

## Supporting Information

### Engineering Hypoxia-Responsive 6-Aminonicotinamide Prodrugs for On-Demand NADPH Depletion and Redox Manipulation

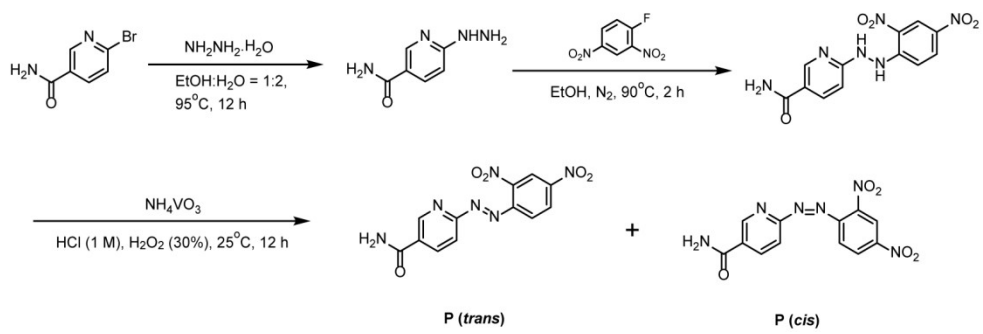
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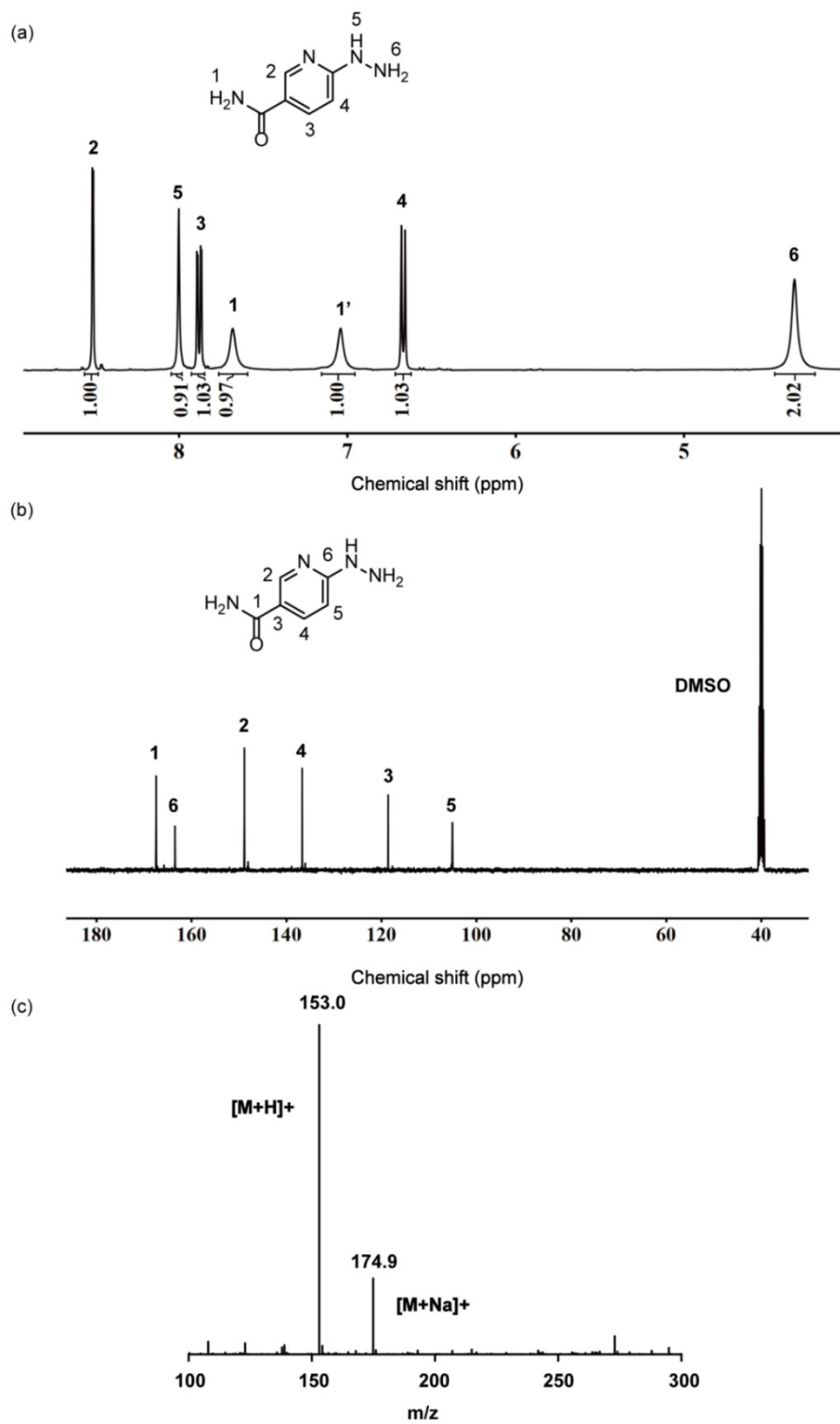
