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Note

Synthesis and cytotoxicity studies of thiazole analogs of the anticancer marine alkaloid dendrodoine

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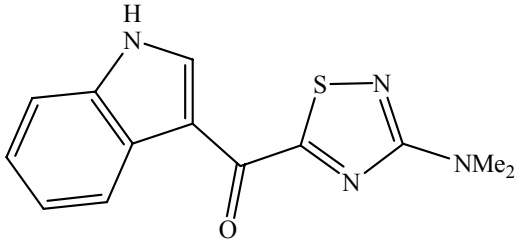
The synthesis and cytotoxicity evaluation of 2-*N*,*N*-dimethylamino-5-indol-3-oylthiazole as the first member of a new portfolio of the thiazole analogs of the cytotoxic marine alkaloid dendrodoine (3-*N*,*N*-dimethylamino-5-indol-3-oyl-1,2,4-thiadiazole) is described. Exploiting the opportunity arising from the replacement of the thiadiazole ring of dendrodoine by a thiazole ring which allowed further substitution on the five-membered ring, 2-*N*,*N*-dimethylamino-5-indol-3-oyl-4-phenylthiazole has also been synthesized. Structural diversity is further extended by synthesizing 5-fur-2-oyl- and 5-coumarin-3-oyl-2-*N*,*N*-dimethylaminothiazoles, as well as 5-fur-2-oyl, 5-thiophen-2-oyl, 5-(1-methylbenzimidazol-2-oyl) and 5-benzothiazol-2-oyl derivatives of 2-*N*,*N*-dimethylamino-4-phenylthiazoles. Among these new *N*,*N*-dimethylaminothiazoles, 2-*N*,*N*-dimethylamino-5-indol-3-oyl-4-phenylthiazole shows significant *in vitro* cytotoxicity against a panel of human cancer cell lines.

Keywords: Dendrodoine, marine alkaloid, thiazole analog, 2-aminothiazole, cytotoxicity

Dendrodoine, 3-*N*,*N*-dimethylamino-5-indol-3-oyl-1,2,4-thiadiazole **1**, is a marine alkaloid isolated from the tunicate *Dendrodoa grossularia*. It contains a 1,2,4-thiadiazole unit, quite uncommon either in terrestrial or in marine natural products[1].

Dendrodoine also belongs to the indole class of marine alkaloids. Indole derivatives of marine origin such as topsentins and nortopsentins exhibit a spectrum of bioactivity including cytotoxicity towards cancer cells[2-4]. Dendrodoine has been reported to be cytotoxic to lymphoma cells L1210 in culture[1]. Though its synthesis has been reported[5], further studies on it or on its analogs have not appeared. It appears that not only the preparation of dendrodoine analogs with a variety of substituents is difficult, but the prospect for substituent manipulation in **1** is also rather limited since only two carbons are available for functionalization in the five-membered heterocyclic ring. We noted that the substitution of a thiazole ring in place of the thiadiazole ring in **1** would provide additional opportunities for introducing structural diversity on **1**. Thiazole moiety is present in a variety of natural and synthetic bioactive molecules. Recent studies on the *in vitro* cytotoxicity of thiazole derivatives include reports on the significant cytotoxic activitiy of bis(indolyl)thiazoles[6] and indolylthiazoles[7]. In this context, we now report on the synthesis and cytotoxicity evaluation of a novel portfolio of thiazole-based dendrodoine analogs such as 2-*N*,*N*-dimethylamino-5-indol-3-oylthiazole **2a** (**Scheme I**). These studies are expected to throw some light on the relationship between the structure and bioactivity of dendrodoine and its thiazole analog.

