# Science Advances

AAAS

## Supplementary Materials for

## An anticancer drug suppresses the primary nucleation reaction that initiates the production of the toxic Aβ42 aggregates linked with Alzheimer's disease

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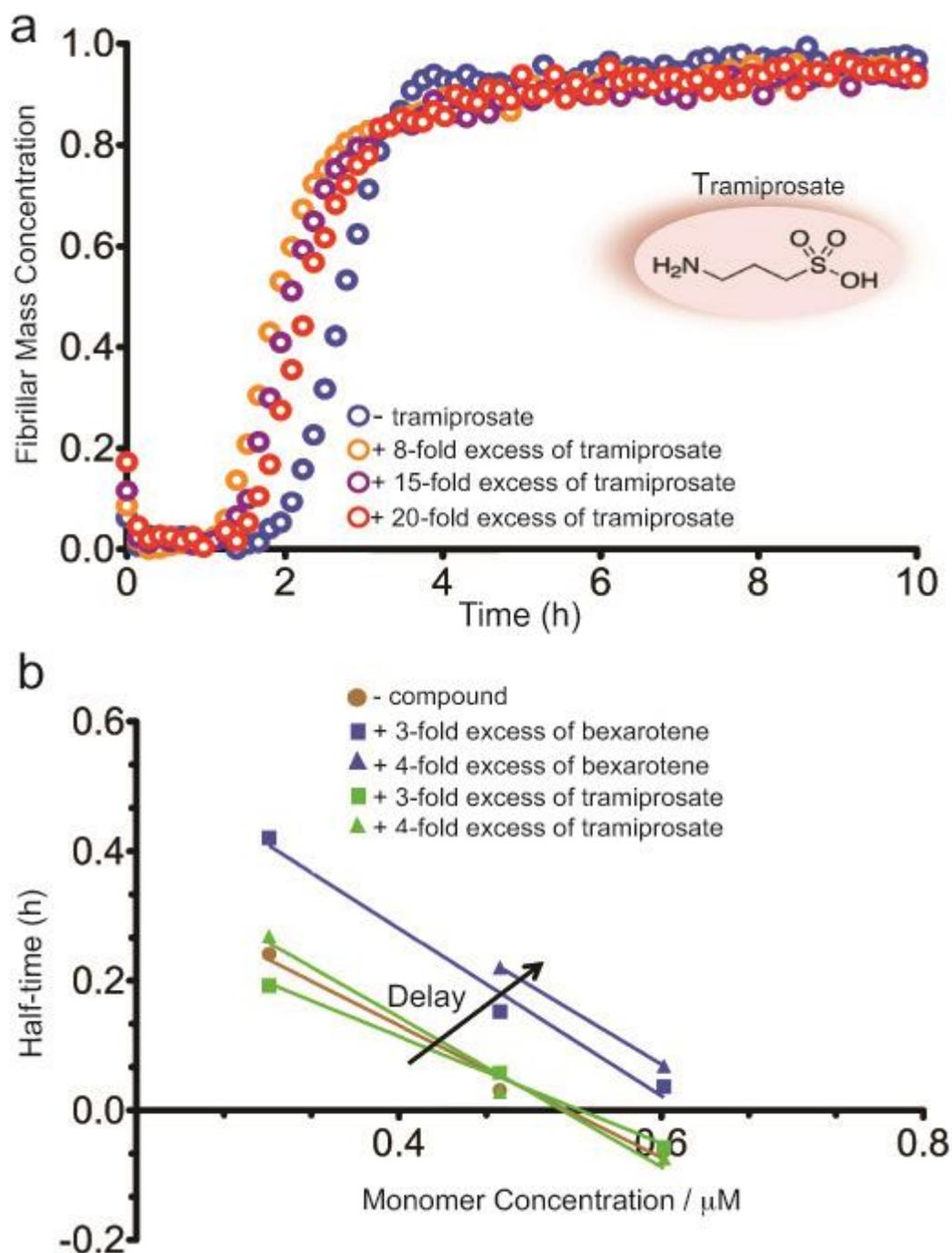
**Figure S1: Comparison of the effects of bexarotene and tramiprosate on Aβ42 aggregation. (a)** Kinetics profiles of the aggregation of 2 μM Aβ42 under quiescent conditions in the absence and in the presence of either 8-fold, or 15-fold or 20-fold excess of tramiprosate. **(b)** Average half-time as a function of the initial monomer concentration in the absence or the presence of either 3-fold or 4-fold excess of bexarotene or tramiprosate. Note that the delay in Aβ42 aggregation is observed only in the presence of bexarotene.
