Regular Article

Synthesis and Cytotoxic Evaluation of Pyran, Dihydropyridine and Thiophene Derivatives of 3-Acetylcoumarin

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A series of coumarin analogues bearing 4*H*-pyran rings 2a–d, 11a–d and 1,4-dihydropyridine rings 3a–d, 12a–d at position 3 were synthesized starting from either 3-acetyl coumarin (1) or the coumarin acetohydrazide derivative 4. Condensation of 3-acetylcoumarin (1) with 2-cyanoacetohydrazide afforded 2-cyano-*N*′-{1-[2-oxo-2*H*-chromen-3-yl]ethylidene}acetohydrazide (4). Reaction of compound 4 with elemental sulfur and either malononitrile or ethyl cyanoacetate afforded the thiophene derivatives 8 and 9, respectively. The structures of the newly synthesized compounds were confirmed on the basis of their spectral data and elemental analyses. All synthesized compounds were screened for their *in vitro* anticancer activity against six human cancer cell lines and normal fibroblasts. Several compounds showed potent inhibition with an IC_{50} value of <870 nM. Compound 3d exhibited equivalent cytotoxic effect as the standard CHS 828 against a breast cancer cell line (IC_{50} value=18 nM). Normal fibroblast cells (WI38) were affected to a much lesser extent (IC_{50} value >10000 nM).

Key words coumarin; 4*H*-pyran; dihydropyridine; thiophene; 2-cyanoacetohydrazide; anticancer activity

Coumarins (2*H*-chromen-2-one) have been established as well known naturally occurring heterocyclic compounds that can be either isolated from various plants including edible vegetables and fruits[1,2] or can be carried out in the laboratory.[3]

Among the oxygen heterocycles, coumarin derivatives are an important class of natural, synthetic compounds and pharmacologically active substances displaying a broad range of biological activities including anticancer,[4] anti-human immunodeficiency virus (HIV),[5] antituberculosis,[6] anti-influenza,[7] anti-Alzheimer[8] and anti-inflammatory.[9] They have also been shown to be novel lipid lowering agents that possess moderate triglyceride lowering activity.[10] Certain coumarin derivatives have been shown to function as HIV integrase inhibitors and evaluated in the treatment of HIV infection,[11] whereas others evaluated as anti-invasive compounds due to their inhibitory activity against some serine proteases and matrix metalloproteases (MMPs).[12] 7-*O*-Alkoxy-4-methylumbelliferone derivatives with longer chains, especially nonyl and decyl have good inhibitory activity against *Mycobacterium tuberculosis*.[13,14]

Recently coumarin derivatives have been reported to possess the potent anticancer effect through different mechanisms. The tricyclic coumarin sulfamate (STX64) (IC_{50}=8 nM), a nonsteroid-based irreversible aromatase-steroid sulfatase (STS) inhibitor provides remarkable activity for the cure of prostate cancer, and most encouragingly, its clinical trials have been accomplished in 2011.[15–17] For instance, 3,8-dibromo-7-hydroxy-4-methyl coumarin (DBC) (IC_{50}=100 nM) is treated as a CK2 inhibitor to suppress neoplastic growth.[18] Novobiocin, a known DNA gyrase inhibitor, binds to a nucleotide-binding site located on the Hsp90-C terminus and induces degradation of Hsp90-dependent client proteins at *ca.* 700 μM in breast cancer cells.[19,20] Some biologically active anticancer agents, such as Geipavarin,[21] Auraptene, Collinin[22] and Scopoletin[23] having substituted coumarin moiety are presented in Fig. 1. Moreover, 7-hydroxycoumarin (Fig. 1) was shown to inhibit the release of cyclin D1, which is over expressed in many types of cancers.[24] In addition,

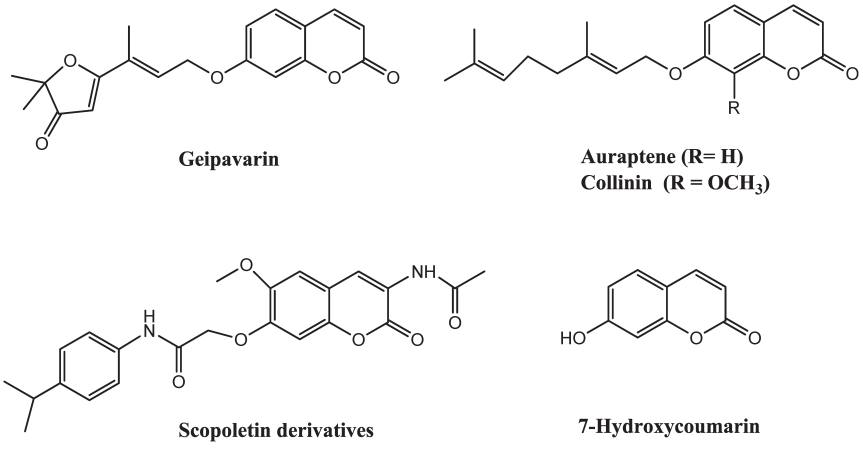

Fig. 1. Structure of Some Potent Anticancer Compounds

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3-acetylcoumarin received considerable attention as a target molecule for the synthesis of pyridine, thiazole and other heterocyclic derivatives.[25–28)]

In this work, we are demonstrating the reaction of 3-acetyl coumarin with 2-cyanoacetohydrazide to give the 2-cyano-*N*′-(1-(2-oxo-2*H*-chromen-3-yl)ethylidene)acetohydrazide (**4**). Reaction of **4** with elemental sulfur and either malononitrile or ethyl cyanoacetate in ethanol using triethylamine as a catalyst produced the (2-oxo-2*H*-chromen-3-yl)ethylidene)-hydrazinyl)thiophene derivatives **8** and **9**, respectively. Moreover, the (2-oxo-2*H*-chromen-3-yl)-4*H*-pyran derivatives **2a**–**d**, **11a**–**d** and the (2-oxo-2*H*-chromen-3-yl)-1,4-dihydropyridine derivatives **3a**–**d**, **12a**–**d** have been prepared by condensation of either 3-acetyl coumarin (**1**) or coumarin acetohydrazide derivative **4** with substituted aromatic aldehyde and malononitrile in presence of either triethylamine or ammonium acetate as catalyst, respectively.

The design and development of new bioactive agents based on the molecular hybridization strategy, involving the integration of two or more pharmacophoric units having different mechanisms of action in the same molecule, is a rationally attractive approach.[29,30)] These combined pharmacophores probably offer some advantages such as in overcoming drug resistance[31)] as well as improving their biological potency.[32)] Therefore, in the present study it was planned to synthesize hybrid compounds that comprise 3-acetylcoumarin and the aforementioned heterocyclic ring systems in order to identify new candidates that may be of value in designing new, potent, selective and less toxic anticancer agents. All the synthesized compounds were evaluated for their *in vitro* cytotoxicity against six human cancer cell lines and normal fibroblast cells.

Results and Discussion

Chemistry The multicomponent reaction of 3-acetylcoumarin with either of benzaldehyde, 4-methoxybenzaldehyde, 4-chlorobenzaldehyde or furfural in ethanol containing a catalytic amount of triethylamine gave the 4*H*-pyran derivatives **2a**–**d**,[33,34)] respectively. The analytical and spectral data of **2a**–**d** were consistent with their respective structures. Thus, the ^{1}H-NMR spectrum of **2c** (as an example) showed the presence of two singlets at δ 2.49 (D_2O exchangeable) and 6.97 ppm due to the presence of NH_2 group and coumarin H-4 beside another two singlets at δ 6.94 and 8.39 ppm corresponding to the presence of pyran H-4 and H-5, respectively. The ^{13}C-NMR spectrum revealed the presence of signals at δ 88.1 (pyran C-4), 116.0 (CN) and 162.1 (CO) (C=O). On the other hand, carrying the same reaction but using a catalytic amount of ammonium acetate gave the 1,4-dihydropyridine derivatives **3a**–**d**, respectively (Chart 1). The structures of the latter products were confirmed on the basis of their respective ^{1}H-NMR and ^{13}C-NMR spectra. Thus, the ^{1}H-NMR spectrum of **3a** (as an example) displayed the presence of two signals (D_2O exchangeable) at δ 2.89 and 9.04 ppm due to the presence of NH_2 and pyridine-NH beside the presence of another three signals at δ 6.97 (pyridine H-4), 7.14 (coumarin H-4), 8.67 (pyridine H-5), respectively. In addition, the ^{13}C-NMR spectrum revealed the presence of signals δ 84.8 (pyridine C-4), 116.4 (CN) and 164.8 (C=O).

