ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Synthesis, cytotoxic activity and molecular docking study of Bis-Rhodanine derivative

P.K. Harshitharaj, R. Kumar and S. Ravi*
Department of Chemistry, Karpagam University, Coimbatore-28, Tamilnadu, India.
***Corresponding author: E-Mail: ravisubban@rediffmail.com**

ABSTRACT

A bis-rhodanine was prepared from 1, 2 diaminopropane by microwave assisted synthesis and characterized by spectral data. The cytotoxic activity of the compound was studied by MTT assay. It showed a dose dependent cytotoxic activity and exhibited an IC_{50} value of 34.5 µg/ml against the HeLa cell lines. Molecular docking was carried out against the protein HPV 16 E2 present in HeLa cell line to study the binding capacity of the bis-rhodanine with the protein. It showed a docking score of -4.5 Kcal/mol involving hydrogen bonds and hydrophobic interactions.

KEYWORDS: 1, 2 diaminopropane, bis-rhodanine, MTT assay, HeLa cell line, Molecular docking, HPV 16 E2 protein

1. INTRODUCTION

It is known that cancer is one of a serious health problem throughout the world. Thus, in the last few years researchers have been challenged by the task of finding efficient clinical ways for the cancer treatment. Apart from the use of radiotherapy and surgery, chemotherapy dominates as an important choice for cancer treatment (De, 2008). While many advances have been made for the prevention and treatment of cancer, chemotherapeutic molecules normally affect metabolically active or rapidly proliferating cells, and cannot differentiate between cancer and normal cells (Kumar, 2007). While many anticancer agents like 5-fluorouracil, cisplatin, paclitaxel and docetaxel are currently used for cancer treatment, these diseases still remain tenacious and deadly (Curran, 2002). Thus, identifying new anticancer agents is a highly active research field, motivated by the invention of novel biological targets and by the possibility of identifying new anticancer agents with high efficiency, minimum side effects and low toxicity. (Sawyers, 2004; Li, 2005). In search of key chemotherapeutic agents, considerable effort has been focused on the development of anticancer agents that contain heterocyclic structures as their main structural shape. Rhodanins are an attractive scaffold due to its impressive position in the medicinal chemistry as it is responsible for numerous pharmacological and biological activities like antimycobacterial (Taniyama, 1959; Singh, 2008). Antifungal (Allan, 1960; 1962; 1963; 1964; Orchard, 2002). pesticidal (Dovlatyan and Avetisyan, 1973; Muro, 1996). Antihypertensive (Frankov, 1985). Antineoplastic (Singh, 2004), anticancer (Friebe, 2001; Kumar, 2015; Patil, 2010). Antidiabetic (Rakowitz, 2006). Antimicrobial, (Habib, 1997), Anti-Inflammatory (Cutshall 2005) activities. Rhodanine derivatives have many characteristic features like ease of starting materials, built in biocidal unit, and enhanced lipid solubility with hydrophilitcity and simple metabolism of compounds.

2. EXPERIMENTAL

All melting points reported were uncorrected. FT-IR spectrum was recorded in a Shimadzu (1650) model instrument. ^{1}H-NMR and ^{13}C-NMR were recorded in a Bruker (300) instrument. Elemental analysis was recorded in a Perkin Elmer 240C model instrument. All the chemicals used for synthesis are of AR grade. Microwave oven (CEM Discover Benchmate., USA) was used for microwave assisted synthesis.

Synthesis of a bis-rhodanine:

Conventional method: A suspension of diamine (1mole) was dissolved in aqueous NaOH (40%). The solution was cooled, carbon disulphide (2 mol) was added and stirred for 3 hours. To this was then added chloroacetic acid (2 mol) which had been neutralized with sodium carbonate. Stirring was continued for another three hours. The mixture was then made acidic to congo red and stood overnight. Further the mixture was heated on a steam bath for one and half hour to yield an oily substance. The product solidified out on cooling. It was filtered, then washed with water and dried.

