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Comprehensive, Characterization and Molecular Docking of Novel 1,4-Diaryl /Alkyl Substituted Diazine Analogues for Antimicrobial Application

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Abstract

A new class of phthalazinone derivatives was synthesized by the reaction of 1-(4-(4-oxo-3,4-dihydrophthalazin-1-yl) phenyl)-pyrrolidine-2,5-dione **(2)** and acetohydrazide **(5),** with several chemical reagents. According to their elemental analysis and spectrum data, all synthesized derivative structures were characterized. Certain prepared compounds were screened versus some bacteria and fungi strains to assess their antimicrobial efficacy. The newly derivatives demonstrated antimicrobial activity toward the selected microbes compared with the used references. Notably, compound **7** revealed activities versus *Escherichia coli* (inhibition zone 23±1 mm, and *Staphylococcus aureus* (inhibition zone 20.3±0.6mm) as compared to Gentamicin (inhibition zone 27±0.1 mm) & Ampicillin (inhibition zone 21.3±0.6 mm), and exhibiting potent inhibitory activity toward *Candida albicans* and *Asperagillus nigar* fungi (inhibition zone: 30.3±0.6 mm, 15±1.0 mm) respectively, compared to Nystatin (inhibition zone: 21.6±0.6 mm, 19.3±0.6 mm). Moreover, compounds **5** and **9** displayed anti-bacterial and anti-fungal activities against some tested strains. Also, compounds **6, 14,** and **18** exhibited commendable efficacy towards the examined bacterial strains. A molecular docking study was conducted for the most potent compounds **5, 6, 7, 9, 14,** and **18,** the results demonstrating significant binding affinities with essential microbial enzymes, consistent with their observed inhibitory effects.

Keywords: Phthalazinone, acetohydrazide, Antimicrobial activity, Molecular docking

Introduction

Heterocyclic compounds have been employed in treating several illnesses due to their distinctive structure. Notably, Diazines and their benzo derivatives are an important class of nitrogen-containing heterocyclic compounds because of their various pharmacological activities [1-8]. Phthalazine (Benzopyridazine), and its oxygenated derivative phthalazinone (benzopyridazinone), are promising fused-ring compounds with two nearby nitrogen atoms in their ring structure [9-10]., demonstrated a wide array of biological characteristics, encompassing anti-cancer, -diabetic, -asthmatic, -histaminic, -hypertensive, -thrombotic, -depressant, -inflammatory, -proliferative, -antiviral, -antibacterial, -antifungal, -antiparasitic properties, and analgesic impact [12-21]. It is noteworthy that benzopyridazine and benzopyridazinone derivatives constitute the core of specific pharmaceutical drugs, as shown in Figure 1 [22], among which is azelastine [23]. Zopolrestat [24]. As well as other chemicals, 1-(4-chlorophenyl)-3-(4-((4-chlorophthalazin-1-yl) amino) phenyl) urea (**A**) and 1-(4-chloro-3-(trifluoromethyl) phenyl)-3-(4-((4-chlorophthalazin-1-yl)oxy) phenyl)urea (**B**) exhibited significant inhibitory effects [25].

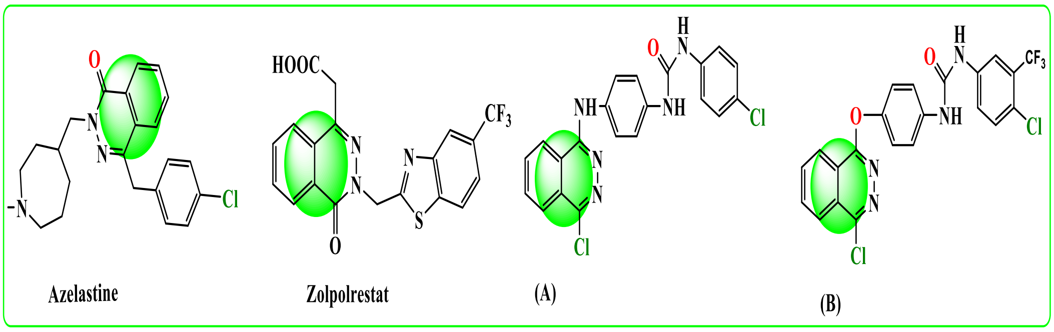

Figure 1: Some commercial drugs based on phthalazinone structures

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Since DNA is the intracellular target for numerous anti-microbial drugs, research on how drug molecules interact with DNA has exploded in the last few years. Discovering drug-DNA interactions and developing novel, promising medications for clinical usage can both be substantially aided by these investigations. In this context, Molecular docking is a prevalent computational technique employed to forecast interactions between small medicinal compounds and their protein targets, offering vital understanding into binding affinity and biological activity. It is crucial for the rational design and development of drugs. Owing to its substantial biological and pharmacological importance, tremendous progress has been achieved in optimizing docking algorithms to improve the precision of interaction predictions [26]. Docking studies are essential for assessing the binding modes and possible biological impacts of synthesized drugs on protein receptors, establishing a basis for comprehending their therapeutic potential [27-29]. Considering the aforementioned facts, to prepare new derivatives of phthalazinone to evaluate their mechanisms of action and enhance their pharmacological applications. Consequently, authors have endeavored to design a new series of phthalazine derivatives to acquire more precise insights into the reaction pathways and to evaluate their antimicrobial activity against different strains of bacteria and fungi. The molecular docking was conducted to evaluate their pharmacological activity.

2. Materials and Methods

2. Materials

All chemical reagents utilized in this study were sourced from Sigma-Aldrich, while analytical-grade solvents were procured from El-Nasr Chemicals Company. The melting points in Celsius degrees of the synthesized compounds were determined using a Gallen-Kamp melting point apparatus, with all reported values remaining uncorrected. We documented IR spectra (KBr) utilizing the Thermo Fisher Nicolete iS10. In DMSO–d_6 solvent, the ^{1}H and $^{13C\text{-}NMR}$ spectra were acquired using a Jeol Resonance 500 and 125 *MHz* apparatus, with TMS serving as the internal standard. The δ values in ppm were employed to represent chemical alterations. Mass spectrometry was performed *via* the direct inlet apparatus of the Thermo Scientific GC/MS model ISQ mass analyzer. The antibacterial activities were conducted at the Micro Analytical Centre, Cairo University.

2.2 Synthesis

4-(4-Aminophenyl) phthalazin-1-(2*H*)-one (1)

A solution of 2-(4-aminobenzoyl)-benzoic acid (10 mmol) in 15 ml of absolute ethanol was added (240 mmol) hydrazine hydrate. The reaction mixture was heated under reflux for 6 hours [15-16]. After cooling, the reaction mixture was filtered off and crystallized from DMF/H_2O mixture to give **1** as pale brown crystalline. Yield 50%. M p 250 – 252°C. IR [KBr, cm^{-1}]: 3400, 3254, 3156, 3100 (NH~OH, NH_2), 1666 (C=O). ^{1}H-NMR [DMSO-d_6, 500 *MHz*, δ, ppm]: 7.24 – 7.57 (m, 8H, aromatic protons), 9.96 (s, 2H, NH_2, exch..), 13.15 (s, 1H, NH~OH, exch..). ^{13}C NMR [DMSO-d_6, 125 *MHz*, δ, ppm]: 159.15, 137.14, 134.74, 134.58, 133.09, 131.92, 131.56, 128.90, 128.36, 128.12, 127.47, 126.55, 125.56, 123.46(aromatic carbons). Ms, m/z = 237 ($M^{\bullet+}$, 21.44%). *Anal.* Calcd. for $C_{14}H_{11}N_3O$ (237.26): C, 70.87 %; H, 4.67%; N,17.71%; O, 6.74 %. Found, 70.85%.; H, 4.69%.; N, 17.70; %. O, 6.79 %.

1-(4-(4-Oxo-3,4-Dihydrophthalazin-1-yl) phenyl)-pyrrolidine-2,5-dione (2)

An equimolar amount of compound 1 (10 mmol) was fused with succinic anhydride (10 mmol) at 250°C for 2 hours. After cooling, the precipitate was triturated with H_2O, the resultant mixture was filtered off and recrystallized from ethanol to give **2** as pale yellow crystals. Yield 90%. M p 203 - 205°C. IR [KBr, cm^{-1}]: 3467, 3158 (OH, NH), 1780, 1668 (C=O), 1595 (C=N). ^{1}H NMR [DMSO-d_6, 500 *MHz*, δ, ppm]: 2.02 (m, 4H, $2CH_2$), 6.98 –7.56 (m, 8H, aromatic protons), 9.92 (s, 1H, NH, exch..), 12.88 (s, 1H, OH, exch.). ^{13}C NMR [DMSO-d_6, 100 *MHz*, δ, ppm]: 167.48, 159.58, 137.57, 135.18, 135.02, 133.52, 132.38, 132.01, 129.32, 128.82, 128.54, 127.88, 126.99, 125.99, 123.89 (CO and aromatic carbons), 29.26 ($2CH_2$). Ms, m/z = 319 ($M^{\bullet+}$, 36.26%). *Anal.* Calcd. for $C_{18}H_{13}N_3O_3$ (319.32):C, 67.71%.; H, 4.10%.; N, 13.16%; O, 15.03%. Found C, 67.65%.; H, 4.20%.; N, 13.20%.; O, 15.115%.

2-(4-(4-(2,5-Dioxopyrrolidin-1-yl)phenyl)-1-oxophthalazin-2(1*H*)-yl)acetic acid (3)

A mixture of monochloroacetic acid (0.94 g, 0.01 mmol) and compound **2** (3.19 g, 0.01 mmol) was refluxed in 20 mL of 10% aqueous NaOH for 4 hours. After cooling, the reaction mixture was diluted with water, extracted using ether, washed well, and recrystallized from a proper solvent to give **3**. Yield [%] = 75. M p 216 - 218°C. IR [KBr, cm^{-1}] υ max = 3291 (OH), 3048 (CH-aliphatic), 2919, 2845 (CH-aliphatic), 1710 -1658 (C=O), 1595 (C=N). ^{1}H-NMR [DMSO-d_6, 500 *MHz*, δ, ppm] = 2.04 (m, 4H, $2CH_2$), 4.45 (s, 2H, CH_2), 7.43 – 8.27 (m, 8H, aromatic protons), 1 (s, 1H, OH exch.). ^{13}C NMR [DMSO-d_6, 125 *MHz*, δ, ppm] = 167.07, 163.30, 159.58,153.41, 136.77, 135.19, 135.04, 132.38, 132.02, 129.33, 129.27, 128.54, 127.84, 126.07, 125.29, 123.90 (CO and aromatic carbons), 58.41(CH_2), 29.26($2CH_2$). Ms, m/z = 377($M^{\bullet+}$). *Anal.* calcd for $C_{20}H_{15}N_3O_5$ (377.10): C, 63.66; H, 4.01; N, 11.14; O, 21.20, %. Found: C, 63.70; H, 4.05; N, 11.20; O, 21.25, %.

Ethyl2-(4-(4-(2,5-dioxopyrrolidin-1-yl) phenyl)-1-oxophthalazin-2(1*H*)-yl) acetate (4)

A solution of phthalazinone **2** (3.19 g, 0.01 mmol), ethyl chloroacetate (3.67 g, 0.03 mol), and potassium carbonate (4.14 g, 0.03 mol) in dry acetone (50 ml) was refluxed for 24 hrs. After cooling, the solid obtained was poured into water and filtered off to give **4**. Yield 60 %. M p 210 - 212°C IR [KBr, cm^{-1}] υ max = 3002 (CH- aromatic), 2939, 2869 (CH -aliphatic), 1735, 1706, 1660 (3C=O), 1585 (C=N), 1065 (COC). ^{1}H-NMR [DMSO-d_6, 500 *MHz*, δ, ppm] = 1.18 (t, 3H, CH_3), 2.05 (m, 4H, 2CH_2), 4.16 (s, 2H, CH_2), 4.73 - 4.93 (q, 2H, CH_2), 7.43 -7.96 (m, 8H, aromatic protons). ^{13}C NMR [DMSO-d_6, 125 *MHz*, δ, ppm] = 167.50, 166.77, 134.50, 132.78, 131.89, 131.79, 131.38, 128.75, 128.65, 127.83, 127.14, 126.87, 126.50, 123.41(CO and aromatic carbons), and 63.17, 61.44, 61.20, 61.07, 60.48, 51.77, 28.75(aliphatic carbons). Ms, m/z = 405 (M$^{\bullet+}$, 53.37 %). *Anal.* calcd for $C_{22}H_{19}N_3O_5$ (405.13), C, 65.18; H, 4.72, N, 10.37, O, 19.73%. Found C, 65.25; H, 4.75; N, 10.40; O, 19.78; %.

