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Il Farmaco 58 (2003) 1163–1169
IL FARMACO
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A novel sunscreen agent having antimelanoma activity☆

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Received 14 February 2003; accepted 10 July 2003

Abstract

A novel series of eight dibenzoylmethane derivatives having both sunscreen and cytotoxic activity has been obtained by derivatizing commercial dibenzoyl methanes. Four human cancer cell lines (MCF 7 (breast), NCI ADR (breast expressing the multidrug resistance phenotype), NCI 460 (lung) and UACC 62 (melanoma)) were used for the cytotoxic assay. Eight among the 19 dibenzoylmethane derivatives showed cytotoxicity against these four cell lines. Absorption spectroscopies revealed that these compounds can be used as sunscreens against UV radiation.

Keywords: Dibenzoylmethane derivatives; UV sunscreen; Cancer; Melanoma

1. Introduction

Skin cancer is one of the common malignancies in man, showing increasing occurrence in Europe and in the United States. There are three recognized types of skin cancer: basal cell carcinoma, squamous carcinoma, and melanoma. There is evidence from analytical studies that the increase in melanoma incidence is the result of intermittent recreational sun exposure and severe sunburns, which are particularly harmful in childhood [1]. These cancers frequently occur on the sun-exposed areas (face, ears, scalp, etc.). Consequently, broad-spectrum sunscreens displaying appropriate UVA (320–400 nm), UVB (280–320 nm) and UVC (200–280 nm) protection and novel mechanisms of action are in demand to combat this disease [1].

Dibenzoylmethane (DBM, 1,3-diphenyl-propanedione), shown in Fig. 1, is a small β-diketone compound and sunscreen, which display anti-inflammatory and anti-tumor activities. In female rats, DBM was an effective inhibitor of in vivo mammary tumors promoted by 7,12-dimethylbenz[*a*]anthracene and of 7,12-tetradenoylphorbol-13-acetate-induced skin tumors in mice [2]. Recently, the effect of DBM on prostate cancer cell growth has been reported [3]. The sunscreen derivatives from DBM, Eusolex® 8020 and Parsol® 1789, are more efficient in the UVA range because they prevent the penetration of the radiation to vital cell components and block over production of oxygen-derived free radicals [4]. The use of sunscreen products has been recommended by many health care organizations as a means to reduce skin damage produced by UV radiation from sunlight [1].

In the natural products realm, DBM and its derivatives have been found as constituents of some plants belonging to the Leguminosae family, as minor constituent of licorice [5], and are classified as a rare kind of flavonoid. The natural DBM **4** was reported for the first time in *Lonchocarpus latifolius* (Wild) DC and showed biological activity [6,7].

Compounds protecting human cells against UV radiation (sunscreen) and against skin cancer would certainly find application in both medicine and cosmetics. This prompted us to search for 2-alkyl-1,3-

☆ Patent request: INPI 6583-8 (Brazil).

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0014-827X/03/$ - see front matter
doi:10.1016/S0014-827X(03)00195-2

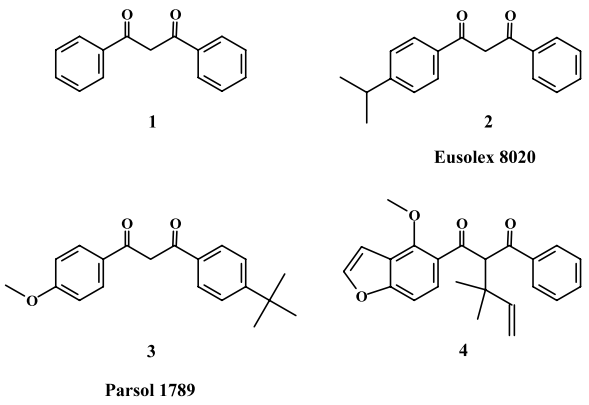

Fig. 1. Chemical structures of DBM (**1**) and derivatives.

diphenyl-propanedione derivatives (DBM derivatives) with chemopreventive and photoprotection effects against skin cancer (melanoma) and UVB/UVC radiation, respectively. This paper describes the synthesis of various DBM derivatives (**5**–**22**), shown in Fig. 2, and the evaluation of their anticancer activity.

2. Chemistry

Compounds **5**–**13** were obtained by alkylation of DBM (**1**), Eusolex® 8020 (**2**) and Parsol® 1789 (**3**) using potassium carbonate in acetone (Araújo, 1996). These conditions were chosen viewing future scale up of active compounds. The products were characterized by high-resolution mass spectrometry (HRMS) and ^{1}H and ^{13}C NMR spectroscopies. Alkylation of the DBMs **1**–**3** drastically inhibited the keto-enolic equilibrium present in these compounds. The major spectroscopic evidences are the lack of a chelated enol hydrogen at δ 12–17 in the ^{1}H NMR spectra and the hypsochromic shift of the UV maximum absorption bands, which shifted from $\lambda_{max} \approx 350$ nm (for **1**–**3**) to $\lambda_{max} \approx 250$ nm (for **5**–**13**). This phenomenon was assigned to the steric strain of the enolic form that now has two substituents located on the double bond, as shown in Fig. 3, thereby diminishing the enol contribution in the keto-enolic equilibrium, which is physically evident in the hypsochromic shift in the UV. This effect limited the application of compounds (**5**, **6**, **7**, **10**, **13**) to UVB/UVC sunscreen agents while maintaining their potentiality as cytotoxic compounds.

