

Evaluation of biological activity of new 1,2,4-triazole derivatives containing propionic acid moiety

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Table S1. A summary of the yield and melting point of compounds **3a-3g** obtained under different reaction conditions (methods A-D)

compound	method	solvent	temp.	time	yield	melting point
3a	A	diethyl ether	ambient	2 days	55.39%	197-199 °C
3a	B	toluene	boiling	4 h	69.36%	199-202 °C
3a	C	chloroform	boiling	1 h	29.90%	198-200 °C
3b	A	diethyl ether	ambient	2 days	85.76%	178-180 °C
3b	B	toluene	boiling	1 h	52.94%	181-183 °C
3b	C	chloroform	boiling	1.5 h	44.12%	183-185 °C
3c	A	diethyl ether	ambient	2 days	73.53%	157-160 °C
3c	B	toluene	boiling	1 h	31.58%	157-159 °C
3c	C	chloroform	boiling	1 h	35.29%	158-160 °C
3d	B	toluene	boiling	2.5 h	32.83%	186-189 °C
3d	D	alkaline cyclization *	boiling	2 h	31.88%	189-191 °C
3e	A	diethyl ether	ambient	2 days	80.82%	235-237 °C
3e	B	toluene	boiling	1 h	85.71%	237-240 °C
3e	C	chloroform	boiling	1.5 h	66.47%	237-239 °C
3f	A	diethyl ether	ambient	2 days	64.30%	208-211 °C
3f	B	toluene	boiling	1 h	53.79%	208-211 °C
3g	A	diethyl ether	ambient	2 days	76.47%	251-254 °C
3g	B	toluene	boiling	1.5 h	74.93%	250-252 °C

* alkaline cyclization of intermediate obtained in diethyl ether

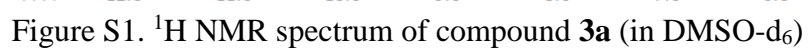


Figure S2. ^1H NMR spectrum of compound **3b** (in DMSO- d_6)

(5.762 ppm – impurity of dichloromethane formed in the NMR study, 2.091 – impurity of acetone in the NMR study)

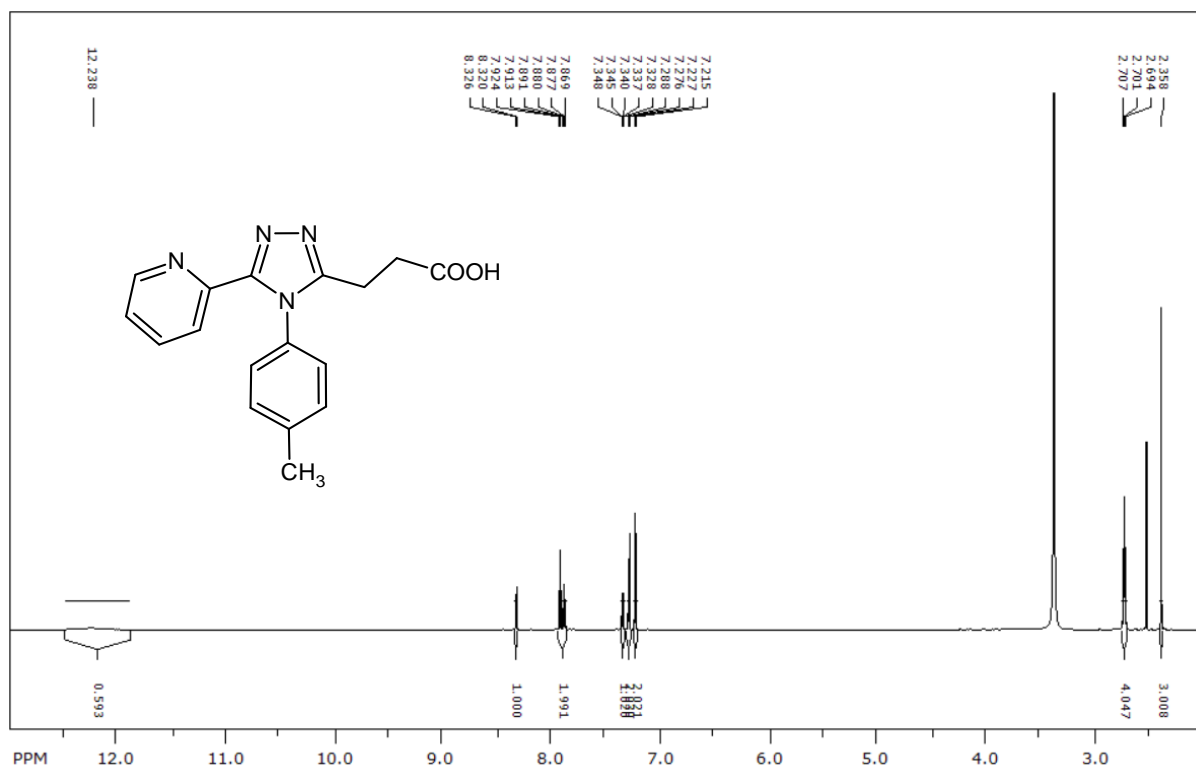


Figure S3. ^1H NMR spectrum of compound **3c** (in DMSO- d_6)

