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(*E*)- and (*Z*)-1,2,4-Triazolylchromanone oxime ethers as conformationally constrained antifungals

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Abstract—A series of 1,2,4-triazolylchromanone oxime ethers were synthesized and tested for in vitro antifungal activity. Many of these derivatives exhibit high activity against *Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus niger* and *Microsporum gypseum*.

1. Introduction

In recent years, fungal infection became an important complication and a major cause of morbidity and mortality in immunocompromised individuals such as those suffering from tuberculosis, cancer or AIDS and in organ transplant case.[1,2] Furthermore, systemic mycoses are being recognized more frequently as serious infections in a diverse and emergent group of patients. In particular, mycotic infections have flourished in response to major medical and surgical achievement, such as potent antibacterial agents and intravascular catheters, which represent the major predisposing factors for opportunistic infections.[3] Amphotericin B has been widely used to treat fungal infections, but it can cause serious side effects notably due to its renal toxicity.[4,5] However, more recently, there has been an expansion in the number of antifungal drug available. Five major classes of antifungal compounds are currently in clinical use: polyenes, azole derivatives, allylamines, thiocarbamates and fluoropyrimidines. Despite this growing list of antifungal agent, their clinical value has been limited by their relatively high risk of toxicity, the emergence, of drug resistance, pharmacokinetic deficiencies and/or insufficiencies in their antifungal activity. This situation has led to an ongoing search for potent broad spectrum antifungal agents with fewer side effects, which can be administered both orally and parenterally.[6–10] Among antifungal agents, azole derivatives still remain a viable lead structure in pursuit of a more efficacious, broad spectrum, systemic antifungal drug. Moreover, it is one of the few classes of compounds offering a clear identity of the target enzyme and adequate specificity for fungal organisms.[11] The azoles, which include the imidazole and triazole compounds inhibit the synthesis of sterols in fungi by inhibiting cytochrome P450-dependent 14α-lanosterol demethylase (P-450$_{14DM}$), which removes the methyl group on C-14 of lanosterol, a key intermediate step in the formation of ergosterol in the fungal cell membrane.[12,13] Figure 1 summarized the principal structural modifications introduced by the SAR (structure–activity relationships) of antifungal azoles. These studies reveal the presence in all of these molecules of one common pharmacophoric portion, which is characterized by a phenyl ring linked by an ethane chain to a nitrogen of azole ring (imidazole, A = CH, or triazole, A = N).[14] The ethane chain is often substituted on its C-2 by ether group, as in miconazole **1**, by the 1,3-spirodioxolane ring, as in ketoconazole **2**, or oxime ether group as in oxiconazole **3** (Fig. 2).

The present work is a follow up of our past efforts to design new antifungal agents in azolylchromanone

Keywords: Azole antifungals; 1,2,4-Triazole; Chromanone oxime ether; Antifungal activity.
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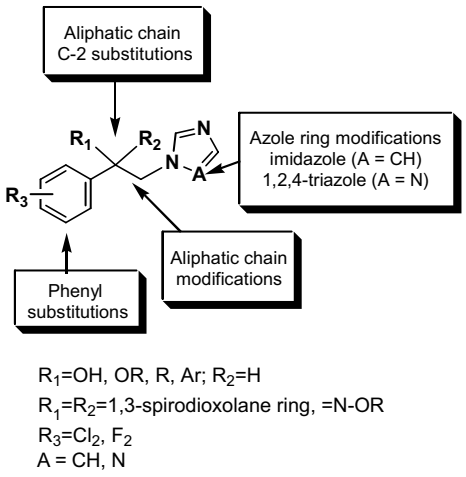


Figure 1. Common pharmacophoric structure and SAR studies of azole antifungals.

