Novel bis(dihydropyrano[3,2-*c*]chromenes): Synthesis, Antiproliferative Effect and Molecular Docking Simulation

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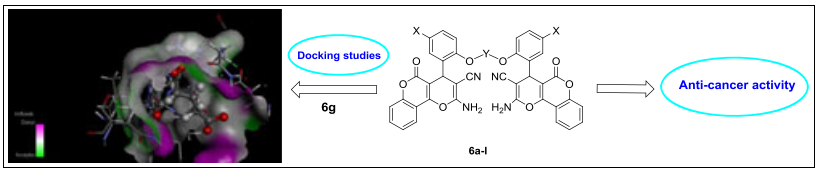

An efficient and convenient route for the synthesis of novel bis dihydropyrano[3,2-*c*]chromenes is reported. The synthetic pathway involves one-pot, multicomponent reaction of bis-aldehydes, malononitrile, and 4-hydroxycoumarin in the presence of pyridine or acetic acid/sodium acetate. A stepwise approach for the synthesis of the target compounds was also investigated. The anticancer activity of the synthesized products against MCF7, HEPG2, and A549 cell lines was assessed. Attempts to detect the molecular action of 6g, docking simulation was done using DHFR PDB:ID (1DLS). The study revealed that compound 6g was strongly fit into the active sites of the target protein through six bindings, and hence, it was considered as promising inhibitor for cancer proliferation.

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INTRODUCTION

Dihydropyrano[3,2-*c*]chromenes are very interesting compounds due to their biological and pharmaceutical activities, for instance, as anti-HIV, antimicrobial, antibacterial, anticancer, spasmolytic, and anticoagulant [1–3]. Dihydropyrano[3,2-*c*]chromene derivatives have likewise been accounted for as potential medications for the treatment of neurodegenerative diseases, including Alzheimer's sickness, Parkinson's ailment, amyotrophic lateral sclerosis, AIDS-associated dementia, Down's syndrome, and Huntington's disease and in addition, schizophrenia and myoclonus [1–3].

Moreover, multicomponent reactions, which include more than two components are very elegant and fastest route for the synthesis of complex molecules. They reduce the number of reaction steps, minimize by-products formation, and thus result in both atom and step economy [4–10]. Recently, we reviewed the use of multicomponent reactions in the synthesis of fused heterocyclic systems [11–14].

Besides, bis-heterocyclic compounds were found to have various applications as electrical materials [15], chelating agents, and metal ligands [16]. Attention has also progressively been paid lately to the synthesis of heterocyclic compounds, especially when two pharmacologically active heterocyclic moieties are present in the same molecule [17–19]. These compounds were encountered in numerous bioactive natural product [20], and recent reports demonstrated that among libraries of derivatized heterocycles, the most active library compounds had a bis-heterocyclic structure [21–24].

In continuation of these studies, we report on the first synthesis of novel bis(2-amino-5-oxo-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile) derivatives in which the two dihydropyrano[3,2-*c*]chromene moieties are linked to aliphatic or aromatic spacer. The anticancer activity of the novel synthesized products against MCF7, HEPG2, and A549 cell lines was also investigated.

RESULTS AND DISCUSSION

Synthetic chemistry. Previously, impressive endeavors have been made in the synthesis of pyrano[3,2-*c*]chromenes **4** as an important class of compounds. Several methods have been accounted for to the synthesis of this class of compounds, including the reaction of aromatic aldehydes **1**, malononitrile **2**, and 4-hydroxycoumarin **3** in the presence of homogeneous or heterogeneous catalysts under conventional heating, microwave irradiation, or ultrasound irradiation (Scheme 1) [25–37].

As part of the continuing interest in this area, we investigated here two possible synthetic approaches, path A and path B for bipodal dihydropyrano[3,2-*c*]chromenes **6**, as outlined in Scheme 2. If there should arise an occurrence of pathway A, the desired dihydropyrano[3,2-*c*]chromene **4** has initially been synthesized, which may then be reacted with the appropriate dihalo compounds **5** to give the desired bipodals by using a mild base. On the

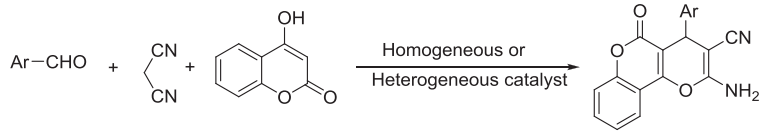
other hand, this can likewise be accomplished by means of path B, where bis-aldehydes **7** have to be synthesized in a first step, which can further be reacted with malononitrile, and 4-hydroxycoumarin to give the desired bipodals **6** (Scheme 2).

As indicated by the first methodology, the precursor monopodal dihydropyrano[3,2-*c*]chromene **4a** was prepared *via* initial Knoevenagel condensation of the appropriate 4-hydroxybenzaldehyde **1a** with malononitrile in ethanol in the presence of piperidine as a catalyst to give the corresponding arylidenemalononitrile derivative followed by reaction with 4-hydroxycoumarin **3** *via* Michael addition reaction [36,38]. The potassium salt (obtained upon treatment of **4a** with ethanolic KOH) was then allowed to react with the appropriate dihalo compound **5** in refluxing DMF. The reaction, unfortunately, did not lead to the formation of the corresponding bis(4*H*-chromene-3-carbonitrile) **6** and gave instead a mixture of products that were not easily separated and have not been characterized yet (Scheme 3). It is noteworthy to mention that our attempts to synthesize 2-amino-4-(2-hydroxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile **4b** *via* multicomponent reaction of salicylaldehyde **1b** with malononitrile and 4-hydroxycoumarin under basic conditions were unsuccessful, and instead, 3-cyanocoumarin **8** was obtained in 74% yield as a sole product [39,40].

