

Supporting information for:

The Spectrophysics of Warfarin - Implications for Protein Binding

Björn C. G. Karlsson,[†] Annika M. Rosengren,[†] Per Ola Andersson,[‡] Ian A. Nicholls^{†*}

[†] *Bioorganic and Biophysical Chemistry Laboratory, School of Pure and Applied Natural Sciences, University of Kalmar, SE-391 82 Kalmar, Sweden* [‡] *Department of Environment and Protection, FOI NBC Defence, SE-901 82 Umeå, Sweden.*

Correspondence to:

Ian A. Nicholls

Email: ian.nicholls@hik.se

Tel: +46-480 446258

Fax: +46-480 446244

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Lippert-Mataga Plot Data

Table S1. Lippert-Mataga plot data.

Solvent	$\lambda_{\text{abs.}}$ (nm)	$\tilde{\nu}_{\text{abs.}}$ (cm ⁻¹)	$\log \epsilon_{\text{abs.}}$ (M ⁻¹ × cm ⁻¹)	$\lambda_{\text{em.}}$ (nm)	$\tilde{\nu}_{\text{em.}}$ (cm ⁻¹)	Stokes' shift (cm ⁻¹)	ϵ_{D}	n^b	Δf
HEP ^a	305	32787	<i>n.d.</i>	349	28653	4134	1.92	1.3876	0 (-0.001)
CHCl ₃	307	32573	3.92	356	28090	4483	4.80	1.4459	0.148
EtOAc	305	32787	3.97	354	28249	4538	6.04	1.3720	0.200
AcOH	306	32680	3.99	351	28490	4190	6.21	1.3719	0.203
THF	306	32680	3.98	354	28249	4431	7.49	1.4070	0.209
DCM	306	32680	3.98	354	28249	4431	9.00	1.4242	0.218
2PrOH	306	32680	4.02	385	25974	6706	20.0	1.3772	0.276
EtOH	306	32680	4.08	389	25707	6973	25.0	1.3614	0.289
ACN	306	32680	3.99	407	24570	8110	36.4	1.3436	0.305

^a Due to low absorption, the fluorescence emission maximum was estimated using a 1.4×10^{-4} M solution of warfarin.

^b Values taken from the Handbook of Chemistry and Physics, CRC Press, 60th Edition (1979-1980), determined at 293K.

