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A hybrid between the antifungal azole eberconazole and the alkaloid onychine

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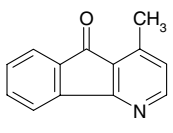
The 9-imidazolylazafluorene **3**, a hybrid between the antifungal azole eberconazole and the alkaloid onychine, was prepared from onychine in two steps. This product shows moderate antifungal activity, but, in contrast to other azoles, is not an inhibitor of ergosterol biosynthesis.

1. Introduction

The need for new antifungal drugs is obvious because of the increasing number of fungal infections, especially systemic mycoses, which mainly occur in immunocompromised patients (Fernandes 1992). Only very few new antifungals have come to the market in the last few years, among them the new triazole voriconazole (Chandrasekar 2001), and the semisynthetic lipopeptide caspofungin (Keating 2001). Thus, still large efforts are made to develop new antifungals.

Natural products have been shown to be a rich source of new antifungal compounds, e.g. the echinocandines, sordarines, and nikkomycines (Ernst 2001). Investigations performed in our (Bracher 1993) and other groups (Hufford 1987, Peterson 1992) showed that polycyclic aromatic alkaloids from Annonaceae like onychine (**1**) and sampangine (**2**) exhibit potent antifungal activities. Up to now the molecular mechanism of action of these alkaloids is not known.

In continuation of our research on alkaloids from Annonaceae (Bracher 1992) and analogues thereof, we intended to prepare a hybrid **3** between onychine and one new imidazole antifungal, eberconazole (**4**) (N. N. 1996). The rationale of this attempt was to investigate the effect of a replacement of the dibenzocycloheptyl substituent of **4** by the structurally related tricyclic azafluorene moiety of onychine.



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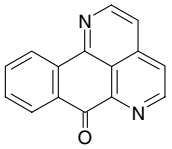

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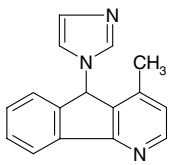

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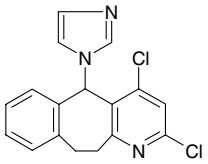

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2. Investigations, results and discussion

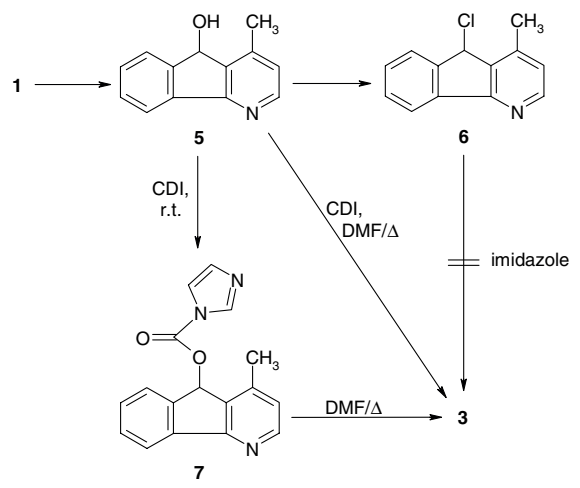
2.1. Chemistry

Alcohol **5** was obtained by reduction of onychine (**1**) (Bracher 1989) with $NaBH_4$ in an almost quantitative manner (Hufford 1987). Subsequent reaction with thionyl chloride gave the chloro compound **6** in 81% yield. The yield could be raised to 93% by adding catalytic amounts of $ZnCl_2$ (Squires 1975) and 4-dimethylaminopyridine to the reaction mixture.

First attempts to convert the chloro compound **6** the target product **3** by nucleophilic substitution with imidazole, as described for the synthesis of eberconazole (Andreoli 1990), gave disappointing results. Reactions of equimolar amounts of **6** and imidazole resulted in very poor conversion. Using a large excess of imidazole, complete conversion was accomplished, but product **3** could not be separated from excess imidazole under various conditions (extraction with water, column chromatography, crystallization).

Finally, hybrid **3** was obtained using a methodology for the direct conversion of alcohols to *N*-alkylimidazoles with *N*,*N*′-carbonyldiimidazole (CDI) (Njar 2000). Thus, alcohol **5** was reacted with CDI in acetonitrile to give one single product within 2 h. The spectroscopic data of the product, however, showed that it is not the desired imidazolylazafluorene **3**, but the carbamate **7**. The constitution of **7** could unambiguously be determined by IR spectroscopy (carbonyl resonance at 1751 cm^{-1}), mass spectroscopy (m/z = 291) and ^{13}C NMR spectroscopy (carbonyl resonance at 149.4 ppm). In order to convert this carbamate to the target compound **3**, we first carried out the

Scheme



reaction in acetonitrile under reflux, but a complex mixture of products was obtained. The only reaction product we could identify, was the ketone onychine (**1**).

