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# Synthesis of new quinoxaline, pyrimidine, and pyrazole furochromone derivatives as cytotoxic agents

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**Abstract** A series of novel quinoxaline, pyrimidine, and pyrazole furochromone derivatives were synthesized for the first time. These derivatives were prepared under mild conditions using a stepwise efficient methodology. The developed protocol led to the synthesis of furochromone derivatives in moderate to good yields (60–75%). The structures of the prepared derivatives were identified using several spectroscopic techniques including IR, NMR, and mass spectrometry. The cytotoxic activity of the synthesized derivatives was evaluated using in vitro Ehrlich ascites assay. Pyrazolobenzofurans exhibited the most potent effect suggesting the importance of pyrazole nucleus for the cytotoxic activity.

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Graphical abstract



**Keywords** Furochromones · Visnagenone · Khellinone · Quinoxalines · Pyrimidines · Pyrazolobenzofurans · Cytotoxic activity

#### Introduction

Furochromones such as khellin and visnagin represents one of the oldest classes of secondary metabolites isolated from a well-known medicinal plant, Ammi visnaga L. (Apiaceae) (Fig. 1). Their structures were elucidated during the first half of the last century [1, 2]. The medicinal uses of A. visnaga were documented in ancient scrolls and papyri [3]. It was prescribed for the treatment of urinary infection and renal colics. Following the isolation of A. visnaga active constituents, furochromones, the therapeutic applications of the herb and its components entered a new era. Furochromones were not only isolated from A. visnaga but also from other plants [4]. The activities of furochromones against different ailments were evaluated. It was found that these components possess potent phototoxic properties which led to their application in the treatment of vitiligo [5] and certain microbial infections [6]. Also furochromones



Fig. 1 Chemical structures of visnagin, khellin and visnagenone, khellinone

were found to exhibit potent anti-atherosclerotic and lipidaltering activity [7]. The interesting biological activities of this class of compounds promoted the synthesis of different derivatives which showed a plethora of important biological activities. Furochromones with pyrimidine moiety exhibited anti-inflammatory and analgesic activities [8]. Visnaginone derivatives were prepared using different protocols and demonstrated potent antibacterial activity [9-11]. Benzofuran derivatives showed potent vasodilatatory, hypotensive, antibacterial, antifungal, and antiparasitic activities [11–14]. Pyrazoline derivatives were synthesized and exhibited bacteriostatic, fungicidal and anticancer activity [15–17].

The synthesis of molecules with highly strained rings represents one of the most challenging tasks in synthetic organic chemistry. Certain rings such as the six and five membered rings are kinetically favorable and can be prepared easily in high yields.

In a continuation of our work on the synthesis of new heterocyclic compounds derived from the naturally occurring furochromones (visnagin and khellin) [2, 8, 18, 19], we prepared and characterized a series of derivatives containing a benzodifuran nucleus (visnaginone, khellinone) combined with quinoxaline, pyrimidine, or pyrazole moiety. The cytotoxic activity of the prepared compounds was evaluated.

#### **Results and discussion**

#### Chemistry

In the current research, visnagin (1a) or khellin (1b) was hydrolyzed with aqueous KOH yielding visnagenone (2a) or khellinone (2b) [18]. Treatment of 2a, 2b with absolute methanol containing 2–3 drops of concentrated H<sub>2</sub>SO<sub>4</sub> or refluxing of 2a, 2b in ethanolic potassium hydroxide solution with methyl iodide provided 1-(4,6-di- or 4,6,7trimethoxybenzofuran-5-yl)ethan-1-one (3a, 3b), respectively. The <sup>1</sup>H NMR spectrum of compound 3b showed three singlet signal at  $\delta = 3.84$ , 3.85, 3.91 ppm indicative of three methoxy groups and the mass spectra of **3a** and **3b** showed molecular ion peaks at m/z = 220 (M<sup>+</sup>, 12%) and 250 (M<sup>+</sup>, 15%), respectively. Similar derivatives were prepared by Hafez et al. using acetone as the solvent which led to lower yields [20].

Bromination [21] of **3a** or **3b** in acetic acid furnished the corresponding 2-bromo-1-(7-bromo-4,6-dimethoxy- or 4,6,7-trimethoxybenzofuran-5-yl)ethan-1-one (**4a**, **4b**). <sup>1</sup>H NMR spectrum of **4a** or **4b** showed one singlet signal at 4.65 or 4.62 ppm indicative of two methylene (CH<sub>2</sub>) groups and the molecular ion peaks at m/z = 378 (M<sup>+</sup>, 100%) and 329 (M<sup>+</sup>, 86%), respectively.

Moreover, the treatment of **4a** or **4b** with *o*-phenylenediamine [22] in boiling absolute methanol resulted in the formation of 2-(7-bromo-4,6-dimethoxy- or 4,6,7trimethoxybenzofuran-5-yl)-1,4-dihydroquinoxaline (**5a**, **5b**). IR spectra of compounds **5a** and **5b** revealed the presence of a broad band absorption at 3420–3400 cm<sup>-1</sup> indicative of two (NH) groups. Additionally, the <sup>1</sup>H NMR spectra of **5a** showed a singlet broad signal at 8.30 and 9.20 ppm corresponding to the two protons of the two (NH) groups, which were D<sub>2</sub>O exchangeable. Closely related derivatives were reported by Mohamed et al. with similar yields [23].

Alkylation of **5a** or **5b** with methyl iodide yielded 2-(7bromo-4,6-dimethoxy- or 4,6,7-trimethoxybenzofuran-5yl)-1,4-dimethyl-1,4-dihydroquinoxaline (**6a**, **6b**). The mass spectra of **5a**, **5b**, **6a**, and **6b** showed molecular ion peaks at m/z = 387 (M<sup>+</sup>, 90%), 338 (M<sup>+</sup>, 92%), 415 (M<sup>+</sup>, 88%), and 366 (M<sup>+</sup>, 82%), respectively (Scheme 1).

Condensation of visnagenone (2a) or khellinone (2b) with thiourea in acetic acid afforded 1-[1-(6-hydroxy-4-methoxy- or 4,7-dimethoxybenzofuran-5-yl)ethylidene]thiourea (7a, 7b). IR spectra of 7a and 7b showed absorption of a broad band at 3400–3200 cm<sup>-1</sup> corresponding to NH<sub>2</sub> and OH groups. Also, peaks at 1618 cm<sup>-1</sup> for (C=N) and 1355 cm<sup>-1</sup> for (CS) were detected in the IR spectrum. <sup>1</sup>H NMR spectrum of 7a showed one singlet at 6.68 ppm corresponding to the two protons of NH<sub>2</sub> group and one a broad singlet at 12.46 ppm for OH proton (D<sub>2</sub>O exchangeable).

Similar derivatives were reported by Ragab et al. but the products were formed after 72 h in lower yields [24]. Mohamed et al. also synthesized similar compounds through condensation of thiourea with ethoxycoumarin derivatives forming the corresponding thiomaide derivatives in good yields using DMF as the refluxing solvent [23].

Refluxing of **7a** and **7b** in dimethylformamide yielded 5-methoxy- or 5,9-dimethoxy-4-methylfuro[2,3-b]benzo[5',6'-e]pyrimidine-2(1*H*)-thione (**8a**, **8b**). The reaction of **7a** or **7b** with secondary aliphatic amines [25], namely piperazine in glacial acetic acid yielded 1-[1-[4-methoxy- or 4,7-dimethoxy-



6-(piperazin-1-yl]benzofuran-5-yl]ethylidene]thiourea (9a, 9b). Furthermore, 9a or 9b reacted with malononitrile in boiling ethanol solution and triethylamine producing 2-[amino-[[1-[4-methoxy- or 4,7-dimethoxy-6-(piperazin-1-yl)benzofuran-5-yl]ethylidene]amino]methylene]malononitrile (10a, 10b). The IR spectrum of 10a and 10b revealed the presence of a wide band at 3355–3220 cm<sup>-1</sup> for NH and NH<sub>2</sub> groups as well as two bands (2210 and 2205 cm<sup>-1</sup>) corresponding to two cyano groups. Mass spectra of 7a, 7b, 8a, 8b, 9a, 9b, 10a, and 10b displayed molecular ion peaks at m/z = 264 (M<sup>+</sup>, 100%), 294 (M<sup>+</sup>, 100%), 246 (M<sup>+</sup>, 100%), 276 (M<sup>+</sup>, 42%), 332 (M<sup>+</sup>, 73%), 362 (M<sup>+</sup>, 95%), 364 (M<sup>+</sup>, 82%), 394 (M<sup>+</sup>, 12%), respectively (Scheme 2).

