# Synthesis and cytotoxic activity of new furanochromone derivatives

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**Abstract:** New furanochromone derivatives have been synthesized and evaluated for cytotoxicity in HEPG2 (liver cancer) cell line by SRB (Sulphorhodamine B) assay. Among the tested compounds, the cytotoxic activity of compounds **2k, 5** and **6c** was the most prominent, revealed by, $IC_{50}$ of 2.1, 2.3 and 2.5 µg/ml, respectively. The titled semisynthetic compounds were obtained by condensation of khellin (extracted from *Ammi visnaga* L. fruits) with different aldehydes followed by reaction with different primary amines and malononitril. The detailed synthesis, spectroscopic and biological data are reported.

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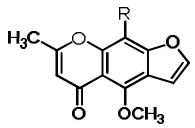
## 1. Introduction

Chromones and their derivatives are well known naturally occurring oxygen-containing heterocyclic compounds that perform important biological functions in nature. Natural and synthetic chromone derivatives have attracted a great deal of interest due to their wide spectrum of pharmacological activity, such as antitumor (McClure et al., 1975, Atassi et al., 1985, Bhatnagar et al., 2010) antiviral, antioxidant (Hudson and Towers 1999, Alves et al., 1999, Ungwitayatorn et al., 2004 ) anti-inflammatory (Middleton et al., 1994, Hutter et al., 1996) antispasmolytic, estrogenic (Bruneton 1995) and antibacterial activities (Harborne and Williams 2000). Chromones and coumarins comprise a large group of compounds widely distributed in the plant kingdom especially in families *Apiaceae*, *Rutaceae*, *Fabaceae* and *Asteraceae* (Razavi et al., 2010) with attractive pharmacological activities. For example, chroman-4-one 2-carboxylic acids are spasmolytic agents, and disodium chromoglycate acts as anti-allergenic drug (Pawar et al., 2009). Khellin and visnagen (Figure. 1) are natural furochromone derivatives, obtained from the fruits of *Ammi visnaga* L. Khellin and visnagen have been widely employed as herbal medicine in the treatment of angina (Dewar and Grimson 1950). Moreover, they significantly prolong the induction time of nucleation of calcium oxalate (Abdel-Aal et al., 2009) and have been used for photochemo-therapeutic treatment of vitiligo and psoriasis (Vedaldi et al., 1988). Also, khellin is used as a spasmolytic agent and for kidney stone treatment (Vanachayangkul et al., 2010). The photodynamic properties of khellin and visnagen in their photoreaction with DNA have been studied (Trabalzini et al., 1990). A literature survey has revealed that new polycyclic compounds derived from benzofurans and furochromones exhibited antitumor activities (Atta et al., 2010). Moreover, the antitumor activity of some furochromenly pyrazoles were reported (Asmaa et al., 2010). In addition, new heterocyclic benzofuran derivatives obtained from naturally occurring visnagin were assessed against HEPG2 (liver cancer) cell line and showed promising activity (El-Nakkady et al., 2012). liver cancer remains the fifth most common malignancy in men and the eighth in women worldwide (Ribes et al., 2004). In view of the previous observations, the present work has been endeavored aiming at determining the cytotoxic effect of khellin against HEPG2 cell line. So, new semisynthetic derivatives containing furanochromone scaffold and bearing new functionalities on the pyran ring have been synthesized in an attempt to further optimize the anticancer activity of khellin.