It is well known that the hydrazide-hydrazones play an important role for the antitumor activity.[35–37)] With the aim of obtaining new hydrazide-hydrazones with such wide spectrum of pharmaceutical applications,[38–48)] we report here the synthesis of a series of hydrazide-hydrazones *via* the reaction of 3-acetylcoumarine (**1**) with 2-cyanoacetohydrazide followed by heterocyclizations of the reaction product. Moreover, the cytotoxic evaluations of the synthesized products were measured. Thus, the reaction of compound **1** with 2-cyanoacetohydrazide in 1,4-dioxane under the reflux conditions gave the hydrazide-hydrazone derivative **4**. The structure of compound **4** was confirmed on the basis of its ^{1}H-NMR and ^{13}C-NMR spectra. The ^{1}H-NMR spectrum revealed the presence of two singlets at δ 2.15 and 5.08 ppm for the CH_3 and CH_2 groups beside the presence of two signals at δ 6.63 ppm and δ 8.92 ppm equivalent to the coumarin H-4 and NH (D_2O exchangeable) of the acetohydrazide moiety. Moreover, the ^{13}C-NMR spectrum showed the presence of signals at δ 28.3 (CH_3), 64.2 (CH_2), 117.3 (CN), 160.1, 164.3 (2CO) and 172.1 (C=N), respectively. Compound **4** was a good candidate in

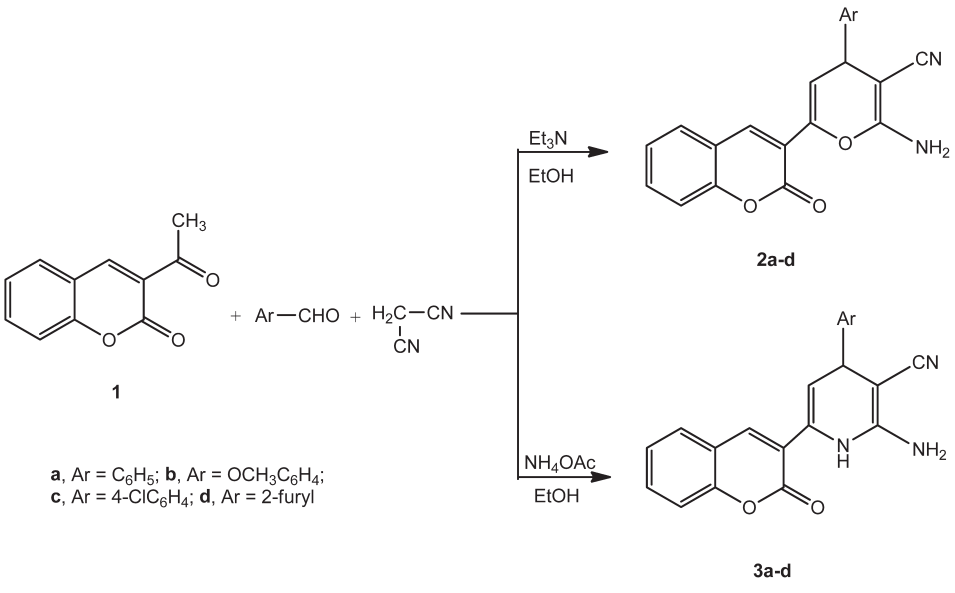

Chart 1. Synthesis of Compounds **2a**–**d** and **3a**–**d**

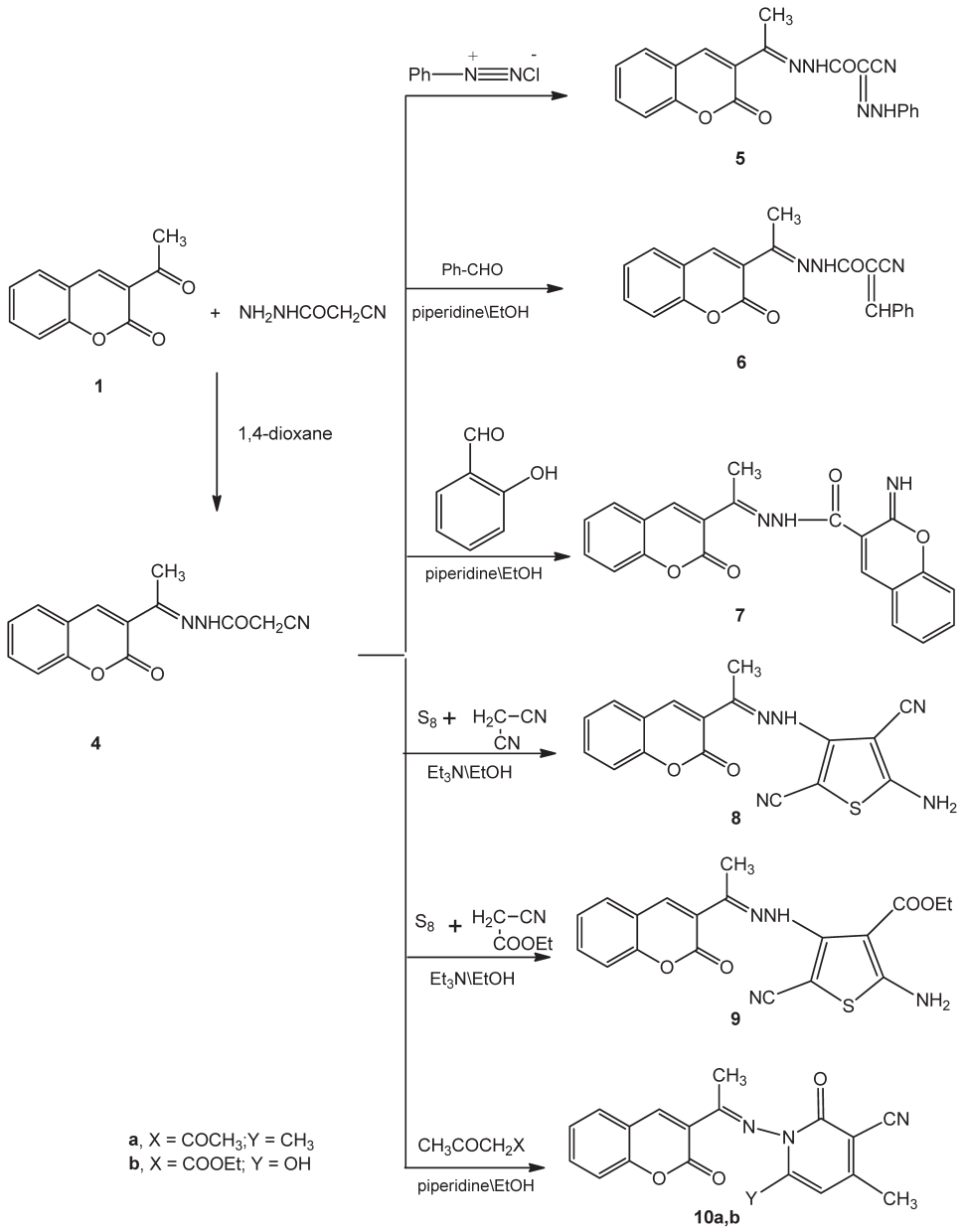

Chart 2. Synthesis of Compounds **5**–**9**, and **10a**, **b**

synthesizing heterocyclic compounds and their fused derivatives with potential antitumor activities. Thus, compound **4** reacted with benzenediazonium chloride at 0–5°C to afford the phenylhydrazone derivative **5**. On the other hand, it reacted with benzaldehyde in the presence of a catalytic amount of piperidine to give the benzylidene derivative **6**. In addition, compound **4** reacted with salicylaldehyde to afford the 2-iminobenzo[*b*]pyran derivative **7**. The analytical and spectral data of compounds 5–**7** are in agreement with their respective structures (see Experimental).

