Synthesis of Novel Series of Phthalazine Derivatives with Antibacterial and Antifungal Evaluation

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Abstract:

The oxirane derivative (2) was allowed to react with 4-amino benzoic acid to yield β-amino derivative (3). The hydrazide (5) was reacted with glucose, aromatic aldehyde (benzaldehyde, p-chloro benzaldehyde) and benzoyl chloride to give the phthalazine derivative (6), (9, 10) and (14). A new heterocyclic molecule was synthesized using acetylacetoneto give (12). Reaction with phthalic anhydride yielded compounds (7) and (8).

The new compounds were synthesized with the objective of studying their antifungal and antibacterial activity. Some of them gave positive results. The newly synthesized compounds were characterized on the basis of their spectral (¹H-NMR, Mass spectrum, IR and Elementary analysis).

Keywords: Epichlorohydrin, Pyrazole, Glucose, Lactone.

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1. Introduction:

Phthalazin-1(2H) – one of the most considerable interest due to their antidiabetic [1], antiallergic [2], vasorelaxant [3], PDE₄ inhibitors [4], VEGF (Vascular endothelial group factor) receptor tyrosine kinase for the treatment of cancer [5, 6], antiasthmatic agents with dual activities of thromboxane A₂ (TXA₂) Synthetase inhibition, bronchodilation [7], and herbicidal [8]. A number established drug molecules like hydralazine [9, 10], Budalazine [11, 12], Azelastine [13, 14], ponalrest [15], and zopolrest [16] are prepared from the corresponding phthalazinones.

Several phthalazine derivatives have been reported to possess antitumor [17, 21], antihypertensive [22, 23], anticoagulant [24, 25], antimicrobial [26], antiparasomal [27], and anti-inflammatory drugs (NSAIDs) show serious side effects including gastrointestinal disorders and kidney damage. These studies for developing safer NSAIDs lacking the gastrointestinal and renal side effects of current used ones have recently been of interest for many researches. Most of the classical NSAIDs exerts their side effects by inhibitions of COX-1 enzyme since the COX-1 isoform is the constitutive one that is responsible for regulation of physiological processes, and the COX-2 isoform is discovered to be the enzyme induced by an inflammatory stimuli, selective inhibition of COX-2 provides a rationale for developing anti-inflammatory and analgesic agents. Although the diaryl heterocyclic compounds are mainly studied as new class of NSAIDs without gastric side effects, many studies have also focused on a different type of compounds to develop safer NSAIDs [28]. Also in terms of this aspect, many studies have been focused on pyridazin-(3H)-ones, which are characterized to possess good analgesic and anti-inflammatory activities [29].

Beside pyridazinones, these studies have indicated that the heterocyclic ring substitutions at the six position, and the presence of acetamide side chain when linked to the lactam nitrogen of pyridazinone ring at the two position of the pyridazinone ring, improve the analgesic and anti-inflammatory activity along with nil or very low ulcerogenicity [30-34]. In view of the aforementioned facts, it seemed most interesting to synthesize some 4-(4-ethylphenyl)-2-substituted phthalazine-1(2H)-one derivatives with the aim to obtain more precise information about the course of reactions and biological activities.

2. Results and Discussion:

Upon reacting (1) [35] with epichlorohydrin in the presence of anhydrous potassium carbonate in dry acetone on heating water bath afforded 2-(oxiran-2-ylmethyl)-4-(4-ethylphenyl)phthalazin-1(2H)- one (2).

The structure of (2) was inferred from correct microanalytical data as well as IR spectrum which revealed strong absorption band at ν [1176, 1222], 1612, 1658 cm⁻¹ due to [epoxide], νC=N, νC=O respectively.
The EIMS showed M/Z at 264

\[ \text{M}^+ + \text{CH}_2\text{CH}_2 \]

\[ 245(\text{M}^+ + \text{CH}_2\text{CH}_2 \text{CH}_2 \text{CH}_2) \text{Br} \]

are the major daughters produced from the loss of \( \text{C}_6\text{H}_5\text{C}_2\text{H}_5 \) (P), CO, N\(_2\) yielded finally the M/Z 91 formulated as tropolium radical cation.