A second series of derivatives was obtained by reducing the diketones **5**–**13** with $NaBH_4$ at $-10\,^{\circ}C$ in methanol producing ketols (**14**–**19**) and diols (**20**–**22**). The ^{1}H NMR spectra of all derivatives showed absorption at ca. δ 4.00 corresponding to the carbinolic protons. The ketols (**14**–**19**) were produced as *syn* and *anti* mixtures with variable diastereomeric ratios, with a predominance of the *anti* isomer. The relative stereochemistry of the major diol **22** was determined by ^{13}C NMR as 1,2-*syn* and 2,3-*anti*. DBM asymmetric reduction, relative stereochemistry and full ^{1}H NMR and ^{13}C NMR details will be published elsewhere.

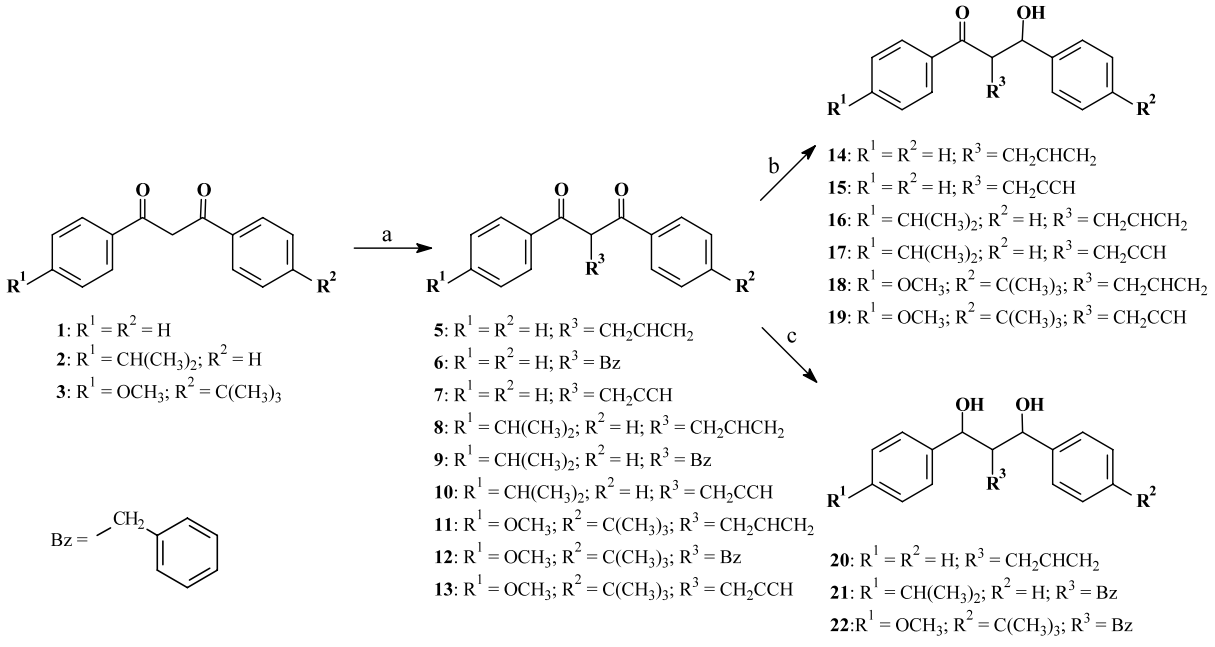

Fig. 2. Obtention of DBM derivatives. [Reaction conditions: (a) R^3Br, K_2CO_3, $(CH_3)_2CO$; (b) $NaBH_4$ (1 equiv.), MeOH, $-10\,^{\circ}C$; (c) $NaBH_4$ (excess), MeOH, ta.].

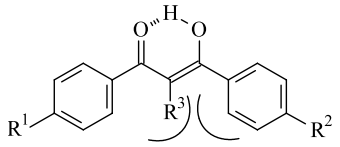

Fig. 3. The steric strain of the enolic form that now has two substituents located on the double bond.

3. Experimental

^{1}H and ^{13}C NMR spectra were obtained on a Varian Inova-500 spectrometer (11.74 T) with standard pulse sequences operating at 499.88 and 125.7 MHz for ^{1}H and ^{13}C, respectively. The chemical shifts (δ) are reported in ppm using TMS (δ 0) and residual $CHCl_3$ (δ 77.00) signals as internal references for ^{1}H and ^{13}C NMR, respectively. The coupling constant (J) are in hertz. All NMR samples (0.6 ml) were prepared in $CDCl_3$. IR data were measured on a Perkin–Elmer model 1600 (FTIR) spectrometer using KBr pellets or film. Mass spectra (HRMS) were recorded on a VG Auto Spec (10 000) instrument using a 70 eV ionizing potential.

