# **Supplementary Material**

## O-Benzyl-N-(9'-acridinyl)hydroxylamines

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### **General Procedures**

Benzyl chloride, substituted benzyl chlorides, N-hydroxyphthalimide, 9-chloroacridine (**5**) and other reagents were obtained commercially from Sigma Aldrich, and were used after a determination of purity via <sup>1</sup>H NMR spectroscopy. Compounds **3** and **4** were prepared using the method of Bonaccorsi and Giorgi (ref. 9). All solvents used were dried prior to use and their purity verified via spectroscopic methods. Hydrogen chloride gas was generated as needed through the addition of concentrated sulfuric acid to sodium chloride. Radial chromatography was performed using a Harrison Associates Chromatotron<sup>®</sup> on 2mm-thick silica gel plates containing fluorescent indicator that were pre-cleaned with methanol and stored at elevated temperatures prior to use. NMR spectra were obtained on a Bruker Avance II (400 MHz for <sup>1</sup>H) multinuclear FT-NMR. Infrared spectra were collected using a Thermo Scientific iD<sub>5</sub> ATR ZnSe cell. All UV-visible data were measured using an Agilent UV-visible diode-array spectrophotometer with a Peltier-temperature controller. MTT assay data were collected using published procedures (ref. 15).

### General procedure for preparation of O-benzyl-N-(9'-acridinyl)-hydroxylamines (6a-l)

The appropriate salt, **4a-l**,  $(7.02 \times 10^{-4} \text{ mol})$  was treated with commercially available 9-chloroacridine (**5**) (4.68×10<sup>-4</sup> mol). The reaction was carried out in molten phenol using 3.0 grams of phenol per gram of 9-chloroacridine (**5**). The reaction was heated between 80-100 °C for a period of 6-8 hours, then cooled to room temperature and dissolved in CH<sub>2</sub>Cl<sub>2</sub>. The resulting orange or red organic solution was washed repeatedly with 0.25 M NaOH until greater than a 1:1 molar ratio of hydroxide to phenol was used. The organic phase was then washed with water (once) and brine (once). The organic layer was dried over anhydrous sodium sulfate, gravity filtered, and concentrated to a final volume of approximately 1 mL. This sample was then transferred to the top of a 5-cm column of silica gel constructed from a 10-mL syringe barrel and eluted with ethyl acetate. The orange filtrate was collected, concentrated to a final volume of 0.5-1.0 mL, and subjected to radial chromatography (2mm plate, silica gel, CH<sub>2</sub>Cl<sub>2</sub>:Et<sub>2</sub>O 100:0 to 90:10 gradient elution). Compounds **6a-l** were obtained in pure form by evaporation of the solvent from the bands that eluted.



### O-(3-methoxybenzyl)-N-(9'-acridinyl)-hydroxylamine, 6a

Yield: 64%. <sup>1</sup>HNMR (acetone-d<sub>6</sub>)  $\delta$ , in ppm: 9.32 (s, 1H); 9.03 (d, 1H); 8.10 (d, 1H); 7.34 (m, 3H); 7.13 (m, 4H); 6.97 (m, 2H); 6.88 (m, 1H); 5.30 (s, 2H); 3.78 (s, 3H). <sup>13</sup>CNMR (acetone-d<sub>6</sub>)  $\delta$ , in ppm: 159.9; 143.5; 140.5; 140.4; 140.3; 138.1; 138.0; 131.9; 130.9; 129.8; 129.3; 124.6; 120.7; 120.0; 119.1; 118.1; 118.1; 115.4; 115.3; 115.0; 115.0; 114.9; 113.4; 113.0; 76.5; 54.5. IR (ATR-ZnSe) in cm<sup>-1</sup>: 747.39; 964.12; 1157.11; 1265.46; 1474.23; 1598.05; 1614.45.  $\Delta$ T<sub>m</sub> = 9.1°C.