Microwave method: A suspension of diamine (1 mol), sodium hydroxide (2 mol) and carbon disulfide (2 mol) in water (10 ml) was allowed to react in a microwave synthesizer for 5 min at 100°C. After cooling to 40°C chloroacetic acid (2 mol) was added and subjected to 100°C for 5 min. After cooling concentrated HCl (3 ml) was added and the mixture was concentrated, extracted with ethyl acetate and dried. The crude product was purified by column chromatography.

Compound 1: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.5 (3H, d, J=6.8Hz, H3'), 4.5 (1H, m, H-2'), 4.05 (2H, m, H-5'), 4.15(2H, m, H-5), 4.20(2H, m, H-1').

^{13}C-NMR (100 MHz, $CDCl_3$): δ 203.47 and 204.41 (C=S), 174.62 and 174.54 (C=O), 51.02(S─CH_2), 35.58 (N─CH_2), 34.28 (N─CH), 14.46 (CH_3).

Anticancer Activity:

Cell line: The HeLa cervical cancer cell line obtained from National Centre for Cell Science (NCCS), Pune was grown in a Medium having 10% fetal bovine serum (FBS). The cells were kept at 37^0C, 95% air, 5% CO_2 and 100% relative humidity. The culture used for maintenance passaged every week and changed two times a week.

Cell treatment procedure: The monolayer cells were detached to make single cell suspensions using trypsin-ethylene diamine tetra acetic acid (EDTA). The viable cells present were counted by means of a hemocytometer. Then it is diluted with 5% FBS to give final density of $1x10^5$ cells/ml. One hundred micro liters per well of cell suspension was seeded into a 96-well plate with a plating density of 10,000 cells/well. Then it is incubated so that the cell attachment takes place at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After the completion of 24 h the cells were treated with serial concentrations of the test samples. Initially they were dissolved in dimethylsulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the required final test concentration with a medium free from the serum. Additionally, four serial dilutions were made to give a total of five sample concentrations. Aliquots of 100 µl of these various sample solutions were added to the respective wells already having 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for 48 h at 37^0C, 5% CO_2, 95% of air and 100% relative humidity. The medium without samples served as a control and triplicate was maintained for all concentrations.

MTT assay: 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow tetrazolium salt soluble in water. A mitochondrial enzyme present in living cells, succinate-dehydrogenase, goes away from the tetrazolium ring, to convert MTT in to an insoluble purple formazan. Therefore, the quantity of formazan obtained is directly proportional to the number of viable cells.

After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and kept at 37^0C for 4h. The medium having MTT was then flicked off and the resulted formazan crystals were solubilized in 100µl of DMSO. Then the absorbance was measured at 570 nm using micro plate reader.

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

% Cell viability = [A] Test / [A]control x 100

% Cell Inhibition = 100- Abs (sample)/Abs (control) x100.

The IC_{50} value was determined using the nonlinear regression graph which was plotted between % cell inhibition on one axis and log concentration on the other axis.

Docking studies: The target protein selected for the present study is HPV 16 E2 protein. The synthesized bis-rhodanine was used for molecular docking.

Methods:

Active Site Prediction: The structure of the human papillomavirus type 16 E2 protein was downloaded from the Protein Data Bank, (PDB-ID: 1 DTO) (Alfred 2000). Since the protein HPV 16 E2 TAD do not have any bound ligands, therefore the binding site residues were identified before docking. It was identified using Q Site Finder. The binding pocket includes a deep as well as a narrow cavity and Tyr 32 sterically hinders the binding pocket. Active site residues identified in HPV 16 E2 TAD are Ile 15, Tyr 19, Asp 28, His 29, Ile 30, Tyr 32, Trp 33, Met 36, Glu 39, Ala 63, Val 64, Ser 65, Asn 67, Lys 68, Ala 69, Gln 71, Ala 72, Leu 79, Thr 93, Leu 94, Gln 95, Val 97, Ser 98, Leu 99, Glu 100, Val 101.

Preparation of the Protein and the Binding Site: After detection of active site of the TAD domain, a 'clean input file' was generated by removing water molecules, ions and subunits which are not involved in the binding of the ligand from the original structure file. Local minimization was then carried out in the presence of restraints to get rid of potential bad contacts, at the same time protein conformation was maintained very close to that observed in the original structure. The obtained receptor model was saved to a PDB file.

