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Article

Limonin Derivatives: Synthesis Using Methodology in Solution and Heterogeneous Medium and Evaluation of the Antimicrobial Activity

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We herein described the preparation of a novel series of limonin derivatives (modification in A-ring), which was synthesized efficiently using methodology in solution as well as in heterogeneous medium (K-10). In addition, we obtained derivatives by inserting the 1,2,3-triazole nucleus via click reaction and also prepared derivatives from reactions with limonin-7-oxime. All compounds were submitted to investigation of the antimicrobial activity against a collection of microorganisms. The results of the antimicrobial activity, in general, demonstrated that a relevant number of synthetic derivatives presented higher activity than the natural product.

Keywords: limonin derivatives, methodology in solution, heterogeneous medium, antimicrobial activity

Introduction

Limonoids are secondary metabolites also known as tetranortriterpenoids. This large class of C_{26} degraded triterpenes is found in plant families such as Rutaceae and Meliaceae, which have been shown to possess a wide spectrum of biological properties.[1-4] Limonin (**1**) (Figure 1), the most abundant limonoid from citrus, is a highly oxygenated compound known to present various biological activities, including the ability to inhibit HIV-1 replication,[5] anticarcinogenic,[6,7] antinociceptive and anti-inflammatory properties.[8] Studies have described that changes in the B-ring of **1** (at C-7 position) greatly affect biological activities, such as antifeedant,[9] anti-proliferative,[10] antiinflammatory and analgesic.[11] Literature has demonstrated a positive influence on the induction of phase II enzymes by limonin-7-methoxime (**1** modified on B-ring). Phase II enzymes are associated with the initiation of most types of cancers.[12] Other changes in the limonin skeleton are described, such as in the D-ring. The D-ring of the limonin nucleus has a furan ring attached to its C-3 position. Its modified forms such as defuran limonin exhibit loss of cytotoxicity in human breast cancer cells (MDA-MB-231),[10] and loss of p38 mitogen-activated protein (MAP) kinase activity.[13] Moreover, the complete hydrogenation of the furan ring **1** resulted in a lower antifeedant activity against *S. frugiperda*.[9] Another synthetic limonin derivative from the modification of D-ring is the desoxylimonin that exhibited less analgesic and anti-inflammatory efficacy than limonin, suggesting the importance of the epoxy group for these activities.[11]

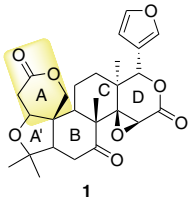

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Figure 1. Structure of limonin (**1**).

On the other hand, literature reports few studies from the modification in A-ring of **1** and its investigation of the antimicrobial activity.[14-17] Based on these aspects, this study reports the obtaining of limonin derivatives from changes in A-ring and the investigation of antimicrobial activity of all compounds. The modifications in the A-ring were made through aminolysis reactions with different primary amines

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in homogeneous and heterogeneous media. In addition, we obtained new derivatives by inserting the 1,2,3-triazole nucleus via click reaction and also from *O*-alkylation and *O*-acylation reactions of limonin-7-oxime.

Experimental

Reagents and equipments

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a Bruker DPX-400 spectrometer (^{1}H at 400.1 MHz and ^{13}C at 100.6 MHz) in $CDCl_3$, CD_3OD or in $CDCl_3$-CD_3OD with tetramethylsilane (TMS) as the internal standard. Chemical shifts (δ) are reported in ppm and the coupling constants (*J*) are expressed in Hertz (Hz). Melting points were determined with a MQAPF-301 apparatus and are uncorrected. Electrospray ionization (ESI) high-resolution mass spectra (HRMS) were recorded on a Waters-Xevo G2 QTof mass spectrometer. An ultrasound bath (water), Bandelin Sonorex RK510S (50-60 Hz, 220 V, 9.5 A), was used. Reactions were performed using a microwave (MW) oven (model Multiwave 3000, Anton Paar), equipped with a rotor for eight high-pressure quartz vessels (capacity of 80 mL, maximum pressure and operation temperature of 80 bar and 280 °C, respectively). Reactions were monitored using thin layer chromatography (TLC), performed using Merck DC aluminum plates coated with silica gel GF-254. Flash chromatography was carried out with silica gel (200-300 mesh). Compounds were detected by short and long wavelength ultraviolet light, by spraying with 5% H_2SO_4, followed by heating. All commercially available reagents were purchased from Sigma-Aldrich. Ampicillin, azithromycin, levofloxacin and nystatin, purchased from Sigma-Aldrich, were used as control antibiotics. All solvents were of analytical grade and freshly distilled prior to use.

General procedure for extraction of limonin (**1**) from citrus seeds

The following procedure was employed for the extraction of **1**. Dried and crushed citrus seeds (1.0 kg) were extracted in a 3 L round-bottom flask equipped with a Soxhlet apparatus with acetone (1.0 L) in reflux by 8 h. The resulting acetone extract was concentrated in vacuum to obtain a crude residue. The residual extract was washed with light petroleum (b.p. 30-60 °C). The solid crude limonin was solubilized in dichloromethane (250 mL) and precipitated by slow addition of acetone, its solid was then filtered out and dried under reduced pressure to give the pure **1** (12 g) as a white solid that was characterized by corresponding spectroscopic data ^{1}H and ^{13}C NMR listed below.

Limonin (**1**)

White solid; m.p. 296-297 °C (lit.[18] 298 °C); ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 1.08 (s, 3H), 1.18 (s, 6H), 1.29 (s, 3H), 1.45-1.54 (m, 1H), 1.70-1.85 (m, 2H), 1.85-1.97 (m, 1H), 2.23 (dd, 1H, *J* 15.7, 3.3 Hz), 2.45 (dd, 1H, *J* 14.5, 3.3 Hz), 2.56 (dd, 1H, *J* 12.2, 2.9 Hz), 2.67 (dd, 1H, *J* 16.8, 2.1 Hz), 2.85 (dd, 1H, *J* 15.7, 14.7 Hz), 2.96 (dd, 1H, *J* 16.8, 3.7 Hz), 4.02 (br s, 1H), 4.06 (s, 1H), 4.47 (d, 1H, *J* 13.1 Hz), 4.76 (d, 1H, *J* 13.1 Hz), 5.47 (s, 1H), 6.34 (br s, 1H), 7.38-7.42 (m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 17.6. 18.9, 20.6, 21.4, 30.2, 30.8, 35.6, 36.4, 38.1, 46.1, 48.1, 51.4, 54.0, 60.7, 65.4, 65.8, 77.8, 79.2, 80.3, 109.7, 120.1, 141.2, 143.2, 166.5, 168.9, 206.0; HRMS (ESI) calcd. for $C_{26}H_{31}O_8$ $[M + H]^+$: 471.2013; found: 471.2015.

General procedure for the synthesis of derivatives **3a-o**

Reaction condition *i* (microwave-assisted)

To a solution of **1** (2.0 mmol) in absolute EtOH (8.0 mL) in a glass tube was added dropwise the appropriate amine (3.6 mmol) and K-10 (0.3 g mmol^{-1}); the quartz tube was sealed with reaction mixture and introduced into a microwave oven. The flask was irradiated for 30 min (150 W) the temperature of 80 °C. After completion of the reaction the mixture was filtered, the organic phase was dried with Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to give the crude products. All the compounds were purified by column chromatography on silica gel using 2-5% EtOH-CH_2Cl_2 as eluent to give analytically pure products **3a**-**m**. The products were characterized by corresponding spectroscopic data (^{1}H and ^{13}C NMR, and HRMS).

Reaction condition *ii* (reflux)

To a solution of **1** (2.0 mmol) in absolute EtOH (8.0 mL) in round-bottom flask (equipped with a reflux condenser and recirculating chiller) was added dropwise the appropriate amine (3.6 mmol) and K-10 (0.3 g mmol^{-1}) and stirred. The reaction mixture was then heated at reflux and the progress of the reaction was monitored by TLC.

After completion of the reaction (12-36 h), the mixture was filtered, the organic phase was dried with Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to give the crude products. All compounds were purified by column chromatography on silica gel using 2-5% EtOH-CH_2Cl_2 as eluent to give analytically pure products **3a**-**o**.

Reaction condition *iii* (ultrasound)

To a round-bottom flask was added montmorillonite K-10 (0.3 g $mmol^{-1}$), and **1** (2.0 mmol) in CH_2Cl_2 was dispersed on K-10. Then the appropriate amine (3.6 mmol) was added dropwise and the mixture was sonicated in an ultrasonic bath; the progress of the reaction was monitored by TLC and after completion of the reaction (10-12 h), the products were extracted by washing the K-10 with CH_2Cl_2. The organic phase was dried with Na_2SO_4, filtered and the solvent was removed *in vacuo* to yield the crude products. The crude products were purified by column chromatography over silica gel using 2-5% EtOH-CH_2Cl_2 as eluent to give analytically pure products **3a**-**o**. The products were characterized by corresponding spectroscopic data (^{1}H and ^{13}C NMR, and HRMS).

N-Benzyl-2-((1*S*,3a*S*,4a*R*,4b*R*,9a*R*,11a*S*)-1-(furan-3-yl)-9a-(hydroxymethyl)-4b,7,7,11a-tetramethyl-3,5-dioxotetradecahydroisobenzofuro[5,4-*f*]oxireno[2,3-*d*]isochromen-9-yl)acetamide (**3a**)

White crystal; m.p. 216-217 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$ + CD_3OD) δ 1.01 (s, 3H), 1.12 (s, 3H), 1.20 (s, 3H), 1.32 (s, 3H), 1.39-1.46 (m, 1H), 1.68 (dd, 1H, *J* 13.7, 7.5 Hz), 1.89-2.14 (m, 3H), 2.26-2.35 (m, 2H), 2.67 (dd, 1H, *J* 15.5, 9.6 Hz), 2.79-2.91(m, 2H), 3.80 (s, 1H), 3.85 (d, 1H, *J* 8.1 Hz), 4.07 (s, 2H), 4.39 (d, 1H, *J* 15.0 Hz), 4.47 (d, 1H, *J* 15.0 Hz), 5.45 (s, 1H), 6.35 (s, 1H), 7.27-7.34 (m, 5H), 7.38-7.43 (m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$ + CD_3OD) δ 15.9. 21.0, 22.3, 23.3, 29.7, 33.4, 36.5, 39.1, 37.7, 43.5, 48.8, 51.0, 52.6, 53.2, 60.4, 61.3, 65.7, 78.5, 78.6, 82.9, 109.7, 120.3, 127.4, 127.5 (2C–Ar), 128.6 (2C–Ar), 138.0, 141.0, 143.1, 167.9, 171.9, 208.5; HRMS (ESI) calcd. for $C_{33}H_{40}NO_8$ $[M + H]^+$: 578.2748; found: 578.2753.

N-(4-Chlorobenzyl)-2-((1*S*,3a*S*,4a*R*,4b*R*,9a*R*,11a*S*)-1-(furan-3-yl)-9a-(hydroxymethyl)-4b,7,7,11a-tetramethyl-3,5-dioxotetradecahydroisobenzofuro[5,4-*f*]oxireno[2,3-*d*]isochromen-9-yl)acetamide (**3b**)

Yellowish white solid; m.p. 189-190 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 1.01 (s, 3H), 1.13 (s, 3H), 1.22 (s, 3H), 1.32 (s, 3H), 1.39-1.46 (m, 1H), 1.66-1.72 (m, 1H), 1.95-2.11 (m, 3H), 2.27-2.35 (m, 2H), 2.70 (dd, 1H, *J* 15.5, 8.3 Hz), 2.78-2.96 (m, 2H), 3.83 (s, 1H), 3.81 (s, 1H), 4.14 (s, 2H), 4.39 (dd, 1H, *J* 14.6, 5.4 Hz), 4.47 (dd, 1H, *J* 14.6, 5.1 Hz), 5.45 (s, 1H), 6.35 (s, 1H), 6.75 (br s, 1H), 7.22-7.31 (m, 4H), 7.37-7.44 (m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 16.2. 21.3, 22.4, 23.5, 29.9, 33.6, 36.7, 37.9, 39.7, 43.1, 48.9, 51.4, 52.7, 53.5, 61.1, 61.5, 65.8, 78.5, 78.8, 83.0, 109.9, 120.5, 128.9 (2C–Ar), 129.1 (2C–Ar), 133.5, 136.9, 141.2, 143.2, 167.2, 171.5, 207.7; HRMS (ESI) calcd. for $C_{33}H_{38}ClNNaO_8$ $[M + Na]^+$: 634.2178; found: 634.2169.

