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Synthesis and Quantitative Structure−Antifungal Activity Relationships of Clovane Derivatives against *Botrytis cinerea*†

LIANE SAIZ-URRA,§ JUAN C. RACERO,# ANTONIO J. MACÍAS-SÁNCHEZ,# ROSARIO HERNÁNDEZ-GALÁN,# JAMES R. HANSON,⊥ MAYKEL PEREZ-GONZALEZ,§ AND ISIDRO G. COLLADO*,#

Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, Apartado 40, 11510 Puerto Real, Cádiz, Spain; Department of Chemistry, University of Sussex, Brighton, Sussex BN1 9QJ, United Kingdom; and Chemical Bioactive Center, Central University of Las Villas, Santa Clara, Villa Clara, C.P. 54830, Cuba

Twenty-three clovane derivatives, nine described here for the first time, bearing substituents on carbon C-2, have been synthesized and evaluated for their in vitro antifungal activity against the phytopathogenic fungus *Botrytis cinerea*. The results showed that compounds **9**, **14**, **16**, and **18** bearing nitrogen atoms in the chain attached at C-2 displayed potent antifungal activity, whereas mercapto derivatives **13**, **19**, and **22** displayed low activity. The antifungal activity showed a clear structure−activity relationship (SAR) trend, which confirmed the importance of the nature of the C-2 chain on the antifungal activity. On the basis of these observations, the metabolism of compounds **8** and **14** by the fungus *B. cinerea*, and the metabolism of other clovanes by this fungus, described previously, a pro-drug action mechanism for 2-alkoxyclovane compounds is proposed. Quantitative structure−activity relationship (QSAR) studies were performed to rationalize the results and to suggest further optimization, using a topological sub-structural molecular design (TOPS-MODE) approach. The model displayed good fit and predictive capability, describing 85.5% of the experimental variance, with a standard deviation of 9.502 and yielding high values of cross-validation determination coefficients ($q^2_{CV\text{-}LOO} = 0.784$ and $q^2_{boot} = 0.673$). The most significant variables were the spectral moments weighted by bond dipole moment (Dip), hydrophobicity (Hyd), and the combined dipolarity/polarizability Abraham molecular descriptor (Ab-π_2^H).

KEYWORDS: ***Botrytis cinerea*; sesquiterpenoid; clovane; synthesis; antifungal activity; QSAR; spectral moments; crop-protection agents**

INTRODUCTION

Botrytis cinerea is a plant pathogen that causes serious economic losses. *B. cinerea* has been identified as a pathogen of more than 235 plant species including grapes, lettuce, tomatoes, tobacco, and strawberries, producing a gray powdery mold on the infected crops. Other relatives such as *Botrytis allii*, *Botrytis byssoides* and *Botrytis squamosa* are pathogens on onions, the latter causing stalk breakage. *Botrytis fabae* is a pathogen on beans, causing lesions on the leaves, *Botrytis gladioli* is a pathogen of gladioli and lilies, and *Botrytis tulipae* affects tulips and saffron (*1*).

Several strains have developed resistance to some commercial fungicides (*2*−*4*). There is a considerable need to develop fungicides with a novel mode of action to combat resistant strains of organisms and with specific rather than general antifungal activity. A further aim is to produce novel fungicides that do not impede the role of beneficial organisms in plant development and which do not persist in the environment and food chain.

There is evidence that the production of characteristic secondary metabolites during the idiophase of fungal growth has a limiting effect on fungal growth (*6*). Botrydial, one of the most important chemical weapons used by *B. cinerea* during plant infection (*5*), has this effect on the growth of *B. cinerea* (*6*−*8*). Furthermore, the addition of the analogue metabolites to botrydial, during the earlier growth phase of the organism, may have the consequence of restricting its development. Another approach to the design of rational fungicides is

* Corresponding author (telephone 34 956 016371; fax 34 956 016193; e-mail isidro.gonzalez@uca.es).

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§ Central University of Las Villas.
# Universidad de Cádiz.
⊥ University of Sussex.

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to block the biosynthesis of botrydial to prevent the fungus from exerting its pathogenicity (*9*).

This method of fungal control has been explored by our research group in the development of novel antifungal agents. The effect of analogues of biosynthetic intermediates on the formation of botrydial has recently been reviewed (*9*).

A series of clovane derivatives were prepared and incubated with *B. cinerea* (*10*−*12*). The effectiveness of the inhibition of the growth of the fungus was evaluated and found to be of the order of 83% for 2*β*-methoxyclovan-9α-ol, at a concentration of 200 ppm, after 6 days (*12*).

The existence of a microbial detoxification pathway for these fungistatic agents suggests that they might not persist in the environment for a prolonged period (*12*). An increase in the chain length of the ether at C-2, and the presence of certain functional groups within it, increased the effectiveness of the inhibitors (*13*).

To rationalize the results and to suggest further optimization on the activity clovanes, a QSAR study has been carried out. In this paper we report the synthesis of 9 new clovanol derivatives and the QSAR study on 23 2-alkyloxyclovan-9-ol derivatives designed to get more active clovane derivatives as efficient, selective, and rational fungicides.

MATERIALS AND METHODS

General Experimental Procedures. Melting points were determined with a Reichert-Jung Thermovar hot-stage microscope. ^{1}H (400 MHz) NMR spectra were recorded on a Varian Inova 400 spectrometer, and ^{13}C (50 MHz) NMR spectra were recorded on a Varian Gemini 200 spectrometer. Chemical shifts are reported in parts per million from TMS (δ scale), but were measured against the solvent signal ($CDCl_3$: $\delta_H = 7.24$; $\delta_C = 77.0$). Electron impact (EI) mass spectra were obtained at 70 eV on a Hewlett-Packard 5973 MSD spectrometer. FAB and HRMS were recorded at VG AutoSpec (Micromass Instrument).

Preparation of Haloethoxyclovanols. Compounds **11** and **12** were prepared by solvolysis of caryophyllene oxide, either in bromoethanol or iodoethanol, respectively, and catalyzed by tetracyanoethylene (TCNE), according to a previously described procedure (*10*).

*2β-(2-Bromoethoxy)clovan-9α-ol (**11**):* yield 43%; oil; $[\alpha]^{25}_D$ +12 (*c* 4 $CHCl_3$); IR (neat, KBr) ν_{max} 3424 (OH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.81 (s, 3H, H-13α), 0.92 (s, 3H, H-15), 0.96 (m, 1H, H-12a), 0.99 (s, 3H, H-14*β*), 1.09 (m, 1H, H-11*β*), 1.10 (m, 1H, H-7a,), 1.4−1.2 (4H, H-5*β*, H-6a, H-6b, H-7b), 1.46 (m, 1H, H-3a), 1.6−1.52 (2H, H-12b, H-10α), 1.74−1.61 (2H, H-3b, H-11α), 1.92 (m, 1H, H-10*β*), 3.27 (bs, 1H, H-9*β*), 3.37−3.43 (3H, H-2′a, H-2′b, H-2α), 3.67−3.77 (2H, H-1′a, H-1′b); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.5 (C-6), 25.3 (C-13), 26.0 (C-10), 26.7 (C-11), 28.3 (C-15), 30.9 (C-2′), 31.2 (C-14), 33.0 (C-7), 34.6 (C-8*), 36.2 (C-12), 37.1 (C-4*), 44.5 (2C, C-3, C-1*), 50.3 (C-5), 70.3 (C-1′), 75.0 (C-9), 89.0 (C-2) (*, interchangeable signals); EIMS *m*/*z* (%) 346 (12) $[M + 2]^+$, 344 (12) $[M]^+$ 331 (20), 329 (20), 272 (19), 270 (19), 220 (80), 193 (99), 191 (100); HREIMS (*m*/*z*) $[M]^+$ calcd for $C_{17}H_{29}O_2Br$, 344.1351; found, 344.1341.

