# The Synthesis and Antifungal Activity of Nitrogen Containing Hemiaminal Ethers of LY303366

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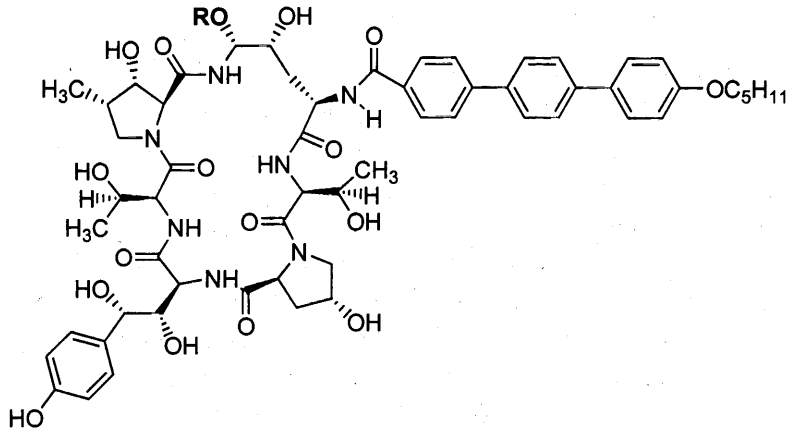
LY303366 (Fig. 1), currently in phase II clinical trials, is a semisynthetic, broad spectrum, fungicidal agent derived through extensive structure-activity relationship (SAR) studies based on the echinocandin B (ECB) nucleus[1~3]. Possession of such potent activity against clinically important fungi such as *Aspergillus fumigatus*, *Candida albicans* and *Candida parapsilosis* has prompted subsequent SAR studies focused on two major properties. First, improving the water solubility (<0.1 mg/ml) associated with the molecule and secondly, addressing the instability which is observed under strongly basic conditions. This instability is attributed to the hemiaminal group and results in a ring opened, inactive product as shown previously[4]. To this end, derivatization of the hemiaminal to form an ether linkage with amino alcohols would address both areas *via* formation of a stable hemiaminal ether possessing a pendant basic amine for salt formation. A similar approach has been taken in the pneumocandin $B_0$ class of antifungal agents[5].

## Chemistry

Selective ether formation at the hemiaminal hydroxy group of LY303366 was realized due to its increased reactivity over all other hydroxy groups in the molecule. A large excess of the desired amino alcohol (initially protected with a benzyloxycarbonyl group in some cases) was added to a solution of LY303366 in dioxane or DMSO. The reaction was catalyzed with either *p*-toluenesulfonic acid or HCl gas. Such conditions allowed the reaction to go to completion in approximately 48 hours. Careful monitoring of the reaction showed consumption of LY303366 and formation of the product at a longer retention time. Longer reaction times resulted in undesired ether formation at the benzylic alcohol site in additon to the hemiaminal ether initially formed.

Fig. 1. Hemiaminal derivatives of LY303366.



| | OR | | OR |
|---|---|---|---|
| LY303366 | OH | 4 | $O(CH_2)_2N(CH_3)_2$ |
| 1 | $O(CH_2)_2NH_2$ | 5 | $O(CH_2)_2N(CH_2CH_3)CH_3$ |
| 2 | $O(CH_2)_4NH_2$ | 6 | $O(CH_2)_2N(n\text{-}Bu)CH_3$ |
| 3 | $O(CH_2)_6NH_2$ | 7 | $O(CH_2)_2$–N⟨ ⟩ |

Table 1. Biological evaluation of hemiaminal derivatives.

| Compound | MIC[a] (μg/ml) | | | $ED_{50}$[b] (mg/kg) |
|---|---|---|---|---|
| | *C. albicans* | *C. parapsilosis* | *A. fumigatus* | |
| LY303366 | 0.005 | 0.312 | 0.156 | 0.312 |
| **1** | 0.02 | 0.312 | 0.156 | 0.312 |
| **2** | 0.312 | 1.25 | 0.312 | 1.25 |
| **3** | 0.312 | 2.5 | 0.625 | 1.67 |
| **4** | 0.156 | 1.25 | 0.625 | 0.312 |
| **5** | 0.156 | 0.625 | 0.625 | 0.34 |
| **6** | 0.625 | 5.0 | 10 | 0.47 |
| **7** | 0.312 | 5.0 | 10 | 0.34 |

[a] MIC performed in a dilution assay in Antibiotic 3 broth (DIFCO), pH 7.0.
[b] i.p. administration in mouse *C. albicans* model.

