Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 909–912

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Anti-tumor-promoting Activity of Glycoglycerolipid Analogues Lacking the Glycerol Backbone

Diego Colombo,[a,*] Fiamma Ronchetti,[a] Antonio Scala,[a] Lucio Toma,[b] Harukuni Tokuda[c] and Hoyoku Nishino[c]

[a]*Dipartimento di Chimica, Biochimica e Biotecnologie per la Medicina, Università di Milano, Via Saldini 50, 20133 Milan, Italy*
[b]*Dipartimento di Chimica Organica, Università di Pavia, Via Taramelli 10, 27100 Pavia, Italy*
[c]*Department of Biochemistry, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602-0841, Japan*

Received 31 May 2002; accepted 22 October 2002

Abstract—Glycoglycerolipid analogues lacking the glycerol backbone were prepared through a lipase catalyzed transesterification of β-D-galactosylethylene glycol. The inhibitory effect of the resultant isomeric hexanoates at the primary alcoholic positions, β-D-galactosylethylene glycol itself and nonyl β-D-galactopyranoside, was tested on Epstein–Barr virus early antigen (EBV-EA) activation in Raji cells promoted by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA), as a primary screening test for inhibitors of tumor promotion. All the compounds assayed were found to be less active than the reference 2-*O*-β-D-galactopyranosylglycerol derivatives, of which they are simplified models, indicating that the anti-tumor-promoting activity is very closely related to the presence of a free hydroxymethylene group on the glycerol-like aglycone moiety.

Introduction

In the course of a study aimed at clarifying the structure–anti-tumor-promoting activity relationship of glycoglycerolipids active in cancer chemoprevention,[1–7] synthetic 1-*O*- and 6′-*O*-hexanoyl derivatives **1b** and **1c** of 2-*O*-β-D-galactopyranosylglycerol (**1a**) have shown a remarkable inhibitory effect in vitro against Epstein–Barr virus early antigen (EBV-EA) activation induced by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA),[4] and in vivo against mouse skin tumor promotion in a two-stage carcinogenesis test.[5]

To elucidate the minimal structural requirements needed for inhibitory activity we have planned the synthesis of simple analogues of the glycoglycerolipids **1b** and **1c**, namely the β-D-galactopyranosylethylene glycol hexanoates **2b** and **2c**, the aglycone in such hexanoates lacking a hydroxymethylene group of glycerol and thus being devoid of chirality. Moreover, given the significant in vitro and in vivo activity[8] of an alkane isoster of **1b,** compound **3**, we also prepared a simplified model of **3** without the hydroxymethylene group, nonyl β-D-galactopyranoside **4**. Here we describe the synthesis of compounds **2b–c** and **4,** and their anti-tumor promoting activity based on the short term in vitro bioassay for the inhibition of EBV-EA activation induced by TPA, together with that of the non-acylated compound **2a**.

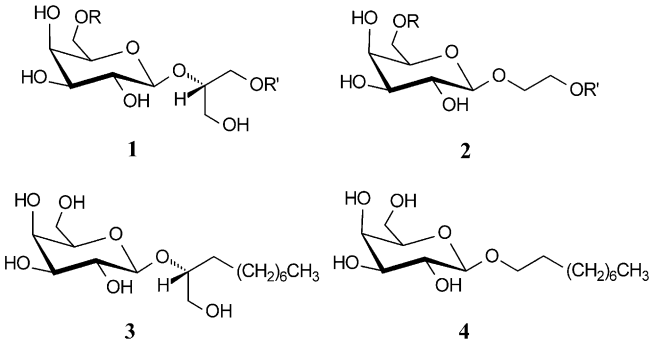
Results and Discussion

Chemistry

Compound **2a** was prepared according to literature procedures,[9] whereas the two isomeric hexanoates **2b** and **2c** were obtained in one step and with good yields from **2a** through an efficient chemoenzymatic synthesis that made use of lipases of opposite regioselectivity. In a first approach to the synthesis of **2b** and **2c**, we decided to use the enzymatic method we had recently employed to obtain compounds **1b** and **1c.** In that case, we had managed, in a single experiment performed with *Pseudomonas cepacia* lipase (LPS),[10] to simultaneously produce 1- and 6′-monoesters in almost equimolar amounts through the transesterification of **1a**. However, when β-D-galactopyranosylethylene glycol (**2a**) was underwent to LPS catalyzed transesterification under the

