SUPPLEMENTARY INFORMATION

Structural Diversity and Similar Bioactivity in Synthetic Bicyclo Nonanes

Liliana E. Luna¹, Pamela S. Forastieri¹, Patricia Marchiaro², Adriana Limansky², Raquel M. Cravero^{1*}

¹ *IQUIR (Instituto de Química Rosario), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531 (S2002LRK) Rosario, Argentina,* ² *IBR (Instituto de Biología Molecular y Celular de Rosario). Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531 (S2002LRK) Rosario, Argentina.* E-mail: cravero@iquir-conicet.gov.ar

Contents:

Description	Page N°
Experimental	1-4
Microbiological Assay	4-5
¹ H and ¹³ C NMR Spectra of new compounds	6-17

Experimental

Compounds **2**,^[11] **3**,^[15] **4**,^[13] **8**,^[14] **10**,^[12] and **14**^[24] were described previously.

Michael reaction on solid support. A soln. of cyclohexane-1,3-dione (**1**, 112 mg, 1 mmol) in CH₂Cl₂ (1 mL) was added to a two-necked flask containing 500 mg of neutral Al₂O₃ (previously activated under vacuum at 80°C for 4 h) at r.t. The solvent was evaporated with the help of a stream of dry N₂ and the mixture was left stirring at r.t. for 20 min.. Then MVK (85 µL, 1 mmol) was added dropwise maintaining good agitation. After 40 min. at r.t., the solid phase was purified by *filtration* through a *silica gel pad* with CH₂Cl₂ (5 × 10 mL). The collected org. soln. was dried (anh. Na₂SO₄), filtered and evaporated under reduced pressure. Triketone **3** (66%) was obtained as oil after purification by flash chromatography, while tetraketone **4** (33%) was obtained as a white solid.

3-Allyloxy-2-(3-oxo-butyl)-cyclohex-2-enone (7) and 2-Allyl-2-(3-oxobutyl)cyclohexane -1,3-dione (8).^[20]

K₂CO₃ (209 mg, 1.5 mmol) was added portionwise to a flask containing the triketone **3** (200 mg, 1.1 mmol) dissolved in 5.5 mL of acetone and left at 50°C for 50 min. with magnetic stirring. Then, allyl bromide (170 µL, 1.5 mmol) was added slowly and the mixture was heated under reflux for 3 h. Once the reaction reached the r.t., acetone was removed under vacuum and then the crude was dissolved in Et₂O and extracted with a sat. aq. soln. of NaCl (3 × 5 mL). The aq. phase was extracted with Et₂O (3 × 5 mL), twice with EtAcO (3 × 5 mL), and dried (anh. Na₂SO₄). The residual oil was purified by flash chromatography to give oils **7** and **8** in a ratio of 1:4.

Compound 7: Colorless oil, Hex/EtAcO 50 : 50, R_f = 0.34. IR (film): 2844, 1715 (C=O ketone), 1655 (C=C), 1602 (conjugated C=C). $^1\text{H-NMR}$ (CDCl_3): δ ABX system 5.93 (*ddt*, 1H, J = 17.9, 10.5, 4.6, H_X); 5.33 (*ddd*, 1H, J = 15.0, 3.0, 1.7, H_A); 5.27 (*ddd*, 1H, J = 10.4, 1.5, 1.3, H_B); 4.55 (*d*, 2H, J = 5.8, CH_2O); 2.60-2.51 (*complex signal*, 4H, H-C(2'), H-C(6)); 2.40-2.36 (*m*, 2H, H-C(4)); 2.13 (*s*, 3H, CH_3); 2.04-1.84 (*m*, 2H, H-C(1')), 1.77-1.62 (*m*, 2H, H-C(5)). $^{13}\text{C-NMR}$ (CDCl_3) 209.2 (C=O side chain); 198.0 (C=O); 171.5 (C(3)); 132.7 (CH vinyl); 118.4; 117.6 (CH_2 vinyl, C(2)); 68.1 (CH_2 allyl); 42.6 (C(2')); 36.2 (C(6)); 29.4 (CH_3); 25.2 (C(1')); 20.8 (C(4)); 17.1 (C(5)). EIMS: 182 (17), 140 (10), 139 (85), 84 (14), 69(19), 55 (43), 44 (88), 43 (100). ESI-HRMS: calcd for ($M+\text{Na}^+$) $\text{C}_{13}\text{H}_{18}\text{NaO}_3$ 245.11536; found 245.11326.

