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Bioorganic & Medicinal Chemistry Letters 13 (2003) 191–196

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design, Synthesis and Antifungal Activity of a Novel Water Soluble Prodrug of Antifungal Triazole

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Received 21 August 2002; accepted 17 October 2002

Abstract—A highly potent water soluble triazole antifungal prodrug, RO0098557 (**1**), has been identified from its parent, the novel antifungal agent RO0094815 (**2**). The prodrug includes a triazolium salt linked to an aminocarboxyl moiety, which undergoes enzymatic activation followed by spontaneous chemical degradation to release **2**. Prodrug **1** showed high chemical stability and water solubility and exhibited strong antifungal activity against systemic candidiasis and aspergillosis as well as pulmonary aspergillosis in rats.

Introduction

There is a high medical need for an injectable agent with a broad spectrum for the treatment of severe deep mycoses of hospitalized patients. Only fluconazole (FCZ) and amphotericin B are currently available for parenteral use, but they have limitations in antifungal spectra and safety, respectively.[1] Most of the azoles under development have broader spectrum but cannot be administered parenterally due to high lipophilicity.[2] There have been some efforts to overcome this problem by using a prodrug approach.[3] For example, we recently reported a novel prodrug of azole antifungals and Takeda Chemical Industries, Ltd. has identified a water soluble quaternary triazolium prodrug, TAK-457.

Previously, we identified RO0094815 (**2**) which has a broad antifungal spectrum covering *Aspergillus* spp., FCZ-resistant *Candida* spp. and has a good safety profile including low drug–drug interaction.[4] Since the water solubility of **2** was, however, too low for parenteral formulation and the existing prodrug approaches were not satisfactory in stability and/or water solubility, we conducted a study on a new prodrug of **2**, which should have sufficient water solubility, stability in aqueous solution and quantitative bioconversion.

Compound **2** obviously has two functional groups, namely a tertiary alcohol and a triazole group, with potential to be linked to a pro-moiety. However, the high steric hindrance around the hydroxyl group may limit the modification. This prompted us to design a triazolium salt type prodrug.[5] The general concept of prodrugs is depicted in Scheme 1. The prodrug **3** contains a [*N*-(3-acetoxypropyl)-*N*-methylamino]carboxymethyl group, and an ester group of which can be rapidly hydrolyzed by a nonspecific enzyme, serum esterase, to generate an alcohol intermediate, and then undergoes rapid and spontaneous intra-molecular cyclization to release **2**, a cyclic carbamate and an aldehyde. Further optimization of the pro-moiety of **3** by its conformational restriction to adjust the conversion rate to **2** led to the identification of **1**, a new injectable prodrug of highly potent antifungal azole **2**. In this paper, we describe the design, synthesis and biological profile of RO0098557 (**1**) (Fig. 1).

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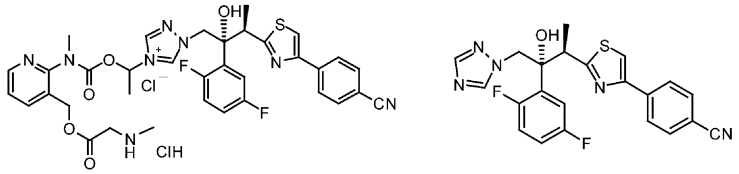
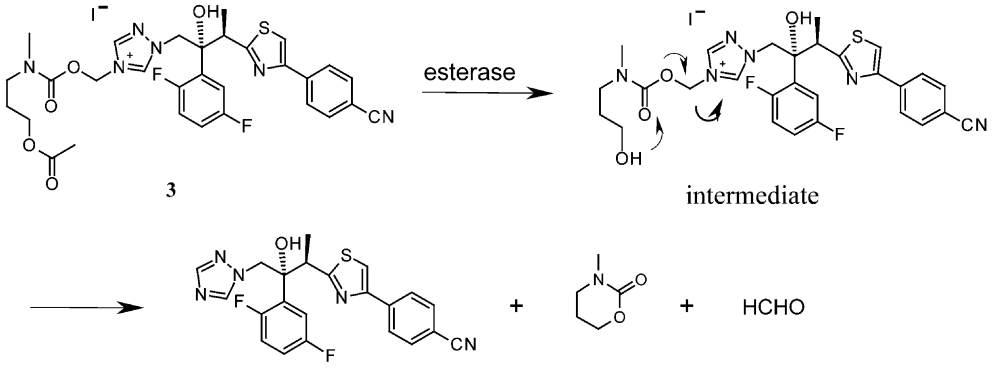
PII: S0960-894X(02)00892-2

