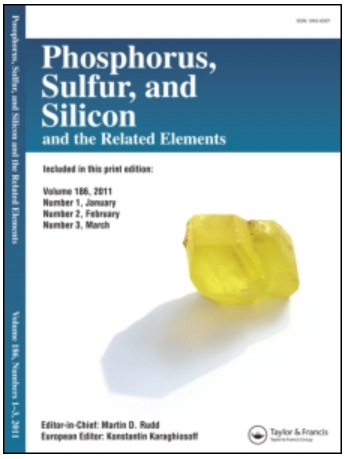


Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

The Synthesis and Biological Evaluation of Benzamide Riboside and Its Phosphordiamidates Prodrugs

Jianning Zhou[ab]; Chunyan Tan[a]; Nan Zhang[a]; Jian Fan[a]; Chun Guo[b]; Yuyang Jiang[ac]
[a] The Key Laboratory of Chemical Biology, Guangdong Province, The Graduate School at Shenzhen, Tsinghua University, Shenzhen, China [b] School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang, China [c] School of Medicine, Tsinghua University, Belijing, China

To cite this Article Zhou, Jianning , Tan, Chunyan , Zhang, Nan , Fan, Jian , Guo, Chun and Jiang, Yuyang(2008) 'The Synthesis and Biological Evaluation of Benzamide Riboside and Its Phosphordiamidates Prodrugs', Phosphorus, Sulfur, and Silicon and the Related Elements, 183: 2, 787 — 790
To link to this Article: DOI: 10.1080/10426500701808135
URL: http://dx.doi.org/10.1080/10426500701808135

Phosphorus, Sulfur, and Silicon, 183:787–790, 2008

ISSN: 1042-6507 print / 1563-5325 online
DOI: 10.1080/10426500701808135

Taylor & Francis
Taylor & Francis Group

The Synthesis and Biological Evaluation of Benzamide Riboside and Its Phosphordiamidates Prodrugs

Jianning Zhou,[1,2] Chunyan Tan,[1] Nan Zhang,[1] Jian Fan,[1] Chun Guo,[2] and Yuyang Jiang[1,3]
[1]The Key Laboratory of Chemical Biology, Guangdong Province, The Graduate School at Shenzhen, Tsinghua University, Shenzhen, China
[2]School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang, China
[3]School of Medicine, Tsinghua University, Belijing, China

*In order to overcome the drug resistance and enhance the membrane penetration ability of benzamide riboside [BR, (1-β-D-ribofuranosyl) benzene-3-carboxamide], which is a novel C-glycoside analogue of nicotinamide riboside and has excellent cytotoxic activity, we designed and synthesized two phosphordiamidates (**1a** and* **1b***) as prodrugs to deliver phosphorylated BR into cells. However, the bioactivity evaluation shows that **1a and 1b** have lower biological activity (IC_{50} > 200 μM and 173 μM, respectively) compared to BR. This might be due to the fact that **1a and 1b** could only serve as the BR depot form and thus could not be metabolized to the phosporylatd benzamide riboside.*

Keywords Benzamide riboside prodrug; benzylphosphordiamidate

Nucleosides and nucleotides have demonstrated excellent antiviral and anticancer activities. However, the application of nucleosides and nucleotides as therapeutics has been limited due to their disadvantages including poor cell penetration and poor stability in the extracellular medium. Thus, the development of the prodrug methodology has been proposed to overcome these hurdles and achieve the *in vivo* delivery of these types of drugs. Benzamide riboside [BR, (1-β-*D*-ribofuranosyl) benzene-3-carboxamide], a novel *C*-glycoside analogue of nicotinamide riboside, has been studied because of its excellent cytotoxic activity against a diverse group of human tumor cells, specifically more active

The authors gratefully acknowledge the National Natural Science Foundation of China (NSFC20132020, 20572060) and National Department of Science and Technology (2005CCA03400) for financial support of this work.

