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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of some novel *N*-substituted rhodanines for their anticancer activity

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Abstract Novel *N*-substituted rhodanines **2a–g** were synthesized by conventional and microwave-assisted methods and tested for their anticancer activity. Structure–activity relationship of the synthesized rhodanine **2a–g** as antiproliferative agents was investigated. The results revealed that all the seven compounds showed potent antiproliferative activity in a concentration-dependent manner on leukemic cell line K562. Among the tested compounds, **2b** was found to be more potent when compared by trypan blue and MTT assay. IC_{50} values of **2b** using trypan blue and MTT assay were found to be 11.1 and 20.3 μg/ml, respectively. A dose-dependent increase in the LDH release was also observed upon treatment with **2a–g**. Cell cycle analysis revealed that **2b** affects DNA replication and leads to accumulation of cells in G_0 and decline of G_2/M, G_1 and *S* phases which indicates apoptosis. The selective cytotoxic activity against human chronic myelogenous cell line (K562), via apoptosis, suggests that compound **2b** is a promising scaffold for the development of novel anticancer drug.

Keywords Rhodanine · Microwave-assisted synthesis · Cytotoxicity · Apoptosis

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Introduction

Cancer is one of the difficult diseases to be cured, and only very few effective ways are available. The development of novel, efficient and less toxic anticancer agents remains an important and challenging goal in medicinal chemistry. Understanding the molecular mechanism involved in cancers should lead to the identification of novel anticancer agents. Leukemia is one of the major types of cancer affecting a significant segment of the population. In fact, leukemia is the most frequent childhood cancer with 26 % of all cases and 30 % mortality (Brown *et al.*, 1956; Lesyk and Zimenkovsky, 2004; Moorthy *et al.*, 2010; Ravi *et al.*, 2010). Although the incidence rate for this disease remains relatively unchanged, some success has no doubt been attained in its treatment. The current treatments, however, have many limitations. This includes side effects and the development of acquired drug resistance (Brown *et al.*, 1956; Moorthy *et al.*, 2010). Therefore, there is a need for the development of effective anticancer drugs with well-defined pharmacokinetic properties.

Rhodanine derivatives have been proven to be attractive compounds due to their outstanding biological activities and have undergone rapid development as anticonvulsant, antibacterial, antiviral and antidiabetic agents (Brown and Bradsher, 1951; Nitsche and Klein, 2012; Prashantha Kumar *et al.*, 2012; Kamila *et al.*, 2012). At the same time, these have also been reported as inhibitors of hepatitis C virus (HCV) protease (Frankov *et al.*, 1985), HIV-1 integrase inhibitors (Kavya *et al.*, 2010a, b) and also used as inhibitors of uridinediphospho-*N*-acetylmuramate/*L*-alanine ligase (Alizadeh *et al.*, 2009). Recently, substituted rhodanines were investigated for tau aggregation inhibitor properties (Jacobine and Posner, 2011). Rhodanines are classified as nonmutagenic (Alizadeh and Zohreh, 2009),

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and a long-term study on the clinical effects of the rhodanine-based Epalrestat as an antidiabetic has demonstrated that it is well tolerated (Alizadeh *et al.*, 2009). Additionally, rhodanines have been designed as inhibitors of various enzymes such as bacterial β-lactamase and Mur ligases (Jacobine and Posner, 2011). Due to various possibilities of chemical derivatization of the rhodanine ring, rhodanine-based compounds will probably remain a privileged scaffold in drug discovery. The synthesis of these compounds is of considerable interest. The anticancer properties of rhodanine derivatives have not been investigated extensively.

Earlier we have synthesized and evaluated 5-isopropylidene-3-ethyl rhodanine (**Ia**) for its cytotoxicity (Brown *et al.*, 1956; Ravi *et al.*, 2010; Nitsche and Klein, 2012) and found that the compound shows cytotoxicity in leukemic cell line, CEM and induces apoptosis. These encouraging results make rhodanine a promising starting point for further synthesis and optimization by structure–activity relationship studies. To compare them with their O and N counterparts, we also synthesized and evaluated (Brown *et al.*, 1956; Ravi *et al.*, 2010; Nitsche and Klein, 2012) three novel compounds, mainly 5-isopropylidene derivatives of 3-methyl-2-thio-hydantoin **Ib**, 3-ethyl-2-thio-2,4-oxazolidinedione **Ic** and 5-benzylidene-3-ethyl rhodanine **Id** (Fig. 1). The results revealed that **Id** was more potent than **Ia, Ib** and **Ic** with an IC_{50} value around 10 μM. Furthermore, we also showed that **Id** affects DNA replication by inducing a block at G_0/G_1 followed by induction by cell death by apoptosis. This showed the importance of the presence of a benzylidene moiety in position C-5 of the rhodanine nucleus. Now, in order to optimize the N-3 position, in the present study we have synthesized different *N*-substituted rhodanines and tested them for their anticancer properties.

Chemistry

We designed some novel rhodanines incorporated with amino acid residues like glycine, alanine, phenylalanine, valine and glutamic acid residues in addition to ethyl and allyl residues. The *N*-substituted rhodanines **2a–g** were synthesized from amine/amino acid and carbon disulfide adopting base-catalyzed process in the conventional (Scheme 1) and microwave-assisted methods as shown in Scheme 2. The addition of sodium hydroxide in the reaction serves to support the nucleophilic attack of amine at carbon disulfide. The hypothesis here is that the amino acid moiety will change the polarity and acidity near the thiazolidine ring due to the freely exposed carboxyl group and is also relatively safe with respect to its metabolism in the body.