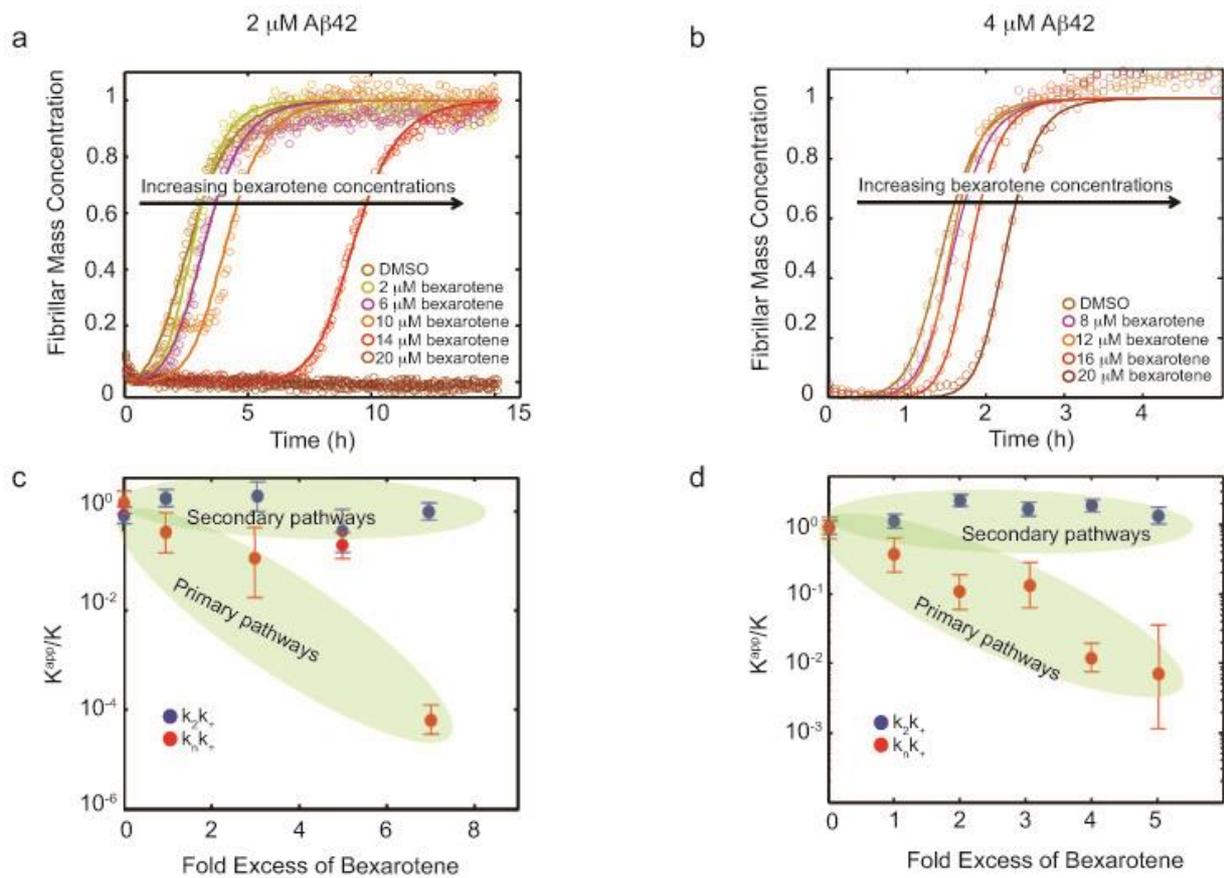
**Figure S2: Quantitative evaluation of the effect of bexarotene on the rate constants of Aβ42 aggregation.** Kinetic profiles of the aggregation of 2 μM **(a)** and 4 μM **(b)** Aβ42 in the absence or in the presence of increasing concentrations of bexarotene (represented by different colors). The global experimental profiles are well described by a kinetic model (represented by solid lines) that considers a specific inhibition of the primary nucleation rate constant, $k_n$. **(c, d)**, Plots of the evolution of the apparent reaction rate constants with increasing fold excesses of bexarotene ($k_n$ for primary nucleation, $k_+$ for elongation and $k_2$ for secondary nucleation). Note the significant decrease in primary pathways, $k_n k_+$, when compared to secondary pathways, $k_2 k_+$.
