



Original article

Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents

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ABSTRACT

Five series of thiourea derivatives bearing benzothiazole moiety (20 compounds) were efficiently synthesized and evaluated for antimicrobial and anticancer activities. The results indicated that the compounds possessed a broad spectrum of activity against the tested microorganisms and showed higher activity against fungi than bacteria. Compounds **1b**, **2b**, **3b**, **4b** and **5b** exhibited the greatest antimicrobial activity. Preliminary study of the structure–activity relationship revealed that electronic factors in benzothiazole rings had a great effect on the antimicrobial activity of these compounds. In preliminary MTT cytotoxicity studies, the thiourea derivatives (**2d**, **5c** and **5d**) were found most potent. In MCF-7 and HeLa cells, the IC₅₀ values were observed in the range of 18–26 μ M and 38–46 μ M, respectively.

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1. Introduction

The development of new antimicrobial and anticancer therapeutic agents is one of the fundamental goals in medicinal chemistry. Cytotoxicity and genotoxicity of anticancer drugs to the normal cells are major problems in cancer therapy and engender the risk of inducing secondary malignancy [1]. A dose of anticancer drug sufficient to kill tumor cells is often toxic to the normal tissue and leads to many side effects, which in turn, limits its treatment efficacy. In recent years, there has been a concerned search for the discovery and development of novel selective anti-tumor agents, devoid of many of the unpleasant side effects of conventional anti-tumor agents.

In the efforts to develop drugs with such capabilities, scientists have focused upon many different aspects of cancer biology during their research. Among the anti-tumor drugs discovered in the recent years, various benzothiazoles [2–4] as well as urea and thiourea derivatives [5–8] possess potent anticancer properties. The combinations of urea and thiourea derivatives with

benzothiazoles have produced DNA topoisomerase [9,10] or HIV reverse transcriptase inhibitors [11,12]. Thiourea derivatives display a wide range of biological activity including antibacterial, anti-fungal, antitubercular, antithyroid, antihelmintic, rodenticidal, insecticidal, herbicidal, and plant growth regulator properties [13–18].

The importance of such work lies in the possibility that the next generation thiourea derivatives might be more efficacious as antimicrobial and anticancer agents. However, a thorough investigation relating the structure and the activity of the thiourea derivatives as well as their stability under biological conditions is required. These detailed investigations could be helpful in designing more potent antimicrobial and anticancer agents for the therapeutic use.

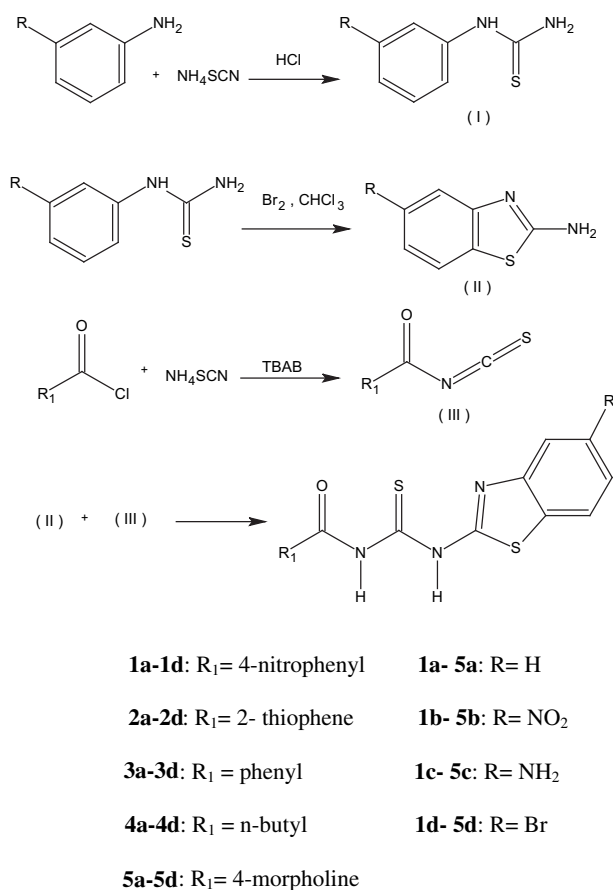
Since varying substituents is a common method for drug design in medicinal chemistry and a useful medical value of substituted thiourea derivatives containing benzothiazole moiety, we aimed to synthesize new thiourea derivatives and to investigate their antimicrobial and anti-tumor activities. Based on these reports, we herein report the synthesis, characterization, antibacterial, anti-fungal and *in vitro* evaluation of anti-tumor activity of five different series of novel thiourea derivatives bearing benzothiazole moiety.

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2. Chemistry

In the present study thiourea derivatives bearing benzothiazole moiety were synthesized as presented in Scheme 1. These compounds were prepared according to our published procedure [19,20] with minor modifications. The use of phase transfer catalyst (PTC) as a method of agitating a heterogeneous reaction system is gaining recognition [21,22]. In search of improving methods to prepare the target thiourea by reacting isothiocyanates with nucleophiles, we have found the use of tetrabutyl ammonium bromide (TBAB) as PTC can afford isothiocyanates in good yield. In this paper, we have conducted our reaction using TBAB as PTC to synthesize the thiophenoyl, butanoyl, benzoyl, para-nitrobenzoyl and morpholinoyl thiourea derivatives bearing benzothiazole moiety. All the structures of newly synthesized compounds were assigned on the basis of their elemental analysis and spectroscopic data, IR and ^1H NMR. All the compounds were soluble in DMF, DMSO, ethanol and ethyl acetate. IR (KBr) spectrum of all the synthesized compounds had strong N–H absorptions at about 3300 cm^{-1} , and displayed absorptions at about $1670\text{--}1710\text{ cm}^{-1}$, 1440 cm^{-1} , which were assigned to $\text{C}=\text{O}$, and $\text{C}=\text{S}$ functions respectively. The medium strong $\nu_{\text{C}=\text{O}}$ band in the IR spectra of all the compounds appeared at $1670\text{--}1710\text{ cm}^{-1}$, which is lower than that of the ordinary carbonyl absorption (1730 cm^{-1}). The formation of H-bond leads an increase of their polarity, so the strength of their double bond decreased, and absorption moved to lower wave number. The ^1H NMR spectrum exhibited broad signals at $10.50\text{--}13.75\text{ ppm}$, which were assigned to the N–H protons. ^{13}C NMR showed peaks at δ 168.29–169.09, 178.05–180.01 ppm for $\text{C}=\text{O}$ (amide) and $\text{C}=\text{S}$ (thioamide), respectively for all the target compounds.



Scheme 1. Preparation of thiourea derivatives bearing benzothiazole moiety.

3. Results and discussion

3.1. Antimicrobial activities

In the light, interesting antimicrobial activities of thiourea derivatives were screened for antibacterial and anti-fungal activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Candida albicans*, *Candida glabrata* and *Candida tropicalis* by the broth micro-dilution procedure. The Gram-positive antibacterial agent, amikacin, the Gram-negative antibacterial agent, gentamycin, and the anti-fungal agent, nystatin, were used as controls. The *in vitro* antimicrobial properties against a number of Gram-negative and Gram-positive bacteria, and yeasts are presented in Tables 1–3, respectively.

All the compounds inhibited the growth of bacteria with MIC values ranging between 10 and $100\text{ }\mu\text{g/ml}$ and showed anti-yeast activity with MICs between 5 and $50\text{ }\mu\text{g/ml}$. Compounds which showed MIC of $>100\text{ }\mu\text{g/ml}$ or above have not been included for the discussion. According to the antimicrobial studies, all the compounds showed such activity, albeit lower than their anti-yeast efficacy. This difference may be due to the differences between the cell structure of bacteria and yeast. While the cell wall of fungi contain chitin, the cell wall of bacteria contains murein [17]. In addition, fungi contain ergosterol in their cell membranes instead of the cholesterol found in the cell membranes of animals [23]. When all the anti-yeast MIC values are compared, four of twenty compounds show good activity against *C. glabrata* and ten of twenty compounds showed low activity against *C. tropicalis*. According to the antibacterial studies, the efficacy against Gram-negative is higher than Gram-positive bacteria. Seven of twenty compounds showed good activity against *S. epidermidis*. In addition, **2b** and **5b** showed high activity against Gram-positive, Gram-negative bacteria and fungi. The value of the investigated compounds in this research were higher than that reported for other thiourea derivatives [24–26]. The main difference in the thiourea derivatives reported in this paper is the presence of the benzothiazole moiety.

