Supporting information:

Substituted 4,5'-Bithiazoles as Catalytic Inhibitors of Human DNA Topoisomerase IIα

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1. Tested commercially available substituted 4,5'-bithiazoles

Table S1. List of compounds tested for their inhibitory activity on human topo IIα

Number	Chemical code	Vendor
1	**STK163157**	VitasM Labs[1]
2	**7872757**	ChemBridge[2]
3	**7858097**	ChemBridge[2]
4	**7211760312**	Otava[3]
5	**STK015148**	VitasM Labs[1]
6	**7859208**	ChemBridge[2]
7	**5936031**	ChemBridge[2]
8	**5934630**	ChemBridge[2]
9	**5935030**	ChemBridge[2]
10	**5932095**	ChemBridge[2]
11	**STK070262**	VitasM Labs[1]
12	**5932282**	ChemBridge[2]
13	**5935989**	ChemBridge[2]
14	**7211760371**	Otava[3]

2. Validation of the docking procedure and molecular docking of substituted 4,5'-bithiazoles

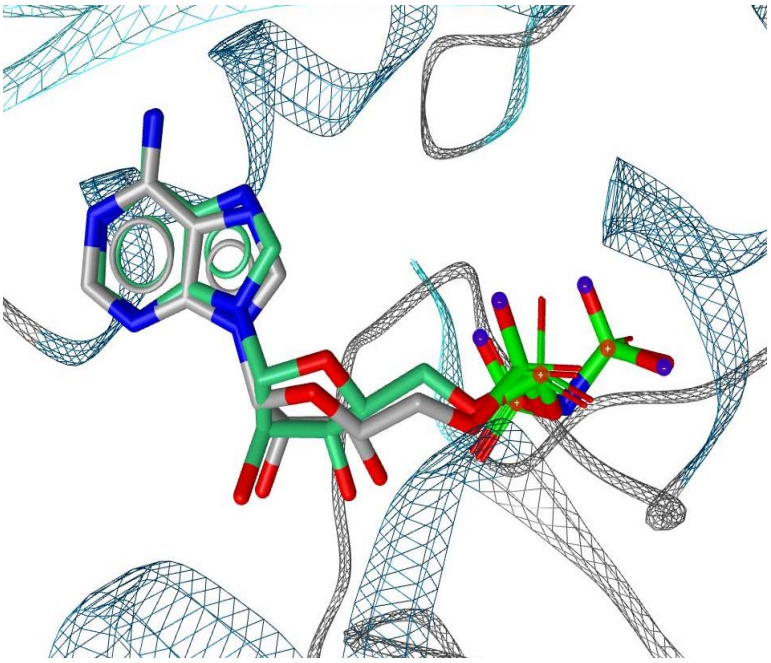
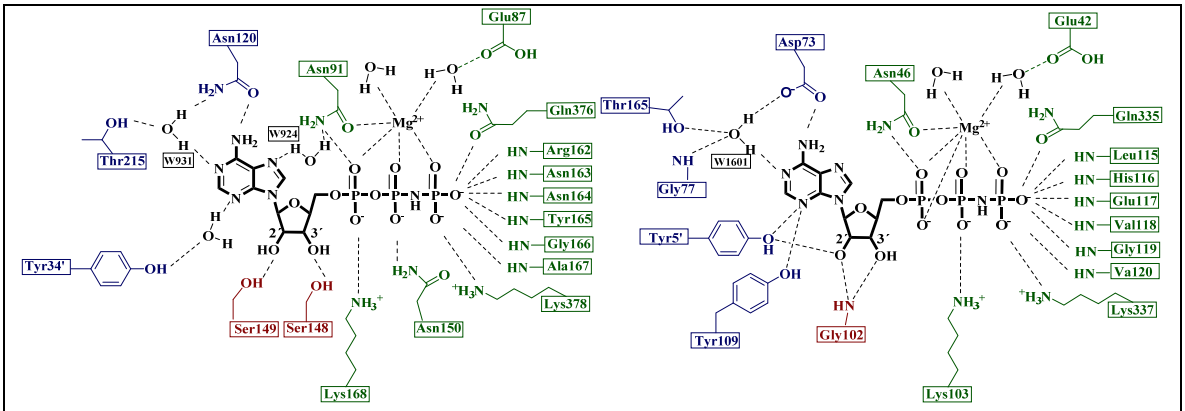


Figure S1. Comparison of the docked and x-ray conformation of AMP-PNP in the ATP human topo IIα binding site using GoldScore scoring function (PDB: 1ZXM). RMSD of 0.9 Å between heavy atoms of both poses was obtained.

Table S2. (above) 2D Intermolecular interaction pattern of the AMP-PNP ligand in the ATP bindings sites of both type II topoisomerases. (bellow) Selected amino acid pairs, important for ATP binding, that are equal/similar or different in the ATP binding sites of human topo IIα and DNA gyrase.



Amino acids, important for binding, that are equal/similar in both binding sites		**Amino acids, important for binding, that are different in both binding sites**	
Human topo IIα	***DNA Gyrase***	***Human topo IIα***	***DNA Gyrase***
Asn120	Asp73	*Ser148*	*Gly101*
Asn91	Asn46	*Ser149*	*,Gly102*
Lys378	Lys337	*Asn150*	*Lys103*
Gln376	Gln335	*Pro126**	*Arg136**

*residue pair Pro126(htIIα)/Arg136(DNA Gyrase) is listed since ARG136 plays a role in binding of 4,5'-bithiazole compound **13** to the DNA Gyrase ATP binding site.

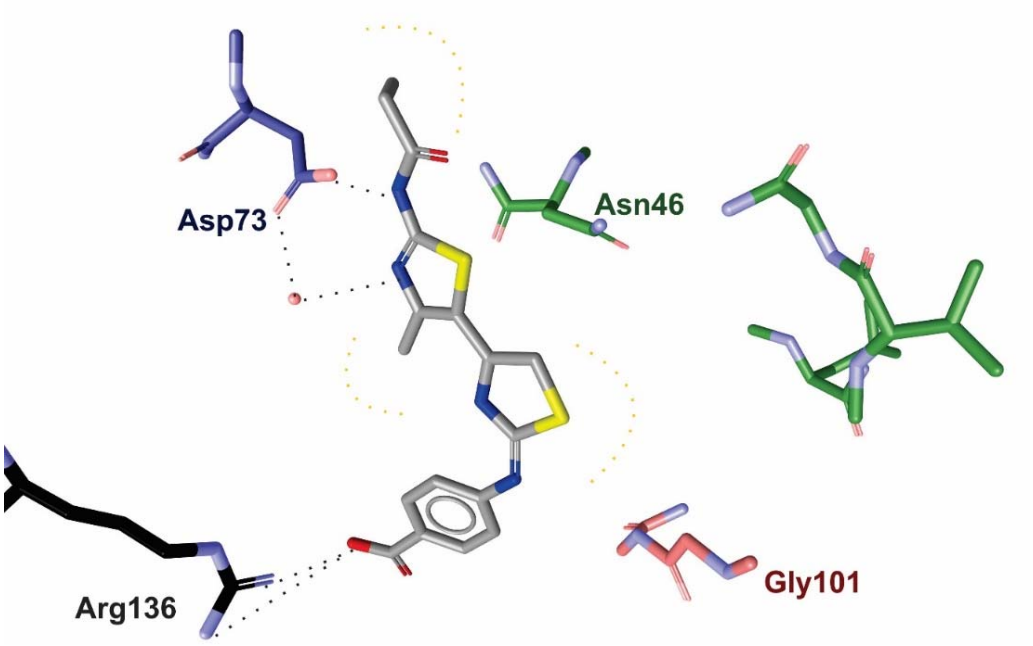


Figure S2. Docking binding mode of compound **13** in the DNA gyrase ATP binding site generated using gyrase crystal structure with the bound AMP-PNP substrate (PDB: 1EI1).

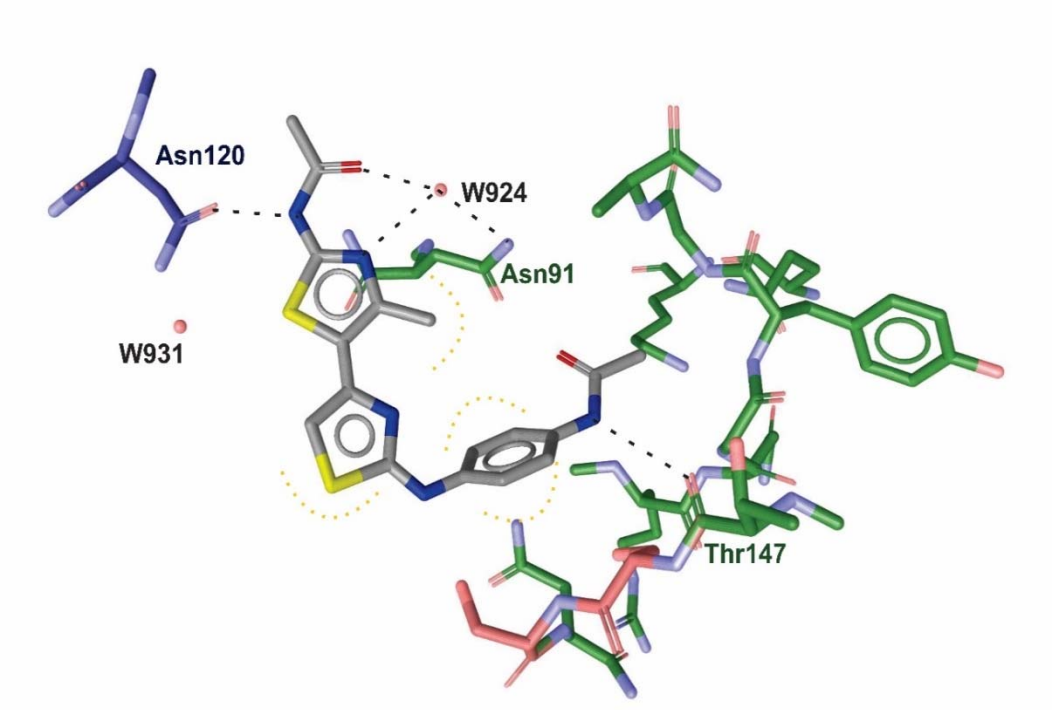


Figure S3. Proposed docking binding modes of active substituted 4,5'-bithiazole **9** in the human topo IIα **ATP** binding site (PDB: 1ZXM).

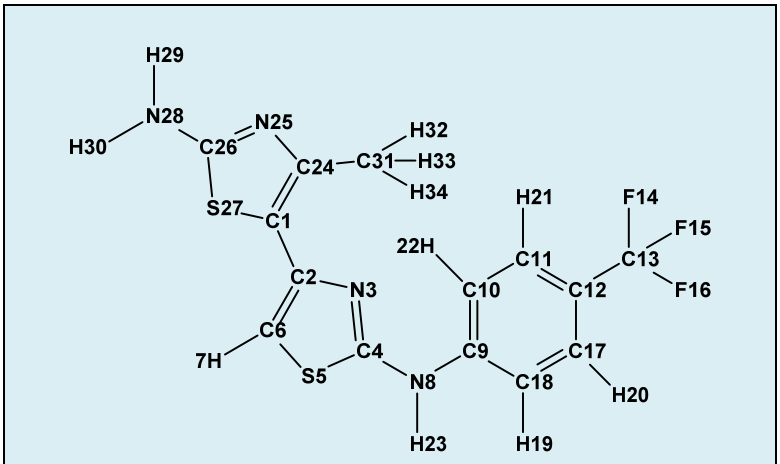
3. Comparison of the bacterial DNA gyrase vs. human topoisomerase IIα inhibition activities for compounds selected by both virtual screenings

Table S3. Comparison of the human topo IIα and bacterial DNA Gyrase inhibition vales for compounds that were selected in this screening assays as well as also by our previous virtual screening campaign on bacterial DNA Gyrase [ref 39].

Compound	IC_{50} [μM] bacterial DNA gyrase	IC_{50} [μM] human topo IIa
5	8.2	70.1
6	67	37.7
8	5.5	119.7
12	30.0	192.9
13	1.1	123.0

4. Molecular dynamics simulation and dynophore calculations

Table S4. Assigned atom types and partial atomic charges of compound **1** using CHARMM General Force Filed (CGenFF).



Name	Type	q	Name	Type	q
C1	CG2R57	-0,121	**C18**	CG2R61	-0,115
C2	CG2R57	0,277	**H19**	HGR61	0,155
N3	NG2R50	-0,638	**H20**	HGR61	0,250
C4	CG2R53	0,341	**H21**	HGR61	0,250
S5	SG2R50	0,015	**H22**	HGR61	0,115
C6	CG2R51	-0,239	**H23**	HGPAM1	0,376
H7	HGR52	0,210	**C24**	CG2R51	0,276
N8	NG311	-0,310	**N25**	NG2R50	-0,624
C9	CG2R61	-0,034	**C26**	CG2R53	0,280
C10	CG2R61	-0,115	**S27**	SG2R50	0,020
C11	CG2R61	-0,196	**N28**	NG321	-0,574
C12	CG2R61	-0,432	**H29**	HGPAM2	0,334
C13	CG302	0,803	**H30**	HGPAM2	0,334
F14	FGA3	-0,159	**C31**	CG331	-0,195
F15	FGA3	-0,159	**H32**	HGA3	0,090
F16	FGA3	-0,159	**H33**	HGA3	0,090
C17	CG2R61	-0,196	**H34**	HGA3	0,090

4.1 Measured time-dependant distances between selected protein atoms and Compound 1

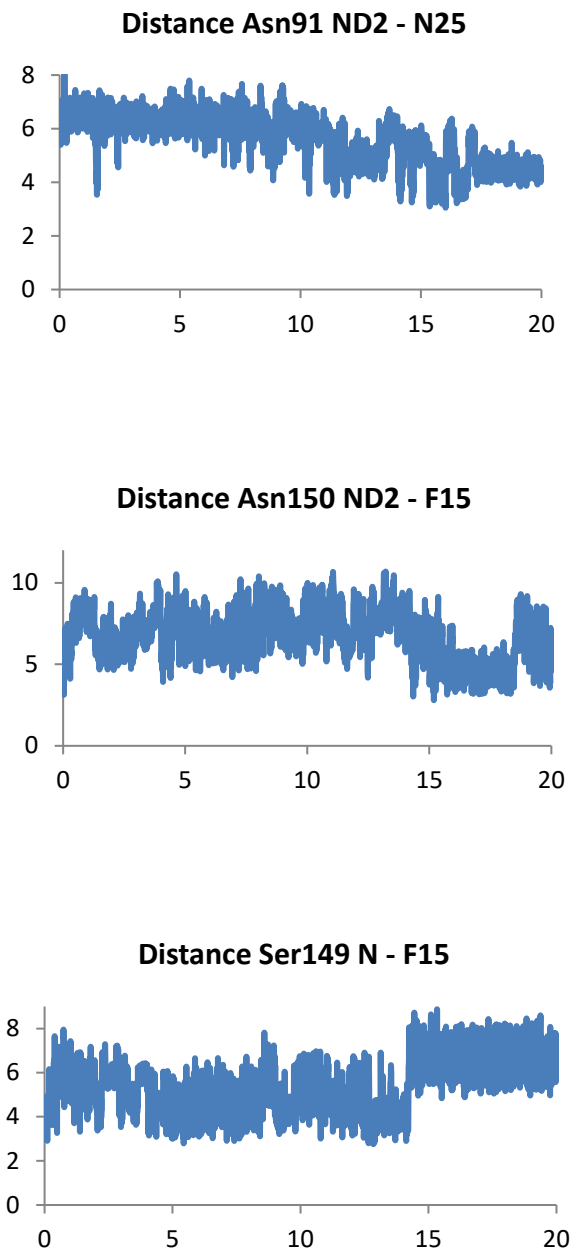


Figure S4. Measured time-dependant distances between selected protein atoms and compound **1** during the MD simulation.

