JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Synthesis and Antifungal Activity of a Series of *N*-Substituted [2-(2,4-Dichlorophenyl)-3-(1,2,4-triazol-1-yl)]propylamines

ANNA ARNOLDI,[†] SABRINA DALLAVALLE,*[,†] LUCIO MERLINI,[†] LOANA MUSSO,[†]
GANDOLFINA FARINA,[‡] MADDALENA MORETTI,[‡] AND LALITH JAYASINGHE[§]

Dipartimento di Scienze Molecolari Agroalimentari and Istituto di Patologia Vegetale, Università di Milano, Via Celoria 2, 20133 Milano, Italy, and Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka

A series of *N*-mono- or *N*,*N*-disubstituted [2-(2,4-dichlorophenyl-3-(1,2,4-triazol-1-yl)]propylamines and *N*-[2-(2,4-dichlorophenyl-3-(1,2,4-triazol-1-yl)propyl]amides were synthesized and tested for their fungicidal activity in vitro and in vivo against a group of plant pathogenic fungi. Some compounds exhibited a fairly good in vitro activity. The replacement of the ether group of tetraconazole with a secondary or tertiary amino group leads to compounds that maintain the antifungal activity on several phytopathogenic fungi, provided that the substituents are not too bulky or lipophilic. The allyl, propargyl, and cyclopropyl groups appear particularly suitable. Although these compounds have some structural similarities with terbinafine and naftifine, which act as squalene epoxidase inhibitors, they maintain the usual mechanism of action of the other triazoles.

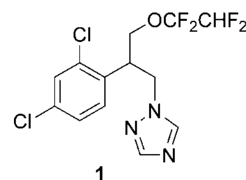
KEYWORDS: Triazole; ergosterol biosynthesis inhibitors; fungicide; synthesis; tetraconazole

INTRODUCTION

The agrochemical industry is continuously searching for new active pesticide compounds. The main goal of this research is to develop substances with lower application doses, increased selectivity and reduced undesired ecological impact (*1*).

Azole fungicides were synthesized for the first time in the early 1970s and quickly gained a great importance in the protection of various crops, because they represented a significant progress in the chemical control of fungal diseases. In fact, this class includes several excellent systemic fungicides with long protective and curative activity against a broad spectrum of foliar, root, and seedling diseases caused by many Ascomycetes, Basidiomycetes, and Deuteromycetes (*1*). Their mechanism of action is related to the inhibition of the sterol biosynthesis (*2, 3*) in fungi. In the past decades this class of fungicides has been thoroughly explored and many active structures have been reported (*4–6*).

Tetraconazole (**1**) (Eminent, 2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-propyl-1,1,2,2-tetrafluoroethylether) is currently among the most employed agricultural fungicides. The mechanism of action of **1** has been studied in detail (refs *7–9* and preceding papers quoted therein), but little is known about the structure–activity relationships of tetraconazole analogues. Although **1** is characterized by an efficacy against biotrophes higher than that of most commercial compounds (*7, 10*), only a few analogues of this fungicide have been synthesized and tested for their activity (*11*).

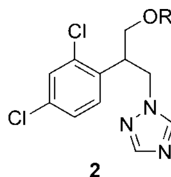


In a previous paper, we reported studies of a series of ethers (**2**) related to tetraconazole (*11*). A QSAR study showed that for this class of compounds lipophilicity was a major positive parameter affecting the activity. To gain a further insight into the most relevant physicochemical features affecting fungitoxicity in tetraconazole analogues, we planned to replace the ether oxygen with an amino group. The advantage of such a replacement could be the functionalization of the nitrogen atom with either one or two alkyl and arylalkyl chains differing in size, shape, and lipophilicity. In addition, as the designed amines showed structural similarity with the antifungal allylamines, such as terbinafine or naftifine (*12*), which act as inhibitors of squalene epoxidase, we investigated whether our compounds might also exhibit such a mechanism of inhibition.

Moreover, the presence of the amino group allowed us to prepare some representative amido derivatives and to evaluate the effect of this moiety on the fungicidal activity. Here we

* To whom correspondence should be addressed. Phone: +39-2-50316818. Fax: +39-2-50316801. E-mail: sabrina.dallavalle@unimi.it.
[†] Dipartimento di Scienze Molecolari Agroalimentari.
[‡] Istituto di Patologia Vegetale.
[§] Institute of Fundamental Studies.

10.1021/jf071631g CCC: $37.00 © 2007 American Chemical Society
Published on Web 10/03/2007



report the synthesis and antifungal activity of this new series of amines and amides.

MATERIALS AND METHODS

Chemistry. All reagents and solvents were reagent grade or were purified by standard methods before use. Melting points were determined in open capillaries on a melting point apparatus and are uncorrected. Column chromatography was carried out on flash silica gel (Merck 230–400 mesh). TLC analysis was conducted on silica gel plates (Merck $60F_{254}$). ^1H NMR spectra were recorded at 300 MHz. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz, respectively. Mass spectra were recorded on a Finnigan-MAT TSQ70 spectrometer. Solvents were routinely distilled prior to use. All reactions requiring anhydrous conditions were performed under a positive nitrogen flow, and glassware were oven-dried and/or flame-dried. All of the compounds synthesized had satisfactory elemental analysis data.

2-(2,4-Dichlorophenyl)-3-[1,2,4]triazol-1-yl-propylamine (4). 2-(2,4-Dichlorophenyl)-3-[1,2,4]triazol-1-yl-propionitrile (**3**) (kindly provided by Isagro, Milano) (2.18 g) was dissolved in 50 mL of methanol by gentle heating, and then $CoCl_2 \cdot 6H_20$ (3.56 g, 14.98 mmol) and $NaBH_4$ (2.83 g, 74.9 mmol) were added in portions. The mixture was stirred for 2 h, and the pH was adjusted to 2 with 2 N HCl. The solvent was evaporated, and the aqueous phase was extracted with dichloromethane. The aqueous phase was then basified with ammonia and extracted with dichloromethane. Evaporation of dichloromethane gave a brown oil (1.64 g). The crude material was purified by chromatography on silica gel with dichloromethane/methanol 9:1 to give 1.04 g of an oil (yield 52%). ^1H NMR ($CDCl_3$): δ 2.95–3.10 (m, 2H, H-1), 3.75–3.80 (m, 1H, H-2), 4.45–4.55 (m, 2H, H-3), 6.98 (d, 1H, J = 8.19 Hz, H-6′), 7.25 (dd, 1H, J = 2.23, 8.19 Hz, H-5′), 7.40 (d, 1H′, J = 2.23 Hz, H-3), 7.76 (s, 1H, triazole), 7.85 (s, 1H, triazole).

