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Short communication

Synthesis and antiproliferative activity of novel sugiol β-amino alcohol analogs

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Abstract

A series of β-amino alcohol analogs of sugiol were synthesized in a straightforward manner. The in vitro antiproliferative activities were examined in the human solid tumor cell lines A2780, SW1573 and WiDr. The most potent analogs induced considerably growth inhibition in the range 1.5–6.7 μM. The results showed that β-amino alcohol analogs are more potent than the parent compound. In addition, the derivatives with secondary amine fragments showed more active than those bearing tertiary amines.

Keywords: Sugiol; Solid tumors; Anticancer drugs; Drug design; Structure–activity relationship

1. Introduction

From the chemical point of view, the plants of the genus *Salvia* have been subjected to intensive studies focused on the isolation of several types of secondary metabolites such as sterols, flavonoids sesquiterpenes, diterpenes and triterpenes [1]. A number of these natural products have shown interesting biological activities, including anti-inflammatory [2], antimalarial [3], antileukemic [4], antiviral [5] and antimicrobial [6]. In particular, sugiol (**1**) is an abietane diterpene isolated from several plants, including *Salvia* [7]. This natural product has been proposed as an interesting starting material for the synthesis of new compounds with different biological properties [8]. In a recent study, sugiol (**1**) was reported to exhibit modest growth inhibitory activity below 166 μM (the exact value was not reported) against human breast, lung and colon cancer cell lines [9]. In addition, sugiol (**1**) does not present hemolytic activity [10].

In a previous work, a set of antiplasmodial β-amino alcohol analogs of totarol (**2**) (Fig. 1) showed cytotoxic activity against chinese hamster ovarian cells [11]. The reported β-amino alcohol analogs were prepared exclusively from secondary amines. Due to our interest in the development of anticancer compounds, we explored the possibility as antiproliferative agents of β-amino alcohol analogs of sugiol, which were derived from commercially available primary and secondary amines.

We report herein on the synthesis of a series of β-amino alcohol analogs of sugiol (**1**). The antiproliferative profile of the obtained derivatives was evaluated in vitro against a panel of three human solid tumor cell lines: A2780 (ovarian cancer),

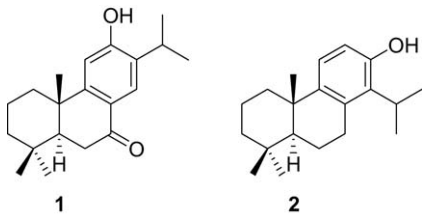

Fig. 1. Chemical structures of the natural products sugiol (**1**) and totarol (**2**).

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SW1573 (non-small cell lung cancer, NSCLC) and WiDr (colon cancer).

2. Results and discussion

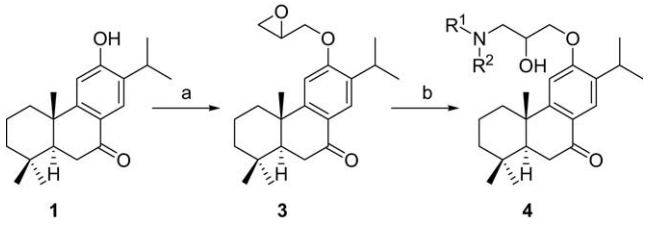
2.1. Chemistry

Our strategy to synthesize β-amino alcohol analogs of sugiol was based on a previous preparation of antiplasmodial totarol derivatives [11] Scheme 1 outlines the general synthetic pathway. The starting material sugiol (**1**) [8] was treated with sodium hydride and then racemic (±)-epibromohydrin was added. The resulting epoxide **3** was obtained in high yields (93%). The epoxide ring opening of **3** was performed with a set of diverse commercially available primary and secondary amines. With this strategy we obtained the corresponding derivatives **4a**–**k** in yields ranging 55–86% (Table 1).

2.2. Drug lipophilicity

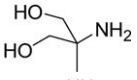
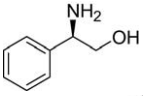
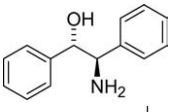
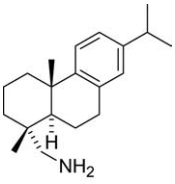
The lipophilicities of the compounds described in this study were calculated to correlate their values with the antiproliferative activity. Lipophilicity is given as ClogP and the values (Table 2) were calculated using the computer program ClogP®. This program is designed to determine the partition coefficient of the non-ionized form of a given compound. In a recent comparative study ClogP® appeared the most accurate predictor of ClogP values [12].

The ClogP values obtained for sugiol (**1**) and its derivatives were in the range 4.93–11.95. Compounds **4a** and **4f**, which showed large ClogP values (>10), were not soluble enough in DMSO to go through the biological experiments.



Scheme 1. Reagents and conditions: (a) i. NaH, DMF, 0 °C; ii. (±)-epibromohydrin, 55 °C, 20 h, 93%; (b) R^1R^2NH, MeOH, 70 °C, 20 h.

