Imidazole Analogues of Fluoxetine, a Novel Class of Anti-*Candida* Agents

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Abstract: Imidazole analogues of fluoxetine have been obtained by replacing the methylamino terminus of aminopropane chain with the imidazole ring. The newly designed imidazoles showed potent anti-*Candida* activity, superior to those of miconazole and other antifungal agents of clinical interest. 1-(4-Chlorophenyl)-1-(2,4-dichlorophenoxy)-3-(1*H*-imidazol-1-yl)propane (**16**), the most active among test imidazoles, was about 2-fold more active and as much less cytotoxic than miconazole. High increase of activity was observed with methyl, nitro, fluorine, and chlorine (Cl > F > CH_3 > NO_2 > CF_3).

Over the past two decades a significant increase in fungal infections has been observed. Among these, the widespread diffusion of topical and systemic infectious diseases caused by the opportunistic pathogen *Candida albicans* is often related to the use of broad-spectrum antibiotics, immunosuppressive agents, anticancer, and anti-AIDS drugs.[1,2] One of the principal problems in the treatment of *C. albicans* infections is the spread of antifungal drug resistance mainly in patients chronically subjected to antimycotic therapy such as HIV-infected people.[3,4]

Treatment of *Candida* infections is based on azoles, such as ketoconazole (**1**), itraconazole (**2**), econazole (**3**), miconazole (**4**), fluconazole (**5**), and nonazole antifungal agents, i.e., the allylamines naftifine (**6**) and terbinafine (**7**), polyene antibiotics, and flucytosine (Chart 1).

Despite appreciable antifungal activities have been registered in the in vitro test against *C. albicans*, candidemia remains an unfulfilled matter with maintenance of the mortality rate.[5] Azoles are endowed with fungistatic activity and are responsible of nausea and hepatotoxicity as side effects. Allylamines are regarded as active as azoles and polyenes. The latter cause adverse side effects such as chills, nausea, and vomiting during acute treatment and nephrotoxicity and anemia during chronic treatment.[6]

The increasing number of therapeutic failures due to the widespread diffusion of *C. albicans* infections together with the concomitant increase in drug resistant strains foregrounded the need for new effective anti-*Candida* agents. Efforts in this direction were well documented by about 200 patents registered within 1998−2000.

Chart 1. Commonly Used Antifungal Drugs

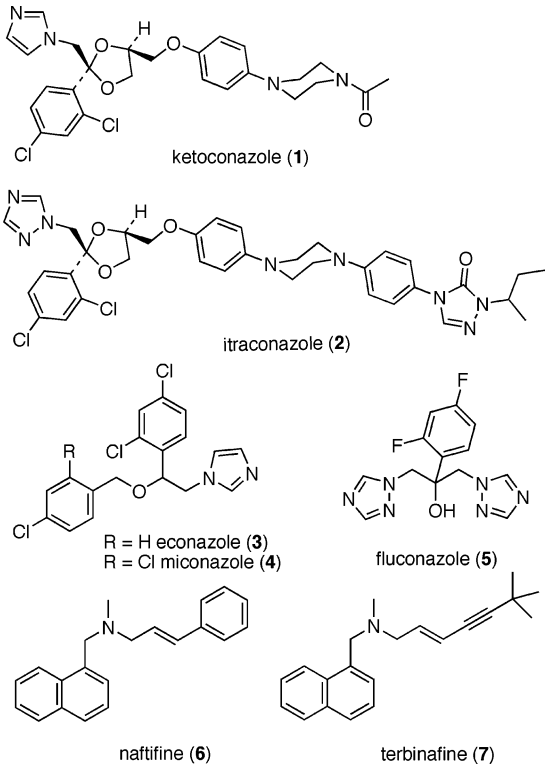
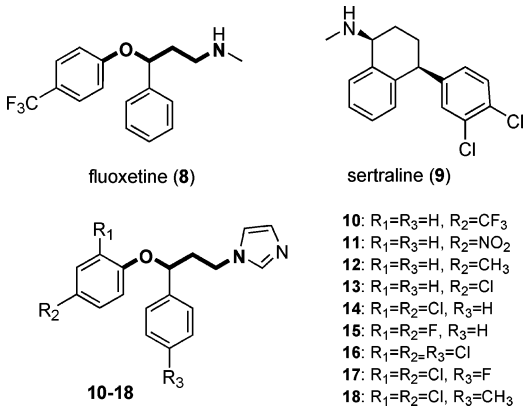


Chart 2



Recently, Lass-Flörl et al. reported the in vitro antifungal properties of selective serotonin reuptake inhibitors (SSRIs) against *Aspergillus* species and *Candida parapsilosis*.[7] Among five tested SSRIs, fluoxetine (**8**) and sertraline (**9**) showed the highest activity against these fungi with differences in susceptibility of the various isolates tested.

