Mechanistic insights into carbonic anhydrase IX inhibition by coumarins from *Calendula officinalis*: *in vitro* and *in silico* approaches

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Cartesian coordinates of DFT-optimized geometries of isolated coumarins

6,7-di-O-glucopyranosyl esculetin

C	-8.25383	4.47979	-0.15492
C	-7.04713	4.92899	0.61158
C	-5.93553	4.19609	0.71378
C	-5.91443	2.90529	0.04358
C	-7.03393	2.47549	-0.67442
O	-8.18553	3.20079	-0.79832
C	-7.01973	1.23739	-1.32002
C	-5.88903	0.42329	-1.25202
C	-4.77003	0.84719	-0.53742
C	-4.78173	2.08528	0.10928
O	-9.24412	5.16775	-0.22765
O	-5.87862	-0.77756	-1.87963
O	-3.67054	0.05814	-0.46998
C	-3.8677	-1.13254	-1.18344
C	-2.54575	-1.8837	-1.27588
O	-2.71802	-3.01996	-2.0789
C	-2.07296	-2.31654	0.10785
O	-1.69439	-1.18277	0.84056
C	-3.18761	-3.04446	0.85182
O	-3.41684	-4.28381	0.23775
C	-4.47415	-2.22942	0.82699
O	-4.85211	-1.92093	-0.51472
C	-4.25799	-0.9323	1.59527
O	-5.43808	-0.17555	1.57738
H	-7.07842	5.90748	1.11315
H	-5.06668	4.55574	1.28452
H	-7.90297	0.90299	-1.884

H	-3.89716	2.41733	0.67255
H	-4.22614	-0.91206	-2.21382
H	-1.78084	-1.20815	-1.72008
H	-1.8853	-3.45891	-2.11453
H	-1.20773	-3.00701	-0.00784
H	-1.40922	-1.48558	1.68576
H	-2.87605	-3.18876	1.91053
H	-4.10777	-4.70482	0.72017
H	-5.28554	-2.8282	1.29802
H	-3.43786	-0.35101	1.1175
H	-3.98345	-1.16629	2.6482
H	-5.26422	0.61434	2.0603
C	-6.72456	-1.00046	-3.01083
C	-7.9197	-1.70431	-2.86015
C	-8.74466	-1.92234	-3.96338
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H	-7.4278	0.22832	-5.77187
O	-6.35496	-0.51415	-4.2648
H	-9.16404	-0.79507	-5.55335
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H	-6.87836	-1.50477	-7.34956
H	-6.15557	-2.47603	-6.08968
O	-5.09922	-0.79659	-6.61265
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O	-7.58973	-2.98634	-2.31937
H	-8.38954	-3.50808	-2.22086

Aesculin

C	0	-8.25383	4.47979	-0.15492
C	0	-7.04713	4.92899	0.61158
C	0	-5.93553	4.19609	0.71378
C	0	-5.91443	2.90529	0.04358
C	0	-7.03393	2.47548	-0.67442
O	0	-8.18553	3.20079	-0.79832
C	0	-7.01973	1.23739	-1.32002
C	0	-5.88903	0.42329	-1.25202
C	0	-4.77003	0.84719	-0.53742
C	0	-4.78173	2.08528	0.10928
O	0	-9.24412	5.16775	-0.22765
O	0	-5.87862	-0.77756	-1.87963
O	0	-3.67054	0.05814	-0.46998
C	0	-3.8677	-1.13254	-1.18344
C	0	-2.54575	-1.8837	-1.27588
O	0	-2.71802	-3.01996	-2.0789
C	0	-2.07296	-2.31654	0.10785
O	0	-1.69439	-1.18277	0.84056
C	0	-3.18761	-3.04446	0.85182
O	0	-3.41684	-4.28381	0.23775
C	0	-4.47415	-2.22942	0.82699
O	0	-4.85211	-1.92093	-0.51472

C	0	-4.25799	-0.9323	1.59527
O	0	-5.43808	-0.17555	1.57738
H	0	-7.07842	5.90748	1.11315
H	0	-5.06668	4.55574	1.28452
H	0	-7.90297	0.90299	-1.884
H	0	-3.89716	2.41733	0.67255
H	0	-5.01469	-1.19299	-1.71891
H	0	-4.22614	-0.91206	-2.21382
H	0	-1.78084	-1.20815	-1.72008
H	0	-1.8853	-3.45891	-2.11453
H	0	-1.20773	-3.00701	-0.00784
H	0	-1.40922	-1.48558	1.68576
H	0	-2.87605	-3.18876	1.91053
H	0	-4.10777	-4.70482	0.72017
H	0	-5.28554	-2.8282	1.29802
H	0	-3.43786	-0.35101	1.1175
H	0	-3.98345	-1.16629	2.6482
H	0	-5.26422	0.61434	2.0603

Isobaisseoside

C	0	-0.90001	-0.00858	-0.8776
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C	0	-5.92701	6.26079	0.43319
O	0	-8.124	6.49956	-0.40604
C	0	-7.13371	5.81159	-0.33331
C	0	2.71095	-2.33382	2.63058
O	0	0.91804	-1.83141	1.10907
C	0	1.29487	-1.79238	2.4854
O	0	0.69007	-2.4984	4.68125
C	0	0.3486	-2.63735	3.32859
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C	0	-1.09605	-2.19138	3.12938
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C	0	-1.43411	-2.11676	1.64414
C	0	-0.38966	-1.30057	0.89378
O	0	-0.67892	-1.33401	-0.47765
H	0	0.0144	0.59494	-0.68164
H	0	-1.75475	0.41427	-0.30369
H	0	-0.46605	0.6358	-2.9111

