ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

MEDICINAL CHEMISTRY

SYNTHESIS AND *IN VITRO* ANTITUMOR ACTIVITY OF NEW NICOTINYL-RHODANINE DERIVATIVES

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ABSTRACT

A series of new nicotinyl-rhodanine derivatives were synthesized by condensing various chloronicotinaldehydes with rhodanine and substituted rhodanines. The *in vitro* antitumor activity for these compounds was screened against MCF-7, A549 and HT29 human cancer cell lines. The results show that compounds **1**, **3**, **5**, **7**, **8** and **9** are more potent against MCF-7 cell lines; compounds **9** and **11** are more potent against A549 cell lines; compound **3** is more potent against HT29 cell lines amongst the 14 nicotinyl-rhodanine compounds synthesized. The relationships between structure and antitumor activity were elucidated.

ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011

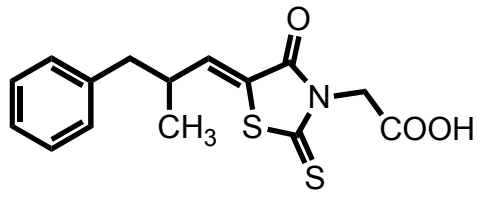
KEYWORDS

Rhodanine Derivatives, Nicotinaldehydes, Synthesis, Antitumor Activity, Structure-Activity Relations.

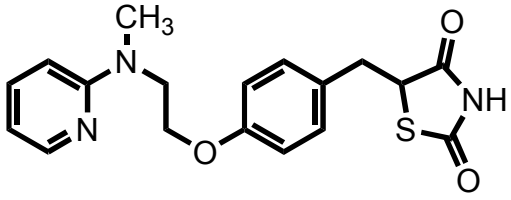
INTRODUCTION

Cancer is considered to be the most fatal disease and till today, cure of cancer is very challenging although there are several anticancer agents yet to be explored from literature and a lot are in clinical trials.[1] Effective strategies in combating the disease include targeting proliferation pathways,[2] and signal transduction mechanisms including Akt,[1,3] apoptotic,[4] JNK stimulation pathways,[2,5] to accelerate cell demises. But the emergence of resistance,[6] physiological destructive consequences of therapy,[7] and toxicity of diversified anticancer agents leads to limitation of their use. These limitations have paved the way to search for biologically promising new chemical entities (NCEs),[8-13] against deadly cancer disease. Major sources of bioactive NCEs are identified from or inspired by natural products,[14,15] marine metabolites,[16,17] and random screening of chemical library.[18-20] In discovering small antitumor molecules, a notable role is played by heterocyclic structures,[21] and among these, a growing attention focuses on the synthesis and study of the biological properties of compounds containing various combinations of pyridine **and /or** rhodanine moieties.[22] A wide spectrum of pharmacological activities has been reported for these compounds. These include fungal protein mannosyl transferase-1 inhibitors,[23] PDE4 inhibitors,[24] Protease inhibitors,[25] JNK-stimulating phosphatase-1 (JSP-1) inhibitors,[26] UDP-N-acetylmuramate/L-alanine ligase,[27] antimalerials,[28] HIV-1 Integrase inhibitors,[29] aldose reductase,[30] β-lactamase,[31] antidiabetic agents,[32] HCV NS3 Protease inhibitor,[33] and histidine decarboxylase,[34] *etc*. Epalrestat, marketed drug as aldose reductase inhibitor, is an analogue of rhodanine and Rosiglitazone, PPAR-γ agonist, is an isostere of rhodanine scaffold signifies its importance (Chart 1). On the other hand rhodanine derivatives also possess wide range of pharmacological action, especially as antitumor agents,[22e-p] and an ability to inhibit the JNK stimulating phosphatase-1 inhibition. Tomasiae and Masie recently published a review on rhodanine as a privileged scaffold in drug discovery whose functionalization and appropriate modifications led to compounds endowed with various biological activities.[35] It is also reported that, groups like pyridine and aryl substituted pyridine contribute to the biological activities,[26] and these would result in highly potent and selective antitumor agents. The design, synthesis and biological study of new compounds with enhanced activity is an ongoing research project in our group.[36] This article is the outcome of the intension to bring rhodanine-pyridine (nicotinyl) based NCEs as antitumor agents and it deals with the synthesis, *in vitro* antitumor activity and structure-activity relationships.

