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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, cytotoxicity against cancer and normal cell lines of novel hydrazide–hydrazone derivatives bearing 5H-chromen-5-one

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Abstract

The reaction of cyclohexan-1,3-dione with either of malononitrile or ethyl cyanoacetate gave the 4-amino-6,7-dihydro-5H-chromen-5-one derivatives **3a** and **3b**, respectively. The reaction of the latter compounds with cyanoacetylhydrazine gave the hydrazide–hydrazone derivatives **5a** and **5b**, respectively. Compounds **5a,b** were used as the key starting materials for the synthesis of thiophen, coumarin, and pyridine derivatives. The newly synthesized compounds were evaluated against three human cancer cell lines, including HCT116 (colon carcinoma cell), MGC803 (gastric carcinoma cell), and Huh7 (hepatoma carcinoma cell). The results showed that **3b**, **5b**, **6b**, **6d**, **8b**, **8c**, **8d**, **8f**, **10b**. **12a–h**, **14a–d**, **15a–h**, and **16b–h** displayed higher cytotoxic activity than 5-FU against HCT116 and MGC803 cell lines. Compounds **14d** and **16f** were the most promising compounds with IC_{50}'s 0.25 and 0.09 µM against HCT116 cell line. The most potent compounds were selected for the in vitro against peripheral blood lymphocytes (PBL) from healthy donors. All compounds were practically devoid of significant cytotoxic activity in quiescent lymphocytes, with GI_{50}'s of 42–68 µM, while with the mitogenic stimulus phytohaemagglutinin (PHA), the GI_{50}'s were reduced to about 20–32 µM.

Keywords Cyclohexan-1,3-dione · Chromen · Hydrazide–hydrazone · Cytotoxicity · Cancer cell lines · Normal cells

Introduction

A number of hydrazide–hydrazone derivatives have been claimed to possess interesting bioactivity such as antibacterial–antifungal (Popiołek and Biernasiuk 2017; Loncle et al. 2004), anticonvulsant (Küçükgüzel et al. 2003), antiinflammatory (Todeschini et al. 1998), antimalarial (Melnyk et al. 2006), analgesic (Leite et al. 1999; Lima et al. 2000), antiplatelets (Cunha et al. 2003), anti-tuberculosis (Bedia et al. 2006), and anticancer activities (Terzioglu and Gürsoy 2003). Aroylhydrazide–hydrazones containing hetero-ring such as pyridine, indole ring has attracted special attention (Galić et al. 2001; Kaynak et al. 2005). A few of thiophene carbohydrazide hydrazone derivatives have also been reported (Rostom et al. 2003; Bernardino et al. 2006). In our effort to discover and develop apoptosis inducers as potential new anticancer agents, we recently reported a novel synthesized steroidal carbohydrazide hydrazone and effects of them on different cancer cell lines (Mohareb et al. 2016). Literature survey revealed that the hydrazide–hydrazone (–CO–NH–N=CH–) moiety has significant role as antitumor agent (Vicini et al. 2006; Üstündağ et al. 2016; Abadi et al. 2003; Kumar et al. 2012). On the other hand, Nerkar et al. reported that the in vitro anticancer activity of some carbohydrazide derivatives is due to their ability to inhibit dihydrofolate reductase enzyme (Xia et al. 2008; Nerkar et al. 2009). In continuation of this work we report here the synthesis of hydrazide–hydrazone derivatives from the 5H-chromen-5-one derivatives **3a,b** through its reaction with cyanoacetylhydrazine. Originally compounds **3a,b** were obtained through the reaction of cyclohexan-1,3-dione with the cyanomethylene reagents **2a,b**.

Results and discussion

The synthesis of the 4-amino-6,7-dihydro-5H-chromen-5-one derivatives **3a,b** has been accomplished as outlined in

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Scheme 1 starting from cyclohexan-1,3-dione and either of malononitrile (**2a**) or ethyl cyanoacetate (**2b**) in ethanol containing triethylamine. Compounds **3a,b** were used the key starting compounds for the synthesis of hydrazide–hydrazone derivatives. Thus, the reaction of either of compound **5a** or **5b** with cyanoacetylhydrazine (**4**) in 1,4-dioxan under reflux gave the hydrazide–hydrazone derivatives **5a,b**. The structures of the hydrazide–hydrazone derivatives were determined by IR, ^{1}H NMR and mass mass spectrometry. Thus, for example **5a**, obtained as white crystal, gave a [M^{+}]-ion peak at m/z 259 in the ESI-MS, in accord with the molecular formula $C_{12}H_{13}N_5O_2$. In the IR spectra, the carbonyl group absorptions in hydrazide moiety and NH bands in CONH were observed in the 1689 and 3473–3380 cm^{-1} region, respectively. The ^{1}H NMR spectra indicated the chemical shift of the NH proton at $\delta =$ 8.36 ppm in the form of singlet peak. Moreover, the ^{13}C NMR spectrum revealed the presence of signals at δ 155.3, 145.9, 135.8, 128.9, 120.8, 105.6 assigned to the pyran and cyclohexene carbons (C-2, C-3, C-4, C-4a, C-8, C-8a) and two signals at δ 172.8 and 166.3 assigned to the CO and C=N groups.

Following successful synthesis of thiophene derivatives recently reported by our research group (Mohareb et al. 2017, 2018a, 2018b, 2019) either of compound **5a** or **5b** reacted with elemental sulfur and either of malononitrile (**2a**) or ethyl cyanoacetate (**2b**) to afford the thiophene derivatives **6a–d**, respectively (Scheme 1). The structures of compounds **6a–d** were elucidated on the basis of their respective analytical and spectral data (see experimental section).

Compounds **5a,b** containing the active CH_2 group capable for coupling with diazonium salts to form arylhydrazone derivatives. Thus, the reaction of either of compound **5a** or **5b** with any of the diazonium salts namely benzenediazonium chloride (**7a**), 4-chlorobenzenediazonium chloride (**7b**) or 4-methoxybenzenediazonium chloride (**7c**) in ethanol containing sodium acetate to give the arylhydrazone derivatives **8a–f**, respectively. Moreover, the reaction of either compound **5a** or **5b** with salicylaldehyde gave the coumarin derivatives **10a** and **10b**, respectively. The IR, ^{1}H NMR, and ^{13}C NMR spectra of the resulting products **8a–f** and **10a,b** were consistent with their respective structures. In addition, the reaction of either of **5a** or **5b** with any of the aromatic aldehydes, benzaldehyde (**11a**), 4-chlorobenzaldehyde (**11b**), 4-bromobenzaldehyde (**11c**) or 4-methoxybenzaldehyde (**11d**) gave the arylidene derivatives **12a–h**, respectively (Scheme 2).

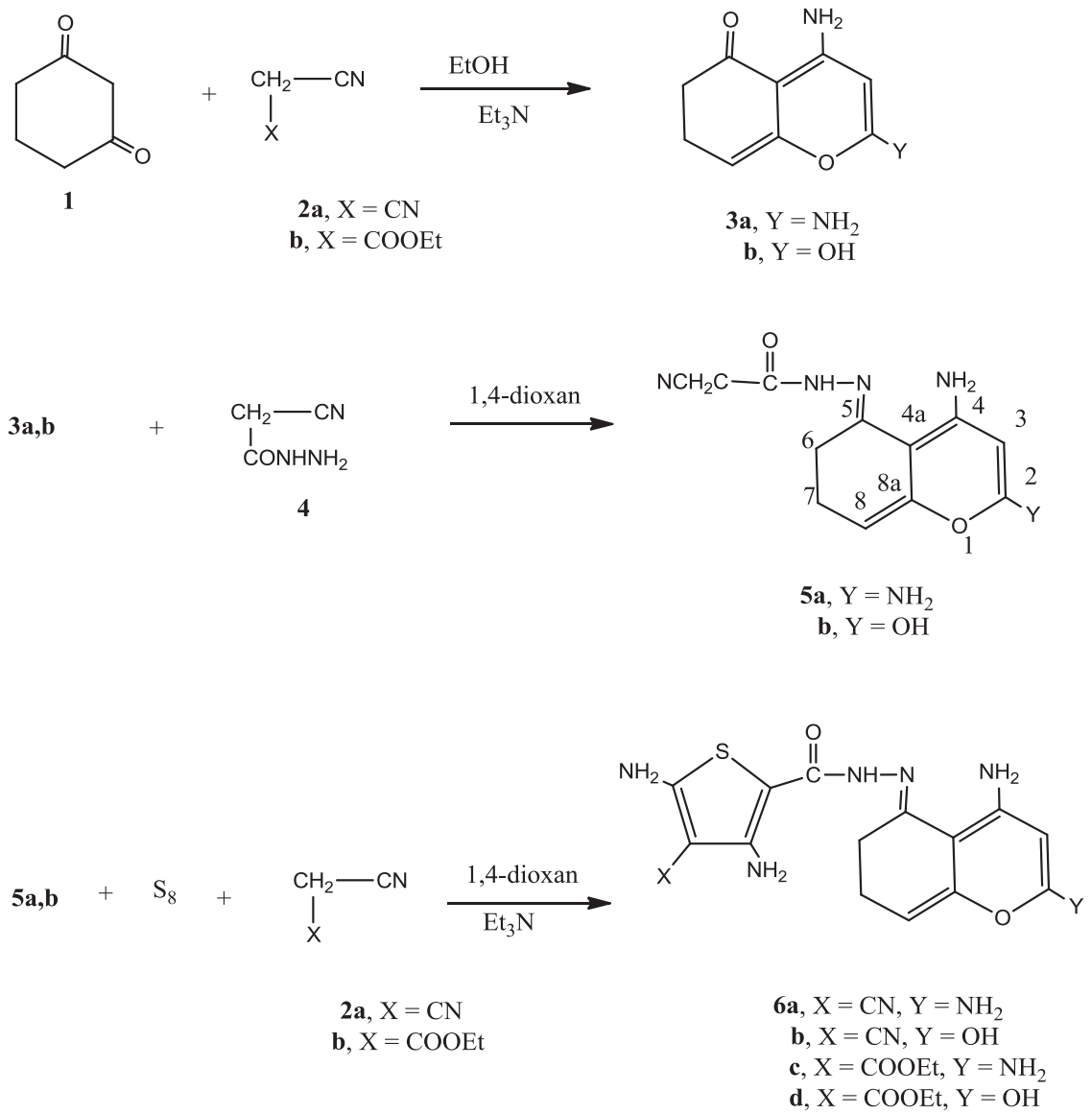
Next, we moved toward the uses of compounds **5a,b** for the synthesis of pyridine derivatives. Thus, the reaction of either compound **5a** or **5b** with either acetylacetone (**13a**) or ethyl acetoacetate (**13b**) in 1,4-dioxan solution in the presence of a catalytic amount of triethylamine under the reflux conditions gave the 2-oxo-1,2-dihydropyridine-3-carbonitrile derivatives **14a–d**, respectively. The structures of the latter compounds were based on their analytical and spectral data. Thus, the ^{1}H NMR spectrum of compound **14a**, as an examples, showed the presence of a singlet at δ 6.09 ppm assigned for the pyridine H-5 and a singlet at δ 2.66, 2.42 ppm indicating the presence of the two CH_3 groups and the ^{13}C NMR spectrum revealed the presence of the pyridine an ethylenic carbons at δ 153.6, 142.8, 134.5, 133.4, 130.1, 129.5, 128.2, 127.2, and two signals at δ 172.3 and 168.9 corresponding to the CO and C=N groups. Similarly, the reaction of either compound **5a** or **5b** with either of malononitrile (**2a**) or ethyl cyanoacetate (**2b**) gave the pyridine derivatives **15a–h** and **16a–h**, respectively (Scheme 3). The structures of all new synthesized compounds **15a–h** and **16a–h** were confirmed by ^{1}H NMR, ^{13}C NMR, IR, and mass spectra (see experimental section). The addition of either malononitrile or ethyl cyanoacetate to the benzylidene bonding present in compounds **12a** and **12b**, followed by cyclization and autooxidation gave the 1,2-dihydropyridine derivatives **15a–h** or **16a–h**, respectively.