Table 1
β-Amino alcohol analogs of sugiol (**1**)

Compound	Amine (R^1R^2NH)	Yield (%)
4a	n-$C_{12}H_{25}NH_2$	80
4b	$PhCH_2CH_2NH_2$	85
4c		86
4d		68
4e		62
4f		55
4g	Bn_2NH	75
4h	$(n\text{-}C_5H_{11})_2NH$	69
4i	$(c\text{-}C_6H_{11})_2NH$	68
4j	Morpholine	82
4k	Adenine	71

2.3. Chemosensitivity testing

We screened growth inhibition and cytotoxicity against A2780, WiDr and SW1573 cancer cells after 48 h of exposure using the sulforhodamine B (SRB) assay [13,14]. In this method, for each drug a dose–response curve is generated. The effect is defined as percentage of growth (PG), where 50% growth inhibition (GI_{50}), represents the drug concentration at which PG is +50 [15]. The sensitivities expressed as GI_{50} are listed in Table 2.

From the results, it appears that the in vitro biological activity of these sugiol analogs does not correlate with the calculated ClogP values. Although the series of β-amino alcohols synthesized is relatively small, a number of interesting preliminary generalizations can be made on the basis of the data presented. All analogs were more active than the parent compound sugiol (**1**). This effect was observed against all cell lines. The most potent derivatives were **4b**–**e**, which were obtained from primary amines. Their antiproliferative activity was similar in the three cell lines, showing GI_{50} values within the range 1.5–6.7 μM (Table 2).

The derivatives **4h**–**k** that were prepared from secondary amines showed a decreased activity against the A2780 and the SW1573 cell lines. Interestingly, these compounds were more potent against WiDr cells and the activity against colon cancer cells was comparable to that of the analogs **4b**–**e**. This

Table 2
Lipophilicity and in vitro antiproliferative activity of sugiol (**1**) and its derivatives against human solid tumor cells [a]

Compound	ClogP	A2780 (ovarian cancer)	SW1573 (non-small cell lung cancer)	WiDr (colon cancer)
1	6.17	> 50	> 50	23 (± 4.6)
3	5.93	15 (± 1.3)	18 (± 1.8)	17 (± 5.4)
4a	10.86	nt	nt	nt
4b	7.14	2.4 (± 0.8)	2.4 (± 0.8)	1.6 (± 0.2)
4c	4.93	6.4 (± 3.5)	6.7 (± 3.7)	2.0 (± 1.4)
4d	6.14	1.8 (± 0.3)	2.3 (± 0.5)	1.5 (± 0.2)
4e	7.71	2.2 (± 0.7)	3.0 (± 0.8)	1.6 (± 0.1)
4f	11.95	nt	nt	nt
4g	9.21	> 100	91	> 100
4h	9.87	13 (± 6.6)	24 (± 2.4)	3.0 (± 1.9)
4i	9.70	17 (± 4.9)	26 (± 5.2)	4.4 (± 2.3)
4j	5.62	17 (± 2.3)	17 (± 7.3)	2.8 (± 0.2)
4k	4.80	11 (± 7.2)	21 (± 2.7)	2.2 (± 0.1)

[a] Values are given in μM and are means of two to four experiments, standard deviation is given in parentheses (nt = not tested).

is a surprising effect, since colon cancer cells are more drug resistant than ovarian cancer cells to conventional anticancer drugs [16].

With the exception of compound **4g** the results indicate that the nature of the functional group on the amino side chain is not relevant for the activity. Although the mechanism of action of these compounds remains unclear, the results suggest a significant role of the β-amino alcohol fragment in the enhancement of the antiproliferative activity of sugiol (**1**).

3. Conclusions

We have prepared a series of β-amino alcohol analogs of sugiol (**1**) in a straightforward manner. Although preliminary, the in vitro experiments show that the analogs containing secondary amine fragments induce considerable growth inhibition in a panel of three diverse human solid tumor cells. Based on these results, it is anticipated that these compounds will be active against both sensitive and resistant solid tumors. In addition, the wide range of amines commercially and readily available presents opportunities for potential generation of diverse libraries of novel sugiol derived β-amino alcohols.

In conclusion, β-amino alcohol analogs of sugiol (**1**) appear as good lead molecules for the development of new antitumor agents.

4. Materials and methods

4.1. General

All reagents and solvents used were obtained from commercially available sources and used with no further purification. Sugiol (**1**) was obtained from ferruginol according to literature procedures [8]. Reactions were monitored by thin-layer chromatography carried out on 0.25 mm Schleicher & Schuell plates (G-1510/LS 254) using UV light as visualizing agent and sprayed with 7% ethanolic phosphomolybdic acid followed by heating. Flash chromatography was performed using Merck silica gel 60 (0.04–0.063 mm) with solvents distilled prior to use. FTIR spectra were recorded on Perkin-Elmer 781 spectrophotometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter and melting points were determined on a Buchi 535 apparatus and are uncorrected. NMR spectra were recorded on a Bruker AM400 instrument operating at 400 MHz. All chemical shifts are reported in ppm relative to $CDCl_3$ with TMS as an internal reference. High resolution mass spectra (HRMS) were recorded on a VG Micromass ZAB-2F mass spectrometer operating at 70 eV.