H	0	-1.36037	-2.24298	-4.6107
H	0	-0.2083	-1.8518	-2.67365
H	0	-2.68785	-2.98313	-0.57921
H	0	-2.36113	-3.20714	-2.83015
H	0	-4.87701	-1.05147	-3.75007
H	0	-4.38312	-2.07753	-1.76618
H	0	-3.27922	-0.13154	-0.78036
H	0	-6.78284	2.23479	-2.06239
H	0	-2.7307	0.5797	-1.15413
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H	0	-3.94656	5.88755	1.10613
H	0	-5.9583	7.23928	0.93476
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H	0	2.70224	-3.21712	3.30769
H	0	3.09702	-2.63518	1.63112
H	0	1.24472	-0.7385	2.8398
H	0	0.08796	-3.03145	5.17184
H	0	0.44137	-3.7007	3.01321
H	0	-2.83171	-2.80679	3.60852
H	0	-1.22538	-1.18125	3.57845
H	0	-2.86582	-1.48166	0.56355
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H	0	-0.41658	-0.25346	1.27008

Skimmin

C	0	-9.04893	4.29659	1.95119
C	0	-7.84223	4.7458	2.71769
C	0	-6.73063	4.0129	2.81989
C	0	-6.70953	2.7221	2.14969

C	0	-5.57683	1.9021	2.21539
C	0	-5.56513	0.664	1.56869
C	0	-6.68413	0.2401	0.85409
C	0	-7.81483	1.05419	0.78609
C	0	-7.82903	2.2923	1.43169
O	0	-8.98063	3.0176	1.30779
O	0	-10.03922	4.98456	1.87846
O	0	-6.67372	-0.96075	0.22648
O	0	-4.42293	-0.89863	-0.26841
C	0	-5.44832	-1.61356	0.42099
C	0	-5.5551	-3.04102	-0.09988
C	0	-5.78156	-3.04982	-1.60793
C	0	-4.74973	-2.17341	-2.31009
C	0	-4.68728	-0.79383	-1.66747
C	0	-6.02097	-0.08739	-1.87173
O	0	-6.88947	-0.92463	-2.58611
O	0	-3.49001	-2.78158	-2.21612
O	0	-7.06587	-2.55972	-1.88351
O	0	-4.36688	-3.72621	0.19039
H	0	-7.87352	5.72429	3.21926
H	0	-5.86178	4.37256	3.39063
H	0	-4.69225	2.23414	2.77866
H	0	-4.67255	0.02344	1.62344
H	0	-8.69806	0.7198	0.22211
H	0	-5.19446	-1.64653	1.50415
H	0	-6.41939	-3.5384	0.39448
H	0	-5.67945	-4.09364	-1.98046
H	0	-5.04521	-2.05794	-3.37692

H	0	-3.86496	-0.21463	-2.14402
H	0	-5.85822	0.8529	-2.44456
H	0	-6.46868	0.15314	-0.88155
H	0	-7.6987	-0.45405	-2.69125
H	0	-2.87945	-2.21647	-2.65797
H	0	-7.17137	-2.57904	-2.81939
H	0	-4.4667	-4.60123	-0.14387

Xeroboside

C	-6.8	7.46818	1.78691
C	-5.5933	7.91738	2.55341
C	-4.4817	7.18447	2.65561
C	-4.4606	5.89367	1.98541
C	-3.3279	5.07367	2.05111
C	-3.3162	3.83558	1.40441
C	-4.4352	3.41168	0.68981
C	-5.5659	4.22578	0.62181
C	-5.5801	5.46387	1.26741
O	-6.7317	6.18918	1.14351
O	-7.79029	8.15615	1.71419
O	-2.2167	3.04653	1.47185
O	-4.42479	2.21083	0.06221
O	-3.4282	0.15211	0.35088
C	-3.19938	1.55802	0.25671
C	-2.27308	1.8775	-0.90959
C	-0.96509	1.10293	-0.78792
C	-1.23936	-0.37959	-0.55829
C	-2.22261	-0.57471	0.58855
O	-0.03538	-1.02675	-0.2465

O	-0.22714	1.25693	-1.97
O	-1.99486	3.25162	-0.91241
C	-2.5505	-2.05635	0.71806
O	-1.84698	-2.77828	-0.25635
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C	-2.18868	-4.12736	-0.08658
C	-2.80223	-4.67302	-1.35773
C	-2.41607	-6.12826	-1.2889
C	-1.02926	-6.05448	-0.68798
C	-2.3859	-6.75903	-2.67481
O	-2.02339	-8.10904	-2.56678
O	-3.31118	-6.91817	-0.55375
O	-4.19531	-4.5151	-1.35964
H	-5.62459	8.89587	3.05498
H	-3.61285	7.54414	3.22636
H	-2.44332	5.40572	2.61438
H	-6.44913	3.89138	0.05784
H	-2.77656	1.58257	-1.85737
H	-0.39136	1.50062	0.07894
H	-1.68109	-0.80932	-1.4851
H	-1.75699	-0.19743	1.52643
H	-0.24033	-1.93622	-0.11149
H	0.57051	0.76791	-1.86054
H	-1.42243	3.41361	-1.64278
H	-3.6437	-2.20781	0.57402
H	-2.25558	-2.41436	1.7298
H	-2.905	-4.19149	0.76286
H	-2.45534	-4.15858	-2.28174

