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Stereoselective synthesis and antifungal activity of (*Z*)-*trans*-3-azolyl-2-methylchromanone oxime ethers

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Abstract—A series of (*Z*)-*trans*-3-azolyl-2-methylchromanone oxime ethers were stereoselectively 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*.
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1. Introduction

During the past two-decades the frequencies and type of life threatening fungal infection have increased dramatically among cancer patients, transplant recipients, patients with AIDS, and patients receiving broad-spectrum antibiotic or parental nutrition. There has been a constant effort to develop more effective and safe antifungal drug to combat these infections.[1–5] Furthermore the development of resistant fungal strains in response to the widespread use of current antifungal drugs is likely to cause serious problems in future. Therefore there is an urgent need to develop safe, efficacious, and nontoxic antimycotic agents with a broad spectrum of antifungal activity.[6–8]

Up to only 30 years ago the choice of systemically available antimycotics was between two drugs, amphotericin B and 5-flurocytosine, neither of which was satisfactory. Then a series of inhibitors of the biosynthesis of ergosterol the major sterol of the fungal cell membrane, were found to have excellent antifungal activity, improved safety, and some were also active after oral or parenteral application. Starting with miconazole **1** and ketoconazole **2**, and improving through fluconazole **3** and itraconazole **4** (Fig. 1), the imidazole and triazole inhibitors of cytochrome P450-dependent 14α-lanosterol demethylase ($P\text{-}450_{14DM}$) have been the most successful.[6,9]

A critical structure survey of azole class of antifungals revealed that most of them possess *N*-(phenethyl)azole moiety linked with either 1,3-dioxolane or hydroxy group at position-2 in their molecular make up that seems to be the pharmacophore for this activity (Fig. 2).[10] Furthermore, numerous ethers of 1-(4-chlorophenyl)- and 1-(2,4-dichlorophenyl)-2-(1*H*-imidazolyl)ethanol or ethanone oxime (e.g., oxiconazole **5**) have been developed as antifungal agents or show promise for clinical use.[10–13] In an effort to find new antifungals, we have recently designed and synthesized a novel class of azoles such as compound **6** as potential antifungal agents (Fig. 1).[14,15] These compounds are halogen-substituted azolylchromanone oxime ethers, and have a more constrained conformation than that of oxiconazole **5**. Oxiconazole is a well-known antifungal agent with a broad spectrum of activity, which is characterized by having an oxime ether group in the (*Z*)-configuration.[10,16] The results of our previous study revealed that some of the (*Z*)- and (*E*)-azolylchromanone oxime ethers **6** exhibited a comparable or better antifungal activity than reference drugs (fluconazole and oxiconazole).[14,15] In particular, 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,

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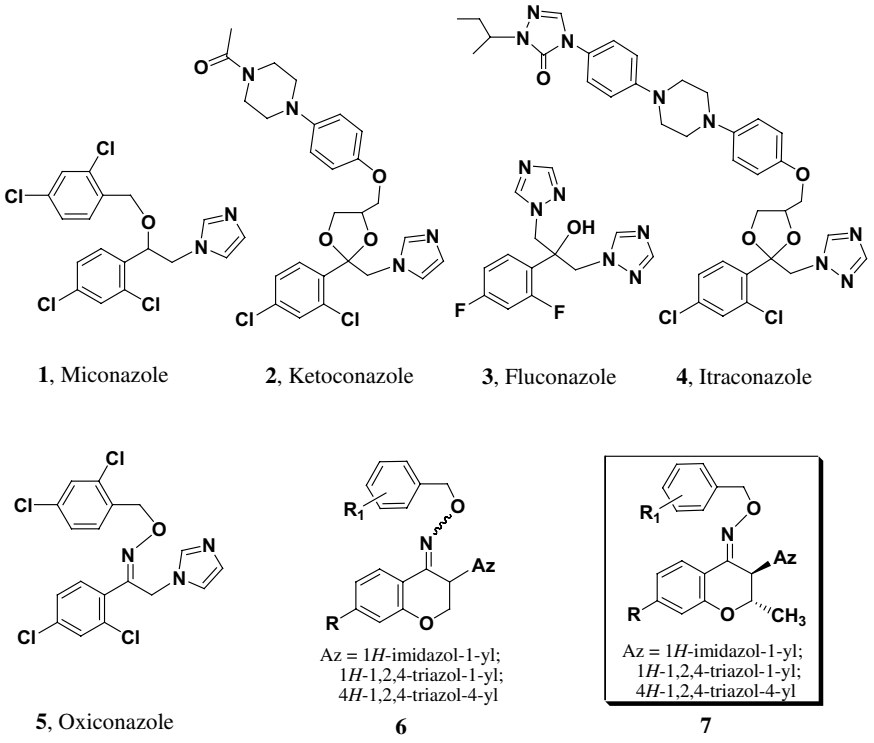


Figure 1. Azole antifungals.