Next, we moved towards studying the reactivity of compound **4** towards thiophene synthesis through the well-known Gewald's thiophene synthesis.[49,50]

Thus, the reaction of compound **4** with either malononitrile or ethyl cyanoacetate gave the thiophene derivatives **8** and **9**,[34] respectively. The structures of **8** and **9** were confirmed on the basis of their ^{1}H-NMR and ^{13}C-NHR spectra. The ^{1}H-NMR spectrum of **8** (as an example) showed the presence of two signals (D_2O exchangeable) at δ 3.83 and 8.95 ppm corresponding to NH_2 and NH groups beside another two signals at δ. 3.05 and 6.83 ppm corresponding to CH_3 and coumarin H-4, respectively. Moreover, the ^{13}C-NMR spectrum revealed the presence of signals at δ 28.8 (CH_3), 116.6, 117.3 (2CN), 163.8 (C=O) and 170.3 (C=N).

The reaction of compound **4** with either acetylacetone or ethyl acetoacetate gave the 1,2-dihydropyridine[34] derivatives **10a**, **b**, respectively (Chart 2). The ^{1}H-NMR and ^{13}C-NMR spectra were used to confirm the structures of **10a**, **b**. Thus the ^{1}H-NMR spectrum of **10a** (for example) showed the presence of three singlets at δ 2.09, 3.01, 3.48 ppm due to the presence of three CH_3 groups beside two singlets at δ 6.99 and 9.01 ppm corresponding to the presence of coumarin H-4 and pyridine H-5, respectively. Moreover, the ^{13}C-NMR spectrum revealed the presence of signals at δ 30.0, 33.9, 42.1 ppm for three (CH_3) groups beside the presence of signals at δ 116.2 (CN), 160.1, 164.1 (2C=O), 168.3 (C=N).

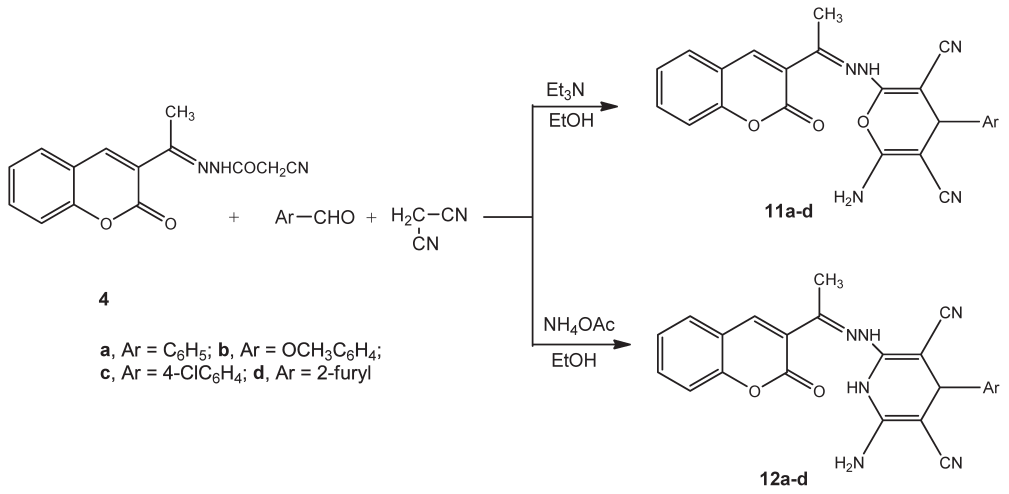

Chart 3. Synthesis of Compounds **11a**–**d** and **12a**–**d**

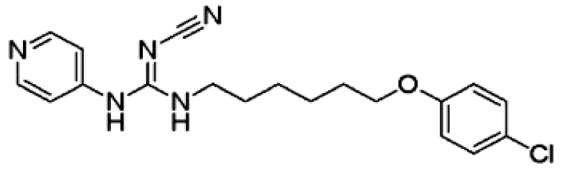

Fig. 2. Chemical Structure of CHS 828

Finally, the multicomponent reactions of compound **4** with either of benzaldehyde, 4-methoxybenzaldehyde, 4-chlorobenzaldehyde or furfural in ethanol containing a catalytic amount of triethylamine gave the 4*H*-pyran derivatives **11a**–**d**, respectively. On the other hand carrying the same reaction but using ammonium acetate instead of triethylamine afforded the 1,4-dihydropyridine derivatives **12a**–**d**, respectively (Chart 3). The structures of **11a**–**d** and **12a**–**d** were based on analytical and spectral data (see Experimental).

***In Vitro* Cytotoxicity**

Effect on the Growth of Human Cancer Cell Lines

The heterocyclic compounds, prepared in this study, were evaluated according to standard protocols for their *in vitro* cytotoxicity against six human cancer cell lines including cells derived from human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), nasopharyngeal carcinoma (HONE1), human breast cancer (MCF) and normal fibroblast cells (WI38). For comparison purposes, CHS 828, a pyridyl cyanoguanidine, was used as standard antitumor drug[51] (Fig. 2). All of the IC_{50} values (the sample concentration that produces 50% reduction in cell growth) in nanomolar (nM) are listed in Table 1. Several compounds showed potent inhibition with an IC_{50} Values <870 nM and the results are represented graphically in (Figs. 3, 4). All the synthesized compounds were tested for their cytotoxicity against normal fibroblast cells. The results obtained showed that normal fibroblast cells (WI38) were affected to a much lesser extent ($IC_{50}>10000$ nM).

Structure–Activity Relationship

In this study, when correlating the structures of the synthesized compounds with their anticancer activity, it has been observed that several compounds showed significant cytotoxic effect with IC_{50} values <870 nM. Normal fibroblast cells (WI38) were affected to a much lesser extent ($IC_{50}>10000$ nM). Among the 4*H*-pyran derivatives **2a**–**d**, compounds **2a** and **c** are the most potent. The latter compound showed high potency towards the six cancer cell lines, while compound **2a** was potent only against four cancer cell lines namely: NUGC, DLD1, HA22T and MCF with IC_{50}'s 48, 59, 122 and 480 nM, respectively. The high potency of **2c** is attributed to the presence of the 4-chloro group. Considering the 1,4-dihydropyridine derivatives **3a**–**d**, each one of these derivative revealed selective activity against certain cancer cell lines. Compound **3a** showed selective higher activity against liver cancer HEPG2 ($IC_{50}=22$ nM) than **3b**, **c** and **d**. The introduction of 4-methoxy group in **3b** exhibited remarkable increase in the activity against NUGC and HA22T than **3a**, **c** and **d**. Moreover, the presence of furan moiety in **3d** is responsible for its high potency against breast cancer MCF, it showed equivalent cytotoxic effect to the standard CHS 828 ($IC_{50}=18$ nM).

Comparing the cytotoxicity of the hydrazide-hydrazone **4** with its condensation products **5** and **6**, all of them showed low cytotoxicity. On the other hand the thiophene derivatives **8** and **9** showed optimal cytotoxic activity against the six cancer cell lines. Moreover compound **8** exhibited two fold higher activity ($IC_{50}=48$ nM) against NUGC compared to the standard CHS 828 ($IC_{50}=25$ nM). The remarkable activity of **8** and **9** is due to the presence of the thiophene ring.[29]

Considering the 1,2-dihydropyridine derivatives **10a**, **b**, it is clear that the cytotoxicity of **10b** is higher than that of **10a**. Compound **10b** showed more potency towards the three cancer cell lines namely: NUGC, HEPG2 and MCF with IC_{50}'s 239, 125 and 36 nM, respectively. Such high cytotoxicity of **10b** is attributed to the presence of the electronegative OH group.

Considering the 4*H*-pyran derivatives **11a**–**d**, compound **11c** substituted with 4-chloro group showed the highest cytotoxicity among the four compounds with remarkable activity against the six human cancer cell lines. Thus it is obvious that while some of the compounds were not the most potent, their specific activity against particular cell lines makes them of interest for further development as anticancer drugs.