From the results of the antimicrobial activity of the synthesized substituted thiourea derivatives, the following structure–activity relationships (SAR) can be derived:

- In general, it was observed that most of the compounds with substituted benzothiazole rings showed better antimicrobial activity than the ones with a non-substituted benzothiazole ring.
- The high antimicrobial activity of compounds **2b** and **5b** in comparison to compounds **1a–5a** may be attributed to the

Table 1

MIC values ($\mu\text{g/ml}$) of the synthesized thiourea derivatives against the tested Gram-negative bacteria.

Compound code	<i>E. cloacae</i> (ATCC 13047)	<i>P. aeruginosa</i> (ATCC 27853)	<i>E. coli</i> (ATCC 25922)	<i>P. vulgaris</i> (ATCC 13315)
1b	25	50	50	25
1c	25	50	25	25
2a	50	50	25	50
2b	15	20	15	20
2c	50	50	20	25
2d	50	25	25	50
3b	25	20	20	15
3c	50	50	50	50
4b	20	20	25	20
4d	50	50	25	50
5a	25	50	25	25
5b	15	25	15	20
5c	25	50	20	20
5d	50	50	25	50
Gentamycin	2	1	0.5	2

Table 2MIC values ($\mu\text{g/ml}$) of the synthesized thiourea derivatives against the tested Gram-positive bacteria.

Compound code	<i>S. epidermidis</i> (ATCC 12228)	<i>S. aureus</i> (ATCC 29213)	<i>E. faecalis</i> (ATCC 29212)	<i>S. aureus</i> (ATCC 25923)
1b	25	50	50	50
1c	50	50	50	50
1d	50	50	50	50
2a	50	50	50	50
2b	10	25	15	25
3b	20	25	50	15
3c	50	50	50	50
3d	50	50	50	50
4b	50	50	50	50
4c	50	50	50	50
4d	50	50	50	50
5a	25	50	50	50
5b	10	25	10	20
5c	20	25	50	50
5d	25	25	50	50
Amikacin	0.5	2	4	2

presence of nitro functional groups on benzothiazole ring. These electron withdrawing groups may decrease electron density on benzene ring through resonance effect. The synthesized compounds containing nitro functional groups on benzothiazole ring are highly active not only against Gram-positive, but also against Gram-negative bacteria and fungi (**2b** and **5b**).

- c) In contrast to point (b) mentioned above, the presence of electron donating amino group also showed significant antifungal activity against *C. glabrata*, *C. albicans*, and *C. tropicalis* in case of **5c**. Here the electronic factors is not justified. The other reason may be due to low lipophilic character ($\log P_{ow} \sim 0.39 \pm 0.97$) of the compound.

However, the thiophene and morpholine based thiourea derivatives (**2b** and **5b**) containing nitro group as electron withdrawing group on benzothiazole nucleus showed good antimicrobial activity. These electron withdrawing groups are also present in benzothiazole rings for **3b** and **4b** thiourea derivatives but their antimicrobial activity is comparatively lower than **2b** and **5b**. Lipophilicity is another factor, which correlates well with the bioactivity of chemicals, is a very important molecular descriptor and different lipophilic behavior of compounds plays an important role in their biological activity mechanisms. The *n*-octanol/water partition coefficient ($\log P_{ow}$) is widely used as a general measure of

Table 3MIC values ($\mu\text{g/ml}$) of the synthesized thiourea derivatives against the tested fungi.

Compound code	<i>C. glabrata</i> (ATCC32554)	<i>C. albicans</i> (ATCC 90028)	<i>C. tropicalis</i> (ATCC 20336)	<i>C. krusei</i> (ATCC 6268)
1b	25	25	25	25
1c	50	50	25	50
1d	50	50	50	50
2a	25	50	50	25
2b	10	15	15	20
2c	20	25	25	25
3a	25	50	50	50
3b	15	25	50	50
3c	25	25	50	25
3d	25	25	50	50
4a	50	20	50	25
4b	25	20	50	25
4c	50	20	50	50
4d	50	25	50	50
5a	25	25	25	25
5b	5	15	5	20
5c	15	20	20	25
5d	25	50	25	50
Nystatin	2	1	4	0.5

Table 4

Preliminary MTT cytotoxicity screening of synthesized thiourea derivatives at 24 h of drug exposure.

Compounds code	IC ₅₀ (μM)	
	MCF-7	HeLa
1a	33.81	62.56
1b	35.23	68.47
1c	33.20	63.11
1d	32.40	56.53
2a	32.63	68.25
2b	35.85	72.56
2c	28.41	56.10
2d	24.15	46.46
3a	37.27	57.47
3b	41.64	75.13
3c	38.61	70.09
3d	32.11	64.56
4a	36.55	56.70
4b	>100 μM	NT
4c	44.73	NT
4d	45.72	60.18
5a	29.31	51.80
5b	31.00	54.80
5c	26.43	45.29
5d	18.10	38.85
Doxorubicin	2.62	3.24

The known numbers of cells (1.0×10^4) were incubated for 24 h in a 5% CO₂ incubator at 37 °C in the presence of different concentrations of test compounds. After 24 h of drug incubation the MTT solution was added and supernatant was discarded and 100 μl DMSO was added in each well and absorbance was recorded at 540 nm by ELISA reader.

NT: Not Tested.

lipophilicity. Compounds with 4-nitrophenyl (**1b**) and phenyl group (**3b**) have relatively higher $\log P_{ow}$ values of 3.37 ± 0.91 and 3.33 ± 0.92 , respectively and hence show more lipophilic character [27]. The calculated value of partition coefficient ($\log P_{ow}$) for *n*-butyl derivative (**4b**) is 2.68 ± 0.91 . Its antibacterial activity is comparatively lower than the compounds (**5b**) and (**2b**) due to its higher partition coefficient ($\log P_{ow}$) value. The compounds with a morpholine ring (**5b**) and thiophene ring (**2b**), which have the $\log P_{ow}$ value of 0.62 ± 0.97 and 2.97 ± 0.93 , respectively show higher antibacterial activity than other investigated compounds due probably to their lower lipophilic character.

3.2. Anticancer studies

3.2.1. Cytotoxicity studies on cancerous MCF-7 and HeLa cell lines

In vitro cytotoxicity of synthesized compounds was assessed by standard 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) bioassay in different cancer cells at 24 h of drug exposure. To assess the efficacy and to select the promising compounds human cancer cells (HeLa cells and MCF-7) were used. In MCF-7 cells, the IC₅₀ values for thiourea derivatives bearing benzothiazole moiety were in the range of 18.10–45.72 μM (Table 4). Similarly, in HeLa cells, the IC₅₀ values for thiourea derivatives were found in the range of 38.85–75.13 μM . The compounds **4b** and **4c** do not show 50% inhibition even at a concentration of 100 μM ; hence

Table 5

Cytotoxic activity of promising thiourea derivatives in MCF-7 cells at different time points of drug exposure by MTT assay.

Compounds code	IC ₅₀ (μM)		
	24 h	48 h	72 h
5d	18.10	20.33	6.95
2d	24.15	15.65	12.10
5c	26.43	19.18	17.05

The IC₅₀ values of promising compounds at 48 h and 72 h were significantly reduced in comparison with 24 h values.

