Electronic Supplementary Information

Thioflavin-based molecular probes for application in Alzheimer's Disease: from *in silico* to *in vitro* models[†]

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Complete Reference

(57) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J., J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A.; Gaussian 09, Revisions B.01 and C.01 ed.; Gaussian, Inc.: Wallingford CT, 2009.

Synthesis

2-(Benzo[d]oxazol-2-yl)-4-iodophenol (HBXI)

HBXI was prepared according to a previously reported method.[1] ^{1}H NMR (CD_2Cl_2, 300 MHz): δ 6.95 (d, *J*=8.7 Hz, 1H), 7.46-7.44 (m, 2H), 7.66 (dd, *J* =8.7, 1.8 Hz, 1H), 7.79-7.65 (m, 2H), 8.36 (d, *J* =1.8 Hz, 1H), 11.5 (s, 1H). ^{13}C NMR (CD_2Cl_2, 75 MHz): δ 81.0, 111.0, 112.6, 119.6, 119.4, 125.1, 125.8, 135.0, 139.5, 141.7, 149.1, 158.3, 161.3. MS (+ESI-MS) *m*/*z* (relative intensity): 338.1 ($[L + H]^+$, 100). Elemental analysis: calcd (found) for $C_{13}H_8INO_2 \cdot 0.25H_2O$: C, 45.71 (45.60); H, 2.51 (2.35); N, 4.10 (4.14).

2-(Benzo[d]thiazol-2-yl)-4-iodophenol (HBTI)

HBTI was prepared according to a previously reported method.[1] ^{1}H NMR (CD_2Cl_2, 300 MHz): δ 6.93 (d, *J*=8.6 Hz, 1H), 7.55-7.46 (m, 2H), 7.65 (dd, *J*=8.6, 1.9 Hz, 1H), 7.95-7.99 (m, 2H), 8.03 (d, *J*=1.9 Hz, 1H), 12.6 (s, 1H). ^{13}C NMR (CD_2Cl_2, 75 MHz): δ 80.3, 120.3, 121.5, 122.2, 122.3, 125.8, 126.1, 126.8, 132.4, 136.3, 140.9, 156.6, 167.5. MS (+ESI-MS) *m*/*z* (relative intensity): 354.0 ($[L + H]^+$, 100). Elemental analysis: calcd (found) for $C_{13}H_8INSO \cdot 0.3H_2O$: C, 43.54 (43.35); H, 2.42 (2.22); N, 3.91 (3.96).