Conclusion

The present study reports the successful synthesis, charac-

Table 1. Cytotoxicity of Compounds **2a–d**; **3a–d**; **4**; **5**; **6**; **7**; **8**; **9**; **10a**, **b**; **11a–d**, and **12a–d** against a Variety of Cancer Cell Lines[a] [IC_{50}[b] (nM)]

Compound No.	Cytotoxicity (IC_{50} in nM)						
	NUGC	DLDI	HA22T	HEPG2	HONE1	MCF	WI38
2a	48	59	122	2334	3289	480	na
2b	1128	1892	2377	1328	1290	2673	360
2c	862	207	380	282	206	264	660
2d	2101	2458	2258	350	2180	1140	428
3a	1288	2187	2530	22	2135	1729	650
3b	122	3210	59	1245	1140	1130	na
3c	1289	2266	351	2328	2612	430	na
3d	2265	2139	2257	2177	2250	18	262
4	1232	1166	2225	2216	326	1286	na
5	1280	2419	2160	1284	2130	2073	872
6	3138	2366	2228	2130	1584	326	650
7	2210	2433	1650	2560	1544	2457	520
8	48	55	128	128	248	128	838
9	135	158	278	279	206	668	829
10a	1126	2168	1312	1232	1824	2330	549
10b	389	1220	1480	125	1620	36	na
11a	2120	2055	2173	1359	2149	2580	883
11b	3242	2150	1165	4321	4273	2533	na
11c	470	80	31	132	168	447	276
11d	1040	2763	2469	3146	1342	2293	370
12a	1278	1830	2067	2634	1970	2263	1179
12b	1488	1259	1224	3120	1680	2328	na
12c	2210	2186	1160	2178	2562	1179	na
12d	1175	2340	1169	1273	2181	2834	na
CHS 828	25	2315	2067	1245	15	18	na

a) NUGC, gastric cancer; DLDI, colon cancer; HA22T, liver cancer; HEPG2, liver cancer; HONEI, nasopharyngeal carcinoma; MCF, breast cancer; WI38, normal fibroblast cells. *b*) The sample concentration produces a 50% reduction in cell growth.

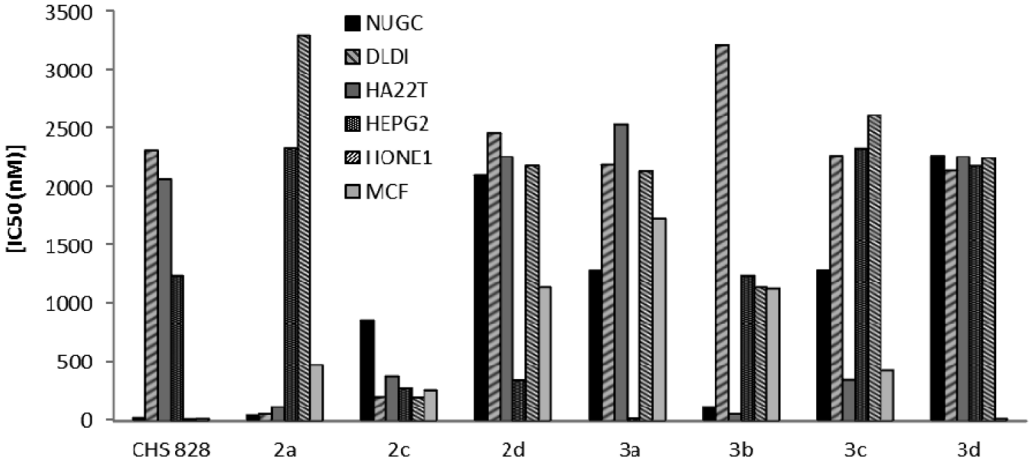

Fig. 3. Cytotoxicity of Compounds **2a**, **c**, **d**, **3a–d** and CHS 828 against NUGC, Gastric Cancer; DLDI, Colon Cancer; HA22T, Liver Cancer; HEPG2, Liver Cancer; HONEI, Nasopharyngeal Carcinoma; MCF, Breast Cancer

terization and anticancer activity of new series of 4*H*-pyran, dihydropyridine and thiophene derivatives starting from either 3-acetyl coumarin (**1**) or coumarin acetohydrazide derivative **4**. Several compounds showed potent inhibition with an IC_{50} <870 nM. Among these derivatives compound **3d** exhibited equivalent cytotoxic effect to the standard CHS 828 against breast cancer cell line (IC_{50}=18 nM). Normal fibroblast cells (WI38) were affected to a much lesser extent (IC_{50}>10000 nM). The obtained results suggest that these compounds may serve as lead chemical entities for further modification in the search of new classes of potential anticancer agents.

Experimental

Chemistry All melting points were determined on a Stuart apparatus and the values given are uncorrected. IR spectra (KBr, cm^{-1}) were determined on a Shimadzu IR 435 spectrophotometer (Faculty of Science, Cairo University, Egypt). ^{1}H-NMR spectra were recorded on Varian Gemini 300 MHz (Microanalysis Center, Cairo University, Egypt) using tetramethylsilane (TMS) as internal standard. Chemical shift values are recorded in ppm on δ scale. The electron impact (EI) mass spectra were recorded on a Hewlett Packard 5988 spectrometer (Microanalysis Center, Cairo University, Egypt).

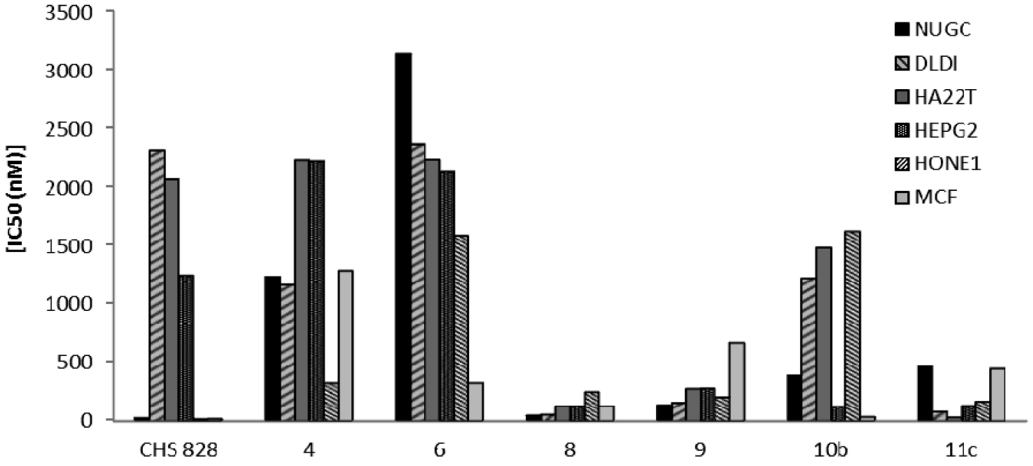

Fig. 4. Cytotoxicity of Compounds **4**, **6**, **8**, **9**, **10b**, **11c**, and CHS 828 against NUGC, Gastric Cancer; DLDI, Colon Cancer; HA22T, Liver Cancer; HEPG2, Liver Cancer; HONEI, Nasopharyngeal Carcinoma; MCF, Breast Cancer

Elemental analyses were carried out at the Microanalysis Center, Cairo University, Egypt; found values were within ±0.35% of the theoretical ones. Progress of the reactions was monitored using thin layer chromatography (TLC) sheets re-coated with UV fluorescent silica gel Merck 60F 254 and were visualized using UV lamp.

General Procedure for the Synthesis of Compounds 2a–d A mixture of 3-acetyl-2*H*-chromen-2-one (**1**) (1.88 g, 0.01 mol), the appropriate arylaldehyde (0.1 mol) and malononitrile (0.66 g, 0.1 mol) were heated under reflux in ethanol (40 mL) containing triethylamine (1.0 mL) for 3h. The reaction mixture was left to cool, poured onto ice water and neutralized by hydrochloric acid. The solid product was precipitated, filtered, washed with water, and crystallized from ethanol.