Table 6

The effect of **2d** and **5d** on DNA damaging in MCF-7 cells at 24 h of drug exposure by comet assay.^a

Compounds	Tail length	Tail DNA	Tail moment	OTM
2d				
10 μ M	103.25 \pm 7.016	15.36 \pm 1.42	16.87 \pm 2.21	21.87 \pm 1.09
20 μ M	107.56 \pm 7.87	17.65 \pm 1.85	18.09 \pm 2.89	23.35 \pm 1.85
40 μ M	134.10 \pm 5.24	22.31 \pm 1.63	86.63 \pm 4.26	102 \pm 15.57
50 μ M	305.30 \pm 32.12 ^c	31.10 \pm 2.01 ^c	105.52 \pm 14.75 ^c	139.80 \pm 24.09 ^c
5d				
10 μ M	97.10 \pm 4.78	14.63 \pm 1.105	15.01 \pm 1.96	18.35 \pm 9.95
20 μ M	110.27 \pm 6.46	16.10 \pm 1.41 ^c	18.11 \pm 3.0	21.01 \pm 5.98
40 μ M	155.03 \pm 7.81	18.78 \pm 1.58 ^c	31.87 \pm 4.21 ^b	24.20 \pm 7.25
50 μ M	195.27 \pm 7.11 ^c	23.62 \pm 2.21 ^c	55.15 \pm 4.96 ^c	32.33 \pm 2.24 ^c
Control ^d	65.56 \pm 3.23	9.75 \pm 1.16	7.18 \pm 1.05	8.83 \pm 0.83

^a All values are means \pm SEM.

^b $P < 0.05$ compared to control.

^c $P < 0.01$ compared to control.

^d Doxorubicin as control.

they were not evaluated in HeLa cells. Present study reveals that among the human cancer cell lines tested, MCF-7 cells are more sensitive to all the tested compounds than HeLa cells. Many anti-cancer drugs are effective against MCF-7 and HeLa cells by causing apoptosis through the expression of caspase-3, generating reactive oxygen species (ROS) and damaging DNA [28]. Cisplatin causes cytotoxicity in MCF-7 and HeLa cells by a similar mechanism [29]. Chemotherapeutic agents such as doxorubicin, mitoxantrone and bleomycin cause cytotoxicity by generating ROS [30]. Hence like other cytotoxic drugs (doxorubicin, mitoxantrone and cisplatin), the synthesized compounds may act as effective anticancer drugs by similar mechanism. Previous studies have shown that strong electronegative atom substitution such as chloro/bromo at the para position of the aromatic ring increases the lipophilicity of molecules and is responsible for enhanced cytotoxicity in MTT model [31]. Similar substitutions are present in the compounds **1d**, **2d**, **3d**, **4d** and **5d**. We have also observed enhanced cytotoxicity in these molecules. Hence, these molecules were taken up to assess the cytotoxic potency at different intervals in MCF-7 cells.

In the MTT time course study, the selected compounds (**2d**, **5c**, **5d**) showed dose-dependent and time-dependent activities. The previous study reported that the most potent fluorinated benzothiazole, 5F 203, produced apoptosis and DNA damage in MCF-7 cells, which is characteristic of cytotoxic activity [32,33]. In our present study, also MTT assays revealed substantial cytotoxicity in MCF-7 cells with increasing exposure to drug concentration, the IC₅₀ values of promising compounds at 48 and 72 h were significantly reduced as compared with 24 h values (Table 5)

3.2.2. DNA damaging studies

To determine the DNA damaging activity of synthesized compounds, alkaline comet assay was performed in MCF-7 cells. Comet assays is a rapid and inexpensive method for measuring DNA single-strand breaks (SSBs). It also has an advantage over the other

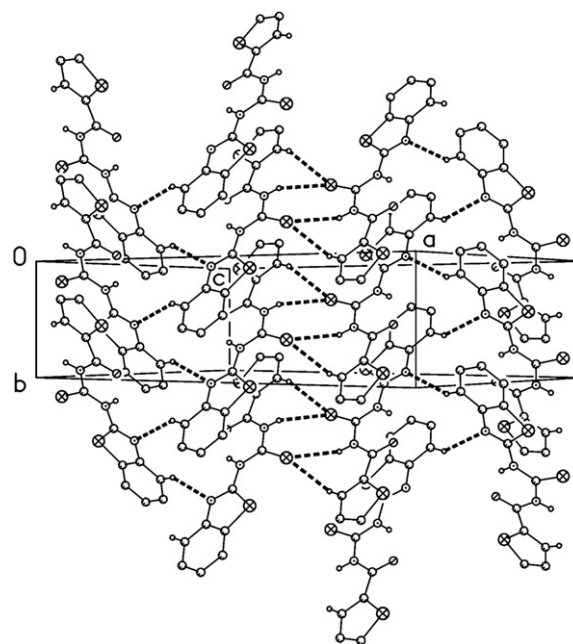


Fig. 2. Packing diagram of 1-(1,3-benzothiazol-2-yl)-3-(thiophene-5-carbonyl)thiourea (**2a**) viewed perpendicular to (101). Hydrogen bonds are indicated by thick dashed lines.

DNA damage-detecting methods, such as sister chromatid exchange, alkali elution, and micronucleus assay, because of its high sensitivity [34]. A number of cytotoxic compounds (doxorubicin, cisplatin etc.) act as anticancer drugs by causing DNA damage and subsequently inducing apoptosis in cancerous cells. 24 h treatment with **2d** and **5d** produced a dose dependent increase in tail moment. The damage produced by **2d** was quite prominent as at 50 μ M it produced apoptotic bodies and tail moment was 105.52. Elongated tail length and reduced DNA content in head are sufficient indicators of DNA damage, and higher degree of damage would result in greater number of smaller fragments, ending up

Table 7

Crystallographic data, data collection and refinement of **2a** structure.

CCDC	738482	
Empirical formula	C ₁₃ H ₉ N ₃ OS ₃	
Formula weight	319.41	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	C2/c	
Unit cell dimensions	$a = 24.6305(6)$ Å $b = 5.88932(11)$ Å $c = 18.7899(4)$ Å	$\alpha = 90^\circ$ $\beta = 102.506(3)^\circ$ $\gamma = 90^\circ$
Volume	2660.93(10) Å ³	
Z	8	
Density (calculated)	1.595 g/cm ³	
Absorption coefficient	0.554 mm ⁻¹	
$F(000)$	1312	
Crystal size	0.20 \times 0.15 \times 0.05 mm ³	
Theta range for data collection	2.22–30.03°	
Index ranges	–34 $\leq h \leq$ 34, –8 $\leq k \leq$ 8, –26 $\leq l \leq$ 26	
Reflections collected	33722	
Independent reflections	3851 [R(int) = 0.0291]	
Completeness to theta = 30.03°	98.9%	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	1.00000 and 0.98475	
Refinement method	Full-matrix least-squares on F^2	
Data/restraints/parameters	3851/0/189	
Goodness-of-fit on F^2	0.994	
Final R indices [I > 2sigma(I)]	R1 = 0.0258, wR2 = 0.0670	
R indices (all data)	R1 = 0.0344, wR2 = 0.0685	
Largest diff. peak and hole	0.409 and –0.333 e Å ⁻³	

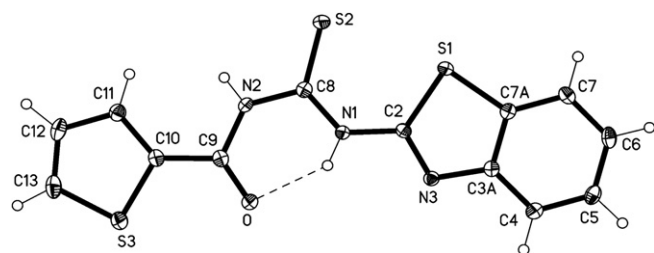


Fig. 1. An ORTEP drawing of 1-(1,3-benzothiazol-2-yl)-3-(thiophene-5-carbonyl)thiourea (**2a**) with displacement ellipsoids plotted at 50% probability level. The dotted line shows the intramolecular H-bonding interaction.

Table 8

Selected bond distances (Å), angles (°) and torsion angles (°).

