Supporting Information

Title: 7-Methoxytacrine and 2-Aminobenzothiazole Heterodimers: Structure–Mechanism Relationship of Amyloid Inhibitors Based on Rational Design

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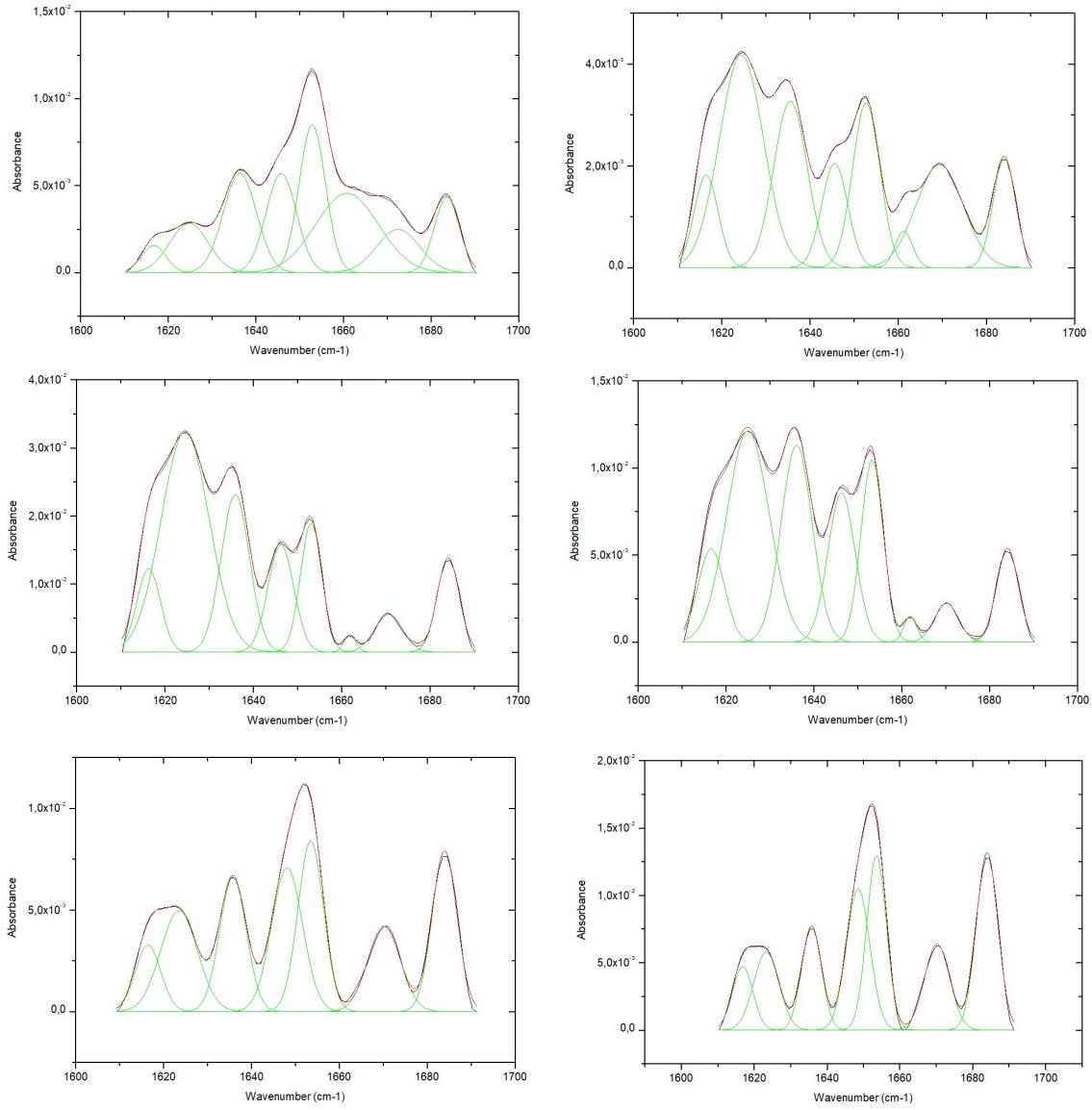

Figure S1. FTIR peak analysis for (A) native HEWL, (B) HEWL amyloid fibrils, HEWL aggregates formed in the presence of parent molecules (C) 7-MEOTA, (D) BTZ and synthesized heterodimers (E) HK 1068 and (F) HK 1072. All deconvolutions were done in Origin 8.