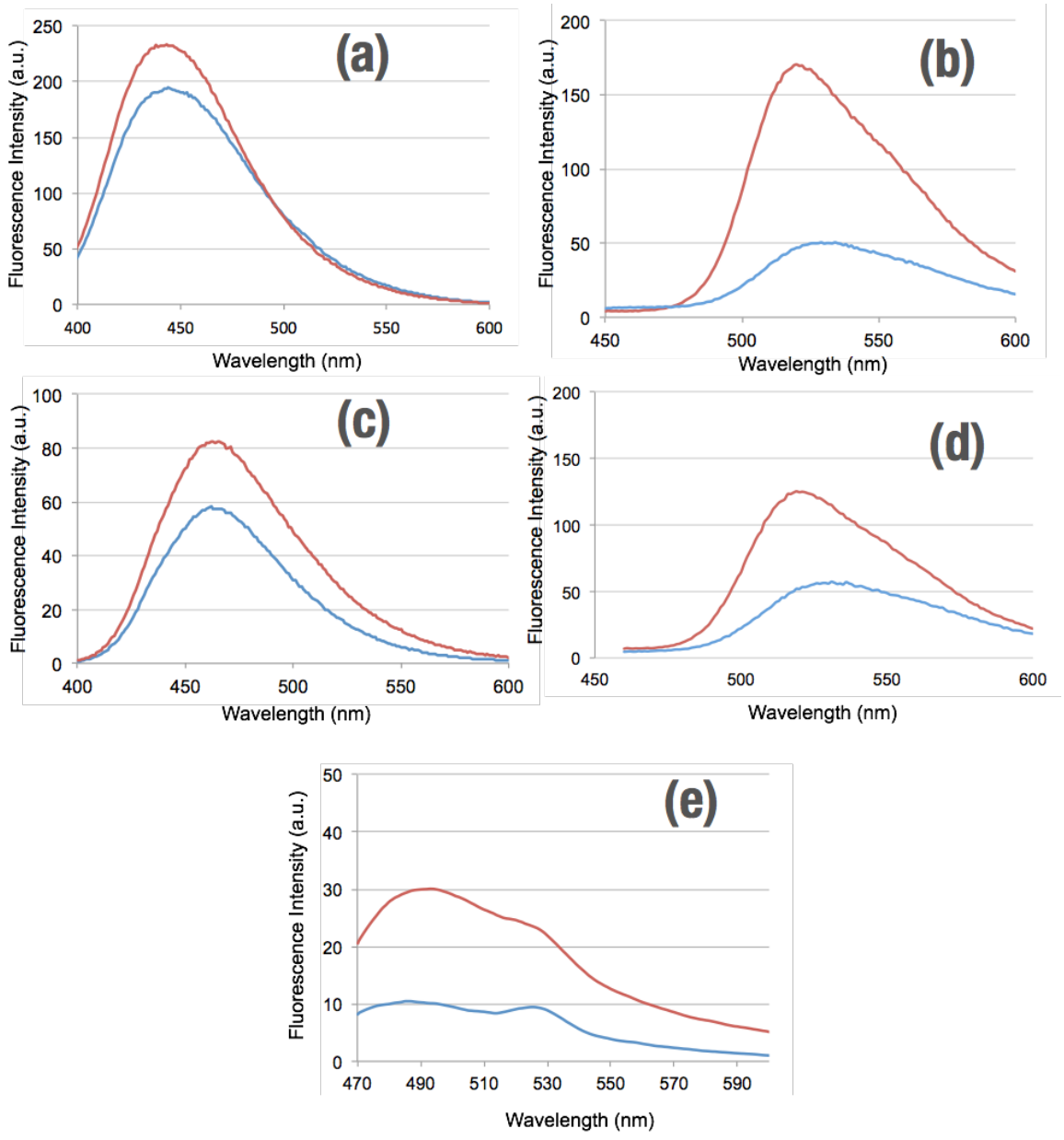

Fig. S1. Fluorescence spectra of (a) **HBX**, (b) **HBXI**, (c) **HBT**, (d) **HBTI** and (e) ThT, in the presence (red line) of $A\beta_{1\text{-}40}$ fibrils. Excitation wavelength for **HBX** and **HBT** was 320 nm, for **HBXI** and **HBTI** 340 nm and for ThT 450 nm.

