ± 1.41	14 ± 0.00	14 ± 0.00	12 ± 1.41	10 ± ± 0.00	12±0.70	
6.25	11 ± 0.00	12 ± 0.00	13 ± 1.41	10 ± 0.00	12 ± 1.41	10 ± 0.00	10 ± 0.70	10 ± 0.70	–	–	
100	18 ± 1.41	16 ± 0.00	14 ± 0.70	17 ± 0.70	15 ± 0.70	16 ± 1.40	16 ± 0.00	14 ± 0.00	14 ± 0.70	14 ± 1.40	3
50	16 ± 1.41	14 ± 0.70	12 ± 1.40	15 ± 0.00	14 ± 0.00	14 ± 0.00	14 ± 1.40	12 ± 0.00	12 ± 1.40	12 ± 0.00	
25	14 ± 1.41	12 ± 0.00	10 ± 0.00	13 ± 0.00	13 ± 0.00	12 ± 1.40	12 ± 0.00	10 ± 0.70	10 ± 0.00	10 ± 0.70	
12.5	12 ± 0.00	10 ± 0.70	10 ± 0.00	11 ± 0.70	11 ± 0.00	10 ± 0.70	10 ± 0.00	–	–	–	
6.25	10 ± 0.00	–	–	10 ± 1.41	10 ± 1.40	–	–	–	–	–	
-VeControl	–	–	–	–	–	–	–	–	–	–	
+Ve control	36 ± 1.41	38 ± 1.41	36 ± 1.41	38 ± 1.41	38 ± ± 0.00	40 ± 0.00	26 ± 1.41	28 ± ± 0.00	28 ± 0.00	26 ± 4.24	Controls

Note: *S. aureus* = *Staphylococcus aureus* (Gram-positive bacteria), *E.coli* = *E. coli* (Gram-negative bacteria), *B. subtilis.*= *Bacillus subtilis* (Gram-positive bacteria), *P. aeruginosa* = *Pseudomonas aeruginosa* (Gram-negative bacteria), *S. typhi* = *S. typhi* (Gram-negative bacteria), *S. viridians* = *Stregitococcus viridians* (Gram-positive bacteria), *C. albicans.*= *Candida albicans* (fungi), *A. niger* = *Aspergillus niger* (fungi), *P. notatum.* = *P. notatum* (fungi),), *R. studonite=Rhizopus stolonifer* fungi).
Negative control = Dimethyl sulfoxide (DMSO).
Positive control = Gentamicin 10 µg/ml (bacteria), Triconazole 70% (fungi).
The result represents mean ± SD of triplicate analysis.

Table 2b
Antimicrobial Result of N-acylhydrazone Derivatives in millimeter (mm).

Conc. mg/ml	S. aureus	E. coli	B. subtilis	P. aeruginosa	S. typhi	S. viridians	C. albicans	A. niger	P. notatum	R. studonite	Compound
100	26 ± 1.41	28 ± 0.00	24 ± 1.41	25 ± 1.41	26 ± 0.70	24 ± 1.41	20 ± 1.41	18 ± 1.41	18 ± 0.00	18 ± ± 0.00	4
50	24 ± 0.70	26 ± 0.70	21 ± 0.00	22 ± ± 1.41	24 ± 1.41	18 ± 0.00	18 ± 1.41	16 ± 0.00	16 ± ± 0.00	16 ± 1.41	
25	21 ± 1.41	21 ± 0.00	19 ± 0.00	18 ± 0.00	21 ± 1.41	16 ± 0.00	16 ± 0.00	14 ± 0.00	14 ± 0.00	14 ± 0.00	
12.5	15 ± 0.00	18 ± 1.41	16 ± 1.41	14 ± 1.41	17 ± 0.00	14 ± 0.00	14 ± 1.41	12 ± 1.41	12 ± 0.00	12 ± 0.00	
6.25	12 ± 0.00	14 ± 0.70	14 ± 0.00	10 ± 0.00	15 ± 0.70	10 ± 0.00	10 ± 0.00	10 ± 0.00	10 ± 0.00	10 ± 0.70	
100	20 ± 0.00	18 ± 0.70	19 ± 0.00	19 ± 0.70	17 ± 0.00	18 ± 0.00	19 ± 1.41	17 ± 0.00	19 ± 0.00	16 ± 0.00	5
50	18 ± 1.41	16 ± 0.00	17 ± 1.41	16 ± 0.00	15 ± 0.70	16 ± 0.70	17 ± 1.41	15 ± 0.70	17 ± 0.70	14 ± 0.00	
25	16 ± 0.00	14 ± 0.70	15 ± 0.00	14 ± 0.00	13 ± 0.70	14 ± 0.00	15 ± 0.00	13 ± ± 1.41	15 ± 1.41	12 ± 0.70	
12.5	14 ± 0.70	12 ± 0.70	12 ± 0.70	12 ± 0.70	11 ± 0.00	12 ± 0.00	11 ± 1.41	10 ± 0.00	12 ± 1.41	10 ± 0.70	
6.25	10 ± 0.00	10 ± 0.70	10 ± 0.00	10 ± 1.41	10 ± 0.70	10 ± 0.00	10 ± 0.00	10 ± 0.00	10 ± 0.70	10 ± 0.00	
100	26 ± 0.00	28 ± 0.70	24 ± 0.00	25 ± 0.00	26 ± 0.00	24 ± 0.00	20 ± 0.00	18 ± 0.00	18 ± 0.00	18 ± 0.00	6
50	24 ± 0.00	26 ± 0.00	21 ± 0.00	22 ± 0.00	24 ± 0.70	18 ± 0.00	18 ± 0.00	16 ± 1.41	16 ± 0.00	16 ± 0.70	
25	21 ± 0.00	22 ± 0.00	19 ± 0.70	18 ± 1.41	21 ± 0.00	16 ± 0.00	16 ± 0.00	14 ± 0.00	14 ± 1.41	14 ± 0.00	
12.5	15 ± 0.00	18 ± 0.70	16 ± 0.00	14 ± 0.00	17 ± 0.00	14 ± 0.70	14 ± 0.00	12 ± 0.70	12 ± 0.70	12 ± 0.70	
6.25	12 ± 0.00	14 ± 0.00	14 ± 0.70	10 ± 0.70	15 ± 0.70	10 ± 0.00	10 ± 0.70	10 ± 0.00	10 ± 0.00	10 ± 0.00	
-Ve control	–	–	–	–	–	–	–	–	–	–	
+Ve control	36 ± 1.41	38 ± 1.41	36 ± 1.41	38 ± 1.41	38 ± 0.00	40 ± 0.00	26 ± 1.41	28 ± 0.00	28 ± 0.00	26 ± 4.24	Controls

Note: *S. aureus* = *Staphylococcus aureus* (Gram-positive bacteria), *E.coli* = *E. coli* (Gram-negative bacteria), *B. subtilis.*= *Bacillus subtilis* (Gram-positive bacteria), *P. aeruginosa* = *Pseudomonas aeruginosa* (Gram-negative bacteria), *S. typhi* = *S. typhi* (Gram-negative bacteria), *S. viridians* = *Stregitococcus viridians* (Gram-positive bacteria), *C. albicans.*= *Candida albicans* (fungi), *A. niger* = *Aspergillus niger* (fungi), *P. notatum.* = *P. notatum* (fungi),), *R. studonite*=*Rhizopus stolonifer* fungi).
Negative control = Dimethyl sulfoxide (DMSO).
Positive control = Gentamicin 10 µg/ml (bacteria), Triconazole 70% (fungi).
The result represents mean ± SD of triplicate analysis.

