

In Vitro Anti-Inflammatory Activity of Methyl Derivatives of Flavanone

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Table S1. The effect of methyl-derivatives of flavanone on the production IL-1 β in compared to control in LPS stimulated RAW264.1 cells (n=3). Statistical significance was analysed using Fisher's LSD test. Results marked in red are statistically significant in Fisher's LSD test. Multivariate Tests of Significance ($F = 7.499$, $p < 0.05$).

Table S2. The effect of methyl-derivatives of flavanone on the production IL-6 in compared to control in LPS stimulated RAW264.1 cells (n=3). Statistical significance was analysed using Fisher's LSD test. Results marked in red are statistically significant in Fisher's LSD test. Multivariate Tests of Significance ($F = 7.499$, $p < 0.05$).

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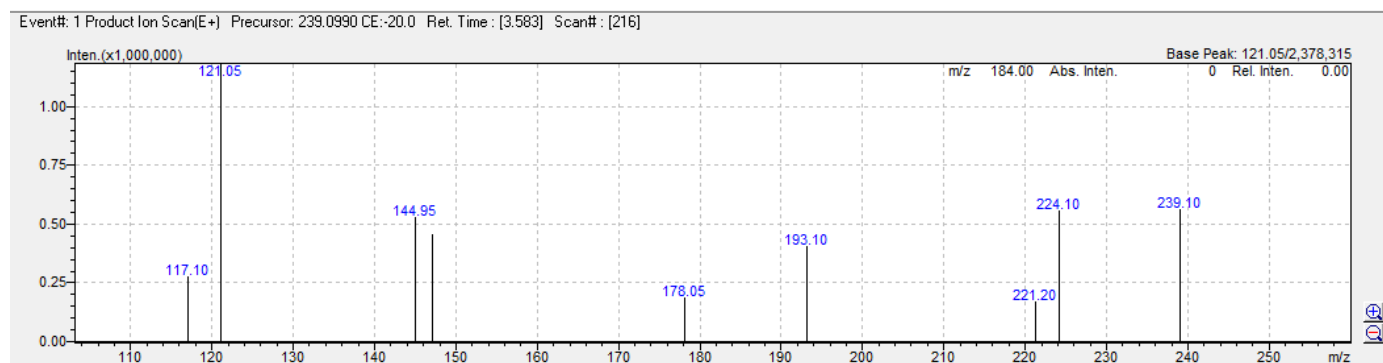
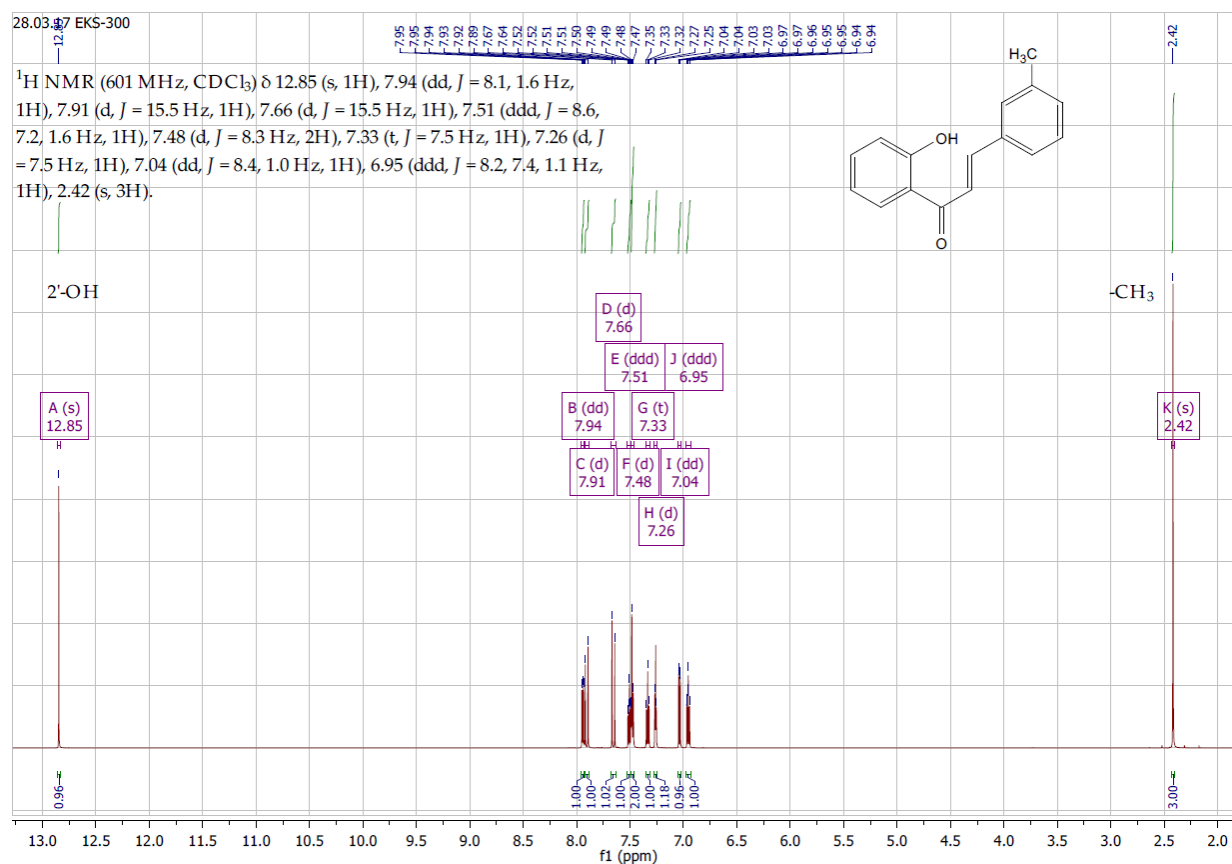
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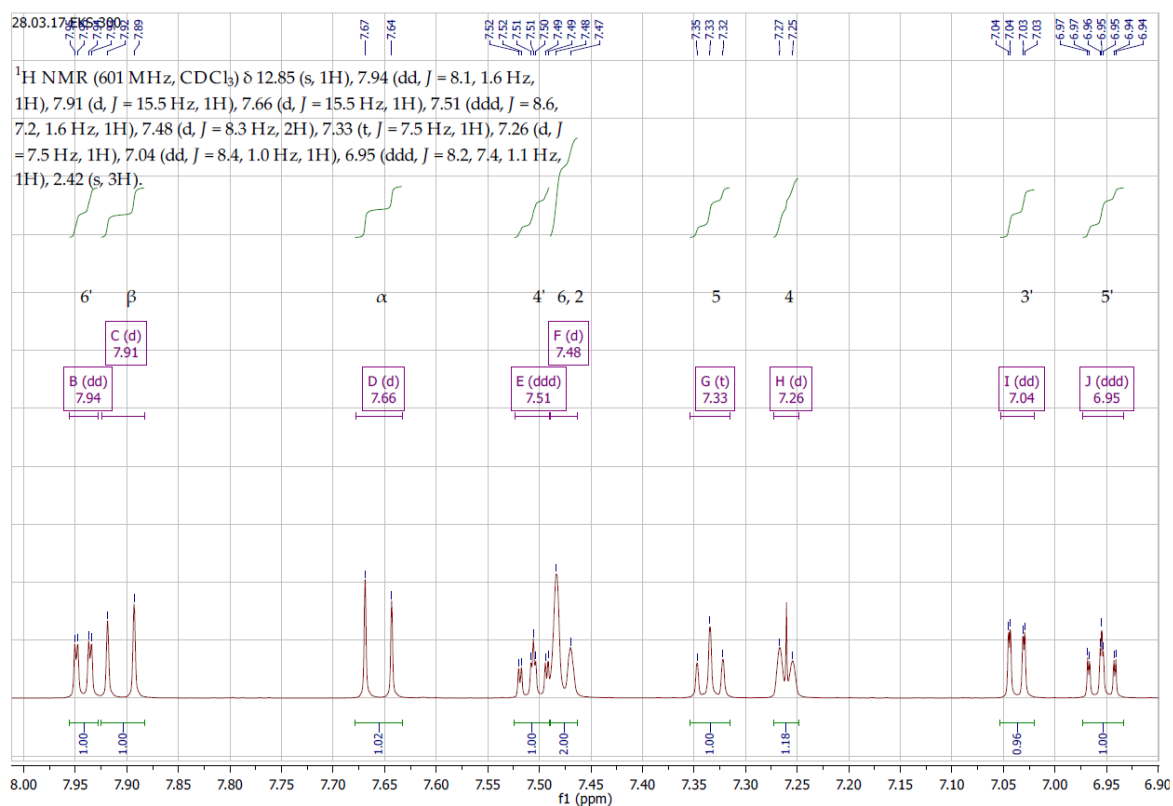
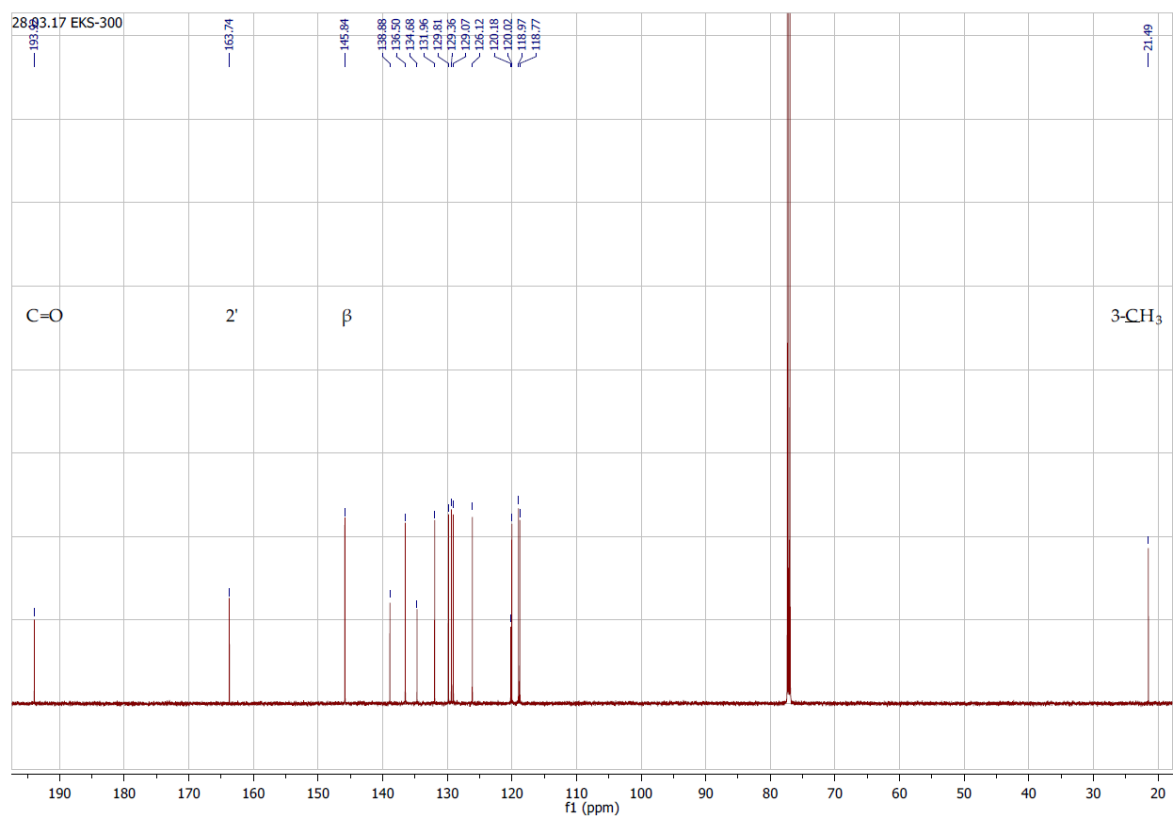
