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Synthesis and Cytotoxicity of New 3-Alkyl-1-(1-methyl-2-phenylethyl)ureas Related to Ceramide

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A series of new 3-alkyl-1-(1-methyl-2-phenylethyl)ureas related to ceramide was synthesized and evaluated for their *in vitro* cytotoxic activity against five human tumor cell lines. The urea analogue (**2b**) of B13 showed comparable or slightly more potent cytotoxic activity as compared to B13, indicating that urea does appear to serve as a bioisostere of amide.

Key words: Cytotoxic activity, Ceramide, B13, Urea

INTRODUCTION

Ceramide, a bioactive sphingolipid, has been recognized as a second messenger that regulates a variety of key biologic functions, such as cell growth, differentiation, transformation, and apoptosis (Obeid *et al.*, 1995; Jarvis *et al.*, 1996; Hannun, 1996; Kolesnick *et al.*, 1998; Perry *et al.*, 1998). Interest in this area has been stimulated recently by the disclosure that ceramide analogues induce apoptosis in tumor cells (Lucci *et al.*, 1999; Bieberich *et al.*, 2000; Senchenkov *et al.*, 2001; Radin *et al.*, 2001; Selzner *et al.*, 2001). Because the deficiency of apoptosis is one of the key elements in the proliferation and resistance of tumor cells to anticancer agents apoptosis-inducing ceramide analogues may serve as leads for the development of new therapeutic agents (Galve-Roperh *et al.*, 2000; Perry *et al.*, 2000). B13 (Fig. 1), a ceramide analogue possessing the phenyl group instead of the alkenyl chain and (1*R*,2*R*) configuration instead of (2*S*,3*R*) of natural ceramides (Bielawska *et al*, 1996) exhibited potent cytotoxic activity in human colon cancer cells with the induction of apoptotic cell death (Selzner *et al.*, 2001). Its cytotoxic activity is associated with the inhibition of ceramidase, a critical scavenging enzyme of ceramide, thereby leading to the accumulation of intracellular ceramide. Intracellular targets of ceramide are known to be ceramide-activated phosphatases and ceramide-activated kinases, which in turn activate the apoptotic pathways, SAPK/JNK and the caspase cascade (Haimovitz-Friedman *et al.*, 1997; Perry *et al.*, 1998). Interestingly, B13 displays selective toxicity toward malignant but not normal cells. Furthermore, it prevents colon cancer cells from metastasis to liver in a nude mouse model (Selzner *et al.*, 2001).

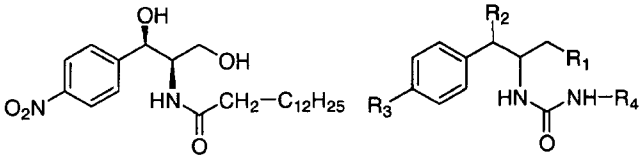

Fig. 1. Structure of B13 and 3-alkyl-(1-methyl-2-phenylethyl)ureas, **2a-2n**

In the present study, we synthesized and evaluated new B13 analogues, 3-alkyl-1-(1-methyl-2-phenylethyl)ureas and investigated the influence of the structural features including replacement of the amide group in B13 with the urea, the presence or the absence of *p*-nitro group at the phenyl ring and two hydroxyl groups at the backbone, stereochemistry at the backbone, and the length of the alkyl chain at urea moiety.

MATERIALS AND METHODS

^{1}H-NMR spectra were run at 300 MHz on a varian Gemini 2000 spectrometer, and chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane as

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internal standard. ^{1}H-NMR multiplicity data are denoted by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and b (broad). Fast atom bombardment mass spectra were recorded on an AutoSpec EBE spectrometer using glycerol as matrix. IR spectra were recorded using a Nicolet Magna 750 infrared spectrometer. Optical rotation was measured using a JASCO DIP-370 digital polarimeter. Silica gel 60 (230-400) mesh was used for column chromatography, and analytical thin-layer chromatography was conducted using Kieselgel 60 F_{254} plates. Solvents and reagents were obtained from commercial sources and used without further purification.

