SUPPLEMENTAL INFORMATION

Supplemental Data

Preparation of 1-hydroxy-1,8-naphthyridin-2(1*H*)-one derivatives.

The bromo naphthyridone intermediate was prepared using published procedures (Int. Patent WO 2008/010964 A1 (2008)) and as described in Figures S3 and S4. Commercially available boronic acids were purchased from Sigma Aldrich. Suzuki coupling reactions were used to prepare *N*-benzyloxynaphthyridone adducts. These were purified by silica gel flash chromatography. The resulting adducts were reduced with H_2/10% Pd on carbon giving the resulting debenzylated naphthyridone compounds.

General Procedure for Suzuki Couplings: To a solution of the 6-bromonaphthyridone intermediate (0.171 mmol) in DMF (3 mL) was added a boronic acid (0.298 mmol), anhydrous K_2CO_3 (54 mg, 0.436 mmol) and water (0.7 mL). The resulting solution was purged with a stream of argon for 10 min and then treated with $Pd(dppf)Cl_2$ (18 mg, 0.014 mmol) and heated in a sealed vial using *microwaves* at 110 °C for 20 min. Flash chromatography on silica gel eluting with a gradient of hexane/ethyl acetate (0 – 10% ethyl acetate in hexanes) gave pure adducts (20-30% yield).

General Procedure for Reduction of *N*-benzyloxynapthyridones: A suspension of *N*-benzyloxynaphthyridone adducts (0.03 mmol), in EtOH/THF (50/50) (12 mL) was treated with 10% Pd /C (2 mg) and then hydrogenated at 1 atm overnight. The catalyst was removed by filtration through Celite and evaporated *in vacuo* giving pure products (80-100% yield).

Analytical Data:

6-(Biphenyl-3-yl)-1,4-dihydroxy-3-phenyl-1,8-naphthyridin-2(1*H*)-one, Sodium salt (1). ^{1}H NMR (300 MHz) (CD_3OD) δ 8.86 (d, 1H, *J* = 2.6 Hz), 8.83 (d, 1H, *J* = 2.6 Hz), 7.96 (t, 1H, *J* = 1.65 Hz), 7.73-7.69 (m, 3H), 7.65-7.61 (m, 1H), 7.59-7.54 (m, 3H), 7.50-7.43 (m, 2H), 7.39-7.31 (m, 3H), 7.20-7.14 (m, 1H). MS: m/z (relative intensity) (ESI, negative ion) 405 (M-H, 100).

1,4-Dihydroxy-3-phenyl-6-(4-(trifluoromethyl)phenethyl)-1,8-naphthyridin-2(1*H*)-one (2). ^{1}H NMR (300 MHz) (CD_3OD) δ 8.36 (s, 1H), 8.25 (s, 1H), 7.22-7.60 (m, 9H), 3.15-3.00 (m, 4H). MS: m/z (relative intensity) (ESI, negative ion) 425 (M-H, 100).

(*E*)-1-(Benzyloxy)-4-hydroxy-3-phenyl-6-(4-(trifluoromethyl)styryl)-1,8-naphthyridin-2(1*H*)-one (3). ^{1}H NMR (300 MHz) ($CDCl_3$) δ 8.87 (d, 1H, *J* = 2.3 Hz), 8.46 (d, 1H, *J* = 2.3 Hz), 7.74-7.70 (m, 2H), 7.68-7.64 (m, 4H), 7.62-7.49 (m, 5H), 7.40-7.36 (m, 3H), 6.89-6.76 (m, 2H), 5.35 (s, 2H). MS: m/z (relative intensity) (ESI, negative ion) 513 (M-H, 100).

1,4-Dihydroxy-6-(4-methoxyphenethyl)-3-phenyl-1,8-naphthyridin-2(1*H*)-one (4). ^{1}H NMR (300 MHz) (CD_3OD) δ 8.35 (s, 1H), 8.25 (s, 1H), 3.74 (s, 3H), 3.06-2.91 (m, 4H). MS: m/z (relative intensity) (ESI, negative ion) 387 (M-H, 100).

