Synthesis, Biological Valuation and Molecular Docking Analysis of New 5-Benzylidene Bis-Rhodanine Derivatives

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http://dx.doi.org/10.13005/ojc/360609

(Received: October 13, 2020; Accepted: November 17, 2020)

ABSTRACT

The synthesis of 5-benzylidene bis-rhodanine derivatives are reported from bis-rhodanine (III) and different aromatic aldehydes (IV) via Knoevenagel condensation reactions. All the Derivatives (V) and (Va-m) were deep-rooted by NMR spectroscopic techniques and elemental analysis. The antiproliferative study of the compounds on HeLa human cervical cancer cell line, K562 leukemic cell line and MDAMB231 breast cancer cell line were performed by MTT assay. Docking studies were carried out against the protein HPV 16E2 present in the HeLa cell line. It show good docking scores. The results indicate that the bis-rhodanine derivatives could serve as potential molecules for the development of new anticancer agents.

Keywords: 5-benzylidene bis-rhodanine, Knoevenagel condensation, Cytotoxic studies, docking studies.

INTRODUCTION

Heterocyclic compounds are a major part of synthetic therapeutic chemistry. They offer a high level of structure variety and have demonstrated to be generally utilized as therapeutic agents. They are distributed widely as a natural product. Heterocyclic compounds have great potential and the most encouraging molecule for the discovery of new drugs. Ever since the introduction of Cigitazole and Epalrestat for the treatment of diabetes, rhodanine has attracted medicinal chemists showing favourable biological properties. The chemical derivatization of rhodanine gives rise to molecules with a broad range of therapeutic activities like antiviral, antifungal, antibacterial, antitumor, aldose reductase inhibitors and anti-inflammatory activities. Rhodanine has also been reported as inhibitors of uridine diphosphate-N-acetyl/L-alanine ligase, hepatitis C virus protease inhibitors and also inhibitors of cancer cell migration. From the laboratory, it has been reported 5-benzylidene-3-ethyl rhodanine and 5-isopropylidene-3-ethyl rhodanine induced cytotoxicity in a time and concentration-dependent manner with an IC50 value of <10 µM. Recently focus has been given on the mixture of bis-heterocyclic compounds, which showed various biological activities. As part of researcher’s growing interest in synthesizing bis-rhodanine, we describe herein an easy and
Experimental procedure

General procedure for the synthesis of Bis-rhodanine(III)

1,2-diamino propane (0.01 mol) was dissolved in water and while stirring then sodium Hydrosside (0.04 mol) was added. The solution was cooled at 0°C, the solution of carbon disulfide (0.02 mol) was mixed into it and kept for stirring for about 4 hours. Then the water solution of sodium chloroacetate (0.02 mol) was mixed with it and stirred for 3 hours. Subsequently, the solution of HCl was added and refluxed for 2-3 hours. While cooling a precipitate appeared and then it was filtered and dried to the corresponding product(III).

3,3’-(propane-1,2-diyl)bis(2-thioxothiazolidin-4-one): (III)

Red colour solid, 58%, m.p. 296°C. 1H-NMR (400 MHz, CDCl₃): δ 1.5 (3H, d, J=6.8 Hz, H-1”), 4.5 (1H, m, H-2”), 4.05 (2H, m, H-3”), 4.15 (2H, m, H-5), 4.20 (2H, m, H-5’). 13C-NMR (100 MHz, CDCl₃): δ 203.47 and 204.38 (C=S), 174.52 and 174.44 (C=O), 114.28 and 114.73 (S-C), 150.24 and 148.78 (C-6 & 6' benzylic carbon), 125.36 to 134.9 (Aromatic carbon), 45.14 (C=N), 51.02 (N-CH₂), 35.58 and 34.28 (S-C), 151.02 and 149.82 (C-6 & 6' benzylic carbon), 125.36 to 134.9 (Aromatic carbon), 51.77 (N-CH₂), 14.91 (CH₃); Found: C-50.07, H-2.93, Cl-12.85, N-5.07,O-5.82, S-23.24.

Synthesis of 5-benzylidene bis-rhodanine derivatives(V) & (Va-m)

The mixture of bis-rhodanine (III) (0.002 mol) and substituted aromatic aldehyde (IV) and (IVa-n) (0.004 mol) were refluxed in glacial acetic acid (0.004 mol) and sodium acetate (0.004 mol) for 4-6 hours. The resultant mixture on cooling yielded a precipitate which was filtered, washed, dried and recrystallised (ethanol) to afford the corresponding product (V) & (Va-m).