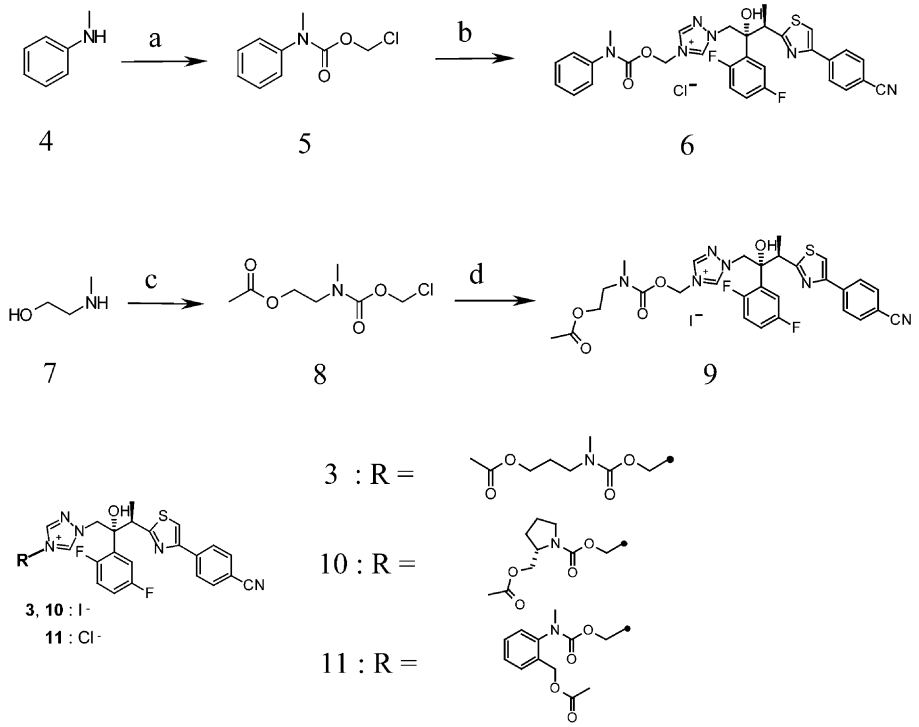


RO0098557 (1) **RO0094815 (2)**

Figure 1. Structure of RO0098557 and RO0094815.



Scheme 1.