Chart 1



Epalrestat: Aldose Reductase Inhibitor



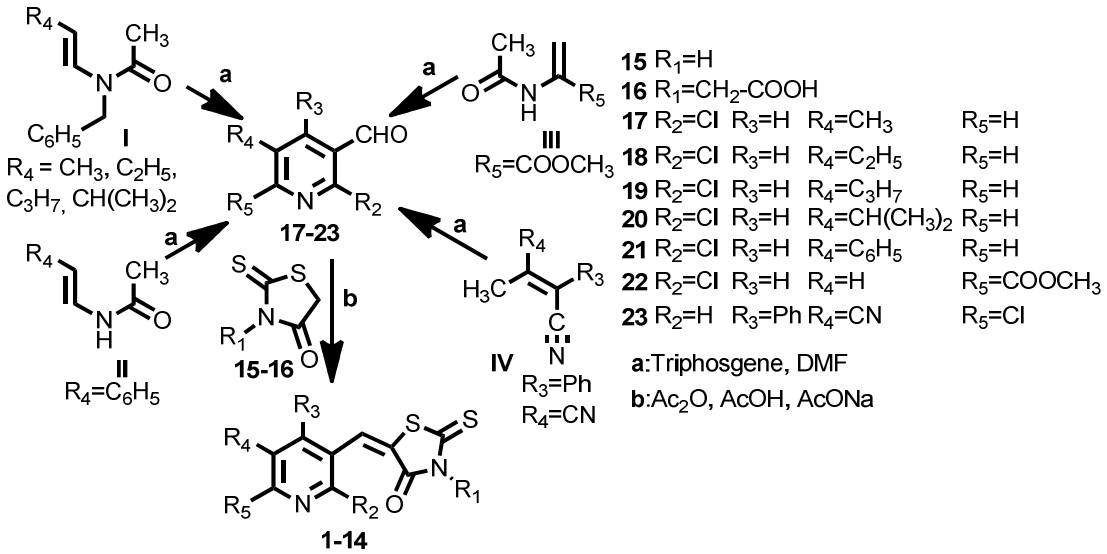
Rosiglitazone: PPAR receptors

Chemistry:

Substituted nicotinaldehydes **17**-**23** were prepared via reported procedures [37]. Nicotinyl-rhodanines **1-14** were synthesized in good yields by treating rhodanines **15**-**16** with various nicotinaldehydes **17-23** using acetic acid and sodium acetate under reflux conditions and the corresponding route is presented in **Scheme 1**. All the synthesized compounds are well characterized by ^{1}H-NMR, ^{13}C-NMR, Mass, IR etc. There are two sets of final products w. r. t nitrogen of rhodanine, one being secondary amide in ring (-NH-) and other is substituted with acetic acid (tertiary). Structure-activity relationship studies focused primarily on two regions of inhibitors: substitution on pyridine and rhodanine skeleton.

Scheme 1

General synthetic strategy of nicotinyl-rhodanine derivatives (1-14)

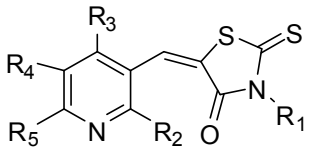


***Biological Activity*:**

All compounds synthesized were evaluated for their *in vitro* activity against three human tumor cell lines *viz.* MCF-7 (Breast cancer), A549 (Lung cancer), HT-29 (Colon cancer). [3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) assays, of respective cell lines, for reach compound were conducted in triplicates at 10^{-4} M and 10^{-5} M concentrations and the results of these studies are represented in **Table 1.**

