Synthesis, Docking and Anti-Tumor Activity of β-L-1,3-Thiazolidine Pyrimidine Nucleoside Analogues

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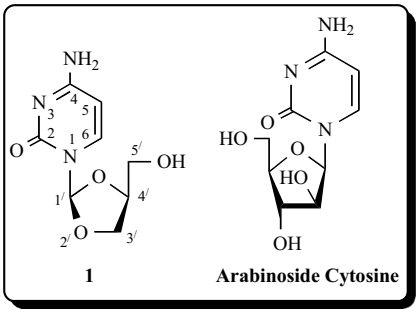
Abstract: In the search for effective, selective, and nontoxic antiviral and antitumor agents, a variety of strategies have been devised to design nucleoside analogues. Here we have described the versatile synthesis of β-L-1,3-thiazolidine nucleoside analogues. These analogues are all derived from the key stereochemically defined intermediate N-tert-butoxy-carbonyl-4-hydroxymethyl-1,3-thiazolidine-2-ol which was accessible in 57% yield starting from L-Cysteine methylester hydrochloride. *N*-tert-butoxycarbonyl-2-acyloxy-4-trityloxymethyl-1,3-thiazolidine was coupled with the pyrimidine bases in the presence of Lewis acids stannic chloride or trimethyl silyl triflate following Vorbruggen procedure. Proof of the structure and configuration was obtained through ^{1}H NMR, ^{13}C NMR, Mass, elemental analysis and NOE experiments. Docking and antitumor activity of these nucleoside analogues are also reported.

Key Words: β-L-1,3-thiazolidine nucleoside analogues, docking, EAC model, anti-tumor activity.

INTRODUCTION

The synthesis and biological activity of nucleosides have been of interest for some time, particularly as antiviral and anticancer agents. β-L-(-)-dioxolane-cytidine [(-)-OddC] **1** is the first L-nucleoside analogue ever shown to have anticancer activity [1].



1 **Arabinoside Cytosine**

In addition to having potent anti-HBV and anti-HIV activities, it is also extremely cytotoxic. When its anti-cancer potential was evaluated in comparison with arabinoside cytosine, the most effective drug clinically available for the acute leukemia, the L-nucleoside was found to be effective against both solid and lymphoid tumors [2]. This compound was a good substrate for replicative and repair DNA polymerases but resistant to inactivation by cytidine deaminase, which increased its effectiveness. Toxicity experiments indicated that very high doses of the drug were well tolerated by the mice [3,4]. All these factors encouraged us to design the nucleoside analogues, which are structural resemblance with (-)-OddC.

Thymidylate synthase (TS) is a well-validated target for cancer chemotherapy [5-7]. TS catalyze the reductive methylation of deoxy uridine monophosphate (dUMP) by N5, N10-methylene tetrahydrofolate. The product of TS catalysis, dTMP, is readily phosphorylated to dTTP, one of the four principal building blocks required for DNA synthesis. dTTP is also a negative regulator of dCMP deaminase and ribonucleotide reductase [8]. Since dTTP is required for DNA synthesis, inhibition of TS effectively limits DNA replication and cell division to what can be achieved using dTMP obtained *via* thymidine kinase and the salvage pathway [9]. TS inhibition is an important strategy for anticancer drug design because rapidly proliferating malignant cells require the *de novo* synthesis of deoxynucleotides to a greater extent than do cells that are less highly proliferative [10]. In this connection, we have selected TS as our target for the docking experiment.

Computational studies on the possible binding mode of β-L-1,3-thiazolidine pyrimidine derivatives to the Thymidylate synthase (TS) target was carried out to analyze the effect of replacement of furanose 2′-carbon atom by sulphur atom and oxygen atom by nitrogen atom . It has been proposed by the authors that a slight change in size and electro negativity of the heteroatom would lead to the compounds that would increase the biological activity and metabolic stability with decreased toxicity. Moreover positive charges associated with protonation to the nitrogen atom on 1,3-thiazolidine ring would be expected to play a very important role on the inhibition of viral or cellular enzymes which are essential for viral replication [11].

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The 1,3-thiazolidines exhibit different biological activities such as anticancer, antithyroid, anti-inflammatory, cardiovascular, antiviral drugs. Due to their various biological activities 1,3-thiazolidines can be used as powerful "building blocks" for the forming of the heterocyclic combinatorial rows and structure modeling of the potential biologically active compounds [12].

Based on the above observations, we have carried out the synthesis of the new class of β-L-1,3-thiazolidine nucleoside analogues using Vorbruggen procedure [13] and reported the docking and anti-tumor activity of these nucleoside analogues.