[2-(2,4-Dichlorophenyl)-3-[1,2,4]triazol-1-yl-propyl]dimethylamine (6). Compound **3** (0.5 g, 1.84 mmol) was stirred with formic acid (0.47 g, 10 mmol) and 37% aqueous formaldehyde (0.35 g, 4.3 mmol) at 120 °C. After 6 h, the mixture was acidified with 1 mL of 1 N HCl and extracted with dichloromethane. The aqueous phase was basified with 2 N NaOH and extracted with dichloromethane. The organic extracts were dried, filtered, and evaporated to give a crude that was purified by flash chromatography on silica gel (dichloromethane/methanol 9:1) to give 250 mg (45%) of an oil (**6**). ^1H NMR ($CDCl_3$): δ 2.29 (s, 6H, $N(CH_3)_2$), 2.45–2.55 (*m*, 2H, H-1), 3.85–4.00 (m, 1H, H-2), 4.41 (dd, 1H, J = 13.02, 7.50 Hz, H-3_A), 4.55 (dd, 1H, J = 13.02, 5.80 Hz, H-3_B), 6.92 (d, 1H, J = 8.19 Hz, H-6′), 7.19 (dd, 1H, J = 2.23, 8.19 Hz, H-5′), 7.36 (d, 1H, J = 2.23 Hz, H-3′), 7.70 (s, 1H, triazole), 7.88 (s, 1H, triazole). MS *m/z* (%): 301 (1), 299 (2), 204 (2), 58 (100). Anal. Calcd for $C_{13}H_{16}Cl_2N_4$: C, 52.19; H, 5.39; N, 18.73. Found: C, 52.32; H, 5.30; N, 18.64.

Synthesis of Compounds 7a–d. To a solution of **4** (1.84 mmol) in dry ethanol (10 mL) were added K_2CO_3 (11 mmol) and the appropriate bromide (3.68 mmol). The mixture was stirred at room temperature for 5–20 h and filtered at a reduced pressure. The filtrate was purified by flash chromatography on silica gel.

[2-(2,4-Dichlorophenyl)-3-[1,2,4]triazol-1-yl-propyl]allylamine (7a). Eluted with dichloromethane/methanol 98:2, oil (yield 33%). ^1H NMR (300 MHz, $CDCl_3$): δ 2.85–2.95 (m, 2H, H-1), 3.18–3.30 (m, 2H, -NC H_2CH=CH_2), 3.95–4.01 (m, 1H, H-2), 4.44 (dd, 1H, J = 6.48, 14.11 Hz, H-3_A), 4.58 (dd, 1H, J = 6.48, 14.11 Hz, H-3_B), 5.06–5.20 (m, 2H, -CH_2CH=C H_2), 5.74–5.90 (m, 1H, -CH_2C H=CH_2), 7.00 (d, 1H, J = 8.77 Hz, H-6′), 7.19 (dd, 1H, J = 2.29, 8.77 Hz, H-5′), 7.39 (d, 1H, J = 2.29 Hz, H-3′), 7.77 (s, 1H, triazole), 7.87 (s, 1H, triazole). MS *m/z* (%): 313 (2), 311 (4), 243 (66), 241 (100), 174 (35), 172 (53). Anal. Calcd for $C_{14}H_{16}Cl_2N_4$: C, 54.03; H, 5.18; N, 18.00. Found: C, 54.11; H, 5.27; N, 17.91.

[2-(2,4-Dichlorophenyl)-3-[1,2,4]triazol-1-yl-propyl]prop-2-ynylamine (7b). Eluted with dichloromethane/methanol 95:5, oil (yield 33%). ^1H NMR (300 MHz, $CDCl_3$): δ 2.23–2.20 (m, 1H, -C≡CH), 2.90– 3.10 (m, 2H, H-1), 3.43 (m, 2H, C H_2C≡CH), 3.90 (m, 1H, H-2), 4.48 (dd, 1H, J = 6.75, 15.00 Hz, H-3_A), 4.58 (dd, 1H, J = 7.50, 15.00 Hz, H-3_B), 7.02 (d, 1H, J = 7.50 Hz, H-6′), 7.21 (dd, 1H, J = 3.75, 7.50 Hz, H-5′), 7.43 (d, 1H, J = 3.75 Hz, H-3′), 7.80 (s, 1H, triazole), 7.90 (s, 1H, triazole). MS *m/z* (%) 311 (6), 309 (9), 243 (51), 241 (77), 206 (27) 174 (50), 172 (70), 68 (100). Anal. Calcd for $C_{14}H_{14}Cl_2N_4$: C, 54.38; H, 4.56; N, 18.12. Found: C, 54.27; H, 4.50; N, 18.01.

(*E*)-[2-(2,4-Dichlorophenyl)-3-[1,2,4]triazol-1-yl-propyl]-(3-phenylallyl)amine (7c). Eluted with ethyl acetate, oil (yield 27%). ^1H NMR (300 MHz, $CDCl_3$): δ 2.90–3.10 (m, 2H, H-1), 3.45 (d, 2H, J = 8.00 Hz, -NC H_2CH=CH_2), 3.90–4.00 (m, 1H, H-2), 4.50 (dd, 1H, J = 6.00, 14.00 Hz, H-3_A), 4.62 (dd, 1H, J = 8.00, 14.00 Hz, H-3_B), 6.25 (dt, 1H, J = 8.00, 16.00 Hz, -C H=CHPh), 6.50 (d,1H, J = 16.00 Hz, -CH=C HPh), 6.95 (d, J = 8.80 Hz, H-6′), 7.20–7.50 (m, 7H, 5Ar + H-5′ + H-3′), 7.80 (s, 1H, triazole), 7.90 (s, 1H, triazole). MS *m/z* (%): 387 (24), 385 (37), 316 (5), 262 (97), 172 (9), 117 (100), 91 (26). Anal. Calcd for $C_{20}H_{20}Cl_2N_4$:C, 62.02; H, 5.20; N, 14.47. Found: C, 62.11; H, 5.14; N, 14.41.

[2-(2,4-Dichlorophenyl)-3-[1,2,4]triazol-1-yl-propyl]benzylamine (7d). Eluted with dichloromethane/methanol 98:2, oil (yield 20%) ^1H NMR ($CDCl_3$): δ 2.85–3.05 (*m*, 2H, H-1), 3.78 (d, 2H, J = 5.09 Hz, CH_2Ph), 3.85–3.98 (m, 1H, H-2), 4.45 (dd, 1H, J = 6.24, 13.87 Hz, H-3_A), 4.59 (dd, 1H, J = 6.24, 13.87 Hz, H-3_B), 6.96 (d, 1H, J = 8.21 Hz, H-6′), 7.20 (dd, 1H, J = 2.08, 8.21 Hz, H-5′), 7.25–7.39 (m, 5H, 5Ar), 7.39 (d, 1H, H-3′, J = 2.08 Hz), 7.69 (s, 1H, triazole), 7.87 (s, 1H, triazole). Anal. Calcd for $C_{18}H_{18}Cl_2N_4$: C, 59.84; H, 5.02; N, 15.51. Found: C, 59.77; H, 5.10; N, 15.39.