It is known that reports with the straightforward synthesis of the *N*-substituted rhodanine precursors with a 'free' 5-position are very scarce (Alizadeh and Zohreh, 2009). Only a small number of these *N*-substituted rhodanine synthons are commercially available. There is a need for a straightforward synthetic approach to make these key building blocks. Reported protocols for *N*-substituted rhodanines require long reaction time under aqueous conditions (Vivek and Rajender, 2008). Considering these aspects, we used an interesting approach for the synthesis of rhodanine derivatives and report an aqueous, microwave-assisted and one-pot protocol for the rapid and efficient synthesis of *N*-substituted rhodanines using carbon disulfide. This protocol enables the synthesis of *N*-substituted rhodanines from the corresponding amino acids under environmentally friendly conditions. Our procedure employed sodium hydroxide as the reagent in the first two reaction steps and hydrochloric acid in the last step (Alizadeh *et al.*, 2009). After some optimization using alanine as a model substrate, we identified the conditions for a

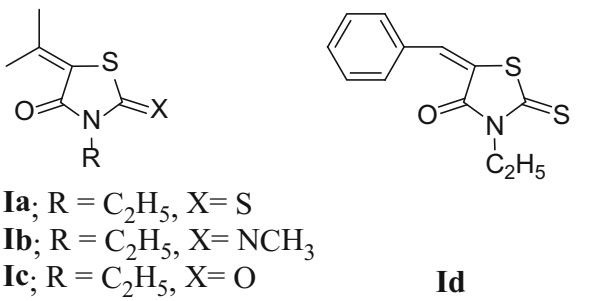
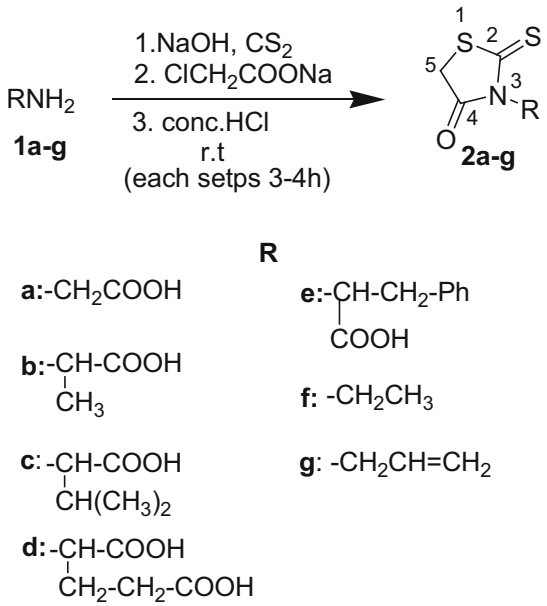
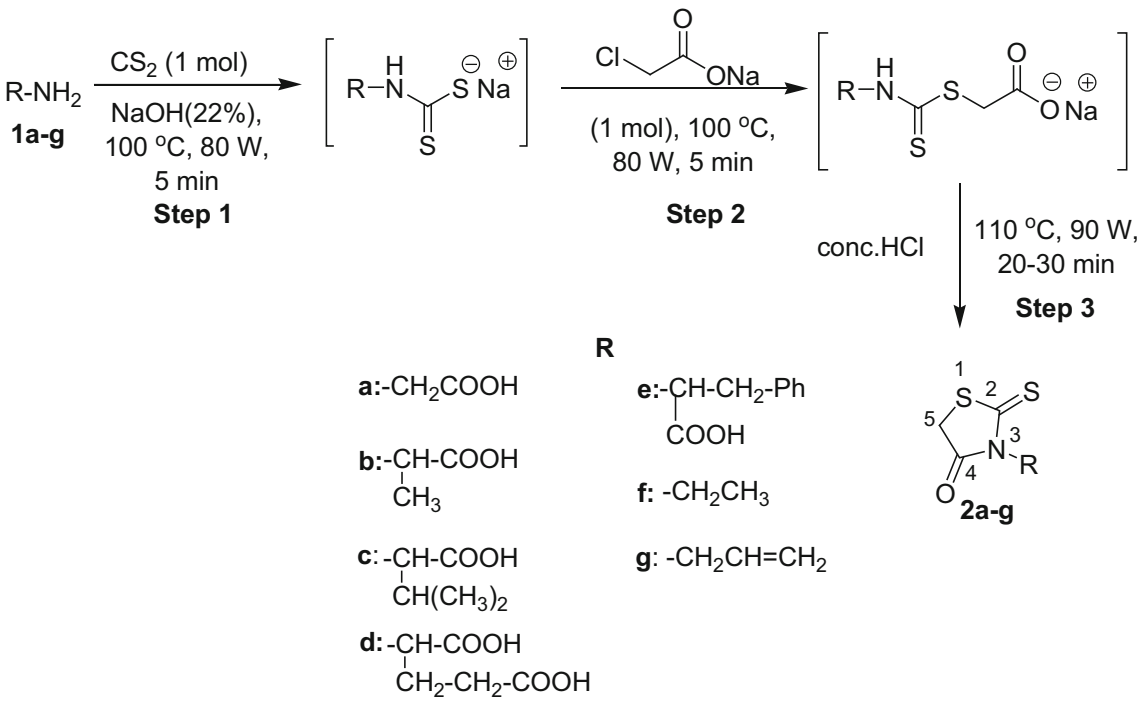


Fig. 1 The five-membered heterocyclic core; rhodanine (X = S), thiohydantoin (X = NCH_3) and thioxooxazolidine (X = O)



Scheme 1 Conventional synthesis of **2a–g**



Scheme 2 Microwave-assisted synthesis of **2a–g**

one-pot synthetic approach as shown in Table 1 as the starting point for further investigations. Alanine and carbon disulfide were allowed to react in water under basic conditions in a microwave reactor for 5 min at 80 W and 100 °C, followed by the addition of chloroacetic acid and a second reaction for 5 min at 80 W and 100 °C. An excess of aqueous hydrochloric acid was then added, and final reaction step was executed at 80 W and 110 °C for 30 min. The same conditions were used for the preparation of all other *N*-substituted rhodanines. For comparison purposes, the compounds were also prepared by conventional method.