2-((1*S*,3a*S*,4a*R*,4b*R*,9a*R*,11a*S*)-1-(Furan-3-yl)-9a-(hydroxymethyl)-4b,7,7,11a-tetramethyl-3,5-dioxotetradecahydroisobenzofuro[5,4-*f*]oxireno[2,3-*d*]isochromen-9-yl)-*N*-(4-methoxybenzyl)acetamide (**3c**)

Yellowish solid; m.p. 132 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.97 (s, 3H), 1.10 (s, 3H), 1.18 (s, 3H), 1.30 (s, 3H), 1.34-1.46 (m, 1H), 1.67 (dd, 1H, *J* 13.4, 7.2 Hz), 1.81 (br s, 1H), 1.90-2.15 (m, 3H), 2.24-2.33 (m, 2H), 2.68 (dd, 1H, *J* 15.6, 9.2 Hz), 2.80-2.95 (m, 2H), 3.78 (s, 5H), 4.10 (s, 2H), 4.33 (dd, 1H, *J* 14.7, 5.4 Hz), 4.39 (dd, 1H, *J* 14.7, 5.8 Hz), 5.42 (s, 1H), 6.34 (s, 1H), 6.82-6.88 (m, 3H), 7.20 (d, 2H, *J* 8.6 Hz), 7.35-7.44 (m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 16.0, 21.3, 22.5, 23.5, 29.9, 33.7, 36.6, 37.8, 39.5, 43.2, 48.9, 51.2, 52.7, 53.3, 55.4, 60.9, 61.4, 65.7, 78.5, 78.8, 82.9, 109.8, 114.2 (2C–Ar), 120.4, 128.9 (2C–Ar), 130.3, 141.1, 143.2, 159.1, 167.4, 171.6, 207.9; HRMS (ESI) calcd. for $C_{34}H_{41}NNaO_9$ $[M + Na]^+$: 630.2674; found: 630.2656.

2-((1*S*,3a*S*,4a*R*,4b*R*,9a*R*,11a*S*)-1-(Furan-3-yl)-9a-(hydroxymethyl)-4b,7,7,11a-tetramethyl-3,5-dioxotetradecahydroisobenzofuro[5,4-*f*]oxireno[2,3-*d*]isochromen-9-yl)-*N*-(4-(trifluoromethyl)benzyl)acetamide (**3d**)

Yellowish solid; m.p. 130-132 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.93 (s, 3H), 1.03 (s, 3H), 1.14 (s, 3H), 1.23 (s, 3H), 1.29-1.37 (m, 1H), 1.61 (dd, 1H, *J* 13.6, 7.2 Hz), 1.84-2.03 (m, 3H), 2.23 (dd, 2H, *J* 14.0, 3.3 Hz), 2.45 (br s, 1H), 2.59-2.70 (m, 1H), 2.73-2.88 (m, 2H), 3.72 (s, 1H), 3.75 (br s, 1H), 4.06 (s, 2H), 4.38 (dd, 1H, *J* 15.4, 5.5 Hz), 4.49 (dd, 1H, *J* 15.4, 6.1 Hz), 5.35 (s, 1H), 6.26 (s, 1H), 6.86 (br s, 1H), 7.27-7.39 (m, 4H), 7.46-7.53 (m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 16.1, 21.3, 22.5, 23.5, 29.9, 33.6, 36.6, 37.8, 39.7, 43.1, 48.8, 51.3, 52.7, 53.3, 61.0, 61.4, 65.7, 78.5, 78.9, 82.9, 109.8, 120.4, 124.2 (q, $^1J_{CF}$ 272.2 Hz), 125.7 (q, 2C, $^3J_{CF}$ 3.7 Hz), 127.8 (2C–Ar), 129.9 (q, $^2J_{CF}$ 32.6 Hz), 141.1, 142.5, 143.2, 167.4, 171.8, 207.8; HRMS (ESI) calcd. for $C_{34}H_{38}F_3NNaO_8$ $[M + Na]^+$: 668.2442; found: 668.2479.

2-((1*S*,3a*S*,4a*R*,4b*R*,9a*R*,11a*S*)-1-(Furan-3-yl)-9a-(hydroxymethyl)-4b,7,7,11a–tetramethyl-3,5-dioxotetradecahydroisobenzofuro[5,4-*f*]oxireno[2,3-*d*]isochromen-9-yl)-*N*-phenethylacetamide (**3e**)

White solid; m.p. 231.5 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$ + CD_3OD) δ 0.91 (s, 3H), 1.04 (s, 6H), 1.23 (s, 3H), 1.26-1.38 (m, 1H), 1.62 (dd, 1H, *J* 13.6, 6.9 Hz), 1.77-1.91 (m, 2H), 1.91-2.06 (m, 1H), 2.13-2.26 (m, 2H), 2.50 (dd, 1H, *J* 15.8,

9.9 Hz), 2.62-2.82 (m, 4H), 3.36-3.50 (m, 2H), 3.64 (d, 1H, *J* 8.2 Hz), 3.72 (s, 1H), 3.96 (br s, 2H), 5.37 (s, 1H), 6.28 (s, 1H), 7.11-7.16 (m, 3H), 7.19-7.24 (m, 2H), 7.30-7.36 (m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$ + CD_3OD) δ 15.9, 21.0, 22.2, 23.2, 29.6, 33.4, 35.3, 36.5, 37.7, 39.0, 40.5, 48.8, 50.9, 52.6, 53.2, 60.3, 61.2, 65.7, 78.5, 78.6, 82.8, 109.7, 120.2, 126.5, 128.6 (2C–Ar), 128.7 (2C–Ar), 138.9, 141.0, 143.1, 168.1, 172.0, 208.4; HRMS (ESI) calcd. for $C_{34}H_{41}NNaO_8$ $[M + Na]^+$: 614.2724; found: 614.2726.

2-((1*S*,3a*S*,4a*R*,4b*R*,9a*R*,11a*S*)-1-(Furan-3-yl)-9a-(hydroxymethyl)-4b,7,7,11a-tetramethyl-3,5-dioxotetradecahydroisobenzofuro[5,4-*f*]oxireno[2,3-*d*]isochromen-9-yl)-*N*-((*S*)-1-phenylethyl)acetamide (**3f**)

Yellowish solid; m.p. 127.0 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.94 (s, 3H), 1.11 (s, 3H), 1.22 (s, 3H), 1.30 (s, 3H), 1.37-1.41 (m, 1H), 1.47 (d, 3H, *J* 6.8 Hz), 1.63-1.68 (m, 1H), 1.90-2.08 (m, 4H), 2.28 (d, 2H, *J* 11.5 Hz), 2.65 (dd, 1H, *J* 15.4, 8.3 Hz), 2.77-2.90 (m, 2H), 3.77 (d, 1H, *J* 6.7 Hz), 3.81 (s, 1H), 4.04 (s, 2H), 5.03-5.16 (m, 1H), 5.42 (s, 1H), 6.33 (s, 1H), 6.78 (d, 1H, *J* 7.3 Hz), 7.28-7.41 (m, 7H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 16.0, 21.3, 22.3, 22.5, 23.4, 29.9, 33.7, 36.6, 37.8, 39.4, 48.9, 49.2, 51.3, 52.7, 53.3, 60.8, 61.7, 65.7, 78.5, 78.8, 83.6, 109.9, 120.2, 126.2 (2C–Ar), 127.5, 128.8 (2C–Ar), 141.1, 143.1, 143.3, 167.5, 171.0, 207.9; HRMS (ESI) calcd. for $C_{34}H_{41}NNaO_8$ $[M + Na]^+$: 614.2724; found: 614.2719.

2-((1*S*,3a*S*,4a*R*,4b*R*,9a*R*,11a*S*)-1-(Furan-3-yl)-9a-(hydroxymethyl)-4b,7,7,11a–tetramethyl-3,5-dioxotetradecahydroisobenzofuro[5,4-*f*]oxireno[2,3-*d*]isochromen-9-yl)-*N*-((*R*)-1-phenylethyl)acetamide (**3g**)

Yellowish solid; m.p. 126-127 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.94 (s, 3H), 1.11 (s, 3H), 1.22 (s, 3H), 1.30 (s, 3H), 1.38-1.42 (m, 1H), 1.47 (d, 3H, *J* 6.8 Hz), 1.63-1.69 (m, 1H), 1.90-2.09 (m, 4H), 2.28 (d, 2H, *J* 11.5 Hz), 2.62-2.71 (m, 1H), 2.77-2.91 (m, 2H), 3.77 (d, 1H, *J* 6.7 Hz), 3.81 (s, 1H), 4.04 (s, 2H), 5.04-5.14 (m, 1H), 5.42 (s, 1H), 6.33 (s, 1H), 6.78 (d, 1H, *J* 7.3 Hz), 7.28-7.46 (m, 7H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 16.1, 21.3, 22.4, 22.6, 23.5, 29.9, 33.7, 36.6, 37.9, 39.4, 48.9, 49.3, 51.3, 52.7, 53.5, 61.1, 61.5, 65.6, 78.4, 78.7, 82.8, 109.9, 120.4, 126.8 (2C–Ar), 127.7, 128,9 (2C–Ar), 141.1, 142.3, 143.2, 167.2, 171.3, 207.8; HRMS (ESI) calcd. for $C_{34}H_{41}NNaO_8$ $[M + Na]^+$: 614.2724; found: 614.2720.

2-((1*S*,3a*S*,4a*R*,4b*R*,9a*R*,11a*S*)-1-(Furan-3-yl)-9a-(hydroxymethyl)-4b,7,7,11a–tetramethyl-3,5-dioxotetradecahydroisobenzofuro[5,4-*f*]oxireno[2,3-*d*]isochromen-9-yl)-*N*-(pyridin-2-ylmethyl)acetamide (**3h**)

White crystal; m.p. 214°C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.96 (s, 3H), 1.03 (s, 3H), 1.19 (s, 3H), 1.25 (s, 3H), 1.27-1.39 (m, 1H), 1.59 (dd, 1H, *J* 13.6, 6.9 Hz), 1.74-2.10 (m, 4H), 2.23 (d, 2H, *J* 11.7 Hz), 2.64-2.95 (m, 3H), 3.72 (s, 1H), 3.80 (d, 1H, *J* 8.9 Hz), 4.05 (d, 1H, *J* 11.5 Hz), 4.11 (d, 1H, *J* 11.5 Hz), 4.45 (dd, 1H, *J* 16.3, 4.4 Hz), 4.53 (dd, 1H, *J* 16.3, 5.2 Hz), 5.37 (s, 1H), 6.26 (s, 1H), 7.07-7.15 (m, 1H), 7.16-7.23 (m, 2H), 7.31 (d, 2H, *J* 4.1 Hz), 7.53-7.62 (m, 1H), 7.74 (br s, 1H), 8.33-8.53 (m, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 16.1, 21.2, 22.5, 23.5, 29.9, 33.6, 36.7, 37.8, 39.4, 44.8, 48.9, 51.2, 52.6, 53.3, 60.9, 61.5, 65.7, 78.4, 78.7, 82.9, 109.8, 120.4, 122.1, 122.5, 136.9, 141.0, 143.2, 149.0, 156.5, 167.4, 171.9, 208.1; HRMS (ESI) calcd. for $C_{32}H_{38}N_2NaO_8$ $[M + Na]^+$: 601.2520; found: 601.2508.

N-(Furan-2-ylmethyl)-2-((1*S*,3a*S*,4a*R*,4b*R*,9a*R*,11a*S*)-1-(furan-3-yl)-9a-(hydroxymethyl)-4b,7,7,11a-tetramethyl-3,5-dioxotetradecahydroisobenzofuro[5,4-*f*]oxireno[2,3-*d*]isochromen-9-yl)acetamide (**3i**)

White solid; m.p. 225-226 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.98 (s, 3H), 1.11 (s, 3H), 1.21 (s, 3H), 1.30 (s, 3H), 1.34-1.45 (m, 1H), 1.70 (dd, 1H, *J* 13.8, 6.3 Hz), 1.90-2.17 (m, 4H), 2.25-2.36 (m, 2H), 2.66 (dd, 1H, *J* 15.5, 8.5 Hz), 2.75-3.01 (m, 2H), 3.76 (br s, 1H), 3.80 (s, 1H), 4.10 (s, 2H), 4.40 (dd, 1H, *J* 15.5, 5.5 Hz), 4.47 (dd, 1H, *J* 15.5, 5.4 Hz), 5.44 (s, 1H), 6.23 (dd, 1H, *J* 3.2, 0.7 Hz), 6.32 (dd, 1H, *J* 3.2, 1.9 Hz), 6.34 (dd, 1H, *J* 1.8, 0.8 Hz), 6.79 (br s, 1H), 7.34 (dd, 1H, *J* 1.8, 0.8 Hz), 7.37-7.43 (m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 16.2, 21.3, 22.4, 23.5, 29.9, 33.6, 36.6, 37.9, 39.6, 48.3, 48.9, 51.4, 52.7, 53.5, 60.9, 61.7, 66.0, 78.0, 78.5, 82.9, 109.8, 109.9, 110.6, 120.4, 141.2, 142.2, 143.2, 143.4, 167.3, 171.3, 207.9; HRMS (ESI) calcd. for $C_{31}H_{37}NNaO_9$ $[M + Na]^+$: 590.2361; found: 590.2527.

