Egypt. J. Chem. **Vol. 6**, No. 8 pp. 351 - 366 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Synthesis and Characterization of Novel Phthalazin-1(2*H*)-one Hybrids with Anticipated Antibacterial Activity

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Abstract

Herein, a phthalazine acetohydrazide derivative **4** was assembled and its behavior towards carbon nucleophiles was thoroughly investigated. Subsequently, phthalazine derivative **4** was used as a valuable scaffold for synthesizing assorted heterocycles such as pyrazol, thiazolidine, and azetidine. Diversified reaction conditions and reagents were explored. The assigned structures of the assembled heterocycles were elucidated utilizing IR, ^{1}H NMR, ^{13}C NMR, MS, and elemental analysis. Finally, the newly assembled heterocycles were evaluated *in vitro* for their antibacterial activities.

Keywords: Acetohydrazide; Thiazolidine; 2-Azetidinone; Pyrazole; Antibacterial

1. Introduction

Nitrogen-containing heterocyclic frameworks are commonly found in many active pharmaceuticals and natural products. They have a pivotal role in drug design because they are frequently utilized as a central backbone in complex structures with chemical, biological, and industrial significance [1-5].

Nitrogen-based heterocycles possess very unique structural features that enable them to interact selectively with various Gram-positive and Gram-negative bacteria [6]. Pyrazole and thiazole derivatives are of utmost importance as antibacterial agents [7-11]. They serve as valuable building blocks for the design and synthesis of novel antibacterial drugs. As pyrazoles are well known for having strong antibacterial properties [7-9],numerous antibiotics, such as sulfaphenazole and PNU-172576, comprise a pyrazole ring (Figure 1a). Moreover, pyrazole is the main heterocyclic motief in several avilable drugs, such as the pain killer antipyrine, the non-steroidal anti-inflammatory drug (NSAIDs) lonazolac, and the arthritis medication phenylbutazone (Figure 1 b) [12].

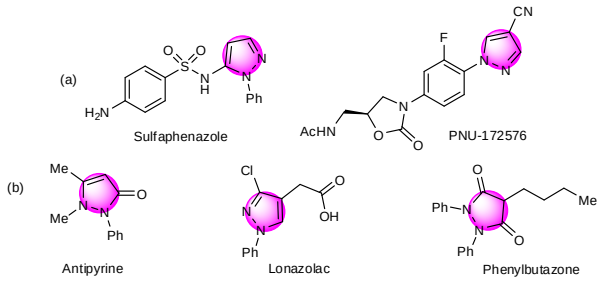

Figure 1:Examples of (a) pyrazole-based antibiotics and (b) drugs based on pyrazoles.

Also, thiazoles possess amphiphilic characteristics; this inherent amphiphilicity enables thiazoles to exhibit antibacterial activity by effectively interacting with the bacterial cell wall [13]. Accordingly, thiazole is found in various commercially available antibiotics like cefdinir and sulfathiazole (Figure 2a) [14]. Notably, peptide thiazole antibiotics such as thiostrepton and siomycin

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Received date 25 December 2023; revised date 09 January 2024; accepted date 09 January 2024
DOI: 10.21608/EJCHEM.2024.258130.9071

A have revealed anti-tumour activity by promoting apoptosis in human cancer cells [15, 16]. Moreover, thiazole is a core structure pharmacodynamic group in numerous FDA-approved drugs, including pramipexole for Parkinson's disease, ritonavir for AIDS, dabrafenib for melanoma, and alpelisib for breast cancer (Figure 2b) [17].

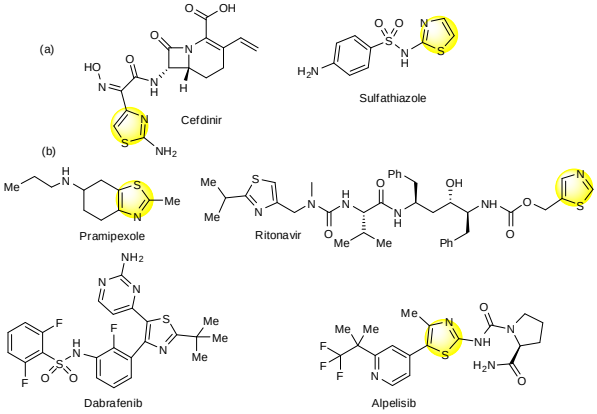


Figure 2: Examples of (a) thiazole-based antibiotics and (b) drugs based on thiazole.

Additionally, phthalazine is a diazaheterobicyclic compound, also referred to as benzo[d]pyridazine and 2,3-diazanaphthalene. Living organisms facing difficulties in assembling N-N bonds, therefore phthalazine derivatives are found to be very rare and limited in nature. Despite the existence of two adjacent nitrogen atoms is not a habitual scaffold in natural products, as the aforementioned, phthalazine exhibits a variety of pharmacological activities [18, 19] including anticancer [20, 21], anticonvulsant [22], antihypertensive [23], vasorelaxant [24], antidiabetic [18], analgesic [25], antimicrobial [18, 26-28], antithrombotic [29], anti-inflammatory [30-32], antitrypanosomal [33-34], and antileishmanial [35]. Moreover, phthalazine containing heterocycles can act as powerful inhibitors for assorted enzymes such as phosphodiesterase (PDE) [36], poly-[ADP-ribose] polymerase (PARP) [37, 38], and aldose reductase (AR) [39].

Phthalazine is the main constituent of various important synthetic molecules correlated to various branches of chemistry. In drug discovery, the phthalazinone nucleus is one of the most valuable pharmacophores, as it is comprised in a plethora of marketed therapeutic drugs. For instance, olaparib is in clinical use for treating ovarian cancer, breast cancer, prostate cancer, and pancreatic cancer, as it acts like a poly ADP-ribose polymerase (PARP) inhibitor (Figure 3) [19].

Another FDA approved phthalazinone-based drug is azelastine, also known as optivar, which acts as an H1 receptor-blocking medication for the treatment of allergic rhinitis (hay fever) and allergic conjunctivitis [40, 41]. Moreover, the phthalazinone motif is the core of zopolrestat drug, which currently undergoes rigorous evaluation in phase III clinical trials. Zopolrestat exhibits robust inhibitory activity against aldose reductase, rendering it a promising therapeutic agent for addressing the secondary complications associated with diabetes (Figure 3) [42].

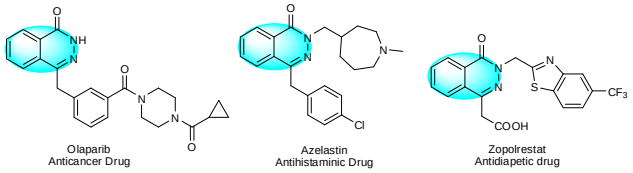


Figure 3: Commercially available phthalazin-1(2*H*)-one drugs.

In spite of the impressive advancements in antimicrobial therapy, the World Health Organization (WHO) has warned that antibiotic-resistant bacteria are becoming a severe threat to public health world-wide [43]. Moreover, the Centers for Disease Control and Prevention considered the antibiotic-resistant microbes as an instant global public health concern. They are not only responsible for 1.27 million deaths worldwide but alsocorrelate to about 5 million fatalities per year [44].Therefore, in the absence of innovative and effective antibiotics, the annual mortality rate could potentially escalate to 10 million by the year 2050 [45].

Molecular hybridization is a potent technique utilized in the field of drug development, where two active pharmacophores are merged within a single molecular framework. This technique has demonstrated notable advantages, such as enhanced effectiveness, the ability to overcome drug resistance, and reduced toxicity compared to the their parent compounds[46, 47]. By hybridizing phthalazin derivatives with various antibacterial pharmacophores, such as pyrazoles and thiazoles, it becomes feasible to create more potent potential drugs for combating bacterial infections.

Motivated by the preceding essential facts and as a continuation of our persistent efforts to establish efficient synthetic pathways for the construction of diverse heterocycles for subsequent biological assessment [48, 49], a new series of assorted phthalazin-1(2*H*)-one hybrids was assembled and tested for their antibacterial activity. Further, effort

was spent to develop simple and straightforward synthetic routes to construct targeted phthalazin-1(2*H*)-one motiffrom readily available and inexpensive starting materials.

