



## Utility of 4-(isatin-3-ylideneamino)benzohydrazide in the synthesis of bioactive *N*-heterocyclic compounds

M. M. H. Arief\*, A. A. Aly, A. A. Khalil and H. I. Mohamed

Department of Chemistry, Faculty of Science, Benha University, Benha, Egypt

### ABSTRACT

4-(Isatin-3-ylideneamino)benzoic acid, was synthesized and converted into 4-(isatin-3-ylideneamino)benzoyl chloride. Hydrazinolysis of the acid chloride afforded 4-(isatin-3-ylideneamino)benzohydrazide. The latter compound was used as a key precursor for the synthesis of pyrazole, triazole, phthalazine, thiadiazole, and oxadiazole derivatives via its reaction with different electrophilic reagents. Structures of all synthesized compounds were elucidated from FT-IR, <sup>1</sup>H-NMR, mass spectroscopy and elemental analyses. Some of the synthesized heterocycles were screened against selected microbes to test their antimicrobial activity.

**Keywords:** Isatin schiff's bases, benzohydrazide, thiadiazole, pyrazole, antimicrobial activity.

### INTRODUCTION

Isatin, its Schiff and Mannich bases are reported to exhibit a broad range of biological and pharmacological properties. They are widely used as starting materials for the synthesis of a variety of heterocyclic compounds and as substrates for drug synthesis[1]. Isatin moiety shows biological activities such as antioxidant and anti-inflammatory[2], antimicrobial[3-5], antituberculosis[6,7], anticancer[8], anti-HIV[9], antiviral[10,11], anticonvulsant activities[12,13]. Numerous isatin derivatives have shown *in vitro* anti-tumor activity against T-47D(breast), NCI-H322M(lung) and SNB-75(CNS) cancer cell lines<sup>[14]</sup>. Also, the anti-tumor effects of isatins *in vitro* and *in vivo* against (SH-SY5Y) cells were investigated [15]. Promoted by the above facts and in continuation of our program of identification of new candidates that may be valuable in design and synthesis of biologically active leads [16-18], we report a facile synthesis of new isatin derivatives bearing different *N*-heterocyclic moieties using 4-(2-oxoindolin-3-ylideneamino)- benzohydrazide **3** as a starting material.

### EXPERIMENTAL SECTION

#### Instrumentation

Melting points were determined in open capillaries using GALENKAMP melting point apparatus and are uncorrected. FT-IR spectra (KBr disc) were recorded on a Perkin-Elmer model 1720 FT-IR spectrometer. <sup>1</sup>H-NMR spectra were recorded on a Varian Gemini NMR Spectrometer 300 MHz using TMS as internal standard. All reactions were monitored by TLC using aluminum silica gel plates 60 F245. Elemental analyses and antimicrobial activity were carried out at the Micro Analytical Center, Faculty of Science, Cairo University, Egypt.

#### Chemistry

##### 4-(2-Oxoindolin-3-ylideneamino)benzoic acid (1)

A mixture of isatin (10 mmol) and 4-aminobenzoic acid (10mmol) in absolute ethanol (20 mL) containing acetic acid (0.5 mL) was heated under reflux for 4h. The reaction mixture was concentrated, cooled, and the formed precipitate was filtered, washed with water, dried and recrystallized from ethanol to give **1**. Orange (93%), mp 288-

290°C; IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$ 3500-2500 (br, OH, NH), 1725 (C=O, isatin ring), 1686 (C=O, acid), 1616 (C=N). EI-MS:  $m/z$  266 [ $\text{M}^+$ ]. Anal. calcd. for  $\text{C}_{15}\text{H}_{10}\text{N}_2\text{O}_3$ ; C, 67.67; H, 3.79; N, 10.52. Found: C, 67.62; H, 3.81; N, 10.55.

#### 4-(2-Oxoindolin-3-ylideneamino)benzoyl chloride (2)

A mixture of **1** (10 mmol) and thionyl chloride (10 mL) was refluxed on a water bath for 2h. The excess thionyl chloride was removed under reduced pressure to give **2** which was used *in situ*.

#### 4-(2-Oxoindolin-3-ylideneamino)benzohydrazide (3)

To a stirred solution of **2** (10 mmol) in absolute ethanol (20 mL), (10 mmol) of hydrazine hydrate was added dropwisely over a period of 30 min., while maintain the temperature below 15°C. The reaction mixture was then refluxed for 1h. After cooling and concentration, the obtained solid was filtered, dried and recrystallized from ethanol to afford **3**. Light brown (86%), mp 195-197°C; IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$  3435, 3185 (OH/NH,  $\text{NH}_2$ ), 1714 (C=O), 1605 (C=N).  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  (ppm) 5.45 (br, 2H,  $\text{NH}_2$ , exchangeable), 6.91-7.53 (m, 8H, Ar-H), 8.28 (s, 1H, NH, exchangeable), 10.98 (s, 1H, NH, exchangeable). EI-MS:  $m/z$  280 [ $\text{M}^+$ ]. Anal. calcd. for  $\text{C}_{15}\text{H}_{12}\text{N}_4\text{O}_2$ ; C, 64.28; H, 4.32; N, 19.99. Found: C, 64.33; H, 4.29; N, 20.03.