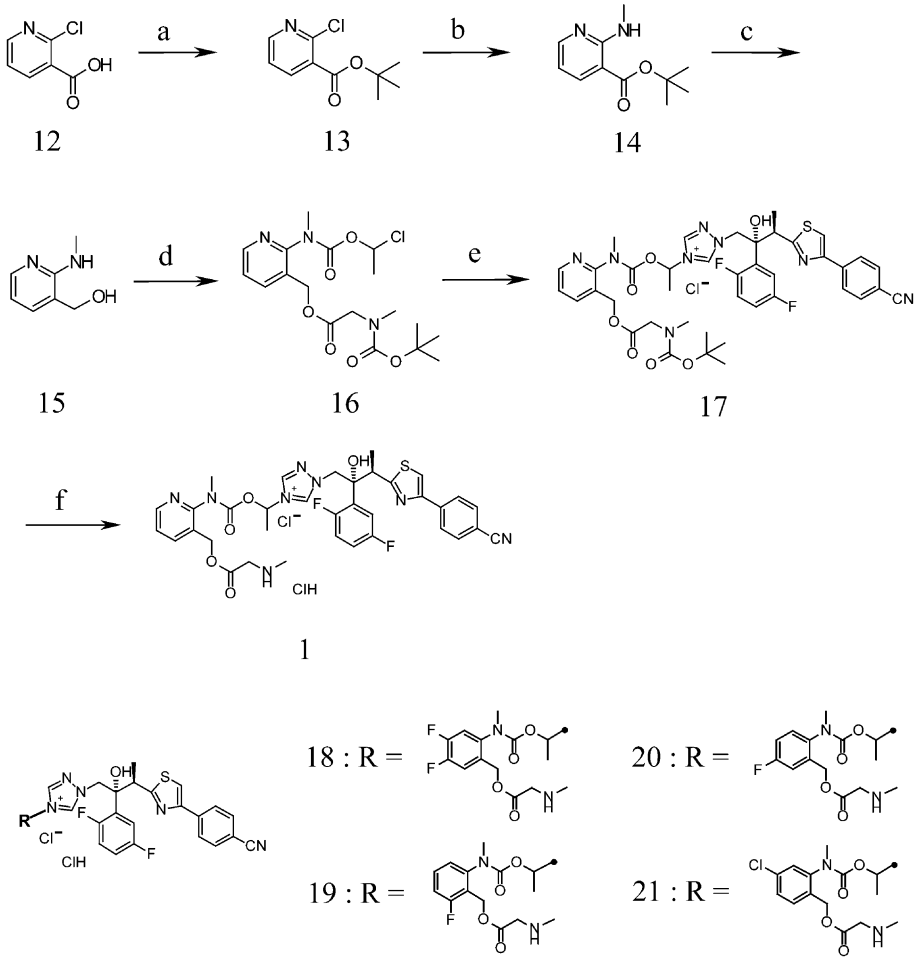
Scheme 2. (a) $ClCOOCH_2Cl$, diisopropylethylamine, CH_2Cl_2, rt (quant); (b) **2**, CH_3CN, 80 °C (60%); (c) (1) $ClCOOCH_2Cl$, Et_3N, CH_2Cl_2, rt; (2) Ac_2O, pyridine, rt (30%, two steps); (d) (1) NaI, CH_3CN, 50 °C ; (2) **2**, CH_3CN, 50 °C (88%, two steps); Synthesis of **3**: (1) *N*-3-hydroxypropyl-*N*-methylamine, $ClCOOCH_2Cl$, Et_3N, CH_2Cl_2, rt; (2) AcCl, Et_3N, CH_2Cl_2, rt (20%, two steps); (3) **2**, NaI, CH_3CN, 50 °C (82%); Synthesis of **10**: (1) L-prolinol, $ClCOOCH_2Cl$, Et_3N, CH_2Cl_2, rt; (2) Ac_2O, pyridine, rt (<10%, 2 steps); (3) **2**, NaI, CH_3CN, 50 °C (92%); Synthesis of **11**: (1) 2-hydroxymethyl-*N*-methylaniline, $ClCOOCH_2Cl$, diisopropylethylamine, CH_2Cl_2, rt; (2) Ac_2O, diisopropylethylamine, rt (20%, two steps); (3) **2**, cat. NaI, CH_3CN, reflux (63%).

Chemistry

We synthesized a series of new triazolium derivatives of **2**. Compounds **3**, **6**, **9**, **10** and **11** were first prepared as outlined in Scheme 2 in order to analyze their stability and ability to release **2**. Next, aromatic analogues **18**, **19**, **20**, **21** and **1** were synthesized for optimization of **11** to increase its water solubility and conversion rate. Compounds in the second series had sarcosine esters[6] to make them water soluble, and they were also designed to generate acetaldehyde[7] instead of formaldehyde for a better safety profile. The synthetic procedures for the second series of the derivatives are outlined in Scheme 3.

Compound **5** was prepared from *N*-methylaniline by *N*-acylation with chloromethyl chloroformate. The coupling of **2** with **5** was carried out in acetonitrile to give a triazolium salt **6**. Compound **9** was synthesized in four steps: (i) *N*-acylation of 2-methylaminoethanol, (ii) *O*-acetylation, (iii) iodination of **8** with sodium iodide in acetonitrile, and (iv) coupling with **2** in acetonitrile at 50 °C. The derivatives **3**, **10** and **11** were similarly obtained from corresponding aminoalcohols as described in Scheme 2.