2. Experimental

Synthesis of 2-(4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl)acetohydrazide (4) [50]

Hydrazine hydrate (0.50 mL, 0.01 mol) was added to a solution of ester **3** (3.36 g, 0.01 mol) in 30 mL absolute ethanol. The resulting mixture was refluxed for 24 h. After cooling to ambient temperature, the separated solid was filtered and subsequently recrystallized from ethanol, affording the acetohydrazide derivative **4** in a yield of 21%. The resulting crystals were colourless and exhibited a melting point of 238-240ºC. IR (KBr): 1650 (2CO), 2919 (CH aliph.), 3208, 3289 cm^{-1} (NH) and (NH_2).

Synthesis of 2-(2-(5-amino-2,3-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-4-(3,4-dimethylphenyl)phthalazin-1(2H)-one (5)

A reaction was conducted by refluxing a mixture of acetic acid hydrazide **4** (0.5 g, 0.0015 mol) in 15 mL pyridine and 0.1 mL of acrylonitrile (0.0015 mol) for 10 h. The reaction mixture was cooled to ambient temperature before being poured into ice-cold water and acidified with concentrated hydrochloric acid. The obtained precipitate was separated by filtration and subjected to crystallization from ethanol, yielding grey crystals of compound **5** with a 51% yield. Derivative **5** exhibited a melting point of 340-342 ℃. IR **(**KBr**)**: 1619 (C=N), 1666 (2CO), 2954 (CH aliph.), 3181, 3417 cm^{-1} (NH) and (NH_2). ^{1}H NMR (DMSO-d_6), δ_H (ppm):2.29 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 3.65 (d, J = 4.2 Hz, 2H, CH_2 pyrazole), 4.81 (s, 2H, CH_2CO), 4.88 (t, J = 4.2 Hz, 1H, CH pyrazole), 4.96 (s, 2H, NH_2, D_2O-exchangeable), 7.32- 7.94 (m, 7 H, arom. H), and 10.29 (s,1H, NH, D_2O-exchangeable).Analysis calcd for $C_{21}H_{21}N_5O_2$: C, 67.18; H, 5.64; N, 18.65. Found: C, 67.38; H, 5.74; N, 18.70. MS (70 eV) *m/z* (%): 375 (7.38) (M^+), 291 (56.26), 263 (100).

Synthesis of ethyl 3-(2-(2-(4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl)acetyl)hydrazineylidene)butanoate (6)

Equimolar amounts of acetohydrazide derivative **4** (1.0 g, 0.003 mol) and ethyl acetoacetate (0.38 mL, 0.003 mol) in 30 mL dioxane were heated under reflux for 10 h. After cooling to ambient temperature, the separated precipitate was collected by filteration and subjected to crystallization from ethanol, resulting in colourless crystals of compound **6** with a 65% yield. The compound exhibited a melting point of 266-268 ℃. IR **(**KBr**)**: 1581 (C=N), 1658 (CO amide), 1735 (CO ester), 2923 (CH aliph.), 3189 cm^{-1}(NH). ^{1}H NMR (DMSO-d_6), δ_H (ppm):1.18 (t, J = 7.4 Hz, 3H, CH_3), 1.94 (s, 3H, CCH_3), 2.27 (s, 6H, $2CH_3$), 3.56 (s, 2H, CH_2COO), 4.07 (q, J = 7.5 Hz, 2 H, CH_2), 5.11 (s, 2H, CH_2CO), 7.27- 8.31 (m, 7 H, arom. H), and 10.75 (s, 1H, NH, D_2O-exchangeable).Analysis calcd for$C_{24}H_{26}N_4O_4$: C, 66.34; H, 6.03; N, 12.89. Found: C, 66.54; H, 6.07; N, 12.95. MS (70 eV) *m/z* (%):434 (14.94) (M^+), 390 (34.51), 44 (100).

Synthesis of 4-(3,4-dimethylphenyl)-2-(2-(3-methyl-5-oxo-2,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)phthalazin-1(2H)-one (7)

A solution of derivative **6** (0.5 g, 0.001 mol) in 20 mL dioxane was refluxed for 30 h. After cooling to ambient temperature, the solid precipitate was separated by filtration and subsequently recrystallized from ethanol, affording derivative **7** in a 52% yield as faint brown crystals. The compound exhibited a melting point of 350-352 ℃. IR **(**KBr**)**: 1627 (C=N), 1662 (3CO), 2950 (CH aliph.), 3178 cm^{-1} (NH). ^{1}H NMR (DMSO-d_6), δ_H (ppm): 2.13 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 4.88 (s, 2H, CH_2CO), 4.96 (s, 1H, CH pyrazole), 7.30- 8.36 (m, 7 H, arom. H), and 10.30 (s, 1H, NH, D_2O-exchangeable),^{13}C NMR (DMSO-d_6), δ_C (ppm): 10.10, 19.71, 19.79, 66.82, 79.20, 126.93, 127.26, 127.93, 129.37, 130.77, 132.52, 134.16, 137.90, 140.50, 146.98. 149.40, 150.37, 158.82, 166.29, 169.59. Analysis calcd for $C_{22}H_{20}N_4O_3$: C, 68.03; H, 5.19; N, 14.42. Found: C, 68.13; H, 5.24; N, 14.12. MS (70 eV) *m/z* (%): 388 (4.06) (M^+), 265 (63.06), 194 (100).

Synthesis of 4-(3,4-dimethylphenyl)-2-(2-oxo-2-(5-oxo-3-phenyl-2,5-dihydro-1H-pyrazol-1-yl)ethyl)phthalazin-1(2H)-one (8)

After being heated under reflux for 30 h, a mixture of acetohydrazide **4** (0.5 g, 0.0015 mol) and ethyl benzoylacetate (0.26 mL, 0.0015 mol) in 20 mL dioxane was allowed to cool. After filtering the resulting solid, it was subjected to crystallization from dioxane, yielding colourless crystals of compound **8** with a 65% yield. The compound exhibited a melting point above 360 ℃.IR **(**KBr**)**: 1619 (C=N), 1666 (3CO), 2954 (CH aliph.), 3181 cm^{-1} (NH). ^{1}H NMR (DMSO-d_6), δ_H (ppm): 2.32 (s, 6H, $2CH_3$), 4.89 (s, 2H, CH_2CO), 6.22 (s, 1H, CH pyrazole), 7.33- 8.43 (m, 12 H, arom. H), and 10.31 (s, 1H, NH, D_2O-exchangeable). Analysis calcd for$C_{27}H_{22}N_4O_3$: C, 71.99; H, 4.92; N, 12.44. Found: C, 71.95; H, 5.02; N, 12.49. MS (70 eV) *m/z* (%): (450) (1.61) (M^+-1), 377 (50.22), 86 (100).

Synthesis of 2-(4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl)-N'-(4-oxopentan-2-ylidene)acetohydrazide (9) [50]

Method 1: A mixture of acetohydrazide derivative **4** (1.0 g, 0.003 mol) and acetylacetone (0.30 mL, 0.003 mol) in dioxane (30 mL) was refluxed for 10 h. The resultant mixture was concentrated, cooled down, and

then filtered off. The obtained solid was crystallized from ethanol to produce derivative **9** in 56% yield.

Method 2: In an oil bath maintained at a temperature of 200 ℃, acetic acid hydrazide **4** (1.0 g, 0.003 mol) was fused with acetylacetone (0.30 mL, 0.003 mol) and 2 drops of piperidine for a duration of 30 minutes. The resulting precipitate obtained upon cooling was separated by filtration, desiccated, and subjected to recrystallization using ethanol, yielding compound **9** in a 44% yield as colourless crystalline solid. The compound exhibited a melting point of206-209℃. IR **(**KBr**)**: 1619 (C=N), 1662 (3 CO), 2946 (CH aliph.), 3178 cm^{-1} (NH).

Synthesis of 2-(2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethyl)-4-(3,4-dimethylphenyl)phthalazin-1(2H)-one (10)

Method 1: In a clean and dried round bottom flask, a solution of acetohydrazide derivative **9** (0.5 g, 0.001 mol) in 20 mL dioxane was heated under refluxed for 30 h. After cooling down to ambient temperature, the resulting solid obtained upon cooling was separated by filtration, desiccated, and subjected to recrystallization using methanol, providing compound **10** in a 85% yield.

