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Phosphonooxymethyl Prodrugs of the Broad Spectrum Antifungal Azole, Ravuconazole: Synthesis and Biological Properties

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Abstract—Synthesis of phosphonooxymethyl derivatives of ravuconazole, **2** (BMS-379224) and **3** (BMS-315801) and their biological evaluation as potential water-soluble prodrugs of ravuconazole are described. The phosphonooxymethyl ether analogue **2** (BMS-379224) and *N*-phosphonooxymethyl triazolium salt **3** (BMS-315801) were both prepared from ravuconazole (**1**) and bis-*tert*-butyl chloromethylphosphate, but under two different conditions. Both derivatives were highly soluble in water and converted to the parent in alkaline phosphatase, and also in vivo (rat). However, BMS-315801 was found to be less stable than BMS-379224 in water at neutral pH. BMS-379224 (**2**) has proved to be one of the most promising prodrugs of ravuconazole that we tested, and it is currently in clinical evaluation as an intravenous formulation of the broad spectrum antifungal azole, ravuconazole.

Ravuconazole **1** (BMS-207147) licensed to Bristol-Myers Squibb Company from Eisai (ER-30346) is a potent and broad spectrum antifungal agent, exhibiting excellent antifungal activity against fungal pathogens such as *Candida albicans*, *Cryptococcus neformans* with exceptional activity against *Aspergillus* species.[1] It is currently under clinical evaluation by BMS as an oral agent, but to optimize its therapeutic potential, development of an intravenous (iv) formulation was deemed necessary. This is essential for therapy of serious systemic fungal infections such as pulmonary *Aspergillus* infections, particularly for patients to whom oral medication cannot be readily administered. The poor aqueous solubility of ravuconazole (0.6 μg/mL) precludes its development for intravenous administration.[2] Thus, an approach of a water-soluble prodrug was employed to develop an iv formulation.

The hydroxyl group and the triazole moiety present in ravuconazole (**1**) were thought to be most logical sites for derivatization, leading to potentially water-soluble prodrugs. Phosphonooxymethyl derivatives of alcohols and cyclic imides have shown to be successful water-soluble prodrugs for poorly water-soluble drugs such as paclitaxel and phenytoin.[3] These phosphonooxymethyl prodrugs are known to be hydrolyzed by the ubiquitous mammalian enzyme, alkaline phosphatase (ALP) to chemically labile hemiacetal derivatives which are spontaneously cleaved to the parent alcohols or cyclic imides. However, the sterically hindered hydroxyl group in ravuconazole **1**, coupled with the presence of the reactive neighboring groups posed a formidable challenge for functionalizing this hydroxyl group. The preparation of quaternary ammonium salts and triazolium salts as potential prodrugs of tertiary amine- or triazole-containing drugs has been reported,[4] but there was no report on the preparation of quaternary phosphonooxymethyl triazolium salt of antifungal triazoles. Here, we would like to report our unique synthesis of phosphonooxymethyl ether of ravuconazole, **2** (BMS-379224) and the preparation of *N*-quaternary phosphonooxymethyl

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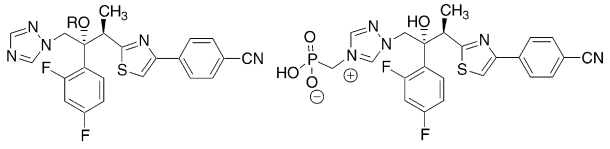
triazolium derivative of ravuconazole, **3** (BMS-315801) as well as their physico-chemical and biological properties as potential prodrugs.