Compound **1** was prepared from 2-chloronicotinic acid in seven steps. **12** was treated with oxalyl chloride followed by potassium tert-butoxide in THF to give *tert*-butyl ester **13**. Substitution of the chlorine atom in **13** with methylamine, followed by reduction of the ester group of **14** by lithium aluminium hydride, gave aminoalcohol **15** as a colourless crystal. *N*-Acylation of **15** with 1-chloroethyl chloroformate followed by *N*-Boc-sarcosine gave sarcosine ester **16**. It was then coupled with **2** in the presence of NaI in acetonitrile followed by anion exchange chromatography with DOWEX-1 to give **17** as an amorphous powder.



Scheme 3. (a) (1) oxalyl chloride, DMF, 0 °C; (2) KO′Bu, THF, −5 °C (97%, two steps); (b) CH_3NH_2, MeOH, rt (90%); (c) $LiAlH_4$, THF, 0 °C (80%); (d) (1) $ClCOOCH(CH_3)Cl$, diisopropylethylamine, CH_2Cl_2, 0 °C; (2) Boc-Sarcosine, WSCI, DMAP, CH_2Cl_2, 0 °C (84%, two steps); (e) (1) **2**, NaI, CH_3CN, 50 °C; (2) DOWEX-1 Cl^- form, aqueous MeOH, rt (65%, two steps); (f) (1) HCl, EtOAc, rt; (2) lyophilization (69%, two steps); Synthesis of **18**: (1) (i) (4,5-difluoro-2-methylaminophenyl)methanol, $ClCOOCH(CH_3)Cl$, diisopropylethylamine, CH_2Cl_2, 0 °C; (ii) Boc-Sarcosine, WSCI, DMAP, CH_2Cl_2, 0 °C (quant, two steps); (2) **2**, cat. NaI, CH_3CN, 80 °C; (50%,); (3) HCl, EtOAc, rt (90%); Synthesis of **19**: (1) (i) 2-fluoro-6-methylaminophenyl)methanol, $ClCOOCH(CH_3)Cl$, diisopropylethylamine, CH_2Cl_2, 0 °C; (ii) Boc-Sarcosine, WSCI, DMAP, CH_2Cl_2, 0 °C (74%, two steps); (2) **2**, cat. NaI, CH_3CN, reflux; (3) HCl, EtOAc, rt (29%, two steps); Synthesis of **20**: (1) (i) (5-fluoro-2-methylaminophenyl)methanol, $ClCOOCH(CH_3)Cl$, diisopropylethylamine, CH_2Cl_2, 0 °C; (ii) Boc-Sarcosine, WSCI, DMAP, CH_2Cl_2, 0 °C (91%, two steps); (2) **2**, cat. NaI, CH_3CN, 70 °C (72%); (3) HCl, EtOAc, rt (88%); Synthesis of **21**: (1) (i) (4-chloro-2-methylaminophenyl)methanol, $ClCOOCH(CH_3)Cl$, diisopropylethylamine, CH_2Cl_2, 0 °C; (ii) Boc-Sarcosine, WSCI, DMAP, CH_2Cl_2, 0 °C (71%, two steps); (2) **2**, CH_3CN, 65 °C; (3) HCl, EtOAc, rt (65%, two steps).

Table 1. Solubility in water and conversion rate of the prodrugs in rat plasma

Compd	Solubility in water (mg/mL)	Conversion rate in rat plasma ($T_{1/2}$)	
		Prodrug	Intermediate
6	<1	29 h	–
9	<1	<2 min[a]	>10 h
3	<1	<2 min[a]	>10 h
10	<1	<2 min[a]	3.8 h
11	1	<2 min[a]	13 min

[a]Hydrolysis of the acetate group.

Finally, removal of Boc group of **17** by HCl in ethyl acetate and lyophilization gave **1** as a white powdery solid.[8] The derivatives **18**, **19**, **20** and **21** were obtained from corresponding aminoalcohols in a similar manner as described above (Scheme 3).

All of the prodrugs prepared were sufficiently stable for biological evaluation.

