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Semi-synthesis and Antimicrobial activities of some new Euphorbioside derivatives

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Abstract: The synthesis of a series of euphorbioside derivatives is described, starting from euphorbioside 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 *Staphyloccus aureus* strains and antifungal activity against *Candida albicans* and *Aspergillus niger* strains by using serial dilution method.
Keywords : semisynthesis; euphorbiosidel; derivatives; antibacterial activities; antifungal activity.

1. Introduction

The synthesis of new compounds to dead 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 derivatives from euphorbioside (**1**). The results of this study are discussed in this paper.

2. Material and Methods

2.1. General experimental procedures

Reactions were monitored by TLC on Merck 60 F_{254} (0.25 mm) plates, which were viewed by UV inspection and/or staining with 5% H_2SO_4 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 ^{1}H-NMR and 75 MHz for ^{13}C-NMR, the multiplicities were determined through DEPT. Mass spectra were recorded on a Varian MAT 311 spectrometer.

2.2. 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-,6].

2.3. 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 euphorbioside was extracted and isolated from fresh latex of *Euphorbia resinifera* Berg., as described[6]. (Ernesto Fattorusso, Virginia Lanzotti, Orazio Taglialatela-Scafati, Gian Cesare Tron, and Giovanni Appendino. Bisnorsesquiterpenoids from *Euphorbia resinifera* Berg. and an Expeditious Procedure to Obtain Resiniferatoxin from Its Fresh Latex. *Eur. J. Org. Chem.* 2002, 71-78)

2.4. Semisynthesis of euphorbioside derivatives

2.4.1. **Semisynthesis of euphorbioside-9-(3-dimethylamino-benzoate) (2):** To a solution of euphorbioside (200 mg, 214.14 g/mol, 0.93 mmol) in dry toluene (7 mL), 3-dimethylamino-benzoic acid (153 mg, 165.08 g/mol, 0.93 mmol, 1 equivalents), DMAP (230 mg, 1.86 mmol, 2 equivalents) and DCC (398 mg, 1.86 mmol, 2 equivalents) were added. After stirring at 60°C for 4 h, the reaction was worked up by filtration. The precipitate was washed with toluene, and the filtrates were treated with 5% $NaHCO_3$ and dried (Na_2SO_4). After evaporation, the residue was purified by gravity CC on silica gel (15 g, petroleum ether-EtOAc 9:1 as eluant) to afford 182 mg (54%) of compound (**2**) as a brown-yellowish powder Rf : 0.25 (petroleum ether-EtOAc 1:9); IR (KBr): 3700; 3374, 1674, 1605, 1577, 1520, 1262, 1238, 1172, 1127, 1082 cm^{-1}; IR (KBr): 3700, 3374, 1674, 1605, 1577, 1520, 1262, 1238, 1172, 1127, 1082 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD): δ = 1.03 (s, 3 H); 1.17 (s, 3 H); 1.28 (d, 3 H); 1.67 (dd, 1 H); 1.93 (dd, 1 H); 2.19 (d, 1 H); 3.34 (s, 1 H); 3.53 (d, 1 H); 3.73 (ddd, 1 H); 4.01 (d, 1 H); 4.31 (dq, 1 H); 5.69 (dd, 1 H); 5.79 (dd, 1 H); 3.02 (3H, s, *N*-Me), 3.00 (3H, s, *N*-Me), 6.73 (1H, br t, H-5’), 6.87 (1H, br d, H-4’), 7.43 (1H, td, H-2’), 7.93 (1H, dd, H-6’). ^{13}C NMR (CD_3OD): δ= 18.5 (C-12); 23.0 (C-13); 24.3 (C-10); 42.1 (C-4); 43.2 (N-CH_3), 43.0 (N-CH_3), 50.0 (C-1); 61.5 (C-6); 68.8 (C-9); 70.5 (C-3); 74.3 (C-2); 75.0 (C-11); 82.8 (C-5); 123.5 (C-7); 142.1 (C-8); 109.8 (C-1’), 150.1 (C-3’), 114.7 (C-5’), 131.5 (C-6’), 111.3 (C-4’), 133.6 (C-2’), 168.1 (C=O). CI-EIMS: m/z [M+ H]$^+$ 362 [$C_{20}H_{27}NO_5$ + H]$^+$.