Table 2c
Antimicrobial Result of N-acylhydrazone Derivatives in millimeter (mm).

Conc. mg/ml	S. aureus	E. coli	B. subtilis	P. aeruginosa	S. typhi	S. viridians	C. albicans	A. niger	P.notatum.	R. studonite	Compd
100	20 ± 0.00	21 ± 0.70	18 ± 0.00	21 ± 0.00	20 ± 0.00	21 ± 0.00	18 ± 0.00	18 ± 0.00	16 ± 0.00	14 ± 0.00	7
50	18 ± 0.00	19 ± 0.00	16 ± 0.00	18 ± 0.70	18 ± 0.00	18 ± 0.00	16 ± 0.70	16 ± 0.00	14 ± 0.00	12 ± 0.70	
25	16 ± 0.70	17 ± 0.00	14 ± 1.41	16 ± 0.00	16 ± 0.70	16 ± 0.00	14 ± 0.00	14 ± 0.00	12 ± 0.00	10 ± 0.00	
12.5	14 ± 1.41	13 ± 1.41	12 ± 0.00	14 ± 1.41	14 ± 0.00	14 ± 0.70	12 ± 0.00	10 ± 0.00	10 ± 0.00	–	
6.25	10 ± 1.41	10 ± 1.41	10 ± 0.70	10 ± 0.00	10 ± 0.00	10 ± 0.00	–	–	–	–	
100	24 ± 1.41	26 ± 1.41	24 ± 1.41	22 ± 1.41	26 ± 0.70	24 ± 0.70	20 ± 1.41	18 ± 0.00	16 ± 0.00	18 ± 1.41	8
50	21 ± 0.00	23 ± 0.70	22 ± 1.41	18 ± 1.41	22 ± 0.70	22 ± 0.00	18 ± 1.41	16 ± 0.00	14 ± 0.70	16 ± 0.70	
25	18 ± 0.00	21 ± 1.41	18 ± 1.41	14 ± 0.00	18 ± 0.00	18 ± 1.41	16 ± 0.70	14 ± 0.70	12 ± 1.41	14 ± 0.70	
12.5	14 ± 1.41	17 ± 1.41	16 ± 0.70	12 ± 0.00	15 ± 0.00	16 ± 1.41	14 ± 0.70	12 ± 1.41	10 ± 0.00	10 ± 0.00	
6.25	10 ± 1.41	14 ± 0.00	12 ± 0.70	10 ± 0.00	12 ± 0.00	12 ± 1.41	10 ± 0.70	10 ± 1.41	10 ± 0.70	10 ± 0.00	
100	18 ± 0.70	20 ± 0.00	21 ± 0.00	19 ± 0.00	18 ± 0.00	21 ± 0.00	18 ± 0.00	18 ± 0.00	18 ± 0.00	16 ± 0.00	9
50	16 ± 0.00	18 ± 0.70	18 ± 0.70	16 ± 0.00	16 ± 0.00	19 ± 0.00	16 ± 0.00	14 ± 0.00	16 ± 0.70	14 ± 0.00	
25	14 ± 1.41	16 ± 0.00	16 ± 0.00	14 ± 1.41	14 ± 1.41	16 ± 0.70	14 ± 0.70	12 ± 0.00	14 ± 1.41	10 ± 0.00	
12.5	12 ± 0.00	14 ± 1.41	14 ± 0.00	12 ± 1.41	10 ± 0.00	13 ± 0.00	12 ± 1.41	10 ± 0.00	10 ± 0.00	10 ± 0.00	
6.25	10 ± 1.41	10 ± 0.00	10 ± 0.00	10 ± 0.00	–	10 ± 0.00	10 ± 1.41	–	–	–	
-Ve control	–	–	–	–	–	–	–	–	–	–	
+Ve control	36 ± 1.41	38 ± 1.41	36 ± 1.41	38 ± 1.41	38 ± 0.00	40 ± 0.00	26 ± 1.41	28 ± 0.00	28 ± 0.00	26 ± 4.24	Controls

Note: *S. aureus* = *Staphylococcus aureus* (Gram-positive bacteria), *E.coli* = *E. coli* (Gram-negative bacteria), *B. subtilis.*= *Bacillus subtilis* (Gram-positive bacteria), *P. aeruginosa* = *Pseudomonas aeruginosa* (Gram-negative bacteria), *S. typhi* = *S. typhi* (Gram-negative bacteria), *S. viridians* = *Stregitococcus viridians* (Gram-positive bacteria), *C. albicans.*= *Candida albicans* (fungi), *A. niger* = *Aspergillus niger* (fungi), *P. notatum.* = *P. notatum* (fungi),), *R. studonite*=*Rhizopus stolonifer* fungi).
Negative control = Dimethyl sulfoxide (DMSO).
Positive control = Gentamicin 10 µg/ml (bacteria), Triconazole 70% (fungi).
The result represents mean ± SD of triplicate analysis.

Table 2d
Antimicrobial Result of N-acylhydrazone Derivatives in millimeter (mm).

Conc. mg/ml	S. aureus	E. coli	B. subtilis	P. aeruginosa	S. typhi	S. viridians	C. albicans	A. niger	P. notatum	R. studonite	Compound
100	18 ± 0.00	16 ± 0.00	18 ± 0.00	19 ± 0.00	16 ± 1.41	17 ± 0.00	16 ± 1.40	14 ± 0.70	16 ± 0.00	16 ± 0.00	10
50	16 ± 0.00	14 ± 0.70	16 ± 0.70	17 ± 0.00	14 ± 0.00	15 ± 1.41	14 ± 0.00	12 ± 0.00	14 ± 0.70	14 ± 0.70	
25	14 ± 1.41	12 ± 0.00	14 ± 0.00	15 ± 0.70	12 ± 0.70	12 ± 1.41	12 ± 0.70	10 ± 0.00	12 ± 0.70	12 ± 0.70	
12.5	12 ± 0.70	10 ± 0.00	12 ± 0.00	12 ± 0.00	10 ± 0.00	10 ± 0.70	10 ± 0.00	–	10 ± 0.00	10 ± 1.41	
6.25	10 ± 0.00	10 ± ± 0.70	10 ± 0.00	10 ± 0.00	10 ± 0.00	–	–	–	10 ± 0.00	–	
100	16 ± 0.00	16 ± 0.00	16 ± 0.00	17 ± 0.00	14 ± 0.00	14 ± 0.00	12 ± 0.00	12 ± 0.00	12 ± 0.70	–	11
50	14 ± 0.70	14 ± 0.70	14 ± 1.41	15 ± 0.00	12 ± 0.70	12 ± 0.00	10 ± 0.70	10 ± 0.00	10 ± 0.00	–	
25	12 ± 0.00	12 ± 0.00	12 ± 0.00	12 ± 0.70	10 ± 0.00	10 ± 0.00	–	–	–	–	
12.5	10 ± 0.00	10 ± 1.41	10 ± 0.70	10 ± 0.00	–	–	–	–	–	–	
6.25	–	–	–	–	–	–	–	–	–	–	
-Ve control	–	–	–	–	–	–	–	–	–	–	
+Ve control	36 ± 1.41	38 ± 1.41	36 ± 1.41	38 ± 1.41	38 ± 0.00	40 ± 0.00	26 ± 1.41	28 ± 0.00	28 ± 0.00	26 ± 4.24	Controls