### O-(2-methylbenzyl)-N-(9'-acridinyl)-hydroxylamine, 6c

Yield: 49%. <sup>1</sup>HNMR (acetone-d<sub>6</sub>)  $\delta$ , in ppm: 9.31 (s, 1H); 8.95 (m, 1H); 8.09 (d, 1H); 7.47 (d, 1H); 7.34 (m, 2H); 7.23 (m, 3H); 7.12 (m, 2H); 6.98 (m, 2H); 5.33 (s, 2H); 2.45 (s, 3H). <sup>13</sup>CNMR (acetone-d<sub>6</sub>)  $\delta$ , in ppm: 143.3; 140.4; 140.3; 138.1; 138.0; 136.8; 136.4; 131.7; 130.9; 130.0; 129.7; 129.2; 127.9; 125.7; 124.6; 120.6; 119.1; 118.2; 118.1; 115.4; 115.3; 115.0; 114.9; 114.9; 75.2. IR (ATR-ZnSe) in cm<sup>-1</sup>: 1473. HRMS: M-1, 315.1496 (C<sub>21</sub>H<sub>19</sub>N<sub>2</sub>O).  $\Delta$ T<sub>m</sub> = 15.5°C. MTT IC<sub>50</sub> = 17.7±0.2µM







### O-(3-methylbenzyl)-N-(9'-acridinyl)-hydroxylamine, 6d

Yield: 71%. <sup>1</sup>H NMR (acetone-d<sub>6</sub>)  $\delta$ , in ppm: 9.31 (s, 1H); 8.99 (d, 1H); 8.09 (d, 1H); 7.33 (m, 5H); 7.10 (m, 3H); 6.98 (m, 2H); 5.27 (s, 2H); 2.34 (s, 3H). <sup>13</sup>C NMR (acetone-d<sub>6</sub>)  $\delta$ , in ppm: 143.3; 140.4; 140.4; 138.6; 138.1; 138.0; 137.7; 131.9; 130.9; 129.7; 128.7; 128.3; 128.2; 125.2; 124.6; 120.7; 119.1; 118.2; 118.1; 115.4; 115.3, 114.98, 115.0; 114.9; 76.8. IR (ATR-ZnSe) in cm<sup>-1</sup>: 745; 770; 964, 1156; 1472; 1486; 1598; 1614. HRMS: M-1, 315.1477 (C<sub>21</sub>H<sub>19</sub>N<sub>2</sub>O).  $\Delta T_m = 19.0^{\circ}$ C. MTT IC<sub>50</sub> = 20.7±0.5µM







### O-(4-methylbenzyl)-N-(9'-acridinyl)-hydroxylamine, 6e

Yield: 40%. <sup>1</sup>HNMR (acetone-d<sub>6</sub>) δ, in ppm: 9.31 (s, 1H); 8.98 (m, 1H); 8.11 (m, 1H); 7.37 (m, 4H); 7.14 (m, 4H); 6.96 (m, 2H); 5.27 (s, 2H); 2.33 (s, 3H). <sup>13</sup>CNMR (acetone-d<sub>6</sub>) δ, in ppm: 143.2; 140.4; 138.1; 138.0; 137.1; 135.6; 131.8; 130.9; 129.7; 128.9; 128.2; 124.6; 120.7; 119.1; 119.1; 118.2; 115.4; 115.3; 115.0; 114.9; 76.6. IR (ATR-ZnSe) in cm<sup>-1</sup>: 746, 965, 1157, 1472. HRMS: M-1, 315.1490 (C<sub>21</sub>H<sub>19</sub>N<sub>2</sub>O).  $\Delta T_m = 19.0^{\circ}$ C. MTT IC<sub>50</sub> = 22.2±1.7µM.







### O-benzyl-N-(9'-acridinyl)-hydroxylamine, 6f

Yield: 16%. <sup>1</sup>H NMR (acetone-d<sub>6</sub>) δ, in ppm: 9.33 (s, 1H); 9.00 (m, 1H); 8.09 (m, 1H); 7.54 (m, 2H); 7.35 (m, 5H); 7.14 (m, 1H); 7.08 (m, 2H); 6.95 (m, 2H); 5.31 (s, 2H).