H	-0.81693	-6.97572	-0.10061
H	-0.25337	-5.97188	-1.48167
H	-1.64268	-6.22544	-3.30859
H	-3.39436	-6.68189	-3.13938
H	-2.01616	-8.46139	-3.44037
H	-3.3518	-6.5515	0.31301
H	-4.51032	-4.87271	-2.1722
C	-2.51258	1.88195	2.24716
H	-3.48694	1.52125	1.99138
H	-1.78633	1.12326	2.04254
H	-2.48585	2.12992	3.28769
H	-2.7548	1.90506	1.166

DFT-optimized geometries of isolated coumarins

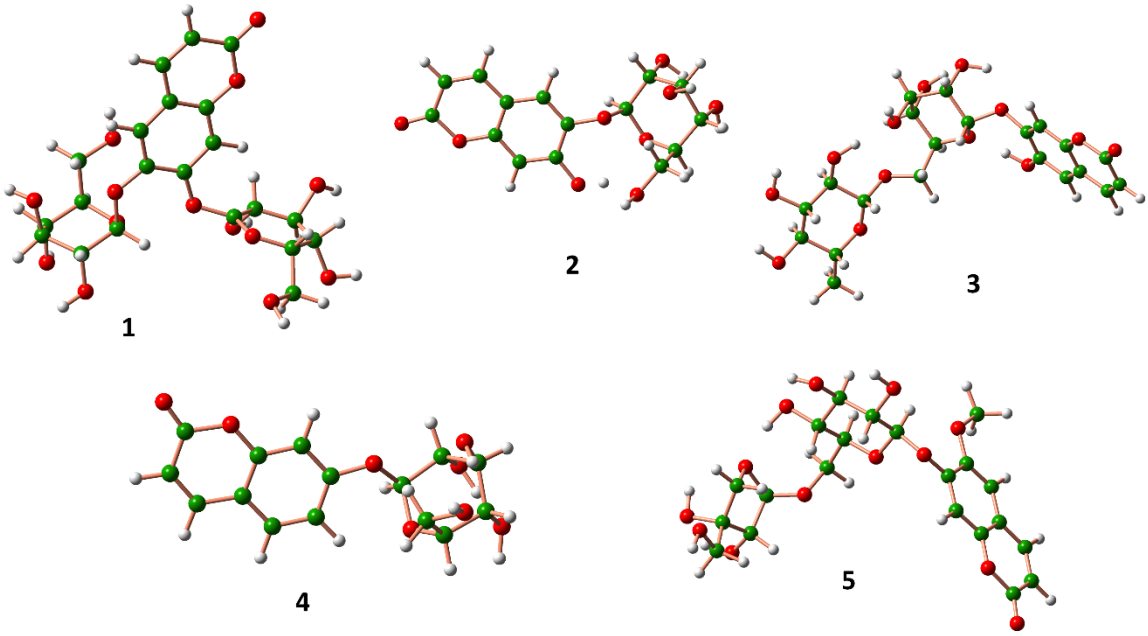

Figure S1. DFT optimized geometries of isolated coumarins.

Molecular docking data

The dimensions of the grid box are center_x = 13.955, center_y = -24.907, and center_z = 59.633; size_x = 30, size_y = 30, and size_z = 28, spcaing 0.375.

Spectral data of isolated coumarins

6,7-di-O-glucopyranosyl esculetin

^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 7.92 (d, J = 8.5 Hz, 1H, H-3 of esculetin), 7.53 (d, J = 8.5 Hz, 1H, H-4 of esculetin), 6.96 (s, 1H, H-6 of esculetin), 5.30 (d, J = 7.8 Hz, 1H, H-1' of Glc), 5.01 (d, J = 7.8 Hz, 1H, H-1" of Glc), 4.21 - 3.00 (m, H-2' to H-6' of Glc and H-2" to H-6" of Glc). ^{13}C NMR (DMSO-d_6, 125 MHz): δ (ppm) 162.0 (C-1 of esculetin), 150.0 (C-2 of esculetin), 114.0 (C-3 of esculetin), 145.0 (C-4 of esculetin), 117.0 (C-5 of esculetin), 156.5 (C-6 of esculetin), 104.0 (C-1' of Glc), 77.0 - 60.0 (C-2' to C-6' of Glc and C-1" to C-6" of Glc).

Aesculin

^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 7.89 (d, J = 8.6 Hz, 1H, H-3 of coumarin), 7.47 (d, J = 8.6 Hz, 1H, H-4 of coumarin), 6.91 (s, 1H, H-6 of coumarin), 5.31 (d, J = 7.5 Hz, 1H, H-1' of Glc), 5.04 (d, J = 7.5 Hz, 1H, H-1" of Glc), 4.27 - 3.25 (m, H-2' to H-6' of Glc). ^{13}C NMR (DMSO-d_6, 125 MHz): δ (ppm) 161.5 (C-1 of coumarin), 150.2 (C-2 of coumarin), 115.0 (C-3 of coumarin), 145.0 (C-4 of coumarin), 116.7 (C-5 of coumarin), 157.0 (C-6 of coumarin), 103.0 (C-1' of Glc), 77.0 - 62.0 (C-2' to C-6' of Glc).