General procedure for the synthesis of 3-alkyl-1-(methyl-2-phenylethyl)ureas (2a-2n)

To a solution of an amine (1.2 mmol in a 1:1 mixture of ethanol/$CHCl_3$ (50 mL) was added dropwise isocyanate (1.4 mmol) over a period of 4 h at room temperature. The mixture was diluted with ethyl acetate (200 mL), washed with 5% citric acid (400 mL) four times, dried over Na_2SO_4, and filtered. The filtrate was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography eluting with dichloromethane to afford 3-alkyl-1-(methyl-2-phenylethyl)ureas (**2a-2n**) as a white solid.

(1*R*,2*R*) 3-Octyl-1-(2-hydroxy-1-hydroxymethyl-2-(4-nitrophenyl)ethyl)urea (2a)

Yield 53%; white powder. R_f = 0.39 (ethyl acetate); $[\alpha]_D^{20}$ -52.09 (*c* 0.1, CH_3OH); IR (KBr) 1610, 1540 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 0.65 (t, *J* = 7.2 Hz, 3H), 0.84-1.17 (m, 12H), 2.59-2.73 (m, 2H), 3.33 (dd, *J* = 10.8, 5.7 Hz, 1H), 3.45 (dd, *J* = 10.8, 7.9 Hz, 1H), 3.62-3.76 (m, 1H), 4.87 (broad s, 1H) 5.61 (broad s, 1H), 5.64 (broad s, 1H), 7.39 (d, *J* = 9.0 Hz, 2H), 7.94 (d, *J* = 8.4 Hz, 2H); FABMS *m/z* 368 $(M+H)^+$.

(1*R*,2*R*) 3-Dodecyl-1-(2-hydroxy-1-hydroxymethyl-2-(4-nitroophenyl)ethyl)urea (2b)

Yield 80%; white powder. R_f = 0.14 (ethyl acetate); $[\alpha]_D^{20}$ -50.98 (*c* 0.4, CH_3OH); IR (KBr) 1620, 1550 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 0.90 (t, *J* = 6.9 Hz, 3H), 1.38-1.17 (m, 20H), 2.84-3.01 (m, 2H), 3.57 (dd, *J* = 10.5, 5.7 Hz, 1H), 3.70 (dd, *J* = 10.8, 7.8 Hz, 1H), 3.87-3.96 (m, 1H), 5.12 (d, *J* = 2.1 Hz, 1H) 5.81 (broad s, 2H), 7.64 (d, *J* = 9.0 Hz, 2H), 8.18 (d, *J* = 8.7 Hz, 2H); FABMS *m/z* 424 $(M+H)^+$.

(1*S*,2*S*) 3-Dodecyl-1-(2-hydroxy-1-hydroxymethyl-2-(4-nitroophenyl)ethyl)urea (2c)

Yield 75%; white powder. R_f = 0.12 (dichloromethane: methanol=9:1); $[\alpha]_D^{20}$ +52.15 (*c* 0.3, CH_3OH); IR (KBr) 1610, 1540 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 0.92 (t, *J* = 6.9 Hz, 3H), 1.21-1.41 (m, 20H), 2.84-3.04 (m, 2H), 3.60 (dd, *J* = 11.4, 5.7 Hz, 1H), 3.72 (dd, *J* = 11.4, 7.8 Hz, 1H), 3.89-3.98 (m, 1H), 5.14 (d, *J* = 1.8 Hz, 1H) 5.94 (broad s, 2H), 7.67 (d, *J* = 8.7 Hz, 2H), 8.20 (d, *J* = 8.7 Hz, 2H); FABMS *m/z* 424 $(M+H)^+$.

(1*R*,2*R*) 3-Octyl-1-(2-hydroxy-1-hydroxymethyl-2-phenylethyl)urea (2d)

Yield 76%; white powder. R_f = 0.21 (ethyl acetate); $[\alpha]_D^{20}$ -88.00 (*c* 0.1, CH_3OH); IR (KBr) 1620, 1540 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 0.73 (t, *J* = 6.9 Hz, 3H), 1.09-1.19 (m, 10H), 1.19-1.30 (m, 2H), 2.91 (t, *J* = 7.2 Hz, 2H), 3.42 (dd, *J* = 11.1, 5.4 Hz, 1H), 3.49 (dd, *J* = 11.1, 4.8 Hz, 1H), 3.62-3.78 (m, 1H), 4.72 (d, *J* = 5.4 Hz, 1H), 6.01 (broad s, 2H), 7.02-7.31 (m, 5H); FABMS *m/z* 323 $(M+H)^+$.

