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RO0098557, a Novel Water Soluble Azole Prodrug for Parenteral and Oral Administration (II) Prodrug Principle and Broad Spectrum Antifungal

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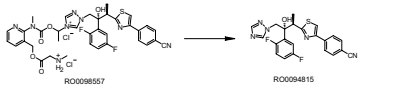
Abstract

Background: Currently available azoles with a broad spectrum are better suited for oral administration than for parenteral administration due to their limited solubility. RO0098557, is a new water soluble prodrug of RO0094815. **Method:** *In vitro* antifungal activity against 122 reference strains and 83 clinical isolates was tested by microdilution method. *In vivo* efficacies against systemic or pulmonary infections due to *C. albicans* or *A. fumigatus* in normal and immunosuppressed rats were evaluated. Treatments were performed by IV or PO. The minimal effective concentration (MEC) in plasma against systemic candidiasis or aspergillosis was estimated using a rat infusion model. PK profiles of RO0094815 after administration of RO0098557 to rats and monkeys were evaluated.

Results: RO0094815 showed a broad antifungal spectrum with *C. albicans* (Geometric Mean of MIC = 0.008 µg/ml), *C. glabrata* (0.09), *C. krusei* (0.10), and *A. fumigatus* (0.1). *In vivo*, RO0098557 showed efficacy superior to voriconazole (VCZ) and equivalent to itraconazole (ICZ) against systemic candidiasis in normal rats. ED_{50}s (µmol/kg) were in the range of 1.5-4.2 by PO or IV [FCZ (PO) 0.85-1.8; ICZ (PO) 1.7-4.7; VCZ (PO or IV) 2.3->22]. Against aspergillosis, RO0098557 was comparably effective to VCZ or ICZ with ED_{50}s ranges of 6-14 (systemic) and 6.8-11 (pulmonary). Its MEC against both systemic infections was 0.05 µg/ml in plasma. RO0094815 in monkey after RO0098557 administration showed t1/2 = 9.8h (IV), 12.8h (PO), and 87% oral bioavailability. **Conclusion:** The combination of a high water solubility, good oral bioavailability, t1/2, broad spectrum will allow to treat once-a-day mycoses both orally and parenterally. These results warrant further evaluation of RO0098557 for clinical studies.



RO0098557 RO0094815

Introduction

RO0098557 was specifically designed to have a water-soluble azole derivative. The molecule is converted to hydrophobic active metabolite by esterases from sera and tissues with high distribution profile, after administration of either iv or oral route. The active metabolite RO0094815 has strong inhibitory activity against ergosterol synthesis. It has a high bioavailability and is rapidly bioconverted to active one. PK profile supports once a day treatment. In the clinical dose range expected, any significant toxicity findings have not been observed so far.

Broad spectrum, including against FCZ resistant *Candida* strains and *Aspergillus fumigatus*, has been proven *in vitro* and various *in vivo* models with constant effective dose range. A water-soluble azole derivative, RO0098557, could be a potent and safe antifungal agent with broad spectrum.

Materials and Methods (1)

Antifungal drugs.
RO0094815, ICZ, VCZ and FCZ were dissolved with dimethyl sulfoxide (DMSO) for *in vitro* studies. For *in vivo* studies, RO0098557 was dissolved and diluted with KN-1B (electrolyte infusion, Ohtsuka Phamaceuticals). Fungizone® was dissolved and diluted with 5% dextrose solution. ICZ was dissolved and diluted with 40% β-HPCD, VCZ was dissolved and diluted with 10% β-HPCD. FCZ was dissolved and diluted with saline.

Fungal strains
As reference strains, including the quality control strains of the National Committee for Clinical Laboratory Standards (NCCLS), ATCC strains from the American Type Culture Collection (Rockville, MD, USA), IFO strains from the Institute for Fermentation Osaka (Osaka, Japan) and IAM strains from the Institute of Applied Microbiology, University of Tokyo, (Tokyo, Japan) were purchased. IFM strains were kindly provided by Prof. Nishimura, Chiba University (Chiba, Japan). KULM strains were kindly provided by Prof. Kume, Kitasato University (Kanagawa, Japan). Fluconazole resistant *Candida albicans* strains were kindly provided by Prof. J. Graybill, University of Texas Health Science Center (San Antonio, TX, USA). Other clinical isolates were obtained from hospitals in Japan.