Compound V: Yellow solid, 58%, m.p. 296°C. 1H-NMR (400 MHz, CDCl₃): δ 1.64 (3H, d, J=7.5 Hz, H-1”), 4.75 (1H, m, H-2”), 3.84 (2H, m, H-3”), 7.78 and 7.84 (2H, S, H-6 and 6’), 7.98-8.21 (10H, m, H-8 to 12 and 8'-12”). 13C-NMR (100 MHz, CDCl₃): δ 203.18 and 201.14 (C=S), 174.50 and 174.52 (C=O), 114.77 (S-C), 150.13 and 148.83 (C-6 & 6’ benzylic carbon), 125.16 to 133.7 (Aromatic carbon), 44.21 (N-CH₂), 50.70 (N-CH₃), 14.77 (CH₃); Found: C-57.24, H-3.77, N-5.79, O-6.62, S-26.56.

Compound Va: Yellow solid, 63%, m.p. 296°C. 1H-NMR (400 MHz, CDCl₃): δ 1.62 (3H, d, J=6.7 Hz, H-1”), 4.65 (1H, m, H-2”), 3.94 (2H, m, H-3”), 3.66 (3H, S, OCH₃) 7.76 and 7.82 (2H, S, H-6 and 6’), 7.92-8.24 (8H, d, J=7.8 Hz, H-8 to 12 and 8’-12”). 13C-NMR (100 MHz, CDCl₃): δ 203.18 and 200.85 (C=S), 174.23 and 174.44 (C=O), 114.28 and 114.73 (S-C), 150.24 and 148.78 (C-6 & 6’ benzylic carbon), 125.38 to 134.6 (Aromatic carbon), 56.3 (OCH₃), 45.54 (N-CH₂), 50.10 (N-CH₃), 14.13 (CH₃); Found: C-55.34, H-4.08, N-5.17, O-11.78, S-23.62.

Compound Vb: Yellow solid, 66%, m.p. 296°C. 1H-NMR (400 MHz, CDCl₃): δ 1.56 (3H, d, J=6.8 Hz, H-1”), 4.81 (1H, m, H-2”), 3.77 (2H, m, H-3”), 7.58 and 7.67 (2H, S, H-6 and 6’), 7.91-8.29 (8H, d, J=7.7 Hz, H-8,9,11,12 and 8’,9’,11’,12’). 13C-NMR (100 MHz, CDCl₃): δ 203.28 and 201.14 (C=S), 172.42 and 172.33 (C=O), 116.28 and 115.77 (S-C), 151.12 and 149.64 (C-6 & 6’ benzylic carbon), 125.35 to 134.9 (Aromatic carbon), 45.14 (N-CH₂), 51.77 (N-CH₂), 14.91 (CH₃); Found: C-50.07, H-2.93, Cl-12.85, N-5.07,O-5.82, S-23.24.

Compound Vc: Yellow solid, 65%, m.p. 294°C. 1H-NMR (400 MHz, CDCl₃): δ 1.62 (3H, d, J=6.7 Hz, H-1”), 4.71 (1H, m, H-2”), 3.92 (2H, m, H-3”), 7.88 and 7.97 (2H, S, H-6 and 6’), 7.92-8.35 (8H, d, J=7.5 Hz, H-8,9,11,12 and 8’,9’,11’,12’). 13C-NMR (100 MHz, CDCl₃): δ 204.38 and 203.85 (C=S), 174.02 and 174.21 (C=O), 115.11 and 114.63 (S-C), 151.02 and 149.82 (C-6 & 6’ benzylic carbon), 125.36 to 134.7 (Aromatic carbon), 44.25 (N-CH₂), 50.70 (N-CH₂), 15.05 (CH₃); Found: C-43.15, H-2.54,Br-24.98, N-4.34, O-5.03, S-20.05.

