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Research Article

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Synthesis of novel [4, 9-dimethoxy-5H-furo [3, 2-g] chromen-5-one] derivatives with antiproliferative potency towards breast and hepatic cancer cell lines

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Abstract Chromen derivative (2) was obtained by the reaction of [cyclohexyl isocyanide, furochromone carbaldehyde (1), cyclohexane-1, 3-dione]. Also, reaction of compound (2) in the presence of semicarbazide hydrochloride to proceed the compound (3) which react with selenium dioxide to form compounds (4). In one-step, the condensation of cyclohexyl isocyanide, furochromone carbaldehyde (1) and pentane-2-dionein pipredine was given compound (5). The mixture of furochromone carbaldehyde (1), barbaturic/or thio-barbaturic acid and cyclohexyl isocyanide was afforded the corresponding compounds ($6_{a,b}$). While the reaction of furochromonecarbaldehyde (1), Meldrum acid and cyclohexyl isocyanide was yielded the compound (7). The condensation of furochromonecarbaldehyde (1), 1, 3-dicarbonyl compound (cyclohexane-1, 3-dione) with acid derivatives (carboxylic, chlorocarboxylic and cinnamic acid) afforded the corresponding compounds (8a-c). The reaction of furochromonecarbaldehyde (1), dimethyl acetylene dicarboxylate, cyclohexyl isocyanide with 1, 3-dicarbonyle was afforded compound (9). Some of the newly synthesized derivatives showed a reasonable antiproliferative activity towards breast (MCF7) and liver (HEPG2) cancer cell lines in comparison to traditional anticancer agents: 5-Fluorouracil & Doxorubicin.

Keywords Chromen derivatives, formyl furochromone, semicarbazide hydrochloride, cyclohexyl isocyanide, HEPG2 & MFC7 cancer cell lines

Introduction

It has been important for the preparation of organic compounds [1] with using new methods, with expected biologically activities, which lead for new compounds in short time and good yield and easy method to yield the desired products without waste. The reactions with more than one compound [2] have an important role in these reactions. These reactions have been used in both liquid and solid phase and developed heterocyclic compounds with pharmaceutical effect using formyl furochromone [3-6]. Isocyanide-new method for the synthesis of hetero compounds. The potency of some traditional antitumor drugs is somewhat limited to their toxicity in rapidly growing cancer cells that might gain resistance to those drugs. For that and with the objective of synthesizing new anticancer agents with significant activity. Cancer is one of the most widespread serious diseases. It is characterized by uncontrolled growth of abnormal cells. The growth and metastasis of cancer cells are dependent on angiogenesis. Therefore, affecting angiogenesis will be of great importance in inhibition of tumor growth, invasion, and metastasis [7]. The present work studies the antiproliferative effect of the newly synthesized compounds towards hepatic and breast cultured cell lines (HEPG2 and MFC7).

Material and methods

A-Chemistry

All melting points are uncorrected and were taken on electro-thermal capillary melting point apparatus. The melting points were measured in degrees centigrade and determined using Bauchi 510 apparatus. Elemental analyses were carried out in the micro analytical unit of the National Research Centre. IR spectra were recorded on a Mattson-5000 FTIR spectrometer using KBr Wafer technique. 1H-NMR spectra were determined on a Varian-Gemini-300 MHz and Jeol-Ex-300 MHz NMR spectrometer using TMS as an internal standard with (chemical shift. δ= 0 ppm). Mass Spectra were determined on Finnegan MatSSQ 7000 mode: EI, 70Ev (Thermo Inst. Sys. Inc., USA). The purity of the synthesized compounds was tested by thin layer chromatography (TLC), Merck plates. TLC Silica gel 60 F254 25 Aluminum sheets 20 x 20 cm.

General procedure for the preparation of 2-(cyclohexylamino)-6, 7-dihydro-3-(4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl)-6, 6-dimethyl-1H-indol-4(5H)-one (2)

The reaction mixture ofcyclohexane-1, 3-dione (10mmol), furochromone carbaldehyde (1) (10mmol), cyclohexyl isocyanide (10mmol), ammonium acetate (15mmol) and KHSO4 in acetonitrile (5ml) was refluxed for 4h. After completion of the reaction, acetonitrile was removed in vacuo and then diethyl ether was added to the solid residue to separate the catalyst from the mixture. The filtrate was washed with $NaHCO_3$ (5ml) and dried over $MgSO_4$. The product was recrystallized from CH_2Cl_2-EtOH (1:2) to give the pure product (2).

Orange solid, m.p. 120-125 ^oC. yield (75%). Analysis for: $C_{29}H_{32}N_2O_6$, Mol. Wt.: 504.6, calc.: C, 69.03; H, 6.39; N, 5.55, found: C, 69.05; H, 6.38; N, 5.54; IR (KBr, cm^{-1}): 1613; 1687 (2C=O) and 3220, 3336 (2NH). ^1H NMR (DMSO-d6, δ, ppm): 1.01 (ss, 6H, $2CH_3$); 1.87-2.49 (m, 14H, $5CH_2$ cyclohexane, $2CH_2$); 3.34 (d, 1H, NCH, cyclohexane); 3.80, 3.85 (ss, 6H, $2OCH_3$); 6.93, 7.31(dd, 2H, J=2.0, furan ring); 8.23(s, 1H, H_7) and 8.99, 9.55 (dd, 2H, 2NH exchangeable with D_2O).

General procedure of synthesis of compounds (3, 4):

(12mmol) of Semi carbazide hydrochloride and(15mmol) of sodium acetate were dissolved in (35ml) dist. water, 10mmol of **2**, dissolved in ethanol then added to the previous mixture drop by drop, warm to 80^oC for 6-8h, poured the product on ice to get the product filtrate it (3) then crystallized from ethanol. Dissolve comp. (3) in glacial acetic and warm under stirring to >60^oC add Selenium hydroxide (7mmol) during 30 min under stirring and heating for 1-2h to get compound (4).

1-(2-(cyclohexylamino)-6, 7-dihydro-3-(4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl)-1H-indol-4(5H)-ylidene) semi carbazide (3)

Crystallized from ethanol, beige solid, and m.p.135ºC yield (60%). Analysis for $C_{28}H_{31}N_5O_6$, Mol. Wt.: 533.58, found: 533.23 calcd: C, 68.05; H, 5.92; N, 5.88, found, 68.03; C, 5.93; N, 5.89 IR (KBr, cm^{-1}): 1613, 1687 (2C=O)) and 3127, 3336 (2NH), 3455 (NH_2). ^1H NMR (DMSO-d6, δ, ppm):1.01-1.75 (m, 16H, $8CH_2$ cyclohexane), 2.65 (m, 1H, NCH, cyclohexane) ,3.86, 3.96, (ss, 6H, $2OCH_3$), 6.93, 7.31, (dd, 2H, J=2.0, furan ring), 8.23 (s, 1H, H_7) and 4.01; 8.34; 9.11 (sss, 4H, 2NH, $1NH_2$ exchangeable with D_2O).

6-(4-(cyclohexylamino)-5a,6,7,8-tetrahydro-3aH- [1,2,3] selenadiazino[6,5,4-cd] indol-3a-yl)-4,9-dimethoxy-5H-furo[3,2-g] chromen-5-one (4)

Crystallized from ethanol, brown solid, and m.p. 2100 ºC yield (60%). Analysis for $C_{27}H_{28}N_4O_5Se$, Mol. Wt. 568.12, found 567.5 calcd: C, 57.14; H, 4.94; N, 9.87; Se, 13.91, found: C, 57.15; H, 4.92; N, 9.88; Se, 13.88. IR (KBr, cm^{-1}): 1613, (C=O)) and 3312 (NH). ^1H NMR (DMSO-d6, δ, ppm): 1.01-1.75 (m, 16H, $8CH_2$ cyclohexane), 2.00; 2.75 (ss, 2H, NCH, cyclohexane), 3.96, 4.06, (ss, 6H, $2OCH_3$), 6.63, 7.01, (dd, 2H, J=2.0, furan ring), 8.21 (s, 1H, H7) and 4.00; (s, 1H, 1NH, exchangeable with D_2O).

4-acetyl-1-cyclohexyl-5-hydroxy-3-(4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl)-5-methyl-1H-pyrrol-2(5H)-one (5)

To a mixture of 1,3-dicarbonyl compound (pentane-2-dione), (1.0 mmol) and pipredine (0.5 mmol) in 3 mL dry toluene at -15 to -10 ºC, a solution of formyl furochromone (1) (1.0 mmol) in 1 ml dry toluene was added slowly and the mixture was stirred at -10 ºC for 12 h. Then, the resulting solution will be warm and a solution of cyclohexyl

isocyanide (0.5 mmol) in 1 mL dry toluene was added. The reaction mixture was heated at 100 ºC for 12 h. The solvent and most of the pipredine were evaporated under reduced pressure and the residue was purified by diethyl ether.

Buff solid, m.p. 192ºC yield (80%). Analysis for: $C_{26}H_{27}NO_8$, Mol. Wt.: 481.49, calc.: C, 64.86; H, 5.65; N, 2.91, found: C, 64.88; H, 5.64; N, 2.90. IR (KBr, cm^{-1}): 1658; 1545; 1735 (3C=O) and 3454 (OH). 1H-NMR (DMSO-d6, δ, ppm): 1.13, 2.46 (ss, 6H, $2CH_3$); 1.16- 1.24 (m, 10H ,5 CH_2- cyclohexane); 3.43 (s, 3H, CH, O=C-CH_3); 3.93, 4.08 (ss, 6H, $2OCH_3$); 7.03, 7.81 (dd, 2H, J=2.0, furan ring) and 8.53 (s, 1H, H_7); 8.80 (s, 1H OH exchangeable with D_2O)

General procedure of synthesis of compounds (6a, b, 7)

To a solution of (0.5g) of barbaturic and/ or thiobarbituric and/or Meldrum acid, (10mmol) of furochromone carbaldehyde (1) and (10mmol) of cyclohexyl isocyanide were dissolved in (0.3g) Toluene-4-sulfonic acid monohydrate in dichloromethane. The resulting mixture was stirred at room temperature till compilation of the reaction as indicated by TLC, the reaction mixture washed with water then recrystallized from CH_2Cl_2/n-hexane (2:1) to give the product.

6-(cyclohexylamino)-5-(4, 9-dimethoxy-5-oxo-5H- furo [3,2-g] chromen-6-yl) furo [2,3-d] pyrimidine-2,4 (1H, 3H) -dione (6a):

Crystallized from CH_2Cl_2/n-hexane (2:1), break red solid, m. p. 112 ºC yield (95%). Analysis for: $C_{25}H_{23}N_3O_8$, Mol. Wt. 493.47, found, 493.15 calcd C, 66.95; H, 6.05; N, 2.90, found: C, 66.96; H, 6.06; N, 2.88. IR (KBr, cm_{-1}): 1617.02; 1648.84, 1742.37 (3C=O), 3210.32; 3300.06; 3433.00 (3NH). ^{1}H NMR (DMSO-d6, δ, ppm): 1.20- 1.84 (m, 8H, 4 CH_2- cyclohexane), 2.48 (m, 1H, CH, cyclohexane), 3.87; 4.00 (ss, 6H, $2OCH_3$), 4.90; 5.72, 11.10 (sss, 3H, 3NH exchangeable with D_2O), 7.02; 8.10, (dd, 2H, J=2.0, furan ring) and 8.55(s, 1H, H_7)

6-(cyclohexylamino)-2, 3-dihydro-5-(4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl)-2-thioxofuro [2, 3-d] pyrimidin-4(1H)-one (6b):

Crystallized from CH_2Cl_2/n-hexane (2:1), orange solid, m.p. 147 ºC yield 90%). Analysis for: $C_{25}H_{23}N_3O_7S$ Exact Mass: 509.13 Mol. Wt.: 509.53 found, 509.13 calcd C, 66.95; H, 6.05; N, 2.90, found: C, 66.94; H, 6.05; N, 2.91. IR (KBr, cm^{-1}): 1619.92(C=S), 1678.04; 1702.10 (2C=O), 3119.30; 3397.96; 3420.14 (3NH). ^{1}H NMR (DMSO-d6, δ, ppm): 1.18- 1.64 (m, 8H, 4 CH_2- cyclohexane), 2.58 (m, 1H, CH, cyclohexane), 3.97; 4.01 (ss, 6H, $2OCH_3$), 4.60; 6.00; 10.00 (sss, 3H, 3NH exchangeable with D_2O), 7.02; 8.10, (dd, 2H, J=2.0, furan ring) and 8.55 (s, 1H, H_7).

6-(2-(cyclohexylamino)-4, 5-dihydro-5-oxofuran-3-yl)-4, 9-dimethoxy-5H-furo [3, 2-g] chromen-5-one (7)

Buff solid, m.p. 205ºC yield (90%). Analysis for: $C_{23}H_{23}NO_7$, Mol. Wt. 425.4, calc.: C, 64.93; H, 5.45; N, 3.29, found: C, 64.90; H, 5.44; N, 3.30. IR (KBr, cm^{-1}): 1588, 1684 (2C=O); 3431(NH). ^{1}H NMR (DMSO-d6, δ, ppm): 1.10- 1.76 (m, 10H ,5 CH_2- cyclohexane); 3.93 (d, 1H ,CH-N, cyclohexane); 3.32 (ss, 2H, CH_2); 4.07, 4.09 (ss, 6H, $2OCH_3$); 7.28, 8.13 (dd, 2H, J=2.0, furan ring); 8.76 (s, 1H, H_7) and 10.08 (dd, 1H, NH exchangeable with D_2O).m/e: 425 (15%), 246(90%), 181(9%), 98(10%), 82(7%).

General procedure to preparation of 2-(cyclohexylamino)-3-arylindeno [1,2-b] furan-4-ones (8a-c)

A mixture of formyl furochromone (1) (10 mmol), 1, 3-indandione (1mmol) and acid derivatives (1mmol) in water (5ml) in the presence of tetraethyl ammonium chloride (10mol %) was stirred at 80 ◦C for 5 h. The progress of the reactions was monitored by TLC (ethyl acetate: n-hexanes 1:3). After cooling to room temperature, the resulting precipitate was filtered and washed with water. The solid was dried and crystallized from CH_2Cl_2: EtOH (1:2) to give desired product. TLC analysis of the final products (ethyl acetate: n-hexanes 1:3) indicated the compounds were of high purity for all analytical purposes.

2-(1-(4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl)-2-benzaldehyde)-5, 5-dimethyl cyclohexane-1, 3-dione (8a)

Pale yellow solid, m.p.150-154 ºC yield (90%). Analysis for: $C_{29}H_{24}O_8$, Mol. Wt.: 500.5, calc.: C, 69.59; H, 4.83, found: C, 69.61; H, 4.81. IR (KBr, cm^{-1}): 1614; 1657, 1694 (4C=O); 1H-NMR (DMSO-d6, δ, ppm): 0.86, 0.98 (ss, 6H, $2CH_3$); 2.11- 2.39 (m, 4H ,$2CH_2$- dimidone); 3.77, 4.31 (ss, 6H, $2OCH_3$); 7.47, 7.88 (dd, 2H, J=2.0, furan ring); 7.42- 7.86 (m, 5H, arom); 7.91 (s, 1H, H_7), m/e: 500(12%), 256(10%), 246(15%), 140(8%), 106(90%).

2-(1-(4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl)-2-chlorobenzaldehyde)-5, 5-dimethyl cyclohexane-1, 3-dione (8b):

Buff solid, m.p.176-180 ºC yield (90%). Analysis for: $C_{29}H_{23}ClO_8$, Mol. Wt.: 534.94, calc.: C, 65.11; H, 4.33; Cl, 6.63, found: C, 65.13; H, 4.31; Cl, 6.63. IR (KBr, cm^{-1}): 1614, 1656, 1696 (4C=O); ^{1}H NMR (DMSO-d6, δ, ppm): 0.84, 0.99 (ss, 6H, $2CH_3$); 4.05, 4.09 (ss, 6H, $2OCH_3$); 7.26, 8.00 (dd, 2H, J=2.0, furan ring); 7.29-7.66 (m, 4H, arom.); 8.01 (s, 1H, H_7) and 10.00 (s, 1H, OH exchangeable with D_2O).

2-(1-(4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl)-2-cinnamaldehyde)-5, 5-dimethyl cyclohexane-1, 3-dione (8c):

Buff solid, m.p.148-150^{0}C yield (55%). Analysis for: $C_{31}H_{26}O_8$, Mol. Wt.: 526.53, found: C, 70.71; H, 4.98, calc.: C, 70.72; H, 4.97. IR (KBr, cm^{-1}) 1619,1658, 1712, (4C=O), ^{1}H NMR (DMSO-d6, δ, ppm): 0.88, 0.97 (ss, 6H, 2 CH_3) 2.49- 2.41 (m, 4H ,$2CH_2$- cyclohexane); 3.75, 3.99 (ss, 6H, $2OCH_3$); 3.97 (m, 1H ,CH, cyclohexane); 5.80, 6.61 (ss, 2H, CH= CH); 7.02, 8.00 (dd, 2H, J=2.0, furan ring); 7.47-7.88 (m, 5H, arom.); 7.91 (s, 1H, H_7).

General procedure of synthesis of comp. (9)

A mixture of 2-aminophenol (0.25mmol) and (0.3g) of formyl furochromone (1) dissolved in methanol was added in a 25ml round flask and the mixture was stirred for 30 min at room temperature. Then adding (0.25g) of the acid to the mixture followed by stirring for another 5 mmin. Finally, (0.23 mmol) of isocyanides was added. After the resultant mixture was stirred at room temperature for 1-7days, solid K_2CO_3 (50 mg) was added. After the resultant mixture was stirred at room temperature for 8-12 h the reaction mixture was quenched by water and the organic layer was extracted with EtOAc.

Dimethyl 2-(N-cyclohexyl-N-((4,9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl) methyl) amino) -5, 6, 7, 8-tetrahydro-5-oxo-4H-chromene-3, 4- (9):

Crystallized from EtOAc., brown solid, m. p. 210 ºC yield (55%). Analysis for: $C_{33}H_{35}NO_{11}$, Mol. Wt.: 621.63 found, 621.22 calcd C, 66.95; H, 6.05; N, 2.90, found: 66.94; H, 6.04; N, 2.92. IR (KBr, cm^{-1}): 1613.16, 1647.88; 1865.79, (4C=O). ^{1}H NMR (DMSO-d6, δ, ppm): 1.39- 2.64 (m, 16H, 8 CH_2-cyclohexane), 2.75 (m, 1H, CH, cyclohexane), 3,20 (s, 2H, CH_2), 3,90 (s, 1H, CHCO), 3.87; 4.00 (ss, 12H, $4OCH_3$), 7.02; 8.00 (dd, 2H, J=2.0, furan ring), 8.05 (s, 1H, H_7) and 2.00 (s, 1H, OH exchangeable with D_2O).

B- Biological activity

Materials and Methods

1-Pharmacological Screening

Measurement of Potential Cytotoxicity by Sulforhodamine B (SRB) Assay

The selected derivatives (compounds 2, 4, 5, 6a and 7) were subjected to a screening system for evaluation of their antitumor activity against liver and breast (HEPG2 and MFC7) cancer cell lines in comparison to 5-FU and DOX as reference drugs. Potential cytotoxicity of the compounds in this study was investigated using the method of Skehan *et al* [8]. Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the compounds to allow attachment of cells to the wall of the plate. Different concentrations of the compound under test (0, 1, 2.5, 5, 10 µg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in an atmosphere of 5% CO_2. Cultures were then fixed with trichloro acetic acid and stained for 30 min with 0.4% (w/v) Sulforhodamine B (SRB) dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and protein-bound dye was extracted with 10 mm unbuffered tris base (tris hydroxyl methyl amino methane, Sigma-Aldrich, Taufkirchen, Germany) for determination of optical density in a computer-interfaced, 96-well micro titer plate reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve of both cancer cell lines after the specified compound.

2-Biochemical Analysis

Male albino mice weighing 18–20 g was used in the present study. Mice were divided into three main groups as follows: Untreated or control group (5 mice each), the second group is, divided into two subgroups (5 mice for each

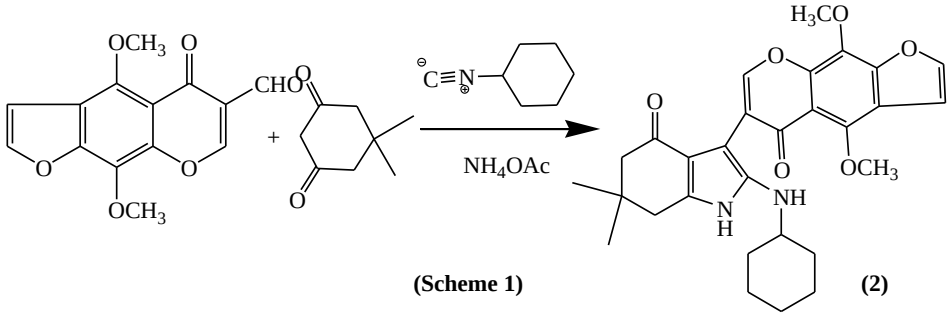
subgroup) and treated with 5-FU or DOX as reference anticancer drugs and the third group is divided into nine subgroups (5 mice for each subgroup) which was treated with the selected derivatives. In the control group each mouse was given a single intraperitoneal (i.p.) injection of 0.1 mL DMSO while the second and the third groups were given a single i.p. injection of 0.1 mL containing 12 mg/kg body weight of the standard or tested compounds. 5-FU or DOX was dissolved in sterile water and the synthesized.

Compounds were dissolved in DMSO. Blood was collected after 7 days from all mice groups. The biochemical effects of selected compounds which shows antiproliferative potency, on some liver enzymes such as aspartate, alanine aminotransferases (AST and ALT) [9] and alkaline phosphatase (ALP) [10], were analyzed using a blood auto analyzer (Olympus AV 400, Tokyo, Japan). Moreover, albumin [11], globulins [12], creatinine [13], total lipids [14], cholesterol [15], triglycerides and bilirubin [16] in serum of mice were evaluated in comparison to 5-FU and DOX. Statistical analysis of the results was performed using Chi-square values (SPSS computer program, IBM Corporation, New York, United States.

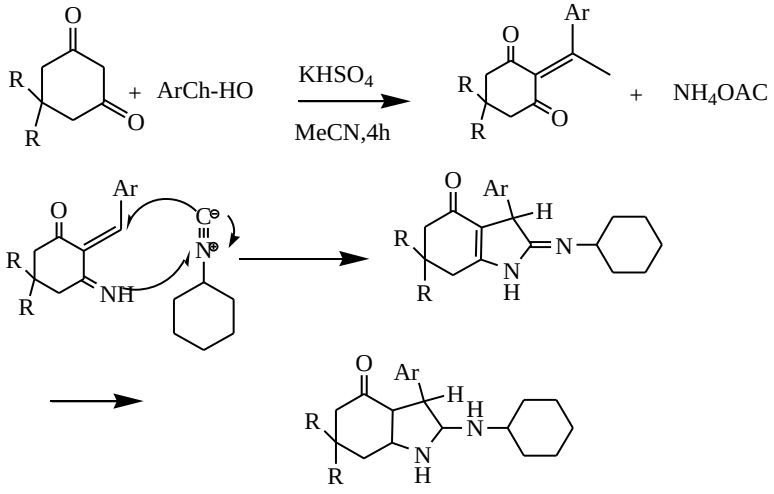
Result and Discussion

A-Chemistry

There had been interest for the preparation of organic compounds [2] with expected biologically activity. This prompted us to design and synthesis new furochromone derivatives to study their antitumor activities. A synthesis compound (2) was obtained at the reaction of isocyanide derivative, furochromone carbaldehyde (1), cyclohexane-1, 3-dione (scheme 1). The IR spectrum was showed the absence of peak for the carbonyl band of the (CHO gp). ^{1}H NMR spectra were characterized by the presence of 2H proton for (2NH gps). The advantages of the present procedure were experimental simplicity, easy work-up, use of an easy to handle and safe catalyst, and high yields of products.

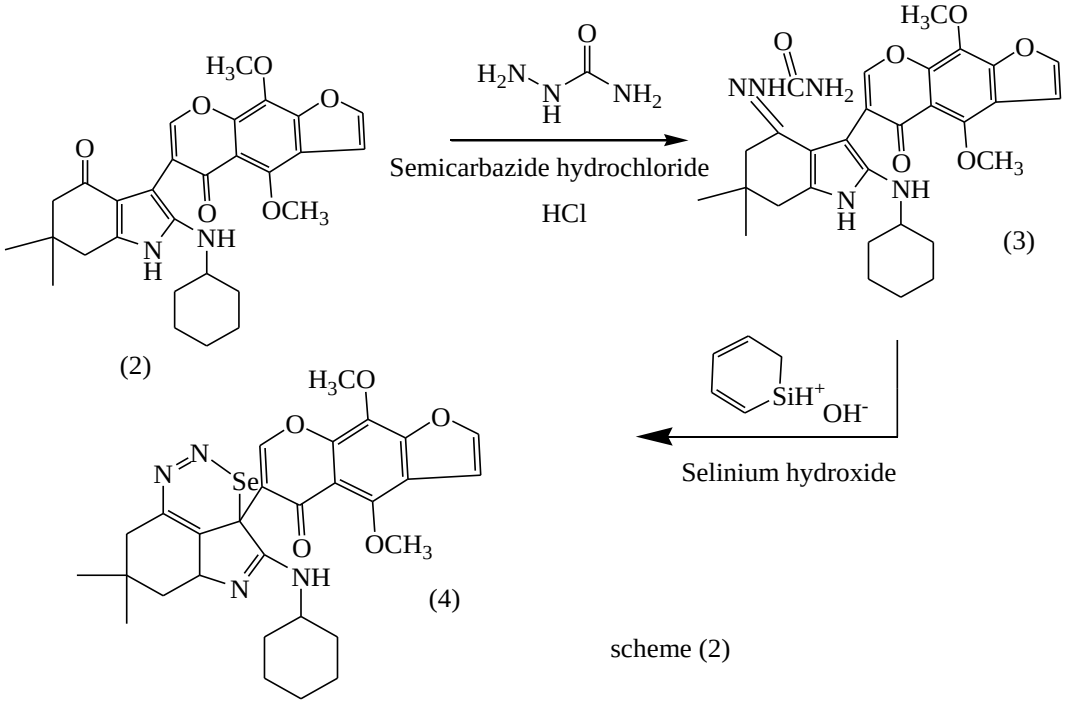


(Scheme 1) **(2)**



mechanism of compound 2

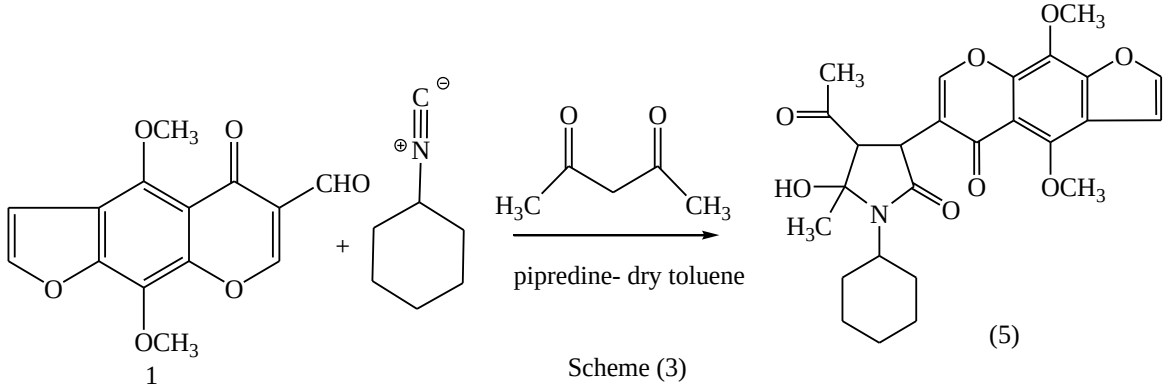
A side reaction on compound (2) with semi carbazide hydrochloride (3) followed by reaction with selenium dioxide to get compounds (4) (scheme 2).



(2) (3) (4)

scheme (2)

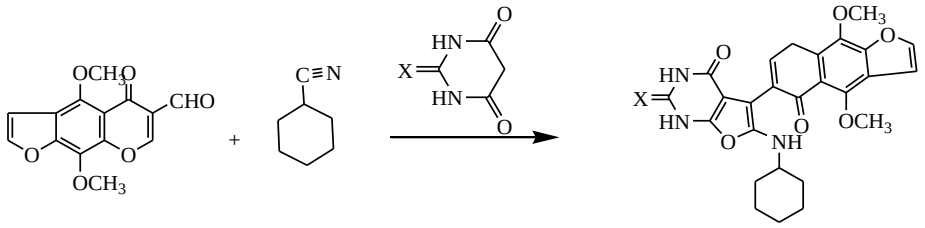
The reaction of more than two material in one step occurred at the using of [cyclohexyl isocyanide and formyl furochromone and pentane-2-dione] was reacted together in the presence of pipredine to give the corresponding [5-hydroxy-2H-pyrrol-2-one derivatives] (5) (scheme 3). The structure of (5) was deduced from its IR and ^{1}H NMR spectroscopic data. The IR spectrum was showed the absence of the peak for (CHO gp); ^{1}H NMR spectra were characterized by the presence of 3H proton for (CH_3CO gps), 3H proton of (CH_3 gp) and 1Hproton of (OH gp)



1 (5)

Scheme (3)

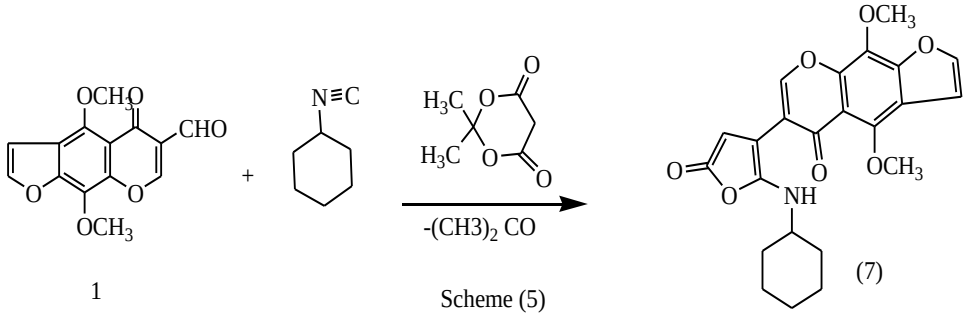
The pyrimidine derivatives have been a wide range of biological activities [17]. So, the synthesis of furo pyrimidine and 2-aminofuran derivatives has received little attention, A reaction of formyl-furochromone (1) with acid derivatives (barbaturic and /or thiobarbituric) and cyclohexyl isocyanide were afforded compounds (6**a, b**) (scheme 4). The IR characterized by presence of (3NH gps) also ^{1}H NMR spectra were characterized by the presence of 3H proton for (3NH gps).



1 6a, x= O, 6b,= S

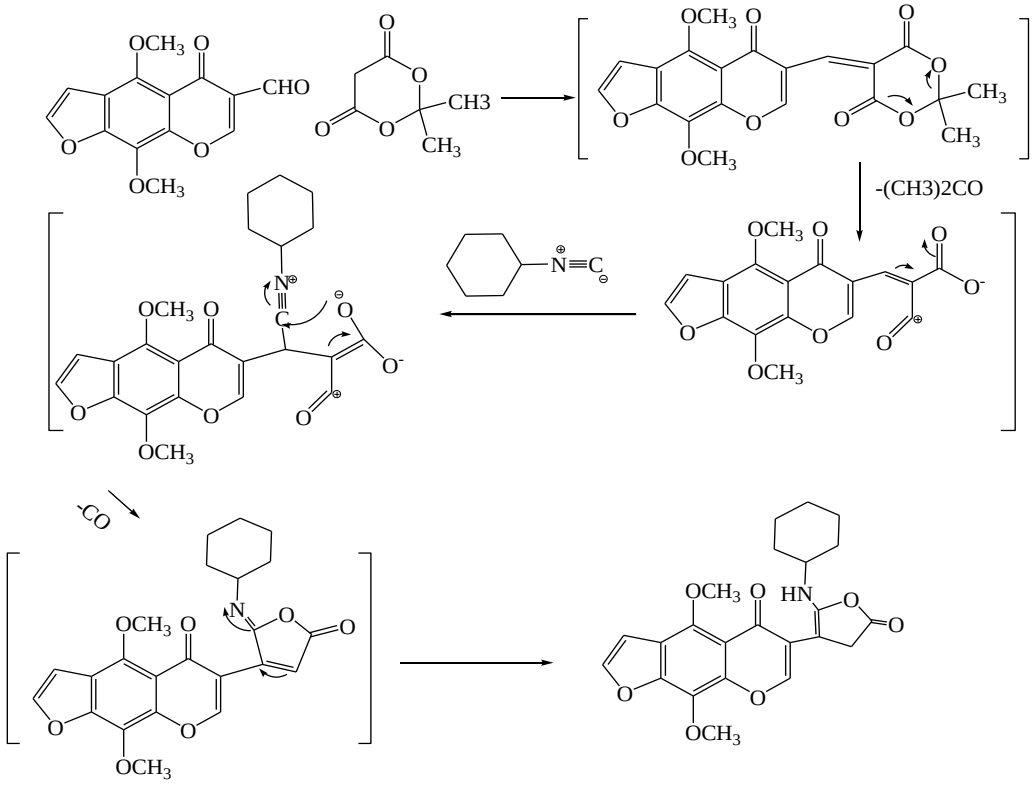
scheme (4)

The reaction of cyclohexyl isocyanide and furochromone carbaldehyde (1), with Meldrum acid the compound was afforded (7) (scheme5) in one pot reaction. This new procedure was given many advantages such as simplicity, mild reaction condition and improved yield. The IR spectra showed the presence of (2CO gp.), (NH gp). ^{1}H NMR spectra were characterized by the presence of 1H proton for (NH gp). Also the structure of the compound 7 was confirmed by mass spectroscopy.



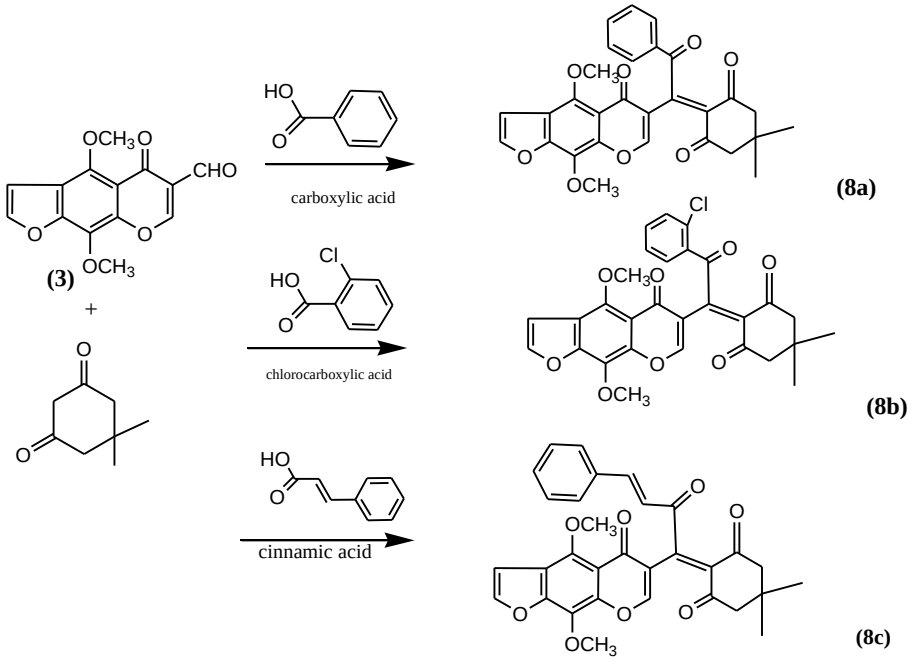
1 (7)

Scheme (5)

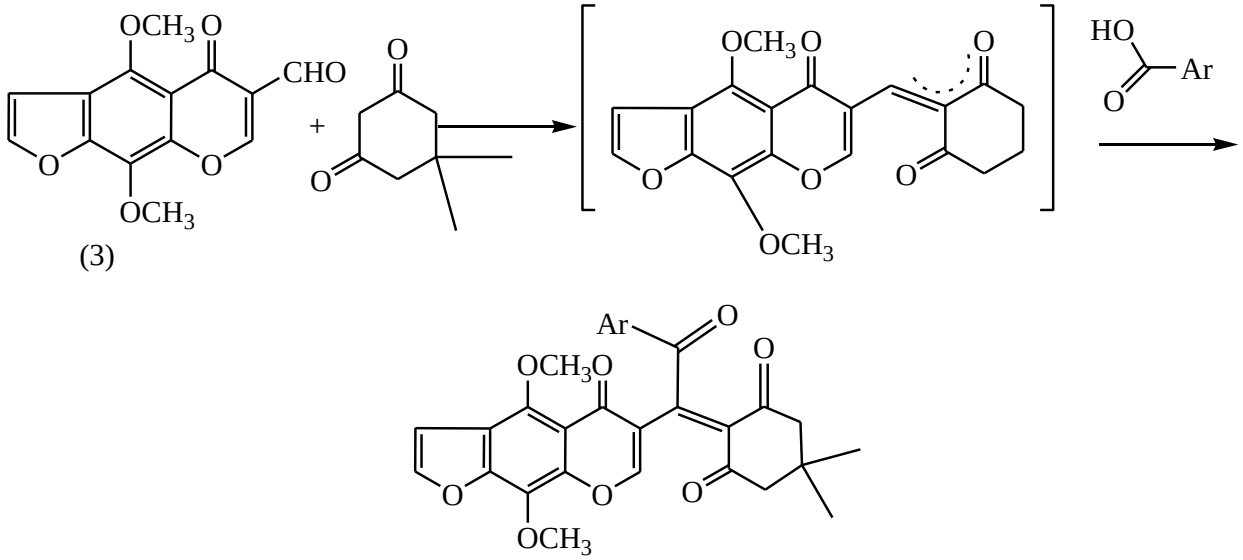


Possible mechanism for the formation of product 7

As a part of the current studies on the development of a novel method (Roya, 2008), for the synthesis of compound derivatives (8a-c) (Scheme 6) by means of a three-component reaction between 5, 5-dimethylcyclohexane-1, 3-dione, carboxylic acid, chlorocarboxylic acid and cinnamic acid with furochromone carboxaldehyde (1), this method was simple and was convenient to prepare a wide range of acid derivatives in a single-step operation. The IR was showed peak for (4CO gps) of (16a-c) and ^{1}H NMR spectrum of (16a-c) exhibited 4 protons identified as ($2CH_2$) and 6H proton for ($2CH_3$ gps).

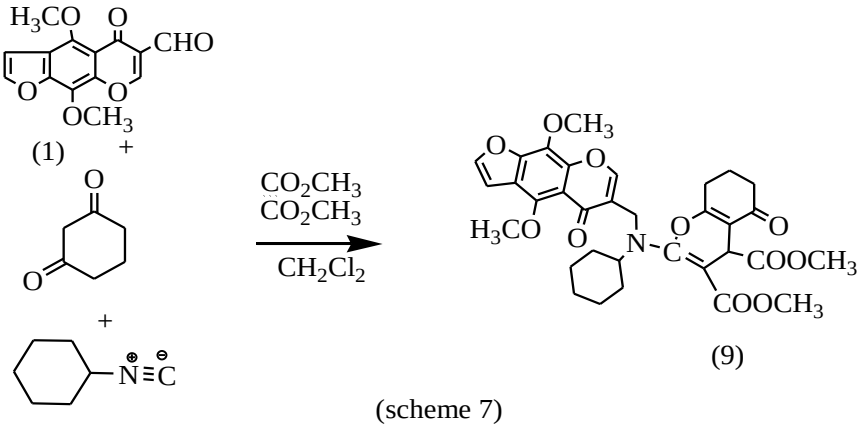


(Scheme 6)



Possible mechanism for the formation of product 8a-c

The reaction of aldehyde, dimethyl acetylene dicarboxylate, cyclohexyl isocyanide with 1, 3-dicarbonyle to get compound (9) (scheme7).



(scheme 7)

All the products were characterized by IR and ^{1}H NMR spectral data.

B- Biological activity

Biological evaluation of the newly synthesized compounds:

The newly synthesized furochromone derivatives gave a reasonable antiproliferative potency towards current investigated liver and breast cultured cell lines (HEPG2 and MFC7) between 4.88 – 10.07 µg/ml concentrations compared to traditional antitumor agents: Doxorubicin and 5-Fluorouracil (table 1).

Biochemical parameters induced by effect of newly synthesized furochromone derivatives:

Results demonstrated in tables 2, 3 and 4 represents the effect of newly synthesized furochromone derivatives on different biochemical parameters induced in serum of treated and untreated groups of mice in comparison to antitumor agents: Doxorubicin and 5-Fluorouracil. It is clear from the results that most of the investigated biochemical parameters were significantly higher ($P < 0.001$) in Doxorubicin and 5-Fluorouracil treated groups of mice than the untreated, while treatment with the newly synthesized furochromone derivatives showed different effects, where some of the estimated values of were non-significant (n's.) or slightly higher ($P < 0.01$) compared to untreated.

Table 1: The antiproliferative effects of furochromone derivatives against hepatic and breast cancer cell lines (HEPG2 and MCF7).

Compound	Cell Lines	
	HEPG2 (IC_{50})	**MCF7 (IC_{50})**
5 fluorouracil	5	0.67
Doxorubicin	3.56	6.71
2	33.2	-ve
4	44.9	-ve
5	37.3	-ve
6a	10.3	10.7
7	20.7	8.93

Table 2: Biochemical effects (Mean ± SD) of treatment with 5-flurouracil (5-FU), doxorubicin (DOX), and the furochromone derivatives on serum ALT, AST, and ALP in mice

Compounds	**ALT (IU/mL)**	**AST (IU/mL)**	**ALP (k.k./dL)**
Control	43.50 ± 2.03	108.32 ± 4.19	17.70 ± 1.10
5-Flurouracil	51.47 ± 9.02 *	130.43 ± 8.92 *	25.49 + 6.03 *
Doxorubicin	59.26 ± 12.03 *	147.23 ± 16.34 *	30.32 ± 5.14 *
2	42.5 ± 7.3 ***	114.6 ± 11.2 **	18.4 ± 5.1 ***

4	69.7 ± 12.6 *	149.4 ± 23.1 *	47.9 ± 12.5 *
5	72.5 ± 17.3 *	151.8 ± 21.4 *	42.7 ± 10.4 *
6a	52.7 ± 10.4 **	117.2 ± 18.2 **	27.9 ± 7.8 **
7	41.6 ± 5.7***	110.1 ± 11.5***	25.4 ± 5.8**

* p< 0.001: Highly significant; ** p < 0.01: Significant; *** n.s.: Non-significant; ALT: Alanine amino transferase; AST: Aspartate amino transferase; and ALP: Alkaline phosphatase

Table 3: Biochemical effects (Mean ± SD) of treatment with 5-FU, DOX, and furochromone derivatives on total lipids, cholesterol, triglycerides, and bilirubin in mice

Compounds	Total Lipids (mg/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Bilirubin (mg/dL)
Control	323.41 ± 27.1	94.32 ± 13.5	108.70 ± 16.8	0.63 ± 0.04
5-Flurouracil	378.20 ± 31.4 *	105.90 ± 11.7 *	126.50 ± 19.4 *	0.75 ± 0.1 *
Doxorubicin	366.70 ± 6.1 *	109.30 ± 14.2 *	137.80 ± 17.1 *	0.81 ± 0.19 *
2	326.4 ± 18.3 ***	99.2 ± 7.8 ***	120.1 ± 11.3 **	0.64 ± 0.02 ***
4	378.9 ± 33.6 *	123.4 ± 28.3 *	141.5 ± 37.9 *	0.92 ± 0.03 *
5	320.9 ± 28.4 ***	92.1 ± 18.6 ***	112.4 ± 13.8 ***	0.65 ± 0.04 ***
6a	319.5 ± 23.2***	95.3 ± 13.5***	114.9 ± 12.3***	0.66 ± 0.04***
7	319.5 ± 23.2***	95.3 ± 13.5***	114.9 ± 12.3***	0.66 ± 0.04***

* p< 0.001: Highly significant; ** p < 0.01: Significant; and *** n.s.: Non-significant.

Table 4: Biochemical effects of treatment with 5-FU, DOX, and furochromone derivatives on serum albumin, globulin and creatinine in mice

Biochemical Parameters	Albumin (mg/dL)	Globulin (mg/dL)	A/G Ratio	Creatinine (mg/dL)
Control	5.63 ± 0.51	4.32 ± 0.9	1.3	0.69 ± 0.03
5-FU	6.49 ± 0.92 **	5.75 ± 0.8 **	1.13 **	0.81 ± 0.06 **
DOX	6.37 ± 0.85 **	5.91 ± 0.63 **	1.078 **	0.78 ± 0.04 **
2	5.74 ± 0.3 ***	5.4 ± 0.65 ***	1.46 ***	0.67 ± 0.01 ***
4	8.52 ± 0.4 **	7.46 ± 0.5 **	1.001 **	0.83 ± 0.05 **
5	5.71 ± 0.6 ***	5.11 ± 0.4 ***	1.13 ***	0.68 ± 0.06 ***
6a	7.2 ± 0.9 **	6.5 ± 0.8 **	1.006 **	0.9 ± 0.04 **
7	5.34 ± 0.8***	4.8 ± 0.6***	1.12***	0.67 ± 0.1***

* p< 0.001: Highly significant; ** p < 0.01: Significant; and *** n.s.: Non-significant.

Conclusion

Synthesis of novel heterocyclic compounds such as chromen derivative (2-9) with pharmaceutical effect. The present results indicated that some of the synthesized compounds may constitute a potential antitumor activity against MCF7 and HEPG2 cancer cell lines in vitro in comparison to known anticancer drugs Doxorubicin and 5-fluorouracil. Moreover, the studied biochemical parameters showed that the newly synthesized compounds may induce lower side effects than the traditional anticancer drugs used in this investigation. However, more experiments are necessary to identify the mode of action of these compounds.

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