4.2. Compound 3: 12-(2-oxiranylmethoxy)abieta-8,11,13-trien-7-one (3)

To a solution of sugiol (**1**) (54 mg, 0.18 mmol) in DMF (1 ml) under nitrogen and at 0 °C, were sequentially added NaH (80%, 59 mg, 0.36 mmol) and (±)-epibromohydrin (38 μl, 0.45 mmol). The reaction mixture was heated at 55 °C and stirred for 20 h. After cooling down to room temperature the excess of (±)-epibromohydrin was removed in vacuo. Then, the residue was extracted with ethyl acetate, washed with water, and the combined organic layers were dried over anhydrous sodium sulfate, and the solvent was evaporated to dryness. The resulting crude was dissolved in dichloromethane and purified by column chromatography using EtOAc/hexane (2:8) as eluent to give compound **3** as a white solid (60 mg, 93% yield). m.p. 115–116 °C. IR (NaCl) 2960, 1672, 1600, 1561, 1459, 1260, 756 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.90 (3H, s), 1.00 (3H, s), 1.24 (9H, brs), 1.30 (1H, m), 1.55 (2H, m), 1.70 (1H, brs), 1,78 (1H, brs, $J = 13.8$ Hz), 1.87 (1H, dd, $J_1 = 4.3$ Hz, $J_2 = 13.4$ Hz), 2.28 (1H, d, $J = 11.9$ Hz) 2.62 (1H, dd, $J_1 = 13.6$ Hz, $J_2 = 17.8$ Hz), 2.69 (1H, dd, $J_1 = 3.7$ Hz, $J_2 = 17.8$ Hz), 2.81 (1H, d, $J = 2.0$), 2.94 (1H, t, $J = 4.3$ Hz), 3.28 (1H, heptet, $J = 7.0$ Hz), 3.39 (1H, brs), 4.05 (1H, dd, $J_1 = 5.4$ Hz, $J_2 = 10.9$ Hz), 4.34 (1H, d, $J = 10.9$ Hz), 6.77 (1H, s), 7.90 (1H, s). ^{13}C RMN ($CDCl_3$) δ 18.9, 21.3, 22.3, 22.4, 23.2, 26.7, 32.5, 33.2, 36.0, 37.9, 38.2, 41.3, 44.5, 49.6, 50.1, 68.7, 105.6, 124.5, 125.8, 135.3, 156.3, 160.4, 198.4. EIMS *m/z* 356 $[M]^+$ (100), 341 (65), 299 (18), 285 (10), 273 (30), 259 (25), 203 (10), 123 (8), 69 (8), 55 (5). HREIMS *m/z* 356.2363 (calcd for $C_{23}H_{32}O_3$, 356.2351).

4.3. General procedure for the synthesis of sugiol β-amino alcohol derivatives

To a solution of epoxide **3** (0.140 mmol) in methanol (5 ml) was added the appropriate amine (0.150 mmol). The reaction mixture was refluxed for 15 h and then allowed to cool down to room temperature. The resulting crude was concentrated under reduced pressure and purified by silica column chromatography, to yield the corresponding β-amino alcohol derivative.

4.3.1. 12-[3-(Dodecylamino)-2-hydroxypropoxy]abieta-8,11,13-trien-7-one (4a)

The general ring opening procedure was applied to epoxide **3** (50 mg, 0.140 mmol) and dodecylamine (28 mg, 0.150 mmol) to give amino alcohol **4a** (61 mg, 80% yield) as a colorless oil. TLC [acetone/hexane (4:6), R_f 0.83]. IR (NaCl) 3418, 2926, 1670, 1599, 1495, 1464, 1260, 1180, 756 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.89 (3H, t, $J = 6.3$ Hz), 0.94 (3H, s), 1.00 (3H, s), 1.20 (29H, m), 1.40 (2H, m), 1.50 (1H, m), 1.55 (1H, m), 1.68 (2H, m), 1.87 (1H, dd, $J_1 = 3.3$ Hz, $J_2 = 13.3$ Hz), 2.28 (1H, brd, $J = 12.1$ Hz), 2.70 (2H, m), 2.80 (3H, m), 2.90 (1H, m), 4.10 (2H, m), 4.18 (1H, m), 6.76 (1H, s), 7.91 (1H, s). ^{13}C RMN ($CDCl_3$) δ 14.0, 18.8, 21.3, 22.3, 22.5, 23.2, 26.7, 27.0, 27.3, 31.8, 32.5, 33.2, 36.0, 37.9, 38.2, 41.2, 49.5, 57.9, 58.4, 68.0, 70.0, 105.3, 124.4, 125.7, 134.9, 156.4, 160.4, 198.4. EIMS *m/z* 540 $[M^+ - 1]$ (3), 462 (4), 301 (7), 254 (13), 212 (32), 210 (23), 198 (11), 154 (23), 136 (18), 100 (32), 86 (27), 69 (32), 57 (50). HREIMS *m/z* 540.2589 (calcd for $C_{35}H_{58}NO_3$, 540.2539).