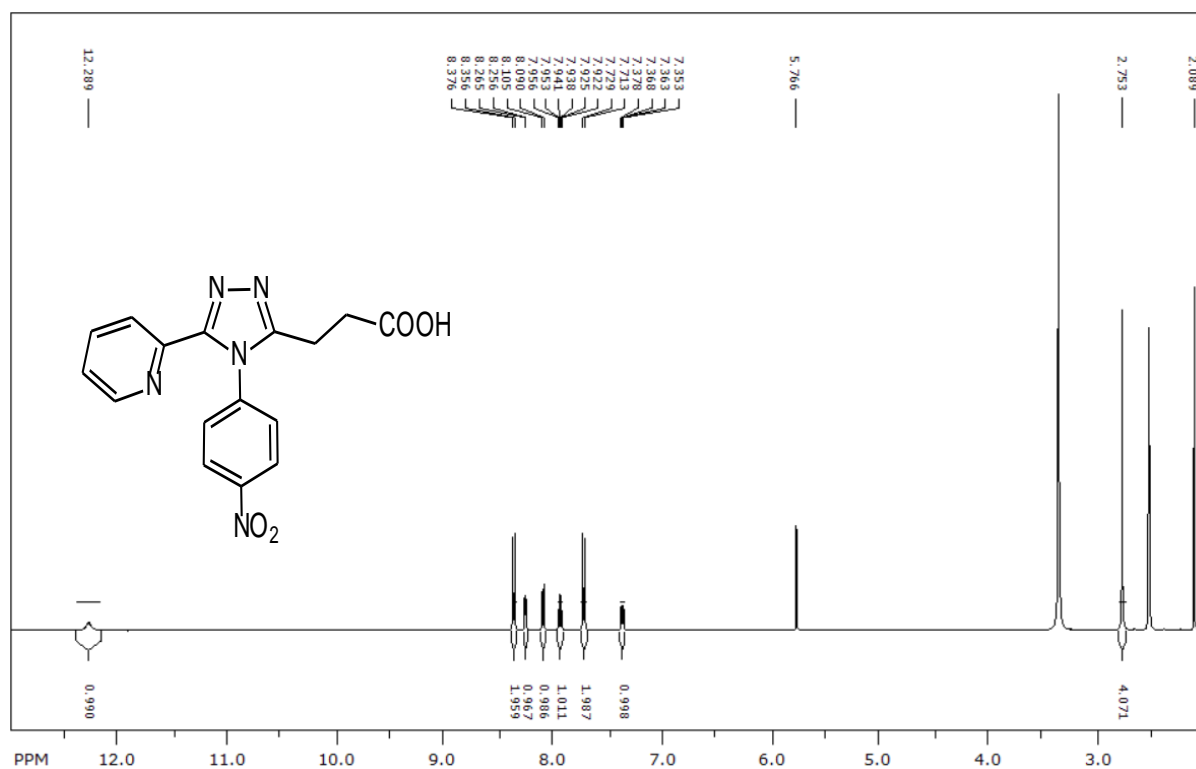


Figure S4. ^1H NMR spectrum of compound **3d** (in DMSO- d_6)

(5.762 ppm – impurity of dichloromethane formed in the NMR study, 2.089 – impurity of acetone in the NMR study)

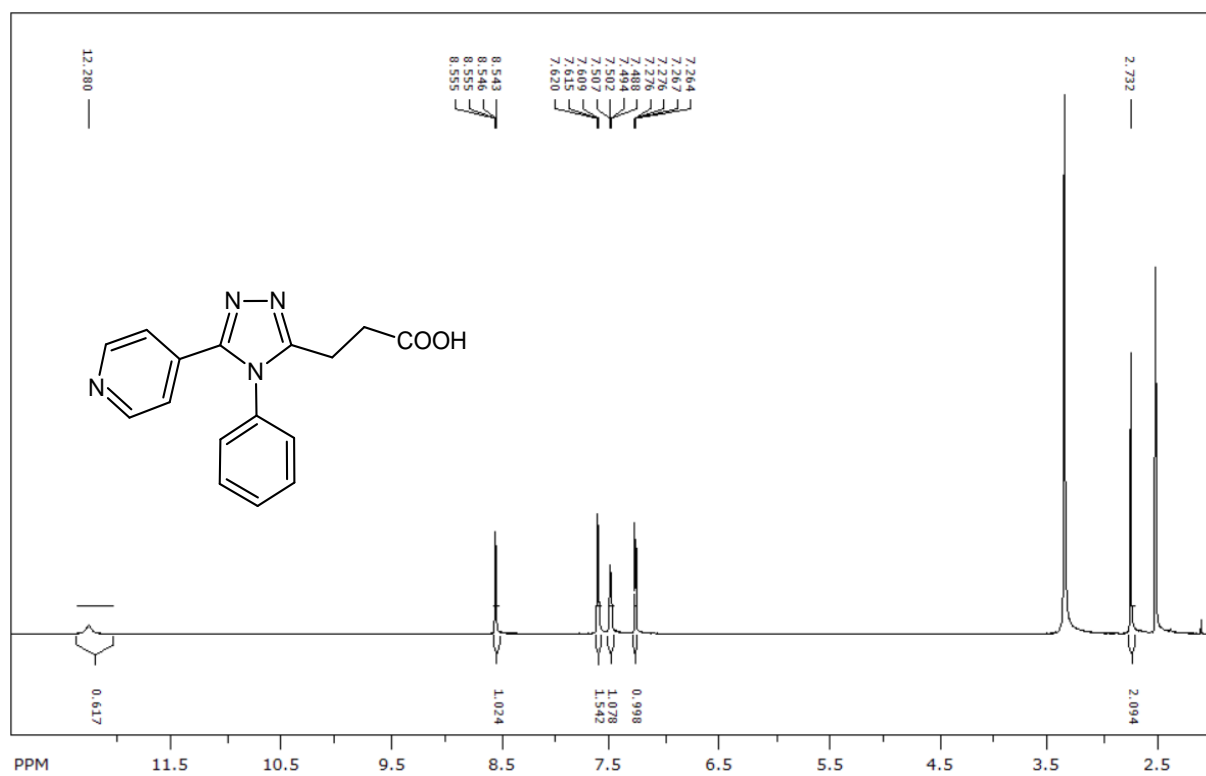


Figure S5. ¹H NMR spectrum of compound **3e** (in DMSO-d₆)

