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Synthesis of 5-benzylidine Rhodanines and Their Cytotoxicity on HeLa Cell Lines.

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ABSTRACT

A series of 5-benzylidine rhodanine analogues were synthesized using 3-carboxy methyl rhodanine and different substituted aromatic aldehydes based on Konevanagal condensation reaction. Further the synthesized compounds were characterized by UV, IR, NMR and MS spectral data. All the synthesized compounds were tested for cytotoxic activity on HeLa cell line. Among the compound **3c** exhibited highest potential against HeLa cell line with an IC_{50} 8.2 µl.

Keywords: 3-carboxy methylrhodanine, HeLa cell line, MTT assay.

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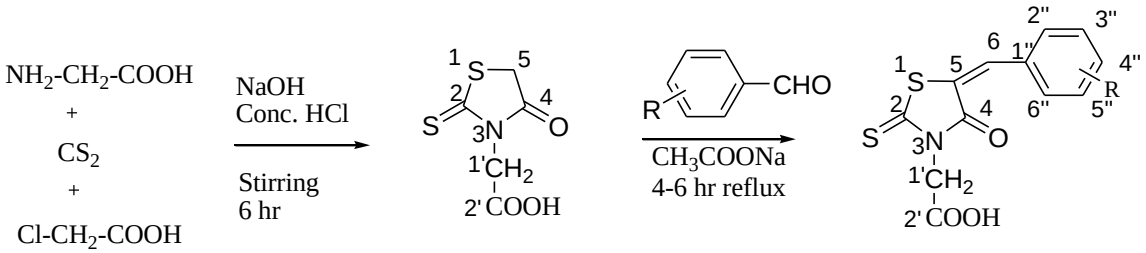
INTRODUCTION

Worldwide, cervical cancer is second most common and the fifth deadliest cancer in women. It affects about 16 per 1, 00,000 women per year and kills about 9 per 1, 00,000 per year. Approximately 80% of cervical cancers occur in developing countries. Worldwide, in 2008, it was estimated that there were 4, 73,000 cases of cervical cancer, and 2, 53,500 deaths per year. Infection with specific types of high-risk human papilloma viruses (HPV), particularly types 16 and 18, can lead to the development of cervical intraepithelial neoplasia (CIN) and cervical cancer, possibly through the actions of viral on coproteins E6 and E7[1, 2]. Rhodanine and its analogs are important class of heterocyclic compounds exhibiting a variety of biological activities such as fungal protein mannosyl transferase-1 inhibitors [3], PDE4 inhibitors [4], protease inhibitors [5], JNK stimulating phosphatase-1 (JSP-1) inhibitors [6], UDP-N-acetylmuramate/L-alanine ligase [7], antimalerials [8], HIV-1 Integrase inhibitors [9], aldose reductase inhibitors[10], β-lactamase inhibitors[11], antidiabetic agents [12], etc. In the present work, we synthesized a series of 5-benzylidine rhodanines and evaluated cytotoxic activity on HeLa cell lines.

RESULTS AND DISCUSSION

Chemistry

The 3-carboxy methylrhodanine was prepared by the reaction of glycine and carbon disulphide as summarized in Scheme 1. Compounds (**3a–m)** were prepared by Knoevenagel condensation [13] of 3- carboxy methylrhodanine **2** with different substituted aromatic aldehydes (**a-m**). The structures of the desired compounds were determined by UV, IR, ^{1}H-NMR, ^{13}C-NMR, and elemental analysis. The synthesized compounds **3a–m** has a benzylidene rhodanine moiety. All the compounds **3a–m** were analyzed as a mixture of enantiomers. In the IR spectrum of compound **2** showed the presence of a broad band in the region 3,454 cm^{-1} for the carboxylic OH group and a strong band at 1,734 cm^{-1} indicated C=O group. The ^{1}H-NMR spectra, the signal at δ 4.41 indicating the S-CH_2 proton appeared as singlet and the signal at δ 4.56 confirmed the N-CH_2 proton. It was confirmed by their ^{13}C-NMR spectra by exhibiting six signals at δ 202.80 (C-2), 173.72 (C-4), 167.29 (C-2'), 44.77 (C-1'), and at 35.94 (C-5). In compound **3b**, the signal at δ 4.41 present in compound **2** disappeared. The benzylidene proton H-6 appeared at δ 7.86 as a singlet and the aromatic protons appeared as a pair of doublets, each integrating for two protons at δ 7.55 (d, 2H, J = 8.0 Hz, H-3'' and 5'') and 7.38 (d, 2H, J = 8.0 Hz, H-2'' and 6''). The methyl protons H-7'' resonated as a singlet at δ 2.37.



