Targeting the Multifaceted Neurotoxicity of Alzheimer's Disease by Tailored Functionalisation of the Curcumin Scaffold

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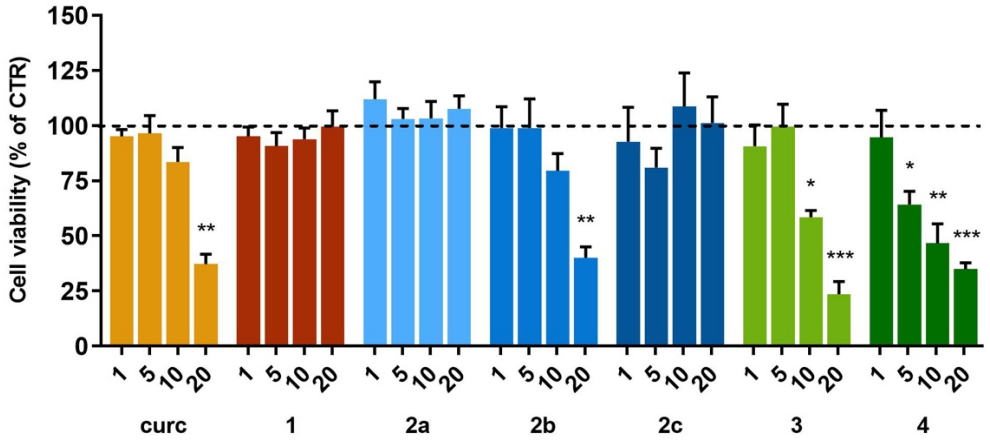


Figure S1. Effects of **1**, **2a-c**, **3**, and **4** and **curc** on microglia cell viability.
Microglia were treated with **curc** and the compounds (1-20 μM) for 24 h. Results are expressed as a percentage of cell viability relative to vehicle-treated cells. Data are reported as mean ± SEM (n = 3 independent experiments, in triplicate). *p < 0.05, **p < 0.01, and ***p < 0.001 versus control cells (dashed line). One-way ANOVA followed by Holm-Sidak's multiple comparison test.

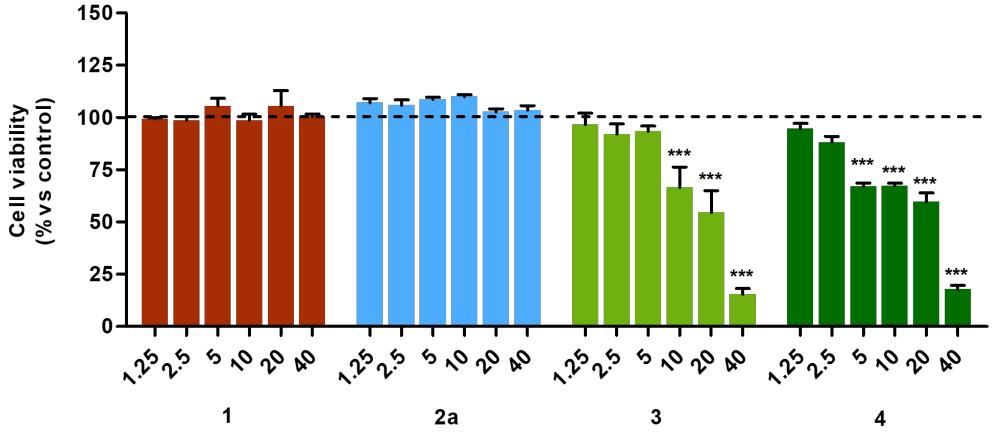


Figure S2. Effects of **1**, **2a**, **3**, and **4** and **curc** on SH-SY5Y cell viability.
Cells were incubated with different concentrations of the derivatives (1.25-40 μM). At the end of incubation, the cell viability was measured by MTT assay as described in the Experimental Section. Data are reported as mean ± SEM of three independent experiments. ***p < 0.001 versus untreated cells (dashed line). One-way ANOVA with Dunnett's Post Hoc Test.

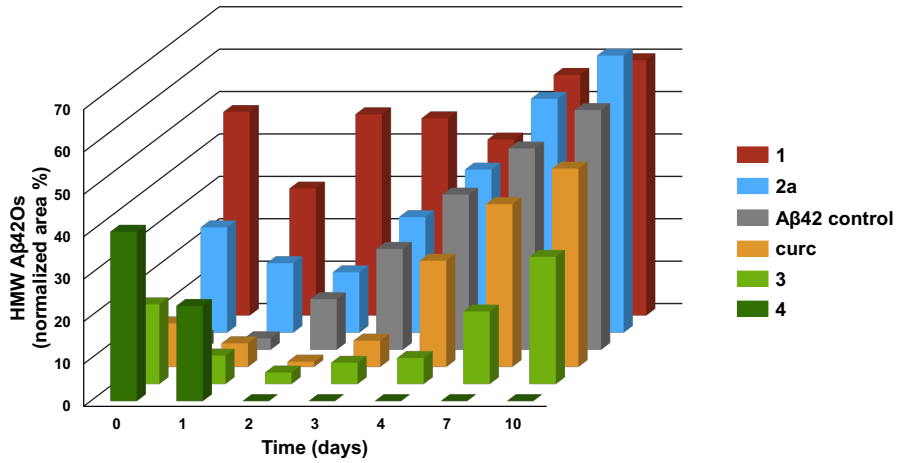


Figure S3. Effect of curcumin-based analogues **1**, **2a**, **3**, and **4** and **curc** on Aβ42 oligomerization.
Normalized area percentage plots of toxic HMW Aβ42Os from a 221 μM solution of Aβ42 monomer, in the absence (Aβ42 control) and in the presence of **curc**, **1**, **2a**, **3** (5 μM) and **4** (2.5 μM).

