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SYNTHESIS OF 1-{4-[4-(ADAMANT-1-YL)PHENOXYMETHYL]-2-(4-BROMOPHENYL)-1,3-DIOXOLAN-2-YLMETHYL}IMIDAZOLE WITH EXPECTED ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY

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Abstract: A multistep synthesis of a new analogue of ketoconazole, which was expected to show antifungal and antibacterial activity, has been described.

Keywords: imidazole, adamantane derivatives, ketoconazole, antibacterial activity, antifungal activity

The excessive use or misuse of broad-spectrum antibiotics and corticosteroids or cytostatic drugs, hospitalization of patients after surgical treatment or in the critical care units and also AIDS (1, 2) cause a frequent occurrence of mycoses, which have been treated with difficulties by nowadays approached drugs. AIDS, a disease that for the first time appeared in the 1980s', debilitates the immune system.

The subsequent immunological defects cause an increase in the occurrence and extent of fungal infections as well. In particular, opportunistic systemic fungal infections are increasing in number throughout developed countries. The etiology of systemic infections is mostly connected with *Candida* spp. and *Aspergillus* spp. Many newer species are increasingly responsible for these invasive infections, e.g. *Fusarium* spp., *Acremonium* spp., *Scedosporium* spp., *Alternaria* spp., *Trichosporon* spp., *Malassezia* spp., *Rhodotorula rubra*, etc. Many recent studies show dramatic increase in the frequency of invasive aspergillosis and other systemic infections due to non-*albicans Candida* species. Because of changes in pathogens responsible for systemic fungal infection and many cases connected with antifungal drug resistance, the mycological pharmacotherapy is still developing (2 – 4). A lot of new antifungal drugs have been used in the treatment of invasive fungal infections. Consequently, there is a growing need for the next pharmaceutical products to create the efficient and safe antifungal medication. Particularly, there is a shortage of the antifungal drugs showing systemic activity, which could be applied orally (5).

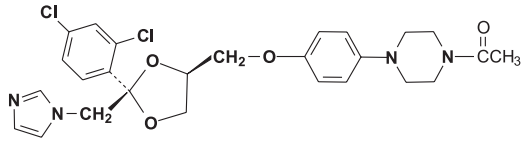
The most active antifungal drugs contain a triazole or imidazole ring (6). For many years interest has been directed toward the synthesis and biological evaluation of imidazole derivatives (7, 8). A significant progress made the advent to the clinic of the first antifungal imidazoles: clotrimazole (9), miconazole and econazole (10), and several other azole derivatives, most notably ketoconazole (11-18), which have been successful as antifungal agents. Ketoconazole was the first orally active antifungal medicine that was effective against a broad array of systemic and superficial fungal infections (17).

The N-substituted azole drugs are known to interfere with the cytochrome P_{450} dependent on 14α-demethylation of lanosterol or 24-methylenedihydrolanosterol, a key step in the biosynthesis of ergosterol, which is the main sterol in the vast majority of yeasts and fungi (19, 20).

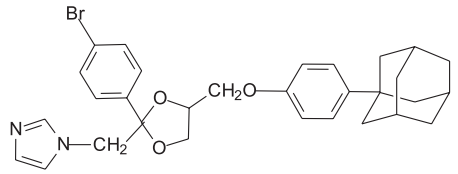
The most active ketoconazole contains imidazole ring and 1,3-dioxolane skeleton. The dioxolane ring in position 4 is bound with 1-acetylpiperazin-4-phenoxymethyl group.

After examination of the structure of ketoconazole, we decided to synthesise a similar structure containing a 1,3-dioxolane ring connected with 4-(adamant-1-yl)phenoxymethyl group in this position and 4-bromophenyl group in 2 position (Scheme 1).

It is known from the literature that some of the adamantane derivatives presented also antiviral, antibacterial (21 – 27) and antifungal (28, 29) activity. The analogues of ketoconazole in which adamantane would be bound by the phenoxymethyl group with 1,3-dioxolane ring in 4 position have not been synthesized up to now.