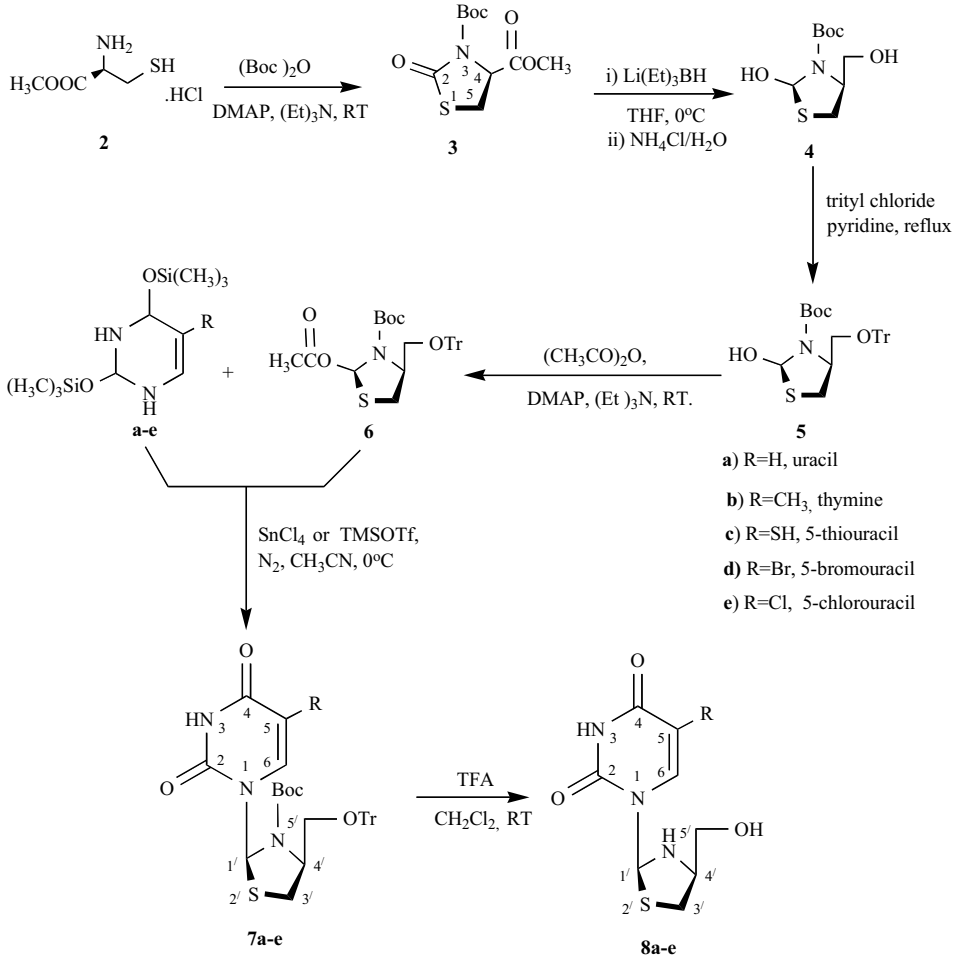
CHEMISTRY

Our synthetic strategy was based on the preparation of *N*-tert-butoxycarbonyl-4-methoxycarbonyl-1,3-thiazolidine-2-one **3** followed by the reduction and acylation gave *N*-tert-butoxycarbonyl-2-acyloxy-4-trityloxymethyl-1,3-thiazolidine **6**.

The compound **3** was prepared by condensing di-tert-butyl-di-carbonate with L-cysteine methyl ester hydrochloride in the presence of dimethylaminopyridine (DMAP) and triethylamine [14]. The lactam and the ester functional groups were reduced by using lithium triethylborohydride ($Li(Et)_3BH$) at 0^o C gave *N*-tert-butoxycarbonyl-4-hydroxymethyl-1,3-thiazolidine-2-ol **4** in quantitative yield [15]. Protection of primary alcoholic group using trityl chloride in the presence of pyridine gave *N*-tert-butoxycarbonyl-4-trityloxymethyl-1,3-thiazolidine-2-ol **5** which upon treatment with acetic anhydride, dimethylaminopyridine and triethylamine gave compound **6** (Scheme **1**).

Coupling of compound **6** with silylated pyrimidine bases in the presence of Lewis acids stannic chloride or trimethylsilyl triflate (TMS triflate) gave the nucleoside analogues **7a-e** in moderate yield [16]. The *N*-tert-butoxycarbonyl (Boc) and trityl (Tr) groups were deprotected by stirring it with trifluoroacetic acid at room temperature gave the desired products **8a-e** in excellent yield.

In general, the IR Spectra of **8a-e** showed the absorption peak in the region 1698-1710 cm^{-1} due to carbonyl group of pyrimidine moiety and 3300-3320 cm^{-1} due to OH and NH protons. The proton NMR spectra of the nucleoside analogue **8b** showed singlet due to the 6H protons in the region at δ 7.63. The signals due to the NH protons of the pyrimidine

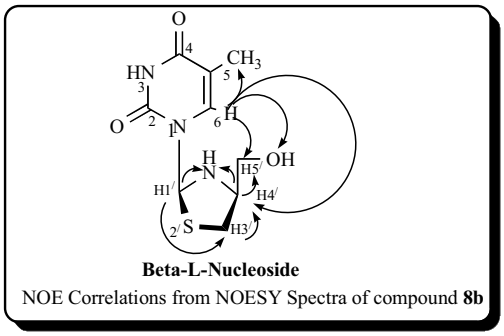


Synthesis of 1-(2*R*,4*S*-4-hydroxymethyl-1,3-thiazolidine-2-yl)-pyrimidine nucleoside analogues 8a-e.

