Supplementary information

A focused structure-activity relationship study of psoralen-based immunoproteasome inhibitors

Eva Shannon Schiffrer[1], Izidor Sosič[1], Andrej Šterman[1], Janez Mravljak[1], Irena Mlinarič Raščan[2], Stanislav Gobec[1], Martina Gobec[2,*]

[1] University of Ljubljana, Faculty of Pharmacy, Chair of Pharmaceutical Chemistry, Aškerčeva 7, 1000 Ljubljana

[2] University of Ljubljana, Faculty of Pharmacy, Chair of Clinical Biochemistry, Aškerčeva 7, 1000 Ljubljana

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1. General chemistry methods

Reagents and solvents were obtained from commercial sources (i.e., Acros Organics, Aldrich, TCI Europe, Merck, Alfa Aesar, Fluorochem). DPLG-3 was synthesized according to the literature procedure (E. S. Karreci, et al. Proc Natl Acad Sci USA, 2016, 113, E8425-E8432.) and as presented in Scheme S1. Solvents and chemicals were used as received. In specific cases, solvents were distilled before use. Reactions were monitored using analytical thin-layer chromatography plates (Merck 60 F254, 0.20 mm), and the components were visualized under UV light and/or through staining with the relevant reagent. Flash column chromatography was performed on Merck Silica Gel 60 (particle size 0.040-0.063 mm; Merck, Germany). Melting points were determined on a Reichelt hot-stage apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer at 295 K. The chemical shifts (δ) are reported in parts per million (ppm) and are referenced to the deuterated solvent used. The coupling constants (*J*) are given in Hz, and the splitting patterns are designated as follows: s, singlet; br s, broad singlet; d, doublet; dd, double doublet; t, triplet; app q, apparent quartet; dq, doublet of quartets; m, multiplet. Reactions using microwaves were performed on a standard monomode microwave reactor MONOWAVE 200 (Anton Paar). Mass spectra data and high-resolution mass measurements were performed on a VG-Analytical Autospec Q mass spectrometer at the Jožef Stefan Institute, Ljubljana, Slovenia, and on a Thermo Scientific Q Exactive Plus mass spectrometer at the Faculty of Pharmacy, University of Ljubljana. Elemental analyses were performed on a 240 C Perkin-Elmer C, H, N analyzer. Analytical normal-phase HPLC for the test compounds was performed on an Agilent 1100 LC modular system that was equipped with a photodiode array detector set to 254 nm. A Kromasil 3-CelluCoat column (150 × 4.6 mm) was used, with a flow rate of 1.0 mL/min and a sample injection volume of 10 µL. An eluent system of A (hexane) and B (isopropanol) was used according to the general method of: 0-18 min, 80% A). The purities of the test compounds used for the biological evaluations were ≥95%, as determined by HPLC.

2. Proteasome activity measurements

2.1 Residual activity of β5c, β2c, β1c, β2i, and β1i

The residual activity determination was performed at 10 µM final concentrations. To 50 µL of the desired compound, 25 µL 0.8 nM human iCP (Boston Biochem, Inc., Cambridge/MA, USA) was added. After 30 min of incubation at 37 °C, the reaction was initiated by the addition of the relevant flourogenic substrate: Suc-LLVY-AMC for β5c, Boc-LRR-AMC for β2i and β2c, Z-LLE-AMC for β1c (all from Bachem, Bubendorf, Switzerland), and Ac-PAL-AMC for β1i (Boston Biochem, Inc., Cambridge/MA, USA). The fluorescence was monitored at 460 nm (λ_{ex} = 360 nm) for 120 min at 37 °C. The initial linear ranges were used to calculate the velocity and to determine the residual activity.

2.2 Determination of IC_{50} values for inhibition of β5i

The final assay mixtures contained 0.2 nM human iCP (Boston Biochem, Inc., Cambridge/MA, USA) in assay buffer (0.01% SDS, 50 mM Tris-HCl, 0.5 mM EDTA, pH 7.4). Inhibitors were dissolved in DMSO and added to black 96-well plates for at least eight different concentrations (the final concentration of DMSO did not exceed 1%). After 30 min of incubation at 37 °C, the reaction was initiated by the addition of the Suc-LLVY-AMC (Bachem, Bubendorf, Switzerland). The fluorescence was monitored at 460 nm (λ_{ex} = 360 nm) for 120 min at 37 °C. The progress of the reactions was recorded and the initial linear ranges were used to calculate the velocity. IC_{50} values were calculated in Prism (GraphPad Software, CA, USA) and are means from at least three independent determinations.

2.3 Cell culture

HeLa cells (ATCC, LGC Standards, UK) were cultured in Dulbecco's modified Eagle's Medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin (all from Sigma-Aldrich). THP-1 cells (ATCC) were cultured in RPMI 1640 medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA), 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin and 50 µM 2-mercaptoethanol. Cells were maintained in a humidified chamber at 37 °C and 5% CO_2.

2.4 Inhibition of chymotrypsin-like (β5) activity in cell lysates

THP-1 or HeLa cells (5 ×10^6) were lysed with ice-cold 5 mM EDTA (pH 8.0). The suspensions were frozen at –80 °C for 15 min, thawed on ice, and this cycle was repeated three times. Centrifugation at 10,000× *g* for 10 min followed. Afterwards, the supernatants were collected and the protein concentrations were determined using the *DC*™ Protein Assay (BioRad Laboratories, CA, USA). Then 10 µg of extracted protein was incubated with different concentrations of the compound of interest in the assay buffer (50 mM Tris-HCl, 0.5 mM EDTA, pH 7.4) with 6 nM PA28α (Boston Biochem Inc., Cambridge/MA, USA) for 30 min at 37 °C. The reactions were initiated by adding 15 µM Ac-ANW-AMC (Boston Biochem) for β5i, or 100 µM Suc-LLVY-AMC for total β5 activities. The fluorescence was measured on a BioTek Synergy HT microplate reader for 90 min at 37 °C. IC_{50} values were calculated using Prism software (GraphPad Software) and are means from at least three independent determinations.

2.5 Inhibition of 26S chymotrypsin-like (β5) activity in intact cells

THP-1 and HeLa cells were plated in 96-well plates (2 ×10^5 cells/mL) and incubated with the compound of interest at the indicated concentrations for 2 h at 37 °C. The activity of the 26S proteasome activity was measured *in situ* by monitoring hydrolysis of the β5 substrate Suc-LLVY-aminoluciferin in the presence of luciferase using the Proteasome-Glo™ assay reagents (Promega, WI, USA), according to the manufacturer instructions. The luminescence was measured using a BioTek Synergy HT microplate reader. RA values are means from at least three independent determinations.

2.6 Cytokine secretion

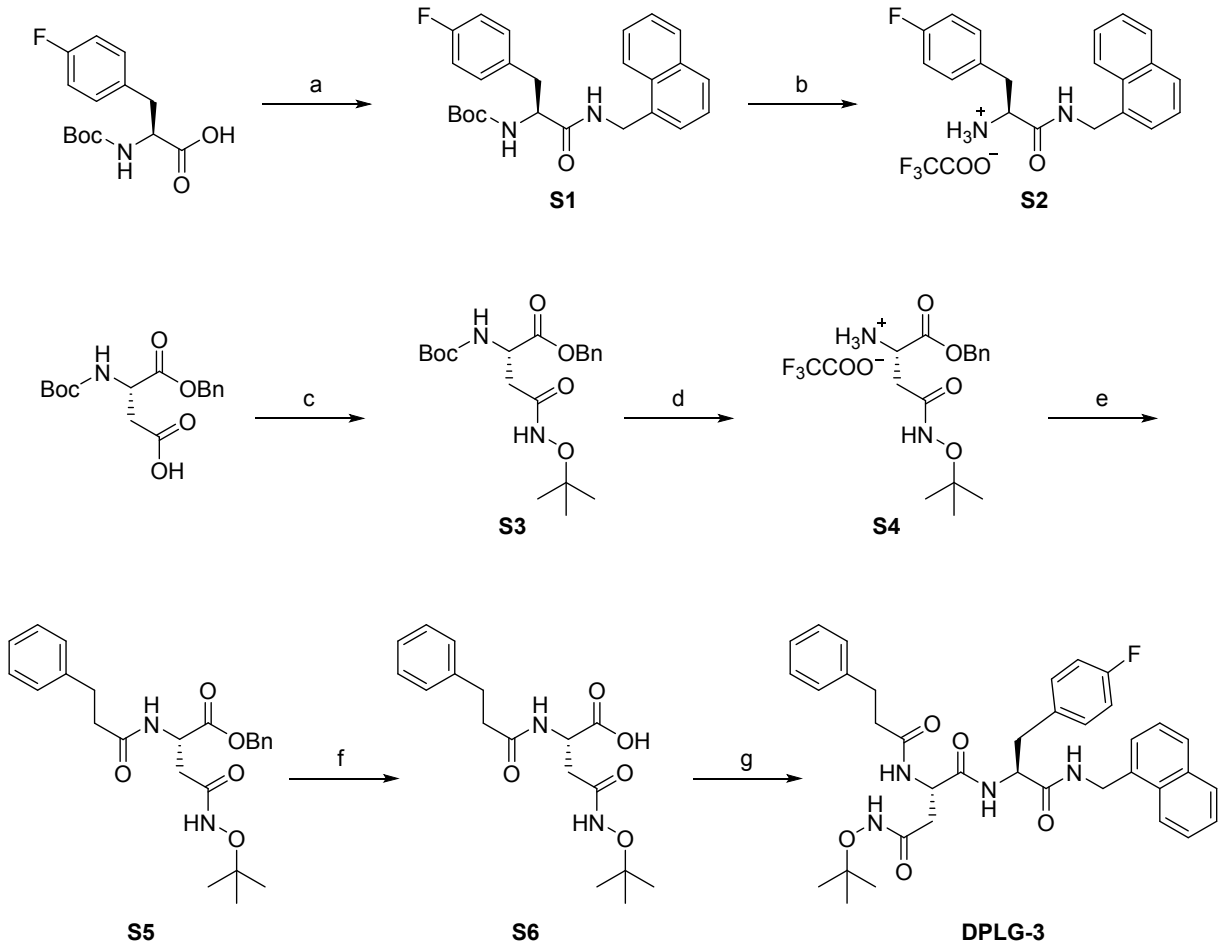
Human PBMC from healthy blood donors were isolated by density gradient centrifugation with Ficoll-Paque (Pharmacia, Sweden). The cells were cultured in RPMI 1640 (Sigma, Germany) supplemented with 100 U/mL penicillin (Sigma), 100 µg/mL streptomycin (Sigma), 2 mM L-glutamine (Sigma), 50 µM 2-mercaptoethanol (Sigma) and 10% heat-inactivated foetal bovine serum (Gibco, USA). 1×10^6 cells were plated on 48-well culture treated with selected compounds or medium alone for 1 h, followed by 24 h stimulation with 10 ng/mL LPS (E. coli O111:B4, Sigma). Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air. The cell-free supernatants were collected and the cytokine production was assessed by BD Cytometric Bead Array (CBA) Human Inflammatory Cytokine Kit (Content: IL-

6, IL-10, TNF-α, IL-8, IL12p70, IL-1β). Analysis was performed using Attune® NxT Acoustic Focusing Flow Cytometer (Thermo Fisher Scientific, USA). The data were analysed with FlowJo software (Tree Star, Inc., Ashland, OR). Results are expressed relative to LPS treated cells. The results are expressed as means of duplicates ± SEM of two independent experiments.

2.7 Rapid Dilution Assay

The iCP at 100-fold final concentration (20 nM) was incubated with inhibitors **42**, **44**, **47**, or **DPLG-3** at a concentration of 10-fold the IC_{50}, for 0 or 30 min at room temperature (volume, 2 µL). This mixture was diluted 100-fold with the substrate (25 µM Suc-LLVY-AMC) to a final volume of 200 µL. In the case of reversibility of the inhibitor, the recovery of the enzyme activity is expected to be more than 90%.

3. Supplementary scheme



Scheme S1: Synthesis of DPLG-3

Reagents and conditions: (a) 1-naphthylmethylamine, HATU, HOBt, DIPEA, DMF, 0 °C, 90 min; (b) TFA, CH_2Cl_2, 0 °C to rt, 20 h; (c) O-(*tert*-butyl)hydroxylammonium chloride, HATU, HOBt, DIPEA, DMF, 0 °C, 90 min; (d) TFA, CH_2Cl_2, 2 h; (e) 3-phenylpropionic acid, HATU, HOBt, DIPEA, DMF, 0 °C, 90 min, then rt 18 h; (f) H_2, Pd-C, MeOH, rt, 2 h; (g) **S2**, HATU, HOBt, DIPEA, DMF, 0 °C to rt, 16 h.

4. Supplementary tables

Table S1. Residual activities (%) of the other catalytically active subunits of iCP (β2i, β1i) and cCP (β5c, β2c and β1) in the presence of 10 µM compound.

Cpd	β2i	β1i	β5c	β2c	β1c
	Boc-LRR-AMC	Ac-PAL-AMC	Suc-LLVY-AMC	Boc-LRR-AMC	Z-LLE-AMC
38	69±6	54±1	84±9	74±3	82±3
39	117±3	66±3	97±5	77±5	101±4
40	39±12	60±1	90±8	53±2	87±4
41	60±6	47±4	82±16	66±4	83±3
42	105±5	102±8	69	72±13	77±9
43	21±7	96±9	84	27±1	87±11
44	76±5	116±10	100	40±1	89±16
45	29±5	44±6	77±9	67±7	102±1
46	37±10	21±9	72±7	57±1	83±9
47	67±7	108±3	82±5	68±11	90±3

Suc-LLVY-AMC, succinyl-Leu-Leu-Val-Tyr-7-amino-4-methylcoumarin; Boc-LRR-AMC, *t*-butyloxycarbonyl-Leu-Arg-Arg-7-amino-4-methylcoumarin; Ac-PAL-AMC, acetyl-Pro-Ala-Leu-7-amino-4-methylcoumarin; Z-LLE-AMC, benzyloxycarbonyl-Leu-Leu-Glu-7-amino-4-methylcoumarin.

Table S2. Cell-based IC_{50} or RA (%) values against β5 activity in THP-1 and HeLa cells and lysates. The provided data is from three independent biological repeats given as a mean value with S.E.M.

Compound	RA (%) THP-1 cells	RA (%) HeLa cells	IC_{50} THP-1 lysates (μM)	RA (%) HeLa lysates
44	74 ± 8	101 ± 7	1.53 ± 0.37	68 ± 4[a]
47	65 ± 10	98 ± 16	1.01 ± 0.07	99 ± 11[a]

[a] IC_{50} values could not be determined due to solubility problems, thus the RA (%) values are provided at 25 μM concentration for both compounds.

Table S3. Determination of the IC_{50} shift for compounds **42**, **44**, and **47** for the inhibition of β5i activity without or with 30 min pre-incubation.

Pre-incubation (min)	Compound IC_{50} (nM)[a]		
	42	**44**	**47**
0	1300±200	1500±150	2100±200
30	141±6	174±5	106±4

[a] IC_{50} values are means from at least three independent determinations.

Table S4. Determination of the recovery of β5i activity after 30 min incubation with 10-fold IC_{50} concentrations of **42**, **44**, **47**, and **DPLG-3** followed by a 100-fold dilution with substrate.

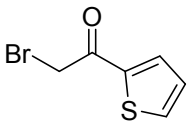
Compound	42	44	47	DPLG-3
Recovery (%)	29	32	22	93

Table S5. The effect of compounds **44**, **47**, **DPLG-3** and **PR-957** on the secretion of cytokines in PBMCs. The results present a relative value to LPS treated cells. The cells were pre-treated for 1 h with either **DPLG-3** (50 nM), **44** (10 μM), **47** (10 μM) or **PR-957** (50 nM). Afterwards, 10 ng/mL LPS was added and the concentrations of cytokines were determined in the supernatants after additional 24 h treatment. The results are expressed as means of duplicates ± SEM of at least three independent experiments.

Cytokine (% of LPS)	**Compound**			
	DPLG-3 (50 nM)	**44 (10 μM)**	**47 (10 μM)**	**PR-957 (50 nM)**
IL-12p70	101 ± 6	91 ± 0	98 ± 4	96 ± 6
TNFα	95 ± 11	93 ± 5	104 ± 19	72 ± 9
IL-10	73 ± 3	77 ± 8	65 ± 16	25 ± 7
IL-6	78 ± 9	79 ± 11	89 ± 2	62 ± 14
IL-1β	108 ± 3	123 ± 8	98 ± 19	510 ± 89
IL-8	99 ± 3	97 ± 9	95 ± 15	98 ± 13

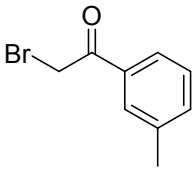
5. Spectroscopic analyses of remaining compounds

2-Bromo-1-(thiophen-2-yl)ethan-1-one 2



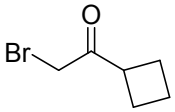
Brown oil; yield 100% (crude, unpurified product); ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.67 (s, 2H, C$\underline{H}_2$), 7.25 (t, J = 3.8 Hz, 1H, Ar-$\underline{H}$), 7.96 (dd, J_1 = 8.9 Hz, J_2 = 3.8 Hz, 1H, Ar-$\underline{H}$), 8.02 (dd, J = 8.9 Hz, 3.8 Hz, 1H, Ar-$\underline{H}$). HRMS (*m/z*) (ESI): calcd for C_6H_5BrOS $[M+H]^+$: 204.9323, found: 204.9326.

2-Bromo-1-(*m*-tolyl)ethan-1-one 3



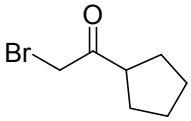
Green oil; yield 81% (crude, unpurified product); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.40 (s, 3H, C$\underline{H}_3$), 4.93 (s, 2H, C$\underline{H}_2$), 7.42-7.52 (m, 2H, Ar-H), 7.76-7.83 (m, 2H, Ar-$\underline{H}$). HRMS (*m/z*) (ESI): calcd for C_9H_9BrO $[M+H]^+$: 212.9915, found: 212.9913.

2-Bromo-1-cyclobutylethan-1-one 5



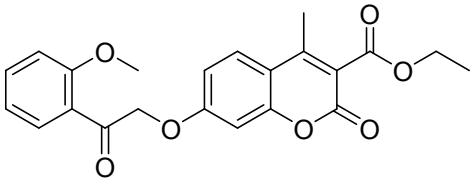
Brown liquid; yield 100 % (crude, unpurified product); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.50-1.92 (m, 4H, cyclobutyl-C$\underline{H}_2$), 2.20-2.30 (m, 2H, cyclobutyl-C$\underline{H}_2$), 2.92-3.00 (m, 1H, C$\underline{H}$), 4.45 (s, 2H, C$\underline{H}_2$).

2-Bromo-1-cyclopentylethan-1-one 6



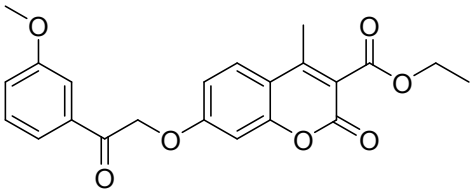
Yellow liquid; yield 87 % (crude, unpurified product); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.67-1.84 (m, 8H, cyclopentyl-C<u>H</u>$_2$), 2.30-2.35 (m, 1H, C<u>H</u>), 4.33 (s, 2H, C<u>H</u>$_2$).