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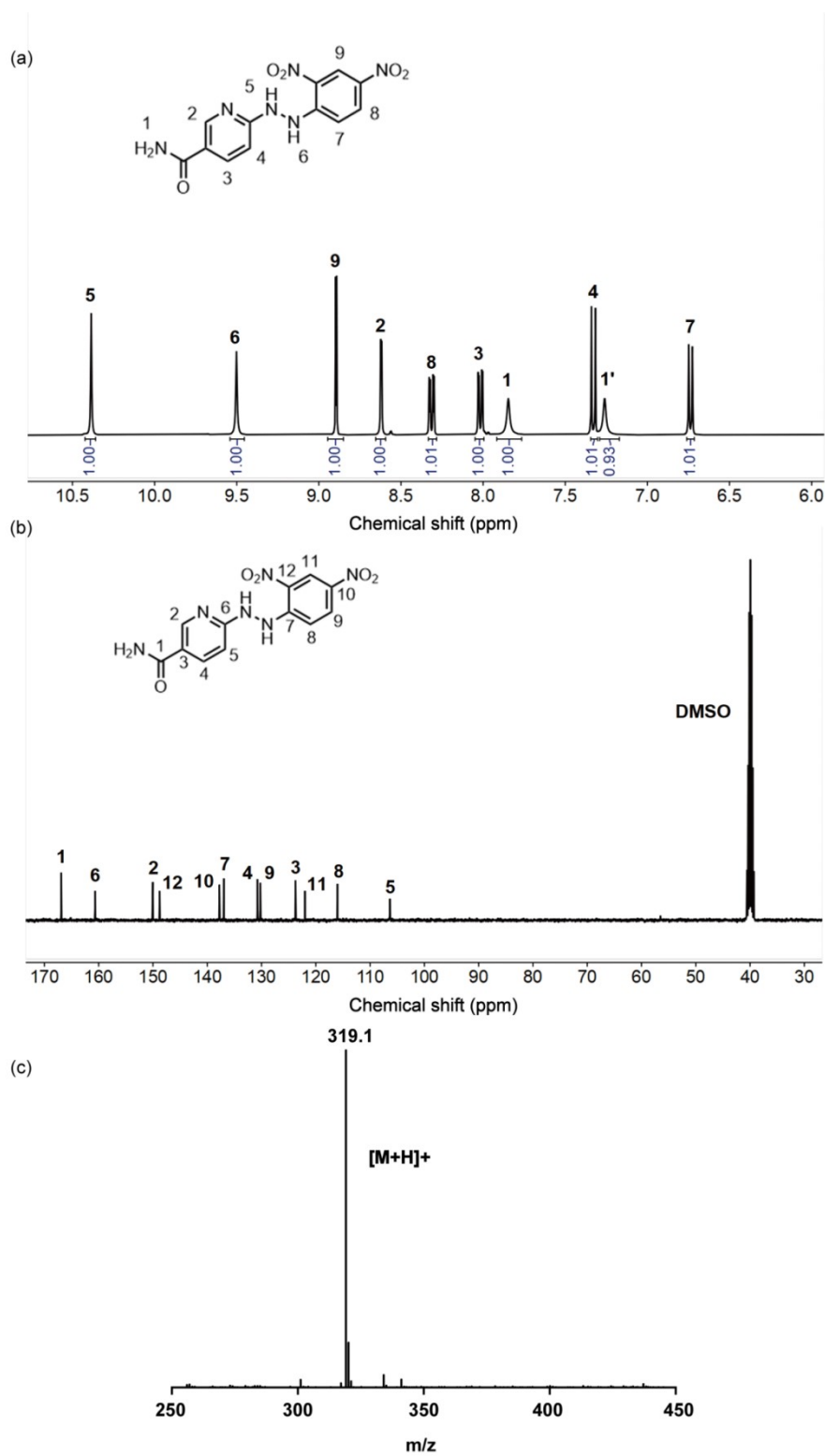
\*Corresponding author E-mail: zhaoyj@tju.edu.cn; (Zhao Y); yjdai@126.com (Dai Y); z197218275@yeah.net (Zhang B)