Scheme (1).

moiety appeared downfield in the region at δ 11.27. The 1'-H proton of the 1, 3-thiazolidine ring appeared as a singlet in the region at δ 6.8 indicating the attachment of the 1, 3-thiazolidine moiety to the pyrimidine base. While triplet due to the 5'-OH protons appeared at δ 4.98 [17]. The 5-methyl group appeared in the region at δ 1.71. The 3' H and 4' H appeared in the region between δ 5.9-6.4. The NH proton of the 1.3-thiazolidine moiety appeared in the region at δ 4.75. ^{13}C NMR spectrum of **8b** showed signals at δ 80.8, 34.5, 64.0, 137.5, 110.9, 163.8, 150.9, 65.0, 15.5 ppm due to 1'-C, 3'-C, 4'-C, 6C, 5C, C=O of pyrimidine moiety, C=O, 5'-C, 5-CH_3 gp respectively. The mass spectrum of **8b** exhibited the molecular ion peak at 243 (M^+, 92) in addition to four major fragment ions at 175 (15), 167.5 (5), 109 (35), and 82 (7).

This modification is envisaged by the formation of nucleosides in enhanced yield and if the sugar moiety carries a 2-α-acyloxy function, the reaction reaches absolute stereospecificity, resulting in the β-ribo or deoxyribonucleosides in contrast to the anomeric mixture routinely obtained by using other methods [18].



Beta-L-Nucleoside

NOE Correlations from NOESY Spectra of compound **8b**

The structure and configuration was obtained through NOE experiment. According to NOE, 6 H is giving cross peak to 5-CH_3, 5'-H, 4'-H and OH. 6 H to 5'-H cross peak is stronger compared to 6H to 4'-H. So 5'-H is more nearer to 6H compared to 4'-H. This is possible only in β configuration [19]. The NOE between 6H to 5'-H and 6H to OH suggests that it is L-configuration. In this connection the structure is β-L-Nucleoside.

RESULTS &DISCUSSION

Docking Studies

Compounds **8a-e** were individually docked onto the grid encompassing the binding site of human thymidylate synthase, both in the presence and the absence of N5,N10-methylene tetrahydrofolate, a co-factor of the enzyme. The control docking exercise of the substrate itself indicated that the orientation of the substrate as seen in the crystal structure was best reproduced in the presence of the co-factor analogue (rmsd: 0.91).When the co-factor is not considered for computation during docking, the binding pockets of the co-factor as well as the substrate which are adjacent to each other, together appear as one large binding pocket. The ligand therefore has a much larger pocket to sample and can be theoretically trapped into local minima corresponding to sub-optimal orientations, due to the high reactivity of the phosphate groups. This problem is avoided when docking is carried out in the presence of the folate co-factor. It is not surprising therefore, the docking of dUMP as a control exercise was reproduced best in the presence of the folate co-factor. For the ligands 8a-e however, which lack the phosphate groups, understandably, there was no significant difference in binding orientations with and without the co-factor analogues. For consistency, only the results in the presence of co-factor are discussed further. Positions and orientations of the docked ligands that resemble one another closely (as judged by rmsd) are grouped into clusters by the clustering analysis module implemented in Autodock [20]. The larger the cluster size, higher the confidence of docking. The histograms of clustering indicate that the most populous clusters by and large were the most stable as reflected by lowest energies for each ligand .

Anti-Tumor Activity

The compounds were tested using the short term *in vitro* cytotoxicity towards EAC (Ehrlich Ascites Carcinoma) cells as a preliminary screening technique of tryphan Blue exclusion method (Cell Viability Test) for their cytotoxic potential. All the compounds were found to have considerable cytotoxicity in the Cell Viability test. Tumor induction led to an increase in body weight. This elevation was significantly reduced by the test compounds and cisplatin, pointing to their antitumor activity [21]. There was also a reversal in the altered haematological parameters in the treated animals when compared against control tumor induced animals, but the recovery was far from complete. The mean survival time significantly improved in comparison to the control group. The results obtained in the treated animals showed a reversal towards normal values of all parameters monitored viz., % increase in body weight, % increase in life span and hematological parameters (total and DLC of WBC, total RBC and Hemoglobin content) were comparable as that of the standard drug cisplatin. The results of the antitumor activity were summarized in the Table **1**.

Results of the short term *in vitro* cytotoxicity of the compounds **(8a-e)** are shown in Fig. (**1**). These preliminary experiments were carried out mainly with five different concentrations of the compounds. All the compounds were found to be cytotoxic and produced 50% cell death at a concentration of 12.5 μg/ml. Cisplatin was found to be the most cytotoxic.

The effect of compounds **8a-e** on survival of tumor-bearing mice is shown in Table **1** and Fig (**2**). Cisplatin significantly prolonged the Median and Mean Survival times ($p<0.05$) with respect to its control. It showed a significant increase in the percentage life span of animals (ILS>50). On the other hand, all the compounds significantly prolonged the Mean Survival Times. The influence of all the compounds on %ILS was more than 25%. By convention, a 25% increase in Life Span is considered as possible anticancer activity of a test compound.

The effect of compounds **8a-e** on haematological parameters is shown in Table **2**. Tumor induction significantly ($p<0.05$) increased total number of WBC almost 4 times. Cisplatin administration reversed this effect significantly ($p<0.05$). All the compounds significantly ($p<0.05$) reversed

Table 1. Effect of Drugs on the Survival Time in Tumor Induced Mice

Group	Dose (mg/kg)	Median Survival Time (Days)			Mean Survival Time (Days)		
		MST	%T/C	%ILS	(Mean ± SEM)	%T/C	% ILS
Control	-	17	-	-	17.00 ±1.09	-	-
Cisplatin	3.5	33	194.12	94.12	33.33 ±0.76[a]	196.06	96.06
8a	50	24	141.18	41.18	24.17 ± 0.47[a]	142.18	42.18
8b	50	26	152.94	52.94	25.50 ± 0.67[a]	150.00	50.00
8c	50	25	147.06	47.06	24.33 ± 0.95[a]	143.12	43.18
8d	50	29	170.58	70.58	28.66 ± 0.56	168.59	68.58
8e	50	27	158.82	58.82	26.66 ± 0.76[a]	156.82	56.82

a= p<0.05 Vs Control Groups, MST=Median Survival Time.