3a, R = H
3b, R = 4 -CH_3
3c, R = 4 -OCH_3
3d, R = 2-NO_2
3e, R = 3-NO_2
3f, R = 4 -NO_2
3g, R = 2-Cl
3h, R = 3-Cl
3i, R = 4 -Cl
3j, R = 2,3-Cl
3k, R = 3-Br
3l, R = 4 -Br
3m, R= 4 -F
3n, R = 2,3-OCH_3

Scheme 1 synthesis of 3-carboxy methylrhodanine and its 5-benzylidinerhodanines

In the ^{13}C-NMR spectra, the peak at δ 35.94 of the compound **2** disappeared and a new peak at 121.05 was generated. In addition to the signals of the rhodanine moiety, a bunch of signals appeared between δ 100 and 160 for the benzylidine moiety. The two intense signals at δ 131.36 and 130.68, each for two carbon atoms, were attributed to the pair of carbons, C-3'' and 5'' and C-2'' and 6'', respectively. The two quarternary carbon atoms of the aromatic ring appeared at δ 130.57 (C-1'') and 134.59 (C-4''). The methyl carbon resonated at δ 21.66 (C-7'') and the C-6 carbon atom signal was exhibited at δ 142.00.

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Cytotoxic activity

In the present work, we have reported the synthesis of 3-carboxy methylrhodanine derivatives (**3a-n**) and screened for their anticancer activity. As a part of our ongoing research, we report the structure-based design using **2** as the lead compound, in which the modification of **2** was focused on reserving the rhodanine moiety and changing the benzylidene moiety by introducing different substituents into the phenyl ring. This design is aimed to get more optimized structure binding to receptor easily, consequently results in their more potent activity.

Table 1: *In vitro* cytotoxic activity by MTT assay for the compounds 3a-n

Compounds	R	IC_{50} Value
2	-	162.7
3a	H	62.62
3b	4-CH_3	78.43
3c	O-CH_3	8.2
3d	2-NO_2	92.58
3e	3-NO_2	42.60
3f	4-NO_2	21.86
3g	2-Cl	72.14
3h	3-Cl	74.21
3i	4-Cl	28.23
3j	2,3-Cl	20.58
3k	3-Br	28.11
3l	4-Br	17.42
3m	4-F	80.12
3n	2,3-OCH_3	22.43
Cisplatin		5.33

The synthesized compounds **3a-n** was screened for the *in vitro* anticancer activity against human cervical cancer cell line HeLa by MTT assay method. All the compounds exhibited the activity (**Table 1**). The electron withdrawing groups chloro, bromo and fluro compounds and electron donating substituent's methyl and methoxy groups in the para position were taken up for the SAR study. The results suggested that the electron donating groups have more potency than the electron withdrawing groups.

