



ANTIMICROBIAL AND ANTIFUNGAL OF SOME NEW SEMISYNTHETIC TERPENOID DERIVATIVES FROM EUPHORBOL

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ABSTRACT

The synthesis of a series of euphorbol derivatives is described, starting from euphorbol isolated from fresh latex of *Euphorbia resinifera* Berg. Their structures have been established on the basis of spectral data. All compounds were evaluated for antibacterial activities against *Escherichia coli* and *Staphylococcus aureus* strains and antifungal activity against *Candida albicans* and *Aspergillus niger* strains by using serial dilution method.

Keywords: Synthesis; euphorbol; latex; *Euphorbia resinifera* Berg, derivatives; antibacterial activities; antifungal activity

INTRODUCTION

The synthesis of new compounds to deal with resistant bacteria and fungi has become one of the most important areas of antibacterial and antifungal research today, since resistance of pathogenic bacteria and fungi toward available antimicrobial drug is rapidly becoming a major problem worldwide. So the discovery of novel and potent antibacterial as well as antifungal agent is more demanding. Despite great effort from the pharmaceutical industry to manage the resistance problem, the discovery and development of new mechanistic classes of antibiotics has found with very little success [1]. The difficulty of this task is demonstrated by the fact that only two antibiotics of new classes, linezolid and daptomycin, have been successfully developed in the past three decades [2]. As part of our ongoing valorisation of *Euphorbia* species native to Morocco, we have investigated the antimicrobial and antifungal semisynthetic terpenoid derivatives from euphorbol (1). The results of this study are discussed in this paper.

MATERIAL AND METHODS

General experimental procedures: Reactions were monitored by TLC on Merck 60 F₂₅₄ (0.25 mm) plates, which were viewed by UV inspection and/or

staining with 5% H₂SO₄ in ethanol and heating. Merck silica gel was used for column chromatography (CC). Melting points were determined on a Boëtius hot plate microscope and are uncorrected. The IR spectra were recorded on a Nicolet Impact 410 spectrometer, in KBr pellets. The NMR spectra were recorded on a Varian Gemini 300 BB instrument, operating at 300 MHz for ¹H-NMR and 75 MHz for ¹³C-NMR, the multiplicities were determined through DEPT. Mass spectra were recorded on a Varian MAT 311 spectrometer.

Plant material: Latex from *Euphorbia resinifera* Berg., was collected in the area of Demnat (Morocco), and identified by Dr. A. Echchahad (National Institute of Medicinal and Aromatic Plants of Taounate, Morocco). Latex was obtained as described [3,4,5].

Extraction and isolation: The latex of *Euphorbia resinifera* Berg., is strongly irritant to skin and mucous membranes. Handling of these substances should be carried out wearing latex gloves and face protection, and avoiding contact with the skin. The use of disposable plastic "glassware" is advisable for all operations involving either the latex. The triterpene euphorbol was extracted and isolated from fresh latex of *Euphorbia resinifera* Berg., as described [6].

Semisynthesis of euphorbol derivatives:

Semisynthesis of euphorbol-3-benzoate (2): To a solution of euphorbol (500 mg, 469.44 g/mol, 1.06 mmol) in dry toluene (7 mL), Benzoic acid (259 mg, 122.04 g/mol, 2.12 mmol, 2 equivalents), DMAP (262 mg, 2.12 mmol, 2 equivalents) and DCC (454 mg, 2.12 mmol, 2 equivalents) were added. After stirring at 90°C for 4 h, the reaction was worked up by filtration. The precipitate was washed with toluene, and the filtrates were treated with 5% NaHCO₃ and dried (Na₂SO₄). After evaporation, the residue was purified by gravity CC on silica gel (25 g, petroleum ether-EtOAc 9:1 as eluant) to afford 484 mg (84%) of compound (2) as a brown-yellowish powder R_f : 0.42 (petroleum ether-EtOAc 9:1). Boiling point: 133 °C; IR (KBr): 1720 (CO), 889 (C=C); ¹³C NMR (75 MHz, CDCl₃): C-1 (35.3), C-2 (27.9), C-3 (85.4), C-4 (38.9), C-5 (51.0), C-6 (18.9), C-7 (27.7), C-8 (133.5), C-9 (134.1), C-10 (37.3), C-11 (21.5), C-12 (30.8), C-13 (44.1), C-14 (50.0), C-15 (29.8), C-16 (28.1), C-17 (50.1), C-19 (20.2), C-20 (36.3), C-21 (18.8), C-22 (35.0), C-23 (31.3), C-24 (156.9), C-25 (33.8), C-26 (22.0), C-27 (21.9), C-28 (28.0), C-29 (15.5), C-30 (24.4), C-31 (105.9), C=O (167.2), C-1'(130.5), C-2'(129.3), C-3'(128.2), C-4'(132.4), C-5'(127.2), C-6'(128.2). ¹H NMR (CDCl₃, 300 MHz): H-1 [1.21 (α), 1.77 (β)], H-2 [1.67 (α), 1.57 (β)], H-3 [3.24 (dd, J = 4.3, 11.6 Hz)], H-5 [1.12 (dd, J) 1.8, 12.5 Hz)], H-6 [1.69 (α), 1.42 (β)], H-7 [2.08 (2H)], H-11 [2.06 (α), 1.92 (β)], H-12 [1.72 (2H)], H-15 [1.52 (α), 1.18 (β)], H-16 [1.32 (α), 1.94 (β)], H-18 [0.76 (s)], H-19 [0.96 (s)], H-21 [0.93 (d, J) 6.4 Hz)], H-22 [1.12, 1.55], H-23 [1.88, 2.12], H-25 [2.24 (s, J= 6.7 Hz)], H-26 [1.02 (d, J = 6.8 Hz)], H-27 [1.03 (d, J) 7.0 Hz)], H-28 [1.00 (s)], H-29 [0.80 (s)], H-30 [0.88 (s)], H-31 [4.66 and 4.72 (s)], H-2'(7.86), H-3'(7.38), H-4'(7.48), H-5'(7.31), H-6'(7.88); CI-EIMS: m/z [M+ H]⁺ 545 [C₃₈H₅₆O₂ + H]⁺.