the tumor-induced rise in total counts of WBC. However, they were not as efficacious as cisplatin in reversing the tumor induced total counts. On differential counts, tumor induction caused a significant reduction in lymphocyte and a significant ($p<0.05$) increase in neutrophil counts. This was significantly ($p<0.05$) reversed towards normal by cisplatin and the test compounds. However, the compounds were less efficacious than cisplatin in their effects. Tumor induction caused significant decrease in RBC and Hb almost to the half of the normal animals [22]. This was significantly ($p<0.05$) reversed towards normal by cisplatin and the test compounds.

In conclusion, we have presented short and efficient preparations of the β-L-1, 3-thiazolidine nucleoside analogues, which as a consequence of their low toxicity should prove to be important anti-tumor agents. Visual inspection of the docking hits showed that the best clusters contained ligands **8a-e** interacting with the active site of TS in all the cases. All the compounds **8a-e** were found to have considerable cytotoxicity in the Cell Viability test and significantly prolonged the Mean survival times. Tumor induction caused significant decrease in RBC almost to the half of the normal animals. The compounds with free NH group in pyrimidine moiety showed significant cytotoxic activity. Since amines are strongly basic, compounds carry positive charge at physiological pH. These compounds may prove useful as enzyme inhibitors and cytotoxic agents.

EXPERIMENTAL SECTION

All reagents used were AR grade. THF was distilled from sodium/benzophenone prior to use. Melting points were determined using a Thomas Hoover melting point apparatus and are uncorrected. The ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker 300 NMR spectrometer in $CDCl_3$ and DMSO-d_6 (with TMS for ^{1}H and DMSO-d_6 for ^{13}C as internal references) unless otherwise

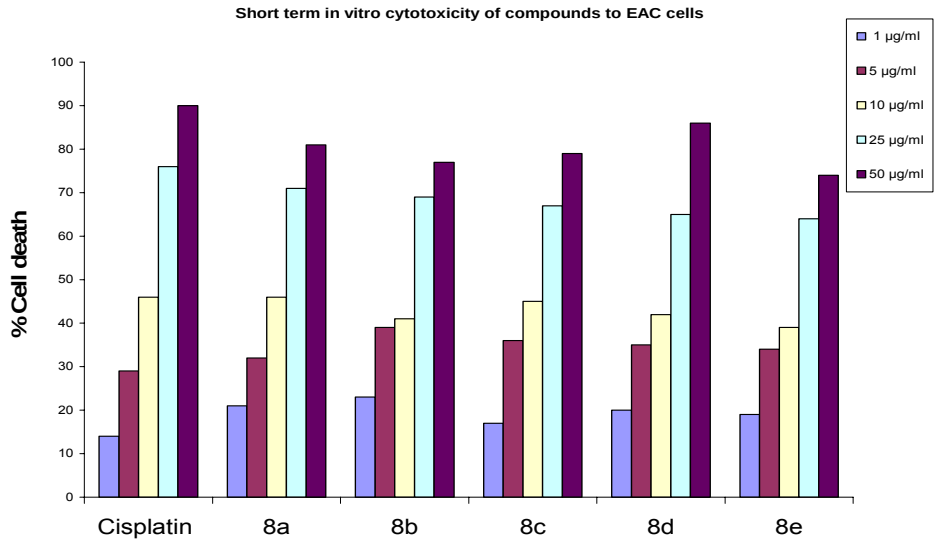


Fig. (1). Effect of different concentration of (8a-e) on tumor living cell number in Ascitic fluid of tumor induced mice.

Table 2. Effect of the (8a-e) on Hematological Parameters

Groups	Dose (mg/kg)	RBC (Mean ± SE) (Millions /mm^3)	Hb (Mean ± SE) (g%)	WBC (Mean ± SE) (10^{3}cells/ mm^3)	Differential Leucocyte Count %		
					Lymphocytes	Neutrophils	Monocytes
Normal	-	5.09 ± 0.18	15.80 ±0.62	8.66 ±0. 26	87.3±1.27	11.7 ±1.23	1.0±0
Control	-	2.15 ± 0.22[a]	9.60 ±0.52[a]	32.32 ± 1.12[a]	42.5±1.28[a]	56 ±1.01[a]	1.5± 0.22[a]
Cisplatin	3.5	3.23 ± 0.34[a]	13.01 ±0.24[a]	10.93 ± 1.32[b]	78.2 ±1.31 [a,b]	21.2 ± 0.96[a,b]	1.5±0.24[a]
8a	50	4.92 ± 0.81[a]	12.48 ±0.23 [a]	23.73 ±2.10[a,b,c]	65.2 ±1.49[a,b]	33.6±1.14 [a,b]	1.2 ±0.34[a,b]
8b	50	3.85 0.53[a]	12.5 ±0.37 [a]	17.25 ± 1.27[a,b,c]	72.6 ±2.17[a,b]	25.7±1.14 [a,b]	1.7 ±0.31[a,b]
8c	50	4.81 ± 0.22[a]	11.98 ±0.19 [a]	21.16 ± 1.82[a,b,c]	66.6 ±1.93[a,b]	31.9±1.14 [a,b]	1.5 ±0.32[a,b]
8d	50	3.83 ± 0.27[a]	12.90 ±0.25 [a]	16.34 ± 1.42[a,b,c]	75.6 ±1.67[a,b]	22.8 ±1.14 [a,b]	1.6 ±0.27[a,b]
8e	50	4.63 ± 0.35[a]	13.40 ±0.21 [a]	19.75 ± 1.67[a,b,c]	69.3 ±2.12[a,b]	29.3 ±1.14 [a,b]	1.4 ±0.21[a,b]

a=p<0.05 Vs Normal, b=p<0.05 Vs Control mice, c=p<0.05 Vs Cisplatin.