(1*R*,2*R*) 3-Dodecyl-1-(2-hydroxy-1-hydroxymethyl-2-phenylethyl)urea (2e)

Yield 75%; white powder. R_f = 0.63 (dichloromethane: methanol=9:1); $[\alpha]_D^{20}$ -51.43 (*c* 0.1, CH_3OH); IR (KBr) 1640, 1540 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 0.8 (t, *J* = 7.2 Hz, 3H), 1.10-1.21 (m, 18H), 1.24-1.38 (m, 2H), 3.01 (t, *J* = 7.2 Hz, 2H), 3.52 (dd, *J* = 11.1, 5.1 Hz, 1H), 3.6 (dd, *J* = 11.1, 4.2 Hz, 1H), 3.73-3.81 (m, 1H), 4.80 (d, *J* = 5.4 Hz, 1H), 5.73 (broad s, 2H), 7.20-7.90 (m, 5H); FABMS *m/z* 379 $(M+H)^+$.

(1*S*,2*S*) 3-Octyl-1-(2-hydroxy-1-hydroxymethyl-2-phenylethyl)urea (2f)

Yield 75%; white powder. R_f = 0.32 (dichloromethane: methanol=20:1); $[\alpha]_D^{20}$ +92.50 (*c* 0.1, CH_3OH); IR (KBr) 1625, 1540 cm–^{1}; ^{1}H-NMR (CD_3OD) δ 0.93 (t, *J* = 6.6 Hz, 3H), 1.21-1.40 (m, 10H), 1.40-1.52 (m, 2H), 3.03 (t, *J* = 7.2 Hz, 2H), 3.52 (dd, *J* = 10.8, 5.4 Hz, 1H), 3.69 (dd, *J* = 8.4, 7.2 Hz, 1H), 3.84-3.92 (m, 1H), 4.97 (d, *J* = 3.3 Hz, 1H), 5.91 (broad s, 2H), 7.21-7.45 (m, 5H); FABMS *m/z* 323 $(M+H)^+$.

(1*S*,2*S*) 3-Dodecyl-1-(2-hydroxy-1-hydroxymethyl-2-phenylethyl)urea (2g)

Yield 40%; white powder. R_f = 0.36 (dichloromethane: methanol=9:1); $[\alpha]_D^{20}$ +49.18 (*c* 0.1, CH_3OH); IR (KBr) 1620, 1550 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 0.81 (t, *J* = 7.2 Hz, 3H), 1.15-1.30 (m, 18H), 1.30-1.39 (m, 2H), 3.00 (t, *J* = 6.9Hz, 2H), 3.53 (dd, *J* = 11.4, 5.7 Hz, 1H), 3.65 (dd, *J* = 11.4, 4.5 Hz, 1H), 3.72-3.82 (m, 1H), 4.44 (d, *J* = 5.4 Hz, 1H), 5.79 (broad s, 2H), 7.19-7.36 (m, 5H); FABMS *m/z* 379 $(M+H)^+$.

(1*S*,2*R*) 3-Octyl-1-(2-hydroxy-1-methyl-2-phenylethyl) urea (2h)

Yield 45%; white powder. R_f = 0.20 (*n*-hexane:ethyl acetate =1:2); $[\alpha]_D^{20}$ -19.45 (*c* 0.4, CH_3OH); IR (KBr) 1630, 1560 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 0.92 (t, *J* = 7.2 Hz, 3H), 0.98 (d, *J* = 6.6 Hz, 3H), 1.26-1.37 (m, 10H), 1.37-1.51 (m, 2H),

3.10 (t, J = 6.9 Hz, 2H), 3.92-4.06 (m, 1H), 4.73 (d, J = 3.9 Hz, 1H), 5.52 (broad s, 2H), 7.19-7.40 (m, 5H); FABMS *m/z* 307 $(M+H)^+$.