3.1. Synthesis of the DBM derivatives: general procedures

3.1.1. Alkylation of DBM derivatives (*1*–*3*) [8]

Potassium carbonate (3.0 equiv.) and DBM (1.0 equiv.) were suspended in acetone (20 ml) and stirred for 30 min at room temperature (r.t.). To this mixture the alkylating reagent (alkyl bromide or benzyl bromide or propargyl bromide, 1.0 equiv.) was added and the mixture was stirred at r.t. overnight. The mixture was filtered, the solvent was evaporated and the product was purified by preparative TLC (SiO_2) using *n*-hexane/AcOEt 80:20 v/v as eluent.

3.1.2. Reduction of DBM derivatives (*5*–*13*)

DBM derivatives **5**–**13** (1.0 equiv.) were suspended in MeOH (3 ml). To this mixture, $NaBH_4$ (1.0 equiv. for monoreduction or excess amounts for total reduction) was added and it was stirred at -10 °C for 30 min. The mixture was monitored by TLC (CH_2Cl_2), evaporated and purified by preparative TLC using CH_2Cl_2 as eluent.

3.1.3. Spectroscopic data of the eight compounds (Fig. 4) that showed cytotoxicity against the human cancer cell lines evaluated

(±)-1,3-Diphenyl-2-allyl-1,3-propanedione (**5**) was obtained as a colorless solid in 76% yield; m.p.: 64.9–65.7; $R_f = 0.55$ (*n*-hexane:ethyl acetate 8:2); IR (KBr, cm^{-1}): 1694.8, 1670.2, 1007.7, 962.2; ^{1}H NMR: δ_H (500 MHz; $CDCl_3$; TMS) 2.88 (2H, tt, J 6.7 and 1.3, H-1″), 5.03 (1H, dtt, J 10.1, 1.5 and 1.3, H-3b″), 5.10 (1H, dtt, J 17.1, 1.5 and 1.3, H-3a″), 5.30 (1H, t, J 6.7, H-2), 5.87 (1H, ddt, J 17.1, 10.1 and 6.7, H-2″), 7.45 (4H, tt, 7.8 and 1.3, H-3′ and H-5′), 7.57 (2H, tt, 7.5 and 1.3, H-4′), 7.96 (4H, dd, J 8.5 and 1.3, H-2′ and H-6′); ^{13}C NMR: δ_C (125.8 MHz; $CDCl_3$; TMS) 33.57 (CH_2, C-1″), 56.76 (CH, C-2), 117.26 (CH_2, C-3″), 128.61 (4CH, C-3′ and C-5′), 128.91 (4CH, C-2′ and C-6′), 133.57 (2CH, C-4′), 135.08 ($2C_o$, C-1′), 135.98 (CH, C-2″), 195.55 ($2C_o$, C-1 and C-3); MS [m/z (% rel. int.)]: 159.07 (12), 142.70 (6), 105.02 (100); HRMS: Calc. for $C_{18}H_{16}O_2$ 264.1150, observed 264.1153.

(±)-1,3-Diphenyl-2-benzyl-1,3-propanedione (**6**) was obtained as a colorless solid in 85% yield; m.p.: 58.3–58.7; $R_f = 0.74$ (*n*-hexane:ethyl acetate 8:2); IR (KBr, cm^{-1}): 3031.33, 1694.20, 1664.60, 999.3; ^{1}H NMR: δ_H (500 MHz; $CDCl_3$; TMS) 3.45 (2H, d, J 6.7, $CH_{2\ Bz}$), 5.52 (1H, t, J 6.7, H-2), 7.16 (1H, tt, 6.6 and 2.0, H-4″), 7.24 (4H, m, H-2″, H-3″, H-5″ and H-6″), 7.40 (4H, tt, J 7.9 and 1.3, H-3′ and H-5′), 7.53 (2H, tt, 7.5 and 1.3, H-4′), 7.89 (4H, dd, J 8.5 and 1.3, H-2′ and H-6′); ^{13}C NMR: δ_C (125.8 MHz; $CDCl_3$; TMS) 35.21 ($CH_{2\ Bz}$), 58.99 (CH, C-2), 126.63 (CH, C-4″), 128.60 (4CH, C-3′ and C-5′), 128.84 (4CH, C-2″, C-3″, C-5″ and C-6″), 128.98 (4CH, C-2′ and C-6′), 133.51 (C_o, C-1″), 135.98 (2CH, C-4′), 139.05 ($2C_o$, C-1′), 195.58 ($2C_o$, C-1 and C-3); MS [m/z (% rel. int.)]: 209.09 (100), 131.05 (7), 105.03 (90); HRMS: Calc. for $C_{22}H_{18}O_2$ 314.1307, observed 314.1306.