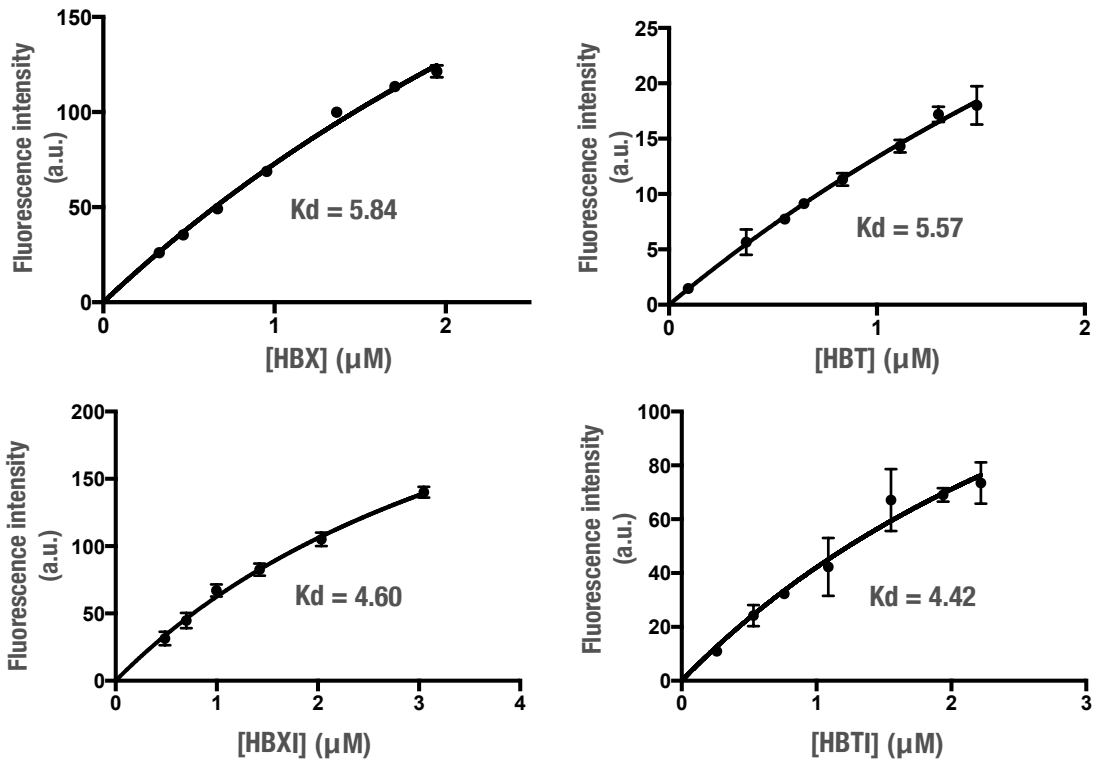

Fig. S2. Binding constant, $K_{d,}$ for the ligands **HBX**, **HBXI**, **HBT** and **HBTI** under the studied conditions. Data is an average of three trials with relative standard deviations.

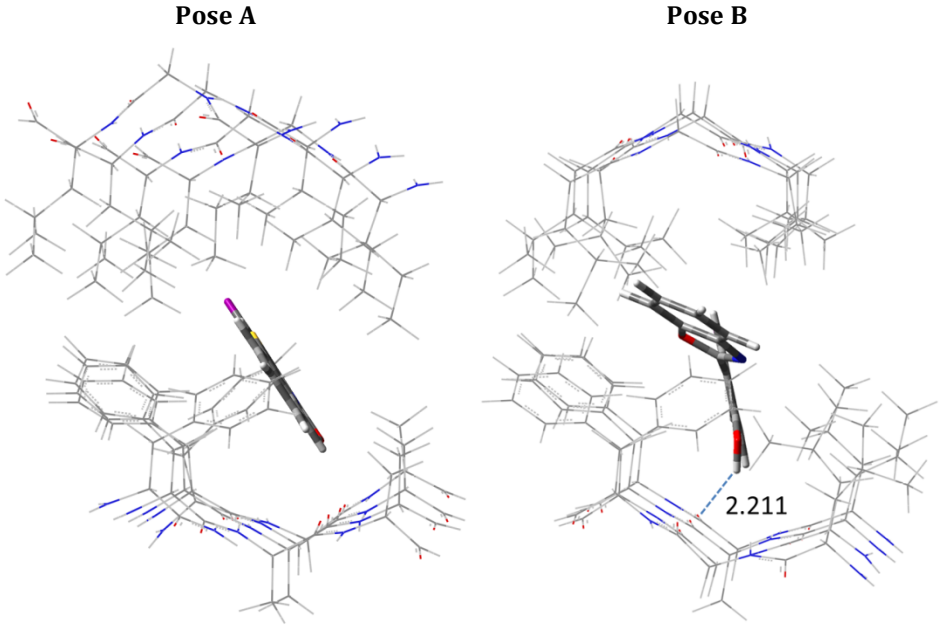

Fig. S3. ONIOM optimized geometries for **HBTI** (poses A) and **HBXI** (pose B) bound to $A\beta_{1\text{-}40}$. The initial structures were obtained from molecular docking as detailed in the text.