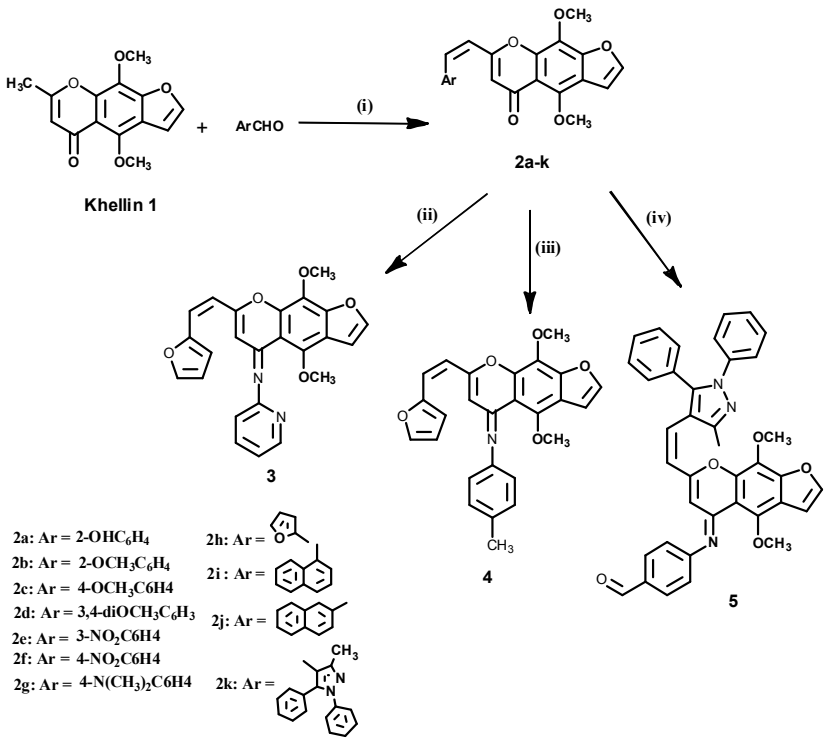

Visnagen, R= H
Khellin, R= $OCH_3$

**Figure 1.** Chemical structures of Visnagen and Khellin.

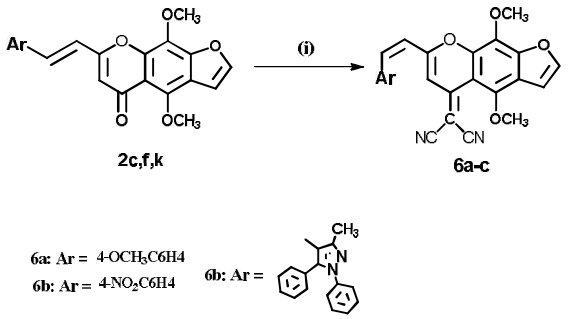
## 2. Results and discussion

### Chemistry

The conversion of the natural furochromone (khellin **1**), extracted from *Ammi visnaga* L. fruits, to the target compounds was accomplished as depicted in Schemes 1 and 2.




**Scheme 1** Reaction protocol for the synthesis of **2a-k** and **3-5** (i) 3% NaOH,$CH_3OH$ (ii), (iii), (iv) Absolute $C_2H_5OH$, glacial acetic acid




**Scheme 2** Reaction protocol for the synthesis of **6a-c** (i) Malononitrile, $CH_3COONa$, DMF

Reaction of different aromatic aldehydes with khellin in methanol in the presence of 3% sodium hydroxide gave **2a-k** via condensation of the aldehydes with the active methyl group at position 7 of khellin. The structures of the isolated products were established on the basis of their elemental and spectral analyses. For example, formation of compound **2d** was indicated by the absence of $CH_3$ protons at C7 and the appearance of aromatic and vinyl protons at 7.52-7.01 ppm in the $^1$H-NMR spectrum and the structure was further supported by $^{13}$C NMR spectrum. IR spectrum of compound **2f** showed characteristic absorption bands at 1532 and 1372 cm$^{-1}$ representing $NO_2$ group. Moreover, a molecular ion peak of *m/z* 393.20 (29.48%) was clearly identified in the mass spectrum of compound **2f**. The carbonyl functional group in compounds **2h** and **2k** has been utilized to synthesize the corresponding imine derivatives **3-5** through reaction with different primary amines in absolute ethanol in the presence of glacial acetic acid. The formation of Schiff base **4** was indicated by the presence of C=N band at 1595 cm$^{-1}$ in the IR spectrum and combined with the disappearance of the carbonyl group band. The preparation of dicyano derivatives **6a-c** was achieved through Knoevenagel condensation of arylvinyl derivatives **2c**, **2f** and **2k** with malononitrile in dimethylformamide in the presence of sodium acetate. The structures of the prepared compounds were substantiated by elemental and spectral analyses. IR data for compound **6a** exhibited characteristic absorption bands at 2211, 2205 cm$^{-1}$

assigned for (CN) groups and characterized by the disappearance of the absorption band of the carbonyl group. The protons of **6a** appeared at the expected regions of the $^1$H-NMR spectrum.