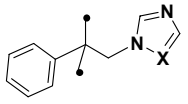


Figure 2. Common structural unit of azole antifungals.

the potential 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 the result of rigid steric and/or electronic requirements operative at the enzyme or enzyme active center. In addition, the overall antifungal activity observed for azolylchromanone oxime ethers against fungal species is probably due to the chroman moiety, which occurs in several antifungal agents.[17,18] The fused six-membered hetero-ring of chroman moiety can exist in the form of two energetically different half-chairs,[19,20] and azole ring occupies pseudo-axial or pseudo-equatorial positions (Fig. 3). It is to be noted that the introduction of a 2-substituent into the chroman ring influences the conformation of chroman ring and stabilizes more than one conformer. With these in mind, we have designed and synthesized (*Z*)-*trans*-3-azolyl-2-methylchromanone oxime ethers **7**, to investigate the influence of a methyl group at position-2 on chroman nucleus and to optimize the structure–activity relationships and development of the more potent antifungal agent.

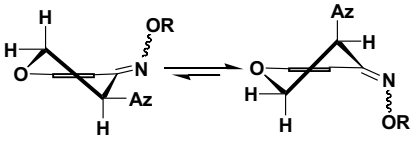
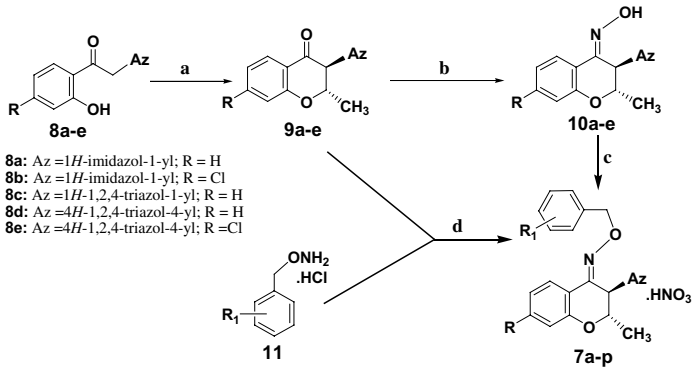


Figure 3. Possible conformations for azolylchromanone oxime ethers.

2. Chemistry

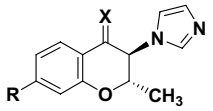
Our synthetic pathway to target compounds **7a**–**p** is presented in Scheme 1. *trans*-3-Azolyl-2-methylchromanones **9** were obtained from 2-azolyl-2′-hydroxy-acetophenones **8** according to the method reported in the literature.[21–23] Compound **9** was converted to the pure (*Z*)-oxime derivatives **10** by stirring with 3 equiv of $HONH_2$·HCl in methanol. *O*-Alkylation of **10** with substituted benzyl halides in the presence of NaH in DMF gave the corresponding (*Z*)-oxime ethers **7** (Method A). Alternatively, the (*Z*)-oxime ethers **7** were synthesized by the reaction of compounds **9** with *O*-(arylmethyl)-hydroxylamine·HCl **11** (Method B). Thus, treatment of compounds **9** with **11** in methanol gave oxime ethers derivatives mainly in the (*Z*)-configuration. In the latter procedure, the work-up of the crude product led to the practically pure (*Z*)-oxime ethers **7** in moderate yield. Physiochemical and spectroscopic characterization of compounds **9c**–**e**, **10c**–**e**, and **7m**–**p** have been described by us elsewhere.[22] The compounds **10a**,**b** and **7a**–**l**, here newly described (Tables 1 and 2).

According to our previous papers, the configurational assignment of the oxime geometry was simple due to the strong anisotropic deshielding by the oxime oxygen on the H-3 proton on chroman ring (Tables 3



Scheme 1. Stereoselective synthesis of (±)-(*Z*)-*trans*-3-azolyl-2-methylchroman-4-one oxime ethers **7**. Reagents and conditions: (a) CH_3CHO, AcOH, 90 °C; (b) $NH_2OH{\cdot}HCl$, K_2CO_3, MeOH, rt; (c) substituted benzyl halide, NaH, DMF, rt, and then HNO_3; (d) K_2CO_3, MeOH, rt, and then HNO_3.

Table 1. Chemical, physical, and ^1H NMR spectral data of the compounds **9a**,**b** and **10a**,**b**