Finally, we found that heating of carbamate **7** in DMF gives the desired imidazole **3** in 76% yield. The conversion of alcohol **5** to **3** could also be performed in a one pot reaction with CDI in refluxing DMF in 62% yield.

2.2. Antifungal activity

Hybrid **3** and the intermediates onychine (**1**) and **7** were tested for antifungal activity against *Aspergillus niger*, *Candida glabrata*, and *Yarrowia lipolytica* in an agar diffusion assay. As a reference substance we used the known imidazole antifungal clotrimazole. The results are presented in the Table.

These results clearly indicate that **3** has moderate activity against *A. niger* and *C. glabrata*, but only very poor activity against *Y. lipolytica*. Carbamate **7** was found to be almost inactive, whereas the parent alkaloid onychine (**1**) inhibits the growth of *C. glabrata* and *Y. lipolytica*. The reference substance clotrimazole was found to be the most active compounds against the three strains.

Nevertheless we performed investigations to find out, whether **3** is an inhibitor of ergosterol biosynthesis using a screening system recently developed in our group (Bracher 2003).

Thus, the strain *Y. lipolytica* was incubated with **3**, and the sterol pattern was analyzed by GLC-MS and HPLC-MS. Inhibitors of ergosterol biosynthesis, like the C14-demethylase inhibitors eberconazole (**4**) and clotrimazole, result in significant and typical changes in the sterol pattern (accumulation of lanosterol). Compound **3**, despite reducing the biomass of the yeast up to 30% compared to the blank experiment, did not cause any changes in the sterol pattern of *Y. lipolytica*. Thus, we can conclude that the antifungal activity of this eberconazole-onychine hybrid is not due to an inhibition of ergosterol biosynthesis.

Table: Antifungal activities determined in an agar diffusion assay

Compd.	Strain		
	A. niger	*C. glabrata*	*Y. lipolytica*
1	(−)	10	12
3	8	14	(±)
7	7	(−)	(−)
Clotrimazole	21	15	26

Diameters of zones of inhibition in mm; determined at concentrations of 50 μg/plate; (±) = poor inhibition, (−) = no inhibition

3. Experimental

3.1. General

Melting points were determined with a Büchi B-50 apparatus. IR spectra were run on a Perkin Elmer IR-881 spectrometer as KBr plates. ^{1}H NMR and ^{13}C NMR spectra were recorded on Joel GSX 400 and Joel GSX 500 instruments in $CDCl_3$ solution. Chemical shifts are expressed in ppm with reference to TMS as an internal standard. Mass spectra were measured on a Hewlett Packard 5989 A mass spectrometer. Elemental analyses were run on a Vario EL analysator. All the results were in an acceptable range. Flash column chromatography (CC) was carried out on silica gel (Merck, Kieselgel 60).

3.2. Synthesis of the compounds

3.2.1. Synthesis of (R,S)-5-chloro-4-methyl-5H-indeno[1,2-b]pyridine (**6**)

A solution of **5** (395 mg, 2.00 mmol) in 30 ml benzene was treated with 10 mg anhydrous zinc chloride and 10 mg 4-dimethylaminopyridine. Thionyl chloride (0.40 ml, 5.4 mmol) was added dropwise to this solution with vigorous stirring. After 30 min the precipitate was collected by filtration and washed with diethyl ether. The residue (mainly the hydrochloride of **6**) was dissolved in water (40 ml) and treated with 20 ml saturated $NaHCO_3$ solution, followed by extraction with ethyl acetate (3 × 30 ml). The combined organic layers were dried over Na_2SO_4, evaporated, and purified by CC (ethyl acetate : hexane, 1 : 1) to give 401 mg (93%) of **6** as a white solid; m.p. of the hydrochloride 219 °C. MS (70 eV) : m/z 217 (9) [M^+], 215 (24) [M^+], 182 (29), 180 (100); IR (KBr): (hydrochloride): ν_{max} = 3074, 3036, 2871, 2552, 2040, 1975, 1631, 1618, 1453, 1354, 1252, 1029, 828, 773, 750 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.45 (d, J = 5.2 Hz, 1 H, 2-H), 7.98 (d, J = 5.3 Hz, 1 H, 6-H), 7.68 (d, J = 5.1 Hz, 1 H, 9-H), 7.45–7.50 (m, 2 H, 7-H and 8-H), 7.01 (d, J = 5.2 Hz, 1 H, 3-H), 5.80 (s, 1 H, 5-H), 2.54 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$): δ 158.8 (C-9b), 150.3 (C-2), 145.2 (C-4a), 144.3 (C-5a), 139.5 (C-9a), 135.8 (C-4), 129.9 and 129.8 (C-7, C-8), 125.8 (C-6), 124.1 (C-3), 121.2 (C-9), 54.9 (C-5), 18.3 (CH_3).
$C_{13}H_{10}ClN$

