EXPERT OPINION

Evaluation of WO2012177707 and WO2012097269: Vertex's phosphate prodrugs of gyrase and topoisomerase antibacterial agents

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The two patent applications describe two novel compounds in the benzimidazole class of GyrB/ParE antibacterial agents and multiple phosphate prodrugs derived from these compounds. The new benzimidazole compounds have excellent antibacterial activity on Gram-positive strains. But like previous benzimidazoles, they have limited solubility and are highly protein bound. The phosphate prodrugs offer a drug substance with high aqueous solubility that should aid both intravenous and oral formulations. The potential utility of the prodrugs was demonstrated in efficacy studies.

Keywords: antibacterial, DNA gyrase, prodrugs, topoisomerase IV

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1. Introduction

The emergence of bacterial resistance to the major classes of antibacterial drugs is causing significant public health problems [1-3]. To address this challenge, researchers have actively pursued the discovery of new antibacterial classes that act on novel targets for the past two decades [4]. This effort has proven to be difficult and the majority of these programs failed to identify promising candidates [5].

One of the few programs to show progress has been the discovery of novel classes of antibacterial agents that target the GyrB and ParE subunits of bacterial DNA gyrase and topoisomerase IV [6]. Bacterial DNA gyrase and topoisomerase IV both form heterodimeric complexes and are validated antibacterial targets inhibited by the fluoroquinolone (FQ) antibiotics [7]. The GyrB/ParE ATPase sites are distal from the FQ-binding site and their value as novel antibacterial targets has long been appreciated. Inhibition of GyrB is the mechanism of action of the natural product, novobiocin, discovered in the 1950s [8]. Roche in the late 90s pioneered the use of structure-based drug design (SBDD) to engineer GyrB-based antibacterial agents [9,10]. This effort encouraged other groups to target GyrB using SBDD [11].

In 2001, the first Vertex patent application disclosing the benzimidazole series of antibacterial agents was published [12]. Details of the potency of the two lead compounds in this series, VRT-125853 and VRT-752586, were published [13-15]. These compounds are notable for their high enzymatic and antibacterial potency on Gram-positive strains (VRT-752586 Minimum Inhibitory Concentration (MIC) values on *Staphylococcus aureus* range between 0.06 and 0.25 µg/ml). In addition, the Vertex team designed these compounds to inhibit both GyrB and ParE, a feature missing in previous bacterial gyrase inhibitors. This design strategy led to compounds with a low frequency for the emergence of new resistance [16].

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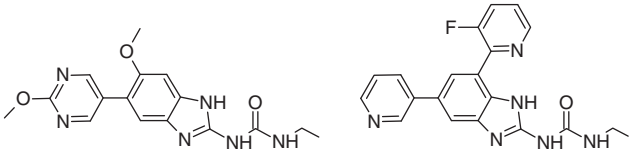
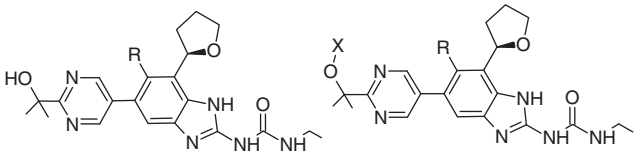
Despite impressive antibacterial potency, compounds like VRT-752586 suffer from high protein binding and presumably low aqueous solubility. The high protein binding results in significant increases in the MIC values when the compounds are tested in the presence of serum. For example, VRT-752586 has its MIC value against *S. aureus* increased by 16-fold in the presence of 50% human serum. The two recent patent applications that are the subject of this report, WO2012/177707 and WO2012/097269, detail new antibacterial members of the benzimidazaole series Formula I [17,18]. While these compounds contain the potentially solubilizing hydroxyl and cyclic ether functionalities, the main advantage of these new compounds is the ability to be converted to phosphate prodrugs that have high aqueous solubility and potentially advantageous pharmacokinetics.



VRT-125853 **VRT-752586**



Formula I

12 R = H
23 R = F

R = H, F;
X = H, PO_3^{-2}, PO_3H_2, $PO(O^-)OR'$

2. Chemistry

2.1 WO2012097269

Claims compounds of Formula I where:

R is hydrogen or fluorine;

X is hydrogen, $-PO(OH)_2$; $-PO(OH)O^-M^+$, $-PO(O^-)_2{*}2M^+$, $-PO(O^-)_2{*}D^{2+}$;

M^+ is a pharmaceutical acceptable monovalent cation; and

D^{2+} is a pharmaceutical acceptable divalent cation; or a pharmaceutically acceptable salt.

