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Synthesis of a new nitrogenated drimane derivative with antifungal activity

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ABSTRACT

Drimane sesquiterpenes are highly valuable due to their strong biological activity. In this work, we report the enhanced antifungal activity of 11-guanidinodrimene, a new compound derived from drimenol. The binding of the guanidine group at the C-11 carbon increased the antifungal activity when tested against *Candida albicans*, one of the most commonly found human pathogens.

The search for better antifungal compounds with increased specificity for fungal enzymes has become an important research area in medicine.[1] Nitrogenated organic compounds containing guanidinium[2] and azole[3] groups have shown important antifungal activity. One of the targets more used in the screening of antifungal compounds is the inhibition of $P450_{14}$demethylase ($P450_{14}$DM), which catalyzes the C-14 methyl group oxidation of lanosterol **1**. The inhibition involves the blockage of the oxidative reaction catalyzed by the $P450_{14}$DM enzyme, upon the coordination of one of the nitrogen atoms to the iron carried by the porphyrin unit of the enzyme.[4]

Drimanes skeleton **2** belong to a family of natural sesquiterpenes isolated from terrestrial and marine sources with proven biological activity.[5] These compounds mimic the A and B rings of lanosterol **1**, thus the binding of functional groups at the C-11 and C-12 simulates the C ring of lanosterol interacting with the enzyme (Fig. 1).

In order to evaluate the antifungal capacity of guanidino drimenes, we have designed and synthesized the guanidino drimene **4** and tested it against cultures of *Candida albicans*, an opportunistic fungus of the intestinal tract. The steps followed during the synthesis of the new antifungal compound **4** are shown in Scheme 1.

The preparation of 11-guanidinodrimene **4** was based on the initial preparation of aminodrimene **7** from drimenol **5**. Oxidation of drimenol **5**, obtained from *Drimys winteri* bark, yielded 90% drimenal[6] **6**. The aminodrimene **7** was prepared from a mixture of oximes obtained from a condensation reaction of drimenal **6** with hydroxylamine. The in situ reduction of this mixture with $LiAlH_4$ in Et_2O generated the aminodrimene **7** with an 80% yield. The aminodrimene **7** showed two diasterotopic protons for the C-11 methylene at δ 3.0 (1H, dd, J = 13.2, 2.4 Hz) and 2.8 (1H, dd, J = 13.2, 6.5 Hz) in a 250 MHz ^{1}H NMR spectrum. In addition, a signal observed in ^{13}C NMR DEPT for this methylene carbon at δ 40.2 ppm suggests that this methylene group supports the amine functionality. The reaction of aminodrimene **7** with sodium cyanamide in ethanol/water solution produced 11-guanidinodrimene **4**. This compound showed a signal in ^{1}H NMR spectrum for the hydrogens of C-11 methylene shifted to δ 3.2 (1H, dd, J = 12.7, 3.0 Hz) and 3.0 (1H, dd, J = 12.7; 7.2 Hz) and a ^{13}C NMR DEPT signal for this methylene at 42.0 ppm, indicating that this group now supports a nitrogen atom of the guanidine group. A signal at 157.9 ppm confirms the presence of a guanidine group. This assignment is consistent with the reported NMR data for other guanidine compounds.[7]

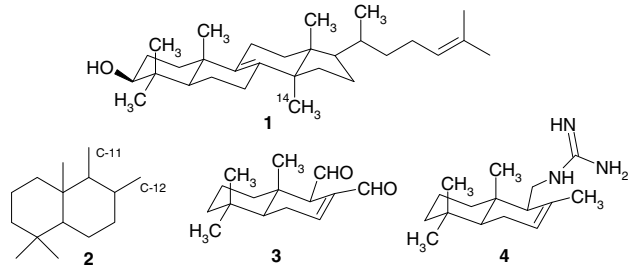
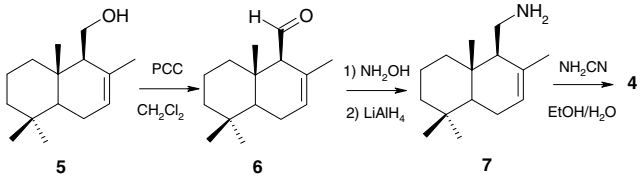


Figure 1. Structure of drimane sesquiterpene derivatives.



Scheme 1.

