Supplementary Information

On the use of the experimentally determined enzyme inhibition constant as a measure of absolute binding affinity

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Table of Contents

Chemicals. NaH_2PO_4, Na_2HPO_4, acetylthiocholine chloride (ATCh), and Triton X-100 were purchased from ACROS (Morris Plains, NJ). 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) and 9-amino-1,2,3,4-tetrahydroacridinium monohydrochloride (tacrine) were ordered from Sigma-Aldrich (St. Louis, MO). 1,7-*N*-heptylene-bis-9,9'-amino-1,2,3,4-tetrahydro-acridinium dihydrochloride [Bis(7)-tacrine] was synthesized according to a published scheme[1].

Inhibitor Purity. Tacrine: Anal. Calcd. for $C_{13}H_{17}ClN_2O$: C, 61.78; H, 6.78; N, 11.08. Found: C, 61.57; H, 7.20; N, 11.17. Bis(7)-tacrine: Anal. Calcd. for $C_{32}H_{44}Cl_2N_4O_2$: C, 65.41; H, 7.55; N, 9.53. Found: C, 65.81; H, 7.63; N, 9.34. Both elemental Analyses were performed at NuMega (San Diego, CA).

Enzymes. *Electrophorus electricus* acetylcholinesterase (*ee*AChE) was purchased from Sigma-Aldrich (St. Louis, MO; catalog # of C2888 with log #s of SLBN0954V and SLBS4398 and specific enzyme activity of ≥1000 U/mg; catalog # of 3389 with log # of SLBL3186V and specific enzyme activity of 200–1000 U/mg).

General lab supplies. The 1.5-mL or 2.0-mL microcentrifuge tubes were purchased from Fisher Scientific (Asheville, NC; catalog #s of 02-682-550 for 1.5-mL and 02-681-258 for 2.0-mL). The 7.4-mL general-purpose borosilicate glass vials were purchased from Fisher Scientific (Asheville, NC; catalog # of 03-339-22D). The 15-mL conical centrifuge tubes were purchased from Fisher Scientific (Asheville, NC; catalog # of 352095). The 0.2-mL MicroAmp® 8-tube strips were purchased from Applied Biosystems (Foster City, CA; catalog # of N8010580). The 100-mL and 250-mL reagent bottles were purchased from Fisher Scientific (Asheville, NC; product #s of S02286 for 100-mL and S02286A for 250-mL). The 16-mL dark-glass reagent bottles were purchased from Fisher Scientific (Asheville, NC; catalog # of 03-339-23D). Natural, non-sterile, low-retention, 0.1–10-µL pipet tips were

purchased from Fisher Scientific (Asheville, NC; catalog # of 02717-134). Natural, non-sterile 100–300-µL and 100–1000-µL pipet tips were purchased from Molecular BioProducts (San Diego, CA; catalog # of 3580 and 3771, respectively). The non-sterile, flat bottom, clear, 96-well plates were purchased from Fisher Scientific (Asheville, NC; catalog # of 12565501). The 25-mL disposable Matrix™ Reagent Reservoirs were purchased from Fisher Scientific (Asheville, NC; catalog # of 14-387-071).

General lab tools and equipment for enzyme inhibition assay. Sodium phosphates, Ellman assay reagents, and inhibitors were weighed using either a three-digit analytical balance from Denver (Bohemia, NY; Model: RS232) or an analytic plus electronic balance from Ohaus (Florham Park, NJ; Model: AP250D). The Ellman assay reagent and inhibitor solutions were prepared using a 40-mm octagonal magnetic stir bar with integral pivot ring from Fisher Scientific (Asheville, NC; catalog # of 14-513-56) and a stirrer from CORNING (Tewksbury, MA; Model: PC420) for stirring at a speed of 4–6 revolutions per minute at 26 ± 1 °C and transferred using an Eppendorf Reference series adjustable-volume 0.5–10-µL pipette from Eppendorf (Hauppauge, NY; catalog # of 022470051), a Finnpipette F1 1–10-µL 8-channel pipette from Fisher Scientific (Asheville, NC; catalog # of 03-339-22D), an Eppendorf Research 8-channel 30–300-µL pipette from Eppendorf (Hauppauge, NY; catalog # of 022452100), a 1000-µL Pipetman P1000 pipette from Gilson (Middleton, WI; catalog # of EF13988P), a 20–200-µL Eppendorf Research pipette from Eppendorf (Hauppauge, NY; catalog # ES-200), or an Eppendorf easypet electronic pipette filler from Eppendorf (Hauppauge, NY; catalog # of 4421 000.013), or a disposable Falcon® 25-mL serological pipette from CORNING (Tewksbury, MA; catalog #357525). The pH values of all sodium phosphate buffer solutions were determined using a pH meter from Fisher Scientific (Asheville, NC; Model: AP63). *ee*AChE activity or ATCh hydrolysis rate was determined using a SpectraMax Plus 384 Absorbance Microplate Reader with SoftMax Pro 4.7.1 from Molecular Devices (Sunnyvale, CA).

Inhibitor solutions. The 2.4 mM tacrine stock solution was freshly prepared by adding an appropriate amount of distilled water using a 1000-µL Pipetman P1000 pipette to a 2.0-mL microcentrifuge tube (or a 7.4-mL glass vial) with ~1.0 mg of tacrine. Subsequent two-fold and 10-fold serial dilutions of the stock using distilled water yielded (1) a tacrine solution (1.8 mL for each enzyme inhibition assay) of 6.00 µM, 0.60 µM, or 0.06 µM in a 2.0-mL microcentrifuge tube or a 7.4-mL glass vial using a 20–200 µL Eppendorf Research pipette and a 1000-µL Pipetman P1000 pipette, and (2) a tacrine solution (3.6 mL for each adsorption study) of 30.0 µM, 20.0 µM, 15.0 µM, 10.0 µM, 7.5 µM, or 5.0 µM in two 2.0-mL microcentrifuge tubes or a 7.4-mL glass threaded vial using a 20–200-µL Eppendorf Research pipette and a 1000-µL Pipetman P1000 pipette. The 1.2 mM bis(7)-tacrine stock solution was freshly prepared by adding an appropriate amount of distilled water using a 1000-µL Pipetman P1000 pipette to a 2.0-mL microcentrifuge tube (or a 7.4-mL glass vial) with ~1.0 mg of bis(7)-tacrine. Subsequent two-fold and 10-fold serial dilutions of the stock using distilled water yielded (1) a bis(7)-tacrine solution (1.8 mL for each enzyme inhibition assay) of 600 nM, 60 nM, 6 nM, 600 pM, or 60 pM in a 2.0-mL microcentrifuge tube or a 7.4-mL glass vial using a 20–200 µL Eppendorf Research pipette and a 1000-µL Pipetman P1000 pipette, and (2) a bis(7)-tacrine solution (3.6 mL for each adsorption study) of 30.0 µM, 20.0 µM, 15.0 µM, 10.0 µM, 7.5 µM, or 5.0 µM in two 2.0-mL microcentrifuge tubes or a 7.4-mL general-purpose borosilicate glass threaded vial using a 20–200-µL Eppendorf Research pipette and a 1000-µL Pipetman P1000 pipette.

The phosphate buffer for preparing Ellman assay reagents[2]. To make 250 mL of 50 mM sodium phosphate buffer pH 8.0 at 26 °C, 1.5 g of NaH_2PO_4 was added to ~230 mL distilled water in a 500-mL beaker. After stirring at 4–6 revolutions per minute for 10 minutes, the pH of the solution with a distilled-water-rinsed electrode immersed in the NaH_2PO_4 solution under stirring was adjusted at 26 °C from initially 4.5 to 8.0 by adding drop-wise 1N NaOH. The volume of the solution was then

brought up to 250 mL with distilled water. Note: The buffer solution must be used within 12 hours and discard the remaining of the buffer solution after 12 hours.

DTNB solution. To prepare 2.5 mM DTNB solution, 9.9 mg of DTNB was dissolved in 10 mL of the freshly prepared 50 mM sodium phosphate buffer pH 8.0 that was stored in a 16-mL dark-glass reagent bottle. The resulting solution was stored in a refrigerator at 4 °C for 2–3 hours before transferring 2.0 mL of the DTNB solution to a 25-mL disposable Matrix™ Reagent Reservoir that was located in the vicinity of the Microplate Reader for measuring *ee*AChE activity. Note: Discard the remaining of the DTNB solution after each enzyme inhibition assay.

ATCh solutions. To prepare a stock solution of 30 mM ATCh, 86.8 mg ATCh was dissolved in 10 mL of the freshly prepared 50 mM sodium phosphate buffer pH 8.0 that was stored in a 15-mL conical centrifuge tube. Two-fold serial dilutions using 50 mM sodium phosphate buffer pH 8.0 in five 15-mL conical centrifuge tubes led to five working solutions of ATCh (5 mL) at concentrations of 15.000, 7.500, 3.750, 1.875, and 0.938 mM ATCh. All five ATCh working solutions were stored in a refrigerator at 4 °C for 2–3 hours before transferring 150 µL for each of these solutions to five tubes of a 0.2-mL MicroAmp® 8-tube strip that was located in the vicinity of the Microplate Reader for measuring *ee*AChE activity. Note: Discard the remaining of the ATCh solution after each enzyme inhibition assay.

The phosphate buffer for preparing enzyme stock solution. A stock of 500 mL of 20 mM NaH_2PO_4 was prepared by dissolving 1.20 g of NaH_2PO_4 in 500 mL distilled water in a 500-mL beaker and stirring at 4–6 revolutions per minute for 10 minutes. Another stock of 500 mL of 20 mM Na_2HPO_4 was prepared by dissolving 1.42 g of Na_2HPO_4 in 500 mL distilled water in a 500-mL beaker and stirring at 4–6 revolutions per minute for 10 minutes. After mixing the NaH_2PO_4 stock with the Na_2HPO_4 stock in 4:6 ratio and stirring at 4–6 revolutions per minute for 10 minutes, the pH of the

combined solution was adjust at 26 °C, using a distilled-water-rinsed electrode immersed while the solution was kept stirring, to pH 7.0 by drop-wise addition of the NaH_2PO_4 or Na_2HPO_4 stock solution. The resulting solution was then stored in a refrigerator at 4 °C for three hours before the enzyme solution preparation. Note: The buffer solution must be used within 12 hours and discard the remaining of the buffer solution after 12 hours.

Enzyme stock solution. To the glass vial received from Sigma-Aldrich that contained 0.5 mg (or a fraction of 1.0 mg) of *ee*AChE as a lyophilized powder, 1.0 mL (or an appropriate amount) of a freshly prepared 20 mM sodium phosphate buffer solution pH 7.0 was added. The vial was then kept in a refrigerator at 4 °C for 2–4 hours to ensure homogeneity. The resulting solution was then diluted with 3.0 mL of the 20 mM sodium phosphate buffer solution pH 7.0, mixed by gently withdrawing and expelling the solution five times using a 1000-µL Pipetman P1000 pipette. Then 4.0 mL of the mixed solution was transferred to a 250-mL or 100-mL reagent bottle containing an appropriate amount of the 20 mM sodium phosphate buffer solution pH 7.0 to make an *ee*AChE stock solution with a concentration of 0.625 µg/mL, 2.500 µg/mL, 5.000 µg/mL, 7.500 µg/mL, or 15.000 µg/mL. The enzyme solution was then mixed by gently withdrawing and expelling the solution 20 times using a 1000-µL Pipetman P1000 pipette and kept in a refrigerator at 4 °C for 2–4 hours to ensure homogeneity. The enzyme solution was lastly distributed to 50 1.5-mL microcentrifuge tubes that were then stored in a refrigerator at 4 °C for at least three hours for subsequent enzyme kinetics studies that must be performed within 30 days.

The phosphate buffer for preparing enzyme solutions. To a 100-mL reagent bottle with 100 mL of 50 mM sodium phosphate buffer pH 8.0, 0.11 g of Triton X-100 was added. The resulting solution was stirred for 30 minutes before enzyme inhibition assay and kept stirring during the entire period of the assay. Note: Discard the remaining of the buffer solution after each enzyme inhibition assay.