(±)-1,3-Diphenyl-2-propargyl-1,3-propanedione (**7**) was obtained as a yellow solid in 82% yield; m.p.: 95.2–95.9; $R_f = 0.47$ (*n*-hexane:ethyl acetate 8:2); IR (KBr, cm^{-1}): 3300.0, 1685.40, 1655.60, 977.8; ^{1}H NMR: δ_H (500 MHz; $CDCl_3$; TMS) 1.98 (1H, t, J 2.7, H-3″), 3.01 (2H, dd, J 7.0 and 2.7, H-1″), 6.49 (1H, t, J 7.0, H-2), 7.45 (4H, tt, 7.8 and 1.3, H-3′ and H-5′), 7.58 (2H, tt, 7.5 and 1.3, H-4′), 7.98 (4H, dd, J 8.5 and 1.3, H-2′ and H-6′); ^{13}C NMR: δ_C (125.8 MHz, $CDCl_3$; TMS) 19.05 (CH_2, C-1″), 55.56 (CH, C-2), 70.90 (CH, C-3″), 81.02 (C_o, C-2″), 128.78 (4CH, C-3′ and C-5′), 128.92 (4CH, C-2′ and C-6′), 133.80 (2CH, C-4′), 135.75 ($2C_o$, C-1′), 194.43 ($2C_o$, C-1 and C-3); MS [m/z (% rel. int.)]: 262.10 (26), 105.03 (100), 77.03 (43); HRMS: Calc. for $C_{18}H_{14}O_2$ 262.0994, observed 262.0999.

(±)-1-[(4-Isopropyl)phenyl]-2-allyl-3-phenyl-1,3 propanedione (**8**) was obtained as a colorless oil in 80% yield; $R_f = 0.59$ (*n*-hexane:ethyl acetate 8:2); IR (Film, cm^{-1}): 1693.64, 1668.49, 1000.72; ^{1}H NMR: δ_H (500 MHz; $CDCl_3$; TMS) 1.25 (6H, d, J 7.0, H-8′), 2.87 (2H, m, H-1‴), 2.95 (1H, hept., J 7.0, H-7′), 5.02 (1H, dtt, J 10.1, 1.7 and 1.3, H-3b‴), 5.10 (1H, dtt, J 17.1, 1.7 and 1.3, H-3a‴), 5.28 (1H, t, J 6.7, H-2), 5.88 (1H, ddt, J 17.1, 10.1 and 6.7, H-2‴), 7.31 (2H, d, J 8.4, H-3′ and H-5′), 7.46 (2H, t, J 7.7, H-3″ and H-5″), 7.58 (1H, tt, J 7.5 and 1.3, H-4″), 7.92 (2H, d, J 8.4, H-2′ and H-6′), 7.98 (2H, dd, J 8.5 and 1.3, H-2″ and H-6″); ^{13}C NMR: δ_C (125.8 MHz, $CDCl_3$; TMS) 23.58 (CH_3, C-8′), 33.58

(CH_2, C-1‴), 34.23 (CH, C-7′), 56.63 (CH, C-2), 117.11 (CH_2, C-3‴), 127.03 (2CH, C-3′ and C-5′), 128.59 (2CH, C-2″ and C-6″), 128.86 (2CH, C-3″ and C-5″), 128.89 (2CH, C-2′ and C-6′), 133.49 (CH, C-4″), 133.74 (C_o, C-1′), 135.22 (C-2‴), 136.03 (C_o, C-1″), 155.18 (C_o, C-4′), 195.13 (C_o, C-1), 195.65 (C_o, C-3); MS [*m*/*z* (% rel. int.)]: 147.07 (100), 105.03 (35), 77.03 (16); HRMS: Calc. for $C_{21}H_{22}O_2$ 306.1619, observed 306.1620.

(±)-1-[(4-Isopropyl)phenyl]-2-propargyl-3-phenyl-1,3-propanedione (**10**) was obtained as a colorless oil in 60% yield; $R_f = 0.49$ (*n*-hexane:ethyl acetate 8:2); IR (Film, cm^{-1}): 3297.22, 1693.99, 1666.24, 1323.57, 836.60; ^1H NMR: δ_H (500 MHz; $CDCl_3$; TMS) 1.25 (6H, d, *J* 7.0, H-8′), 1.98 (1H, t, *J* 2.7, H-3‴), 2.95 (1H, hept., *J* 7.0, H-7′), 3.00 (2H, dd, *J* 7.0 and 2.7, H-1‴), 5.46 (1H, t, *J* 7.0, H-2), 7.30 (2H, d, *J* 8.4, H-3′ and H-5′), 7.45 (2H, tt, *J* 7.7 and 1.3, H-3″ and H-5″), 7.57 (1H, tt, *J* 7.5 and 1.3, H-4″), 7.92 (2H, d, *J* 8.4, H-2′ and H-6′), 7.99 (2H, dd, *J* 8.5 and 1.3, H-2″ and H-6″); ^{13}C NMR: δ_C (125.8 MHz, $CDCl_3$; TMS) 19.05 (CH_2, C-1‴), 23.57 (CH_3, C-8′), 34.26 (CH_3, C-7′), 55.53 (CH, C-2), 70.79 (CH, C-3‴), 81.15 (C_o, C-2‴), 127.05 (2CH, C-3′ and C-5′), 128.78 (2CH, C-3″ and C-5″), 128.88 (2CH, C-2″ and C-6″), 129.07 (2CH, C-2′ and C-6′), 133.46 (C_o, C-1′), 133.72 (C_o, C-4″), 135.79 (C_o, C-1″), 155.49 (C_o, C-4′), 193.95 (C_o, C-1), 194.55 (C_o, C-3); MS [*m*/*z* (% rel. int.)]: 147.08 (100), 105.03 (48) 304.14 (6); HRMS: Calc. for $C_{21}H_{20}O_2$ 304.1463, observed 304.1463.