Isobaisseoside

^{1}H NMR (DMSO-d6, 400 MHz): δ (ppm) 7.70 (s, 1H, H-4 of coumarin), 7.58 (d, J = 9.5 Hz, 1H, H-3 of coumarin), 7.20 (s, 1H, H-5 of coumarin), 5.12 (d, J = 7.9 Hz, 1H, H-1' of GlcA), 4.89 (d, J = 7.7 Hz, 1H, H-1" of Rha), 4.18-3.02 (m, H-2' to H-6' of GlcA, H-2" to H-5" of Rha), 1.16 (d, J = 6.0 Hz, 3H, H-6" of Rha, methyl group). 13C NMR (DMSO-d6, 100 MHz): δ (ppm) 160.2 (C-1 of coumarin), 153.8 (C-2 of coumarin), 112.0 (C-3 of coumarin), 144.0 (C-4 of coumarin), 118.1 (C-5 of coumarin), 152.5 (C-6 of coumarin), 143.9 (C-7 of coumarin), 112.9 (C-8 of coumarin), 148.5 (C-9 of coumarin), 112.2 (C-10 of coumarin), 103.5 (C-1' of GlcA), 103.2 (C-1" of Rha), 74.9-60.7 (C-2' to C-6' of GlcA, C-2" to C-5" of Rha), 17.9 (C-6" of Rha, methyl group).

Skimmin

^{1}H NMR (DMSO-d6, 500 MHz): δ (ppm) 7.86 (d, J = 8.4 Hz, 1H, H-3 of coumarin), 7.63 (d, J = 8.4 Hz, 1H, H-4 of coumarin), 6.92 (s, 1H, H-6 of coumarin), 5.23 (d, J = 7.8 Hz, 1H, H-1' of Glc), 4.99 (d, J = 7.8 Hz, 1H, H-1" of Glc), 4.15 - 3.05 (m, H-2' to H-6' of Glc). 13C NMR (DMSO-d6, 125 MHz): δ (ppm) 162.2 (C-1 of coumarin), 150.5 (C-2 of coumarin), 114.3 (C-3 of coumarin), 146.0 (C-4 of coumarin), 116.5 (C-5 of coumarin), 156.0 (C-6 of coumarin), 102.5 (C-1' of Glc), 76.0 - 60.5 (C-2' to C-6' of Glc).

Xeroboside

^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 7.82 (d, J = 8.5 Hz, 1H, H-2), 7.72 (d, J = 8.5 Hz, 1H, H-4), 6.98 (s, 1H, H-3), 6.63 (d, J = 8.0 Hz, 1H, H-5), 6.57 (d, J = 8.0 Hz, 1H, H-6), 4.55 (t, J = 5.0 Hz, 2H, -CH_2OH), 4.35 (m, 2H, OCH_2), 3.90 (s, 3H, 7-OCH_3), 3.20 - 4.00 (m, 6H, Oxane ring protons); ^{13}C NMR (DMSO-d_6, 125 MHz): δ (ppm) 185.0 (C=O), 163.0 (C-7), 133.0 (C-5), 126.0 (C-3), 119.0 (C-6), 103.0 (C-4), 95.0 (C-2), 85.0 - 70.0 (C-1' to C-6' of oxane ring), 61.5 (Hydroxymethyl carbon), 55.0 (7-OCH_3).

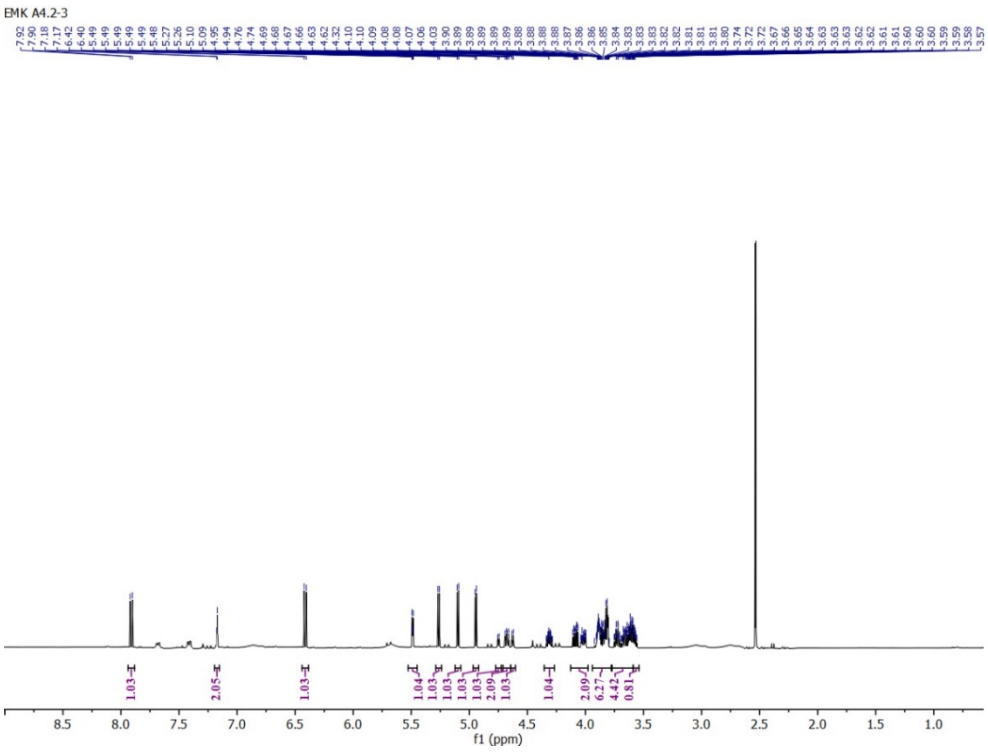

Figure S1. ^{1}H-NMR (DMSO-d_6) spectrum of compound **1**.