Enzyme solutions. To a 25-mL disposable Matrix™ Reagent Reservoir that was located in the vicinity of the Microplate Reader for measuring *ee*AChE activity, 13.5 mL of 50 mM sodium phosphate buffer pH 8.0 with 0.1% (v/v) Triton X-100 was transferred using a 25-mL disposable pipette and an Eppendorf easypet electronic pipette filler. Then 250 µL of an *ee*AChE stock solution with an appropriate concentration (0.625 µg/mL, 2.500 µg/mL, 5.000 µg/mL, 7.500 µg/mL, or 15.000 µg/mL), which had been kept in a refrigerator at 4 °C for at least three hours, was added to the Reservoir using a 1000-µL Pipetman P1000 pipette. The resulting solution was mixed by gently withdrawing and expelling the solution 10 times using the P1000 pipette.

Specific enzyme activity and K_i determination. Briefly, to each of 40 wells in a flat-bottom, clear, 96-well plate was added at 26 °C sequentially 270 µL 50 mM sodium phosphate buffer (pH 8.0) with 0.1% (v/v) Triton X-100, 5 µL *ee*AChE solution (15.000, 7.5000, 5.000, 2.500, or 0.625 µg/mL), 5 µL of inhibitor solutions (for tacrine: 3.0 µM, 1.5 µM, and 0.6 µM for 0.625 µg/mL of *ee*AChE or 6.0 µM, 3.0 µM, and 1.5 µM for 15.000 µg/mL of *ee*AChE; for bis(7)-tacrine: 0.6 nM, 0.3 nM, and 0.15 nM for 0.625 µg/mL of *ee*AChE or 90 nM, 60 nM, and 30 nM for 15.000 µg/mL of *ee*AChE) or distilled water (for control and the specific enzyme activity determination), 10 µL 2.5 mM DTNB, and 10 µL ATCh solutions (15.000, 7.500, 3.750, 1.875, and 0.938 mM). The resulting solutions were left on the bench at 26 °C for equilibration for 2 minutes and then measured for ATCh hydrolysis rate (v) at a microplate reader temperature of 26 ± 2 °C. The specific enzyme activity (SEA) for *ee*AChE was calculated according to $SEA = (A \bullet V)/(\varepsilon \bullet L \bullet T \bullet W_E)$, where A was the UV absorption of the ATCh hydrolysis product (0.21–1.26 x 10^{-3} OD); V was the volume of the assay solution (300 µL); ε was molar absorptivity at 405 nm (13.3 $L \bullet cm^{-1} \bullet mol^{-1}$)[3]; L was the length of the light path of the flat-bottom, clear, 96-well plate (0.75 cm); T was the time over which the hydrolysis product was generated (10 minutes); W_E was the weight of the enzyme (10.4 x 10^{-6}–250 x 10^{-6} mg); 1U is defined as converting 1

μmol of substrate to its product in a minute[4]. K_i was obtained from 1/*v*, 1/[ATCh], and [I] using Prism 4 with the Lineweaver-Burk plot[5] (see details below).

For each new batch of *ee*AChE, UV absorbance of the assay solution was measured at 10-second intervals to determine whether *v* reached plateau over the 10-minute period (or determine whether $r^2<1.0$ for the curve of the absorbance over time) first using *ee*AChE at a stock concentration of 15 μg/mL and ATCh at a stock concentration of 15.000 mM. If a plateau was observed, the 15 μg/mL *ee*AChE solution was repeatedly diluted by two fold until *v* remained constant (or determine whether $r^2=1.0$ for the curve of the absorbance over time) and in the range of 30–80 x 10^{-3} OD/min. Then, the UV absorbance was measured at 10-second intervals over the 10-minute period to determine whether *v* was in the range of 10–20 x 10^{-3} OD/min using a diluted *ee*AChE solution (15.000, 7.500, 5.000, 2.500, or 0.625 μg/mL) and five ATCh concentrations (15.000, 7.500, 3.750, 1.875, and 0.938 mM). Any ATCh concentration (typically the 0.938 mM concentration) that causes $v<10$ x 10^{-3} OD/min was excluded. Consequently, *v* was determined using either five ATCh concentrations (15.000, 7.500, 3.750, 1.875, and 0.938 mM) or four ATCh concentrations (15.000, 7.500, 3.750, and 1.875 mM). Three inhibitor concentrations (initially 0.1, 1.0, and 10.0 times the estimated K_i concentration) were used to determine the percentage of enzyme inhibition. The three concentrations were modified repeatedly until the ATCh hydrolysis rates in the presence of the three modified inhibitor concentrations were reduced to ~20%, ~50%, and ~80% of the rate in the absence of an inhibitor, respectively.

Under the assay conditions described above (temperature at 26 °C, pH of 8.0, ionic strength of 50 mM sodium phosphate buffer, and ATCh concentration of 15.000 mM), the ATCh hydrolysis rates in the absence of *ee*AChE of two identical Ellman assay solutions (two duplicates) were found to be 0.375 x 10^{-3} OD/min and 0.243 x 10^{-3} OD/min, whereas the corresponding ones in the presence of *ee*AChE were 32.872 x 10^{-3} OD/min and 31.633 x 10^{-3} OD/min. Therefore, as described below the

ATCh hydrolysis rate in the absence of *ee*AChE were not measured because of its insignificant contribution to the ATCh hydrolysis rate in the presence of *ee*AChE.

Step 1: 5 µL of distilled water was added to all wells of Columns 2–3 and Rows 2–6 of a flat-bottom, clear, 96-well plate using a Finnpipette F1 1–10-µL 8-channel pipette. Step 2: 5 µL of the low inhibitor concentration was added well by well to all wells of Columns 4–5 and Rows 2–6 using an Eppendorf Reference series adjustable-volume 0.5–10 µL pipette. Step 3: 5 µL of the median inhibitor concentration was added well by well to all wells of Columns 6–7 and Rows 2–6 using the 0.5–10 µL pipette. Step 4: 5 µL of the high inhibitor concentration was added well by well to all wells of Columns 8–9 and Rows 2–6 using the 0.5–10 µL pipette. Step 5: 275 µL of an enzyme solution (0.625 µg/mL, 2.500 µg/mL, 5.000 µg/mL, 7.500 µg/mL, or 15.000 µg/mL) was added column by column starting from Column 2 to all wells of Columns 2–9 and Rows 2–6 using an Eppendorf Research 8-channel 30–300-µL pipette (it took ~3 seconds to complete the enzyme addition to each column). Step 6: A timer was set to count the incubation time of the Column-2 wells upon adding the enzyme solution. Step 7: The solutions in each row were stirred in circle 6 times, starting from Row 2 to Row 9, using a Finnpipette F1 1–10-µL 8-channel pipette with the same set of Fisherbrand low-retention pipette tips. Step 8: When the timer reached 4 minutes, 10 µL of DTNB was added column by column starting from Column 2 to all wells of Columns 2–9 and Rows 2–6 using a Finnpipette F1 1–10-µL 8-channel pipette with the same set of Fisherbrand low-retention pipette tips (it took ~3 seconds to complete the DTNB addition to each column). Step 9: When the timer reached 5 minutes, 10 µL of five ATCh solutions (15.000 mM to Row 2, … and 0.938 mM to Row 6) were added column by column starting from Column 2 to all wells of Columns 2–9 and Rows 2–6 using a Finnpipette F1 1–10-µL 8-channel pipette with a new set of Fisherbrand low-retention pipette tips for each row (it took ~10 seconds to complete the ATCh addition and mixing of each column). Upon addition of ATCh, all solutions of each column were mixed by withdrawing and expelling the

solutions 5 times using the same set of pipette tips as those for adding ATCh followed by stirring the solution in circle 5 times using the same set of pipette tips. Step 10: The timer was reset to count the reaction time of the first column wells upon adding ATCh to the last column cells. Step 11: When the timer reached 2 minutes, the plate of solutions was placed in a microplate reader. The ATCh hydrolysis rates of the plate were determined by measuring the UV absorbance in optical density (OD) of an assay solution at 405 nm at 10-second intervals over a period of 10 minutes at plate reader temperature of 26 ± 2 °C using (1) autocalibration, (2) no automix, (3) no blanking, and (4) the basic kinetic assay protocol available in the SoftMax Pro 4.7.1 program. An average of two ATCh hydrolysis rates of duplicated assay solutions was used for K_i calculation. Each K_i listed in Tables 1 and 2 was an average of five independent and consecutively performed K_i determinations using freshly prepared solutions of enzyme, inhibitor, and Ellman assay reagents.

UV absorptions of inhibitor solutions that were prepared using glass or plastic vials. To a single quartz cuvette that was washed with distilled water and dried by blowing N_2 gas, 3.0 mL of a tacrine or bis(7)-tacrine solution of 30.0 μM, 20.0 μM, 15.0 μM, 10.0 μM, 7.5 μM, or 5.0 μM was added using a 1000-μL Pipetman P1000 pipette. The cuvette with the highest tacrine or bis(7)-tacrine concentration that was prepared using two 2.0-mL microcentrifuge tubes was first placed in the SpectraMax Plus 384 Absorbance Microplate Reader to scan for λ_{max} in the range of 190–400 nm. The λ_{max}s for tacrine and bis(7)-tacrine were found to be 242 nm and 244 nm, respectively. The UV absorption of an inhibitor solution that was prepared using two 2.0-mL microcentrifuge tube or a 7.4-mL glass vial was then determined by the observed absorbance of an inhibitor solution with or without 0.4% (v/v) Polysorbate 20 subtracted by the observed absorbance of distilled water with 0.4% (v/v) Polysorbate 20 or distilled water without 0.4% (v/v) Polysorbate 20. The UV absorbance data are listed in Table S2. The UV absorbance of an inhibitor at each concentration shown in Figure 1 was an average of at least three measurements each of which used a freshly prepared inhibitor solution.

Table S1. ATCh hydrolysis rate (v), inhibition constant (K_i), and specific enzyme activity (SEA) for tacrine (THA) and bis(7)-tacrine (B7T) against *Electrophorus electricus* acetylcholinesterase (*ee*AChE).

