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Supporting Information

Azobioisosteres of Curcumin with Pronounced Activity against Amyloid Aggregation, Intracellular Oxidative Stress, and Neuroinflammation

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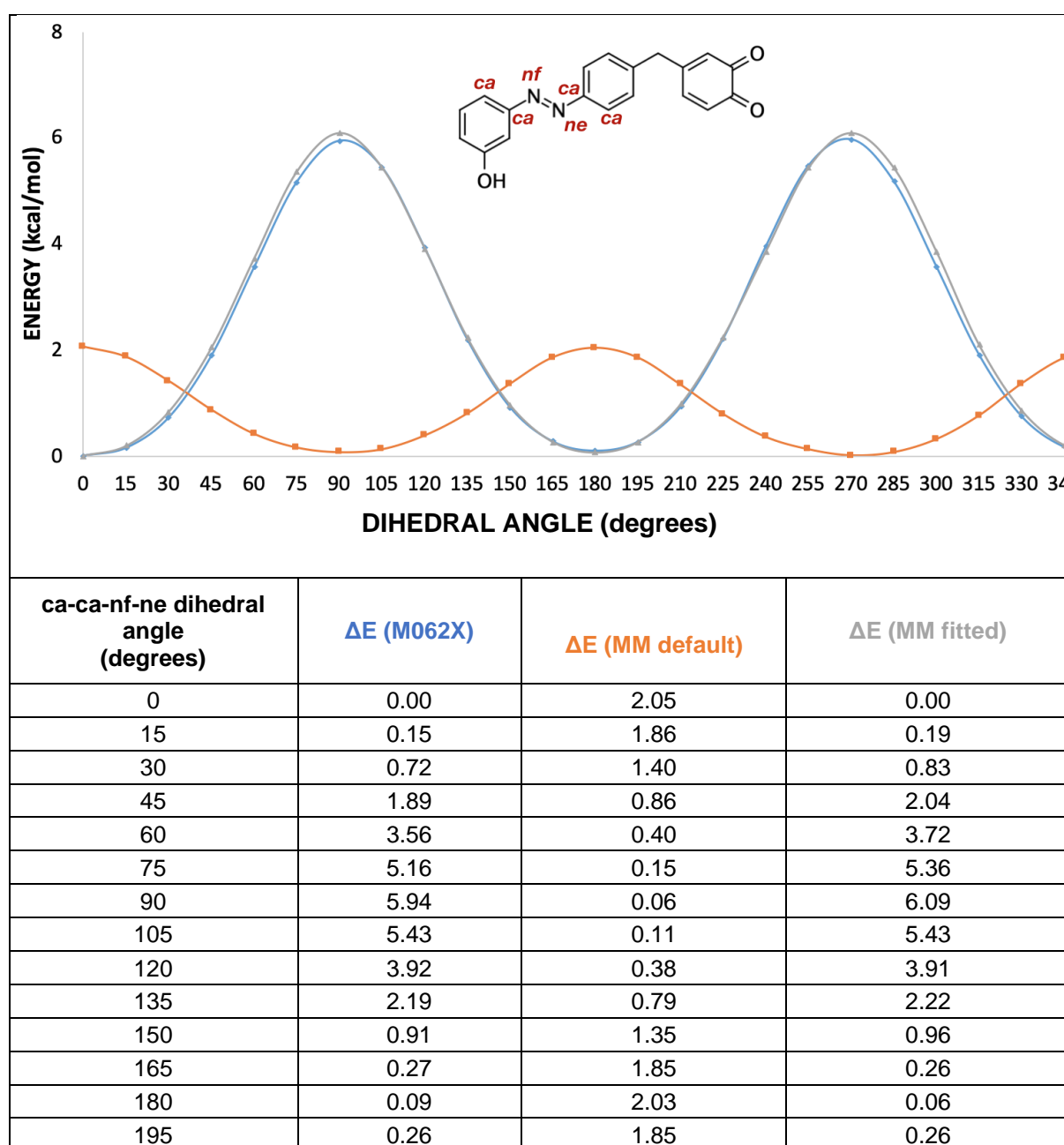
Supporting Information

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1. Parametrization of the C-C-N-N dihedral angle

The capability of the general Amber force field (GAFF) to properly describe the chemistry of the azobioisosteres investigated in this study was evaluated through preliminary MD simulations (data not shown). Accordingly, since no energy barrier was observed for the Car=Car-N=N torsional, a proper parametrization of this torsional was carried out prior to proceed to MD simulations with Aβ42. MM parameters were generated by fitting the MM potential energy of the Car=Car-N=N torsional on the QM-derived potential energy profile for the same torsional, derived from a relaxed scan performed at the M062X/6-31G(d) level in vacuo (see **Table S1**).