2-Amino-6-(2-oxo-2*H*-chromen-3-yl)-4-phenyl-4*H*-pyran-3-carbonitrile (**2a**)

Yield: 62%; mp: 98–100°C; IR (KBr, cm^{-1}): 3436, 3328 (NH_2), 3066 (CH, aromatic), 2198 (CN), 1724 (C=O); ^{1}H-NMR (dimethyl sulfoxide (DMSO)-d_6) δ: 2.88 (s, 2H, NH_2, D_2O exchangeable), 6.76 (s, 1H, pyran H-4), 6.89 (s, 1H, coumarin H-4), 7.38–7.96 (m, 9H aromatic), 8.65 (s, 1H, pyran H-5); ^{13}C-NMR (DMSO-d_6) δ: 86.3, 116.6, 120.2, 122.1, 122.9, 123.9, 124.7, 125.1, 127.5, 129.0, 130.7, 132.6, 133.4, 136.8, 138.9, 139.3, 140.1, 142.0, 164.6; MS electron impact (EI): *m/z* (%) 342 (M^+). *Anal*. Calcd for $C_{21}H_{14}N_2O_3$: C, 73.68; H, 4.12; N, 8.18. Found: C, 73.87; H, 4.18; N, 7.93.

2-Amino-4-(4-methoxyphenyl)-6-(2-oxo-2*H*-chromen-3-yl)-4*H*-pyran-3-carbonitrile (**2b**)

Yield: 65%; mp: >300°C; IR (KBr, cm^{-1}): 3440, 3342 (NH_2), 3089 (CH, aromatic), 2212 (CN), 1722 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.57 (s, 2H, NH_2, D_2O exchangeable), 3.81 (s, 3H, OCH_3), 6.79 (s, 1H, pyran H-4), 7.01 (s, 1H, coumarin H-4), 7.39–8.57 (m, 8H aromatic), 8.90 (s, 1H, pyran H-5); ^{13}C-NMR (DMSO-d_6) δ: 33.2, 86.2, 116.8, 120.8, 121.3, 122.8, 123.2, 124.2, 125.2, 126.3, 128.2, 129.4, 130.2, 133.1, 134.6, 138.6, 139.1, 140.1, 142.3, 163.8; MS (EI): *m/z* (%) 372 (M^+). *Anal*. Calcd for $C_{22}H_{16}N_2O_4$: C, 70.96; H, 4.33; N, 7.52. Found: C, 70.63; H, 4.65; N, 7.83.

2-Amino-4-(4-chlorophenyl)-6-(2-oxo-2*H*-chromen-3-yl)-4*H*-pyran-3-carbonitrile (**2c**)

Yield: 61%; mp: >300°C; IR (KBr, cm^{-1}): 3430, 3410 (NH_2), 3087 (CH, aromatic), 2220 (CN), 1719 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.49 (s, 2H, NH_2, D_2O exchangeable), 6.94 (s, 1H, pyran H-4), 6.97 (s, 1H, coumarin H-4), 7.41–7.81 (m, 8H aromatic), 8.39 (s, 1H, pyran H-5); ^{13}C-NMR (DMSO-d_6) δ: 88.1, 116.0, 120.5, 120.6, 122.8, 123.7, 124.6, 125.4, 126.1, 127.3, 128.5, 129.2, 129.5, 133.1, 134.5, 139.6, 140.3, 142.9, 162.1; MS (EI): *m/z* (%) 376 (M^+). *Anal*. Calcd for $C_{21}H_{13}ClN_2O_3$: C, 66.94; H, 3.48; N, 7.43. Found: C, 66.83; H, 3.35; N, 7.08.

2-Amino-4-(furan-2-yl)-6-(2-oxo-2*H*-chromen-3-yl)-4*H*-pyran-3-carbonitrile (**2d**)

Yield: 66%; mp: 118–120°C; IR (KBr, cm^{-1}): 3427, 3389 (NH_2), 3077 (CH, aromatic), 2214 (CN), 1722 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.88 (s, 2H, NH_2, D_2O exchangeable), 6.69 (s, 1H, pyran H-4), 6.81 (s, 1H, coumarin H-4), 7.05–7.95 (m, 7H aromatic), 8.65 (s, 1H, pyran H-5); ^{13}C-NMR (DMSO-d_6) δ: 88.3, 116.8, 120.9, 121.2, 122.8, 123.9, 124.6, 125.4, 126.5, 127.9, 129.2, 129.5, 133.1, 134.9, 139.6, 140.8, 142.6, 149.9, 162.1. MS (EI): *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, 68.49; H, 3.31; N, 8.72.

General Procedure for the Synthesis of Compounds 3a–d A mixture of 3-acetyl-2*H*-chromen-2-one (**1**) (1.88 g, 0.01 mol), the appropriate arylaldehyde (0.1 mol) and malononitrile (0.66 g, 0.1 mol) were heated under reflux in ethanol (40 mL) containing ammonium acetate (0.5 g) for 2–4 h. The reaction mixture was left to cool, poured onto ice water and neutralized by hydrochloric acid. The solid product was precipitated, filtered, washed with water, and crystallized from ethanol.

2-Amino-6-(2-oxo-2*H*-chromen-3-yl)-4-phenyl-1,4-dihydropyridine-3-carbonitrile (**3a**)

Yield: 70%; mp: 178–180°C; IR (KBr, cm^{-1}): 3423–3254 (NH_2, NH), 3089 (CH, aromatic), 2204 (CN), 1719 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.89 (s, 2H, NH_2, D_2O exchangeable), 6.97 (s, 1H, pyridine H-4), 7.14 (s, 1H, coumarin H-4), 7.42–7.81 (m, 9H aromatic), 8.67 (s, 1H, pyridine H-5), 9.04 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 84.8, 116.4, 119.6, 120.7, 121.8, 122.3, 123.2, 124.5, 125.4, 126.3, 128.3, 129.8, 132.6, 134.6, 136.8, 138.2, 139.1, 140.2, 164.8; MS (EI): *m/z* (%) 341 (M^+). *Anal*. Calcd for $C_{21}H_{15}N_3O_2$: C, 73.89; H, 4.43; N, 12.31. Found: C, 73.58; H, 4.41; N, 11.98.

2-Amino-4-(4-methoxyphenyl)-6-(2-oxo-2*H*-chromen-3-yl)-1,4-dihydropyridine-3-carbonitrile (**3b**)

Yield: 74%; mp: 169–171°C; IR (KBr, cm^{-1}): 3428–3263

(NH_2, NH), 3067 (CH, aromatic), 2191 (CN), 1722 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.95 (s, 2H, NH_2, D_2O exchangeable), 3.94 (s, 3H, OCH_3), 6.83 (s, 1H, pyridine H-4), 6.95 (s, 1H, coumarin H-4), 7.05–7.48 (m, 8H aromatic), 8.89 (s, 1H, pyridine H-5), 9.97 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 32.8, 86.1, 116.4, 120.3, 120.9, 121.6, 123.1, 123.9, 124.3, 125.8, 127.4, 129.4, 130.3, 132.5, 133.9, 137.3, 139.5, 140.2, 141.9, 165.0; MS (EI): *m/z* (%) 371 (M^+). *Anal*. Calcd for $C_{22}H_{17}N_3O_3$: C, 71.15; H, 4.61; N, 11.31. Found: C, 71.43; H, 4.39; N, 11.52.

2-Amino-4-(4-chlorophenyl)-6-(2-oxo-2*H*-chromen-3-yl)-1,4-dihydropyridine-3-carbonitrile (**3c**)

Yield: 75%; mp: 188–190°C; IR (KBr, cm^{-1}): 3455–3240 (NH_2, NH), 3078 (CH, aromatic), 2213 (CN), 1735 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 3.09 (s, 2H, NH_2, D_2O exchangeable), 6.80 (s, 1H, pyridine H-4), 7.21 (s, 1H, coumarin H-4), 7.41–8.59 (m, 8H aromatic), 8.96 (s, 1H, pyridine H-5), 11.20 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 88.2, 116.1, 120.6, 121.0, 121.3, 122.1, 123.1, 124.2, 125.3, 126.0, 127.9, 129.5, 129.8, 133.0, 136.1, 138.5, 140.0, 145.3, 163.1; MS (EI): *m/z* (%) 375 (M^+). *Anal*. Calcd for $C_{21}H_{14}ClN_3O_2$: C, 67.12; H, 3.75; N, 11.18. Found: C, 67.43; H, 3.47; N, 11.51.