Results and Discussion

We initially compared the conversion rate of the derivatives **3**, **6**, **9**, **10** and **11** in rat plasma and their solubility in water. As shown in Table 1, conformational restriction had a definitive effect on the conversion rate of the intermediates in rat plasma. Compounds **3**, **9**, **10** and **11** were rapidly hydrolyzed by esterase in rat plasma to give corresponding primary alcohol intermediates. However, there was a considerable difference in the cyclization rate of releasing the active substance **2**. The intermediates generated from **3**, **9** and **10** were slowly converted to **2** ($T_{1/2}$: >10 h, >10 h and >3.8 h, respectively). In contrast, the intermediate generated from **11** rapidly converted to **2** with a half life of 13 min. Compound **6** was resistant to direct hydrolysis by esterase in plasma, indicating the carbamate linkage to be stable in plasma. Thus, the high conversion rate of **11** into **2** can be explained by facile intra-molecular cyclization of the intermediate alcohol as a result of conformational restriction of the promoiety.

Calculated preferred conformations of the intermediates for **10** and **11** are shown in Figure 2. The faster conversion rate of **11** as compared with that of **10** is explained by the fact that the carbamate group of the intermediate from **11** locates perpendicular to the phenyl ring due to steric repulsion between the *N*-methyl group and the ortho substituent, the hydroxymethyl group in the intermediate, which forces the nucleophile, OH, close to the carbonyl carbon. On the other hand, the carbamate group in **10** is almost coplanar with the proline ring, which is not favorable for intramolecular cyclization between the carbonyl group and the primary hydroxy group. We surmised that prodrugs should be even more rapidly cleaved in human plasma in order to not affect the PK profile of **2** and have higher water solubility for parenteral formulation. Thus, we synthesized additional analogues of **11** having both electron withdrawing groups and a solubilizing moiety, sarcosine ester, on the

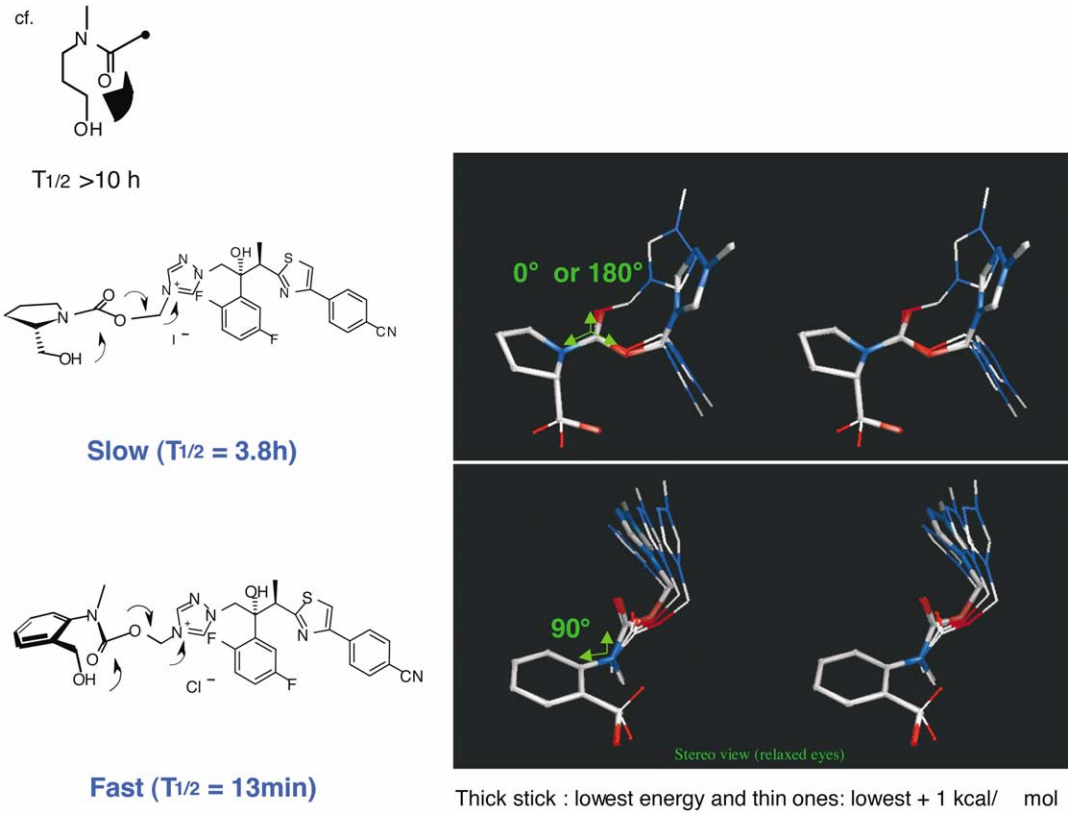


Figure 2. Conformational analysis of two intermediates by MOPAC (RO0094815 was replaced by 1-methyl-1,2,4-triazole in this calculation).