*2β-(2-Iodoethoxy)clovan-9α-ol (**12**):* yield 62%; oil; $[\alpha]^{25}_D$ +12 (*c* 2 $CHCl_3$); IR (neat, KBr) ν_{max} 3432 (OH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.76 (s, 3H, H-13α), 0.87 (s, 3H, H-15), 0.94 (s, 3H, H-14*β*), 1.32−1.15 (5H, H-11*β*, H-7a, H-6a, H-6b, H-7b), 1.43 (dd, 1H, *J* = 9.9, 2 Hz; H-5*β*), 1.49 (d, 1H, *J* = 12.6 Hz; H-12a), 1.67−1.42 (4H, H-10α, H-11α, H-3α, H-3*β*), 1.89 (m, 1H, H-10*β*), 3.18−3.11 (2H, H-2′a, H-2′b), 3.21 (bs, 1H, H-9*β*), 3.36 (dd, 1H, *J* = 9.8, 5.4 Hz; H-2α), 3.66−3.57 (2H, H-1′a, H-1′b); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 3.9 (C-2′), 20.4 (C-6), 25.2 (C-13), 25.8 (C-10), 26.6 (C-11), 28.3 (C-15), 31.2 (C-14), 32.9 (C-7), 34.4 (C-8*), 36.0 (C-12), 37.0 (C-4*), 44.2 (C-1*), 44.3 (C-3), 50.1 (C-5), 70.8 (C-1′), 74.7 (C-9), 88.6 (C-2) (*, interchangeable signals); EIMS *m*/*z* (%) 392 (13) $[M]^+$, 377 (36), 336 (12), 318 (14), 305 (10), 239 (81), 220 (74), 163 (79), 155 (100); HREIMS (*m*/*z*) $[M]^+$ calcd for $C_{17}H_{29}O_2I$, 392.1213; found, 392.1220.

*2β-(2-(1,3-Dioxoisoindolin-2-yl)ethoxy)clovan-9α-ol (**23**).* Compound **11** (100 mg) was dissolved in dry DMF (5 mL) and stirred under N_2 atmosphere. Potassium phthalamide (66 mg) was added, the reaction mixture was stirred for 16 h, and the progress of the reaction was monitored by TLC. The resulting reaction mixture was diluted with ethyl acetate (50 mL), washed with brine (2 × 50 mL), and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded a crude reaction product, which was purified by column chromatography on silica gel, with increasing gradients of ethyl acetate on petroleum ether, to give compound **23** (69 mg) (58%): oil; $[\alpha]^{25}_D$ +11 (*c* 2.1 $CHCl_3$); IR (neat, KBr) ν_{max} 3354 (OH), 1778, 1735, 1368 (phthalimide) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.79 (s, 3H, H-13α), 0.82(m, 1H, H-12a), 0.85 (s, 3H, H-15), 0.95 (s, 3H, H-14*β*), 0.98−1.05 (3H, H-11a, H-6a, H-7a) 1.25−1.36 (3H, H-6b, H-7b, H-5*β*), 1.35−1.59 (4H, H-11b, H-12b, H-3a, H-10α), 1.60 (m, 1H, H-3b) 1.90 (m, 1H, H-10*β*), 3.22 (s, 1H, H-9*β*), 3.34 (dd, 1H, *J*= 5.6, 4.2 Hz; H-2α), 3.63 (m, 1H, H- 2′a), 3.73 (m, 1H, H-2′b), 3.94−3.81 (2H, H-1′a, H-1′b), 7.70 (dd, 2H, *J*= 5.42, 2.2 Hz; H-6′, H-7′), 7.85 (dd, 2H, *J*= 5.4, 2.4 Hz; H-5′, H-8′); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.5 (C-6), 25.3 (C-13), 26.0 (C-10), 26.5 (C-11), 28.3 (C-15), 31.2 (C-14), 33.0 (C-7), 34.6 (C-8*), 36.1 (C-12), 37.3 (C-4*), 38.2 (C-2′), 44.2 (C-3), 44.4 (C-1*), 50.3 (C-5), 66.9 (C-1′), 75.1 (C-9), 89.1 (C-2), 123.2 (2C, C-6′, C-7′), 132.1 (2C, C-4′, C-9′), 133.9 (2C, C-5′, C-8′), 168.4 (2C, C-3′, C-10′) (*, interchangeable signals); EIMS *m*/*z* (%) 411 (1) $[M]^+$, 393 (3), 310 (2), 237 (5), 220 (11), 192 (74), 174 (100); HREIMS (*m*/*z*) $[M + Na]^+$ calcd for $C_{25}H_{33}O_4N$ + Na, 434.2307; found, 434.2309.

*2β-(2-Aminoethoxy)clovan-9α-ol (**9**).* A solution of compound **23** (48 mg), dissolved in methanol (10 mL), was treated with hydrazine (5 mg), and the reaction was magnetically stirred for 30 min. Then, solvent was evaporated under reduced pressure, and the resulting reaction crude was purified by column chromatography on silica gel, with increasing gradients of methanol on ethyl acetate, to give 2*β*-(2′-aminoethoxy)clovan-9α-ol (**9**) (21 mg) (65%): oil; $[\alpha]^{25}_D$ +6 (*c* 0.7 $CHCl_3$); IR (neat, KBr) ν_{max} 3467 (OH, NH_2) cm^{-1}; ^{1}H NMR (CD_3OD, 200 MHz) δ 0.75 (s, 3H, H-13α), 0.80 (s, 3H, H-15), 0.91 (s, 3H, H-14*β*), 0.86−0.92 (2H, H-11a, H-12a), 0.99 (m,1H, H-11b), 1.31−1.17 (5H, H-6a, H-7a, H-6b, H-7b, H-5*β*), 1.40−1.55 (3H, H-3a, H-10α, H-12b), 1.7−1.59 (2H, H-3b, H-11b), 1.83 (m, 1H, H-10*β*), 2.66−2.59 (2H, H-2′a, H-2′b), 3.11 (bs, 1H, H-9*β*), 3.41−3.29 (3H, H-2α, H-1′a, H-1′b); ^{13}C NMR (CD_3OD, 50 MHz) δ 21.7 (C-6), 25.7 (C-13), 26.9 (C-10), 28.1 (C-11), 29.1 (C-15), 31.8 (C-14), 34.4 (C-7), 35.8 (C-8*), 37.5 (C-12), 38.1 (C-4*), 42.6 (C-3), 45.6 (C-2′), 45.7 (C-1*), 52.1 (C-5), 72.6 (C-1′), 79.9 (C-9), 90.1 (C-2) (*, interchangeable signals); EIMS *m*/*z* (%) 282 (6) $[M + 1]^+$, 237 (15), 236 (14), 221 (9), 218 (10), 203 (48), 161 (62), 147 (62), 133 (69), 105 (100); HREIMS (*m*/*z*) $[M + 1]^+$ calcd for $C_{17}H_{32}O_2N$, 282.2434; found, 282.2400.

*2β-(2-Carbamimidoylthioethoxy)clovan-9α-ol (**18**).* Compound **11** (101 mg), dissolved in MeOH (10 mL), was treated with thiourea (247 mg), and the reaction was left heating under reflux for 24 h. Then, solvent was evaporated under reduced pressure, and the resulting crude reaction mixture was purified by column chromatography on silica gel, with increasing gradients of methanol on ethyl acetate, to give compound **18** (75 mg) (76%): oil; $[\alpha]^{25}_D$ +11 (*c* 2.1 $CHCl_3$); IR (neat, KBr) ν_{max} 3476 (OH, NH_2), 1612, 1589, (RSC═NHNH$_2$) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.81 (s, 3H, H-13α), 0.95 (s, 3H, H-15), 1.01 (s, 3H, H-14*β*), 1.07 (m, 1H, H-12a), 1.16 (m, 1H, H-11a), 1.23−1.35 (2H, H-6a, H-7a), 1.37−1.44 (3H, H-6b, H-7b, H-5*β*), 1.51−1.63 (3H, H-3a, H-3b, H-10α), 1.67 (m, 1H, H-12), 1.69 (m, 1H, H-11b), 1.90 (m, 1H, H-10*β*), 3.24−3.20 (2H, H-2′a, H-2′b), 3.22 (bs, 1H, H-9*β*), 3.42 (dd, 1H, *J* = 5.6, 9.2 Hz; H-2α), 3.68−3.64 (2H, H-1′a, H-1′b); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.3 (C-6), 25.1 (C-13), 25.4 (C-10), 27.0 (C-11), 28.5 (C-15), 30.9 (C-14), 32.7 (C-2′), 33.3 (C-7), 33.6 (C-8*), 36.4 (C-12), 37.4 (C-4*), 44.1 (C-3), 44.4 (C-1*), 50.6 (C-5), 70.5 (C-1′), 74.9 (C-9), 90.1 (C-2), 213.1 (C-3′) (*, interchangeable signals); FABMS (thioglycerol matrix) *m*/*z* (%) 363 (20) $[M + Na]^+$ 341 (100) $[M + 1]^+$, 203 (8); HRFABMS (*m*/*z*) $[M + 1]^+$ calcd for $C_{18}H_{33}O_2N_2S$, 341.2257; found, 341.2263.