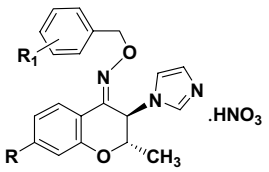
Compd	R	X	Yield (%)	Mp (°C)	^1H NMR	Formula
9a	H	O	67	143–144	1.37 (d, 3H, *J* = 6.0 Hz, CH_3), 4.74 (dq, 1H, *J* = 12.0, 6.0 Hz, H-2), 4.88 (d, 1H, *J* = 12.0 Hz, H-3), 6.89 (br s, 1H, imidazole H), 7.05 (dd, 1H, *J* = 8.0, 1.0 Hz, H-8), 7.12 (dt, 1H, *J* = 8.0, 1.0 Hz, H-6), 7.19 (br s, 1H, imidazole H), 7.57 (s, 1H, imidazole H), 7.58 (dt, 1H, *J* = 8.0, 1.6 Hz, H-7), 7.93 (dd, 1H, *J* = 8.0, 1.6 Hz, H-5) (a)	$C_{13}H_{12}N_2O_2$
9b	Cl	O	47	140–141	1.18 (d, 3H, *J* = 5.6 Hz, CH_3), 5.12 (dq, 1H, *J* = 12.0, 5.6 Hz, H-2), 5.65 (d, 1H, *J* = 12.0 Hz, H-3), 6.91–7.62 (m, 5H, H-6, H-8, and imidazole H), 7.83 (d, 1H, *J* = 8.0 Hz, H-5) (a)	$C_{13}H_{11}ClN_2O_2$
10a	H	NOH (*Z*)	83	227–228	1.11 (d, 3H, *J* = 6.4 Hz, CH_3), 4.42 (dq, 1H, *J* = 6.4, 2.4 Hz, H-2), 5.80 (d, 1H, *J* = 2.4 Hz, H-3), 6.60 (br s, 1H, imidazole H), 7.04 (m, 3H, H-6, H-8, and imidazole H), 7.37 (dt, 1H, *J* = 8.0, 2.0 Hz, H-7), 7.53 (br s, 1H, imidazole H), 7.84 (dd, 1H, *J* = 8.0, 2.0 Hz, H-5), 11.75 (s, 1H, OH) (b)	$C_{13}H_{13}N_3O_2$
10b	Cl	NOH (*Z*)	79	188–189	1.11 (d, 3H, *J* = 6.4 Hz, CH_3), 4.45 (dq, 1H, *J* = 6.4, 2.4 Hz, H-2), 5.85 (d, 1H, *J* = 2.4 Hz, H-3), 6.80–7.40 (m, 5H, H-6, H-8, and imidazole H), 7.84 (d, 1H, *J* = 9.0 Hz, H-5), 11.89 (s, 1H, OH) (b)	$C_{13}H_{12}ClN_3O_2$

NMR solvents: (a) $CDCl_3$; (b) DMSO-d_6.

and 4).[14,15,22,23] All described compounds; namely, ketones **9**, oximes **10**, and oxime ethers **7**, which possess two chiral center on their chroman ring on C-2 and C-3 positions, are racemates, but for the sake of clarity only one enantiomer is shown in each case.

During the course of this study we observed an unusual decrease in the $J_{2,3}$ coupling constants of chroman ring upon the formation of oximes and oxime ethers (Tables 3 and 4). Thus, the small $J_{2,3}$ observed in the (*Z*)-*trans*-3-azolyl-2-methyl chromanone oxime derivatives required explanation. The half-chair conformation of the hetero-ring in the chromans principally arises from the restricted rotation imposed by the benzene ring and is analogous to the preferred conformation of the dihydropyran ring in the flavan derivatives described by Clark-Lewis et al.[19,20] As previously discussed by Clark-Lewis et al., variation in vicinal coupling constants may be due to changes in conformational equilibria.[19] The results confirm the stereochemistry of the chromans and, in agreement with earlier work, favor the half-chair conformation for the heterocyclic ring as shown in Figure 4. Thus, relatively large $J_{2,3}$ value (11.6–12.0 Hz) in compound **9** confirms the preferred pseudo-axial conformation of H-2 and H-3. Examination of the ^1H NMR coupling constant information of the oxime derivatives **7** and **10** in Tables 3 and 4 clearly shows the small $J_{2,3}$ value (2.4–2.6 Hz) and suggests a preference for the conformation in which H-2 and H-3 are in pseudo-equatorial orientation (Fig. 4). Thus, by analysis of vicinal interproton coupling constants it is believed that compounds **7** and **10** exist predominantly in the diaxial half-chair conformation and formation of oxime derivatives results in a remarkable conformational inversion for methyl and azole systems on chroman ring. According to the relationship between the axial azole

Table 2. Chemical, physical, and ^{1}H NMR spectral data of the compounds **7a**–**l**



