ChemistryOPEN

Supporting Information

Structural Determination of Antioxidant and Anticancer Flavonoid Rutin in Solution through DFT Calculations of ^{1}H NMR Chemical Shifts

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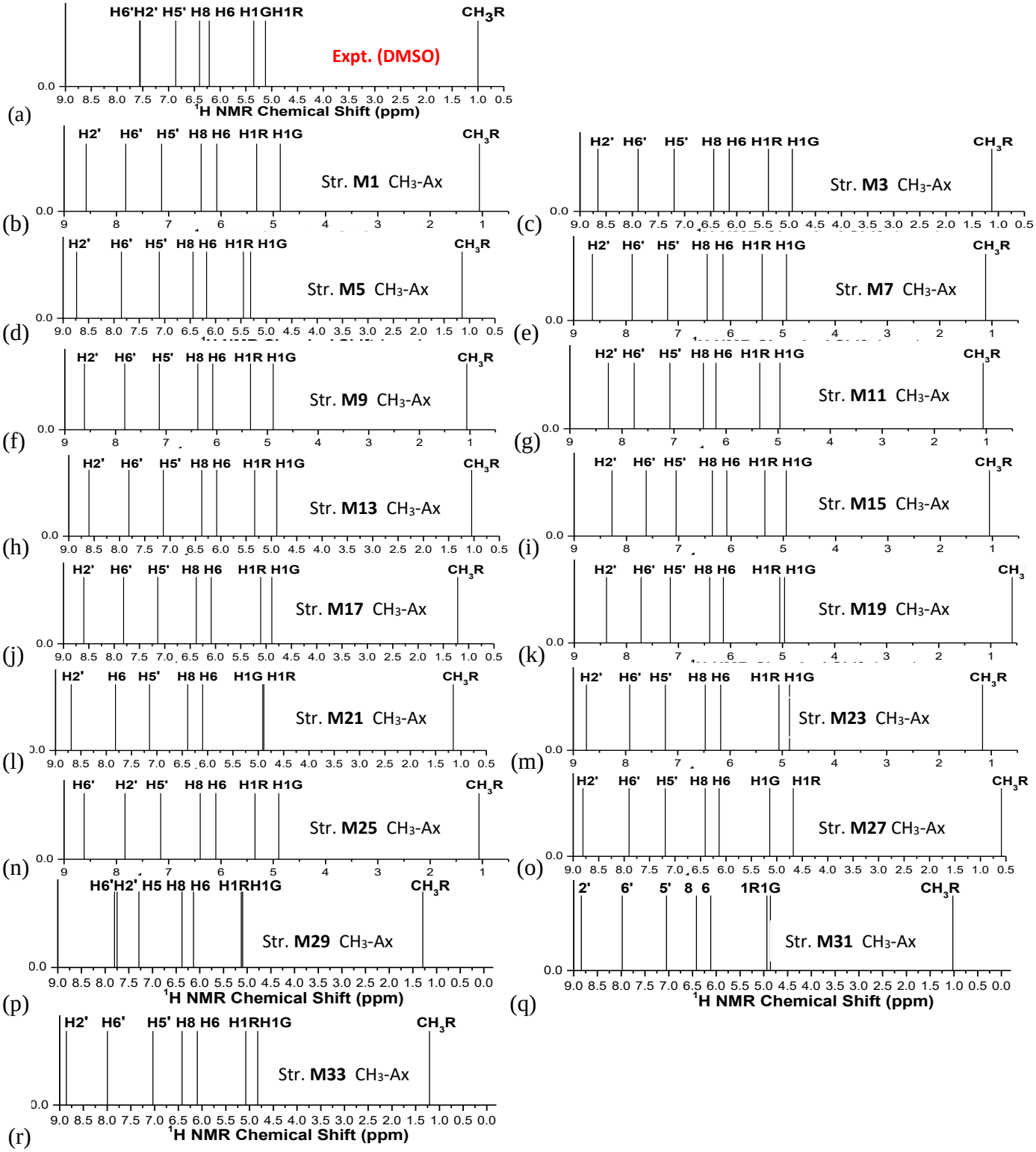


Figure S1. Experimental ^{1}H NMR spectrum (in DMSO-*d6*) from ref. [46] (a) and B3LYP/6-31G(d,p)-PCM(DMSO) (b-r) ^{1}H NMR spectra for 17 Rutin optimized structures with the CH_3-C5''' group in axial position.

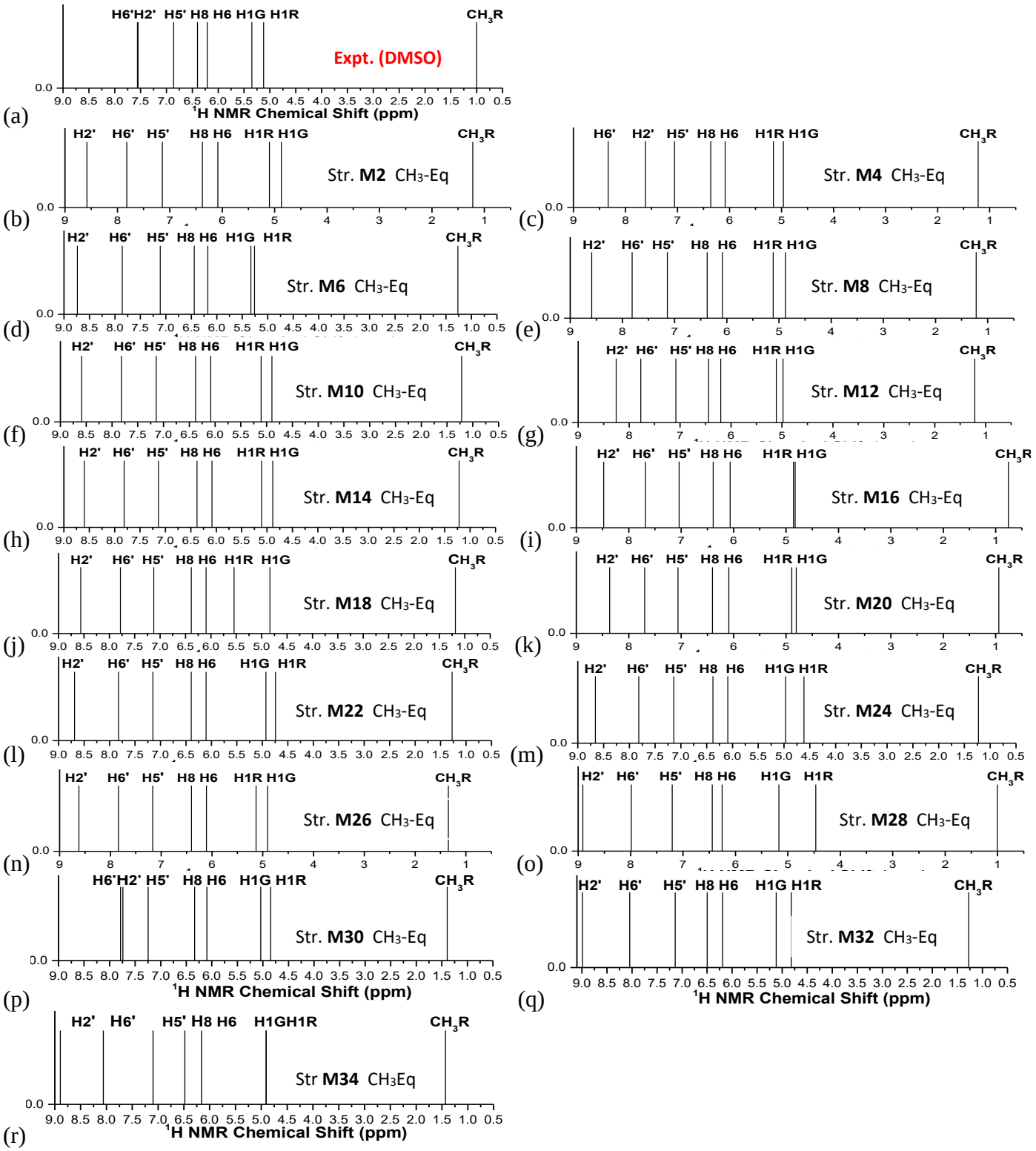


Figure S2. Experimental ^{1}H NMR spectrum (in DMSO-*d6*) from ref. [46] (a) and B3LYP/6-31G(d,p)-PCM(DMSO) (b-r) ^{1}H NMR spectra for 17 rutin optimized structures with the CH_3-C5''' group in equatorial position.