2-((1*S*,3a*S*,4a*R*,4b*R*,9a*R*,11a*S*)-1-(Furan-3-yl)-9a-(hydroxymethyl)-4b,7,7,11a-tetramethyl-3,5-dioxotetradecahydroisobenzofuro[5,4-*f*]oxireno[2,3-*d*]isochromen-9-yl)-*N*-(thiophen-2-ylmethyl)acetamide (**3j**)

Yellowish white solid; m.p. 228 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.97 (s, 3H), 1.11 (s, 3H), 1.20 (s, 3H), 1.30 (s, 3H), 1.37-1.44 (m, 1H), 1.68-1.74 (m, 1H), 1.95-2.11 (m, 4H), 2.30 (dd, 2H, *J* 14.0, 3.3 Hz), 2.66 (dd, 1H, *J* 15.4, 8.6 Hz), 2.78-2.92 (m, 2H), 3.77 (br s, 1H), 3.80 (s, 1H), 4.11 (s, 2H), 4.61 (d, 2H, *J* 5.6 Hz), 5.44 (s, 1H), 6.34 (d, 1H, *J* 0.9 Hz), 6.77 (br s, 1H), 6.93-7.00 (m, 2H), 7.21 (dd, 1H, *J* 5.0, 1.2 Hz), 7.38-7.42 (m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 16.0, 21.0, 22.3, 23.4, 29.9, 33.7, 36.5, 37.6, 39.0, 48.4, 48.6, 51.4, 52.5, 53.4, 60.3, 61.4, 65.8, 78.4, 78.6, 82.9, 109.6, 120.3, 125.1, 126.1, 126.9, 140.3, 140.9, 143.1, 167.0, 171.5, 208.2; HRMS (ESI)

calcd. for $C_{31}H_{37}NNaO_8S$ [M + Na]$^+$: 606.2132; found: 606.2154.

N-(Benzo[*d*][1,3]dioxol-5-ylmethyl)-2-((1*S*,3a*S*,4a*R*,4b*R*,9a*R*,11a*S*)-1-(furan-3-yl)-9a-(hydroxymethyl)-4b,7,7,11a-tetramethyl-3,5-dioxotetradecahydroisobenzofuro[5,4-*f*]oxireno[2,3-*d*] isochromen-9-yl)acetamide (**3k**)

Yellowish white solid; m.p. 210 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.99 (s, 3H), 1.21 (s, 3H), 1.12 (s, 3H), 1.31 (s, 3H), 1.40-1.46 (m, 1H), 1.68-1.75 (m, 1H), 1.97-2.11 (m, 4H), 2.31 (dd, 2H, *J* 14.2, 3.3 Hz), 2.63-2.72 (m, 1H), 2.78-2.91 (m, 2H), 3.80 (s, 1H), 3.82 (br s, 1H), 4.13 (s, 2H), 4.32 (dd, 1H, *J* 14.7, 5.5 Hz), 4.40 (dd, 1H, *J* 14.7, 5.9 Hz), 5.44 (s, 1H), 5.94 (s, 2H), 6.35 (d, 1H, *J* 1.0 Hz), 6.64 (br s, 1H), 6.75 (s, 2H), 6.80 (br s, 1H), 7.39 (dd, 1H, *J* 2.5, 0.9 Hz), 7.41 (br s, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 16.0, 21.3, 22.5, 23.5, 29.9, 33.7, 36.6, 37.8, 39.6, 43.5, 48.9, 51.2, 52.7, 53.3, 60.9, 61.4, 65.7, 78.5, 78.8, 82.9, 101.2, 108.3, 108.4, 109.8, 120.4, 120.9, 132.2, 141.1, 143.2, 147.0, 148.1, 167.3, 171.6, 207.9; HRMS (ESI) calcd. for $C_{34}H_{39}NNaO_{10}$ [M + Na]$^+$: 644.2466; found: 644.2476.

N-Allyl-2-((1*S*,3a*S*,4a*R*,4b*R*,9a*R*,11a*S*)-1-(furan-3-yl)-9a-(hydroxymethyl)-4b,7,7,11a-tetramethyl-3,5-dioxotetradecahydroisobenzofuro[5,4-*f*]oxireno[2,3-*d*]isochromen-9-yl)acetamide (**3l**)

White solid; m.p. 143-145 °C; ^{1}H NMR (400.1 MHz, CD_3OD) δ 1.10 (s, 3H), 1.20 (s, 3H), 1.26 (s, 3H), 1.40 (s, 3H), 1.43-1.54 (m, 1H), 1.81 (dd, 1H, *J* 13.8, 7.2 Hz), 1.95-2.21 (m, 3H), 2.33 (dd, 1H, *J* 14.1, 3.2 Hz), 2.44 (d, 1H, *J* 11.7 Hz), 2.68 (dd, 1H, *J* 14.6, 9.9 Hz), 2.86 (d, 1H, *J* 14.6 Hz), 3.03-3.18 (m, 1H), 3.84 (s, 1H), 3.87 (br s, 1H), 4.01 (d, 1H, *J* 9.4 Hz), 4.16 (d, 1H, *J* 11.2 Hz), 4.23 (d, 1H, *J* 11.2 Hz), 5.15 (d, 1H, *J* 10.3 Hz), 5.31 (d, 1H, *J* 17.2 Hz), 5.56 (s, 1H), 5.79-5.98 (m, 1H), 6.50 (s, 1H), 7.56 (d, 2H, *J* 13.7 Hz), 7.95 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 16.3, 21.5, 23.6, 23.8, 29.9, 34.5, 37.6, 38.9, 40.1, 42.7, 49.9, 52.1, 53.9, 54.3, 61.3, 62.6, 67.0, 79.3, 79.9, 84.3, 110.9, 116.0, 121.9, 135.3, 142.6, 144.3, 169.8, 174.1, 210.5; HRMS (ESI) calcd. for $C_{29}H_{37}NNaO_8$ [M + Na]$^+$: 550.2411; found: 550.2513.

2-((1*S*,3a*S*,4a*R*,4b*R*,9a*R*,11a*S*)-1-(Furan-3-yl)-9a-(hydroxymethyl)-4b,7,7,11a-tetramethyl-3,5-dioxotetradecahydroisobenzofuro[5,4-*f*]oxireno[2,3-*d*] isochromen-9-yl)-*N*-(prop-2-yn-1-yl)acetamide (**3m**)

Yellowish white solid; m.p. 141 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 1.05 (s, 3H), 1.11 (s, 3H), 1.26 (s, 3H), 1.31 (s, 3H), 1.35-1.44 (m, 1H), 1.64-1.76 (m, 1H), 1.92-2.10 (m, 4H), 2.23 (t, 1H, *J* 2.8 Hz), 2.27-2.36 (m, 2H), 2.61-2.74 (m, 1H), 2.80-2.94 (m, 2H), 3.80 (br s, 2H), 4.04 (dd, 2H, *J* 5.3, 2.8 Hz), 4.14 (br s, 2H), 5.44 (s, 1H), 6.34 (s, 1H), 6.87 (t, 1H, *J* 5.3 Hz), 7.36-7.44 (m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 16.1, 21.3, 22.5, 23.5, 29.3, 29.9, 33.7, 36.6, 37.8, 39.2, 48.9, 51.2, 52.7, 53.3, 60.9, 61.4, 65.7, 71.6, 78.4, 78.9, 79.5, 82.7, 109.8, 120.4, 141.1, 143.2, 167.4, 171.5, 207.9; HRMS (ESI) calcd. for $C_{29}H_{35}NNaO_8$ [M + Na]$^+$: 548.2255; found: 548.2242.

2-((1*S*,3a*S*,4a*R*,4b*R*,9a*R*,11a*S*)-1-(Furan-3-yl)-9a-(hydroxymethyl)-4b,7,7,11a-tetramethyl-3,5-dioxotetradecahydroisobenzofuro[5,4-*f*]oxireno[2,3-*d*] isochromen-9-yl)-*N*-isopropylacetamide (**3n**)

Yellowish white solid; m.p. 124-126 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 1.04 (s, 3H), 1.06 1.20 (m, 9H), 1.24 (s, 3H), 1.32 (s, 3H), 1.38-1.45 (m, 1H), 1.63-1.73 (m, 1H), 1.92-2.17 (m, 4H), 2.30 (d, 2H, *J* 11.5 Hz), 2.59-2.73 (m, 1H), 2.78-2.90 (m, 2H), 3.24-3.31 (m, 1H), 3.77 (d, 1H, *J* 8.1 Hz), 3.81 (s, 1H), 4.13 (s, 2H), 5.43 (s, 1H), 6.34 (s, 1H), 6.54 (br s, 1 H), 7.31-7.48 (m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 11.4, 16.0, 21.3, 22.5, 22.7, 23.5, 29.9, 33.7, 36.6, 37.8, 39.5, 41.4, 48.9, 51.3, 52.7, 53.3, 60.8, 61.4, 65.7, 78.5, 78.9, 83.1, 109.8, 120.4, 141.1, 143.2, 167.4, 172.1, 208.0; HRMS (ESI) calcd. for $C_{29}H_{39}NNaO_8$ [M + Na]$^+$: 552.2568; found: 552.2642.

N-Ethyl-2-((1*S*,3a*S*,4a*R*,4b*R*,9a*R*,11a*S*)-1-(furan-3-yl)-9a-(hydroxymethyl)-4b,7,7,11a–tetramethyl-3,5-dioxotetradecahydroisobenzofuro[5,4-*f*]oxireno[2,3-*d*]isochromen-9-yl)acetamide (**3o**)

Yellowish solid; m.p. 128-130 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.92 (t, 3H, *J* 7.4 Hz), 1.03 (s, 3H), 1.10 (s, 3H), 1.23 (s, 3H), 1.31 (s, 3H), 1.36-1.44 (m, 1H), 1.67 (dd, 1H, *J* 13.8, 7.1 Hz), 1.92-2.15 (m, 4H), 2.21-2.34 (m, 2H), 2.66 (dd, 1H, *J* 15.8, 9.4 Hz), 2.78-2.91(m, 2H), 3.12-3.27 (m, 2H), 3.73 (d, 1H, *J* 8.8 Hz), 3.79 (s, 1H), 4.12 (s, 2H), 5.41 (s, 1H), 6.33 (s, 1H), 6.72 (br s, 1H), 7.33-7.43 (m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.7, 16.1, 21.3, 22.4, 23.5, 29.9, 33.7, 34.6, 36.7, 37.9, 39.5, 49.0, 51.3, 52.7, 53.4, 60.9, 61.54, 65.8, 78.5, 78.8, 83.1, 109.9, 120.5, 141.1, 143.2, 167.3, 171.7, 207.9; HRMS (ESI) calcd. for $C_{28}H_{37}NNaO_8$ [M + Na]$^+$: 538.2411; found: 538.2462.

General procedure for synthesis of limonin-7-oxime (**4**)

To a solution of **1** (235.0 mg) in absolute C_2H_5OH (6.0 mL) was added hydroxylamine hydrochloride ($NH_2OH.HCl$, 240.0 mg). Pyridine (6.0 mL) was then added subsequently and the solution was refluxed for 5 h. The reaction was cooled and a saturated solution of NaCl

added. The mixture was then extracted with AcOEt to obtain pure limonin-7-oxime (**4**) in 91% yield. Spectral data for the product prepared are listed below.