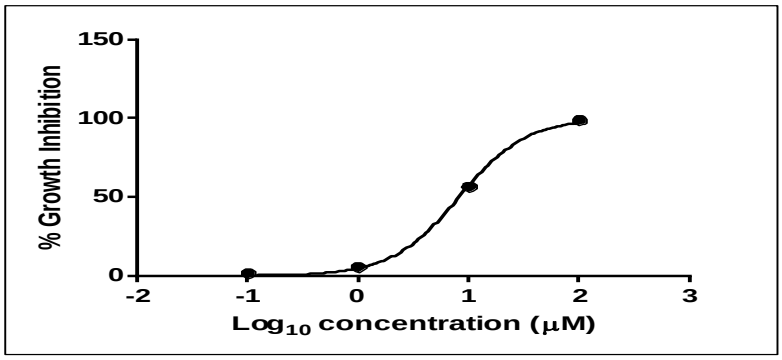

Figure 1: Cytotoxicity of compound 3c on HeLa cell line with an IC_{50} value of 8.2 µM

The compound **3c** exhibited highest potential against HeLa cell line with an IC_{50} 8.2 µM (**Fig 1**). The chloro substituent's **3g**, **3h** and **3i** exhibited cytotoxicity with an IC_{50} value of 72.14, 74.21 and 28.23 µl respectively. In the series compound **3f** with an electron withdrawing group in the para position showed more activity with an IC_{50} 21.86 µl than the nitro substituent's in the ortho and meta position **3d** and **3e** respectively. Similarly the bromo substituent in the para position compound **3l** which is exhibited more activity with an IC_{50}17.42 µl than the meta substituent **3k** with an IC_{50} in 28.11 µl. Among the tested electron withdrawing groups NO_2, Cl and Br the para substituted compounds **3f**, **3i** and **3l** showed higher activity compared to ortho and meta substituents. Also the results suggested that the electron donating substituent OCH_3 group showed good activity and the efficiency is more when the group is in para position. In addition the activity of halogen

compounds decreases in the following order Br > Cl > F and the reason may be larger size of the compound showed higher activity than other compounds. On the other hand, when increasing electro negativity the activity would be decreased. Cisplatin was used as a positive control and exhibited IC_{50} at 5.33 µl against HeLa cell line. When compared to the positive control the compound **3c** showed nearest activity with cisplatin.

CONCLUSION

The present work, we synthesized series of 3-carboxy methyl rhodanine derivatives (**3a-n**) and evaluated them for their *in vitro* cytotoxicity activitiy against HeLa cell lines. All the synthesized compounds showed cytotoxic activity with an IC_{50} range from 8 µl to 100 µl and found to be better than compound **2**. The compound **3c** exhibited very good activity with an IC_{50} 8.2 µl and on compared with the standard compound cisplatin with an IC_{50} value of 5.33 µl.

EXPERIMENTAL

General considerations

Melting points were determined in a XT-5 digital melting point instrument and are uncorrected. IR spectra were recorded (in KBr) on a Shimadzu 360 FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were measured at 400 MHz on a Bruker-400 spectrometer using TMS as internal standard and DMSO-d_6 as solvent. MS spectra were obtained on a Shimadzu MS instrument. Elemental analyses for C, H, N and S were ±0.04% of the theoretical values and determined using a PerkinElmer 240C Elemental Analyzer.

General procedure for synthesis of 3-α-carboxy ethyl rhodanine (2)

Compound **3** was prepared by the method reported [14]. 0.001 mol of glycine was dissolved in a mixture of 160 cc of water and 84 cc of percent aqueous sodium hydroxide solution. The whole was chilled in an ice water bath and 45.6g of carbon disulfide was then stirred in the cold for about four hours. To this was then added a solution made up of 56.4 g chloroacetic acid, 100 cc of water and enough sodium carbonate to give a neutral solution. The whole was stirred for a further three hours and when allowed standing overnight. The mixture was then acidified with conc. Hydrochloric acid and heated two hours on a steam bath. The product then separated on chilling and was filtered and dried. It as recrystallized from ethanol.