2-(4-(4-(2,5-Dioxopyrrolidin-1-yl)phenyl)-1-oxophthalazin-2(1*H*)-yl)acetohydrazide (5)

A mixture of compound **4**(4.05 g, 0.01 mol) and hydrazine hydrate (0.03 mol), in 30 ml of ethanol, was refluxed for 3 hours. After cooling, the resultant solid was filtered off and crystallized from a proper solvent to afford **5**. Yield [%] = 74. M. p 195-197°C. IR [KBr, cm^{-1}] υ max = 3338, 3263 (NH_2), 3181 (NH), 3020 (CH-aromatic), 2978, 2899 (CH - aliphatic), 1780, 1661,1621(3C=O), 1595 (C=N). ^{1}H-NMR [DMSO-d_6, 500 *MHz*, δ, ppm] = 2.02 (m, 4H, 2CH_2), 4.50 (s, 2H, $N\underline{CH_2}CO$), 9.96 (s, 2H, NH_2, exch.), 7.28 -7.94 (m, 8H, aromatic protons), 13.15 (s, 1H, NH exch.). Ms, m/z = 391 (M$^{\bullet+}$, 26.90%). *Anal.* calcd for $C_{20}H_{17}N_5O_4$ (391.39): C, 61.38; H, 4.38; N, 17.89; O, 16.35; %. Found: C, 61.35; H, 4.40; N, 17.90; O, 16.40%.

1-(4-(2-(Piperidin-1-ylmethyl)-4-oxo-3,4-dihydrophthalazin-1-yl) phenyl)pyrrolidine-2,5-dione (6)

A mixture of compound **2** (3.91 g, 0.01 mol), piperidine (0.02 mol), formaldehyde solution (2 ml), and one drop of HCl, in ethanol was refluxed for 4 hours. The solvent was evaporated under vacuum, the solid obtained was filtered off and crystallized from ethanol to give compound **6**. Yield [%] = 80. M p 204-206°C. IR [KBr, cm^{-1}] υ max = 3054 (CH - aromatic), 2928, 2854 (CH - aliphatic), 1702, 1630 (C=O), 1593 (C=N). ^{1}H-NMR [DMSO-d_6, 500 *MHz*, δ, ppm] = 1.52 - 1.64 (m, 6H, CH_2), 2.94 (m, 4H, 2CH_2), 4.40 (t, 4H, N-(2CH_2)), 5.36 (s, 2H, CH_2), 7.43 – 7.97 (m, 8H, aromatic protons). ^{13}C NMR [DMSO-d_6, 125 MHz, δ, ppm] = 167.07, 159.13, 157.97, 134.85, 134.79, 131.54, 128.91, 128.34, 128.19, 128.13, 127.47, 123.47(CO and aromatic carbons), 72.67, 53.4, 30.03, 22.43, 22.04, 21.74 (aliphatic carbons). Ms, m/z = 416 (M$^{\bullet+}$,100 %). *Anal.* calcd for $C_{23}H_{22}N_4O_4$ (416.48): C, 69.21; H, 5.81; N, 13.45; O, 11.52; %. Found: C, 69.15; H, 5.88; N, 13.50; O, 11.55; ***%.***

1-(4-(3-((2-Hydroxy-naphthalen-1-yl)methyl)-4-oxo-3,4-dihydrophthalazin-1-yl)phenyl)pyrrolidine-2,5-dione (7)

A mixture of compound **2** (3.91 g, 0.01 mol) and ß-naphthol (2.88 g, 0.02 mol), in ethanol–acetone (1:1, v/v, 30 mL) containing hydrochloric acid (2 mL), and formaldehyde (2 mL), the reaction mixture was refluxed for 16 hours. The solid that was obtained was filtered and crystallized from a proper solvent to give **7**. Yield [%] = 80, M p 210 - 212°C. IR [KBr, cm^{-1}] υ max = 3333 (OH), 3072 (CH - aromatic), 2945, 2897 (CH - aliphatic), 1715 - 1649 (C=O), 1598 (C=N). ^{1}H-NMR [DMSO-d_6, 500 *MHz*, δ, ppm] = 2.07 (m, 4H, 2CH_2), 3.92 (s, 2H, CH_2), 6.83 - 8.08(m, 14H, aromatic protos), 12.87 (s, 1H, OH exch.). ^{13}C NMR [DMSO-d_6, 125 *MHz*, δ, ppm] = 167.08, 159.18, 153.17, 137.19, 134.57, 134.4, 133.09, 128.91, 127.48, 127.41, 126.47, 126.13, 124.59, 123.47, 122.01, 118.35 (CO and aromatic carbons), 50.01, 28.56 (aliphatic carbons). Ms, m/z = 475 (M$^{\bullet+}$). *Anal.* calcd for $C_{29}H_{21}N_3O_4$ (475.50): C, 73.25; H, 4.45; N, 8.84; O, 13.46; %. Found: C, 73.30; H, 4.50; N, 8.80; O, 13.44; %.

Ethyl (E)-3-amino-3-(4-(4-(2,5-dioxopyrrolidin-1-yl)phenyl)-1-oxophthalazin-2(1*H*)-yl)acrylate (8)

A mixture of phthalazinone **2** (3.91 g, 0.01 mol) and ethyl cyanoacetate (3.39 g, 0.03 mol) in 30 ml of ethanol was refluxed for 3 hours. The reaction mixture was filtered off and crystallized from ethanol to furnish **8**. Yield [%] = 60. M p 195 - 197°C. IR [KBr, cm^{-1}] υ max = 3398 - 3295 (NH_2), 3069 (CH-aromatic), 2956, 2871 (CH-aliphatic), 1734,1706, 1655 (3C=O), 1601 (C=N), 1051 (COC). ^{1}H-NMR [DMSO-d_6, 500 *MHz*, δ, ppm] = 1.04 – 1.08 (t, 3H, CH_3), 2.01 (m, 4H, 2CH_2), 3.29 (s, 2H, NH_2, exchangeable), 4.17 - 4.27 (q, 2H, CH_2), 5.98 (s, 1H, CH), 6.90 - 7.97 (m, 8H, aromatic proton). ^{13}C NMR [DMSO-d_6, 125 *MHz*, δ, ppm] = 167.07, 163.90, 159.10, 134.70, 134.54, 133.05, 131.54, 128.83, 128.34, 128.05, 127.40, 126.51, 125.52, 123.41, 90.09, 50.59, 29.56, 14.46. Ms, m/z = 432 (M$^{\bullet+}$, 32.53%). *Anal.* calcd for $C_{23}H_{20}N_4O_5$ (432.14): C, 63.88; H, 4.66; N, 12.96; O, 18.50; %. Found: C, 63.90; H, 4.60; N, 12.90; O, 18.55; %.

(E)-3-Amino-3-(4-(4-(2,5-dioxopyrrolidin-1-yl)phenyl)-1-oxophthalazin-2(1*H*)-yl)-N-phenylacrylohydrazide (9)

A mixture of compound **8** (4.32 g, 0.01 mol), phenyl hydrazine (0.03 mol) in 30 ml of ethanol was heated under reflux for 3h. The resultant solid was filtered off and crystallized from ethanol to give **9**. Yield 70%, M p. 208-210, [KBr, cm^{-1}] υ max = 3466 - 3320 (NH_2), 3155 (NH), 3001 (CH-aromatic), 2935, 2894 (CH-aliphatic), 1776, 1708, 1665 (C=O), 1591 (C=N). ^{1}H-NMR [DMSO-d_6, 500 *MHz*, δ, ppm] = 2.03 (m, 4H, 2CH_2), 4.57 (s, 2H, NH_2, exch.), 5.45 (s, 1H, CH), 6.85 - 7.57 (m, 13H, aromatic protons), 10.14 (s, 1H, NH exch.). ^{13}C NMR [DMSO-d_6, 125 *MHz*, δ, ppm] = 167.80, 166.64, 159.16, 157.37,149.42, 146.86, 135.05, 134.59, 133.10, 129.10, 128.68, 128.47, 128.37, 126.56, 126.02, 125.57, 123.53, 112.4 (CO and aromatic carbons), 30.08 (2CH_2). Ms, m/z = 495 (M+1). *Anal. calcd for* $C_{27}H_{22}N_6O_4$ (494.17): C, 65.58; H, 4.48; N, 16.99; O, 12.94; %. Found: C, 65.55; H, 4.50; N, 16.95; O, 12.95; %.

(Z)-2-(4-(4-(2,5-Dioxopyrrolidin-1-yl)phenyl)-1-oxophthalazin-2(1*H*)-yl)-N'-(4-oxopentan-2-ylidene) acetohydrazide (10)

A mixture of compound **5** (3.91 g, 0.01 mmol) and acetylacetone (1 g, 0.01 mol) in 20 ml of ethanol was heated under reflux for 3 hours. The obtained solid was filtered off and crystallized from ethanol to afford **10** as pale white crystals. Yield [%] = 88, M p 248 - 250 °C. IR [KBr, cm^{-1}] υ max = 3267 (NH), 2940, 2899 (CH-aliphatic), 1712, 1669, 1602 (3C=O), 1556 (C=N). ^{1}H-NMR [DMSO-d_6, 500 *MHz*, δ, ppm] = 1.49 (s, 6H, CH_3), 2.02 (m, 4H, 2CH_2), 3.60 (s, 2H, CH_2), 4.50 (s, 2H, $N\underline{CH_2}CO$), 7.50 - 7.94 (m, 8H, Aromatic proton), 10.57 (s. 1H, NH exch.).^{13}C NMR [DMSO-d_6, 125 *MHz*, δ, ppm] = 181.12, 167.08, 159.15, 153.41, 137.18, 134.77, 134.64, 133.14, 131.59, 128.92, 128.39, 128.14, 127.50, 126.58, 125.61, 123.49(CO and aromatic carbons), 53.85, 42.38, 31.05, 29.65, 11.07(aliphatic carbons). Ms, m/z = 473 (M$^{•+}$, 12.56 %). *Anal.* calcd for $C_{25}H_{23}N_5O_5$ (473.49). C, 63.42; H, 4.90; N, 14.79; O, 16.88; %. Found: C, 63.45; H, 4.95; N, 14.75; O, 16.90; %.

N-(1,3-Dioxoisoindolin-2-yl)-2-(4-(4-(2,5-dioxopyrrolidin-1-yl)phenyl)-1-oxophthalazin-2(1*H*)-yl)acetamide (11)

A mixture of **5** (3.91 g, 0.01 mol) and phthalic anhydride (2.22 g, 0.015 mol) was heated in an oil bath at 150°C for 2 hours. The reaction mixture was diluted with water, the solid obtained filtered off, and crystallized from ethanol to give **11**. Yield [%] = 80. M p. 188 - 190°C. IR [KBr, cm^{-1}] υ max = 3155 (NH), 3029, 3001 (CH-aromatic), 2939, 2894 (CH-aliphatic), 1767, 1712, 1667 (3C=O),1595 (C=N). ^{1}H-NMR [DMSO-d_6, 500 *MHz*, δ, ppm] = 2.03 (m, 4H, 2CH_2), 4.25 (s, 2H, $N\underline{CH_2}CO$), 7.43 - 8.64 (m, 12H, Aromatic protons), 12.50 (s, NH exch.).^{13}C NMR [DMSO-d_6, 125 *MHz*, δ, ppm] = 168.08, 167.08, 159.16, 137.02, 136.91, 135.54, 130.71, 129.37, 129.02, 128.85, 128.62, 128.06, 127.41, 123.42, 122.93 (CO and aromatic carbons), 54.23, 29.26 (aliphatic carbons). Ms, m/z = 521 (M$^{•+}$, 21.34 %). *Anal.* calcd for $C_{28}H_{19}N_5O_6$ (521.49). C: 64.49; H: 3.67; N: 13.43; O: 18.41; %. Found: C, 64.51; H, 3.62; N, 13.39; O, 18.45; %.

