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Synthesis of novel pyrrole and pyrrolo[2,3-*d*]pyrimidine derivatives bearing sulfonamide moiety for evaluation as anticancer and radiosensitizing agents

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ABSTRACT

Pyrroles and pyrrolo[2,3-*d*]pyrimidines were reported to act as potent anticancer agents, in this work, a series of novel 2-substituted-3-cyano-4-phenyl-pyrrole **5**, **6**, **11–18**, and 5-phenyl-pyrrolo[2,3-*d*]pyrimidine derivatives **7–10**, **19–24** bearing either sulfathiazole or sulfapyridine were synthesized. The structures of these compounds were confirmed by elemental analysis, IR, ^{1}H NMR and mass spectral data. All the newly synthesized compounds were evaluated for their in vitro cytotoxicity against liver and breast cancer cell line (HEPG2 and MCF7). Most of the screened compounds showed interesting cytotoxic activities compared with the used reference drug (doxorubicin). The radiosensitizing ability of some of the synthesized compounds was studied and the results showed an increase in the cell killing effect of γ-radiation after combination with the tested compounds.

Pyrrole and pyrrolo[2,3-*d*]pyrimidine derivatives have aroused recent attention as potent anticancer agents.[1–3] Several mechanisms are involved in their cytotoxic activities as being dihydrofolate reductase inhibitors,[4] tyrosine kinase inhibitors,[5] cyclin dependant kinase inhibitors[6] or adenosine receptor antagonist.[7] A series of N_7-substituted and 5-aryl-pyrrolo[2,3-*d*]pyrimidines **I–IV** represent a class of potent antitumor agents acting as tyrosine kinase inhibitors.[1–3,8] On the other hand, a host of structurally novel sulfonamide derivatives have recently been reported to show substantial anticancer activity in vitro and/or in vivo.[9–14] Since the discovery of E-7010 **V**,[15] sulfonamides have emerged as an important class of anticancer agents which interact with a wide range of different cellular targets as disruption of microtubules assembly and carbonic anhydrase inhibition,[16–18] and some of these compounds having the NH of the sulfonamide group substituted by an aryl group showed potent anticancer activity such as **VI**, KD5170 **VII**, and PXD101 **VIII**.[19–21]

In the light of these facts and as a continuation of previous work on anticancer agents,[22–29] we report the synthesis of a series of 2-substituted-3-cyano-4-phenyl-pyrrole and 5-phenyl-pyrrolo[2,3-*d*]pyrimidine derivatives substituted at *N*-pyrrole by either sulfathiazole or sulfapyridine moiety (Fig. 1) to be screened as anticancer agents. Additionally, some of the synthesized compounds were evaluated as radiosensitizing agents to prove their ability to enhance the cell killing effect of γ-radiation.

The synthesis of 4-(2-amino-3-cyano-4-phenyl-pyrrol-1-yl)-benzenesulfonamide derivatives **5** and **6** is described in Scheme 1, where, the reaction of sulfathiazole **1**, sulfapyridine **2**, with phenacyl bromide furnished 4-(2-oxo-2-phenyl-ethylamino)-benzenesulfonamide derivatives **3**, **4**, which upon reaction with malononitrile in sodium ethoxide gave pyrrole derivatives **5**, **6**. (Scheme 1).

Pyrrolo[2,3-*d*]pyrimidine-4-ones **7**, **8** and 4-amino- pyrrolo[2,3-*d*]pyrimidines **9**, **10** were obtained via condensation of the pyrroles **5** and **6** with formic acid or formamide, respectively. The reactivity of pyrrole *o*-amino carbonitriles **5** or **6** towards different nucleophiles was studied in order to obtain novel pyrrole or pyrrolo[2,3-*d*]pyrimidines. Thus, interaction of **5** or **6** with urea in refluxing ethanol containing sodium ethoxide afforded the corresponding pyrrole derivatives **11** or **12**, respectively. While their fusion with either urea or thiourea for 15 min yielded the corresponding pyrrolopyrimidine derivatives **21–24**, respectively. On the other hand, reaction of **5** or **6** with succinic anhydride in ethanol yielded the corresponding pyrrole derivatives **15** or **16**, respectively. While, under condition of fusion, gave the corresponding pyrrolopyrimidine derivatives **17** or **18**, respectively. Finally, acetylation of **5** or **6** with acetic anhydride for 2 h, afforded the corresponding monoacetyl pyrrole derivatives **13** or **14**, respectively, while, upon conducting the same reaction for long time (10 h), cyclization occurred to give the corresponding pyrrolopyrimidine derivatives **19** or **20**, respectively (Scheme 2).

