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Anticancer activity of new coumarin substituted hydrazide–hydrazone derivatives

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ABSTRACT

Drug resistance is a major impediment for cancer treatment, to overcome it we designed and synthesized sixteen coumarins bearing hydrazide–hydrazone moiety and evaluated them against human drug-resistant pancreatic carcinoma (Panc-1) cells and drug-sensitive (hepatic carcinoma; Hep-G2 and leukemia; CCRF) cell lines *in vitro*. The 6-brominated coumarin hydrazide–hydrazone derivatives (BCHHD) **7c**, **8c** and **10c** were more potent than doxorubicin (DOX) against resistant Panc-1 cells. BCHHD **7c** showed significant cytotoxicity against all tested cells (IC_{50}: 3.60–6.50 μM) on comparison with all other coumarin hydrazide–hydrazone derivatives (CHHD), whereas BCHHD's **8c** and **10c** showed significant antiproliferative activity only against resistant Panc-1 cells with IC_{50} of 2.02 μM and 2.15 μM, respectively. All the investigated BCHHD's were able to activate caspases 3/7 and they could induce apoptosis in resistant Panc-1 cells. Microarray analysis showed that BCHHD **7c** induced the expression of apoptotic- and cell cycle arrest (G2/M)- genes in resistant Panc-1 cells. Moreover, BCHHD **7c** induced the up-regulation of CDKN1A, DDIT4, GDF-15 and down-regulation of CDC2, CDC20, CDK2 genes. Based on our results, we conclude that **7c** could be a potent anticancer drug to overcome drug resistance in cancer and it could be highly beneficial for patients in the clinic.

1. Introduction

Drug resistance constitutes lack of response to many chemically and mechanically unrelated anticancer agents by cancer cells. It is one of the main causes for failure of chemotherapy and can lead to recurrence of disease or even death [1]. Clinical administration of high doses of anticancer drugs to overcome resistance leads to drug-induced toxicities [2]. Hence newer anticancer agents need to be synthesized and tested for its efficacy both *in vitro* and *in vivo* to overcome drug resistance.

The natural and synthetic coumarins attract great attention due to their wide range of biological properties, including anticancer [3], anti-HIV [4], anti-inflammatory [5] and antibacterial [6] activities. Furthermore, their cancer chemopreventive properties have been recently emphasized [3,7]. The apoptosis and differentiation-induced activities of coumarins extend to several different cell line models *in vitro*, and they appear to be the most promising in terms of cancer treatment [8].

Coumarins could exert their anticancer activity by different mechanisms; either by inhibiting the telomerase enzyme [9], inhibiting protein kinase activity and down regulating oncogene expression [10] or by inducing the caspase-9 mediated apoptosis. Additionally, researchers showed that coumarins are able to suppress cancer cell proliferation by arresting cell cycle in G0/G1 [9], G2/M phases [11], and through affecting the p-gp of the cancer cells [12,13]. It was also reported that hydroxycoumarins might exert their anticancer activity by generating free radical species in cancer cells producing oxidative stress leading to pro-apoptic effect [7]. It was proven that the δ-lactone ring of the coumarinic system has a fundamental role in both the generation and stabilization of such species as well as in the pro-apoptotic action of hydroxycoumarins [7]. Moreover, the antiproliferative activity of 7-hydroxycoumarin derivatives could be due to their effect on the mitochondrial thiol compounds of cancer cells [14].

Literature survey revealed that the hydrazide–hydrazone (–CO–NH–N=CH–) moiety has significant role as antitumor

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agent [15–18]. 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 [19]. It was also reported that furan, thiophene, pyrrole and isatin derivatives have cytotoxic activities against several cancer cell lines [18].

Based on the afore-mentioned findings, and in an attempt to find new potent anticancer agents; novel hybrid compounds having coumarin hydrazide–hydrazone backbone have been designed to evaluate their cytotoxic activity against several tumor cell lines (Fig. 1). Moreover, the possible underlying mechanisms of action have been also investigated.

2. Results and discussion

2.1. Chemistry

The synthesis of the target compounds is summarized in Scheme 1. To synthesize the CHHD's **7**–**10**, several 2-cyanoacetohydrazones **2**–**5** were prepared by condensing 2-hetaryl aldehydes with 2-cyanoacetohydrazide (**1**) or by combining isatin with 2-cyanoacetohydrazide (**1**) [20–23]. The chemical structure of the newly synthesized *N*′-((1*H*-pyrrol-2-yl) methylene)-2-cyanoacetohydrazide (**4**) was established on the basis of analytical and spectral data. Its IR spectrum displayed absorption bands at 3325, 3265, 2260, 1665, 1635 cm^{-1} due to the presence of two NH, CN, amidic C=O, and C=N groups, respectively. The ^1H NMR spectrum of **4** revealed four singlet signals at δ 3.67, 6.75, 8.43, 8.93 ppm assigned to the methylene group, amidic NH, azomethine CH=N, and pyrrole NH, besides three aromatic protons centered around 7.22 ppm due to the pyrrole ring residue. The Knoevenagel condensation of 2-hydroxybenzaldehydes **6a**–**d** with 2-cyanoacetohydrazones **2**–**5** in refluxing ethanol containing a catalytic amount of piperidine followed by treating the product with dilute HCl afforded CHHD's **7**–**10** in high chemical yield. The structures of the latter CHHD's **7**–**10** were elucidated on the basis of their spectra (IR, ^1H NMR, ^{13}C NMR, and MS) and elemental analyses. For example, their IR spectra showed the absence of CN absorption bands present in IR spectrum of their precursors **2**–**5**, and revealed the presence of coumarin C=O absorption bands in the region 1679–1690 cm^{-1}. Their ^1H NMR spectra, in addition to the expected signals due to the aromatic protons, exhibit three singlet signals near δ 8.54, 8.63, and 9.23 ppm assignable to the protons of azomethine (CH=N), chromene-H_4 and amidic NH, respectively. Analysis of the ^{13}C NMR spectra of the coumarins revealed the presence of a new signal for the carbonyl group of coumarin at approximately 160 ppm. CHHD's **7**–**10** exhibited peaks corresponding to their molecular ions $[M + H]^+$ in the ESI-mass spectrum.