2-(4-(4-(2,5-Dioxopyrrolidin-1-yl) phenyl)-1-oxophthalazin-2(1H)-yl)-N'-formylacetohydrazide (12)

A solution of 5 (3.91 g, 0.01 mol) in formic acid (15 ml) was heated under reflux for 8 hours. The obtained solid was filtered off and washed several times with water. Dried and crystallized from ethanol to give 12. Yield [%] = 83. M p 263 - 265°C. IR [KBr, cm-1] υ max = 3245, 3166 (2NH), 3017 (CH-aromatic), 2960, 2898 (CH-aliphatic), 1702, 1668, 1603 (C=O), 1556 (C=N). ^{1}H-NMR [DMSO-d_6, 500 MHz, δ, ppm] = 2.02 (m, 4H, 2CH2), 3.94 (s, 2H, NCH_2CO), 7.43 - 8.34 (m, 8H aromatic protons), 9.02 (s, 1H, NH, NHCO exch.), 11.55 (s, 1H, NH exch. Ms, m/z = 419 (M•+, 65.34%). *Anal.* calcd for $C_{21}H_{17}N_5O_5$ (419.40): C, 60.14; H, 4.09; N, 16.70; O, 19.07; %. Found: C, 60.17; H, 4.11; N, 16.73; O, 19.10; %.

N'-Acetyl-2-(4-(4-(2,5-dioxopyrrolidin-1-yl)phenyl)-1-oxophthalazin-2(1*H*)-yl)acetohydrazide (13)

A solution of compound **5** (3.91 g, 0.01 mmol) and acetic anhydride (15ml) was heated under reflux for 3 hours. the reaction mixture was left overnight, then filtered off, washed several times with water, and crystallized from a proper solvent to give **13**. Yield [%]=73. M p 204 - 206°C. IR [KBr, cm^{-1}] υ max = 3445, 3153 (2NH), 3097, 3001 (CH-aromatic), 2938, 2894 (CH-aliphatic), 1715, 1665, 1604 (C=O), 1587 (C=N). ^{1}H-NMR [DMSO-d_6, 500 *MHz*, δ, ppm] = 2.04 (m, 4H, 2CH_2), 3.87 (s, 2H, $N\underline{CH_2}CO$), 4.52 (s, 2H, $COCH_3$), 7.50 -7.80 (m, 8H, aromatic protons), 10.80 (s, 1H, NH exch.). ^{13}C NMR [DMSO-d_6, 125 *MHz*, δ, ppm] = 167.80, 159.18, 153.45, 151.21, 137.12, 136.14, 135.54, 130.14, 129.40, 129.02, 128.90, 128.14, 128.02, 127.45, 122.13, (aromatic carbons), 58.41, 29.26, 21.22 (aliphatic carbons). Ms, m/z = 475 (M$^{•+}$, 12.13%). *Anal.* calcd for $C_{22}H_{19}N_5O_5$ (475.33): C, 60.97; H, 4.42; N, 16.16; O, 18.46; %. Found: C, 60.95; H, 4.45; N, 16.20; O: 18.40%.

1-(2-(4-(4-(2,5-Dioxopyrrolidin-1-yl)phenyl)-1-oxophthalazin-2(1*H*)-yl)acetyl)pyrazolidine-3,5-dione (14)

Compound **5** (3.91 g, 0.01 mol) and diethylmalonate (1.60 g, 0.01 mol) in ethanol (20 ml) were refluxed for 6 hours. After cooling, the solid obtained was filtered off, dried, and crystallized from methanol to afford **14**. Yield [%] =70, M p 230 - 232°C. IR [KBr, cm^{-1}] υ max = 3451 (NH), 3016 (CH-aromatic), 2950, 2898 (CH-aliphatic), 1749, 1661, 1603 (C=O), 1556 (C=N). ^{1}H-NMR [DMSO-d_6, 500 *MHz*, δ, ppm] = 1.85 (s, 2H, CH_2), 2.01 (m, 4H, 2CH_2), 3.90 (s, 2H, $N\underline{CH_2}CO$), 6.83 - 6.86 (m, 8H, aromatic protons), 10.95 (s, 1H, NH exch.). ^{13}C NMR [DMSO-d_6, 125 *MHz*, δ, ppm] = 167.07, 159.16, 153.41, 151.01, 137.21, 133.64, 132.86, 130.41, 128.85, 127.41, 127.06, 123.36 (aromatic carbons), 55.10, 45.21, 30,03 (aliphatic carbons). Ms, m/z = 459 (M$^{•+}$, 12.65%). *Anal.* calcd for $C_{23}H_{17}N_5O_6$ (459.42): C, 60.13; H, 3.73; N, 15.24; O, 20.89; %. Found: C, 60.15; H, 3.75; N, 15.20; O, 20.90%.

N'-(2-(4-(4-(2,5-Dioxopyrrolidin-1-yl)phenyl)-1-oxophthalazin-2(1H)-yl)acetyl)benzohydrazide (15)

An equimolar amount of hydrazide **5** (3.91 g, 0.01 mol) and benzoyl chloride (2.10 g, 0.015 mol) in 20 ml pyridine, underwent refluxing for 1 hour, followed by pouring on ice/HCl. The resultant solid was filtered and crystallized from ethanol to furnish **15**. Yield [%] = 73. M p 232 - 234°C. IR [KBr, cm^{-1}] υ max =3153 (NH), 3029 (CH-aromatic), 2939, 2894 (CH-aliphatic), 1777, 1707, 1665 (3C=O), 1588 (C=N). ^{1}H-NMR [DMSO-d_6, 500 *MHz*, δ, ppm] = 2.02 (m, 4H, 2CH_2), 3.98 (s, 2H, $N\underline{CH_2}CO$), 7.86 - 8.28 (m, 13H, aromatic protons), 11.57 (s, 1H, NH exch.). ^{13}C NMR [DMSO-d_6, 125 *MHz*, δ, ppm] = 167.08, 159.18, 153.18, 151.40, 139.09, 134.64, 131.86, 131.49, 128.76, 127.94, 127.31, 124.83, 124.57, 123.36 (CO and aromatic carbons), 50.08, 30,03 (aliphatic carbons). Ms, m/z = 495 (M$^{•+}$, 11.43 %). *Anal.* calcd for $C_{27}H_{21}N_5O_5$ (495.50): C, 62.53; H, 4.25; N, 17.36; O, 15.86; %. Found: C, 62.50; H, 4.20; N, 17.30; O, 15.82; %.

(E)-2-(4-(4-(2,5-Dioxopyrrolidin-1-yl)phenyl)-1-oxophthalazin-2(1H)-yl)-N'-(2,3,4,5,6-pentahydroxyhexylidene) acetohydrazide (16)

Acetohydrized **5** (3.91gm, 0.01 mol) and D-glucose (1.8 gm, 0.01 mol) in (30 ml) ethanol, heated under reflux for 3 hrs., the resultant solid filtered off, and crystallized from ethanol to afford **16**. Yield [%] = 60%. M p 253-255°C. IR [KBr, cm^{-1}] ʋ max = 3483 (OH), 3161 (NH), 3005 (CH-aromatic), 2945, 2896 (CH-aliphatic), 1715, 1700, 1603 (3C=O), 1556 (C=N). ^{1}H-NMR [DMSO-d_6, 500 *MHz*, δ, ppm] = 2.04 (m, 4H, $2CH_2$), 3.50 (s, H, CH), 3.92 (s,1H, N$\underline{CH_2}$CO), 5.67 (s, H, OH exch..), 7.46 - 8.13 (m, 8H, aromatic protons), 10.08 (s, 2NH exch.). ^{13}C NMR [DMSO-d_6, 125 *MHz*, δ, ppm] = 167.07, 159.10, 153.05, 151.55, 136.70, 135.54, 133.05, 131.54, 129.83, 128.34, 128.05, 128.40, 126.51, 124.52, 123.41(CO and aromatic carbons), 80.3, 77.10, 70.51, 65.89, 62.45, 56.60, 29.55(aliphatic carbons). Ms, m/z = 553($M^{\bullet+}$, 9.50 %). *Anal.* calcd for $C_{26}H_{27}N_5O_9$. (553.53): C, 56.42; H, 4.92; N, 12.65; O, 26.01; %. Found: C, 56.45; H, 4.95; N, 12.60; O, 256.05; %.

E)-6-(2-(2-(4-(4-(2,5-dioxopyrrolidin-1-yl)phenyl)-1-oxophthalazin-2(1H)-yl)acetyl)hydrazono)hexane-1,2,3,4,5-pentayl pentaacetate (17)

A mixture of compound **16** (5.53 g, 0.01 mol) and acetic anhydride (5 mL) was heated under reflux for 3 hrs. The resultant solid was poured into cold water. The precipitate was filtered off and crystallized from ethanol to give **17**. Yield [%] = 65. M p 209 - 211°C. IR [KBr, cm^{-1}] ʋ max = 3166 (NH), 3019 (CH-aromatic), 2950, 2898 (CH aliphatic), 1777, 1668, 1602 (C=O), 1555 (C=N). ^{1}H-NMR [DMSO-d_6, 500 *MHz*, δ, ppm] = 1.24 (s, H, $-CH_3$), 2.01 (m, 4H, $2CH_2$), 3.86 (s, 2H, N$\underline{CH_2}$CO) 4.42 - 4.47 (m, methine & methylene CH_2O), 6.45 (s, 1H, CH=N), 7.98 - 8.56 (m, 8H, aromatic protons), 11.54 (s, 1H, NH exch.).^{13}C NMR [DMSO-d_6, 125 *MHz*, δ, ppm] = 167.08, 166.56, 166.05, 165.78, 163.09, 160.65, 159.41, 153.18, 136.18, 135.58, 133.62, 131.15, 129.21, 128.18, 127.48, 126.51, 124.52, 122.43 (CO and aromatic carbons), 78.70, 76.32, 72.89, 62.2, 53.45, 29.54, 21.05, 20.91, 20.50, 20.01, 19.98 (aliphatic carbons). Ms, m/z = 763 ($M^{\bullet+}$, 10.50 %). *Anal.* calcd for $C_{36}H_{37}N_5O_{14}$ (763.71): C, 56.62; H, 4.88; N, 9.17; O, 29.33; %. Found: C, 56.58; H, 4.85; N, 9.20; O, 29.35; %.

(Z)-N'-(2-(4-(4-(2,5-Dioxopyrrolidin-1-yl)phenyl)-1-oxophthalazin-2(1H)-yl)acetyl)-2,3,4,5,pentahydroxyhex-2-enehydrazide (18)

A mixture of compound **5** (3.91 g, 0.01 mol) and L-Ascorbic acid (1.76 g, 0.01 mol) in anhydrous ethanol under reflux for 3 hrs. The resultant solid was allowed to dry and recrystallized from ethanol to afford **18**. Yield [%] = 85. M p 205 - 207°C. IR [KBr, cm^{-1}] ʋ max = 3528 – 3413 (OH), 3219 - 3318 (2NH), 2916, 2850 (CH aliphatic), 1755, 1662 (C=O), 1554 (C=N). ^{1}H-NMR [DMSO-d_6, 500 *MHz*, δ, ppm] = 2.01 (m, 4H, $2CH_2$), 2.95 (s, 2H, N$\underline{CH_2}$CO), 3.42 - 3.71 (d, 2H, CH_2 methylene), 4.70 - 4.90 (m, 1H, CH methine), 7.11 - 8.08 (m, 8H, aromatic protons), 11.08 (s, 1H, NH exch.); 12.56 (s, 1H, OH exch.). ^{13}C- NMR [DMSO-d_6, 125 *MHz*, δ, ppm] = 171.79, 167.08, 154.61, 153.06, 147.86, 134.70, 134.54, 133.05, 131.54, 128.83, 128.34, 128.05, 127.40, 126.51, 125.52, 123.41(aromatic carbons),74.67, 68.44, 62.03, 52.50, 28.45. Ms, m/z = 568(M+ 1, 13.56 %). *Anal.* calcd for $C_{26}H_{25}N_5O_{10}$ (567.51): C, 55.03; H, 4.44; N, 12.34; O, 28.19; %. Found: C, 55.08; H, 4.99; N, 12.30; O, 28.21, %.

2. B Biological assessment

Antimicrobial assay

The antibacterial effectiveness of the synthesized compounds was assessed in vitro against *Staphylococcus Aureus* (ATCC:13565) (Gram-positive bacteria (G+Ve), and *Escherichia coli* (ATCC:10536) (Gram-negative bacteria (G-Ve)) using nutrient agar as the culture medium. Their antifungal efficacy was assessed against *Candida albicans* (ATCC:10231) and *Aspergillus niger*(ATCC:16404) on Sabouraud dextrose agar medium. Standard medications for Gram-negative bacteria and Gram-positive bacteria were Gentamicin and Ampicillin, respectively. While Nystatin was used for *Candida Albicans* and *Asperagillus Nigar***.** The compounds were evaluated against bacteria and fungi at a dose of 15 mg/ml, with DMSO as a solvent (negative control).