Note: *S. aureus* = *Staphylococcus aureus* (Gram-positive bacteria), *E.coli* = *E. coli* (Gram-negative bacteria), *B. subtilis.*= *Bacillus subtilis* (Gram-positive bacteria), *P. aeruginosa* = *Pseudomonas aeruginosa* (Gram-negative bacteria), *S. typhi* = *S. typhi* (Gram-negative bacteria), *S. viridians* = *Stregitococcus viridians* (Gram-positive bacteria), *C. albicans.*= *Candida albicans* (fungi), *A. niger* = *Aspergillus niger* (fungi), *P. notatum.* = *P. notatum* (fungi),), *R. studonite*=*Rhizopus stolonifer* fungi).
Negative control = Dimethyl sulfoxide (DMSO).
Positive control = Gentamicin 10 µg/ml (bacteria), Triconazole 70% (fungi).
The result represents mean ± SD of triplicate analysis.

M^+at *m/z* 291. The cleavage of the Indole-CO bond gave a fragment at *m/z* 116; $C_8H_6N^+$. A fragmentation at NH-N bridge gave fragment ion at *m/z* 161; $C_9H_8N_2O^+$, called 1*H*-indole-2- carboxamide cation and a loss of proton from $C_9H_8N_2O^+$, gave a fragment ion at *m/z* 160; $C_9H_7N_2O^+$. The base peak was observed at *m/z* 143; $C_9H_5NO^+$. All the synthesised compounds (**1–11**) are similar in structure, hence, show similarities in their spectral features (^{1}H NMR, and mass spectrometry).

Spectral data of synthetic compounds (2-11)

Characterization of N'-(3, 5-dichloro-2-hydroxybenzylidene)-1H-indole-2-carbohydrazide (Compd. 2)

White solid powder, yield of 80.4% (0.19 g) with a melting point of 211–213 °C and a 0.58 (ethyl acetate/MeOH, 9.0: 1.0) R_f value. ^{1}H NMR spectrum (500 MHz, DMSO–d_6) δ (ppm): 12.54 (s, 1H, NH $_{\text{ring}}$), 12.44 (s, 1H, OH-2′), 11.91 (s, 1H, NH-CO), 8.56 (s, 1H, N=CH$_{(\text{imine proton})}$, 7.69 (sd, 2H, H-4, H-6′), 7.63 (d, $J_{4',6'}$ = 2.5 Hz, 1H, H-4′), 7.35 (d, $J_{3,NH}$ = 1.5 Hz, 1H, H-3), 7.24 (m, 1H, H- 6), 7.08 (m, 1H, H-5). The EI-MS spectrum: *m/z* (relative abundance%), 349 (M^++ 2, 22), 347 (M^+, 39), 160 (18), 161 (30), 145 (22), 144 (100), 143 (26), 116 (21), 89 (31).

Characterization of N'-(2-chloro-3-methoxybenzylidene)-1H-indole-3-carbohydrazide (Compd. 3)

The white solid compound was obtained in a yield of 80.4% (0.19 g) with a melting point of 205–207 °C and a 0.58 (ethyl acetate/MeOH, 9: 1) R_f value. ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ (ppm): 11.74 (s, 1H, NH $_{\text{ring}}$), 11.59 (s, 1H, NH-CO), 8.71 (s, 1H, N=CH$_{(\text{imine proton})}$, 8.25 (s, 1H, H-2), 8.19 (d, $J_{4,5}$ = 7.5 Hz, 1H, H-4), 7.61 (d, $J_{7,6}$ = 8.0 Hz, 1H, H-7), 7.48 (d, $J_{6',5'}$ = 8.0 Hz, 1H, H-6′), 7.39 (t, $J_{5',6'}$ = 8.0 Hz, 1H, H-4), 7.17 (m, 3H, H-5′, H-5, H-6), 3.89 (s, 3H, OCH_3–3′).

The EI-MS spectrum (Fig. 4.3c) showed the following peak: *m/z* (relative abundance%), 327 (M^+, 4), 160 (55), 161 (34), 145 (19), 144 (100), 116 (18), 89 (11).

Molecular docking evaluation

Binding free energy is the sum of all the intermolecular interactions that is present between the ligand and the target. Free energies of binding with a more negative value indicates a stronger binding capacities. Compound **10** was predicted to exhibit highest negative binding energy of −9.7 kcal/mole with the 6SPC antibacterial receptor (Table 3); followed by compound **1** with −9.2 kcal/mole. However, compound **9** showed a predicted binding energy of −7.8 kcal/mole. All the remaining compound have higher binding energies higher than the standards. Result show that compound **8** with binding energy of −8.4 kcal/mole with 3CR7 antibacterial receptor (Table 3) while the remaining compounds have energies which were comparable with the standards. Compounds **1–3, 5–8** and **10–11** exhibited higher predictive binding energies against 3FHV antibacterial receptor (Table 3) while all the compounds have comparable predictive binding energies against 6L1L antibacterial receptor in comparison with the selected standards (Table 3).

Compounds that showed good fit to the binding sites of all the four receptors compared to the standard drugs includes Compound **1, 8, 10** and **11** (Table 3).

Where blue values in the Table 3 are the predicted standards' stability, while those in green are values having a higher stability value than the selected standards.

Table 3
Binding energies of compounds binding with the four antibacterial receptors.