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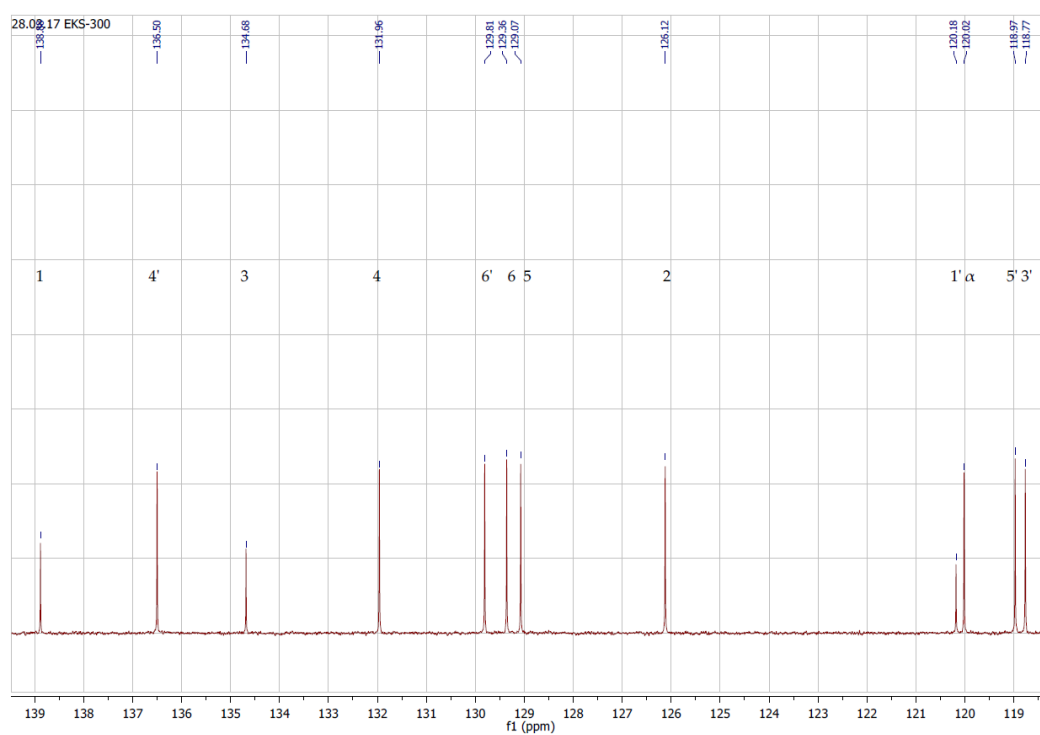
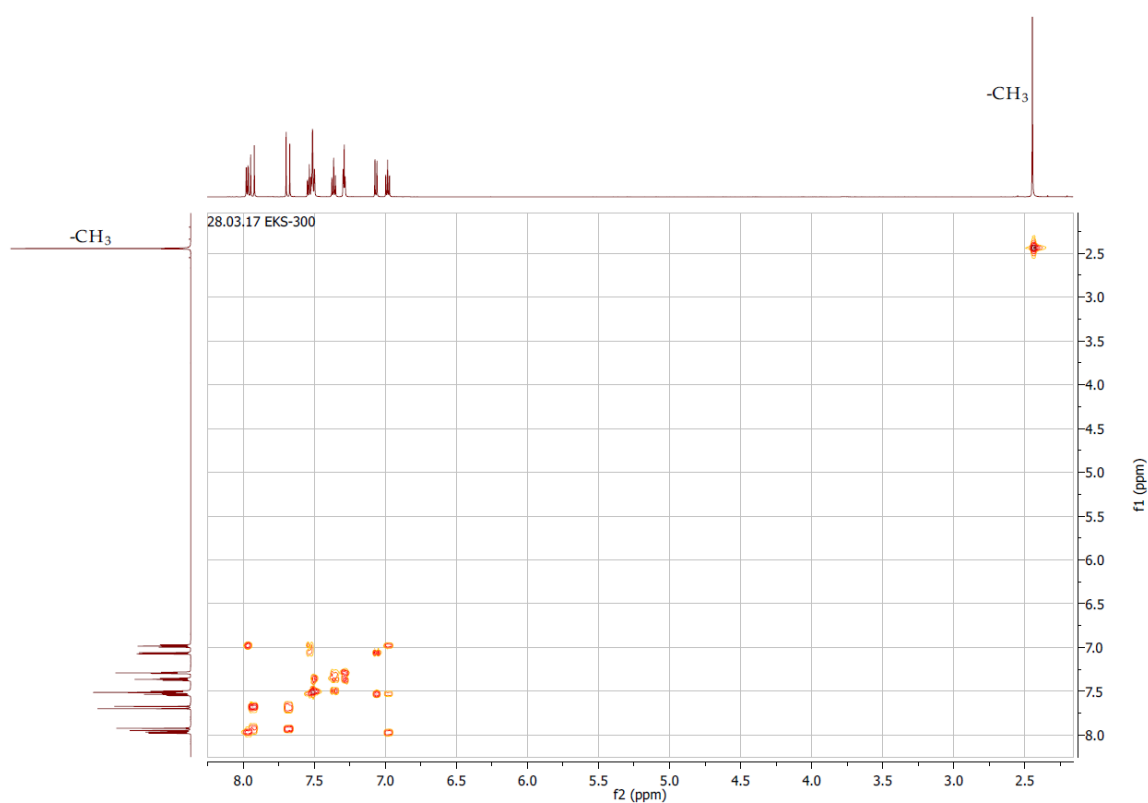
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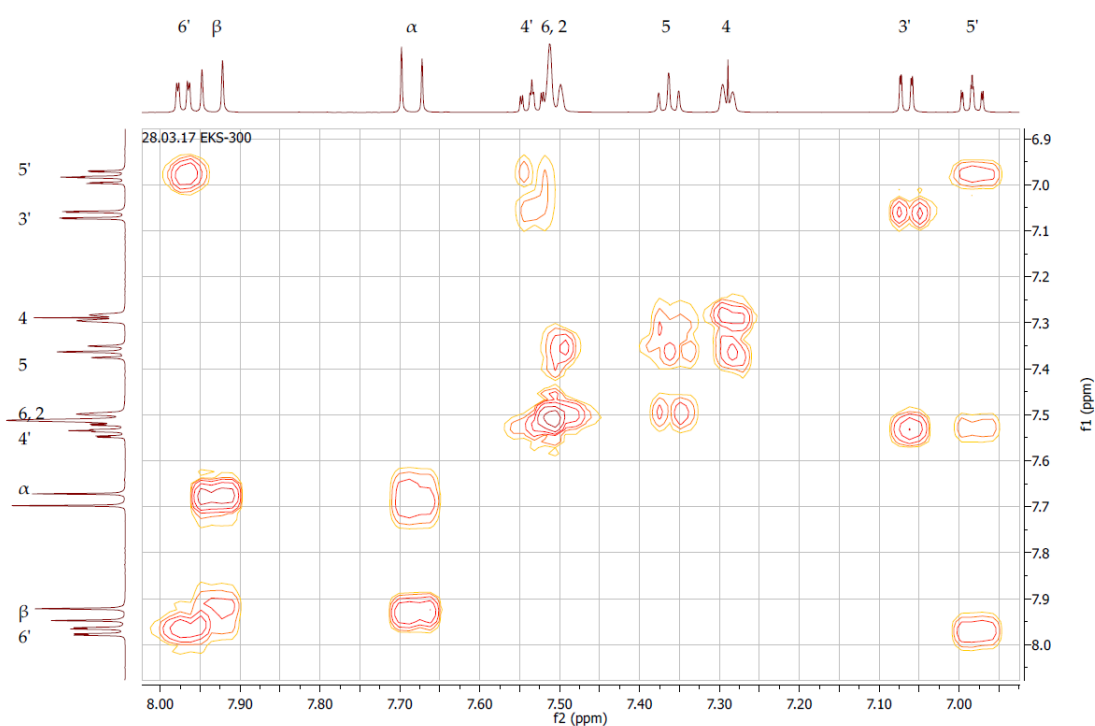
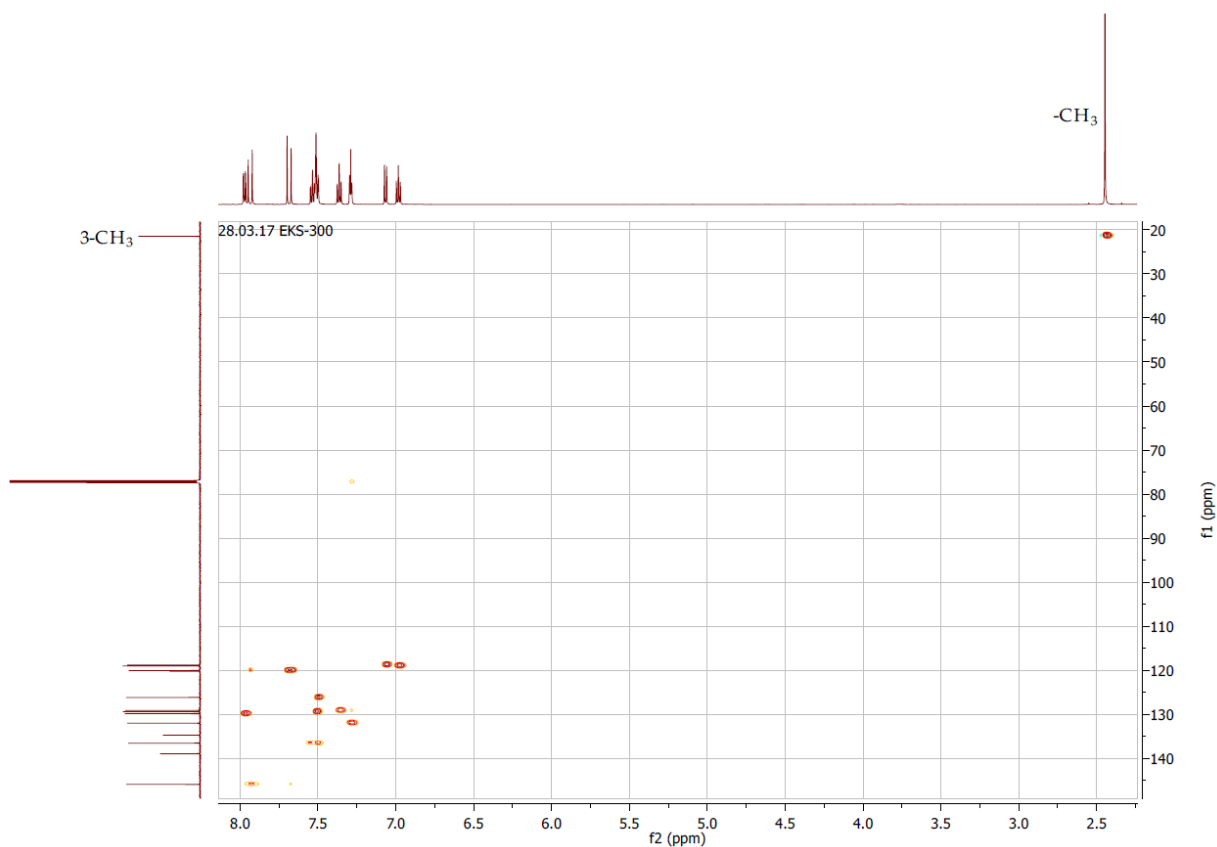
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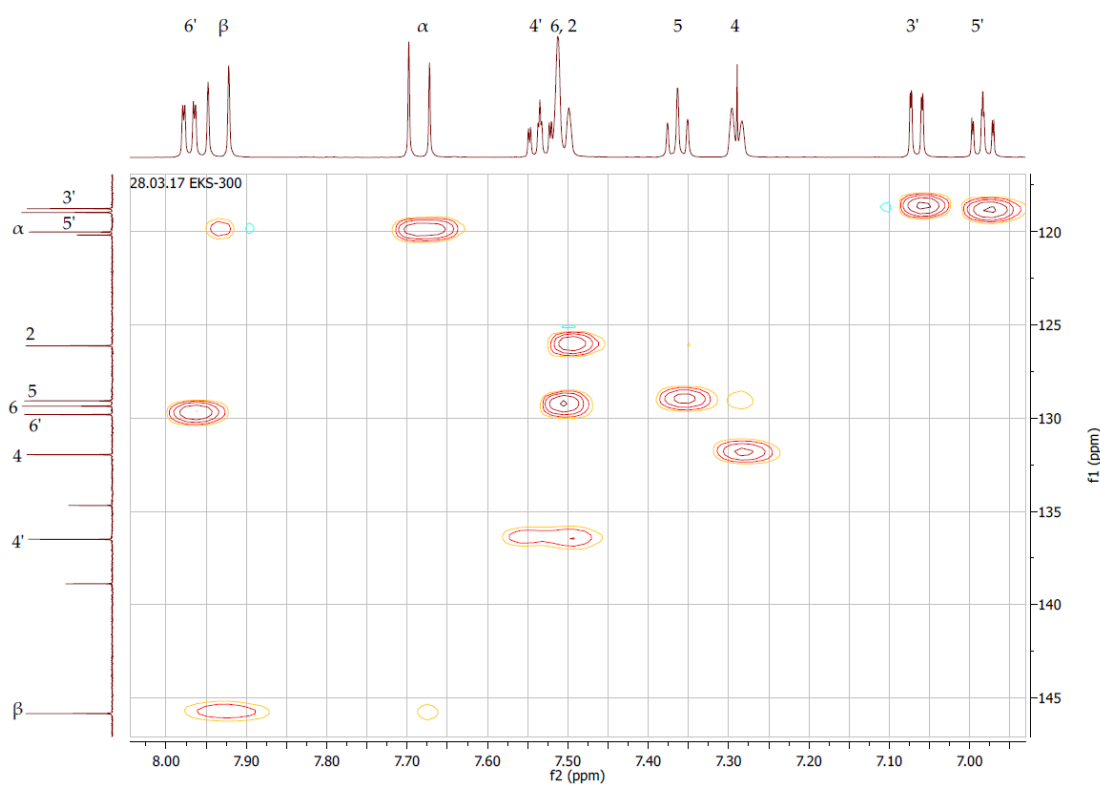
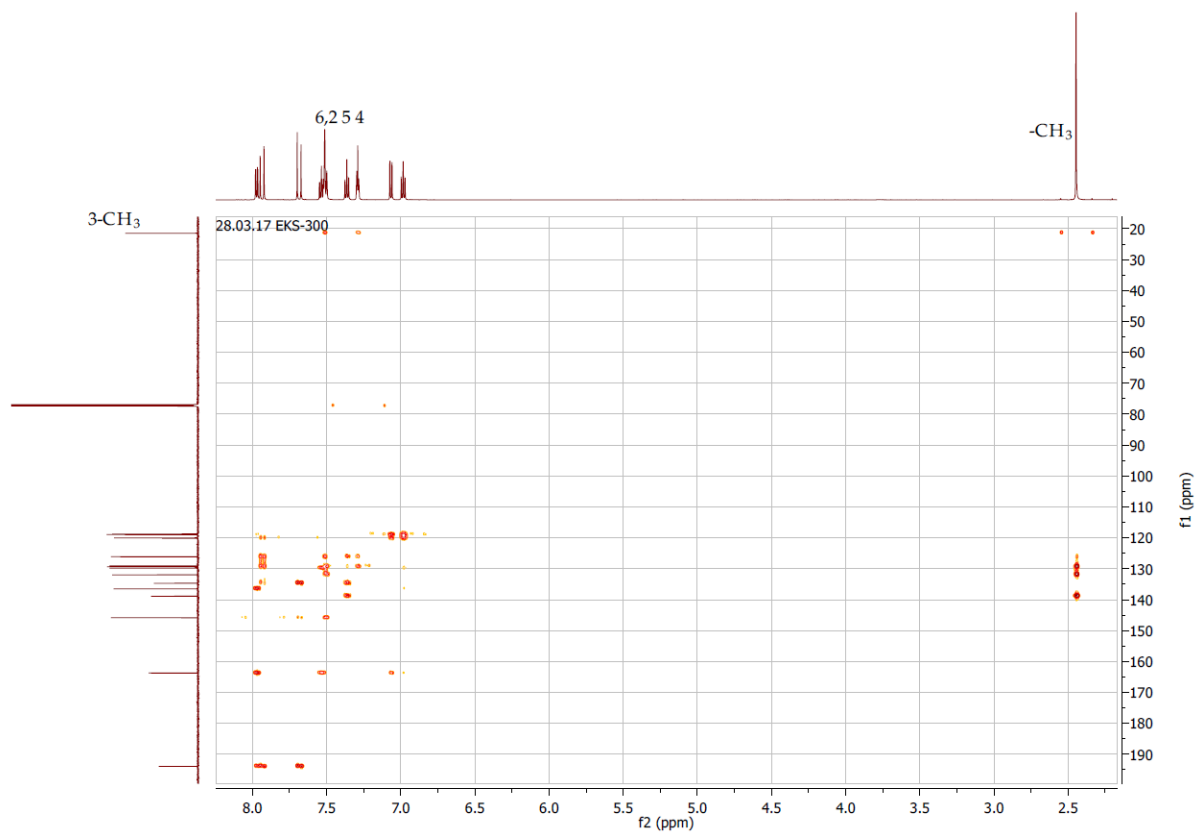
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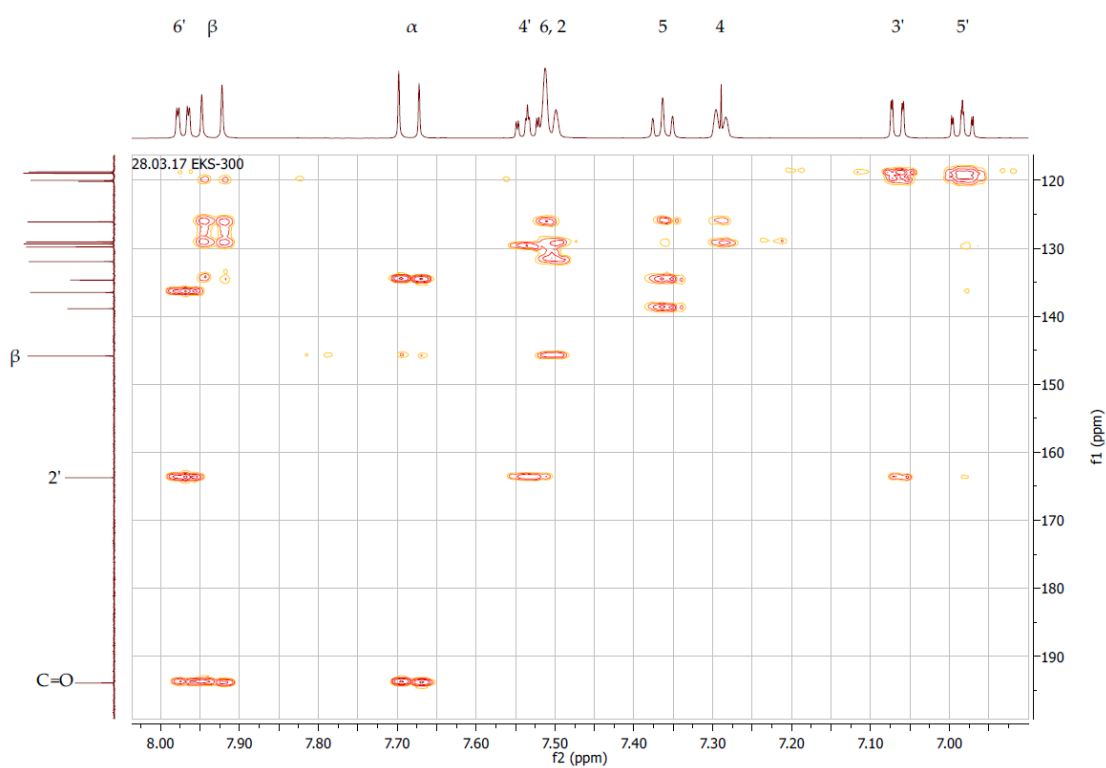
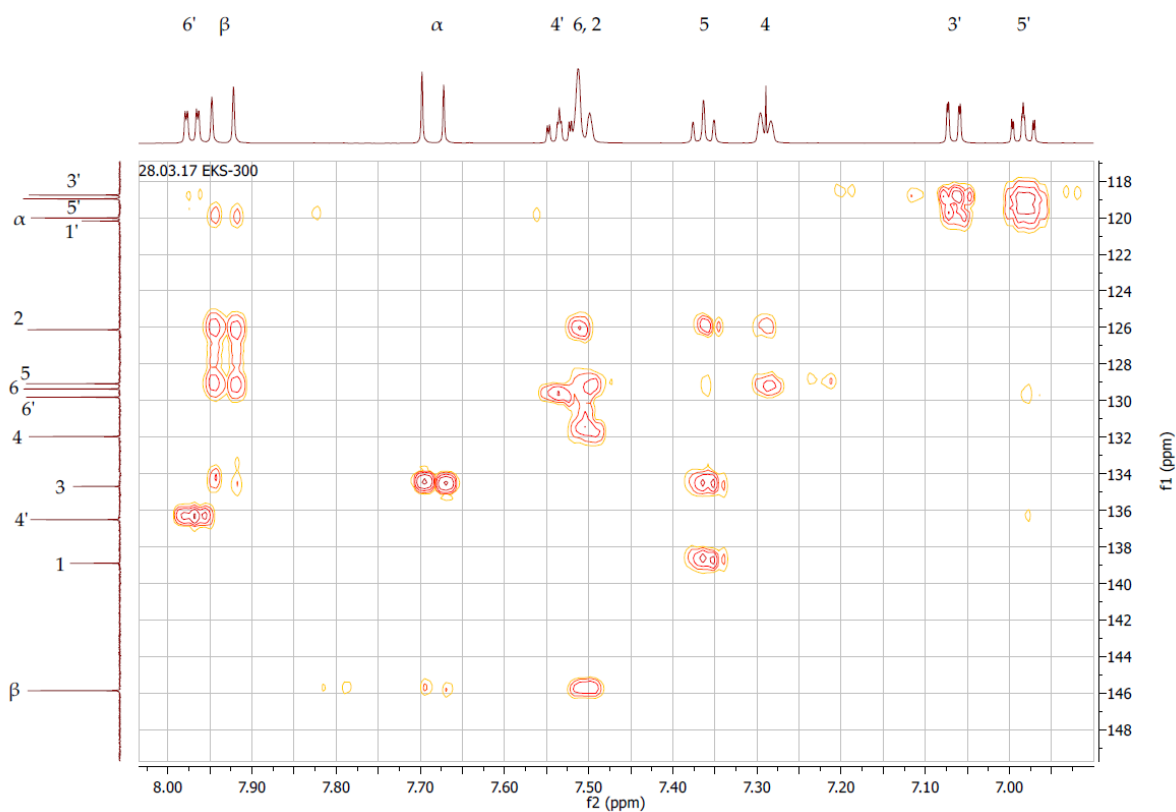
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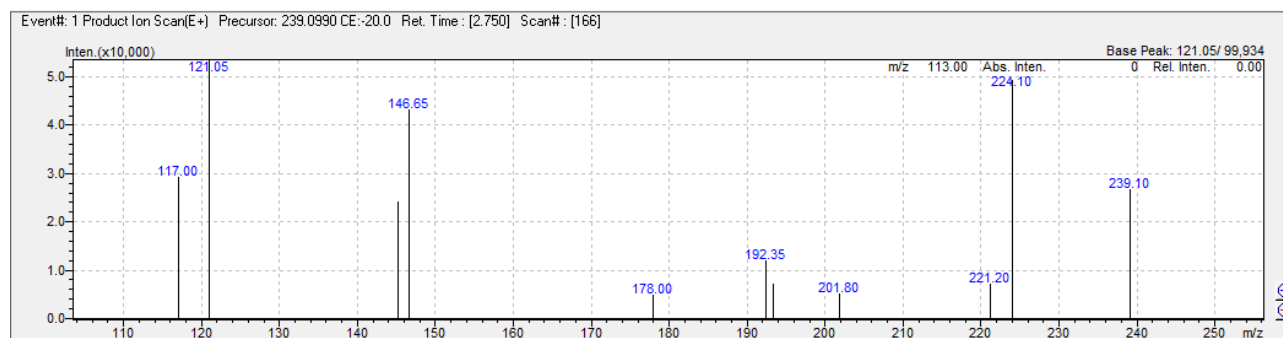