Semisynthesis of euphorbol-3-(3-methoxybenzoate) (3): To a solution of euphorbol (500 mg, 469.44 g/mol, 1.06 mmol) in dry toluene (7 mL), 3-methoxy benzoic acid (381 mg, 180.04 g/mol, 2.12 mmol, 2 equivalents), DMAP (262 mg, 2.12 mmol, 2 equivalents) and DCC (454 mg, 2.12 mmol, 2 equivalents) were added. After stirring at 90°C for 4 h, the reaction was worked up by filtration. The precipitate was washed with toluene, and the filtrates were treated with 5% NaHCO₃ and dried (Na₂SO₄). After evaporation, the residue was purified by gravity CC on silica gel (25 g, petroleum ether-EtOAc 9:1 as eluant) to afford 505 mg (83%) of compound (3) as a brown-yellowish powder R_f : 0.35 (petroleum ether-EtOAc 9:1). Boiling point: 131 °C; IR (KBr): 1720 (CO), 889 (C=C); ¹H NMR (CDCl₃, 300 MHz): H-1 [1.21

(α), 1.77 (β)], H-2 [1.67 (α), 1.57 (β)], H-3 [3.24 (dd, J = 4.3, 11.6 Hz)], H-5 [1.12 (dd, J) 1.8, 12.5 Hz)], H-6 [1.69 (α), 1.42 (β)], H-7 [2.08 (2H)], H-11 [2.06 (α), 1.92 (β)], H-12 [1.72 (2H)], H-15 [1.52 (α), 1.18 (β)], H-16 [1.32 (α), 1.94 (β)], H-18 [0.76 (s)], H-19 [0.96 (s)], H-21 [0.93 (d, J) 6.4 Hz)], H-22 [1.12, 1.55], H-23 [1.88, 2.12], H-25 [2.24 (s, J= 6.7 Hz)], H-26 [1.02 (d, J = 6.8 Hz)], H-27 [1.03 (d, J) 7.0 Hz)], H-28 [1.00 (s)], H-29 [0.80 (s)], H-30 [0.88 (s)], H-31 [4.66 and 4.72 (s)], H-2'(7.5), H-4'(6.86), H-5'(7.2), H-6'(7.53), H-OCH₃ (3.73); ¹³C NMR (75 MHz, CDCl₃): C-1 (35.3), C-2 (27.9), C-3 (85.4), C-4 (38.9), C-5 (51.0), C-6 (18.9), C-7 (27.7), C-8 (133.5), C-9 (134.1), C-10 (37.3), C-11 (21.5), C-12 (30.8), C-13 (44.1), C-14 (50.0), C-15 (29.8), C-16 (28.1), C-17 (50.1), C-19 (20.2), C-20 (36.3), C-21 (18.8), C-22 (35.0), C-23 (31.3), C-24 (156.9), C-25 (33.8), C-26 (22.0), C-27 (21.9), C-28 (28.0), C-29 (15.5), C-30 (24.4), C-31 (105.9), C=O (169.2), C-1'(132.5), C-2'(116.3), C-3'(165.2), C-4'(119.4), C-5'(130.2), C-6'(123.2), C-OCH₃ (59.2); CI-EIMS: m/z [M+ H]⁺ 575 [C₃₉H₅₈O₃ + H]⁺.