	BINDING AFFINITY (ΔG), kcal/mol			
Compound	3CR7	3FHV	6L1L	6SPC
1	-7.9	-7.1	-8	-9.2
2	-7.5	-6.9	-7.6	-8.9
3	-7.8	-6.7	-8.3	-8.4
4	-7.4	-6.5	-7.9	-8.3
5	-7.6	-7.1	-8.6	**-9**
6	-7.4	-7.2	-8.6	-8.4
7	-7.3	-6.8	-7.8	-8.3
8	-8.4	-7.6	-8.6	-8.6
9	-7.5	-6.6	-7.6	-7.8
10	-8	-7.2	-8.5	-9.7
11	-7.8	**-7**	-8.2	-8.4
12*	-8.2	-6.6	**-8**	-7.8
13*	-7.8	-6.5	**-9**	**-7**

**Where 12* (Amoxicillin) and 13* (Ciprofloxacin) are selected standards for comparative studies

Compound 1 was found to exhibit conventional hydrogen bonding with Lys38, of 3CR7 receptor, Asn45, Gly76 of 3FHV receptor and Lys295 of 6LIL receptor. Pi-alkyl interaction was found between Pro150, Phe75, in 3CR7 receptor (Table 4 .0), the HB interactions (Table 5 .0).

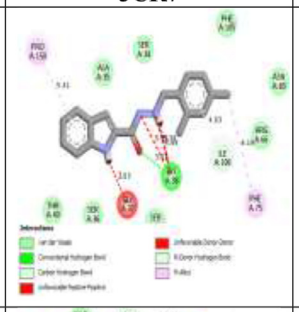
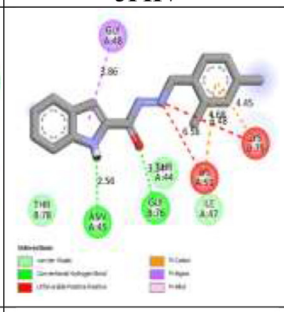
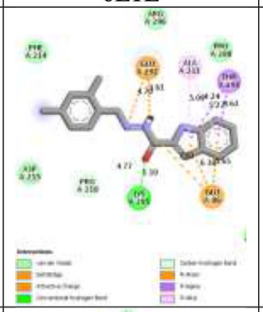
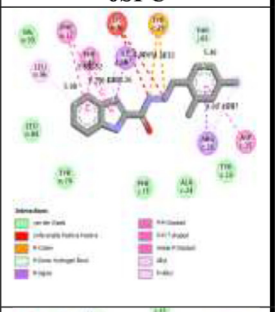
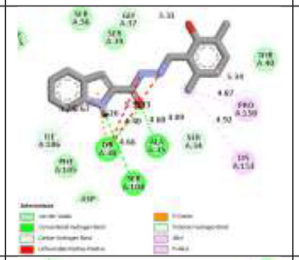
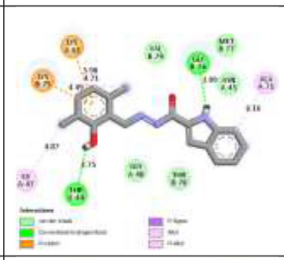
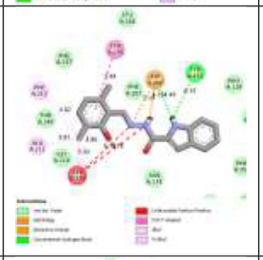
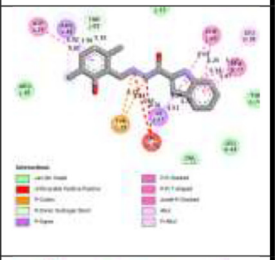
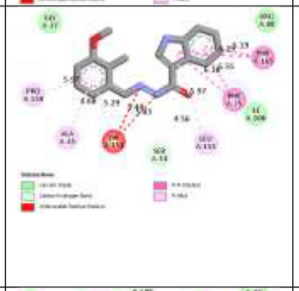
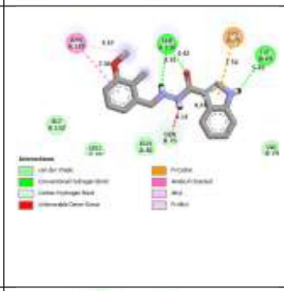
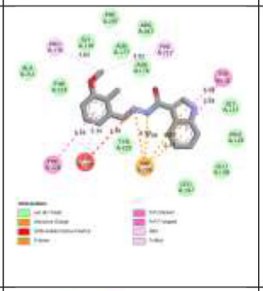
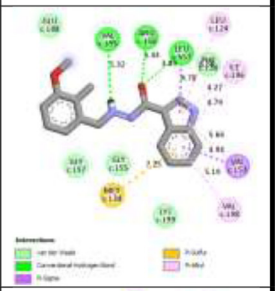
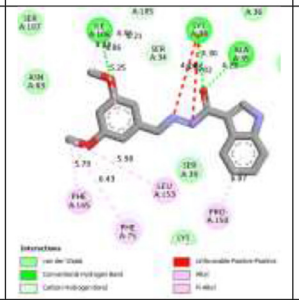
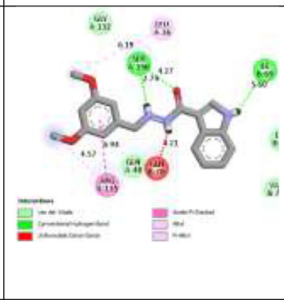
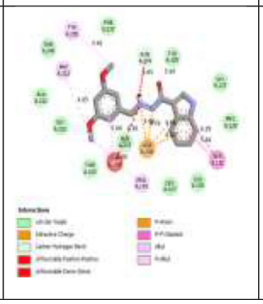
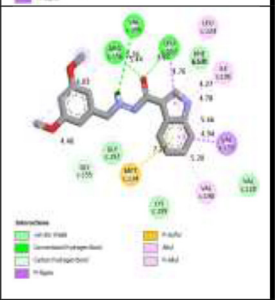
Compound 2 showed conventional hydrogen bonding with Lys38, Ser104 and Ala35 with 3CR7, Thr44 and Gly76 with 3FHV and Tyr131 in 6LIL (Table 4.0), the HB interactions (Table 5.0).

Compound 3 displayed hydrogen bonding with Ser136, Ile69 of 3FHV and Val195, Arg156 and Leu157 of 6SPC (Table 4.0), the HB interactions (Table 5.0).

Compound 4 showed conventional hydrogen bonding with Ile106, Lys38, Ala35 with 3CR7, Ser136, Ile69 with 3FHV and Val195, Leu157, Arg156 in 6SPC (Table 4.0), the HB interactions (Table 5.0).