Biological activity

The in vitro antitumor activity of the newly synthesized compounds was evaluated against a panel of three human cancer cell lines, including HCT116 (colon carcinoma cell), MGC803 (gastric carcinoma cell), and Huh7 (hepatoma carcinoma cell) by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. 5-Fluorouracil was used as positive control, and the results expressed as half-maximal inhibitory concentration (IC_{50}) values and are presented in Table 1, as mean values of experiments performed in triplicate. From the screening results in Table 1, it was observed that most of the synthesized compounds exhibited potent cytotoxic activities against the three human cancer cell lines. Cytotoxicity of the newly synthesized compounds was performed through the Cancer Research Center at Cairo University together with National Research Center, AR, Egypt.

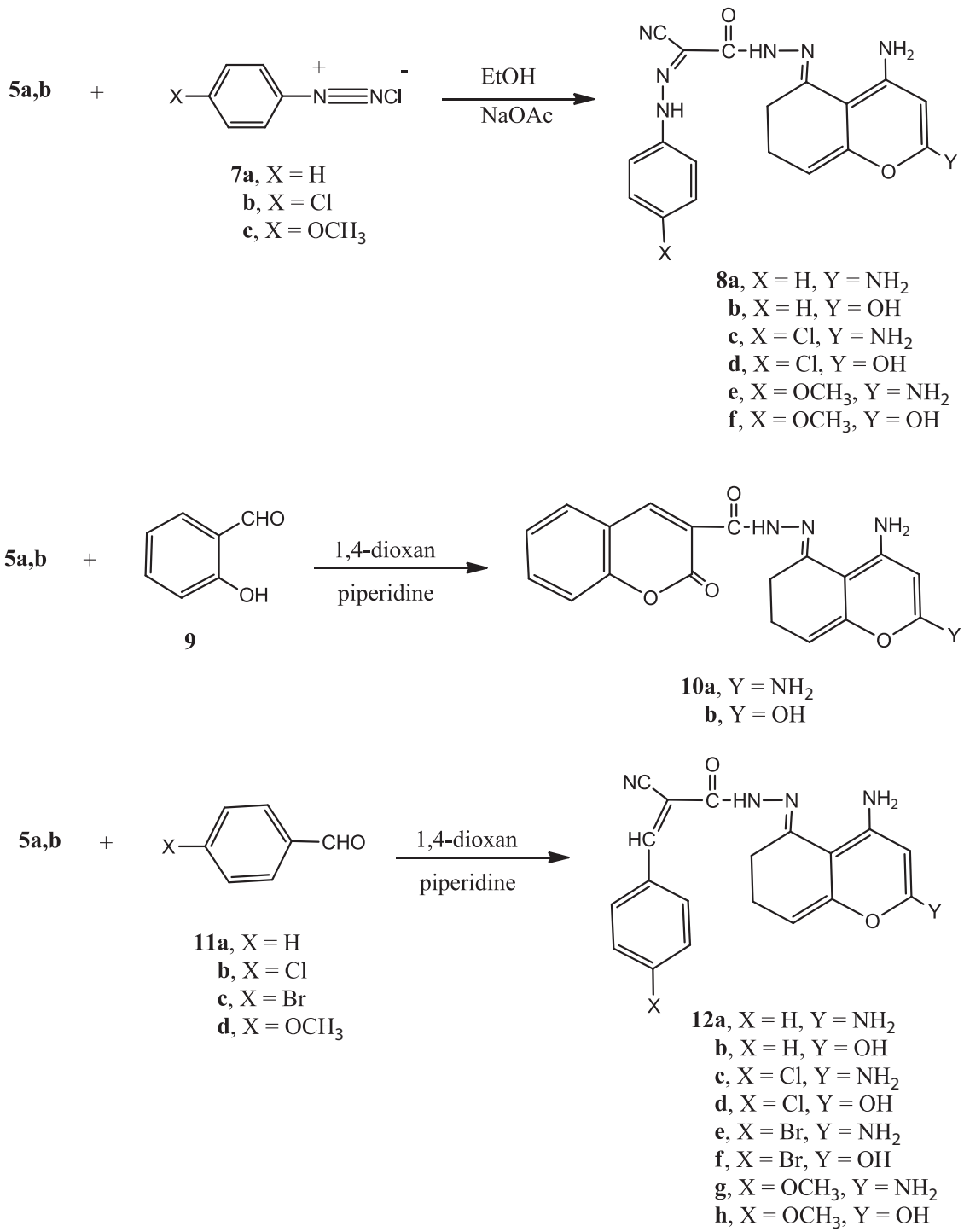
Structure–activity relationship

It is clear from Table 1 that compounds **3b**, **5b**, **6b**, **6d**, **8b**, **8c**, **8d**, **8f**, **10b**, **12a–h**, **14a–d**, **15a–h**, and **16b–h** displayed higher cytotoxic activity than 5-FU against HCT116 and MGC803 cell lines. Compounds **14d** and **16f** were the most promising compounds with IC_{50}'s 0.25 and 0.09 µM against HCT116 cell line. Although most of the compounds showed potent antitumor activity, some compounds exhibited selectivity between the three human cancer cell lines. Such as compounds **15b** and **16e** showed the more potent inhibitory activity against HCT116 and MGC803 cell line and less inhibitory activity against Huh7 cell line. From the



Scheme 1 Synthesis of compounds **3a, b; 5a, b** and **6a–d**

antitumor activity against three human cancer cell lines, preliminary structure–activity relationships of the synthesized compounds were achieved. For the 5H-chromen-5-one derivatives **3a,b** and the hydrazide–hydrazone derivatives **5a,b** it is clear that the presence of the electronegative OH group in compound **3b** and **5b** is responsible for their reactivity's. For the thiophene derivatives **6a–d**, compound **6d** (X=COOEt, Y=OH) was the most potent compound among the four compounds. For the arylhydrazone derivatives **8a–f**, it is obvious that compounds **8b** (X=H, Y=OH), **8c** (X=Cl, Y=NH_2) and **8d** (X=Cl, Y=OH) were most inhibit compounds, moreover, compound **8b** was of the highest inhibitions due to the presence of the two electronegative groups the Cl and the OH. Considering the coumarin derivatives **10a, b**, where compound **10b** with Y=OH was more potent than the compound **10a** with Y=NH_2. On the other hand, for compounds **12a–h** only compound **12a** (X=H, Y=NH_2) showed low cytotoxicity relative to the rest of such series of compounds. It is of great value to mention that compounds **12d** (X=Cl, Y=OH) and **12f** (X=Br, Y=OH) were the most cytotoxic compounds and this was attributed to the presence of either of the Cl or Br together with the OH groups through these compounds. For the pyridine derivatives **14a–d**, compounds **14b** and **14d** revealed the most cytotoxic activities due to the presence of the NH_2 and OH in case of **14b** and two OH groups for **14d**. However, the presence of CH_3 group together with the OH group in case of **14c** slightly decreased the

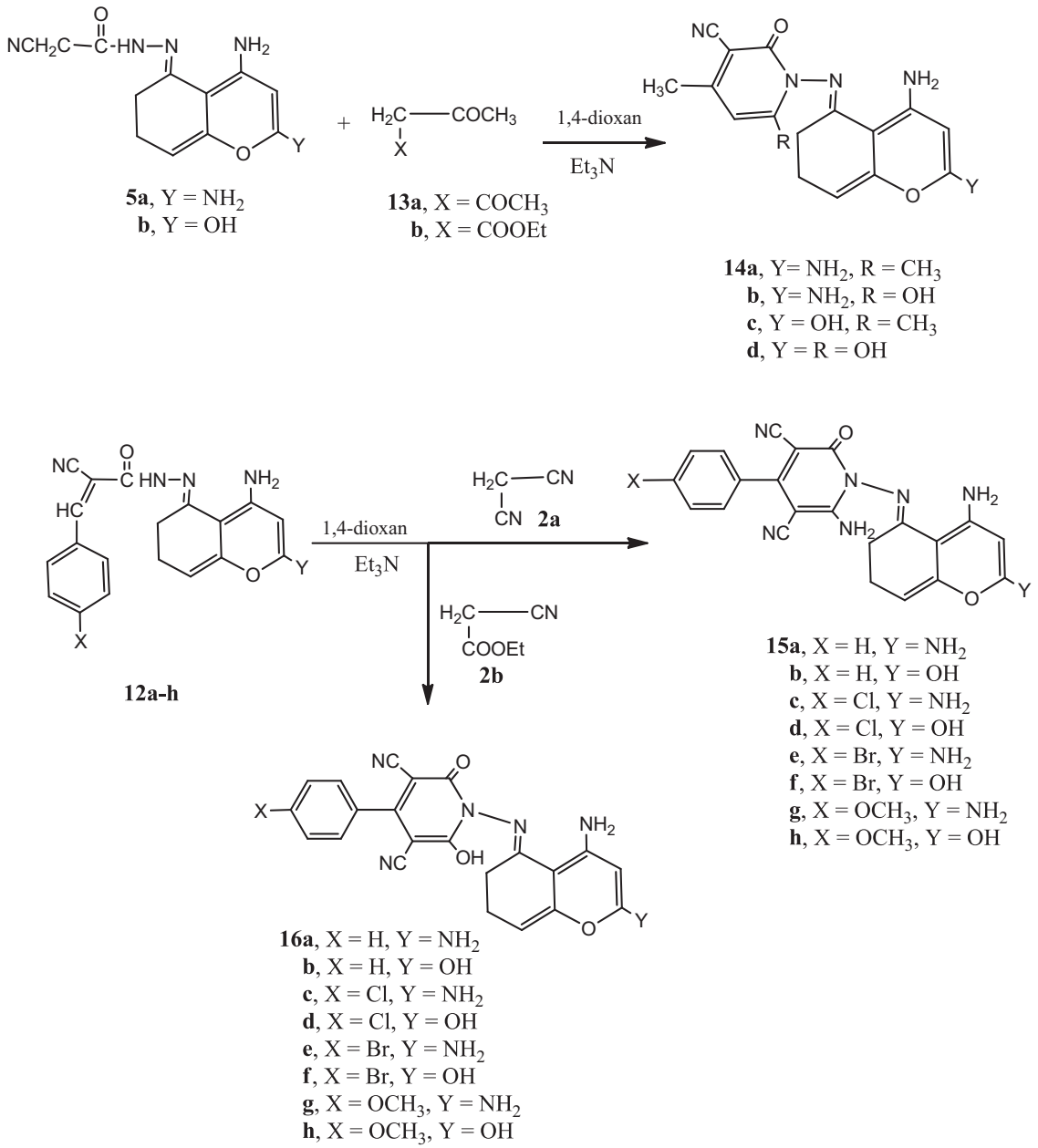


Scheme 2 Synthesis of compounds **8a–f**; **10a, b** and **12a–h**

cytotoxicity. The pyridine derivatives **15a–h** and **16a–h** showed interesting inhibitions toward the three cancer cell lines. Compounds **15b**, **15c**, **15d**, **15e**, **15f**, and **15h** showed high reactivity and the same also for the compounds **16b**, **16c**, **16d**, **16e**, **16f**, and **16h**. The high inhibitions of the last twelve were attributed to the presence of the OH, NH_2, and/or halogen groups.

Evaluation of cytotoxicity of selected compounds toward human noncancer cells

To obtain a preliminary indication of the cytotoxic potential of these derivatives in normal human cells, thirteen of the most active compounds (**12d, 12e, 12f, 12h, 14b, 14d, 15d, 15e, 15f, 15h, 16d, 16f**, and **16h**) were evaluated in vitro



Scheme 3 Synthesis of **14a–d**; **15a–h** and **16a–h**

against peripheral blood lymphocytes from healthy donors (Table 2). All compounds were practically devoid of significant cytotoxic activity in quiescent lymphocytes, with GI_{50}'s of 42–68 µM, while with the mitogenic stimulus phytohaemagglutinin, the GI_{50}'s were reduced to about 20–31 µM. These values, even under proliferation conditions, were >100 times than those found in the two cancer cell lines human colon carcinoma HCT116 and the human hepatocellular carcinoma MGS 803 shown in Table 1. These results indicate that these compounds have little effect in rapidly proliferating normal cells and even less in quiescent cells.