2-Amino-4-(furan-2-yl)-6-(2-oxo-2*H*-chromen-3-yl)-1,4-dihydropyridine-3-carbonitrile (**3d**)

Yield: 75%; mp: 105–107°C; IR (KBr, cm^{-1}): 3424–3267 (NH_2, NH), 3079 (CH, aromatic), 2215 (CN), 1718 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.90 (s, 2H, NH_2, D_2O exchangeable), 6.61 (s, 1H, pyridine H-4), 6.78 (s, 1H, coumarin H-4), 6.80–7.96 (m, 7H aromatic), 8.29 (s, 1H, pyridine H-5), 9.08 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 86.8, 116.8, 120.6, 120.9, 122.3, 123.9, 125.7, 127.4, 129.8, 130.5, 132.8, 134.3, 137.3, 137.8, 139.5, 140.2, 142.3, 144.9, 163.9; MS (EI): *m/z* (%) 331 (M^+). *Anal*. Calcd for $C_{19}H_{13}N_3O_3$: C, 68.88; H, 3.95; N, 12.86. Found: C, 68.59; H, 3.61; N, 12.64.

Synthesis of 2-Cyano-*N*′-(1-(2-oxo-2*H*-chromen-3-yl)-ethylidene)acetohydrazide (4) A mixture of 3-acetyl-2*H*-chromen-2-one (**1**) (1.88 g, 0.01 mol) and 2-cyanoacetohydrazide (0.99 g, 0.01 mol) in 1,4-dioxane was heated under reflux for 2 h. The reaction mixture was left to cool, poured onto ice water and neutralized by hydrochloric acid. The solid product was precipitated, filtered, washed with water, and crystallized from ethanol.

Yield: 65%; mp: 268–270°C; IR (KBr, cm^{-1}): 3348–3259 (NH), 3063 (CH, aromatic), 2236 (CN), 1734, 1685 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.15 (s, 3H, CH_3), 5.08 (s, 2H, CH_2), 6.63 (s, 1H, coumarin H-4), 6.88–7.83 (m, 4H aromatic), 8.92 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 28.3, 64.2, 117.3, 120.4, 120.9, 124.3, 126.2, 128.5, 130.3, 133.2, 142.3, 160.1, 164.3, 172.1; MS (EI): *m/z* (%) 269 (M^+). *Anal*. Calcd for $C_{14}H_{11}N_3O_3$: C, 62.45; H, 4.12; N, 15.61. Found: C, 62.23; H, 4.29; N, 15.69.

Synthesis of 2-Oxo-2-(2-(1-(2-oxo-2*H*-chromen-3-yl)ethylidene)hydrazinyl)-*N*′-phenylacetohydrazonoyl Cyanide (5) To a cold solution of the hydrazide-hydrazone derivative **4** (2.69 g, 0.01 mol) in ethanol (30 mL) containing sodium acetate (2.5 g), a cold solution benzenediazonium chloride (0.01 mol) [prepared by the addition of sodium nitrite solution (0.7 g, 0.01 mol) to a cold solution of aniline (0.01 mol) in concentrated hydrochloric acid (3 mL, 18 N) with continuous stirring] was added while stirring. The reaction mixture was kept at room temperature for 1 h and the formed solid product, was collected by filtration and crystalized from ethanol.

Yield: 75%; mp: 278–280°C; IR (KBr, cm^{-1}): 3444–3104 (2NH), 3044 (CH, aromatic), 2230 (CN), 1730, 1680 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.49 (s, 3H, CH_3), 7.04 (s, 1H, coumarin H-4), 7.44–7.83 (m, 9H aromatic), 8.95 (s, 2H, 2 NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 22.8, 116.7, 120.8, 121.3, 123.7, 124.6, 125.4, 126.5, 127.3, 129.2, 130.1, 132.8, 133.2, 142.9, 162.2, 164.1, 169.8, 170.3; MS (EI): *m/z* (%) 373 (M^+). *Anal*. Calcd for $C_{20}H_{15}N_5O_3$: C, 64.34; H, 4.05; N, 18.76. Found: C, 64.08; H, 4.22; N, 18.59.

General Procedure for the Synthesis of Compounds 6 and 7 The mixture of hydrazide-hydrazone derivative (**4**) (2.69 g, 0.01 mol) with either of benzaldehyde or salicylaldehyde (0.01 mol) in absolute ethanol containing piperidine (1 mL) was heated under reflux for 1 h. The reaction mixture was left to cool, poured onto ice water and neutralized by hydrochloric acid. The solid product was precipitated, filtered, washed with water, and crystallized from ethanol.

2-Cyano-*N*′-(1-(2-oxo-2*H*-chromen-3-yl)ethylidene)-3-phenylacrylohydrazide (**6**)

Yield: 62%; mp: 230°C; IR (KBr, cm^{-1}): 3413–3328 (NH), 3013 (CH, aromatic), 2217 (CN), 1704, 1687 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.14 (s, 3H, CH_3), 6.68 (s, 1H, coumarin H-4), 7.12 (s, 1H, C=CH) 7.44–7.92 (m, 9H aromatic), 10.09 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 22.8, 116.8, 120.8, 121.4, 122.8, 123.7, 124.6, 125.8, 126.4, 127.2, 128.0, 129.5, 132.1, 134.5, 140.3, 164.8, 166.2, 169.5, 170.1; MS (EI): *m/z* (%) 357 (M^+). *Anal*. Calcd for $C_{21}H_{15}N_3O_3$: C, 70.58; H, 4.23; N, 11.76. Found: C, 70.69; H, 4.39; N, 12.05.

2-Imino-*N*′-(1-(2-oxo-2*H*-chromen-3-yl)ethylidene)-2*H*-chromene-3-carbohydrazide (**7**)

Yield: 60%; mp: 291°C; IR (KBr, cm^{-1}): 3434–3328 (2 NH), 3023 (CH, aromatic), 1714, 1681 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.92 (s, 3H, CH_3), 6.78 (s, 1H, coumarin H-4), 6.81 (s, 1H, coumarin H-4), 6.83–8.76 (m, 8H aromatic), 9.98 (s, 1H, NH, D_2O exchangeable), 11.19 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 32.2, 120.6, 121.0, 121.4, 122.1, 122.5, 123.4, 124.2, 125.0, 126.2, 127.7, 128.3, 129.4, 130.6, 131.9, 132.1, 140.1, 160.3, 164.5, 166.1, 168.9; MS (EI): *m/z* (%) 373 (M^+). *Anal*. Calcd for $C_{21}H_{15}N_3O_4$: C, 67.56; H, 4.05; N, 11.25. Found: C, 67.29; H, 4.37; N, 11.58.

General Procedure for the Synthesis of Compounds 8 and 9 A mixture of hydrazide-hydrazone derivative (**4**) (2.69 g, 0.01 mol), sulphur metal (0.32 g, 0.01 mol), and either malononitrile or ethyl cyanoacetate (0.01 mol) in absolute ethanol containing triethylamine (1 mL) was heated under reflux for 1 h. The reaction mixture was left to cool, poured onto ice water and neutralized by hydrochloric acid. The solid product was precipitated, filtered, washed with water, and crystallized from ethanol.

5-Amino-3-(2-(1-(2-oxo-2*H*-chromen-3-yl)ethylidene)-hydrazinyl)thiophene-2,4-dicarbonitrile (**8**)

Yield: 71%; mp: 173–175°C; IR (KBr, cm^{-1}): 3422–3278 (NH_2, NH), 3091 (CH, aromatic), 2205 (CN), 1706 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 3.05 (s, 3H, CH_3), 3.83 (s, 2H, NH_2, D_2O exchangeable), 6.83 (s, 1H, coumarin H-4), 6.97–7.93 (m, 4H aromatic), 8.95 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 28.8, 116.6, 117.3, 120.3, 120.7, 121.6, 122.8, 126.8, 128.4, 129.9, 132.8, 133.2, 140.2, 143.6, 144.0, 163.8, 170.3; MS (EI): *m/z* (%) 349 (M^+). *Anal*. Calcd for $C_{17}H_{11}N_5O_2S$: C, 58.44; H, 3.17; N, 20.05; S, 9.18. Found: C,

58.79; H, 3.49; N, 20.39; S, 9.04.