*Synthesis of N-substituted rhodanine (**2a–g**)*

A mixture of amino acid, sodium hydroxide in water and carbon disulfide was stirred for 4 h at room temperature, followed by the addition of sodium chloroacetate and

Table 1 Evaluation of conditions for the synthesis of *N*-substituted rhodanine **2b** by microwave-assisted synthesis

S. no	Solvent	Step 1		Time	Step 2		Time	Step 3		Time	Yield (%)
		Conditions			Conditions			Conditions			
		Temp (°C)	MW (W)		Temp (°C)	MW (W)		Temp (°C)	MW (W)		
1	–	100	80	10	100	80	10	100	80	10	55
2	Ethanol	100	80	10	100	80	10	100	80	10	61
3	Water	100	80	10	100	80	10	100	80	10	69
4	Water	100	60	10	100	60	10	100	60	10	48
5	Water	100	80	10	100	80	10	100	80	10	74
6	Water	100	100	10	100	100	10	100	100	10	72
7	Water	100	80	5	100	80	5	100	80	5	76
8	Water	100	80	10	100	80	10	100	80	10	71
9	Water	100	80	15	100	80	15	100	80	15	74
10	Water	100	80	5	100	80	5	100	80	10	72
11	Water	100	80	5	100	80	10	100	80	20	79
12	Water	100	80	5	100	80	15	100	80	30	84

further stirred for 3 h. Conc. HCl was then added, and the product was separated and dried (Wang *et al.*, 2012; Xing *et al.*, 2007; Kaminsky *et al.*, 2012; Bernardo *et al.*, 2009, 2011) (Scheme 1).

In order to develop a greener synthesis, the above mixture was treated in a microwave oven at 100 °C, 80 W for 45 min using water as the solvent, and the product was recovered as above. The yield was comparable to the conventional method, but the reaction time was shorter (Scheme 2). The compounds were characterized by UV, IR, NMR and elemental analysis.

Results and discussion

Compounds related to **2a** have been reported to behave as an imaging agent for neurofibrillary tangles (NFTs) detection in Alzheimer's disease and as GSK-3 inhibitors (Martinez *et al.*, 2003; Anumala *et al.*, 2013). Compounds related to **2b** have been tested and found to alter the life span of eukaryotic organisms (David, 2009). Compounds related of **2c** have been reported as selective inhibitors for antiapoptotic Bcl-2 proteins from BHI-1 by molecular modeling studies (Xing *et al.*, 2007). However, in none of these studies cytotoxicity was studied in detail.

Antiproliferative activity in human chronic myelogenous leukemia cell line in a dose-dependent manner

In order to evaluate the antiproliferative activity of synthesized rhodanines, **2a–g**, we used trypan blue assay. Cancer cell line K562 (human chronic myelogenous leukemia) was treated with 6.25, 12.5, 25, 50 and 100 μg/ml of compounds **2a–g** (Table 2).

Since the compounds dissolved in 0.1 % DMSO, the cells with DMSO were used as vehicle control. Following the addition of compounds, cells were counted after 24 h till the control cells attained stationary phase. Results showed that cell growth was affected by the increase in concentration. The effect was limited when 25 μg/ml of **2b** was used. However, concentrations of 50 and 100 μg/ml resulted in increased cell death (Fig. 2). These results suggest that **2b** induces antiproliferative effect in human cancer cell line K562.

The IC_{50} of values obtained for **2a–g** after 48 h of treatment is shown in Table 2. The data revealed that compounds **2a–c** and **2f** are more potent with an IC_{50} of 14.60, 11.10, 19.98, 21.48 μg/ml, respectively, and are twice or three times more potent than compounds **2d, 2e** and **2g** with an IC_{50} of 29.23, 36.52 and 23.72 μg/ml, respectively. The compounds **2a–c** and **2f** with small groups such as $-CH_2COOH$, $-CH-(CH_3)-COOH$, $-CH(CH(CH_3)_2)-COOH$ and $-C_2H_5$ as *N*-substituents showed good cytotoxic activity. In the other three molecules **2d, 2e** and **2g,** the low activity is due to steric hindrance of the larger groups present as *N*-substituents. The groups may have different orientation in space and may not fit into the receptors.

Table 2 Trypan blue exclusion test for compound **2a–g** against the human chronic myelogenous cell line K562

Compound	IC_{50} (μg/ml)[a,b]
2a	14.60 ± 3.5
2b	11.10 ± 2.0
2c	19.98 ± 2.9
2d	29.21 ± 3.8
2e	36.52 ± 2.6
2f	21.48 ± 2.4
2g	23.74 ± 3.4
Cisplatin	4.78 ± 3.2

[a] Determined by trypan blue exclusion method

[b] All experiments were conducted in triplicate

The antiproliferative effect induced by rhodanines on proliferation of cancer cells was further verified using MTT assay on K562 cancer cell line. The cancer cells treated with the 6.25, 12.5, 25, 50 and 100 μg/ml concentrations of the compounds **2a–g** were harvested after 48 h and were subjected to MTT assay. Results showed that cell viability (Table 3) was affected upon treatment with **2a–g**.

Compounds **2a**, **2b** and **2f** showed good antiproliferative effect with an IC_{50} value of 27.73, 20.31 and 28.18 μg/ml, respectively, and compounds **2c–e** and **2 g** also exhibited cytotoxic effect against K562 cell line with an IC_{50} value of 87.07, 51.35, 40.77 and 35.84 μg/ml, respectively. Compounds **2a**, **2b** and **2f** are seen to be twice potent than **2c–e** and **2 g**. The most potent compound was found to be **2b** with an IC_{50} value of 20.31 μg/ml.

To further check the antiproliferative effect of **2a–g,** LDH release in the cell culture suspension was measured using LDH assay (Fig. 3). The DMSO-treated cells were used as vehicle control, and **2a–g**-treated cells showed LDH release suggesting antiproliferative effect on K562 cells. The membrane of K562 cells was disrupted due to antiproliferative effect of **2a–g** causing release of LDH (lactate dehydrogenase) into the supernatant. Results showed a dose-dependent increase in the LDH release upon treatment of various concentrations of **2a–g** further confirming the above results (Fig. 3).

