Bioorganic & Medicinal Chemistry Letters 19 (2009) 2013–2017

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Amide analogs of antifungal dioxane–triazole derivatives: Synthesis and in vitro activities

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ARTICLE INFO

Article history:
Received 8 January 2009
Revised 6 February 2009
Accepted 7 February 2009
Available online 12 February 2009

Keywords:
Triazole
Antifungal
Amide-analog
Acid-stability

ABSTRACT

A new series of triazole compounds possessing an amide-part were efficiently synthesized and their in vitro antifungal activities were investigated. The amide analogs showed excellent in vitro activity against *Candida*, *Cryptococcus* and *Aspergillus* species. The MICs of compound **23d** against *C. albicans* ATCC24433, *C. neoformans* TIMM1855 and *A. fumigatus* ATCC26430 were ⩽0.008, 0.031 and 0.031 μg/mL, respectively, (MICs of fluconazole: 0.5, >4 and >4 μg/mL; MICs of itraconazole: 0.125, 0.25, 0.25 μg/mL). Furthermore, compound **23d** was stable under acidic conditions.

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 an olefin part, as depicted by general formula **A** (Fig. 1).[4] We varied the length of the side chains ($n = 0, 1, 2$) and the substituents on aromatic ring Ar. From these compounds, CS-758 was chosen as a candidate compound on the basis of minimum inhibitory concentrations (MICs), solubility and chemical/metabolic stability. CS-758 is currently under development as an antifungal agent against systemic mycosis.

In parallel to the development study of CS-758, we continued to explore additional compounds with excellent antifungal activity and good pharmacokinetics. Although there is a fear of acid instability in the 1,3-dioxane ring in structure **A**, the ring is crucial to the antifungal activity, and the extent of the acid stability varied enormously between compounds.[5] We assumed that the acid-stability of CS-758 could be ascribed to its electron-withdrawing CN and F groups on the ring Ar, and designed a novel series of compounds as depicted by general structure **B**, wherein electron-withdrawing groups **X** such as an amide group or a sulfonyl group are situated in closer proximity to the 1,3-dioxane ring. In this Letter, we describe the synthesis and the in vitro antifungal activities of such a novel series of triazole derivatives.

First, we synthesized compounds **1a**–**d**, which have various electron-withdrawing groups **X**, and compared their MICs (Fig. 2). The Ar group was fixed to the 4-fluorophenyl group. Synthesis of **1a** was conducted as shown in Scheme 1. Alcohol **4**, which was synthesized from ethyl bromocrotonate **2** in two steps, was oxidized with MnO_2 to give corresponding aldehyde **5**. The aldehyde **5** was coupled with triol **6**[4a] in the presence of *p*-toluenesul-

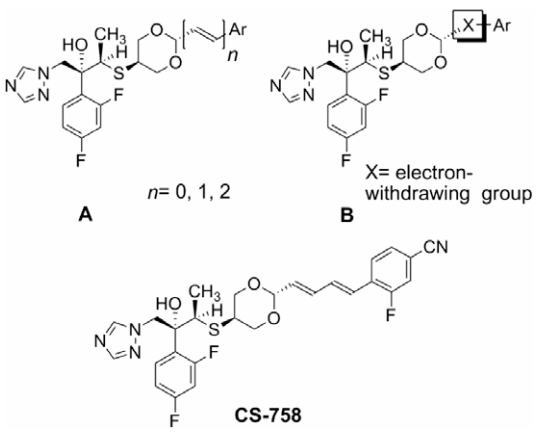

Figure 1. Structural formulas of dioxane–triazole derivatives.

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doi:10.1016/j.bmcl.2009.02.036

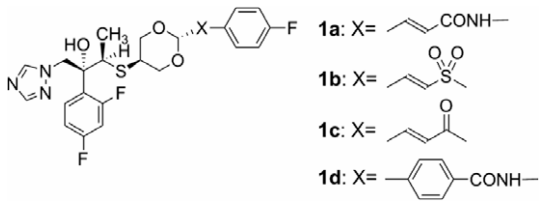


Figure 2. Structural formulas of **1a**–**d**.

fonic acid hydrate and molecular sieves 4 Å to give **1a**. The *trans* dioxane isomer **1a** was predominantly produced over its cis isomer, and was easily separated by silica gel column chromatography.[6]