Table S1. Secondary structure percentage distributions of samples measured by FTIR with corresponding peak positions and adjusted R^2 coefficient representing precision of the deconvolution.

HEWL	**Native**		**Fibrils**		**+ 7-MEOTA**		**+ BTZ**		**+ HK 1068**		**+ HK 1072**	
Secondary structure	peak [cm^{-1}]	%	peak [cm^{-1}]	%	peak [cm^{-1}]	%	peak [cm^{-1}]	%	peak [cm^{-1}]	%	peak [cm^{-1}]	%
α-helical	1653 1661	41	1653 1661	15	1653 1662	12	1653 1662	15	1653	20	1654	19
β-sheet	1625 1636	24	1624 1636	46	1625 1636	58	1625 1636	52	1623 1636	29	1623 1636	22
β-turn	1670 1683	17	1669 1684	23	1671 1684	12	1670 1684	10	1670 1684	27	1670 1684	33
Side chain	1617	3	1616	8	1616	7	1617	8	1616	7	1617	7
Random coil	1646	14	1645	8	1646	11	1646	14	1648	18	1648	18
Adj. R^2	0.998		0.998		0.998		0.998		0.997		0.997	

Table S2. List of residues forming non-bonded contacts with six HK compounds. Red refers to common residues in the binding site for all of compounds.

Compound	**List of residues forming non-bonded contact with six compounds**
BTZ	Ile98, Trp108, Trp63, Asn59, Asp52, Gln57, Ala107
7-MEOTA	Ile98, Trp108, Asn46, Asp48, Trp63, Asn59, Asp52, Gln57, Ala107
HK 1066	Ile98, Trp108, Asp48, Phe34, Asn46, Asn44, Gln35, Val109, Trp63, Asn59, Asp52, Gln57, Ala107
HK 1068	Ile98, Trp108, Arg114, Phe34, Asp48, Asn46, Gln35, Val109, Ala110, Trp63, Asn59, Asp52, Gln57, Ala107
HK 1070	Arg61, Ash101, Leu75, Asp48, Gln35, Val109, Trp62, Trp63, Asn59, Asp52, Gln57, Ala107

HK 1072	Asn44, Asn46, Ile98, Trp108, Val109, Gln35, Trp63, Asn59, Asp52, Gln57, Ala107