Antifungal susceptibility *in vitro*
The modified broth microdilution methods of NCCLS M27-A with the spectrophotometric method; RPMI 1640 medium buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) was used for yeasts and RPMI-MOPS medium solidified with 0.2% low-melting-point agarose (LMPA) was used for filamentous fungi. The inoculum size for all strains was 1 to 3 x 10^3 conidia/ml in final concentration. Microtestplates were then incubated at 35 °C for a suitable period for each species. The MIC endpoint of azoles were determined by the concentration of a score 2 (prominent decrease in turbidity) or by the concentration of drug that produced an 80 % reduction of turbidity (IC80) compared to that of the drug free control measured by the spectrophotometer at 600 nm.

Efficacy of antifungals *in vivo*
Male Fisher rats (F344/Du-Crj; age 4.5 to 5 weeks, body weight 50-60g, Charles River Japan Inc) were used for all examinations of efficacy. The treatment volume for either i.v or p.o was 0.5 ml for all experiments except studies by infusion. The efficacy of the drug was assessed by its ED_{50}, the dose required to keep half of the animal group alive during the observation period. Each group consisted of 5 rats. To compare the efficacy among azoles used which was comprised of either prodrug or active form, ED_{50}s were calculated in molecular units as µmol/kg. For immunosuppressed conditions, rats were received doxycycline via drinking water throughout the course of the tests for preventing bacteria infection.

1. Systemic candidiasis model caused by *Candida albicans* or *C. tropicalis*.
C. albicans CY 1002, CY 3003, H-12 or UTHS 93-2067 (FCZ resistant) and *C. tropicalis* CY5042 or CY962312 was grown on 1GYP agar plate at 37 °C for 1 or 2 days, and conidial suspension for inoculation was prepared to 6 to 10 x 10^6 conidia /ml with saline. Rats were infected via the tail vein with 0.5 ml conidial suspension to be 3 to 5 x 10^6 conidia per rat. Treatments were performed at 0, 4, 24, 48 h after infection.

2. Systemic candidiasis caused by *C. albicans* in immunosuppressed rats. Conditions of immunosuppression were discribed in Tables 4 in detail. A conidial suspension of *C. albicans* CY 1002 was prepared to 6-10 x 10^6 conidia /ml with saline. Rats were infected via the tail vein with 0.5 ml conidial suspension to be 3 to 5 x 10^6 conidia per rat. Treatments were performed at 0, 4, 24, 28, 48 and 52 h after infection (b.i.d x3).

3. Systemic candidiasis caused by *C. parapsilosis* in immunosuppressed rats. Conditions of immunosuppression were discribed in Tables 4 in detail. A conidial suspension of *C. parapsilosis* KULM219C was prepared to 3 x 10^6 conidia /ml with saline. Rats were infected via the tail vein with 0.5 ml conidial suspension to be 1.5 x 10^6 conidia per rat. Treatments were performed at 0, 4, 24 and 48 h after infection.

4. Systemic aspergillosis caused by *Aspergillus fumigatus* in normal rats.
A.fumigatus CF1003, CF1083 or CF924390 was grown on CY20S agar plate at 27 °C for 1 or 2 weeks, a conidial suspension of *A. fumigatus* was prepared to 1.2 x 10^6 conidia /ml with saline. Rats were infected via the tail vein with 0.5 ml conidial suspension to be 6 x 10^5 conidia per rat. Treatments were performed at 0, 4, 24, 28, 48, 52, 72 and 76 h after infection (b.i.d x 4).

5. Systemic aspergillosis caused by *A. fumigatus* in immunosuppressed rats. Conditions of immunosuppression were discribed in Tables 5 in detail. A conidial suspension of *A. fumigatus* CF924390 was prepared to 4 x 10^4 conidia /ml with saline. Rats were infected via the tail vein with 0.5 ml conidial suspension to be 2 x 10^4 conidia per rat. Treatments were performed at 0, 4, 24, 28, 48, and 52 h after infection (b.i.d x 3).