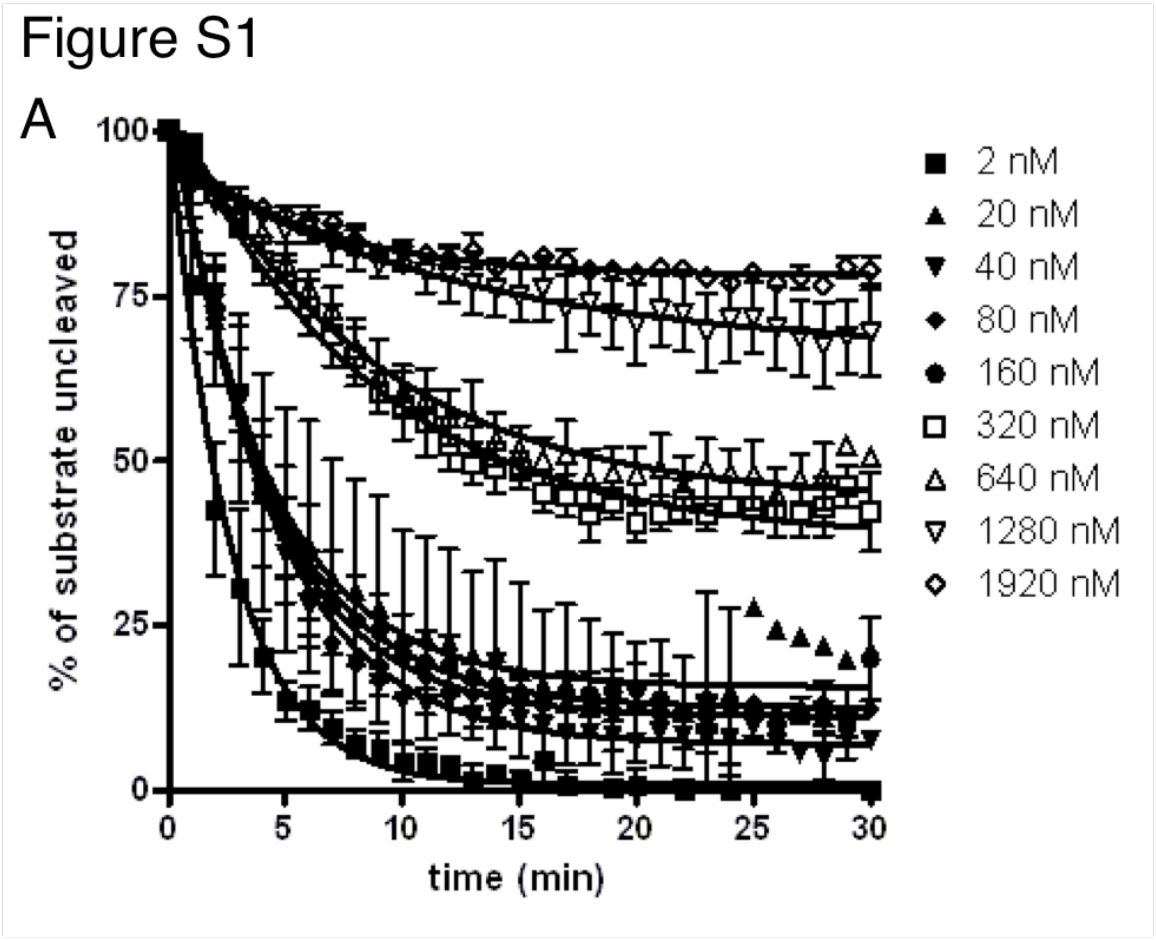
Ethyl 1,4-dihydroxy-2-oxo-6-(4-(trifluoromethyl)phenethyl)-1,2-dihydro-1,8-naphthyridine-3-carboxylate (5). ^{1}H NMR (300 MHz) (CD_3OD) δ 8.50 (d, 1H, *J* = 1.7 Hz), 8.35 (d, 1H, *J* = 1.7 Hz), 7.55 (d, 1H, *J* = 8.1 Hz), 7.37 (d, 1H, *J* = 8.1 Hz), 4.47 (q, 2H, *J* = 7.2 Hz), 3.13-3.08 (m, 4H), 1.42 (t, 3H, *J* = 7.2 Hz). MS: m/z (relative intensity) (ESI, negative ion) 421 (M-H, 100).

(*E*)-Ethyl 1-(benzyloxy)-4-hydroxy-2-oxo-6-(4-(trifluoromethyl)styryl)-1,2-dihydro-1,8-naphthyridine-3-carboxylate (6). ^{1}H NMR (300 MHz) ($CDCl_3$) δ 8.90 (d, 1H, *J* = 2.3 Hz), 8.57 (d, 1H, *J* = 2.3 Hz), 7.75-7.69 (m, 2H), 7.68-7.62 (m, 4H), 7.43-7.35 (m, 3H), 7.27-7.21 (m, 3H), 5.30 (s, 2H) 4.56 (q, 2H, *J* = 7.1 Hz), 1.51 (t, 3H, *J* = 7.1 Hz).. MS: m/z (relative intensity) (ESI, negative ion) 509 (M-H, 100).