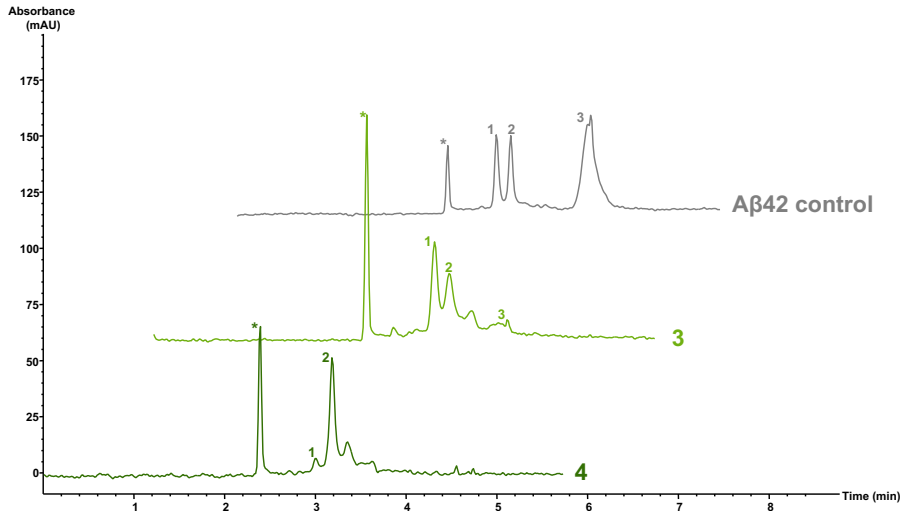


Figure S4. Electrophoretic profiles of 221 μM Aβ42 control and 221 μM Aβ42 control in the presence of 1 μM **3** and **4**.
Peak * refers to the electroosmotic flow. Peaks 1 and 2 are related to the migration of non-toxic LMW Aβ42Os (from trimers up to dodecamers), while peak 3 is related to the migration of toxic HMW Aβ42Os (aggregates smaller than 22-mers and larger than dodecamers) [1,2]. Electropherograms are taken at 10 days from sample dissolution.

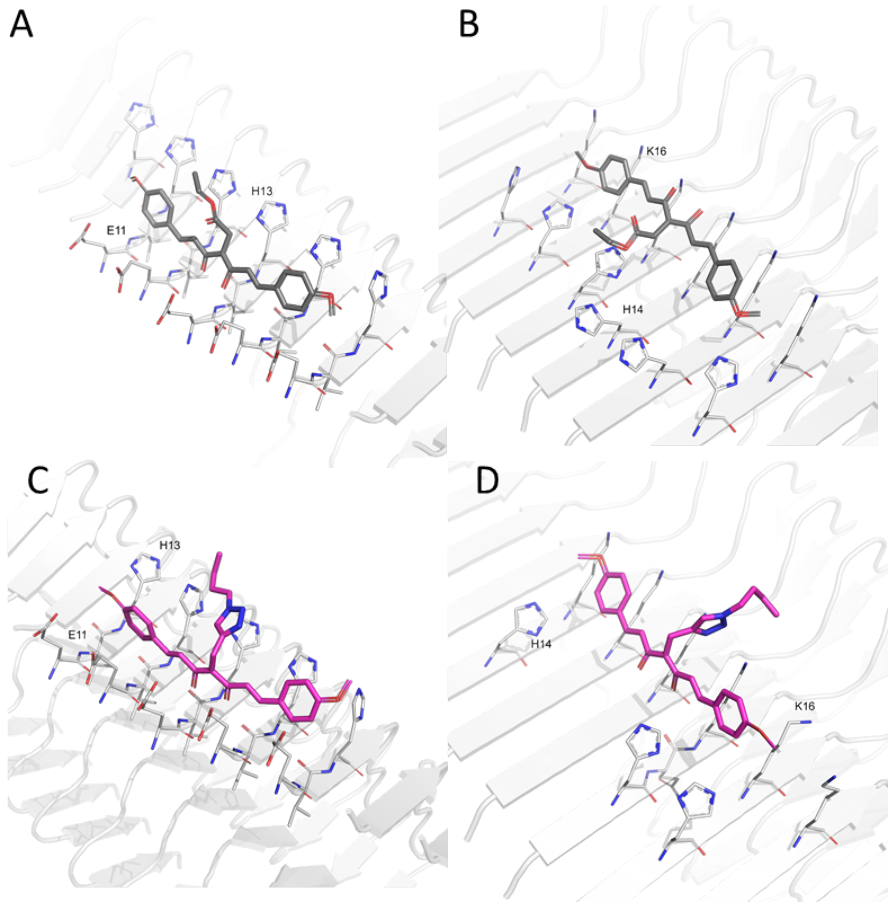


Figure S5. Docking study: putative complexes.
Putative complexes as obtained for Spot1 and Spot2 for the pro-aggregating compound **2a** (A and G for Spot1 and Spot2, respectively) and the anti-aggregating derivative **4** (C and D for Spot1 and Spot2, respectively).