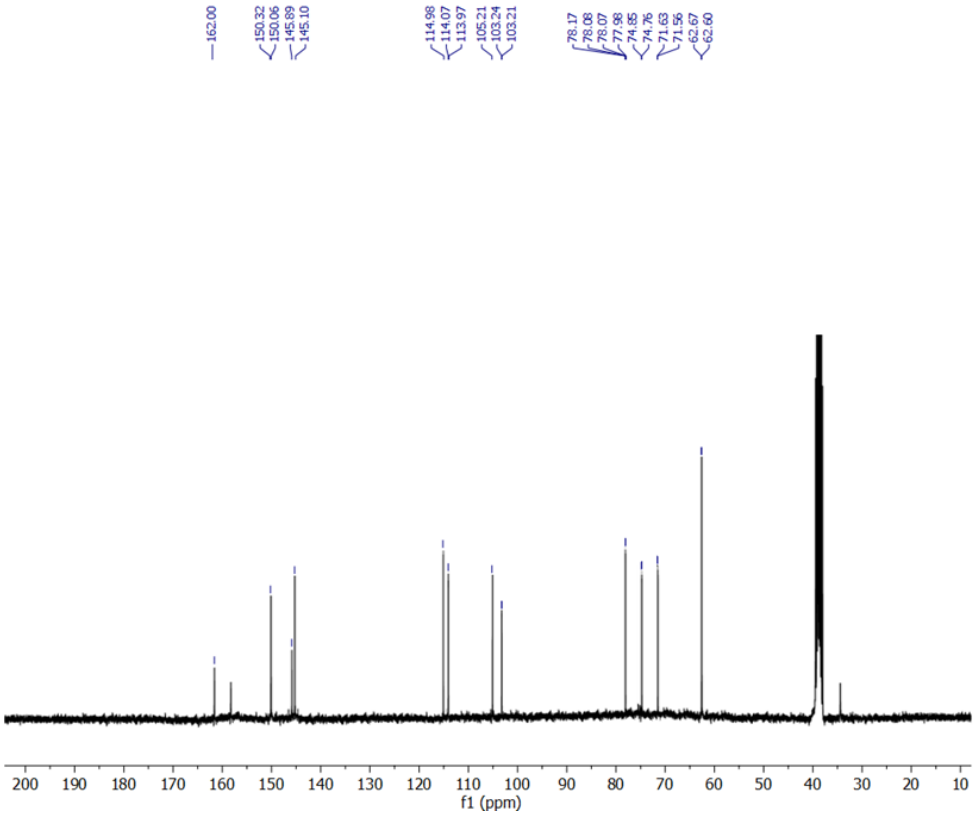

Figure S2. ^{13}C-NMR (DMSO -d_6) spectrum of compound **1**.

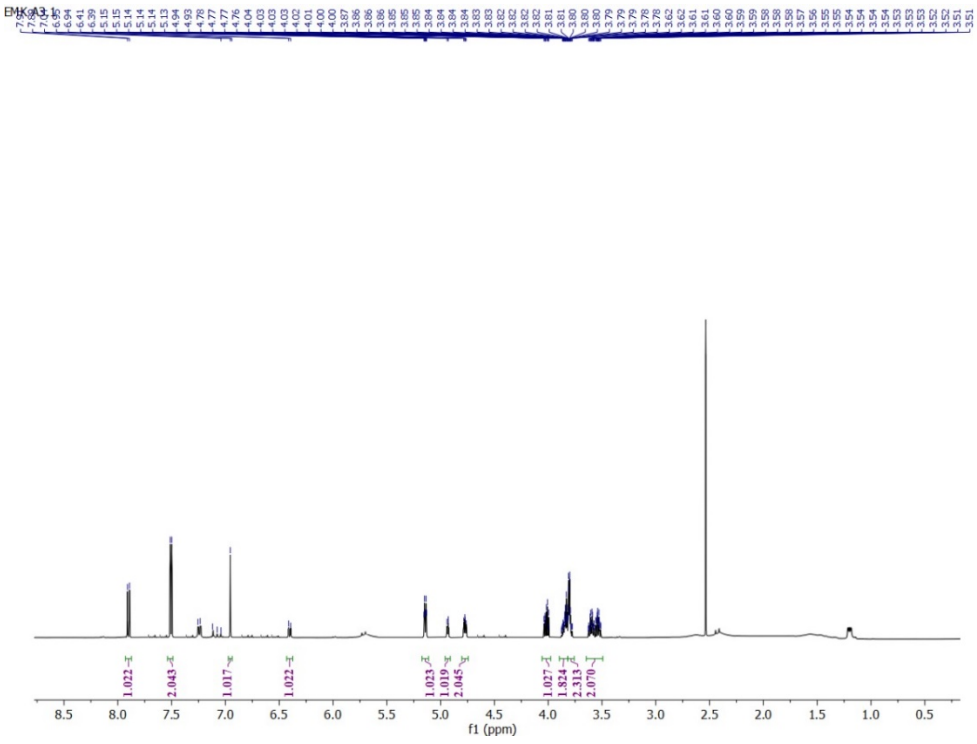

Figure S3. ^{1}H-NMR (DMSO-d_6) spectrum of compound **2**.