**Cytotoxic activity**

Khellin and fifteen of the newly synthesized compounds were screened for their *in vitro* cytotoxic and growth inhibitory activities against HEPG2 cell line in comparison with the activity of the known anticancer Doxorubicin (DXR) as a reference drug. The *in vitro* SRB assay of cytotoxic activity was employed and the results are shown in Table 1, and presented graphically in Figure. 1. The cytotoxic activities of the tested compounds were expressed as $IC_{50}$ which is the concentration required for 50% inhibition of cell viability. It is evident that the tested compounds showed antitumor activities with $IC_{50}$ values ranging from 2.1 to 12.2 µg/ml and reaching about one third that of DXR ($IC_{50}$ = 0.6 µg/ml) in the case of compound **2k** ($IC_{50}$ = 2.1 µg/ml). In general, among the arylvinyl derivatives of khellin, compounds containing 1,5-diphenyl pyrazolyl, 4-nitrophenyl, furanyl and 1(2)-naphthalenyl moieties showed pronounced cytotoxic activities confirmed by the least $IC_{50}$ values (2.1-4.3 µg/ml ). Interestingly, It was reported that many compounds containing 1,5-diphenyl pyrazolyl moiety in their molecular structures displayed potent antitumor activities (Farag et al., 2010). Accordingly, the increased cytotoxic activities of compounds **2k** ($IC_{50}$ =2.1 µg/ml), **5** ($IC_{50}$ =2.3 µg/ml) and **6c** ($IC_{50}$ =2.5 µg/ml) relative to khellin and other tested compounds, can be correlated with presence of 1,5-diphenylpyrazolyl vinyl sustituent at C7 of the furochromone structure. Therefore, these results demonstrate the potential of the furochromone structure as a scaffold for anticancer drug discovery and the importance of developing different derivatives through introduction of new functionalities on the pyran ring to deepen the structure activity relationship study.

**3. Conclusion**

From the results of the present study, it is concluded that, khellin was used as a starting material for the synthesis of new furanochromone derivatives. The cytotoxic activity of khellin and its semisynthetic products against HEPG2 (liver cancer) cell line was evaluated using SRB assay. Compounds **2k, 5** and **6c** containing 1,5-diphenylpyrazolyl moiety exhibited enhanced cytotoxic activity revealed by $IC_{50}$ of 2.1, 2.3 and 2.5 µg/ml, respectively comparing to khellin ($IC_{50}$ = 4.65).

**4. Experimental**

**Plant material**

The *Ammi visnaga* L. fruits were purchased from a local market in El Mansoura, Egypt in 2011, air dried and finely powdered.

**Table 1** *In vitro* Cytotoxic activity of khellin, semisynthetic derivatives and Doxorubicin against liver cell line (HEPG2)[a]

| Compound | $IC_{50}$ (µg/ml)[b] |
|---|---|
| Doxorubicin | 0.6 |
| 2k | 2.1 |
| 5 | 2.3 |
| 6c | 2.5 |
| 2f | 3.0 |
| 3 | 3.9 |
| 2h | 4.2 |
| 2i | 4.2 |
| 2b | 4.3 |
| Khellin | 4.65 |
| 2j | 5.7 |
| 2c | 7.5 |
| 2g | 8.0 |
| 6a | 10.2 |
| 2a | 12.0 |
| 2e | 12.0 |
| 2d | 12.2 |

[a] Arranged according to their descending orders of activities.

[b] $IC_{50}$ value is the compound concentration required to produce 50% inhibition of cell growth.