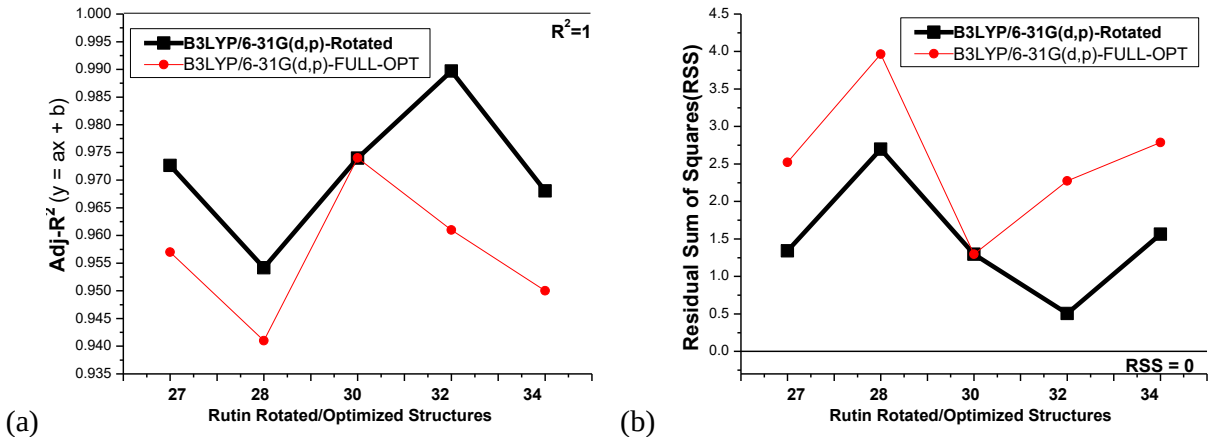


Figure S3. B3LYP/6-31G(d,p)-PCM(DMSO) ^{1}H NMR chemical shifts (in ppm) regression analysis for rutin molecule. Rotated and Fully-optimized almost planar (FULL-OPT) structures.

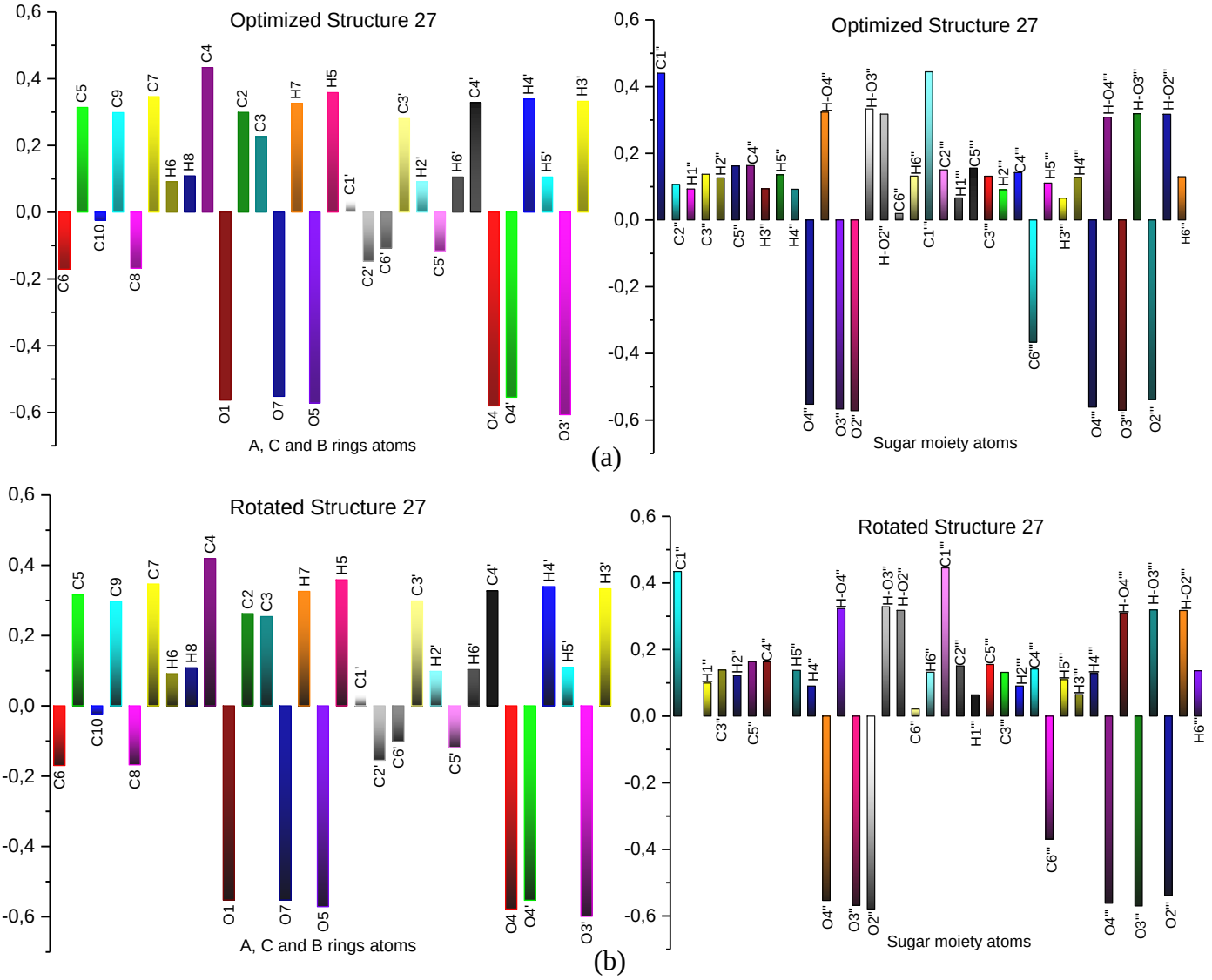
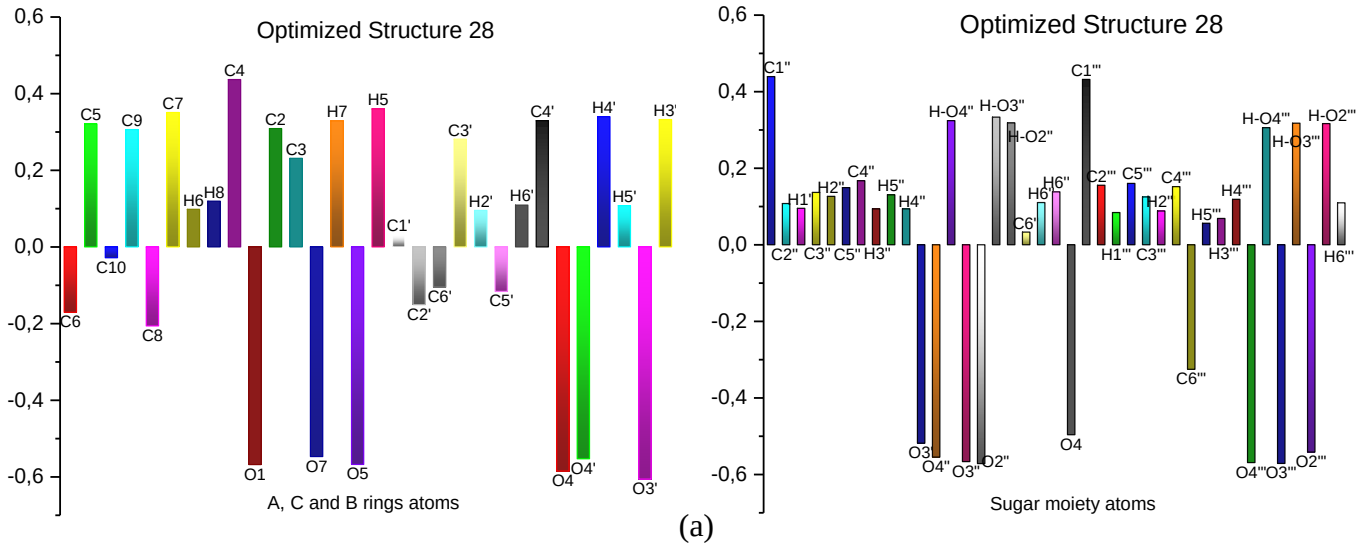
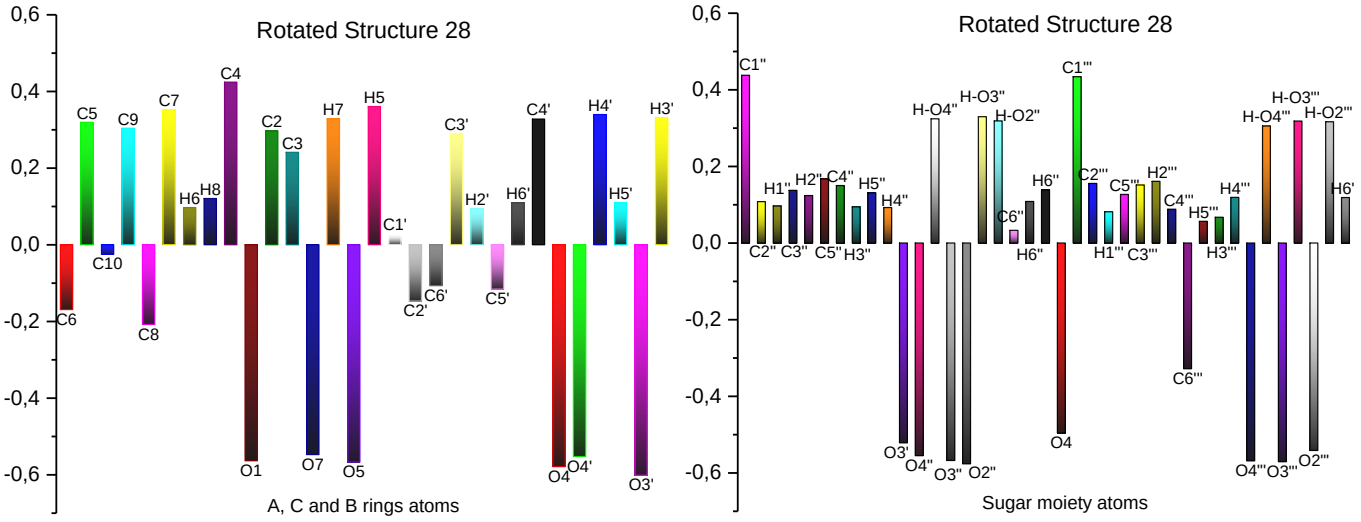


