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Supporting Information

Elucidating new structural features of the triazole scaffold for the development of mPGES-1 inhibitors

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1. Docking Calculations

The compound chemical structures (**1-33**) were processed with LigPrep (version 2.8)[1] generating all the possible tautomers and protonation states at a pH of 7.4 ± 1.0, and finally minimized using OPLS 2005 force field. Protein 3D model was prepared using the Schrödinger Protein Preparation Wizard,[1] starting from the mPGES-1 X-ray structure in the active form (PDB code: 4AL0; conformation "A" chosen for residues with alternate positions).[2] Docking calculations were performed using Glide software[3] with extra precision (XP) mode.[4] For all the compounds, all open-chain bonds were treated as active torsional bonds.

The binding site between A and B chains explored during the docking calculations with a inner box of 24×24×30 Å, an outer box of 39×39×45 Å, centered at 10.527 (x), -13.702 (y), and -6.774 (z). Default settings, except for the number of structure to save (150 in this case), were used for the docking studies. Post docking minimization was performed to optimize the ligand geometries. Docking calculation were performed on 4 × AMD Opteron SixCore 2.4 GHz, results and illustrations of the 3D models were generated with Maestro (version 9.6)1 and AutoDockTools 1.5.6.

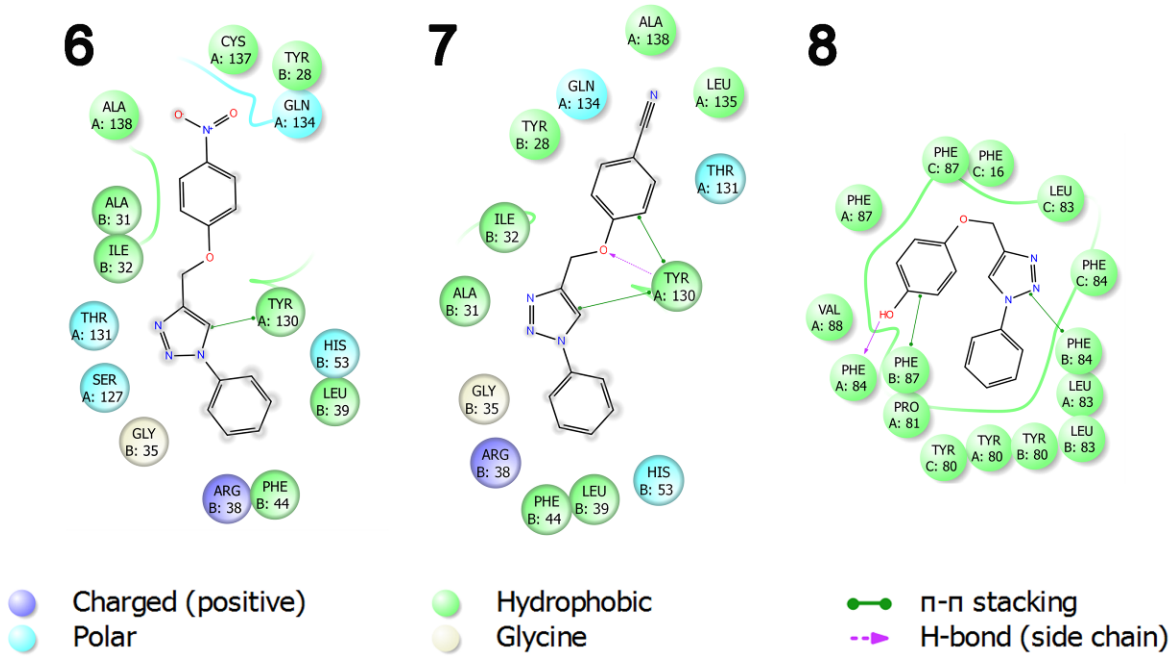


Figure S1. 2D diagram interactions of **6-8** with the mPGES-1 catalytic binding site.

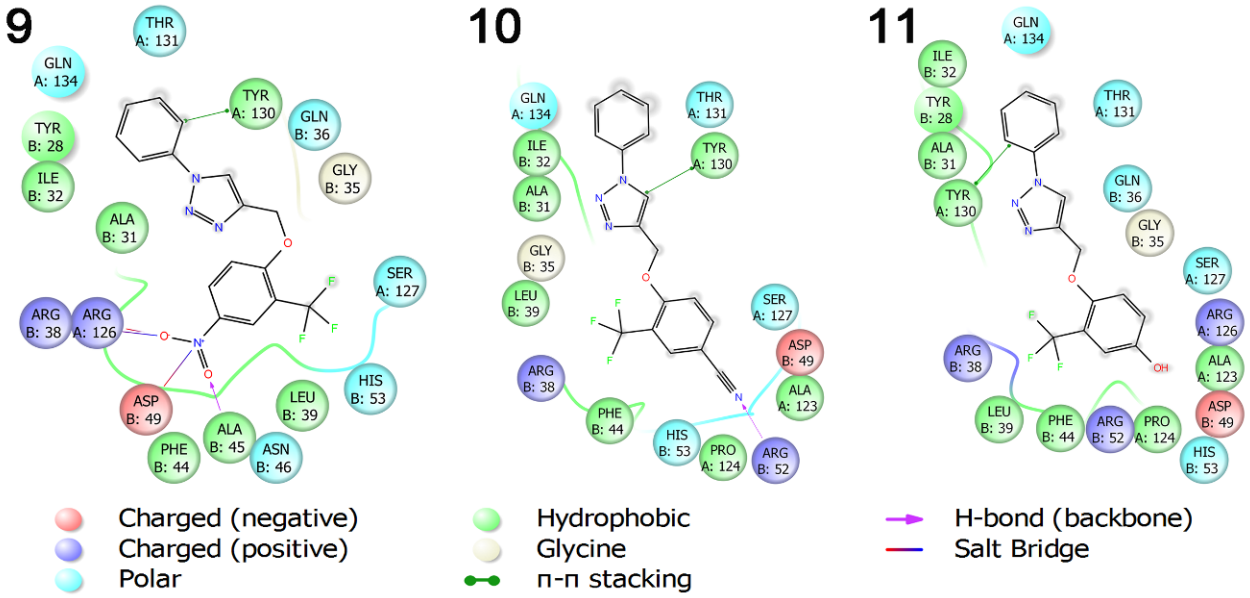


Figure S2. 2D diagram interactions of **9-11** with the mPGES-1 catalytic binding site.

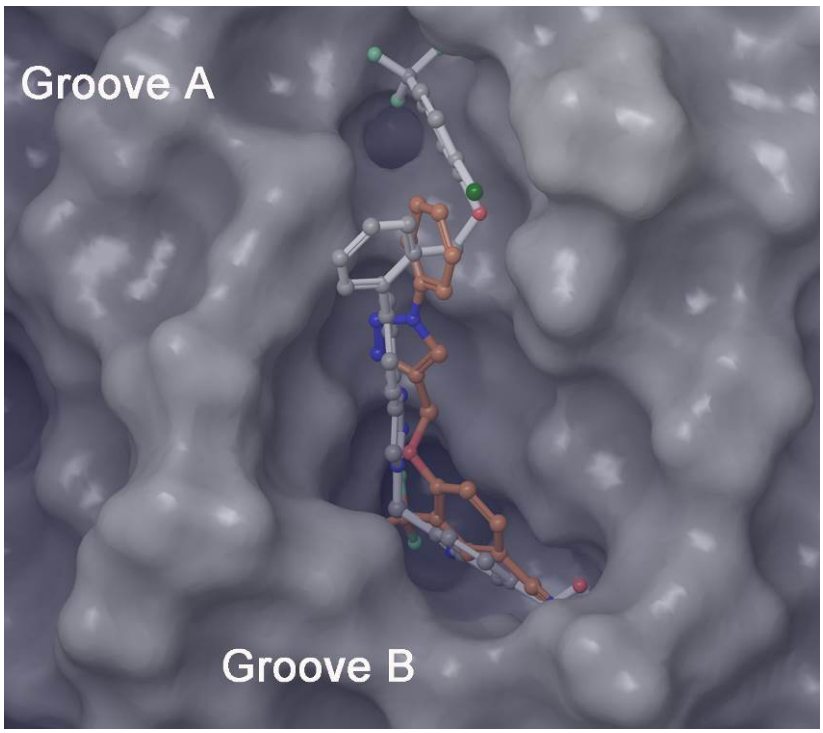


Figure S3 Superimposition of **4** with **9** in the mPGES-1 binding site.

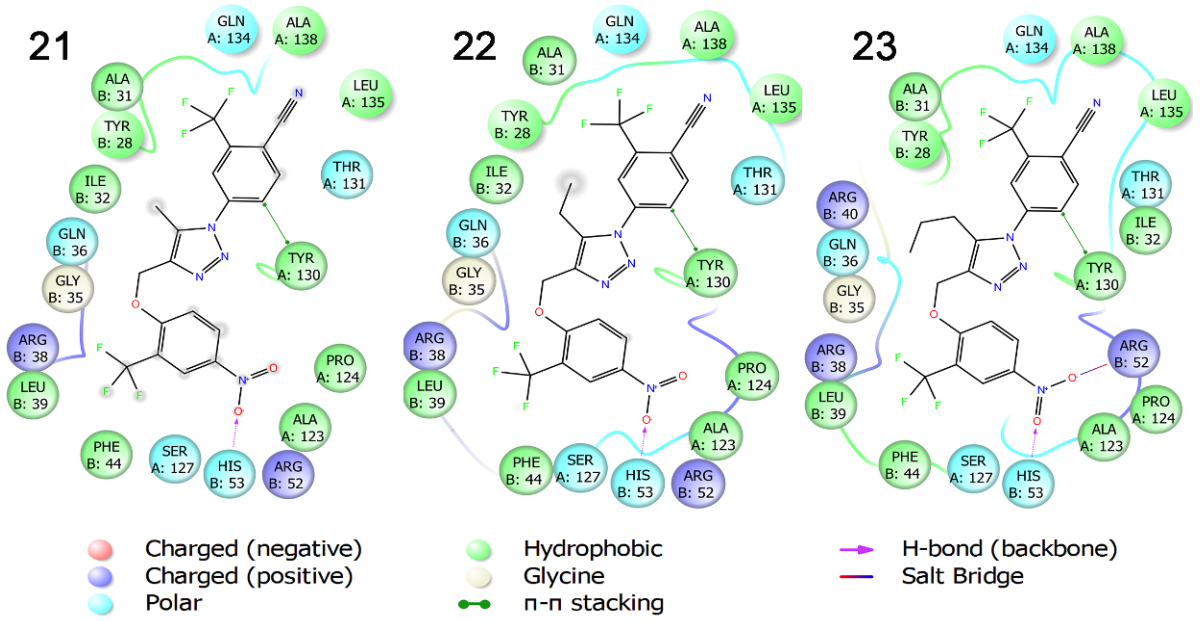


Figure S4. 2D diagram interactions of **21-23** with the mPGES-1 catalytic binding site.

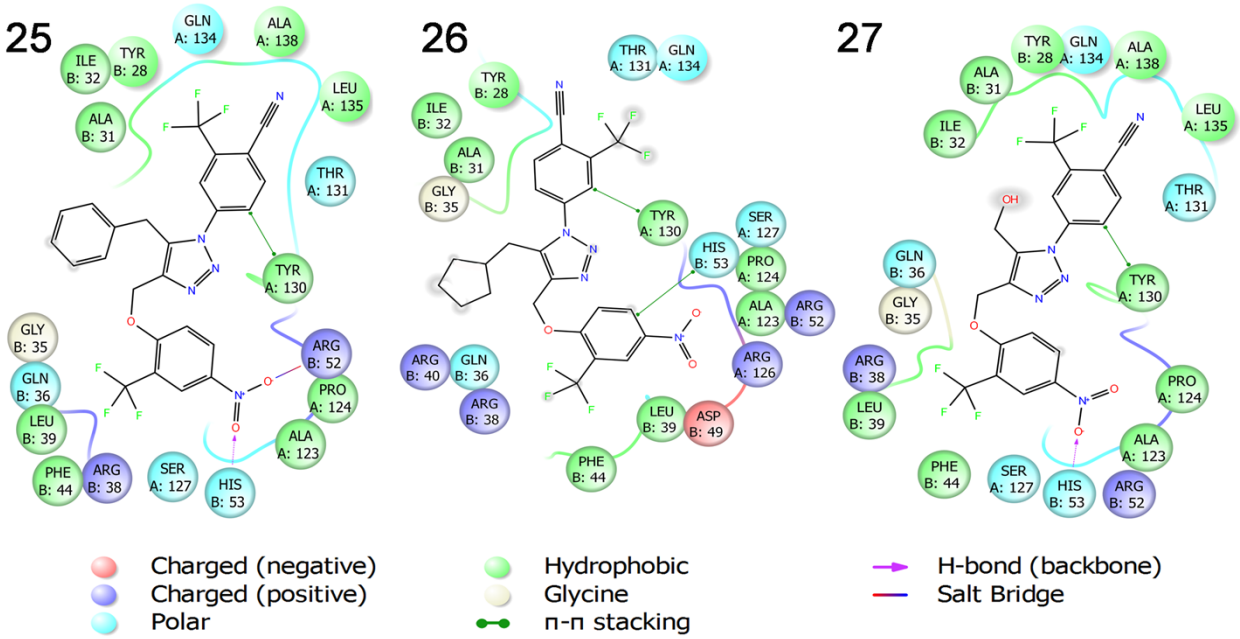


Figure S5. 2D diagram interactions of **25-27** with the mPGES-1 catalytic binding site.