Ethyl 2-Amino-5-cyano-4-(2-(1-(2-oxo-2*H*-chromen-3-yl)-ethylidene)hydrazinyl)thiophene-3-carboxylate (**9**)

Yield: 68%; mp: 273–275°C; IR (KBr, cm^{-1}): 3433–3252 (NH_2, NH), 3025 (CH, aromatic), 2220 (CN), 1734, 1685 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 1.15 (t, 3H, J=7.2 Hz, CH_2-$\underline{CH_3}$), 3.08 (s, 3H, CH_3), 3.77 (s, 2H, NH_2, D_2O exchangeable), 3.85 (q, 2H, J=7.2 Hz, $\underline{CH_2}$-CH_3), 6.96 (s, 1H, coumarin H-4), 7.38–7.83 (m, 4H aromatic), 8.95 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 28.1, 30.0, 65.3, 116.9, 120.6, 121.4, 122.4, 123.4, 124.2, 126.1, 127.7, 129.4, 130.6, 140.0, 146.9, 147.9, 160.3, 163.4, 168.2; MS (EI): *m/z* (%) 396 (M^+). *Anal*. Calcd for $C_{19}H_{16}N_4O_4S$: C, 57.57; H, 4.07; N, 14.13; S, 8.09. Found: C, 57.36; H, 4.29; N, 13.97; S, 7.99.

General Procedure for the Synthesis of Compounds 10a and b The reaction of hydrazide-hydrazone derivative (**4**) (2.69 g, 0.01 mol) with either acetylacetone or ethyl acetoacetate (0.01 mol) in absolute ethanol containing piperidine (1 mL) was heated under reflux for 8 h. The reaction mixture was left to cool, poured onto ice water and neutralized by hydrochloric acid. The solid product was precipitated, filtered, washed with water, and crystallized from ethanol.

4,6-Dimethyl-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)-ethylidene)amino)-1,2-dihydropyridine-3-carbonitrile (**10a**)

Yield: 59%; mp: 200–202°C; IR (KBr, cm^{-1}): 3045 (CH, aromatic), 2231 (CN), 1726, 1680 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.09 (s, 3H, CH_3), 3.01 (s, 3H, CH_3), 3.48 (s, 3H, CH_3), 6.99 (s, 1H, coumarin H-4), 7.34–7.69 (m, 4H aromatic), 9.01 (s, 1H, pyridine H-5); ^{13}C-NMR (DMSO-d_6) δ: 30.0, 33.9, 42.1, 116.2, 120.4, 120.6, 121.1, 122.8, 124.3, 125.8, 126.9, 128.3, 129.4, 130.6, 140.0, 148.4, 160.1, 164.1, 168.3; MS (EI): *m/z* (%) 333 (M^+). *Anal*. Calcd for $C_{19}H_{15}N_3O_3$: C, 68.46; H, 4.54; N, 12.61. Found: C, 68.73; H, 4.66; N, 12.72.

6-Hydroxy-4-methyl-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)-ethylidene)amino)-1,2-dihydropyridine-3-carbonitrile (**10b**)

Yield: 61%; mp: 243–245°C; IR (KBr, cm^{-1}): 3427 (OH), 3034 (CH, aromatic), 2210 (CN), 1724, 1687 (2C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.42 (s, 3H, CH_3), 3.61 (s, 3H, CH_3), 6.78 (s, 1H, coumarin H-4), 6.83–7.68 (m, 4H aromatic), 9.01 (s, 1H, pyridine H-5), 11.14 (s, 1H, OH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 22.8, 30.0, 116.9, 120.5, 120.9, 122.8, 123.9, 124.6, 125.4, 126.1, 127.9, 129.2, 130.2, 134.8, 136.6, 162.8, 164.0, 169.6; MS (EI): *m/z* (%) 335 (M^+). *Anal*. Calcd for $C_{18}H_{13}N_3O_4$: C, 64.47; H, 3.91; N, 12.53. Found: C, 64.69; H, 4.22; N, 12.67.

General Procedure for the Synthesis of Compounds 11a–d A mixture of hydazide-hydrazone derivative (**4**) (2.69 g, 0.01 mol, the appropriate arylaldehyde (0.1 mol) and malononitrile (0.66 g, 0.1 mol) were heated under reflux in 1,4-dioxane (30 mL) containing triethylamine (1.0 mL) for 3 h. The reaction mixture was left to cool, poured onto ice water and neutralized by hydrochloric acid. The solid product was precipitated, filtered, washed with water, and crystallized from ethanol.

2-Amino-6-(2-(1-(2-oxo-2*H*-chromen-3-yl)ethylidene)-hydrazinyl)-4-phenyl-4*H*-pyran-3,5-dicarbonitrile (**11a**)

Yield: 70%; mp: 123–125°C; IR (KBr, cm^{-1}): 3431–3254 (NH_2, NH), 3065 (CH, aromatic), 2212 (CN), 1723 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.49 (s, 3H, CH_3), 3.48 (s, 2H, NH_2, D_2O exchangeable), 5.12 (s, 1H, pyran H-4), 6.77 (s, 1H, coumarin H-4), 6.79–7.83 (m, 9H aromatic), 8.95 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 29.3, 86.9, 116.8, 117.4, 120.8, 121.3, 122.4, 123.7, 124.6, 125.8, 127.1, 128.4, 130.3, 132.2, 136.9, 137.0, 138.2, 140.2, 142.8, 143.6, 164.3, 171.6; MS (EI): *m/z* (%) 423 (M^+). *Anal*. Calcd for $C_{24}H_{17}N_5O_3$: C, 68.08; H, 4.05; N, 16.54. Found: C, 68.31; H, 4.40; N, 16.72.

2-Amino-4-(4-methoxyphenyl)-6-(2-(1-(2-oxo-2*H*-chromen-3-yl)ethylidene)hydrazinyl)-4*H*-pyran-3,5-dicarbonitrile (**11b**)

Yield: 75%; mp: 138–140°C; IR (KBr, cm^{-1}): 3441–3104 (NH_2, NH), 3043 (CH, aromatic), 2223 (CN), 1729 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.93 (s, 3H, CH_3), 3.72 (s, 2H, NH_2, D_2O exchangeable), 3.89 (s, 3H, OCH_3), 5.02 (s, 1H, pyran H-4), 6.96 (s, 1H, coumarin H-4), 7.02–7.99 (m, 8H aromatic), 8.95 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 30.0, 56.3, 88.0, 116.0, 117.2, 120.6, 121.3, 122.3, 123.1, 124.2, 125.4, 126.5, 127.6, 129.4, 129.8, 133.0, 136.0, 138.6, 140.1, 144.5, 148.4, 160.0, 168.1; MS (EI): *m/z* (%) 453 (M^+). *Anal*. Calcd for $C_{25}H_{19}N_5O_4$: C, 66.22; H, 4.22; N, 15.44. Found: C, 66.52; H, 3.99; N, 15.29.

2-Amino-4-(4-chlorophenyl)-6-(2-(1-(2-oxo-2*H*-chromen-3-yl)ethylidene)hydrazinyl)-4*H*-pyran-3,5-dicarbonitrile (**11c**)

Yield: 77%; mp: 159–161°C; IR (KBr, cm^{-1}): 3441–3282 (NH_2, NH), 3054 (CH, aromatic), 2214 (CN), 1715 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.68 (s, 3H, CH_3), 3.57 (s, 2H, NH_2, D_2O exchangeable), 5.09 (s, 1H, pyran H-4), 6.82 (s, 1H, coumarin H-4), 7.15–7.84 (m, 8H aromatic), 8.94 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 28.8, 84.2, 116.4, 117.1, 119.8, 120.3, 122.4, 123.9, 124.0, 125.8, 126.4, 128.6, 132.7, 136.3, 138.4, 139.8, 140.4, 143.9, 144.1, 148.3, 164.4, 171.2; MS (EI): *m/z* (%) 457 (M^+). *Anal*. Calcd for $C_{24}H_{16}ClN_5O_3$: C, 62.96; H, 3.52; N, 15.30. Found: C, 62.91; H, 3.29; N, 15.02.