Compound Vd: Yellow solid, 65%, m.p. 290°C. 1H-NMR (400 MHz, CDCl₃): δ 1.65 (3H, d, J=6.8 Hz, H-1”), 4.85 (1H, m, H-2”), 3.85 (2H, m, H-3”), 7.79 and 7.83 (2H, S, H-6 and 6’), 7.96-8.25 (8H, d, J=7.5 Hz, H-8,9,11,12 and 8’,9’,11’,12’). 13C-NMR (100 MHz, CDCl₃): δ 203.38 and 201.85 (C=S), 174.52 and 174.34 (C=O), 115.18 and 114.83 (S-C), 151.14 and 149.94 (C-6 & 6’ benzylic carbon), 125.38 to 134.7 (Aromatic carbon), 45.15 (N-CH₂), 51.80 (N-CH₂), 14.85 (CH₃); Found: C-48.25, H-2.83, N-9.77, O-16.77, S-22.42.

Compound Ve: Yellow solid, 62%, m.p. 291°C. 1H-NMR (400 MHz, CDCl₃): δ 1.64 (3H, d, J=6.7 Hz, H-1”), 4.84 (1H, m, H-2”), 3.76 (2H, m,
**Compound Vf:** Yellow solid, 63%, m.p. 302°C, $^{13}$C-NMR (400 MHz, CDCl$_3$): $\delta$ 1.46 (3H, d, $J$=6.7 Hz, H-1'), 4.63 (1H, m, H-2'), 3.77 (2H, m, H-3'), 7.72 and 7.85 (2H, S, H-6 and 6'). 15C-NMR (100 MHz, CDCl$_3$): 20038 and 20076 (C=S), 17433 and 17401 (C=O), 11517 and 11481 (S-C), 15111 and 14990 (C-6 & 6' benzylcarbon), 12530 to 1346 (Aromatic carbon), 4418 (N-CH$_2$), 5186 (N-CH$_3$), 1481 (CH$_3$); Found: C-53.27, H-3.12, F-7.34, N-5.43, O-6.18, S-24.72.

**Compound Vg:** Yellow solid, 63%, m.p. 288°C, $^{1}$H-NMR (400 MHz, CDCl$_3$): $\delta$ 1.56 (3H, d, $J$=6.8 Hz, H-1'), 4.80 (1H, m, H-2'), 3.63 (2H, m, H-3'), 5.44 (1H, S, OH) 7.73 and 7.81 (2H, S, H-6 and 6'), 7.95-8.26 (8H, d, $J$=7.8 Hz, H-8, 9, 11, 12 and 8', 9', 11', 12'). $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 20311 and 20385 (C=S), 17042 and 17103 (C=O), 11498 and 11421 (S-C), 15013 and 15014 (C-6 & 6' benzylcarbon), 12535 to 1346 (Aromatic carbon), 4415 (N-CH$_2$), 5066 (N-CH$_3$), 1479 (CH$_3$); Found: C-53.68, H-3.53, N-5.44, O-12.43, S-24.92.

**Compound Vh:** Yellow solid, 59%, m.p. 287°C, $^{1}$H-NMR (400 MHz, CDCl$_3$): $\delta$ 1.63 (3H, d, $J$=6.8 Hz, H-1'), 4.72 (1H, m, H-2'), 3.81 (2H, m, H-3'), 7.70 and 7.81 (2H, S, H-6 and 6'), 7.95-8.29 (8H, d, $J$=7.6 Hz, H-8 to 10, 12 and 8'-10', 12'). $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 20330 and 20266 (C=S), 17051 and 17124 (C=O), 11533 and 11478 (S-C), 15123 and 14999 (C-6 & 6' benzylcarbon), 12530 to 1346.5 (Aromatic carbon), 4211 (N-CH$_2$), 5101 (N-CH), 1477 (CH$_3$); Found: C-50.08, H-2.93, Cl-12.85, N-5.09, O-5.80, S-23.25.

**Compound Vf:** Yellow solid, 64%, m.p. 287°C, $^{1}$H-NMR (400 MHz, CDCl$_3$): $\delta$ 1.64 (3H, d, $J$=6.8 Hz, H-1'), 4.86 (1H, m, H-2'), 3.86 (2H, m, H-3'), 7.74 and 7.80 (2H, S, H-6 and 6'), 7.99-8.21 (8H, d, $J$=7.7 Hz, H-8 to 10, 12 and 8'-10', 12').