*4.3.2. 12-{2-Hydroxy-3-[(2-phenylethyl)amino] propoxy} abieta-8,11,13-trien-7-one (**4b**)*

The general ring opening procedure was applied to epoxide **3** (50 mg, 0.140 mmol) and 2-phenylethylamine (18 μl, 0.150 mmol) to give amino alcohol **4b** (57 mg, 85% yield) as an amorphous white solid. TLC [EtOAc/hexane (1:1), R_f 0.71]. IR (NaCl) 3351, 2960, 1667, 1599, 1455, 1274, 762 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.93 (3H, s), 1.00 (3H, s), 1.24 (9H, brs), 1.30 (1H, m), 1.53 (2H, m), 1.70 (1H, m), 1.81 (1H, m), 1.86 (1H, dd, J_1 = 3.9 Hz, J_2 = 13.3 Hz), 2.27 (1H, brd, J = 12.3 Hz) 2.55 (2H, m), 2.80 (1H, m), 2.90 (1H, m), 2.98 (2H, m), 3.00 (2H, m), 3.23 (1H, heptet, J = 7.0 Hz), 4.02 (1H, dd, J_1 = 5.2 Hz, J_2 = 9.3 Hz), 4.10 (1H, dd, J_1 = 5.2 Hz, J_2 = 9.1 Hz), 4.17 (1H, m), 6.76 (1H, s), 7.20 (1H, m), 7.21 (2H, d, J = 7.04 Hz), 7.31 (2H, d, J = 7.04 Hz),7.90 (1H, s). ^{13}C RMN ($CDCl_3$) δ 18.9, 21.4, 22.3, 22.5, 23.2, 26.7, 32.5, 33.2, 35.8, 36.0, 37.9, 38.2, 41.3, 49.6, 50.8, 51.7, 67.7, 70.3, 105.4, 124.5, 125.2, 126.4, 128.6, 128.7, 135.0, 139.1, 156.4, 160.5, 198.6. EIMS *m/z* 477 $[M]^+$ (2), 387 (32), 386 (100), 343 (9), 300 (4), 134 (30), 105 (19), 86 (15), 69 (3), 55 (2). HREIMS *m/z* 477.3172 (calcd for $C_{31}H_{43}NO_3$ 477.3243).

*4.3.3. 12-(2-Hydroxy-3-{[2-hydroxy-1-(hydroxymethyl)-1-methylethyl]amino}propoxy)abieta-8,11,13-trien-7-one (**4c**)*

The general ring opening procedure was applied to epoxide **3** (50 mg, 0.140 mmol) and 2-amino-2-methyl-1,3-propanediol (16 mg, 0.150 mmol) to give amino alcohol **4c** (56 mg, 86% yield) as a colorless oil. TLC (acetone, R_f 0.31). IR (NaCl) 3353, 2928, 1667, 1598, 1494, 1261, 1048, 766 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.93 (3H, s), 0.99 (6H, s), 1.25 (9H, m), 1.30 (1H, m), 1.55 (2H, m), 1.70 (2H, m), 1.85 (1H, dd, = 3.0 Hz, J_2 = 13.4 Hz), 2.28 (1H, brd, J = 12.1 Hz), 2.65 (1H, dd, J_1 = 12.6 Hz, J_2 = 17.0 Hz), 2.69 (1H, dd, J_1 = 14.0 Hz, J_2 = 17.0 Hz), 2.83 (1H, brd, J = 8.5 Hz), 2.96 (1H, brd, J = 10.0 Hz), 3.23 (1H, m), 3.60 (4H, m), 4.10 (2H, m), 4.19 (1H, m), 6.74 (1H, s), 7.93 (1H, s). ^{13}C RMN ($CDCl_3$) δ 18.1, 18.8, 21.2, 22.3, 22.4, 23.1, 26.6, 32.5, 33.2, 35.9, 37.9, 38.2, 41.2, 44.2, 49.5, 56.9, 66.7, 67.6, 69.1, 70.2, 105.4, 124.2, 125.7, 135.0, 156.5, 160.5, 198.6. EIMS *m/z* 461 $[M^+]$ (5), 430 (31), 301 (10), 219 (6), 154 (72), 136 (49), 107 (20), 89 (16), 77 (20), 69 (17). HREIMS *m/z* 461.3158 (calcd for C_{27} $H_{43}NO_5$ 461.3141).

