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Synthesis and cytotoxic evaluation of thiourea and *N*-bis-benzothiazole derivatives: A novel class of cytotoxic agents

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ABSTRACT

Benzothiazolyl thiocarbamides has been achieved using a catalytic amount of 4-dimethylaminopyridine (DMAP) followed by its chemoselective oxidative cyclization with 1,3-di-*n*-butylimidazolium tribromide[bbim][Br_3] to afford the *N*-bis-benzothiazole derivatives. All the synthesized compounds were evaluated for cytotoxic activity against two human monocytic cell lines (U 937, THP-1) and a mouse melanoma cell line (B16-F10). Based on their IC_{50} values, the majority of the benzothiazolyl thiocarbamides and *N*-bis-benzothiazoles had significant antiproliferative activity on U 937 and B16-F10 cells, the compounds **3b**, **3e**, **3f**, **3k**, **6c** and **6h** were found to be the most active. The present findings indicate clearly that the compound **3e** exhibited more antiproliferative activity on U 937 cells than the standard molecule, etoposide. Nevertheless, these compounds have shown comparatively less cytotoxicity towards THP-1 cells.

Benzothiazoles are an important class of heterocycles, which can serve as unique and versatile scaffolds for experimental drug design.[1] 2-Aminobenzothiazoles have received considerable attention because of their interesting pharmacological activities including anticonvulsant,[2] analgesic,[3] anti-tumor,[4,5] antibacterial,[6,7] antimicrobial[8,9] and muscle relaxant agents.[10] The combinations of urea and thiourea derivatives with benzothiazole have produced DNA topoisomerase[11,12] or HIV reverse transcriptase inhibitors[13,14] for example frentizole (**1**) (Fig. 1) is a nontoxic antiviral and immunosuppressive agent used clinically in rheumatoid arthritis and systemic lupus erythematosus[15] and thiourea derivatives YH3945 (**2**) (Fig. 1), a selective and potent inhibitor of farnesyl-protein transferase, is being developed for the treatment of cancer.[16] Similarly, substituted benzothiazoles such as 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (PMX 610) (**3**) (Fig. 1) has been shown to exhibit exquisitely potent (GI_{50} <0.1 nM) and selective in vitro antitumor properties in human cancer cell (e.g., colon, nonsmall-cell lung and breast subpanels) lines of the National Cancer Institute (NCI) 60 human cancer cell line screen.[17] Other benzothiazoles such as, 2-(4-amino-3-methylphenyl) benzothiazole (DF 203) and the 2-(4-amino-3-methylphenyl)-5-fluoro benzothiazole (5F 203) (**4** and **5** of Fig. 1) were found to activate on arylhydrocarbon receptor (AhR) via translocation from the cytosol to the nucleus. Therein the induced cytochrome P450 CYP1A1 enzyme activity was determined, which subsequently leads to the generation of a reactive chemical intermediate that selectively generates DNA adducts only in sensitive tumor types (e.g., mammary and ovarian tumor cell lines).[18] Especially interesting are bis-benzothiazole derivatives which exhibit their importance in amyloid-imaging,[19] vulcanization accelerators[20] and starting materials for various pharmaceutical industries.[21] In continuation of our previous studies for the design of novel anticancer agents[22–24] and importance to keep in mind the application of thiourea and

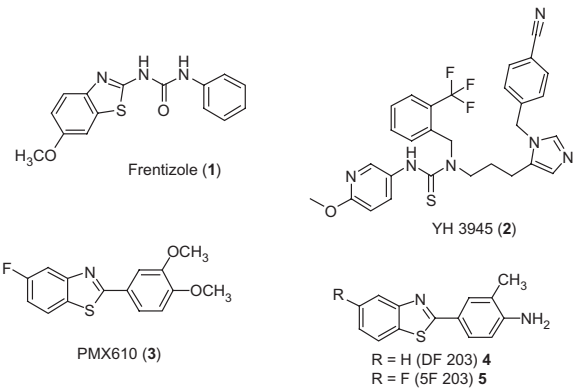

Figure 1. Chemical structures of biologically important compounds.

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benzothiazole derivatives, a new series of benzothiazolyl thiocarbamide (**3a**–**3l**) and *N*-bis-benzothiazole (**6a**–**6l**) motifs has been synthesized as anticancer agents.