#### 5,6,7,8-Tetrabromo-2-[4-(2-oxoindolin-3-ylideneamino)benzoyl]-2,3-dihydrophthalazine-1,4-dione (4)

A mixture of **3** (10 mmol) and tetrabromophthalic anhydride (10 mmol) in dioxane (20 mL) containing acetic acid (0.5 mL) was refluxed for 7h. The reaction mixture was concentrated under reduced pressure and the deposited solid was filtered, dried, and recrystallized from (DMF/water) mixture to give **4**. Brown (62%), mp 151-153°C; IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$ 3432, 3200 (OH, NH), 1721 (C=O), 1610 (C=N).  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  (ppm) 6.82-8.14 (m, 8H, Ar-H), 9.07 (s, 1H, NH, exchangeable), 11.60 (s, 1H, NH, exchangeable). Anal. calcd. for  $\text{C}_{23}\text{H}_{10}\text{Br}_4\text{N}_4\text{O}_4$ ; C, 38.05; H, 1.39; N, 7.72. Found: C, 38.06; H, 1.42; N, 7.68.

#### 1-[4-(2-Oxoindolin-3-ylideneamino)benzoyl]pyrazolidine-3,5-dione (5)

(10 mmol) of compound **3** and (10 mmol) of diethyl malonate in absolute ethanol (20 mL) were refluxed for 6h. The obtained solid, after cooling, was collected by filtration, dried and recrystallized from (methanol/water) mixture to give **5**. Yellow (71%), mp 220-222°C; IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$  3396, 3255 (OH, NH), 2933 (CH, aliphatic), 1717, 1658 (C=O), 1607 (C=N). EI-MS:  $m/z$  348 [ $\text{M}^+$ ]. Anal. calcd. for  $\text{C}_{18}\text{H}_{12}\text{N}_4\text{O}_4$ ; C, 62.07; H, 3.47; N, 16.09. Found: C, 62.04; H, 3.44; N, 16.13.

#### 3-[4-(3,5-Dimethyl-1H-pyrazole-1-carbonyl)phenylimino]indolin-2-one (6)

Equimolar quantities (10 mmol) of **3** and acetylacetone in absolute ethanol (20 mL) containing few drops of triethylamine were refluxed for 12h. The reaction mixture was concentrated and poured onto ice-HCl, and the resultant solid was filtered, washed with dilute ethanol, dried and recrystallized from ethanol to give **6**. Yellow (78%), mp 190-192°C; IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$ 3385, 3244 (OH/NH), 2981 (CH, aliphatic), 1716 (C=O), 1606 (C=N). EI-MS:  $m/z$  344 [ $\text{M}^+$ ]. Anal. calcd. for  $\text{C}_{20}\text{H}_{16}\text{N}_4\text{O}_2$ ; C, 69.76; H, 4.68; N, 16.27. Found: C, 69.80; H, 4.69; N, 16.24.

#### 3-[4-(3-Methyl-5-oxo-4,5-dihydro-1H-pyrazole-1-carbonyl)phenylimino]indolin-2-one (7)

A mixture of **3** (10 mmol) and ethyl acetoacetate (10 mmol) in absolute ethanol (20 mL) was heated under reflux for 4h. The reaction mixture was concentrated and the formed precipitate was filtered, washed with water, dried and recrystallized from ethanol to give **7**. Brown (69%), mp 132-134°C; IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$ 3420, 3250 (OH/NH), 2980 (CH, aliphatic), 1719, 1657 (C=O), 1611 (C=N).  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  (ppm) 1.35 (s, 3H,  $\text{CH}_3$ ), 4.37 (s, 2H,  $\text{CH}_2$ ), 7.24-8.27 (m, 8H, Ar-H), 11.59 (s, 1H, NH, exchangeable). Anal. calcd. for  $\text{C}_{19}\text{H}_{14}\text{N}_4\text{O}_3$ ; C, 65.89; H, 4.07; N, 16.18. Found: C, 65.86; H, 4.10; N, 16.21.

#### 3-[4-(5-Imino-3-oxopyrazolidine-1-carbonyl)phenylimino]indolin-2-one (8)

A mixture of **3** (10 mmol) and ethyl cyanoacetate (10 mmol) in absolute ethanol (20 mL) was refluxed for 8h. The reaction mixture was concentrated and the deposited solid was filtered, dried, and recrystallized from methanol to give **8**. Bright yellow (74%), mp 200-202 °C; IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$ 3388, 3229 (OH/NH), 2982, 2928 (CH, aliphatic), 1715, 1660 (C=O), 1605 (C=N).  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  (ppm) 4.36 (s, 2H,  $\text{CH}_2$ ), 5.90 (s, 1H, C=NH, exchangeable), 7.24-8.07 (m, 8H, Ar-H), 11.59 (s, 1H, NH, exchangeable), 11.75 (s, 1H, NH, exchangeable). Anal. calcd. for  $\text{C}_{18}\text{H}_{13}\text{N}_5\text{O}_3$ ; C, 62.24; H, 3.77; N, 20.16. Found: C, 62.26; H, 3.76; N, 20.19.

#### N'-(2-Oxoindolin-3-ylidene)-4-(2-oxoindolin-3-ylideneamino)benzohydrazide (9)

A mixture of **3** (10 mmol) and isatin (10 mmol) in absolute ethanol (20 mL) containing glacial acetic acid (0.5 mL) was refluxed for 4h. The reaction mixture was concentrated, cooled and the obtained solid was filtered, dried, and recrystallized from ethanol to give **9**. Deep brown (56%), mp 146-148°C; IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$  3434, 3250 (OH, NH),

1728 (C=O), 1614 (C=N).EI-MS:  $m/z$  409 [ $M^+$ ].Anal.calcd. for  $C_{23}H_{15}N_5O_3$ ; C, 67.48; H, 3.69; N, 17.11. Found: C, 67.41; H,3.74; N, 17.08.