Conclusions

In summary, in order to develop potent antitumor agents, we have designed and synthesized a series of novel hydrazide–hydrazone derivatives containing 5H-chromen-

Table 1 In vitro antiproliferative activity of the newly synthesized product

Compound	X	Y	R	IC_{50}[a] (μM)		
				HCT116[b]	MGS 803[b]	Huh7[b]
3a	–	NH_2	–	18.25 ± 2.64	12.42 ± 3.19	10.22 ± 2.09
3b		OH	–	6.36 ± 1.58	8.31 ± 2.74	5.08 ± 1.32
5a	–	NH_2	–	14.36 ± 3.84	10.16 ± 2.57	16.49 ± 2.63
5b	–	OH	–	3.61 ± 0.89	6.37 ± 1.42	4.59 ± 1.82
6a	CN	NH_2	–	12.38 ± 2.29	18.51 ± 3.42	16.42 ± 4.26
6b	CN	OH	–	8.47 ± 2.42	6.72 ± 2.58	7.41 ± 1.69
6c	COOEt	NH_2	–	6.58 ± 3.83	8.49 ± 2.80	9.25 ± 2.41
6d	COOEt	OH	–	2.01 ± 0.42	3.19 ± 1.46	3.27 ± 1.46
8a	H	NH_2	–	12.85 ± 2.74	14.70 ± 3.52	11.39 ± 2.41
8b	H	OH	–	4.31 ± 1.27	3.25 ± 1.41	3.16 ± 1.48
8c	Cl	NH_2	–	3.26 ± 1.58	3.18 ± 1.07	6.42 ± 2.51
8d	Cl	OH	–	2.40 ± 0.15	2.17 ± 0.83	3.28 ± 1.51
8e	OCH_3	NH_2	–	16.32 ± 4.62	17.31 ± 3.27	14.27 ± 3.62
8f	OCH_3	OH	–	8.16 ± 2.53	6.75 ± 2.38	6.39 ± 1.56
10a	–	NH_2	–	16.53 ± 4.37	15.26 ± 2.41	14.73 ± 4.62
10b	–	OH	–	5.61 ± 1.27	6.24 ± 1.46	8.58 ± 2.31
12a	H	NH_2	–	8.25 ± 2.47	6.38 ± 1.53	9.51 ± 3.58
12b	H	OH	–	2.30 ± 1.04	3.25 ± 1.61	2.19 ± 0.92
12c	Cl	NH_2	–	1.44 ± 0.86	1.72 ± 0.69	2.53 ± 0.96
12d	Cl	OH	–	0.39 ± 0.19	0.43 ± 0.29	0.63 ± 0.18
12e	Br	NH_2	–	1.28 ± 0.87	0.96 ± 0.52	0.69 ± 0.29
12f	Br	OH	–	0.28 ± 0.04	0.34 ± 0.11	0.43 ± 0.08
12g	OCH_3	NH_2	–	4.33 ± 1.26	3.28 ± 1.46	5.16 ± 1.62
12h	OCH_3	OH	–	0.63 ± 0.24	0.41 ± 0.27	0.39 ± 0.13
14a	–	NH_2	CH_3	8.44 ± 2.39	6.42 ± 1.68	5.28 ± 1.08
14b	–	NH_2	OH	1.62 ± 0.93	0.57 ± 0.31	0.39 ± 0.21
14c	–	OH	CH_3	2.36 ± 0.94	1.04 ± 0.69	1.26 ± 0.92
14d	–	OH	OH	0.25 ± 0.03	0.31 ± 0.18	0.28 ± 0.04
15a	H	NH_2	–	6.73 ± 1.04	5.80 ± 2.42	8.09 ± 2.53
15b	H	OH	–	1.36 ± 0.93	0.72 ± 0.27	6.85 ± 0.38
15c	Cl	NH_2	–	1.66 ± 0.82	0.79 ± 0.58	0.42 ± 0.15
15d	Cl	OH	–	0.69 ± 0.42	0.33 ± 0.19	0.41 ± 0.25
15e	Br	NH_2	–	0.82 ± 0.37	0.59 ± 0.17	0.41 ± 0.08
15f	Br	OH	–	0.05 ± 0.008	0.17 ± 0.09	0.14 ± 0.03
15g	OCH_3	NH_2	–	6.49 ± 2.38	5.81 ± 1.07	7.33 ± 2.80
15h	OCH_3	OH	–	1.37 ± 0.93	0.82 ± 0.19	0.55 ± 0.21
16a	H	NH_2	–	8.48 ± 2.30	7.93 ± 2.46	8.05 ± 2.71
16b	H	OH	–	1.80 ± 0.68	2.69 ± 1.83	2.69 ± 0.85
16c	Cl	NH_2	–	1.58 ± 0.94	1.69 ± 0.38	0.89 ± 0.26
16d	Cl	OH	–	0.16 ± 0.07	0.28 ± 0.04	0.37 ± 0.27
16e	Br	NH_2	–	0.69 ± 0.42	0.58 ± 0.31	5.73 ± 0.36
16f	Br	OH	–	0.09 ± 0.007	0.21 ± 0.09	0.19 ± 0.04
16g	OCH_3	NH_2	–	6.48 ± 1.83	5.29 ± 1.76	8.69 ± 2.72
16h	OCH_3	OH	–	1.28 ± 0.83	0.69 ± 0.31	0.83 ± 0.29
5-FU	–	–	–	11.29 ± 1.06	25.54 ± 0.05	5.63 ± 0.14

[a]IC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared with an untreated control. Each value represents the mean ± S.E. of these experiments

[b]HCT116 cells were the human colon carcinoma cells, MGC803 cells were the human gastric carcinoma and Huh7 cells were the human hepatocellular carcinoma cell

5-one and evaluate their in vitro antitumor activities against HCT116, MGC803, and Huh7 human tumor cell lines by MTT assay. Some of the compounds inhibited the proliferation better than positive control 5-Fluorouracil. In particular, compound **14d** showed the best inhibitory effect against MGC803, HCT116, and Huh7 cells, with IC_{50} value of 0.25 ± 0.03, 0.31 ± 0.18 μM, 0.28 ± 0.04, respectively. Therefore, the results laid a foundation for further improving the potency and the selectivity of such series of compounds.

Table 2 Cytotoxicity of the compounds **12d, 12e, 12f, 12h, 14b, 14d, 15d, 15e, 15f, 15h, 16d, 16f**, and **16h** for human peripheral blood lymphocytes (PBL)

Compound	X	Y	R	GI_{50} (μM)[a]	
				$PBL_{resting}$[b]	PBL_{PHA}[c]
12d	Cl	OH	–	68.38 ± 12.47	28.59 ± 6.26
12e	Br	NH_2	–	54.38 ± 13.28	32.28 ± 3.36
12f	Br	OH	–	44.39 ± 8.52	22.68 ± 2.91
12h	OCH_3	OH	–	59.21 ± 14.69	26.83 ± 3.63
14b	–	NH_2	OH	51.62 ± 15.28	31.03 ± 4.33
14d	–	OH	OH	42.61 ± 12.72	20.29 ± 2.42
15b	H	OH	–	62.53 ± 13.63	26.72 ± 3.70
15d	Cl	OH	–	42.59 ± 8.82	28.95 ± 5.28
15e	Br	NH_2	–	56.73 ± 15.70	32.72 ± 8.40
15f	Br	OH	–	47.38 ± 12.70	28.06 ± 4.93
15h	OCH_3	OH	–	52.37 ± 14.55	26.26 ± 5.84
16d	Cl	OH	–	63.93 ± 15.62	25.74 ± 2.69
16f	Br	OH	–	52.80 ± 16.25	31.36 ± 5.72
16h	OCH_3	OH		46.43 ± 12.28	30.76 ± 3.52

Values are the mean ± SEM from two separate experiments

[a]Compound concentration required to reduce cell growth inhibition by 50%

[b]PBL not stimulated with PHA

[c]PBL stimulated with PHA

Materials and methods

Chemistry

The solvents used through this work were dried prior to their use. All melting points of the synthesized compounds were recorded on Buchi melting point apparatus D-545; IR spectra (KBr discs) were recorded on Bruker Vector 22 instrument. ^{13}C NMR and ^{1}H NMR spectra were recorded on Bruker DPX200 instrument in DMSO-d_6 with TMS as internal standard. Chemical shifts are mentioned in δ (ppm). Mass spectra were measured using EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. Elemental analyses were carried out using the Microanalytical Data center at Cairo University. The completion of all reactions was monitored by TLC on 2.5 cm precoated silica gel 60 F254 plates of thickness 0.25 mm (Merck).

General procedure for the synthesis of the 5H-chromen-5-one derivatives 3a,b

To a solution of cyclohexan-1,3-dione (1.12 g, 0.01 mol) in absolute ethanol (40 mL) containing triethylamine (0.50 mL) either of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then poured onto ice/water containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

2,4-Diamino-6,7-dihydro-5H-chromen-5-one (3a) Orange crystals from ethanol, yield (1.25 g, 70%), m.p. 166–168 °C, IR (KBr) υ max cm^{-1}: 3477–3362 ($2NH_2$), 3055 (CH, aromatic), 1686 (CO), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 7.87, 8.03 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 5.15 (*s*, 1H, H-8), 4.58 (*s*, 1H, H-3), 2.86, 2.23 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): 168.4 (C-5), 155.6, 148.2, 138.4, 128.6 (C-4, C-4a, C-8, C-8a), 120.8, 105.6 (C-2, C-3), 34.8 (C-6), 19.6 (C-7). 3Anal. Calculated for $C_9H_{10}N_2O_2$: C, 60.66; H, 5.66; N, 15.72. Found: C, 60.79; H, 5.74; N, 15.82. MS: m/e 178 (M^+, 40%).

4-Amino-2-hydroxy-6,7-dihydro-5H-chromen-5-one (3b) Orange crystals from 1,4-dioxan, yield (1.43 g, 80%), m.p. 133–135 °C, IR (KBr) υ max cm^{-1}: 3546–3337 (OH, NH_2), 3055 (CH, aromatic), 1688 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 9.31 (*s*, 1H, D_2O exchangeable, OH), 7.87 (*s*, 2H, D_2O exchangeable, NH_2), 5.17 (*s*, 1H, H-8), 4.56 (*s*, 1H, H-3), 2.85, 2.24 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): 168.8 (C-5), 155.8, 146.3, 136.9, 128.8 (C-4, C-4a, C-8, C-8a), 120.8, 105.6 (C-2, C-3), 34.6 (C-6), 19.8 (C-7). Anal. Calculated for $C_9H_9NO_3$: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.51; H, 5.31; N, 7.93. MS: m/e 179 (M^+, 36%).

Synthesis of the hydrazide–hydrazone derivatives 5a,b

Equimolar amounts of either of compound **3a** (1.78 g, 0.01 mol) or **3b** (1.79 g, 0.01 mol) and cyanoacetylhydrazine (1.00 g, 0.01 mol) in 1,4-dioxan (40 mL) was heated under reflux for 3 h. The reaction mixture was left to cool to room temperature and the formed solid product was collected by filtration.

2-Cyano-N′-(2,4-diamino-6,7-dihydro-5H-chromen-5-ylidene)acetohydrazide (5a) Orange crystals from 1,4-dioxan, yield (1.43 g, 80 %), m.p. 133–135 °C, IR (KBr) υ max cm^{-1}: 3473–3380 (NH, NH_2), 3055 (CH, aromatic), 2256 (CN), 1689 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 8.36 (*s*, 1H, D_2O exchangeable, NH), 8.11, 7.86 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 5.19 (*s*, 1H, H-8), 4.54 (*s*, 1H, H-3), 3.84 (*s*, 2H, CH_2), 2.89, 2.27 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): 172.8 (C-5), 166.3 (C=O), 155.3, 145.9, 135.8, 128.9 (C-4, C-4a, C-8, C-8a), 116.8 (CN), 120.8, 105.6 (C-2, C-3), 34.8 (C-6), 29.3 (CH_2), 19.4 (C-7). Anal calculated for $C_{12}H_{13}N_5O_2$: C, 55.59; H, 5.05; N, 27.01. Found: C, 55.38; H, 5.29; N, 26.83. MS: m/e 259 (M^+, 48%).