Dendrodoine has been synthesised[5] by a 1,3-dipolar cycloaddition of a nitrile sulfide to a nitrile group. The nitrile sulfide was generated by the thermolysis of a 1,3,4-oxathiazol-2-one which in turn was obtained by the reaction of 1,1-dimethylurea with chlorocarbonylsulphenyl chloride. The nitrile sulfide reacted *in situ* with the dipolarophile indoloylcyanide to afford **1**. We realised that the reported synthesis of dendrodoine is restricted to the preparation of 3-*N*,*N*-dialkylamino derivatives alone because mono or unsubstituted ureas would react with Cl-CO-S-Cl



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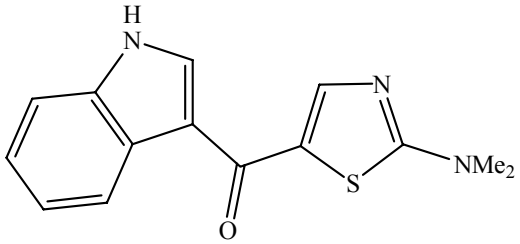
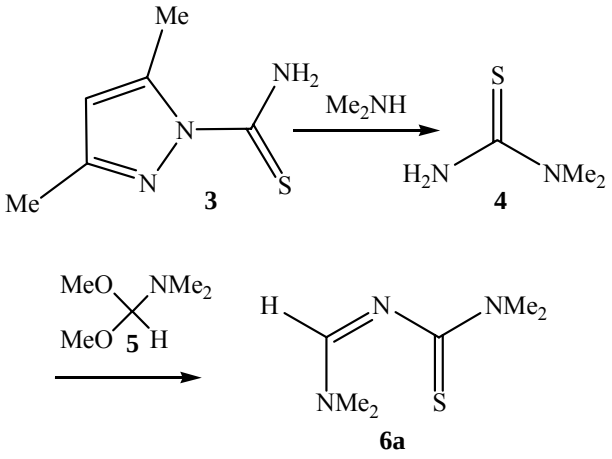


2a

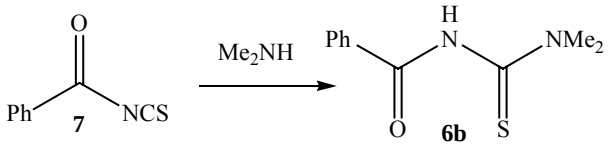
Scheme I

differently. In addition, hetaroyl or aroyl cyanides are not easy to access. Further, no other direct cyclisation route exists to 3-amino-5-aroyl-1,2,4-thiadiazoles. Thus, the preparation of dendrodoine analogs with a variety of substituents appears to be not easy.

In this context, the versatility of the 2-amino-5-ketothiazole synthesis based on imidoyl[8], acyl[9] and amidinothioureas[10,11] appeared promising. A variety of substituents could be placed on C-2 and C-4 carbons of the thiazole ring by choosing the appropriate thiourea synthons from those mentioned above. The 5-keto substituent of such 2-amino-5-ketothiazoles could be accessed through different α-haloketones. We therefore felt it worthwhile to synthesize the corresponding thiazole analog of dendrodoine and screen it for cytotoxic activity. It was also desirable to broaden the scope of our study by preparing and screening a variety of 5-hetaroyl substituted 2-aminothiazole derivatives **2b-h** as additional thiazole analog of dendrodoine.



