

CHEMICAL MODIFICATIONS OF A NATURAL XANTHONE AND ANTIMICROBIAL ACTIVITY AGAINST MULTIDRUG RESISTANT *Staphylococcus aureus* AND CYTOTOXICITY AGAINST HUMAN TUMOR CELL LINES

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A series of 15 ω -aminoalkoxyloxanthones containing methyl, ethyl, propyl, *tert*-butylamino and piperidinyl moieties were synthesized from a natural xanthone isolated from a lichen species. These compounds were tested for their *in vitro* antibacterial properties against Gram-positive and Gram-negative bacteria and cytotoxicity against a number of human tumor cell lines was too evaluated. The newly synthesized derivatives revealed selective activity against *Staphylococcus aureus* (Gram-positive), and the most promising results are for a multidrug resistant strain, for which six of these compounds showed good activity (MICs 4 μ g/mL). Many derivatives inhibited tumor cells growth and most compounds were active on multiple lines.

Keywords: xanthone; antimicrobial activity; cytotoxic activity.

INTRODUCTION

Nature continues to be a rich source of structurally diverse small organic molecules, and natural products play a significant role in the discovery and development of new drugs for the treatment of human diseases. Over 60% of approved drugs are either natural products, derivatives or based on their structures. Seventy-eight percent of antibacterials and 74% of antitumor compounds are related to natural products, and almost half of the best-selling pharmaceuticals are natural or are related to them.¹ Natural products can be highly functionalized and possess multi-functional groups with well-balanced polarity and hydrophobicity, frequently with high oxygen content. Therefore, when it's necessary chemical and biological methods are often applied to modify these natural products to optimize their desired activity and physical properties before they can be further developed as a therapeutic agent.²

Lichens are one important source of biologically active natural compounds. They are spread world-wide and live in symbiotic relationship with fungi (mycobiont), algae (phycobiont) and/or cyanobacteria (photobiont)³ and have been used in folk medicine for centuries, as traditional medicines.⁴ Many different secondary metabolites have been isolated from lichens including aliphatic, cycloaliphatic, aromatic and terpenic components that are unique with respect to those found in higher plants.⁵ Screening of lichen extracts has revealed the frequent occurrence of metabolites with antibiotic, antimycobacterial, antiviral, antitumor, analgesic,

antiallergic and antipiretic properties, and also substances that inhibit plant growth as well as several enzymes.^{4,6}

Xanthone compounds represent a class of these lichen substances, but they are widely distributed in nature,² and can also be isolated from higher plants and microorganisms.⁷ In recent years, xanthones and xanthone derivatives have been reported to have significant pharmacological activities based on their diverse structures.⁸⁻²⁰ Their interesting structural scaffold and the significant biological activities have led many scientists to isolate, modify or synthesize different xanthones for the development of prospective new drug candidates.⁷

Many xanthones bearing nitrogenated side chains connected to the phenolic core in different positions by C, O or N atoms have been synthesized during the last 10 years, and this group of molecules have showed interesting biological effects^{14,18,21-25} including antibacterial²⁶ and cytotoxic activities.^{27,28}

Based on this information and as a part of our efforts to enhance biological activities of lichen substances, we have decided to synthesize five series (dimethyl, diethyl, dipropyl, *t*-butylamine and piperidinyl) of (ω -aminoalkoxy)-xanthone derivatives from lichexanthone (**1**), a naturally occurring xanthone isolated from the lichen *Parmotrema lichexanthonicum*, and evaluate their cytotoxic activity against human tumor cell lines and their antimicrobial activity against selected bacterial strains, including multidrug resistant *Staphylococcus aureus*, aiming to establish a relationship between side chains characteristics and biological activity.

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EXPERIMENTAL

General

Silica gel (Carlo Erba 70-270 mesh) was used for column chromatography. All solvents and chemicals were analytical grade. NMR spectra were taken in a Bruker DPX-300 spectrometer (solvent is indicated for each compound; C and H atoms from side chains were consecutively numbered from 10 (for the group attached to oxygen) to 17. Mass spectra (EI, 70 eV) were run on a Shimadzu CGMS QP2010 Plus gas chromatography mass spectrometer, in direct injection mode, and melting points were recorded in a Uniscience do Brasil apparatus, model 498.

Isolation and synthesis

Isolation and demethylation (Scheme 1) of lichexanthone (**1**) were carried out as a previous described procedure.²⁹

General procedure for ω -bromoalkoxyxanthenes (**3**), (**4**) and (**5**)

ω -bromoalkoxyxanthenes (**3**), (**4**) and (**5**) were prepared by treatment of (**2**) (50 mg, 0.2 mmol) with 0.5 mL of 1,3-dibromopropane, 1,4-dibromobutane or 1,5-dibromopentane, according to the method described by Sousa *et al.*³⁰ Chromatographic column separations (hexane/ethyl acetate 2% (v/v) as eluent) gave desired compounds in good yields (Scheme 1).

3,6-bis(3-bromopropoxy)-1-hydroxy-8-methyl-9H-xanthen-9-one (**3**)

Yield 65%; Mp: 139-140 °C (from CHCl₃); ¹H NMR (300 MHz; CDCl₃): δ = 2.35 (H-11, m, 4H), 2.81 (CH₃-Ph, s, 3 H), 3.60 (H-12, m, 4H), 4.17 (H-10, m, 4H), 6.26 (H-2, br s, 1H), 6.29 (H-4, br s, 1H), 6.63 (H-7, br s, 1H), 6.65 (H-5, br s, 1H), 13.33 (OH, s, 1H); ¹³C NMR (75 MHz; CDCl₃): δ = 23.4 (CH₃-Ph); 29.6 (C-11); 32.0 (C-12); 65.7 (C-10); 92.4 (C-4); 97.2 (C-2); 99.0 (C-5); 104.2 (C-9a); 113.1 (C-8a); 115.7 (C-7); 143.5 (C-8); 156.9 (C-4a); 159.3 (C-10a); 162.8 (C-6); 163.7 (C-1); 164.8 (C-3); 182.3 (C-9); MS (EI) *m/z* (%): 498 (22) [M - 2]⁺, 500 (42) [(M - 2)+2]⁺, 502 (22) [(M - 2)+4]⁺, 421 (97), 419 (100), 391 (22), 229 (21).