Computationally Derived Molecular Structures

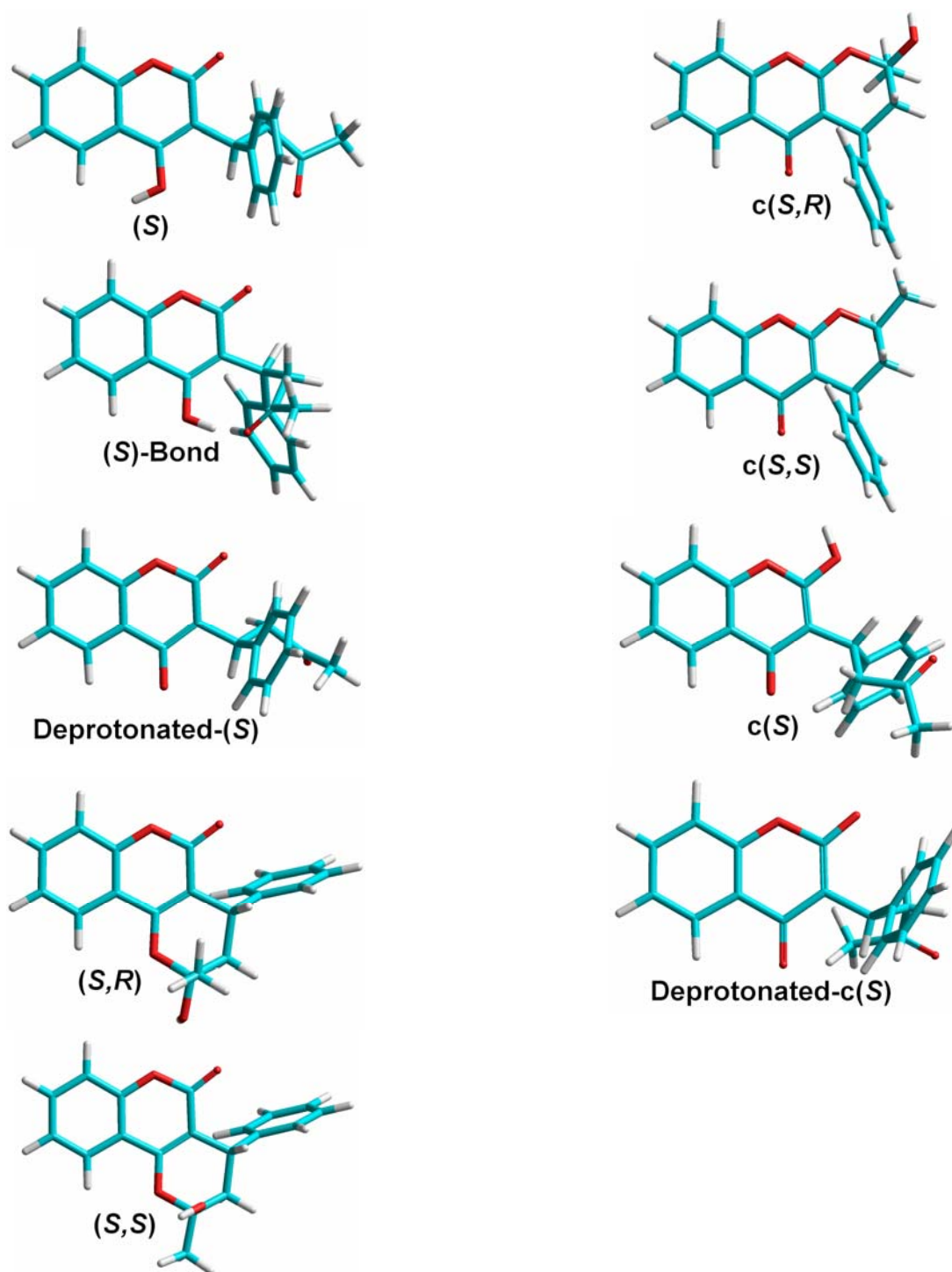


Figure S1. B3LYP optimized molecular structures of warfarin. Chromone structures in this figure are denoted with the letter C.

XYZ-coordinates for warfarin isomers and related structures

(S)-Warfarin

39

C	-2.444965	1.593582	-0.976865
C	-1.851246	0.811232	0.021729
C	-1.972621	1.222953	1.357935
C	-2.666119	2.389885	1.680045
C	-3.251642	3.163777	0.676231
C	-3.140050	2.760229	-0.654265
C	-1.099671	-0.466459	-0.365020
C	-1.813576	-1.736317	0.143935
C	-3.055092	-2.076993	-0.675236
O	-3.237310	-1.638845	-1.796718
C	0.378815	-0.402691	0.001938
C	0.820224	-0.768204	1.339951
O	2.195091	-0.736489	1.599819
C	3.106257	-0.312529	0.688237
C	2.718265	0.099075	-0.598254
C	1.302679	0.021369	-0.915903
C	3.718928	0.550981	-1.479702
C	5.051751	0.571358	-1.094763
C	5.414413	0.142802	0.189966
C	4.446392	-0.296142	1.083750
O	0.116337	-1.118477	2.264604
O	0.868676	0.380268	-2.147108
C	-4.048629	-3.017161	-0.018757
H	6.456629	0.158548	0.492628
H	5.809351	0.923540	-1.786749
H	4.696151	-0.625368	2.086148
H	3.463877	0.907055	-2.475310
H	-1.139364	-0.527916	-1.454320
H	-1.518928	0.628850	2.144703
H	-2.748663	2.694796	2.719738
H	-3.792091	4.071408	0.929831
H	-3.596447	3.350710	-1.443936
H	-2.371611	1.275304	-2.012318
H	-1.146262	-2.607255	0.060324
H	-2.064822	-1.669424	1.204554
H	-4.778035	-3.362711	-0.752632
H	-3.541989	-3.870894	0.444253
H	-4.571187	-2.483705	0.784712
H	1.616365	0.540911	-2.736124