Limonin-7-oxime (**4**)

White crystal; m.p. 237 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.96 (s, 3H), 1.19 (s, 3H), 1.23 (s, 3H), 1.32 (s, 3H), 1.47-1.55 (m, 1H), 1.75-1.88 (m, 3H), 1.97 (br s, 1H), 1.99 (br s, 1H), 2.42 (d, 1H, *J* 10.3 Hz), 2.95 (dd, 1H, *J* 16.8, 3.7 Hz), 2.71 (d, 1H, *J* 16.8 Hz), 3.58 (d, 1H, *J* 10.5 Hz), 3.81 (s, 1H), 4.01 (br s, 1H), 4.38 (d, 1H, *J* 13.0 Hz), 4.69 (d, 1H, *J* 13.0 Hz), 5.46 (s, 1H), 6.34 (br s, 1H), 7.36-7.42 (m, 2H), 8.41 (s, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 18.4, 18.8, 19.6, 21.3, 21.5, 30.4, 33.0, 35.9, 38.1, 45.9, 46.3, 49.7, 54.4, 60.3, 65.4, 65.9, 78.6, 79.4, 80.7, 109.8, 120.3, 141.1, 143.3, 159.1, 167.8, 170.0; HRMS (ESI) calcd. for $C_{26}H_{31}NNaO_8$ $[M + Na]^+$: 508.1942; found: 508.1941.

General procedure for synthesis of limonin-7-oxime ether derivatives **5a** and **5b** and limonin-7-oxime ester **5c**

Synthesis of limonin-7-oxime ether derivatives **5a** and **5b**

To a solution of **4** (1.0 mmol) in *N*,*N*-dimethylformamide (DMF, 10.0 mL) was added dropwise the appropriate alkyl bromide (1.3 eq). The reaction mixture was cooled to 0 °C, and sodium hydride (1.5 eq) was added portionwise over a period of 10 min. The reaction mixture was slowly warmed to room temperature and stirred for 8 h. The reaction was then quenched with water and DMF was removed *in vacuo*; the aqueous layer was extracted with EtOAc (3 × 10.0 mL). The organic layers were combined, washed with brine (3 mL) and dried over Na_2SO_4. The solvent was removed under vacuum and the product isolated by column chromatography over silica gel using 5% EtOH-CH_2Cl_2 as eluent to afford the desired products **5a** and **5b** in yields of 72% for **5a** and 79% for **5b**. The products were characterized by corresponding spectroscopic data (^{1}H and ^{13}C NMR, and HRMS).

(8a*S*,8b*S*,9a*S*,12*S*,12a*S*,14b*R*,*E*)-8-((Allyloxy)imino)-12-(furan-3-yl)-6,6,8a,12a-tetramethyldodecahydrooxireno[2,3-*d*]pyrano[4',3':3,3a]isobenzofuro[5,4-*f*]isochromene-3,10(1*H*,6*H*)-dione (**5a**)

Yellowish solid; m.p. 137-138 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.96 (s, 3H), 1.19 (s, 3H), 1.24 (s, 3H), 1.33 (s, 3H), 1.48-1.55 (m, 1H), 1.72-1.90 (m, 3H), 1.90-2.07 (m, 2H), 2.41 (d, 1H, *J* 10.4 Hz), 2.68 (dd, 1H, *J* 16.7, 1.6 Hz), 2.97 (dd, 1H, *J* 16.7, 3.8 Hz), 3.51 (dd, 1H, *J* 13.2, 1.8 Hz), 3.81 (s, 1H), 3.98 (br s, 1H), 4.34 (d, 1H, *J* 13.0 Hz), 4.57 (d, 2H, *J* 5.8 Hz), 4.69 (d, 1H, *J* 13.0 Hz), 5.25 (d, 1H, *J* 11.3 Hz), 5.32 (dd, 1H, *J* 17.2, 1.4 Hz), 5.47 (s, 1H), 5.90-6.04 (m, 1H), 6.37 (br s, 1H), 7.39-7.45 (m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 18.2, 19.7, 19.8, 21.5, 21.7, 30.4, 33.2, 35.9, 38.1, 46.1, 46.3, 50.0, 54.4, 60.6, 64.9, 65.9, 75.4, 78.4, 79.4, 80.5, 109.9, 118.5, 120.4, 134.2, 141.1, 143.3, 158.4, 167.0, 169.5; HRMS (ESI) calcd. for $C_{29}H_{35}NNaO_8$ $[M + Na]^+$: 548.2255; found: 548.2257.

(8a*S*,8b*S*,9a*S*,12*S*,12a*S*,14b*R*,*E*)-8-(((4-Bromobenzyl)oxy)imino)-12-(furan-3-yl)-6,6,8a,12a-tetramethyldodecahydrooxireno[2,3-*d*]pyrano[4',3':3,3a]isobenzofuro[5,4-*f*]isochromene-3,10(1*H*,6*H*)-dione (**5b**)

Yellowish solid; m.p. 210-212 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.90 (s, 3H), 1.17 (s, 4H), 1.26 (s, 3H), 1.31 (s, 3H), 1.38-1.45 (m, 1H), 1.67-1.86 (m, 3H), 1.88-2.02 (m, 2H), 2.29 (d, 1H, *J* 9.5 Hz), 2.63 (dd, 1H, *J* 17.0, 1.5 Hz), 2.90 (dd, 1H, *J* 17.0, 3.7 Hz), 3.51 (d, 1H, *J* 13.9 Hz), 3.69 (s, 1H), 3.92 (br s, 1H), 4.30 (d, 1H, *J* 13.2 Hz), 4.63 (br s, 3H), 5.38 (s, 1H), 6.31 (s, 1H), 7.19-7.22 (m, 2H), 7.35-7.39 (m, 2H), 7.46-7.50 (m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 18.1, 19.8, 19.9, 21.0, 21.5, 30.5, 33.4, 35.9, 38.0, 46.3, 46.4, 50.1, 54.4, 60.9, 64.8, 65.9, 75.6, 78.4, 79.5, 80.4, 109.9, 120.3, 128.7, 130.6 (2C–Ar), 131.8 (2C–Ar), 137.3, 141.1, 143.3, 159.4, 167.0, 169.4; HRMS (ESI) calcd. for $C_{33}H_{36}BrNNaO_8$ $[M + Na]^+$: 676.1517; found: 676.1521.

Synthesis of limonin-7-oxime ester (**5c**)

To a solution of **4** (1.0 mmol) in DMF (10.0 mL) was added dropwise benzoyl chloride (1.3 eq). The reaction mixture was cooled to 0 °C, and sodium hydride (1.5 eq) was added portionwise over a period of 10 min. The reaction mixture was slowly warmed to room temperature and stirred for 3 h. The mixture was quenched with aqueous sodium bicarbonate (5.0%) and extracted with dichloromethane (4 × 30.0 mL). The combined organic layers were washed with saturated aqueous NaCl (5.0 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give crude solid. The crude product was purified by column chromatography over silica gel using 10% EtOH-CH_2Cl_2 as eluent to give pure product **5c** in 80% yield. The product was characterized by corresponding spectroscopic data (^{1}H and ^{13}C NMR, and HRMS).

(8a*S*,8b*S*,9a*S*,12*S*,12a*S*,14b*R*,*E*)-8-((Benzoyloxy)imino)-12-(furan-3-yl)-6,6,8a,12a-tetramethyldodecahydrooxireno[2,3-*d*]pyrano[4',3':3,3a]isobenzofuro[5,4-*f*]isochromene-3,10(1*H*,6*H*)-dione (**5c**)

Yellowish solid; m.p. 215-216 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 1.10 (s, 3H), 1.18 (s, 3H), 1.27 (s, 3H), 1.37 (s, 3H), 1.48-1.60 (m, 1H), 1.75-1.95 (m, 3H), 2.09 (dd, 1H,

J 15.1, 2.3 Hz), 2.24-2.36 (m, 1H), 2.59 (d, 1H, *J* 11.7 Hz), 2.69 (d, 1H, *J* 16.7 Hz), 2.93-2.98 (m, 1H), 3.36 (dd, 1H, *J* 14.3, 2.6 Hz), 4.02 (br s, 2H), 4.38 (d, 1H, *J* 13.1 Hz), 4.69 (d, 1H, *J* 13.1 Hz), 5.47 (s, 1H), 6.37 (s, 1H), 7.39-7.44 (m, 2H), 7.45-7.53 (m, 2H), 7.58-7.66 (m, 1H), 7.98-8.07 (m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 18.4, 19.6, 21.4, 21.5, 22.1, 30.2, 32.6, 35.9, 38.2, 46.2, 47.4, 49.8, 54.6, 60.3, 65.0, 65.6, 78.2, 79.3, 80.2, 109.9, 120.3, 127.14, 128.8 (2C–Ar), 129.7 (2C–Ar), 133.7, 141.1, 143.2, 163.3, 166.4, 169.3, 169.4; HRMS (ESI) calcd. for $C_{33}H_{35}NNaO_8$ $[M + Na]^+$: 596.2255; found: 596.2246.

General procedure for synthesis of 1,2,3-triazolyl limonins **6a** and **6b** by click reaction

To a solution of **3m** (0.3 mmol) previously synthesized as described, in tetrahydrofuran (THF, 1.0 mL) were added dropwise the respective organic azide (0.3 mmol). Then a fresh solution of $Cu(OAc)_2.H_2O$ (0.0006 g, 1 mol%) in distilled H_2O (0.5 mL) and sodium ascorbate (0.0012 g, 2 mol%) in distilled H_2O (0.5 mL) was added and the mixture stirred under air for 10 h. The solvent was evaporated under vacuum and brine (3 mL) was added and the mixture was then extracted with CH_2Cl_2 (3 × 5 mL). The organic layers were combined, washed with brine (3 mL) and dried over Na_2SO_4. The solvent was removed under vacuum and the product isolated by column chromatography on silica gel using 5% MeOH-CH_2Cl_2 as eluent to afford the desired products **6a** and **6b** in yields of 78% for **6a** and 71% for **6b**. The products were characterized by corresponding spectroscopic data (^{1}H and ^{13}C NMR, and HRMS). Spectral data for the products prepared are listed below.

N-((1-(4-Bromobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-((1*S*,3a*S*,4a*R*,4b*R*,9a*R*,11a*S*)-1-(furan-3-yl)-9a-(hydroxymethyl)-4b,7,7,11a-tetramethyl-3,5-dioxotetradecahydroisobenzofuro[5,4-*f*]oxireno[2,3-*d*]isochromen-9-yl)acetamide (**6a**)

Yellowish solid; m.p. 245-246 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.91 (s, 3H), 1.10 (s, 3H), 1.17 (s, 3H), 1.28 (s, 3H), 1.39-1.46 (m, 1H), 1.73-1.66 (m, 1H), 1.91-2.04 (m, 3H), 2.25-2.33 (m, 2H), 2.63 (dd, 1H, *J* 15.4, 8.5 Hz), 2.73-2.88 (m, 2H), 3.76 (d, 1H, *J* 6.0 Hz), 3.80 (s, 1H), 4.01 (s, 2H), 4.44 (dd, 1H, *J* 15.2, 5.6 Hz), 4.52 (dd, 1H, *J* 15.2, 5.8 Hz), 5.43 (s, 3H), 6.33 (s, 1H), 7.02 (br s, 1H), 7.10-7.18 (m, 2H), 7.35-7.40 (m, 2H), 7.45-7.54 (m, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 16.0, 21.2, 22.3, 23.3, 29.7, 33.5, 35.0, 36.4, 37.7, 39.2, 48.7, 51.1, 52.5, 53.2, 53.5, 60.9, 61.3, 65.5, 78.2, 78.5, 82.5, 109.7, 120.3, 122.0, 123.1, 129.7 (2C–Ar), 132.3 (2C–Ar), 133.4, 140.9, 143.0, 145.4, 167.5, 171.4, 207.6; HRMS (ESI) calcd. for $C_{36}H_{41}BrN_4NaO_8$ $[M + Na]^+$: 759.2000; found: 759.2004.