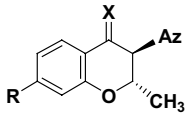
Compd	R	R_1	Method (Yield %)	Mp (°C)	^{1}H NMR (DMSO-d_6)	Formula
7a	H	H	B(63)	166–167 (a)	1.11 (d, 3H, J = 6.4 Hz, CH_3), 4.65 (dq, 1H, J = 6.4, 2.6 Hz, H-2), 5.20 (s, 2H, CH_2), 6.13 (d, 1H, J = 2.6 Hz, H-3), 7.01–7.69 (m, 10H, H-6, H-7, H-8, aromatic H, and imidazole H), 7.86 (dd, 1H, J = 8.0, 2.0 Hz, H-5), 9.09 (s, 1H, imidazole H-2)	$C_{20}H_{19}N_3O_2{\cdot}HNO_3$
7b	H	4-Cl	A(52)	169–170 (b)	1.12 (d, 3H, J = 6.4 Hz, CH_3), 4.63 (dq, 1H, J = 6.4, 2.6 Hz, H-2), 5.19 (s, 2H, CH_2), 6.13 (d, 1H, J = 2.6 Hz, H-3), 7.00–7.70 (m, 9H, H-6, H-7, H-8, aromatic H, and imidazole H), 7.85 (dd, 1H, J = 8.0, 2.0 Hz, H-5), 9.10 (s, 1H, imidazole H-2)	$C_{20}H_{18}ClN_3O_2{\cdot}HNO_3$
7c	H	2,4-Cl_2	A(50)	144–145 (b)	1.12 (d, 3H, J = 6.4 Hz, CH_3), 4.63 (dq, 1H, J = 6.4, 2.4 Hz, H-2), 5.24 (s, 2H, CH_2), 6.12 (d, 1H, J = 2.4 Hz, H-3), 6.93–8.03 (m, 9H, H-5, H-6, H-7, H-8, aromatic H, and imidazole H), 9.12 (s, 1H, imidazole H-2)	$C_{20}H_{17}Cl_2N_3O_2{\cdot}HNO_3$
7d	H	3,4-Cl_2	B(36)	151–153 (a)	1.11 (d, 3H, J = 6.4 Hz, CH_3), 4.64 (dq, 1H, J = 6.4, 2.6 Hz, H-2), 5.18 (s, 2H, CH_2), 6.14 (d, 1H, J = 2.6 Hz, H-3), 7.00–7.73 (m, 8H, H-6, H-7, H-8, aromatic H, and imidazole H), 7.83 (dd, 1H, J = 8.0, 2.0 Hz, H-5), 9.11 (s, 1H, imidazole H-2)	$C_{20}H_{17}Cl_2N_3O_2{\cdot}HNO_3$
7e	H	2,6-Cl_2	B(63)	121–122 (a)	1.12 (d, 3H, J = 6.4 Hz, CH_3), 4.64 (dq, 1H, J = 6.4, 2.4 Hz, H-2), 5.38 (s, 2H, CH_2), 6.13 (d, 1H, J = 2.4 Hz, H-3), 6.94–8.01 (m, 9H, H-5, H-6, H-7, H-8, aromatic H, and imidazole H), 9.10 (s, 1H, imidazole H-2)	$C_{20}H_{17}Cl_2N_3O_2{\cdot}HNO_3$
7f	H	4-Br	B(66)	167–168 (a)	1.14 (d, 3H, J = 6.4 Hz, CH_3), 4.63 (dq, 1H, J = 6.4, 2.6 Hz, H-2), 5.17 (s, 2H, CH_2), 6.13 (d, 1H, J = 2.6 Hz, H-3), 7.02–7.77 (m, 9H, H-6, H-7, H-8, aromatic H, and imidazole H), 7.87 (dd, 1H, J = 8.0, 2.0 Hz, H-5), 9.09 (s, 1H, imidazole H-2)	$C_{20}H_{18}BrN_3O_2{\cdot}HNO_3$
7g	H	4-F	A(74)	160–162 (a)	1.12 (d, 3H, J = 6.4 Hz, CH_3), 4.63 (dq, 1H, J = 6.4, 2.4 Hz, H-2), 5.17 (s, 2H, CH_2), 6.12 (d, 1H, J = 2.4 Hz, H-3), 6.95–7.70 (m, 9H, H-6, H-7, H-8, aromatic H, and imidazole H), 7.89 (d, 1H, J = 8.0 Hz, H-5), 9.06 (s, 1H, imidazole H-2)	$C_{20}H_{18}FN_3O_2{\cdot}HNO_3$
7h	H	3-F	A(70)	140–141 (a)	1.14 (d, 3H, J = 6.4 Hz, CH_3), 4.64 (dq, 1H, J = 6.4, 2.4 Hz, H-2), 5.21 (s, 2H, CH_2), 6.17 (d, 1H, J = 2.4 Hz, H-3), 6.87–7.73 (m, 9H, H-6, H-7, H-8, aromatic H, and imidazole H), 7.88 (d, 1H, J = 8.0 Hz, H-5), 9.08 (s, 1H, imidazole H-2)	$C_{20}H_{18}FN_3O_2{\cdot}HNO_3$
7i	H	2-F	A(72)	158–160 (a)	1.12 (d, 3H, J = 6.4 Hz, CH_3), 4.63 (dq, 1H, J = 6.4, 2.4 Hz, H-2), 5.22 (s, 2H, CH_2), 6.10 (d, 1H, J = 2.4 Hz, H-3), 6.88–7.68 (m, 9H, H-6, H-7, H-8, aromatic H, and imidazole H), 7.84 (d, 1H, J = 8.0 Hz, H-5), 9.06 (s, 1H, imidazole H-2)	$C_{20}H_{18}FN_3O_2{\cdot}HNO_3$
7j	Cl	H	B(58)	168–170 (a)	1.13 (d, 3H, J = 6.4 Hz, CH_3), 4.70 (dq, 1H, J = 6.4, 2.6 Hz, H-2), 5.18 (s, 2H, CH_2), 6.15 (d, 1H, J = 2.6 Hz, H-3), 7.05–7.95 (m, 10H, H-5, H-6, H-8, aromatic H, and imidazole H), 9.13 (s, 1H, imidazole H-2)	$C_{20}H_{18}ClN_3O_2{\cdot}HNO_3$
7k	Cl	4-Cl	B(61)	146–148 (a)	1.12 (d, 3H, J = 6.4 Hz, CH_3), 4.70 (dq, 1H, J = 6.4, 2.6 Hz, H-2), 5.19 (s, 2H, CH_2), 6.14 (d, 1H, J = 2.6 Hz, H-3), 7.12–7.70 (m, 8H, H-6, H-8, aromatic H, and imidazole H), 7.83 (d, 1H, J = 9.0 Hz, H-5), 9.11 (s, 1H, imidazole H-2)	$C_{20}H_{17}Cl_2N_3O_2{\cdot}HNO_3$