The synthesized compounds were evaluated for their in vitro cytotoxicity against human liver and breast cancer cell lines

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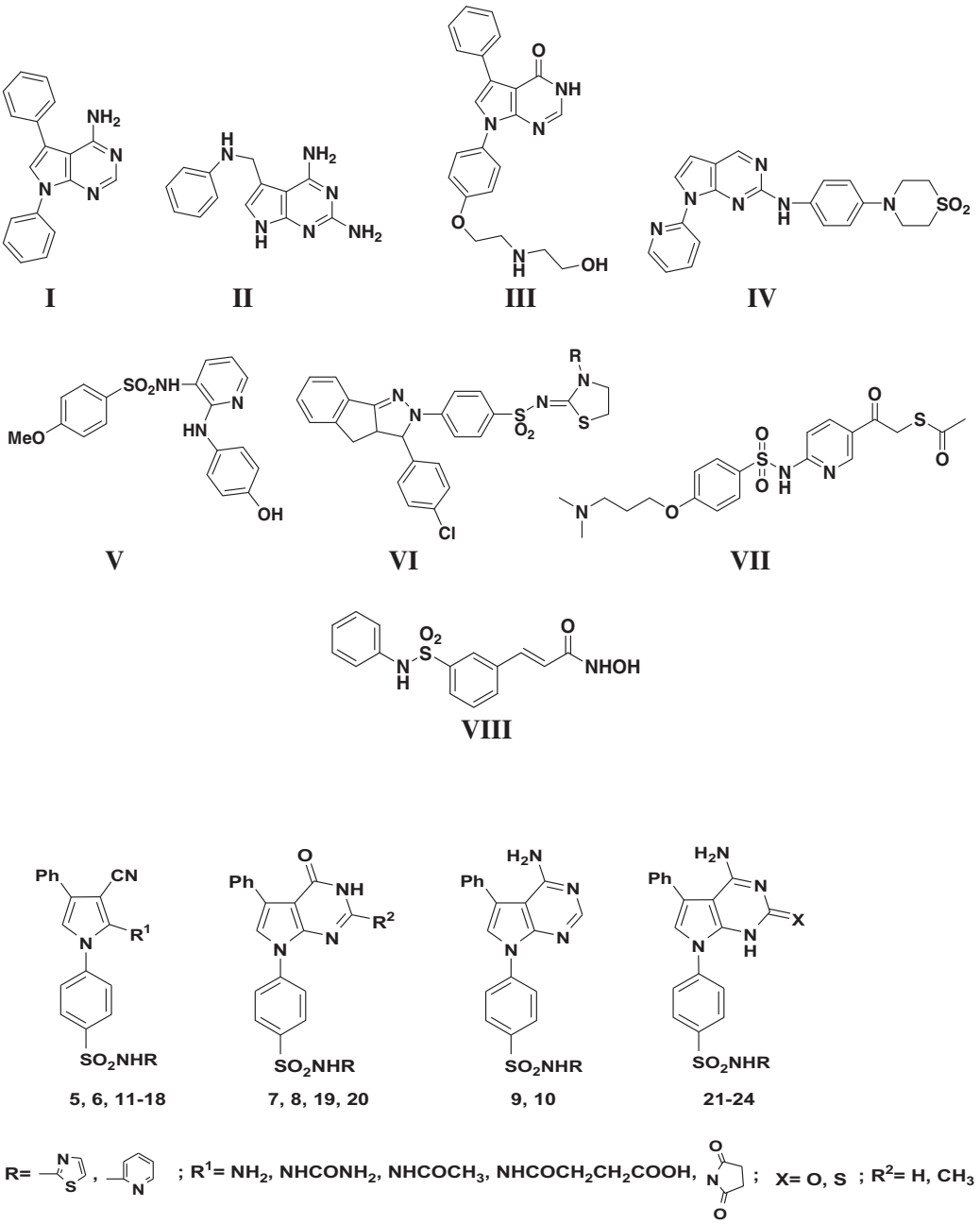
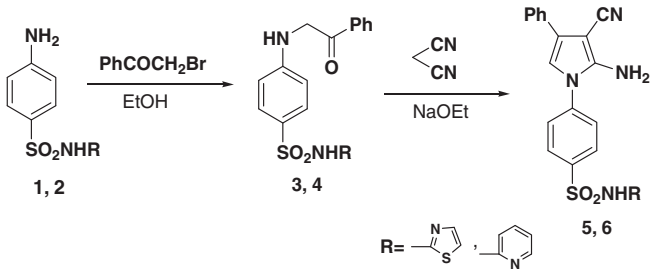
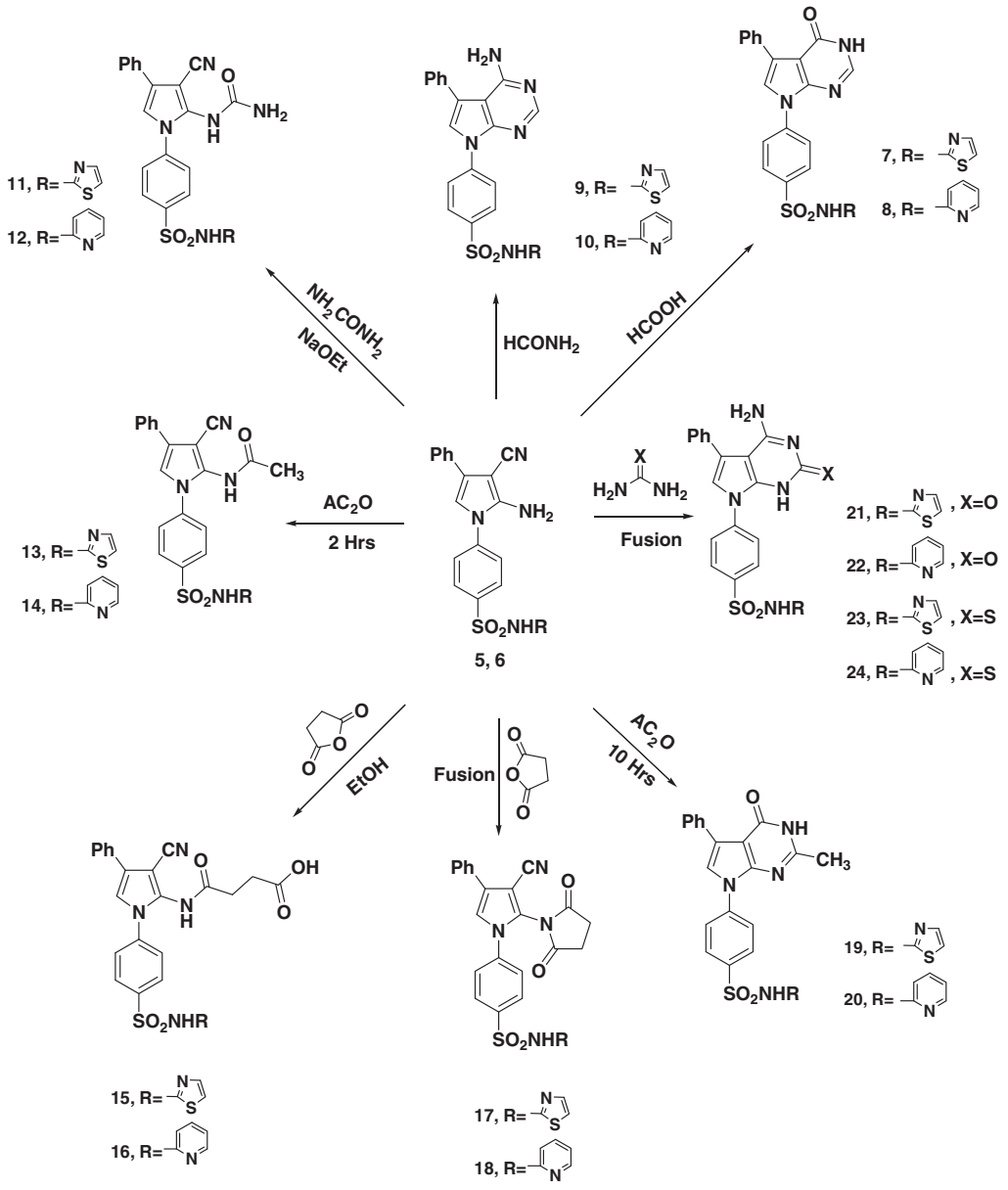


Figure 1. Structure of the target compounds.