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The antifungal activity of 11-guanidinodrimene **4**, measured against a culture of *C. albicans*, showed that this compound is active at a minimal inhibitory concentration (MIC) of 32 μg/mL; compared to 125 μg/mL shown by the natural product **5** and 3.13 μg/mL by the natural compound polygodial **3**.[8] The increased basicity constant of the guanidine group suggests that their antifungal activity can be different from that of the triazoles. Current studies have been undertaken to compare the antifungal activity of imidazol and triazol compounds derived from drimenol with respect to 11-guanidinodrimene.

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 250 MHz spectrometer in $CDCl_3$ solution using TMS as internal standard. Carbon substitution degrees were established by DEPT pulse sequences. High-resolution mass spectra (HRMS) and fast atom bombardment mass spectra (FAB MS) were obtained with a JEOL JMS-DX 303 spectrometer. IR spectra were recorded using a JASCO FT/IR-300 spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. Silica Gel (Kieselgel 60) was employed for column chromatography.

The PCC reagent (500 mg) in 10 mL of dichloromethane was added to a solution of drimenol **5** (300 mg, 1.35 mmol) in dichloromethane (5 mL) at 0 °C and was stirred at room temperature for 20 min. The reaction mixture was diluted with 20 mL of water and extracted. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated to give a crude residue, which was flash chromatographed on silica gel with hexane to give **6** (270 mg, 90%), IR (neat): 1735 cm^{-1} (CHO). ^{1}H NMR: 0.87 (3 H, s), 0.91 (3H, s), 0.92 (3H, s), 1.55 (3H, s, br), 1.05 (9H, m), 2.30 (2H, m), 2.59 (1H, s, br), 5.7 (1H, s, br), 9.7 (1H, J = 5.1 Hz). ^{13}C NMR: 15.6 (q), 18.2 (t), 21.5 (q), 22.0 (q), 23.6 (t), 32.9 (s), 33.2 (q), 36.9 (s), 40.3 (t), 41.9 (t), 49.0 (d), 67.5 (d), 125.4 (d), 127.7 (s), 206.5 (d). Anal. Calcd for $C_{16}H_{26}O$: C, 80.4; H, 11.0. Found: C, 81.85; H, 11.20.

A solution of drimenal **6** (250 mg, 1.13 mmol) and hydroxylamine (75 mg) in ethanol (20 mL) was stirred for 5 h at room temperature. After the reaction, the mixture was quenched with H_2O (5 mL), acidified with 2.0 M aqueous HCl (1 mL), and extracted with dichloromethane (10 mL). The organic solution was then basified with $NaHCO_3$ 0.1 M (5 mL) and washed with brine and dried over $MgSO_4$. The solvent was evaporated and the residue chromatographed on silica gel (n-hexane/AcOEt = 4:1) to give a 7:3 mixture of oximes (245 mg, 98%), as a colorless oil, IR (neat): 1637 cm^{-1} (C=N). ^{1}H NMR: 0.83 (3H, s), 0.95 (3H, s), 0.98 (3H, s), 1.55 (3H, s, br), 1.03 (9H, m), 2.30 (2H, m), 2.6 (1H, d, 9.8 Hz), 5.6 (1H, s, br), 7.3 (1H, J = 9.8 Hz). ^{13}C NMR: 15.0 (q), 18.4 (t), 21.9 (q), 22.1 (q), 23.6 (t), 33.0 (s), 33.1 (q), 36.1 (s), 40.1 (t), 42.2 (t), 49.3 (t), 55.8 (d), 123.6 (d), 130.5 (s), 153.2 (d).

$LiAlH_4$ (152 mg, 4 mmol) in THF (20 mL) was added to the mixture of oximes (200 mg, 0.89 mmol) in anhydrous THF (20 mL) at 0 °C and this was stirred overnight at room temperature. The reaction mixture was quenched with H_2O (5 mL), acidified with 2.0 M aqueous HCl (2 mL), and extracted with ether. The organic solution was then basified with $NaHCO_3$ 0.1 M (10 mL) and extracted with ether. The ether layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic layer gave a crude residue, which was chromatographed on silica gel (n-hexane/AcOEt = 4:1) to give **7**, as an oil (160 mg, 80%) $[\alpha]_D^{24}$ +6.98 (c 0.50, $CHCl_3$). ^{1}H NMR: 0.78 (3H, s), 0.84 (3H, s), 0.87 (3H, s), 1.76 (3H, s), 1.11–2.45 (12H, m), 2.7 (1H, dd, J = 6.5, 13.2 Hz), 2.9 (1H, d, J = 2.4, 13.2 Hz), 5.5 (1H, br). ^{13}C NMR: 14.0 (q), 18.7 (t), 21.9 (q), 21.8 (q), 23.5 (t), 23.7 (s), 39.2 (t), 33.0 (q), 42.0 (s), 40.1 (t), 41.9 (t), 49.5 (d), 53.8 (d), 124.1 (d), 134.3 (s). Anal. Calcd for $C_{15}H_{26}N$: C, 81.8; H, 11.8; N, 6.4. Found: C, 80.1; H, 11.3; N, 6.1.