Ketoconazole

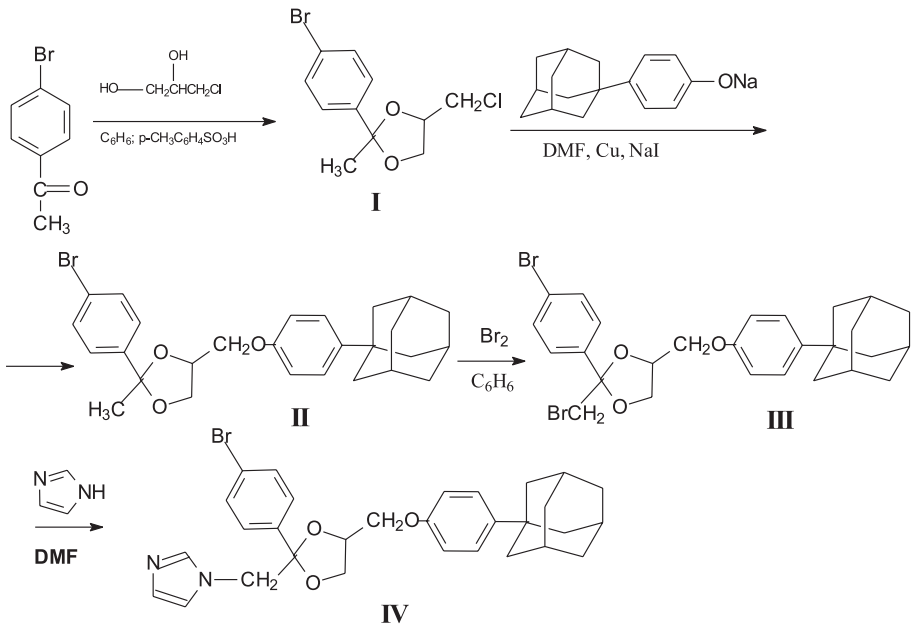


Analogue (**IV**)

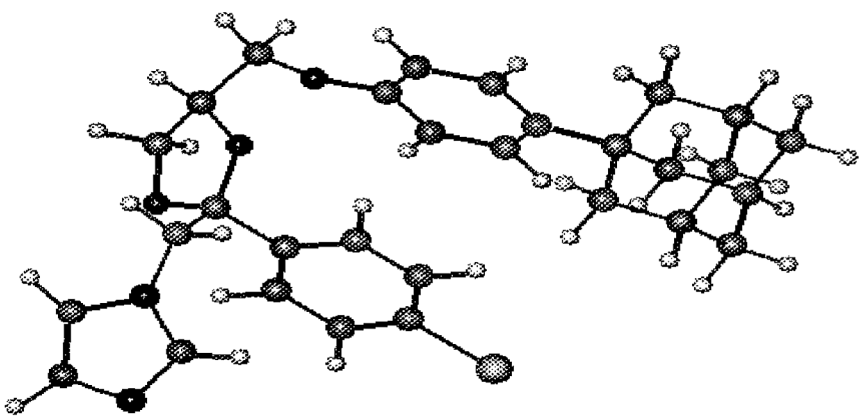
Scheme 1.

EXPERIMENTAL

4-(Adamant-1-yl)phenol was received in the reaction of electrophilic substitution of 1-bromoadamantane with phenol (30). Its sodium salt was

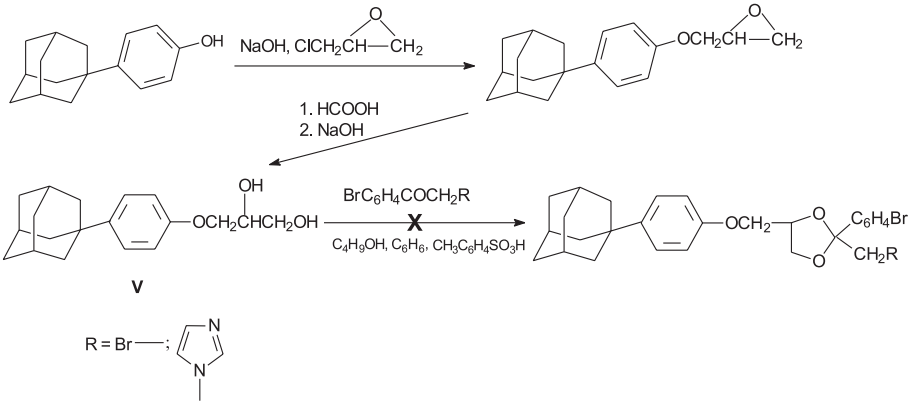


Scheme 2.