Figure S4.1 Comparison of B3LYP/6-31G(d,p) Mulliken charges for all atoms of the optimized and rotated structure **27** of the rutin molecule showed in Figure 6.



(a)



(b)

Figure S4.2 Comparison of B3LYP/6-31G(d,p) Mulliken charges for all atoms of the optimized and rotated structure **28** of the rutin molecule showed in Figure 6.

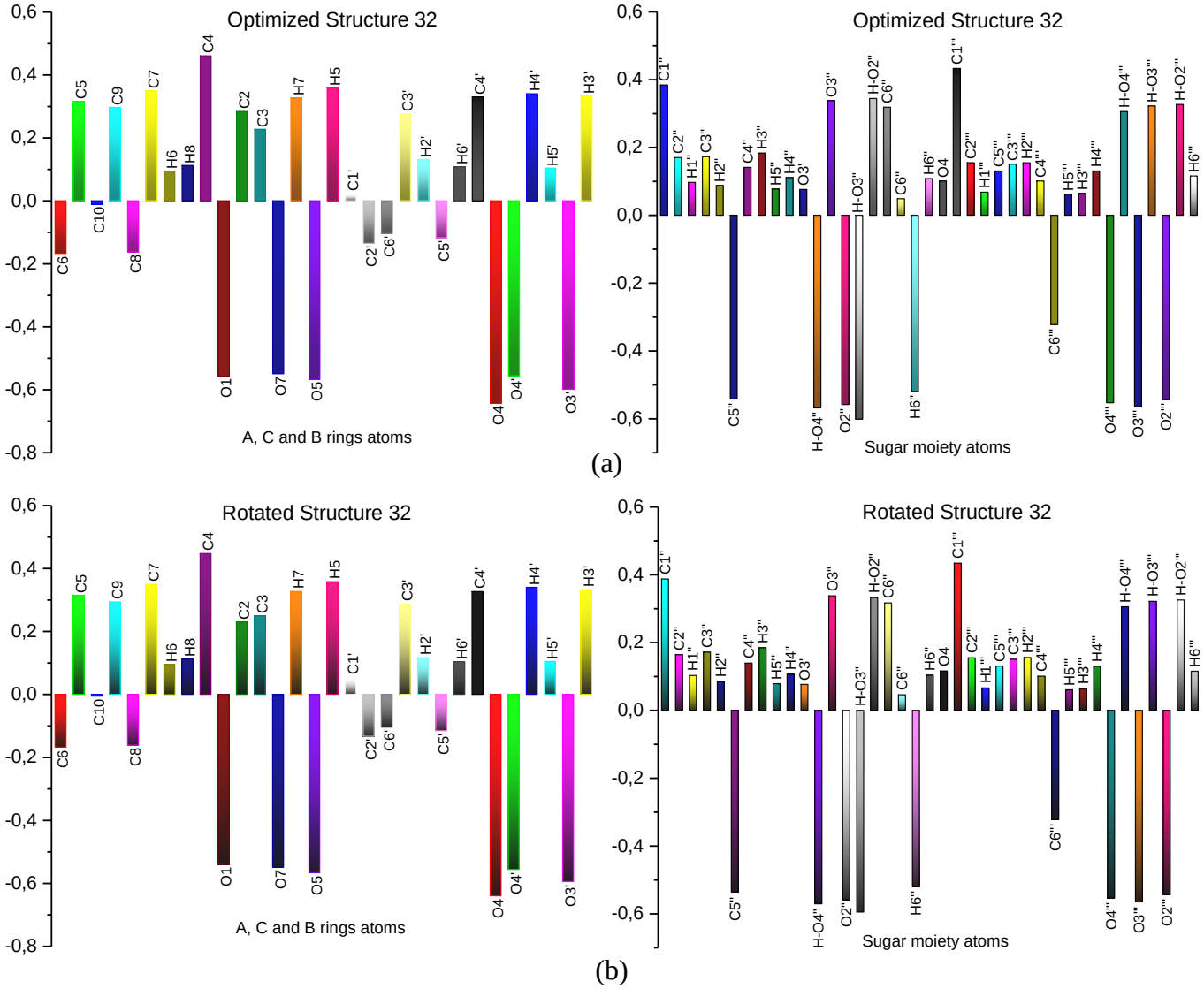


Figure S4.3 Comparison of B3LYP/6-31G(d,p) Mulliken charges for all atoms of the optimized and rotated structure **32** of the rutin molecule showed in Figure 6.

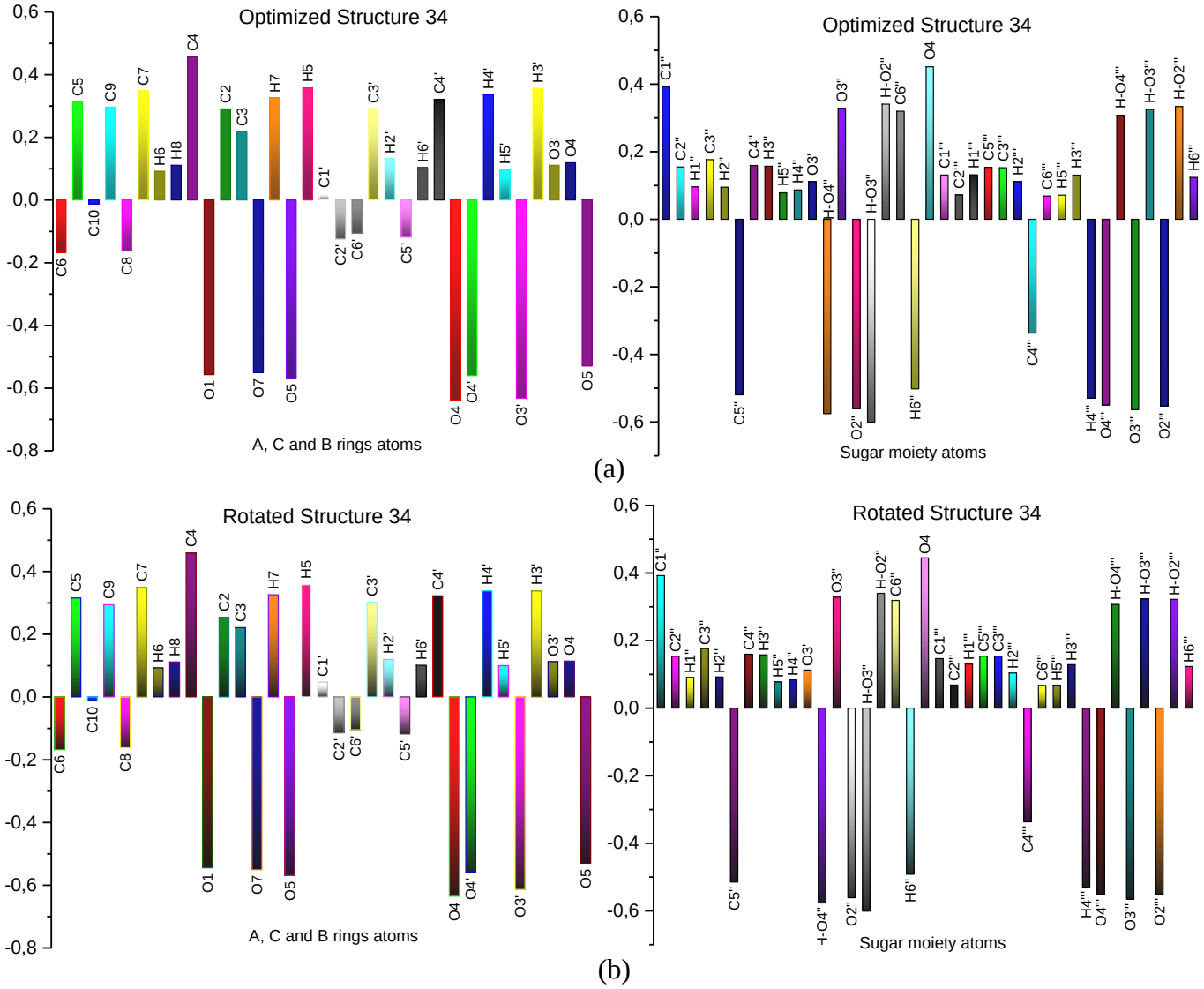


Figure S4.4 Comparison of B3LYP/6-31G(d,p) Mulliken charges for all atoms of the optimized and rotated structure **34** of the rutin molecule showed in Figure 6.