Thiazole ring in benzothiazole moiety is frequently constructed via an oxidative cyclization of thiobenzanilides using various oxidants, including Jacobson's and traditional Hugershoff's methods.[25–27]

Methods for synthesis of *N*-bis-benzothiazoles are scarce in literature. Chemoselective oxidative cyclization of unsymmetrical thioureas with *N*-bromosuccinimide (NBS) as an oxidizing agent for the synthesis of *N*-bis-benzothiazole is also effective[28] but, this method is not explored much for different functionalities. Still, there have been no reports on ionic liquid mediated chemoselective oxidative cyclization of benzothiazolyl thiocarbamides. In the view of this, there remains a wide scope to develop a general, efficient and green method for the synthesis of more functionalized *N*-bis-benzothiazol-2-yl amine derivatives. Recently ionic liquid has gained more attention in synthetic organic chemistry as an alternative green reaction media.[29] Hence, a total of 24 derivatives were synthesized and evaluated for their in vitro cytotoxic activities against two human monocytic cell lines (U 937, THP-1) and a mouse melanoma cell line (B16-F10).

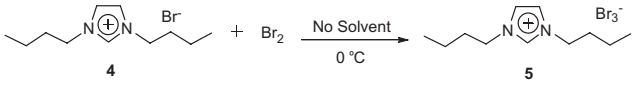
The appropriate substituted 2-aminobenzothiazole **1** and commercially available substituted phenyl isothiocyanate **2** were reacted in the presence of 4-dimethylaminopyridine (5 mol %) in *N*,*N*-dimethyl formamide to furnish substituted benzothiazolyl thiocarbamides derivative **3** Scheme 1.

Next, we examined the oxidative cyclization of benzothiazolyl thiocarbamides using new tribromide-based ionic liquid 1,3-di-*n*-butylimidazolium tribromide[bbim][Br_3] as a reagent of choice. 1,3-Di-*n*-butylimidazolium bromide **4** on drop wise addition of molecular bromine under stirring formed exothermally red liquid 1,3-di-*n*-butylimidazolium tribromide **5** Scheme 2.

Subsequently, the reaction of benzothiazolyl thiocarbamide **3a** was performed in 1,3-di-*n*-butylimidazolium tribromide[bbim][Br_3] **5**. The reaction was sluggish at room temperature. Surprisingly reaction proceeded well at 70 °C giving *N*-bis-benzothiazole **6a** exclusively in 92% yield via C–S bond formation. Thus the oxidative cyclization was found to be chemoselective Scheme 3.

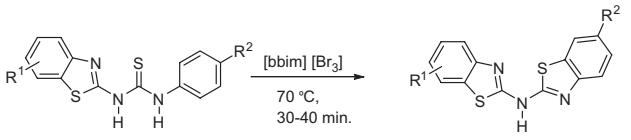
The structure of product **6a** was determined on the basis of ^{1}H NMR spectrum. It shows only two doublets and two triplets with same coupling constant (J = 7.5 Hz) which are consistent with structure of **6a**. Other benzothiazolyl thiocarbamides also participated well in this reaction. The scope of above reaction is illustrated with respect to different functionalities.

The biological activities of benzothiazolyl thiocarbamide (**3a**–**3l**) and *N*-bis-benzothiazole (**6a**–**6l**) were evaluated to investigate their antiproliferative activities in different types of human leukemia (THP-1 and U 937) and mouse melanoma (B16-F10) cell lines. It is evident from the results that the several of the test



4 5

Scheme 2. Preparation of 1,3-di-*n*-butylimidazolium tribromide[bbim][Br_3].



3 6

6a: R^1 = H; R^2 = H
6b: R^1 = H; R^2 = Cl
6c: R^1 = 6-F; R^2 = F
6d: R^1 = 6-F; R^2 = Cl
6e: R^1 = 4-Cl; R^2 = F
6f: R^1 = 6-Me; R^2 = H
6g: R^1 = 6-OEt; R^2 = H
6h: R^1 = 6-OEt; R^2 = F
6i: R^1 = 6-F; R^2 = H
6j: R^1 = 4-Cl; R^2 = H
6k: R^1 = H; R^2 = F
6l: R^1 = 6-Me; R^2 = F

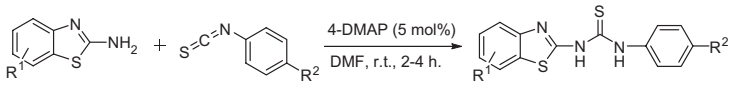
Scheme 3. Chemoselective oxidative cyclization of thiourea using ionic liquid.

derivatives have shown significant decrease in cell viability in all the test cell lines on concentration dependent manner (Table 1).