Ethyl 7-(2-(2-methoxyphenyl)-2-oxoethoxy)-4-methyl-2-oxo-2H-chromene-3-carboxylate 8



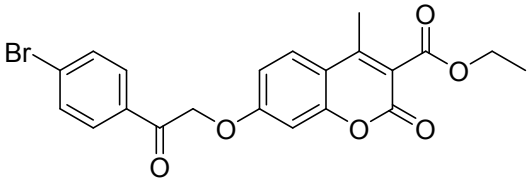
Off-white solid; yield 100%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30 (t, *J* = 7.1 Hz, 3H, CH$_2$C<u>H</u>$_3$), 2.43 (s, 3H, Ar-C<u>H</u>$_3$), 3.98 (s, 3H, OC<u>H</u>$_3$), 4.33 (q, *J* = 7.1 Hz, 2H, C<u>H</u>$_2$CH$_3$), 5.51 (s, 2H, C<u>H</u>$_2$), 7.01-7.05 (m, 2H, Ar-<u>H</u>), 7.09-7.12 (m, 1H, Ar-<u>H</u>), 7.27 (d, *J* = 8.2 Hz, 1H, Ar-<u>H</u>), 7.63-7.68 (m, 1H, Ar-<u>H</u>), 7.76 (dd, *J* = 7,7 Hz, 1,8 Hz, 1H, Ar-<u>H</u>), 7.81 (d, *J* = 8,9 Hz, 1H, Ar-<u>H</u>). HRMS (*m/z*) (ESI): calcd for $C_{22}H_{20}O_7$ [M+H]$^+$: 397.1287, found: 397.1281.

Ethyl 7-(2-(3-methoxyphenyl)-2-oxoethoxy)-4-methyl-2-oxo-2H-chromene-3-carboxylate 9



Orange solid; yield 74%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30 (t, *J* = 7.0 Hz, 3H, CH$_2$C<u>H</u>$_3$), 2.44 (s, 3H, Ar-C<u>H</u>$_3$), 3.85 (s, 3H, OC<u>H</u>$_3$), 4.33 (q, *J* = 7.0 Hz, 2H, C<u>H</u>$_2$CH$_3$), 5.79 (s, 2H, C<u>H</u>$_2$), 7.11 (dd, *J* = 8.9 Hz, 2.5 Hz, 1H, Ar-<u>H</u>), 7.16 (d, *J* = 2.5 Hz, 1H, Ar-<u>H</u>), 7.30 (ddd, *J* = 7.9 Hz, 2.5 Hz, 1.0 Hz, 1H, Ar-<u>H</u>), 7.50-7.54 (m, 2H, Ar-<u>H</u>), 7.64 (dt, *J* = 7.9 Hz, 1.0 Hz, 1H, Ar-<u>H</u>), 7.83 (d, *J* = 8.9 Hz, 1H, Ar-<u>H</u>). HRMS (*m/z*) (ESI): calcd for $C_{22}H_{20}O_7$ [M+H]$^+$: 397.1287, found: 397.1286.

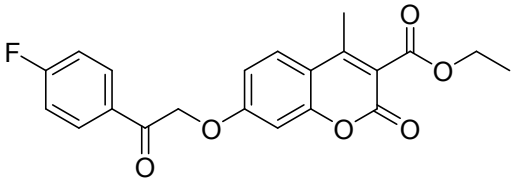
Ethyl 7-(2-(4-bromophenyl)-2-oxoethoxy)-4-methyl-2-oxo-2H-chromene-3-carboxylate 10



Yellow solid; yield 69%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30 (t, *J* = 7.1 Hz, 3H, CH$_2$C<u>H</u>$_3$), 2.44 (s, 3H, Ar-C<u>H</u>$_3$), 4.33 (q, *J* = 7.1 Hz, 2H, C<u>H</u>$_2$CH$_3$), 5.77 (s, 2H, C<u>H</u>$_2$), 7.11 (dd, *J* = 8.9 Hz, 2.7 Hz,

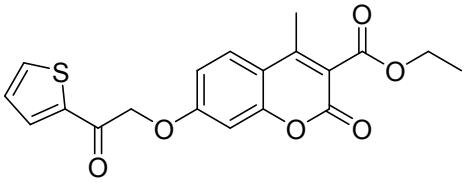
1H, Ar-H), 7.18 (d, *J* = 2.7 Hz, 1H, Ar-H), 7.82-7.84 (m, 3H, Ar-H), 7.98 (dt, *J* = 8.9 Hz, 1,9 Hz, 2H, Ar-H). HRMS (*m/z*) (ESI): calcd for $C_{21}H_{17}BrO_6$ $[M+H]^+$: 445.0287, found: 445.0285.

Ethyl 7-(2-(4-fluorophenyl)-2-oxoethoxy)-4-methyl-2-oxo-2H-chromene-3-carboxylate 11



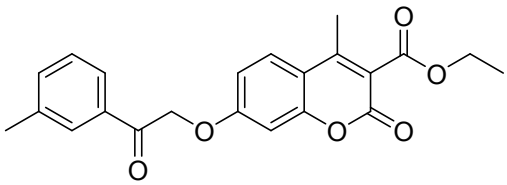
Off-white solid; yield 92%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30 (t, *J* = 7.1 Hz, 3H, CH_2CH_3), 2.44 (s, 3H, Ar-CH_3), 4.33 (q, *J* = 7.1 Hz, 2H, CH_2CH_3), 5.78 (s, 2H, CH_2), 7.11 (dd, *J* = 8.9 Hz, 2.5 Hz, 1H, Ar-H), 7.17 (d, *J* = 2.5 Hz, 1H, Ar-H), 7.42-7.47 (m, 2H, Ar-H), 7.83 (d, *J* = 8.9 Hz, 1H, Ar-H), 8.11-8.15 (m, 2H, Ar-H). HRMS (*m/z*) (ESI): calcd for $C_{21}H_{17}FO_6$ $[M+H]^+$: 385.1087, found: 385.1081.

Ethyl 4-methyl-2-oxo-7-(2-oxo-2-(thiophen-2-yl)ethoxy)-2H-chromene-3-carboxylate 12



Brown solid; yield 53%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30 (t, *J* = 7.1 Hz, 3H, CH_2CH_3), 2.44 (s, 3H, Ar-CH_3), 4.33 (q, *J* = 7.1 Hz, 2H, CH_2CH_3), 5.69 (s, 2H, CH_2), 7.09-7.16 (m, 2H, Ar-H), 7.35 (t, *J* = 4.2 Hz, 1H, Ar-H), 7.84 (d, *J* = 7.8 Hz, 1H, Ar-H), 8.15 (dd, *J* = 7.8 Hz, 4.2 Hz, 2H, Ar-H). HRMS (*m/z*) (ESI): calcd for $C_{19}H_{16}O_6S$ $[M+H]^+$: 373.0746, found: 373.0750.

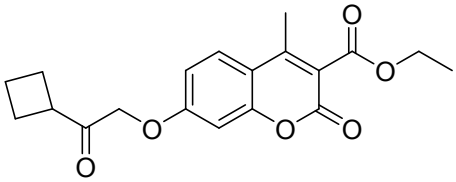
Ethyl 4-methyl-2-oxo-7-(2-oxo-2-(m-tolyl)ethoxy)-2H-chromene-3-carboxylate 13



Brown solid; yield 73%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30 (t, *J* = 7.2 Hz, 3H, CH_2CH_3), 2.42 (s, 3H, Ar-CH_3), 2.44 (s, 3H, Ar-CH_3), 4.33 (q, *J* = 7.2 Hz, 2H, CH_2CH_3), 5.78 (s, 2H, CH_2), 7.08-

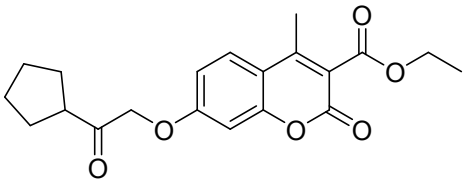
7.14 (m, 1H, Ar-H), 7.14-7.17 (m, 1H, Ar-H), 7.45-7.56 (m, 2H, Ar-H), 7.81-7.89 (m, 3H, Ar-H). HRMS (*m/z*) (ESI): calcd for $C_{22}H_{20}O_6$ $[M+H]^+$: 381.1338, found: 381.1345.

Ethyl 7-(2-cyclobutyl-2-oxoethoxy)-4-methyl-2-oxo-2H-chromene-3-carboxylate 15



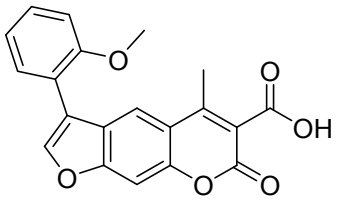
Brown oil; yield 77%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (t, *J* = 7.3 Hz, 3H, CH_2CH_3), 2.12-2.37 (m, 6H, cyclobutyl-CH_2), 2.45 (s, 3H, Ar-CH_3), 3.47-3.57 (m, 1H, cyclobutyl-CH), 4.42 (d, *J* = 7.3 Hz, 2H, CH_2CH_3), 4.67 (s, 2H, CH_2), 6.73 (d, *J* = 2.5 Hz, 1H, Ar-H), 6.91 (dd, *J* = 9.0 Hz, 2.5 Hz, 1H, Ar-H), 7.60 (d, *J* = 9.0 Hz, 1H, Ar-H). HRMS (*m/z*) (ESI): calcd for $C_{18}H_{18}O_6$ $[M+H]^+$: 345.1338, found: 345.1343.

Ethyl 7-(2-cyclopentyl-2-oxoethoxy)-4-methyl-2-oxo-2H-chromene-3-carboxylate 16



Brown oil; yield 52%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (t, *J* = 7.1 Hz, 3H, CH_2CH_3), 1.60-1.94 (m, 8H, cyclopentyl-CH_2), 2.45 (s, 3H, Ar-CH_3), 3.05-3.15 (m, 1H, cyclobutyl-CH), 4.42 (d, *J* = 7.1 Hz, 2H, CH_2CH_3), 4.75 (s, 2H, CH_2), 6.73 (d, *J* = 2.5 Hz, 1H, Ar-H), 6.93 (dd, *J* = 8.9 Hz, 2.5 Hz, 1H, Ar-H), 7.60 (d, *J* = 8.9 Hz, 1H, Ar-H). HRMS (*m/z*) (ESI): calcd for $C_{19}H_{20}O_6$ $[M+H]^+$: 359.1495, found: 359.1497.

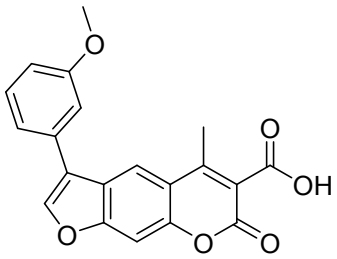
3-(2-Methoxyphenyl)-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-carboxylic acid 18



Brown solid; yield 100% (crude, unpurified product); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.39 (s, 3H, Ar-CH_3), 3.87 (s, 3H, OCH_3), 7.21-7.23 (m, 1H, Ar-H), 7.62-7.67 (m, 3H, Ar-H), 7.68 (s, 1H,

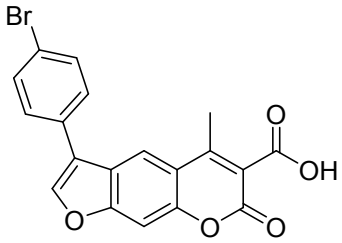
Ar-H), 7.87 (s, 1H, Ar-H), 8.28 (s, 1H, Ar-H). HRMS (*m/z*) (ESI): calcd for $C_{20}H_{14}O_6$ $[M+H]^+$: 351.0869, found: 351.0871.

3-(3-Methoxyphenyl)-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-carboxylic acid 19



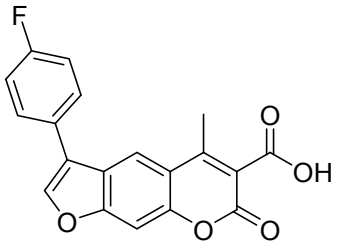
Orange solid; yield 100% (crude, unpurified product); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.44 (s, 3H, Ar-CH_3), 3.85 (s, 3H, OCH_3), 6.95-6.97 (m, 1H, Ar-H), 7.27-7.30 (m, 2H, Ar-H), 7.64-7.66 (m, 1H, Ar-H), 7.72 (s, 1H, Ar-H), 8.08 (s, 1H, Ar-H), 8.47 (s, 1H, Ar-H). HRMS (*m/z*) (ESI): calcd for $C_{20}H_{14}O_6$ $[M+H]^+$: 351.0869, found: 351.0865.

3-(4-Bromophenyl)-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-carboxylic acid 20



Orange solid; yield 100% (crude, unpurified product); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.48 (s, 3H, Ar-CH_3), 7.72-7.81 (m, 5H, Ar-H), 8.10 (s, 1H, Ar-H), 8.51 (s, 1H, Ar-H). HRMS (*m/z*) (ESI): calcd for $C_{19}H_{11}BrO_5$ $[M+H]^+$: 398.9868, found: 398.9865.

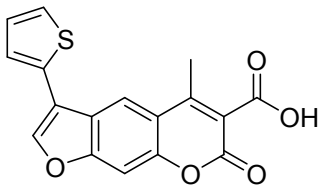
3-(4-Fluorophenyl)-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-carboxylic acid 21



Orange solid; yield 100% (crude, unpurified product); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.44 (s, 3H, Ar-CH_3), 7.37-7.41 (m, 2H, Ar-H), 7.72 (s, 1H, Ar-H), 7.86-7.89 (m, 2H, Ar-H), 8.06 (s, 1H,

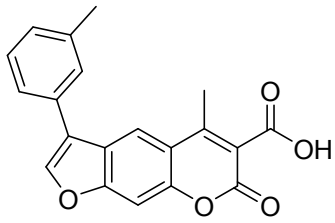
Ar-H), 8.45 (s, 1H, Ar-H). HRMS (*m/z*) (ESI): calcd for $C_{19}H_{11}FO_5$ $[M+H]^+$: 339.0669, found: 339.0662.

5-Methyl-7-oxo-3-(thiophen-2-yl)-7H-furo[3,2-g]chromene-6-carboxylic acid 22



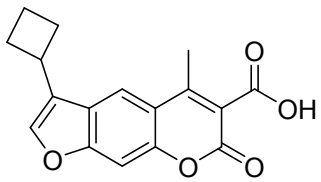
Brown oil; yield 100% (crude, unpurified product); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.30 (s, 3H, Ar-CH_3), 6.36-6.41 (m, 1H, Ar-H), 6.58 (s, 1H, Ar-H), 6.96-6.99 (m, 1H, Ar-H), 7.78 (s, 1H, Ar-H), 8.12-8.15 (m, 1H, Ar-H), 8.27 (s, 1H, Ar-H). HRMS (*m/z*) (ESI): calcd for $C_{17}H_9O_5S$ $[M+H]^-$: 325.01762, found: 325.01709.

5-Methyl-7-oxo-3-(m-tolyl)-7H-furo[3,2-g]chromene-6-carboxylic acid 23



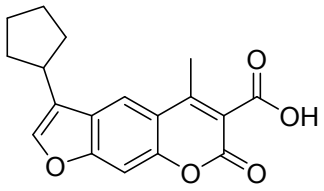
Brown oil; yield 100% (crude, unpurified product); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.34 (s, 3H, Ar-CH_3), 3.45 (s, 3H, Ar-CH_3), 7.58-7.66 (m, 3H, Ar-H), 7.88 (s, 1H, Ar-H), 8.14 (s, 1H, Ar-H), 8.26 (s, 1H, Ar-H), 8.51 (s, 1H, Ar-H). HRMS (*m/z*) (ESI): calcd for $C_{20}H_{14}O_5$ $[M+H]^+$: 335.0919, found: 335.0924.

3-Cyclobutyl-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-carboxylic acid 25



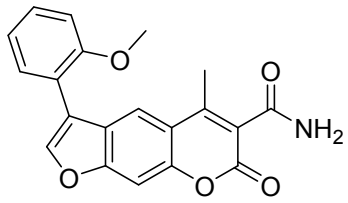
Brown oil; yield 55% (crude, unpurified product); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.29-2.36 (m, 4H, cyclobutyl-CH_2), 2.53 (s, 3H, Ar-CH_3), 2.69-2.78 (m, 2H, cyclobutyl-CH_2), 3.11-3.18 (m, 1H, cyclobutyl-CH), 7.21 (s, 1H, Ar-H), 7.43 (s, 1H, Ar-H), 7.66 (s, 1H, Ar-H). HRMS (*m/z*) (ESI): calcd for $C_{17}H_{14}O_5$ $[M+H]^+$: 299.0919, found: 299.0913.

3-Cyclopentyl-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-carboxylic acid 26



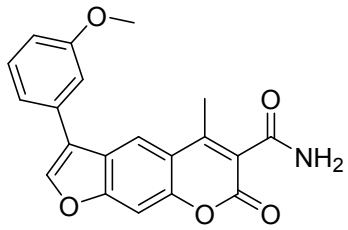
Orange solid; yield 79% (crude, unpurified product); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.51-1.86 (m, 6H, cyclopentyl-C<u>H</u>$_2$), 2.12-2.23 (m, 2H, cyclopentyl-C<u>H</u>$_2$), 2.43 (s, 3H, Ar-C<u>H</u>$_3$), 3.20-3.26 (m, 1H, cyclopentyl-C<u>H</u>), 7.70 (s, 1H, Ar-<u>H</u>), 7.95 (d, *J* = 1.1 Hz, 1H, Ar-<u>H</u>), 8.11 (s, 1H, Ar-<u>H</u>). HRMS (*m/z*) (ESI): calcd for $C_{18}H_{16}O_5$ $[M+H]^+$: 313.1076, found: 313.1084.

3-(2-Methoxyphenyl)-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-carboxamide 28



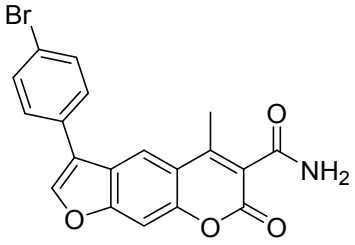
Yellow solid; yield 30%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.53 (s, 3H, Ar-C<u>H</u>$_3$), 3.87 (s, 3H, OC<u>H</u>$_3$), 7.01-7.02 (m, 1H, Ar-<u>H</u>), 7.21-7.25 (m, 1H, Ar-<u>H</u>), 7.61-7.64 (m, 2H, Ar-<u>H</u>), 7.80-7.82 (m, 2H, CON<u>H</u>$_2$), 7.83 (s, 1H, Ar-<u>H</u>), 8.03 (s, 1H, Ar-<u>H</u>), 8.34 (s, 1H, Ar-<u>H</u>). HRMS (*m/z*) (ESI): calcd for $C_{20}H_{15}NO_5$ $[M+H]^+$: 350.1028, found: 350.1023.

3-(3-Methoxyphenyl)-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-carboxamide 29



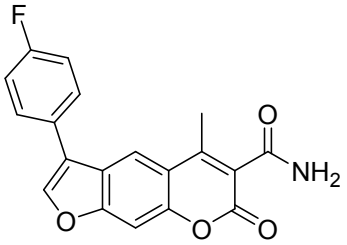
Off-white solid; yield 11%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.56 (s, 3H, Ar-C<u>H</u>$_3$), 3.86 (s, 3H, OC<u>H</u>$_3$), 7.34-7.35 (m, 2H, Ar-<u>H</u>), 7.68-7.71 (m, 3H, Ar-<u>H</u> and CON<u>H</u>$_2$), 7.87 (s, 1H, Ar-<u>H</u>), 7.90-7.91 (m, 1H, Ar-<u>H</u>), 8.24 (s, 1H, Ar-<u>H</u>), 8.54 (s, 1H, Ar-<u>H</u>). HRMS (*m/z*) (ESI): calcd for $C_{20}H_{15}NO_5$ $[M+H]^+$: 350.1028, found: 350.1021.

