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Structural Diversity and Similar Bioactivity in Synthetic Bicyclo Nonanes

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Abstract

Simple syntheses of diverse bicyclo[3.3.1]nonanes and related compounds as the minimal substructure of bioactive natural products via Michael, aldol, and alkylation reactions from diketones, are described herein. The structures of the synthesized compounds were determined by IR, NMR (^{1}H and ^{13}C) and ESI-HRMS. We also show the in vitro antimicrobial activity against Gram-positive and Gram-negative bacteria. The qualitative analysis has revealed that the new synthesized compounds **5**, **6**, **9** and **11** present antibacterial properties.

Simple syntheses and *in vitro* antimicrobial activity of bicyclo nonanes and related compounds as the minimal substructure of bioactive natural products, via Michael, aldol and alkylation reactions from diketones.
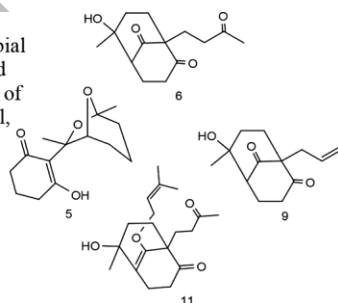
KEYWORDS: bicyclo nonanes, diketones, Michael-aldol-alkylation, antimicrobial activity

INTRODUCTION

Substituted bicyclo [3.3.1] nonanes are characteristic carbon skeletons of natural products. They belong to a class of interesting compounds with a remarkable diversity of biological activities against conditions such as cancer, HIV, bacterial infections and depression, among others.[1,2]

Members of prenylated acyl phloroglucinols derivated from prolifenone, nemorosone, clusianone,[3,4] and particularly hyperforin isolated from *Hypericum* species, possess potent antibacterial activity and moderate cytotoxicity, (Fig. 1).[5, 6] Recently new cytotoxic and anti-inflamatory prenylated benzoylphloroglucinols were isolated from *Garcinia esculenta* and examined against human cancer and hepatic cells.[7] Due to manifest bioactivities of these compounds, many and innovative strategies have been developed towards the [3.3.1] bridged bicycle class of phloroglucinol natural products.[8-10]

Exploring synthetic strategies to build oxygenated polinuclear heterocycles of biological interest, first we found a methodology on solid support for the chromenone **2** *via* Michael reaction on 1,3-cyclohexanedione **1**. Biological evaluation of the heterocycle **2** led to its consideration as antifeedant compound which was compared with related natural and semi-synthetic products.[11] (Scheme 1).

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In this work we report the synthesis of bicyclic analogs of [3.3.1] bridged phloroglucinol natural products from 1,4-addition, alkylation and cyclization reactions on 1,3-cyclohexanediones **1** and **12**, and their biological activities to disclose minimal functional requirements as potential antibacterial agents, Fig. 1.

In spite of the existence of related synthetic studies,[11-15] the originality of this work is based on the low number of synthetic pathways to create functional diversity in highly oxygenated-structures with similar biological activity.

RESULTS AND DISCUSSION

Chemistry. The Michael addition continues to be a valuable synthetic method which, in combination with alkylation and condensation reactions, may be used to obtain a wide variety of complex molecules from relatively simple starting materials. Consequently, new structures containing acetal, hemiketal and carbonyl functions linked to a common ring have been isolated and identified. These structures are related to prolifenone A and B,[16] hyperforin, clusianone[17] as well as the advanced intermediary phomoidride B, among others.[18]

Michael addition of **1**, methyl vinyl ketone (MVK) and NaOH in MeOH at room temperature led to compounds **3**,[15] **4**[16] and the ketal **5** with 25:35:30% yield, respectively (Scheme 1). The same reaction carried out on neutral alumina as solid support in CH_2Cl_2 at room temperature, also afforded adducts **3** (66%) and **4** (33%).

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Alternatively, **3** could be transformed into **4** with prior isolation of the mixture, under the same reaction conditions.

As shown in Scheme 2, the reaction pathways to give a new compound **5** involves a dimerization of the methyl vinyl ketone followed by an aldol reaction between the resulting cyclic hemiacetal **A** and cyclohexane-1,3-dione. The formation of hemiacetal **A** was evidenced by literature data[19] and its structure was confirmed by spectroscopic characterization of the methylketal derivative **B**, as we discussed. The ^{1}H-NMR spectrum shows a double doublet at 4.02 ppm corresponding to the CH axial coupled with the near CH$_2$ (^3J = 2.6 (H$_{ax}$-H$_{ec}$) and ^3J = 12.0 (H$_{ax}$-H$_{ax}$)), and signals at 3.22 (CH$_3$O), 2.20 (CH$_3$C=O), 1.35 (CH$_3$) and at 1.90-1.30 appears a multiplet for other methylene groups of the ring.