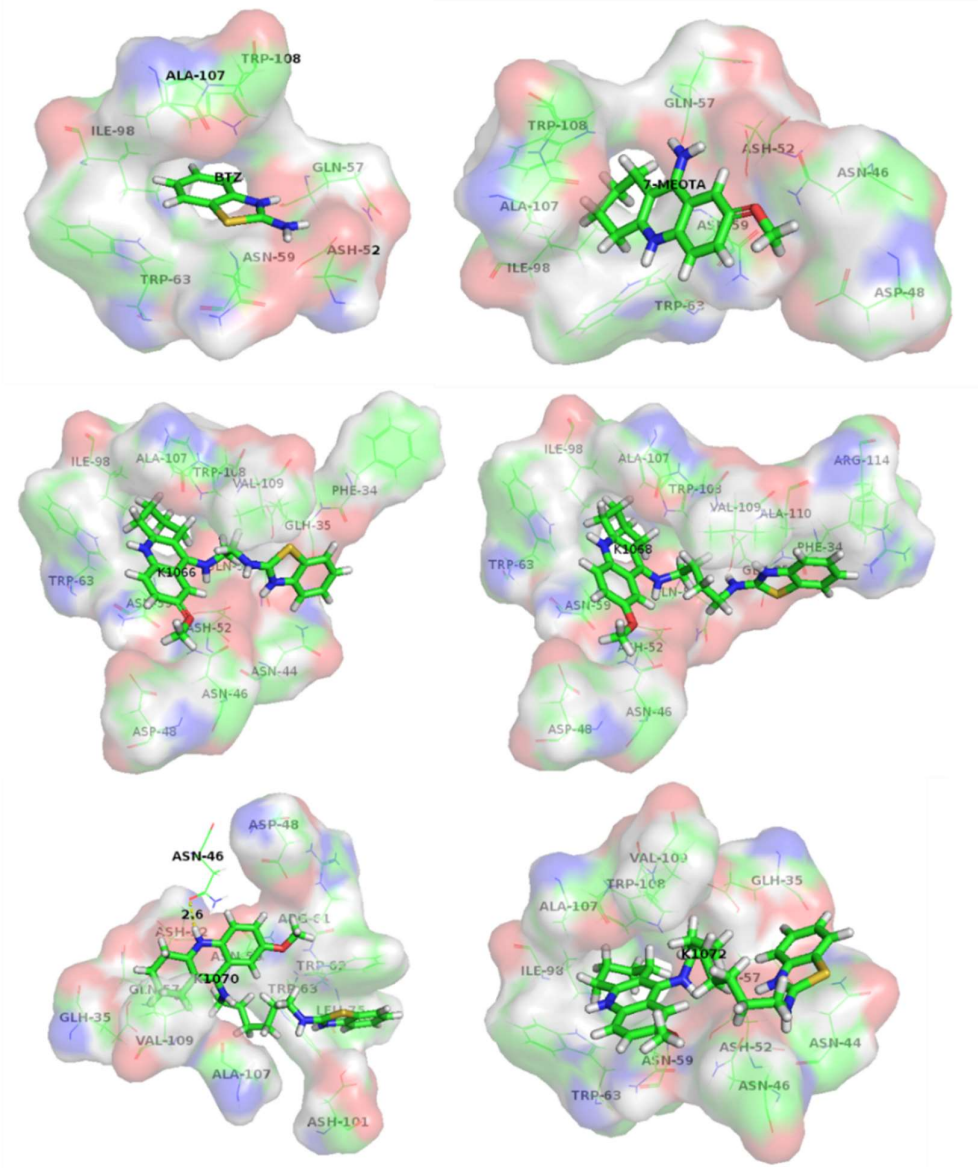

Figure S2. 3D images of docking poses of six HK compounds in lysozyme.

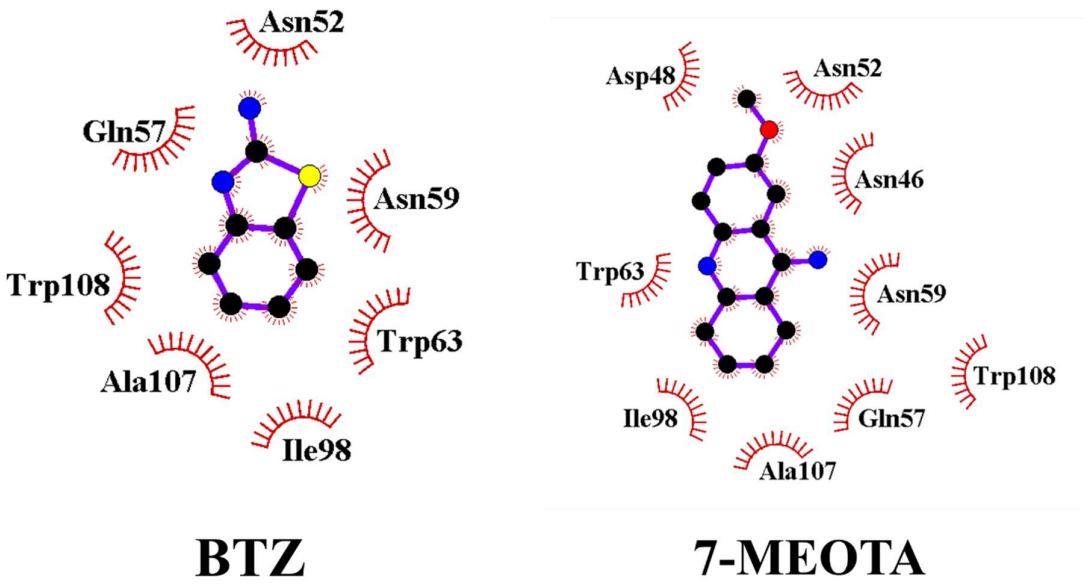

Figure S3. Contact networks of parent compounds 7-MEOTA and BTZ in the best docking mode. Red "eyes' refer to non-bonded contacts. The plots were created by using LigPlot+ version 1.4.4.