Semisynthesis of euphorbol-3-(3,5-dimethoxybenzoate) (4): To a solution of euphorbol (500 mg, 469.44 g/mol, 1.06 mmol) in dry toluene (7 mL), 3,5-dimethoxy-benzoic acid (386 mg, 182.06 g/mol, 2.12 mmol, 2 equivalents), DMAP (262 mg, 2.12 mmol, 2 equivalents) and DCC (454 mg, 2.12 mmol, 2 equivalents) were added. After stirring at 90°C for 4 h, the reaction was worked up by filtration. The precipitate was washed with toluene, and the filtrates were treated with 5% NaHCO₃ and dried (Na₂SO₄). After evaporation, the residue was purified by gravity CC on silica gel (25 g, petroleum ether-EtOAc 9:1 as eluant) to afford 525 mg (82%) of compound (4) as a brown-yellowish powder R_f : 0.29 (petroleum ether-EtOAc 9:1). Boiling point: 134 °C; IR (KBr): 1720 (CO), 889 (C=C); ¹H NMR (CDCl₃, 300 MHz): H-1 [1.21 (α), 1.77 (β)], H-2 [1.67 (α), 1.57 (β)], H-3 [3.24 (dd, J = 4.3, 11.6 Hz)], H-5 [1.12 (dd, J) 1.8, 12.5 Hz)], H-6 [1.69 (α), 1.42 (β)], H-7 [2.08 (2H)], H-11 [2.06 (α), 1.92 (β)], H-12 [1.72 (2H)], H-15 [1.52 (α), 1.18 (β)], H-16 [1.32 (α), 1.94 (β)], H-18 [0.76 (s)], H-19 [0.96 (s)], H-21 [0.93 (d, J) 6.4 Hz)], H-22 [1.12, 1.55], H-23 [1.88, 2.12], H-25 [2.24 (s, J= 6.7 Hz)], H-26 [1.02 (d, J = 6.8 Hz)], H-27 [1.03 (d, J) 7.0 Hz)], H-28 [1.00 (s)], H-29 [0.80 (s)], H-30 [0.88 (s)], H-31 [4.66 and 4.72 (s)], H-2'(7.06), H-4'(6.51), H-6'(7.1), H-OCH₃ (3.73), H-OCH₃ (3.73); ¹³C NMR (75 MHz, CDCl₃): C-1 (35.3), C-2 (27.9), C-3 (85.4), C-4 (38.9), C-5 (51.0), C-6 (18.9), C-7 (27.7), C-8 (133.5), C-9 (134.1), C-10 (37.3), C-11 (21.5), C-12 (30.8), C-13 (44.1), C-14 (50.0), C-15 (29.8), C-16 (28.1), C-17 (50.1), C-19 (20.2), C-20 (36.3), C-21 (18.8), C-22 (35.0), C-23 (31.3), C-24 (156.9), C-25 (33.8),

C-26 (22.0), C-27 (21.9), C-28 (28.0), C-29 (15.5), C-30 (24.4), C-31 (105.9), C=O (168.2), C-1'(132.5), C-2'(107.3), C-3'(162.2), C-4'(104.4), C-5'(162.2), C-6'(107.2), C-OCH₃ (56.2), C-OCH₃ (56.2); CI-EIMS: m/z [M+ H]⁺ 605 [C₄₀H₆₀O₄ + H]⁺.

Semisynthesis of euphorbol-3-phenylacetate (5):

To a solution of euphorbol (500 mg, 469.44 g/mol, 1.06 mmol) in dry toluene (7 mL), phenylacetic acid (385 mg, 182.06 g/mol, 2.12 mmol, 2 equivalents), DMAP (262 mg, 2.12 mmol, 2 equivalents) and DCC (454 mg, 2.12 mmol, 2 equivalents) were added. After stirring at 90°C for 4 h, the reaction was worked up by filtration. The precipitate was washed with toluene, and the filtrates were treated with 5% NaHCO₃ and dried (Na₂SO₄). After evaporation, the residue was purified by gravity CC on silica gel (25 g, petroleum ether-EtOAc 9:1 as eluant) to afford 497 mg (84%) of compound (5) as a brown-yellowish powder Rf : 0.41 (petroleum ether-EtOAc 9:1). Boiling point: 138 °C; IR (KBr): 1720 (CO), 889 (C=C); ¹H NMR (CDCl₃, 300 MHz): H-1 [1.21 (α), 1.77 (β)], H-2 [1.67 (α), 1.57 (β)], H-3 [3.24 (dd, J = 4.3, 11.6 Hz)], H-5 [1.12 (dd, J) 1.8, 12.5 Hz], H-6 [1.69 (α), 1.42 (β)], H-7 [2.08 (2H)], H-11 [2.06 (α), 1.92 (β)], H-12 [1.72 (2H)], H-15 [1.52 (α), 1.18 (β)], H-16 [1.32 (α), 1.94 (β)], H-18 [0.76 (s)], H-19 [0.96 (s)], H-21 [0.93 (d, J) 6.4 Hz], H-22 [1.12, 1.55], H-23 [1.88, 2.12], H-25 [2.24 (s, J = 6.7 Hz)], H-26 [1.02 (d, J = 6.8 Hz)], H-27 [1.03 (d, J) 7.0 Hz], H-28 [1.00 (s)], H-29 [0.80 (s)], H-30 [0.88 (s)], H-31 [4.66 and 4.72 (s)], H-CH₂ (3.49), H-2'(7.86), H-3'(7.38), H-4'(7.48), H-5'(7.31), H-6'(7.88); ¹³C NMR (75 MHz, CDCl₃): C-1 (35.3), C-2 (27.9), C-3 (85.4), C-4 (38.9), C-5 (51.0), C-6 (18.9), C-7 (27.7), C-8 (133.5), C-9 (134.1), C-10 (37.3), C-11 (21.5), C-12 (30.8), C-13 (44.1), C-14 (50.0), C-15 (29.8), C-16 (28.1), C-17 (50.1), C-19 (20.2), C-20 (36.3), C-21 (18.8), C-22 (35.0), C-23 (31.3), C-24 (156.9), C-25 (33.8), C-26 (22.0), C-27 (21.9), C-28 (28.0), C-29 (15.5), C-30 (24.4), C-31 (105.9), C=O (167.2), C-CH₂ (39.9), C-1'(130.5), C-2'(129.3), C-3'(128.2), C-4'(132.4), C-5'(127.2), C-6'(128.2); CI-EIMS: m/z [M+ H]⁺ 559 [C₃₉H₅₈O₂ + H]⁺.