Synthesis of Compounds 8a,b,c,e. To a solution of **4** (1.84 mmol) in dry ethanol (10 mL)were added K_2CO_3 (11 mmol) and the appropriate bromide (3.68 mmol). The mixture was refluxed for 5 h and filtered at a reduced pressure. The filtrate was purified by flash chromatography on silica gel.

[2-(2,4-Dichlorophenyl)-3-[1,2,4]triazol-1-yl-propyl]diallylamine (8a). Eluted with dichloromethane/methanol 98:2. Yield 16%. ^1H NMR (300 MHz, $CDCl_3$): δ 2.55–2.72 (m, 2H, H-1), 3.0–3.12 (m, 4H, -N(C H_2CH=CH_2)$_2$), 3.85–3.95 (m, 1H, H-2), 4.30 (dd, 1H, J = 7.50, 15.00 Hz, H-3_B), 4.55 (dd, 1H, J = 5.00, 15.00 Hz, H-3_A), 5.07–5.12 (m, 4H, -CH=C H_2), 5.65–5.75 (m, 2H, -C H=CH_2), 6.92 (d,1H, J = 8.19 Hz, H-6′), 7.17 (dd, 1H, J = 2.23, 8.19 Hz, H-5′), 7.27 (d, 1H, J =2.23 Hz, H-3′), 7.62 (s, 1H, triazole), 7.75 (s, 1H, triazole). MS *m/z* (%) 355 (35), 353 (94), 351 (100), 258 (22), 256 (26), 97 (46), 91 (13). Anal. Calcd for $C_{17}H_{20}Cl_2N_4$: C, 58.13; H, 5.74; N, 15.95. Found: C, 58.19; H, 5.66; N, 16.06.

[2-(2,4-Dichlorophenyl)-3-[1,2,4]triazol-1-yl-propyl]-di-prop-2-ynylamine (8b). Eluted with dichloromethane/methanol 97:3, oil (yield 44%). ^1H NMR (300 MHz, $CDCl_3$): δ 2.22 (m, 1H, -C≡CH), 2.89 (m, 2H, H-1), 3.40–3.51 (m, 4H, -NC H_2C≡CH), 3.92 (m, 1H, H-2), 4.47 (dd, 1H, J = 6.75, 14.25 Hz, H-3_B), 4.60 (dd, 1H, J = 5.25, 14.25 Hz, H-3_A), 6.95 (d, 1H, J = 7.44 Hz, H-6′), 7.19 (dd, 1H, J = 2.23, 7.44 Hz, H-5′), 7.36 (s, 1H, J = 2.23 Hz, H-3′), 7.72 (s, 1H, triazole), 7.86 (s, 1H, triazole). MS *m/z* (%) 349 (8), 347 (18), 311 (7), 266 (10), 264 (15), 106 (100). Anal. Calcd for $C_{17}H_{16}Cl_2N_4$: C, 58.80; H, 4.64; N, 16.13. Found: C, 58,72; H, 4,66; N, 16,18.

(*E*)-[2-(2,4-Dichlorophenyl)-3-[1,2,4]triazol-1-yl-propyl]-bis(3-phenylallyl)amine (8c). Eluted with dichloromethane/methanol 97:3, oil (yield 36%). ^1H NMR (300 MHz, $CDCl_3$): δ 2.75–2.85 (m, 2H, H-1), 3.45 (d, 4H, J =8.00 Hz, -NC H_2CH=CH_2), 4.00–4.10 (m, 1H, H-2), 4.40–4.65 (m, 2H, H-3), 6.19 (m, 2H, -C H=CHPh), 6.48 (d,2H, J = 16.00 Hz, -CH=C HPh), 6.95 (d, J = 8.50 Hz, H-6′), 7.12 (dd, J = 2.23, 8.50 Hz, H-5′), 7.20–7.50 (m, 11H, 10Ar + H-3′), 7.80 (s, 1H, triazole), 7.90 (s, 1H, triazole). MS CI *m/z* (%) 505 (5), 503 (7), 387 (4), 154 (48), 117 (9), 91 (100). Anal. Calcd for $C_{29}H_{28}Cl_2N_4$: C, 69.18; H, 5.61; N, 11.13. Found: C, 69.08; H, 5.67; N, 11.19.

Bis(4-*tert*-butyl-benzyl)-[2-(2,4-dichlorophenyl)-3-[1,2,4]triazol-1-yl-propyl]amine (8e). Eluted with hexane/ethyl acetate 1:1, waxy solid (yield 66%), mp 84–85 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 1.41 (s, 18H, *t*But), 2.60–2.70 (m, 2H, H-1), 3.60 (m, 4H, -CH_2Ph),

3.90–4.40 (m, 3H, H-2 + H-3), 6.70 (d, 1H, J = 8.50 Hz, H-6′), 7.00–7.60 (m, 11H, Ar + H3′ + H-5′ triazole), 7.80 (s, 1H, triazole). MS CI m/z (%) 564(4), 565 (17), 563 (36), 415 (11), 322 (100), 147 (49). Anal. Calcd for $C_{33}H_{40}Cl_2N_4$: C, 70.32; H, 7.15; N, 9.94. Found: C, 71.04; H, 7.11; N, 10.03.

Synthesis of [2-(2,4-Dichlorophenyl)-3-[1,2,4]triazol-1-yl-propyl]-dibenzylamine (8d). Compound **4** (1.84 mmol) was dissolved in *N,N′*-dimethylpropyleneurea (5 mL, DMPU) and treated with K_2CO_3 (3.68 mmol) and the appropriate bromide (3.68 mmol). The mixture was heated and stirred for 4 h at 100 °C under nitrogen. The cooled mixture was diluted with water (15 mL) and extracted with ethyl acetate. The organic extracts were purified by chromatography on silica gel eluting with a solution of dichloromethane/methanol 98:2. Yield 47%. ^1H NMR (300 MHz, $CDCl_3$): δ 2.70 (d, 2H, J = 6.00 Hz, H-1), 3.54 (s, 4H, -CH_2Ph), 3.90–4.00 (m, 1H, H-2), 4.15 (dd, 1H, J = 9.00, 15.00 Hz, H-3_A), 4.55 (dd, 1H, J = 6.00, 15.00 Hz, H-3_B), 6.70 (d, 1H, J = 8.25 Hz, H-6′), 7.06 (dd, 1H, J = 2.25, 8.25 Hz, H-5′), 7.20–7.40 (m, 11H, -CH_2Ph + H-3′), 7.42 (s, 1H, triazole), 7.80 (s, 1H, triazole). MS m/z (%) 455 (3), 453 (19), 451 (33), 210 (100), 91 (56). Anal. Calcd for $C_{25}H_{24}Cl_2N_4$: C, 66.52; H, 5.36; N, 12.41. Found: C, 66.01; H, 5.30; N, 12,98.