Table 2. Solubility in water and conversion rate of the prodrugs in plasma

Compd	Solubility in water (mg/mL)	Conversion rate in plasma ($T_{1/2}$) (min)	
		Prodrug	Intermediate
18	>10	Rat <2 Monkey 6 Human 6	5 6 8
19	>10	Rat <2 Monkey 2 Human 2	3 3 2
20	>10	Rat <2 Monkey 2 Human 2	9 8 8
21	>10	Rat <2 Monkey 2 Human 3	4 5 4
1	>100	Rat <1 Monkey <1 Human <1	Not detected Not detected Not detected

phenylring of the pro-moiety. Conversion rates and water solubility of the new derivatives, **18**, **19**, **20**, **21** and **1** are summarized in Table 2. At this stage, we evaluated the conversion rate in rat, monkey and human plasma to determine whether or not species difference exists. All compounds in Table 2 showed a more rapid conversion rate and higher water solubility compared to **11**. Especially compound **1**, having a pyridine ring, quickly converted into **2** in the plasma of the three species ($T_{1/2}$: <1 min), and exhibited extremely high solubility in water (>100 mg/mL). Furthermore, **1** was found to stoichiometrically release **2** and the cyclized compound **22**[9] in human plasma as shown in Figure 3, suggesting that **1** underwent a rapid and quantitative intramolecular cyclization.

Prodrug **1** was quite stable in an aqueous buffer solution at pH 1 to 4 (>99% of **1** remained after 7 h), suggesting sufficient stability for parenteral use. The PK profile after intravenous (iv) bolus and oral (po) administration of **1** to monkey at a dose of 3 mg/kg is shown in Figure 4. In

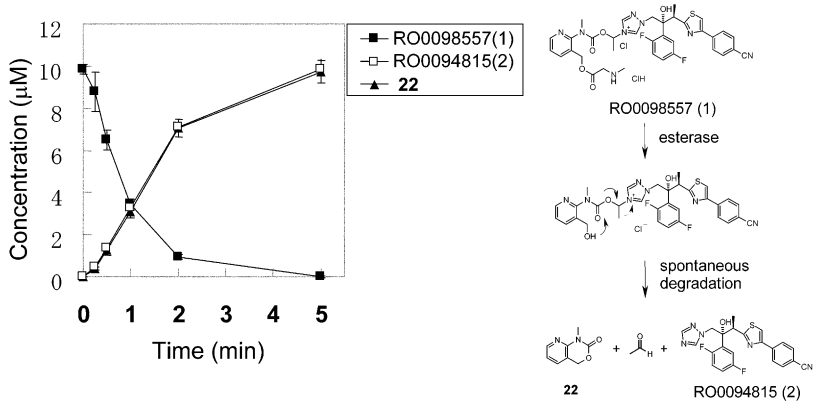


Figure 3. Conversion of **1** in human plasma.