This report and our personal interest on azole antifungal agents[8−10] has induced us to investigate how the antifungal activity of **8** would be influenced by the replacement of the $NHCH_3$ terminus with an imidazole ring. Thus, we planned the synthesis of derivatives **10**−**18** which are imidazole analogues of fluoxetine (Chart 2).

Compounds **10**−**18** were prepared starting from 1-aryl-3-chloropropanones **19**−**22** which were reduced to 1-aryl-

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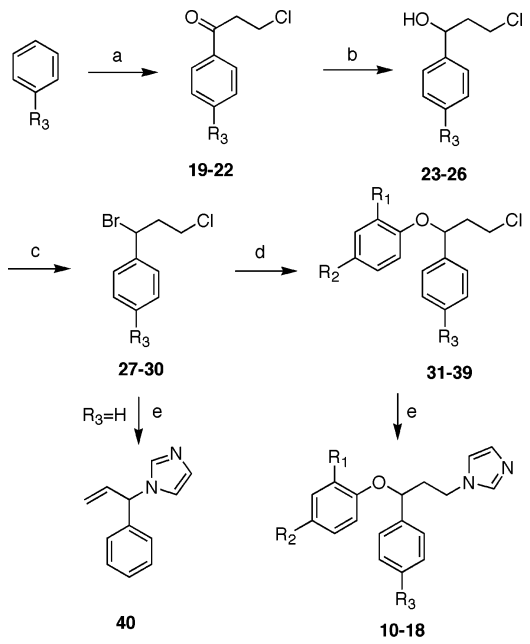
Table 1. Antifungal Activity of Compounds **10**−**18** against 20 *Candida Albicans* Strains

compd	CC_{50}[a]	CC_{50}[b]	MIC ± SD[c]	MIC_{50}[d]	MIC_{90}[e]	MIC range	QC[f] MIC	MFC ± SD[g]	MFC_{50}[h]	MFC_{90}[i]	MFC range	QC[j] MFC
10	86	71	38.4 ± 27.2	64	128	16−128	4	76.8 ± 57.2	64	128	32−128	16
11	97.2	102.3	26.8 ± 12.2	32	32	8−64	4	67.2 ± 66.4	32	>128	16−128	32
12	140.8	95.9	9.4 ± 4.16	8	16	4−16	2	13.6 ± 3.7	16	16	8−16	16
13	84.9	221	3.15 ± 1.9	2	4	1−8	4	9.8 ± 9.6	4	16	2−32	32
14	56.5	96.7	5.25 ± 2.4	4	8	1−8	2	8 ± 2.25	8	8	4−16	8
15	219.2	298.3	44.2 ± 19.6	32	64	4−64	8	88 ± 34.2	64	128	32−128	32
16	61	54	2.1 ± 1.24	2	4	0.5−4	0.5	3.62 ± 2.06	4	4	1−8	8
17	43.3	33.5	2.55 ± 1.4	2	4	0.5−4	0.5	6.25 ± 3.7	8	8	2−16	4
18	40	31.4	2.65 ± 1.3	2	8	1−4	1	7.12 ± 4.95	4	16	2−16	4
1	70	61.7	5.9 ± 3.1	4	8	2−16	0.06	16.3 ± 5.6	16	32	8−32	0.5
3	60	54.7	4.3 ± 1.7	4	8	2−8	2	15.04 ± 7.4	16	32	8.32	2
4	40	39.3	3.25 ± 1.6	4	4	1−8	1	12.2 ± 6.2	8	16	4−32	0.5
5	316	385.2	15.32 ± 23.2	2	64	0.125−64	0.5	>64	- -	- -	>64	8
8	33.3	25	76.8 ± 31.8	64	128	32−128	8	112 ± 28.4	128	128	64−128	64

