Unraveling Molecular Mechanisms of β-glucuronidase Inhibition by Flavonoids from *Centaurea scoparia*: Integrated *in silico* and *in vitro* insights

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Contents

Cartesian coordinates of DFT-calculated isolated phenolics at the B3LYP level of theory

2'-hydroxychrysin (1)

C	-5.81028	-1.92477	0.00026
C	-4.43748	-1.92477	0.00026
C	-3.71554	-0.69965	0.00026
C	-4.43341	0.52422	0.00063
C	-5.85483	0.49485	0.00079
C	-6.52633	-0.70313	0.0005
C	-2.29381	-0.6701	0.
H	-6.40207	1.4493	0.00087
C	-2.3378	1.74901	0.00048
C	-1.62179	0.52712	0.00002
O	-3.71066	1.74895	0.00075
O	-1.58246	-1.91062	0.00003
O	-3.70348	-3.15202	-0.00003
O	-7.95592	-0.73741	0.00063
H	-8.25413	-1.64991	0.00416
H	-2.76245	-2.96207	0.00001
C	-1.5528	3.07392	0.00023
C	-2.23677	4.28993	0.00067
C	-0.15806	3.05829	-0.00033
C	-1.52607	5.48998	-0.00013
C	0.553	4.25863	-0.00013
H	0.38117	2.09998	-0.0005
C	-0.13073	5.47437	-0.00017
H	-2.06511	6.44849	-0.00041
H	1.65269	4.24599	-0.00016
H	0.42941	6.4207	-0.00074
O	-3.66668	4.30551	0.00023
H	-3.9768	5.21368	-0.02537
H	-6.35572	-2.84531	0.00019
O	-0.19185	0.54039	-0.00043
H	0.13488	-0.11361	0.62176

Jaceosidin (2)

C	4.15356	-0.25876	-0.12101
C	3.33787	0.86513	-0.22919
C	1.93066	0.7225	-0.19368
C	1.4314	-0.58826	-0.07671
C	2.23888	-1.72092	-0.00983
C	3.61452	-1.54772	-0.03754
C	0.98303	1.85821	-0.27881
H	1.80322	-2.70781	0.05721
C	-0.82233	0.1709	-0.12775
C	-0.42014	1.45478	-0.25749
O	0.08663	-0.84281	-0.03246
O	1.31568	3.0327	-0.36313
O	4.43889	-2.6233	0.01841
H	5.33073	-2.30852	-0.18545
C	-2.20758	-0.32043	-0.08495
C	-2.49058	-1.68028	-0.24588

C	-3.27622	0.57718	0.11321
C	-3.80325	-2.14377	-0.22447
C	-4.57945	0.11531	0.13299
C	-4.85448	-1.25851	-0.0378
O	5.5369	-0.10423	-0.14432
H	-1.68021	-2.37934	-0.39579
C	6.11808	0.08736	1.16544
H	5.9185	-0.77513	1.8074
H	7.19148	0.19333	1.01113
H	5.72033	0.98954	1.63782
O	3.9127	2.0785	-0.36631
H	4.85323	1.91947	-0.53214
H	-1.14167	2.25231	-0.35968
O	-6.13398	-1.70411	-0.01311
H	-6.70401	-0.93571	0.12866
H	-3.07538	1.62763	0.26656
O	-5.70407	0.87717	0.32037
C	-5.55409	2.28127	0.5001
H	-6.56083	2.67765	0.61818
H	-4.96736	2.50397	1.39724
H	-5.08059	2.74236	-0.37275
H	-4.02886	-3.19513	-0.35375

5,7,4’,5’-tetrahydroxy-6,3’-dimethoxyflavone (3)