Synthesis of Compounds 9a and 9b. To a solution of the amine **4** (5.8 mmol) in methanol (10 mL) were added acetic acid (5.8 mmol), 1-ethoxy-1-trimethylsilylcyclopropane (2.32 mmol), and sodium cyanoborohydride (2.32 mmol). The mixture was stirred and refluxed for 5 h. The solvent was removed at a reduced pressure to give a dense oil that was partitioned between ethyl acetate and a 2 N NaOH solution. The organic phase was dried and evaporated to obtain a crude that was purified by flash column chromatography on silica gel (eluent, ethyl acetate).

[2-(2,4-Dichlorophenyl)-3-[1,2,4]triazol-1-yl-propyl]dicyclopropylamine (9a). Yield 32%. ^1H NMR (300 MHz, $CDCl_3$): δ 0.38–0.58 (m, 8H, cyclopropyl), 1.85–1.95 (m, 2H, cyclopropyl-N), 3.10–3.20 (m, 2H, H-1), 4.05–4.20 (m, 1H, H-2), 4.30–4.50 (m, 2H, H-3), 6.98 (d, 1H, H-6′, J = 8.53 Hz), 7.10 (dd, 1H, J = 2.23, 8.53 Hz, H-5′), 7.30 (d, 1H, J = 2.23 Hz, H-3′), 7.61 (s, 1H, triazole), 7.82 (s, 1H, triazole). MS m/z (%): 353 (3), 351 (5), 317 (18), 315 (43), 270 (43), 268 (76), 256 (19), 218 (26), 186 (18), 159 (26), 110 (100). Anal. Calcd for $C_{17}H_{20}Cl_2N_4$: C, 58.13; H, 5.74; N, 15.95. Found: C, 58.01; H, 5.66; N, 16.08.

Allyl-cyclopropyl-[2-(2,4-dichlorophenyl)-3-[1,2,4]triazol-1-yl-propyl]amine (9b). Obtained from compound **7a**. Yield 39%. ^1H NMR (300 MHz, $CDCl_3$): δ 0.38–0.58 (m, 4H, cyclopropyl), 1.75–1.85 (m, 1H, cyclopropyl-N), 3.18–3.32 (m, 2H, H-1), 4.05–4.18 (m, 1H, H-2), 4.40 (dd, 1H, J = 6.70, 13.80 Hz, H-3_B), 4.54 (dd, 1H, J = 4.50, 13.80 Hz, H-3_A), 5.10–5.20 (m, 2H, -CH=C H_2), 5.75–5.90 (m, 1H, -C H=CH_2), 6.99 (d, 1H, J = 8.19 Hz, H-6′), 7.18 (dd, 1H, J = 2.23, 8.19 Hz, H-5′), 7.36 (d, 1H, J = 2.23 Hz, H-3′), 7.65 (s, 1H, triazole), 7.85 (s, 1H, triazole). MS m/z (%): 353 (1), 351 (3), 317 (12), 315 (29), 270 (14), 268 (24), 256 (19), 254 (32), 240 (12) 218 (11), 176 (15), 159 (17), 110 (100). Anal. Calcd for $C_{17}H_{20}Cl_2N_4$: C, 58.13; H, 5.74; N, 15.95. Found: C, 58.24; H, 5.59; N, 15.14.

***N*-[2-(2,4-Dichlorophenyl)-3-[1,2,4]triazol-1-yl-propyl]acetamide (5a).** Compound **4** (150mg, 0.55 mmol) was dissolved in pyridine (1 mL) and acetic anhydride (1 mL) The solution was stirred overnight at room temperature and then evaporated to dryness. The residue was purified by flash chromatography with dichloromethane as eluent to give 168 mg of the title compound.Yield 98%. ^1H NMR (300 MHz, $CDCl_3$): δ 1.96 (s, 3H, Ac), 3.50–3.56 (m, 2H, H-1), 3.95–4.01 (m, 1H, H-2), 4.45 (d, 2H, J = 6.33 Hz, H-3), 5.78 (brs, 1H, NH), 6.92 (d, 1H, J = 8.19 Hz, H-6′), 7.20 (dd, 1H, J = 2.23, 8.19 Hz, H-5′), 7.41 (d, 1H, J = 2.23 Hz, H-3′), 7.82 (s, 1H, triazole), 7.91 (s, 1H, triazole). Anal. Calcd for $C_{13}H_{14}Cl_2N_4O$: C, 49.86; H, 4.51; N, 17.89. Found: C, 49.77; H, 4.61; N, 18.02.

***N*-[2-(2,4-Dichloro-phenyl)-3-[1,2,4]triazol-1-yl-propyl]-benzamide (5b).** A solution of freshly distilled benzoyl chloride (81 mg, 0.58 mmol, 66 μL) in dichloromethane (2 mL) was added dropwise to a stirred solution of **4** (150 mg, 0.55 mmol) and triethylamine (58 mg, 0.577 mmol, 81 μL) at 0 °C under nitrogen. The resulting mixture was stirred at room temperature for 3 h, and then water (10 mL) was added. The organic layer was separated, dried with Na_2SO_4, and evaporated at a reduced pressure to give the crude product. Purification by flash chromatography (dichloromethane/methanol 9:1) afforded 151 mg (yield 71%) of the title compound as a sticky solid. ^1H NMR (300 MHz, $CDCl_3$): δ 3.70–3.86 (m, 2H, H-1), 3.95–4.15 (m, 1H, H-2), 4.50 (d, 2H, J = 5.95 Hz, H-3), 6.86 (t, 1H, J = 6.33 Hz, NH), 6.96 (d, 1H, J = 8.56 Hz, H-6′), 7.17 (dd, 1H, J = 2.23, 8.56 Hz, H-5′), 7.38–7.58 (m, 3H, 3Ar), 7.71 (d, 2H, J = 8.93 Hz, 2Ar), 7.85 (s, 1H, triazole), 7.90 (s, 1H, triazole). Anal. Calcd for $C_{18}H_{16}Cl_2N_4O$: C, 57.61; H, 4.30; N, 14.93. Found: C, 57.74; H, 4.27; N, 14.99.