[a,b] CC_{50}: Drug concentration (μM) required to reduce the human histiocytic lymphoma U937 (*a*) and chronic myeloid leukemia K562 (*b*) cells viability by 50%. [c] MIC ± SD: Arithmetic mean of minimum inhibitory concentration (μg/mL) values ± standard deviation. [d,e] MIC_{50} and MIC_{90}: MICs at which 50% and 90% of isolates, respectively, are inhibited. [f] QC *C. parapsilosis* ATCC22019 MIC values. [g] MFC ± SD: Arithmetic mean of minimum fungicidal concentration (μg/mL) values ± standard deviation; when MIC/MFC ratio is lower than three double dilutions, the drug is regarded as fungicidal. [h,i] MFC_{50} and MFC_{90}: MFCs required to cause 99% reduction of surviving cells in 50% and 90%, respectively, of isolates. [j] QC *C. parapsilosis* ATCC22019 MFC values.

Scheme 1[a]



19, **23**, **27**: R_3=H; **20**, **24**, **28**: R_3=Cl; **21**, **25**, **29**: R_3=F; **22**, **26**, **30**: R_3=CH_3; **31**: R_1=R_3=H, R_2=CF_3; **32**: R_1=R_3=H, R_2=NO_2; **33**: R_1=R_3=H, R_2=CH_3; **34**: R_1=R_3=H, R_2=Cl; **35**: R_1=R_2=Cl, R_3=H; **36**: R_1=R_2=F, R_3=H; **37**: R_1=R_2=R_3=Cl; **38**: R_1=R_2=Cl, R_3=F; **39**: R_1=R_2=Cl, R_3=CH_3.

[a] Reagents: (a) R_3-Ph, $ClCOCH_2CH_2Cl$, $AlCl_3$, r.t., 2 h; (b): $NaBH_4$, THF−H_2O, r.t., 2 h; (c): PBr_3, Et_2O, r.t., 2 h; (d): 2-R_1-4-R_2-PhOH, NaH, DMF, r.t., 60 h; (e): imidazole, DMF, reflux, 24 h.

3-chloro-propanols **23**−**26** with sodium borohydride and then transformed into the corresponding 1-aryl-1-bromo-3-chloropropanes **27**−**30** with phosphorus tribromide. Reaction of **27**−**30** with proper phenols in the presence of sodium hydride afforded the corresponding 1-(2-R_1,4-R_2-aryloxy)-1-(4-R_3-aryl)-3-chloropropanes **31**−**39** which were reacted with imidazole in DMF at 100 °C to give the required compounds **10**−**18**. 1-Phenyl-1-(1*H*-imidazol-1-yl)-2-propene (**40**) was obtained by heating **27** with imidazole in DMF at 100 °C (Scheme 1).

Test compounds were evaluated in vitro for their antifungal activity against 20 strains of *C. albicans*, a pathogenic opportunistic dimorphic fungus, according to a microdilution tray as described in NCCLS document.[11] The compounds were tested at different concentrations ranging from 0.25 to 128 μg/mL. Ketoconazole, econazole nitrate, miconazole nitrate, fluconazole, and fluoxetine were used as reference drugs. Cytotoxicity of test compounds was evaluated on human histiocytic lymphoma (U937) and chronic myeloid leukemia (K562) cell lines obtained from American type culture Collection (ATCC, Rockville; MD). The significance of differences in mean values was determined by using a paired *t* test (SigmaStat 2.03 statistical software for Windows). *P* values of <0.05 were considered statistically significant.

First we focused our attention mainly on the synthesis of 3-phenyl-3-(4-trifluomethylphenoxy)-1-(1*H*-imidazol-1-yl)propane (**10**), the fluoxetine-like imidazole necessary for a direct comparison between its anti-*Candida* activity and that of the lead fluoxetine (**8**).

When tested against *C. albicans* (Table 1) compound **10** showed minimum inhibitory concentration (MIC) about 2-fold greater than that of fluoxetine ($P < 0.001$), thus suggesting the imidazole as the moiety responsible for the increase of activity. However, the inhibitory potency of **10** differed greatly from those of econazole (**3**) and miconazole (**4**), two chloro derivatives of the imidazole family largely used for clinical treatment of antifungal disease. We therefore attempted to improve the antifungal potency of **10** by replacing the 4-trifluoromethyl substituent with a 4-nitro group (compound **11**), but only a very little variation of the inhibitory potency was observed as a result of this chemical modification. On the contrary, a remarkable increase in activity was obtained when methyl (**12**) or chlorine (**13**) were inserted instead of the trifluoromethyl group of **10** ($P < 0.001$).

