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Synthesis and biological evaluation of novel triazoles and isoxazoles linked 2-phenyl benzothiazole as potential anticancer agents

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ABSTRACT

A new series of isoxazoles and triazoles linked 2-phenyl benzothiazole were synthesized and evaluated for their anticancer activity. These compounds have been tested for their cytotoxicity against three cancer cell lines. Among the compounds tested, compound **5d** showed good cytotoxicity against Colo-205 and A549 cells in comparison to standard control PMX 610(**1**). Further compound **5d** has been tested for its apoptotic activity and its inhibitory activity against caspase and PARP proteins. Hence this compound has the potential that it can be selected for further biological studies.

Over the last two decades, the synthesis and functionalization of benzothiazole has become a major area of focus for synthetic organic chemists because of their several pharmacological functions including antitumour activity,[1–8] neurotransmission blockage,[9–11] calmodulin antagonists[12] and neuroprotective activity.[13,14] In recent years, extensive research has been carried out for modifying benzothiazole nucleus to improve their antitumour activities. Among the modified structures, especially interesting are 2-phenyl

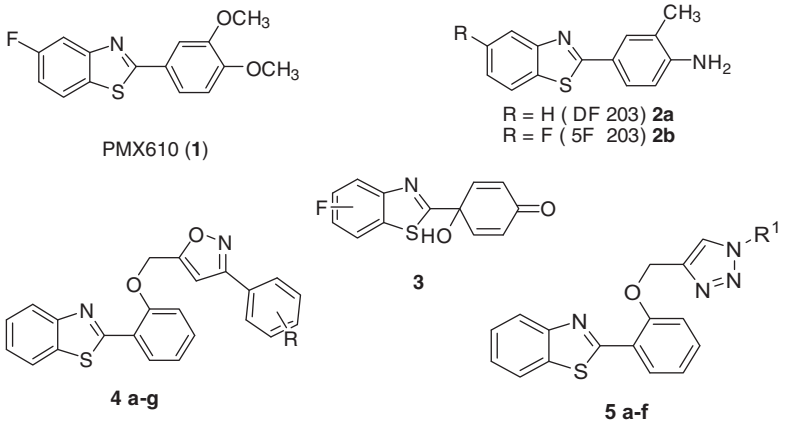

Figure 1. Chemical structure of anticancer phenylbenzothiazole derivatives.

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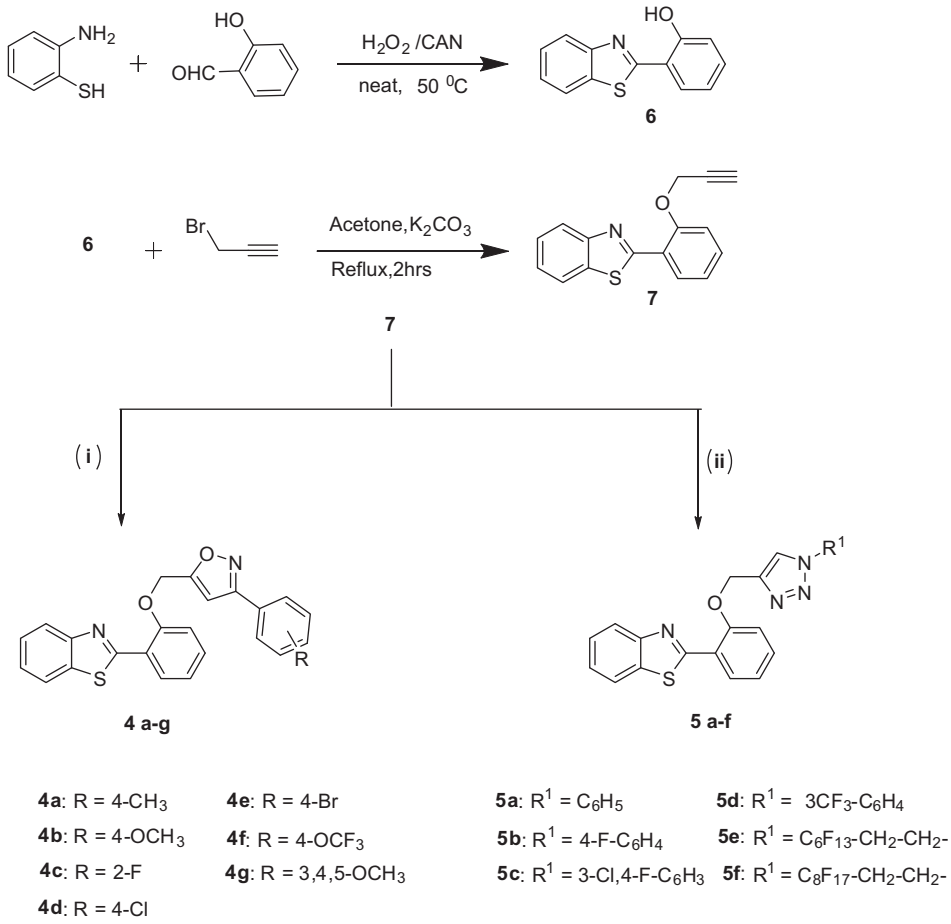
benzothiazoles that exhibit potent and selective antitumour activity. Some of the structurally related benzothiazoles such as 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (PMX 610)[15] (**1**) (Fig. 1), 2-(4-amino-3-methylphenyl) benzothiazole (DF 203) and the 2-(4-amino-3-methylphenyl)-5-fluoro benzothiazole (5F 203)[16] (**2a** and **2b** of Fig. 1) have been reported to possess potent and selective in vitro antitumour properties in human cancer cell lines particularly against colon, non-small cell lung and breast cancer lines of the National Cancer Institute (NCI) 60 human cancer cell line screen. Other benzothiazole derivatives such as 4-(benzothiazol-2-yl)-4-hydroxycyclohexa-2,5-dienones[17] (PMX 464) (**3**) (Fig. 1) exhibited potent and selective antitumour activity concentrated in certain colon, renal and breast cancer cell lines, that acts via inhibition of the cellular redox protein thioredoxin (Trx-1). On the other hand, isoxazole and triazole derivatives have been reported for their anticancer activity against human colon cancer cell lines.[18]