By virtue of some substructures of natural products that contain prenyl side chains, we explored the incorporation of allyl and prenyl groups. After several attempts, introduction of the allylic function was exemplified by the base-catalyzed aldol process of the triketone **3** dissolved in acetone with K$_2$CO$_3$ at 50ºC, and then addition of allyl bromide under reflux.[20] This reaction afforded products of O-alkylation (**7**) and C-alkylation (**8**) in a ratio 1:4, in quantitative yield (Scheme 3). The mixture of **7** and **8** could be easily converted into **8** by a Claisen rearrangement in boiling xylene during 5 h.[21] The same reaction conditions also allowed to transform **7** and **8** to the bicyclic allylic compound **9** in good yield by an aldol reaction on the side chain carbonyl group. When the reaction of **8** was carried out in the presence of L-pro in MeCN at r.t., the known allyl Wieland-

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Miescher ketone **10** is formed by means of an aldol condensation on the carbonyl group of the ring.[12]

Another approach was explored using tetraketone **4** as starting material, which led to two more advanced bicyclic substructures of interest (Scheme 4). Thus, reaction of **4** with K_2CO_3, dissolved in MeOH at -18°C at r.t.[22] rendered triketone **6** *via* intramolecular aldol condensation in quantitative yield. Then, treatment of **6** with LDA in THF and subsequent addition of prenyl bromide furnished only the O-alkylated **11** in good yield.[9]

All compounds required a detailed analysis of their structures by ^{1}H and ^{13}C NMR. The structure elucidation of compounds **5** and **6** was performed by extensive spectroscopic analysis; including 1D (BB and DEPT), 2D NMR (HSQC and HMBC) and their relative stereochemistries were readily determined by NOE measurements.

In case of the compound **5**, from HSQC spectrum shows that the methylene carbon at 24.7 ppm correlates with proton resonances at 1.63 and 1.35 and the methylene carbon at 17.2 ppm correlates with proton resonances at 1.55 and 1.45 showing the diatereostereotopic character of this methylene group. Besides, the irradiation of methine C-1' gave a nuclear Overhauser enhancement of de methylic protons on C-7' and the methylene protons of C-2'.

From the HSQC spectrum of **6**, we observed the correlation between the methylene carbon at 39.4 ppm with proton resonances at 2.57 and 2.31, the methylene carbon at 38.7

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ppm with proton resonances at 2.59 and 2.25 and the methylene carbon at 18.9 ppm also

correlates with proton resonances at 2.09 and 1.70. Thus, we have assigned the chemical

shifts for all de protons of **6** and correlated them with the resonance from ^{13}C spectrum

with the proposal to define the diastereotopic protons for each of the methylene groups.

In addition, the irradiation of methyl at peak δ = 1.35 ppm (**CH₃**C-OH) gave a nuclear

Overhauser enhancement of de methine H-5 (δ = 2.65 ppm) and axial proton of

methylene H-8 (δ = 1.70 ppm) (Fig. 3.)

In particular, structure **11** was elucidated by comparison with related Michael products

previously reported by Hajos and Parrish in the Michael reaction between 2-methyl-1,3-

cyclopentanedione and MVK in refluxing MeOH and catalytic amounts of KOH.[23]

Treatment of **12** with MVK, DBU and prenyl bromide at room temperature resulted in

the formation of the O-alkylated product **14** (78%) along with the C- and O-alkylated **13**

(20%) and the methyl vinyl ketone dimer (2%), in a one-pot reaction (Scheme 5). These

products of alkylations reactions **13** and **14** were used as representative simple models of

functional groups for the *in vitro* evaluation of biological activity.

Antimicrobial Activity. Fig. 4 shows the active compounds resulting from *in vitro* assays

of antibacterial activity against *Gram*-positive organisms such as *Enterococcus faecalis*

ATCC 29212 (E. fa), *Staphylococcus aureus* ATCC 25923 (S. au), and *Gram*-negative

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bacteria *Escherichia coli* ATCC 25922 (E. co), and *Pseudomonas aeruginosa* ATCC 27853 (P. ae).[25,26]

Monocyclic compounds **13** and **14**, substituted with a gem-methyl on the ring and prenyl chains, did not exhibit activity in the *in vitro* tests.

The results observed in the microbiological assays indicate antibacterial activity of compounds **5**, **6** and **9** on *E. fa* but not on *E. co*., and equivalent antimicrobial susceptibility testing results were obtained with *S. au* and *P. ae*, respectively.

The more advanced substructure of prenylated natural products, this is, the prenylated-analog **11**, maintained its *Gram*-positive activity on *E. fa* (10^4 dilution) and also diminished bacterial growth as seen on *E. co*.

