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, IC50 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.


Keywords: Furanochromones; Ammi visnaga L.; Khellin; Synthesis; Cytotoxicity.

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) antispasmodic, estrogenic (Brunton 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 spasmytic agents, and disodium chromoglycate acts as anti-allergic drug (Pawar et al., 2009). Khellin and visnagen (Figure 1) are natural furochrome 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 photochemotherapeutic treatment of vitiligo and psoriasis (Vedaldi et al., 1988). Also, khellin is used as a spasmytic 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 furochromenyl 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.

![Figure 1. Chemical structures of Visnagen and Khellin.](http://www.jofamericanscience.org)
clearly identified in the mass spectrum of compound 1. The molecular ion peak of 1372 cm$^{-1}$ showed characteristic absorption bands in the IR spectrum and the structure was further supported by vinyl protons at 7.52 ppm. Protons at C7 and the appearance of aromatic and 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 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 '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 substituent at C7 of the furochrome structure. Therefore, these results demonstrate the potential of the furochrome 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)$^a$

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (µg/ml)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>0.6</td>
</tr>
<tr>
<td>2k</td>
<td>2.1</td>
</tr>
<tr>
<td>5</td>
<td>2.3</td>
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<tr>
<td>6c</td>
<td>2.5</td>
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<td>2f</td>
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<td>3</td>
<td>3.9</td>
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<tr>
<td>2h</td>
<td>4.2</td>
</tr>
<tr>
<td>2i</td>
<td>4.2</td>
</tr>
<tr>
<td>2b</td>
<td>4.3</td>
</tr>
<tr>
<td>Khellin</td>
<td>4.65</td>
</tr>
<tr>
<td>2j</td>
<td>5.7</td>
</tr>
<tr>
<td>2c</td>
<td>7.5</td>
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<tr>
<td>2e</td>
<td>12.0</td>
</tr>
<tr>
<td>2d</td>
<td>12.2</td>
</tr>
</tbody>
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$^a$ 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-5H-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-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. 1H NMR (400 MHz, DMSO-d₆): δ 8.06 (br.s., 1H, furan CH=CHO); 7.18 (br.s., 1H, furan CH=CHO); 6.02 (s, 1H, γ-pyron H); 4.07 (s, 3H, OCH₃); 3.89 (s, 3H, OCH₃); 2.32 (s, 3H, CH₃). 13C 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 C₁₃H₁₂O₃: 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 1H and 13C 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 1H and 100 MHz for 13C. 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-5H-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-5H-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-pyron H); 4.10 (s, 3H, OCH₃); 3.94 (s, 3H, OCH₃). Anal. Calcd. for C₂₄H₁₇O₅: C, 69.23; H, 4.43. Found: C, 69.60; H, 4.50.

4,9-Dimethoxy-7-(2-methoxystyryl)-5H-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 C₁₃H₁₅O₅: C, 69.83; H, 4.79. Found: C, 69.41; H, 5.12.

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

Yield, 65%; mp 155–156 °C; IR (KBr) \( \nu_{max}/cm^{-1} \); 1620 (C=O). 1H NMR δ 8.11 (d, 1H, furan CH=CHO, J = 2.3 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-pyron H); 4.00 (s, 3H, OCH₃); 3.84 (s, 3H, OCH₃); 3.80 (s, 3H, OCH₃). 13C NMR δ 173.7; 163.0; 160.4; 151.0; 149.6; 149.5; 149.2; 146.7; 136.3; 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 C₁₃H₁₅O₅: C, 67.64; H, 4.94. Found: C, 67.21; H, 5.22.

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

Yield, 55%; mp 170–172 °C; IR (KBr) \( \nu_{max}/cm^{-1} \); 1635 (C=O); 1542, 1382 (NO). 1H 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-pyron H); 4.10 (s, 3H, OCH₃); 3.94 (s, 3H, OCH₃). Anal. Calcd. for C₁₃H₁₅NO₅: 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)-5H-furo[3,2-g]chromen-5-one (2e)

Yield, 60%; mp 246–247 °C; IR (KBr) \( \nu_{max}/cm^{-1} \); 1630 (C=O); 1532, 1372 (NO). 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 C₁₃H₁₅NO₅: 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-5H-furo[3,2-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 C₂₃H₂₂NO₅ (%): C, 70.58; H, 5.41; N, 3.58. Found: C, 71.01; H, 6.02; N, 4.22.

7-(2-(Furan-2-ylvinyl)-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-ylidene)malononitrile (6a) (Scheme 2)

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.

7-(2-(2-Furanylvinyl)-1H-pyrazol-4-yl)-4,9-dimethoxy-5H-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 C₂₃H₂₄N₂O₅ (%): C, 69.56; H, 4.38; N, 6.76. Found: C, 69.91; H, 4.90; N, 7.02.