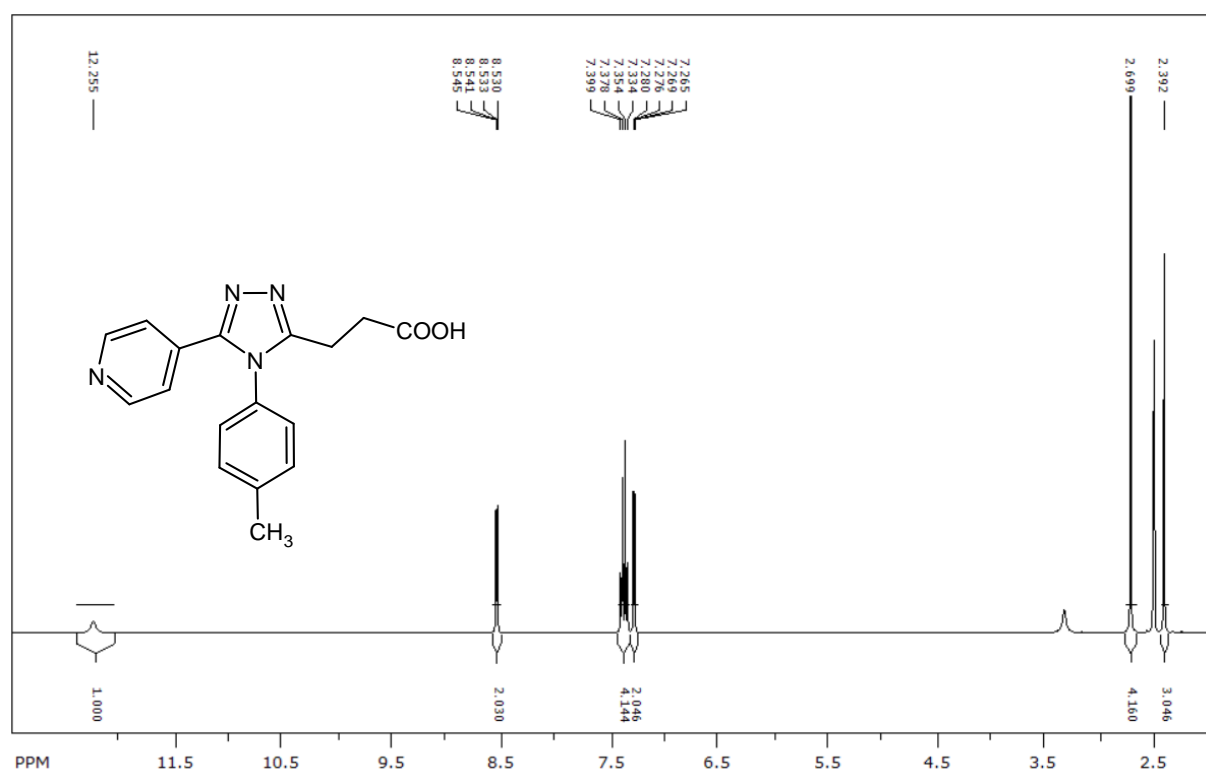


Figure S6. ¹H NMR spectrum of compound **3f** (in DMSO-d₆)

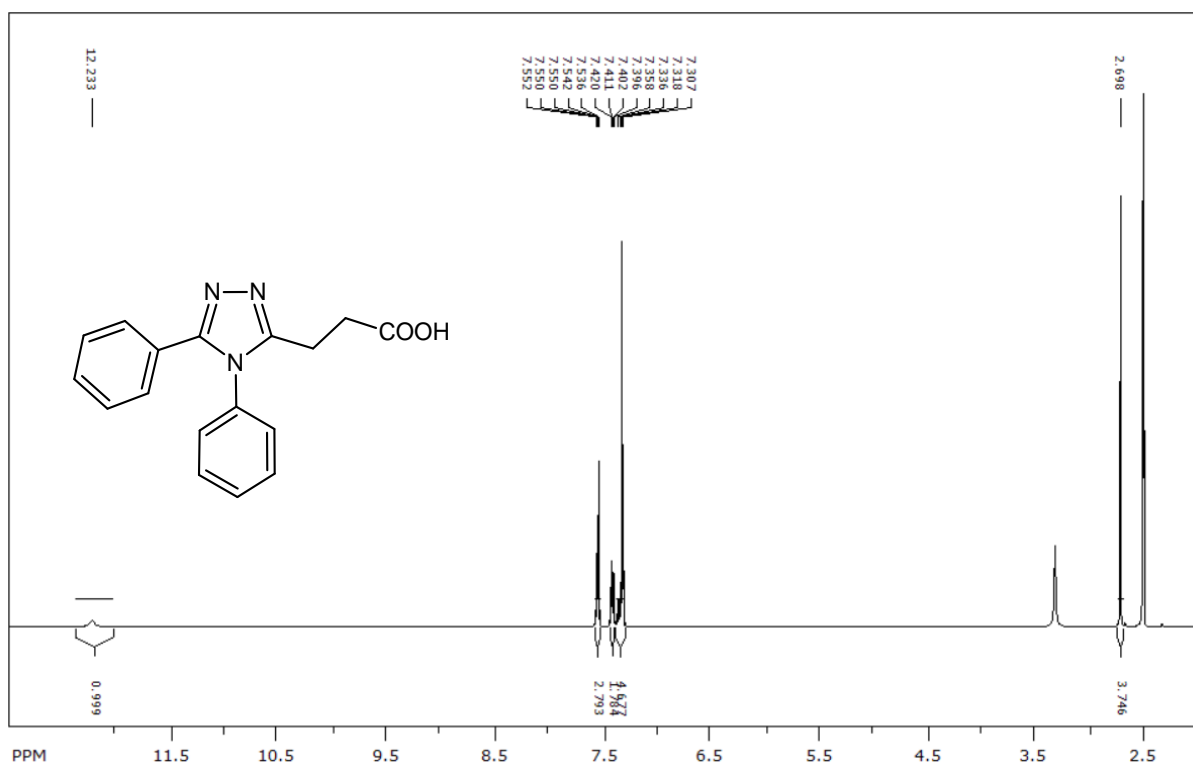


Figure S7. ¹H NMR spectrum of compound **3g** (in DMSO-d₆)