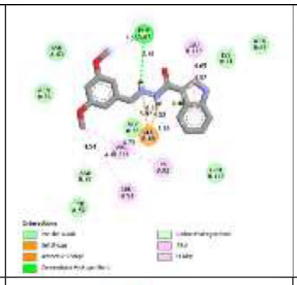
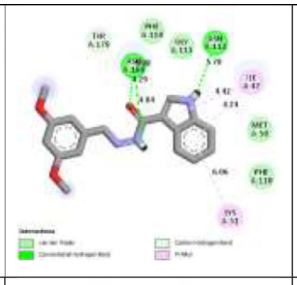
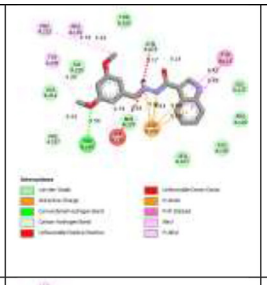
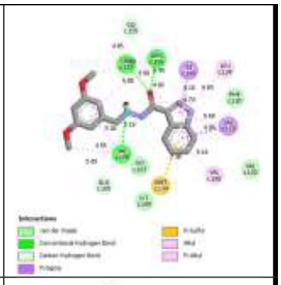
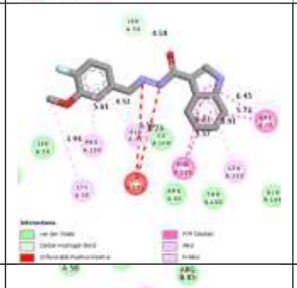
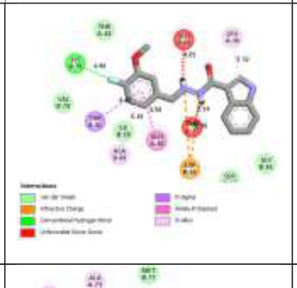
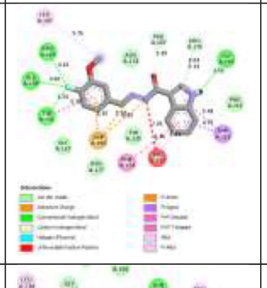
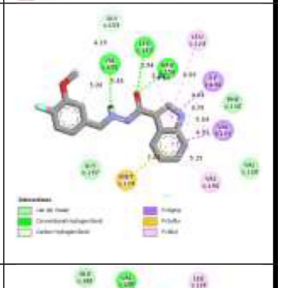
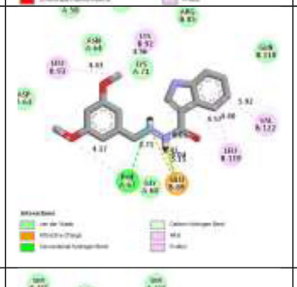
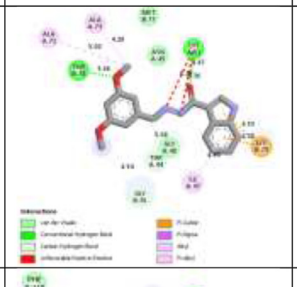
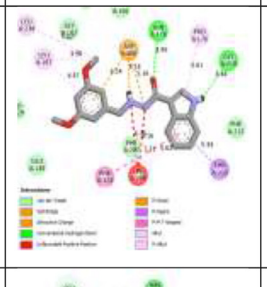
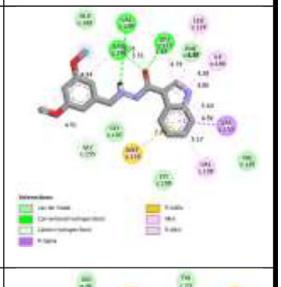
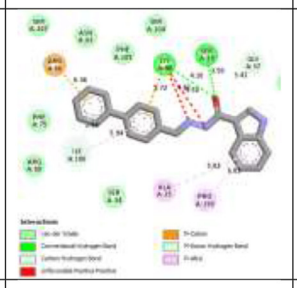
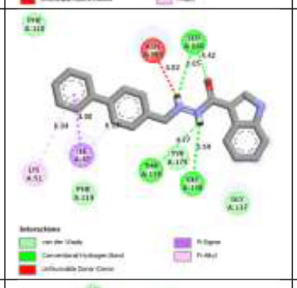
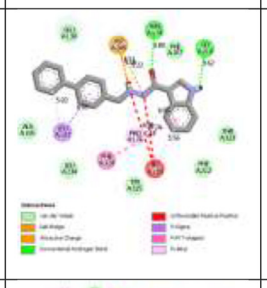
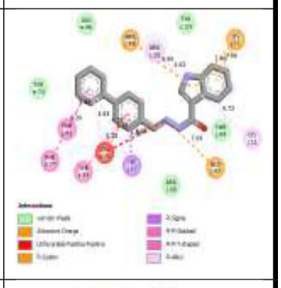
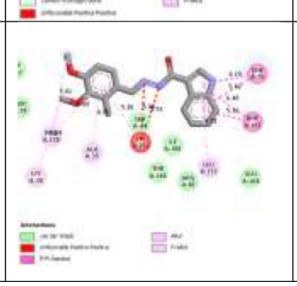
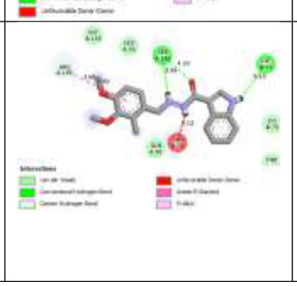
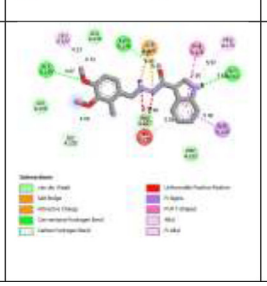
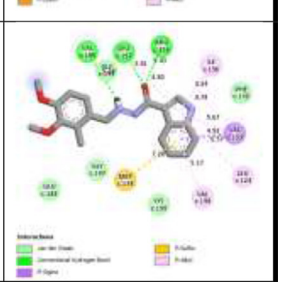
Compound 5 exhibited conventional hydrogen bonding with Phe67 in 3CR7, Asn180, Asn112 with 3FHV, Thr240 with 6LIL, Leu157, Val195, Arg154 with 6SPC (Table 4.0), the HB interactions (Table 5.0).

Table 4
3D interaction of the docked structures with their interaction with the receptor's binding site.

Compd.	3D INTERACTION			
	3CR7	3FHV	6L1L	6SPC
1				
2				
3				
4				

(*continued on next page*)

Table 4 (*continued*)

5				
6				
7				
8				
9				

(*continued on next page*)