*Corresponding author. Tel.: +39-02-5031-6045; fax: +39-02-5031-6036; e-mail: diego.colombo@unimi.it

0968-0896/03/$ - see front matter
PII: S0968-0896(02)00543-6



a: R=R'=H; **b**: R=H, R'=$CO(CH_2)_4CH_3$; **c**: R=$CO(CH_2)_4CH_3$, R'=H; **d**: R=R'=$CO(CH_2)_4CH_3$

same reaction conditions used for the acylation of **1a**, the reaction was found to be very regioselective (Table 1). The acylation of the primary hydroxyl of the sugar gave 1-*O*-(6-*O*-hexanoyl-β-D-galactopyranosyl)ethylene glycol (**2c**) as the main monoacylation product, and only very small amounts of **2b** being derived from the acylation at the aglycone site, as ascertained by ^{1}H NMR analysis of the monoesters fraction.

To obtain the 1-hexanoate **2b** in appreciable yields we turned our attention to *Candida antarctica* lipase (LCA), known to acylate **1a** mainly at the glycerol moiety.[10] In fact, when, in the previously reported reaction conditions, **2a** was submitted to LCA catalyzed transesterification, the reaction showed a regioselectivity opposite that of the LPS catalyzed reaction, yielding, almost exclusively, the desired 1-*O*-hexanoyl-2-*O*-(β-D-galactopyranosyl)ethylene glycol (**2b**) (Table 1). The two 1- and 6′-hexanoyl derivatives were obtained pure by flash chromatography of the respective monoester fractions. Both the LPS and LCA catalyzed reactions afforded some diester (Table 1), identified by ^{1}H NMR analysis as 1-*O*-hexanoyl-2-*O*-(6-*O*-hexanoyl-β-D-galactopyranosyl)ethylene glycol (**2d**). Lastly, the alkane isoster, nonyl β-D-galactopyranosyde (**4**), was obtained through a completely stereoselective glycosylation reaction (no traces of the α anomer were detected) using β-D-galactose pentaacetate as glycosyl donor, and 1-nonanol as acceptor in the presence of boron trifluoride diethyl etherate. The β configuration of the glycosidic bond was indicated by the characteristic value (8.0 Hz) of the trans-diaxial $J_{1,2}$ coupling constant measured by ^{1}H NMR analysis. The acetyl groups of the obtained nonyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside were removed by Zemplén reaction to afford **4**.

Table 1. Lipase catalyzed transesterification of **2a**[a]

Enzyme	Time (min)	Monoesters[b] (%)	Diesters[b] (%)	Monoester ratio[c] **2b**/**2c**
LPS	60	69	14	3:97
LCA	30	70	30	97:3

[a]No acylation took place in the absence of the enzyme.
[b]Isolated yield from flash chromatography.
[c]Determined through ^{1}H NMR analysis of the monoester fraction.

