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Research Article

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New Studying for One-pot Multicomponent Reactions to Prepare Novel Furochromone Compounds with Antitumor Activity

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Abstract The refluxing of furochromone carboxaldehyde (1) and dimethyl acetylene dicarboxylate with amine derivatives namely *m*-nitroanilline, *p*-methylanilline, *o*-aminophenol, *o*-anisidine and *p*-anisidine to give compounds (2a-e). While the reaction of mixture of furochromone carboxaldehyde (1) with dimethyl acetylendicarboxylate in the presence of cyclohexyl isocyanide to afford compound (3). The reaction mixture of furochromone carboxaldehyde (1), cyclohexyl isocyanide with cinnamic acid, chlorocarboxylic acid and/ or benzoic acid respectively to give compounds (4a-c). Also, the condensation of furochromone carboxaldehyde (1), cyclohexyl isocyanide and *o*-aminophenol/and or *o*-phenylenediamine 5a, b respectively with chlorocarboxylic acid, carboxylic acid and cinnamic acid give compounds (6a, b - 8a, b). The addition of aldehyde with cyclohexyl isocyanide afforded compound (9) which when react with p-anisidine give compound (10). Some of the newly synthesized derivatives shows a reasonable antiproliferative activity towards liver and breast (HEPG2 & MCF7) tumor cell lines compared to traditional anticancer agents: 5-Fluorouracil & Doxorubicin. Moreover, compound 6a showed anticancer activity against NMU induced breast tumor *in vivo.*

Keywords dimethyl acetylene dicarboxylate, cyclohexyl isocyanide, *o*-aminophenol, p-anisidine, breast and liver cancer cell lines, N-Methyl-N-Nitrosourea (NMU).

1. Introduction

Many types of tumors are mainly from the most serious diseases around the world. Cancer characterized by the uncontrolled growing abnormal cells [1-2]. Metastasis of tumor cells mainly depend on angiogenesis; for that, angiogenesis should be affected to help the inhibition of tumor invasion, rapidly growing cells and metastasis [3].

It is well known that the newly synthesized organic compounds have significant role in new drugs development [4]. Heterocyclic compounds [5] are the most important in the discovery and development of new drugs, large numbers of heterocyclic compounds having important applications and intermediates in organic synthesis. Heterocyclic compounds [6] have useful pharmacological activities. Also, Multi-component method allows more than two molecules to be combined in practical, one-pot operations [7]. Recently, multi-component reactions have synthetic of modern drug discovery. Recent observations show that 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 [8]. So, in this study, the new chromone derivatives are prepared.

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.

Synthesis of (2a-e)

Toluene-4-sulfonic acid monohydrate (0.1mmol) was dissolved in toluene stirred with (10mmol) from aniline derivatives(3-nitroaniline, 4-methylaniline, aminophenol, o-anisidine and 4-anisidin for 2min and then(1mmol)of diethyl acetylene dicarboxylate followed by (10mmol) of furochromone carboxaldehyde (1), after the reaction mixture was heated at 60-70^{o}C until completion of the reaction as indicated by TLC the reaction mixture was cooled to the room temperature the aqueous phase was extracted with ethyl acetate.

Methyl 4-(3-nitrophenylamino)-2, 5-dihydro-2-(4-methoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl)-5-oxofuran-3-carboxylate (2a)

Yellow solid, m.p.220ºC yield (75%). Analysis for: $C_{25}H_{18}N_2O_{11}$, Mol. Wt. 522.42: calc.: C, 57.48; H, 3.47; N, 5.36, found: C, 57.46; H, 3.49; N, 5.36; IR (KBr, cm^{-1}): 1533 (NO_2), 1636, 1738 (3CO) and 3433 (NH). ^{1}H NMR (DMSO-d6, δ, ppm, 3.35, 3.42 (s, 9H, $3OCH_3$); 4.20 (s, 1H, CH), 7.09; 7.84(dd, 2H, J=2.0, furan ring); 6.46-7.45(m, 4H, arom); 8.28 (s, 1H, H_7) and 8.44(s, 1H, NH exchangeable with D_2O)

Methyl 4-(p-tolylamino)-2, 5-dihydro-2-(4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl)-5-oxofuran-3-carboxylate (2b)

Yellow solid, m. p220ºC yield (75%). Analysis for: $C_{26}H_{21}NO_9$, Mol. Wt.: 491.45, calc.: C, 63.54; H, 4.31; N, 2.85; found: C, 63.52; H, 4.31; N, 2.86; IR (KBr, cm^{-1}): 1646, 1755 (3CO) and 3423 (NH). ^{1}H NMR (DMSO-d6, δ, ppm): 1.19 (st, 3H, CH_3-), 3.78; 3.89 (s, 9H, $3OCH_3$), 5.45 (s, 1H, CH), 7.00; 7.45 (dd, 2H, J=2.0, furan ring), 6.67-7.96 (m, 4H, arom), 8.00 (s, 1H, H_7) and 11.44 (s, 1H, NH exchangeable with D_2O).

3-(methoxy carbonyl)-2-(2-hydroxyphenylimino)-4-(4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl) but-3-enoic acid (2c):

Brown solid, m.p.140ºC yield (75%). Analysis for: $C_{25}H_{19}NO_{10}$, Mol.Wt. 493.42 calc.: C, 60.61; H, 3.88; N, 2.84, found: C, 60.63; H, 3.86; N, 2.81 IR (KBr, cm^{-1}): 1615, 1734 (3CO) and 3421 broad band for (NH, OH). ^{1}H NMR (DMSO-d6, δ, ppm): 3.64, 3.68, 3.83 (ss, 9H, $3OCH_3$); 4.66 (s,1 H, OH exchangeable with D_2O) : 5.67(s, 1H, CH); 6.76; 7.82 (dd, 2H, J=2.0, furan ring); 6.67 -7.43(m,4H, arom); 8.16 (s, 1H, H_7) and 9.25 (s, 1H, NH exchangeable with D_2O).

Methyl 4-(2-methoxyphenylamino)-2, 5-dihydro-2-(4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl)-5-oxofuran-3-carboxylate (2d)

Brown solid, m.p.175 ºC yield (75%). Analysis for: $C_{26}H_{21}NO_{10}$, Mol. Wt.: 507.12, calc.: C, 61.54; H, 4.17; N, 2.76, found: C, 61.55; H, 4.18; N, 2.74. IR (KBr, cm^{-1}): 1632; 1700. (3CO) and 3411 (NH). ^{1}H NMR (DMSO-d6, δ, ppm): 3.76, 3.87 (ss, 12H, $4OCH_3$); 5.55 (s, 1H, CH); 7.00, 7.44 (dd, 2H, J=2.0, furan ring); 6.66-7.68 (m, 4H, arom); 8.00 (s, 1H, H_7) and 9.94 (s, 1H, NH exchangeable with D_2O).

Methyl 4-(4-methoxyphenylamino)-2, 5-dihydro-2-(4, 9-dimethoxy-5-oxo-5H-furo [3,2-g] chromen-6-yl)-5-oxofuran-3-carboxylate (2e)

Brown solid, m.p.140ºC yield (75%). Analysis for: $C_{26}H_{21}NO_{10}$, Mol. Wt.: 507.45, calc.: C, 61.54; H, 4.17; N, 2.76, found: C, 61.55; H, 4.01; N, 2.19 IR (KBr, cm^{-1}): 1617, 1730 (3CO) and 3434 (NH). ^{1}H NMR (DMSO-d6, δ,

ppm): 3.66; 3.69; 3.72 (ss, 12H, $4OCH_3$); 5.45 (s, 1H, CH); 6.95; 7.43(dd, 2H, J=2.0, furan ring); 6.54-7.52 (m, 4H, arom); 7.93 (s, 1H, H_7) and 9.40 (s, 1H, NH exchangeable with D_2O).