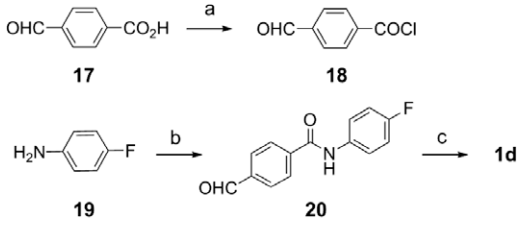
Synthesis of **1b** was conducted as shown in Scheme 2. Sodium 4-fluorophenylsulfinate obtained from **7** was allowed to react with epichlorohydrin to afford alcohol **9**.[7] **9** was oxidized to give corresponding aldehyde **10**. Though **10** did not react with triol **6** in the presence of *p*-toluenesulfonic acid hydrate and molecular sieves 4 Å in dichloromethane, compound **1b** was afforded by acetalization using trimethylsilyl chloride and a catalytic amount of trimethylsilyl trifluoromethanesulfonate.

Synthesis of **1c** was conducted as shown in Scheme 3. Compound **13**, obtained in two steps from **11**, was treated with *n*-BuLi and 4-fluorobenzoyl chloride to afford **14**. The tosyl group and the dimethylacetal group in **14** were removed in a single step by treatment with hydrochloric acid at 60 °C to give aldehyde **16**. The aldehyde **16** was acetalyzed with triol **6** by treatment with *p*-toluenesulfonic acid hydrate and molecular sieves 4 Å.

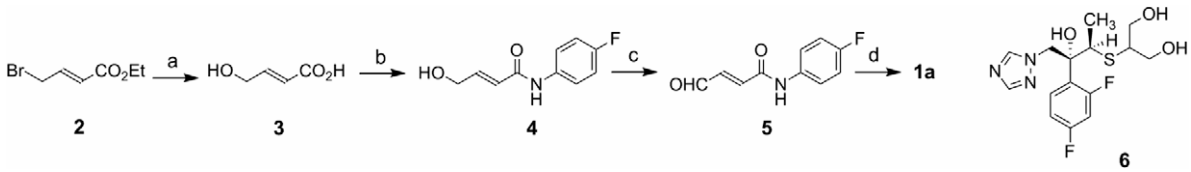
Synthesis of **1d** was conducted as shown in Scheme 4. The amine **19** was coupled with acid chloride **18** to give amide **20**. Compound **20** was acetalyzed with triol **6** to give compound **1d**. The acetalization reaction was driven in the presence of *p*-toluenesulfonic acid in tetrahydrofuran using a rotary evaporator to remove the water. The *trans* dioxane isomer **1d** was predominantly produced over its cis isomer.

The MICs of compounds **1a**–**d** were determined[8] against *Candida*, *Cryptococcus* and *Aspergillus* species and compared with those of our former compound **1e** (Table 1). The MICs of compounds **1a**–**c** were higher than those of **1e**. This difference was most clear in the activity against *Aspergillus flavus* SANK18497. Compound **1d**, which has an aryl-amide group, showed good MICs, which are almost comparable to those of **1e**. In particular, the MICs against *Candida albicans* TIMM3164 (fluconazole resistant strain) and *C. tropicalis* ATCC750 were remarkable. Against *C. glabrata*, the MIC of **1d** was slightly inferior to that of **1e**.

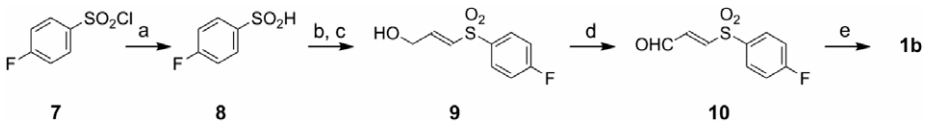
We then fixed **X** to the aryl-amide group, and the substituents on the terminal benzene ring Ar were examined. These compounds (**23a**–**k**) were synthesized in a manner similar to that shown in Scheme 4 or according to the route shown in Scheme 5, wherein common intermediate **22** was prepared by an acetalization reaction of **21** with triol **6**. The intermediate **22** was condensed with the appropriate amine using trimethyaluminum to afford desired