#### General procedure for synthesis of compounds(10a,b)

A mixture of **3** (10 mmol) and carbonyl compounds (10 mmol) namely; acetophenone and cinnamaldehyde in absolute ethanol (20 mL) was heated under reflux for 6-8h. The reaction mixture was refrigerated overnight. The formed precipitate was filtered, washed with water, dried and recrystallized from proper solvent to afford **10a,b**.

#### 4-(2-Oxoindolin-3-ylideneamino)-N'-(1-phenylethylidene)benzohydrazide(10a)

Reddish-brown (48%, acetic acid), mp 174-176°C; IR (KBr,  $cm^{-1}$ ):  $\nu$  3411, 3190 (OH, NH), 2978, 2925 (CH, aliphatic), 1717 (C=O), 1605 (C=N).  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  (ppm) 2.24 (s, 3H,  $CH_3$ ), 7.11-8.18 (m, 13H, Ar-H), 8.45 (s, 1H, NH, exchangeable), 11.55 (s, 1H, NH, exchangeable).Anal. calcd. for  $C_{23}H_{18}N_4O_2$ ; C, 72.24; H, 4.74; N, 14.65. Found: C, 72.26; H,4.69; N, 14.57.

#### 4-(2-Oxoindolin-3-ylideneamino)-N'-(3-phenylallylidene)benzohydrazide(10b)

Brown (63%, methanol), mp 230-232°C; IR (KBr,  $cm^{-1}$ ):  $\nu$  3380, 3218 (OH, NH), 3066 (CH, aromatic/olefinic), 1720 (C=O), 1618 (C=N).Anal. calcd. for  $C_{23}H_{18}N_4O_2$ ; C, 73.08; H, 4.60; N, 14.20. Found: C, 73.03; H, 4.56; N, 14.23.

#### Potassium 2-[4-(2-oxoindolin-3-ylideneamino)benzoyl]hydrazinecarbodithioate(11)

To a stirred solution of **3** (10 mmol) in absolute ethanol (20 mL), potassium hydroxide (20 mmol) and carbon disulphide (40 mmol) were added within half an hour. The mixture was diluted with absolute ethanol (15 mL) and stirred at room temperature for 12h. Dry ether (20 mL) was then added and the separated solid was filtered, washed with portions of ether and recrystallized from ethanol to give **11**. Brown (64%), mp > 300 °C; IR (KBr,  $cm^{-1}$ ):  $\nu$  3448, 3370 (OH, NH),1703 (C=O), 1599 (C=N). Anal. calcd. for  $C_{16}H_{11}KN_4O_2S_2$ ; C, 48.71; H, 2.81; N, 14.20. Found: C, 48.68; H,2.85; N, 14.18.

#### 3-[4-(4-Amino-5-mercapto-4H-1,2,4-triazol-3-yl)phenylimino]indolin-2-one (12)

**Method A:** A mixture of carbodithioate **11** (10 mmol) and hydrazine hydrate (10 mmol) in acetic acid (80%, 20 mL) was heated under reflux for 2h. The separated solid was filtered, washed cold water, dried and recrystallized from acetic acid to afford **12**.

**Method B:** (10 mmol) of the acid derivative **1** and thiocarbohydrazide (10 mmol) were fused in a sand bath at 240 °C for 2h. Hot water was added and the product was collected by filtration, dried and recrystallized from acetic acid to afford **12**. Brown (86%), mp 209-211 °C; IR (KBr,  $cm^{-1}$ ):  $\nu$  3442, 3364, 3228 (OH, NH,  $NH_2$ ), 1712 (C=O), 1618 (C=N).EI-MS:  $m/z$  336 [ $M^+$ ].Anal. calcd. for  $C_{16}H_{12}N_6OS$ ; C, 57.13; H, 3.60; N, 24.98. Found: C, 57.09; H, 3.58; N, 25.00.

#### 3-[4-(5-Mercapto-1,3,4-oxadiazol-2-yl)phenylimino]indolin-2-one (13)

A solution of **11** (10 mmol) in ethanol (20 mL) containing hydrochloric acid(5 mL) was refluxed for 1h. The obtained solid, after cooling, was collected by filtration, dried and recrystallized from ethanol to give **13**. Dark brown (61%), mp 268-270°C; IR (KBr,  $cm^{-1}$ ):  $\nu$  3425, 3250 (OH, NH), 1708 (C=O), 1605 (C=N), 1275 (C=S).EI-MS:  $m/z$  322 [ $M^+$ ]. Anal. calcd. for  $C_{16}H_{10}N_4O_2S$ ; C, 59.62; H, 3.13; N, 17.38. Found: C, 59.59; H,3.17; N, 17.39.