S (1)–C (2)	1.7528(11)
C (2)–N (3)	1.2973(14)
C (2)–N (1)	1.3913(14)
N (3)–C (3A)	1.3898(15)
C (3A)–C (4)	1.4024(16)
S (3)–C (13)	1.7018(13)
S (3)–C (10)	1.7171(12)
C (9)–O	1.2249(14)
C (10)–C (11)	1.3804(16)
C (7A)–S (1)–C (2)	87.69(5)
N (3)–C (2)–N (1)	117.47(10)
C (10)–C (11)–C (12)	112.63(11)
C (13)–S (3)–C (10)	91.71(6)
C (7A)–S (1)–C (2)–N (3)	–0.65(10)
S (1)–C (2)–N (1)–C (8)	6.54(17)
N (1)–C (2)–N (3)–C (3A)	–178.72(10)
C (2)–N (3)–C (3A)–C (4)	–179.26(11)
N (3)–C (2)–N (1)–C (8)	–174.31(11)
C (7A)–S (1)–C (2)–N (1)	178.49(10)
S (1)–C (2)–N (3)–C (3A)	0.49(12)
N (1)–C (8)–N (2)–C (9)	–0.31(18)

with longer tails. The compound **5d** significantly increased the tail moment and tail length in MCF-7 cells. Previous studies have shown that cytotoxicity of benzothiazoles is mediated via activation of the AhR signaling pathway in sensitive MCF-7 cells [35]. The activation of AhR pathway leads to generation of reactive electrophilic species by inducing CYP1A1 expression. The generated highly reactive intermediates cause DNA damage, ultimately resulting in cell death by activation of apoptotic machinery [36]. Hence our compounds may be acting by similar mechanisms, which, however, need to be confirmed by gene expression studies. (Table 6)

4. Crystal structure of 2a

The molecular structure and packing diagram of the compound **2a** are shown in Figs. 1 and 2, respectively. Crystallographic data and refinement are presented in Table 7. Selected bond distances and angles are listed in Table 8. Compound **2a**, C₁₃H₉N₃OS₃, crystallizes in the thioamide forms. The hydrogens at N1 and N2 are *anti* and *syn* respectively with respect to the C = S bond. Bond lengths and angles may be regarded as normal. The entire molecule is planar to within a mean deviation of 0.04 Å (for non-H atoms). The C9–O and C8–S2 bonds show a typical double bond character with bond lengths of 1.2249(14) and 1.6648(12) Å, respectively. All the C–N bonds, C8–N1 = 1.3426(15), C8–N2 = 1.3837(15), C2–N3 = 1.2973(14) Å display at least partial double bond character. Thiophene and benzimidazole units are planar. There is a strong intramolecular hydrogen bond N1–H01...O, with H01...O = 1.962(16) Å, forming a 6-membered ring. The molecular packing is three-dimensional, but can be interpreted reasonably well by viewing perpendicular to the (101) plane (Fig. 2); the weak H bonds H02...LS2 2.92 Å and H11...LS2 2.78 Å can be recognised connecting columns molecules in the centre of the Figure and H4...LN3 2.55 Å left and right of the central column.

5. Conclusions

An *in vitro* screen led to the identification of compounds **2d**, **5c** and **5d** as potential anticancer candidates worthy of further structural modification and pharmacological evaluation. Moreover, the antimicrobial activity of this series suggests the benzothiazole-thiourea core offers a novel template for the development of a new class of antimicrobial agents.

6. Experimental protocols

6.1. Chemistry

Synthetic starting material, reagents and solvents were of analytical reagent grade or of the highest quality commercially available and were purchased from Aldrich Chemical Co., Merck Chemical Co. and were dried when necessary. Melting points were recorded on Electrothermal IA9000 series digital melting point apparatus. The proton NMR and ¹³C spectra were recorded in DMSO-*d*₆ solvent on Jeol ECS-400 and 300 MHz spectrophotometer using tetramethylsilane as an internal reference, respectively. The apparent resonance multiplicity is described as s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet) and m (multiplet). Infrared measurements were recorded in the range 400–4000 cm^{–1} on spectrum 2000 by Perkin Elmer. Elemental analysis was carried out using Perkin Elmer CHNS/O 2400. Obtained results were within 0.4% of the theoretical values. Mass spectra were recorded on a MAT-112-S spectrometer at 70 eV. X-ray diffraction data were collected on Oxford Diffraction Xcalibur Nova diffractometer. Thin layer chromatography (TLC) analysis was carried out on 5 × 20 cm plate coated with silica gel GF₂₅₄ type 60 (25–250 mesh) using an ethyl acetate–petroleum ether mixture (1:2) as solvent.

6.2. X-ray crystallography

A crystal of **2a** in the form of a yellow lath was mounted in inert oil on a glass fibre. The intensity data were collected at 100 K on an Oxford Diffraction Xcalibur E diffractometer using ω -scan mode with graphite monochromatized MoK α radiation. The structure was solved by direct methods and refined by full-matrix least-squares techniques on *F*² using the program SHELXL-97 [41]. Hydrogens of NH groups were refined freely, other H atoms using a riding model.

6.3. General procedure for synthesis

All the synthesized compounds were prepared according to the following two-step procedures:

- Step 01: To substituted aniline (25 mL), concentrated hydrochloric acid (25 mL) was added and the solution was warmed for 30 min. A saturated solution of ammonium thiocyanate in water (30 g in 60 mL) was added slowly in above solution. The mixture was boiled until the solution got turbid. The turbid solution was poured in cold water. The resulting precipitate was filtered and re-crystallized from aqueous ethanol (80%) to provide pure phenylthiourea. The substituted phenylthiourea (26 mmol) in chloroform (75 mL) was brominated by using bromine solution in chloroform (5%) till the orange-yellow color appeared. The slurry was kept overnight. The precipitate obtained was filtered and washed with chloroform until the color disappeared. The precipitate, as hydrobromide, was dissolved in rectified spirit (150 mL) and basified with ammonia solution. The precipitated was filtered, washed with water, dried and re-crystallized using ethanol:dichloromethane mixture (1:2). Substituted or unsubstituted 2-amino-benzothiazole prepared by this method was named as (II)
- Step 02: A solution of substituted carbonyl chloride (26 mmol) in anhydrous acetone (80 mL) and 3% TBAB in acetone was added drop wise to a suspension of ammonium thiocyanate in acetone (50 mL) and the reaction mixture was refluxed for 30 min. After cooling at room temperature, a solution of substituted or unsubstituted 2-amino-benzothiazole (26 mmol) in acetone (25 mL) was added

and the resulting mixture refluxed for 1.5 h. The reaction mixture was poured into five times its volume of cold water when the thiourea precipitated as a solid. The solid product was washed with water and purified by re-crystallization from an ethanol–dichloromethane mixture (1:2).

6.3.1. 1-(Benzo[d]thiazol-2-yl)-3-(4-nitrobenzoyl) thiourea (**1a**)

Elemental analysis for $C_{15}H_{10}N_4O_3S_2$ (MW = 358.39) in wt% calc. C = 50.27, H = 2.79, N = 15.64, S = 17.87 and found to be C = 50.35, H = 2.95, N = 15.63, S = 17.84. m.pt. 278 °C, yield 89%. IR (KBr pellet) in cm^{-1} : 3310 (free NH), 3206 (assoc NH), 1670 (C=O), 1525 (benzene ring), 1404 (C–N stretching), 1512 (NO₂), 1140 (C=S). ¹H NMR (400 MHz, DMSO-*d*₆) in δ (ppm) and *J* (Hz): 13.53 (1H, s, broad, NH), 10.25 (1H, s, broad, NH), 7.75 (2H, d, *J* = 8.2 Hz), 7.65 (2H, d, *J* = 6.9 Hz), 7.34 (H, d, *J* = 8.7 Hz); ¹³C NMR (300 MHz, DMSO-*d*₆) in δ (ppm): 179.02 (C=S), 173.3 (C=N), 168.29 (C=O), 151.8, 149.2, 144.5, 130.6, 128.2, 122.7, 120.9; EI MS (70 eV) *m/z* (%): 358.41

6.3.2. 1-(5-Nitrobenzo[d]thiazol-2-yl)-3-(4-nitrobenzoyl) thiourea (**1b**)