2.B.1.Test Method [32]

The sterilized medium was carefully dispensed into sterile Petri dishes (20–25 mL per dish) and allowed to set at 25°C. Sterile saline was used to prepare a microbiological suspension, adhering to the "McFarland 0.5 standard solution" (1.5 x 10^5 CFU/ml), and the turbidity was adjusted to an optical density of 0.13 at 625 nm using a spectrophotometer. After a 15-minute interval, a cotton swab was immersed in the prepared suspension, streaked across the agar surface, and allowed to dry under a cover for an additional 15 minutes. Wells of 6 mm diameter were formed in the solidified media employing a sterile borer. Afterward, the solution under analysis (100 µl) was dispensed into each well with a micropipette. Incubation of the plates at 37°C overnight was performed for bacterial analysis. All tests were performed in triplicate to ensure reproducibility. The zones of inhibition were measured on a millimeter (mm) scale using a standard ruler. throughout this experiment.

-2B.2. Statistical analysis

statistical differences between samples in the same type of bacteria (or fungi) were analyzed using one-way analysis of variance (ANOVA), followed by Duncan multiple range test for post hoc comparisons, using SPSS software package(version "22", IBM Corp, Armonk, NY, USA). Values are represented as mean ± standard deviation(X±SD). A $p<0.05$ was considered statistically significant, while $p<0$ was considered highly significant and $p<0.001$ was considered very highly significant. The observed inhibition zones are systematically presented in Table 2.

2. C Molecular Docking

Computational method

Protein receptor 3D structures were obtained from the RCSB Protein Data Bank (Table 1). PyMOL was employed to preprocess the target protein structures, removing water molecules, ions, and bound ligands. Compound structures were generated with BIOVIA Draw and converted with Open Babel [33], followed by conversion to the mol2 file format. These structures were subsequently transformed into the pdbqt format using AutoDock Tools. Ligand-centric grid maps for docking simulations were produced with AutoDock Vina [34]. Discovery Studio was employed to analyze and illustrate the two-dimensional protein–ligand binding interactions.

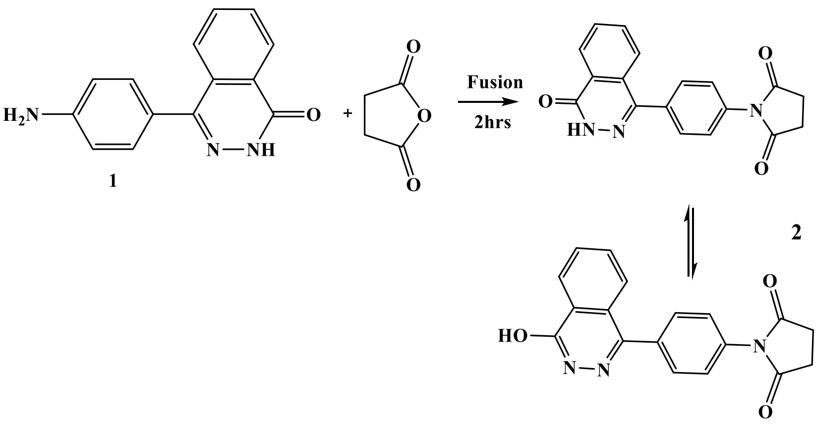
Table 1. Molecular docking targets of antimicrobial PDB IDs, active site coordinates

Organism		Protein Targets	PDB ID	Active site coordinates:			Reference Ligands
				X	Y	Z	
S. aureus	**G+ve**	Dihydropteroate synthase	**1AD4**	36.04	4.35	36.35	Ampicillin
E. coli	**G-ve**	DNA Gyrase	**7P2M**	-17.55	-4.58	12.77	Gentamicin
A.niger	**Fungi**	fdc1of A.niger	**4ZA5**	19.95	5.08	20.12	Nystain
C. albicans	**Fungi**	sterol 14-alpha demethylase	**5TZ1**	62.22	66.67	2.67	Nystain

3. Results and Discussion

3.A Chemistry

Herein, the authors present the preparation of phthalazinone **(2),** as a key precursor, by fusion of succinic anhydride with aminophthalazinone **(1).** Precursor chemical **1** was produced by reacting hydrazine hydrate with γ-keto acids in ethanol, as previously recorded. [30-31] (*Scheme 1*). Structural elucidation indicated that phthalazine **2** exists in two tautomeric forms [35-38]. Thus, the IR spectrum of **2** showed the presence of absorption bands for the NH and OH groups at υ 3158, 3467 cm^{-1} and a band for C=O at 1780, 1668 cm^{-1}. Also, the ^1H NMR spectrum showed signals attributed to NH and OH at δ 9.92 and 12.88 *ppm*, respectively.



Scheme 1. Synthesis of 1-(4-(4-oxo-3,4-dihydrophthalazin-1-yl)phenyl) pyrrolidine-2,5-dione (2)

The reaction of compound **2** with chloroacetic acid [39], facilitated by NaOH solution, gave phthalazinacetic acid derivative **3**. The IR spectrum of compound **3** exhibited significant absorption bands at υ 3291, 2919 to 2845, 1710 to 1658, 1595 cm^{-1} attributed to OH, CH, C=O, C=N, respectively. The ^{1}HNMR displayed signals at δ 4.7 (s, 2H, CH_2), 11.55 (s, 1H, OH exchangeable with D_2O), 7.40 - 8.04 (m, 8H, aromatic protons). Also, the ^{13}C NMR showed signals ppm at δ 58.41 c corresponding to CH_2, and ($2CH_2$) at 29.26 *ppm*. The mass spectra detected the molecular ion peak at m/z 377.

On the other hand, treatment of phthalazinone **2** with ethyl chloroacetate [40], in the presence of anhydrous K_2CO_3 and dry acetone, give phthalazine-2-acetate **4**. The IR spectrum of compound **4** showed distinct absorption bands at υ 2939 to 2869, 1735, 1585, 1065 cm^{-1}, attributed to CH, C=O of ester, C=N, C-O-C, respectively. The ^{1}H NMR showed signals at δ 1.18 (t, 3H, CH_3), 2.01 (m, 4H, $2CH_2$), 4.16 (s, 2H, CH_2), 4.73- 4.93 (q, 2H, CH_2), 7.43 to 7.96 (m, 8H, Aromatic protons). Also, the ^{13}C NMR displayed signals attributed to aliphatic carbons at δ 60.01, 49.99, 29.56, 14.10 *ppm*. The mass spectra detected a molecular ion peak at m/z 405. Furthermore, the hydrazinolysis [40] of compound **4** with hydrazine hydrate afforded phthalazinacetohydrazide **5**. The IR spectrum showed absorption peaks at υ 3338 to 3263, 3181, 1780,1661, 1621, 1595 cm^{-1} attributed to NH_2, NH, (3C=O), and C=N, respectively. The ^{1}HNMR displayed signals at δ 4.50 (s, 2H, $N\underline{CH_2}CO$), 9.96 (s, 2H, NH_2 exchangeable with D_2O), 7.28 -7.94 (m, 8H, aromatic protons), 13.15 (s, 1H, NH exchangeable with D_2O). The 13C NMR and mass spectra were in good agreement with the desired structure (c.f. experimental **5**).

Under Mannich reaction conditions, the authors examined the reactivity of phthalazinone **2** with piperidine [41], and ß-naphthol [42] in the presence of formaldehyde and hydrochloric acid. The results were the formation of Mannich bases **6** and **7,** respectively. The IR spectrum displayed significant absorption bands for ($-CH_2-$) at υ 2928 to 2854 and 2945 to 2897 cm^{-1}, and (C=N) at 1593 and 1598 cm^{-1}, with no detectable NH group peak in the phthalazinone derivatives **6** and **7**, indicating that the substitution in position-2 formed the Mannich bases. Also, ^{1}HNMR of compound **6** exhibited signals at δ 1.52-1.64 (m, 6H, CH_2), 3.40 (t, 4H, N-($2CH_2$)), 5.36 (s, 2H, CH_2), 6.89 - 9.04 (m, 8H, aromatic protons). The ^{13}CNMR showed signals attributed to aliphatic carbons at δ 72.67, 53.4, 30.03, 22.43, 22.04, 21.74 ppm. Mass spectra showed a molecular ion peak at m/z 416.

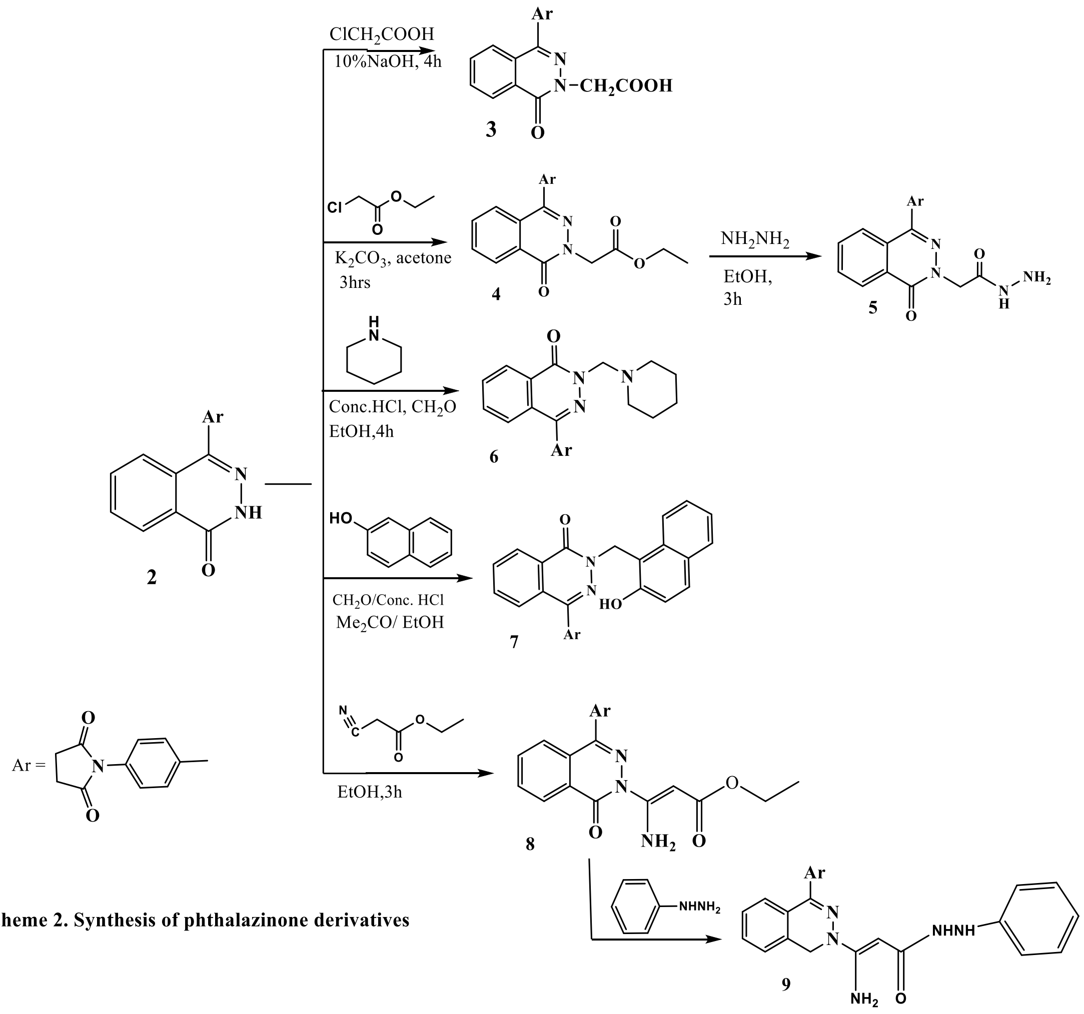
On the other hand, the IR spectrum of compound **7** exhibited absorption peaks at υ 3333, 1715,1649 cm^{-1}, attributed to (OH), (C=O). The ^{1}HNMR showed signals at δ 3.92 (s, 2H, CH_2), 7.83 - 8.08(m, 14H, aromatic protos), 12.87 (s, 1H, OH exchangeable with D_2O). The ^{13}CNMR displayed signals at δ 167.08 (C=O imide), 159.18 (C=O phthal), 153.17 (C-OH), and 50.01, 28.56 *ppm*, attributed to aliphatic carbons. The mass spectra of compounds **7** gave a molecular ion peak at m/z 475.