Table S1. Energy profiles for the selected torsion in azobenzenes determined by fitting quantum mechanical data. All energy values are in kcal/mol.

ca-ca-nf-ne dihedral angle (degrees)	ΔE (M062X)	ΔE (MM default)	ΔE (MM fitted)
0	0.00	2.05	0.00
15	0.15	1.86	0.19
30	0.72	1.40	0.83
45	1.89	0.86	2.04
60	3.56	0.40	3.72
75	5.16	0.15	5.36
90	5.94	0.06	6.09
105	5.43	0.11	5.43
120	3.92	0.38	3.91
135	2.19	0.79	2.22
150	0.91	1.35	0.96
165	0.27	1.85	0.26
180	0.09	2.03	0.06
195	0.26	1.85	0.26

210	0.92	1.33	0.97
225	2.21	0.78	2.24
240	3.95	0.35	3.85
255	5.47	0.11	5.43
270	5.97	0.00	6.09
285	5.17	0.06	5.43
300	3.57	0.31	3.85
315	1.90	0.75	2.09
330	0.74	1.36	0.85
345	0.16	1.84	0.20
360	0.00	2.03	0.02

Fitted torsional parameters used in this study for the C-C-N-N torsion:

```
1. ca-ca-ne-nf  2   4.100    180.000      -2.000
1. ca-ca-ne-nf  2   0.300    360.000       4.000

2. ca-ca-nf-ne  2   4.100    180.000      -2.000
2. ca-ca-nf-ne  2   0.300    360.000       4.000
```
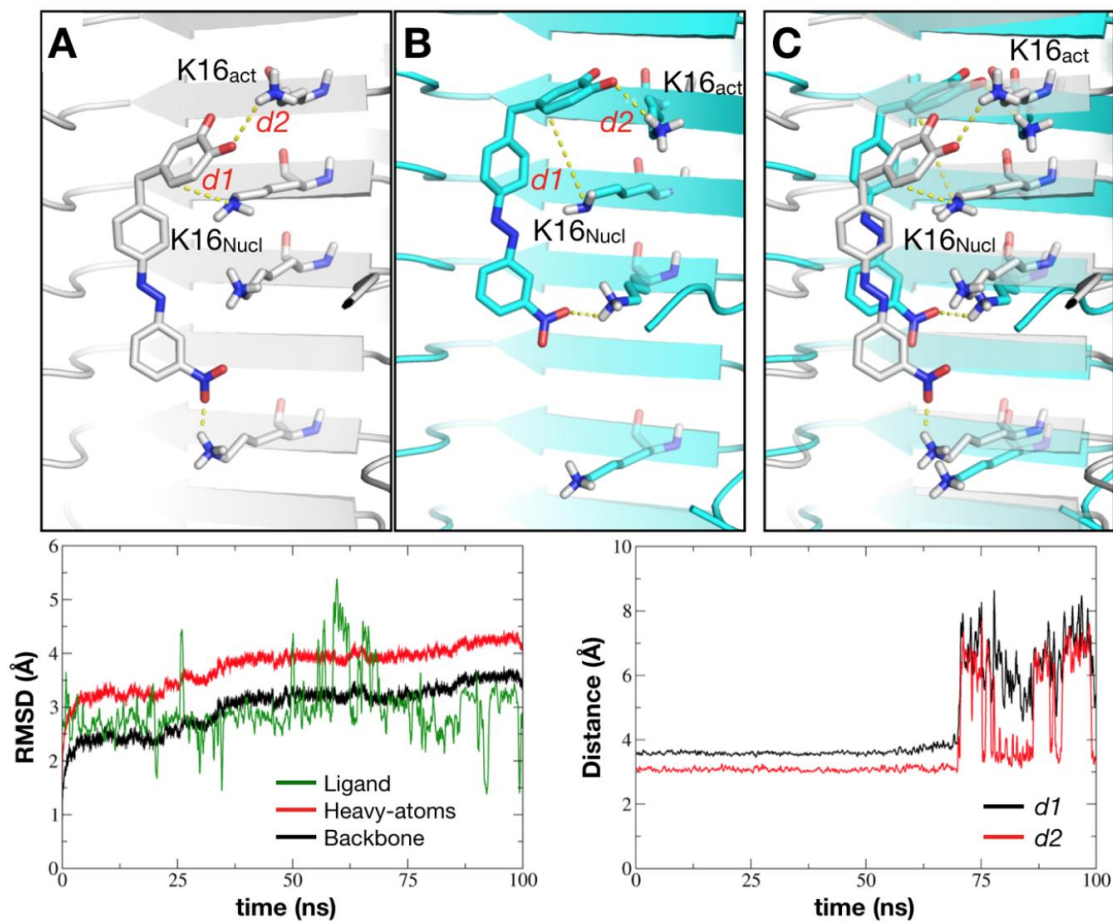
Figure S1. Initial (**A**), final (**B**) complex and their superposition (**C**) from one of the three MD simulation of **8c** with Aβ42. RMSD for protein backbone (in black), heavy atoms (in red) and for the ligand (in green) and distance analysis for Cβ•••NH$_2$-K16$_{Nucl}$ (*d1*) and C=O•••NH$_3$-K16$_{Act}$ (*d2*). No data was reported for the other two simulated systems since the ligand left the cavity during the unrestrained MD simulation.
