Supporting Information

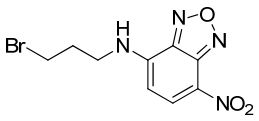
1. Chemical Synthesis

1.1. General Considerations

Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded on a Bruker Avance 300 (300 and 75 MHz, respectively) or a Bruker Avance III 500 MHz spectrometer (500 and 126 MHz, respectively). All chemical shifts are quoted on the δ scale in ppm using residual solvent peaks as the internal standard. Coupling constants (*J*) are reported in Hz with the following splitting abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad signal. Thin layer chromatography (TLC) was carried out using Merck aluminum backed sheets coated with 60 F254 silica gel. Visualization of the silica plates was achieved using a UV lamp (λmax = 254 nm). Flash chromatography was performed using a Combi Flash RF-200 device from Teledyne Isco with RediSepbnormal-phase silica flash columns and using gradients of hexane and ethyl acetate. All reagents were purchased from Sigma-Aldrich or AlfaAesar and used without further purification.
Low-resolution mass spectra were recorded using Micromass Quattro Micro API mass spectrometer, using electrospray ionization. Mass spectra were analyzed using MassLynx V4.1 software. High resolution mass spectrum for compound **2** was gently performed by Prof. Paula Gomes at Faculdade de Ciências do Porto on an LTQ Orbitrap XL hybrid mass spectrometer (Thermo Fischer Scientific, Bremen, Germany) controlled by *LTQ Tune Plus 2.5.5* and *Xcalibur 2.1.0*.

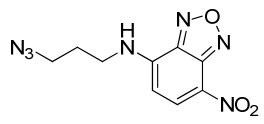
1.2. Synthesis of N-(3-azidopropyl)-7-nitrobenzo[c][1,2,5]oxadiazol-4-amine **5**) [as described by Ruivo et al.[35]]

1.2.1. N-(3-bromopropyl)-7-nitrobenzo[c][1,2,5]oxadiazol-4-amine **6**



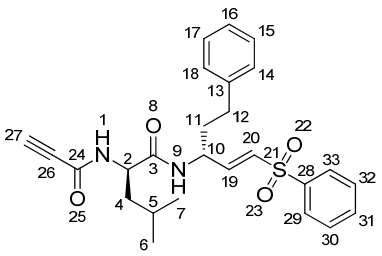
4-chloro-7-nitrobenzo[c][1,2,5]oxadiazol (500.0 mg, 2.51 mmol), was dissolved in DMF (25 mL). 3-bromopropan-1-amine hydrobromide (604.0 mg, 2.76 mmol) was added to the reaction, followed by triethylamine (0.45 mL, 3.2 mmol). The reaction was stirred for two hours at room temperature. 40 mL of water were added to the reaction and the product was extracted with 4 x 10 mL of ethyl acetate. The organic fractions were combined, dried with anhydrous sodium sulfate and concentrated under reduced pressure. Purification by combi flash chromatography (gradient of 100% hexane to 50% hexane and 50% ethyl acetate) yielded the product **6** as an orange solid (552 mg, 1.83 mmol, 73%).

1.2.2. N-(3-azidopropyl)-7-nitrobenzo [c] [1,2,5] oxadiazol-4-amine **5**



Compound **6** (552mg, 1.83 mmol) was dissolved in DMSO (20 mL). Sodium azide (155 mg, 2.38 mmol) was added and the reaction was stirred overnight at room temperature. The reaction was diluted with 40 mL of water and the product was extracted with 4 x 15 mL of ethyl acetate. The organic fractions were combined, dried with anhydrous sodium sulfate and concentrated under reduced pressure. The product **5** was used in the following reactions without further purification.

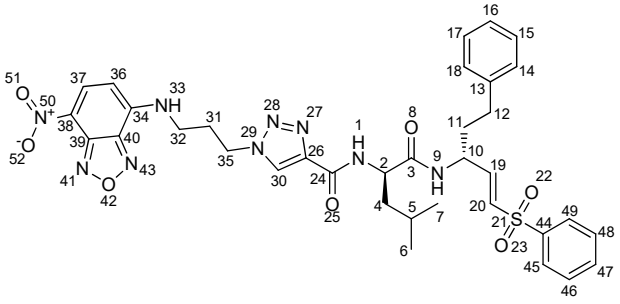
1.3. Synthesis of (R)-4-methyl-N-((R,E)-5-phenyl-1-(phenylsulfonyl)pent-1-en-3-yl)-2-propiolamidopentanamide **4**.



Propiolic acid (30µL, 0.49mmol) and TEA (60µL, 0.43mmol) were dissolved in DMF (12mL) and stirred at 0ºC. HoBt (60mg, 0.39mmol) and TBTU (120mg, 0.37mmol) were added and the mixture was stirred for 30 minutes. Compound **3** (180.4mg, 0.34mmol) in DMF (12mL) and TEA (60µL) was added to the reaction, which was allowed to reach room temperature and stirred overnight. The reaction was diluted with 20mL of HCl 3M and extracted with 3 x 10mL of ethyl acetate. The organic fractions were combined, dried with anhydrous sodium sulfate and concentrated under reduced pressure. Purification by combi flash chromatography ((gradient of 100% hexane to 100% ethyl acetate)) yielded compound **4** as a colorless oil (73mg, 0.15mmol, 38%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 – 7.79 (m, 2H, H-30, H-32), 7.50 (m, 3H, H-29, H-31, H-33), 7.24 – 7.11 (m, 3H, H-14, H-16, H-18), 7.06 – 7.01 (m, 2H, H-15, H-17), 6.90 (dd, *J* = 15.1, 5.5 Hz, 1H, H-19), 6.46 (dd, *J* = 15.1, 1.4 Hz, 1H, H-20), 4.66 – 4.50 (m, 2H, H-2, H-10), 2.78 (s, 1H, H-27), 2.63 – 2.52 (m, 2H, H-12), 1.95 – 1.82 (m, 2H, H-11), 1.61 – 1.46 (m, 3H, H-4, H-5), 0.84 (d, *J* = 5.9 Hz, 3H, H-6), 0.80 (d, *J* = 6.0 Hz, 3H, H-7).

1.4. Synthesis of N-((R)-4-methyl-1-oxo-1-(((R,E)-5-phenyl-1-(phenylsulfonyl)pent-1-en-3-yl)amino)pentan-2-yl)-1-(3-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)propyl)-1H-1,2,3-triazole-4-carboxamide **2**.

