Antimycotic Imidazoles. Part 4. Synthesis and Antifungal Activity of Ketoconazole, a New Potent Orally Active Broad-Spectrum Antifungal Agent

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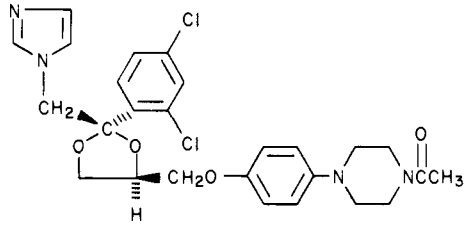
The preparation and antifungal properties of *cis*-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine (I) are described. Ketoconazole has, at low oral doses, a high in vivo activity against vaginal candidosis in rats and against cutaneous candidosis in guinea pigs.

Miconazole, econazole,[1] and clotrimazole[2] are potent broad-spectrum antimycotics which show high in vitro activity against almost all fungi of clinical interest. They have also been successfully applied for topical use in many clinical trials, particularly in mucocutaneous candidosis of the vagina and in dermatophytosis.

The major disadvantage of miconazole, econazole, and clotrimazole is that serum, urine, and body fluid levels after oral doses are disappointingly low, at best hardly sufficient to inhibit fungal growth.[3]

In some cases, however, intravenous miconazole therapy can be successfully applied for the treatment of systemic fungal infections in man.[4,5] In this paper, we report the synthesis and antifungal properties of ketoconazole (I), a



cis-I

new 1,3-dioxolanylmethylimidazole derivative, which is at present undergoing clinical investigation.[6]

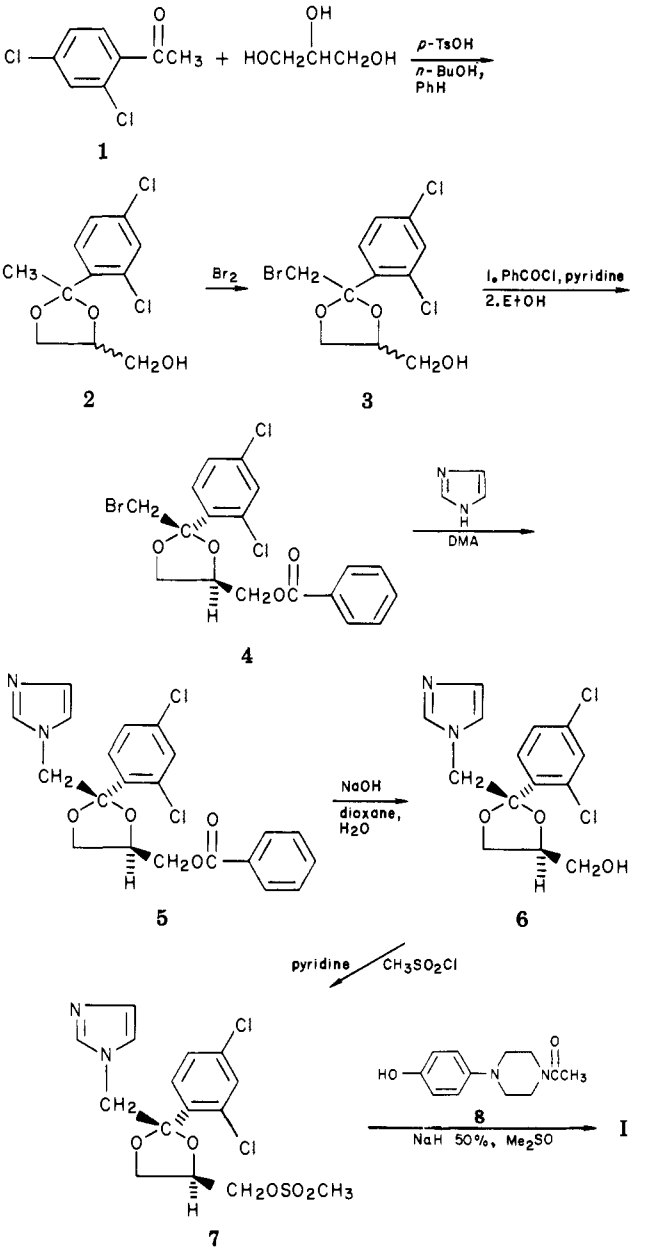
Chemistry. The synthesis, starting from 2,4-dichloroacetophenone, is outlined in Scheme I. Ketalization of 1 with glycerine was performed in a benzene–1-butanol medium with azeotropic removal of water in the presence of a catalytic amount of *p*-toluenesulfonic acid. Without isolation, the ketal **2** was brominated at 30 °C to bromo ketal **3**.