3-(4-Bromophenyl)-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-carboxamide 30



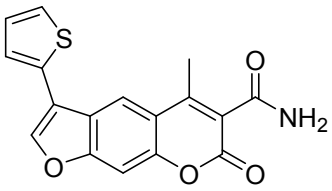
Off-white solid; yield 12%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.56 (s, 3H, Ar-C<u>H</u>$_3$), 7.75-7.76 (m, 2H, Ar-<u>H</u> and CON<u>H</u>$_2$), 7.81-7.83 (m, 3H, Ar-<u>H</u>), 7.88 (s, 1H, Ar-<u>H</u>), 7.96-8.00 (m, 1H, CON<u>H</u>$_2$), 8.24 (s, 1H, Ar-<u>H</u>), 8.58 (s, 1H, Ar-<u>H</u>). HRMS (*m/z*) (ESI): calcd for $C_{19}H_{12}BrNO_4$ $[M+H]^+$: 398.0028, found: 398.0027.

3-(4-Fluorophenyl)-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-carboxamide 31



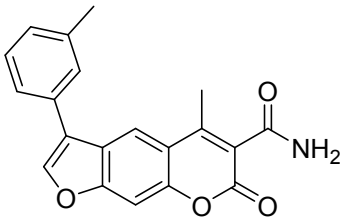
Off-white solid; yield 11%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.56 (s, 3H, Ar-C<u>H</u>$_3$), 7.37-7.44 (m, 3H, Ar-<u>H</u> or CON<u>H</u>$_2$), 7.87-7.91 (m, 4H, Ar-<u>H</u> or CON<u>H</u>$_2$), 8.23 (s, 1H, Ar-<u>H</u>), 8.52 (s, 1H, Ar-<u>H</u>). HRMS (*m/z*) (ESI): calcd for $C_{19}H_{12}FNO_4$ $[M+H]^+$: 338.0829, found: 338.0824.

5-Methyl-7-oxo-3-(thiophen-2-yl)-7H-furo[3,2-g]chromen-6-carboxamide 32



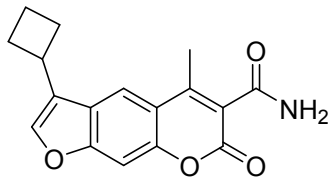
Off-white solid; yield 13%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.93 (s, 3H, Ar-C<u>H</u>$_3$), 7.09-7.12 (m, 1H, Ar-<u>H</u>), 7.20-7.23 (m, 1H, Ar-<u>H</u>), 7.37-7.44 (m, 1H, Ar-<u>H</u>), 7.52-7.55 (m, 3H, Ar-<u>H</u> and CON<u>H</u>$_2$), 7.93 (s, 1H, Ar-<u>H</u>), 8.30 (s, 1H, Ar-<u>H</u>). HRMS (*m/z*) (ESI): calcd for $C_{17}H_{11}NO_4S$ $[M+H]^+$: 326.0487, found: 326.0489.

5-Methyl-7-oxo-3-(m-tolyl)-7H-furo[3,2-g]chromen-6-carboxamide 33



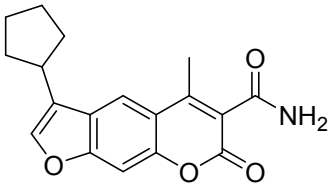
Orange solid; yield 13%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.47 (s, 3H, Ar-C<u>H</u>$_3$), 2.90 (s, 3H, Ar-C<u>H</u>$_3$), 7.33-7.35 (m, 2H, CON<u>H</u>$_2$), 7.42-7.46 (m, 2H, Ar-<u>H</u>), 7.53 (s, 1H, Ar-<u>H</u>), 7.57-7.61 (m, 2H, Ar-<u>H</u>), 7.84 (s, 1H, Ar-<u>H</u>), 8.19 (s, 1H, Ar-<u>H</u>). HRMS (*m/z*) (ESI): calcd for $C_{20}H_{15}NO_4$ $[M+H]^+$: 334.1079, found: 334.1086.

3-Cyclobutyl-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-carboxamide 35



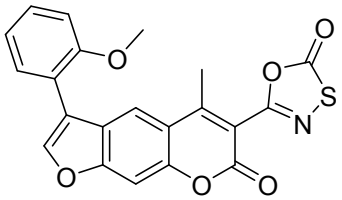
Yellow solid; yield 53%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79-0.91 (m, 2H, cyclobutyl-C<u>H</u>$_2$), 1.95-2.04 (m, 2H, cyclobutyl-C<u>H</u>$_2$), 2.15-2.25 (m, 2H, cyclobutyl-C<u>H</u>$_2$), 2.45-2.55 (m, 1H, cyclobutyl-C<u>H</u>), 2.91 (s, 3H, Ar-C<u>H</u>$_3$), 7.21-7.23 (m, 1H, CON<u>H</u>$_2$), 7.43 (s, 1H, Ar-<u>H</u>), 7.50-7.53 (m, 2H, Ar-<u>H</u> and CON<u>H</u>$_2$), 7.89 (s, 1H, Ar-<u>H</u>). HRMS (*m/z*) (ESI): calcd for $C_{17}H_{14}O_5$ $[M+H]^+$: 298.1079, found: 298.1080.

3-Cyclopentyl-5-methyl-7-oxo-7H-furo[3,2-g]chrome-6-carboxamide 36



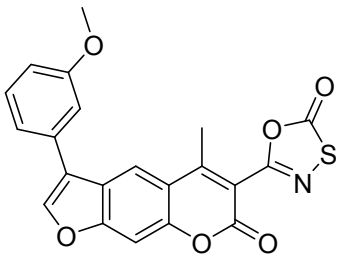
Off-white solid; yield 56%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.72-1.88 (m, 6H, cyclopentyl-C<u>H</u>$_2$), 2.17-2.25 (m, 2H, cyclopentyl-C<u>H</u>$_2$), 2.93 (s, 3H, Ar-C<u>H</u>$_3$), 3.15-3.24 (m, 1H, cyclopentyl-C<u>H</u>), 7.21-7.23 (m, 1H, CON<u>H</u>$_2$), 7.43 (s, 1H, Ar-<u>H</u>), 7.48 (d, *J* = 1.3 Hz, 1H, CON<u>H</u>$_2$), 7.52 (s, 1H, Ar-<u>H</u>), 7.98 (s, 1H, Ar-<u>H</u>). HRMS (*m/z*) (ESI): calcd for $C_{18}H_{17}NO_4$ $[M+H]^+$: 312.1236, found: 312.1232.

5-(3-(2-Methoxyphenyl)-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-yl)-1,3,4-oxathiazol-2-one 38



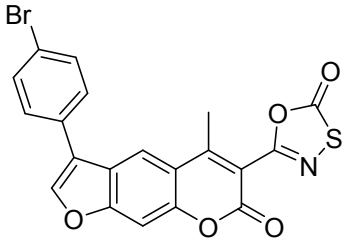
Yellow solid; yield 8%; mp 215.5-218.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.65 (s, 3H, Ar-C<u>H</u>$_3$), 3.92 (s, 3H, OC<u>H</u>$_3$), 7.11-7.18 (m, 2H, Ar-<u>H</u>), 7.47 (td, *J* = 7.9 Hz, 1.8 Hz, 1H, Ar-<u>H</u>), 7.57-7.59 (m, 2H, Ar-<u>H</u>), 7.99 (s, 1H, Ar-<u>H</u>), 8.05 (s, 1H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.77, 163.88, 163.74, 163.62, 162.99, 158.89, 146.60, 130.77, 130.24, 130.08, 130.03, 128.07, 121.16, 117.46, 116.11, 112.38, 111.50, 108.83, 105.97, 96.22, 46.80, 21.24. HRMS (*m/z*) (ESI): calcd for $C_{21}H_{13}NO_6S$ $[M+H]^+$: 408.0542, found: 408.0550. Purity by HPLC: 95%.

5-(3-(3-Methoxyphenyl)-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-yl)-1,3,4-oxathiazol-2-one 39



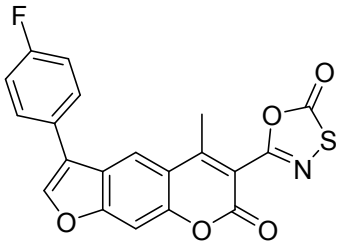
Yellow solid; yield 7%; mp 188.8-191.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.68 (s, 3H, Ar-C<u>H</u>$_3$), 3.92 (s, 3H, OC<u>H</u>$_3$), 7.02-7.04 (m, 1H, Ar-C<u>H</u>$_3$), 7.16-7.17 (m, 1H, Ar-<u>H</u>), 7.22-7.24 (m, 1H, Ar-<u>H</u>), 7.50 (t, *J* = 7.9 Hz, 1H, Ar-<u>H</u>), 7.60 (s, 1H, Ar-<u>H</u>), 7.91 (s, 1H, Ar-<u>H</u>), 8.15 (s, 1H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.09, 160.28, 159.36, 169.06, 149.94, 143.72, 136.75, 130.50, 124.98, 124.84, 124.73, 120.05, 117.75, 117.65, 116.06, 113.80, 113.33, 110.59, 100.63, 55.44, 17.19. HRMS (*m/z*) (ESI): calcd for $C_{21}H_{13}NO_6S$ $[M+H]^+$: 408.0542, found: 408.0541. Purity by HPLC: 100%.

5-(3-(4-Bromophenyl)-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-yl)-1,3,4-oxathiazol-2-one 40



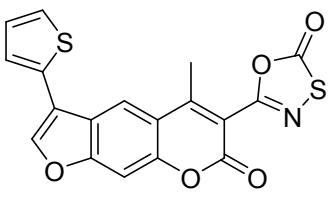
Yellow solid; yield 17%; mp 237.5-238.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.68 (s, 3H, Ar-C<u>H</u>$_3$), 7.50-7.54 (m, 2H, Ar-<u>H</u>), 7.61 (s, 1H, Ar-<u>H</u>), 7.69-7.72 (m, 2H, Ar-<u>H</u>), 7.91 (s, 1H, Ar-<u>H</u>), 8.08 (s, 1H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.85, 158.10, 155.99, 153.45, 151.53, 143.68, 132.64, 132.59 (2C), 129.42, 129.20 (2C), 124.65, 122.42, 121.53, 117.32, 115.72, 112.97, 100.74, 17.16. HRMS (*m/z*) (ESI): calcd for $C_{20}H_{10}BrNO_5S$ $[M+H]^+$: 455.9541, found: 455.9546. Purity by HPLC: 100%.

5-(3-(4-Fluorophenyl)-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-yl)-1,3,4-oxathiazol-2-one 41



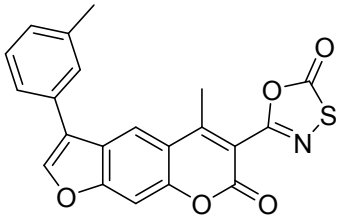
Off-white solid; yield 8%; mp 217.5-218.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.68 (s, 3H, Ar-C<u>H</u>$_3$), 7.25-7.30 (m, 2H, Ar-<u>H</u>), 7.60-7.63 (m, 3H, Ar-<u>H</u>), 7.87 (s, 1H, Ar-<u>H</u>), 8.08 (s, 1H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.88, 163.98, 158.07, 156.04, 153.48, 151.50, 143.50, 129.42 (2C), 129.34, 126.50, 124.95, 121.59, 117.31, 116.59 (2C), 116.38, 115.65, 100.69, 17.16. HRMS (*m/z*) (ESI): calcd for $C_{20}H_{10}FNO_5S$ $[M+H]^+$: 396.0342, found: 396.0340. Purity by HPLC: 98%.

5-(5-Methyl-7-oxo-3-(thiophen-2-yl)-7H-furo[3,2-g]chromen-6-yl)-1,3,4-oxathiazol-2-one 42



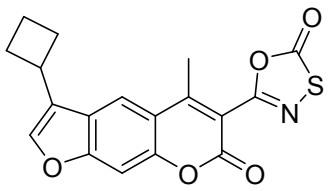
Yellow solid; yield 5%; mp 215.7-216.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.79 (s, 3H, Ar-C<u>H</u>$_3$), 7.26-7.29 (m, 1H, Ar-<u>H</u>), 7.67-7.70 (m, 1H, Ar-<u>H</u>), 7.74-7.76 (m, 1H, Ar-<u>H</u>), 7.96 (s, 1H, Ar-<u>H</u>), 8.40 (s, 1H, Ar-<u>H</u>), 8.62 (s, 1H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.68, 165.73, 165.14, 156.93, 153.15, 146.15, 139.77, 138.41, 133.67, 127.60, 127.56, 126.54, 123.05, 122.88, 122.75, 110.79, 92.99, 24.50. HRMS (*m/z*) (ESI): calcd for $C_{18}H_{10}NO_5S_2$ $[M+H]^+$: 383.99949, found: 383.99899. Purity by HPLC: 97%.

5-(5-Methyl-7-oxo-3-(m-tolyl)-7H-furo[3,2-g]chromen-6-yl)-1,3,4-oxathiazol-2-one 43



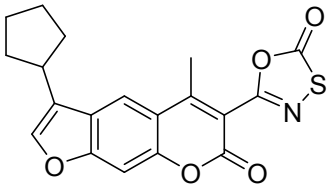
Yellow solid; yield 15%; mp 64.5-65.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.47 (s, 3H, Ar-C<u>H</u>$_3$), 2.65 (s, 3H, Ar-C<u>H</u>$_3$), 7.40-7.46 (m, 3H, Ar-<u>H</u>), 7.57 (s, 1H, Ar-<u>H</u>), 7.86 (s, 1H, Ar-<u>H</u>), 8.12 (s, 1H, Ar-<u>H</u>), one aromatic proton overlapping with solvent. ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.35, 158.17, 157.50, 156.22, 151.44, 143.57, 139.20, 130.42, 129.33, 129.29, 129.18, 128.46, 125.11, 124.78, 122.56, 119.71, 117.64, 115.55, 100.59, 21.60, 17.18. HRMS (*m/z*) (ESI): calcd for $C_{21}H_{14}NO_5S$ $[M+H]^+$: 392.05872, found: 392.05805. Purity by HPLC: 100%.

5-(3-Cyclobutyl-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-yl)-1,3,4-oxathiazol-2-one 45



Yellow solid; yield 6%; mp 137.7-138.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98-2.07 (m, 1H, cyclobutyl-C<u>H</u>$_2$), 2.15-2.26 (m, 3H, cyclobutyl-C<u>H</u>$_2$), 2.47-2.55 (m, 2H, cyclobutyl-C<u>H</u>$_2$), 2.65 (s, 3H, Ar-C<u>H</u>$_3$), 3.64-3.71 (m, 1H, cyclobutyl-C<u>H</u>), 7.47 (s, 1H, Ar-<u>H</u>), 7.52 (d, *J* = 1.4 Hz, 1H, Ar-<u>H</u>), 7.81 (s, 1H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.97, 158.15, 157.92, 156.24, 153.66, 151.34, 142.91, 125.97, 125.36, 116.87, 114.86, 112.37, 100.26, 30.11, 28.76 (2C), 19.31, 17.10. HRMS (*m/z*) (ESI): calcd for $C_{18}H_{14}NO_5S$ $[M+H]^+$: 356.05872, found: 356.05797. Purity by HPLC: 94%.

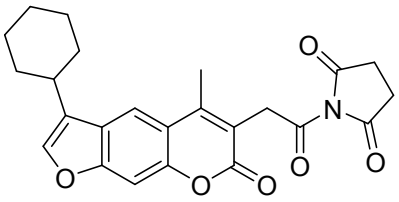
5-(3-Cyclopentyl-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-yl)-1,3,4-oxathiazol-2-one 46



Off-white solid; yield 6%; mp 134.0-135.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60-1.82 (m, 6H, cyclopentyl-C<u>H</u>$_2$), 2.14-2.23 (m, 2H, cyclopentyl-C<u>H</u>$_2$), 2.74 (s, 3H, Ar-C<u>H</u>$_3$), 3.22-3.28 (m, 1H, cyclopentyl-C<u>H</u>), 7.79 (s, 1H, Ar-<u>H</u>), 8.00 (d, *J* = 1.2 Hz,1H, Ar-<u>H</u>), 8.24 (s, 1H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.97, 158.22, 157.93, 156.26, 153.68, 151.27, 142.42, 126.60, 125.08, 117.16, 114.81, 112.36, 100.22, 35.20, 32.40 (2C), 25.06 (2C), 17.12. HRMS (*m/z*) (ESI): calcd for $C_{19}H_{16}NO_5S$ $[M+H]^+$: 370.07437, found: 370.07361. Purity by HPLC: 95%.

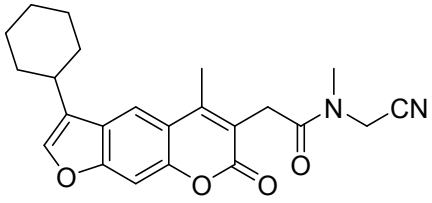
Spectroscopic analyses of compounds 48-53 were reported previously.[1]

2,5-Dioxopyrrolidin-1-yl 2-(3-cyclohexyl-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-yl)acetate 54



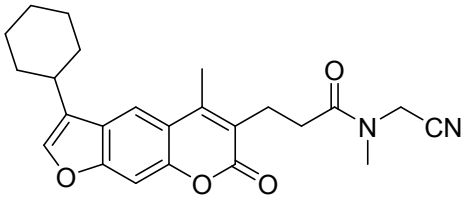
White solid; yield 34%; mp 161.0-162.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.22-1.30 (m, 1H, cyclohexyl-<u>H</u>), 1.37-1.50 (m, 4H, cyclohexyl-<u>H</u>), 1.70-1.77 (m, 1H, cyclohexyl-<u>H</u>), 1.78-1.83 (m, 2H, cyclohexyl-<u>H</u>), 2.01-2.09 (m, 2H, cyclohexyl-<u>H</u>), 2.61 (s, 3H, Ar-C<u>H</u>$_3$), 2.80 (rs, 4H, COC<u>H</u>$_2$C<u>H</u>$_2$CO), 2.81-2.90 (m, 1H, cyclohexyl-<u>H</u>), 4.14 (s, 2H, C<u>H</u>2COO), 7.71 (s, 1H, Ar-<u>H</u>), 7.88 (s, 1H, Ar-<u>H</u>), 8.15 (s, 1H, Ar-<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.02, 166.38, 160.41, 155.88, 151.42, 149.78, 142.65, 126.27, 124.72, 116.85, 115.66, 115.10, 99.26, 33.01, 32.48, 29.77, 25.97, 25.68, 25.37, 15.84. HRMS (*m/z*) (ESI): calcd for $C_{24}H_{24}NO_7$ $[M+H]^+$: 438.1553, found: 438.1548. Elemental analysis: found: C, 65.48; H, 5.14; N, 3.21 calc. for $C_{24}H_{23}NO_7$: C, 65.90; H, 5.30; N, 3.20%.

***N*-(Cyanomethyl)-2-(3-cyclohexyl-5-methyl-7-oxo-7*H*-furo[3,2-*g*]chromen-6-yl)-N-methylacetamide 56**



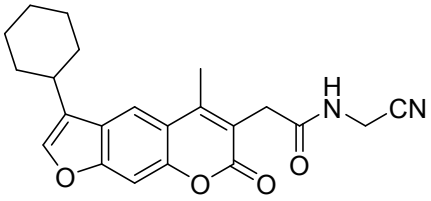
Yellow-white solid; yield 29%; mp 177.2-179.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22-1.34 (m, 1H, cyclohexyl-H), 1.37-1.52 (m, 4H, cyclohexyl-H), 1.70-1.86 (m, 3H, cyclohexyl-H), 2.02-2.10 (m, 2H, cyclohexyl-H), 2.49 (s, 3H, Ar-CH_3), 2.72-2.82 (m, 1H, cyclohexyl-H), 3.35 (s, 3H, NCH_3), 3.81 (s, 2H, CH_2CO), 4.39 (s, 2H, CH_2CN), 7.26 (s, 1H, Ar-H), 7.42 (s, 1H, Ar-H), 7.79 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.88, 162.04, 159.73, 156.57, 150.44, 141.71, 126.47, 125.18, 117.64, 116.36, 115.54, 115.31, 99.70, 35.71, 35.62, 34.13, 33.07 (2C), 31.82, 26.51 (2C), 26.25, 16.38. HRMS (*m/z*) (ESI): calcd for $C_{23}H_{25}N_2O_4$ $[M+H]^+$: 392.1745, found: 392.1741. Purity by HPLC: 93%.