N-(7-(2-(Furan-2-ylvinyl)-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-ylidene)-4-methylamino)benzaldehyde (5)

Yield, 65%; mp 175–176 °C; IR (KBr) \( \nu_{\text{max}}/\text{cm}^{-1} \) 1595 (C=N). \( ^1H \) NMR (DMSO-d₆); \( \delta \) 8.01 (br.s., 1H, furan H-CHO); 7.9 (br.s., 1H, furan H-CHO); 7.66–7.87 (m, 8H, Ar-H, CH=CH), furan H-CHO); 6.53 (s, 1H, \( \gamma \)-pyrone H); 6.32 (m, 1H, furan CH); 3.91 (s, 3H, OCH₃); 3.85 (s, 3H, OCH₃); 2.3 (s, 3H, CH₃). Anal. Calcd for C₂₃H₂₅N₂O₅ (%): C, 73.06; H, 4.95; N, 3.28. Found: C, 73.42; H, 5.02; N, 3.70.

General method for synthesis of 2-(7-(arylvinylnyl)-4,9-dimethoxy-5H-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-methoxy styryl)-5H-furo[3,2-g]chromen-5-ylidene)malononitrile (6a)

Yield, 55%; mp 120–121 °C; IR (KBr) \( \nu_{\text{max}}/\text{cm}^{-1} \) 2211, 2205 (CN). \( ^1H \) NMR \( \delta \) 8.11 (d, 1H, furan CH=CHO); 7.69–7.21 (m, 13H, Ar-H, CH=CH, furan H-CHO); 5.98 (s, 1H, \( \gamma \)-pyrone H); 4.01 (s, 3H, OCH₃); 3.83 (s, 3H, OCH₃); 2.3 (s, 3H, CH₃). \( ^13C \) NMR \( \delta \) 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 C₂₃H₂₄N₂O₅ (%): 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.

\( \nu_{\text{max}}/\text{cm}^{-1} \) 1595 (C=N). \( ^1H \) NMR (DMSO-d₆); \( \delta \) 8.01 (br.s., 1H, furan H-CHO); 7.9 (br.s., 1H, furan H-CHO); 7.66–7.87 (m, 8H, Ar-H, CH=CH), furan H-CHO); 6.53 (s, 1H, \( \gamma \)-pyrone H); 6.32 (m, 1H, furan CH); 3.91 (s, 3H, OCH₃); 3.85 (s, 3H, OCH₃); 2.3 (s, 3H, CH₃). Anal. Calcd for C₂₃H₂₅N₂O₅ (%): C, 73.06; H, 4.95; N, 3.28. Found: C, 73.42; H, 5.02; N, 3.70.
3. Following 24, 48 and 72 hours treatment, the percentage of cell survival was calculated as follows:

\[
\text{Survival fraction} = \frac{\text{O.D. (treated cells)}}{\text{O.D. (control cells)}}
\]

4. 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\text{50}) was calculated.

5. The experiment was repeated three times for each cell line. The mean background absorbance was calculated. The mean background absorbance was measured spectrophotometrically at 564 nm with an ELIZA microplate reader. The mean background absorbance was calculated.

6. Cells were treated with vehicle alone. For each drug concentration (5.0, 12.5, 25.0 and 72 hours) control cells were treated with vehicle alone. For each drug concentration (5.0, 12.5, 25.0 and 72 hours) control cells were treated with vehicle alone.

7. 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\text{50}) was calculated.

**Biological Activities**

Sulphorhodamine B (SRB) assay of cytotoxic activity

This method was carried out according to the reported method (Skene et al., 1990) at Cancer Biology Department, National Cancer Institute, Cairo University. Cells were plated 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 5×10⁴–10⁵ 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 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 0.2% SRB dissolved in 1% acetic acid. The wells were then washed 4 times with 1% acetic acid. The plates were air-dried at 4 °C 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 were calculated. The experiment was repeated three times for each cell line. The percentage of cell survival was calculated as follows:

**Acknowledgement**

We are grateful to the Cancer Biology Department, National Cancer Institute, Cairo University for the pharmacological evaluation.

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**References**

10. Farag AM; Mayhoub AS; Eldebss TM; Amr AG; Ali KA; Abdel-Hafez NA; Abdulla MM.


17. Pawar M J; Burungale A B; Karale B K. Synthesis and antimicrobial activity of spiro[chromeno[4,3-d][1,2,3]thiadiazole-4,1'-cyclohexane, spirolchromeno[4,3-d][1,2,3]selenadiazole-4,1'-cyclohexane and spirolchroman-2,1'-cyclohexan]-4-one-5-spiro-

4-acetyl-2-(acetylamino)-\Delta^2\text{-}1,3,4-thiadiazoline compounds. Arkivoc, (xiii), 2009, 97-107.


20. Skehan P; Storeng R; Scudiero D; Monks A; McMahon J; Vistica D; Warren JT; Bokesch H; Kenney S; Boyd M R. New colorimetric cytotoxicity assay for anticancer drug screening. J Natl Cancer Inst1990: 82: 1107-12.


24. Vedaldi D; Caflleri S; Dall’Acqua F; Andrea L; Bovalini L; Martelli P. Khellin, a naturally occurring furochromone, used for the photochemotherapy of skin diseases: mechanism of action. Farmaco 1988: 4: 333-46.