5. Results of the human topo IIα and human topo IIβ decatenation assay

Table S5: Results of the decatenation assay catalysed by human topo IIα for compounds **1** and **9** and positive control etoposide. Results are represented as % of decatenated kDNA.

Compound	% Decatenated Assay 1				% Decatenated Assay 2				% Decatenated Average			
	Concentration (µM)				Concentration (µM)				Concentration (µM)			
	3.9	**31.5**	**125**	**500**	**3.9**	**31.5**	**125**	**500**	**3.9**	**31.5**	**125**	**500**
Etoposide	100	91	21.6	1.5	100	96.7	44	7.2	100	93.9	32.8	4.4
1	100	72.4	0	0	91	89.4	0	0	95.5	80.9	0	0
9	98.7	99.4	89.5	34	99.2	97.9	86.7	31.3	98.9	98.7	88.1	32.7

Table S6: Results of the decatenation assay catalysed by human topo IIβ for compounds **1** and **9** and positive control etoposide). Results are represented as % of decatenated kDNA.

Compound	% Decatenated Assay 1				% Decatenated Assay 2				% Decatenated Average			
	Concentration (µM)				Concentration (µM)				Concentration (µM)			
	3.9	**31.5**	**125**	**500**	**3.9**	**31.5**	**125**	**500**	**3.9**	**31.5**	**125**	**500**
Etoposide	100.0	94.0	24.6	15.0	100.0	92.8	23.1	14.4	100.0	93.4	23.9	14.7
1	87.3	78.6	0	0	95.5	79.7	0	0	91.3	79.2	0	0
9	87.0	80.1	30.5	17.5	75.6	73.3	21.7	5.3	81.3	76.7	26.1	11.4

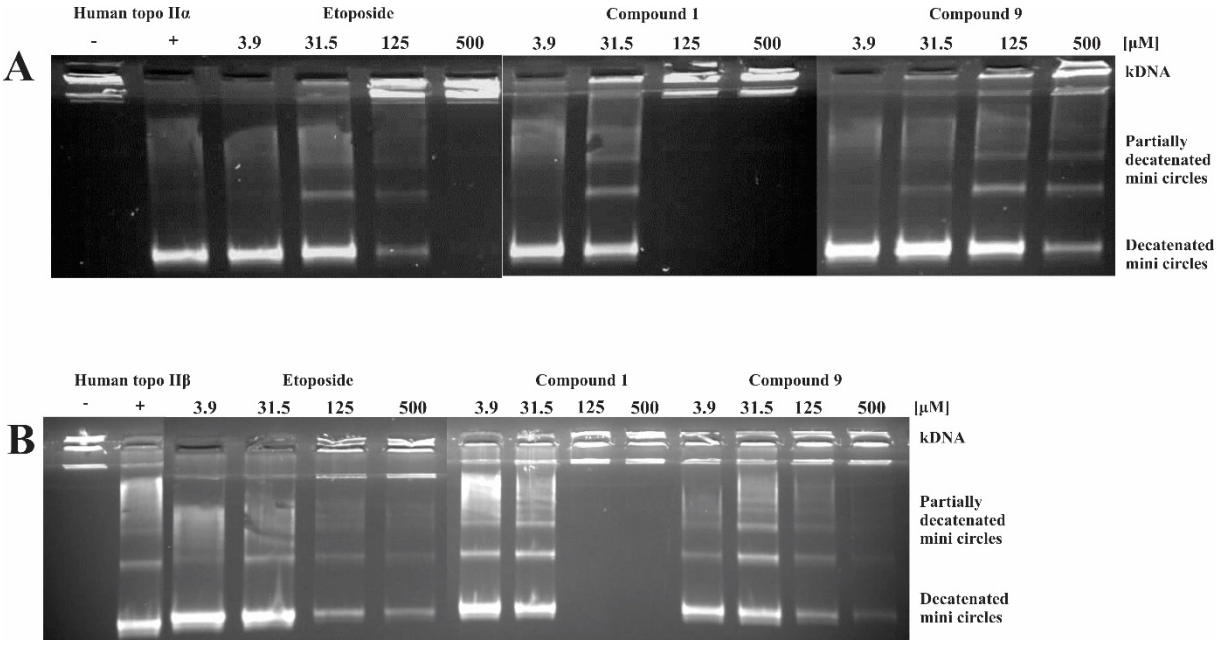


Figure S5: (**A**) Results of the second run of the decatenation assay of human topo IIα for compounds **1**, **9** and etoposide. The assay was performed at 4 different concentrations (3.9, 31.5, 125 and 500 µM) of compound **1** and **9** and reference compound etoposide. (**B**) Results of the second run of the decatenation assay of human topo IIβ for compounds **1**, **9** and

etoposide. The assay was performed at 4 different concentrations (3.9, 31.5, 125 and 500 μM) of compound **1**, **9** and reference compound etoposide.

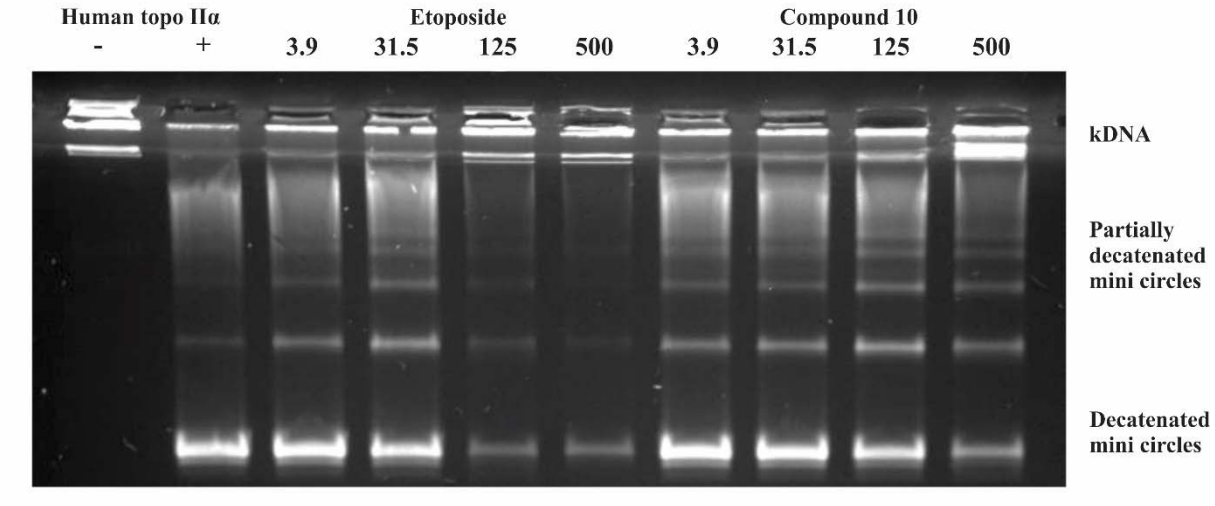


Figure S6. Results of the first run of the human topo IIα decatenation assay for compound **10** and etoposide. The assay was performed at 4 different concentrations (3.9, 31.5, 125 and 500 μM) of compound **10** and reference compound etoposide. Compound **10** inhibited 64% decatenation at concentration 500 μM and 26% at 125 μM. Experiment was performed in duplicates (second run is not shown).

6. Results of the human topo IIα cleavage assay

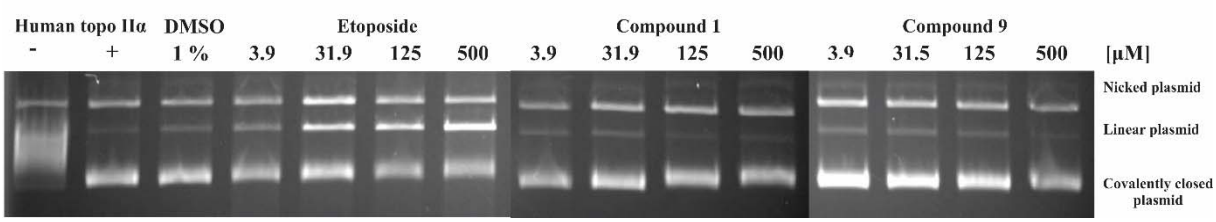


Figure S7. Results of the second run of the topoisomerase IIα cleavage assay. The assay was performed at 4 different concentrations (3.9, 31.5, 125 and 500 μM) of compound **1** and **9** and reference compound etoposide. Linear band was measured as a % of the total DNA, with 100% representing the amount of DNA in track 1 (DNA alone).

Table S7. % of linear DNA, determined in the cleavage assay for compounds **1** and **9** and etoposide at four concentrations (500, 125, 31.5 and 3.9 μM).

Compound	% Linear	% Linear	% Linear Average
DNA alone	0	0	0
DNA + topo II	55.5	4.08	4.81
DNA + topo II + DMSO	7.45	8.77	8.11
Etoposide 3.9 μM	22.76	11.88	17.32
Etoposide 31.5 μM	33.00	36.26	34.63
Etoposide 125 μM	45.35	36.91	41.13
Etoposide 500 μM	43.03	48.40	45.71
Compound 1 3.9 μM	8.05	5.57	6.81
Compound 1 31.5 μM	6.32	7.77	7.04
Compound 1 125 μM	3.02	2.85	2.94
Compound 1 500 μM	2.45	2.98	2.72
Compound 9 3.9 μM	5.49	7.03	6.26
Compound 9 31.5 μM	7.77	6.75	7.26
Compound 9 125 μM	4.84	4.60	4.72
Compound 9 500 μM	2.95	2.25	2.60

7. Results of the human topo IIα ATPase activity

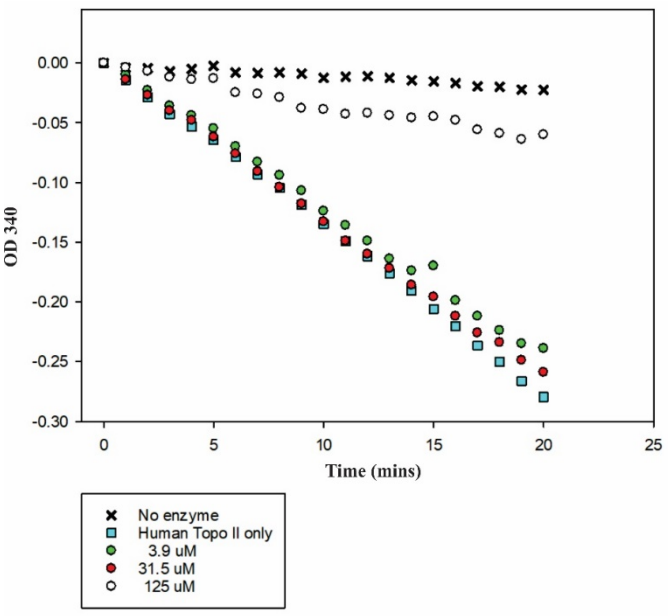


Figure S8. Results of the ATPase assay (second run) of compound **1**. It inhibited 85% of ATPase activity at 125 μM. The reaction was performed at three concentrations (3.9, 31.5 and 125 μM).

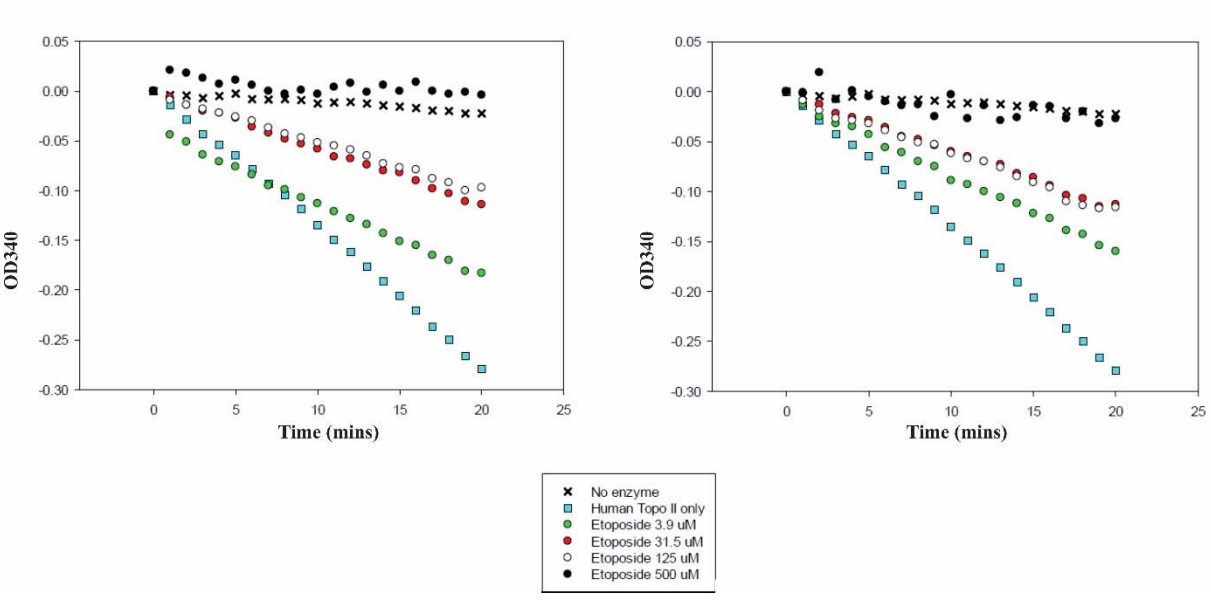


Figure S9. Results of the ATPase assay for etoposide (two runs). Results of the ATPase assay for the second run of compound **1.** The reaction was performed at four concentrations (3.9, 31.5, 125 and 500 μM). Etoposide inhibited 70% (first run) or 63% (second run) of ATPase activity at 125 μM.

Table S8. Results of the ATPase assay given as % activity which were calculated as follows: The spectrophotometer produces an output as change in OD340 with time. After 40 or 60 minutes the blank reading (no enzyme) was subtracted from the readings for each compound and divided by the positive reading (no inhibitor, 100% activity) minus the blank to give the percentage ATPase activity for each compound. There were solubility problems with compound **1** at 500 μM (erratic readings). 2 negative controls and 5 positive controls were run along with a 10 μM etoposide control.

