

## **Supplementary information**

### **Repurposing antitubercular agent isoniazid for treatment of prostate cancer**

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## Synthesis and Characterization of INH Conjugates

**3*H*-Indolium, 1-(5-carboxypentyl)-2,3,3,5-tetramethyl-, bromide (II).** A solution of I (2g, 11.6mmol) and 6-bromohexanoic acid (9.0 g, 46.2mmol) in acetonitrile was refluxed for 40 h under a nitrogen condition. The solvent was removed *in vacuo*. The oily residue was purified by a silica gel to give II (3.2 g, 75.1%). MS (ESI) *m/z*: 288 [M-Br]<sup>+</sup>.

**1-(5-carboxypentyl)-2-(2-(3-(-2-(1-(5-carboxypentyl)-3,3,5-trimethylindolin-2-ylidene)ethylidene)-2-chlorocyclohex-1-en-1-yl)vinyl)-3,3,5-trimethyl-3*H*-indol-1-ium bromide (IV).** Compound III (0.5g, 2.9 mmol) was added to a solution of Compound II (2.13 g, 5.8 mmol) and sodium acetate (0.24 g, 2.9 mmol) in acetic anhydride. The reaction mixture was heated at 70 °C under vigorous stirring for 40 min, and then cooled to room temperature; the mixture was added to water and stirred for additional 3 h, followed by extraction with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to obtain compound IV (1.6g, 69.9%). MS (ESI) *m/z*: 711.3 [M-Br]<sup>+</sup>.

**2-[2-[3-[2-[1-(5-carboxypentyl)-1,3-dihydro-3,3,5-trimethyl-2*H*-indol-2-ylidene]ethylidene]-2-chloro-1-cyclohexen-1-yl]ethenyl]-1-(6-(2-isonicotinoylhydrazinyl)-6-oxohexyl)-3,3,5-trimethyl-3*H*-indoliumbromide (NIC-1).** PyBOP (263 mg, 0.506 mmol) was added to a solution of Compound IV (400mg, 0.506 mmol) in dry DCM in ice-salt bath. The mixture was allowed to room temperature. Isoniazide (58 mg, 0.422 mmol) was added, the mixture was stirred for additional 15 hours at room temperature. The residue was filtered, and the filtrate was evaporated under reduced

pressure and purified by a silica gel to give NIC-1 (100 mg, 27%) as green solid. MS (ESI)  $m/z$ : 830.6  $[M-Br]^+$ .  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.64 (s, 2H), 8.31 (d,  $J$  = 14.1 Hz, 1H), 8.16 (d,  $J$  = 13.6 Hz, 1H), 7.93 (s, 2H), 7.17 – 7.09 (m, 4H), 6.93 (d,  $J$  = 7.9 Hz, 1H), 6.31 (d,  $J$  = 14.3 Hz, 1H), 5.92 (d,  $J$  = 13.8 Hz, 1H), 4.23 – 4.15 (br, 2H), 3.96 – 3.90 (br, 2H), 2.67 – 2.63 (br, 2H), 2.52 – 2.47 (br, 2H), 2.46 – 2.42 (br, 2H), 2.36 (d,  $J$  = 7.4 Hz, 6H), 2.34 (s, 2H), 1.87 – 1.83 (br, 2H), 1.78 – 1.73 (br, 4H), 1.69 (s, 2H), 1.65 (s, 6H), 1.64 (s, 6H), 1.48 (d,  $J$  = 6.7 Hz, 2H), 1.38 – 1.33 (m, 4H).

**3*H*-Indolium, 1-(4-ethoxy-4-oxobutyl)-2,3,3,5-tetramethyl-, bromide (V).**

Compound V (3.0 g, 70.4%) was obtained by the same synthetic route of compound II with ethyl 4-bromobutanoate as a reactant. MS (ESI)  $m/z$ : 288  $[M-Br]^+$ .

**2-6-(*N*-phenylacetamido)hexa-1,3,5-trien-1-yl)-1-(4-ethoxy-4-oxobutyl)-3,3,5-trimethyl-3*H*-indol-1-ium bromide (VII).** Compound V (1.5 g, 4.1 mmol) and VI (1.16 g, 4.1 mmol) were added to acetic anhydride and stirred at 50 °C for 50 min. The resulting mixture was added into ether, and the particulate was filtered to obtain Compound VII (2.0 g, 87%) as brownish solid. MS (ESI)  $m/z$ : 485  $[M-Br]^+$ .