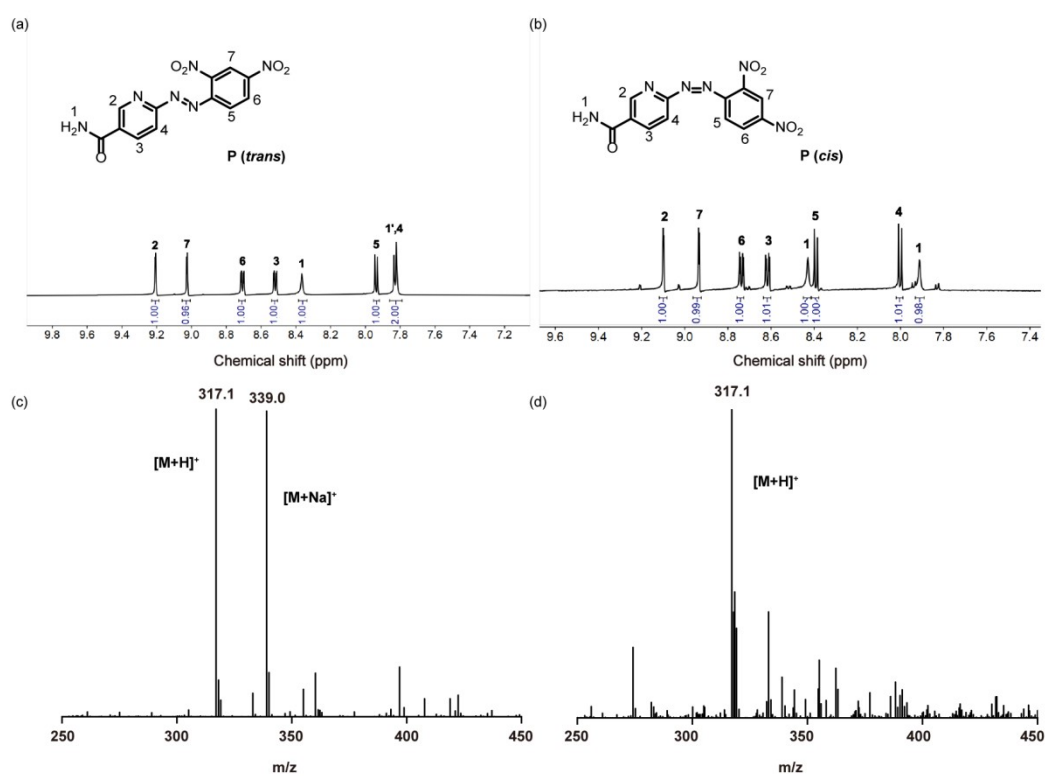
**Scheme S1.** The synthetic route of P (*trans*) and P (*cis*).



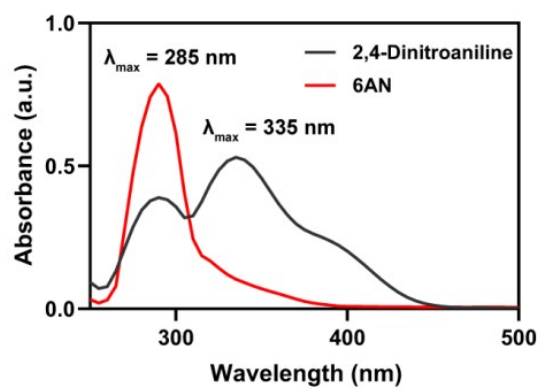
**Figure S1.** Characterization of 6-hydrazineynicotinamide. (a)  $^1\text{H}$  NMR spectrum (DMSO- $d_6$ ). (b)  $^{13}\text{C}$  NMR spectrum. (c) LC-MS analysis.



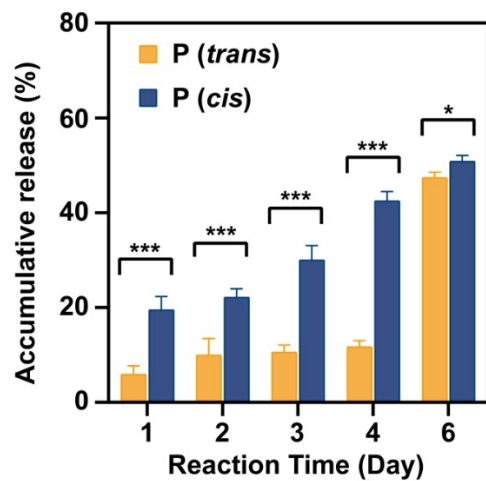
**Figure S2.** Characterization of 6-(2-(2,4-dinitrophenyl)hydrazine)nicotinamide. (a)  $^1\text{H}$  NMR spectrum (DMSO- $d_6$ ). (b)  $^{13}\text{C}$  NMR spectrum. (c) LC-MS analysis.