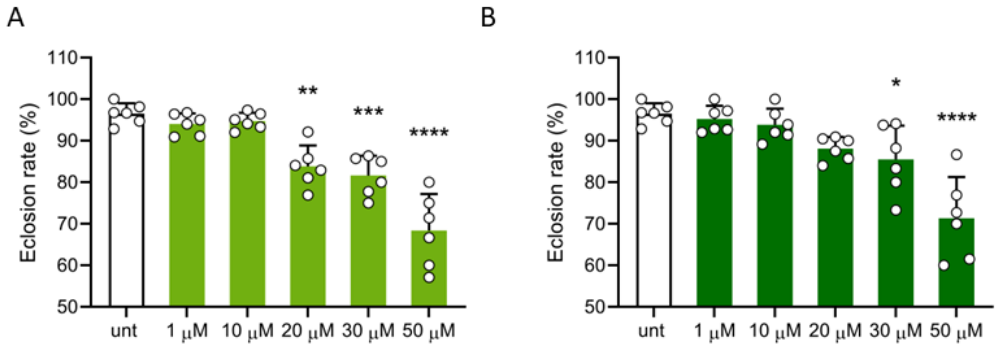


Figure S6. Identification of the non-toxic concentrations of **3** and **4** in the control *Drosophila* line.
Eclosion rate of the control line W1118 in standard food (unt), in (**A**) compound **3** and (**B**) compound **4** dissolved in DMSO and added to standard food at the following concentrations: 1, 10, 20, 30, 50 µM. The % of eclosion is expressed as the number of adult flies on the total number of pupae. One-way ANOVA with Tukey's *post hoc* test; * vs W1118 unt (n=6).

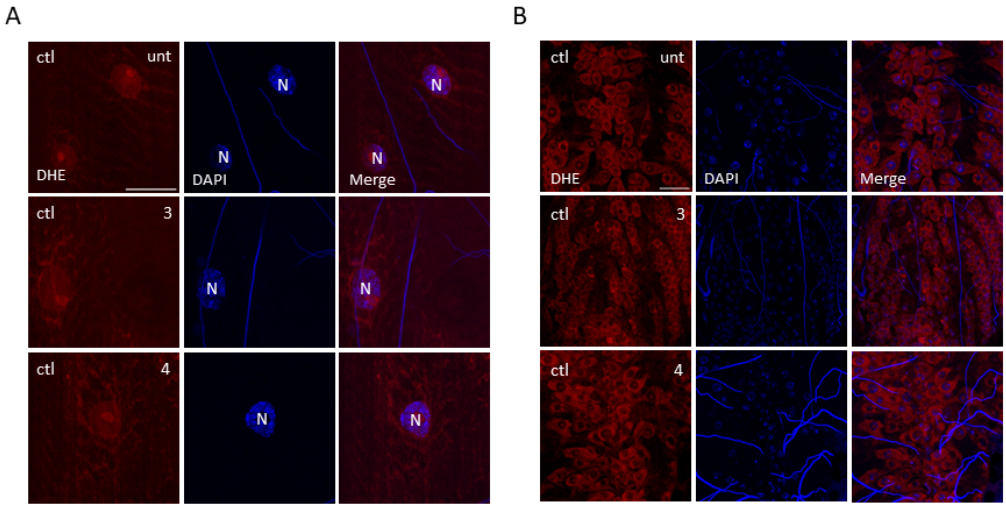


Figure S7. Effect of compounds **3** and **4** on muscle and brain ROS levels in control *Drosophila* line.
Control line (*Tubulin-Gal4/+*) was raised in standard, and **3** or **4** enriched food, larvae were dissected and stained with DHE to detect ROS levels and DAPI to visualize nuclei. Images show (**A**) larval muscles and (**B**) ventral nerve cords of larval brain. Scale bar = 20 µm.