The reaction possibly takes place via the following mechanism (Figure 1). The mechanism involves opening of the more reactive oxirane nucleus followed by ring closure via SN\(_2\) mechanism. The function of KCO\(_3\) is to augment the removal of leaving group (chloride ion).

Also, the structure of 2 was verified chemically via its reaction with nitrogen nucleophiles namely p-aminobenzoic acid. Thus, when 2 was reacted with p-aminobenzoic acid in boiling ethanol yielded 2-(2-hydroxy-3-(1-oxo-4-(4-ethyl phenyl) phthalazine-2(1H)-yl)propyl amino) benzoic acid (3). Structure of compound 3 was inferred from (i) its correct microanalytical data (ii) its IR spectrum revealed strong absorption bands at \( \nu \) 3453, 3376, 2961, 1680, 1660, 1604 cm\(^{-1}\) attributable to \( \nu \text{OH}, \nu \text{NH}, \nu \text{CH}, \) and \( \nu \text{ max of two carbonyl groups and } \nu \text{C}=\text{N}, \) respectively. The reaction exhibited regioselective hetero ring opening at carbon atom. Since the β-amino alcohols are versatile compounds with pharmaceutical and biological importance, this prompted us to extend the reaction of oxirane derivative (2).

Interaction of compound 1 with ethyl chloroacetate in boiling dry acetone in the presence of anhydrous potassium carbonate furnished ethyl 2-(4-(4-ethylphenyl)-1-oxophthalazin-2(1H)-yl)acetate (4). Structure of compound 4 was inferred from (i) its correct microanalytical data (ii) its IR spectrum revealed strong
absorption bands at 1649 and 1751 cm\(^{-1}\) due to u\(\text{max}\) of two carbonyl groups. The \(^1\)H-NMR spectrum of compound 4 when run in DMSO shows signals at \(\delta\) (ppm): 1.3 (t, 6H, 2 CH\(_3\) for methyl alkyl and methyl ester), 2.7 (q, 2H, CH\(_2\) of ethyl alkyl), 4.1 (q, 2H, CH\(_2\) of ester), 4.9 (S, 2H, CH\(_2\)CO), 7.1–8.5 (m, 8H, Ar-H). On other hand, the structure of compound 4 was inferred chemically from reaction with hydrazide in boiling ethanol to afford 2-(4-(4-ethylphenyl)-1-oxophthalazin-2(1H)-yl)acetohydrazide (5) [35, 36]. Structure of compound 5 was inferred from (i) its correct micro- analytical data (ii) its IR spectrum revealed strong absorption bands at \(\upsilon\)max of two carbonyl groups, \(\upsilon\)CH and \(\upsilon\)NH respectively.

The \(^1\)H–NMR spectrum of compound 5 when run in DMSO exhibits the following signals at \(\delta\) (ppm): 1.3 (t, 3H, CH\(_3\) of ethyl), 2.7 (q, 2H, CH\(_2\) of ethyl), 4.9 (S, 2H, CH\(_2\)CO), 7.8-8.5 (m, 8H, Ar-H). The previously prepared hydrazide 5 was allowed to react with glucose yielded Z-2-(1-oxo-4-(4-ethylphenyl)phthalazine-2(1H)-yl) N\(^1\)-(2R, 3S, 4S, 5S)- 2, 3, 4, 5, 6-pentahydroxyhexylidene)acetohydrazide (6). Structure of compound 6 was inferred from (i) correct microanalytical data (ii) its IR spectrum revealed strong absorption bands at \(\upsilon\)1664, 2439, 3323 and 3406 cm\(^{-1}\) attributable to \(\upsilon\)C=O, \(\upsilon\)NH and \(\upsilon\)OH, respectively. When the hydrazide 5 was allowed to react with phthalic anhydride in oil bath at 150\(^\circ\)C afforded the lactone derivative (7). Structure of compound 7 was inferred from (i) its correct microanalytical data (ii) its IR spectrum revealed strong absorption bands at 1665, 1747, 3253 and 3432 cm\(^{-1}\) attributable to \(\upsilon\)max C=O and \(\upsilon\)NH respectively. The reaction takes place via condensation with carbonyl group of anhydride.