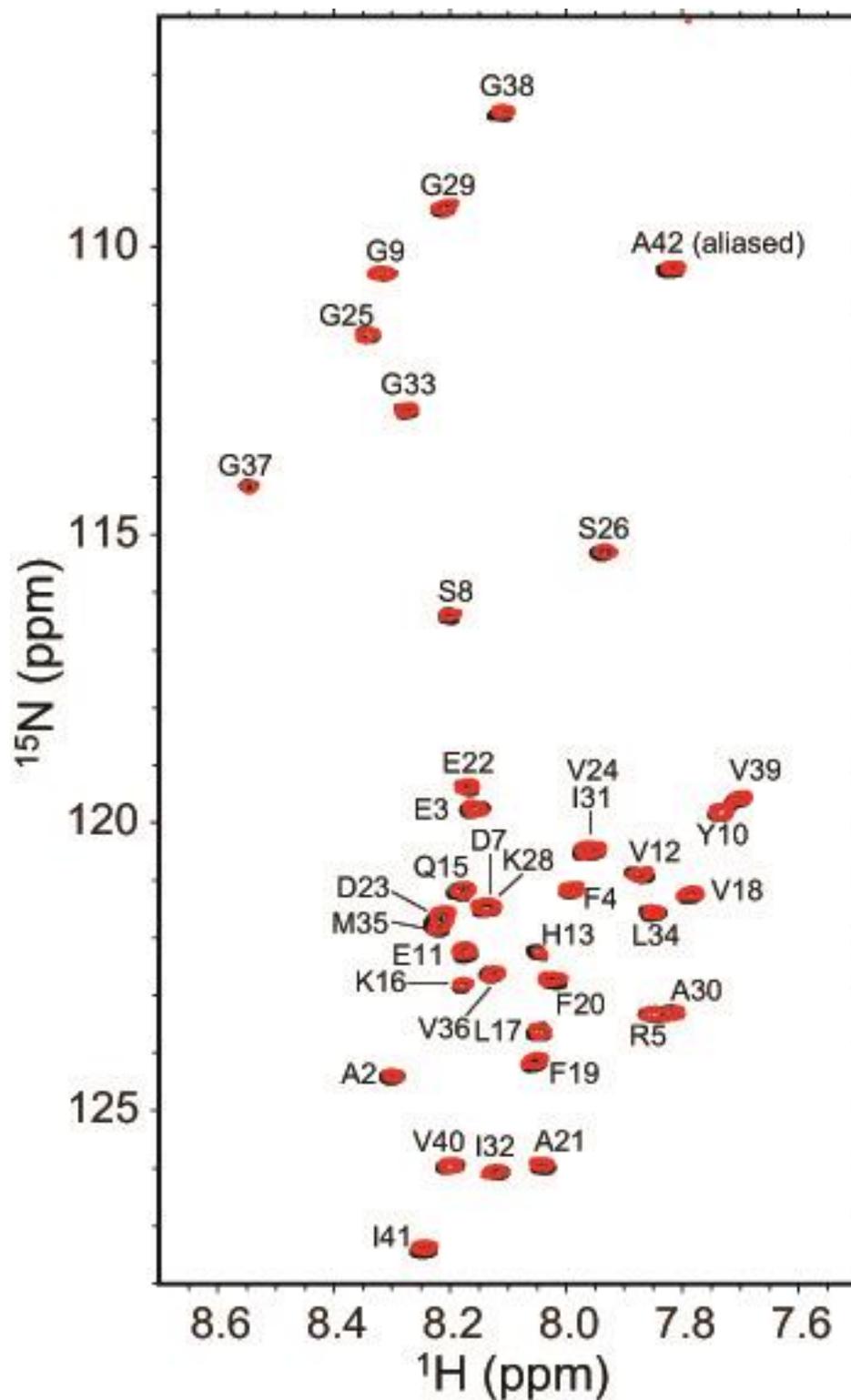
**Figure S3: HSQC spectra of $^{15}$N-A$\beta$42 in the absence and the presence of fivefold excess of bexarotene.** Superimposition of the $^1$H -$^{15}$N HSQC spectra of 25 $\mu$M monomeric $^{15}$N-A$\beta$42 in the absence (black) and in the presence of 5-fold excess of bexarotene (red). The spectra were obtained at a $^1$H frequency of 500 MHz and 278 K.
