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Letter

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Discovery and Characterization of a Water-Soluble Prodrug of a Dual Inhibitor of Bacterial DNA Gyrase and Topoisomerase IV

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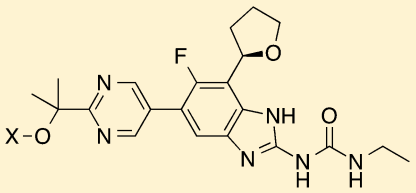
Supporting Information

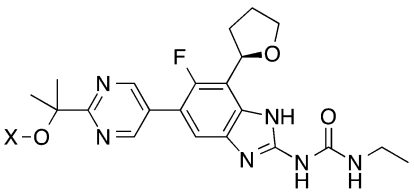
ABSTRACT: Benzimidazole **1** is the lead compound resulting from an antibacterial program targeting dual inhibitors of bacterial DNA gyrase and topoisomerase IV. With the goal of improving key drug-like properties, namely, the solubility and the formulability of **1**, an effort to identify prodrugs was undertaken. This has led to the discovery of a phosphate ester prodrug **2**. This prodrug is rapidly cleaved to the parent drug molecule upon both oral and intravenous administration. The prodrug achieved equivalent exposure of **1** compared to dosing the parent in multiple species. The prodrug **2** has improved aqueous solubility, simplifying both intravenous and oral formulation.



1, X = H
2, X = $PO(OH)_2$

KEYWORDS: *Prodrug, DNA gyrase, topoisomerase IV, water soluble*

Recently, we reported the discovery and characterization of compound **1**,[1] a dual inhibitor of bacterial DNA gyrase and topoisomerase IV[2] with no cross resistance with the extensively used fluoroquinolone antibiotics.[3−6] Compound **1** was shown to possess potent *in vitro* antibacterial activity versus clinically important pathogens and *in vivo* efficacy was demonstrated versus *S. aureus* in a neutropenic rat thigh infection model.[1] For nosocomial bacterial infections, where drug-resistance is particularly pronounced, the option of intravenous drug delivery is highly desirable.[7−11] Due to the extremely poor aqueous solubility of **1**, the identification of a water-soluble prodrug became a priority. In this letter we describe the discovery and characterization of the phosphate ester prodrug **2** of compound **1** (Figure 1).



1, X = H
2, X = $PO(OH)_2$

Figure 1. Compound **1** and its phosphate ester prodrug **2**.

The clinical use of phosphate esters as prodrugs is precedented;[12,13] noteworthy examples include HIV protease inhibitor fosamprenivir,[14] anticonvulsant fosphenytoin,[15] antiemetic fosaprepitant,[16] and antifungal fosfluconazole[17] (Figure 2).

Compound **1** offers multiple points of attachment for a phosphate containing promoiety, and initially, attachment to the benzimidazole NH was considered. Phosphonooxymethyl derivatives of benzimidazole anthelmintic drugs have been reported.[18,19] Attempts to apply the same strategy to **1** were not successful, as none of the desired phosphonooxymethyl adducts were observed upon reaction of **1** with either dibenzyl chloromethyl phosphate or di-*tert*-butyl chloromethyl phosphate under a variety of conditions. Furthermore, it was recognized that even if successful this approach would likely suffer from a lack of regioselectivity with respect to the benzimidazole nitrogens and potentially the urea nitrogens. Accessing the phosphate ester of the tertiary alcohol proved more straightforward. Following the reported synthesis of fosfluconazole,[17,20] **1** was reacted with dibenzyl diisopropyl phosphoramidite, and the resulting phosphite ester intermediate was oxidized *in situ* to provide the dibenzylphosphate