6. Pulmonary aspergillosis caused by *A. fumigatus* in immunosuppressed rats. Conditions of immunosuppression were discribed in Tables 5 in detail. A conidial suspension of *A. fumigatus* CF924390 was prepared to 1.2 x 10^6 conidia /ml with BSG80, respectively. Rats were infected via trachea with 50 µl of conidial suspension to be 6 x 10^5 conidia per rat. Treatments were performed at 0, 4, 24, 28, 48 and 52 h after infection (b.i.d x3) or two additional dosing at 72 and 76h after infection (b.i.d x 4).

7. Infusion treatment test for systemic candidiasis caused by *C. albicans* CY1002 and systemic aspergillosis caused by *A. fumigatus* CF924390. Rats that had a catheter installed in the external jugular vein received drugs or vehicle solution via the constant infusion with a flow rate of 0.3ml per hour for 1 week by a micro speed syringe pump (Model KDS220, Kd scientific INC.). Both of *C. albicans* and *A. fumigatus* were infected in the same manner as the bolus treatment with 4 x 10^6 conidia per rat and 1x10^7 conidia per rat respectively. Plasma revel of RO0094815 after the constant infusion of RO0098557 was determined by LC/MS/MS.

Results and Discussion

Table 1a), b) Antifungal spectrum of RO0094815
MICs of Azoles against laboratory reference strains

a) Yeasts (µg/ml)

		FCZ	ICZ	VCZ	RO0094815
Candida albicans	ATCC48130(=CY1002)	0.39	0.003	0.0062	0.0054
C. albicans	ATCC10231	0.31	0.003	0.016	0.0060
C. albicans	KB	6.0	0.059	0.095	0.082
C. albicans	AD	24	0.28	0.35	0.06
C. albicans	ATCC24433	0.19	0.0031	0.0056	0.0041
C. albicans	ATCC90028	0.2	0.0027	0.0031	0.0031
C. albicans	CY3003	0.049	0.0028	0.0016	0.0013
C. albicans	Basel412	0.078	0.0011	0.0015	0.0011
C. glabrata	ATCC2001	5.0	0.041	0.081	0.051
C. glabrata	IFO0005	5.4	0.15	0.13	0.16
C. guilliermondii	CY1017	2.9	0.047	0.071	0.094
C. guilliermondii	ATCC9058	1.9	0.022	0.079	0.053
C. tropicalis	ATCC13803	0.25	0.0054	0.021	0.019
C. tropicalis	ATCC750	0.54	0.0064	0.02	0.02
C. tropicalis	CY5042	>200	>200	28	3.3
C. tropicalis	IFO0199	1.4	23	5.9	5.7
C. krusei	ATCC20579	33	0.64	0.21	0.19
C. krusei	IFO0011	19	0.023	0.095	0.089
C. krusei	ATCC6258	41	0.026	0.18	0.085
C. kefyr	IFO 0586	0.27	0.0064	0.0027	0.0013
C. parapsilosis	ATCC90018	0.34	0.0018	0.0026	0.0012
C. parapsilosis	ATCC22019	1.0	0.017	0.025	0.02
C. parapsilosis	IFM0640	2.5	0.017	0.046	0.032
C. lusitaniae	IFO1019	0.1	0.0014	0.0027	0.0027
C. lusitaniae	IFO10059	0.32	0.0031	0.0061	0.0095
Cryptococcus neoformans	MTU13001	3.1	0.012	0.042	0.043
C. neoformans	Basel422	4.3	0.018	0.044	0.042
C. neoformans	ATCC35673	1.2	0.012	0.019	0.02
C. neoformans	ATCC28957	0.7	0.0083	0.01	0.0085
C. neoformans	ATCC28958	1.2	0.0083	0.016	0.0098
C. neoformans	ATCC34871	2.1	0.019	0.021	0.028
C. neoformans	1841	4.1	0.015	0.028	0.024
C. neoformans	Basel140/1	2.9	0.0096	0.021	0.020
Malassezia furfur	IFO0656	9.3	0.18	0.067	0.42
M. furfur	ATCC44344	29	0.14	0.17	3.0
M. furfur	KULM	8.4	0.094	0.051	1.1
M. pachydermatis	IFO10064	2.5	<0.0004	0.023	0.0095
M. pachydermatis	IFO0995	4.0	0.0044	0.02	0.0028
Trichosporon beigelii	IFO1500	0.56	0.0047	0.018	0.009
T. beigelii	IFO116	0.04	0.021	0.0031	0.01
T. asahii	IFO10844	8.2	0.09	0.11	0.57