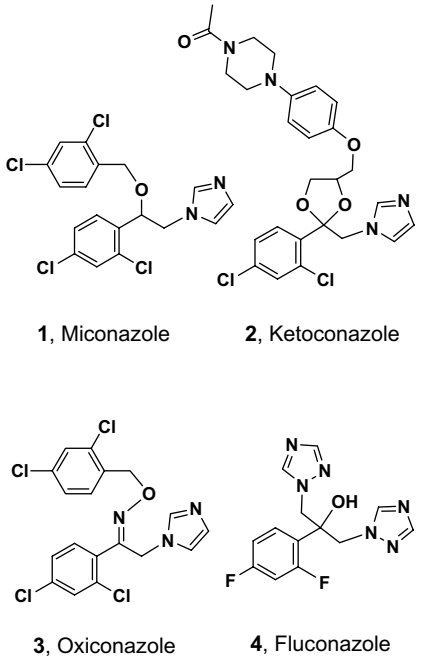


Figure 2. Azole antifungals.

oxime ether series that has produced few potentially active imidazolylchromanone oxime ethers **5**[15] (Fig. 3). These compounds can be considered as conformationally constrained or cyclic analogues of oxiconazole **3** and consisted of an chroman ring that, in itself, shows some antifungal activity.[16,17] These encouraging results prompted us to prepare and evaluate 1,2,4-triazolylchromanone oxime ethers **6** and **7** (Fig. 3) as antifungal agents and their structure–activity relationship.

2. Chemistry

The 1,2,4-triazolylchromanone oxime ethers **6** and **7** (Table 1) were prepared by reacting oximes (*E*)-**9**, (*Z*)-**9** and (*Z*)-**11** with substituted benzyl halides in DMF, in the presence of NaH at room temperature or K_2CO_3 at 50 °C (Fig. 4). Two stereoselective synthetic pathways were used to obtain the (*E*)- or (*Z*)-stereoisomers of oximes (*E*)-**9**, (*Z*)-**9** and (*Z*)-**11**.[18] Ring closure of 2-(1,2,4-triazolyl)-2′-hydroxyacetophenones **8** and **10**, followed by reaction with $HONH_2{\cdot}HCl$ gave the corresponding (*Z*)-oximes (*Z*)-**9** and (*Z*)-**11**. On the other hand, reaction of (*Z*)-3-bromo-2,3-dihydro-4*H*-1-benzopyran-4-one oxime (**12**) with 1,2,4-triazole afforded (*E*)-oxime (*E*)-**9** as a major product.[18]

The configuration of the oxime ethers (*Z*)-**6**, (*Z*)-**7** and (*E*)-**6** was assigned by ^1H NMR spectroscopy. In particular, the *syn* relationship between the oxime ether oxygen and the hydrogen at the C-5 [(*E*)-isomer] or the hydrogen at the C-3 [(*Z*)-isomer] of chroman ring may be confirmed by the fact that the protons, in this type of spatial arrangement, resonate at a lower field with respect to the same type of protons of the corresponding isomers.[15,18] This fact is due to the paramagnetic effect of the proximal oxime ether oxygen.[19,20] Physicochemical and spectroscopic characterization of the oximes (*Z*)-**9**, (*Z*)-**11**, (*E*)-**9** and of (*Z*)-oxime ethers (*Z*)-**6a–c**, (*Z*)-**6j,k**, (*Z*)-**7a**, (*Z*)-**7c,d** and (*Z*)-**7f** as well as of (*E*)-oxime ethers (*E*)-**6a–c** have been previously described by us.[18] The compounds (*Z*)-**6d–i**, (*Z*)-**6l**, (*Z*)-**7b**, (*Z*)-**7e**, (*E*)-**6d** and (*E*)-**6f–i**, here newly described, were obtained in generally good yield (45–82%) and purified by recrystallization, using 2-propanol. Physicochemical data of these compounds are shown in Table 2.