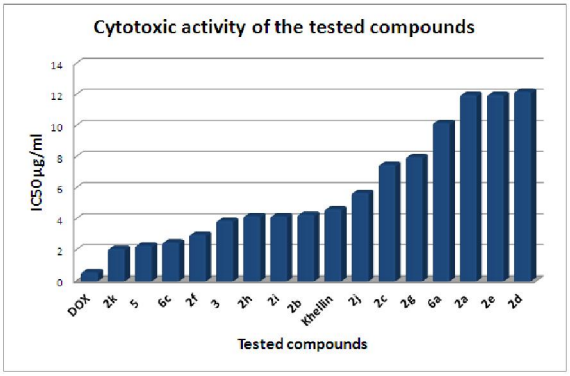



**Figure 1**

**Extraction and isolation of 4,9-dimethoxy-7-methyl-5*H*-furo[3,2-*g*]chromen-5-one (khellin 1)**

Five hundred grams of the finely powdered fruits of *Ammi visnaga* L. (F. *Apiaceae*) were subjected to exhaustive extraction with methanol. The extract was evaporated under reduced pressure at 40 °C. The residue (53g) was suspended in distilled water and extracted with petroleum ether followed by chloroform. The chloroform soluble fraction was submitted to silica gel column chromatography and eluted with petroleum ether followed by petroleum ether-ethyl acetate in increasing polarity. The eluted fractions (250 ml each) were collected, concentrated and examined by TLC and similar fractions were combined together. Fractions (43-49) eluted with

petroleum ether-ethyl acetate (70 %) were purified by crystallization from methanol to afford compound **1** (2.5 g) as a white-buff crystals . Mp 155-156°C; UV (MeOH, $\lambda_{max}$): 273 and 322 nm.$^1$H NMR (400 MHz, DMSO-$d_6$); δ 8.06 (br.s., 1H, furan CH=CHO); 7.18 (br.s., 1H, furan CH=CHO), 6.02 (s, 1H, γ-pyrone H); 4.07 (s, 3H, $OCH_3$); 3.89 (s, 3H,$OCH_3$); 2.32 (s, 3H, $CH_3$). $^{13}$C NMR (100 MHz, pyridine-*d5*) δ 177.1, 164.8, 148.7, 147.1, 147.0 (2C),129.8 (2C),119.3, 110.5, 105.6, 62.3, 61.7, 20.0. Anal. calcd. For $C_{14}H_{12}O_5$ (%): C, 64.61; H, 4.65. Found: C, 64.51; H, 4.60.

**Chemistry**

Melting points were determined using Fisher–Johns melting point apparatus and are uncorrected. UV spectra were measured in methanol using Shimadzu 1601 PC (TCC240, Japan) spectrophotometer. IR (KBr-discs) spectra were measured by Nicolet MX-1 FT-IR spectrometer. Chromatographic separations were performed using silica gel (E-Merck, Germany). TLC was performed on silica gel G60F254 (E-Merck, Germany). Normal phase chromatography was performed using silica gel (70-230 mesh) (E-Merck, Germany). The $^1$H and $^{13}$C NMR spectra were recorded in DMSO-d6 using TMS as an internal standard, on a JEOL Eclipse-400 NMR spectrometer, operating at 400 MHz for $^1$H and 100 MHz for $^{13}$C. Chromatograms were visualized under UV light (Ultra-Violet Lamp, Desaga, Germany). Mass spectra were measured on JEOL JMS-600H spectrometer. Elemental analysis was carried out for C, H and N at the Microanalytical Centre of Cairo University. All reagents were purchased from the Aldrich chemical company. The well-known compound, 3-methyl-1,5-diphenyl-1H-pyrazole-4-carbaldehyde was prepared following the procedure reported in the literature (Genin et al., 2000).

**General method for synthesis of 7-( arylvinyl)-4,9-dimethoxy-5*H*-furo[3,2-*g*]chromen-5-one (2a–k) (Scheme 1)**

A mixture of compound **1** (2.60 g, 10 mmol) and the appropriate aldehyde (10 mmol) in methanolic sodium hydroxide solution (3%, 40 mL) was stirred at room temperature for 4 h. The reaction mixture was poured onto ice/cold water and the formed precipitate was filtered and crystallized from ethanol to give compounds **2a–k**.

**7-(4-Hydroxystyryl)-4,9-dimethoxy-5*H*-furo[3,2-*g*] chromen-5-one (2a)**

Yield, 70%; mp 142–143 °C; 1H NMR (DMSO-d6); δ 9.71 ( br.s., 1H, OH); 7.81 (br.s., 1H, furan CH=CHO); 7.62-7.00 (m, 7H, Ar-H, CH=CH, furan CH=CHO ); 6.21 (s, 1H, γ-pyrone H ); 4.10 (s, 3H,OCH3); 3.94 (s, 3H,OCH3). Anal. Calcd for $C_{21}H_{16}O_6$ (%): C, 69.23; H, 4.43. Found: C, 69.60; H, 4.50.