Similarly, treatment of compound **2** with ethyl cyanoacetate [40] in boiling ethanol afforded **8**. The IR spectrum displayed absorption peaks at υ 3398 – 3295 to 3269, 2956 - 2871, 1734, 1707, 1655, 1601, 1544 cm^{-1}, attributed to NH_2, CH, (3C=O), C=N, respectively. The ^{1}H-NMR showed signals δ 1.04 - 108 (t, 3H, CH_3), 3.29 (s, 2H, NH_2), 4.17 - 4.27 (q, 2H, CH_2), 5.98 (s, 1H, CH). The ^{13}C NMR exhibited signals related to aliphatic carbons at δ 90.09, 50.59, 29.56, 14.46 *ppm*. Additionally, Treatment of compound **8** with phenyl hydrazine [40] in ethanol furnished **9**. The IR spectrum of **9** showed peaks at υ 3466 to 3320, 3155, 1776,1708, 1665, 1591 cm^{-1}, attributed to NH_2, (NH), C=O, C=N, respectively. The ^{1}H-NMR displayed signals at δ 4.57 (s, 2H, NH_2), 5.45 (s, 1H, CH), 6.85 - 7.57 (m, 13H, aromatic protons), 10.14 (s, 1H, 2NH exch..). The ^{13}C NMR and mass spectra agreed well with the proposed structure (c.f. experimental **9**) (*scheme* 2).

The Hydrazide **5** reacted with acetylacetone [43] in boiling ethanol for 3 hours to give acetone derivative **10**. The IR spectrum exhibited absorption bands at υ 3267, 1712, 1669, 1602 cm^{-1} due to NH, (3C=O). The ^{1}HNMR spectrum showed a singlet at δ 1.49 (s, 6H, $2CH_3$), 3.60 (s, 2H, CH_2). Also, ^{13}CNMR showed signals at δ 181.12 *ppm* attributed to (C=O ketone), and aliphatic carbons appeared at δ 53.85, 42.38, 31.05, 29.65, 11.07 *ppm*.

Treatment of hydrazide 5 with phthalic anhydride [43] in an oil bath at 150°C afforded phthalimide compound **11**. The IR spectrum of **11** revealed strong absorption bands at υ 3155, 1767, 1712, 1667 cm^{-1} attributed to NH and (3C=O) respectively. The ^{1}HNMR displayed signals at δ 4.25 ppm, attributed to NCH_2CO, while 12.50 ppm corresponds to NH exchangeable with D_2O, and 7.43 to 8.64 (m, 12H, aromatic protons). The ^{13}C NMR and mass spectra agreed well with the proposed structure (c.f. experimental **11**).

Additionally, the *N*-formyl derivative **12** was synthesized from the reaction of formic acid [44] with hydrazine **5**. The IR spectrum displayed absorption bands at υ 3245 to 3166, 1702 cm^{-1}, indicative of (2NH), and C=O of formyl, respectively. The ^{1}HNMR exhibited signals at δ 9.02 (s, 1H, NH, NHCO exchangeable with D_2O) and 11.55 (s, 1H, NHCO phth, exchangeable with D_2O). The ^{13}C NMR supported the structure that displayed signals at δ 167.10 (C=O imide).



Scheme 2. Synthesis of phthalazinone derivatives

The reaction of compound **6** [41] presumably occurs through the subsequent mechanism, as illustrated in ***Figure 2***.

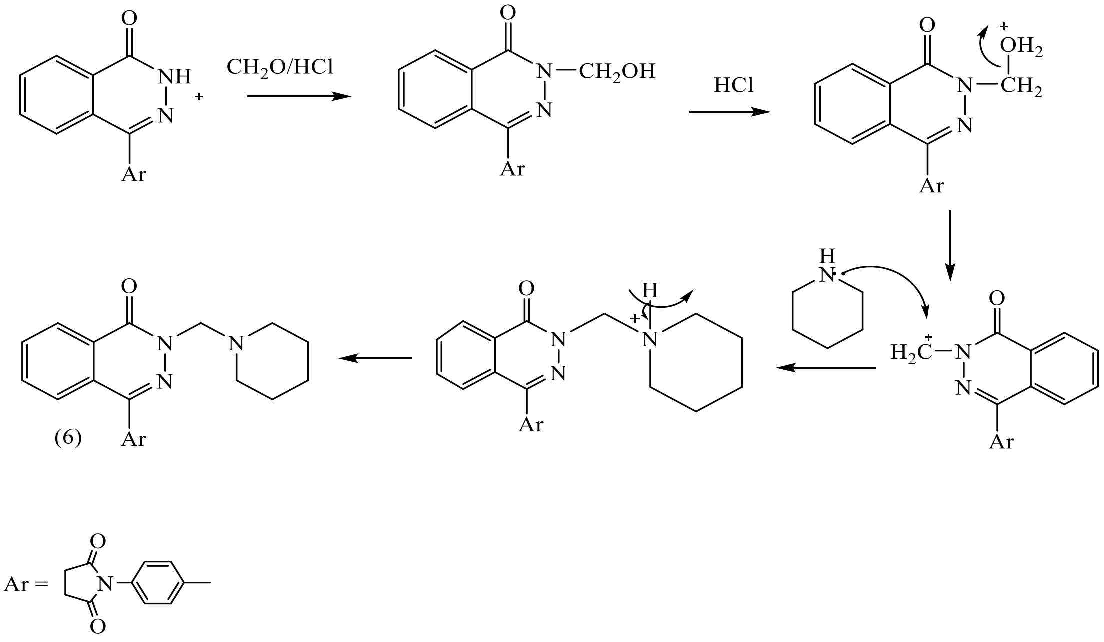
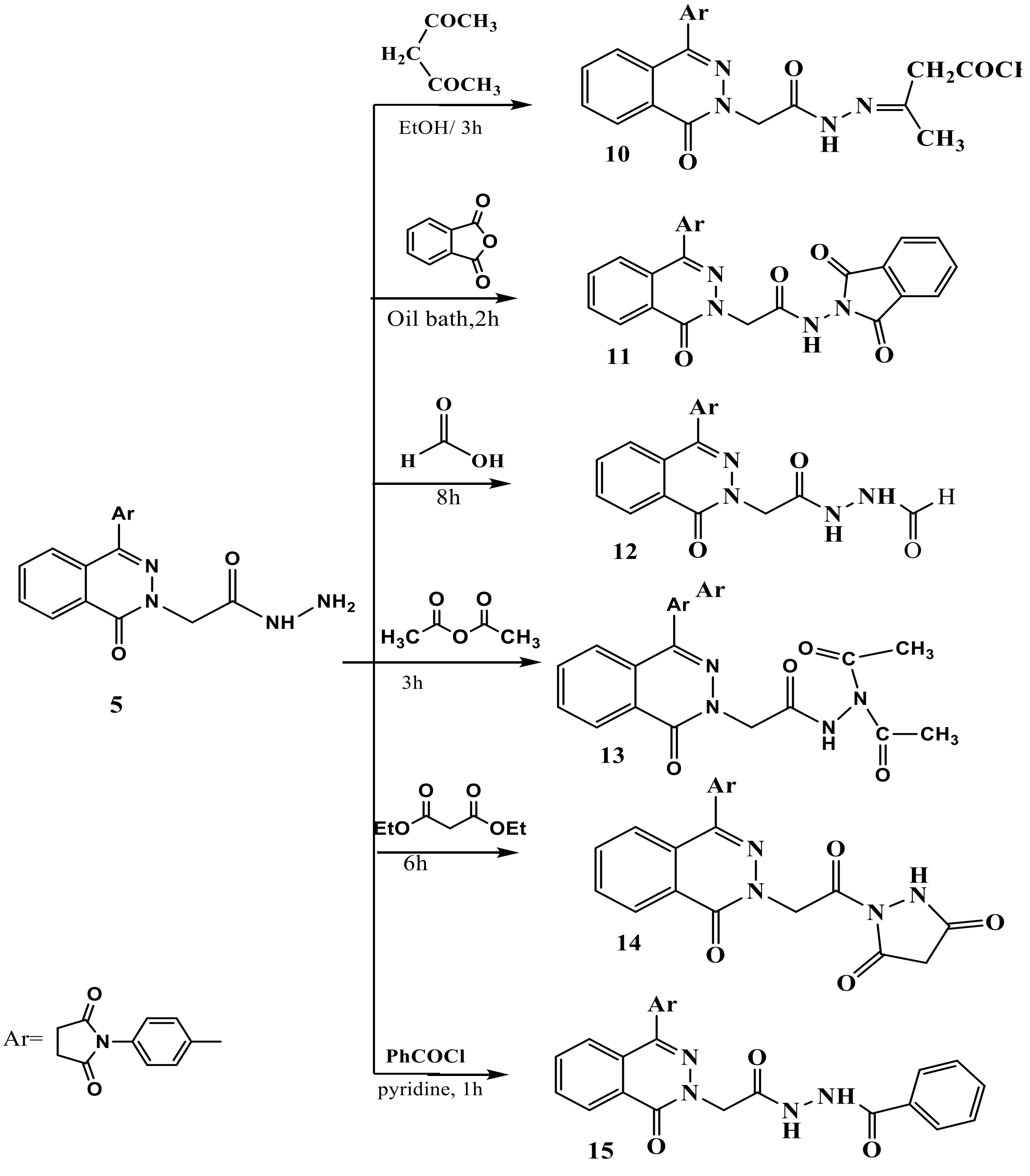


Figure 2. Outline the proposal mechanism of compound 6 synthesized

Conversely, the condensation of hydrazide **5** with acetic anhydride [45], furnished derivative N, N-diacetyl derivative **13**. The IR spectrum showed bands at υ 3445 to 3153, 2938 to 2894 cm-1 attributed to (2NH), and CH aliphatic, respectively. The ^{1}H-NMR displayed signals at δ 3.87 (s, 3H, CH_3), 4.52 (s, 2H, $N\underline{CH_2}CO$), 7.50 -7.80 (m, 8H, aromatic protons), 10.80 (s, 1H, NH exch..). The ^{13}C NMR exhibited a signal of methyl at 20.4 *ppm*.

The reaction of compound **5** with active methylene group-containing compounds, such as diethylmalonate [44], afforded pyrazole derivative **14**. The IR spectrum showed bands at υ 3451, 1749, 1661, 1603 attributed to NH and C=O, respectively. The ^{1}HNMR displayed signals at δ 1.85 (s, 2H, CH_2), and 10.95 (s, 1H, NH exchangeable with D_2O). Also, ^{13}C NMR exhibited signals at δ 55.10, 45.21, 30.03 *ppm* attributed to aliphatic carbons. Mass spectra detected a molecular ion peak at m/z 459.

Consequently, hydrazide **5** reacted with benzoyl chloride [43], giving acetyl benzo hydrazide **15**. The IR spectrum of **15** revealed strong absorption bands at υ 3153, 2939 to 2894, 1777, 1707, 1665, attributed to NH, CH, C=O, respectively. The ^{1}HNMR displayed signals at δ 3.98 (s, 2H, $N\underline{CH_2}CO$), 7.86 - 8.04 (m, 13H, aromatic protons), 11.57 (s, 1H, NH exchangeable with D_2O). The ^{13}C NMR exhibited the existence of a peak of carbonyl groups at δ 167.08, 159.18, 153.18, 151.40*ppm*. Mass spectra agreed well with the proposed structure **(*scheme* 3).**