These data show an antibacterial effect of the compounds **5**, **6**, **9** against *Gram*-positive bacteria; although no effect against *Gram*-negative bacteria was observed under the conditions tested, and the comparison between the compound **9** (allyl) and **11** (prenyl chain) shown the latter has a more broad spectrum of activity.

CONCLUSIONS

By means of simple reactions and few steps in respect to the known synthesis, we synthesized new bicycle[3.3.1]nonanes of interest as core of prenylated acyl phloroglucinols to further our understanding of structure-activity relationships in

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bioactivity profiles. *In vitro* analysis of antimicrobial activity allowed us to elucidate minimal functional requirements of the structural core to display bioactivity. The results demonstrate that the oxidation degree, this is *β*-hydroxyenone, *β*-hydroxyketone, *β*-diketone and ketal moieties of the compounds **5**, **6**, **9** and **11,** have a significant role similar to that of alkenyl chains in **9** and **11**, which are present in the original structures. In addition, the negative tests for antibacterial activity of the monocyclic compounds **13** and **14,** although with functional groups similar to those in **5**, **6**, **9** and **11**, suggest that their antibacterial activity is also dependent on the number of rings and the three-dimensional arrangement (conformation) of these kind of molecules. Therefore molecular arrangements such as two separate rings (**5**) or fused ring systems (**6**, **9**, and **11**), along with the above mentioned structural features are needed for antibacterial activity. Further synthetic manipulations will be improve the biological activity of these compounds.

EXPERIMENTAL

General. All solvents were dried and distilled before use. All reactions were carried out under anhydrous conditions under N_2 atmosphere. All the organic extracts were dried over anh. Na_2SO_4. Reactions were monitored by thin layer chromatography (TLC) on aluminum-foil plates coated with *Merck* Kieselgel 60 F254, and spot visualization performed under UV light. Column chromatography (CC): Analtech silica gel for flash chromatography, under low N_2 pressure. Elution was carried out with hexane/AcOEt mixtures. IR Spectra (in cm^{-1}) were recorded on a *Shimadzu Prestige-21 FTIR* spectrophotometer. ^{1}H- and ^{13}C-NMR spectra (300 MHz and 75 MHz) were recorded on a *Bruker AC-300* spectrometer. Samples were dissolved in CDCl$_3$ as solvent and Me$_4$Si

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as internal standard; δ in ppm, *J* in Hz. High-resolution mass spectrometry (HR-MS) was performed using a *Bruker MicrOTOF-Q II 10223* instrument. Melting points were measured on a *Ernst Leitz* hot-stage microscope; uncorrected.

Preparation Of 2-(5,7-Dimethyl-6,8-Dioxabicyclo[3.2.1]Oct-7-Yl)-3-Hydroxy-Cyclohex-2-Enone (5).

Michael reaction in solution. MVK (125 μl, 1.5 mmoles) was added slowly to a mixture of **1** (112 mg, 1 mmol), in anh. MeOH (1mL) and NaOH (1.2 mg, 0.03 mmol) at r.t., and the mixture was left at r.t. for 2 h. MeOH was removed under vacuum and the resulting solid was suspended in CH_2Cl_2, washed with a sat. aq. soln. of NaCl, dried (anh. Na_2SO_4), filtered and concentrated. Purification of the residue obtained by flash chromatography rendered **3** (25%) as oil, **4** (35%) as a white solid, and compound **5** (30%) as white crystals.
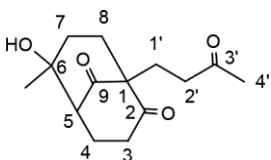
Compound 5
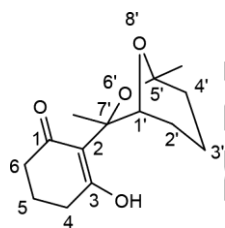
White crystals (30%), Hex/EtAcO 50:50, R_f = 0.90, mp: 93.5-94.0°C. IR (film): 3109 (OH vinylogous acid), 2941, 1685 (C=O ketone), 1645, 1615, 1360, 1182, 1113, 1044, 969, 837. ^{1}H-NMR (CDCl$_3$): δ 10.98 (*s*, 1H, OH); 4.66 (*bs*, 1H, H-C(1′)); 2.48 (*t*, 2H, *J* = 6.2, H-C(4)); 2.32 (d*t*, 2H, *J* = 6.6, 2.0, H-C(6)); 2.01-1.87 (*m*, 2H, H-C(5)); 1.74-1.35 (*m*, 6H, H-C(2′), H-C(3′), H-C(4′)); 1.49 (*s*, 3H, H$_3$C-C(5′)), 1.41 (*s*, 3H, H$_3$C-C(7′)). [13]C-