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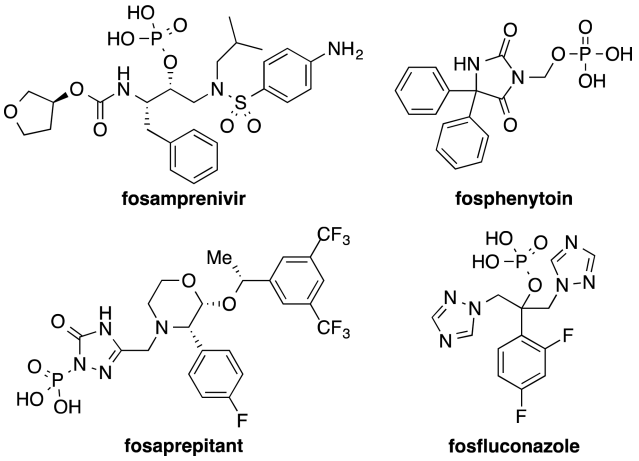
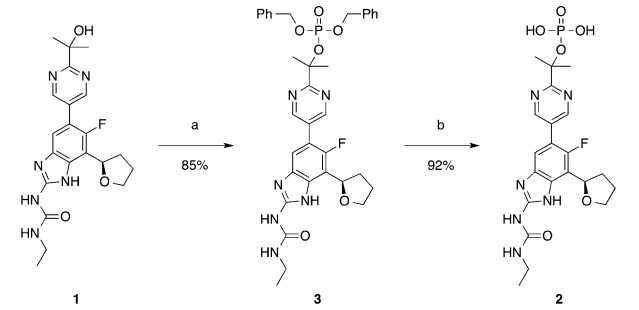


Figure 2. Phosphate prodrugs fosamprenivir, fosphenytoin, fosaprepitant, and fosfluconazole.

derivative **3** (Scheme 1). Hydrogenolysis of the benzyl groups in the presence of sodium hydroxide gave the disodium salt of

Scheme 1. Synthesis of Phosphate Ester Prodrug 2 from Compound 1[a]



[a]Reaction conditions: (a) $(BnO)_2P{-}N^iPr_2$ (1.5 equiv), tetrazole (2 equiv), DMF, MeCN, rt for 18 h, then *m*-CPBA (1.6 equiv), 0 °C to rt, 40 min, 85%; (b) (i) H_2, Pd/C (0.07 equiv), aq NaOH (2 equiv), EtOH, rt; (ii) aq HCl (2.2 equiv), MeOH, rt, 92%.

the phosphate ester **2**; a solvent mixture of ethanol and aqueous sodium hydroxide was necessary to ensure that both the starting dibenzyl phosphate ester and deprotected phosphate ester would remain in solution and allow removal of the palladium catalyst. Conversion to the free acid form was accomplished via treatment of the disodium salt with aqueous hydrochloric acid in methanol.

As anticipated, the phosphate prodrug was much more water-soluble than the parent. At pH 7, the aqueous solubility of the prodrug **2** was approximately 75 mg/mL, >30,000-fold higher than that of **1** (Figure 3).

Appending the phosphate moiety to compound **1** renders the resulting prodrug much less potent *in vitro* versus all pathogens tested (Table 1). Interestingly, at the target-level, the prodrug **2** showed similar activity to that of the parent **1**; K_i values versus both *S. aureus* gyrase and topoisomerase IV enzymes[21] are shown in Table 1. This finding was rationalized based on the established binding mode of the benzimidazole urea class in both DNA gyrase and topoisomerase IV, i.e., the phosphate moiety extends toward solvent.[1,3] The lack of whole cell activity is likely a result of the altered physicochemical properties of the prodrug prohibiting it from reaching the desired targets, which reside in the bacterial cytoplasm.

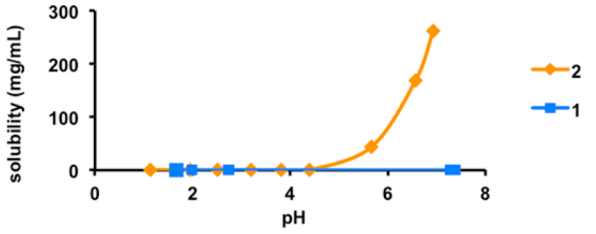


Figure 3. Aqueous solubility of **1** (blue) and phosphate prodrug **2** (orange) as a function of pH.