Biological evaluation

Epstein–Barr virus (EBV) is activated by tumor promoters to produce viral early antigens (EA), and EBV inhibition is often evaluated in vitro as a primary screening for anti-tumor-promoting activity.[11] The present study evaluated the inhibitory effect of compounds **2a–c** and **4** using a short term in vitro assay for EBV-EA activation in Raji cells induced by the tumor promoter TPA.[12,13] Each compound was assayed in triplicate, and it was found that the cytotoxicity against Raji cells was, for all the compounds, quite weak, even at a concentration of 1×10^3 mol ratio. Table 2 shows the in vitro tumor inhibitory activity of compounds **2a–c** and **4**, and the reference compounds **1b–c**[4] and **3**.[8]

Note that all the tested compounds (**2a–c** and **4**) inhibited the TPA promoted EBV activation (Table 2). The four compounds, almost equiactive, showed small differences in activity that appear to be mainly related to the presence, or absence of a primary hydroxyl on the aglycone moiety of the molecule. In fact, the hydroxyl carrying compounds **2a** and **2c** were more active than **2b** and **4** that lack it, **2b** and **4** not being able to completely inhibit EBV activation even at 1000 mol ratio/TPA (Table 2). Furthermore, as previously described,[2,4] the presence of a hexanoyl chain seemed to increase the inhibitory activity. In fact, on comparing **2a** and **2c**, both carrying the primary hydroxyl on the aglycone, **2a**, with no hexanoyl chain, was less active than **2c**, with a hexanoyl chain, this latter also being the most active compound of this series of simple analogues (Table 2). However, as evidenced by the data of Table 2, the galactosylethylene glycol derivatives **2b–c** and nonyl β-D-galactopyranoside (**4**), the isoster of **2b**, showed a markedly lower inhibitory potency than the galactosylglycerol derivatives **1b–c**[4] and the galactoside **3**,[8] the isoster of **1b**, to which they are referred.

In conclusion, the data indicate that a necessary requisite for significant anti tumor-promoting activity in glycoglycerolipid analogues is not only the presence of one

Table 2. Inhibitory effects of **1b–c**, **2a–c**, **3** and **4** on TPA-induced EBV-EA activation

	Mol ratio (test compound/TPA)[a]			
	1000	500	100	10
	% to positive control[b] ± SE ($n=3$) (% viability)			
1b[c]	0.0±0.0 (70)	11.4±0.3 (>80)	32.1±0.9 (>80)	63.4±1.3 (>80)
1c[c]	0.0±0.0 (70)	10.7±0.1 (>80)	30.1±0.9 (>80)	67.8±1.2 (>80)
2a	0.0±0.3 (70)	46.2±1.5 (>80)	76.0±2.2 (>80)	100±0.2 (>80)
2b	6.7±0.5 (70)	49.5±1.4 (>80)	78.5±2.5 (>80)	100±0.2 (>80)
2c	0.0±0.4 (70)	41.3±1.3 (>80)	71.0±2.3 (>80)	96.9±0.4 (>80)
3[d]	0.0±0.5 (70)	20.3±1.3 (>80)	38.5±1.5 (>80)	72.1±0.4 (>80)
4	12.4±0.3 (70)	50.6±1.7 (>80)	80.5±2.3 (>80)	100±0.3 (>80)

[a]TPA = 32 pmol; Test compounds = 32, 16, 3.2 and 0.32 nmol.
[b]Values represent EBV-EA activation (%) in the presence of test compound relative to the positive control (TPA only).
[c]See ref 4.
[d]See ref 8.

hexanoyl chain, already suggested by previous results,[2,4] but also integrity in the glycerol-like backbone, a key structural feature being the presence of one hydroxymethylene group on the aglycone.