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NMR (CDCl$_3$) 197.2 (C=O); 174.6 (C(3)); 115.1 (C(2)); 108.4 (C(5′)); 87.4 (C(7′)); 82.1 (C(1′)); 37.5 (C(6)); 34.1 (C(4′)); 29.7 (C(4)); 27.4 (CH$_3$(7′)); 25.8 (CH$_3$(5′)); 24.7 (C(3′)); 20.7 (C(5)); 17.2 (C(2′)). EIMS: 252 (M^+), 237 (M^+- CH$_3$, 5), 177 (18), 163 (33), 139 (15), 98 (43), 82 (25), 79 (28), 77 (28), 69 (40), 67 (37), 65 (25), 55 (100). ESI-HRMS: calcd for (M+H$^+$) C$_{14}$H$_{21}$O$_4$ 253.14344; found 253.14261.

Preparation Of 6-Hydroxy-6-Methyl-1-(3-Oxobutyl)Bicyclo[3.3.1]Nonane-2,9-Dione (6).


Compound **4** (30 mg, 0.12 mmol) in MeOH (1.5 mL) was added to a soln. of K$_2$CO$_3$ (30 mg, 0.12 mmol) in MeOH (0.3 mL), maintained at -18°C, and left in the freezer overnight. The mixture was filtered through a funnel containing a pad of celite and washed with EtAcO, dried and concentrated. Purification by flash chromatography of the crude rendered compound **6** in quantitative yield. White solid, mp: 113–114°C. IR (film): 3446 (OH), 2946, 2931, 2878, 1701 (C=O), 1375, 1166. ^{1}H-NMR (CDCl$_3$): δ 2.65-2.51 (*m*, 3H); 2.41-2.27 (*m*, 2H); 2.10 (*s*, 3H, CH$_3$CO); 2.04-1.94 (*m*, 5H); 1.72-1.64 (*m*, 3H); 1.35 (*s*, 3H, CH$_3$C-OH). ^{13}C-NMR (CDCl$_3$) 211.6, 211.2 (C=O, C(2), C(9)); 208.2 (C(3′)); 78.9 (C-OH); 64.4 (C(1)); 57.0 (C(5)); 39.4 (C(3)); 38.7 (C(2′)); 36.3(C(4)); 32.0 (C(7)); 29.8 (*C*H$_3$CO); 27.7 (*C*H$_3$C-OH); 25.7 (C(1')); 18.9 (C(8)). EIMS: 222 (M^+, 8) 204 (M^+ - H$_2$O, 25), 166 (10), 155 (20), 95 (36), 67(50) 55 (55), 43 (CH$_3$CO$^+$, 100). ESI-HRMS: calcd for (M+Na$^+$) C$_{14}$H$_{20}$NaO$_4$ 275.12538; found 275.12475.

Preparation Of 1-Allyl-6-Hydroxy-6-Methylbicyclo[3.3.1]Nonane-2,9-Dione (**9**)**.**
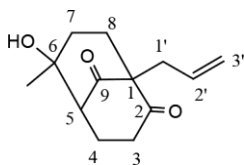
Compound **7** (180 mg, 0.81 mmoles) dissolved in xylene (18 mL) was heated under

reflux for 10 h. Solvent evaporation under vacuum and further flash chromatography of

crude furnished **9** (92%) as oil. Compound **8** could also be converted into **9** by refluxing

in xylene for 5 h. Yellow oil (87%). IR (film): 3420 (OH), 3080, 2933, 1703 (C=O

ketone), 1639 (C=C), 1000, 910. ^{1}H-NMR (CDCl$_3$)): δ ABCDX spin system 5.83

(*complex signal*, 1H, H$_X$); 5.08 (*complex signal,* 1H, H$_B$); 5.03-4.98 (*m*, 1H, H$_A$); 4.50

(*bs*, 1H, OH); 2.64 (*t*, 1H, J = 1.0, H-C(5)); 2.54-1.92 (*m*, 6H, H-C(4), H-C(7), H-C(8));

1.72-1.62 (*m*, 4H, H-C(3), H-C(1'), CD spin system); 1.35 (*s*, 3H, CH$_3$). ^{13}C-NMR

(CDCl$_3$) 211.1 (C=O); 211.0 (C=O); 133.2 (C(2')); 118.2 (C(3')); 78.7 (C-OH)); 65.1

(C(1)); 56.8 (C(5)); 39.8 (C(3)); 35.8 (C(1')); 35.3 (C(7)); 31.5 (C(8)); 27.5 (CH$_3$);

18.7(C(4)). ESI-HRMS: calcd for (M+Na$^+$) C$_{13}$H$_{18}$NaO$_3$ 245.11482; found 245.11108.