Compounds with pyrazole moiety showed potent biological activity and were successfully synthesized using different protocols [26]. o-Halogenated alkanoylphenones, benzophenones, and arylcarboxylic acids were successfully converted to pyrazole derivatives via copper-catalyzed amination [27]. Metal free protocols were also developed converting *o*-aminobenzoximes to pyrazole derivatives using methanesulfonyl chloride and triethylamine [28], or triphenylphosphine, I<sub>2</sub>, and imidazole [26]. To prepare pyrazole derivatives of furochromones, 2a or 2b was treated with phenyl hydrazine in the presence of absolute ethanol (EtOH) providing 4-methoxy-3-methyl-1-phenyl-1H-furo[3,2-f]indazole (11a), or 4,8-dimethoxy-3-methyl-1-phenyl-1*H*-furo[3,2-*f*]indazole (**11b**), respectively, as a yellowish white powder. Moreover, the reaction of 2a or 2b with hydrazine hydrate afforded 4-methoxy-3-methyl-1H-furo[3,2-f]indazole (12a) or 4,8-dimethoxy-3-methyl-1*H*-furo[3,2-f]indazole (12b), respectively, as a yellowish brown powder. The reaction of 12a or 12b with sodium ethoxide (NaOEt) yielded the corresponding sodium salt, which upon treatment with acetic anhydride (Ac<sub>2</sub>O) provided the acetylated derivatives (**13a** or **13b**) and the treatment with methyl iodide (MeI) yielded the methylated derivatives (**14a** or **14b**) (Scheme 3). In all of the prepared pyrazole derivatives the characteristic <sup>13</sup>C imine signal (130.1–145.9 ppm) was consistent with the reported data for similar derivatives [27, 29]. Assignment of the new synthesized compounds was based on elemental analyses, IR, <sup>1</sup>H, <sup>13</sup>C NMR, and mass spectral data (c.f. Exp. Part, Scheme 3).

#### Evaluation of cytotoxic activity using in vitro Ehrlich ascites assay

The newly synthesized compounds were screened for their cytotoxic activity using in vitro Ehrlich ascites assay (Table 1). 5-Fluorouracil was used as the positive control causing 99.5% mortality. The viability of the cells used in the control experiments exceeded 95%. The use of compounds **13b** and **13a** resulted in 94.44 and 92.90% mortality, respectively. Other compounds also resulted in high percentage of mortality including **11b**, **11a**, **14b**, **14a**, **12b**, and **12a** (76.90–90.84%). Mediocre percentages of mortality were observed after the treatment with **8a**, **8b**, **10a**, **10b**, **9a**, **9b**, **6a**, **6b**, **5a**, and **5b** (52.25–74.50%). Other compounds showed weaker activity.

#### Structural activity relationship (SAR)

The cytotoxic results indicated that the pyrazole moiety is important for the activity. Compounds **13b** and **13a** with



Yield 14a (78%); 14b (76%)

Table 1 Cytotoxic evaluation   of the prepared compound using	Compounds no. <sup>a</sup>	Mortality/%	SEM
Ehrlich in vitro assay	Control (no drugs)	0	_
	5-Fluorouracil <sup>b</sup>	99.50	$\pm 0.005$
	1a	20.75	$\pm 0.048$
	1b	22.64	$\pm 0.044$
	2a	24.92	±0.041
	2b	27.80	$\pm 0.039$
	3a	35.45	$\pm 0.024$
	3b	38.50	$\pm 0.027$
	4a	42.40	$\pm 0.022$
	4b	44.20	$\pm 0.042$
	5a	52.25	$\pm 0.052$
	5b	55.60	$\pm 0.045$
	6a	58.15	$\pm 0.032$
	6b	60.55	$\pm 0.026$
	7a	48.24	$\pm 0.035$
	7b	50.10	$\pm 0.020$
	8a	70.15	$\pm 0.024$
	8b	74.50	$\pm 0.022$
	9a	62.33	$\pm 0.040$
	9b	64.40	$\pm 0.028$
	10a	66.35	$\pm 0.030$
	10b	69.10	$\pm 0.020$
	11a	88.52	$\pm 0.014$
	11b	90.84	$\pm 0.013$
	12a	76.90	$\pm 0.018$
	12b	80.30	$\pm 0.017$
	13a	92.90	$\pm 0.009$
	13b	94.44	$\pm 0.007$
	14a	82.10	$\pm 0.016$
	14b	85.45	$\pm 0.015$

<sup>a</sup> 1 mg cm<sup>-3</sup> in DMSO/RPMI-1640 (1:10)

 $^{\rm b}$  25 mg cm  $^{-3}$  in DMSO/RPMI-1640 (1:10). All the values are mean  $\pm$  SEM of three samples

this nucleus showed the highest activity. Also, compounds 14a and 14b exhibited potent activity albeit lower than the acetylated derivatives (13b and 13a) highlighting the importance of the acetyl group for the cytotoxic activity. The importance of the pyrazole moiety was also supported by the high activity demonstrated by 11a and 11b. One the other hand, pyrazole derivatives without substitution on the nitrogen atom showed lower activity (12a and 12b). Compounds carrying pyrazole moiety showed potent toxicity in previous studies [30]. Furobenzopyrimidinethione (8a, 8b); piperazinbenzofuran, malononitrile thiourea (10a, 10b and 9a, 9b) and benzofuranquinoxaline (6a, 6b and 5a, derivatives resulted mediocre 5b) in mortality (52.25–74.50%). The natural furochromone derivatives (1a and 1b) as well as their hydrolyzed products (2a and 2b) exhibited weak cytotoxic activity suggesting the future potential of the synthesized derivatives as cytotoxic agents.

### Conclusion

Furochromones (visnagin and khellin) were converted into polyfunctionalized heterocycles with promising cytotoxic activity. Different derivatives were prepared including benzofuranquinoxalines, furobenzopyrimidinethione, piperazinbenzofurans, and pyrazolo[4,5-f]benzofuran derivatives. Compounds with pyrazole moiety and substituted nitrogen atom showed potent cytotoxic activity. The simplicity of the synthetic procedures and the potent activity of the prepared compounds render these derivatives interesting targets for future development as cytotoxic agents.

# Experimental

All melting points were taken on an Electrothermal IA 9100 series digital melting point apparatus (Shimadzu, Japan). Elemental analyses were performed on Vario EL (Elementar, Germany). Microanalytical data were processed in the microanalytical center, Faculty of Science, Cairo University and National Research Center. The IR spectra (KBr disc) were recorded using a Perkin-Elmer 1650 spectrometer (USA). NMR spectra were determined using JEOL 270 MHz and JEOL JMS-AX 500 MHz (JEOL, Japan) spectrometers with Me<sub>4</sub>Si as an internal standard. Mass spectra were recorded on an EI Ms-QP 1000 EX instrument (Shimadzu, Japan) at 70 eV. Pharmacological evaluation was done by the Pharmacology Unit, Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Egypt. All starting materials and solvents were purchased from Sigma Aldrich (Saint Lewis, USA).

# *1-(4,6-Di- or 4,6,7-trimethoxybenzofuran-5-yl)ethan-1-one* (*3a, 3b*)

Method A: compounds **1a** or **1b** were prepared according to the reported procedures [31]. Previous studies reported the synthesis of quinoxaline derivatives which were followed with slight modification [31]. A mixture of 2.06 g visnaginone (**2a**, 0.01 mol) or 2.36 g khellinone (**2b**, 0.01 mol) in 30 cm<sup>3</sup> absolute methanol containing a few drops of concentrated sulfuric acid was refluxed for 3 h. The solid formed was filtered off, dried, and crystallized from the proper solvent to give **3a** and **3b**, respectively.