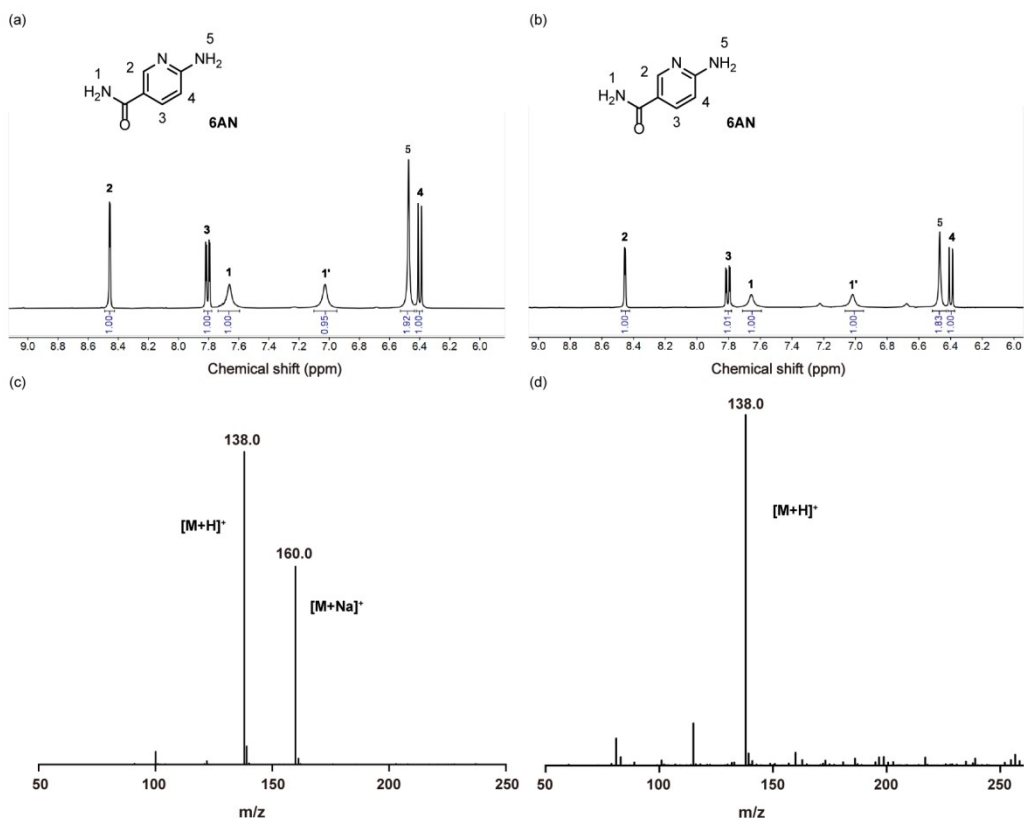
**Figure S3.** Verification diagram of 6AN prodrug structure. (a) <sup>1</sup>H NMR spectrum of P (*trans*) (DMSO-*d*<sub>6</sub>), (b) LC-MS analysis of P (*trans*), (c) <sup>1</sup>H NMR spectrum of P (*cis*) (DMSO-*d*<sub>6</sub>), (d) LC-MS analysis of P (*cis*).



**Figure S4.** The UV-vis absorption spectra of 6AN and 2,4-dinitroaniline.

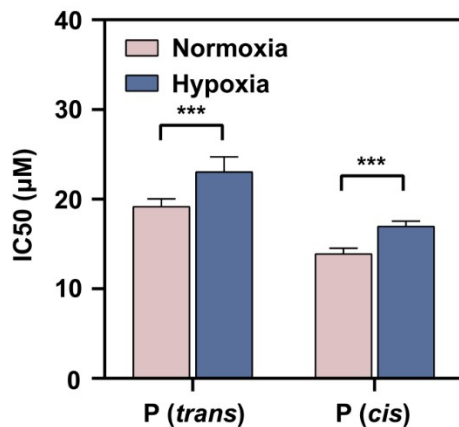


**Figure S5.** The reaction rate of P (*trans*) and P (*cis*) reduced by Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> at 25°C. Comparison of the percentage of cumulative release of 6AN by P (*cis*) and P (*trans*) at Day 1 to Day 6 (n = 3, \**p* < 0.05, \*\*\**p* < 0.001).