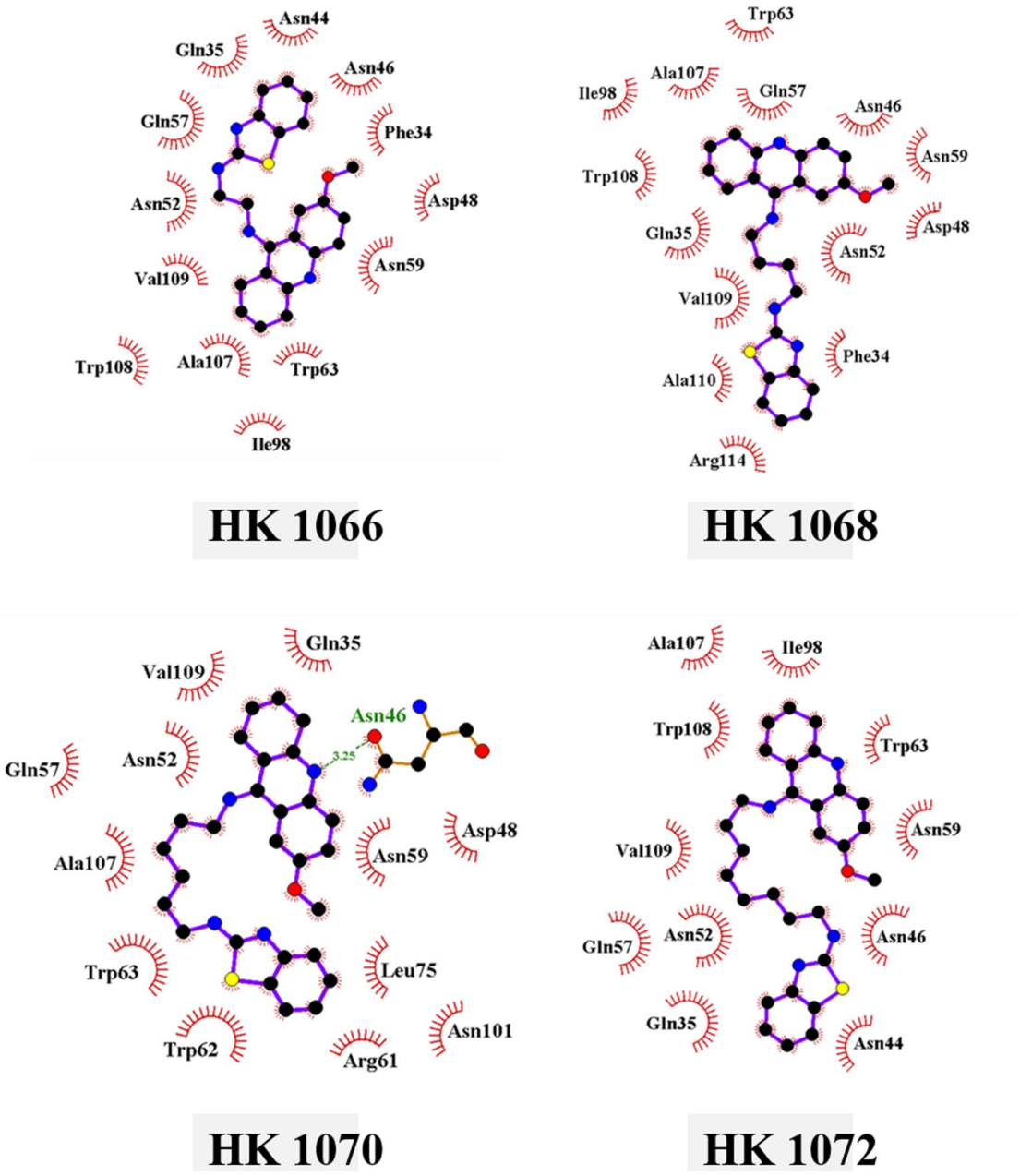

Figure S4. Contact networks of heterodimers HK 1066, HK 1068, HK 1070, and HK 1072 in the best docking mode. Red "eyes" refer to non-bonded contacts, while the green dashed line denotes hydrogen bonds. The plots were created by using LigPlot+ version 1.4.4.

Table S3. Binding energy of six ligands in lysozyme was obtained by the docking method. Non-bond contacts and hydrogen bonds were obtained by Ligplot software. The number of carbons that link BTZ and 7-MEOTA groups is in the fourth column.

