Supplementary data on BENA435 and related molecules 1, 2, 3, 5, 6, 7, 8, 9, 10, 11 and 14

BENA435 (Free base, pale yellow crystals) 88% yield, mp 162°C (softens) to 210°C (dec.) (Et_2O): 1H NMR (DMSO-d6) δ 1.95 (m, 2 H, CH2-β), 2.32 (s, 6 H, N(CH3)2), 2.50 (m, 2 H, CH2-γ), 2.97 (s, 3 H, 12-CH3), 3.76 (m, 2 H, CH2-α), 3.99 & 3.96 (2*s, 2*3 H, 2*OCH3), 7.45-7.60 (m, 3 H, H-3 + H-9 + H-10), 7.75 (d, 1 H, H-1, J = 1.8 Hz), 7.85 (t, 1 H, N-H), 8.93 (m, 2 H, H-4 + H-7), 9.09. Anal. (C24H28N4O2. 0.5 H2O) Calcd: C, 69.71; H, 7.07; N, 13.55 ; Found : C, 70.01; H, 7.09; N, 13.73

Compound **1** (*6-Methyl-dibenzo[c,h][1,5]naphthyridine*): A mixture of 6-chloro-12-methyldibenzo [*c*,*h*][1,5]naphthyridine (1.10 g, 3.9 mmol) in DMF (100 mL) was hydrogenated at 100°C under 1 atmosphere H_2 pressure using 10% Pd-C (300 mg) as the catalyst. Once hydrogen uptake was complete, the catalyst was removed by filtration, while the mixture was still hot, and washed with hot DMF. The filtrate was evaporated to dryness. Compound **1**, a yellow crystalline solid (250 mg, 28%), was obtained after silica gel column chromatography (CH_2Cl_2) and recrystallization from ethanol (mp 153°C): ^{1}H NMR ($CDCl_3$**)** δ 3.13 (s, 3 H, CH_3), 7.72 (m, 2 H, H-2 + H-3), 7.88 (m, 2 H, H-8 + H-9), 8.07 (d, 1 H, H-1, J = 8.0 Hz), 8.21 (d, 1 H, H-7, J = 8.3 Hz), 9.24 (m, 2 H, H-4 + H-10), 9.39 (s, 1 H, H-12). Anal. ($C_{17}H_{12}N_2$) Calcd: C, 83.58; H, 4.95; N, 11.47 ; Found : C, 83,09; H, 4,86; N, 11,40.

Compounds **2, 5, 6** and **7 (***6-Aminomethyl substituted dibenzo[c,h][1,5]naphthyridine derivatives*):
Step 1: SeO_2 oxidation: To a solution of **1** (667 mg; 3 mmol) in dioxane (90 ml) was slowly added SeO_2 (380 mg). The reaction mixture was refluxed for 3 h and the hot solution was then filtered and washed with hot dioxane. The solvent was removed under reduced pressure and the residue was recrystallized in toluene giving dibenzo[*c*,*h*][1,5]naphthyridine-6-carbaldehyde (450 mg, 57%), Anal. ($C_{17}H_{10}N_2O$) Calcd: C, 79.06; H, 3.90; N, 10.85 ; Found : C, 78,61; H, 3,77; N, 10,41.
Step 2: Reductive amination: N'-Dibenzo[c,h][1,5]naphthyridin-6-ylmethyl-N,N-diethyl-propane-1,3-diamine (compound **2**) : Example of the general method.
$NaBH_4$ (293 mg, 7.8 mmol) was added portionwise to a refluxing solution of the above aldehyde intermediate (500 mg, 2 mmol) and N,N-diethylaminopropyl-1,3-diamine (620 mg, 6 mmol) in MeOH (10 mL). The mixture was refluxed for 1 h. Cold water was then added and the resulting mixture extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, concentrated, and the residue was neutral alumina (7% water) column chromatographed (CH_2Cl_2/EtOH: 100/0 to 95/5 gradient). The free base of compound **2**, obtained as yellow oil, was dissolved in HCl/MeOH and concentrated to dryness. Ether was added and the yellow precipitate of **2**·HCl was collected (610 mg, 30%), mp 145°C (softens) ^{1}H NMR (DMSO-d_6) δ 1.29 (t, 6 H, 2*CH_3), 2.43 (m, 2 H, CH_2-β), 3.19 (m, 4 H, 2*C*H*$_2$$CH_3$), 3.31 (m, 2 H, CH_2-γ), 3.43 (m, 2 H, CH_2-α), 5.29 (br s, 2 H, CH_2-6), 8.02 (m, 2 H, H-2 + H-3), 8.16 (m, 2 H, H-8 + H-9), 8.47 (m, 2 H, H-1 + H-7), 9.35 (d, 1 H, H-4, J = 8.2 Hz), 9.54 (d, 1 H, H-10, J = 8.2 Hz), 9.75 (s, 1 H, H-12), 10.00 (br s, 2 H, H-Hydrochloride salt), 10.92 (br s, 1 H, H-Hydrochloride salt). Anal ($C_{24}H_{29}N_4$· 3 HCl· 1.5 H_2O) Calcd: C, 56.64; H, 6.73; N, 11.01 ; Found : C, 56.43; H, 6.38; N, 10.78·
Using the requisite amines compounds **5, 6** and **7** were also prepared by reductive amination.