The application describes the synthesis of two novel benzimidazoles (Formula I R is H or F, and X is H) that have good antibacterial activity. These compounds were prepared as racemates or as optical isomers of high enantiomeric purity. The resolution was accomplished by chromatography on a column packed with a chiral stationary phase (CHIRALPAK®IC®) to provide the more active (R) isomer. **Scheme 1** shows the preparation of compounds **13** and **23A** and their conversion to the phosphate prodrugs **1B** and **W**. In this scheme, the chiral resolution is conducted at an early stage with the 2-aryl-tetrahydrofuran intermediate. Experimental details also demonstrate that the stereoisomers of the final benzimidazole antibacterial agent (the racemic versions of **12** and **23**) can be separated on this column.

2.2 WO2012177707

Claims phosphate diesters of Formula I where:

R is fluorine;

X is $-PO(OH)-OR^1$, $-PO(O^-M^+)-OR^1$;

M^+ is a pharmaceutically acceptable monovalent cation; and R^1 is (C_1-C_{20})-alkyl, (C_2-C_{20})-alkenyl, $(CH_2CH_2O)_nCH_3$, or $CH_2CH_2R^2$, where n is an integer 1 – 5 and the key R^2 group is a 5 – 6-membered heterocyclic aliphatic ring system; such as a morpholine.

The application describes the synthesis of three phosphate diester prodrugs. **Scheme 2** shows the preparation of these three compounds (**X**, **Y**, and **Z**) from compound **23**.

3. Biology

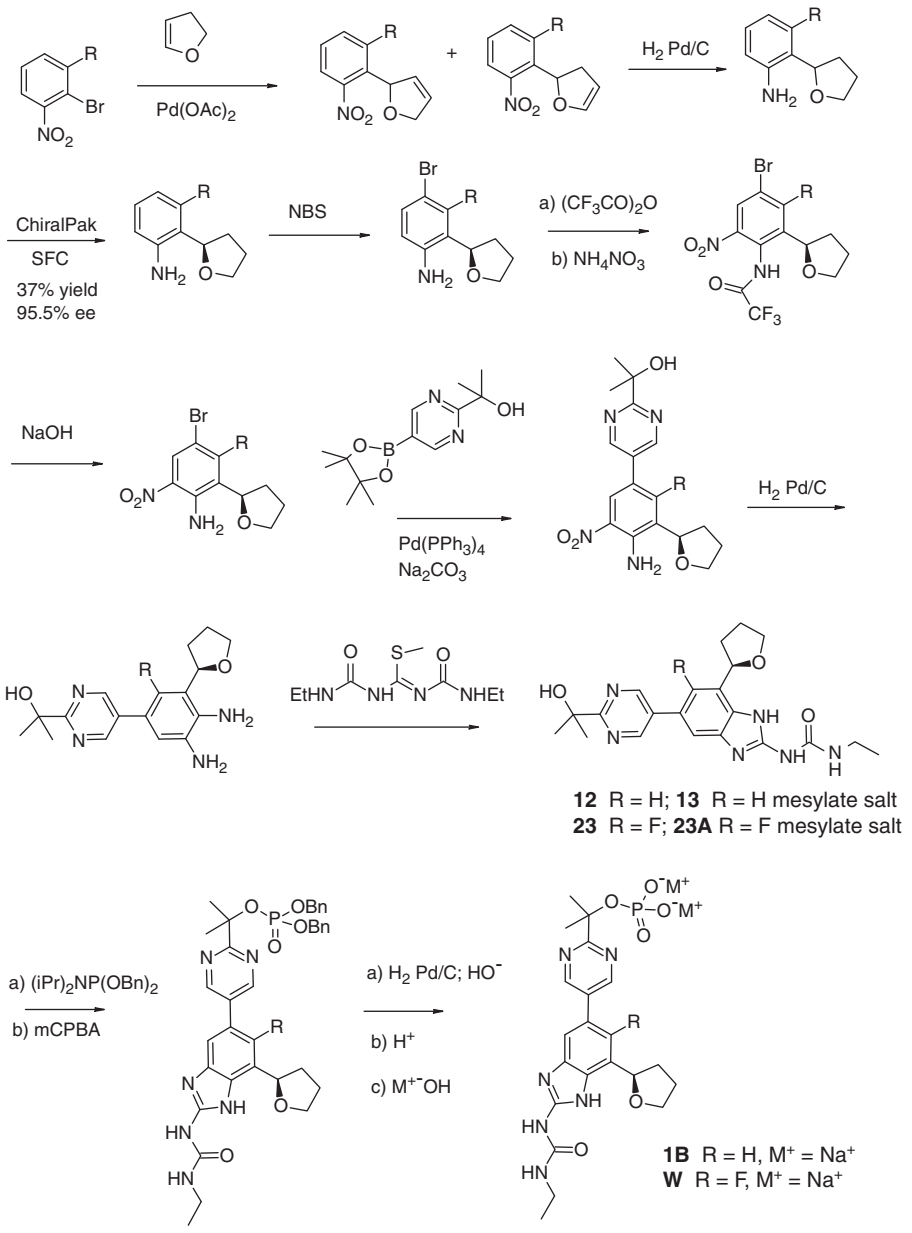
The WO2012/097269 application provides extensive details regarding the *in vitro* and *in vivo* activities of the lead compounds. This includes enzymology, microbiology, pharmacokinetics, efficacy, and safety.