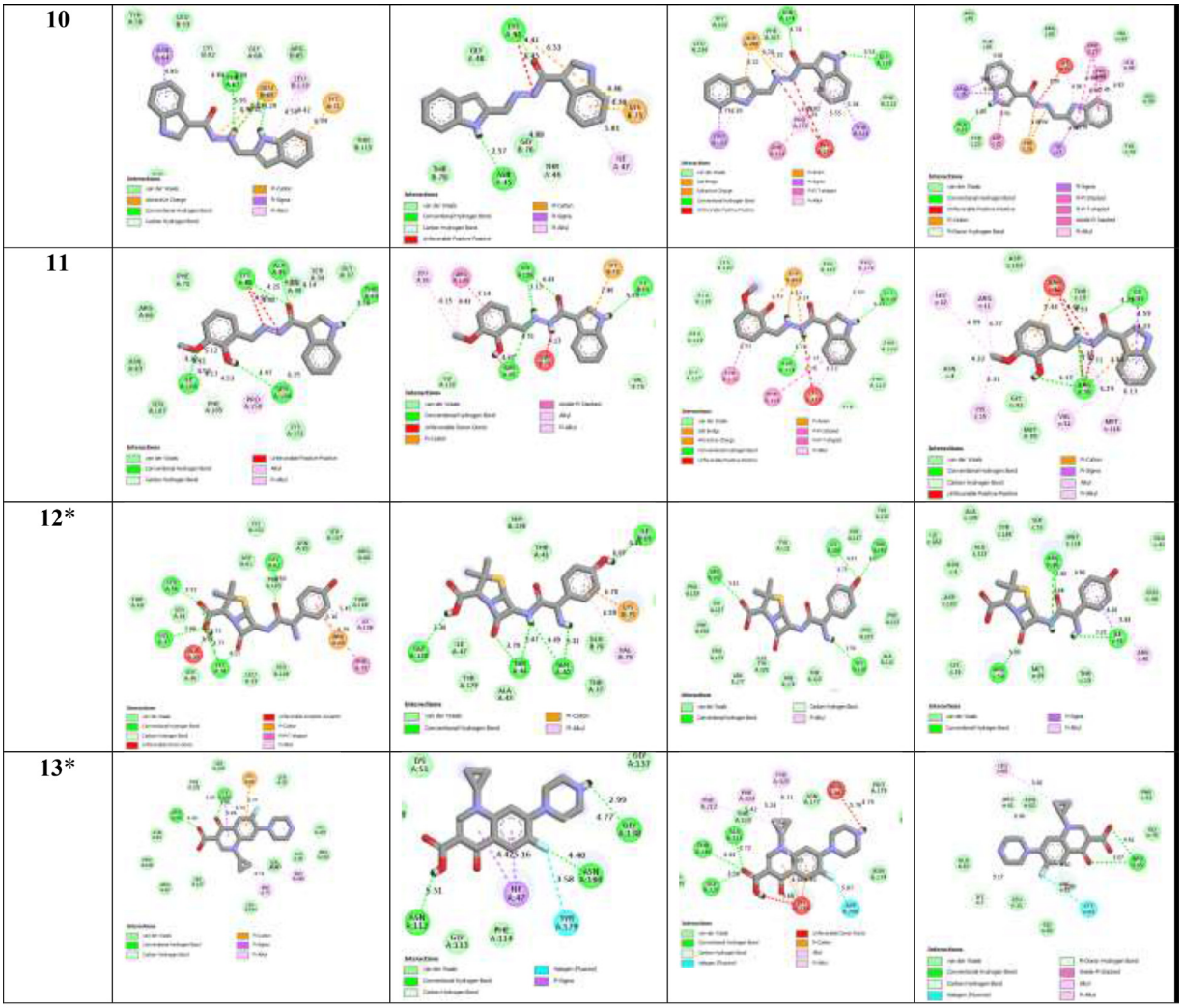
Compound 6 displayed hydrogen bonding with Lys75 in 3CR7 receptor, Pro129, Glu130, Tyr131, Gly210 in 6LIL, Leu124, Val198, Leu157 in 6SPC (Table 4.0), the HB interactions (Table 5.0).

Compound 7 exhibited hydrogen bonding with Phe67 in 3CR7 receptor, Tyr78, Lys51 with 3FHV, Asn174, Gly210 in 6LIL and Val195, Arg156, Leu157 in 6SPC (Table 4.0), while the HB interactions (Table 5.0).

Compound 8 exhibited conventional hydrogen bonding with Ser39, Lys38 in Phe67 in 3CR7, Ser140, Thr139, Gly138 with 3FHV, Asn174, Gly210 in 6LIL (Table 4.0), the HB interactions (Table 5.0).

Compound 9 was found to exhibited conventional hydrogen bonding with Ser156, Ile69 in 3FHV, Ala105, Asn176, Gly210 in 6LIL, Val195, Leu157, Arg156 in 6SPC (Table 4.0), the HB interactions (Table 5.0).

Table 4 (*continued*)



**Where 12* (Amoxicillin) and 13* (Ciprofloxacin) are selected standards for comparative studies.

**Where 12* (Amoxicillin) and 13* (Ciprofloxacin) are selected standards for comparative studies.

Compound 10 showed conventional hydrogen bonding with Phe67 in 3CR7 receptor, Lys51, Asn45 in 3FHV, Asn174, Gly210 in 6LIL, Ala24 in 6SPC (Table 4.0), the HB interactions (Table 5.0).

Compound 11 displayed conventional hydrogen bonding with Lys38, Ala35, Thr40, Ser104, Ile106 in 3CR7, Ser136, Ile69, Gln40 in 3FHV, Gly210, Asn174 in 6LIL and Ile55, Arg90 in 6SPC (Table 4.0), the HB interactions (Table 5.0).

These hydrogen bonding interactions with the binding sites of each receptor was found to be comparable with the binding site of Amoxicillin and Ciprofloxacin used in the docking studies.