**4,9-Dimethoxy-7-(2-methoxystyryl)-5*H*-furo[3,2-*g*] chromen-5-one (2b)**

Yield, 60%; mp 234–235 °C; MS *m/z* (%); 378.00 (5.24, $M^+$); 364.20 (1.37); 350.20 (6.06); 293.20 (21.48); 240.15 (10.11); 205.05 (100.00); 177.05 (10.22); 175.10 (38.56); 77.05 (34.83). Anal. Calcd for $C_{22}H_{18}O_6$ (%): C, 69.83; H, 4.79. Found: C, 69.41; H, 5.12.

**4,9-Dimethoxy-7-(4-methoxystyryl)-5*H*-furo[3,2-*g*] chromen-5-one (2c)**

Yield, 65%; mp 155–156 °C; IR (KBr) $\nu_{max}$/cm$^{-1}$ , 1620 (C=O). $^1$H NMR δ 7.91 (d, 1H, furan CH=CHO, *J* = 2.4 Hz); 7.62-7.21 (m, 6H, Ar-H, CH=CH); 7.33 (d, 1H, furan CH=CHO, *J* = 2.4 Hz); 6.23 (s, 1H, γ-pyrone H ); 4.00 (s, 3H,$OCH_3$); 3.84 (s, 3H,$OCH_3$); 3.80 (s, 3H,$OCH_3$). Anal. Calcd for $C_{22}H_{18}O_6$ (%): C, 69.83; H, 4.79. Found: C, 69.41; H, 5.12.

**7-(3,4-Dimethoxystyryl)-4,9-dimethoxy-5*H*-furo [3,2-*g*]chromen-5-one (2d)**

Yield, 60%; mp 266–267 °C; $^1$H NMR δ 8.11 (d, 1H, furan CH=CHO, *J* = 2.3 Hz); 7.52-7.01 (m, 5H, Ar-H, CH=CH), 7.23 (d, 1H, furan CH=CHO, *J* = 2.3 Hz); 6.23 (s, 1H, γ-pyrone H); 4.17 (s, 3H, $OCH_3$); 3.94 (s, 3H, $OCH_3$); 3.84 (s, 3H, $OCH_3$); 3.80 (s, 3H, $OCH_3$). $^{13}$C NMR δ 177.3; 163.0; 160.4; 151.0; 149.6; 149.5; 149.2;146.7; 136.6; 136.0;128.4; 128.3; 122.8; 119.4; 118.6; 114.2; 112.2; 110.4; 109.9; 62.4; 62.2; 56.2; 56.1. Anal. Calcd for $C_{23}H_{20}O_7$ (%): C, 67.64; H, 4.94. Found: C, 67.21; H, 5.22.

**4,9-Dimethoxy-7-(3-nitrostyryl)-5*H*-furo[3,2-*g*] chromen-5-one (2e)**

Yield, 55%; mp 170–172 °C; IR (KBr) $\nu_{max}$/cm$^{-1}$ 1635 (C=O); 1542, 1382 ($NO_2$). $^1$H NMR δ 7.71 (d, 1H, furan CH=CHO *J* = 2.4 Hz); 7.72-7.22 (m, 6H, Ar-H, CH=CH); 7.23 (d, 1H, furan CH=CHO, *J* = 2.4 Hz); 6.26 (s, 1H, γ-pyrone H ); 4.10 (s, 3H,$OCH_3$); 3.94 (s, 3H,$OCH_3$). Anal. Calcd for $C_{21}H_{15}NO_7$ (%): C, 64.12; H, 3.84; N, 3.65. Found: C, 64.61; H, 3.42; N, 4.22