Compound **5** (yellow powder, 56% yield) mp 160°C (softens) 1H NMR ($CDCl_3$) δ 2.22 (s, 6 H, 2*CH_3), 2.54 (t, 2 H, CH_2-β, J = 2.9 Hz), 2.91 (m, 2 H, CH_2-α), 4.58 (s, 2 H, CH_2-6), 7.69 (m, 2 H, H-2 + H-3), 7.86 (m, 2 H, H-8 + H-9), 8.05 (d, 1 H, H-1, J = 7.8 Hz), 8.20 (d, 1 H, H-7, J = 8.1 Hz), 9.23 (m, 2 H, H-4 + H-10), 9.37 (s, 1 H, H-12). Anal. ($C_{21}H_{22}N_4$·3H_2O) Calcd: C, 65.60; H, 7.34; N, 14.57 ; Found : C, 65.84; H, 6.95; N, 14.60.

Compound **6** (yellow powder, 50% yield): mp 45°C (softens); ^{1}H NMR (DMSO-d_6) δ 1.72 (m, 2 H, CH_2-β), 2.15 (s, 6 H, N(CH_3)$_2$), 2.32 (t, 2 H, CH_2-γ), 2.82 (m, 2 H, CH_2-α), 4.58 (s, 2 H, CH_2-6), 7.93 (m, 2 H, H-2 + H-3), 8.09 (m, 2 H, H-8 + H-9), 8.40 (d, 1 H, H-1, J = 8.0 Hz), 8.59 (d, 1 H, H-7, J = 8.3 Hz), 9.27 (m, 2 H, H-4 + H-10), 9.65 (s, 1 H, H-12). Anal. ($C_{22}H_{24}N_4$· 3H_2O) Calcd: C, 66.31; H, 7.59; N, 14.06 ; Found : C, 66.62; H, 7.57; N, 14.12.

Compound **7** (pale pink powder, 57% yield): mp 156°C; ^{1}H NMR ($CDCl_3$) δ 1.15 (s, 3 H, CH_3), 3.40-3.65 (m, 4 H, 2*C*H*$_2$-OH), 4.67 (s, 2 H, CH_2-6), 7.72 (m, 2 H, H-2 + H-3), 7.92 (m, 2 H, H-8 + H-9), 8.09 (d, 1 H, H-1, J = 7.8 Hz), 8.22 (d, 1 H, H-7, J = 7.9 Hz), 9.10 (d, 1 H, H-4, J = 8.4 Hz), 9.28 (d, 1 H, H-10, J = 8.4 Hz), 9.41 (s, 1 H, H-12). Anal. ($C_{21}H_{23}N_3O_3$·H_2O) Calcd: C, 69.02; H, 6.34; N, 11.50 ; Found : C, 69.15; H, 6.32; N, 11.33.