Table 1

***The in vitro cell growth inhibitory effect of nicotinyl-rhodanine derivatives (1-14) in various cell lines with MTT assays*.**



1 - 14

Compound No.	R_1	R_2	R_3	R_4	R_5	IC_{50} (µg/ml) MCF-7	A549	HT-29
1	**-H**	**-Cl**	**-H**	**$-CH_3$**	**-H**	44	106	115
2	**$-CH_2COOH$**	**-Cl**	**-H**	**$-CH_3$**	**-H**	130	NA[a]	NA[a]
3	**-H**	**-Cl**	**-H**	**$-CH_2CH_3$**	**-H**	18	97	27
4	**$-CH_2COOH$**	**-Cl**	**-H**	**- CH_2CH_3**	**-H**	82	187	136
5	**-H**	**-Cl**	**-H**	**- CH_2 CH_2CH_3**	**-H**	14	125	136
6	**$-CH_2COOH$**	**-Cl**	**-H**	**- CH_2 CH_2CH_3**	**-H**	109	174	84
7	**-H**	**-Cl**	**-H**	**- $CH(CH_3)_2$**	**-H**	53	101	NA[a]
8	**$-CH_2COOH$**	**-Cl**	**-H**	**- $CH(CH_3)_2$**	**-H**	36	193	NA[a]
9	**-H**	**-Cl**	**-H**	**-Ph**	**-H**	46	41	184
10	**$-CH_2COOH$**	**-Cl**	**-H**	**-Ph**	**-H**	87	131	NA[a]
11	**-H**	**-Cl**	**-H**	**-H**	**$-COOCH_3$**	70	42	84
12	**$-CH_2COOH$**	**-Cl**	**-H**	**-H**	**$-COOCH_3$**	141	164	134
13	**-H**	**-H**	**-Ph**	**-CN**	**-Cl**	104	69	NA[a]
14	**$-CH_2COOH$**	**-H**	**-Ph**	**-CN**	**-Cl**	191	108	89

[a]NA- IC_{50} values are beyond 200 µg/ml

RESULTS AND DISCUSSION

It has been observed from the results presented in Table 1 that most of the compounds are active against three cell lines. Some of these compounds have been found to be highly cell line specific and show appreciable inhibition of a particular cell line at low concentration. Free –NH- in rhodanine series of compounds show good activity than that of substituted derivatives (substitution on NH with $-CH_2COOH$ group) except very few compounds. This is quite interesting because one may expect more interactions with –

CH_2COOH group than secondary amide. In MCF-7 cell lines, compound **1** exhibited good anti tumor activity with an IC_{50} value of 44 µg/mL. Presence of ethyl and propyl group on 5th position of pyridine ring, **3** and **5** respectively**,** is more potent compared to methyl group **1.** Substitution of isopropyl group in place of *n*-propyl group **7** on pyridine gave diminished activity and it may be the consequence of steric hindrance caused by bulkiness (volume) of isopropyl group. This shows that the presence of linear alkyl chain is essential to have very good activity. At same position, replacement of isopropyl with phenyl group **9** exhibited similar activity as **1** and **7**. Other compounds **11** and **13** are less potent compared to the rest of the compounds in the same series. In this particular cell lines, presence of alkyl groups on pyridine ring helped to have better antitumor activity compared to other groups. Further, presence of CH_2COOH group on rhodanine nitrogen in most of the compounds also leads to less activity.