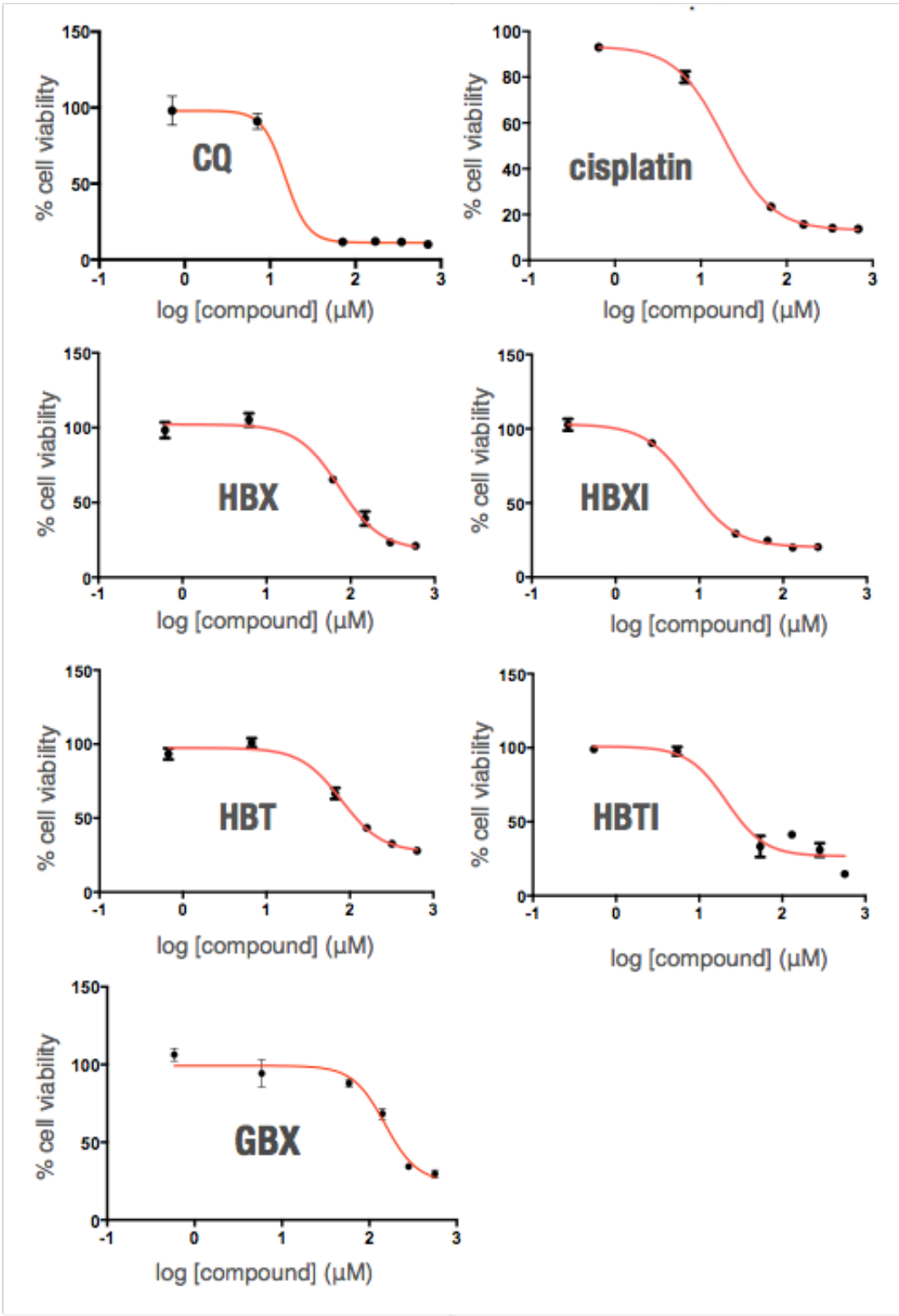

Fig. S4. BEnd.3 cell line viability in the presence of compounds **HBX**, **HBT**, **HBXI**, **HBTI** and the prodrug **GBX**. Clioquinol(CQ) and cisplatin are added for comparison. Values are an average of three trials with standard relative deviation.