Benzoylation of **3** in pyridine afforded the ester as a cis/trans mixture, from which the cis form **4** could be isolated by crystallization from EtOH. The pure trans isomer could be obtained by liquid chromatography of the mother liquor.

Coupling of bromo ketal **4** in dry DMA with imidazole gave the imidazole derivative **5**. The ester **5** was saponified at reflux with NaOH in dioxane–water medium to the alcohol **6**. This alcohol was converted to methanesulfonate **7**, which was coupled with the sodium salt of **8** to give ketoconazole I. From the NMR spectra of ketoconazole I and its imidazole precursors **5**–**7**, stereochemical information could be obtained which showed them to be the cis isomers.[7] The NMR data were confirmed by X-ray diffraction.[8]

Biological Data. The in vitro results, obtained by the procedure described by Godefroi et al.,[9] are roughly comparable with those of related antifungal imidazoles. In preliminary experiments, ketoconazole showed complete or marked inhibition of growth at the concentration (μg/mL) indicated against a number of fungi and bacteria (Table I). In Sabouraud broth containing 10% inactivated bovine serum, the antifungal activity of ketoconazole was markedly enhanced. The antifungal effect of ketoconazole

Scheme I



in vitro depends on the test medium used and this will be described more extensively elsewhere.

In vivo ketoconazole showed high activity after oral treatment against experimental cutaneous candidosis in guinea pigs and vaginal candidosis in rats. For oral treatment, the compound was dissolved in PEG 200 and administered by gavage.

The in vivo activity in vaginal candidosis was evaluated experimentally by induced vaginal infection with *C. albicans* in rats.[10] Female Wistar rats weighing 100 g were

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Table I. Complete or Marked Inhibition of Growth in Sabouraud Broth after 14 Days of Incubation (μg/mL)

	ketoconazole		miconazole	
	no inact. bovine serum	10% inact. bovine serum	no inact. bovine serum	10% inact. bovine serum
Microsporum canis	10	1	1	10
Trichophyton mentagrophytes	0.1	0.1	0.01	0.1
Trichophyton rubrum	1		1	
Cryptococcus neoformans	1		1	
Candida tropicalis	1	0.1	1	>10
Candida albicans	10	1	10	>10
Aspergillus fumigatus	100	10	10	>10
Sporothrix schenkii	1	1	1	10
Saprolegnia species	1		10	
Phialophora verrucosa	1		100	
Erysipelothrix insidiosa	10		0.01	
Staphylococcus hemolyticus	100		10	
Streptococcus pyogenes	10		0.01	

Table II. Vaginal Candidosis in Rats: Oral Treatment with Ketoconazole

treatment	dose, mg/kg	days of treat.	effectiveness[a] ratio
prophylactic	1.25	14	7/26
	2.5	14	21/26
	5	14	22/22
curative	2.5	11	2/24
	5	5	11/12

[a] Ratio of negative animals vs. total number of animals.

Table III. Cutaneous Candidosis in Guinea Pigs

dose, mg/kg	effectiveness[a] ratio
placebo	0/22
0.63	0/12
2.5	10/17
10	20/22

[a] Ratio of negative animals vs. total number of animals at day 21.

ovariectomized and hysterectomized under thalamonal anaesthesia. Three weeks later and weekly thereafter during the whole experiment, 100 μg of oestradiol undecanoate in sesame oil was injected subcutaneously into each rat. The induced pseudooestrus was checked by microscopic examinations of vaginal smears. Only animals in pseudooestrus were infected intravaginally with 8×10^5 cells of *C. albicans* (strain B 2630), diluted in 0.2 mL of saline. Control of infection or cure was done by taking vaginal smears with sterile cotton swabs 7, 11, and 14 days after infection.