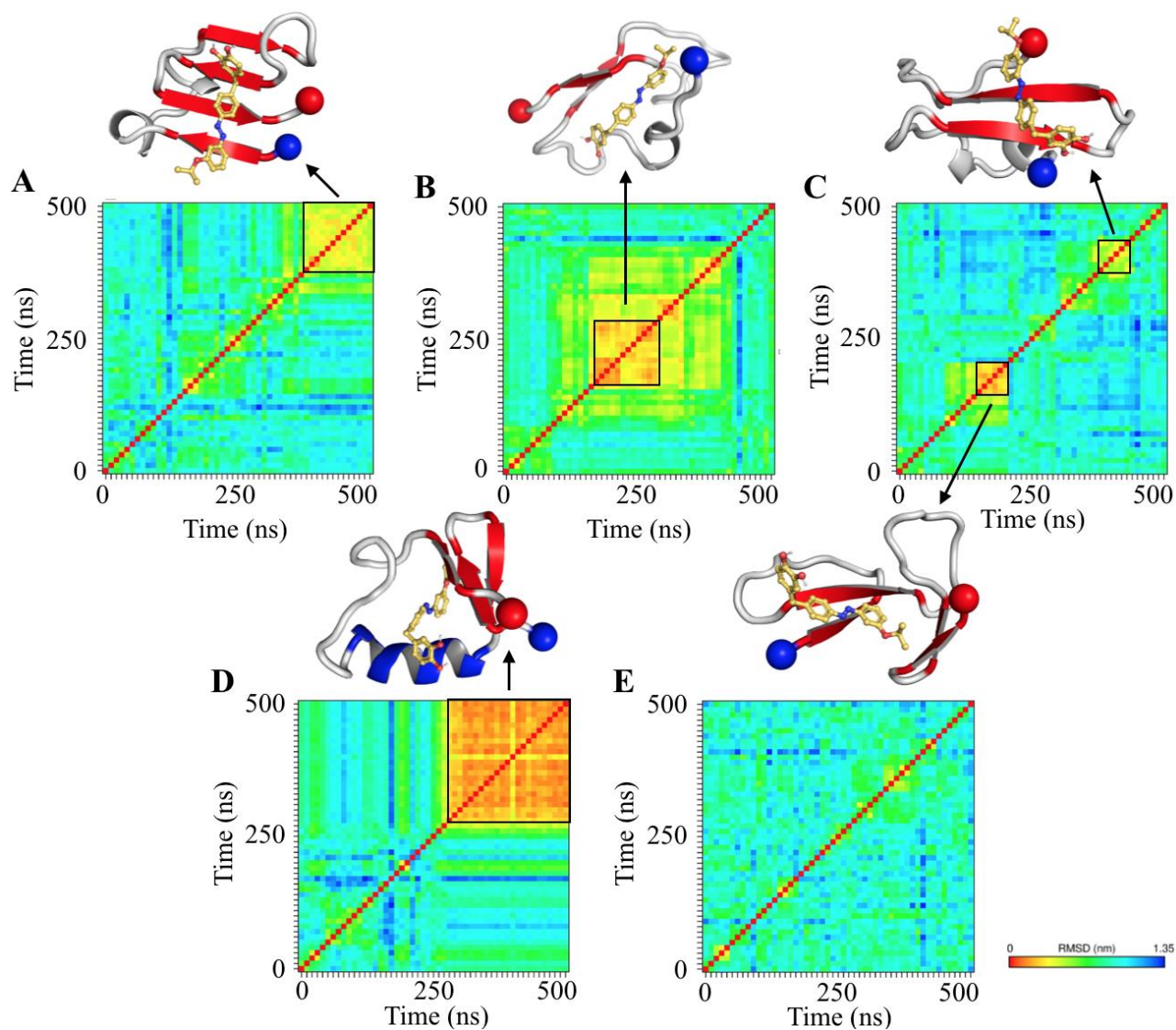
Figure S2. 2D-RMS analysis for the first five T-replicas of Aβ42$_{mon}$-**8f**. representative conformers relative to the most populated clusters are also reported. Colour code for the Aβ42$_{mon}$ sequence is set according to the secondary structure analysis shown in **Figure 2** of the main text while **8f** is reported as yellow sticks. N- and C-terminal edges of the Aβ42$_{mon}$ were reported as blue and red spheres, respectively.
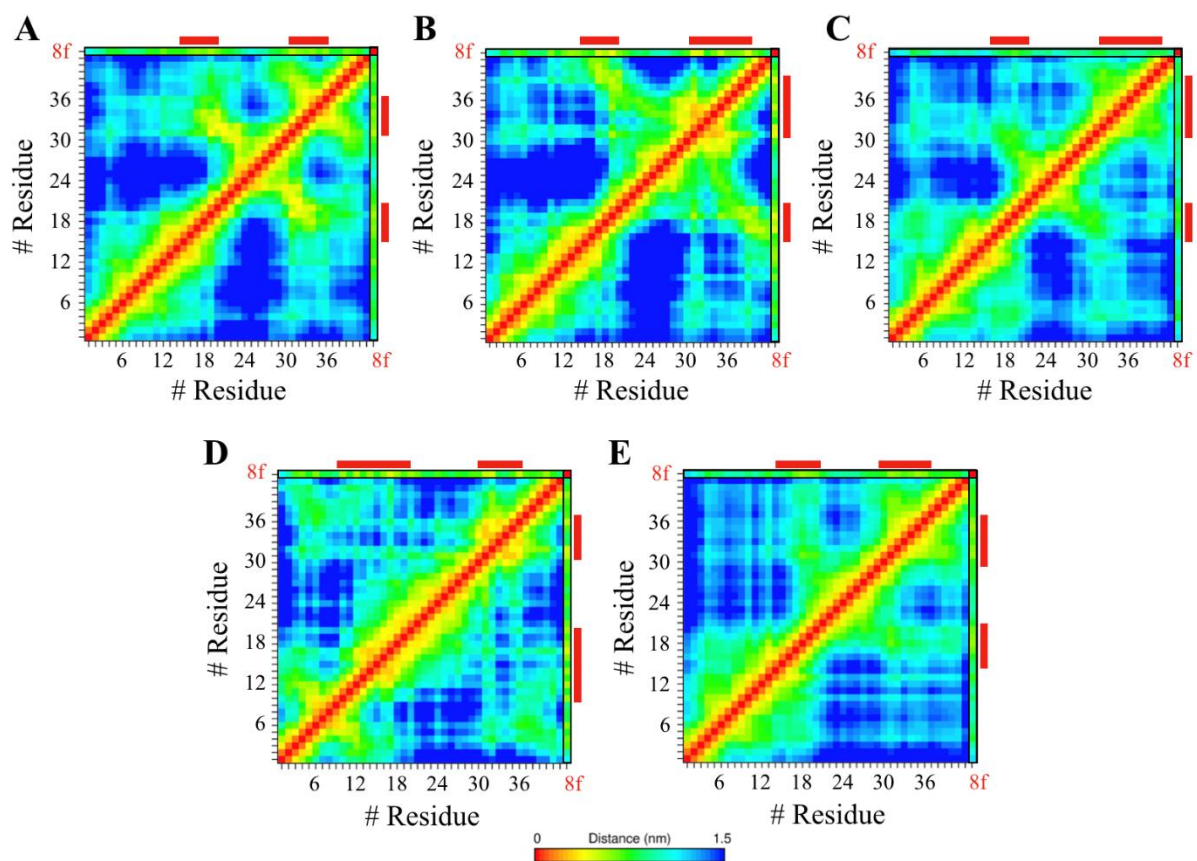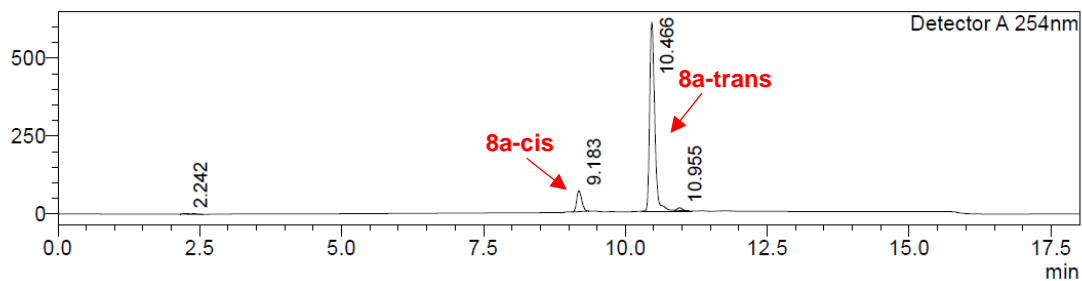
Figure S3. Contact map for the five T-replicas of the Aβ42$_{mon}$-**8f** complex. Contacts between Cβ atoms of Aβ42$_{mon}$ and compound **8f** are marked by red rectangles.