Cell cycle analysis by flow cytometry

We performed fluorescence-activated cell sorting (FACS) analysis to determine the effect of the most potent

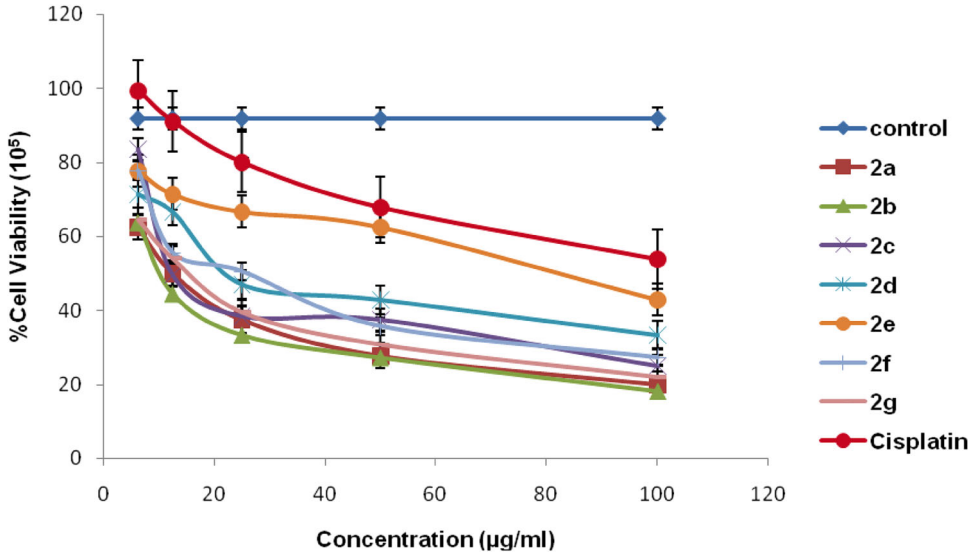


Fig. 2 Evaluation of cell viability using trypan blue assay following **2a–g** treatment. K562 cells were treated with 6.25, 12.5, 25, 50 and 100 µg/ml. The data presented is result of three independent experiments and error bar are indicated

Table 3 MTT assay for compound **2a–g** against human chronic myelogenous cell line K562

Compound	IC_{50} (µg/ml)[a,b]
2a	27.73 ± 4.7
2b	20.31 ± 5.3
2c	87.07 ± 6.4
2d	51.35 ± 3.2
2e	40.77 ± 4.3
2f	28.18 ± 5.9
2g	35.84 ± 5.1
Cisplatin	5.947 ± 2.3

[a] Determined by MTT assay

[b] All experiments were conducted in triplicate

compound **2b** on cell cycle progression. K562 cells were stained with propidium iodide after 72 h of treatment with **2b** (25 µg/ml) and subjected to FACS. Histogram of control cells showed a standard cell cycle pattern, which includes G_1 and G_2 separated by S phase (Fig. 4). The G_0 phase (mostly dead cells) was not prominent. Interestingly, upon addition of **2b**, a concentration of 25 µg/ml change was observed in the cell cycle pattern (Table 4) leading to the accumulation of cells in G_0 and decline of G_2/M, G_1 and S phases indicating apoptosis.

These results of the antiproliferative effect of rhodanine **2b** on cancer cells were recognized mainly due to the induction of apoptosis with less or no contribution from cell cycle arrest. The results obtained thus confirm our

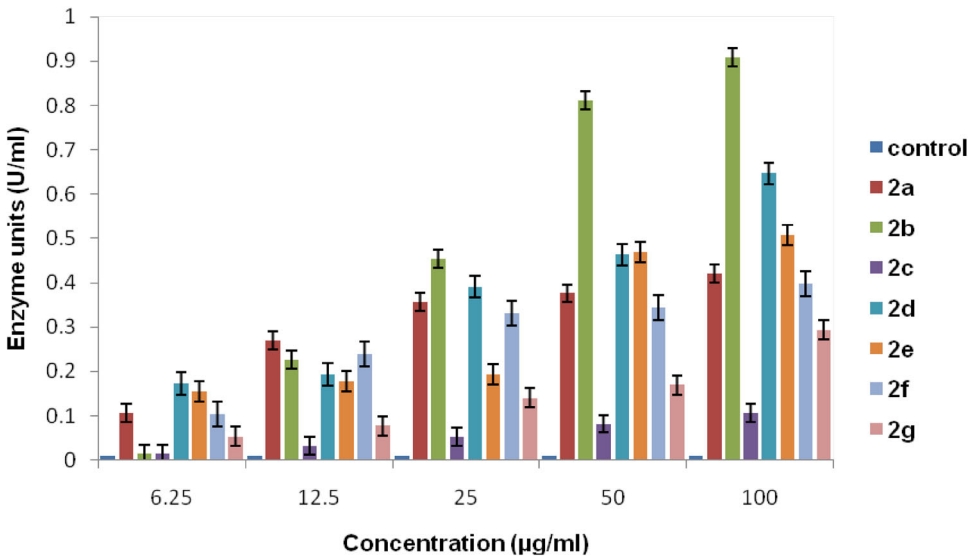


Fig. 3 Measurement of LDH release following treatment with **2a–g**. After the contact of K562 with **2a–g** at different concentrations (6.25, 12.5, 50 and 100 µg/ml) for 24 h, the release of LDH was measured at 490 nm. The data presented is result of three independent experiments and error bar are indicated