Table 2 (*continued*)

Compd	R	R_1	Method (Yield %)	Mp (°C)	^{1}H NMR (DMSO-d_6)	Formula
7l	Cl	2,4-Cl_2	B(48)	165–166 (a)	1.12 (d, 3H, J = 6.4 Hz, CH_3), 4.70 (dq, 1H, J = 6.4, 2.4 Hz, H-2), 5.25 (s, 2H, CH_2), 6.13 (d, 1H, J = 2.4 Hz, H-3), 7.00–7.69 (m, 7H, H-6, H-8, aromatic H, and imidazole H), 7.83 (d, 1H, J = 9.1 Hz, H-5), 9.10 (s, 1H, imidazole H-2)	$C_{20}H_{16}Cl_3N_3O_2 \cdot HNO_3$

Recrystallization solvents: (a) 2-propanol; (b) ethanol.

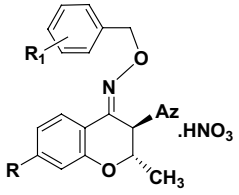
Table 3. Selected ^{1}H NMR spectral data of the compounds **9** and **10**



Compd	Az	R	X	δ H-2 (ppm)	δ H-3 (ppm)	$J_{2,3}$ (Hz)
9a	Im-1-yl[a]	H	O	4.74	4.88	12.0
9b	Im-1-yl	Cl	O	5.12	5.65	12.0
9c	Tz-1-yl[b]	H	O	5.09	5.13	12.0
9d	Tz-4-yl[c]	H	O	4.75	5.03	11.6
9e	Tz-4-yl	Cl	O	4.77	5.03	12.0
10a	Im-1-yl	H	NOH (*Z*)	4.42	5.80	2.4
10b	Im-1-yl	Cl	NOH (*Z*)	4.45	5.85	2.4
10c	Tz-1-yl	H	NOH (*Z*)	4.45	6.04	2.4
10d	Tz-4-yl	H	NOH (*Z*)	5.01	6.49	2.4
10e	Tz-4-yl	Cl	NOH (*Z*)	5.08	6.52	2.4

[a] 1*H*-Imidazol-1-yl.
[b] 1*H*-1,2,4-Triazol-1-yl.
[c] 4*H*-1,2,4-Triazol-4-yl.

Table 4. Selected ^{1}H NMR spectral data of the compounds **7**



Compd	Az	R	R_1	δ H-2 (ppm)	δ H-3 (ppm)	$J_{2,3}$ (Hz)
7a	Im-1-yl[a]	H	H	4.65	6.13	2.6
7b	Im-1-yl	H	4-Cl	4.63	6.13	2.6
7c	Im-1-yl	H	2,4-Cl_2	4.63	6.12	2.4
7d	Im-1-yl	H	3,4-Cl_2	4.64	6.14	2.6
7e	Im-1-yl	H	2,6-Cl_2	4.64	6.13	2.4
7f	Im-1-yl	H	4-Br	4.63	6.13	2.6
7g	Im-1-yl	H	4-F	4.63	6.12	2.4
7h	Im-1-yl	H	3-F	4.64	6.17	2.4
7i	Im-1-yl	H	2-F	4.63	6.10	2.4
7j	Im-1-yl	Cl	H	4.70	6.15	2.6
7k	Im-1-yl	Cl	4-Cl	4.70	6.14	2.6
7l	Im-1-yl	Cl	2,4-Cl_2	4.70	6.13	2.4
7m	Tz-1-yl[b]	H	4-Cl	4.52	6.10	2.6
7n	Tz-1-yl	H	2,4-Cl_2	4.53	6.10	2.6
7o	Tz-4-yl[c]	Cl	2,4-Cl_2	4.69	6.12	2.6
7p	Tz-4-yl	H	2,4-Cl_2	4.61	6.13	2.6

[a] 1*H*-Imidazol-1-yl.
[b] 1*H*-1,2,4-Triazol-1-yl.
[c] 4*H*-1,2,4-Triazol-4-yl.