The 4-methyl derivative **12** was found about 8- and 4-fold more active in MIC values than fluoxetine (**8**) ($P < 0.001$) and **10** ($P < 0.001$), respectively. The best activity was displayed by the 4-chloro derivative **13** which was about 24-times more active than fluoxetine and showed against *C. albicans* the same inhibitory activity of miconazole (**4**). Compared with ketoconazole (**1**) compound **13** was found to be 2-times more active ($P = 0.002$, $n = 20$).

The surprisingly high degree of activity showed by the monochloro derivative **13** suggested to attempt a progressive introduction of chlorine atoms in the phenyl rings in order to gradually improve the antifungal activity of this compound. In fact, previous studies on antifungal imidazoles claimed the maximum of activity for compounds bearing three/four chlorine atoms, as proved with the couple econazole/miconazole.

Unexpectedly, the introduction of a second atom of chlorine in the phenoxy moiety resulted in a slight decrease of activity (compare **14** with **13** $P = 0.004$), which was restored when a third chlorine atom was linked to the 4 position of the phenyl bound directly to the propane chain (compare **16** with **13** and **14**, $P = 0.005$ and $P < 0.001$ respectively).

Due to the high chemotherapeutical interest shown by some fluoro derivatives, such as fluconazole, flutrimazole and fluoroquinolones, we synthesized **15**, the difluorocounterpart of **14**, but without success with regard to the antifungal activity. Therefore the preparation of difluorophenoxy derivatives was discontinued indefinitely.

Replacement of the third chlorine of **16** with fluorine (**17**) or methyl (**18**) gave new derivatives as potent as **16**. It is noteworthy that the 2,4-dichlorophenoxy moiety led to potent antifungal derivatives independently from the nature of the substituents at 4 position of the phenylpropane moiety (compare **16**−**18** with **14** $P < 0.001$). This result is clearly different from that observed for the couple econazole/miconazole, which showed the highest antifungal activity in the presence of three/four chlorine atoms.

In conclusion, the introduction of substituents different from trifluoromethyl in the structure of **10** led to a substantial increase of activity with the maximum potency for chlorine (**13**), followed in decreasing order by methyl (**12**) and nitro (**11**). In general, all new derivatives were more active than fluoxetine, the compound selected as lead compound for the present search.

As a rule, monosubstituted derivatives were less active than those bearing two or three substituents. Data against *C. albicans* showed five compounds more active or as potent as reference clinical drugs ketoconazole, econazole, and miconazole (compare **13**, **16**−**18** with **1** and **14** with **3** and **4**), the trichloro derivative **16** being the most potent. As regards the fungicidal activity (MFC_{90}), it is worthy to note that compound **16** was 4 (miconazole, $P < 0.001$) to 8 (ketoconazole, $P < 0.001$) times more potent than the references. Furthermore, imidazole **13** and **16** were less cytotoxic than reference compounds, including fluconazole, with a selectivity index CC_{50}/MIC_{50} of 76.5 and 28.75, the highest among test derivatives **10**−**18**.

As shown by the examples reported here, proper manipulations of the number, nature, and position of substituents in the phenyl ring and phenoxy groups of imidazole derivative **13** can be useful for the design and synthesis of novel potent antifungal agents active against *C. albicans*. Trichloro substitution seems at the present to be the most favorable chemical modification with the formation of the potent econazole-like derivative **16**, but other substituents than chlorine, for example methyl and fluorine, can act as suitable bioisosters.

Our preliminary results on the antifungal activity of new compounds against a strain of *Aspergillus fumigatus* and a strain of *Aspergillus niger* demonstrate that **14, 16**, and **17** were active at a concentration of 16 μg/mL and more active than fluoxetine (100 μg/mL for *A. fumigatus* and 32 μg/mL for *A. niger*).

Besides the high potency displayed by some members of the novel class of antifungal imidazoles discovered in the present preliminary work, we are aware that further progress is obtainable to acquire compounds much more active than miconazole. For this reason, searches are now ongoing on novel fluoxetine-like azoles for developing more wide and deep structure−activity relationships (SAR) investigations also guided by molecular modeling and QSAR studies. Furthermore, comparative microbiological tests between title compounds and other classical azoles against a panel of different fungal pathogens will be performed soon to complete the present search.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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