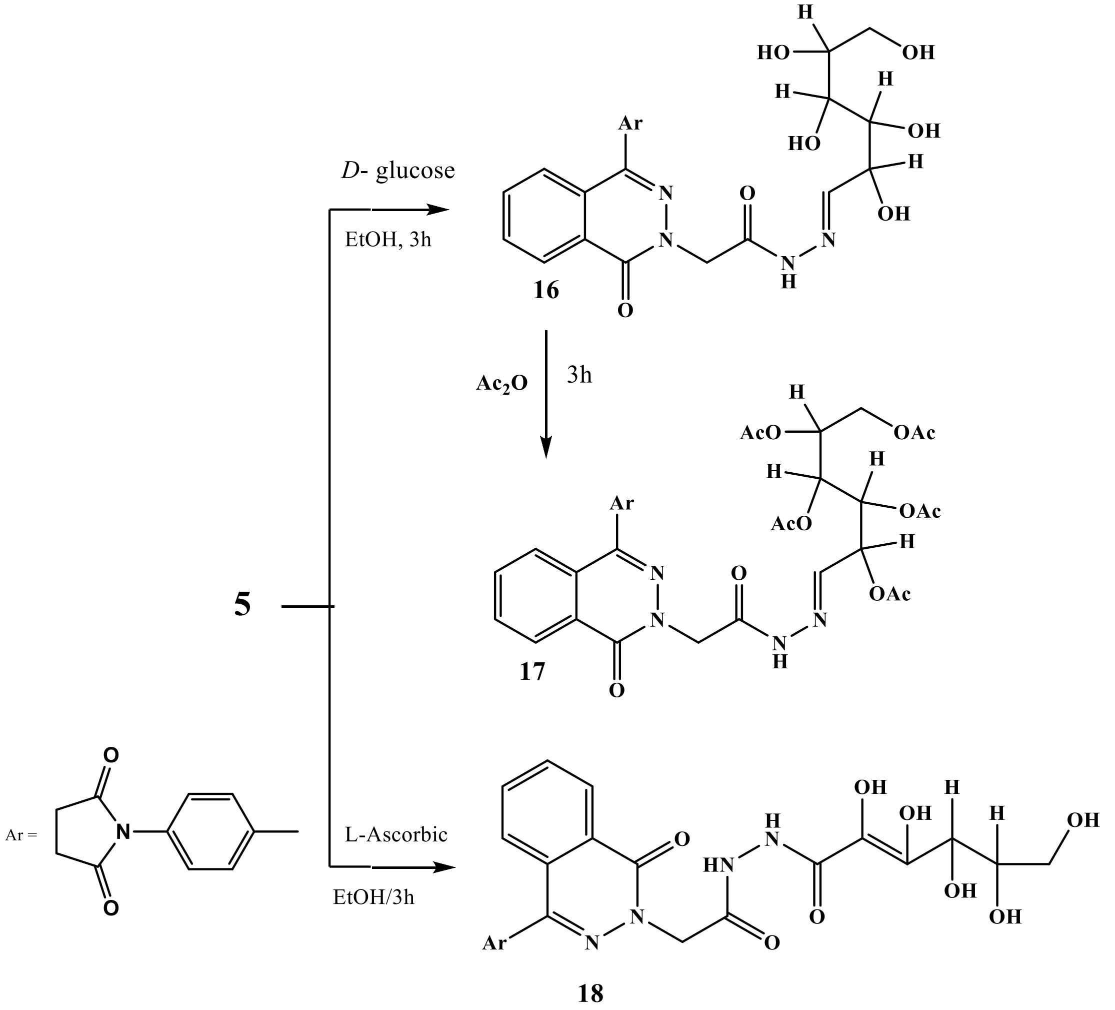


Scheme 3. Reaction of hydrazide 5 with some reagents

When hydrazide **5** reacted with glucose [31], it was then coupled with acetic anhydride [31] to produce the respective sugar hydrazones **16** and **17**. The IR spectrum of product **16** showed significant absorption bands at υ 3483, 3161, 1715,1700, 1603, 1556 cm^{-1}, relating to OH, NH, C=O, C=N, respectively. Also, the ^{1}HNMR and ^{13}CNMR agreed well with the proposed structure (c.f. Experimental 16). The mass spectra showed a molecular ion peak at m/z 553.

On the other hand, the IR spectrum of compound **17** displayed peaks at υ 3166, 3019, 2950 to 2898, 1744, 1668, 1602, 1555. attributed to NH, CH, C=O, C=N, and devoid any bands for OH. Also, the ^{1}HNMR showed signals at δ 1.24 (s, 15H, 5CH$_3$), 3.86 (s, 2H, $N\underline{CH_2}CO$), 4.42-4.47 (m, methine & methyl), 6.45 (s, 1H, CH=N), 11.54 (s, NH exchangeable with D$_2$O). The ^{13}CNMR was in good agreement with the desired structure (c.f. experimental 17). Mass spectra showed a molecular ion peak at m/z 763.

Additionally, treatment of compound **5** with L-Ascorbic acid [31] in ethanol resulted in compound **18**. The IR spectrum of **18** exhibited strong absorption bands at peaks at υ 3528, 3413- 3219, 1755,1662 cm$^{-1,}$ indicative of OH, (2NH), C=O, respectively. Also, the ^{1}H and ^{13}C- NMR were in good agreement with the desired structure (c.f. experimental 18). Whereas, Mass spectra exhibited a molecular ion peak at m/z 567 (scheme 4).



Scheme 4. Reaction of compound 5 with some aldohexoses and L-ascorbic acid

3. B Antimicrobial assay

In vitro, assessment of antimicrobial activity of synthesized compounds (1-18) against some of the pathogenic bacteria and fungi

The newly synthesized compounds evaluated against two reference drugs, Gentamicin and Ampicillin in *vitro* of Gram-negative and positive bacteria, in addition to one reference drug, Nystatin against fungi pathogens, and as the results are presented in Table 2, a small number of compounds exhibited positive effects on the examined organisms and the inhibition zones ranging from (10 to 20 mm) as compared to Gentamicin and Ampicillin. while a few numbers of the examined compounds offered fungal inhibition zones ranging from 21 to 30, as compared to Nystatin. On the other hand, other compounds gave no reaction with most of the tested organisms.

Table 2. The antimicrobial results of the examined compounds were measured by inhibition zone (mm),

	Escherichia Coli (ATCC:10536)	**Staphylococcus Aureus (ATCC:13565)**	**Candida Albicans (ATCC:10231)**	**Aspergillus Nigar (ATCC:16404)**
Compound No.	**Inhibition Zone(mm)**			
1	**NA**	**NA**	**NA**	**NA**
2	**NA**	**NA**	**NA**	**NA**
3	**NA**	**NA**	**NA**	**NA**
4	**NA**	**NA**	**NA**	**NA**
5	**20.0±1.0**	**14±0.6**	**21±1.0**	**NA**
6	**17.30±0.6**	**12±0.6**	**NA**	**NA**
7	**23.0±1.0**	**20.3±0.6**	**30.3±0.6**	**15.0±1.0**
8	**10±0.6**	**NA**	**NA**	**NA**
9	**20.7±0.6**	**13.3±0.6**	**25.0±1.0**	**NA**
10	**10±0.6**	**NA**	**NA**	**NA**
11	**12.0±1.0**	**NA**	**NA**	**NA**
12	**11.0±1.0**	**NA**	**NA**	**NA**
13	**NA**	**NA**	**NA**	**NA**
14	**13.3±0.6**	**13.0±1.0**	**NA**	**NA**
15	**NA**	**NA**	**NA**	**NA**
17	**10±0.6**	**NA**	**NA**	**NA**
18	**10±0.6**	**15.0±1.0**	**NA**	**NA**
Gentamicin	**27.0±1.0**			
Ampicillin		**21.3±0.6**		
Nystatin			**21.6±0.6**	**19.3±0.6**

NA: no activity, The expression for zone of inhibition is represented by mean± standard deviation (mm).

- As listed in **Table 2**, the anti-microbial activity results of the evaluated compounds demonstrated that many synthesized compounds displayed different inhibition in zones versus the examined bacteria and fungi. Notably, compound **7** displayed activities against *Escherichia coli* (inhibition zone 23±1 mm, and *Staphylococcus aureus* (inhibition zone 20.3±0.6mm) as compared to Gentamicin (inhibition zone 27±0.1 mm) and Ampicillin (inhibition zone 21.3±0.6 mm), also revealed potent inhibitory activity toward *Candida albicans* and *Asperagillus nigar* fungi (inhibition zone: 30.3±0.6 mm, 15±1.0 mm) respectively, compared to Nystatin (inhibition zone: 21.6±0.6 mm, 19.3±0.6 mm). On the other hand, compounds **5** and **9** revealed good bacterial inhibitory activity against Gentamicin and Ampicillin; both compounds showed fungal zone of inhibition with the values (21±1.0) and (25.0±1.0 mm) against Candida albicans as compared to Nystatin (21.6±0.6mm &19.3±0.6mm). In addition, Compounds (6, 14, 18) showed commendable efficacy against *Escherichia coli* and *Staphylococcus aureus* strains (17.30, 13.3, 10 ±0.6), and (12±0.6mm, 13, 15±1mm) as compared to Gentamicin (inhibition zone 27±0.1 mm) and Ampicillin (inhibition zone 21.3±0.6 mm), while demonstrating inactivity against antifungal bacteria. Conversely, a distinct trend in antibacterial activity against *Escherichia coli* was noted with compounds (**10, 11, 17**), displayed inhibition zone values (10±0.6, 12 ±1.0, 10 ±0.6 mm) as compared to Gentamicin (inhibition zone 27±0.1 mm), also exhibited inactivity against antifungal strains.

3.3 Molecular Docking studies

3.3.1 Docking and interaction method with Dihydropteroate synthase (DHPS) of *S. aureus*:

DHPS is a key enzyme in the biosynthesis of folate pathway, and its suppression is vital for impeding bacterial growth in this study, compounds **7, 9, 5, 6, 14, and 18** demonstrated significant binding energies of 7.90, -7.90, -7.10, -7.10, -8.10, and -6.60 kcal/mol, respectively, exceeding Ampicillin's binding energy of -6.40 kcal/mol, as outlined in the (**Table 3, figure 3**). These derivatives established H-bonds with many catalytic residues, encompassing His241, Arg52, Asn11, Arg239, His55, Arg204, Lys203, Ser50, Val49, and Arg239. Moreover, they participated in numerous hydrophobic interactions within the enzyme's active site, encompassing alkyl bonds with Pro216, Lys203, Ala199, Met128, Phe172, and His241; carbon-hydrogen bonds with Val49 and Lys203; pi-cation interactions with Asp84, Arg52, and Arg239; and pi-pi T-shaped interactions with Phe172. The residues His241, Asn11, Arg52, and Gln105 in the catalytic region were recognised as critical for enhancing the binding affinity of the derivatives. The findings reveal that the derivatives likely exert their antibacterial effects by inhibiting DHPS in *S. aureus*. This aligns with the endeavours of [46]. To assess the suppressive interactions between small molecules and *S. aureus* DHPS, they employed molecular docking. The results combined highlight the potential of these compounds as effective DHPS inhibitors, offering a promising strategy to combat bacterial resistance.

Table 3: Molecular interactions result with amino acids of DHPS of *S. aureus* (PDB: ID 1AD4):

Protein	**Ligand**	**Hydrophilic Interactions**		**Hydrophobic Contacts**		**No. of H-Bonds**	**No. of Total Bonds**	**affinity kcal mol-1**
		Residue (H- Bond)	**Length**	**Residue (Bond type)**	**Length**			
Dihydropteroate synthase of *S. aureus*	**7**	**His241 Arg52 Asn11**	**2.09 2.22 2.30**	**Asp84, (Pi-cation) Pro216, (Pi-alkyl) Lys203, (Pi-alkyl) Arg239, (Pi-cation)**	**4.70 5.18 5.34 4.15**	**3**	**9**	**-7.90**
	9	**His241 Arg52 Asn11 Arg239**	**2.02 2.36 2.05 2.02**	**Pro216, (Pi-alkyl) Lys203, (Pi-alkyl) Ala199, (Pi-alkyl) Met128, (Pi-alkyl) Arg239, (Pi-cation) Phe172, (Pi-pi T-shaped)**	**5.05 5.22 4.88 4.86 3.60 5.49**	**4**	**10**	**-7.90**
	5	**His55 Arg204 Asn11 Arg52 Asn105**	**3.09 2.95 2.21 2.61 2.92**	**Arg52, (Pi-cation) Val49, (C-H bond) Lys203, (Pi-alkyl) Phe172, (Pi-alkyl)**	**3.86 3.13 5.32 5.00**	**5**	**9**	**-7.10**
	6	**His241 Arg52 Asn11**	**1.96 2.57 2.04**	**Arg52, (Pi-cation) Pro216, (Pi-alkyl) Lys203, (Pi-alkyl) His241, (Pi-alkyl)**	**3.82 5.47 5.25 5.49**	**3**	**7**	**-7.10**
	14	**His241 Gln105 Asn11 Arg52 Lys203**	**2.11 2.53 2.24 2.99 2.67**	**Arg52, (Pi-cation) Lys203, (C-H bond) Lys203, (Pi-alkyl) Pro216, (Pi-alkyl)**	**3.95 3.59 5.24 5.12**	**5**	**9**	**-8.10**
	18	**Ser50 Val49 Arg239 Asn11**	**2.00 2.87 2.10 2.34**	**-**	**-**	**4**	**0**	**-6.60**
	Ampicillin	**Arg239 Asn11 Arg52 His241**	**2.12 2.01 2.90 2.20**	**Pro216, (Pi-alkyl) Arg202, (Pi-alkyl) His55, (Pi-alkyl) Phe172, (Pi-alkyl) His241, (Pi-Pi-T-shaped)**	**4.30 5.35 4.60 5.40 5.02**	**4**	**9**	**-6.40**