Ethyl 1,4-dihydroxy-6-(6-methoxypyridin-2-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxylate (7). ^{1}H NMR (300 MHz) (CD_3OD) δ 8.99 (s, 1H), 8.72 (s, 1H), 8.50 (s, 1H), 8.15-7.90 (m, 1H), 6.99-6.90 (m, 1H), 4.49 (q, 2H, *J* = 7.1 Hz), 3.97 (s, 3H), 1.43 (t, 3H). MS: m/z (relative intensity) (ESI, negative ion) 356 (M-H, 100).

Ethyl 1-(benzyloxy)-4-hydroxy-6-(6-methoxypyridin-2-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxylate (8). ^{1}H NMR (300 MHz) ($CDCl_3$) δ 8.95 (d, 1H, *J* = 2.3 Hz), 8.55 (d, 1H, *J* = 2.3 Hz), 8.44 (d, 1H, *J* = 2.1 Hz), 7.83 (dd, 1H, *J* = 2.1 Hz, *J* = 8.4 Hz), 7.76-7.68 (m, 2H), 7.46-7.36 (m, 3H), 6.89 (d, 1H, *J* = 8.4Hz), 5.31 (s, 2H), 4.56 (q, 2H, *J* = 6.9 Hz), 4.01 (s, 3H), 1.51 (t, 3H). MS: m/z (relative intensity) (ESI, negative ion) 446 (M-H, 100).

Figure S1



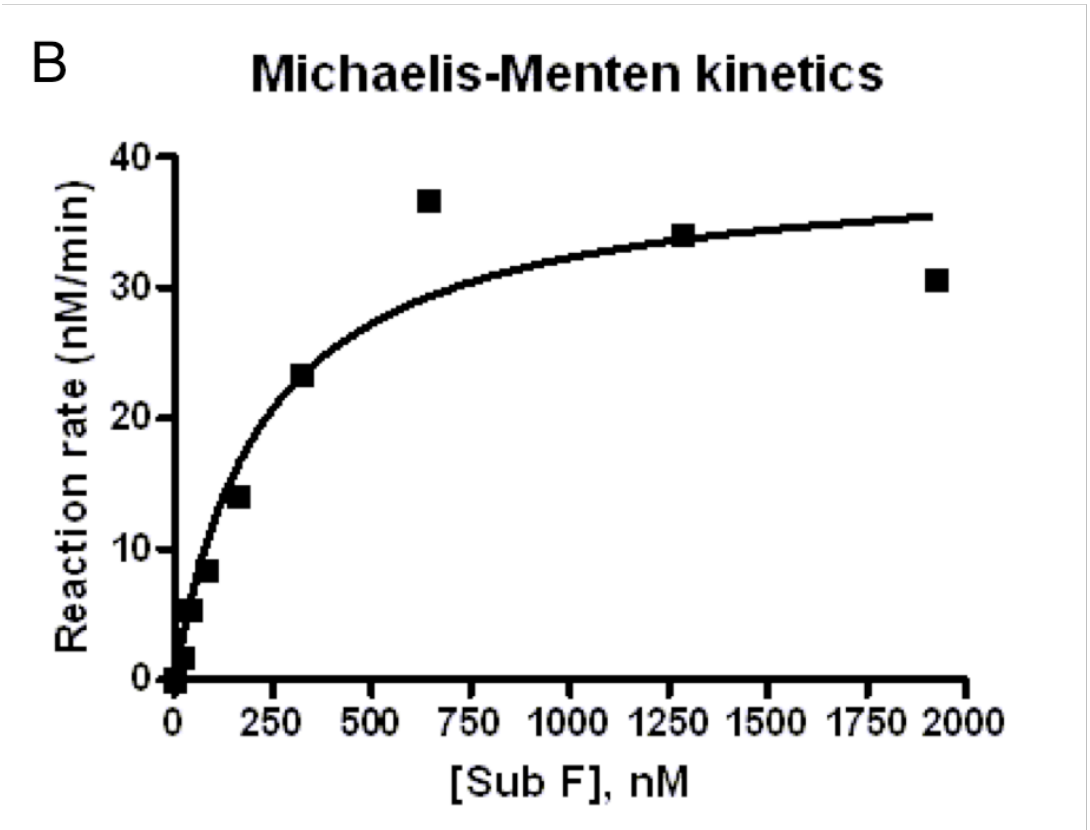


Figure S1. Michaelis-Menten analysis of cleavage by fowlpox resolvase. A) Fits to kinetic plots of reaction progression profiles at different concentrations of substrate. B) Plot of rates at different substrate concentrations.

