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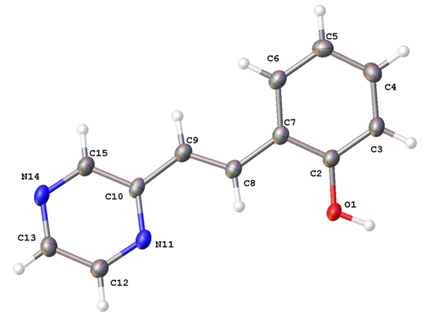
A Phosphate Prodrug of Pyrazinib: Improving Solubility and Antiproliferative Activity



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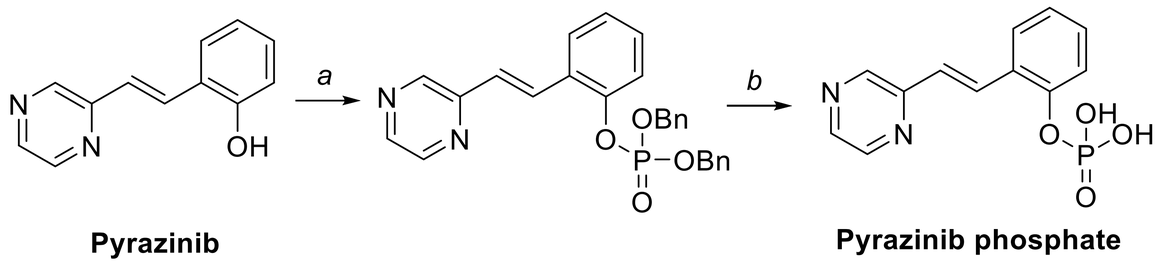
INTRODUCTION

Pyrazinib [(E)-2-(2-(pyrazin-2-yl)vinyl)phenol, *Scheme 1*] is a novel small molecule with anti-angiogenic and anti-metabolic activity. It enhances radiosensitivity in models of esophageal adenocarcinoma. Despite its promising activity, pyrazinib is limited by extremely poor aqueous solubility. To overcome this, we designed a phosphate prodrug of pyrazinib. Herein, we describe the following for pyrazinib phosphate:

Synthesis → Physiochemical Properties → Enzymatic Hydrolysis → Antiproliferative Activity

SYNTHESIS OF PYRAZINIB PHOSPHATE



Scheme 1. Synthesis of pyrazinib phosphate from pyrazinib. Reagents and conditions: (*a*) Dibenzylphosphite (1.4 eq.), CCl_4 (3 eq.), DMAP (0.1 eq.), DIPEA (2 eq.), anhyd. ACN, under N_2, 60% yield; (*b*) BBr_3 (1 eq), anhydrous toluene, -10°C to 80°C, 2 hr, 40% yield.

SOLUBILITY and LogP MEASUREMENTS

Solubility of pyrazinib and pyrazinib phosphate was measured by the shake-flask method. Pyrazinib phosphate improved the solubility of pyrazinib almost 600-fold. The measured logP values confirm the improved hydrophilicity of the phosphate prodrug of pyrazinib (*Table 1*).

Compound	Solubility (µg/mL)	LogP
Pyrazinib	0.26	1.55
Pyrazinib phosphate	155	-2.34

Table 1. Experimentally determined solubility and logP values for pyrazinib and pyrazinib phosphate. Solubility was determined by the shake-flask method for 24 hr at 20 °C. LogP was determined by HPLC by calculation of the capacity factor in comparison to standards of known logP [adenine (-0.45), colchicine (1.14), glipizide (1.91), indole (2.14), and naproxen (2.78)].

ENZYMATIC HYDROLYSIS BY ALKALINE PHOSPHATASE

The cleavage of the phosphate moiety of pyrazinib phosphate by the alkaline phosphatase enzyme was demonstrated at two different concentrations at 37 °C (*Figure 1A and B*).

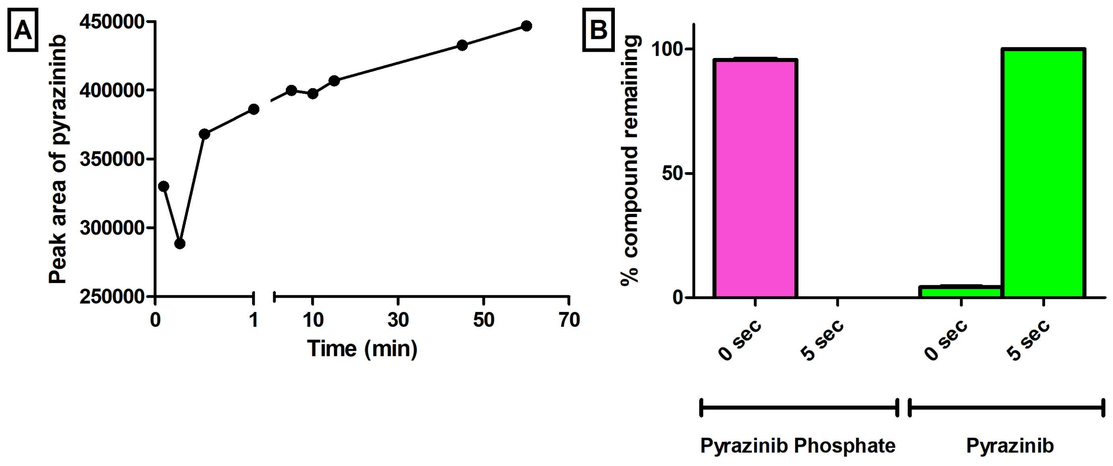


Figure 1. Conversion of pyrazinib phosphate to pyrazinib by alkaline phosphatase at 37 °C. Alkaline phosphatase (5 mL; 25 DEA units) was added to pyrazinib phosphate [**A.** 0.3 mM (10 mL) or **B.** 0.3 mM (5 mL)]. Samples were withdrawn at the indicated timepoints and reaction was terminated with a stop solution.