(S)-Warfarin-bond

39

C	5.012192	-0.765368	-0.558571
C	4.240076	-1.197003	-1.647571
C	4.414532	-0.139720	0.529169
C	3.030457	0.048463	0.527135
C	2.240280	-0.381241	-0.549058
C	2.866171	-1.002072	-1.644776
O	2.482826	0.682270	1.602444
C	1.112607	0.894551	1.715646
C	0.247756	0.400233	0.652025
C	0.806602	-0.153521	-0.476353
O	0.732721	1.487726	2.707724
C	-1.245584	0.555404	0.938325
C	-2.103807	-0.653973	0.548946
C	-1.674296	-1.934862	0.933206
C	-2.451007	-3.062885	0.684497
C	-3.687170	-2.938812	0.046136
C	-4.130973	-1.675279	-0.335361
C	-3.347141	-0.545511	-0.084796
C	-1.788290	1.940906	0.530223
C	-1.839104	2.287570	-0.943592
C	-2.231174	3.707464	-1.292056
O	-1.587599	1.483698	-1.833074
O	0.119529	-0.541653	-1.554078
H	6.087445	-0.916397	-0.562646
H	4.717427	-1.680708	-2.493679
H	4.989596	0.209009	1.379752
H	2.251147	-1.322531	-2.477563
H	-1.281460	0.594166	2.033738
H	-0.716304	-2.044889	1.433065
H	-2.091558	-4.040675	0.992160
H	-4.294883	-3.817133	-0.149936
H	-5.089608	-1.560240	-0.833131
H	-3.722326	0.421767	-0.399475
H	-2.803659	2.085104	0.927296
H	-1.182059	2.701660	1.038172
H	-1.437248	4.393117	-0.973128
H	-2.376128	3.800276	-2.368785
H	-3.140766	4.006695	-0.760864
H	-0.722188	-0.031618	-1.608153

(S)-Warfarin-Deprotonated

38

C	-2.065662	1.570737	-1.205166
C	-1.843214	0.720735	-0.108485
C	-2.287541	1.142843	1.154831
C	-2.956145	2.359321	1.306139
C	-3.191343	3.184821	0.204329
C	-2.732308	2.787265	-1.053204
C	-1.083958	-0.587732	-0.323608
C	-1.730333	-1.843797	0.332108
C	-3.008001	-2.353567	-0.312676
O	-3.107839	-3.511207	-0.693247
C	0.384699	-0.425808	0.049852
C	0.764415	-0.468685	1.415590
O	2.150404	-0.318499	1.723861
C	3.070047	-0.105309	0.750610
C	2.715449	-0.010654	-0.603116
C	1.291508	-0.154047	-1.013513
C	3.722255	0.217460	-1.550102
C	5.053306	0.345175	-1.167302
C	5.392995	0.246633	0.189854
C	4.408430	0.023456	1.147729
O	0.050812	-0.621729	2.405133
O	0.975202	-0.043386	-2.223899
C	-4.199579	-1.412331	-0.436419
H	6.429971	0.345231	0.502072
H	5.825800	0.520303	-1.911581
H	4.644993	-0.055110	2.204262
H	3.403168	0.286915	-2.585459
H	-1.054029	-0.755235	-1.405342
H	-2.063880	0.533084	2.023284
H	-3.290432	2.665897	2.294715
H	-3.716250	4.129072	0.325889
H	-2.892300	3.425591	-1.919075
H	-1.677175	1.274013	-2.175201
H	-1.013818	-2.666823	0.280832
H	-1.913178	-1.649409	1.393777
H	-5.055356	-1.973882	-0.816551
H	-4.437695	-0.962549	0.533661
H	-3.968572	-0.577628	-1.104757