Method 2: In an agate mortar, acetohydrazide **4** (1.0 g, 0.003 mol) and acetylacetone (0.30 mL, 0.003 mol) were combined together, and 0.50 mL of acetic acid was then added. The resulting solution was gently ground with a pestle for a duration of 30 minutes while monitoring the reaction progress using TLC. Subsequently, the reaction mixture was poured into water, filtered, and washed with water. After the crude product was refined by recrystallization using methanol, compound **10** was obtained as a colourless crystalline solid with a 30% yield: m.p. 102-206℃, IR **(**KBr**)**: 1585 (C=N), 1666, 1739 (2CO), 2962 cm^{-1} (CH aliph.). ^{1}H NMR (DMSO-d_6), δ_H (ppm): 2.06 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 5.69 (s, 2H, CH_2CO), 6.47 (s, 1H, CH pyrazole), 7.32-7.94 (m, 7 H, arom. H). Analysis calcd for$C_{23}H_{22}N_4O_2$: C, 71.48; H, 5.74; N, 14.50. Found: C, 71.58; H, 5.94; N, 14.55. MS (70 eV) *m/z* (%): 386 (21.17) (M^+), 291 (69.86), 236 (100).

Synthesis of ethyl 3-(2-(2-(4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl)acetyl)hydrazineyl)-3-oxopropanoate (11)

In 20 mL of dioxane, 0.5 g of acetohydrazide derivative **4** (0.0015 mol) was dissolved, and diethyl malonate (0.23 mL, 0.0015 mol) was added. Subsequently, the reaction was refluxed for 10 h. The separated-out solid upon cooling was filtered off and recrystallized utilizing ethanol to furnish **11** in a 65% yield as faint yellow crystals: m.p. 298-299 ℃, IR **(**KBr**)**: 1616 (C=N),1658 (CO amide), 1735 (CO ester), 2923 (CH aliph.), 3185, 3286 cm^{-1} (2NH). ^{1}H NMR (DMSO-d_6), δ_H (ppm): 1.16 (t, J = 7.2 Hz, 3H, CH_3), 2.29 (s, 6H, $2CH_3$), 3.26 (s, 2H, $COCH_2CO$), 4.07 (q, J = 7.5 Hz, 2 H, CH_2), 4.73 (s, 2H, CH_2CO), 7.29- 8.32 (m, 7 H, arom. H), 9.27 (s, 1H, NH, D_2O-exchangeable), and 10.35 (s, 1H, NH, D_2O-exchangeable).Analysis calcd for$C_{23}H_{24}N_4O_5$: C, 63.29; H, 5.54; N, 12.84. Found: C, 66.54; H, 6.07; N, 12.95. MS (70 eV) *m/z* (%): (436) (14.61) (M^+), 390 (24.57), 94 (100).

Synthesis of 1-(2-(4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl)acetyl)pyrazolidine-3,5-dione (12)

Method 1: In a mortar,a mixture of 0.5 g of acetohydrazide derivative **4** (0.0015 mol) and diethyl malonate (0.23 mL, 0.0015 mol) was ground together with a pestle in the presence of a few drops of acetic acid for 30 min at room temperature. The precipitated solid was collected by filtration, washed with water, dried well, and recrystallized using methanol to submit **12** in a 42% yield.

Method 2: After boiling under reflux for 30 h, a solution of **11** (0.5 g, 0.001 mol) in 20 mL dioxane was cooled to ambient temperature. After cooling, the resulting precipitate was filtered, thoroughly dried, and subsequently subjected to recrystallization using ethanol, resulting in the formation of compound **12** in a 50% yield as colourless crystalline solid. The compound exhibited a melting point above 360 ℃.IR **(**KBr**)**: 1546 (C=N), 1650 (4CO), 2950 (CH aliph.), 3421 (NH) cm^{-1}. ^{1}H NMR (DMSO-d_6), δ_H (ppm): 2.33 (s, 6H, $2CH_3$), 3.66 (s, 2H, CH_2 pyrazole), 4.77 (s, 2H, CH_2CO), 7.32- 8.37 (m, 7 H, arom. H), and 9.29 (s, 1H, NH, D_2O-exchangeable), ^{13}C NMR (DMSO-d_6), δ_C (ppm): 19.72, 19.85, 41.25, 52.87, 126.71, 126.87, 126.88, 127.28, 128.01, 130.01, 130.73, 132.28, 133.99, 134.01, 137.03, 137.88, 146.90, 156.90, 163.45, 166.84. Analysis calcd for$C_{21}H_{18}N_4O_4$: C, 64.61; H, 4.65; N, 14.35. Found: C, 64.67; H, 4.75; N, 14.15. MS (70 eV) *m/z* (%): (394) (10.5) (M^++4), 324 (100), 282 (9.56).

Synthesis of 2-(2-(3,5-diamino-1H-pyrazol-1-yl)-2-oxoethyl)-4-(3,4-dimethylphenyl)phthalazin-1(2H)-one (13)

In a clean and dried round bottom flask, malnonitrile (0.1 mL, 0.0015 mol) was introduced to a dioxane solution (20 mL) containing acetohydrazide **4** (0.5 g, 0.0015 mol). The resulting reaction mixture was refluxed for a duration of 30 h. After cooling to ambient temperature, the solid product obtained was filtered, thoroughly dried, and subjected to recrystallization using ethanol, yielding compound **13** in a 60% yield as a brown crystalline solid. The compound exhibited a melting point above 360 ℃.IR **(**KBr**)**: 1623 (C=N), 1666 (2CO), 2950 (CH aliph.), 3181- 3440 ($2NH_2$). ^{1}H NMR (DMSO-d_6), δ_H (ppm):2.31 (s, 6H, $2CH_3$), 4.77 (s, 2H, NH_2, D_2O-exchangeable), 5.15 (s, 1H, CH pyrazole), 5.29 (s,

2H, CH_2CO), 6.07 (s, 2H, NH_2, D_2O-exchangeable), and 7.31-8.37 (m, 7H, arom. H). Analysis calcd for$C_{21}H_{20}N_6O_2$: C, 64.94; H, 5.19; N, 21.64. Found: C, 64.96; H, 5.29; N, 21.85. MS (70 eV) *m/z* (%):388 (39.80) (M^+), 264 (54.97), 136 (100).

Synthesis of 2-(4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl)-N'-(2-oxoindolin-3-ylidene)acetohydrazide (14)

In a boiling water bath, a mixture of acetohydrazide derivative **4** (0.5 g, 0.0015 mol), isatin (0.22 g, 0.0015 mol) in ethyl alcohol (20 mL), and a few drops of acetic acid was heated for 8 h. After cooling, the obtained solid was poured over crushed ice, collected by filtration, dried well, and subjected to recrystallization from acetic acid to produce **14** in a 45% yield as yellow crystals: m.p. 268-270℃, IR (KBr): 1581(C=N), 1639, 1707 (3CO), 3197cm^{-1} (2NH). ^{1}H NMR (DMSO-d_6), δ_H (ppm): 2.27 (s, 6H, $2CH_3$), 4.55 (s, 2H, CH_2CO), 6.96- 8.41 (m, 11 H, arom. H), 11.28 (s, 1H, NH isatin, D_2O-exchangeable), and 11.65 (s, 1H, NH, D_2O-exchangeable), ^{13}C NMR (DMSO-d_6), δ_C (ppm): 19.72, 19.84, 52.41, 111.66, 120.07, 121.31, 123.10, 125.27, 126.93, 127.29, 127.69, 129.34, 130.04, 130.75, 131.26, 132.32, 132.41, 134.30, 136.45, 137.09, 138.00, 139.14, 143.07, 158.89, 163.01, 165.82. Analysis calcd for$C_{26}H_{21}N_5O_3$: C, 69.17; H, 4.69; N, 15.51. Found: C, 69.07; H, 4.49; N, 15.31. MS (70 eV) *m/z* (%): 451 (12.01) (M^+), 263 (43.99), 45 (100).