Scheme II



Scheme III

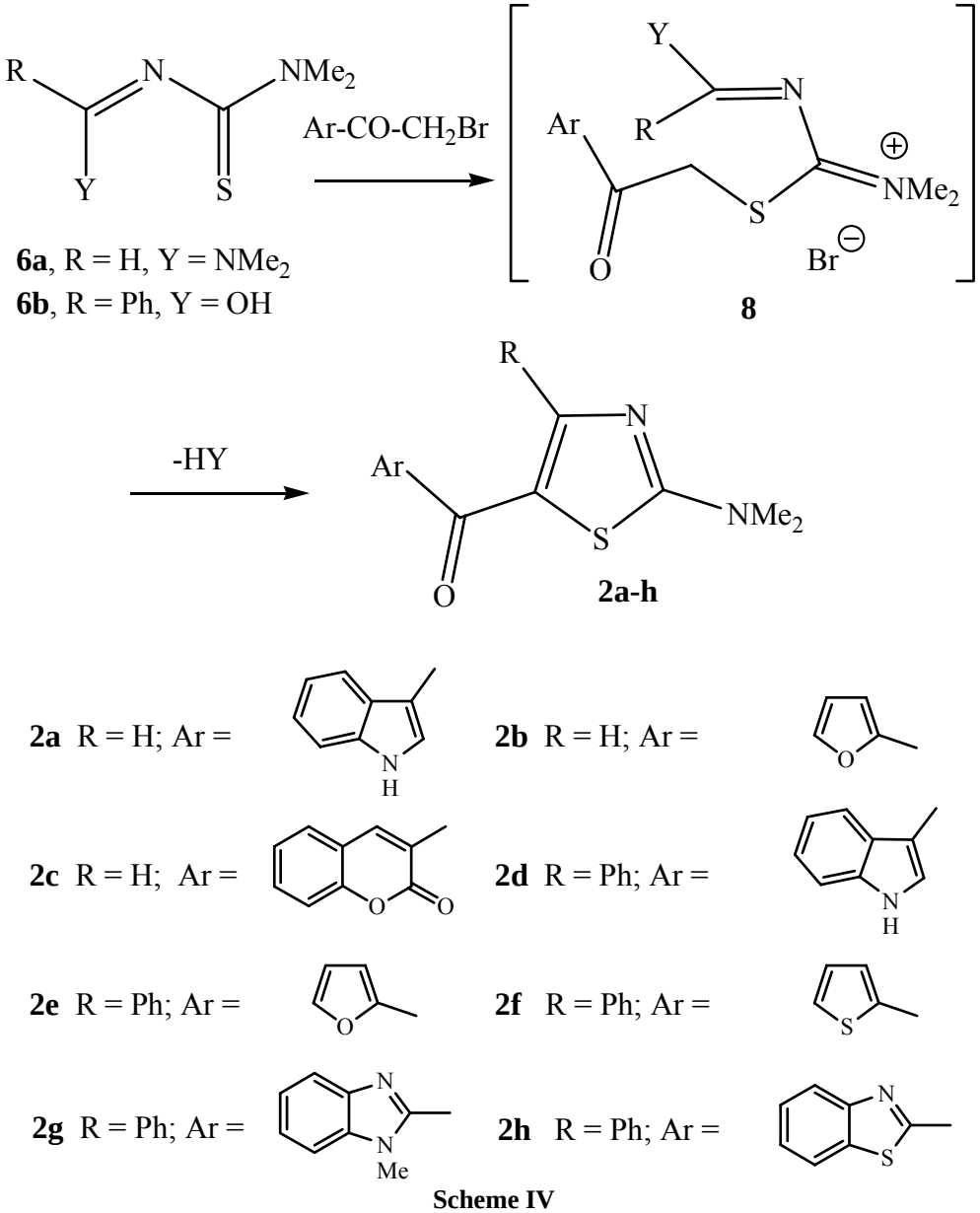
Results and Discussion

Based on a retrosynthetic analysis, a [4+1] thiazole ring construction strategy was chosen which involves the fusion of a thiourea-derived [C4-N3-C2-S1] synthon, such as the imidoylthiourea **6a** and the acylthiourea **6b**, with another synthon that provides the remaining C5 of the thiazole ring. The latter usually is a reactive halomethyl reagent such as α-haloketone. For the synthesis of **2a**, the required imidoylthiourea **6a** was obtained by the reaction of 1,1-dimethylthiourea **4** with *N*,*N*-dimethylformamide dimethylacetal **5** (**Scheme II**). 1,1-Dimethylthiourea **4** was in turn obtained by reacting 3,5-dimethyl-1-thiocarbamoylpyrazole **3** with dimethylamine. The imidoylthiourea **6a** on reaction with 3-bromoacetylindole in the presence of triethylamine gave the target molecule **2a** through the open chain intermediate **8** (R = H; Y = NMe_2) which underwent base catalysed cyclisation followed by elimination of dimethylamine. The use of 2-bromoacetylfuran and 3-bromoacetylcoumarin led to the furan-2-oylthiazole **2b** and coumarin-3-oylthiazole **2c**.