*4.3.4. 12-{2-Hydroxy-3-[(2-hydroxy-1-phenylethyl) amino] propoxy}abieta-8,11,13-trien-7-one (**4d**)*

The general ring opening procedure was applied to epoxide **3** (50 mg, 0.140 mmol) and (*R*)-(–)-2-amino-2-phenylethanol (18 mg, 0.150 mmol) to give amino alcohol **4d** (46.5 mg, 68% yield) as an amorphous solid: TLC (EtOAc, R_f 0.79). IR (NaCl) 3390, 2928, 1667, 1598, 1494, 1454, 1260, 756 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.93 (3H, s), 1.00 (3H, s), 1.15 (3H, m), 1.17 (3H, m), 1.23 (3H, s), 1.30 (2H, m), 1.50 (1H, m), 1.67 (1H, m), 1.80 (1H, m), 1.85 (1H, dd, J_1 = 3.8 Hz, J_2 = 13.3 Hz), 2.25 (1H, brd, J = 10.8 Hz), 2.61 (1H, d, J_1 = 13.5 Hz, J_2 = 18.0 Hz), 2.69 (1H, d, J_1 = 4.0 Hz, J_2 = 13.5 Hz), 2.75 (2H, m), 3.12 (1H, m), 3.70 (1H, m), 3.80 (1H, dd, J_1 = 10.6 Hz, J_2 = 3.65 Hz), 3.90 (1H, m), 4.04 (1H, m), 4.24 (2H, m), 6.71(1H, s), 7.30 (5H, m), 7.88 (1H, s). ^{13}C RMN ($CDCl_3$) δ 18.4, 20.8, 21.8, 21.9, 22.7, 26.1, 32.0, 32.7, 35.5, 37.4, 37.7, 40.8, 48.9, 49.0, 64.7, 66.4, 68.8, 69.6, 104.8, 123.8, 125.1, 126.8, 127.3, 128.2, 134.5, 140.0, 155.9, 159.8, 198.1. EIMS *m/z* 494 $[M^+ + 1]$ (64), 462 (20), 374 (5), 307 (20), 289 (11), 155 (24), 154 (100), 136 (72), 121 (25), 107 (21), 91 (27), 77 (19), 69 (12). HREIMS *m/z* 494.3317 (calcd for $C_{31}H_{44}NO_4$ 494.3270).

*4.3.5. 12-{2-Hydroxy-3-[(2-hydroxy-1,2-diphenylethyl) amino] propoxy}abieta-8,11,13-trien-7-one (**4e**)*

The general ring opening procedure was applied to epoxide **3** (50 mg, 0.140 mmol) and (1*S*, 2*R*)-(+)-2-amino-1,2-diphenylethanol (32.5 mg, 0.150 mmol) to give amino alcohol **4e** (48.4 mg, 62% yield) as a colorless oil. TLC [acetone/hexane (1:1), R_f 0.60]. IR (NaCl) 3381, 2960, 1666, 1598, 1494, 1454, 1260, 756 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.94 (3H, s), 1.00 (3H, s), 1.12 (3H, d, J = 6.9 Hz), 1.15 (3H, d, J = 6.9 Hz), 1.22 (3H, s), 1.30 (1H, m), 1.54 (1H, m), 1.55 (1H, m), 1.70 (1H, m), 1.83 (1H, m), 1.85 (1H, dd, J_1 = 4.0 Hz, J_2 = 13.4 Hz), 2.25 (1H, d, J = 12.1 Hz), 2.65 (1H, m), 2.70 (1H, m), 2.73 (1H, m), 2.85 (1H, dd, J_1 = 4.0 Hz, J_2 = 7.8 Hz), 3.06 (1H, m), 3.94 (1H, m), 4.00 (2H, m), 4.16 (1H, m), 5.11 (1H, d, J = 4.5 Hz), 6.66 (1H, s), 7.25 (10H, m), 7.86 (1H, s). ^{13}C RMN ($CDCl_3$) δ 18.6, 21.0, 22.0, 22.2, 22.9, 26.4, 32.3, 32.0, 35.7, 37.7, 37.9, 41.0, 49.3, 49.8, 68.3, 69.5, 69.6, 75.9, 105.1, 124.2, 125.5, 126.4, 127.7, 127.9, 128.0, 128.2, 128.4, 134.8, 139.9, 156.1, 160.0, 198.2. EIMS *m/z* 551 $[M^+ - 18]$ (1), 463 (34), 462 (100), 343 (2), 300 (3), 162 (12), 148 (4), 118 (9), 91 (8), 77 (2). HREIMS *m/z* 551.3339 (calcd for $C_{37}H_{45}NO_3$ 551.3399).