***N*-(Cyanomethyl)-3-(3-cyclohexyl-5-methyl-7-oxo-7*H*-furo[3,2-*g*]chromen-6-yl)-*N*-methylpropanamide 57**



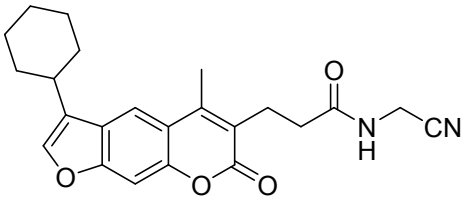
Yellow-brown solid; yield 24%; mp 77.3-78.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43-1.51 (m, 4H, cyclohexyl-H), 1.77-1.92 (m, 4H, cyclohexyl-H), 2.07-2.15 (m, 2H, cyclohexyl-H), 2.61 (s, 3H, Ar-CH_3), 2.70 (t, J = 7.2 Hz, 2H, CH_2CH_2CO), 2.73-2.80 (m, 1H, cyclohexyl-H), 3.03 (t, J = 7.2 Hz, 2H, CH_2CH_2CO), 3.17 (s, 3H, NCH_3), 4.35 (s, 2H, CH_2CN), 7.40 (s, 1H, Ar-H), 7.41 (s, 1H, Ar-H), 7.77 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.24, 173.32, 173.04, 170.62, 157.77, 149.37, 142.73, 139.06, 126.46, 115.62, 112.92, 110.87, 103.89, 47.67, 46.56, 43.94, 41.86, 33.07 (2C), 29.72, 26.49 (2C), 26.23, 15.66. HRMS (*m/z*) (ESI): calcd for $C_{24}H_{27}N_2O_4$ $[M+H]^+$: 406.1895, found: 406.1894 . Purity by HPLC: 95%.

***N*-(Cyanomethyl)-2-(3-cyclohexyl-5-methyl-7-oxo-7*H*-furo[3,2-*g*]chromen-6-yl)acetamide 58**



Yellow-brown solid; yield 21%; mp 204.9-206.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22-1.34 (m, 1H, cyclohexyl-H), 1.40-1.52 (m, 4H, cyclohexyl-H), 1.70-1.75 (m, 1H, cyclohexyl-H), 1.78-1.91 (m, 2H, cyclohexyl-H), 2.08-2.13 (m, 2H, cyclohexyl-H), 2.69 (s, 3H, Ar-CH_3), 2.72-2.80 (m, 1H, cyclohexyl-H), 3.69 (s, 2H, CH_2CO), 4.13 (d, J = 5.8 Hz, 2H, NHCH_2), 7.08 (t, J = 5.8 Hz, 1H, NHCH_2), 7.44 (s, 2H, Ar-H), 7.81 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.77, 163.31, 156.70, 151.19, 150.02, 142.00, 126.46, 125.60, 116.89, 116.14, 115.84, 115.78, 99.77, 35.64, 34.06, 33.02, 27.50, 26.44, 26.18, 16.21. HRMS (*m/z*) (ESI): calcd for $C_{22}H_{23}N_2O_4$ $[M+H]^+$: 379.1658, found: 379.1661. Purity by HPLC: 95%.

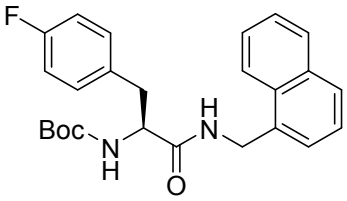
N-(Cyanomethyl)-3-(3-cyclohexyl-5-methyl-7-oxo-7H-furo[3,2-g]chromen-6-yl)propanamide 59



Yellow-brown solid; yield 26%; mp 119.8-123.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21-1.28 (m, 4H, cyclohexyl-H), 1.88-1.95 (m, 4H, cyclohexyl-H), 2.02-2.15 (m, 2H, cyclohexyl-H), 2.61 (s, 3H, Ar-CH_3), 2.62 (t, *J* = 7.4 Hz, 2H, CH_2CH_2CO), 2.77 (sim m, 1H, cyclohexyl-H), 3.05 (t, *J* = 7.4 Hz, 2H, CH_2CH_2CO), 4.15 (d, *J* = 5.8 Hz, 2H, NHCH_2), 6.68 (t, *J* = 5.8 Hz, 1H, NHCH_2), 7.32 (s, 1H, Ar-H), 7.38 (s, 1H, Ar-H), 7.77 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.72, 162.78, 156.14, 149.78, 149.53, 141.54, 126.36, 125.21, 121.65, 116.26, 116.21, 115.56, 99.14, 34.45, 34.01, 33.01, 27.41, 26.45, 26.21, 24.01, 15.77. HRMS (*m/z*) (ESI): calcd for $C_{23}H_{25}N_2O_4$ $[M+H]^+$: 392.1741, found: 392.1740. Purity by HPLC: 94%.

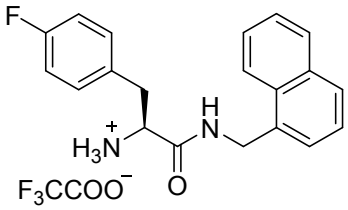
Synthesis of DPLG-3

***tert*-Butyl-(*S*)-(3-(4-fluorophenyl)-1-((naphthalen-1-ylmethyl)amino)-1-oxopropan-2-yl)carbamate (S1)**



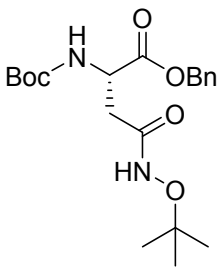
White solid. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 1.30 (s, 9H, C(C$\underline{H}_3$)$_3$), 2.77 (dd, *J* = 14,0 Hz, 10,1 Hz, 1H, Ar-C$\underline{H}_a$), 2.93 (dd, *J* = 13,6 Hz, 5,1 Hz, 1H, Ar-C$\underline{H}_b$), 4.15 – 4.24 (m, 1H, C$\underline{H}$), 4.74 (d, *J* = 5,5 Hz, 2H, C$\underline{H}_2$NH), 7.01 – 7.09 (m, 3H, Ar-$\underline{H}$), 7.24 – 7.29 (m, 2H, Ar-$\underline{H}$), 7.35 – 7.38 (m, 1H, Ar-$\underline{H}$), 7.40 – 7.45 (m, 1H, Ar-$\underline{H}$), 7.52 – 7.56 (m, 2H, Ar-$\underline{H}$), 7.85 (d, *J* = 8,0 Hz, 1H, Boc-N$\underline{H}$), 7.94 (dd, *J* = 6,2 Hz, 3,4 Hz, 1H, Ar-$\underline{H}$), 8.03 (dd, *J* = 6,3 Hz, 3,4 Hz, 1H, Ar-$\underline{H}$), 8.48 (t, *J* = 5,6 Hz, 1H, CON$\underline{H}$).

(*S*)-3-(4-Fluorophenyl)-1-((naphthalen-1-ylmethyl)amino)-1-oxopropan-2-aminium 2,2,2-trifluoroacetate (S2)



White solid. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 2.95 – 3.06 (m, 2H, C$\underline{H}_2$NH), 4.03 (t, *J* = 7,0 Hz, 1H, C$\underline{H}$), 4.67 (dd, *J* = 15,1, 5,1 Hz, 1H, Ar-C$\underline{H}_a$), 4.80 (dd, *J* = 15,1, 5,8 Hz, 1H, Ar-C$\underline{H}_b$), 7.03 – 7.09 (m, 2H Ar-$\underline{H}$), 7.17 – 7.22 (m, 2H, Ar-$\underline{H}$), 7.27 (d, *J* = 6,8 Hz, 1H, Ar-$\underline{H}$), 7.43 (dd, *J* = 8,5 Hz, 7,0 Hz, 1H, Ar-$\underline{H}$), 7.53 – 7.58 (m, 2H, Ar-$\underline{H}$), 7.88 (d, *J* = 8,3 Hz, 1H, Ar-$\underline{H}$), 7.93 – 7.99 (m, 2H, Ar-$\underline{H}$), 8,30 (bs, 3H, N$\underline{H}_3^+$), 8,90 (t, , *J* = 5,5 Hz, 1H, CON$\underline{H}$).

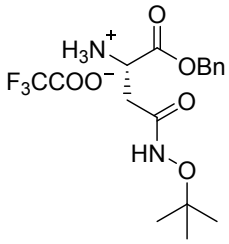
Benzyl *N*^{4}-(*tert*-butoxy)-*N*^{2}-(*tert*-butoxycarbonyl)-L-asparaginate (S3)



Colourless oil. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 1.13 (s, 9H, C(C$\underline{H}_3$)$_3$), 1.36 (s, 9H, C(C$\underline{H}_3$)$_3$), 2.40 (dd, *J* = 15,1 Hz, 8,0 Hz, 1H, COC$\underline{H}_a$), 2.55 (dd, *J* = 14,8 Hz, 5,9 Hz, 1H, COC$\underline{H}_b$), 4.36 – 4.43 (m,

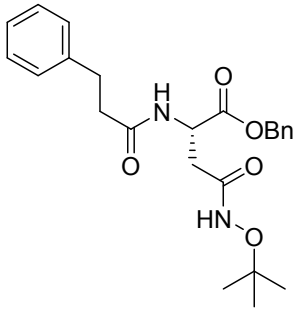
1H, C<u>H</u>), 5.11 (s, 2H, Ar-C<u>H</u>$_2$), 7.23 (d, *J* = 8,3 Hz, 1H, Boc-N<u>H</u>), 7.30 – 7.39 (m, 5H, Ar-<u>H</u>), 10.34 (s, 1H, CON<u>H</u>O).

(*S*)-1-(Benzyloxy)-4-(*tert*-butoxyamino)-1,4-dioxobutan-2-aminium 2,2,2-trifluoroacetate (S4)



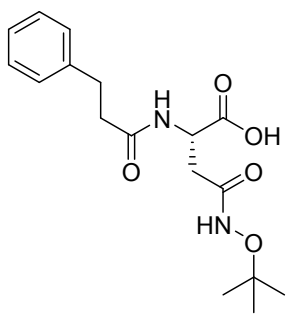
White solid. The compound was used in the next step without further characterisation.

Benzyl *N*^{4}-(*tert*-butoxy)-*N*^{2}-(3-phenylpropanoyl)-L-asparaginate (S5)



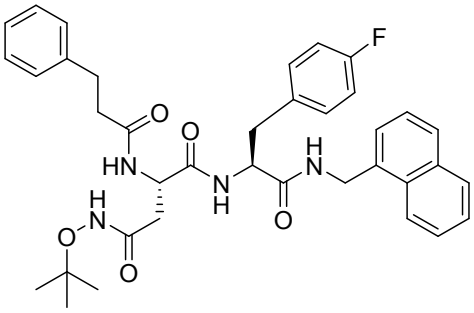
White solid. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 1.12 (s, 9H, C(C<u>H</u>$_3$)$_3$), 2.37 – 2.44 (m, 3H, overlapping COC<u>H</u>$_a$ and C<u>H</u>$_2$CH$_2$), 2.57 (dd, *J* = 15.0, 6.2 Hz, 1H, COC<u>H</u>$_b$), 2.77 (t, *J* = 8,0 Hz, 2H, C<u>H</u>$_2$CH$_2$), 4.61 - 4.68 (m, 0.9H, C<u>H</u> (major)), 4.75 - 4.81 (m, 0.1H, C<u>H</u> (minor)), 5.10 (s, 2H, Ar-C<u>H</u>$_2$), 7.14 – 7.20 (m, 3H, Ar-<u>H</u>), 7.22 - 7.28 (m, 2H, Ar-<u>H</u>), 7.38 - 7.31 (m, 5H, Ar-<u>H</u>), 8.30 (m, 0.1H, CON<u>H</u> (minor)), 8.39 (d, *J* = 7,9 Hz, 0.9H, CON<u>H</u> (major)), 10.16 (s, 0.1H, CON<u>H</u>O (minor)), 10.38 (s, 0.9H, CON<u>H</u>O (major)).

***N*^{4}-(*tert*-butoxy)-*N*^{2}-(3-phenylpropanoyl)-L-asparagine (S6)**



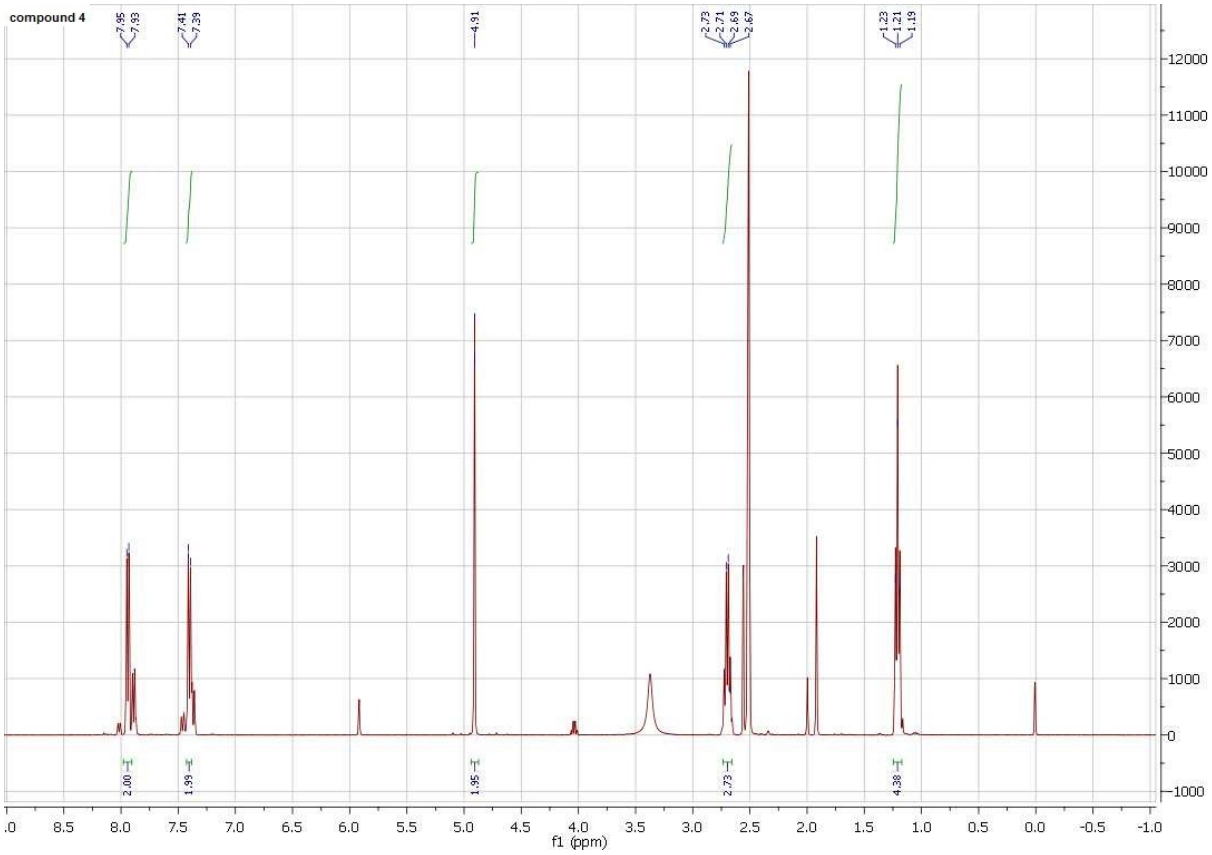
Off-white solid. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 1.13 (s, 9H, C(C<u>H</u>$_3$)$_3$), 2.30 – 2.42 (m, 3H, overlapping COC<u>H</u>$_a$ and C<u>H</u>$_2$CH$_2$), 2.51 – 2.54 (m, 1H, COC<u>H</u>$_b$, partially overlapping with residual solvent signal), 2.75 – 2.81 (m, 2H, C<u>H</u>$_2$), 4.48 – 4.55 (m, 1H, C<u>H</u>), 7.14 – 7.21 (m, 3H, Ar-<u>H</u>), 7.23 – 7.29 (m, 2H, Ar-<u>H</u>), 8.18 (d, *J* = 8.0 Hz, 1H, CON<u>H</u>), 10.35 (s, 1H, CON<u>H</u>O), 12.73 (bs, 1H, COO<u>H</u>).

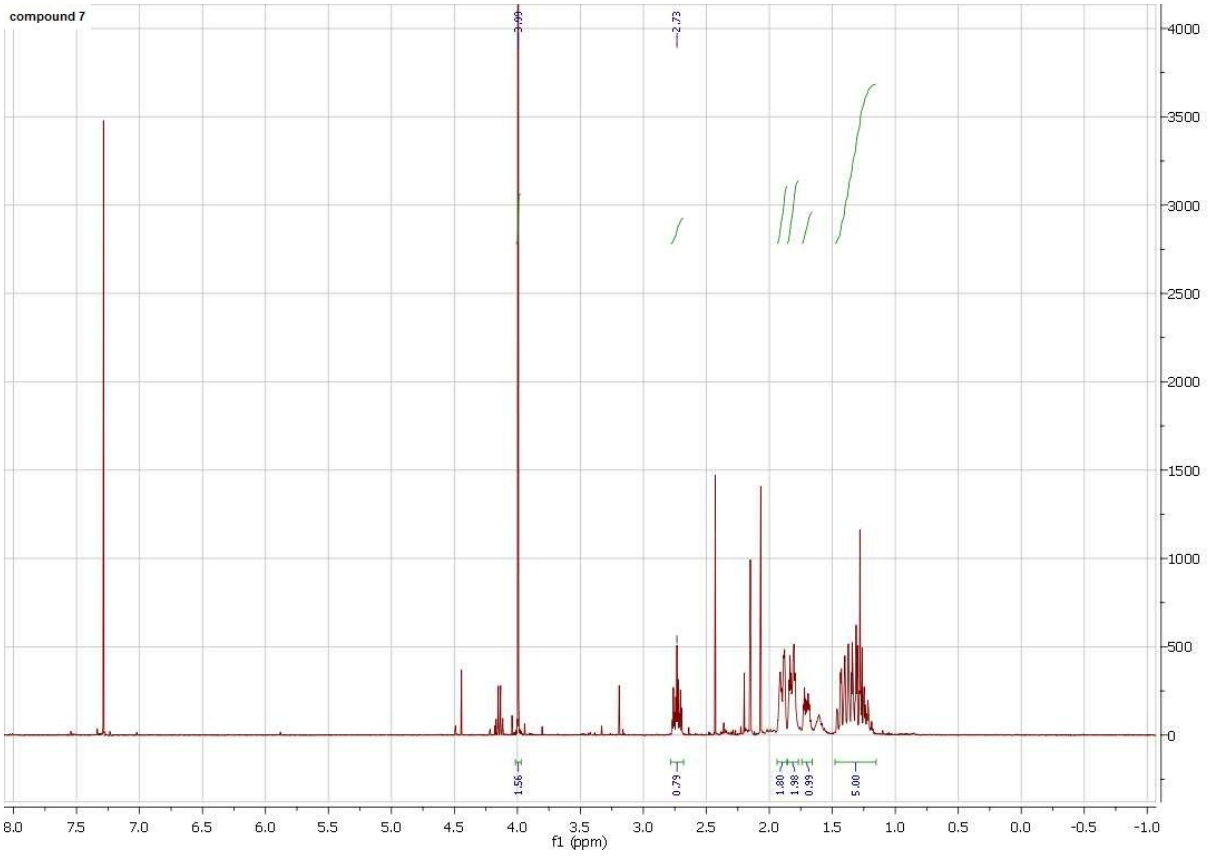
(*S*)-*N*^{4}-(*tert*-butoxy)-*N*^{1}-((*S*)-3-(4-fluorophenyl)-1-((naphthalen-1-ylmethyl)amino)-1-oxopropan-2-yl)-2-(3-phenylpropanamido)succinamide (DPLG-3)