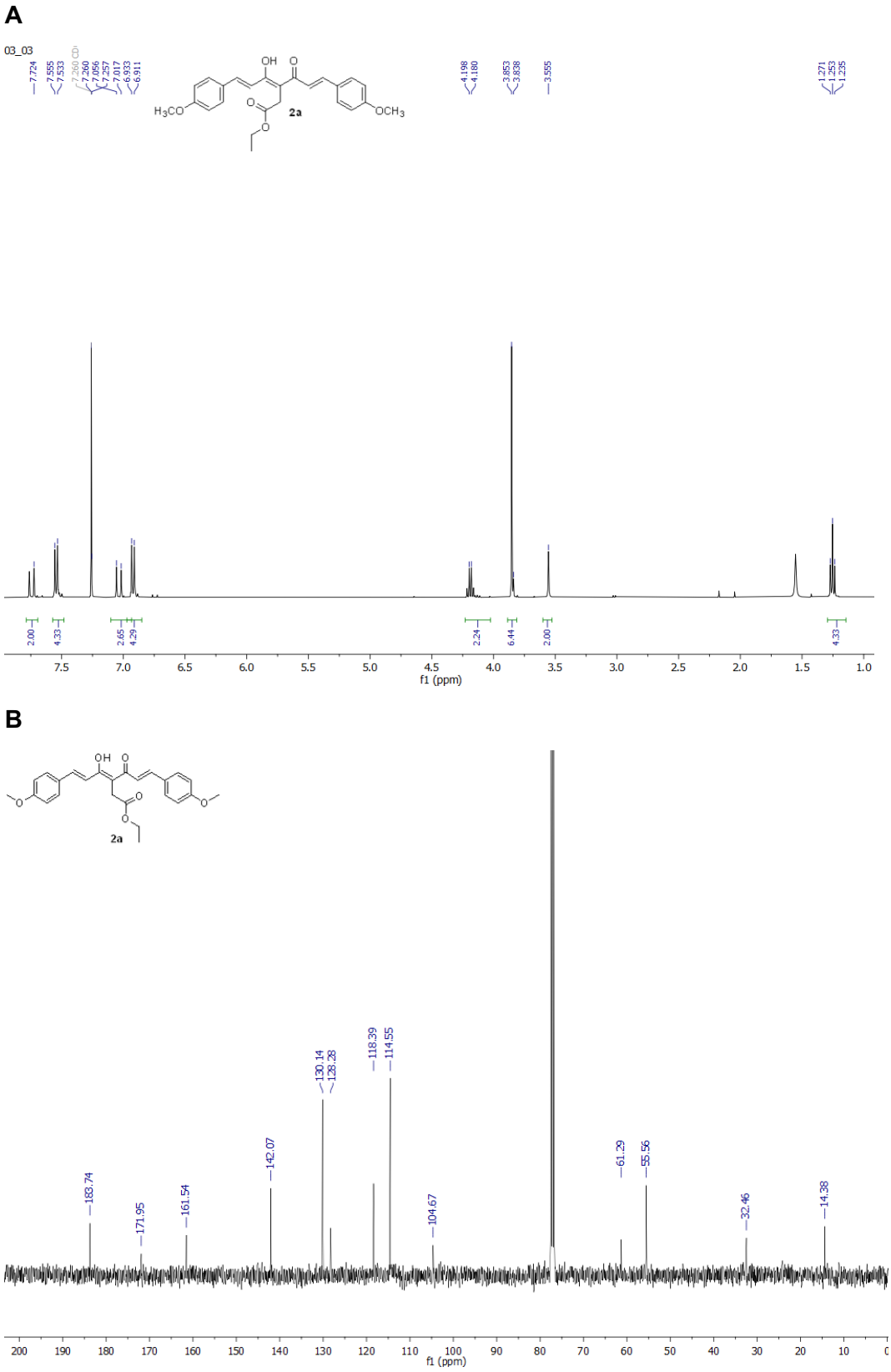


Figure S8. ^{1}H-NMR and ^{13}C-NMR Spectra of compound **2a.**
(**A**) ^{1}H-NMR and (**B**) ^{13}C-NMR ($CDCl_3$, 400 MHz) spectra of compound **2a.**