General procedure for the preparation of dimethyl 2-(Cyclohexylimino)-5-(4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl) furan-3, 4-dicarboxylate compound (3):

A mixture of furochromone carboxaldehyde (1) (1mmol), dimethyl acetylendicarboxylate (DMAD) (1mmol) and cyclohexyl isocyanide (1mmol) in water (5mml) in presence of benzyl triethyl ammonium chloride (10mol %) was stirred at 80oC for appropriate time (1-2h). After completion of the reaction as indicated by TLC, the reaction mixture was extracted with ethyl acetate. The combined organic extracts were concentrated in vacuo, and then purified with diethyl ether.

Brown solid, m.p. 115ºC yield (75%). Analysis for: $C_{27}H_{27}NO_{10}$, Mol. Wt.: 525.5, calc.: C, 61.71; H, 5.18; N, 2.67, found: C, 61.72; H, 5.19; N, 2.65; IR (KBr, cm^{-1}), 1615;1735 (3CO) and 3409 (NH).^{1}H NMR (DMSO-d6, δ, ppm): 1.26-1.65 (m, 10H, $5CH_2$ cyclohexane), 2.46 (dd, 2H, CH-NH); 3.74, 4.12, 414, 4.15(ss, 12H, $4OCH_3$); 7.02, 7.91 (dd, 2H, J=2.0, furan ring); 8.00 (s, 1H, H_7) and 9.65 (d, 1H, NH exchangeable with D_2O).

General procedure for the preparation of comp(s) (4a-c)

To a magnetically stirred solution of acid derivatives namely, chlorobenzoic acid and benzoic acid (1.0mmol) and furochromone carboxaldehyde (3) (1.0mmol) in toluene (25ml) was added cyclohexyl isocyanide (1.0mmol) and the mixture was heated to reflux for 4h. The solvent was removed under reduced pressure, the residue was washed with toluene (5ml) and re-crystalized from diethyl ether/hexane (1:3) to give the products.

2 - (Cyclohexylcarbamoyl) (4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl) methyl cinnamate (4a)

Beige solid, mp.108 ^oC, Yield (55 %). Analysis for: $C_{30}H_{29}NO_8$, Mol. Wt.: 531.55 found: C, 67.79; H, 5.50; N, 2.64, calc: C, 67.67; H, 5.51; N, 2.65. IR (KBr, cm^{-1}): 1618, 1654, 1688 (3C=O), 3329 (NH). ^{1}H NMR (DMSO-d6, δ, ppm) : 1.19-1.173 (m, 10H, $5CH_2$ cyclohexane) ; 3.93, 4.07 (s, 6H, $2OCH_3$); 3.54 (dd, 2H, CH-NH); 6.39 (ss,2H, CH=CH); 6.67, 7.84(dd, 2H, J=2.0, furan ring); 6.67-8.04(m,5H, arom); 8.13 (s, 1H, H_7) and 8.22 (d, 1H, NH exchangeable with D_2O).

Cyclohexylcarbamoyl) (4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl) methyl 2-chlorobenzoate (4b):

Yellow must solid, mp.215^{o}C. Yield (75%). Analysis for: $C_{28}H_{26}ClNO_8$, Mol. Wt.: 539.96, calc.: C, 62.28; H, 4.85; Cl, 6.57; N, 2.59, found: C, 62.30; H, 4.84; N, 2.58, IR (KBr, cm^{-1}): 1600, 1684, 1797 (3C=O); 3431 (NH). ^{1}H NMR (DMSO-d6, δ, ppm): 1.19 -1.174 (m, 10H, $5CH_2$ cyclohexane); 3.93, 4.07 (s, 6H, $2OCH_3$); 6.19 (s, 1H, CH-NH); 6.67, 7.84(dd, 2H, J=2.0, furan ring) ,6.88-7.82(m,5H, arom); 8.13 (s, 1H, H_7) and 8.22 (d, 1H, NH exchangeable with D_2O), m/e: 539(15%), 274(17%), 246(18%), 171(10%), 156(85%).

(Cyclohexylcarbamoyl)(4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl) methyl benzoate (4c):

Buff Solid, mp. 154^{o}C Yield (70%).Analysis for: $C_{28}H_{27}NO_7$, Mol. Wt.: 505.52, calc.: C, 66.53; H, 5.38; N, 2.77; found: C,66.55; H,5.37; N, 2.76, IR (KBr, cm^{-1}): 1618, 1654; 1693 (3CO) and 3434 (NH). 1H-NMR (DMSO-d6, δ, ppm, 1.12-1.71 (m, 10H, $5CH_2$ cyclohexane); 3.91, 4.04 (s, 6H, $2OCH_3$); 6.06 (d, 1H, CH-NH); 6.65; 7.67(dd, 2H, J=2.0, furan ring); 7.22, 7.63 (m,4H, arom), 8.01 (s, 1H, H_7) and 8.24 (d, 1H, NH exchangeable with D_2O).

General procedure for the preparation of comp(s). (6a, b-8a, b)

To a 25ml round flask was added (0.25mmol) of o-aminophenol and/or o-phenylenediamine (5a, b) (0.3g) of furochromone carboxaldehyde (3) in methanol and the mixture was stirred for 30min at room temperature. Add (0.25g) of the acid to the mixture followed by stirring for another 5mmin. Finally, (0.23mmol) of isocyanides was added. After the resultant mixture was stirred at room temperature for 1-7days, solid K2CO3 (50mg) 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. Crystallized from pet ether

N-((cyclohexylcarbamoyl)(4,9-dimethoxy-5-oxo-5H-furo[3,2-g] chromen-6-yl) methyl)-2-chloro-N-(2-hydroxyphenyl) benzamide (6a):

Dark brown solid, m.p.195ºC yield (80%).Analysis for: $C_{34}H_{31}ClN_2O_8$, Mol. Wt.: 631.07, calc.:C, 64.71; H, 4.95; Cl, 5.62; N, 4.44, found: C, 64.72; H, 4.94; Cl, 5.01; N, 4.13., IR (KBr, cm^{-1}) :1586, 1655, 1739 (3CO) and 3278 ,

3396 (NH,OH) .^{1}H NMR (DMSO-d6, δ, ppm): 1.00-1.17 (m, 10H , 5CH_2 cyclohexane); 3.36 (d, 1H, CH-NH); 3.90 (s, 6H, 2OCH_3); 3.89 (s, 1H, OH exchangeable with D_2O), 4.36 (d, 1H, CH-) , 6.63; 7.52 (dd, 2H, J=2.0, furan ring),6.88-7.45 (m 8H, arom.); 7.91 (s, 1H, H_7) and . 8.22 (d, 1H, NH exchangeable with D_2O).

N-((cyclohexylcarbamoyl)(4,9-dimethoxy-5-oxo-5H-furo[3,2-g] chromen-6-yl) methyl)-N-(2-aminophenyl)-2-chlorobenzamide (6b:)

Yellow solid, m.p.245^{o}C. Yield (75%). Analysis: $C_{34}H_{32}ClN_3O_7$, Mol. Wt.: 630.09. calc.: C, 64.81; H, 5.12; Cl, 5.63; N, 6.67 found: C, 64.83; H, 5.10; Cl, 5.32; N, 6.04. IR (KBr, cm^{-1}) :1615, 1735, 1756 (3CO) and 3183, 3448 (NH, NH_2) .^{1}H NMR (DMSO-d6, δ, ppm): 1.26-1.45 (m, 10H , 5CH_2 cyclohexane); 3.31 (d, 1H, CH-NH); 3.88 (s, 6H, 2OCH_3); 5.25 (s, 2H, NH2 exchangeable with D_2O); 4.35 (d, 1H, CH-); 6.60,7.36 (dd, 2H, J=2.0, furan ring); 6.3-7.53 (m 8H, arom.); 7.56 (s, 1H, H7) and 7.65 (d, 1H, NH exchangeable with D_2O).