2.2. Biology

2.2.1. Antitumor evaluation

Currently, we are studying the synthesis and antiproliferative activity of compounds having coumarin and/or hydrazide–hydrazone pharmacophores. The initial antiproliferative screening showed that compounds having coumarin hydrazide–hydrazone pharmacophore were more potent than compounds having coumarin or hydrazide–hydrazone pharmacophores (data not shown).

All CHHD's **7**–**10** were screened for their *in vitro* cytotoxic and growth inhibitory activities against three different tumor types, namely resistant Panc-1, Hep-G2 and CCRF, in comparison with the activity of the known anticancer reference drug DOX. The cytotoxic activities of our tested compounds were expressed as IC_{50} μM value (the dose that reduces survival to 50%) (Table 1).

Regarding the activity of CHHD's **7**–**10** against resistant Panc-1 cell line, the results in Table 1 showed that BCHHD's **8c** and **10c** possessed the highest degree of cytotoxicity. They were three times more active than DOX (Fig. 2). On the other hand, BCHHD **7c** was almost equipotent to DOX (IC_{50}: 6.50 μM). CHHD's **8a** (IC_{50}: 7.87 μM) and **7b** (IC_{50}: 8.75 μM) were quite less potent than DOX. The activity of the tested CHHD's against resistant Panc-1 cell line had the following descending order: (**8c** > **10c** > **7c** > **8a** > **7b** > **8b** > **7a** > **10a** > **10b** > **9c** > **9a** > **7d** > **8d** > **9b** > **10d** > **9d**).

Concerning Hep-G2 cell, it is evident that all of the tested CHHD's **7**–**10** showed antitumor activities with IC_{50} values ranging from 3.60 to 40.30 μM. Interestingly, BCHHD's **7c** (IC_{50}: 3.60 μM) and **9c** (IC_{50}: 4.16 μM) were more potent than reference drug DOX (IC_{50}: 5.43 μM) (Fig. 3). On the other hand, the CHHD **9b** had moderate activity (IC_{50}: 9.29 μM). The activity of the tested compounds against Hep-G2 cell line had the following descending order: (**7c** > **9c** > **9b** > **10c** > **9d** > **7b** > **8b** > **8d** > **9a** > **10d** > **7a** > **10a** > **10b** > **7d** > **8c** > **8a**).

The CHHD's **7**–**10** were also screened against CCRF cancer cell line. The results showed that most of the tested CHHD's were weaker than DOX and BCHHD **7c** was the most active. Although, **7c** is fairly less potent than DOX against CCRF cell line (IC_{50} 5.15 μM *vs* 1.05 μM respectively), it is still promising antitumor agent against leukemia since the chemotherapeutic DOX has known cardiotoxic side effect [24].

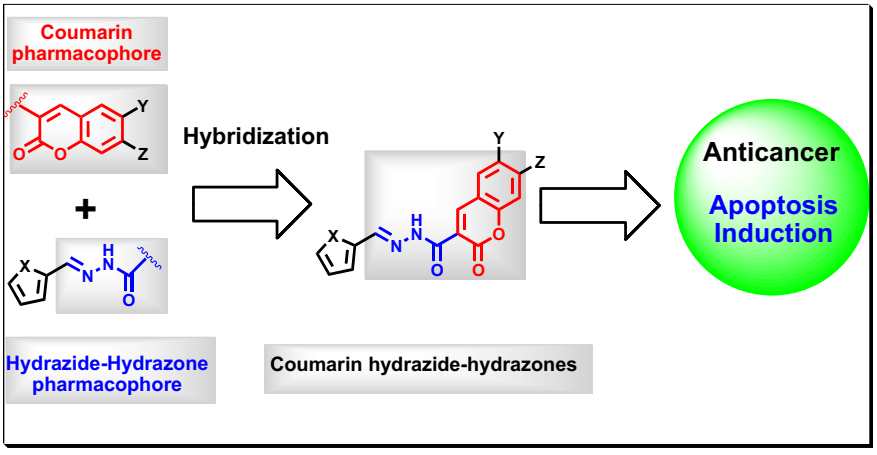

Fig. 1. Design of CHHD's as anticancer agents.