3,6-bis(3-bromobutoxy)-1-hydroxy-8-methyl-9H-xanthen-9-one (**4**)

Yield 67.7%; Mp: 117-118 °C (from CHCl₃); ¹H NMR (300 MHz; CDCl₃): δ = 2.00 (H-11, H-12, m, 8H), 2.77 (CH₃-Ph, s, 3 H), 3.47 (H-13, 2t, *J* = 6.2 Hz, 4H), 4.02 (H-10, m, 4H), 6.21 (H-2, d, *J* = 2.2 Hz, 1H), 6.23 (H-4, d, *J* = 2.2 Hz, 1H), 6.57 (H-5, H-7, br s, 2H), 13.31 (OH, s, 1H); ¹³C NMR (75 MHz; CDCl₃): δ = 23.4 (CH₃-Ph); 27.6 (C-11); 29.3 (C-12); 33.2 (C-13); 67.3 (C-10); 92.4 (C-4); 97.1 (C-2); 98.9 (C-5); 104.1 (C-9a); 113.0 (C-8a); 115.7 (C-7); 143.5 (C-8); 156.9 (C-4a); 159.4 (C-10a); 163.0 (C-6); 163.7 (C-1); 165.1 (C-3); 182.3 (C-9); MS (EI) *m/z* (%): 526 (19) [M - 2]⁺, 528 (37) [(M - 2)+2]⁺, 530 (19) [(M - 2)+4]⁺, 447 (80), 449 (80), 395 (33), 394 (46), 393 (35), 393 (35), 259 (22), 229 (30), 136 (38), 135 (41), 55 (100).

3,6-bis(3-bromopentoxy)-1-hydroxy-8-methyl-9H-xanthen-9-one (**5**)

Yield 77.4%; Mp: 89-90 °C (from CHCl₃); ¹H NMR (300 MHz; CDCl₃): δ = 1.61 (H-12, m, 4H), 1.79 (H-13, m, 4H), 1.89 (H-11, m, 4H), 2.71 (CH₃-Ph, s, 3H), 3.42 (H-14, 2t, *J* = 6.6 Hz, 4H), 3.94 (H-10, m, 4H), 6.15 (H-2, H-4, br s, 2H), 6.48 (H-5, H-7, s, 2H), 13.27 (1H, s, OH); ¹³C NMR (75 MHz; CDCl₃): δ = 23.4 (CH₃-Ph); 24.7 (C-12); 28.2 (C-11); 32.4 (C-13); 33.6 (C-14); 68.0 (C-10); 92.2 (C-

4); 97.0 (C-2); 98.7 (C-5); 103.8 (C-9a); 112.6 (C-8a); 115.6 (C-7); 143.1 (C-8); 156.7 (C-4a); 159.1 (C-10a); 163.0 (C-6); 163.5 (C-1); 165.9 (C-3); 182.1 (C-9); MS (EI) *m/z* (%): 554 (19) [M - 2]⁺, 556 (37) [(M - 2)+2]⁺, 558 (19) [(M - 2)+4]⁺, 477 (100), 475 (99), 408 (79), 406 (75), 380 (30), 378 (30), 258 (40), 229 (43), 69 (87).

General procedure for the synthesis of (ω -aminoalkoxy)-xanthone derivatives (**6**) – (**20**)

Each haloalkanolic derivative [20 mg, (**3**) - 0.04 mmol, (**4**) - 0.038 mmol, (**5**) - 0.036 mmol] was stirred with 0.5 mL of the corresponding amine (dimethyl, diethyl, dipropyl, *t*-butylamine or piperidine) in acetone for about 48 h at room temperature. After solvent evaporation, the mixture was dissolved in an HCl aqueous solution (3%) and shaken out with ethyl acetate. The pH of the aqueous layer was raised to 10 with aqueous KOH and then it was shaken out again with ethyl acetate. The organic layer was separated and dried over Na₂SO₄ and the residue after the evaporation of the solvent could be characterized as the desired compound. In this way the ω -aminoalkoxyxanthenes (**6**) – (**20**) were obtained.

3,6-bis[3-(dimethylamino)propoxy]-1-hydroxy-8-methyl-9H-xanthen-9-one (**6**)²⁹

Yield 98%.

3,6-bis[4-(dimethylamino)butoxy]-1-hydroxy-8-methyl-9H-xanthen-9-one (**7**)

Yield 98%; Mp: 80-82 °C (dec.) (from MeOH); ¹H NMR (300 MHz; methanol-d₄): δ = 1.70 (H-12, br s, 4H), 1.82 (H-11, br s, 4H), 2.28 (H-14, s, 12H), 2.42 (H-13, t, *J* = 5.9 Hz, 4H), 2.79 (CH₃-Ph, s, 3 H), 4.09 (H-10, m, 4H), 6.24 (H-3, br s, 1H), 6.39 (H-4, br s, 1H), 6.70 (H-7, br s, 1H), 6.77 (H-5, br s, 1H); ¹³C NMR (75 MHz; DMSO-d₆): δ = 23.0 (C-12); 23.5 (CH₃-Ph); 26.3 (C-11); 45.2 (C-14); 58.6 (C-13); 68.3 (C-10); 92.3 (C-4); 97.3 (C-2); 99.3 (C-5); 103.2 (C-9a); 111.9 (C-8a); 116.0 (C-7); 142.7 (C-8); 156.6 (C-4a); 158.9 (C-10a); 163.3 (C-1 and C-6); 165.2 (C-3); 181.7 (C-9); MS (EI) *m/z* (%): 456 (16) [M]⁺, 441 (6), 357 (14), 100 (34), 58 (100).

3,6-bis[5-(dimethylamino)pentyl]oxy]-1-hydroxy-8-methyl-9H-xanthen-9-one (**8**)

Yield 99%; Mp: 65-67 °C (dec.) (from MeOH); ¹H NMR (300 MHz; CDCl₃): δ = 1.50 (H-12, H-13, br s, 8H), 1.82 (H-11, m, 4H), 2.21 (H-15, s, 12H), 2.28 (H-14, t, *J* = 7.1 Hz, 4H), 2.79 (CH₃-Ph, s, 3 H), 3.99 (H-10, m, 4H), 6.23 (H-2, br s, 1H), 6.25 (H-4, br s, 1H), 6.60 (H-5, H-7, br s, 2H), 13.35 (OH, br s, 1H); ¹³C NMR (75 MHz; CDCl₃): δ = 23.4 (CH₃-Ph); 23.9 (C-12); 27.3 (C-13); 28.9 (C-11); 45.4 (C-15); 59.6 (C-14); 68.3 (C-10); 92.4 (C-4); 97.1 (C-2); 98.8 (C-5); 103.9 (C-9a); 112.8 (C-8a); 115.8 (C-7); 143.3 (C-8); 156.9 (C-4a); 159.4 (C-10a); 163.2 (C-6); 163.6 (C-1); 165.3 (C-3); 182.3 (C-9); MS (EI) *m/z* (%): 484 (1) [M]⁺, 469 (2), 384 (2), 114 (45), 58 (100).