Table 1. MICs versus Select Pathogens; Gyrase and Topoisomerase K_is[a]

	MIC (μg/mL)				K_i (μM)	
compd	*S. aureus* (29213)	*E. faecalis* (29212)	*E. faecium* (49624)	*S. pneumoniae* (10015)	*S. aureus* gyrase	*S. aureus* topo IV
1	0.016	0.016	0.063	≤0.008	<0.009	0.012
2	8	1	4	0.25	<0.009	0.030

[a]ATCC #s in parentheses.

Despite inhibiting both enzymatic targets, the less potent MICs of **2** indicated that bioconversion to **1** would be necessary for *in vivo* antibacterial activity. The assumption at the outset was that the phosphate promoiety would be cleaved by alkaline phosphatase (AP), which is present in high abundance in both the intestine and the liver.[22] Intravenous administration of the prodrug would rely on AP cleavage in the liver for conversion to the parent. Initially, the phosphate prodrug pharmacokinetics were evaluated in rat; when administered intravenously (1 mg/kg) the prodrug form was rapidly converted to **1**; **2** was below the level of quantitation at 2 h, while the presence of **1** in plasma was detectable at 4 h postdose (Figure 4). Further pharmacokinetic studies in rat,

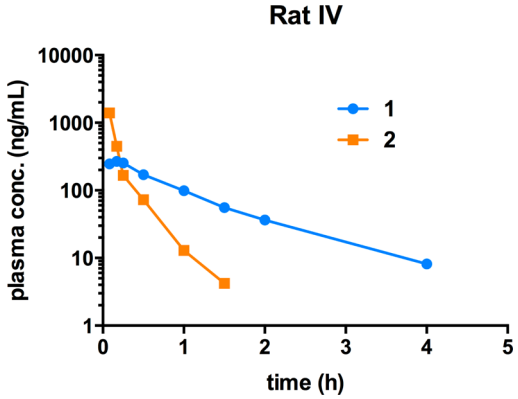


Figure 4. Conversion of prodrug **2** (orange) to **1** (blue) following intravenous administration of **2** in rat (1 mg/kg).

dog, and monkey demonstrated that a similar level of plasma exposure (area under the curve, AUC) to the parent was achievable dosing the prodrug intravenously versus dosing the parent (Figure 5).

While the phosphate prodrug was designed primarily to overcome issues with developing an intravenous formulation of **1**, improving solubility via phosphate prodrugging has been shown to result in improved oral bioavailability.[14,23,24] Additionally, development of a single agent suitable for both oral and IV administration versus developing two drug substances is

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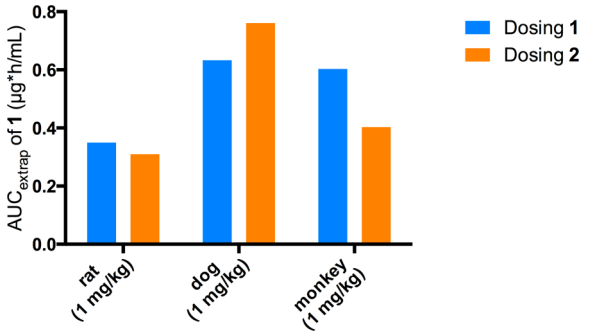

Figure 5. Plasma exposure of the parent **1** administered IV as **1** (blue bar) versus as the prodrug **2** (orange bar) in rat, dog, and monkey (1 mg/kg nominal dose).

preferred. Oral delivery would require AP cleavage prior to intestinal epithelial absorption, the phosphate ester being too polar to be orally bioavailable;[25−27] evaluation of the prodrug in MDCK cells[28] confirmed this to be the case (Table 2). Plasma