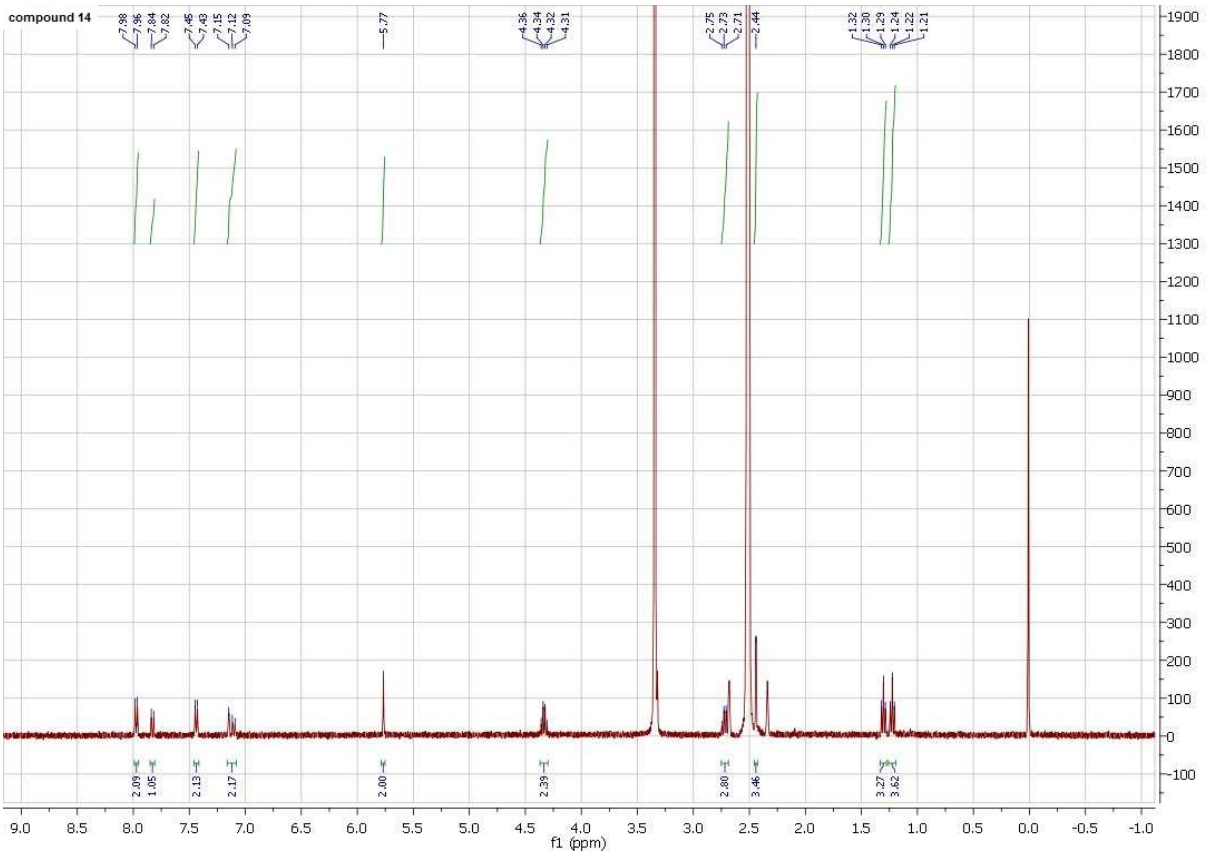


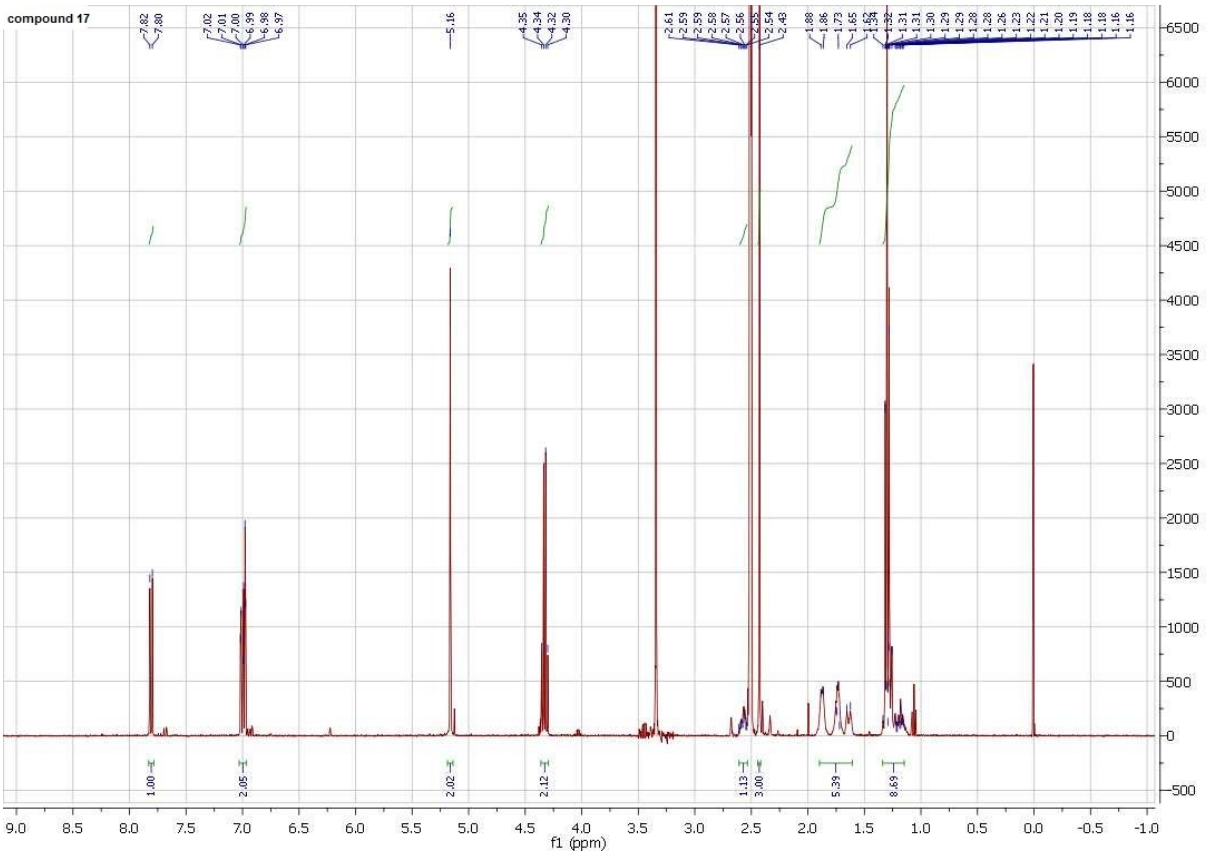
White solid. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 1.11 (s, 9H, C(C<u>H</u>$_3$)$_3$), 2.26 (dd, *J* = 14,7, 7,8 Hz, 1H, COC<u>H</u>$_a$), 2.31 – 2.39 (m, 2H, C<u>H</u>$_2$CH$_2$), 2.46 (dd, *J* = 14.9, 6.5 Hz, 1H, COC<u>H</u>$_b$), 2.71 – 2.77 (m, 2H, C<u>H</u>$_2$CH$_2$), 2.81 (dd, *J* = 13,7, 9,3 Hz, 1H, Ar-C<u>H</u>$_a$), 3.04 (dd, *J* = 13,9, 4,7 Hz, 1H Ar-C<u>H</u>$_b$), 4.43 – 4.49 (m, 1H, C<u>H</u>), 4.55 – 4.62 (m, 1H, C<u>H</u>), 4.70 (dd, *J* = 15.0, 5.7 Hz, 1H, Ar-C<u>H</u>$_a$), 4.72 (dd, *J* = 15.3, 5.8 Hz, 1H, Ar-C<u>H</u>$_b$), 6.95 – 7.02 (m, 2H, Ar-<u>H</u>), 7.14 – 7.22 (m, 5H, Ar-<u>H</u>), 7.23 – 7.29 (m, 2H, Ar-<u>H</u>), 7.36 (d, *J* = 7.0 Hz, 1H, CON<u>H</u>CH), 7.40 – 7,46 (m, 1H, Ar-<u>H</u>), 7.51 – 7.58 (m, 2H, Ar-<u>H</u>), 7.84 (d, *J* = 8.1 Hz, 1H, CON<u>H</u>CH), 7.91 – 7.97 (m, 1H, Ar-<u>H</u>), 8.05 – 8.13 (m, 3H, Ar-<u>H</u>), 8.54 (t, *J* = 5,6 Hz, 1H, CON<u>H</u>CH$_2$), 10.35 (s, 1H, CON<u>H</u>O). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 171.26, 170.64, 170.38, 167.53, 160.85 (d, *J* = 241,5 Hz), 141.20, 134.15, 133.78 (d, *J* = 3,4 Hz), 133.16, 130.91 (d, *J* = 7,4 Hz), 130.75, 128.41, 128.25, 128.05, 127.43, 126.15, 125.81, 125.71, 125.33, 125.31, 123.38, 114.62 (d, *J* = 20,7 Hz), 80.50, 54.22, 49.49, 40.12, 36.73, 36.34, 34.59, 30.90, 26.18. HRMS (*m/z*) (ESI): calcd for $C_{37}H_{41}FN_4O_5$ [M-H]$^-$: 639.2988, found: 639.2996. Melting point: 222.3 – 224.5 °C. Purity by HPLC: 98%.

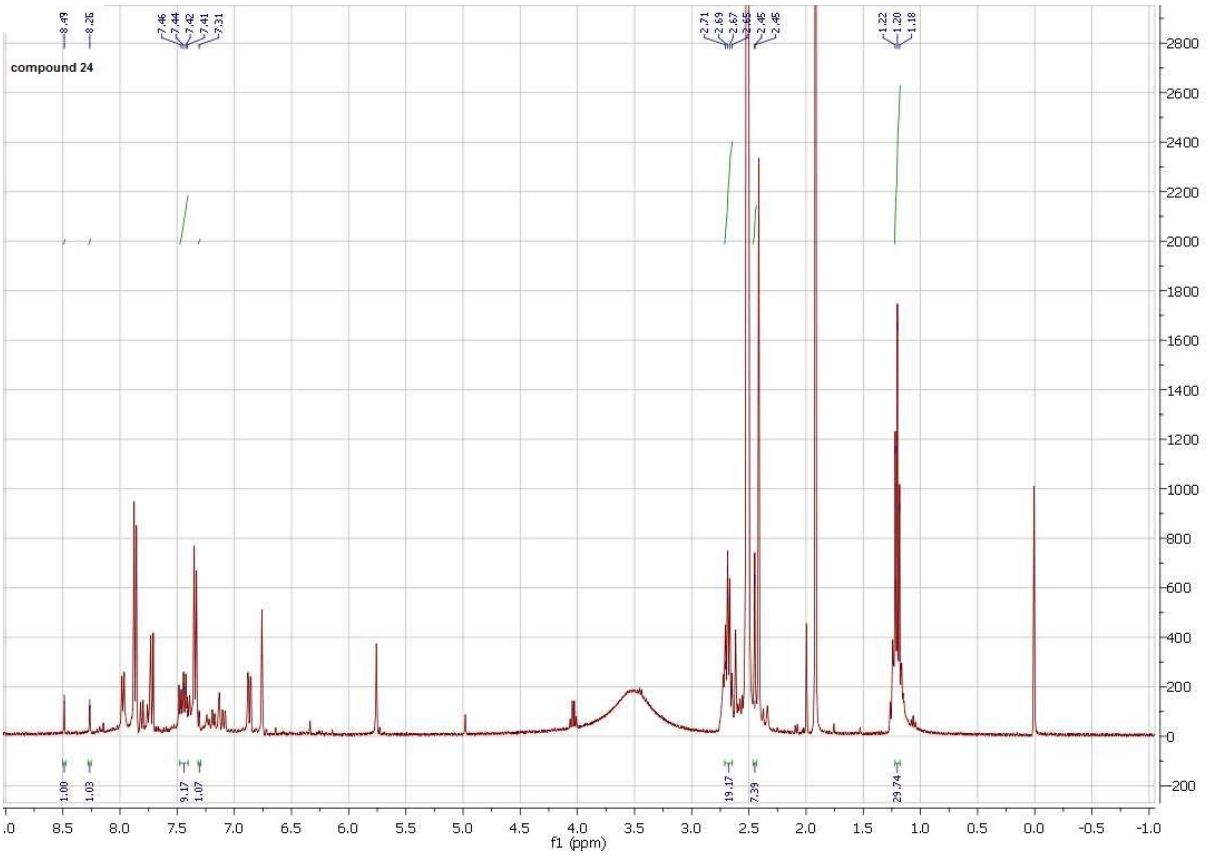
6. Selected ^{1}H and ^{13}C NMR spectra