C	-4.32489	0.32103	-0.11074
C	-3.56686	-0.83892	-0.25108
C	-2.15424	-0.76861	-0.21031
C	-1.59007	0.51152	-0.0546
C	-2.33938	1.68117	0.04423
C	-3.72191	1.57845	0.01009
C	-1.26528	-1.94751	-0.32837
H	-1.85387	2.64207	0.14027
C	0.6214	-0.35864	-0.12338
C	0.15676	-1.61597	-0.2938
O	-0.23437	0.69625	-0.0002
O	-1.65592	-3.10086	-0.44801
O	-4.49144	2.6919	0.09606
H	-5.39731	2.42856	-0.11861
C	2.03223	0.05811	-0.06263
C	2.37079	1.40678	-0.20895
C	3.04259	-0.90089	0.13799
C	3.70439	1.80331	-0.16995
C	4.36973	-0.49833	0.17314
C	4.70507	0.8524	0.01808
O	-5.71418	0.23697	-0.13951
H	1.61031	2.15869	-0.36052
C	-6.3069	0.0384	1.164
H	-6.06703	0.87237	1.82942
H	-7.38386	-0.01095	1.00596
H	-5.95448	-0.8946	1.61187
O	-4.20193	-2.01727	-0.42397
H	-5.13276	-1.80591	-0.58607
H	0.83875	-2.4442	-0.42023
O	6.0018	1.28371	0.05134
H	6.56441	0.51018	0.18947
H	2.78512	-1.93903	0.28371

O	5.4593	-1.30859	0.36307
C	5.24418	-2.70723	0.525
H	6.23115	-3.14986	0.64581
H	4.64096	-2.91306	1.41494
H	4.75722	-3.13574	-0.35671
O	4.02539	3.11718	-0.31926
H	4.98657	3.19661	-0.26778

5,4'-dihydroxy-6,7-dimethoxyflavone (4)

C	3.44532	-0.05771	0.15725
C	2.64244	1.0835	0.17634
C	1.22795	0.96067	0.11827
C	0.67101	-0.31948	0.05483
C	1.44727	-1.47022	0.03007
C	2.83871	-1.33162	0.0702
C	0.36515	2.13185	0.14985
H	0.96187	-2.43223	-0.03218
C	-1.52754	0.57851	0.03267
C	-1.05229	1.84824	0.11211
O	-0.68998	-0.49304	0.0049
O	0.81908	3.29092	0.21362
C	-2.94188	0.18164	-0.01589
C	-3.32736	-1.14103	0.26063
C	-3.9446	1.10786	-0.33634
C	-4.65887	-1.5216	0.2332
C	-5.28001	0.73478	-0.36726
H	-3.68358	2.12903	-0.58371
C	-5.64609	-0.58373	-0.07963
H	-6.04022	1.46606	-0.62475
O	4.80394	0.0236	0.27432
H	-2.57013	-1.87299	0.5064
H	-1.72806	2.68898	0.16783
O	3.21804	2.29126	0.25082
O	-6.93708	-1.01046	-0.09374
H	-7.51835	-0.27554	-0.31665
H	2.47616	2.95194	0.25567
H	-4.95611	-2.53938	0.4534
O	3.69872	-2.37421	0.03461
C	3.171	-3.69429	-0.01313
H	2.58121	-3.85796	-0.92147
H	4.03583	-4.35485	-0.02049
H	2.55659	-3.91014	0.86718
C	5.49155	0.53027	-0.87623
H	5.18989	1.55743	-1.09361
H	6.55196	0.50621	-0.62664
H	5.3107	-0.10693	-1.74881