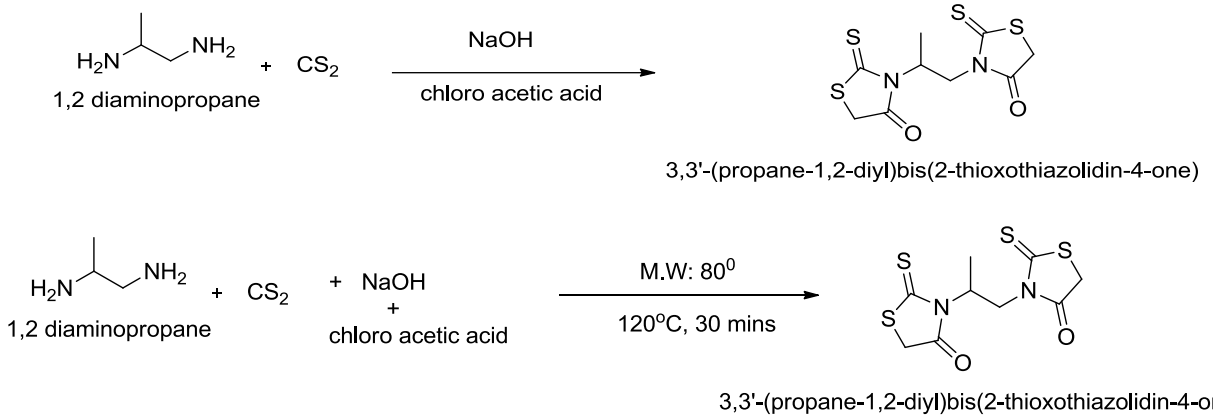
Molecular Docking: The molecular docking program Autodock was used to perform the docking studies. For the study, the binding pocket on the TAD was the region targeted for docking. Dockings were carried out under 'Standard default settings' mode. PYMOL (TM) software was used to analyse the docking results which permits visualization of the protein-ligand docking and calculation of several parameters such as feasible hydrogen bonding between the protein and the ligand. The scores were determined and presented in the table.

3. RESULTS ND DISCUSSION

In the present work for the preparation of bis-rhodanine the reaction begins with the nucleophiliic addition of carbon of carbon disulfide with the amino group to give a thioamide. The resulting sulfur nucleophiles then react with sodium chloro acetate followed by cyclization with loss of water to give rhodanine. Due to the fascinating chemistry and biology of novel bis-rhodanine, this compound will continue to attract, the organic and medicinal chemists for further research. The present work deals with synthesis of a novel bis-rhodanine by conventional and microwave method (Scheme 1). The mechanism for the formation of bis-rhodanine is given in Scheme 2.

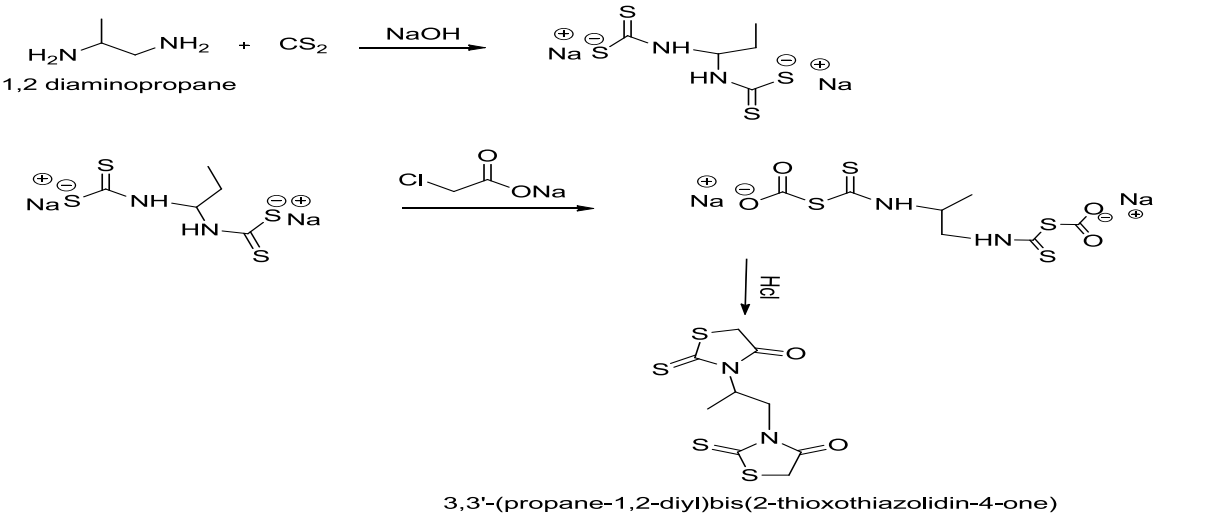
Synthesis of Bis Rhodanine:

Conventional Method: 0.8 ml (1 mole) of 1, 2 diaminopropane was dissolved in aqueous NaOH and to the resulted cooled solution carbon disulphide was added with stirring. To this was then added a solution of chloroacetic which had been neutralized with sodium carbonate and stirred for three hours. The resulted mixture was concentrated and cooled to yield the compound.



Scheme.1. Synthesis of bis-rhodanine by conventional and microwave assisted methods

Microwave Method: 1 mole of 1, 2 diaminopropane, sodium hydroxide (2 mol) and carbon disulfide (2 mol) were reacted in a microwave synthesizer for 15 min at 120° C. After cooling to 40° C chloroacetic acid (2 mol) was added and the mixture was allowed to react at 120° C for 15 min. After cooling concentrated hydrochloric acid (3 ml) was added and the reaction mixture was concentrated to yield the product.



Scheme.2. Mechanism for the formation of bis-rhodanine

Table: 1 Physical and analytical date of bis-rhodanine

Empirical formula	Molecular weight	Yield		Melting Point ºC	Colour	Elemental analysis found %C,%H,%N,%O,,%S
		Conventional method %	Microwave method %			
$C_9H_{10}N_2O_2S_4$	306.45	59	86	232	Red colour	C-35.27, H-32.9, N-9.14, O-10.44, S-41.85

In the ^{1}H-NMR spectra the doublet signal appeared at δ 1.15 (3H,d, J=6.8 Hz) is due to the methyl group. The multiplet for one proton δ 4.5 is due to the methine proton attached with nitrogen, the doublet of doublets at 4.05 and 4.15 for the two protons are due to the methylene protons in the rhodanine ring system. The signal at δ 4.20 is due to the methylene protons joined with the nitrogen atom.

In the ^{13}C-NMR spectra nine signals appeared indicating the presence of nine carbon atoms. The pair of signals at δ 174.87 and 174.62 are due to carbonyl carbon of the rhodanine ring systems. The methylene carbons inside the rhodanine ring system appeared at δ 51.02 and 44.84. The methyl carbon appeared at δ 14.46 and the methylene and methine carbons attached to the nitrogen resonated at 35.58 and 34.28.

Both the conventional and microwave assisted synthesis were carried out to ascertain the advantages of microwave heating in reducing energy requirement and in improving yield. It was found that higher yield (86%) was obtained in microwave activated method when compared to (59%) yield in conventional method (table-1). In addition the reaction time for microwave assisted method was 48 times shorter than the conventional reaction. Since the reaction time was reduced, thermal decomposition was minimized which results, resulting in higher yield.

Cytotoxic Activity: Human cervical cancer is one of the most common and critical cancer in women caused by human papilloma virus (HPV). There are over 100 types of Human Papilloma Virus (HPV), many of which infect the genital tract. The genital HPVs can be subdivided into two groups. Low-risk HPV types, like HPV 6, cause benign warts. In contrast, low-risk HPV types, like HPV 16 and HPV 18, are associated with cervical cancer. Almost 80% of the cases occur in low income or developing countries. Till now no effective drugs are there for cervical cancer. Developing a less toxic anticancer drug is a challenging task. So we need to enhance the effective less anticancer drugs. Chemotherapy is considered to be permanent and used for cancer treatments while traditional cytotoxic agents are not satisfactory due to diverse toxic side effects. It is necessary to develop novel anticancer agents with high potency and low toxicity.