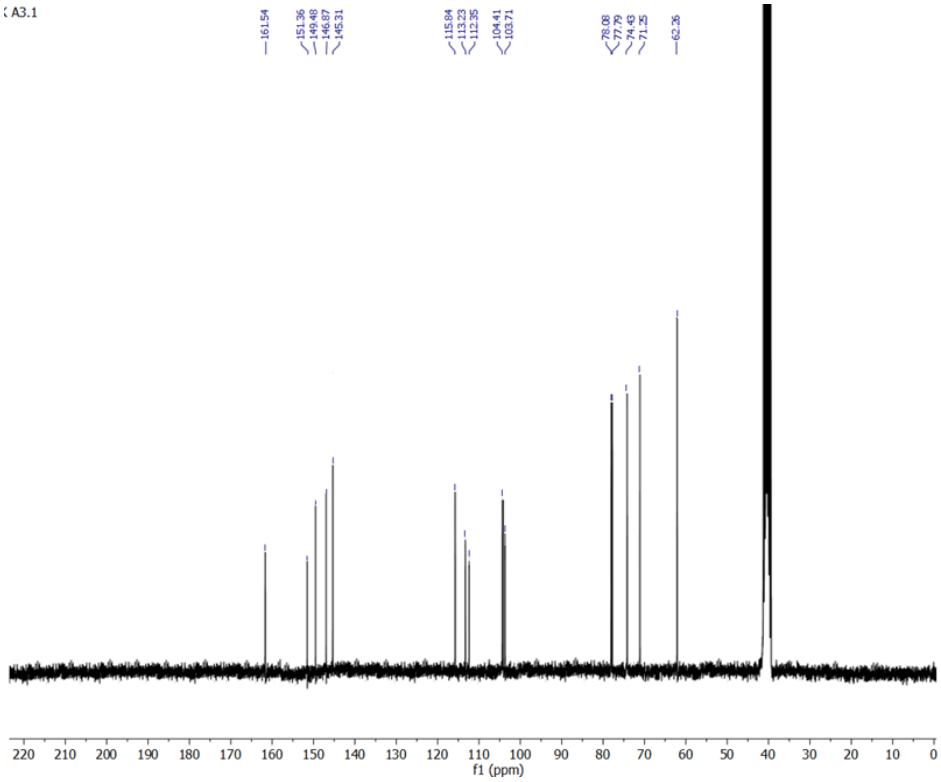

Figure S4. ^{13}C-NMR (DMSO -d_6) spectrum of compound **2**.

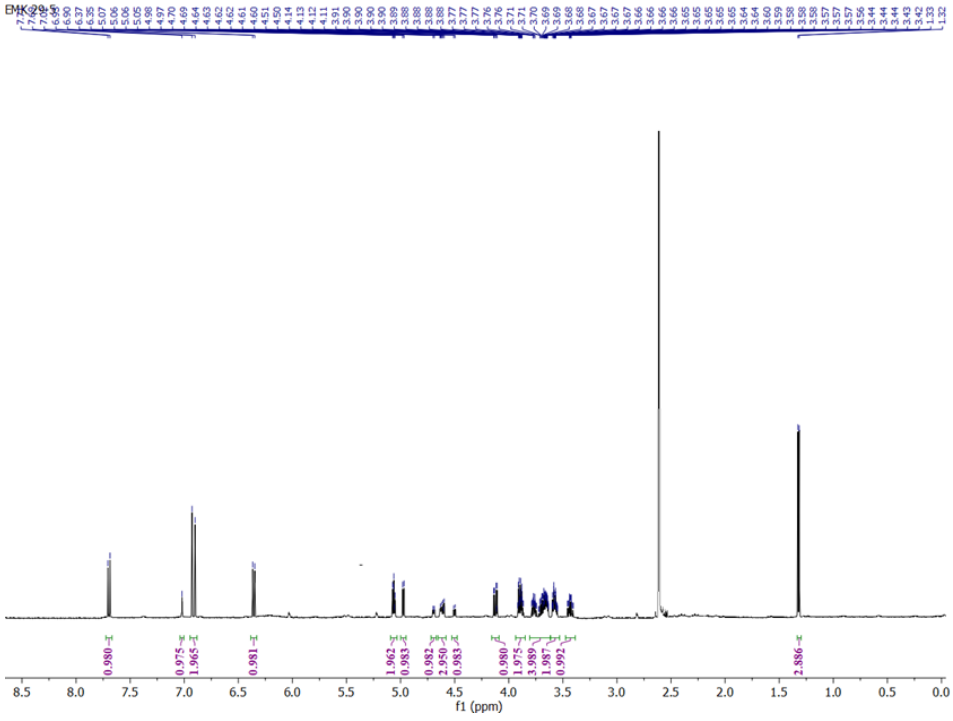

Figure S5. ^{1}H-NMR (DMSO-d_6) spectrum of compound **3**.