#### 2-[4-(2-Oxoindolin-3-ylideneamino)benzoyl]hydrazinecarbothioamide(14)

A mixture of **3**(10 mmol), ammonium thiocyanate (30 mmol) and hydrochloric acid (5 mL) in ethanol (25 mL) was refluxed for 16h. The solvent was evaporated and the residue was added to crushed ice with stirring. The resulted precipitate was filtered, dried and recrystallized from ethanol to give **14**. Shiny grey (52%), mp 155-157 °C; IR (KBr,  $cm^{-1}$ ):  $\nu$  3422, 3203 (OH, NH,  $NH_2$ ), 1717 (C=O), 1609 (C=N), 1278 (C=S).EI-MS:  $m/z$  339 [ $M^+$ ].Anal.calcd. for  $C_{16}H_{13}N_5O_2S$ ; C, 56.63; H, 3.86; N, 20.64. Found: C, 56.66; H, 3.84; N, 20.67.

#### 3-[4-(4,5-Diamino-4H-1,2,4-triazol-3-yl)phenylimino]indolin-2-one (15)

A mixture of thiosemicarbazide derivative **14** (10 mmol) and hydrazine hydrate (5 mL) was refluxed for 2h. The mixture was cooled, poured onto crushed ice, and the formed solid was filtered, washed with water, dried and recrystallized from ethanol to give **15**. Brown (72%), mp 225-227 °C; IR (KBr,  $cm^{-1}$ ):  $\nu$  3358, 3264 (OH, NH,  $NH_2$ ), 1720 (C=O), 1613 (C=N). $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  (ppm) 5.55 (s, 2H,  $NH_2$ , exchangeable), 6.10 (s, 2H,  $NH_2$ , exchangeable), 6.91-8.05 (m, 8H, Ar-H) 11.74 (s, 1H, NH, exchangeable). Anal. calcd. for  $C_{16}H_{13}N_7O$ ; C, 60.18; H, 4.10; N, 30.70. Found: C, 60.15; H,4.09; N, 30.75.

**3-[4-(5-Amino-1,3,4-thiadiazol-2-yl)phenylimino]indolin-2-one (16)**

**Method A:** A mixture of isatin Schiff's base **1** (10 mmol) and thiosemicarbazide (10 mmol) in phosphorus oxychloride (10 mL) was refluxed on water bath for 7h. After cooling, the reaction mixture was poured onto crushed ice dropwisely. The formed precipitate was filtered, dried and recrystallized from ethanol to afford **16**.

**Method B:** A mixture of thiosemicarbazide derivative **14** (10 mmol) and concentrated sulphuric acid (6 mL) was stirred at room temperature for 1h then heated on water bath at 90°C for 2h. The reaction mixture was poured onto crushed ice, neutralized with concentrated ammonium hydroxide. The precipitate was filtered, washed with water, dried and recrystallized from ethanol to afford **16**. Brownish-black (82%) mp 171-173 °C; IR (KBr, cm<sup>-1</sup>):  $\nu$  3379, 3210 (OH, NH, NH<sub>2</sub>), 1708 (C=O), 1600 (C=N).EI-MS:  $m/z$  321 [M<sup>+</sup>]. Anal. calcd. for C<sub>16</sub>H<sub>11</sub>N<sub>5</sub>O<sub>3</sub>; C, 59.80; H, 3.45; N, 21.79. Found: C, 59.84; H, 3.42; N, 21.82.

**General procedure for synthesis of compounds(17a,b)**

A suspension of **3** (10 mmol) in absolute ethanol (15 mL) was added to a well-stirred solution of the respective monosaccharide namely; glucose or galactose (10 mmol) in water (2 mL) and glacial acetic acid (0.5 mL). The mixture was heated under reflux for 5-7h, concentrated and left to cool. The formed precipitate was filtered, washed with water, dried and recrystallized from ethanol to give **17a,b**.

**4-(2-Oxoindolin-3-ylideneamino)-N'-[(2S,3R,4R,5R)-2,3,4,5,6-pentahydroxyhexylidene]- benzohydrazide (17a)**

Brown (77%) mp 160-162°C; IR (KBr, cm<sup>-1</sup>):  $\nu$ 3412, 3227 (OH, NH),3060 (CH, aromatic), 2979 (CH, aliphatic), 1717 (C=O), 1606 (C=N).EI-MS:  $m/z$  442 [M<sup>+</sup>]. Anal. calcd. for C<sub>21</sub>H<sub>22</sub>N<sub>4</sub>O<sub>7</sub>; C, 57.01; H, 5.01; N, 12.66. Found: C, 56.98; H, 4.99; N, 12.70.

**4-(2-Oxoindolin-3-ylideneamino)-N'-[(2S,3R,4S,5R)-2,3,4,5,6-pentahydroxyhexylidene]- benzohydrazide (17b)**

Brown (81%) mp 165-167°C; IR (KBr, cm<sup>-1</sup>):  $\nu$ 3427 (OH, NH), 3085 (CH, aromatic), 2976, 2929 (CH, aliphatic), 1717 (C=O), 1608 (C=N).Anal.calcd. for C<sub>21</sub>H<sub>22</sub>N<sub>4</sub>O<sub>7</sub>; C, 57.01; H, 5.01; N, 12.66. Found: C, 57.00; H, 5.03; N, 12.68.

**N'-Formyl-4-(2-oxoindolin-3-ylideneamino)benzohydrazide(18)**

A solution of **3** (10 mmol) in formic acid (15 mL) was refluxed for 8h. The deposited solid, after cooling, was filtered, washed several times with water, dried and recrystallized from aqueous ethanol to give **18**. Brown (76%) mp 234-236°C; IR (KBr, cm<sup>-1</sup>):  $\nu$ 3398, 3276 (OH, NH), 1714 (C=O), 1604 (C=N).EI-MS:  $m/z$  308 [M<sup>+</sup>]. Anal. calcd. for C<sub>16</sub>H<sub>12</sub>N<sub>4</sub>O<sub>3</sub>; C, 62.33; H, 3.92; N, 18.17. Found: C, 62.35; H,3.89; N, 18.14.