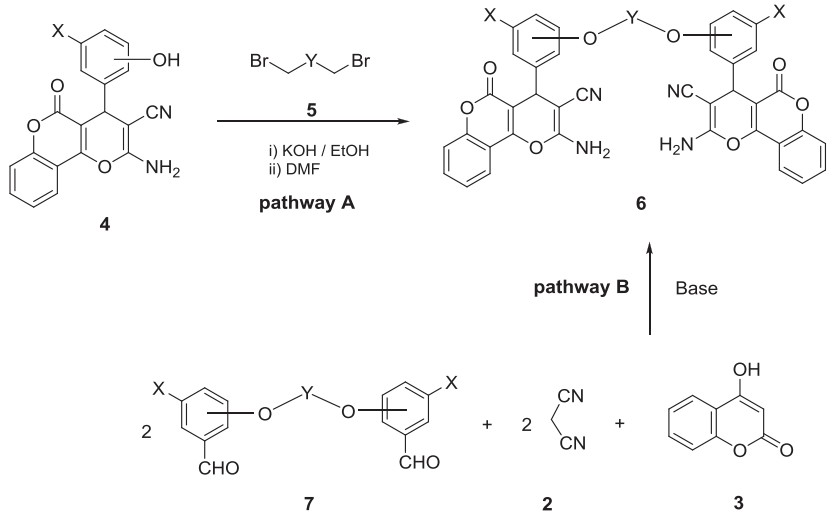
Searching for an expedient pathway to prepare the target compounds **6**, we turned to path B, and the bis(aldehydes) **7** were chosen as key intermediates. The latter compounds should then react effectively with two equivalents of both malononitrile **2** and 4-hydroxycoumarin **3** under basic conditions to give the bis(4*H*-chromene-3-carbonitrile) **6**.

Our preliminary investigations were focused on systematic evaluation of different catalysts for the model reaction of 2,2′-(ethane-1,2-diylbis(oxy))dibenzaldehyde **7a** with two equivalents of both malononitrile **2** and 4-hydroxycoumarin **3** (Scheme 4 and Table 1). At first, we attempted to utilize chitosan in dioxane as an ecofriendly basic catalyst for the multicomponent reaction. Unfortunately, the reaction stopped at the ylidene stage **9**, and no traces of the of target bis(4*H*-chromene-3-carbonitrile) **6** were detected even after prolonged heating**.** Similar results were obtained when we explored the catalytic activity of phthalimide-*N*-oxyl anion as an effective and readily available Lewis base for the above reaction. When the reaction was carried out in refluxing dioxane for 6 h using 1,4-diazabicyclo[2.2.2]octan (DABCO) as an inexpensive, ecofriendly, and nontoxic base catalyst, the ^{1}H–NMR indicated the presence of a

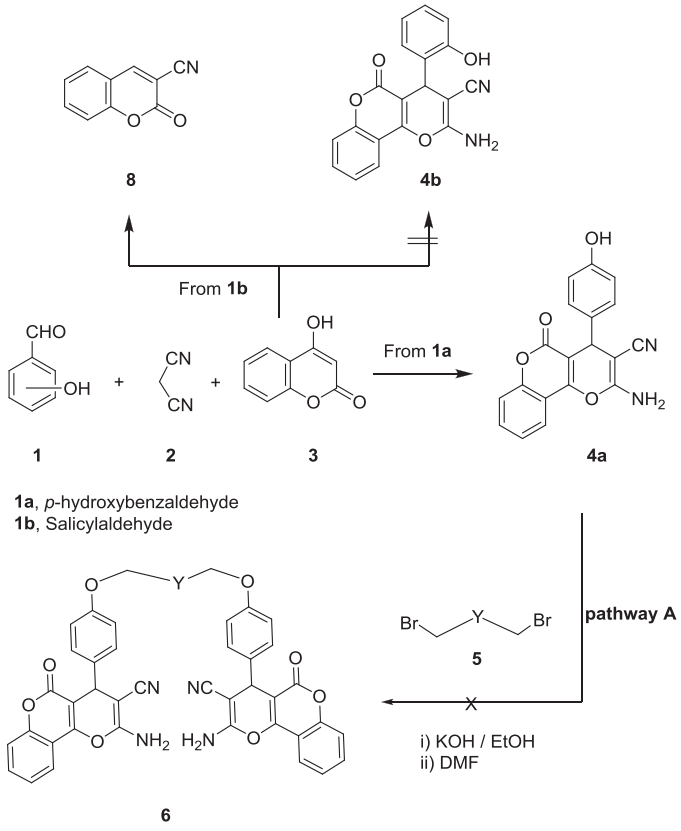
Scheme 1. The synthesis of pyrano[3,2-*c*]chromenes **4**.



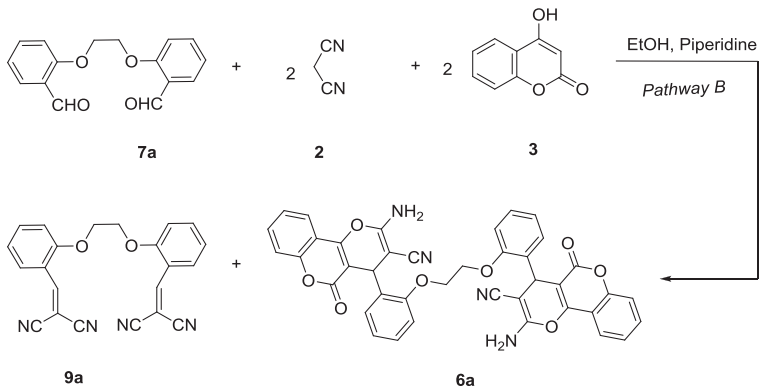
Scheme 2. The possible synthetic approaches for bipodal dihydropyrano[3,2-*c*]chromenes **6**.