Elemental analysis for $C_{15}H_9N_5O_5S_2$ (MW = 403.39) in wt% calc. C = 44.66, H = 2.23, N = 17.36, S = 15.88 and found to be C = 44.63, H = 2.25, N = 17.35, S = 15.89. m.pt. 298 °C, yield 73%. IR (KBr pellet) in cm^{-1} : 3313 (free NH), 3203 (assoc NH), 1678 (C=O), 1520 (benzene ring), 1405 (C–N stretching), 1512 (NO₂), 1141 (C=S). ¹H NMR (400 MHz, DMSO-*d*₆) δ : 13.85 (1H, s, broad, NH), 10.40 (1H, s, broad, NH), 8.12 (H, d, *J* = 8.63 Hz), 7.74 (2H, d, *J* = 8.1 Hz), 7.66 (2H, d, *J* = 6.7 Hz); ¹³C NMR (300 MHz, DMSO-*d*₆) in δ (ppm): 179.01 (C=S), 173.3 (C=N), 168.51 (C=O), 150.6, 148.3, 145.2, 128.8, 122.9, 114.5; EI MS (70 eV) *m/z* (%): 403.38

6.3.3. 1-(5-Aminobenzo[d]thiazol-2-yl)-3-(4-aminobenzoyl) thiourea (**1c**)

This compound was prepared from **1b** by the following reduction procedure. **1b** (4.13 g, 10 mmol), 5 ml hydrazine monohydrate, 70 ml ethanol and 0.03 g of 10% Pd–C was transferred into 250 ml two necked round bottom flask and refluxed for 18 h. The reaction was monitored by TLC. After completion the reaction, it was allowed to stand for one day and then filtered the reaction mixture. Removed the solvent by rotary evaporator. The crude product was re-crystallized in ethanol. Elemental analysis for $C_{15}H_{11}N_5O_3S_2$ (MW = 373.42) in wt% calc. C = 48.25, H = 2.94, N = 18.76, S = 17.15 and found to be C = 47.98, H = 2.96, N = 18.85, S = 17.12. m.pt. 232 °C, yield 70%. IR (KBr pellet) in cm^{-1} : 3310 (free NH), 3206 (assoc NH), 1670 (C=O), 1525 (benzene ring), 1404 (C–N stretching), 1140 (C=S). ¹H NMR (400 MHz, DMSO-*d*₆) in δ (ppm) and *J* (Hz): 13.11 (1H, s, broad, NH), 10.25 (1H, s, broad, NH), 7.73 (2H, d, *J* = 8.0 Hz), 7.63 (2H, d, *J* = 6.8 Hz), 6.62 (H, d, *J* = 8.44 Hz), 5.18 (2H, s, NH₂); ¹³C NMR (300 MHz, DMSO-*d*₆) in δ (ppm): 178.05 (C=S), 173.0 (C=N), 168.29 (C=O), 151.3, 148.5, 145.8, 130.7, 128.4, 122.1, 120.8, 116.9; EI MS (70 eV) *m/z* (%): 373.45

6.3.4. 1-(5-Bromobenzo[d]thiazol-2-yl)-3-(4-nitrobenzoyl) thiourea (**1d**)

Elemental analysis for $C_{15}H_9BrN_4O_3S_2$ (MW = 437.29) in wt% calc. C = 41.18, H = 2.05, N = 12.81, S = 14.64 and found to be C = 41.17, H = 2.08, N = 12.83, S = 14.63. m.pt. 247–248 °C, yield 79%. IR (KBr pellet) in cm^{-1} : 3309 (free NH), 3206 (assoc NH), 1669 (C=O), 1525 (benzene ring), 1404 (C–N stretching), 1511 (NO₂), 1140 (C=S). ¹H NMR (400 MHz, DMSO-*d*₆) in δ (ppm) and *J* (Hz): 13.75 (1H, s, broad, NH), 12.40 (1H, s, broad, NH), 7.75 (2H, d, *J* = 8.2 Hz), 7.65 (2H, d, *J* = 6.9 Hz), 6.61 (H, d, *J* = 8.40 Hz); ¹³C NMR

(300 MHz, DMSO-*d*₆) in δ (ppm): 179.02 (C=S), 173.3 (C=N), 168.29 (C=O), 150.9, 129.1, 128.4, 125.0, 124.1, 123.1, 116.5; EI MS (70 eV) *m/z* (%): 437.30

6.3.5. 1-(1, 3-Benzothiazol-2-yl)-3-(thiophene-5-carbonyl) thiourea (**2a**)

Elemental analysis for $C_{13}H_9N_3OS_3$ (MW = 319.41) in wt% calc. C = 45.08, H = 2.82, N = 13.16, S = 30.09 and found to be C = 45.10, H = 2.85, N = 13.15, S = 30.20. m.pt. 185 °C, yield 90%. IR (KBr pellet) in cm^{-1} : 3309 (free NH), 3206 (assoc NH), 1672 (C=O), 1523 (benzene ring), 1405 (C–N stretching), 1140 (C=S), 1450, 1250, 1020 (benzothiazole stretching). ¹H NMR (400 MHz, DMSO-*d*₆) in δ (ppm) and *J* (Hz): 12.40 (1H, s, broad, NH), 10.25 (1H, s, broad, NH), 8.10 (H, d, *J* = 8.41 Hz), 7.34 (H, d, *J* = 8.7 Hz), 7.31 (H, d, *J* = 8.0 Hz); ¹³C NMR (300 MHz, DMSO-*d*₆) in δ (ppm): 179.02 (C=S), 172.2 (C=N), 168.29 (C=O), 149.4, 136.5, 128.5, 125.7, 122.3; EI MS (70 eV) *m/z* (%): 319.40

6.3.6. 1-(5-nitro[d]benzothiazol-2-yl)-3-(thiophene-5-carbonyl) thiourea (**2b**)

Elemental analysis for $C_{13}H_8N_4O_3S_3$ (MW = 364.37) in wt% calc. C = 42.85, H = 2.19, N = 15.38, S = 26.37 and found to be C = 42.81, H = 2.20, N = 15.36, S = 26.35. m.pt. 225 °C, yield 85%. IR (KBr pellet) in cm^{-1} : 3315 (free NH), 3204 (assoc NH), 1670 (C=O), 1525 (benzene ring), 1512 (NO₂), 1404 (C–N stretching), 1142 (C=S). ¹H NMR (400 MHz, DMSO-*d*₆) in δ (ppm) and *J* (Hz): 12.40 (1H, s, broad, NH), 10.25 (1H, s, broad, NH), 8.12 (H, d, *J* = 8.63 Hz), 7.34 (2H, d, *J* = 8.7 Hz), 7.31 (2H, d, *J* = 8.0 Hz); ¹³C NMR (300 MHz, DMSO-*d*₆) in δ (ppm): 180.01 (C=S), 174.2 (C=N), 168.29 (C=O), 149.7, 145.5, 137.0, 135.7, 130.3, 128.4, 123.5, 120.7; EI MS (70 eV) *m/z* (%): 364.39

6.3.7. 1-(5-Aminobenzo[d]thiazol-2-yl)-3-(thiophene-5-carbonyl) thiourea (**2c**)