Hence in continuation of our efforts for the structural modifications on benzothiazole moiety and thus to improve their anticancer activity,[19,20] new series of benzothiazoles have been synthesized by



Scheme 1. Reagents and conditions: (i) aq NaOCl (9–12%), DCM, Et_3N, oxime, rt, 24 h, 65–75%; (ii) dry THF, CuI (5 mol %), R^1-N_3, rt, 24 h, 85–89%.

Table 1
IC_{50} values for compounds (**4a**–**5f**) in selected cancer cell lines (A549, colo-205, MCF-7) as well as normal cells MCF-10A

S. No.	Compound	IC $_{50}$ in MCF-10A	IC $_{50}$ in A549	IC $_{50}$ in Colo-205	IC $_{50}$ in MCF-7
1.	Std	25.22 ± 0.80	15.88 ± 0.93	13.94 ± 0.24	20.22 ± 0.97
2.	**4a**	26.37 ± 0.74	16.37 ± 0.56	14.16 ± 0.91	27.52 ± 1.28
3.	**4b**	34.13 ± 1.82	24.13 ± 1.12	20.70 ± 0.68	22.14 ± 1.28
4.	**4c**	42.54 ± 0.50	22.54 ± 0.56	18.28 ± 0.68	23.87 ± 0.96
5.	**4d**	38.8 ± 1.65	18.8 ± 0.75	16.45 ± 0.68	23.8 ± 1.66
6.	**4e**	29.2 ± 0.70	19.2 ± 0.57	16.449 ± 0.36	20.03 ± 0.96
7.	**4f**	43.87 ± 2.57	23.87 ± 0.57	19.87 ± 0.62	26.62 ± 1.28
8.	**4g**	42.87 ± 2.37	22.87 ± 0.37	17.5 ± 0.69	25.6 ± 0.96
9.	**5a**	36.41 ± 2.32	16.41 ± 1.12	16.58 ± 0.69	26.24 ± 1.28
10.	**5b**	33.32 ± 3.72	23.32 ± 0.37	18.87 ± 0.69	26.24 ± 1.28
11.	**5c**	26.28 ± 1.54	16.28 ± 0.56	14.40 ± 0.69	27.26 ± 1.33
12.	**5d**	31.07 ± 2.73	11.07 ± 0.62	10.78 ± 0.69	18.94 ± 0.97
13.	**5e**	35.05 ± 3.67	15.05 ± 0.75	14.16 ± 0.45	19.84 ± 1.28
14.	**5f**	33.1 ± 3.92	13.1 ± 0.74	12.21 ± 0.32	19.2 ± 0.36

IC_{50} is concentration at which 50% of cells were undergo cytotoxic cell death due to compound treatment.