**2-(7-(1-(5-carboxypentyl)-3,3,5-trimethylindolin-2-ylidene)hepta-1,3,5-trien-1-yl)-1-(4-ethoxy-4-oxobutyl)-3,3,5-trimethyl-3*H*-indol-1-ium bromide (VIII).** Compound II (0.65 g, 1.77 mmol) and compound VII (1g, 1.77 mmol) was added to pyridine. The solution was stirred at 80 °C for 40 min. After being cooled to room temperature, ether was added to the mixture, and the particulate was filtered. The crude product was purified by a silica gel to give compound VIII (0.25g, 20%). MS (ESI)  $m/z$ : 637  $[M-Br]^+$ .

**2-(7-(1-(4-ethoxy-4-oxobutyl)-3,3,5-trimethyl-3*H*-indolin-2-ylidene)hepta-1,3,5-trien-1-yl)-1-(6-(2-isonicotinoylhydrazinyl)-6-oxohexyl)-1,3-dihydro-3,3,5-trimethyl-3*H*-indolium bromide (NIC-2).** NIC-2 (99 mg, 40.6%) was obtained by the same synthetic strategy of compound NIC-1 with compound VIII as a reactant. MS (ESI) *m/z*: 756.6 [M-Br]<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.69 (d, *J* = 5.6 Hz, 2H), 7.70 (d, *J* = 5.8 Hz, 3H), 7.67 – 7.60 (br, 1H), 7.41 – 7.32 (br, 1H), 7.16 (d, *J* = 8.1 Hz, 1H), 7.13 (d, *J* = 6.2 Hz, 1H), 7.10 (s, 2H), 7.00 (t, *J* = 8.8 Hz, 1H), 6.63 – 6.56 (m, 1H), 6.42 (t, *J* = 12.5 Hz, 1H), 6.23 (d, *J* = 13.4 Hz, 1H), 6.01 (d, *J* = 13.2 Hz, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 3.98 (dt, *J* = 15.3, 7.7 Hz, 4H), 2.49 (t, *J* = 6.6 Hz, 2H), 2.10 – 2.02 (m, 2H), 1.84 – 1.75 (m, 4H), 1.67 (s, 2H), 1.63 (d, *J* = 3.4 Hz, 12H), 1.59 – 1.53 (m, 2H), 1.27 (t, *J* = 7.1 Hz, 3H).

**2-[2-[3-[2-[1-(6-(2-isonicotinoylhydrazinyl)-6-oxohexyl)-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-ylidene]ethylidene]-2-chloro-1-cyclohexen-yl]ethenyl]-1-(6-(2-isonicotinoylhydrazinyl)-6-oxohexyl)-3,3-dimethyl-3*H*-indoliumbromide (NIC-3).** NIC-3 was obtained by the same synthetic strategy of NIC-1 with MHI-148 as a reactant which was synthesized as reported earlier [1]. MS (ESI) *m/z*: 921 [M-Br]<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.61 – 8.56 (br, 4H), 8.24 (d, *J* = 13.9 Hz, 2H), 7.82 – 7.76 (br, 4H), 7.72 – 7.67 (br, 1H), 7.56 – 7.52 (br, 1H), 7.36 (d, *J* = 7.6 Hz, 2H), 7.33 (d, *J* = 7.7 Hz, 2H), 7.21 (t, *J* = 7.5 Hz, 2H), 6.13 (d, *J* = 14.0 Hz, 2H), 4.06 – 3.99 (br, 4H), 2.56 – 2.50 (br, 4H), 2.41 – 2.35 (br, 4H), 1.84 – 1.77 (br, 4H), 1.76 – 1.72 (m, 2H), 1.68 (s, 12H), 1.57 – 1.49 (m, 6H).