Scheme 3. Trials to synthesize the bis(4*H*-chromene-3-carbonitrile) **6** through the bis alkylation of the potassium slat of compound **4a** with the di-bromo compounds.



Scheme 4. Attempted synthesis of the bis(4*H*-chromene-3-carbonitrile) **6** in EtOH / piperidine.



mixture of the corresponding bis-arylidenemalononitriles **9** and the target compound **6** at a ratio of 1:1 based on the area of their characteristic signals. The product ratio did not change with further standing up to 12 h. The same results were obtained using piperidine as a basic catalyst in either refluxing ethanol or refluxing dioxane. The reaction was also investigated in the presence of a catalytic amount of hexamethylenetetramine as a very cheap, nontoxic, and stable reagent followed the method recently reported by Wang et al. [31] to prepare a variety of dihydropyrano[3,2-*c*]chromene derivatives in high to excellent yields within short times. We also tried the ecofriendly methodology recently reported by Sajadikhah et al. [41] in which NaCl in water–ethanol media (3:1) was used as an inexpensive catalyst for the one-pot synthesis of dihydropyrano[3,2-*c*]chromene derivatives [41]. Unfortunately, in both cases, no traces of the target bis(dihydropyrano[3,2-*c*]chromenes) **6** were obtained. On

Table 1

Optimizing the yield of compound **6a**.

Entry	Solvent	Catalyst	6:9	Conversion (%)[a]
1	Dioxane	Chitosan	0:100	80
2	Water	PINO	0:100	78
3	Dioxane	DABCO	50:50	85
4	Dioxane	Piperidine	50:50	87
5	Ethanol	Piperidine	50:50	89
6	Dioxane	HMT	0:100	82
7	Water–Ethanol	NaCl	0:100	76
8	Pyridine		100:0	88
9	Acetic acid	Sodium acetate	100:0	82

[a]Based on % conversion of **7a**, **2**, and **3** into products **6a** and **9a** on ^{1}H NMR spectroscopic analysis of crude reaction mixtures.

the other hand, the reaction takes another course when performed in refluxing pyridine for 15 min, and the bis(4*H*-dihydrobenzo[*b*]pyrans) **6a** could be obtained in 88% yield as a sole product. Compound **6a** was alternatively obtained in 82% yield by carrying out the reaction in refluxing acetic acid containing a catalytic amount of sodium acetate for 1 h.

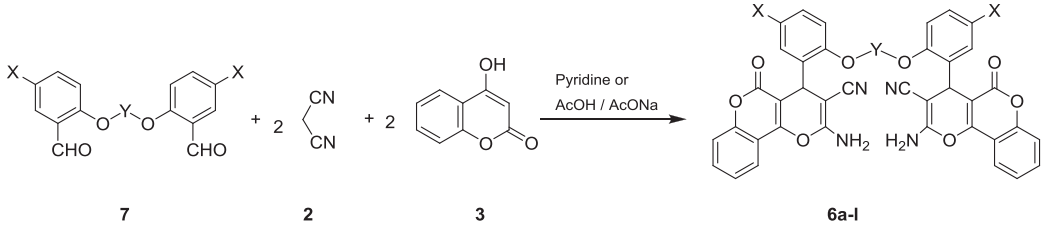
Subsequently, with optimal condition in hand, the generality and synthetic scope of this reaction were demonstrated by synthesizing a series of bis(4*H*-chromene-3-carbonitriles) **6a–l** (linked to aliphatic or aromatic cores *via* ether linkage). Thus, different bis-aldehydes **7a–f** [42–44] were well tolerated under the optimized reaction conditions and furnished the corresponding bis(4*H*-chromene-3-carbonitriles) **6** in good yields (Scheme 5 and Table 2).

It is even possible to carry out these reactions in a stepwise fashion in which the arylidenemalononitrile derivatives **9**, [45] containing the electron-poor *C–C* double bond, are firstly produced by Knoevenagel condensation of the bis-aldehydes **7** with two moles of malononitrile. Subsequent reaction of **9** two moles of 4-hydroxycoumarin **3** afforded the target molecules **6** good yields (Scheme 6, Table 2, method C).

The infrared (IR) spectra of compound **6a** indicated the presence of amino group at 3402 cm^{-1}. In addition, it revealed the cyano band at 2202 cm^{-1}. The carbonyl group appeared as a broad band at 1671 cm^{-1}. The constitutions of compounds **6a–l** were established based on their elemental analysis and spectral data. The ^{1}H NMR spectrum of **6a** indicated the presence of the pyran-H4 as singlet signal at δ 4.62 ppm. Moreover, compounds **6** also featured the methylene ether linkage OCH_2 as multiplet signals in the region 3.50–5.27 ppm, although their precursors **7** or **9** exhibit singlet signals for these protons. This suggests that the generated asymmetric center (in the dihydropyran rings) is close enough to this CH_2 group to affect such splitting. All other protons were seen at the expected chemical shifts and integral values (See Experimental section and Supporting Information).