Table 2. Permeability of 1 versus Phosphate Ester Prodrug 2 in MDCK Cells

	P_{app} (1×10^{-6} cm/sec) in MDCK wild-type	
	apical-to-basolateral	basolateral-to-apical
1	9.5	16.3
2	not permeable	not permeable

exposure of **1** via oral administration of the phosphate prodrug was determined in rat, dog, and monkey (Figure 6). As

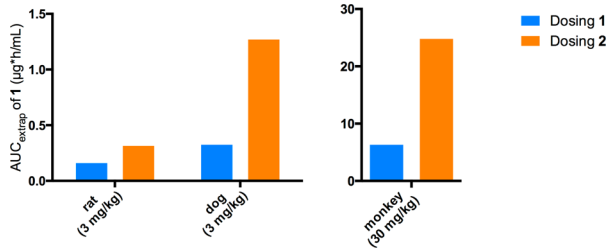

Figure 6. Plasma exposure of the parent **1** administered PO as **1** (blue bar) versus as the prodrug **2** (orange bar) in rat (3 mg/kg), dog (3 mg/kg), and monkey (30 mg/kg).

anticipated minimal exposure of the prodrug was observed when delivered orally (data not shown), improved oral exposure of **1** was observed when dosing the prodrug **2** versus dosing as the parent in rat, dog, and monkey. In the higher species, dog and monkey, greater than 3-fold improvement in exposure was demonstrated dosing the same nominal dose of prodrug versus the parent.

Liver and intestinal S9 fractions were used to evaluate the conversion of phosphate ester prodrug to parent[29] and the likelihood of prodrug conversion in humans. Prodrug **2** was incubated over a concentration range from 1 to 1000 μM in liver and intestinal S9 fractions from rat, monkey, dog, and human. The concentration−time profiles of the parent **1** formed at various concentrations of the prodrug **2** were plotted to give velocity of formation of the parent. The velocity of formation of parent at each prodrug concentration was fitted to the Michaelis−Menten equation to yield kinetic constants (Table 3). Rank order of conversion in liver S9 fractions was rat ≈ human > monkey > dog. The rate of conversion of the prodrug to parent in intestinal S9 fractions was higher. The V_{max} and K_m in human intestinal S9 could not be accurately determined since the velocity of formation of **1** did not saturate over the range of concentrations in the experiment. However, the rate of formation was clearly higher in human and monkey than in rat and dog.

Formulation for both IV and oral delivery was simplified for the prodrug versus **1**. Table 4 shows the formulations used in

Table 4. Comparison of formulations used in PK studies

	IV		PO	
	1	**2**	**1**	**2**
rat	25% DMA/30% PG/ 5% tween	D5W	10% vitamin E-TPGS	0.5% MC
dog	20% captisol	D5W	10% vitamin E-TPGS	0.5% MC
monkey	20% captisol	D5W	20% cavitron/1% HPMC-AS	0.5% MC

the PK studies presented above (Figures 3 and 4). For IV delivery of the prodrug, formulation with 5% dextrose in water (D5W) was sufficient to attain a stable solution. Methylcellulose (MC, 0.5%) was found to be suitable for oral delivery.

An unmet medical need that could potentially be addressed by an agent with the antibacterial spectrum of **1** is nosocomial bacterial pneumonia caused by methicillin-resistant *S. aureus*.[30] The antibacterial efficacy of the prodrug was evaluated in a neutropenic mouse model of lung infection[31] versus two methicillin-resistant *S. aureus* (MRSA) isolates (Figure 7). Neutropenic female CD-1 mice (6 per group) were infected with the *S. aureus* strain of interest; 3 h postchallenge the treatment phase was initiated, dosing **2** at 7.5, 15, and 30 mg/kg BID PO. After 24 h of drug treatment the lungs were evaluated for bacterial burden, as measured by bacterial colony-forming units (CFU). Levofloxacin, a fluoroquinolone used to treat pneumonia in the clinic, served as the control antibacterial and was dosed TID subcutaneously (SC) to approximate clinical dosing. The prodrug showed a dose-dependent decrease in bacterial burden; at 30 mg/kg BID, the prodrug achieved >3 log reduction in CFU. The prodrug **2** is efficacious versus both