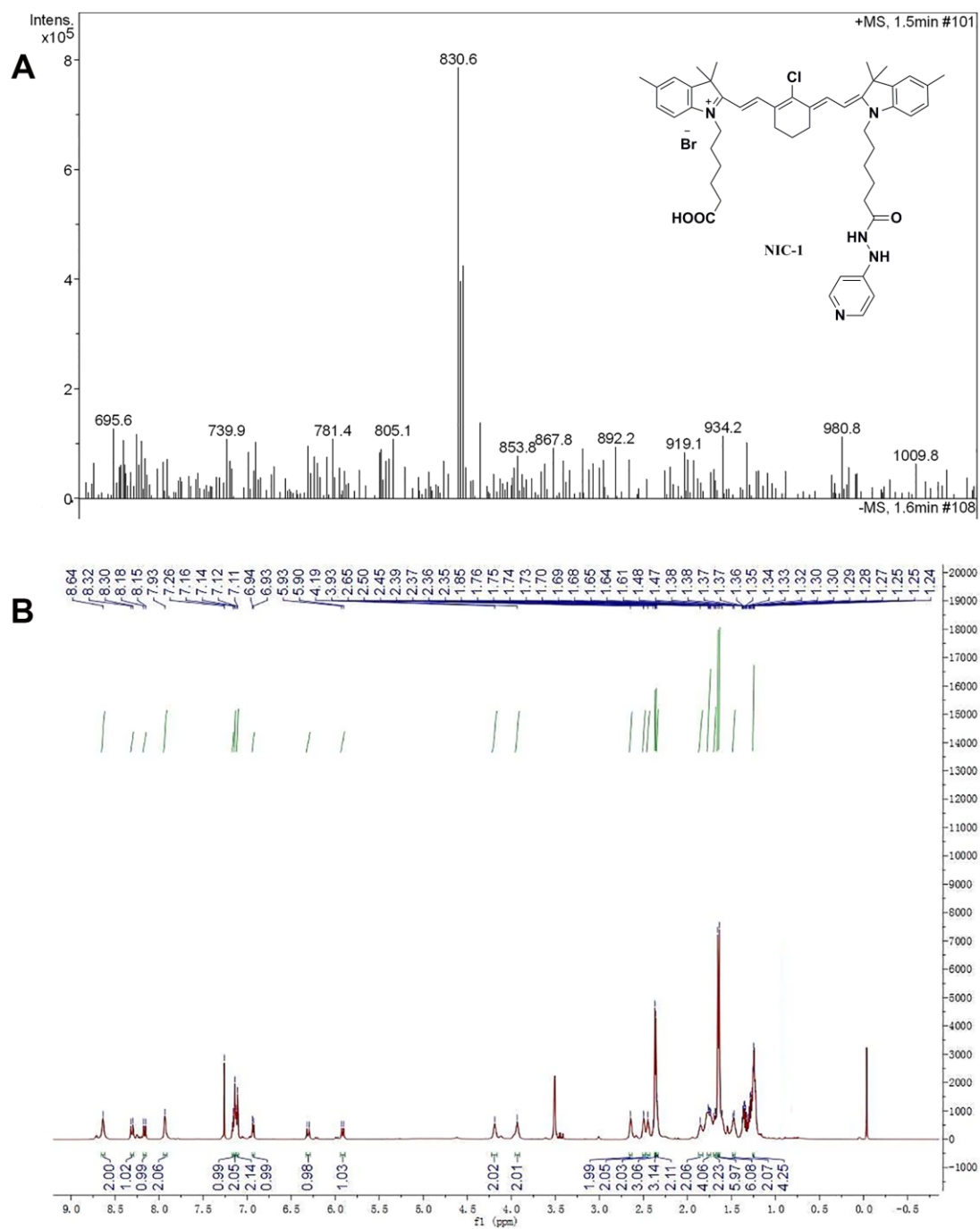


Figure S1 MS (A) and <sup>1</sup>H NMR (B) spectrum of compound NIC-1

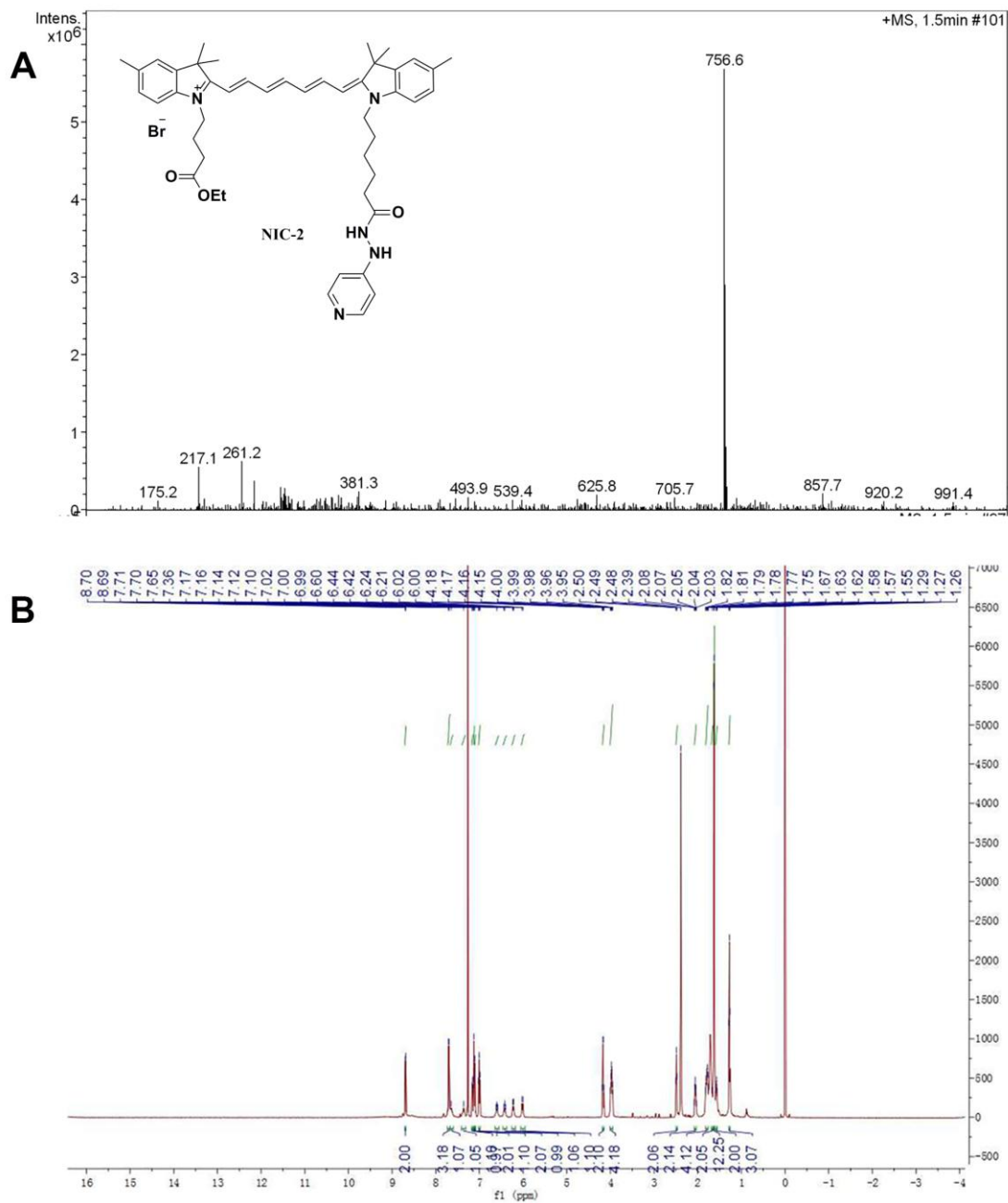


Figure S2 MS (A) and <sup>1</sup>H NMR (B) spectrum of compound NIC-2

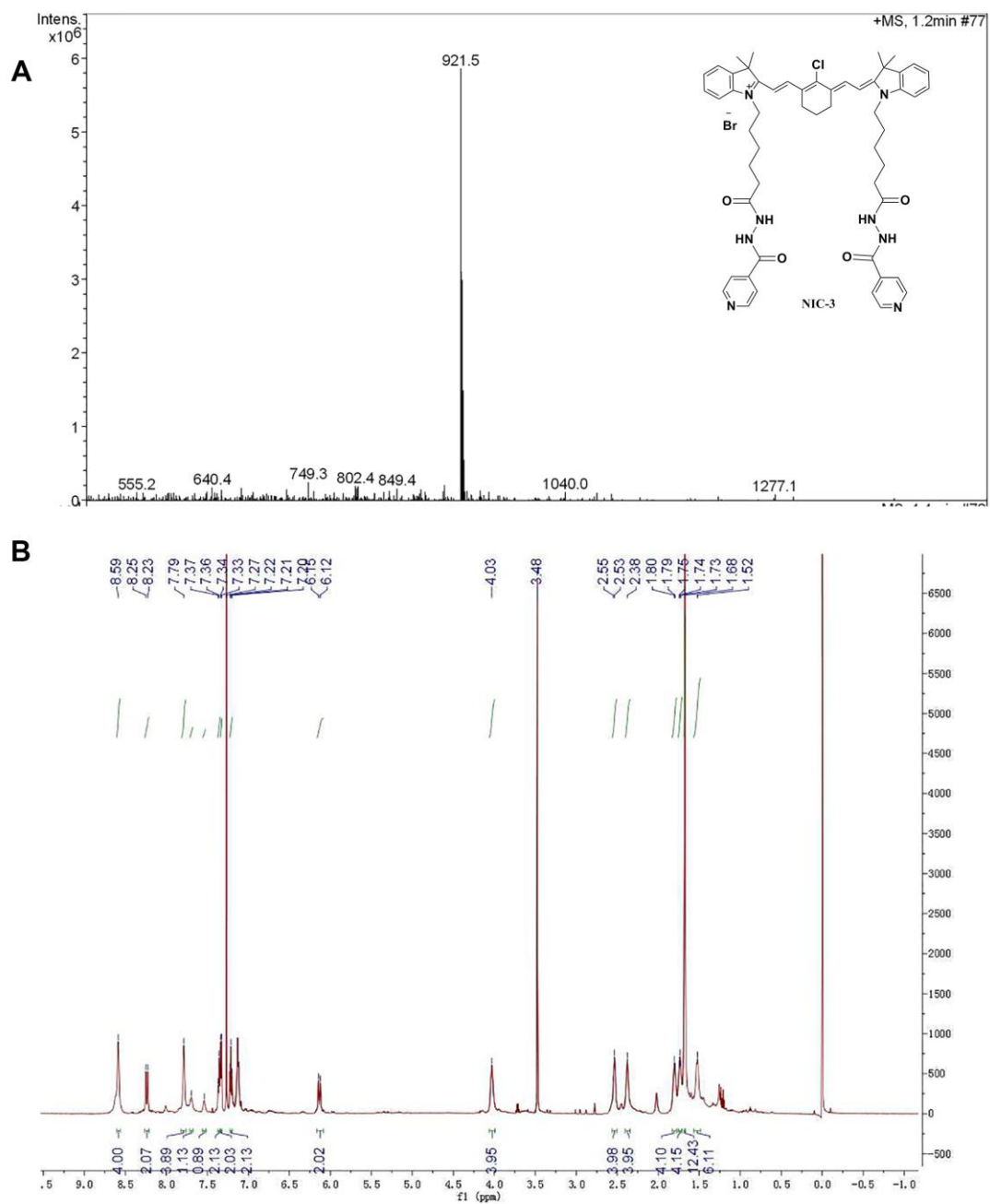