Molecular weight of 549.48 Capacity: 466.00 Å

Scheme 3. Presentation of the computer modelled three-dimensional structure of compound **IV**.



Scheme 4.

Table 1. The physico-chemical data of compounds **II – V**.

No.	Formula Molecular weight	M.p. [°C] solvent	Time of reaction [h]	Yield [%]	Analysis Calculated/Found			
					%C	%H	%N	%Br
II	$C_{27}H_{31}BrO_3$ 483.2	134-136 EtOH	20	39.5	67.11 67.12	6.41 6.40	–	16.54 16.44
III	$C_{27}H_{30}Br_2O_3$ 562.3	106-107 MeOH	11.5	85.2	57.66 57.21	5.38 5.43	–	28.42 27.96
IV	$C_{27}H_{33}BrN_2O_3$ 549.5	143-145 EtOH	150	51.7	65.57 65.16	6.05 5.95	5.10 5.32	14.54 14.12
V	$C_{19}H_{26}O_3$ 302.2	196-197 AcOEt/AcOBu	15	55	75.51 75.16	8.66 8.47	–	–

Table 2. ^{1}H-NMR spectra of compounds **II – V**.

No	^{1}H-NMR I, III, IV in $CDCl_3$, V in DMSO
II	Ad: 1.765[6H]m, 1.889[6H]pd, 2.090[3H]ps; Ad-Ar: 6.882[2H]d ^3J = 8.8, 7.286[2H]d; Ar-O-C**H**$_2$: 3.965m; O-CH: 4.322q ^3J = 5.2; O-C**H**$_2$-CH: 3.840[1H]pt, 4.120[1H]dd ^2J = 9.2, ^3J = 5.2; Br-Ar: 7.391[2H]d ^3J = 8.4, 7.485[2H]d; CH_3: 1.669s.
III	Ad: 1.759[6H]m, 1.884[6H]ps, 2.085[3H]ps; Ad-Ar: 6.884[2H]d ^3J = 8.8, 7.516[2H]d; Ar-O-C**H**$_2$: 4.093[1H]m, 4.225[1H]dd ^2J = 9.6, ^3J = 5.6; O-CH: 4.423q ^3J = 5.6; O-C**H**$_2$-CH:3.981[1H]pt, 4.093[1H]m; Br-Ar: 7.413[2H]d ^3J = 8.4, 7.516[2H]d; CH_2-Br: 3.629s.
IV	Ad: 1.756[6H]m, 1.877[6H]ps, 2.082[3H]ps; Ad-Ar: 6.769[2H]d ^3J = 8.4, 7.267[2H]d; Ar-O-CH_2: 4.157pd; O-CH: 4.338q ^3J = 4.8; O-C**H**$_2$-CH: 3.316[1H]pt, 3.843[1H]pt; Br-Ar: 7.368[2H]d ^3J = 8.4, 7.520[2H]d; CH_2-N: 3.698[1H]d ^2J = 6.8, 3.734[1H]d; N-CH=N: 7.469ps; CH_2-N-C**H**:6.949ps; N-CH: 7.005ps.
V	Ad: 1.714[6H]ps, 1.813[6H]ps, 2.032[3H]ps; Ad-Ar: 6.859[2H]ps, 7.229[2H]ps; Ar-O-CH_2: 3.775d ^3J = 9.2; C**H**-OH,C**H**$_2$-OH: 3.324[3H]ps.

Ad – adamantane, Ar – aromatic, s – singlet, ps – pseudosinglet, d – doublet,
pd – pseudodoublet, dd – doublet of doublets, pt – pseudotriplet, m – multiplet.

Table 3. IR spectra of compounds **II – V**.