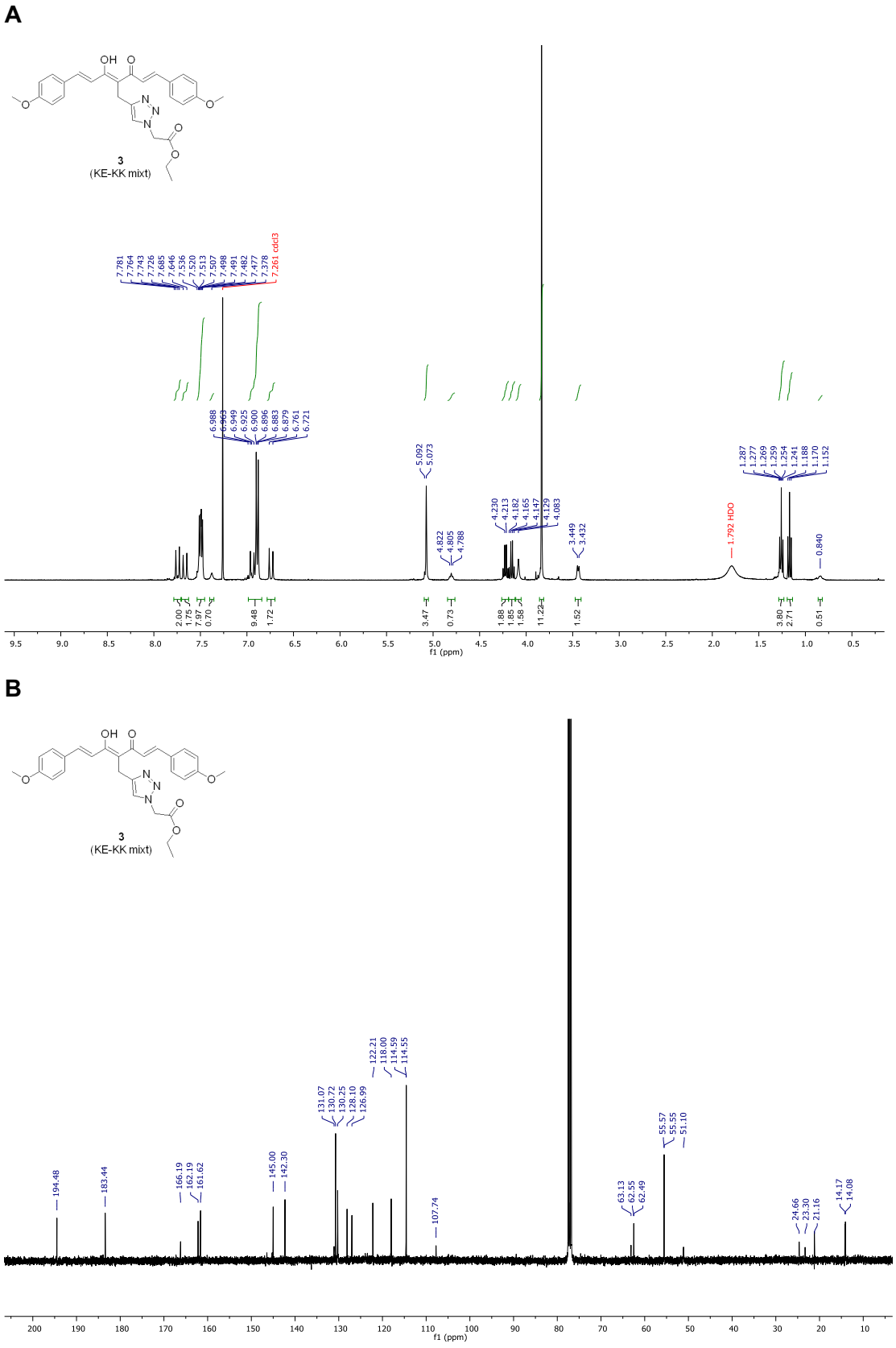


Figure S9. ^{1}H-NMR and ^{13}C-NMR Spectra of compound **3.**
(**A**) ^{1}H-NMR and (**B**) ^{13}C-NMR ($CDCl_3$, 400 MHz) spectra of compound **3.**

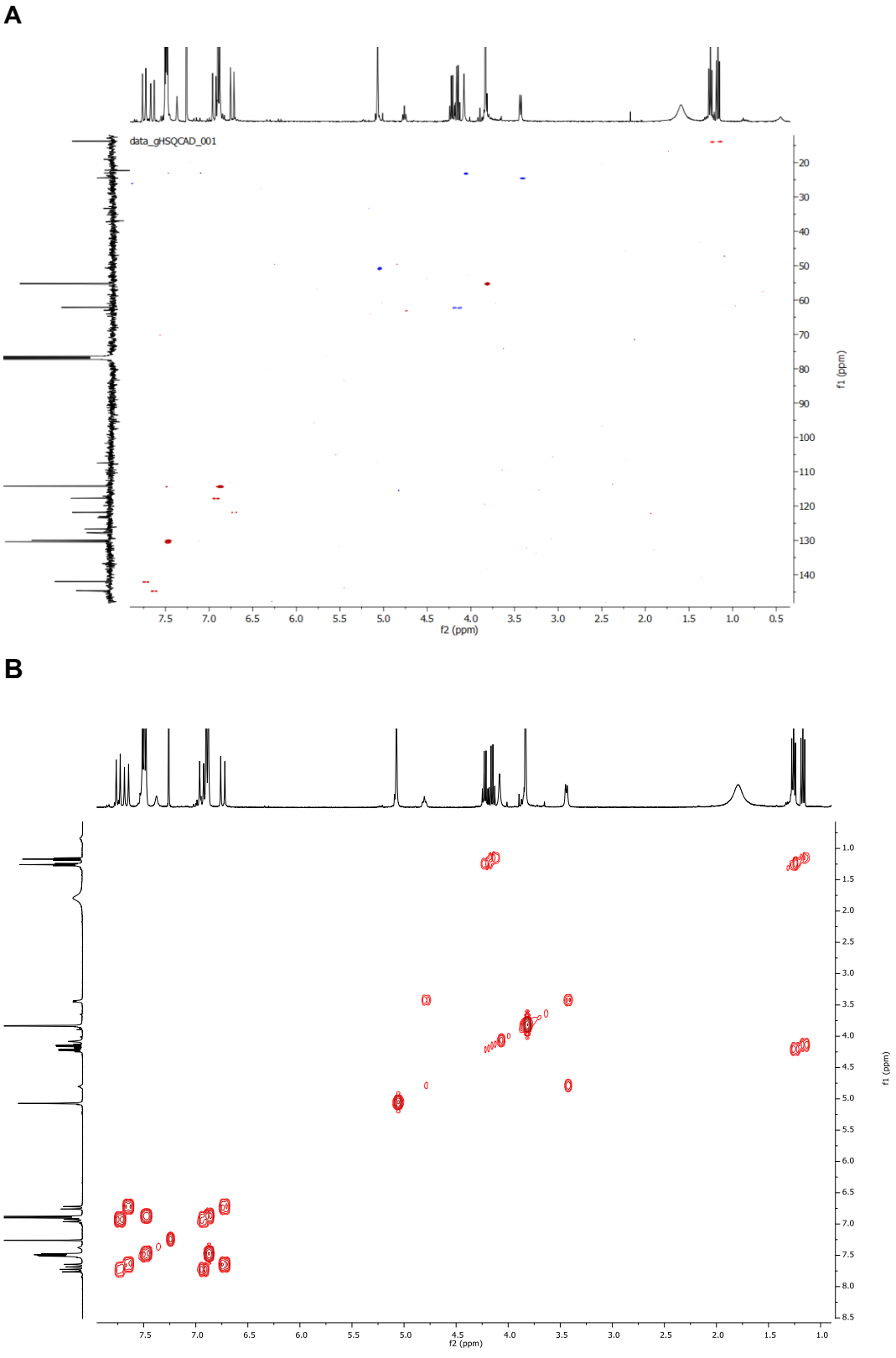


Figure S10. 2D ^{1}H-^{1}H COSY and 2D ^{1}H-^{13}C HSQC spectra of compound **3**. (**A**) 2D^{1}H-^{1}H COSY and (**B**) 2D ^{1}H-^{13}C HSQC spectra of compound **3**.