Tacrine in plastic vial with 10.4 pg *ee*AChE										
Experiment 1										
[ATCh] (mM)	v (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		10 nM of THA		25 nM of THA		50 nM of THA			
15.00	34.174	36.900	25.261	25.253	15.859	17.029	10.665	8.667	8.46 nM	342.0
7.50	33.280	32.234	21.628	19.588	12.910	12.231	8.107	8.304		
3.75	26.781	22.933	15.407	14.499	8.696	8.613	5.804	6.125		
1.88	18.805	16.850	10.436	9.880	5.815	5.375	3.536	3.061		
0.94	11.220	10.811	6.628	5.877	3.368	3.318	1.790	2.275		
Experiment 2										
[ATCh] (mM)	v (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		10 nM of THA		25 nM of THA		50 nM of THA			
15.00	35.679	38.069	25.103	26.987	15.360	19.001	11.975	14.698	12.42	354.9
7.50	36.293	31.537	21.496	19.610	13.989	14.347	9.033	9.794		
3.75	27.704	25.956	16.914	15.826	11.427	10.134	7.012	6.993		
1.88	19.078	18.265	11.620	11.304	7.299	6.624	4.257	4.469		
0.94	11.728	11.811	7.212	6.812	4.409	4.182	2.531	2.819		
Experiment 3										
[ATCh] (mM)	v (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		10 nM of THA		25 nM of THA		50 nM of THA			
15.00	31.680	34.521	23.879	22.506	16.709	16.874	11.207	11.012	12.90	318.6
7.50	31.650	30.283	17.375	18.727	13.114	11.488	8.131	7.867		
3.75	26.684	24.739	15.722	15.330	9.191	9.362	5.764	6.920		
1.88	18.434	17.286	10.811	10.233	6.126	6.045	4.029	3.937		
0.94	11.962	10.181	6.783	6.529	3.979	3.518	2.384	2.633		
Experiment 4										
[ATCh] (mM)	v (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		10 nM of THA		25 nM of THA		50 nM of THA			
15.00	38.326	35.205	22.415	23.356	15.157	13.232	9.948	9.089	8.93 nM	353.8
7.50	35.941	33.166	20.243	18.479	11.496	10.112	6.650	6.675		
3.75	29.594	27.291	16.256	14.332	8.614	7.692	4.428	4.838		
1.88	20.012	19.041	11.713	10.012	5.839	5.279	3.291	3.276		
0.94	12.635	12.191	7.409	6.929	3.772	3.432	2.256	2.255		
Experiment 5										
[ATCh] (mM)	v (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		10 nM of THA		25 nM of THA		50 nM of THA			
15.00	36.835	33.803	22.989	19.017	14.723	14.400	8.440	12.151	12.32	339.9
7.50	36.740	30.337	22.409	18.839	11.741	13.504	6.830	8.818		
3.75	28.203	26.592	14.794	14.675	9.267	8.807	5.706	5.551		
1.88	18.805	18.299	10.437	9.325	5.531	5.817	3.698	3.433		
0.94	11.873	11.275	6.574	6.097	4.515	4.098	2.386	2.409		
Tacrine in plastic vial with 41.7 pg *ee*AChE										
Experiment 1										
[ATCh] (mM)	v (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		10 nM of THA		12.5 nM of THA		25 nM of THA			
15.00	126.328	126.145	112.203	104.781	82.016	85.872	67.134	67.690	13.47	303.7
7.50	112.459	107.673	85.795	80.536	71.672	62.947	53.996	53.746		
3.75	67.271	67.992	47.698	51.831	44.626	39.330	35.064	36.478		
1.88	43.416	45.105	29.271	27.898	23.385	23.426	17.045	17.600		
Experiment 2										
[ATCh] (mM)	v (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		10 nM of THA		12.5 nM of THA		25 nM of THA			
15.00	96.323	97.120	51.656	49.279	43.457	45.131	37.716	33.494	15.3	232.7
7.50	80.372	80.603	44.711	40.868	37.275	32.324	32.103	30.394		
3.75	58.174	58.255	32.904	34.624	32.409	31.431	25.478	25.949		
1.88	30.689	37.785	19.083	19.050	16.104	16.769	12.859	12.369		
Experiment 3										
[ATCh] (mM)	v (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		10 nM of THA		12.5 nM of THA		25 nM of THA			
15.00	94.613	84.734	67.364	63.211	54.668	55.663	43.900	42.055	14.54	215.8
7.50	74.025	73.543	57.983	54.464	47.302	48.202	35.462	33.446		
3.75	47.499	47.829	40.028	38.386	34.190	36.438	27.973	28.169		

1.88	30.191	32.948	21.643	20.490	18.675	19.297	12.070	13.736		
Experiment 4										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		10 nM of THA		12.5 nM of THA		25 nM of THA			
15.00	85.145	88.917	66.132	64.117	48.951	49.861	43.905	42.555	14.94	209.4
7.50	70.976	68.905	55.633	54.282	49.636	47.944	35.777	31.424		
3.75	43.724	44.279	36.931	37.672	34.744	33.527	24.883	21.959		
1.88	20.623	23.950	18.845	18.444	16.746	15.258	9.476	10.395		
Experiment 5										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		10 nM of THA		12.5 nM of THA		25 nM of THA			
15.00	71.627	65.127	50.1835	47.682	41.827	40.1835	33.157	31.062	14.87	164.5
7.50	55.6175	56.607	40.456	43.682	34.682	36.827	25.1185	25.0025		
3.75	36.178	35.3445	29.482	29.1805	27.6575	26.1835	21.0175	21.111		
1.88	23.178	24.3435	15.7945	15.9925	13.999	14.0127	9.656	9.6175		
Tacrine in plastic vial with 83.3 pg *ee*AChE										
Experiment 1										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		12.5 nM of THA		25.0 nM of THA		50.0 nM of THA			
15.00	92.616	87.109	67.311	65.749	55.839	57.102	39.613	41.090	31.19 nM	108.1
7.50	87.347	84.078	60.412	58.810	45.326	45.586	32.368	33.022		
3.75	63.608	59.347	43.785	42.045	32.764	33.382	24.239	24.583		
1.88	42.463	40.789	30.224	29.125	22.780	22.168	16.270	16.759		
Experiment 2										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		12.5 nM of THA		25.0 nM of THA		50.0 nM of THA			
15.00	117.283	108.629	84.339	82.532	59.749	58.562	44.253	42.716	31.21 nM	135.9
7.50	114.115	101.242	71.712	71.812	53.885	56.045	38.631	36.789		
3.75	85.132	66.856	61.590	58.181	42.409	42.130	29.830	29.462		
1.88	59.306	60.132	44.061	43.154	32.121	32.195	23.067	23.065		
Experiment 3										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		12.5 nM of THA		25.0 nM of THA		50.0 nM of THA			
15.00	116.124	119.042	99.358	91.154	74.473	71.030	53.891	50.782	31.4 nM	141.5
7.50	107.186	105.368	84.959	78.655	62.214	57.706	39.127	41.579		
3.75	80.265	79.382	63.999	60.783	46.553	44.578	29.915	31.378		
1.88	56.675	53.762	42.907	43.453	33.579	31.457	22.164	22.879		
Experiment 4										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		12.5 nM of THA		25.0 nM of THA		50.0 nM of THA			
15.00	96.685	89.941	75.897	72.361	61.062	61.101	46.476	48.888	38.11 nM	112.3
7.50	86.094	84.450	61.189	63.428	42.652	48.514	39.479	39.509		
3.75	63.179	60.482	43.382	45.452	36.950	30.762	24.863	29.594		
1.88	43.747	42.190	32.925	31.617	27.298	24.828	19.103	20.264		
Experiment 5										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		12.5 nM of THA		25.0 nM of THA		50.0 nM of THA			
15.00	107.437	91.984	72.311	71.418	57.033	54.914	39.443	38.944	29.66 nM	120.0
7.50	93.065	83.547	62.612	59.724	47.043	43.859	32.100	32.580		
3.75	66.045	63.627	45.445	45.244	34.759	34.958	24.355	24.152		
1.88	46.617	45.212	31.859	31.946	24.748	24.462	17.108	17.268		
Tacrine in glass vial with 83.3 pg *ee*AChE										
Experiment 1										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		12.5 nM of THA		25.0 nM of THA		50.0 nM of THA			
15.00	112.471	109.176	104.028	104.608	95.644	104.271	88.453	91.411	38.34 nM	133.3
7.50	124.437	116.819	105.279	108.668	92.646	93.805	78.058	81.122		
3.75	88.878	89.444	75.691	78.446	68.874	67.656	56.996	57.342		
1.88	71.059	71.059	59.631	61.082	52.727	52.402	41.187	42.279		
Experiment 2										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		12.5 nM of THA		25.0 nM of THA		50.0 nM of THA			
15.00	89.770	70.787	64.628	62.845	56.718	55.148	41.938	46.501	36.22 nM	96.6
7.50	82.644	79.399	69.603	79.330	68.149	68.342	50.868	48.621		
3.75	50.808	54.250	48.361	53.602	46.584	46.813	37.231	33.737		
1.88	49.435	46.546	34.415	39.288	34.336	33.223	23.654	23.368		
Experiment 3										

[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		12.5 nM of THA		25.0 nM of THA		50.0 nM of THA			
15.00	109.273	108.514	93.352	91.403	82.079	78.659	63.312	61.502	42.88 nM	131.0
7.50	96.075	106.552	84.560	85.681	73.517	72.952	53.027	48.802		
3.75	69.512	78.260	61.157	59.509	50.644	52.083	38.855	36.161		
1.88	50.062	55.470	43.129	43.373	37.914	38.007	26.176	27.622		
Experiment 4										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		12.5 nM of THA		25.0 nM of THA		50.0 nM of THA			
15.00	99.742	99.742	92.107	93.344	83.449	81.433	64.119	66.093	32.09 nM	120.0
7.50	84.275	84.275	77.607	76.976	64.941	63.508	48.489	52.277		
3.75	60.256	60.256	50.324	48.935	45.315	44.705	33.855	35.823		
1.88	49.349	49.349	35.252	34.157	30.111	31.282	22.449	23.544		
Experiment 5										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		12.5 nM of THA		25.0 nM of THA		50.0 nM of THA			
15.00	112.249	100.939	95.599	105.538	90.135	98.478	74.572	64.618	27.67 nM	128.2
7.50	114.370	90.131	87.209	91.114	68.086	75.571	54.262	45.487		
3.75	84.340	67.113	60.035	53.497	42.937	44.401	29.728	28.738		
1.88	43.506	43.506	38.074	33.301	27.646	26.743	19.633	18.496		
Tacrine in plastic vial with 125.0 pg *ee*AChE										
Experiment 1										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		16.67 nM of THA		33.33 nM of THA		66.67 nM of THA			
15.00	62.804	47.128	41.149	43.312	39.302	39.617	31.254	29.366	42.12 nM	44.1
7.50	44.964	35.936	34.335	37.962	34.011	33.211	21.908	22.125		
3.75	28.530	27.805	22.210	22.883	19.876	19.552	13.293	14.722		
1.88	24.532	22.404	18.303	18.693	16.058	15.923	10.168	11.586		
Experiment 2										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		16.67 nM of THA		33.33 nM of THA		66.67 nM of THA			
15.00	36.770	30.636	26.243	32.066	26.563	26.818	16.896	18.273	44.10 nM	27.0
7.50	34.847	35.850	22.014	28.107	21.524	21.285	15.008	15.264		
3.75	25.630	26.113	16.305	18.394	11.678	10.435	9.632	9.823		
1.88	13.812	14.838	10.097	11.909	7.657	9.733	6.254	6.121		
Experiment 3										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		16.67 nM of THA		33.33 nM of THA		66.67 nM of THA			
15.00	49.874	46.297	32.138	36.806	27.317	25.653	23.362	20.067	46.42 nM	38.6
7.50	40.727	36.943	29.357	30.104	24.911	20.362	19.456	18.058		
3.75	26.661	34.521	19.110	21.922	17.311	17.504	13.533	14.204		
1.88	18.431	19.418	13.684	11.626	10.169	10.322	7.507	8.036		
Experiment 4										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		16.67 nM of THA		33.33 nM of THA		66.67 nM of THA			
15.00	72.256	74.932	51.630	51.709	41.897	43.794	36.225	40.637	44.40 nM	59.0
7.50	51.839	55.217	35.361	39.133	30.585	33.840	33.259	29.778		
3.75	46.694	40.504	27.981	29.784	26.177	28.239	22.309	22.338		
1.88	33.493	31.747	21.255	20.174	18.022	18.013	15.801	14.312		
Experiment 5										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		16.67 nM of THA		33.33 nM of THA		66.67 nM of THA			
15.00	83.027	72.257	52.591	55.459	45.430	42.637	39.483	39.972	46.20 nM	62.3
7.50	52.913	54.894	34.151	32.598	33.758	29.520	27.721	28.352		
3.75	42.208	45.359	31.989	32.116	28.645	29.249	23.684	27.288		
1.88	34.873	34.156	22.436	20.325	18.666	19.932	15.784	15.708		
Tacrine in plastic vial with 250.0 pg *ee*AChE										
Experiment 1										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		25 nM of THA		50 nM of THA		100 nM of THA			
15.00	70.503	77.549	74.195	59.114	42.685	43.240	37.128	42.021	65.94 nM	29.7
7.50	45.766	45.549	39.603	39.680	25.491	24.116	23.531	20.931		
3.75	23.723	29.695	20.315	16.834	13.078	13.392	8.924	13.019		
1.88	15.241	17.935	11.673	12.084	8.637	7.899	6.145	7.434		
Experiment 2										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		25 nM of THA		50 nM of THA		100 nM of THA			

