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In Silico Molecular Docking and Synthetic Elaboration of New Phthalazine Scaffolds Functionalized with a Phthalyl Moiety for Putative Antimicrobial Potency

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Abstract
This study investigated the synthesis and evaluation of novel phthalazine derivatives bearing phthalyl amino acid moieties as potential antibacterial and antifungal agents . A series of phthalazine derivatives (3 a-g) were synthesized by condensing phthalazin -1(2H) – one with various phthalyl amino acids (glutamine , methionine , tyrosine , histidine , alanine , phenylalanine and glycine) using N,N`-dicyclohexylcarbodiimide (DCC) as a dehydrating agent . Subsequent removal of the phthalyl protecting group yielded the corresponding free amino acid derivatives (4 a-g) The bioactivity of the synthesized compounds was scrutinized for antimicrobial efficacy, with certain candidates exhibiting auspicious potential. Computational molecular docking analyses were executed to decipher the intricate binding affinities and interaction dynamics between the synthesized compounds and their respective targets and their putative targets, revealing a correlation between binding affinity and antibacterial activity. The molecular architectures of the synthesized compounds were unequivocally authenticated through advanced spectroscopic modalities, including ^{1}H-NMR, mass spectrometry, and infrared (IR) spectroscopy, alongside comprehensive elemental composition analysis.
Keywords: phthalazinone, phthalyl amino acid, Anti-bacterial, ati-fungal, molecular docking

1. Introduction

Heterocyclic compounds constitute a broad class of cyclic organic molecules that incorporate at least one heteroatom typically nitrogen, oxygen, or sulfurwithin their ring structure. These frameworks play a fundamental role in medicinal chemistry due to their widespread occurrence in biologically significant molecules such as vitamins, enzymes, and natural products. They exhibit a diverse range of biological activities, including antifungal, anti-inflammatory, antibacterial, antioxidant, anticonvulsant, antiallergic, enzyme inhibitory, herbicidal, anti-HIV, antidiabetic, anticancer, and insecticidal effects [1,2].Among these, nitrogen-containing fused heterocyclesparticularly diaza-heterocycleshave garnered considerable attention as core pharmacophores in drug development. Notable examples include benzodiazine derivatives such as cinnoline (1,2-), quinazoline (1,3-), quinoxaline (1,4-), and phthalazine (2,3-), which feature two nitrogen atoms embedded within a fused aromatic ring system. These structures are commonly found in natural bioactive compounds and synthetic therapeutic agents, and they display a wide spectrum of pharmacological properties [3–6]. Phthalazine (benzopyridazine), with the molecular formula $C_8H_6N_2$, is considered a privileged scaffold in drug design. Its derivatives have shown a broad range of biological activities, including cardiotonic [7–9], anticonvulsant [10–15], vasorelaxant [16,17], anti-inflammatory [18,19], anticancer [20–23], antibacterial [24–29], antifungal [30–34], antihypertensive [35–38], and carbonic anhydrase inhibitory effects [39–41], as well as applications in agrochemistry [42–45].Owing to its versatile biological potential and synthetic flexibility, the phthalazine scaffold continues to be a focal point in medicinal chemistry. In this study, a series of 4-(2,4-dimethylphenyl)phthalazine-1(2H)-one derivatives were synthesized and evaluated for their antibacterial and antifungal activities. The findings were further supported by molecular docking studies to explore their potential mechanisms of action.

2. Material and method

Materials

All melting points were ascertained without correction utilizing an electrothermal melting point apparatus. Elemental composition assessments were executed via a Heraeus CHN Rapid Analyzer within the Microanalytical Unit at Cairo University. Thin-layer chromatography (TLC) analyses were conducted on Merck TLC aluminum plates coated with silica gel $60F_{254}$, with visualization facilitated through UV quenching at 254 nm. Infrared (IR) spectral data were acquired using a Unicam SP-1200 spectrophotometer, employing the KBr pelletization technique. ^{1}H-NMR spectra were recorded in DMSO-d_6 using a Varian Plus spectrometer (300 MHz). Mass spectrometric profiles were generated on a Shimadzu GC-MS QP 1000 EX system, operating at 70 eV under electron ionization (EI) conditions.

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Synthesis

4-(2,4 di methyl phenyl)phthalazin-(1H)- one (1)

Hydrazine hydrate (0.01 mol) was added to a stirred solution of 2-(2,4-dimethylbenzoyl)benzoic acid (A) (0.01 mol) in absolute ethanol (50 mL). The reaction mixture was then heated under reflux for 3 hours. After completion of the reaction, the mixture was allowed to cool to room temperature, resulting in the formation of a solid precipitate. The solid was collected by filtration, washed with cold ethanol, and recrystallized from ethanol to afford pure phthalazinone (1).

The obtained phthalazinone (1) was isolated as colorless crystalline solids in 60% yield; m.p. 230–232 °C.IR (cm^{-1})ν_{max}: 3320 (NH) ,3161and 2908(CH) ,1671(CO).^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.31–2.40 (s, 6H, 2 × CH_3, m-xylene), 7.10–8.10 (m, 7H, Ar–H), 12.40 (s, 2H, NH_2, exchangeable with D_2O). ^{13}C NMR(DMSO-d_6, 300MHz) δ ppm: 159.4(C=O), 138.8, 136.9, 134.7, 132.2, 131.1,131, 130.4, 130.8, 129, 127.5, 126.2, 123.7 (aromatic carbons), 21.6, 19.5 (aliphatic carbons). Anal. Calcd. for $C_{16}H_{14}N_2O$: C, 76.78; H, 5.64.; N, 11.19. Found C,76.2; H,5.5; N,11.02%.

General procedure for the synthesis of N-phthaloyl amino acid(2a-g)

A mixture of 0.01mol of amino acid (a-g) and 0.01mol of finally ground phthalic anhydride was placed in beaker,then the beaker was then immersed in an oil bath preheated to 180-185 °C for 15 minutes. The mixture was stirred occasionally during the first 10 minutes. After the reaction, the beaker was removed and allowed to cool until the liquid mass solidified. The solid material was recrystallized from methanol-water.

General procedure for synthesis of N-phthalyl amino acid-phthalazinone (3a-g)

A mixture of 4-(2,4-di methyl phenyl) phthalazin-1H-one 1 (0.01mole) and phthalyl amino acid 2a-g (0.01mole) was dissolved in 30mlTHF. The conglomerate of reactants was allowed to cool down to a cryogenic state of 0°C and N,N`-dicyclohexylcarbodiimideDCC(0.01mole) dissolved in THF and added to reaction mixture . The cohort of reactants was subject to stirring for a full 24-hour period at 0°C, transitioned to a further 24 hours of agitation at room temperature, and ultimately maintained at 0°C throughout the overnight interval.The precipitated N,N'-dicyclohexylurea was separated via filtration, and the resulting filtrate underwent successive washing with 1N hydrochloric acid, followed by 1N sodium bicarbonate, and subsequently with a solution that has reached the threshold concentration of sodium chloride as a solute, beyond which additional solute will no longer dissolveof 1N sodium chloride. This mixture was then dried using anhydrous sodium sulfate and allowed to stand overnight. Following this, it was subjected to filtration to eliminate any remaining solids, after which excess solvent was evaporated. The resultant residue was dissolved in benzene and set aside for a duration of two hours, leading to the re-precipitation of dicyclohexylurea, which was then removed by filtration. The solvent was subsequently eliminated under reduced pressure, and the resultant residue was crystallized from an appropriate solvent to yield compound (3 a-g) [47].