On the other hand, when the reaction was carried out in refluxing ethanol yield the acid derivative (8). Structure of compound 8 was inferred from (i) its correct microanalytical data (ii) its IR spectrum revealed strong absorption bands at 1621, 1651, 1721, 3187, 3255 and 3435 cm\(^{-1}\) due to \(\upsilon\)max of C=\(\text{N}\), \(\upsilon\)C=O, \(\upsilon\)OH and \(\upsilon\)NH respectively. The hydrazide 5 was reacted with benzaldehyde, the reaction was carried out in refluxing ethanol afforded N’-benzylidene-2-(4-(4-ethylphenyl)phthalazine-2(1H)-yl)acetohydrazide (9). Structure of compound 9 was inferred from (i) its correct microanalytical data (ii) its IR spectrum revealed strong absorption bands at 1655 and 3436 cm\(^{-1}\) due to \(\upsilon\)C=O and \(\upsilon\)NH respectively. In the same manner, hydrazide reacted with p-chlorobenzaldehyde afforded (E)-N’-(4-chlorobenzylidene)-2-(4-(4-ethylphenyl)-1-oxophthalazin-2(1H)-yl)acetohydrazide (10).

Structure of 10 was inferred from (i) its correct microanalytical data (ii) its IR spectrum revealed strong absorption bands at \(\upsilon\) 1664 and 3431 cm\(^{-1}\) due to \(\upsilon\)C=O and \(\upsilon\)NH. EIMS shows M/Z at 445 (M\(^+\)) corresponding to molecular ion peak. Also, the hydrazide 5 was reacted with acetylacetone in boiling ethanol for 3 hrs yield the lactone derivative (9). Structure of compound 11 revealed strong absorption bands at \(\upsilon\) 1654 cm\(^{-1}\) due to carbonyl group and \(\upsilon\)NH. The structure of compound 11 was inferred from (i) correct microanalytical data (ii) its IR spectrum revealed strong absorption bands at \(\upsilon\) 1665, 1737 and 3111 cm\(^{-1}\) due to \(\upsilon\)max of two carbonyl groups and \(\upsilon\)NH. On the other hand, when the reaction was carried out in boiling ethanol for 9 hrs the pyrazole derivative 2-(2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethyl)-4-(4-ethylphenyl)phthalazin-1(2H)one (12) was obtained.

The IR spectrum revealed strong absorption bands at \(\upsilon\) 1654 cm\(^{-1}\) due to carbonyl group and devoid any band for \(\upsilon\)NH.

The \(^1\)H-NMR of compound 12 when its spectrum run in DMSO shows the following signals at \(\delta\) (ppm): 1.3 (t, 3H, CH\(_3\) of ethyl group), 1.9 (S, 3H, CH\(_3\)-C= of pyrazole moiety), 2.1 (S, 3H, CH\(_3\)-C=N of pyrazole moiety), 2.8 (q, 2H, CH\(_2\) of ethyl group), 4.3 (S, 2H, CH\(_2\)CO), 5.8 (S, 1H of pyrazole moiety), 7.2 – 8.5 (m, 8H, Ar-H). Interaction of hydrazide 5 with ethyl acetoacetate in boiling ethanol for 3 hrs afforded the ester derivative ethyl (Z)-3-(2-(2-(4-(4-ethylphenyl)-1-oxophthalazin-2(1H)-yl)acetyl)hydrazono)butanoate (13). Structure of compound 13 was inferred from (i) correct microanalytical data (ii) its IR spectrum revealed strong absorption bands at \(\upsilon\) 1651, 1744, 2964, 3043 and 3429 cm\(^{-1}\) attributable to \(\upsilon\)max of two carbonyl groups, \(\upsilon\)CH, and \(\upsilon\)NH respectively; the EIMS shows M/Z 434 (M\(^+\)) and many fragments daughters.