Biology. ***Anticancer evaluations.*** In the present investigation, all the synthesized compounds were screened against the three cell lines MCF7, HEPG2, and A549 (Fig. 1). The concentration causing 50% cell growth inhibition (IC_{50}) was determined as shown in (Table 3). It was shown that the breast line was the most sensitive one toward most of our derivatives. Also, it was noted that compound **6g** was the most active compound in this series with IC_{50} values (0.03, 0.08, and 0.22 mM) against MCF7, HEPG2, and A549 cell lines, respectively. On the other hand, compound **6b** was the least active one toward HEPG2 and A549 lines with IC_{50} (9.99 and 4.9 mM), respectively. Regarding compounds **6c** (IC_{50} = 1.07, 0.31, and 0.73 mM) and **6d** (IC_{50} = 2.38, 0.77, and 0.78), it was noted that the presence of unsaturated butene group in **6d** decreases the biological activity as indicated in the IC_{50} values against A549, MCF7, and HEPG2, respectively. Unfortunately, the activity of **6f** was decreased to (8.9 mM) using HEPG2 cell line. The data also indicate that compound **6f** with *para* substitution (IC_{50} = 0.04 and 0.65 mM) is more favored than **6e** with *ortho* substitution (IC_{50} = 0.06 and 4.03 mM) toward the two cell lines MCF7 and A549, respectively. Moreover, it is clear that the addition of bromines into the benzene ring increases the activity as indicated in the IC_{50} values [**6f** (IC_{50} = 0.65, 0.04, and 8.9 mM), **6g** (IC_{50} = 0.22, 0.03, and 0.08 mM)] against A549, MCF7, and HEPG2, respectively. Compound **6h** with meta substitution showed better cytotoxicity to MCF7 and HEPG2, cell lines (IC_{50} = 0.52 and 0.57) than

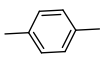
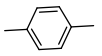
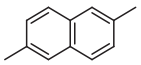
Scheme 5. Synthesis of a series of bis(4*H*-chromene-3-carbonitrile) **6a–i** under the optimized reaction conditions.



7 2 3 6a-l

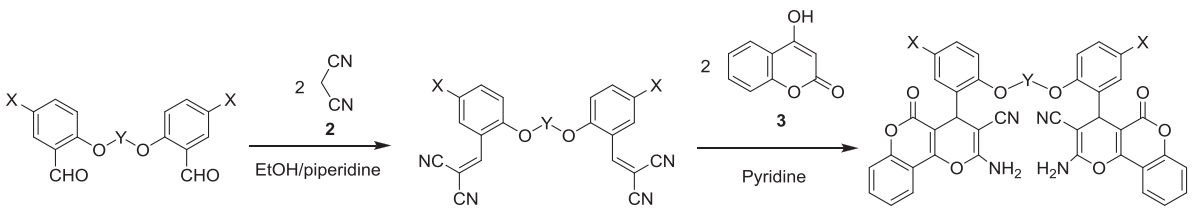
Table 2

The % yields of the bis(4*H*-chromene-3-carbonitriles) **6** in different reaction conditions.

Compound	X	Y	% Yield		
			Method A[a]	Method B[b]	Method C[c]
6a	H	-$(CH_2)_2$-	88	82	85
6b	H	-$(CH_2)_3$-	86	82	84
6c	H	-$(CH_2)_4$-	89	84	86
6d	H		86	75	82
6e	H		89	81	86
6f	H		87	80	82
6g	Br		88	83	-
6h	Br		87	80	-
6i	H		90	82	-

[a]Bisaldehyde (1 mmol) 7, malononitrile 2 (2 mmol), and 3 (2 mmol) in pyridine.
[b]Bisaldehyde (1 mmol) 7, malononitrile 2 (2 mmol), and 3 (2 mmol) in AcOH – AcONa.
[c]Bis-arylidenemalononitrile (1 mmol) **9**, and **3** (2 mmol) in pyridine.
-, position of attachment of the two rings.

Scheme 6. Stepwise synthesis of the bis(4*H*-chromene-3-carbonitrile) **6**.



for A549 (IC_{50} = 1.19), respectively. Finally, it is clear that while the naphthalene ring in **6i** decreases the biological activity against the respective MCF7 and HEPG2 (IC50 = 3.72 and 0.16 mM), it increases the activity in case of A549 (IC50 = 0.13 mM).

Molecular docking simulation. Molecular modeling was performed on human dihydrofolate reductase enzyme to predict the binding mode of **6g** within the binding site of target proteins (human dihydrofolate reductase enzyme). Protein was selected and downloaded from the Protein Data Bank (PDB ID: 1DLS). The protein was optimized, removing the water molecule and cofactors from the proteins and deleting the ligand present in the crystal structure. The solvent molecules were deleted, and the bond order of the crystal ligand and protein was adjusted. The ligand was built using the chembiodraw ultra 10.0, protonate 3D and subjected to energy minimization. Docking study of compound **6g** into the active site of human dihydrofolate reductase enzyme showed six interactions as showed in Figure 2. The first interaction was hydrogen bonding between the amino acid lys 63 and *N* atom of CN group with bond distance 2.64A°. The second binding was hydrogen bonding between ser 59 and H atom of NH_2 group with bond distance 2.03A°. The third and fourth bindings were H-bonding between Arg 28 and the two oxygen