Method B: to a warmed ethanolic potassium hydroxide solution (prepared by dissolving 0.56 g of potassium hydroxide (10 mmol) in 50 cm<sup>3</sup> ethanol) was added 2.06 g visnaginone (**2a**, 0.01 mol) or 2.36 g khellinone (**2b**, 0.01 mol) and heating was continued for 40 min. The mixture was allowed to cool to room temperature, and 2 cm<sup>3</sup> methyl iodide was added. The mixture was stirred under reflux for 6 h and then allowed to cool to room temperature and finally poured into 100 cm<sup>3</sup> cold water. The solid product precipitated was filtered off and washed with 100 cm<sup>3</sup> water and crystallized.

# $\begin{array}{l} 1\text{-}(4,6\text{-}Dimethoxybenzofuran\text{-}5\text{-}yl)ethan\text{-}1\text{-}one\\ \textbf{(3a, }C_{12}H_{12}O_{4})\end{array}$

The compound was obtained from the reaction of 2.06 g visnaginone (**2a**, 0.01 mol) and methanol/conc. H<sub>2</sub>SO<sub>4</sub> or methyl iodide, as a yellowish powder, crystallized from ethanol (85%). M.p.: 133–135 °C; IR (KBr):  $\bar{\nu} = 3040$ 

(CH-aryl), 2950 (CH-aliph), 1695 (CO- acetyl) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta = 2.39$  (s, 3H, CH<sub>3</sub>), 3.85 (s, 3H, OCH<sub>3</sub>), 3.95 (s, 3H, OCH<sub>3</sub>), 6.27 (s, 1H, H-7), 7.04 (d, 1H, J = 2.30 Hz, furan), 7.24 (d, 1H, J = 2.33 Hz, furan) ppm; <sup>13</sup>C NMR (DMSO- $d_6$ ):  $\delta = 33.1$ , 56.9, 61.8, 92.7, 106.5, 109.5, 115.9, 146.2, 155.9, 156.9, 163.1, 179.8 ppm; MS (70 eV): m/z = 221 ([M+1]<sup>+</sup>, 18%), 220 (M<sup>+</sup>, 12%).

# 1-(4,6,7-*Trimethoxybenzofuran*-5-yl)*ethan*-1-one (**3b**, C<sub>13</sub>H<sub>14</sub>O<sub>5</sub>)

The compound was obtained from the reaction of 2.36 g khellinone (**2b**, 0.01 mol) and methanol/conc. H<sub>2</sub>SO<sub>4</sub> or methyl iodide, as a pale yellow powder, crystallized from methanol (80%). M.p.: 125–127 °C; IR (KBr):  $\bar{v} = 3038$  (CH-aryl), 2948 (CH-aliph), 1692 (CO-acetyl) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta = 2.38$  (s, 3H, CH<sub>3</sub>), 3.84 (s, 3H, OCH<sub>3</sub>), 3.85 (s, 3H, OCH<sub>3</sub>), 3.91 (s, 3H, OCH<sub>3</sub>), 7.06 (d, 1H, J = 2.32 Hz, furan), 7.25 (d, 1H, J = 2.34 Hz, furan) ppm; <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta = 33.5$ , 56.5, 61.2, 61.7, 106.8, 110.3, 118.2, 135.1, 144.3, 146.4, 149.4, 150.5, 178.9 ppm; MS (70 eV): m/z = 250 (M<sup>+</sup>, 15%).

### 2-Bromo-1-(7-bromo-4,6-dimethoxy- or 4,6,7-trimethoxybenzofuran-5-yl)ethan-1-one (4a, 4b)

A solution of 2.20 g **3a** (10 mmol) or 2.50 g **3b** (10 mmol) in 10 cm<sup>3</sup> acetic acid was stirred with 1 cm<sup>3</sup> (20 mmol) or 0.50 cm<sup>3</sup> (10 mmol) bromine for 2–4 h in direct sun-light. The solid formed was collected by filtration, washed with acetic acid, then ethanol, and dried. The product was recrystallized from the proper solvent to give **4a** and **4b**, respectively.

# $\begin{array}{l} 2\text{-}B\text{romo-1-(7-bromo-4,6-dimethoxybenzofuran-5-yl)ethan-1-one} & (\textbf{4a}, C_{12}H_{10}Br_2O_4) \end{array}$

The compound was obtained from the reaction of 2.20 g **3a** (10 mmol) and 1 cm<sup>3</sup> bromine (20 mmol), as a yellow powder, crystallized from dioxane (75%). M.p.: 266–268 °C; IR (KBr):  $\bar{\nu} = 3035$  (CH-aryl), 2946 (CH-aliph), 1700 (CO-acetyl) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta = 3.81$  (s, 3H, OCH<sub>3</sub>), 3.86 (s, 3H, OCH<sub>3</sub>), 4.65 (s, 2H, CH<sub>2</sub>), 7.09 (d, 1H, J = 2.30 Hz, furan), 7.22 (d, 1H, J = 2.31 Hz, furan) ppm; <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta = 32.4$ , 61.4, 61.9, 85.6, 105.7, 110.8, 111.5, 146.1, 155.8, 160.6, 169.3, 180.5 ppm; MS (70 eV): m/z = 378 (M<sup>+</sup>, 100%).

# 2-*Bromo-1-(4,6,7-trimethoxybenzofuran-5-yl)ethan-1-one* (**4b**, C<sub>13</sub>H<sub>13</sub>BrO<sub>5</sub>)

The compound was obtained from the reaction of 2.50 g **3b** (10 mmol) and 0.50 cm<sup>3</sup> bromine (10 mmol), as a yellowish powder, crystallized from benzene (70%). M.p.: 242– 244 °C; IR (KBr):  $\bar{\nu} = 3037$  (CH-aryl), 2945 (CH-aliph), 1705 (CO-acetyl) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta = 3.81$ (s, 3H, OCH<sub>3</sub>), 3.88 (s, 3H, OCH<sub>3</sub>), 3.91 (s, 3H, OCH<sub>3</sub>), 4.62 (s, 2H, CH<sub>2</sub>), 7.04 (d, 1H, J = 2.36 Hz, furan), 7.33 (d, 1H, J = 2.37 Hz, furan) ppm; <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta = 32.8, 61.5, 61.7, 97.2, 108.3, 108.6, 123.9, 135.3, 146.6, 152.7, 155.03, 168.1 ppm; MS (70 eV): <math>m/z = 329$  (M<sup>+</sup>, 86%).

### 2-(7-Bromo-4,6-dimethoxy- or 4,6,7-trimethoxybenzofuran-5-yl)-1,4-dihydroquinoxaline (**5a**, **5b**)

A solution of 3.78 g **4a** (10 mmol) or 3.29 g **4b** (10 mmol) and 1.08 g *o*-phenylenediamine (10 mmol) in 40 cm<sup>3</sup> absolute methanol was refluxed for 4–6 h. The solid obtained was filtered, washed with ethanol, and dried under vacuum. The crude product was recrystallized from the proper solvent to give **5a** or **5b**.