2.4.2. **Semisynthesis of euphorbioside-9-(4-dimethylamino-benzoate) (3):** To a solution of euphorbioside (200 mg, 214.14 g/mol, 0.93 mmol) in dry toluene (7 mL), 4-dimethylamino-benzoic acid (153 mg, 165.08 g/mol, 0.93 mmol, 1 equivalents), DMAP (230 mg, 1.86 mmol, 2 equivalents) and DCC (398 mg, 1.86 mmol, 2 equivalents) were added. After stirring at 60°C for 4 h, the reaction was worked up by filtration. The precipitate was washed with toluene, and the filtrates were treated with 5% $NaHCO_3$ and dried (Na_2SO_4). After evaporation, the residue was purified by gravity CC on silica gel (15 g, petroleum ether-EtOAc 9:1 as eluant) to afford 202 mg (60%) of compound (**3**) as a brown-yellowish powder Rf : 0.24 (petroleum ether-EtOAc 1:9). IR (KBr): 3700, 3374, 1674, 1605, 1577, 1520, 1262, 1238, 1172, 1127, 1082 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD): δ = 1.03 (s, 3 H); 1.17 (s, 3 H); 1.28 (d, 3 H); 1.67 (dd, 1 H); 1.93 (dd, 1 H); 2.19 (d, 1 H); 3.34 (s, 1 H); 3.53 (d, 1 H); 3.73 (ddd, 1 H); 4.01 (d, 1 H); 4.31 (dq, 1 H); 5.69 (dd, 1 H); 5.79 (dd, 1 H); 3.02 (3H, s, *N*-Me), 3.00 (3H, s, *N*-Me), 6.73 (1H, br t, H-5’), 6.87 (1H, br d, H-3’), 7.43 (1H, td, H-2’), 7.93 (1H, dd, H-6’). ^{13}C NMR (CD_3OD): δ= 18.5 (C- 12); 23.0 (C-13); 24.3 (C-10); 42.1 (C-4); 43.2 (N-CH_3), 43.0 (N-CH_3), 50.0 (C-1); 61.5 (C-6); 68.8 (C-9); 70.5 (C-3); 74.3 (C-2); 75.0 (C-11); 82.8 (C-5); 123.5 (C-7); 142.1 (C-8); 109.8 (C-1’), 111.7

(C-3'), 114.7 (C-5'), 131.5 (C-6'), 149.1 (C-4'), 133.6 (C-2'), 168.1 (C=O). CI-EIMS: m/z $[M+ H]^+$ 362 $[C_{20}H_{27}NO_5 + H]^+$.

2.4.3. **Semisynthesis of euphorbioside-9-(2-methylamino-benzoate) (4):** To a solution of euphorbioside (200 mg, 214.14 g/mol, 0.93 mmol) in dry toluene (7 mL), 2-methylamino-benzoic acid (140 mg, 151.06 g/mol, 0.93 mmol, 1 equivalents), DMAP (230 mg, 1.86 mmol, 2 equivalents) and DCC (398 mg, 1.86 mmol, 2 equivalents) were added. After stirring at 60°C for 4 h, the reaction was worked up by filtration. The precipitate was washed with toluene, and the filtrates were treated with 5% $NaHCO_3$ and dried (Na_2SO_4). After evaporation, the residue was purified by gravity CC on silica gel (15 g, petroleum ether-EtOAc 9:1 as eluant) to afford 178.61 mg (55%) of compound (**4**) as a brown-yellowish powder Rf : 0.21 (petroleum ether-EtOAc 1:9); IR (KBr): 3700, 3374, 1674, 1605, 1577, 1520, 1262, 1238, 1172, 1127, 1082 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ = 1.03 (s, 3 H); 1.17 (s, 3 H); 1.28 (d, 3 H); 1.67 (dd, 1 H); 1.93 (dd, 1 H); 2.19 (d, 1 H); 3.34 (s, 1 H); 3.53 (d, 1 H); 3.73 (ddd, 1 H); 4.01 (d, 1 H); 4.31 (dq, 1 H); 5.69 (dd, 1 H); 5.79 (dd, 1 H); 3.02 (3H, s, *N*-Me), 6.73 (1H, br t, H-5'), 6.87 (1H, br d, H-3'), 7.43 (1H, td, H-4'), 7.93 (1H, dd, H-6'). ^{13}C NMR (CD_3OD): δ= 18.5 (C- 12); 23.0 (C-13); 24.3 (C-10); 42.1 (C-4); 43.2 (N-CH_3); 50.0 (C-1); 61.5 (C-6); 68.8 (C-9); 70.5 (C-3); 74.3 (C-2); 75.0 (C-11); 82.8 (C-5); 123.5 (C-7); 142.1 (C-8); 109.8 (C-1'), 111.7 (C-3'), 114.7 (C-5'), 131.5 (C-6'), 134.4 (C-4'), 151.9 (C-2'), 168.1 (C=O). CI-EIMS: m/z $[M+ H]^+$ 348$[C_{19}H_{25}NO_5 + H]^+$.