But-2-enoic Acid [2-(2,4-Dichlorophenyl)-3-[1,2,4]triazol-1-yl-propyl]amide (5c). Compound **4** (150 mg; 0.55 mmol) was allowed to react with triethylamine (58 mg; 0.557 mmol; 81 μL) and crotonoylchloride (60.3 mg; 0.577 mmol; 55 μL). The mixture was stirred at room temperature for 3 h, and then water (10 mL) was added. The organic layer was separated, dried with Na_2SO_4, and evaporated at a reduced pressure to give the crude product. Purification by flash chromatography (dichloromethane/methanol 9:1) gave 155 mg (yield 64%) of pure compound **5c**. ^1H NMR (300 MHz, $CDCl_3$): δ 1.85 (dd, 3HJ = 1.49 Hz, CH_3-,), 3.54–3.74 (m, 2H, H-1), 3.90–4.10 (m, 1H, H-2), 4.45 (d, 2H, J = 5.95 Hz, H-3), 5.75 (dd, 1H, J = 1.49, 15.26 Hz, CH=), 6.80 (dq, 1H, J = 15.26, 6.70 Hz, CH=), 6.91 (d, 1H, J = 8.56 Hz, H-6′), 7.19 (dd, 1H, J = 1.86, 8.56 Hz, H-5′), 7.40 (d, 1H, J = 1.86 Hz, H-3′), 7.83 (s, 1H, triazole), 7.90 (s, 1H, triazole). Anal. Calcd for $C_{15}H_{16}Cl_2N_4O$: C, 53.11; H, 4.75; N, 16.52. Found: C, 53.02; H, 4.70; N, 16.61.

Partition Coefficients. The log P (where P is the octanol–water partition coefficient) was obtained as described in ref *13*, using reversed-phase chromatography (*14*) by comparison with seven reference compounds whose log P are known (*15*). Retention times were determined on a Hewlett-Packard HPLC equipped with a quaternary pump HP-1050 with a Rheodyne injector (20 L loop) with an UV–vis detector HP-1050 or a Waters Lambda Max Model 481. Methanol for HPLC was purchased from Baker, and water for HPLC was produced with a Milli-Q Water purification system (Millipore). Analyses were performed on a Merck column LiChrospher 100 RP18 (250 mm × 4 mm, 5 m), the flow rate was 1 mL/min, using methanol/ammonium phosphate buffer at pH 7.0 80:20 as eluent.

Biological Assays. Compounds were tested in vitro and in vivo against a number of phytopathogenic fungi. The results were compared with those obtained with tetraconazole, used as a standard compound.

Test Pathogens. Botrytis cinerea Pers., *Cercospora beticola* Sacc., *Cercosporella herpotricoides* Fron., *Fusarium roseum* Link., and *Helminthosporium teres* Sacc. of the IPV Collection (Milan, Italy) were maintained on potato dextrose agar Difco (PDA); *Ustilago maydis* (DC) Corda, strain ATCC 14826 was cultured on Czapek Agar Difco (CZA) and Coursen and Sisler broth.

Erysiphe graminis DC f. sp. *tritici*Marchel and *Puccinia recondita* Rob. ex Desm. f. sp. *tritici* were grown on stock plants. *Phytophtora infestans* (Mont.) De Bary kindly provided by Isagro was maintained on V8 juice agar and on tomato stock plants.

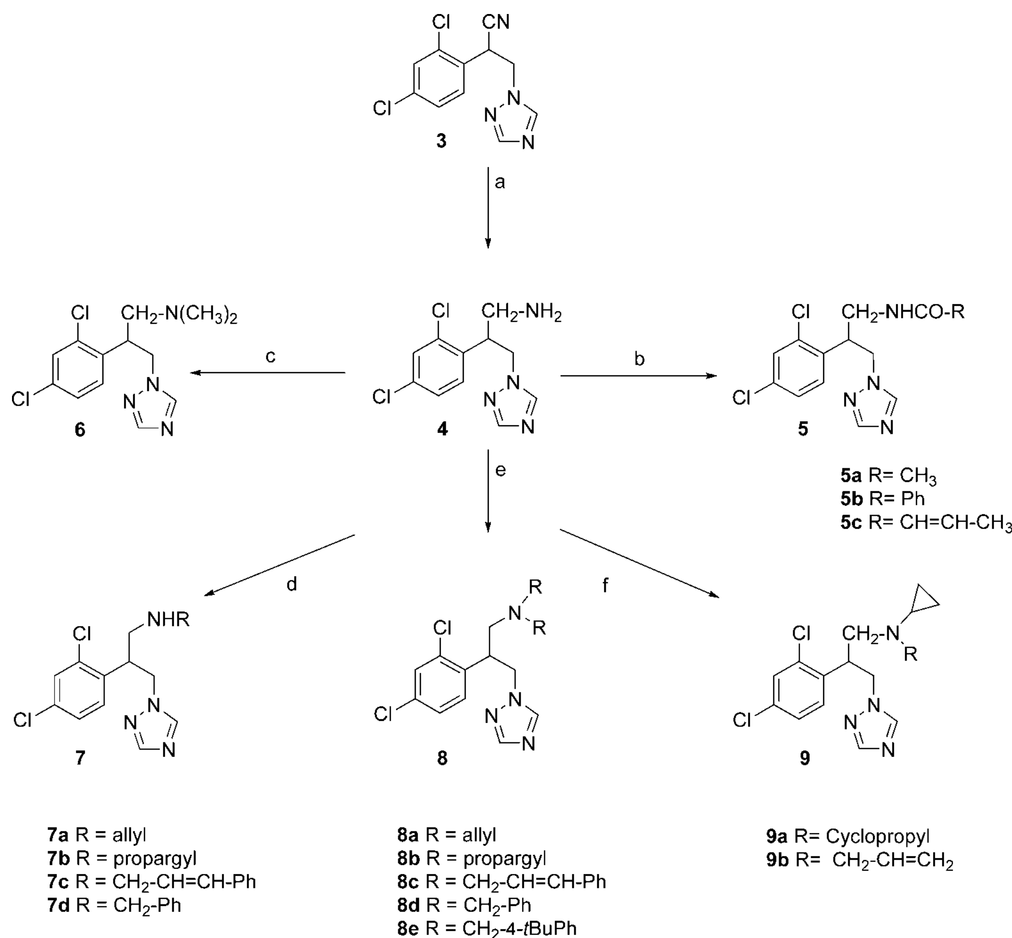
Among the pathogens selected, some were chosen because they are typically controlled by azoles, others because they are generally less sensitive.

Plants. *Lycopersicon esculentum* L. cv Marmande and *Triticum aestivum* L. cv Gemini used in this study were grown in plastic pots (diameter 11 cm) in a growth room or greenhouse at 21 ± 1 °C and 70 ± 10 % RH.

In Vitro Fungicidal Activity. The activity of the new compounds against *B. cinerea*, *C. beticola*, *C. herpotricoides*, *F. roseum*, *H. teres,* and *U. maydis* was determined in vitro on PDA or CZA as inhibition of the radial growth at different concentrations (100–0.1 mg L^{-1}). Growth inhibition was calculated from the mean difference between treated and control cultures as a percentage of the latter. Percentage growth inhibition was plotted on a probit scale against compound doses in order to obtain EC_{50} values (50% inhibitory concentration). Compound **7** was also tested on *U. maydis* broth cultures (*7*). Growth was estimated by cell count at the end of the treatment, and the results were expressed as percentage inhibition compared to the control. These cultures were used for sterol and squalene analysis.