	Rate ATPase activity (μmol/min)							
	Run 1				**Run 2**			
	No htII #1		0.26		**No htII #2**		0.21	
	htII only #1		3.35		**htII only #2**		3.12	
	htII only #3		3.33		**htII only #4**		3.14	
	htII onliy #5		3.19		**+ novobiocin 10μM**		0.14	
	Average No htII		0.24		**Average htII only**		3.22	
	Percentage ATPase activity of compounds				**Percentage ATPase activity of compounds**			
	Concentration (μM) Run 1				**Concentration (μM) Run 2**			
Compound	**3.9**	**31.5**	**125**	**500**	**3.9**	**31.5**	**125**	**500**
Etoposide	52.71	36.43	30.23	0	51.94	37.21	37.21	4.65
Compound 1	92.25	96.12	24.81	65.12	88.37	94.57	16.28	36.43

8. Human topoisomerase II ATPase assays at different concentration of the ATP – Competitive ATPase assay

The spectrophotometer produces an output as change in OD340 with time (data not shown). The data from the runs after the addition of the ATP were plotted after adjusting the starting OD340 to zero. Lines are fitted to these by linear regression and the rates (OD change/min) calculated. The rates for each ATP and inhibitor concentration were then calculated as µM ATP hydrolysed/min using an extinction coefficient of 6.22 M^{-1} cm^{-1} and a path length of 0.5 cm. The rates were then plotted against the ATP concentration and curves fitted to the data using the hyperbolic equation y=(ax/(b+x) where y = rate, x = [ATP], a = Vmax and b=Km. Using the equation y=y0+(ax/(b+x)) it was possible to solve it and obtain values.

Table S9: The table shows the rates (µM /min) at different compound and ATP concentrations after subtraction of the background rate and calculated Km (mM ATP) and Vmax (µM ATP/min) values. They were calculated from equation y = (ax /(b+x) where y = rate, x = [ATP], a = Vmax.

Rates for controls without enzyme in the presence of 2 mM ATP (µmol/min)									
100 µM	**75 µM**		**50 µM**		**31 µM**		**3.9 µM**		**0 µM**
1.125	**0.515**		**0.354**		**0.354**		**0.257**		**0.386**
Rates (µmol/min) at different ATP concentrations (mM) after subtraction of background									
[Cp 1] µM	**2**	**1**	**0.75**	**0.5**	**0.25**	**0.1**	**0.075**	**0.05**	**0.025**
100	0.772	0.354	0.289	0.418	0.064	0.032	0.000	0.032	0.064
75	0.740	0.354	0.386	0.322	0.225	-0.064	-0.096	0.032	0.096
50	1.222	0.772	0.707	0.450	0.193	0.032	0.129	0.096	0.000
31	1.286	1.061	0.868	0.707	0.450	0.161	0.096	-0.032	-0.129
3.9	2.444	1.897	1.801	1.479	1.158	0.450	0.354	0.257	0.064
0	2.990	2.605	2.508	1.994	1.350	0.707	0.482	0.322	0.129

Calculated Km (mM ATP) and Vmax (µM ATP/min) values						
[Cp 1] µM	**100**	**75**	**50**	**31**	**3.9**	**0**
Km	6.14	3.35	2.5	0.88	0.49	0.41
Vmax	3.07	1.94	2.77	1.9	2.97	3.72

9. Cytotoxicity on MCF-7 and HepG2 cell lines using MTS assay

Table S10. Determined cytotoxicity of the substituted 4,5′-bithiazoles represented by EC_{50} values and value ranges on HepG2 and MCF-7 cancer cell lines (24h vs. 72h).

Compound	EC_{50} [μM] **MCF-7** (24h)	EC_{50} [μM] **MCF-7** (72h)	EC_{50} [μM] **HepG2** (24h)	EC_{50} [μM] **HepG2** (72h)
1	65.0 (58.1-70.9)	59.5 (58.1-59.3)	72.8 (64.2-86.1)	46.8 (47.1-49.3)
7	12.3 (10.4-14.9)	6.6 (4.6-7.6)	45.6 (32.8-53.7)	32.3 (29.7-37.2)
9	15.0 (11.2-16.2)	13.8 (9.7-15.9)	73.5 (59.6-80.8)	23.5 (17.1-32.1)
10	16.4 (14.6-17.0)	4.5 (3.4-5.9)	34.7 (31.5-38.9)	14.6 (12.7-19.5)
14	32.3 (28.2-34.3)	7.5 (5.2-6.7)	47.7 (31.7-60.4)	28.2 (20.5-29.9)
Etoposide	320.2 (226.9-358.9)	12.6 (10.1-15.9)	196.2 (169.4-201.5)	25.8 (15.1-35.3)

10. Investigation of the effect of 4,5'-bithiazoles on the cell cycle and cell proliferation

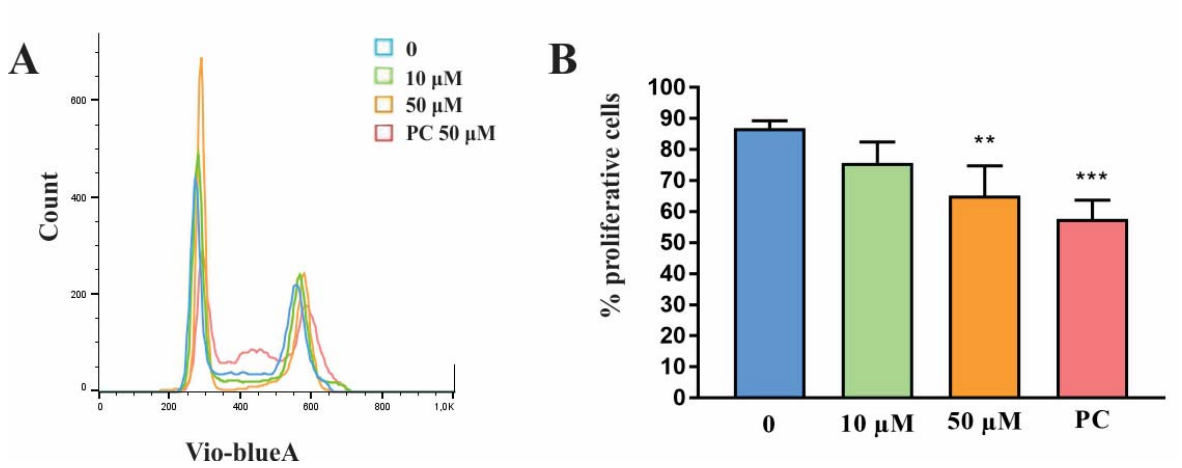


Figure S10. A) Representative histograms for non-labelled cells, vehicle control (0), compound **1** at 10 µM and 50 µM and etoposide at 50 µM for cell cycle analysis. (B) Percent of proliferative cells after 24h treatment to compound **1** at 50 µM and 10 µM determined with the Ki67 antibody (** $p < 0.01$ and *** $p < 0.001$).

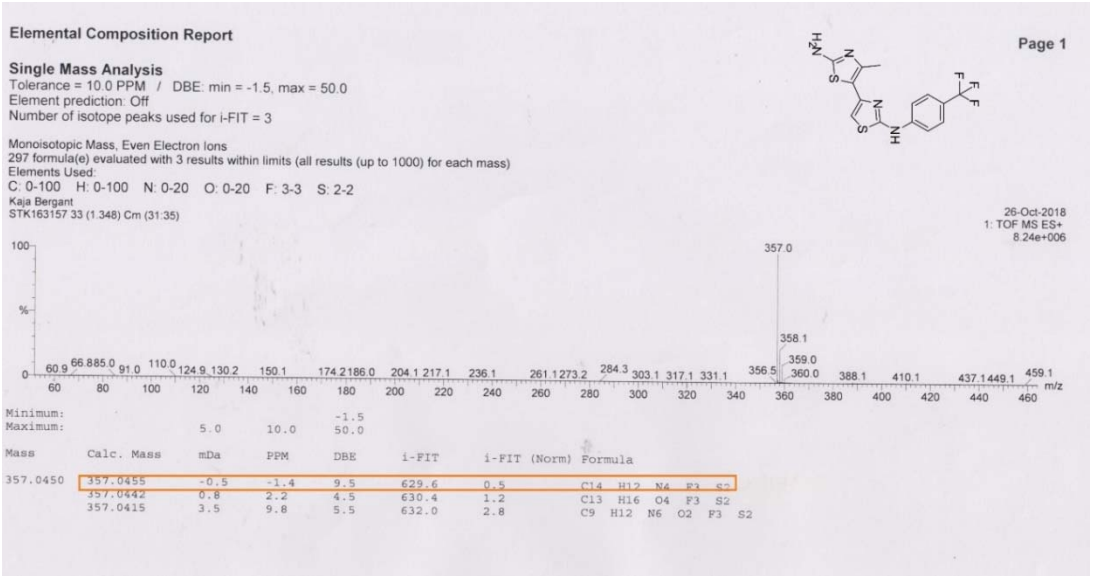
Table S11. Results of cell cycle analysis for compound 1. Experiments were performed in 3 independent measurements and SD values were calculated. Significant differences between the vehicle control (0) and treated cells (compound 1) as well as PC (etoposide; 50 µM) were calculated using ANOVA (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

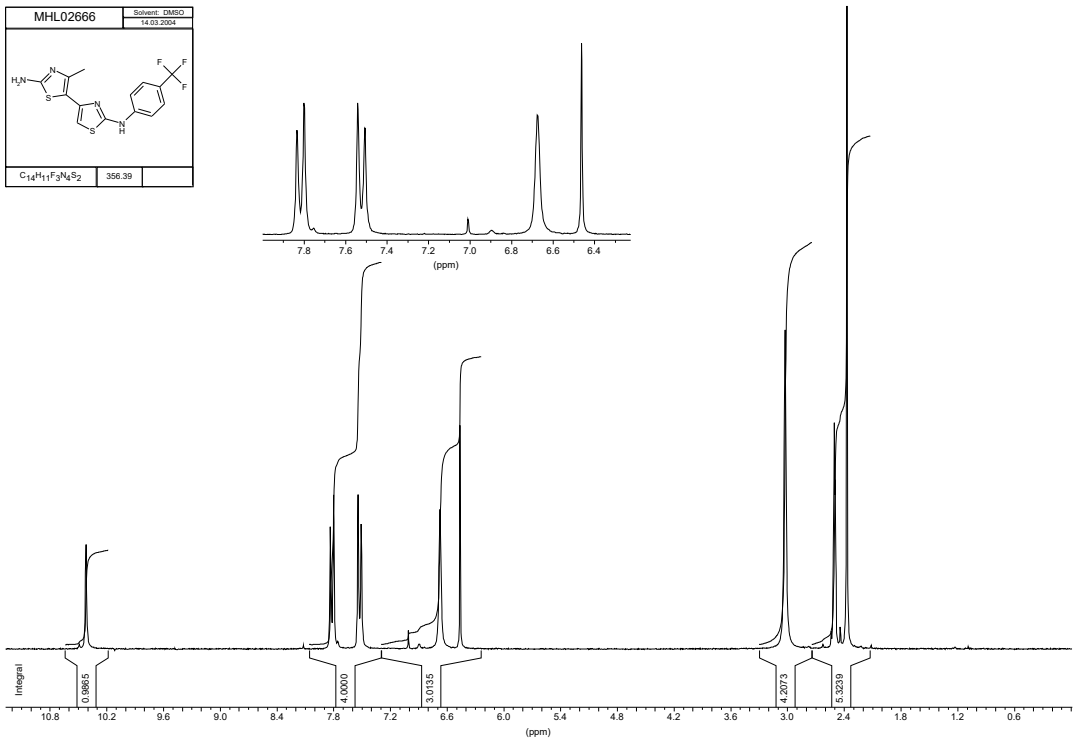
Compound	0	1 (10 µM)	1 (50 µM)	PC
G1	46.7 ± 4.6	56.5 ± 6.4	60.6 ± 7.0*	33.1 ± 2.4*
S	25.9 ± 3.3	17.5 ± 1.1***	11.8 ± 4.5**	44.0 ± 1.5***
G2	25.4 ± 4.9	24.8 ± 4.5	26.2 ± 2.5	22.9 ± 3.5
>G1, G2<	2 ± 1.1	1.2 ± 1.0	1.4 ± 0.6	0

11. NMR, HR-MS, elemental analysis and HPLC purity of active compounds

1) The HR-MS analysis were performed at the Centre for Mass spectroscopy, Josef Stefan Institute, Ljubljana.
2) The 1H-NMR spectra were supplied by vendors.
3) Elemental analysis was performed at the National Institute of Chemistry, Ljubljana using Perkin-Elmer C, H, N, S analyser.
4) HPLC purity measurements were performed at National Institute of Chemistry Slovenia

Compound 1





Compound 2

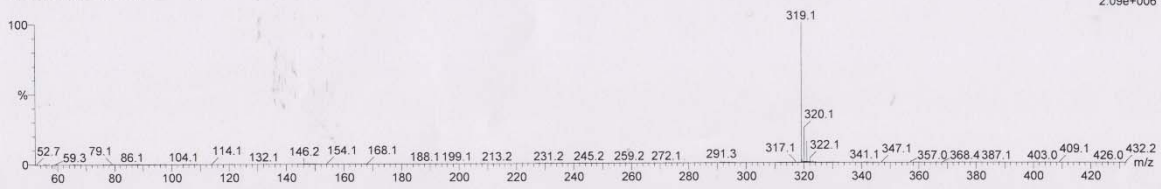
Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

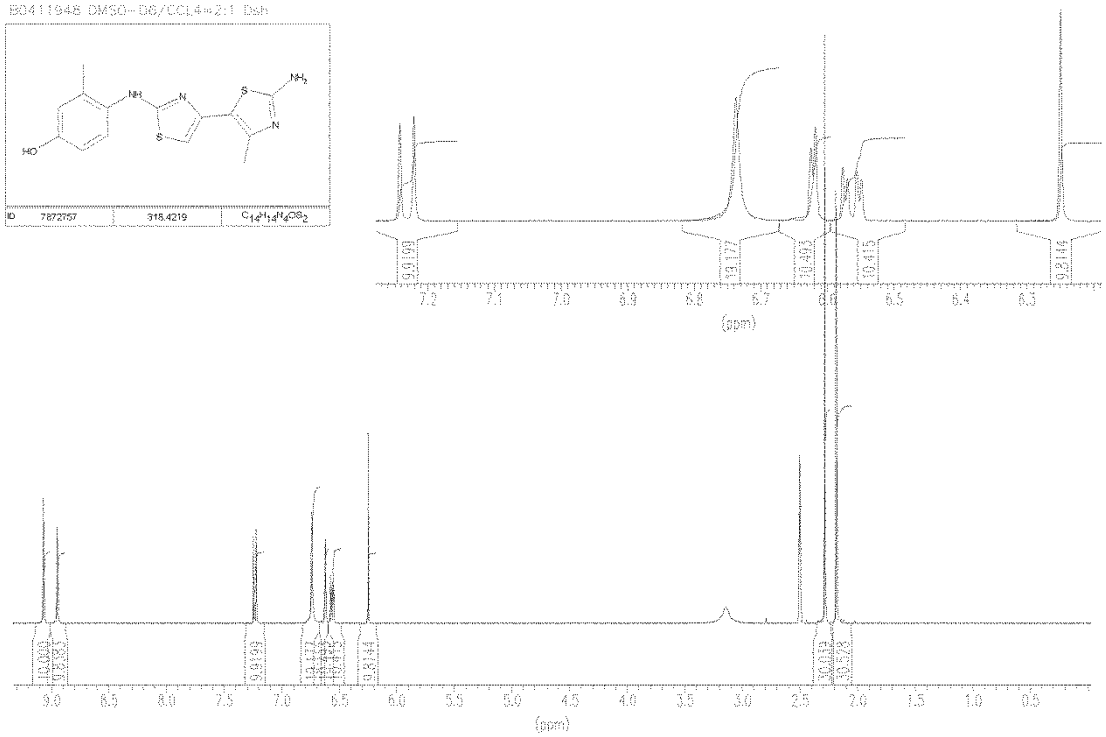
Monoisotopic Mass, Even Electron Ions
404 formula(e) evaluated with 8 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-20 O: 0-20 S: 2-2