3. Results and discussion

Compounds (*Z*)-**6a–l**, (Z)-**7a–f**, (*E*)-**6a–d** and (*E*)-**6f–i** were evaluated for their in vitro antifungal activity

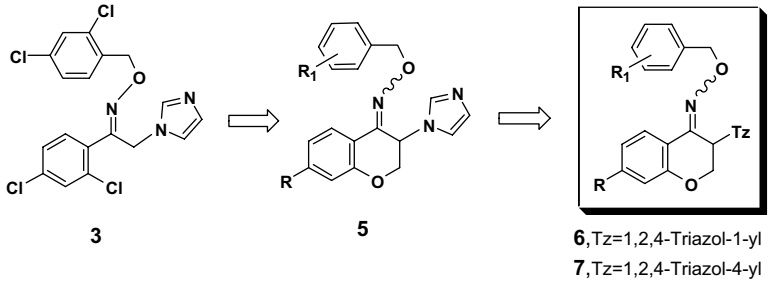
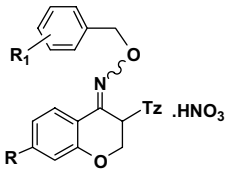


Figure 3.

Table 1. Structures and in vitro antifungal activity for (*E*)- and (*Z*)-1,2,4-triazolylchromanone oxime ethers



Compd	Tz[a]	R	R_1	*C. albicans*[b]		*S. cerevisiae*		*A. niger*		*M. gypseum*	
				MIC[c]	MFC[d]	MIC	MFC	MIC	MFC	MIC	MFC
(*Z*)-**6a**	-1-yl	H	H	64	>64	>64	>64	32	>64	16	>64
(*Z*)-**6b**	-1-yl	H	4-Cl	16	64	32	64	16	>64	4	32
(*Z*)-**6c**	-1-yl	H	2,4-Cl_2	64	>64	>64	>64	16	64	4	32
(*Z*)-**6d**	-1-yl	H	3,4-Cl_2	>64	>64	>64	>64	4	nt	1	nt
(*Z*)-**6e**	-1-yl	H	2,6-Cl_2	>64	>64	>64	>64	64	>64	16	32
(*Z*)-**6f**	-1-yl	H	4-Br	16	64	32	>64	16	16	8	32
(*Z*)-**6g**	-1-yl	H	4-F	64	>64	64	>64	32	64	32	64
(*Z*)-**6h**	-1-yl	H	3-F	32	>64	32	>64	16	32	8	16
(*Z*)-**6i**	-1-yl	H	2-F	>64	>64	>64	>64	32	64	8	16
(*Z*)-**6j**	-1-yl	Cl	4-Cl	32	>64	>64	>64	64	64	8	32
(*Z*)-**6k**	-1-yl	Cl	2,4-Cl_2	4	>64	8	>64	64	64	8	8
(*Z*)-**6l**	-1-yl	Cl	3,4-Cl_2	16	64	16	64	32	64	32	>64
(*E*)-**6a**	-1-yl	H	H	16	>64	>64	>64	2	16	2	64
(*E*)-**6b**	-1-yl	H	4-Cl	8	>64	32	>64	16	16	4	8
(*E*)-**6c**	-1-yl	H	2,4-Cl_2	64	>64	>64	>64	2	16	4	32
(*E*)-**6d**	-1-yl	H	3,4-Cl_2	16	>64	>64	>64	1	16	1	16
(*E*)-**6f**	-1-yl	H	4-Br	8	64	16	64	16	16	4	8
(*E*)-**6g**	-1-yl	H	4-F	64	>64	64	>64	8	16	8	32
(*E*)-**6h**	-1-yl	H	3-F	64	>64	64	>64	2	nt	4	nt
(*E*)-**6i**	-1-yl	H	2-F	>64	>64	>64	>64	16	32	8	16
(*Z*)-**7a**	-4-yl	H	H	64	>64	>64	>64	>64	>64	64	>64
(*Z*)-**7b**	-4-yl	H	4-Cl	32	>64	>64	>64	16	>64	16	>64
(*Z*)-**7c**	-4-yl	H	2,4-Cl_2	64	>64	>64	>64	64	64	32	>64
(*Z*)-**7d**	-4-yl	Cl	H	64	>64	64	>64	64	>64	16	>64
(*Z*)-**7e**	-4-yl	Cl	4-Cl	16	>64	32	32	64	64	16	32
(*Z*)-**7f**	-4-yl	Cl	2,4-Cl_2	64	>64	>64	>64	>64	>64	16	32
Fluconazole				8	64	32	>64	16	32	32	>64
Oxiconazole				16	32	2	32	4	16	16	64