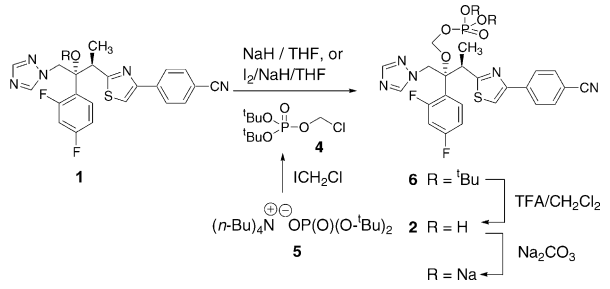


1, Ravuconazole, R = H

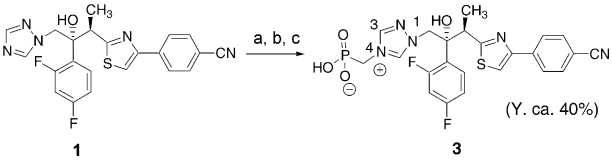
3, BMS-315801

2, BMS-379224, R = $-CH_2OP(O)(OH)_2$

The phosphonooxymethyl ether derivative of ravuconazole **2** (BMS-379224) was prepared in two steps from ravuconazole (**1**) by *O*-alkylation with di-*tert*-butyl chloromethyl phosphate **4** followed by cleavage of the protecting *tert*-butyl ester moiety. The first step, *O*-alkylation was successfully achieved under rather unconventional conditions. The methods reported for the preparation of *O*-phosphonooxymethyl ether of alcohols[3a] were not successful for this particular alcohol-containing ravuconazole. After many attempts to alkylate this hydroxyl moiety, a direct alkylation reaction of oxy-anion, which was generated from ravuconazole and NaH in THF, with a semi-purified di-*tert*-butyl chloromethylphosphate **4**, at room temperature to 50 °C, provided a modest but variable yield (10–55%) of the desired *tert*-butyl phosphate ester product **6**.[5] The phosphate **4** was prepared by the reaction of chloromethyliodide with ammonium salt of di-*tert*-butyl phosphoric acid **5**.[6] The yield of **6** was dependent on the purity of the alkylating agent **4**. When highly purified di-*tert*-butyl chloromethylphosphate **4** was used, very little or no desired product was observed. Later, it was found that addition of iodine and an excess amount of NaH as an iodide source provided more consistent and much higher yield of the phosphonooxymethyl ether derivative **6**.[8] Deprotection of the *tert*-butyl group was accomplished by use of trifluoroacetic acid in CH_2Cl_2 to produce the phosphonooxymethyl ether **2**[9] in good yield (>80%). The sodium salt was prepared by treatment with sodium carbonate and purified by C-18 reverse phase column. The structure of **2** was confirmed by single-crystal X-ray analysis of the bis-lysine salt (Scheme 1).[8]



Scheme 1.



Scheme 2. (a) $ClCH_2OP(O)(O^tBu)_2$, **4**, THF; (b) neat, 75–85 °C; (c) C-18 column.

BMS-315801, an *N*-phosphonooxymethyl quaternary derivative of ravuconazole, **3** was prepared from ravuconazole (**1**) by direct *N*-alkylation with bis-*tert*-butyl chloromethylphosphate **4** at ~80 °C (neat)[10] as shown in Scheme 2. Interestingly, the tertiary butyl groups were cleaved concomitantly during this process to provide modest yield (ca. 40%) of **3**[11] as the quaternary salt after C-18 reverse-phase column chromatography. The structure of **3** was supported by its ^1H NMR (DMSO-d_6) which indicated the presence of the methylene protons ($-OCH_2N^+$) at 5.60–5.76 ppm as a multiplet and its MS which indicated *m*/*z* 548 (M + H). The location of the quaternary group at the 4-nitrogen was supported by the NOE experiment where NOEs were observed between the quaternary methylene protons and both triazole protons (C-3 and C-5).

Solubility and Stability

The amorphous sodium salt and the crystalline bis-lysine salt of **2** were soluble in water at pH 7 (>30 mg/mL). The amorphous zwitterion **3** was also soluble in water (>30 mg/mL) at pH 6.6.

The phosphonooxymethyl ether **2** was stable in solution with concentration of 1–10 mg/mL at pH ranges of 3.6–8.9, generating less than 1% of ravuconazole after 13 days at room temperature. However, quaternary triazolium salt **3** was not as stable as the phosphonoooxymethyl ether **2** in solution at pH 7, generating 5–6% of ravuconazole at room temperature during 24-h period. It also became cloudy during this period due to precipitation of insoluble ravuconazole.

The phosphonooxymethyl ether **2** was also stable as crystalline bis-lysine salt at 40 °C and 75% relative humidity for 3 weeks. No degradation and no generation of ravuconazole were observed. Whereas, the *N*-quaternary phosphonooxymethyl triazolium salt **3** was hygroscopic with moisture uptake of 5–7% at 25 °C and 40% relative humidity. It was also susceptible to hydrolysis under accelerated conditions of heat and humidity (e.g., 40 °C and 75% relative humidity).