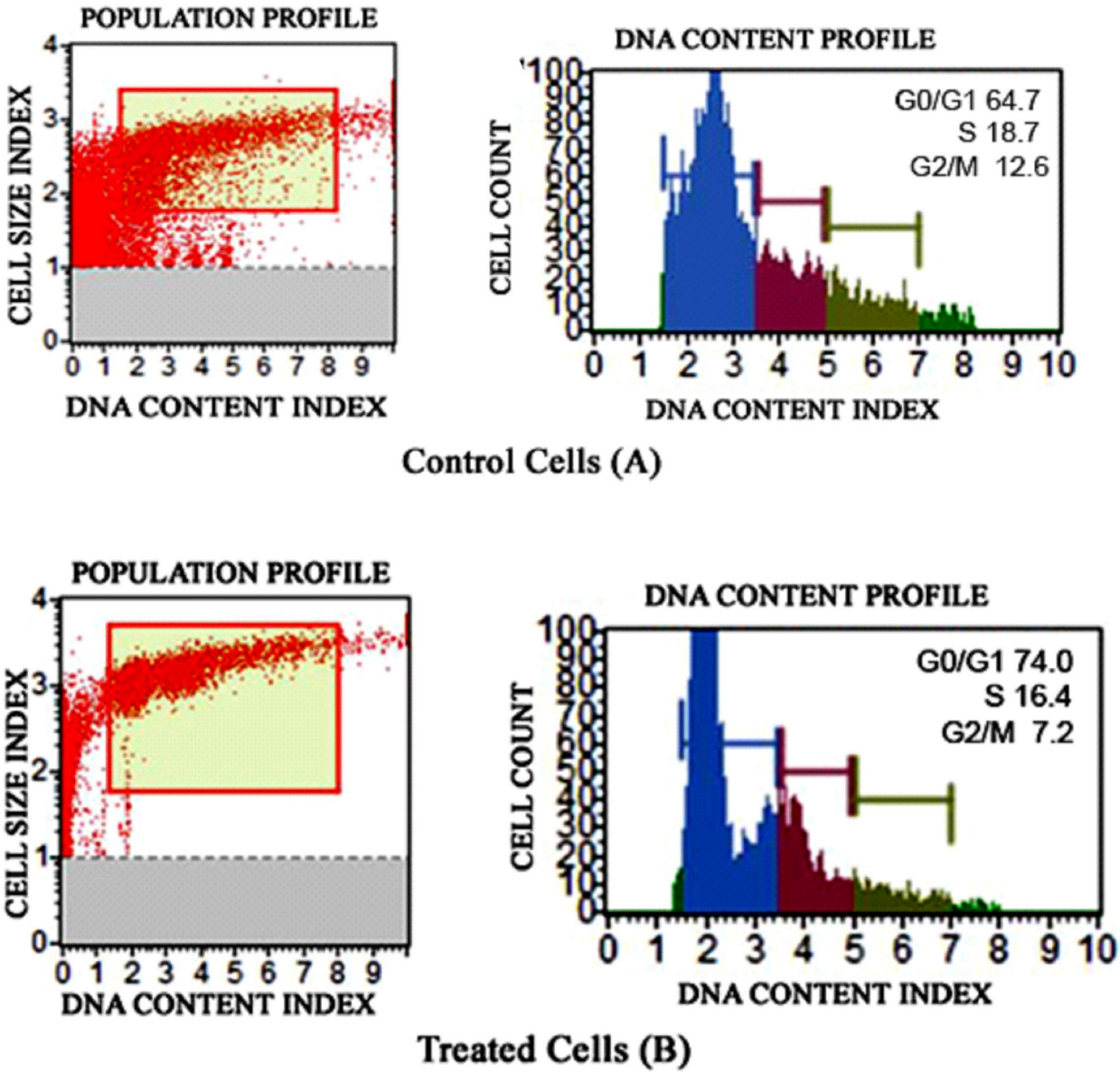


Fig. 4 Cell cycle analysis of K562 cells (A is control, and B is 25 μg of **2b** treated) following **2b** treatment. K562 cells were treated with 25 μg/ml of **2b** for 24 h, harvested and stained with propidium iodide and quantified by flow cytometry. The histograms show the percentage of cells in the G_0/G_1, G_1, S and G_2/M phase of the cell cycle, and 10,000 cells were used for sorting

earlier observation of rhodanine **2b**-induced cytotoxicity (Table 5).

Structure–activity relationship

The molecules were designed in such a way that they have different moieties in the N-3 position of the rhodanine nucleus. The compound **2a** possesses a hydrophilic moiety ($-CH_2COOH$), whereas the compounds **2b**, **2c** and **2e** have a hydrophobic methyl, isopropyl and benzyl moieties, respectively, along with a hydrophilic carboxylic acid group. The compounds **2f** and **2g** have only a hydrophobic ethyl and allyl group, respectively, in the N-3 position, and the compound **2d** has two hydrophilic carboxylic acid groups. By the introduction of larger groups, the steric effect increases.

The compound **2a** with a hydrophilic group in the N-3 position showed a marked cytotoxic activity with an IC_{50} value of 27.3 μg/ml in the MTT assay. By the introduction of hydrophobic methyl, isopropyl and benzyl groups into **2a**, to form compounds **2b, 2c** and **2e,** respectively, the activity increased in **2b** (IC_{50} value of 20.3 μg/ml) but decreased in **2c** (IC_{50} value of 51.35 μg/ml) and **2e** (IC_{50} value of 87.07 μg/ml). The activity decreases as the

Table 4 LDH release of human chronic myelogenous cell line K562 against **2a–g**

Concentration (μg/ml)	Enzyme units (U/ml)							
	Control[a]	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**
	0.0096 ± 0.0010							
6.25		0.106 ± 0.028	0.0149 ± 0.0023	0.014 ± 0.0016	0.173 ± 0.024	0.154 ± 0.011	0.103 ± 0.014	0.053 ± 0.025
12.5		0.27 ± 0.031	0.227 ± 0.022	0.033 ± 0.024	0.193 ± 0.021	0.178 ± 0.016	0.24 ± 0.026	0.077 ± 0.024
25		0.357 ± 0.022	0.454 ± 0.014	0.053 ± 0.016	0.391 ± 0.018	0.193 ± 0.021	0.33 ± 0.044	0.14 ± 0.031
50		0.376 ± 0.019	0.811 ± 0.023	0.082 ± 0.013	0.463 ± 0.027	0.468 ± 0.022	0.343 ± 0.024	0.169 ± 0.028
100		0.42 ± 0.028	0.908 ± 0.023	0.106 ± 0.025	0.647 ± 0.018	0.507 ± 0.028	0.398 ± 0.026	0.294 ± 0.034