Synthesis of N'-acetyl-2-(4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl)acetohydrazide (15)

A mixture comprising acetohydrazide derivative**4** (0.5 g, 0.0015 mol), acetic acid (0.1 mL, 0.0015 mol), and phosphorous oxychloride (2 mL) was heated under reflux for 6 h, followed by cooling. After cooling, the resulting mixture was then poured into ice-cold water and neutralized using sodium bicarbonate. The formed crude product was filtered, dried, and subjected to recrystallization from ethyl acetate to produce reddish-brown crystals of compound **15** in a 54% yield. The compound exhibited a melting point of 260-262 ℃.IR **(**KBr**)**: 1573 (C=N), 1654 (3CO), 2919 (CH aliph.), 3413 cm^{-1} (2NH). ^{1}H NMR (DMSO-d_6), δ_H (ppm): 1.92 (s, 3H, $COCH_3$), 2.26 (s, 6H, 2 CH_3), 4.57 (s, 2H, CH_2CO), 7.19-8.35 (m, 7 H, arom. H), and 12.79 (s, 2H, 2NH, D_2O-exchangeable). Analysis calcd for$C_{20}H_{20}N_4O_3$: C, 65.92; H, 5.53; N, 15.38. Found: C, 65.94; H, 5.59; N, 15.68. MS (70 eV) *m/z* (%): 364 (44.84) (M^+), 319 (51.65), 84 (100).

Synthesis of acetic N-acetyl-2-(4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl) acetohydrazonic anhydride (16)

A solution of acetohydrazide derivative **15** (0.36 g, 0.001 mol) in 2 mL acetic anhydride was heated under reflux for 6 h. After cooling to ambient temperature, the formed solid poured into ice-cold water, and then left to stand at room temperature for the entire night. The solid obtained was collected, dried, and subjected to recrystallization utilizing methanol, resulting in the formation of brown crystals of compound **16** with a 50% yield. The compound exhibited a melting point of 136-138 ℃.IR **(**KBr**)**: 1577 (C=N), 1658 (CO amide), 1727 (CO ester**)**, 2919 (CH aliph.), 3436 cm^{-1} (NH). ^{1}H NMR (DMSO-d_6), δ_H (ppm): 1.92 (s, 3H, $COCH_3$), 2.20 (s, 3H, $OCOCH_3$), 2.29 (s, 6H, 2 CH_3), 4.98 (s, 2H, CH_2CO), 7.30- 8.34 (m, 7 H, arom. H), and 12.79 (s, 1H, NH, D_2O-exchangeable), ^{13}C NMR (DMSO-d_6), δ_C (ppm): 19.85, 28.65, 31.01, 54.34, 124.15, 127.77, 128.09, 130.86, 131.38, 131.93, 132.02, 132.70, 133.16, 134.10, 134.19, 134.23, 134.70, 145.19, 163.14, 167.85, 174.50. Analysis calcd for$C_{22}H_{22}N_4O_4$: C, 65.01; H, 5.46; N, 13.78. Found: C, 65.06; H, 5.66; N, 13.84. MS (70 eV) *m/z* (%):406 (44.94) (M^+), 244 (100), 174 (50.55).

Synthesis of 2-(4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl)-N'-formylacetohydrazide (17)

Acetohydrazide derivative **4** (0.5 g, 0.0015 mol) and 20 mL of formic acid were mixed together and refluxed for 24 h. After cooling to ambient temperature, the resulting crude product was recrystallized from ethanol, yielding colourless crystals of compound **17** with a 51% yield. The compound exhibited a melting point of 150-151 ℃.IR **(**KBr**)**: 1581 (C=N), 1654, 1727 (3CO), 2996 (CH aliph.), 3120 cm^{-1} (2NH). ^{1}H NMR (DMSO-d_6), δ_H (ppm): 2.28 (s, 6H, $2CH_3$), 4.92 (s, 2H, CH_2CO), 7.26- 8.33 (m, 8 H, 7 arom. H + aldehyde proton CHO), 9.27 (s, 1H, NHCHO, D_2O-exchangeable), and 9.52 (s, 1H, NH, D_2O-exchangeable). Analysis calcd for$C_{19}H_{18}N_4O_3$: C, 65.13; H, 5.18; N, 15.99. Found: C, 65.18; H, 5.38; N, 16.01. MS (70 eV) *m/z* (%):350 (4.54) (M^+), 321 (12), 83 (100).

Synthesis of (E)-N'-(4-chlorobenzylidene)-2-(4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl)acetohydrazide (18)

Equimolar amounts of acetohydrazide derivative **4** (1.0 g, 0.003 mol), *p*-chlorobenzaldehyde (0.42 g, 0.003 mol), and a few drops of piperidine in 20 mL pyridine were refluxed for 6 h. After cooling to ambient temperature, the resulting mixture was then poured into ice-cold water and acidified using concentrated hydrochloric acid. The separated solid product was filtered and subjected to crystallization from ethanol, resulting in faint yellow crystals of compound **18** in a 65% yield. The compound exhibited a melting point of 140-142 ℃.IR **(**KBr**)**: 1577 (C=N), 1662 (2CO), 2939 (CH aliph.), 3197 cm^{-1} (NH). ^{1}H NMR (DMSO-d_6), δ_H (ppm):2.31 (s, 6H, $2CH_3$), 4.97 (s, 2H, CH_2CO), 7.34- 8.37 (m, 11 H, arom. H), 8.39 (s, 1H, imine-CH), and 11.78 (s,

1H, NH, D_2O-exchangeable). Analysis calcd for $C_{25}H_{21}ClN_4O_2$:C, 67.49; H, 4.76; N, 12.59. Found: C, 67.69; H, 4.86; N, 12.60.

Synthesis of N-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)-2-(4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl)acetamide (19)

Under a calcium chloride guard tube, a reaction was performed by refluxing a mixture of acetohydrazide derivative **18** (0.5 g, 0.001 mol), thioglycolic acid (0.1 mL, 0.001 mol), and anhydrous aluminum chloride (0.50 g) in 20 mL DMF with a few drops of piperidine for 24 h. The resulting mixture was then poured into ice-cold water, and the solid precipitate was separated by filtration. The crude product was washed with water and subsequently subjected to recrystallization from acetic acid, affording compound **19** in a 60% yield as pale yellow crystals with a melting point of 216-218 °C. IR **(**KBr**)**: 1608 (C=N), 1643 (3 CO), 2935 (CH aliph.), 3239 cm^{-1} (NH). ^{1}H NMR (DMSO-d_6), δ_H (ppm): 2.27 (s, 6H, $2CH_3$), 3.47 (s, 2H, thiazolidin-CH_2), 4.84 (s, 2H, CH_2CO), 5.30 (s, 1 H, thiazolidin-CH), 7.28- 8.30 (m, 11 H, arom. H), and 11.85 (s, 1H, NH, D_2O-exchangeable), ^{13}C NMR (DMSO-d_6), δ_C (ppm): 19.69, 19.82, 21.50, 53.05, 53.62, 126.90, 127.25, 127.81, 129.01, 129.28, 130.02, 130.72, 131.96, 132.33, 133.37, 134.07, 135.05, 137.06, 137.89, 143.22, 146.36, 146.69, 158.87, 168.79, 172.50. Analysis calcd for $C_{27}H_{23}ClN_4O_3S$:C, 62.48; H, 4.47; N, 10.80; S, 6.18. Found: C, 62.58; H, 4.67; N, 10.95; S, 6.40. MS (70 eV) *m/z* (%):519 (0.26) (M^+), 250 (100), 235 (91.73).

Synthesis of N-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-2-(4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl)acetamide (20)

Over a period of 30 minutes, 0.32 mL of chloroacetyl chloride (0.004 mol) was added dropwise to a stirring mixture of 0.5 g of benzylidene derivative **18** (0.001 mol) and 0.42 mL of triethylamine (0.003 mol) in 20 mL dioxane. The stirring was continued for an additional 3 h. The solid product was isolated by filtration and washed with dioxane. The crude solid was subsequently purified by recrystallization from acetic acid, yielding compound **20** in a 55% yield as yellow crystals with a melting point of 206-207 °C. IR **(**KBr**)**: 1608 (C=N), 1662 (3 CO), 2915 (CH aliph.), 3185 cm^{-1} (NH). ^{1}H NMR (DMSO-d_6), δ_H (ppm):2.28 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 4.09 (s, 2H, CH_2CO), 4.91 (d, J = 4.1Hz, 1 H, β-lactam-CH), 5.31 (d, J = 4.1Hz, 1H, β-lactam-CHCl), 7.29- 8.00 (m, 11 H, arom. H), and 10.37 (s, 1H, NH, D_2O-exchangeable),^{13}C NMR (DMSO-d_6), δ_C (ppm): 19.77, 19.90, 53.12, 60.07, 64.67, 126.59, 127.00, 127.34, 129.13, 129.43, 130.12, 130.82, 132.44, 132.65, 133.52, 134.18, 134.96, 137.15, 137.68, 137.98, 143.30, 146.77, 158.94, 165.21, 168.85. Analysis calcd for $C_{27}H_{22}Cl_2N_4O_3$: C, 62.20; H, 4.25; N, 10.75. Found: C, 62.40; H, 4.35; N, 10.95. MS (70 eV) *m/z* (%): 521 (0.12) (M^+), 250 (100), 235 (59.88).