3,6-bis[3-(diethylamino)propoxy]-1-hydroxy-8-methyl-9H-xanthen-9-one (**9**)

Yield 97%; gum; ¹H NMR (300 MHz; acetone-d₆): δ = 0.99 (H-15, t, *J* = 7.2 Hz, 12H), 1.92 (H-11, m, 4H), 2.51 (H-13, m, 8H), 2.59 (H-12, 2t, *J* = 6.7 Hz, 4H), 2.77 (CH₃-Ph, s, 3 H), 4.18 (H-10, m, 4H), 6.24 (H-2, br s, 1H), 6.36 (H-4, br s, 1H), 6.72 (H-7, br s, 1H), 6.76 (H-5, br s, 1H); ¹³C NMR (75 MHz; methanol-d₄): δ = 11.5 (C-14); 23.6 (CH₃-Ph); 26.9 (C-11); 47.8 (C-13); 50.2 (C-12); 67.9 (C-10); 93.2 (C-4); 98.2 (C-2); 99.9 (C-5); 104.8 (C-9a); 113.5 (C-8a); 116.7 (C-7); 144.2 (C-8); 158.2 (C-4a); 160.5 (C-10a); 164.5 (C-6); 164.6 (C-1); 166.6 (C-3); 183.2 (C-9); MS (EI) *m/z* (%): 484 (10) [M]⁺, 469 (9), 455 (13), 113 (20), 86 (100), 72 (23).

3,6-bis[4-(diethylamino)butoxy]-1-hydroxy-8-methyl-9H-xanthen-9-one (10)

Yield 94%; Mp: 61-62 °C (from MeOH); ¹H NMR (300 MHz; methanol-d₄): δ= 1.07 (H-15, t, *J* = 7.3 Hz, 12H), 1.68 (H-12, m, 4H), 1.78 (H-11, m, 4H), 2.59 (H-13, H-14, m, 12H), 2.71 (CH₃-Ph, s, 3 H), 4.03 (H-10, m, 4H), 6.15 (H-2, br s, 1H), 6.26 (H-4, br s, 1H), 6.57 (H-7, br s, 1H), 6.61 (H-5, br s, 1H); ¹³C NMR (75 MHz; methanol-d₄): δ= 11.2 (C-15); 23.6 (CH₃-Ph); 23.7 (C-12); 28.2 (C-11); 47.7 (C-14); 53.4 (C-13); 69.4 (C-10); 93.2 (C-4); 96.1 (C-2); 100.0 (C-5); 104.8 (C-9a); 113.5 (C-8a); 116.8 (C-7); 144.3 (C-8); 158.2 (C-4a); 160.6 (C-10a); 164.5 (C-6); 164.8 (C-1); 166.7 (C-3); 183.3 (C-9); MS (EI) *m/z* (%): 512 (2) [M]⁺, 497 (3), 483 (8), 128 (13), 86 (100).

3,6-bis[[5-(diethylamino)pentyl]oxy]-1-hydroxy-8-methyl-9H-xanthen-9-one (11)

Yield 98%; Mp: 75-76 °C (dec) (from MeOH); ¹H NMR (300 MHz; CDCl₃): δ= 1.00 (H-16, t, *J* = 7.2 Hz, 12H), 1.48 (H-12, H-13, m, 8H), 1.81 (H-11, m, 4H), 2.43 (H-14, t, *J* = 7.2 Hz, 4H), 2.52 (H-15, q, *J* = 7.1 Hz, 8H), 2.80 (CH₃-Ph, s, 3 H), 4.61 (H-10, m, 4H), 6.25 (H-2, br s, 1H), 6.27 (H-4, br s, 1H), 6.62 (H-5, H-7, 1H), 13.36 (OH, s, 1H); ¹³C NMR (75 MHz; CDCl₃): δ= 11.6 (C-15); 23.4 (CH₃-Ph); 24.1 (C-12); 26.8 (C-13); 29.0 (C-11); 46.9 (C-15); 52.8 (C-14); 68.4 (C-10); 92.4 (C-4); 97.2 (C-2); 98.9 (C-5); 103.9 (C-9a); 112.8 (C-8a); 115.8 (C-7); 143.3 (C-8); 156.9 (C-4a); 159.4 (C-10a); 163.3 (C-6); 163.7 (C-1); 165.3 (C-3); 182.3 (C-9); MS (EI) *m/z* (%): 540 (1) [M]⁺, 525 (4), 511 (10), 412 (6), 142 (19), 85 (100).

3,6-bis[3-(dipropylamino)propoxy]-1-hydroxy-8-methyl-9H-xanthen-9-one (12)

Yield 97%; gum; ¹H NMR (300 MHz; acetone-d₆/methanol-d₄): δ= 0.86 (H-15, t, *J* = 7.3 Hz, 12H), 1.43 (H-14, m, 8H), 1.92 (H-11, m, 4H), 2.37 (H-13, t, *J* = 7.2 Hz, 8H), 2.59 (H-12, 2t, *J* = 6.6 Hz, 4H), 2.80 (CH₃-Ph, s, 3 H), 4.21 (H-10, m, 4H), 6.27 (H-2, d, *J* = 2.1 Hz, 1H), 6.42 (H-4, d, *J* = 2.1 Hz, 1H), 6.77 (H-7, br s, 1H), 6.82 (H-5, br s, 1H); ¹³C NMR (75 MHz; acetone-d₆/methanol-d₄): δ= 12.2 (C-15); 21.2 (C-14); 23.4 (CH₃-Ph); 27.8 (C-11); 51.0 (C-12); 57.0 (C-13); 67.6 (C-10); 93.0 (C-4); 97.9 (C-2); 99.8 (C-5); 104.4 (C-9a); 113.2 (C-8a); 116.8 (C-7); 144.0 (C-8); 157.9 (C-4a); 160.3 (C-10a); 164.3 (C-6); 164.7 (C-1); 166.6 (C-3); 183.1 (C-9); MS (EI) *m/z* (%): 541 (2) [M+1]⁺, 540 (1) [M]⁺, 512 (34), 241 (4), 114 (60), 86 (100).

3,6-bis[4-(dipropylamino)butoxy]-1-hydroxy-8-methyl-9H-xanthen-9-one (13)

Yield 98%; Mp: 70-72 °C (from MeOH); ¹H NMR (300 MHz; CDCl₃): δ= 0.85 (H-16, t, *J* = 7.3 Hz, 12H), 1.43 (H-15, m, 8H), 1.59 (H-12, m, 4H), 1.80 (H-11, m, 4H), 2.35 (H-14, t, *J* = 7.3 Hz, 8H), 2.45 (H-13, t, *J* = 7.2 Hz, 4H), 2.80 (CH₃-Ph, s, 3 H), 4.01 (H-10, m, 4H), 6.24 (H-2, br s, 1H), 6.26 (H-4, br s, 1H), 6.61 (H-7, H-5, br s, 2H); ¹³C NMR (75 MHz; CDCl₃): δ= 12.0 (C-16); 20.2 (C-15); 23.4 (CH₃-Ph); 23.7 (C-12); 27.0 (C-11); 53.8 (C-14); 56.2 (C-13); 68.4 (C-10); 92.4 (C-4); 97.1 (C-2); 98.9 (C-5); 104.0 (C-9a); 112.7 (C-8a); 115.8 (C-7); 143.3 (C-8); 156.9 (C-4a); 159.4 (C-10a); 163.3 (C-6); 163.6 (C-1); 165.3 (C-3); 182.3 (C-9); MS (EI) *m/z* (%): 568 (2) [M]⁺, 553 (0.3), 539 (45), 525. (1), 156 (19), 128 (32), 114 (100).