Endpoint: 80% inhibitory concentration by spectrophotometry (600nm)
Method: NCCLS M27-A microdilution method
Medium: RPMI1640-MOPS+2%glucose w/wo LMPA (pH7.0)
Incubation: 35° C

b) Molds (µg/ml)

		FCZ	ICZ	VCZ	RO0094815
Aspergillus fumigatus	MTU06002(CF=1003)	96	0.032	0.085	0.068
A. fumigatus	CF1025	>100	0.086	0.16	0.28
A. fumigatus	CF1027	69	0.018	0.081	0.097
A. fumigatus	Basel437	>100	0.023	0.083	0.095
A. fumigatus	Basel638	45	0.044	0.098	0.17
A. fumigatus	IFO5840	97	0.022	0.088	0.094
A. fumigatus	IAM13869	>100	0.038	0.099	0.19
A. fullutans	IFO5860	7.8	0.0059	0.015	0.035
A. flavus	NR6591	>100	0.046	0.23	0.29
A. niger	IFO6342	>100	0.097	0.33	0.35
A. oryzae	NR6399	>100	0.084	0.36	0.29
A. terreus	NR6427	>100	0.047	0.18	0.18
A. terreus	IAM13909	180	0.031	0.18	0.16
A. terreus	IAM13910	184	0.023	0.18	0.18
Fusarium solani	IFM1531	>100	82	1.9	12
F. solani	IFM1533	>100	>100	1.2	10
F. solani	NR7144	>100	73	2.0	15
F. solani	IFO7164	>100	>100	2.9	23
F. solani	IFO8505	>100	17	1.8	4.0
F. solani	IFO8509	>100	>100	2.3	11
F. moniliforme	IFM1529	>100	27	0.5	2.8
F. moniliforme	ATCC38159	>100	0.69	0.45	1.2
F. oxysporum	IFM1530	>200	>200	1.3	2.3
Paecilomyces variotii	IFO4855	>200	0.0018	0.71	0.66
Scedosporium prolificans	ATCC64913	>200	>200	5.5	5.5
S. prolificans	ATCC200543	>200	>200	8.7	10
S. prolificans	ATCC201214	>200	>200	6.1	6.0
S. apiospermum	IFM40731	7.8	0.16	0.052	0.26
Pseudallescheria boydii	IFM41584	27	0.60	0.19	0.77
P. boydii	CY3007	16	0.18	0.10	0.12
Absidia corymbifera	IFM0894	>100	0.021	10	0.20
A. corymbifera	IFO32279	>100	0.072	19	1.3
A. corymbifera	ATCC46773	>100	0.071	11	0.52
A. corymbifera	ATCC46775	>100	0.078	11	0.27
A. corymbifera	IFO8802	>100	0.048	2.7	0.18
Mucor circinelloides	IFO4554	>100	0.085	4.9	0.98
M. rouxianus	IFO6773	>100	>100	>100	>100
M. ramosissimus	ATCC28933	>100	0.11	13	2.5
Rhizomucor pusillus	IFO4578	>100	33	9.2	4.3
R. pusillus	IFO8856	>100	0.018	2.8	0.31
R. pusillus	ATCC36606	90	0.012	2.7	0.24
Rhizopus oryzae	IFO4705	>100	0.048	3.7	0.23
R. oryzae	IFO4706	>100	0.045	1.4	0.18
R. oryzae	ATCC34965	>100	0.24	9.6	1.0
R. oryzae	ATCC66275	>100	2.2	12	2.9
R. microsporus	ATCC14050	>100	0.33	5.9	0.89
R. microsporus	ATCC42406	>100	0.19	5.3	0.65
R. microsporus	ATCC66276	>100	0.33	6.0	0.59
Cunninghamella bertholletiae	ATCC42113	>100	0.33	11	5.9
C. bertholletiae	ATCC42115	>100	0.15	10	5.9
Alternaria alternata	TIMM1289	180	0.17	0.73	1.3
A. mali	IFO6984	23	0.075	0.35	0.74
Cladosporium trichoides	CF1047	25	0.0015	0.088	0.023
C. carrionii	CF1048	2.9	<0.0004	0.0057	0.0036
C. herbarum	IFO4458	48	0.037	0.59	0.43
Fonsecaea pedrosoi	CF1036	19	0.016	0.034	0.054
F. compacta	IFM41704	33	0.018	0.064	0.044
Phialophora parasitica	IFM4924	170	>200	0.17	0.30
P. verrucosa	CF1044	11	0.01	0.022	0.021
Exophiala dermatitidis	IFO 6421	22	0.024	0.16	0.15
E. dermatitidis	IFO8194	26	0.014	0.024	0.052
E. jeanselmei	MTU23016	20	0.0009	0.048	0.021
E. jeanselmei	NCMH123	18	0.011	0.14	0.32
E. spinifei	CF1043	36	0.0083	0.11	0.28
E. moniliae	NCMH721	25	0.00077	0.022	0.022
Trichophyton mentagrophytes	CF1007	3.6	0.011	0.02	0.028
T. mentagrophytes	CF1071	22	<0.0004	0.021	0.003
T. mentagrophytes	IFO7522	15	0.011	0.024	0.038
T. rubrum	IFO6807	0.72	0.0016	0.011	0.011
T. rubrum	IFO9185	0.39	0.0056	0.011	0.012
T. tonsurans	IFO5928	8.3	0.0014	0.047	0.017
T. tonsurans	IFO5946	2.2	<0.0004	0.011	0.003
T. tonsurans	IFO5971	3.8	0.0051	0.041	0.049
Microsporum canis	CF1008	77	0.047	0.03	0.025
M. gypseum	IFO6231	12	0.007	0.023	0.015
Epidermophyton floccosum	IFO6045	1.1	0.0014	0.0074	0.023
Paecilium marneffei	CF1097	2.7	0.00075	0.0089	0.008
P. roseilocum	CF1093	>200	0.062	0.025	0.13
Sporothrix schenckii	IFO5984	>200	0.025	10	8.2
S. schenckii	IFO6073	>200	0.18	11	4.2
S. schenckii	IFO8158	>200	>200	11	8.0