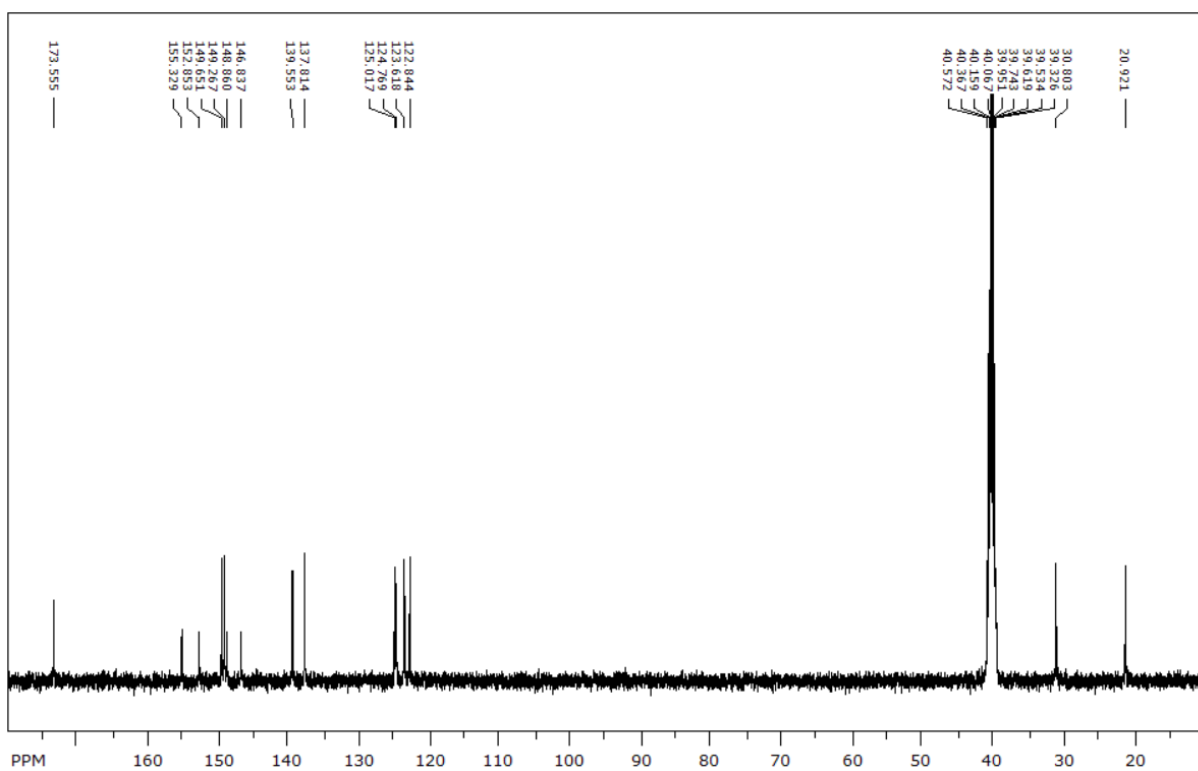


Figure S8. ¹³C NMR spectrum of compound **3a** (in DMSO-d₆)

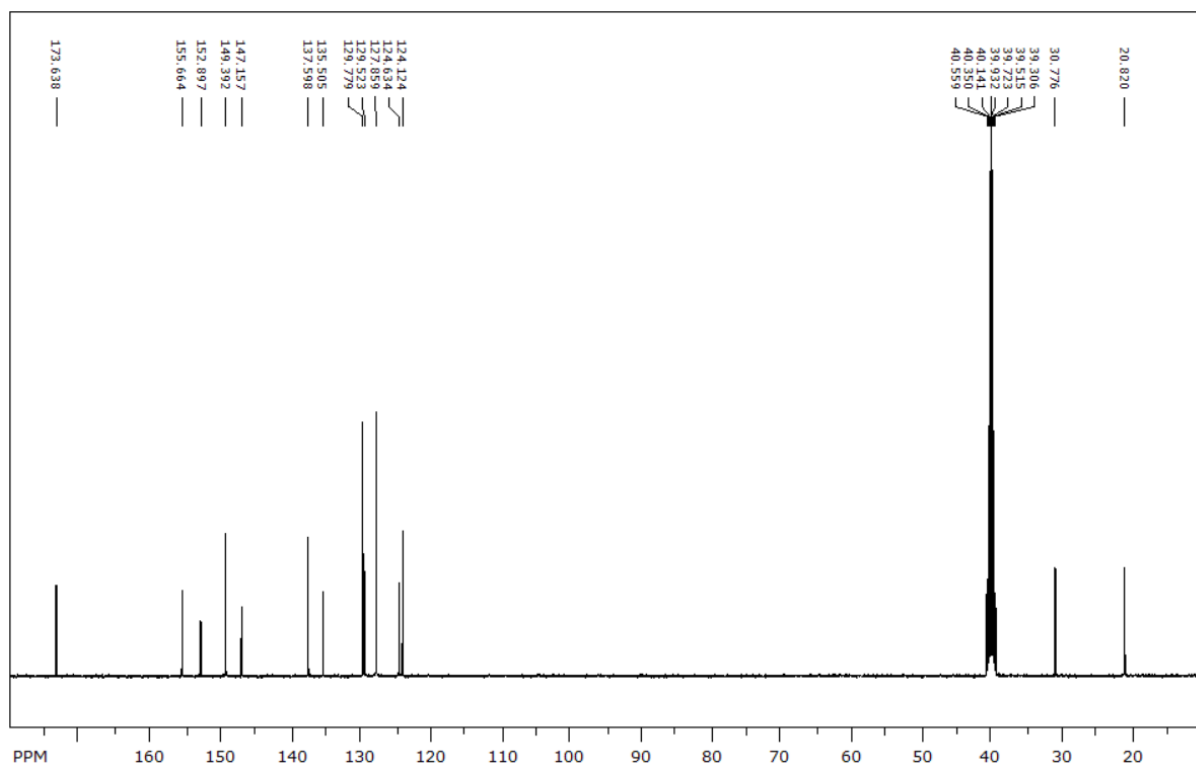


Figure S9. ¹³C NMR spectrum of compound **3b** (in DMSO-d₆)