Preparation Of 6-Hydroxy-6-Methyl-9-(3-Methylbut-2-Enyloxy)-1-(3-Oxobutyl)
Bicyclo [3.3.1]Non -5(9)-En-2-One (**11**)**.**
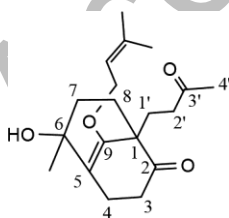
A soln. of **6** (50 mg, 0.2 mmol) in anh. THF/HMPA (7 : 1, 0.25 mL) was slowly added to

a soln. of LDA at −78ºC, (prepared by treating a soln. of diisopropylamine (0.05 mL) in

anh. THF (0.3 mL) with *n*-BuLi (9.9 mL), 1.6 M in hexane for 20 min). The resulting

11

soln. was stirred for 30 min at the same temperature. Prenyl bromide (0.05 mL) in anh.

THF (0.07 mL) was subsequently added and the temperature was slowly raised up to

20ºC over a 12 h period. The reaction was quenched with sat. aq. NH_4Cl (1 mL) and

diluted with Et_2O (3 mL). Layers were separated and the org. phase was washed with

brine (40 mL) and dried (Na_2SO_4). The solvent was removed *in vacuo* and the crude

residue was purified by flash column chromatography (silica gel, EtAcO/Hexane 1 : 3) to

furnish the desired product **11**. Colorless oil (78%). IR (film): 3390 (OH), 3082, 2940,

2843, 1715 (C=O side chain), 1705 (C=O), 1610 (C=C), 1220, 1130. ^{1}H-NMR ($CDCl_3$)):

δ 5.34 (*t*, 1H, *J* = 7.3, H-C=); 4.57 (*d*, 2H, *J* = 7.5, OCH_2); 2.74 (*s*, 1H, OH); 2.55 (*t*, 2H,

J = 7.5, H-C(3)); 2.40 (*t*, 2H, *J* = 5.3, H-C(2´)); 2.11 (*s*, 3H, CH_3C=O); 2.00-1.84 (*m*,

8H, H-C(1´), H-C(4), H-C(7), H-C(8)); 1.66 (*s*, 3H, CH_3C=C), 1.62 (*s*, 3H, CH_3C=C);

1.22 (*s*, 3H, CH_3COH). ^{13}C-NMR ($CDCl_3$) 211.1 (C=O side chain); 209.3 (C=O); 165.2

(=C-O); 138.2 (=$C(CH_3)_2$); 117.5 (=C); 113.1 (C(5)); 79.2 (C(OH)); 68.1 (C(1)); 67.4

(C(allyl)); 39.7 (C(3)); 37.9 (C(2´)); 35.2 (C(7)); 30.8 (C(8)); 29.2 (CH_3C=O); 27.5

(CH_3COH); 25.7 (C(1´)); 25.6 (($CH_3)_2C$=); 20.0 (C(4)); 17.9 (($CH_3)_2C$=). ESI-HRMS:

calcd for (*M*+H$^+$) $C_{19}H_{29}O_4$ 321.20659; found 321.20232.

REFERENCES

12

[1]   Grossman, R. B.; Ciochina, R. *Chem. Rev.* **2006**, *106*, 3963-3986.

[2]   Socolsky, C.; Borkosky, S. A.; Hernández de Terán, M.; Asakawa, Y.; Bardón, A.

J. Nat. Prod. **2010**, *73*, 901-904.

[3]   Takagi, R.; Inoue, Y; Ohkata, K. *J. Org. Chem.* **2008**, *73*, 9320-9325.

[4]   Qi, J.; Porco, J. A. *J. Am. Chem. Soc.* **2007**, *129*, 12682-12683.

[5]   Verotta, L. *Phytochem. Rev.* **2002**, *1*, 389-407.

[6]   Albert, D.; Zundorf, I.; Dingermann, T.; Muller, W. E.; Steinhilber, D.; Werz, O.

Biochem. Pharmacol. **2002**, *64*, 1767-1775.

[7]   Zhang, H.; Zhang, D.-D.; Lao, Y-Z.; Fu, W.-W.; Liang, S.; Yuan, Q.-H.; Yang,

L.; Xu, H.-Xi *J. Nat. Prod.* **2014**, *77*, 1700-1707.

[8]   Garnsey, M. R.; Lim, D.; Yost, J. M.; Coltart, D. M.*Org. Lett.* **2010**, *12*, 5234-

5237.

[9]   Snider, B. B.; O'Neil, S. V. *Tetrahedron* **1995**, *51*, 12983-12994.

[10]   Njardarson, J. T. *Tetrahedron* **2011**, *67*, 7631-7666.