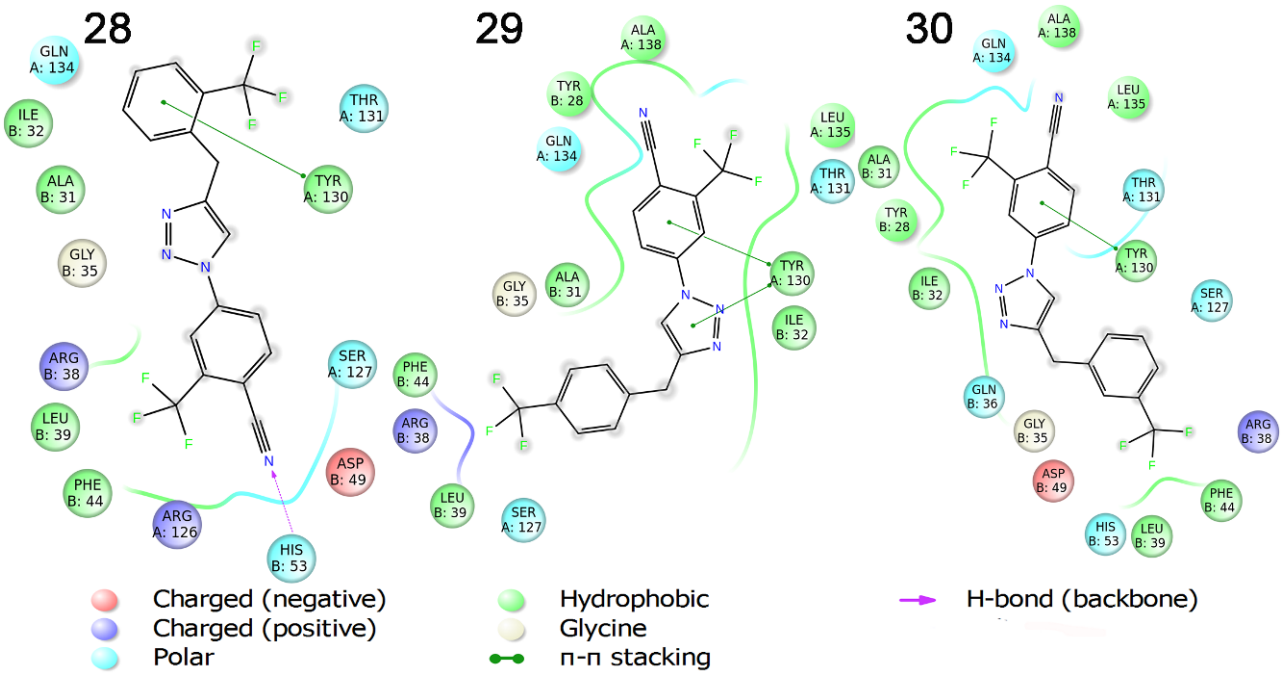


Figure S6. 2D diagram interactions of **28-30** with the mPGES-1 catalytic binding site.

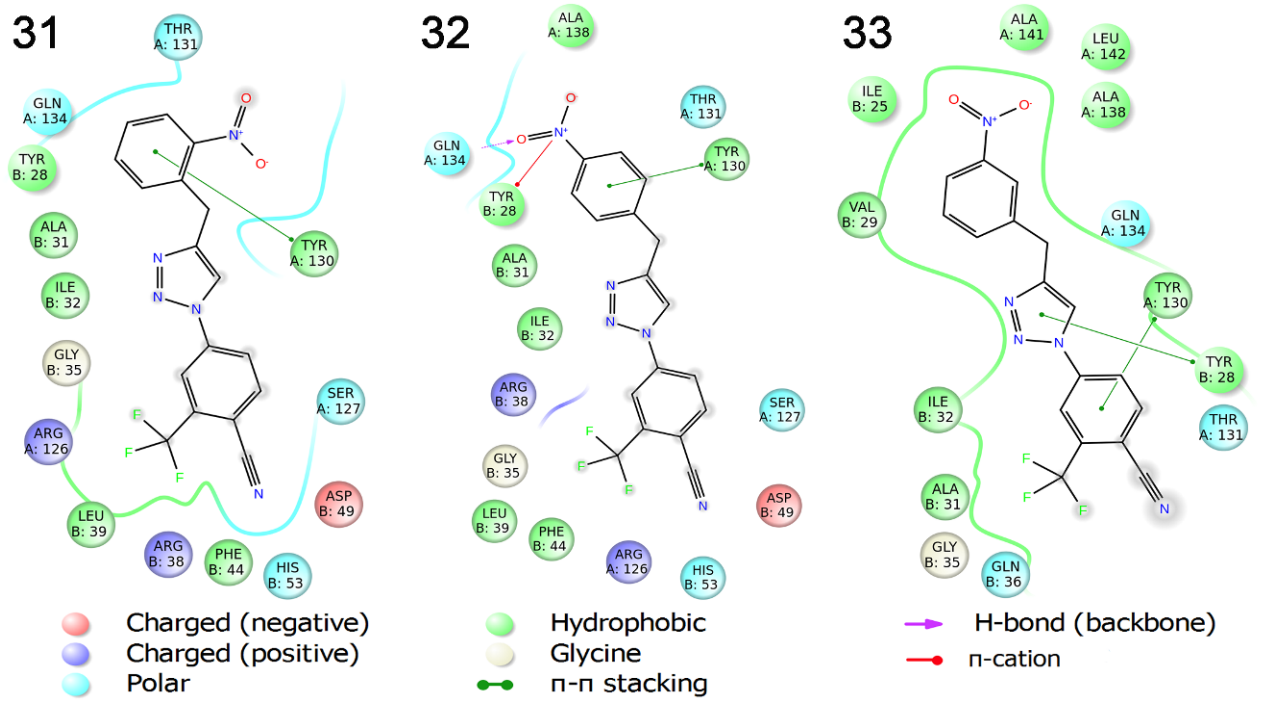


Figure S7.2D diagram interactions of **31-33** with the mPGES-1 catalytic binding site.

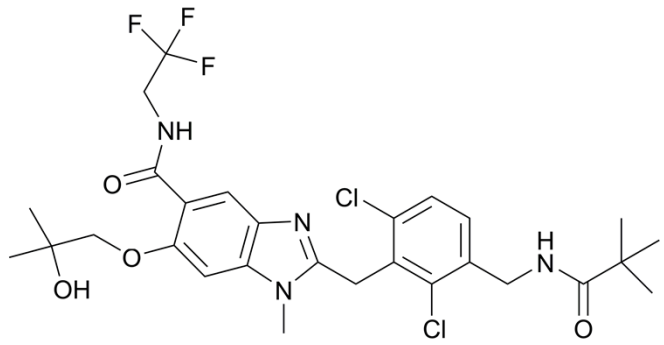
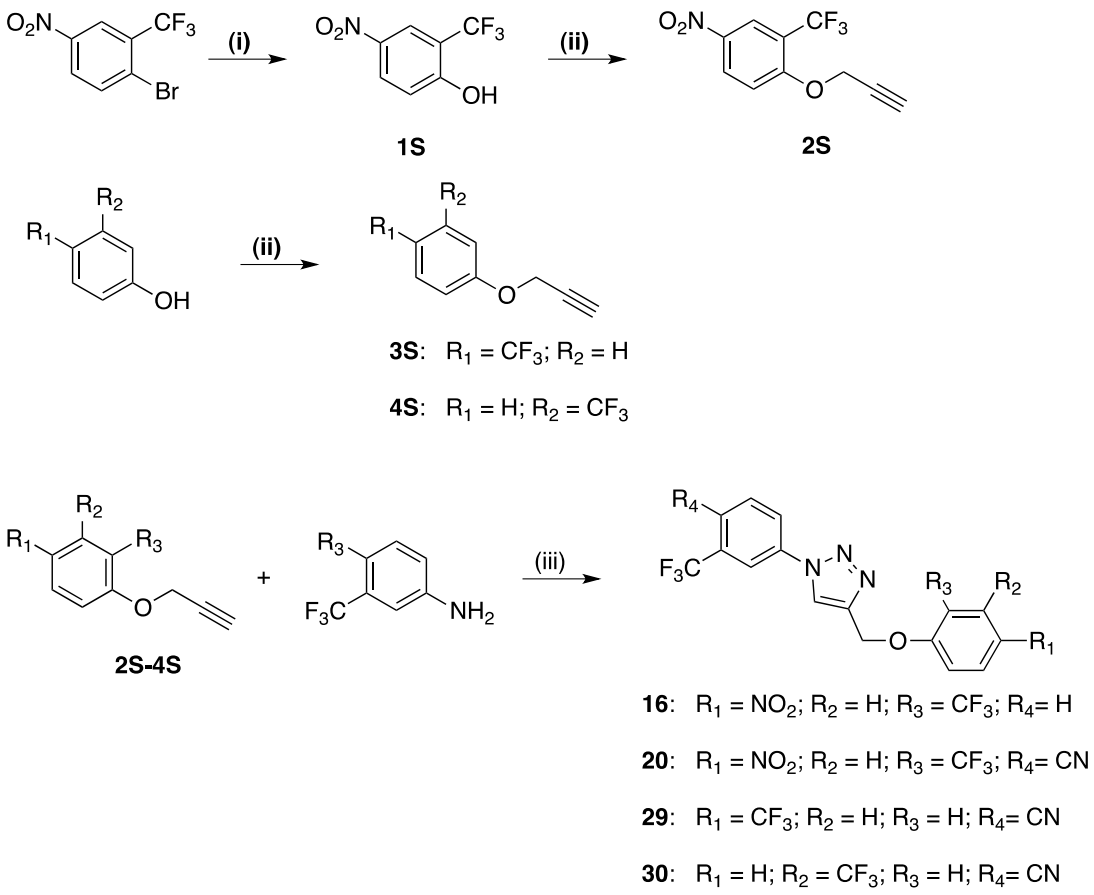


Figure S8. Chemical structure of LVJ (**34**).

2. Chemical experimental section: procedures and characterization data

All chemicals were purchased from Aldrich. Solvents were dried over standard drying agents and freshly distilled prior to use. Deionized water was obtained with milli-Q Millipore Water Purification System and is referred as milliQ water throughout the paper. Flash chromatography was performed on Teledyne Isco CombiFlash® R_f 200 using RediSep® Normal-phase Silica Flash Columns (230-400 mesh). Reactions were monitored by TLC on silica gel 60 F254 with detection by charring with $KMnO_4$. NMR spectra were recorded in $CDCl_3$ solutions, unless otherwise stated, at 25°C on a Mercury Varian spectrometer operating at 400 MHz (^1H) and 100.5 MHz (^{13}C) equipped with a Varian AutoSwitchable probe. UV/Vis spectra were registered on a Agilent Cary 100 spectrophotometer. HPLC/UV chromatograms were collected on a Merck-Hitachi LaChrom HPLC system equipped with a Merck-Hitachi L-7400 UV detector). Chromatograms were elaborated using DataApex Clarity Lite software. Mass spectra were registered with a Thermo Scientific TSQ Quantum Access Max mass spectrometer equipped with an APCI probe.



(**i**) CuI, 1,10-phenanthroline, KOH, DMSO/H_2O; (**ii**) propargyl bromide, K_2CO_3, acetone;
(**iii**) a. tBuONO, $TMSN_3$, acetonitrile; b. $CuSO_4$, Na-L-ascorbate, H_2O.

Scheme 1S. Synthesis of compounds **16**, **20**, **29** and **30**.

2.1 Synthesis of 4-nitro-2-(trifluoromethyl)phenol (**1S**)[5]

A round bottom flask with a magnetic stirring bar was charged with CuI (38 mg, 0.2 mmol), 1,10-phenanthroline (72 mg, 0.4 mmol), KOH (394 mg, 7 mmol), 1-bromo-4-nitro-2-(trifluoromethyl)benzene (540 mg, 2 mmol) and DMSO/H_2O (1:1, 1.6 mL) under N_2. The system was evacuated twice and back filled with N_2. The reaction mixture was stirred for 10 minutes at room temperature, and then heated at 100 ºC for 24 h. The reaction mixture was then allowed to cool to room temperature, diluted with 4-5 mL of ethyl acetate and water, filtered through a plug of silica gel and washed with 10-20 mL of ethyl acetate. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under vacuum. The resulting residue was purified by column chromatography on silica gel (petroleum ether/ethylacetate 1/1) to provide the desired product as a yellow solid (178 mg, Yield = 43%).

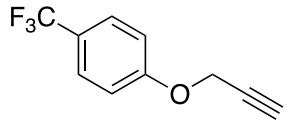
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.62 (br s, 1H), 8.47 (d, J = 2.4 Hz, 1H), 8.30 (dd, J = 9.2, 2.8 Hz, 1H), 7.07 (d, J = 8.8 Hz, 1H).

2.2. Synthesis of 4-nitro-1-(prop-2-yn-1-yloxy)-2-(trifluoromethyl)benzene (**2S**)[6]

A round bottom flask, equipped with a magnetic stir bar, was charged with phenol **1S** (128 mg, 0.62 mmol), K_2CO_3 (343 mg, 2.48 mmol), propargyl bromide (80% wt toluene, 0.27 mL, 2.48 mmol) and acetone (1.2 mL). The mixture was heated to reflux for 24 h and then cooled to rt. The solvent was removed under pressure and water was added. The heterogeneous mixture was extracted twice with EtOAc and the combined organic layers were dried over Na_2SO_4, filtered and concentrated. The yellow solid did not need further purification (152 mg, quantitative yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.53 (d, *J* = 2.7 Hz, 1H), 8.45 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.30 (d, *J* = 9.2 Hz, 1H), 4.94 (d, *J* = 2.4 Hz, 2H), 2.64 (t, *J* = 2.4 Hz, 1H).