To exploit the opportunity for introducing a third substituent, acylthioureas were selected next as the [C4-N3-C2-S1] synthon to access 4-substituted thiazole analogs of **2a**. Accordingly, the reaction of 1-benzoyl-3,3-dimethylthiourea **6b**, obtained from benzoyl isothiocyanate **7** and dimethylamine **(Scheme III)**, with 3-bromoacetylindole afforded 2-*N*,*N*-dimethylamino-5-indol-3-oyl-4-phenylthiazole **2d** by the cyclisation of the intermediate **8** (R = Ph, Y = OH) followed by elimination of water. The furan-2-oyl-, thiophen-2-oyl-, 1-methylbenzimidazol-2-oyl- and benzothiazol-2-oylthiazoles **2e-h** were also obtained similarly in good yield. The reaction steps are illustrated in a generalized manner in **Scheme IV**. For generalization, the acylthiourea **6b** is shown in the reaction scheme as an enol tautomer.

The cytotoxicity of the thiazole analogs **2a-c** was subjected to a preliminary evaluation at a concentration of at 10^{-4}M against a panel of three human tumor cell lines using the National Cancer Institute primary anticancer screen[12] which included NCI-H460 (lung cancer), MCF7 (breast cancer) and SF-268 (CNS cancer). The results revealed that the thiazole analog **2a** of dendrodoine **1** and its 5-furo-2-yl or 5-coumarin-3-oyl derivatives **2b-c** were rather inactive under this screening protocol. However, the 4-phenylthiazole analog **2d** of dendrodoine **1** showed significant activity as could be seen from the data on percentage growth presented in **Table I** in which only those compounds that showed growth less than 60% for at least one of the three cell lines tested are included.

In a subsequent round of screening using a panel of sixty cell lines, it was found that **2d** showed the GI_{50} value (the minimum concentration needed to inhibit the cell growth by 50% *in vitro*) of 0.77μM against the human cancer cell line Leukaemia SR. The GI_{50}



Scheme IV

Table I — Growth percentages of cells treated *in vitro* at 10^{-4} M

Compd	Cancer cell line growth (%) (Untreated = 100)		
	NCI-H460 (Lung)	NCF7 (Breast)	SF-268 (CNS)
2a	57	62	82
2b	98	42	94
2d	12	7	10
2e	56	56	56
2f	72	80	35

values against other cell lines for **2d** were: cell line, (GI_{50} value in μM): lung cancer, NCI-H 522 (2.76), NCI-H460 (3.18); colon cancer, KM12 (3.47), HT29 (3.32); CNS cancer, SNB-75 (2.67), SF-268 (6.70); melanoma, UACC-257 (3.66); ovarian cancer, OVCAR-3 (3.51), IGROV1 (2.96); breast cancer, MCF-7 (4.19); renal cancer TK-10 (3.04).

Thus we have now found that the replacement of a thiazole ring in place of the 1,2,4-thiadiazole ring in dendrodoine **1** results in an analog **2a** devoid of cytotoxic activity. However, the introduction of a phenyl ring at the C-4 of **2a** leading to **2d** resulted in a dendrodoine analog that has significant cytotoxic

activity against human cancer cell lines. Based on this observation, further studies on 4-aryl-5-indoloyl-2-(disubstituted amino)thiazoles seem warranted.