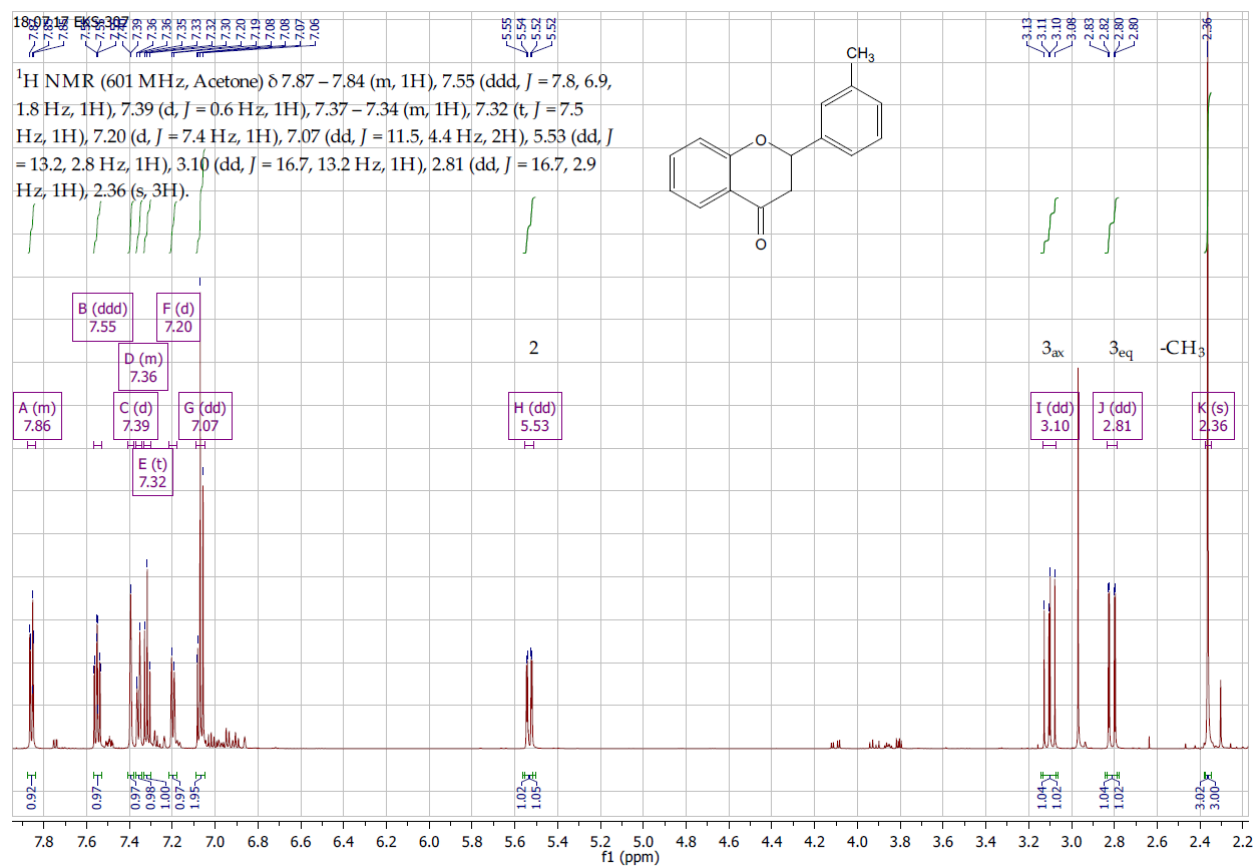
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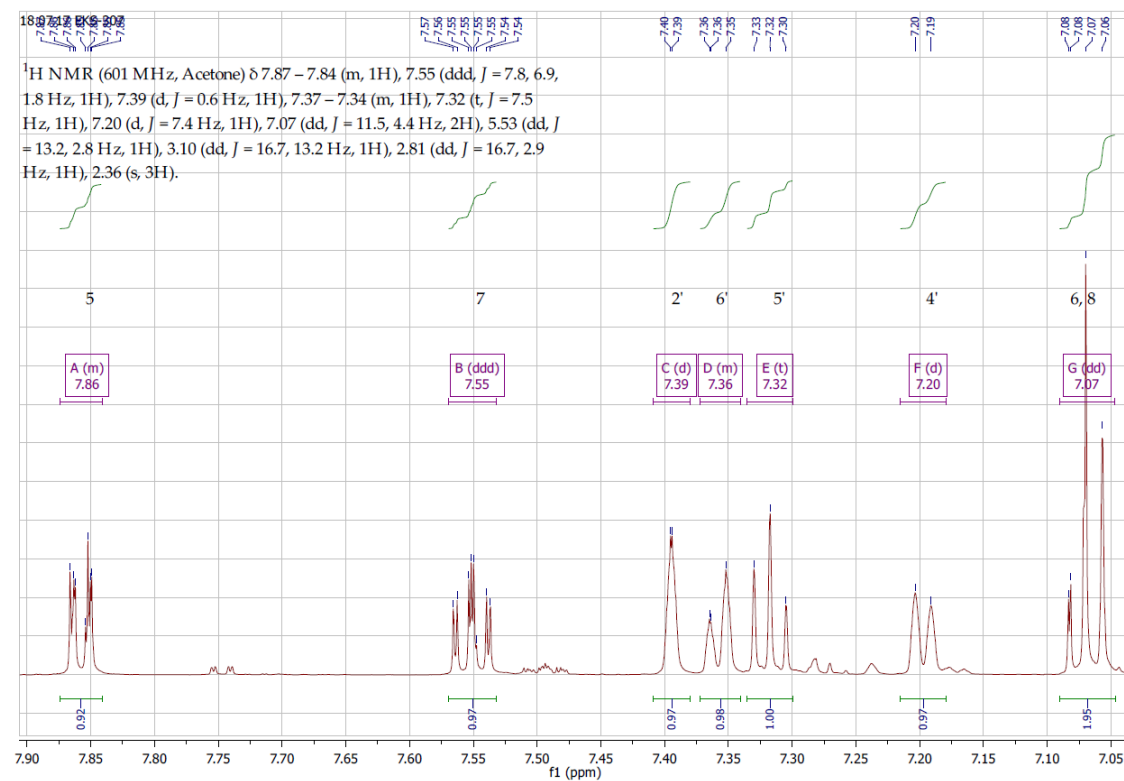
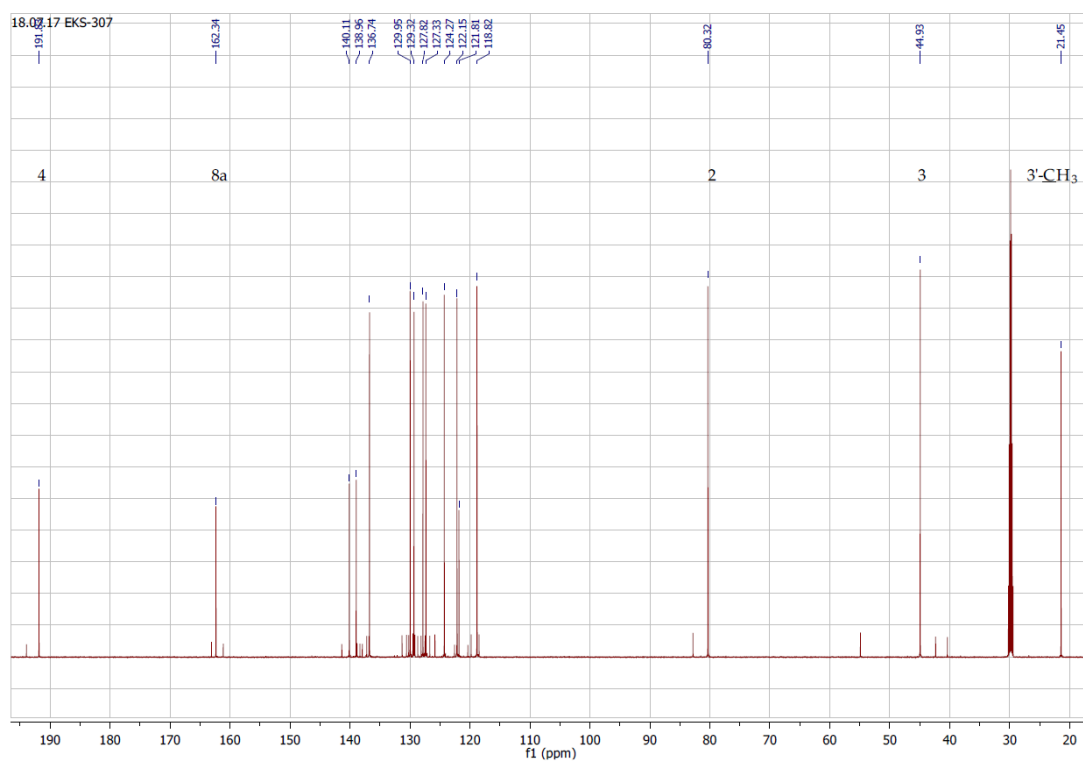
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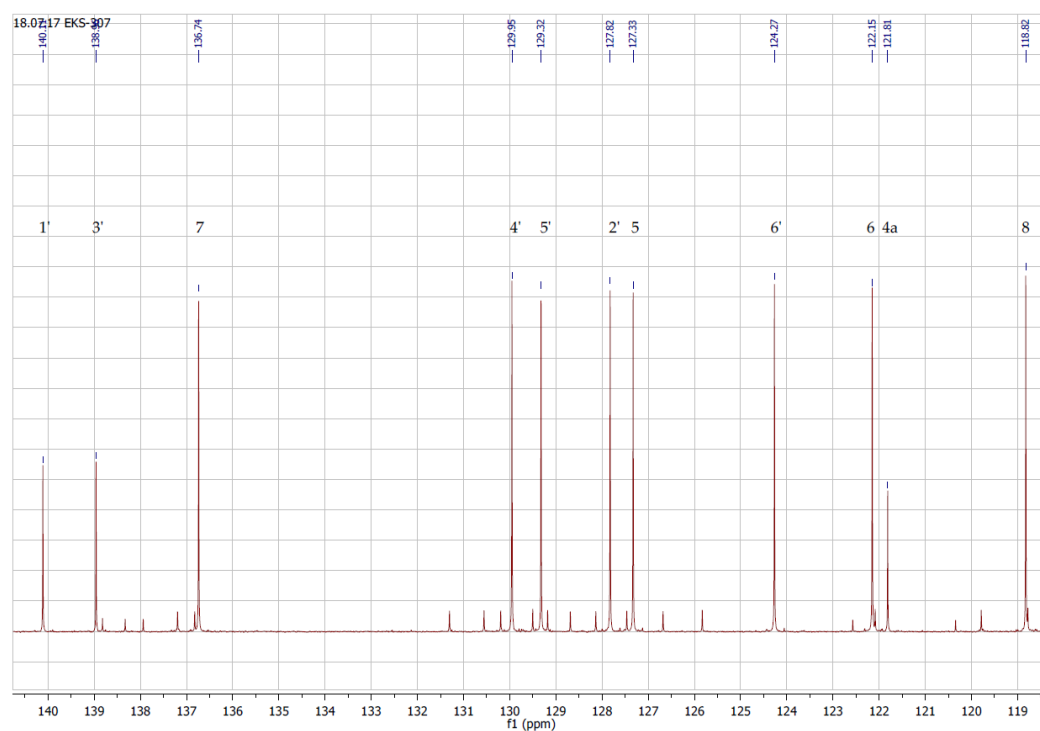
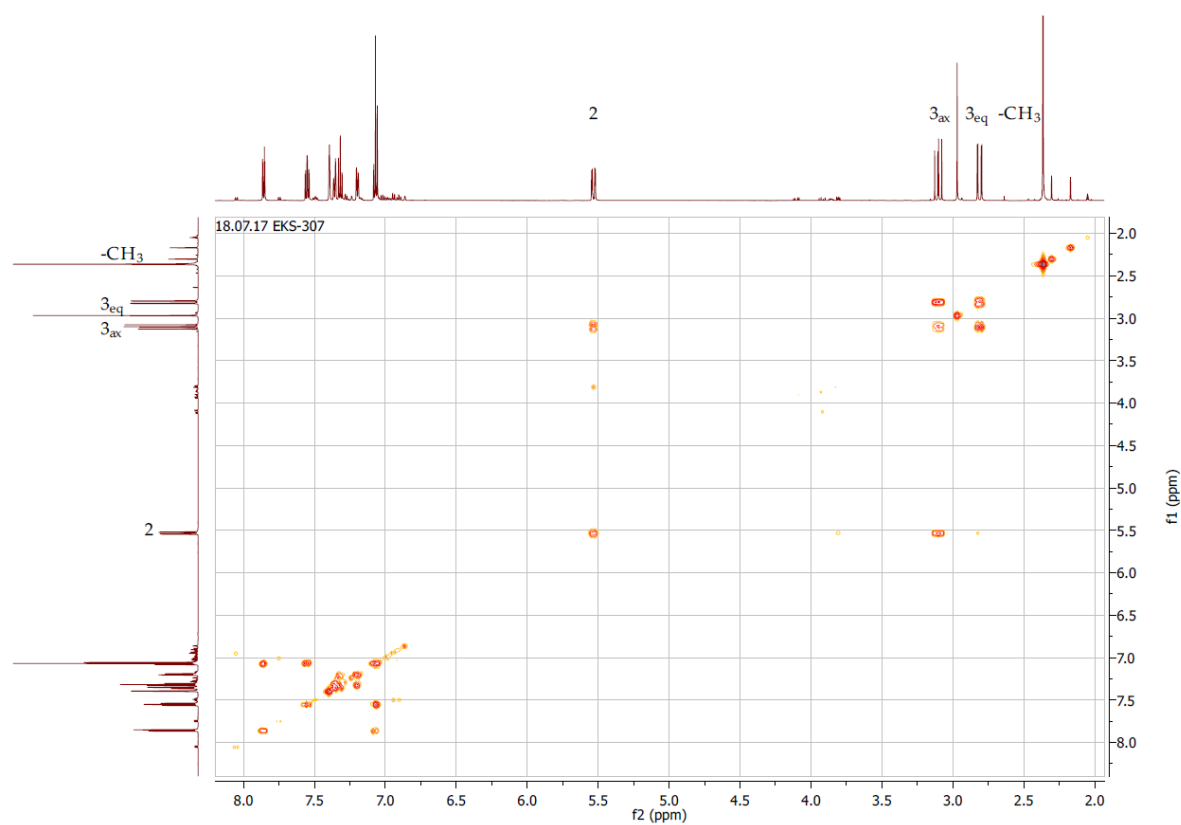
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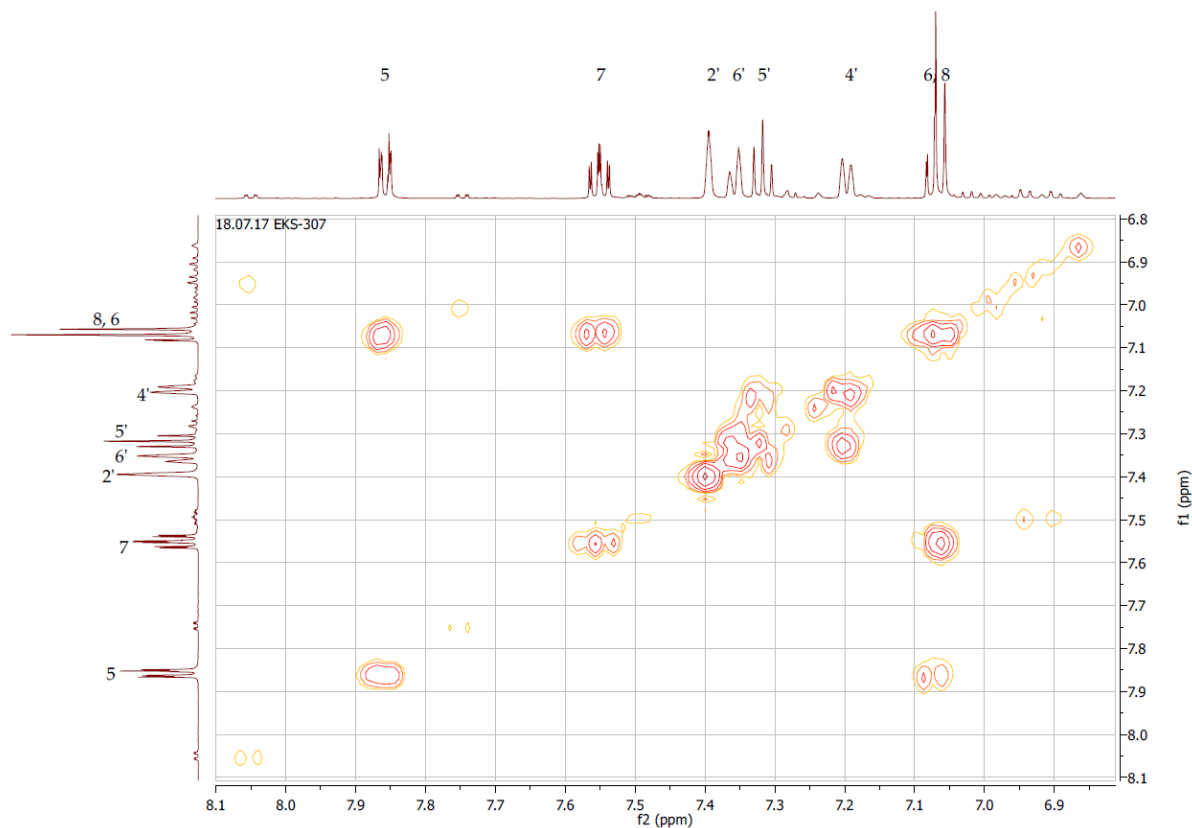
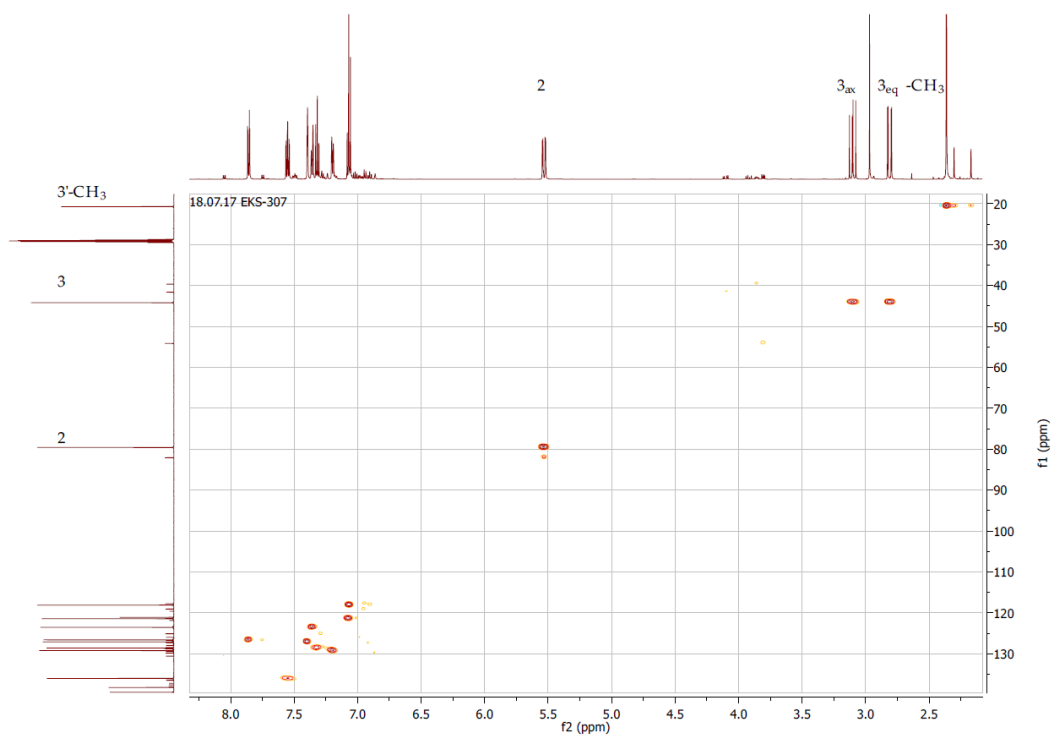
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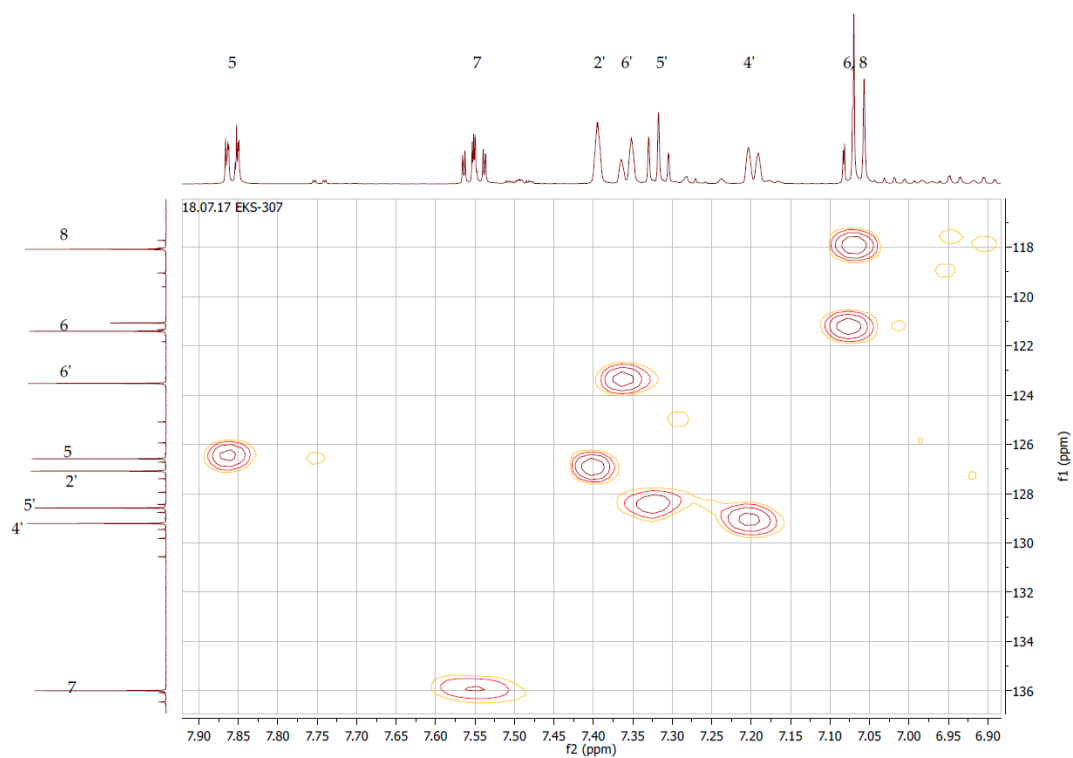