Kaja Bergant
7872757 26 (1.071) Cm (26:27-1:16)

25-Oct-2018
1: TOF MS ES+
2.09e+006

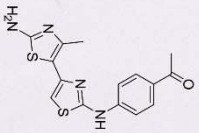


Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
319.0683	319.0687	-0.4	-1.3	9.5	535.9	0.7	C14 H15 N4 O S2
	319.0674	0.9	2.8	4.5	536.4	1.1	C13 H19 O5 S2
	319.0647	3.6	11.3	5.5	538.0	2.8	C9 H15 N6 O3 S2
	319.0634	4.9	15.4	0.5	538.4	3.1	C8 H19 N2 O7 S2
	319.0746	-6.3	-19.7	0.5	538.8	3.5	C7 H19 N4 O6 S2
	319.0620	6.3	19.7	6.5	539.5	4.3	C5 H11 N12 O S2
	319.0733	-5.0	-15.7	6.5	539.9	4.6	C4 H11 N14 S2
	319.0719	-3.6	-11.3	1.5	540.2	4.9	C3 H15 N10 O4 S2



Compound 3

Elemental Composition Report Page 1

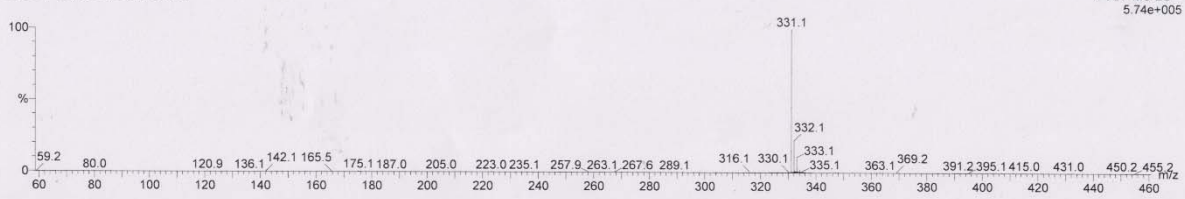


Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

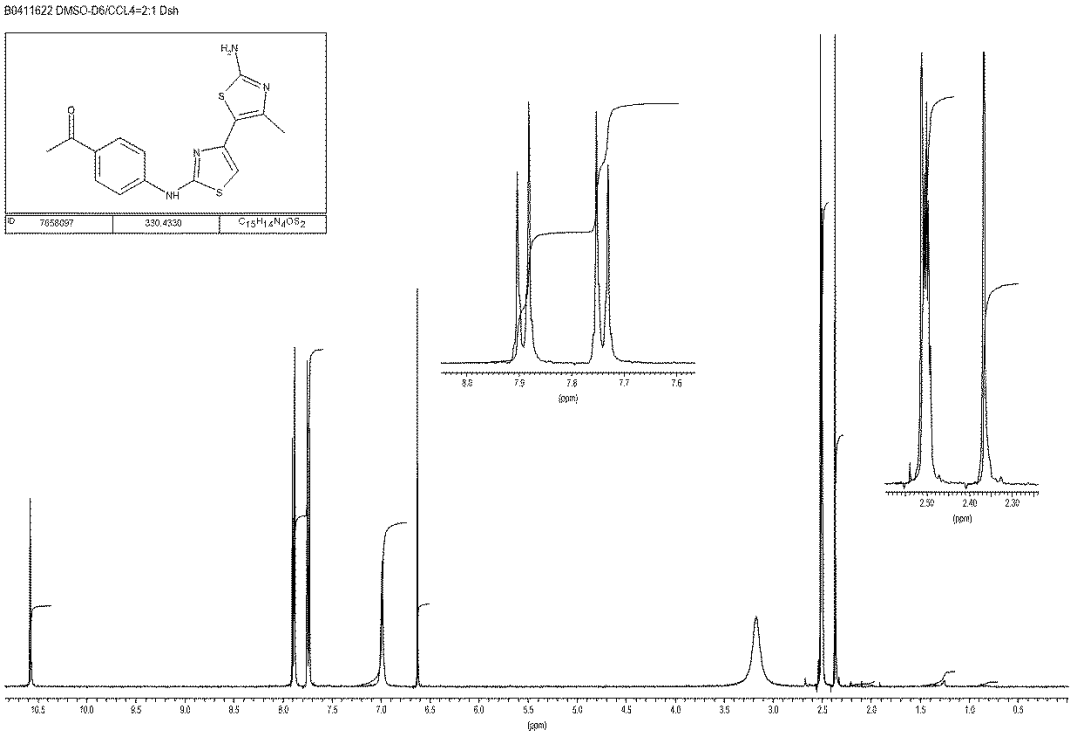
Monoisotopic Mass, Even Electron Ions
455 formula(e) evaluated with 9 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-20 O: 0-20 S: 2-2

Kaja Bergant
7858097 50 (2.031) Cm (50-2:22)

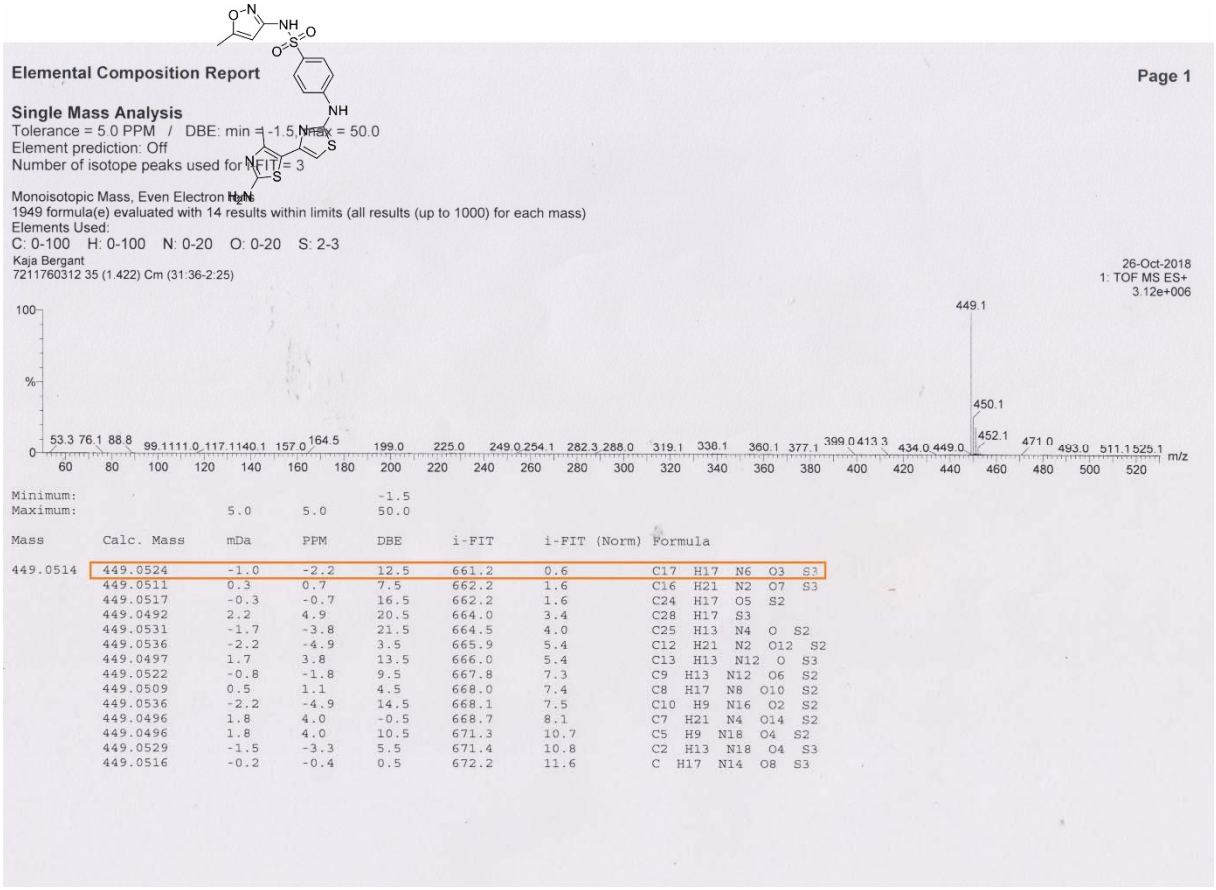
25-Oct-2018
1: TOF MS ES+
5.74e+005



Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
331.0685	331.0687	-0.2	-0.6	10.5	494.1	0.1	C15 H15 N4 O S2
	331.0674	1.1	3.3	5.5	497.2	3.1	C14 H19 O5 S2
	331.0647	3.8	11.5	6.5	499.0	4.9	C10 H15 N6 O3 S2
	331.0634	5.1	15.4	1.5	500.2	6.2	C9 H19 N2 O7 S2
	331.0746	-6.1	-18.4	1.5	500.6	6.5	C8 H19 N4 O6 S2
	331.0620	6.5	19.6	7.5	501.1	7.0	C6 H11 N12 O S2
	331.0733	-4.8	-14.5	7.5	501.4	7.3	C5 H11 N14 S2
	331.0719	-3.4	-10.3	2.5	502.5	8.4	C4 H15 N10 O4 S2
	331.0692	-0.7	-2.1	3.5	504.8	10.8	H11 N16 O2 S2



Compound 4



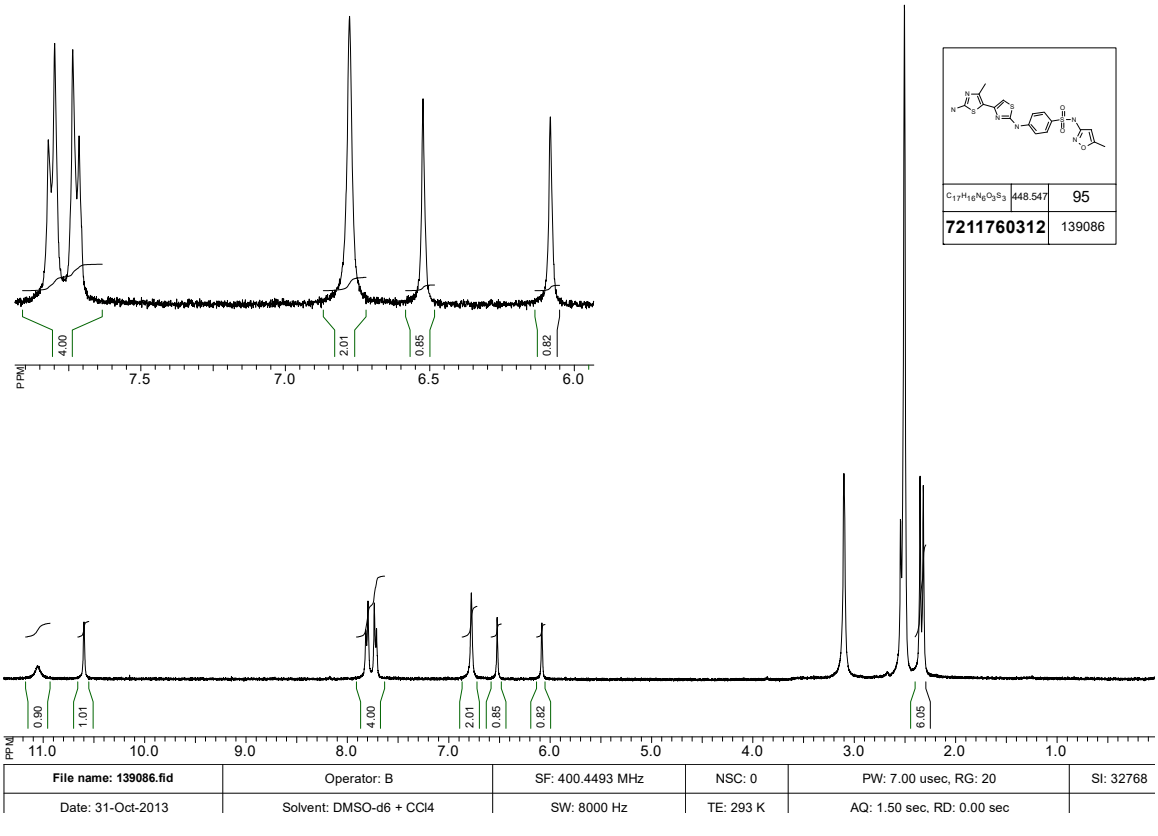
Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for FIT = 3

Monoisotopic Mass, Even Electron Ions
1949 formula(e) evaluated with 14 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-20 O: 0-20 S: 2-3
Kaja Bergant
7211760312 35 (1.422) Cm (31:36-2:25)

26-Oct-2018
1: TOF MS ES+
3.12e+006

Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
449.0514	449.0524	-1.0	-2.2	12.5	661.2	0.6	C17 H17 N6 O3 S3
	449.0511	0.3	0.7	7.5	662.2	1.6	C16 H21 N2 O7 S3
	449.0517	-0.3	-0.7	16.5	662.2	1.6	C24 H17 O5 S2
	449.0492	2.2	4.9	20.5	664.0	3.4	C28 H17 S3
	449.0531	-1.7	-3.8	21.5	664.5	4.0	C25 H13 N4 O S2
	449.0536	-2.2	-4.9	3.5	665.9	5.4	C12 H21 N2 O12 S2
	449.0497	1.7	3.8	13.5	666.0	5.4	C13 H13 N12 O S3
	449.0522	-0.8	-1.8	9.5	667.8	7.3	C9 H13 N12 O6 S2
	449.0509	0.5	1.1	4.5	668.0	7.4	C8 H17 N8 O10 S2
	449.0536	-2.2	-4.9	14.5	668.1	7.5	C10 H9 N16 O2 S2
	449.0496	1.8	4.0	-0.5	668.7	8.1	C7 H21 N4 O14 S2
	449.0496	1.8	4.0	10.5	671.3	10.7	C5 H9 N18 O4 S2
	449.0529	-1.5	-3.3	5.5	671.4	10.8	C2 H13 N18 O4 S3
	449.0516	-0.2	-0.4	0.5	672.2	11.6	C H17 N14 O8 S3



