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Short communication

Anticancer and radiosensitizing evaluation of some new pyranothiazole-Schiff bases bearing the biologically active sulfonamide moiety

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ABSTRACT

The present work reports the synthesis of some new Schiff bases, 5-(substituted benzylideneamino)-6-cyano-7*H*-7-(4-methoxyphenyl)-2-(4-sulphamoylphenylamino) pyrano[2,3-*d*]thiazole (**5**–**15**). The design of the structures of these compounds complies with the general pharmacophoric requirements for CA inhibiting anticancer drugs. The newly synthesized compounds were evaluated for their *in vitro* anticancer activity against human breast cancer cell line (MCF7). Most of the screened compounds showed interesting cytotoxic activities compared to doxorubicin as a reference drug. Compounds **4**, **6**–**8** and **11** (IC_{50}: 27.51, 10.25, 9.55, 9.39 and 9.70 μM, respectively) exhibited higher cytotoxic activities than the reference drug doxorubicin (IC_{50}: 32.00 μM). Additionally, the previously mentioned compounds were evaluated again for their ability to enhance the cell killing effect of γ-radiation.

1. Introduction

Sulfonamides are currently an important group of organic compounds that possess several types of biological activities [1–4] including anticancer activity [5–10]. The anticancer activity of sulfonamides was found to take place through a variety of mechanisms such as cell cycle perturbation in the G1phase, disruption of microtubule assembly, angiogenesis inhibition, and functional suppression of the transcriptional activator NF-Y, and carbonic anhydrase inhibition [11–14] which was reported to be the most prominent mechanism [15]. Carbonic anhydrases (CAs) are zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide to give bicarbonate and a proton. CAs are involved in pH regulation, secretion of electrolytes, respiration [16,17], biosynthetic reactions which require CO_2/bicarbonate as substrate such as gluconeogenesis, lipogenesis, ureagenesis, and pyrimidines synthesis [18]. The catalytic domain of CAs contains an active site Zn^{2+}; a strong Lewis acid that binds to and activates a substrate H_2O molecule to catalyze the reversible hydration reaction of carbon dioxide. The hydration of CO_2 does not proceed at an appreciable rate under physiological conditions in the absence of CA enzymes [19]. CA inhibition has been found to have an important role in cancer treatment through reducing the provision of bicarbonate for the synthesis of nucleotides and other cell components such as membrane lipids [20]. E7070 II (Fig. 1) is an example of carbonic anhydrase inhibitors.

Furthermore, among the compounds that had shown significant anticancer activity were the thiazole derivatives [21–26]. Also, combination of several pyranothiazole with sulfonamide moiety was reported to have significant anticancer activity [27–29]. In addition, Schiff bases are important class of compounds in medicinal and pharmaceutical field that show vast biological applications including antitumor activity [30,31]. In the light of these facts, and in a hope to obtain some novel compounds with significant anticancer activity, this work reports the synthesis of a novel series of Schiff base derivatives containing pyranothiazole bearing a sulfonamide moiety and the testing of these compounds for their *in vitro* anticancer activity. We also aimed to evaluate the most potent compounds for their *in vitro* anticancer activity in combination with γ-radiation, to evaluate their ability to enhance the cytotoxic activity of γ-radiation.

2. Result and discussion

2.1. Chemistry

The newly synthesized compounds were obtained starting with 4-(4-oxo-4,5-dihydrothiazol-2-ylamino)benzenesulfonamide

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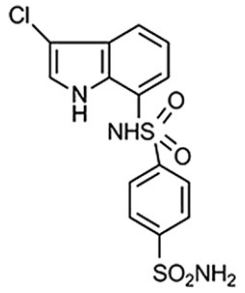

Fig. 1. Compound E7070, a sulfonamide compound in advanced clinical trials as anticancer agent.

3, which was prepared according to the reported procedure [32], by refluxing the *N*-chloroacetyl derivative **2** with ammonium thiocyanate in ethanol, through intramolecular cyclization and elimination of 1 mol of ammonium chloride. Treatment of compound **3** with 2-(4-chlorobenzylidene) malononitrile in ethanol containing a catalytic amount of piperidine, as a base catalyst, resulted in intramolecular cyclization affording the 4-(5-amino-6-cyano-7-(2-methoxyphenyl)-7*H*-pyrano[2,3-*d*]thiazol-2-ylamino) benzenesulfonamide **4**. The structure of compound **4** was confirmed by its microanalytical and spectral data; where the IR spectrum showed bands at 2187 attributed to the presence of (C≡N) group and the ^{1}H NMR spectrum showed a peak at δ 4.70 ppm attributed to the presence of C-4 pyran H (Scheme 1).

Treatment of compound **4** with aromatic aldehydes in acetic acid yielded the corresponding Schiff bases **5**–**15**. The structures of synthesized compounds **5**–**15** were supported from their microanalytical and spectral data. The ^{1}H NMR spectra of compounds **5**–**15** displayed the disappearance of NH_2 absorption of the precursor **4** and the presence of singlets at the range δ 8.23–8.79 ppm for (N=CH–), in addition to the presence of the expected methoxyphenyl and sulphamoylphenyl protons signals together with other signals assigned to N-substituted benzylidene groups (Scheme 2).