(±)-1-(4-Methoxyphenyl)-2-propargyl-3-[(4-tertbutyl)phenyl]-1,3-propanedione (**13**) was obtained as a yellow solid in 61% yield; m.p.: 79.3–79.7; $R_f = 0.53$ (*n*-hexane:ethyl acetate 8:2); IR (KBr, cm^{-1}): 1604.78, 1508.01, 1024.50, 843–795; ^1H NMR: δ_H (500 MHz; $CDCl_3$; TMS) 1.31 (9H, s, H-8″), 1.97 (1H, t, *J* 2.7, H-3‴), 2.97 (1H, ddd, *J* 17.1; 7.0 and 2.7, H-1a‴), 3.01 (1H, ddd, *J* 17.1; 7.0 and 2.7, H-1b‴), 3.85 (3H, s, H-7′), 5.39 (1H, t, *J* 7.0, H-2), 6.93 (2H, d, *J* 9.0, H-3′ and H-5′), 7.45 (2H, d, *J* 8.6, H-3″ and H-5″), 7.91 (2H, d, *J* 8.6, H-2″ and H-6″), 8.00 (2H, d, *J* 9.0, H-2′ and H-6′); ^{13}C NMR: δ_C (125.8 MHz, $CDCl_3$; TMS) 19.11 (CH_2, C-1‴), 31.00 (CH_3, C-8″), 35.17 (C_o, C-7″), 55.49 (CH, C-2), 55.54 (CH_3, C-7′), 70.67 (CH, C-3‴), 81.36 (C_o, C-2‴), 114.11 (2CH, C-3′ and C-5′), 125.86 (2CH, C-3″ and C-5″), 128.72 (2CH, C-2″ and C-6″), 128.78 (C_o, C-1′), 131.27 (2CH, C-2′ and C-6′), 133.16 (C_o, C-1″), 157.55 (C_o, C-4″), 164.05 (C_o, C-4′), 193.09 (C_o, C-1), 194.03 (C_o, C-3); MS [*m*/*z* (% rel. int.)]: 310.15 (100), 253.08 (10), 135.03 (95), 161.08 (49), 108.04 (27); HRMS: Calc. for $C_{23}H_{24}O_3$ 348.1725, observed 348.1725.

1,3-Diphenyl-2-allyl-1,3-propanediol (**20**) was obtained as colorless crystal in 70% yield; m.p.: 118.6–120.3; $R_f = 0.47$ (*n*-hexane:ethyl acetate 8:2); IR (KBr, cm^{-1}): 3286.49, 3026.05, 2904.39, 1638.23, 1599.08, 1305.53, 1197.31, 1082.99, 1059.28, 997.71. ^1H NMR: δ_H (500 MHz; $CDCl_3$; TMS) 1.99 (1H, dt, *J* 13.5, 5.8 and 1.7, H-1a″), 2.03 (1H, ddd, *J* 9.3, 5.0 and 1.7, H-2), 2.30 (1H, dt, *J* 13.5 and 8.3, H-1a″), 5.02 (1H, dd, *J* 10.1 and 1.5, H-3b‴), 5.04 (1H, dt, *J* 17.1 and 1.5, H-3a‴), 5.68 (1H, dddd, *J* 17.1, 10.1, 8.1 and 5.8, H-2‴), 7.19 (4H, t, 7.8, H-3′, H-5′, H-3″ and H-5″), 7.28 (2H, t, 7.5, H-4′ and H-4″), 7.38 (4H, d, *J* 8.5, H-2′, H-6′, H-2″ and H-6″); ^{13}C NMR: δ_C (125.8 MHz, $CDCl_3$; TMS) 28.96 (CH_2, C-1‴), 51.09 (CH, C-2), 72.72 (CH, C-3), 74.90 (CH, C-1), 116.89 (CH_2, C-3‴), 125.74 (2CH, C-3″ and C-5″), 125.80 (2CH, C-3′ and C-5′), 128.06 (2CH, C-2″ and C-6″), 128.51 (2CH, C-2′ and C-6′), 126.94 (CH, C-4″), 127.40 (CH, C-4′), 137.14 (CH, C-2‴), 142.52 (C_o, C-1″), 143.55 (C_o, C-1′); MS [*m*/*z* (% rel. int.)]: 209.09 (23), 143.09 (26), 129.07 (100), 107.05 (28), 77.04 (30); HRMS: Calc. for C_5H_7O 107.04969, observed 107.04944.