Endpoint: 80% inhibitory concentration by spectrophotometry (600nm)
Method: NCCLS M27-A microdilution method
Medium: RPMI1640-MOPS+2%glucose w/wo LMPA (pH7.0)
Incubation: 35° C

RO0094815 showed a broad antifungal spectrum which contained FCZ' *C.albicans*, non-*albicans Candida* spp. *C. neoformans*, *Malassezia* spp. *Aspergillus* spp. Zygomycetes fungi, Dematiaceous fungi and Dermatophytes fungi.

Table 2 Antifungal activity of RO0094815 against FCZ resistant *C. albicans*

(µg/ml)

Strains	UTHS number	FCZ	ICZ	VCZ	RO0094815	AMPH
C.albicans	93-1591	25	0.1	0.1	0.2	0.013
	93-1595	50	0.39	0.39	0.39	0.025
	93-1604	6.25	0.025	0.05	0.05	0.025
	93-1618	50	0.05	0.2	0.1	0.025
	93-1706	50	0.1	0.2	0.2	0.025
	93-1729	25	0.0063	0.025	0.0016	0.013
	93-1835	>200	>200	100	25	0.025
	93-1870	100	0.05	0.1	0.1	0.05
	93-1949	100	1.56	0.78	0.39	0.025
	93-1997	50	0.05	0.39	0.1	0.025
	93-2022	50	0.1	0.1	0.39	0.025
	93-2036	50	0.39	0.39	0.39	0.025
	93-2067	50	0.025	0.05	0.05	0.025
	R2477	>200	>200	50	12.5	0.013