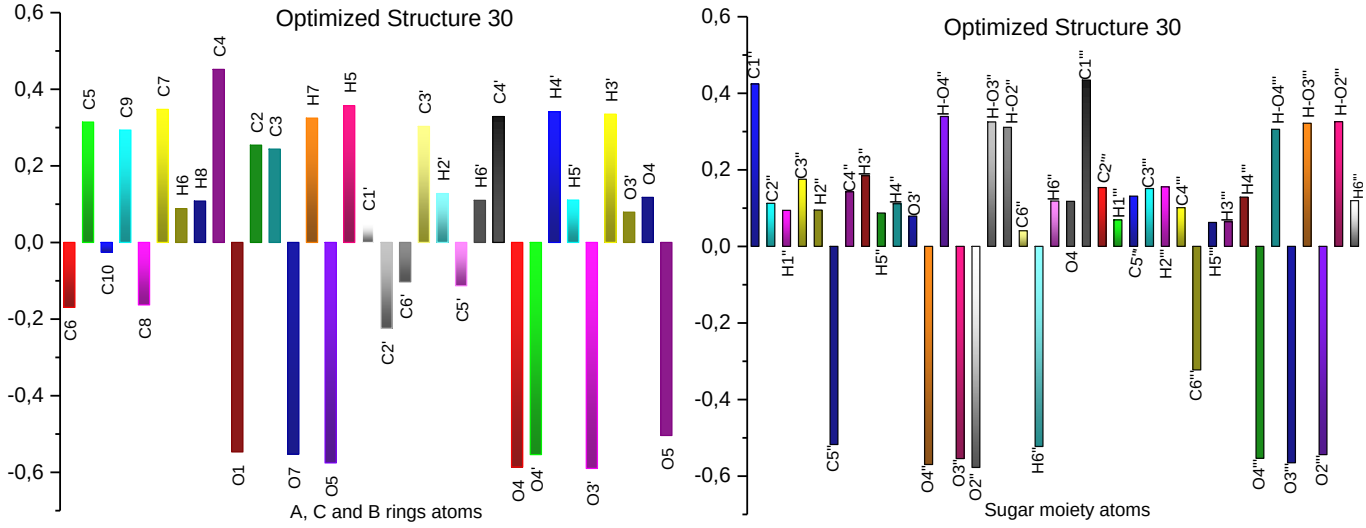


Figure S4.5 Comparison of B3LYP/6-31G(d,p) Mulliken charges for all atoms of the optimized and rotated structure **30** of the rutin molecule showed in Figure 6.

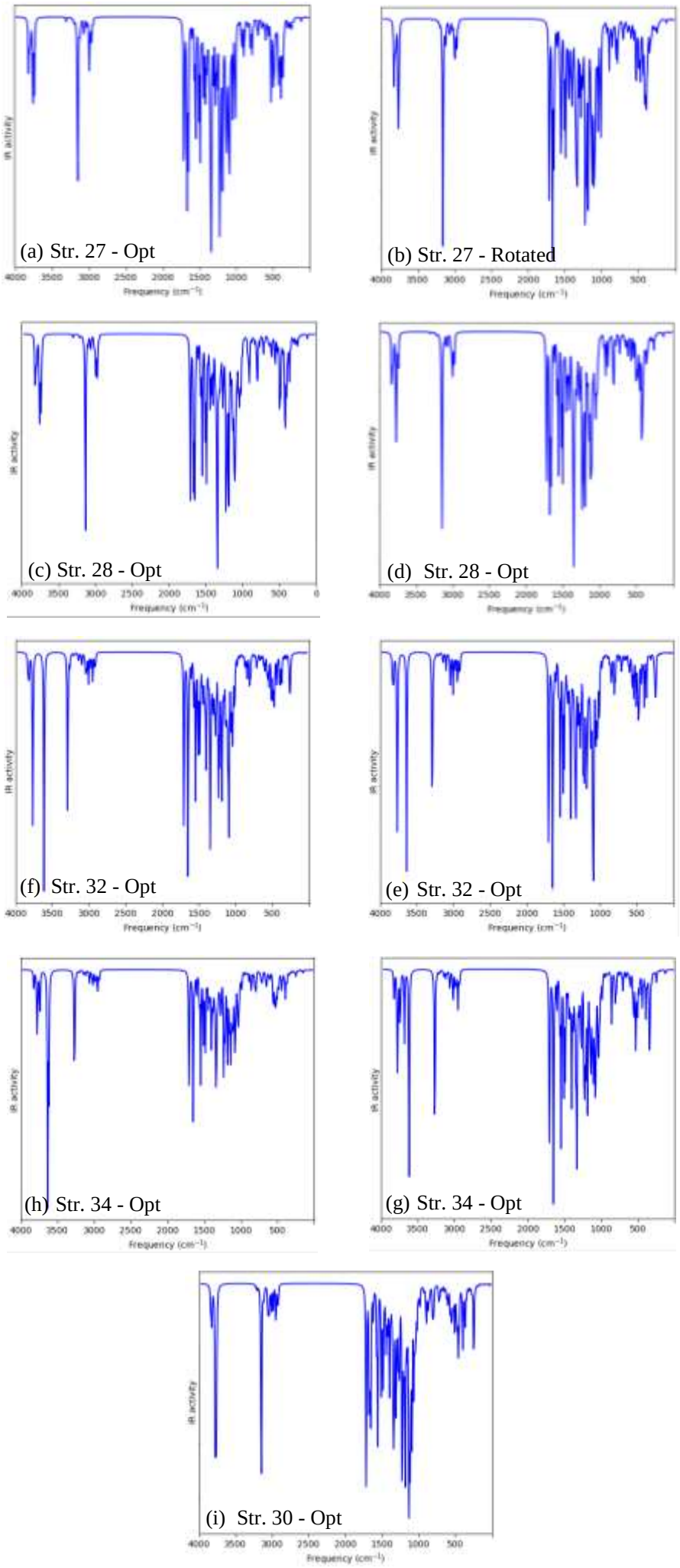


Figure S5. Simulation of the IR spectra calculated for rutin structures shown in Figure 6. The main absorption bands are described in Table S2.