*4.3.6. 12-[3-Abieta-8,11,13-trien-18-ylamino)-2-hydroxypropoxy]abieta-8,11,13-trien-7-one (**4f**)*

The general ring opening procedure was applied to epoxide **3** (50 mg, 0.140 mmol) and abieta-8,11,13-trien-18-amine (42 mg, 0.150 mmol) to give amino alcohol **4f** (49 mg, 55% yield) as an amorphous solid. TLC [EtOAc/hexane (1:1), R_f 0.71]. IR (NaCl) 3351, 2960, 1667, 1599, 1455, 1274, 762 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.94 (3H, s), 0.97 (3H, d), 1.01 (3H, s), 1.23 (18H, m), 1.30 (1H, m), 1.40 (2H, m), 1.46 (1H, m), 1.50 (2H, m), 1.55 (1H, m), 1.60 (1H, m), 1.70 (6H, m),1.88 (1H, dd, J_1 = 3.8 Hz, J_2 = 13.4 Hz), 2.30 (1H, d, J = 12.4 Hz), 2.41 (1H, dd, J_1 = 11.0 Hz, J_2 = 12.0 Hz), 2.60 (3H, m), 2.80 (1H, m), 2.88 (2H, m), 2.95 (2H, m), 3.24 (1H, m), 4.12 (1H, m), 4.15 (2H, m), 6.78 (1H, s), 6.89 (1H, s), 7.01 (1H, brd, J = 8.1 Hz), 7.19 (1H, d, J = 8.1 Hz), 7.90 (1H, s). ^{13}C RMN ($CDCl_3$) δ 18.4, 18.8, 21.3, 22.4, 23.2, 23.9, 25.3, 26.7, 30.2, 32.5, 33.2, 33.4, 35.9, 36.2, 37.0, 37.3, 37.9, 38.2, 38.4, 41.3, 45.2, 49.5, 52.6, 61.5, 67.6, 70.3, 105.4, 123.8, 123.9, 125.6, 126.7, 134.5, 135.1, 145.5, 147.2, 156.3, 160.6, 198.5. EIMS *m/z* 641 $[M]^+$, 412 (3), 387 (30), 386 (100), 300 (6), 239 (5), 173 (6), 86 (14), 70 (10), 55 (4). HREIMS *m/z* 641.4818 (calcd. for $C_{43}H_{43}NO_3$ 641.4808).

*4.3.7. 12-[3-Dibenzylamino)-2-hydroxypropoxy]abieta-8,11,13-trien-7-one (**4g**)*

The general ring opening procedure was applied to epoxide **3** (50 mg, 0.140 mmol) and *N,N*-dibenzylamine (28.5 μl, 0.150 mmol) to give amino alcohol **4g** (58 mg, 75% yield) as a colorless oil. TLC [EtOAc/hexane (2.5:7.5), R_f 0.62]. IR (NaCl) 3448, 3062, 2960, 1667, 1599, 1494, 1453, 1260, 752 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.94 (3H, s), 1.01 (3H, s), 1.18 (6H, m), 1.24 (3H, s), 1.35 (1H, m), 1.50 (1H, m), 1.60 (1H, m), 1.68 (2H, m), 1.86 (1H, dd, $J_1 = 4.1$ Hz, $J_2 = 13.4$ Hz), 2.26 (1H, brd, $J = 12.8$ Hz), 2.65 (2H, m), 2.70 (2H, m), 3.09 (1H, m), 3.56 (2H, d, $J = 13.3$ Hz), 3.89 (2H, d, $J = 13.3$ Hz), 3.98 (2H, brd, $J = 2.7$ Hz), 4.15 (1H, m), 6.69 (1H, s), 7.30 (2H, m), 7.36 (8H, brs), 7.88 (1H, s). ^{13}C RMN ($CDCl_3$) δ 18.4, 20.8, 21.8, 21.9, 22.7, 26.1, 32.0, 32.7, 35.5, 37.4, 37.7, 40.8, 49.1, 55.5, 58.2, 65.7, 69.5, 104.7, 123.7, 125.0, 126.9, 128.0, 128.5, 134.5, 137.8, 155.8, 160.1, 198.0. EIMS *m/z* 553 $[M]^+$(1), 285 (2), 210 (100), 118 (1), 91 (30), 70 (10), 55 (2). HREIMS *m/z* 553.3348 (calcd for $C_{37}H_{47}NO_3$ 553.3331).