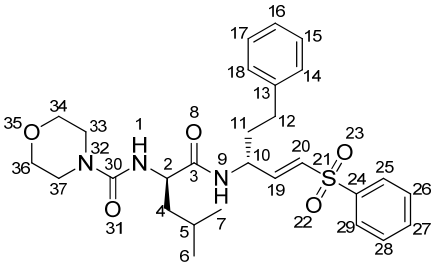


Compound **4** (73mg, 0.15mmol) was dissolved in DMF (0.75mL). $CuSO_4$ (0.1M, 10% eq. mol) and sodium ascorbate (0.1M, 10% eq. mol) were added to the solution, which was stirred under nitrogen atmosphere for 10 minutes. Compound **5** (60mg, 0.23mmol) was added to the reaction, which was stirred overnight. 10 mL of water were added to the reaction medium and the product was extracted with 3 x 5 mL of ethyl acetate. The organic fractions were combined, dried with anhydrous sodium sulfate and concentrated under reduced pressure. Purification by combi flash chromatography (gradient of 100% hexane to 20% hexane and 80% ethyl acetate)) yielded compound **2** as an orange solid (30mg, 0.04mmol, 28%).

^{1}H NMR (500 MHz, Acetone) δ 8.51 (d, *J* = 8.8 Hz, 1H, H-37), 8.48 (s, 1H, H-30), 8.35 (s, 1H, H-33), 7.90 – 7.86 (m, 2H, H-46, H-48), 7.86 – 7.81 (m, 1H, H-1), 7.78 (d, *J* = 8.2 Hz, 1H, H-9), 7.73 – 7.69 (m, 1H, H-47), 7.65 – 7.61 (m, 2H, H-45, H-49), 7.22 (t, *J* = 7.4 Hz, 2H, H-14, H-18), 7.18 – 7.13 (m, 3H, H-15-H-17), 6.98 (dd, *J* = 15.1, 4.9 Hz, 1H, H-19), 6.70 (d, *J* = 15.1 Hz, 1H, H-20), 6.48 (d, *J* = 8.7 Hz, 1H, H-36), 4.78 – 4.68 (m, 3H, H-10, H-35), 4.70 – 4.62 (m, 1H, H-2), 3.79 (s, 2H, H-32), 2.79 – 2.60 (m, 2H, H-12), 2.59 – 2.52 (quint, J = 6.9 Hz, 2H, H-31), 1.92 – 1.66 (m, 5H, H-4, H-5, H-11), 0.94 (dd, J = 16.0, 6.1 Hz, 6H, H-6, H-7). ^{13}C NMR (126 MHz, Acetone) δ 172.6 (C-3), 160.9 (C-24), 147.4 (C-19), 145.6 (C-34), 145.2 (C-38), 143.7 (C-26), 142.2 (C-13), 142.0 (C-44), 137.8 (C-37), 134.3 (C-47), 131.6 (C-20), 130.3 (C-45, C-49), 129.3 (C-15, C-17), 129.3 (C-14, C-18), 128.3 (C-46, C-48), 127.2 (C-30), 126.8 (C-16), 52.8 (C-10), 50.1 (C-2), 48.7

(C-35), 42.0 (C-32), 36.2 (C-11), 32.8 (C-12), 25.7 (C-5), 23.4 (C-6), 22.2 (C-7). HRMS (m/z): found 730.2777 [M+1].