Figure S2

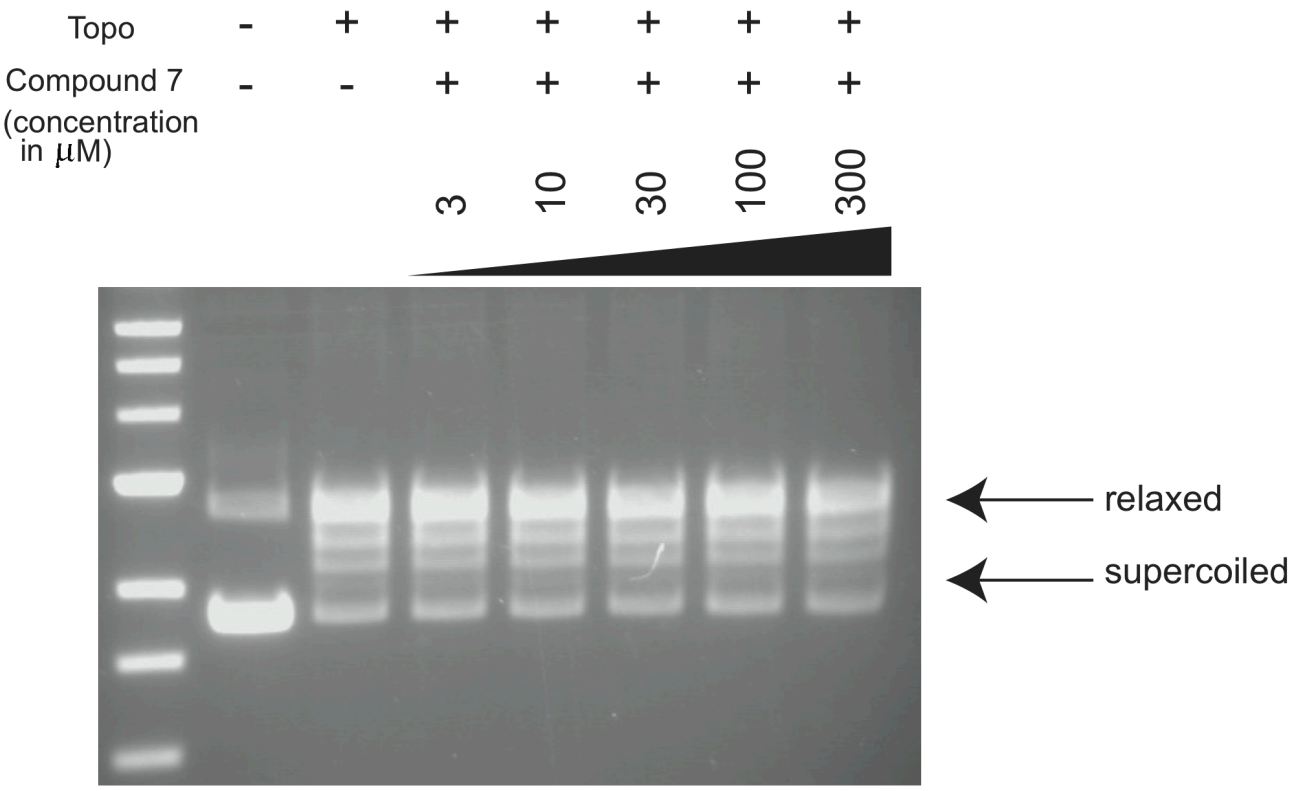
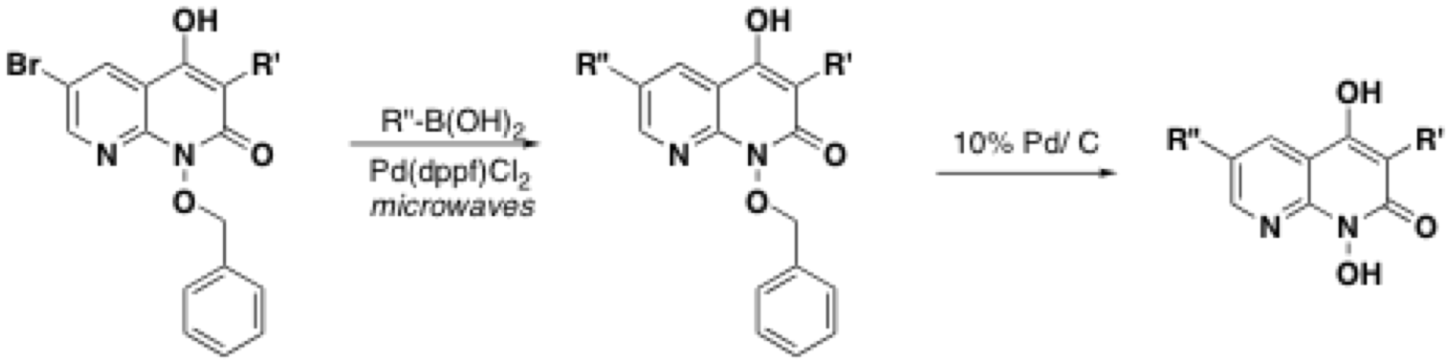