3,6-bis[[5-(dipropylamino)pentyl]oxy]-1-hydroxy-8-methyl-9H-xanthen-9-one (14)

Yield 96%; Mp: 65-66 °C (from MeOH); ¹H NMR (300 MHz; CDCl₃): δ= 0.85 (H-17, t, *J* = 7 Hz, 12H), 1.44 (H-12, H-13, H-16, m, 16H), 1.81 (H-11, m, 4H), 2.35 (H-15, t, *J* = 7.6 Hz, 8H), 2.41 (H-14, br s, 4H), 2.81 (CH₃-Ph, s, 3H), 4.00 (H-10, m, 4H), 6.25 (H-2,

br s, 1H), 6.27 (H-4, br s, 1H), 6.62 (H-5, H-7, br s, 2H), 13.36 (OH, br s, 1H); ¹³C NMR (75 MHz; CDCl₃): δ= 12.0 (C-17); 20.2 (C-16); 23.4 (CH₃-Ph); 24.0 (C-12); 26.9 (C-13); 29.0 (C-11); 54.0 (C-14); 56.3 (C-15); 68.4 (C-10); 92.4 (C-4); 97.1 (C-2); 98.8 (C-5); 104.0 (C-9a); 112.8 (C-8a); 115.8 (C-7); 143.3 (C-8); 156.9 (C-4a); 159.4 (C-10a); 163.3 (C-6); 163.6 (C-1); 165.3 (C-3); 182.3 (C-9); MS (EI) *m/z* (%): 596 (2) [M]⁺, 567 (67), 553 (8), 440 (5), 269 (8), 170 (13), 142 (17), 114 (100), 86 (14).

3,6-bis[3-(tert-butylamino)propoxy]-1-hydroxy-8-methyl-9H-xanthen-9-one (15)

Yield 96%; Mp: 85-87 °C (dec.) (from MeOH); ¹H NMR (300 MHz; acetone-d₆): δ= 1.07 (H-14, s, 18H), 1.90 (H-11, m, 4H), 2.72 (H-12, 2t, *J* = 6.6 Hz, 4H), 2.76 (CH₃-Ph, s, 3H), 4.20 (H-10, m, 4H), 6.22 (H-2, d, *J* = 2.3 Hz, 1H), 6.34 (H-4, d, *J* = 2.3 Hz, 1H), 6.70 (H-7, br s, 1H), 6.73 (H-5, br s, 1H); ¹³C NMR (75 MHz; methanol-d₄): δ= 24.3 (CH₃-Ph); 28.6 (C-15); 30.7 (C-11); 40.4 (C-13); 51.9 (C-12); 68.9 (C-10); 913.2 (C-4); 98.1 (C-2); 100.0 (C-5); 104.7 (C-9a); 113.5 (C-8a); 116.6 (C-7); 144.1 (C-8); 158.1 (C-4a); 160.2 (C-10a); 164.4 (C-6); 164.5 (C-1); 166.5 (C-3); 183.1 (C-9); MS (EI) *m/z* (%): 484 (1) [M]⁺, 469 (62), 413 (42), 372 (11), 356 (16), 259 (17), 227 (15), 98 (100).

3,6-bis[4-(tert-butylamino)butoxy]-1-hydroxy-8-methyl-9H-xanthen-9-one (16)

Yield 94%; Mp: 154-155 °C (dec.) (from MeOH); ¹H NMR (300 MHz; methanol-d₄): δ= 1.27 (H-15, s, 18H), 1.79 (H-12, m, 4H), 1.90 (H-11, m, 4H), 2.68 (CH₃-Ph, s, 3H), 2.86 (H-13, 2t, *J* = 7.4 Hz, 4H), 4.05 (H-10, br s, 4H), 6.13 (H-2, br s, 1H), 6.23 (H-4, br s, 1H), 6.53 (H-7, br s, 1H), 6.57 (H-5, br s, 1H); ¹³C NMR (75 MHz; methanol-d₄): δ= 23.6 (CH₃-Ph); 26.3 (C-12); 27.3 (C-15); 27.5 (C-11); 42.8 (C-14); 54.8 (C-13); 69.2 (C-10); 93.2 (C-4); 98.2 (C-2); 100.0 (C-5); 104.8 (C-9a); 113.5 (C-8a); 116.7 (C-7); 144.3 (C-8); 158.2 (C-4a); 160.5 (C-10a); 164.5 (C-6); 164.6 (C-1); 166.6 (C-3); 183.2 (C-9); MS (EI) *m/z* (%): 512 (1) [M]⁺, 497 (17), 370 (9), 259 (9), 112 (100).

3,6-bis[[5-(tert-butylamino)pentyl]oxy]-1-hydroxy-8-methyl-9H-xanthen-9-one (17)

Yield 98%; Mp: 78-79 °C (from MeOH); ¹H NMR (300 MHz; DMSO-d₆): δ= 1.01 (H-16, s, 18H), 1.43 (H-12, H-13, br s, 8H), 1.73 (H-11, br s, 4H), 2.50 (H-14, br s, 4H), 2.75 (CH₃-Ph, s, 3H), 4.10 (H-10, br s, 4H), 6.30 (H-2, br s, 1H), 6.47 (H-4, br s, 1H), 6.81 (H-7, br s, 1H), 6.88 (H-5, br s, 1H); ¹³C NMR (75 MHz; DMSO-d₆): δ= 23.4 (CH₃-Ph); 23.8 (C-12); 28.8 (C-13); 29.0 (C-16); 30.4 (C-11); 42.1 (C-15); 50.4 (C-14); 68.8 (C-10); 92.8 (C-4); 97.6 (C-2); 99.6 (C-5); 103.5 (C-9a); 112.3 (C-8a); 116.4 (C-7); 143.1 (C-8); 156.9 (C-4a); 159.3 (C-10a); 163.3 (C-6); 163.7 (C-1); 165.6 (C-3); 182.1 (C-9); MS (EI) *m/z* (%): 540 (1) [M]⁺, 525 (52), 483 (30), 469 (41), 142 (51), 126 (100), 86 (49).