N-((cyclohexylcarbamoyl)(4,9-dimethoxy-5-oxo-5H-furo[3,2-g] chromen-6-yl) methyl)-N-(2-hydroxyphenyl) benzamide (7a):

Brown solid, m.p.225ºC yields (75%).Analysis for: $C_{34}H_{32}N_2O_8$, Mol. Wt.: 596.60, calc.: C, 68.45; H, 5.41; N, 4.70, found: C, 68.43; H, 5.42; N, 4.71. IR (KBr, cm^{-1}): 1600, 1685 (3CO) and 3124 ,3368 (NH,OH). ^{1}H NMR (DMSO-d6, δ, ppm): 1.26-1.65 (m, 10H , 5CH_2 cyclohexane); 3.32 (d, 1H, CH-NH); 3.88, 4.00 (s, 6H, 2OCH_3); 3.89 (s, 1H, OH exchangeable with D_2O); 4.36 (d, 1H, CH-); 7.54, 7.91(dd, 2H, J=2.0, furan ring); 7.00-7.89 (m 9H, arom.); 8.06 (s, 1H, H_7) and 10.00 (d, 1H, NH exchangeable with D_2O).

N-((cyclohexylcarbamoyl) (4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl) methyl)-N-(2-aminophenyl) benzamide (7b)

Yellow solid, m.p. 205 ^{o}C, yield (75%). Analysis: $C_{34}H_{33}N_3O_7$, Mol. Wt.: 595.64, calc.: C, 68.56; H, 5.58; N, 7.05, found: C, 68.55; H, 5.57; N, 7.07, IR (KBr, cm^{-1}): 1610, 1676 (3CO) and 3100 ,3439 (NH, NH_2) .^{1}H NMR (DMSO-d6, δ, ppm): 1.19-1.91 (m, 10H , 5CH_2 cyclohexane); 3.13 (d, 1H, CH-NH); 3.38, 3.92 (s, 6H, 2OCH3); 4.74 (s, 2H, NH_2 exchangeable with D_2O); 6.32 (d, 1H, CH-), 7.52, 7.57 (dd, 2H, J=2.0, furan ring); 6.44-7.88 (m 9H, arom.); 7.89 (s, 1H, H_7) and 9.40 (d, 1H, NH exchangeable with D_2O).

N-((cyclohexylcarbamoyl) (4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl) methyl)-N-(2-hydroxyphenyl) cinnamamide (8a)

Mustered solid, m.p.215ºC yields (75%).Analysis for: $C_{36}H_{34}N_2O_8$, Mol. Wt 622.66, calc.: C, 69.44; H, 5.50; N, 4.50, found: C, 69.43; H, 5.52; N, 4.49, IR (KBr, cm-1) :1611, 1650 (3CO) and 3123 , 3424 (NH,OH) .1H-NMR (DMSO-d6, δ, ppm): 1.24-1.76 (m, 10H , 5CH_2 cyclohexane), 3.31 (d, 1H, CH-NH); 3.96 (s, 6H, 2OCH_3); 4.03 (s, 1H, OH exchangeable with D_2O); 4.16 (d, 1H, CH-); 6.41 (d,2H,CH=CH); 7.38; 7.66 (dd, 2H, J=2.0, furan ring); 7.48-7.65 (m, 9H, arom.); 7.71 (s, 1H, H_7) and . 9.00 (s, 1H, NH exchangeable with D_2O).

(N-((cyclohexylcarbamoyl)(4,9-dimethoxy-5-oxo-5H-furo[3,2-g] chromen-6-yl) methyl)-N-(2-aminophenyl) cinnamamide (8b)

Yellow solid, m.p.265 ºC yields (75%).Analysis for: $C_{36}H_{35}N_3O_7$, Mol. Wt. 621.25, calc.: C, 69.55; H, 5.67; N, 6.76, found: C, 69.54; H, 5.66; N, 6.78, IR (KBr, cm^{-1}) : 1610, 1660 (3CO) and 3126 , 3445 (NH,NH_2) .^{1}H NMR (DMSO-d6, δ, ppm): 1.46-1.75 (m, 10H , 5CH_2 cyclohexane); 4.00 (d, 1H, CH-NH); 3.63 (ss, 6H, 2OCH3); 4.00 (d, 1H, CH-); 5.10 (s, 2H, NH_2 exchangeable with D_2O); 6.33,7.53 (d, 2H, CH=CH); 7.57, 7.52(dd, 2H, J=2.0, furan ring); 6.43-7.88(m, 9H, arom.); 7.40 (s, 1H, H_7); and . 9.30 (d, 1H, NH exchangeable with D_2O).

3-(Cyclohexylimino)-6-[(4,9-dimethoxy-6 (furan-5H-furo [3,2-g] chromen-5-one- 1-methyl 6-(5-amino-2-methoxybenzyl)-4\,9\-dimethoxy-5H-furo[3,2-g]chromen-5-one (10)

Yellow solid m.p.98 ºC yield (80%). Analysis $C_{42}H_{36}N_2O_{12}$, Mol. Wt 760.74, calc.: C, 66.31; H, 4.77; N, 3.68, found: C, 66.30; H, 4.78; N, 3.66; IR (KBr, cm^{-1}): 1646, 1671 (2C=O) and 3454 (NH_2). ^{1}H NMR (DMSO-d6, δ, ppm): 1.19-1.87 (m, 10H, 5CH_2); 3.53 (d, 1H, CH-N, cyclohexane); 3.61 (s, 2H, CH_2); 3.85 (s, 2H, NH_2 exchangeable with D_2O); 3.98, 3.99, 4.00 (sss, 15 H, 5OCH_3), 7.20- 6.42 (m, 3H, arom.); 7.31; 6.93 (dd, 4H, J=2.0, furan ring).

B- Bioactivity

***In vitro* anticancer activity**

Measurement of Potential Cytotoxicity by Sulforhodamine B (SRB) Assay

The selected derivatives (compounds 2a,2b,2c,2e,4a,4b,6a,10), 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 the known anticancer drugs: 5-FU and DOX. Potential cytotoxicity of the compounds in this study was investigated using the method of (Skehan *et al*., 1990). 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% CO2. Cultures were then fixed with trichloroacetic 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.

Biochemical Analysis

Male albino mice weighing 18–20 g were 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).

***In vivo* anticancer activity**

The aim of this part of the study was to investigate the anticancer activity of the tested compound *in vivo* (N-Methyl-N-Nitrosourea (NMU)- induced breast cancer in rats) [17-18]. To achieve this aim, the following studies were conducted into its inhibitory effect on the tumor volume, and some of the biological effects of the tested compound on hematological and biochemical alterations caused by chemically-induced mammary carcinogenesis in Wistar rats.

In this experimental study, we choose the tested synthesized compound 6a for performing *in vivo* anticancer activity as it shows the most anticancer activity against breast carcinoma cell line MCF7. In this part, female albino Wistar rats (n=40) 50-59 days old maintained at temperature 24±2ºC with a 12-hour light/dark cycle and 60%±5% humidity. They were provided with standard pellet diet and water ad libitum. They were acclimated for about two weeks before the start of the study.

Tumor induction

The animals were weighed weekly. Animals in control group (n=10) only received vehicle injections (Group-A), in the tested compound (6a) control group (n=10) received vehicle injection and the tested compound at the dose of 15 mg/kg body weight (Group-B), in NMU group (n=10) received only NMU at the dose rate of 60 mg/kg body weight intraperitoneally (i.p) five times at 50, 70, 80, 90 and 110 days of age (Group-C), in the test compound treatment group (n=10) administered with NMU and after appearance of palpable tumors, treated with the test compound for 30 days (Group-D).