4-(2,4-dimethylphenyl) phthalazin-1-yl-2-acetate derived from 1,3-dioxoisoindolin-2-yl (3a) .

Yield ,61%, m.p 181-183°C, IR(cm^{-1})ν_{max}: 2910(CH),1735,1719 (imidic CO), 1672 (C=O),1630(C=N).. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.30–2.40 (s, 6H, 2 × CH_3, m-xylene), 4.80 (s, 2H, CH_2, $COCH_2N$), 7.10–8.40 (m, 11H, Ar–H).^{13}C NMR(DMSO-d_6,300MHz) δ ppm:171(C-O-C),168.2(2C=O), 168(C=O), 150, 138.3, 136.8, 133. 5,132.6,132.2, 131.4, 127.5,127.3,126.7,126.6,125.5,123.7,119.7(aromaticcarbon),43,1,21.6,19.5(aliphatic carbons) Anal.Calcd . for $C_{26}H_{19}N_3O_4$:C,71.39;H,4.38;N,9.61%.Found C,71.19; H,4.18; N,9.41%.

5-Amino-2-(1,3-dioxoisoindolin-2-yl)-5-oxo pentanoate of 4-(2,4-dimethylphenyl)phthalazin-1-yl. (3b).

Yield ,63%, m.p 163-165°C, IR(cm^{-1})ν_{max}:2916(CH),1730,1719 (imidic CO), 1667 (C=O),1660(C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.05–2.10 (m, 4H, CH_2CH_2, $COCHCH_2CH_2$), 2.30–2.40 (s, 6H,2 × CH_3, m-xylene), 4.30 (t, J = 6.6 Hz, 1H, CH,COCHN),7.10–8.30(m,11H,Ar–H),7.10(brs,2H,NH_2,exchangeablewithD_2O).Anal.Calcd.for $C_{29}H_{24}N_4O_5$:C,68.5;H,4.7;N,11.02%.Found C,68.3; H,4.5; N,10.82%.

3-(4-Hydroxyphenyl)-2-(1,3-dioxoisoindolin-2-yl) propanoate of 4-(2,4-dimethylphenyl)phthalazin-1-yl (3c).

Yield ,81%, m.p 180-182°C, IR(cm^{-1})ν_{max}: 3325(OH),1771,1730(imidic CO), 1714(C=O),1668(C=N) .^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.30–2.40 (s, 6H,2 × CH_3, m-xylene), 3.10 (d, J = 6.8 Hz, 2H, CH_2–Ph), 4.80 (t, J = 6.8 Hz, 1H, CH, COCHN), 6.90–8.40(m,15H,Ar–H),9.08(s,1H,OH, exchangeable with D_2O).Anal. Calcd. for $C_{33}H_{25}N_3O_5$:C,72.9;H,4.64;N,7.7%.Found C,72.6;H,4.31;N,7.4%.

2-(1,3-Dioxoisoindolin-2-yl) propanoate of 4-(2,4-dimethylphenyl) phthalazin-1-yl (3d).

Yield ,52%, m.p 180-182°C, IR(cm^{-1}) ν_{max} :1725,1718(imidic CO), 1673 (C=O ester),1610(C=N).^{1}H NMR (300 MHz, DMSO-d_6) δ:1.50 (d,J = 6.6 Hz, 3H, CH_3,$HCCH_3$), 2.30–2.40 (s, 6H,2×CH_3, m-xylene), 5.30 (q,J = 6.6 Hz, 1H, CH, COCHN),7.10–8.40 (m,11H,Ar–H).^{13}C NMR(DMSO-d_6, 300MHz) δ ppm:171(C-O-C), 168.3(C=O), 167.9(2C=O), 150, 138.3, 136.8, 133. 5,132.6,132.2, 131.4, 127.5,127.3, 126.7,126.6, 125.5,123.7, 119.7 (aromatic carbons),55.6, 43,1, 21.6, 19.5,13.2 (aliphatic carbons). Anal. Calcd. for $C_{27}H_{21}N_3O_4$:C,71.8; H,4.65; N,9.3%. Found C,71.6; H,4.45;N,9.1%.

3-Phenyl-2-(1,3-dioxoisoindolin-2-yl) propanoate of 4-(2,4-dimethylphenyl) phthalazin-1-yl (3e).

Yield ,75%, m.p 182-184°C, IR(cm^{-1}) ν_{max} :1720,1714(imidic CO), 1667 (C=O ester),1610(C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.30–2.40 (s, 6H, 2 × CH_3, m-xylene), 3.50 (d, J = 6.8 Hz, 2H, CH_2–Ph), 4.80 (t, J = 6.8 Hz, 1H, CH, COCHN), 7.10–7.90 (m, 16H, Ar–H). Anal.Calcd.for $C_{33}H_{25}N_3O_4$:C,75.14; H,4.74; N,7.96%. Found C,74.94; H,4.54; N,7.76%.

3-(1H-Imidazol-4-yl)-2-(1,3-dioxoisoindolin-2-yl) propanoate of 4-(2,4-dimethylphenyl) phthalazin-1-yl(3f).
Yield: 72%; m.p. 190-192°C. IR (cm^{-1}) ν_{max}: 1735, 1709 (imidic C=O), 1672 (C=O ester), 1627 (C=N). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 2.3–2.4 (2s, 6H, 2 × CH_3, m-xylene), 3.3 (d, J = 7.2 Hz, 2H, $CHCH_2$), 4.8 (t, J = 7.8 Hz, 1H, COCHN), 7.1–8.3 (m, 11H, aromatic protons), 7.8 and 8.3 (2s, 2H, two CH groups from the imidazole ring), 12.7 (s, 1H, NH, exchangeable with D_2O). Analytical data calculated for $C_{30}H_{23}N_5O_4$: C, 69.62; H, 4.48; N, 13.53%. Found: C, 69.4; H, 4.32; N, 13.2%.