1-hydroxy-8-methyl-3,6-bis(3-piperidin-1-ylpropoxy)-9H-xanthen-9-one (18)

Yield 93%; Mp: 98-100 °C (from MeOH); ¹H NMR (300 MHz; CDCl₃/methanol-d₄): δ= 1.37 (H-15, m, 4H), 1.52 (H-14, br s, 8H), 1.92 (H-11, m, 4H), 2.40 (H-12, H-13, m, 12H), 2.71 (CH₃-Ph, s, 3H), 3.97 (H-10, m, 4H), 6.15 (H-2, d, *J* = 1.9 Hz, 1H), 6.21 (H-4, d, *J* = 1.9 Hz, 1H), 6.54 (H-7, br s, 1H), 6.57 (H-5, br s, 1H); ¹³C NMR (75 MHz; CDCl₃/methanol-d₄): δ= 23.4 (CH₃-Ph); 24.3 (C-15); 25.8 (C-14); 26.4 (C-11); 54.6 (C-13); 55.7 (C-12); 67.0 (C-10); 92.4 (C-4); 97.2 (C-2); 98.9 (C-5); 104.0 (C-9a); 112.8 (C-8a); 115.8 (C-7); 143.3 (C-8); 156.9 (C-4a); 159.4 (C-10a); 163.2 (C-6); 163.4 (C-1); 165.2 (C-3); 182.3 (C-9); MS (EI) *m/z* (%): 508 (4) [M]⁺, 395 (4), 126 (13), 98 (100).

1-hydroxy-8-methyl-3,6-bis(4-piperidin-1-ylbutoxy)-9H-xanthen-9-one (19)

Yield 95%; Mp: 84-85 °C (dec.) (from MeOH); ¹H NMR (300 MHz; DMSO-d₆): δ= 1.54 (H-16, br s, 4H), 1.72 (H-15, br s, 8H), 1.79 (H-11, H-12, br s, 8H), 2.76 (CH₃-Ph, s, 3H), 3.06 (H-13, H-14, br s, 12H), 4.14 (H-10, m, 4H), 6.34 (H-2, br s, 1H), 6.50 (H-4, br s, 1H), 6.83 (H-7, br s, 1H), 6.92 (H-5, br s, 1H), 13.31 (OH, s, 1H); ¹³C NMR (75 MHz; DMSO-d₆): δ= 20.7 (C-16); 21.9 (C-12); 23.1 (C-15); 23.4 (CH₃-Ph); 26.1 (C-11); 52.6 (C-14); 56.0 (C-13); 68.2 (C-10); 92.9 (C-4); 97.7 (C-2); 99.8 (C-5); 103.6 (C-9a); 112.4 (C-8a); 116.3 (C-7); 143.2 (C-8); 156.9 (C-4a); 159.3 (C-10a); 163.3 (C-6); 163.6 (C-1); 165.5 (C-3); 182.1 (C-9); MS (EI) *m/z* (%): 536 (3) [M]⁺, 397 (6), 140 (16), 98 (100).

1-hydroxy-8-methyl-3,6-bis([5-piperidin-1-ylpentyl]oxy)-9H-xanthen-9-one (20)

Yield 95%; Mp: 72-74 °C (from MeOH); ¹H NMR (300 MHz; CDCl₃): δ= 1.41 (H-12, H-17, br s, 8H), 1.54 (H-13, H-16, m, 12H), 1.79 (H-11, m, 4H), 2.30 (H-14, H-15, m, 12H), 2.77 (CH₃-Ph, s, 3H), 3.97 (H-10, m, 4H), 6.21 (H-2, br s, 1H), 6.23 (H-4, br s, 1H), 6.58 (H-5, H-7, br s, 2H) 13.33 (OH, s, 1H); ¹³C NMR (75 MHz; CDCl₃): δ= 23.3 (CH₃-Ph); 24.1 (C-17); 24.4 (C-12); 25.9 (C-16); 26.8 (C-13); 28.9 (C-11); 54.6 (C-15); 59.3 (C-14); 68.3 (C-10); 92.3 (C-4); 97.1 (C-2); 98.8 (C-5); 103.9 (C-9a); 112.7 (C-8a); 115.7 (C-7); 143.2 (C-8); 156.8 (C-4a); 159.3 (C-10a); 163.2 (C-6); 163.6 (C-1); 165.2 (C-3); 182.2 (C-9); MS (EI) *m/z* (%): 564 (1) [M]⁺, 424 (8), 154 (44), 126 (46), 98 (100), 84 (59), 60 (99), 45 (98).

Antibiotic assay*Microorganisms and media*

The test organisms used in this study were as follows: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and clinical isolated *S.aureus* resistant to ciprofloxacin, chloramphenicol, penicillin, clindamycin, erythromycin, sulphamethoxazol + trimetoprim and oxacillin. All media were purchased from Oxoid.

Disc diffusion method

Stock solutions (10 mg/mL) of each substance were prepared in dimethylsulfoxide (DMSO). The inoculum was an overnight culture of each bacterial species in Mueller-Hinton agar diluted in saline sterile solution (0.45%) to a concentration of approximately 10⁸ CFU/mL. The inoculum was spread on Mueller Hinton agar (MHA) and air-dried at room temperature. A 6-mm sterile paper disc was placed on the agar and 20 µL of each solution was poured over a disc, and the plates were incubated at 37 °C for 24 h. As a control, a disc impregnated with gentamycine (10 µg) was used. All disc diffusion tests were performed in triplicate and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by the compounds.

Determination of minimum inhibitory concentrations

The 96-well plates were prepared by dispensing 100 µL Mueller-Hinton broth into each well. A stock solution was prepared at a concentration of 2 mg/mL and serial dilutions were performed to reach a final concentration within a 1 µg/mL to 1.000 µg/mL range, with a 100 µL final volume in each well. For gentamycine, final concentration ranged from 64 to 0.5 µg/mL. The inoculum was, again, an overnight culture of each bacterial species in Mueller-Hinton agar diluted in saline sterile solution (0.45%) to a concentration of approximately 10⁸ CFU/mL. This solution was diluted 1/10 in saline solution (0.45%) and 5 µL (10⁴ CFU/mL) were added to each well containing the test samples.

All experiments were performed in triplicate and the microdilution trays were incubated at 36 °C for 18 h. Then, 20 µL of an aqueous solution (0.5%) of triphenyltetrazolium chloride (TTC) were added to each well and the trays were again incubated at 36 °C for 2 h. Afterwards, in those wells where bacterial growth did occur, TTC changed from colorless to red. MIC was defined as the lowest concentration of each substance at which no color change occurred, and was expressed in µg/mL.