The optimized geometries of isolated phytochemicals at the B3LYP level of theory

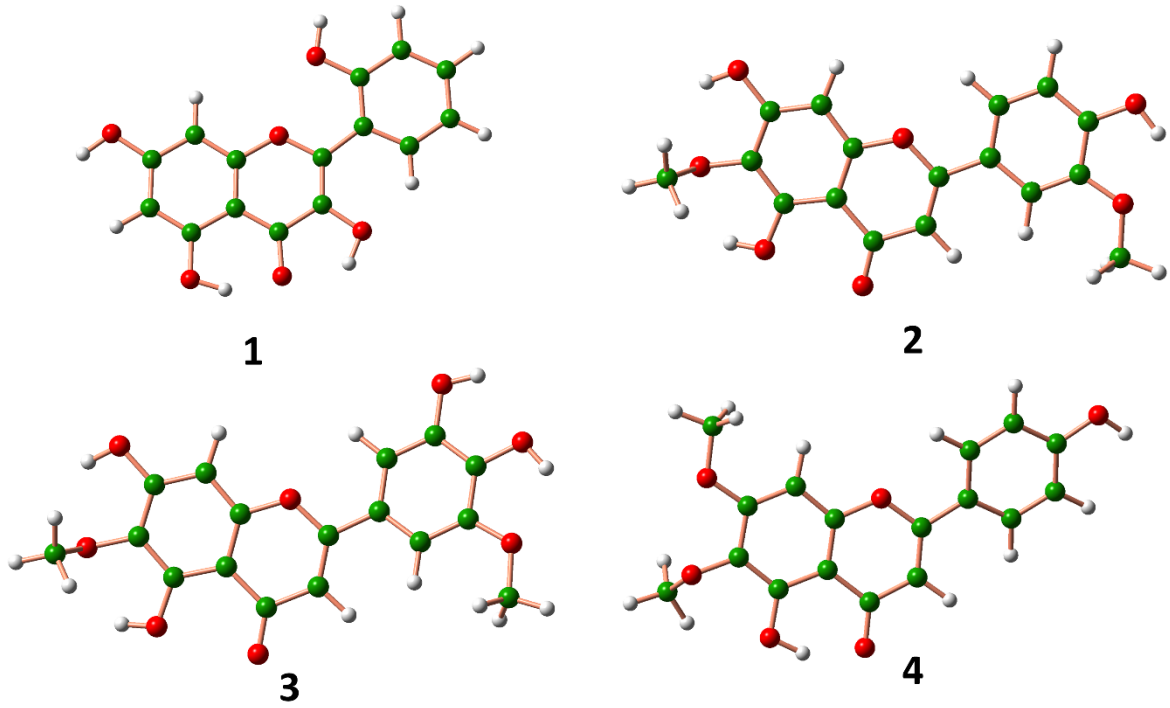

Figure S1. DFT optimized geometries of isolated phenolics at the B3LYP level of theory.

Molecular docking data

The dimensions of the grid box are center_x = -26.061, center_y = -23.252, and center_z = 1.935; size_x = 34, size_y = 38, and size_z = 30