No	IR, KBr, cm^{-1}
II	νCH/Ar/3040, νCH 2980-2940, νC=C/Ar/1610, 1570, δCH_2 1460, 1370, νC-O-C 1250, 1240, 1180.
III	νCH/Ar/3050, 3020, νCH 2990-2845, νC=C/Ar/1609, 1578, 1510, δCH_2 1483, 1460, νC-O-C 1256, 1240, 1188.
IV	νCH/Ar/3020, νCH 2980-2840, νC=C/Ar/1605, 1508, δCH_2 1460, 1380, νC-O-C 1250, 1230.
V	νOH 3566, 3384, νCH/Ar/3057, 3034, νCH 2932-2843, νC=C/Ar/1609, 1557, 1508 δCH_2 1452, νC-O-C 1259, 1184.

Table 4. Influence of compound **IV** on sensitivity of the selected antifungal medicines.

Strains	Diameter of the growth inhibition zone [mm]					
	Econazole (+ IV)*	Miconazole (+ IV)*	Tioconazole (+ IV)*	Ketoconazole (+ IV)*	Itraconazole (+ IV)*	Fluconazole (+ IV)*
C. albicans ATCC 90028	35 (24)	25 (20)	43 (39)	49 (46)	25 (20)	36 (36)
C. parapsilosis ATCC 22019	26 (14)	20 (15)	31 (19)	42 (38)	25 (18)	resist. (resist.)
C. tropicalis IBA 171	21 (24)	20 (20)	38 (39)	45 (46)	22 (20)	24 (23)
C. krusei IBA 161	28 (13)	22 (12)	39 (30)	37 (31)	23 (18)	resist. (resist.)
C. guillermondii IBA 155	24 (10)	18 (10)	32 (21)	55 (47)	19 (13)	28 (28)
S. cerevisiae IBA 198	31 (18)	27 (18)	43 (34)	31 (27)	resist. (resist.)	resist. (resist.)

***** Diameter of the fungal growth inhibition zone around a disc including the antifungal medicine and 400 μg of compound **IV**.

Table 5. Influence of compound **IV** on sensitivity of *Enterococcus faecalis* on the selected medicines.

Strains	Diameter of the growth inhibition zone [mm]										
	GM (+ IV)*	S (+ IV)*	Va (+ IV)*	TEC (+ IV)*	NA (+ IV)*	CIP (+ IV)*	OFX (+ IV)*	NOR (+ IV)*	SPA (+ IV)*	AM (+ IV)*	TE (+ IV)*
E. faecalis ATCC 29212	19 (19)	20 (19)	19 (15)	16 (16)	resist. (resist.)	16 (13)	13 (11)	14 (11)	15 (15)	20 (20)	12 (12)

***** Diameter of the fungal growth inhibition zone around a disc including the antifungal medicine and 400 μg of compound **IV**.
GM – gentamycin (120 μg/ disc), S – streptomycin (300 μg/ disc), Va – vancomycin (30 μg/ disc), TEC – teicoplanin (30 μg/ disc), NA – nalidixic acid (30 μg/ disc), CIP – ciprofloxacin (5 μg/ disc), OFX – ofloxacin (5 μg/ disc), NOR – norfloxacin (10 μg/ disc), SPA – sparfloxacin (5 μg/ disc), AM – ampicillin (10 μg/ disc), Te – tetracycline (30 μg/ disc).

obtained by the reaction of 4-(adamant-1-yl)phenol with sodium hydroxide in methanol (29).

4-Chloromethyl-2-(4-bromophenyl)-2-methyl-1,3-dioxolane (**I**) was received in the reaction of 4-bromoacetophenone with 3-chloro-1,2-propanediol in the presence of catalytic amount of 4-toluenesulfonic acid in benzene with azeotropic removal of water according to the methods described in the literature (31 – 33).

2-(4-Bromophenyl)-2-methyl-4-[4-(adamant-1-yl)phenoxymethyl]-1,3-dioxolane (**II**) was obtained by the unconventional reaction of **I** with 4-(adamant-1-yl)phenol sodium salt in the presence of catalytic amounts of copper and sodium iodide in DMF.

As the result of selective bromination of **II**, 2-(4-bromophenyl)-2-bromomethyl-4-[4-(adamant-1-yl)phenoxymethyl]-1,3-dioxolane (**III**) was received with high yield.

The reaction of **III** with a significant excess of imidazole in boiling DMF gave the title compound (**IV**) as shown in Scheme 2. The three-dimensional structure of compound IV is presented in Scheme 3.