2. Spectral data for compound 1 [as indicated by Oliveira et al.[31]]

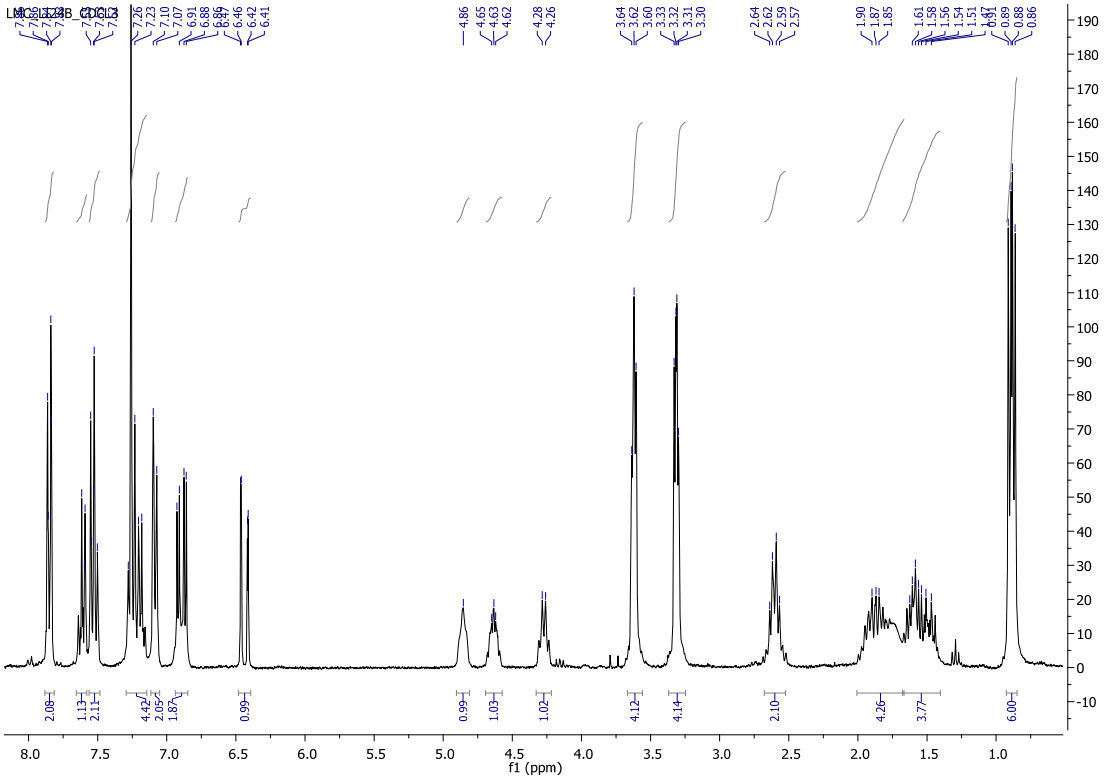


^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 – 7.81 (m, 2H, H-26, H-28), 7.66 – 7.57 (m, 1H, H-27), 7.57 – 7.48 (m, 2H, H-25, H-29), 7.29 – 7.22 (m, 2H, H-14, H-18), 7.21 – 7.14 (m, 1H, H-16), 7.09 (d, 2H, H-15, H-17), 6.89 (dd, *J* = 15.1, 5.1 Hz, 1H, H-19), 6.44 (dd, *J* = 15.1, 1.6 Hz, 1H, H-20), 4.86 (br, 1H, H-1), 4.68 – 4.57 (m, 1H, H-10), 4.34 – 4.20 (m, 1H, H-2), 3.67 – 3.57 (m, 2H, H-34, H-36), 3.36 – 3.25 (m, 2H, H-33, H-37), 2.67 – 2.53 (m, 2H, H-12), 2.03 – 1.39 (m, 5H, H-4, H-5, H-11), 0.90 (d, *J* = 6.1 Hz, 1H, H-6), 0.87 (d, *J* = 6.0 Hz, 1H, H-7). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.7 (C-3), 157.7 (C-30), 145.8 (C-19), 140.6 (C-13), 140.3 (C-24), 133.7 (C-27), 131.0 (C-20), 129.5 (C-25, C-29), 128.8 (C-14, C-18), 128.5 (C-34, C-36), 127.8 (C-26, C-28), 126.5 (C-16), 66.5 (C-33, C-37), 53.1 (C-2), 49.2 (C-10), 44.2 (C-34, C-36), 40.4 (C-4), 35.8 (C-11), 31.9 (C-12), 25.0 (C-5), 22.9 (C-6), 22.4 (C-7).

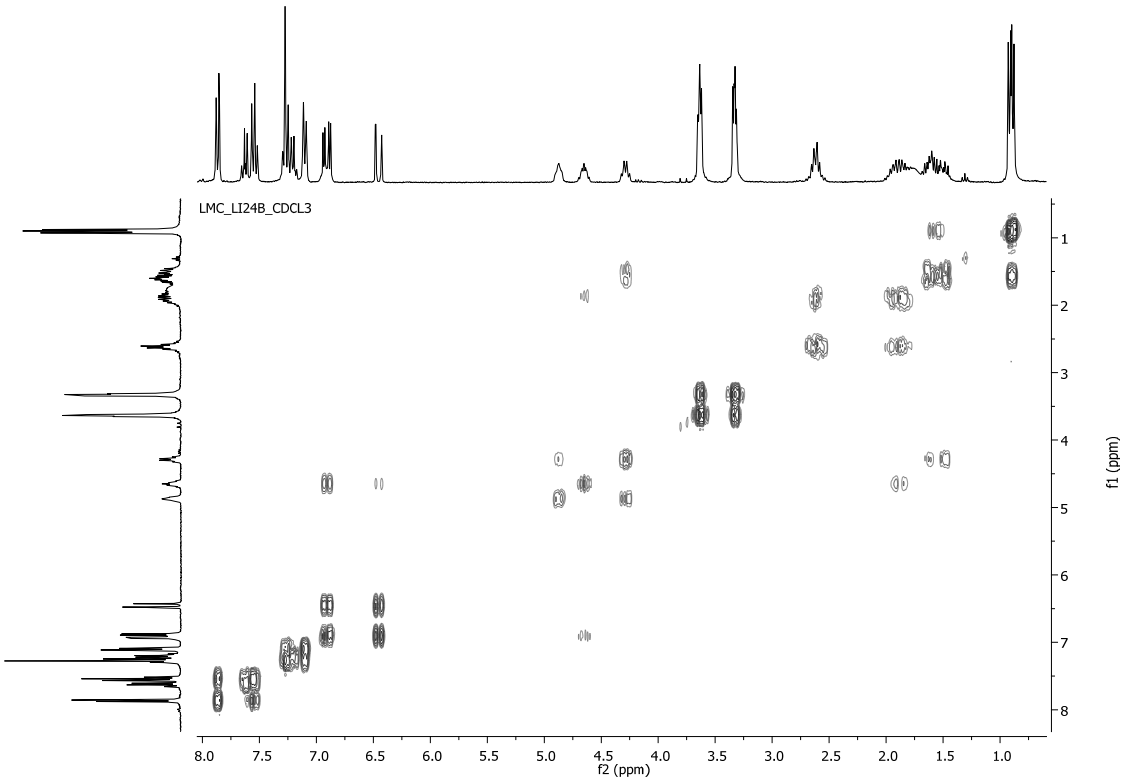
3. NMR spectra:

3.1. Compound **1**

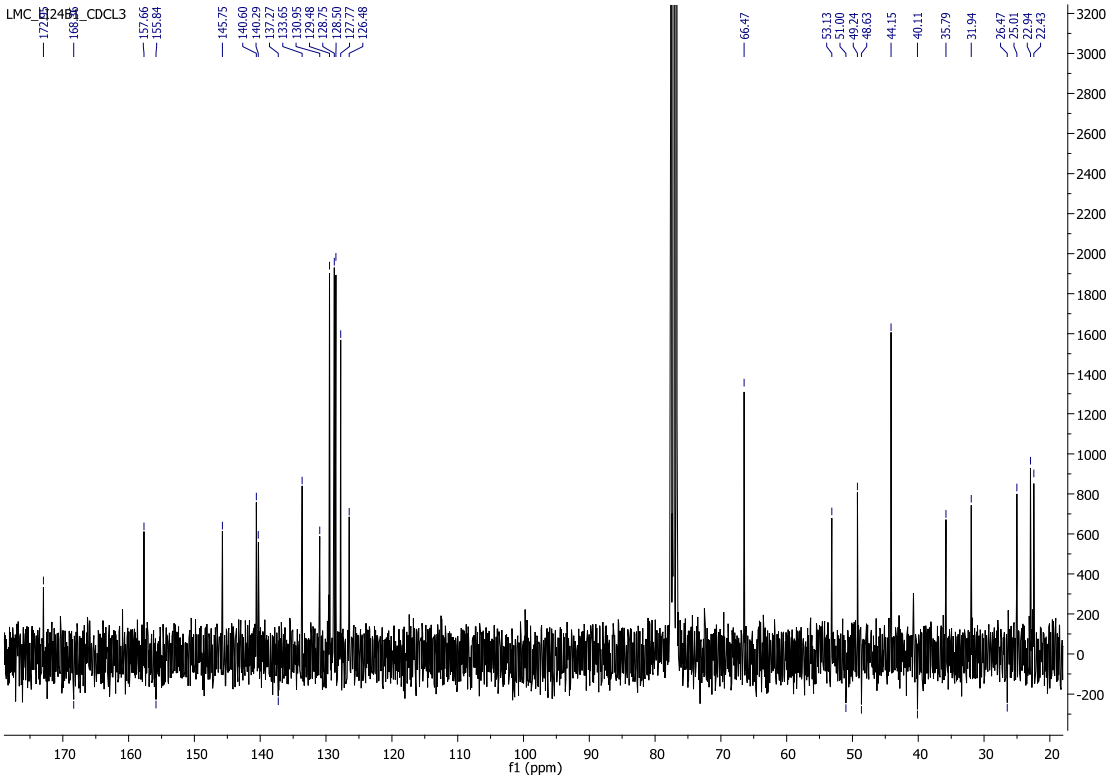
3.1.1. ^{1}H NMR spectrum of compound **1**.