Table 3. Michaelis−Menten Parameters for the Formation of 1 from Phosphate Ester Prodrug 2 in Both Liver and Intestine S9 Fractions

	liver		intestine	
	V_{max} (pmol/min/mg)	K_m (μM)	V_{max} (pmol/min/mg)	K_m (μM)
rat	45.5 ± 23	49.8 ± 38	958 ± 111	736 ± 158
dog	19.3 ± 0.66	256 ± 16	1162 ± 387	998 ± 556
monkey	25.2 ± 4.1	122 ± 45	1974 ± 362	5177 ± 1098
human	45.8 ± 16	137 ± 95	*>1000*	ND

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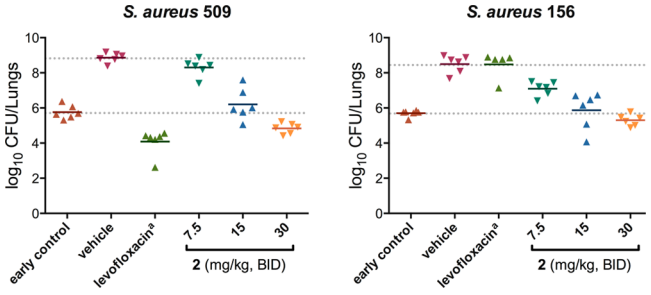

Figure 7. Oral efficacy of prodrug **2** versus two MRSA strains in a mouse pneumonia model. Gray dotted lines indicate mean burden from early control and vehicle treated groups. [a]Dosed 10.6 mg/kg TID SC.

MRSA isolates, including the levofloxacin-resistant strain, *S. aureus* 156. The MICs for **1** and levofloxacin, the control, versus the two methicillin-resistant strains used are shown in Table 5.

Table 5. MICs (μg/mL) of 1 and Levofloxacin versus MRSA Isolates Used in Mouse Pneumonia Model

	1	levofloxacin
S. aureus 509	0.06	0.25
S. aureus 156 (levo^R)	0.06	8

In conclusion, we identified a water-soluble phosphate ester prodrug of a highly potent, yet poorly soluble bacterial gyrase/topoisomerase IV inhibitor, which considerably simplified the development path of this novel antibacterial. The prodrug **2** is several orders of magnitude more water-soluble than the parent **1** at physiological pH; this has enabled the identification of simple formulations suitable for both IV and PO delivery. The phosphate moiety of **2** is rapidly cleaved in rat, dog, and monkey *in vivo* upon both IV and PO administration; it achieves similar or improved plasma exposure compared to dosing the parent **1**. *In vitro* assessment in liver and intestine S9 fractions suggests that the conversion of prodrug **2** to parent **1** would occur upon dosing in human at a similar rate or faster to that observed in the preclinical species. In a mouse model of pneumonia, the prodrug exhibited dose-dependent decrease in bacterial burden upon oral administration to MRSA-challenged mice. The prodrug **2** has been selected as a candidate for further preclinical evaluation. Data will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details for the synthesis of **2** from **1**, *in vitro* ADME, and *in vivo* PK/PD protocols. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00196.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

ADME, absorption, distribution, metabolism, and excretion; AP, alkaline phosphatase; ATCC, American Type Culture Collection; AUC, area under curve; BID, "*bis in die*", twice a day; CFU, colony forming units; DMA, dimethylacetamide; HPMC-AS, hydroxypropyl methylcellulose acetate succinate; IV, intravenous; MDCK, Madin−Darby canine kidney epithelial cell line; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *S. aureus*; PD, pharmacodynamics; PG, propylene glycol; PK, pharmacokinetics; PO, "*per os*", by mouth; SC, subcutaneous; TID, "*ter in die*", three times a day; vitamin E-TPGS, D-α-tocopherol polyethylene glycol 1000 succinate

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