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

Rafat Milad Mohareb and Nadia Youssef Megally Abdo

Chemistry Department, Faculty of Science, Cairo University; Giza, Cairo 12613, Egypt; and Chemistry Department, Faculty of Education, Alexandria University; Alexandria 21526, Egypt.

Received February 4, 2015; accepted May 21, 2015

A series of coumarin analogues bearing 4H-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-2H-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 IC50 value of <870 nM. Compound 3d exhibited equivalent cytotoxic effect as the standard CHS 828 against a breast cancer cell line (IC50 value = 18 nM). Normal fibroblast cells (WI38) were affected to a much lesser extent (IC50 value >10000 nM).

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

Coumarins (2H-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 metalloproteinases (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<sub>50</sub>=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<sub>50</sub>=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,
3-acetyl coumarin received considerable attention as a target molecule for the synthesis of pyridine, thiazole and other heterocyclic derivatives.\textsuperscript{29–30}

In this work, we are demonstrating the reaction of 3-acetyl coumarin with 2-cyanoacetohydrazide to give the 2-cyano-N‘-(1-(2-oxo-2H-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-2H-chromen-3-yl)ethylidene)hydrazinylthiophene derivatives 8 and 9, respectively. Moreover, the (2-oxo-2H-chromen-3-yl)-4H-pyran derivatives 2a–d, 11a–d and the (2-oxo-2H-chromen-3-yl)-1,4-dihydropyridine derivatives 3a–d, 12a–d have been prepared by condensation of either 3-acetyl coumarin (1) or coumarin acetylhydrazide 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.\textsuperscript{29,30} These combined pharmacophores probably offer some advantages such as in overcoming drug resistance\textsuperscript{31} as well as improving their biological potency.\textsuperscript{32} Therefore, in the present study it was planned to synthesize hybrid compounds that comprise 3-acetyl coumarin 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 selectivity and less toxic anticancer agents. All the synthesized compounds were consistent with their respective structures.

Results and Discussion

Chemistry The multicomponent reaction of 3-acetyl coumarin with either of benzaldehyde, 4-methoxybenzaldehyde, 4-chlorobenzaldehyde or furfural in ethanol containing a catalytic amount of triethylamine gave the 4H-pyran derivatives 2a–d, 33,34 respectively. The analytical and spectral data of 2a–d were consistent with their respective structures.

Thus, the \textsuperscript{1}H-NMR spectrum of 2c (as an example) showed the presence of two singlets at δ 2.49 (D\textsubscript{2}O exchangeable) and 6.97 ppm due to the presence of NH\textsubscript{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 \textsuperscript{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 \textsuperscript{1}H-NMR and \textsuperscript{13}C-NMR spectra. Thus, the \textsuperscript{1}H-NMR spectrum of 3a (as an example) displayed the presence of two signals (D\textsubscript{2}O exchangeable) at δ 2.89 and 9.04 ppm due to the presence of NH\textsubscript{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 \textsuperscript{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.\textsuperscript{35–37} With the aim of obtaining new hydrazide-hydrazones with such wide spectrum of pharmaceutical applications,\textsuperscript{38–40} we report here the synthesis of a series of hydrazide-hydrazones via the reaction of 3-acetyl coumarine (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 \textsuperscript{1}H-NMR and \textsuperscript{13}C-NMR spectra. The \textsuperscript{1}H-NMR spectrum revealed the presence of two singlets at δ 2.15 and 5.08 ppm for the CH\textsubscript{2} and CH\textsubscript{3} 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\textsubscript{2}O exchangeable) of the acetylhydrazide moiety. Moreover, the \textsuperscript{13}C-NMR spectrum showed the presence of signals at δ 28.3 (CH\textsubscript{3}), 64.2 (CH\textsubscript{2}), 117.3 (CN), 160.1, 164.3 (2CO) and 172.1 (C=O), respectively. Compound 4 was a good candidate in