Semisynthesis of euphorbol-3-(3-methoxy-phenylacetate) (6):

To a solution of euphorbol (500 mg, 469.44 g/mol, 1.06 mmol) in dry toluene (7 mL), 3-methoxy-phenylacetic acid (352 mg, 166.06 g/mol, 2.12 mmol, 2 equivalents), DMAP (262 mg, 2.12 mmol, 2 equivalents) and DCC (454 mg, 2.12 mmol, 2 equivalents) were added. After stirring at 90°C for 4 h, the reaction was worked up by filtration. The precipitate was washed with toluene, and the filtrates were treated with 5% NaHCO₃ and

dried (Na₂SO₄). After evaporation, the residue was purified by gravity CC on silica gel (25 g, petroleum ether-EtOAc 9:1 as eluant) to afford 511 mg (82%) of compound (6) as a brown-yellowish powder Rf : 0.41 (petroleum ether-EtOAc 9:1). Boiling point: 131 °C; IR (KBr): 1720 (CO), 889 (C=C); ¹H NMR (CDCl₃, 300 MHz): H-1 [1.21 (α), 1.77 (β)], H-2 [1.67 (α), 1.57 (β)], H-3 [3.24 (dd, J = 4.3, 11.6 Hz)], H-5 [1.12 (dd, J) 1.8, 12.5 Hz], H-6 [1.69 (α), 1.42 (β)], H-7 [2.08 (2H)], H-11 [2.06 (α), 1.92 (β)], H-12 [1.72 (2H)], H-15 [1.52 (α), 1.18 (β)], H-16 [1.32 (α), 1.94 (β)], H-18 [0.76 (s)], H-19 [0.96 (s)], H-21 [0.93 (d, J) 6.4 Hz], H-22 [1.12, 1.55], H-23 [1.88, 2.12], H-25 [2.24 (s, J = 6.7 Hz)], H-26 [1.02 (d, J = 6.8 Hz)], H-27 [1.03 (d, J) 7.0 Hz], H-28 [1.00 (s)], H-29 [0.80 (s)], H-30 [0.88 (s)], H-31 [4.66 and 4.72 (s)], H-CH₂ (3.52), H-2'(7.5), H-4'(6.86), H-5'(7.2), H-6'(7.53), H-OCH₃ (3.73); ¹³C NMR (75 MHz, CDCl₃): C-1 (35.3), C-2 (27.9), C-3 (85.4), C-4 (38.9), C-5 (51.0), C-6 (18.9), C-7 (27.7), C-8 (133.5), C-9 (134.1), C-10 (37.3), C-11 (21.5), C-12 (30.8), C-13 (44.1), C-14 (50.0), C-15 (29.8), C-16 (28.1), C-17 (50.1), C-19 (20.2), C-20 (36.3), C-21 (18.8), C-22 (35.0), C-23 (31.3), C-24 (156.9), C-25 (33.8), C-26 (22.0), C-27 (21.9), C-28 (28.0), C-29 (15.5), C-30 (24.4), C-31 (105.9), C=O (169.2), C-CH₂ (39.1), C-1'(132.5), C-2'(116.3), C-3'(165.2), C-4'(119.4), C-5'(130.2), C-6'(123.2), C-OCH₃ (59.2); CI-EIMS: m/z [M+ H]⁺ 589 [C₄₀H₆₀O₃ + H]⁺.

Semisynthesis of euphorbol-3-(3,5-dimethoxy-phenylacetate) (7):

To a solution of euphorbol (500 mg, 469.44 g/mol, 1.06 mmol) in dry toluene (7 mL), 3,5-dimethoxy-phenylacetic acid (416mg, 196.07 g/mol, 2.12 mmol, 2 equivalents), DMAP (262 mg, 2.12 mmol, 2 equivalents) and DCC (454 mg, 2.12 mmol, 2 equivalents) were added. After stirring at 90°C for 4 h, the reaction was worked up by filtration. The precipitate was washed with toluene, and the filtrates were treated with 5% NaHCO₃ and dried (Na₂SO₄). After evaporation, the residue was purified by gravity CC on silica gel (25 g, petroleum ether-EtOAc 9:1 as eluant) to afford 544 mg (83%) of compound (7) as a brown-yellowish powder Rf : 0.36 (petroleum ether-EtOAc 9:1). Boiling point: 135 °C; IR (KBr): 1720 (CO), 889 (C=C); ¹H NMR (CDCl₃, 300 MHz): H-1 [1.21 (α), 1.77 (β)], H-2 [1.67 (α), 1.57 (β)], H-3 [3.24 (dd, J = 4.3, 11.6 Hz)], H-5 [1.12 (dd, J) 1.8, 12.5 Hz], H-6 [1.69 (α), 1.42 (β)], H-7 [2.08 (2H)], H-11 [2.06 (α), 1.92 (β)], H-12 [1.72 (2H)], H-15 [1.52 (α), 1.18 (β)], H-16 [1.32 (α), 1.94 (β)], H-18 [0.76 (s)], H-19 [0.96 (s)], H-21 [0.93 (d, J) 6.4 Hz], H-22 [1.12, 1.55], H-23 [1.88, 2.12], H-25 [2.24 (s, J = 6.7 Hz)], H-26 [1.02 (d, J = 6.8 Hz)], H-27 [1.03 (d, J) 7.0 Hz], H-28 [1.00 (s)], H-29 [0.80 (s)], H-30 [0.88 (s)], H-31 [4.66 and