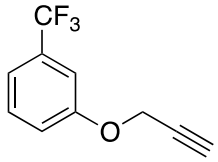
2.3. Synthesis of 1-(prop-2-yn-1-yloxy)-4-(trifluoromethyl)benzene (**3S**)[6]



A round bottom flask, equipped with a magnetic stir bar, was charged with 4-(trifluoromethyl)phenol (325 mg, 2 mmol), K_2CO_3 (1.10 g, 8 mmol), propargyl bromide (80% wt toluene, 0.89 mL, 8 mmol) and acetone (5 mL). The mixture was heated to reflux overnight and cooled to rt. The solvent was removed under pressure and water was added. The heterogeneous mixture was extracted twice with dichloromethane and the combined organic layers were dried over Na_2SO_4, filtered and concentrated. The yellow oil did not need further purification (398 mg, quantitative yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.62 - 7.54 (m, 2H), 7.09 - 7.03 (m, 2H), 4.75 (d, *J* = 2.4 Hz, 2H), 2.57 (t, *J* = 2.4 Hz, 1H).

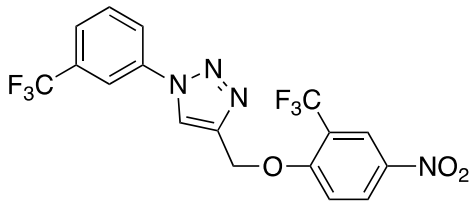
2.4. Synthesis of 1-(prop-2-yn-1-yloxy)-3-(trifluoromethyl)benzene (**4S**)[6]



A round bottom flask, equipped with a magnetic stir bar, was charged with 3-(trifluoromethyl)phenol (0.25 mL, 2 mmol), K_2CO_3 (1.10 g, 8 mmol), propargyl bromide (80% wt toluene, 0.89 mL, 8 mmol) and acetone (10 mL). The mixture was heated to reflux overnight and cooled to rt. The solvent was removed under pressure and water was added. The heterogeneous mixture was extracted twice with dichloromethane and the combined organic layers were dried over Na_2SO_4, filtered and concentrated. The yellow oil did not need further purification (401 mg, quantitative yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.46 (t, *J* = 8.0 Hz, 1H), 7.33 – 7.29 (m, 1H), 7.28 – 7.26 (m, 1H), 7.21 (dd, *J* = 8.2, 2.6 Hz, 1H), 4.78 (d, *J* = 2.6 Hz, 2H), 2.60 (t, *J* = 2.6 Hz, 1H).

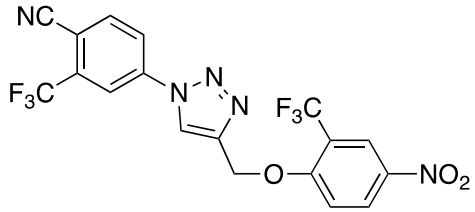
2.5 Synthesis of 4-((4-nitro-2-(trifluoromethyl)phenoxy)methyl)-1-(3-(trifluoromethyl)phenyl)-1H-1,2,3-triazole (**16**).[7]



3-(trifluoromethyl)aniline (0.025 mL, 0.19 mmol) was dissolved in CH_3CN (1 mL) in a three-necked flask and cooled to 0°C in an ice bath. To this stirred mixture was added *t*BuONO (0.035 mL, 0.29 mmol) followed by $TMSN_3$ (0.03 mL, 0.23 mmol) dropwise. The resulting solution was stirred at room temperature for 2 h. Alkyne **2S** (70 mg, 0.29 mmol), an aqueous solution of $CuSO_4 \cdot 5H_2O$ (5 mg, 0.02 mmol) and sodium L-ascorbate (20 mg, 0.1 mmol) were added and the reaction was stirred overnight at rt. The reaction mixture was diluted with EtOAc and then extracted. The combined organic extracts were dried over Na_2SO_4 and concentrated. The tail solid was purified by flash chromatography on silica gel (petroleum ether/ethylacetate 3/1) to provide the desired product as a white solid (13 mg, 0.03 mmol, Yield = 16%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.52 (d, *J* = 2.7 Hz, 1H), 8.45 (dd, *J* = 9.2, 2.7 Hz, 1H), 8.15 (s, 1H), 8.06 – 8.03 (m, 1H), 7.98 – 7.94 (m, 1H), 7.77 - 7.68 (m, 2H), 7.44 (d, *J* = 9.2 Hz, 1H), 5.56 (s, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$) δ ppm 160.4 (q, *J* = 1.3 Hz), 143,6, 137.0, 132.8* (q, *J* = 32.0 Hz), 130.7, 129.3, 125.8 (q, *J* = 4.0 Hz), 123.8 (q, *J* = 5.4 Hz), 123.7 (q, *J* = 1.3 Hz), 123.3**, 122.2**, 121.1,119.9* (q, *J* = 35.4 Hz), 117.7 (q, *J* = 4.0 Hz), 113.6, 110.0, 63.4. (*: from ^{19}F-^{13}C HSQC; **: from ^{19}F-^{13}C HMBC). UV: λ_{max} 293 nm. HPLC: t_R 11.10 min, 94.8% (293 nm). MS/APCI ($C_{17}H_{10}F_6N_4O_3$): *m/z* 432.94 (MH^+, 100).

2.6 Synthesis of 4-(4-((4-nitro-2-(trifluoromethyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)-2-(trifluoromethyl)benzonitrile (**20**).[7]

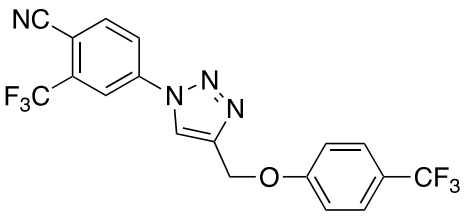


4-amino-2-(trifluoromethyl)benzonitrile (39 mg, 0.21 mmol) was dissolved in CH_3CN (1 mL) in a three-necked flask and cooled to 0°C in an ice bath. To this stirred mixture was added *t*BuONO (0.04 mL, 0.32 mmol) followed by $TMSN_3$ (0.035 mL, 0.26 mmol) dropwise. The resulting solution was stirred at room temperature for 2 h. Alkyne **2S** (78 mg, 0.32 mmol), an aqueous solution of $CuSO_4 \cdot 5H_2O$ (5 mg, 0.02 mmol) and sodium L-ascorbate (21 mg, 0.1 mmol) were added and the reaction was stirred overnight at rt. The reaction mixture was diluted with EtOAc and then extracted. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated. The tail solid was purified by flash chromatography

on silica gel (hexanes/ethylacetate 1/1) to provide the desired product as a white solid (56 mg, 0.12 mmol, Yield = 58%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.55 (d, *J* = 2.7 Hz, 1H), 8.48 (dd, *J* = 9.2, 2.7 Hz, 1H), 8.30 (d, *J* = 2.0 Hz, 1H), 8.23 (s, 1H), 8.13 (dd, *J* = 8.4, 2.2 Hz, 1H), 8.07 (m, *J* = 8.4 Hz, 1H), 7.41 (d, *J* = 9.3 Hz, 1H), 5.57 (s, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$) δ ppm 160.1 (q, *J* = 1.3 Hz), 144.4, 141.2, 139.4, 136.6, 135.2 (q, *J* = 3.3 Hz), 129.3, 123.8 (q, *J* = 5.6 Hz), 123.1, 122.2 (q, *J* = 273.2 Hz), 121.6 (q, *J* = 274.7 Hz), 120.9, 119.9 (q, *J* = 32.8 Hz), 118.5 (q, *J* = 5.0 Hz), 114.3, 113.4, 110.3 (q, *J* = 1.9 Hz), 63.2. UV: λ_{max} 274 nm. HPLC: t_R 10.67 min, 98.7% (274 nm). MS/APCI ($C_{18}H_9F_6N_5O_3$): *m/z* 458.04 (MH^+, 100).

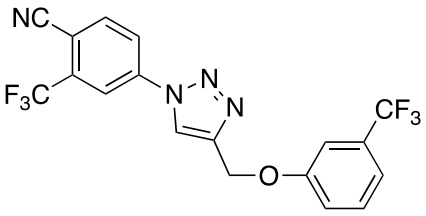
2.7. Synthesis of 2-(trifluoromethyl)-4-(4-((4-(trifluoromethyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)benzonitrile (**29**).[7]



4-amino-2-(trifluoromethyl)benzonitrile (93 mg, 0.5 mmol) was dissolved in CH_3CN (1 mL) in a three-necked flask and cooled to 0°C in an ice bath. To this stirred mixture was added *t*BuONO (0.09 mL, 0.75 mmol) followed by $TMSN_3$ (0.08 mL, 0.6 mmol) dropwise. The resulting solution was stirred at room temperature for 2 h. Alkyne **3S** (150 mg, 0.75 mmol), an aqueous solution of $CuSO_4 \cdot 5H_2O$ (13 mg, 0.05 mmol) and sodium L-ascorbate (50 mg, 0.25 mmol) were added and the reaction was stirred overnight at rt. The reaction mixture was diluted with EtOAc and then extracted. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated. The tail solid was purified by flash chromatography on silica gel (hexanes/ethylacetate 4/1) to provide the desired product as a white solid (138 mg, 0.34 mmol, Yield = 67%).

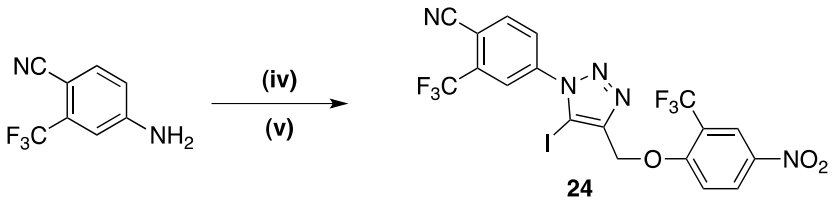
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.27 (d, *J* = 1.6 Hz, 1H), 8.23 (s, 1H), 8.15 (dd, *J* = 8.4, 2.0 Hz, 1H), 8.05 (d, *J* = 8.4 Hz, 1H), 7.59 (d, *J* = 8.8 Hz, 2H), 7.10 (d, *J* = 8.8 Hz, 2H), 5.38 (s, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$) δ ppm 160.2 (q, *J* = 1.7 Hz), 145.7, 139.5, 136.6, 135.1 (q, *J* = 33.6 Hz), 127.1 (q, *J* = 3.5 Hz), 124.2 (q, *J* = 271.3 Hz), 123.9 (q, *J* = 32.8 Hz), 123.0, 121.6 (q, *J* = 274.4 Hz), 120.6, 118.3 (q, *J* = 4.6 Hz), 114.7, 114.3, 110.0 (q, *J* = 1.7 Hz), 61.8. UV: λ_{max} 269 nm. HPLC: t_R 10.92 min, 97.9% (269 nm). MS/APCI ($C_{18}H_{10}F_6N_4O$): *m/z* 412.96 (MH^+, 100).

2.8. Synthesis of 2-(trifluoromethyl)-4-(4-((3-(trifluoromethyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)benzonitrile (**30**).[7]



4-amino-2-(trifluoromethyl)benzonitrile (93 mg, 0.5 mmol) was dissolved in CH_3CN (1 mL) in a three-necked flask and cooled to 0°C in an ice bath. To this stirred mixture was added *t*BuONO (0.09 mL, 0.75 mmol) followed by $TMSN_3$ (0.08 mL, 0.6 mmol) dropwise. The resulting solution was stirred at room temperature for 2 h. The alkyne **4S** (150 mg, 0.75 mmol), an aqueous solution of $CuSO_4 \cdot 5H_2O$ (13 mg, 0.05 mmol) and sodium L-ascorbate (50 mg, 0.25 mmol) were added and the reaction was stirred overnight at rt. The reaction mixture was diluted with EtOAc and then extracted. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated. The tail solid was purified by flash chromatography on silica gel (hexanes/ethylacetate 1/1) to provide the desired product as a white solid (150 mg, 0.36 mmol, Yield = 73%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.27 (d, *J* = 2.0 Hz, 1H), 8.21 (s, 1H), 8.14 (dd, *J* = 8.4, 2.0 Hz, 1H), 8.05 (d, *J* = 8.4 Hz, 1H), 7.47 - 7.41 (m, 1H), 7.30 - 7.24 (m, 2H), 7.20 (dd, *J* = 8.4, 2.4 Hz, 1H), 5.36 (s, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$) δ ppm 158.0, 145.7, 139.5, 136.6, 135.1 (q, *J* = 33.7 Hz), 132.1 (q, *J* = 32.1 Hz), 130.3, 123.7 (q, *J* = 272.5 Hz), 122.9, 121.6 (q, *J* = 274.5 Hz), 120.6, 118.3 (q, *J* = 3.8 Hz), 118.3 (q, *J* = 4.6 Hz), 117.9 (q, *J* = 1.5 Hz), 114.3, 111.8 (q, *J* = 3.8 Hz), 110.0 (q, *J* = 2.3 Hz), 61.9. UV: λ_{max} 271 nm. HPLC: t_R 10.80 min, 98.8% (271 nm). MS/APCI ($C_{18}H_{10}F_6N_4O$): *m/z* 412.91 (MH^+, 100).



(iv) tBuONO, $TMSN_3$, acetonitrile; **(v)** b. **2S**, CuI, DIPEA, NBS, THF.

Scheme 2S. Synthesis of compound **24**.

2.9 Synthesis of 4-(5-iodo-4-((4-nitro-2-(trifluoromethyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)-2-(trifluoromethyl)benzonitrile (**24**).[8]

4-amino-2-(trifluoromethyl)benzonitrile (38 mg, 0.2 mmol) was dissolved in CH_3CN (2.5 mL) in a three-necked flask and cooled to 0°C in an ice bath. To this stirred mixture was added *t*BuONO (0.036 mL, 0.31 mmol) followed by $TMSN_3$ (0.033 mL, 0.25 mmol) dropwise. The resulting solution was stirred at room temperature for 2 h. The solvent was removed under pressure and the azide was used without further purification.