In Vivo Fungicidal Activity. Compounds **7c**, **8a**, **8b**, and **9a** were assayed in preventive application tests. They were dissolved in acetone/

Scheme 1[a]



[a] Reagents and conditions: (a) $NaBH_4$, $CoCl_2$, MeOH, 2 h, rt, 52%; (b) acetic anhydride, Py, rt, overnight, quantitative; PhCOCl or crotonylchloride, TEA, CH_2Cl_2, rt, 3 h; (c) HCOOH, HCHO, 120 °C, 6 h, 45%; (d) K_2CO_3, R-Br, EtOH, rt, 5–20 h; (e) K_2CO_3, R-Br, EtOH, reflux, 5 h or DMPU, K_3CO_3, benzyl bromide, 100 °C 4 h (47% for **8d**); (f) **4** or **7a**, methanol, glacial acetic acid, (1-Ethoxycyclopropoxy)trimethylsilane, $NaCNBH_3$, reflux, 5 h.

distilled water (1:4, v/v) containing Tween 20 (0.3 mg mL^{-1}) and sprayed on wheat (7 days old) and tomato plant (six leaves stage) until moist, at the standard concentration of 125 μg mL^{-1}. One day later, wheat plants were inoculated with a water suspension of *P. recondita* conidia (1 mg mL^{-1} plus 0.1 % Tween 20), and tomato plants with a suspension of *P. infestans* zoosporangia (5×10^4 mL^{-1}). After inoculation, wheat and tomato plants were kept 24 h in an incubation cabin at 20 ± 1 °C and 100% RH and then transferred to a greenhouse (23 ± 1 °C and 70 ± 10 % RH). Inoculation of wheat plants with *E. graminis* was obtained by strongly stirring infected plants with abundant sporification on the test plants, then moving the plants to a growth room (20 ± 1 °C and 70 ± 10 % RH). The fungicidal activity was evaluated on wheat plants 10 days and on tomato plants 7 days after inoculation. The area of inoculated treated leaves covered by disease symptoms was assessed and compared with that of nontreated ones to determine the percentage inhibition of disease.

Squalene and Sterol Analysis of *U. maydis* Sporidia. Cultures of *U. maydis* grown in Coursen and Sisler broth amended with different concentrations of compound **8a** (0.01–100 mg L^{-1}) were used for sterol and squalene analysis. After 18 h treatment, sporidia were harvested by centrifugation and added with a known amount of dihydrocholesterol, and then heated with 2 mL of 20% KOH in 60% ethanol for 1 h at 90 °C. The unsaponifiable fraction was extracted with heptane (3 × 3 mL), dried with anhydrous sodium sulfate, and concentrated in vacuo. A known amount of cholestane was added as a second internal standard before derivatizing samples as trimethylsilyl ethers with a 1:1 mixture of pyridine/*N*,*O*-bis(trimethylsilyl)trifluoroacetamide (1:1, v/v). Gas chromatographic analyses were performed on a DANI 3800 gas chromatograph equipped with a FID detector and PTV injector. A capillary column SPB5 Supelco, 30 m × 0.25 mm i.d., 0.25-μm film thickness, was used. Analysis conditions were carrier gas, helium; flow, 0.75 mL/min; oven temperature, 275 °C; injector from 30 to 290 °C in 30 s; splitless injection; after 30 s split 30 mL min^{-1}; detector 300 °C. Data were recorded by a Shimadzu CR2A integrator. Gas chromatography–mass spectrometry analyses were performed on a Finnigan TSQ70 instrument, equipped with the same column. Analysis conditions were oven, 265 °C; injector, 280 °C; transfer line, 280 °C; electron energy, 70 eV; electromultiplier, 900V. The results are the average of two replicates per test.

RESULTS AND DISCUSSION

Chemistry. The key intermediate for the preparation of all new compounds was amine **4** (**Scheme 1**).

It was obtained by reduction of the corresponding nitrile (**3**) (kindly provided by Isagro, Milano) with $NaBH_4$ and $CoCl_2 \cdot 6H_2O$. This procedure, despite the formation of stable emulsions that complicated the workup, gave better yields than diborane without catalysts. Standard acetylation methods gave the amides **5a–c**. The *N*,*N*′-dimethylderivative **6** was prepared by Leuckart methylation of **4** with formic acid and formaldehyde. The secondary amines **7a–d** were obtained by alkylation of **4** with the corresponding alkylbromides. The reactions were performed at room temperature in the presence of potassium carbonate as a base. The corresponding tertiary amines **8a–c** and **8e** were obtained after refluxing the ethanolic solutions for about 5 h. Compound **8d** was obtained with a better yield using *N*,*N*-dimethylpropyleneurea (DMPU) as a solvent, as reported by Juaristi et al. (*16*).

As far as the synthesis of cyclopropyl derivatives is concerned, a survey of the literature revealed few methods to

Table 1. In Vitro Fungicidal Activity Expressed as 50% Effective Concentration (EC_{50}, μg mL^{-1})

	log *P*	*B. cinerea*	*C. beticola*	*C. herpotricoides*	*F. roseum*	*H. teres*	*U. maydis*
4	1.92[a]	>100	>100	>100	>100	>100	70.30
5a	1.85[a]	>100	>100	>100	>100	>100	60.00
5b	3.75[a]	~100	2.5	80.3	>100	11.5	1.90
5c	2.90[a]	>100	60.5	>100	>100	>100	20.70
6	2.54	75.0	8.0	6.5	>100	42.0	2.50
7a	3.13	>100	31.7	91.3	>100	>100	19.9
7b	2.15	38.0	4.0	11.0	>100	38.0	1.30
7c	3.67	42.0	2.4	7.5	26.0	12.0	1.40
7d	4.17	>100	2.7	23.4	>100	30.3	11.3
8a	3.92	10.0	2.6	3.6	>100	6.5	0.22
8b	3.48	4.9	1.1	2.8	9.0	2.1	0.14
8c	5.00	>100	12.5	>100	>100	6.0	0.65
8d	4.60	6.0	4.0	17.0	>100	1.9	0.09
8e	9.69	>100	7.0	>100	>100	>100	9.00
9a	2.86	7.5	0.4	1.2	55.0	5.5	0.02
9b	2.86	4.4	0.2	0.8	55.0	3.2	0.01
Tetra[b]	3.99[a]	0.04	0.12	0.45	4.00	0.40	0.25

[a] Calculated (ChemDraw 7.0 package). [b] Tetraconazole.

cyclopropylate primary or secondary amines (*17–20*). Moreover, these routes could not be extended to aliphatic amines, which were of interest to us. To the best of our knowledge, only Gillaspy et al. reported an efficient, one-step method to cyclopropylate aliphatic amines, based on the reductive amination with (1-ethoxycyclopropoxy)trimethylsilane and $NaCNBH_3$ (*21*). Following this procedure we obtained cyclopropylamines **9a** and **9b** in fair yield. Attempts to isolate the monosubstituted cyclopropyl derivative of **4** failed, as the reaction gave only the disubstituted derivative **9a**.