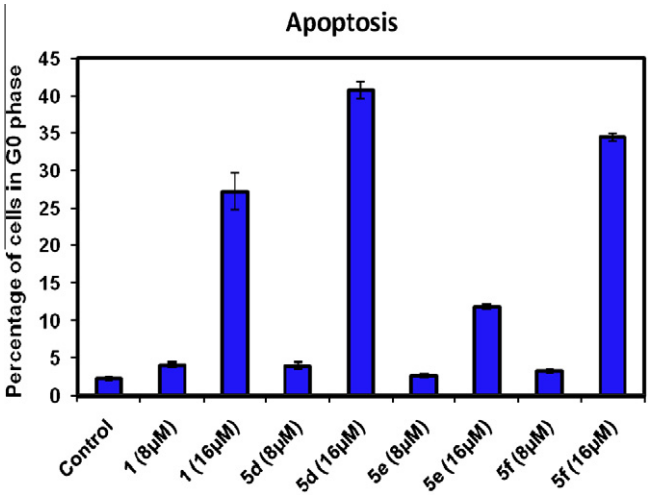


Figure 2. The graph representing the percentage of apoptotic cells accumulated after the treatment of colo-205 cells with compounds **1**, **5d**, **5e** and **5f** at 8 and 16 μM concentrations for 24 h. control indicates the untreated cells.

Table 2
The cell cycle distribution of compounds

Compound	G0	G1	S	G2/M
Control	2.28 ± 0.25	71.02 ± 1.00	3.56 ± 0.40	23.09 ± 1.01
1 (8 μM)	4.15 ± 0.30	70.04 ± 0.96	3.21 ± 0.25	22.5 ± 0.92
1 (16 μM)	27.17 ± 2.45	52.82 ± 2.45	4.31 ± 0.59	15.6 ± 0.54
5d (8 μM)	4.02 ± 0.46	70.91 ± 1.01	3.37 ± 0.33	21.68 ± 0.59
5d (16 μM)	40.70 ± 1.12	45.77 ± 0.69	2.68 ± 0.27	10.67 ± 0.58
5e (8 μM)	2.72 ± 0.25	73.66 ± 0.57	3.09 ± 0.15	20.53 ± 0.84
5e (16 μM)	11.91 ± 0.37	61.36 ± 1.18	4.42 ± 0.51	22.3 ± 1.08
5f (8 μM)	3.30 ± 0.26	72.0 ± 0.56	4.43 ± 0.51	20.22 ± 0.69
5f (16 μM)	34.45 ± 0.50	46.57 ± 1.91	3.79 ± 0.35	15.18 ± 1.40

FACS analysis of compounds(**1**, **5d**, **5e** and **5f**) at 8 and 16 μM concentration in Colo-205 cells for 24 h. Cell cycle distribution was conducted in three independent experiments. Average and standard deviation was derived.

incorporating isoxazole and triazole moieties at the 2-phenyl group of 2-phenyl benzothiazole. Further, these new series of compounds have been tested for their in vitro cytotoxicity against three human cancer cell lines. Representatives of some biologically important phenylbenzothiazole compounds have been illustrated in Figure 1.

The synthesis of 2-(2′-hydroxyphenyl) benzothiazole **6** was carried out from the compounds thiophenol and 2-hydroxybenzaldehyde which were prepared by the literature method.[21] Compound **6** coupled with propargyl bromide to provide compound **7** which were reacted with oximes and aromatic/aliphatic azides produced corresponding isoxazoles (**4a–g**) and triazoles (**5a–f**) respectively as shown in Scheme 1.

The new series of isoxazoles and triazoles linked 2-phenyl benzothiazole derivatives (**4a–g** and **5a–f**) were evaluated for their cytotoxic activity against three cancer cell lines such as human lung adenocarcinoma (A549), human colon cancer cells (Colo-205) and breast carcinoma cell line (MCF-7) by MTT assay. These cancer cell lines were treated with compounds (**4a–g** and **5a–f**) along with PMX 610 (**1**) as a standard positive control at a concentration ranging from 1 to 64 μM for 24 h .Most of these benzothiazoles exhibited higher anticancer activity than **1**, particular against colon-205 and A549 cell lines. (Table 1 and Supplementary Figs. 1 and 3a–4c). The structure–activity relationship studies revealed that the introduction of a fluorine atom particular the $-CF_3$ group in the 3rd position of the target compounds **5d** enhanced the cytotoxic activity. Similarly, the triazole derivatives of phenyl benzothiazole series also lead to an increase in cytotoxic activity.