This compound was prepared from **2b** by the following reduction procedure. **2b** (3.65g, 10 mmol), 5 ml hydrazine monohydrate, 70 ml ethanol and 0.03 g of 10% Pd–C was transferred into 250 ml two necked round bottom flask and refluxed for 18 h. The reaction was monitored by TLC. After completion the reaction, it was allowed to stand for one day and then filtered the reaction mixture. Removed the solvent by rotary evaporator. The crude product was re-crystallized in ethanol. Elemental analysis for $C_{13}H_{10}N_4O_3S_3$ (MW = 334.12) in wt% calc. C = 46.70, H = 2.99, N = 16.76, S = 28.74 and found to be C = 46.85, H = 3.01, N = 16.75, S = 28.75. m.pt. 191 °C, yield 80%. IR (KBr pellet) in cm^{-1} : 3315 (free NH), 3204 (assoc NH), 1671 (C=O), 1525 (benzene ring), 1407 (C–N stretching), 1142 (C=S). ¹H NMR (400 MHz, DMSO-*d*₆) in δ (ppm) and *J* (Hz): 12.40 (1H, s, broad, NH), 10.25 (1H, s, broad, NH), 7.35 (2H, d, *J* = 8.5 Hz), 7.30 (2H, d, *J* = 8.1 Hz), 6.60 (H, d, *J* = 8.42 Hz), 5.18 (2H, s, NH₂), 4.28 (aromatic C–NH); ¹³C NMR (300 MHz, DMSO-*d*₆) in δ (ppm): 180.01 (C=S), 174.2 (C=N), 168.29 (C=O), 149.7, 145.5, 137.0, 135.7, 130.3, 128.4, 122.3, 120.7; EI MS (70 eV) *m/z* (%): 334.15

6.3.8. 1-(5-Bromobenzo[d]thiazol-2-yl)-3-(thiophene-5-carbonyl) thiourea (**2d**)

Elemental analysis for $C_{13}H_8BrN_3OS_3$ (MW = 398.68) in wt% calc. C = 39.19, H = 2.01, N = 10.55, S = 24.12 and found to be C = 39.23, H = 2.36, N = 10.56, S = 24.27. m.pt. 182 °C, yield 69%. IR (KBr pellet) in cm^{-1} : 3315 (free NH), 3204 (assoc NH), 1670 (C=O), 1525 (benzene ring), 1404 (C–N stretching), 1142 (C=S). ¹H NMR (400 MHz, DMSO-*d*₆) in δ (ppm) and *J* (Hz): 12.04 (1H, s, broad, NH), 10.32 (1H, s, broad, NH), 7.34 (2H, d, *J* = 8.7 Hz), 7.31 (2H, d, *J* = 8.0 Hz), 6.61 (H, d, *J* = 8.40 Hz); ¹³C NMR (300 MHz, DMSO-*d*₆) in δ (ppm): 180.01 (C=S), 174.2 (C=N), 168.29 (C=O), 149.5, 145.5, 137.0, 135.7, 130.3, 128.4, 122.8, 120.1; EI MS (70 eV) *m/z* (%): 398.69

6.3.9. 1-(1, 3-Benzothiazol-2-yl)-3-benzoylthiourea (**3a**)

Elemental analysis for $C_{15}H_{11}N_3OS_2$ (MW = 313.39) in wt% calc. C = 57.50, H = 3.51, N = 13.41, S = 20.44 and found to be C = 57.43, H = 3.53, N = 13.40, S = 20.45. m.pt. 203–205 °C, yield 78%. IR (KBr pellet) in cm^{-1} : 3310 (free NH), 3206 (assoc NH), 1670 (C=O), 1525 (benzene ring), 1404 (C–N stretching), 1140 (C=S). 1H NMR (400 MHz, DMSO- d_6) in δ (ppm) and J (Hz): 12.40 (1H, s, broad, NH), 10.25 (1H, s, broad, NH), 7.75 (2H, d, J = 8.2 Hz), 7.65 (2H, d, J = 6.9 Hz), 7.34 (H, d, J = 8.7 Hz), 7.31 (H, d, J = 8.0 Hz); ^{13}C NMR (300 MHz, DMSO- d_6) in δ (ppm): 179.02 (C=S), 168.29 (C=O), 151.7, 148.9, 144.5, 142.1, 136.2, 128.4, 127.3; EI MS (70 eV) m/z (%): 313.37

6.3.10. 1-Benzoyl-3-(5-nitrobenzo[d]thiazol-2-yl) thiourea (**3b**)

Elemental analysis for $C_{15}H_{10}N_4O_3S_2$ (MW = 358.39) in wt% calc. C = 50.27, H = 2.79, N = 15.64, S = 17.87 and found to be C = 50.31, H = 2.84, N = 15.64, S = 17.85. m.pt. 203–205 °C, yield 72%. IR (KBr pellet) in cm^{-1} : 3310 (free NH), 3206 (assoc NH), 1671 (C=O), 1527 (benzene ring), 1406 (C–N stretching), 1512 (NO₂), 1140 (C=S). 1H NMR (400 MHz, DMSO- d_6) in δ (ppm) and J (Hz): 12.40 (1H, s, broad, NH), 10.20 (1H, s, broad, NH), 8.12 (d, H, J = 8.63 Hz), 7.75 (2H, d, J = 8.2 Hz), 7.65 (2H, d, J = 6.9 Hz), 7.34 (2H, d, J = 8.7 Hz); ^{13}C NMR (300 MHz, DMSO- d_6) in δ (ppm): 179.02 (C=S), 168.29 (C=O), 151.8, 149.2, 144.0, 142.8, 134.6, 128.2, 127.9; EI MS (70 eV) m/z (%): 358.41

6.3.11. 1-Benzoyl-3-(5-aminobenzo[d]thiazol-2-yl) thiourea (**3c**)

This compound was prepared from **3b** by the following reduction procedure. **3b** (3.60 g, 10 mmol), 5 ml hydrazine monohydrate, 70 ml ethanol and 0.03 g of 10% Pd–C was transferred into 250 ml two necked round bottom flask and refluxed for 18 h. The reaction was monitored by TLC. After completion the reaction, it was allowed to stand for one day and then filtered the reaction mixture. Removed the solvent by rotary evaporator. The crude product was re-crystallized in ethanol. Elemental analysis for $C_{15}H_{12}N_4OS_2$ (MW = 328.41) in wt% calc. C = 54.87, H = 3.65, N = 17.07, S = 19.51 and found to be C = 54.90, H = 3.70, N = 17.10, S = 19.48. m.pt. 203–205 °C, yield 82%. IR (KBr pellet) in cm^{-1} : 3313 (free NH), 3206 (assoc NH), 1672 (C=O), 1523 (benzene ring), 1404 (C–N stretching), 1141 (C=S). 1H NMR (400 MHz, DMSO- d_6) in δ (ppm) and J (Hz): 12.45 (1H, s, broad, NH), 10.20 (1H, s, broad, NH), 7.73 (2H, d, J = 8.1 Hz), 7.62 (2H, d, J = 6.8 Hz), 6.63 (H, d, J = 8.40 Hz), 5.18 (2H, s, NH₂); ^{13}C NMR (300 MHz, DMSO- d_6) in δ (ppm): 179.02 (C=S), 168.29 (C=O), 151.8, 149.3, 144.5, 142.7, 125.4, 128.4, 127.0; EI MS (70 eV) m/z (%): 328.45

6.3.12. 1-Benzoyl-3-(5-bromobenzo[d]thiazol-2-yl) thiourea (**3d**)

Elemental analysis for $C_{15}H_{10}BrN_3OS_2$ (MW = 392.29) in wt% calc. C = 45.91, H = 2.55, N = 10.71, S = 16.32 and found to be C = 46.01, H = 2.85, N = 10.72, S = 16.35. m.pt. 203–205 °C, yield 83%. IR (KBr pellet) in cm^{-1} : 3310 (free NH), 3204 (assoc NH), 1670 (C=O), 1526 (benzene ring), 1402 (C–N stretching), 1140 (C=S). 1H NMR (400 MHz, DMSO- d_6) in δ (ppm) and J (Hz): 12.15 (1H, s, broad, NH), 10.70 (1H, s, broad, NH), 7.70 (2H, d, J = 8.2 Hz), 7.65 (2H, d, J = 6.9 Hz), 6.60 (H, d, J = 8.41 Hz); ^{13}C NMR (300 MHz, DMSO- d_6) in δ (ppm): 179.02 (C=S), 168.29 (C=O), 151.3, 149.2, 144.2, 142.2, 134.2, 128.6, 127.2; EI MS (70 eV) m/z (%): 392.28

6.3.13. 1-(1, 3-Benzothiazol-2-yl)-3-butyryl thiourea (**4a**)