*4.3.8. 12-[3-(Dipentylamino)-2-hydroxypropoxy]abieta-8,11,13-trien-7-one (**4h**)*

The general ring opening procedure was applied to epoxide **3** (50 mg, 0.140 mmol) and *N,N*-dipentylamine (31.6 μl, 0.150 mmol)to give amino alcohol **4h** (49 mg, 69% yield) as a colorless oil. TLC [acetone/hexane (2:3), R_f 0.74]. IR (NaCl) 3417, 2957, 1672, 1599, 1495, 1463, 1259, 1180 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.92 (6H, t, $J = 7.0$ Hz), 0.94 (3H, s), 1.01 (3H, s), 1.24 (9H, m), 1.30 (1H, m) 1.34 (8H, m), 1.50 (5H, m), 1.55 (1H, m), 1.65 (2H, m), 1.87 (1H, dd, $J_1 = 2.7$ Hz, $J_2 = 13.4$ Hz), 2.28 (1H, brd, $J = 11.6$ Hz), 2.55 (2H, m), 2.62 (4H, m), 2.73 (2H, m), 3.24 (1H, m), 4.10 (2H, m), 4.13 (1H, m), 6.82 (1H, s), 7.90 (1H, s). ^{13}C RMN ($CDCl_3$) δ 14.03, 18.8, 21.3, 22.3, 22.4, 22.5, 23.2, 26.3, 26.8, 29.4, 32.5, 33.2, 36.0, 37.9, 38.2, 41.3, 49.6, 54.4, 57.4, 65.6, 70.1, 105.3, 124.3, 125.7, 135.1, 156.4, 160.7, 198.5. EIMS *m/z* 512 $[M^+ - 1]$ (1), 456 (7), 300 (2), 285 (3), 170 (100), 114 (8), 58 (2). HREIMS *m/z* 512.4174 (calcd for $C_{33}H_{54}NO_3$ 512.4104).

*4.3.9. 12-[3-(Dicyclohexylamino)-2-hydroxypropoxy] abieta-8,11,13-trien-7-one (**4i**)*

The general ring opening procedure was applied to epoxide **3** (50 mg, 0.140 mmol) and *N,N*-dicyclohexylamine (30.7 μl, 0.150 mmol) to give amino alcohol **4i** (51 mg, 68% yield) as a colorless oil. TLC [EtOAc/hexane (3:7), R_f 0.65]. IR (NaCl) 3390, 2929, 1669, 1599, 1495, 1455, 1260, 755 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.95 (3H, s), 1.01 (3H, s), 1.17–1.48 (30H, m), 1.50 (1H, brd, $J = 12.7$ Hz), 1.55 (1H, m), 1.72 (2H, m), 1.88 (1H, dd, $J_1 = 3.8$ Hz, $J_2 = 13.4$ Hz), 2.29 (1H, brd, $J = 12.3$ Hz), 2.63 (1H, dd, $J_1 = 13.6$ Hz, $J_2 = 18.0$ Hz), 2.70 (1H, dd, $J_1 = 4.0$ Hz, $J_2 = 18.0$ Hz), 2.80 (2H, m), 3.25 (1H, m), 3.62 (2H, m), 4.12 (2H, m), 4.22 (1H, m), 6.80 (1H, s), 7.91 (1H, s). ^{13}C RMN ($CDCl_3$) δ 18.8, 21.3, 22.4, 22.5, 23.2, 26.7, 27.7, 31.5, 32.5, 33.2, 36.0, 37.9, 38.2, 41.3, 49.6, 58.5 68.8, 68.9, 73.4, 105.4, 124.4, 125.7, 135.1, 156.4, 160.4, 198.5. EIMS *m/z* 537 $[M^+]$ (1), 388 (10), 285 (9), 215 (3), 194 (100), 138 (4), 112 (8), 83 (5), 55 (9). HREIMS *m/z* 537.4105 (calcd for $C_{35}H_{55}NO_3$, 537.4182).

*4.3.10. 12-[2-Hydroxy-3-(4-morpholinyl)propoxy] abieta-8,11,13-trien-7-one (**4j**)*

The general ring opening procedure was applied to epoxide **3** (50 mg, 0.140 mmol) and morpholine (13.8 μl, 0.150 mmol) to give amino alcohol **4j** (51.3 mg, 82% yield) as a colorless oil. TLC [acetone/hexane (1:1), R_f 0.57]. IR (NaCl) 3440, 2959, 1669, 1599, 1494, 1456, 1260, 1118, 755 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.93 (3H, s), 0.99 (3H, s), 1.24 (9H, m), 1.27 (1H, m), 1.50 (2H, m), 1.70 (1H, m), 1.78 (1H, m), 1.86 (1H, dd, $J_1 = 3.5$ Hz, $J_2 = 13.5$ Hz) , 2.28 (1H, brd, $J = 12.1$ Hz), 2.52 (1H, m), 2.61 (4H, m), 2.65 (2H, m), 2.73 (1H, m), 3.23 (1H, m), 3.76 (4H, m), 4.06 (1H, m), 4.11 (1H, dd, $J_1 = 2.4$ Hz, $J_2 = 7.2$ Hz), 4.16 (1H, m), 6.76 (1H, s), 7.93 (1H, s). ^{13}C RMN ($CDCl_3$) δ 18.8, 21.3, 22.2, 22.4, 23.2, 26.7, 32.5, 33.2, 35.9, 37.9, 38.1, 41.2, 49.5, 53.7, 61.3, 65.2, 66.8, 70.1, 105.4, 124.3, 125.7, 135.1, 156.3, 160.6, 198.4. EIMS *m/z* 443 $[M^+]$ (2), 342 (2), 285 (5), 130 (3), 100 (100), 69 (5), 56 (5). HREIMS *m/z* 443.3048 (calcd for $C_{27}H_{41}NO_4$ 443.3036).