Experimental

Chemistry

P. cepacia lipase (LPS, lipase PS, specific activity 30.5 triacetin units/mg solid), a generous gift from Amano Pharmaceutical Co (Mitsubishi Italia), was supported on Celite[14] and kept overnight under vacuum prior to use. *Candida antarctica* lipase SP 435 L, (Novozym® 435, LCA, specific activity 9.5 PL units/mg solid), was a generous gift from Novo Nordisk A/S. Pyridine was distilled over calcium hydride prior to use. The acyl carrier 2,2,2-trifluoroethyl *n*-hexanoate was synthesized from hexanoyl chloride and 2,2,2-trifluoroethanol.[15] β-D-Galactopyranosylethylene glycol (**2a**) was synthesized according to literature procedures.[9] β-D-Galactose pentaacetate and 1-nonanol were purchased from Fluka. Evaporation under reduced pressure was always effected with a bath temperature below 40 °C. All the new compounds were characterized by ^{1}H NMR analysis at 500.13 MHz, ^{13}C NMR at 125.76 MHz (Bruker AM500 spectrometer at 303 K) and chemical ionization mass spectrometry (CI-MS) as described in ref 15 by LC particle beam introduction with a quadrupolar mass spectrometer Hewlett-Packard HP 5988A equipped with an interface PB 59980A and a low pressure HPLC HP 1050. The elemental analyses were consistent with the theoretical ones. Optical rotations were determined on a Perkin-Elmer 241 polarimeter as methanol solutions, unless otherwise stated, in a 1 dm cell at 20 °C. Melting points were recorded on a Büchi 510 capillary melting point apparatus and were uncorrected.

1-*O*-Hexanoyl-2-*O*-(β-D-galactopyranosyl)ethylene glycol (2b) and 1-*O*-(6-*O*-hexanoyl-β-D-galactopyranosyl)ethylene glycol (2c). β-D-Galactopyranosylethylene glycol (**2a**) (0.30 g, 1.34 mmol) was dissolved in pyridine (5 mL) and 2,2,2-trifluoroethyl hexanoate (4.00 mmol) and LCA (1.00 g) or LPS (1.50 g) were added in the order. The mixture was stirred at 45 °C and the reaction was stopped, after 0.5 h for LCA and after 1.0 h for LPS, by filtering-off the enzyme and washing with pyridine. The solvent was removed under vacuum and the crude product was submitted to flash chromatography. Flash chromatography (dichloromethane–methanol 85:15, v/v) of LCA catalyzed transesterification yielded 0.17 g (30%) of the diester **2d**, and 0.30 g (70%) of a monoester fraction. Compound **2d**, mp: 64–65 °C (diisopropyl ether); $[\alpha]_D^{20}$: −0.5 (c = 1.0); ^{1}H NMR (CD_3OD): δ 4.30 (dd, 1H, $J_{6'a,\ 6'b}$ = 11.0 Hz, $J_{5',\ 6'a}$ = 7.0 Hz, H-6′a), 4.28–4.23 (m, 3H, H-1a, H-1b and H-1′), 4.21 (dd, 1H, $J_{5',\ 6'b}$ = 5.0 Hz, H-6′b), 4.00 (m, 1H, H-2a); 3.84–3.76 (m, 2H, H-2b and H-4′), 3.73 (dd, 1H, H-5′), 3.53 (dd, 1H, $J_{1',\ 2'}$ = 8.0 Hz, $J_{2',\ 3'}$ = 9.0 Hz, H-2′), 3.47 (dd, 1H, $J_{3',\ 4'}$ = 3.0 Hz, H-3′), 2.34 (m, 2H, CH_2), 2.33 (m, 2H, CH_2), 1.62 (m, 4H, 2CH_2), 1.39–1.28 (m, 8H, 4CH_2), 0.92 (m, 6H, 2CH_3). ^{13}C NMR (CD_3OD): δ 175.5; 175.2; 105.1; 74.7; 74.0; 72.2; 70.3; 68.6; 64.7 (2C); 34.9 (2C); 32.4 (2C); 25.7 (2C); 23.4 (2C); 14.3 (2C); MS: m/z 438 $[M + NH_4]^+$. Flash chromatography (dichloromethane–methanol 85:15, v/v) of the above monoester fraction yielded 0.29 g (68%) of pure **2b** as a foam, mp: 55–56 °C; $[\alpha]_D^{20}$: −7.7 (c 1.0). ^{1}H NMR (CD_3OD): δ 4.30–4.22 (m, 3H, H-1a, H-1b and H-1′), 4.06 (m, 1H, H-2a); 3.84 (d, 1H, $J_{3',\ 4'}$ = 3.0 Hz, H-4′), 3.80 (m, 1H, H-2b), 3.77–3.69 (m, 2H, H-6′a and H-6′b), 3.56–3.50 (m, 2H, H-5′ and H-2′), 3.47 (dd, 1H, $J_{2',\ 3'}$ = 9.0 Hz, H-3′), 2.34 (m, 2H, CH_2), 1.63 (m, 2H, CH_2), 1.38–1.28 (m, 4H, 2CH_2), 0.92 (m, 3H, CH_3). ^{13}C NMR (CD_3OD): δ 175.6; 105.2; 76.7; 74.9; 72.5; 70.3; 68.5; 64.8; 62.5; 34.9; 32.4; 25.7; 23.4; 14.3; MS: m/z 340 $[M + NH_4]^+$.