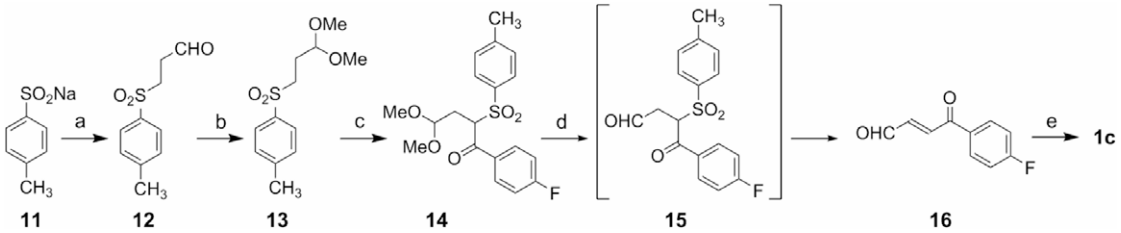
Scheme 4. Synthesis of **1d**. Reagents and conditions: (a) $(COCl)_2$, cat. *N*,*N*-dimethylformamide, THF, rt, 100%; (b) 1.6 equiv Et_3N, 1.5 equiv **18**, THF, rt, 78%; (c) 0.9 equiv **6**, 3.2 equiv *p*-toluenesulfonic acid hydrate, THF, rt, evaporation, 49%.



Scheme 1. Synthesis of **1a**. Reagents and conditions: (a) 2 N KOH, H_2O, reflux, 76%; (b) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, Et_3N, 4-fluoroaniline, THF, 0 °C to rt, 47%; (c) MnO_2, CH_2Cl_2, 70%; (d) **6**, *p*-toluenesulfonic acid hydrate, molecular sieves 4 Å, CH_2Cl_2, 39%.

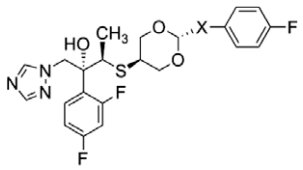


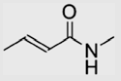
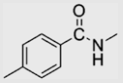
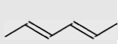
Scheme 2. Synthsis of **1b**. Reagents and conditions: (a) Na_2SO_3, NaOH, H_2O, 0–40 °C, 58%; (b) NaOH, H_2O, DMF, rt; (c) epichlorohydrin, reflux, 60% (2 steps); (d) MnO_2, CH_2Cl_2, 40%; (e) **6**, *i*-Pr_2NEt, Me_3SiCl, cat. Me_3SiOTf, CH_2Cl_2, *i*-PrOH, 15%.



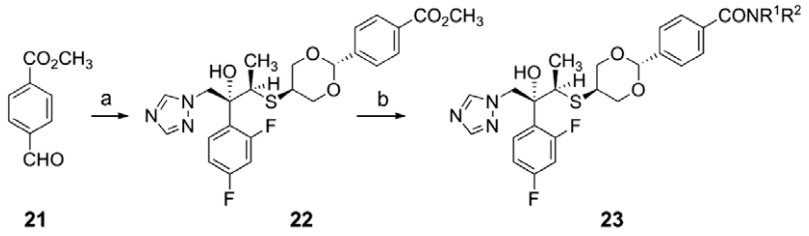
Scheme 3. Synthesis of **1c**. Reagents and conditions: (a) 0.8 equiv acrolein, AcOH, rt; (b) 2.0 equiv $CH(OMe)_3$, cat. *p*-toluenesulfonic acid hydrate, MeOH rt, 42% (2 steps); (c) 2.0 equiv *n*-BuLi, 1 equiv 4-F-BzCl, THF, −78 °C to rt, 65%; (d) 2 N HCl, THF, 60 °C, 72%; (e) **6**, *p*-toluenesulfonic acid hydrate, molecular sieves 4 Å, CH_2Cl_2, 35%.