**4,9-Dimethoxy-7-(4-nitrostyryl)-5*H*-furo[3,2-*g*] chromen-5-one (2f)**

Yield, 60%; mp 246–247 °C; IR (KBr) $\nu_{max}$/cm$^{-1}$ 1630 (C=O); 1532, 1372 ($NO_2$). MS *m/z* (%); 396.20 (0.24, $M^+$+3), 395.20 (1.37, $M^+$+2); 394.20 (6.06, $M^+$+1); 393.20 (29.48, $M^+$); 350.15 (15.11); 205.05 (38.10),; 177.05 (100.00); 176.10 (38.56); 77.05 (33.83). Anal. Calcd for $C_{21}H_{15}NO_7$ (%): C, 64.12; H, 3.84; N, 3.65. Found: C, 64.51; H, 4.12; N, 4.20

**7-(4-(Dimethylamino)styryl)-4,9-dimethoxy-5*H*-furo3,2-*g*]chromen-5-one (2g)**

Yield, 55%; mp 75–76 °C; MS *m/z* (%); 391.11 (6.24, $M^+$); 390.20 (1.37); 350.20 (6.56); 312.20 (28.48); 210.15 (100.00); 205.05 (38.10); 177.05 (0.56);150.10 (38.56); 77.05 (23.83). Anal. Calcd for $C_{23}H_{21}NO_5$ (%): C, 70.58; H, 5.41; N, 3.58. Found: C, 71.01; H, 6.02; N, 4.22.

**7-(2-(Furan-2-yl)vinyl)-4,9-dimethoxy-5*H*-furo [3,2-*g*]chromen-5-one (2h)**

Yield, 60%; mp 205–206 °C; $^1$H NMR δ 8.11 (d, 1H, furan HC=CHO, *J* = 2.3 Hz); 7.86 (d, 1H, furan HC=CHO ,*J* = 2 Hz); 7.38 (d, 1H, HC=CH, *J* = 16 Hz); 7.23 (d, 1H, furan HC=CHO, *J* = 2.3 Hz); 6.94 (d, 1H, furan HC=CHO ,*J* = 2 Hz); 6.86 (d, 1H, HC=CH, *J* = 16 Hz); 6.66 (m, 1H, furan CH); 6.34 (s, 1H, γ-pyrone H); 4.15 (s, 3H,$OCH_3$); 3.92 (s, 3H,$OCH_3$). $^{13}$C NMR δ 177.4; 174.1; 159.6; 151.6(2C); 147.4; 147.3; 129.9(2C); 123.3; 119.4; 118.1; 114.9; 114.1; 113.4; 110.8; 105.8; 62.35; 62.13. Anal. Calcd for $C_{19}H_{14}O$ (%): C, 67.41; H, 4.17. Found: C, 67.01; H, 5.52.

**4,9-Dimethoxy-7-(2-(naphthalen-1-yl)vinyl)-5*H*-furo[3,2-*g*]chromen-5-one (2i)**

Yield, 70%; mp 176–177 °C; MS *m/z* (%); 401.05 (0.02, $M^+$+3); 400.20 (0.68, $M^+$+2); 399.20 (3.97, $M^+$+1); 398.20 (12.96, $M^+$); 383.15 (15.47); 250.10 (18.25); 249.15 (100.00); 177.05 (31.38); 176.10 (24.85). Anal. Calcd for $C_{25}H_{18}O_5$ (%): C, 75.37; H, 4.55. Found: C, 75.75; H, 5.12.

**4,9-Dimethoxy-7-(2-(naphthalen-2-yl)vinyl)-5*H*-furo[3,2-*g*]chromen-5-one (2j)**

Yield, 65%; mp 190–191 °C; IR (KBr) $\nu_{max}$/cm$^{-1}$ 1635 (C=O). Anal. Calcd for $C_{25}H_{18}O_5$ (%): C, 75.37; H, 4.55. Found: C, 75.78; H, 5.02.

**4,9-Dimethoxy-7-(2-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)vinyl)-5*H*-furo[3,2-*g*]chromen-5-one (2k)**

Yield, 55%; mp 152–153 °C; $^1$H NMR δ 8.01 (br. S., 1H, furan HC=CHO); 7.69-7.21 (m, 13H, Ar-H, CH=CH, furan HC=CHO ); 5.98 (s, 1H, γ-pyrone H); 4.01(s, 3H,$OCH_3$); 3.83 (s, 3H,$OCH_3$); 2.3 (s, 3H, $CH_3$). $^{13}$C NMR δ 186.1 (2C); 177.1; 164.7; 150.4; 149.1; 147.1(2C); 138.8 (2C); 130.8(2C); 130.2(2C); 129.6(2C); 129.2(2C);128.7(2C); 127.6(2C); 125.9; 120.04; 119.6(2C); 110.5; 105.7; 62.3; 61.7; 13.9. Anal. Calcd for $C_{31}H_{24}N_2O_5$ (%): C, 73.80; H, 4.79; N, 5.55. Found: C, 74.01; H, 5.02; N, 5.92.