stated. MS were recorded on Agluent 1100 ES-MS, Karlsruhe, Germany. Infrared spectra (υ_{max}) were recorded on Perkin Elmer FTIR spectrophotometer as thin films on KBr plates (for oils) or KBr discs (for solids). Column chromatography was performed on silica gel (230–400 mesh). Microanalysis was obtained with an Elemental Analysensysteme GmbH VarioEL V3.00 element analyzer. The reactions were monitored by thin layer chromatography (TLC) using aluminum sheets with silica gel 60 F_{254} (Merck). All the reactions were carried out under nitrogen atmosphere.

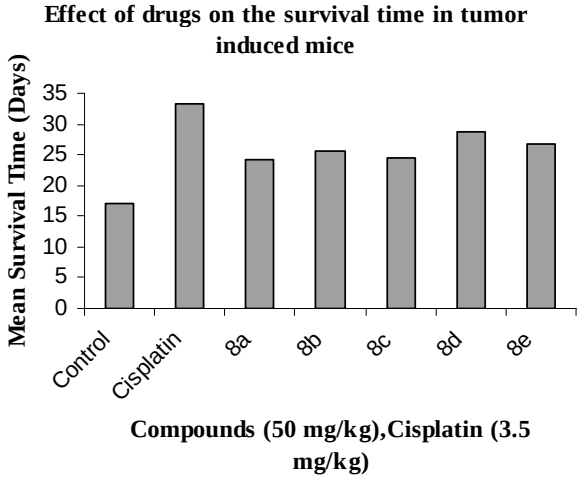


Fig. (2).

N-tert-butoxycarbonyl-4-hydroxyl methyl-1,3-thiazolidin-2-ol (4)

Compound **2**(1gm, 3.83 mmol) was dissolved in THF (20ml), cooled to 0^o C was added 3 eq of 1M soln of Li(et)$_3$ BH. The reaction requires 10 to 60 minutes to go to completion. Excess of reagent was destroyed by the addition of saturated solution of NH_2Cl aq. Solution at 0^o C and the reaction mixture was extracted with 15 ml dichloromethane and dried over anhydrous $MgSO_4$ and evaporated to dryness followed by the purification by flash chromatography (hexane-ethyl acetate 7:2) to afford the compound **3** as light yellow oil.

Light Yellow oil, (from EtOAc/hexanes), (0.513 gm, 67%).

IR (nujol): 1710-1720 (C=O of Boc-ester group), 3200-3220 cm^{-1} (OH). ^{1}H NMR ($CDCl_3$): δ, 4.24 (dd, J= 8.5, 2.25 Hz,1H,), 3.64 (dd, J=11.75, 8.5 Hz, 1H), 3.32(dd, J=11.75, 2.25 Hz,1H), 1.51,(s,9H, Boc ester),3.88 (t,2H), 5.2 (2H, s, OH), 6.68 (1H, s, CH) : ^{13}CNMR ($CDCl_3$δ 66.0, 32.3, 86.0, 154.1, 79.8, 28.5, 28.5, 68.5: LCMS (*m/z*) 219.2 (M + 1, 55%). Anal Calcd for $C_9H_{17}NO_4S$: C, 45.95; H, 7.23; N, 5.95; S, 13.6. Found; C, 44.94; H, 7.05; N, 5.16; S, 12.9.

N-tert-butoxycarbonyl-4-trityloxy methyl-1,3-thiazolidine-2-ol (5)

Compound **3** (1g, 4.25mmol) and trityl chloride (triphenylmethylchloride) (1.39g, 5mmol) were dissolved in 20 ml of pyridine. The mixture was heated at 100^o C (steam bath) with swirling for 30 minutes.The reaction mixture was cooled at room temperature and then poured in to the 100 ml of ice water. The slurry was stirred vigorously during quenching. The solid was filtered and it was washed thoroughly with water until it is free from pyridine. The solid was dried separately. The white product O-trityl derivative was purified by recrystallization from acetone-toluene.

White Solid (from EtOAc/hexanes), (1.15g, 55%); m.p. 154–156°C;

IR (nujol): 1710-1720 (C=O of Boc-ester group), 3200-3220 (OH), 1500 cm^{-1}(ArH).

