Bioorganic & Medicinal Chemistry Letters 18 (2008) 6538–6541

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Carbon analogs of antifungal dioxane-triazole derivatives: Synthesis and in vitro activities

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ARTICLE INFO

Article history:
Received 16 July 2008
Revised 26 September 2008
Accepted 10 October 2008
Available online 15 October 2008

Keywords:
Triazole
Antifungal
Grignard reaction

ABSTRACT

A new series of triazole compounds possessing a carbon atom in place of a sulfur atom were efficiently synthesized and their in vitro antifungal activities were investigated. The carbon analogs showed excellent in vitro activity against *Candida*, *Cryptococcus*, and *Aspergillus* species. The MICs of compound **1c** against *C. albicans* ATCC24433, *C. neoformans* TIMM1855, and *A. fumigatus* ATCC26430 were 0.016, 0.016, and 0.125 μg/mL, respectively (MICs of fluconazole: 0.5, >4, and >4 μg/mL; MICs of itraconazole: 0.125, 0.25, and 0.25 μg/mL).

The growing population of immunocompromised patients due to transplantation, AIDS and cancer chemotherapy, has resulted in an increase in severe fungal infections.[1] In many cases, it is not the AIDS or cancer itself but the mycoses that are lethal to these patients. Triazole compounds are an important class of antifungal agents because of their generally broad antifungal spectrum, high potency and low toxicity.[2] Triazole derivatives displace lanosterol from lanosterol 14-demethylase, a cytochrome P450-dependent enzyme, and block the biosynthesis of an essential component of the fungal cell membrane, ergosterol.[3] Previously, we synthesized a series of dioxane-triazole compounds possessing a sulfur atom, as depicted by general formula **A** (Fig. 1).[4]

They showed excellent in vitro antifungal activity against various pathogenic fungi including *Candida*, *Cryptococcus*, and *Aspergillus* species. The structure–activity relationship studies on **A**[4,5] led us to further investigate the role of the sulfur atom in the antifungal activity. Herein, we report the synthesis and in vitro antifungal activities of triazole derivatives **B**, in which the sulfur atom was replaced by a carbon atom.

For comparison of the antifungal activities with those of the previous derivatives, we planned to synthesize **1a–d** having various lengths of olefinic chains and a *p*-(trifluoromethyl)phenyl group (Fig. 2). Aldehydes **2a–d** were prepared from 4-(trifluoromethyl)benzaldehyde stereoselectively as described previously.[4] The precursor triol **3** was prepared in three different routes, shown as follows.

Route A (Scheme 1) uses optically active epoxide **4**.[6,7] Following Tsuruoka's protocol,[8] epoxide **4** was treated with lithium cyanide

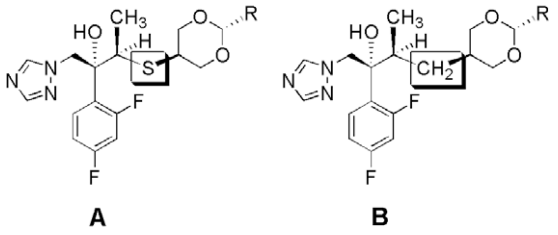

Figure 1. Structural formulas of dioxane-triazole derivatives.

generated in situ from acetone cyanohydrin and lithium hydride to afford nitrile **5**, in 75% yield. Nitrile **5** was converted, via amide **6** (Na_2CO_3, H_2O_2, acetone, H_2O, 82%) and ester **7** (trifluoromethanesulfonic acid, PrOH, 74%), to diol **8** ($LiBH_4$, EtOH, 74%). Treatment of diol **8** with triethylamine and thionyl chloride gave cyclic sulfite ester **9**, and successive oxidation with sodium periodate in the presence of a catalytic amount of ruthenium trichloride afforded cyclic sulfate ester **10** in 52% yield.[9,10] The cyclic sulfate was opened by a sodium diethyl malonate attack and successive one-pot acid treatment gave diester **11** in 71% yield. Finally, $LiBH_4$ reduction gave triol **3** in 81% yield.