### 2-(7-Bromo-4,6-dimethoxybenzofuran-5-yl)-1,4-dihydroquinoxaline (**5a**, C<sub>18</sub>H<sub>15</sub>BrN<sub>2</sub>O<sub>3</sub>)

The compound was obtained from the reaction of 3.78 g **4a** (10 mmol) and 1.08 g *o*-phenylenediamine (10 mmol), as a brownish crystals, crystallized from toluene (72%). M.p.: >350 °C; IR (KBr):  $\bar{\nu} = 3415$  broad (2NH), 3055 (CH-aryl), 2960 (CH-aliph) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta = 3.86$  (s, 3H, OCH<sub>3</sub>), 3.88 (s, 3H, OCH<sub>3</sub>), 5.81 (d, 1H, CH, pyrazine), 7.03 (d, 1H, J = 2.35 Hz, furan), 7.16 (d, 1H, J = 2.34 Hz, furan), 7.55–7.76 (m, 4H, phenyl), 8.35 (br, NH, D<sub>2</sub>O exchangeable), 9.25 (br, NH, D<sub>2</sub>O exchangeable) ppm; <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta = 61.6$ , 62.4, 85.1, 99.3, 103.4, 106.2, 111.5, 117.3, 119.5, 119.8, 120.2, 122.5, 131.1, 131.4, 146.1, 150.6, 157.2, 160.1 ppm; MS (70 eV): m/z = 387 (M<sup>+</sup>, 90%).

### 2-(4,6,7-*Trimethoxybenzofuran*-5-yl)-1,4-dihydroquinoxaline (**5b**, C<sub>19</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>)

The compound was obtained from the reaction of 3.29 g **4b** (10 mmol) and 1.08 g *o*-phenylenediamine (10 mmol), as a brown crystals, crystallized from benzene (67%). M.p.: >350 °C; IR (KBr):  $\bar{\nu} = 3418$  broad (2NH), 3058 (CH-aryl), 2965 (CH-aliph) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta = 3.89$  (s, 3H, OCH<sub>3</sub>), 3.91 (s, 3H, OCH<sub>3</sub>), 3.95 (s, 3H, OCH<sub>3</sub>), 5.83 (d, 1H, CH, pyrazine), 7.01 (d, 1H, J = 2.31 Hz, furan), 7.12 (d, 1H, J = 2.32 Hz, furan), 7.63–7.80 (m, 4H, phenyl), 8.23 (br, NH, D<sub>2</sub>O exchangeable), 9.16 (br, NH, D<sub>2</sub>O exchangeable) ppm; <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta = 61.8$ , 61.9, 97.3, 100.5, 115.5, 115.6, 115.8, 116.7, 123.9, 126.4, 129.9, 131.9, 132.5, 132.9, 135.3, 141.9, 148.8, 155.1 ppm; MS (70 eV): m/z = 338 (M<sup>+</sup>, 92%).

### 2-(7-Bromo-4,6-dimethoxy- or 4,6,7-trimethoxybenzofuran-5-yl)-1,4-dimethyl-1,4-dihydroquinoxaline (**6a**, **6b**)

To a warmed ethanolic potassium hydroxide solution (prepared by dissolving 0.56 g of potassium hydroxide (10 mmol) in 45 cm<sup>3</sup> ethanol), 3.87 g **5a** (0.01 mol) or 3.38 g **5b** (0.01 mol) was added and heating was continued for 30 min. The mixture was allowed to cool to room temperature, and methyl iodide (20 mmol) was added. The mixture was stirred under reflux for 5–7 h and then allowed

to cool to room temperature and finally poured into  $100 \text{ cm}^3$  cold water. The solid product precipitated was filtered off and washed with  $100 \text{ cm}^3$  water. The products were recrystallized from the proper solvent to give **6a** and **6b**.

# 2-(7-Bromo-4,6-dimethoxybenzofuran-5-yl)-1,4-dimethyl-1,4-dihydroquinoxaline (6a, C<sub>20</sub>H<sub>19</sub>BrN<sub>2</sub>O<sub>3</sub>)

The compound was obtained from the reaction of 3.87 g **5a** (0.01 mol) and methyl iodide (20 mmol), as a pale yellow crystals, crystallized from dioxane (66%). M.p.: >350 °C; IR (KBr):  $\bar{v} = 3052$  (CH-aryl), 2954 (CH-aliph) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta = 3.25$  (s, 3H, CH<sub>3</sub>), 3.30 (s, 3H, CH<sub>3</sub>), 3.82 (s, 3H, OCH<sub>3</sub>), 3.85 (s, 3H, OCH<sub>3</sub>), 5.58 (d, 1H, CH, pyrazine), 7.05 (d, 1H, J = 2.38 Hz, furan), 7.30 (d, 1H, J = 2.37 Hz, furan), 7.50–7.62 (m, 4H, phenyl) ppm; <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta = 35.2$ , 45.8, 62.1, 62.5, 84.4, 99.1, 103.2, 106.1, 111.9, 117.6, 117.8, 118.5, 118.6, 122.1, 129.6, 129.7, 146.2, 150.8, 160.1, 160.3 ppm; MS (70 eV): *m/z* = 415 (M<sup>+</sup>, 88%).

## 1,4-Dimethyl-2-(4,6,7-trimethoxybenzofuran-5-yl)-1,4-dihydroquinoxaline (**6b**, $C_{21}H_{22}N_2O_4$ )

The compound was obtained from the reaction of 3.38 g **5b** (0.01 mol) and methyl iodide (20 mmol), as a yellowish crystals, crystallized from hexane (64%). M.p.: >350 °C; IR (KBr):  $\bar{v} = 3050$  (CH-aryl), 2952 (CH-aliph) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta = 3.31$  (s, 3H, CH<sub>3</sub>), 3.35 (s, 3H, CH<sub>3</sub>), 3.80 (s, 3H, OCH<sub>3</sub>), 3.84 (s, 3H, OCH<sub>3</sub>), 3.91 (s, 3H, OCH<sub>3</sub>), 5.61 (d, 1H, CH, pyrazine), 7.02 (d, 1H, J = 2.35 Hz, furan), 7.26 (d, 1H, J = 2.34 Hz, furan), 7.45–7.58 (m, 4H, phenyl) ppm; <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta = 35.1, 46.2, 61.6, 61.8, 62.1, 98.6, 102.2, 106.3, 111.7, 117.5, 117.7, 118.6, 118.8, 122.4, 129.5, 129.6, 134.6, 142.2, 143.8, 144.3, 146.3 ppm; MS (70 eV):$ *m*/*z*= 366 (M<sup>+</sup>, 82%).

### 1-[1-(6-Hydroxy-4-methoxy- or 4,7-dimethoxybenzofuran-5-yl)ethylidene]thiourea (7a, 7b)

Following reported procedures with slight modification [24]. A solution of visnaginone 2.06 g (2a, 0.01 mol) or 2.36 g khellinone (2b, 0.01 mol) and 0.76 g thiourea (10 mmol) in 40 cm<sup>3</sup> glacial acetic acid the mixture was refluxed for 3–5 h After cooling the solid mass was collected by filtration, washed with water, dried, and recrystallized from appropriate solvent to give compounds 7a and 7b, respectively.

#### *1-[1-(6-Hydroxy-4-methoxybenzofuran-5-yl)ethylidene*]*thiourea* (**7a**, C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>S)

The compound was obtained from the reaction of 2.06 g visnaginone (**2a**, 10 mmol) and 0.76 g thiourea (10 mmol), as a yellow crystals, crystallized from methanol (75%). M.p.: 186–188 °C; IR (KBr):  $\bar{\nu} = 3400–3200$  (br. NH<sub>2</sub>, OH), 3045 (CH-aryl), 2955 (CH-aliph), 1618 (C = N), 1355 (C = S) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta$  = 1.68 (s, 3H, CH<sub>3</sub>), 3.83 (s, 3H, OCH<sub>3</sub>), 6.34 (s, 1H, H-7), 6.68 (s, 2H, NH<sub>2</sub>), 7.04 (d, 1H, J = 2.32 Hz, furan), 7.33 (d, 1H, J = 2.31 Hz, furan), 12.46 (brs, OH D<sub>2</sub>O exchangeable) ppm; <sup>13</sup>C NMR (DMSO- $d_6$ ):  $\delta$  = 18.2 (1C, CH<sub>3</sub>), 61.6 (1C, OCH<sub>3</sub>), 95.4 (1C, C<sub>7</sub>), 99.1, 106.2, 109.8, 146.1, 151.3, 155.2, 160.4 (Ar–C), 164.5 (1C, C=N), 191.6 (1C, C=S) ppm; MS (70 eV): m/z = 264 (M<sup>+</sup>, 100%).