Experimental Section

Reagents and solvents were from Merck India and Fluka. ^{1}H NMR spectra were run on Bruker Specrtospin-90, Bruker AC 300F or JEOL DRX 300 and mass spectra on JEOL SX 102 or D-300 spectrometers. Melting points are uncorrected. Elemental analysis was done at Central Drug Research Institute, India. Literature methods were used for the preparation of 3,5-dimethyl-1-thiocarboxamidopyrazole **3** (Ref.13), 3-bromoacet- ylindole[7], 2-bromoacetyl-1-methylbenzimidazole[14] and 2-bromoacetylbenzothiazole[15]. Bromination of 2-acetylfuran and 2-acetylthiophen were done using Cu(II)Br_2 in refluxing ethyl acetate and the crude bromoacetyl derivatives were purified on a short column of silica gel (60-120 mesh, chloroform). The 2-bromoacetyl derivatives of furan and thiophen were obtained as yellow pasty mass in 65 and 76% yield respectively and were used without delay. (CAUTION! Both 2-bromoacetylfuran and thiophene may cause skin irritation). The bromination of 3-acetylcoumarin was done in glacial acetic acid at 70-80°C for 1hr using equimolar amount of bromine followed by cooling and dilution with cold water to obtain 3-bromoacetylcoumarin which was crystallised from EtOH-H_2O. Yield 88%, m.p. 162-3°C; lit. m.p. 162-3°C[16].

Synthesis of 2-N,N-dimethylamino-5-indol-3-oyl-thiazole 2a

Dimethylamine (5 mmol, 0.57 mL, 40% in water) was added to 3,5-dimethyl-1-thiocarbamoylpyrazole 3 (5 mmol, 0.775 g), the mixture was stirred well and kept at RT for 15 hr. The reaction mixture was heated and the excess amine was removed under reduced pressure. The crude material was purified by column chromatography (silica gel 60-120 mesh, chloroform) to obtain colourless needles of 1,1-dimethylthiourea **4**. Yield: 75%, m.p. 158-9°C; lit. m.p. 159°C[1]. 1,1-Dimethylthiourea **4** and N,N-dimethylformamide dimethylacetal **5** (1 mmol each) were heated in a closed vessel at 90-95°C for 2 hr, followed by evaporation under reduced pressure to afford 1-N,N-dimethyl-3-[(N,N-dimethylamino)methylene]thiourea **6a.** Yield: 95%; m.p. 89-91°C. IR (KBr): 2923, 1621, 1507, 1429, 1373, 1333, 1288, 1180, 1141, 1102, 1056, 986, 921, 871, 752, 664 cm^{-1}. The product was used directly in the next step immediately.

To a solution of the thiourea **6a** (1 mmol, 0.159 g) in N,N-dimethylformamide (5 mL), 3-bromoacetyl-indole (1 mmol, 0.238 g) was added. The reaction mixture was warmed on a water bath at 80-85°C for 5 min. To this, triethylamine (1 mmol, 0.15 mL) was added and heating was continued for another 15 min. The above mixture was cooled and poured into ice-cold water with stirring. A yellow precipitate thus obtained was filtered, washed with water and air-dried. This crude material was purified by column chromatography (silica gel, 60-120 mesh, EtOAc) to obtain **2a**. Yield: 85%, m.p. 264-65°C. Anal. Calcd. for $C_{14}H_{13}N_3OS$ (271.35): C, 62.0; H, 4.8; N, 15.5. Found: C, 61.9; H, 4.9; N, 15.4%. IR (KBr): 3140, 2895, 1725, 1710, 1691, 1658, 1562, 1460, 1372, 1312, 1296, 1242, 1113, 1010, 935, 820, 771, 654 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.22 (s, 6H, NMe_2), 7.25 (m, 2H, indole H-5, H-6), 7.46 (d, J = 5.7 Hz, 1H, indole H-7), 7.87 (s, 2H, overlapped indole H-2 and thiazole H-4), 8.28 (d, J = 8 Hz, 1H, indole H-4), 10.81 (s, 1H, NH); EIMS: *m/z* (%) 271 (M^+, 100).