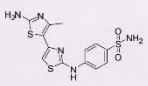
$C_{17}H_{16}N_6O_3S_3$	448.547	95		
7211760312	139086			

File name: 139086.fid	Operator: B	SF: 400.4493 MHz	NSC: 0	PW: 7.00 usec, RG: 20	SI: 32768
Date: 31-Oct-2013	Solvent: DMSO-d6 + CCl4	SW: 8000 Hz	TE: 293 K	AQ: 1.50 sec, RD: 0.00 sec	

Compound 5

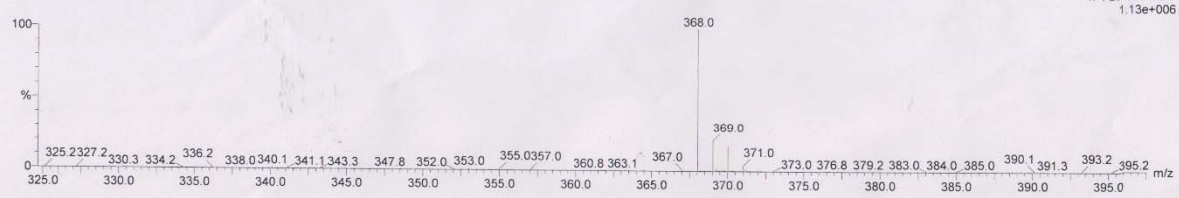
Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

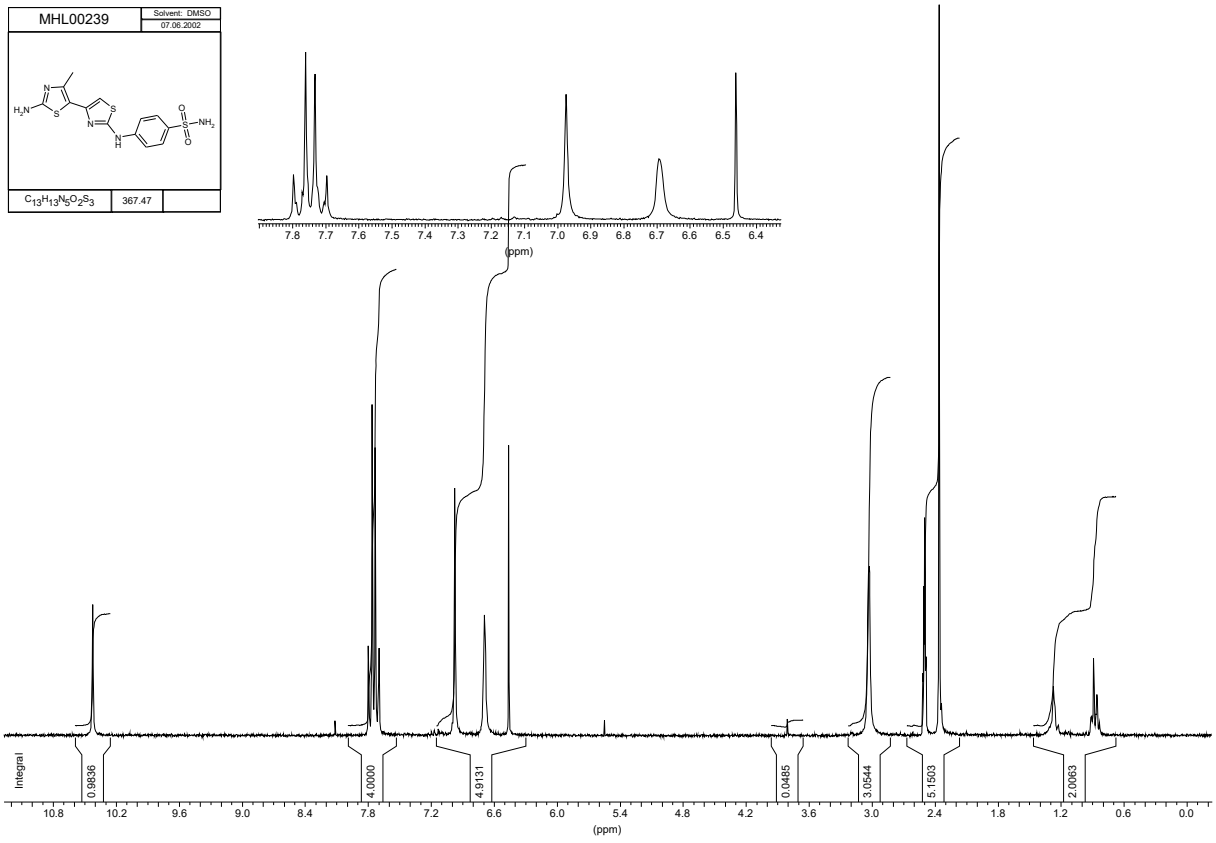


Monoisotopic Mass, Even Electron Ions
1070 formula(e) evaluated with 12 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-20 O: 0-20 S: 2-3
Kaja Bergant
STK015148 28 (1.145) Cm (28:33)

17-Dec-2018
1: TOF MS ES+
1.13e+006



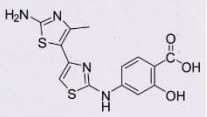
Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
368.0313	368.0310	0.3	0.8	9.5	626.1	0.2	C13 H14 N5 O2 S3
	368.0296	1.7	4.6	4.5	627.9	2.1	C12 H18 N O6 S3
	368.0316	-0.3	-0.8	18.5	630.0	4.1	C21 H10 N3 S2
	368.0321	-0.8	-2.2	0.5	630.1	4.3	C8 H18 N O11 S2
	368.0335	-2.2	-6.0	5.5	631.6	5.7	C9 H14 N5 O7 S2
	368.0283	3.0	8.2	10.5	632.0	6.2	C9 H10 N11 S3
	368.0348	-3.5	-9.5	10.5	632.6	6.7	C10 H10 N9 O3 S2
	368.0321	-0.8	-2.2	11.5	635.3	9.4	C6 H6 N15 O S2
	368.0308	0.5	1.4	6.5	635.6	9.7	C5 H10 N11 O5 S2
	368.0294	1.9	5.2	1.5	636.0	10.1	C4 H14 N7 O9 S2
	368.0342	-2.9	-7.9	1.5	637.2	11.3	C2 H14 N11 O5 S3
	368.0281	3.2	8.7	7.5	639.3	13.4	C H6 N17 O3 S2



Compound 6

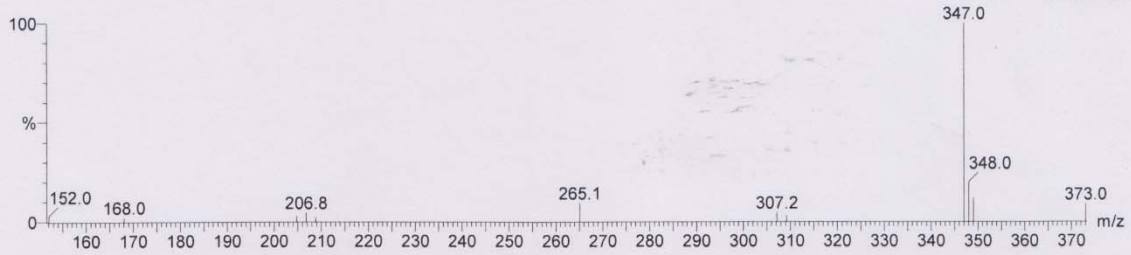
Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

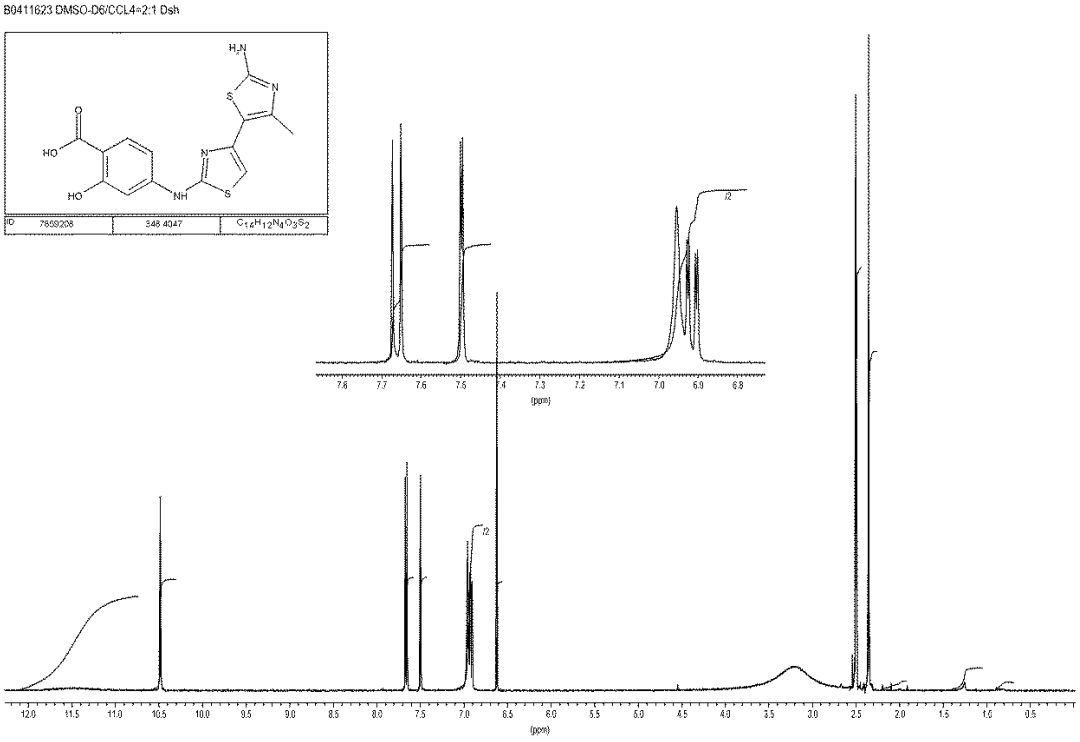


Monoisotopic Mass, Even Electron Ions
524 formula(e) evaluated with 6 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-20 O: 0-40 S: 2-2
Kaja Bergant
7859208 21 (0.868) Cm (20:21-4:8)

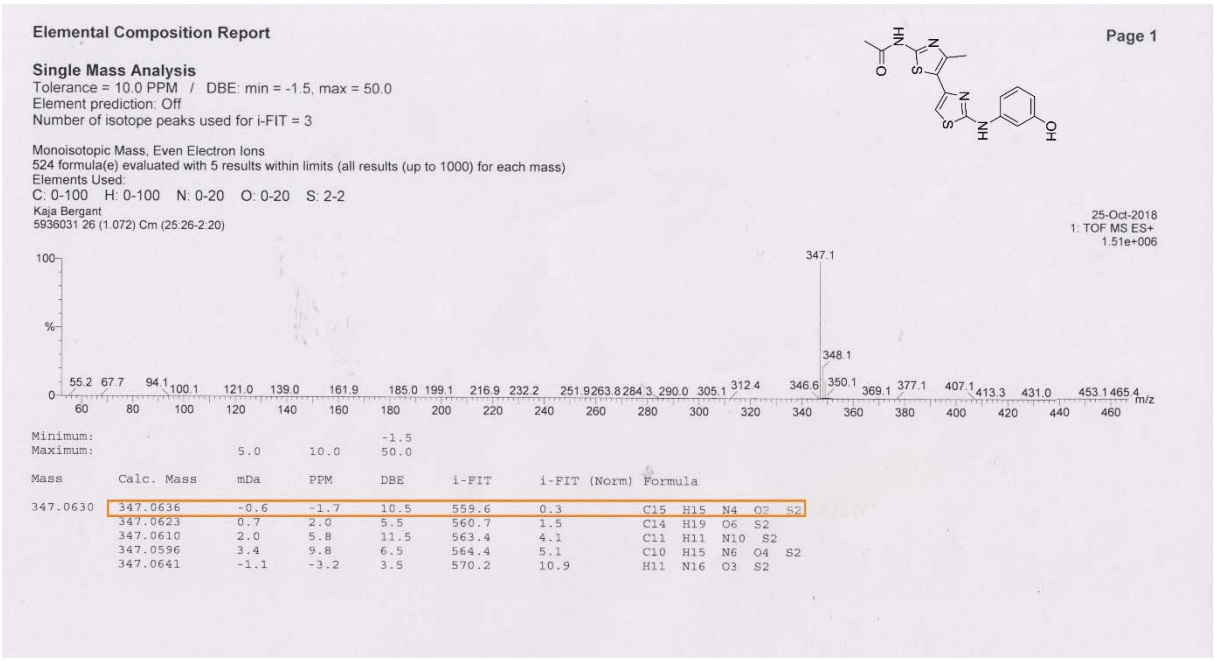
1: TOF MS ES-
8.48e+003



Minimum:				-1.5			
Maximum:		10.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
347.0272	347.0273	-0.1	-0.3	11.5	12.5	0.0	C14 H11 N4 O3 S2
	347.0259	1.3	3.7	6.5	16.2	3.8	C13 H15 O7 S2
	347.0246	2.6	7.5	12.5	18.1	5.6	C10 H7 N10 O S2
	347.0304	-3.2	-9.2	3.5	23.1	10.6	C3 H11 N10 O6 S2
	347.0291	-1.9	-5.5	-1.5	23.6	11.2	C2 H15 N6 O10 S2
	347.0291	-1.9	-5.5	9.5	24.5	12.1	H3 N20 S2



Compound 7



Elemental Composition Report Page 1

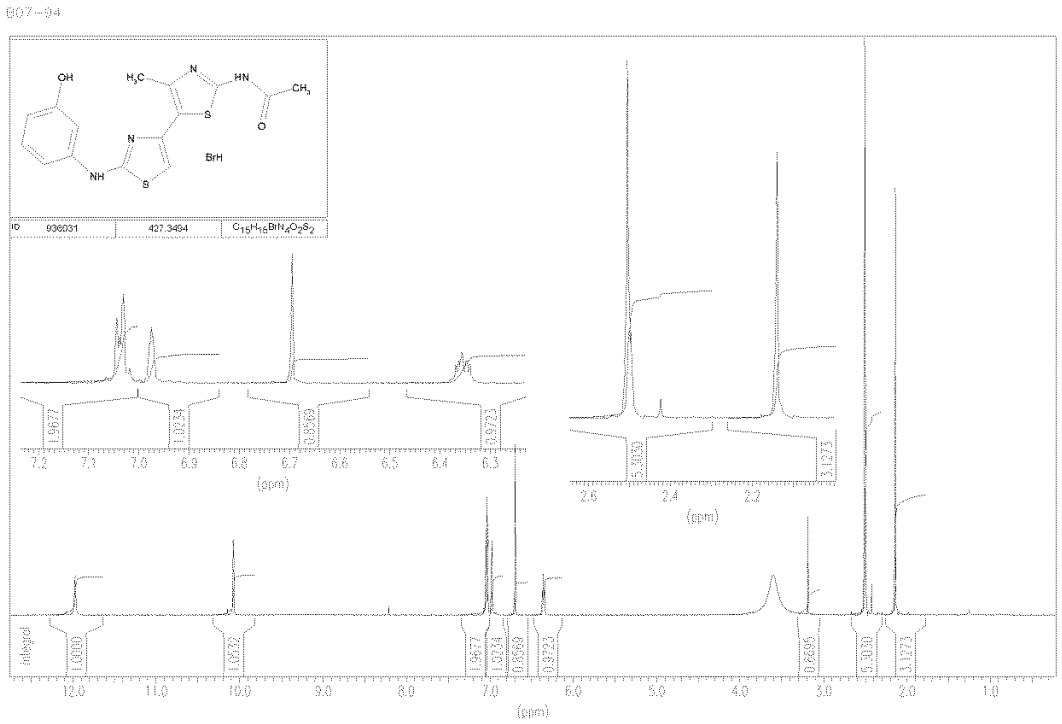
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
524 formula(e) evaluated with 5 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-20 O: 0-20 S: 2-2