4-(2,4- di methylphenyl) phthalazin-1-yl -2-(1,3-dioxoisoindolin-2-yl) -4-(methyl thio)butanoate(3g)
Yield: 77%; Melting Point: 170-172°C. IR (cm^{-1}) ν_{max}: 1730, 1716 (imidic C=O), 1669 (C=O ester), 1580 (C=N).^{1}H-NMR (DMSO-d_6, 400 MHz): δ 2.1 (m, 3H, overlapping signals from SCH_3 and $CHCH_2CH_2$), 2.3–2.4 (3s, 6H, 2 × CH_3, m-xylene), 2.6 (t, J = 7.6 Hz, 2H, CH_2CH_2S), 4.3 (t, J = 8.1 Hz, 1H, COCHN), 7.1–7.9 (m, 11H, aromatic protons).Anal. Calcd . for $C_{29}H_{25}N_3O_4S$: C, 68.08; H, 4.93; S, 6.26; N, 8.21%. Found values: C, 67.88; H, 4.73; S, 6.16; N, 8.01%

General procedure for the synthesis of amino acid-phthalazinone (4a-g).
To a solution of compound 3a-g (0.01 moles) in an appropriate alcoholic solvent, hydrazine hydrate (0.01 moles) was meticulously introduced. The resultant reaction mixture was subjected to reflux conditions for a span of three hours. Following the completion of the reaction, the mixture was allowed to cool, leading to the precipitation of a solid product. This solid was then collected by filtration and underwent recrystallization from ethanol, resulting in the formation of the desired compounds (4a-g).

4-(2,4- di methyl phenyl) phthalazin-1-yl-glycinate(4a)
Yield ,80%, m.p 238-240°C, IR(cm^{-1}) ν_{max}: 3436,3300,3161(NH_2 group),1671(C=O ester),1609(C=N). ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 2.3–2.4 (2s, 6H, 2 × CH_3 of m-xylene), 4.1 (s, 2H, $COCH_2NH_2$), 7.1–7.8 (m, 7H, Ar-H), 8.3 (s, 1H, NH, exchangeable with D_2O).^{13}C NMR(DMSO-d_6, 300MHz) δ ppm: 171(C-O-C), 168(C=O), 150, 138.3, 136.8, 133.5, 131.4, 127.5,127.3, 126.7,126.6 119.7 (aromatic carbons),45,5, 21.6, 19.5 (aliphatic carbons). Anal. Calcd .for $C_{18}H_{17}N_3O_2$:C,70.34;H,5.58;N,13.67%. Found C,67.68;H,5.38;N,13.47%.

4-(2,4- di methyl phenyl) phthalazin-1-yl-glutaminate(4b).
Yield,83% , m.p. 218–220 °C, IR (cm^{-1}) ν_{max}: 3437, 3303 (NH_2), 1672 (C=O, ester), 1610 (C=N).^{1}H-NMR (DMSO-d_6, 300 MHz): δ 2.05–2.1 (m, 4H, $COCHCH_2CH_2$, J = 7.8 Hz, with interference), 2.3–2.4 (2s, 6H, 2 × CH_3 of m-xylene), 3.4 (t, J = 7.5 Hz, 1H, $COCHNH_2$), 7.1 (s, 2H, $CONH_2$, exchangeable with D_2O), 8.4 (s, 2H, NH_2, exchangeable with D_2O), 7.1–7.9 (m, 7H, aromatic protons, Ar-H).^{13}C NMR(DMSO-d_6, 300MHz) δ ppm: 173.6(C=O), 171(C-O-C), 168.3(C=O), 150, 138.3, 136.8, 133. 5, 131.4, 127.5,127.3, 126.7,126.5,119.7 (aromatic carbons),52.2, 33, 28, 21, 19.1 (aliphatic carbons). Anal. Calcd. For$C_{21}H_{22}N_4O_3$: C,66.65;H,5.86;N,14.81%. Found C,66.45;H,5.66;N,14.61%.

4-(2,4-di methyl phenyl) phthalazin-1-yl-tyrosinate(4c).
Yield ,76%, m.p 238-240°C, IR(cm^{-1})$\boldsymbol{\nu}_{max}$:3431broad band for(OH group), 3163(NH_2 group),1669(C=O ester). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 2.3–2.4 (2s, 6H, 2 × CH_3 of m-xylene), 3.4 (d, J = 7.2 Hz, 2H, CH_2–Ph), 4.2 (t, J = 7.6 Hz, 1H, $COCHNH_2$), 6.6–7.9 (m, 11H, aromatic protons, Ar–H), 8.9 (s, 2H, NH_2, exchangeable with D_2O), 9.1 (s, 1H, OH, exchangeable with D_2O).Anal. Calcd .for $C_{25}H_{23}N_3O_3$:C,72.62;H,5.61;N,10.16%. Found C,72.42.45;H,5.41;N,9.96%

.4-(2,4-di methyl phenyl)phthalazin-1-yl alaninate(4d)
Yield ,89%, m.p 239-241°C, IR(cm^{-1})$\boldsymbol{\nu}_{max}$: 3326,3160(NH_2 group),1670(C=O ester). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 1.2 (d, J = 6.8 Hz, 3H, $CHCH_3$), 2.3–2.4 (s, 6H, 2 × CH_3 from m-xylene), 3.5 (q, J = 6.8 Hz, 1H, $COCHNH_2$), 7.1–7.9 (m, 7H, aromatic protons, Ar–H), 8.7 (s, 2H, NH_2, exchangeable with D_2O). Anal. Calcd .for $C_{19}H_{19}N_3O_2$:C,71.01;H,5.96;N,13.08%. Found C,70.81;H,5.61;N,12.8%.

4-(2,4-di methyl phenyl) phthalazin-1-yl phenylalaninate(4e).
Yield ,71%, m.p 200°C, IR(cm^{-1}) ν_{max} :3327,3162(NH_2 group),1670(C=O ester),1628(CN).^{1}H-NMR (DMSO-d_6, 300 MHz): δ 2.3–2.4 (2s, 6H, 2 × CH_3 of m-xylene), 3.2 (d, J = 7.2 Hz, 2H, CH_2–Ph), 4.1 (t, J = 7.6 Hz, 1H, $COCHNH_2$), 7.1–7.9 (m, 12H, Ar–H), 8.7 (s, 2H, NH_2, exchangeable with D_2O).Anal. Calcd. for $C_{25}H_{23}N_3O_2$:C,75.55; H 5. 83;N,10.57%. Found C,75.32;H,5.61;N,10.2%.

4-(2,4-di methyl phenyl) phthalazin-1-yl histidinate(4f).
Yield ,78%, m.p 220°C, IR(cm^{-1}) $\boldsymbol{\nu}_{max}$: 3327,3162(NH_2 group),1671(C=O ester),1626(CN). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 2.3–2.4 (2s, 6H, 2 × CH_3 from m-xylene), 3.2 (d, J = 7.2 Hz, 2H, $CHCH_2$), 4.1 (t, J = 7.6 Hz, 1H, $COCHNH_2$), 7.1–7.9 (m, 7H, Ar–H), 7.6 and 8.6 (2s, 2H, imidazole CH), 8.7 (s, 2H, NH_2, exchangeable with D_2O), 12.7 (s, 1H, imidazole NH, exchangeable with D_2O).Anal. Calcd. For $C_{22}H_{21}N_5O_2$: C,68.20; H,5.46;N,18.08%. Found C,68.1;H,5.11;N,17.9%.