2-((1*S*,3a*S*,4a*R*,4b*R*,9a*R*,11a*S*)-1-(Furan-3-yl)-9a-(hydroxymethyl)-4b,7,7,11a-tetramethyl-3,5-dioxotetradecahydroisobenzofuro[5,4-*f*]oxireno[2,3-*d*]isochromen-9-yl)-*N*-((1-(4-methylbenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (**6b**)

Yellowish solid; m.p. 209 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.91 (s, 3H), 1.10 (s, 3H), 1.17 (s, 3H), 1.26 (s, 3H), 1.37-1.42 (m, 1H), 1.67-1.69 (m, 1H), 1.87-2.04 (m, 3H), 2.27 (d, 2H, *J* 13.6 Hz), 2.33 (s, 3H), 2.63 (dd, 1H, *J* 15.5, 8.4 Hz), 2.72-2.87 (m, 2H), 3.77 (d, 1H, *J* 5.9 Hz), 3.80 (s, 1H), 4.01 (s, 2H), 4.43 (dd, 1H, *J* 15.3, 5.5 Hz), 4.51 (dd, 1H, *J* 15.3, 5.8 Hz), 5.43 (s, 3H), 6.34 (s, 1H), 7.07 (br s, 1H), 7.16 (s, 2H), 7.26 (s, 2H), 7.35-7.41 (m, 2H), 7.43 (s, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 16.0, 21.1, 21.1, 22.2, 23.3, 29.7, 33.4, 35.0, 36.4, 37.7, 39.2, 48.6, 51.0, 52.5, 53.2, 54.0, 60.8, 61.3, 65.5, 78.2, 78.5, 82.5, 109.7, 120.3, 121.9, 128.3 (2C–Ar), 129.8 (2C–Ar), 131.4, 138.8, 140.9, 143.0, 144.8, 167.1, 171.4, 207.7; HRMS (ESI) calcd. for $C_{37}H_{44}N_4NaO_8$ $[M + Na]^+$: 695.3051; found: 695.3066.

X-Ray crystallography

Single crystal X-ray measurements were made on a crystal glued to a fine glass fiber in a Bruker X8 Kappa APEX II CCD diffractometer using MoKα graphite monochromatized radiation (λ = 0.71073 Å) either at room temperature or at 100 K with a cold nitrogen stream. The individual images were integrated using SAINT[19] to 0.70 Å resolution for all crystal structures. Data were corrected for absorption effects using the multiscan method using SADABS.[20] The structure was solved and refined using the Bruker SHELXTL software package.[21]

Crystal data of limonin (**1**)

Molecular formula: $C_{26}H_{30}O_8$, MM: 470.51, orthorhombic, $P2_12_12_1$ (No. 19), a = 8.7938(11) Å, b = 14.4208(19) Å, c = 17.653(3) Å, V = 2238.6(5) Å^3, T = 100 K, Z = 4, 20128 reflections measured, 5945 independent (R_{int} = 0.0762) which were used in all calculations. The final $wR(F_2)$ was 0.0964. Flack x determined using 1243 quotients by the Parsons method[22] was 0.6(8). The chirality of the compound was based on the structure of **4**.

Crystal data of limonin derivative **3a**

Molecular formula: $C_{33}H_{39}NO_8$, MM: 577.65, orthorhombic, $P2_12_12_1$ (No. 19), a = 9.5605(4) Å, b = 12.1844(5) Å, c = 24.9809(10) Å, V = 2910.0(2) Å^3,

T = 296 K, Z = 4, 56267 reflections measured, 8895 independent (R_{int} = 0.0925) which were used in all calculations. The final $wR(F_2)$ was 0.1179. Flack x determined using 1208 quotients by the Parsons method[22] was 0.2(6). The chirality of the compound was based on the structure of **4**.

Crystal data of limonin derivative **3h**

Molecular formula: $C_{32}H_{38}N_2O_8$, MM: 578.64, orthorhombic, $P212121$ (No. 19), a = 9.4401(4) Å, b = 13.0432(5) Å, c = 23.2182(8) Å, V = 2858.84(19) Å^3, T = 296 K, Z = 4, 69806 reflections measured, 8643 independent (R_{int} = 0.1309) which were used in all calculations. The final $wR(F^2)$ was 0.1385. Flack x determined using 1026 quotients by the Parsons method[22] was 0.3(7). The chirality of the compound was based on the structure of **4**.

Crystal data of limonin-7-oxime (**4**) as ethanol solvate

Molecular formula: $C_{26}H_{31}NO_8 \cdot C_2H_6O$, MM: 485.53, monoclinic, $P2_1$ (No. 4), a = 9.093(3) Å, b = 11.227(3) Å, c = 12.984(4) Å, β = 106.847(14)°, V = 1268.6(7) Å^3, T = 100 K, Z = 2, 30536 reflections measured, 7586 independent (R_{int} = 0.0196) which were used in all calculations. The final $wR(F^2)$ was 0.0838. Flack x determined using 3254 quotients by the Parsons method[22] was –0.07(13); a value close to zero indicates the correct enantiomorph. The correct chirality was confirmed by the Bayesian method[23] in PLATON [version 301214];[24] with a probability P2(true) = 1.000 with 3544 Bijvoet pairs.

Antimicrobial test methods

For the antimicrobial evaluation, strains from the American Type Culture Collection (ATCC) were used. Fungi: *Candida albicans* ATCC 10231, *Candida tropicalis* ATCC 18803, *Candida krusei* ATCC 6258, *Candida parapslosis* ATCC 22018, *Cryptococcus neoformans* ATCC 28952, and *Cryptococcus gatti* ATCC 2601; Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 33019, *Enterococcus spp*. ATCC 6589, *Enterobacter aerogenes* ATCC 13048, *Listeria innocua* ATCC 33090, and *Listeria monocytogenes* ATCC 19112; Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 1304, *Burkholderia cepacia* ATCC 17759, *Pseudomonas aeruginosa* ATCC 27853, *Shigella sonnei* ATCC 25931, *Salmonella typhimurium* ATCC 14028, and *Morganella morganii* ATCC 25829. Ampicillin, azithromycin and levofloxacin were included as antibacterial controls. Nystatin was used as antifungal control.

Broth microdilution method

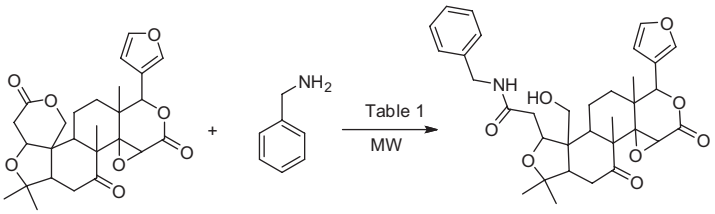
The minimal inhibitory concentration (MIC) was determined on 96 well culture plates by a microdilution method using a microorganism suspension at a density of 105 colony-forming unit (CFU) mL^{-1} with casein soy broth incubated for 24 h at 37 °C for bacteria, and Sabouraud broth incubated for 72 h at 25 °C for fungi. The cultures that did not present growth were used to inoculate plates of solid medium (Muller Hinton agar and Sabouraud agar) in order to determine the minimal lethal concentration (MLC). Proper blanks were assayed simultaneously and samples were tested in triplicate. Technical data have been described previously (National Committee for Clinical Laboratory Standards, NCCLS).[25]

Results and Discussion

This study began with the use of a small amount of limonin (**1**) which was isolated from *Helietta apiculata Benth* in our laboratories. However, we decided to investigate other sources in order to obtain compound **1** in an amount consistent with the present study. We selected *Citrus sinensis* (orange) seeds, which are easily accessible and provide a greater yield in the isolation of **1**. The strategy used to prepare the derivatives starting from changes in A-ring of **1** involved its aminolysis with different primary amines in homogeneous and heterogeneous media using microwave or ultrasound sonication. Initially, we investigated the role of the solvent and the use of montmorillonite K-10 clay under microwave energy on the reaction yield. The reaction between compound **1** and benzylamine (**2a**) generated the desired product **3a** in good yields (63-84%) at a reaction time of 30 min at 80 °C, as shown in Table 1. Obtaining this new limonin derivative **3a** employing the K-10 was effective with all solvents used and the reaction condition EtOH/K-10/microwave was the best since the reaction yield was 84% (Table 1).

The montmorillonite K-10 clay is widely studied and found to be useful in many reactions, such as the synthesis of polyfunctionalized heterocyclic systems,[26,27] the obtaining of β-enamine compound from β-dicarbonyl compound,[28] the protection of functional groups such as alcohols, thiols, phenols and amines,[29] the protection of carbonyl compounds,[30] the transesterification of β-ketoesters,[31] and the aminolysis of epoxides.[32] This mineral clay catalyzes reactions and provides easy isolation of reactions. Reactions with the other primary amines **2b**-**m** were performed using EtOH/K-10/microwave oven at a reaction time of 30 min at 80 °C

Table 1. Optimization of the reaction conditions



1 **2a** **3a**

Solvent	Catalyst	time (80 °C) / min	Yield[a] / %
CH_3CN	K-10	30	63
THF	K-10	30	67
EtOH	K-10	30	84
1,4-Dioxane	K-10	30	55
CH_3CN	–	30	48
THF	–	30	53
EtOH	–	30	47
1,4-Dioxane	–	30	42

[a]Yield after chromatography. MW: microwave; THF: tetrahydrofuran.

(condition *i*, Table 2). All the new derivatives **3b**-**m** were obtained in good yields of 67-85%. The derivative with *p*-OMeBn substituent **3c** was obtained in higher yield (entry 2) and the derivative with 2-thiophenemethyl substituent **3j** was generated in lower yield (entry 10). The structures of the products obtained are shown in Table 2.

Utilizing the condition EtOH/K-10 under reflux (condition *ii*, Table 2), the limonin derivatives **3a**-**o** were also synthesized with good yields of 60-70%, although with a reaction time higher than when these reactions were associated with microwave oven, as described in Table 2. In order to investigate the obtaining of these new derivatives **3a**-**o** from the heterogeneous methodology, we conducted aminolysis reactions employing montmorillonite K-10 as a solid support associating the use of ultrasound sonication under solvent free conditions (condition *iii*, Table 2). This series of compounds **3a**-**o** was efficiently obtained (yields 60-80%) at a reaction time between 10-12 h (see Table 2). In conditions *ii* and *iii*, other primary amines with a low boiling point were also used such as isopropylamine **2n** (entry 10) and ethylamine **2o** (entry 11).

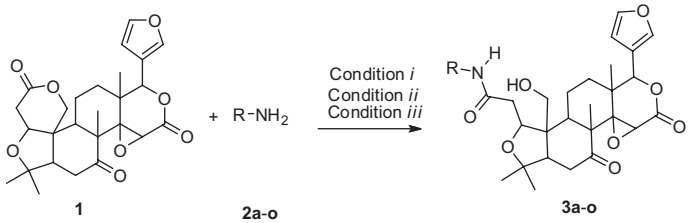
The structure of each product **3a**-**o** was identified from spectroscopic data. In the ^{1}H NMR spectra of the compounds **3a**-**e**, **3h**-**m** and **3o**, the signals assigned to methylene protons bonded to NH appeared in the ranges of ca. 3.36-4.14 ppm. On the other hand, the signals attributed to the methine protons bonded to NH of compounds **3f**, **3g** and **3n** were registered as multiplets in the ranges of ca. 5.03-5.16 ppm for **3f** and **3g** and at 3.24-3.31ppm for **3n**.

In addition to the signals assigned to the furan ring observed in the characteristic region of aromatic protons, we have also observed other signals corresponding to the aromatic moiety of the derivatives **3a**-**k** at around 6.23-8.53 ppm. The ^{13}C NMR spectra showed the signals of the respective amides formed through the opening of the lactone (A-ring of **1**) due to deshielding of the carbonyl carbon from 168.9 to 171.0-174.0 ppm in derivatives **3a**-**o**. Besides elucidating structures by the use of spectroscopic techniques, crystals were obtained and the structures **1**, **3a** and **3h** were reconfirmed by crystallographic methods.

These crystalline structures were obtained from slow evaporation of the solvent mixture CH_2Cl_2/diisopropyl ether (1:2) for **1**, and CH_2Cl_2/diisopropyl ether (1:1) for limonin derivatives **3a** and **3h**. The absolute configuration of structures of **1**, **3a** and **3h** are based on that of **4**, which was confirmed by the single crystal X-ray diffraction experiment (Figure 2). The structure of **1** is identical to that reported before[33] and is included here as it is a better determination made at 100 K.