The phthalazine acetic acid hydrazide 5 used as versatile starting material for the synthesis of several heterocyclic derivatives through its reactions with a variety of activated reagents. Thus, the hydrazide 5 was reacted with benzyl chloride in pyridine yield N’-(2-(4-(4-ethylphenyl)-1-oxophthalazin-2(1H)-yl)acetonylbenzohydrazide (14). Structure of compound 14 was inferred from (i) its correct microanalytical data (ii) its IR spectrum revealed strong absorption bands at \(\upsilon\) 1660, 3281, 3329 and 3435 cm\(^{-1}\) attributable to \(\upsilon\)C=O and \(\upsilon\)NH, respectively (Scheme 1).
Antimicrobial activity:

The antibacterial activity of the synthesized compounds was tested against B. cereus and S. aureus using nutrient agar medium.

The antifungal activity of the compounds was tested against A. niger and R. oryzae using sabouraud dextrose agar medium.

Agar diffusion Medium:

All compounds were screened in vitro for their antimicrobial activity by agar diffusion method [37]. A suspension of the organisms were added to sterile nutrient agar media at 45°C and the mixture was transferred to sterile petri dishes and allowed to solidify. Holes of 10 mm in diameter were made using a cork borer. An amount of 0.1 mL of every synthesized compound was poured inside the holes. A hole filled with DMF was also used as control. The plates were left for 1hr at room temperature as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of the different solutions the plates were then incubated at 37°C for 24 hrs and observed for antimicrobial activity. The diameters of zone of inhibition were measured and compared with that of the standard.

Ciprofloxacin (50 mg/mL) and fusidic acid (50 mg/mL) were used as standard for antibacterial and antifungal activity, respectively. The observed zone of inhibition is presented in Table (1).
Table 1: Antibacterial and antifungal activities of the newly synthesized compounds.

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>Microorganism</th>
<th>B. cereus</th>
<th>S. aureus</th>
<th>A. niger</th>
<th>R. oryzae</th>
</tr>
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<tbody>
<tr>
<td>3</td>
<td>+++ ve</td>
<td>++ ve</td>
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<tr>
<td>7</td>
<td>+++ ve</td>
<td>++++ ve</td>
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<tr>
<td>8</td>
<td>+ ve</td>
<td>+++ ve</td>
<td>+ ve</td>
<td>- ve</td>
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<td>10</td>
<td>+++ ve</td>
<td>+++ ve</td>
<td>+++ ve</td>
<td>++ ve</td>
<td>- ve</td>
</tr>
<tr>
<td>13</td>
<td>+ ve</td>
<td>+++ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>- ve</td>
</tr>
</tbody>
</table>

- Highly active (++++) = (inhibition zone > 20 mm).
- Moderately active (+++) = (inhibition zone 16-20 mm).
- Slightly active (+) = (inhibition zone 13-15 mm).
- Inactive = (inhibition zone < 11 mm).

3. Experimental:

All melting points are uncorrected and were measured on an electrothermal melting point apparatus. Elemental analyses were performed using a Heraeus CHN Rapid analyzer at the microanalytical unit, Cairo University. Thin–layer chromatography (TLC) was performed on Merck TLC aluminum sheets gel 60F254 with detection by UV quenching at 254 nm. IR spectra were measured on a unicam SP-1200 spectrophotometer using KBr wafer technique. 1H-NMR spectra were measured in DMSO-d6 on a varian plus instrument (300 MHz). Mass spectra were recorded on a shimadzu GC-MS Qp 1000 EX instrument operating at 70 eV in El mode.