(±)-1-(4-Methoxyphenyl)-2-benzyl-3-[(4-tertbutyl)phenyl]-1,3-propanediol (**23**) was obtained as a colourless oil in 90% yield; $R_f = 0.34$ (*n*-hexane:ethyl acetate 8:2); IR (KBr, cm^{-1}): 3443.89, 2961.70, 2923.17, 2866.57, 1655.14, 1601.69, 1571.48, 1455.05, 1250.71, 1175.96, 1033.17, 944.48; ^1H NMR: δ_H (500 MHz; $CDCl_3$; TMS) 1.31 (9H, s, H-8″), 2.23 (1H, dtd, *J* 10.2; 4.5 and 2.7, H-2), 2.54 (1H, dd, *J* 13.6 and 4.5, H-7a‴), 2.82 (1H, dd, *J* 13.6 and 10.2, H-7b‴), 3.24 (1H, d, *J* 4.5, OH-3), 3.80 (3H, s, H-7′), 3.96* (1H, d, *J* 2.7, H-3), 4.75* (1H, t, *J* 4.5, H-1), 4.96 (1H, sl, OH-1), 6.86 (2H, d, *J* 9.0, H-3′ and H-5′), 7.06 (2H, d, *J* 7.5, H-2‴ and H-6‴), 7.15 (1H, t, *J* 7.5, H-4‴), 7.18 (2H, d, *J* 8.6, H-3″ and H-5″), 7.22 (2H, d, *J* 8.6, H-2″ and H-6″), 7.23 (2H, t, *J* 7.5, H-3‴ and H-5‴), 7.34 (2H, d, *J* 9.0, H-2′ and H-6′); ^{13}C NMR: δ_C (125.8 MHz, $CDCl_3$; TMS) 31.36 ($3CH_3$, C-8″), 30.44 (C_o, C-7″), 53.19 (CH, C-2), 55.25 (CH_3, C-7′), 72.43* (CH, C-3), 74.29* (CH, C-1), 113.49 (2CH, C-3′ and C-5′), 125.31** (2CH, C-3″ and C-5″), 125.34** (2CH, C-3‴ and C-5‴), 126.89 (2CH, C-2″ and C-6″), 128.36 (2CH, C-2‴ and C-6‴), 129.02 (2CH, C-2′ and C-6′), 134.73 (C_o, C-1‴), 140.38# (C_o, C-1′), 140.72# (C_o, C-1″), 150.19 (C_o, C-4″), 158.51 (C_o, C-4′); MS [*m*/*z* (% rel. int.)]: 241.12 (15), 209.10 (13), 161.09 (27), 135.05 (100); HRMS: Calc. for $C_{18}H_{18}O_2$ 241.12285, observed 241.12291.

3.2. Cytotoxic activity

3.2.1. Cell culture

The experiments were performed using the following human cancer cell lines: MCF 7 (breast), NCI ADR (breast expressing the multidrug resistance phenotype), NCI 460 (lung) and UACC 62 (melanoma). The National Cancer Institute, Washington, USA (NCI), kindly donated these cell lines; and stock cultures were kept in liquid nitrogen.

Cells were cultured in 25-cm^2 flasks (Nunc Brand Products) containing 5 ml of RPMI 1640 (Gibco BRL,