Although Route A furnished desired triol **3** in an efficient manner, it uses toxic reagent ruthenium trichloride, and therefore alternative synthetic routes were searched for. Route B (Scheme 2) involves a stereoselective Evans aldol reaction. Following the report of Bartroli et al.,[11] epoxide **14** was formed from commercially available (*S*)-(−)-4-benzyl-2-oxazolidinone. Treatment of epoxide **14** with sodium bis(2-methoxyethoxy)aluminum hydride afforded epoxy alcohol **15** in 85% yield. Epoxy alcohol **15** was activated with

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doi:10.1016/j.bmcl.2008.10.055

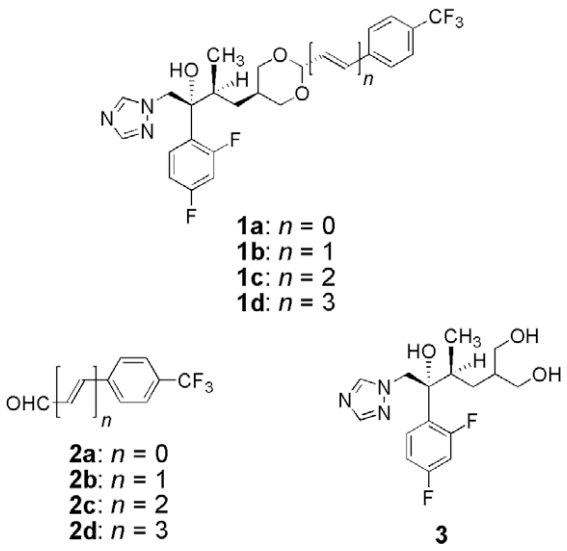
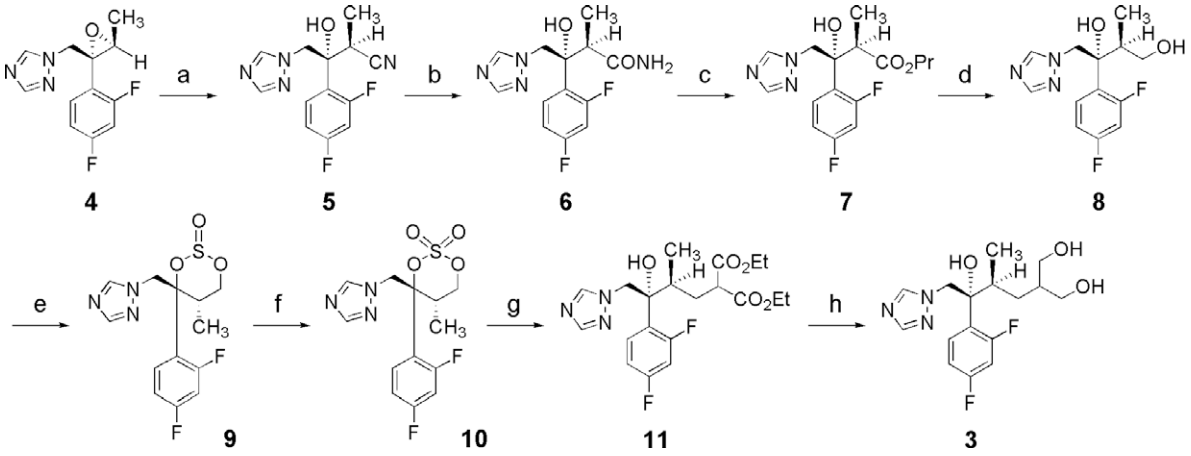


Figure 2. Structural formulas of carbon analogs **1a**–**d** and their precursors **2a**–**d**, **3**.

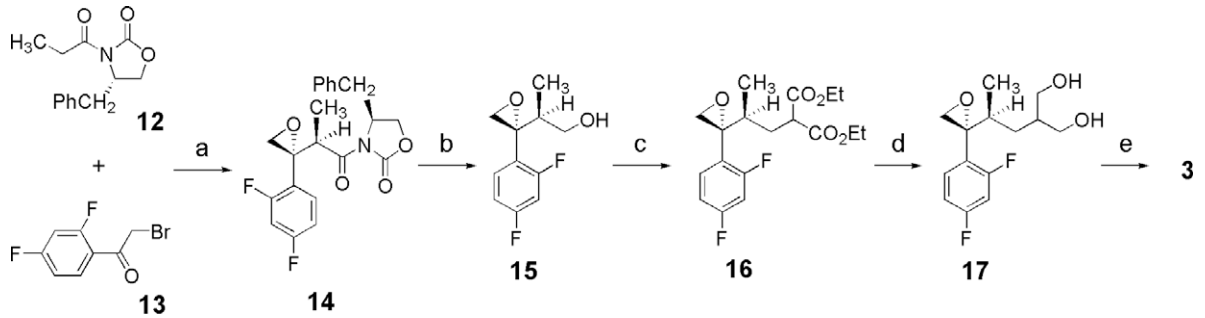
trifluoromethanesulfonic anhydride and allowed to react with sodium diethyl malonate to give epoxy diester **16** in 83% yield. Reduction of the diester moiety of **16** with $LiBH_4$ gave epoxy diol **17** in 86% yield. Finally, the epoxy ring was opened by an attack of sodium salt of triazole and triol **3** was obtained in 73% yield.