**3-[4-(1,3,4-Oxadiazol-2-yl)phenylimino]-1-acetylindolin-2-one(19)**

A mixture of **18** (10 mmol) and freshly distilled acetic anhydride (10 mL) was refluxed for 6h, concentrated, and the excess solvent was evaporated under reduced pressure. The oil residue was solidified in petroleum ether 40-60 (10 mL). The formed precipitate was separated by filtration, dried and recrystallized from acetic acid to give **19**. Dark brown (67%) mp 181-183°C; IR (KBr, cm<sup>-1</sup>):  $\nu$  1714 (C=O), 1599 (C=N), 1174 (C-O).EI-MS:  $m/z$  332 [M<sup>+</sup>]. Anal. calcd. for C<sub>18</sub>H<sub>12</sub>N<sub>4</sub>O<sub>3</sub>; C, 65.06; H, 3.64; N, 16.86. Found: C, 65.02; H,3.63; N, 16.89.

**3-[4-(1,3,4-Thiadiazol-2-yl)phenylimino]indolin-2-one(20)**

A mixture of **18** (10 mmol) and phosphorus pentasulphide (10 mmol) in pyridine (10 mL) was heated under reflux for 11h. After cooling, the reaction mixture was poured onto ice-HCl and stirred for 10 min. The formed precipitate was collected by filtration, washed several times with acidified water, dried and recrystallized from benzene to give **20**. Light brown (84%) mp227-229°C; IR (KBr, cm<sup>-1</sup>):  $\nu$  3422, 3225 (OH/NH), 3060 (CH, aromatic), 1706 (C=O), 1602 (C=N). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>):  $\delta$  (ppm) 6.60-8.10 (m, 9H, Ar-H andCH of thiadiazole), 11.30 (s, 1H, NH, exchangeable). Anal.calcd. forC<sub>16</sub>H<sub>10</sub>N<sub>4</sub>O<sub>3</sub>; C, 62.73; H, 3.29; N, 18.29. Found: C, 62.68; H, 3.33; N, 18.23.

**N'-(2-Chloroacetyl)-4-(2-oxoindolin-3-ylideneamino)benzohydrazide(21)**

To a well-stirred solution of **3** (10 mmol) in DMF (15 mL), chloroacetyl chloride (10 mmol) was added through 10 min. Stirring was continued for further 2h, then the reaction mixture was refrigerated overnight. The resulted solid was filtered, dried and recrystallized from (DMF/water) mixture to give **21**. Brownish-violet (72%) mp 296-298°C; IR (KBr, cm<sup>-1</sup>):  $\nu$  3442, 3277 (OH, NH). 1722 (C=O), 1614 (C=N), 753 (C-Cl). EI-MS:  $m/z$  356/358 [M<sup>+</sup>/ M<sup>+</sup> + 2]. Anal. calcd. for C<sub>17</sub>H<sub>13</sub>ClN<sub>4</sub>O<sub>3</sub>; C, 57.23; H, 3.67; N, 15.70. Found: C, 57.22; H, 3.63; N, 15.76.

**3-[4-[5-(Chloromethyl)-1,3,4-thiadiazol-2-yl]phenylimino]indolin-2-one(22)**

A mixture of**21**(10 mmol) and phosphorus pentasulphide (10 mmol) in pyridine (10 mL) was refluxed for 9h. After cooling, the reaction mixture was poured onto ice-HCl. The formed precipitate was filtered, washed several times with acidified water, dried and recrystallized from ethanol to give **22**. Yellowish-brown (56%) mp271-273°C; IR

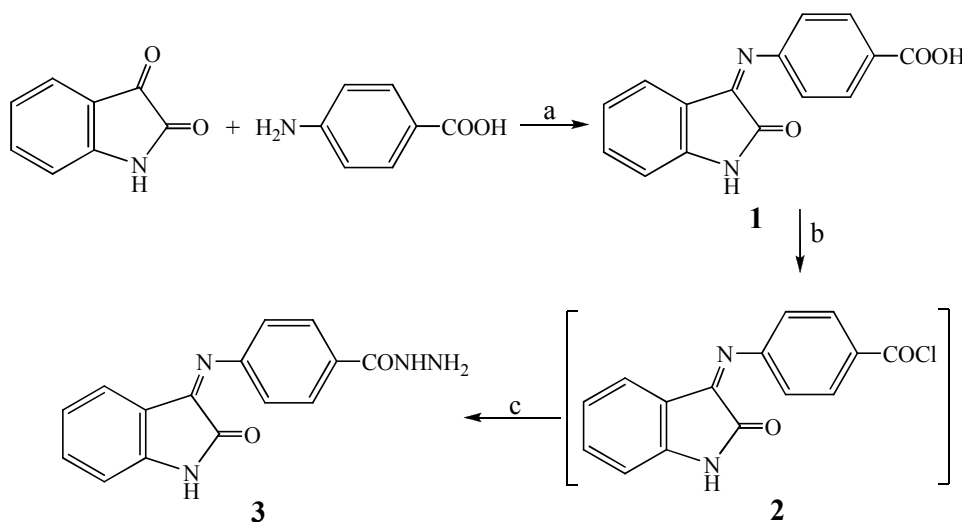
(KBr,  $\text{cm}^{-1}$ ):  $\nu$  3418, 3232 (OH/NH), 3062 (CH, aromatic), 2975 (CH, aliphatic), 1716 (C=O), 1614 (C=N).  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  (ppm) 4.24 (s, 2H,  $\text{CH}_2$ ), 6.90-8.24 (m, 8H, Ar-H), 10.96 (s, 1H, NH, exchangeable). Anal. calcd. for  $\text{C}_{17}\text{H}_{11}\text{ClN}_4\text{OS}$ ; C, 57.55; H, 3.12; N, 15.79. Found: C, 57.57; H, 3.10; N, 15.81.