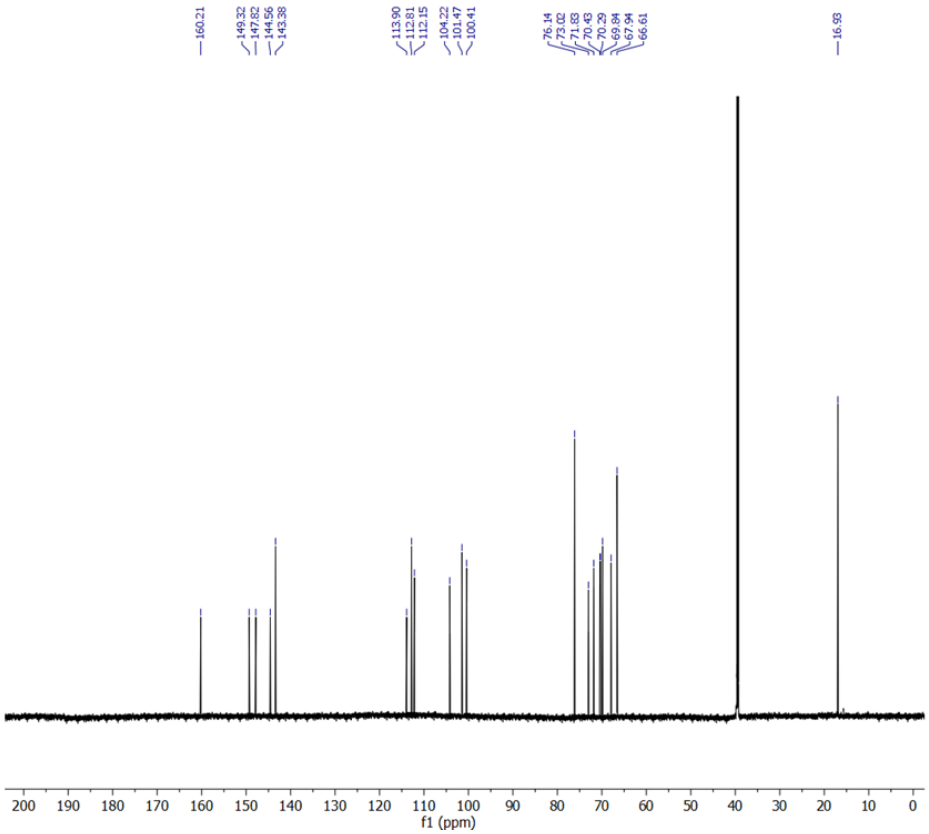

Figure S6. ^{13}C-NMR (DMSO -d_6) spectrum of compound **3**.

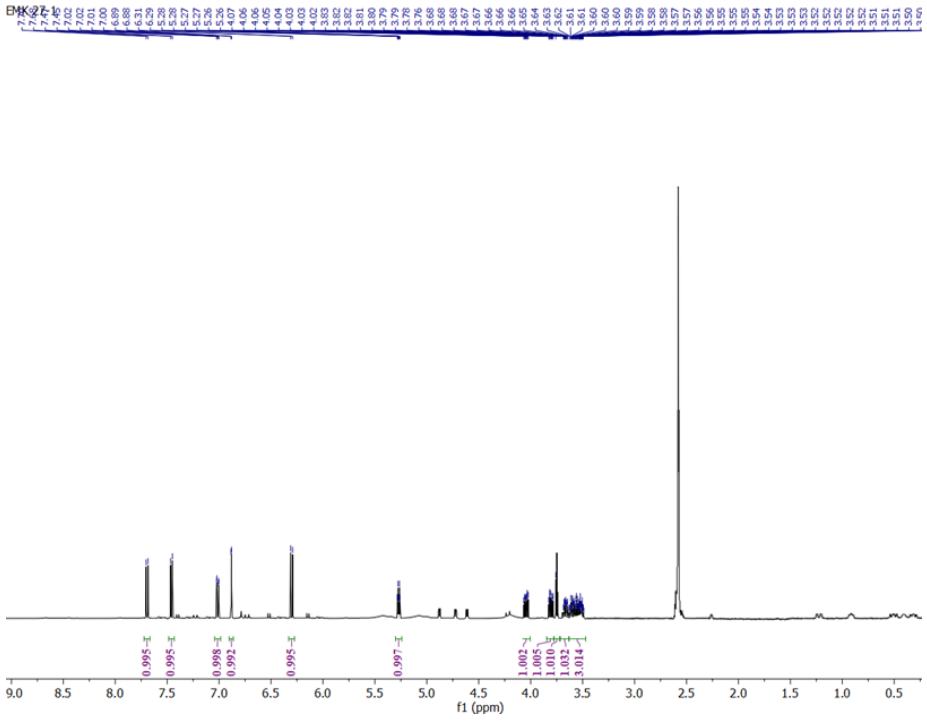

Figure S7. ^{1}H-NMR (DMSO-d_6) spectrum of compound **4**.