# 1-[1-(6-Hydroxy-4,7-dimethoxybenzofuran-5-yl)ethylidene]thiourea (**7b**, C<sub>13</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>S)

The compound was obtained from the reaction of 2.36 g, khellinone (**2b**, 0.01 mol) and 0.76 g thiourea (10 mmol), as a yellowish crystals, crystallized from ethanol (70%). M.p.: 200–202 °C; IR (KBr):  $\bar{v} = 3405-3210$  (br. NH<sub>2</sub>, OH), 3048 (CH-aryl), 2956 (CH-aliph), 1615 (C=N), 1354 (C=S) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta = 1.92$  (s, 3H, CH<sub>3</sub>), 3.80 (s, 3H, OCH<sub>3</sub>), 3.84 (s, 3H, OCH<sub>3</sub>), 6.74 (s, 2H, NH<sub>2</sub>), 7.05 (d, 1H, J = 2.37 Hz, furan), 7.32 (d, 1H, J = 2.38 Hz, furan), 12.55 (brs, OH D<sub>2</sub>O exchangeable) ppm; <sup>13</sup>C NMR (DMSO- $d_6$ ):  $\delta = 18.4$  (1C, CH<sub>3</sub>), 61.7 (1C, OCH<sub>3</sub>), 61.8 (1C, OCH<sub>3</sub>), 99.8, 106.3, 112.4, 129.5, 146.2, 147.5, 152.3, 154.1 (Ar–C), 164.7 (1C, C=N), 191.8 (1C, C=S) ppm; MS (70 eV): m/z = 294 (M<sup>+</sup>, 100%).

### 5-Methoxy- or 5,9-dimethoxy-4-methylfuro[2,3b]benzo[5',6'-e]pyrimidine-2(1H)-thione (**8a**, **8b**)

A solution of 2.64 g **7a** (0.01 mol) or 2.94 g **7b** (0.01 mol) in 40 cm<sup>3</sup> DMF was refluxed for 10–12 h. The product formed was filtered off, washed with ethanol, dried, and recrystallized from appropriate solvent to give compounds **8a** or **8b**.

# 5-Methoxy-4-methylfuro[2,3-b]benzo[5',6'-e]pyrimidine-2(1H)-thione (**8a**, C<sub>12</sub>H<sub>10</sub>N<sub>2</sub>O<sub>2</sub>S)

The compound was obtained from the reaction of 2.64 g **7a** (0.01 mol) and 40 cm<sup>3</sup> DMF, as a pale yellow crystals, crystallized from benzene (70%). M.p.: 288–290 °C; IR (KBr):  $\bar{\nu} = 3250$  (NH), 3040 (CH-aryl), 2951 (CH-aliph), 1620 (C=N), 1360 (C=S) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta = 1.92$  (s, 3H, CH<sub>3</sub>), 3.82 (s, 3H, OCH<sub>3</sub>), 7.10 (d, 1H, J = 2.39 Hz, furan), 7.35 (d, 1H, J = 2.38 Hz, furan), 8.05 (s, 1H, CH<sub>phenyl</sub>), 10.85 (brs, NH D<sub>2</sub>O exchangeable) ppm; <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta = 23.5$  (1C, CH<sub>3</sub>), 61.7 (1C, OCH<sub>3</sub>), 99.9 (1C, CH<sub>phenyl</sub>), 106.1, 108.5, 113.5, 137.2, 146.2, 154.1, 160.3, (Ar–C),164.7 (1C, C=N), 180.5 (1C, C=S) ppm; MS (70 eV): m/z = 246 (M<sup>+</sup>, 100%).

# 5,9-Dimethoxy-4-methylfuro[2,3-b]benzo[5',6'-e]pyrimidine-2(1H)-thione (**8b**, $C_{13}H_{12}N_2O_3S$ )

The compound was obtained from the reaction of 2.94 g **7b** (0.01 mol) and 40 cm<sup>3</sup> DMF, as a yellowish crystals, crystallized from methanol (72%). M.p.: 328–330 °C; IR (KBr):  $\bar{v} = 3255$  (NH), 3042 (CH-aryl), 2954 (CH-aliph),

1622 (C=N), 1357 (C=S) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta = 1.90$  (s, 3H, CH<sub>3</sub>), 3.83 (s, 3H, OCH<sub>3</sub>), 3.84 (s, 3H, OCH<sub>3</sub>), 7.11 (d, 1H, J = 2.36 Hz, furan), 7.36 (d, 1H, J = 2.37 Hz, furan), 10.88 (brs, NH D<sub>2</sub>O exchangeable) ppm; <sup>13</sup>C NMR (DMSO- $d_6$ ):  $\delta = 23.2$  (1C, CH<sub>3</sub>), 56.5 (1C, OCH<sub>3</sub>), 61.6 (1C, OCH<sub>3</sub>), 106.3, 109.7, 115.9, 122.8, 134.6, 146.1, 146.4, 150.2 (Ar–C), 164.5 (1C, C=N), 180.3 (1C, C=S) ppm; MS (70 eV): m/z = 276 (M<sup>+</sup>, 42%).

# 1-[1-[4-Methoxy- or 4,7-dimethoxy-6-(piperazin-1-yl)benzofuran-5-yl]ethylidene]thiourea (**9a**, **9b**)

Freshly distilled piperazine (0.9 cm<sup>3</sup>, 10 mmol) was added to a warm solution of 2.64 g **7a** (10 mmol) or 2.94 g **7b** (10 mmol) in 45 cm<sup>3</sup> glacial acetic acid. The reaction mixture was stirred under reflux for 4 h, then allowed to cool to 0 °C for 5 h, and the solid obtained was filtered, washed with 100 cm<sup>3</sup> water, dried, and recrystallized from appropriate solvent to produce **9a**, **9b**.

# $\label{eq:linear} \begin{array}{l} 1-[1-[4-Methoxy-6-(piperazin-1-yl)benzofuran-5-yl]ethylene] thiourea~(\textbf{9a},~C_{16}H_{20}N_4O_2S) \end{array}$

Compound **9a** was obtained from the reaction of 2.64 g **7a** (10 mmol) and 0.9 cm<sup>3</sup> piperazine (10 mmol), as a yellow crystals, crystallized from *n*-hexane (62%). M.p.: 212–214 °C; IR (KBr):  $\bar{\nu} = 3360-3200$  (br. NH, NH<sub>2</sub>), 3050 (CH-aryl), 2960 (CH-aliph), 1617 (C=N), 1352 (C=S) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta = 1.82$  (s, 3H, CH<sub>3</sub>), 2.79–2.87 (m, 8H, piperazine), 3.85 (s, 3H, OCH<sub>3</sub>), 6.80 (s, 2H, NH<sub>2</sub>), 7.08 (d, 1H, J = 2.34 Hz, furan), 7.35 (d, 1H, J = 2.32 Hz, furan), 8.01 (s, 1H, CH<sub>phenyl</sub>), 9.55 (brs, NH D<sub>2</sub>O exchangeable) ppm; <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta = 21.2$  (1C, CH<sub>3</sub>), 46.5 (2C, 2CH<sub>2</sub>, piperazine), 51.7 (2C, 2CH<sub>2</sub>, piperazine), 61.5 (1C, OCH<sub>3</sub>), 88.2 (1C, CH <sub>phenyl</sub>), 96.4, 107.2, 107.6, 137.4, 140.5, 148.5, 152.8 (Ar–C),160.5 (1C, C=N), 190.8 (1C, C=S) ppm; MS (70 eV): m/z = 334 ([M+2]<sup>+</sup>, 76%), 333 ([M+1]<sup>+</sup>, 25%), 332 (M<sup>+</sup>, 73%).