### 3-{4-[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]phenylimino}indolin-2-one (23)

To a solution of **3** (10 mmol) and 4-chlorobenzaldehyde (10 mmol) in dry dichloromethane (25 mL) was added ceric ammonium nitrate (10 mmol). The reaction mixture was heated under reflux and stirring for 14h. Water (15 mL) was added and the mixture was extracted with chloroform (3 x 15 mL). The organic layer was separated and dried. The solvent was evaporated and the crude product was recrystallized from dichloromethane to give **23**. Brown (83%) mp 142-144°C; IR (KBr,  $\text{cm}^{-1}$ ):  $\nu$  3398, 3247 (OH/NH), 3062 (CH, aromatic), 1723 (C=O), 1608 (C=N). EI-MS:  $m/z$  400 [ $\text{M}^+$ ]. Anal. calcd. for  $\text{C}_{22}\text{H}_{13}\text{ClN}_4\text{O}_2$ ; C, 65.92; H, 3.27; N, 13.98. Found: C, 65.88; H, 3.23; N, 14.02.

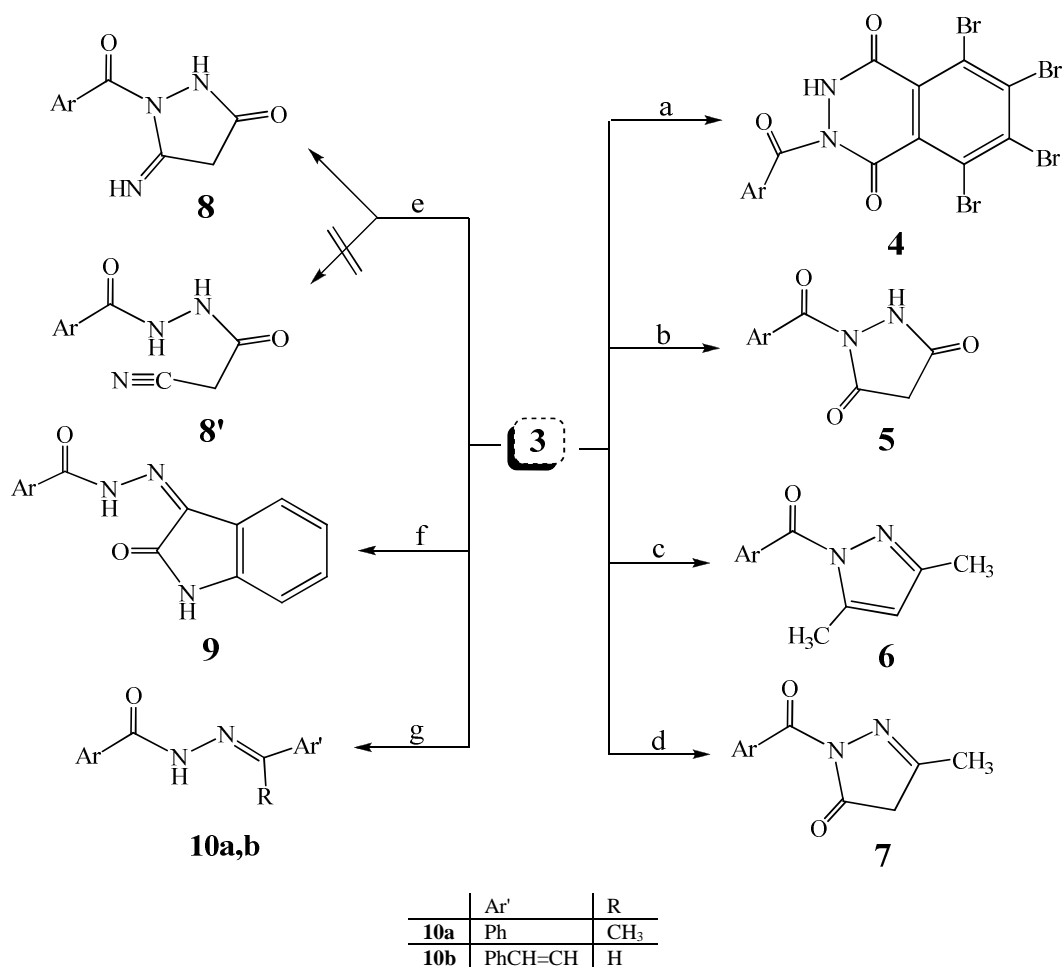
## RESULTS AND DISCUSSION

As a part of our ongoing studies in developing novel antimicrobials, we report the synthesis of new class of isatin derivatives. Thus, 4-(2-oxoindolin-3-ylideneamino)benzoic acid (**1**) was prepared by Condensation of isatin with 4-aminobenzoic acid. Reaction of compound **1** with thionyl chloride gave 4-(2-oxoindolin-3-ylideneamino)benzoyl chloride (**2**) which was reacted with hydrazine hydrate in absolute ethanol and gave the acid hydrazide **3**, (Scheme 1).