SAR of rhodanine derivatives against A549 cell lines is different than that of MCF-7 cell lines. Alkyl substituted rhodanine derivatives **1**, **3**, **5** and **7** has moderate activity with IC_{50} value range of 125 – 97 µg/mL. Compound **9** where phenyl group has been substituted in place of alkyl group has better activity with IC_{50} value of 41 µg/mL. Compound **11** has similar activity as **9** where phenyl on 5th position and hydrogen on 6th position of pyridine is replaced with hydrogen and -$COOCH_3$, electron with drawing group, respectively. Compound **13**, where positions of phenyl, CN (electron with drawing group), and chlorine are present at altered positions, exhibited moderate activity. In this cell lines, presence of phenyl groups, and or electron with drawing groups on pyridine ring help to have better antitumor activity compared to alkyl groups. On the other hand, all the compounds bearing CH_2COOH group has lower activity compared to its counterpart, secondary amide in rhodanine.

Compounds possessing free –NH- group on rhodanine skeleton, have better antitumor activity against HT-29 cell lines except for **5** and **13**. Compound **3**, where 5th position of pyridine ring is substituted with ethyl, is found to have better activity than its methyl counterpart **1.** This is the potent compound amongst the screened compounds against HT29 cell lines. Increment in chain length from ethyl **3** to either linearly to *n*-propyl **5** or with branching to isopropyl **7,** exhibited lower activity. Substitution of phenyl group and or electron withdrawing group, like results against MCF-7 cell lines, demonstrated lower activity compared to ethyl substitution. Therefore, a suitable combination of the substituents and their appropriate position in the molecule significantly control the activity/function of the molecule.

CONCLUSION

In conclusion, novel nicotinaldehyde attached rhodanine derivatives were synthesized in good yields for their antitumor activity. Compounds possessing alkyl chain on pyridine ring, in particular increment of chain length linearly rather than branched, demonstrated the better activity against the MCF-7 cell lines. In contrast, presence of phenyl or electron with drawing group on pyridine and secondary amide of rhodanine skeleton exhibits increased antitumor activity in A549 cell lines. Compound **3**, where 5th position of pyridine ring is substituted with ethyl, is most potent amongst the screened compounds against HT29 cell lines. Either

increment or decrease of alkyl chain length exhibited relatively lower activity. Presence of –CH_2COOH group on rhodanine skeleton showed least activity except few cases against three cell lines. Present investigation highlight the role of various groups along with the position in the molecule toward their tumor cell growth inhibitory properties and could be useful in the further tailoring of the molecules for improving their antitumor activity.

EXPERIMENTAL

All reactions were carried out under an open atmosphere. Rhodanine and rhodanine 3-acetic acid from Sigma-Aldrich and the solvent were obtained from commercial suppliers and used without further purification. Melting points were determined on a Mel-apparatus and are uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian inova 400 MHz spectrometer with tetramethylsilane (TMS) as internal standard. Chemical shifts values are given in (δ) ppm, coupling constants (*J*) are in hertz, and splitting patterns are designated as follows: s, Singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet. HRMS (ESI) data were recorded on a QSTAR XL high- resolution mass spectrometer. IR spectra were taken on a Thermo Nicolet nexus 670 FT-IR spectrophotometer.

General procedure for synthesis of Rhodanine derivatives

0.01 Mole (1 equivalent) of chloronicotinaldehyde, 0.01 mole (1equivalent), of rhodanine derivatives, 0.03 mole (3 equivalents) of freshly fused sodium acetate in 25 ml. of acetic acid, to which 0.008 moles (0.8 equivalents) of acetic anhydride had been added, was refluxed for 3 hours, and allowed to cool. Water (200 mL) was then added to the solution where precipitate was formed. The precipitate was collected via filtration, re-crystallized from methanol and dried to give the desired compound. Yields (75-80%)

5-[(Z)-1-(2-chloro-5-methyl-3-pyridyl) methylidene]-2-thioxo-1,3-thiazolan-4-one (1):

Light brown solid (2.04g, 76%); mp 245-247 °C; IR (KBr) 3204, 2814, 2660, 1719, 1595, 1470, 1387, 1222, 1166, 1052 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.37 (s, 3H), 7.59 (s, 1H), 7.70 (d, *J* = 1.8 Hz, 1H), 8.31 (d, *J* = 1.8 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 17.0, 124.4, 127.0, 130.7, 133.7, 137.7, 148.1, 150.6, 168.9, 195.0; MS (ESI, -ve): m/z: 269 $[M-H]^-$; HRMS: m/z $[M-H]^-$ Calcd for $C_{10}H_6N_2OS_2Cl$: 268.9610, found: 268.9617.