A round bottom flask, equipped with a magnetic stir bar, was charged with azide, alkyne **2S** (41 mg, 0.17 mmol), CuI (38 mg, 0.2 mmol), DIPEA (0.034 mL, 0.2 mmol) and THF (2 mL). To this stirred mixture was added NBS (36 mg, 0.2 mmol) in THF (1 mL) dropwise The reaction mixture was stirred for 24 h, then the solvent was removed under pressure. The oily residue was purified by flash chromatography on silica gel (cyclohexanes/ethylacetate 3/1) to provide the desired product as yellow oil (35 mg, 0.06 mmol, Yield = 35%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.53 (d, *J* = 2.7 Hz, 1H), 8.48 (dd, *J* = 9.1, 2.7 Hz, 1H), 8.16 (d, *J* = 2.0 Hz, 1H), 8.11 (d, *J* = 8.4 Hz 1H), 8.04 (dd, *J* = 8.2, 2.0 Hz 1H), 7.56 (d, *J* = 9.3 Hz, 1H), 5.49 (s, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$) δ ppm 160.3, 147.7, 141.2, 139.5, 136.1, 134.7 (q, *J* = 33.3 Hz), 129.2, 128.9, 124.1 (q, *J* = 4.3 Hz), 123.8 (q, *J* = 5.1 Hz), 122.1 (q, *J* = 273.6 Hz), 121.5 (q, *J* = 275.3 Hz), 119.7 (q, *J* = 33.3 Hz), 114.1, 113.6, 111.9 (q, *J* = 1.7 Hz), 81.4, 62.7. UV: λ_{max} 251 nm. HPLC: t_R 10.64 min, 96.4% (251 nm). MS/APCI ($C_{18}H_8F_6IN_5O_3$): *m/z* 583.76 (MH^+, 100).

3. Bioactivity assays

3.1 Assay systems and materials.

Dulbecco's Modified Eagle Medium (DMEM)/high glucose (4.5 g/L) medium, penicillin, streptomycin, trypsin/ethylenediaminetetraacetate (EDTA) solution, and LSM 1077 lymphocyte separation medium were obtained from PAA (Pasching, Austria). IL-1β was obtained from ReproTech (Hamburg, Germany). Fetal calf serum (FCS), phenylmethylsulfonylfluoride (PMSF), leupeptin, soybean trypsin inhibitor (STI), glutathione (reduced), PGB_1, lysozyme, Ca^{2+}-ionophore A23187, and arachidonic acid were obtained from Sigma–Aldrich (Deisenhofen, Germany). MK886 and 11β-PGE_2 were obtained from Cayman Chemical (Ann Arbor, MI). PGH_2, adenosine triphosphate (ATP), isopropyl-β-d-1-thiogalactopyranoside (IPTG), and dextrane were obtained from Larodan (Malmoe, Sweden), Roche Diagnostics (Mannheim, Germany), AppliChem (Darmstadt, Germany), and Fluka (Neu-Ulm, Germany), respectively. A549 cells were provided by the Karolinska Institute (Stockholm, Sweden). Leukocyte concentrates from human healthy volunteers were provided by Institute of Transfusion Medicine, University Hospital Jena, Germany.

3.2 Cell culture.

A549 cells were grown in DMEM/high glucose (4.5 g/mL) medium supplemented with heat-inactivated FCS (10%, v/v), penicillin (100 U/mL) and streptomycin (100 µg/mL). After three days, confluent cells were detached using 1x trypsin/EDTA and reseeded with a density of 1×10^5 cells/ml medium.

3.3 Preparation of crude mPGES-1 in microsomes of A549 cells and determination of $mPGE_2$ synthase activity.

Preparation of A549 cells and determination of mPGES-1 activity was performed as described previously.[9] In brief, A549 cells were treated with 1 ng/mL interleukin-1β for 48 h at 37 °C and 5% CO_2. After sonification, the homogenate was subjected to differential centrifugation at 10,000×*g* for 10 min and 174.000×*g* for 1 h at 4 °C. The pellet (microsomal fraction) was resuspended in 1 mL homogenization buffer (0.1 M potassium phosphate buffer pH 7.4, 1 mM phenylmethanesulphonyl fluoride, 60 μg/mL soybean trypsin inhibitor, 1 μg/mL leupeptin, 2.5 mM glutathione, and 250 mM sucrose), and the total protein concentration was determined. Microsomal membranes were diluted in potassium phosphate buffer (0.1 M, pH 7.4) containing 2.5 mM glutathione. Test compounds or vehicle were added, and after 15 min at 4 °C, the reaction (100 μl total volume) was initiated by addition of PGH_2 (20 μM, final concentration, unless stated otherwise). After 1 min at 4 °C, the reaction was terminated using stop solution (100 μl; 40 mM $FeCl_2$, 80 mM citric acid, and 10 μM of 11β-PGE_2 as internal standard). PGE_2 was separated by solid phase extraction and analyzed by RP-HPLC as described.[9]

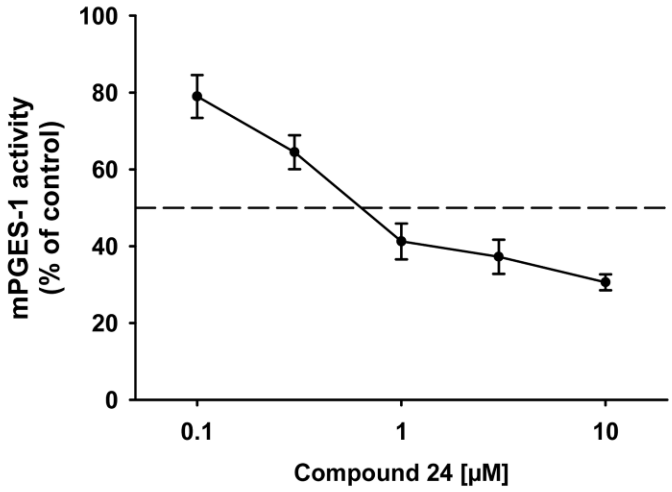


Figure S9. Inhibition of mPGES-1 activity by compound **24** in a cell-free assay. Data are means ± S.E.M., n = 3.

3.4 Statistics.

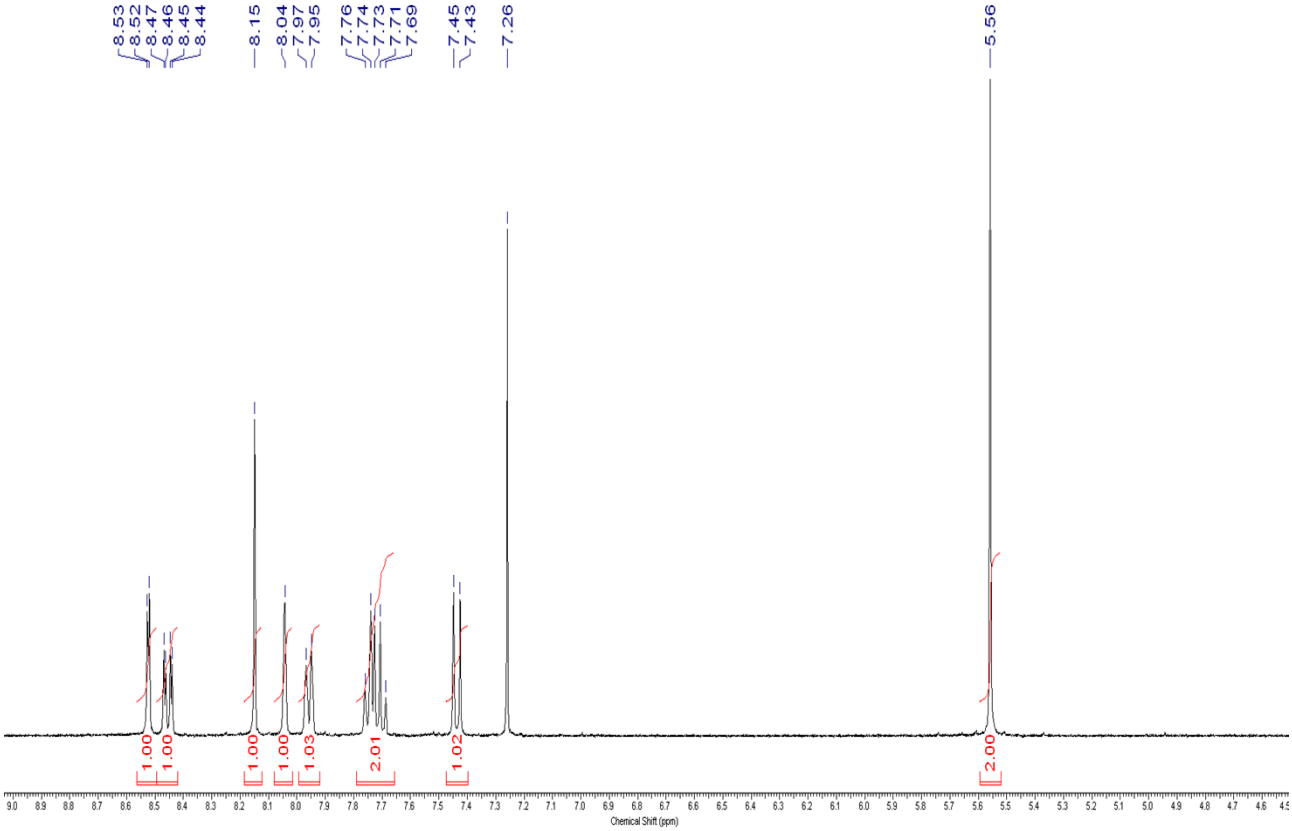
Data are expressed as mean ± SE. IC_{50} values were graphically calculated from measurements at 4-5 different concentrations of the compounds using SigmaPlot 9.0 (Systat Software Inc., San Jose, USA). The program Graphpad Instat (Graphpad Software Inc., San Diego, CA) was used for

statistical comparisons. Statistical evaluation of the data was performed by one-way ANOVAs for independent or correlated samples followed by Tukey HSD post-hoc tests. Where appropriate, Student's *t* test for paired and correlated samples was applied. A *P* value of <0.05 was considered significant.

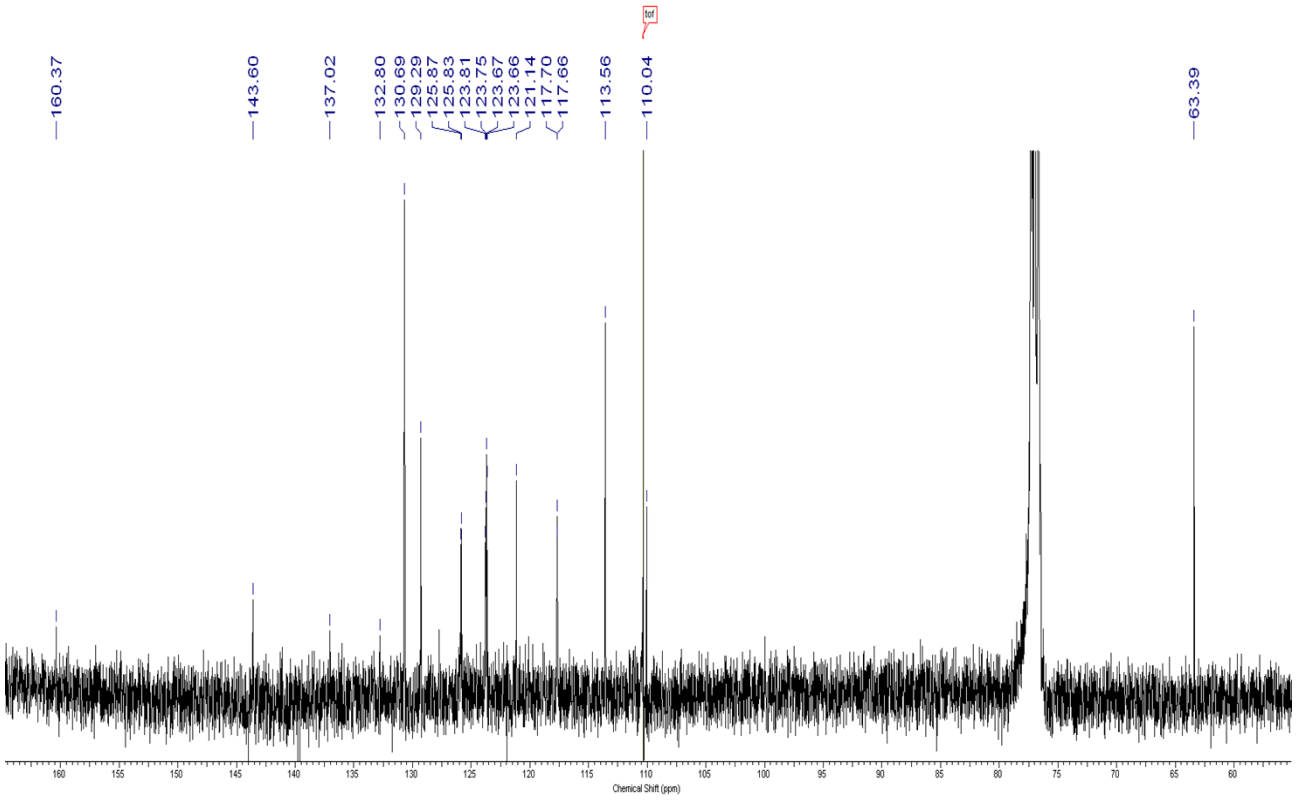
4. ^{1}H NMR, ^{13}C NMR, HPLC/UV and MS of compounds 16, 20, 24, 29 and 30