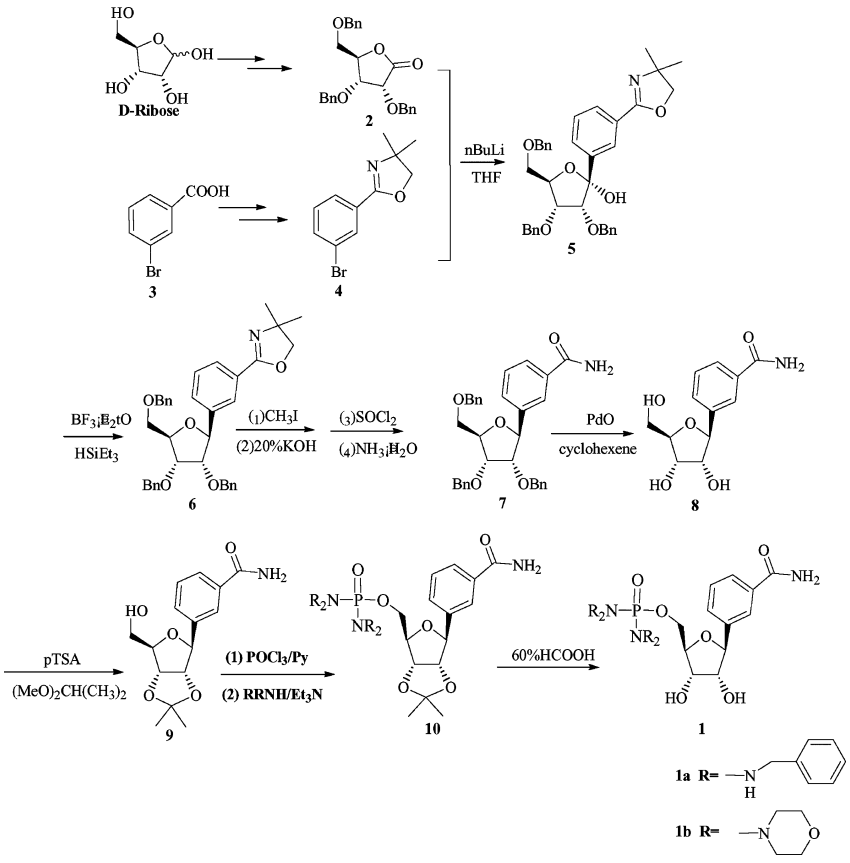
Address correspondence to Dr. Yu-yang Jiang, The Key Laboratory of Chemical Biology, Guangdong Province, The Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, China. E-mail: jiangyy@sz.tsinghua.edu.cn

in sarcomas and central nervous system (CNS) neoplasms compared to tiazofurin (TR) or selenazofurin (SR).[1] BR shares similar mechanism of action with TR and SR, and is metabolized intracellularly in malignant cells to its active metabolite benzamide adenine dinucleotide (BAD), which inhibits the key enzyme of guanylate biosynthesis inosine 5′-monophosphate dehydrogenase (IMPDH), resulting in the inhibition of cell proliferation. The cytotoxicity and biochemical activity of BR shows that it is a promising candidate for a phase I clinical study.[2] The ideal prodrug design for BR should principally meet the following three requirements: (1) penetrate the cell membrane intact, (2) not require enzymatic activation, and (3) generate BR-55′-phoaphate *in situ*.[3,4] In the present study, we reported the synthesis and bioactivity evaluation of two recently synthesized BR prodrugs.

The synthesis of BR has been discussed in literature.[5,6] In our study, BR was synthesized using an improved synthetic method starting from a commercially available chemical D-ribose.[7] The key intermediate 2,3,5-O-benzyl-D-ribonolactone **2** was obtained through a straightforward 4-step synthesis with an overall yield at about 45%. The coupling reaction took place between the intermediate ribonolactone **2** and bromo-oxazoline **4,** which was synthesized through a 3-step route with an overall yield at about 75% starting from 3-bromobenzoic acid. Followed by stereoselective reduction, cleavage of the oxazoline group, and hydrolysis the benzamide tribenzyl ether **7** was obtained, which was then deprotected using PdO/cyclohexene to give the product BR **8**. The total yield is about 45%.