4-(2,4-di methyl phenyl) phthalazin-1-yl methioninate(4g).
Yield ,50%, m.p 210°C, IR(cm^{-1}) $\boldsymbol{\nu}_{max}$:3437,3326(NH_2 group),1671(C=O ester),1630(CN). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 2.1 (m, 3H, overlapping signals from SCH_3 and $CHCH_2CH_2$), 2.3–2.4 (2s, 6H, 2 × CH_3 of m-xylene), 2.6 (t, J = 7.4 Hz, 2H, CH_2SCH_3), 3.4 (t, J = 7.2 Hz, 1H, $COCHNH_2$), 7.1–7.9 (m, 7H, Ar–H), 8.7 (s, 2H, NH_2, exchangeable with D_2O).Anal. Calcd. For $C_{21}H_{23}N_3O_2S$:C,66.12; H,6.08;S,8.39;N,11.01%. Found C,66.01.32;H,6.01;S,8.25;N,10.9%.

Antimicrobial assay

The antimicrobial activity of the synthesized compounds was evaluated using the agar diffusion method. All compounds were screened *in vitro* for their antibacterial activity against **Staphylococcus aureus** (Gram-positive bacteria), and **Klebsiella pneumoniae** (Gram-negative bacteria) using nutrient agar as the culture medium.The antifungal activity was assessed against **Candida albicans** and **Aspergillus niger** using Sabouraud dextrose agar medium. **Ampicillin** and **gentamicin** served as standard reference drugs for Gram-positive and Gram-negative bacteria, respectively, while **nystatin** was used as the standard antifungal agent. **Dimethyl sulfoxide (DMSO)** was employed as a negative control. All compounds were tested at a final concentration of **15 mg/mL** against both bacterial and fungal strains.

Method of Testing[48]

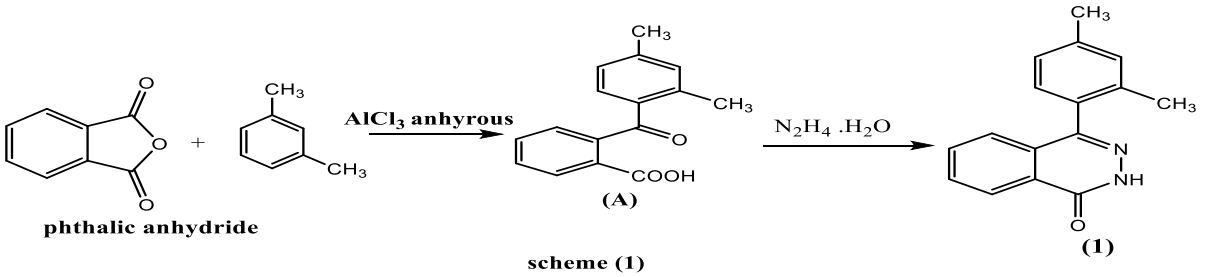
Sterilized nutrient agar medium (20–25 mL) was aseptically poured into sterile Petri dishes and allowed to solidify at room temperature. A microbial suspension was prepared in sterile normal saline to match the turbidity of a 0.5 McFarland standard, equivalent to approximately 1.5×10^{8}CFU/mL. The turbidity was adjusted to an optical density (OD) of 0.13 using a spectrophotometer at 625 nm. Within 15 minutes of turbidity adjustment, a sterile cotton swab was immersed in the microbial suspension and used to evenly inoculate the surface of the solidified agar. The plates were left to dry for 15 minutes with the lids in place to ensure proper adherence of the inoculum. Subsequently, wells of 6 mm diameter were aseptically punched into the agar using a sterile cork borer. Each well was filled with 100 µL of the test compound solution using a micropipette. The plates were then incubated at 37 °C for 24 hours to assess antibacterial activity. The experiment was performed in triplicate, and the resulting zones of inhibition were measured in millimeters (mm) using a standard ruler.

Molecular Docking Investigations

Molecular docking is a sophisticated computational method utilized to anticipate the interaction between a protein (receptor) and a small molecule (ligand) within the protein's binding site. This information is crucial in drug discovery and design .

3. Results and Discussion

The synthesis of new phthalazinone derivatives was successfully achieved through a Friedel-Crafts acylation reaction. The reaction between phthalic anhydride and m-xylene in the presence of $AlCl_3$ as a catalyst led to the formation of 2-(2,4-dimethylbenzoyl) benzoic acid (compound A), as depicted in Scheme 1. The structure of compound A was confirmed through infrared (IR) spectroscopy, which displayed a characteristic carbonyl stretch (ν C=O) at 1673 cm^{-1}, confirming the presence of the acylated moiety. Subsequently, compound A was subjected to a nucleophilic cyclization reaction using hydrazinium hydroxide in ethanol, resulting in the formation of 4-(2,4-dimethylphenyl)phthalazine-1(2H)-one (1)[24,49,50,51] , as illustrated in Scheme (1).

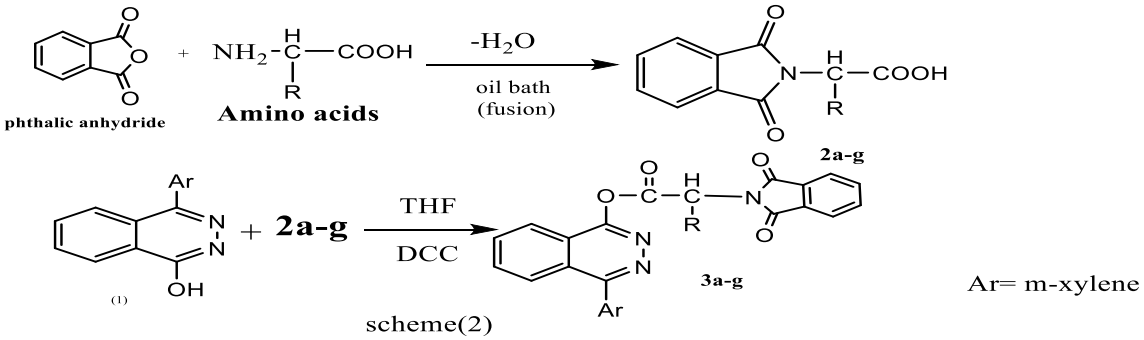


scheme (1)

Infrared (IR) spectroscopic analysis of compound (1) demonstrated the complete disappearance of the characteristic hydroxyl group absorption band, accompanied by the emergence of a strong carbonyl stretching band at 1671 cm^{-1}, consistent with the presence of a cyclic amide functionality. These findings confirm the successful formation of the phthalazinone core structure.

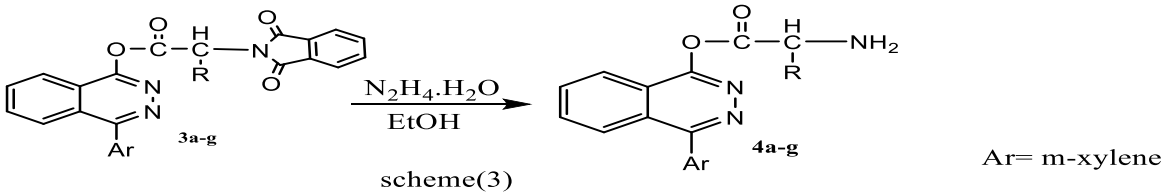
Compound **(1)** was subsequently employed as a key synthetic intermediate for the generation of a structurally diverse array of amino acid-conjugated derivatives. These derivatives were synthesized through the coupling of **(1)** with a series of *N*-protected amino acids. The rationale behind this structural diversification lies in the well-documented enhancement of biological activity imparted by the introduction of amino acid residues to heterocyclic cores such as phthalazinones.