Animals were observed daily to assess their general health. After NMU administration, right pectoral area of all rats was followed up for the tumoral development. The volume of every tumor was measured weekly using calipers. Tumor volume was calculated using the formula: Tumor volume (cc) (D x d2 x π) / 6 (D= big diameter, d= small diameter). The results are expressed as the mean ± Standard Error. When the nodule reached to a mean volume of 230±3.2 mm^3 (± S.E.), fine needle aspiration biopsy was performed from the nodules. Nodules reached that size in a mean of 8±2 weeks. Histopathological examination was performed from that biopsy for each nodule. After NMU, rats were sacrificed and tumors were removed from the animals. The tumor volume inhibition ratio (%) was calculated by the following formula: Inhibition Ratio (%) = [(A - B) / A] x 100, where A is the average tumor volume of the control group, and B is the tumor volume of the treated group.

Hematology

Red Blood Cells(RBC), hemoglobin (Hb), Packed Cell Volume (PCV), total White Blood Cells(WBC), Differential Leukocyte Count (DLC) and thrombocyte count was estimated using an automated blood analyzer (Cell Dyn®3700, Abbott Dignostic, USA).

Serum biochemistry

Blood was collected in sterile vial without anticoagulant for serum separation. Sera samples were analyzed for biochemical parameters such as total protein, albumin, calcium, alanine amino teransferases, (ALT/SGPT), aspartate amino teransferases (AST/SGOT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) using standard commercial kits.

Statistical analysis

All experiments were done in triplicate, independently, and the results were expressed as the mean ± SD. Statistical analyses of the data were performed using the SPSS software, version 9.01. Results were expressed as mean ± SD of the indicated number of independent experiments. Analysis of variance (ANOVA) was used to compare the mean value of data and $P<0.05$ were considered as significant.

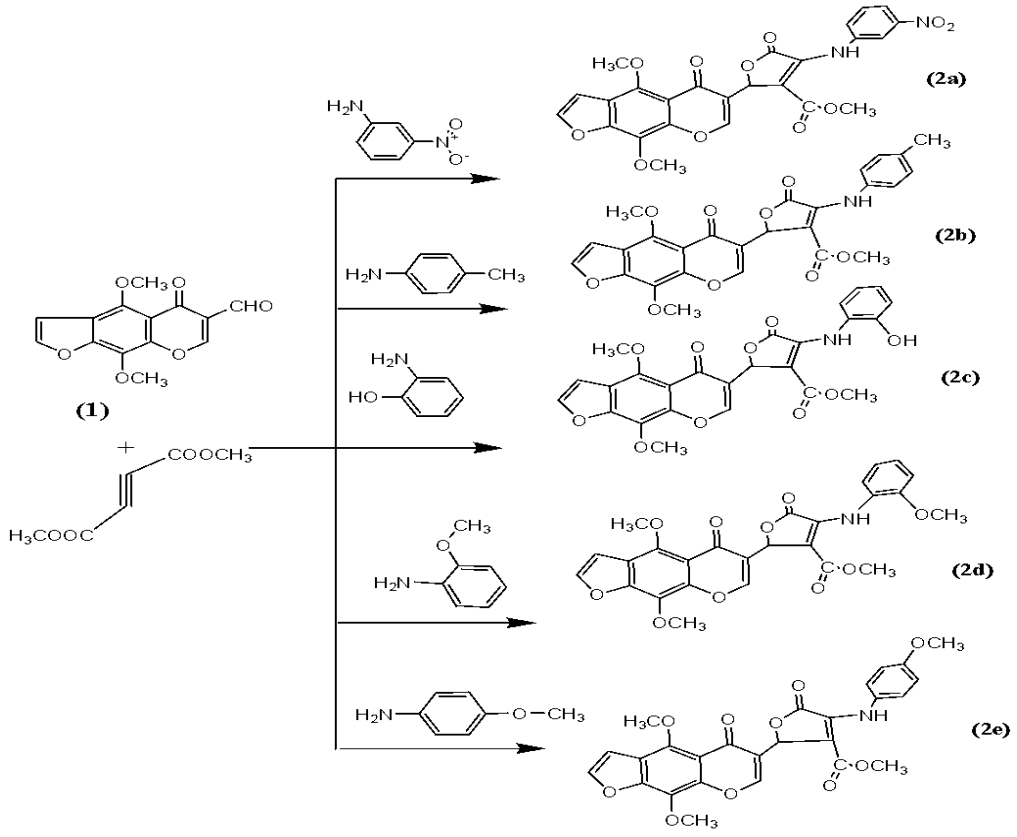
Results and Discussion

A- Chemistry

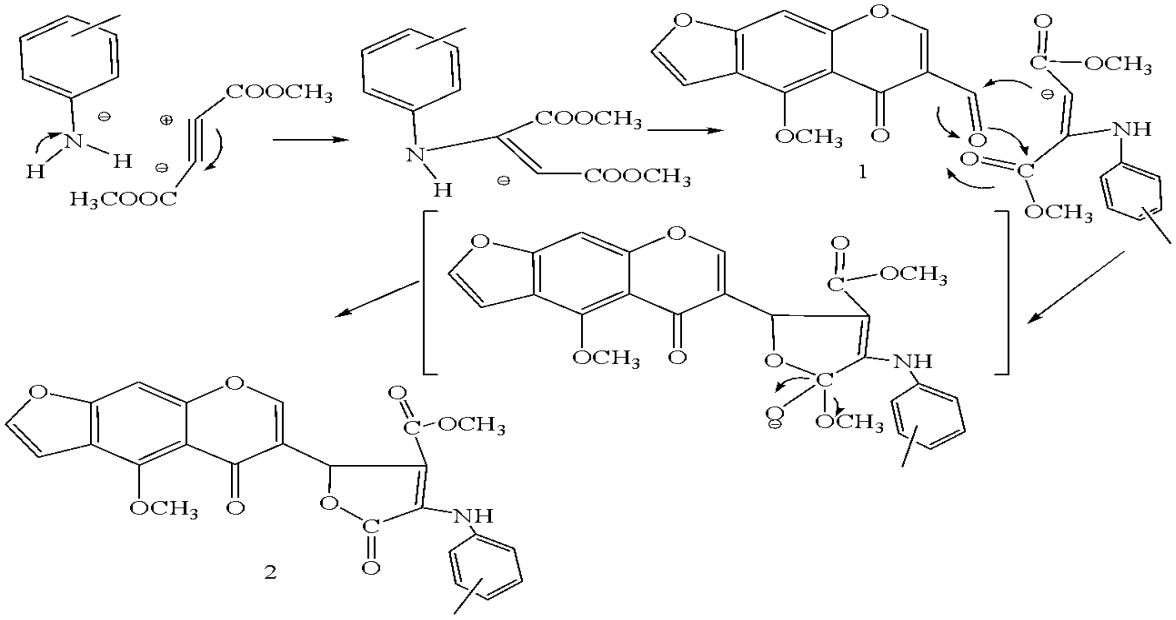
In general, model reaction was carried out to yield derivatives **(3a-e)** through the reaction of [aniline derivatives (2a-e) (namely, m-nitroanilline, *p*-methylanilline, *o*-aminophenol, *o*-anisidine and p-anisidine), followed by successive addition of dimethyl acetylene dicarboxylate [23-26] (DMAD) and furochromone carboxaldehyde (1)] while stirring. The reaction mixture was heated at 60-70^oC until completion of the reaction after 12 h (Scheme 1). The final ring-closing reaction was performed by a classical intermolecular N-aryl amidation of secondary amides. This reaction was agreed with [19]. In the IR spectrum, the (CHO gp) disappeared. In the ^1H NMR spectra showed the aromatic protons of aniline, ester and tertiary amine.