*4.3.11. 12-[3-(6-Amino-9H-purin-9-yl)-2-hydroxy propoxy]abieta-8,11,13-trien-7-one (**4k**)*

A solution of adenine (17 mg, 0.1234 mmol) and DMF (1 ml) was treated with sodium hydride (80%, 4 mg, 0.1234 mmol). The mixture was allowed to react at 110 °C for 2 h under nitrogen. After cooling to room temperature, epoxide **3** (40 mg, 0.1121 mmol) in DMF (3 ml) was added to the solution and the resulting mixture was heated to 75 °C, under N_2 for 20 h. Upon conclusion, the reaction mixture was cooled to room temperature and the solvent was evaporated under reduced pressure. The extract was purified by silica column chromatography using ethanol as eluent. The product **4k** (38.7 mg, 71% yield) was obtained as an amorphous solid. TLC (acetone, R_f 0.36). IR (NaCl) 3326, 3182, 2927, 1660, 1598, 1470, 1260, 756 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.94 (3H, s), 1.01 (3H, s), 1.25 (9H, m), 1.30 (1H, m), 1.55 (2H, m), 1.75 (2H, m), 1.80 (1H, m), 2.24 (1H, brd, $J = 12.3$ Hz), 2.65 (2H, m), 3.24 (1H, m), 3.90 (1H, m), 4.20 (1H, m), 4.47 (1H, m), 4.49 (1H, m), 4.61 (1H, d, $J = 13.5$ Hz), 6.72 (1H, s), 7.81 (1H, s), 7.92 (1H, s), 8.36 (1H, s). ^{13}C RMN ($CDCl_3$) δ 18.8, 21.3, 22.4, 22.6, 23.2, 26.7, 32.5, 33.2, 36.0, 37.9, 38.2, 41.2, 48.7, 49.5, 68.6, 69.1, 105.4, 120.0, 124.4, 125.9, 134.8, 141.5, 150.1, 152.6, 155.6, 156.9, 159.9, 198.5. EIMS *m/z* 491 $[M^+]$ (14), 300 (4), 192 (45), 178 (100), 174 (63), 149 (15), 136 (9), 69 (3). HREIMS *m/z* 491.2904 (calcd for $C_{28}H_{37}N_5O_3$, 491.2896).

4.4. Drug lipophilicity

The octanol/water partition coefficient expressed in logarithmic form (ClogP) is been widely used for calculating drug lipophilicity. It is usually calculated from the sum of partition coefficients of the chemical fragments composing the mole-

cule. Software-predicted lipophilicity of the compounds was calculated with the program ClogP® accessible via Internet (www.daylight.com/daycgi/clogp) working with the Hansch–Leo's ''fragment constant'' method.

4.5. Cell cultures and plating

Three human cancer cell lines originating from diverse tissues were used in this study: A2780, ovarian; SW1573, non-small cell lung cancer; and WiDr, colon. These cell lines were a kind gift from Professor Godefridus J. Peters (VU Medical Center, Amsterdam, The Netherlands). Cells were maintained in 25 cm^2 culture flasks in RPMI 1640 supplemented with 5% heat inactivated fetal calf serum and 2 mM L-glutamine in a 37 °C, 5% CO_2, 95% humidified air incubator. Exponentially growing cells were trypsinized and resuspended in antibiotic containing medium (100 units penicillin G and 0.1 mg of streptomycin per ml). Single cell suspensions displaying >97% viability by trypan blue dye exclusion were subsequently counted. After counting, dilutions were made to give the appropriate cell densities for inoculation onto 96-well microtiter plates. Cells were inoculated in a volume of 100 μl per well at densities of 7000 (A2780), 5000 (SW1573) and 10,000 (WiDr) cells per well, based on their doubling times.

4.6. Chemosensitivity testing

Chemosensitivity tests were performed using the SRB assay of the National Cancer Institute (NCI) with slight modifications [14]. Pure compounds were initially dissolved in DMSO at 400 times the desired final maximum test concentration. Control cells were exposed to an equivalent concentration of DMSO (0.25% v/v). Each agent was tested in duplicates at five different ten-fold dilutions. Drug incubation times were 48 h, after which cells were precipitated with 25 μl ice-cold 50% (w/v) trichloroacetic acid and fixed for 60 min at 4 °C. Then the SRB assay was performed. The optical density (OD) of each well was measured at 490 nm using Bio-Tek's Elx800 NB 96-well plate reader. The percentage growth was calculated at each of the drug concentration levels based on the difference in OD at the start and end of drug exposure. Values were corrected for background OD from wells only containing medium.

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