In order to study the effect of these benzothiazole derivatives on cell cycle progression and apoptosis in human colon cancer cells (Colo-205) were treated with compounds **1**, **5d**, **5e** and **5f** analyzed using fluorescence activated cell sorter (FACS shown in Supplementary Figs. 2, 5a and b). We have observed 3–4% of apoptotic cells as indicated by G0 phase at 8 μM concentration of phenylbenzothiazoles.

Interestingly the percentage of apoptosis was observed to be 27, 40, 12 and 34 in case of compounds **1**, **5d**, **5e** and **5f** treated cells respectively. Thus compounds at a concentration of 16 μM was considered as effective concentration and compounds have apoptotic inducible nature (Fig. 2, Table 2 and Supplementary Figs. 2, 5a and b).

One of the important characteristic features of apoptosis is the degradation of procaspase-3 to active caspase-3 and cleavage of DNA repair enzyme PARP [Poly (ADP-ribose) Polymerase] by caspase-3.[22] Early literature has shown that caspase-3 and caspase-9 plays a prominent role in mediating drug induced apoptosis.[23] Caspase-3 is considered to be the effector caspase and also considered as the therapeutic target for the treatment of cancer.[24] It was observed in apoptosis pro-caspases are cleaved to active caspases.[25] Since compounds have shown apoptotic cell death at 16 μM concentration, the role of caspases and PARP was examined using the effective compound of the series (**5d**). The positive control used in this study is **1**. Colo-205 cells were treated with compounds **1** and **5d** at 8 and 16 μM for 24 h. The cell lysates was isolated and Western blot analysis was carried out using antibodies against procaspase-8, procaspase-9, active caspase-3 and cleaved PARP. It was

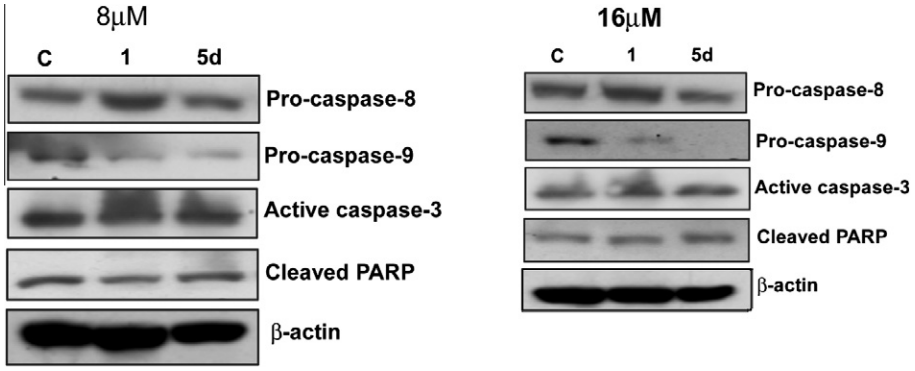


Figure 3. Effect of phenylbenzothiazole compounds on caspase and PARP proteins. The colo-205 cells were treated with **1**, **5d** compounds at 8 and 16 μM concentration for 24 h. The cell lysates extracted were subjected to Western blot analysis using antibodies against procaspases-8, 9, active caspase-3 and active PARP (cleaved) proteins. β-Actin was used as loading control. Compound **1** indicates the positive control. Here 16 μM concentration was highly effective.

observed from the study that compound **5d** caused increased expression of active caspase-3 and PARP with degradation of pro-caspase-8 and 9 proteins. This suggests the existence of caspase mediated apoptotic cell death (Fig. 3).

In conclusion, in the present study, a series of new triazole and isoxazole were synthesized. All these benzothiazole derivatives (**4a–g** and **5a–f**) showed significant anticancer activity, with IC_{50} values ranging from 26 to 43 μM in MCF-7 cells, 11–24 μM in A549 cells and 11–21 μM in Colo-205 cells. The effective compound (**5d**) has shown IC_{50} of 11 μM in colon cancer cells as deduced from MTT assay. MTT assay identified the three most promising compounds **5d**, **5e** and **5f** which have shown higher cytotoxicity in Colo-205 cells than the other cell lines tested. Flow cytometry (FACS) analysis showed a greater cell population in the G0 phase, indicating that these phenyl benzothiazole possess the ability to cause apoptosis. Further biological assay such western analysis indicated the role of caspases in cell death when cell treated with the compound **5d**. From these studies it may be concluded that compound **5d** which could be a promising compound can be taken up for further in vivo cancer studies that may be of interest in cancer chemoprevention.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.07.041.

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