[a] Control use as cells without drug

Table 5 FACS analysis to determine the effect of **2b** on cell cycle progression

Compound	Cell cycle[a,b]		
	G_0/G_1	S	G_2/M
Control	64.7	18.7	12.6
2b	74.0	16.4	7.2

[a] Determined by flow cytometry

[b] Experiments were conducted in 25 μg/ml concentration

bulkiness of the group increases in the N-3 position from **2c** to **2e**. Compared to **2b,** the cytotoxic activity was less in compounds which possess only hydrophobic groups as in **2f** (IC_{50} value of 28.18 μg/ml) and **2 g** (IC_{50} value of 35.84 μg/ml). The cytotoxic activity of compound **2d** with two hydrophilic groups was less than **2f** and **2 g** with hydrophobic groups. It clearly indicates that the compound with hydrophilic (−COOH) and a small hydrophobic ($-CH_3$) groups is required at N-3 position of the rhodanine nucleus to exhibit a good cytotoxic activity. Further, it indicates that irrespective of the hydrophilic or hydrophobic nature of the groups, the activity decreases with the increase in size.

The same trend was observed in the trypan blue assay. The IC_{50} values are marginally different in this assay. The trypan blue assay required evaluation using a microscope and a human observer, while the MTT assay is a colorimetric assay performed by a spectrophotometer. This explains, at least partly, why the IC_{50} values assessed by trypan blue and MTT are so different.

Further, it was reported in the literature that 5-(5-phenylfuran-2-ylmethylene)-substituted 3-α-carboxy ethyl rhodanine having the same rhodanine nucleus as in **2b** has shown a good in vitro ASK1 inhibitory activity with an IC_{50} value of 0.65 μM (Volynets *et al.*, 2013). 5-Aryloxypyrazole derivatives bearing a rhodanine-3-(3-phenyl propanoic acid) and 3-α-carboxy isobutyl rhodanine derivatives bearing a quinoline moiety in the fifth position exhibited cytotoxicity against HeLa cell lines with an IC_{50} value of 8.84 and 9.83 μg/mL, respectively (Guo *et al.*, 2013). N-(4-chlorophenyl)-2-{5-[(5-nitro-2-furyl)methylene]-rhodanine-3-amino-2-thioxoacetamide and N-(4-methoxyphenyl)-2-{5-[(5-nitro-2-furyl)methylene]-rhodanine-3-amino-2-thioxoacetamide were found to possess anticancer activity against colon cancer cell line (HCT 116) and a pancreatic cell line (Panc-1) with an GI_{50} value of less than 10 μM concentration (Kavya *et al.*, 2010a, b). All these results indicate that if the rhodanines are substituted in the fifth position, the anticancer activity of the molecule increases. Potential anticancer activity of the above rhodanines prepared from amino acids and their amenability to structural modification in the fifth position will help in designing novel anticancer agents with *N*-α-carboxyethyl rhodanine nucleus.

Conclusion

We have described the synthesis, antiproliferative activity and apoptosis of novel rhodanine derivatives. Synthesis was conducted by both conventional and microwave-assisted methods. Microwave synthesis gets completed in shorter reaction time when compared with conventional method and also results in good yield. We also report the antiproliferative activity of the synthesized rhodanines against human cancerous cell line K562. From the results, we have obtained a simplified analogue **2b** with a considerable cytotoxicity for the further work. FACS analysis confirmed the effect of **2b** in the change of cell cycle profile. The structure–activity relationship of **2a–g** as

antiproliferative agents against human chronic myelogenous leukemia cell (K562) has been investigated.

Experimental

Cell culture

Human chronic myelogenous leukemia cell line (K562) purchased from NCCS, Pune, was maintained in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10 % FBS (Invitrogen) and grown to confluency at 37 °C in 5 % CO_2 (NBS, Eppendorf, Germany) in a 90 % air in a CO_2 incubator. The cells were trypsinized (500 µl of 0.025 % trypsin in PBS/0.5 mM EDTA solution (HiMedia)) for 2 min and passaged to T flasks in complete aseptic conditions.

Cell viability by trypan blue exclusion

The effect of compounds **2a–g** on the viability of cancer cells (K562) was determined by trypan blue dye exclusion assay (Arung *et al.*, 2009; Shahabuddin *et al.*, 2009). Briefly, the cells were plated at a density of 1×10^5 in six-well plates followed by the addition of different concentrations of compound (6.25, 12.5, 50 and 100 µg in 1 ml of DMSO) or untreated cell. After incubation for every 24 h, cells were collected and diluted in equal volume of media and mixed with 20 ml of trypan blue. Cells were counted under the microscope using hemocytometer. All the experiments were performed in triplicate. IC_{50} value calculated using GraphPad Prism software 6.01 version.

Cell proliferation by MTT assay

Extracts were added to grown cells at concentrations of 6.25, 12.5, 25, 50 and 100 µg from a stock of 1 mg/ml 0.1 % DMSO and incubated for 24 h. The % difference in viability was determined by standard MTT assay after 24 h of incubation (Mosmann, 1983; Arung *et al.*, 2009).

MTT is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, colored (dark purple) formazan product. The cells are then solubilized with an organic solvent, dimethyl sulfoxide (Sigma-Aldrich), and the released, solubilized formazan product was measured at 570 nm. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

The cell culture suspension was washed with 1x PBS and then added 30 µl of MTT solution to the culture (MTT −5 mg/ml dissolved in PBS). It was then incubated at 37 °C for 3 h. MTT was removed by washing with 1× PBS, and 200 µl of DMSO was added to the culture. Incubation was carried out at room temperature for 30 min until the cell got lysed and color was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2 min to precipitate cell debris. Optical density was measured at 570 nm using DMSO as blank in an ELISA reader (LISASCAN, Erba). All the experiments were performed in triplicate. IC_{50} value calculated using GraphPad Prism software 6.01 version.