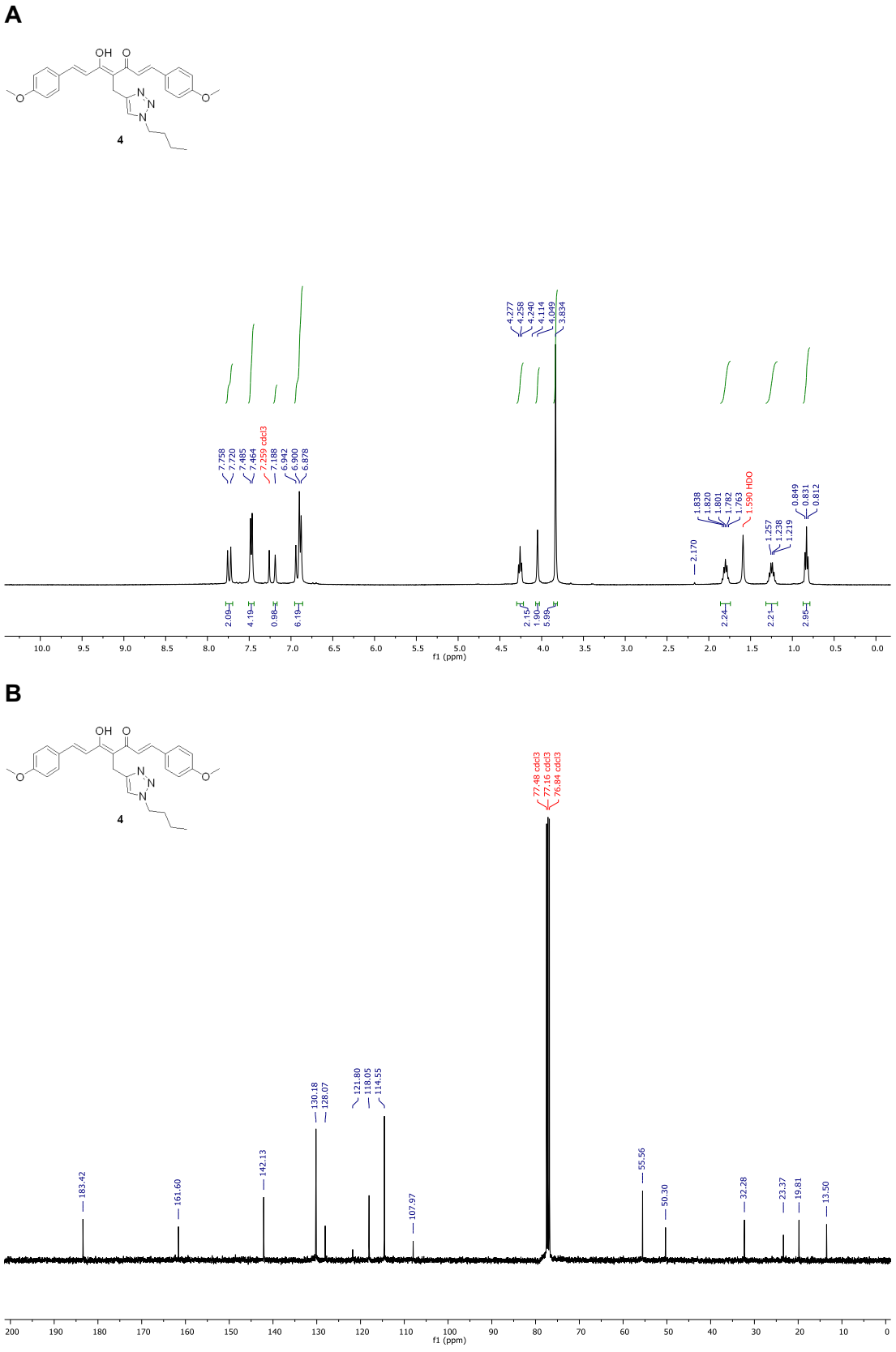


Figure S11. ^{1}H-NMR and ^{13}C-NMR Spectra of compound **4**.
(**A**) ^{1}H-NMR and (**B**) ^{13}C-NMR ($CDCl_3$, 400 MHz) spectra of compound **4**.