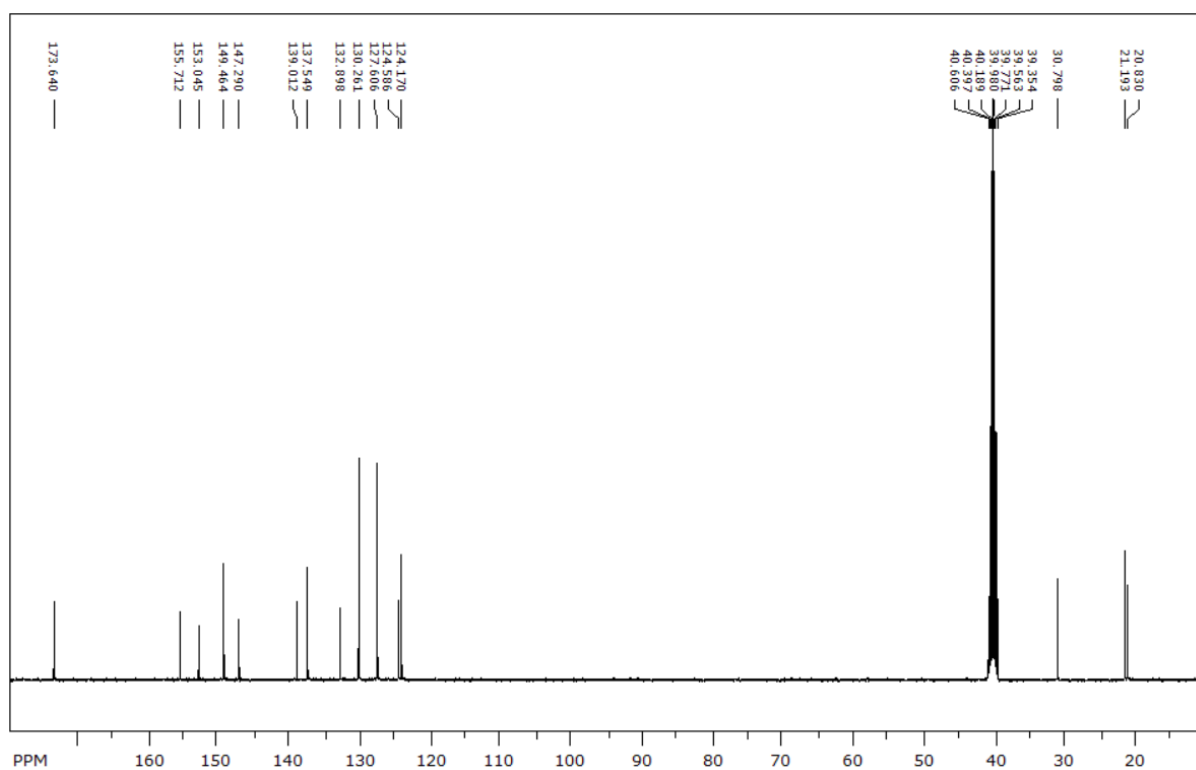


Figure S10. ¹³C NMR spectrum of compound **3c** (in DMSO-d₆)

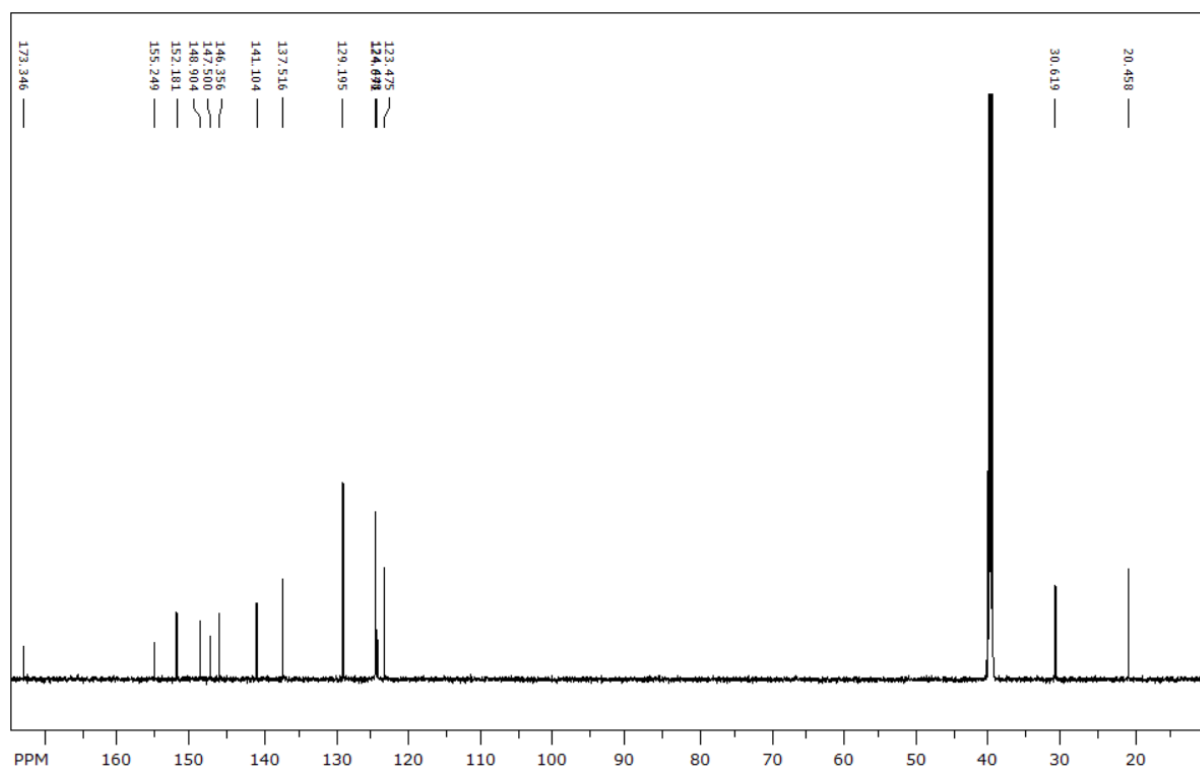


Figure S11. ¹³C NMR spectrum of compound **3d** (in DMSO-d₆)