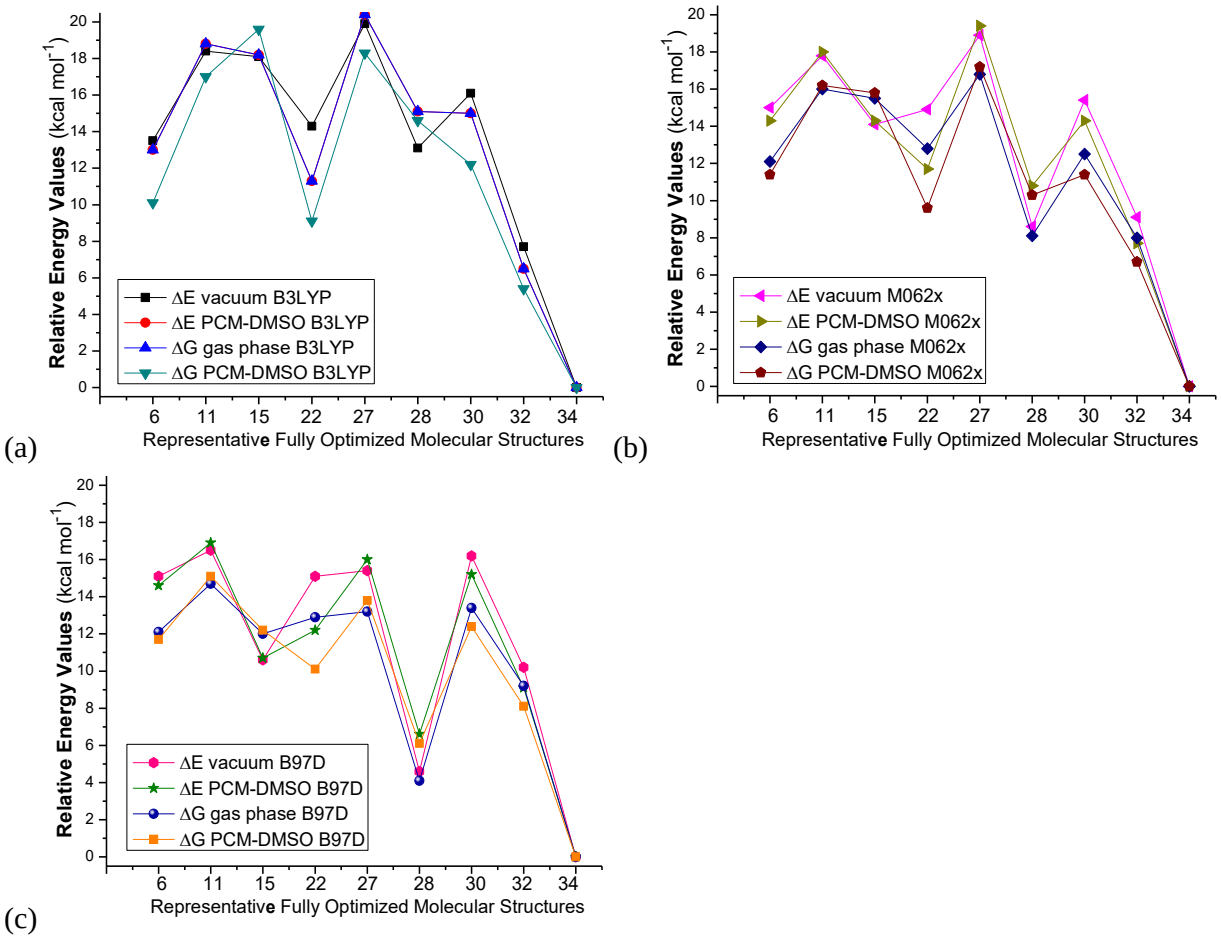


Figure S6. DFT/6-31G(d,p) relative energies ($\mathbf{\Delta E_{rel}}$) and Gibbs free energy ($\mathbf{\Delta G_{rel}}$) values (in kcal mol^{-1}) for fully optimized rutin structures: **6**, **11**, **15**, **22**, **27**, **28**, **30**, **32** and **34**. Gas phase and PCM-DMSO data are shown.

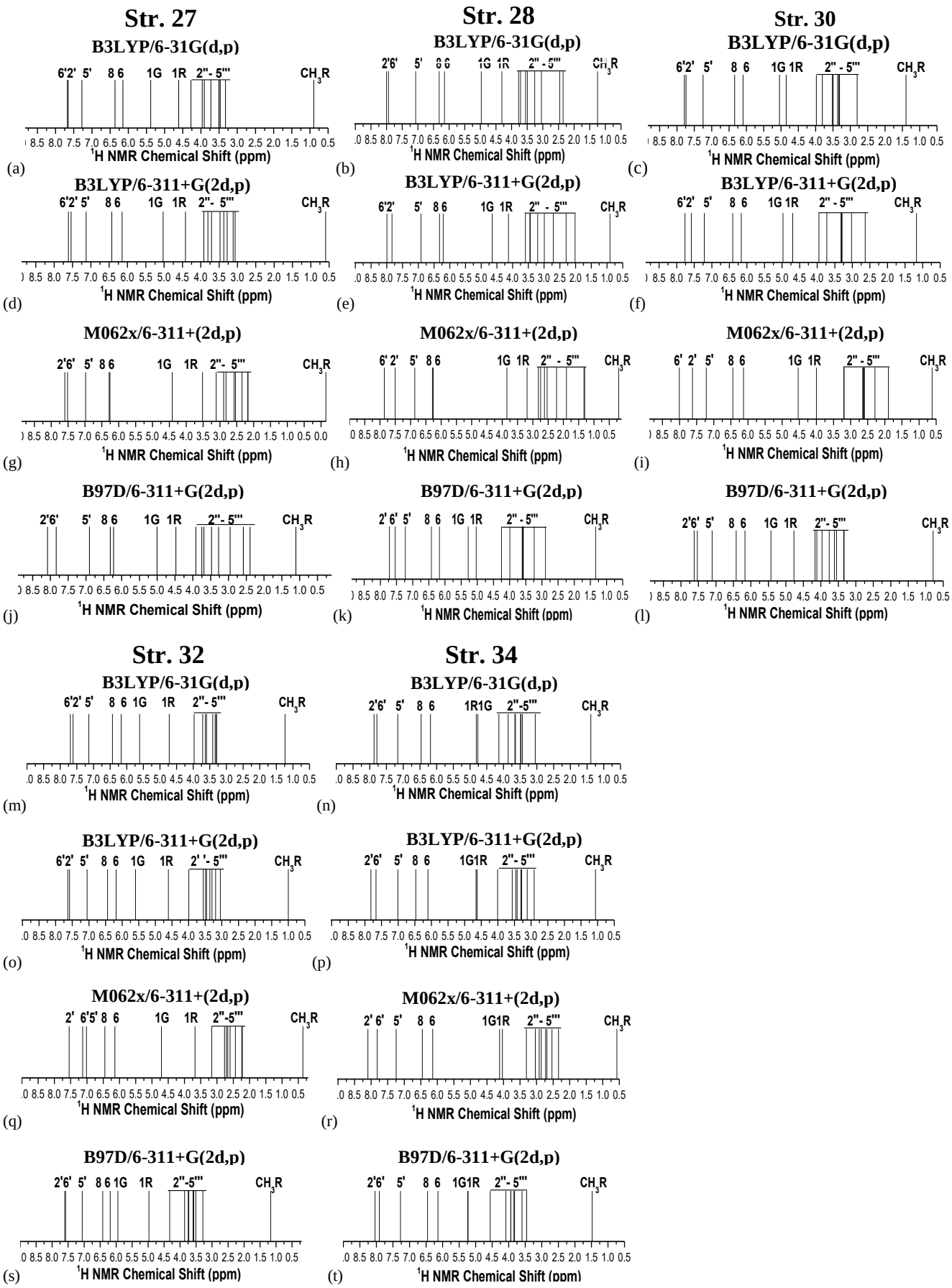


Figure S7. Theoretical PCM-DMSO ^{1}H NMR spectra for five representative rutin ϕ_1 ,ϕ_2 rotated structures (see Figure 6) calculated with B3LYP, M062x and B97D functionals using 6-31G(d,p) and 6-311+G(2d,p) basis sets and PCM model to describe DMSO solvent effects. Structure **30** (ϕ_1 = -146º) did not need to be rotated to describe correctly the H2' and H6' NMR signals.