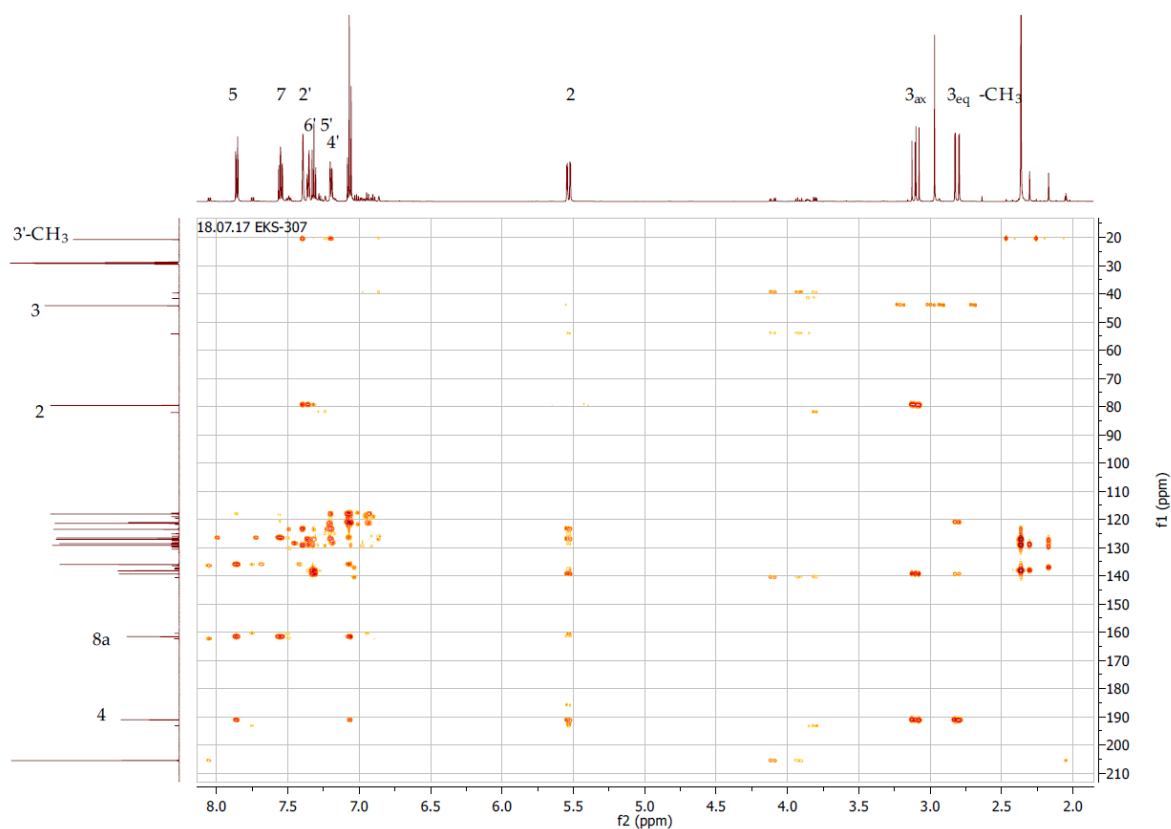
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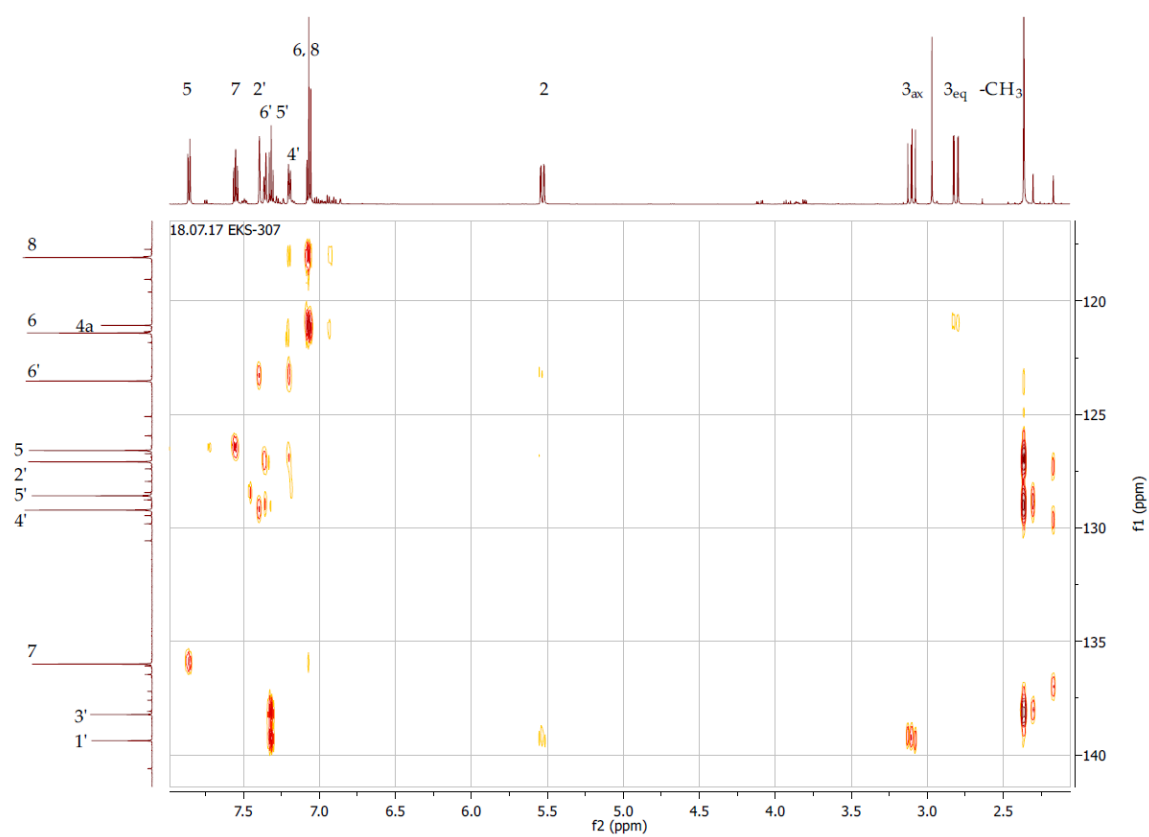
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Table S1. The effect of methyl-derivatives of flavanone on the production IL-1 β in compared to control in LPS stimulated RAW264.1 cells (n=3). Statistical significance was analysed using Fisher's LSD test. Results marked in red are statistically significant in Fisher's LSD test. Multivariate Tests of Significance (F = 7.499, p < 0.05).