Ligands	**IC_{50} [μM]**	**Binding energy [kcal.mol^{-1}] (ΔE_{bind})**	**Linking carbons**	**Non-bond contacts (NBCs)**	**Hydrogen bonds (HBs)**
7-MEOTA	66.8 ± 4.4	-7.0	-	9	0
BTZ	871.4 ± 64.3	-5.7	-	7	0
HK 1066	115.6 ± 11.3	-8.7	2	13	0
HK 1068	48.7 ± 7.6	-8.5	4	14	0
HK 1070	15.4 ± 0.9	-7.3	6	12	1
HK 1072	1.6 ± 0.3	-8.3	8	11	0

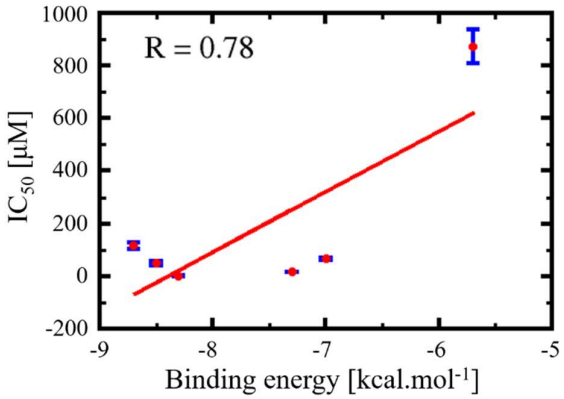

Figure S5. Correlation between IC_{50} values and the docking binding energy.