Flash chromatography (dichloromethane–methanol 85:15, v/v) of LPS catalyzed transesterification yielded 0.08 g (14%) of the diester **2d**, 0.30 g (69%) of a monoester fraction and 0.025 g (8%) of unreacted **2a**. Flash chromatography (dichloromethane–methanol 85:15, v/v) of the monoester fraction afforded 0.29 g (67%) of pure **2c**, mp: 113–114 °C (methylene chloride–methanol); $[\alpha]_D^{20}$: −0.7 (c 1.0); ^{1}H NMR (CD_3OD): δ 4.30 (dd, 1H, $J_{6'a,\ 6'b}$ = 11.0 Hz, $J_{5',\ 6'a}$ = 7.0 Hz, H-6′a), 4.26 (d, 1H, $J_{1',\ 2'}$ = 8.0 Hz, H-1′), 4.22 (dd, 1H, $J_{5',\ 6'b}$ = 5.0 Hz, H-6′b), 3.91 (m, 1H, H-1a); 3.80 (d, 1H, $J_{3',\ 4'}$ = 3.0 Hz, H-4′), 3.74 (dd, 1H, H-5′), 3.72–3.63 (m, 3H, H-1b, H-2a and H-2b), 3.55 (dd, 1H, $J_{2',\ 3'}$ = 9.0 Hz, H-2′), 3.50 (dd, 1H, H-3′), 2.34 (m, 2H, CH_2), 1.63 (m, 2H, CH_2), 1.38–1.28 (m, 4H, 2CH_2), 0.92 (m, 3H, CH_3). ^{13}C NMR (CD_3OD): δ 175.2; 105.1; 74.6; 74.0; 72.5 (2C); 70.3; 64.7; 62.4; 34.9; 32.4; 25.7; 23.4; 14.3; MS: m/z 340 $[M + NH_4]^+$.