![Chart 1. Synthesis of Compounds 2a–d and 3a–d](Image)
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-iminobenzopyran 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-NMR spectra. The \(^1\)H-NMR spectrum of 8 (as an example) showed the presence of two signals (D\(_2\)O exchangeable) at \(\delta\) 3.83 and 8.95 ppm corresponding to NH\(_2\) and NH groups beside another two signals at \(\delta\) 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 \(\delta\) 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 \(\delta\) 2.09, 3.01, 3.48 ppm due to the presence of three CH\(_3\) groups beside two singlets at \(\delta\) 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 \(\delta\) 30.0, 33.9, 42.1 ppm for three (CH\(_3\)) groups beside the presence of signals at \(\delta\) 116.2 (CN), 160.1, 164.1 (2C=O), 168.3 (C=N).
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 4H-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). Among the 4H-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 4H-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-
terization and anticancer activity of new series of 4H-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<sub>50</sub> < 870 nM. Among these derivatives compound 3d exhibited equivalent cytotoxic effect to the standard CHS 828 against breast cancer cell line (IC<sub>50</sub> = 18 nM). Normal fibroblast cells (WI38) were affected to a much lesser extent (IC<sub>50</sub> > 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 Stu-art apparatus and the values given are uncorrected. IR spectra (KBr, cm<sup>-1</sup>) were determined on a Shimadzu IR 435 spectrophotometer (Faculty of Science, Cairo University, Egypt). ¹H-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).

**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<sup>a,b</sup> [IC<sub>50</sub> (nM)]**

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

<sup>a</sup> NUGC, gastric cancer; DLDI, colon cancer; HA22T, liver cancer; HEPG2, liver cancer; HONE1, nasopharyngeal carcinoma; MCF, breast cancer; WI38, normal fibroblast cells.

<sup>b</sup> 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; HONE1, 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 recoated 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-2H-chromen-2-one (1) (1.88 g, 0.01 mol), the appropriate arylaldehyde (0.1 mol) and malononitrile (0.66 g, 0.01 mol) were heated under reflux in ethanol (40 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-oxo-2H-chromen-3-yl)-4-phenyl-4H-pyran-3-carbonitrile (2a)**

Yield: 62%; mp: 98–100°C; IR (KBr, cm⁻¹): 3436, 3328 (NH), 3066 (CH, aromatic), 2198 (CN), 1724 (C=O); ¹H-NMR (DMSO-d₆): δ: 2.88 (s, 2H, NH₂, D₂O exchangeable), 6.76 (s, 1H, pyran H-4), 6.89 (s, 1H, coumarin H-4), 7.38–7.86 (m, 9H aromatic), 8.65 (s, 1H, pyran H-5); ¹³C-NMR (DMSO-d₆): 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 C₁₂H₁₂N₂O₃: C, 67.82; H, 5.57; N, 11.99; Found: C, 67.78; H, 5.49; N, 11.96.

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

Yield: 66%; mp: 118–120°C; IR (KBr, cm⁻¹): 3427, 3389 (NH), 3077 (CH, aromatic), 2214 (CN), 1722 (C=O); ¹H-NMR (DMSO-d₆): δ: 2.88 (s, 2H, NH₂, D₂O 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); ¹³C-NMR (DMSO-d₆): δ: 88.3, 116.8, 120.9, 121.2, 122.8, 123.9, 124.9, 125.4, 126.5, 127.9, 129.2, 129.5, 131.3, 134.9, 139.6, 140.8, 142.6, 149.9, 161.2, 162.1; MS electron impact (EI): m/z (%): 332 (M⁺). Anal. Calcd for C₁₂H₁₀ClN₂O₃: 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-2H-chromen-2-one (1) (1.88 g, 0.01 mol), the appropriate arylaldehyde (0.1 mol) and malononitrile (0.66 g, 0.01 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-2H-chromen-3-yl)-4-phenyl-1,4-dihydropyridine-3-carbonitrile (3a)**

Yield: 70%; mp: 178–180°C; IR (KBr, cm⁻¹): 3423–3254 (NH, NH₂), 3089 (CH, aromatic), 2214 (CN), 1719 (C=O); ¹H-NMR (DMSO-d₆): δ: 2.89 (s, 2H, NH₂, D₂O exchangeable), 6.97 (s, 1H, pyridine H-4), 7.41–7.81 (m, 8H aromatic), 8.39 (s, 1H, pyran H-5); ¹³C-NMR (DMSO-d₆): 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 C₁₂H₁₃N₂O₃: C, 68.72; H, 3.41; N, 11.48; Found: C, 68.39; H, 3.42; N, 11.46.