**Compound Vf:** Yellow solid, 65%, m.p. 299°C, $^{1}$H-NMR (400 MHz, CDCl$_3$): $\delta$ 1.64 (3H, d, $J$=6.8 Hz, H-1'), 4.89 (1H, m, H-2'), 3.86 (2H, m, H-3'), 7.79 and 7.88 (2H, S, H-6 and 6'), 7.94-8.22 (8H, d, $J$=7.6 Hz, H-8 to 10, 12 and 8'-10', 12'). $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 20239 and 20183 (C=S), 17451 and 17434 (C=O), 11517 and 11482 (S-C), 15113 and 14998 (C-6 & 6' benzylcarbon), 12539 to 1349 (Aromatic carbon), 4518 (N-CH$_2$), 5185 (N-CH), 1484 (CH$_3$); Found: C-53.22, H-3.11, F-7.36, N-5.43, O-6.19, S-24.71.
125.38 to 134.7 (Aromatic carbon), 45.15 (N-CH2), 51.80 (N-CH), 14.85 (CH3); Found: C-48.25, H-2.83, N-9.77, O-16.77, S-22.42.

Anticancer activity

Cell Line

The cervical cell line HeLa, leukemic cell line K562 and the cell line MDAMB231 for breast cancer were received from NCCS-National Center for Cell Science, Pune and grown.

MTT assay

MTT assay was followed by the standard protocol according to Rajendran Kumar et al., The worth of IC50 was calculated with a nonlinear regression graph that strategized amid current inhibition of cells in one axis and concentration of logs one another.

LDH assay

Lactate dehydrogenase release assay liberates. Lactate dehydrogenase (LDH) is a pointer of membrane integrity and henceforth cell injury. Following treatment with compound Vf (3.12, 6.25, 12.5, 25, 50 and 100 µg/mL) on HeLa, K562, MDAMB231 cancer cells for 48 h and it was conducted to measure the proclamation of LDH on the way to the media and measured using standard protocols (Moorthy et al., 2010). The intracellular LDH was determined by fast congelation and melting in liquid nitrogen after losing the cells. The proclamation of LDH was calculated at an absorption rate of 490 nm. The releasing proportion of LDH was measured for example (media LDH activity)/(Media LDH activity) X 100%. Outcomes are exposed as a proportion of LDH release that substrates switch standards from the preserved ones.

FACS analysis

Analysis of the FACS was performed to determine compound 1 had any effect on the development of cell cycles. After 48 h of 50 and 100 µg/mL treatments, MDAMB231 cells stained with propidium iodide and endangered to FACS. The DMSO treated cells showed a regular pattern for the cell rotation. Cells were treated with compound Vf absorptions of 50 and 100 µg/mL and incubated for further 24 hours. At room temperature, cells were gathered then centrifuged for 5 min at 2000rpm. The supernatant was carefully removed, preserving the cell pellet. The cell pellet washed through re-dangling in 2 mL of 1XPBS. With the same conditions, the washing was repeated for another time. The supernatant abandoned, with the pellet was retained. Cells set by resuspending in 300 µL of Sheath fluid. Preceded by adding 1 mL of chilled 70% EtOH dropwise with constant gentle shaking, and adding another 1 mL again.

Docking studies

The mark protein nominated for the current research is HPV 16 E2 protein. The manufactured bis-rhodanine derivatives were practiced for molecular docking study.

Molecular Docking

The study of Docking under the default setting was done using Autodock with a binding pocket on the TAD. The result was analyzed using PYMOL (TM) by visualizing the protein-ligand and calculating parameters like hydrogen bonding amid protein and the ligand thus, determining scores are tabulated.

RESULT AND DISCUSSION

While doing the literature survey to the best of our knowledge, there is only one report available for the synthesis of bis-rhodanine using diamines, Carbon disulfide and dialkylacetylenedi carboxylates. In the present work, diamines, Carbon disulfide, and chloroacetic acid for the synthesis of bis-rhodanine are used. To found the optimal reaction conditions for the integration of bis-rhodanine, it is decided to begin with the conventional method. In this method, one mole of diamine and two moles of carbon disulfide and chloroacetic acid are used. The dithioamide is formed initially by the reaction of carbon disulfide and diamine serves as a nucleophile and reacts further with chloroacetic acid followed by cyclization yield bis-rhodanine (III). This method have been reported earlier, but the mechanism was not explained adequately. Now the device is shown in Scheme 2.

