Lead Optimization of Prodrug PABA/NO: Targeting the Drug to Cancer Cells

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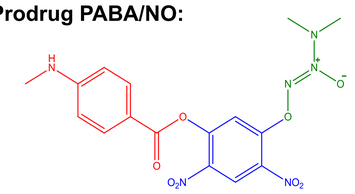
1. Introduction

Prodrug PABA/NO:



dimethylamino diazeniumdiolate (DMA/NO)

- Arylated diazeniumdiolate
- Specifically designed for targeted NO delivery

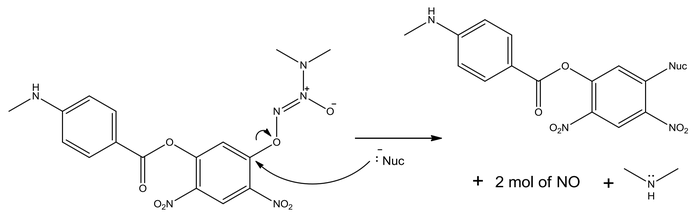
N-methyl-p-aminobenzoic acid (PABA)

dinitrobenzene ring

PABA/NO

(Saavedra, Joseph E. et al., *J. Med. Chem.* 2006:49, 1157-64.)

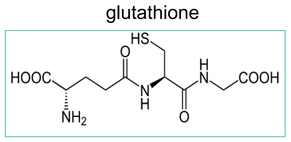
Activation of PABA/NO for NO release:



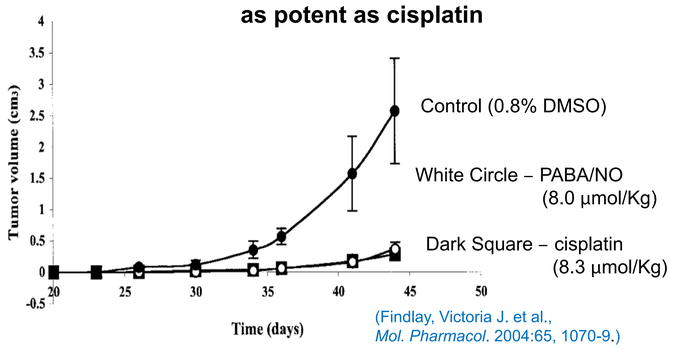
Targeted delivery of NO:

- Co-substrate: glutathione (GSH)
- Catalyst: glutathione S-transferase (GST)
- Many isoforms of GST, including GSTα, π, and μ.
- Overexpression of GSTπ in cancer cells

glutathione

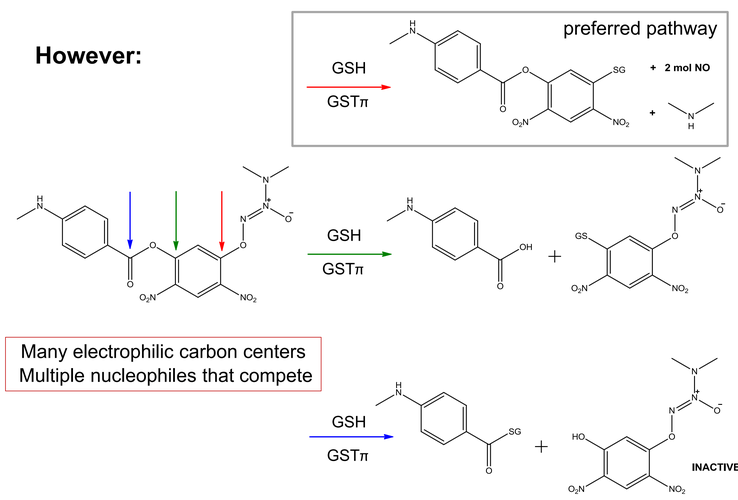


A270 human ovarian xenograft: as potent as cisplatin



(Findlay, Victoria J. et al., *Mol. Pharmacol.* 2004:65, 1070-9.)

However:



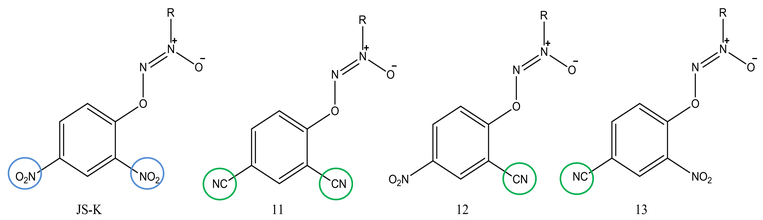
Objectives:

Overcome the various negative aspects of PABA/NO.