To develop new methods for the preparation of biologically active compounds from readily available building blocks, It was used a simple new method for the synthesis of ***chromen*** derivatives (4a-c) from a three-component reaction. At heating a mixture of [furochromone carboxaldehyde (1), cyclohexyl isocyanide with cinnamic acid, chlorobenzoic acid and/ or benzoic acid respectively], the isolated products (4a-c) (Scheme3) were characterized

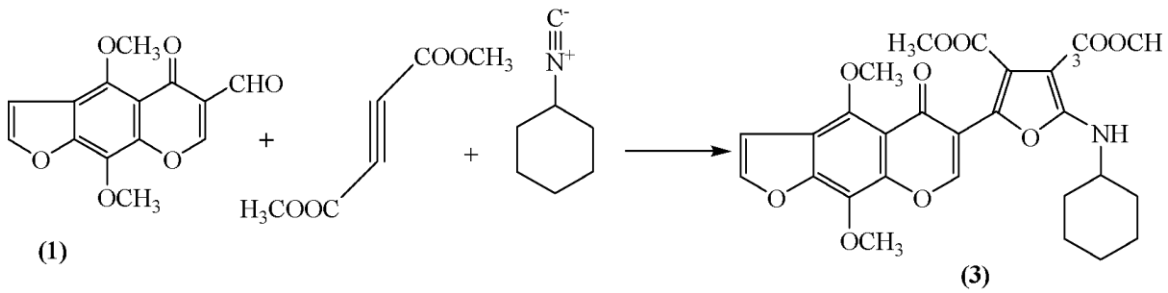
based on IR and ^{1}HNMR spectroscopy. The peaks of IR spectrum of (3CO gps) and (NH gp) were shown. The ^{1}H NMR spectrum of (21a-c) exhibited 1H proton for (CH- gp) and 1H proton for (NH-gp).



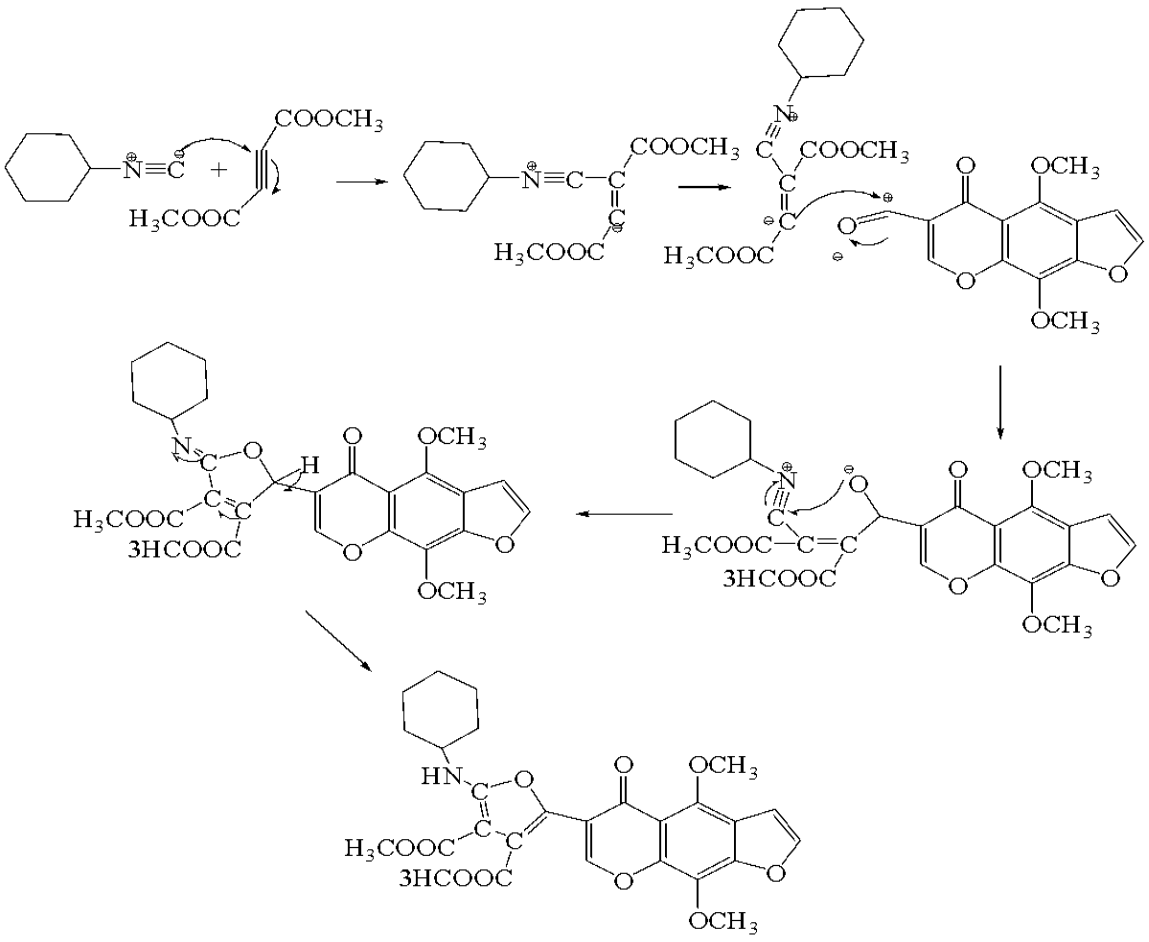
(Scheme1)



Mechanism of compounds 2a-e

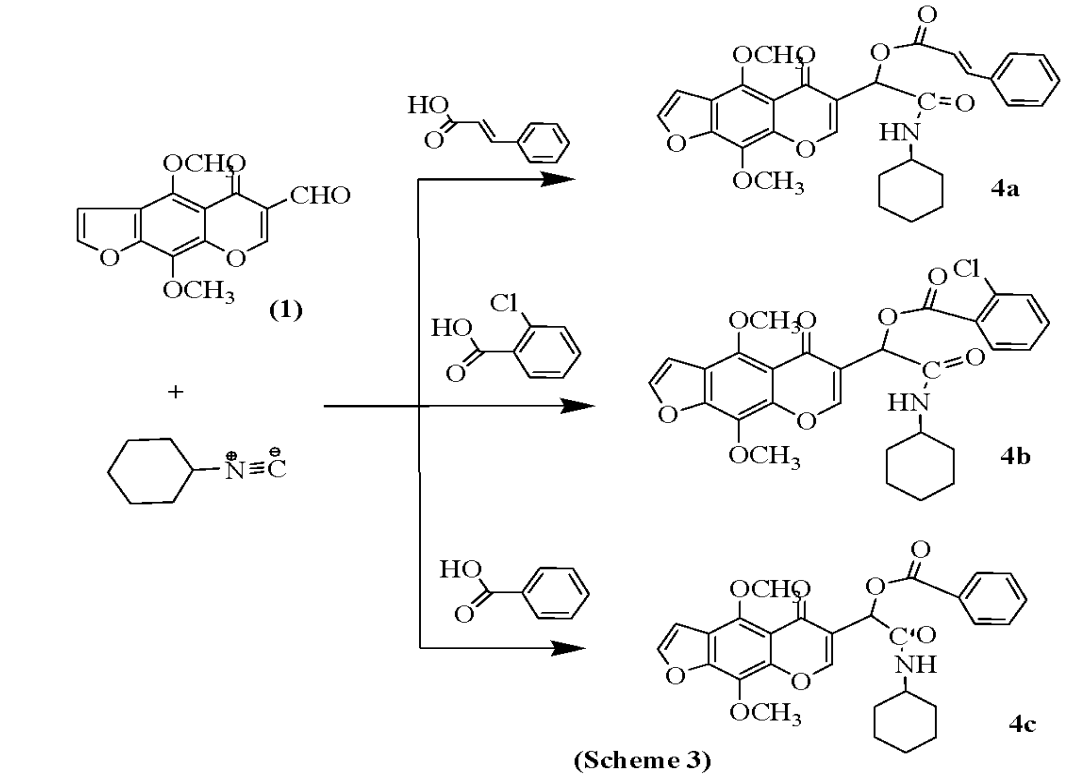


(Schem 2)