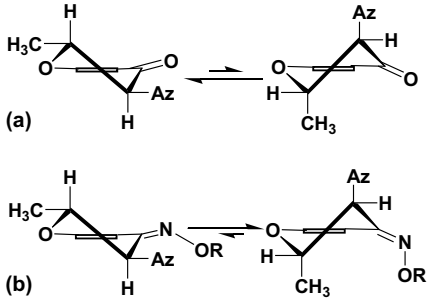


Figure 4. Preferential conformations in ketones **9** (a) and in oxime derivatives **7** and **10** (b).

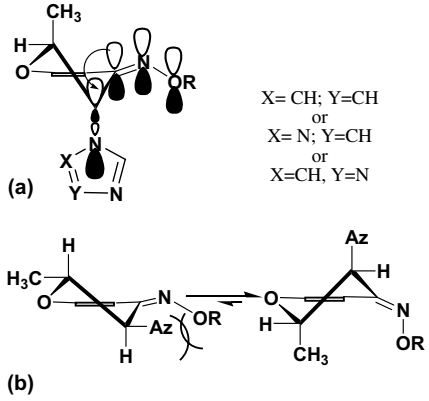


Figure 5. Preferential stabilization of the diaxial conformation of oxime derivatives **7** and **10** by 'vinylogous anomeric effect' (a) and '1,3-allylic strain' (b). The vinylogous anomeric effect is due to π–σ^*_{C-N} hypercojugation. The (*Z*)-configuration of oxime and *syn* relation between the oxime oxygen and the azole ring causes a 1,3-allylic strain.[22]

ring and oxime oxygen atom in oxime derivatives we suggested that a vinylogous anomeric-type effect[20] and/or an 1,3-allylic strain[20,24] might be responsible for the added stability of the diaxial conformer (Fig. 5).[22] These results can also be compared to those reported on benzotriazole-substituted dihydropyrans[25] and 3-alkyl-2-chlorocyclohexanone oxime derivatives.[26]

3. Results and discussion

Compounds **7a**–**p** were evaluated for their antifungal activity against the pathogenic fungi *Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Microsporum gypseum* (Table 5). The MIC (minimum inhibitory concentration) and the MFC (minimum fungicidal concentration)[27] values were determined by comparison to oxiconazole and fluconazole as reference drugs.

The MIC values of the test derivatives against *C. albicans* indicate that most compounds possessed a comparable or better activity (MIC = 0.25–16 μg/mL) with respect to the oxiconazole (MIC = 16 μg/mL) and fluconazole (MIC = 8 μg/mL). Compound **7j** was the most active compound against *C. albicans*, its activity was found to be 32–64 times better than fluconazole and oxiconazole, respectively.

Most compounds showed significant activity against *S. cerevisiae*. The compound **7j** was the most potent against *S. cerevisiae*, with MIC value of 0.5 μg/mL. Compound **7j** was also more potent than reference drugs against *S. cerevisiae* (4–64 times more potent than oxiconazole and fluconazole, respectively).

Derivatives **7a**, **7g**, and **7k** were the most active against *A. niger*, showing MIC values of 4 μg/mL, their activities were equal to oxiconazole and 4-fold more than fluconazole.

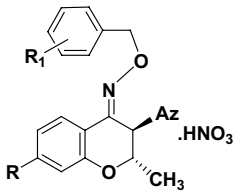
The MIC values of the test derivatives against *M. gypseum* indicate that most compounds possessed a comparable or better activity with respect to reference drugs. In fact, most active compounds were **7g** and **7j** (MIC = 1 μg/mL) being 16–32-fold more active than oxiconazole and fluconazole, respectively.

As noted in Table 5, the MFC values of the tested compounds against *C. albicans* indicated that compounds **7b**, **7d**, **7j**, and **7k** exhibited higher activity than reference drugs. The MFC values for these compounds, with the exception of **7d** were 3-fold higher than the MIC values and they were fungistatic.[28]

The MFC values of all compounds and fluconazole for *S. cerevisiae* were $\geqslant$64 μg/mL with the exception of **7j** and **7k**. The MFC values of compound **7j** was lower than the reference drugs. Nearly all of the tested compounds **7** showed a fungistatic character against the yeasts. Compounds **7a**, **7b**, **7d**, and **7k** showed lower or equal MFC values with respect to reference drugs against *A. niger*. In addition, most of the test derivatives, as well as the reference drugs, were cidal against *A. niger*. Some derivatives, which displayed a good growth inhibition against *M. gypseum* (MIC $\leqslant$ 32 μg/mL), showed cidal properties against this fungi, while compound **7j** (one of the most potent compounds, 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 **7a**, **7b**, and **7k** showed comparable or more potent antifungal activity with respect to reference drugs against all tested fungal species. Compounds **7d** and **7f** exhibited equal or better activity than fluconazole. Derivatives **7d** and **7g** showed comparable or more potent antifungal activity in comparison to oxiconazole except for antifungal activity against *S. cerevisiae*. Compound **7j** showed more potent antifungal activity with respect to reference drugs except for antifungal activity against *A. niger*.