Since the above described Evans aldol reaction requires a low temperature and the diastereoselectivity was unsatisfactory, we searched for further synthetic routes. In Route C (Scheme 3), the same intermediate **16** used in route B could be synthesized starting from methyl (*S*)-(+)-3-hydroxy-2-methylpropionate (**18**). Treatment of **18** with a mixture of sulfuric acid and acetic acid afforded carboxylic acid **19**. The carboxylic acid **19** was transformed into acyl chloride **20** by treatment with oxalyl chloride and subjected to a Friedel–Crafts reaction to afford propiophenone **21** in 55% yield (3 steps). The acetyl protecting group was removed using sulfuric acid to give keto alcohol **22** in 90% yield. A Grignard reaction of **22** furnished a diastereomeric mixture of 1,2-oxasilinanes **23** and **24**, which were treated with H_2O_2 under a basic condition[12] to afford triol **25** in 78% yield (2 steps), after chromatographic separation. The diastereomeric ratio observed in triol **25** was >20:1. In contrast, a similar Grignard reaction of the acetic acid ester **21** or the tetrahydropyranyl ether analog gave almost no diastereoselectivity (ratio both ca. 3:2; determined after converting to triol **25**). The high diastereoselectivity observed in the Grignard reaction of alcohol **22** was presumably brought about by either of the following mechanisms (Fig. 3). Nucleophilic attack occurred selectively from the less hindered face of the carbonyl group of the magnesium-chelated intermediate **X**, or, after exchange of the alkoxy group on the silicon atom in the Grignard reagent, the attack proceeded through the Felkin-Anh transition state **Y** with chair-like conformation.[13] Triol **25** was treated with trifluoromethanesulfonic anhydride to afford a ditriflate intermediate, which was treated with sodium bis(trimethylsilyl)amide and then with sodium diethyl malonate to give diester **16** in 43% yield.

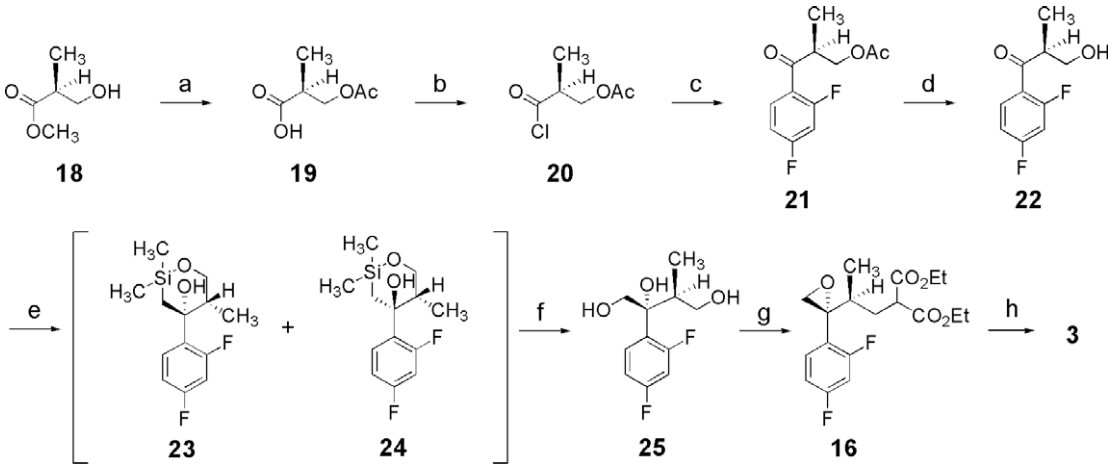
The triol **3** thus obtained was coupled with aldehydes **2a**–**d** to give **1a**–**d**. The reaction was driven in the presence of *p*-toluenesulfonic acid and molecular sieves 4A in dichloromethane. The *trans*