**General method for synthesis of Schiff bases (3–5) (Scheme 1)**

A mixture of compounds **2h** and **2k** (0.1 mol) and the appropriate primary amine (0.1 mol) in absolute ethanol (20 mL) containing 5 drops of glacial acetic acid was refluxed for 8 h. The solvent was evaporated under reduced pressure and the obtained residue was treated with ice water . The formed precipitate was collected by filtration, washed with water, dried and Crystallized from aqueous ethanol to give compounds **3-5.**

***N*-(7-(2-(Furan-2-yl)vinyl)-4,9-dimethoxy-5*H*-furo [3,2-*g*]chromen-5-ylidene)pyridine-2-amine (3)**

Yield, 65%; mp 201–202 °C; MS *m/z* (%); 414.30 (0.25, $M^+$); 413.40 (0.48); 236.25 (14.06); 167.05 (12.24); 149.05 (35.77); 123.15 (14.22); 111.15 (19.77); 71.10 (34.55); 55.05 (100.00). Anal. Calcd for $C_{24}H_{18}N_2O_5$ (%): C, 69.56; H, 4.38; N, 6.76. Found: C, 69.91; H, 4.90; N, 7.02.

***N*-(7-(2-(Furan-2-yl)vinyl)-4,9-dimethoxy-5*H*-furo [3,2-*g*]chromen-5-ylidene)-4-methylaniline (4)**

Yield, 55%; mp 175–176 °C; IR (KBr) $\nu_{max}$/cm$^{-1}$ 1595 (C=N). $^1$H NMR (DMSO-$d_6$); δ 8.01 ( br.s., 1H, furan HC=CHO ); 7.9 ( br.s., 1H, furan HC=CHO); 7.7–6.9 (m, 8H, Ar-H, CH=CH, furan 2HC=CHO); 6.53 (s, 1H, γ-pyrone H); 6.32 (m, 1H, furan CH); 3.91(s, 3H,$OCH_3$); 3.85 (s, 3H,$OCH_3$); 2.3 (s, 3H, $CH_3$). Anal. Calcd for $C_{26}H_{21}NO_5$ (%): C, 73.06; H, 4.95; N, 3.28. Found: C, 73.42; H, 5.02; N, 3.70.

**4-(4,9-Dimethoxy-7-(2-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)vinyl)-5*H*-furo[3,2-*g*]chromen-5-ylideneamino) benzaldehyde (5)**

Yield, 65%; mp 145–146 °C; MS *m/z* (%); 608.05 (0.02, $M^+$+1); 607.21 (0.68, $M^+$); 599.20 (3.97); 508.20 (2.96); 483.62 (15.47); 250.12 (19.25); 240.15 (100.00);179.05 (26.38); 176.10 (25.85). Anal. Calcd for $C_{38}H_{29}N_3O_5$ (%): C, 75.11; H, 4.81; N, 6.92. Found: C, 75.60; H, 5.02; N, 7.22.

**General method for synthesis of 2-(7-( arylvinyl)-4,9-dimethoxy-5*H*-furo[3,2-*g*]chromen-5-ylidene) malononitrile (6a–c) (Scheme 2)**

A mixture of compounds **2c**, **2f** and **2k** (0.01 mol), malononitrile ( 0.66 g, 0.01 mol) and sodium acetate (3 g), in DMF (20 ml) was refluxed for 5 h. On cooling, the precipitated solid was filtered, dried and crystallized from chloroform to yield compound **6a-c**.