2-(4-oxo-2-thioxo thiozolidine-3-yl) acetic acid (2)

UV (MeOH) λmax (nm): 294.50, 260.50; IR (KBr)cm^{-1} : 3454 (br. band, OH) and 1734 (C=O); ^{1}H NMR(DMSO-d6): δ 4.41(s, 2H, H-5), 4.56 (s, 2H, H-1'). ^{13}C NMR(DMSO-d6): δ 35.94 (C-5), 44.77 (C-1), 167.24 (C-2'), 173.72 (C-4) and 202.10 (C-2). Anal. Calcd. for $C_6H_7NO_3S_2$: C, 35.11%; H, 3.44%; N, 6.82%; found: C, 35.08%; H, 3.46%; N, 6.83%.

General procedure for the preparation of compounds (3a–n)

To a solution of compound **2** (0.001 mol) and anhydrous sodium acetate (0.001 mol) in glacial acetic acid, were added the respective aldehydes (**a–h**). The mixture was stirred under reflux for 4–6 h and then poured into ice-cold water. The precipitate was filtered and washed with water. The dried product was recrystallized with ethanol.

(E)-2-(5-(4-methylbenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (3b)

^{1}H NMR (DMSO-d6): δ 2.37(s, 3H, H-7"), 4.74(s, 2H, H-1'), 7.38(d, 2H, *J*= 8.0Hz, H-2", 6"), 7.55(d, 2H, *J*= 8.0Hz, H-3", 5"), 7.86(s, 1H, H-6). ^{13}C NMR (DMSO-d6): δ 21.66(C-7"), 45.47(C-1'), 121.05(C-1"), 130.68(C-2", 6"), 131.36(C-3", 5"), 134.59(C-4"), 142.32(C-6), 166.87(C-2'), 167.76(C-4), 193.71(C-2). MS: *m/z* 293.36 (M+1); Anal. Calcd. for $C_{13}H_{11}NO_3S_2$: C, 53.22%; H, 3.78%; N, 4.77%; O, 16.36%; S, 21.86%; found: C, 53.20%; H, 3.77%; N, 4.81%; O, 16.39%; S, 21.83%.

All other compounds showed similar spectral value. Compound **3b** was taken as a model compound for spectral discussion.

MTT assay for cytotoxicity screening

The human cervical cancer cell line was obtained from National Centre for Cell Science (NCCS), Pune, and grown in Eagle's minimum essential medium containing 10 % fetal bovine serum (FBS). Cells were maintained at 37 ˚C, 5 % CO_2, 95 % air, and 100 % relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week. The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions, and viable cells were counted using a hemocytometer and diluted with medium containing 5 % FBS to give the final density of 1 9 105 cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at a plating density of 10,000 cells/well and incubated to allow for cell attachment at 37 ˚C, 5 % CO_2, 95 % air, and 100 % relative humidity. After 24 h, the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) to prepare the stock (200 mM) and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with serum-free medium. An additional four, 10-fold serial dilutions were made to provide a total of five drug concentrations. Aliquots of 100 µl of these different drug dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final drug concentrations. Following the drug addition, the plates were incubated for an additional 48 h at 37 ˚C, 5 % CO_2, 95 % air, and 100 % relative humidity. The medium containing no samples served as control and triplicates were maintained for all concentrations. After the 48 h incubation, 15 µl of MTT (5 mg/ml) in phosphate buffer saline (PBS) was added to each well and incubated at 37 ˚C for 4 h. The medium with MTT was then flicked off and the formed Formosan crystals were solubilized in 100 µl of DMSO and then measured for absorbance at 570 nm using a micro-plate reader.

The percentage cell viability was then calculated with respect to control as follows

$$\% \text{ Cell viability} = [A] \text{ Test} / [A]\text{control} \times 100$$

The percentage cell inhibition was determined using the following formula.

$$\% \text{ Cell Inhibition} = 100 - \text{Abs (sample)}/\text{Abs (control)} \times 100.$$

A nonlinear regression graph was plotted between % cell inhibition and log10 concentration, and IC_{50} was determined using Graph Pad Prism software.

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