*2β-(2-Mercaptoethoxy)clovan-9α-ol (**13**).* Compound (**18**) (230 mg), dissolved in methanol (20 mL), was treated with diisopropylamine (76 mg) and heated under reflux for 3 h. Then solvent was evaporated under reduced pressure, and the resulting crude reaction mixture was purified

by column chromatography on silica gel, with increasing gradients of ethyl acetate on petroleum ether, to give compound **13** (125 mg) (62%): oil; $[\alpha]^{25}_D$ +4 (*c* 2.1 $CHCl_3$); IR (neat, KBr) ν_{max} 3398 (OH) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.81 (s, 3H, H-13α), 0.92 (s, 3H, H-15), 0.99 (s, 3H, H-14β), 0.95 (m, 1H, H-12a), 1.10 (m, 1H, H-11a), 1.17−1.31 (5H, H-6a, H-7a, H-6b, H-7b, H-5β), 1.40−1.55 (3H, H-3a, H-10α, H-12b), 1.6−1.7 (2H, H-3b, H-11b), 1.92 (m, 1H, H-10β), 2.65−2.59 (2H, H-2′a, H-2′b), 3.27 (bs, 1H, H-9β), 3.39 (dd, 1H, *J* = 5.6, 4.2 Hz, H-2α), 3.62−3.50 (2H, H-1′a, H-1′b); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.6 (C-6), 24.9 (C-2′), 25.4 (C-13), 26.0 (C-10), 26.8 (C-11), 28.3 (C-15), 31.3 (C-14), 33.1 (C-7), 34.6 (C-8*), 36.3 (C-12), 37.1 (C-4*), 44.3 (C-1*), 44.4 (C-3), 50.4 (C-5), 71.9 (C-1′), 75.0 (C-9), 88.6 (C-2) (*, interchangeable signals); EIMS *m*/*z* (%) 298 (4) $[M]^+$, 280 (20), 220 (49), 204 (74), 203 (100); HRFABMS (thioglycerol matrix) (*m*/*z*) $[M + Na]^+$ calcd for $C_{17}H_{30}O_2SNa$, 321.1864; found, 321.1862.

*2β-(2-(Imidazol-1-yl)ethoxy)clovan-9α-ol (**16**).* Sodium imidazole (195 mg) was added to a solution of compound **11** (330 mg) in dry dimethylformamide (10 mL), and the reaction mixture was left stirring under nitrogen atmosphere for 48 h. The resulting reaction mixture was diluted with ethyl acetate (50 mL), washed with brine (2 × 50 mL), and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded a crude reaction product, which was purified by column chromatography on silica gel, with increasing gradients of methanol on ethyl acetate, to give compound **16** (220 mg) (70%); oil; $[\alpha]^{25}_D$ +13 (*c* 5.4 $CHCl_3$); IR (neat, KBr) ν_{max} 3239 (OH) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.75 (s, 3H, H-13α), 0.82 (m, 1H, H-12a), 0.89 (s, 3H, H-15), 0.92 (s, 3H, H-14β), 1.1−0.95 (2H, H-7a, H-11a), 1.1−1.25 (5H, H-3a, H-5β, H-6a, H-6b, H-7a), 1.25−1.6 (4H, H-3b, H-10a, H-11b, H-12b), 1.88 (m, 1H, H-10b), 3.25 (bs, 1H, H-9β), 3.29 (dd, 1H, *J* = 5.6, 9.6 Hz; H-2α), 3.63−3.58 (2H, H-2′a, H-2′b), 4.02−3.97 (2H, H-1′a, H-1′b), 6.92 (bs, 1H, H-3′), 6.95 (bs, 1H, H-4′), 7.47 (bs, 1H, H-5′); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.5 (C-6), 25.2 (C-13), 26.0 (C-10), 26.7 (C-11), 28.3 (C-15), 31.1 (C-14), 32.9 (C-7), 34.5 (C-8*), 36.2 (C-12), 37.1 (C-4*), 44.0 (C-3), 44.4 (C-1*), 47.4 (C-2′), 50.3 (C-5), 69.4 (C-1′), 74.6 (C-9), 89.1 (C-2), 119.4 (C-4′), 128.71 (C-5′), 137.48 (C-3′) (*, interchangeable signals); EIMS *m*/*z* (%) 333 (97) $[M + H]^+$, 275 (96), 203 (10), 164 (5), 149 (5), 112 (60), 96 (95), 82 (100); HREIMS (*m*/*z*) $[M + 1]^+$ calcd for $C_{20}H_{33}O_2N_2$, 333.2542; found, 333.2533.

*2β-(2-(1,2,4-Triazol-1-yl)ethoxy)clovan-9α-ol (**20**).* Sodium triazole (60 mg) was added to a solution of compound **11** (200 mg) in dry dimethylformamide (10 mL), and the reaction mixture was left stirring under nitrogen atmosphere for 30 h. The resulting reaction mixture was diluted with ethyl acetate (50 mL), washed with brine (2 × 50 mL), and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded a crude reaction product, which was purified by column chromatography on silica gel, with increasing gradients of methanol on ethyl acetate, to give compound **20** (160 mg) (75%): oil; $[\alpha]^{25}_D$ +12 (*c* 4 $CHCl_3$); IR (neat, KBr) ν_{max} 3422 (OH) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.73 (s, 3H, H-13α), 0.80 (s, 3H, H-15), 0.82 (m, 1H, H-12a), 0.86 (s, 3H, H-14β), 0.95−1.65 (11H, H-3a, H-3b, H-5β, H-6a, H-6b, H-7a, H-7b, H-10a, H-11a, H-11b, H-12b), 1.85 (m, 1H, H-10b), 3.10 (bs, 1H, H-9β), 3.20 (dd, 1H, *J* = 5.6, 9.6 Hz; H-2α), 3.71−3.66 (2H, H-2′a, H-2′b), 4.30−4.24 (2H, H-1′a, H-1′b), 7.86 (s, 1H, H-4′), 8.34 (s, 1H, H-3′); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 21.8 (C-6), 25.8 (C-13), 27.1 (C-10), 28.1 (C-11), 29.1 (C-15), 31.8 (C-14), 34.4 (C-7), 35.7 (C-8*), 37.3 (C-12), 38.3 (C-4*), 45.2 (C-3), 46.0 (C-1*), 51.4 (C-2′), 51.9 (C-5), 68.7 (C-1′), 75.7 (C-9), 90.4 (C-2), 145.7 (C-4′), 151.9 (C-3′) (*, interchangeable signals); EIMS *m*/*z* (%) 334 (18) $[M + 1]^+$, 316 (5), 276 (24), 237 (5), 203 (7), 180 (4), 163 (7), 114 (25), 97 (100); HREIMS (*m*/*z*) $[M + 1]^+$ calcd for $C_{19}H_{32}O_2N_3$, 334.2495; found, 334.2496.