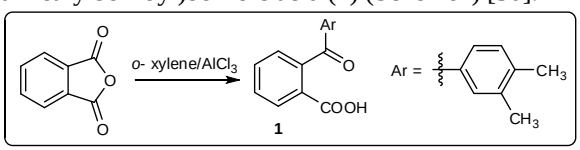
Antibacterial activity assay

Disc diffusion technique employing sterile Whatman-No.5 filter paper discs (11 mm in diameter) was utilized to measure the antibacterial activity of the newly assembled heterocycles against selected bacterial strains. The newly synthesized heterocycles were dissolved in DMSO. Subsequently, 10 mg/mL of the tested material (50 µL) was loaded on filter paper discs (11 mm), and then the discs were carefully placed under a hot air stream until complete dryness was achieved. For preparing the test plates, 10 mL of Muller-Hinton agar medium was poured into the plates and seeded with the organism under investigation. On the surface of agar plates, discs were carefully placed and then incubated at 5 °C for 1 h to assure adequate diffusion. After that, each plate was incubated for 24 h at 37 °C. Following incubation, the outgrowth of the microorganism was observed.

The plates were prepared in triplicate, and the antibacterial activity was assessed using the mean inhibitory zone diameters, which were expressed in millimetres. The size of the clear zone observed is equivalent to the inhibitory effect of the heterocycles under investigation. In similar conditions, a negative control disc containing our pure solvent was introduced in all the experiments in addition to a disc containing amoxicillin (a standard drug) for antibacterial activity comparison.

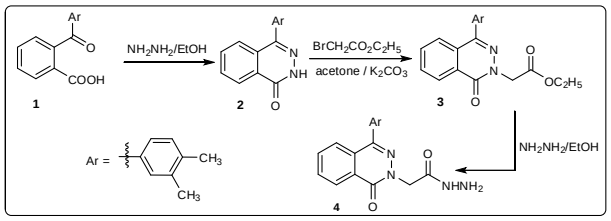
3. Results and Discussion

To commence, Friedel Craft's reaction conditions were exploited for *o*-xylene aroylation reaction by phthalic anhydride to afford 2-(3,4-dimethylbenzoyl)benzoic acid (**1**) (Scheme1) [50].



Scheme 1: Synthesis of *o*-benzoic acid derivative **1**.

According to Goudah *et al*. procedure, *o*-benzoyl benzoic acid **1** and hydrazine hydrate readily underwent a condensation reactionin boiling ethanol to produce phthalazin-1(2*H*)-one derivative **2** (Scheme 2) [50].

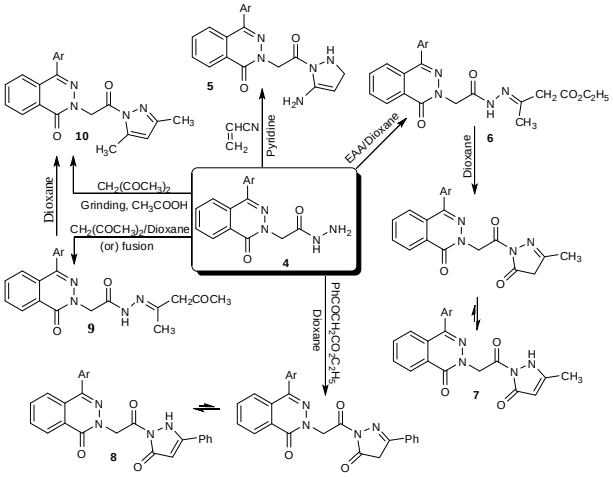


Scheme 2: Synthesis of acetohydrazide derivative **4.**

Subsequently, phthalazine ethyl acetate derivative **3** was accessible *via* the treatment of compound **2** with ethyl bromoacetate. Finally, the target acetohydrazide derivative **4** was acquired in a good yield through refluxing phthalazine ethyl acetate derivative **3** with hydrazine hydrate in boiling ethanol [50].

It is well-known that hydrazide derivatives are versatile organic compounds that can be used as scaffolds for constructing valuable heterocycles. Accordingly, the behaviour of acetohydrazide derivative **4** towards assorted carbon nucleophiles, namely acrylonitrile, ethyl acetoacetate, ethyl benzoylacetate, acetylacetone, and diethyl malonatewas thoroughlyinvestigated.

Interestingly, the reaction of acetohydrazidederivative **4** with acrylonitrile in pyridine gave rise to a new attached 2,3-dihydro-1*H*-pyrazole ring, which was contrary to the reported procedure by Goudah *et al.* in which the open-chain cyanoethyl hydrazide was acquired in a longer reaction time (Scheme 2) [50].



Scheme 3: Reactions of acetohydrazide derivative **4**.

The structure of phthalazinone derivative **5** was verified using spectral and analytical data. The infrared spectrum of compound **5** displayed absorption frequencies at *ύ* 3181 and 3417 cm^{-1} due to (NH) and (NH_2) moieties. Also, ^{1}H NMR spectrum exhibited distinct peaks at δ 3.65 ppm assigned for dihydropyrazole methylene (C*H*$_2$) and at δ 4.88 ppm corresponding to dihydropyrazole (C*H*) proton.Two peaks, exchangeable with D_2O, were observed at δ 4.96 and 10.29 ppm, representing the (NH_2) and (N*H*) groups, respectively. Moreover, the molecular ion peak of derivative **5** was detected at *m/z* = 375 (7.38%), consistent with the molecular formula $C_{21}H_{21}N_5O_2$.

In another attempt to build up more heterocycles attached to phthalazine acetohydrazide derivative **4**, it was subjected to reflux with ethyl acetoacetate in dioxane. Surprisingly, and after 10 h, only the open-chain derivative **6** was isolated (Scheme 2). The IR spectrum of phthalazine derivative **6** exhibited a significant absorption band at *ύ* 1735 cm^{-1} suggesting that derivative **6** still possesses an ester carbonyl group and no cyclization reaction took place. Moreover, the ^{1}H NMR spectrum of derivative **6** not only exhibited the distinctive splitting pattern of the ethyl group, characterized by a triplet-quartet arrangement at δ 1.18 and 4.07 ppm, but also displayed a discernible peak corresponding to the (N*H*) moiety at δ 10.75 ppm. Finally, the molecular ion peak of phthalazine derivative **6** was recorded at *m/z* = 434 (14.94%), aligned with the proposed molecular formula $C_{24}H_{26}N_4O_4$.

Additionally, compound **6** was chemically proven when a subsequent cyclization was accomplished and 4-(3,4-dimethylphenyl)-2-(2-(3-methyl-5-oxo-2,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethyl)phthalazin-1(2*H*)-one (**7**) was attained*via* refluxing the open-chain isolated intermediate **6** in dioxane for an extra 30 h (Scheme 2). The reaction took place at the ester carbonyl, followed by a successive 5-*exo-trig* ring closure [51].

The structure of derivative **7** was elucidated utilizing spectroscopic data. The ^{1}H NMR spectrum of phthalazine derivative **7** lacked the characteristic ethyl group triplet-quartet splitting pattern, but peaks for dihydropyrazol (C*H*) and (N*H*) were observed at δ 4.96 and 10.30 ppm, respectively. Moreover, the ^{13}C NMR spectrum of derivative **7** was in adequate agreement with the prospective structure **7** in which dihydropyrazol (*C*H) appeared at δ 79.20 ppm along with the methyl carbon (*C*H$_3$) at 10.10 ppm and the three carbonyl carbons (*C*O) at 158.82, 166.29, and 169.59 ppm. Also, the mass spectrum of phthalazine derivative **7**revealed the prospective molecular ion peak at *m/z* = 388(4.06%).

Likewise, another 5-*exo-trig* ring closure was observed, and a new dihydropyrazolone ring was attained through the reaction of phthalazine acetohydrazide derivative **4** with ethyl benzoylacetate in boiling dioxane for 30 h (Scheme 2). The IR spectrum of derivative **8** showed the amide carbonyl stretching frequency at *ύ* 1666 cm^{-1} and (NH) stretching frequency at *ύ* 3181 cm^{-1}. An extra piece of proof for the prospective chemical structure of derivative **8** was acquired from the ^{1}H NMR data,

where the new peaks of dihydropyrazol (C*H*) and (N*H*) appeared at δ 6.22 and δ 10.31, respectively.