Synthesis of 5-furan-2-oyl-2-N,N-dimethylamino-thiazole 2b

Using a similar procedure as above, the thiourea **6a** was reacted with 2-bromoacetylfuran (1 mmol each) to obtain crude **2b** which was purified by column chromatography as above. Yield: 76%, m.p. 133-35°C. Anal. Calcd. for $C_{10}H_{10}N_2O_2S$ (222.27): C, 54.0; H, 4.5; N, 12.6. Found: C, 54.1; H, 4.5; N, 12.7%. IR (KBr): 3122, 3107, 2923, 1554, 1468, 1393, 1283, 1177, 1142, 1061, 1002, 941, 882, 825, 752 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.21 (s, 6H, NMe_2), 6.82 (t, 1H, furan H-4), 7.25 (d, 1H, furan H-3), 7.92-7.95 (m, 2H, furan H-5, thiazole H-4); EIMS: *m/z* (%) 222 (M^+, 100).

Synthesis of 5-coumarin-3-oyl-2-*N,N*-dimethyl-aminothiazole 2c

The compound **2c** was synthesized using 3-bromo-acetylcoumarin and purified by chromatography (silica gel, EtOAc) as described above. Yield: 88%, m.p. 230-33°C. Anal. Calcd. for $C_{15}H_{12}N_2O_3S$ (300.34): C, 60.0; H, 4.0; N, 9.3. Found C, 60.1; H, 4.1; N, 9.5%. IR (KBr): 3070, 1725, 1620, 1607, 1587, 1564, 1509, 1453, 1348, 1321, 1297, 1197, 1138, 1124, 1006, 958, 894, 761, 724, 687 cm^{-1}; ^{1}H NMR ($CDCl_3$+DMSO-d_6): δ 3.20 (s, 6H, NMe_2),

7.35-7.85 (m, coumarin 4H), 7.95 (s, 1H, thiazole H-4), 8.25 (s, 1H, coumarin H-4); EIMS: *m/z* (%) 300 (M^+, 100).

Synthesis of 2-*N,N*-dimethylamino-5-indol-3-oyl-4-phenylthiazole 2d

To a stirred solution of benzoyl chloride (7.5 mmol, 0.85 mL) in benzene (4 mL), tetrabutylammonium bromide (0.2 g) was added. To this mixture, an aqueous solution of potassium thiocyanate (33%, 5.5 mL) was added during 15 min and the stirring was continued for another 30 min. The aqueous layer was removed and the benzene layer containing the isothiocyanate **7** was quickly dried. Dimethylamine (7.5 mmol, 0.85 mL, 40%) was added, the mixture was stirred at RT for 2 hr and then diluted with petroleum ether to obtain 1-benzoyl-3,3-dimethylthiourea **6b** which was crystallised from EtOH-H_2O. Yield 75%, m.p. 138-139°C, lit. m.p. 138°C[17].

Equimolar amounts of the thiourea **6b** and 3-bromoacetylindole (1 mmol each) were reacted in *N,N*-dimethylformamide (5 mL) on a water bath at 80-85°C for 30 min and worked up to obtain a crude product which was purified by column chromatography (silica gel, $CHCl_3$-EtOAc, 3:1) to afford the compound **2d**. Yield: 89%, m. p. 260-261°C. Anal. Calcd. for $C_{20}H_{17}N_3OS$ (347.41): C, 69.1; H, 4.9; N, 12.1. Found: C, 69.0; H, 5.1; N, 12.2%. IR (KBr): 3276, 1568, 1515, 1474, 1434, 1370, 1342, 1325, 1311, 1244, 1203, 1120, 938, 897, 770, 751, 696 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.15 (s, 6H, NMe_2), 7.13-7.27 (m, 5H, indole H-5, H-6, phenyl 3H), 7.32-7.41 (m, 1H, indole H-7), 7.45-7.54 (m, 2H, phenyl 2H), 7.59 (s, 1H, indole H-2), 8.09 (d, 1H, indole H-4), 11.69 (s, 1H, NH); FABMS: (NBA matrix) *m/z* (%) 348 (MH^+).