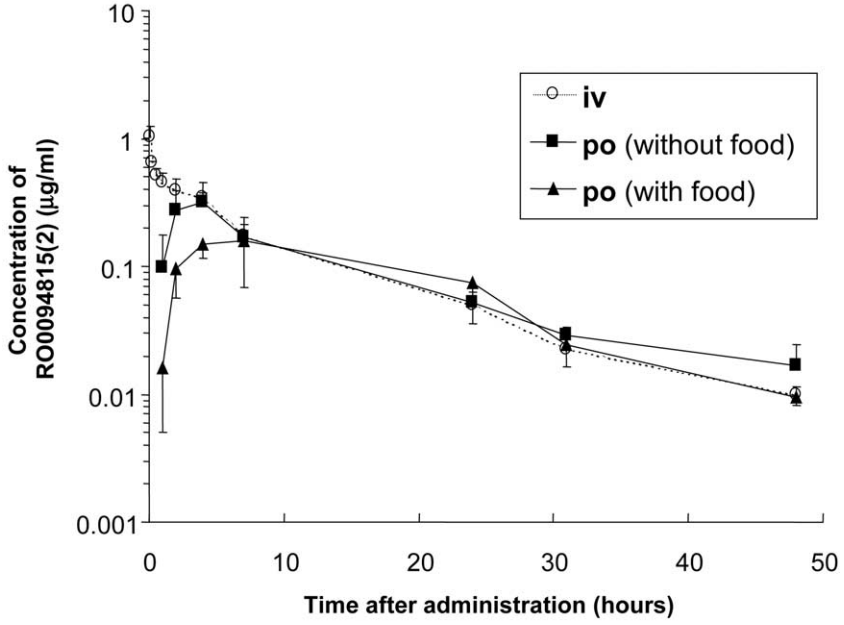


Figure 4. Plasma level of **2** after administration of **1** to monkey at a dose of 3 mg/kg.

Table 3. In vivo efficacy of **1** against systemic candidiasis in rat

Rat systemic candidiasis; ED50 (μmol/kg) on day 14				
		C. albicans[a]		*C. tropicalis*[a]
		CY1002	CY3003	CY5042
RO0098557	iv	4.0	1.5	0.9
	po	4.0	2.6	0.8
ITCZ	po	4.7	1.7	1.7

[a]Treatments: 0, 4, 24, and 48 h after infection (b.i.d.×1 + q.d.×2).

Table 4. In vivo efficacy of **1** against aspergillosis in rat

Rat aspergillosis; ED50 (μmol/kg) on day 14				
		Systemic[a]	Systemic[b]	Pulmonary[c]
		A. fumigatus		*A. fumigatus*
		CF1003[d]	CF924390[e]	CF924390[e]
RO0098557	iv	12	6.0	6.8
	po	14	9.9	8.9
ITCZ	po	8.9	7.2	2.5

[a]Normal host.
[b]Immunosuppression: cyclophophamide 100 mg/kg, ip on day −4 and cortisone acetate 125 mg/kg, sc on day −1.
[c]Immunosuppression: cortisone acetate 125 mg/kg, sc on days −6, −4, and −1 and low protein diet.
[d]Treatments: 0, 4, 24, 28, 48, 52, 72, 76 h after infection (b.i.d. ×4).
[e]Treatments: 0, 4, 24, 28, 48 and 52 h after infection (b.i.d. ×3).

both administrations, the active drug **2** was quickly formed and slowly eliminated with $T_{1/2}$ of 9.8 h (iv) and 12.8 h (po). Oral bioavailability of **2** was fairly good in monkey (87%) after oral administration of **1**. The in vivo efficacy of **1** in various infection models in rats is summarized in Tables 3 and 4. All the ED_{50} values were measured on Day 14. In the systemic candidiasis model in rats, **1** exhibited almost equal to or better activity than ITCZ against *Candida albicans* CY1002, CY3003, and a fluconazole resistant strain, *C. tropicalis,* in both iv and oral administration. **1** was also strongly active in the systemic and pulmonary aspergillosis model in rats in both administrations (ED_{50}: 6.0–14 μmol/kg).

In summary, we developed a widely applicable prodrug technique for solubilization of compounds having the nitrogen containing hetero-aromatic ring. We identified the triazolium salt prodrug RO0098557 (**1**) that showed potent antifungal activity against both systemic candidiasis and aspergillosis as well as pulmonary aspergillosis in rat in both iv and po administration. Because there is few injectable antifungal azole agent with a broad spectrum, **1** could be a promising drug for treatment of systemic fungal infections both parenterally and orally.

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6. Among several amino acid esters, sarcosine ester was chosen from its physicochemical and safety property.
7. Acetaldehyde is most likely safe at the clinical dose of the prodrug.
8. Compound **1** was determined to be mono HCl salt. Anal. calcd for $C_{35}H_{35}F_2N_8O_5S{\cdot}Cl.HCl$: Cl, 8.98. Found: Cl, 9.27.
9. Compound **22** as well as **1** showed no toxicity at the clinical dose in rat and monkey, respectively.