# *1-[1-[4,7-Dimethoxy-6-(piperazin-1-yl)benzofuran-5-yl]ethylidene]thiourea* (**9b**, C<sub>17</sub>H<sub>22</sub>N<sub>4</sub>O<sub>3</sub>S)

Compound **9b** was obtained from the reaction of 2.94 g **7b** (10 mmol) and 0.9 cm<sup>3</sup> piperazine (10 mmol), as a yellowish crystals, crystallized from toluene (64%). M.p.: 232–234 °C; IR (KBr):  $\bar{v} = 3365-3205$  (br. NH, NH<sub>2</sub>), 3052 (CH-aryl), 2961 (CH-aliph), 1616 (C=N), 1351 (C=S) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta = 1.84$  (s, 3H, CH<sub>3</sub>), 2.80–2.88 (m, 8H, piperazine), 3.80 (s, 3H, OCH<sub>3</sub>), 3.82 (s, 3H, OCH<sub>3</sub>), 6.84 (s, 2H, NH<sub>2</sub>), 7.12 (d, 1H, J = 2.30 Hz, furan), 7.44 (d, 1H, J = 2.31 Hz, furan), 9.60 (brs, NH D<sub>2</sub>O exchangeable) ppm; <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta = 21.3$  (1C, CH<sub>3</sub>), 46.9 (2C, 2CH<sub>2</sub>, piperazine), 51.9 (2C, 2CH<sub>2</sub>, piperazine), 54.4 (1C, OCH<sub>3</sub>), 61.8 (1C, OCH<sub>3</sub>), 100.5, 108.4, 108.9, 125.8, 129.8, 144.5, 146.6,146.9 (Ar–C), 154.5 (1C, C=N), 192.3 (1C, C=S) ppm; MS (70 eV): *m*/z = 362 (M<sup>+</sup>, 95%).

### 2-[Amino-[[1-[4-methoxy- or 4,7-dimethoxy-6-(piperazin-1-yl)benzofuran-5-yl]ethylidene]amino]methylene]malononitrile (**10a**, **10b**)

A solution of 3.32 g **9a** (10 mmol) or 3.62 g **9b** (10 mmol) in 35 cm<sup>3</sup> ethanol was treated with 0.66 g malononitrite (10 mmol) and 1 cm<sup>3</sup> triethylamine was added. The reaction mixture was refluxed for 4 h and then evaporated in vacuo. The solid product was collected by filtration and crystallized from appropriate solvent to produce **10a**, **10b**.

### 2-[Amino-[[1-[4-methoxy-6-(piperazin-1-yl)benzofuran-5yl]ethylidene]amino]methylene]malononitrile (10a, C<sub>19</sub>H<sub>20</sub>N<sub>6</sub>O<sub>2</sub>)

Compound **10a** was obtained from the reaction of 3.32 g **9a** (10 mmol) and 0.66 g malononitrite (10 mmol), as a brownish crystals, crystallized from methanol (60%). M.p.: 165–167 °C; IR (KBr):  $\bar{v} = 3355-3220$  (br. NH, NH<sub>2</sub>), 3056 (CH-aryl), 2958 (CH-aliph), 2210, 2205 (2CN), 1619 (C=N) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta = 1.86$  (s, 3H, CH<sub>3</sub>), 2.83–2.97 (m, 8H, piperazine), 4.09 (s, 3H, OCH<sub>3</sub>), 6.28 (s, 2H, NH<sub>2</sub>), 7.28 (d, 1H, *J* = 2.35 Hz, furan), 7.39 (d, 1H, *J* = 2.36 Hz, furan), 8.01 (s, 1H, CH<sub>phenyl</sub>), 9.32 (brs, NH D<sub>2</sub>O exchangeable) ppm; <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta = 18.6$ (1C, CH<sub>3</sub>), 45.7 (2C, 2CH<sub>2</sub>, piperazine), 51.4 (2C, 2CH<sub>2</sub>, piperazine), 52.1 (1C, C(CN)<sub>2</sub>), 61.8 (1C, OCH<sub>3</sub>), 88.4 (1C, CH<sub>phenyl</sub>), 96.2, 106.1, 107.2, 113.5, 142.3, 146.2, 154.7, 160.9 (Ar–C),164.8 (1C, C=N), 186.5 (1C, C–NH<sub>2</sub>) ppm; MS (70 eV): *m/z* = 364 (M<sup>+</sup>, 82%).

### 2-[Amino-[[1-[4,7-dimethoxy-6-(piperazin-1-yl)benzofuran-5-yl]ethylidene]amino]methylene]malononitrile (**10b**, C<sub>20</sub>H<sub>22</sub>N<sub>6</sub>O<sub>3</sub>)

Compound 10b was obtained from the reaction of 3.62 g 9b (10 mmol) and 0.66 g malononitrite (10 mmol), as a brown crystals, crystallized from dioxane (61%). M.p.: 176–178 °C; IR (KBr):  $\bar{v} = 3352-3222$  (br. NH, NH<sub>2</sub>), 3054 (CH-aryl), 2956 (CH-aliph), 2212, 2208 (2CN), 1617 (C=N) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta = 1.82$  (s, 3H, CH<sub>3</sub>), 2.82-2.95 (m, 8H, piperazine), 3.84 (s, 3H, OCH<sub>3</sub>), 3.85 (s, 3H, OCH<sub>3</sub>), 6.42 (s, 2H, NH<sub>2</sub>), 7.18 (d, 1H, J = 2.34 Hz, furan), 7.44 (d, 1H, J = 2.33 Hz, furan), 9.55 (brs, NH D<sub>2</sub>O exchangeable) ppm; <sup>13</sup>C NMR (DMSO- $d_6$ ):  $\delta = 18.5$ (1C, CH<sub>3</sub>), 45.5 (2C, 2CH<sub>2</sub>, piperazine), 51.6 (2C, 2CH<sub>2</sub>, piperazine), 52.2 (1C, C(CN)<sub>2</sub>), 56.4 (1C, OCH<sub>3</sub>), 61.7 (1C, OCH<sub>3</sub>), 97.5, 106.3, 109.5, 113.8, 122.5, 127.9, 146.1, 146.9, 150.5 (Ar-C), 164.7 (1C, C=N), 186.8 (1C, C-NH<sub>2</sub>) ppm; MS (70 eV):  $m/z = 395 ([M+1]^+, 10\%), 394 (M^+,$ 12%).

#### 4-Methoxy-3-methyl-1-phenyl-1H-furo[3,2-f]indazole

## (**11a**) or 4,8-dimethoxy-3-methyl-1-phenyl-1H-furo[3,2f]indazole (**11b**)

A mixture of 2.06 g **2a** (0.01 mol) or 2.36 g **2b** (0.01 mol) and 1.08 cm<sup>3</sup> phenylhydrazine (10 mmol) in 40 cm<sup>3</sup> absolute ethanol was refluxed for 4 h and then left to cool. The precipitate formed was filtered off, dried, and crystallized from the proper solvent to give **11a** or **11b** [27, 29].

# $\label{eq:4-Methoxy-3-methyl-1-phenyl-1H-furo[3,2-f]indazole} (11a, C_{17}H_{14}N_2O_2)$

Compound **11a** was obtained from the reaction of 2.06 g visnaginone (**2a**, 10 mmol) and 1.08 cm<sup>3</sup> phenylhydrazine (10 mmol) as a yellowish powder, crystallized from dioxane (75%). M.p.: 220–222 °C; IR (KBr):  $\bar{v} = 3032$  (CH-aryl), 2948 (CH-aliph) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta = 2.81$  (s, 3H, CH<sub>3</sub>), 3.82 (s, 3H, OCH<sub>3</sub>), 7.11 (d, 1H, J = 2.30 Hz, furan), 7.15 (d, 1H, J = 2.30 Hz, furan), 7.15 (d, 1H, J = 2.30 Hz, furan), 7.46–7.97 (m, 5H, phenyl), 8.12 (s, 1H, H-8) ppm; <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta = 15.5$ , 61.7, 100.1, 108.3, 108.9, 118.2, 124.5, 125.6, 126.3, 129.2, 139.5, 145.7, 146.1, 155.3, 157.8 ppm; MS (70 eV): m/z = 278 (M<sup>+</sup>, 95%).