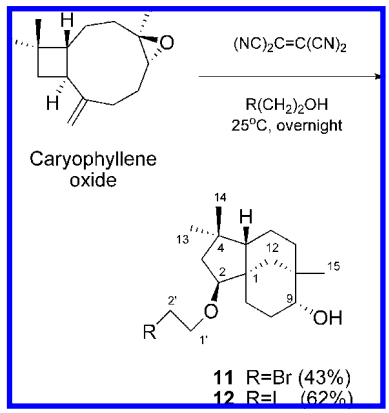
*2β-(2-(4-Nitrophenoxy)ethoxy)clovan-9α-ol (**17**).* *p*-Nitrophenol (278 mg) and NaH (49 mg) were added to a solution of compound **11** (135 mg) in dry DMF (5 mL) and left stirring under N_2 atmosphere for 24 h. The resulting reaction mixture was diluted with ethyl acetate (50 mL), washed with brine (2 × 50 mL), and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded a crude reaction product, which was purified by column chromatography on silica gel, with increasing gradients of ethyl acetate on petroleum ether, to give compound **17** (148 mg) (94%): oil; $[\alpha]^{25}_D$ −4 (*c* 6.4 $CHCl_3$); IR (neat, KBr) ν_{max} 3456 (OH), 1601, 1598, 1431 (C═C aromatic, NO_2) 1369 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.82 (s, 3H, H-13α), 0.91 (s, 3H, H-15), 0.98 (s, 3H, H-14β), 0.96 (d, 1H, *J* = 12.6 Hz, H-12a), 1.08 (m, 1H, H-11β), 1.30−1.22 (3H, H-6a, H-7a, H-11a), 1.38−1.31 (3H, H-6b, H-7b, H-5β), 1.54−1.50 (3H, H-3a, H-10α, H-3b), 1.56 (d, 1H, *J* = 12.6 Hz, H-12b), 1.66 (m, 1H, H-11b), 1.93 (m, 1H, H-10β), 3.28 (bs, 1H, H-9β), 3.51 (dd, 1H, *J* = 5.6, 4.4 Hz, H-2α), 3.84−3.78 (2H, H-2′a, H-2′b), 4.18−4.14 (2H, H-1′a, H-1′b), 6.95 (d, 2H, *J* = 9.4 Hz, H-4′, H-8′), 8.15 (d, 2H, *J* = 9.4 Hz, H-5′, H-7′); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.5 (C-6), 25.3 (C-13), 25.9 (C-10), 26.6 (C-11), 28.3 (C-15), 31.2 (C-14), 32.9 (C-7), 34.6 (C-8*), 36.4 (C-12), 37.0 (C-4*), 44.3 (C-1*), 44.4 (C-3), 50.4 (C-5), 68.4 (C-1′), 68.5 (C-2′) 75.0 (C-9), 89.2 (C-2′), 114.6 (2C, C-4′, C-8′), 125.7 (2C, C-5′, C-7′) 140.2 (C-3′†), 164.0 (C-6′†) (*, †, interchangeable signals); EIMS *m*/*z* (%) 404 (16) $[M + 1]^+$, 358$[M + 1 - 46]^+$ (50), 266 (11), 262 (15), 220 (10), 190 (60), 176 (100); HREIMS (*m*/*z*) $[M + 1]^+$ calcd for $C_{23}H_{34}NO_5$, 404.2438; found, 404.2441.

*2β-(2-(Phenylthio)ethoxy)clovan-9α-ol (**19**).* Thiophenol (200 mg) and NaH (47 mg) were added to a solution of compound **11** (129 mg) in dry DMF (5 mL) and left stirring under N_2 atmosphere for 48 h. The resulting reaction mixture was diluted with ethyl acetate (50 mL), washed with brine (2 × 50 mL), and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded a crude reaction product, which was purified by column chromatography on silica gel, with increasing gradients of ethyl acetate on petroleum ether, to give compound **19** (128 mg) (92%): oil; $[\alpha]^{25}_D$ −3 (*c* 3.5 $CHCl_3$); IR (neat, KBr) ν_{max} 3399 (OH), 1523, 1442 (C═C aromatic); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.82 (s, 3H, H-13α), 0.93 (s, 3H, H-15), 1.00 (s, 3H, H-14β), 0.95 (m, 1H, H-12a), 1.2−1.4 (6H, H-5β, H-6a, H-7a, H-6b, H-7b, H-11a), 1.40−1.55 (3H, H-3a, H-10α, H-12b), 1.55−1.7 (2H, H-3b, H-11b), 1.94 (m, 1H, H-10β), 3.10−3.06 (2H, H-2′a, H-2′b), 3.27 (bs, 1H, H-9β), 3.39 (dd, 1H, *J* = 5.6, 4.5 Hz; H-2α), 3.65−3.61 (2H, H-1′a, H-1′b), 7.15 (t, 1H, *J* = 7.0 Hz; H-6′), 7.26 (t, 2H, *J* = 7.0 Hz; H-5′, H-7′), 7.35 (d, 2H, *J* = 7.0 Hz; H-4′, H-8′); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.8 (C-6), 25.4 (C-13), 26.3 (C-10), 27.0 (C-11), 28.6 (C-15), 31.5 (C-14), 33.3 (C-7), 33.7 (C-2′), 34.9 (C-8*), 36.6 (C-12), 37.3 (C-4*), 44.6 (C-1*), 44.7 (C-3), 50.7 (C-5), 69.4 (C-1′), 75.3 (C-9), 89.0 (C-2), 126.1 (C-6′), 129.1 (2C, C-5′, C-7′), 129.3 (2C, C-4′, C-5′), 136.7 (C-3′) (*, interchangeable signals); EIMS *m*/*z* (70 eV) 374 $[M]^+$ (20), 203 (33), 156 (40), 138 (58), 110 (100); HREIMS (*m*/*z*) $[M]^+$ calcd for $C_{23}H_{34}SO_2$, 374.2281; found, 374.2269.

*2β-(2-Mercaptoethylthio)clovan-9α-ol (**22**).* $BF_3 \cdot Et_2O$ (518 mg, 0.45 mL) was added dropwise to a solution of caryophyllene oxide (201 mg) in dry Et_2O (4 mL). The resulting solution was kept stirred, under nitrogen atmosphere, at −63 °C for 2 h. Then, 1,2-ethanedithiol (782 mg) was added, and the reaction was kept stirring for a further 1 h and then allowed to reach room temperature. The resulting reaction mixture was diluted with diethyl ether (50 mL), washed with brine (2 × 50 mL), and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded a crude reaction product, which was purified by column chromatography on silica gel, with increasing gradients of ethyl acetate on petroleum ether, to give compound **22** (40 mg) (8%): oil; $[\alpha]^{25}_D$ +4 (*c* 2.0 $CHCl_3$); IR (neat, KBr) ν_{max} 3390 (OH) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.83 (s, 3H, H-13α), 0.88 (d, 1H, *J* = 12.8 Hz, H-12a), 0.96 (s, 3H, H-15), 0.99 (m, 1H, H-11a), 1.03 (s, 3H, H-14β), 1.08 (m, 1H, H-7a), 1.22−1.44 (3H, H-6a, H-6b, H-7b), 1.52−1.60 (3H, H-3a, H-5β, H-10α), 1.64 (d, 1H, *J* = 12.8 Hz,; H-12b), 1.68−1.78 (2H, H-3b, H-11b), 1.93 (m, 1H, H-10β), 2.66−2.78 (5H, H-2′a, H-2′b, H-1′a, H-1′b, H-2α), 3.28 (bs, 1H, H-9β); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.6 (C-6), 24.5 (C-13), 25.1 (C-2′), 25.9 (C-10), 28.3 (C-15), 28.7 (C-11), 30.8 (C-14), 33.0 (C-7), 34.8 (C-8*), 35.8 (C-12), 36.6 (C-1′), 39.1 (C-4*), 44.7 (C-1*), 48.3 (C-3), 51.2 (C-5), 55.8 (C-2), 74.8 (C-9) (*, interchangeable signals); EIMS *m*/*z* (%) 314 (18) $[M]^+$, 296 (36) $[M - 18]^+$, 281 (10) $[M + 1 - 34]^+$, 253 (22), 235 (16), 203 (100); HRFABMS (thioglycerol matrix) (*m*/*z*) $[M - H]^+$ calcd for $C_{17}H_{29}OS_2$, 313.1660; found, 313.1657.