In summary, we have developed a novel protocol for the synthesis of symmetrical as well as unsymmetrical *N*-bis-benzothiazol-2-yl amines derivatives. An ionic liquid, 1,3-di-*n*-butylimidazolium tribromide[bbim][Br_3] is found to be an efficient chemoselective reagent for the oxidative cyclization of benzothiazolyl thiocarbamides. This method can be classified as 'green' as it utilizes mild reaction conditions, eliminates toxic bromine vapours, and avoids the use of organic solvents.

Comparison with the IC_{50} values of positive control (etoposide) indicated that the THP-1 cells are more sensitive (approximately 9 times) than the other two (U 937 and B16-F10) cell lines. However, several of these benzothiazolyl thiocarbamides (**3a**–**3l**) and *N*-bis-benzothiazole (**6a**–**6l**) exhibited relatively high activities against these drug-resistant cancerous cell lines. Comparatively, about 75% of the test compounds (**3** and **6** series) have shown cytotoxic activity with IC_{50} less than 50 μM against THP-1 cells, followed by 46% against U 937 and 30% against B16-F10 cells. However, considering the IC_{50} values of the standard molecule, the order of sensitivity of these cell lines towards benzothiazolyl thiocarbamides and *N*-bis-benzothiazole derivatives is U 937 > B16-F10 > THP-1.

This study provides a convenient green method for synthesis of various benzothiazolyl thiocarbamide (**3a**–**3l**) and *N*-bis-benzothiazole (**6a**–**6l**) as novel anticancer agents. Further lead optimization is ongoing.



1 2 3

R^1 = H, 6-F, 6-OMe, 6-OC_2H_5, 4-Cl
R^2 = H, F, Cl

3a: R^1 = H; R^2 = H
3b: R^1 = H; R^2 = F
3c: R^1 = H; R^2 = Cl
3d: R^1 = 6-F; R^2 = H
3e: R^1 = 6-F; R^2 = F
3f: R^1 = 6-F; R^2 = Cl
3g: R^1 = 4-Cl; R^2 = H
3h: R^1 = 4-Cl; R^2 = F
3i: R^1 = 6-Me; R^2 = H
3j: R^1 = 6-Me; R^2 = F
3k: R^1 = 6-OEt; R^2 = H
3l: R^1 = 6-OEt; R^2 = F

Scheme 1. Synthesis of benzothiazolyl thiocarbamides.

Table 1
In vitro cytotoxicity of benzothiazolyl thiocarbamides (**3a**–**3l**) and *N*-bis-benzothiazole (**6a**–**6l**) derivatives (structural analogues) against U 937, THP-1 and B16-F10 cancer cells

Compounds	IC_{50}[b] values in micro molar			Compounds	IC_{50}[b] values in micro molar		
	U 937	B16-F10	THP-1		U 937	B16-F10	THP-1
3a	–	–	36.49 ± 1.83	**6a**	40.04 ± 2.0	–	28.76 ± 1.44
3b	32.05 ± 1.61	–	26.73 ± 1.34	**6b**	–	–	45.43 ± 2.27
3c	–	–	38.97 ± 1.95	**6c**	27.40 ± 1.37	47.46 ± 2.37	17.46 ± 0.87
3d	–	–	46.60 ± 2.33	**6d**	–	41.94 ± 2.09	41.01 ± 2.05
3e	16.23 ± 0.81	47.73 ± 2.39	34.58 ± 1.73	**6e**	–	–	36.18 ± 1.81
3f	18.69 ± 0.94	48.13 ± 2.41	31.16 ± 1.56	**6f**	48.42 ± 2.42	–	34.18 ± 1.71
3g	–	–	31.79 ± 1.59	**6g**	–	–	37.09 ± 1.86
3h	–	–	–	**6h**	26.58 ± 1.33	33.16 ± 1.66	38.99 ± 1.95
3i	–	–	–	**6i**	40.63 ± 2.03	–	–
3j	–	–	36.91 ± 1.85	**6j**	–	29.46 ± 1.47	–
3k	37.72 ± 1.89	–	43.77 ± 2.18	**6k**	43.52 ± 2.18	–	–
3l	28.13 ± 1.41	–	39.54 ± 1.98	**6l**	–	29.59 ± 1.48	–
Etoposide[a]	17.94 ± 0.89	18.69 ± 0.94	2.16 ± 0.11	Etoposide[a]	17.94 ± 0.89	18.69 ± 0.94	2.16 ± 0.11

– Indicates IC_{50} valves are more than 50 μM.
[a] Standard drug (positive control).
[b] IC_{50} is defined as the concentration, which results in a 50% decrease in cell number as compared with that of the control cultures in the absence of an inhibitor. The values represent the mean ± SE of four individual observations.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.106.

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