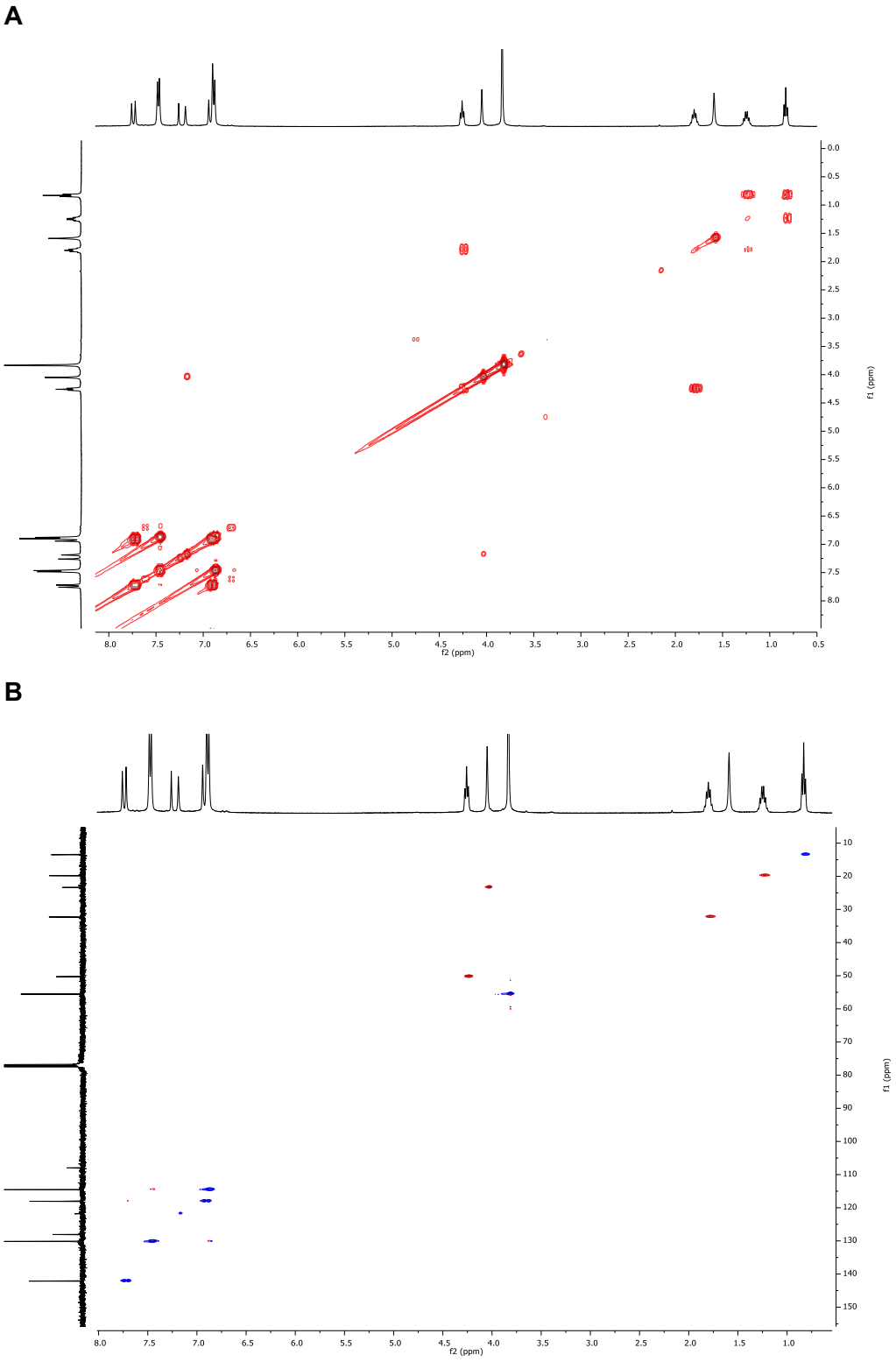
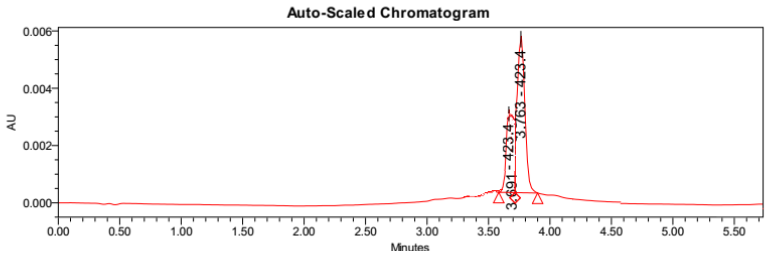


Figure S12. 2D ^{1}H-^{1}H COSY and 2D ^{1}H-^{13}C HSQC spectra of compound **4**. (**A**) 2D ^{1}H-^{1}H COSY and (B**)** 2D ^{1}H-^{13}C HSQC spectra of compound **4**.

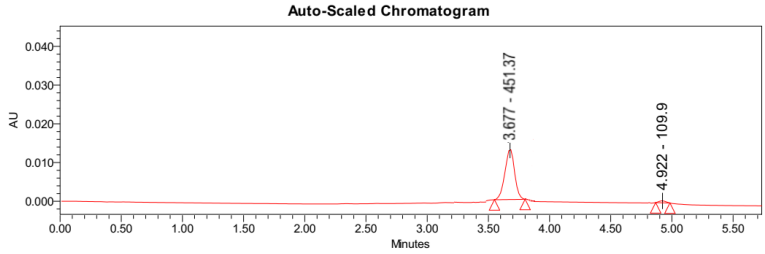
2a: Formula: $C_{25}H_{26}O_6$, Molecular Weight: 422.48



Peak Results

	Name	RT	Area	% Area	Height
1		3.691	11798	33.10	2945
2		3.763	23841	66.90	5376

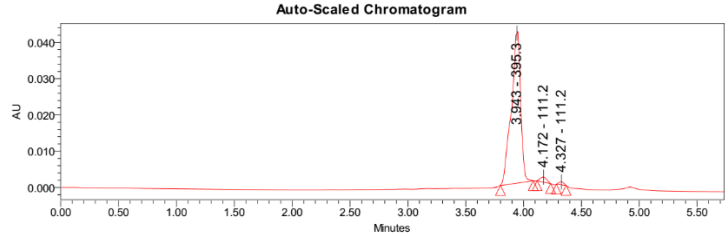
2b: Formula: $C_{27}H_{30}O_6$, MW: 450.53



Peak Results

	Name	RT	Area	% Area	Height
1		3.677	71111	97.31	12949
2		4.922	1967	2.69	492

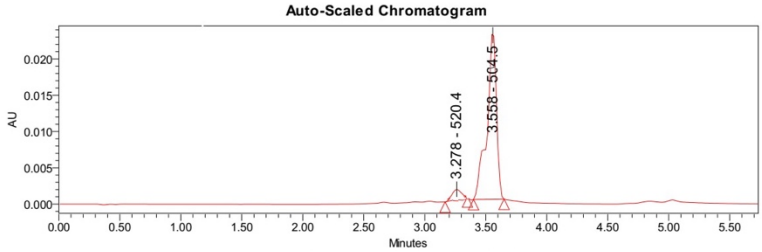
2c: Formula: $C_{23}H_{22}O_6$, MW: 394.42