(1*S*,2*R*) 3-Dodecyl-1-(2-hydroxy-1-methyl-2-phenylethyl) urea (2i)

Yield 76%; white powder. R_f = 0.46 (*n*-hexane:ethyl acetate =2:1); $[\alpha]_D^{20}$ -27.27 (*c* 0.1, CH_3OH); IR (KBr) 1620, 1550 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 0.84 (t, J = 6.9 Hz, 3H), 0.92 (d, J = 7.2 Hz, 3H), 1.18-1.30 (m, 18H), 1.39-1.46 (m, 2H), 3.10 (t, J = 7.5 Hz, 2H), 4.05-4.15 (m, 1H), 4.71 (d, J = 2.4 Hz, 1H), 5.83 (broad s, 2H), 7.09-7.29 (m, 5H); FABMS *m/z* 363 $(M+H)^+$.

(1*R*,2*S*) 3-Octyl-1-(2-hydroxy-1-methyl-2-phenylethyl) urea (2j)

Yield 49%; white powder. R_f = 0.40 (*n*-hexane:ethyl acetate =1:3); $[\alpha]_D^{20}$ +6.15 (*c* 0.1, CH_3OH); IR (KBr) 1620, 1540 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 0.90 (t, J = 6.9 Hz, 3H), 0.96 (d, J = 6.6 Hz, 3H), 1.26-1.38 (m, 10H), 1.39-1.46 (m, 2H), 3.08 (t, J = 6.9 Hz, 2H), 3.92-4.02 (m, 1H), 4.72 (d, J = 3.9 Hz, 1H), 5.91 (broad s, 2H), 7.19-7.39 (m, 5H); FABMS *m/z* 307 $(M+H)^+$.

(1*R*,2*S*) 3-Dodecyl-1-(2-hydroxy-1-methyl-2-phenylethyl) urea (2k)

Yield 78%; white powder. R_f = 0.46 (*n*-hexane:ethyl acetate =1:1; $[\alpha]_D^{20}$ +15.81 (*c* 0.2, CH_3OH); IR (KBr) 1610, 1540 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 0.88 (t, J = 6.9 Hz, 3H), 0.93 (d, J = 6.9 Hz, 3H), 1.18-1.37 (m, 18H), 1.38-1.45 (m, 2H), 3.06 (t, J = 6.9 Hz, 2H), 3.93-4.01 (m, 1H), 4.69 (d, J = 4.2 Hz, 1H), 6.01 (broad s, 2H), 7.19-7.38 (m, 5H); FABMS *m/z* 363 $(M+H)^+$.

(*S*)-3-Octyl-1-(1-hydroxymethyl-2-phenylethyl)urea (2l)

Yield 70%; white powder. R_f = 0.34 (dichloromethane: methanol=20:1); $[\alpha]_D^{20}$ -40.26 (*c* 0.1, CH_3OH); IR (KBr) 1620, 1550 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 0.93 (t, J = 6.9 Hz, 3H), 1.24-1.40 (m, 10H), 1.40-1.50 (m, 2H), 2.74 (dd, J = 13.0, 7.8 Hz, 1H), 2.90 (dd, J = 13.8, 6.6 Hz, 1H), 3.08 (t, J = 7.2 Hz, 2H), 3.51 (d, J = 5.1 Hz, 2H), 3.89-3.98 (m, 1H), 5.82 (broad s, 2H), 7.19-7.30 (m, 5H); FABMS *m/z* 307 $(M+H)^+$.

(*S*)-3-Dodecyl-1-(1-hydroxymethyl-2-phenylethyl)urea (2m)

Yield 71%; white powder. R_f = 0.30 (dichloromethane: methanol=20:1); $[\alpha]_D^{20}$ -26.30 (*c* 0.1, CH_3OH); IR (KBr) 1610, 1550 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 0.89 (t, J = 6.9 Hz, 3H), 1.21-1.35 (m, 18H), 1.35-1.42 (m, 2H), 2.71 (dd, J = 13.8, 7.8 Hz, 1H), 2.88 (dd, J = 13.5, 6.3 Hz, 1H), 3.04 (t, J = 6.9 Hz, 2H), 3.48 (d, J = 4.8 Hz, 2H), 3.85-3.91 (m, 1H), 5.69 (broad s, 2H), 7.14-7.30 (m, 5H); FABMS *m/z* 363 $(M+H)^+$.