Isolation of the preferred product was performed *via* reverse phase HPLC to yield the TFA salt of the amine. Characterization of products was performed using HR MS (FAB).

## Results and Discussion

All derivatives were tested *in vitro* and *in vivo* for comparison to LY303366 (Table 1). Introduction of the primary amino ether group onto LY303366 (compounds **1**~**3**) exhibited increased minimum inhibitory concentrations (MICs) with increasing chain length against all fungal pathogens compared to those of LY303366. The MICs of *C. parapsilosis* and *A. fumigatus* are particularly sensitive to the increasing lipophilicity associated with these derivatives. *In vivo* efficacy of compounds **1**~**3** decreases 5 fold with increasing lipophilicity associated with the tether length of the aminal ether. Similar derivatization leading to a tertiary amino ether group (compounds **4**~**7**) on LY303366 at the hemiaminal site again led to decreased *in vitro* activity as compared to the parent compound with particular sensitivity towards *C. parapsilosis* and *A. fumigatus* as seen in the primary amine series. Interestingly, compounds **4**~**7** did not exhibit the same decrease of *in vivo* efficacy associated with increased lipophilicity as shown in compounds **1**~**3**. Within the entire group, compound **1** was seen as having the most comparable *in vitro* and *in vivo* data to that of LY303366. The water solubility of compound **1** was found to be $>5$ mg/ml as compared to $<0.1$ mg/ml for LY303366.

All compounds were shown to be stable to the same basic conditions (aqueous $NaHCO_3$) that degraded the parent compound into its ring opened form over time.

## Experimental

### Compounds

All derivatives were dissolved in methanol and diluted in aqueous broths for *in vitro* studies. Each compound was suspended in β-cyclodextrin (Sigma, St. Louis, MO) for intraperitoneally (i.p.) treated *in vivo* studies.

### Animals

Outbred, male ICR mice (mean weight, 18~20 g; Harlan-Sprague Dawley, Indianapolis, IN) were used for systemic candidiasis survival studies.

### Survival Studies

Systemic candidiasis mice were X-irradiated with a sublethal dose of 400 r 24 hours prior to infection with a Gammacell 40 (Atomic Energy of Canada Limited Commercial Products, Ottawa, Canada). The mice were then infected intravenously (i.v.) through the lateral tail vein with a 0.1 ml saline suspension of *C. albicans* A26 ($2\times10^6$ conidia/mouse). The mean day of death for untreated control mice was 3.0~3.5 days. Morbidity and mortality were recorded for 7 days. All derivatives were administered i.p. at 0, 4, 24 and 48 hours post-infection. Compounds were tested at titrated concentrations using serial two-fold dilutions. Ten mice were used per drug level. Ten untreated infected control mice were administered only the vehicle.

### *In Vitro* Susceptibility Studies

Antifungal susceptibility studies were conducted using a microdilution assay. Compounds were diluted serially using 2-fold dilutions equivalent to 2X the final concentration (20~0.0006 μg/ml) desired. Aliquots of 100 μl

of the diluted compound were placed in the wells serially. The 12th well received 100 $\mu$l of broth only and served as a positive growth control. Broths were adjusted to contain $2.0 \times 10^5$ cells/ml with a spectrophotometer (optical density at 660 nm). Aliquots of 100 $\mu$l of the inoculated broth were added to each well. Plates were incubated at 35°C for 48 hours in ambient air. *C. albicans* was tested in Antibiotic 3 broth (DIFCO Laboratories, Detroit, MI). MICs were interpreted as the lowest concentration of an antifungal which inhibited the growth of the organism detected visually with the naked eye.