Microorganism and Antifungal Assays. The culture of *B. cinerea* employed in this work, *B. cinerea* (UCA 992), was obtained from grapes from the Domecq vineyard, Jerez de la Frontera, Cádiz, Spain. This culture of *B. cinerea* has been deposited at the Mycological Herbarium

Scheme 1



Collection (UCA), Facultad de Ciencias, Universidad de Cádiz. Bioassays were performed by measuring inhibition of radial growth on agar medium in a Petri dish. Test compounds were dissolved in EtOH to give a final compound concentration in the culture medium of 50−200 mg L^{-1}. Solutions of test compounds were added to glucose−malt−peptone−agar medium (61 g of glucose−malt−peptone−agar/L, pH 6.5−7.0). The final EtOH concentrations were identical in both the control and treated cultures. The medium was poured in 6 or 9 cm diameter sterile plastic Petri dishes, and a 5 mm diameter mycelial disk of *B. cinerea* cut from an actively growing culture was placed in the center of the agar plate. Inhibition of radial growth was measured for 6 days.

General Culture Conditions. B. cinerea (UCA 992) was grown on surface culture in Roux bottles at 25 °C for 4 days on a Czapek−Dox medium (150 mL per flask) comprising (per liter of distilled water), glucose (40 g), yeast extract (1 g), potassium dihydrogen phosphate (5 g), sodium nitrate (2 g), magnesium sulfate (0.5 g), ferrous sulfate (10 mg), and zinc sulfate (5 mg). The substrate dissolved in ethanol was added to each flask and the fermentation continued for a further period (see below). The mycelium was filtered and washed with brine and EtOAc. The broth was saturated with sodium chloride, acidified (pH 2), and extracted with EtOAc. The extracts were separated into acidic and neutral fractions with aqueous sodium hydrogen carbonate. The acid fraction was recovered in EtOAc. The extracts were dried over sodium sulfate, the solvent was evaporated, and the residues were chromatographed on silica gel in a gradient mixture of petroleum ether−EtOAc of increasing polarity. The acidic fractions were methylated with diazomethane prior to chromatography. Chromatography of the acidic fractions yielded inseparable mixtures.

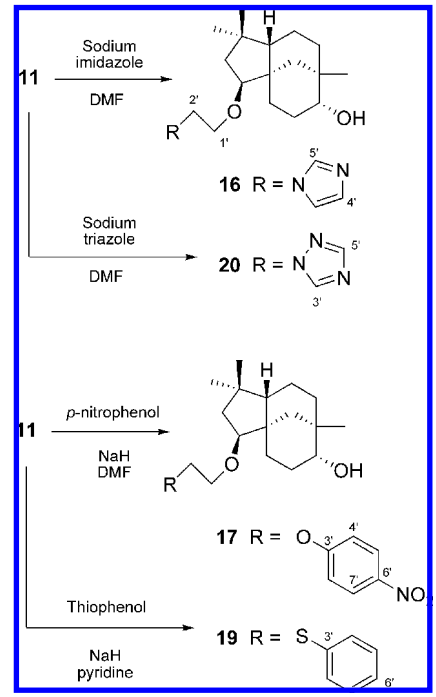
*Biotransformation of 2β-(Prop-2-enyloxy)clovan-9α-ol (**8**) by B. cinerea.* Compound **8** (151 mg) (*10*) was distributed over 21 flasks of *B. cinerea* and the fermentation grown for a further 5 and 10 days. Chromatography of the neutral fraction (5 days, 15 flasks) gave 2β-(prop-2-enyloxy)clovan-9α-ol (**8**) (*10*) (27 mg), clovan-2β,9α-diol (**2**) (*12*) (9 mg), clovan-2β,9β-diol (**3**) (*12*) (1 mg), and dihydrobotrydial (*12*) (5 mg). Chromatography of the neutral fraction (10 days, 6 flasks) gave 2β-(prop-2-enyloxy)clovan-9α-ol (**8**) (3 mg), clovan-2β,9α-diol (**2**) (11 mg), clovan-2β,9β-diol (**3**) (1 mg), and dihydrobotrydial (10 mg).

*Biotransformation of 2β-(2-Nitroethoxy)clovan-9α-ol (**14**) by B. cinerea.* Compound **14** (*10*) (110 mg) was distributed over 15 flasks of *B. cinerea* and the fermentation grown for a further 5 and 10 days. Chromatography of the neutral fraction (5 days, 10 flasks) gave 2β-(2-nitroethoxy)clovan-9α-ol (**8**) (10 mg), clovan-2β,9α-diol (**2**) (6 mg), clovan-2β,9β-diol (**3**) (1 mg), and dihydrobotrydial (3 mg). Chromatography of the neutral fraction (10 days, 5 flasks) gave clovan-2β,9α-diol (**2**) (9 mg) and dihydrobotrydial (**10**) (8 mg).

Computational Strategy To Develop the QSAR Study. The QSAR model was derived by multiple regression analysis (MRA) and employing MobyDigs software (*14*). The database contained the 23 2-alkyloxyclovan-9-ol derivatives stated in **Schemes 1** and **2**.

First, three different models were developed by taking into account the percentage of the fungal growth inhibition reported at three different concentrations, 200, 100, and 50 μg/mL, to find the one that best describes the antifungal activity of this set of compounds.

Scheme 2



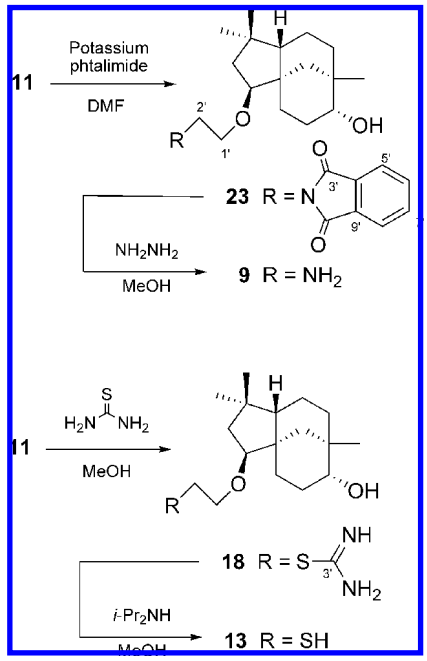
The first 15 spectral moments ($\mu_1-\mu_{15}$) for each bond weight as well as the number of bonds in the molecules (μ_0) were calculated by Modeslab 1.5 software (*15*) for the further selection of the most significant ones to be included in the model. The above methodology has been successfully used for modeling a broad spectrum of biological activities as well as physical chemistry properties of molecules (*16*−*21*). Owing to the nonlinearity of the biological process under study (fungicidal activity) the interactions of μ_0 and μ_1 with all variables (descriptors) was evaluated. Spectral moments were calculated by considering the molecules in the absence of hydrogen atoms. The genetic algorithm (GA) (*14*, *22*−*24*) was used as the variable selection strategy including in the equation the most significant parameters from the data set. The bond dipole moment (Dip), hydrophobicity (Hyd), and combined dipolarity/polarizability Abraham molecular descriptor (Ab-π_2^H) resulted in the best bond weights from the pool. Also, the dependent variable was evaluated as the %*I*.

Once the statistical significance of the models was determined, their robustness and predictivity were evaluated by both q^2 "leave-one-out" (LOO) cross-validation and bootstrap.

An analysis of the applicability domain of the model was carried out to explore the presence of potential outliers and compounds from the training set that influence model parameters to a marked extent, resulting in an unstable model. On the one hand, a training compound with a leverage value greater than the warning leverage *h** can be considered as a single data point with great influence on the model, resulting in a less robust model. On the other hand, if a test compound displays this behavior, its predicted response can be extrapolated from the model and, therefore, the predicted value must be used with great care. Only predicted data for chemicals belonging to the chemical domain of the training set should be proposed (*25*, *26*).