^{1}H NMR ($CDCl_3$): δ, 4.24 (dd, J= 8.5, 2.25 Hz, 1H), 3.58 (dd, J=11.75, 8.5 Hz, 1H), 3.35 (dd, J=11.75, 2.25 Hz, 1H), 4.2 (t, 2H), 1.48, (s, 9H, Boc ester), 6.75 (1H, s, CH), 5.6 (2H, s, OH), 7.19 (m, 15H, ArH). ^{13}CNMR ($CDCl_3$) δ: δ, 66.0,32.3,86.0,154.1,79.8,28.5,85.9,143.9,128.3,129.3,126.3, 128.3,143.3,143.9,128.3,129.3,126.3,129.3,129.3,70.1. LCMS (*m/z*) 429 (M + 1, 65%). Anal Calcd for $C_{27}H_{30}NO_4S$: C,

70.41; H, 6.54; N, 2.93;S, 6.71: Found; C, 69.42; H, 5.53; N, 2.04; S, 6.22.

_N_-tert-butoxycarbonyl-4-trityloxymethyl-2-acyloxy-1,3-thiazolidine (6)

A solution of trityl derivative **4** (1gm, 2.09 mmol) in CH_2Cl_2 was treated with acetic anhydride (2.6 gm, 26.3 mmol), Triethylamine (2.6 gm, 26.3 mmol) and a catalytic amount of 4-DMAP (Dimethyl Amino Pyridine) at room temperature for 3 hours. The resultant mixture was washed with 5% HCl and extracted with CH_2Cl_2 and evaporated to dryness and purified by silicagel column chromatography with 5% chloroform: Ethyl acetate 7:2 to get white solid.

White Solid (from EtOAc/hexanes), (0.7493g, 65%); m.p. 164–167°C;

IR (nujol): 1710-1720 (C=O of Boc-ester group), 1690 (C=O), 1500 cm^{-1} (Ar H).

^{1}H NMR ($CDCl_3$)**:** δ, 4.24 (dd, J= 8.5, 2.25 Hz, 1H) 3.64 (dd, J=11.75, 8.5 Hz, 1H), 3.34 (dd, J=11.75, 2.25 Hz,1H), 3.38 (t,2H), 1.51,(9H s, Boc ester), 6.68 (s,1H,CH), 3.0 (s,3H,CH_3),7.19 (m,15H,ArH). ^{13}CNMR ($CDCl_3$) δ 64.6, 29.0,89.4,154.1,79.8,28.5,28.5,28.5,85.9,128.3,129.3,126.3,129.3,128.3,129.3,126.3,128.3,143.3,143.9,128.3,129.3,126.3, 129.3,129.3,170.3,20.7; LCMS (*m/z*) 471.6 (M + 1, 70%). Anal Calcd For $C_{30}H_{33}NO_5S$: C, 69.34; H, 6.40; N, 2.70; S, 6.17. Found; C, 68.35; H, 5.42; N, 2.12; S, 6.05.

General Procedure for the Synthesis of 1-(2R, 4S-4-hydroxymethyl-1,3-thiazolidine-2-yl)-Pyrimidine Nucleoside Analogues (8a-e)

A mixture of pyrimidine base (2.35 mmol) in HMDS (Hexamethyldisilazane) (10 ml) and CH_3CN was heated under reflux for 5 hours. After removal of solvent by vacuum pump, a solution of acetate **6** (1gm, 1.926 mmol) in 15 ml of CH_3CN was added to the reaction flask containing the silylated pyrimidine bases and then $SnCl_4$ (0.364gm, 1.4 mmol) was added drop wise at room temperature. After 16 hours the reaction was quenched with 1 ml of saturated solution of $NaHCO_3$ and the resultant mixture was concentrated. The crude mixture was diluted with 100 ml of CH_2Cl_2, washed with 5% $NaHCO_3$, dried over $MgSO_4$, filterd and concentrated. The crude product was purified by silica gel column chromatography with 30% ethyl acetate in hexane to give nucleoside analogues **(7a-e)**.

Procedure for the Deprotection of Boc and Trityl Groups in Compounds 7a-e

To a solution of **7a-e**(2.34 mmol) in CH_2Cl_2 (2.5 ml) was added TFA (2ml) under nitrogen atmosphere at RT. After stirring at the same temperature for 24 hours, the solvent was evaporated under reduced pressure. Purification of the residue by washing with ether, afforded the targeted nucleoside analogues **8a-e** in excellent yield.

1-(2R, 4S-4-hydroxymethyl-1,3-thiazolidine-2-yl)-uracil (8a)

$[\alpha]_D^{25^oC}$ =-146 (C=2.5, CH_3OH)

White needles (from EtOAc/hexanes), (1.36gm, 67%) m.p. 167°-169°C;

IR (nujol): 1890 (C=O), 1690 (C=O), 2950 (NH), 3557 cm^{-1} (OH).^{1}H NMR (DMSO d_6): δ,3.10-3.50 (m, 2H, 2'-H), 3.95-4.10 (m,2H, 5'-H),5.01 (t,1H, 5'-OH), 5.20 (t, 1H, 4'-H), 5.50 (d, J=7.9 Hz, 1H, 5H), 5.88 (s, 1H, NH), 6.33 (m, 1H, 1'-H), 7.95 (d, J=7.9 Hz, 1H, 6-H), 11.20 (br s, 1H, NH). ^{13}CNMR (DMSO d_6)δ: 80.5, 34.5, 64.0, 141.3, 102.4, 163.6, 150.9, 65.0. LCMS (*m/z*) 229 (M^+, 87), 175 (15), 153 (7), 95 (38), 83 (8). Anal Calcd. For $C_8H_{11}N_3O_3S$: C, 41.91; H, 4.84; N, 18.33; S, 13.99. Found: C, 41.84; H, 4.75; N, 18.20; S, 13.88.