2. HPLC-Chromatograms for purity control

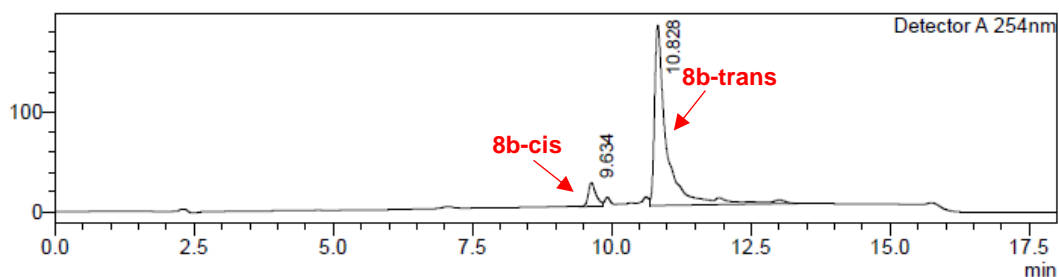*(E)-4-(4-(phenyldiazenyl)benzyl)benzene-1,2-diol* **(8a)**

<Chromatogram>

mV

Detector A 254nm				
Peak#	Ret. Time	Area	Height	Area%
1	2.242	23546	1450	0.546
2	9.183	420138	66690	9.737
3	10.466	3822013	603335	88.580
4	10.955	49057	8647	1.137
Total		4314754	680122	100.000

(E)-4-(4-((3-ethoxyphenyl)diazenyl)benzyl)benzene-1,2-diol **(8b)**

<Chromatogram>

mV

Detector A 254nm				
Peak#	Ret. Time	Area	Height	Area%
1	9.634	228633	23643	7.728
2	10.828	2729853	180339	92.272
Total		2958487	203982	100.000

8

(E)-4-(4-((3-nitrophenyl)diazenyl)benzyl)benzene-1,2-diol **(8c)**

<Chromatogram>

mV
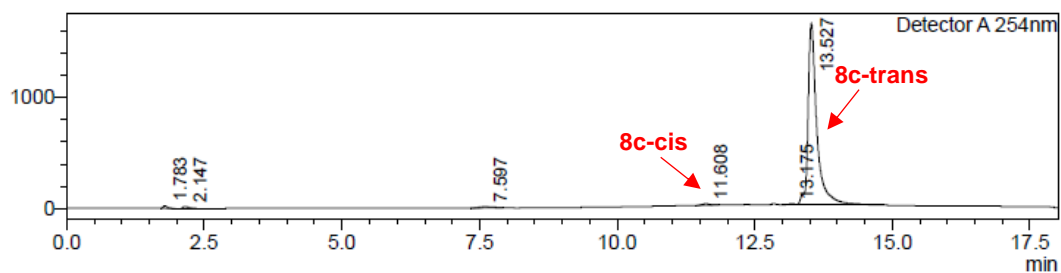
Detector A 254nm

Detector A 254nm

Peak#	Ret. Time	Area	Height	Area%
1	1.783	108048	23313	0.567
2	2.147	139768	19661	0.734
3	7.597	194872	11746	1.023
4	11.608	124705	12670	0.655
5	13.175	116605	11775	0.612
6	13.527	18363965	1633112	96.409
Total		19047961	1712277	100.000

(E)-4-(4-((3-chlorophenyl)diazenyl)benzyl)benzene-1,2-diol **(8d)**

<Chromatogram>

mV
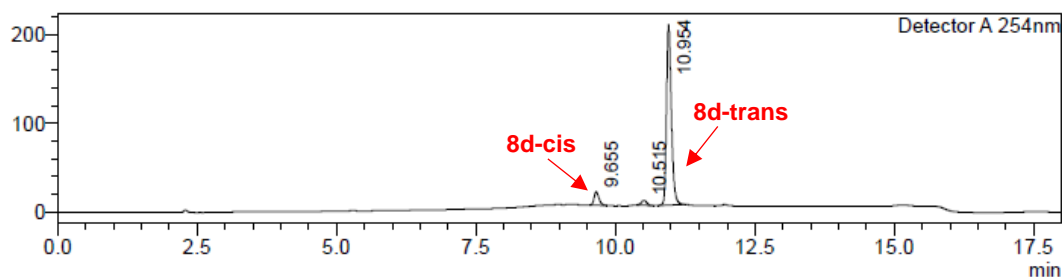
Detector A 254nm

Detector A 254nm

Peak#	Ret. Time	Area	Height	Area%
1	9.655	91003	15057	6.737
2	10.515	35427	5431	2.623
3	10.954	1224333	202168	90.640
Total		1350763	222657	100.000