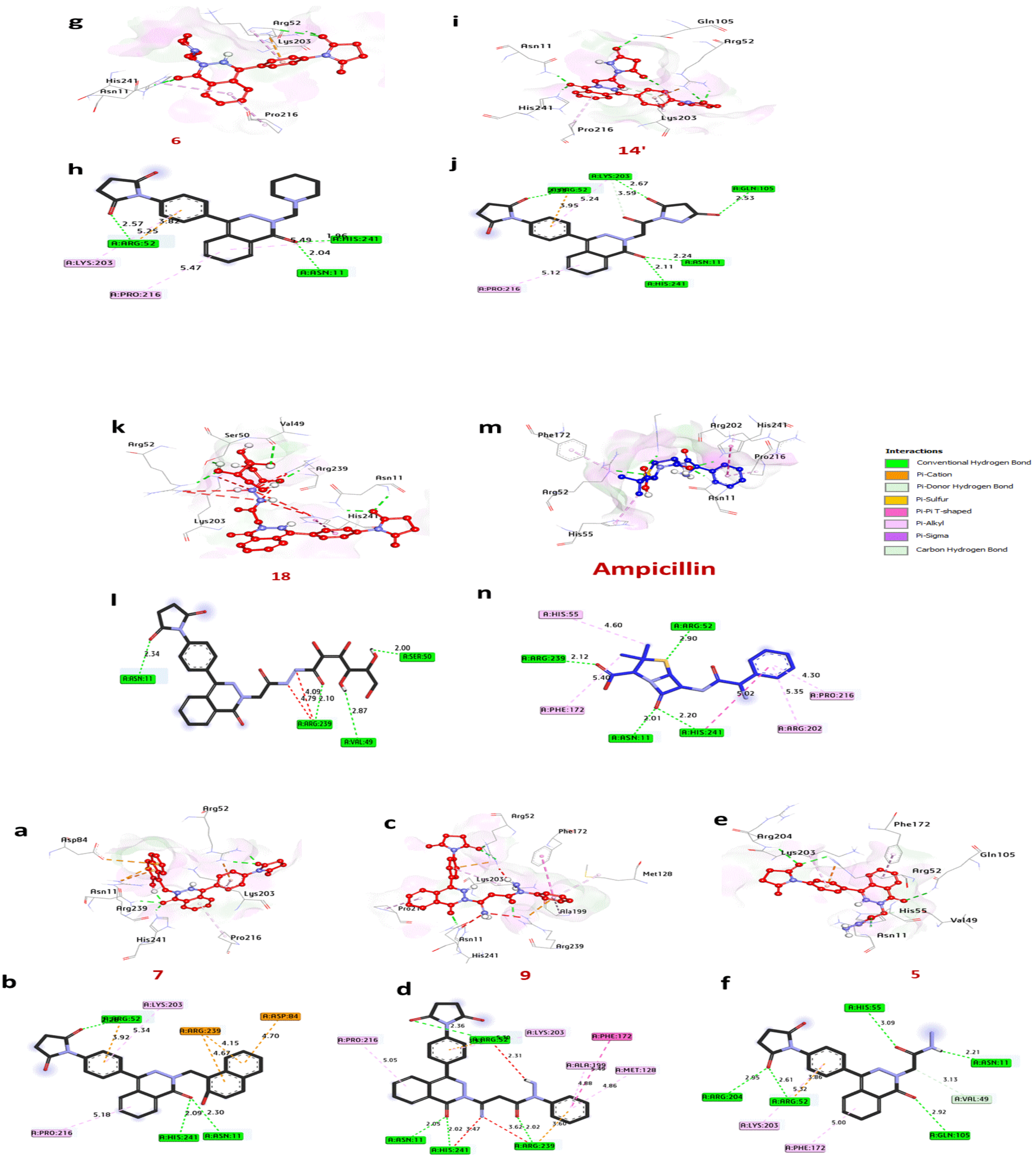


Figure 3. 3D representations of the conformations of compounds at the binding pocket of DHPS in *S. aureus* (PDB: ID 1AD4) are as follows: (a and b) **7,** (c and d) **9,** (e, and f) **5,** (g and h) **6,** and (I and j) **14**, (k, and l) **18**, (m, and n) **Ampicillin**.

3.3.2 Docking and interaction method with receptor, DNA Gyrase of *E. coli* :

This work examined DNA gyrase, a vital enzyme necessary for DNA replication and transcription in *E. coli,* as a target for possible inhibitors. Molecular docking analyses, as elaborated in **Table 4** and **Figure 4**, Compounds **7**, **9, 5, 6, 14,** and **18** demonstrated robust binding energies of 8.60, -7.90, -7.80, -8.80, -7.90, and -7.70 kcal/mol, respectively, surpassing Gentamicin's binding energy of -5.70 kcal/mol. Compounds **5, 14**, and **18** established hydrogen bonds with critical catalytic residues, including Asp49, Asn46, Glu42, Gly117, Val118, and Ser121. Furthermore, the enzyme's active site exhibited hydrophobic interactions, including alkyl bonds with Val120, Ile78, Pro79, Ile94, Val167, and Val43; pi-pi T-shaped interactions with Asn46; carbon-hydrogen bonds with Gly119, Asp73, Ile78, Val43, and Gly119; pi-sigma interactions with Ile78 and Ile94; and pi-cation interactions with Glu50 and Glu42. The residues Asp49, Glu42, and Asn46 in the catalytic site substantially enhanced ligand binding stability, highlighting their significance for inhibitor effectiveness. Compounds **9, 5, 6, 14**, and **18** collectively exhibit considerable potential as inhibitors of DNA gyrase, presenting a promising approach to combat bacterial resistance. These results correspond with the research of [47]. They utilised molecular docking to examine inhibitor interactions with the DNA gyrase enzyme.

Table 4: The molecular interactions result between ligands and amino acids of DNA receptor Gyrase in *E. coli*

Protein	Ligand	Hydrophilic Interactions		Hydrophobic Contacts		No. of H-Bonds	No. of Total Bonds	affinity kcal mol-1
		Residue (H- Bond)	Length	Residue (Bond type)	Length			
DNA Gyrase of *E. coli* (PDB: ID 7P2M)	7	-	-	Val120, (alkyl) Ile78, (alkyl) Ile78, (pi-sigma) Asn46, Pi Pi-T shaped)	4.99 4.35 3.50 5.06	0	4	-8.60
	9	-	-	Val120, (alkyl) Pro79, (alkyl) Ile94, (alkyl) Ile94, (pi-sigma) Ile78, (pi-sigma) Gly119, (C-H bond)	5.77 5.31 5.02 3.74 3.86 3.56	0	6	-7.90
	5	Asp49, Asp49 Asn46 Glu42	2.44 3.54 2.23 2.03	Ile94, (alkyl) Ile94, (alkyl) Ile78, (C-H bond) Gly119, (C-H bond)	5.35 4.37 3.63 3.79	4	8	-7.80
	6	-	-	Val167, (alkyl) Val120, (alkyl) Val43, (alkyl) Ile78, (alkyl) Pro79, (alkyl) Glu50, (pi-Cation) Gly119, (C-H bond) Asp73, (C-H bond)	4.89 5.04 5.55 4.78 5.24 4.16 3.56 3.12	0	8	-8.80
	14	Gly117 Asn46	2.69 2.14	Ile94, (alkyl) Ile78, (alkyl) Ile94, (pi-sigma)	5.17 5.43 3.90	2	6	-7.90
	18	Val118 Val118 Asn46 Ser121	2.20 2.87 2.77 3.00	Glu42, (pi-Cation) Ile78, (alkyl)	5.49 4.87	4	6	-7.70
	Gentamicin	Asp49 Asn46	2.19 2.34	Ile94, (alkyl) Ile94, (alkyl) Ile78, (alkyl)	4.24 5.08 5.05	2	6	-5.70

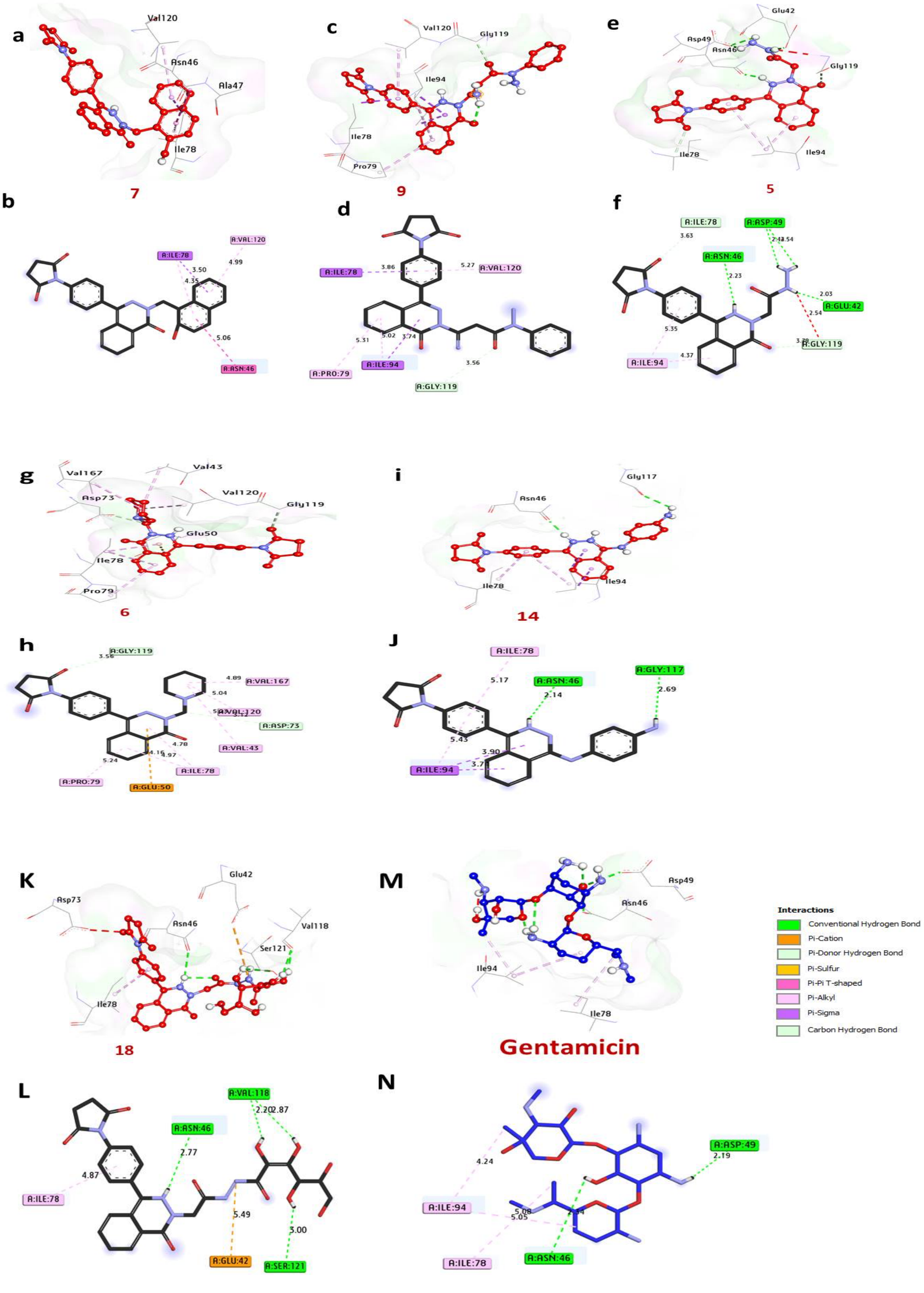


Fig. 4: 3D representations of compounds at the binding pocket of DNA Gyrase in *E. coli* (PDB: ID 7P2M) are as follows: (a and b) 7**,** (c and d) **9,** (e, and f) **5,** (g and h) **6,** (I and j) **14**, (k, and l) **18**, (m, and n). **Gentamicin**.

3.3.3 Docking and interaction with fdc1of *A. niger* (PDB:ID 4ZA5)*:*

The FDC1 protein in *A. niger* is crucial for the aromatic compounds' breakdown, a process that significantly influences the characteristics of fermented beverages. Molecular docking, depicted in **Figure 5** and **Table 5,** Compound **7** exhibited a strong binding affinity of. -13.70 kcal/mol, surpassing that of Nystatin at -10.80 kcal/mol. Compound **7** established H-bonds with critical catalytic residues Ile171, Ser224, and Cys316, thereby enhancing its binding stability. Furthermore, it participated in multiple hydrophobic interactions within the enzyme's active site, encompassing alkyl bonds with Met326, Ile327, Leu185, Arg173, and Ala172; pi-cation interactions with Arg173; pi-sulfur bonds with Cys316; and carbon-hydrogen bonds with Ser170. Residues Ile171, Trp166, and Cys316 in the catalytic region were identified as essential for augmenting binding affinity. The results indicate that chemical **7** is a promising inhibitor of fungal FDC1 in A. niger, necessitating additional research. The findings correspond with the research of [48], who highlighted the antibacterial efficacy of drugs aimed at *A. niger* FDC1 by molecular docking. This consistency highlights the potential of these compounds as effective FDC1 inhibitors, presenting exciting opportunities for addressing fungal infections and enhancing fermentation processes in food and beverage manufacturing.