Figure S3 MS (A) and <sup>1</sup>H NMR (B) spectrum of compound NIC-3

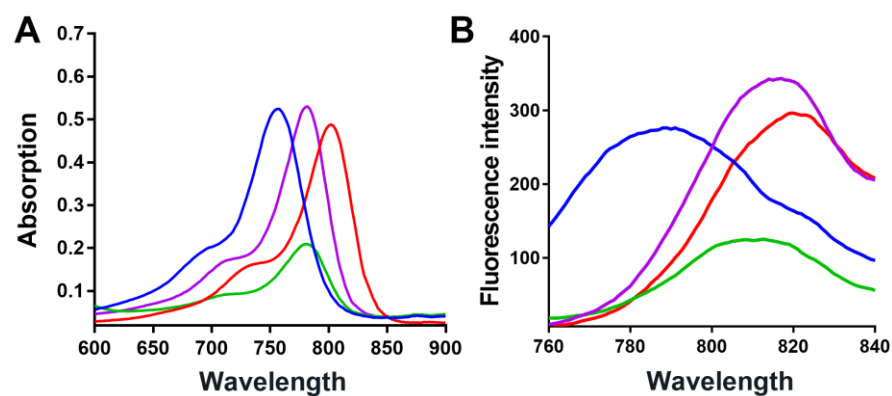


Figure S4. Optical properties of three INH conjugates in MeOH. (A) The absorption spectra of MHI-148 dye and INH conjugates in MeOH. (B) The emission spectra of MHI-148 dye and INH conjugates in MeOH.

## Reference

[1] Lv Q, Yang X, Wang M, Yang J, Qin Z, Kan Q, et al. Mitochondria-targeted prostate cancer therapy using a near-infrared fluorescence dye-monoamine oxidase A inhibitor conjugate. *J Control Release*. 2018;279:234.