Also, the presence of outliers can be detected by examining several statistics such as standard residual, Mahalanobis distance, deleted residual, and Cook's distance values. From these statistics, standard residual values are one of the most used in the literature (*17*, *27*), and it has been established that values outside the range of ±2 point to compounds considered to be outliers. For these reasons, in this study we plotted the leverage values calculated for every compound (*X*-axis) versus standard residuals (*Y*-axis). Then, the domain of applicability of the model was defined as a squared area within the ±2 range for standard residuals and the leverage threshold *h** (*25*, *26*). This kind of

Scheme 3



plot allows a graphical detection of both the outliers and the influential chemicals in a model.

To overcome the correlation of the descriptors involved in the study, given the small size of the data set and the large number of potential descriptors being considered, an orthogonalization process of molecular descriptors was carried out. The above methodology was introduced by Randić (*28*−*30*) as a way of improving the statistical interpretation of the model, which had been built by using inter-related indices and has been described in detail in several publications (*31*).

RESULTS AND DISCUSSION

Compounds **1**−**23**, bearing different chains at carbon C-2 on the clovane skeleton, have been synthesized and evaluated for their in vitro antifungal activity against the phytopathogenic fungus *B. cinerea*. As mentioned above, we had shown that 2β-methoxyclovan-9α-ol (**5**), a readily available cyclization and rearrangement product of caryophyllene oxide, is an active fungistatic agent against *B. cinerea* (*10*−*12*). Its detoxification by this fungus was studied and suggests that active products with a clovane skeleton should not persist for long after application (*12*, *13*, *32*).

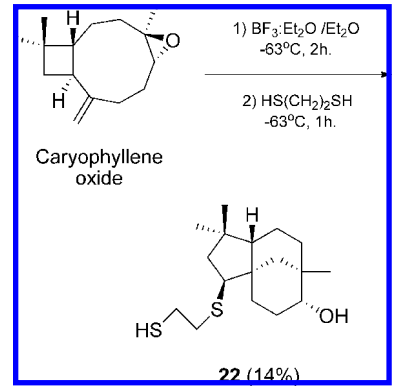
The introduction of other alkylated chains at C-2 might noticeably increase the potency of this class of compounds as inhibitors of the plant pathogen *B. cinerea* by releasing the potentially toxic chain attached at C-2.

To evaluate this hypothesis, compounds **1**−**8**, **10**−**12**, **14**, and **15** were obtained as previously reported (*10*−*13*). The new clovane derivatives **9**, **13**, **16**−**20**, **22**, and **23** were synthesized by the chemical transformations summarized in **Schemes 1**−**4**.

Caryophyllene-4β,5α-oxide, free from isomeric epoxides, was stirred with either 2-bromoethanol or 2-iodoethanol, following a previously published procedure (*10*). As a result, either compound **11** or compound **12**, respectively, was obtained as main product (**Scheme 1**). These compounds showed physical and spectroscopic characteristics consistent with the structures assigned to them as 2β-(2-bromoethoxy)clovan-9α-ol (**11**) and 2β-(2-iodoethoxy)clovan-9α-ol (**12**), respectively.

Compound **11** was employed to obtain a number of clovane derivatives with different functionalities on the ethylene side chain, which were unattainable by direct solvolysis of caryophyllene oxide (*10*, *11*).

Scheme 4



Treatment of 2β-(2-bromoethoxy)clovan-9α-ol (**11**) in dry dimethylformamide with either sodium imidazole, sodium triazole, or potassium phthalimide led to the preparation of compounds **16**, **20**, and **23**, respectively (**Schemes 2** and **3**). HREIMS of compound **16** gave a quasi-molecular ion peak at *m*/*z* 333.2533 $[M + 1]^+$, which is consistent with the formula $C_{20}H_{33}O_2N_2$ for the molecular ion; HREIMS of compound **20** gave a quasi-molecular ion peak at *m*/*z* 334.2496 $[M + 1]^+$, which accounts for the formula $C_{19}H_{32}O_2N_3$ for the molecular ion, whereas HREIMS of compound **23** gave a quasi-molecular ion peak at *m*/*z* 434.2309 $[M + Na]^+$, which is consistent with a molecular ion $C_{25}H_{33}O_4N$ + Na. This, together with the presence of signals in the ^{1}H NMR spectrum for compound **16** at δ_H 6.92 (bs, 1H), 6.95 (bs, 1H), and 7.47 (bs, 1H) and at δ_H 7.86 (s, 1H) and 8.34 (bs, 1H) for compound **20** and at δ_H 7.70 (dd, 2H, J = 5.4, 2.2 Hz), 7.85 (dd, 2H, J = 5.4, 2.2 Hz) for compound **23** led to the assignation of the structure for these compounds as 2β-(2-(imidazol-1-yl)ethoxy)clovan-9α-ol (**16**), 2β-(2-(1,2,4-triazol-1-yl)ethoxy)clovan-9α-ol (**20**), and 2β-(2-(1,3-dioxoisoindolin-2-yl)ethoxy)clovan-9α-ol (**23**), respectively.

Compounds **17** and **19** were prepared from 2β-(2-bromoethoxy)clovan-9α-ol (**11**) by treatment of *p*-nitrophenol or thiophenol in the presence of sodium hydride (**Scheme 2**). Compound **17** showed ^{1}H NMR signals at δ_H 6.95 (d, 2H, J = 9.4 Hz) and 8.15 (d, 2H, J = 9.4 Hz) and IR absorptions at 1601, 1598, 1431, and 1369 cm^{-1}, which suggested the presence of a nitro-aromatic moiety. HREIMS of compound **17** gave a quasi-molecular ion peak at *m*/*z* 404.2441 $[M + 1]^+$, which is consistent with the formula $C_{23}H_{34}O_5N$ for the molecular ion. These data led to the assignment of the structure for compound **17** as 2β-(2-(4-nitrophenoxy)ethoxy)clovan-9α-ol. On the other hand, HREIMS of compound **19** gave a molecular ion peak at *m*/*z* 374.2269 $[M]^+$, which is consistent with the formula $C_{23}H_{34}SO_2$. In addition, ^{1}H NMR signals at δ_H 7.15 (t, 1H), 7.26 (t, 2H), and 7.35 (d, 2H) supported the assignment of the structure of compound **19** as 2β-(2-(phenylthio)ethoxy)clovan-9α-ol.

Amino alcohol **9** and mercapto alcohol **13** were prepared from bromo clovanol **11** via previous formation of intermediates **23** and **18** (**Scheme 3**), respectively, which were thoroughly characterized and their biological activities evaluated. Heating under reflux of compound **11** with thiourea in methanol gave a compound with a molecular ion of $C_{18}H_{33}O_2N_2S$, obtained through HREIMS, from quasi-molecular ion *m*/*z* 341.2263 $[M + 1]^+$. In addition, infrared absorptions at 3476, 1612, and 1589 cm^{-1} and ^{13}C NMR resonance at δ_C 213.1 supported the assignation of the structure of compound **18** as 2β-(2-carbamimidoylthioethoxy)clovan-9α-ol.

Table 1. Results of Antifungal Bioassays of Compounds **1**−**23** against *Botrytis cinerea*

	% inhibition[a] ± SD		
compd	200 μg/mL	100 μg/mL	50 μg/mL
1	36 ± 2	25 ± 1	21 ± 3
2	63 ± 1	50 ± 2	36 ± 1
3	30 ± 2	24 ± 2	21 ± 1
4	24 ± 2	9 ± 2	2 ± 1
5	83 ± 3	60 ± 2	49 ± 0
6	65 ± 2	43 ± 2	29 ± 1
7	76 ± 1	42 ± 1	17 ± 2
8	82 ± 2	72 ± 2	63 ± 4
9	97 ± 1	82 ± 3	50 ± 3
10	66 ± 2	64 ± 4	56 ± 1
11	77 ± 3	73 ± 2	68 ± 4
12	70 ± 3	67 ± 2	65 ± 1
13	28 ± 2	27 ± 1	27 ± 3
14	90 ± 2	86 ± 1	80 ± 2
15	60 ± 1	54 ± 2	48 ± 1
16	98 ± 2	84 ± 1	67 ± 1
17	81 ± 2	79 ± 2	76 ± 1
18	97 ± 1	83 ± 2	70 ± 1
19	24 ± 0	12 ± 1	6 ± 1
20	86 ± 1	57 ± 1	34 ± 2
21	78 ± 1	66 ± 6	59 ± 1
22	38 ± 3	34 ± 2	32 ± 4
23	60 ± 3	55 ± 3	50 ± 3

[a] % inhibition = 100 − [(growth on treated/growth on control) × 100] ± SD; results are the means of at least three independent experiments conducted in triplicate.