(E)-4-(4-((3-methoxyphenyl)diazenyl)benzyl)benzene-1,2-diol **(8e)**

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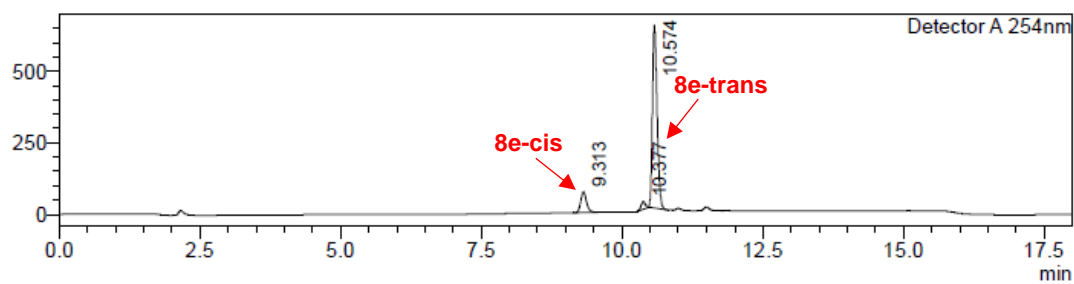
Detector A 254nm

Peak#	Ret. Time	Area	Height	Area%
1	9.313	524822	71424	11.939
2	10.377	142458	26276	3.241
3	10.574	3728487	635583	84.820
Total		4395768	733282	100.000

(E)-4-(4-((3-isopropoxyphenyl)diazenyl)benzyl)benzene-1,2-diol **(8f)**

<Chromatogram>
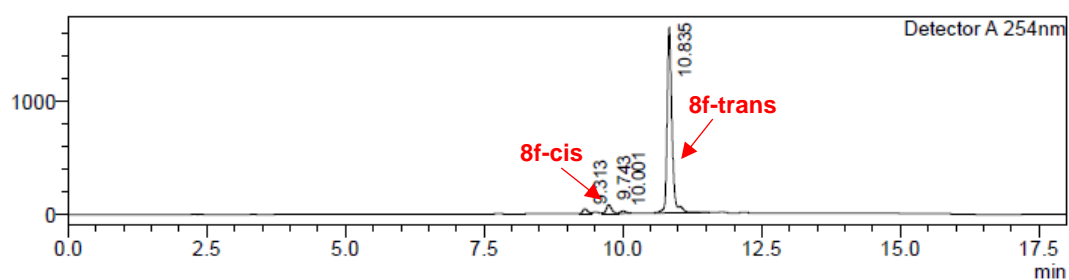
Detector A 254nm

Peak#	Ret. Time	Area	Height	Area%
1	9.313	238187	41003	2.162
2	9.743	483583	76927	4.390
3	10.001	132954	20649	1.207
4	10.835	10160992	1630878	92.241
Total		11015717	1769456	100.000

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(E)-1-(4-(3,4-dimethoxybenzyl)phenyl)-2-phenyldiazene **(8g)**

