Development of a Pterin-based Fluorescent Probe for Screening Dihydropteroate Synthase

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SUPPORTING INFORMATION

Content: **page**

Kd fit equation for competition experiments:

The following equation derived from the definition of dissociation constant with the approximation of a negligible concentration of fluorescent probe:

$$FP = FP^{0} + \Delta FP \bullet \left\{ \frac{-\left[K_{comp} \bullet \left([comp] - [DHPS]\right) + 2 \bullet [DHPS] + K_{D}\right] + \sqrt{\left[K_{comp} \bullet \left([comp] - [DHPS]\right) + 2 \bullet [DHPS] + K_{D}\right]^{2} + 4 \bullet [DHPS] \bullet \left[K_{comp} \bullet \left([DHPS] - [comp]\right) + K_{comp} \bullet K_{D} - [DHPS] - K_{D}\right]}}{2 \bullet \left[K_{comp} \bullet \left([DHPS] - [comp]\right) + K_{comp} \bullet K_{D} - [DHPS] - K_{D}\right]} \right\}$$

where K_D is the dissociation constant between the probe and DHPS, K_{comp} is the ratio between dissociation constant between the K_D and the K_d of the competitor.

Method for HPLC/UV/ELSD/MS

LC-MS chromasolv grade methanol and ammonium bicarbonate were obtained from Sigma-Aldrich (St. Louis, MO). Milli-Q water as an ultrapure laboratory grade water was used in aqueous mobile phase.

Chromatographic separation was performed on an Acquity UPLC BEH C18 1.7 μm, 2.1 x 50 mm column (Waters Corporation, Milford, MA) using an Acquity ultra performance liquid chromatography system. Data were acquired using Masslynx v. 4.1 and analyzed using the Openlynx software suite. This was coupled to an Acquity photodiode array detector, which acquired UV data from 210-400 nm. The flow was then split, with half directed to an evaporative light scattering detector (ELSD) and half to an SQ mass spectrometer. The total flow rate was 0.7 mL/min. The sample injection volume was 2 μL. The UPLC column was maintained at 50 °C and the gradient program started at 90% A (10 mM ammonium bicarbonate in MilliQ H_2O), changed to 70% A over 0.2 min, to 95 % B (100% methanol) over 1.4 minutes, held for 0.35 minutes, then to 90% A over 0.05 minutes. The mass spectrometer was operated in positive-ion mode with electrospray ionization. The conditions were as follows: capillary voltage 3.4 kV, cone voltage 30 V, source temperature 130 °C, desolvation temperature 400 °C, desolvation gas 800 L/hr, cone gas 100 L/hr. A full scan range from m/z = 110-1000 in 0.2 s was used to acquire MS data. The ELSD-drift tube temperature was set at 52 °C.