# 4,8-Dimethoxy-3-methyl-1-phenyl-1H-furo[3,2-f]indazole (11b, $C_{18}H_{16}N_2 O_3$ )

The compound was obtained from the reaction of 2.36 g khellinone (**2b**, 10 mmol) and 1.08 cm<sup>3</sup> phenylhydrazine (10 mmol) as a white powder, crystallized from *n*-hexane (76%). M.p.: 255–257 °C; IR (KBr):  $\bar{\nu} = 3035$  (CH-aryl), 2944 (CH-aliph) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta = 2.88$  (s, 3H, CH<sub>3</sub>), 3.83 (s, 3H, OCH<sub>3</sub>), 4.09 (s, 3H, OCH<sub>3</sub>), 7.12 (d, 1H, J = 2.30 Hz, furan), 7.25 (d, 1H, J = 2.30 Hz, furan), 7.55–7.98 (m, 5H, phenyl) ppm; <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta = 15.9$ , 61.5, 108.4, 108.9, 112.5, 118.8, 124.6, 126.5, 129.7, 137.8, 139.9, 145.8, 146.3, 147.2, 147.8 ppm; MS (70 eV): *m*/*z* = 309 ([M+1]<sup>+</sup>, 16%), 308 (M<sup>+</sup>, 13%), 307 ([M-1]<sup>+</sup>, 38%).

### 4-Methoxy-3-methyl-1H-furo[3,2-f]indazole (12a) or 4,8dimethoxy-3-methyl-1H-furo[3,2-f]indazole (12b)

A mixture of 2.06 g **2a** (10 mmol) or 2.36 g **2b** (10 mmol) and 0.5 cm<sup>3</sup> hydrazine monohydrate (NH<sub>2</sub>NH<sub>2</sub>.H<sub>2</sub>O, 0.01 mol) in 40 cm<sup>3</sup> absolute ethanol was heated at reflux for 4 h and then left to cool. The precipitate formed was filtered off, dried, and crystallized from the proper solvent to give **12a** or **12b**.

# 4-Methoxy-3-methyl-1H-furo[3,2-f]indazole

# $({\bf 12a},\,C_{11}H_{10}N_2O_2)$

The compound was obtained from the reaction of 2.06 g visnaginone (**2a**, (10 mmol) and 0.5 cm<sup>3</sup> NH<sub>2</sub>NH<sub>2</sub>.H<sub>2</sub>O (0.01 mol) as a yellow powder, crystallized from methanol (78%). M.p.: 278–280 °C; IR (KBr):  $\bar{\nu} = 3320$  (NH), 3032(CH-aryl), 2942 (CH-aliph) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta = 2.11$  (s, 3H, CH<sub>3</sub>), 3.73 (s, 3H, OCH<sub>3</sub>), 7.10 (d, 1H, J = 2.30 Hz, furan), 7.24 (d, 1H, J = 2.30 Hz, furan), 8.15 (s, 1H, H-8), 9.04 (br, 1H, NH, D<sub>2</sub>O exchangeable) ppm; <sup>13</sup>C NMR (DMSO- $d_6$ ):  $\delta = 15.6, 61.8, 101.2, 108.4, 108.8, 117.3, 125.7, 138.5, 146.4, 155.2, 157.6 ppm; MS (70 eV): <math>m/z = 202$  (M<sup>+</sup>, 92%).

4,8-Dimethoxy-3-methyl-1H-furo[3,2-f]indazole (12b,  $C_{12}H_{12}N_2O_3$ )

The compound was obtained from the reaction of 2.36 g khellinone (**2b**, (10 mmol) and 0.5 cm<sup>3</sup> hydrazine monohydrate (0.01 mol) as a brown powder, crystallized from acetone (75%). M.p.: 298–300 °C; IR (KBr):  $\bar{\nu} = 3335$ (NH), 3030 (CH-aryl), 2936 (CH-aliph) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta = 2.14$  (s, 3H, CH<sub>3</sub>), 3.80 (s, 3H, OCH<sub>3</sub>), 4.08 (s, 3H, OCH<sub>3</sub>), 7.09 (d, 1H, J = 2.30 Hz, furan), 7.27 (d, 1H, J = 2.30 Hz, furan), 9.08 (br, 1H, NH, D<sub>2</sub>O exchangeable) ppm; <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta = 15.8$ , 61.6, 108.2, 108.7, 111.4, 118.2, 137.8, 138.5, 146.5, 147.1, 147.8 ppm; MS (70 eV): m/z = 232 (M<sup>+</sup>, 96%).

## 1-(4-Methoxy-3-methyl-1H-furo[3,2-f]indazol-1-yl)ethan-

1-one (**13a**), 1-(4,8-dimethoxy-3-methyl-1H-furo[3,2-f]indazol-1-yl)ethan-1-one (**13b**), 4-methoxy-1,3-dimethyl-1Hfuro[3,2-f]indazole (**14a**), or 4,8-dimethoxy-1,3-dimethyl-1H-furo[3,2-f]indazole (**14b**)

To a warm (50–70 °C) solution of sodium ethoxide (prepared by dissolving 0.23 g of sodium metal in 30 cm<sup>3</sup> ethanol), 2.02 g **12a** (10 mmol) or 2.32 g **12b** (10 mmol) was added and heating (50–70 °C) was continued for 30 min. The mixture was allowed to cool to room temperature and acetic anhydride or methyl iodide (10 mmol) was added. The mixture was stirred under reflux for 6–8 h, allowed to cool to room temperature and finally poured into 100 cm<sup>3</sup> cold water. The solid product precipitated was filtered off and washed with 100 cm<sup>3</sup> water. The solid obtained was filtered off, dried, and crystallized from the proper solvent to give **13a**, **13b**, **14a**, and **14b**.

### 1-(4-Methoxy-3-methyl-1H-furo[3,2-f]indazol-1-yl)ethan-1-one (**13a**, C<sub>13</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>)

The compound was obtained from the reaction of 2.02 g **12a** (10 mmol) and 1 cm<sup>3</sup> acetic anhydride (0.01 mol), as a white powder, crystallized from dioxane (82%). M.p.: 308–310 °C; IR (KBr):  $\bar{\nu} = 3030$  (CH-aryl), 2938 (CH-aliph), 1785 (C=O) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta = 2.29$  (s, 3H, COH<sub>3</sub>), 2.32 (s, 3H, CH<sub>3</sub>), 3.82 (s, 3H, OCH<sub>3</sub>), 7.09 (d, 1H, J = 2.30 Hz, furan), 7.39 (d, 1H, J = 2.30 Hz, furan), 8.17 (s, 1H, H-8) ppm; <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta = 15.9$ , 22.4, 61.5, 100.8, 108.1, 108.6, 117.4, 126.1, 138.3, 146.2, 155.2, 157.4, 170.9 ppm; MS (70 eV): m/z = 245 ([M+1]<sup>+</sup>, 4%), 244 (M<sup>+</sup>, 8%), 243 ([M-1]<sup>+</sup>, 35%), 242 ([M-2]<sup>+</sup>, 100%).

# $\label{eq:l-(4,8-Dimethoxy-3-methyl-1H-furo[3,2-f]indazol-1-yl)e-than-1-one~(\textbf{13b},~C_{14}H_{14}N_2O_4)$

The compound was obtained from the reaction of 2.32 g **12b** (10 mmol) and 1 cm<sup>3</sup> acetic anhydride (0.01 mol) as a white powder, crystallized from dioxane (82%). M.p.: 318–320 °C; IR (KBr):  $\bar{\nu} = 3035$  (CH-aryl), 2936 (CH-aliph),

1780 (C=O) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta = 2.28$  (s, 3H, COH<sub>3</sub>), 2.33 (s, 3H, CH<sub>3</sub>), 3.84 (s, 3H, OCH<sub>3</sub>), 3.98 (s, 3H, OCH<sub>3</sub>), 7.13 (d, 1H, J = 2.30 Hz, furan), 7.38 (d, 1H, J = 2.30 Hz, furan) ppm; <sup>13</sup>C NMR (DMSO- $d_6$ ):  $\delta = 19.7, 23.3, 55.6, 58.4, 108.4, 108.9, 117.7, 120.02, 132.9, 135.4, 141.9, 148.9, 155.03, 167.6 ppm; MS (70 eV): <math>m/z = 275$  ([M+1]<sup>+</sup>, 10%), 274 (M<sup>+</sup>, 74%), 273 ([M-1]<sup>+</sup>, 100%).