Amine **7** (100 mg, 0.45 mmol) was dissolved in a 5:1 alcohol–water mixture and neutralized slowly with acetic acid 1.0 M. A solution prepared with 0.1 g sodium cyanamide dissolved in 2 mL of water was added dropwise to this mixture; the resulting solution was allowed to react for 24 h. The product was extracted with ethyl ether from a basic medium and the extract was concentrated and subjected to purification by column chromatography. The compound obtained is a colorless oil (322 mg, 81%): $[\alpha]_D^{24}$ +26.5 (c 0.50, $CHCl_3$). ^{1}H NMR: 0.86 (3H, s), 0.89 (3H, s), 0.92 (3H, s), 1.68 (3H, br s), 1.03–2.0 (9H, m), 2.30–2.50 (2H, m), δ 3.2 (1H, dd, J = 12.7, 3.0 Hz) and 3.0 (1H, dd, J = 12.7, 7.2 Hz), 5.47 (1H, br s). ^{13}C NMR: 14.0 (q), 18.7 (t), 21.9 (q), 21.8 (q), 23.5 (t), 32.8 (s), 33.1 (t), 36.1 (q), 39.3 (s), 40.0 (t), 41.9 (t), 49.4 (d), 53.8 (d), 124.0 (d), 132.5 (s), 157.9 (s). EI MS m/z: 263.2326 (M+).

The minimal inhibitory concentration (MIC) of compounds **4** and **5** was determined by using broth microdilution techniques according to the guidelines of the National Committee for Clinical Laboratory Standards for yeasts (M27-A2) and for filamentous fungi (M38-A).[9] The starting inocula were 1×10^5–5×10^5 CFU/mL. Microliter trays were incubated at 35 °C for yeast and MICs were visually recorded at 48 h. The MIC was defined as the minimum inhibitory concentration of the pure compound required for total inhibition.

Acknowledgments

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References and notes

1. Fridkin, S. K.; Gaynes, R. P. *Clin. Chest Med.* **1999**, *20*, 203.
2. Jana, G. H.; Jain, S.; Arora, S. K.; Sinha, N. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3592–3595.
3. (a) Block, J. H.; Belae, M. J., Jr. *Organic Medicinal and Pharmaceutical Chemistry*, 11th ed.; Lippincott Williams-Wilkins, 2004. pp 240–245; (b) Hlasta, D. J. et al. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1545–1548; (c) Tanitame, A. et al *Bioorg. Med. Chem. Lett.* **2004**, *12*, 5515–5524.
4. (a) Port, A. et al *Arch. Int. Med.* **1984**, *144*, 2150; (b) Vanden Bosssche, H. *Drug Dev. Res.* **1986**, *8*, 287.
5. Jansen, B. J. M.; Groot, Ae. *Nat. Prod. Rep.* **2004**, *21*, 449–477.
6. (a) Cortés, M. J.; Razmilic, I.; Sierra, J. R.; Lopéz, J. *Chem. Ind.* **1985**, *21*, 735; (b) Razmilic, I.; Lopéz, J.; Sierra, J. *Synth. Commun.* **1987**, *17*, 95–103.
7. Lavorenti, A. Doctoral Thesis, The Polytechnic of North London, 1988.
8. (a) Kubo, I.; Fujira, K.; Lee, S. H. *J. Agric. Food. Chem.* **2001**, *49*, 1607–1611; (b) Lee, S. H.; Lee, J. R.; Lunde, C. S.; Kubo, I. *Planta Med.* **1999**, *65*, 204–208.
9. Taniguchi, M.; Chapya, A.; Kubo, I.; Nakanishi, K. *Chem. Pharm. Bull.* **1978**, *26*, 1913–2910.