In Vitro Assays. The in vitro fungitoxicity of the new compounds expressed as EC_{50} is reported in **Table 1** with tetraconazole used as a standard.

Some of the compounds (**6**; **7b–d**; **8a**,**b**,**d**; **9a**,**b**) showed fairly good activity, in particular against *U. maydis,* which seems to be the most sensitive one among the tested fungi. In fact, EC_{50} values of compounds **9a,b**, **8b**, and **8d** against this pathogen are lower than the EC_{50} of the standard tetraconazole. *C. beticola* and *H. teres* growth was also inhibited by various compounds at low concentrations.

On the contrary, *F. roseum* was the pathogen less sensitive to this group of compounds, followed by *B. cinerea* and *C. herpotricoides*.

The most active compounds were **7c**, **8b**, an **9a,b**, followed by **8d**. Compound **8e** seemed to act selectively against *C. beticola* and *U. maydis*. Interestingly, compound **8b** showed a significant activity against *F. roseum*, having an EC_{50} value comparable to that of the standard tetraconazole, despite the generally very low efficacy of triazole fungicides against this pathogen. This is a promising result, as rot and wilt agents of many economically important crops belong to the genus *Fusarium*. Some species are also responsible for mycotoxin contamination of cereals and cereal-derived foods and feeds.

When the amino moiety is involved in an amidic bond (**5a–c**), there is a dramatic decrease of activity.

To test the effect of lipophilicity on the antifungal activity, the log *P* for most compounds was measured with a HPLC method (**Table 1**). The most active compounds showed a log *P* in the range 2.54–3.92. Compounds **8c,d** and **8e**, characterized by log *P* values higher than 4.60, showed a decrease in activity. On the other hand, the unsubstituted amine **4** (log $P = 1.85$) was substantially inactive.

A comparison of the disubstituted amines (**7a–d**) with the corresponding trisubstituted ones (**8a–d**) revealed that the activity is mainly correlated to log *P*. When the introduction of further alkyl groups leads to compounds with an intermediate log *P* (see **7a**,**b**,**d** versus **8a**,**b**,**d**), the activity increases going from a secondary to the corresponding tertiary amine. On the contrary, when the introduction of the third alkyl group leads to compounds having a log *P* higher than 4.60 (see **7c** versus **8c**), a drop in the biological activity is observed, with the only exception of *H. teres* and *U. maydis*.

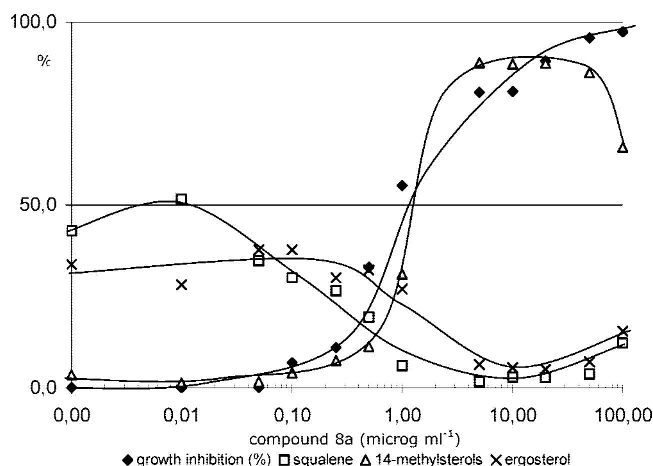


Figure 1. Growth inhibition of *U. maydis* by count of sporidia versus percentage composition of unsaponifiable fraction after incubation with increasing concentrations of compound **8a**.

The introduction of an amide moiety is detrimental to the activity (compounds **5a** and **5c**). It is likely that the increase in polarity makes it difficult for the compounds to be carried through the cell membrane. In fact some activity is restored in the less polar **5b**. All of these results corroborate the hypothesis that lipophilicity plays an important role in determining antifungal activity. This is in agreement with the results we obtained in our previous work with a similar series of compounds. (*11*)

The compounds were also tested in vitro against a series of human fungal pathogens, such as some strains of *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus niger*, *Trichophyton mentagrophytes*, and *Microsporum canis*. No remarkable activity was found except for compounds **7c** and **8b**, which were active against *C. albicans* strain SKF2270 (MIC 0.13 and 0.25 μg mL^{-1}, respectively, compared with the standard fluconazole 0.25 μg mL^{-1}) and against a strain of fluconazole- and itraconazole-resistant *C. albicans* (MIC 2.0 and 16 μg mL^{-1}, respectively).

In Vivo Assays. Some of the compounds most active in vitro (**7c**, **8a**, **8b**, **9a**) were also assayed for their preventive activity in vivo on two pathogen–host combinations, i.e., *Erysiphe graminis* f. sp . *tritici–Triticum aestivum* (wheat powdery mildew) and *Puccinia recondita* f. sp . *tritici–Triticum aestivum* (wheat brown rust). As already mentioned, our compounds show a structure similar to that of allylamines, well-known inhibitors of squalene biosynthesis. For this reason, we selected a further pathogen–host combination (*Phytophthora infestans–Lycopersicon esculentum)* to rule out squalene biosynthesis inhibition as a possible side mechanism of action for this class of compounds. In fact, the last pathogen does not synthesize sterols and is therefore insensitive to triazole fungicides.

Results are reported in **Table 2**. All compounds were more active in protecting wheat plants from powdery mildew than from rust. Compound **9a** induced the highest inhibition, controlling powdery mildew at 60% at 125 μg mL^{-1}. As expected, the tested compounds were completely inactive against tomato late blight.

Table 2. In Vivo Protectant Activity Expressed as Disease Percentage Inhibition at One Standard Concentration (125 μg mL^{-1})

compound	*P. infestans/ L. esculentum*	*E. graminis/ T. aestivum*	*P. recondita/ T.aestivum*
7c	0	25	20
8a	0	30	25
8b	0	35	15
9a	0	60	10
tetraconazole	0	100	100

Squalene and Sterol Analysis. Broth cultures of *U. maydis* treated with different concentrations of compound **8a** were used to check the activity of this amine as a squalene epoxidase inhibitor, as shown by some related molecules (allylamines) with antifungal activity (*22*). Interestingly, squalene percentage decreased as long as growth inhibition increased (**Figure 1**). Therefore the squalene epoxidase inhibition could be ruled out as a possible side mechanism of action for this compound. Furthermore, growth inhibition was strictly related to an increase in the 14-methyl sterols percentage, due to the inhibition of the 14α-demethylase, a key enzyme of the ergosterol biosynthesis pathway. The inhibition of this enzyme is the typical mechanism of action for triazoles in general. (*23*)

In conclusion, the replacement of the ether group of tetraconazole with a secondary or tertiary amino group maintains the antifungal activity on several phytopathogenic fungi, provided that the substituents are not too bulky or lipophilic. The allyl, propargyl, and cyclopropyl groups appear particularly suitable. Although these compounds have some structural similarities with terbinafine and naftifine, which act as squalene epoxidase inhibitors, they maintain the usual mechanisms of action of the other triazoles.