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

Yield: 74%; mp: 169–171°C; IR (KBr, cm⁻¹): 3428–3263.
(NH₂, NH), 3067 (CH, aromatic), 2191 (CN), 1722 (C=O); 1H-NMR (DMSO-d₆) δ: 2.95 (s, 2H, NH₂, D₂O exchangeable), 3.94 (s, 3H, OCH₃), 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, D₂O exchangeable); 13C-NMR (DMSO-d₆) 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 C₁₇H₁₄N₃O₃: C, 68.88; H, 4.05; N, 16.59.

**Synthesis of 2-Oxo-2-(1-(2-oxo-2H-chromen-3-yl)ethylene)acetohydrazide** (4) A mixture of 3-acetyl-2H-chromen-2-one (1) (1.88 g, 0.01 mol) and 3-acetoxyacetohydrazine (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.

**Synthesis of 2-Oxo-2-(1-(2-oxo-2H-chromen-3-yl)ethylene)diacylhydrazinyl)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%) 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 crystallized from ethanol.

**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-2H-chromen-3-yl)ethylene)3-phenoxyacrylohydrozide** (6) Yield: 62%; mp: 230°C; IR (KBr, cm⁻¹): 3413–3328 (NH), 3013 (CH, aromatic), 2217 (CN), 1704, 1687 (2C=O); 1H-NMR (DMSO-d₆) δ: 2.14 (s, 3H, CH₃), 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, D₂O exchangeable), 13C-NMR (DMSO-d₆) δ: 22.8, 116.8, 120.8, 121.4, 122.8, 123.7, 124.6, 125.8, 126.4, 127.2, 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 C₂₄H₂₂N₃O₇: 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-2H-chromen-3-yl)ethylene)-2H-chromene-3-carboxyhydrazone** (7) Yield: 60%; mp: 291°C; IR (KBr, cm⁻¹): 3434–3328 (2 NH), 3023 (CH, aromatic), 1714, 1681 (2C=O); 1H-NMR (DMSO-d₆) δ: 2.92 (s, 3H, CH₃), 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, D₂O exchangeable), 11.19 (s, 1H, NH, D₂O exchangeable), 13C-NMR (DMSO-d₆) 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 C₂₄H₂₂N₃O₇: C, 76.56; H, 4.05; N, 11.25. Found: C, 76.79; 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-2H-chromen-3-yl)ethylene)hydrazinyl)thiophene-2,4-dicarbonitrile** (8) Yield: 71%; mp: 173–175°C; IR (KBr, cm⁻¹): 3422–3278 (NH₂, NH), 3091 (CH, aromatic), 2205 (CN), 1706 (C=O); 1H-NMR (DMSO-d₆) δ: 3.05 (s, 3H, CH₃), 3.83 (s, 2H, NH₂, D₂O exchangeable), 6.83 (s, 1H, coumarin H-4), 6.97–7.93 (m, 4H aromatic), 8.95 (s, 1H, NH, D₂O exchangeable); 13C-NMR (DMSO-d₆) 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 C₂₅H₂₁N₃O₇S: C, 58.44; H, 3.17; N, 20.05; S, 9.18. Found: C, 2.05 H, N, 70.99; S, 9.06.
58.79; H, 3.49; N, 20.39; S, 9.04.

Ethyl 2-Amino-5-cyano-4-(2-(1-(2-oxo-2H-chromen-3-yl)-ethylidene)hydrazinyl)thiophene-3-carboxylate (11a)

Yield: 68%; mp: 273–275°C; IR (KBr, cm⁻¹): 3431–3254 (NH, NH), 3065 (CH, aromatic), 2212 (CN), 1732 (C=O);
\[ ^1H \]NMR (DMSO-d₆): \( \delta \) 1.55 (t, J=7.2 Hz, CH₂-CH₃), 3.08 (s, 3H, CH₃), 3.77 (s, 2H, NH₂, D₀ exchangeable), 3.85 (q, 2H, J=7.2 Hz, CH₂-CH₃), 6.96 (s, 1H, coumarin H-4), 7.36–7.83 (m, 4H aromatic), 2231 (CN), 1723 (C=O), 1685 (2C=O); IR (KBr, cm⁻¹): 3047–3019 (CH, aromatic), 1685 (2C=O)

13C-NMR (DMSO-d₆): \( \delta \) 28.3, 30.0, 116.3, 117.2, 120.6, 121.0, 122.3, 124.6, 124.7, 125.3, 125.4, 126.1, 127.9, 129.2, 132.0, 134.8, 138.6, 162.8, 164.0, 167.0; MS (EI): m/z (%) 535 (M⁺).