New derivatives were synthesized by Knoevenagel condensation of bis-rhodanine(III) with different substituted aldehydes (IV) in the presence of sodium acetate and acetic acid to yield the products (V) and (Va-m). The newly synthesized 5-benzylidenebis-rhodanine derivatives were fully characterized by proton NMR, carbon NMR spectral data and elemental analysis.
In the $^1$H-NMR spectra of a representative compound Ve in the upfield region the N-methylene protons resonated at $\delta$ 3.76 (2H, m, H-3$''$), the methyl group appeared as a doublet at $\delta$ 1.64 (3H, J = 6.7 Hz), and the corresponding methine proton appeared as a multiplet at $\delta$ 4.8 (1H, m, H-2$''$). The two singlets at $\delta$ 7.68 (1H, s, H-6) and 7.79 (1H, s, H-6') were attributed to the benzylic protons one on each side. The Phenyl protons appeared as a pair at $\delta$ 7.96 (4H, d, J = 7.8 Hz) and 8.15 (4H, d, J = 7.8 Hz). The CH$_3$ group attached to the phenyl ring resonated at 2.51 (3H, s, CH$_3$). The above data was complemented by its carbon NMR spectra. The pair of signals at $\delta$ 115.88 and 114.97 are due to the =C=S group of the rhodanine system. The CH$_3$ carbon appeared at $\delta$ 14.05, and the CH$_2$ and CH carbons attached to the nitrogen resonated at 50.54 and 43.26. The signals at $\delta$ 151.32 and 150.27 are assigned to the benzylic carbons (C-6 & C-6$'$). The methyl carbon attached to the aromatic system appeared at $\delta$ 28.4. The bunches of signals between 125.33 to 134.60 are attributed to the phenyl carbon atoms. All the other messages of the compound (V) and (Va-m) coincided with signs of compound(Ve).

**Mechanism**

![Scheme 1. Synthesis of bis-rhodanine (III)](image1)

Scheme 1. Synthesis of bis-rhodanine (III)

![Scheme 2. Synthesis of 5-benzylidene bis-rhodanine derivatives (V) and (Va-m)](image2)

Scheme 2. Synthesis of 5-benzylidene bis-rhodanine derivatives (V) and (Va-m)
Cytotoxic activity

The effect of compounds V and Va-m on the proliferation of HeLa, MDAMB231 and K562 cells were carried out using MTT assay. HeLa, MDAMB231, and K562 cells were added with 3.12, 6.25, 12.5, 25, 50 and 100 µM of compounds V and Va-m and after 48 h the assay was performed. IC\textsubscript{50} values were calculated and presented in the Table 1. The viability of the cells were affected at a moderate concentration in all the derivatives V and Va-m with HeLa, MDAMB231 and K562 cells and was more pronounced in the case of the compound Vf against MDAMB231 breast cancer cell lines. The compound V without any substituents should be an IC\textsubscript{50} value of 75.0, 85.4, and 72 µM concentration against HeLa, MDAMB231, and K562 cells. The IC\textsubscript{50} value of all the other compounds Va-m which has substituents in the aromatic system has varied substantially from the IC\textsubscript{50} value of compound V against the three cell lines. This indicates that the substituents affect the proliferation activity against the three cell lines. If we divide these substituents into two groups like electron-withdrawing substituents (NO\textsubscript{2}, F, Cl and Br) and electron-pumping substituents (-OCH\textsubscript{3}, OH and CH\textsubscript{3}) and consider the IC\textsubscript{50} value concerning HeLa cell lines, the activity in compounds with electron-withdrawing groups Vb, Vc, Vd, Vf showed lower IC\textsubscript{50} costs than the compounds with electron-donating substituents Va, Ve and Vg. As an example, the IC50 value of compound Va with an electron-donating substituent - OCH\textsubscript{3} is 81.2 µM.