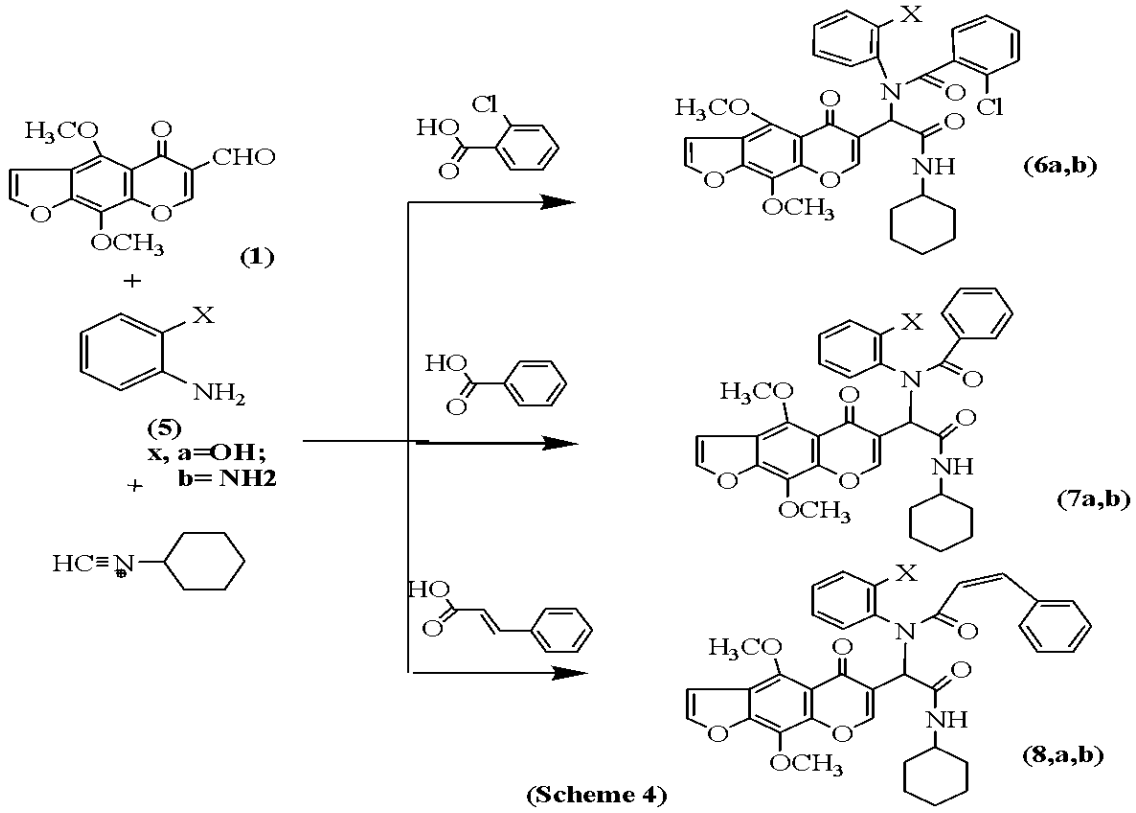


Mechanism of compound 3

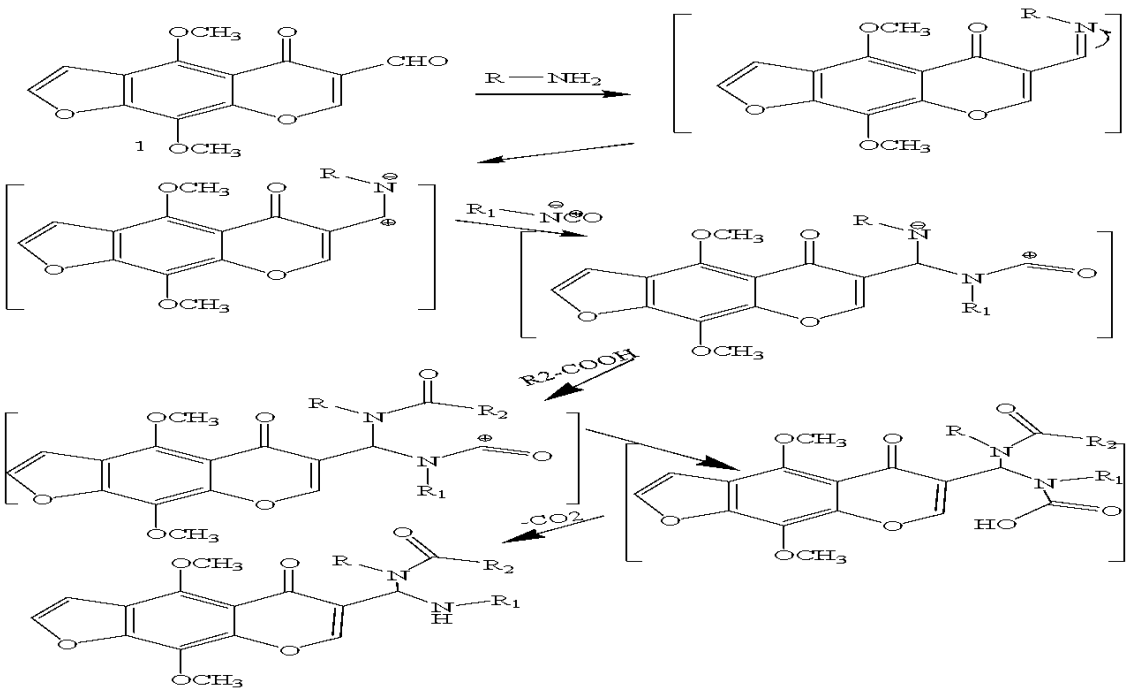
The reaction was formed in a procedure by [amine, carbonyl, carboxylic acid and isocyanides] were mixed in polar parotic solvents (methanol (, the expected N-heterocyclic compounds were successfully isolated with moderate to good yields. They were characterized by ^{1}H NMR, IR and mass spectrum. All the synthesized compounds had purities >95% .The reaction of furochromone carboxaldehyde (1), cyclohexyl isocyanide and *o*-aminophenol and/ or *o*-phenylenediamine (5a, b) with acid derivatives (6a-e) (namely, chlorobenzoic acid and benzoic acid) to give chromen derivatives (7a, b, 8a, b) (schem4). This reaction was agreed with [20-21].



(Scheme 3)