N′-(4-Amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)-2-cyanoacetohydrazide (5b) Orange crystals from 1,4-dioxan, yield (1.89 g, 73%), m.p 184–187 °C, IR (KBr) υ max cm^{-1}: 3562–3358 (OH, NH, NH_2), 3055 (CH, aromatic), 2255 (CN), 1688 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 9.69$ (*s*, 1H, D_2O exchangeable, OH), 8.34 (*s*, 1H, D_2O exchangeable, NH), 7.89 (*s*, 2H, D_2O exchangeable, NH_2), 5.14 (*s*, 1H, H-8), 4.58 (*s*, 1H, H-3), 3.83 (*s*, 2H, CH_2), 2.29, 2.85 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): 172.5 (C-5), 166.8 (C=O), 155.1, 145.5, 135.4, 128.7 (C-4, C-4a, C-8, C-8a), 120.8, 105.6 (C-2, C-3), 116.7 (CN), 34.3 (C-6), 29.5 (CH_2), 19.8 (C-7). Anal. calculated for $C_{12}H_{12}N_4O_3$: C, 55.38; H, 4.65; N, 21.53. Found: C, 55.46; H, 4.70; N, 21.79. MS: m/e 260 (M^+, 36%).

General procedure for the synthesis of the thiophene derivatives 6a-d

To a solution of either of compound **5a** (2.59 g, 0.01 mol) or **5b** (2.60 g, 0.01 mol) in 1,4-dioxan (40 mL) containing triethylamine (1.0 mL), either of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) and elemental sulfur (0.32 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 3 h then poured onto ice/water containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

3,5-Diamino-4-cyano-N′-(2,4-diamino-6,7-dihydro-5H-chromen-5-ylidene)thiophene-2-carbohydrazide (6a) Orange crystals from acetic acid, yield (2.03 g, 55%), m.p. 211–214 °C, IR (KBr) υ max cm^{-1}: 3464–3358 (NH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d6, 200 MHz): $\delta = 2.28$, 2.86 (2t, 4H, $2CH_2$), 4.56 (*s*, 1H, H-3), 5.16 (*s*, 1H, H-8), 8.13, 7.87, 5.26, 5.04 (4*s*, 8H, D_2O exchangeable, $4NH_2$), 8.42 (*s*, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d6, 75 MHz): 172.8 (C-5), 167.6 (C=O), 138.3, 136.0, 135.6, 130.2 (thiophene C), 155.6, 145.3, 135.4, 128.7 (C-4, C-4a, C-8, C-8a), 120.8, 105.6 (C-2, C-3), 116.8 (CN), 34.7 (C-6), 19.8 (C-7). Anal. calculated for $C_{15}H_{15}N_7O_2S$: C, 50.41; H, 4.23; N, 27.43; S, 8.97. Found: C, 59.28; H, 4.39; N, 27.52; S, 9.16. MS: m/e 357 (M^+, 32%).

3,5-Diamino-N′-(4-amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)-4-cyanothiophene-2-carbohydrazide (6b) Orange crystals from acetic acid, yield (2.43 g, 68%), m.p. 177–180 °C, IR (KBr) υ max cm^{-1}: 3573–3321 (OH, NH, NH_2), 3055 (CH, aromatic), 2221 (CN), 1689 (CO), 1632 (C=C); ^{1}H NMR (DMSO-d6, 200 MHz): $\delta = 9.78$ (*s*, 1H, OH), 8.48 (*s*, 1H, D_2O exchangeable, NH), 8.14, 7.85, 5.28 (3*s*, 6H, D_2O exchangeable, $3NH_2$), 5.19 (*s*, 1H, H-8), 4.58 (*s*, 1H, H-3), 2.88, 2.25 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d6, 75 MHz): 172.6 (C-5), 167.9 (C=O), 138.6, 136.0, 135.2, 130.4 (thiophene C), 155.6, 145.3, 135.6, 128.3, (C-4, C-4a, C-8, C-8a), 120.5, 105.5 (C-2, C-3), 116.9 (CN), 34.9 (C-6), 19.8 (C-7). Anal. calculated for $C_{15}H_{14}N_6O_3S$: C, 50.27; H, 3.94; N, 23.45; S, 8.95. Found: C, 50.42; H, 4.28; N, 23.68; S, 8.62. MS: m/e 358 (M^+, 40%).

Ethyl 2,4-diamino-5-(2-(2,4-diamino-6,7-dihydro-5H-chromen-5-ylidene)hydrazinecarbonyl)thiophene-3-carboxylate (6c) Pale yellow crystals from acetic acid, yield (2.98 g, 74%), m.p. 144–147 °C, IR (KBr) υ max cm^{-1}: 3569–3353 (NH, NH_2), 3055 (CH, aromatic), 1689 (CO), 1632 (C=C); ^{1}H NMR (DMSO-d6, 200 MHz): $\delta = 8.43$ (s, 1H, D_2O exchangeable, NH), 8.16, 7.85, 5.36, 5.27 (4 s, 8H, D_2O exchangeable, $4NH_2$), 5.01 (*s*, 1H, H-8), 4.59 (*s*, 1H, H-3), 4.22 (q, 2H, $J = 6.89$ Hz, CH_2), 2.23, 2.84 (2t, 4H, $2CH_2$), 1.12 (t, 3H, $J = 6.89$ Hz, CH_3); ^{13}C NMR (DMSO-d6, 75 MHz): 172.8 (C-5), 167.7, 164.2 (2C=O), 138.5, 136.2, 135.5, 130.6 (thiophene C), 155.9, 145.8, 135.2, 128.4 (C-4, C-4a, C-8, C-8a), 120.6, 105.53 (C-2, C-3), 52.6 ($O\underline{CH_2}CH_3$), 34.7 (C-6), 19.8 (C-7), 16.8 ($OCH_2\underline{CH_3}$). Anal. calculated for $C_{17}H_{20}N_6O_4S$: C, 50.48; H, 4.98; N, 20.78; S, 7.93. Found: C, 50.68; H, 4.72; N, 20.58; S, 8.26. MS: m/e 404 (M^+, 36%).

Ethyl 2,4-diamino-5-(2-(4-amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)hydrazinecarbonyl)thiophene-3-carboxylate (6d) Pale yellow crystals from acetic acid, yield (2.98 g, 74%), m.p. 144–147 °C, IR (KBr) υ max cm^{-1}: 3569–3353 (NH, NH_2), 3055 (CH, aromatic), 1689 (CO), 1632 (C=C); ^{1}H NMR (DMSO-d6, 200 MHz): $\delta = 9.29$ (*s*, 1H, D_2O exchangeable OH), 8.46 (*s*, 1H, D_2O exchangeable, NH), 8.13, 5.33, 5.29 (3*s*, 6H, D_2O exchangeable, $3NH_2$), 5.04 (*s*, 1H, H-8), 4.57 (*s*, 1H, H-3), 4.24 (q, 2H, $J = 7.29$ Hz, CH_2), 2.88, 2.26 (2t, 4H, $2CH_2$), 1.13 (t, 3H, $J = 7.29$ Hz, CH_3); ^{13}C NMR (DMSO-d6, 75 MHz): 172.8 (C-5), 167.7, 164.2 (2C=O), 138.5, 136.2, 135.5, 130.6 (thiophene C), 155.8, 145.5, 135.5, 128.3, (C-4, C-4a, C-8, C-8a), 120.8, 105.7 (C-2, C-3), 52.4 ($O\underline{CH_2}CH_3$), 34.5 (C-6), 16.6 ($OCH_2\underline{CH_3}$), 19.7 (C-7). Anal. calculated for $C_{17}H_{19}N_5O_5S$: C, 50.36; H, 4.72; N, 17.27; S, 7.91. Found: C, 50.44; H, 4.58; N, 17.41; S, 8.23. MS: m/e 405 (M^+, 42%).

General procedure for the synthesis of the arylhydraone derivatives 8a-f

To a solution of either of compound **5a** (2.59 g, 0.01 mol) or **5b** (2.60 g, 0.01 mol) in ethanol (50 mL) containing sodium acetate (3.5 g), any of the diazonium salts, namely benzenediazonium chloride (0.01 mol), 4-chlorodiazonium chloride (0.01 mol), or 4-methoxydiazonium chloride (0.01 mol) [prepared via the addition of sodium nitrite solution (0.69 g, 0.01 mol) in water (10 mL) to a cold solution (0–5 °C) of the appropriate aromatic amine

dissolved in concentrated hydrochloric acid (10 mL, 18 mol) with continuous stirring], was added with continuous stirring. The whole reaction, in each case, was stirred at room temperature for 2 h and the formed solid product was collected by filtration.

2-(2-(2,4-Diamino-6,7-dihydro-5H-chromen-5-ylidene)hydrazinyl)-2-oxo-N′-phenylacetohydrazonoyl cyanide (8a) Orange crystals from 1,4-dioxan, yield (2.79 g, 77%), m.p. 241–245 °C, IR (KBr) υ max cm^{-1}: 3483–3340 (NH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO-d6, 200 MHz): $\delta = 8.38$, 8.24 (2*s*, 2H, D_2O exchangeable, 2NH), 8.13, 7.86 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 7.38–6.26 (m, 5H, C_6H_5), 5.18 (*s*, 1H, H-8), 4.54 (*s*, 1H, H-3), 2.28, 2.86 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d6, 75 MHz): 178.2, 172.3 (C=N, C-5), 166.7 (C=O), 155.5, 145.9, 135.5, 128.3 (C-4, C-4a, C-8, C-8a), 124.4, 121.8, 120.5, 119.4 (C_6H_5), 116.8 (CN), 120.7, 105.5 (C-2, C-3), 34.9 (C-6), 19.6 (C-7). Anal. calculated for $C_{18}H_{17}N_7O_2$: C, 59.50; H, 4.75; N, 26.98. Found: C, 59.42; H, 4.83; N, 26.71. MS: m/e 363 (M^+, 28%).

2-(2-(4-amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene) hydrazinyl)-2-oxo-N′-phenylacetohydrazonoyl cyanide (8b) Orange crystals from 1,4-dioxan, yield (2.80 g, 77%), m.p. 188–190 °C, IR (KBr) υ max cm^{-1}: 3469–3352 (NH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1689 (CO), 1630 (C=C); ^1H NMR (DMSO-d6, 200 MHz): $\delta = 9.59$ (*s*, 1H, D_2O exchangeable, OH), 8.30, 8.22 (2*s*, 2H, D_2O exchangeable, 2NH), 7.83 (*s*, 2H, D_2O exchangeable, NH_2), 7.40–6.23 (m, 5H, C_6H_5), 4.56 (*s*, 1H, H-3), 5.16 (*s*, 1H, H-8), 2.83, 2.25 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d6, 75 MHz): 178.1, 172.6 (C=N, C-5), 166.4 (C=O), 155.2, 145.9, 135.6, 128.1 (C-4, C-4a, C-8, C-8a), 128.2, 125.8, 122.1, 120.8 (C_6H_4), 116.8 (CN), 120.7, 105.8 (C-2, C-3), 34.9 (C-6), 19.4 (C-7). Anal. calculated for $C_{18}H_{16}N_6O_3$: C, 59.34; H, 4.43; N, 23.07. Found: C, 59.51; H, 4.58; N, 22.83. MS: m/e 364 (M^+, 52%).