	Multivariate Tests of Significance											
	Sigma-restricted parameterization											
	Effective hypothesis decomposition											
Effect	Test	Value	F	Effect	Error	p						
Intercept	Wilks	0.001581	2273.118	5	18.00000	0.000000						
sample	Wilks	0.000462	7.499	50	85.45684	0.000000						
LSD test; variable IL-1β (sta-zabrze)												
Probabilities for Post Hoc Tests												
Error: Between MS = 6,1378, df = 22,000												
Cell No.	sample	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}
1	K DMSO + LPS		0.880882	0.318805	0.009652	0.006865	0.757877	0.963008	0.032619	0.612231	0.875184	0.538352
2	5B 1μM + LPS	0.880882		0.253881	0.006813	0.004823	0.647391	0.917569	0.044695	0.720358	0.994230	0.640541
3	5B 20μM + LPS	0.318805	0.253881		0.083296	0.062462	0.486448	0.297571	0.003257	0.139229	0.251016	0.114164
4	6B 1μM + LPS	0.009652	0.006813	0.083296		0.883452	0.019403	0.008671	0.000040	0.002907	0.006699	0.002231
5	6B 20μM + LPS	0.006865	0.004823	0.062462	0.883452		0.013973	0.006158	0.000028	0.002040	0.004741	0.001562
6	7B 1μM + LPS	0.757877	0.647391	0.486448	0.019403	0.013973		0.722984	0.016612	0.417480	0.642240	0.358826
7	7B 20μM + LPS	0.963008	0.917569	0.297571	0.008671	0.006158	0.722984		0.035993	0.644880	0.911834	0.569024
8	8B 1μM + LPS	0.032619	0.044695	0.003257	0.000040	0.000028	0.016612	0.035993		0.091209	0.045369	0.112020
9	8B 20μM + LPS	0.612231	0.720358	0.139229	0.002907	0.002040	0.417480	0.644880	0.091209		0.725753	0.912729
10	flawanon 1μM + LPS	0.875184	0.994230	0.251016	0.006699	0.004741	0.642240	0.911834	0.045369	0.725753		0.645685
11	flawanon 20μM + LPS	0.538352	0.640541	0.114164	0.002231	0.001562	0.358826	0.569024	0.112020	0.912729	0.645685	