To investigate this effect further, compound (1) was reacted with *phthalyl*-protected derivatives of amino acids including glutamine, alanine, phenylalanine, tyrosine, methionine, glycine, and histidine. The coupling reactions were carried out in the presence of N,N′-dicyclohexylcarbodiimide (DCC), a widely used dehydrating agent, to facilitate amide bond formation. This procedure afforded a series of new phthalazinone derivatives, designated as compounds (3a–g), as illustrated in Scheme 2.



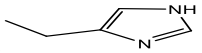
scheme(2)

The *N*-deprotection of compounds **3a–g** was accomplished via thermal reflux in anhydrous ethanol. Each compound was subjected to continuous reflux under boiling conditions for a duration of **3 hours**, ensuring complete removal of the phthalyl protecting group from the amino moiety. This deprotection strategy efficiently afforded the corresponding free aminoacyl derivatives **(4a–g)** in good yields. The transformation is depicted in **Scheme 3**, highlighting the successful conversion of protected intermediates to their respective unprotected analogues.



scheme(3)

The success of the deprotection process was unambiguously confirmed by infrared spectroscopic analysis. The spectra of compounds **4a–g** exhibited the appearance of a characteristic **N–H stretching vibration**, indicative of a free amino group, typically observed in the region of **3300–3500 cm⁻¹**. Concurrently, the **disappearance of the imide-related absorption band** confirmed the complete removal of the *N*-phthalyl protecting group. These spectral changes collectively provide definitive evidence for the successful generation of the deprotected aminoacyl-phthalazinone derivatives.

R=a,H;b,$CH_2CH_2CONH_2$;c,p-OHC_6H_5-CH_2;d,CH_3;e,$C_6H_5CH_2$; f ,  ;g,$CH_2CH_2SCH_3$.

Statistical analysis :-

Statistical differences among samples of the same bacterial or fungal type were assessed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for post hoc comparisons, utilizing the SPSS software package (version 22.0, IBM Corp., Armonk, NY, USA). Data are presented as mean ± standard deviation ($\bar{X}$ ± SD). A p-value of less than 0.05 was considered statistically significant, while p-values less than 0.01 and 0.001 were regarded as highly significant and very highly significant, respectively. The observed inhibition zones are systematically presented in Tables 1 and 2

Table (1) delineates the antibacterial and antifungal bioactivity profiles of the newly synthesized compounds (3a–g), providing a comprehensive evaluation of their inhibitory efficacies.

Sample / Microorganism	3a	3b	3c	3d	3e	3f	3g	Standard antibiotic
Gram negative bacteria								Gentamicin
Klebsiella pneumonia (ATCC: 10031)	11±1.0 +Ve	NA	15.3±0.6 ++Ve	14.7±0.6 ++Ve	22.0±1.0 ++++Ve	9.7±0.6 +Ve	12.3±0.6 ++Ve	25.3±0.6[a]
Gram positive bacteria								Ampicillin
Staphylococcus aureus (ATCC: 13565)	NA	NA	NA	NA	NA	NA	NA	21.3±0.6[a]
Fungi								Nystatin
Candida albicans (ATCC: 10231)	NA	NA	NA	NA	NA	NA	NA	21.6±0.6
Asperagillus Nigar (ATCC: 16404)	NA	NA	NA	NA	NA	NA	NA	19.3±0.6

Table (2) delineates the antibacterial and antifungal efficacies of the newly synthesized compounds (4a–g), offering an intricate comparative analysis of their inhibitory potentials against targeted microbial strains .

Sample / microorganism	4a	4b	4c	4d	4e	4f	4g	Standard antibiotic
Gram negative bacteria								**Gentamicin**
Klebsiella pneumonia (ATCC: 10031)	**28.3±0.6 ++++Ve**	**14 ±1.0 ++Ve**	**20±1.0 + ++Ve**	**25±0.6 ++++Ve**	**19.0±0.6 +++Ve**	**11.7±0.6 +Ve**	**13±1.0 ++Ve**	**25.3±0.6[a]**
Gram positive bacteria								**Ampicillin**
Staphylococcus aureus (ATCC: 13565)	**16.7±0.6**	**NA**	**NA**	**20.3±0.6**	**NA**	**NA**	**NA**	**21.3±0.6[a]**
Fungi								**Nystatin**
Candida albicans (ATCC: 10231)	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**21.6±0.6**
AsperagillusNigar (ATCC: 16404)	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**19.3±0.6**

- **Zone of inhibition is expressed in the form of mean± Standard deviation (mm) .**
- **NA: Noactivity.**
- **Well diameter (6mm).**
- **100 µ was tested.**