Antiproliferative assay

All compounds were solubilized in DMSO and tested at four concentrations (0.25, 2.5, 25, and 250 µg/mL), each in triplicate wells. The final content of DMSO was lower than 0.4%. Doxorubicin, an anticancer drug, was used as positive control for all cell lines tested.

Cell lines and culture

The human tumoral B16-F10 (murine melanoma) were donated by A. Nomizo from Faculdade de Ciências Farmacêuticas de Ribeirão Preto-USP and MCF-7 (breast), 786-0 (kidney), OVCAR03 (ovarian), NCI-ADR (ovarian expressing the resistance phenotype for adriamycin) and HT-29 (colon) were donated by Prof. Dr. J. E. de Carvalho from Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas, Unicamp.

The cells were maintained in RPMI-1640 medium (Sigma Chemical Co., St. Louis, MO, USA), supplemented with 10% fetal calf serum, 100 U/mL penicillin, 0.1 mg/mL streptomycin and 0.25 µg/mL amphotericin (complete medium) at 37 °C with 5% CO₂.

The adherent cell lines were detached from the culture flasks by adding 0.5 mL trypsin solution (0.25% + EDTA 1 mM). Then they were transferred to conical tubes containing complete culture medium and after centrifugation at low speed, the culture medium and trypsin were discarded and cells resuspended in small volume of complete medium. After coating, the cells were plated in 96-well microtiter plates (100,000 cells/mL) with a fixed volume of 100 µL per well. The microtiter plates containing cells were pre-incubated for 24 h at 37 °C to allow stabilizations prior to addition of testing compounds as well as doxorubicin (DOX) (100 µL). The plates were incubated with the test substances for 48 h at 37 °C and 5% CO₂.^{31,32}

Sulforhodamine B (SRB) assay

The SRB assay was done as described by Skehan and coworkers.³¹ Briefly, the cells were fixed with 20% TCA (sigma) at 4 °C (100 µL/well) for 30 min. The supernatant was then discarded and the plates were washed five times with tap water. The cells were stained for 30 min with 0.1% SRB in 1% acetic acid (50 µL/well) and subsequently washed four times with 1% acetic acid to remove unbound dye. The plates were air-dried and protein-bound dye was solubilized with 100 µL (10 mM) of Trizma buffer (Sigma). The plates were shaken for 15 min and the resulting optical density was read in a multiwell plate reader at 540 nm.

The growth inhibition - IC (%) - of each test sample was calculated as Monks *et al.*³² using Excell® program (Microsoft Office package). The dose that inhibits 50% of cell growth (IC₅₀) was determined graphically by the program for graphics and data analysis (Microcal Origin Version 6.0) Excell®.

RESULTS AND DISCUSSION

We obtained five series (dimethyl, diethyl, dipropyl, *t*-butylamine and piperidiny) groups with spacers of 3, 4 and 5 carbon atoms between N and O, in order to establish a relationship between the characteristics of side chains and the biological activity. The preparation of

ω -aminoalkoxyxanthenes is illustrated in Scheme 1. Lichexanthone (**1**) was isolated from the lichen *Parmotrema lichexanthonicum*, and then was *O*-demethylated by BBr_3 to produce norlichexanthone (**2**) in 60% yield.²⁹ ω -bromoalkoxyxanthenes (**3**, 65%), (**4**, 67.7%) and (**5**, 77.4%) were obtained by treatment of (**2**) with an appropriate 1, ω -dihaloalkane³⁰ and were used to prepare the nitrogenated derivatives (**6**) – (**20**) by reaction with different amines. This last step was very profitable and it was possible to produce these derivatives in good yields, ranging from 93 to 99%.

The structure of compounds (**3**) – (**20**) was established on the basis of NMR and MS techniques. Compounds (**3**), (**4**) and (**5**) showed similar NMR features. Their ¹H NMR spectra showed all characteristic signals for the scaffold and, besides them, signals in 3.9, and 3.4 ppm, that refer to methylene groups bearing an oxygen and a bromine atoms, respectively, and others at about 2 ppm, for the remaining CH₂ groups (1, 2 or 3 depending on the length of the ω -bromoalkyl chain). Integration areas of these ¹H-NMR signals showed that two alkyl chains were introduced in the hydroxyl groups in positions 3 and 6 of the xanthenone core, what was confirmed by 2D-NMR techniques. The hydroxyl group in position 1 does not react under the conditions used because of the intramolecular hydrogen bond established between the hydroxyl H and the carbonyl oxygen. Actually, it can be confirmed by the presence of a sharp singlet at 13.3 ppm in ¹H NMR of (**3**), (**4**) and (**5**) corresponding to this group. ¹³C-NMR spectra of these substances presented scaffold characteristic signals referring to the ω -bromoalkoxy chains, including these two methylene groups bearing heteroatoms (68 ppm, for the group CH₂-O-, and 33 ppm for CH₂-Br-). Aminoalkanoic derivatives (**6**) – (**20**) had ¹H and ¹³C NMR spectra very close to the profile exhibited by starting materials, but with signals at 2.6-2.7 and 50-60 ppm in ¹H and ¹³C NMR spectra, respectively, that can be attributed to CH₂ group connected to nitrogen, were the S_N2 reaction took place.

All compounds were tested for antibiotic activity against *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and oxacillin resistant *S. aureus*. Inhibition zones and MIC values are summarized in Table 1.

All tested substances were much more active for Gram-positive than Gram-negative bacteria. For *E. coli* (Gram-negative) the best activity was achieved for compounds (**6**) – (**9**), including the three dimethylamino derivatives. For standard and clinical *S. aureus* strains, there is a similar behavior, and the most active compounds are that

containing *tert*-butylamino moieties, and the ones that have 4 or 5 member chains between N and O atoms. This pattern was even more evident for the clinical strain, for which the most potent compounds, with MIC value of 4 μ g/mL, are the three ones with *N-tert*-butylamino groups (**15** – **17**), and the ones which have five member chains between those heteroatoms (**8**, **14**, **20**).