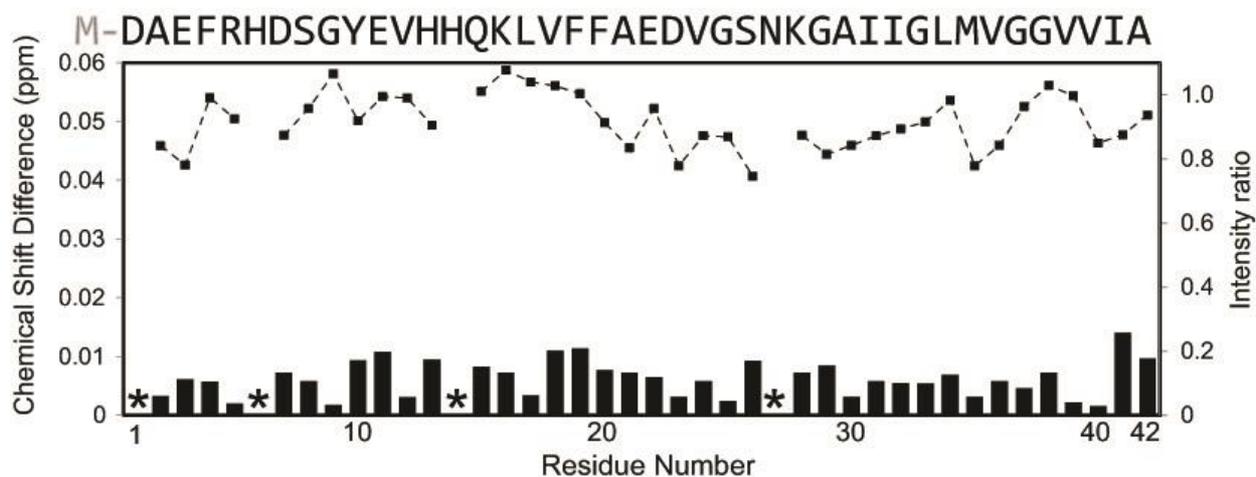
**Figure S4: Interaction between ^{15}N-Aβ42 and bexarotene.** Chemical shifts (bar graph) and normalized intensity (dotted line) of 25 μM monomeric ^{15}N-Aβ42 in the presence of 5-fold excess of bexarotene in 2.5% DMSO are similar to those observed for Aβ42 in the absence of bexarotene suggesting that persistent binding events are unlikely to occur.
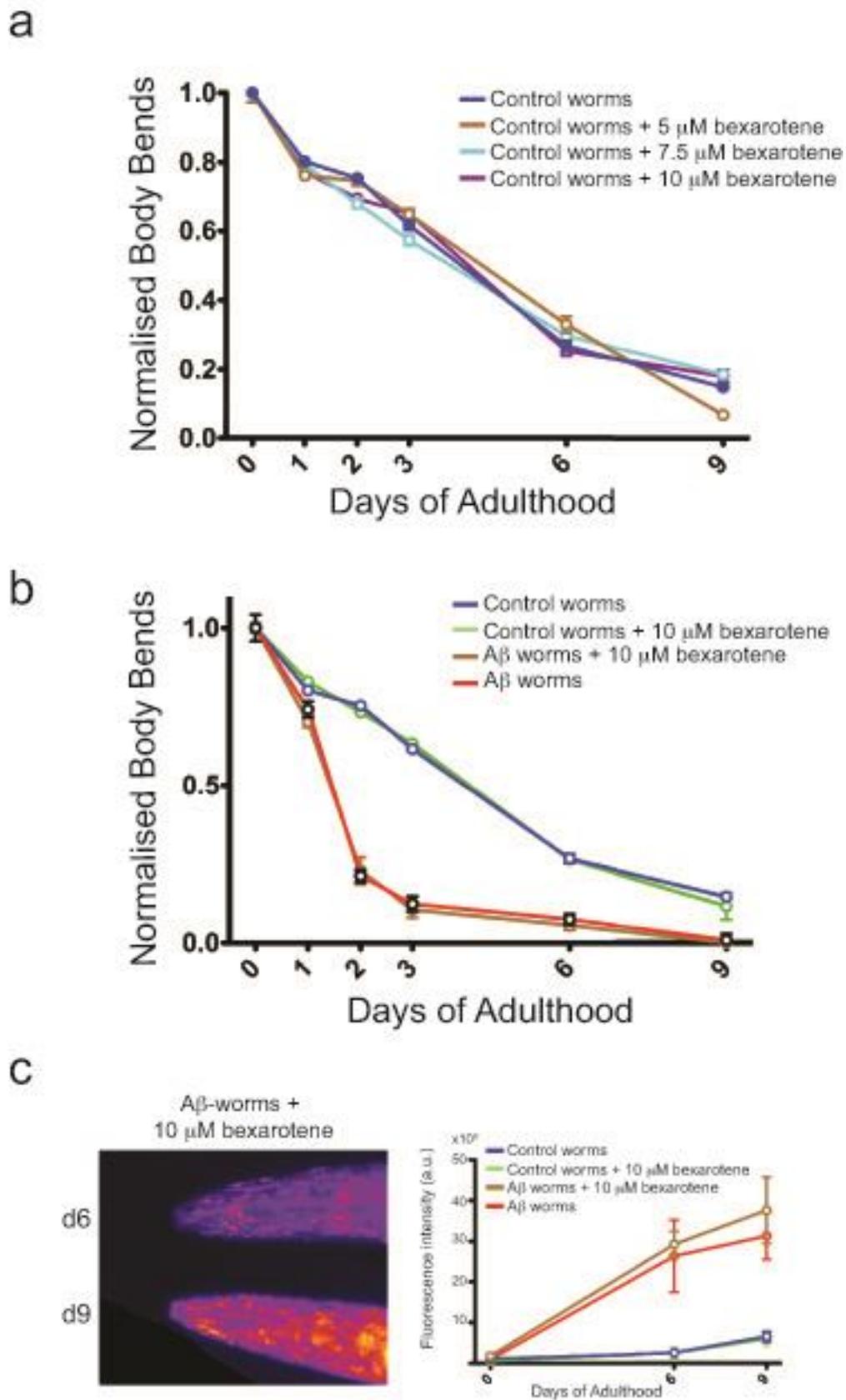
**Figure S5: Effect of bexarotene on Aβ42 aggregation in *C. elegans*.**
**(a)** Measurements of the effect of increasing concentrations of bexarotene ranging from

5 to 10 $\mu$M on the frequency of body bends of control models of *C. elegans.* Normalized values with respect to day 0 are shown. One experiment is shown as a representative of four independent experiments (n ≥ 111). Note the absence of effect of bexarotene at concentrations as high as 10 $\mu$M on the control worm model, thus supporting a specific A$\beta$-bexarotene interaction. **(b)** Effect of bexarotene at 10 $\mu$M concentration on the frequency of body bends given