(*R*)-3-Dodecyl-1-(1-hydroxymethyl-2-phenylethyl)urea (2n)

Yield 68%; white powder. R_f = 0.30 (dichloromethane: methanol=20:1); $[\alpha]_D^{20}$ = +28.00 (*c* 0.1, CH_3OH); IR (KBr) 1620, 1540 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 0.89 (t, J = 7.2 Hz, 3H), 1.21-1.35 (m, 18H), 1.35-1.42 (m, 2H), 2.71 (dd, J = 13.8, 8.1 Hz, 1H), 2.87 (dd, J = 13.8, 6.3 Hz, 1H), 3.04 (t, J = 6.9 Hz, 2H), 3.48 (d, 2H, J = 4.8 Hz), 3.85-3.91 (m, 1H), 6.01 (broad s, 2H), 7.16-7.27 (m, 5H); FABMS *m/z* 363 $(M+H)^+$

Cytotoxicity assay

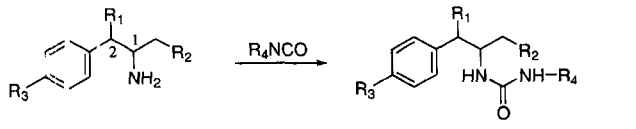
The compounds were tested for their cytotoxic activity on the following human tumor cell lines: colon cancer HT-29, renal cancer Caki-2, lung cancer A549, prostate cancer PC-3, and leukemic cancer HL-60. The cytotoxicity assay was performed by a modification of the MTT method (Everitt *et al.*, 1987; Skehon *et al.*, 1990). Briefly, the cells were plated at a density of approximately 1×10^4 cells/well in 96-well flat-bottomed microplates, and after 24 h the fractions to be tested were added, appropriately dissolved in DMSO and diluted with PBS. After 96 h incubation, the medium was removed and 50 mL of MTT solution (1 mg/mL) was added. After 4 h of incubation, the MTT-formazan was solubilized in DMSO, and the optical density was measured with a microplate analyzer at a wavelength of 570 nm. The results represent the mean of three independent experiments and are expressed as IC_{50}, the concentration that reduced by 50% the optical density of treated cells with respect to untreated controls.

RESULTS AND DISCUSSION

The preparation of 3-alkyl-1-(1-methyl-2-phenylethyl)ureas **2a-2n** was performed by the addition of the required amines **1a-1h** to octylisocyanate or dodecylisocyanate in 40-80% yields (Scheme 1).

The synthesized compounds **2a-2n** and B13 were evaluated for cytotoxic activity in five human tumor cell lines including HT-29 (human colon cancer), Caki-2 (human renal cancer), A549 (human lung cancer), PC-3 (human prostate cancer), and HL-60 (human leukemia). To get an idea of relative potency of compounds in this series, the averages of the IC_{50} values are used and the data are presented in Table I.

B13 showed a modest cytotoxicity at the value of IC_{50} = 28 μM against HL-60 leukemic cell under our assay conditions, while it showed weak activity against other four tumor cells. The urea analogue (**2b**) of B13 showed comparable or slightly more potent cytotoxicity as compared to B13, suggesting that urea group would serve as a



1	Config.	R_1	R_2	R_3
a	1*R*,2*R*	OH	OH	NO_2
b	1*S*,2*S*	OH	OH	NO_2
c	1*R*,2*R*	OH	OH	H
d	1*S*,2*S*	OH	OH	H
e	1*S*,2*R*	OH	H	H
f	1*R*,2*S*	OH	H	H
g	*S*	H	OH	H
h	*R*	H	OH	H

2	Config.	R_1	R_2	R_3	R_4	Yield(%)
a	1*R*,2*R*	OH	OH	NO_2	C_8H_{17}	53
b	1*R*,2*R*	OH	OH	NO_2	$C_{12}H_{25}$	80
c	1*S*,2*S*	OH	OH	NO_2	$C_{12}H_{25}$	75
d	1*R*,2*R*	OH	OH	H	C_8H_{17}	76
e	1*R*,2*R*	OH	OH	H	$C_{12}H_{25}$	75
f	1*S*,2*S*	OH	OH	H	C_8H_{17}	75
g	1*S*,2*S*	OH	OH	H	$C_{12}H_{25}$	40
h	1*S*,2*R*	OH	H	H	C_8H_{17}	45
i	1*S*,2*R*	OH	H	H	$C_{12}H_{25}$	76
j	1*R*,2*S*	OH	H	H	C_8H_{17}	49
k	1*R*,2*S*	OH	H	H	$C_{12}H_{25}$	78
l	*S*	H	OH	H	C_8H_{17}	70
m	*S*	H	OH	H	$C_{12}H_{25}$	71
n	*R*	H	OH	H	$C_{12}H_{25}$	68