Lactate dehydrogenase (LDH) release assay

Lactate dehydrogenase (LDH) release is an indicator of membrane breaking, thus resulting in cell injury. LDH assay was performed to evaluate the LDH release to the media following treatment with the **2a–g** (6.25, 12.5, 25, 50 and 100 µg) on K562 for 24 h and measured using standard protocols (Korzeniewski and Callewaert, 1983). The intracellular LDH was determined after lysing the cells by rapid freezing and thawing in liquid nitrogen. The LDH release was measured at an absorbance of 490 nm. The percentage of LDH release was calculated as: (LDH activity in media)/(LDH activity in media/intracellular LDH activity) × 100 %. Results are presented as percentage of LDH release subtracting the control values from treated ones. All the experiments were performed in triplicate (Korzeniewski and Callewaert, 1983).

Cell cycle analysis by fluorescence-activated cell sorting (FACS) method

K562 cell was cultured and treated with 25 µg of compound **2b,** and the treated cells were incubated for 24 h. After overnight incubation cells were trypsinized by adding 100 µl of 0.25 % trypsin (Invitrogen) and allowed the cells to detach. The detached cells were pipetted out and spun at 300×*g* (RPM of rotor × gravity constant) for 5 min and washed once with 1× PBS. The cells were fixed with 1 ml of ice-cold ethanol and incubated at −20 °C for overnight. The ethanol fixed cells were washed once with PBS followed by 200 µl of Muse cell cycle reagent. The tubes were incubated for 30 min at dark and analyzed on Muse flow cytometer (Millipore, USA, 2012).

Chemistry

All compounds synthesized were characterized by IR, ^1H NMR and ^{13}C NMR elemental analyses and are described in experimental section. Melting points were determined in a BUNA melting point instrument and are uncorrected. IR spectra were recorded on a Shimadzu Affinity I FT-IR

spectrometer. NMR spectra were measured at 400 MHz on a Bruker 400 spectrometer using TMS as internal standard and DMSO-d_6 as solvent. Elemental analyses were determined using a PerkinElmer 240c elemental analyzer. Microwave reaction was carried out using CEM Discover, Benchmate, USA.

General procedure for the synthesis of ***2a, 2b, 2f*** *and* ***2 g***

In a 22 % aqueous NaOH, 1 mol of glycine/dl-alanine/ethyl amine or allyl amine was dissolved. The solution was cooled, and 1 mol of carbon disulfide was added with stirring which was continued for 3 h. To this, a solution of 1 mol of sodium chloroacetate was added and stirring was continued for another 3 h. The mixture was then made acidic with conc. HCl and kept overnight. Then the mixture was heated on a water bath for 90 min till the product separated as oil which was solidified on cooling. It was collected on a filter, washed with water and dried (Brooker *et al.*, 1950; Bernardo *et al.*, 2009, 2011).

General procedure for the synthesis of ***(2d)***

In a 22 % aqueous NaOH, 1 mol of L-glutamic acid hydrochloride was dissolved. The solution was cooled, and 1 mol of carbon disulfide was then added with stirring which was continued for 4 h. To this, a solution of 1 mol of sodium chloroacetate was added and stirring was continued another 3 h. The mixture was kept overnight and then made acidic with dil. H_2SO_4. The mixture was heated on a water bath for 2 h till the product separated as an oil which solidified on cooling. It was collected on a filter, washed with water and dried, and the crystallized product was collected (Brooker *et al.*, 1950).

General procedure for the synthesis of ***2c*** *and* ***2e***

In a 22 % aqueous NaOH, 1 mol of dl-valine or L-phenylalanine was dissolved. The solution was cooled, and 1 mol of carbon disulfide was then added with stirring which was continued for 6 h. To this, a solution of 1 mol of sodium chloroacetate was added and stirring was continued another 3 h. The mixture was kept overnight and then made acidic with conc. HCl. The mixture was refluxed for 8 h. The product separated as oil which was solidified on cooling. It was collected on a filter by column chromatography (Bernardo *et al.*, 2009, 2011).

General procedure for the microwave-assisted synthesis of N-substituted rhodanines ***(2a–g)***

Mixture of amine or amino acid (1 mol), sodium hydroxide (22 %) and carbon disulfide (1 mol) in 3 ml water was reacted in a microwave reactor (CEM Discover, Benchmate, USA) for 5 min at 100 °C at 80 W. After automated cooling to 40 °C, sodium chloroacetate (1 mol) was added and the mixture was reacted again at 100 °C for 5 min. After cooling (40 °C), conc. HCl (3 ml) was added and the reaction was completed at 110 °C for 20–30 min. The crude product was extracted with ethyl acetate and purified (Nitsche and Klein, 2012).

2-(4-Oxo-2-thioxothiazolidin-3-yl)acetic acid (**2a**) Yield; 82.5 %; mp 245–247 °C; UV λ_{max} 266, 215 nm; IR(KBr) ν_{max} 3439, 1663, 1512, 1321, 896 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 4.56$ (2H, s, N–C$\underline{H_2}$), 4.41 (2H, s, H-5); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 202.80$ (C = S, C-2), 173.72 (C = O, $\underline{C}$OOH), 167.29 (C = O, C-4), 44.77 (CH_2, N–$\underline{CH_2}$), 35.94 (CH_2, C-5); Anal.calcd. for $C_5H_5NO_3S_2$: C, 31.40; H, 2.64; N, 7.32. Found: C, 31.55; H, 2.53; N, 7.22.