COMPOUND 16

^{1}HNMR

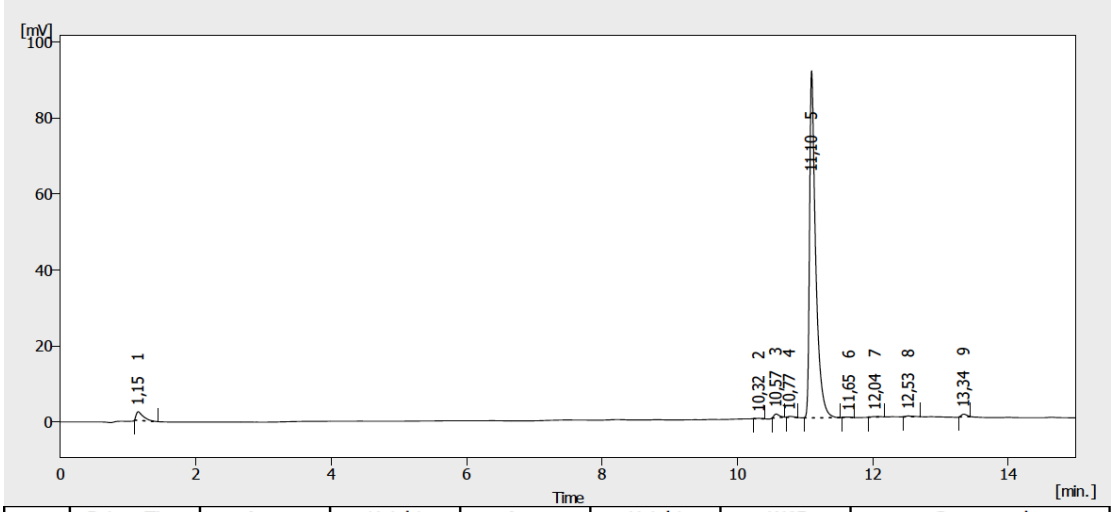


^{13}CNMR


160.37
143.60
137.02
132.80
130.69
129.29
125.87
125.83
123.81
123.75
123.67
123.66
121.14
117.70
117.66
113.56
110.04
63.39
Chemical Shift (ppm)

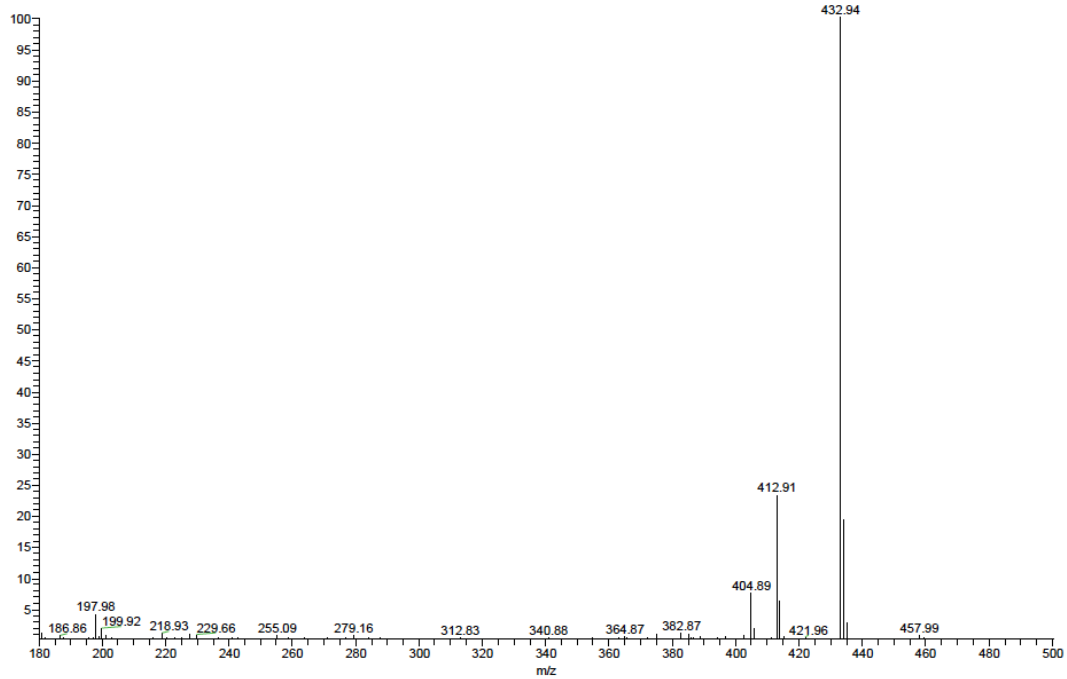
HPLC/UV

HPLC column: Waters Nova-Pak C18 60Å 4µm 3.9x150mm. **Detection wavelength**: 293 nm. **Eluents**: A = milliQ water; B = MeOH. **Gradient**: t = 0.0 min, 100% A; form 100% A to 100% B in 10.0 min; from t = 10.0 min to t = 14.0 min, 100% B; form 100% B to 100% A in 0.5 min; from t = 14.5 min to t = 15.0 min, 100% A.



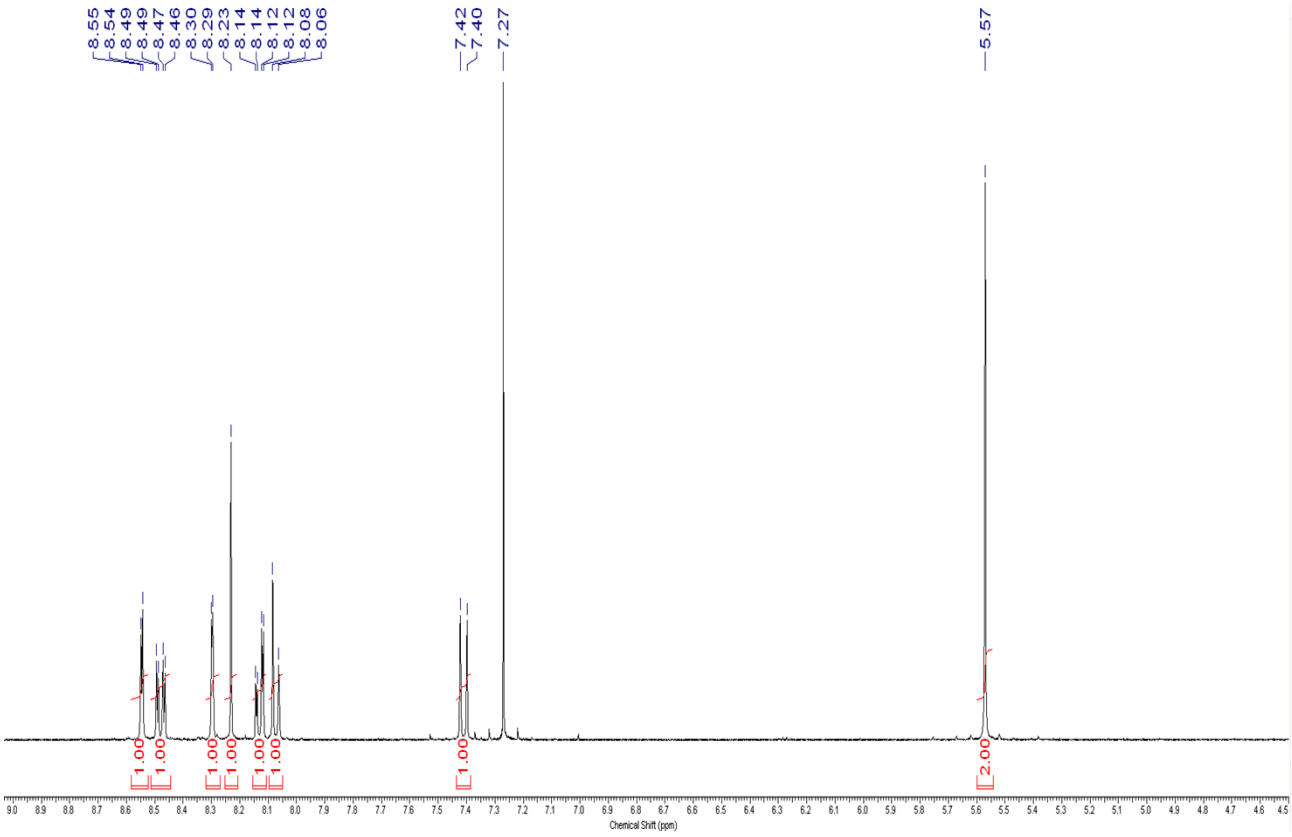
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1,149	16,872	2,286	2,8	2,4	0,11	
2	10,320	0,559	0,123	0,1	0,1	0,07	
3	10,573	5,378	0,996	0,9	1,0	0,09	
4	10,771	1,519	0,260	0,3	0,3	0,10	
5	11,096	574,851	91,377	94,8	95,0	0,09	
6	11,651	0,636	0,092	0,1	0,1	0,14	
7	12,037	1,127	0,134	0,2	0,1	0,18	
8	12,531	1,605	0,215	0,3	0,2	0,13	
9	13,344	3,627	0,691	0,6	0,7	0,09	
	Total	606,173	96,173	100,0	100,0		