Kaja Bergant
5936031 26 (1.072) Cm (25:26-2:20)

25-Oct-2018
1: TOF MS ES+
1.51e+006

Minimum:		5.0	10.0	-1.5			
Maximum:				50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
347.0630	347.0636	-0.6	-1.7	10.5	559.6	0.3	C15 H15 N4 O2 S2
	347.0623	0.7	2.0	5.5	560.7	1.5	C14 H19 O6 S2
	347.0610	2.0	5.8	11.5	563.4	4.1	C11 H11 N10 S2
	347.0596	3.4	9.8	6.5	564.4	5.1	C10 H15 N6 O4 S2
	347.0641	-1.1	-3.2	3.5	570.2	10.9	H11 N16 O3 S2

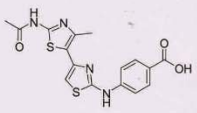


h

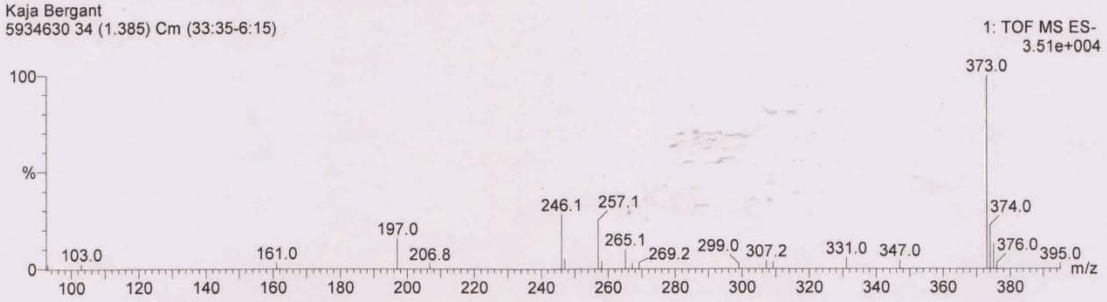
Compound 8

Elemental Composition Report Page 1

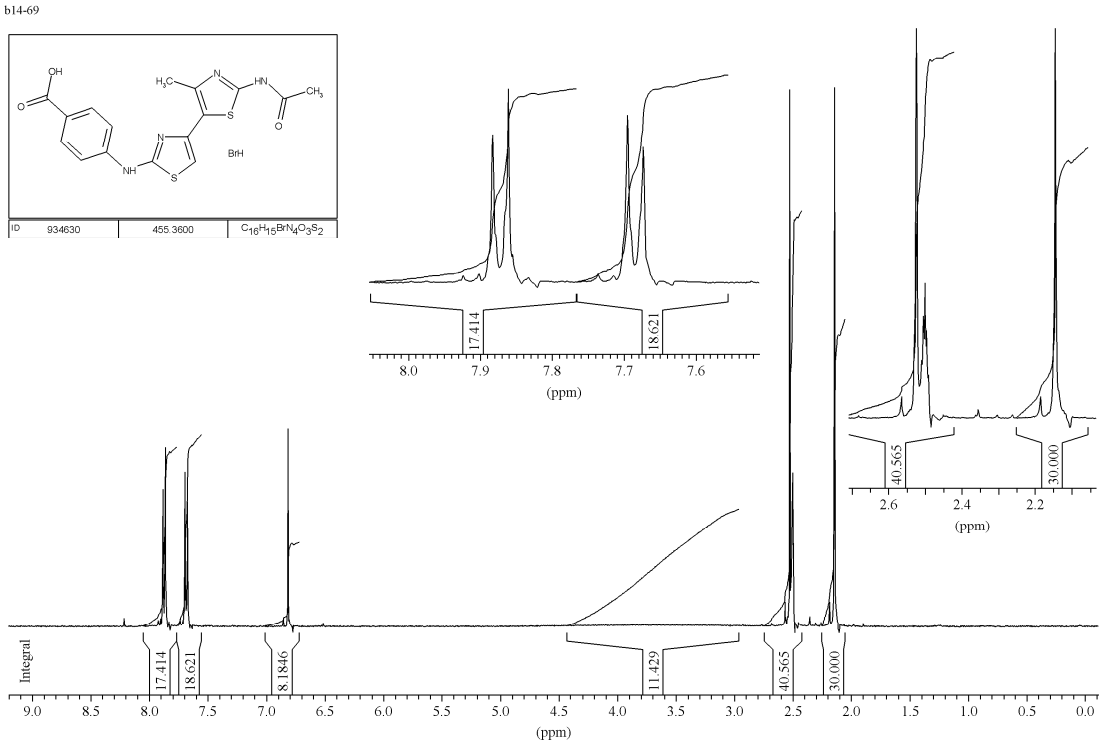
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3



Monoisotopic Mass, Even Electron Ions
650 formula(e) evaluated with 8 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-20 O: 0-40 S: 2-2
Kaja Bergant
5934630 34 (1.385) Cm (33:35-6:15)

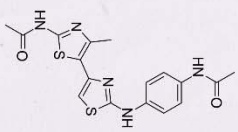


Minimum:				-1.5			
Maximum:		10.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
373.0420	373.0429	-0.9	-2.4	12.5	20.9	0.2	C16 H13 N4 O3 S2
	373.0416	0.4	1.1	7.5	22.7	1.9	C15 H17 O7 S2
	373.0402	1.8	4.8	13.5	24.4	3.7	C12 H9 N10 O S2
	373.0389	3.1	8.3	8.5	25.6	4.8	C11 H13 N6 O5 S2
	373.0448	-2.8	-7.5	-0.5	29.1	8.3	C4 H17 N6 O10 S2
	373.0447	-2.7	-7.2	10.5	29.6	8.9	C2 H5 N20 S2
	373.0434	-1.4	-3.8	5.5	30.3	9.6	C H9 N16 O4 S2
	373.0421	-0.1	-0.3	0.5	31.0	10.3	H13 N12 O8 S2



Compound 9

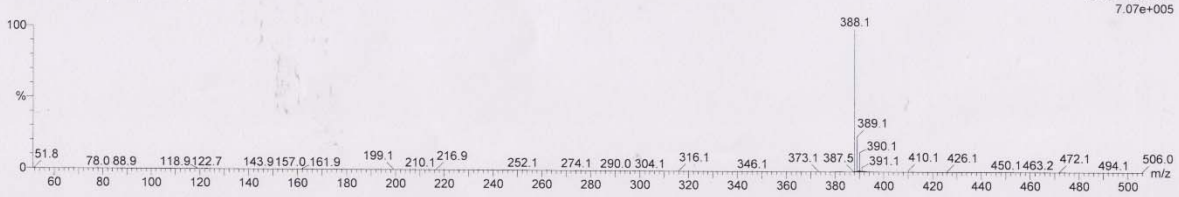
Elemental Composition Report Page 1



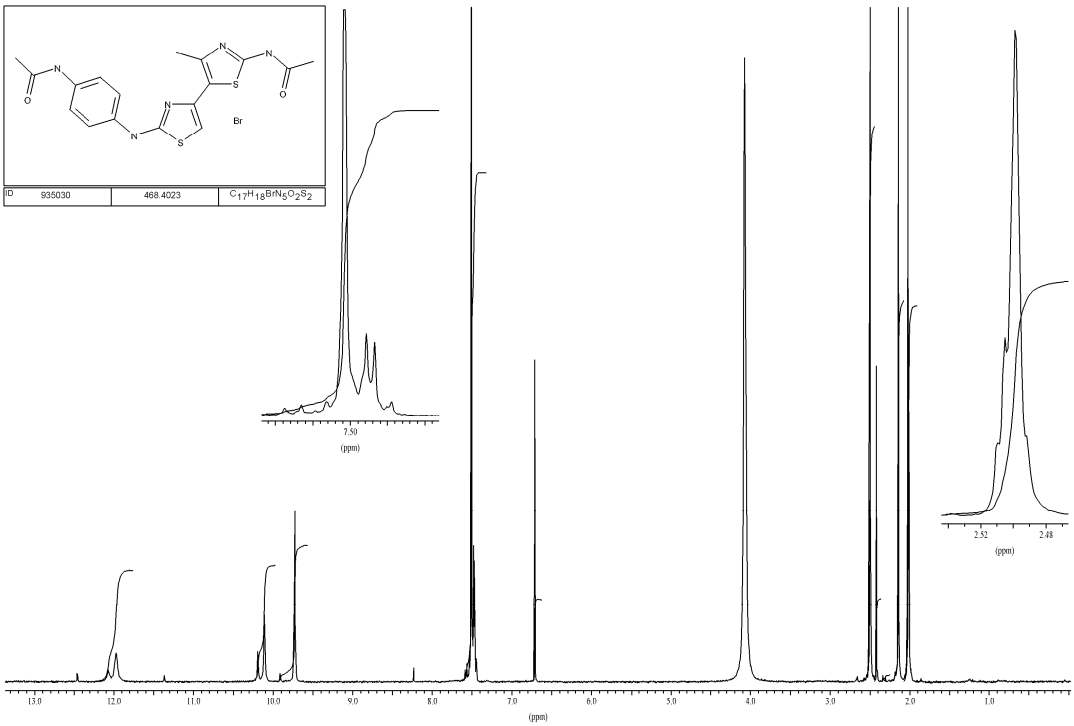
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
710 formula(e) evaluated with 8 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-20 O: 0-20 S: 2-2
Kaja Bergant
5935030 40 (1.625) Cm (40-9:24)

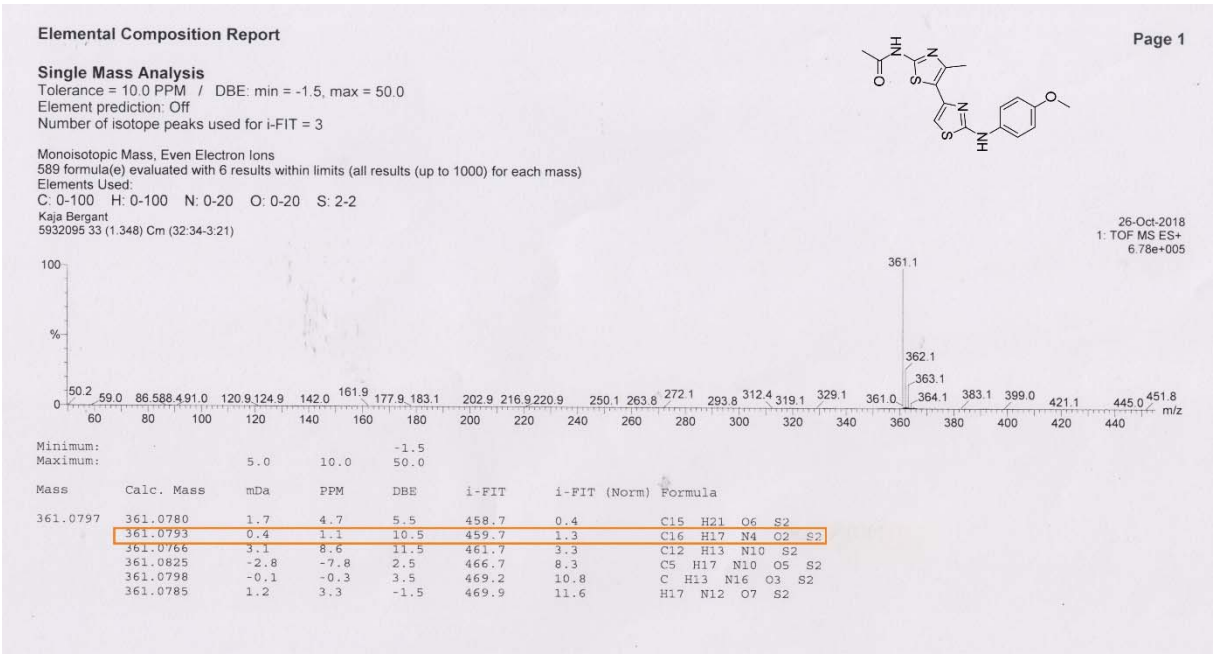
26-Oct-2018
1: TOF MS ES+
7.07e+005



Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
388.0907	388.0902	0.5	1.3	11.5	398.5	0.6	C17 H18 N5 O2 S2
	388.0942	-3.5	-9.0	15.5	399.0	1.0	C22 H18 N3 S2
	388.0889	1.8	4.6	6.5	400.5	2.6	C16 H22 N O6 S2
	388.0875	3.2	8.2	12.5	403.4	5.5	C13 H14 N11 S2
	388.0934	-2.7	-7.0	3.5	407.3	9.3	C6 H18 N11 O5 S2
	388.0920	-1.3	-3.3	-1.5	407.4	9.5	C5 H22 N7 O9 S2
	388.0907	0.0	0.0	4.5	409.7	11.8	C2 H14 N17 O3 S2
	388.0894	1.3	3.3	-0.5	410.4	12.5	C H18 N13 O7 S2