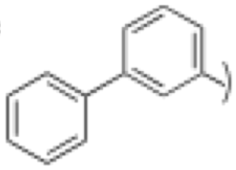
Figure S2. Lack of inhibition of variola topoisomerase activity by compound 7. Supercoiled DNA was exposed to purified variola topoisomerase in vitro. Products were then separated by electrophoresis on a native agarose gel and stained with ethidium bromide. The relaxation products (upper bands) were not reduced in intensity by the presence of compound 7. Methods were as described in Hwang et al., J. Biol. Chem. 281, 38052-38060 (2006).

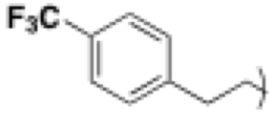
Figure S3

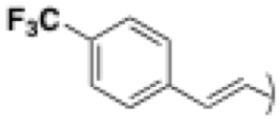


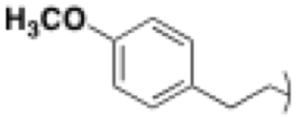
R' = phenyl, -CO_2Et

R" =









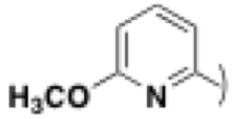


Figure S3. General synthetic scheme.

Figure S4

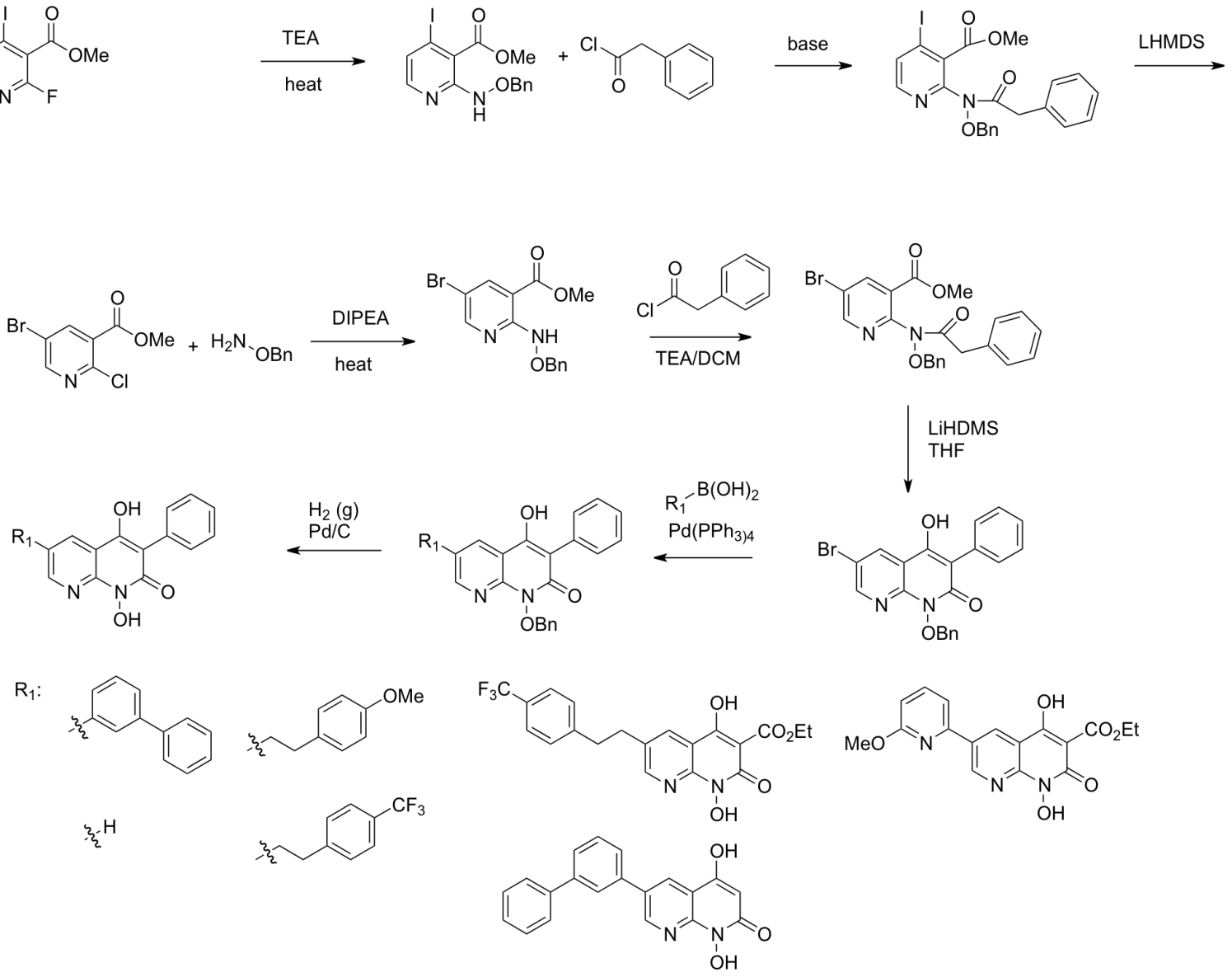


Figure S4. Scheme for synthesis of 1-hydroxy-1,8-naphthyridin-2(1*H*)-ones.

Table S1 Oligonucleotides used for the assay substrates and size markers.

Substrate	Oligonucleotide sequence	use