2.4.4. **Semisynthesis of euphorbioside -9-(4-methylamino-benzoate) (5):** To a solution of euphorbioside (200 mg, 214.14 g/mol, 0.93 mmol) in dry toluene (7 mL), 4-methylamino-benzoic acid (385 mg, 182.06 g/mol, 2.12 mmol, 2 equivalents), DMAP (230 mg, 1.86 mmol, 2 equivalents) and DCC (398 mg, 1.86 mmol, 2 equivalents) were added. After stirring at 60°C for 4 h, the reaction was worked up by filtration. The precipitate was washed with toluene, and the filtrates were treated with 5% $NaHCO_3$ and dried (Na_2SO_4). After evaporation, the residue was purified by gravity CC on silica gel (15 g, petroleum ether-EtOAc 2:8) to afford 142 mg (44%) of compound (**5**) as a brown-yellowish powder Rf : 0.19 (petroleum ether-EtOAc 1:9). IR (KBr): 3700, 3374, 1674, 1605, 1577, 1520, 1262, 1238, 1172, 1127, 1082 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ = 1.03 (s, 3 H); 1.17 (s, 3 H); 1.28 (d, 3 H); 1.67 (dd, 1 H); 1.93 (dd, 1 H); 2.19 (d, 1 H); 3.34 (s, 1 H); 3.53 (d, 1 H); 3.73 (ddd, 1 H); 4.01 (d, 1 H); 4.31 (dq, 1 H); 5.69 (dd, 1 H); 5.79 (dd, 1 H); 3.02 (3H, s, *N*-Me), 6.73 (1H, br t, H-5'), 6.87 (1H, br d, H-3'), 7.43 (1H, td, H-2'), 7.93 (1H, dd, H-6'). ^{13}C NMR (CD_3OD): δ= 18.5 (C-12); 23.0 (C-13); 24.3 (C-10); 42.1 (C-4); 43.2 (N-CH_3); 50.0 (C-1); 61.5 (C-6); 68.8 (C-9); 70.5 (C-3); 74.3 (C-2); 75.0 (C-11); 82.8 (C-5); 123.5 (C-7); 142.1 (C-8); 109.8 (C-1'), 111.7 (C-3'), 114.7 (C-5'), 131.5 (C-6'), 149.1 (C-4'), 133.6 (C-2'), 168.1 (C=O). CI-EIMS: m/z $[M+ H]^+$ 348$[C_{19}H_{25}NO_5 + H]^+$.

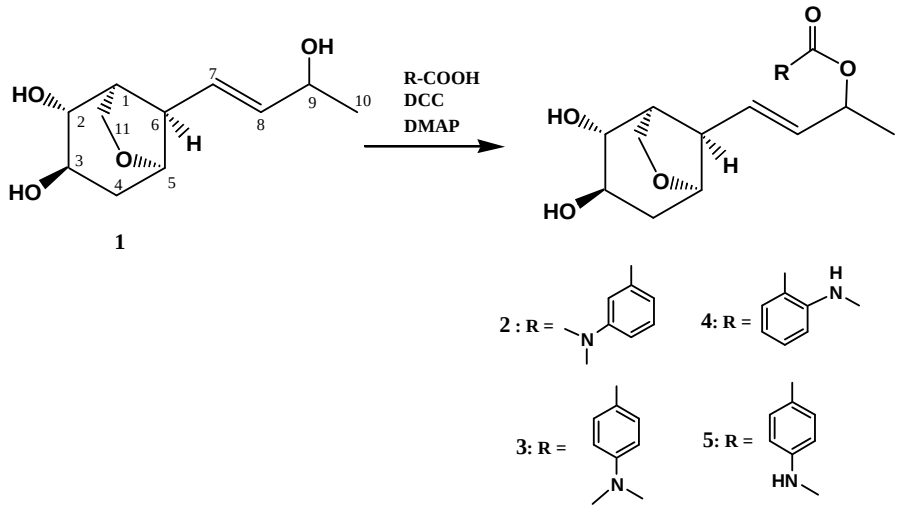
2.5. Determination of the in vitro antimicrobial activity

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 µg/disk). The disks containing the compounds under test, the antimicrobial antibiotic ampicillin trihydrate (100 µg/disk) and antifungal drug clotrimazole (100 µg/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 dimethylsufoxide at concentration 800 µg/mL. The twofold dilutions of the solution were prepared (400, 200, 100, … 6.25 µg/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.

3. Results and discussion

3.1. Chemistry

The preparation of the target compounds is outlined in schema 1. The chemical modifications of natural euphorbioside (**1**) were focused mainly on positions 9 with esterification with various aromatic amino acids as 3-dimethylamino-benzoic acid, 4-dimethylamino-benzoic acid, 2-methylamino-benzoic acid, and 4-methylamino-benzoic acid in presence of acylating agent (DCC and DMAP) to give successively euphorbioside-9-(3-dimethylamino-benzoate) (**2**), euphorbioside-9-(4-dimethylamino-benzoate) (**3**), euphorbioside-9-(2-methylamino-benzoate) (**4**) and euphorbioside -9-(4-methylamino-benzoate) (**5**).