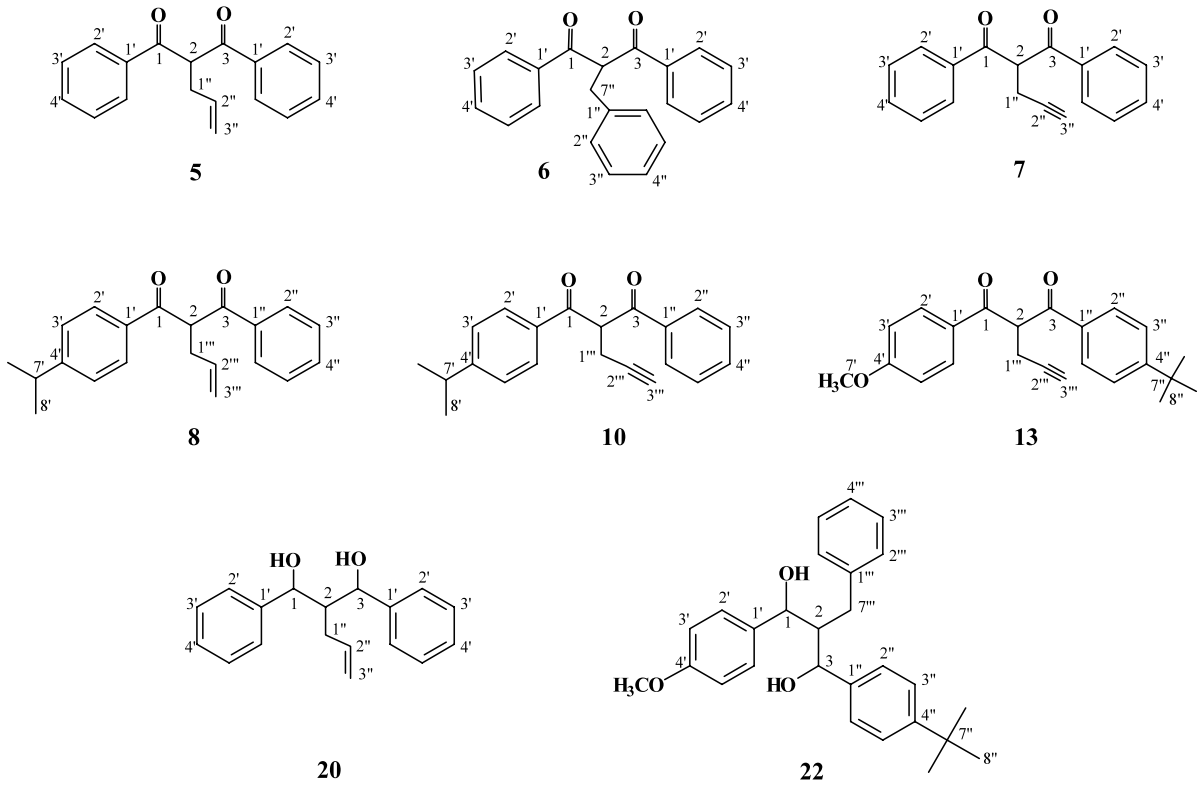

Fig. 4. Compounds that showed activity against human cancer cell line K 562 (leukemia), MCF 7 (breast), NCI ADR (breast expressing the multidrug resistance phenotype), NCI 460 (lung) and UACC 62 (melanoma).

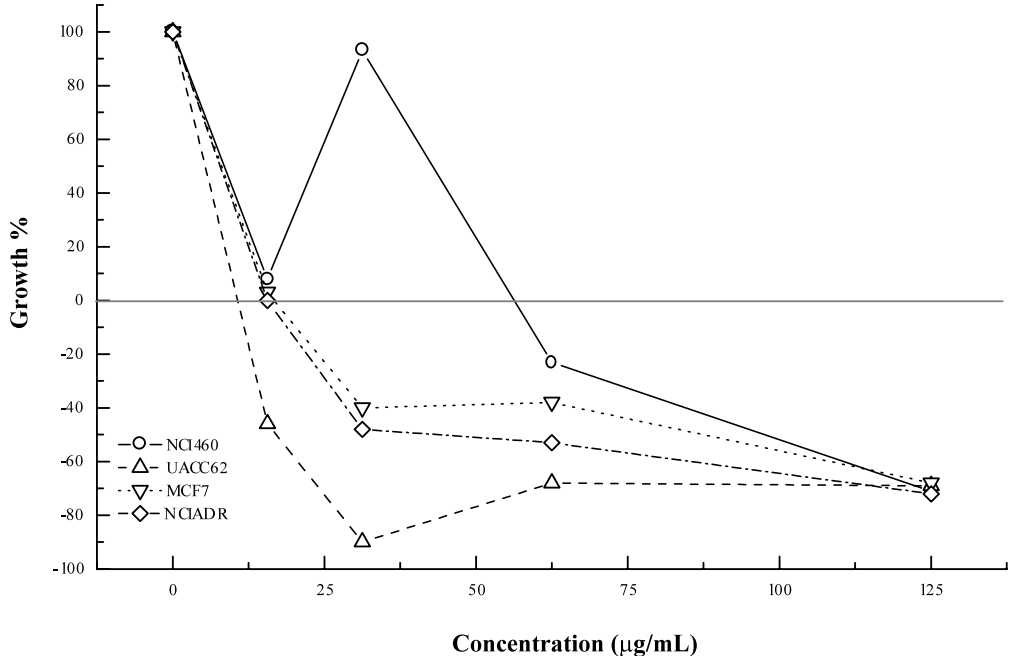

Fig. 5. Growth inhibitory effects of DMB derivative **22** against human cancer cell line MCF 7 (breast), NCI ADR (breast expressing the multidrug resistance phenotype), NCI 460 (lung) and UACC 62 (melanoma) as a function of increasing concentration. Viable cells were measured using an MTT assay and expressed as corrected units at 590 nm ($n = 3$).

Table 1
IC_{50} of 1,3-propanedione and 1,3-propanediol derivatives against human cancer cells

Comp.	MCF 7 mama cancer cells	NCI 460 lung cancer cells	UACC 62 melanome cancer cells	NCI ADR multidrug resistant breast cancer cells
8		55.2	198.2	74.9
7			37.66	
10	207.0		68.19	665.4
20	197.7	132.9	241.7	103.9
5	222.3		446.4	171.4
6		231.9		179.3
22	11.82	9.126	5.8	8.66
Doxorubicine	6.75	6.25	7.01	6.14

Life Technologies) with 5% fetal bovine serum (Gibco BRL, Life Technologies). Cells were diluted once a week, discarded after 20 consecutive dilutions, and then replaced by newly thawed stock from the liquid nitrogen.