4.72 (s)], H-CH₂ (3.56), H-2'(7.06), H-4'(6.51), H-6'(7.1), H-OCH₃ (3.73), H-OCH₃ (3.73); ¹³C NMR (75 MHz, CDCl₃): C-1 (35.3), C-2 (27.9), C-3 (85.4), C-4 (38.9), C-5 (51.0), C-6 (18.9), C-7 (27.7), C-8 (133.5), C-9 (134.1), C-10 (37.3), C-11 (21.5), C-12 (30.8), C-13 (44.1), C-14 (50.0), C-15 (29.8), C-16 (28.1), C-17 (50.1), C-19 (20.2), C-20 (36.3), C-21 (18.8), C-22 (35.0), C-23 (31.3), C-24 (156.9), C-25 (33.8), C-26 (22.0), C-27 (21.9), C-28 (28.0), C-29 (15.5), C-30 (24.4), C-31 (105.9), C=O (168.2), C-CH₂ (40), C-1'(132.5), C-2'(107.3), C-3'(162.2), C-4'(104.4), C-5'(162.2), C-6'(107.2), C-OCH₃ (56.2), C-OCH₃ (56.2); CI-EIMS: m/z [M+ H]⁺ 619 [C₄₁H₆₂O₄ + H]⁺.

Semisynthesis of euphorbol-3- phenylpropionate

(8): To a solution of euphorbol (500 mg, 469.44 g/mol, 1.06 mmol) in dry toluene (7 mL), phenylpropionic acid (318 mg, 150.07 g/mol, 2.12 mmol, 2 equivalents), DMAP (262 mg, 2.12 mmol, 2 equivalents) and DCC (454 mg, 2.12 mmol, 2 equivalents) were added. After stirring at 90°C for 4 h, the reaction was worked up by filtration. The precipitate was washed with toluene, and the filtrates were treated with 5% NaHCO₃ and dried (Na₂SO₄). After evaporation, the residue was purified by gravity CC on silica gel (25 g, petroleum ether-EtOAc 9:1 as eluant) to afford 509 mg (84%) of compound (8) as a brown-yellowish powder R_f : 0.40 (petroleum ether-EtOAc 9:1). Boiling point: 137 °C; IR (KBr): 1720 (CO), 889 (C=C); ¹H NMR (CDCl₃, 300 MHz): H-1 [1.21 (α), 1.77 (β)], H-2 [1.67 (α), 1.57 (β)], H-3 [3.24 (dd, J = 4.3, 11.6 Hz)], H-5 [1.12 (dd, J) 1.8, 12.5 Hz], H-6 [1.69 (α), 1.42 (β)], H-7 [2.08 (2H)], H-11 [2.06 (α), 1.92 (β)], H-12 [1.72 (2H)], H-15 [1.52 (α), 1.18 (β)], H-16 [1.32 (α), 1.94 (β)], H-18 [0.76 (s)], H-19 [0.96 (s)], H-21 [0.93 (d, J) 6.4 Hz], H-22 [1.12, 1.55], H-23 [1.88, 2.12], H-25 [2.24 (s, J= 6.7 Hz)], H-26 [1.02 (d, J = 6.8 Hz)], H-27 [1.03 (d, J) 7.0 Hz], H-28 [1.00 (s)], H-29 [0.80 (s)], H-30 [0.88 (s)], H-31 [4.66 and 4.72 (s)], H-CH₂ (2.59), H-CH₂ (2.94), H-2'(7.86), H-3'(7.38), H-4'(7.48), H-5'(7.31), H-6'(7.88); ¹³C NMR (75 MHz, CDCl₃): C-1 (35.3), C-2 (27.9), C-3 (85.4), C-4 (38.9), C-5 (51.0), C-6 (18.9), C-7 (27.7), C-8 (133.5), C-9 (134.1), C-10 (37.3), C-11 (21.5), C-12 (30.8), C-13 (44.1), C-14 (50.0), C-15 (29.8), C-16 (28.1), C-17 (50.1), C-19 (20.2), C-20 (36.3), C-21 (18.8), C-22 (35.0), C-23 (31.3), C-24 (156.9), C-25 (33.8), C-26 (22.0), C-27 (21.9), C-28 (28.0), C-29 (15.5), C-30 (24.4), C-31 (105.9), C=O (167.2), C-CH₂ (36.1), C-CH₂ (30.9) C-1'(130.5), C-2'(129.3), C-3'(128.2), C-4'(132.4), C-5'(127.2), C-6'(128.2); CI-EIMS: m/z [M+ H]⁺ 573 [C₄₀H₆₀O₂ + H]⁺.