2-(Oxiran-2-ylmethyl)-4-(4-ethylphenyl)phthalazin-1(2H)-one [2]

A mixture of I (2.59 g, 0.01 mol), epichlorohydin (2.775 g 0.03 mol) and potassium carbonate (2.949 g, 0.03 mol) in 30 mL dry acetone was refluxed for 24 hrs. The excess solvent was removed by distillation and the reaction mixture was diluted with water and the solid that separated filtered and crystallized from ethanol to give (2), 71% yields as yellow crystals m.p. 120-122°C. IR spectrum band at ν [1176, 1222], 1612, 1658 due to (epoxide), νC=O and νC=O, MS, m/z at 306 corresponding to the molecular ion. Anal calcd for C_{10}H_{13}N_{2}O_{2} C (74.49%), H (5.92 %), N (9.14%). Found: C, 74.19; H, 5.81; N, 8.99.

4-(2-Hydroxy-3-(1-oxo-4-(4-ethylphenyl)phthalazin-2(1H)-yl)-propylamino)benzoic acid [3]

A mixture of 2 (0.306 g, 0.001 mol) and p-aminobenzoic acid (0.2055 g, 0.0015 mol) in 30 mL ethanol was refluxed for 3 hrs. The resultant solid was filtered and crystallized from ethanol to give (3), 60% yield as brown crystal m.p. 180-182°C. The IR spectrum at ν 3453, 3376, 2961, 1680, 1660 and 1604 cm⁻¹ due to νOH, νCH, umax of two carbonyl group and νC=N, respectively. Anal calcd for C_{14}H_{15}N_{3}O_{2}: C (70.41%), H (5.68%), N (9.47%). Found: C, 70.11; H, 5.55; N, 9.23.

Ethyl 2-(4-(4-ethylphenyl)-1-oxophthalazin-2(1H)-yl)acetate [4]

A mixture of I (2.5 g, 0.01 mol), ethyl chloroacetate (3.585 g, 0.03 mol) and potassium carbonate (2.94 g, 0.03 mol) in 50 mL dry acetone was reflux for 24 hrs. The excess solvent was removed by distillation. The reaction mixture was diluted with water and the solid that separated filtered off and crystallized from ethanol to give (4), 55% yield as colorless crystal, m.p. 160-162°C. IR spectrum at ν 1649 and 1751 cm⁻¹ due to umax of two carbonyl groups. 1H-NMR (DMSO-d6, 300 MHz) δ: 1.3 (t, 6H, 2CH₃ for methyl alkyl and methyl ester), 2.7 (q, 2H, CH₂ of ethyl alkyl), 4.1 (q, 2H, CH₂ of ester), 4.9 (s, 2H, CH₂CO), 7.1–8.5 (m, 8H, ArH). Anal calcd for C_{20}H_{22}N_{2}O_{2}: C (71.41%), H (5.92 %), N (9.14%). Found: C, 71.22; H, 5.89; N, 8.19.

2-(4-(4-ethylphenyl)-1-oxophthalazin-2(1H)-yl)acetohydrazide [5]