Table 1
MICs of **1a**–**d**



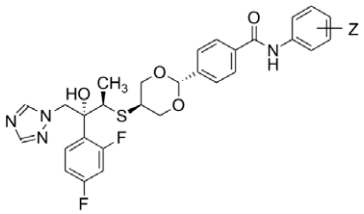
Strain[b]	MIC[a] (μg/mL)				
Cmpd.	**1a**	**1b**[c]	**1c**[c]	**1d**	**1e**
X					
C. albicans SANK51486	⩽0.008	⩽0.008	⩽0.008	⩽0.008	⩽0.008
C. albicans TIMM3164	0.125	0.125	0.125	0.016	0.063
C. glabrata ATCC90030	4	2	1	2	1
C. tropicalis ATCC750	0.125	0.5	0.5	0.016	0.25
C. neoformans TIMM1855	0.125	0.125	0.125	0.031	0.016
A. fumigatus SANK10569	0.5	4	1	0.125	0.063
A. flavus SANK18497	4	>4	>4	0.25	0.25

[a] 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 concentration of the test compounds that inhibit the growth of the fungi by 80%.
[b] *C. albicans, Candida albicans; C. glabrata, Candida glabrata; C. tropicalis, Candida tropicalis; C. neoformans, Cryptococcus neoformans; A. fumigatus, Aspergillus fumigatus; A. flavus, Aspergillus flavus*.
[c] For **1b** and **1c**, their maleic acid salts were subjected to the test.



Scheme 5. Synthesis of **23**. Reagents and conditions: (a) 0.9 equiv **6**, 1.6 equiv *p*-toluenesulfonic acid hydrate, THF, rt, evaporation, 67%; (b) 4 equiv trimethylaluminum, 4 equiv R^1R^2NH, toluene, 71–96%.

Table 2
MICs of **1d**, and **23a**–**k**



Strain[b]	MIC (μg/mL)[a]											
Cmpd.	**1d**	**23a**	**23b**	**23c**	**23d**	**23e**	**23f**	**23g**	**23h**	**23i**	**23j**	**23k**
Z	4-F	4-Cl	4-Br	4-Me	4-CN	4-Ac	4-OH	4-$OCH_2CF_2CHF_2$	4-SCF_3	3,4-$(CN)_2$	3-Cl-4-CN	2-F-4-CN
C. albicans SANK51486	⩽0.008	⩽0.008	⩽0.008	⩽0.008	⩽0.008	⩽0.008	0.016	0.016	⩽0.008	⩽0.008	⩽0.008	⩽0.008
C. albicans TIMM3164	0.016	⩽0.008	0.016	0.016	⩽0.008	0.031	0.063	0.031	0.031	0.016	0.016	0.016
C. glabrata ATCC90030	2	>4	>4	2	2	>4	>4	>4	1	>4	1	>4
C. tropicalis ATCC750	0.016	0.016	⩽0.008	⩽0.008	0.016	0.125	0.25	0.125	0.031	0.031	0.063	0.031
C. neoformans TIMM1855	0.031	0.016	0.016	0.031	0.031	0.125	0.25	0.063	0.016	0.063	0.031	0.063
A. fumigatus SANK10569	0.125	0.063	0.063	0.063	0.031	0.063	0.5	0.125	0.25	0.25	0.125	0.031
A. flavus SANK18497	0.25	0.125	0.125	0.25	0.125	0.25	1	0.5	0.5	0.5	0.25	0.125

[a] 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 concentration of the test compounds that inhibit the growth of the fungi by 80%.
[b] *C. albicans*, *Candida albicans*; *C. glabrata*, *Candida glabrata*; *C. tropicalis*, *Candida tropicalis*; *C. neoformans*, *Cryptococcus neoformans*; *A. fumigatus*, *Aspergillus fumigatus*; *A. flavus*, *Aspergillus flavus*.

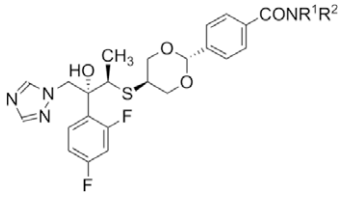
amide **23** in good yield (71–96%). The MICs of these compounds are listed in Tables 2 and 3.