In contrast, for the compound with electron-withdrawing substituent fluorine in Vf, the IC\textsubscript{50} value is 54 µM. The same trend is followed in MDAMB231 and K562 cells too. Further the compounds are divided into two groups depending upon the position of the substituent placed in the aromatic ring-like para-substituted compounds Va to Vg and meta substituted compounds Vh, Vi, Vj, and Vm, the IC\textsubscript{50} value of para-substituted compounds and relatively lower than the meta substituted compounds. The IC\textsubscript{50} value of Vf with a para-substituted fluorine group has an IC\textsubscript{50} value of 54 µM, whereas the corresponding compound with meta substituted fluorine group showed an IC\textsubscript{50} amount of 61 µM. Overall concerning all the three HeLa, MDAMB231, and K562 cell lines, the mixture Vf with a para-substituted fluorine atom performed well. It showed an IC\textsubscript{50} value of 54.0, 32.0, and 58.0 against HeLa, MDAMB231, and K562 cell lines, respectively. So compound Vf was taken for further studies.

### Table 1: Inhibitory effect of compounds (V) and (Va-m) on cytotoxic activity by MTT assay

| S No | Compound R | IC\textsubscript{50} µM HeLa | IC\textsubscript{50} µM MDAMB231 | IC\textsubscript{50} µM K562 | Binding energy, kcal/mol |
|------|------------|-----------------------------|-------------------------------|---------------------------|-------------------------|
| 2    | (V) H      | 75.0                        | 85.0                          | 72.0                      | -8.6                    |
| 3    | (Va) p-OCH\textsubscript{3} | 81.2                      | 82.5                          | 78.2                      | -9.5                    |
| 4    | (Vb) p-Cl  | 77.0                        | 78.0                          | 79.0                      | -9.5                    |
| 5    | (Vc) p-Br  | 55.5                        | 65.2                          | 60.5                      | -8.9                    |
| 6    | (Vd) p-NO\textsubscript{2} | 66.5                      | 76.5                          | 70.5                      | -8.8                    |
| 7    | (Ve) p-CH\textsubscript{3} | 77.5                      | 82.2                          | 74.5                      | -9.3                    |
| 8    | (Vf) p-F   | 54.0                        | 32.0                          | 58.0                      | -9.1                    |
| 9    | (Vg) p-CH\textsubscript{3} | 66.5                      | 69.5                          | 69.5                      | -9.2                    |
| 10   | (Vh) m-Cl  | 72.5                        | 82.5                          | 75.5                      | -8.8                    |
| 11   | (Vl) m-Br  | 69.0                        | 76.0                          | 70.0                      | -8.7                    |
| 12   | (Vj) m-F   | 61.0                        | 44.0                          | 64.0                      | -9.6                    |
| 13   | (Vk) m-p-Cl | 62.5                      | 68.4                          | 68.5                      | -9.5                    |
| 14   | (Vl) m-p-OCH\textsubscript{3} | 65.5                      | 69.3                          | 67.5                      | -9.0                    |
| 15   | (Vm) m-NO\textsubscript{2} | 80.0                      | 83.0                          | 82.0                      | -9.1                    |
| 16   | Cis platin | -                          | 23.60                         | 24.86                     | 29.32                   |

Lactate dehydrogenase discharge examine was performed to test the cell membrane harm incited by Compound Vf for this, the MDAMB231 cell line was refined with 10, 50, 100, and 250 µM concentration of compound V, and LDH discharged was estimated at 24, 48, and 72 hours. Predictable with the above outcomes a portion and time-subordinate increment in LDH discharge was monitored and confirming the cytotoxic capability of Compound Vf Fig. 1 was confirmed.

![Fig. 1. LDH assay of compound Vf against MDAMB231 cell line](image)

### FACS analysis

Fluorescence-activated Cell Sorting investigation was performed to decide if compound Vf has any impact on cell cycle movement. MDAMB231 cells were recolored with propidium iodide after 48 h of treatment 50 and 100 µM and exposed to FACS. Histogram of the vehicle (DMSO) rewarded cells demonstrated a standard cell cycle design, which incorporates G1 and G2 isolated by the S stage. The subG1 step (for the most part dead cells) was not noticeable. Endless supply of compound 1, a subordinate focus change was seen in the cell cycle design Figures 2, 3 and 4).
Fig. 2. Flow Cytometry Control plots of MDAMB231 cells

Fig. 3. Flow Cytometry plots of MDAMB231 cells treated with 50µg/mL of compound Vf
Compound Vf has shown S arrest from 19.94% and 32.39%, respectively. G2M phase arrest was found to be 6.38% and 10.44% in MDAMB231 cells. Thus, these outcomes show that compound 1 may meddle with cell division by initiating S stage capture followed by apoptosis. So, more researches are required to comprehend the mechanism of cell cycle capture.