[a] 1,2,4-Triazol-.
[b] Fungi tested: *C. albicans* ATCC 10231, *S. cerevisiae* PTCC 5177, *A. niger* ATCC 16401, *M. gypseum* ATCC×191.
[c] MIC in μg/mL.
[d] MFC in μg/mL.

against the pathogenic fungi *Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus niger* and *Microsporum gypseum* (Table 1). The MIC (minimum inhibitory concentration) and the MFC (minimum fungicidal concentration) values were determined by comparison to fluconazole and oxiconazole as the reference drugs. The MIC values of the test derivatives indicate that the most active compounds were 1,2,4-triazol-1-yl derivatives (*Z*)-**6** and (*E*)-**6**, which showed generally good activity against all tested fungal species. In contrast, compounds (*Z*)-**7** did not show significant antifungal activity, although some of them exhibited good activity comparable to the reference drugs against some fungal species.

The compound (*Z*)-**6k** was the most potent against the yeasts, with MIC values of 4 μg/mL for *C. albicans* and 8 μg/mL for *S. cerevisiae*. Compound (*Z*)-**6k** was also more potent than reference drugs against *C. albicans* and more potent than fluconazole against *S. cerevisiae*. In general, there was no appreciable difference between the (*E*)- and (*Z*)-isomers as regards to the activity against the yeasts with the exception of (*E*)-**6a** and (*E*)-**6d** (MIC = 16 μg/mL), which were four-fold more active than their (*Z*)-isomers (MIC $\geqslant$ 64 μg/mL).

The MIC values of the test derivatives against *A. niger* and *M. gypseum* indicate that most compounds possessed a comparable or better activity with respect to reference drugs. In fact, the most active compound was (*E*)-**6d** (MIC = 1 μg/mL) being 16–32-fold more active than fluconazole and 4–16 times more active than oxiconazole against *A. niger* and *M. gypseum*, respectively. In addition (*Z*)-**6d**, stereoisomer of (*E*)-**6d**, was the most potent compound in (*Z*)-isomer series against *M. gypseum*, its activity was found to be equal to (*E*)-**6d**. Comparison between MIC values of the (*E*)- and (*Z*)-isomers against *A. niger* revealed appreciable differences, with the exception of **6b** and **6f**, which their stereoisomers showed equal activity (MIC = 16 μg/mL). Actually, the (*E*)-isomer of **6a**, **6c**, **6d** and **6g–i** were 2–16 times more potent than the corresponding (*Z*)-isomers.