2-5-[(Z)-1-(2-chloro-5-methyl-3-pyridyl) methylidene]-4-oxo-2-thioxo-1, 3-thiazolan-3-ylacetic acid (2)

Light brown solid (2.46g, 75%); mp 211-213 ^{o}C; IR (KBr) 3426, 3040, 1722, 1594, 1411, 1330, 1206, 1107, 1057 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.43 (s, 3H), 4.75 (s, 2H), 7.66 (s, 1H), 7.91 (s, 1H), 8.24 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 17.4, 44.6, 95.7, 126.7, 127.0, 127.2, 132.8, 137.6, 148.9, 150.4, 166.6, 191.4; MS (ESI, -ve): m/z: 327 $[M-H]^-$; HRMS: m/z $[M-H]^-$ Calcd for $C_{12}H_8N_2O_3S_2Cl$: 326.9664, found: 326.9678.

5-[(Z)-1-(2-chloro-5-ethyl-3-pyridyl) methylidene]-2-thioxo-1,3-thiazolan-4-one (3)

Light brown solid (2.27g, 80%); mp 222-224 ^{o}C; IR (KBr) 3004, 2935, 2711, 2494, 1712, 1616, 1402, 1330, 1222, 1195 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.23 (t, *J* = 8.1 Hz, 3H), 2.73 (q, *J* = 8.1 Hz, 2H), 7.63 (s, 1H), 7.75

(d, *J* = 2.7 Hz, 1H), 8.38 (d, *J* = 2.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.9, 24.3, 124.6, 127.4, 131.0, 136.8, 139.5, 148.2, 150.0, 169.0, 195.1; MS (ESI, -ve): m/z: 283 $[M-H]^-$; HRMS: m/z $[M-H]^-$ Calcd for $C_{11}H_8N_2OS_2Cl$: 282.9766, found: 282.9779.

2-5-[(Z)-1-(2-chloro-5-ethyl-3-pyridyl) methylidene]-4-oxo-2-thioxo-1,3-thiazolan-3-ylacetic acid (4)

Yellow solid (2.7g, 79%); mp 223-225 °C; IR (KBr) 3415, 2970, 2821, 1723, 1594, 1460, 1396, 1219, 1170, 1059 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.23 (t, *J* = 7.8 Hz, 3H), 2.73 (q, *J* = 7.8 Hz, 2H), 4.75 (s, 2H), 7.84 (s, 1H), 7.85 (d, *J* = 2.7 Hz, 1H), 8.40 (d, *J* = 2.7 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 15.0, 24.4, 45.1, 127.3, 127.4, 137.2, 139.7, 148.2, 148.3, 150.5, 165.8, 167.2, 192.7; MS (ESI, -ve): m/z: 341 $[M-H]^-$; HRMS: m/z $[M-H]^-$ Calcd for $C_{13}H_{10}N_2O_3S_2Cl$: 340.9821, found: 340.9835.

5-[(Z)-1-(2-chloro-5-propyl-3-pyridyl) methylidene]-2-thioxo-1,3-thiazolan-4-one (5)

Light brown solid (2.2g, 77%); mp 196-198 °C; IR (KBr) 2959, 2871, 1705, 1650, 1539, 1393, 1334, 1198, 1110, 1060 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.88 (t, *J* = 6.8 Hz,3H), 1.54-1.61 (m, *J* = 7.6 Hz, *J* =6.8 Hz, 2H), 2.53 (t, *J* = 7.6 Hz, 2H), 7.14 (s, 1H), 7.45 (s, 1H), 7.79 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.3, 23.6, 32.9, 124.8, 127.4, 131.0, 137.4, 138.0, 148.3, 150.5, 168.9, 195.2; MS (ESI, -ve): m/z: 297 $[M-H]^-$; HRMS: m/z $[M-H]^-$ Calcd for $C_{12}H_{10}N_2OS_2Cl$: 296.9923, found: 296.9930

