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Antitumour Activity of Sphingoid Base Adducts of Phenethyl Isothiocyanate

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Abstract—*N*-(*N*′-Phenethylthiocarbamoyl) derivatives of sphingosine and sphinganine were prepared. They had antitumour activity: GC_{50} values of 0.64 ± 0.02 μM ($N = 18$) and 1.6 ± 0.01 μM ($N = 18$), respectively, with human leukaemia 60 cells in vitro. This antitumour effect may contribute to the suppression of carcinogenesis associated with dietary phenethyl isothiocyanate and sphingolipid bases. © 1999 Elsevier Science Ltd. All rights reserved.

Sphingosine-derived second messenger molecules coordinate signalling for cell proliferative and apoptosis responses.[1,2] Ceramide derivatives are of interest for development of therapeutic agents in the treatment of cancer, allergy and other diseases.[3] The balance of ceramide and sphingosine-1-phosphate signalling may be modified pharmacologically with synthetic ceramide derivatives, *N*-acetyl- *N*-butyryl- and *N*-hexanoyl-sphingosine, and by inhibitors of sphingosine kinase–*N*,*N*-dimethylsphingosine. Recent research has suggested that ceramides and ceramide metabolites derived from the diet may be inhibitors of colon carcinogenesis.[4]

Isothiocyanates, such as phenethyl isothiocyanate (PEITC), have recently been of intense interest for their anti-carcinogenic activities and potential use in the chemoprevention of cancer.[5] They also have anticancer activity in vitro. PEITC inhibited the growth of human leukaemia 60 (HL60) cells in vitro and induced apoptosis.[6] Sphingoid base adducts of PEITC may be functionally involved in the induction of apoptosis.

D-Sphingosine or DL-sphinganine (20 mM) and PEITC (20 mM) in methanol (0.8 ml) was incubated at 37°C for 2 days. ^{1}H NMR analysis indicated the formation of PETC-sphingosine (**1**) and PETC-sphinganine (**2**): the α-CH_2 of PEITC had a chemical shift of 2.97 ppm and changed to 2.87 ppm and 2.88 ppm in the PETC adducts, respectively. MALDI-MS gave M + 1/z values of 463 and 465, and the yields were 87 and 92%, respectively. These compounds, PEITC, sphingosine and sphinganine were evaluated for anti-tumour activity, determining the median growth inhibitory concentration GC_{50} and median toxic concentration TC_{50} values with human leukaemia (HL-60) cells in vitro as described.[6] The anti-proliferative effects of sphingosine and sphinganine[7,8] were confirmed. PETC-sphingosine was a more potent inhibitor of HL60 cell growth than either PEITC or sphingosine, whereas PETC-sphinganine was less potent than either PEITC or sphinganine. All the compounds had similar potency but PEITC lost anti-proliferative activity after 3 h in culture medium due to spontaneous hydrolysis to phenethylamine. PEITC and sphingoid bases have both been associated with the prevention of cancer (Table 1).[5,9] Antitumour effects may suppress the growth of pre-clinical tumours and make additional contributions to the well-established decreased cancer incidence associated with a vegetable-rich diet.[10] In PETC-sphingosine, the methylene-amide moiety –CH_2C=O–NH– found in ceramide derivatives has been replaced by a thiourea group –NH–C=S–NH–. These classical isosteric modifications may be used to readily produce ceramide analogues with different biological half-lives and pharmacological activities to ceramide itself. The sphinganine isomer Safingol (L-*threo*-dihydrosphingosine) is currently under clinical evaluation for antitumour activity.[11]

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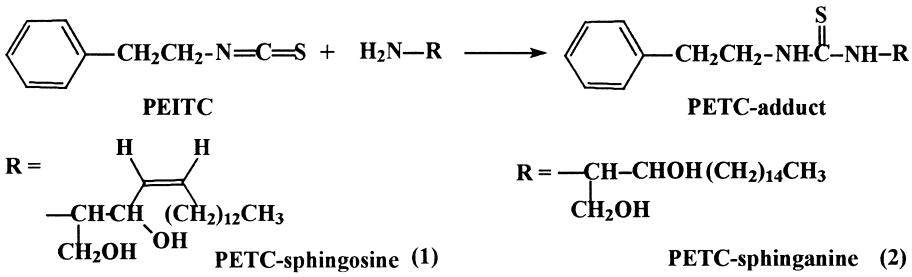

Table 1. Inhibition of human leukaemia 60 cell growth in vitro by PETC-sphingoid base derivatives

Compound	GC_{50} (μM) Mean ± S.D. (*N*)	TC_{50} (μM) Mean ± S.D. (*N*)
PEITC	0.84 ± 0.01 (18)	1.37 ± 0.02 (18)
PETC-sphingosine	0.64 ± 0.02 (18)	2.53 ± 0.07 (18)
PETC-sphinganine	1.60 ± 0.01 (18)	2.37 ± 0.17 (18)
Sphingosine	1.24 ± 0.01 (18)	2.42 ± 0.09 (18)
Sphinganine	0.49 ± 0.01 (18)	2.55 ± 0.01 (18)

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