MIC endpoint: Concentration of score 2, prominent decrease in turbidity
Method: NCCLS M27-A microdilution method
Medium: RPMI1640-MOPS+2%glucose (pH7.0)
Incubation: 35° C

Table 5 Efficacies of RO0098557 against rat systemic and pulmonary aspergillosis

(µmol/kg)

		systemic			pulmonary	
		Normal[1]	Immunosuppressed[2]		Immunosuppressed[2]	
		CF924390[3]	CF1003[3]	CF923450[4]	CF924390[3]	CF924390[3]
RO0098557	i.v.	14	12	6.0	11	6.8
	p.o	8.8	14	9.9	7.1	8.9
ICZ	p.o	4.9	8.9	7.2	2.2	2.5
VCZ	i.v.	3.0	8.7	8.0	-	-
	p.o	3.0	7.4	8.0	2.7	2.2
AMPH	i.v.	-	-	-	0.2	0.6

[1]Normal host, [2]Immunosuppression:Cyclophosphamide 100mg/kg, i.p. on the day -4 and Cortisone acetate 125mg/kg, s.c. on the day -1.
[3]Immunosuppression: Cortisone acetate 125mg/kg, s.c. on days -6, -4, and -1 and low protein diet.
[4]Treatments: 0,4,24,28,48, 52, 72 and 76 h after infection (b.i.d.x4)
[5]Treatments: 0,4,24,28,48 and 52 h after infection (b.i.d.x3)

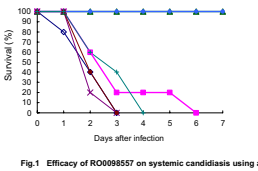


Fig.1 Efficacy of RO0098557 on systemic candidiasis using a rat infusion model

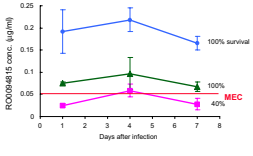


Fig.2 RO0094815 conc. in plasma during the constant infusion of RO0098557 in systemic candidiasis rat model

MEC of RO0094815 against systemic candidiasis or systemic aspergillosis (data not shown) would be 0.05 µg/ml in plasma

Table 3 *In vitro* activity against clinical isolates
Geometric mean (range) / MIC50 / MIC90 (µg/ml)

species	n	FCZ GM	FCZ MIC50	FCZ MIC90	ICZ GM	ICZ MIC50	ICZ MIC90	VCZ GM	VCZ MIC50	VCZ MIC90	RO0094815 GM	RO0094815 MIC50	RO0094815 MIC90
C. albicans	12	0.59 (0.1->200)	0.39	3.13	0.0054 (0.0016 - 0.051)	0.0063	0.013	0.0040 (0.00164-0.015)	0.003	0.013	0.0022 (0.00078-0.0063)	0.0016	0.0016
C. glabrata	12	5.0 (0.78 - 12.5)	6.25	12.5	0.13 (0.0063 - 0.39)	0.2	0.39	0.07 (0.00634-0.2)	0.1	0.1	0.08 (0.0031-0.39)	0.1	0.2
C. tropicalis	11	14 (0.1->200)	3.13	200	1.5 (0.0016 - >200)	0.025	200	1.6 (0.0063-100)	0.05	100	0.33 (0.00156-25)	0.025	12.5
C. guilliermondii	9	5.0 (3.13 - 12.5)	6.25	6.25	0.17 (0.05 - 0.39)	0.2	0.39	0.093 (0.05-0.2)	0.1	0.2	0.13 (0.05-0.39)	0.1	0.2
C. krusei	8	42 (25 - 50)	50	50	0.032 (0.025 - 0.1)	0.025	0.05	0.10 (0.1)	0.1	0.1	0.046 (0.025-0.1)	0.05	0.1
C. parapsilosis	7	0.32 (0.2 - 0.78)	0.39	0.78	0.0021 (0.00078 - 0.0031)	0.0031	0.0031	0.0029 (0.00154-0.0063)	0.0063	0.0063	0.0011 (0.0004-0.0031)	0.0008	0.0031
C. lusitaniae	8	0.20 (0.05 - 1.56)	0.2	1.56	0.003 (0.0078 - 0.025)	0.0016	0.025	0.001 (0.004-0.0016)	0.0008	0.0063	0.003 (<0.0004-0.025)	0.0016	0.025
T. beigelii	2	4.4 (1.56 - 12.5)	1.56	12.5	0.07 (0.05-0.1)	0.05	0.1	0.04 (0.013-0.1)	0.013	0.1	0.10 (0.03-0.2)	0.05	0.2
C. neoformans	14	2.1 (0.78 - 3.13)	1.56	3.13	0.005 (0.0004-0.013)	0.0063	0.013	0.01 (0.0052-0.013)	0.013	0.025	0.01 (0.0052-0.013)	0.013	0.013