Molecular docking results of EGCG with β-glucuronidase

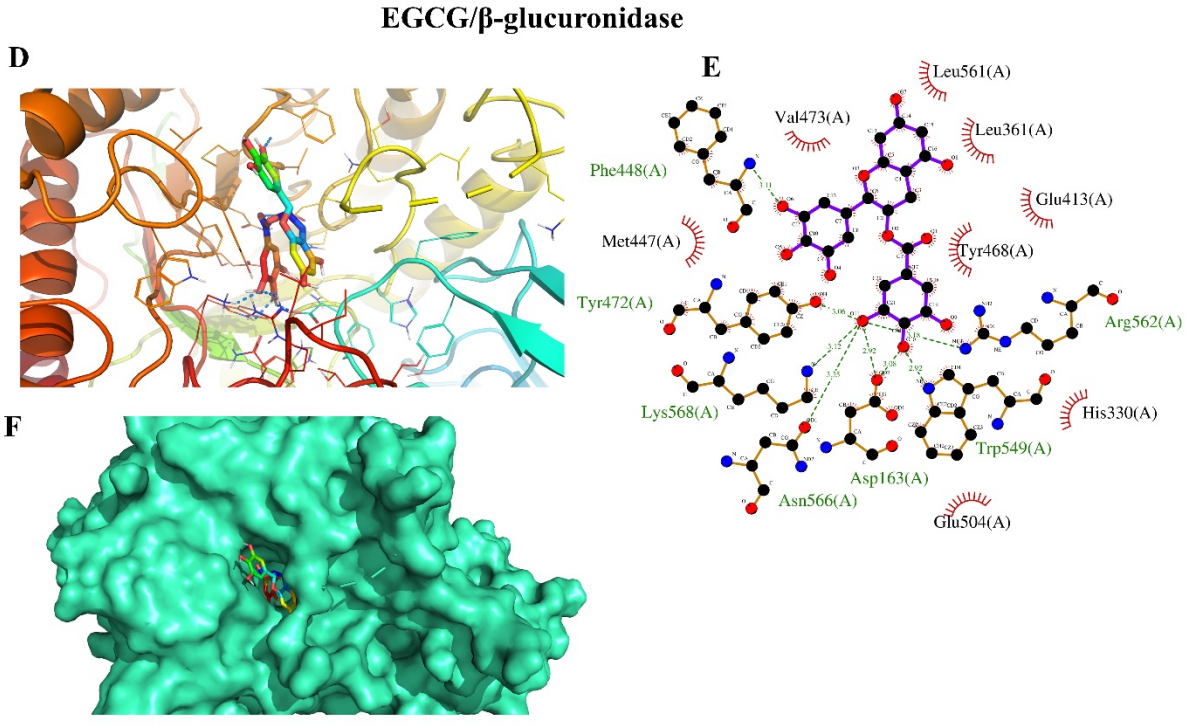

Figure S2. Results of Molecular docking analysis of the reference drug EGCG

Molecular docking results of the native ligand with β-glucuronidase

D-Glucaro-delta-lactam/β-glucuronidase

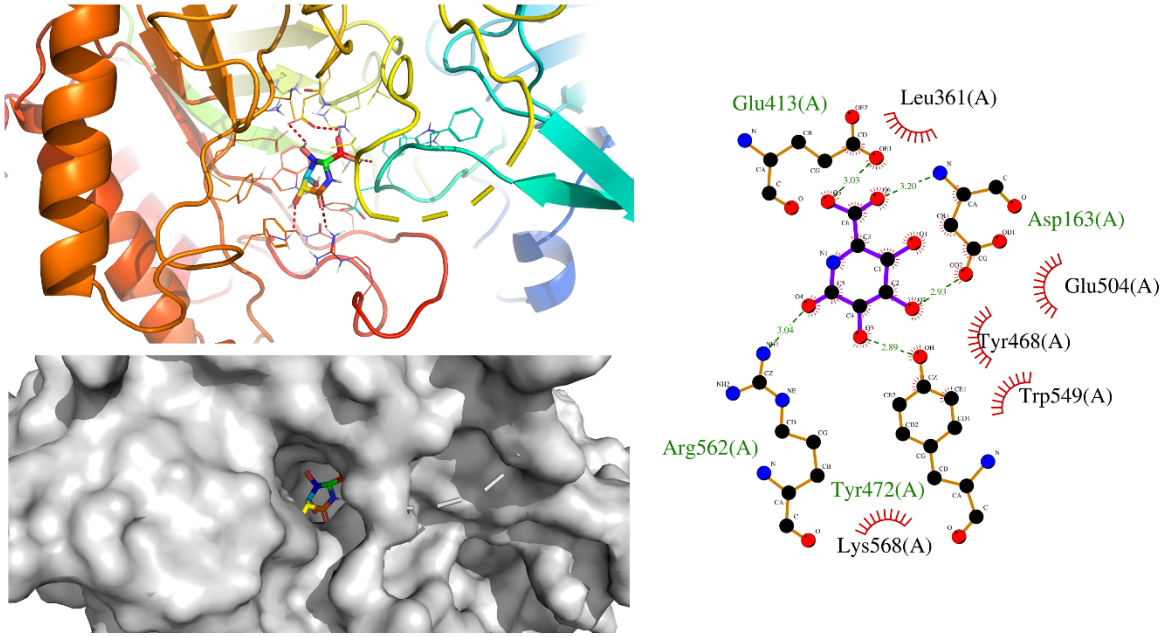

Figure S3. Results of Molecular docking analysis of the PDB native drug with β-glucuronidase (binding affinity of -6.7 kcal/mol)

The purity of the tested compounds was confirmed using PC, TLC, and NMR spectral data.