only at day 2 of the A$\beta$ and control-worm models. Data are shown for a single experiment but are representative of three independent experiments. Normalized values with respect to day 0 are shown. (**c**) Time course *in vivo* imaging and quantification of amyloid aggregates formed in A$\beta$ and control-worm models in the absence and in the presence of bexarotene at a concentration of 10 $\mu$M that was given only at day 2. Images of aggregates were taken *in vivo* with a Zeiss Axio Observer fluorescence microscope after staining with NIAD-4 as an amyloid-specific dye. Quantification of fluorescence intensity was performed using ImageJ software (see Methods). In all panels, error bars represent the SEM.
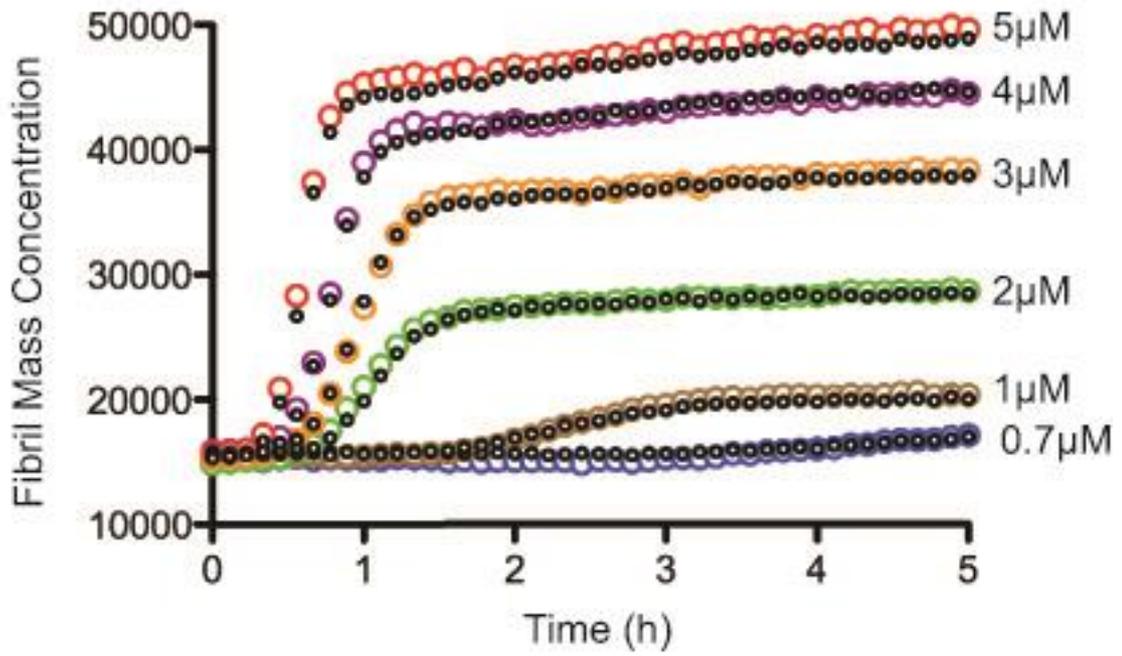
**Figure S6: Effect of DMSO on Aβ42 aggregation.** Superimposition of the kinetic profiles of Aβ42 aggregation at 6 initial monomer concentrations in the absence (shown in colors) or in the presence (shown in black) of 1% DMSO. The addition of 1% DMSO affects neither the slope nor the lag time of the aggregation of Aβ42.