The synthesized bis-rhodanine was screened for its anticancer activity using MTT assay against HeLa cell lines. The medium without the sample of the HeLa cell lines served as control. The experiments were carried out in triplicate. The percentage cell inhibition was determined by its IC_{50} value. The IC_{50} value is found to be 34.5 µM. However, the value is higher when compared with the standard compound cisplatin with an IC_{50} value of 6.2 µM.

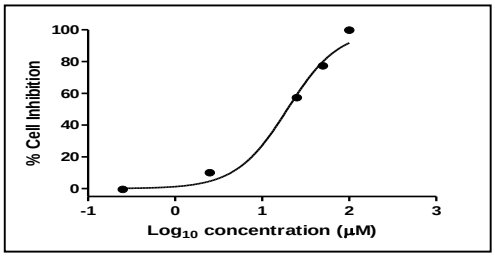


Fig.1. cytotoxic effect of Compound 1

Molecular Docking studies: The application of docking offers the possibility of virtually testing the ability of a molecule to bind to a target. Docking tools use the crystal structure of a target protein and find different binding modes for the molecules of interest. Before starting the Screening one has found out the binding pocket residues of the target protein because its X-ray crystallographic structure obtained from the PDB do not have any bound inhibitors. Therefore, in the present case, the binding pocket was detected by Site Finder module. The genetic algorithm-based docking program Autodock was used to screen the molecule, flexibly docking the synthesized compound to HPV E2 TAD. The targeted region (HPV E2 TAD) defined in our screening covers Ser 98, Glu 100, Leu 99, residues in active site. As given in the literature we belive that a small molecule that binds to this region will compete with the above residues, consequently blocking the transactivation of domain.

Docking score of the synthesized compound: From the docking study it was learned that the synthesized compound has a reasonable score of -4.5 Kcal/mole and have very good interactions. Since HPV is the major cause of cervical cancer, an inhibitor may prevent progression to invasive cancer by inhibiting HPV replication. To find such some effective inhibitors the x-ray structure of the trans activation domain of HPV E2 was chosen as a molecular target. The carbonyl oxygen of the one of the rhodanine moiety forms hydrogen bonds with GLU100 and LEU99 and the carbonyl oxygen of the other rhodanine moiety forms hydrogen bond with TYR32. It exhibited hydrophobic interactions with SER98, TRP33, MET36, TYR19, LEU94 and GLN95.

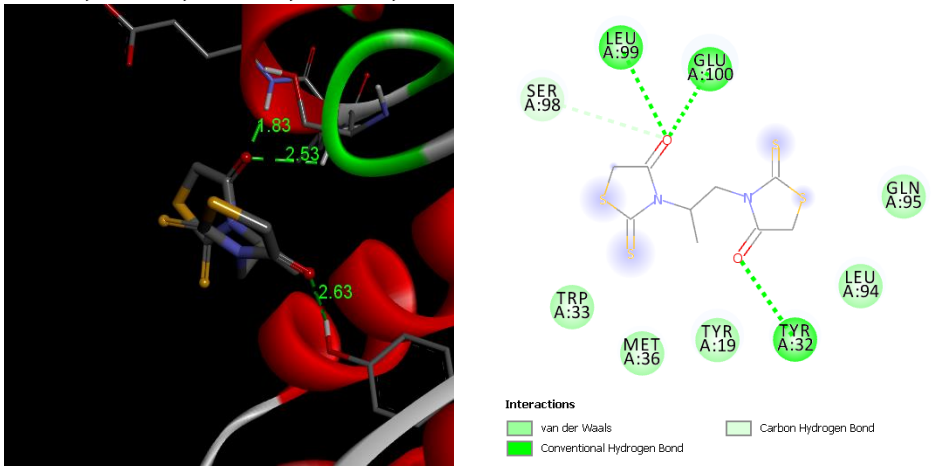


Figure.2. interaction of bis-rhodanine with SER98, TRP33, MET36, TYR19, LEU94 and GLN95

4. CONCLUSION

In conclusion it has been realized that the synthesized bis-rhodanine is an effective inhibitor. Hence, this compound can act as a template and can be taken to the wet laboratory in order to carry out further derivatives to optimize the properties.