MS

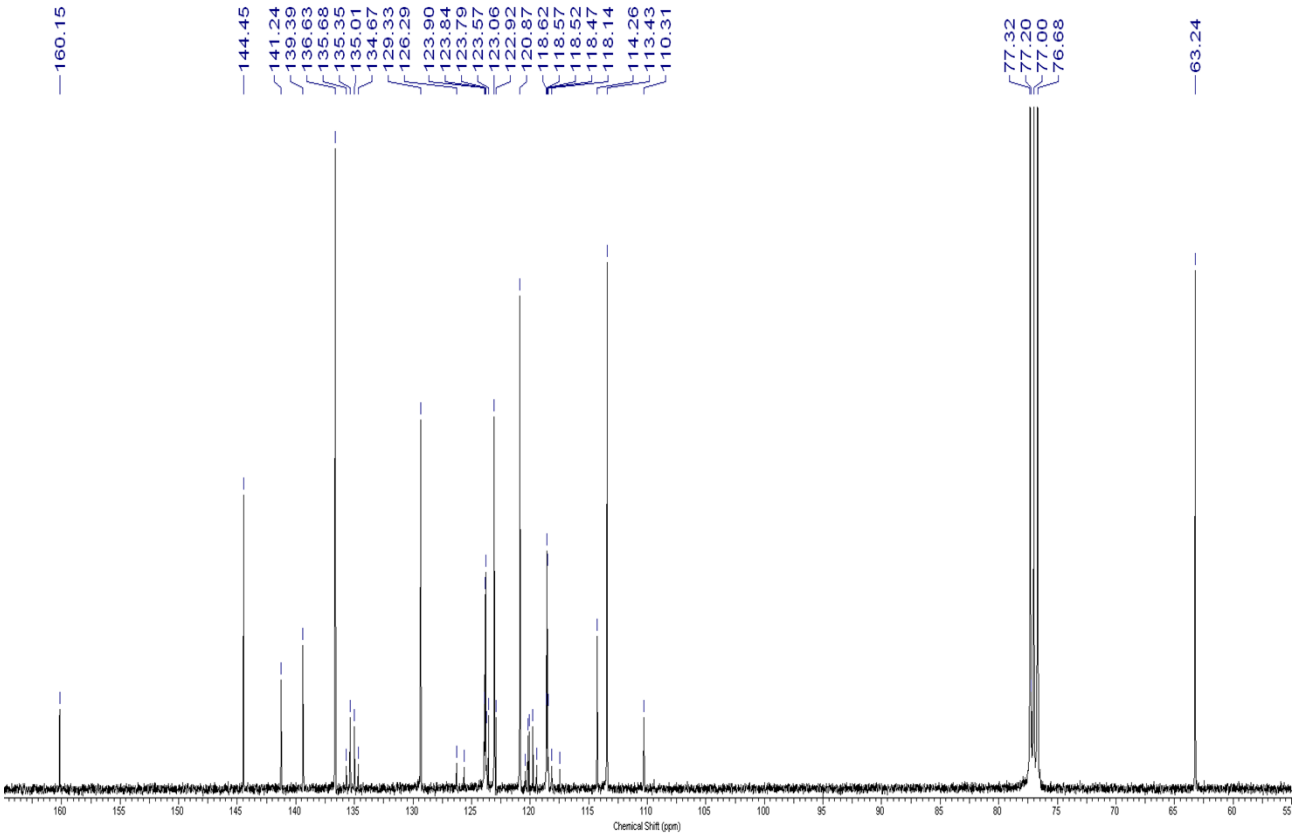


COMPOUND 20

^{1}HNMR

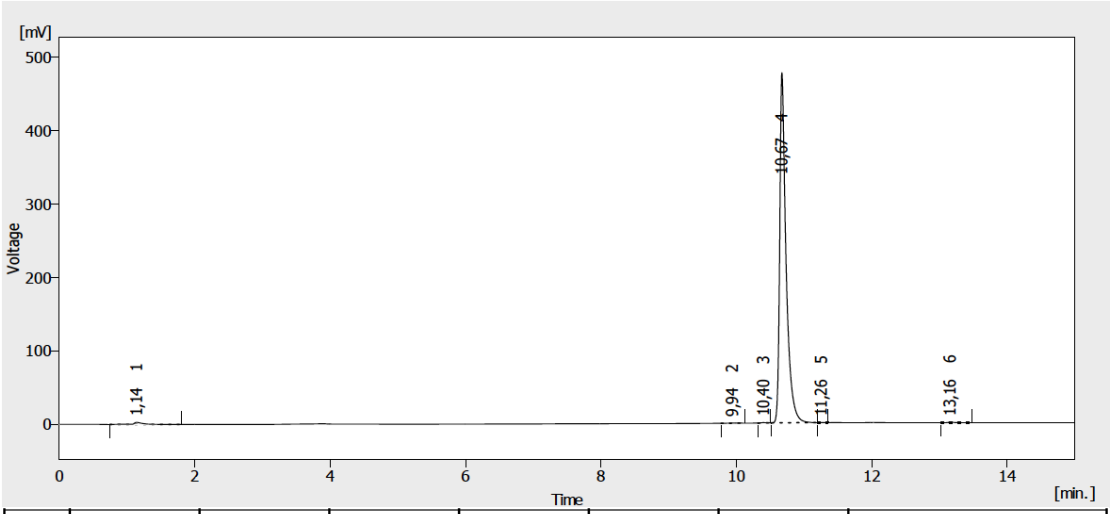


^{13}CNMR



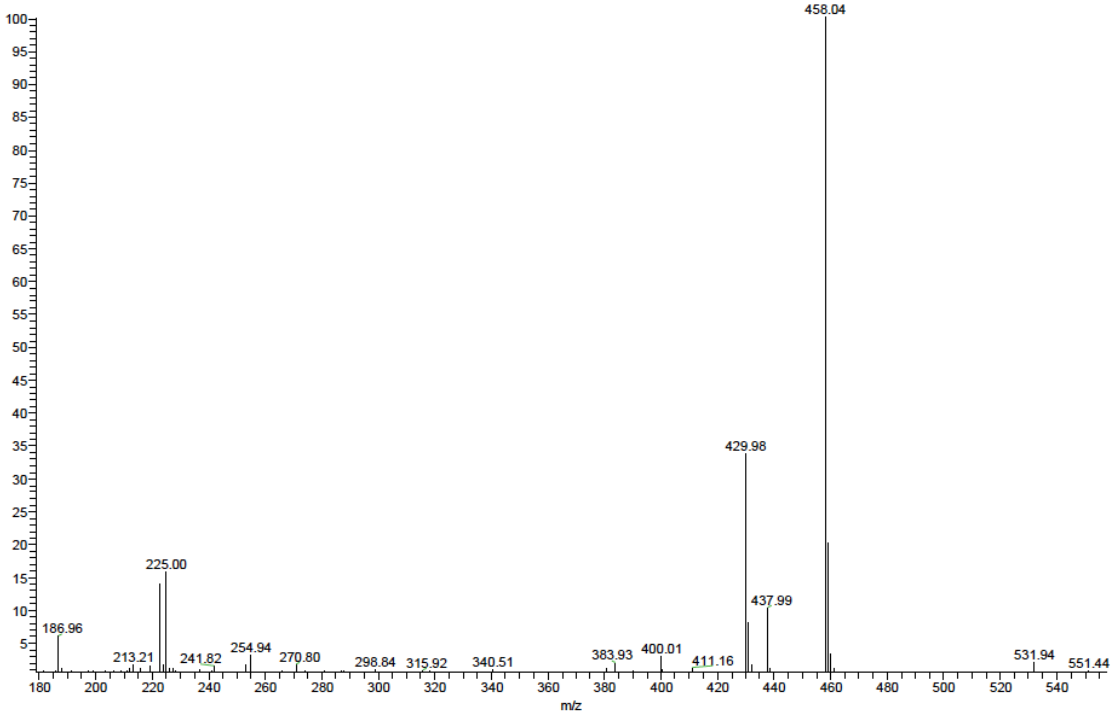
HPLC/UV

HPLC column: Waters Nova-Pak C18 60Å 4µm 3.9x150mm. **Detection wavelength**: 274 nm. **Eluents**: A = milliQ water; B = MeOH. **Gradient**: t = 0.0 min, 100% A; form 100% A to 100% B in 10.0 min; from t = 10.0 min to t = 14.0 min, 100% B; form 100% B to 100% A in 0.5 min; from t = 14.5 min to t = 15.0 min, 100% A.



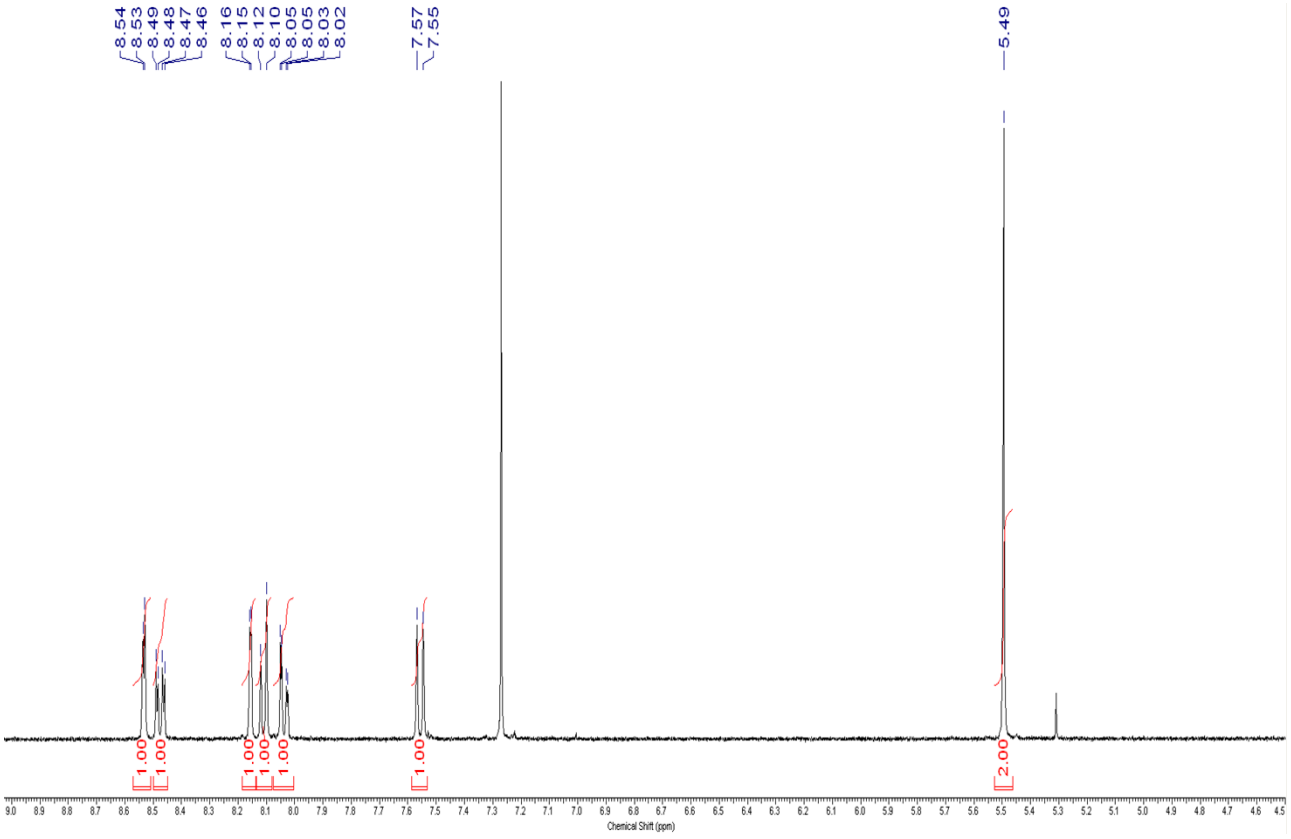
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1,144	29,990	2,803	1,0	0,6	0,12	
2	9,944	2,542	0,269	0,1	0,1	0,23	
3	10,404	2,261	0,439	0,1	0,1	0,10	
4	10,672	3095,853	476,950	98,7	99,1	0,10	
5	11,256	0,881	0,181	0,0	0,0	0,10	
6	13,160	5,230	0,728	0,2	0,2	0,10	
	Total	3136,757	481,370	100,0	100,0		