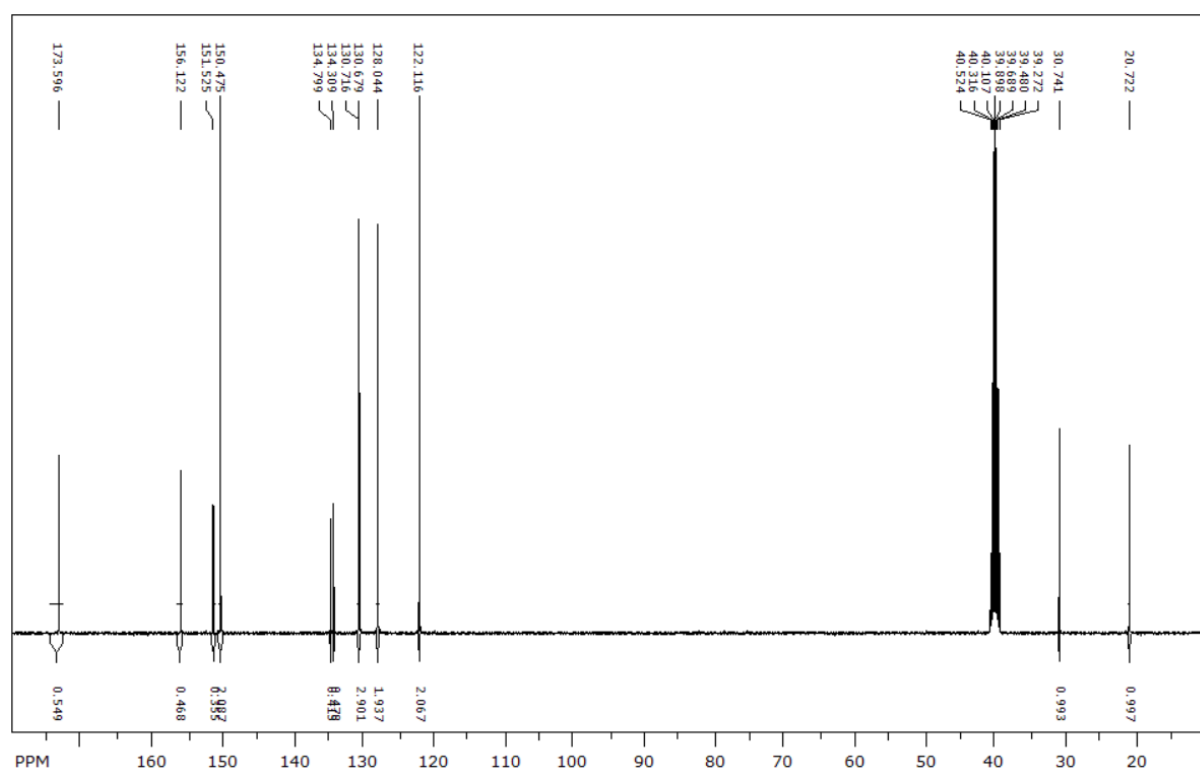


Figure S12. ¹³C NMR spectrum of compound **3e** (in DMSO-d₆)

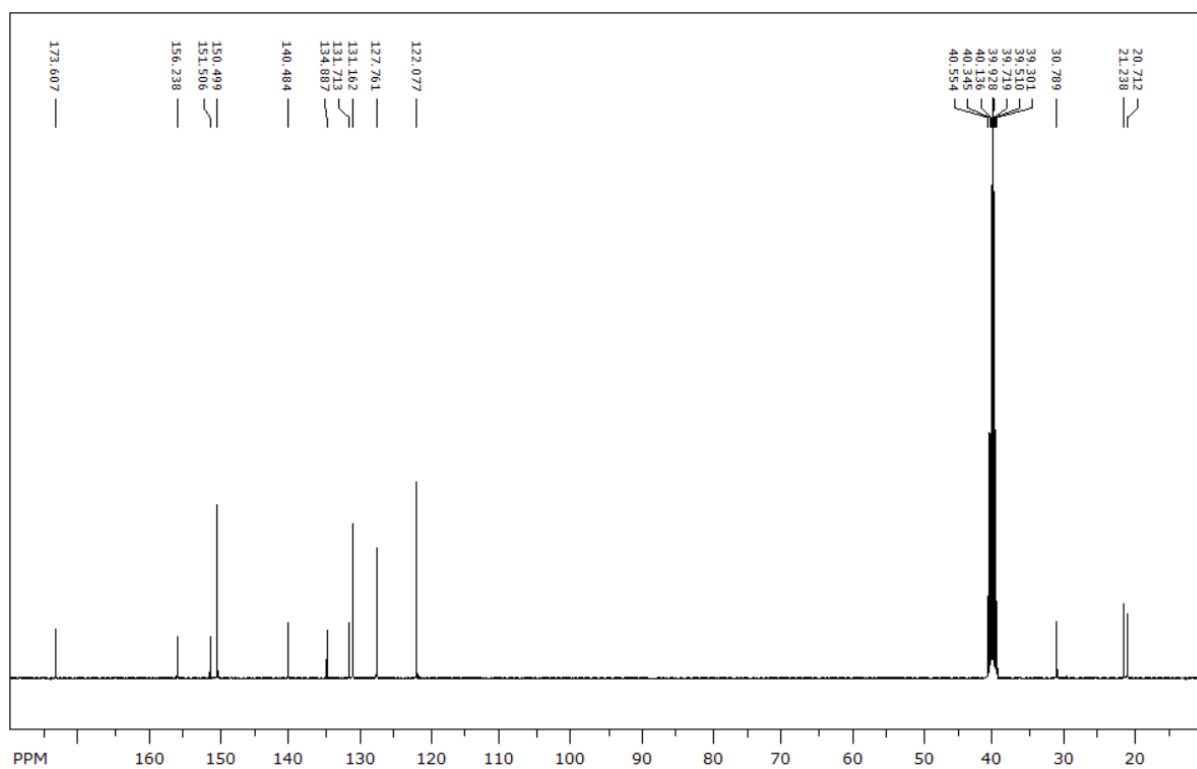


Figure S13. ¹³C NMR spectrum of compound **3f** (in DMSO-d₆)