**Docking Score**

From the docking study, it was learned that the synthesized 5-benzylidene bis-rhodanine derivatives have a reasonable score and outstanding interactions. The compound (Vc) has an excellent binding score and Compound (Vc), sulphur and the C=O is one of the bisrhodanine moiety form H-bonds with LEU99 and TYR32. It exhibited hydrophobic interactions with GLU100, SER98, ILE15, MET36, GLN12, TYR19, LEU94, LYS68, and GLN95.

CONCLUSION

In the present work, fourteen new 5-benzylidene bis-rhodanine derivatives (V) and (Va-m) were have been prepared, characterized by proton NMR, carbon NMR spectroscopy, elemental analysis and evaluated for their anticancer
activities against HeLa cell lines by MTT assay, leukemic cell line K562, breast cancer cell line MDAMB231 and molecular docking studies showed better binding scores. Screening of (Vc) and (Vf) compounds was carried for their potential anticancer activity. The results proposed that further study of such compounds with 5-benzylidenebisrhodanine moiety may be interest.

ACKNOWLEDGEMENT

The Authors Acknowledge Central Instruments Facility, Karpagam Academy of Higher Education for providing all the equipments, instruments, chemicals and materials during this research work.

Conflicts of Interest

We declare that we have no conflict of interest.

REFERENCES

1. Whiting, E.; Raje, M. R.; Chauhan, J.; Wilder, P. T.; Eker, D. V.; Hughes, S. J.; Bowen, N. G.; Vickers, G. E. A.; Fenimore, I. C.; Fletcher, S. Bioorg. Med. Chem. Lett., 2018, 28, 523-528.
2. Mendgen, T.; Steuer, C.; Klein, C. D. J. Med. Chem., 2012, 55, 743-747.
3. Hotta, N.; Akanuma, Y.; Kawamori, R.; Matsuoka, K.; Oka, Y.; Shichiri, M.; Toyota, T.; Nakashima, M.; Yoshimura, I.; Sakamoto, N.; Shigeta, Y. Diabetes Care, 2006, 29, 1538-1544.
4. Nagahara, K.; Anderson, J. D.; Kini, G. D.; Dalley, N. K.; Larson, S. B.; Smeee, D. F; Jin, A.; Sharma, B. S.; Jolley, W. B. J. Med. Chem., 1990, 33, 407-415.
5. Nazaktabar, A.; Lashkenari, M. S.; Araghi, A.; Ghorbani, M.; Golshahi, H. Int. J. Biol. Macromol., 2017, 103, 379-384.
6. Sortino, M.; Delgado, P.; Juarez, S.; Quiroga, J.; Abonia, R.; Insuasty, S.; Nogueras, M.; Rodero, L.; Garibotto, F. M.; Enriz, R. D.; Zacchino, S. A. Bioorg. Med. Chem., 2006, 15, 484-494.
7. Kartsev, V.; Shikhaliev, K. S.; Geronikaki, A.; Medvedeva, S. M.; Ledenyova, I. V.; Krysin, M. Y.; Petrou, A.; Ciric, A.; Glamoclija, J.; Sokovic, M. Eur. J. Med. Chem., 2019, 175, 201-214.
8. Shaikh, M. S.; Kanhed, A. M.; Chandrasekaran, B.; Palkar, M. B.; Agrawal, N.; Lherbet, C.; Hampannavar, G. A.; Karpoormath, R. Bioorg. Med. Chem. Lett., 2019, 29, 2338-2344.
9. Chao, L.; Jia-Chun, L.; Ya-Ru, L.; Cheng, G.; Mei-Ling, Z.; Hong-Yan, L.; Xiao-Zhen, L.; Chang-Ji, Z.; Hu-Ri, P. Bioorg. Med. Chem. Lett., 2015, 25, 3052-3057.
10. Liu, H.; Sun, D.; Du, H.; Zheng, C.; Li, J.; Piao, H.; Li, J.; Sun, L. Eur. J. Med. Chem., 2019, 172, 163-173.
11. Trotsko, N.; Kosikowska, U.; Paneth, A.; Wu, M.; Malm, A. Saudi Pharm. J., 2018, 26, 568-577.
12. Tarahomi, M.; Baharfar, R.; Mohseni, M. Clin. Microbiol. Infect., 2019, 4, 1-5.
13. Rostam, A. B.; Peyravi, M.; Ghorbani, M. Appl. Surf. Sci., 2018, 427, 17-28.
14. Kratky, M.; Vinsova, J.; Iko, J. I. S. Bioorg. Med. Chem., 2017, 25, 1839–1845.
15. Bhath, H. B.; Sharma, S. Arab. J. Chem., 2013, 10, 1590-1596.
16. Abdel Hafez, N. A.; Elsayed, M. A.; El-Shahawi, M. M.; Awad, G. E. A.; Ali, K. A. J. Heterocycl. Chem., 2018, 55, 1729-1737.
17. Wacothon, K. C.; Ludovic, P.; Anouabi, B.; Yves-Alain, B.; Rémy, L. G.; Myriam, R.; Anne, C.; Jean, P. B. Med. Chem. Res., 2015, 24, 1653-1661.
18. Celestina, S. K.; Sundaram, K.; Ravi, S. Bioorg. Chem., 2020, 97, 103640.
19. Khan, N.; Gautam, G.; Gupta, A. k. J. D. D. T., 2019, 9, 161-167.
20. Andleeb, H.; Tehseen, Y.; Shah, S. J. A.; khan, I.; Iqbal, J.; Hameed, S. RSC Advances., 2016, 6(81), 77688–77700.
21. Sim, M. M.; Ng, S. B.; Buss, A. D.; Crasta, S. C.; Goh, K. L.; Lee, S. K. Bioorg. Med. Chem. Lett., 2002, 12, 697-699.
22. Talele, T. T.; Arora, P.; Kulkarni, S. S.; Patel, M. R.; Singh, S.; Chudayeu, M.; Kaushik-Basu, N. Bioorg. Med. Chem., 2010, 18, 4630-4638.
23. Ahn, J. H.; Kim, S. J.; Park, W. S.; Cho, S. Y.; Ha, J. D.; Kim, S. S.; Kang, S. K.; Jeong, D. G.; Jung, S. K.; Lee, S. H.; Kim, H. M.; Park, S. K.; Lee, K. H.; Lee, C. W.; Ryu, S. E.; & Choi, J. K. Bioorg. Med. Chem. Lett., 2006, 16, 2996-2999.