1. Decrease the rate of hydrolysis. (striving for $t_{1/2}$ >> 25 min in phosphate buffer, pH = 7.4 at 37 °C)
2. Increase the catalytic efficiency of GSTπ.
3. Achieve solubility > 3 μM in phosphate buffer.

2. Hypothesis

As the introduction of "ortho-CN" to a structurally similar drug, JS-K, showed an enhancement in stability without sacrificing drug efficacy, we hypothesize that "Cyano-PABA/NO" may also exhibit a similar trend.



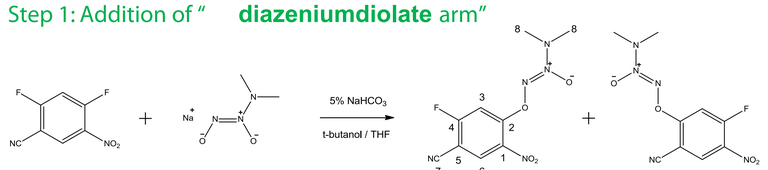
	JS-K	11	12	13
$t_{1/2}$(min)	**2.8**	**918.0**	**33.6**	**22.1**
IC_{50} (μM)	1.08	> 20.00	0.55	0.73

H1703 human NSCLC line — 4 mM GSH medium

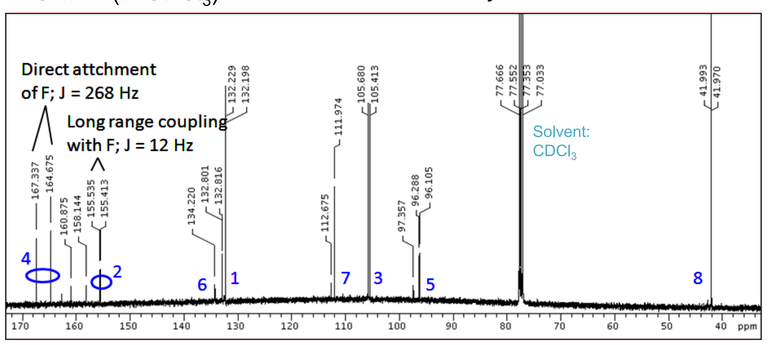
(Nandurdikar, Rahul S. et al., *Bioorg. Med. Chem.* 2012:20(9), 3094-39.)

3. Synthesis of Cyano-PABA/NO

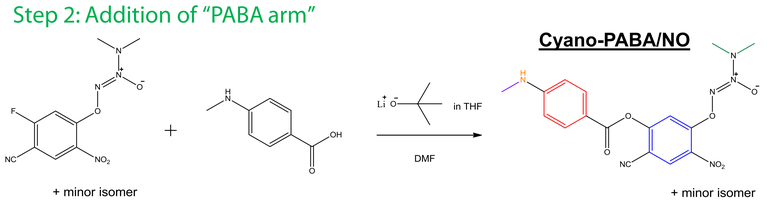
Step 1: Addition of " **diazeniumdiolate** arm"

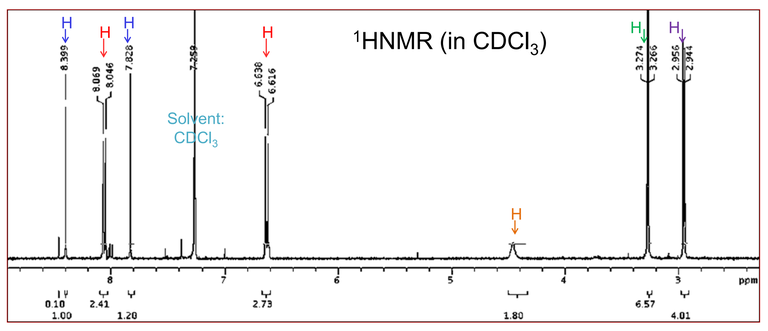


^{13}CNMR (in $CDCl_3$)



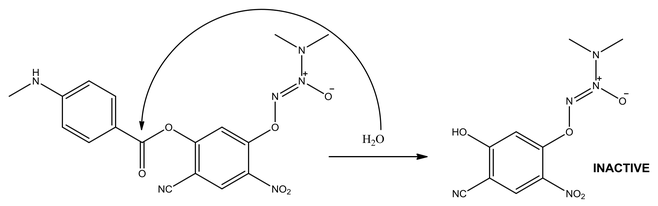
Step 2: Addition of "PABA arm"