Table 2. Statistical Parameters Derived from the QSAR Models Built at 50, 100, and 200 μg/mL for Modeling the Percentage of Fungal Growth Inhibition (*I*%)[a]

	model 1	model 2	model 3
variables	$\mu_0\mu_2^{Dip}$	μ_{12}^{Std}	$\mu_0\mu_1^{Ab\text{-}\Sigma B_2^H}$
	$\mu_1\mu_3^{Dip}$	$\mu_1\mu_5$	$\mu_0\mu_5^{Ab\text{-}\Sigma B_2^O}$
	$\mu_0\mu_2^{Hyd}$	$\mu_1\mu_6^{Std}$	$\mu_0\mu_6^{Ab\text{-}\Sigma B_2^O}$
	$\mu_{11}^{Ab\text{-}\pi_2^H}$	$\mu_0\mu_5^{Std}$	$\mu_8^{Ab\text{-}\log L_{16}}$
concn	50 μg/mL	100 μg/mL	200 μg/mL
N	23	23	23
R^2	0.855	0.778	0.676
S	9.502	12.31	15.36
F	26.49	15.72	9.39
p	$<10^{-6}$	$<10^{-5}$	$<10^{-5}$
$Q^2_{CV\text{-}LOO}$	0.784	0.724	0.598

[a] The different weights are Std = distance, Ab-ΣB_2^H= the summation solute hydrogen bond basicity, Ab-ΣB_2^O = for certain functional groups (e.g., pyridines, sulfoxides) the basicity of which is found to change substantially between wet and dry solvents (*35*), Ab-logL_{16} the solute gas−hexadecane partition coefficient.

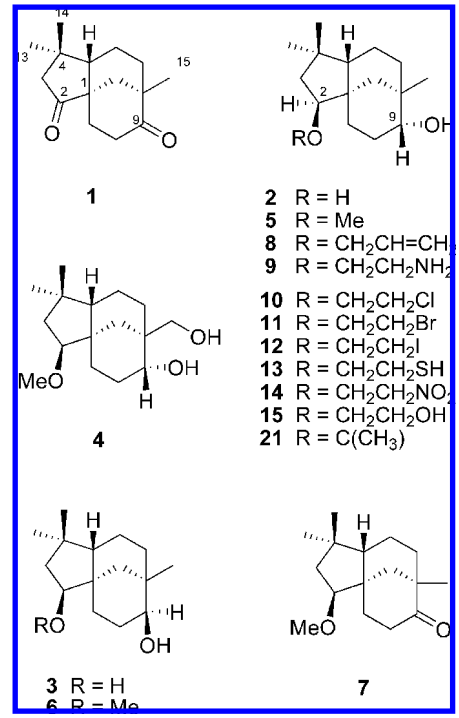
On the one hand, heating under reflux of compound **18** with diisopropylamine in methanol gave a compound of formula $C_{17}H_{30}O_2S$, obtained through HRFABMS, from quasi-molecular ion *m*/*z* 321.1862 $[M + Na]^+$. Analysis of the physical and spectroscopic characteristics of this compound led to the assignment of its structure as 2β-(2-mercaptoethoxy)clovan-9α-ol (**13**). Treatment of compound **23** with hydrazine in methanol led to the preparation of compound **9**, the physical and spectroscopic properties of which led to the assignment of its structure as 2β-(2-aminoethoxy)clovan-9α-ol (**9**).

All of the previously described compounds have, as a common structural feature, an oxygen atom directly attached to position C-2 on the clovane skeleton. Attempts have been made by our research group to overcome this structural limitation (*12*), which arguably can be fundamental in the study of the structure−activity relationships for clovane compounds (*13*).

In this context, we have carried out the preparation of 2β-(2-mercaptoethylthio)clovan-9α-ol (**22**), obtained by treatment of caryophyllene-4β,5α-oxide with $BF_3 \cdot Et_2O$ and 1,2-ethanedithiol in Et_2O at −63 °C for 2 h (**Scheme 4**).

The antifungal properties of compounds **1**−**23** were determined against the growth of *B. cinerea* using the poisoned food technique (*33*). The commercial fungicide Euparen was used as a standard for comparison in this test. Several levels of inhibition were observed.

According to **Table 1** and as expected (*13*), a general decrease of the activity of compounds with clovane skeleton was observed when a second hydroxyl group was added to the structure, even if an ether group is present at C-2, as shown in compound **4**. Along the same line, poor results were obtained for the epimeric and oxidized derivatives at C-2 and C-9, compounds **1**−**4**, **6**, and **7**.



Furthermore, sulfur-containing derivatives **13**, **19**, and **22** displayed a weak antifungal activity. A moderate activity was observed for compounds **10**−**12**, **15**, **21**, and **23**. The results of the antifungal activity screening test indicated that in general compounds including nitrogen atoms displayed the best antifungal activity (see **Table 1**) with compounds **20**, **14**, **18**, **9**, and **16** being the most active against the fungus. A 97% inhibition rate after 6 days at 200 μg/mL was observed for compounds **9** and **18**, and a 98% inhibition rate was observed for compound **16**. Interestingly, compounds **16** and **18** displayed 67 and 70% inhibition at 50 μg/mL, respectively.

To extend these results, the previously described observations have been analyzed by a QSAR study based on the synthesized compounds. The biological activity was referred to the different concentrations assessed experimentally, 50, 100, and 200 μg/mL, at 6 days of observation.

Model selection was subjected to the principle of parsimony (*34*) to search for a function with a high statistical significance but having as few parameters as possible. The resulting best models for each concentration are contained in **Table 2**, where *N* is the number of compounds included in the model, R^2 is the square of the correlation coefficient, *S* is the standard deviation of the regression, *F* is the Fisher ratio, *p* is the significance of

Table 3. Statistical Parameters Derived from the QSAR Models Built at 50, 100, and 200 μg/mL for Modeling the Percentage of Fungal Growth Inhibition (*I*%)[a]

	model 1	model 4	model 5
variables	$\mu_0\mu_2^{Dip}$ $\mu_1\mu_3^{Dip}$ $\mu_0\mu_2^{Hyd}$ $\mu_{11}^{Ab\text{-}\pi_2^H}$	$\mu_0\mu_2^{Dip}$ $\mu_1\mu_3^{Dip}$ $\mu_0\mu_2^{Hyd}$ $\mu_{11}^{Ab\text{-}\pi_2^H}$	$\mu_0\mu_2^{Dip}$ $\mu_1\mu_3^{Dip}$ $\mu_0\mu_2^{Hyd}$ $\mu_{11}^{Ab\text{-}\pi_2^H}$
concn	50 μg/mL	100 μg/mL	200 μg/mL
N	23	23	23
R^2	0.855	0.776	0.554
S	9.502	12.34	18.02
F	26.49	15.63	5.59
p	$<10^{-5}$	$<10^{-4}$	$<4.2 \times 10^{-3}$
$Q^2_{CV\text{-}LOO}$	0.784	0.657	0.325

[a] The variables from model 1 in **Table 2** were kept at this time.

Table 4. Values of Percentage of Inhibition (*I*%) Observed at 50 μg/mL and Predicted by Model 1 as well as the Standard Residuals for Each Compound in the Data Set

compd	observed	predicted	residual	standard residual
1	21	11.84	9.16	0.96
2	36	32.29	3.71	0.39
3	21	32.29	−11.29	−1.19
4	2	7.13	−5.13	−0.54
5	49	34.38	14.62	1.54
6	29	34.38	−5.38	−0.57
7	17	21.51	−4.51	−0.47
8	63	50.15	12.85	1.35
9	50	52.19	−2.19	−0.23
10	56	55.59	0.41	0.04
11	68	63.98	4.02	0.42
12	65	71.34	−6.34	−0.67
13	27	31.75	−4.75	−0.50
14	80	66.42	13.58	1.43
15	48	54.09	−6.09	−0.64
16	67	51.89	15.11	1.59
17	76	81.30	−5.30	−0.56
18	70	69.82	0.18	0.02
19	6	4.75	1.25	0.13
20	34	51.87	−17.87	−1.88
21	59	61.88	−2.88	−0.30
22	32	38.57	−6.57	−0.69
23	50	46.62	3.38	0.36

the model, and $Q^2_{CV\text{-}LOO}$ is the cross-validated squared regression coefficient.