**2-(4,9-Dimethoxy-7-(4-methoxystyryl)-5*H*-furo[3,2-*g*]chromen-5-ylidene)malononitrile (6a)**

Yield, 55%; mp 120–121 °C; IR (KBr) $\nu_{max}$/cm$^{-1}$ 2211, 2205 (CN). $^1$H NMR δ 8.11 (d, 1H, furan CH=CHO, *J* = 2.5 Hz); 7.90-7.31 (m, 6H, Ar-H, CH=CH); 7.23 (d, 1H, furan CH=CHO, *J* = 2.5 Hz); 6.63 (s, 1H, γ-pyrone H ); 4.12 (s, 3H,$OCH_3$); 3.94 (s, 3H,$OCH_3$); 3.80 (s, 3H,$OCH_3$). Anal. Calcd for $C_{25}H_{18}N_2O_5$ (%): C, 70.42; H, 4.25; N, 6.57. Found: C, 70.81; H, 4.62; N, 7.02.

**2-(4,9-Dimethoxy-7-(4-nitrostyryl)-5*H*-furo[3,2-*g*] chromen-5-ylidene)malononitrile (6b)**

Yield, 75%; mp 257–258 °C; $^1$H NMR δ 7.81 (br.s., 1H, furan CH=CHO); 7.72-7.22 (m, 7H, Ar-H, CH=CH, furan CH=CHO); 6.56 (s, 1H, γ-pyrone H ); 4.20 (s, 3H,$OCH_3$); 4.00 (s, 3H,$OCH_3$).

Anal. Calcd for $C_{24}H_{15}N_3O_6$ (%): C, 65.31; H, 3.43; N, 9.52. Found: C, 65.71; H, 3.82; N, 10.02.

**2-(4,9-Dimethoxy-7-(2-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)vinyl)-5*H*-furo[3,2-*g*]chromen-5-ylidene)malononitrile (6c)**

Yield, 55%; mp 127–128 °C; IR (KBr) $\nu_{max}/cm^{-1}$ 2235, 2225 (CN). MS *m/z* (%); 553.01 (0.24, $M^+$+1), 552.20 (1.37, $M^+$); 395.20 (12.06); 360.20 (25.48); 343.15 (16.11); 205.25 (38.10); 201.05 (100.00); 176.11 (3.56); 76.05 (33.83). Anal. Calcd for $C_{34}H_{24}N_4O_4$ (%): C, 73.90; H, 4.38; N, 10.14. Found: C, 74.21; H, 4.63; N, 9.82.

**Biological Activities**

**Sulphorhodamine B (SRB) assay of cytotoxic activity**

This method was carried out according to the reported method (Skehan and Storeng, 1990) at Cancer Biology Department, National Cancer Institute, Cairo University. Cells were used when 90% confluence was reached in T25 flasks. Adherent cell lines were harvested with 0.025% trypsin. Viability was determined by trypan blue exclusion using the inverted microscope. Cells were seeded in 96-well micro titer plates at a concentration of 5x104-105 cell/well in a fresh medium and left to attach to the plates for 24 hours. After 24 hours, cells were incubated with the appropriate concentration ranges of drugs, completed to total of 200 µl volume/well using fresh medium and incubation was continued for 24, 48 and 72 hours. Control cells were treated with vehicle alone. For each drug concentration (5.0, 12.5, 25.0 and 50.0 µg/ml), 4 wells were used. Doxorubicin was used as a positive control at the same concentration range. Following 24, 48 and 72 hours treatment, the cells were fixed with 50 µl cold 50% trichloroacetic acid for 1 hour at 4 °C. Wells were washed 5 times with distilled water and stained for 30 min. at room temperature with 50 µl 0.4% SRB dissolved in 1% acetic acid. The wells were then washed 4 times with 1% acetic acid. The plates were air-dried and the dye was solubilized with 100 µl/well of 10 mM tris base (PH 10.5) for 5 min. on a shaker at 1600 rpm. The optical density (O.D.) of each well was measured spectrophotometrically at 564 nm with an ELIZA microplate reader. The mean background absorbance was automatically subtracted and mean values of each drug concentration was calculated. The experiment was repeated three times for each cell line. The percentage of cell survival was calculated as follows:

Survival fraction= O.D. (treated cells) / O.D. (control cells)

The relation between surviving fraction and drug concentration was plotted to get the survival curve. The concentration required for 50% inhibition of cell viability ($IC_{50}$) was calculated.

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