N′-(4-chlorophenyl)-2-(2-(2,4-diamino-6,7-dihydro-5H-chromen-5-ylidene)hydrazinyl)-2-oxoacetohydrazonoyl cyanide (8c) Orange crystals from 1,4-dioxan, yield (2.62 g, 66%), m.p. 177–180 °C, IR (KBr) υ max cm^{-1}: 3474–3358 (NH, NH_2), 3055 (CH, aromatic), 2221 (CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO-d6, 200 MHz): $\delta = 8.38$, 8.22 (2 s, 2H, D_2O exchangeable, 2NH), 8.15, 7.83 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 7.49–6.24 (m, 4H, C_6H_4), 5.15 (*s*, 1H, H-8), 4.53 (*s*, 1H, H-3), 2.27, 2.88 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d6, 75 MHz): 178.6, 172.2 (C=N, C-5), 166.8 (C=O), 155.5, 145.6, 135.8, 128.2 (C-4, C-4a, C-8, C-8a), 127.8, 124.6, 120.7, 119.7 (C_6H_4), 116.6 (CN), 120.6, 105.5 (C-2, C-3), 34.3 (C-6), 19.8 (C-7). Anal. calculated for $C_{18}H_{16}ClN_7O_2$: C, 54.34; H, 4.05; N, 24.65. Found: C, 54.42; H, 4.29; N, 24.55. MS: m/e 397 (M^+, 30%).

2-(2-(4-Amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene) hydrazinyl)-N′-(4-chlorophenyl)-2-oxoacetohydrazonoyl cyanide (8d) Orange crystals from 1,4-dioxan, yield (2.80 g, 60%), m.p. 220–223 °C, IR (KBr) υ max cm^{-1}: 3580–3339 (OH, NH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO-d6, 200 MHz): $\delta =$ 9.62 (*s*, 1H, D_2O exchangeable, OH), 8.23, 8.32 (2*s*, 2H, D_2O exchangeable, 2NH), 7.86 (*s*, 2H, D_2O exchangeable, NH_2), 7.48–6.21 (m, 4H, C_6H_4), 5.18 (s, 1H, H-8), 4.54 (s, 1H, H-3), 2.85, 2.23 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d6, 75 MHz): 178.4, 172.3 (C=N, C-5), 166.6 (C=O), 155.1, 145.7, 135.8, 128.3 (C-4, C-4a, C-8, C-8a), 128.2, 123.6, 121.6, 120.4 (C_6H_4), 116.9 (CN), 120.7, 105.8 (C-2, C-3), 34.5 (C-6), 19.6 (C-7). Anal. calculated for $C_{18}H_{15}ClN_6O_3$: C, 54.21; H, 3.79; N, 21.07. Found: C, 54.33; H, 4.08; N, 21.24. MS: m/e 398 (M^+, 30%).

2-(2-(2,4-Diamino-6,7-dihydro-5H-chromen-5-ylidene) hydrazinyl)-N′-(4-methoxyphenyl)-2-oxoacetohydrazonoyl cyanide (8e) Pale brown crystals from 1,4-dioxan, yield (2.79 g, 71%), m.p. 266–268 °C, IR (KBr) υ max cm^{-1}: 3459–3328 (NH, NH_2), 3054 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO-d6, 200 MHz): $\delta = 8.34$, 8.25 (2*s*, 2H, D_2O exchangeable, 2NH), 8.19, 7.88 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 7.46–6.26 (m, 4H, C_6H_4), 5.12 (*s*, 1H, H-8), 4.55 (*s*, 1H, H-3), 3.68 (*s*, 3H, OCH_3), 2.86, 2.24 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d6, 75 MHz): 178.9, 172.4 (C=N, C-5), 166.6 (C=O), 155.3, 145.1, 135.3, 128.5 (C-4, C-4a, C-8, C-8a), 126.3, 123.9, 120.8, 119.3 (C_6H_4), 116.9 (CN), 120.5, 105.6 (C-2, C-3), 50.4 (OCH_3), 34.6 (C-6), 19.6 (C-7). Anal. calculated for $C_{19}H_{19}N_7O_3$: C, 58.01; H, 4.87; N, 24.92. Found: C, 57.82; H, 4.92; N, 24.73. MS: m/e 393 (M^+, 46%).

2-(2-(4-Amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)hydrazinyl)-N′-(4-methoxy-phenyl)-2-oxoacetohydrazonoyl cyanide (8f) Orange crystals from 1,4-dioxan, yield (2.67 g, 68%), m.p. 255–258 °C, IR (KBr) υ max cm^{-1}: 3562–3384 (OH, NH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO-d6, 200 MHz): $\delta = 9.68$ (*s*, 1H, D_2O exchangeable, OH), 8.36, 8.25 (2 s, 2H, D_2O exchangeable, 2NH), 7.88 (s, 2H, D_2O exchangeable, NH_2), 7.46–6.28 (m, 4H, C_6H_4), 5.18 (*s*, 1H, H-8), 4.56 (*s*, 1H, H-3), 3.70 (*s*, 3H, OCH_3), 2.86, 2.21 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d6, 75 MHz): 178.2, 172.1 (C=N, C-5), 166.8 (C=O), 155.7, 145.7, 135.6, 128.1 (C-4, C-4a, C-8, C-8a), 128.1, 123.8, 121.8, 120.6 (C_6H_4), 116.6 (CN), 120.9, 105.7 (C-2, C-3), 50.8 (OCH_3), 34.7 (C-6),

19.8 (C-7). Anal. calculated for $C_{19}H_{18}N_6O_4$: C, 57.86; H, 4.60; N, 21.31. Found: C, 54.52; H, 4.43; N, 21.41. MS: m/e 394 (M^+, 24%).

General procedure for the synthesis of the coumarin derivatives 10a,b

To a solution of either of compound **5a** (2.59 g, 0.01 mol) or **5b** (2.60 g, 0.01 mol) in 1,4-dioxan (40 mL) containing piperdine (0.50 mL) salicylaldehyde (1.22 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 1 h then was left to cool and the solid product formed was collected by filtration.

N′-(2,4-diamino-6,7-dihydro-5H-chromen-5-ylidene)-2-oxo-2H-chromene-3-carbohydr-azideide (10a) Orange crystals from 1,4-dioxan, yield (2.54 g, 70%), m.p. 183–186 °C, IR (KBr) υ max cm^{-1}: 3473–3358 (NH, NH_2), 3054 (CH, aromatic), 1688, 1686 (2CO), 1630 (C=C); ^{1}H NMR (DMSO-d6, 200 MHz): $\delta = 8.26$ (*s*, 1H, D_2O exchangeable, NH), 8.49, 7.82 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 7.48–6.22 (m, 4H, C_6H_4), 6.02 (*s*, 1H, coumarin H-4), 5.14 (*s*, 1H, H-8), 4.53 (*s*, 1H, H-3), 2.88, 2.22 (2t, 4H, $2CH_2$), ^{13}C NMR (DMSO-d6, 75 MHz): 172.8 (C-5), 166.9, 164.3 (2C=O), 155.3, 145.1, 135.3, 128.5 (C-4, C-4a, C-8, C-8a), 128.9, 125.5, 123.8, 122.6 (C_6H_4), 98.3 (coumarin C-4), 120.9, 105.5 (C-2, C-3), 34.2 (C-6), 19.6 (C-7). Anal. calculated for $C_{18}H_{16}N_4O_4$: C, 61.36; H, 4.58; N, 15.90. Found: C, 61.44; H, 4.67; N, 15.63. MS: m/e 352 (M^+, 34%).

N′-(4-amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)-2-oxo-2H-chromene-3-carbohydrazide (10b) Orange crystals from 1,4-dioxan, yield (2.84 g, 78%), m.p. 222–225 °C., IR (KBr) υ max cm^{-1}: 3584-3337 (OH, NH, NH_2), 3054 (CH, aromatic), 1688 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 9.45$ (*s*, 1H, D_2O exchangeable OH), 8.26 (*s*, 1H, D_2O exchangeable, NH), 7.84 (*s*, 2H, D_2O exchangeable, NH_2), 7.48–6.22 (m, 4H, C_6H_4), 6.04 (*s*, 1H, coumarin H-4), 5.15 (*s*, 1H, H-8), 4.50 (*s*, 1H, H-3), 2.86, 2.24 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): 172.5 (C-5), 166.7, 164.3 (2C=O), 155.4, 145.6, 134.1, 128.6 (C-4, C-4a, C-8, C-8a), 128.7, 125.5, 124.2, 122.8 (C_6H_4), 98.1 (coumarin C-4), 120.7, 105.6 (C-2, C-3), 34.3 (C-6), 19.8 (C-7). Anal. calculated for $C_{18}H_{15}N_3O_5$: C, 61.19; H, 4.28; N, 11.89. Found: C, 61.36; H, 4.29; N, 11.73. MS: m/e 353 (M^+, 48%).

General procedure for the synthesis of the cinnamonitrile derivatives 12a-h

To a solution of either of compound **5a** (2.59 g, 0.01 mol) or **5b** (2.60 g, 0.01 mol) in 1,4-dioxan (40 mL) containing piperdine (0.50 mL) any of benzaldehyde (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (1.40 g, 0.01 mol), 4-bromobenzaldehyde (1.84 g, 0.01 mol) or 4-methoxybenzaldehyde (1.36 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 1 h then was left to cool and the formed solid product was collected by filtration.

2-Cyano-N′-(2,4-diamino-6,7-dihydro-5H-chromen-5-ylidene)-3-phenylacrylohydrazide (12a) Orange crystals from 1,4-dioxan, yield (2.35 g, 68%), m.p. 190–192 °C, IR (KBr) υ max cm^{-1}: 3468–3353 (NH, NH_2), 3054 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 8.23$ (*s*, 1H, D_2O exchangeable, NH), 7.83, 8.16 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 7.38–6.26 (m, 5H, C_6H_5), 6.18 (*s*, 1H. CH=C), 5.17 (*s*, 1H, H-8), 4.52 (s, 1H, H-3), 2.83, 2.25 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d6, 75 MHz): 172.6 (C-5), 166.3 (C=O), 155.5, 145.9, 135.5, 128.3, (C-4, C-4a, C-8, C-8a), 126.5, 121.4, 120.1, 119.8 (C_6H_5), 120.9, 105.5 (C-2, C-3), 116.8 (CN), 102.3, 94.2 (CH=C), 34.8 (C-6), 19.3 (C-7). Anal. calculated for $C_{19}H_{17}N_5O_2$: C, 65.69; H, 4.93; N, 20.16. Found: C, 65.42; H, 4.53; N, 20.28. MS: m/e 347 (M^+, 58%).

N′-(4-amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)-2-cyano-3-phenylacrylo-hydrazide (12b) Orange crystals from 1,4-dioxan, yield (2.35 g, 75%), m.p. 190–192 °C, IR (KBr) υ max cm^{-1}: 3568–3353 (NH, NH_2), 3054 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 9.49$ (*s*, 1H, D_2O exchangeable, OH), 8.26 (*s*, 1H, D_2O exchangeable, NH), 7.86 (*s*, 2H, D_2O exchangeable, NH_2), 7.39–6.25 (m, 5H, C_6H_5), 6.17 (s, 1H, CH=C), 5.13 (*s*, 1H, H-8), 4.57 (*s*, 1H, H-3), 2.86, 2.22 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): 172.4 (C-5), 166.8 (C=O), 155.6, 145.3, 135.8, 128.6 (C-4, C-4a, C-8, C-8a), 126.9, 123.2, 120.8, 119.4 (C_6H_5), 116.9 (CN), 120.7, 105.6 (C-2, C-3), 102.6, 94.0 (CH=C), 34.6 (C-6), 19.5 (C-7). Anal. calculated for $C_{19}H_{16}N_4O_3$: C, 65.51; H, 4.63; N, 16.08. Found: C,65.42; H, 4.78; N, 15.85. MS: m/e 348 (M^+, 28%).

3-(4-chlorophenyl)-2-cyano-N′-(2,4-diamino-6,7-dihydro-5H-chromen-5-ylidene)acrylohydrazide (12c) Orange crystals from 1,4-dioxan, yield (2.05 g, 54%), m.p. 158–161 °C, IR (KBr) υ max cm^{-1}: 3475–3342 (NH, NH_2), 3058 (CH, aromatic), 2220 (CN), 1689 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 8.26$ (*s*, 1H, D_2O exchangeable, NH), 8.18, 7.80 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 7.38–6.26 (m, 4H, C_6H_4), 6.18 (*s*, 1H. CH=C), 5.17 (*s*, 1H, H-8), 4.52 (*s*, 1H, H-3), 2.83, 2.25 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): 172.4 (C-5), 166.8 (C=O), 155.9, 145.2, 135.3, 128.6 (C-4, C-4a, C-8, C-8a), 125.8, 124.1, 120.6, 119.5 (C_6H_4), 120.7, 105.5 (C-2, C-3), 116.6 (CN), 102.6, 94.2 (CH=C), 34.8 (C-8), 19.6 (C-7). Anal.

calculated for $C_{19}H_{16}ClN_5O_2$: C, 59.77; H, 4.22; N, 18.34. Found: C,59.63; H, 4.09; N, 18.68. MS: m/e 381 (M^+, 42%).