5,5-Dimethyl-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)cyclohex-2-enone (13) and 5,5-Dimethyl-3-(3-methylbut-2-enyloxy)cyclohex-2-enone (14).^[9]

DBU (1.7 mL, 10.7 mmol) was added to a soln. of dimedone (490 mg, 3.5 mmol) in dry THF (7.5 mL) and the mixture was left stirring at r.t. for 15 min. Methyl vinyl ketone (0.435 mL, 5.35 mmol) was added and the mixture was maintained at r.t. for 3 h. After starting material was fully consumed (TLC), prenyl bromide (0.650 mL, 5.35 mmol) was added and the mixture was left stirring for 3 h at r.t. The reaction was quenched with H_2O (2×2.5 mL) and the product extracted with EtAcO (3×30 mL). Removal of the solvent produced a yellowish oil, which was purified by column chromatography to give pure **13** (20%) and **14** (78%).

Compound 13: Colorless oil (20%). IR (film): 2960, 2871, 1655 (C=O ketone), 1602(C=C), 1372, 1210, 1150. $^1\text{H-NMR}$ (CDCl_3) : δ 5.31 (*complex signal*, 1H, H-C=);

5.02 (*complex signal*, 1H, HC=); 4.49 (*d*, 2H, $J = 6.7$, OCH₂); 2.93 (*d*, 2H, $J = 7.0$, C-CH₂-C=); 2.38 (*s*, 2H, CH₂CO); 2.19 (*s*, 2H, CH₂C-O); 1.75, 1.69, 1.66, 1.65 (4*s*, 3H each, (CH₃)₂C=C); 1.04 (*s*, 6H, (CH₃)₂C). ¹³C-NMR (CDCl₃): 199.6 (C=O); 176.1 (C(3)); 139.5, 135.2 (=C(CH₃)₂); 116.7 (CH₂-C=C(CH₃)₂); 101.7 (C(2)); 65.5 (CH₂O); 50.7 (C(6)); 42.3 (C(4)); 34.1 (CH₂ (allyl)); 32.5 (C(5)), 28.2 (*gem*CH₃ C(5)), 25.7, 18.2 (=C(CH₃)₂). ESI-HRMS: calcd for (M+H⁺) C₁₈H₂₉O₂ 277.21621; found 277.21553.

Compound 14:^[24] ¹³C-NMR (CDCl₃): 199.6 (C=O); 176.2 (C(3)); 139.5 (=C(CH₃)₂); 117.9(CH₂-C=C(CH₃)₂); 101.7 (C(2)); 65.5 (CH₂O); 50.7 (C(6)); 42.9 (C(4)); 32.5 (C(5)), 28.2 (*gem*CH₃ C(5)), 25.7, 18.2 (=C(CH₃)₂).

Microbiological Assay

Microbial strains. Antibacterial activity was evaluated against two Gram-positive, *i.e.*, *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923, and two Gram-negative bacteria, *i.e.*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853.

Microbiological assay. To test *in vitro* the antibacterial activity of above the mentioned compounds, different Gram-negative and Gram-positive bacterial strains were employed. Bacterial cells were previously grown at 37°C during 18 to 24 h on a nutritive agar plate. Next, an inoculum of each microorganism was prepared by making a direct saline suspension of isolated colonies selected from the solid medium. Suspensions were then adjusted to match the 0.5 McFarland turbidity standard (1×10^8 CFU/mL), using sterile saline solution and a vortex mixer.^[25] Next, 10-fold serial dilutions of each bacterial suspension were made and 2 μ l corresponding to dilutions 10^8 to 10^4 CFU/mL were applied

to the surface of Mueller-Hinton agar without supplement or supplemented with 1000 µg/mL of compound **5**, 1000 µg/mL of compound **9**, 1000 µg/mL of compound **6** and 1000 µg/mL of compound **11**, as Viale *et al*^[26] with modifications. Developing colonies were observed after a 24 h incubation period at 37°C. These assays were carried out in duplicate.

^1H and ^{13}C NMR Spectra of new compounds

Figure S1: ^1H NMR Spectrum of compound 5

Figure S2: ^{13}C NMR Spectra of compound 5

Figure S3: HSQC-NMR Spectrum of compound 5

Figure S4: ^1H NMR Spectrum of compound 6

Figure S5: ^{13}C NMR Spectra of compound 6

Figure S6: HSQC-NMR Spectrum of compound 6

Figure S7: ^1H NMR Spectrum of compound 7

Figure S8: ^{13}C NMR Spectra of compound 7

Figure S9: ^1H NMR Spectrum of compound 9

Figure S10: ^{13}C NMR Spectra of compound 9

Figure S11: ^1H NMR Spectrum of compound 11

Figure S12: ^{13}C NMR Spectra of compound 11

Figure S13: ^1H NMR Spectrum of compound 13

Figure S14: ^{13}C NMR Spectra of compound 13