It is worth noting that all models are suitable for the prediction of antifungal activity according to the statistical parameters, especially taking into account that all of the models show an appropriate value for the Q^2 because many authors consider a value of >0.5 to be an indicator of the predictive capacity of the model (*36*). However, the model related to the percentage of the fungal growth inhibition at 50 μg/mL concentration is the best because it shows the best statistical values by describing 85.5% of the variance and the greatest predictive capability (78.4%).

In addition, QSAR models for fungal growth inhibition were developed at 100 and 200 μg/mL, keeping the same variables that resulted in the best model for 50 μg/mL in order to make a comparison.

From **Table 3** can be seen the effect of the variables involved in model 1 on modeling the inhibition at the other different concentrations. For example, model 4 yields results similar to those of model 2 but its predictive capability decreases notably from a value of $Q^2_{CV\text{-}LOO}$ equal to 0.724 to 0.657. On the other hand, model 5 shows the worst statistical results, especially taking into account that its predictive capability is lower than the critical value of 0.5 (*36*). Therefore, these variables are not well-correlated with the antifungal activity displayed by these compounds and are not suitable to model the antifungal activity against *B. cinerea* at the other two concentrations.

The applicability domain of a QSAR model is the response and chemical structure space in which the model makes predictions with certain reliability. The chart resulting from this study displayed that compound **23** has the largest leverage value ($h_{23} = 0.693 > h^* = 0.652$) not being, however, a potential outlier because its value of standardized residual is not greater than 2 standard deviation units (for more details, see **Table 4**).

It follows that the influence of this compound is not critical for the model, and it was not excluded. However, it should be considered in further studies that the value of activity for chemical structures similar to compound **23** predicted by this model could be laid out of the domain as well.

To overcome the inter-relation among the descriptors, the orthogonalization process was performed. The final QSAR model obtained with the spectral moments after orthogonalization and standardization is given below, together with the statistical parameters of regression analysis.

$$\%I = 6.35(\pm 2.03) \times {}^1\Omega\mu_0\mu_2^{Hyd} - 6.76(\pm 2.03) \times {}^2\Omega\mu_0\mu_2^{Dip} + 12.26(\pm 2.03) \times {}^3\Omega\mu_1\mu_3^{Dip} - 14.10(\pm 2.03) \times {}^4\Omega\mu_{11}^{Ab\text{-}\pi_2^H} + 44.61(\pm 1.98) \quad (1)$$

$N = 23$, $R^2 = 0.855$, $S = 9.502$, $F = 26.49$, $p < 10^{-6}$, AIC = 140.44, FIT = 2.699, $q^2_{CV\text{-}LOO} = 0.784$, $q^2_{boot} = 0.673$, $a(R^2) = 0.119$, $a(q^2) = -0.62$, and LOF = 166.12. Besides the statistical parameters stated in the tables, it is shown that Akaike's information criterion (*37*, *38*) (AIC) is the Kubinyi function (FIT) (*39*, *40*). Furthermore, we calculated the validation parameters from the bootstrapping (q^2_{boot}) and *Y* scrambling ($a(R^2)$ and $a(q^2)$) procedures. Finally, Friedman's lack of fit (LOF) factor (*41*) is given, which takes into account the number of terms used in the equation and is not biased, as are other indicators toward large numbers of parameters.

The variables in the model involved in eq 1 are related to the bond hydrophobicity, dipole moment, and combined dipolarity/polarizability Abraham molecular descriptor describing the relationship between this physicochemical property and the antifungal activity in question. In general, an increase in the hydrophobicity on the structure causes a favorable effect on the fungal inhibition.

It is important to highlight the contrary contribution of $\mu_0\mu_2^{Dip}$ and $\mu_1\mu_3^{Dip}$ despite being weighted by the same property. The main difference is the order of the spectral moments involved in the interactions and the significance of these variables in the model equation, $\mu_0\mu_2^{Dip}$ being more significant than $\mu_1\mu_3^{Dip}$.

These results suggest that the introduction of alkylated chains at C-2 increase the potency of this class of compounds as inhibitors of the plant pathogen *B. cinerea*. To extend these observations, compounds **8** and **14**, which both display activity higher than that of **5** (see **Table 1**), were incubated separately with *B. cinerea* for 5−10 days on surface culture following the methodology previously described by us (*13*). As a result, compounds **2** and **3** were isolated from the culture, confirming that the chain attached at C-2 on the clovane skeleton was released during metabolism. These observations are consistent with our previous observations for compound **5**, and support our hypothesis by which the alkylated chains at C-2 increase the potency of this class of compounds as inhibitors of the plant pathogen *B. cinerea* (*12*, *13*). The 2-alkoxyclovane derivatives

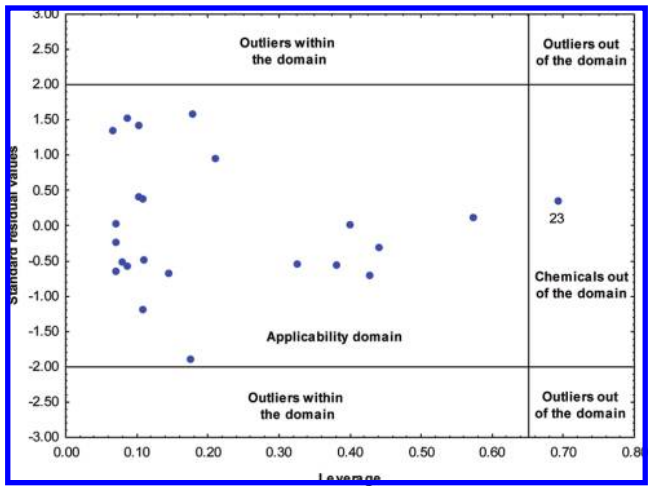


Figure 1. Applicability domain of the model.

seem to work as prodrugs, carrying the potentially toxic chain into the fungus cell.

In conclusion, the QSAR study yielded as the most important weights to take into account for further design of new and more potent botrycides the bond dipole moment, the combined dipolarity/polarizability Abraham molecular descriptor, and hydrophobicity, the last being the most significant in the model. Also, in this study is reported for the first time the combination of Abraham solute descriptors with spectral moments for predicting antifungal activity against *B. cinerea*.

The *r*th spectral moments are the sum of all self-returning walks of length *r* weighted by different bond properties in the line graph of the molecular graph, beginning and ending in the same vertex (*42*). Its interpretation is highly related to the topology of the molecule but taking into account the specific weight. In this case, the use of this simple descriptor in the absence of hydrogen atoms described successfully the relationship between activity and chemical structure. Therefore, such a model can be used as a tool for predicting the fungal growth inhibition of *B. cinerea*.

The outlined results are consistent with our previous findings, which show that the introduction of alkylated chains at C-2 can lead to an increase of the potency of this class of compounds as inhibitors of the plant pathogen *B. cinerea*, probably by releasing the potentially toxic chain attached at C-2 (*12*, *13*). Furthermore, the results discussed above together with the facile dealkylation of the 2β-methyl ether (**5**) to yield dione **1** and the diols **2** and **3**, releasing the carbon chain attached at C-2 (*12*), permit us to propose a mechanism of action for this family of clovane derivatives similar to that of a prodrug, where the clovanol skeleton carries the potential toxic chain into the fungus cell.

Works are in progress to study the degradation of the carbon chain attached at C-2 and the clovane skeleton during the metabolism of these compounds by *B. cinerea* and the use of three-dimensional descriptors to improve the capability of the model to describe the structure−activity relationships considering the different configurations of the molecules.

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