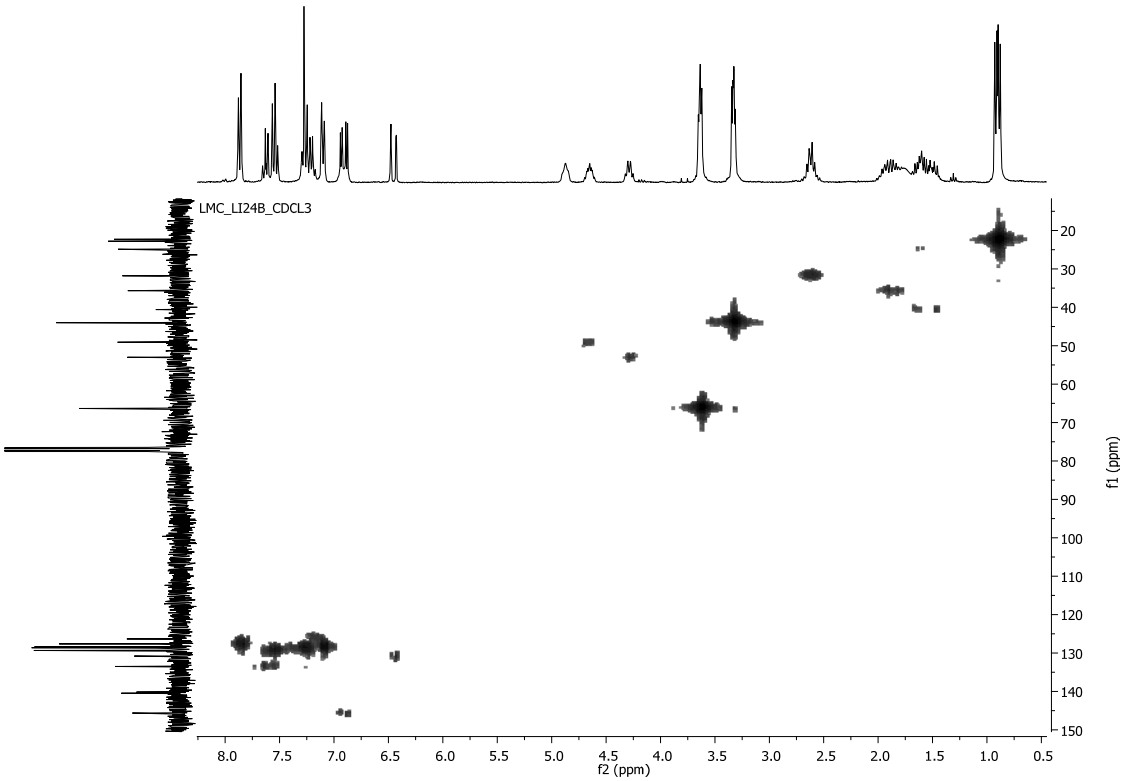
3.1.2. COSY spectrum of compound **1**.



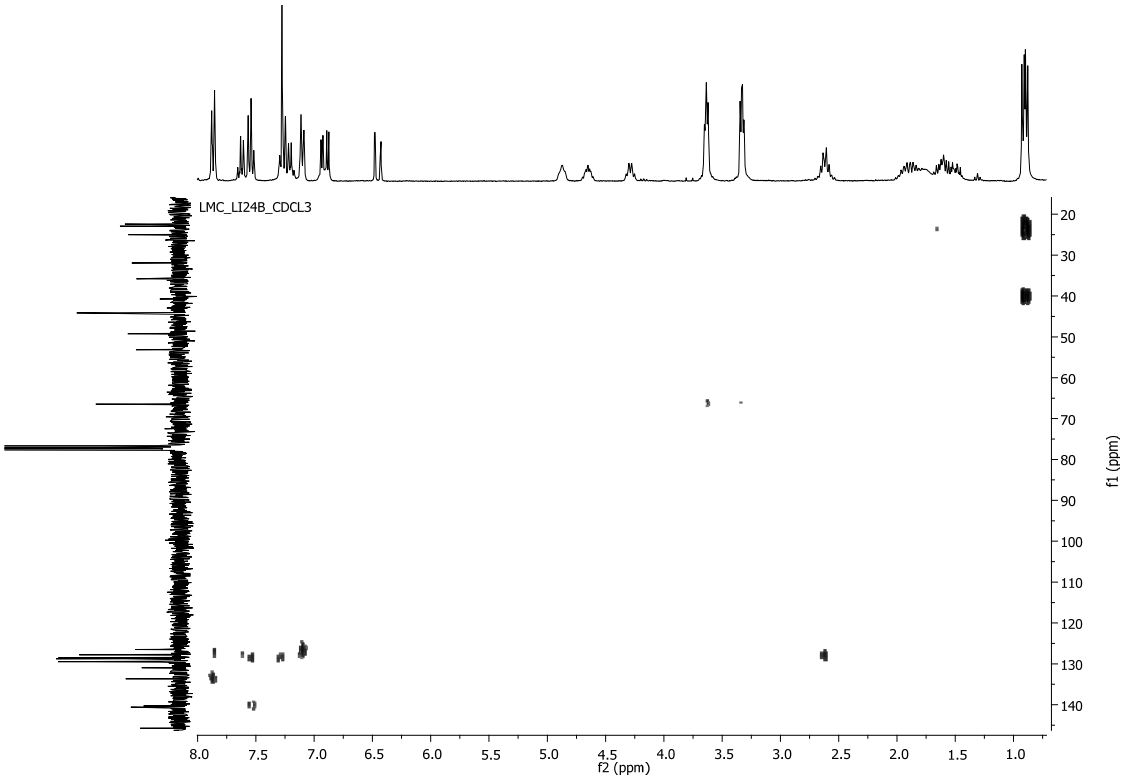
3.1.3. ^{13}C NMR spectrum of compound **1**.