These results on the relative stability in solution and solid-state indicate the phosphonooxymethyl ether **2** (BMS-379224) is much more promising than the quaternary triazolium salt **3** (BMS-315801).

In Vitro Conversion

Both compounds, **2** (BMS-379224) and **3** (BMS-315801) were readily converted to ravuconazole (**1**) by ALP. In a typical experiment, the disappearance of **3** (BMS-315801) and formation of ravuconazole (**1**) after incubation of **3** (BMS-315801) with human placentral ALP is shown in Figure 1. The results demonstrate that these phosphates are substrates for ALP, an enzyme of wide

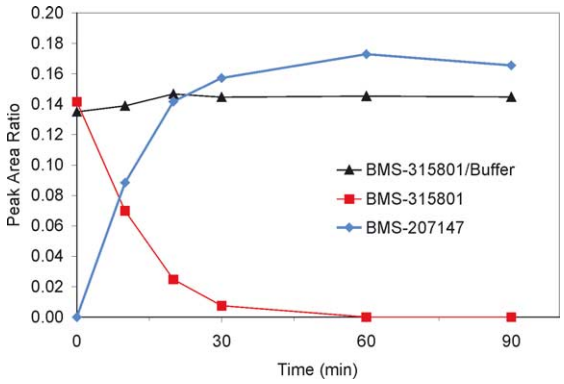


Figure 1. Disappearance of BMS-315801 (■) and formation of ravuconazole (BMS-207147) (◆) as a function of time after incubation of BMS-315801 in alkaline phosphatase solution. BMS-315801 in buffer solution (▲) is shown as a control.

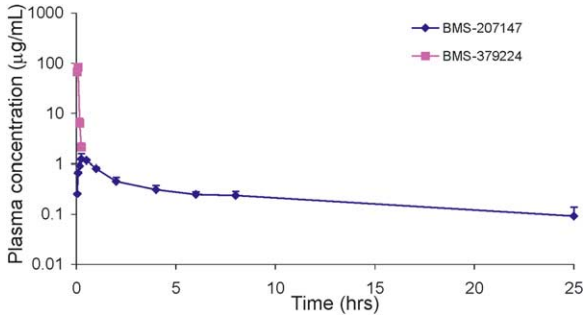


Figure 2. Plasma concentrations of BMS-379224 (■) and ravuconazole (BMS-207147) (◆) in rats following iv administration of BMS-379224 at 6.76 mg/kg (mean ± S.D., $n = 3$).

distribution in various mammalian tissues. These phosphate prodrugs were also converted to ravuconazole after incubation in liver S9 homogenates from rat dog and human tissues.[12] However, these prodrugs were not rapidly converted to ravuconazole in rat, dog or human plasma. We believe this is due to high binding of plasma proteins such as albumin to these phosphates of highly lipophilic molecules.[13]

In Vivo Conversion

The rapid in vivo conversion of the phosphate prodrugs **2** (BMS-379224) and **3** (BMS-315801) to ravuconazole (**1**) upon iv administration to rats is shown in Figures 2 and 3, respectively. A similar rapid conversion of **2** (BMS-379224) to ravuconazole was also demonstrated in dogs and monkeys.

In vivo antifungal efficacy

In vivo efficacy of the water-soluble phosphate **2** (BMS-379224) was evaluated in the systemic *C. albicans* infection model in mice. The results are summarized in Figure 4. The iv administered prodrug **2** (BMS-379224) showed comparable efficacy as orally administered ravuconazole.

In summary, two phosphonooxymethyl derivatives of ravuconazole, **2** and **3** were synthesized and evaluated as

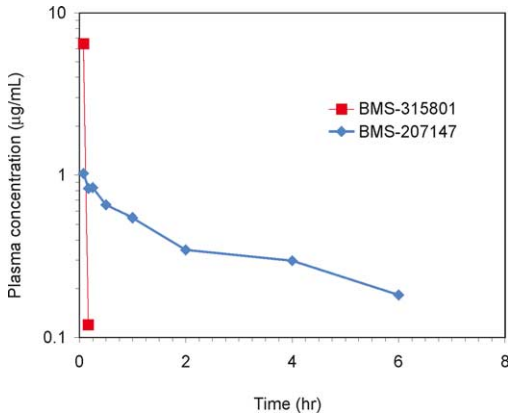


Figure 3. Plasma concentrations of BMS-315801 (■) and ravuconazole (BMS-207147) (◆) in rats following iv infusion of BMS-315801 at 5.9 mg/kg (average of two rats).