N′-(4-amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)-3-(4-chlorophenyl)-2-cyanoacrylohydrazide (12d) Yellow crystals from 1,4-dioxan, yield (2.01 g, 75%), m.p. 212–215 °C, IR (KBr) υ max cm^{-1}: 3528–3342 (OH, NH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 9.46$ (*s*, 1H, D_2O exchangeable, OH), 8.23 (*s*, 1H, D_2O exchangeable, NH), 7.83 (*s*, 2H, D_2O exchangeable, NH_2), 7.49–6.22 (m, 4H, C_6H_4), 6.19 (s, 1H. CH=C), 5.12 (*s*, 1H, H-8), 4.55 (*s*, 1H, H-3), 2.88, 2.20 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): 172.8 (C-5), 166.6 (C=O), 155.9, 143.8, 134.5, 128.4 (C-4, C-4a, C-8, C-8a), 126.3, 124.1, 123.6, 121.8 (C_6H_4), 120.7, 105.6 (C-2, C-3), 116.7 (CN), 102.4, 94.2 (CH=C), 34.3 (C-6), 19.8 (C-7).Anal. calculated for $C_{19}H_{15}ClN_4O_3$: C, 59.61; H, 3.95; N, 14.64. Found: C, 59.48; H, 4.15; N, 14.80. MS: m/e 382 (M^+, 20%).

N′-3-(4-bromophenyl)-2-cyano-N′-(2,4-diamino-6,7-dihydro-5H-chromen-5-ylidene)acrylohydrazide (12e) Orange crystals from 1,4-dioxan, yield (2.89 g, 68%), m.p. 199–203 °C, IR (KBr) υ max cm^{-1}: 3487–3352 (NH, NH_2), 3054 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 8.26$ (*s*, 1H, D_2O exchangeable, NH), 8.18, 7.80 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 7.47–6.26 (m, 4H, C_6H_4), 6.17 (*s*, 1H. CH=C), 5.13 (*s*, 1H, H-8), 4.54 (*s*, 1H, H-3), 2.89, 2.25 (2t, 4H, $2CH_2$), ^{13}C NMR (DMSO-d_6, 75 MHz): 172.3 (C-5), 166.4 (C=O), 155.6, 143.2, 134.5, 128.1 (C-4, C-4a, C-8, C-8a), 127.6, 123.3, 122.7, 120.6 (C_6H_4), 120.9, 105.5 (C-2, C-3), 116.6 (CN), 102.6, 94.3 (CH=C), 34.1 (C-8), 19.8 (C-7). Anal. calculated for $C_{19}H_{16}BrN_5O_2$: C, 53.54; H, 3.78; N, 16.43. Found: C, 53.28; H, 4.04; N, 16.80. MS: m/e 426 (M^+, 30%).

N′-(4-amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)-3-(4-bromophenyl)-2-cyanoacrylohydrazide (12f) Yellow crystals from 1,4-dioxan, yield (2.98 g, 75%), m.p. 249–253 °C., IR (KBr) υ max cm^{-1}: 3548–3362 (OH, NH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 9.49$ (*s*, 1H, D_2O exchangeable, OH), 8.26 (*s*, 1H, D_2O exchangeable, NH), 7.85 (*s*, 2H, D_2O exchangeable, NH_2), 7.52–6.21 (m, 4H, C_6H_4), 6.16 (s, 1H. CH=C), 5.10 (*s*, 1H, H-8), 4.57 (*s*, 1H, H-3), 2.85, 2.23 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d6, 75 MHz): 172.5 (C-5), 166.2 (C=O), 155.3, 143.2, 134.7, 128.2 (C-4, C-4a, C-8, C-8a), 127.7, 125.3, 122.8, 121.3 (C_6H_4), 116.5 (CN), 120.8, 105.5 (C-2, C-3), 102.6, 94.2 (CH=C), 34.1 (C-6), 19.5 (C-7). Anal. calculated for $C_{19}H_{15}BrN_4O_3$: C, 53.41; H, 3.54; N, 13.11. Found: C, 53.29; H, 3.68; N, 12.94. MS: m/e 427 (M^+, 32%).

N′-2-cyano-N′-(2,4-diamino-6,7-dihydro-5H-chromen-5-ylidene)-3-(4-methoxyphenyl)acrylohydrazide (12g) Yellowish brown crystals from 1,4-dioxan, yield (2.78 g, 74%), m.p. 155–157 °C, IR (KBr) υ max cm^{-1}: 3457–3383 (NH, NH_2), 3054 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 8.27$ (*s*, 1H, D_2O exchangeable, NH), 8.15, 7.82 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 7.49–6.23 (m, 4H, C_6H_4), 6.14 (s, 1H. CH=C), 5.16 (*s*, 1H, H-8), 4.53 (*s*, 1H, H-3), 3.68 (*s*, 3H, OCH_3), 2.89, 2.22 (2t, 4H, $2CH_2$), ^{13}C NMR (DMSO-d_6, 75 MHz): 172.2 (C-5), 166.1 (C=O), 153.4, 142.8, 134.5, 128.3 (C-4, C-4a, C-8, C-8a), 124.6, 123.4, 122.8, 120.6 (C_6H_4), 116.3 (CN), 120.7, 105.5 (C-2, C-3), 102.4, 94.1 (CH=C), 52.8 (OCH_3), 34.5 (C-8), 19.3 (C-7). Anal. calculated for $C_{20}H_{19}N_5O_2$: C, 63.65; H, 5.07; N, 18.56. Found: C, 63.80; H, 4.92; N, 18.73. MS: m/e 377 (M^+, 21%).

N′-(4-amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)-2-cyano-3-(4-methoxyphenyl)-acrylohydrazide (12h) Yellowish brown crystals from 1,4-dioxan, yield (2.78 g, 74%), m.p. 155–157 °C, IR (KBr) υ max cm^{-1}: 3457–3383 (NH, NH_2), 3054 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 9.52$ (*s*, 1H, D_2O exchangeable, OH), 8.27 (*s*, 1H, D_2O exchangeable, NH), 7.82 (s, 2H, D_2O exchangeable, NH_2), 7.46–6.25 (m, 4H, C_6H_4), 6.13 (s, 1H. CH=C), 5.18 (*s*, 1H, H-8), 4.53 (s, 1H, H-3), 3.68 (*s*, 3H, OCH_3), 2.89, 2.22 (2t, 4H, $2CH_2$);^{13}C NMR (DMSO-d_6, 75 MHz): 172.6 (C-5), 166.5 (C=O), 153.6, 142.8, 134.5, 127.2 (C-4, C-4a, C-8, C-8a), 126.7, 123.4, 123.5, 120.6 (C_6H_4), 120.8, 105.5 (C-2, C-3), 116.3 (CN), 102.8, 94.3 (CH=C), 52.8 (OCH_3), 34.5 (C-8), 19.3 (C-7). Anal. calculated for $C_{20}H_{18}N_4O_4$: C, 63.48; H, 4.79; N, 14.81. Found: C, 63.62; H, 4.58; N, 15.16. MS: m/e 378 (M^+, 42%).

General procedure for the synthesis of the pyridine derivatives 14a-d

Equimolar amounts of either of compound **5a** (2.59 g, 0.01 mol) or **5b** (2.60 g, 0.01 mol) in 1,4-dioxan (40 mL) containing piperdine (0.50 mL) and either of acetylacetone (1.00 g, 0.01 mol) or ethyl acetoacetate (1.30 g, 0.01 mol) were heated under reflux for 4 h. The solid product, in each case, formed upon pouring onto ice water containing a few drops of hydrochloric acid was collected by filtration.

1-((2,4-Diamino-6,7-dihydro-5H-chromen-5-ylidene)amino)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (14a) Yellowish brown crystals from 1,4-dioxan, yield (2.58 g, 80%), m.p. 148–151 °C, IR (KBr) υ max cm^{-1}: 3462–3380 (NH_2), 3055 (CH, aromatic), 2220 (CN), 1689 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 8.17$, 7.80 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 6.09 (*s*, 1H. Pyridine H-5),

5.15 (*s*, 1H, H-8), 4.51 (*s*, 1H, H-3), 2.66, 2.42 (2*s*, 6H, $2CH_3$), 2.86, 2.24 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): 172.3 (C-5), 168.9 (C=O), 153.6, 142.8, 134.5, 133.4, 130.1, 129.5, 128.2, 127.2 (pyridine, C-4, C-4a, C-8, C-8a), 120.7, 105.5 (C-2, C-3), 116.3 (CN), 94.3, 102.9 (CH=C), 34.5 (C-8), 21.2, 20.8 ($2CH_3$), 19.4 (C-7). Anal. calculated for $C_{17}H_{17}N_5O_2$: C, 63.15; H, 5.30; N, 21.66. Found: C, 63.27; H, 5.28; N, 21.84. MS: m/e 323 (M^+, 36%).

1-((2,4-Diamino-6,7-dihydro-5H-chromen-5-ylidene)amino)-6-hydroxy-4-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (14b) Yellowish brown crystals from 1,4-dioxan, yield (2.50 g, 77%), m.p. 113–116 °C, IR (KBr) υ max cm^{-1}: 3530–3372 (OH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1689 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 10.26$ (*s*, 1H, D_2O exchangeable, OH), 8.19 7.82 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 6.12 (s, 1H. Pyridine H-5), 5.13 (*s*, 1H, H-8), 4.48 (*s*, 1H, H-3), 2.85, 2.24 (2t, 4H, $2CH_2$), 2.42 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): 172.4 (C-5), 168.7 (C=O), 153.1, 142.4, 133.9, 132.8, 130.5, 129.3, 128.6, 126.1 (pyridine, C-4, C-4a, C-8, C-8a), 120.7, 105.8 (C-2, C-3), 116.8 (CN), 102.6, 94.1 (CH=C), 34.6 (C-8), 21.3 (CH_3), 19.6 (C-7). Anal. calculated for $C_{17}H_{16}N_4O_3$: C, 59.07; H, 4.65; N, 21.53. Found: C, 59.25; H, 4.38; N, 21.48. MS: m/e 325 (M^+, 42%).

1-((4-Amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)amino)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (14c) Yellowish brown crystals from 1,4-dioxan, yield (2.10 g, 65%), m.p. 210–213 °C, IR (KBr) υ max cm^{-1}: 3541–3363 (OH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1689 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 10.28$ (*s*, 1H, D_2O exchangeable, OH), 7.86 (*s*, 2H, D_2O exchangeable, NH_2), 6.12 (*s*, 1H. Pyridine H-5), 5.16 (*s*, 1H, H-8), 4.45 (*s*, 1H, H-3), 2.68, 2.46 (2*s*, 6H, $2CH_3$), 2.86, 2.21 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): 172.6 (C-5), 168.5 (C=O), 153.3, 142.8, 134.5, 132.4, 130.9, 128.9, 127.8, 126.5 (pyridine, C-4, C-4a, C-8, C-8a), 120.7, 105.5 (C-2, C-3), 116.9 (CN), 102.7, 94.4, (CH=C), 34.3 (C-8), 21.4, 20.7 ($2CH_3$), 19.4 (C-7). Anal. calculated for $C_{17}H_{16}N_4O_3$: C, 62.95; H, 4.97; N, 17.27. Found: C, 63.13; H, 4.72; N, 17.52. MS: m/e 324 (M^+, 26%).