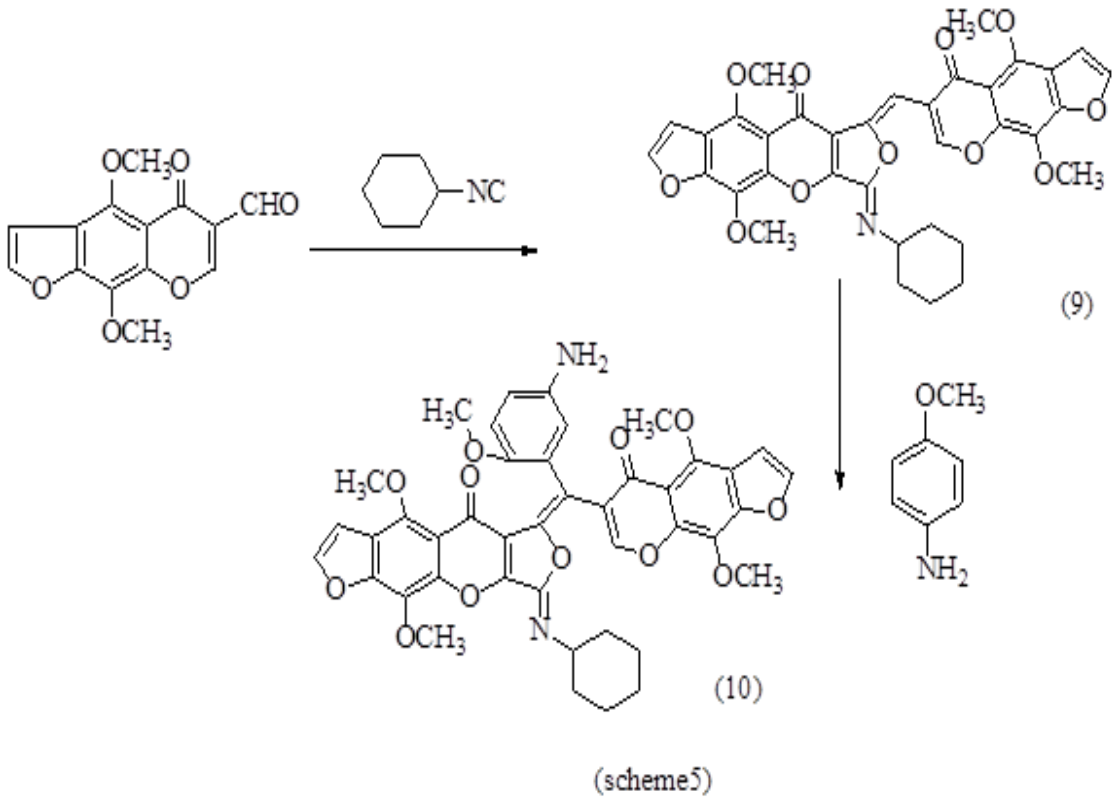


(Scheme 4)



Mechanism of compounds 6-8a,b

In the present investigation chromen (10) was obtained by the reaction of [aldehyde with cyclohexyl isocyanide] afforded compound (9) which when react with p-anisidine give compound (10) (scheme 5). Compound (10) was stable yellow solids whose structure was established by IR and ^{1}HNMR and mass spectroscopy. The IR showed the absence of carbonyl band of the (CHO gp), ^{1}H NMR spectrum exhibited 2H protons for (-NH_2) and disappearance of 1H proton for (CHO gp).



(scheme5)

***B*- Bioactivity**

***C*- *In vitro* anticancer activity**

The newly synthesized furochromone derivatives indicated a reasonable antiproliferative activity towards current tested breast and liver cultured cell lines (MCF7 and HEPG2) between 4.88 – 10.6 µg/ml concentrations in comparison to anticancer agents: 5-Fluorouracil & Doxorubicin as demonstrated in table 1.

Induced biochemical parameters through the effect of newly synthesized furochromone derivatives:

Tables 2, 3 & 4 demonstrates the effect of newly synthesized furochromone derivatives on some biochemical parameters which induced in serum of treated and nontreated groups of mice in comparison to anticancer agents: 5-Fluorouracil & Doxorubicin. Results declared that most of the investigated biochemical parameters gave high significance ratio of $P < 0.001$ in mice groups with 5-Fluorouracil & Doxorubicin than that of non-treated group, while in mice groups treated with the newly synthesized derivatives of furochromone there were different biochemical effects, where some of the estimated values of the induced biochemical parameters were indicates a non-significant (n.s.) or a little bit higher significance ($P < 0.01$) compared to non-treated group.

Based on these findings in the present work, some of the newly synthesized derivatives may have reasonable biological activity as anti-proliferative agents towards some cancer cell lines with the advantage of less toxic side effects.

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

Cell Lines		**Compound**
MCF7 (IC_{50})	**HEPG2 (IC_{50})**	
0.67	5	5 flurouracil
6.71	3.56	Doxorubicin
16	14.3	2a
9.08	8.3	2b
8.63	18.1	2c

8.48	10.6	2e
10.7	10.3	4a
8.93	20.7	4b
4.88	18.1	6a
11.6	16.7	10

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

ALP (k.k./dL)	AST (IU/mL)	ALT (IU/mL)	Compounds
17.70 ± 1.10	108.32 ± 4.19	43.50 ± 2.03	**Control**
25.49 + 6.03 *	130.43 ± 8.92 *	51.47 ± 9.02 *	**5-Flurouracil**
30.32 ± 5.14 *	147.23 ± 16.34 *	59.26 ± 12.03 *	**Doxorubicin**
23.5 ± 4.9 **	109.4 ± 14.8 ***	46.8 ± 7.6 ***	2a
41.3 ± 11.7 *	157.9 ± 23.4 *	69.3 ± 17.1 *	2b
38.6 ± 14.5 *	148.9 ± 35.4 *	77.4 ± 11.3 *	2c
19.9 ± 5.6 ***	115.9 ± 7.3 ***	47.7 ± 8.6 ***	2e
27.9 ± 7.8 **	117.2 ± 18.2 **	52.7 ± 10.4 **	4a
25.4 ± 5.8**	110.1 ± 11.5***	41.6 ± 5.7***	4b
45.7 ± 9.9*	152.3 ± 29.1*	61.4 ± 9.2*	6a
19.8 ± 5.8 ***	116.9 ± 17.3 **	38.2 ± 6.9 ***	10

* $p < 0.001$: 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 the new derivatives on total lipids, cholesterol, triglycerides, and bilirubin in mice.

Bilirubin (mg/dL)	Triglycerides (mg/dL)	Cholesterol (mg/dL)	Total Lipids (mg/dL)	Compounds
0.63 ± 0.04	108.70 ± 16.8	94.32 ± 13.5	323.41 ± 27.1	**Control**
0.75 ± 0.1 *	126.50 ± 19.4 *	105.90 ± 11.7 *	378.20 ± 31.4 *	**5-Flurouracil**
0.81 ± 0.19 *	137.80 ± 17.1 *	109.30 ± 14.2 *	366.70 ± 6.1 *	**Doxorubicin**
0.51 ± 0.09 **	109.7 ± 24.7 ***	96.3 ± 13.5 ***	317.4 ± 21.8 ***	2a
1.01 ± 0.9 *	96.9 ± 11.4 ***	115.3 ± 12.7 **	381.2 ± 38.4 *	2b
0.76 ± 0.04 **	162.2 ± 25.8 *	110.3 ± 28.7 **	365.4 ± 37.3 *	2c
0.53 ± 0.02 **	112.1 ± 17.3 ***	96.9 ± 20.4 ***	326.7 ± 19.5 ***	2e
0.74 ± 0.1 **	113.4 ± 9.5 **	112.5 ± 17.7 **	371.5 ± 31.8 *	4a
0.66 ± 0.04***	114.9 ± 12.3***	95.3 ± 13.5***	319.5 ± 23.2***	4b
0.97± 0.05*	141.8 ± 36.8*	145.3± 28.4*	363.7 ± 23.7*	6a
0.65 ± 0.07***	124.9 ± 11.3***	96.8 ± 16.1***	317.3 ± 9.5***	4
0.66 ± 0.01 ***	111.5 ± 9.3 ***	95.4 ± 8.8 ***	327.2 ± 11.2 ***	10

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

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

Creatinine (mg/dL)	A/G Ratio	Globulin (mg/dL)	Albumin (mg/dL)	Biochemical Parameters
0.69 ± 0.03	1.3	4.32 ± 0.9	5.63 ± 0.51	**Control**
0.81 ± 0.06 **	1.13 **	5.75 ± 0.8 **	6.49 ± 0.92 **	**5-FU**
0.78 ± 0.04 **	1.078 **	5.91 ± 0.63 **	6.37 ± 0.85 **	**DOX**

0.74 ± 0.05 ***	1.15 ***	5.21 ± 0.8 ***	5.82 ± 0.7 ***	2a
1.72 ± 0.09 *	1.02 **	6.11 ± 0.4 **	6.94 ± 0.3 **	2b
0.75 ± 0.05 ***	1.14 *	8.94 ± 0.7 *	10.46 ± 1.1 *	2c
0.67 ± 0.09 ***	1.13 ***	4.63 ± 1.01 ***	5.72 ± 0.1 ***	2e
0.9 ± 0.04 **	1.006 **	6.5 ± 0.8 **	7.2 ± 0.9 **	4a
0.67 ± 0.1***	1.12***	4.8 ± 0.6***	5.34 ± 0.8***	4b
0.76 ± 0.03**	1.001**	6.2 ± 0.9**	6.7 ± 0.51**	6a
0.79 ± 0.03 **	1.15 ***	4.93 ± 0.7 ***	5.32 ± 0.8 ***	10

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

***In vivo* anticancer activity**

Tumor volume

Table 5 demonstrate the inhibition of tumor volume in Group D by 30% compared to Group A. Ratio differences between both Groups were found to be statistically significant ($p < 0.05$). Other signs of toxicity (loss in weight, changes in behavior) were not observed in the tested compound treated Groups (B and D).