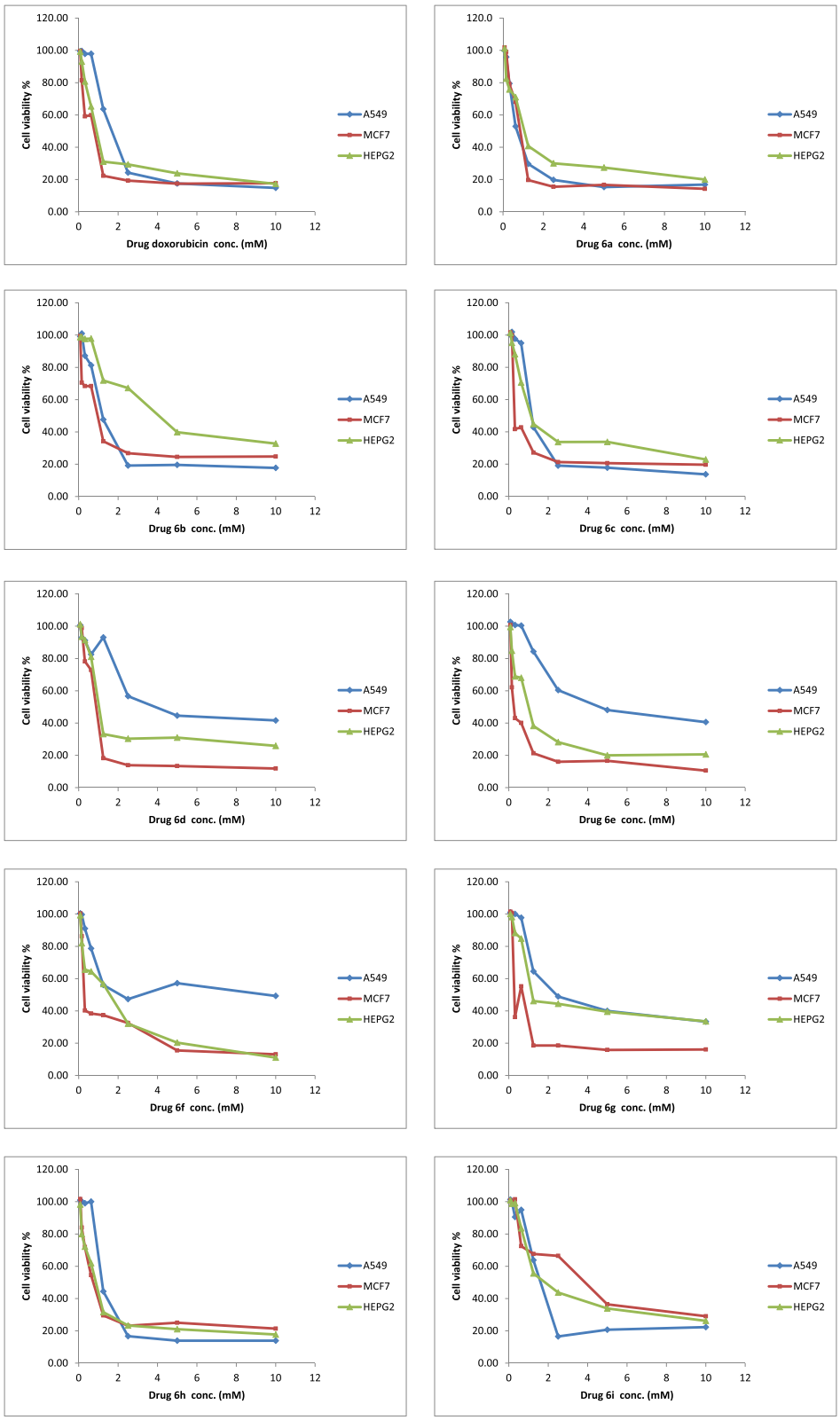


Figure 1. Cytotoxic activity of the new derivatives (Doxorubicin, **6a–i**) against the tumor A549, MCF7, and HEPG2 cell lines after 48-h exposure. Doxorubicin is used as a standard agent against the same lines. Each point is the mean ± standard deviation of three independent experiments performed in triplicate, using prism software program (Graphpad software incorporated, version 3). [Color figure can be viewed at wileyonlinelibrary.com]

Table 3

The IC_{50} values (the drug concentrations that inhibited 50% of cell proliferation) of the selected nine compounds on the different human cell lines A_{549}, MCF7, and $HEPG_2$.

	IC50 values (mM)		
Sample	A549	MCF7	HEPG2
6a	0.52	0.77	0.68
6b	4.9	0.39	9.99
6c	1.07	0.31	0.73
6d	2.38	0.77	0.78
6e	4.03	0.06	0.56
6f	0.65	0.04	8.9
6g	0.22	0.03	0.08
6h	1.19	0.52	0.57
6i	0.13	3.72	0.16

atoms of the carbonyl groups in the two pyran rings with bond distances 2.5A° and 1.97A°. The fifths binding was arene–cation interaction between Arg 28 and benzene ring. The last one is arene–arene interaction between the amino acid Ph 31 and benzene ring. All these interactions enhanced the binding activity and fitting of compound **6g** into the active site of the target protein and hence cancer proliferation inhibition. The data obtained by the docking program MOE were saved as PDP file, where the 3D structure of **6g** and the 3D structure of our domains were entered into SCIGRESS version 3.0 and visualized through BIOVIA Discovery Studio V6.1.0.15350 (Fig. 3).

EXPERIMENTAL AND ANALYTICAL DATA

Chemistry. General. Melting points were measured with a Stuart melting point apparatus and are uncorrected. The IR spectra were recorded using an FTIR Bruker–vector 22 spectrophotometer as KBr pellets. The ^{1}H NMR spectra were recorded in DMSO–d_6 as solvent on Varian Gemini NMR spectrometer at 300 MHz using TMS as internal standard. Chemical shifts are reported as δ values in ppm. Mass spectra were recorded with a Shimadzu GCMS–QP–1000 EX mass spectrometer in EI (70 eV) model. The elemental analyses were performed at the Micro analytical center, Cairo University.

Synthesis of compounds 4a and **8**. To a mixture of the appropriate aryl aldehydes **1a** or **1b** (0.003 mol), dimedone **3** (0.003 mol) and malononitrile **2** (0.0033 mol) in ethanol was added (0.2 mL) as a catalyst, and the mixture was set at reflux for 3 h. The reaction mixture was cooled, and the resulting solid was filtered to afford the crude product, which then recrystallized from ethanol to give pure **4a** or **8**.