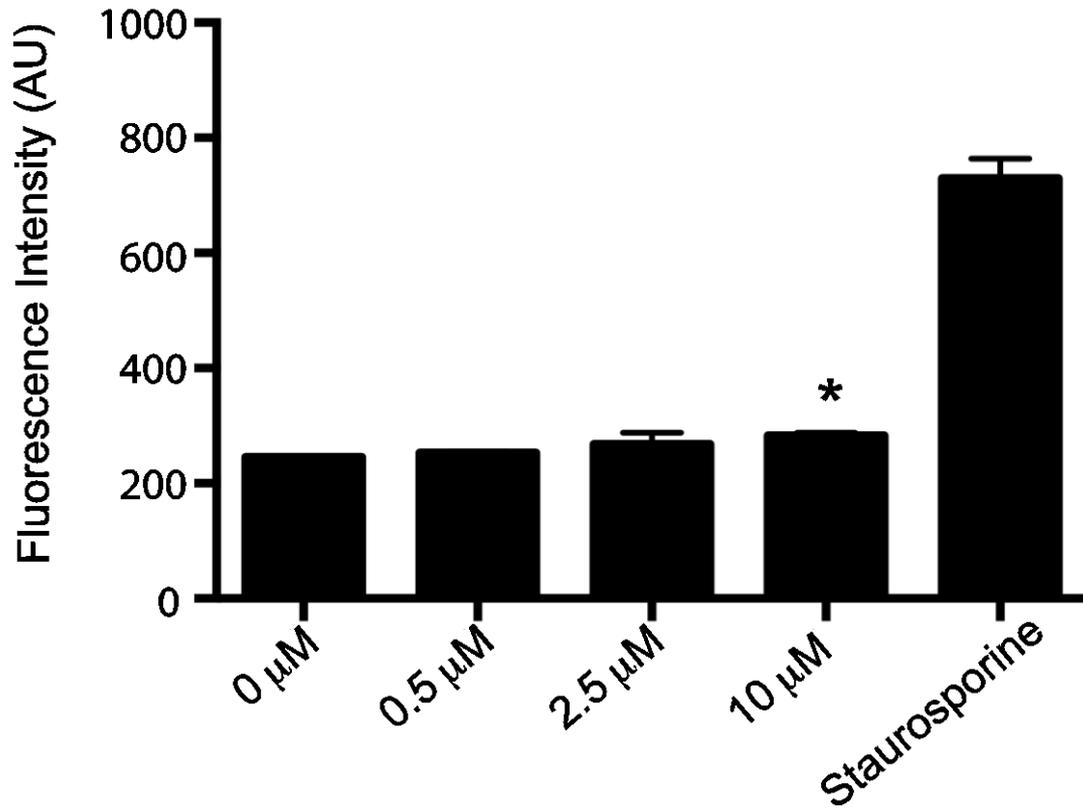
**Figure S7: Toxicity induced by bexarotene in SH-SY5Y human neuroblastoma cells.** No toxicity was detected for DMSO-solubilized (i.e. 1% DMSO final concentration) bexarotene in the concentration range of 1 $\mu$M to 10 $\mu$M. 10 $\mu$M (labeled with a star) is the concentration of bexarotene that was used in the toxicity assays.