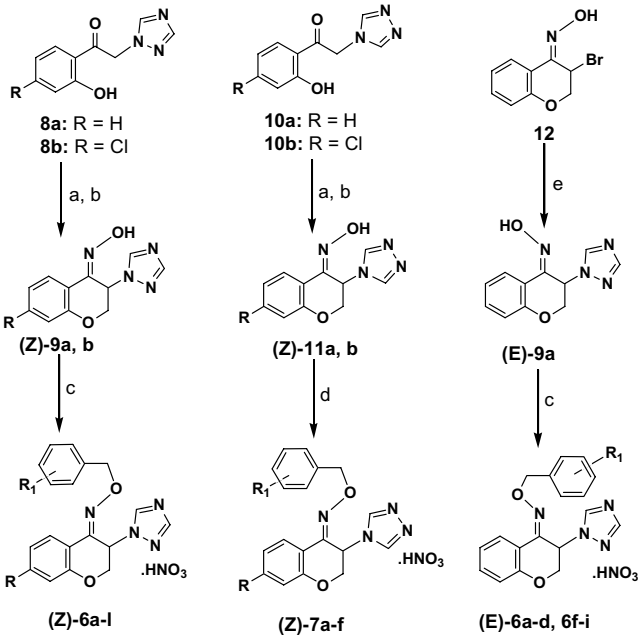


Figure 4. Stereoselective synthesis of (*E*)- and (*Z*)-triazolylchromanone oxime ethers. Reagents and conditions: (a) $(CH_2O)_n$, AcOH, 90–100 °C; (b) $NH_2OH{\cdot}HCl$, MeOH, rt; (c) substituted benzyl halide, NaH, DMF, rt; (d) substituted benzyl halide, K_2CO_3, DMF, 50 °C; (e) 1,2,4-triazole, K_2CO_3, CH_3CN, rt.

Comparison between MIC_S of the (*E*)- and (*Z*)-isomers against *M. gypseum* revealed equal activity except (*Z*)-**6a** and (*E*)-**6a**. In the latter, (*E*)-**6a** (MIC = 2 μg/mL) was eight times more active than (*Z*)-**6a** (MIC = 16 μg/mL).

As noted in Table 1, the MFC values of all compounds and fluconazole for yeasts were ⩾ 64 μg/mL. The MFC values of compound (*Z*)-**6k** (possessing lowest MIC against yeasts) were higher than its MIC values and this compound showed a fungistatic character against yeasts.[21] In addition, some derivatives, which displayed a good growth inhibition against *A. niger* and *M. gypseum* (MIC ⩾ 16 μg/mL), showed cidal properties against these fungal species, while compound (*E*)-**6d** (the most potent compound, MIC = 1 μg/mL) was clearly fungistatic.

In general, the results of antifungal evaluation of test compounds in comparison with reference drugs indicated that compounds (*E*)-**6b** and (*E*)-**6f** showed comparable or more potent antifungal activity with respect to fluconazole against all tested fungal species. Compounds (*Z*)-**6b**, (*Z*)-**6f** and (*Z*)-**6h** showed comparable or more potent antifungal activity with respect to fluconazole except for antifungal activity against *C. albicans*. Compounds (*E*)-**6a** and (*E*)-**6d** showed comparable or more potent antifungal activity with respect to oxiconazole except for antifungal activity against *S. cerevisiae*.

In terms of structure–activity relationship, as expected the 1,2,4-triazol-1-yl derivatives **6** showed more potent antifungal activity than 1,2,4-triazol-4-yl derivatives **7**. In general, among the test strains, only against *A. niger*,

Table 2. Physical and spectral data of new 1,2,4-triazolylchromanone oxime ethers