[11]   Vera, N.; Popich, S.; Luna, L. E.; Cravero, R. M.; González Sierra, M.; Bardón,

A. *Chem. Biodiversity* **2006**, *3,* 21-26.

[12]   Hiroya, K.; Takahashi, T.; Shimomae, K.; Sakamoto, T. *Chemical and*

Pharmaceutical Bulletin **2005**, *53*, 207-213.

[13]   Balasubramanian, K.; John, J. P.; Swaminathan, S. *Synthesis* **1974**, 51-53.

[14]   Bradshaw, B.; Etxebarria-Jardi, G.; Bonjoch, J.; Viozquez, S. F.; Guillena, G.;

Najera, C. *Advanced Synthesis and Catalysis,* **2009**, *351*, 2482–2490.

[15]   Noël, R.; Vanucci-Bacqué, C.; Fargeau-Bellassoued, M. C.; Lhommet, G. *J. Org.*

Chem. **2005**, *70*, 9044-9047.

13

[16]   Henry, G. E.; Raithore, S.; Zhang, Y.; Jayakaprasam, B.; Nair, M. G.; Heber, D.;

Seeram, N. P. *J. Nat. Prod.* **2006**, *69,* 1645-1648.

[17]   Rodeschini, V.; Ahmad, N.; Simpkins, N. S. *Org. Lett.* **2006**, *8*, 5283-5285.

[18]   Ohmori, N. *Chem. Commun.* **2001**, 1552-1553.

[19]   Alder, K.; Offermanns, H; Ruden, E. *Chem. Ber*. **1941**, *74B,* 905-920.

[20]   Johnson, A. W.; Markhan, E.; Price, R. *Org. Syn.* **1962**, *42*, 75.

[21]   Bennett, G. B. *Synthesis* **1977**, 589-606.

[22]   Bacigaluppo, J. A.; Colombo, M. I.; Preite, M. D.; Zinczuk, J.; Rúveda, E. A.

Pure Appl. Chem. **1996**, *68*, 683-686.

[23]   Hajos, Z. G.; Parrish, D. R. *J. Org. Chem*., **1974**, *39,* 1612-1615.

[24]   Tamura, Y.; Ishibashi, H.; Ikeda, M. *J. Org. Chem*. **1976**, *41,* 1277-1279.

[25]   Clinical and Laboratory Standards Institute. *Performance standards for

antimicrobial susceptibility testing*: Twenty-Third Informational Supplement **2013**,

M100-S23, V.*33* Nº 1. ISBN1-56238-865-7. Wayne. PA. USA

[26]   Morán-Barrio, J.; Limansky, A. S.; Viale, A. M. *Antimicrob. Agents Chemother*.
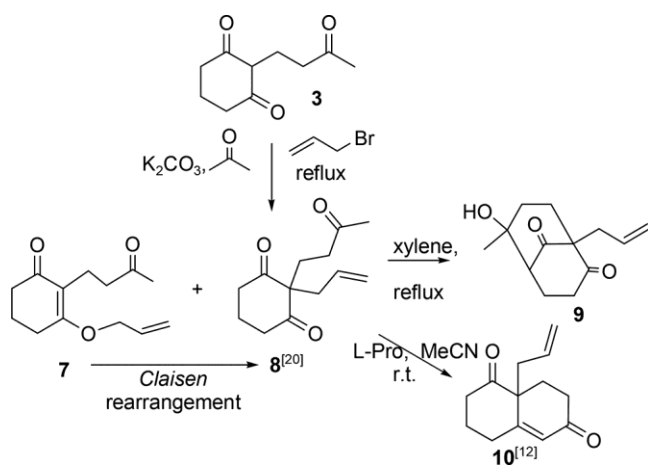
2009, *53*, 2908-2017.

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Scheme 1. Products derived from the Michael reaction of cyclohexane-1,3-dione in solution or on solid support.
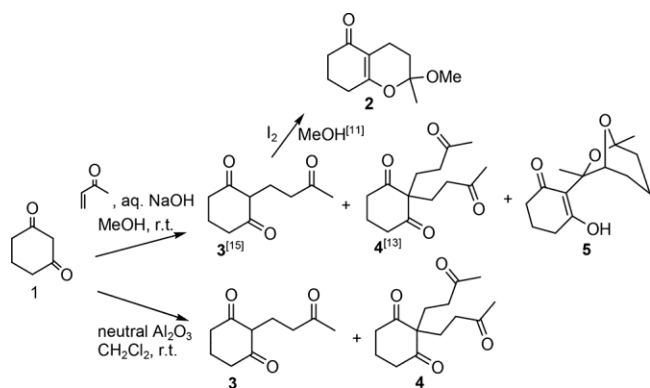
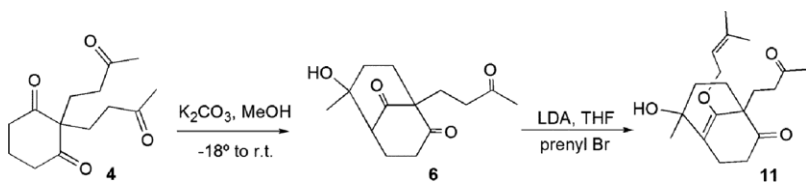Scheme 2. Sequence of chemical reactions for the formation of **5**.