Scheme 1.

(HEPG2 and MCF7) and some of the tested compounds were equipotent while the others were more potent compared with doxorubicin. From Table 1 we can observe that, for the liver cell line (HEPG2), considering the pyrrole series **5**, **6**, **11–18**, the pyrrole derivatives **5** and **6** showed increased activity (IC_{50} = **5.36** and **5.3** μM) when compared to compounds **3** and **4** (IC_{50} = **6.78** and **6.75** μM) and they were found to be nearly as active as doxorubicin (IC_{50} = **5.23** μM). While, substitution on the amino group at position 2 of the pyrrole ring showed increase in activity especially for compounds **14** (IC_{50} = **3.49** μM), **15** (IC_{50} = **3.75** μM), and **16** (IC_{50} = **3.75** μM) which are the most potent compounds in this series. Concerning the pyrrolo[2,3-*d*]pyrimidine derivatives **7–10**, **19–24**, the most potent of this series were compounds **19** (IC_{50} = **3.39** μM), **20** (IC_{50} = **3.15** μM), and **21** (IC_{50} = **3.49** μM). For the breast cell line (MCF7), The *O*-amino carbonitriles **5** and **6** were the most potent in the pyrrole series (IC_{50} = **3.49** and **4.6** μM) and they were found to be more potent than doxorubicin



Scheme 2.

(IC_{50} = **3.22** μM), while, substitutions on the amino group resulted in a drop in their anticancer activity which may give an idea about the possible importance of the free amino group on activity. Finally, for the pyrrolopyrimidine derivatives, the most potent compounds were **22** (IC_{50} = **3.9** μM), **23** (IC_{50} = **3.12** μM), and **24** (IC_{50} = **3.8** μM). Considering the activity on both cell lines when using either sulfathiazole or sulfapyridine, the activity doesn't change significantly which indicate that these sulfonamides may be equipotent on both cell lines. These preliminary results of biological screening of the tested compounds could offer an encouraging framework in this field that may lead to the discovery of potent anticancer agent.

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.[30] Consequently, the ability of some of the most potent compounds resulted from in vitro cytotoxic activity **14**, **15**, **16**, and **21**, to enhance the cell killing effect of γ-irradiation was studied. From Tables 2 and 3 and Figure 2, we can observe that the effect of radiation alone was not significant when compared to control group which contains the cell lines without radiation or drug. On the other hand, the surviving fraction of the tested compounds decreased significantly from the control after being subjected to radiation which indicate the synergism due to combining drugs with radiation.

Table 1
Anticancer screening of the newly synthesized compounds against human liver cell line (HEPG2) and human breast cell line (MCF7)

Compound	Cytotoxicity[a,b] (IC_{50}) (μM)	
	HEPG2	MCF7
3	6.78	8.89
4	6.75	7.56
5	5.36	**3.49**
6	5.3	**4.6**
7	5.3	6.21
8	4.67	5.32
9	4.24	6.45
10	4.09	6.34
11	4.02	8.05
12	4.12	7.34
13	4.02	7.24
14	**3.49**	11.82
15	**3.75**	9.12
16	**3.75**	8.32
17	4.56	6.71
18	5.36	9.93
19	**3.39**	5.8
20	**3.15**	5.1
21	**3.49**	8.32
22	4.3	**3.9**
23	4.12	**3.12**
24	4.1	**3.8**
Doxorubicin	**5.23**	**3.22**

[a] IC_{50}, compound concentration required to inhibit tumor cell proliferation by 50%.
[b] Values are means of three experiments.

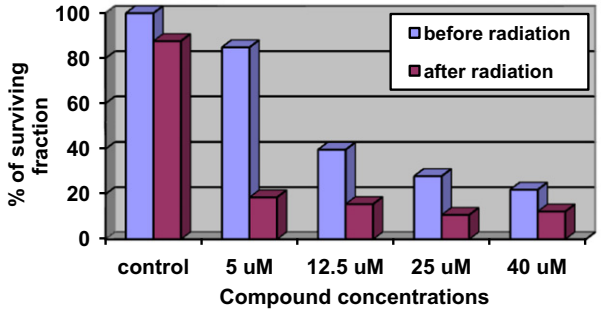


Figure 2. The effect of radiation on anticancer activity of different concentrations of compound **16** on HEPG2 cell line.