Synthesis of 5-furan-2-oyl-2-*N,N*-dimethylamino -4-phenylthiazole 2e

The reaction of 2-bromoacetylfuran with **6b** and work up as above followed by crystallisation from benzene-petroleum ether afforded **2e**. Yield: 94%, m.p. 115-17°C. Anal. Calcd. for $C_{16}H_{14}N_2O_2S$ (298.37): C, 64.4; H, 4.7; N, 9.4. Found: C, 64.5; H, 4.6; N, 9.4%. IR (KBr): 2360, 1615, 1564, 1501, 1469, 1436, 1421, 1347, 1220, 1182, 1107, 1072, 1025, 1011, 829, 743, 732 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.22, (s, 6H, NMe_2), 6.31-6.32, (m, 1H, H-4), 6.86 (d, 1H, H-3), 7.23-7.29 (m, 4H, 4ArH), 7.51-7.56 (m, 2H, H-5); EIMS: *m/z* (%) 298 (M^+, 100).

Synthesis of 2-*N,N*-dimethylamino-4-phenyl-5-thiophen-2-oylthiazole 2f

The reaction of 2-bromoacetylthiophen with the thiourea **3b** and purification as above afforded the thiazole **2f.** Yield: 72%, m.p. 180-82°C. Anal. Calcd. for $C_{16}H_{14}N_2OS_2$ (314.4): C, 61.1; H, 4.5; N, 8.9. Found: C, 61.2; H, 4.5; N, 8.8%. IR (KBr): 3093, 2284, 1564, 1509, 1413, 1346, 1228, 1157, 1101, 1075, 1038, 937, 829, 755, 703, 630 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 3.25 (s, 6H, NMe_2), 7.0-7.12 (m, 2H, H-4, ArH), 7.25-7.32 (m, 4H, 4ArH), 7.46 (d, 1H, H-5), 7.7 (d, 1H, H-3); EIMS: m/z (%) 314 (M^+, 100).

Synthesis of 2-*N,N*-dimethylamino-5-(1-methylbenzimidazol-2-oyl)-4-phenylthiazole 2g

The reaction of 2-bromoacetyl-1-methylbenzimidazole with **6b** afforded the thiazole **2g**. Yield: 58%, m.p. 165-66°C. Anal. Calcd. for $C_{20}H_{18}N_4OS$ (362.4): C, 66.3; H, 5.0; N, 15.5. Found: C, 66.5; H, 5.3; N, 15.6%. IR (KBr): 2935, 1620, 1566, 1490, 1458, 1410, 1357, 1310, 1290, 1236, 963, 865, 811, 764, 717 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.21, s, 6H; 3.97, s, 3H; 7.20-7.35, m, 4H, ArH; 7.36-7.45, t, *J*=7.5 Hz, 1H; 7.47-7.59, m, 2H; 7.60-7.68, d, *J*=8 Hz, 1H; 7.76, d, *J*=8 Hz, 1H; FABMS (NBA matrix): *m/z* 363 [MH^+].

Synthesis of 5-benzothiazol-2-oyl-2-*N,N*-dimethylamino-4-phenylthiazole 2h

The reaction of 2-bromoacetylbenzothiazole with **3b** afforded the thiazole **2h**. Yield: 63%, m.p. 156°C. Anal. Calcd. for $C_{19}H_{15}N_3OS_2$ (365.4): C, 62.5; H, 4.1; N, 11.5. Found C, 62.3; H, 4.0; N, 11.3%. IR (KBr): 3063, 2935, 2369, 1692, 1566, 1499, 1459, 1371, 1303, 1155, 1075, 947, 906, 818, 771, 722, 710 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.25, s, 6H, 7.32-7.40, m, 3H, ArH, 7.54-7.70, m, 4H, 8.14-8.23, m, 2H; FABMS (NBA matrix): *m/z* 366 [MH^+].

Cytotoxicity studies

The cytotoxicity assays were done at National Cancer Institute, USA as part of developmental therapeutics program at the preliminary level using a three-cell panel. Those compounds that reduced the cell growth below 32% were further evaluated against a panel of sixty cell lines over a 5-log dose range to determine efficacy.

Acknowledgement

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^{1}H NMR and MS data and National Cancer Institute, USA for cytotoxic evaluations.

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