A R=H
B R= CH$_3$

1

−H$_2$O

5

16
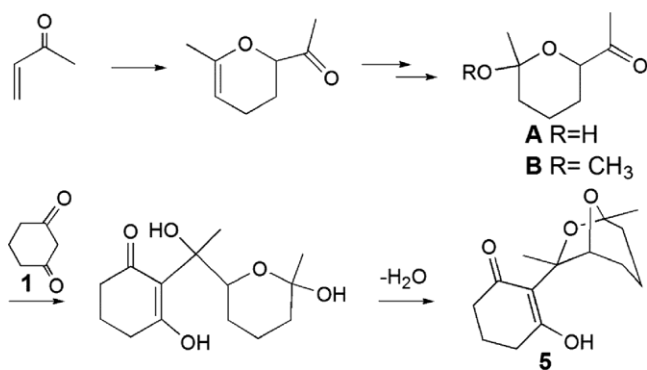
Scheme 3. Introduction of the allylic function into compound **3**

17

Scheme 4. Introduction of the prenyl function into compound **4.** Preparation of compound

11.

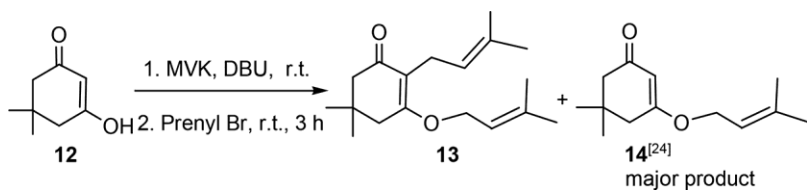Scheme 5. C- and O-alkylation of 5,5-dimethyl cyclohexane-1,3-dione with prenyl bromide.

Figure 1. Prenylated acyl phloroglucinol derivative**s** and **S**ynthetic bicyclic analogs of

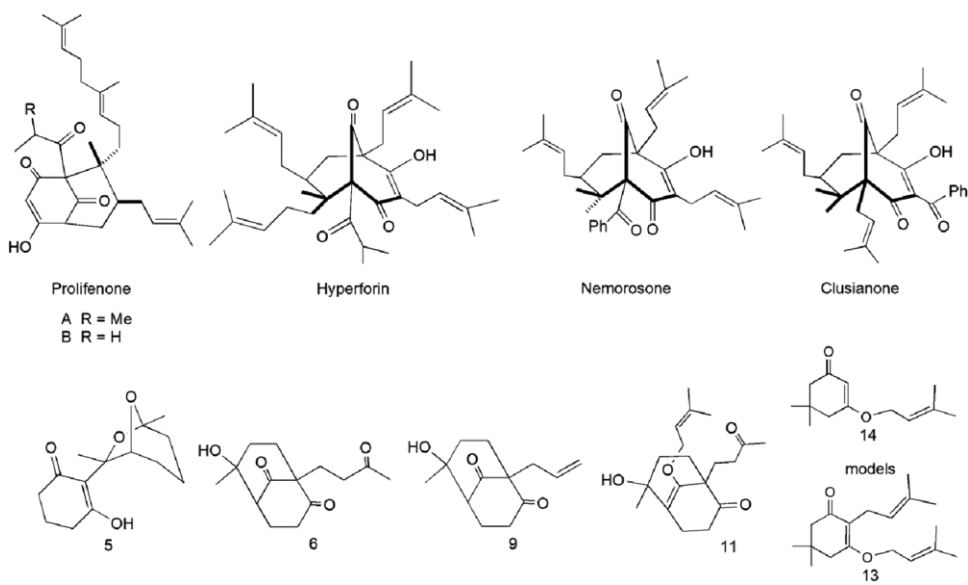[3.3.1] bridged phloroglucinol natural products from cyclohexane-1,3-diones.
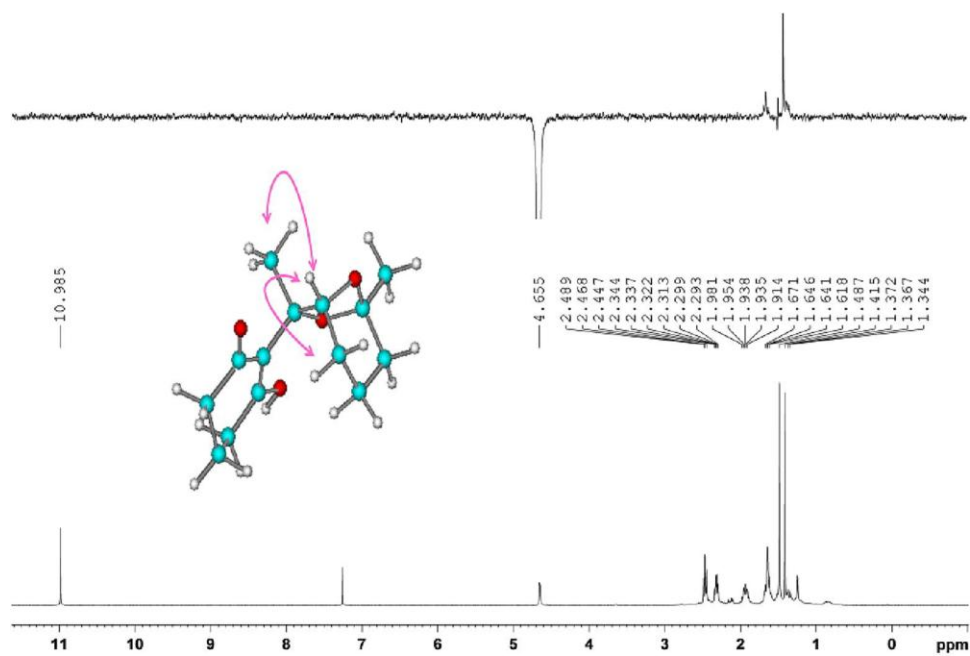
Figure 2. NOE measurement of the compound **5.**