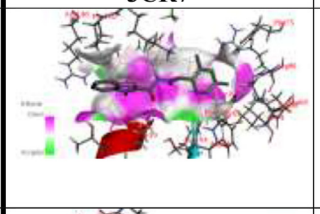
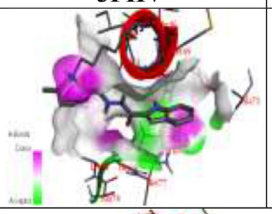
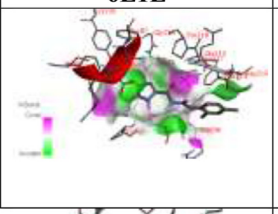
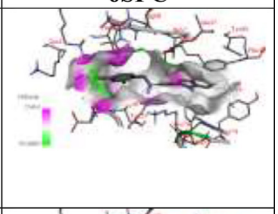
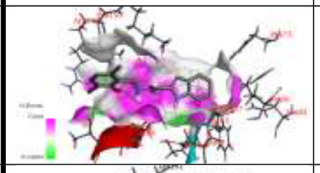
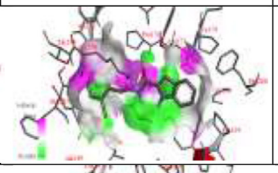
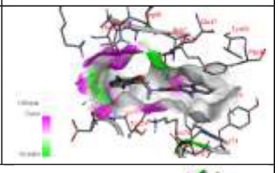
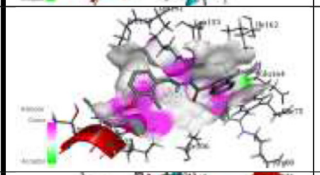
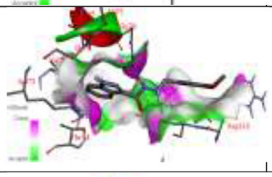
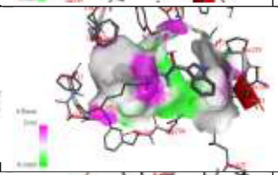
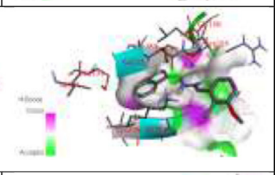
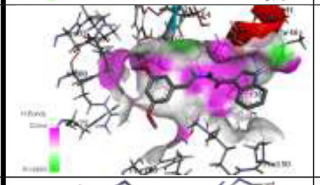
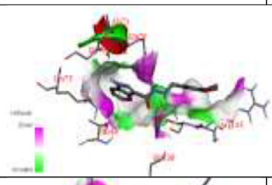
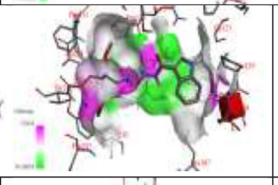
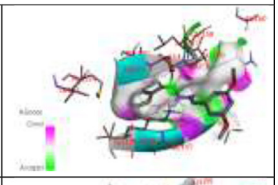
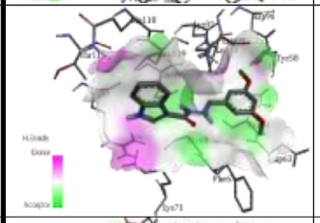
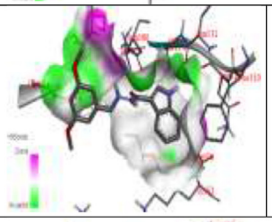
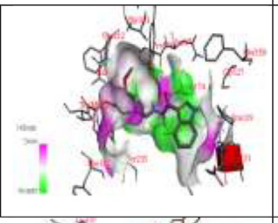
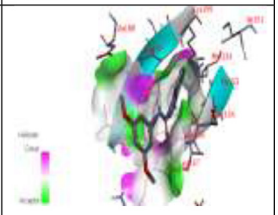
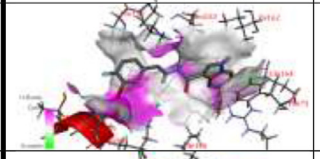
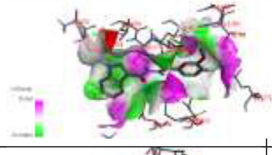
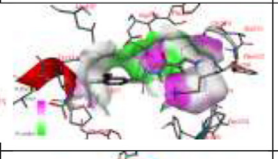
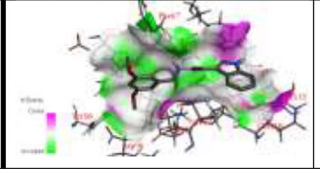
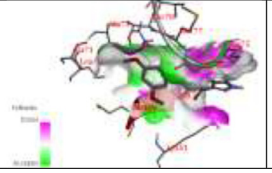
The synthesis of N-acylhydrazone derivatives (compound 1–11) was carried out from 1*H*-indole-2 and 3-carboxylic acid, as starting materials in the search of compounds with good biological activity.

The docking results revealed that all synthesised N-acylhydrazone derivatives exhibited a good number of hydrophobic interactions, classical and non-classical hydrogen bonding with the docked protein enzymes. The synthesis, in silico and antimicrobial analysis in search for potential antimicrobial (bacterial and fungal) inhibitors resulted in eleven good ligands (compounds 1–11) against 3CR7, 3FHV, 6L1L and 6SPC receptors based on predictive binding energies. Hence, results showed the best potential of N-acylhydrazone derivatives to be developed into an antimicrobial drugs against selected bacterial and fungi. All compounds docked has similar binding which indicates the same biochemistry, pathway and mechanism of biological action.

Limited structure-activity relationship

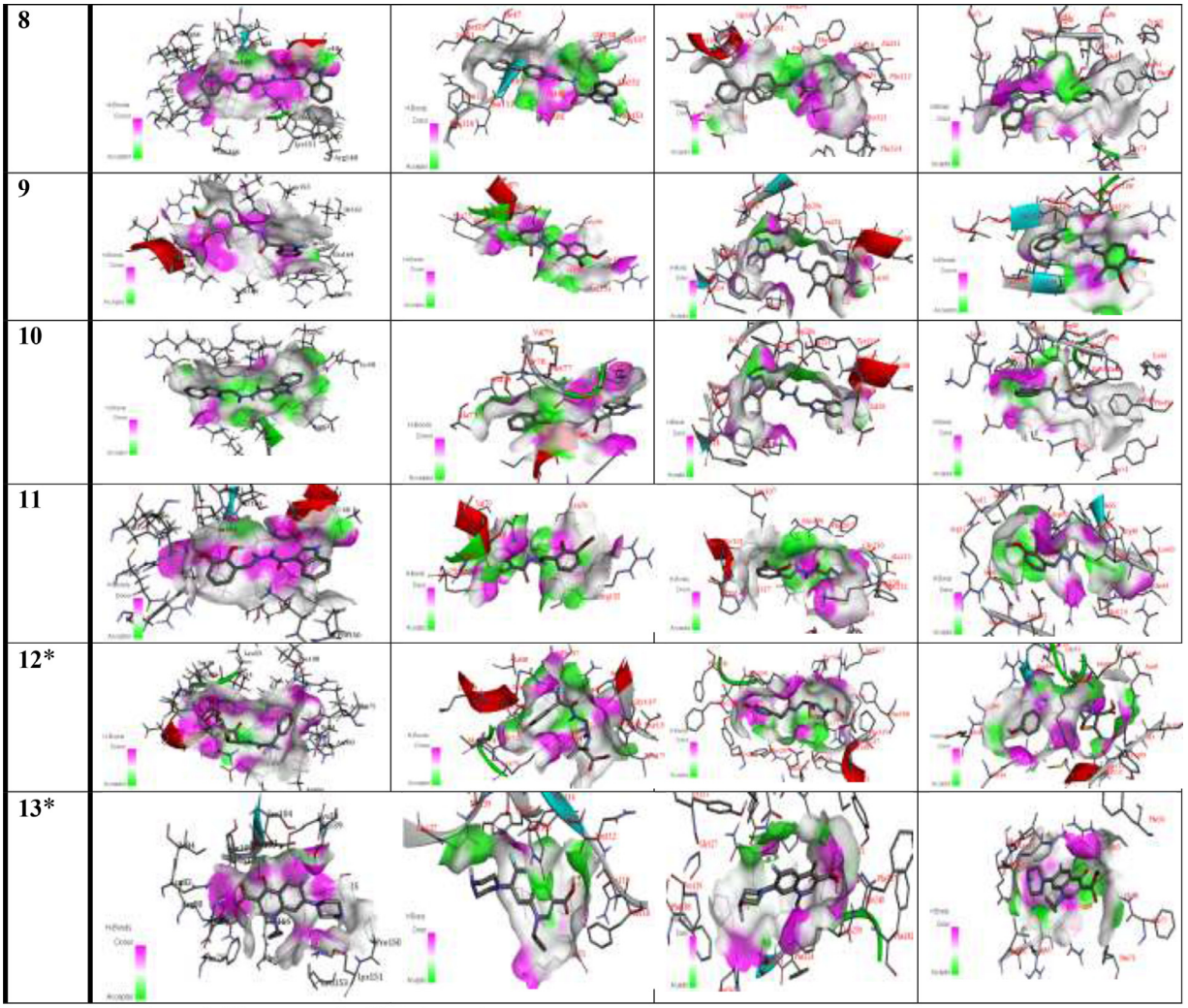
Compound **1** with two electron donating CH_3 groups at ortho and para positions similar in structure with compound **4** having two electron donating methoxyl (OCH_3) groups at the meta positions possessed good antimicrobial activities (Fig. 2). Compound **8** and **10** with electron rich pi-electrons (ring activator) possessed moderately good antimicrobial results. However, the di-ortho substitution observed in compound **5** may be responsible for the de creased antimicrobial activities (Fig. 2). Compound **2** with moderate activity due to the hydroxyl (OH) electron donating group, but the activity reduced due to the presence of chloro group at the ortho and meta positions, the activity observed with compound **3** was reduced probably due to the ortho deactivating chloro group. Compound **11** with two electron donating groups at the ortho and