Nonyl β-D-galactopyranoside (4). β-D-Galactose pentaacetate (2.00 g, 5.12 mmol) was dissolved in dry dichloromethane (40 mL) together with nonanol (2.7 mL, 15.5 mmol) and boron trifluoride diethyl etherate (2.1 mL, 16.7 mmol) was added dropwise at 0 °C. The reaction mixture was kept under stirring overnight at room temperature, and then quenched by addition of saturated $NaHCO_3$ solution (30 mL). The aqueous layer was extracted with dichloromethane (3 × 15 mL) and the combined organic layers were then washed with brine, dried, and evaporated under reduced pressure. Flash chromatography (petroleum ether–ethyl acetate 75:25, v/v) of the crude reaction mixture yielded 1.30 g (54%) of nonyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside, oil; $[\alpha]_D^{20}$: −12.2 (c 1.0, chloroform). ^{1}H NMR ($CDCl_3$): δ 5.33 (d, 1H, $J_{3',\ 4'}$ = 3.0 Hz, H-4′), 5.14 (dd, 1H, $J_{1',\ 2'}$ = 8.0 Hz, $J_{2',\ 3'}$ = 10.0 Hz, H-2′), 4.96 (dd, 1H, H-3′), 4.40 (d, 1H, H-1′), 4.13 (dd, 1H, $J_{6'a,\ 6'b}$ = 12.0 Hz, $J_{5',\ 6'a}$ = 6.0 Hz, H-6′a), 4.07 (dd, 1H, $J_{5',\ 6'b}$ = 7.0 Hz, H-6′b), 3.88–3.79 (m, 2H, H-5′ and OCHa), 3.42 (m, 1H, OCHb), 2.09 (s, 3H, CH_3CO), 1.98 (s, 6H, 2CH_3CO), 1.92 (s, 3H, CH_3CO), 1.51 (m, 2H, CH_2), 1.30–1.16 (m, 12H, 6CH_2), 0.82 (m, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 171.0; 170.9; 170.8; 170.0; 102.0; 71.6; 71.2; 70.9; 69.6; 67.7; 61.9; 32.5; 30.2; 30.0 (2C); 29.9; 26.4; 23.3; 21.3 (4C); 14.7; MS: m/z 492 $[M + NH_4]^+$.

The obtained tetraacetyl galactoside (1.30 g, 2.76 mmol) was dissolved in methanol (4 mL) and a solution (12 mL, 0.70 M) of sodium methoxide in methanol was added under stirring at room temperature. After 2 h the reaction mixture was neutralized by adding an ion exchange resin (Dowex 50 × H^+). The resin was filtered off and the solvent removed under vacuum. Crystallization of the crude residue (0.85 g) yielded 0.75 g (89%) of the desired nonyl β-D-galactopyranoside (**4**), mp: 147–148 °C (acetone); $[\alpha]_D^{20}$: −15.8 (*c* 1.0). ^{1}H NMR (CD_3OD): δ 4.21 (d, 1H, $J_{1',2'}$ = 7.4 Hz, H-1′), 3.88 (m, 1H, OCHa), 3.83 (d, 1H, $J_{3',4'}$ = 3.0 Hz, H-4′), 3.75 (dd, 1H, $J_{6'a,6'b}$ = 11.0 Hz, $J_{5',6'a}$ = 6.6 Hz, H-6′a), 3.73 (dd, 1H, $J_{5',6'b}$ = 5.8 Hz, H-6′b), 3.54 (m, 1H, OCHb), 3.52–3.47 (m, 2H, H-2′ and H-5′), 3.46 (dd, 1H, $J_{2',3'}$ = 9.6 Hz, H-3′), 1.62 (m, 2H, CH_2), 1.44–1.24 (m, 12H, 6CH_2), 0.90 (m, 3H, CH_3). ^{13}C NMR (CD_3OD): δ 105.0; 76.6; 75.1; 72.6; 70.8; 70.3; 62.5; 33.1; 30.8; 30.7 (2C); 30.5; 27.1; 23.7; 14.5; MS: *m*/*z* 324 $[M + NH_4]^+$.

Biological evaluation

Short-term in vitro bioassay for anti-tumor promoters. All the compounds were tested for their anti-tumor promoting activity using a short-term in vitro assay for EBV-EA activation in Raji cells, cultivated in RPMI 1640 medium containing 10% fetal calf serum, as described in refs 12 and 13. Raji cells (1×10^6/mL) were incubated at 37 °C for 48 h in 1 mL of a medium containing *n*-butyric acid (4 mM), TPA (32 pmol) and a known amount of the test compound. The inhibitory activity of the test compounds was expressed by comparison of the average EBV-EA induction of each experiment, observed by indirect immunofluorescence,[12,13] with that of positive control experiments (100%) carried out with *n*-butyric acid (4 mM) and TPA (32 pmol) only, in which EBV-EA induction was ordinarily around 30%. In each assay at least 500 cells were counted and the assays were performed in triplicate for each compound.