2-Amino-4,5-dihydro-4-(4-hydroxyphenyl)-5-oxopyrano[3,2-c]chromene-3-carbonitrile (4a). Off-white solid (EtOAc/Pet ether); Mp = 258–260°C; IR (KBr): ν = 3504, 3407 (br)(NH_2), 2197 (s) (CN), 1690 (s) (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.29 (s, 1H, CH), 6.65 (d, 2H, J = 8.1 Hz, Ar-H), 7.01 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.29 (s, 2H, NH_2), 7.38–7.46 (m, 2H, Ar-H.), 7.65 (t, 1H, *J* = 7.8 Hz, Ar-H), 7.84 (d, 1H, *J* = 7.8 Hz, Ar-H), 9.31

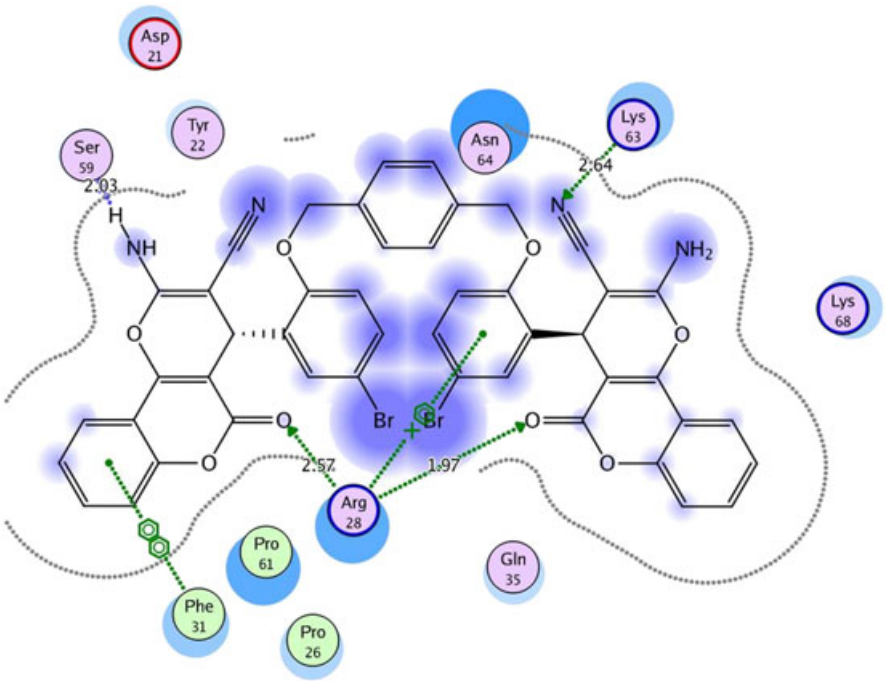


Figure 2. Two-dimensional representation of compound **6g** into the active site of human dihydrofolate reductase enzyme, respectively. [Color figure can be viewed at wileyonlinelibrary.com]

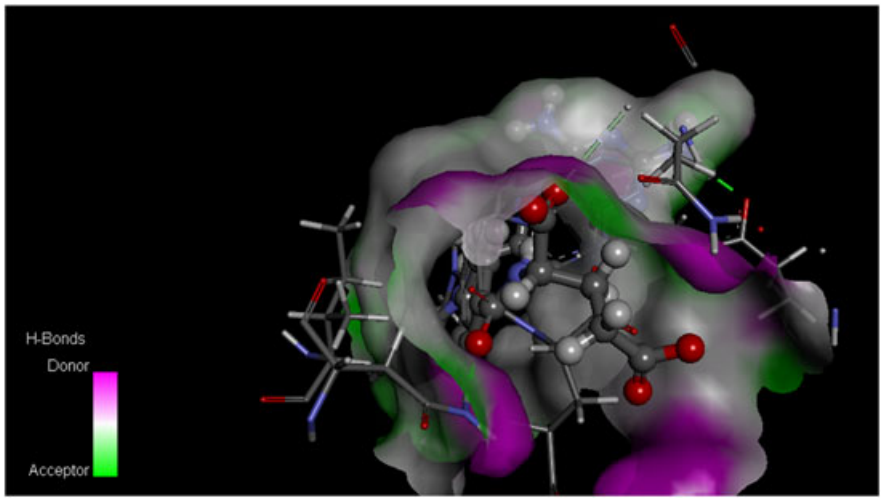


Figure 3. Three-dimensional representation of compound **6g** into the active sites of human dihydrofolate reductase enzyme, respectively. [Color figure can be viewed at wileyonlinelibrary.com]

(s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 36.2, 58.5, 104.6, 113.1, 115.3, 116.6, 119.4, 122.5, 124.7, 128.7, 132.8, 133.7, 152.1, 153.0, 156.5, 158.0, 159.6, 161.9. MS (EI, 70 eV): m/z = 332 [M^+], Anal. Calcd for $C_{19}H_{12}N_2O_4$: C, 68.67; H, 3.64; N, 8.43%. Found: C, 68.71; H, 3.66; N, 8.46.

2-Oxo-2H-chromene-3-carbonitrile (8). Off-white solid (EtOH-dioxane); Mp = 183–185°C (lit. [19] 184–185°C); IR (KBr): ν = 2220 (s) (CN), 1712 (s) (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.44–7.75 (m, 4H, Ar-H), 8.30 (s, 1H, H4); ^{13}C NMR (75 MHz, DMSO-d_6): δ 103.61, 112.00, 117.56, 117.22, 128.15, 128.21, 136.50, 152.11, 155.20, 156.88. MS (EI, 70 eV): m/z = 171 [M^+], $C_{10}H_5NO_2$: C, 70.18; H, 2.94; N, 8.18. Found (%): C, 70.23; H, 2.95; N, 8.21.