A mixture of 4 (3.36 g, 0.01 mol) and hydrazine hydrate (0.75 g, 0.015 mol) in 30 mL ethanol was refluxed for 3 hrs. After cooling the solid was obtained, filtered off and crystallized from ethanol to give (5), 50% yield as colorless crystals m.p. 233-235°C. IR spectrum 1666, 2961, 3226 and 3332 cm⁻¹ due to νC=O, νCH and νNH respectively. 1H-NMR (DMSO-d6, 300 MHz) δ: 1.3 (t, 3H, CH₃ of ethyl), 2.7 (q, 2H, CH₂ of ethyl), 4.9 (s, 2H, CH₂CO), 7.8–8.5 (m, 8H, ArH). Anal calcd for C_{20}H_{22}N_{2}O_{2}: C (67.07%), H (5.51%); N (17.38%). Found: C, 66.92; H, 5.51; N, 17.52.
Z-2-(1-oxo-4-(4-ethylphenyl)phthalazin-2(1H)-yl)-N'-((2R,3S,4S,5S)-2,3,4,5,6-pentahydroxyhexylidene)acetohydrazide [6]  
A mixture of 5 (3.22 g, 0.01 mol) and glucose (2.7 g, 0.015 mol) in 30 mL ethanol was refluxed for 3 hrs. The resultant solid was filtered off and crystallized from ethanol to give (6), 70% yield as colorless crystal m.p. 150-152°C. IR spectrum: 1664, 2939, 3323 and 3406 cm⁻¹ attributable to υC=O, CH, NH and OH, respectively. Anal calc. for C₂₃H₂₄N₂O₆: C (67.49%), H (4.76%), N (12.59%). Found: C; 67.12; H; 4.61; N; 12.33.

(Z)-2-(4-(4-ethylphenyl)-1-oxophthalazin-2(1H)-yl)-N'-((3-oxoisobenzofuran-1(3H)-ylidine) acetohydrazide [7]  
A mixture of 5 (0.322 g, 0.001 mol) and phthalic anhydride (0.22 g, 0.0015 mol) was heated in oil bath for 2hs. The reaction mixture was diluted with water, the solid filtered off and crystallized from ethanol to give (7), 65% yield as colorless crystals m.p. 165-167°C. IR spectrum at v 1665, 1747, 3253 and 3432 cm⁻¹ attributable to υmax C=O and υNH respectively. Anal calc. for C₂₃H₂₀N₂O₆: C (69.02%), H (4.46%), N (12.38%). Found: C; 68.91; H; 4.31; N; 12.12.

2-(2-(2-(4-(4-ethylphenyl)-1-oxophthalazin-2(1H)-yl)(acetyl)hydrazine-1-carbonyl) benzoic acid [8]  
A mixture of 5 (3.22 g, 0.01 mol) and phthalic anhydride (2.22 g, 0.015 mol) in 30 mL ethanol was refluxed for 3 hrs. The resultant solid was filtered and crystallized from ethanol to give (8), 59% yield as colorless crystals m.p. 125-128°C. IR spectrum at v 1621, 1651, 1721, 3187, 3255, 3435 cm⁻¹ due to υmax of C=O, C=O, NH and OH respectively. Anal calc. for C₂₃H₂₁N₂O₆: C (66.37%), H (4.71%), N (11.91%). Found: C; 65.99; H; 4.61; N; 11.72.

N'-benzhydridene-2-(4-(4-ethylphenyl)phthalazin-2(1H)-yl) acetohydrazide [9]  
A mixture of 5 (0.322 g, 0.001 mol) and benzaldehyde (0.159 g, 0.015 mol) in 30 mL ethanol was refluxed for 3 hrs and cooled at room temperature. The resultant solid was filtered and crystallized from ethanol to give (9), 70% yield as colorless crystals m.p. 140-142°C. IR spectrum: 1655 and 3426 cm⁻¹ attributable to υC=O and υNH, respectively. Anal calc. for C₂₃H₂₁N₂O₆: C (73.15%), H (5.40%), N (13.65%). Found: C; 72.99; H; 5.50; N; 13.71.

(E)-N'-((4-chlorobenzylidene)-2-(4-(4-ethylphenyl)-1-oxophthalazin-2(1H)-yl)acetohydrazide [10]  
A mixture of 5 (3.22 g, 0.01 mol) and p-chlorobenzaldehyde (2.1075 g, 0.015 mol) in 30 mL ethanol was refluxed for 3 hrs. The resultant solid was filtered and crystallized from ethanol to give (10), 54% yield as colorless crystals m.p. 173-175°C. IR spectrum: 1664 and 3431 cm⁻¹ due to υC=O and υNH respectively.