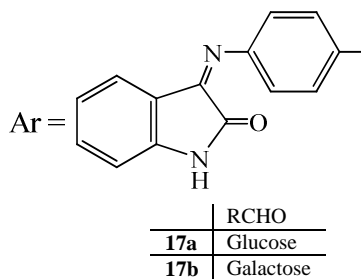
Reagents and conditions: a) EtOH, AcOH; b)  $\text{SOCl}_2$ ; c)  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ , EtOH

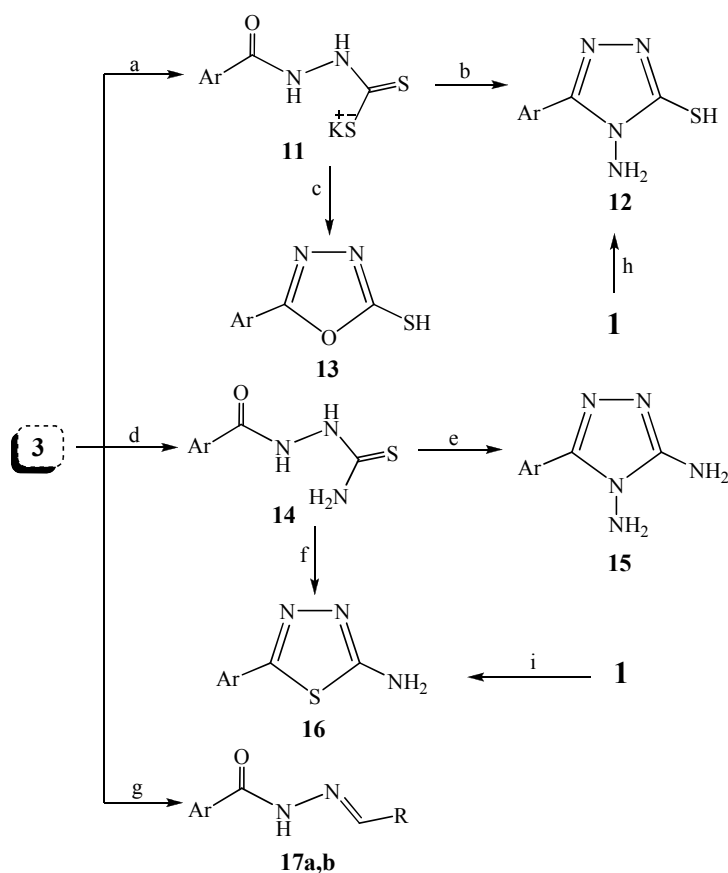
Scheme 1

**Scheme 2**

Reagents and conditions: a) Tetrabromophthalic anhydride, dioxane; b) Diethyl malonate, EtOH; c) Acetylacetone, EtOH, Et<sub>3</sub>N; d) Ethyl acetoacetate, EtOH; e) Ethyl cyanoacetate, EtOH; f) Isatin, EtOH, AcOH; g) Acetophenone/ cinnamaldehyde, EtOH, AcOH

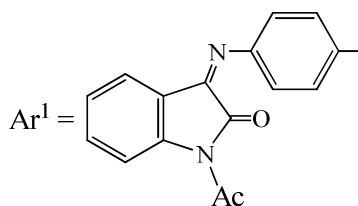
Hydrazides are useful intermediate for synthesis of various heterocyclic compounds [19-22]. The reactivity of **3** towards some electrophilic reagents was studied with the aim of preparation of biologically active heterocycles. Thus, the reaction of the hydrazide **3** with tetrabromophthalic anhydride in dioxane gave 5,6,7,8-tetrabromo-2-[4-(2-oxoindolin-3-ylidene-amino)benzoyl]-2,3-dihydrophthalazine-1,4-dione **4**. While the reaction of **3** with active methylene group containing-compounds namely; diethyl malonate, acetylacetone, ethyl acetoacetate and/or ethyl cyanoacetate afforded pyrazole derivatives **5**, **6**, **7** and **8**. It was deduced that the reaction between **3** and ethyl cyanoacetate gave the cyclized product **8** not the open one **8'** as the FT-IR spectrum didn't show any absorption peak for the cyano group (C≡N). Also, condensation of **3** with isatin, acetophenone and/or cinnamaldehyde yielded the hydrazone derivatives **9** and **10a,b**, respectively, (Scheme 2).