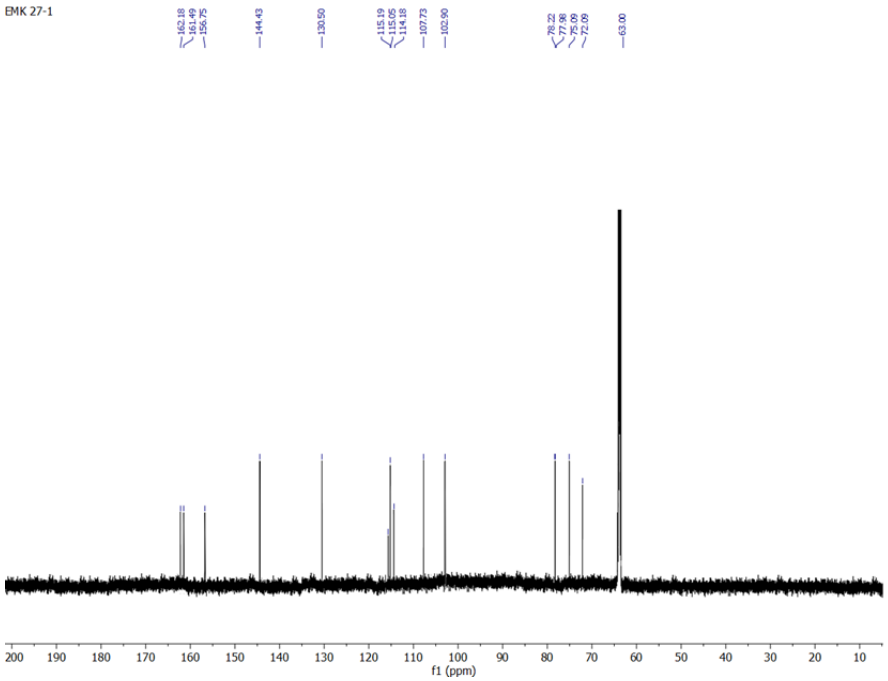

Figure S8. ^{13}C-NMR (DMSO -d_6) spectrum of compound **4**.

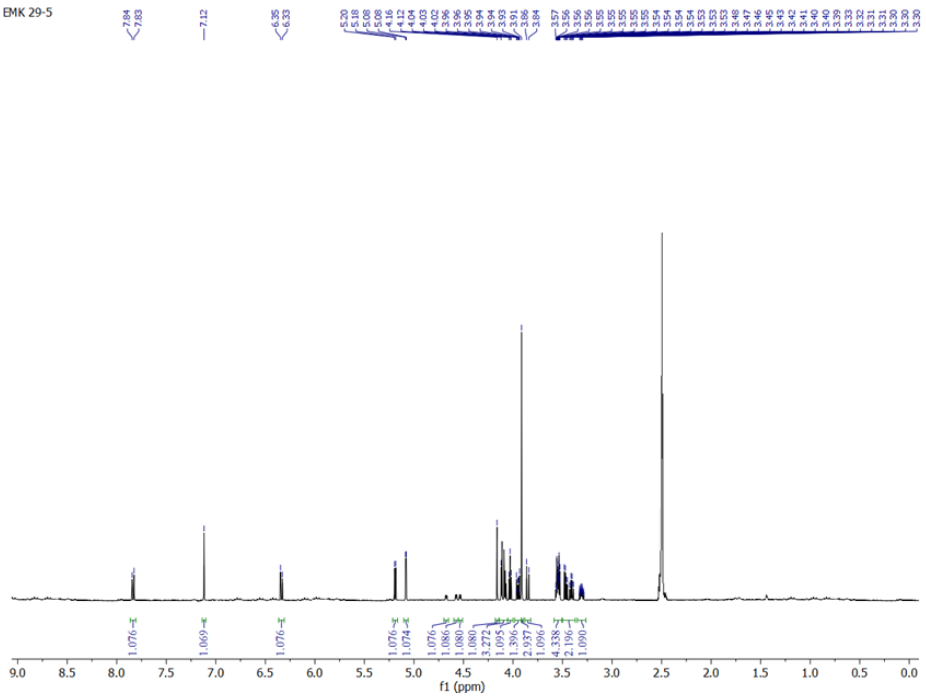

Figure S9. ^{1}H-NMR (DMSO-d_6) spectrum of compound **5**.

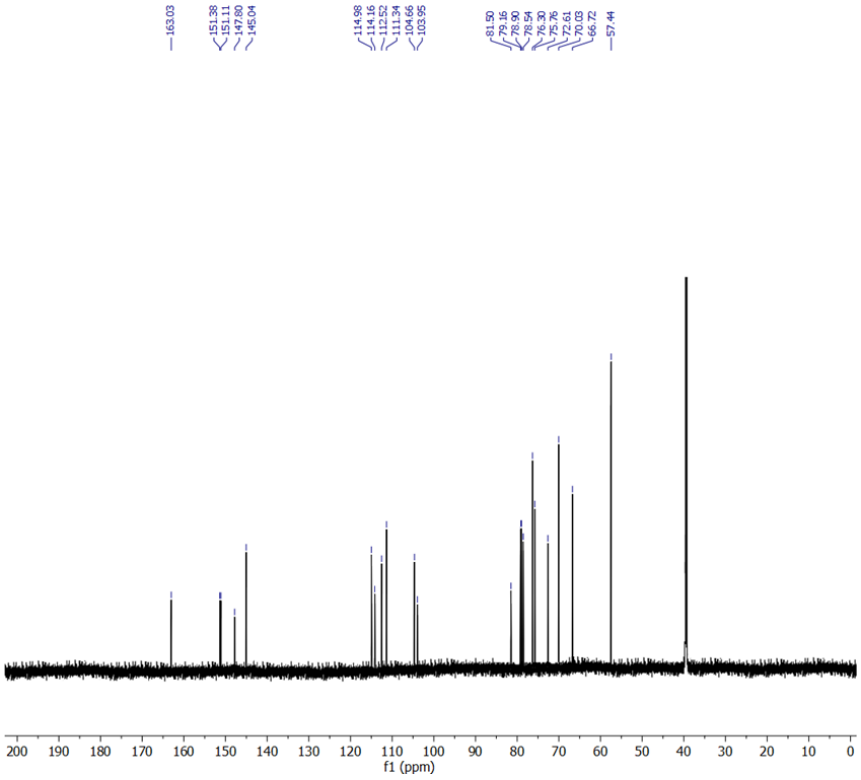

Figure S10. ^{13}C-NMR (DMSO -d_6) spectrum of compound **5**.