(S,R)-Warfarin

39

C	-0.073420	2.710900	-0.048800
O	-1.166103	1.829092	0.385972
C	-1.118302	0.530980	0.016756
C	-0.001894	-0.089111	-0.473753
C	1.333224	0.622484	-0.579238
C	1.246405	1.973291	0.167079
C	-2.366530	-0.190651	0.178657
C	-2.389929	-1.533643	-0.231358
O	-1.302886	-2.126180	-0.798865
C	-0.096807	-1.445696	-0.997619
C	-3.531044	0.375777	0.726368
C	-4.679340	-0.390083	0.870190
C	-4.682217	-1.732531	0.462978
C	-3.544144	-2.308026	-0.089761
O	0.775197	-2.033414	-1.595348
C	2.513500	-0.180892	-0.036122
C	2.455545	-0.789552	1.224058
C	3.559938	-1.466047	1.740215
C	4.744503	-1.538927	1.005007
C	4.812803	-0.934102	-0.250055
C	3.703886	-0.261603	-0.764806
O	-0.173923	3.848287	0.746319
C	-0.343529	3.147324	-1.481881
H	-5.581533	-2.330535	0.574822
H	-5.575062	0.050442	1.295929
H	-3.524411	-3.340803	-0.419459
H	-3.508991	1.415437	1.032116
H	1.539152	0.814044	-1.640505
H	1.534091	-0.745951	1.799489
H	3.494213	-1.939833	2.715583
H	5.604597	-2.067709	1.405092
H	5.726956	-0.991160	-0.833988
H	3.759753	0.196150	-1.749180
H	2.074511	2.625126	-0.124876
H	1.346649	1.788309	1.244243
H	0.031514	3.597797	1.658651
H	-0.390659	2.287613	-2.153358
H	-1.295411	3.680910	-1.517938
H	0.450463	3.818531	-1.818742

(S,S)-Warfarin

39

C	-4.705148	-0.374871	0.818996
C	-4.700677	-1.728919	0.452660
C	-3.551564	-2.319942	-0.059298
C	-2.394697	-1.549627	-0.202120
C	-2.378763	-0.193992	0.164861
C	-3.553874	0.386503	0.674147
O	-1.295946	-2.158648	-0.725611
C	-0.086008	-1.482763	-0.925447
C	-0.000243	-0.110039	-0.440291
C	-1.127253	0.519745	0.003221
C	1.326829	0.612445	-0.570480
O	-1.182090	1.833650	0.336862
C	-0.083074	2.679923	-0.109364
C	1.238986	1.973165	0.156481
C	2.522453	-0.169960	-0.034862
C	3.694361	-0.268279	-0.791123
C	4.818682	-0.921889	-0.285718
C	4.785274	-1.490026	0.987887
C	3.619933	-1.398897	1.751209
C	2.500146	-0.741024	1.244075
O	0.793809	-2.089224	-1.490837
O	-0.181879	2.873078	-1.495321
C	-0.261253	3.978167	0.665894
H	-5.608240	0.077738	1.215665
H	-5.601868	-2.323863	0.565645
H	-3.524610	-3.362639	-0.355607
H	-3.538280	1.433603	0.953536
H	1.501114	0.802093	-1.636236
H	1.329434	1.823041	1.236769
H	2.064143	2.618735	-0.155912
H	3.722291	0.160986	-1.789296
H	5.717853	-0.993088	-0.891108
H	5.657582	-2.004022	1.380928
H	3.581475	-1.843335	2.741803
H	1.594225	-0.681724	1.842506
H	-1.022992	3.316247	-1.677064
H	0.499490	4.698038	0.355451
H	-1.251394	4.401496	0.466220
H	-0.176042	3.807516	1.741589