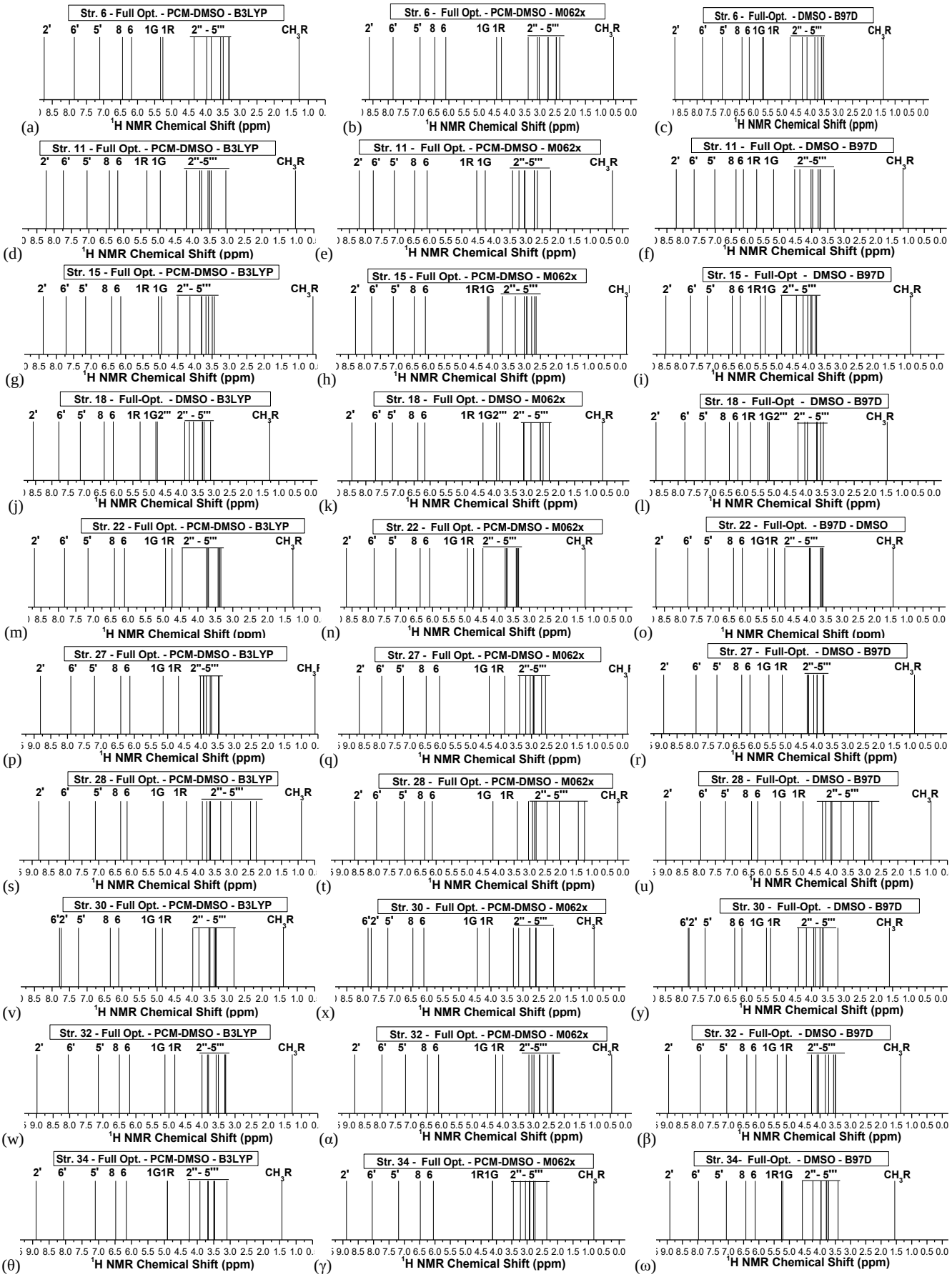


Figure S8. Comparison between B3LYP/6-31G(d,p), M062x/6-31G(d,p)//B3LYP/6-31G(d,p) and B97D/6-31G(d,p)//B3LYP/6-31G(d,p) PCM-DMSO ^{1}H NMR spectra for ten rutin fully optimized structures: **6**, **11**, **15**, **18**, **22**, **27**, **28**, **30**, **32** and **34**.

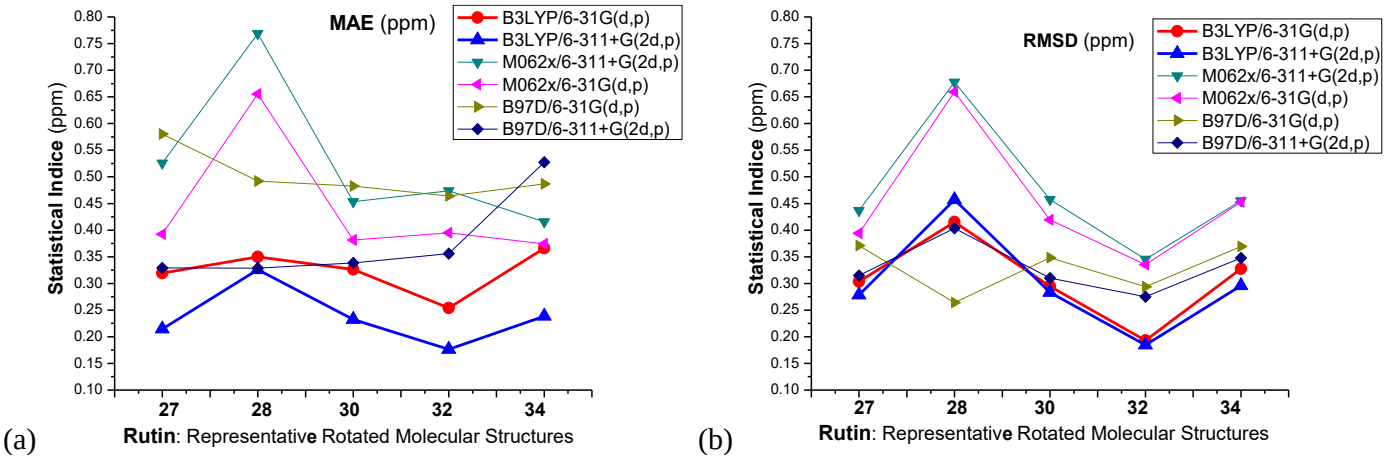


Figure S9. Comparison between B3LYP/6-31G(d,p), M062x/6-31G(d,p)//B3LYP/6-31G(d,p) and B97D/6-31G(d,p)//B3LYP/6-31G(d,p) PCM-DMSO ^{1} H NMR spectra for ten rutin fully optimized structures: **6**, **11**, **15**, **18**, **22**, **27**, **28**, **30**, **32** and **34**. (Continuation)

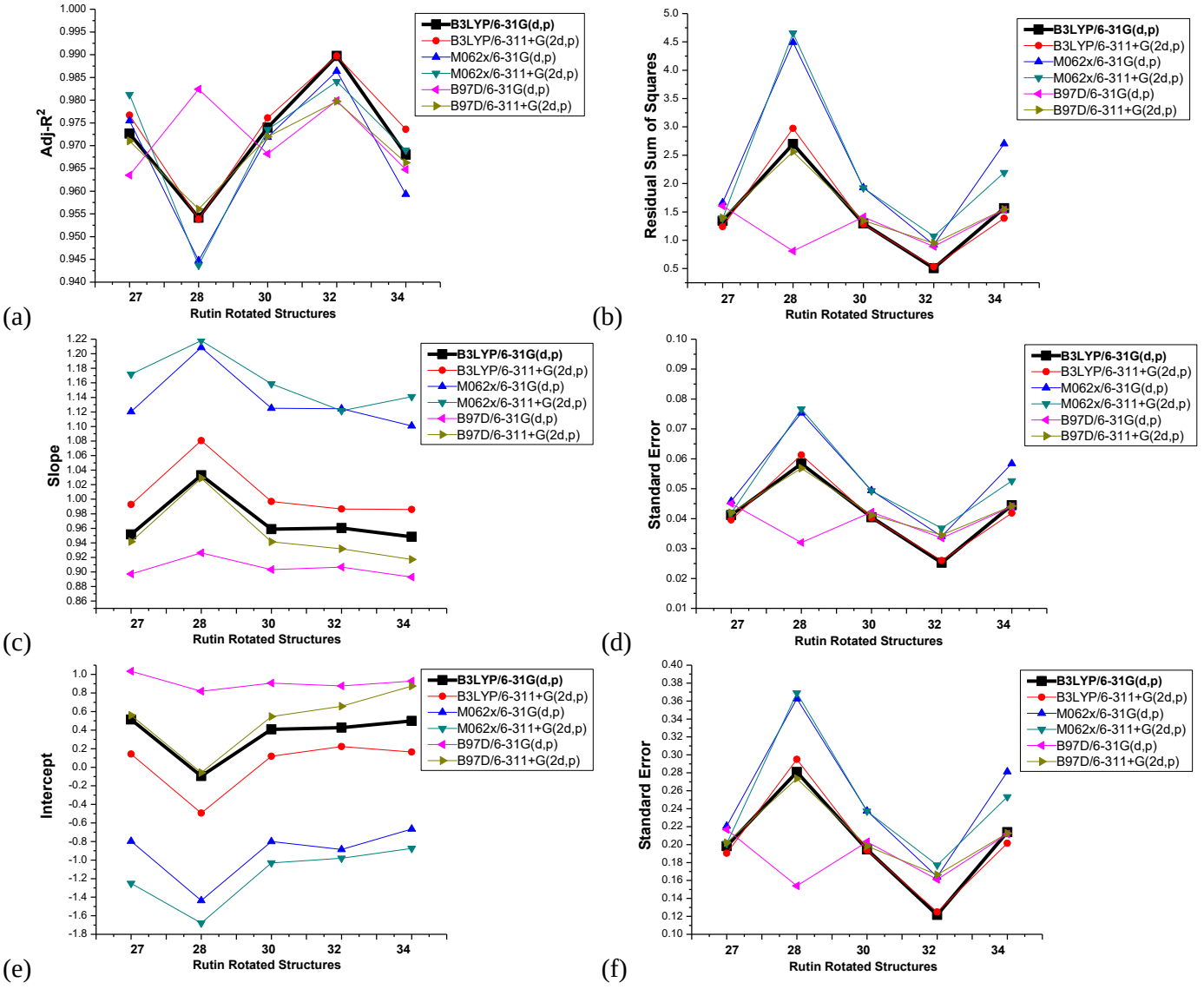


Figure S10. DFT-PCM(H_2O) ^{1}H NMR chemical shift regression analysis for five representative rutin rotated structures (**27**, **28**, **30**, **32** and **34**).