15.00	36.770	30.636	26.243	32.066	26.563	26.818	16.896	18.273	66.14 nM	13.5
7.50	34.847	35.850	22.014	28.107	21.524	21.285	15.008	15.264		
3.75	25.630	26.113	16.305	18.394	11.678	10.435	9.632	9.823		
1.88	13.812	14.838	10.097	11.909	7.657	9.733	6.254	6.121		
Experiment 3										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		25 nM of THA		50 nM of THA		100 nM of THA			
15.00	77.077	68.433	57.570	54.857	49.325	49.350	42.128	42.021	71.30 nM	29.2
7.50	68.437	70.891	48.460	44.274	31.894	36.733	23.531	24.931		
3.75	26.819	31.812	25.907	25.351	18.148	18.358	15.924	13.019		
1.88	25.432	25.665	18.458	17.902	13.335	15.698	11.145	10.434		
Experiment 4										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		25 nM of THA		50 nM of THA		100 nM of THA			
15.00	76.989	75.239	49.991	64.601	56.098	48.012	43.720	44.258	68.32 nM	30.5
7.50	65.181	61.110	43.135	47.886	41.706	36.170	30.745	31.487		
3.75	58.060	55.019	34.591	33.879	25.064	25.802	20.971	18.568		
1.88	22.673	22.976	15.504	15.272	12.911	12.181	9.411	9.979		
Experiment 5										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of THA		25 nM of THA		50 nM of THA		100 nM of THA			
15.00	77.059	75.383	50.798	63.086	54.774	47.428	42.646	43.053	89.96 nM	30.6
7.50	50.900	49.470	39.214	41.698	37.861	33.800	22.599	22.121		
3.75	28.678	20.482	24.677	24.634	22.860	21.061	15.368	15.166		
1.88	16.205	15.152	10.778	10.805	10.760	9.773	7.038	7.188		
1.25										
Bis(7)-tacrine in glass vial with 10.4 pg *ee*AChE										
Experiment 1										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of B7T		0.83 nM of B7T		1.67 nM of B7T		3.3 nM of B7T			
15.00	24.415	25.740	21.561	21.257	18.569	17.222	14.663	15.335	3.16 nM	344.8
7.50	19.934	19.405	15.374	15.913	13.039	12.920	10.495	10.850		
3.75	13.743	14.189	11.657	11.311	9.445	9.530	7.646	7.964		
1.88	9.740	9.150	7.467	7.805	6.258	6.361	4.797	4.171		
1.25	7.727	7.650	6.096	6.205	5.346	4.991	4.208	3.983		
Experiment 2										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of B7T		0.83 nM of B7T		1.67 nM of B7T		3.3 nM of B7T			
15.00	24.170	21.920	18.541	18.840	15.941	15.250	13.126	13.074	3.66 nM	316.8
7.50	18.926	17.794	13.004	14.217	12.346	11.358	9.555	9.682		
3.75	13.189	12.250	10.028	9.430	8.458	7.435	6.511	6.471		
1.88	7.101	7.747	6.505	6.431	5.646	4.760	3.922	4.132		
1.25	5.989	6.003	5.081	5.124	4.312	3.691	3.292	3.229		
Experiment 3										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of B7T		0.83 nM of B7T		1.67 nM of B7T		3.3 nM of B7T			
15.00	27.832	25.684	20.251	20.823	18.136	17.695	15.037	14.427	3.35 nM	367.9
7.50	21.839	20.382	16.731	16.655	13.679	14.349	11.066	11.518		
3.75	14.363	13.187	10.835	10.188	8.955	9.470	8.012	8.069		
1.88	10.753	10.242	7.934	7.728	6.167	6.443	5.434	5.102		
1.25	8.463	8.158	6.300	6.307	5.110	4.891	4.484	4.072		
Experiment 4										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of B7T		0.83 nM of B7T		1.67 nM of B7T		3.3 nM of B7T			
15.00	22.236	21.770	17.265	17.680	14.061	16.206	12.584	13.389	2.87 nM	302.5
7.50	15.060	17.561	9.562	13.586	11.009	10.673	9.149	10.064		
3.75	12.464	11.276	9.544	9.580	7.603	7.759	6.816	6.839		
1.88	9.007	8.576	6.383	6.523	5.597	5.740	4.570	4.601		
1.25	7.219	6.896	5.017	5.086	4.290	4.382	3.566	3.328		
Experiment 5										
[ATCh] (mM)	*v* (mOD/min)								K_i (nM)	SEA (U/mg)
	0 nM of B7T		0.83 nM of B7T		1.67 nM of B7T		3.3 nM of B7T			
15.00	24.698	24.498	20.752	19.556	16.918	17.740	13.864	13.522	3.00 nM	338.2
7.50	18.558	18.729	15.787	15.045	12.618	12.798	10.186	10.305		
3.75	12.684	14.079	11.815	11.157	8.978	9.063	7.129	6.846		
1.88	7.818	10.096	7.491	7.217	5.993	5.930	4.344	4.525		
1.25	6.467	7.126	5.436	5.367	4.429	4.300	3.411	3.369		

Bis(7)-tacrine in plastic vial with 10.4 pg *ee*AChE										
Experiment 1										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		1.25 pM of B7T		2.5pM of B7T		5.0 pM of B7T			
15.00	35.773	35.174	26.509	26.203	20.275	20.111	13.586	13.557	2.53 pM	341.4
10.00	36.203	37.939	22.867	22.542	18.065	18.350	11.643	11.631		
7.50	30.091	32.957	19.820	19.612	14.944	14.765	10.279	10.565		
3.75	21.575	23.518	14.254	14.819	10.791	10.211	7.020	8.076		
1.88	14.764	15.888	7.776	9.818	6.578	6.211	7.020	8.076		
Experiment 2										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		1.25 pM of B7T		2.5pM of B7T		5.0 pM of B7T			
15.00	38.545	36.973	25.166	22.094	17.428	17.071	15.738	16.053	2.87 pM	363.4
10.00	35.754	32.711	21.429	20.131	15.133	16.758	13.506	13.404		
7.50	27.992	27.243	18.537	16.804	13.342	14.259	10.942	11.457		
3.75	23.057	22.188	13.950	11.161	9.570	9.744	7.954	5.881		
1.88	15.035	14.820	8.849	9.165	6.570	5.923	5.895	5.058		
Experiment 3										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		1.25 pM of B7T		2.5pM of B7T		5.0 pM of B7T			
15.00	38.821	34.716	15.386	13.391	11.611	15.312	11.345	11.905	2.39 pM	353.9
10.00	25.871	34.326	18.969	11.993	10.007	12.772	9.290	9.250		
7.50	29.426	29.218	12.257	10.383	8.366	11.150	8.285	8.232		
3.75	22.756	21.306	10.171	10.820	7.950	8.595	6.446	6.721		
1.88	15.596	14.933	7.355	6.115	4.962	5.879	4.116	4.502		
Experiment 4										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		1.25 pM of B7T		2.5pM of B7T		5.0 pM of B7T			
15.00	32.365	30.642	23.639	23.940	14.630	12.760	11.688	11.539	4.39 pM	303.2
10.00	29.379	28.257	20.156	18.162	12.760	12.954	11.823	9.335		
7.50	26.697	25.433	18.142	18.189	12.998	12.912	9.765	10.396		
3.75	12.083	12.646	11.343	12.271	9.256	9.285	6.741	6.611		
1.88	13.276	12.868	8.663	9.894	6.326	6.585	5.102	5.379		
Experiment 5										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		1.25 pM of B7T		2.5pM of B7T		5.0 pM of B7T			
15.00	36.689	36.147	28.995	25.920	20.649	15.863	11.313	11.775	2.52 pM	350.5
10.00	33.208	32.235	22.339	23.028	15.461	13.155	9.300	9.310		
7.50	30.106	29.680	21.429	21.011	13.021	12.243	8.199	8.333		
3.75	20.849	20.229	14.625	14.953	9.434	9.510	6.664	6.598		
1.88	13.786	13.552	9.764	9.195	6.472	6.713	4.310	4.351		
Bis(7)-tacrine in plastic vial with 41.7 pg *ee*AChE										
Experiment 1										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		25.0 pM of B7T		50.0 pM of B7T		100 pM of B7T			
15.00	101.314	110.419	112.961	102.609	84.747	87.093	55.525	56.528	77.35 pM	254.7
7.50	78.508	78.616	78.974	76.949	60.179	61.539	34.803	40.111		
3.75	56.580	56.029	50.697	46.700	38.647	31.030	23.138	24.759		
1.88	26.560	23.481	25.080	21.663	18.502	15.982	12.230	11.664		
Experiment 2										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		25.0 pM of B7T		50.0 pM of B7T		100 pM of B7T			
15.00	112.879	113.048	87.433	100.587	99.448	92.107	47.152	49.586	82.88 pM	271.8
7.50	85.021	77.867	57.794	70.665	73.657	49.802	27.680	34.249		
3.75	52.164	50.123	39.010	44.391	43.884	34.458	18.963	18.659		
1.88	24.992	25.301	21.104	22.752	21.349	15.364	11.695	11.524		
Experiment 3										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		25.0 pM of B7T		50.0 pM of B7T		100 pM of B7T			
15.00	104.313	104.419	86.737	91.983	75.391	62.832	64.868	52.591	84.71 pM	251.1
7.50	75.575	76.725	61.018	56.723	59.057	64.987	43.546	38.168		
3.75	47.758	48.123	40.950	33.521	39.328	37.618	24.155	26.779		
1.88	27.117	24.040	22.338	21.921	19.301	20.776	12.353	13.418		
Experiment 4										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		25.0 pM of B7T		50.0 pM of B7T		100 pM of B7T			
15.00	100.470	98.050	98.047	95.745	72.685	83.710	33.733	34.467	82.97 pM	238.8

7.50	79.828	78.500	79.484	75.764	58.418	68.301	28.855	22.755		
3.75	49.690	48.100	48.007	44.051	42.476	47.197	19.411	17.825		
1.88	26.837	24.817	24.199	22.453	22.255	22.035	11.966	10.133		
Experiment 5										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		25.0 pM of B7T		50.0 pM of B7T		100 pM of B7T			
15.00	100.043	100.700	102.778	106.192	94.724	75.927	38.370	45.911	76.58 pM	241.5
7.50	100.959	94.172	95.219	83.940	65.644	63.585	38.392	37.933		
3.75	70.647	71.073	68.880	63.633	45.021	43.693	27.812	21.408		
1.88	39.346	39.342	36.809	36.702	31.853	31.248	17.886	17.168		
Bis(7)-tacrine in plastic vial with 125.0 pg *ee*AChE										
Experiment 1										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		0.25 nM of B7T		0.5 nM of B7T		1.0 nM of B7T			
15.00	65.721	63.231	58.462	55.816	43.211	39.639	26.331	25.843	545.4 pM	51.7
7.50	57.109	53.983	43.025	42.062	28.708	32.097	18.979	20.656		
3.75	42.793	31.909	28.183	28.050	20.436	23.590	13.975	14.899		
1.88	27.127	23.520	18.794	20.491	15.809	13.036	9.645	9.128		
Experiment 2										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		0.25 nM of B7T		0.5 nM of B7T		1.0 nM of B7T			
15.00	55.607	54.076	47.936	49.056	39.087	36.624	25.636	18.996	441.4 pM	44.0
7.50	50.182	44.461	38.614	36.904	25.540	26.499	16.974	15.840		
3.75	28.288	30.757	25.605	26.270	16.360	14.064	12.966	13.863		
1.88	22.476	22.982	19.554	19.064	12.000	15.126	7.201	9.323		
Experiment 3										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		0.25 nM of B7T		0.5 nM of B7T		1.0 nM of B7T			
15.00	63.203	53.775	46.564	46.123	44.387	42.346	29.180	28.006	411.1 pM	46.9
7.50	49.174	45.032	32.154	33.815	32.620	32.877	16.107	16.832		
3.75	24.448	28.835	21.539	23.679	17.951	17.478	12.966	13.863		
1.88	22.827	22.847	17.538	16.665	15.283	14.189	7.201	9.323		
Experiment 4										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		0.25 nM of B7T		0.5 nM of B7T		1.0 nM of B7T			
15.00	53.391	58.507	43.649	46.428	41.046	40.071	23.741	22.090	445.6 pM	44.9
7.50	43.881	44.880	34.635	34.846	30.262	30.509	19.173	18.868		
3.75	24.448	28.835	19.232	21.229	19.799	18.477	14.950	14.939		
1.88	22.827	17.847	16.715	15.060	11.207	12.681	6.891	7.652		
Experiment 5										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		0.25 nM of B7T		0.5 nM of B7T		1.0 nM of B7T			
15.00	47.208	51.985	48.068	41.580	37.751	36.063	29.189	28.341	439.7 pM	39.8
7.50	41.383	39.566	36.538	38.085	28.743	26.149	19.084	21.529		
3.75	30.471	27.812	25.868	23.132	16.393	17.101	15.266	13.735		
1.88	22.474	23.469	19.586	19.780	13.324	13.996	11.974	7.768		
Bis(7)-tacrine in plastic vial with 250.0 pg *ee*AChE										
Experiment 1										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		0.5 nM of B7T		1.0 nM of B7T		1.5 nM of B7T			
15.00	50.356	66.265	38.899	41.264	31.244	28.611	20.509	19.342	591.6 pM	23.4
7.50	40.255	48.755	29.842	32.327	21.471	18.932	15.428	16.469		
3.75	22.012	27.373	17.915	20.699	13.562	10.804	9.623	10.174		
1.88	17.542	19.445	10.817	15.588	9.349	8.095	7.296	5.091		
Experiment 2										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		0.5 nM of B7T		1.0 nM of B7T		1.5 nM			
15.00	81.303	78.159	54.300	53.348	30.550	30.006	29.926	28.177	624.2 pM	32.0
7.50	53.991	51.363	37.749	40.099	20.434	20.297	15.815	15.585		
3.75	30.074	27.295	21.115	22.331	12.071	11.389	10.135	9.167		
1.88	19.571	20.567	15.814	14.449	8.815	7.689	6.770	6.714		
Experiment 3										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		0.5 nM of B7T		1.0 nM of B7T		1.5 nM of B7T			
15.00	65.636	79.451	57.878	53.320	44.522	37.627	25.305	26.798	701.5 pM	29.1
7.50	51.187	59.260	47.771	43.864	37.782	33.882	22.182	20.671		
3.75	29.801	30.833	22.556	20.307	18.118	19.219	13.806	11.856		