Anal. Calcd for C₆H₆N₂O₄S: C, 61.64; H, 3.87; S, 18.9.

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 acetylace tone or ethyl acetocetate (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-2H-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);
\[ ^1H \]NMR (DMSO-d₆): \( \delta \) 2.09 (s, 3H, CH₃), 3.01 (s, 3H, CH₃), 3.48 (s, 3H, CH₃), 6.99 (s, 1H, coumarin H-4), 7.36–7.76 (m, 4H aromatic), 9.01 (s, 1H, pyridine H-5);
\[ ^13C \]NMR (DMSO-d₆): \( \delta \) 30.0, 33.9, 42.1, 116.2, 120.4, 121.1, 122.8, 124.3, 125.8, 126.9, 128.3, 129.4, 130.6, 140.9, 146.9, 147.9, 160.3, 163.4, 168.2; MS (EI): m/z (%) 535 (M⁺).

Anal. Calcd for C₁₀H₁₀N₂O₄S: C, 59.33; H, 4.26; S, 12.75.

6-Hydroxy-4-methyl-2-oxo-1-((1-(2-oxo-2H-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);
\[ ^1H \]NMR (DMSO-d₆): \( \delta \) 2.42 (s, 3H, CH₃), 3.61 (s, 3H, CH₃), 6.78 (s, 1H, coumarin H-4), 8.37–8.78 (m, 4H aromatic), 9.01 (s, 1H, pyridine H-5), 11.14 (s, 1H, OH, D₀ exchangeable);
\[ ^13C \]NMR (DMSO-d₆): \( \delta \) 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, 138.6, 162.8, 164.0, 169.6; MS (EI): m/z (%) 535 (M⁺).

Anal. Calcd for C₁₀H₁₀N₂O₄S: 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 hydrazide-hydrazone derivative (4) (2.69 g, 0.01 mol), the appropriate arylaldehyde (0.1 mol) and malononitrile (0.066 g, 1.0 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-2H-chromen-3-yl)-ethylidene)hydrazinyl)-4-phenyl-3,5-dicarbonitrile (11a)

Yield: 72%; mp: 238–240°C; IR (KBr, cm⁻¹): 3398–3274 (NH₂, NH), 3044 (CH, aromatic), 2212 (CN), 1720 (C=O);
\[ ^1H \]NMR (DMSO-d₆): \( \delta \) 2.92 (s, 3H, CH₃), 3.61 (s, 3H, CH₃), 6.78 (s, 1H, coumarin H-4), 8.37–8.78 (m, 4H aromatic), 9.01 (s, 1H, pyridine H-5), 11.14 (s, 1H, OH, D₀ exchangeable), 12.82 (s, 1H, pyridine H-5), 11.14 (s, 1H, OH, D₀ exchangeable);
\[ ^13C \]NMR (DMSO-d₆): \( \delta \) 22.8, 30.0, 116.4, 120.5, 120.9, 123.0, 123.9, 124.6, 125.4, 126.1, 127.9, 129.2, 130.2, 134.8, 138.6, 162.8, 164.0, 169.6; MS (EI): m/z (%) 535 (M⁺).

Anal. Calcd for C₁₀H₁₀N₂O₄S: 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 hydrazide-hydrazone derivative (4) (2.69 g, 0.01 mol), the appropriate arylaldehyde (0.1 mol) and malononitrile (0.066 g, 1.0 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-2H-chromen-3-yl)-ethylidene)hydrazinyl)-4-phenyl-1,4-dihydropyridine-3,5-dicarbonitrile (11d)
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 (AGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and normal fibroblast cells (W138) 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, 2mM glutamine and antibiotics (penicillin 100U/mL, streptomycin 100lg/mL), at 37°C in a humidified atmosphere containing 5% CO₂. Exponentially growing cells were obtained by plating 1.5×10⁵ cells/mL for the six human cancer cell lines followed by 24h 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.

References