2-5-[(Z)-1-(2-chloro-5-propyl-3-pyridyl) methylidene]-4-oxo-2-thioxo-1,3-thiazolan-3-ylacetic acid (6)

Light brown solid (2.77g, 78%); mp 158-159 °C; IR (KBr) 3420, 2960, 1706, 1650, 1540, 1394, 1335, 1200, 1109, 1061 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87 (t, *J* = 7.6 Hz, 3H), 1.50-1.54 (m, *J* = 7.6 Hz, 2H), 2.36 (t, *J* = 7.6 Hz, 2H), 4.69 (s, 2H), 7.48 (s, 1H), 7.62 (s, 1H), 7.86 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.2, 23.2, 32.1, 44.9, 120.1, 121.3, 122.3, 131.6, 137.5, 148.3, 160.2, 166.9, 167.4, 196.2; MS (ESI, -ve): m/z: 355 $[M-H]^-$; HRMS: m/z $[M-H]^-$ Calcd for $C_{14}H_{12}N_2O_3S_2Cl$: 354.9977, found: 354.9976.

5-[(Z)-1-(2-chloro-5-isopropyl-3-pyridyl) methylidene]-2-thioxo-1,3-thiazolan-4-one (7)

Light brown solid (2.26g, 76%); mp 243-245 °C; IR (KBr) 2960, 2832, 1724, 1647, 1595, 1445, 1215, 1172, 1061 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.27 (d, *J* = 6.3 Hz, 6H), 3.05-3.12 (m, *J* = 6.3 Hz, 1H), 7.64 (s, 1H), 7.75 (d, *J* = 1.8 Hz, 1H), 8.43 (d, *J* = 1.8 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.2, 30.3, 125.0, 127.6, 131.2, 135.5, 143.9, 148.3, 149.3, 168.9, 195.1; MS (ESI, -ve): m/z: 297 $[M-H]^-$; HRMS: m/z $[M-H]^-$ Calcd for $C_{12}H_{10}N_2OS_2Cl$: 296.9923, found: 296.9933.

2-5-[(Z)-1-(2-chloro-5-isopropyl-3-pyridyl) methylidene]-4-oxo-2-thioxo-1,3-thiazolan-3-ylacetic acid (8)

Yellow solid (2.81g, 79%); mp 163-165 °C; IR (KBr) 3411, 2966, 2714, 2506, 1852, 1714, 1615, 1328, 1198, 1055 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.28 (d, *J* = 7.2 Hz, 6H), 3.07-3.13 (m, *J* = 7.2 Hz, 1H), 4.76 (s, 2H), 7.86 (s, 1H), 7.87 (d, *J* = 1.8 Hz, 1H), 8.4 (d, *J*

= 1.8 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.0, 30.2, 45.0, 127.4, 127.5, 135.8, 135.9, 143.9, 148.1, 149.6, 165.7, 167.1, 192.6; MS (ESI, -ve): m/z: 355 $[M-H]^-$; HRMS: m/z $[M-H]^-$ Calcd for $C_{14}H_{12}N_2O_3S_2Cl$: 354.9977, found: 354.9976.