1.88	20.697	20.941	17.173	16.494	13.356	13.588	8.617	8.471		
Experiment 4										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		0.5 nM of B7T		1.0 nM of B7T		1.5 nM of B7T			
15.00	51.731	45.302	36.026	39.138	34.133	31.114	29.528	27.178	989.0 pM	19.5
7.50	38.887	37.291	27.281	27.777	23.675	16.575	22.125	19.439		
3.75	24.315	24.025	18.610	18.492	15.320	15.417	11.145	11.118		
1.88	15.070	16.331	12.341	12.533	10.039	9.493	8.328	8.438		
Experiment 5										
[ATCh] (mM)	*v* (mOD/min)								K_i (pM)	SEA (U/mg)
	0pM of B7T		0.5 nM of B7T		1.0 nM of B7T		1.5 nM of B7T			
15.00	61.400	61.028	48.224	48.531	37.041	32.436	23.231	22.383	764.3 pM	24.6
7.50	40.257	40.984	35.235	33.565	19.538	18.890	15.634	15.068		
3.75	23.815	22.042	16.711	16.037	15.271	14.422	10.170	12.894		
1.88	14.424	15.936	11.470	12.211	7.559	8.884	7.553	5.654		

Table S2A. Absorbance data of bis(7)-tacrine solutions with or without 0.4% Polysorbate 20 that were prepared using glass or plastic vials.

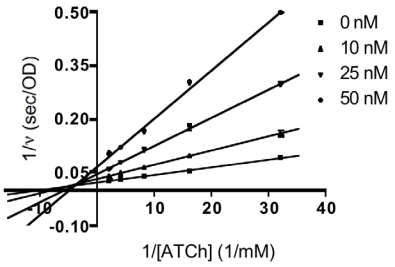
Bis(7)-tacrine solution that was prepared using plastic vials							
[B7T] (μM)	Absorbance at λ 244 nm (mOD)						
	Exp. 1	Exp. 2	Exp. 3		Mean	SEM	N
30.0	1.499	1.52	1.559		1.526	0.018	3.00
20.0	0.997	1.018	1.033		1.016	0.010	3.00
15.0	0.736	0.757	0.775		0.756	0.011	3.00
10.0	0.503	0.496	0.522		0.507	0.008	3.00
7.5	0.378	0.372	0.398		0.383	0.008	3.00
5.0	0.259	0.24	0.252		0.250	0.006	3.00
Bis(7)-tacrine solution that was prepared using glass vials							
[B7T] (μM)	Absorbance at λ 244 nm (mOD)						
	Exp. 1	Exp. 2	Exp. 3		Mean	SEM	N
30.0	1.34	1.274	1.245		1.286	0.028	3.00
20.0	0.899	0.877	0.853		0.876	0.013	3.00
15.0	0.628	0.589	0.61		0.609	0.011	3.00
10.0	0.406	0.403	0.362		0.390	0.014	3.00
7.5	0.276	0.259	0.265		0.267	0.005	3.00
5.0	0.179	0.171	0.173		0.174	0.002	3.00
Bis(7)-tacrine solution that was prepared using glass vials in presence of 0.4% P20							
[B7T] (μM)	Absorbance at λ 244 nm (mOD)						
	Exp. 1	Exp. 2	Exp. 3		Mean	SEM	N
30.0	1.419	1.356	1.443		1.406	0.026	3.00
20.0	0.912	0.913	0.927		0.917	0.005	3.00
15.0	0.688	0.672	0.709		0.690	0.011	3.00
10.0	0.451	0.445	0.443		0.446	0.002	3.00
7.5	0.331	0.324	0.337		0.331	0.004	3.00
5.0	0.216	0.203	0.21		0.210	0.004	3.00

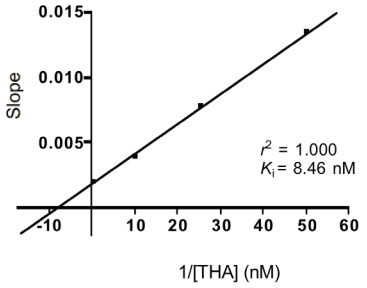
Table S2B. Absorbance data of tacrine solutions were prepared using glass or plastic vials.

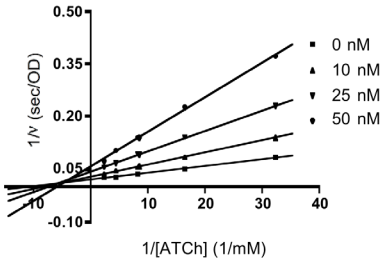
Tacrine solution that was prepared using plastic vials							
[THA] (μM)	Absorbance at λ 242 nm (mOD)						
	Exp. 1	Exp. 2	Exp. 3		Mean	SEM	N
30.0	1.141	1.111	1.07		1.107	0.021	3.00
20.0	0.734	0.744	0.721		0.733	0.007	3.00
15.0	0.566	0.557	0.547		0.557	0.005	3.00
10.0	0.378	0.372	0.365		0.372	0.004	3.00
7.5	0.275	0.278	0.275		0.276	0.001	3.00
5.0	0.185	0.186	0.183		0.185	0.001	3.00
Tacrine solution that was prepared using glass vials							
[THA] (μM)	Absorbance at λ 242 nm (mOD)						
	Exp. 1	Exp. 2	Exp. 3		Mean	SEM	N
30.0	1.104	1.072	1.094		1.090	0.009	3.00
20.0	0.753	0.722	0.717		0.731	0.011	3.00
15.0	0.556	0.542	0.54		0.546	0.005	3.00
10.0	0.376	0.369	0.362		0.369	0.004	3.00
7.5	0.277	0.276	0.297		0.283	0.007	3.00
5.0	0.186	0.189	0.186		0.187	0.001	3.00

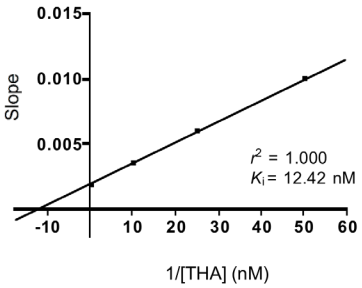
Figure S1. Plots for K_i determination under different assay conditions. Left: reciprocal hydrolysis rate (1/v in second per optical density) was plotted against reciprocal substrate concentration (1/[ATCh] in 1/mM) in the absence and presence of an inhibitor at varying concentration; right: the slope of the double reciprocal plot was plotted against inhibitor concentration ([I] μM or nM or pM). K_i was obtained from the negative x intercept of the slope replot.

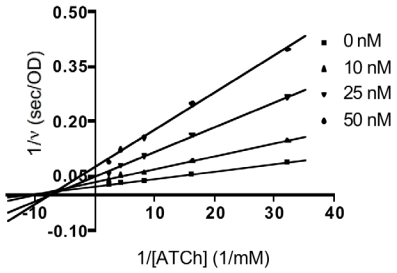
Figure S1A. Tacrine in plastic vial with 10.4 pg *ee*AChE.

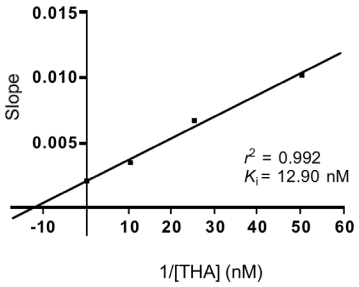


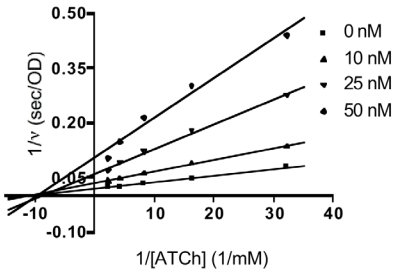


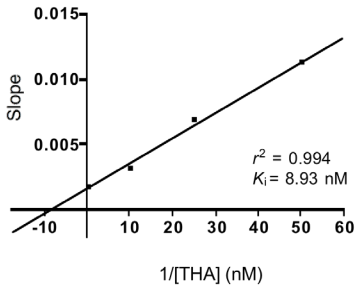


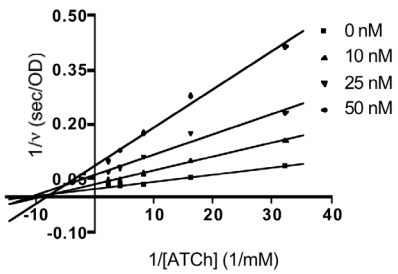












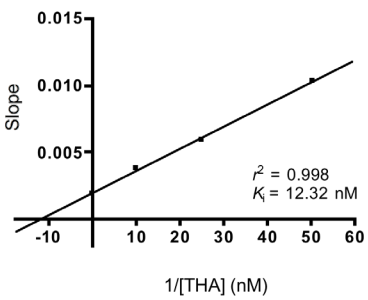
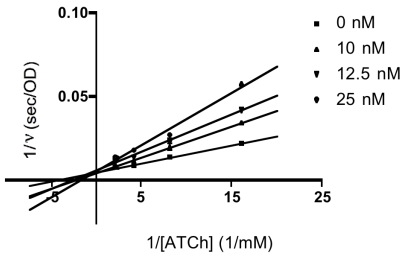
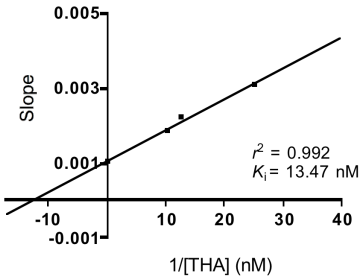
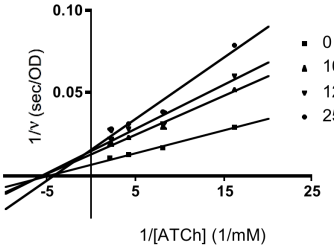
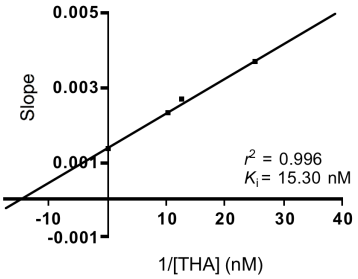


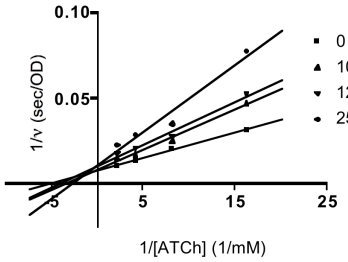
Figure S1B. Tacrine in plastic vial with 41.7 pg *ee*AChE.