Compd	Mp (°C)	Yield (%)	^{1}H NMR (δ ppm)[a]	Formula
(*Z*)-**6d**	145–146	76	4.24–4.72 (m, 2H, H-2), 5.20 (s, 2H, CH_2), 6.14 (m, 1H, H-3), 6.88–7.55 (m, 6H, aromatic H, H-6, H-7 and H-8), 7.84 (d, 1H, J = 8.0, H-5), 8.00 (s, 1H, triazole H), 8.65 (s, 1H, triazole H)	$C_{18}H_{14}Cl_2N_4O_2{\cdot}HNO_3$
(*Z*)-**6e**	141–143	61	4.23–4.70 (m, 2H, H-2), 5.39 (s, 2H, CH_2), 6.00 (m, 1H, H-3), 6.85–7.60 (m, 6H, aromatic H, H-6, H-7 and H-8), 7.84 (d, 1H, J = 8.0, H-5), 7.98 (s, 1H, triazole H), 8.50 (s, 1H, triazole H)	$C_{18}H_{14}Cl_2N_4O_2{\cdot}HNO_3$
(*Z*)-**6f**	149–151	48	4.35 (dd, 1H, J = 12.8, 2.4, H-2a), 4.62 (dd, 1H, J = 12.8, 2.0, H-2b), 5.16 (s, 2H, CH_2), 6.12 (m, 1H, H-3), 6.85–7.60 (m, 7H, aromatic H, H-6, H-7 and H-8), 7.83 (d, 1H, J = 8.0, H-5), 8.01 (s, 1H, triazole H), 8.60 (s, 1H, triazole H)	$C_{18}H_{15}BrN_4O_2{\cdot}HNO_3$
(*Z*)-**6g**	132–134	50	4.37 (dd, 1H, J = 12.8, 2.4, H-2a), 4.63 (dd, 1H, J = 12.8, 1.9, H-2b), 5.16 (s, 2H, CH_2), 6.10 (m, 1H, H-3), 6.88–7.49 (m, 7H, aromatic H, H-6, H-7 and H-8), 7.85 (d, 1H, J = 8.0, H-5), 7.96 (s, 1H, triazole H), 8.51 (s, 1H, triazole H)	$C_{18}H_{15}FN_4O_2{\cdot}HNO_3$
(*Z*)-**6h**	133–135	72	4.39 (dd, 1H, J = 12.8, 2.6, H-2a), 4.63 (dd, 1H, J = 12.8, 2.0, H-2b), 5.21 (s, 2H, CH_2), 6.13 (m, 1H, H-3), 6.85–7.50 (m, 7H, aromatic H, H-6, H-7 and H-8), 7.83 (dd, 1H, J = 8.0, 2.0, H-5), 7.96 (s, 1H, triazole H), 8.56 (s, 1H, triazole H)	$C_{18}H_{15}FN_4O_2{\cdot}HNO_3$
(*Z*)-**6i**	124–126	67	4.25–4.79 (m, 2H, H-2), 5.23 (s, 2H, CH_2), 6.10 (m, 1H, H-3), 6.85–7.52 (m, 7H, aromatic H, H-6, H-7 and H-8), 7.85 (d, 1H, J = 8.0, H-5), 8.01 (s, 1H, triazole H), 8.51 (s, 1H, triazole H)	$C_{18}H_{15}FN_4O_2{\cdot}HNO_3$
(*Z*)-**6l**	133–135	45	4.32–4.75 (m, 2H, H-2), 5.20 (s, 2H, CH_2), 6.18 (m, 1H, H-3), 6.95–7.63 (m, 5H, H-6, H-8 and aromatic H), 7.80 (d, 1H, J = 8.0, H-5), 7.99 (s, 1H, triazole H), 8.66 (s, 1H, triazole H)	$C_{18}H_{13}Cl_3N_4O_2{\cdot}HNO_3$
(*E*)-**6d**	135–137	45	4.53 (dd, 1H, J = 12.8, 2.6, H-2a), 4.92 (dd, 1H, J = 12.8, 2.6, H-2b), 5.27 (s, 2H, CH_2), 5.51 (t, 1H, J = 2.6, H-3), 6.90–7.70 (m, 6H, H-6, H-7, H-8 and aromatic H), 8.12 (s, 1H, triazole H), 8.51 (d, 1H, J = 8.0, H-5), 8.73 (s, 1H, triazole H)	$C_{18}H_{14}Cl_2N_4O_2{\cdot}HNO_3$
(*E*)-**6f**	141–143	53	4.55 (dd, 1H, J = 12.0, 3.0, H-2a), 4.96 (dd, 1H, J = 12.0, 3.0, H-2b), 5.24 (s, 2H, CH_2), 5.48 (t, 1H, J = 3.0, H-3), 7.02 (m, 2H, H-6 and H-8), 7.30–7.65 (m, 5H, H-7 and aromatic H), 8.08 (s, 1H, triazole H), 8.51 (d, 1H, J = 9.0, H-5), 8.65 (s, 1H, triazole H)	$C_{18}H_{15}BrN_4O_2{\cdot}HNO_3$

Table 2 (*continued*)