General

HR MS (FAB) mass spectra were obtained using a V6 ZAB-2SE mass spectrometer. Analytical reverse-phase HPLC work was done using the Varian 2050 or Waters 600E systems equipped with Waters $\mu$Bondapak® (C18, $3.9 \times 300$ mm) columns (1 : 1 acetonitrile/0.1% aqueous TFA solvent system) with a flow rate of 2 ml/minute and using UV detection at 280 nm. Preparative HPLC work was performed with a Waters Prep 2000 system using Waters 3X preppak Nova-pak® (C18, $40 \times 100$) column and identical solvent systems as used in the analytical HPLC system. All final products were >95% pure as determined by analytical HPLC.

Method A

HCl gas was bubbled through an ethereal solution of the amino alcohol (excess) until precipitation of the HCl salt of the amine ceased. The solvent was then removed *in vacuo* to yield an oil. To this oil was added DMSO (0.05 M) and LY303366 (1 equiv.). The reaction was catalyzed by applying a brief stream of HCl gas over the reaction mixture and capping the reaction vessel. The reaction mixture was allowed to stir until analytical HPLC showed consumption of starting material (approx. 48 hours) at which time the reaction mixture was filtered to remove any solids and directly injected into the preparative HPLC system which afforded the TFA salt of the product as a white solid after lyophilization.

Method B

The benzyl carbamate (Cbz) of the amine (10 equiv.) was preformed *via* benzyl chloroformate using standard conditions[6] and added to a solution of LY303366 (1 equiv.) in dioxane (0.01 M). To this mixture was added catalytic *p*-toluenesulfonic acid. The reaction had similar reaction times and identical isolation techniques as in method A. The resultant N-Cbz protected analog was deprotected *via* hydrogenolysis to provide the free amine using 10% Pd-charcoal (stoichiometric) and $H_2$ at atomospheric temperature and pressure with ethanol as the solvent. HPLC purification yielded the TFA salt of the product after lyophilization.

*O*-(2-Aminoethyl) 303366 (**1**):

Method A using commercially available (Aldrich) ethanolamine hydrochloride (1.71 g, 17.5 mmol) and LY303366 (0.500 g, 0.438 mmol) yielded 53.6 mg (4.5%) of **1**. Analytical HPLC retention time, 4.76 minutes; HR MS (FAB) calcd for $C_{60}H_{79}N_8O_{17}$ $(M+H)^+$ 1183.5563, found 1183.5577.

*O*-(4-Amino-*n*-butyl) 303366 (**2**):

Method B using 4-(*N*-benzyloxycarbonyl)-*n*-butanol (1.12 g, 5.02 mmol), LY303366 (0.557 g, 0.489 mmol) and *p*-toluenesulfonic acid (0.352 g, 0.185 mmol) yielded 79.5 mg (6.6%) of **2** after hydrogenation. Analytical HPLC retention time, 4.83 minutes; HR MS (FAB) calcd for $C_{62}H_{83}N_8O_{17}$ $(M+H)^+$ 1211.5876, found 1211.5884.

*O*-(6-Amino-*n*-hexyl) 303366 (**3**):

Method B using 6-(*N*-benzyloxycarbonyl)-*n*-hexanol (1.12 g, 4.46 mmol), LY303366 (0.553 g, 0.485 mmol) and *p*-toluenesulfonic acid (0.432 g, 0.227 mmol) yielded 102.6 mg (8.3%) of **3** after hydrogenation. Analytical HPLC retention time, 5.60 minutes; HR MS (FAB) calcd for $C_{64}H_{87}N_8O_{17}$ $(M+H)^+$ 1239.6189, found 1239.6193.

*O*-[2-(*N*,*N*-Dimethylamino)ethyl] 303366 (**4**):

Method A using 2-(*N*,*N*-dimethylamino)ethanol (15.6 g, 175.4 mmol) and LY303366 (2.00 g, 1.75 mmol) yielded 559.0 mg (26%) of **4**. Analytical HPLC retention time, 5.39 minutes; HR MS (FAB) calcd for $C_{62}H_{83}N_8O_{17}$ $(M+H)^+$ 1211.5876, found 1211.5883.

*O*-[2-(*N*-Ethyl-*N*-methylamino)ethyl] 303366 (**5**):

Method A using 2-(*N*-ethyl-*N*-methylamino)ethanol (20.0 g, 273.4 mmol) and LY303366 (2.00 g, 1.75 mmol) yielded 975 mg (46%) of **5**. Analytical HPLC retention time, 4.80 minutes; HR MS (FAB) calcd for $C_{63}H_{85}N_8O_{17}$ $(M+H)^+$ 1225.6033, found 1225.5995.