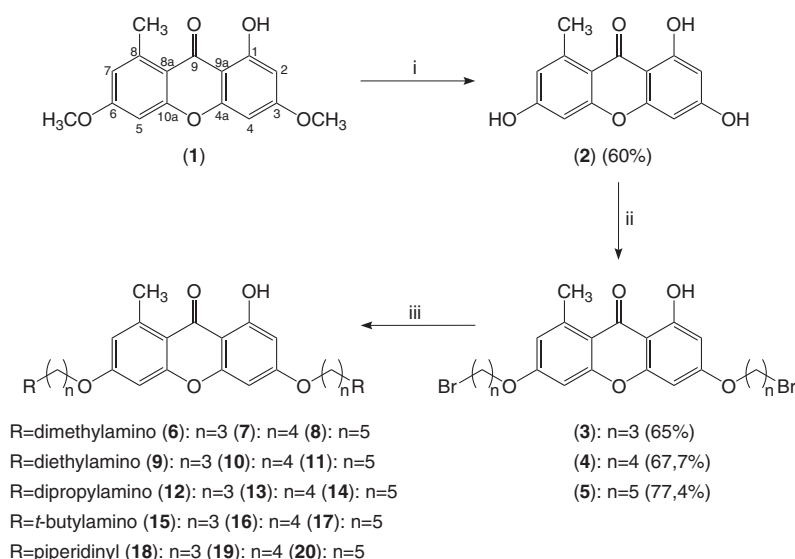
Some natural xanthenes are described as important anti-staphylococcal agents, including for resistant strains, having MIC values ranging from 8 until 1.95 μ g/mL.^{10,33-35} Thus, some of the semi synthetic ω -aminoalkoxyxanthenes presented herein are potential antimicrobials and can be considered relevant specially taking into account the growing problem of resistant *S. aureus* strains.¹

Studies have reported the synthesis and cytotoxic activity of bromoalkoxyxanthenes³⁰ and xanthenes with nitrogenated side chains^{27,28} including arylhydrazones,³⁶ with promising results, showing IC₅₀ values between 2 and 30 μ M.

Aiming at evaluating derivatives for their antitumor potential, cytotoxicity on cell lines of various histological origin {B16-F10 (murine melanoma), MCF-7 (breast carcinoma), 786-0 (kidney carcinoma), OVCAR03 (ovarian carcinoma), NCI-ADR (ovarian expressing the resistance phenotype for adriamycin) and HT-29 (colon carcinoma)} was determined. Cell proliferation was determined by spectrophotometric assay using sulforhodamine B as protein-binding dye. Concentrations that elicits 50% inhibition of cell growth (IC₅₀) are summarized in Table 2.

Synthesized substances were in general active against multiple cell lines. For almost all lines, at least 50% of compounds were active, showing IC₅₀ values below 20 μ M (about 10 μ g/mL for these substances). Compound (**15**), containing, *tert*-butylamino moieties was the most active, with IC₅₀= 4.3, 7.6 and 8.5 μ M for MCF-7 (breast), 786-0 (kidney) and HT-29 (colon) lines respectively. Compounds (**10**) and (**11**), with four or five member chains between N and O atoms and diethylamino moieties were more active than DOX over the resistant line NCI-ADR. It is notable a tendency of lower activity for substances with dipropylamino moieties (**12-14**).

Starting materials, lichexanthone (**1**) and norlichexanthone (**2**) were tested before for antibiotic (disc diffusion method) and cytotoxic activities,²⁹ but they showed low activity against bacterial strains and cells tested. On this basis we can say that the changes made were relevant and useful to achieve this biological profile.



Scheme 1. Reagents and conditions: i) BBr_3 , CH_2Cl_2 , N_2 , rt, 16 h; ii) K_2CO_3 , acetone, $Br(CH_2)_nBr$, n=3, 4 or 5, rt, 24 h; iii) amine (dimethyl, diethyl, dipropyl, *t*-butylamine and piperidine), acetone, rt, 48 h

Table 1. Antimicrobial activity for compounds (3) – (20) and gentamycin (GENTA)

Compounds	Microorganism					
	<i>S. aureus</i> ATCC (25923)		Resistant <i>S. aureus</i>		<i>E. coli</i> ATCC (25922)	
	Inhibition zone (mm)	MIC ($\mu\text{g/mL}$)	Inhibition zone (mm)	MIC ($\mu\text{g/mL}$)	Inhibition zone (mm)	MIC ($\mu\text{g/mL}$)
(3)	0	NT	0	NT	0	NT
(4)	0	NT	0	NT	0	NT
(5)	0	NT	0	NT	0	NT
(6)	20 ^[29]	62.5	13	125	12 ^[29]	31.25
(7)	15	31.25	16	15.6	13	31.25
(8)	14	7.8	16	4	0	31.25
(9)	14	15.6	19	31.25	12	31.25
(10)	19	7.8	20	7.8	10	250
(11)	14	15.6	16	15.6	10	125
(12)	18	15.6	19	15.6	8	125
(13)	12	7.8	14	15.6	0	250
(14)	12	7.8	15	4	10	250
(15)	17	7.8	18	4	10	62.6
(16)	20	2	21	4	10	62.5
(17)	16	4	17	4	8	250
(18)	15	15.6	15	31.25	20	62.5
(19)	17	7.8	17	15.6	8	125
(20)	13	4	15	4	12	62.5
GENTA	27	<0.5	14	64	24	<0.5

NT = not tested

Table 2. IC₅₀ values, given in μM , for compounds (3) – (20) and doxorubicin (DOX) required for inhibiting tumor cell proliferation

Compounds	IC ₅₀ (μM)					
	OVCAR	NCLADR	MCF-7	786-0	B16-F10	HT-29
(3)	> 500	> 500	> 500	> 500	> 500	> 500
(4)	> 500	> 500	> 500	387.6	> 500	> 500
(5)	> 500	> 500	> 500	313.3	> 500	> 500
(6)	NT	NT	4.7	13.3	17.5	13.8
(7)	15.5	32.0	17.3	15.1	16.4	28.5
(8)	16.5	108.1	19.4	15.5	17.1	126.3
(9)	NT	114.8	150.1	117.4	122.8	166.5
(10)	NT	17.7	13.8	13.5	15.8	15.8
(11)	NT	13.5	15.2	12.2	12.8	15.0
(12)	NT	NT	112.4	125.4	131.8	NT
(13)	> 500	92.3	131.2	71.4	109.7	> 500
(14)	38.2	40.1	30	12.1	13.6	NT
(15)	NT	NT	4.3	7.6	15.7	8.5
(16)	7.4	> 500	> 500	> 500	> 500	11.3
(17)	NT	NT	13.9	13.1	15.7	15.3
(18)	NT	NT	29.5	13.8	NT	30.7
(19)	> 500	74.3	14.3	13.6	16.0	12.8
(20)	NT	NT	14.5	12.9	14.5	14.1
DOX	1.57	22.8	0.40	0.88	0.69	2.33

NT = not tested

CONCLUSION

Many xanthone derivatives were synthesized and described for the first time, including a series of aminoalkanoic derivatives. Promising results were obtained in the evaluation of antimicrobial activity for these compounds, specially related to activity against multidrug resistant *S. aureus*, a growing serious problem worldwide. Good cytotoxic activities were achieved as well, confirming the great biological potential of this compound class.

SUPPLEMENTARY MATERIAL

Available at <http://quimicanova.sbq.org.br>, in format PDF, with free access.