Scheme 1. Semisynthesis of euphorbioside derivatives

The reaction was investigated with 3-dimethylamino-benzoic acid. A first upgrade to 34% yield was achieved by delaying (ca. 30 min) the addition of euphorbioside to the acid–DCC–DMAP mixture, while a 1:1 acid to euphorbioside ratio accelerated the reaction with beneficial effect in yield. The insolubility of 3-dimethylamino-benzoic acid in toluene was a further point of improvement, since the elevation of temperature of reaction to 60°C for 4 h 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 euphorbioside-9-(3-dimethylamino-benzoate) (**2**) in 50% yield. Alternatively, unreacted euphorbioside could be removed by chromatography on silica gel, affording compound **2** in 54% yield. The optimized protocol was next applied to a variety of aromatic amino acids (scheme 1). The identification of the various euphorbioside derivatives (**2-5**) was based on spectroscopic data.

3.2. Antimicrobial activity

The results of the preliminary antimicrobial testing of compounds **1-5** (200 µg/disk) and the broad-spectrum antibacterial antibiotic ampicillin trihydrate (100 µg/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 **2, 3, 4** and **5** 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 **5** was moderately active against *C. albicans* and compounds **3** and **2** showed moderate activity against *E. coli*. However, compound **4** 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 natural euphorbioside (**1)** is completely inactive against the tested strains.

Table 1. Antimicrobial activity of compounds (200 µg/8 mm disk), the broad-spectrum antibacterial drug ampicillin trihydrate (100 µg/8 mm disk) and the antifungal drug Clotrimazole (100 µg/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 (µg/mL)				
	SA	BS	EC	PA	CA
2	76	75	73	128	124
3	77	75	73	132	124
4	37	38	71	75	123
5	31	42	74	121	74
Ampicillin	< 10	< 10	< 10	< 10	NT
Clotrimazole	NT	NT	NT	NT	14

NT, not tested.

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References

1. Taun, P.T., Edmund, L.E., Joseph, P.S., Brian, M.W., Michael, A.S., Hollis, S., John, M.D., Martin, A.S., Themis, J., Stephen, J.G., Dai, Q.N., Paul, B., Judy, Y., Anurag, S., Chan, H., Saeed, R., Xiujuan, W., Rajeshwar, S., Structure activity relationshipsof 3-aminoquinazolinediones, a new class of bacterial type-2 topoisomerase (DNA gyrase and topo IV) inhibitors. *Bioorg. Med. Chem., Lett*. 2007, 17, 1312–1320.
2. Weigelt, J., Itani, K., Stevens, D., Lau, W., Dryden, M., Knirsch, C., Linzolid versus vancomycin in treatment of complicated skin and soft tissue infections. *Antimicrob. Agents Chemother*. 2005, 49, 2260–2266.
3. Mazoir, N., Liazid, A., Auhmani, A., Daoubi, M., Dakir, M., Benharref, A., Kenz, A., Pierrot, M.,. 1H and 13C NMR structural study of products obtained by semisynthesis on the triterpenes isolated from latex of Euphorbia officinarum. Phys. Chem. News. 2005, 21, 123–128.
4. Daoubi, M., Marquez, N., Mazoir, N., Benharref, A., Gala´n, R.H., Mun˜oz, E., Collado, I.G. Isolation of new phenylacetylingol derivatives that reactivate HIV-1 latency and a novel spirotriterpenoid from Euphorbia officinarum latex. Bioorg. Med. Chem. 2007, 15, 4577–4584.
5. Daoubi, M., Macias-Sanchez, A.J., Hernandez-Galan, R., Benharref, A., Collado, I.G.Two novel steroids from Euphorbia officinarum latex. Nat. Prod. Res. 2004, 18, 177–181.
6. Ernesto Fattorusso, Virginia Lanzotti, Orazio Taglialatela-Scafati, Gian Cesare Tron, and Giovanni Appendino. Bisnorsesquiterpenoids from *Euphorbia resinifera* Berg. and an Expeditious Procedure to Obtain Resiniferatoxin from Its Fresh Latex. *Eur. J. Org. Chem.* 2002, 71-78
7. Patrick, P.M., Ellen, B.J., Michael, A.P., Fred, C.T., Robert, H.Y., Clinical laboratory standards. In: Wood, G.L., Washington, (Eds.), Manual of Clinical Microbiology. Am Soc Microbiol, Washington, DC, 1995, pp. 273–286.

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