<sup>13</sup>C NMR (acetone-d<sub>6</sub>) δ, in ppm: 143.40; 140.45; 138.76; 138.14; 131.84; 130.91; 129.74; 128.28; 128.03; 127.57; 124.61; 120.68; 119.11; 118.12; 115.39; 115.30; 114.97; 114.89; 76.65. IR (ATR-ZnSe) in cm<sup>-1</sup>: 1473. HRMS: M-1, 301.1341 (C<sub>20</sub>H<sub>17</sub>N<sub>2</sub>O). UV  $\lambda_{max}$ : 259 nm, A<sub>259</sub> = 0.36471.  $\Delta T_m$  = 6.6°C. MTT IC<sub>50</sub> = 33.2±0.6µM.









### O-(2-chlorobenzyl)-N-(9'-acridinyl)-hydroxylamine, 6g

Yield: 15%. <sup>1</sup>HNMR (acetone-d<sub>6</sub>)  $\delta$ , in ppm: 9.37 (s, 1H); 9.02 (m, 2H); 8.05 (m, 2H); 7.61 (m, 2H); 7.36 (m, 8H); 7.15 (m, 2H); 7.09 (m, 1H); 6.97 (m, 3H); 5.05 (s, 2H). <sup>13</sup>CNMR (acetone-d<sub>6</sub>)  $\delta$ , in ppm: 143.9; 140.4; 140.4; 138.1; 138.0; 136.4; 132.9; 131.9; 131.0; 130.1; 129.8; 129.2; 129.2; 127.0; 124.6; 120.7; 119.2; 117.9; 115.5; 115.4; 115.0; 114.9; 114.8; 73.7. IR (ATR-ZnSe) in cm<sup>-1</sup>: 746 1474. HRMS: M-1, 335.0952 (C<sub>20</sub>H<sub>16</sub>N<sub>2</sub>OCl).  $\Delta T_m = 18.2^{\circ}C$ . MTT IC<sub>50</sub> = 17.4±0.2µM.





acridine w/O-(2-chlorobenzyl)hydroxylamine hydrochloride Before C2 crystals  $\hfill \hfill \$ 





### O-(3-chlorobenzyl)-N-(9'-acridinyl)-hydroxylamine, 6h

Yield: 46%. <sup>1</sup>HNMR (acetone-d<sub>6</sub>)  $\delta$ , in ppm: 9.37 (s, 1H); 9.00 (m, 1H); 8.08 (m, 1H); 7.40 (m, 6H); 7.10 (m, 2H); 6.98 (m, 2H); 5.32 (s, 2H). <sup>13</sup>CNMR (acetone-d<sub>6</sub>)  $\delta$ , in ppm: 143.8; 141.5; 140.4; 140.4; 138.1; 138.0; 133.6; 131.8; 131.0; 130.0; 129.8; 127.8; 127.5; 126.3; 124.6; 120.7; 119.2; 117.9; 117.9; 115.5; 115.4; 115.04; 115.0; 114.8; 114.8; 75.6. IR (ATR-ZnSe) in cm<sup>-1</sup>: 747, 1474. HRMS: M-1, 335.0948 (C<sub>20</sub>H<sub>16</sub>N<sub>2</sub>OCl).  $\Delta T_m = 18.5^{\circ}$ C. MTT IC<sub>50</sub> = 18.0±0.2µM







### O-(4-chlorobenzyl)-N-(9'-acridinyl)-hydroxylamine, 6i

Yield: 30%. <sup>1</sup>HNMR (acetone-d<sub>6</sub>) δ, in ppm: 9.34 (s, 1H); 8.98 (m, 1H); 8.06 (m, 1H); 7.54 (m, 2H); 7.40 (m, 4H); 7.14 (m, 1H); 7.08 (m, 1H); 6.97 (m, 2H); 5.30 (s, 2H). <sup>13</sup>CNMR (acetone-d<sub>6</sub>) δ, in ppm: 143.7; 140.4; 140.4; 138.1; 138.0; 137.8; 132.8; 131.8; 131.0; 129.8; 129.8; 128.3; 124.6; 120.7; 119.2; 118.0; 118.0; 115.4; 115.4; 115.0; 114.9; 114.8; 114.8; 75.6. IR (ATR-ZnSe) in cm<sup>-1</sup>: 747; 964; 1473; 1489; 1598. HRMS: M-1, 335.0951 (C<sub>20</sub>H<sub>16</sub>N<sub>2</sub>OCl).  $\Delta T_m = 20.2^{\circ}$ C. MTT IC<sub>50</sub> = 17.0±0.4µM.





