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Design, synthesis and cytotoxic studies on the simplified oxy analog of eleutherobin ☆

S. Chandrasekhar,[a,*] V. Jagadeshwar,[a] Ch. Narsihmulu,[a] M. Sarangapani,[b] D. R. Krishna,[b] J. Vidyasagar,[b] Dolly Vijay[c] and G. Narahari Sastry[c,*]

[a]*Organic Division-I, Indian Institute of Chemical Technology, Hyderabad 500 007, India*
[b]*Cancer and Aging Research Division, University College of Pharmaceutical Sciences, Kakatiya University, Warangal 506 009, India*
[c]*Molecular Modelling Group, Organic Chemical Sciences, Indian Institute of Chemical Technology, Hyderabad 500 007, India*

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Abstract—A straight forward entry into nine membered B ring of eleutherobin as oxy analog and its cyctotoxic properties on HBL cell lines is described. Molecular modeling studies were carried out to ascertain the binding of the model compound to the active site of β-tubulin.

Eleutherobin (**1**) and Sarcodictyins A and B (**2**)[1] (generally referred as Eleutheside family of microtubule stabilizing natural products) are derived from membrane precursors having common 4,7-oxaeunicellane oxatricyclic ring system. These compounds have shown highest activity against paclitaxel resistant tumor cell lines and are likely to lead second generation microtubule stabilizing anti-cancer drugs.[2] The scarce availability of these complex diterpene natural products has initiated major programmes of total synthesis across the world. Even though the total synthesis of eleutherobin and sarcodictyins is achieved by the groups of Nicolaou et al.,[3] Danishefsky and co-workers,[4] and several synthetic approaches by various groups,[5] none of the methods are useful for a large scale synthesis (Fig. 1).

While Eleutherobin is a potent β-tubulin inhibitor molecule, it has a complex architecture and the undesirable aspect of the available synthetic procedures is that they fail to provide quantitative yields. Therefore, we felt that attempts towards designing simpler analogs of eleutherobin are worthwhile. As part of a project aimed to synthesize eleutheside and their analogs, we

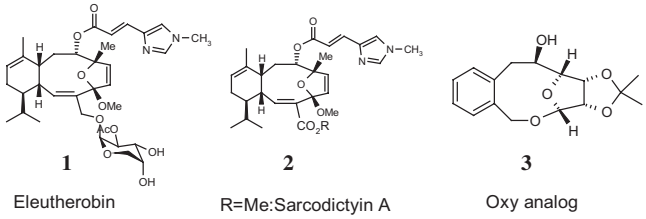

Figure 1.

were interested in synthesizing simple and easily accessible tricyclic core, so that large quantities of potent cytotoxic molecules would be accessible for further studies. Herein our preliminary studies on synthesis of oxy-B-ring analog grafted into A-ring benzene and furano-C-ring with appendages for further analoging is reported.

Keeping in view the complex 6–9–5 natural eleutherobin, we conceived a simple 6–9–5 scaffold **3**. In this approach, the five membered furan ring is originated from glucofuranose, the six membered cyclohexyl moiety was replaced with benzene ring and the nine membered B ring was grafted with *–O–* link. We employed the in vitro cytotoxicity studies and molecular modeling approaches to validate whether such an analog retains the biological activity.

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* Corresponding authors. Tel.: +91-40-27193434; fax: +91-40-27160512; e-mail addresses: srivaric@iict.res.in; gnsastry@iict.res.in

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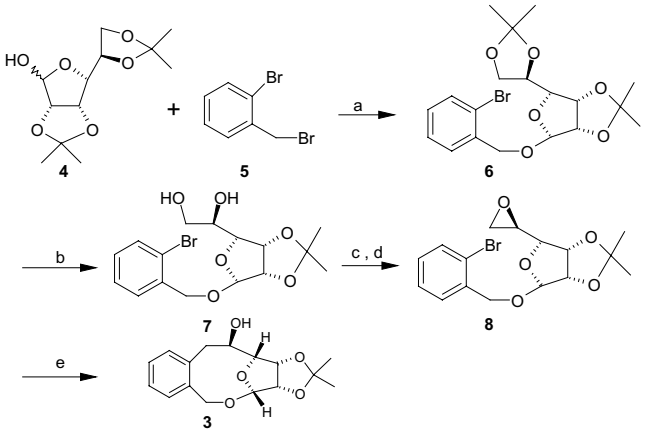

Scheme 1. Reagents and conditions: (a) NaH, THF, reflux, 12 h, 85%; (b) 60% AcOH, 8 h, 90%; (c) TsCl, DMAP (cat), triethylamine, CH_2Cl_2, 12 h, 95%; (d) K_2CO_3, MeOH, 2 h 98%; (e) *n*-BuLi, $BF_3{\cdot}OEt_2$, THF, 4 h 60%.

The synthesis of this analog begins from diacetone mannose, which has been thoroughly utilized as a versatile building block in chiral synthesis. Anomeric alkylation with *ortho*-bromobenzyl bromide in THF as solvent and NaH as base furnished the benzyl ether **6** in 85% yield. Exposure of **6** to 60% AcOH yielded diol **7** in 90% yield. Selective monotosylation and exposure to K_2CO_3 in methanol furnished the epoxide **8** in 98% yield. Intramolecular cyclization was triggered by using *n*-BuLi in presence of $BF_3{\cdot}OEt_2$ at −78 °C to furnish the 6–9–5 system **3** whose scaffold is architecturally close to the natural product (Scheme 1).