Elemental analysis for $C_{12}H_{13}N_3OS_2$ (MW = 279.38) in wt% calc. C = 51.61, H = 4.65, N = 15.05, S = 22.93 and found to be C = 51.56, H = 4.85, N = 15.10, S = 22.96. m.pt. 156 °C, yield 75%. IR (KBr pellet) in cm^{-1} : 3311 (free NH), 3203 (assoc NH), 1670 (C=O), 1523 (benzene ring), 1401 (C–N stretching), 1140 (C=S). 1H NMR

(400 MHz, DMSO- d_6) in δ (ppm) and J (Hz): 12.40 (1H, s, broad, NH), 10.25 (1H, s, broad, NH), 8.13 (H, d, J = 8.2 Hz), 2.17 (t, $-CH_2$, J = 7.3 Hz), 1.56 (m, $-CH_2$), 0.95 (t, $-CH_3$, J = 7.1 Hz); ^{13}C NMR (300 MHz, DMSO- d_6) in δ (ppm): 179.02 (C=S), 173.9 (C=N), 168.29 (C=O), 148.2, 126.6, 124.8, 121.2, 60.0, 45.3, 23.1; EI MS (70 eV) m/z (%): 279.36

6.3.14. 1-Butyryl-3-(5-nitrobenzo[d]thiazol-2-yl) thiourea (**4b**)

Elemental analysis for $C_{12}H_{12}N_4O_3S_2$ (MW = 324.38) in wt% calc. C = 44.44, H = 3.70, N = 17.28, S = 19.75 and found to be C = 44.21, H = 3.87, N = 17.26, S = 19.76. m.pt. 197–198 °C, yield 71%. IR (KBr pellet) in cm^{-1} : 3318 (free NH), 3205 (assoc NH), 1669 (C=O), 1525 (benzene ring), 1403 (C–N stretching), 1512 (NO₂), 1140 (C=S). 1H NMR (400 MHz, DMSO- d_6) in δ (ppm) and J (Hz): 13.55 (1H, s, broad, NH), 10.28 (1H, s, broad, NH), 8.12 (d, H, J = 8.63 Hz), 2.17 (t, $-CH_2$, J = 7.3 Hz), 1.56 (m, $-CH_2$), 0.95 (t, $-CH_3$, J = 7.1 Hz); ^{13}C NMR (300 MHz, DMSO- d_6) in δ (ppm): 179.02 (C=S), 174.3 (C=N), 168.29 (C=O), 149.8, 146.2, 130.6, 122.9, 120.4, 117.5, 60.6, 45.3, 23.3; EI MS (70 eV) m/z (%): 324.37

6.3.15. 1-Butyryl-3-(5-aminobenzo[d]thiazol-2-yl) thiourea (**4c**)

This compound was prepared from **4b** by the following reduction procedure. **4b** (3.30g, 10 mmol), 5 ml hydrazine monohydrate, 70 ml ethanol and 0.03 g of 10% Pd–C was transferred into 250 ml two necked round bottom flask and refluxed for 18 h. The reaction was monitored by TLC. After completion the reaction, it was allowed to stand for one day and then filtered the reaction mixture. The crude product was re-crystallized in ethanol. Elemental analysis for $C_{12}H_{14}N_4OS_2$ (MW = 294.39) in wt% calc. C = 48.97, H = 4.76, N = 19.04, S = 21.76 and found to be C = 48.84, H = 4.89, N = 19.02, S = 21.85. m.pt. 165–166 °C, yield 69%. IR (KBr pellet) in cm^{-1} : 3318 (free NH), 3210 (assoc NH), 1672 (C=O), 1525 (benzene ring), 1403 (C–N stretching), 1140 (C=S). 1H NMR (400 MHz, DMSO- d_6) in δ (ppm) and J (Hz): 12.40 (1H, s, broad, NH), 10.25 (1H, s, broad, NH), 8.13 (H, d, J = 8.41 Hz), 5.13 (2H, s, NH₂), 2.17 (t, $-CH_2$, J = 7.3 Hz), 1.56 (m, $-CH_2$), 0.95 (t, $-CH_3$, J = 7.1 Hz); ^{13}C NMR (300 MHz, DMSO- d_6) in δ (ppm): 179.02 (C=S), 174.0 (C=N), 168.29 (C=O), 149.0, 145.5142.5, 122.6, 115.8, 60.6, 45.0, 23.4; EI MS (70 eV) m/z (%): 294.39

6.3.16. 1-Butyryl-3-(5-bromobenzo[d]thiazol-2-yl) thiourea (**4d**)

Elemental analysis for $C_{12}H_{12}BrN_3OS_2$ (MW = 358.27) in wt% calc. C = 40.22, H = 3.35, N = 11.73, S = 17.87 and found to be C = 40.56, H = 3.51, N = 12.00, S = 17.81. m.pt. 158 °C, yield 79%. IR (KBr pellet) in cm^{-1} : 3310 (free NH), 3206 (assoc NH), 1670 (C=O), 1525 (benzene ring), 1404 (C–N stretching), 1140 (C=S). 1H NMR (400 MHz, DMSO- d_6) in δ (ppm) and J (Hz): 12.40 (1H, s, broad, NH), 10.25 (1H, s, broad, NH), 8.12 (H, d, J = 8.40 Hz), 2.17 (t, $-CH_2$, J = 7.3 Hz), 1.56 (m, $-CH_2$), 0.95 (t, $-CH_3$, J = 7.1 Hz); ^{13}C NMR (300 MHz, DMSO- d_6) in δ (ppm): 179.02 (C=S), 174.8 (C=N), 168.29 (C=O), 151.8, 129.1, 125.7, 124.1, 123.6, 116.8, 60.1, 45.1, 23.5; EI MS (70 eV) m/z (%): 358.28

6.3.17. 1-(1, 3-Benzothiazol-2-yl)-3-(morpholine-4-carbonyl) thiourea (**5a**)

Elemental analysis for $C_{13}H_{14}N_4O_2S_2$ (MW = 322.40) in wt% calc. C = 48.48, H = 4.34, N = 17.39, S = 19.87 and found to be C = 48.46, H = 4.37, N = 17.36, S = 19.85. m.pt. 172–173 °C, yield 88%. IR (KBr pellet) in cm^{-1} : 3310 (free NH), 3206 (assoc NH), 1670 (C=O), 1525 (benzene ring), 1404 (C–N stretching), 1140 (C=S). 1H NMR (400 MHz, DMSO- d_6) in δ (ppm) and J (Hz): 13.10 (1H, s, broad, NH), 11.05 (1H, s, broad, NH), 7.75 (2H, d, J = 8.2 Hz), 7.65 (2H, d, J = 6.9 Hz), 6.61 (H, d, J = 8.40 Hz); ^{13}C NMR (300 MHz, DMSO- d_6) in δ (ppm): 179.02 (C=S), 168.29 (C=O), 151.8, 149.2, 144.5, 142.2, 134.8, 128.1, 127.3; EI MS (70 eV) m/z (%): 322.42

6.3.18. 1-(Morpholine-4-carbonyl)-3-(5-nitrobenzo[d]thiazol-2-yl)thiourea (**5b**)

Elemental analysis for $C_{13}H_{13}N_5O_4S_2$ (MW = 367.73) in wt% calc. C = 42.50, H = 3.54, N = 19.07, S = 17.43 and found to be C = 42.62, H = 3.85, N = 18.97, S = 17.47. m.pt. 217–218 °C, yield 82%. IR (KBr pellet) in cm^{-1} : 3313 (free NH), 3201 (assoc NH), 1670 (C=O), 1523 (benzene ring), 1512 (NO₂), 1406 (C–N stretching), 1140 (C=S). ¹H NMR (400 MHz, DMSO-*d*₆) in δ (ppm) and *J* (Hz): 13.15 (1H, s, broad, NH), 12.40 (1H, s, broad, NH), 8.12 (d, H, *J* = 8.63 Hz), 7.75 (2H, d, *J* = 8.2 Hz), 7.65 (2H, d, *J* = 6.9 Hz); ¹³C NMR (300 MHz, DMSO-*d*₆) in δ (ppm): 180.01 (C=S), 169.25 (C=O), 150.2, 149.1, 144.5, 142.1, 134.8, 128.2, 127.2; EI MS (70 eV) *m/z* (%): 367.74