Table 5
Hydrogen bonding interactions with the binding sites of each receptor.

	HB INTERACTIONS			
	3CR7	3FHV	6L1L	6SPC
1				
2				
3				
4				
5				
6				
7				

(*continued on next page*)

Table 5 (*continued*)



**Where 12* (Amoxicillin) and 13* (Ciprofloxacin) are selected standards for comparative studies.

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Table 6
Receptors used in this study and their description.

S/N	PDB CODE	DESCRIPTION
1	3CR7	Crystal structure of n-terminal truncation of aps kinase from *penicillium chrysogenum*
2	3FHV	Structural basis of *S. typhi* type ivb pils and cystic fibrosis trans membrane conductance regulator (cftr) interaction
3	6L1L	Apo-bacf structure from *bacillus subtillis*
4	6SPC	*Pseudomonas aeruginosa* 30 s ribosome from an aminoglycoside resistant clinical isolate

para positions with an electron withdrawing (ring deactivator) at the ortho position, this might account for why compound **11** is not a good candidate for fungi and bacteria at lower concentrations (Fig. 2). Compound **6** with a para-chloro ring deactivator and an ortho-methoxy ring activator possessed good activity. Compound **7** with di-meta-substituted methoxy groups and a para-substituted electron withdrawing bromo group displayed moderate antimicrobial activity. Compound **9 is** similar in structure to compound **7** but with reduced activity (Fig. 2) might be due to the ortho-substituted chloro group.

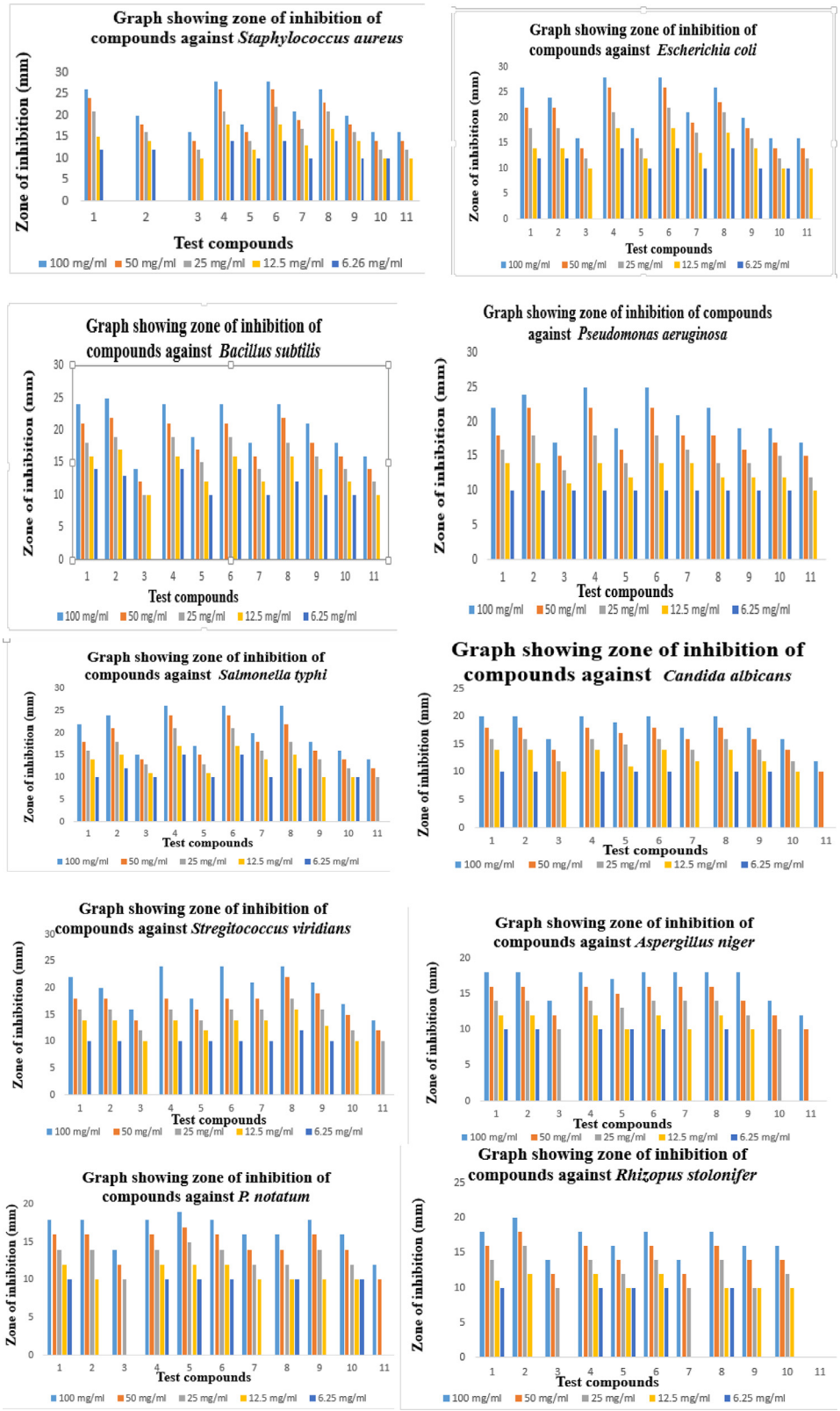


Fig. 2. Antimicrobial results of the N-acylhydrazone derivatives.

Conclusion

The results showed that the synthesised N-acylhydrazone derivatives have the potential of inhibiting microbial growth and showed good binding parameters with the tested enzymes, they could be effectively developed into antimicrobial agents useful for treating a wide range of microbial infections. Further research should be carried out on drugability, toxicity, minimal inhibitory concentration and minimal bactericidal concentrations of the drug compounds.

Data availability statement

Supporting information on the spectroscopic data for the synthesised compounds are found in the [supplementary data].

Declaration of Competing Interest

The authors declared that there is no competing interest.

Acknowledgments

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2022.e01463.

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