5-[(Z)-1-(2-chloro-5-phenyl-3-pyridyl) methylidene]-2-thioxo-1,3-thiazolan-4-one (9)

Light brown solid (2.49g, 75%); mp 246-248 °C; IR (KBr) 3029, 2822, 1701, 1591, 1443, 1386, 1222, 1167, 1056 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.56-7.67 (m, 3H), 7.75 (s, 1H), 7.84 (d, *J* = 7.1 Hz, 2H), 8.15 (d, *J* = 2.3 Hz, 1H), 8.86 (d, *J* =2.3 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 124.4, 126.9, 127.9, 128.9, 129.1, 129.3, 31.6, 134.7, 135.2, 135.4, 148.2, 149.3, 168.7, 194.8, 206.4; MS (ESI, -ve): m/z: 331 $[M-H]^-$; HRMS: m/z $[M-H]^-$ Calcd for $C_{15}H_8N_2OS_2Cl$: 330.9766, found: 330.9760.

2-5-[(Z)-1-(2-chloro-5-phenyl-3-pyridyl) methylidene]-4-oxo-2-thioxo-1,3-thiazolan-3-ylacetic acid (10)

Light brown solid (2.92g, 75%); mp 236-238 °C; IR (KBr) 3420, 2936, 1720, 1596, 1390, 1333, 1207, 1178, 1110, 1058 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.76 (s, 2H), 7.48 (t, *J* = 7.2 Hz, 2H), 7.55 (t, *J* = 7.2 Hz, 2H), 7.78 (d, *J* = 7.2 Hz, 2H), 7.87 (s, 1H), 8.17 (d, *J* = 2.7 Hz, 1H), 8.22 (d, *J* =2.7 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.0, 127.0, 127.1, 127.9, 128.0, 128.9, 129.3, 129.6, 134.6, 135.5, 135.6, 148.6, 149.3, 165.6, 167.1, 192.5, 206.4; MS (ESI, -ve): m/z: 389 $[M-H]^-$; HRMS: m/z $[M-H]^-$ Calcd for $C_{17}H_{10}N_2O_3S_2Cl$: 388.9821, found: 388.9820.

Methyl 6-chloro-5-[(4-oxo-2-thioxo-1, 3-thiazolan-5-yliden) methyl]-2-pyridinecarboxylate (11)

Light brown solid (2.44 g,78%); mp 227-229 °C; IR (KBr) 3176, 1717, 1595, 1426, 1322, 1237, 1198, 1143, 1064 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.87 (s, 3H), 7.57 (s, 1H), 8.06 (d, *J* = 8.05 Hz, 1H), 8.11 (d, *J* = 8.05 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 52.8, 123.0, 124.6, 131.2, 133.0, 139.1, 146.8, 150.5, 163.2, 168.9, 194.8; MS (ESI, -ve): m/z: 313 $[M-H]^-$; HRMS: m/z $[M-H]^-$ calcd for $C_{11}H_6N_2O_3S_2Cl$: 312.9508, found: 312.9506.

5-(Z)-1-[2-chloro-6-(methoxy carbonyl)-3-pyridyl] methylidene-4-oxo-2-thioxo-1, 3-thiazolane-3-carboxylic acid (12)

Light brown solid (2.28 g, 76%); mp 214-216 °C; IR (KBr) 3477, 3021, 2939, 1722, 1554, 1404, 1311, 1212, 1109, 1048 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.88 (s, 3H), 4.73 (s, 2H), 7.82 (s, 1H), 8.14 (d, *J* = 8.1 Hz, 1H), 8.19 (d, *J* = 8.05 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.0, 52.9, 124.6, 125.7, 129.1 131.1, 139.5, 147.2, 150.5, 163.2, 165.7, 167.0, 192.3; MS (ESI, -ve): m/z: 371 $[M-H]^-$; HRMS: m/z $[M-H]^-$ calcd for $C_{13}H_8N_2O_5S_2Cl$: 370.9563, found: 370.9577.