In terms of structure–activity relationship, the results of MIC tests against yeasts showed that no substitution or chlorine substitution on the benzyl moiety of the pharmacophoric portion, substituent R_1, seem to be preferred instead of the dichloro-ones, which are present in oxiconazole. Nevertheless, in the case of *M. gypseum*, better results are obtained with the hydrogen or 4-fluoro

Table 5. In vitro antifungal activity of compounds **7a–p**



Compd	Az	R	R_1	*C. albicans*[a]		*S. cerevisiae*		*A. niger*		*M. gypseum*	
				MIC[b]	MFC[c]	MIC	MFC	MIC	MFC	MIC	MFC
7a	Im-1-yl[d]	H	H	1	64	1	64	4	8	2	>64
7b	Im-1-yl	H	4-Cl	2	16	4	>64	8	8	32	32
7c	Im-1-yl	H	2,4-Cl_2	16	>64	64	>64	16	64	4	32
7d	Im-1-yl	H	3,4-Cl_2	8	16	16	64	8	8	4	8
7e	Im-1-yl	H	2,6-Cl_2	64	>64	32	>64	32	>64	32	>64
7f	Im-1-yl	H	4-Br	8	nt[e]	32	>64	8	nt	16	nt
7g	Im-1-yl	H	4-F	8	nt	16	>64	4	nt	1	nt
7h	Im-1-yl	H	3-F	16	>64	16	>64	16	>64	16	64
7i	Im-1-yl	H	2-F	16	>64	16	>64	16	64	8	nt
7j	Im-1-yl	Cl	H	0.25	16	0.5	16	8	64	1	64
7k	Im-1-yl	Cl	4-Cl	0.5	16	2	32	4	16	8	>64
7l	Im-1-yl	Cl	2,4-Cl_2	16	>64	8	>64	32	64	8	64
7m	Tz-1-yl[f]	H	4-Cl	8	>64	32	>64	64	>64	16	64
7n	Tz-1-yl	H	2,4-Cl_2	8	>64	>64	>64	64	>64	32	>64
7o	Tz-4-yl[g]	Cl	2,4-Cl_2	16	>64	>64	>64	64	>64	16	>64
7p	Tz-4-yl	H	2,4-Cl_2	>64	>64	>64	>64	>64	>64	16	>64
Fluconazole				8	64	32	>64	16	32	32	>64
Oxiconazole				16	32	2	32	4	16	16	64

[a] Fungi tested: *C. albicans* ATCC 10231, *S. cerevisiae* PTCC 5177, *A. niger* ATCC 16401, *M. gypseum* ATCC x191.
[b] MIC in μg/mL.
[c] MFC in μg/mL.
[d] 1*H*-Imidazol-1-yl.
[e] Not tested.
[f] 1*H*-1,2,4-Triazol-1-yl.
[g] 4*H*-1,2,4-Triazol-4-yl.

substitution on the benzyl moiety. Furthermore, the type, number and position of halogene 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. In general, triazoles were less potent than the imidazole analogs. Nevertheless, the most active triazole **7m** was equipotent to fluconazole except for antifungal activity against *A. niger*. Even triazol-4-yl analog **7o** was also found to be active comparable to reference drugs against *C. albicans* and *M. gypseum*. The 2-methyl triazoles **7m–o** were evidently more active than the corresponding nonmethyl substituted analogs against *C. albicans*.[15] In the 2-methyl imidazole series **7a–l** comparison with the corresponding nonmethyl substituted derivatives **6**[14] brought to our attention that this pharmacomodulation in many cases exerted a positive effect: for example, compound **7j** was more active than nonmethyl substituted analog against all of the tested fungal strains (about 16-fold against yeasts and 4-fold against *A. niger* and *M. gypseum*). This compound was the most potent antifungal against *C. albicans*, *S. cerevisiae*, and *M. gypseum* among the studied 2-methyl series **7** and nonmethyl substituted series **6**.[14,15]

These results demonstrated that the introduction of methyl group at 2-position of chroman ring in azolylchromanone oxime ethers improved the overall antifungal activity. Nevertheless, the effect of methyl group dependent on the other substituents.