REFERENCES

Allan FJ, Allan GG, Crank G, and Jack J, The condensation of rhodanine and derivatives with benzaldehyde sulphonic acids, Recueil des Travaux Chimiques des Pays-Bas, 79, 1960, 247–254.

Allan FJ, and Allan GG, Izoxazolylmethylenerhodanines, Recueil des Travaux Chimiques des Pays-Bas, 83, 1964, 1299– 1300.

Allan FJ, and Allan GG, The condensation of rhodanine and derivatives with some indole aldehydes, Monatshefte f¨ur Chemie, 94, 1963, 1434–4475.

Allan F.J, and Allan G.G, The condensation of rhodanine and derivatives with aromatic aldehydes containing iodine, Recueil des Travaux Chimiques des Pays Bas, 82, 1962, 177–181.

Cutshall NS, O'Day C, and Prezhdo M, Rhodanine derivatives as inhibitors of JSP-1, Bioorganic & Medicinal Chemistry Letters, 15 (14), 2005, 3374–3379.

Dovlatyan VV, and Avetisyan F.V, 2-Thioxo-4-imino (oxo) - 1,s 3-thiazolidin-3-ylacetic acid and its alkyl esters, Armyanskii Khimicheskii Zhurnal, 26, 1973, 494–498.

Frankov IA, Kirillov MV, Sokolova TN, Skupskaya R, Kharitonovich AN, and Chizhevskaya II, Recent Pharmacological Developments on Rhodanines and 2,4-Thiazolidinediones, Khimiko- Farmatsevticheskii Zhurnal, 19, 1985, 943–946.

Friebe WG, Krell HW, Woelle S, and Wolff HP, Thiazolidine carboxylic acid derivatives and their use in the treatment of cancer, WOP at 0157006, 2001.

Habib NS, Ridal SM, Badaweyl EAM, Fahmyl HTY, and Ghozlan HA, Synthesis and antimicrobial activity of rhodanine derivatives, European Journal of Medicinal Chemistry, 32 (9), 1997, 759–762.

Kumar R, Ravi S, Sundaram K, Venkatachalapathi S, and Ali Muhammad S, Conventional and Microwave Assisted Synthesis of 2-Aminothiazoles and Oxazoles and their Anticancer activity, Indo American Journal of Pharmaceutical Research, 5, 2015, 555-561.

Muro C, Yasuda M and Sakagami Y, Inhibitory activities of rhodanine derivatives on plant growth, Bioscience, Biotechnology and Biochemistry, 60 (8), 1996, 1368–1371.

Orchard MG, Neuss JC, and Galley CMS, Benzylidene thiazolidinediones and their use as antimycotic agents, WOP at 02017915, 2002.

Patil V, Tilekar K, Mehendale-Munj S, Mohan R, and Ramaa C.S, Synthesis and primary cytotoxicity evaluation of new 5-benzylidene-2,4- thiazolidinedione derivatives, European Journal of Medicinal Chemistry, 45 (10), 2010, 4539– 4544.

Rakowitz D, Maccari R, Ottana R, and Vigorita MG, *In vitro* aldose reductase inhibitory activity of 5-benzyl-2, 4-thiazolidinediones, Bioorganic & Medicinal Chemistry, 14 (2), 2006, 567–574.

Singh J, Nathan C.F, Bryk R, Samy R, Pupek K, and Gurney, Cyclic carboxylic acid rhodanine derivatives for the treatment and prevention of tuberculosis, WOP at 2008005651, 2008.

Singh R, Ramesh U.V, and Goff D, Rhodanine derivatives and pharmaceutical compositions containing them, WO Pat 2004043955, 2004.

Taniyama H, Yasui B, Takehara N, and Uchida H, Chemotherapeutics for mycobacterium tuberculosis, Synthesis and antibacterial activity of some 3-substituted rhodanines, Yakugaku Zasshi, 79, 1959, 1465–1468.