2-chloro-5-[(4-oxo-2-thioxo-1, 3-thiazolan-5-yliden) methyl]-4-phenyl-3-pyridyl cyanide (13)

Yellow solid (2.82g, 79%); mp 290-292 °C; IR (KBr) 3157, 3055, 2992, 2853, 2235, 1701, 1611, 1544, 1435, 1360, 1229, 1068 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.00 (s, 1H), 7.50-7.63 (m, 5H), 8.79 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 110.9, 114.1, 123.7, 128.2, 128.7, 128.8, 129.2, 129.3, 130.5, 131.4, 132.7, 150.8, 152.1, 155.8, 168.4, 195.0; MS (ESI, -ve): m/z: 356 $[M-H]^-$; HRMS: m/z $[M-H]^-$ Calcd for $C_{16}H_7N_3OS_2Cl$: 355.9719, found: 355.9720.

5-[(Z)-1-(6-chloro-5-cyano-4-phenyl-3-pyridyl) methylidene]-4-oxo-2-thioxo-1,3-thiazolane-3-carboxylic acid (14)

Yellow solid (3.32g, 80%); mp 172-174 ^{o}C; IR (KBr) 3517, 3279, 3037, 2942, 2554, 2233, 1718, 1607, 151, 1324, 1197, 1051 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.70 (s, 2H), 7.23 (s, 1H), 7.49 (d, J = 6.6 Hz, 2H), 7.61-7.63 (m, J = 6.6 Hz, 3H), 8.85 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 54.6, 120.6, 123.7, 135.8, 136.2, 137.3, 137.7, 138.5, 138.8, 140.2, 142.4, 160.7, 162.1, 165.6, 175.0, 176.6, 202.3; MS (ESI, -ve): m/z: 414 [M-H]$^{-}$; HRMS: m/z [M-H] $^{-}$ Calcd for $C_{18}H_9N_3O_3S_2Cl$: 413.9773, found: 413.9775.

Biology:

HT-29 (Colon cancer), A549 (Lung cancer), MCF-7 (Breast cancer) cell line was obtained from National center for Cell science (NCCS), Pune, India. DMEM (Dulbeccos Modified Eagles Medium), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], Trypsin, EDTA were purchased from Sigma Chemicals Co (st.Louis, MO), Fetal bovine serum were purchased from Arrow labs,96 well flat bottom tissue culture plates were purchased from Tarson.

Method

a) Maintenance of cell lines.

HT-29 (Colon cancer), A549 (Lung cancer), MCF-7 (Breast cancer), all cell lines were grown as adherent in DMEM media supplemented with 10% fetal bovine serum, 100 µg / ml penicillin, 200 µg/ml streptomycin, 2mM L-glutamine, and culture was maintained in a humidified atmosphere with 5% CO_2.

b) Preparation of samples for cytotoxicity

Stock solution of 10mg/ml stock solution in DMSO, from the above stock various dilutions was made with sterile water to get required concentration.

c) MTT assay

1. HT-29 (Colon cancer), A549 (Lung cancer), MCF-7 (Breast cancer) cell lines were seeded at a density of 1x 10^4 cells (cell number was determined by Trypan blue exclusion dye method) per each well in 100µl of DMEM supplemented with 10% FBS

2. 24 hrs after seeding, above media was replaced with fresh DMEM supplemented with 10% FBS then 10µl sample from above stock solutions were added to each well in triplicates which gives final concentration of 200, 100, 50,10 µg/well.

3. The above cells were incubated for 48 hrs at at 37 ^{0}C with 5% CO_2

4. After 48 hrs, incubation the above media was replaced with 100 µl of fresh DMEM without FBS and to this 10 µl of MTT (5mg dissolved in 1ml of PBS) was added and incubated for 3 hrs at 37^{0}C with 5% CO_2.

5. After 3 hrs incubation the above media was removed with multi channel pipette, then 200 µl of DMSO was added to each well and the incubated at 37 ^{0}C for 15min.

6. Finally the plate was read at 570 nm using spectrophotometer (Spectra Max, Molecular devices).

ACKNOWLEDGEMENTS

We thank Director IICT, Project Director NIPER-Hyderabad and Heads of Divisions for the support. BAR and VGMN thanks CSIR, New Delhi, India for research fellowship.

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