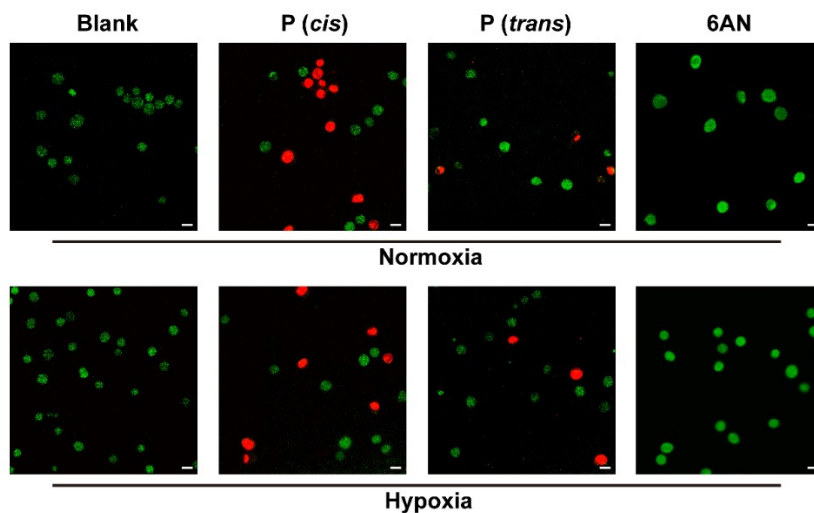


**Figure S6.** Verification that the 6AN prodrug can be reduced to 6AN under simulated hypoxic environmental conditions with  $\text{Na}_2\text{S}_2\text{O}_4$ . (a)  $^1\text{H}$  NMR spectrum of reduction product of P (*trans*) (DMSO- $d_6$ ). (b) MS analysis of reduction product of P (*trans*). (c)  $^1\text{H}$  NMR spectrum of reduction product of P (*cis*) (DMSO- $d_6$ ). (d) MS analysis of reduction product of P (*cis*).

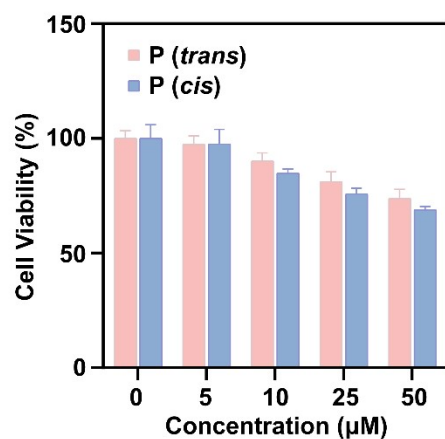




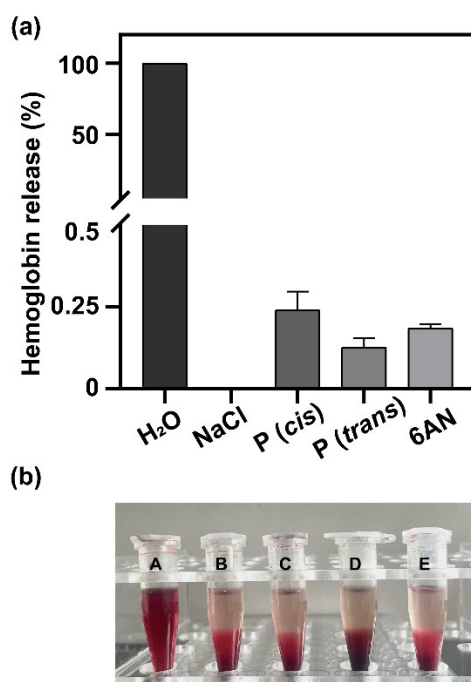
**Figure S7.** Half maximal inhibitory concentration of P (*trans*) and P (*cis*) to HepG2 cells for 24h (n = 4, \*\*\* $p < 0.001$ ).



**Figure S8.** Live-dead cell imaging by confocal laser scanning microscopy upon HepG2 cells treatment by 6AN (13.90 µM) and 6AN prodrugs (16.97 µM) under normoxia and hypoxia for 24 h. The cells were stained with Calcein AM (green, live cells) and PI (red, dead cells). Scale bar: 20 µm.



**Figure S9.** The viability of 3T3 cells post incubation with 6AN, P (*trans*) and P (*cis*) for 12 h (n = 5).



**Figure S10.** (a) Summary of hemolysis degree upon three different samples treatment (n = 3). (b) Hemolysis test of (A) deionized H<sub>2</sub>O (positive control), (B) 0.9% NaCl (negative control), (C) P (*cis*), (D) P (*trans*) and (E) 6AN.