Compound 10



Elemental Composition Report

Page 1

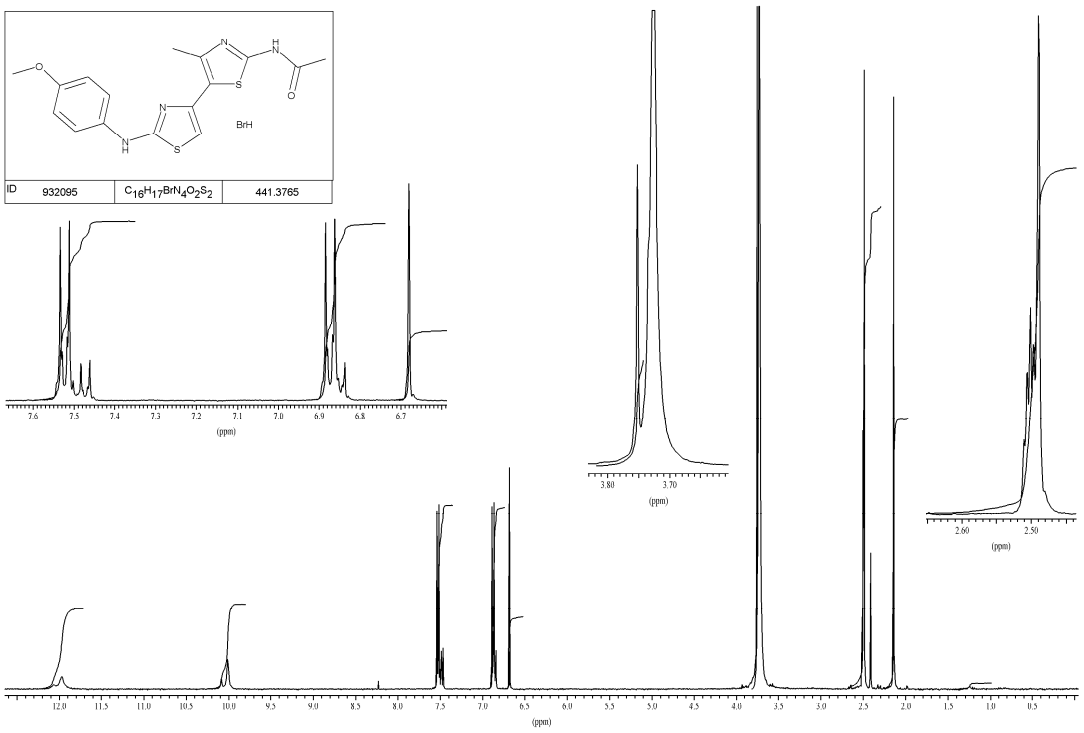
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
589 formula(e) evaluated with 6 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-20 O: 0-20 S: 2-2

Kaja Bergant
5932095 33 (1.348) Cm (32:34-3:21)

26-Oct-2018
1: TOF MS ES+
6.78e+005

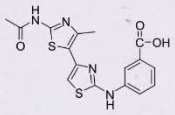
Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
361.0797	361.0780	1.7	4.7	5.5	458.7	0.4	C15 H21 O6 S2
	361.0793	0.4	1.1	10.5	459.7	1.3	C16 H17 N4 O2 S2
	361.0766	3.1	8.6	11.5	461.7	3.3	C12 H13 N10 S2
	361.0825	-2.8	-7.8	2.5	466.7	8.3	C5 H17 N10 O5 S2
	361.0798	-0.1	-0.3	3.5	469.2	10.8	C H13 N16 O3 S2
	361.0785	1.2	3.3	-1.5	469.9	11.6	H17 N12 O7 S2



Compound 11

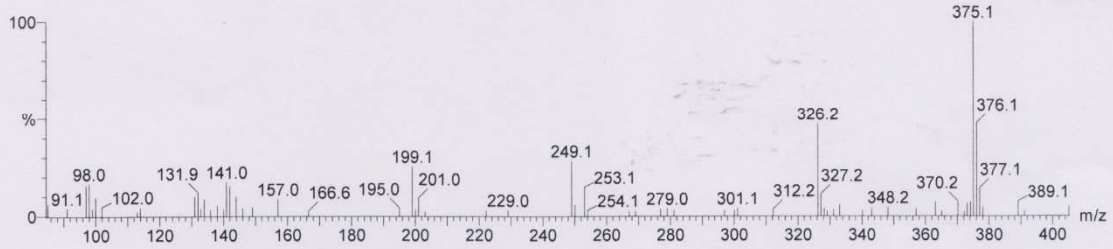
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

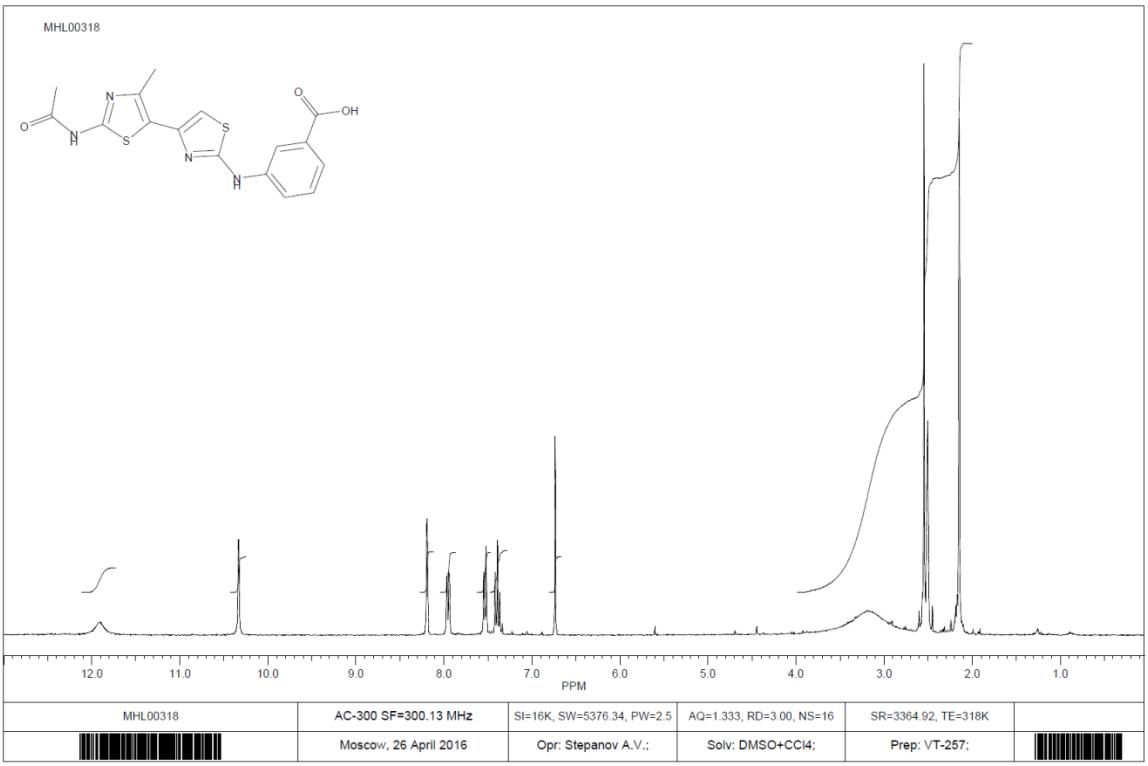


Monoisotopic Mass, Even Electron Ions
661 formula(e) evaluated with 9 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-20 O: 0-40 S: 2-2
Kaja Bergant
STK070262 37 (1.514) Cm (36:38-22:30)

1: TOF MS ES+
5.15e+004



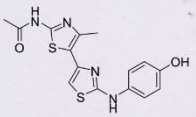
Minimum:				-1.5			
Maximum:		10.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
375.0581	375.0586	-0.5	-1.3	11.5	29.3	0.8	C16 H15 N4 O3 S2
	375.0572	0.9	2.4	6.5	29.7	1.2	C15 H19 O7 S2
	375.0559	2.2	5.9	12.5	31.0	2.5	C12 H11 N10 O S2
	375.0545	3.6	9.6	7.5	31.4	2.9	C11 H15 N6 O5 S2
	375.0617	-3.6	-9.6	3.5	31.7	3.2	C5 H15 N10 O6 S2
	375.0604	-2.3	-6.1	-1.5	31.9	3.4	C4 H19 N6 O10 S2
	375.0604	-2.3	-6.1	9.5	33.3	4.8	C2 H7 N20 S2
	375.0591	-1.0	-2.7	4.5	33.8	5.3	C H11 N16 O4 S2
	375.0577	0.4	1.1	-0.5	34.3	5.8	H15 N12 O8 S2



Compound 12

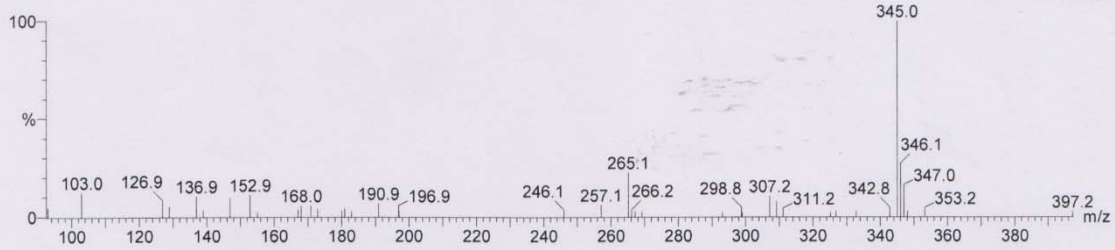
Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

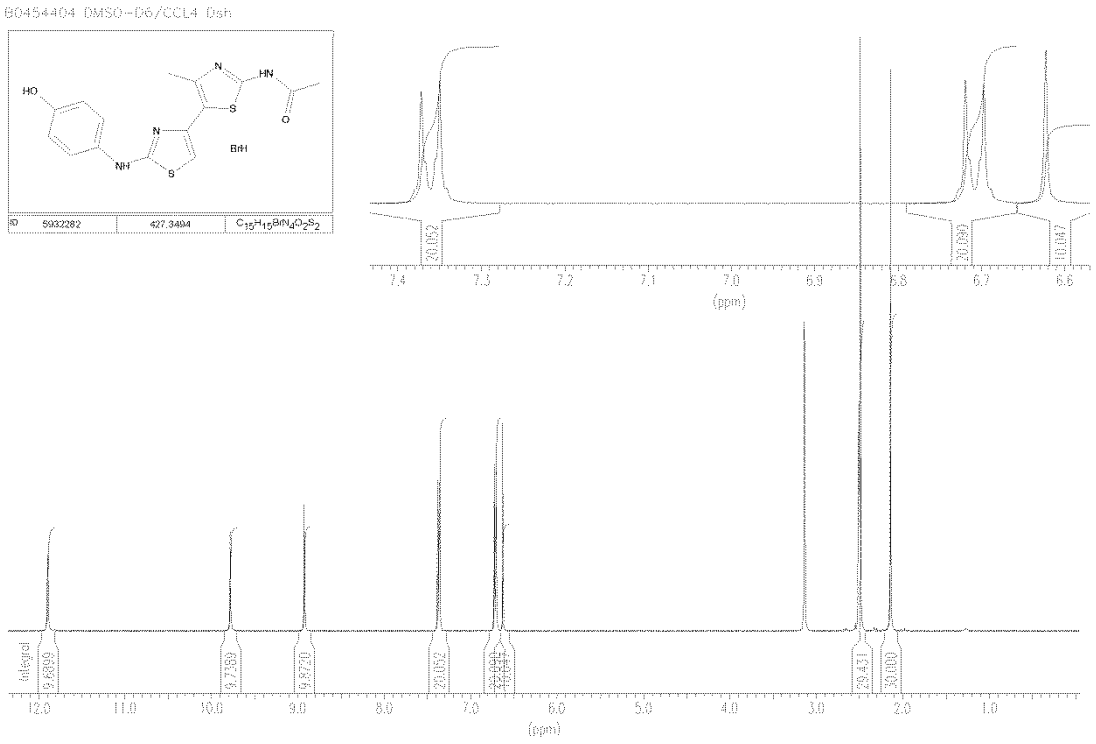


Monoisotopic Mass, Even Electron Ions
515 formula(e) evaluated with 7 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-20 O: 0-40 S: 2-2
Kaja Bergant
5932282 43 (1.754) Cm (43:45-66:81)

1: TOF MS ES-
1.32e+004



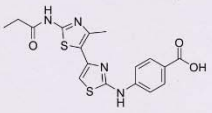
Minimum:				-1.5								
Maximum:		10.0	10.0	50.0								
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula					
345.0486	345.0520	-3.4	-9.9	15.5	24.8	0.3	C20	H13	N2	S2		
	345.0480	0.6	1.7	11.5	26.6	2.0	C15	H13	N4	O2	S2	
	345.0467	1.9	5.5	6.5	27.1	2.6	C14	H17	O6	S2		
	345.0453	3.3	9.6	12.5	27.8	3.3	C11	H9	N10	S2		
	345.0512	-2.6	-7.5	3.5	30.1	5.6	C4	H13	N10	O5	S2	
	345.0498	-1.2	-3.5	-1.5	30.4	5.9	C3	H17	N6	O9	S2	
	345.0485	0.1	0.3	4.5	31.2	6.7	H9	N16	O3	S2		



Compound 13

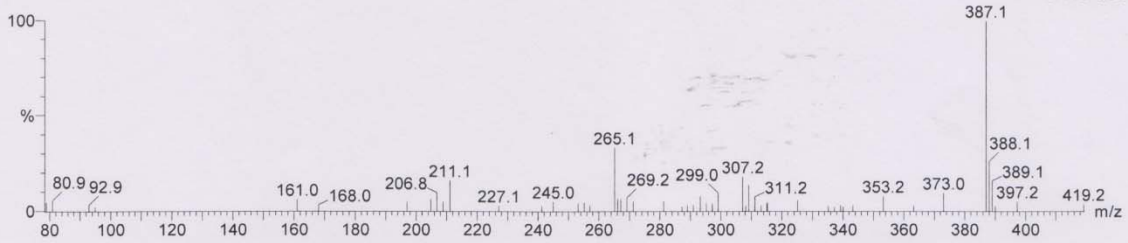
Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

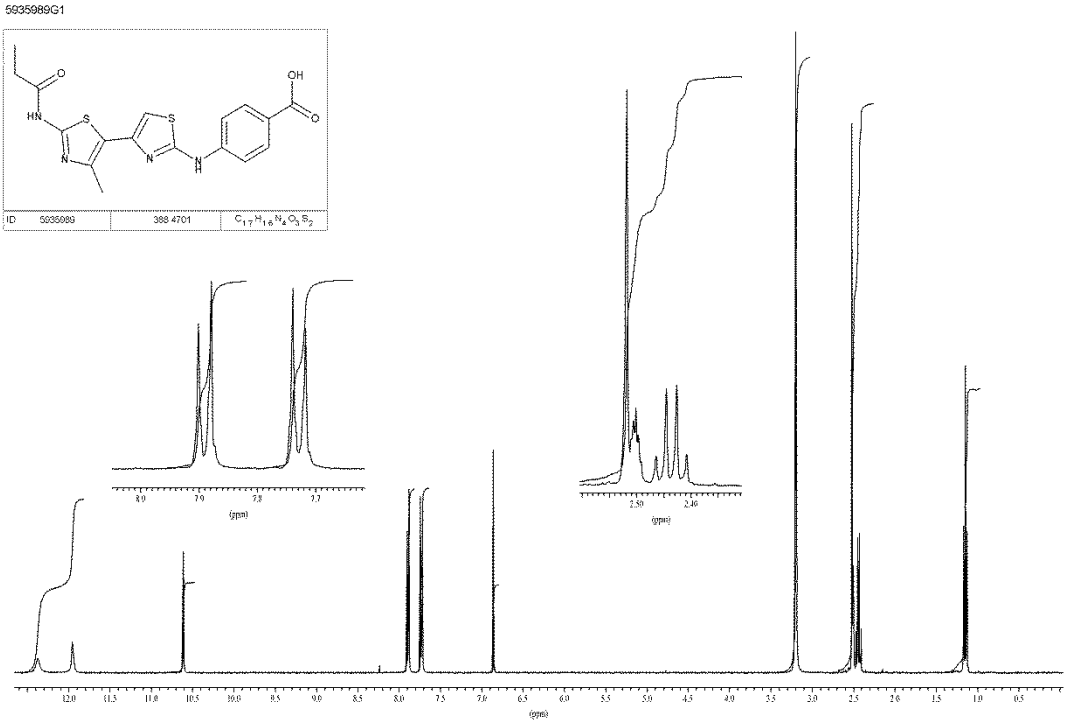


Monoisotopic Mass, Even Electron Ions
724 formula(e) evaluated with 9 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-20 O: 0-40 S: 2-2
Kaja Bergant
5935989 33 (1.348) Cm (32:34-11:17)