c(S,R)-Warfarin

39

C	5.084167	-0.467898	0.629428
C	4.709276	-1.692483	0.054176
C	4.169067	0.572153	0.740948
C	2.869803	0.371293	0.270858
C	2.470575	-0.838050	-0.305847
C	3.414018	-1.871634	-0.409832
O	1.995756	1.428682	0.399127
C	0.723208	1.278025	-0.025252
C	0.212439	0.147181	-0.583227
C	1.081810	-1.004088	-0.801151
O	0.081666	2.438130	0.169241
C	-1.372989	2.445627	-0.008645
C	-1.764709	1.545740	-1.181112
C	-1.218007	0.101445	-1.065110
O	-1.968267	1.938400	1.149094
C	-1.711184	3.915021	-0.227731
C	-2.130914	-0.842435	-0.276610
C	-1.898772	-1.193688	1.056223
C	-3.254830	-1.377341	-0.919328
C	-2.768375	-2.051406	1.730255
C	-3.889444	-2.571325	1.082655
C	-4.130876	-2.230093	-0.248759
O	0.704324	-2.031337	-1.366594
H	6.097923	-0.326424	0.991717
H	5.434281	-2.496229	-0.026761
H	4.435029	1.527770	1.179495
H	3.086740	-2.801555	-0.863055
H	-1.367359	2.007593	-2.091232
H	-2.854921	1.540909	-1.257220
H	-1.183002	-0.308198	-2.081755
H	-1.735306	2.521997	1.885558
H	-1.227577	4.301056	-1.127809
H	-2.793457	4.029413	-0.323225
H	-1.366193	4.509818	0.624538
H	-1.033325	-0.789930	1.570502
H	-3.440646	-1.131327	-1.962808
H	-2.567547	-2.314797	2.765115
H	-4.564345	-3.241167	1.607707
H	-4.993693	-2.636193	-0.769346

c(S,S)-Warfarin

39

C	-4.924251	-1.071063	0.436565
C	-4.342304	-2.191303	-0.177802
C	-4.174049	0.073587	0.678626
C	-2.830437	0.082791	0.299206
C	-2.225725	-1.019329	-0.311156
C	-3.005656	-2.161341	-0.549119
O	-2.126174	1.242211	0.538153
C	-0.811300	1.278869	0.235798
C	-0.092415	0.247105	-0.284960
C	-0.791448	-0.968818	-0.692720
O	-0.349903	2.503586	0.533014
C	0.929708	2.872686	-0.077299
C	1.907310	1.714312	0.078428
C	1.386888	0.413106	-0.574017
O	0.739416	3.086111	-1.447379
C	1.341703	4.141345	0.655285
C	2.248635	-0.757564	-0.108986
C	2.119043	-1.288550	1.180514
C	3.247516	-1.267519	-0.944269
C	2.962816	-2.307368	1.621048
C	3.957486	-2.808182	0.779050
C	4.096668	-2.284365	-0.506366
O	-0.247592	-1.888306	-1.304741
H	-5.970448	-1.093459	0.726353
H	-4.939918	-3.078749	-0.360928
H	-4.601373	0.952918	1.148165
H	-2.520332	-3.005585	-1.027660
H	2.066376	1.552014	1.149116
H	2.869764	1.995242	-0.357574
H	1.503930	0.500570	-1.660621
H	0.129296	3.829957	-1.554814
H	0.566150	4.906684	0.545977
H	1.480977	3.949924	1.721567
H	2.275454	4.520089	0.233332
H	1.341160	-0.910438	1.839263
H	3.353525	-0.871529	-1.951075
H	2.841613	-2.712607	2.621838
H	4.614271	-3.602986	1.120553
H	4.862446	-2.671255	-1.172818

c(S)-Warfarin

39

C	5.385289	-0.217734	-0.187450
C	4.900840	0.171301	-1.446141
C	3.542627	0.387407	-1.630988
C	2.643336	0.222720	-0.565855
C	3.155072	-0.164429	0.673119
C	4.515482	-0.389509	0.883229
C	1.184733	0.443965	-0.747498
C	0.365789	0.263806	0.446692
C	0.987027	-0.122841	1.596816
O	2.313724	-0.342449	1.752471
O	0.731760	0.762478	-1.852965
O	0.330720	-0.337231	2.745539
C	-1.146870	0.466606	0.454357
C	-1.583102	1.749131	-0.285794
C	-2.951561	2.250249	0.164398
C	-3.659232	3.200769	-0.783310
O	-3.446849	1.926202	1.228794
C	-1.909922	-0.766888	-0.035363
C	-1.746004	-1.270472	-1.335303
C	-2.456536	-2.395470	-1.754323
C	-3.343630	-3.035429	-0.886328
C	-3.517040	-2.539327	0.405594
C	-2.805468	-1.414097	0.825411
H	6.447753	-0.388121	-0.043374
H	5.590898	0.301150	-2.273737
H	3.131131	0.685423	-2.589390
H	4.864950	-0.690621	1.864792
H	0.973003	-0.586706	3.426745
H	-1.441850	0.618287	1.495057
H	-0.876175	2.565523	-0.074386
H	-1.557249	1.622234	-1.370053
H	-4.514423	3.659276	-0.284795
H	-2.976933	3.973353	-1.154354
H	-4.009958	2.639903	-1.658280
H	-1.055477	-0.778864	-2.013171
H	-2.315925	-2.773348	-2.763478
H	-3.896234	-3.910910	-1.215659
H	-4.208912	-3.024640	1.088530
H	-2.954412	-1.022500	1.827259