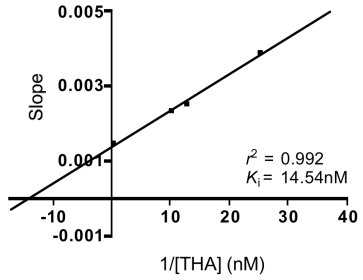


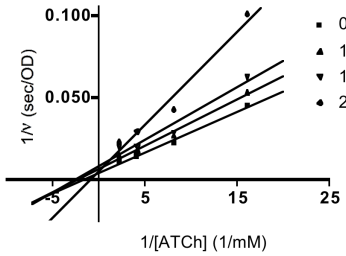


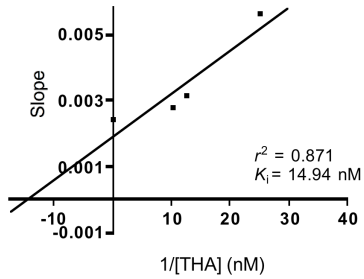


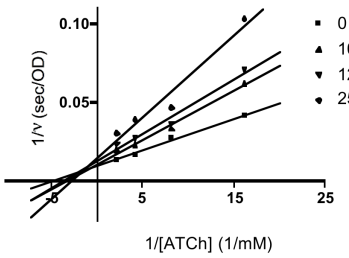












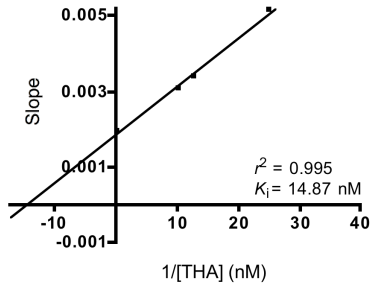
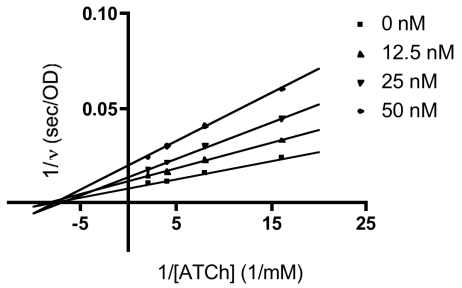
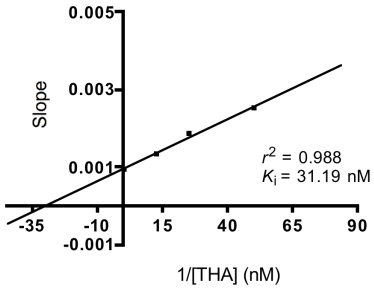
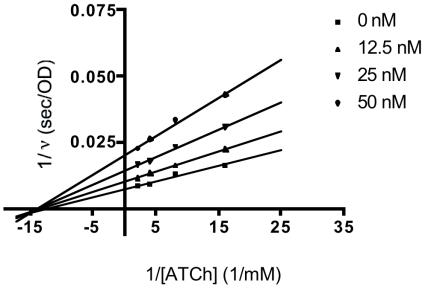
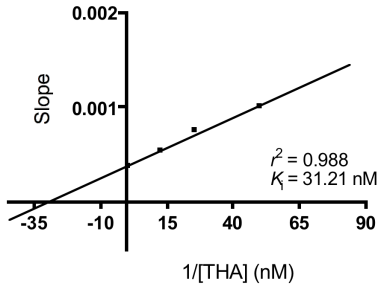


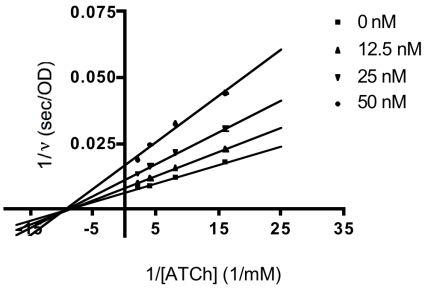
Figure S1C. Tacrine in plastic vial with 83.3 pg *ee*AChE.

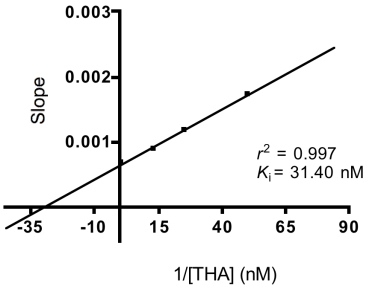


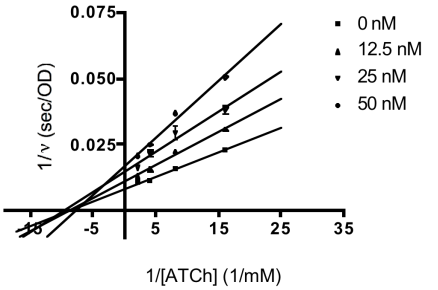


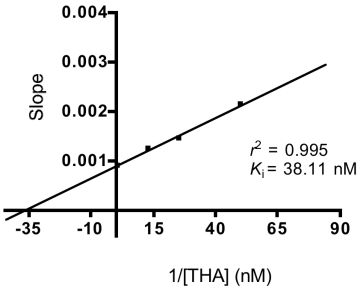


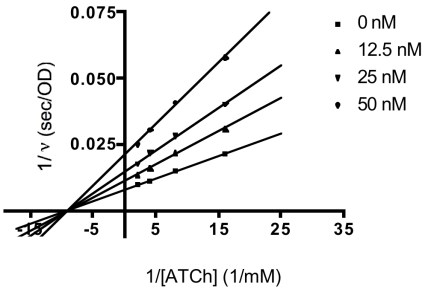












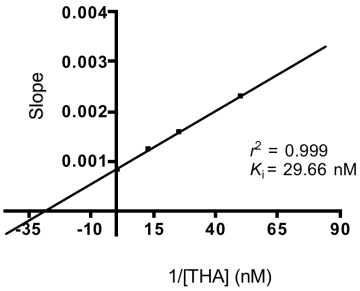


Figure S1D. Tacrine in glass vial with 83.3 pg *ee*AChE.

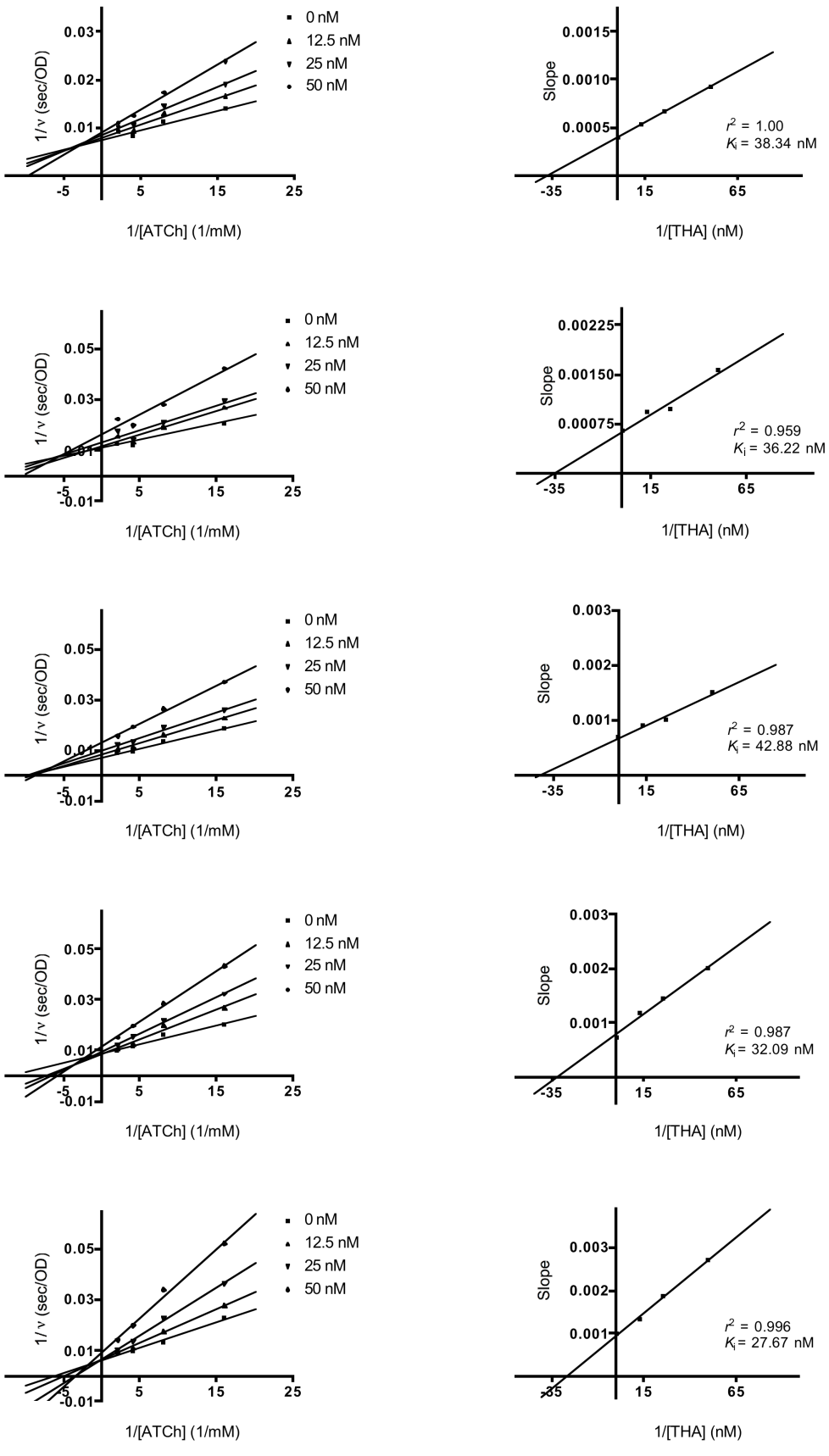
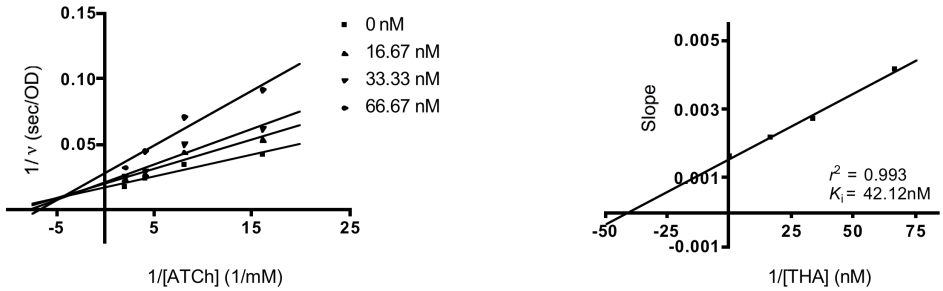
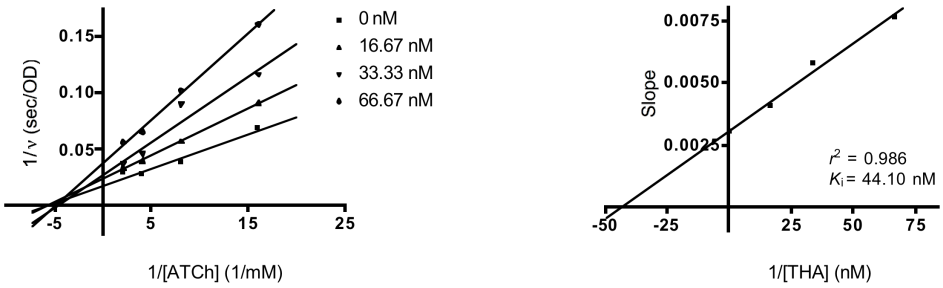
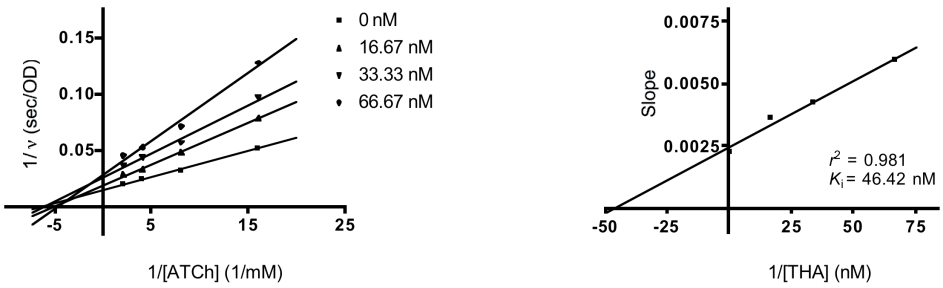
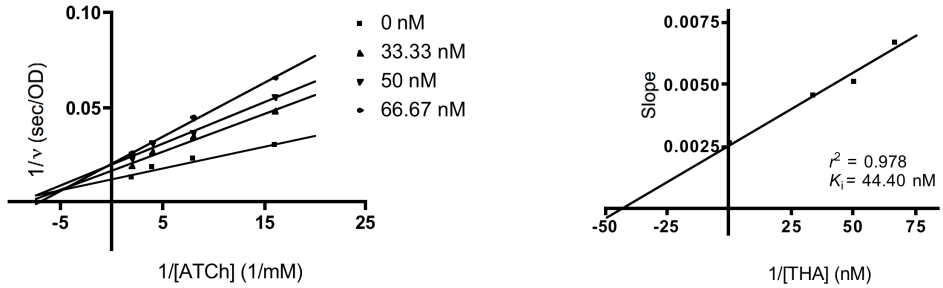


Figure S1E. Tacrine in plastic vial with 125.0 pg *ee*AChE.