1-(2R, 4S-4-hydroxymethyl-1,3-thiazolidine-2-yl)-thymine (8b)

$[\alpha]_D^{25^oC}$ =-121 (C=2.5, CH_3OH)

White Solid (from EtOAc/hexanes), (1.31gm, 68%); m.p. 148–150°C;

IR (nujol): 1890 (C=O), 1690 (C=O), 2950 (NH), 3557 cm^{-1} (OH). ^{1}H NMR (DMSO d_6): δ, 1.71 (s, 3H, 5-CH_3), 3.59 (m, 2H, 5'-H), 4.98 (t, 1H, 5'-OH), 4.79 (s, 1H, 6^1 NH), 6.8 (s, 1H, 1'-H), 6.38(t, 1H, 4'-H), 5.9 (dd, J=6.3 and 8.8 Hz, 2H, 3'-H), 7.63 (s, 1H, 6-H), 11.27 (brs, 1H, NH); ^{13}CNMR (DMSO d_6)δ: 80.8, 34.5, 64.0, 137.5, 110.9, 163.8, 150.9, 65.0. LCMS (*m/z*) 243 (M^+, 92), 175 (15), 167.5 (5), 109 (35), 82 (7). Anal Calcd. For $C_8H_{11}N_3O_3S$: C, 44.43; H, 5.39; N, 17.27; S, 13.18. Found**:** C, 44.35; H, 5.25; N, 17.20; S, 13.10.

1-(2R, 4S-4-hydroxymethyl-1,3-thiazolidine-2-yl)-5-thiouracil (8c)

$[\alpha]_D^{25^oC}$ = -98 (C=2.5, CH_3OH)

Yellow solid (from EtOAc/hexanes), (1.12gm, 62%); m.p. 174°-176°C

IR (nujol): 1890 (C=O), 1690 (C=O), 3557 (OH, NH), 2600 cm^{-1} (SH).^{1}H NMR (DMSO d_6)**:** δ 1.5 (s,1H, SH), 3.10-3.50 (m, 2H, 2'-H), 3.95-4.05 (m,2H, 5'-H),5.00 (t,1H, 5'-OH), 5.20 (t, 1H, 4'-H),5.85 (s, 1H, NH), 5.95 (m, 1H, 1'-H), 7.95 (s, 1H, 6-H), 11.20 (br s, 1H, NH).^{13}CNMR δ: 80.1, 34.5, 64.0, 142, 100, 162.4, 150.9, 65.0. LCMS (*m/z*) 261 (M^+, 89), 175 (15), 148.8 (15), 126.9 (35), 98 (6). Anal Calcd. For $C_8H_{11}N_3O_3S_2$**:** C, 36.77; H, 4.24; N, 16.08; S, 24.54. Found: C, 36.65; H, 4.12; N, 15.98; S, 24.43.

1-(2R,4S-4-hydroxymethyl-1,3-thiazolidine-2-yl)-5-bromouracil (8d)

$[\alpha]_D^{25^oC}$ =-152 (C=2.5, CH_3OH)

Yellow oil (from EtOAc/hexanes), (1.05gm, 65%);

IR (nujol): 1890 (C=O), 1690 (C=O), 2950 (NH), 3557 cm^{-1} (OH). ^{1}H NMR (DMSO d_6)**:** δ 3.10-3.50 (m, 2H, 2'-H), 3.95-4.05 (m, 2H, 5'-H), 5.00 (t, 1H, 5'-OH), 5.20 (t, 1H, 4'-

H), 5.85 (s, 1H, NH), 5.95 (m, 1H, 1'-H), 8.10 (s, 1H, 6-H), 11.20 (brs, 1H, NH). ^{13}CNMR (DMSO d_6) δ: 79.8, 34.5, 64.0, 143.5, 94.8, 159.9, 150.9, 65.0. LCMS (*m/z*) 308 (M^+, 75), 232 (8), 175 (15), 168 (35), 147 (6). Anal Calcd. For $C_8H_{10}BrN_3O_3S$: C, 35.04; H, 3.68; N, 20.43; S, 11.69. Found: C, 34.91; H, 3.56; N, 20.31; S, 11.55.

Docking Studies

All molecular modeling calculations and manipulations were performed using the software packages of Accelrys (INSIGHT-II modules; Accelrys inc, http://www.accelrys.com). The Biopolymer module in Tripos software suite (SYBYL7.0):Tripos inc, http://www.tripos.com)was used to assign charges to the protein and the ligand. The possibility of binding of **8a-e** and the precise location of binding to TS was evaluate by an automated docking algorithm, AutoDock 3.05.The possible binding conformations and orientations were also analyzed by clustering methods, embedded in Autodock. The protein-drug interactions such as hydrogen bonding, hydrophobic interactions and aromatic stacking were evaluated using the LPC software (Sobolev *et al*., 1999).

For docking studies the molecular structures of **8a-e** were built and energy minimized, gasteiger charges were assigned to the minimized ligands using sybyl7.0. The crystal structure of human thymidylate synthase complexed with an cofactor inhibitor LYA (pyrrolo(2,3d)pyrimidine) and substrate UMP (2'-deoxyuridine 5'-monophosphate (PDB:1JU6),was used as the template in all cases. All water atoms were removed. Polar Hydrogens were added to the protein using sybyl7.0 and the atomic partial charges were computed using the Kollman united method. Autotors utility was used to define the rotatable bonds in the ligands. A grid of 60Å^3, 0.375 spacing was first computed such that the known ligand binding and catalytic sites of TS was covered. Each ligand was individually docked into this grid using the Lamarckian genetic algorithm and its interactions monitored using detailed energy estimates.100 cycles of docking with about 25,00,000 energy evaluations and 500 generations in each cycle were carried out, which sampled all possibilities of conformations of the ligand in each cycle. Clustering was performed based on the similarities in binding modes and strengths in these cycles.