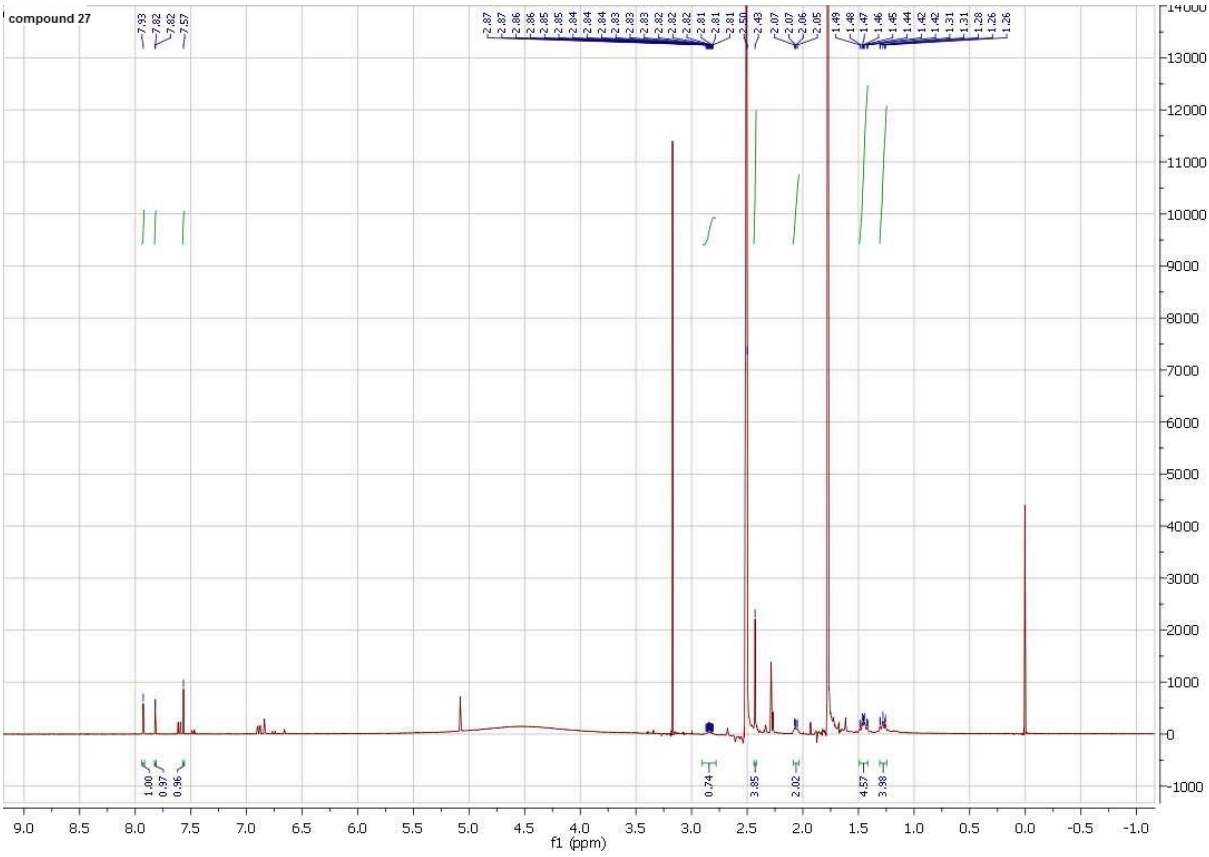


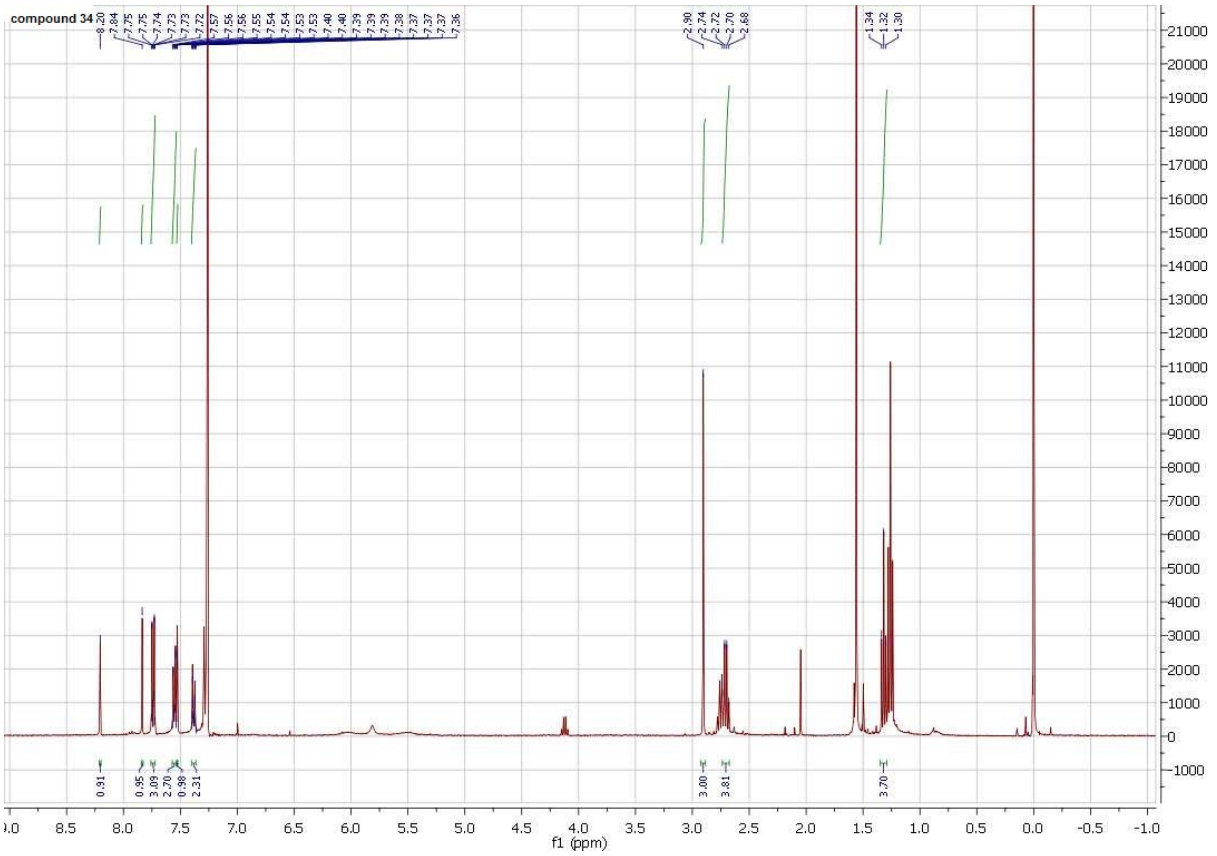


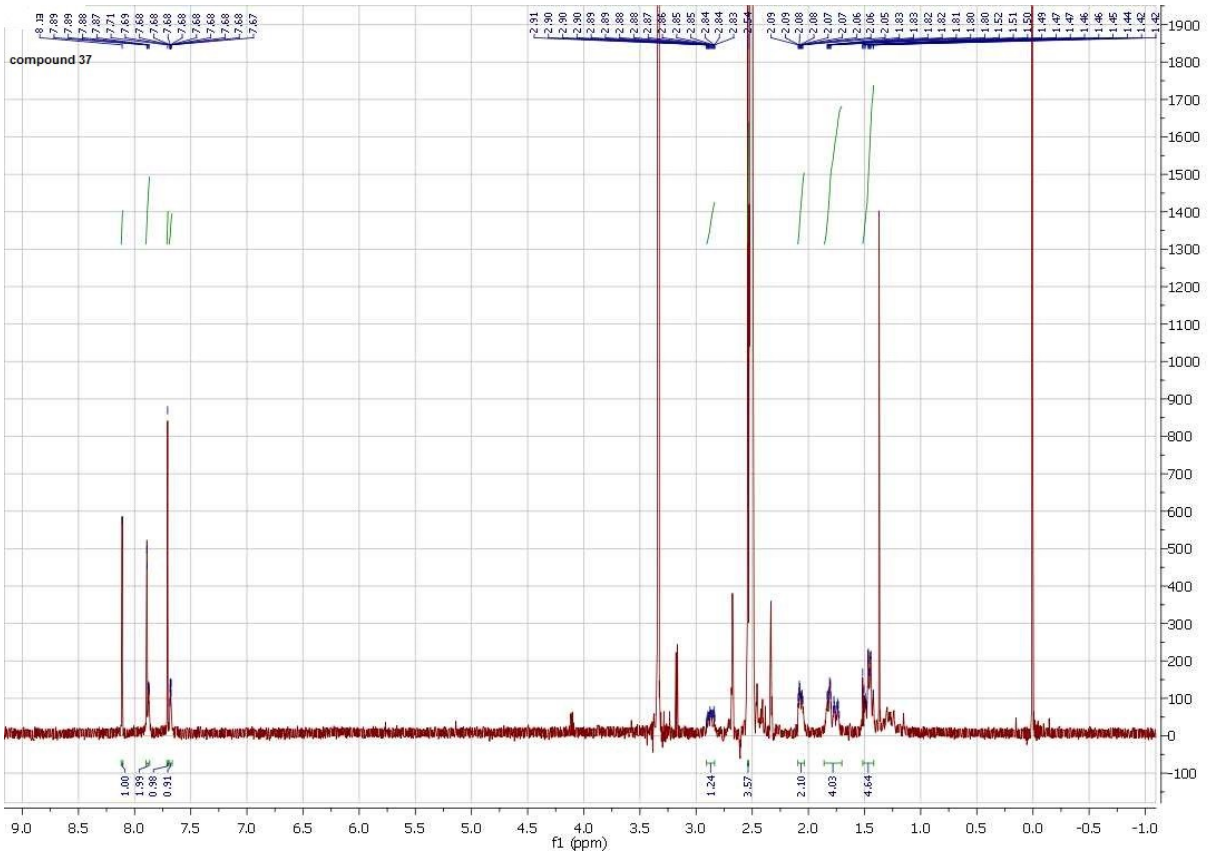

compound 14
f1 (ppm)

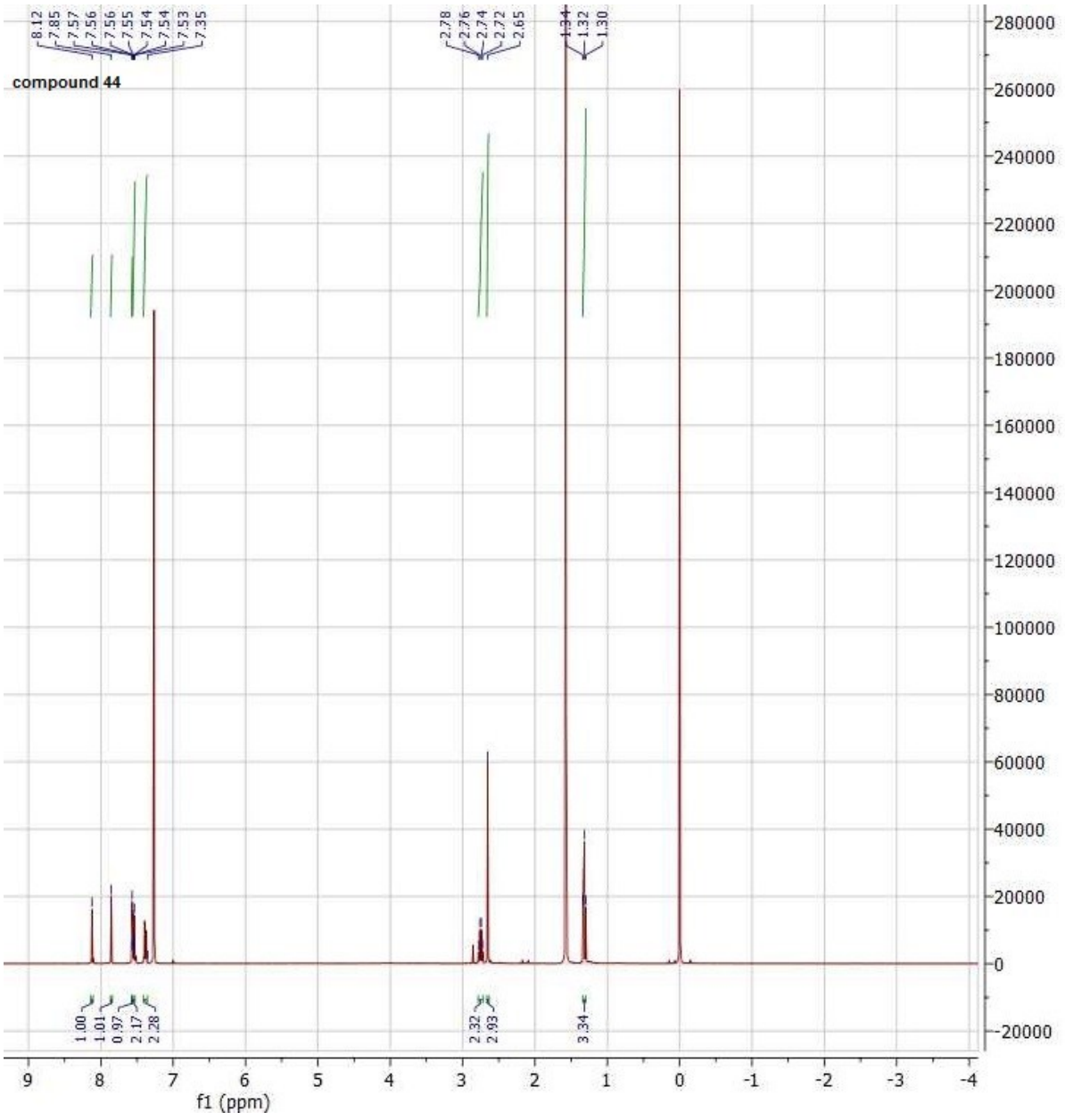

compound 17
f1 (ppm)

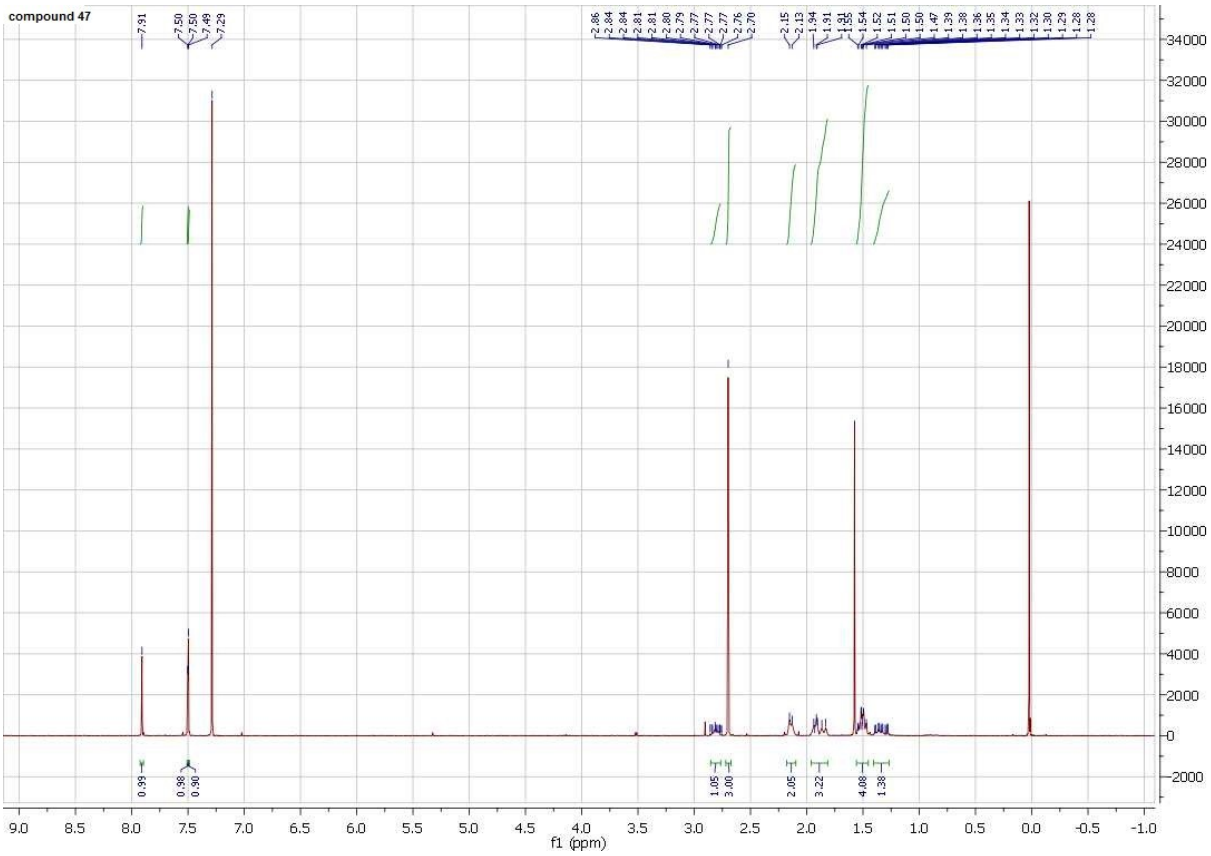

compound 24
f1 (ppm)

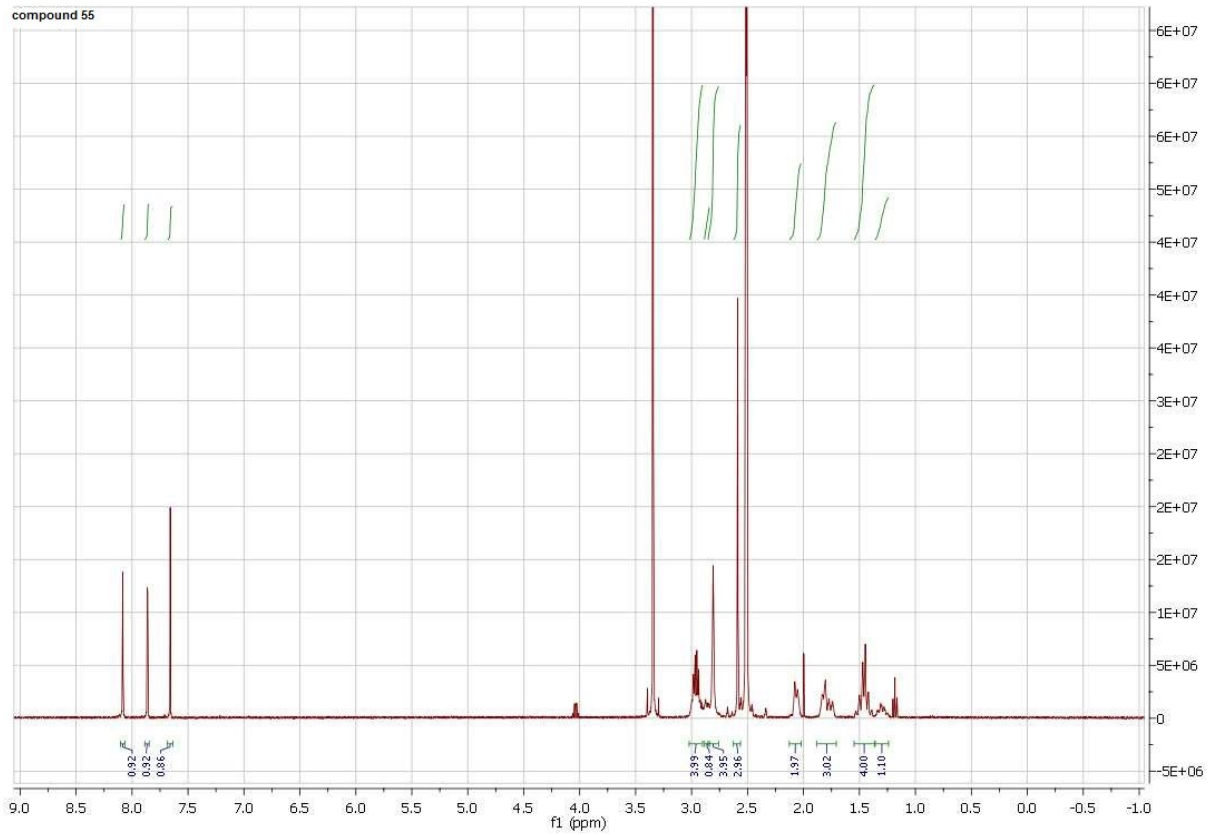

compound 27
f1 (ppm)

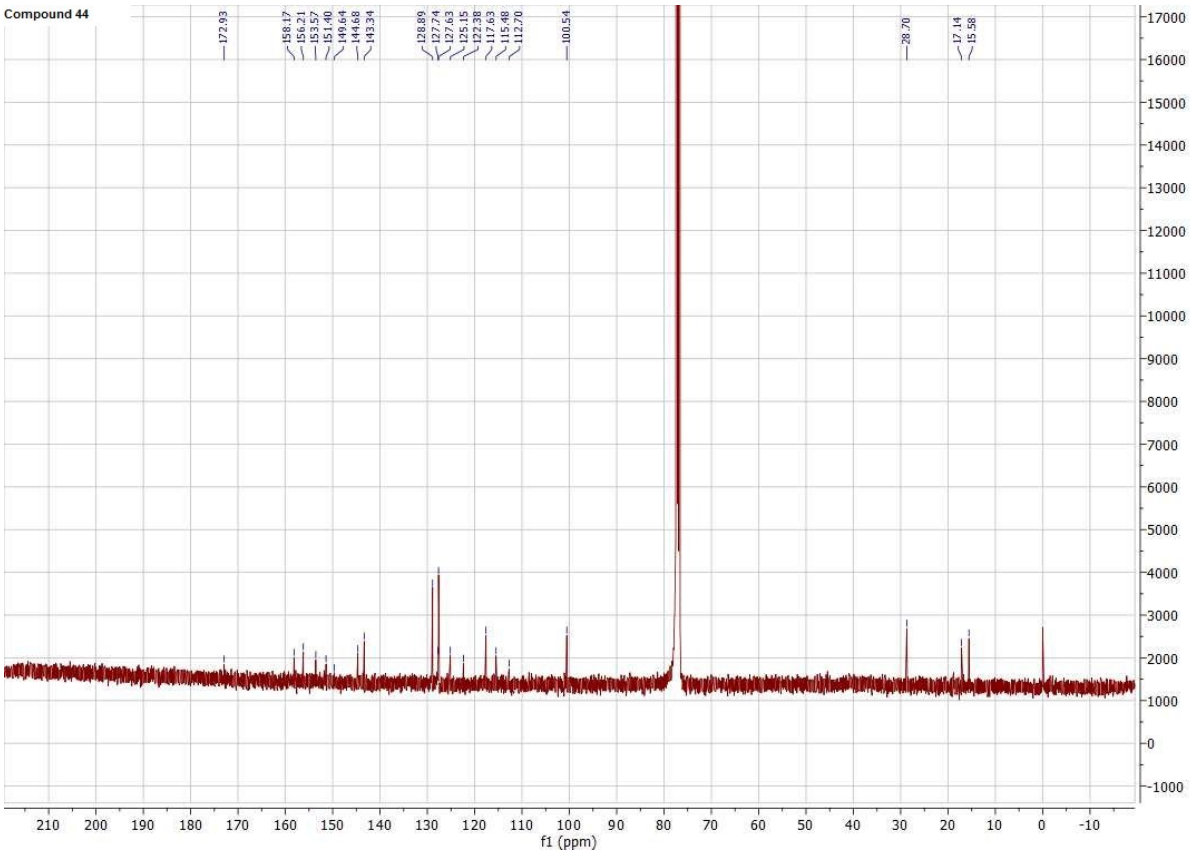

compound 34
f1 (ppm)

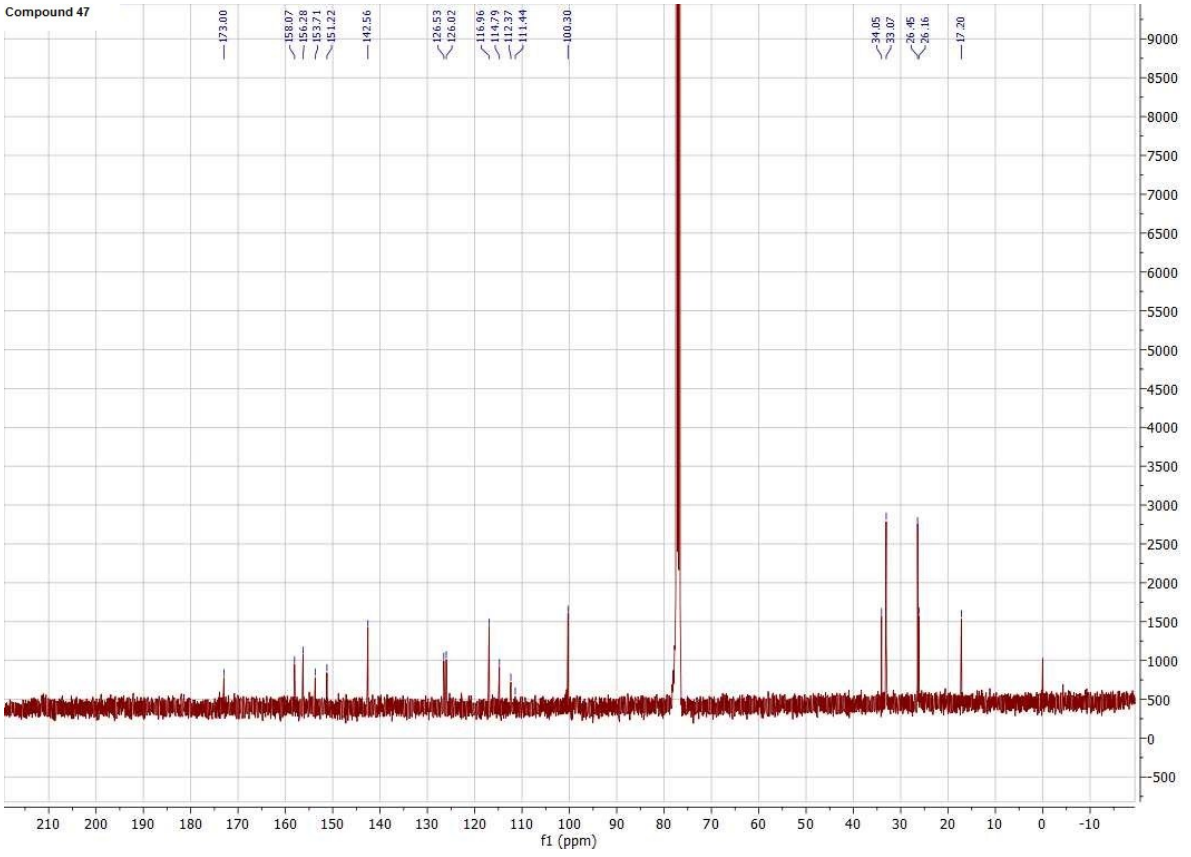

compound 37
f1 (ppm)