\<Chromatogram\>

mV
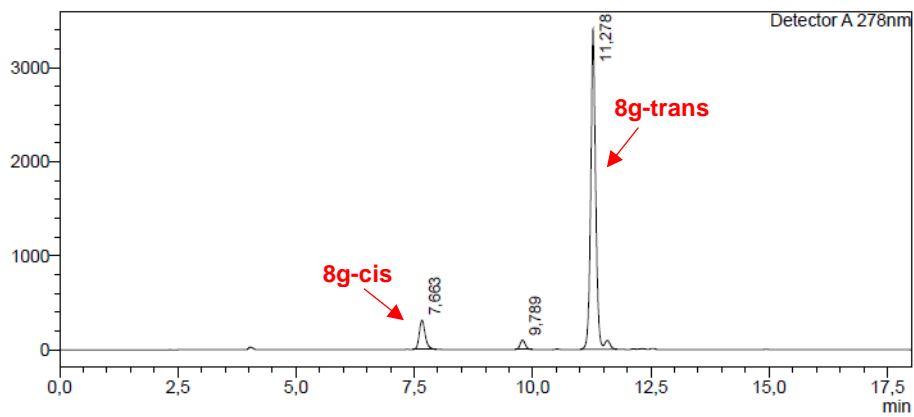
Detector A 278nm

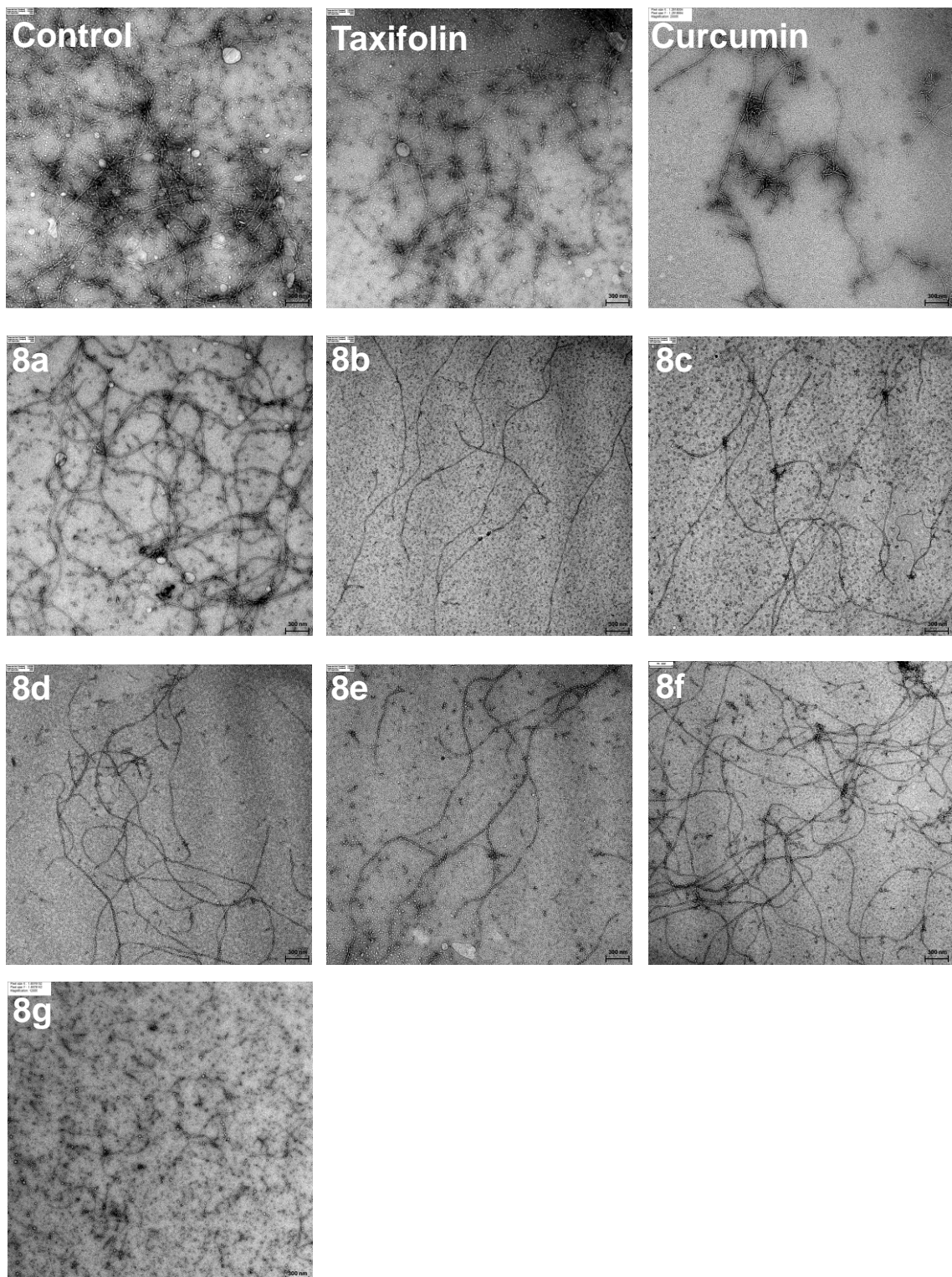| Peak#	Ret. Time	Area	Height	Area%
1	7.663	2688643	310342	9.351
2	9.789	738512	98967	2.569
3	11.278	25324315	3390073	88.080
Total		28751470	3799381	100.000