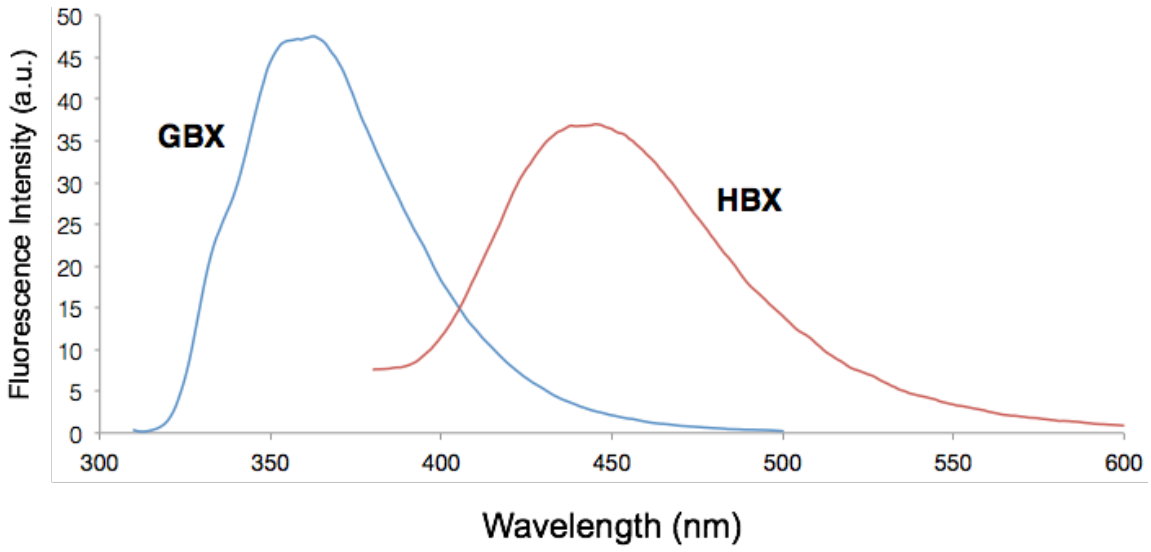

Fig. S5. Fluorescence spectra of **GBX**, and **HBX**. Excitation wavelength for **GBX** 300 nm, and for **HBX** 320 nm.

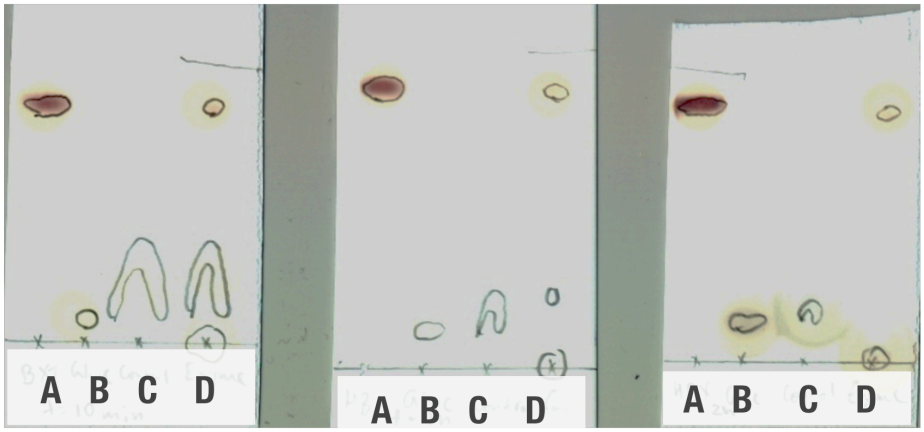

Fig. S6. TLC plates monitoring enzyme activity (95% dichloromethane/5% methanol) A. **HBX**, B. **GBX,** C. control: **GBX** in DMSO/water solution, D. **GBX** and glucosidase in DMSO/water solution. Incubation times: **1.** 10 min., **2.** one hour, **3.** two hours. **HBX** and glucose spots are discoloured due to contact with Fe(III)/methanol and H_2SO_4/ethanol, respectively.

Table S1. Main interaction distances between the different ligands and the fibrils. R(CH–π) refers to the distance between the C atom of the closest CH group and the centroid of the closest five or six-membered ring.

Ligand	R(CH- π) distances	H-Bond
HBT	4.324, 3.631, 3.747, 4.185, 4.332	
HBX	3.673, 3.235, 4.381, 3.912	2.017
HBTI	4.286, 3.625, 3.846, 4.484	
HBXI	3.222, 3.965, 4.408, 3.526	2.211

Table S2. EC_{50} values with their respective 95% confidence intervals, as calculated by GraphPad Prism for cisplatin, **1**, **2**, and **3**.

Ligand	**EC_{50} (μM)**	**95% Confidence Intervals (μM)**
HBX	73.28	56.63 to 94.83
HBT	78.93	62.07 to 100.4
HBXI	7.75	5.974 to 10.05
HBTI	21.23	8.011 to 56.25
GBX	150.90	117.1 to 194.4
CQ	14.97	5.195 to 43.33
cisplatin	18.58	15.52 to 22.24

Bibliography for Supporting Information

(1) Rodríguez-Rodríguez, C.; Sánchez de Groot, N.; Rimola, A.; Álvarez-Larena, A.; Lloveras,V.; Vidal-Gancedo, J.; Ventura, S.; Vendrell, J.; Sodupe, M.; González-Duarte, P. Design, selection, and characterization of thioflavin-based intercalation compounds with metal chelating properties for application in Alzheimer's disease, *J. Am. Chem. Soc.* **2009,** *131*, (4), 1436-1451.