In 2010, Gouda *et al.* reported a two-step procedure for the synthesis of pyrazole derivative **10**[50]. The aforementioned procedure included a condensation reaction between hydrazide derivative **4** and acetyl acetone in ethanol for 13 h to obtain derivative **9**, then cyclization to the corresponding pyrazole derivative **10** using the strongly alkaline sodium ethoxide solution [50].

In our attempts to develop better reaction profiles, various reaction conditions were explored to synthesize pyrazole derivative **10**. First, phthalazine acetohydrazide derivative **4** was subjected to reflux with acetylacetone in dioxane for a duration of 10 h to have access to the acetohydrazide derivative **9** (Scheme 2). An alternative pathway to assemble compound **9** was also developed by the fusion of acetohydrazide**4** with acetylacetone and 2 drops of piperidine at 200℃for only 30 min. Noteworthy, despite the considerable enhancement of the reaction time in case of fusion, a better reaction profile and yield were observed in case of refluxing in dioxane. Later, the cyclization of the isolated intermediate **9** to the target pyrazole derivative **10** took place through the prolonged heating of **9** in dioxane without the strong basic conditions utilized by Gouda *et al.* (Scheme 2).

Importantly, and after various trials, pyrazole derivative **10** could be synthesized from phthalazine acetohydrazide derivative **4** in a one-step procedure utilizing a solvent-free green chemistry grinding approach. Our new optimized procedure included the grinding of acetohydrazide **4** and acetylacetone with a few drops of acetic acid at room temperature for only 30 min. The IR spectrum revealed stretching frequencies at *ύ* 1585 cm^{-1} implying the existence of imine groups (C=N), and at *ύ* 1666 and 1739cm^{-1} for the two carbonyl groups (CO). Additionally, two singlet peaks at δ 2.06 and 2.32 ppm, corresponding to three protons each, were recorded in the ^{1}H NMR spectrum of pyrazole derivative **10**, suggesting the existence of two methyl groups (CH_3). Furthermore, a singlet signal was detected at δ 6.47 ppm, which was identified as the (C*H*-pyrazole) moiety. At *m/z* = 386 (21.17%), the molecular ion peak was identified corresponding to the chemical formula $C_{23}H_{22}N_4O_2$.

Likewise, the same behavior was observed in case of treating phthalazine acetohydrazide derivative **4** with another active methylene compound, namely diethyl malonate, and pyrazolidine-3,5-dione derivative **12** was acquired *via* two routs (Scheme 4). Either by refluxing acetohydrazide scaffold **4** with diethyl malonate in dioxane for 10 h to obtain the isolable hydrazineyl oxopropanoate derivative **11** and then cyclizing the isolated intermediate **11** by refluxing it in dioxane for an extra 30 h, or *via* grinding of acetohydrazide derivative **4** with diethyl malonate and a few drops of acetic acid at room temperature for only 30 min (Scheme 2). Noteworthy, pyrazolidine-3,5-dione derivative **12** was isolated in a better yield and reaction profile using the solvent-free grinding approach (Scheme 4).

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In our attempts to develop better reaction profiles, various reaction conditions were explored to synthesize pyrazole derivative **10**. First, phthalazine acetohydrazide derivative **4** was subjected to reflux with acetylacetone in dioxane for a duration of 10 h to have access to the acetohydrazide derivative **9** (Scheme 2). An alternative pathway to assemble compound **9** was also developed by the fusion of acetohydrazide**4** with acetylacetone and 2 drops of piperidine at 200℃for only 30 min. Noteworthy, despite the considerable enhancement of the reaction time in case of fusion, a better reaction profile and yield were observed in case of refluxing in dioxane. Later, the cyclization of the isolated intermediate **9** to the target pyrazole derivative **10** took place through the prolonged heating of **9** in dioxane without the strong basic conditions utilized by Gouda *et al.* (Scheme 2).

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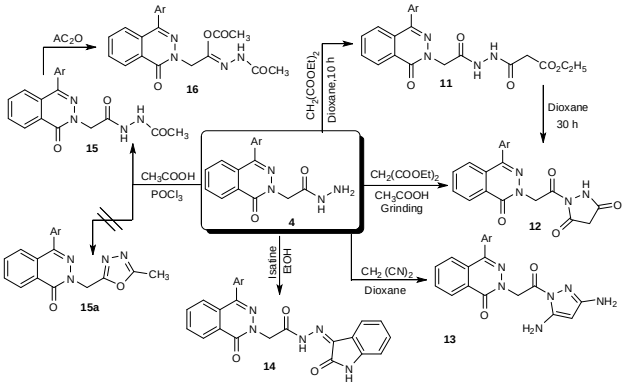
As well, the same behavior was observed in case of treating phthalazine acetohydrazide derivative **4** with another active methylene compound, namely diethyl malonate, and pyrazolidine-3,5-dione derivative **12** was acquired *via* two routs (Scheme 4). Either by refluxing acetohydrazidescaffold **4** with diethyl malonate in dioxane for 10 h to obtain the isolable hydrazineyl oxopropanoate derivative **11** and then cyclizing the isolated intermediate **11** by refluxing it in dioxane for an extra 30 h, or *via* grinding of acetohydrazide derivative **4** with diethyl malonate and a few drops of acetic acid at room temperature for only 30 min (Scheme 4). Noteworthy, pyrazolidine-3,5-dione derivative **12** was isolated in a better yield and reaction profile using the solvent-free grinding approach (Scheme 4).



Scheme 4: Reactions of acetohydrazide derivative **4**.

The IR spectrum of the hydrazineyl oxopropanoate derivative **11** revealed a distinctive absorption at *ύ* 1735 cm^{-1} implying the introduction of a new ester carbonyl group and confirming that no cyclization reaction occurred. On the other hand, the infrared (IR) spectrum of pyrazolidine-3,5-dione derivative **12** lacked characteristic bands associated with ester carbonyls, but exhibited a distinctive absorption band at *ύ* 1650 cm^{-1}, which most likely attributed to the amide carbonyl groups. Additionally, an absorption band at *ύ* 3421 cm^{-1} due to NH was detected.

Furthermore, the ^{1}H NMR spectrum of derivative **11** revealed a characteristic ethyl group splitting pattern, displaying a triplet-quartet arrangement at δ 1.16 and 4.07 ppm and characteristic bands exchangeable with D_2O at δ 9.27 and 10.35 for the two amino (N*H*) groups. However, ^{1}H NMR spectrum of pyrazolidine-3,5-dione derivative **12** exhibited peaks at δ 3.66 ppm attributed to pyrazolidine (CH_2) in addition to a singlet peak exchangeable with D_2O at 9.29 ppm attributed to the proton of the (N*H*) group. Also, ^{13}C

NMR spectrum of pyrazolidine-3,5-dione derivative **12** revealed peaks at δ 41.25 ppm for pyrazolidine carbon (*C*H_2) and at δ 156.90, 163.45, and 166.84 ppm for four carbonyl carbons.

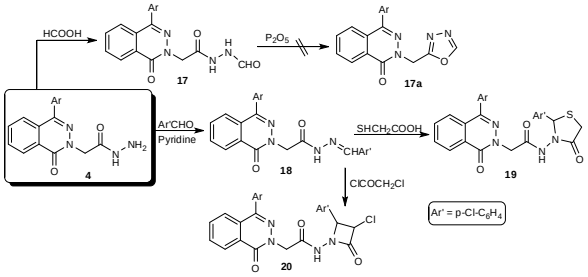
In the same context, and in our exploration of the effect of active methylene nucleophiles on acetohydrazide scaffold **4**, the latter compound was refluxed with malononitrile in dioxane to give the cyclized product **13** (Scheme 4). Formation of the cyclized derivative **13** was asserted by spectral data in which IR spectrum exhibited an imine (C=N) absorption band at *ύ* 1623 cm^{-1}, carbonyl absorption bands at *ύ* 1666 cm^{-1}, and two amino groups (NH_2) absorption bands around *ύ* 3181-3440 cm^{-1}. Furthermore, ^{1}H NMR spectrum of heterocyclic derivative **13** showed two D_2O exchangeable peaks at δ 4.77 and 6.07 ppm assigned to two (N*H*$_2$) groups along with a singlet peak at δ 5.15 ppm attributed to pyrazole (C*H*). Besides, the molecular ion peak was recorded at *m/z* = 388(39.80%).