3. Transmission Electron Microscopy

Figure S4. TEM analysis of the inhibitory effect on Aβ42. The Aβ monomer (100 µM) was incubated at 37°C in PBS for 24 h with or without 50 µM of the respective compound. Scale bar 300 nm.
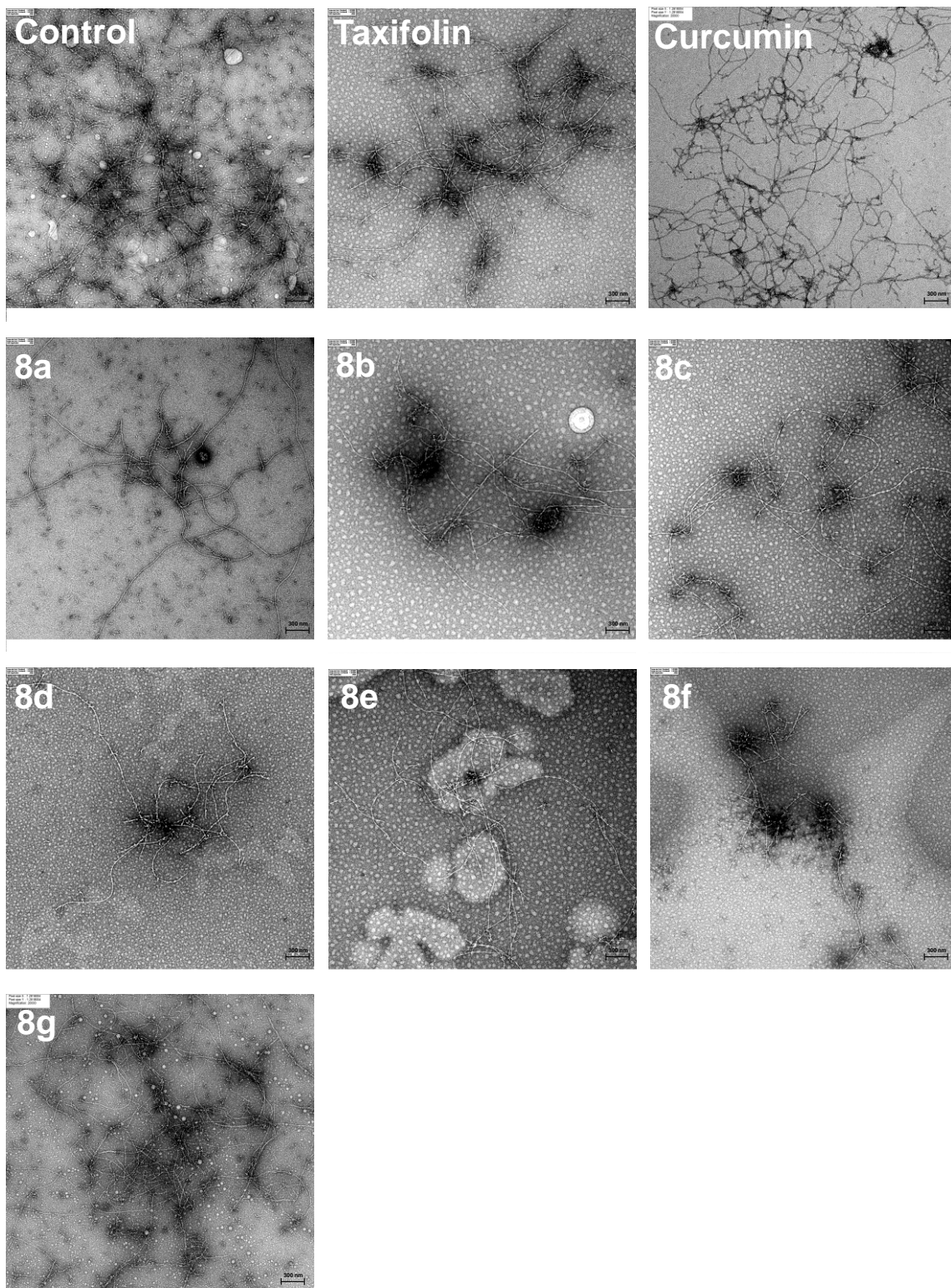
Figure S5. TEM analysis of the inhibitory effect on Aβ42. The Aβ monomer (100 μM) was incubated at 37°C in PBS for 24 h with or without 10 μM of the respective compound. Scale bar 300 nm.

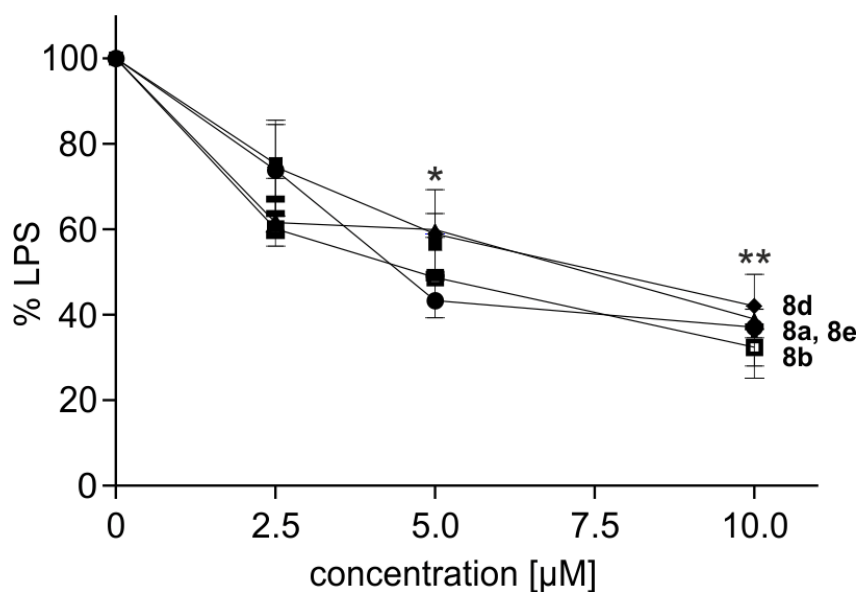## 4. Anti-Inflammatory Effect on BV-2 cells

Figure 6: Effect of compounds **8a**, **8b**, **8d** and **8e** on the production of NO as inflammation marker. BV-2 cells were treated with 50 ng/mL LPS alone or with the respective compound. NO was determined by the Griess assay in the supernatant. Data is presented as means ± SEM of three independent experiments and results refer to LPS treated cells. Statistical analysis was performed using One-Way ANOVA followed by Dunnett's multiple comparison posttest using GraphPad Prism 5. Level of significance: ** $p < 0.01$, *$p < 0.05$.