# *4-Methoxy-1,3-dimethyl-1H-furo[3,2-f]indazole* (**14a**, C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>)

The compound was obtained from the reaction of 2.02 g **12a** (10 mmol) and 0.7 cm<sup>3</sup> methyl iodide (0.01 mol) as a yellow powder, crystallized from *n*-hexane (78%). M.p.: 338–340 °C; IR (KBr):  $\bar{\nu} = 3046$  (CH-aryl), 2942 (CH-aliph) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta = 2.17$  (s, 3H, CH<sub>3</sub>), 3.82 (s, 3H, OCH<sub>3</sub>), 4.02 (s, 3H, CH<sub>3</sub>), 7.04 (d, 1H, J = 2.30 Hz, furan), 7.33 (d, 1H, J = 2.30 Hz, furan), 8.02 (s, 1H, H-8) ppm; <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta = 15.5$ , 42.1, 61.7, 100.2, 108.1, 108.5, 116.6, 125.4, 143.4, 146.3, 155.1, 157.4 ppm; MS (70 eV): m/z = 216 (M<sup>+</sup>, 88%).

# $\label{eq:2.1} 4,\ 8\ Dimethoxy-1, 3\ dimethyl-1 H\ furo[3,2\ f] indazole$

# $({\bf 14b},\,C_{13}H_{14}N_2O_3)$

The compound was obtained from the reaction of 2.32 g **12b** (10 mmol) and 0.7 cm<sup>3</sup> methyl iodide (0.01 mol) as a yellowish powder, crystallized from ethanol (76%). M.p.: 348–350 °C; IR (KBr):  $\bar{\nu} = 3044$  (CH-aryl), 2928 (CH-aliph) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta = 2.18$  (s, 3H, CH<sub>3</sub>), 3.85 (s, 3H, OCH<sub>3</sub>), 4.04 (s, 3H, CH<sub>3</sub>), 4.15 (s, 3H, OCH<sub>3</sub>), 7.03 (d, 1H, J = 2.30 Hz, furan), 7.35 (d, 1H, J = 2.30 Hz, furan), ppm; <sup>13</sup>C NMR (DMSO- $d_6$ ):  $\delta = 15.8$ , 43.60, 61.6, 108.2, 108.6, 111.4, 118.1, 137.5, 143.2, 146.4, 147.2, 147.8 ppm; MS (70 eV): m/z = 246 (M<sup>+</sup>, 79%).

### Ehrlich cells

Ehrlich cells (Ehrlich ascites carcinoma, EAC) were derived from ascetic fluid from diseased mice (the cells were purchased from the National Cancer institute, Cairo, Egypt).

#### Cytotoxic activity using Ehrlich ascites in vitro assay

Different concentrations of the tested compounds were prepared (100, 50 and 25 g/cm<sup>3</sup> DMSO). Ascites fluid from the peritoneal cavity of the donor animal (contains Ehrlich cells) was aseptically aspirated. The cells were grown partly floating and partly attached in a suspension culture in RPMI 1640 medium, supplemented with 10% fetal bovine serum. They were maintained at 37 °C in a humidified atmosphere with 5% CO<sub>2</sub> for 2 h. The viability of the cells was determined by the microscopical examination using a hemocytometer and using trypan blue stain (stains only the dead cells) [32].

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#### References

- 1. Mustafa A (1967) Furopyrans and furopyrones. John Wiley and Sons, New York
- 2. Abu-Hashem AA, El-Shazly M (2015) Eur J Med Chem 90:633
- 3. Saad B, Said O (2011) Greco-Arab and Islamic herbal medicine: traditional system, ethics, safety, efficacy, and regulatory issues. Wiley, New Jersey
- 4. Kopp B, Kubelka E, Reich C, Robien W, Kubelka W (1991) Helv Chim Acta 74:611
- 5. De Leeuw J, Assen Y, Van Der Beek N, Bjerring P, Martino Neumann H (2011) J Eur Acad Dermatol 25:74
- 6. Towers G, Hudson J (1987) Photochem Photobiol 46:61
- 7. Gammill RB, Day CE, Schurr PE (1983) J Med Chem 26:1672
- 8. Abu-Hashem AA, Youssef MM (2011) Molecules 16:1956
- 9. Ashok M, Holla BS, Kumari NS (2007) Eur J Med Chem 42:380
- Abdou SE, El-Qusy SM, Ghabrial SS, Haggag MI (2011) Mod App Sci 5:140
- El-Nakkady SS, Roaiah HF, El-Serwy WS, Soliman A, Abd El-Moez S, Abdel-Rahman AA (2012) Acta Pol Pharm 69:645
- 12. Kadieva M, Oganesyan E (1997) Chem Heterocycl Compd 33:1245
- Masubuchi M, Ebiike H, Kawasaki K-I, Sogabe S, Morikami K, Shiratori Y, Tsujii S, Fujii T, Sakata K, Hayase M (2003) Bioorg Med Chem 11:4463
- Hou XL, Yang Z, Wong HNC (2002) Furans and Benzofurans. In: Gribble GW, Gilchrist TL (eds) Progress in heterocyclic chemistry, vol 14. Pergamon Press, Oxford, p 139

- Kumar S, Bawa S, Drabu S, Kumar R, Gupta H (2009) Recent Pat Anti-Infect 4:154
- Karthikeyan MS, Holla BS, Kumari NS (2007) Eur J Med Chem 42:30
- Özdemir A, Turan-Zitouni G, Kaplancıklı ZA, Revial G, Güven K (2007) Eur J Med Chem 42:403
- Keshk EM, Abu-Hashem AA, Girges MM, Abdel-Rahman AH, Badria FA (2004) Phosphorus. Sulfur Silicon Relat Elem 179:1577
- Abu-Hashem AA, El-Shehry MF, Badria FA (2010) Acta Pharm 60:311
- 20. Abdel Hafez OM, Ahmed KM, Haggag EE (2001) Molecules 6:396
- Zhuravel IO, Kovalenko SM, Vlasov SV, Chernykh VP (2005) Molecules 10:444
- Zhou J-F, Gong G-X, An L-T, Liu Y, Zhu F-X, Zhu Y-L, Ji S-J (2008) Synlett 3163
- Mohamed HM, Abd El-Wahab AHF, Ahmed KA, El-Agrody AM, Bedair AH, Eid FA, Khafagy MM (2012) Molecules 17:971
- Ragab FA, EL-Sayed NA, Eissa AAM, El-Kerdawy AM (2010) Chem Pharm Bull 58:1148
- El-Gazzar ABA, Youssef MM, Youssef AMS, Abu-Hashem AA, Badria FA (2009) Eur J Med Chem 44:609
- 26. Paul S, Panda S, Manna D (2014) Tetrahedron Lett 55:2480
- Viña D, del Olmo E, López-Pérez JL, San Feliciano A (2007) Org Lett 9:525
- Counceller CM, Eichman CC, Wray BC, Stambuli JP (2008) Org Lett 10:1021
- 29. Turnbull K, George JC (1996) Synth Commun 26:2757
- Sieveking I, Thomas P, Estévez JC, Quiñones N, Cuéllar MA, Villena J, Espinosa-Bustos C, Fierro A, Tapia RA, Maya JD, López-Muñoz R, Cassels BK, Estévez RJ, Salas CO (2014) Bioorg Med Chem 22:4609
- Bodendiek SB, Mahieux C, Hansel W, Wulff H (2009) Eur J Med Chem 44:1838
- Fujita T, Takeda Y, Han-dong S, Minami Y, Marunaka T, Takeda S, Yamada Y, Togo T (1988) Planta Med 54:414