3.1.4. HMQC spectrum of compound **1**.

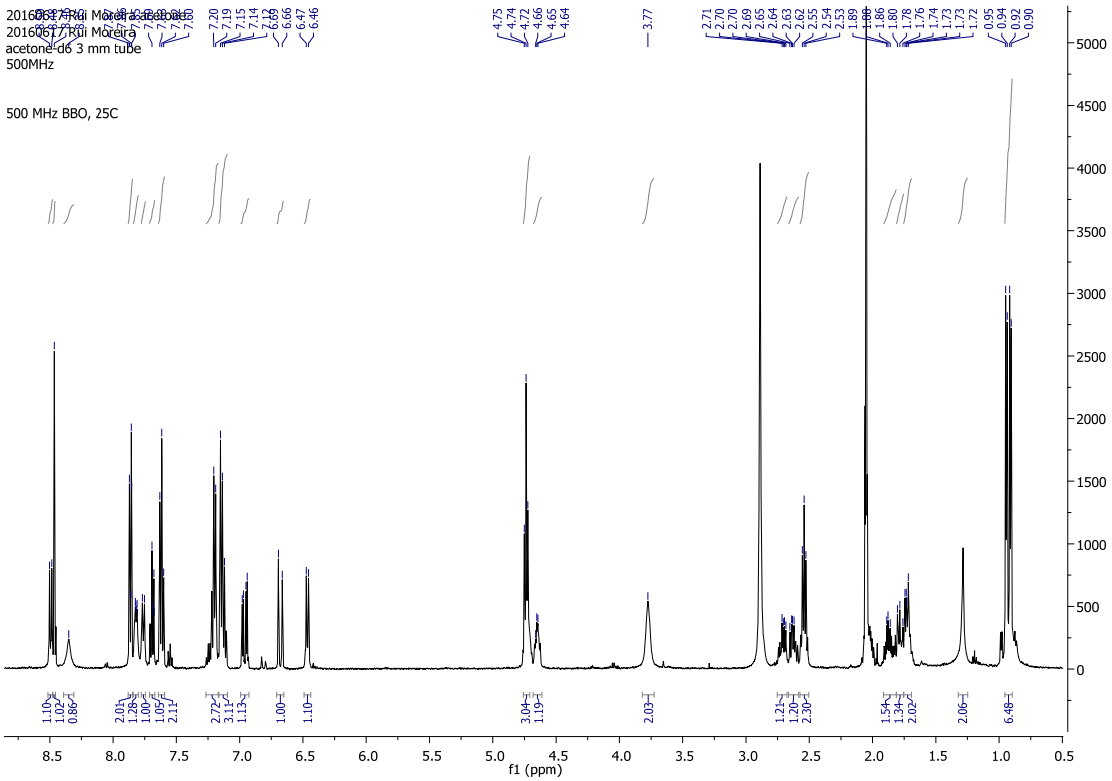


3.1.5. HMBC spectrum of compound **1**.

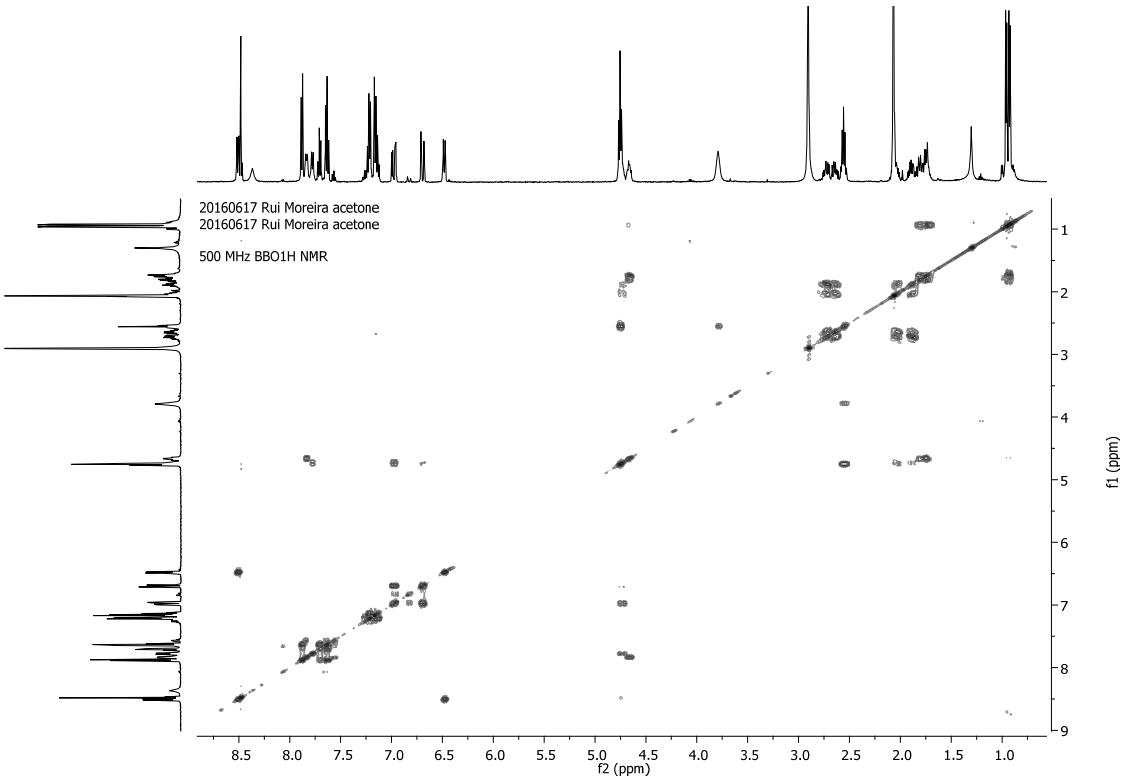


3.2. Compound **2**

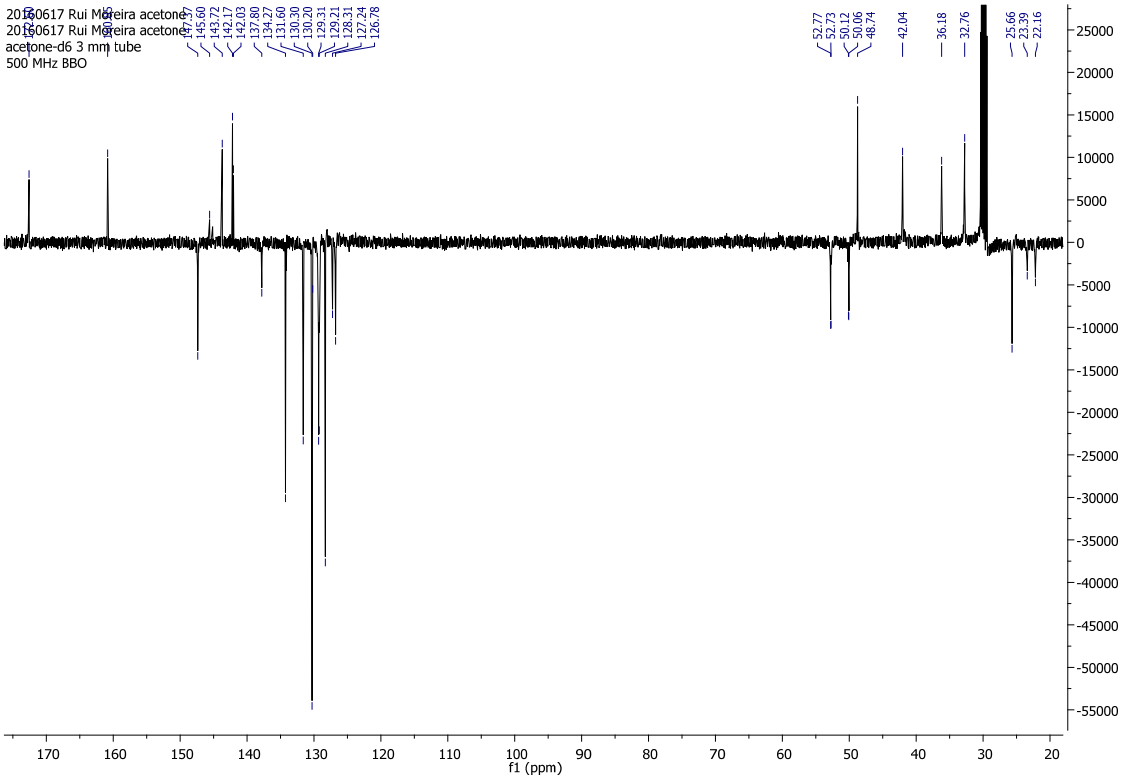