General procedure of synthesis of compounds 9a–f *[45].* To a mixture of bis-aldehydes **7a–f** (1 mmol) and malononitrile **2** (2.2 mmol) in ethanol (20 mL) was added piperidine (0.2 mL), and the mixture was set at reflux for 3 h. The crude solid was isolated and recrystallized from the proper solvent.

General methods for synthesis of compounds 6a–i. ***Method A.*** A mixture of bisaldehyde **7** (1 mmol), malononitrile **2** (2 mmol), and 4-hydroxy-2*H*-chromen-2-one **3** (2 mmol) in pyridine (10 mL) was heated at reflux for 15 min. The crude solid was isolated and recrystallized from the proper solvent.

Method B. A mixture of bisaldehydes **7** (1 mmol), malononitrile **2** (2 mmol), and 4-hydroxy-2*H*-chromen-2-one **3** (2 mmol) in glacial acetic acid (10 mL) was heated at reflux in the presence of sodium acetate (3 mmol) for 1 h. The crude solid was isolated and recrystallized from the proper solvent.

Method C. A mixture of bis-arylidenemalononitrile derivatives **9a–f** (1 mmol) and 4-hydroxy-2*H*-chromen-2-one **3** (2.2 mmol) in pyridine was heated at reflux for 3 h. The crude solid was isolated and recrystallized from the proper solvent.

4,4′-((Ethane-1,2-diylbis(oxy))bis(2,1-phenylene))bis(2-amino-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile) (6a). Pale yellow crystals (DMF); Mp = 288–292°C; IR (KBr): ν = 3402 (br)(NH_2), 2202 (s) (CN), 1676 (s) (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.96–4.14 (m, 4H, OCH_2), 4.627 (s, 2H, pyran H-4), 6.86–7.98 (m, 20H, Ar-H + $2NH_2$). MS (EI, 70 eV): m/z = 690.18 [M^+], Anal. Calcd for $C_{40}H_{26}N_4O_8$: C, 69.56; H, 3.79; N, 8.11. Found: C, 69.77; H, 3.56; N, 8.41.

4,4′-((Propane-1,3-diylbis(oxy))bis(2,1-phenylene))bis(2-amino-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile) (6b). Pale yellow crystals (DMF); Mp = 290–295°C; IR (KBr): ν = 3430 (br)(NH_2), 2195 (s) (CN), 1675 (s) (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.94 (br, 2H, CH_2), 3.50–3.86 (m, 4H, OCH_2), 4.536 (s, 2H, pyran H-4), 5. 6.17–7.93 (m, 20H, Ar-H + $2NH_2$). MS (EI, 70 eV): m/z = 704.19 [M^+], Anal. Calcd for $C_{41}H_{28}N_4O_8$: C, 69.88; H, 4.01; N, 7.95. Found: C, 70.05; H, 4.17; N, 8.11.

4,4′-((Butane-1,4-diylbis(oxy))bis(2,1-phenylene))bis(2-amino-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile) (6c). Pale yellow crystals (DMF); Mp = 290–293°C; IR (KBr): ν = 3406 (s), 3330 (s) (NH_2), 2193 (s) (CN), 1674 (s) (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.51–1.57 (m, 4H, $2CH_2$), 3.64–3.85 (m, 4H, 2-OCH_2), 4.6 (s, 2H, pyran H-4), 6.85–7.95 (m, 20H, Ar-H + $2NH_2$); ^{13}C NMR (75 MHz, DMSO-d_6): δ 25.5 (CH_2), 35.7 (C-4), 67.3 (C-3), 90.9 (CH_2-O), 103.2 (C-4a), 112.2 (C6-phenylene), 113 (C-10a), 116.4 (C-7), 119.3 (CN), 120.1 (C4-phenylene), 120.9 (C2-phenylene), 123.8 (C-10), 124.5 (C-9), 128.5 (C5-phenylene), 130.2 (C-8), 132.6 (C3-phenylene), 151.9 (C-6a), 153.6 (C1-phenylene), 156.8 (C-2), 158.4 (C-10b), 162.3 (CO). MS (EI, 70 eV): m/z = 718.21[M^+], Anal. Calcd for $C_{42}H_{30}N_4O_8$: C, 70.19; H, 4.21; N, 7.80. Found: C, 70.32; H, 4.44; N, 7.58.

4,4′-((But-2-ene-1,4-diylbis(oxy))bis(2,1-phenylene))bis(2-amino-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile) (6d). Pale yellow crystals (DMF); Mp = 296–300°C; IR (KBr): ν = 3408 (s), 3327 (s) (NH_2), 2192 (s) (CN), 1674 (s) (CO) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 4.33 (s, 4H, 2-OCH_2), 4.62 (s, 2H, pyran H-4), 5.72 (s, 2H, vinyl-H), 6.9–7.89 (m, 20H, Ar-H + 2NH_2). MS (EI, 70 eV): m/z = 716.19 [M^+], Anal. Calcd for $C_{42}H_{28}N_4O_8$: C, 70.39; H, 3.94; N, 7.82. Found: C, 70.52; H, 3.71; N, 7.97.

4,4′-(((1,2-Phenylenebis(methylene))bis(oxy))bis(2,1-phenylene))bis(2-amino-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile) (6e). Pale yellow crystals (DMF); Mp = 290–294°C; IR (KBr): ν = 3433 (br) (NH_2), 2194 (s) (CN), 1671 (s) (CO) cm^{-1}; ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.3, 56.8, 67.1, 101.7, 112.1, 112.7, 116.1, 119.4, 120.5, 122.5, 124.3, 128.1, 128.5, 129.1, 130, 130.3, 132.4, 134.6, 151.8, 153.6, 156.3, 158.1, 159.5; ^1H NMR (300 MHz, DMSO-d_6): δ 4.62 (s, 2H, pyran H-4), 4.82–4.92 (m, 4H, 2-OCH_2), 6.87–7.95 (m, 24H, Ar-H + 2NH_2). MS (EI, 70 eV): m/z = 766.21 [M^+], Anal. Calcd for $C_{46}H_{30}N_4O_8$: C, 72.06; H, 3.94; N, 7.31. Found: C, 72.26; H, 4.13; N, 7.51.