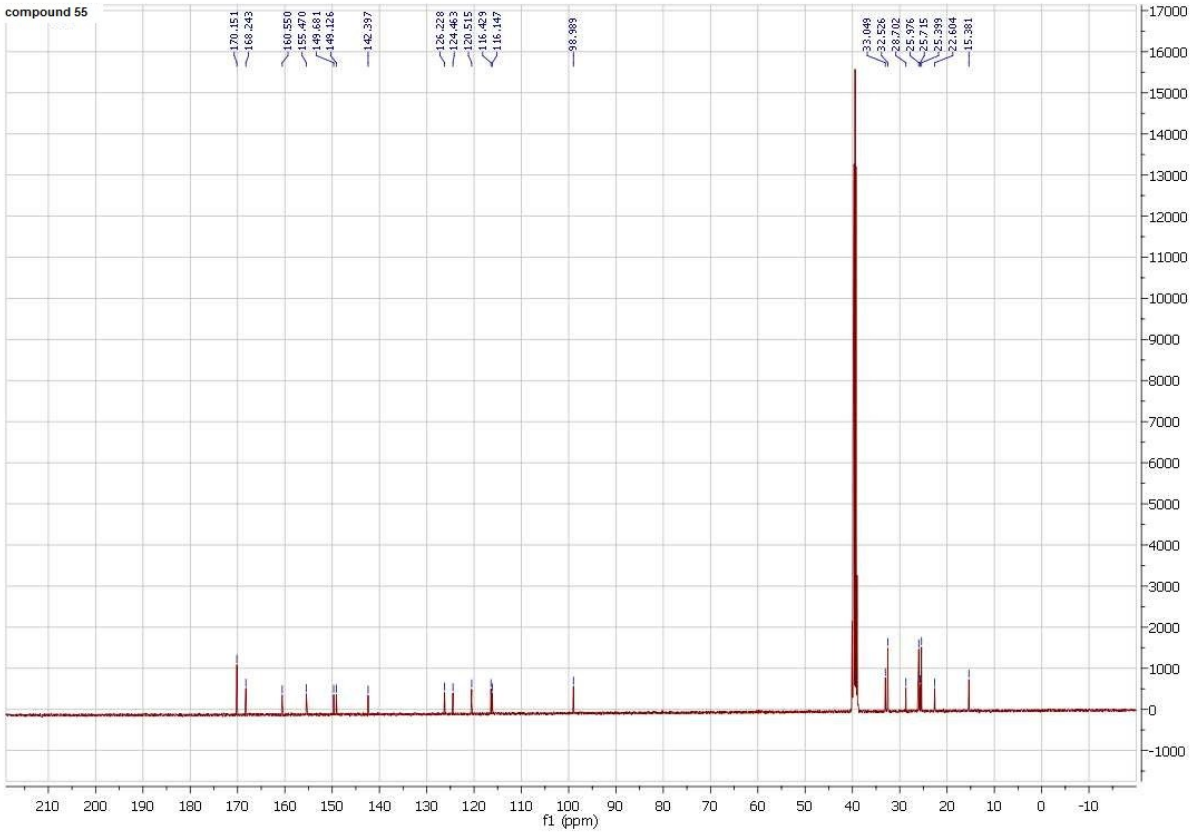

compound 44
8.12
7.85
7.57
7.56
7.56
7.55
7.54
7.54
7.53
7.35
2.78
2.76
2.74
2.72
2.65
1.34
1.32
1.30
1.00
1.01
0.97
2.17
2.28
2.32
2.93
3.34
f1 (ppm)


compound 47
f1 (ppm)


compound 55
f1 (ppm)


Compound 44
172.93
158.17
156.21
153.57
151.40
149.64
144.68
143.34
128.89
127.74
127.63
125.15
122.38
117.63
115.48
112.70
100.54
28.70
17.14
15.58
f1 (ppm)

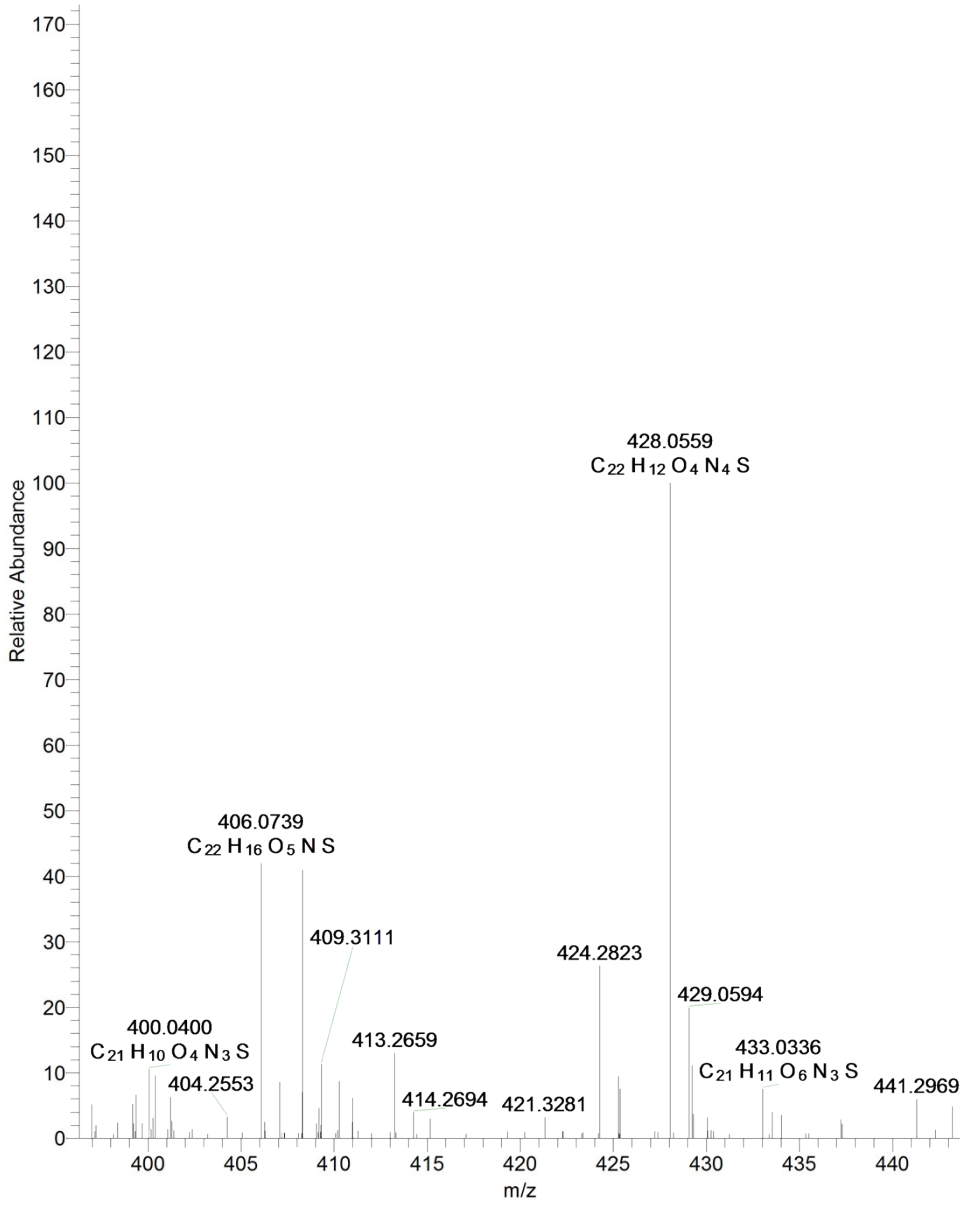

Compound 47
173.00
158.07
156.28
153.71
151.22
142.56
126.53
126.02
116.96
114.79
112.37
111.44
100.30
34.05
33.07
26.45
26.16
17.20
f1 (ppm)


compound 55
170.151
168.243
160.550
155.470
149.681
149.126
142.397
126.228
124.463
120.515
116.429
116.147
98.989
33.049
32.526
28.702
25.976
25.715
25.399
22.604
15.381
f1 (ppm)

7. Selected HRMS spectra

Compound 44:

GES-172-B #48 RT: 0.21 AV: 1 NL: 1.21E7
T: FTMS + c ESI Full ms [100.0000-750.0000]



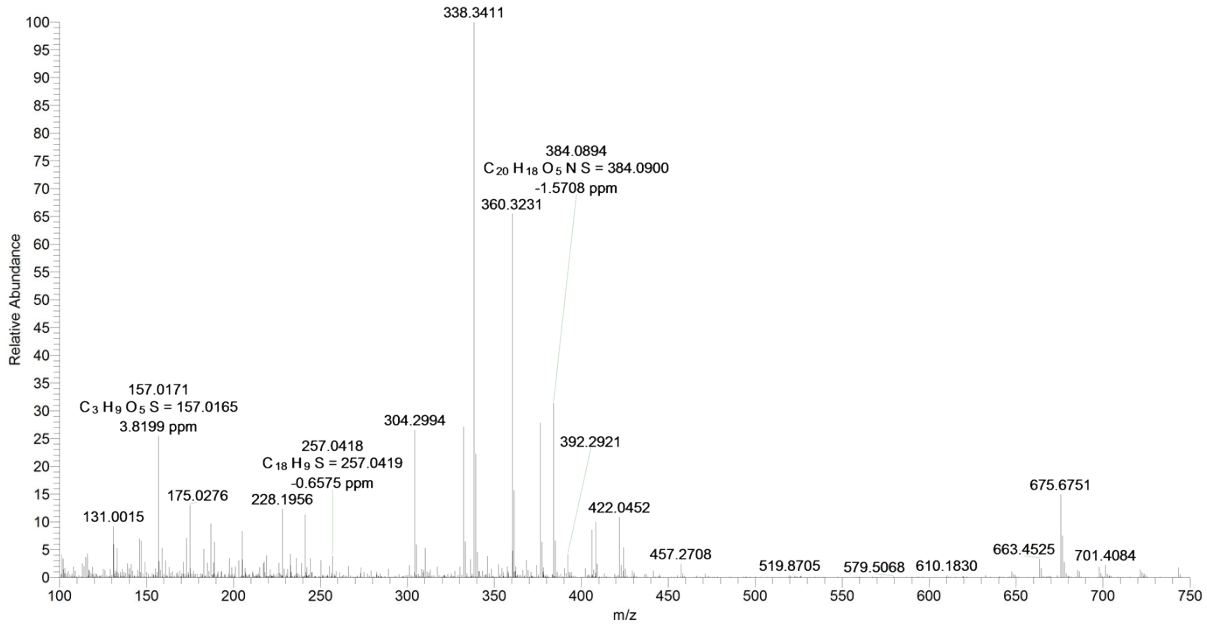
Elemental composition search on mass 406.0738

m/z= 401.0738-411.0738

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
406.07385	406.07437	-1.28	15.5	$C_{22}H_{16}O_5NS$
	406.07303	2.03	16.0	$C_{20}H_{14}O_4N_4S$

Compound 47:

GES-55 #1 RT: 0.00 AV: 1 NL: 3.87E7
T: FTMS + c ESI Full ms [100.0000-750.0000]

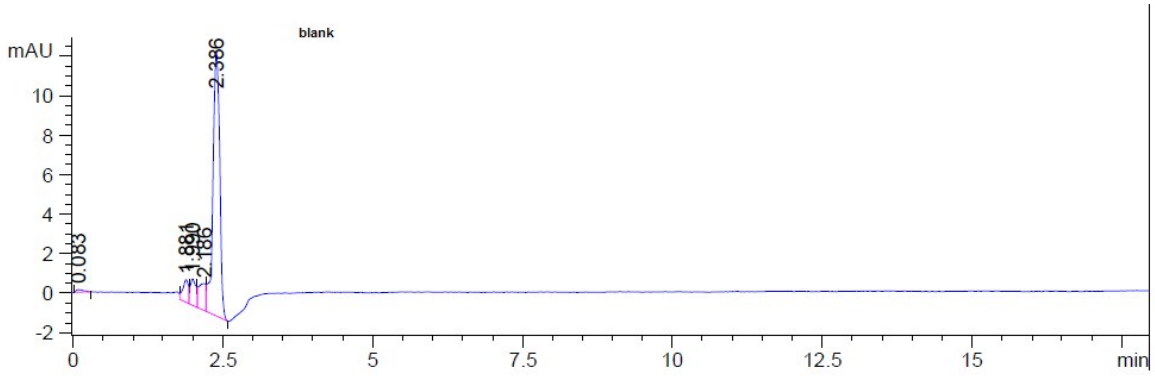


Elemental composition search on mass 384.0894

m/z= 379.0894-389.0894

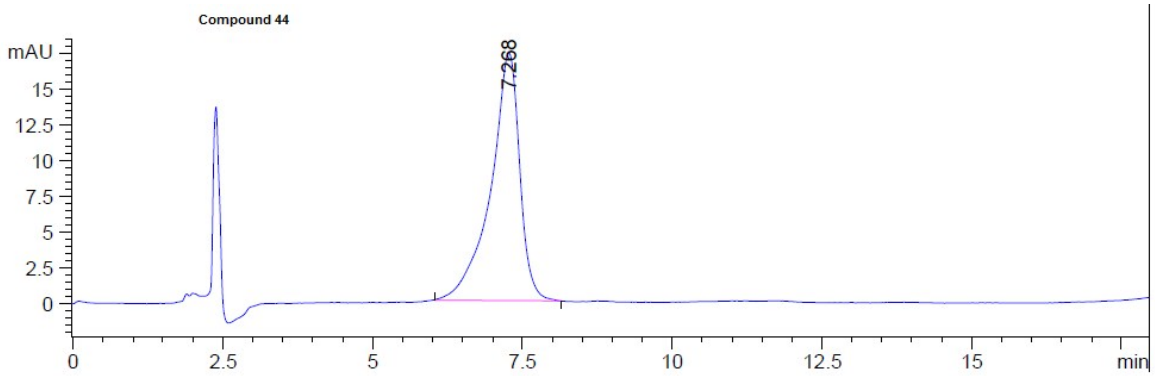
m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
384.08942	384.09002	-1.56	12.5	$C_{20}H_{18}O_5NS$
	384.08868	1.93	13.0	$C_{18}H_{16}O_4N_4S$

8. Selected HPLC spectra



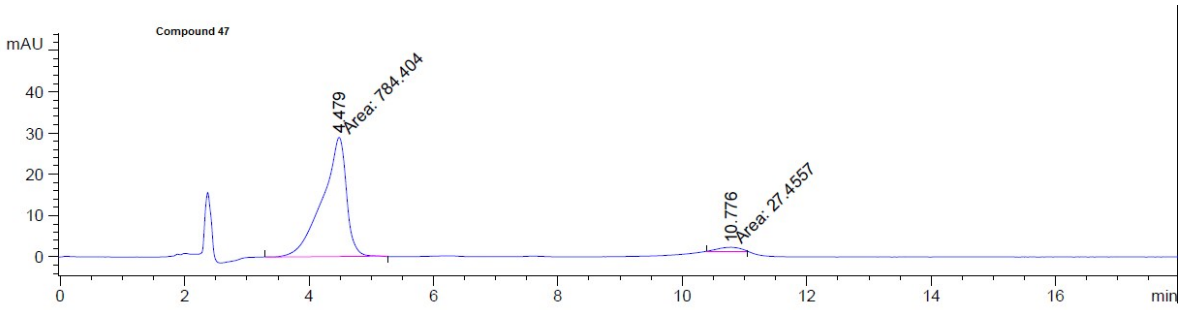
Signal 1: MWD1 B, Sig=254,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.083	BB	0.1285	1.35758	1.40775e-1	1.0154
2	1.881	BV	0.0950	7.54493	1.14312	5.6431
3	1.990	VV	0.0957	9.32327	1.33096	6.9732
4	2.186	VV	0.1210	11.07483	1.36023	8.2833
5	2.386	VV	0.1288	104.40067	13.42113	78.0850
Totals :				133.70129	17.39621	



Signal 1: MWD1 B, Sig=254,16 Ref=off

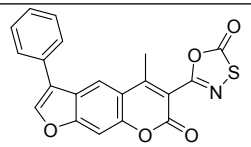
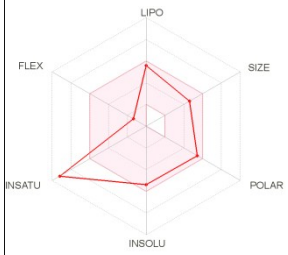
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.268	BB	0.4871	588.59668	17.37942	100.0000
Totals :				588.59668	17.37942	

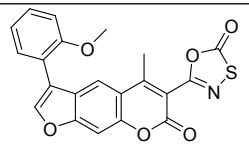
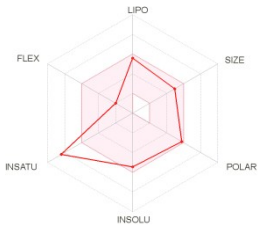
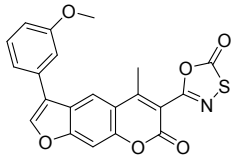
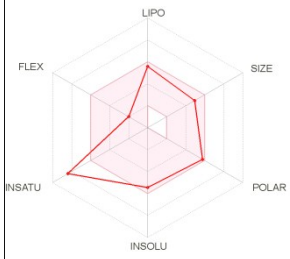


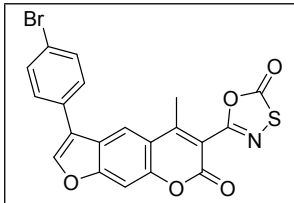
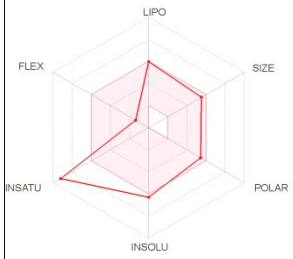
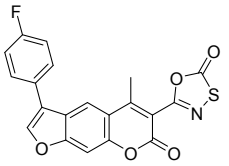
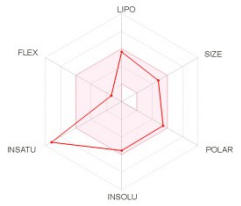
Signal 1: MWD1 B, Sig=254,16 Ref=off

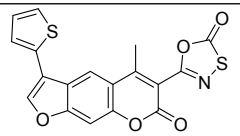
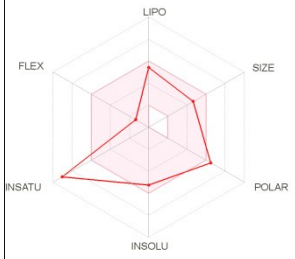
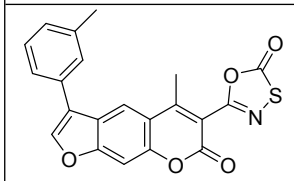
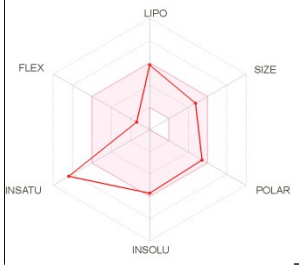
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.479	MM	0.4512	784.40399	28.97399	96.6182
2	10.776	MM	0.4305	27.45573	1.06283	3.3818
Totals :				811.85972	30.03682	