3.2.2. Biological assays

All the adherent cell lines were detached from the culture flasks by addition of 0.5 ml of trypsin (Nutricell Cell Nutrient). Trypsin was inactivated by addition of 5 ml of 5% fetal bovine serum containing RPMI 1640 medium. Cells were separated into single-cell suspensions by a gentle pipetting. After counting and dilution into appropriate seeding densities, the cells were inoculated into 96-wells microtiter plates (Nunc Brand Products). The cells plating volume was 100 μl per well. Seeding densities varied among the cell lines as follows: 6.5×10^4 (MCF 7), 5×10^4 (NCI ADR), 4×10^4 (NCI 460) and 3×10^4 (UACC 62) cells per ml. Microtiter plates containing cells were pre-incubated for ca. 24 h at 37 °C in order to allow stabilization prior to the addition of 100 μl of the test substances in cell culture solutions having concentrations of 0.25, 2.5, 25.0 and 250 μg ml^{-1}. The plates were incubated with the test substance for 48 h at 37 °C and 5% CO_2.

3.2.3. Photochemical study

A Germetec UVA lamp (Cosmetex UVA-Plus), 100 W, was used as light source. Solutions with a concentration of 1.00×10^{-5} mol dm^{-3} of each DBM derivative in 50% v/v ethanol were placed in quartz cells of 1 cm pathlength at 25 °C. UV absorption spectra were recorded on a Hewlett-Packard Spectrophotometer (8452A), with diode array detection, for 540 min [9].

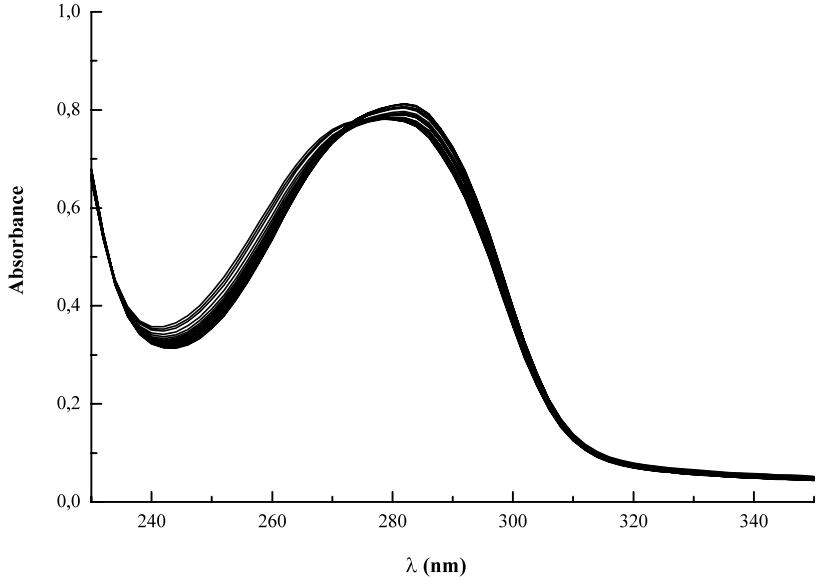

Fig. 6. Photochemical behavior of compound **22** under UVB radiation at different time intervals over 9 h, with an initial concentration of 1.00×10^{-5} mol dm^{-3} in 50% (v/v) ethanol.

4. Results and discussions

Compounds **5**–**22** were submitted to cytotoxic assay using four human cancer cell lines: MCF 7 (breast), NCI ADR (breast expressing the multidrug resistance phenotype), NCI 460 (lung) and UACC 62 (melanoma). The inhibitory effects of DBM derivatives were assessed by varying the concentrations and the results were obtained after 72 h using the MTT assay. All assays were performed in triplicate. Among the 19 compounds tested, only eight (Fig. 4) showed cytotoxicity against these four cell lines. Of these we would like to focus on the activity depicted by compound **22**, which was present against the four cell lines, as shown in Fig. 5 with an IC_{50} that is below doxorubicine for melanome cells (Table 1). Notwithstanding these results we are particularly interested in the antimelanoma results, which indicate **22** as a good UVB/UVC sunscreen with therapeutical action.

As photostability is a desired property for sunscreen agents, the stabilities of compounds **5**, **6**, **7**, **8**, **10**, **13**, **20**, **22** were further evaluated by ultraviolet absorption exposure over 9 h. The photochemical behavior of the eight candidates, measured at different time intervals, indicated that only compound **22** was highly stable under UV radiation for 9 h with a wide band near 280 nm (Fig. 6).

Thus **22** is a leading candidate for use as a UV sunscreen agent, also having therapeutical activity against melanoma cells. As far as we know, this is the first report on the inhibitory effects of DBM derivatives on cancer cell line growth as well as a photochemical evaluation for their application as UVB/UVC radiation sunscreens.

Acknowledgements

This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). M.A.N. is grateful to this foundation for a post-doctoral fellowship.

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