acridine w/O-(4-chlorobenzyl)hydroxylamine hydrochloride after chromatatron F 4-14 pure





### O-(4-bromobenzyl)-N-(9'-acridinyl)-hydroxylamine, 6j

Yield: 51%. <sup>1</sup>HNMR (acetone-d<sub>6</sub>)  $\delta$ , in ppm: 9.34 (s, 1H); 8.97 (m, 1H); 8.06 (m, 1H); 7.57 (m, 2H); 7.48 (m, 2H); 7.37 (m, 2H); 7.14 (m, 1H); 7.08 (m, 1H); 6.96 (m, 2H); 5.28 (s, 2H). <sup>13</sup>CNMR (acetone-d<sub>6</sub>)  $\delta$ , in ppm: 143.7; 140.4; 140.4; 138.3; 138.1; 138.0; 131.8; 131.3; 131.0; 130.1; 129.8; 124.6; 121.0; 120.7; 119.2; 118.0; 117.9; 115.4; 115.4; 115.0; 114.9; 114.8; 75.7 ppm. IR (ATR ZnSe) in cm<sup>-1</sup>: 747; 964; 1008; 1473; 1486. HRMS: M-1, 379.0421 (C<sub>20</sub>H<sub>16</sub>N<sub>2</sub>OBr).  $\Delta T_m = 18.1^{\circ}$ C. MTT IC<sub>50</sub> = 18.5±4.3µM







### O-(3-nitrobenzyl)-N-(9'-acridinyl)-hydroxylamine, 6k

Yield: 41%. <sup>1</sup>HNMR (acetone-d<sub>6</sub>)  $\delta$ , in ppm: 9.40 (s, 1H); 9.01 (s, 1H); 8.39 (s, 1H); 8.18 (m, 1H); 8.05 (m, 1H); 7.95 (m, 1H); 7.69 (m, 1H); 7.39 (m, 2H); 7.16 (m, 1H); 7.10 (m, 1H); 6.98 (m, 2H); 5.46 (s, 2H). <sup>13</sup>CNMR (acetone-d<sub>6</sub>)  $\delta$ , in ppm: 148.36; 144.21; 141.46; 140.46; 138.10; 134.13; 131.83; 131.12; 129.93; 129.64; 124.60; 122.45; 122.34; 120.75; 119.25; 117.79; 117.76; 115.54; 115.46; 115.07; 114.99; 114.69; 114.65; 75.08. IR (ATR ZnSe) in cm<sup>-1</sup>: 963; 1346; 1473; 1524; 1615. HRMS: M-1, 346.1194 (C<sub>20</sub>H<sub>16</sub>N<sub>3</sub>O<sub>3</sub>)..  $\Delta T_m = 15.1^{\circ}$ C. MTT IC<sub>50</sub> = 31.8±0.1µM









### O-(4-nitrobenzyl)-N-(9'-acridinyl)-hydroxylamine, 6l

Yield: 38%. <sup>1</sup>HNMR (acetone-d<sub>6</sub>)  $\delta$ , in ppm: 9.43 (s, 1H); 9.01 (m, 1H); 8.24 (m, 2H); 8.03 (m, 1H); 7.74 (m, 2H); 7.41 (m, 1H); 7.34 (m, 1H); 7.17 (m, 1H); 7.10 (m, 1H); 6.98 (m, 2H); 5.44 (s, 2H).<sup>13</sup>CNMR (acetone-d<sub>6</sub>)  $\delta$ , in ppm: 147.4; 146.9; 144.2; 140.5; 140.4; 138.1; 138.0; 131.8; 131.1; 129.9; 128.4; 123.4; 120.8; 119.3; 117.7; 117.7; 115.6; 115.5; 115.1; 115.0; 114.7; 114.6; 75.1. IR (ATR ZnSe) in cm<sup>-1</sup>: 748; 1342; 1474; 1518. HRMS: M-1, 346.1172 (C<sub>20</sub>H<sub>16</sub>N<sub>3</sub>O<sub>3</sub>). MTT IC<sub>50</sub> = 30.3±1.4 $\mu$ M