c(S)-Warfarin-Deprotonated

38

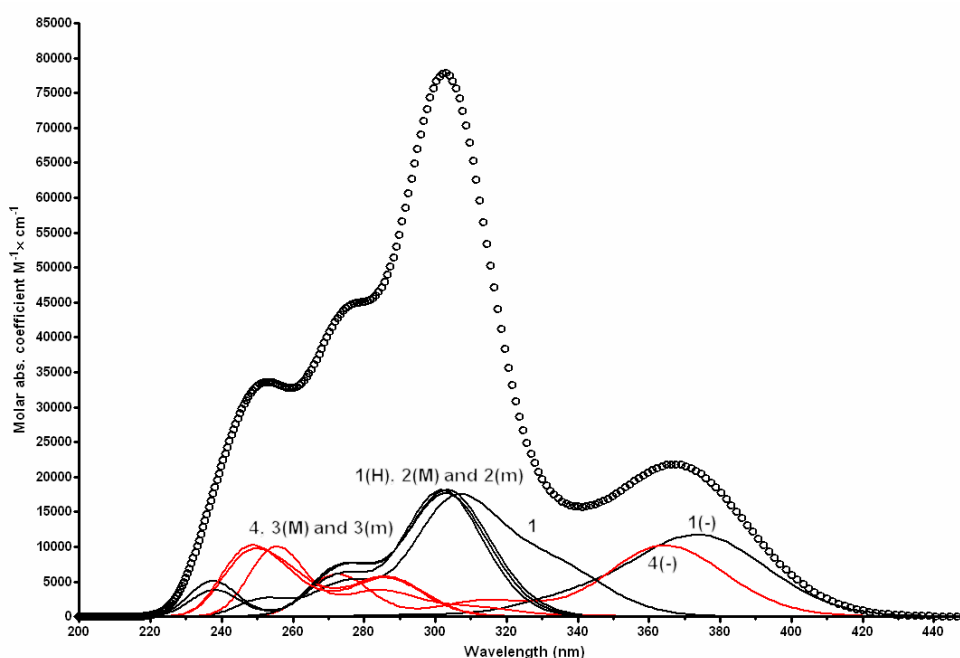
C	-4.748014	-0.763347	-1.093607
C	-4.999928	-1.070971	0.251387
C	-3.984788	-0.975946	1.197882
C	-2.702810	-0.569970	0.799890
C	-2.435730	-0.260663	-0.541102
C	-3.473082	-0.361735	-1.477083
O	-1.750102	-0.485101	1.761005
C	-0.410455	-0.100884	1.448691
C	-0.104156	0.208996	0.099142
C	-1.069294	0.165582	-0.947571
C	1.322932	0.587593	-0.276468
C	2.251890	-0.625789	-0.260803
C	2.863345	-1.101041	0.910142
C	3.698400	-2.218859	0.875732
C	3.939995	-2.889251	-0.325142
C	3.328100	-2.433770	-1.494240
C	2.495167	-1.314874	-1.458751
C	1.907713	1.785241	0.529322
C	1.502233	3.163700	0.031723
C	0.049382	3.412753	-0.328217
O	2.329269	4.060770	-0.061707
O	0.332137	-0.069986	2.429811
O	-0.846246	0.474651	-2.145833
H	-5.545245	-0.838842	-1.828771
H	-5.993526	-1.385985	0.561982
H	-4.155637	-1.207701	2.244701
H	-3.227639	-0.111633	-2.504753
H	1.249946	0.890242	-1.326134
H	2.648562	-0.608077	1.851239
H	4.159567	-2.570976	1.795888
H	4.593298	-3.758373	-0.348454
H	3.501108	-2.948034	-2.437071
H	2.007863	-0.968998	-2.366784
H	1.590129	1.686523	1.575564
H	3.001766	1.768956	0.515803
H	-0.074538	4.462516	-0.603466
H	-0.259016	2.764409	-1.156734
H	-0.602506	3.162710	0.515223