6.3.19. 1-(Morpholine-4-carbonyl)-3-(5-aminobenzo[d]thiazol-2-yl)thiourea (**5c**)

This compound was prepared from **5b** by the following reduction procedure. **5b** (3.70 g, 10 mmol), 5 ml hydrazine monohydrate, 70 ml ethanol and 0.03 g of 10% Pd–C was transferred into 250 ml two necked round bottom flask and refluxed for 18 h. The reaction was monitored by TLC. After completion the reaction, it was allowed to stand for two days and then filtered the reaction mixture. Removed the solvent by rotary evaporator. The crude product was re-crystallized in ethanol. Elemental analysis for $C_{13}H_{15}N_5O_2S_2$ (MW = 337.83) in wt% calc. C = 46.29, H = 4.45, N = 20.77, S = 18.99 and found to be C = 46.31, H = 4.49, N = 20.65, S = 18.95. m.pt. 185–186 °C, yield 81%. IR (KBr pellet) in cm^{-1} : 3310 (free NH), 3204 (assoc NH), 1670 (C=O), 1523 (benzene ring), 1402 (C–N stretching), 1140 (C=S). ¹H NMR (400 MHz, DMSO-*d*₆) in δ (ppm) and *J* (Hz): 13.02 (1H, s, broad, NH), 11.52 (1H, s, broad, NH), 7.75 (2H, d, *J* = 8.2 Hz), 7.65 (2H, d, *J* = 6.9 Hz), 6.61 (H, d, *J* = 8.40 Hz), 5.15 (2H, s, NH₂); ¹³C NMR (300 MHz, DMSO-*d*₆) in δ (ppm): 179.08 (C=S), 169.09 (C=O), 151.5, 148.9, 144.5, 142.1, 134.5, 128.6, 127.0; EI MS (70 eV) *m/z* (%): 337.85

6.3.20. 1-(Morpholine-4-carbonyl)-3-(5-bromobenzo[d]thiazol-2-yl)thiourea (**5d**)

Elemental analysis for $C_{13}H_9BrN_4O_2S_2$ (MW = 397.51) in wt% calc. C = 39.29, H = 2.26, N = 14.10, S = 16.12 and found to be C = 39.23, H = 2.27, N = 14.15, S = 16.11. m.pt. 175 °C, yield 78%. IR (KBr pellet) in cm^{-1} : 3309 (free NH), 3205 (assoc NH), 1671 (C=O), 1521 (benzene ring), 1404 (C–N stretching), 1145 (C=S). ¹H NMR (400 MHz, DMSO-*d*₆) in δ (ppm) and *J* (Hz): 13.05 (1H, s, broad, NH), 11.10 (1H, s, broad, NH), 7.76 (2H, d, *J* = 8.1 Hz), 7.67 (2H, d, *J* = 6.9 Hz), 6.65 (H, d, *J* = 8.43 Hz); ¹³C NMR (300 MHz, DMSO-*d*₆) in δ (ppm): 179.42 (C=S), 168.58 (C=O), 151.4, 149.0, 144.5, 142.2, 134.2, 128.8, 127.6; EI MS (70 eV) *m/z* (%): 397.53

6.4. Evaluation of antimicrobial activity

For the bacterial organisms, both Gram-positive and Gram-negative bacteria were used. Gram-positive and Gram-negative bacteria can be differentiated in the physical appearance of their cell envelopes. The compounds were screened for their *in vitro* antibacterial and anti-yeast activities. Antimicrobial activities were determined by the broth micro-dilution procedures and principles of the Clinical and Laboratory Standards Institute (CLSI) [37,38]. Minimal inhibitory concentrations for each compound were investigated against standard bacterial strains; *S. aureus* (ATCC 25923), *S. aureus* (ATCC 29213), *E. faecalis* (ATCC 29212), *E. coli* (ATCC 25922), *S. epidermidis* (ATCC 12228), *E. cloacae* (ATCC 13047), *P. vulgaris* (ATCC 13315), *P. aeruginosa* (ATCC 27853) and yeast-like fungi, *C. albicans* (ATCC90028), *C. glabrata* (ATCC 32554), *C. tropicalis* (ATCC 20336), *C. krusei* (ATCC 6268). Bacterial and fungal colonies of the test organisms were suspended directly into a small volume of 0.9% saline and further diluted until turbidity

matched the Mc Farland Standard no: 0.5 Petri dishes containing Mueller–Hinton agar for bacteria and Sabouraud Dextrose agar for fungi were impregnated with these microbial suspensions. The stock solutions of the synthesized compounds were prepared in dimethyl sulfoxide (DMSO), which had no effect on the organisms in the concentrations studied. The initial concentration was 200 mg/ml. All of the dilutions were done with distilled water. The concentrations of tested compounds were 100, 50, 25, 12.5, 6.25, 3.125 µg/ml. DMSO was used as negative control. Gentamycin, amikacin and nystatin were used as reference drugs for Gram-negative antibacterial activity, Gram-positive antibacterial activity and anti-fungal activity, respectively. All the inoculated plates were incubated at 37 °C and results were evaluated after 24 h for bacteria and 48 h for fungi. The lowest concentration of the compounds that prevented visible growth was considered minimal inhibitor concentrations (MICs)

6.5. Evaluation of anti-tumor activity

6.5.1. Preliminary *in vitro* cytotoxic activities (MTT assay)

In vitro cytotoxicity was determined using a standard MTT assay [39] with protocol appropriate for the individual test system. Test compounds were prepared prior to the experiment by dissolving in 0.1% DMSO and diluted with medium. The cells were then exposed to different concentrations of the drugs (1–100 µM) in the volume of 100 µM/well. Cells in the control wells received the same volume of medium containing 0.1% DMSO. After 24 h, the medium was removed and cell cultures were incubated with 100 µl MTT reagent (1 mg/ml) for 5 h at 37 °C. The suspension was placed on micro-vibrator for 10 min and absorbance was recorded by the ELISA reader. The experiment was performed in triplicate. Human cancer cell lines, MCF-7 and HeLa cells were cultured in MEM medium supplemented with 10% FBS, 1% glutamine and 50 µM/ml gentamicin sulphate in a CO₂ incubator in a humidified atmosphere of 5% CO₂ and 95% air.

6.5.2. Alkaline comet assay

The effect of synthesized compounds on DNA was assessed by comet assay as described by protocol [40] with slight modifications. After the drug treatment, cells were harvested from each culture flask and counted by trypan blue exclusion method. About 18,000 cells in 50 µl medium were suspended in 150 µl of low melting agarose (0.75%) and layered onto slides pre-coated with an earlier layer of agarose (1.5%). Slides were kept in lying solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris; pH 10–10.5; 1% Triton X-100 and 10% DMSO) overnight at 4 °C in dark, these were subjected to unwinding under alkaline conditions (pH 13) for 1 h to allow DNA supercoils to relax and express DNA single-strand breaks and alkali-labile sites. Electrophoresis was then carried out under highly alkaline (pH 13) conditions for 30 min at 16 V and 300 mA. Three slides were prepared for each concentration. About 50 cells were captured per slide using fluorescent microscope and the images were analyzed using Komet 5.5 software (kinetic imaging systems, UK). A variety of objective measurements like Head DNA, Tail DNA, and Olive Tail moment were made. Tail moment was calculated according to reported procedure [8].

$$\text{Tail moment} = \frac{\text{Tail length} \times \% \text{Tail DNA}}{100}$$

Supplementary crystallographic data

Crystallographic data for the structure reported in this article has been deposited with Cambridge Crystallographic Data Center, CCDC 738482. Copies of this information may be obtained free of

charge from the Director, CCDC, 12 Union Road, Cambridge, CBZ IEZ, UK. Facsimile (44) 01223 336 033, E-mail: deposit@ccdc.cam.ac.uk or <http://www.ccdc.com.ac.uk/deposit>.

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