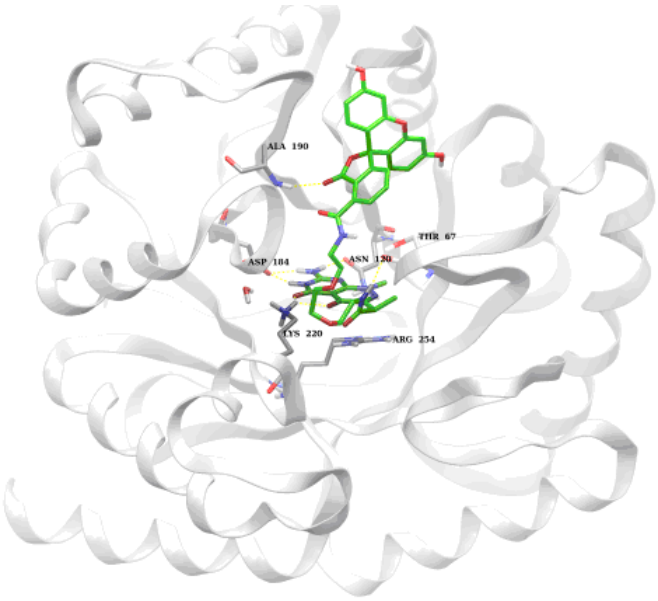


Figure S1 Probe 4 docked into DHPS active site

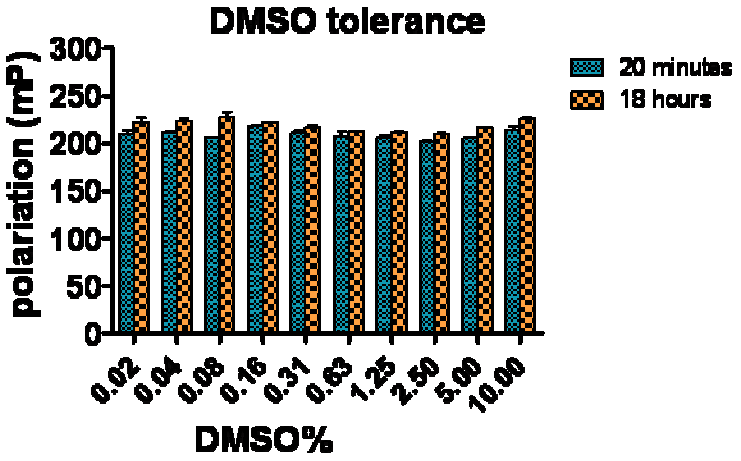
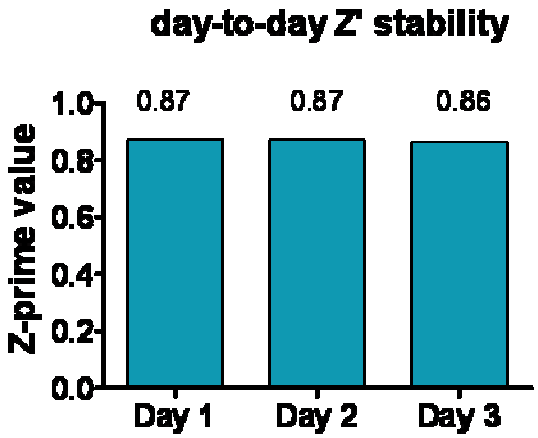


Figure S2: Effect of DMSO on binding experiments. 2.7 μM of **4** and 8 μM of *Ba*DHPS were incubated with 10% in highest, 1-to-2 dilution for 10 concentration level, down to 0.02% of DMSO for 20 min and 18 hr.



$$Z' = 1 - \frac{3\sigma^{+} + 3\sigma^{-}}{Mean^{+} - Mean^{-}}$$

Figure S3: Day-to-day Z' stability: In the equation, σ+ is the standard deviation of high signal group with protein and **4**; σ – is the standard deviation of low signal group with 100 μM of tracer **2**, protein and **4**; $Mean^{+}$ is the mean of high signal group with protein and **4** and $Mean^{-}$ is the mean of low signal group with 100 μM of tracer **2**, protein and **4**.

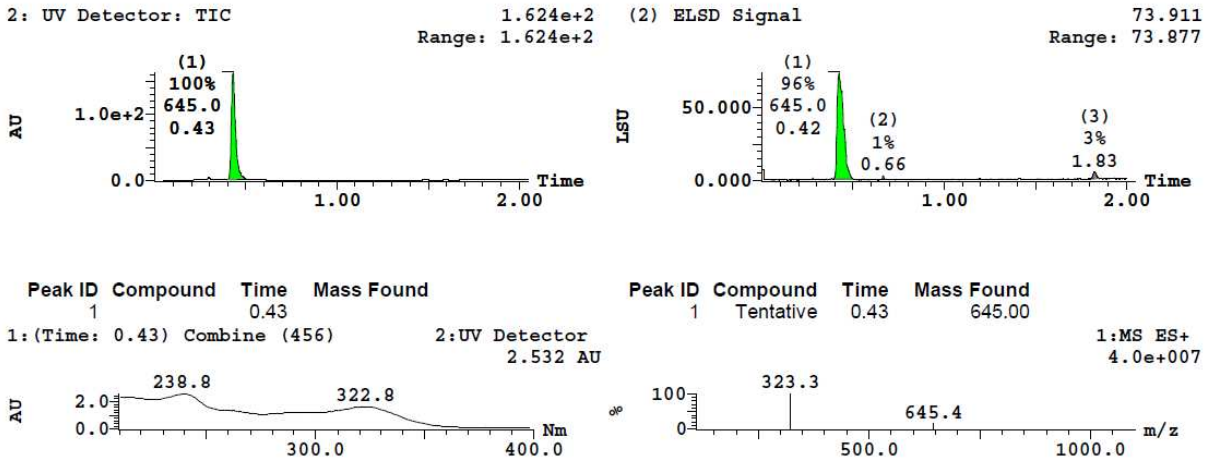
Openlynx Report - Page 1

Sample: 13 Vial:5:F,11 ID:070713
File:3 Date:08-Jul-2010 Time:16:38:03
Description:

Printed: Wed Jun 29 15:53:54 2011

Sample Report:

Sample 13 Vial 5:F,11 ID 070713 File 3 Date 08-Jul-2010 Time 16:38:03 Description



Peak ID	Compound	Time	Mass Found
1		0.43	

Peak ID	Compound	Time	Mass Found
1	Tentative	0.43	645.00

Figure S4: HPLC/UV/ELSD/MS analysis for **3**.

Sample: 14 Vial:5:F,12 ID:070713
File:4 Date:08-Jul-2010 Time:16:41:19
Description:

Printed: Wed Jun 29 15:53:54 2011

Sample Report (continued):

Sample 14 Vial 5:F,12 ID 070713 File 4 Date 08-Jul-2010 Time 16:41:19 Description

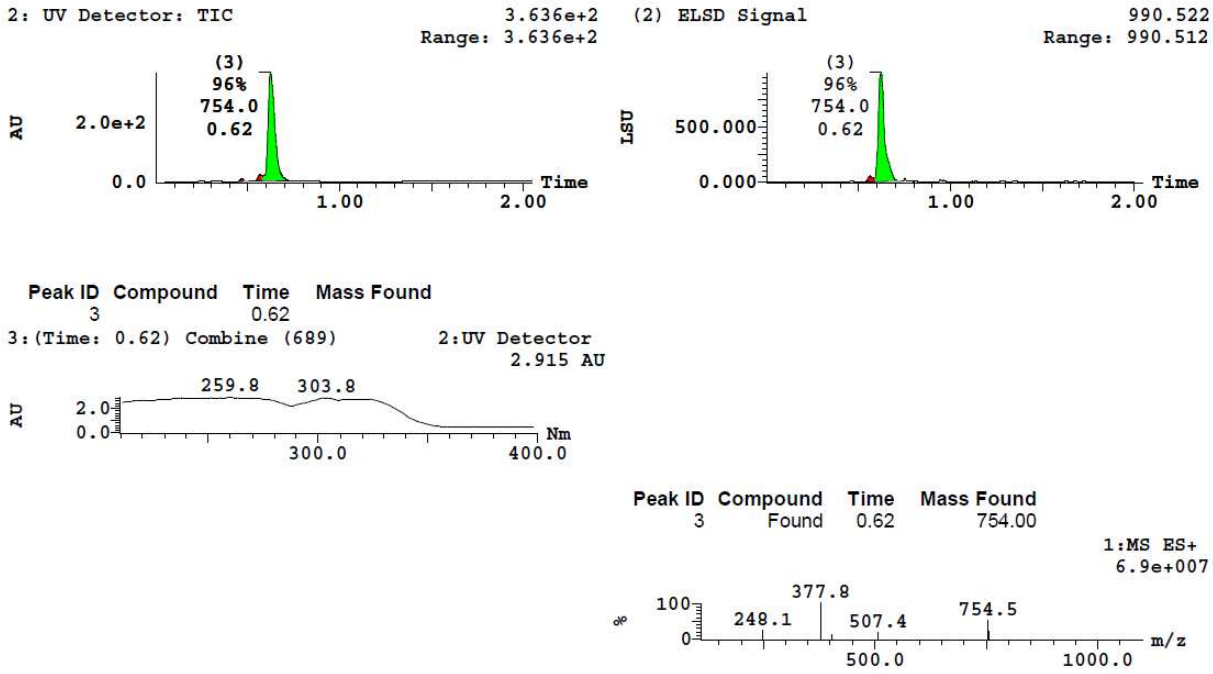


Figure S5: HPLC/UV/ELSD/MS analysis for **4**.