Additionally, a condensation reaction was observed, and acetohydrazide derivative **14** was isolated when phthalazine acetohydrazide derivative **4** was treated with isatin (Scheme 4). The IR spectrum of derivative **14** exhibited distinctive absorption frequencies at *ύ* 1639 and 1707 cm^{-1} attributable to carbonyl groups (CO) and at *ύ* 3197 cm^{-1} attributed to (NH) groups. Also, (N*H*$_2$) group protons were not observed in ^{1}H NMR spectrum of compound **14,** and instead two peaks exchangeable with D_2O at δ 11.28 and 11.65 ppm corresponding to two (N*H*) were recorded.The ^{13}C NMR spectrum exhibited peaks at δ 139.14 ppm for an extra imine carbon (*C*=N) and at δ 165.82 ppm for the istain γ-lactam carbon. The mass spectrometry of compound **14** recorded the molecular ion peak at *m/z* = 451 (12.01%), corresponding to $C_{26}H_{21}N_5O_3$ and in accordance with the molar mass of the proposed structure.

Interestingly, the anticipated cyclized oxadiazol derivative **15a** was not obtained through the reaction of phthalazine acetohydrazide derivative **4** with acetic acid in the presence of phosphorous oxychloride, but rather an *N*-acetylation reaction occurred at the terminal amino of acetohydrazide derivative **4** to afford the *N'*-acetyl acetohydrazide **15** as the sole product (Scheme 4). The infrared spectrum of acetohydrazide derivative **15** exhibited the absence of the amino group and the presence of carbonyl absorption bands at *ύ* 1654 cm^{-1}. Two (NH) stretching vibrations were observed around *ύ* 3413 cm^{-1}. Additionally, the ^{1}H NMR spectrum of *N'*-acetyl acetohydrazide **15** revealed a new singlet peak at δ 1.92 attributed to the new methyl group (C*H*$_3$) along with a singlet peak exchangeable with D_2O at δ 12.79 ppm integrated for two protons of 2 (N*H*).Also, the molecular ion peak of *N'*-acetyl acetohydrazide **15** was recorded at *m/z* = 364(44.84%).

In an additional effort to produce the intended cyclization product **15a**, *N'*-acetyl acetohydrazide derivative **15** was allowed to reflux withacetic anhydride; however, no cyclization reaction was observed. Alternatively, *O*-acylation reaction was reported, and the double acetylation product **16** was attained as the only product (Scheme 4). Accordingly, strong absorption bands were observed in the IR spectrum of *N*-acetyl acetohydrazonic anhydride **16** at *ύ* 1658 cm^{-1}, which corresponded to cyclic amide carbonyl (CO), 1727 cm^{-1} due to ester carbonyl (CO), and 3436 cm^{-1} identified as the amino group (NH). Furthermore, in addition to the (N*H*) singlet peak at δ 12.79 ppm, the ^{1}H NMR spectrum of compound **16** exhibited a new singlet peak integrated for three protons at δ 2.20 ppm and it is attributed to the new methyl group (C*H*$_3$), which was also observed in ^{13}C NMR spectrum at δ 31.01 ppm.However, the new imine (*C*=N) and ester carbonyl (*C*O) carbons were recorded in ^{13}C NMR spectrum at δ 134.70 and 167.85 ppm, respectively. In addition, the molecular ion peak of derivative **16** was recorded at *m/z* = 406(44.94%).

In the same context, and in order to have access to more assorted heterocycles attached to parent scaffold, phthalazine acetohydrazide derivative **4** was refluxed with formic acid to obtain *N'*-formyl acetohydrazide **17**, thereafter **17** was refluxed with phosphorous pentaoxide in dry toluene to obtain the oxadiazol derivative **17a** (Scheme 5). Unfortunately, no cyclization was observed, and the acyclic *N'*-formyl acetohydrazide **17** was recovered unchanged even after 24 h (Scheme 5). In addition tothe disappearance of the amino group observed in the infrared spectrum, a novel carbonyl group at *ύ* 1727 cm^{-1} was detected. Also, two (N*H*) peaks, exchangeable with D_2O, were recorded in ^{1}H NMR at δ 9.27 and 9.52 ppm, while the molecular ion was detectedat *m/z*= 350 (4.54%).



Scheme 5: Reactions of acetohydrazide derivative **4**.

Additionally, the presence of a terminal amino group in hydrazide derivative **4** encourages us to assemble a Schiff's base. Schiff's base **18** was acquired in a good yield *via* a condensation reaction between acetohydrazide derivative **4** and *p*-

chlorobenzaldehyde in pyridine (Scheme 5). IR spectrum of *N*-arylidine derivatives **18** showed no absorption bands for the amino group (NH_2), however a new stretching frequency at $\acute{\upsilon}$ 1577 cm^{-1}, due to the newly formed imine group, was observed. The imine proton (-C*H*=N-) was recorded in ^{1}H NMR at δ 8.39 ppm.

It is well known that thiazolidines are readily available through the cycloaddition reactions of Shiff's bases with thioglycolic acid. However, cycloaddition reactions of Shiff's bases with chloroacetyl chloride give rise to 2-azetidinone derivatives [52, 53]. Accordingly, the aryl methylidene hydrazide derivative **18** was submitted to cycloaddition reactions with both thioglycolic and chloroacetyl chloride (Scheme 5). In case of thioglycolic acid, a new thiazolidine ring was assembled and thiazolidinyl acetamide derivative **19** was accessible through thia addition type on azamethine moiety then a subsequent *5-exo-trig* ring closure reaction. The IR spectrum of thiazolidine derivative **19** did not contain any absorption frequencies associated with the imine group. However, the ^{1}H NMR spectrum revealed peaks atδ3.47 ppm assigned for the thiazolidine methylene group (SCH_2) and atδ 5.30 ppm assigned for thiazolidin (C*H*). Nevertheless, the ^{13}C NMR spectrum showed the thiazolidin-*C*H_2 carbon at δ 21.50 ppm and the thiazolidin-*C*H carbon at δ 53.62 ppm, while the carbonyl carbon was detected at δ 172.50 ppm. Finally, at *m/z* = 519 (0.26%), the molecular ion peak was recorded.

Furthermore, valuable 2-azetidinone ring was attached to scaffold compound **4** through a cycloaddition reaction of chloroacetyl chloride with a solution of Schiff's base **18** in dioxane and triethylamine (Scheme 5). The formation of a new attached *β*-lactam ring in derivative **20** was confirmed *via* assorted techniques. The IR spectrum of derivative **20** exhibited the existence of carbonyl groups (CO) and amino groups (NH) at $\acute{\upsilon}$ 1662 and 3185 cm^{-1}, respectively.Moreover, the ^{1}H NMR exhibited the peaks of the two *β*-lactam-C*H* protons at δ 4.91 and 5.31ppm, which were recorded in ^{13}C NMR at δ 60.07 and 64.67 ppm**,** while carbonyl carbon (*C*O) of the lactam ring was recorded at δ 165.21 ppm. Additionally, the molecular ion peak was recorded at*m/z* = 521 (0.12%).

Biological Activity

The phthalazine scaffold exerts significant influence over a diverse array of intracellular processes, encompassing cellular differentiation, gene transcription, inflammatory responses, mitotic events, and apoptosis [18-28]. Attaching assorted heterocycles incorporating nitrogen atoms to a phthalazine motif imparts a positive charge, facilitating interactions with negatively charged bacterial membranes, leading to membrane disruption and cell death. As several pyrazole derivatives have demonstrated antibacterial activity by targeting DNA gyrase, while others have exhibited potent inhibitory activity against DHFR [54], attaching a pyrazole ring to phthalazine derivative **4** could potentially enhance the antibacterial potency. Likewise, various thiazoles can function as inhibitors for the bacterial type III secretion system (T3SS) and transpeptidase enzymes (PTB) [55], therefore attaching a thiazole ring to phthalazine scaffold **4** could effectively combat bacterial growth.