MS

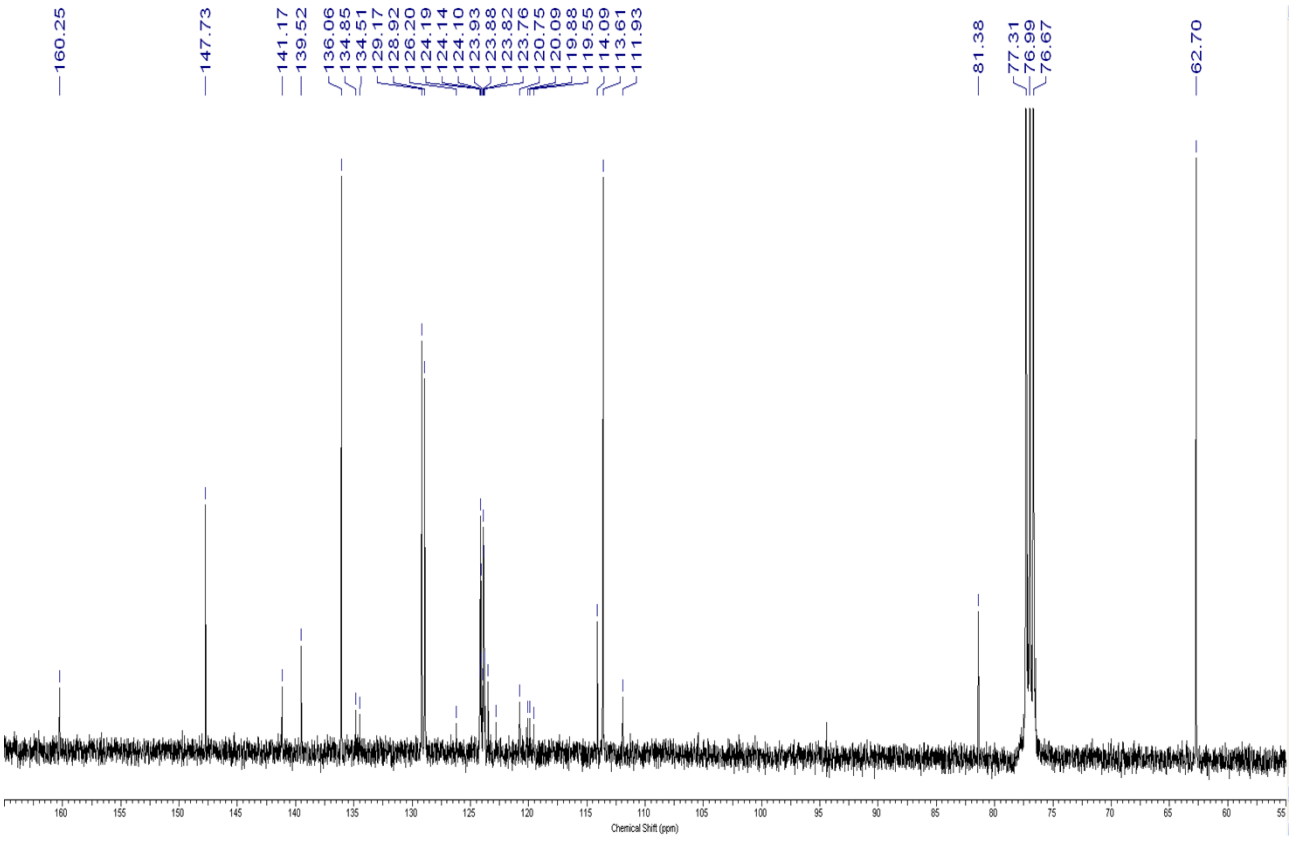


COMPOUND 24

^{1}HNMR

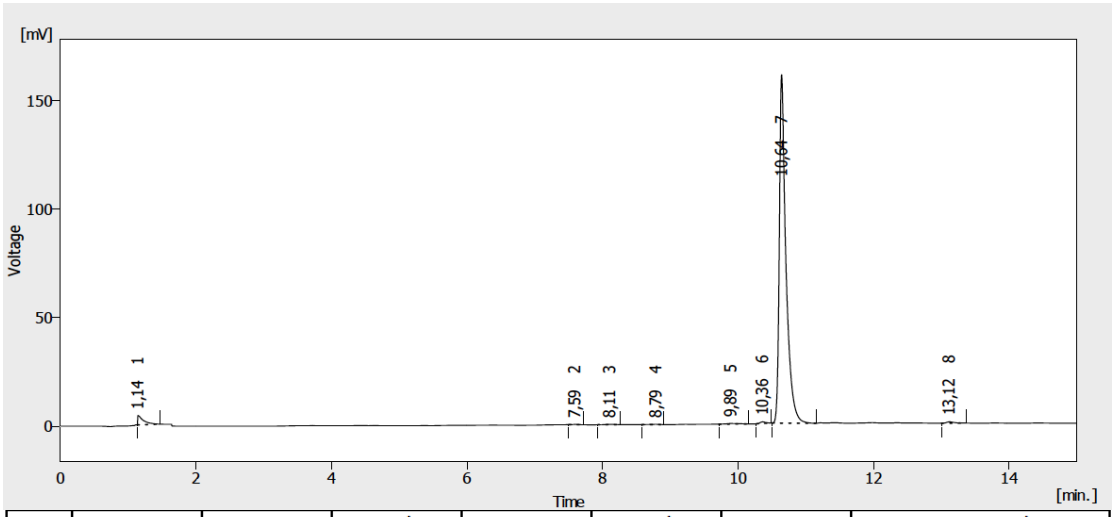


^{13}CNMR



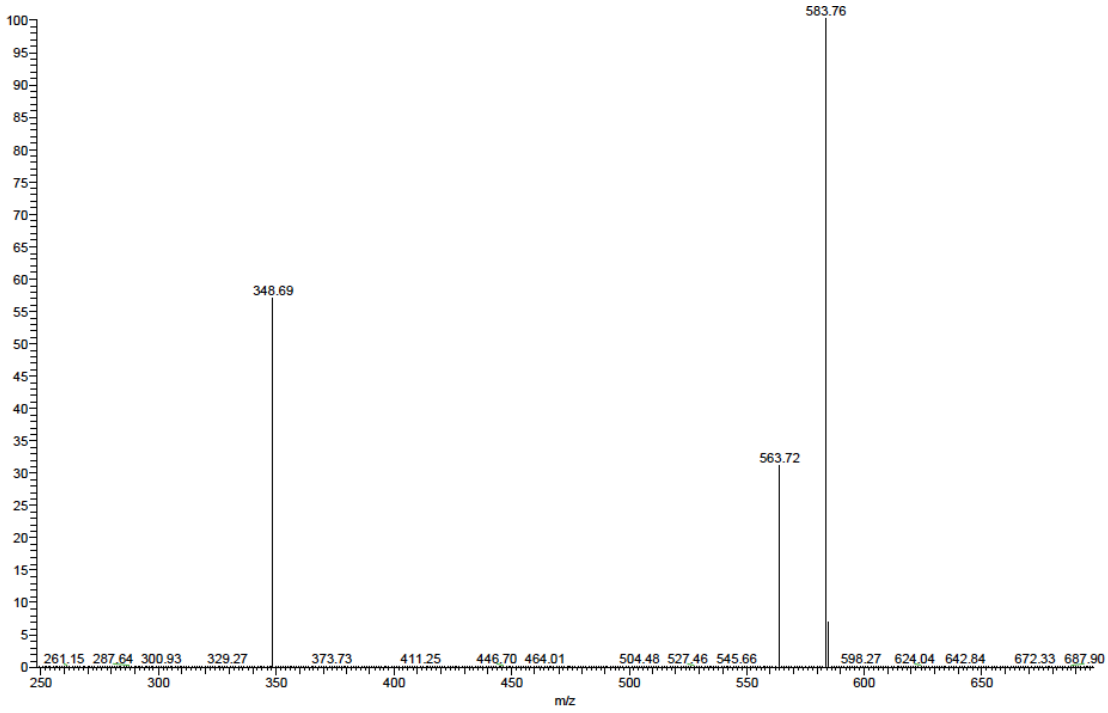
HPLC/UV

HPLC column: Waters Nova-Pak C18 60Å 4µm 3.9x150mm. **Detection wavelength**: 251 nm. **Eluents**: A = milliQ water; B = MeOH. **Gradient**: t = 0.0 min, 100% A; form 100% A to 100% B in 10.0 min; from t = 10.0 min to t = 14.0 min, 100% B; form 100% B to 100% A in 0.5 min; from t = 14.5 min to t = 15.0 min, 100% A.



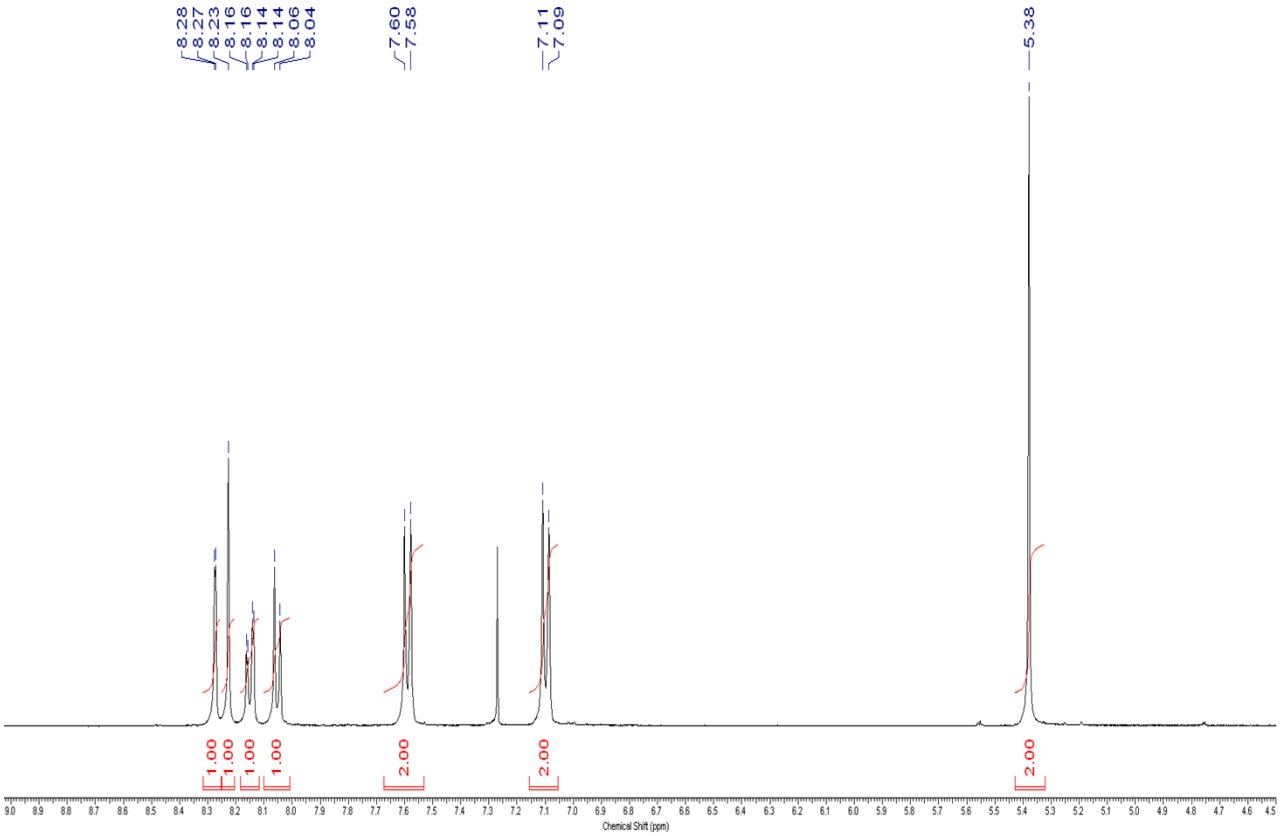
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1,144	25,722	4,336	2,3	2,6	0,08	
2	7,592	0,743	0,122	0,1	0,1	0,10	
3	8,108	1,140	0,121	0,1	0,1	0,17	
4	8,788	1,171	0,124	0,1	0,1	0,14	
5	9,892	3,129	0,256	0,3	0,2	0,24	
6	10,364	4,218	0,741	0,4	0,4	0,10	
7	10,644	1089,435	160,501	96,4	96,2	0,10	
8	13,120	4,560	0,615	0,4	0,4	0,11	
	Total	1130,119	166,817	100,0	100,0		

MS

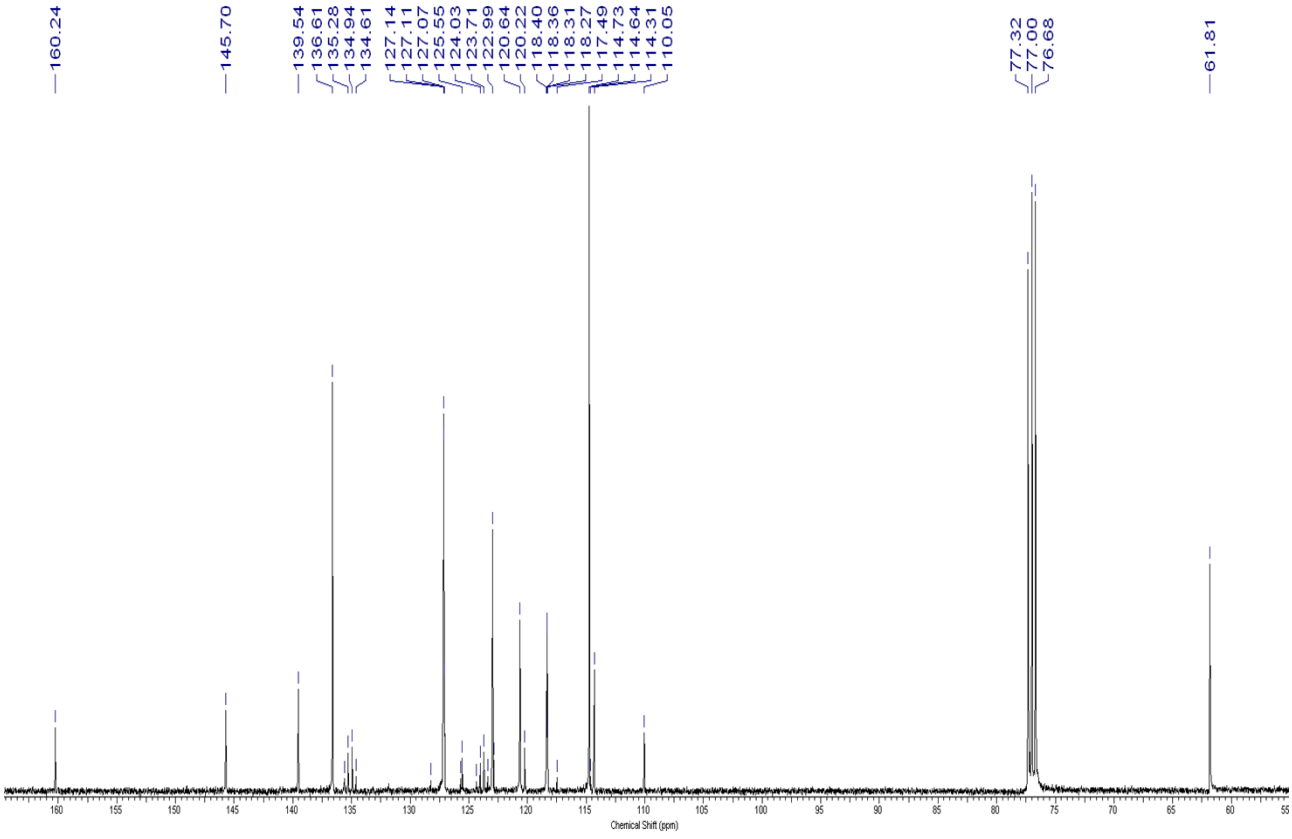


COMPOUND 29

^{1}HNMR

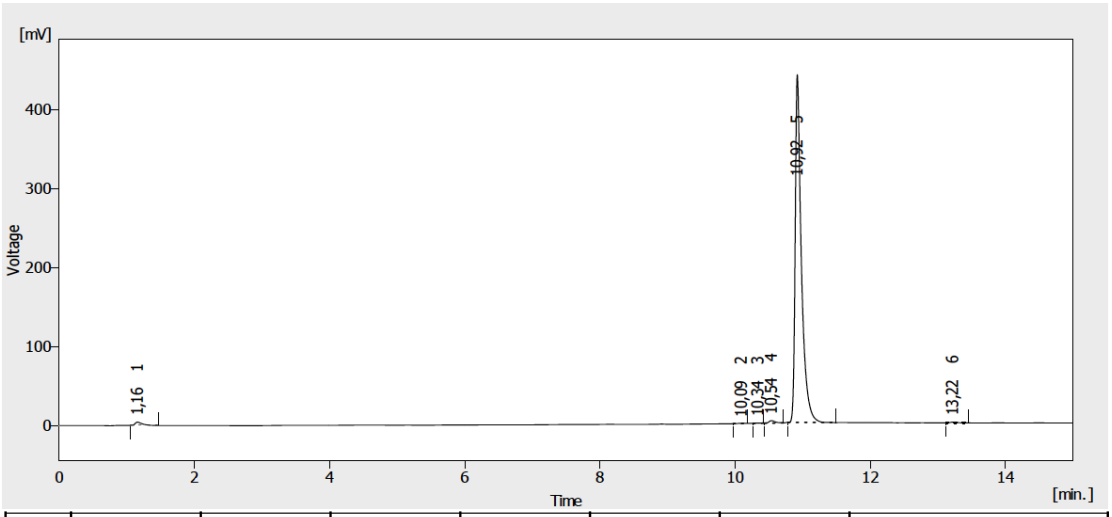


^{13}CNMR



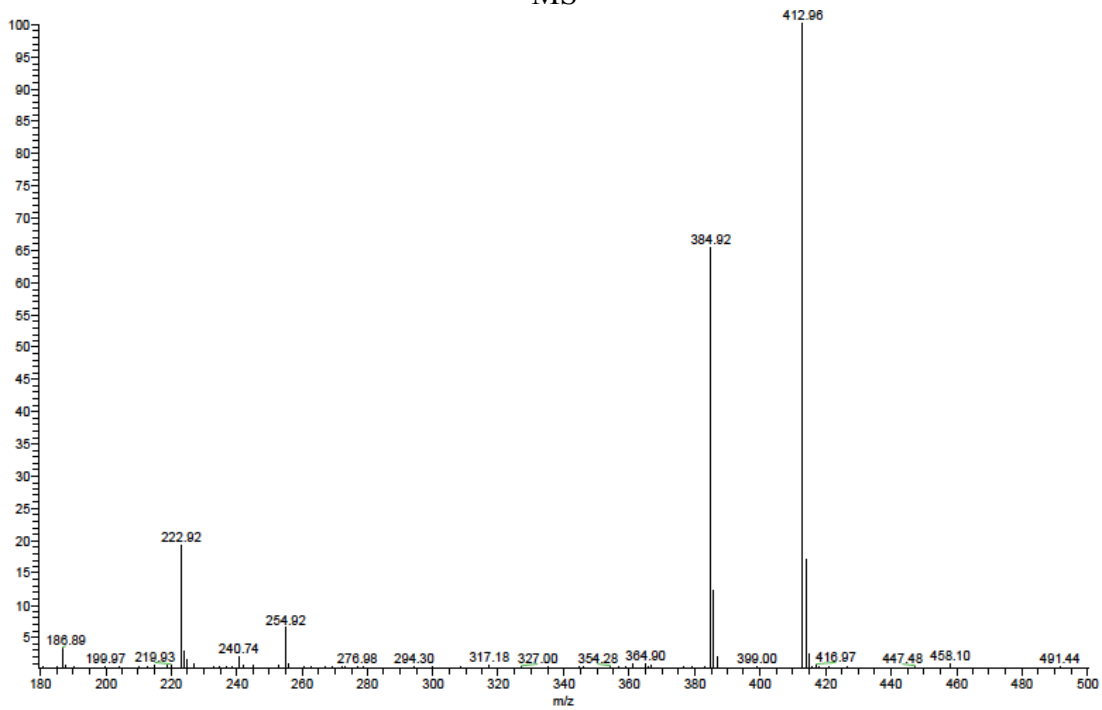
HPLC/UV

HPLC column: Waters Nova-Pak C18 60Å 4µm 3.9x150mm. **Detection wavelength**: 269 nm. **Eluents**: A = milliQ water; B = MeOH. **Gradient**: t = 0.0 min, 100% A; form 100% A to 100% B in 10.0 min; from t = 10.0 min to t = 14.0 min, 100% B; form 100% B to 100% A in 0.5 min; from t = 14.5 min to t = 15.0 min, 100% A.