The two compounds have similar antibacterial spectrum with activity concentrated on Gram-positive strains. Data are provided on the antibacterial activity of **13** and **23A** against both individual strains and modest size MIC_{90} panels. **Table 1** lists the antibacterial potency against some of the key strains in these panels. In general, the fluorinated analog **23A** is 4 – 8-fold more potent than compound **13**. While there is limited spectrum on Gram-negative strains, compound **23A** is active on the respiratory tract pathogens *Haemophilus influenzae* and *Moraxella catarrhalis.* The MIC values for **23A** against *S. aureus* (ATCC 29213) are shifted eightfold in 50% human serum, indicating extensive protein binding.

The alcohols **13** and **23A** and the prodrug **W** are efficacious in multiple animal infection models when given orally. For example, compound **W** gave a 2.25 log cfu reduction (vs control) at 10 mg/kg in the *S. aureus* rat kidney infection model. The performance of **13** and **23A** were similar indicating that there is some bioavailability for both the prodrug and the active moiety.

Compound **23A** was well tolerated in toxicity studies. Oral administration of **23A** to rats for 7 days (twice daily dosing) demonstrated an NOAEL of 600 mg/kg/day (the highest dose tested). Oral administration of **23A** to monkeys for 7 days (once-daily dosing) demonstrated an NOAEL of 200 mg/kg/day (the highest dose tested). No data are given for the tolerability of the prodrug **W**.

PK studies demonstrate that compound **W** is rapidly converted to **23A** when administered either orally or intravenously. Oral bioavailability appears to be high (> 80%) in monkey but not in rat. No data were given for the PK



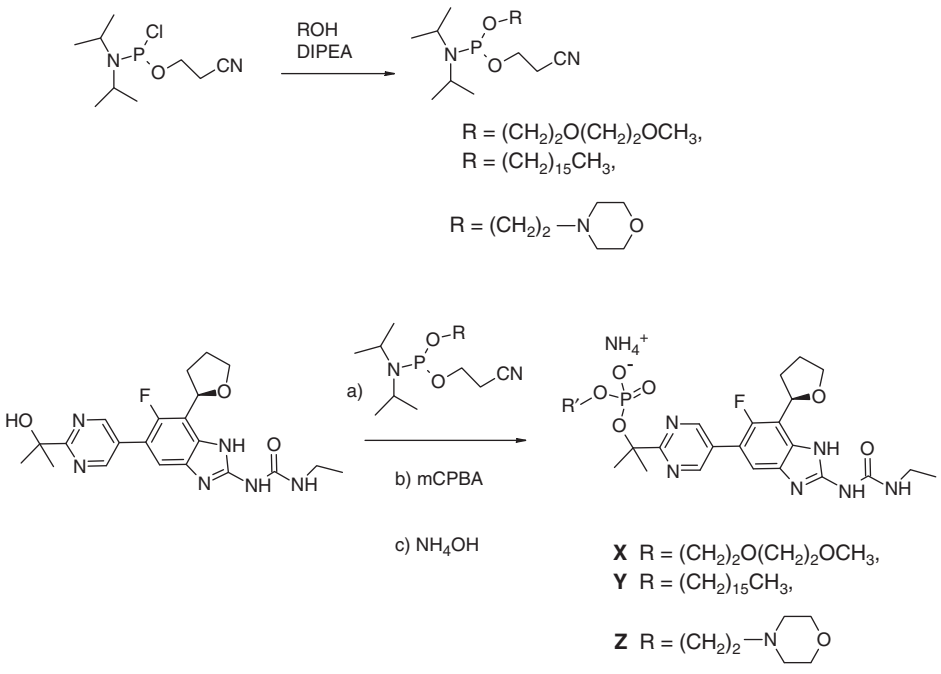
Scheme 1. Preparation of the active antibacterial agents **13** and **23A** and prodrugs **1B** and **W**.

of **23A** (the compound used in the toxicity studies) when dosed as the parent not the prodrug.