Sub A	5′-AGCTTCTGTGCAGCAGCTGCTCTCAACTGCAGTCTAGACT-3′ 5′-AGTCTAGACTGCAGTTGAGAGCTTGCTAGGACGGATCCCT-3′ 5′-AGGGATCCGTCCTAGCAAGCTTTTTGTTTTGATTGCGAGG-3′ 5′-CCTCGCAATCAAAACAAAAAGCAGCTGCTGCACAGAAGCT-3′-F	Holliday junction
Sub B	5′-CCTCGCAATCAAAACAAAAAGCAGCTGCTGCACAGAAGCT-3 F-5′-GTCGTGCTGCTTTTTGTTTTGATTGCGAGG-3′	sprayed duplex
Sub C	5′-TCCTACCACCAGATACACGCCACAGTTTTTTTTTTGATTA-3′-F	single stranded
Sub D	F-5′-TCCTACCACCAGATACACGCCACAGTTTTTTTTTTGATTA-3′	single stranded
Sub E	5′-TCCTACCACCAGATACACGCCACAGTTTTTTTTTTGATTA-3′-F 5′-TAATCAAAAAAAAAACTGTGGCGTGTATCTGGTGGTAGGA-3′	duplex
Sub F	5′-TCCTACCACCAGATACACGCCACAGTTTTTTTTTTGATTA-3′-F 5′-TAATCTTTTTTTTTTCTGTGGCGTGTATCTGGTGGTAGGA-3′	bulged substrate
marker	5′-TCCTACCACCAGATACACGCCACAGTTTTTTTTTTGATTA-3′5′ TTTTTTTTTTCTGTGGCGTGTATCTGGTGGTAGGA-3′	75 base
marker	5′-TCCTACCACCAGATACACGCCACAGTTTTTTTTTTGATTA-3′5′ CTGTGGCGTGTATCTGGTGGTAGGA-3′	65 base
marker	5′TTTTTTTTTTGATTA-3′-F	15 base
marker	5′GAATA-3′-F	5 base

Table S2 results of high throughput screening using the off-center bulge substrate.

Library	Source	Type of compounds	No. of molecules	Hit rate
1	NSRB	diverse	130,540	1.5%
2	Merck & Co.	metal-chelating	2,787	45%