Scheme 1. Preparation of 3-alkyl-1-(1-methyl-2-phenylethyl)ureas

Table 1. *In vitro* cytotoxicity of 3-alkyl-1-(1-methyl-2-phenylethyl) ureas

Compound	Cytotoxicity[1] (IC_{50}[2,3], μM)				
	Caki-2	HT-29	A549	PC-3	HL-60
B13	109	96	64	83	28
2a	198	89	145	150	136
2b	80	55	60	36	30
2c	61	50	45	40	30
2d	>200	139	136	190	148
2e	>200	180	>200	>200	>200
2f	>200	>200	>200	>200	154
2g	>200	170	>200	>200	>200
2h	>200	138	174	>200	94
2i	93	39	36	171	126
2j	>200	163	191	>200	108
2k	79	76	78	>200	101
2l	>200	>200	>200	>200	94
2m	>200	>200	>200	>200	>200
2n	>200	103	>200	>200	>200

[1]Cancer cell lines: Caki-2, human renal cancer; HT-29, human colon cancer; A549, human lung cancer; PC-3, human prostate cancer; HL-60, human leukemia. [2]Mean values of 3 experiments. SD<12% of the mean value. [3]IC_{50}, values were defined by the concentration that reduced by 50% the optical density of treated cells with respect to untreated controls.

suitable replacement for the amide group. This could prove valuable in the further design of novel ceramide analogues since urea group is generally more water-soluble and stable to amidase hydrolysis than amide group (Ng *et al.*, 1999).

Elimination of *p*-nitro group (**2e**) at the phenyl ring of **2b** resulted in a more than 3-fold decrease in cytotoxicity. Comparing the cytotoxicities of compounds having octyl or dodecyl group in the urea moiety (**2a** *vs* **2b**, **2h** *vs* **2i**, and **2j** *vs* **2k**), dodecyl analogues (**2b**, **2i**, **2k**) were significantly more active than octyl analogues (**2a**, **2h**, **2j**), consistent with the reported trend that the biological activity augments with increasing acyl chain length of ceramide (Chang *et al.*, 2002; Bieberich *et al.*, 2002). Other octyl analogues (**2d**, **2f**, **2l**) having different molecular frame and corresponding dodecyl analogues (**2e**, **2g**, **2m**) could not be compared because they were relatively inactive.

The stereochemistry of the backbone may not be essential for a potent cytotoxic activity, although not conclusive yet, as the enantiomers of B13's urea analogues **2b** (1*R*,2*R*) and **2c** (1*S*,2*S*) displayed similar activity. Two enantiomers of 2-hydroxy-1-methyls **2k** (1*R*,2*S*) and **2i** (1*S*,2*R*) also exhibited similar activity throughout. On the other hand, the enantiomers of unsubstituted phenyl ring **2e** (1*R*,2*R*) and **2g** (1*S*,2*S*) and two entiomeric 1-hydroxymethyls **2m** (*S*) and **2n** (*R*) were found to be inactive, therefore making meaningful comparison difficult.

The influence of two hydroxyl groups in B13 analogues is still not clear. It was difficult to compare the activity of diols (**2a-2g**) *vs* the corresponding 2-hydroxy-1-methyls (**2h-2k**) and 1-hydroxymethyls (**2l-2n**) because the data set shown in Table I is not complete and the most of the comparable compounds were inactive. Synthesis of additional analogues is in progress.

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REFERENCES

Bielawska, A., Greensberg, M., Perry, D., Jayadev, S., Shayman, J. A., McKay, C., and Hannun, Y. A., (1*S*,2*R*)-D-Erythro-2-(*N*-myristolamino)-1-phenyl-1-propanol as an inhibitor of ceramidase. *J. Biol. Chem.*, 271, 12646-12654 (1996).