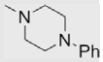
Compounds with a halogen atom at the C4 position on the benzene ring (**1d**, **23a**, **23b**) showed good MICs. Compound **23d**,[9] in which a cyano group was introduced to the C4 position on the benzene ring, showed the best MICs of all the compounds, particularly against *C. albicans* TIMM3164 (fluconazole resistant strain), *A. fumigatus* SANK10569, and *A. flavus* SANK18497. The introduction of a polar subsistent, such as an acetyl group (**23e**) or a hydroxy group (**23f**), reduced in vitro activity. Compounds **23g**, **23h**, which have fluorine atoms, showed slightly higher MICs than **23d**. Because CS-758 has a fluorine atom at the C2 position and a cyano group at the C4 position on the benzene ring, a fluorine atom was introduced to the C2 position on the benzene ring to give **23k**. But the MICs of compound **23k** were higher than the MICs of **23d**.

Compounds with a terminal heterocycle ring instead of a benzene ring, such as pyridine, thiazole, morpholine, piperazine, showed lower in vitro activities (Table 3). The decrease of in vitro activity was particularly obvious in compounds with a non-aromatic ring (**23n**, **23o**, **23p**).

We then fixed the terminal ring Ar to the 4-cyano-phenyl group, and compared the MICs of the derivatives **23d** and **24a–c** (Table 4) with various arylene spacers **A**. These compounds were synthesized in a manner similar to that shown in Scheme 5. The anti-

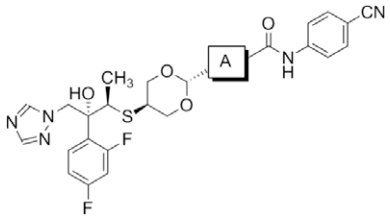
Table 3
MICs of **23l–p**

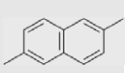


Strain[b]	MIC (μg/mL)[a]				
Cmpd.	**23l**	**23m**	**23n**	**23o**	**23p**
NR^1R^2					
C. albicans SANK51486	⩽0.008	⩽0.008	0.063	0.016	0.016
C. albicans TIMM3164	0.031	0.031	0.25	0.125	0.125
C. glabrata ATCC90030	>4	2	>4	4	2
C. tropicalis ATCC750	0.125	0.125	1	0.5	0.5
C. neoformans TIMM1855	0.125	0.125	0.125	0.031	0.016
A. fumigatus SANK10569	0.25	0.063	4	0.5	1
A. flavus SANK18497	0.25	0.5	>4	4	2

[a] 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 concentration of the test compounds that inhibit the growth of the fungi by 80%.
[b] *C. albicans*, *Candida albicans*; *C. glabrata*, *Candida glabrata*; *C. tropicalis*, *Candida tropicalis*; *C. neoformans*, *Cryptococcus neoformans*; *A. fumigatus*, *Aspergillus fumigatus*; *A. flavus*, *Aspergillus flavus*.

Table 4
MICs of **23d** and **24a–c**



Strain[b]	MIC (μg/mL)[a]			
Cmpd.	**23d**	**24a**	**24b**	**24c**
A				
C. albicans SANK51486	⩽0.008	⩽0.008	⩽0.008	⩽0.008
C. albicans TIMM3164	⩽0.008	0.25	0.031	0.031
C. glabrata ATCC90030	2	2	2	>4
C. tropicalis ATCC750	0.016	1	⩽0.008	0.016
C. neoformans TIMM1855	0.031	1	0.031	0.063
A. fumigatus SANK10569	0.031	2	0.063	0.063
A. flavus SANK18497	0.125	>4	0.25	0.25

[a] 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 concentration of the test compounds that inhibit the growth of the fungi by 80%.
[b] *C. albicans*, *Candida albicans*; *C. glabrata*, *Candida glabrata*; *C. tropicalis*, *Candida tropicalis*; *C. neoformans*, *Cryptococcus neoformans*; *A. fumigatus*, *Aspergillus fumigatus*; *A. flavus*, *Aspergillus flavus*.

Table 5
MICs of **23d**, fluconazole and itraconazole

Strain[b]	MIC (μg/mL)[a]			
	Cmpd. **23d**	CS-758	Fluconazole	Itrazonazole
C. albicans ATCC24433	⩽0.008	0.016	0.5	0.125
C. albicans SANK51486	⩽0.008	⩽0.008	0.25	0.031
C. albicans TIMM3164	⩽0.008	0.063	>4	0.25
C. albicans ATCC64550	0.25	0.5	>4	1
C. parapsilosis ATCC90018	⩽0.008	0.016	0.5	0.125
C. glabrata ATCC90030	2	1	>4	1
C. krusei ATCC6258	0.063	0.25	>4	0.5
C. tropicalis ATCC750	0.016	0.25	2	0.5
C. neoformans TIMM1855	0.031	0.016	>4	0.25
A. fumigatus ATCC26430	0.031	0.063	>4	0.25
A. fumigatus SANK10569	0.031	0.063	>4	0.25
A. flavus SANK18497	0.125	0.25	>4	0.5