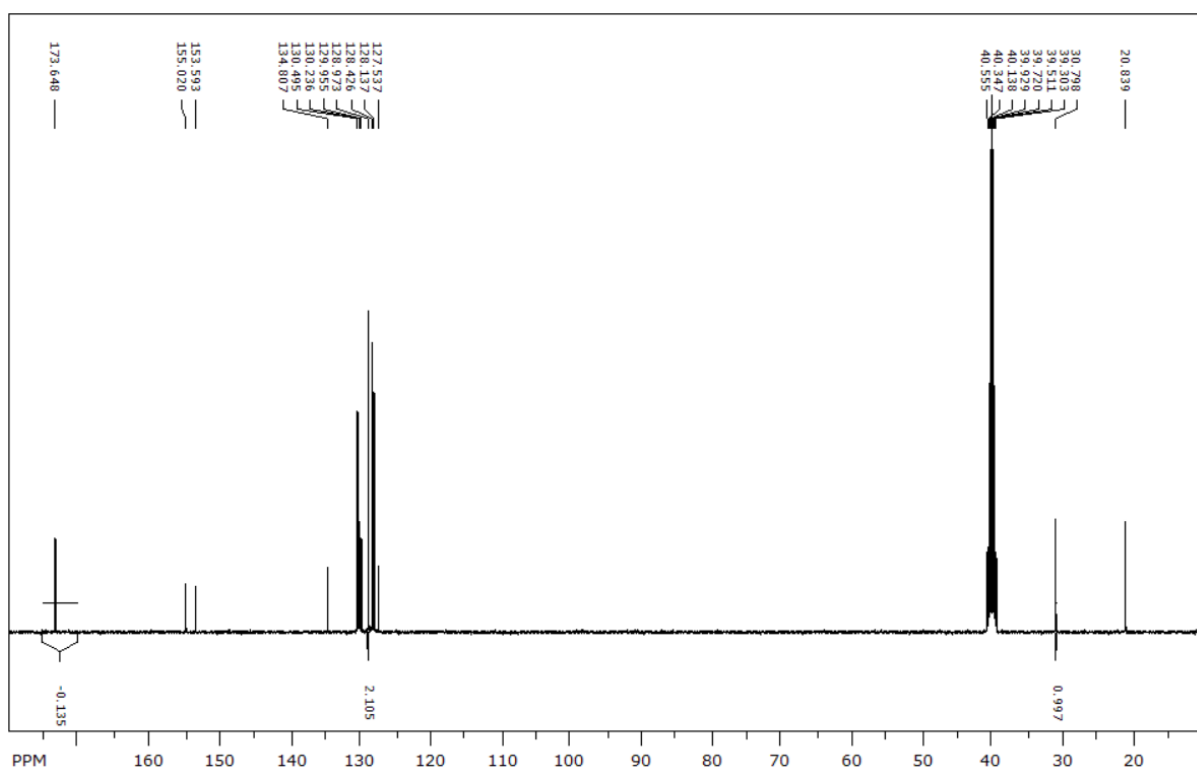


Figure S14. ¹³C NMR spectrum of compound **3g** (in DMSO-d₆)

Elemental Composition Report

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Single Mass Analysis

Tolerance = 7.0 PPM / DBE: min = -1.5, max = 90.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions

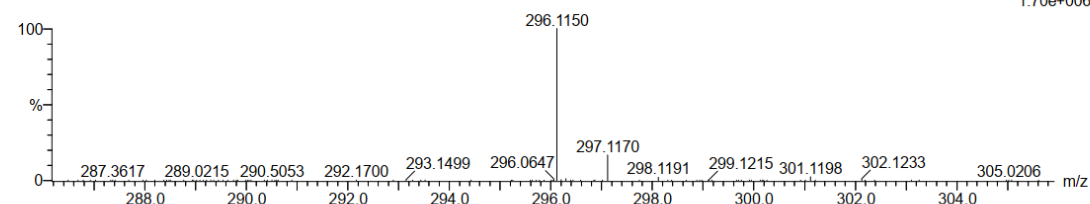
70 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-20 H: 0-25 N: 1-6 O: 0-5

230207_YA 9 (0.147) Cm (8.9-3.6)

TOF MS ES+
1.70e+006



Minimum: -1.5
Maximum: 5.0 7.0 90.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
296.1150	296.1147	0.3	1.0	11.5	345.7	n/a	n/a	C15 H14 N5 O2

Figure S15. HRMS spectrum of compound **3a**

Elemental Composition Report

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Single Mass Analysis

Tolerance = 7.0 PPM / DBE: min = -1.5, max = 90.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions

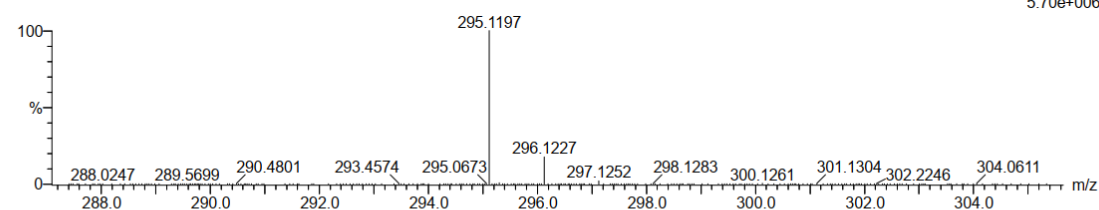
48 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-20 H: 0-25 N: 1-4 O: 0-5

230207_ACA 26 (0.374) Cm (26.29-3.6)

TOF MS ES+
5.70e+006



Minimum: -1.5
Maximum: 5.0 7.0 90.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
295.1197	295.1195	0.2	0.7	11.5	1420.2	n/a	n/a	C16 H15 N4 O2

Figure S16. HRMS spectrum of compound **3b**

Single Mass Analysis

Tolerance = 7.0 PPM / DBE: min = -1.5, max = 90.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 9

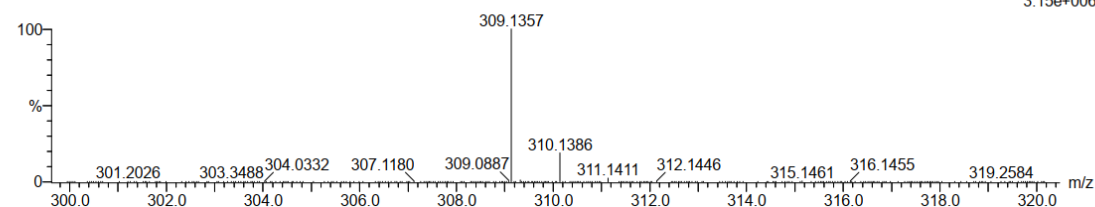
Monoisotopic Mass, Even Electron Ions

43 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-20 H: 0-25 N: 1-4 O: 0-5

230207_XA 40 (0.548) Cm (40:41)

TOF MS ES+
3.15e+006

Minimum:

Maximum:

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
309.1357	309.1352	0.5	1.6	11.5	1340.5	n/a	n/a	C17 H17 N4 O2

Figure S17. HRMS spectrum of compound 3c

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 90.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 9

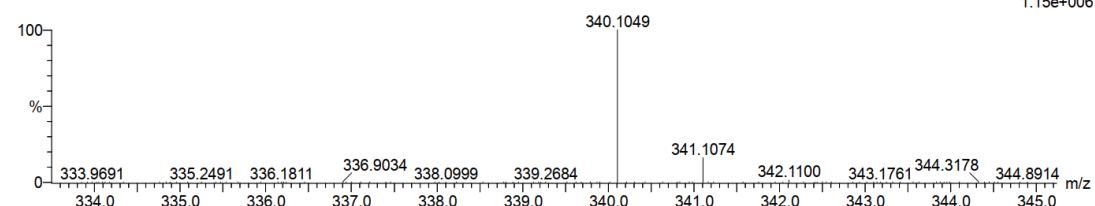
Monoisotopic Mass, Even Electron Ions

185 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-45 H: 0-45 N: 0-6 O: 0-6