2-Amino-4-(furan-2-yl)-6-(2-(1-(2-oxo-2*H*-chromen-3-yl)-ethylidene)hydrazinyl)-4*H*-pyran-3,5-dicarbonitrile (**11d**)

Yield: 72%; mp: 238–240°C; IR (KBr, cm^{-1}): 3398–3274 (NH_2, NH), 3044 (CH, aromatic), 2212 (CN), 1720 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.92 (s, 3H, CH_3), 3.44 (s, 2H, NH_2, D_2O exchangeable), 5.23 (s, 1H, pyran H-4), 6.75 (s, 1H, coumarin H-4), 6.92–7.80 (m, 7H aromatic), 8.97 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 28.6, 84.5, 116.3, 117.0, 119.8, 120.4, 122.3, 123.8, 124.2, 125.3, 126.0, 127.2, 128.3, 129.4, 132.2, 136.1, 138.8, 140.2, 143.6, 143.8, 164.6, 171.8; MS (EI): *m/z* (%) 413 (M^+). *Anal*. Calcd for $C_{22}H_{15}N_5O_4$: C, 63.92; H, 3.66; N, 16.94. Found: C, 63.69; H, 4.01; N, 16.76.

General Procedure for the Synthesis of Compounds 12a–d A mixture of hydazide-hydrazone derivative (**4**) (2.69 g, 0.01 mol), the appropriate arylaldehyde (0.1 mol) and malononitrile (0.66 g, 0.1 mol) were heated under reflux in 1,4-dioxane (30 mL) containing ammonium acetate (0.5 g) for 5 h. The reaction mixture was left to cool, poured onto ice water and neutralized by hydrochloric acid. The solid product was precipitated, filtered, washed with water, and crystallized from ethanol.

2-Amino-6-(2-(1-(2-oxo-2*H*-chromen-3-yl)ethylidene)-hydrazinyl)-4-phenyl-1,4-dihydropyridine-3,5-dicarbonitrile (**12a**)

Yield: 66%; mp: 189–191°C; IR (KBr, cm^{-1}): 3447–3259 (NH_2, 2NH), 3046 (CH, aromatic), 2230 (CN), 1737 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.09 (s, 3H, CH_3), 3.08 (s, 2H, NH_2, D_2O exchangeable), 4.22 (s, 1H, pyridine H-4), 7.20 (s, 1H, coumarin H-4), 7.30–8.03 (m, 9H aromatic), 8.33 (s, 1H, NH, D_2O exchangeable), 10.73 (s, 1H, NH, D_2O exchangeable);

^{13}C-NMR (DMSO-d_6) δ: 28.9, 88.4, 116.8, 117.2, 120.3, 120.9, 121.8, 122.9, 124.4, 125.9, 126.3, 127.4, 128.6, 129.7, 132.8, 136.3, 138.3, 140.6, 142.3, 143.6, 164.8, 170.9; MS (EI): *m/z* (%) 422 (M^+). *Anal*. Calcd for $C_{24}H_{18}N_6O_2$: C, 68.24; H, 4.29; N, 19.89. Found: C, 68.50; H, 3.99; N, 19.63.

2-Amino-4-(4-methoxyphenyl)-6-(2-(1-(2-oxo-2*H*-chromen-3-yl)ethylidene)hydrazinyl)-1,4-dihydropyridine-3,5-dicarbonitrile (**12b**)

Yield: 69%; mp: 159–161°C; IR (KBr, cm^{-1}): 3438–3265 (NH_2, 2NH), 3038 (CH, aromatic), 2222 (CN), 1731 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.35 (s, 3H, CH_3), 2.59 (s, 2H, NH_2, D_2O exchangeable), 2.71 (s, 3H, OCH_3), 3.98 (s, 1H, pyridine H-4), 6.97 (s, 1H, coumarin H-4), 7.08–7.99 (m, 8H aromatic), 8.75 (s, 1H, NH, D_2O exchangeable), 11.12 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 28.2, 54.0, 88.3, 116.3, 117.4, 120.4, 120.6, 121.1, 121.8, 122.8, 124.3, 125.4, 126.2, 127.4, 128.3, 129.5, 133.4, 139.2, 140.5, 146.0, 147.7, 163.9, 168.1; MS (EI): *m/z* (%) 452 (M^+). *Anal*. Calcd for $C_{25}H_{20}N_6O_3$: C, 66.36; H, 4.46; N, 18.57. Found: C, 66.08; H, 4.72; N, 18.49.

2-Amino-4-(4-chlorophenyl)-6-(2-(1-(2-oxo-2*H*-chromen-3-yl)ethylidene)hydrazinyl)-1,4-dihydropyridine-3,5-dicarbonitrile (**12c**)

Yield: 70%; mp: 176–178°C; IR (KBr, cm^{-1}): 3435–3276 (NH_2, 2NH), 3097 (CH, aromatic), 2227 (CN), 1727 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.09 (s, 3H, CH_3), 2.90 (s, 2H, NH_2, D_2O exchangeable), 4.02 (s, 1H, pyridine H-4), 6.92 (s, 1H, coumarin H-4), 7.09–7.92 (m, 8H aromatic), 8.95 (s, 1H, NH, D_2O exchangeable), 10.72 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 28.2, 87.9, 116.5, 117.3, 120.4, 120.9, 122.6, 123.8, 124.7, 125.6, 126.8, 127.4, 128.8, 129.3, 132.8, 136.9, 139.5, 140.9, 142.8, 143.6, 164.1, 172.2; MS (EI): *m/z* (%) 456 (M^+). *Anal*. Calcd for $C_{24}H_{17}ClN_6O_2$: C, 63.09; H, 3.75; N, 18.39. Found: C, 63.39; H, 3.47; N, 18.69.

2-Amino-4-(furan-2-yl)-6-(2-(1-(2-oxo-2*H*-chromen-3-yl)-ethylidene)hydrazinyl)-1,4-dihydropyridine-3,5-dicarbonitrile (**12d**)

Yield: 61%; mp: 193–195°C; IR (KBr, cm^{-1}): 3439–3259 (NH_2, 2NH), 3042 (CH, aromatic), 2226 (CN), 1728 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 2.32 (s, 3H, CH_3), 2.71 (s, 2H, NH_2, D_2O exchangeable), 3.98 (s, 1H, pyridine H-4), 6.97 (s, 1H, coumarin H-4), 7.14–8.00 (m, 7H aromatic), 8.65 (s, 1H, NH, D_2O exchangeable), 11.05 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 26.8, 88.1, 116.2, 117.0, 121.3, 121.8, 122.9, 123.2, 125.1, 126.3, 128.8, 129.3, 132.8, 136.3, 138.9, 140.6, 142.3, 143.6, 144.2, 145.3, 164.3, 172.3; MS (EI): *m/z* (%) 412 (M^+). *Anal*. Calcd for $C_{22}H_{16}N_6O_3$: C, 64.07; H, 3.91; N, 20.38. Found: C, 64.39; H, 3.78; N, 20.11.

***In Vitro* Cytotoxic Assay**

Chemicals

Fetal bovine serum (FBS) and L-glutamine were purchased from Gibco Invitrogen Co. (Scotland, U.K.). RPMI-1640 medium was purchased from Cambrex (New Jersey, NJ, U.S.A.). Dimethyl sulfoxide (DMSO), CHS 828, penicillin, streptomycin and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Cell Cultures

Cell cultures were obtained from the European Collection of cell Cultures (ECACC, Salisbury, U.K.) and human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and normal fibroblast cells (WI38) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They were grown as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 lg/mL), at 37°C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5×10^5 cells/mL for the six human cancer cell lines followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

Conflict of Interest The authors declare no conflict of interest.

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