Semisynthesis of euphorbol-3-(3-methoxy-phenylpropionate) (9): To a solution of euphorbol (500 mg, 469.44 g/mol, 1.06 mmol) in dry toluene

(7 mL), 3-methoxy-phenylpropic acid (382 mg, 180.08g/mol, 2.12 mmol, 2 equivalents), DMAP (262 mg, 2.12 mmol, 2 equivalents) and DCC (454 mg, 2.12 mmol, 2 equivalents) were added. After stirring at 90°C for 4 h, the reaction was worked up by filtration. The precipitate was washed with toluene, and the filtrates were treated with 5% NaHCO₃ and dried (Na₂SO₄). After evaporation, the residue was purified by gravity CC on silica gel (25 g, petroleum ether-EtOAc 9:1 as eluant) to afford 530 mg (83%) of compound (9) as a brown-yellowish powder R_f : 0.31 (petroleum ether-EtOAc 9:1). Boiling point: 139 °C; IR (KBr): 1720 (CO), 889 (C=C); ¹H NMR (CDCl₃, 300 MHz): H-1 [1.21 (α), 1.77 (β)], H-2 [1.67 (α), 1.57 (β)], H-3 [3.24 (dd, J = 4.3, 11.6 Hz)], H-5 [1.12 (dd, J) 1.8, 12.5 Hz], H-6 [1.69 (α), 1.42 (β)], H-7 [2.08 (2H)], H-11 [2.06 (α), 1.92 (β)], H-12 [1.72 (2H)], H-15 [1.52 (α), 1.18 (β)], H-16 [1.32 (α), 1.94 (β)], H-18 [0.76 (s)], H-19 [0.96 (s)], H-21 [0.93 (d, J) 6.4 Hz], H-22 [1.12, 1.55], H-23 [1.88, 2.12], H-25 [2.24 (s, J= 6.7 Hz)], H-26 [1.02 (d, J = 6.8 Hz)], H-27 [1.03 (d, J) 7.0 Hz], H-28 [1.00 (s)], H-29 [0.80 (s)], H-30 [0.88 (s)], H-31 [4.66 and 4.72 (s)], H-CH₂ (2.55), H-CH₂ (2.92), H-2'(7.5), H-4'(6.86), H-5'(7.2), H-6'(7.53), H-OCH₃ (3.73); ¹³C NMR (75 MHz, CDCl₃): C-1 (35.3), C-2 (27.9), C-3 (85.4), C-4 (38.9), C-5 (51.0), C-6 (18.9), C-7 (27.7), C-8 (133.5), C-9 (134.1), C-10 (37.3), C-11 (21.5), C-12 (30.8), C-13 (44.1), C-14 (50.0), C-15 (29.8), C-16 (28.1), C-17 (50.1), C-19 (20.2), C-20 (36.3), C-21 (18.8), C-22 (35.0), C-23 (31.3), C-24 (156.9), C-25 (33.8), C-26 (22.0), C-27 (21.9), C-28 (28.0), C-29 (15.5), C-30 (24.4), C-31 (105.9), C=O (169.2), C-CH₂ (36.2), C-1'(132.5), C-2'(116.3), C-3'(165.2), C-4'(119.4), C-5'(130.2), C-6'(123.2), C-OCH₃ (59.2); CI-EIMS: m/z [M+ H]⁺ 603 [C₄₁H₆₂O₃ + H]⁺.

Semisynthesis of euphorbol-3-(3,5-dimethoxy-phenylpropionate) (10):

To a solution of euphorbol (500 mg, 469.44 g/mol, 1.06 mmol) in dry toluene (7 mL), 3,5-dimethoxy-phenylpropic acid (445 mg, 210.09 g/mol, 2.12 mmol, 2 equivalents), DMAP (262 mg, 2.12 mmol, 2 equivalents) and DCC (454 mg, 2.12 mmol, 2 equivalents) were added. After stirring at 90°C for 4 h, the reaction was worked up by filtration. The precipitate was washed with toluene, and the filtrates were treated with 5% NaHCO₃ and dried (Na₂SO₄). After evaporation, the residue was purified by gravity CC on silica gel (25 g, petroleum ether-EtOAc 9:1 as eluant) to afford 556 mg (83%) of compound (10) as a brown-yellowish powder R_f : 0.28 (petroleum ether-EtOAc 9:1). Boiling point: 141 °C; IR (KBr): 1720 (CO), 889 (C=C); ¹H NMR (CDCl₃, 300 MHz): H-1 [1.21 (α), 1.77 (β)], H-2 [1.67 (α), 1.57 (β)], H-3 [3.24 (dd, J = 4.3, 11.6 Hz)], H-5 [1.12 (dd, J) 1.8, 12.5 Hz], H-6 [1.69 (α), 1.42 (β)], H-7