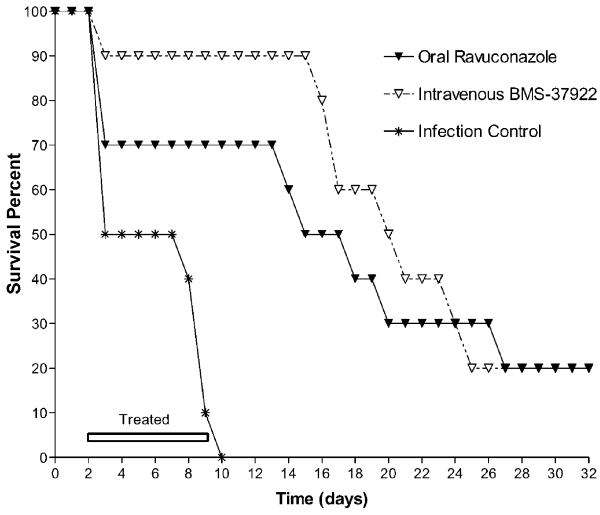


Figure 4. Efficacy of intravenous BMS-379224 and oral ravuconazole in a systemic *Candida albicans* infection model in mice.[14]

potential water-soluble prodrugs. Both compounds, the phosphonooxymethyl ether analogue, **2** (BMS-379224) and the *N*-quaternary phosphonooxymethyl triazolium analogue, **3** (BMS-315801) were soluble in water at neutral pH and both phosphates generated parent ravuconazole (**1**) in in vitro systems (ALP or liver S9) and also in vivo in rats, dogs and in monkeys. However, the prodrug **2** (BMS-379224) was much more stable than the quaternary phosphate **3** (BMS-315801), meeting our requirement of solution and solid-state chemical stability.

The results from pre-clinical evaluations, including animal safety indicate that this phosphonooxymethyl ether derivative **2** (BMS-379224) is one of the most promising prodrugs of ravuconazole we tested, and **2** (BMS-379224) has now advanced to its clinical study as an iv prodrug of ravuconazole.

Acknowledgements

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Science group and the Chemical Process Research group for their support in this program.

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2. Our efforts to formulate parenteral ravuconazole were not successful even by use of solubilizing co-solvents, including cyclodextrin-based agents. It is believed that this is primarily due to its exceptionally poor aqueous solubility.

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5. Compound **6**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.98 (d, 2H, $J=9$ Hz), 7.76 (s, 1H), 7.71 (d, 2H, $J=9$ Hz), 7.63 (s, 1H), 7.36–7.27 (m, 1H), 6.86–6.78 (m, 2H), 5.53 (dd, 1H, $J=28$, 6 Hz), 5.53 (dd, 1H, $J=9$, 6 Hz), 5.17 (d, 1H, $J=15$ Hz), 5.03 (d, 1H, $J=15$ Hz), 4.01 (q, 1H, $J=7$ Hz), 1.47 (s, 9H), 1.45 (s, 9H), 1.37 (d, 3H, $J=7$ Hz). MS (ESI^+) m/z 660 $[M+H]^+$.

6. Compound **5** was prepared from di-*tert*-butyl phosphoric acid[7] and tetrabutyl ammonium hydroxide in methanol.

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8. (a) Ueda, Y.; Matiskella, J. D.; Golik, J.; Hudyma, T. W.; Chen, C.-P. U.S. Patent, US 6,362,172 (March 26, 2002). *Chem. Abstr*. **2001**, *135*, 122628. (b) Chen, C.-P.; Connolly, T. P.; Kolla, L. R.; Matiskella, J. D.; Mueller, R. H.; Pendri, Y.; Petsch, D. T. U.S. Patent, US 6,448,401 (September 10, 2002). *Chem. Abstr*. **2002**, *136*, 386259.