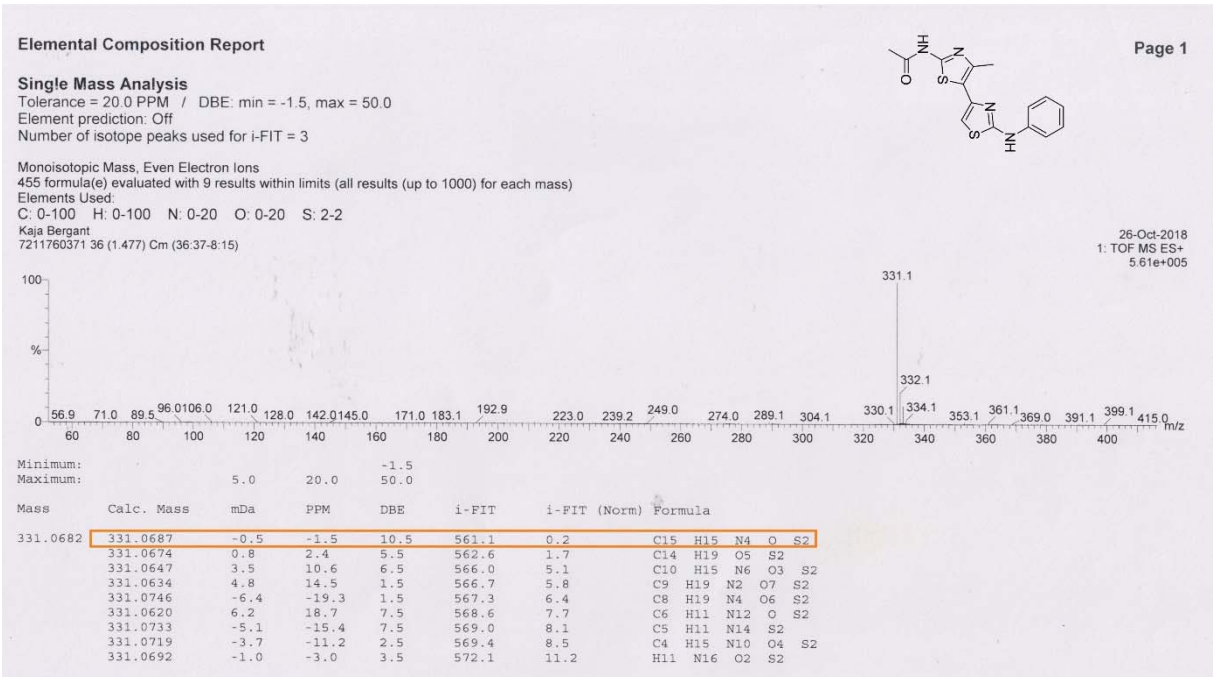
1: TOF MS ES-
9.44e+003



Minimum:				-1.5			
Maximum:		10.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
387.0589	387.0626	-3.7	-9.6	16.5	19.7	0.4	C22 H15 N2 O S2
	387.0586	0.3	0.8	12.5	20.9	1.6	C17 H15 N4 O3 S2
	387.0572	1.7	4.4	7.5	21.5	2.2	C16 H19 O7 S2
	387.0559	3.0	7.8	13.5	22.8	3.5	C13 H11 N10 O S2
	387.0617	-2.8	-7.2	4.5	25.6	6.3	C6 H15 N10 O6 S2
	387.0604	-1.5	-3.9	-0.5	25.9	6.6	C5 H19 N6 O10 S2
	387.0604	-1.5	-3.9	10.5	26.5	7.2	C3 H7 N20 S2
	387.0591	-0.2	-0.5	5.5	26.9	7.6	C2 H11 N16 O4 S2
	387.0577	1.2	3.1	0.5	27.3	8.0	C H15 N12 O8 S2



Compound 14



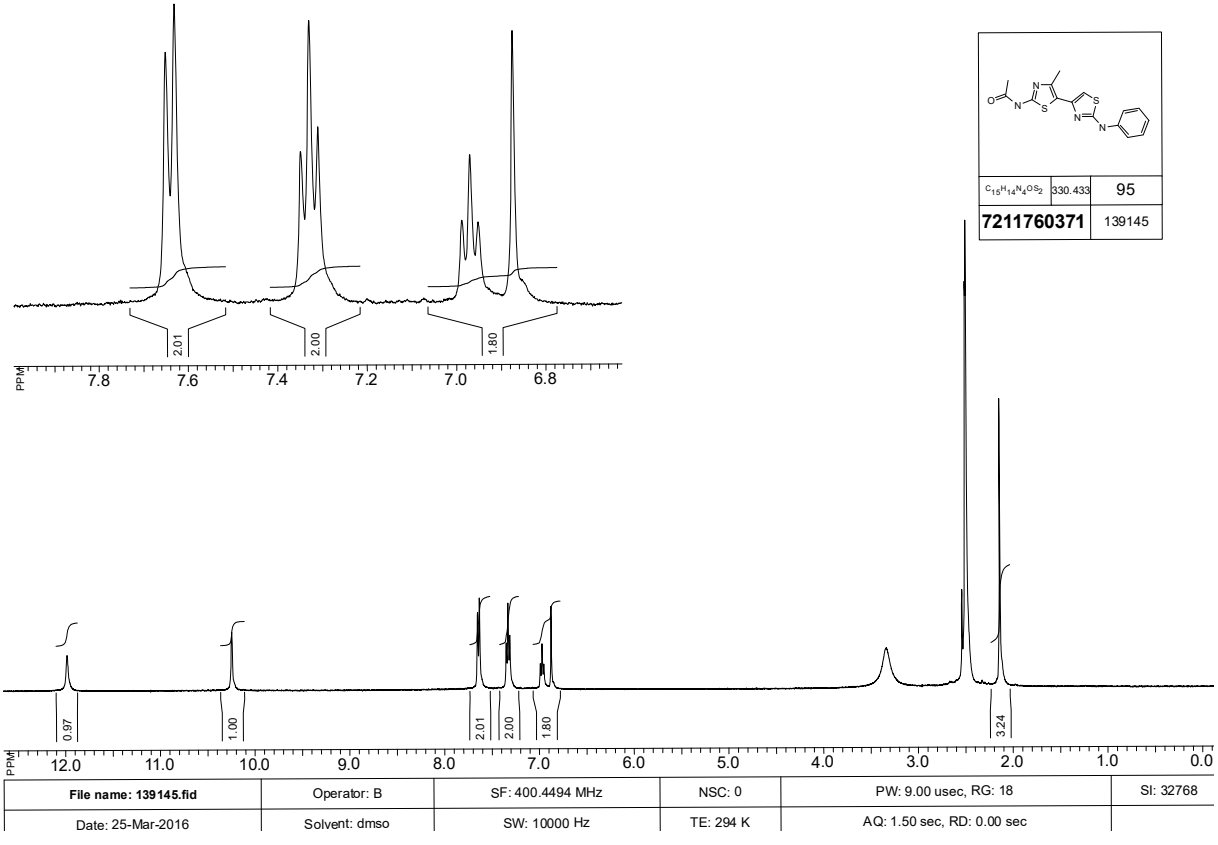
Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
455 formula(e) evaluated with 9 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-20 O: 0-20 S: 2-2
Kaja Bergant
7211760371 36 (1.477) Cm (36:37-8:15)

26-Oct-2018
1: TOF MS ES+
5.61e+005

Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
331.0682	331.0687	-0.5	-1.5	10.5	561.1	0.2	C15 H15 N4 O S2
	331.0674	0.8	2.4	5.5	562.6	1.7	C14 H19 O5 S2
	331.0647	3.5	10.6	6.5	566.0	5.1	C10 H15 N6 O3 S2
	331.0634	4.8	14.5	1.5	566.7	5.8	C9 H19 N2 O7 S2
	331.0746	-6.4	-19.3	1.5	567.3	6.4	C8 H19 N4 O6 S2
	331.0620	6.2	18.7	7.5	568.6	7.7	C6 H11 N12 O S2
	331.0733	-5.1	-15.4	7.5	569.0	8.1	C5 H11 N14 S2
	331.0719	-3.7	-11.2	2.5	569.4	8.5	C4 H15 N10 O4 S2
	331.0692	-1.0	-3.0	3.5	572.1	11.2	H11 N16 O2 S2



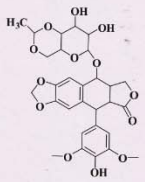
$C_{15}H_{14}N_4OS_2$	330.433	95			
7211760371	139145				

File name: 139145.fid	Operator: B	SF: 400.4494 MHz	NSC: 0	PW: 9.00 usec, RG: 18	SI: 32768
Date: 25-Mar-2016	Solvent: dmso	SW: 10000 Hz	TE: 294 K	AQ: 1.50 sec, RD: 0.00 sec	

Etoposide

Elemental Composition Report **Page 1**

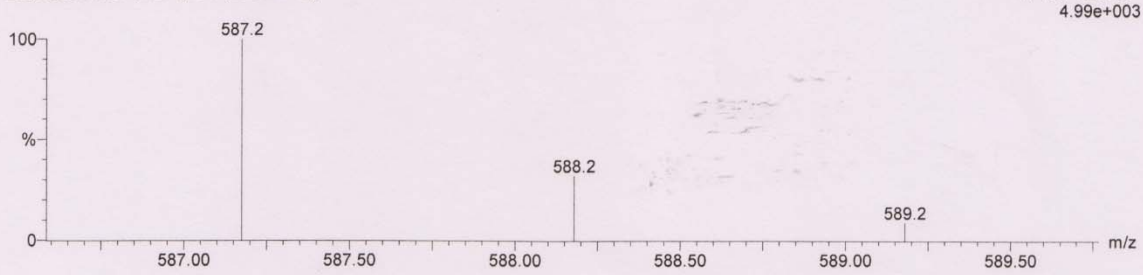
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3



Monoisotopic Mass, Even Electron Ions
2663 formula(e) evaluated with 29 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-20 O: 0-40

Kaja Bergant
Etoposide KI 31 (1.274) Cm (26:31-4:16)

1: TOF MS ES-
4.99e+003



Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				-1.5			
Maximum:		10.0	10.0	50.0			
587.1750	587.1765	-1.5	-2.6	14.5	9.2	0.3	C29 H31 O13
	587.1738	1.2	2.0	15.5	11.4	2.5	C25 H27 N6 O11
	587.1751	-0.1	-0.2	20.5	11.6	2.7	C26 H23 N10 O7
	587.1778	-2.8	-4.8	19.5	11.8	2.9	C30 H27 N4 O9
	587.1724	2.6	4.4	10.5	12.6	3.7	C24 H31 N2 O15
	587.1765	-1.5	-2.6	25.5	12.7	3.8	C27 H19 N14 O3
	587.1738	1.2	2.0	26.5	14.1	5.3	C23 H15 N20 O
	587.1791	-4.1	-7.0	24.5	14.2	5.4	C31 H23 N8 O5
	587.1724	2.6	4.4	21.5	14.4	5.5	C22 H19 N16 O5
	587.1711	3.9	6.6	16.5	15.2	6.4	C21 H23 N12 O9
	587.1706	4.4	7.5	23.5	15.5	6.7	C36 H27 O8
	587.1719	3.1	5.3	28.5	15.6	6.8	C37 H23 N4 O4
	587.1733	1.7	2.9	33.5	15.7	6.9	C38 H19 N8
	587.1805	-5.5	-9.4	29.5	16.2	7.3	C32 H19 N12 O
	587.1760	-1.0	-1.7	32.5	16.2	7.3	C42 H23 N2 O2
	587.1783	-3.3	-5.6	1.5	16.4	7.5	C17 H35 N2 O20
	587.1756	-0.6	-1.0	2.5	16.5	7.6	C13 H31 N8 O18
	587.1698	5.2	8.9	11.5	16.5	7.6	C20 H27 N8 O13
	587.1770	-2.0	-3.4	7.5	16.5	7.6	C14 H27 N12 O14
	587.1692	5.8	9.9	29.5	16.6	7.8	C33 H19 N10 O2
	587.1797	-4.7	-8.0	6.5	16.7	7.8	C18 H31 N6 O16
	587.1783	-3.3	-5.6	12.5	16.9	8.0	C15 H23 N16 O10
	587.1796	-4.6	-7.8	17.5	17.3	8.4	C16 H19 N20 O6
	587.1743	0.7	1.2	8.5	18.5	9.6	C10 H23 N18 O12
	587.1729	2.1	3.6	3.5	19.1	10.2	C9 H27 N14 O16
	587.1800	-5.0	-8.5	36.5	19.4	10.5	C47 H23
	587.1716	3.4	5.8	-1.5	19.8	10.9	C8 H31 N10 O20
	587.1703	4.7	8.0	4.5	22.0	13.1	C5 H23 N20 O14
	587.1802	-5.2	-8.9	-0.5	22.1	13.2	C3 H27 N18 O17

Table S12. Elemental analysis data of active compounds **1, 9** and **10.**

Compound	Molecular formula	Calculated				Found			
		C	**H**	**N**	**S**	**C**	**H**	**N**	**S**
1	$C_{14}H_{11}N_4S_2F_3$	47.2	3.1	15.7	18.0	45.9	3.2	15.2	17.6
9	$C_{17}H_{18}BrN_5O_2S_2$ X 2.5 H_20	39.8	4.5	13.7	12.5	41.2	4.7	13.8	12.0
10	$C_{16}H_{17}BrN_4O_2S_2$ X 4 H_20	37.4	4.9	10.9	12.5	38.9	5.0	11.2	12.5

Table S13. HPLC purity data for active compounds **1**, **7**, **10** and **14**.

	Compound **1**	Compound **7**	Compound **10**	Compound **14**
Area (1%) (0.001 mg/mL)	66680342.00	149750383.00	59760385.00	281812961.00
Area TOTAL (100%) (1 mg/mL)	7084848214.00	4745097290.00	4193933896.00	6488929607.00
Area max (100%) (1 mg/mL)	6705645216.00	4663958201.00	4028846164.00	6101844241.00
Area (impurities 100%; TOTAL)	379202998.00	81139089.00	165087732.00	387085366.00
Purity [%]	94.31	99.46	97.24	98.63