No sample exhibited significant toxicity against Raji cells. The viability of the cells was tested against treated cells using the Trypan blue staining method. For the determination of cytotoxicity, the cell viability was required to be more than 60% 2 days after treatment with the compounds for an accurate result. The results are reported in Table 2.

Acknowledgements

This study was supported in part by the Ministero dell'Università e della Ricerca Scientifica e Tecnologica, Rome, Italy (COFIN 2001: Antitumoral natural and related synthetic compounds) and University of Milan (Italy) and in part by Grants-in-Aid from the Ministry of Education, Science and Culture, and the Ministry of Health and Welfare (Japan).

References and Notes

1. Colombo, D.; Scala, A.; Taino, I. M.; Toma, L.; Ronchetti, F.; Tokuda, H.; Nishino, H.; Nagatsu, A.; Sakakibara, J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1187.
2. Colombo, D.; Scala, A.; Taino, I. M.; Toma, L.; Ronchetti, F.; Tokuda, H.; Nishino, H.; Nagatsu, A.; Sakakibara, J. *Cancer Lett.* **1998**, *123*, 83.
3. Colombo, D.; Compostella, F.; Ronchetti, F.; Scala, A.; Toma, L.; Tokuda, H.; Nishino, H. *Bioorg. Med. Chem.* **1999**, *7*, 1867.
4. Colombo, D.; Compostella, F.; Ronchetti, F.; Scala, A.; Toma, L.; Mukainaka, T.; Nagatsu, A.; Konoshima, T.; Tokuda, H.; Nishino, H. *Cancer Lett.* **1999**, *143*, 1.
5. Colombo, D.; Compostella, F.; Ronchetti, F.; Scala, A.; Toma, L.; Kuchide, M.; Tokuda, H.; Nishino, H. *Cancer Lett.* **2000**, *161*, 201.
6. Colombo, D.; Compostella, F.; Ronchetti, F.; Scala, A.; Toma, L.; Tokuda, H.; Nishino, H. *Eur. J. Med. Chem.* **2000**, *35*, 1109.
7. Colombo, D.; Compostella, F.; Ronchetti, F.; Scala, A.; Tokuda, H.; Nishino, H. *Eur. J. Med. Chem.* **2001**, *36*, 691.
8. Colombo, D.; Compostella, F.; Ronchetti, F.; Reza-Elahi, S.; Scala, A.; Toma, L.; Aoi, W.; Kuchide, M.; Takayasu, J.; Tokuda, H.; Nishino, H. *Cancer Lett.* **2002**, *186*, 37.
9. Prokazova, N. V.; Todriya, K. G.; Vaver, V. A.; Bergelson, L. D. *Bull. Acad. Sci. USSR Div. Chem. Sci.* **1974**, *23*, 2476.
10. Colombo, D.; Ronchetti, F.; Scala, A.; Toma, L. *Tetrahedron: Asymmetry* **1998**, *9*, 2113.
11. Murakami, A.; Ohigashi, H.; Koshimizu, K. *Food Rev. Int.* **1999**, *15*, 335.
12. Shirahashi, H.; Murakami, N.; Watanabe, M.; Nagatsu, A.; Sakakibara, J.; Tokuda, H.; Nishino, H.; Iwashima, A. *Chem. Pharm. Bull.* **1993**, *41*, 1664.
13. Tokuda, H.; Konoshima, T.; Kozuka, M.; Kimura, T. *Cancer Lett.* **1988**, *40*, 309.
14. Colombo, D.; Ronchetti, F.; Scala, A.; Taino, I. M.; Marinone Albini, F.; Toma, L. *Tetrahedron: Asymmetry* **1994**, *5*, 1377.
15. Colombo, D.; Ronchetti, F.; Scala, A.; Taino, I. M.; Toma, L. *Tetrahedron: Asymmetry* **1996**, *7*, 771.