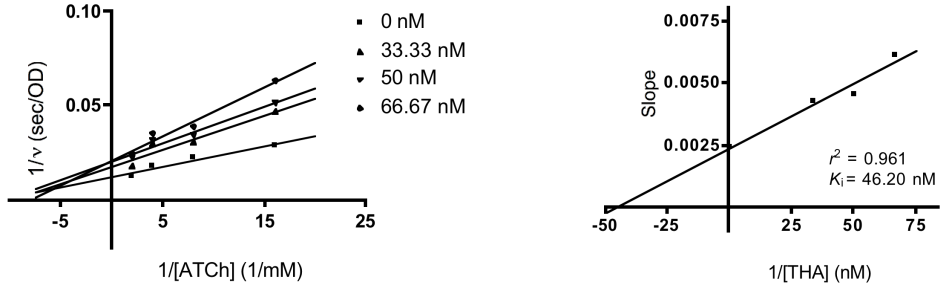


Figure S1F. Tacrine in plastic vial with 250.0 pg *ee*AChE.

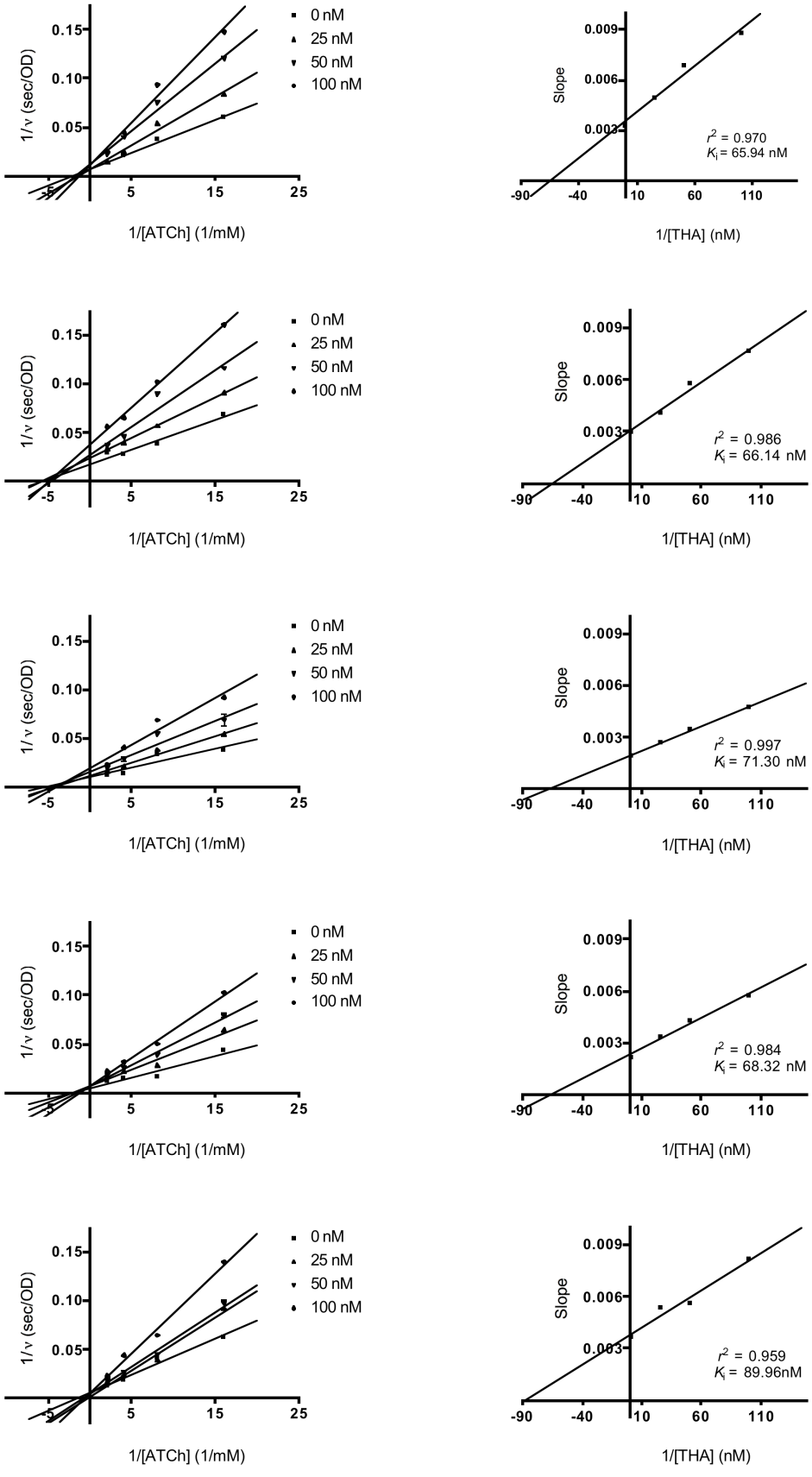
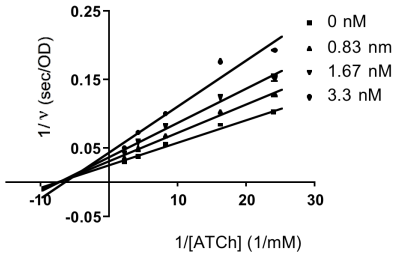
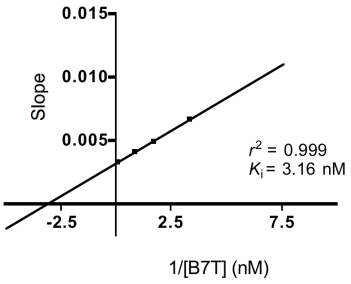
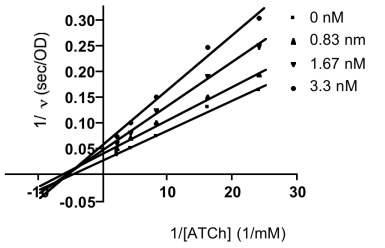
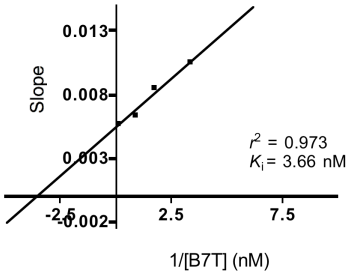


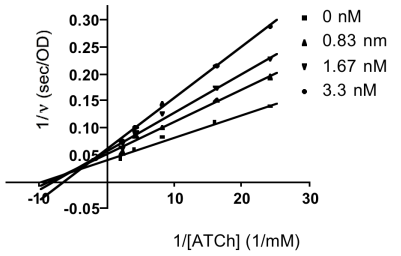
Figure S1G. Bis(7)-tacrine in glass vial with 10.4 pg *ee*AChE.

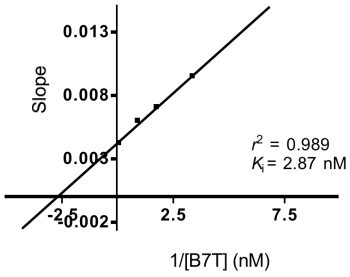


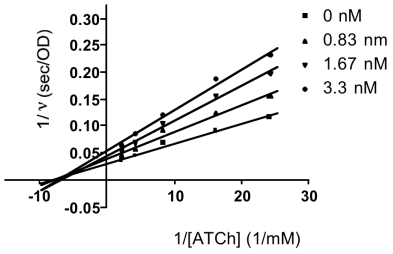


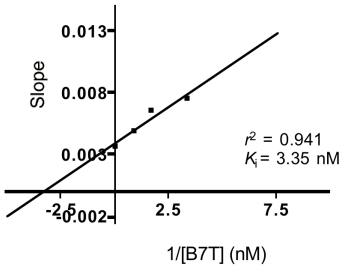


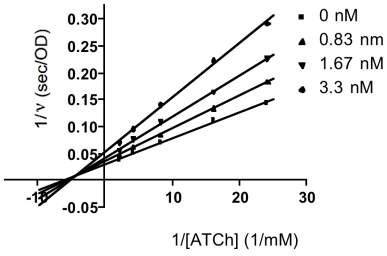












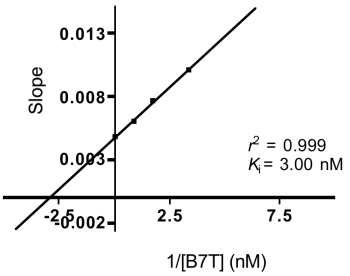
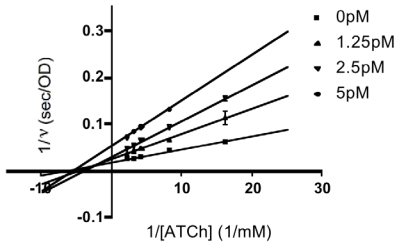
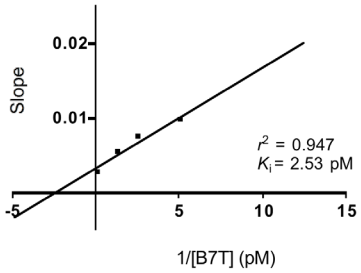
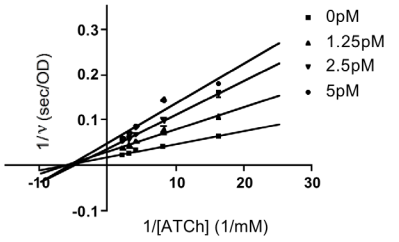
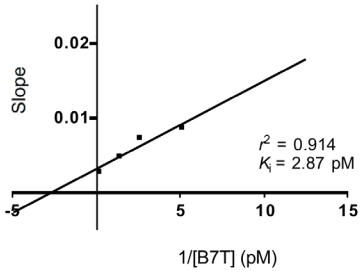


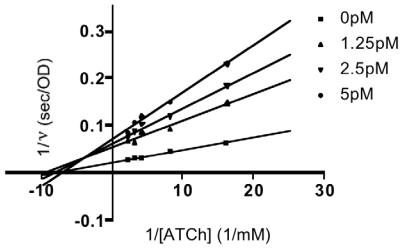
Figure S1H. Bis(7)-tacrine in plastic vial with 10.4 pg *ee*AChE.