Table S1. Optimized B3LYP/6-31G(d,p) dihedral angles (ϕ_1 to ϕ_6) and relative energy (ΔE_{rel}, in kcal mol^{-1}) for 34 true minima located on the PES for rutin molecule. Minimum energy structures from Figure 1 were used as initial guesses input for full geometry optimization.

Rutin strucutures		Dihedral Angles (°)						ΔE_{rel} [a] (kcal mol^{-1})
		ϕ_1	ϕ_2	ϕ_3	ϕ_4	ϕ_5	ϕ_6	PCM-Water [b]
Geometries optimized using minimum energy input structures from rigid 1D Scan								
1	ϕ_1: M1-axial	162.3	-80.2	176.3	-169.3	-68.8	-44.3	20.6 (23.2) [a]
2	ϕ_1: M1-equat	162.6	-80.4	176.7	-168.6	-70.0	-42.2	17.6 (19.7) [a]
3	ϕ_1: M2-axial	163.0	-80.7	176.6	-168.8	-69.1	-44.8	20.5 (23.2) [a]
4	ϕ_1: M2-equat	162.5	-80.3	176.5	-168.7	-69.8	-42.8	20.2 (21.7) [a]
5	ϕ_2: M3-axial	160.4	124.4	134.2	-169.2	-68.9	-43.3	16.0 (17.0) [a]
6	ϕ_2: M3-equat	160.8	124.8	134.3	-167.5	-69.8	-41.5	13.1 (13.5) [a]
7	ϕ_2: M4-axial	162.1	-80.4	176.5	-169.2	-68.9	-44.8	20.5 (23.2) [a]
8	ϕ_2: M4-equat	162.4	-80.2	176.4	-168.7	-69.7	-42.7	17.5 (19.7) [a]
9	ϕ_3: M5-axial	163.0	-80.2	176.2	-168.7	-68.9	-44.0	20.5 (23.3) [a]
10	ϕ_3: M5-equat	162.3	-80.3	176.9	-169.3	-68.8	-43.1	17.6 (19.7) [a]
11	ϕ_3: M6-axial	154.6	-97.2	-64.0	-170.0	-68.0	-46.4	18.8 (18.4) [a]
12	ϕ_3: M6-equat	154.7	-97.3	-64.1	-170.6	-68.4	-43.1	15.8 (14.9) [a]
13	ϕ_4: M7-axial	162.4	-80.3	176.7	-167.9	-70.0	-43.8	20.5 (23.2) [a]
14	ϕ_4: M7-equat	162.4	-80.5	176.7	-169.1	-69.1	-42.1	17.6 (19.7) [a]
15	ϕ_4: M8-axial	156.0	-75.5	170.7	68.9	-83.3	-35.7	18.2 (18.1) [a]
16	ϕ_4: M8-equat	159.5	-79.2	170.4	71.5	-88.0	-75.0	11.4 (12.1) [a]
17	ϕ_4: M9-axial	161.7	-79.7	175.0	-72.4	-102.9	-25.4	18.9 (22.8) [a]
18	ϕ_4: M9-equat	161.7	-80.4	175.4	-71.1	-107.0	-18.2	16.1 (19.1) [a]
19	ϕ_4: M10-axial	157.3	-73.8	170.3	65.7	-83.7	-33.3	17.9 (18.0) [a]
20	ϕ_4: M10-equat	158.6	-74.6	170.8	62.7	-83.0	-45.1	12.2 (12.4) [a]
21	ϕ_5: M11-axial	162.2	-80.8	175.3	173.3	157.3	-83.6	13.8 (17.6) [a]
22	ϕ_5: M11-quat	162.3	-81.0	175.6	172.6	156.6	-83.1	11.3 (14.4) [a]
23	ϕ_5: M12-axial	162.8	-81.0	176.1	162.7	-165.9	-59.9	19.8 (23.5) [a]
24	ϕ_5: M12-equat	162.8	-80.6	175.8	162.9	-164.4	-59.3	17.0 (20.1) [a]
25	ϕ_6: M13-axial	162.6	-80.4	176.4	-169.7	-68.6	-44.0	20.5 (23.2) [a]
26	ϕ_6: M13-equat	162.6	-80.3	176.6	-169.0	-69.3	-42.1	17.6 (19.7) [a]
27	ϕ_6: M14-axial	164.5	-85.1	177.1	116.2	-77.8	-156.6	20.3 (19.9) [a]
28	ϕ_6: M14-equat	167.1	-83.7	177.3	90.7	-59.5	-146.7	15.0 (13.1) [a]
Geometries optimized as input combination of ϕ_1, to ϕ_6 from structures 1 to 28								
29	E-ax (ϕ_5: M11-eq)	-145.4	106.8	-86.7	-170.0	-100.6	177.4	17.6 (19.6) [a]
30	E-eq (ϕ_5: M11-eq)	-145.5	106.7	-84.3	-169.9	-99.7	176.8	15.0 (16.1) [a]
31	G-ax (ϕ_5: M11-eq)	-174.5	114.2	138.2	-168.7	-98.4	177.9	9.4 (11.2) [a]
32	G-eq (ϕ_5: M11-eq)	174.0	119.1	137.3	-168.9	-99.5	176.9	6.4 (7.7) [a]
33	H-ax (ϕ_5: M11-eq)	-171.6	109.1	138.9	-60.9	-125.5	-168.8	3.0 (3.5) [a]
34	H-eq (ϕ_5: M11-eq)	-172.0	109.8	139.8	-60.7	-124.7	-171.1	0 (0) [a]

[a] The gas phase relative energy values are given in parenthesis.

[b] The corresponding PCM-DMSO relative energies are very similar to PCM-Water values, exhibiting precisely the same energetic trend.

Table S2. B3LYP/6-31G(d,p) Mulliken charges (in e) calculated for optimized and rotated (ϕ_1 and ϕ_2 rotated) preferred structures of rutin in the vacuum.

Atoms	Structure 27		Structure 28		Structure 32		Structure 34		Structure 30
	Opt	Rotated	Opt	Rotated	Opt	Rotated	Opt	Rotated	Opt
C6	-0.1708	-0.1697	-0,1706	-0,1691	-0.1671	-0.1673	-0.1681	-0.1679	-0.1696
C5	0.3139	0.3155	0,3211	0,3195	0.3153	0.3147	0.3148	0.3157	0.3144
C10	-0.0244	-0.0227	-0,0282	-0,0248	-0.0116	-0.0053	-0.0132	-0.0130	-0.0254
C9	0.2986	0.2972	0,3062	0,3040	0.2969	0.2940	0.2963	0.2939	0.2933
C8	-0.1685	-0.1681	-0,2063	-0,2079	-0.1623	-0.1614	-0.1625	-0.1616	-0.1633
C7	0.3462	0.3465	0,3508	0,3521	0.3498	0.3499	0.3489	0.3492	0.3473
H6	0.0910	0.0914	0,0981	0,0971	0.0948	0.0949	0.0919	0.0926	0.0882
H8	0.1090	0.1085	0,1194	0,1204	0.1124	0.1131	0.1111	0.1119	0.1081
C4	0.4335	0.4192	0,4368	0,4241	0.4608	0.4475	0.4560	0.4585	0.4519
O1	-0.5637	-0.5524	-0,5681	-0,5637	-0.5566	-0.5403	-0.557	-0.5445	-0.5469
C2	0.2994	0.2625	0,3088	0,2969	0.2838	0.2306	0.2906	0.2534	0.2538
C3	0.2265	0.2539	0,2312	0,2413	0.2275	0.2499	0.2180	0.2209	0.2437
O7	-0.5523	-0.5524	-0,5471	-0,5472	-0.5493	-0.5490	-0.5510	-0.5506	-0.5526
H7	0.3256	0.3257	0,3288	0,3286	0.3271	0.3270	0.3258	0.3260	0.3247
O5	-0.5722	-0.5715	-0,5678	-0,5670	-0.5666	-0.5664	-0.5696	-0.5690	-0.5756
H5	0.3583	0.3588	0,3604	0,3606	0.3584	0.3579	0.3576	0.3579	0.3568
C1'	0.0373	0.0350	0,0326	0,0299	0.0236	0.0556	0.0224	0.0473	0.0508
C2'	-0.1471	-0.1548	-0,1508	-0,1469	-0.1354	-0.1328	-0.1244	-0.1146	-0.2242
C6'	-0.1076	-0.1009	-0,1048	-0,1071	-0.1039	-0.1042	-0.1064	-0.1045	-0.1028
C3'	0.2805	0.2985	0,2813	0,2904	0.2760	0.2878	0.2917	0.3022	0.3029
H2'	0.0908	0.0982	0,0953	0,0949	0.1316	0.1168	0.1322	0.1195	0.1274
C5'	-0.1163	-0.1182	-0,1162	-0,1159	-0.1174	-0.1154	-0.1191	-0.1178	-0.1130
H6'	0.1055	0.1034	0,1090	0,1095	0.1077	0.1030	0.1034	0.1010	0.1099
C4'	0.3281	0.3272	0,3292	0,3278	0.3300	0.3266	0.3213	0.3230	0.3284
O4	-0.5809	-0.5783	-0,5848	-0,5789	-0.6435	-0.6397	-0.6383	-0.6354	-0.5863
O4'	-0.5546	-0.5532	-0,5530	-0,5524	-0.5561	-0.5559	-0.5605	-0.5592	-0.5540
H4'	0.3390	0.3394	0,3397	0,3398	0.3401	0.3404	0.3357	0.3381	0.3410
H5'	0.1054	0.1093	0,1079	0,1093	0.1035	0.1045	0.0975	0.1000	0.1104
O3'	-0.6073	-0.5994	-0,6059	-0,6014	-0.5987	-0.5946	-0.6330	-0.6137	-0.5899
H3'	0.3324	0.3330	0,3322	0,3315	0.3342	0.3330	0.3559	0.3380	0.3349
O3'	-0.5208	-0.5295	-0,5185	-0,5217	0.0761	0.0764	0.1111	0.1127	0.0791
O4	-0.4865	-0.4861	-0,4962	-0,4959	**0.1012**	**0.1162**	**0.1188**	**0.1146**	0.1177
O5	-0.5086	-0.5069	-0,5113	-0,5111	-0.5045	-0.5034	-0.5297	-0.5301	-0.5041