In the prophylactic treatment (starting on the day of infection), a daily dose of 1.25 mg/kg for 14 consecutive days had only a marginal effect, while 2.5 mg/kg was highly active and 5 mg/kg gave a 100% protection. With miconazole, the same effect was obtained with doses of 80 mg/kg (Table II). In the curative treatment (starting 3 days after infection), nearly all the animals were cured at daily dose levels of 5 mg/kg for 5 consecutive days, lower doses being ineffective even on prolonged treatment up to 11 days (Table II). In a similar experiment, the activity against cutaneous candidosis in guinea pigs[11] was evaluated. Ketoconazole was tested orally at daily dose levels of 0.63, 2.5, and 10 mg/kg for 14 consecutive days. At 2.5 mg/kg, a pronounced activity was found, and 10 mg/kg gave an almost complete protection (Table III). Preliminary experiments showed that ketoconazole had a pronounced oral activity against experimental dermatophytosis induced by *M. canis* and *T. mentagrophytes* in guinea pigs.[10] In experimental murine coccidioidomycosis, it was noticed that a dose of 40 mg/kg od for 2.5 weeks was completely protective in mice infected with a number of *C. imitis* cells that kills more than 60% of untreated mice. With higher doses and prolonged therapy, eradication of all pathogens from the host could be achieved.[12,13] Detailed biological data will be published elsewhere.

Experimental Section

Melting points are measured with a Mettler FP1 melting point apparatus and are uncorrected. New compounds were routinely checked for their structure by UV and/or IR and NMR spectrometry (UV, Beckman DK-2A; IR, Perkin-Elmer 421 or 225; NMR, Brucker HX 60-12 and/or WP 80-DS). Where indicated, GC was measured with a gas chromatograph Varian 2100 (column length 2 m, 3% OV-17).

***cis*- and *trans*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-methanol (3).** Glycerine (110 g, 1.2 mol) and 2,4-dichloroacetophenone (189 g, 1.0 mol) in 400 mL of PhH and 200 mL of *n*-BuOH were refluxed in the presence of *p*-toluenesulfonic acid monohydrate (6 g) for 24 h with azeotropic removal of water. After cooling the mixture to 40 °C, bromine (192 g, 1.2 mol) was added dropwise over a period of 2 h. The mixture was stirred for 0.5 h and then evaporated in vacuo. The residue was dissolved in CH_2Cl_2, washed with 6 N NaOH solution (200 mL), dried ($MgSO_4$), and evaporated again in vacuo to leave **3** as an oil: yield 311.2 g (91%); GC 94.3%.

***cis*-[2-(Bromomethyl)-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl]methyl Benzoate (4).** Benzoyl chloride (140.5 g, 1.0 mol) was added dropwise at 5 °C to a solution of **3** (311.2 g, 0.91 mol) in 600 mL of dry pyridine over a period of 1 h. The mixture was stirred for 2.5 h and diluted with water. After extraction of the mixture with $CHCl_3$, the organic layer was washed with 6 N HCl, dried ($MgSO_4$), and evaporated in vacuo to leave an oily residue with solidified on stirring with CH_3OH, giving **5** (225 g, 50%, GC 95.6%). Two crystallizations from EtOH afforded pure **4** (GC 100%, mp 118.3 °C). Anal. ($C_{18}H_{15}BrCl_2O_4$) C, H.

***cis*-[2-(2,4-Dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methyl Benzoate Nitrate (5).** A solution of **4** (220.0 g, 0.492 mol) in dry DMA was refluxed with a threefold excess of imidazole (100.0 g, 1.476 mol) for 4 days. The reaction mixture was cooled, diluted with water, and extracted with ether. The organic layer was dried ($MgSO_4$) and treated with a small excess of HNO_3 (65%). Filtration of the precipitate and crystallization from *i*-PrOH/*i*-Pr_2O gave **5** (134.9 g, 55%, mp 172 °C). Anal. ($C_{21}H_{18}Cl_2N_2O_4$) C, H, N.

***cis*-2-(2,4-Dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolane-4-methanol (6).** A solution of **5** (131.0 g, 0.264 mol) in 1000 mL of dioxane and 200 mL of H_2O was refluxed with 200 mL of 50% NaOH in H_2O for 0.5 h and then the reaction mixture was cooled. The product crystallized on dilution with H_2O, and the precipitate was filtered and taken up in $CHCl_3$. The solution was dried ($MgSO_4$) and evaporated in vacuo, yielding **6** (83.4 g, 96%, mp 140.0 °C). Anal. ($C_{14}H_{14}Cl_2N_2O_3$) C, H, N.

***cis*-[2-(2,4-Dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methyl Methanesulfonate (7).** To a solution of **6** (10.0 g, 0.03 mol) in dry pyridine (50 mL) meth-

anesulfonyl chloride (5.0 g, 0.033 mol) was added dropwise over a period of 20 min while cooling on ice. The reaction mixture was stirred for 5 h. Then H_2O was added and the product crystallized out. The solid was filtered off and recrystallized from PhH, yielding **7** (10.6 g, 87%, mp 111.7 °C). Anal. ($C_{15}H_{16}Cl_2N_2O_5S$) C, H, N.