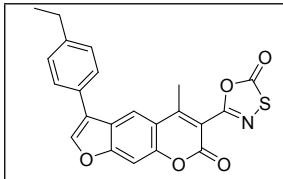
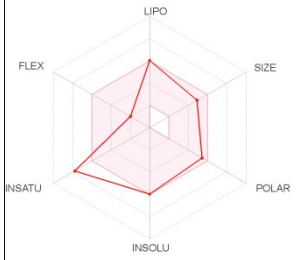
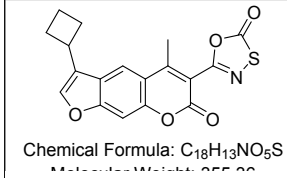
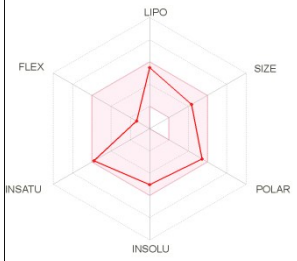
9. Parameters to evaluate pharmacokinetics and drug-likeness and predicted water solubility

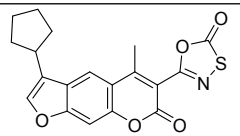
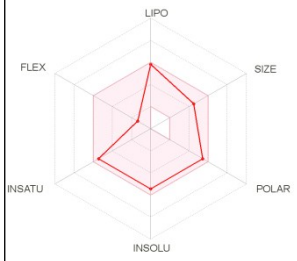
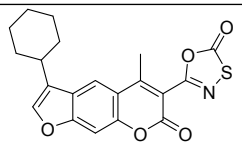
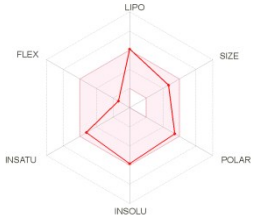
	Physicochemical Properties					Lipophilicity		Water Solubility		
	Frac. Csp3	Num. rotatable bonds	Num. H-bond accep-tors	Num. H-bond donors	TPSA [3]	Log $P_{o/w}$	Cons. Log $P_{o/w}$ [9]	ESOL[10]	Ali[12]	SILICOS-IT[14]
 Chemical Formula: $C_{20}H_{11}NO_5S$ Molecular Weight: 377,37  [2]	0,05	2	6	0	114,69 Å²	Log $P_{o/w}$ (iLOGP)[4] = 2,93 Log $P_{o/w}$ (XLOGP3)[5] = 4,28 Log $P_{o/w}$ (WLOGP)[6] = 4,59 Log $P_{o/w}$ (MLOGP)[7] = 2,27 Log $P_{o/w}$ (SILICOS-IT)[8] = 5,95	4,00	Log S = -5,40 Solubility = 1,50e-03 mg/ml ; 3,96e-06 mol/l Class:[11] Moderately Soluble	Log S = -6,40 Solubility = 1,50e-04 mg/ml ; 3,97e-07 mol/l Class:[13] Poorly Soluble	Log S = -8,30 Solubility = 1,90e-06 mg/ml ; 5,05e-09 mol/l Class:[15] Poorly Soluble

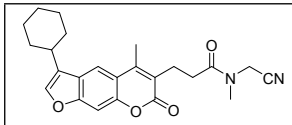
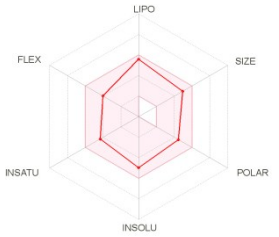
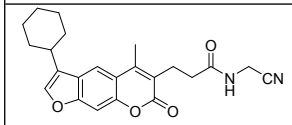
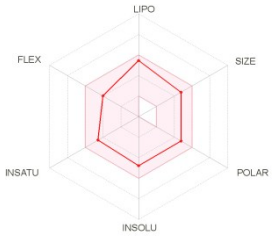
 Chemical Formula: $C_{21}H_{13}NO_6S$ Molecular Weight: 407,40 	0,10	3	7	0	123,92 Å²	Log $P_{o/w}$ (iLOGP) = 3,40 Log $P_{o/w}$ (XLOGP3) = 4,25 Log $P_{o/w}$ (WLOGP) = 4,60 Log $P_{o/w}$ (MLOGP) = 1,96 Log $P_{o/w}$ (SILICOS-IT) = 5,99	4,04	Log S = -5,46 Solubility = 1,42e-03 mg/ml ; 3,49e-06 mol/l Class: Moderately Soluble	Log S = -6,56 Solubility = 1,11e-04 mg/ml ; 2,73e-07 mol/l Class: Poorly Soluble	Log S = -8,40 Solubility = 1,62e-06 mg/ml ; 3,98e-09 mol/l Class: Poorly Soluble
 Chemical Formula: $C_{21}H_{13}NO_6S$ Molecular Weight: 407,40 	0,10	3	7	0	123,92 Å²	Log $P_{o/w}$ (iLOGP) = 3,37 Log $P_{o/w}$ (XLOGP3) = 4,25 Log $P_{o/w}$ (WLOGP) = 4,60 Log $P_{o/w}$ (MLOGP) = 1,96 Log $P_{o/w}$ (SILICOS-IT) = 5,99	4,03	Log S = -5,46 Solubility = 1,42e-03 mg/ml ; 3,49e-06 mol/l Class: Moderately Soluble	Log S = -6,56 Solubility = 1,11e-04 mg/ml ; 2,73e-07 mol/l Class: Poorly Soluble	Log S = -8,40 Solubility = 1,62e-06 mg/ml ; 3,98e-09 mol/l Class: Poorly Soluble

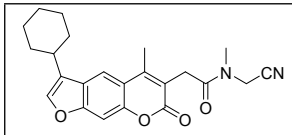
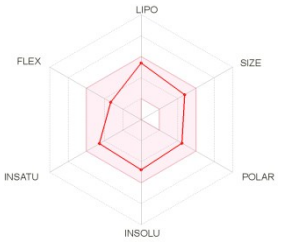
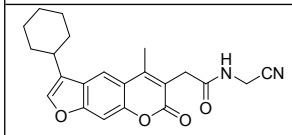
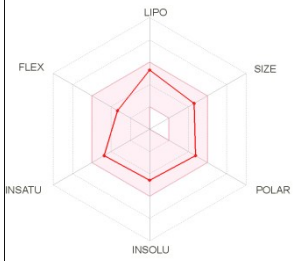
 Chemical Formula: $C_{20}H_{10}BrNO_5S$ Molecular Weight: 456,27 	0,05	2	6	0	114,69 Å²	Log $P_{o/w}$ (iLOGP) = 3,38 Log $P_{o/w}$ (XLOGP3) = 4,97 Log $P_{o/w}$ (WLOGP) = 5,35 Log $P_{o/w}$ (MLOGP) = 2,87 Log $P_{o/w}$ (SILICOS-IT) = 6,62	4,64	Log S = -6,30 Solubility = 2,28e-04 mg/ml ; 4,99e-07 mol/l Class: Poorly Soluble	Log S = -7,12 Solubility = 3,48e-05 mg/ml ; 7,64e-08 mol/l Class: Poorly Soluble	Log S = -9,08 Solubility = 3,81e-07 mg/ml ; 8,36e-10 mol/l Class: Poorly Soluble
 Chemical Formula: $C_{20}H_{10}FNO_5S$ Molecular Weight: 395,36 	0,05	2	7	0	114,69 Å²	Log $P_{o/w}$ (iLOGP) = 3,10 Log $P_{o/w}$ (XLOGP3) = 4,38 Log $P_{o/w}$ (WLOGP) = 5,15 Log $P_{o/w}$ (MLOGP) = 2,65 Log $P_{o/w}$	4,33	Log S = -5,55 Solubility = 1,11e-03 mg/ml ; 2,80e-06 mol/l Class: Moderately Soluble	Log S = -6,50 Solubility = 1,24e-04 mg/ml ; 3,13e-07 mol/l Class: Poorly Soluble	Log S = -8,56 Solubility = 1,08e-06 mg/ml ; 2,74e-09 mol/l Class: Poorly Soluble

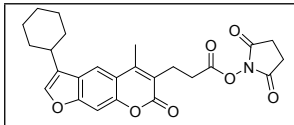
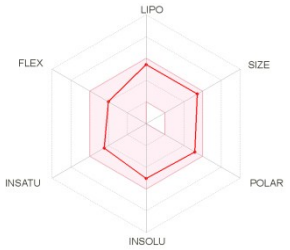
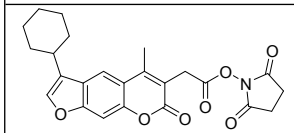
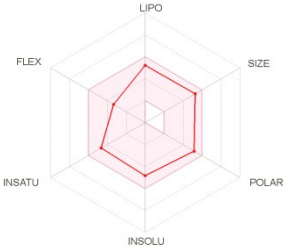
						(SILICOS-IT) = 6,36				
 Chemical Formula: $C_{18}H_9NO_5S_2$ Molecular Weight: 383,39 	0,06	2	6	0	142,93 Å²	Log $P_{o/w}$ (iLOGP) = 2,95 Log $P_{o/w}$ (XLOGP3) = 4,00 Log $P_{o/w}$ (WLOGP) = 4,65 Log $P_{o/w}$ (MLOGP) = 1,84 Log $P_{o/w}$ (SILICOS-IT) = 6,59	4,01	Log S = -5,26 Solubility = 2,11e-03 mg/ml ; 5,50e-06 mol/l Class: Moderately Soluble	Log S = -6,70 Solubility = 7,59e-05 mg/ml ; 1,98e-07 mol/l Class: Poorly Soluble	Log S = -7,56 Solubility = 1,05e-05 mg/ml ; 2,74e-08 mol/l Class: Poorly Soluble
 Chemical Formula: $C_{21}H_{13}NO_5S$ Molecular Weight: 391,40 	0,10	2	6	0	114,69 Å²	Log $P_{o/w}$ (iLOGP) = 3,31 Log $P_{o/w}$ (XLOGP3) = 4,65 Log $P_{o/w}$ (WLOGP) = 4,90 Log $P_{o/w}$ (MLOGP) = 2,49 Log $P_{o/w}$ (SILICOS-IT) = 6,46	4,36	Log S = -5,70 Solubility = 7,84e-04 mg/ml ; 2,00e-06 mol/l Class: Moderately Soluble	Log S = -6,78 Solubility = 6,42e-05 mg/ml ; 1,64e-07 mol/l Class: Poorly Soluble	Log S = -8,67 Solubility = 8,31e-07 mg/ml ; 2,12e-09 mol/l Class: Poorly Soluble

 Chemical Formula: $C_{22}H_{15}NO_5S$ Molecular Weight: 405,42 	0,14	3	6	0	114,69 Å²	Log $P_{o/w}$ (iLOGP) = 3,51 Log $P_{o/w}$ (XLOGP3) = 5,08 Log $P_{o/w}$ (WLOGP) = 5,15 Log $P_{o/w}$ (MLOGP) = 2,71 Log $P_{o/w}$ (SILICOS-IT) = 6,84	4,66	Log S = -5,97 Solubility = 4,36e-04 mg/ml ; 1,08e-06 mol/l Class: Moderately Soluble	Log S = -7,23 Solubility = 2,38e-05 mg/ml ; 5,87e-08 mol/l Class: Poorly Soluble	Log S = -9,07 Solubility = 3,48e-07 mg/ml ; 8,59e-10 mol/l Class: Poorly Soluble
 Chemical Formula: $C_{18}H_{13}NO_5S$ 	0,28	2	6	0	114,69 Å²	Log $P_{o/w}$ (iLOGP) = 2,91 Log $P_{o/w}$ (XLOGP3) = 4,11 Log $P_{o/w}$ (WLOGP) = 4,19 Log $P_{o/w}$ (MLOGP) = 2,02 Log $P_{o/w}$ (SILICOS-IT) = 5,60	3,76	Log S = -5,03 Solubility = 3,29e-03 mg/ml ; 9,26e-06 mol/l Class: Moderately Soluble	Log S = -6,22 Solubility = 2,12e-04 mg/ml ; 5,96e-07 mol/l Class: Poorly Soluble	Log S = -6,67 Solubility = 7,51e-05 mg/ml ; 2,11e-07 mol/l Class: Poorly Soluble

 Chemical Formula: $C_{19}H_{15}NO_5S$ Molecular Weight: 369,39 	0,32	2	6	0	114,69 Å²	Log $P_{o/w}$ (iLOGP) = 3,17 Log $P_{o/w}$ (XLOGP3) = 4,65 Log $P_{o/w}$ (WLOGP) = 4,58 Log $P_{o/w}$ (MLOGP) = 2,25 Log $P_{o/w}$ (SILICOS-IT) = 5,82	4,10	Log S = -5,44 Solubility = 1,34e-03 mg/ml ; 3,63e-06 mol/l Class: Moderately Soluble	Log S = -6,78 Solubility = 6,06e-05 mg/ml ; 1,64e-07 mol/l Class: Poorly Soluble	Log S = -6,94 Solubility = 4,20e-05 mg/ml ; 1,14e-07 mol/l Class: Poorly Soluble
 Chemical Formula: $C_{20}H_{17}NO_5S$ Molecular Weight: 383,42 	0,35	2	6	0	114,69 Å²	Log $P_{o/w}$ (iLOGP) = 3,31 Log $P_{o/w}$ (XLOGP3) = 5,19 Log $P_{o/w}$ (WLOGP) = 4,97 Log $P_{o/w}$ (MLOGP) = 2,48 Log $P_{o/w}$ (SILICOS-IT) = 6,05	4,40	Log S = -5,85 Solubility = 5,44e-04 mg/ml ; 1,42e-06 mol/l Class: Moderately Soluble	Log S = -7,35 Solubility = 1,73e-05 mg/ml ; 4,51e-08 mol/l Class: Poorly Soluble	Log S = -7,12 Solubility = 2,35e-05 mg/ml ; 6,14e-08 mol/l Class: Poorly Soluble

 Chemical Formula: $C_{24}H_{26}N_2O_4$ Molecular Weight: 406,48 	0,46	6	5	0	87,45 Å²	Log $P_{o/w}$ (iLOGP) = 3,50 Log $P_{o/w}$ (XLOGP3) = 4,25 Log $P_{o/w}$ (WLOGP) = 4,81 Log $P_{o/w}$ (MLOGP) = 2,37 Log $P_{o/w}$ (SILICOS-IT) = 5,27	4,04	Log S = -4,96 Solubility = 4,43e-03 mg/ml ; 1,09e-05 mol/l Class: Moderately Soluble	Log S = -5,80 Solubility = 6,47e-04 mg/ml ; 1,59e-06 mol/l Class: Moderately Soluble	Log S = -7,21 Solubility = 2,48e-05 mg/ml ; 6,11e-08 mol/l Class: Poorly Soluble
 Chemical Formula: $C_{23}H_{24}N_2O_4$ Molecular Weight: 392,46 	0,43	6	5	1	96,24 Å²	Log $P_{o/w}$ (iLOGP) = 3,15 Log $P_{o/w}$ (XLOGP3) = 4,07 Log $P_{o/w}$ (WLOGP) = 4,47 Log $P_{o/w}$ (MLOGP) = 2,16 Log $P_{o/w}$ (SILICOS-IT) = 5,31	3,83	Log S = -4,77 Solubility = 6,62e-03 mg/ml ; 1,69e-05 mol/l Class: Moderately Soluble	Log S = -5,80 Solubility = 6,28e-04 mg/ml ; 1,60e-06 mol/l Class: Moderately Soluble	Log S = -7,55 Solubility = 1,10e-05 mg/ml ; 2,79e-08 mol/l Class: Poorly Soluble

 Chemical Formula: $C_{23}H_{24}N_2O_4$ Molecular Weight: 392,46 	0,43	5	5	0	87,45 Å²	Log $P_{o/w}$ (iLOGP) = 3,50 Log $P_{o/w}$ (XLOGP3) = 3,96 Log $P_{o/w}$ (WLOGP) = 4,42 Log $P_{o/w}$ (MLOGP) = 2,16 Log $P_{o/w}$ (SILICOS-IT) = 4,87	3,78	Log S = -4,77 Solubility = 6,67e-03 mg/ml ; 1,70e-05 mol/l Class: Moderately Soluble	Log S = -5,50 Solubility = 1,25e-03 mg/ml ; 3,18e-06 mol/l Class: Moderately Soluble	Log S = -6,82 Solubility = 5,93e-05 mg/ml ; 1,51e-07 mol/l Class: Poorly Soluble
 Chemical Formula: $C_{22}H_{22}N_2O_4$ Molecular Weight: 378,43 	0,41	5	5	1	96,24 Å²	Log $P_{o/w}$ (iLOGP) = 2,84 Log $P_{o/w}$ (XLOGP3) = 3,77 Log $P_{o/w}$ (WLOGP) = 4,08 Log $P_{o/w}$ (MLOGP) = 1,95 Log $P_{o/w}$ (SILICOS-IT) = 4,92	3,51	Log S = -4,57 Solubility = 1,01e-02 mg/ml ; 2,66e-05 mol/l Class: Moderately Soluble	Log S = -5,48 Solubility = 1,24e-03 mg/ml ; 3,28e-06 mol/l Class: Moderately Soluble	Log S = -7,16 Solubility = 2,62e-05 mg/ml ; 6,92e-08 mol/l Class: Poorly Soluble

 Chemical Formula: $C_{25}H_{25}NO_7$ Molecular Weight: 451,48 	0,44	6	7	0	107,03 Å²	Log $P_{o/w}$ (iLOGP) = 3,74 Log $P_{o/w}$ (XLOGP3) = 3,97 Log $P_{o/w}$ (WLOGP) = 4,05 Log $P_{o/w}$ (MLOGP) = 2,95 Log $P_{o/w}$ (SILICOS-IT) = 4,91	3,92	Log S = -5,04 Solubility = 4,16e-03 mg/ml ; 9,12e-06 mol/l Class: Moderately Soluble	Log S = -5,92 Solubility = 5,45e-04 mg/ml ; 1,21e-06 mol/l Class: Moderately Soluble	Log S = -6,92 Solubility = 5,43e-05 mg/ml ; 1,20e-07 mol/l Class: Poorly Soluble
 Chemical Formula: $C_{24}H_{23}NO_7$ Molecular Weight: 437,45 	0,42	5	7	0	107,03 Å²	Log $P_{o/w}$ (iLOGP) = 3,54 Log $P_{o/w}$ (XLOGP3) = 3,67 Log $P_{o/w}$ (WLOGP) = 3,66 Log $P_{o/w}$ (MLOGP) = 2,74 Log $P_{o/w}$ (SILICOS-IT) = 4,51	3,63	Log S = -4,83 Solubility = 6,40e-03 mg/ml ; 1,46e-05 mol/l Class: Moderately Soluble	Log S = -5,61 Solubility = 1,08e-03 mg/ml ; 2,47e-06 mol/l Class: Moderately Soluble	Log S = -6,53 Solubility = 1,29e-04 mg/ml ; 2,96e-07 mol/l Class: Poorly Soluble

10. References

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[5] Calculated by XLOGP program, version 3.2.2, courtesy of CCBG, Shanghai Institute of Organic Chemistry.

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[8] Calculated by FILTER-IT program, version 1.0.2, courtesy of SILICOS-IT, http://www.silicos-it.com.

[9] Consensus Log Po/w: Average of all five predictions.

[10] Method implemented from J. Delaney JS, Journal of Chemical Information and Modeling, 2004, 33 (4), 1000-1005.

[11] Solubility class: Log S scale; Insoluble < -10 < Poorly < -6 < Moderately < -4 < Soluble < -2 < Very < 0 < Highly.

[12] Method implemented from J. Ali, P. Camilleri, M. Brown, A. Hutt and S. Kirton, Journal of Chemical Information and Modeling, 2012, 52 (11), 2950-2957.

[13] Solubility class: Log S scale; Insoluble < -10 < Poorly < -6 < Moderately < -4 < Soluble < -2 < Very < 0 < Highly

[14] Method calculated by FILTER-IT program, version 1.0.2, courtesy of SILICOS-IT, http://www.silicos-it.com.

[15] Solubility class: Log S scale; Insoluble < -10 < Poorly < -6 < Moderately < -4 < Soluble < -2 < Very < 0 < Highly