Bieberich, E., Kawaguchi, T., and Yu, R. K., *N*-acylated serinol is a novel ceramide mimic inducing apoptosis in neuroblastoma cells. *J. Biol. Chem.*, 275, 177-181 (2000).

Bieberich, E., Hu, B., Silva, J., MacKinnon, S., Yu, R. K., Fillmore, H., Broaddus, W. C., and Ottenbrite, R. M., Synthesis and characterization of novel ceramide analogs for induction of apoptosis in human cancer cells. *Cancer Letters*, 181, 55-64 (2002).

Chang, Y, -T., Choi, J., Ding, S., Prieschl, E. E., Baumruker, T., Lee, J. M, Chung, S. K., and Schultz, P. G., The synthesis and biological characterization of a ceramide library. *J. Am. Chem. Soc.*, 124, 1856-1857 (2002).

Everitt, E. and Wohlfart, C., Spectrometric quantitation of anchorage-dependent cell numbers extraction of naphthol blue-black-stained cellular protein. *Anal. Biochem.*, 162, 122-129 (1987).

Galve-Roperh, I., Sanchez, C., Cortes, M. L., Del Pulgar, T. G., Izquierdo, M., and Guzman, M., Anti-tumoral action of cannabinoids: Involvement of sustained ceramide accumula-

tion and extracellular signal-regulated kinase activation. *Nat. Med.* 6, 313-319 (2000).

Haimovitz-Friedman, A., Kolesnick, R. N., and Fuks, Z., Ceramide signaling in apoptosis. *Br. Med. Bull.*, 53, 539-553 (1997).

Hannun, Y., Functions of ceramide in coordination cellular response to stress. *Science* (Washington DC), 274, 18-1859 (1996).

Jarvis, W., Grant, S., and Kolesnick, R. N., Ceramide and the induction of apoptosis. *Clin. Cancer Res.*, 2, 1-6 (1996).

Kolesnick, R. N. and Kronke, M., Regulation of ceramide production and apoptosis. *Annu. Rev. Physiol.,* 60, 643-665 (1998).

Lucci, A., Han, T. Y., Liu, Y. Y., Giuliano, A. E., and Cabot, M. C., Modification of ceramide metabolism increases cancer cell sensitivity to cytotoxics. *Int. J. Oncol.*, 15, 541-546 (1999).

Ng, E. W., Aung, M. M., Abood, M. E., Martin, B. R. and Razdan, R. K., Unique Analogues of Anandamide: Arachidonyl Ethers and Carbamates and Norarachidonyl Carbamates and Ureas. *J. Med. Chem.*, 42, 19751981 (1999).

Obeid, L. and Hannun, Y., Ceramide. A stress signal and mediator of growth suppression and apoptosis. *J. Biol. Chem.*, 58, 191-198 (1995).

Perry, D. K. and Hannun, Y. A., The role of ceramide in cell signaling. *Biochem. Biophys. Acta.*, 1436, 233-243 (1998).

Perry, D. K., Carton, J., Shah, A. K., Meredith, F., Uhlinger, D. J., and Hannun, Y. A., Serine palmitoyltransferase regulates de novo ceramide generation during etoposide-induced apoptosis. *J. Biol. Chem.*, 275, 9078-9084 (2000).

Radin, N. S., Killing cancer cells by poly-drug elevation of ceramide levels: a hypothesis whose time has come? *Eur. J. Biochem.*, 268, 193-204 (2001).

Selzner, M., Bielawska, A., Morse, M. A., Rudiger, H. A., Sindram, D., Hannun, Y. A., and Clavien, P. A., Induction of apoptotic cell death and prevention of tumor growth by ceramide analogues in metastatic human colon cancer. *Cancer Res.*, 61, 1233-1240 (2001).

Senchenkov, A., Litvak, D. A., and Cabot, M. C., Targeting ceramide metabolism a strategy for overcoming drug resistance. *J. Natl Cancer Inst.,* 93, 347-357 (2001).

Skehan, P., Storeng, R., Scudiero, D. A., Monks, A., MacMahon, J., Vista, D. T., Kenny, S., and Boyd, M. R., New colorimetric cytotoxicity assay for anticancer drug screening. *J. Natl. Cancer Inst.*, 82, 1107-1112 (1990).