*O*-[2-(*N*-*n*-Butyl-*N*-methylamino)ethyl] 303366 (**6**):

Method A using 2-(*N*-*n*-butyl-*N*-methylamino)ethanol (10.0 g, 76.2 mmol) and LY303366 (1.00 g, 0.877 mmol) yielded 40.0 mg (3.7%) of **6**. Analytical HPLC retention time, 4.75 minutes; HR MS (FAB) calcd for $C_{65}H_{89}N_8O_{17}$ $(M+H)^+$ 1253.6346, found 1253.6393.

*O*-[2-(*N*-Pyrrolidino)ethyl] 303366 (**7**):

Method A using 2-(*N*-pryrrolidino)ethanol (20.2 g, 175.4 mmol) and LY303366 (2.00 g, 1.75 mmol) yielded

363 mg (17%) of **7**. Analytical HPLC retention time, 3.84 minutes; HR MS (FAB) calcd for $C_{64}H_{85}N_8O_{17}$ $(M+H)^+$ 1237.6033, found 1237.5991.

## References


1) DEBONO, M.; W. W. TURNER, L. M. LAGRANDEUR, F. J. BURKHARDT, J. S. NISSAN, K. K. NICHOLS, M. J. RODRIGUEZ, M. J. ZWEIFEL, D. J. ZECKNER, R. S. GORDEE, J. TANG & T. R. PARR: Semisynthetic chemical modifications of the antifungal lipopeptide echinocandin B (ECB): Structure-activity studies of the lipophilic and geometric parameters of polyarylated acyl analogs of ECB. J. Med. Chem. 38: 3271~3281, 1995
2) DEBONO, M.; B. J. ABBOTT, D. S. FUKUDA, M. BARNHART, K. E. WILLARD, R. M. MOLLOY, K. H. MICHEL, J. R. TURNER, T. F. BUTLER & A. H. HUNT: Synthesis of new analogs of echinocandin B by enzymatic deacylation and chemical reacylation of the echinocandin B peptide: Synthesis of the antifungal agent Cilofungin (LY121019). J. Antibiotics 42: 389~397, 1989
3) DEBONO, M.; B. J. ABBOTT, J. R. TURNER, L. C. HOWARD, R. S. GORDEE, A. S. HUNT, M. BARNHART, R. M. MOLLOY, K. E. WILLARD, D. FUKUDA, T. F. BUTLER & D. J. ZECKNER: Synthesis and evaluation of LY121019, a member of a series of semisynthetic analogues of the antifungal lipopeptide echinocandin B. Antifungal Drugs in Ann. N.Y. Acad. Sci. 544: 152~167, 1988
4) BALKOVEC, J. M.; R. M. BLACK, M. L. HAMMOND, J. V. HOCK, R. A. ZAMBIAS, G. ABRUZZO, K. BARTIZAL, H. KROPP, C. TRAINOR, R. E. SCHWARTZ, D. C. MCFADDEN, K. H. NOLLSTADT, L. A. PITTARELLI, M. A. POWLES & D. M. SCHMATZ: Synthesis, stability, and biological evaluation of water-soluble prodrugs of a new echinocandin lipopeptide. Discovery of a potential clinical agent for the treatment of systemic candidiasis and *Pneumocystis carinii* pneumonia (PCP). J. Med. Chem. 35: 194~198
5) ZAMBIAS, R. A.; C. JAMES, M. L. HAMMOND, G. K. ABRUZZO, K. R. BARTIZAL, K. H. NOLLSTADT, C. DOUGLAS, J. MARRINAN & J. M. BALKOVEC: Antifungal lipopeptides: Structure-activity relationships of 3-hydroxyglutamine modified pneumocandin $B_0$ derivatives. Bioorg. & Med. Chem. Lett. 5: 2357~2362
6) For addition and removal of the benzyloxycarbonyl group see: FIESER, L. F. & M. FIESER: Reagents for organic synthesis 1: 109~110, 1967