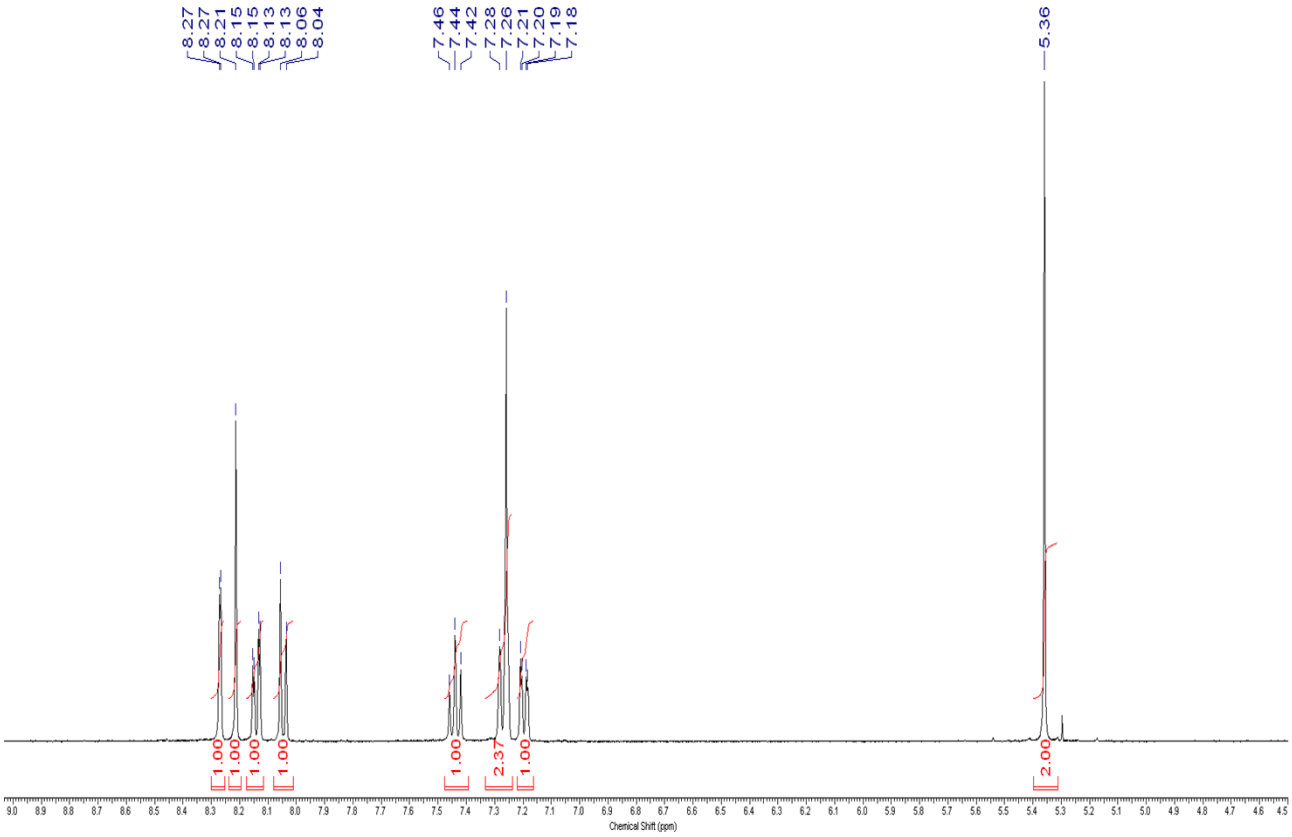
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1,160	31,487	4,183	1,1	0,9	0,11	
2	10,092	1,786	0,291	0,1	0,1	0,13	
3	10,340	1,636	0,352	0,1	0,1	0,08	
4	10,536	19,325	3,105	0,7	0,7	0,10	
5	10,920	2795,245	439,980	97,9	98,0	0,09	
6	13,220	6,191	0,942	0,2	0,2	0,10	
	Total	2855,670	448,853	100,0	100,0		

MS

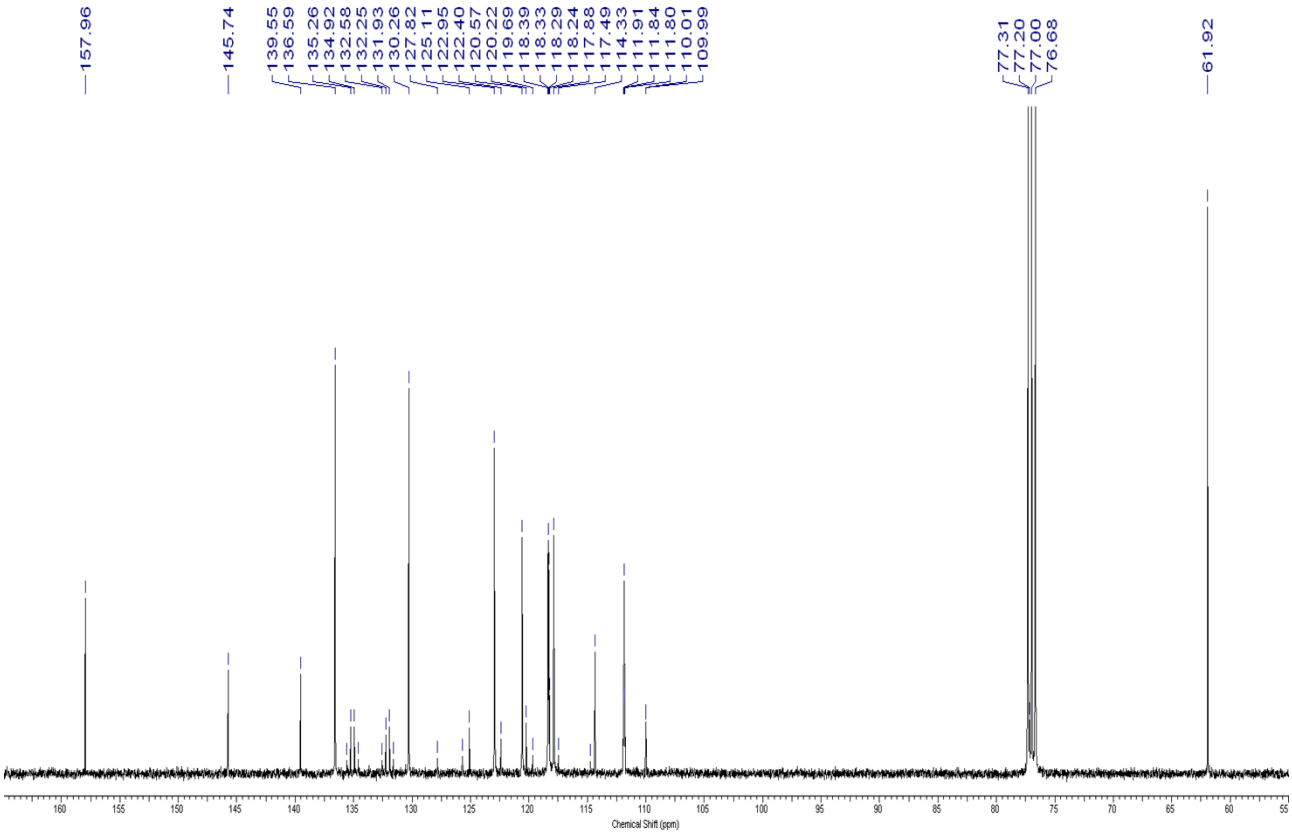


COMPOUND 30

^{1}HNMR

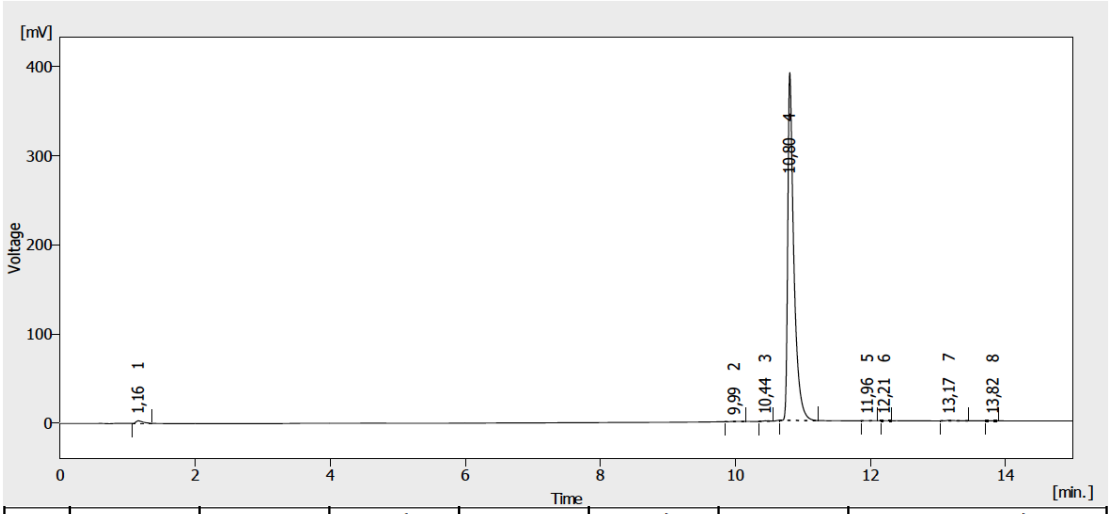


^{13}CNMR



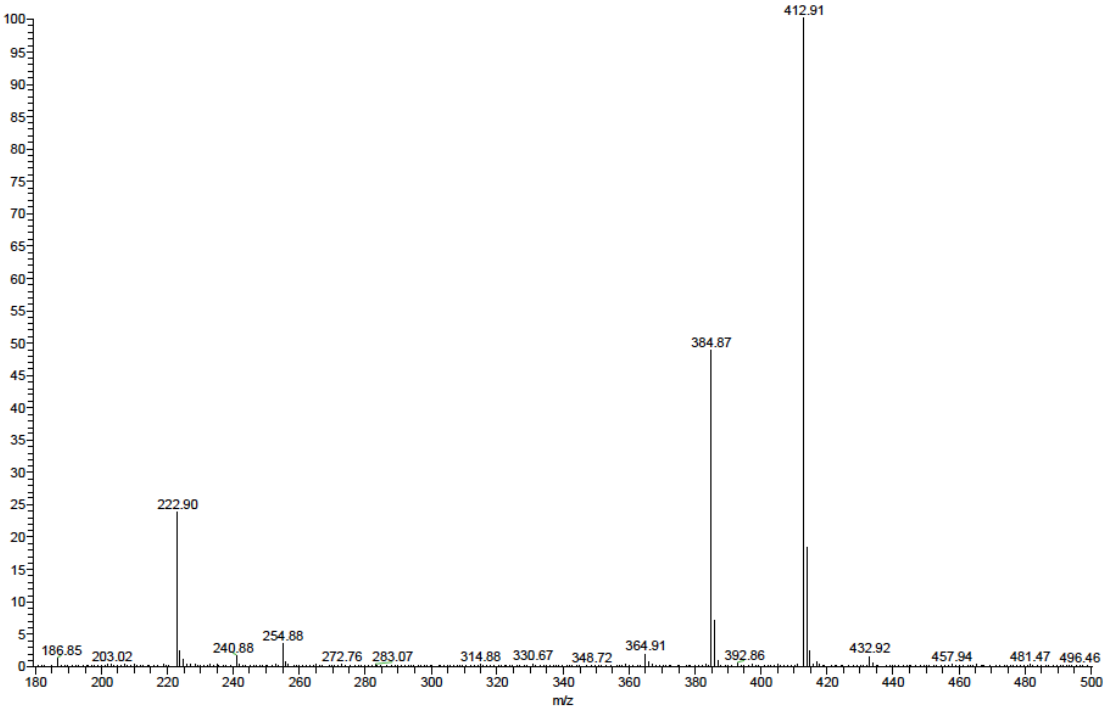
HPLC/UV

HPLC column: Waters Nova-Pak C18 60Å 4µm 3.9x150mm. **Detection wavelength**: 271 nm. **Eluents**: A = milliQ water; B = MeOH. **Gradient**: t = 0.0 min, 100% A; form 100% A to 100% B in 10.0 min; from t = 10.0 min to t = 14.0 min, 100% B; form 100% B to 100% A in 0.5 min; from t = 14.5 min to t = 15.0 min, 100% A.



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1,160	19,749	2,846	0,8	0,7	0,11	
2	9,988	2,347	0,292	0,1	0,1	0,21	
3	10,444	2,404	0,465	0,1	0,1	0,15	
4	10,804	2441,826	390,201	98,8	98,9	0,09	
5	11,960	0,500	0,063	0,0	0,0	0,15	
6	12,208	0,367	0,074	0,0	0,0	0,08	
7	13,168	4,540	0,625	0,2	0,2	0,10	
8	13,820	0,421	0,070	0,0	0,0	0,10	
	Total	2472,155	394,634	100,0	100,0		

MS



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