[2.08 (2H)], H-11 [2.06 (α), 1.92 (β)], H-12 [1.72 (2H)], H-15 [1.52 (α), 1.18 (β)], H-16 [1.32 (α), 1.94 (β)], H-18 [0.76 (s)], H-19 [0.96 (s)], H-21 [0.93 (d, J) 6.4 Hz], H-22 [1.12, 1.55], H-23 [1.88, 2.12], H-25 [2.24 (s, J = 6.7 Hz)], H-26 [1.02 (d, J = 6.8 Hz)], H-27 [1.03 (d, J) 7.0 Hz], H-28 [1.00 (s)], H-29 [0.80 (s)], H-30 [0.88 (s)], H-31 [4.66 and 4.72 (s)], H-CH₂ (2.91), H-CH₂ (2.56), H-2' (7.06), H-4' (6.51), H-6' (7.1), H-OCH₃ (3.73), H-OCH₃ (3.73); ¹³C NMR (75 MHz, CDCl₃): C-1 (35.3), C-2 (27.9), C-3 (85.4), C-4 (38.9), C-5 (51.0), C-6 (18.9), C-7 (27.7), C-8 (133.5), C-9 (134.1), C-10 (37.3), C-11 (21.5), C-12 (30.8), C-13 (44.1), C-14 (50.0), C-15 (29.8), C-16 (28.1), C-17 (50.1), C-19 (20.2), C-20 (36.3), C-21 (18.8), C-22 (35.0), C-23 (31.3), C-24 (156.9), C-25 (33.8), C-26 (22.0), C-27 (21.9), C-28 (28.0), C-29 (15.5), C-30 (24.4), C-31 (105.9), C=O (168.2), C-CH₂ (36.2), C-CH₂ (31.5), C-1' (132.5), C-2' (107.3), C-3' (162.2), C-4' (104.4), C-5' (162.2), C-6' (107.2), C-OCH₃ (56.2), C-OCH₃ (56.2); CI-EIMS: m/z [M+ H]⁺ 633 [C₄₂H₆₄O₄ + H]⁺.

Determination of the in vitro antimicrobial activity: All the synthesized compounds were tested for their in vitro antimicrobial activity against a panel of standard strains of the Gram-positive bacteria, *Staphylococcus aureus* ATCC 19433 (SA) and *Bacillus subtilis* ATCC 6633 (BS), the Gram-negative bacteria, *Escherichia coli* ATCC 25922 (EC) and *Pseudomonas aeruginosa* ATCC 27853 (PA), and the yeast-like pathogenic fungus *Candida albicans* ATCC 753 (CA). The primary screening was carried out using the agar-disk diffusion method using Muller-Hinton agar medium [7]. Sterile filter paper disks (8 mm diameter) were moistened with the test compound solution in dimethylsulfoxide of specific concentration (200 μ g/disk).

The disks containing the compounds under test, the antimicrobial antibiotic ampicillin trihydrate (100 μ g/disk) and antifungal drug clotrimazole (100 μ g/disk), were carefully placed on the agar culture plates that had been previously inoculated separately with the microorganisms suspension at 106 Colony Forming Unit/mL (CFU/mL) concentration. The plates were incubated at 37 °C, and the diameter of the growth inhibition zones was measured after 24 h in case of bacteria and 48 h in case of *C. albicans*. The minimal inhibitory concentration (MIC) for the most active compounds against the same microorganisms used in the primary screening was carried out using the microdilution susceptibility method in Muller-Hinton broth and Sabouraud liquid medium. The compounds, ampicillin trihydrate, and clotrimazole were dissolved in dimethylsulfoxide at concentration 800 μ g/mL. The twofold dilutions of

the solution were prepared (400, 200, 100, ... 6.25 μ g/mL). The microorganism suspensions at 106 CFU/mL concentrations were inoculated to the corresponding wells. The plates were incubated at 37 °C for 24 and 48 h for the bacteria and *C. albicans*, respectively. The MIC values were determined as the lowest concentration that completely inhibited visible growth of the microorganisms as detected by unaided eye.

RESULTS AND DISCUSSION

Chemistry: The preparation of the target compounds is outlined in schema 1. The chemical modifications of natural triterpene euphorbol (**1**) were focused mainly on positions 3 with esterification with various aromatic acids as benzoic acid, 3-methoxy benzoic acid, 3,5-dimethoxybenzoic acid, phenyl acetic acid, 3-methoxy-phenyl acetic acid, 3,5-dimethoxy phenyl acetic acid, phenyl propionic acid, 3-methoxy phenyl propionic acid and 3,5-dimethoxy phenyl propionic acid in presence of acylating agent (DCC and DMAP) to give successively euphorbol-3-benzoate (**2**), euphorbol-3-(3-methoxy-benzoate) (**3**), euphorbol-3-(3,5-dimethoxy-benzoate) (**4**), euphorbol-3-phenylacetate (**5**), euphorbol-3-(3-methoxy-phenylacetate) (**6**), euphorbol-3-(3,5-dimethoxy-phenylacetate) (**7**), euphorbol-3-phenylpropionate (**8**), euphorbol-3-(3-methoxy-phenylpropionate) (**9**) and euphorbol-3-(3,5-dimethoxy-phenylpropionate) (**10**). The reaction was investigated with benzoic acid. A first upgrade to 34% yield was achieved by delaying (ca. 30 min) the addition of euphorbol to the acid-DCC-DMAP mixture, while a 1:2 acid to euphorbol ratio accelerated the reaction with beneficial effect in yield.