The reaction between **1** and hydroxylamine hydrochloride in anhydrous ethanol and pyridine under heating at reflux generates the expected limonin-7-oxime (**4**). In the sequence, compound **4** was subjected to the *O*-alkylation reaction using alkyl bromides (allyl bromide and *p*-bromobenzyl bromide) in the presence of sodium hydride in anhydrous DMF, yielding the oxime ether derivatives **5a** and **5b**. On the other hand, the derivative **5c** was obtained from the *O*-acylation reaction

Table 2. Synthesis of limonin derivatives by aminolysis



1 **2a-o** **3a-o**

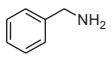
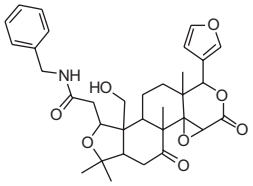
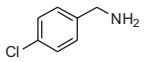
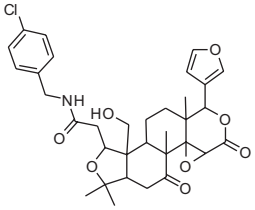
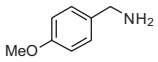
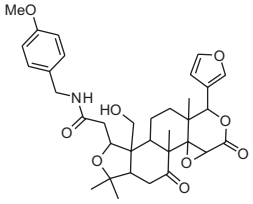
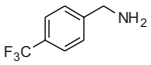
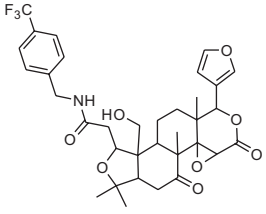
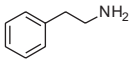
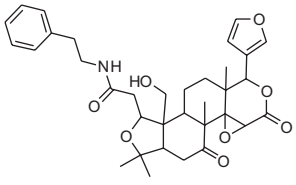
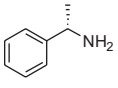
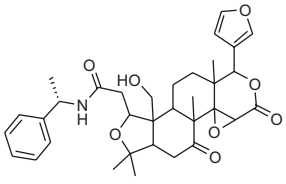
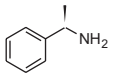
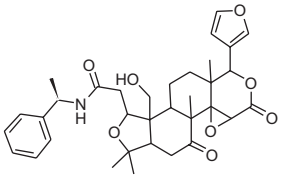
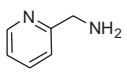
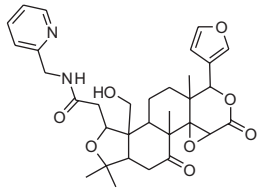
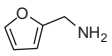
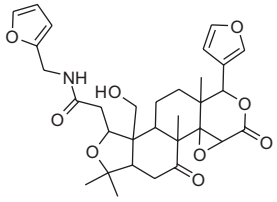
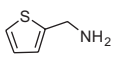
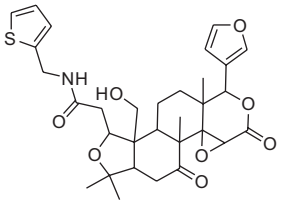
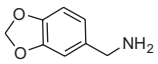
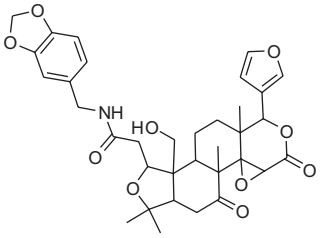
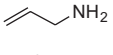
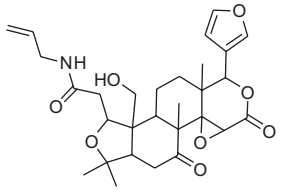
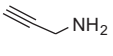
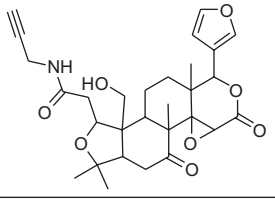
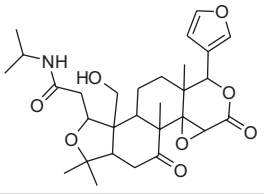
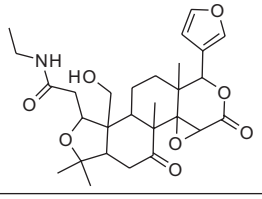
entry	Amine	Product	Yield[a] / %, time / h
1	 **2a**	 **3a**	(*ii*) 67, 24 (*iii*) 63, 12
2	 **2b**	 **3b**	(*i*) 72, 0.5 (*ii*) 65, 20 (*iii*) 70, 12
3	 **2c**	 **3c**	(*i*) 85, 0.5 (*ii*) 70, 24 (*iii*) 80, 10
4	 **2d**	 **3d**	(*i*) 70, 0.5 (*ii*) 60, 36 (*iii*) 67, 10
5	 **2e**	 **3e**	(*i*) 81, 0.5 (*ii*) 67, 24 (*iii*) 79, 12
6	 **2f**	 **3f**	(*i*) 70, 0.5 (*ii*) 61, 24 (*iii*) 70, 10
7	 **2g**	 **3g**	(*i*) 73, 0.5 (*ii*) 65, 24 (*iii*) 69, 10

Table 2. Synthesis of limonin derivatives by aminolysis (cont.)

entry	Amine	Product	Yield[a] / %, time / h
8	 **2h**	 **3h**	(*i*) 69, 0.5 (*ii*) 60, 18 (*iii*) 61, 12
9	 **2i**	 **3i**	(*i*) 73, 0.5 (*ii*) 62, 20 (*iii*) 69, 10
10	 **2j**	 **3j**	(*i*) 67, 0.5 (*ii*) 60, 24 (*iii*) 70, 10
11	 **2k**	 **3k**	(*i*) 78, 0.5 (*ii*) 65, 20 (*iii*) 70, 10
12	 **2l**	 **3l**	(*i*) 75, 0.5 (*ii*) 65, 36 (*iii*) 70, 12
13	 **2m**	 **3m**	(*i*) 68, 0.5 (*ii*) 60, 24 (*iii*) 63, 10
14	 **2n**	 **3n**	(*ii*) 61, 12 (*iii*) 60, 10
15	 **2o**	 **3o**	(*ii*) 66, 24 (*iii*) 61, 12

[a]Yield after chromatography. Reaction conditions: (*i*) K-10 (0.3 g mmol^{-1}) and EtOH (8 mL) associated with microwave oven; (*ii*) K-10 (0.3 g mmol^{-1}), EtOH (8 mL), reflux; (*iii*) K-10 (0.3 g mmol^{-1}), ultrasound.

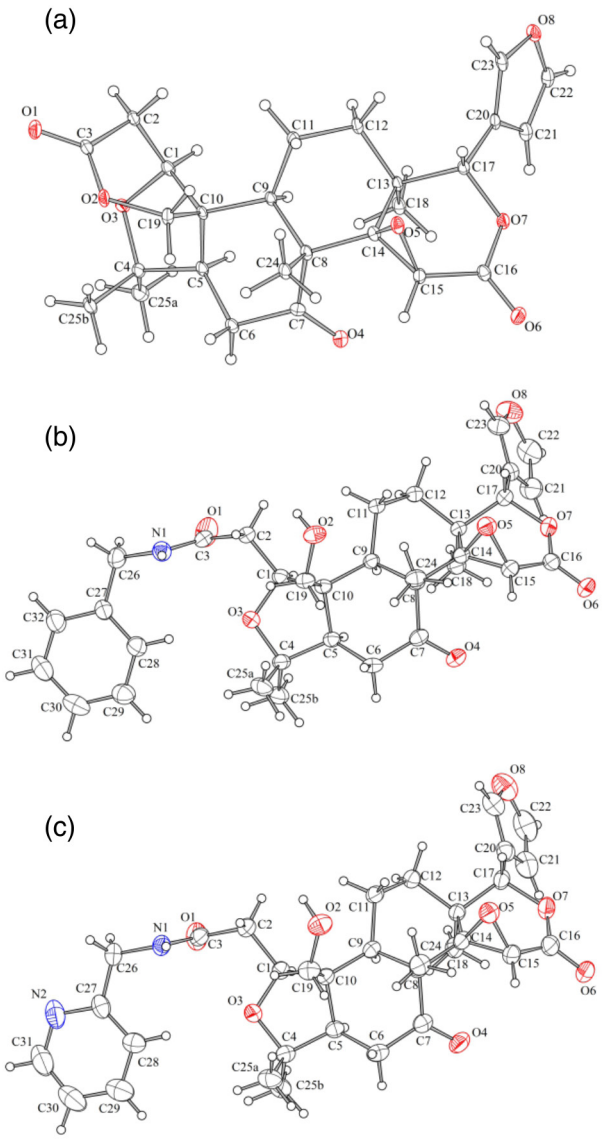
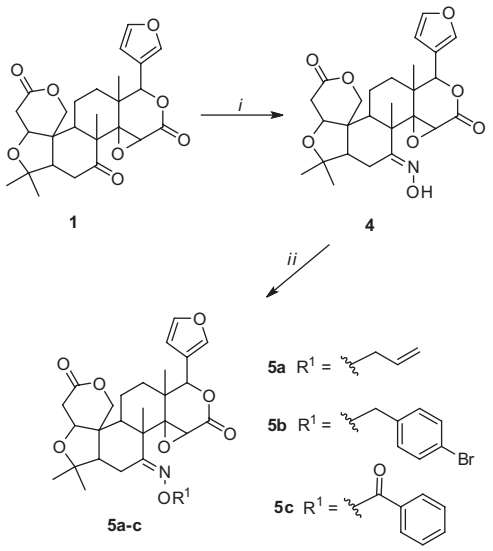


Figure 2. ORTEP plot of **1** (a), derivatives **3a** (b) and **3h** (c).

between compound **4** and benzoyl chloride reagent in the presence of sodium hydride in anhydrous DMF (Scheme 1).

The compounds were obtained in 72-80% yield after column chromatography. The structure of product **4** was identified from spectroscopic data and compared with reported values in the literature.[9] Excellent quality, large crystals of **4** were obtained from slow evaporation of ethanol (Figure 3). The absolute structure using the Parsons method[22] gave a Flack *x* of –0.07(13), which indicates a probably correct absolute structure. Using Bayesian statistics of the Bijvoet pairs[23] the probability of the correct struture for the two possibility case (either the chirality is correct or it is wrong) gave a 1.000 probability that the absolute structure is correct. The X-ray diffraction study was consistent only with an *E* configuration for the C=N double bond.



Scheme 1. Reagents and conditions: (*i*) hydroxylamine hydrochloride, pyridine, ethanol, reflux, 91%; (*ii*) RBr or RCl, NaH, DMF, 0 °C to r.t., 72-80%.

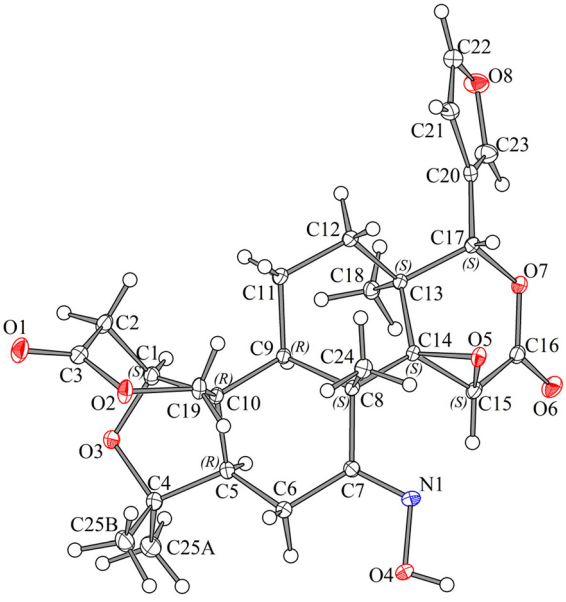
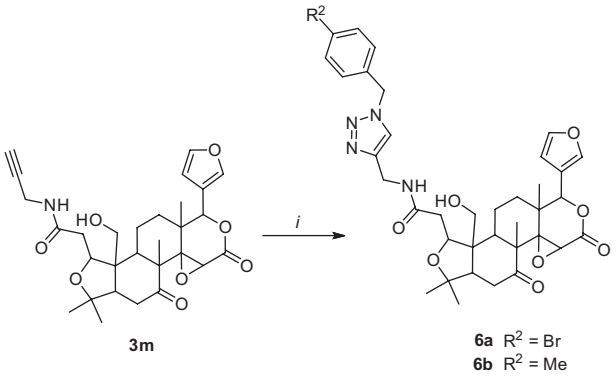


Figure 3. ORTEP plot of limonin-7-oxime (**4**).

The structures of the products **5a-c** were established by the analysis of the ^{1}H and ^{13}C NMR spectra. In the ^{1}H NMR spectra, the disappearance of the signal (C=N–OH), which appeared at 8.41 ppm for compound **4** was observed. The ^{1}H NMR spectra of the derivatives **5b** and **5c** showed signals corresponding to aromatic moiety at around 7.19-8.07 ppm. In order to get the limonin derivatives containing a nitrogen heterocyclic ring, we selected 1,2,3-triazole nucleus. This type of heterocyclic system has a wide range of biological activities and, among these, significant antimicrobial activity.[34-36] The construction of the 1,2,3-triazole moiety was carried out by a click reaction. This reaction occurred

between the propargyl derivative **3m** with the selected benzyl azides 1-(azidomethyl)4-bromobenzene or 1-(azidomethyl)4-methylbenzene using $Cu(OAc)_2.H_2O$ as precatalyst, sodium ascorbate (NaAsc) as reducing agent in a mixture of THF/water (1:1) at room temperature to afford the desired 1,2,3-triazolyl limonins **6a** and **6b** in good yield (Scheme 2).