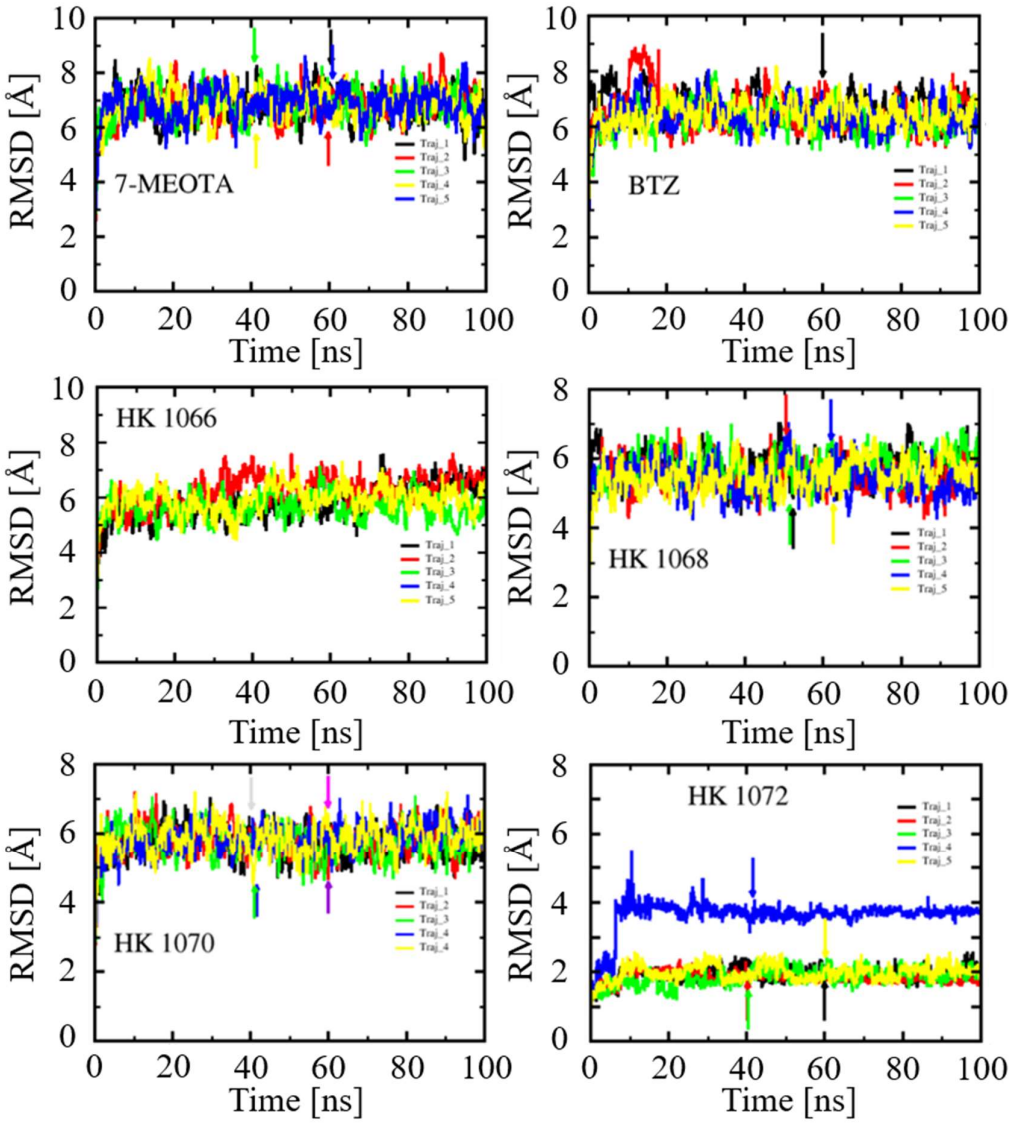

Figure S6. Time dependence of Cα RMSD of lysozyme bound to parent molecules 7-MEOTA, BTZ and heterodimers HK 1066, HK 1068, HK 1070 and HK 1072 at pH 2.7. The arrows refer to the equilibration time.

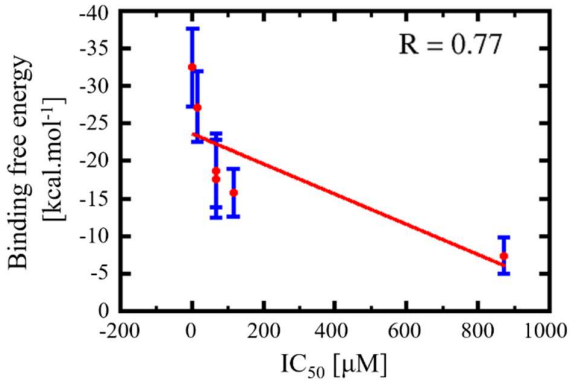

Figure S7. The correlation between binding free energy (MM-PBSA) and IC_{50}.

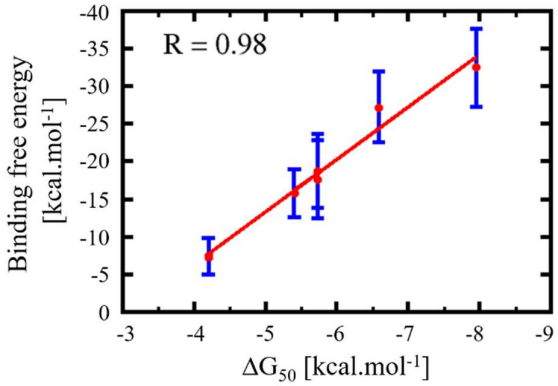

Figure S8. The correlation between binding free energy (MM-PBSA) and ΔG_{exp}.