2-(4-Oxo-2-thioxothiazolidin-3-yl)propanoic acid (**2b**) Yield; 81.2 %; mp 113–114 °C; UV λ_{max} 285, 261 nm; IR (KBr) ν_{max} 3452, 1675, 1458, 1355, 844 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 3.5$ (2H, s, H-5), 5.27 (1H, m, N–C$\underline{H}$), 1.43 (3H, d, $J = 7.2$ H, CH–C$\underline{H_3}$); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 203.01$ (C = S, C-2), 173.75 (C = O, $\underline{C}$OOH), 168.60 (C = O, C-4), 54.31 (CH, N–$\underline{CH}$), 34.71 (CH_2, C-5), 13.73 (CH_3, CH–$\underline{CH_3}$); Anal.calcd. for $C_6H_7NO_3S_2$: C, 35.11; H, 3.44; N, 6.82. Found: C, 35.22; H, 3.48; N, 6.73.

3-Methyl-2-(4-oxo-2-thioxothiazolidin-3-yl)butanoic acid (**2c**) Yield; 79.0 %; mp 115–117 °C; UV λ_{max} 298, 259 nm; IR (KBr) ν_{max} 3428, 1667, 1502, 1413, 1317, 885 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 0.89$ (m, 6H, CH(C$\underline{H_3}$)$_2$), 2.75 (1H, m, N–CH–C$\underline{H}$(CH$_3$)$_2$), 3.56 (2H, m, H-5), 4.20 (1H, m, N–C$\underline{H}$–CH(CH$_3$)$_2$); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 203.30$ (C = S, C-2), 174.41 (C = O, $\underline{C}$OOH), 168.06 (C = O, C-4), 57.59 (CH, N–$\underline{CH}$), 35.76 (CH_2, C-5), 19.06 (CH_3, CH–$\underline{CH_3}$), 17.86 (CH_3, CH–$\underline{CH_3}$); Anal.calcd. for $C_8H_{11}NO_3S_2$: C, 41.18; H, 4.75; N, 6.00. Found: C, 41.24; H, 4.80; N, 6.11.

2-(4-Oxo-2-thioxothiazolidin-3-yl)pentanedioic acid (**2d**) Yield; 89 %; mp 123–127 °C; UV λ_{max} 302, 289 nm; IR (KBr) ν_{max} 3417, 1716, 1676, 1508, 1425, 1271, 819 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.23$ (2H, m, N–CH–C$\underline{H_2}$–CH$_2$–), 2.36 (2H, m, N–CH–CH$_2$–C$\underline{H_2}$–), 4.20 (2H, m, H-5), 4.30 (1H, t, $J = 10.4$ Hz, N–C$\underline{H}$-); ^{13}C NMR (DMSO-d$_6$, 100 MHz,): $\delta = 201.50$ (C = S, C-2), 173.90 (C = O, CH-$\underline{C}$OOH), 173.67 (C = O, CH$_2$-$\underline{C}$OOH), 168.60 (C = O, C-4), 56.47 (CH, N–$\underline{CH}$), 41.38 (CH_2, C-5), 30.03 (CH_2, N–CH–CH$_2$–$\underline{CH_2}$–), 22.58 (CH_2, N–CH–$\underline{CH_2}$–CH$_2$–); Anal.calcd. for $C_8H_9NO_5S_2$: C, 36.49; H, 3.45; N, 5.32. Found: C, 36.56; H, 3. 57; N, 5.44.

2-(4-Oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**2e**) Yield; 84 %; mp 20–23 °C; UV λ_{max} 285, 261 nm; IR (KBr) ν_{max} 3449, 1691, 1558, 1402, 1313, 1149, 835 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 3.76$ (2H, m, H-5), 4.54 (1H, m, N-CH), 3.44, (1H, dd, $J = 11$ & 7.0 Hz CH–CH_2) 3.30 (1H, dd, $J = 11.4$ & 7.2 Hz CH–CH_2), 7.04-7.29 (5H, m, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 202.82$ (C = S), 173.99 (C = O, COOH), 169.7 (C = O), 129.50, 128.9, 128.3, 128.1, 126.4 (C, Ar–C), 59.01 (CH, N–CH), 37.44 (CH_2, C-5), 34.34 (CH_2, CH–CH_2); Anal.calcd. for $C_{12}H_{11}NO_3S_2$: C, 51.23; H, 3.94; N, 4.98. Found: C, 51.34; H, 3.83; N, 4.94; O, 17.10.

3-Ethyl-2-thioxothiazolidin-4-one (**2f**) Yield; 84 %; mp 111–113 °C; UV λ_{max} 265, 211 nm; IR (KBr) ν_{max} 3407, 1687, 1500, 1400, 1149, 827 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 4.06$ (2H, m, H-5), 3.95 (2H, s, H-2′), 1.21 (3H, t, $J = 6.0$ Hz, CH_3, H-1′); ^{13}C NMR (DMSO-d_6,100 MHz): $\delta = 200.9$ (C = S, C-2). 173.6 (C = O, C-4), 39.9 (CH_2, C-5), 35.4(CH_2, N-CH_2), 12.04 (CH_3, CH_2-CH_3); Anal.calcd. for $C_5H_7NOS_2$: C, 37.24; H, 4.38; N, 8.69. Found: C, 37.32; H, 4.51; N, 8.69.

3-Allyl-2-thioxothiazolidin-4-one (**2g**) Yield; 82 %; mp 141–143 °C; UV λ_{max} 275, 223 nm; IR (KBr) ν_{max} 3458, 1674, 1423, 1298, 823 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 3.82$ (2H, m, H-5), 4.63 (2H, s, N-CH_2), 5.32 (3H, m, CH = CH_2), 5.63 (1H, m, CH = CH_2); ^{13}C NMR (DMSO-d_6,100 MHz): $\delta = 202.9$ (C = S, C-2), 172.6 (C = O), 131.78 (CH, CH = CH_2), 116.72(CH_2, CH = CH_2), 43.92 (CH_2, N-CH_2), 39.68 (CH_2, C-5); Anal.calcd. for $C_6H_7NOS_2$: C, 41.59; H, 4.07; N, 8.08. Found: C, 41.78; H, 4.03; N, 8.38.

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