4. Kinetics Studies with Cyano-PABA/NO

I. Rate of hydrolysis (observed using HPLC):

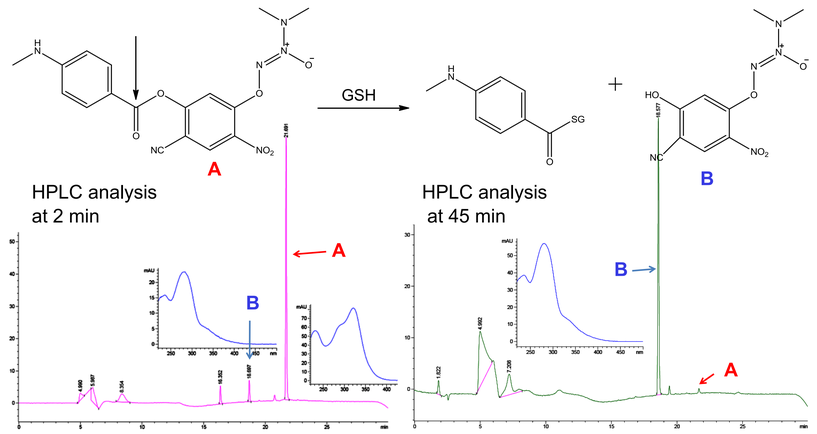


Experimental condition: 2 μM drug in pH 7.4 phosphate buffer at 25 ºC

PABA/NO: $t_{1/2}$ of about **25 min**

Cyano-PABA/NO: **< 2%** hydrolysis over **2 weeks** (~20,000 min)

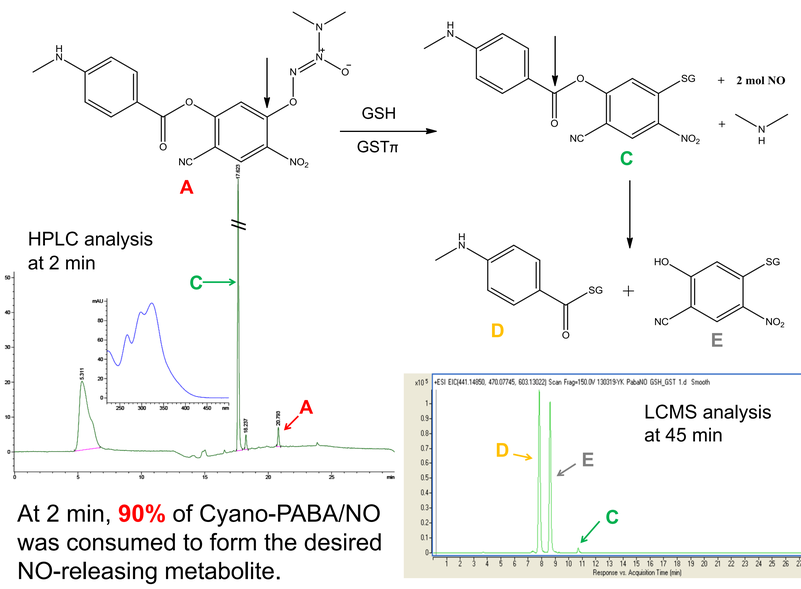
II. Reaction with 4 mM glutathione alone:



PABA/NO: At about 2.5 min, about **50%** reacted with GSH.

Cyano-PABA/NO: At 2 min, only about **14%** reacted with GSH.

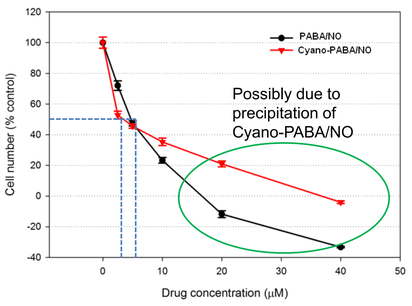
III. Reaction with glutathione and GSTπ:



At 2 min, **90%** of Cyano-PABA/NO was consumed to form the desired NO-releasing metabolite.

5. Biological Studies against PABA/NO

MTT assay A549, 5x10^3/well, 72 h



Cyano-PABA/NO seems to exhibit greater stability (hypothesized according to the preliminary kinetic data) without sacrifice in the drug efficacy at low concentration

6. Conclusion

- Successful synthesis of Cyano-PABA/NO
- Preliminary kinetics study with Cyano-PABA/NO suggests:
  - A. Hydrolysis: dramatic increase in stability
  - B. Reaction with GSH alone:
    - Dramatic decrease in the rate of displacing the diazeniumdiolate
    - Essentially just one reaction (thiolytic cleavage of the PABA arm)
  - C. Reaction with GSH and GSTπ:
    - Dramatic increase in the rate of displacing the diazeniumdiolate

7. Future Plans

- Quantitative comparison of PABA/NO's vs Cyano-PABA/NO's rates of reactions(1. with GSH and 2. with GSH and GSTπ).
- Reactivity of Cyano-PABA/NO with GSH + GSTα.