Compound **3:** (*2-[2-(Dibenzo[c,h][1,5]naphthyridin-6-ylamino)-ethylamino]-ethanol*)
6-Chloro-dibenzo[*c,h*][1,5]naphthyridine (500 mg, 2 mmol) was heated in 2-(2-amino-ethylamino)-ethanol (10 mL) at reflux for 3 h. Excess of diamine was evaporated to dryness under reduced pressure and the residue was extracted (CH_2Cl_2/H_2O). The combined organic layers were dried over $MgSO_4$, concentrated and the residue was neutral alumina (7% water) column chromatographed (EtOH-CH_2Cl_2, 0 to 10% gradient; then CH_2Cl_2/EtOH/NEt_3, 90/10/0.5). Compound **3** was obtained as a beige powder (65 mg, 10%), mp 100°C (softens): 1H NMR ($CDCl_3$) δ 2.86 (t, 2 H, C*H*$_2$CH$_2$OH, J = 5.1 Hz), 3.12 (t, 2 H, C*H*$_2$CH$_2$NHAr, J = 5.7 Hz), 3.67 (t, 2 H, C*H*$_2$NHAr, J = 5.0 Hz), 3.98 (m, 2 H, C*H*$_2$OH), 7.62 (m, 2 H, H-2 + H-3), 7.78 (m, 2 H, H-8 + H-9), 7.88 (d, 1 H, H-1, J = 8.1 Hz), 7.99 (d, 1 H, H-7, J = 8.1 Hz), 9.02 (d, 1 H, H-4, J = 8.0 Hz), 9.12 (m, 2 H, H-10 + H-12). Anal ($C_{20}H_{20}N_4O \cdot 0.33\ H_2O$) Calcd: C, 71.00; H, 6.11 ; Found : C, 71.34; H, 6.33.

Compounds **8**, **9**, **10**, **11** and **14** (*6- Dimethylaminoalkylamino- and 6,12-bis-[dimethylaminoalkylamino]-dibenzo[c,h][1,5]naphthyridines*). General procedure: The requisite chlorointermediate [6-chloro-2-methoxy-12-methyldibenzo[*c,h*][1,5]naphthyridine (for **8**); 6,12-dichloro-2-methoxydibenzo[*c,h*][1,5]naphthyridine (for **9** and **10**); 6,12-dichloro-2,8-dimethoxydibenzo[*c,h*][1,5]naphthyridine (26) (for **11** and **14**)] was heated at reflux in a large excess of either *N,N*-dimethylethane-1,2-diamine or *N,N*-dimethylpropane-1,3-diamine till disappearance of the starting chloro compound (12-48 h). The mixture was then concentrated under reduced pressure, taken up in water and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, concentrated and the residue was neutral alumina (7% water) column chromatographed (EtOH-CH_2Cl_2; 0 to 1% gradient) giving the expected 6,12-diamino substituted dibenzonaphthyridine.

Compound **8** (yellow powder, 33**%** yield): mp 139°C; ^{1}H NMR ($CDCl_3$) δ 1.94 (m, 2 H, CH_2-β), 2.36 (s, 6 H, N(CH_3)$_2$), 2.57 (t, 2 H, CH_2-γ), 3.00 (s, 3 H, 12-CH_3), 3.88 (m, 2 H, CH_2-α), 3.95 (s, 3 H, OCH_3), 7.36 (m, 2 H, H-1 + H-3), 7.52 (m, 1 H, H-8), 7.74 (m, 3 H, H-7 + H-9 +N-H), 9.00 (d, 1 H, H-4, J = 8.9 Hz), 9.09 (d, 1 H, H-10, J = 8.2 Hz). Anal. ($C_{23}H_{26}N_4O$) Calcd: C, 73.77; H, 7.00; N, 14.96 ; Found : C, 73.28; H, 6.92; N, 14.56.

Compound **9** (yellow powder, 18% yield): mp 169°C; ^{1}H NMR (DMSO-d_6) δ 2.29 (2*s, 2*6 H, 2*N(CH_3)$_2$), 2.67 (m, 4 H, 2*CH_2-β), 3.78 (m, 4 H, 2*CH_2-α), 3.96 (s, 3 H, OCH_3), 7.22 (m, 2 H, H-8 + H-9), 7.44 (dd, 1 H, H-3, J = 2.0 & 8.9 Hz), 7.60 (t, 1 H, N-H), 7.70 (d, 1 H, H-1, J = 2.0 Hz), 7.78 (t, 1 H, N-H), 8.24 (d, 1 H, H-7, J = 8.2 Hz), 8.82 (d, 1 H, H-4, J = 8.9 Hz), 8.88 (d, 1 H, H-10, J = 8.2 Hz). Anal. ($C_{25}H_{32}N_6O \cdot 0.5\ H_2O$) Calcd: C, 68.00; H, 7.53; Found : C, 68.39; H, 7.37.