3.2.1. ^{1}H NMR spectrum of compound **2**.



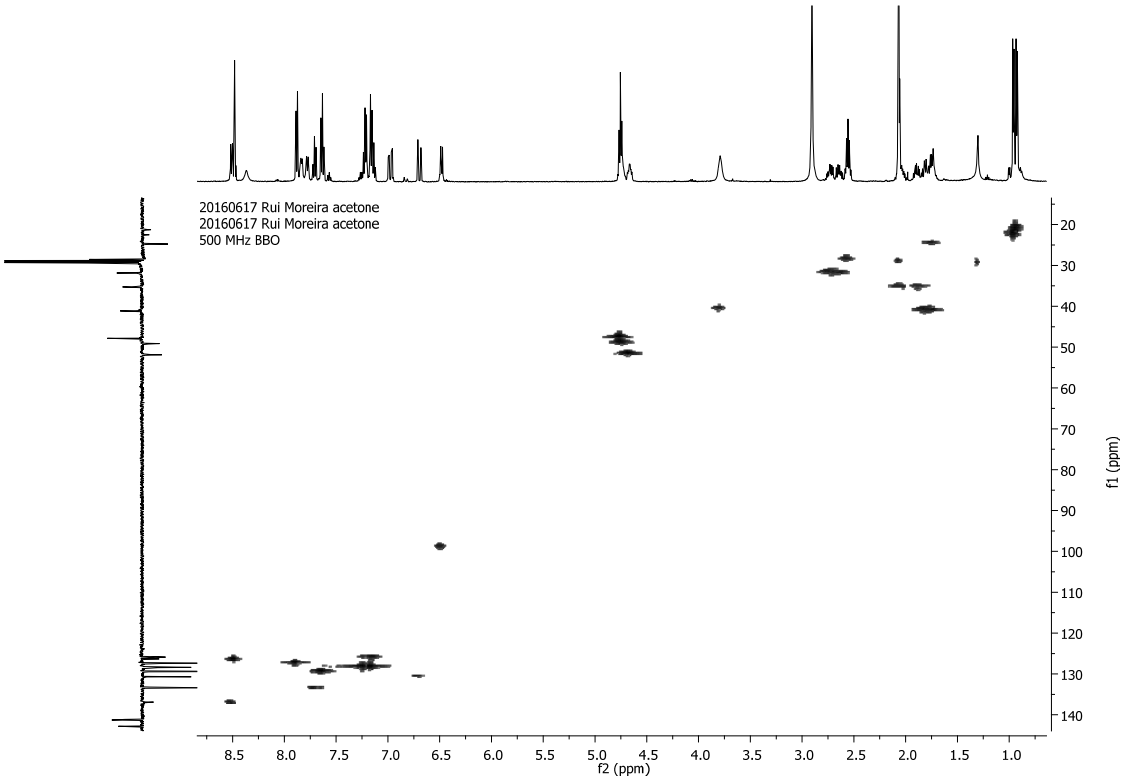
3.2.2. COSY spectrum of compound **2**.



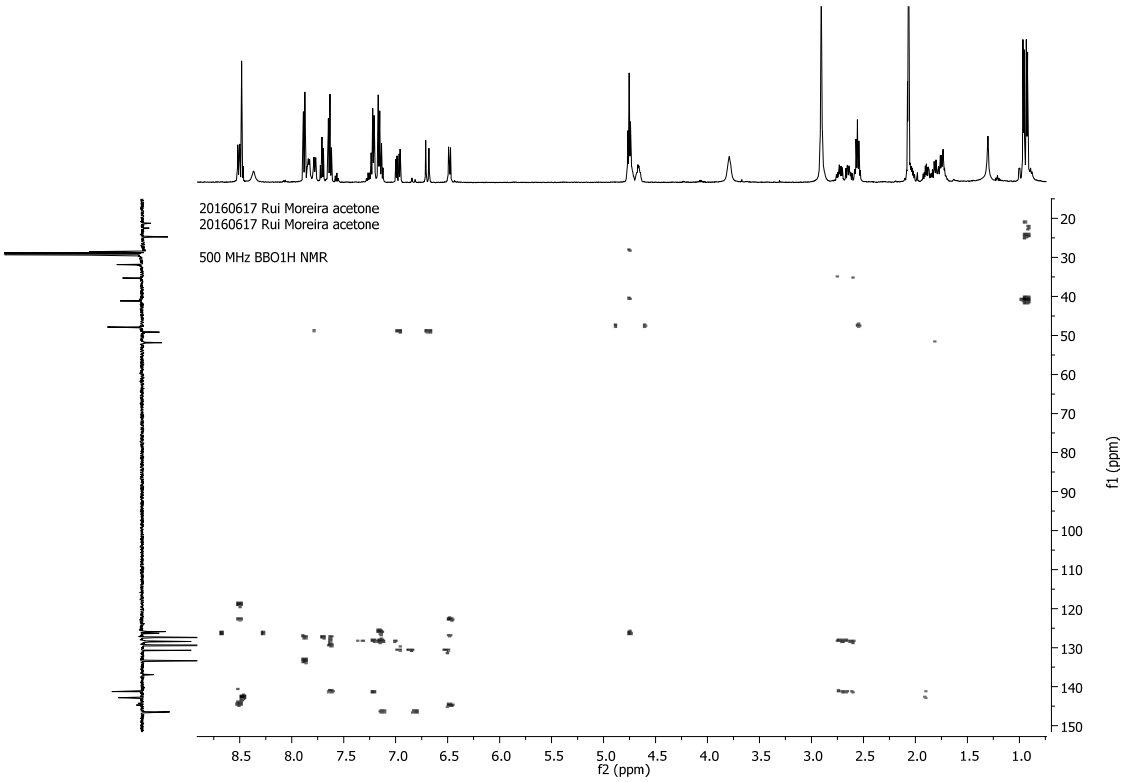
3.2.3. APT carbon spectrum for compound **2**.