24. Ahmed I. Khodaira, A. I.; Awadb, M. K.; Gessonc, J. P.; Elshaierd, Y. A. M. M. Carbohydr. Res., 2020, 487, 107894.

25. Yan, M.; Zhao, J.; Sun, D.; Sun, W.; Wenting, B. Z.; Deng, W.; Zhang, D.; Wang, L. Tetrahedron. Lett., 2017, 73(24), 3355–3362.

26. Dago, C. D.; Ambeu, C. N. T.; Coulibaly, W. K.; Bekro, Y. A.; Bekro, J. A. M.; Guevel, R. L.; Corlu, A.; Pierre Bazureau, J. Chem. Heterocycl. Compd., 2017, 53(3), 341–349.

27. Moorthy B. T.; Ravi S.; Srivastava M.; Chiruvella K. K.; Hemlal H.; Joy O.; Raghavan S. C. Bioorganic & Medicinal Chemistry Letters., 2010, 20, 6297-6301.

28. Ravi, S.; Chiruvella, K. K.; Rajesh, K.; Prabhu, V.; Raghavan, S. C. Eur. J. Med. Chem., 2010, 45, 2748-2752.

29. Didwagh, S. S.; Piste, P. B. J. Chem. Pharm. Res., 2013, 5(5), 271-274.

30. Kamila, S.; Biehl, E. R. Tetrahedron. Lett., 2012, 53, 3998-4003.

31. Braddock, D. C.; Cailleau, T.; Cansell, G.; Hermitage, S. A.; Pouwer, R. H.; Redmond, J. M.; White, A. J. P. Tetrahedron Asymmetry., 2010, 21, 2911-2919.

32. Arafa, W. A. A.; Shaker, R. M.; Rabeh, S. A. Heterocycles., 2016, 92, 1224-1243.

33. Harshitharaj, P. K.; Kumar, R.; Ravi, S. J. Chem. Pharm. Sci., 2016, 9(4), 2478-2482.