Table S2. The effect of methyl-derivatives of flavanone on the production IL-6 in compared to control in LPS stimulated RAW264.1 cells (n=3). Statistical significance was analysed using Fisher's LSD test. Results marked in red are statistically significant in Fisher's LSD test. Multivariate Tests of Significance (F = 7.499, p < 0.05).

LSD test; variable **IL-6**

Probabilities for Post Hoc Tests

Error: Between MS = 25771,, df = 22,000

	sample	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}
1	K DMSO + LPS		0.014881	0.000133	0.018428	0.000066	0.002296	0.804608	0.001515	0.735847	0.253626	0.005065
2	5B 1µM + LPS	0.014881		0.060733	0.923936	0.033538	0.000004	0.008447	0.000003	0.006845	0.000947	0.000009
3	5B 20µM + LPS	0.000133	0.060733		0.050051	0.774094	0.000000	0.000072	0.000000	0.000058	0.000008	0.000000
4	6B 1µM + LPS	0.018428	0.923936	0.050051		0.027329	0.000005	0.010529	0.000003	0.008550	0.001197	0.000011
5	6B 20µM + LPS	0.000066	0.033538	0.774094	0.027329		0.000000	0.000036	0.000000	0.000029	0.000004	0.000000
6	7B 1µM + LPS	0.002296	0.000004	0.000000	0.000005	0.000000		0.004162	0.864110	0.005156	0.032999	0.741529
7	7B 20µM + LPS	0.804608	0.008447	0.000072	0.010529	0.000036	0.004162		0.002761	0.928103	0.366591	0.009041
8	8B 1µM + LPS	0.001515	0.000003	0.000000	0.000003	0.000000	0.864110	0.002761		0.003430	0.022795	0.617078
9	8B 20µM + LPS	0.735847	0.006845	0.000058	0.008550	0.000029	0.005156	0.928103	0.003430		0.415118	0.011128
10	flavanon 1µM + LPS	0.253626	0.000947	0.000008	0.001197	0.000004	0.032999	0.366591	0.022795	0.415118		0.065170
11	flavanon 20µM + LPS	0.005065	0.000009	0.000000	0.000011	0.000000	0.741529	0.009041	0.617078	0.011128	0.065170	

Table S3. The effect of methyl-derivatives of flavanone on the production IL-6 in compared to control in LPS stimulated RAW264.1 cells (n=3). Statistical significance was analysed using Fisher's LSD test. Results marked in red are statistically significant in Fisher's LSD test. Multivariate Tests of Significance (F = 7.499, p < 0.05).

LSD test; variable **IL-12p40**

Probabilities for Post Hoc Tests

Error: Between MS = 10,535, df = 22,000

	sample	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}
1	K DMSO + LPS		0.001031	0.000307	0.038380	0.010886	0.065044	0.232104	0.362711	0.365265	0.205639	0.946897
2	5B 1µM + LPS	0.001031		0.624142	0.129111	0.328996	0.079665	0.018222	0.009310	0.009203	0.021488	0.000875
3	5B 20µM + LPS	0.000307	0.624142		0.050010	0.149056	0.029096	0.005902	0.002917	0.002882	0.007030	0.000260
4	6B 1µM + LPS	0.038380	0.129111	0.050010		0.568785	0.796677	0.340662	0.216194	0.214448	0.378589	0.033339
5	6B 20µM + LPS	0.010886	0.328996	0.149056	0.568785		0.410318	0.134813	0.077516	0.076769	0.153812	0.009340
6	7B 1µM + LPS	0.065044	0.079665	0.029096	0.796677	0.410318		0.483236	0.322293	0.319948	0.530175	0.056918
7	7B 20µM + LPS	0.232104	0.018222	0.005902	0.340662	0.134813	0.483236		0.767478	0.763693	0.940615	0.208327
8	8B 1µM + LPS	0.362711	0.009310	0.002917	0.216194	0.077516	0.322293	0.767478		0.996031	0.711464	0.329661
9	8B 20µM + LPS	0.365265	0.009203	0.002882	0.214448	0.076769	0.319948	0.763693	0.996031		0.707779	0.332055
10	flawanon 1µM + LPS	0.205639	0.021488	0.007030	0.378589	0.153812	0.530175	0.940615	0.711464	0.707779		0.184018
11	flawanon 20µM + LPS	0.946897	0.000875	0.000260	0.033339	0.009340	0.056918	0.208327	0.329661	0.332055	0.184018	

Table S4. The effect of methyl-derivatives of flavanone on the production IL-12p70 in compared to control in LPS stimulated RAW264.1 cells (n=3). Statistical significance was analysed using Fisher's LSD test. Results marked in red are statistically significant in Fisher's LSD test. Multivariate Tests of Significance ($F = 7.499$, $p < 0.05$).