It is conceivable that a conformationally constrained analog that mimics the bioactive conformation might exhibit a higher level of intrinsic antifungal potency such that the analog, or further structural modification of the analog, could produce a candidate from this series for preclinical studies. Therefore our methodology for restricting the conformation of oxiconazole by introducing a chroman ring, would be useful. Although, we first paid attention to the relative stereochemistry of the oximes and the geometry of oxime–activity relationships, our proceeding biological results did not show any good correlation between activity against test fungal strains and the geometry of oxime ether group, with the exception of activity against *A. niger*.[14,15] Therefore, we next turned our attention to the effect of conformational changes of the chroman ring and the configuration of substituents on this ring. Accordingly, we maintained the geometry of oxime group in our molecules same to that of oxiconazole [(*Z*)-geometry] and introduced a small group, such as a methyl group on the position-2 of the chroman ring in order to influence the conformational behaviors of chroman ring. As discussed above (Figs. 4 and 5) it seems that (*Z*)-*trans*-3-azolyl-2-methylchromanone oxime ethers **7** should adopt a conformation in which the 2-methyl group and azole ring are

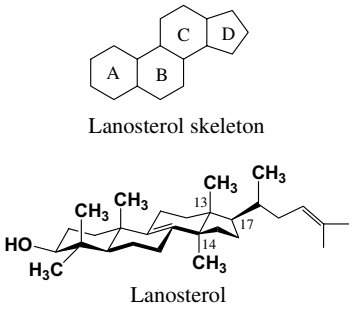


Figure 6.

diaxial. It is speculated that the chroman ring having the 2-methyl group on opposite side of azole (diaxial position) in compounds **7** might be a mimetic of B and C rings or D ring and 17-alkyl side chain of the lanosterol (Fig. 6), and that the 2-methyl group and the 3-azolyl residue of **7** fill the positions of the 13-methyl and 14-methyl groups of lanosterol, respectively. The methyl group on the chroman ring occupies a unique volume within this set of compounds. The additional volume of methyl group relative to nonmethyl substituted compound, the conformational influence of methyl group on chroman ring, or some combination of both could possibly account for the improvement of overall antifungal activity of the new type **7** derivatives.

In conclusion, we have described the stereoselective synthesis of (*Z*)-*trans*-3-azolyl-2-methylchromanone oxime ethers and biological studies have shown that many of these derivatives were highly potent as antifungal agents. First approach in the series of new (*Z*)-*trans*-3-azolyl-2-methylchromanone oxime ethers **7** bearing different structural features on the chroman ring and *O*-benzyl moiety points out that the compound **7j** exert significant in vitro antifungal activity and it was more potent than the reference drugs against *C. albicans*, *S. cerevisiae*, and *M. gypseum*.

4. Experimental

Chemicals and all solvents used in this study were purchased from Merck AG and Aldrich Chemical. The desired 2-azolyl-2′-hydroxyacetophenones **8**,[21–23] and *O*-(arylmethyl)hydroxylamine·HCl **11**[29] were prepared according to the literature. All melting points were determined 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. Yields are of purified product and were not optimized.

4.1. General procedure for the synthesis of *trans*-3-azolyl-2-methylchroman-4-ones 9

A solution of the appropriate precursor **8** (4.9 mmol) and acetaldehyde (3.0 mL) in glacial acetic acid (50 mL) was heated at 90 °C for 12–14 h. The solvent was evaporated under reduced pressure and the residue was dissolved in $CHCl_3$. The organic layer was washed with water, dried (Na_2SO_4), and evaporated. The residue was crystallized from ethanol.

4.2. General procedure for the synthesis of (*Z*)-*trans*-3-azolyl-2-methylchroman-4-one oximes 10

A mixture of **9** (5.0 mmol), $HONH_2$·HCl (1.04 g, 15.0 mmol), and K_2CO_3 (0.69 g, 5.0 mmol) in methanol (20 mL) was stirred at room temperature for 36 h. After concentration under reduced pressure, the mixture was poured into water (20 mL) and neutralized with $NaHCO_3$. The precipitate was filtered, washed with water, and crystallized from methanol.

4.3. General procedure for the synthesis of (*Z*)-*trans*-3-azolyl-2-methylchroman-4-one oxime ethers 7

Method A: A solution of oxime **10** (2.0 mmol) in DMF (4 mL) was added to a suspension of NaH (48 mg, 2.0 mmol) in DMF (2 mL). The reaction mixture was stirred at room temperature for 0.5 h and then a solution of substituted benzyl halide (2.1 mmol) in DMF was added. After stirring at room temperature for 8–10 h, the reaction 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 ethanol or 2-propanol and treated with 70% HNO_3 to give **7**.

Method B: A mixture of **9** (2.0 mmol), *O*-(arylmethyl)hydroxylamine hydrochloride **11** (5.0 mmol), and K_2CO_3 (0.27 g, 2.0 mmol) in methanol (10 mL) was stirred at room temperature for 3–7 days. Water (100 mL) was added 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 ethanol or 2-propanol and treated with 70% HNO_3. The precipitate was filtered and washed with Et_2O to give corresponding **7**.

4.4. 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, 0.25, and 0.125 μg/mL. The minimum inhibitory concentration (MIC) was determined by using the method of 2-fold serial dilution technique.[30] 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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