Table 5. Tumor Volume Inhibition Ratio (%) [Mean Tumor Volume ± Standard Error (mm^3) ($p< 0.05$)].

4 weeks	3 weeks	2 weeks	1 week	Beginning
Control (Group A)				
1197±20.1	969±11.3	731±8.3	452±6.9	250±3.2
Treated Group (Group D)				
837±12.1	737±9.8	598±8.3	388±6.9	250±3.2
Tumor Volume Inhibition ratio (%)				
30	24	18	14	0
(p=0.002)	(p=0.002)	(p=0.002)	(p=0.002)	-
(p< 0.05)	(p< 0.05)	(p< 0.05)	(p< 0.05)	

Effects on survival and body weight

Our findings showed no significant differences in survival between groups, with about 98% of rats surviving to the end of the study. Weight changes of all rats during the experiment as well as the lower weight of rats in the Group C were shown in Fig. 1. The mean body weight of rats in Group B did not differ significantly from animals in Group A. However, the mean body weight of rats exposed to N-Methyl-N-Nitrosourea plus 15 mg/kg body weight of the tested compound group (Group D) was only 78% that of Group A at the end of the study.

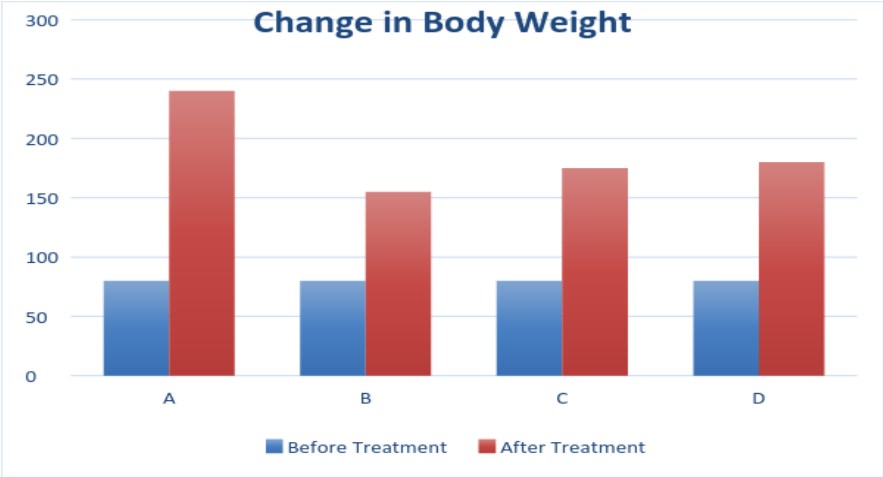


Figure 1: Effects of NMU on rats. Changes in the body weight of rats during the experiment: P<0.05 compared with control. Group A: vehicle control, group B: tested compound control, Group C: NMU control, Group D: tested compound treatment

Hematological Parameters

Hematological parameters in tumor induced groups were found to be significantly altered compared to those of the normal control group. The WBC count was found to be increased in NMU control group-C when compared to Groups B and D. The tested compound treatment group-D showed significant decrease and exhibited normal WBC levels (Table 6). Whereas, RBC count, Hematocrit and hemoglobin levels were significantly decreased in rats in group-C when compared to the normal control group. Treatment with the tested compound showed significant increase in RBC count, hematocrit and Hb level when compared to tumor induced group. Platelets count was significantly ($p<0.05$) decreased in tumor control group-C when compared to group A, B, and D rats. Whereas, the tested compound treatment group-D showed normal levels of platelets count (Table 6).

Table 6: Effect of the tested compound on hematological values in different experimental groups

GROUP IV	Group III	Group II	Group I	CBC PARAMETERS
7.32±0.21	5.37±0.18[a]	7.25±0.21	7.36±0.20	RBC ($x10^6$ / µ L)
10.56± 0.41[b]	14.73±0.59[a]	6.82±0.35	6.65± 0.52	WBC ($x10^3$ / µ L)
13.29± 0.25	11.14±0.19[a]	14.58±0.42	14.12± 0.11	HGB (g/dL)
42.07± 0.77	39.30±0.56[a]	45.10±0.35	45.32± 0.44	PCV (%)
115.60± 0.68[b]	149.90±0.70[a]	83.40±0.51	84.35± 0.55	LYM (%)
16.31± 0.24[b]	22.39±0.45[a]	11.08±0.25	11.03± 0.36	NEU (%)
650.45± 4.30[b]	605.37± 2.80[a]	711.20±3.30	710.43± 3.90	PLT ($x10^3$ / µ L)

Values are presented as mean ± SD; [a]$P<0.05$; in comparison with control group (A); [b]$P<0.05$; in comparison with NMU-treatment group (C); Group A: vehicle control, group B: the tested compound control, Group C: NMU control, Group D: the tested compound treatment

Effects of the tested compound on serum parameters

Table 7 demonstrated the effect of the synthesized tested compound on some biochemical parameters in serum of treated and control groups. Results clearly showed that most of the investigated biochemical parameters were significantly higher ($p < 0.001$) in tumor induced group than the control group. On the other hand, treatment with the tested compound showed different effects, where some of the estimated values of the induced biochemical parameters does not differ significantly or showed significant increase ($p < 0.01$) in comparison to control animal group.

Table 7: Effect of the tested compound on biochemical parameters in different experimental groups

GROUP IV	Group III	Group II	Group I	Biochemical Parameters
730.00±2.80[b]	1150.00±3.80[a]	305.00±2.20	320.00±1.80	LDH (U/L)
320.00±0.20[b]	650.00± 0.50[a]	130.00±0.30	127.00±0.20	ALP (U/L)
135.00±1.50	128.00±1.10[a]	141.00±1.00	140.00±0.85	AST (U/L)
36.00±0.40[b]	34.00±0.25[a]	38.00±0.22	38.00±0.15	ALT (U/L)
3.90±0.18[b]	2.80±0.11[a]	4.60±0.15	4.30±0.09	Albumin (g/dL)
6.90±0.15	5.10±0.20[a]	7.10±0.11	7.10±0.12	Total Proteins (g/dL)

Values are presented as mean ± SD; [a]$P<0.05$; in comparison with control group (A); [b]$P<0.05$; in comparison with NMU-treatment group (C); Group A: vehicle control, group B: the tested compound control, Group C: NMU control, Group D: the tested compound treatment

Conclusion

This study represented a good method for synthesis and characterization of furochromone derivatives with using furochromone carboxaldehyde and different reagents [namely: amine derivatives, or/ and dimethyl acetylendicarboxylate in the present of cyclohexyl isocyanide, or/ and cyclohexyl isocyanide with acid derivatives,

or/ and cyclohexyl isocyanide and o-aminophenol/and or o-phenylenediamine respectively with acid derivatives, or/ and cyclohexyl isocyanide]. The present results indicated that some of the synthesized compounds may constitute a potential *in vitro* antitumor potency towards liver and breast (HEPG2 & MCF7) cancer cell lines in compared to traditional anticancer drugs: Doxorubicin & 5-fluorouracil. Moreover, compound (6a) was found to have anticancer activity in NMU induced breast cancer *in vivo*. More experiments are necessary to identify the mode of action of these compounds.

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