The prodrug strategy resulted in significantly greater solubility. **Table 2** lists the solubility of the different compounds from both patent applications.

4. Expert opinion

It is likely that the poor solubility of the initial benzimidazole leads like VRT-752586 led to problems developing either an intravenous or oral formulation. While the solubility of the new benzimidazole **23** still appears to be low (reported at < 1 μg/ml), the prodrug strategy may have addressed the problem of developing effective formulations. There is ample precedent for phosphate prodrugs providing a soluble drug substance when the parent molecule has low solubility [19-21]. The PK data show that rats dosed with **W** rapidly cleave the phosphate and generate the parent **23** in concentrations well above the MIC values (C_{max} of 16 μg/ml when **W** is given orally at 300 mg/kg and a C_{max} of 9 μg/ml when **W** is given intravenously at 5 mg/kg). The fact that prodrug **W** demonstrated efficacy in animal models suggests that even with significant protein binding, there is



Scheme 2. Preparation of phosphate diesters X, Y, and Z.

Table 1. Antibacterial potency of 13 and 23A against important bacterial pathogens.

		13		23A	
Bacteria	**N**	**MIC range (μg/ml)**	**MIC_{90} (μg/ml)**	**MIC range (μg/ml)**	**MIC_{90} (μg/ml)**
Staphylococcus aureus	67	0.03 – 0.5	0.25	0.008 – 0.06	0.03
Enterococcus faecalis	34	0.03 – 0.25	0.25	0.015 – 0.12	0.06
Streptococcus pneumoniae	67	0.015 – 0.06	0.06	0.008 – 0.03	0.015
Haemophilus influenzae	55	0.25 – 8	2	0.06 – 2	1
Moraxella catarrhalis	26	0.015 – 0.12	0.12	0.004 – 0.03	0.03
Escherichia coli	12	> 8 – > 8	> 8	2 – > 8	> 8
Klebsiella pneumoniae	12	> 8 – > 8	> 8	2 – > 8	> 8
Acinetobacter baumannii	12	> 8 – > 8	> 8	4 – > 8	> 8
Pseudomonas aeruginosa	12	> 8 – > 8	> 8	> 8– > 8	> 8

Table 2. Compound solubility.

Compound	**Solid form**	**pH**	**Solubility (mg/ml)**
23	Crystalline	> 3.0	< 0.001
W	Crystalline	4.39	0.25
X	Crystalline	4.41	12.74
Y	Crystalline	3.01	< 0.001
Z	Amorphous	4.35	> 41

enough **23** free drug concentration to be effective. Additional filings around compound W (a process patent and a solid form) indicate that Vertex has seriously considered advancing W into clinical trials [22,23].

The second prodrug application is interesting. In contrast to phosphate monoesters that are rapidly cleaved by phosphatases *in vivo*, phosphate diesters convert much more slowly. Thus, prodrugs X, Y, and Z are expected to result in a lower C_{max} at a given dose (either intravenously or orally) while still generating a similar area under the curve to that of prodrug W. These slow-release prodrugs could be very useful especially when toxicity is driven by C_{max} (which is likely for solubility-limited compounds). Of the three diester prodrugs, the polyether- and amine-containing prodrugs X and Z have useful aqueous solubility.

Overall, these applications indicate that the Vertex team has made significant progress. Currently, the only drug class that is active on drug-resistant Gram-positive strains like methicillin-resistant *S. aureus* available in intravenous and

oral formulations are the oxazolidinones. Advancement of a benzimidazole drug would provide clinicians with a new drug class for resistant Gram-positive infections and would be a valuable addition to antibacterial therapy.