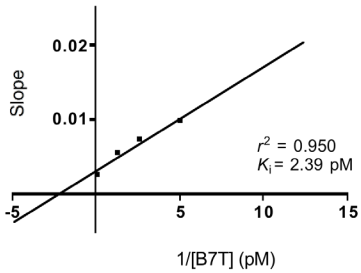


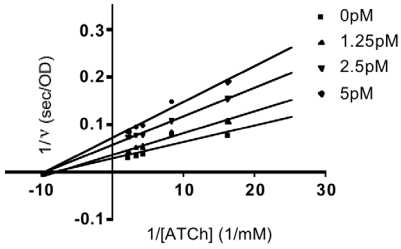


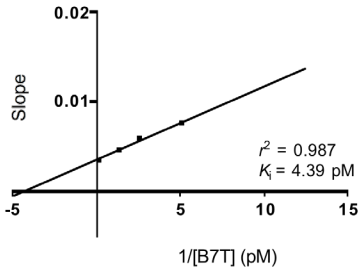


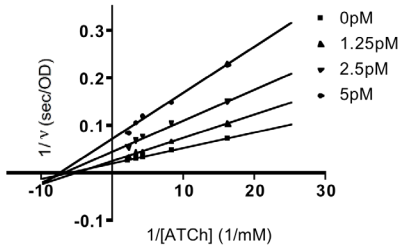












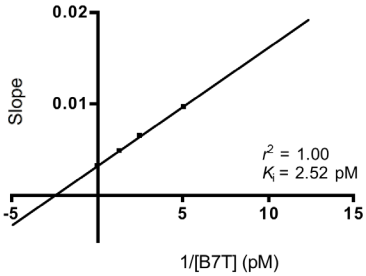
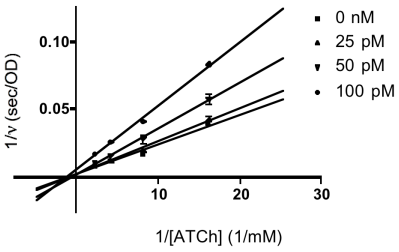
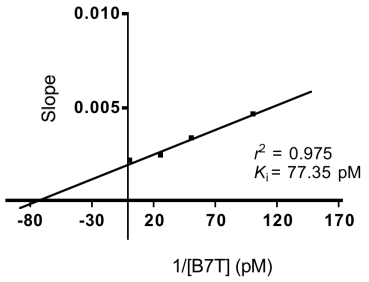
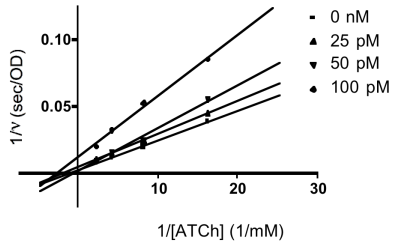
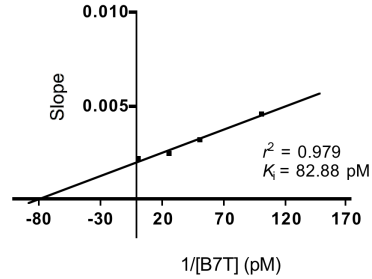


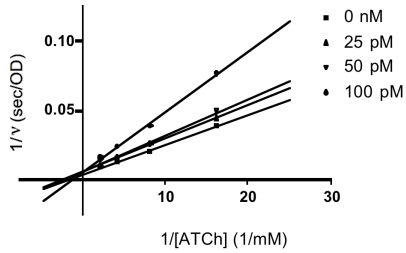
Figure S1I. Bis(7)-tacrine in plastic vial with 41.7 pg *ee*AChE.

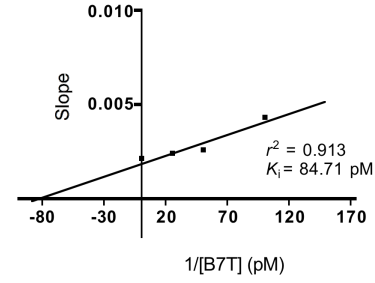


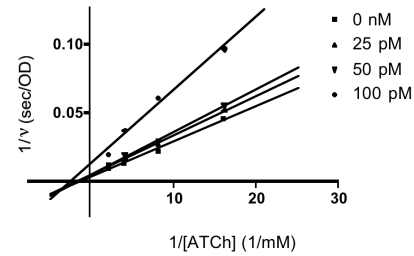


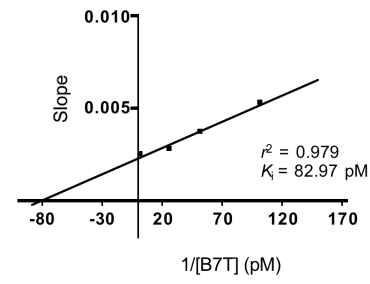


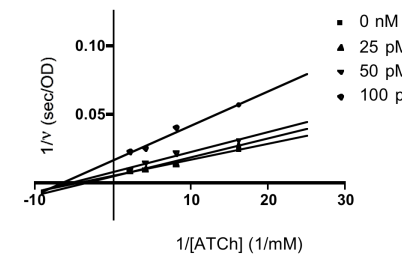












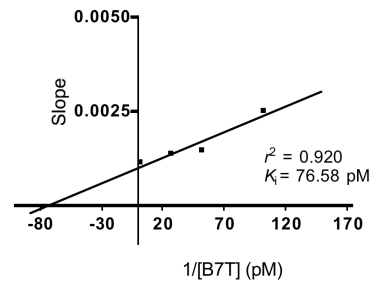
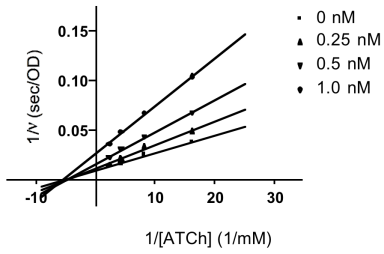
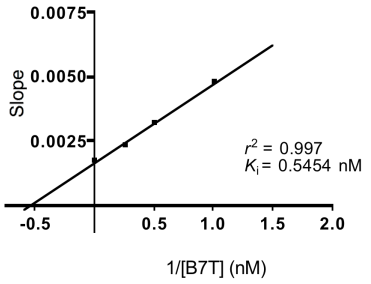
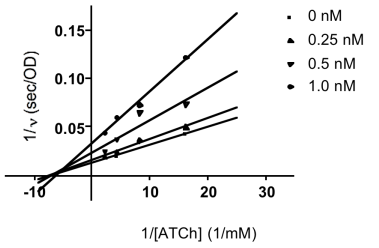
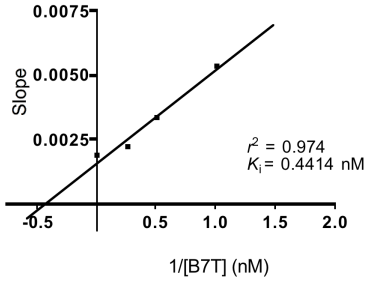


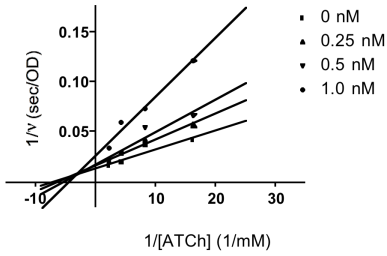
Figure S1J. Bis(7)-tacrine in plastic vial with 125.0 pg *ee*AChE.

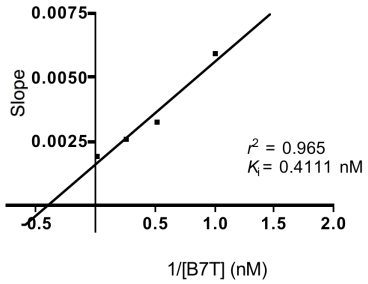


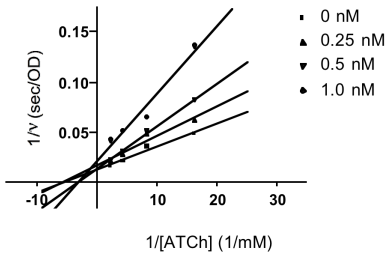


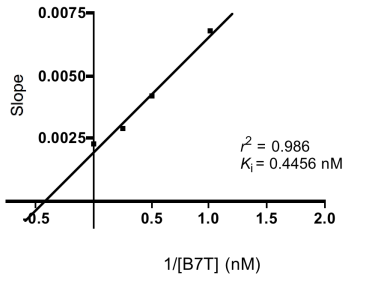


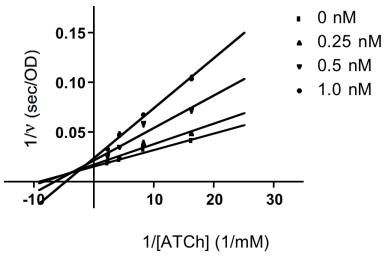












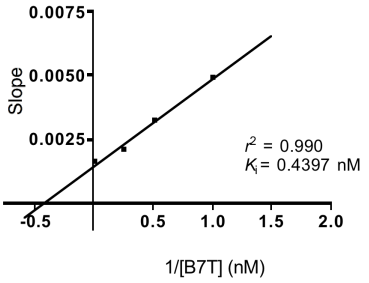
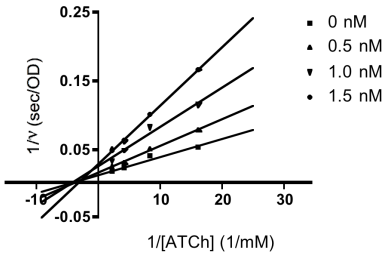
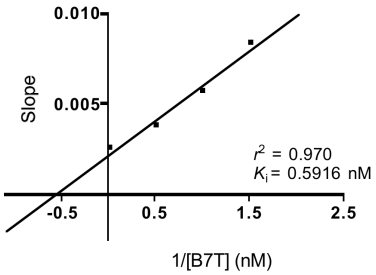
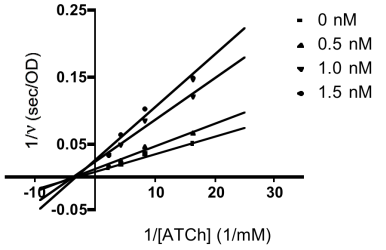
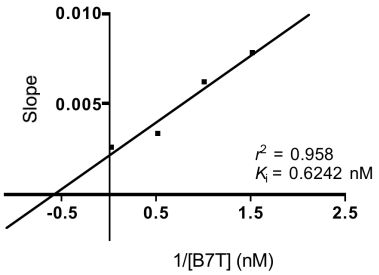


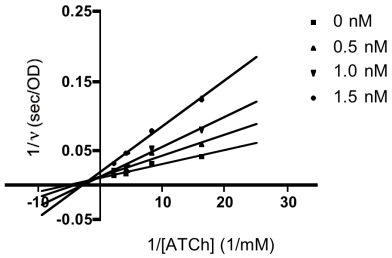
Figure S1K. Bis(7)-tacrine in plastic vial with 250.0 pg *ee*AChE.

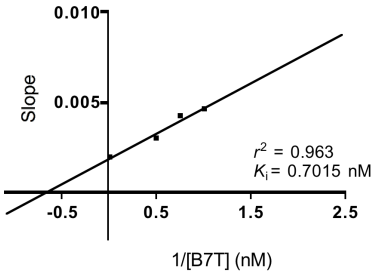


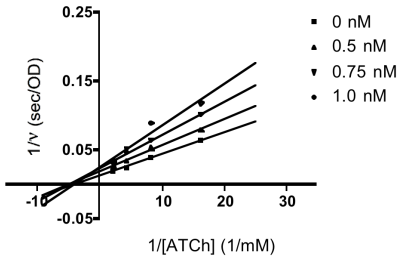


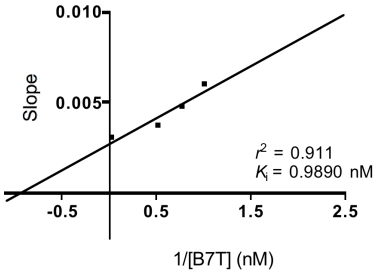


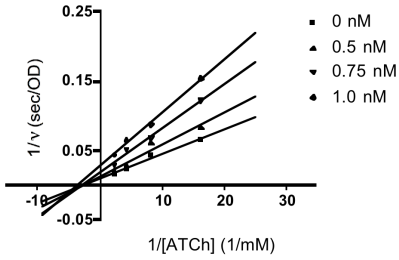


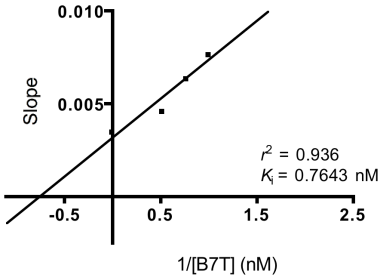












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