**Scheme 3**



Reagents and conditions: a)  $\text{CS}_2$ ,  $\text{KOH}$ ,  $\text{EtOH}$ ; b)  $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$ ,  $\text{AcOH}$ ; c)  $\text{EtOH}$ ,  $\text{HCl}$ ; d)  $\text{NH}_4\text{SCN}$ ,  $\text{EtOH}$ ,  $\text{HCl}$ ; e)  $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$ ; f)  $\text{H}_2\text{SO}_4$ ; g) Glucose/galactose,  $\text{EtOH}$ ,  $\text{AcOH}$ ; h) Thiocarbohydrazide, fusion; i) Thiosemicarbazide,  $\text{POCl}_3$

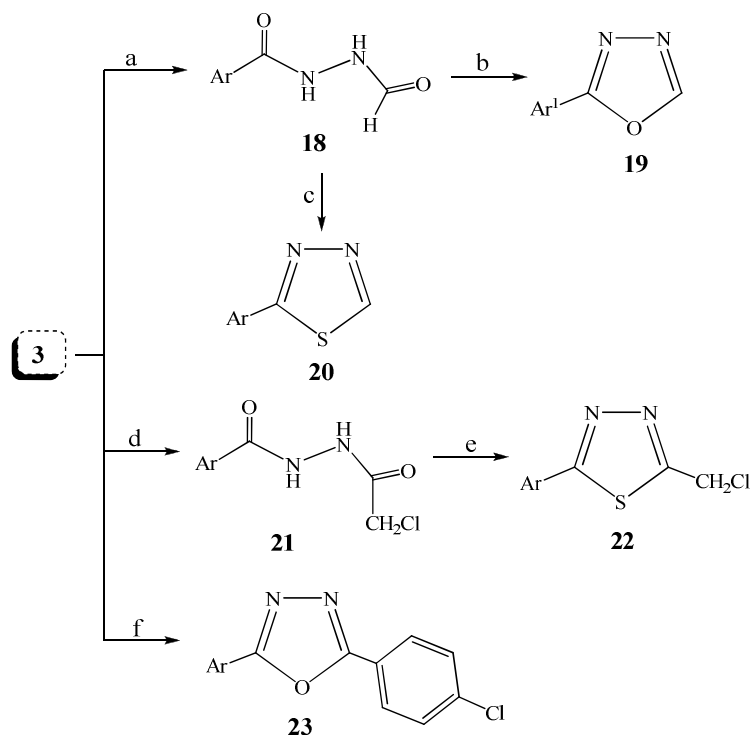
Moreover, addition of carbon disulphide and potassium hydroxide to a well-stirred ethanolic solution of hydrazide **3** afforded the carbodithioate derivative **11**. Compound **11** was cyclized to the 1,2,4-triazole derivative **12** through the reaction with hydrazine hydrate. Compound **12** was synthesized by an alternative method *via* the fusion of the acid **1** with thiocarbohydrazide. Also, carbodithioate derivative **11** was cyclized to 1,3,4-oxadiazole derivative **13** in acidic solution. Thiosemicarbazide derivatives are considered to be important compounds for the construction of a variety of heterocycles [23-25]. Reaction of ammonium thiocyanate with compound **3** yielded thiosemicarbazide derivative **14** which was cyclized to 1,2,4-triazole **15** and 1,3,4-thiadiazole derivatives **16**, respectively. Compound **16** was also synthesized through the reaction between the isatin Schiff's base **1** and thiosemicarbazide in  $\text{POCl}_3$ . Glycosides **17a,b** was synthesized by condensation of **3** with glucose and/or galactose, (Scheme 3).



Scheme 4

Reagents and conditions: a)  $\text{HCOOH}$ ; b)  $\text{Ac}_2\text{O}$ ; c)  $\text{P}_2\text{S}_5$ , pyridine; d) Chloroacetyl chloride,  $\text{DMF}$ ; e)  $\text{P}_2\text{S}_5$ , pyridine; f) 4-chlorobenzaldehyde,  $\text{CH}_2\text{Cl}_2$ ,  $\text{CAN}$

Furthermore, *N*-formyl derivative **18** was obtained from the reaction of formic acid with hydrazide **3**. Compound **18** was cyclized to the 1,3,4-oxadiazole derivative **19** in acetic anhydride and to the 1,3,4-thiadiazole derivative **20** through the reaction with phosphorus pentasulphide in pyridine. Also, reaction of **3** with chloroacetyl chloride in  $\text{DMF}$  gave *N*-chloroacetyl derivative **21** which was cyclized with phosphorus pentasulphide to 1,3,4-thiadiazole derivative **22**. Finally, hydrazide **3** was converted to the 1,3,4-oxadiazole derivative **23** *via* its reaction with 4-chlorobenzaldehyde using ceric ammonium nitrate ( $\text{CAN}$ ) as catalyst, (Scheme 4).



### Antimicrobial activity

The antimicrobial activity (in *vitro*) of some synthesized compounds was determined against some bacteria and fungi using Tetracycline and Amphotericin B as standard antimicrobial agents by using the agar diffusion method [26]. The obtained zones of inhibition were presented in table 1, which indicated that most of the synthesized derivatives have moderate-to-good antibacterial activities and poor antifungal activities.

**Table 1: Antimicrobial activity in *vitro* of some synthesized compounds**

Compd. No.	Zone of inhibition mm/mg of sample			
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Aspergillus flavus</i>	<i>Candida albicans</i>
<b>3</b>	11	12	--	9
<b>4</b>	13	15	--	--
<b>5</b>	11	12	9	--
<b>6</b>	11	11	--	--
<b>7</b>	12	13	--	--
<b>8</b>	10	11	--	--
<b>9</b>	16	18	--	9
<b>12</b>	17	17	9	10
<b>13</b>	13	14	--	--
<b>16</b>	10	13	9	11
<b>17a</b>	15	20	--	--
<b>19</b>	12	12	--	--
Tetracycline	31	28	--	--
Amphotericin B	--	--	16	20

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