From the above results, we can conclude that administration of the tested compounds on human liver and breast (HEPG2 and MCF7) cell lines showed promising cytotoxic activity especially on liver cell line, while, combining these compounds with radiation at the same concentrations enhances their activity which demonstrates the importance of the combination therapy for the patients with cancer to decrease the side effects of both drugs and radiation.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.005.

Table 2
Statistical analysis of the results of in vitro anticancer activity of compounds **5**, **14**, **15**, **16**, **21** against human liver or breast cell line (HEPG2 and MCF7)

Cell line	Compd no.	Control (cells only)	Drug (μM)			
			5	12.5	25	40
			Means ± SE (% change)[#]			
HEPG2	**14**	1.526 ± 0.05680 (100%)	1.063 ± 0.1006[*] (30.34%)	0.71 ± 0.03384[*] (53.47%)	0.5683 ± 0.009735[*] (62.7%)	0.4587 ± 0.02338[*] (69.9%)
	15	1.526 ± 0.05680 (100%)	1.144 ± 0.0788[**] (25.03%)	0.9217 ± 0.08055[*] (39.6%)	0.9773 ± 0.1336[*] (35.95%)	0.7693 ± 0.09242[*] (49.58%)
	16	1.5139 ± 0.03894 (100%)	0.967 ± 0.06185[*] (15.12%)	0.6007 ± 0.01299[*] (60.3%)	0.4227 ± 0.05646[*] (72%)	0.3333 ± 0.01299[*] (78%)
	21	1.526 ± 0.05680 (100%)	1.185 ± 0.0543[**] (22.34%)	0.7987 ± 0.04421[*] (47.66%)	0.5503 ± 0.01810[*] (63.9%)	0.555 ± 0.08602[*] (63.6%)
MCF7	**5**	1.3613 ± 0.05082 (100%)	1.207 ± 0.02674[**] (11.3%)	0.7823 ± 0.01598[*] (42.5%)	0.391 ± 0.03029[*] (71.3%)	0.379 ± 0.01801[*] (72%)

Each value is the mean of three values ± SE.
[#] Percentage of change from control group.
[*] Significant difference from control group at p <0.05.
[**] Non-significant difference from control group at p <0.05.

Table 3
Statistical analysis of the results of in vitro anticancer activity of compounds **5**, **14**, **15**, **16**, **21** against human liver or breast cell line (HEPG2 and MCF7) after irradiation

Cell line	Compd no.	Control (cells only)	Control (radiation)	Drug Irradiated (μM)			
				5	12.5	25	40
				Means ± SE (% change)[#]			
HEPG2	**14**	1.526 ± 0.05680 (100%)	1.2022 ± 0.03297[**] (21.2%)	0.6497 ± 0.04890[*] (57.42%)	0.466 ± 0.01537[*] (69.46%)	0.2667 ± 0.03464[*] (82.5%)	0.2763 ± 0.03552[*] (81.9%)
	15	1.526 ± 0.05680 (100%)	1.2022 ± 0.03297[**] (21.2%)	0.6 ± 0.09200[*] (60.6%)	0.4167 ± 0.03034[*] (72.6%)	0.4287 ± 0.04051[*] (71.8%)	0.3133 ± 0.02671[*] (79.4%)
	16	1.5139 ± 0.03894 (100%)	1.2303 ± 0.04683[**] (18.73%)	0.281 ± 0.09250[*] (81.4%)	0.2363 ± 0.07980[*] (84.4%)	0.162 ± 0.004583[*] (89.2%)	0.1847 ± 0.02278[*] (87.7%)
	21	1.526 ± 0.05680 (100%)	1.2022 ± 0.03297[**] (21.2%)	0.584 ± 0.02835[*] (61.73%)	0.47 ± 0.02793[*] (69.2%)	0.3307 ± 0.03992[*] (78.3%)	0.278 ± 0.02553[*] (81.7%)
MCF7	**5**	1.3613 ± 0.05082 (100%)	1.261 ± 0.03745[**] (7.3%)	0.796 ± 0.03629[*] (41.5%)	0.4727 ± 0.08178[*] (65.3%)	0.241 ± 0.03759[*] (82.3%)	0.1937 ± 0.03320[*] (85.8%)

Each value is the mean of three values ± SE.
[#] Percentage of change from control group.
[*] Significant difference from control group at p <0.05.
[**] Non-significant difference from control group at p <0.05.

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