4,4′-(((1,4-Phenylenebis(methylene))bis(oxy))bis(2,1-phenylene))bis(2-amino-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile) (6f). Pale yellow crystals (DMF); Mp = 298–300°C; IR (KBr): ν = 3419 (br) (NH_2), 2195 (s) (CN), 1674 (s) (CO) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 4.75 (s, 2H, pyran H-4), 4.94–5.06 (m, 4H, 2-OCH_2), 6.88–7.68 (m, 24H, Ar-H + 2NH_2). MS (EI, 70 eV): m/z = 766.21 [M^+], Anal. Calcd for $C_{46}H_{30}N_4O_8$: C, 72.06; H, 3.94; N, 7.31. Found: C, 72.22; H, 3.73; N, 7.53.

4,4′-(((1,4-Phenylenebis(methylene))bis(oxy))bis(5-bromo-2,1-phenylene))bis(2-amino-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile) (6g). Pale yellow crystals (DMF); Mp >300°C; IR (KBr): ν = 3411 (br) (NH_2), 2193 (s) (CN), 1672 (s) (CO) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 4.72 (s, 2H, pyran H-4), 4.91–5.05 (m, 4H, 2-OCH_2), 6.94–7.66 (m, 22H, Ar-H + 2NH_2). MS (EI, 70 eV): m/z = 924.03 [M^+], Anal. Calcd for $C_{46}H_{28}Br_2N_4O_8$: C, 59.76; H, 3.05; N, 6.06. Found: C, 59.61; H, 2.88; N, 6.18.

4,4′-(((1,3-Phenylenebis(methylene))bis(oxy))bis(5-bromo-2,1-phenylene))bis(2-amino-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile) (6h). Pale yellow crystals (DMF); Mp = 280–285°C; IR (KBr): ν = 3433 (br) (NH_2), 2193 (s) (CN), 1671 (s) (CO) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 4.70 (s, 2H, pyran H-4), 4.80–4.94 (m, 4H, 2-OCH_2), 6.95–7.65 (m, 22H, Ar-H + 2NH_2). MS (EI, 70 eV): m/z = 924.03 [M^+], Anal. Calcd for $C_{46}H_{28}Br_2N_4O_8$: C, 59.76; H, 3.05; N, 6.06. Found: C, 59.91; H, 2.84; N, 6.27.

4,4′-(((Naphthalene-2,6-diylbis(methylene))bis(oxy))bis(2,1-phenylene))bis(2-amino-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile) (6i). Pale yellow crystals (DMF); Mp >300°C; IR (KBr): ν = 3403 (br) (NH_2), 2195 (s) (CN), 1672 (s) (CO) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 4.76 (s, 2H, pyran H-4), 5.15–5.27 (m, 4H, 2-OCH_2), 6.87–7.75 (m, 26H, Ar-H + 2NH_2). MS (EI, 70 eV): m/z = 816.22 [M^+], Anal. Calcd for $C_{50}H_{32}N_4O_8$: C, 73.52; H, 3.95; N, 6.86. Found: C, 73.32.; H, 3.68; N, 6.97.

Biology. ***MTT assay.*** The MTT assay is performed according to Mosmann [46]. The assay was modified for the cell lines used (A549, MCF7, and HEPG2). Briefly, these cell lines were exposed to different concentrations of the tested compounds (10, 5, 2.5, 1.25, 0.62, 0.31, 0.15, and 0.07 mM), and for the purpose of the experiments at the end of the incubation time, cells were incubated for 4 h with 0.8 mg/mL of MTT, dissolved in serum free mediums. Washing with phosphate-buffered saline (1 mL) was performed; followed by the addition of DMSO (1 mL), gentle shaking for 10 min so that complete dissolution was achieved. Aliquots (200 µl) of the resulting solutions were transferred in 96-well plates, and absorbance was recorded at 560 nm using the microplates spectrophotometer system. Finally, results of cell viability analysis were analyzed using Prism Software program (Graphpad Software incorporated, version 3).

Modeling simulation. Docking study for the compound **6g** was performed using Molecular Operating Environment (MOE) version 2009.10 (Chemical Computing Group Inc., Montreal, QC, Canada) and Autodock4 program. Regularization and optimization for protein and ligand were performed. Determination of the essential amino acids in binding site was carried out and compared with that present in literature. The performance of the docking method was evaluated by redocking crystal ligand into the assigned active binding site to determine RMSD value. Docked compound was assigned a score according to its fit in the ligand binding pocket and its binding mode.

CONCLUSION

We developed a straightforward methodology for the preparation of novel bis dihydropyrano[3,2-*c*]chromenes. Full characterization of these compounds is reported. The new synthesized compounds are interesting both in their own right as unusual molecules and for their promising pharmacological and biological activities. They offer an advantage of their simple synthesis in a straightforward one-stage or two-stage technique from inexpensive starting materials. Due to the mild reaction conditions, good yields and selectivity, easily accessible starting material, and straightforward product isolation, we think that the new mentioned synthetic approach might offer new viable techniques for novel bis(functionalized) heterocycles of expected biological and pharmaceutical activities. The breast cancer line MCF7 was found to be the most sensitive one toward most of our derivatives. Compound **6g** may have significant and promising

growth inhibitory efficiency against the three cell lines especially MCF7. Docking studies of compounds **6g** showed the best binding mode with the target protein.

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