Declaration of interest

The author states no conflict of interest and has received no payment in preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Arias C, Murray B. Antibiotic-resistant bugs in the 21st century – a clinical super challenge. N Engl J Med 2009;360:439-43
2. Klein E, Smith D, Laxminarayan R. Hospitalizations and deaths caused by methicillin-resistant Staphylococcus aureus, United States 1999-2005. Emerg Infect Dis 2007;13:1840-6
3. Boucher H, Talbot G, Bradley J, et al. Bad bugs, no drugs: no ESKAPE! An update from the infectious diseases society of America. Clin Infect Dis 2009;48:1-12
4. Silver LL. Challenges of antibacterial discovery. Clin Microbiol Rev 2011;24:71-109
5. Payne D, Gwynn M, Holmes D, et al. Drugs for bad bugs: confronting the challenges of antibacterial discovery. Nat Rev Drug Dis 2007;6:29-40
6. Collin F, Karkare S, Maxwell A. Exploiting bacterial DNA gyrase as a drug target: current state and perspectives. Appl Microbiol Biotechnol 2011;92:479-97
   • **A review DNA gyrase as an antibacterial target**
7. Sissi C, Palumbo M. In front of and behind the replication fork: bacterial type IIa topoisomerases. Cell Mol Life Sci 2010;67:2001-24
8. Lambert HP, O'Grady FW. editors. Coumarins. Churchill Livingstone; Edinburgh: 1992
9. Boehm H, Boehringer M, Bur D, et al. Novel inhibitors of DNA gyrase: 3D structure biased needle screening hit validation by biophysical methods, and 3D guided optimization. A promising alternative to random screening. J Med Chem 2000;43:2664-74
   • **One of the first applications of fragment screening in drug discovery**
10. Lubbers T, Angehrn P, Gmunder H, et al. Design, synthesis and structure-activity relationship studies of ATP analogs as DNA gyrase inhibitors. Bioorg Med Chem Lett 2000;10:821-6
11. Oblak M, Kotnik M, Solmajer T. Discovery and development of ATPase inhibitors of DNA gyrase as antibacterial agents. Curr Med Chem 2007;14:2033-47
12. Vertex Pharmaceuticals.Gyrase. Inhibitors and Uses Thereof 2012. WO2002060879
13. Mani N, Gross C, Parsons J, et al. In vitro characterization of the antibacterial spectrum of novel bacterial type II topoisomerase inhibitors of the aminobenzimidazole class. Antimicrob Agents Chemother 2006;50:1228-37
   •• **A paper that details the antibacterial properties of the benzimidazole series.**
14. Grossman T, Bartels D, Mullin S, et al. Dual targeting of GyrB and ParE by a novel Aminobenzimidazole class of antibacterial compounds. Antimicrob Agents Chemother 2007;51:657-66
   • **A paper that details the advantages of a dual-targeting antibacterial agent.**
15. Charifson P, Grillot A, Grossman T, et al. Novel dual-targeting benzimidazole urea inhibitors of DNA gyrase and topoisomerase IV possessing potent antibacterial activity: intelligent design and evolution through the judicious use of structure-guided design and structure-activity relationships. J Med Chem 2008;51:5243-63
16. Silver L. Multi-targeting by monotherapeutic antibacterials. Nat Rev Drug Dis 2007;6:41-55
17. Vertex Pharmaceuticals. Pyrimidine gyrase and topoisomerase IV inhibitors 2012. WO2012097269
18. Vertex Pharmaceuticals. Phosphate esters of gyrase and topoisomerase inhibitors 2012. WO2012177707
19. Rautio J, Kumpulainen H, Heimbach T, et al. Prodrugs: design and clinical applications. Nat Rev Drug Dis 2008;7:255-70
   • **A prodrug review.**
20. Im WB, Choi SH, Park JY, et al. Discovery of torezolid as a novel 5-hydroxymethyl-oxazolidinone antibacterial agent. Eur J Med Chem 2011;46:1027-39
   • **An example of a phosphate prodrug strategy is used to provide a soluble drug substance for an oxazolidinone antibacterial agent of limited solubility.**
21. Baker W, Cai S, Dimitroff M, et al. A prodrug approach toward the development of water soluble fluoroquinolones and structure-activity relationships of quinoline-3-carboxylic acids. J Med Chem 2004;47:4693-709
   • **An example of a phosphate prodrug strategy is used to provide a soluble drug substance for an fluoroquinolone antibacterial agent of limited solubility.**
22. Vertex Pharmaceuticals. Process for making gyrase and topoisomerase IV inhibitors 2012. WO2012097274
23. Vertex Pharmaceuticals. Solid forms of gyrase inhibitor (R)-1-ethyl-3-[6-fluoro-5-[2-(1-hydroxy-1-methyl-ethyl) pyrimidin-5-yl] -7-(tetrahydrofuran-2-yl)-1H-benzimidazol-2-yl]urea 2012. WO2012097273

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