ACKNOWLEDGMENT

We are indebted to Dr. L. Mirenna (ISAGRO Research) for the in vivo tests and Lepetit (then Dow), Gerenzano, for the tests against human pathogenic strains.

LITERATURE CITED

(1) *Modern Crop Protection*; Krämer, W., Schirmer, U., Eds.; Wiley-VCH: Weinheim, Germany, 2007.
(2) Berg, D. Biochemical mode of action of fungicides. Ergosterol biosynthesis inhibitors. In *Fungicide Chemistry: Advances and Practical Applications*; Green, M.B., Spilker, D.A., Eds.; ACS Symposium Series 304; American Chemical Society: Washington, DC, 1986; pp 25–51.
(3) Song, Z.; Nes, W.D. Sterol biosynthesis inhibitors: potential for transition state analogs and mechanism-based inactivators targeted at sterol methyltransferase. *Lipids* **2007**, *42*, 15–33.
(4) Zirngibl, L. *Azoles-Antifungal Active Substances-Synthesis and Uses*; Wiley-VCH: Weinheim, 1998.
(5) Sano, S., Shimoda, S., Hamamura, H., Yokota, C., Sano, H., Saiga, T. In *Development of New Agrochemicals*; Ikura, K., Ed.; C.M.C. Co. Ltd.: Tokyo, 2000; p 23.
(6) Kuck, K.H., Vors, J.P. Sterol Biosynthesis Inhibitors. In *Modern Crop Protection*; Krämer, W., Schirmer, U., Eds.;Wiley-VCH: Weinheim, Germany, 2007; Vol. 2, pp 605–650.
(7) Carzaniga, R.; Carelli, A.; Farina, G.; Arnoldi, A.; Gozzo, F. Comparative antifungal effect and mode of action of tetraconazole on Ustilago maydis. *Pestic. Biochem. Physiol.* **1991**, *40*, 274–283.
(8) Carelli, A.; Farina, G.; Gozzo, F.; Merlini, L. Interaction of tetraconazole and its enantiomers with cytochrome P450 from Ustilago maydis. *Pestic. Sci.* **1992**, *35*, 167–170.
(9) Gozzo, F.; Carelli, A.; Carzaniga, R.; Farina, G.; Arnoldi, A.; Lamb, D.; Kelly, S. L. Stereoselective interaction of tetraconazole with 14α-demethylase in fungi. *Pestic. Biochem. Physiol.* **1995**, *53*, 10–22.
(10) Gozzo, F.; Garavaglia, C.; Mirenna, L. Enantiomers and racemate of M14360: investigation of their antifungal activity. *Tagungsber. Akad. Landwirtschaftswis., DDR* **1990**, *291*, 87–94.
(11) Arnoldi, A.; Carzaniga, R.; Morini, G.; Merlini, L.; Farina, G. Synthesis, fungicidal activity and QSAR of a series of 2-dichlorophenyl-3-triazolylpropyl ethers. *J. Agric. Food Chem.* **2000**, *48*, 2547–2555.
(12) Bracher, F. Point of attack of ergosterol biosynthesis: Active components and effective principles. *Pharm. Unserer Zeit* **2003**, *32*, 118–123.
(13) Arnoldi, A.; Merlini, L. Lipophilicity-antifungal activity relationships for some isoflavonoid phytoalexins. *J. Agric. Food Chem.* **1990**, *38*, 834–838.
(14) Braumann, T. Determination of hydrophobic parameters by reversed-phase liquid chromatography: Theory, experimental techniques, and application in studies on quantitative structure-activity relationships. *J. Chromatogr.* **1986**, *373*, 191–225.
(15) Nys, G. G.; Rekker, R. F. The concept of hydrophobic fragmental constants (f-values). Extensions of its applicability to the calculation of lipophilicities of aromatic and heteroaromatic structures. *Eur. J. Med. Chem. Chim. Ther.* **1974**, *9*, 361–375.
(16) Juaristi, E.; Murer, P.; Seebach, D. Use of *N,N′*-dimethylpropyleneurea (DMPU) as solvent in the efficient preparation of enantiomerically pure secondary amines. *Synthesis* **1993**, 1243–1246.
(17) Maeda, Y.; Ingold, K.U. Kinetic applications of electron paramagnetic resonance spectroscopy. 35. The search for a dialkyl aminyl rearrangement. Ring opening of *N*-cyclobutyl-*N*-*n*-propylaminyl. *J. Am. Chem. Soc* **1980**, *102*, 328–331.
(18) Kang, J.; Kim, K.S. *N*-Cyclopropylation of aromatic amines. *J. Chem. Soc., Chem. Commun.* **1987**, 897–898.
(19) Arvidsson, L.E.; Johansson, A.M.; Hacksell, U.; Nilsson, J.L.G.; Svensson, K.; Hjorth, S.; Magnusson, T.; Carlsson, A.; Lindberg, P.; Andersson, B.; Sanchez, D.; Wikstrom, H.; Sundell, S. *N,N*-Dialkylated monophenolic trans-2-phenylcyclopropylamines: novel central 5-hydroxytryptamine receptor agonists. *J. Med. Chem.* **1988**, *31*, 92–99.
(20) Yoshida, Y.; UmezuK.Hamada, Y.; Atsumi, N.; Tabuchi, F. Practical synthesis of N-cyclopropylanilines via direct condensation of anilines with [(1-ethoxycyclopropyl)oxy]trimethylsilane. *Synlett* **2003**, *14*, 2139–2142.
(21) Gillaspy, M.L.; Lefker, B.A.; Hada, W.A.; Hoover, D.J. A simple method for the formation of cyclopropylamines: the first synthesis of tricyclopropylamine. *Tetrahedron Lett.* **1995**, *36*, 7399–7402.
(22) Kumita, LStudies in antifungal compounds inhibiting squalene epoxidase. *J. Pestic. Sci.* **2002**, *27*, 404–408.
(23) Köller, W. Antifungal agents with target sites in sterol functions and biosynthesis. In *Target Sites of Fungicide Action*; Köller, W., Ed.; CRC Press: Boca Raton, FL, 1992; pp 119–206.

Received for review June 4, 2007. Revised manuscript received July 18, 2007. Accepted July 18, 2007.

JF071631G