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REFERENCES

- Demain, A. L.; *Med. Res. Rev.* **2009**, *29*, 821.
- Liu, Y.; Ke, Z.; Cui, J.; Chen, W.; Ma, L.; Wan, B.; *Bioorg. Med. Chem.* **2008**, *16*, 7185.
- Engel, K.; Schmidt, U.; Reuter, J.; Weckesser, S.; Simon-Haarhaus, B.; Schempp, C. M.; *J. Photochem. Photobiol., B* **2007**, *89*, 9.
- Rankovi, B.; Miši, M.; Sukdolak, S.; *Microbiology* **2007**, *76*, 723.
- Russo, A.; Piovano, M.; Lombardo, L.; Garbarino, J.; Cardile, V.; *Life Sci.* **2008**, *83*, 468.
- Rankovi, B.; Miši, M.; Sukdolak, S.; *World J. Microbiol. Biotechnol.* **2008**, *74*, 1239.
- Woo, S.; Jung, J.; Lee, C.; Kwon, Y.; Na, Y.; *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1163.
- Pinto, M. M. M.; Sousa, M. E.; Nascimento, M. S. J.; *Curr. Med. Chem.* **2005**, *12*, 2517.
- Azebaze, A. G. B.; Ouahou, B. M. W.; Vardamides, J. C.; Valentin, A.; Kuete, V.; Acebey, L.; Beng, V. P.; Kkengfack, A.; E.; Meyer, M.; *Chem. Nat. Comp.* **2008**, *44*, 582.
- Chomnawang, M.; T.; Surassmo, S.; Wongsariya, K.; Bunyaphatsara, N.; *Fitoterapia* **2009**, *80*, 102.
- Ding, L.; Liu, B.; Qi, L.; Zhou, Q.; Hou, Q.; Li, J.; Zhang, Q.; *Toxicol. in Vitro* **2009**, *23*, 408.
- Ha, L. D.; Hansen, P. E.; Vang, O.; Duus, F.; Phan, H. D.; Nguyen, L. D.; *Chem. Pharm. Bull.* **2009**, *57*, 830.
- Chen, L.; Yang, L.; Wang, C.; *Food Chem. Toxicol.* **2008**, *46*, 688.
- Marona, H.; Librowski, T.; Cegla, M.; Erdogan, C.; Sahin, N. Ö.; *Acta Pol. Pharm.* **2008a**, *65*, 383.
- Marona, H.; Szkaradek, N.; Kubacka, M.; Bednarski, M.; Filipek, B.; Cegla, M.; Szneler, E.; *Arch. Pharm. Chem. Life Sci.* **2008b**, *341*, 90.
- Pontius, A.; Krick, A.; Kehraus, S.; Brun, R.; König, G. M.; *J. Nat. Prod.* **2008**, *71*, 1579.
- Zou, J.; Jin, D.; Chen, W.; Wang, J.; Liu, Q.; Zhu, X.; Zhao, W.; *J. Nat. Prod.* **2005**, *68*, 1514.
- Piazzi, L.; Belluti, F.; Bisi, A.; Gobbi, S.; Rizzo, S.; Bartolini, M.; Andrisano, V.; Recanatini, M.; Rampa, A.; *Bioorg. Med. Chem.* **2007**, *15*, 575.
- Urbain, A.; Marston, A.; Grilo, L. S.; Purev, J. B.; Purevsuren, B.; Batsuren, D.; Reist, M.; Carrupt, A.; Hostettman, K.; *J. Nat. Prod.* **2008**, *71*, 895.
- Harkcom, W. T.; Bevan, D. R.; *Biochem. Biophys. Res. Commun.* **2007**, *360*, 401.
- Lin, K.; Fang, S.; Hung, C.; Shieh, B.; Yang, S.; Teng, C.; Lin, C.; *Arch. Pharm. Chem. Life Sci.* **2009**, *342*, 19.
- Marona, H.; Pekala, E.; Antkiewicz-Michaluk, L.; Walczak, M.; Szneler, E.; *Bioorg. Med. Chem.* **2008c**, *16*, 7234.
- Marona, H.; Szkaradek, N.; Rapacz, A.; Filipek, B.; Dybala, M.; Siwek, A.; Cegla, M.; Szneler, E.; *Bioorg. Med. Chem.* **2009a**, *17*, 1345.
- Riscoe, M.; Kelly, J. X.; Winter, R.; *Curr. Med. Chem.* **2005**, *12*, 2539.
- Szkaradek, N.; Stachura, K.; Waszkielewicz, A.; M.; Cegla, M.; Szneler, E.; Marona, H.; *Acta Pol. Pharm.* **2008**, *65*, 21.
- Marona, H.; Szkaradek, N.; Karczewska, E.; Trojanowska, D.; Budak, A.; Bober, P.; Przepiórka, W.; Cegla, M.; Szneler, E.; *Arch. Pharm. Chem. Life Sci.* **2009b**, *342*, 9.
- Kolokythas, G.; Kostakis, I. K.; Pouli, N.; Marakos, P.; Skaltsounis, A.; Pratsinis, H.; *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1443.
- Kostakis, I.; Ghirtis, K.; Pouli, N.; Marakos, P.; Skaltsounis, A.; Leonce, S.; Caignard, D. H.; Atassi, G.; *Il Farmaco* **2000**, *55*, 455.
- Micheletti, A. C.; Beatriz, A.; Lima, D. P.; Honda, N. K.; Pessoa, C. O.; Moraes, M. O.; Lotufo, L. V.; Magalhães, H. I. F.; Carvalho, N. C. P.; *Quim. Nova* **2009**, *32*, 12.
- Sousa, E.; Paiva, A.; Nazareth, N.; Gales, L.; Damas, A. M.; Nascimento, M. S. J.; Pinto, M.; *Eur. J. Med. Chem.* **2009**, *44*, 3830.
- Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R.; *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
- Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Groodich, M.; Campbell, H.; Mayo, J.; Boyd, M.; *J. Natl. Cancer Inst.* **1991**, *83*, 757.
- Pinheiro, L.; Nakamura, C. V.; Dias Filho, B. P.; Ferreira, A. G.; Young, M. C. M.; Cortez, D. A. G.; *Mem. Inst. Oswaldo Cruz*, **2003**, *98*, 549.
- Yasunaka, K.; Abe, F.; Nagayama, A.; Okabe, H.; Lozada-Pérez, L.; López-Villafranco, E.; Muniz, E. E.; Aguilar, A.; Reyes-Chilpa, R.; *J. Ethnopharmacol.* **2005**, *97*, 293.
- Vooturi, S. K.; Cheung, C. M.; Rybak, M. J.; Firestone, S. M.; *J. Med. Chem.* **2009**, *52*, 5020.
- Varache-Lembège, M.; Moreau, S.; Larrouture, S.; Montaudon, D.; Robert, J.; Nuhrich, A.; *Eur. J. Med. Chem.* **2007**, *42*, 1.