Peak Results

	Name	RT	Area	% Area	Height
1		3.943	233664	96.67	42845
2		4.172	5537	2.29	1379
3		4.327	2521	1.04	855

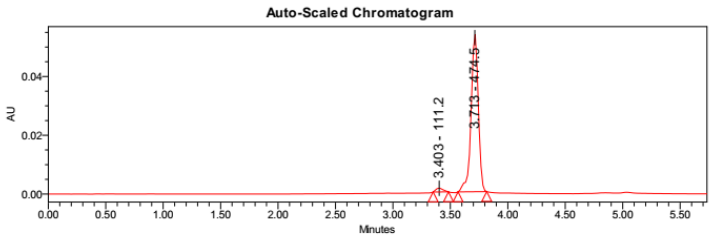
3: Formula: $C_{28}H_{29}N_3O_6$, MW: 503.55



Peak Results

	Name	RT	Area	% Area	Height
1		3.278	6497	4.83	1463
2		3.565	128036	95.17	26196

4: Formula: $C_{28}H_{31}N_3O_4$, MW: 473.57



Peak Results

	Name	RT	Area	% Area	Height
1		3.403	5588	2.34	1281
2		3.713	233684	97.66	53778

Figure S13. RP-UPLC/MS copy of compounds **2a-c**, **3** and **4**.

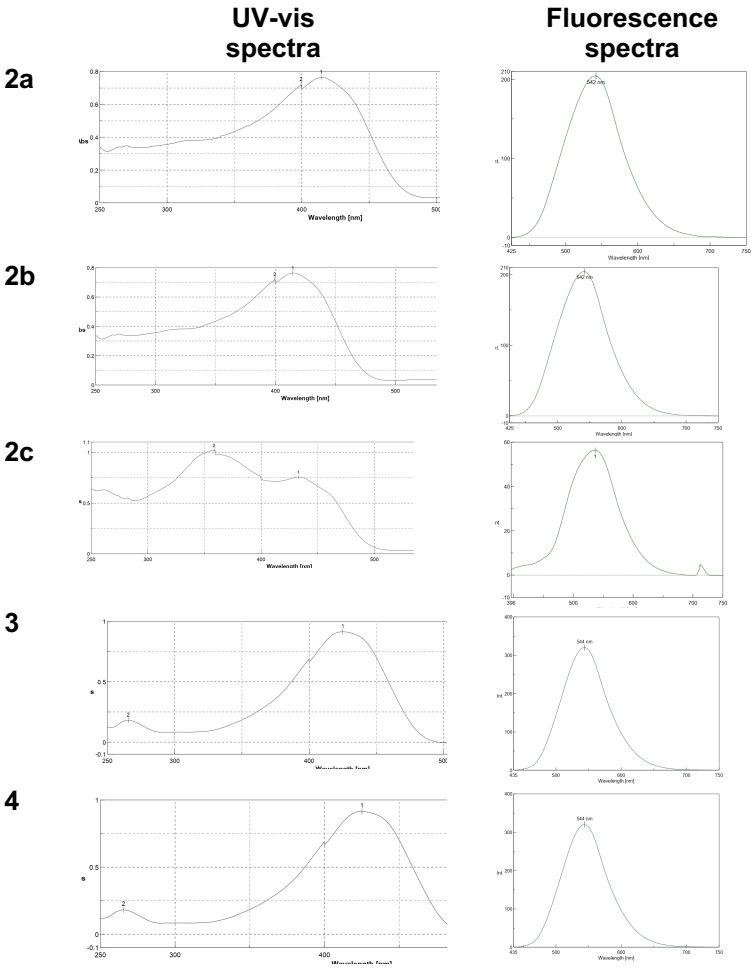


Figure S14. UV-vis and fluorescence spectra of compounds **2a-c**, **3** and **4**.

compound	Percentage of eclosed flies				
	1st test			**2nd test**	
4	1 μM	10 μM	50 μM	20 μM	30 μM
	95.2382 ± 2.9251	93.8310 ± 3.5470	71.3092 ± 9.0779	88.0999 ± 2.5823	85.4616 ± 7.4473
	Correct development timing		Small number of larvae, slow development	Third instar larvae were small	Slow development
3	1 μM	10 μM	50 μM	20 μM	30 μM
	94.0372 ± 2.3268	94.7320 ± 1.8242	68.3730 ± 8.0372	83.8069 ± 4.6219	81.6426 ± 4.3185
	Correct development timing		Less than 5 third instar larva	Slow development	

Table S1. Percentage of eclosed adults/ number of *pupae*.
For both trails, the control *Drosophila* line (W^{1118}) was grown in a standard medium supplemented with the two separate compounds, **3** and **4**. For each compound, crosses were set up between female virgins and males in a 10:5 ratio; three vials per group were used. After 72 h adults were discarded, and larvae were allowed to develop. To assess any toxic effect the development time, the eclosion rate and the total number of larvae were observed. The number of pupae and successfully eclosed adults were scored each day. The percentage of eclosed adults/ number of pupae is shown in the table.

References

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[2] F. Bisceglia, A. Natalello, M.M. Serafini, R. Colombo, L. Verga, C. Lanni, E. De Lorenzi, An integrated strategy to correlate aggregation state, structure and toxicity of Aß 1-42 oligomers, Talanta. 188 (2018) 17–26. https://doi.org/10.1016/j.talanta.2018.05.062.