Table 5: Molecular interactions data with amino acids of fdc1of *A.niger* (PDB: ID 4ZA5)

Protein	**Compounds**	**Hydrophilic Interactions**		**Hydrophobic Contacts**		**No. of H-Bonds**	**No. of Total Bonds**	**affinity kcal mol^{-1}**
		Residue	**Length**	**Residue (Bond type)**	**Length**			
fdc1of *A. niger* (PDB:ID 5TZ1)	**7**	**Ile171, (H- Bond) Ser224, (H- Bond) Cys316, (H- Bond)**	**2.86 2.84 2.67**	**Ile327, (Pi-alkyl) Met326, (Pi-alkyl) Ala172, (Pi-alkyl) Leu185, (Pi-alkyl) Arg173, (Pi-alkyl) Cys316, (Pi-sulfur) Ser170, (Carbon H bond) Arg173, (Pi-cation)**	**4.71 4.77 5.36 5.13 4.64 5.89 3.61 4.46**	**3**	**11**	**-13.70**
	Nystatin	**Val231, (H- Bond) Trp166, (H- Bond) Arg173, (H- Bond)**	**1.93 2.05 2.59**	**Ile142, (Pi-alkyl)**	**4.57**	**3**	**4**	**-10.80**

3.3.4 Docking study with receptor, sterol 14-alpha demethylase of *C. albicans*:

Sterol 14-alpha demethylase, an essential cytochrome P450 enzyme in *C. albicans*, is pivotal in ergosterol biosynthesis, a step vital for preserving fungal cell membrane integrity. The inhibition of this enzyme undermines membrane integrity, leading to lethal cellular damage. Molecular docking analysis, as outlined in **Table 6 (Fig. 6**), found compounds **9** and **5** as potent inhibitors, demonstrating binding affinities of -7.60, -8.10, and -9.00 kcal/mol, respectively, which surpass the affinity of Nystatin (-7.50 kcal/mol). These establish hydrogen bonds with critical catalytic residues (Lys143, Cys470, and Thr311) and participate in various hydrophobic interactions inside the active site. The interactions comprise alkyl bonds with Ile471, Ile131, Lys143, Leu376, Ala146, Ala476, Phe463, Pro375, Leu370, Cys470, and Leu139; C-H bonds with His468 and Cys470; pi-sigma interactions with Leu150, Thr311, and Leu376; sulfur bonds with Cys470; and pi-cation interactions with Lys143. Residues Lys143, Cys470, and Thr311 were identified as essential for stabilising ligand binding, hence enhancing the drugs' inhibitory effectiveness. These results align with the findings of [27], who emphasised the efficacy of docking studies in discovering antifungal drugs that target sterol 14-alpha demethylase. The robust binding characteristics of these compounds highlight their potential as next-generation antifungal medicines, offering a promising approach to combat drug-resistant *C. albicans* infections.

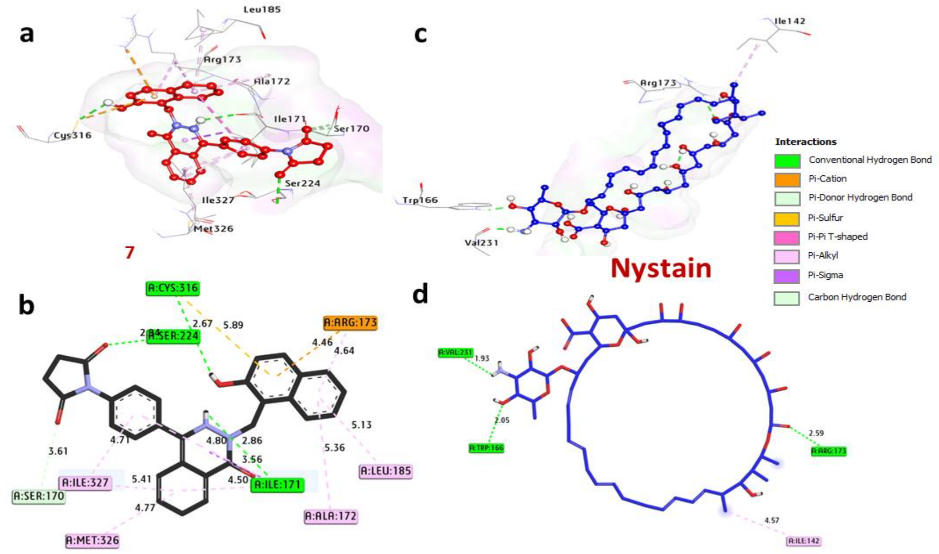


Fig. 5: 3D representations of compounds at the binding pocket of fdc1of *A. niger* (PDB: ID 4ZA5): (a and b) **7**, (c and d) **Nystatin**.

Table (6) Molecular interactions between ligands and amino acids of sterol 14-alpha demethylase in *C.* albicans (PDB: ID 5TZ1)

Protein	Ligand	Hydrophilic Interactions		Hydrophobic Contacts		No. of H-Bon	No. of Total	affinity kcal
		Residue (H- Bond)	Length	Residue (Bond type)	Length			
sterol 14-alpha demethylase of *C. albicans* (PDB: ID 5TZ1)	7	Lys143	2.67	Leu376, (Pi-sigma) Ile471, (Pi-alkyl) Ile471, (Pi-alkyl) Ile131, (Pi-alkyl) Lys143, (Pi-alkyl) Lys143, (Pi-alkyl) Cys470, (Carbon H bond) Cys470, (sulfur) Cys470, (sulfur)	5.26 5.17 5.16 4.65 4.73 5.01 4.12 5.00	1	10	-7.60
	9	Cys470	2.57	Leu376, (Pi-alkyl) Ala146, (Pi-alkyl) Ile471, (Pi-alkyl) Ala476, (Pi-alkyl) Phe463, (Pi-alkyl) Pro375, (Pi-alkyl) Leu370, (Pi-alkyl) Leu150, (Pi-sigma) Ile304, (Pi-sigma) Thr311, (Pi-sigma) Cys470, (sulfur)	5.19 5.15 5.30 5.30 4.18 4.60 4.00 3.51 3.09 3.80 4.28	1	13	-8.10
	5	Cys470 Thr311	3.80 2.17	Cys470, (Pi-alkyl) Ile471, (Pi-alkyl) Leu139, (Pi-alkyl) Ile131, (Pi-alkyl) Lys143, (Pi-alkyl) Lys143, (Pi-cation) His468, (Carbon H bond)	4.54 5.15 5.49 4.58 4.47 4.71 2.66	2	9	-9.00
	Nystatin	Ser312	2.84	Phe105, (Pi-alkyl) Phe105, (Pi-alkyl) Met374, (Pi-alkyl) Ile379, (Pi-alkyl) Pro406, (Pi-alkyl) Phe463, (Carbon H bond)	4.24 4.78 4.94 4.24 4.16 3.48	1	8	-7.50

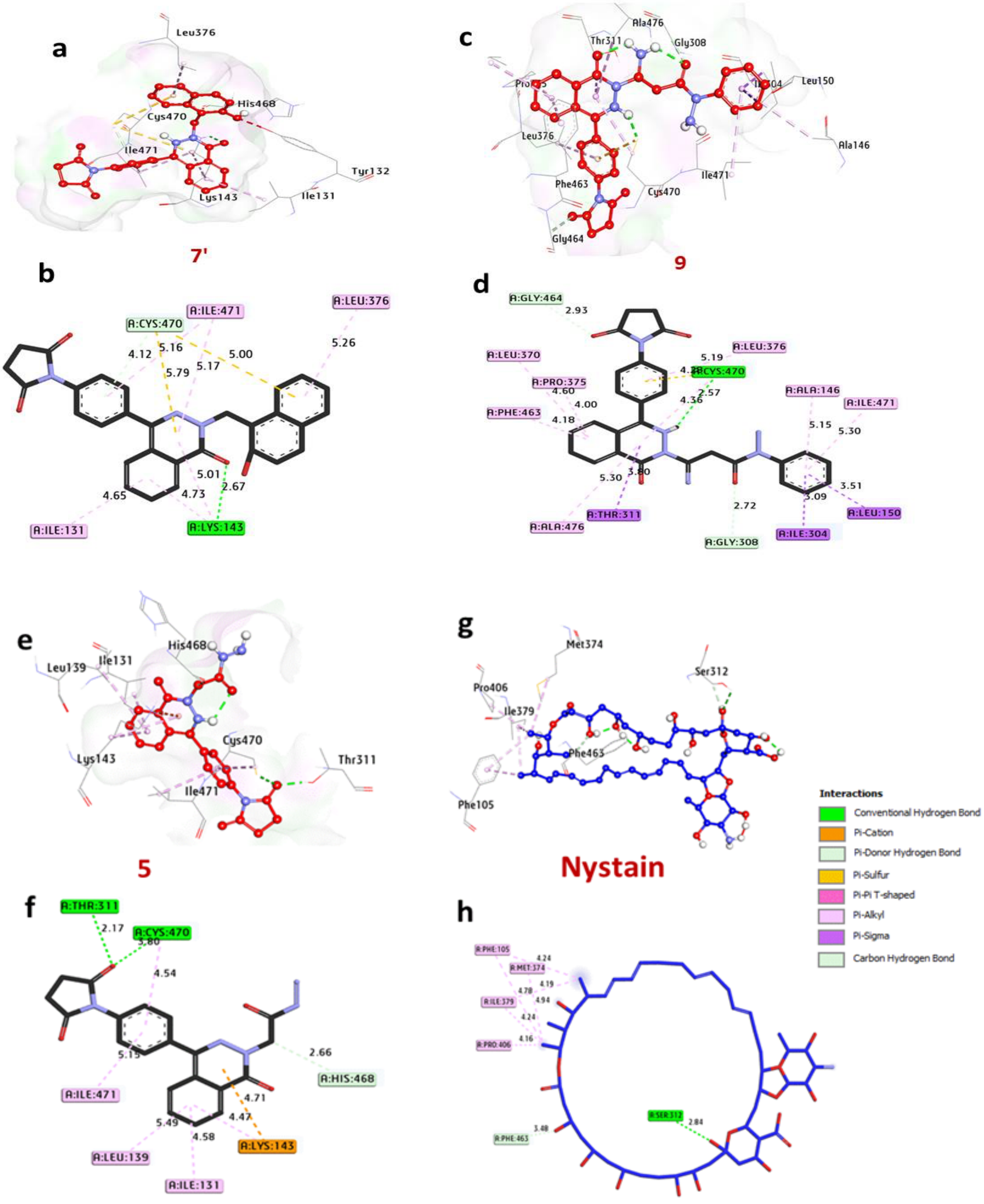


Fig. 6: 3D visualizations of the conformations of the compounds in the binding pocket of the sterol 14-alpha demethylase enzyme in *C. albicans* (PDB: ID 5TZ1): (a and b) **7**, (c and d) **9**, (e and f) **5,** (g and h) Nystatin.

4. Conclusion

A new set of phthalazinone derivatives was synthesized, and the newly prepared compounds were evaluated in *vitro* for their antimicrobial activity. Certain compounds showed potent to weak antimicrobial activity as compared to the used references. compound **7** revealed activities versus *Escherichia coli* (inhibition zone 23±1 mm, and *Staphylococcus aureus* (inhibition zone 20.3±0.6mm) as compared to Gentamicin (inhibition zone 27±0.1 mm) & Ampicillin (inhibition zone 21.3±0.6 mm), and exhibiting potent inhibitory activity toward *Candida albicans* and *Asperagillus nigar* fungi (inhibition zone: 30.3±0.6 mm, 15±1.0 mm) respectively, compared to Nystatin (inhibition zone: 21.6±0.6 mm, 19.3±0.6 mm). Moreover, compounds 5 and 9 displayed anti-bacterial and anti-fungal activities against some tested strains. Molecular docking studies were conducted and reinforce these results by revealing strong interactions with essential microbial enzymes. Collectively, these compounds show potential as promising candidates for the development of multitarget therapeutics against infectious diseases.

Conflicts of interest

No conflicts of interest

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