230310_probka_AA_pos_A 25 (0.365) Cm (25:30-4:6)

TOF MS ES+
1.15e+006

Minimum:

Maximum:

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
340.1049	340.1046	0.3	0.9	12.5	480.0	n/a	n/a	C16 H14 N5 O4

Figure S18. HRMS spectrum of compound 3d

Elemental Composition Report

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Single Mass Analysis

Tolerance = 7.0 PPM / DBE: min = -1.5, max = 90.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions

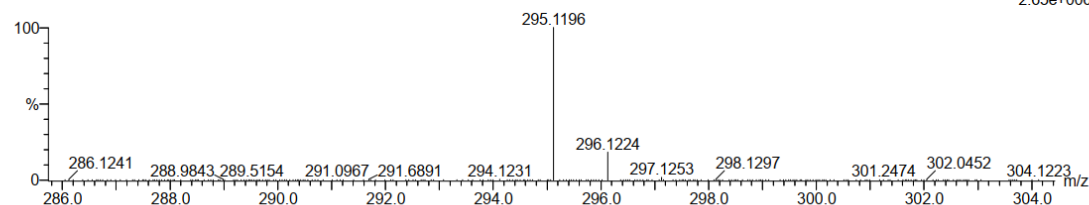
48 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-20 H: 0-25 N: 1-4 O: 0-5

230207_AEA 22 (0.313) Cm (22:27-3:6)

TOF MS ES+
2.65e+006



Minimum: -1.5
Maximum: 5.0 7.0 90.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
295.1196	295.1195	0.1	0.3	11.5	1163.8	n/a	n/a	C16 H15 N4 O2

Figure S19. HRMS spectrum of compound **3e**

Elemental Composition Report

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Single Mass Analysis

Tolerance = 7.0 PPM / DBE: min = -1.5, max = 90.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions

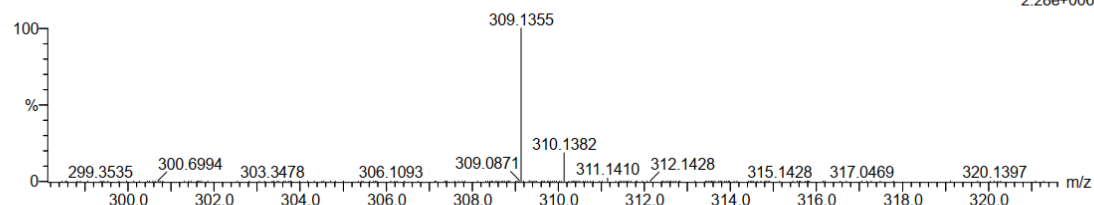
43 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-20 H: 0-25 N: 1-4 O: 0-5

230207_ABA 23 (0.321) Cm (23:24-3:6)

TOF MS ES+
2.28e+006



Minimum: -1.5
Maximum: 5.0 7.0 90.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
309.1355	309.1352	0.3	1.0	11.5	830.5	n/a	n/a	C17 H17 N4 O2

Figure S20. HRMS spectrum of compound **3f**

Single Mass Analysis

Tolerance = 7.0 PPM / DBE: min = -1.5, max = 90.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 9

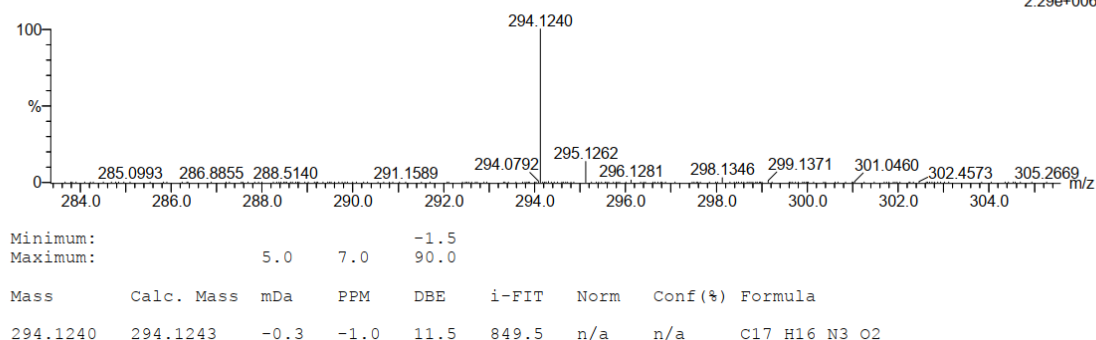
Monoisotopic Mass, Even Electron Ions

45 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-20 H: 0-25 N: 1-4 O: 0-5

230207_ADA 23 (0.321) Cm (23.24-3.6)

TOF MS ES+
2.29e+006Figure S21. HRMS spectrum of compound **3g**Table S2. The results of cell apoptosis assay of compounds **3a-3g** and ibuprofen at concentration 100 μ g/mL in PBMC 24 h cultured measured by flow cytometry.

	viable cells	early apoptotic cells	late apoptotic cells	necrotic cells
control	96.45%	0.58%	0.40%	2.56%
control with 2% DMSO	94.19%	2.08%	0.74%	2.98%
IBU	96.01%	1.39%	0.77%	1.84%
3c	96.72%	1.22%	0.76%	1.31%
3b	94.89%	1.64%	1.51%	1.96%
3c	95.71%	1.68%	1.26%	1.36%
3d	94.71%	1.84%	1.82%	1.62%
3e	95.74%	0.73%	0.47%	3.05%
3f	95.34%	1.57%	0.73%	2.36%
3g	95.62%	1.92%	0.63%	1.84%

Table S3. Minimal inhibitory concentrations of compounds **3a-3g**, ciprofloxacin (CIP) and amphotericin B (AmB).

strain	MIC µg/mL								
	3a	3b	3c	3d	3e	3f	3g	CIP	AmB
<i>E. coli</i> ATCC 25922	>512	512	>512	512	>512	512	256	0.015	
<i>Y. enterocolitica</i> O3	512	512	512	512	512	512	512	0.125	
<i>S. aureus</i> ATCC 25923	>512	>512	>512	>512	>512	>512	256	0.25	
<i>M. smegmatis</i>	128	256	256	512	128	256	512	0.5	
<i>C. albicans</i> ATCC 90028	>512	>512	>512	512	>512	512	512	-	0.125