MIC: Concentration of score 2, prominent decrease in turbidity
Method: NCCLS M27-A microdilution method
Medium: RPMI1640-MOPS+2%glucose (pH7.0)
Incubation: 35° C

Table 4 Efficacies of RO0098557 against systemic candidiasis
Rat systemic candidiasis ; ED_{50} (µmol/kg) on day 14

		C. albicans[1]			FCZ-*C.albicans*	*C. tropicalis*[1]		Immuno-suppressed[2] *C. parapsilosis*	Immuno-suppressed[2] *C. albicans*
		CY1002	CY3003	H-12	UTHS93-2067	CY5042	CY962312	KULM219C	CY1002 *
RO0098557	i.v.	4.0	1.5	4.2	8.0	0.85	3.3	8.5	6.7*
	p.o	4.0	2.6	3.3	11	0.8	3.3	5.9	7.9*
FCZ	p.o	1.8	0.85	1.5	15	1.1	1.1	17*	3.9*
ICZ	p.o	4.7	1.7	2.2	3.9	1.7	2.0	20	5.7*
VCZ	i.v.	>22	4.5	>22	10*	10	>22	20	19*
	p.o	8.4	2.3	>22	5.8*	10	>22	30*	19*

ED_{50} of each strain was derived from an arithmetical mean of two repeated experiments. ED_{50} of immunosuppressed candidiasis due to *C. albicans* CY1002 and the data with asterisk* was calculated by only one examination.
[1]Treatments: 0, 4, 24,and 48h after infection
[2]Immunosuppression: Cyclophosphamide 100 mg/kg, i.p. and prednisolone 20 mg/kg, p.o. on days -4 and -1 after infection. Treatments: 0, 4, 24 and 48 h after infection
[3]Immunosuppression: Cyclophosphamide 50 mg/kg, i.p. on days -3 and 1. Treatments: 0, 4, 24, 28, 48 and 52 h after infection

Table 6 PK parameters of RO0094815 following a single I.V or P.O administration of RO0098557 in monkeys

route		I.V	P.O without food	P.O with food	I.V
Dose of RO0098557	(mg/kg)	2.73	2.73	2.73	-
Dose as RO0094815	(mg/kg)	1.66	1.66	1.66	1
Cmax	(µg/ml)	1.03	0.36	0.175	0.525
Tmax	(h)	0.083	3.33	5.00	11.7
T1/2	(h)	9.84	12.8	9.40	11.7
$AUC_{0-\infty}$	(µg.h/ml)	5.08	4.39	3.54	3.66
in vivo conversion ratio	(%)	84.2	-	-	(100)
Oral bioavailability	(%)	-	86.9	67.9	-
CL/F	(ml/min/kg)	5.54	6.43	8.71	4.64
Vz/F	(L/kg)	4.74	7.28	7.42	3.01

M.W of RO0098557 is 717.8 as a free form, M.W of RO0094815 is 437.5

Conclusions

- Broad antifungal spectrum
- High water solubility (>1g/ml)
- good oral bioavailability (87%)
- preferable t1/2 ; 9.8h (I.V), 12.8h (P.O)

will allow to treat mycoses Once-a-day dosing both P.O and I.V

These results warrant further evaluation of RO0098557 for clinical studies.
Presently this azole is succeeded for development by Basilea Pharmaceutica (Switzerland)