Because phosphorodiamidates are neutral compounds capable of entering cells by passive diffusion, they have been used as potential pronucleotides.[4,8] A series of 5′-phosphorodiamidates of FUdR were synthesized and evaluated for their ability to inhibit the growth of murine leukemia L5178Y cells.[4] For example, it was reported that phosphorodiimidazolate of FUdR had a half-life of 12 h and exhibited only modest growth inhibition potency (16% growth inhibition at 10^{-8} M).[4]

In our study, we designed and synthesized two phosphorodiamidate BR prodrugs **1a** and **1b**. In the synthesis route, 2′, 3′-hydroxy groups of BR **8** were first protected to make the phosphorylation step focusly on 5′-hydroxy of BR. Propylidene was chosen as the protecting group[9,10] by using p-toluene sulfonic acid and dimethoxyl propane as catalysts and acetone as the solvent. This method can preferentially protect two neighbouring hydroxy groups. After the selective hydroxy group protection, 5′-hydroxy group on compound **9** was phosphorylated by POCl3 under basic condition to give BR-5′-phosphorodichloridate. Without further isolation and purification, the reaction mixture was then reacted with the desire amine (benzylamine and morpholine) followed by the



Scheme 1 The synthesis of target molecules **1a** and **1b.**

deprotection of the propylidene protecting group under weak acidic condition to give the target compounds **1a** and **1b**. The synthetic route is shown in Scheme 1.

Compounds **1a** and **1b** were evaluated for the cell growth inhibition against K562 cells using the MTT assay and results are shown in Figure 1. The inhibition rate (IR%) was calculated using such formula as IR% = (ODcontrol–ODsample)/ODcontrol × 100%. Cisplatin was used as the positive control which has the IR% at 60.7 ± 5.38% when the concentration is about 50 μg/mL. Unfortunately, results show that both **1a** and **1b** have lower biological activity compared to BR. For example, our data suggest that at the drug concentration of 10 μM, the values of IR% was 71.28 ± 1.6% for BR, whereas the values were only 1.76 ± 2.6% and 11.05 ± 2.3% for **1a** and **1b**, respectively. The

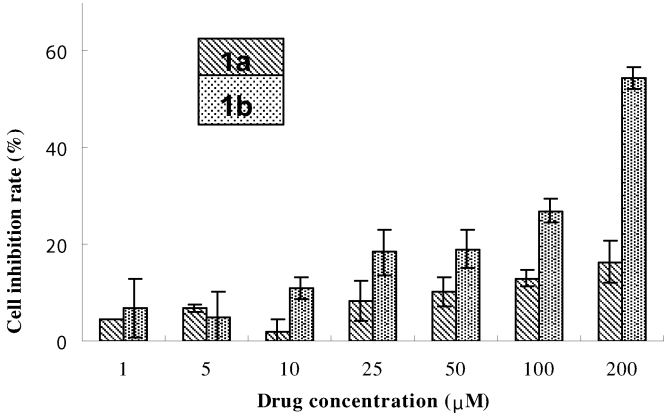


Figure 1 Cell growth inhibition by phosphorodiamidate BR prodrugs **1a** and **1b**.

corresponding IC_{50} values were calculated as greater than 200 μM for **1a** and 173 μM for **1b**. This suggests that **1a** and **1b** could only serve as the BR depot form and could not be metabolized to the monophosphorylatd benzamide riboside. Therefore the more labile linking group would be needed for this structural modification and the study was under investigation.

In conclusion, two phosphordiamidates (**1a** and **1b**) as prodrugs to deliver phosphorylated BR into cells were designed and synthesized. However, the bioactivity evaluation shows that **1a** and **1b** have lower biological activity ($IC_{50} > 200$ μM and 173 μM, respectively) compared to BR.

REFERENCES

[1] H. N. Jayaram, K. Gharehbdghi, and J. Rieser, *Biochem. Biophys. Res. Commun.*, **3,** 1600 (1992).
[2] K. Gharehbaghi, W. Grünberger, and H. N. Jayaram, *Curr. Med. Chem.*, **7,** 743 (2002).
[3] C. R. Wagner, V. V. Iyer, and E. J. Mcintee, *Med. Res. Rev.*, **6,** 417 (2000).
[4] M. E. Phleps, *J. Med. Chem*., **23,** 1229 (1980).
[5] K. Krohn, H. Heins, and K. Wielckens, *J. Med. Chem*., **35,** 511 (1992).
[6] R. Barker and H. G. Fletcher, *J. Org. Chem*., **26,** 4605 (1961).
[7] J. Zhou, C. Tan, N. Zhang, et. al., submitted.
[8] A. Simoncsits and J. Tomasz, *Nucleic Acids Res.*, **2**, 1223 (1975).
[9] P. Taiho, M. T. Eric, and N. Shoji, *J. Am. Chem. Soc*., **1,** 18133 (2005).
[10] T. Moriguchi, N. Asai, and K. Okada, *J. Org. Chem*., **67,** 3290 (2002).