***cis*-1-Acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine (I).** To a suspension of NaH (50%) dispersion (0.6 g, 0.012 mol) in Me_2SO, **7** (2.4 g, 0.011 mol) was added. After stirring the suspension for 1 h, **8** (4.1 g, 0.010 mol) was added and stirring was continued for 5 h at 80 °C. The reaction mixture was cooled and water was added. After extraction of the mixture with CH_2Cl_2, the organic layer was dried ($MgSO_4$) and evaporated to afford an oily residue, which was crystallized from 4-methyl-2-pentanone to afford I (3.2 g, 59%, mp 146.0 °C). Anal. ($C_{26}H_{28}Cl_2N_4O_4$) C, H, N.

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Antimalarials. 11. Synthesis of 3- and 5-Aminoquinolines as Potential Antimalarials

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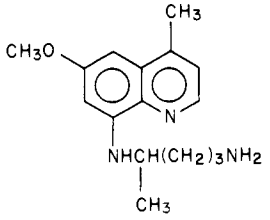
A series of 3-quinolinediamines (**1g**, **2c**, and **3e**) structurally related to primaquine and 4-methylprimaquine have been prepared and tested for antimalarial activity against *Plasmodium berghei* in mice and antileishmanial activity against *Leishmania donovani* in the hamster. All were inactive. In addition, three 5-quinolinediamines (**4b**, **5**, and **6**) were prepared. All were inactive against *Leishmania donovani* in hamsters. One of the examples, **6**, was curative against *Plasmodium cynomolgi* in the rhesus monkey.

In the preceding paper in these series,[1] we reported the preparation of a series of 4-substituted primaquine analogues based in part on a report[2] that 4-methylprimaquine (I) was superior to primaquine itself against *Plasmodium*



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cynomolgi in the rhesus monkey. None of the 4-substituted primaquine analogues was superior to 4-methylprimaquine, however. We then felt that it would be of interest to prepare selected examples in the 3-amino- and 5-aminoquinoline areas. A very limited number of 3- and 5-quinolinediamines were prepared during the World War II program and reported by Wiselogle.[3] All were inactive in the antimalarial tests conducted. However, none contained methyl and/or methoxy substituents, which were subsequently found to enhance antimalarial activity, nor did they contain the highly effective (4-amino-1-methylbutyl)amino side chain.

Chemistry. Three examples were prepared in each series of 3-/5-NHR quinolines. The first analogue (**1g**) closely resembles 4-methylprimaquine, with the exception that the diamine side chain is in the 3 position of the quinoline nucleus. The synthetic sequence is shown in Scheme I and involved previously reported[1,4-7] procedures.

The second example (**2c**) was prepared similarly from 2-amino-4,5-dimethoxyacetophenone.[8] The third example in the 3-quinolinediamine series (**3e**) is the 4-demethyl analogue of **2c** and was prepared as shown in Scheme II. 2-Nitro-4,5-dimethoxybenzaldehyde was converted to the cyclic ethylene acetal **3a**. Catalytic reduction with Raney nickel afforded the intermediate **3b**. This intermediate is presumably formed via partial cleavage of the ethylene acetal to yield a benzaldehyde, which subsequently condenses with the intermediate aniline. We are unable to explain the stability of the second ethylene acetal function. Nevertheless, spectral and analytical data are consistent with the structure proposed for **3b**. Treatment of **3b** with methazonic acid gives rise to the desired 3-nitroquinoline **3c**. This latter intermediate was identical with an authentic sample prepared via condensation of 2-amino-4,5-dimethoxybenzaldehyde and methazonic acid.[9] The remainder of the sequence is identical with that described for the preparation of **1g**.

Three examples (**4b**, **5**, and **6**) were prepared in the 5-aminoquinoline series and all are 8-methoxy-5-quinolinediamines. Condensation of 2-methoxy-5-nitroaniline with acrolein afforded the requisite 5-nitro-8-methoxyquinoline,[10] which was reduced with hydrazine and Raney nickel to yield 5-amino-8-methoxyquinoline. Condensation of the 5-aminoquinoline with the appropriate side-chain intermediates afforded the three target 5-quinolinediamines.

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