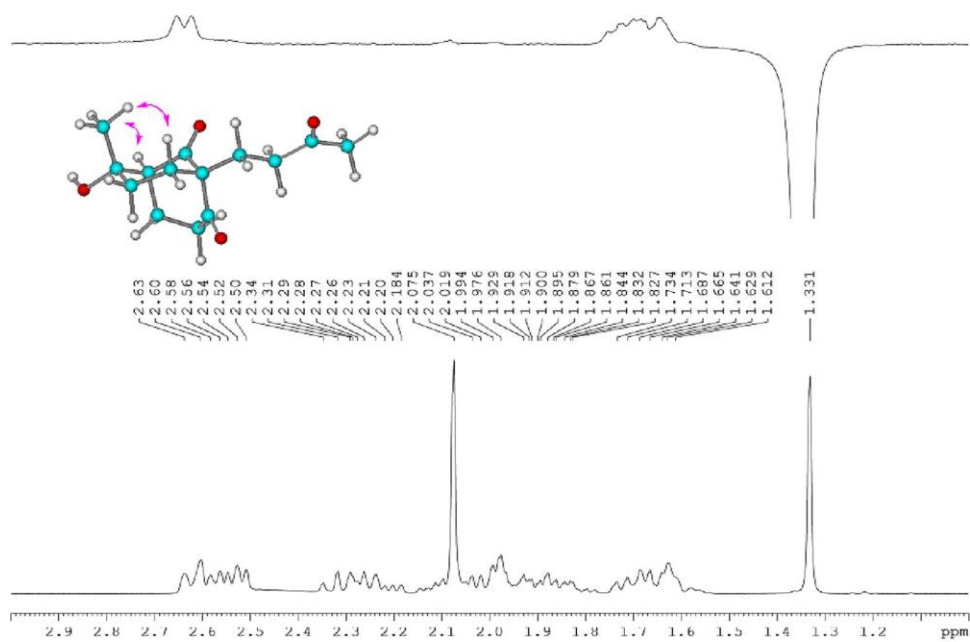Figure 3. NOE measurement of the compound **6.**

Figure 4. **A-E** Microbiological assays on Mueller-Hinton agar. Bacterial inocula of E. faecalis and E. coli cells were prepared by making direct saline suspensions of isolated colonies selected from an 18- to 24-h nutritive agar plate. After that, 10-fold serial dilutions for each microorganism were made. Next, 2 μL aliquots of E. faecalis (top line) and E. coli cells (bottom line) corresponding to dilutions 10^8 to 10^4 CFU/mL were added to the Mueller-Hinton agar without supplement (**A**) or supplemented with 1000 μg/mL of compound 5 (**B**), 1000 μg/mL of compound 9 (**C**), 1000 μg/mL of compound 6 (**D**) and 1000 μg/mL of compound 11 (**E**). Developing colonies were observed after 24 h incubation at 37ºC. In **B-E**, white circle indicates diminished growth of bacterial suspensions.