9. Compound **2** (sodium salt): ^{1}H NMR (500 MHz, D_2O) δ 8.91 (s, 1H), 7.92 (s, 1H), 7.81 (d, 2H, $J=8$ Hz), 7.80 (s, 1H), 7.77 (d, 2H, $J=8$ Hz), 7.21 (dd, 1H, $J=15$, 9 Hz), 6.99 (ddd, 1H, $J=9$, 9, 2 Hz), 6.91 (ddd, 1H, $J=9$, 9, 2 Hz), 5.35 (dd, 1H, $J=6$, 6 Hz), 5.29 (d, 1H, $J=15$ Hz), 5.21 (dd, 1H, $J=6$, 6 Hz), 5.19 (d, 1H, $J=15$ Hz), 3.86 (q, 1H, $J=7$ Hz), and 1.35 (d, 3H, $J=7$ Hz). MS (ESI^-) m/z 546 $[M-H]^-$. Anal. calcd for $C_{23}H_{18}F_2N_5O_5SP/Na_2/3.5H_2O$: C, 42.21: H, 3.85: N, 10.70: Na, 7.03. Found: C, 42.32: H, 3.83: N, 10.60: Na, 7.04; pKa (potentiometric, water): 2.2, 6.1.

10. The mixture dissolved originally in THF was concentrated and maintained at 80 °C, removing THF. See also, Golik, J.; Matiskella, J. D.; Ueda, Y. U.S. Patent, US 6,235,728 (May 22, 2001). *Chem. Abstr*. **2001**, *135*, 5703.

11. Compound **3**: ^{1}H NMR (DMSO-d_6) δ 10.21 (s, 1H), 8.98 (s, 1H), 8.39 (s, 1H), 8.17 (d, 2H, $J=9$ Hz), 7.90 (d, 2H, $J=9$ Hz), 7.38–7.25 (m, 2H), 6.97–6.91 (m, 1H), 5.76–5.60 (m, 2H), 5.00 (d, 1H, $J=14$ Hz), 4.75 (d, 1H, $J=14$ Hz), 4.04 (q, 1H, $J=7$ Hz), 1.16 (d, 3H, $J=7$ Hz). MS ($MH^+=548$). ^{19}F NMR (DMSO-d_6) δ −73.87 (s, 0.1F), −107.5 (s, 1F), −111.3 (s, 1F). Anal. calcd for $C_{23}H_{20}F_2N_5O_5SP/0.05CF_3CO_2H/0.4H_2O$: C 49.51, H 3.75, N 12.50, F 7.29. Found: C 49.39, H3.71, N 12.42, F 7.98 (H_2O 1.21%, Karl Fisher Method). MS (ESI^+) m/z 548 $[M+H]^+$. pK_a (potentiometric, water): 2.0, 4.6.

12. For ALP incubation studies, prodrugs (25 μg/mL, final concentration) were incubated with a solution of human placental ALP (Sigma #P-1391, 50 U/L, final concentration) in TRIS buffer, pH 7.4. For liver homogenate studies, prodrugs (25 μg/mL, final concentration) were incubated with commercially available (GenTest Corporation, Woburn, MA, USA) 9000 *g* supernatant fractions (S9) from rat, dog and human livers fortified with cofactors (NADPH, $MgCl_2$, glucose-6-phosphate, glucose-6-phosphate dehydrogenase). Aliquots were removed at various times, extracted with CH_3CN and analyzed for prodrug and parent using a specific LC/MS method.

13. Ueda, Y.; Mikkilineni, A. B.; Knipe, J.; Rose, W. C.; Casazza, A. M.; Vyas, D. M. *Bioorg. Med. Chem. Lett*. **1993**, *3*, 1761 A separate experiment indicated that the conversion of BMS-315801 to ravuconazole in alkaline phosphatase was significantly retarded by addition of albumin (from 10 to 90%).

14. Female ICR mice (Harlan–Sprague Dawley) were used in these studies. Mice were infected systemically by iv injection of *C. albicans* SC 5314 (inoculum size of 10^5 per animal). Therapy with intravenously administered BMS-379224 or orally administered ravuconazole was begun 2 days post-infection and given once a day for 7 consecutive days. Both compounds were administered at daily doses equivalent to 5 mg/kg ravuconazole. Efficacy was determined by survival for 30 days following infection.