A modification of the ketalization procedure for both 2-bromo-4'-bromoacetophenone and the corresponding 4'-bromo-2-(1*H*-imidazol-1-yl)acetophenone with 3-[4-(adamant-1-yl)phenoxy]-1,2-propanediol in benzene and butanol as already described for 2-bromoacetophenone with phenyl-1,2-ethanodiol (33) was unsuccessful (Scheme 4).

Chemical Part

Melting points were measured using a Boetius apparatus and are given uncorrected.

IR spectra were taken in KBR pellets with a FTIR-8300 Fourier Transform Infrared Shimadzu spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker Avance DMX 400 WB (400, 133 MHz) spectrometer in $CDCl_3$ with TMS as an internal standard.

Elemental analysis for C, H, N and Br was performed on a Perkin-Elmer analyzer and within a satisfactory range (max 0.5%) for each element.

Physicochemical properties and spectral data of the obtained intermediates and the final product are summarized in Tables 1-3.

Synthesis of 4-[4-(adamant-1-yl)phenoxymethyl]-2-(4-bromophenyl)-2-methyl-1,3-dioxolane (**II**)

A mixture of 0.02 mol (5.82 g) 4-chloromethyl-2-(4-bromophenyl)-2-methyl-1,3-dioxolane and 0.02 mol (5.0 g) sodium 4-(adamant-1-yl)phenolate in 140 cm^3 of DMF was refluxed and stirred for 20 h in the presence of catalytic amount of copper dust and sodium iodide. After cooling, it was poured into the mixture of 1 dm^3 of the 20% aqueous solution of NaCl and ice. The product was extracted with 4 × 150 cm^3 of benzene. Anhydrous $MgSO_4$ was used to dry the solution. Benzene was evaporated. The residue was crystallized from ethanol. Yield 3.8 g (39.5%).

Synthesis of 2-(4-bromophenyl)-2-bromomethyl-4-[4-(adamant-1-yl)phenoxymethyl]-1,3-dioxolane (**III**)

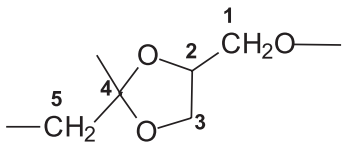
A solution of 0,0046 mol of bromine (0.735 g) in 10 cm^3 of benzene was dropped during 1.5 h to a solution of 0.0046 mol (2.3 g) of compound **II** in 55 cm^3 of benzene. Then, it was stirred at room temperature for 10 h. Benzene was distilled off under reduced pressure. The crystalline residue was eluted with 200 cm^3 of hot hexane. The solvent was evaporated and the residue was crystallized from methanol. Yield 2.2 g (85.2%), m.p. 106-107°C.

Synthesis of 1-{4-[4-(adamant-1-yl)phenoxymethyl]-2-(4-bromophenyl)-1,3-dioxolan-2-ylmethyl}-1*H*-imidazole (**IV**)

The mixture of 0.0018 mol (1.5 g) of **III** and 0.0089 mol (0.92 g) of imidazole and a catalytic amount of powdered Cu and NaI in 30 cm^3 of DMF was refluxed for 150 h. Then, it was cooled and poured into 200 cm^3 of brine. The mixture was extracted with 4 × 50 cm^3 of methylene dichloride and dried with anhydrous $MgSO_4$. The solvent was removed in rotary evaporator under reduced pressure. The remaining precipitate was crystallized from ethanol. Yield 0.9 g (51.7%).

Synthesis of 3-[4-(adamant-1-yl)phenoxy]propane-1,2-diol (**V**)

The mixture of sodium hydroxide 0.025 mol (1.0 g) in 35 cm^3 of water and 4-(adamant-1-yl)phenol 0.024 mol (5.7 g) and 1-chloro-2,3-epoxypropane 0.24 mol (2.22 g) was stirred and refluxed for 2 h. After cooling, the precipitate was filtered off and washed with 50 cm^3 of water and dried. Then, the mixture of the crude solid product (3.0 g) and 86% formic acid (30 cm^3) was stirred and refluxed for 2 h. The excess of formic acid was removed in rotary evaporator under reduced pressure. The solid residue was hydrolyzed by heating it under reflux with 50 cm^3 of 30% aqueous solution of sodium hydroxide for 1.5 h. After cooling, the precipitate of the final product was collected by filtration under suction, washed with water and dried. It was purified by crystallization from the mixture of ethyl acetate and butyl acetate (3:2, v/v). Yield 1.8 g (59.6%).