Owing to the diverse range of biological activities exhibited by phthalazine hybrids, the newly assembled phthalazines were tested as antibacterial agents. The antibacterial activity of the investigated compounds against the Gram-positive bacteria, *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*), as well as the Gram-negative bacteria, *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*), was assessed *in vitro*. The *β*-lactam antibiotic amoxicillin was exploited as a control criterion, and the antibacterial efficacy of the assembled heterocycles against the selected pathogenic bacteria was quantified by measuring the inhibition diameter zones in millimeters (mm) (Table). The compounds under investigation were recrystallized twice and tested in their ultra-pure form indicated by TLC.

As illustrated in Table, the phthalazine motif tested heterocycles showed a notable variation in their antibacterial properties. With the exception of derivatives **5**, **10**, and **12**, which were inactive against *P. aeruginosa* (Table; entries 2, 6, and 8, respectively), derivative **11**, which showed no biological activity against *S. aureus* (Table; entry 7), and phthalazine derivative **18**, which was inactive against *E.coli* (Table; entry 14), nearly all of the compounds under investigation were active against the chosen pathogenic strains.

Table:*In vitro*antibacterial activity of the investigated heterocyclic derivatives.

Entry	Derivative	Gram (+Ve) bacteria				Gram (-Ve) bacteria			
		B. subtilis		*S. aureus*		*P. aeruginosa*		*E.coli*	
		IZ±SD	% Activity index	IZ±SD	% Activity index	IZ±SD	% Activity index	IZ±SD	% Activity index
1	**4**	21±0.29	70	15±0.58	78.9	10±0.29	52.6	10±0.58	41.7
2	**5**	26±0.29	86.6	21±0.50	110.5	NA	-	27±0.29	112.5
3	**6**	28±0.50	93.3	14±0.76	73.6	25±0.58	131.5	25±1.00	104.2
4	**7**	30±0.58	100	25±0.58	131.5	9±0.29	47.3	21±0.29	87.5
5	**8**	28±0.29	93.3	22±0.29	115.7	10±0.29	52.6	10±0.50	41.7
6	**10**	20±0.29	66.6	7±0.50	36.8	NA	-	7±0.29	29.2
7	**11**	21±0.29	70	NA	-	8±0.35	42.1	9±0.29	37.5
8	**12**	28±0.58	93.3	24±0.50	126.3	NA	-	10±0.29	41.7
9	**13**	30±0.76	100	21±0.29	110.5	7±0.29	36.8	19±0.50	79.2
10	**14**	24±0.50	80	25±0.50	131.5	10±0.29	52.6	19±0.76	79.2
11	**15**	29±0.58	96.6	12±0.29	63.2	27±0.50	142.1	10±0.76	41.7
12	**16**	39±1.00	130	29±0.58	152.6	25±0.76	131.5	22±0.58	91.7
13	**17**	30±0.58	100	15±0.29	78.9	15±0.58	78.9	14±0.50	58.3
14	**18**	30±0.29	100	29±0.50	152.6	20±0.29	105.3	NA	-
15	**19**	30±0.58	100	28±0.29	147.3	26±1.00	136.8	25±0.29	104.2
16	**20**	20±0.87	66.6	19±0.58	100	8±0.50	42.1	24±0.50	100
17	Amoxicillin	30	100	19	100	19	100	24	100

NA: No detectable antibacterial activity; IZ: Inhibition diameter zones, given in millimeters (mm); SD: Standard Deviation.

For *B. subtilis* bacteria, only derivative **16** exhibited potency stronger than criterion drug (Table; entry 12), while in the case of *S. aureus*, derivatives **16** and **18** showed activity indexes exceeding amoxicillin (Table; entries 12 and 14, respectively). Compounds **5** and **15** were the most reactive compounds against *E.coli* and *P. aeruginosa*, respectively (Table; entries 2 and 11).

Interestingly, attaching a new 4-thiazolidinone ring to phthalazine scaffold in compound **19** significantly diminished the growth of bacteria (Table; entry 15). Noteworthy, the antibacterial activity index for both derivative **20** and amoxicillin, both include a *β*-lactam ring, was exactly the same (Table; entry 16).

Structure-activity relationship (SAR)

The application of quantitative structure activity relationship (QSAR) analysis to the *in vitro* results of the evaluated heterocycles against Gram-positive bacteria, specifically *B. subtilis* and *S. aureus*, unveiled that the thiazole derivative **19** demonstrated profound antibacterial efficacy in comparison to the indole-2-one **14** and *β*-lactam **20** derivatives (Figure 4a). Additionally, substituted pyrazole-5-one derivatives (**7**, **8** and **12**) exhibited excellent activity compared to mono- and di-substituted pyrazole derivatives (**5**, **10** and **13**) (Figure 4a).

Schiff's base derivative **18** displayed the highest antibacterial activity against Gram-positive bacteria, surpassing that of amoxicillin. However, replacing the benzylidene hydrazide moiety of compound **18** with ethyl-3-hydrazineyl-3-oxo-propanoate residue in compound **11** resulted in a decrease in activity. Similarly, replacement by formohydrazide **17**, hydrazineyl **4**, ethyl-3-hydrazineylidene butanoate **6**, and acetohydrazide **15** moieties also led to decreased activity (Figure 4b).

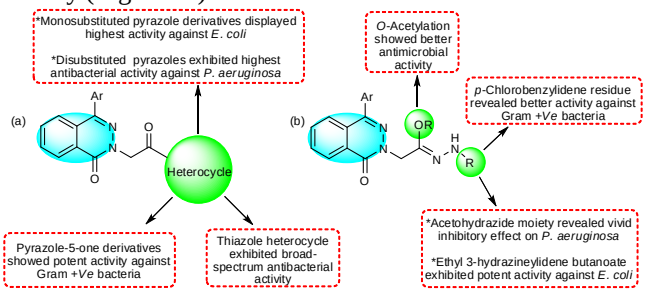


Figure 4: SAR of newly assembled (a) heterocycles and (b) open-chain moieties attached to phthalazine derivative **4**.

Although pyrazole derivative **5** exhibited the highest activity against *E. coli*, the QSAR study demonstrated that thiazole derivative **19** possessed

potent activity against Gram-negative bacteria (*P. aeruginosa* and *E. coli*) compared to all the compounds and was favoured over the *β*-lactam **20**, indole-2-one **14**, and pyrazole (**5**, **7**, **8**, **10**, **12**, and **13**) derivatives. Furthermore, monosubstituted pyrazole derivative **5** showed the highest activity, higher than disubstituted pyrazole derivatives (**7**, **8**, **10**, **12**, and **13**), against *E. coli* but exhibited no activity against *P. aeruginosa* (Figure 4a). Among the open-chain analogues, acetohydrazide derivative **15** demonstrated potent activity against *P. aeruginosa* compared to ethyl-3-hydrazineylidene butanoate derivative **6**, benzylidene hydrazide derivative **18**, formohydrazide **17**, hydrazineyl **4**, and ethyl-3-hydrazineyl-3-oxo-propanoate **11** derivatives. Contrarily, while 4-chlorobenzylidene hydrazide derivative **18** showed no activity against *E. coli*, compound **6** exhibited potent activity compared to formohydrazide **17**, hydrazineyl **4**, acetohydrazide **15**, and ethyl-3-hydrazineyl-3-oxo-propanoate **11** derivatives (Figure 4b).

It is worth mentioning that acetylation of acetohydrazide derivative **15** to produce compound **16** increased its broad-spectrum activity against *E. coli*, *B. subtilis*, and *S. aureus*, while decreasing the activity against *P. aeruginosa* (Figure 4b).

4. Conclusions

In conclusion, 2-(4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1*H*)-yl)acetohydrazide (**4**) was assembled and utilized as a reactive key precursor for the construction of diversified novel heterocyclic and open-chain derivatives attached to a phthalazine motif. Simple and straightforward reactions were utilized to study the effect of different active methylene nucleophiles on acetohydrazide scaffold **4**. Furthermore, the assembled heterocyclic compounds were *in vitro* tested against representative examples of Gram-negative and Gram-positive bacteria for their preliminary antibacterial activity. Several of the evaluated heterocyclic compounds demonstrated highly encouraging antibacterial properties. Finally, the study shed light on the structure-activity relationship (SAR) of the phthalazine hybrids, revealing the potential effectiveness of diverse compounds as antibacterial agents.

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5. Conflicts of interest

The authors report there are no competing interests to declare

6. Formatting of funding sources

No funding was received for conducting this study.

7. Acknowledgments

The authors would like to extend their profound gratitude to Fayoum University and to Dr. Mohammed I. A. Hamed for the valuable discussions.

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