Molecular docking Studies

Binding with hydroxylethylthiazolekinase

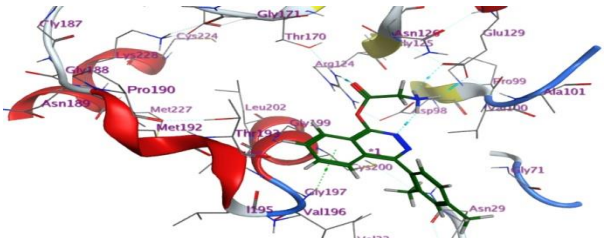
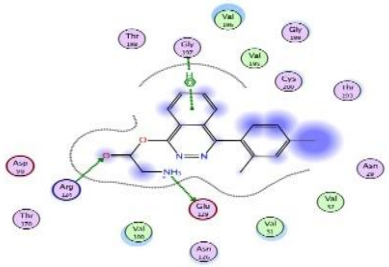
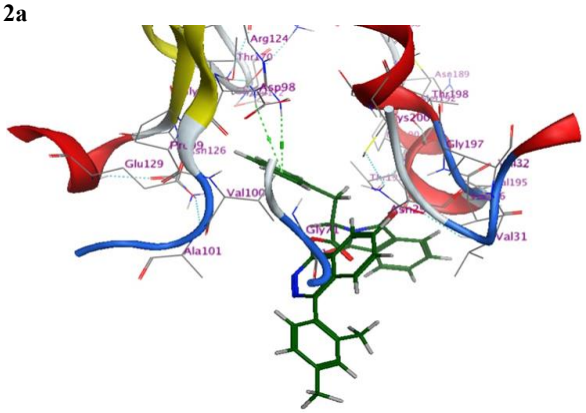
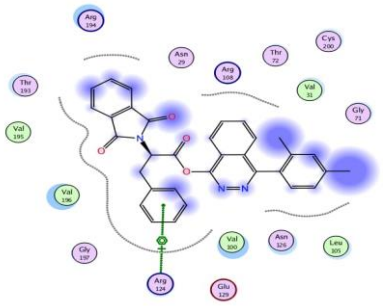
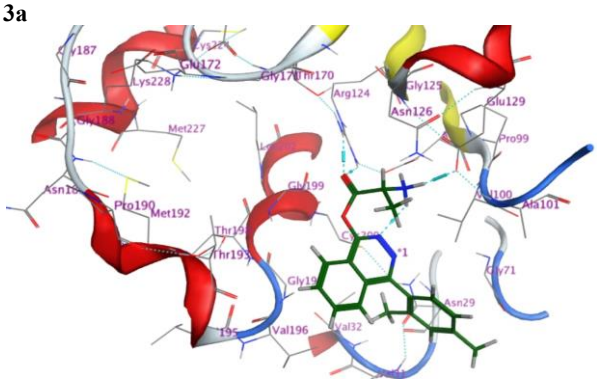
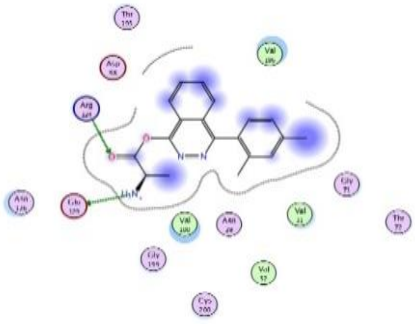
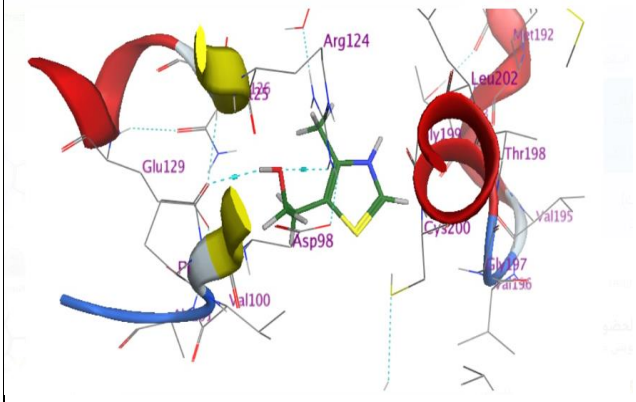
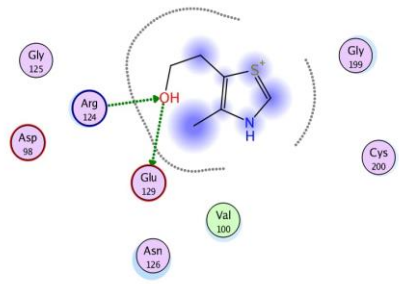
To check the binding ability of compounds with hydroxylethylthiazolekinase, five compounds (4a,4d, 3e,TZE,ampicilin) were docked separately in the active pocket of this enzyme. Strong binding with such enzyme suggests the chance to be used as bacteriocidal and hence the mode of action for their activity as antibiotic agent.

By screening the data depicted in the Table 3 it can be noticed that compound 4a showed binding affinity to hydroxylethylthiazole kinase without forming any Arene-cation, only with one Arene-H with Gly-197 and H-bond with Glu 129 and Arg 124 as show in (fig 1a,1b). We also noticed that compound 3eshowed the strongest binding profile (B.E=-6.049Kcal/mole) to hydroxylethylthiazole kinase without forming any Hydrogen – bond and Arene-H, only one Arene –cation with Arg 129 as show in (fig 2a,2b). Compound 3e had marvelous results as antibiotic compound compared to Ampiciline which has binding energy (B.E= -5.1532Kcal/mole) as show in fig2a,2b, fig5a,5b.

On the other hand compounds 4d,Tzeligand and Ampicline showed binding affinity to hydroxyethylthiazole kinase with H-bond with Glu129, Arg 124, without forming any Arene-H and Arene –cation as show in (fig3a,3b,fig4a,4b, fig5a,5b).Tze ligand showed the worth binding mode (B.E= -3.621932Kcal/mole) although it could form interaction with H-bond with (Glu129 and Arg 124) .This may be attributed to it's small rigid structure and minor flexible functionality.Compund4d has a similar mood withTzeligand , but 4d has more binding energy value(-5.2 Kcal/mole) than binding energy of Tze ligand (-3.6219 Kcal/mole). So,we conclude that 3e has the strongest effect as Anti-biotic.

Table (3). binding data of phthalazine derivative hydroxylethylthiazole kinase B.E= Binding energy , RMSD= root mean square deviation

Ligand	B.E	RMSD	H-Bond	Arene-H	Arenecation
4a	**-5.2343**	**1.7699**	**Glu 129 Arg 124**	**Gly 197**	**-**
4d	**-5.2025**	**1.4853**	**Glu129 Arg124**	**-**	**-**
3e	**-6.0497**	**1.8565**	**-**	**-**	**Arg124**
Tzeligand	**-3.6219**	**1.4534**	**Glu129 Arg124**	**-**	**-**
Ampicillin	-5.1533	1.8978	Gly199 Arg124	-	-

Compound	3D (Three-dimensional)	2D (Two-dimensional)
4a	**1a** 	**1b** 
3e	**2a** 	**2b** 
4d	**3a** 	**3b** 
TZE	**4a** 	**4b** 

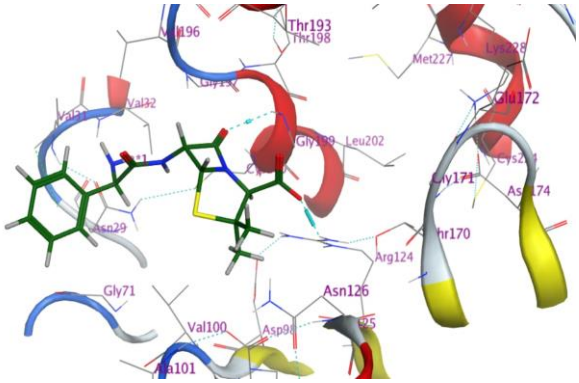
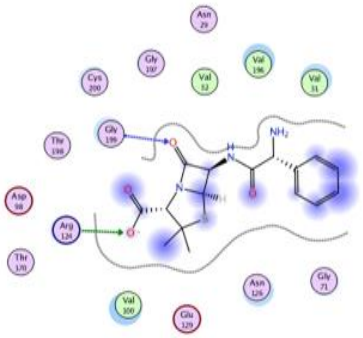
Ampicilline	5a	5b
		

Figure (1a,2a,3a,4a,5a) 2D-(1b,2b,3b,4b,5b) 3D- binding profiles of hydroxylethylthiazole kinase with (3e,4a,4d,ampiciline,tze) respectively showing H-bonds, arene-H and arene- cation .

Conclusion

The present study describes the efficient synthesis of a series of innovative phthalazine-based amino acid conjugates. Their noteworthy antimicrobial activity, supported by molecular docking analyses, underscores their potential as promising lead compounds for the design and development of new antimicrobial therapeutics.

Conflict of interest

The authors unequivocally affirm that no conflicting or divergent interests exist that could be construed as compromising the integrity of this manuscript's publication.

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Reference

1- Al-Mulla, A. (2017). A review: biological importance of heterocyclic compounds. *Der Pharma Chemica*, 9(13), 141-147.
2-Arora, P., Arora, V., Lamba, H. S., & Wadhwa, D. (2012). Importance of heterocyclic chemistry: A review. International Journal of Pharmaceutical Sciences and Research, 3(9), 2947.
3-Lang, D. K., Kaur, R., Arora, R., Saini, B., & Arora, S. (2020). Nitrogen-containing heterocycles as anticancer agents: An overview. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 20(18), 2150-2168.
4-Wasfy, A. A. F. (2002). Studies on quinazolines. Part I. Annelation to the quinazoline ring utilizing amino acid esters. Phosphorus, Sulfur, and Silicon and the Related Elements, 177(5), 1349-1358.
5- Hassan, A. A., Khattab, R. R., Wasfy, A. A., Abuzeid, K. M., & Hassan, N. A. (2018). A Convenient One-Pot Synthesis of Thieno [2′, 3′: 4, 5] Pyrimido [1, 2-b]-[1, 2, 4, 5] Tetrazines. Journal of Heterocyclic Chemistry, 55(4), 907-912.
6-Volzhina, O. N., Gortinskaya, T. V., Gorodetskii, L. S., Sidorova, L. A., Traven', N. I., Shalamova, L. P., & Shvets, Z. I. (1971). Commercial method of preparing phthazine. Pharmaceutical Chemistry Journal, 5(1), 50-51.
7- Yamaguchi, N., Biniecki, K., Jankowski, C. K., &Ciszewski, L. (2022). CARDIOVASCULAR EVALUATION OF THE CHEMICAL STRUCTURE OF N-METHYLPIPERAZINYL PHTHALAZINE ANALOGS. Acta Poloniae Pharmaceutica, 79(1).
8- Yu, H. L., Zhang, F., Lan, T., & Quan, Z. S. (2014). Effects of 6-(4-chlorophenoxy)-tetrazolo [5, 1-a] phthalazine on Anticoagulation in Mice and the Inhibition of Experimental Thrombosis in Rats. Journal of Cardiovascular Pharmacology, 64(6), 560-566.
9-Asif, M. (2016). Study on pyridazinone derivative MCI-154 as a cardiotonic agent. Journal of Scientific and Innovative Research, 5(3), 106-111.
10-Zhang, L., Guan, L. P., Sun, X. Y., Wei, C. X., Chai, K. Y., & Quan, Z. S. (2009). Synthesis and Anticonvulsant Activity of 6-Alkoxy-[1, 2, 4] Triazolo [3, 4-a] Phthalazines. Chemical biology & drug design, 73(3), 313-319.
11-Ayyad, R. A., Sakr, H., & El-Gamal, K. (2016). Synthesis, modeling and anticonvulsant activity of some phthalazinone derivatives. American Journal of Organic Chemistry, 6(1), 29-38.
12-Bian, M., Deng, X. Q., Gong, G. H., Wei, C. X., & Quan, Z. S. (2013). Synthesis and anticonvulsant activity evaluation of 6-substituted-[1, 2, 4] triazolo [3, 4-a](tetrazolo [5, 1-a]) phthalazine derivatives. Journal of Enzyme Inhibition and Medicinal Chemistry, 28(4), 792-800.

13- El-Helby, A. G. A., Ayyad, R. R., Zayed, M. F., Abulkhair, H. S., Elkady, H., & El-Adl, K. (2019). Design, synthesis, in silico ADMET profile and GABA-A docking of novel phthalazines as potent anticonvulsants. Archiv Der Pharmazie, 352(5), 1800387.
14- Sun, X. Y., Wei, C. X., Deng, X. Q., Sun, Z. G., & Quan, Z. S. (2010). Evaluation of the anticonvulsant activity of 6-(4-chlorophenyoxy)-tetrazolo [5, 1-a] phthalazine in various experimental seizure models in mice. Pharmacological reports, 62(2), 273-277.
15- Sun, X. Y., Guan, L. P., Zhang, L., Wei, C. X., Piao, H. R., & Quan, Z. S. (2009). Design, synthesis and anticonvulsant activity evaluation of 7-substituted-4H-[1, 2, 4] triazino [3, 4-a] phthalazin-4-one derivatives. Journal of the Brazilian Chemical Society, 20, 826-831.
16-Awadallah, F. M., El-Eraky, W. I., & Saleh, D. O. (2012). Synthesis, vasorelaxant activity, and molecular modeling study of some new phthalazine derivatives. European journal of medicinal chemistry, 52, 14-21.

17-Munín, J., Quezada, E., Viña, D., Uriarte, E., Delogu, G. L., & Gil-Longo, J. (2019). Discovery of new phthalazinones as vasodilator agents and novel pharmacological tools to study calcium channels. Future Medicinal Chemistry, 11(3), 179-191.
18- Sakib, S. N. (2023). Role of pharmacological active phthalazine scaffolds in medicinal chemistry. Open Science Foundation.
19- Sherif, Y. E., Alansari, R., & Gouda, M. A. (2018). Design, synthesis, characterization, QSAR, docking, anti-inflammatory and analgesic evaluation of some new phthalazinediones. Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents), 17(1), 3-15.
20- Wasfy, A. F., Aly, A. A., Behalo, M. S., & Mohamed, N. S. (2013). Synthesis of novel series of phthalazine derivatives as potential antitumor agents. Synthesis, 10, 20-32.
21- Radwan, T. M., El-Hashash, M. A. A., Wasfy, A. A. H. F., & Abdallah, S. A. (2020). Antitumor, cytotoxic, and antioxidant evaluation of six heterocyclic compounds containing different heterocycle moieties. Journal of Heterocyclic. Chemistry, 57(3), 1111-1122. 22- El-Helby, A. G. A., Sakr, H., Ayyad, R. R., Mahdy, H. A., Khalifa, M. M., Belal, A., ... &Eissa, I. H. (2020). Design, synthesis, molecular modeling, in vivo studies and anticancer activity evaluation of new phthalazine derivatives as potential DNA intercalators and topoisomerase II inhibitors. Bioorganic chemistry, 103, 104233.
23- Elmeligie, S. (2016). DESIGN AND SYNTHESIS OF PHTHALAZINE BASED DERIVATIVES AS POTENTIAL ANTICANCER AGENTS. Al-Azhar Journal of Pharmaceutical Sciences, 53(1), 207-236.
24- Mourad, A. K., Makhlouf, A. A., Soliman, A. Y., & Mohamed, S. A. (2020). Phthalazines and phthalazine hybrids as antimicrobial agents: Synthesis and biological evaluation. Journal of Chemical Research, 44(1-2), 31-41.
25- Rizk, S. A., Abdelwahab, S. S., & El-Badawy, A. A. (2019). Design, regiospecific green synthesis, chemical computational analysis, and antimicrobial evaluation of novel phthalazine heterocycles. Journal of Heterocyclic Chemistry, 56(9), 2347-2357.
26- Azab, M. E., Rizk, S. A., & Mahmoud, N. F. (2016). Facile synthesis, characterization, and antimicrobial evaluation of novel heterocycles, schiff bases, and N-nucleosides bearing phthalazine moiety. Chemical and Pharmaceutical Bulletin, 64(5), 439-450.
27- El-Hashash, M. A. A., Soliman, A. Y., & ELSHAMY, I. E. (2012). Synthesis and antimicrobial evaluation of some annelated phthalazine derivatives and acyclo C-nucleosides from 1-chloro-4-(2, 4, 6-trimethylphenyl) phthalazine precursor. Turkish Journal of Chemistry, 36(3), 347-366.
28- Hemdan, M. M., Taha, S. M., Gabr, A. M., &Elkady, M. Y. (2010). Hydrazine derivatives and o-benzoylbenzoic acid as a source of phthalazines with their antimicrobial activities. Journal of Chemical Research, 34(2), 102-105.
29- Milovanović, V., Petrović, Z., Petrović, V., Simijonović, D., Mladenović, M., Tomaševic, N., ... & Radojevic, I. (2021). In vitro and in silico lipoxygenase inhibition studies and antimicrobial activity of pyrazolyl-phthalazine-diones.
30- El-Hashash, M. A., Guirguis, D. B., AbdEl-Wahed, N. A., &Kadhim, M. A. (2014). Synthesis of novel series of phthalazine derivatives with antibacterial and antifungal evaluation. J. Chem. Eng. Process Technol, 5(4), 1000191-1000196.
31- Asif, M. (2019). Pharmacological activities of various phthalazine and phthalazinone derivatives. Chem. Int, 5(1), 97-108. (fungi)
32- Derita, M., Del Olmo, E., Barboza, B., García-Cadenas, A. E., López-Pérez, J. L., Andújar, S., ... & San Feliciano, A. (2013). Synthesis, bioevaluation and structural study of substituted phthalazin-1 (2 H)-ones acting as antifungal agents. Molecules, 18(3), 3479-3501.
33- Kumar, P. S. V., Suresh, L., &Chandramouli, G. V. P. (2017). Ionic liquid catalysed multicomponent synthesis, antifungal activity, docking studies and in silico ADMET properties of novel fused Chromeno-Pyrazolo-Phthalazine derivatives. Journal of Saudi Chemical Society, 21(3), 306-314.
34- El-Hashash, M. A., Wasfy, A. A., Mohamed, H. I., & Abdel-Rahman, A. S. (2017). Synthesis of novel series of phthalazine derivatives with antibacterial and antifungal evaluation. Journal of Basic and Environmental Sciences, 4, 226-232.
35- Akashi, A., Chiba, T., & Kasahara, A. (1974). Antihypertensive activity of 1-[2-(1, 3-dimethyl-2-butenylidene)-hydrazino]-phthalazine (DJ-1461), a new phthalazine derivative. European Journal of Pharmacology, 29(1), 161-164.
36- Chapleo, C. B., Doxey, J. C., & Myers, P. L. (1982). Synthesis of some potential antihypertensive phthalazinylguanidines and quinoxalinylguanidines. Journal of Medicinal Chemistry, 25(7), 821-824.
37- Omar, A. M. M., Kasem, M. G., Laabota, I. M., & Bourdais, J. (1981). Synthesis and antihypertensive properties of a novel series of 3-substituted amino-s-triazolo [3, 4-α] phthalazine derivatives. Journal of Heterocyclic Chemistry, 18(3), 499-501.
38- Vila, N., Besada, P., Costas, T., Costas-Lago, M. C., & Teran, C. (2015). Phthalazin-1 (2H)-one as a remarkable scaffold in drug discovery. European journal of medicinal chemistry, 97, 462-482.

39-Türkeş, C., Arslan, M., Demir, Y., Cocaj, L., Nixha, A. R., &Beydemir, Ş. (2019). Synthesis, biological evaluation and in silico studies of novel N-substituted phthalazine sulfonamide compounds as potent carbonic anhydrase and acetylcholinesterase inhibitors. Bioorganic chemistry, 89, 103004.
40-Angeli, A., Petrou, A., Kartsev, V. G., Zubenko, A., Divaeva, L. N., Chekrisheva, V., ... &Supuran, C. T. (2024). Phthalazine sulfonamide derivatives as carbonic anhydrase inhibitors. Synthesis, biological and in silico evaluation. ChemMedChem, 19(15), e202400147.
41-Taslimi, P., Turhan, K., Türkan, F., Karaman, H. S., Turgut, Z., &Gulcin, I. (2020). Cholinesterases, α-glycosidase, and carbonic anhydrase inhibition properties of 1H-pyrazolo [1, 2-b] phthalazine-5, 10-dione derivatives: Synthetic analogues for the treatment of Alzheimer's disease and diabetes mellitus. Bioorganic Chemistry, 97, 103647.
42- Imran, M., & Asif, M. (2020). Study of various pyridazine and phthalazine drugs with diverse therapeutical and agrochemical activities. Russian Journal of Bioorganic Chemistry, 46, 745-767.
43- Lamberth, C. (2017). Pyridazine chemistry in crop protection. Journal of Heterocyclic Chemistry, 54(6), 2974-2984.
44- Badiger, K. B., Sannegowda, L. K., &Kamanna, K. (2024). An Agro-Waste Catalyzed Facile Synthesis of 1 H-Pyrazolo [1, 2-b] Phthalazine-5, 10-Dione Derivatives: Evaluation of Antioxidant and Electrochemical Studies. Polycyclic Aromatic Compounds, 44(2), 1128-1150.
45-Asif, M. (2019). Pharmacological activities of various phthalazine and phthalazinone derivatives. Chem. Int, 5(1), 97-108.
46-Calloway, N. O. (1935). The Friedel-Crafts Syntheses. Chemical Reviews, 17(3), 327-392.
47-Rich, D. H., & SINGH, J. (1979). The carbodiimide method. In Major Methods of Peptide Bond Formation (pp. 241-261). Academic press.
48- Vorobets, N. M., &Yavorska, H. V. (2016). Modifications of agar diffusion method to determination of the antimicrobial effect of the herbal medicinal products. Ukrainian biopharmaceutical journal, (2 (43)), 80-84.
49- Nikoofar, K., & Sadathosainy, M. (2023). Phthalic anhydride (PA): a valuable substrate in organic transformations. RSC advances, 13(34), 23870-23946.
50-Mourad, A. K., Soliman, A. Y., Makhlouf, A. A., Mohammed, F. K., & Mohamed, S. A. (2024). Synthesis and Characterization of Novel Phthalazin-1 (2H)-one Hybrids with Anticipated Antibacterial Activity. *Egyptian Journal of Chemistry*, *67*(8), 351-366.
51- Baviskar, P. D., Kale, A. D., Mahire, V. N., Dalal, D. S., & Mahulikar, P. P. (2024). Imidazole as an organocatalyst for the rapid synthesis of 1H-pyrazolo [1, 2-b] phthalazine-5, 10-diones in aqueous medium. Journal of Chemical Sciences, 136(4), 1-11