STABILITY AT DIFFERENT pH VALUES

The stability of pyrazinib and pyrazinib phosphate were determined in phosphate buffers of pH 4, 7.4 and 9. Both compounds were stable at all three pH values until at least 3 weeks (*data for 24 hr and 7 days shown in Figure 2*).

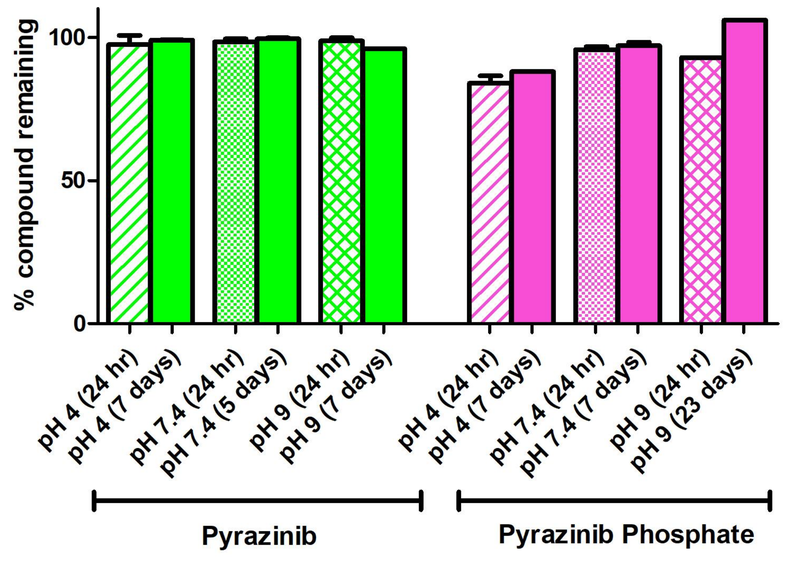


Figure 2. Stability of pyrazinib and pyrazinib phosphate at 24 hr and 7 days in phosphate buffers BP at pH values of 4, 7.4 and 9. Two exceptions noted (pyrazinib pH 7.4 measured at 5 days and pyrazinib phosphate at pH 9 measured at 23 days due to Covid-restricted lab access).

ANTIPROLIFERATIVE ACTIVITY

Pyrazinib phosphate was evaluated alongside pyrazinib in MCF-7 breast cancer cells (*Figure 3*). Pyrazinib phosphate (pink) had significantly more potent antiproliferative activity than pyrazinib (green) at concentrations of 10 and 100 µM. This indicates that the improved solubility increases the availability of drug in cells, but this remains to be determined.

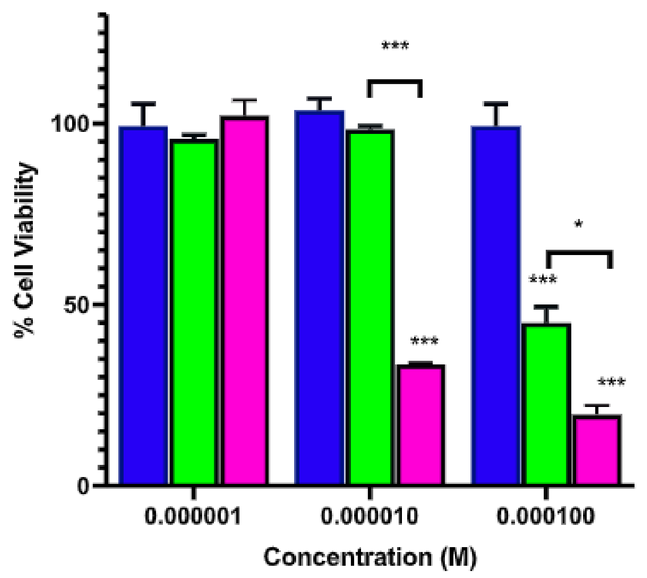


Figure 3: Antiproliferative activity of pyrazinib phosphate (pink) compared to pyrazinib (green) in MCF-7 breast cancer cells. Vehicle control was DMSO (blue). Statistical analysis was performed using multiple t-test comparison test corrected for multiple comparisons using the Holm-Sidak method. Data is presented as mean ± SEM; n=3.

SUMMARY AND OUTLOOK

Pyrazinib phosphate appears to be a promising replacement for pyrazinib, with the phosphate moiety contributing to improved aqueous solubility and a lower logP value. Pyrazinib is rapidly regenerated from the phosphate prodrug in the presence of alkaline phosphatase enzyme. Pyrazinib phosphate has improved antiproliferative activity in MCF-7 breast cancer cells compared to pyrazinib. Further effects, including effects on radiosensitization, remain to be determined.

References: *1. Buckley, AM et al. Sci Rep 10, 12105 (2020); 2. Buckley AM et al. Cancer Lett. 447:115-129 (2019).*

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