EIMS shows m/z at 445 (M⁺) corresponding to molecular ion peak. Anal calc. for C₂₃H₂₁N₂O₆: C (67.49%), H (4.76%), N (12.59%). Found: C; 67.12; H; 4.61; N; 12.33.

(Z)-2-(4-(4-ethylphenyl)-1-oxophthalazin-2(1H)-yl)-N'-(4-oxopentan-2-ylidene) acetohydrazide [11]  
A mixture of 5 (3.22 g, 0.01 mol) and acetylacetone (1.5 g, 0.015 mol) in 20 mL ethanol was refluxed for 3 hrs. The resultant solid was filtered and crystallized from ethanol to give (11). 63% yield as colorless crystals, m.p. 150-152°C. The IR spectrum 1655, 1737 and 3111 cm⁻¹ due to υmax of two carbonyl groups and υNH, respectively. Anal calc. for C₂₃H₂₄N₂O₆; C (70.75 %), H (6.71 %), N (14.35%) Found: C; 70.45; H; 6.56; N; 14.14.

2-(3-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethyl)-4-(4-ethylphenyl) phthalazin-1(2H)-one [12]  
A mixture of 5 (3.22 g, 0.01 mol) and acetylacetone (1.5 g, 0.015 mol) in 30 mL ethanol was refluxed for 9 hrs. The resultant solid was filtered and crystallized from ethanol to give (12). 58% yields as colorless crystals m.p. 192-195°C. IR spectrum 1654 cm⁻¹ due to carbonyl group and devoid any bands for υNH. ¹H-NMR (DMSO-d₆, 300 MHz) δ : 1.3 (t, 3H, ethyl group), 1.9 (S, 3H, CH₃-C= of pyrazole moiety), 2.1 (S, 3H, CH₃-C=N of pyrazole moiety), 2.8 (q, 2H, CH₂ of ethyl group), 4.3 (S, 2H, CH₂CO), 5.8 (s, 1H of pyrazole moiety), 7.2-8.5 (m, 8H, Ar-H). Anal calc. for C₂₃H₂₂N₂O₆; C (74.17%), H (6.49%), N (15.04%). Found: C; 73.96; H; 6.35; N; 14.83.

Ethyl (Z)-3-(2-(2-(4-(4-ethylphenyl)-1-oxophthalazin-2(1H)-yl)acetyl)hydrazono)butanoate [13]  
A mixture of 5 (3.22 g, 0.01 mol) and ethyl acetooxime (1.935 g, 0.015 mol) in 30 mL ethanol was refluxed for 3 hrs. The resultant solid was filtered and crystallized from ethanol to give (13). 62% yield as colorless crystals m.p. 132-134°C. IR spectrum 1651, 1744, 2964, 3043 and 3429 attributable to υmax of two carbonyl groups, υCH and υNH, respectively. EIMS shows m/z at 434 (M⁺) and many fragments daughters. Anal calc. for C₂₃H₂₄N₂O₆; C (66.34%), H (6.03%) N (12.89%). Found: C; 65.98; H; 5.93; N; 12.68.

N'-((2-(4-(4-ethylphenyl)-1-oxophthalazin-2(1H)-yl)acetyl) benzohydrazide [14]  
A mixture of 5 (3.22 g, 0.01 mol) and benzoyl chloride (2.09 g, 0.015 mol) in 20 mL pyridine was refluxed for 1 hr, then poured on ice / HCl. The resultant solid was filtered and crystallized from ethanol to give (14). 50% yield as brown crystals m.p. 190-192°C. IR spectrum 1660, 3281, 3329 and 3435 due to υC=O and υNH, respectively. Anal calc. for C₂₃H₂₄N₂O₆; C (70.41%), H (5.20%), N (13.14%). Found: C; 70.12; H; 5.1; N; 12.99.
References: