Research Article

An Efficient Method for Synthesis of 4,9-dimethoxy-5*H*-furo[3,2-g] chromen-5-one Derivatives via Multicomponent Reactions with Expected Anticancer Activities

Eman A. Elhefny[1*], Sherifa M. Abu-Bakr[2], Nagwa M. Fawzy[2], A. M. Nasef[2], Magdy S. Aly[3]
[1]Photochemistry Department, National Research Center, Dokki, Cairo, Egypt.
[2]Chemistry of Natural and Microbial Products Department, National Research Center, Dokki, Cairo, Egypt.
[3]Zoology Department, Faculty of Science, Beni-Suef University, Egypt.
***Corresponding author's E-mail:** emanelhefny@yahoo.com

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ABSTRACT

A simple one-pot and efficient method was described for the synthesis of 4,9-dimethoxy-5*H*-furo[3,2-g]chromen-5-one derivatives (2_{a-j}). We reported the four-component reaction between furochromonecarbaldehyde**1**, amine derivatives namely: amino pyridine and amino anisidine, isocyanate derivatives namely: (cyclohexane and phenyl) isocyanates and acid derivatives namely: (n-butyric, succinic and anthranilic) acid, we used methanol as solvent. The synthesis via a new Ugi (4CR) was carried out as a one-pot procedure to yield compounds $\mathbf{2}_{a,-j}$ (scheme 1). Also, starting from readily available, between fuochromonecarbaldehyde**1**, *o*-phenylenediamine derivatives (*o*-phenylenediamine, 4-nitrobenzene-1,2-diamine and 3,4-diaminobenzoic acid and isocyanate derivatives namely: cyclohexyl, phenyl isocyanate, a variety of highly substituted (qinoxalin-2-yl)-4,9-dimethoxy-5*H*-furo[3,2-g] chromen-5-one compounds 3_{a-g} were efficiently synthesized (scheme 2). In the present investigation, the newly synthesized products were screened (using the MTT colorimetric assay) for their *in vitro* inhibition capacity in two human cancer cell lines hepatocellular carcinoma (HEPG2) and breast cancer (MCF-7) in comparison to the known anticancer drugs: 5-Flurouracil and Doxorubicin. The anticancer activity results indicated that the synthesized products 2a-c, 2i and 3a-c, 3f showed growth inhibition activity against the tested two cell lines but with varying intensities extents in comparison to the known anticancer drugs, 5-Flurouracil and Doxorubicin.

Keywords: Ugi(4CR), isocyanate, quinazolin-2-yl, qinoxalin-2-yl, cytotoxicity, anticancer activity, human cancer cell lines.

INTRODUCTION

Cancer is a major health problem worldwide. Improvements in treatment and prevention have led to a decrease in cancer deaths, but the number of new diagnoses continues to rise. Chemotherapy is one of the most commonly used treatment options, especially for unrespectable patients. Cytotoxic drugs remain the basis of cancer chemotherapy and are being administered with novel ways of therapy; therefore it is important to discover novel cytotoxic agents with spectra of activity and toxicity that differ from those current agents.

Cytotoxicity assays are used widely in drug discovery research to predict which compounds might have safety concerns in humans before significant times and expense are incurred in their development[1,2]. Other researchers have studied the mechanisms of cytotoxicity as a way of better understanding of the normal and abnormal biological processes controlling cell growth, division, and death[3].

Heterocycles play an important role in the design and discovery of new physiological/pharmacologically active compounds[4]. Chromone (4H-chromen-4-one, 4H-1-benzopyran-4-one) is an important class of oxygen-containing heterocyclic compounds with a benzoannelated g-pyrone ring and they are part of the flavonoid family.

Chromones constitute one of the major classes of naturally occurring compounds, and chemistry continues unabated because of their usefulness as biologically active agents. Chromone derivatives are abundant in nature and exhibit a wide range of pharmacological activity like anti-bacterial, anti-fungal[5,6], anti-cancer[7], anti-oxidant[8], anti-HIV[9], anti-ulcers[10], immunostimulators[11], biocidal[12], wound healing[13], anti-inflammatory[14] and immune-stimulatory[15]. Many chromone derivatives are also photoactive and can be used easily in various photo-induced reactions affording diverse heterocyclic compounds[16].

The chromone and its derivatives are the most important heterocyclic compounds, which is a common and integral feature of a variety of natural products and medicinal agents. These heterocyclic show a variety of pharmacological properties, and change of their structure offer a high degree of diversity that have proven useful for the search of new therapeutic agents[17]. In the present work, multicomponent reactions (MCRs) have drawn considerable interests owing to its exceptional synthetic efficiency[18,19]. Compared with conventional methods, multicomponent process exhibits high levels of efficiency and diversity, as it allows more than two simple and flexible building blocks to be combined in practical, time-saving one-pot operations. Combinatorial chemistry has recently gained much attention in pharmaceutical research, especially in the context of lead finding and lead optimization[20-22]. Multicomponent reactions (MCRs) allow rapid generation of compound libraries containing a variety of different and highly relevant heterocyclic

scaffolds[23-29]. Therefore several research groups described new multicomponent reactions based on the combination of combinatorial and classical chemistry. By using appropriate starting materials in MCRs, a large range of classical post-condensation reactions are tolerated, yielding new interesting scaffolds[30]. The Ugi–Heck strategy reported at the same time by Gracias[31] and Xiang[30] offers a new potential for the Ugi reaction and very many scaffolds based on this strategy can still be envisioned.[32]

Herein we report the synthesis and *in vitro* growth inhibition characterization of new chromone derivatives. The *in vitro* antiproliferative activity of each compound in the study has been determined using the MTT colorimetric assay[33-36] in hepatocellular carcinoma cell line HEPG2 and breast carcinoma cell line MCF-7. The cytotoxic potency of the selected products was studied in comparison to two known anticancer drugs, 5-Flurouracil (5-FU) and Doxorubicin (DOX).

MATERIALS AND METHODS

Chemistry

All starting materials were obtained from commercial suppliers and used without further purification unless otherwise noted. All melting points are uncorrected and were taken on electro-thermal capillary melting point 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. ^{1}H-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 Finnigan MatSSQ 7000 mode: EI, 70Ev (Thermo Inst. Sys. Inc., USA). The melting points were measured in degrees centigrade and determined using BucHI 510 apparatus. The purity of the synthesized compounds was tested by thin layer chromatography (TLC), Merck plates. TLC Silica gel 60 F_{254} 25 Aluminum sheets 20 x 20 cm.

General procedure for the preparation of 4,9-dimethoxy-5H-furo[3,2-g] chromen-6-yl derivatives (2_{a-j})

The desired compounds were synthesized utilizing a 25-ml round bottom flask were added *p*-aminopyridine and/or P-amino-anisidine(1.1 mmol), furochromonecarbaldehyde 1 (1.1 mmol) and in methanol (20.0 ml) and the mixture was stirred for 15-30 min at room temperature. n-butyric and/or succinic, anthranilic acid (1.1 mmol), was added to the mixture followed by stirring for another 5 min. Finally, cyclohexyl and/or phenyl isocyanates (1.1 mmol) were added. After the resultant mixture was stirred at room temperature for 23-168 h, solid K_2CO_3 (0.38 mmol) was added and refluxing for 76-200 h. After the reaction was completed, the crude material was concentrated and re-dissolved in dichloromethane. The resulting organic solution was then washed with 1 M HCl (aq). This was followed by adding a saturated aqueous solution of $NaHCO_3$ (aq) combined with brine. The resulting organic layer was collected, dried by $MgSO_4$, and then concentrated in vacuum at 40°C for 8 h to afford the crude material. The crude material was purified by ethanol to give the desired products **2a-j**.

N-(cyclohexylamino)(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)methyl)-N-(pyridin-4-yl)butyramide 2a

Following the General Procedure, the desired compound was synthesized utilizing *p*-amino-pyridine (1.1 mmol), butyric acid (1.00 mmol), cyclohexylisocyanate (1.10 mmol) and furochromonecarbaldehyde**1** (1.00 mmol) to give compound 2a. Orange solid, m.p.210°C, yield 59%. Mol. formula: $C_{29}H_{33}N_3O_6$, Mol. Wt.: 519.6, elemental anal.: calc: C, 67.04; H, 6.40; N, 8.09 found: C, 67.02; H, 6.38; N, 8.11. IR (KBr, cm^{-1}) bands at 1574, 1626 (2C=O) and 3328 (NH). ^{1}H-NMR (DMSO-d6, δ, ppm): 1.03(t, 3H, CH_3); 1.25-1.64 (m, 12H, $5CH_2$ cyclohexane); 2.20 (t, $2CH_2$, CH_2-CO); 3.10 (dd, H, CH-N cyclohexane); 3.63 (s, 6H, $2OCH_3$); 5.56 (s, 1H, N-CH-N); 644, 6.56 (dd, 2H, J=2.01, furan ring); 8.00 (s, 1H, H_7); 8.02-8.12 (dd, 4H, pyridine) and 9.02 (d, 1H, NH, exchangeable D_2O).

N-(cyclohexylamino)(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)methyl)-N-(4-methoxyphenyl) butyramide 2b

Following the General Procedure, the desired compound was synthesized utilizing *p*-amino-anisidine (1.1 mmol), butyric acid (1.00 mmol), cyclohexylisocyanate (1.10 mmol) and furochromonecarbaldehyde**1** (1.00 mmol) to give compound 2b. Yellow solid, m.p.158°C, yield 85%. Mol. formula: $C_{31}H_{36}N_2O_7$, Mol. Wt.: 548.6, elemental analy.: calcd: C, 67.87; H, 6.61; N, 5.11 found: C, 67.85; H, 6.59; N, 5.13. IR (KBr, cm^{-1}) bands at 1606, 1630 (2CO) and 3307 (NH). ^{1}H-NMR (DMSO-d6, δ, ppm): 1.25 (t, 3H, CH_3); 1.35-1.65 (m, 12H, 5CH2 cyclohexane); 2.26 (t, $2CH_2$, CH_2-CO); 3.34(d, 1H, CH-NH); 3.69 (s, 9H, $3OCH_3$); 5.93 (s, 1H, N-CH-N); 6.54-7.24 (dd, 4H, arom.) and 6.73, 7.36 (dd, 2H, J=2.01, furan ring); 7.89 (s, 1H, H_7) and 8.82 (d, 1H, NH, exchangeable D_2O). m/e: 548.3 (10.0%), 248 (18%), 238 (7.4%), 123 (100%), 108(80%).

N-(benzenamine)(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)methyl)-N-(4-methoxyphenyl)butyramide 2c

Following the General Procedure, the desired compound was synthesized utilizing *p*-amino-anisidine (1.1 mmol), butyric acid (1.00 mmol), phenylisocyanate (1.10 mmol) and furochromonecarbaldehyde**1** (1.00 mmol) to give compound 2c. Yellow solid, m.p. 196°C, yield 74%. Mol. formula: $C_{31}H_{30}N_2O_7$, Mol. Wt.: 542.6, elemental analy.: calcd: C, 68.62; H, 5.57; N, 5.16 found: C, 68.60; H, 5.55; N, 5.18. IR (KBr, cm^{-1}) bands at: 1609, 1656 (2C=O) and 3317 (NH). ^{1}H-NMR (DMSO-d6, δ, ppm): 0.98 (t, 1H, CH3); 2.22 (t, $2CH_2$, CH_2-CO); 3.66, 4.01, 4.03 (sss, 6H, $3OCH_3$); 6.98 (s, 1H, N-CH-N); 7.00, 7.29 (dd, 2H, J=2.00, furan ring); 6.54-7.20 (m, 9H, arom.) ; 8.15 (s, 1H, H_7) and 8.79 (s, 1H, NH exchangeable with D_2O).

N-(benzenamine)(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)methyl)-N-(pyridin-4-yl)butyramide 2d

Following the General Procedure, the desired compound was synthesized utilizing p-amino-pyridine (1.1 mmol), butyric acid (1.00 mmol), phenylisocyanate (1.10 mmol) and furochromonecarbaldehyde 1 (1.00 mmol) to give compound 2d. Yellow solid, m.p. 234°C, yield 65%.

Mol. formula: $C_{29}H_{27}N_3O_6$, calc. Mol. Wt.: 513.5, analy., calcd: C, 67.83; H, 5.30; N, 8.18 found: C, 67.83; H, 5.30; N, 8.18. IR (KBr, cm^{-1}) bands at: 1612, 1642 (2C=O) and 3221 (NH).

^{1}H-NMR (DMSO-d6, δ, ppm): 1.23(t, 3H, CH_3); 2.62 (t, $2CH_2$, CH_2-CO); 3.76, 3.77 (ss, 6H, $2OCH_3$), 6.43 (s, 1H, N-CH-N); 6.54-7.20 (m, 5H, arom); 7.28, 7.56 (dd, 2H, J=2.01, furan ring); 7.94 (s, 1H, H_7); 7.95-8.13 (dd,4H, pyridine), and 9.21 (s, 1H, NH exchangeable with D_2O.

N-(cyclohexylamino)(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)methyl)-N-(pyridin-4-yl)propionamide 2e

Following the General Procedure, the desired compound was synthesized utilizing *p*-amino-pyridine (1.1 mmol), succinic acid (1.00 mmol), cyclohexylisocyanate (1.10 mmol) and furochromonecarbaldehyde**1** (1.00 mmol) to give compound 2e. Brown solid, m.p.146°C, yield 64%.

Mol. formula: $C_{28}H_{31}N_3O_6$, calc. Mol. Wt.: 505.6, analy.: calcd: C, 66.52; H, 6.18; N, 8.31 found: C, 66.50; H, 6.16; N, 8.33. IR (KBr, cm^{-1}) bands at 1610, 1660 (C=O), 3220 (NH) and 3440 (COOH).

^{1}H-NMR (DMSO-d6, δ, ppm): 1,23-1.71 (m, 10H, 5H cyclohexane); 2.39(t, 2H, CH_2-CO); 3.57(d, 1H, CH-NH); 3.86, 3.88 (s, 6H, $2OCH_3$); 5.98 (s, H, N-CH-N); 7.36 (s, 1H, H_7); 6.45, 7.95 (dd, 2H, J=2.01, furan ring); 8.25, 8.27 (dd, 4H, pyridine); 9.11 (d, 1H, NH, exchangeable D_2O) and12.00 (s, 1H, COOH, exchangeable D_2O).

N-(cyclohexylamino)(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)methyl)-N-(4-methoxyphenyl)propion amide 2 f

Following the General Procedure, the desired compound was synthesized utilizing *p*-amino-anisidine (1.1 mmol), succinic acid (1.00 mmol), cyclohexylisocyanate (1.10 mmol) and formylfurochromone (1.00 mmol) to give compound 2f. Yellow solid, m.p.196°C, yield 55%.

Mol. formula: $C_{30}H_{34}N_2O_7$, Mol. Wt.: 534.6, analy.: calcd: C, 67.40; H, 6.41; N, 5.24 found: C, 67.38; H, 6.39; N, 5.26. IR (KBr, cm^{-1}) bands at 1609, 1665 (2C=O), 3222 (NH) and 3430 (COOH).

^{1}H-NMR (DMSO-d6, δ, ppm): 1.27-1.75 (m, 10H, $5CH_2$ cyclohexane); 2.39 (d, 1H, CH-NH); 3.57 (s, 2H, CH_2-CO); 3.76, 3.79,3.98 (3s, 9H, $3OCH_3$), 5.83 (s, 1H, N-CH-N); 7.52, 6.66 (dd, 2H, J=2.01, furan ring), 8.05 (s, 1H, H_7); 6.64-8.44 (dd, 4H, arom.); 9.05 (d, 1H, NH, exchangeable D_2O) and 12.02 (s, 1H, COOH, exchangeable D_2O). m/e: 534.2 (10%), 272 (12%), 257 (9%), 248 (33.8%), 149 (25%), 123 (100%), 108 (75%).

N-((phenylamino)(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)methyl)-N-(4-methoxyphenyl)propionmide 2g

The General Procedure, the desired compound was synthesized utilizing *p*-amino-anisidine (1.1 mmol); succinic acid (150 mg, 1.00 mmol); phenylisocyanate (10 mmol) and furochromonecarbaldehyde***1*** (1.00 mmol) to give compound 2g. brown solid, m.p.>300°C, yield 52%. Mol. formula: $C_{31}H_{31}N_2O_9$, calc. Mol. Wt.: 575, calcd: C, 64.69; H, 5.39; N, 4.87, found: C, 64.70; H, 5.41; N, 4.9. IR (KBr, cm^{-1}) band at: 1615, 1660 (2C=O); 3211 (NH) and 3434 (COOH). ^{1}H-NMR (DMSO-d6, δ, ppm): 2.38(s, 2H, $1CH_2$); 3.78, 3.88, 3.91 (3s, 9H, $3OCH_3$), 6.49(s, 1H, N-CH-N), 7.00, 7.56 (dd, 2H, J=2.01, furan ring), 7.29-7.49 (m, 4H, arom.); 7.95 (s, 1H, H_7); 11.53 (s, 1H, NH exchangeable with D_2O), and 12.11 (s, 1H, COOH, exchangeable D_2O)m/e.

N-((phenylamine)(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)methyl)-N-(pyridin-4-yl)propionamide2h

Following the General Procedure, the desired compound was synthesized utilizing *p*-amino-pyridine (1.1 mmol), succinic acid (1.00 mmol), phenylisocyanate (1.10 mmol) and furochromonecarbaldehyde**1** (1.00 mmol) to give compound 2h. Brown solid, m.p.174°C, yield>60%. Mol. formula: $C_{28}H_{25}N_3O_6$, Mol. Wt.: 499.5, analy.: calcd: C, 67.33; H, 5.04; N, 8.41 found: C, 67.31; H, 5.02; N, 8.43. IR (KBr, cm^{-1}) bands at: 1614. 1654 (2C=O); 3198 (NH) and 3436 (COOHH). ^{1}H-NMR (DMSO-d6, δ, ppm): 2.32 (d, 2H, $1CH_2$); 3.86, 3.89 (s, 6H, $3OCH_3$), 6.49 (s, 1H, N-CH-); 7.01, 7.56 (dd, 2H, J=2.01, furan ring), 7.20-7.60 (m, 5H, arom.) 8.00 (s, 1H, H_7); 7.20-7.60 (m, 5H, arom.); 7.98, 8.33 (dd, 4H, pyridine); 37 (s, 1H, NH exchangeable with D_2O) and 12.12 (s, 1H, COOH, exchangeable D_2O).

2-amino-N-((cyclohexylamino)(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)methyl)-N-(pyridin-4-yl) benzamide 2i

Following the General Procedure, the desired compound was synthesized utilizing *p*-amino-pyridine (1.1 mmol), anthranilic acid (1.00 mmol), cyclohexylisocyanate (1.10 mmol) and formylfurochromone (1.00 mmol) to give compound 2i. brown solid, m.p.264°C, yield 61%. Mol. formula: $C_{32}H_{32}N_4O_6$, Mol. Wt.: 568.6 calcd: C, 67.59; H, 5.67; N, 9.85 found: C, 67.57; H, 5.65; N, 9.87. IR (KBr, cm^{-1}) bands at 1611, 1644 (C=O); 3220 (NH) and 3432 (NH_2). ^{1}H-NMR (DMSO-d6, δ, ppm): 1.50-1.88 (m, 10H, $5CH_2$cyclohexane); 3.88 (s, 6H, $2OCH_3$); 4.20 (s, 2H, NH_2, exchangeable D_2O); 5.59 (s, 1H, N-CH-N); 6.45, 7.51 (dd, 2H, J=2.01, furan ring); 6.76-7.97 (m, 4H, arom.); 8.00 (s, 1H, H_7); 7.98, 8.33 (dd, 4H, pyridine) and 9.70 (d, 1H, NH, exchangeable D_2O).

2-amino-N-(benzenamino)(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)methyl)-N-(pyridin-4-yl)benzamide 2j

Following the General Procedure, the desired compound was synthesized utilizing *p*-amino-pyridine (1.1 mmol),

anthranilic acid (1.00 mmol), phenylisocyanate (1.10 mmol) and formylfurochromone (1.00 mmol) to give compound 2j.

Brown solid, m.p>300°C, yield 54%. Mol. formula: $C_{32}H_{26}N_4O_6$, calc. Mol.Wt.: 562.6, analy.: calcd: C, 68.32; H, 4.66; N, 9.96 found: C, 68.32; H, 4.66; N, 9.96IR (KBr, cm^{-1}) bands at: 1615, 1655 (2C=O); 3210 (NH) and 3433 (NH_2). ^{1}H-NMR (DMSO-d6, δ, ppm): 3.88, 3.91 (ss, 6H, $2OCH_3$); 4.00 (s, 1H, NH_2, exchangeable D_2O); 5.59 (s, 1H, N-CH-N), 6.96, 7.95 (dd, 2H, J=2.01, furan ring), ,7.00-7.34 (m,4H, arom.), 7.91 (s, 1H, H_7); 7.90, 8.36 (dd, 4H,pyridine) and 9.71 (s, 1H, NH exchangeable with D_2O). m/e: 562.2 (11%), 238 (53%), 194 (6%), 146 (36%), 119 (100%).

General procedures for synthesis of compounds (6a-g)

To a solution of 5,9-dihydro-4,9-dimethoxy-5-oxo-4H-furo[3,2-g]chromene-6-carbaldehyde 1 (0.01mol), *o*-phenylenediamine, 1,2-diamino-4-nitrobenzene, and/or 1,2-diamino-4-benzoic acid (0.01mol) and methyl, cyclohexyl and phenylisocyanate (0.01mol) in (20 mL) absolute ethanol and traces of *p*-toluene sulphonic acid was added quickly.

The reaction mixture was stirred at room temperature for 72h., followed by TLC. The collected product was filtered and recrystallized from appropriate solvent to give compound **6a-g** as deep brown powder.

6-(1,2-dihydro-3-(methylamino)quinoxalin-2-yl)-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-one 3a

Mol. formula: $C_{22}H_{19}N_3O_5$, calc. Mol. Wt.: 405.4, analy.: calcd: C, 65.18; H, 4.72; N, 10.37 found: C, 65.16; H, 4.70; N, 10.39. mp: 280°C. IR (KBr/cm_1):1612 (CO) and 3124, 3443 (2NH). ^{1}H NMR (DMSO-d6, δ, ppm): 2.47 (d, 3H, CH_3); (d, H, NH exchangeable with D_2O); 3.84; 4.18 (s, 6H, $2OCH_3$); 3.94 (s, 1H, CH); 7.14, 7.91 (dd, 2H, J=2.01, furan ring);8.55 (s, 1H, H_7); 7.16 (dd, 4H, arom.) and 9.27 (q, H, NH exchangeable with D_2O). m/e: 465 (30%), 421 (45%), 362 (36%).

6-(1,2-dihydro-3-(phenylamino)quinoxalin-2-yl)-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-one 3b

Mol. formula: $C_{27}H_{21}N_3O_5$, calc. Mol. Wt.: 467.5, analy.: calcd: C, 69.37; H, 4.53; N, 8.99 found: C, 69.35; H, 4.51; N, 9.01 mp: 231°C. IR (KBr/cm_1): 1612 (CO) and 3320, 3474 (2NH). ^{1}H NMR (DMSO-d6, δ, ppm): 3.31 (d, 1H, NH exchangeable with D_2O); 3.96 (s, 1H, CH); 3,90; 3.91 (s, 6H, $2OCH_3$); 7.10, 7.39 (dd, 2H, J=2.01, furan ring); 7.19, -7.44 (m,9H, arom.); 7.82 (s, 1H, H_7) and 9.75 (s, H, NH exchangeable with D_2O).

6-(3-(cyclohexylamino)-1,2-dihydro-7-nitroquinoxalin-2-yl)-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-one 3c

Mol. formula: $C_{27}H_{26}N_4O_7$, calc. Mol. Wt.: 518.5, analy.: calcd: C, 62.54; H, 5.05; N, 10.81 found: C, 62.52; H, 5.02; N, 10.83, mp: 169°C. IR (KBr/cm_1): 1611, 1650 (2CO) and 3115, 3150 (2NH). ^{1}H NMR (DMSO-d6, δ, ppm): 1.39-1.78 (m, 10H, 5 CH_2); 3.82, 5.21 (ss, 2H, 2 CH); 3.94, 3.95 (s, 6H, $2OCH_3$); 7.12, 7.33 (dd, 2H, J=2.01, furan ring); 7.73 (s, 1H, H_7); 7.3-7.5 (dd, 2H, arom.); 7.46, 7.82 (dd, 4H, arom.); 3.81 and 9.37 (ss, 2H, 2NH exchangeable with D_2O). m/e: 517 (12%), 434 (77%), 406 (88%).

6-(1,2-dihydro-7-nitro-3-(phenylamino)quinoxalin-2-yl)-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-one 3d

Mol. formula: $C_{27}H_{20}N_4O_7$, calc. Mol. Wt.: ***512.5,*** analy.: calcd: ***C, 63.28; H, 3.93; N, 10.93*** found: ***C, 63.26; H, 3.91; N, 10.95*** mp 202°C. IR (KBr/cm_1): 1610, 1648 (2CO) and 3188, 3153 (2NH).

^{1}H NMR (DMSO-d6, δ, ppm); 3.76 (s, H, NH exchangeable with D_2O) 3.76 (d, 1H, CH); 3.93, 3.95 (s, 6H, $2OCH_3$); 7.52, 6.66 (dd, 2H, J=2.01, furan ring); 8.07 (s, 1H, H_7); 6.62-7.5 (m, 8H, arom.) and 3.76, 9.75 (s, H, NH exchangeable with D_2O). m/e: 511.1 (10%), 421 (55%), 406 (90%).

2-(cyclohexylamino)-3,4-dihydro-3-(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)quinoxaline-6-carboxylic acid 3e

Mol. formula: $C_{28}H_{27}N_3O_7$, calc. Mol. Wt.: 517.5, analy.: calcd: C, 64.98; H, 5.26; N, 8.12 found: C, 64.96; H, 5.24; N, 8.14, mp: 247°C. IR (KBr/cm_1): 1615, 1649 (2CO); 3126, 3267 (2NH) and 3430 (COOH). ^{1}H NMR (DMSO-d6, δ, ppm): 1.39-1.78 (m, 01H, 10 H CH_2); 3.54, 5.24 (s, 2H, 2CH); 3.93; 3.95 (s, 6H, $2OCH_3$), 7.00, 7.44 (dd, 2H, J=2.01, furan ring); 7.3-7.5 (dd,2H, arom.); 7.4(s, 2H, arom.); 7.89 (s, 1H, H_7); 3.72, 9.14 (ss, 2H, 2NH exchangeable with D_2O) and 14.12 (s, 1H, COOH exchangeable with D_2O). m/e: 517 (44%), 489 (90%), 457 (100%).

3,4-dihydro-3-(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)-2-(phenylamino)quinoxaline-6-carboxylic acid 3f

Mol. formula: $C_{28}H_{21}N_3O_7$, calc. Mol. Wt.: 511.5, analy.: calcd: C, 65.75; H, 4.14; N, 8.22 found: C, 65.73; H, 4.12; N, 8.24. Mp: >300°C. IR (KBr/cm_1): 1615.09, 1649 (2CO); 3127, 3158 (2NH) and 3270 (COOH). ^{1}H NMR (DMSO-d6, δ, ppm): 3.32 (d, H, NH exchangeable with D_2O); 3.88 (s, 1H, CH); 3.97, 4.10 (s, 6H, $2OCH_3$); 6.75, 7.10 (dd, 2H, J=2.00, furan ring); 7.92 (s, 1H, H_7); 6.62-7.4 (m, 8H, arom.) and 9.37 (s, H, NH exchangeable with D_2O) and 14.12 (s, 1H, COOH exchangeable with D_2O). m/e: 511 (54%), 465 (25.0%), 406 (86%), 245 (34%).

3,4-dihydro-3-(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)-2-(methylamino)quinoxaline-6-carboxylic acid 3g

Mol. formula: $C_{23}H_{19}N_3O_7$, calc. Mol. Wt.: 449.4, analy.: calcd: C, 61.47; H, 4.26; N, 9.35 found: C, 61.45; H, 4.24; N, 9.37, mp: >300°C. IR (KBr/cm_1): 1615, 1686 (2CO); 3123, 3268 (2NH) and 3270 (COOH). ^{1}H NMR (DMSO-d6, δ, ppm): 3.48 (q, H, NH exchangeable with D_2O); 3.88 (d, 1H, CH); 3.97, 4.10 (s, 6H, $2OCH_3$); 6.75, 7.10 (dd, 2H, J=2.00, furan ring); 6.62-7.4 (ds, 3H, arom.); 7.89 (s, 1H, H_7) and 3.48, 9.24 (s, 2H, NH exchangeable with D_2O) and 14.10 (s, 1H, COOH exchangeable with D_2O). m/e: 436 (20.0%), 406 (90%), 176 (100%).

Determination of Anticancer Activities

Cell Culture

All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, and Sanford, ME, USA). Human hepatocellular carcinoma HepG2 and breast cancer MCF-7 cells were obtained from National Cancer Institute, Cairo University. The cells were grown in DMEM supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 U/ml of penicillin, and 100 µg of streptomycin/ml in a humidified incubator with 5% CO_2 at 37°C.

In vitro Cell Proliferation and Cell Viability Assay—Trypan Blue Exclusion Assay

Trypan blue exclusion assay was performed to assess the effect of newly synthesized products on viability of HEPG2 and MCF7 cells. Approximately 0.75×10^5 cells/ml was seeded in a six well tissue culture plate and different concentrations of compounds were added after 24 h. For the determination of growth rate, smaller aliquots were collected in a 0.5 ml tubes, trypan blue (0.4%) was added to the cell suspension, and the number of cells (viable-unstained and non-viable-blue) was counted using a haemocytometer. The media was not changed during the induction period. Each experiment was repeated a minimum of three times and the results are presented as graphs.

MTT Assay

The synthesized products (2a-c, 2i and3a-c, 3f) were subjected to a screening system for evaluation of their anticancer activity against hepatocellular carcinoma HEPG2 cell line and breast carcinoma MCF-7 cell line in comparison to the known anticancer drugs: 5-FU and DOX.

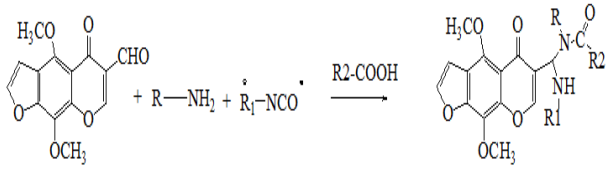
Cell survival was further assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye reduction assay[50] which is based on the ability of viable cells to metabolize a yellow tetrazolium salt to violet form azan product that can be detected spectrophotometrically. Exponentially growing cells (HEPG2 and MCF-7) were plated in triplicate in 96-well sterilized plates at a density of 1×10^4 cells/well. After 24 h, cells were treated with escalating doses of the compound under investigation and incubated in 5% CO_2 atmosphere with high humidity. After 48 and 72 h of compound exposure, the cells were incubated with MTT (0.5 mg/ml) for another 4 h at 37°C. The blue MTT formazan precipitate was then, solubilized in detergent (50% final concentration of N,N-dimethylformamide and 10% of sodium dodecyl sulphate) and incubated for an additional 2 h. Absorbance was measured at 570 nm on a multi-well ELISA plate reader. The mean absorbance of medium control was the blank and was subtracted. IC50 values (concentration of compound causing 50% inhibition of cell growth) were estimated after 72 h exposure of compound. The absorbance of control cells was taken as 100% viability and the values of treated cells were calculated as a percentage of control.

The 5-fluorouracil and doxorubicin anticancer drugs were used as positive control, and cells without samples were used as negative control. The relation between surviving fraction and drug concentration is plotted to get the survival curve of both cancer cell lines with the specified compound. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of products.

RESULTS AND DISCUSSION

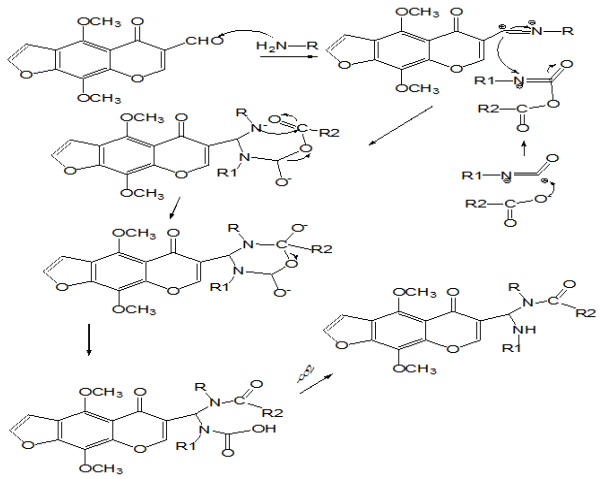
Chemistry

Highly substituted 4,9-dimethoxy-5*H*-furo[3,2-g] chromen-5-one derivatives were synthesized via a new Ugi-reaction. We started our investigation by combining these two reactions sequentially. Thus, the Ugi-reaction was performed in a typical procedure whereby amine derivatives, furochromonecarbaldehyde 1, acid derivatives namely: (n- butyric, succinic and anthranilic) acids, and cyclohexyl and/or phenylisocyanates were mixed in equimolar quantities in polar solvents (methanol)[37]. The Ugi-synthesis generally presented good yields and the purification of the desired secondary amide 2_{a-j} was performed by crystallization. They were characterized by ^{1}H NMR, IR and MS data as well as elemental analyses. All the synthesized compounds had purities >95%. Results prove that our strategy enables the preparation of the desired products. Moreover, MCRs was the isocyanate-based Ugi reaction, where an isocyanate, an aldehyde, an amine, and a carboxylic acid were condensed to afford products 2_{a-j}. The Ugi reaction is an ideal strategy for constructing chemical libraries, the synthesis was carried out as a one-pot procedure (scheme 1). At the reaction of succinic acid at high temperature, carbon dioxide molecule was lost.



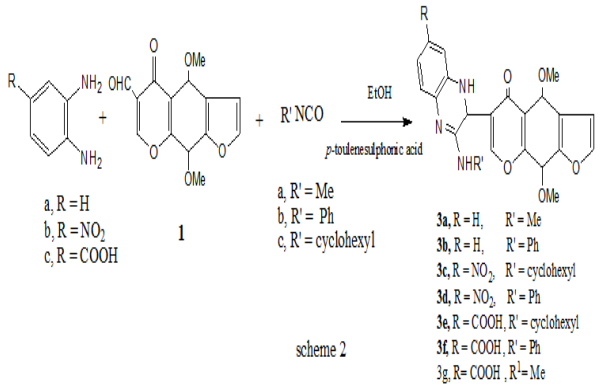
1 2a-j

Product	R	R1	R2
2a			
2b			
2c			
2d			
2e			
2f			
2g			
2h			
2i			
2j			

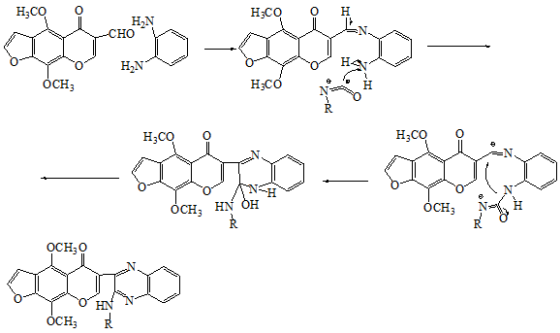


Possible Mechanism of Compound (2)

Multicomponent reactions (MCRs) have emerged as a powerful and bond-forming efficient tool in organic, combinatorial, and medicinal chemistry[38,39]. In our initial experiments, a variety of substituted *o*-phenylenediamines, furochromonecarboxaldehyde 1 and isocyanate derivatives were carried out to establish the scope and the generality of the present transformation (Scheme 2). Several structurally diverse isocyanates including cyclohexyl and phenyl substituted ones were firstly used and the expected products 3_{a-g} were isolated (scheme 2). These results indicate that the excellent reactivity of isocyanate components must play an important role in the whole process as the condensation of *o*-phenylenediamine derivatives with 1equiv. furochromonecarboxaldehyde 1 is very easy to take place[40,41]. In our runs, however, the reactions are quite clean and no other side reactions are observed. Various diamines and furochromonecarboxaldehyde 1 gave satisfactory results. It was important to note that the present reaction was quite region selective. The ^{1}HNMR and IR spectroscopy of compound 3_{a-g} showed that only one isomer was produced. Quinoxaline derivatives are a common occurrence in many pharmacological active substances of natural or synthetic origin.[42] Many known antibiotics including echinomycin, actinomycin, and leromycin possess the basic Scheme 2. Moreover, quinoxaline analogs also serve as dyes, organic semiconductors as well as other useful materials,[43-47] which build up the attractiveness for their syntheses.



scheme 2



Bioactivity

Anticancer Activity

Table 1: IC50 of the newly synthesized products against the two cell lines

Compound	Cell lines	
	MCF-7	HEPG-2
Solvent	76.774a	76.774a
Start	46.023b	41.598b
2i	26.574c	18.782def
3a	25.490c	22.812cd
2a	22.192de	25.982c
2b	24.757c	17.501f
2c	20.625cd	17.785ef
3b	18.699cd	17.505f
3c	14.319de	12.071g
3f	14.278de	19.297def
5 flurouracil	12.410e	
Doxorubicin		11.756g

Means followed by the same letter are not significantly different

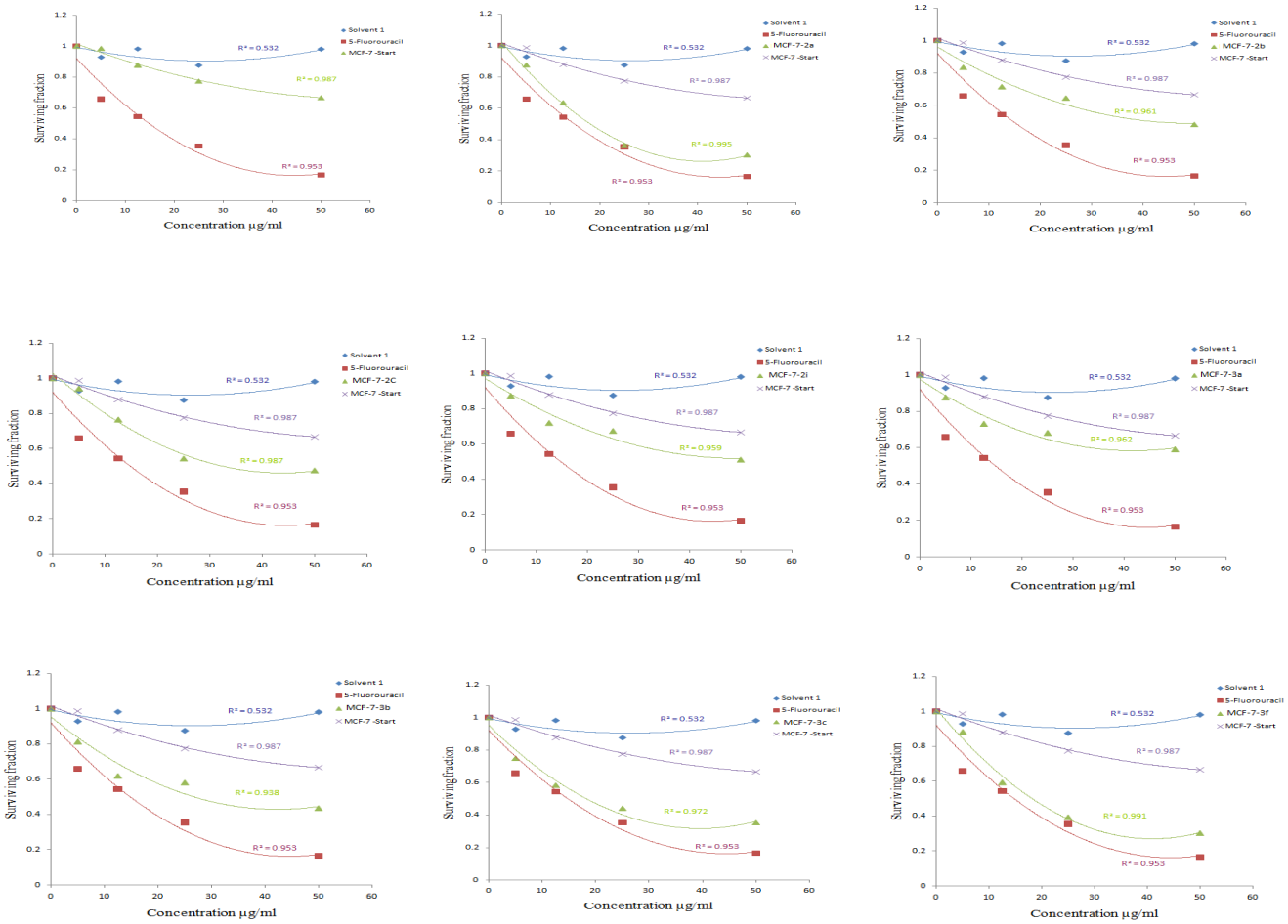
Cancer and other chronic diseases share some common pathogenic mechanisms, such as DNA damage, oxidative stress, and chronic inflammation. These diseases can be controlled by resistant to mutagens/carcinogens and/or to inhibit progression of the disease by administering chemopreventive agents[48]. Chemotherapy and surgery are standard methods for treatment of these diseases, although not been fully effective. Most of the anti-tumor drugs currently used in chemotherapy are toxic to normal cells and cause toxicity for immune cells. So it is important to minimize curing doses to the least amount possible as well as trying to minimize the side effects of these drugs. Therefore, the identification of new anti-cancer drug with low side effects on immune system has become an essential goal in many studies of immunopharmacology[49].