Compd	Mp (°C)	Yield (%)	^{1}H NMR (δ ppm)[a]	Formula
(*E*)-**6g**	134–135	67	4.53 (dd, 1H, J = 12.8, 2.7 H-2a), 4.94 (dd, 1H, J = 12.8, 2.7, H-2b), 5.23 (s, 2H, CH_2), 5.45 (t, 1H, J = 2.7, H-3), 6.90–7.61 (m, 7H, H-6, H-7, H-8 and aromatic H), 7.99 (s, 1H, triazole H), 8.49 (s, 1H, triazole H), 8.52 (dd, 1H, J = 8.8, 2.0, H-5)	$C_{18}H_{15}FN_4O_2{\cdot}HNO_3$
(*E*)-**6h**	117–118	57	4.55 (dd, 1H, J = 12.8, 2.8, H-2a), 4.95 (dd, 1H, J = 12.8, 2.8, H-2b), 5.27 (s, 2H, CH_2), 5.45 (t, 1H, J = 2.8, H-3), 6.89–7.59 (m, 7H, H-6, H-7, H-8 and aromatic H), 7.97 (s, 1H, triazole H), 8.49 (s, 1H, triazole H), 8.55 (d, 1H, J = 8.0, H-5)	$C_{18}H_{15}FN_4O_2{\cdot}HNO_3$
(*E*)-**6i**	119–121	78	4.55 (dd, 1H, J = 12.8, 2.4, H-2a), 4.96 (dd, 1H, J = 12.8, 2.6, H-2b), 5.30 (s, 2H, CH_2), 5.47 (m, 1H, H-3), 6.87–7.62 (m, 7H, H-6, H-7, H-8 and aromatic H), 8.01 (s, 1H, triazole H), 8.48 (d, 1H, J = 8.0, H-5), 8.52 (s, 1H, triazole H)	$C_{18}H_{15}FN_4O_2{\cdot}HNO_3$
(*Z*)-**7b**	146–149	55	4.90 (dd, 1H, J = 12.8, 2.6, H-2a), 5.23 (dd, 1H, J = 12.8, 2.0, H-2b), 5.72 (s, 2H, CH_2), 6.56 (m, 1H, H-3), 7.36–7.60 (m, 2H, H-6 and H-8), 7.70–8.00 (m, 5H, aromatic H and H-7), 8.35 (dd, 1H, J = 8.0, 2.0, H-5), 8.76 (s, 2H, triazole H)	$C_{18}H_{15}ClN_4O_2{\cdot}HNO_3$
(*Z*)-**7e**	138–140	82	4.50 (dd, 1H, J = 12.8, 2.4, H-2a), 4.80 (dd, 1H, J = 12.8, 2.0, H-2b), 5.23 (s, 2H, CH_2), 6.18 (m,1H, H-3), 6.90–7.52 (m, 6H, H-6, H-8 and aromatic H), 7.83 (d, 1H, J = 9.0, H-5), 9.14 (s, 2H, triazole H)	$C_{18}H_{14}Cl_2N_4O_2{\cdot}HNO_3$

[a] Spectra were determined in DMSO-d_6 and J values are in Hz.

a good correlation between activity and the geometry of oxime ether group was observed. Nevertheless, the most active compound against yeasts belong to (*Z*)-isomer series. Furthermore, the type, number and position of halogen substitutions on the *O*-benzyl group and chlorine atom linked to the 7 position of chroman ring seemed to have different influence on the antifungal activity against various fungi strains.

From our biological results, it is evident that 1,2,4-triazol-1-ylchromanone oxime ethers **6** exhibited potent antifungal activity against several fungal species. It is worthwhile observing that, in comparison with imidazolylchromanone oxime ethers **5**, previously described by us,[15] both of them have a comparable or better antifungal activity than oxiconazole and fluconazole. In general, imidazolyl and 1,2,4-triazol-1-ylchromanone oxime ethers (**5** and **6**) have a similar biological profile and structure–activity relationships but as expected, 1,2,4-triazol-1-ylchromanone oxime ethers **6** showed lower in vitro antifungal activity than imidazolylchoromanone oxime ethers **5**.