Determination of Cytotoxicity

Cancer Cell Lines

Cancer cell lines viz. Ehrlich's Ascitic Carcinoma (EAC), to induce cancer in animal model (mice) were obtained from Amala Cancer Research Center, Amala Nagar, Thrissur, Kerala, India. The cells were maintained as ascites tumor in Swiss albino mice by intraperitoneal inoculation [23].

Determination of Cytotoxicity of 1, 3-thiazolidine Nucleoside Analogues (8a-e) to EAC Cells (*In Vitro *Studies)

In vitro short-term cytotoxic activity of drug was determined using EAC cells. The EAC cells that were collected from the animal peritoneum by aspiration, were washed repeatedly with PBS to free from blood. After checking the viability of the cells in a haemocytometer, Cells (1 X 10^6) in 0.1ml PBS, 0.01ml of various concentrations of test compounds (1-50µg/ml) (the test compounds were dissolved in dimethyl sulfoxide (DMSO), the final concentration of DMSO not exceeding 0.1% of the total volume) and phosphate buffered saline (0.1mole/l, pH 7) in a total volume of 0.9ml were incubated in clean sterile tubes for 3h at 37ºC. The control tube had 10µl of solvent. The final volume was made up to 0.9 ml with PBS. To each 100µl of Tryphan blue solution was added. The live (without stain) and dead (with blue stain) cells were counted using haemocytometer and percent cell death was calculated using the formula:

$$\% \text{ Cytotoxicity} = 100 \times (T_{dead} - C_{dead}) / T_{tot}$$

where, T_{dead} is the No. of dead cells in the treated group, C_{dead} is that in the control group and T_{tot} is the total number of dead and live cells in the test compound treated group. Cisplatin was used as the standard.

Antitumor Activity of 1, 3-thiazolidine Nucleoside Analogues (8a-e). On Mouse Ehrlich Ascites Carcinoma (*In Vivo *Studies)

Preparation of Test Solution of Compounds 8a-e

The solutions of the compounds **8a-e** were prepared by suspending them in 4% acacia and administrated intraperitoneally daily for a period of 7 days from the 10 day of tumor inoculation in the volume of 0.1ml/10 g mouse. All the compounds were tested at the dose of 50 mg/kg body weight. The dose of cisplatin selected was 3.5 mg/kg. This was calculated by using Body Mass Index and past experience with the drug [24].

Animals used in the study

Female Swiss albino mice of 6-8 weeks old (25 ± 5 g body weight) were selected from Central animal facility, Manipal academy of Higher Education, Manipal, Karnataka, India. The animals were acclimatized to the experimental room having temperature 23 ± 2°C, controlled humidity conditions, and 12: 12 hour light and dark cycle. The Mice were housed in sterile polypropylene cages containing sterile paddy husk as bedding material with maximum of 4 animals in each cage. The mice were fed on autoclaved standard mice food pellets (Hindustan Lever) and water ad libitum. The animal experiments were performed according to the rules and regulations of the Institutional Animal Ethics Committee (IAEC).

Induction of Ehrlich Ascites Carcinoma

Antitumor activity of test compounds **(8a-e)** were determined using Ehrlich Ascites Carcinoma (EAC) tumor model in mice. Female Swiss albino mice were divided into 7 groups of 6 animals each. The ascitic carcinoma-bearing mice (donor) were used for the study, 15 days after tumor transplantation. The ascitic fluid was drawn using an 18-gauge needle into sterile syringe. A small amount was tested for microbial contamination. Tumor viability was determined by Tryphan blue exclusion test and cells were counted using haemocytometer. The ascitic fluid was suitably diluted in normal saline to get a concentration of 10^6 cells/ml. of tumor cell suspension. This was injected intraperitonealy to obtain ascitic tumor. The mice were weighed on the day of tumor inoculation and then once in three days thereafter.

Treatment was started on the 10th day of tumor inoculation. Cisplatin (one dose) was injected on 10th day intraperitonealy. The compounds were administrated from 10th day for 7 days intraperitonealy. The mortality rate was noted in each group and Median Survival Time (MST) and percentage increase in life span (% ILS) was calculated using the following formula [25].

$$\% \text{ ILS} = \frac{\text{MST of treated group- MST of control group}}{\text{MST of control group}} \times 100$$

Hematological Studies

In order to detect the influence of test compounds (8a-e) on the hematological status of EAC bearing mice, comparison was made amongst eight groups (n=5) of mice on the 14th day after transplantation. These comprised

a) Normal mice

b) EAC bearing mice

c) EAC bearing mice treated with one dose of Cisplatin

d) EAC bearing mice treated with test compounds (8a-e) for 5 days.

Blood was drawn from each mouse by retro orbital puncture under ether anaesthesia and the following parameters-white blood cell total count, differential leukocyte counts, red blood cell total count and haemoglobin (Hb) were determined by standard methods [26].

Statistical Analysis

Results were analyzed by one-way ANOVA by Scheffe's post-hoc test using SPSS computer package.

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