LSD test; variable **IL-12p70**

Probabilities for Post Hoc Tests

Error: Between MS = 15,184, df = 22,000

Cell No.	sample	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}
1	K DMSO + LPS		0.003850	0.000410	0.000050	0.000010	0.264156	0.005071	0.121969	0.000408	0.111074	0.570728
2	5B 1 μ M + LPS	0.003850		0.363357	0.086633	0.021724	0.000241	0.000002	0.000078	0.000000	0.000069	0.014484
3	5B 20 μ M + LPS	0.000410	0.363357		0.396171	0.137294	0.000025	0.000000	0.000008	0.000000	0.000007	0.001660
4	6B 1 μ M + LPS	0.000050	0.086633	0.396171		0.505590	0.000003	0.000000	0.000001	0.000000	0.000001	0.000202
5	6B 20 μ M + LPS	0.000010	0.021724	0.137294	0.505590		0.000001	0.000000	0.000000	0.000000	0.000000	0.000039
6	7B 1 μ M + LPS	0.264156	0.000241	0.000025	0.000003	0.000001		0.061933	0.648135	0.006379	0.612198	0.099198
7	7B 20 μ M + LPS	0.005071	0.000002	0.000000	0.000000	0.000000	0.061933		0.146748	0.306270	0.160439	0.001286
8	8B 1 μ M + LPS	0.121969	0.000078	0.000008	0.000001	0.000000	0.648135	0.146748		0.018187	0.959332	0.039895
9	8B 20 μ M + LPS	0.000408	0.000000	0.000000	0.000000	0.000000	0.006379	0.306270	0.018187		0.020364	0.000100
10	flavanon 1 μ M + LPS	0.111074	0.000069	0.000007	0.000001	0.000000	0.612198	0.160439	0.959332	0.020364		0.035837
11	flavanon 20 μ M + LPS	0.570728	0.014484	0.001660	0.000202	0.000039	0.099198	0.001286	0.039895	0.000100	0.035837	

Table S5. The effect of methyl-derivatives of flavanone on the production TNF- α in compared to control in LPS stimulated RAW264.1 cells (n=3). Statistical significance was analysed using Fisher's LSD test. Results marked in red are statistically significant in Fisher's LSD test. Multivariate Tests of Significance (F = 7.499, p < 0.05).

LSD test; variable **TNF- α**

Probabilities for Post Hoc Tests

Error: Between MS = 3334E4, df = 22,000

	sample	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}
1	K DMSO + LPS		0.000296	0.000095	0.826990	0.974889	0.927038	0.373381	0.531949	0.069246	0.571884	0.008844
2	5B 1 μ M + LPS	0.000296		0.646828	0.000172	0.000274	0.000236	0.000032	0.000063	0.000003	0.000073	0.000000
3	5B 20 μ M + LPS	0.000095	0.646828		0.000056	0.000088	0.000076	0.000011	0.000021	0.000001	0.000024	0.000000
4	6B 1 μ M + LPS	0.826990	0.000172	0.000056		0.851557	0.898877	0.498972	0.682982	0.105365	0.727688	0.014581
5	6B 20 μ M + LPS	0.974889	0.000274	0.000088	0.851557		0.952075	0.390062	0.552539	0.073666	0.593252	0.009512
6	7B 1 μ M + LPS	0.927038	0.000236	0.000076	0.898877	0.952075		0.423234	0.592983	0.082795	0.635104	0.010921
7	7B 20 μ M + LPS	0.373381	0.000032	0.000011	0.498972	0.390062	0.423234		0.786939	0.327509	0.740952	0.062300
8	8B 1 μ M + LPS	0.531949	0.000063	0.000021	0.682982	0.552539	0.592983	0.786939		0.215599	0.951763	0.035704
9	8B 20 μ M + LPS	0.069246	0.000003	0.000001	0.105365	0.073666	0.082795	0.327509	0.215599		0.195135	0.346267
10	flawanon 1 μ M + LPS	0.571884	0.000073	0.000024	0.727688	0.593252	0.635104	0.740952	0.951763	0.195135		0.031395
11	flawanon 20 μ M + LPS	0.008844	0.000000	0.000000	0.014581	0.009512	0.010921	0.062300	0.035704	0.346267	0.031395	

Table S6. Average, standard deviations, statistical significance of cytotoxic activity of methyl-derivatives of flavanone. Statistical significance was calculated using t-test (Table S3).

Sample	RAW264.7 cell viability [%]		
	AVG	SD	<i>p</i>
Control	97.83	3.14	
5B 1 μ M	111.04	12.65	0.114254581
5B 10 μ M	97.93	4.94	0.268060958
5B 20 μ M	92.17	8.00	0.09701206
6B 1 μ M	92.47	1.93	0.182880144
6B 10 μ M	96.81	2.26	0.065944168
6B 20 μ M	89.21	5.46	0.102198598
7B 1 μ M	91.32	1.82	0.001728865
7B 10 μ M	92.95	2.20	0.005413282
7B 20 μ M	89.71	2.74	0.006185175
8B 1 μ M	94.67	5.34	0.140268954
8B 10 μ M	90.45	2.85	0.002481306
8B 20 μ M	88.45	3.41	0.002088066
Flavanone 1 μ M	94.46	2.78	0.020122714
Flavanone 10 μ M	97.94	0.87	0.124969323
Flavanone 20 μ M	97.82	3.10	0.287500787

Table S7. Average, standard deviations, statistical significance of chemiluminescence of methyl-derivatives of flavanone. Statistical significance was calculated using t-test (Table S5).

Sample	RAW264.7 cell viability [%]		
	AVG	SD	<i>p</i>
5B 1μM	107.16	9.42	0.604869355
5B 5μM	102.45	5.02	0.006617773
5B 10μM	65.54	5.15	0.012756791
5B 50μM	28.50	3.16	0.026308649
6B 1μM	94.99	11.99	0.093523114
6B 5μM	89.21	9.56	0.003180751
6B 10μM	58.68	3.73	0.000482798
6B 50μM	18.65	3.41	0.000382919
7B 1μM	99.21	11.79	0.192650358
7B 5μM	97.11	11.36	0.01929824
7B 10μM	68.47	3.39	0.010000
7B 50μM	14.85	2.18	0.000205425
8B 1μM	127.34	7.14	0.063302521
8B 5μM	119.74	5.98	0.52493694
8B 10μM	76.15	6.02	0.538279569
8B 50μM	34.18	5.47	0.562122327
Flavanone 1μM	111.36	12.34	
Flavanone 5μM	123.30	6.87	
Flavanone 10μM	78.80	3.43	
Flavanone 50μM	36.35	4.63	

Table S8. The effect of methyl-derivatives of flavanone on the concentration of nitrite in compared to control in LPS stimulated RAW264.1 cells (n=3). Statistical significance was analysed using Fisher's LSD test. Results marked in red are statistically significant in Fisher's LSD test. Multivariate Tests of Significance (F = 7.499, p < 0).

Effect	Univariate Tests of Significance for Concentration of nitrite																
	SS	Degr. of	MS	F	p												
Intercept	62859.92	1	62859.92	26376.71	0.00												
sample	1885.72	15	125.71	52.75	0.00												
Error	114.39	48	2.38														
LSD test; variable Concentration of nitrite (sta-zabrze)Probabilities for Post Hoc TestsError: Between MS = 2,3832, df = 48,000																	
Cell No.	sample	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}
1	K DMSO + LPS		0.000000	0.000000	0.000000	0.000008	0.000002	0.000000	0.492490	0.981278	0.000639	0.100514	0.207014	0.649640	0.862840	0.118688	0.261753
2	5B 1µM + LPS	0.000000		0.022867	0.271682	0.000000	0.000000	0.000776	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
3	5B 10µM + LPS	0.000000	0.022867		0.221294	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
4	5B 20µM + LPS	0.000000	0.271682	0.221294		0.000000	0.000000	0.000022	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
5	6B 1µM + LPS	0.000008	0.000000	0.000000	0.000000		0.690689	0.000690	0.000084	0.000009	0.188108	0.001753	0.000538	0.000002	0.000005	0.001364	0.000345
6	6B 10µM + LPS	0.000002	0.000000	0.000000	0.000000	0.690689		0.002249	0.000022	0.000002	0.089063	0.000530	0.000154	0.000000	0.000001	0.000407	0.000097
7	6B 20µM + LPS	0.000000	0.000776	0.000000	0.000022	0.000690	0.002249		0.000000	0.000000	0.000009	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
8	7B 1µM + LPS	0.492490	0.000000	0.000000	0.000000	0.000084	0.000022	0.000000		0.507296	0.004740	0.330553	0.559660	0.256340	0.391158	0.374154	0.659055
9	7B 10µM + LPS	0.981278	0.000000	0.000000	0.000000	0.000009	0.000002	0.000000	0.507296		0.000686	0.105262	0.215374	0.632900	0.844442	0.124120	0.271662
10	7B 20µM + LPS	0.000639	0.000000	0.000000	0.000000	0.188108	0.089063	0.000009	0.004740	0.000686		0.053543	0.021606	0.000153	0.000374	0.044337	0.015184
11	8B 1µM + LPS	0.100514	0.000000	0.000000	0.000000	0.001753	0.000530	0.000000	0.330553	0.105262	0.053543		0.694210	0.038174	0.070724	0.931915	0.592385
12	8B 10µM + LPS	0.207014	0.000000	0.000000	0.000000	0.000538	0.000154	0.000000	0.559660	0.215374	0.021606	0.694210		0.088940	0.152791	0.758178	0.886522
13	8B 20µM + LPS	0.649640	0.000000	0.000000	0.000000	0.000002	0.000000	0.000000	0.256340	0.632900	0.000153	0.038174	0.088940		0.778059	0.046271	0.117780
14	flawanon 1µM + LPS	0.862840	0.000000	0.000000	0.000000	0.000005	0.000001	0.000000	0.391158	0.844442	0.000374	0.070724	0.152791	0.778059		0.084365	0.196661
15	flawanon 10µM + LPS	0.118688	0.000000	0.000000	0.000000	0.001364	0.000407	0.000000	0.374154	0.124120	0.044337	0.931915	0.758178	0.046271	0.084365		0.652516
16	flawanon 20µM + LPS	0.261753	0.000000	0.000000	0.000000	0.000345	0.000097	0.000000	0.659055	0.271662	0.015184	0.592385	0.886522	0.117780	0.196661	0.652516	