Compound **10** (yellow powder, 50% yield): mp 155°C; ^{1}H NMR (DMSO-d_6) δ 1.96 (m, 4 H, 2*CH_2-β), 2.24 (2*s, 2*6 H, 2*N(CH_3)$_2$), 2.46 (m, 4 H, 2*CH_2-γ), 3.74 (m, 4 H, 2*CH_2-α), 3.99 (s, 3 H, OCH_3), 7.44 (m, 3 H, H-3 + H-8 + H-9), 7.63 (t, 1 H, N-H), 7.71 (d, 1 H, H-1, J = 2.0 Hz), 7.81 (t, 1 H, N-H), 8.29 (d, 1 H, H-7, J = 8.2 Hz), 8.86 (d, 1 H, H-4, J = 8.9 Hz), 8.95 (d, 1 H, H-10, J = 8.0 Hz). Anal. ($C_{27}H_{36}N_6O$) Calcd: C, 70.40; H, 7.88; Found : C, 70.03; H, 7.66.

Compound **11** (yellow powder, 70% yield): mp 216°C; ^{1}H NMR (DMSO d-$_6$) δ 1.97 (m, 4 H, 2*CH_2-β), 2.25 (s, 12 H, 2*N(CH_3)$_2$), 2.45 (t, 4 H, 2*CH_2-γ), 3.73 (m, 4 H, 2*CH_2-α), 3.98 (s, 6 H, 2*OCH_3), 7.37 (t, 2 H, 2*N-H), 7.45 (dd, 2 H, H-3 + H-9, J = 9.0 & 2.2 Hz), 7.69 (d, 2 H, H-1 + H-7, J = 2.2 Hz), 8.83 (d, 2 H, H-4 + H-10, J = 9.0 Hz). Anal. ($C_{28}H_{38}N_6O_2$) Calcd: C, 68.54; H, 7.81; N, 17.13 ; Found : C, 68.35; H, 7.67; N, 17.07.

Compound **14** (yellow powder, 16% yield): mp 209°C; ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 12 H, 2*N(CH_3)$_2$, 2.73 (t, 4 H, 2*CH_2-β), 3.84 (m, 4 H, 2*CH_2-α), 3.93 (s, 6 H, 2*OCH_3), 5.81 (br s, 2 H, 2*N-H), 7.20 (d, 2 H, H-1 + H-7, J = 2.3 Hz), 7.33 (dd, 2 H, H-3 + H-9, J = 9.0 & 2.3 Hz), 8.90 (d, 2 H, H-4 + H-10, J = 9.0 Hz). Anal. ($C_{26}H_{34}N_6O_2 \cdot H_2O$) Calcd: C, 64.98; H, 7.55; Found : C, 64.43; H, 7.41.

Stability of BENA435: When stored in the dark in anhydrous DMSO at -25°C BENA435 is stable for periods up to 3 years (LC-MS). When stored in DMSO at 4°C in the dark, BENA435 is stable for at least one year. At ambient temperature BENA435 was found to be stable in DMSO solution for at least 48 hours when stored in the dark.

Solubility of BENA435: In DMSO BENA435 (free base) is soluble until saturation at ~7 mM. As much as 1 volume of 7 mM BENA435 in DMSO mixed with 7 volumes of H_2O forms a turbid solution (0.875 mM BENA435 in ~ 12% DMSO). Upon sonication for 1 min this solution becomes limpid and can be further dissolved in aqueous solutions.

Decontamination of BENA435: 2 ml of aqueous solution of BENA435 at 75 mM were incubated for 5 min at RT with 100 mg of activated charcoal. After filtration BENA435 is undetectable by spectrometry.