3.2.2. Synthesis of (R,S)-5-(imidazol-1-ylcarbonyloxy)-4-methyl-5H-indeno[1,2-b]pyridine (**7**)

Compound **5** (95 mg, 0.48 mmol), dissolved in 5 ml acetonitrile under a nitrogen atmosphere, was treated with 102 mg (0.63 mmol) *N,N′*-carbonyldiimidazole. After stirring for 2 h 30 ml ice-water were added and the mixture was stored at 4 °C over night. The resulting precipitate was collected by filtration, washed with ice-cold water, and dried at 120 °C for 15 min to give 125 mg (90%) of **7** as a white solid, m.p. 117 °C.
MS (70 eV) : m/z 291 (3) [M^+], 247 (14), 180 (100); IR (KBr): ν_{max} = 3128, 3118, 3008, 2921, 2852, 1751, 1714, 1601, 1576, 1481, 1454, 1400, 1323, 1279, 1252, 1171, 1001, 752 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.48 (d, J = 5.2 Hz, 1 H, 2-H), 8.13 (br. s, 1 H, 2′-H), 7.96 (d, J = 7.6 Hz, 1 H, 9-H), 7.69 (dd, J = 7.6, 0.6 Hz, 1 H, 6-H), 7.52 (td, J = 7.6, 0.6 Hz, 1 H, 8-H), 7.42 (t, J = 7.6 Hz, 1 H, 7-H), 7.41 (d, J = 1.0 Hz, 1 H, 4′-H), 7.06 (d, J = 1.0 Hz, 1 H, 5′-H), 7.02 (d, J = 5.2 Hz, 1 H, 3-H), 7.01 (s, 1 H, 5-H), 2.39 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$): δ 160.2 (C-9b), 151.1 (C-2), 149.4 (C=O), 145.0 (C-4), 141.0 (C-5a), 140.6 (C-9a), 137.2 (C-2′), 132.6 (C-4a), 131.0 (C-8), 130.8 (C-4′), 130.1 (C-7), 126.2 (C-6), 124.1 (C-3), 121.3 (C-9), 117.2 (C-5′), 76.1 (C-5), 18.1 (CH_3).
$C_{17}H_{13}N_3O_2$

3.2.3. Synthesis of (R,S)-5-(imidazol-1-yl)-4-methyl-5H-indeno[1,2-b]pyridine (**3**)

Method A: A suspension of 82 mg (0.28 mmol) **7** in 4 ml DMF was heated in an oil bath (145 °C) with stirring under nitrogen for 10 min and then, after cooling, treated with 30 ml ice-water and stored at 4 °C over night. After addition of 50 ml brine the mixture was extracted with ethyl acetate (3 × 60 ml). The combined organic layers were dried over Na_2SO_4, and evaporated. The residue was purified by CC (ethyl acetate: hexane, 2 : 3) to give 53 mg (76%) of **3**.
Method B: A suspension of 100 mg (0.51 mmol) **5** in 5 ml DMF was treated with 107 mg (0.66 mmol) *N,N′*-carbonyldiimidazole and refluxed for 1 h. Then 50 ml ice-water were added, followed by 100 ml brine, and extraction with ethyl acetate (3 × 60 ml). Work-up as described above gave 78 mg (62%) of **3** as a pale yellow solid, m.p. 211 °C.
MS (70 eV) : m/z 247 (17) [M^+], 180 (100), 152 (14), 94 (38), 71 (16), 69 (15), 67 (8); IR (KBr): ν_{max} = 1594, 1571, 1503, 1491, 1383, 1224, 1066, 745 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.53 (d, J = 5.2 Hz, 1 H, 2-H), 8.05

(d, J = 7.7 Hz, 1 H, 9-H), 7.71 (br. s, 1 H, 2′-H), 7.52 (dd, J = 7.3, 7.7 Hz, 1 H, 8-H), 7.40 (m, 1 H, 7-H), 7.35 (dd, J = 7.7, 0.8 Hz, 1 H, 6-H), 7.06 (br. s, 1 H, 5′-H), 7.01 (d, J = 5.2 Hz, 1 H, 3-H), 6.65 (br. s, 1 H, 4′-H), 6.19 (s, 1 H, 5-H), 2.09 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$): δ 159.5 (C-9b), 150.8 (C-2), 145.0 (C-4), 143.2 (C-5a), 139.5 (C-9a), 137.0 (C-2'), 134.3 (C-4a), 130.3 (C-4′), 130.1 (C-8), 130.1 (C-7), 124.5 (C-6), 124.3 (C-3), 121.4 (C-9), 117.4 (C-5′), 59.4 (C-5), 17.4 (CH_3).
$C_{16}H_{13}N_3$

3.3. Biological activities

Agar diffusion assays (El-Nakeeb 1976) and screening for ergosterol biosynthesis inhibition in *Yarrowia lipolytica* (Bracher 2003) were performed as previously described.

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