[a] 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 concentration of the test compounds that inhibit the growth of the fungi by 80%.
[b] *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*.

fungal activity of compound **24a**, which has a meta phenylene group, was drastically weakened, whereas the MICs of compound **24b**, in which a fluorine atom was introduced to the inner benzene ring were almost comparable to or slightly higher than those of **23d**.

Finally, the MICs of **23d** were determined against 12 fungal strains and compared with those of CS-758, fluconazole, and itrazonazole (Table 5). The MICs of **23d** surpassed those of fluconazole, and itrazonazole, and were almost comparable to those of CS-758.

In a stability test under acidic conditions, the half-life ($t_{1/2}$) of **23d** in HCl (0.007 mol/L) solution in CH_3CN–H_2O (3:7, v/v at 37 °C) was over 160 min, whereas that of CS-758 was 6.40 min. Thus, compound **23d** showed dramatic improvement in its acid-stability compared with CS-758.[10]

In conclusion, efficient routes were found for the synthesis of our novel aryl-amide analogs of antifungal dioxane–triazole derivatives. Compound **23d**, which has a cyano group at the C4 position on the benzene ring, exhibited higher in vitro activities than fluconazole or itraconazole. Furthermore, compound **23d** has a much longer half-life under acidic conditions than CS-758. Further evaluations of this class of compounds are currently proceeding.

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5. Though itraconazole has a ketal moiety, it is stable under acidic conditions and used for oral administration.
6. 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.
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8. MICs were determined by the broth microdilution methods in accordance with the guidelines in the National Committee for Clinical Laboratory Standards (NCCLS) documents. 1997. M27-A; 1995. M27-T; 1998. M38-P. National Committee for Clinical Laboratory Standards, Wayne, PA.
9. *Data for* **23d**: mp 185–187 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.22 (d, 3H, J = 7 Hz), 3.36 (q, 1H, J = 7 Hz), 3.4–3.6 (m, 1H), 3.76 (t, 1H, J = 11 Hz), 3.79 (t, 1H, J = 11 Hz), 4.42 (ddd, 1H, J = 11, 5, 2 Hz), 4.55 (ddd, 1H, J = 11, 5, 2 Hz), 4.85 (d, 1H, J = 14 Hz), 5.05 (d, 1H, J = 1 Hz), 5.05 (d, 1H, J = 14 Hz), 5.55 (s, 1H), 6.7–6.8 (m, 2H), 7.3–7.4 (m, 1H), 7.65 (d, 2H, J = 8 Hz), 7.68 (d, 2H, J = 8 Hz), 7.80 (s, 2H), 7.80 (d, 2H, J = 8 Hz), 7.89 (d, 2H, J = 8 Hz), 7.93(s, 1H); IR (ν_{max}/cm^{-1}, KBr): 3371, 2225, 1679, 1512, 1319, 1139; MS (FAB) m/z: 592 [M+H]$^+$; $[\alpha]_D^{25}$ −52° (c 0.60, AcOEt); HRMS: calcd for $C_{30}H_{28}F_2N_5O_4S$ [M+H]$^+$ 592.18301, found 592.18186; Anal. Calcd for $C_{30}H_{27}F_2N_5O_4S$: C, 60.90; H, 4.60; N, 11.84; S, 5.42; F, 6.42. Found: C, 61.14; H, 4.35; N, 11.58; S, 5.30; F, 6.39.
10. Contrary to expectation, the absolute bioavailability (BA) of **23d** in rats after oral administration (20 mg/kg) of its polyethylene glycol 400 solution was only 31.7%, whereas that of CS-758 was 113%. Though the reason for the low bioavailability of **23d** was not determined, we reason that hydrolysis of the amide moiety presumably contributed. CS-758 is a compound that can be hydrolyzed in acidic conditions faster than **23d**, but the acid-stability to this degree seems to be sufficient for use in oral administration.