Scheme 2. Reagents and conditions: (*i*) sodium ascorbate (NaAsc), $Cu(OAc)_2.H_2O$, THF:H_2O (1:1), 1-(azidomethyl)4-bromobenzene or 1-(azidomethyl)4-methylbenzene, r.t., 10 h. Yield: 78% (**6a**) and 71% (**6b**).

The structures of the 1,2,3-triazolyl limonins **6a** and **6b** were unambiguously established based on the ^1H and ^{13}C NMR spectra. In the ^1H NMR spectra we observed the disappearance of the triplet at 2.23 ppm with *J* 2.8 Hz assigned to the methine proton of the acetylene and the appearance of a singlet at 7.43 ppm for **6b** and 7.45-7.54 ppm (overlap signal) for **6a** corresponding to 1,2,3-triazolyl moiety. The ^{13}C NMR spectra showed the signals of the respective carbon triazolyl system, whose methine carbon appeared at 122.0 and 121.9 ppm for **6a** and **6b**, respectively. The quaternary carbon appeared at 145.3 ppm for **6a** and 144.8 ppm for **6b**. These chemical shifts confirmed the conversion of derivative **3m** to its corresponding 1,2,3-triazolyl nuclei **6a** and **6b**.

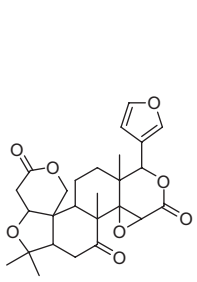
Biological activity

The antimicrobial activity of limonin (**1**), limonin derivatives **3a**-**o**, limonin-7-oxime (**4**), limonin-7-oxime derivatives **5a**-**c** and 1,2,3-triazolyl limonins **6a** and **6b** was evaluated by minimal inhibitory concentration (MIC) from broth microdilution method. The collection of twenty microorganisms used included six fungi: *Candida albicans* (*C. albicans*), *Candida tropicalis* (*C. tropicalis*), *Candida krusei* (*C. krusei*), *Candida parapslosis* (*C. parapslosis*), *Cryptococcus neoformans* (*Crypt. n*), and *Cryptococcus gatti* (*Crypt. gatti*); seven Gram-positive bacteria: *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Bacillus cereus* (*B. cereus*), *Enterococcus spp* (*Enteroc. spp*), *Enterobacter aerogenes* (*E. aerogenes*), *Listeria innocua* (*L. innocua*), and *Listeria monocytogenes* (*L. monocytogenes*); and seven *Gram-negative bacteria: Escherichia coli* (*E. coli*), *Enterobacter cloacae* (*Ent. cloacae*), *Burkholderia cepacia* (*B. cepacia*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Shigella sonnei* (*S. sonnei*), *Salmonella typhimurium* (*S. typhimurium*), and *Morganella morganii* (*M. morganii*). These antimicrobial analyses were performed at concentrations between 6.2-200 μg mL^{-1} and converted to μmol L^{-1}, in order to compare the activity of the investigated compounds. Tables with the results of antimicrobial activities in μg mL^{-1} are in the Supplementary Information.

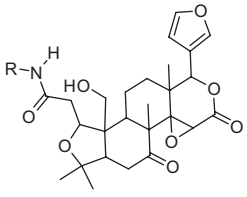
The results observed for the antifungal analysis (Table 3) indicated that among the tested *Candida spp*, the *C. krusei* was the most susceptible to the investigated compounds (**1**, **3a**-**o**, **4**, **5a**-**c**, **6a** and **6b**) with the MIC value between 10-103 μmol L^{-1} (6.2-25 μg mL^{-1}) and minimal fungicidal concentration (MFC) value ranging from 43 to > 190 μmol L^{-1} (25 to > 100 μg mL^{-1}). Limonin (**1**) and some of its derivatives of the series **3a**-**o**, the compounds **3a** (R = Bn), **3f** (R = (*S*)-(+)CH(CH3)Ph), **3g** (R = (*R*)-(–)$CH(CH_3)$Ph), **3j** (R = 2-thiophenemethyl) and **3n** (R = *i*-Pr) exhibited better antifungal activity with MIC value between 10-13 μmol L^{-1} (MIC = 6.2 μg mL^{-1}) against this species of *Candida* (Table 3). Another promising result was the analysis against the fungus *Crypt. neoformans*, since the compounds limonin (**1**), the series **3a**-**o**, limonin-7-oxime (**4**), limonin-7-oxime derivatives **5a**-**c** and 1,2,3-triazolyl limonins (**6a** and **6b**) showed antifungal action with MIC value between 11-53 μmol L^{-1} (6.2-25 μg mL^{-1}) and MFC value between 39-206 μmol L^{-1} (MFC value between 50-100 μg mL^{-1}). The derivatives with 2-thiophenemethyl substituent (**3j**) with MIC = 11 μmol L^{-1} (MIC = 6.2 μg mL^{-1}) and ethyl substituent (**3o**) with MIC = 12 μmol L^{-1} (MIC = 6.2 μg mL^{-1}) exhibited the best antifungal effect. The derivatives **3e** (R = phenethyl) with MIC = 21 μmol L^{-1} (MIC = 12.5 μg mL^{-1}) and **3i** (R = furfuryl) with MIC = 22 μmol L^{-1} (MIC = 12.5 μg mL^{-1}) showed effective antifungal activity against this fungus, as shown in Table 3.

The investigation of the antibacterial activity was performed against a range of Gram-positive and Gram-negative bacteria. Among the employed Gram-positive bacteria, *L. monocytogenes* was the most susceptible to all analyzed compounds with MIC value between 34-169 μmol L^{-1} (25-100 μg mL^{-1}) and minimal bactericidal concentration (MBC) value ranging from 338 to > 425 μmol L^{-1} (200 to > 200 μg mL^{-1}) (Table 4). Limonin (**1**) presented bacterial inhibition against *L. monocytogenes* with MIC = 53 μmol L^{-1}

Table 3. Antifungal activity (MIC and MFC in μmol L^{-1}) for limonin (**1**), limonin derivatives **3a**-**o**, limonin-7-oxime (**4**), limonin-7-oxime derivatives **5a**-**c** and 1,2,3-triazolyl limonins **6a** and **6b**

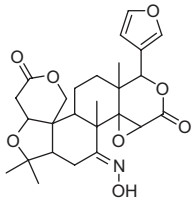


1



3a-o

3a R = Bn, **3b** R = *p*-ClBn, **3c** R = *p*-OMeBn, **3d** R = *p*-CF_3Bn, **3e** R = phenethyl, **3f** R = (*S*)CH(CH_3)Ph, **3g** R = (*R*)CH(CH_3)Ph, **3h** R = 2-picolyl, **3i** R = furfuryl, **3j** R = 2-thiophenemethyl, **3k** R = piperonyl, **3l** R = allyl, **3m** R = propargyl, **3n** R = *i*-pr, **3o** R = ethyl



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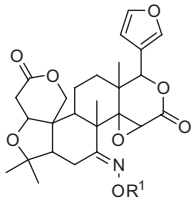
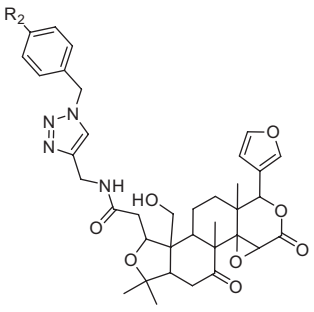


5a-c

5a R^1 = allyl, **5b** R^1 = *p*-BrBn, **5c** R^1 = Bz



6a-b

6a R^2 = Br, **6b** R^2 = Me

Compounds and control	Microorganism MIC and MFC / (μmol L^{-1})											
	Candida albicans		*Candida tropicalis*		*Candida krusei*		*Candida parapslosis*		*Cryptococcus neoformans*		*Cryptococcus gatti*	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
1	212	> 212	212	212	13	53	106	212	53	106	106	106
3a	> 173	–	> 173	–	11	86	173	> 173	43	> 173	86	86
3b	> 163	–	163	> 163	20	163	163	> 163	41	163	82	82
3c	82	82	82	82	41	82	82	82	41	41	41	41
3d	77	77	155	155	39	77	155	> 155	39	39	77	77
3e	169	169	169	169	21	84	84	169	21	84	42	84
3f	169	169	84	169	10	84	84	84	42	84	42	84
3g	169	169	84	84	10	84	84	169	42	> 169	84	84
3h	> 173	–	> 173	–	22	> 173	173	> 173	43	> 173	43	173
3i	176	> 176	176	> 176	22	44	88	> 176	22	176	44	88
3j	171	171	171	171	11	43	86	171	11	86	43	171
3k	80	161	80	80	40	80	80	80	40	40	80	80
3l	189	> 189	189	> 189	24	47	95	189	47	95	47	95
3m	190	> 190	190	190	47	190	95	190	47	47	95	95
3n	189	> 189	189	> 189	12	47	94	> 189	47	94	47	94
3o	194	> 194	194	> 194	24	97	97	194	12	97	48	97
4	103	> 206	206	206	103	103	103	206	51	206	51	103
5a	95	95	95	190	47	> 190	95	190	47	190	47	95
5b	76	76	76	> 153	38	> 153	76	> 153	38	153	38	76
5c	85	170	170	> 170	42	> 170	85	> 170	42	170	85	85
6a	68	68	68	135	34	> 135	68	135	34	135	34	68
6b	74	74	74	> 149	37	> 149	74	149	37	149	37	74
Nystatin	0.8	3.3	1.6	3.3	0.8	0.8	0.8	1.6	1.6	3.3	3.3	3.3

MIC: minimal inhibitory concentration; MFC: minimal fungicidal concentration.

(MIC = 25 μg mL^{-1}) and was less effective against the other utilized Gram-positive bacteria with MIC value between 212-425 μmol L^{-1} (100-200 μg mL^{-1}).

The result of limonin derivatives, series **3a**-**o**, indicated that the compounds **3c** (R = *p*-OMeBn) with MIC = 82 μmol L^{-1} (MIC = 50 μg mL^{-1}), **3d** (R = *p*-CF_3Bn) with MIC = 77 μmol L^{-1} (MIC = 50 μg mL^{-1}), **3m** (R = propargyl) with MIC = 95 μmol L^{-1} (MIC = 50 μg mL^{-1}) showed better antibacterial activity than limonin (**1**) (MIC value between 212-425 μmol L^{-1}) to Gram-positive bacteria *S. aureus*,

B. cereus, *L. innocua* and *Enterococcus spp*. This positive antibacterial activity of compounds **3c**, **3d**, **3m** and also **3g** ((*R*)-(+)CH(CH_3)Ph) with MIC = 84 μmol L^{-1} against *B. cereus* showed better action compared to the control ampicillin (MIC = 143 μmol L^{-1}). Conversely, the derivatives **3c** (R = *p*-OMeBn) with MIC = 82 μmol L^{-1} and **3k** (R = piperonyl) with MIC = 80 μmol L^{-1} against the bacterium *E. aerogenes* were the most active and showed better action compared to the control ampicillin (MIC = 143 μmol L^{-1}). The derivative **3d**, which has a *p*-CF_3Bn substituent, was the only compound in the series **3a**-**o** that presented relevant antibacterial effect (MIC = 77 μmol L^{-1} and MBC > 310 μmol L^{-1}) against the bacterium *B. subtilis*.