1-((4-amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)amino)-6-hydroxy-4-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (14d) Yellowish brown crystals from 1,4-dioxan, yield (2.34 g, 72%), m.p. 177–180 °C, IR (KBr) υ max cm^{-1}: 3558–3342 (OH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 10.27$, 9.80 (2*s*, 2H, D_2O exchangeable, 2OH), 7.89 (*s*, 2H, D_2O exchangeable, NH_2), 6.10 (*s*, 1H. Pyridine H-5), 5.12 (*s*, 1H, H-8), 4.48 (*s*, 1H, H-3), 2.48 (*s*, 3H, CH_3), 2.88, 2.20 (2t, 4H, $2CH_2$), ^{13}C NMR (DMSO-d_6, 75 MHz): 172.4 (C-5), 168.8 (C=O), 154.9, 142.6, 134.4, 131.8, 130.4, 128.8, 127.5, 126.2 (pyridine, C-4, C-4a, C-8, C-8a), 120.7, 105.5 (C-2, C-3), 116.6 (CN), 102.9, 94.2 (CH=C), 34.8 (C-8), 21.5 (CH_3), 19.6 (C-7). Anal. Calculated for $C_{16}H_{14}N_4O_4$: C, 58.89; H, 4.32; N, 17.17. Found: C, 58.72; H, 4.53; N, 17.36. MS: m/e 326 (M^+, 37%).

General procedure for the synthesis of the 2-aminopyridine derivatives 15a-h

To a solution of any of compounds **12a** (3.47 g, 0.01 mol), **12b** (3.48 g, 0.01 mol), **12c** (3.81 g, 0.01 mol), **12d** (3.82 g, 0.01 mol), **12e** (4.26 g, 0.01 mol), **12f** (4.27 g, 0.01 mol), **12g** (3.77 g, 0.01 mol) or **12h** (3.78 g, 0.01 mol) in 1,4-dioxan (40 mL) containing triethylamine (1.0 mL) malononitrile (0.66 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 2 h then was left to cool and the formed solid product, in each case was collected by filtration.

1-((2,4-Diamino-6,7-dihydro-5H-chromen-5-ylidene)amino)-6-hydroxy-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (15a) Yellowish brown crystals from 1,4-dioxan, yield (2.79 g, 68%), m.p. 233–236 °C, IR (KBr) υ max cm^{-1}: 3481–3352 (NH_2), 3055 (CH, aromatic), 2223, 2220 (2CN), 1687 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 8.31$, 7.86 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 7.38- 6.26 (m, 5H, C_6H_5), 5.41 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 5.14 (s, 1H, H-8), 4.43 (s, 1H, H-3), 2.82, 2.25 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): 172.4 (C-5), 168.8 (C=O), 153.7, 141.2, 134.3, 132.6 (C-4, C-4a, C-8, C-8a), 128.4, 125.0, 123.6, 120.4 (C_6H_5), 120.7, 105.5 (C-2, C-3), 116.9, 116.5 (2CN), 102.5, 94.4 (CH=C), 34.6 (C-8), 19.7 (C-7). Anal. calculated for $C_{22}H_{17}N_7O_2$: C, 64.23; H, 4.16; N, 23.83. Found: C, 63.52; H, 4.32; N, 24.03. MS: m/e 411 (M^+, 38%).

6-Amino-1-((4-amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (15b) Pall brown crystals from 1,4-dioxan, yield (3.17 g, 77%), m.p. 195–198 °C, IR (KBr) υ max cm^{-1}: 3456–3339 (NH_2), 3055 (CH, aromatic), 2222, 2220 (2CN), 1689 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 9.27$ (*s*, 1H, D_2O exchangeable, OH), 7.84 (*s*, 2H, D_2O exchangeable, NH_2), 7.39–6.24 (m, 5H, C_6H_5), 5.45 (*s*, 2H, D_2O exchangeable, NH_2), 5.13 (*s*, 1H, H-8), 4.46 (*s*, 1H, H-3), 2.86, 2.23 (2t, 4H, $2CH_2$), ^{13}C NMR (DMSO-d_6, 75 MHz): 172.7 (C-5), 168.5 (C=O), 152.9, 140.5, 133.1, 132.8 (C-4, C-4a, C-8, C-8a), 127.3, 123.9, 122.8, 120.3 (C_6H_5), 120.7, 105.5 (C-2, C-3), 116.8, 116.7 (2CN), 94.3, 102.7 (CH=C), 34.8 (C-8), 19.5 (C-7). Anal. calculated for $C_{22}H_{16}N_6O_3$: C, 64.07; H, 3.91; N, 20.38. Found: C, 63.83; H, 4.18; N, 20.27. MS: m/e 412 (M^+, 58%).

6-Amino-4-(4-chlorophenyl)-1-((2,4-diamino-6,7-dihydro-5H-chromen-5-ylidene)amino)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (15c) Yellow crystals from 1,4-dioxan, yield (3.11 g, 70%), m.p. 168–170 °C, IR (KBr) υ max cm^{-1}: 3458–3326 (NH_2), 3055 (CH, aromatic), 2222, 2220 (2CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 8.35$, 7.83 (2s, 4H, D_2O exchangeable, $2NH_2$), 7.42–6.22 (m, 4H, C_6H_4), 5.44 (*s*, 2H, D_2O exchangeable, NH_2), 5.17 (*s*, 1H, H-8), 4.42 (*s*, 1H, H-3), 2.86, 2.23 (2t, 4H, $2CH_2$), ^{13}C NMR (DMSO-d_6, 75 MHz): 172.2 (C-5), 168.7 (C=O), 153.9, 141.6, 133.2, 132.8 (C-4, C-4a, C-8, C-8a), 130.8, 124.2, 123.3, 120.6 (C_6H_4), 120.3, 105.8 (C-2, C-3), 116.9, 116.6 (2CN), 102.8, 94.6 (CH=C), 34.3 (C-8), 19.4 (C-7). Anal. calculated for $C_{22}H_{16}ClN_7O_2$: C, 59.26; H, 3.62; N, 21.99. Found: C, 59.39; H, 3.80; N, 22.07. MS: m/e 445 (M^+, 42%).

6-Amino-1-((4-amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)amino)-4-(4-chlorophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (15d) Pall brown crystals from 1,4-dioxan, yield (3.12 g, 70%), m.p. 211–213 °C, IR (KBr) υ max cm^{-1}: 3528–3341 (NH_2), 3055 (CH, aromatic), 2223, 2220 (2CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 9.29$ (*s*, 1H, D_2O exchangeable, OH), 7.86 7.46–6.22 (m, 4H, C_6H_4), 5.48 995.16 (*s*, 1H, H-8), 4.43 (*s*, 1H, H-3), 2.89, 2.21 (2t, 4H, $2CH_2$), ^{13}C NMR (DMSO-d_6, 75 MHz): 172.3 (C-5), 168.8 (C=O), 152.4, 140.5, 133.6, 132.5 (C-4, C-4a, C-8, C-8a), 127.3, 124.7, 122.5, 120.4 (C_6H_5), 120.9, 105.6 (C-2, C-3), 116.9, 116.3 (2CN), 102.9, 94.2 (CH=C), 34.9 (C-8), 19.6 (C-7). Anal. calculated for $C_{22}H_{15}ClN_6O_3$: C, 59.13; H, 3.38; N, 18.81. Found: C, 59.06; H, 3.52; N, 19.03. MS: m/e 446 (M^+, 32%).

6-Amino-4-(4-bromophenyl)-1-((2,4-diamino-6,7-dihydro-5H-chromen-5-ylidene)amino)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (15e) Yellow crystals from 1,4-dioxan, yield (3.12 g, 64%), m.p. 188–191 °C, IR (KBr) υ max cm^{-1}: 3479–3336 (NH_2), 3055 (CH, aromatic), 2223, 2220 (2CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 8.38$, 7.82 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 6.20–7.46 (m, 4H, C_6H_4), 5.40 (*s*, 2H, D_2O exchangeable, NH_2), 5.16 (*s*, 1H, H-8), 4.45 (*s*, 1H, H-3), 2.89, 2.21 (2t, 4H, $2CH_2$), ^{13}C NMR (DMSO-d_6, 75 MHz): 172.6 (C-5), 168.9 (C=O), 153.2, 132.8, 141.4 134.5 (C-4, C-4a, C-8, C-8a), 129.3, 125.1, 123.7, 122.3 (C_6H_4), 120.8, 105.5 (C-2, C-3), 116.8, 116.3 (2CN), 102.5, 94.3 (CH=C), 34.6 (C-8), 19.2 (C-7). Anal. calculated for $C_{22}H_{16}BrN_7O_2$: C, 53.89; H, 3.29; N, 20.00. Found: C, 53.61; H, 3.46; N, 19.83. MS: m/e 490 (M^+, 30%).

6-Amino-1-((4-amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)amino)-4-(4-bromophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (15f) Pall brown crystals from 1,4-dioxan, yield (2.84 g, 58%), m.p. 166–168 °C., IR (KBr) υ max cm^{-1}: 3551–3374 (NH_2), 3055 (CH, aromatic), 2223, 2220 (2CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 9.27$ (*s*, 1H, D_2O exchangeable, OH), 7.85 (*s*, 2H, D_2O exchangeable, NH_2), 7.48–6.21 (m, 4H, C_6H_4), 5.42 (*s*, 2H, D_2O exchangeable, NH_2), 5.17 (*s*, 1H, H-8), 4.46 (*s*, 1H, H-3), 2.85, 2.23 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): 172.1 (C-5), 168.6 (C=O), 152.3, 140.7, 133.4, 132.2 (C-4, C-4a, C-8, C-8a), 128.1, 125.2, 123.3, 120.8 (C_6H_5), 116.9, 116.5 (2CN), 102.7, 94.4 (CH=C), 120.6, 105.5 (C-2, C-3), 34.5 (C-8), 19.4 (C-7). Anal. calculated for $C_{22}H_{15}BrN_6O_3$: C, 53.78; H, 3.08; N, 17.11. Found: C, 53.62; H, 3.24; N, 17.26. MS: m/e 491 (M^+, 27%).

6-Amino-1-((2,4-diamino-6,7-dihydro-5H-chromen-5-ylidene)amino)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (15g) Pale yellow crystals from 1,4-dioxan, yield (3.26 g, 74%), m.p. 160–163 °C, IR (KBr) υ max cm^{-1}: 3492–3375 (NH_2), 3055 (CH, aromatic), 2222, 2220 (2CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 8.38$, 7.82, 5.40 (3 s, 6H, D_2O exchangeable, $3NH_2$), 7.49–6.22 (m, 4H, C_6H_4), 5.16 (*s*, 1H, H-8), 4.43 (*s*, 1H, H-3), 3.68 (*s*, 3H, OCH_3), 2.86, 2.25 (2t, 4H, $2CH_2$), ^{13}C NMR (DMSO-d_6, 75 MHz): 172.2 (C-5), 168.4 (C=O), 153.6, 142.0, 134.7, 132.2 (C-4, C-4a, C-8, C-8a), 127.8, 125.6, 122.4, 120.6 (C_6H_4), 116.8, 116.5 (2CN), 102.7, 94.1 (CH=C), 120.9, 105.6 (C-2, C-3), 52.3 (OCH_3), 34.3 (C-8), 19.2 (C-7). Anal. calculated for $C_{23}H_{19}N_7O_2$: C, 62.58; H, 4.34; N, 22.21. Found: C, 62.70; H, 4.51; N, 22.49. MS: m/e 441 (M^+, 30%).

6-Amino-1-((4-amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)amino)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (15h) Pall brown crystals from 1,4-dioxan, yield (3.53 g, 80%), m.p. 176–178 °C, IR (KBr) υ max cm^{-1}: 3581–3348 (NH_2), 3055 (CH, aromatic), 2222, 2220 (2CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 9.24$ (*s*, 1H, D_2O exchangeable, OH), 7.87, 5.45 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 7.49–6.26 (m, 4H, C_6H_4), 5.16 (*s*, 1H, H-8), 4.43 (*s*, 1H, H-3), 3.69 (*s*, 3H, OCH_3), 2.88, 2.22 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): 172.6 (C-5), 168.4 (C=O), 152.6, 141.5, 133.7, 131.8 (C-4, C-4a, C-8, C-8a), 127.4, 124.8, 122.6, 120.3 (C_6H_5), 116.8, 116.7 (2CN), 102.2, 94.6 (CH=C), 120.7, 105.5 (C-2, C-3), 52.3 (OCH_3), 34.8 (C-8), 19.1 (C-7). Anal. calculated for $C_{23}H_{18}N_6O_4$: C,

62.44; H, 4.10; N, 19.00. Found: C, 62.53; H, 3.82; N, 18.94. MS: m/e 442 (M^+, 60%).