3.2.4. HMQC spectrum of compound **2**.



3.2.5. HMBC spectrum of compound 2.



4. Supplementary Figures

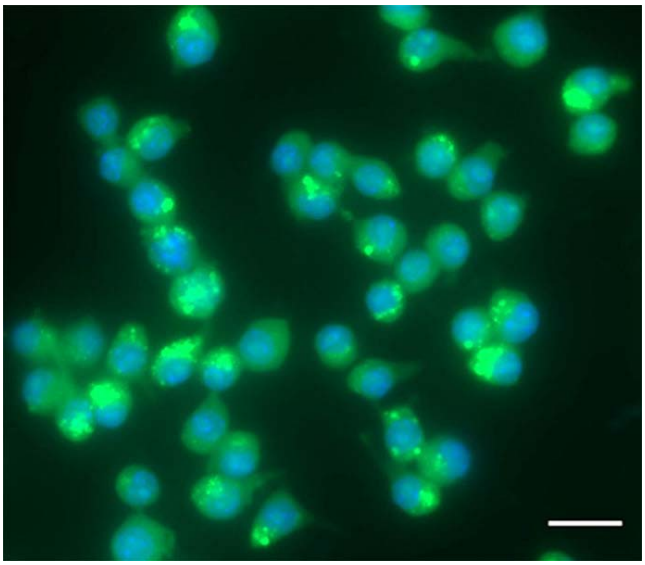


Figure S1. NBD-VS fluorescent compound is able to penetrate the microglial membrane and distributes in the cytoplasm after a 24 h incubation period. N9 cells were treated for 24 h with 20 μM vinyl sulfone linked to a green fluorophore (NBD-VS). Microglial cells nuclei were stained for Hoechst dye (blue). Scale bar represents 20 μm.

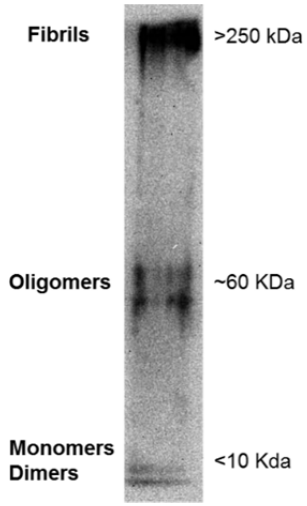


Figure S2. Analysis of the amyloid-β (Aβ) peptide aggregates in the Aβ1-42 stock solution, after 24 h at 37°C. The incubation medium contained a mixture of Aβ 1-42 monomers and dimers, oligomers and fibrils. Aβ1-42 peptide was diluted in DMEM-Ham's F12 culture medium to a stock concentration of 111 µM and allowed to incubate for 24 h at 37 °C to preaggregate the peptides. Image represents immunoblot analysis of aggregation forms of Aβ1-42 present at the time of incubation using a primary antibody raised against 6E10.

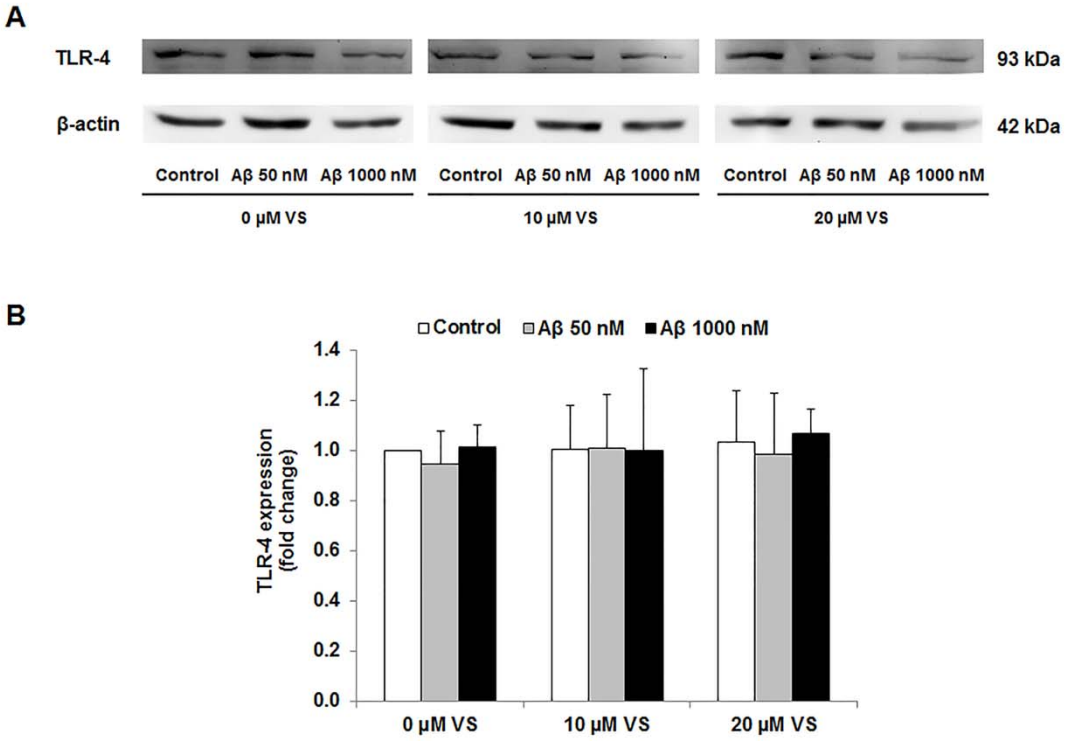


Figure S3. Expression of the toll-like receptor-4 (TLR-4) does not change upon amyloid-β (Aβ) peptide incubation and vinyl sulfone (VS) treatment. N9 cells were treated for 24 h with 50 nM

and 1000 nM Aβ, in the presence or absence of 10 μM and 20 μM VS. Cells not-treated with Aβ served as controls. (A) After treatment, total cell lysates were analyzed by western blot with an antibody specific for TLR-4. Representative results from one experiment are shown. (B) The intensity of the bands was quantified by scanning densitometry, standardized with respect to β-actin protein and graph bars represent the fold change relatively to control without VS (mean ± SEM).