These data demonstrated that the combination of the azolylchromanone oxime ether with chroman ring resulted to potent antifungal agents. In fact, these compounds are skeletal analogs of oxiconazole, but whose conformationally constrained structure allows them to adopt only a limited subset of conformations relative to oxiconazole, possibly including the bioactive conformation. Thus, the improvement of overall antifungal activity of some azolylchromanone oxime ethers might be a result of adopting an energetically accessible bioactive conformation, which is available to active analogs, or result of rigid steric and/or electronic requirements operative at the receptor.

In conclusion, the results obtained from this study revealed that, some of the 1,2,4-triazolylchromanone oxime ethers exhibited a comparable or better antifungal activity than fluconazole and oxiconazole. These results are encouraging to better define and optimize the antifungal effect of these compounds. They could represent new lead compounds for further pharmacomodulation in the series of chroman-based 1,2,4-triazole derivatives.

4. Experimental

All melting points were measured with a Kofler hot stage apparatus and are uncorrected. IR spectra were recorded on a Nicolet FT-IR Magna 550 spectrophotometer. ^{1}H NMR spectra were measured using a Bruker FT-80 spectrometer, and chemical shifts are expressed as δ (ppm) with tetramethylsilane as internal standard. Elemental microanalyses were within ±0.4% of calculated values. Chemicals and all solvents used in this study were purchased from Merck AG and Aldrich Chemical. Yields are of purified product and were not optimized.

4.1. General procedure for the synthesis of 1,2,4-triazol-1-ylchromanone oxime ethers 6

A solution of (*E*)- or (*Z*)-**9** (1.0 mmol) in DMF (2 mL) was added to a suspension of NaH (24 mg, 1.0 mmol) in DMF (1 mL). The reaction mixture was stirred at rt for 30 min and then a solution of substituted benzyl halides (1.0 mmol) in DMF (1 mL) was added. After stirring at room temperature for 6–12 h, the reaction mixture was poured into water and extracted with $CHCl_3$. The organic layer was washed (H_2O), dried (Na_2SO_4) and evaporated. The viscous oily residue was dissolved in 2-propanol and treated with 70% HNO_3 to give (*E*)- or (*Z*)-**6**.

4.2. General procedure for the synthesis of 1,2,4-triazol-4-ylchromanone oxime ethers 7

A stirring suspension of oximes (*Z*)-**11** (1.0 mmol) and K_2CO_3 (1.0 mmol) in DMF (4 mL) was heated at 50 °C and substituted benzyl halides (1.0 mmol) dissolved in

DMF (1 mL) was added dropwise. After 6–10 h the mixture was poured into water and extracted with $CHCl_3$. The organic phase was washed (H_2O), dried (Na_2SO_4) and evaporated. The viscous oily residue was dissolved in 2-propanol and treated with 70% HNO_3 to give (*Z*)-**7**.

4.3. Antifungal activity

For antifungal assays, the compounds were dissolved in DMSO (1 mL) and the solution was diluted with distilled water (9 mL). Further progressive double dilutions with test medium gave the required concentrations of 64, 32, 16, 8, 4, 2, 1, 0.5 and 0.25 μg/mL. The minimum inhibitory concentration (MIC) was determined by using the method of twofold serial dilution technique.[22] Compounds were tested for their in vitro growth inhibitory activity against *C. albicans*, *S. cerevisiae*, *A. niger* and *M. gypseum*. All tested microorganisms were first incubated at 35 °C on Sabouraud dextrose broth for 18 h. Testing was performed in Sabouraud dextrose broth at pH 7.0. Inoculum size was 0.5×10^3 CFU/mL. Reading of MICs were taken after 48 h at 35 °C. The MIC was defined as the lowest concentration of substance at which there was no growth.

MFC values were determined by subculturing 10 and 100 μL of broth from the drug-free control tube, the first tube containing growth and each clear tube on agar Sabouraud plates. To ensure that there was no antifungal agent carry-over, broth samples were centrifuged and resuspended in antifungal agent-free medium. MFC values were defined as the lowest concentration of the drug expressed in μg/mL that killed $\geqslant 99.9\%$ of the initial inoculum.

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