2.2. In vitro anticancer screening

The newly synthesized compounds were evaluated for their *in vitro* cytotoxic activity against human breast cancer cell line, MCF7. Doxorubicin, which is one of the most effective anticancer agents, was used as the reference drug in this study. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of breast cancer cell line (MCF7). The response parameter calculated was the IC_{50} value, which corresponds to the concentration required for 50% inhibition of cell viability. Table 1 shows the *in vitro* cytotoxic activity of the synthesized compounds. Most of the synthesized compounds exhibited significant activity compared to the reference drug.

From the analysis of Table 1, it was found that all compounds showed significant antitumor activities. Interestingly, compounds **6**–**8** and **11** (IC_{50}: 10.25, 9.55, 9.39 and 9.70 μM, respectively) exhibited 3.1–3.4 fold more potent antitumor activity than doxorubicin (IC_{50}: 32.00 μM). Further, the key compound **4** (IC_{50}: 27.5 1 μM) showed better cytotoxicity than doxorubicin. Compounds **9**, **10** and **13** (IC_{50}: 28.85, 23.48, 28.85 μM) showed also better activity than doxorubicin. While compound **5**, **12**, **14** and **15** showed slightly lower activity than that of doxorubicin.

2.3. Radiosensitizing activity

The rationale for combining chemotherapy and radiotherapy is based mainly on two ideas, one being spatial cooperation, which is effective if chemotherapy is sufficiently active to eradicate subclinical metastases and if the primary local tumor is effectively treated by radiotherapy. In this regard, no interaction between radiotherapy and chemotherapy is required. The other idea is the enhancement of radiation effects. Cytotoxic agents can enhance radiation effects by direct enhancement of the initial radiation damage by incorporating drugs into DNA, inhibiting cellular repair, accumulating cells in a radiosensitive phase or eliminating radioresistant phase cells, eliminating hypoxic cells or inhibiting the accelerated repopulation of tumor cells. Virtually, all chemotherapeutic agents have the ability to sensitize cancer cells to the lethal effects of ionizing radiation [33]. The ability of the most five active compounds **4**, **6**–**8** and **11** to enhance the cell killing effect of γ-irradiation was studied. Compounds **4**, **6**–**8** and **11** exhibited (IC_{50}: 27.51, 10.25, 9.55, 9.39 and 9.70 μM, respectively) when used alone (Table 1). The IC_{50} values were decreased to 14.76, 9.11, 8.72, 8.72 and 7.55 μM when the cells were treated with compound **4**, **6**–**8** and **11** in combination with a single dose of γ-radiation at a dose level of 8 Gy (Table 2). The survival curve for MCF7 cell line for compound **11** alone and in combination with γ-irradiation is illustrated in (Fig. 2).

3. Conclusion

The objective of the present study was to synthesize and investigate the anticancer activity of new Schiff bases of pyrano[2,3-*d*] thiazole containing a free sulfonamide moiety. Some of the new synthesized compounds **4**, **6**–**8** and **11** showed promising anticancer activity against human breast cancer cell line [MCF7]. Also, combination of these active compounds with radiation resulted in a remarkable increase of their potency toward cancer cells.

4. Experimental

4.1. Chemistry

Melting points are uncorrected and were determined on a Stuart melting point apparatus (Stuart Scientific, Redhill, UK). Elemental analysis (C, H, N) were performed on Perkin–Elmer 2400 analyser (Perkin–Elmer, Norwalk, CT, USA) at the microanalytical laboratories of the Faculty of Science, Cairo University. All compounds were within ±0.4% of the theoretical values. The IR spectra (KBr) were measured on Shimadzu IR 110 spectrophotometer (Shimadzu, Koyoto, Japan), ^{1}H NMR spectra were obtained on a Bruker proton NMR-Avance 300 (300 MHz) (Bruker, Munich, Germany), in DMSO-d_6 as a solvent, using tetramethylsilane (TMS) as internal standard. Mass spectra were run on JEOL JMS AX-500 mass spectrometer (JEOL JMS, Tokyo, Japan). All reactions were monitored by thin layer chromatograph (TLC) using precoated Aluminum sheets Silica gel Merck 60 F254 and were visualized by UV lamp (Merck, Darmstadt, Germany).

4.1.1. 2-Chloro-N-(4-sulfamoylphenyl)acetamide (**2**)

M.p.: 215–217 °C as reported [31].

4.1.2. 4-(4-Oxo-4,5-dihydrothiazol-2-ylamino)benzenesulfonamide (**3**)

Compound **3** was prepared by another method.

A mixture of compound **2** (2.48 g, 0.01 mol) and ammonium thiocyanate (0.76 g, 0.01 mol) in ethanol (50 mL) was refluxed for 1 h. The solid obtained was crystallized from dioxane. M.p.: 244–246 °C as reported [11].

4.1.3. 5-Amino-6-cyano-7-(4-methoxyphenyl)-7H-2-(4-sulphamoylphenylamino) pyrano[2,3-d]thiazole (**4**)

A mixture of compound **3** (2.71 g, 0.01 mol) with 2-(4-methoxy benzylidene) malononitrile (0.01 mol) in ethanol (30 mL) containing