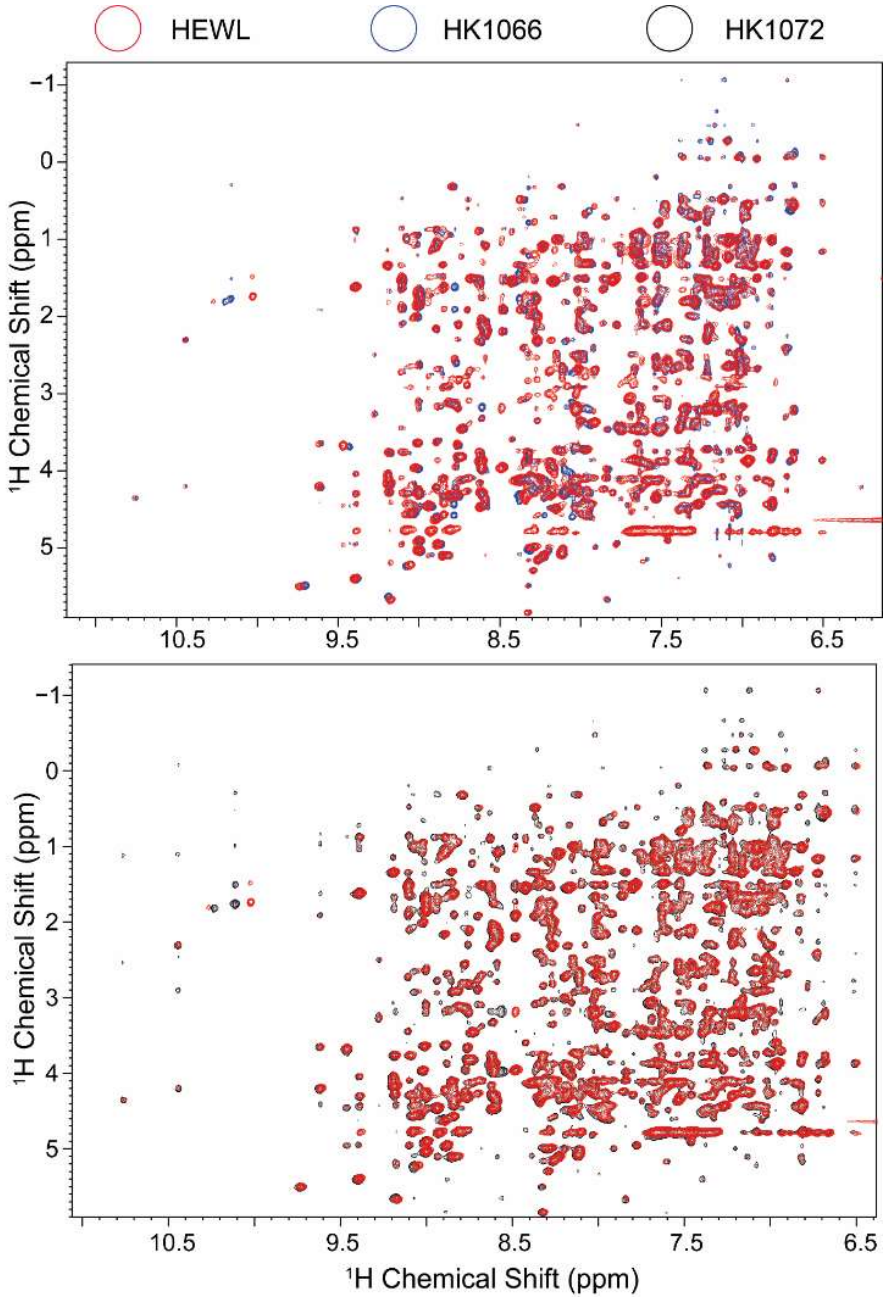

Figure S9. Chemical shift perturbation (CSP) of amide and aromatic protons of HEWL (red) after the addition of HK 1066 (blue) and HK 1072 (black). The overlaid figures depict that the NOESY spectra of HEWL in the presence of compounds got higher cross peaks compared to the free HEWL, indicating interaction with the HK compounds.

Control experiment - clarification of the potential competitive binding among ThT and studied compounds

In figure S10, we show that HEWL amyloid fibrils formed in our experimental conditions bind ThT with resulting fluorescence intensity ~ 75 000 f.u. taken as 100 %. Afterwards, 10 μM HK 1072 and 20 μM ThT (same concentration as in inhibition experiments) were added to the prepared samples. Based on the observed fluorescence (~ 97%) we suggest that ThT was able to bind to amyloid fibrils in the presence of the compounds without any competition. On the other hand, when ThT was added after the incubation of the HEWL in the presence of 10 μM HK 1072 a significant decrease (~ 54 %) of the ThT fluorescence was observed. This indicates that after studied compounds interact with monomeric HEWL species, fewer amyloid aggregates able to bind ThT are formed and therefore the observed fluorescence was lower.

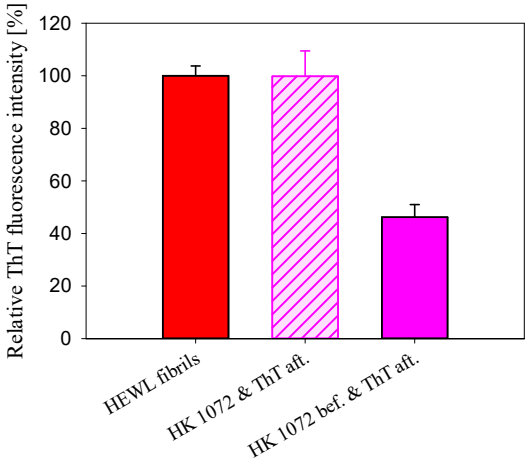

Figure S10. ThT fluorescence of HEWL amyloid aggregates formed without treatment (red column), HEWL amyloid fibrils in the presence of HK 1072 and ThT, both added after the aggregation process was completed (pink patterned column) and aggregates prepared in the presence of the HK 1072 with ThT added after the aggregation (magenta column).