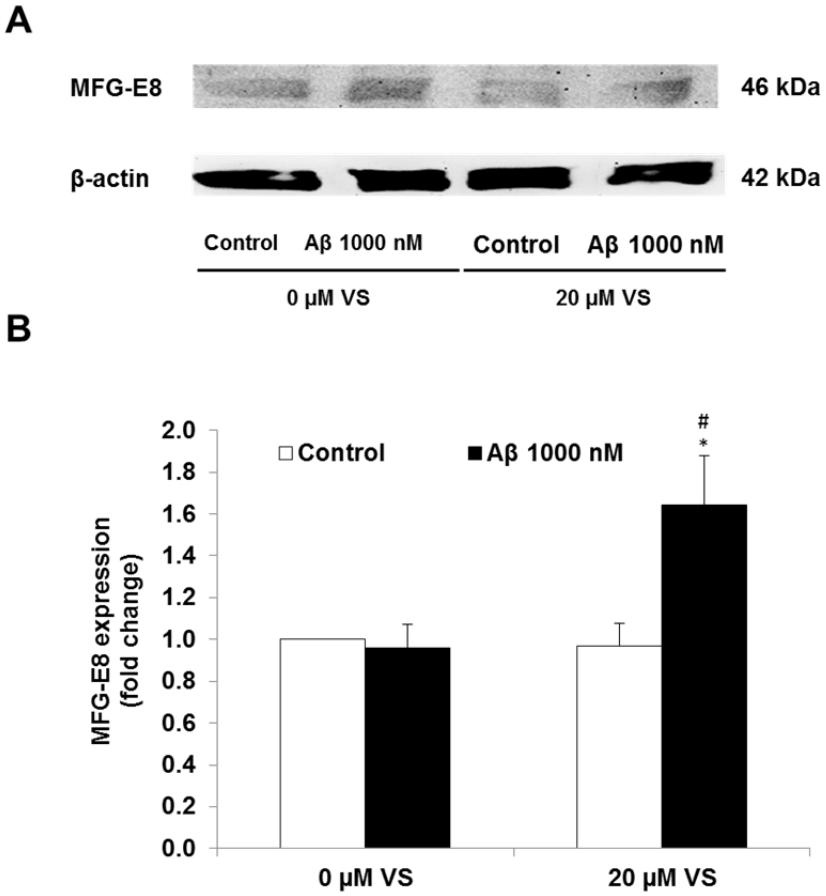


Figure S4. Expression of milk fat globule-EGF factor 8 (MFG-E8) increases in amyloid-β (Aβ) treated cells upon vinyl sulfone (VS) treatment. N9 cells were treated for 24 h with 50 nM and 1000 nM Aβ, in the presence or absence of 10 μM and 20 μM VS. Cells not-treated with Aβ served as controls. (A) After treatment, total cell lysates were analyzed by western blot with an antibody specific for MFG-E8. Representative results from one experiment are shown. (B) The intensity of the bands was quantified by scanning densitometry, standardized with respect to β-actin protein and graph bars represent the fold change relatively to control without VS (mean ± SEM). *$p<0.05$ vs respective control; $^{\#}p<0.05$ vs same experimental condition in the absence of VS.

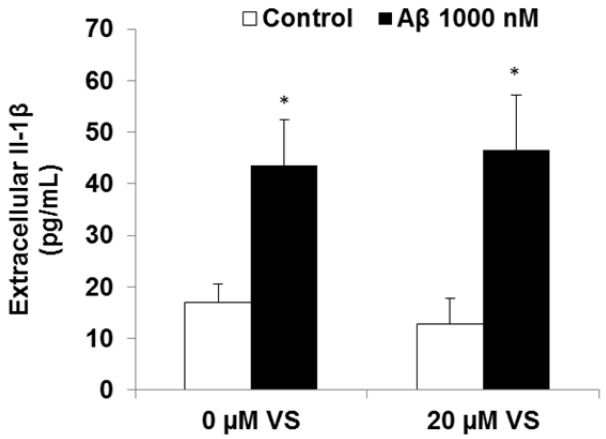


Figure S5. Increased microglial release of interleukin (IL)-1β into the extracellular media upon 24 h treatment with amyloid-β (Aβ) peptide is not modified by vinyl sulfone (VS) co-incubation. N9 cells were treated with 1000 nM Aβ, in the presence and absence of 20 μM VS. Cells not-treated with Aβ served as controls. IL-1β extracellular levels were evaluated by ELISA (mean ± SEM). *$p<0.05$ vs respective control.

5. Supplementary Table

Table S1 - Data on percentage inhibition of cathepsin S, B and L activities with different dipeptidyl vinyl sulfone (VS) concentrations

VS concentration	Cathepsin Activity (%)		
(μM)	Cathepsin S	Cathepsin B	Cathepsin L
80	1.92	0.39	1.82
40	5.83	1.37	14.86
20	6.72	2.22	9.82
10	9.18	2.41	8.21
5	7.86	3.10	14.69
2.5	7.85	2.67	6.52
1.25	7.72	3.00	18.35
0.62	9.14	4.41	25.35
0.31	9.52	4.72	24.37
0.16	9.78	3.89	25.11
0.08	10.66	5.25	20.89
0	100.00	100.00	100.00

The THP-1 (ATCC TIB202) monocytic cell line was used and cultivated following ATCC's instructions. THP-1 monocytes were seeded in 24-well plates at a density of 3 x 10^5 cells per well and differentiated into macrophages by overnight treatment with 20 nM phorbol 12-myristate 13-acetate. Subsequently, the macrophages were incubated with the various concentrations of VS for 24 h. Following treatment, the cells were washed with PBS and recovered with a 5 mM EDTA/PBS solution. Cell lysis and measurement of the enzymatic activity was performed using Cathepsin Activity Fluorometric Assay kits (Biovision), specific for each cathepsin and following the manufacturer's instructions.