The newly synthesized products were evaluated for their *in vitro* cytotoxic activity against human hepatocellular carcinoma (HEPG2) and breast carcinoma (MCF-7) cell lines. Doxorubicin and 5-Fluorouracil, which are two of the most effective anticancer agents, were used as a reference drugs. Our results showed that some of the

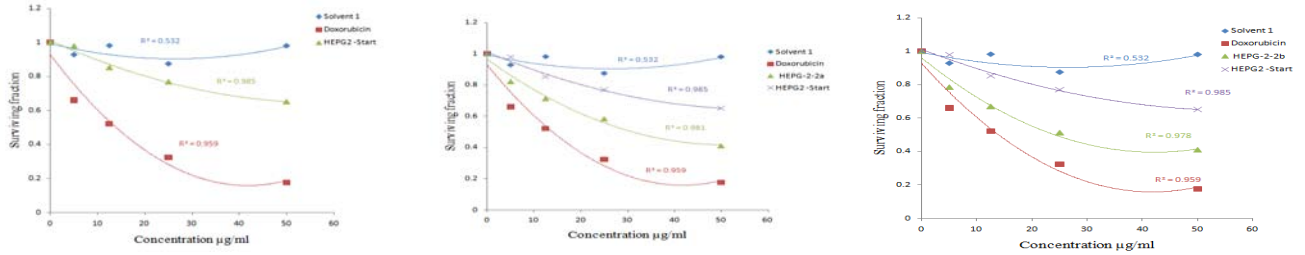
newly synthesized products exhibited a moderate to strong growth inhibition activity on the tested cell lines between 0-50 µg/ml concentrations in comparison to the reference anticancer drugs.

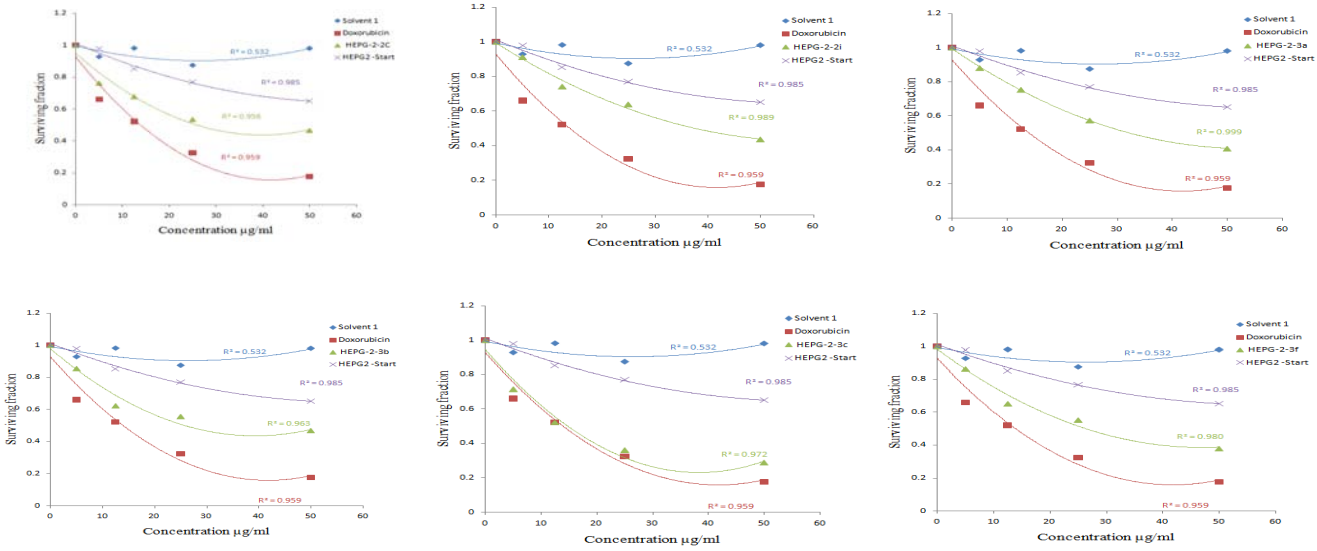
The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of the two cell lines. The response parameter calculated was the IC50 value, which corresponds to the concentration required for 50% inhibition of cell viability.

Table 1 shows the *in vitro* cytotoxic activity of the synthesized compounds, where some compounds exhibited significant activity compared to the reference drug. Figures (1-9) showed the cytotoxic activity of the selected synthesized products (2a-c, 2i/3a-c, 3f) against breast MCF7 cancer cell line and Figures (10-18) showed the activity against HEPG2 cell line in comparison to the reference drugs: 5-FU and DOX. From the results obtained in Table 1, compound 3c and 3f showed an *in vitro* cytotoxic activity with IC50 value of 14.319 and 14.278 respectively for MCF-7 cell line, 12.071 and 19.297 respectively for HEPG-2 cell line when the cells were subjected to different concentrations of the compound. It can be deduced from our results that products 2a-c, 2i and 3a-c, 3f were the most active and induced a reasonable growth inhibition, in a dose-dependent manner against both cell lines when compared to start material and to 5-FU and DOX (Table 1).



Figures 1-9: The cytotoxic activity of the selected synthesized products (2a-c, 2i / 3a-c, 3f) against breast MCF7 cancer cell line





Figures 10-18: The cytotoxic activity of the selected synthesized products (2a-c, 2i/3a-c, 3f) against breast HEPG-2 cancer cell line.

CONCLUSION

Chromone and its analogs have proved for potentially great importance in medicinal chemistry and drug development. They come from a wide variety of natural sources and new chromone derivatives are being discovered or synthesized on a regular basis. Chromone is a simple molecule and many of its derivatives have been known for more than a century. The current study has outlined the chemistry and anticancer activity of some chromone derivatives. It could be concluded from our work that synthesized products have high cytotoxic activity against both types of liver and breast cancer cells subjected in this study. The above mentioned results revealed that the materials under investigation have killing potency to the cancer cells with low concentrations and can be arranged in the following order: 2a > 3f > 3c > 3b.

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