Interestingly, the derivative **3g** that has the (*R*)-(+) CH(CH_3)Ph substituent showed better antibacterial activity against the tested Gram-positive bacteria (MIC value between 42-169 μmol L^{-1}) than its stereoisomer, the compound **3f** (MIC value between 84-338 μmol L^{-1}), which contains the (*S*)-(–)CH(CH_3)Ph substituent. These results suggest that different stereocenters present in these compounds **3f** and **3g** are important for the antibacterial activity observed. Other derivatives of the series **3a**-**o** exhibited good antibacterial action against *L. monocytogenes*, the compounds **3c** (R = *p*-OMeBn) with MIC = 41 μmol L^{-1} (MBC > 329 μmol L^{-1}) and **3k** (R = piperonyl) with MIC = 40 μmol L^{-1} (MBC > 322 μmol L^{-1}). It was also observed with the results shown in Table 4 that compounds **3n** (R = *i*-Pr) with MIC = 94 μmol L^{-1} (MIC = 50 μg mL^{-1}) and **3o** (R = ethyl) with MIC = 97 μmol L^{-1} (MIC = 50 μg mL^{-1}) were selective against both *Listeria*, *L. monocytogenes* and *L. innocua*. Based on the results described above, it can be pointed out that among the series **3a**-**o**, the derivatives **3c** (R = *p*-OMeBn) with the electron donating group to the aromatic ring, **3d** (R = *p*-CF_3Bn) with electron-withdrawing group to the aromatic ring, **3k** with piperonyl group and **3g** chiral group (*R*)(+)CH(CH_3)Ph, in general, presented better antibacterial effect against the tested Gram-positive bacteria. The results of the antibacterial analysis of limonin-7-oxime (**4**), limonin-7-oxime derivatives **5a** and **5b** and 1,2,3-triazolyl limonins **6a** and **6b** indicate good antibacterial effect against *B. cereus* with MIC value between 68-103 μmol L^{-1} (MIC = 50 μg mL^{-1}), which showed better action compared to the control ampicillin with MIC = 143 μmol L^{-1} (MIC = 50 μg mL^{-1}).

In evaluating against all employed Gram-negative bacteria, limonin (**1**) was less active (MIC value ranging from 212 to > 425 μmol L^{-1}) than most of its derivatives (MIC value between 17-388 μmol L^{-1}) as demonstrated in Table 4. The compound **3c** (R = *p*-OMeBn) with MIC = 82 μmol L^{-1} presented good antibacterial effect against *Ent. cloacae* and *B. cepacia*, which showed better action compared to the controls ampicillin, with MIC = 143 μmol L^{-1} (MIC = 50 μg mL^{-1}) and levofloxacin, with MIC = 138 μmol L^{-1} (MIC = 50 μg mL^{-1}) for *Ent. cloacae*. Moreover, the compound **3c** (R = *p*-OMeBn) also exhibited good antibacterial activity against *P. aeruginosa* with MIC = 41 μmol L^{-1} (MIC = 25 μg mL^{-1}) and showed better action compared to the controls ampicillin, with MIC = 71 μmol L^{-1} (MIC = 25 μg mL^{-1}) and levofloxacin, with MIC = 138 μmol L^{-1} (MIC = 50 μg mL^{-1}). Another important result was the antibacterial activity of the compounds **3d** (R = *p*-CF_3Bn), **3g** (R = (*R*)-(+)CH(CH_3)Ph), **3k** (R = piperonyl) and **3m** (R = propargyl) with MIC value between 77-95 μmol L^{-1} (MIC = 50 μg mL^{-1}) against *B. cepacia and P. aeruginosa*, except the compound **3k** which showed MIC = 161 μmol L^{-1} against *B. cepacia*. These results showed better antibacterial effect compared to the controls ampicillin, with MIC = 143 μmol L^{-1} against *B. cepacia*, and levofloxacin, with MIC = 138 μmol L^{-1} against *P. aeruginosa*.

The derivative **3g** (R = (*R*)-(+)CH(CH_3)Ph) showed better antibacterial effect than its stereoisomer **3f** (R = (*S*)-(–)CH(CH_3)Ph) against Gram-negative bacteria, thereby, it reproduced the profile exhibited against Gram-positive bacteria (Table 4). As cited above, the derivatives **3c**, **3d**, **3g**, **3k** and **3m** showed the best results among the series **3a**-**o** against the tested Gram-negative bacteria. The bacterium *P. aeruginosa* was the most sensitive to limonin-7-oxime (**4**), limonin-7-oxime derivatives **5a**-**c** and 1,2,3-triazolyl limonins **6a** and **6b** with MIC value between 17-103 μmol L^{-1} (MBC value ranging from 149 to > 412 μmol L^{-1}). Compounds **4** and **5a**-**c** showed MIC value between 76-103 μmol L^{-1} (MIC = 50 μg mL^{-1}) (MBC value ranging from 76 to > 380 μmol L^{-1}) also to other Gram-negative bacteria *E. coli*, *B. cepacia*, *S. typhimurium*, *M. morganii*, with the exception of the compound **4** (MIC = 206 μmol L^{-1}) against *E. coli*. These results showed better antibacterial action compared to the controls ampicillin, with MIC = 143 μmol L^{-1} against *B. cepacia*, and levofloxacin, with MIC = 138 μmol L^{-1} against *P. aeruginosa*.

The 1,2,3-triazolyl limonin **6a** exhibited MIC = 17 μmol L^{-1} (MIC = 12.5 μg mL^{-1}) against *P. aeruginosa*, thus it was the best antibacterial activity among all the investigated compounds. This result is excellent since this compound **6a** showed better action compared to the controls ampicillin, with MIC = 71 μmol L^{-1} (MIC = 25 μg mL^{-1}), and levofloxacin, with MIC = 138 μmol L^{-1} (MIC = 50 μg mL^{-1}) and it was equivalent to the control azithromycin, with MIC = 16.7 μmol L^{-1} (MIC = 12.5 μg mL^{-1}) (Table 4).

Table 4. Antibacterial activity (MIC and MBC in μmol L^{-1}) for limonin (**1**), limonin derivatives **3a**-**o**, limonin-7-oxime (**4**), limonin-7-oxime derivatives **5a**-**c** and 1,2,3-triazolyl limonins **6a** and **6b**

Compounds and controls	Gram-positive bacteria MIC and MBC / (μmol L^{-1})													
	Staphylococcus aureus		*Bacillus subtilis*		*Bacillus cereus*		*Enterococcus spp*		*Enterobacter aerogenes*		*Listeria innocua*		*Listeria monocytogenes*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	425	> 425	212	> 425	425	> 425	425	> 425	425	> 425	212	> 425	53	> 425
3a	346	> 346	NT	NT	346	> 346	346	> 346	346	> 346	173	> 346	173	> 346
3b	> 327	–	NT	NT	327	> 327	> 327	–	> 327	–	163	> 327	82	> 327
3c	82	164	164	164	82	82	82	164	82	164	82	> 329	41	> 329
3d	77	77	77	> 310	77	> 310	77	> 310	155	> 310	77	> 310	155	> 310
3e	338	> 338	NT	NT	338	> 338	338	> 338	338	338	169	> 338	169	> 338
3f	338	> 338	NT	NT	338	> 338	338	338	338	> 338	169	> 338	84	338
3g	84	> 338	169	> 338	84	> 338	84	> 338	169	> 338	42	338	42	> 338
3h	> 346	–	NT	NT	> 346	–	346	> 346	> 346	–	173	> 346	86	> 346
3i	352	> 352	NT	NT	352	> 352	352	> 352	> 352	–	176	> 352	88	> 352
3j	343	> 343	NT	NT	343	> 343	343	> 343	343	> 343	171	> 343	86	> 343
3k	161	161	161	161	161	161	161	161	80	80	80	> 322	40	> 322
3l	379	> 379	NT	NT	379	> 379	379	> 379	379	> 379	189	379	95	> 379
3m	95	190	190	> 380	95	> 380	95	> 380	190	> 380	95	> 380	95	> 380
3n	378	> 378	NT	NT	378	> 378	378	> 378	378	> 378	94	378	94	> 378
3o	388	> 388	NT	NT	388	> 388	388	> 388	388	> 388	97	388	97	388
4	103	206	206	> 412	103	> 412	103	> 412	206	> 412	206	> 412	103	> 412
5a	95	> 380	190	> 380	95	> 380	95	380	190	> 380	95	> 380	47	> 380
5b	76	> 305	153	> 305	76	> 305	76	> 305	153	> 305	76	> 305	76	> 305
5c	170	> 339	> 339	–	170	> 339	85	> 339	339	> 339	170	> 339	42	> 339
6a	68	135	135	> 271	68	> 271	68	> 271	135	271	68	> 271	34	> 271
6b	74	149	149	> 297	74	> 297	74	> 297	149	> 297	74	> 297	37	> 297
Ampicillin	2.2	143	2.2	143	143	143	71	143	143	143	36	143	36	143
Azithromycin	1.0	67	1.0	1.0	2.0	2.0	2.0	8.3	16.7	16.7	4.1	8.3	4.1	8.3
Levofloxacin	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1
	Gram-negative bacteria MIC and MBC / (μmol L^{-1})													
	Escherichia coli		*Enterobacter cloacae*		*Burkholderia cepacia*		*Pseudomonas aeruginosa*		*Shigella sonnei*		*Salmonella typhimurium*		*Morganella morganii*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	425	> 425	> 425	–	> 425	–	212	> 425	425	425	425	> 425	212	> 425
3a	346	> 346	346	> 346	346	> 346	346	346	346	> 346	346	> 346	173	> 346
3b	> 327	–	> 327	–	> 327	–	327	> 327	> 327	–	> 327	–	> 327	–
3c	82	164	82	164	82	82	41	82	82	82	82	164	82	164
3d	77	> 310	155	> 310	77	77	77	155	77	> 310	77	77	77	> 310
3e	338	> 338	338	> 338	338	> 338	169	338	> 338	–	338	> 338	169	> 338
3f	> 338	–	338	338	338	> 338	338	> 338	> 338	–	> 338	–	160	> 338
3g	84	> 338	169	> 338	84	169	84	338	84	> 338	84	> 338	84	> 338
3h	> 346	–	> 346	–	> 346	–	346	> 346	> 346	–	> 346	–	173	> 346
3i	> 352	–	> 352	–	352	> 352	352	> 352	352	> 352	> 352	–	176	> 352
3j	343	343	343	> 343	343	> 343	171	343	343	> 343	343	> 343	171	> 343
3k	80	161	161	161	161	161	80	80	80	80	80	161	80	161
3l	379	> 379	379	> 379	379	> 379	379	> 379	379	> 379	379	> 379	189	> 379
3m	95	> 380	190	> 380	95	95	95	190	95	> 380	95	> 380	190	> 380
3n	378	378	378	> 378	378	> 378	378	> 378	378	> 378	> 378	–	100	> 378
3o	388	> 388	388	> 388	388	> 388	388	> 388	388	> 388	388	> 388	100	> 388
4	206	> 412	206	> 412	103	103	103	412	103	> 412	103	> 412	103	> 412
5a	95	380	95	380	95	95	95	190	95	> 380	95	> 380	95	> 380
5b	76	> 305	153	> 305	76	76	76	153	153	> 305	76	> 305	76	> 305
5c	85	> 339	170	> 339	85	85	85	170	170	> 339	85	> 339	85	> 339
6a	68	271	68	271	68	68	17	> 271	68	> 271	68	> 271	68	> 271
6b	74	> 297	149	> 297	74	74	74	149	74	> 297	74	149	74	> 297
Ampicillin	8.9	71	143	143	143	143	71	143	71	143	2.2		2.2	2.2
Azithromycin	2.0	4.1	1.0	2.0	2.0	4.1	16.7	33	4.1	4.1	4.1		8.3	4.1
Levofloxacin	8.6	69	138	138	69	138	138	138	69	138	2.1		2.1	2.1

MIC: minimal inhibitory concentration; MBC: minimal bactericidal concentration; NT: not tested.

Conclusions

In our study, a novel series of derivatives was synthesized and characterized from natural limonin (modification in A-ring) using methodology in solution as well as in heterogeneous medium. As a result, it was possible to obtain fifteen compounds. In addition, we obtained two derivatives by inserting the 1,2,3-triazole nucleus via click reaction and prepared three derivatives from reactions with limonin-7-oxime. The results of the antimicrobial activity against a collection of microorganisms, in general, demonstrated that a relevant number of synthetic derivatives presented higher activity than the natural product limonin and showed higher antibacterial effect comparable to employed controls. The present study indicated that the modification in A-ring of the limonin structure and at C-7 position generated compounds that showed to be more active.

Supplementary Information

Supplementary data (^{1}H NMR, ^{13}C NMR, HRMS spectra and tables of the results of antimicrobial activities in μg mL^{-1}) are available free of charge at http://jbcs.sbq.org.br as PDF file. Crystallographic data (compounds **1**, **3a**, **3h**, **4**) have been deposited at the Cambridge Crystallographic Data Centre under CCDC deposition with numbers 1051185-1051188 via www.ccdc.cam.ac.uk/data_request/cif.

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