The model compound synthesized is an analog of the natural product Eleutherobin, which prompted us to do a docking study so as to determine its anti-cancer activity. In the absence of the three dimensional structure of human tubulin we have choosen its close relative, the tubulin of pig (PDB ID:1TUB) which shows a sequence similarity of 85% in the blast search. All modeling studies were carried out using the Molecular Operating Environment (MOE) package.[6] The protein structure was downloaded from the Protein Data Bank (RCSB) which is a heterodimer consisting of two chains α and β. Before venturing to the docking studies we deleted the α-chain as the β-tubulin was known to be the receptor for the anti-cancer natural products, such as taxol, eleutherobin, epothilones, etc.[7] MMFF94 force field was employed on the β-tubulin unit and was subjected to default energy minimization route for optimizing the coordinates of the added hydrogens, which employs the steepest descent initially followed by further refinement with conjugate gradient and Newton–Raphson techniques as implemented in MOE. However, the PDB coordinates of the nonhydrogen atoms were fixed. During the optimization, the RMSD gradient was initially set to 1000 during steepest descent and later the threshold RMSD was fixed at 0.001 in the truncated Newton–Raphson.

Conformational search was performed on the skeleton of the natural product and the model compound using AM1 method[8] implemented in MOPAC package manually. The lowest energy conformation was taken for the docking studies. Figure 2 depicts the lowest energy conformations for the three model systems, which were further used in docking studies.

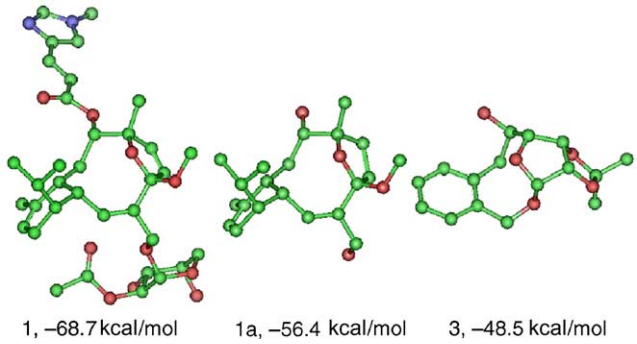

Figure 2. The MOE interaction energies of the three compounds studied.

Docking studies was carried out using the Dock Module of MOE, which utilizes Monte Carlo simulated annealing process for docking the inhibitor into the active site of the target protein. The docking box was arranged such that the residues at a distance of 6.5 Å are taken into consideration. This arrangement ensured that the amino acid residues β1(MET)–β31(ASP) and β217(LEU)–β233(ALA)[9] are in the docking box, which were found to be important areas for β-tubulin targeting anti-cancer drugs. Docking was performed for 25 runs with six cycles in each run and 3000 iterations were carried out. The initial temperature for docking was maintained at 1000 K. The complex thus obtained was minimized using the same force field MMFF94 with a gradient threshold of 0.001. The interaction energy was estimated by considering the contributions of van der Waals and electrostatic energies obtained using the dock module of MOE program. The whole procedure was repeated about 4–5 times to get a consistent picture and the results obtained are based on the best interaction energy values, which are similar for the most stable ligand conformation. The interaction energy values are −68.7, −56.4 and −48.5 kcal/mol for the eleutherobin **1**, the skeleton **1a** and the oxy-analog **3** synthesized, respectively. Thus, the synthesized model compound is expected to have significant binding propensity toward the β-tubulin. The cytotoxicity studies on HBC is shown in Table 1. Although no straightforward correlation exist between the tubulin polymerization and cytotoxic studies,[10] the modeling and the cytotoxicity studies indicate that the structural modification attempted does not lead to loss of activity.

The present study reports a novel structural analog for the eleutherobin skeleton, with a heteroatom substitution in its nine membered ring. Molecular modeling studies indicate that the skeleton obtained with the model is having comparable binding energy with the skeleton of the natural product. The retention of the biological activity of **3** is further supported by the cytotoxic studies on compound **3**. Thus, the combined

Table 1. Cytotoxicity of compound **3** on HBL-100 by MTT assay (human breast cancer cell lines)

Blank	DMSO control	Compound **3**	
		10 μg	100 μg
		0.162	0.095
0.426	0.214	0.170	0.079
0.403	0.262	0.157	0.075
0.265	0.234	0.154	0.073
0.286	0.188	0.148	0.084
0.276	0.195	0.138	0.076
0.270	0.218	0.161	0.078
0.266	0.234	0.135	0.088
0.310[a]	0.220[a]	0.153[a]	0.081[a]
		69.37[b]	36.69[b]
		30.62[c]	63.30[c]

[a] Average absorbance.
[b] %Viability values; %viability = (absorbance of the test/absorbance of control) × 100.
[c] %Inhibition values; %inhibition = 100 − %viability.

synthetic, modeling and biological studies show that the synthesized analog of eleutherobin is a viable strategy.

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