Scheme 1. Synthesis of triol **3** (Route A). Reagents and conditions: (a) Ref. 8; (b) Na_2CO_3, H_2O_2, acetone, H_2O, rt, 82%; (c) CF_3SO_3H, PrOH, reflux, 74%; (d) $LiBH_4$, EtOH, rt, 74%; (e) NEt_3, $SOCl_2$, 0 °C, CH_2Cl_2; (f) $NaIO_4$, $RuCl_3$, CH_3CN, H_2O, rt, 52% (2 steps); (g) 1—NaH, $CH_2(CO_2Et)_2$, DMF, 50 °C, 2—HCl, 1,4-dioxane, EtOH, rt, 71%; (h) $LiBH_4$, EtOH, rt, 81%.



Scheme 2. Synthesis of triol **3** (Route B). Reagents and conditions: (a) Ref. 11; (b) $Na(MeOCH_2CH_2O)_2AlH_2$, THF, −50 °C, 85%; (c) 1—*i*-Pr_2NEt, $(CF_3SO_2)_2O$, toluene, rt, 2—NaH, $CH_2(CO_2Et)_2$, DMF, rt, 83%; (d) $LiBH_4$, EtOH, rt, 86%; (e) NaH, triazole, DMF, 80 °C, 73%.



Scheme 3. Synthesis of triol **3** (Route C). Reagents and conditions: (a) H_2SO_4, AcOH, reflux; (b) $(COCl)_2$, DMF, CH_2Cl_2, 0 °C; (c) 1,3-difluorobenzene, $AlCl_3$, rt, 55% (3 steps); (d) H_2SO_4, MeOH, H_2O, rt, 90%; (e) *i*-PrOSiMe$_2$CH$_2$MgCl, THF, rt, (**23**:**24** = >20:1); (f) $NaHCO_3$, H_2O_2, THF, MeOH, 60 °C, 78% (2 steps); (g) 1—*i*-Pr_2NEt, $(CF_3SO_2)_2O$, toluene, −20 °C, 2—NaHMDS, THF, rt, 3—NaH, $CH_2(CO_2Et)_2$, DMF, rt, 43%; (h) 2 steps as in Scheme 2, 63%.

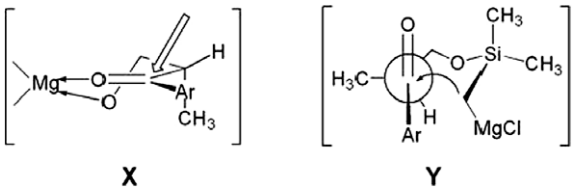


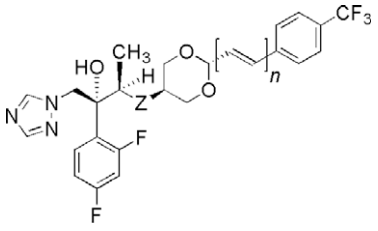
Figure 3. Possible mechanisms of the Grignard reaction of **22**.

dioxane isomers **1a–d** were predominantly produced and were easily separated from the *cis* isomers by silica gel column chromatography.[14]

Minimum inhibitory concentrations (MICs) of the test compounds were determined against *Candida*, *Cryptococcus*, and *Aspergillus* species by the broth microdilution method in accordance with the guidelines in the National Committee for Clinical Laboratory Standards (NCCLS) documents.[15] The activities of **1a–d** were compared with those of our former sulfur derivatives **26a–d**,[4] fluconazole and itraconazole (Table 1).

When the compounds with the same lengths of olefinic chains were compared, most of the MICs of the carbon analogs **1a–d** were about two to eight times better than those of the corresponding sulfur compounds **26a–d**. Among compounds **1a–d**, compound **1c**, which has a side chain with two olefinic double bonds, showed the best MICs. The difference was most clear in the activity against

Table 1
Minimum inhibitory concentrations (MICs) of compounds **1a–d**, **26a–d**, fluconazole (FLCZ), and itraconazole (ITCZ)



