Sarcophine (1) is an epoxy compound isolated in good yields from the Red Sea soft coral Sarcophyton glaucom various biological activities. Most prominent are its antitumor effects. However, since the molecule is primarily a defense toxin for its soft coral, utilizing this lead compound for pharmacological use is conditioned by its toxicity profile that would hinder introducing it for clinical trials if not at safe levels. This study focuses on investigating the toxicity of the molecule following chemical modification of its epoxy functionality to obtain the corresponding derivative, 7(S,8R)-dihydroxydeepsarcophine (2) with optimization of the semisynthesis and purification of the derivative for higher yields and grade of purity against previously reported results. Biochemical assays are conducted with both molecules to assess the resulting biological effects following the structural modification. A cytotoxicology assay using human Embryonic Kidney 293 (HEK293) cells shows almost a four-fold decrease in toxicity with the semisynthetic derivative, asserting the role of the epoxy functionality as a toxopoxiphen. In addition, the effects of both molecules on the activity of the enzyme alcohol dehydrogenase (ADH) are also assessed in a kinetics study showing activation of the enzyme in a concentration dependent manner; and the differences observed with both molecules suggest the involvement of the epoxy functionality in the association and dissociation of sarcophine with the enzyme.

1. Extraction and Isolation

Sarcophine (1) was extracted from 1.5 Kg of fresh coral collected from the Red Sea. A total of 1.8 g of sarcophine were obtained. The melting point of the final product was 133-134°C (lit. 133-134°C). Spectral analysis confirmed the isolation of sarcophine.

2. Semisynthesis

Reaction conditions to cleave the epoxy functionality of sarcophine (1) (100 mg) were optimized. Modifications to the published method by Czarkie and co-workers (1985) resulted in a higher yield of the trans-diol derivative (2) with less elimination side products. TLC examinations of the reaction mixture revealed two additional compounds, not noted in literature, which are suggested to be for elimination and substitution side products. Purification of the major product (2), accomplished over a silica packed column using a Hexane: Ethyl acetate (3:1) mobile phase system. The melting point of (7S,8R)-dihydroxydeepsarcophine (2) was confirmed to be 162-164°C, which is 19°C higher than the value reported in earlier studies (lit. 145°C). Careful TLC examinations and various spectral data confirmed the purity and identity of the diol derivative.

3. Bioassays

3.1. Cytotoxicity assay (MTT Assay)

Human Embryonic Kidney 293 cells (HEK293) were plated in a 24-well culture plate for 24 hours with sarcophine (1) and (7S,8R)-dihydroxydeepsarcophine (2). The test compounds (1) and (2), were dissolved in DMSO and different concentrations were added to a 1% v/v. Triplicate determinations were made for each compound. Positive control was set treated with 1 µl of DMSO, and negative control was treated with 1 µl of DMSO. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (1) was added to each well and incubated for 1 hour after which formazan crystals were formed. The absorbance of the formazan color with different concentrations was determined spectrophotometrically at 490 nm and a VICTOR 3™ Multilabel Counter.

3.2. ADH Activity (Enzyme Kinetics)

The activity of the enzyme alcohol dehydrogenase (ADH) in the absence and presence of sarcophine (1) and the dial diol derivative (2) was measured spectrophotometrically as a function of the formation of NADH which absorbs light at 340 nm using a JENWAY 6305 spectrophotometer. Data were processed and plotted based on the Michaelis-Menten model for enzyme kinetics. The Vmax and Km values for the catalysis of ethanol by ADH the reaction mixtures were determined. Three solutions of the test compounds: (1) and (2), were prepared in DMSO and effects of different concentrations of both compounds were determined. The substrate concentration (ethanol) was maintained constant at 0.05, which is lower than the Km value. Triplicate determinations were made for all reactions.

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