The insolubility of benzoic acid in toluene was a further point of improvement, since the elevation of temperature of reaction to 90°C provided a homogeneous reaction mixture and a beneficial effect in yield. After dilution of the reaction mixture with ethyl acetate, washing with brine, and evaporation, the reaction mixture was crystallized from methanol, affording euphorbol-3-benzoate in 75% yield. Alternatively, unreacted euphorbol could be removed by chromatography on silica gel, affording euphorbol-3-benzoate in 84% yield. The optimized protocol was next applied to a variety of aromatic acids (schema 1). The identification of the various euphorbol derivatives (**2-10**) was based on spectroscopic data.

Antimicrobial activity: The results of the preliminary antimicrobial testing of compounds **1-10** (200 μ g/disk) and the broad-spectrum antibacterial antibiotic ampicillin trihydrate (100

$\mu\text{g}/\text{disk}$) are shown in Table 1. The results revealed that the majority of the synthesized compounds showed varying degrees of inhibition against the tested microorganisms. In general, the inhibitory activity against the tested Gram-positive bacteria was higher than that of the Gram-negative one. Compounds **4–3**, **5–8** and **6** displayed broad-spectrum antimicrobial activity; they possessed excellent activity against the Gram-positive bacteria, moderate activity against *E. coli*, and weak activity against *C. albicans*. The least susceptible organisms were *P. aeruginosa* and *C. albicans*. Only compound **4**, was moderately active against *C. albicans* and compounds **9** and **6** showed moderate activity against *E. coli*. However, compound **7** exhibited moderate activity against *E. coli* and *P. aeruginosa* in addition to weak activity against *C. albicans*. None of the tested compounds were found to be as strong as clotrimazole. The compound **1** is completely inactive against the tested strains.

CONCLUSION

Various new derivatives of euphorbol (**2–10**) have been semisynthesized from the condensation of natural euphorbol isolated from fresh latex of *Euphorbia resinifera* Berg., and the commercial aromatic acids, the reactions were carried out in toluene in presence of DCC and DMAP. To improve the yield, we increased the temperature of reaction to 90°C . The structure of the compounds **2–10** has been characterized by IR, ^1H NMR, ^{13}C NMR and mass spectral data. The novel compounds were subjected to anti-microbial screening. The compounds showed moderate to good antibacterial and antifungal activities.

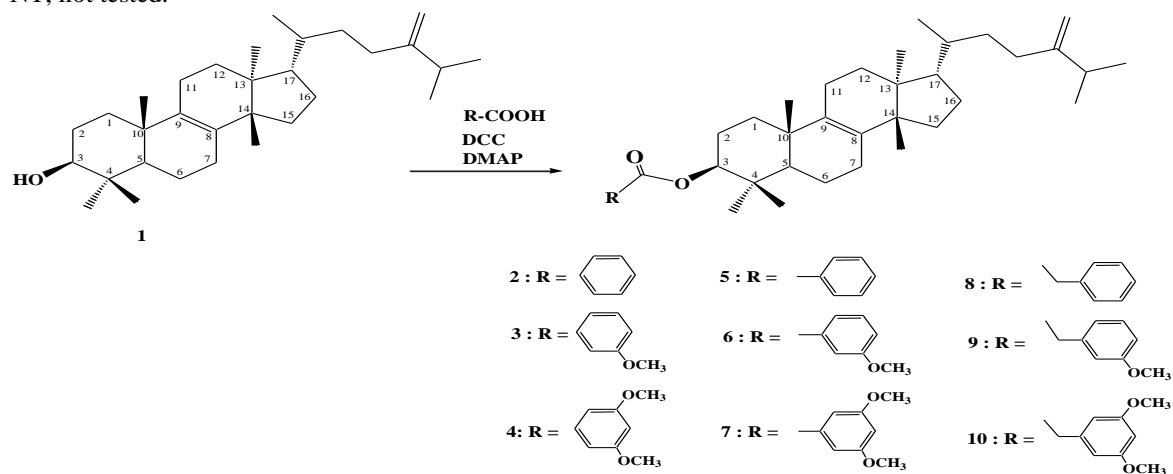
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Table 1. Antimicrobial activity of compounds (200 $\mu\text{g}/8$ mm disk), the broad-spectrum antibacterial drug ampicillin trihydrate (100 $\mu\text{g}/8$ mm disk) and the antifungal drug Clotrimazole (100 $\mu\text{g}/8$ mm disk) against *S. aureus* ATCC 19433 (SA), *B. subtilis* ATCC 6633 (BS), *E. coli* ATCC 25922 (EC), *P. aeruginosa* ATCC 27853 (PA), and *C. albicans* ATCC 753 (CA).

Cpd	MIC ($\mu\text{g}/\text{mL}$)				
	SA	BS	EC	PA	CA
2	100	100	100	100	100
3	55	53	111	116	115
4	17	21	57	106	58
5	56	105	106	113	113
6	58	56	50	110	102
7	19	15	52	55	104
8	57	58	105	106	107
9	54	54	51	105	105
10	19	16	33	102	108
Ampicillin	< 7.5	< 7.5	< 7.5	< 7.5	NT
Clotrimazole	NT	NT	NT	NT	12.8

NT, not tested.



Scheme 1: Semisynthesis of euphorbol derivatives

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