Scheme 5.

Having analyzed a character of the multiplet proton signals of the methylene groups: $C1H_2$, $C3H_2$ and $C5H_2$ (see Scheme 5, e.g. compound **IV**), it could be affirmed that a disclosing magnetic non-equivalence of protons within those groups is connected with a general asymmetry of the molecule. A rigid structure of the dioxolane ring causes a significant diversity of magnetic fields around the outside of protons of the methylene $C3H_2$ group for which the largest chemical shift difference has been noted: $\Delta\delta$ = 0.455 ppm. The methylene proton signals of the methylene $C5H_2$ group give a classical system AB with $\Delta\delta$ = 0.054 ppm and the proton signals of the $C1H_2$ group provide a complicated multiplet resulting from overlapping signals arising as the geminal and vicinal coupling with proton of the C2H group as well as likely with the far range protons of the methylene $C3H_2$ group. The general asymmetry of the molecule causes that even a free revolution round between C4-C5 and C1-C2 bonds do not equalize of magnetic fields and as a result creates different magnetic fields around those protons which have been under discussion.

Microbiological Part

The microorganisms were supplied by the State Institute of Hygiene (Warsaw, Poland), the Children Memorial Health Institute (Warsaw, Poland), and were coming from the own collection of the Department of Pharmaceutical Microbiology, Medical University of Warsaw (Poland).

This research covered testing of influence of compound **IV** on sensitivity of some Gram-positive

and Gram-negative bacteria and fungi to the selected drugs. Antimicrobial activity was examined by the disc-diffusion method under standard conditions using Mueller-Hinton II agar medium (Becton Dickinson) for bacteria or RPMI agar medium (Sigma) with 2% glucose for fungi according to CLSI. For the disc-diffusion assays, sterile filter paper discs supplied by Mast Diagnostics were applied. The first half of discs was provided with the selected drug and the second half of them included the same drug along with 400 μg of compound **IV**. The discs were loaded on the agar plates. The results were read after 18 h of incubation at 35°C in relation to the antibacterial activity and after 24 h of incubation at 35°C in the event of the antifungal activity testing.

RESULTS AND CONCLUSIONS

The growth inhibition zones were not observed for compound **IV** in all tests applied. On the other hand, the tests showed that compound **IV** reduced activity of the imidazole agents (econazole, miconazole, tioconazole and ketoconazole) and itraconazole, which is thiazole derivative (as distinct from fluconazole) against fungi *Candida* spp. and *Saccharomyces cerevisiae*. The biggest decrease in activity to tested fungi strains was observed for econazole, miconazole and tioconazole.

The decrease of the growth inhibition zone for 5 to 10 mm around discs provided with the mentioned drugs and compound **IV** in comparison to those discs including only those antifungal drugs was observed. The presence of compound **IV** did not show any influence on fluconazole activity to fungi strains, which were in use.

Furthermore, the antagonistic activity of vancomycin or fluoroquinolones with compound **IV** towards the reference strain of Gram-positive *Enterococcus faecalis* ATCC 29212 was found.

The decreased growth inhibition zone for 2 to 4 mm around discs provided with compound **IV** and antibacterial agents (vancomycin, ciprofloxacin, ofloxacin or norfloxacin) in comparison with those discs including only those antibacterial medicines was observed. Maybe, the observed antagonistic activity of vancomycin and fluoroquinolones, which were used along with compound **IV** results from the competitive binding of the medicine molecules and compound **IV** into fungi cells receptors. Probably, fluconazole is binding to receptors, which do not identify compound **IV** at the same time. On the other hand, it is not excluded that compound **IV** can diminish distribution of vancomycin and fluoroquinolones to cells of *E. faecalis*.

The results of the above mentioned antimicrobial activity studies are included in Tables 4 and 5**.**

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