Strain[a]	MIC (μg/mL)[b]									
	Compound[c]	**1a**	**1b**	**1c**	**1d**	**26a**	**26b**	**26c**	**26d**	
	n	0	1	2	3	0	1	2	3	
	Z	CH_2	CH_2	CH_2	CH_2	S	S	S	S	FLCZ	ITCZ
C. albicans ATCC24433		0.016	0.031	0.016	0.125	0.016	0.063	0.125	0.25	0.5	0.125
C. albicans SANK51486		⩽0.008	⩽0.008	⩽0.008	0.031	⩽0.008	0.016	0.016	0.031	0.25	0.031
C. albicans TIMM3164		0.016	0.016	0.016	0.125	0.063	0.125	0.125	1	>4	0.25
C. albicans ATCC64550		0.5	1	0.5	0.5	1	2	1	>4	>4	1
C. parapsilosis ATCC90018		0.016	0.031	0.031	0.125	0.031	0.031	0.063	0.25	0.5	0.125
C. glabrata ATCC90030		1	1	1	2	1	1	1	>4	>4	1
C. krusei ATCC6258		0.125	0.063	0.063	0.25	0.25	0.25	0.25	1	>4	0.5
C. tropicalis ATCC750		0.125	0.125	0.125	0.25	0.5	0.031	0.125	2	2	0.5
C. neoformans TIMM1855		0.031	0.016	0.016	0.125	0.031	0.016	0.016	0.5	>4	0.25
A. fumigatus ATCC26430		0.25	0.25	0.125	0.5	1	0.25	0.25	2	>4	0.25
A. fumigatus SANK10569		0.25	0.25	0.125	0.5	1	0.25	0.25	2	>4	0.25
A. flavus SANK18497		1	0.5	0.5	1	2	1	0.5	4	>4	0.5

[a] *C. albicans*, *Candida albicans*; *C. parapsilosis*, *Candida parapsilosis*; *C. glabrata*, *Candida glabrata*; *C. krusei*, *Candida krusei*; *C. tropicalis*, *Candida tropicalis*; *C. neoformans*, *Cryptococcus neoformans*; *A. fumigatus*, *Aspergillus fumigatus*; *A. flavus*, *Aspergillus flavus*.

[b] MICs were determined at 35 °C (30 °C for *Aspergillus* spp.) in RPMI1640 medium (yeast nitrogen base for *C. neoformans*) at pH 7.0. MICs were defined as the minimum concentrations of the test compounds that inhibit the growth of the fungi by 80%.

[c] For compounds **1a–d**, **26c**, and **26d**, their oxalic acid salts were subjected to the test.

A. fumigatus. This tendency was similar to that of the sulfur compounds **26a**–**d**. The activity of the strongest compound, **1c**, was slightly better than of its sulfur analog **26c** with an olefinic side chain of the same length and surpassed that of fluconazole and itraconazole.

In conclusion, we have presented novel carbon analogs of triazole compounds, which could be synthesized stereoselectively. Further investigation of these compounds as antifungal agents for systemic use is currently underway.[16]

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.10.055.

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13. Of the two proposed mechanisms, the one depicted by Y seems more plausible, since, although ester and ether derivatives would also be able to assume a chelated transition state like X, they demonstrated almost no diastereoselectivity. Furthermore, treatment of similar ketone (*RS*)-PhC(=O)CHMeCH$_2$OH with MeMgBr, wherein the mechanism depicted by Y cannot occur, is known to give lower (70% de) diastereoselectivity. See Bartoli, G.; Bellucci, M. C.; Bosco, M.; Marcantoni, E.; Sambri, L. *Chem. Eur. J.* **1998**, *2*, 2154.
14. The stereochemistry of the 1,3-dioxane ring was elucidated by the coupling constants in the ^{1}H NMR spectra. The *trans* isomers showed characteristic signals of the axial methylene protons on the C4 and C6 positions in the 1,3-dioxane ring with large coupling constants (triplet, J = ca. 11 Hz). In contrast, the corresponding signals of *cis* isomers appeared as multiplets.
15. National Committee for Clinical Laboratory Standards. 1997. M27-A; 1995. M27-T; 1998. M38-P. National Committee for Clinical Laboratory Standards, Wayne, PA. See Supplementary data.
16. In a preliminary in vivo study, all the infected mice (ddY strain, n = 10, systemically infected using intravenous invection of *C. albicans* 427, 5.5×10^6 CFU/mouse) survived after 21 days after po or ip administration of **1c** (given 1, 4, 24 h after infection, 20 mg/kg/administration), whereas similar treatment with fluconazole (po) resulted in a survival rate of 60%. In a preliminary toxicity study, all the mice (ddY strain, n = 5) survived after oral dosing of 500 mg/kg of **1c** once daily for 4 days.