General procedure for the synthesis of the 2-hydroxypyridine derivatives 16a-h

To a solution of any of compounds **12a** (3.47 g, 0.01 mol), **12b** (3.48 g, 0.01 mol), **12c** (3.81 g, 0.01 mol), **12d** (3.82 g, 0.01 mol), **12e** (4.26 g, 0.01 mol), **12f** (4.27 g, 0.01 mol), **12g** (3.77 g, 0.01 mol), or **12h** (3.78 g, 0.01 mol) in 1,4-dioxan (40 mL) containing triethylamine (1.0 mL) ethyl cyanoacetate (1.07 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 2 h then was left to cool and the formed solid product, in each case was collected by filtration.

1-((2,4-Diamino-6,7-dihydro-5H-chromen-5-ylidene)amino)-6-hydroxy-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (16a) Yellowish brown crystals from 1,4-dioxan, yield (3.00 g, 73%), m.p. 158–161 °C, IR (KBr) υ max cm^{-1}: 3541–3338 (NH_2), 3055 (CH, aromatic), 2222, 2220 (2CN), 1688 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 10.28$ (*s*, 1H, D_2O exchangeable, OH), 7.80, 7.43 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 7.39–6.23 (m, 5H, C_6H_5), 5.18 (*s*, 1H, H-8), 4.45 (*s*, 1H, H-3), 2.86, 2.23 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): 172.2 (C-5), 168.6 (C=O), 153.3, 141.5, 134.1, 132.3 (C-4, C-4a, C-8, C-8a), 126.7, 124.2, 122.3, 120.8 (C_6H_5), 116.8, 116.4 (2CN), 102.2, 94.6 (CH=C), 120.7, 105.5 (C-2, C-3), 34.9 (C-8), 19.3 (C-7). Anal. calculated for $C_{22}H_{16}N_6O_3$: C, 64.07; H, 3.91; N, 20.38. Found: C, 63.82; H, 4.12; N, 20.58. MS: m/e 412 (M^+, 55%).

1-((4-Amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)amino)-6-hydroxy-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (16b) Brown crystals from 1,4-dioxan, yield (2.80 g, 68%), m.p. 144–147 °C, IR (KBr) υ max cm^{-1}: 3531–3323 (NH_2), 3055 (CH, aromatic), 2224, 2220 (2CN), 1688 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 10.25$, 9.24 (2*s*, 2H, D_2O exchangeable, 2OH), 7.41–6.26 (m, 5H, C_6H_5), 5.41 (*s*, 2H, D_2O exchangeable, NH_2), 5.16 (*s*, 1H, H-8), 4.46 (*s*, 1H, H-3), 2.84, 2.21 (2t, 4H, $2CH_2$), ^{13}C NMR (DMSO-d_6, 75 MHz): 172.2 (C-5), 168.1 (C=O), 152.3, 139.8, 132.9, 132.3 (C-4, C-4a, C-8, C-8a), 125.8, 122.9, 122.1, 120.6 (C_6H_5), 116.9, 116.5 (2CN), 102.4, 94.0 (CH=C), 120.5, 105.5 (C-2, C-3), 34.3 (C-8), 19.1 (C-7). Anal. calculated for $C_{22}H_{15}N_5O_4$: C, 63.92; H, 3.66; N, 16.94. Found: C, 63.62; H, 3.80; N, 17.15. MS: m/e 413 (M^+, 36%).

4-(4-Chlorophenyl)-1-((2,4-diamino-6,7-dihydro-5H-chromen-5-ylidene)amino)-6-hydroxy-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (16c) Yellow crystals from 1,4-dioxan, yield (3.47 g, 78%), m.p. 210–213 °C, IR (KBr) υ max cm^{-1}: 3532–3363 (NH_2), 3055 (CH, aromatic), 2223, 2220 (2CN), 1688 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 10.20$ (*s*, 1H, D_2O exchangeable OH), 7.86, 5.41 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 7.48–6.23 (m, 4H, C_6H_4), 5.14 (*s*, 1H, H-8), 4.46 (*s*, 1H, H-3), 2.88, 2.25 (2t, 4H, $2CH_2$), ^{13}C NMR (DMSO-d_6, 75 MHz): 172.1 (C-5), 168.9 (C=O), 152.4, 140.5, 134.7, 132.2 (C-4, C-4a, C-8, C-8a), 130.3, 126.1, 124.5, 120.2 (C_6H_4), 116.9, 116.3 (2CN), 102.4, 94.2 (CH=C), 120.7, 105.6 (C-2, C-3), 34.6 (C-8), 19.2 (C-7). Anal. calculated for $C_{22}H_{15}ClN_6O_3$: C, 59.13; H, 3.38; N, 18.81. Found: C, 59.25; H, 3.46; N, 19.01. MS: m/e 446 (M^+, 28%).

1-((4-Amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)amino)-4-(4-chlorophenyl)-6-hydroxy-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (16d) Pall brown crystals from 1,4-dioxan, yield (3.03 g, 68%), m.p. 178–180 °C, IR (KBr) υ max cm^{-1}: 3548–3367 (NH_2), 3055 (CH, aromatic), 2222, 2220 (2CN), 1689 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 10.22$, 9.31 (2*s*, 2H, D_2O exchangeable, 2OH), 5.45 (*s*, 2H, D_2O exchangeable, NH_2), 7.49–6.23 (m, 4H, C_6H_4), 5.12 (*s*, 1H, H-8), 4.46 (*s*, 1H, H-3), 2.73, 2.25 (2t, 4H, $2CH_2$), ^{13}C NMR (DMSO-d_6, 75 MHz): 172.7 (C-5), 168.6 (C=O), 152.0, 140.2, 133.4, 132.2 (C-4, C-4a, C-8, C-8a), 128.2, 120.2, 125.1, 124.8 (C_6H_5), 116.9, 116.6 (2CN), 94.4, 102.6 (CH=C), 120.6, 105.3 (C-2, C-3), 34.5 (C-8), 19.3 (C-7). Anal. calculated for $C_{22}H_{14}ClN_{65}O_4$: C, 59.00; H, 3.15; N, 15.64. Found: C, 59.19; H, 3.35; N, 15.39. MS: m/e 447 (M^+, 26%).

4-(4-Bromophenyl)-1-((2,4-diamino-6,7-dihydro-5H-chromen-5-ylidene)amino)-6-hydroxy-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (16e) Yellow crystals from 1,4-dioxan, yield (3.57 g, 73%), m.p. 150–153 °C, IR (KBr) υ max cm^{-1}: 3579–3342 (NH_2), 3055 (CH, aromatic), 2223, 2220 (2CN), 1688 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 10.30$ (*s*, 1H, D_2O exchangeable, OH), 7.83, 5.42 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 7.53–6.23 (m, 4H, C_6H_4), 4.48 (*s*, 1H, H-3), 5.19 (*s*, 1H, H-8), 2.23, 2.84 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): 172.6 (C-5), 168.9 (C=O), 153.6, 141.1, 136.6, 135.5 (C-4, C-4a, C-8, C-8a), 128.1, 125.8, 124.4, 122.6 (C_6H_4), 116.9, 116.6 (2CN), 102.8, 94.1 (CH=C), 120.7, 105.8 (C-2, C-3), 34.8 (C-8), 19.3 (C-7). Anal. calculated for $C_{22}H_{15}BrN_6O_3$: C, 53.78; H, 3.08; N, 17.11. Found: C, 53.68; H, 3.25; N, 17.26. MS: m/e 491 (M^+, 38%).

1-((4-Amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene)amino)-4-(4-bromophenyl)-6-hydroxy-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (16f) Pall brown crystals from 1,4-dioxan, yield (3.14 g, 64%), m.p. 177–179 °C, IR (KBr) υ max cm^{-1}: 3561–3359 (NH_2), 3055 (CH, aromatic), 2223, 2220 (2CN), 1688 (CO), 1630 (C=C); ^{1}H NMR

(DMSO-d_6, 200 MHz): $\delta = 10.27$, 9.29 (2*s*, 2H, D_2O exchangeable, 2OH), 5.40 (*s*, 2H, D_2O exchangeable, NH_2), 2.88, 2.25 (2t, 4H, $2CH_2$), 4.41 (*s*, 1H, H-3), 5.14 (*s*, 1H, H-8), 7.53–6.22 (m, 4H, C_6H_4), ^{13}C NMR (DMSO-d_6, 75 MHz): 172.4 (C-5), 168.2 (C=O), 151.8, 140.8, 133.1, 132.7, (C-4, C-4a, C-8, C-8a), 128.5, 125.8, 124.5, 120.3 (C_6H_5), 116.9, 116.6 (2CN), 102.6, 94.2 (CH=C), 120.8, 105.5 (C-2, C-3), 34.2 (C-8), 19.7 (C-7). Anal. calculated for $C_{22}H_{14}BrN_5O_4$: C, 53.68; H, 2.87; N, 14.23. Found: C, 53.49; H, 3.15; N, 14.40. MS: m/e 492 (M^+, 38%).

1-((2,4-Diamino-6,7-dihydro-5H-chromen-5-ylidene) amino)-6-hydroxy-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (16g) Pale yellow crystals from 1,4-dioxan, yield (2.21 g, 50%), m.p. 255–2258 °C, IR (KBr) υ max cm^{-1}: 3547–3328 (NH_2), 3055 (CH, aromatic), 2222, 2220 (2CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 10.22$ (*s*, 1H, D_2O exchangeable, OH), 7.85, 7.48 (2*s*, 4H, D_2O exchangeable, $2NH_2$), 7.51–6.23 (m, 4H, C_6H_4), 5.15 (*s*, 1H, H-8), 4.41 (*s*, 1H, H-3), 3.69 (s, 3H, OCH_3), 2.89, 2.23 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): 172.6 (C-5), 168.8 (C=O), 152.1, 142.2, 133.3, 130.8 (C-4, C-4a, C-8, C-8a), 128.2, 126.4, 123.7, 120.9 (C_6H_4), 116.9, 116.4 (2CN), 102.9, 94.2 (CH=C), 120.5, 105.7 (C-2, C-3), 52.63 (OCH_3), 34.3 (C-8), 19.5 (C-7). Anal. calculated for $C_{23}H_{18}N_6O_4$: C, 62.44; H, 4.10; N, 19.00. Found: C, 62.58; H, 4.30; N, 18.83. MS: m/e 442 (M^+, 28%).

1-((4-Amino-2-hydroxy-6,7-dihydro-5H-chromen-5-ylidene) amino)-6-hydroxy-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (16h) Pall yellow crystals from 1,4-dioxan, yield (3.01 g, 68%), m.p. 203–206 °C, IR (KBr) υ max cm^{-1}: 3565–3331 (NH_2), 3055 (CH, aromatic), 2222, 2220 (2CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 200 MHz): $\delta = 9.27$, 10.31 (2*s*, 2H, D_2O exchangeable, 2OH), 5.43 (*s*, 2H, D_2O exchangeable, NH_2), 7.53–6.24 (m, 4H, C_6H_4), 5.19 (*s*, 1H, H-8), 4.43 (*s*, 1H, H-3), 3.68 (*s*, 3H, OCH_3), 2.85, 2.26 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): 172.9 (C-5), 168.6 (C=O), 152.1, 141.8, 133.5, 131.2 (C-4, C-4a, C-8, C-8a), 128.9, 120.6, 126.3 123.8 (C_6H_5), 116.9, 116.4 (2CN), 102.7, 94.5 (CH=C), 120.6, 105.8 (C-2, C-3), 52.1 (OCH_3), 34.4 (C-8), 19.5 (C-7). Anal. calculated for $C_{23}H_{17}N_5O_5$: C, 62.30; H, 3.86; N, 15.79. Found: C, 62.49; H, 3.42; N, 15.83. MS: m/e 443 (M^+, 60%).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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