Table S3. B3LYP/6-31G(d,p) IR stretching frequencies (in cm^{-1}) and intensities (in Km mol^{-1}) in parenthesis for relevant rutin structures.

Rutin Structures	**C-H** (C6) str. A-ring	**C-H** (C2') str. B-ring	**O-H** (C5) str. A-ring	**O-H** (C7) str. A-ring	**O-H** (C3') str. B-ring	**O-H** (C4') str. B-ring
Str- 27 Full-opt	3198 (8.2)	3311 (9.1)	3154 (451)	3820 (87)	3742 (156)	3763 (123)
Str. 27 Rotated	3199 (8.4)	3292 (2.3)	3156 (435)	3821 (88)	3754 (98)	3764 (118)
Str- 30 Full-opt	3197 (8.9)	3217 (6.1)	3145 (462)	3821 (84)	3836 (68)	3774 (128)
Str- 32 Full-opt	3200 (4.9)	3261 (31)	3292 (488)	3820 (97)	3835 (61)	3769 (157)
Str. 32 Rotated	3201 (4.8)	3252 (5.4)	3291 (471)	3820 (97)	3835 (61)	3769 (142)

Table S4. Rotated dihedral angles (ϕ_1 and ϕ_2) and relative energies (ΔE_{rel}, ΔH_{rel} and ΔG_{rel}, in kcal mol^{-1}) for ten selected B3LYP/6-31G(d,p) rutin structures showing reasonable agreement with experimental ^1H NMR profile. (T = 298.15 K, p = 1 atm)

	Rutin	**B3LYP/6-31G(d,p)**					**M06-2x/6-31G(d,p)**			**B97D/6-31G(d,p)**		
Structure	**Torsion angle rotated in 1D scan curve**	**Rotated torsion angle values (°)**		ΔE_{rel}	ΔH_{rel}	ΔG_{rel}	ΔE_{rel}	ΔH_{rel}	ΔG_{rel}	ΔE_{rel}	ΔH_{rel}	ΔG_{rel}
		ϕ_1[a,e]	ϕ_2[b,e]	PCM-DMSO	PCM-DMSO	PCM-DMSO	PCM-DMSO	PCM-DMSO	PCM-DMSO	PCM-DMSO	PCM-DMSO	PCM-DMSO
6	ϕ_2	138	- [c]	9.6	9.5	6.3	10.5	10.4	7.1	7.4	7.3	4.0
11	ϕ_3	140	- [c]	12.9	13.2	11.	10.9	11.2	9.1	10.5	10.8	8.7
15	ϕ_4	140	- [c]	15.4	15.7	17.1	13.9	14.2	15.6	7.4	7.7	9.1
18	ϕ_4	145	- [c]	12.5	12.5	12.7	11.5	11.5	11.7	11.8	11.8	12.0
22	ϕ_5	145	- [c]	7.5	7.2	5.6	7.3	6.9	5.4	8.4	8.4	6.5
27	ϕ_6	145	-65	17.0 (13.9)[f]	17.1 (14.0)[f]	14.8 (11.7)[f]	12.4 (10.4)[f]	12.6 (10.6)[f]	10.3 (8.3)[f]	9.5 (6.4)[f]	9.7 (6.6)[f]	7.4 (4.2)[f]
28	ϕ_6	155	-75	11.0 (9.7)[f]	11.3 (10.0)[f]	12.6 (11.3)[f]	4.6 (3.0)[f]	4.8 (3.3)[f]	6.1 (4.6)[f]	**0.6** (**-1.0**)[f]	0.8 (**-0.7**)[f]	**2.1** (0.6)[f]
30	Combined ϕ´s	- [d]	- [d]	8.3 (6.8)[f]	8.1 (6.6)[f]	5.7 (4.1)[f]	6.1 (5.1)[f]	5.9 (4.9)[f]	3.5 (2.5)[f]	8.0 (6.3)[f]	7.8 (6.1)[f]	5.4 (3.7)[f]
32	Combined ϕ´s	**-150**	**130**	**2.7** (1.1)[f]	2.6 (0.9)[f]	**-1.0** (**-2.7**)[f]	**3.2** (2.3)[f]	3.1 (2.2)[f]	**-0.5** (**-1.4**)[f]	5.6 (3.9)[f]	5.5 (3.8)[f]	**1.9** (0.2)[f]
34	Combined ϕ´s	-152	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Rotated ϕ_1 torsion angle to reach agreement with experimental ^1H NMR spectrum in DMSO solution.

[b] Rotated ϕ_2 torsion angle to reach agreement with experimental ^1H NMR spectrum in DMSO solution.

[c] ϕ_2 was optimized.

[d] ϕ_1 and ϕ_2 were optimized.

[e] ϕ_3, ϕ_4, ϕ_5 and ϕ_6 were optimized.

[f] Values calculated with improved 6-311+G(2d,p) basis set are quoted in parenthesis.

Table S5. DFT-PCM-DMSO ^{1}H NMR chemical shifts (in ppm). Regression analysis for rutin molecule.

Structure	**Adjusted R-Squared (Adj. R^2)**							
	Value	**Residual Sum of Squares**	**Value**	**Residual Sum of Squares**	**Value**	**Residual Sum of Squares**	**Value**	**Residual Sum of Squares**
	M062x/6-31G(d,p)		**M062x/6-311+G(2d,p)**		**B97D/6-31G(d,p)**		**B97D/6-311+G(2d,p)**	
27	0.975	±1.664	0.981	±1.384	0.963	±1.607	0.971	±1.395
28	0.945	±4.494	0.944	±4.655	**0.982**	**±0.811**	0.956	±2.560
30	0.972	±1.930	0.974	±1.923	0.968	±1.410	0.972	±1.347
32	**0.986**	**±0.920**	**0.984**	**±1.072**	**0.980**	**±0.889**	**0.980**	**±0.946**
34	0.959	±2.705	0.969	±2.192	0.965	±1.534	0.966	±1.547
Structure	**Regression Slope**							
		Standard Error		**Standard Error**		**Standard Error**		**Standard Error**
	M062x/6-31G(d,p)		**M062x/6-311+G(2d,p)**		**B97D/6-31G(d,p)**		**B97D/6-311+G(2d,p)**	
27	1.120	±0.046	1.172	±0.042	0.897	±0.045	0.942	±0.042
28	1.209	±0.075	1.218	±0.077	0.926	±0.032	1.029	±0.057
30	1.125	±0.049	1.159	±0.049	0.903	±0.042	0.942	±0.041
32	1.125	±0.034	1.122	±0.037	0.907	±0.034	0.932	±0.035
34	1.101	±0.058	1.141	±0.053	0.893	±0.044	0.917	±0.044
Structure	**Regression Intercept**							
		Standard Error		**Standard Error**		**Standard Error**		**Standard Error**
	M062x/6-31G(d,p)		**M062x/6-311+G(2d,p)**		**B97D/6-31G(d,p)**		**B97D/6-311+G(2d,p)**	
27	-0.797	0.221	-1.251	0.201	1.035	0.217	0.560	0.202
28	-1.437	0.363	-1.680	0.369	0.818	0.154	-0.059	0.274
30	-0.800	0.238	-1.030	0.237	0.907	0.203	0.547	0.198
32	-0.885	0.164	-0.980	0.177	0.876	0.161	0.658	0.166
34	-0.666	0.281	-0.874	0.253	0.929	0.212	0.874	0.213