Calculated Solvation Energies and Dipole Moments

Table S2. Data from the B3LYP calculations.

Isomer	Dipole moment, μ (D)		$\Delta\mu$	$-\Delta G_{\text{Solv.}}$ (kcal/mol)
	Vacuo	Water		
(S)	4.878	6.698	1.820	6.33
(S)-H-bond	2.703	3.107	0.403	4.51
Deprotonated-(S)	3.635	5.010	1.375	44.75
(S,R)	3.433	5.026	1.593	8.85
(S,S)	5.431	7.680	2.249	8.50
c(S)	4.812	6.506	1.694	7.04
Deprotonated-c(S)	3.255	4.775	1.520	42.13
c(S,R)	3.413	5.103	1.690	9.36
c(S,S)	4.207	6.143	1.936	9.36

Predicted Singlet and Triplet Excitation Energies

**Figure S2.** Calculated absorption spectra for the different warfarin isomers studied (See Chart 1). In this figure, black solid lines (—) describe absorption by coumarin structures and the corresponding absorption calculated for the chromone structures with red solid lines (—). The additive absorption spectrum is represented by open circles (○).

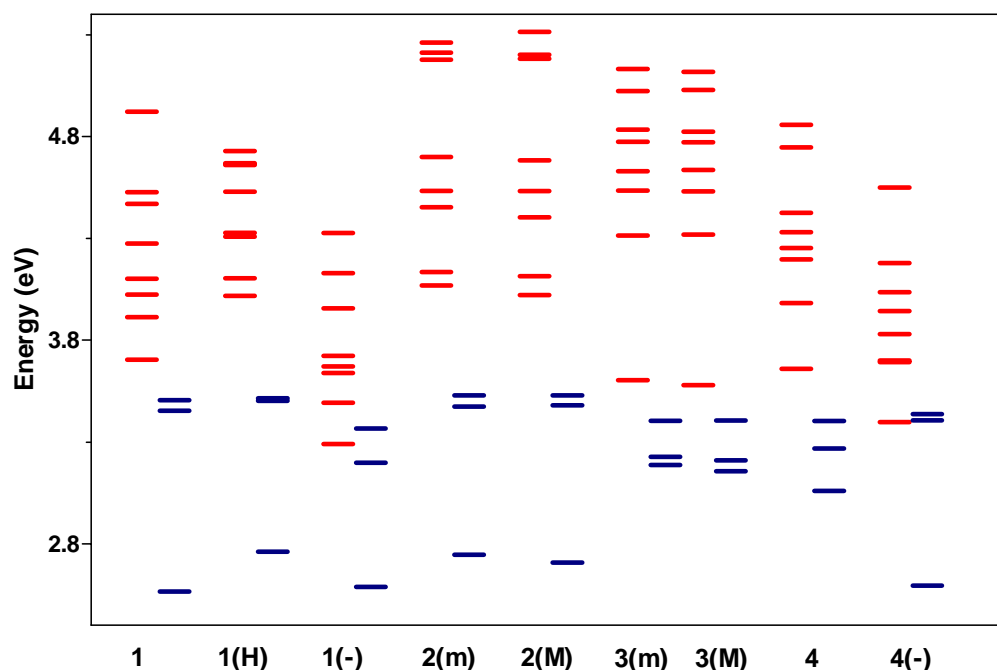


Figure S3. Predicted eight first singlet and the three first triplet states for the warfarin isomers considered. The red lines (—) in the figure correspond to energy levels (eV) for singlet states and blue lines (—) correspond to triplet states. All the calculated energies above have been subtracted by 0.2 eV.

NMR Studies

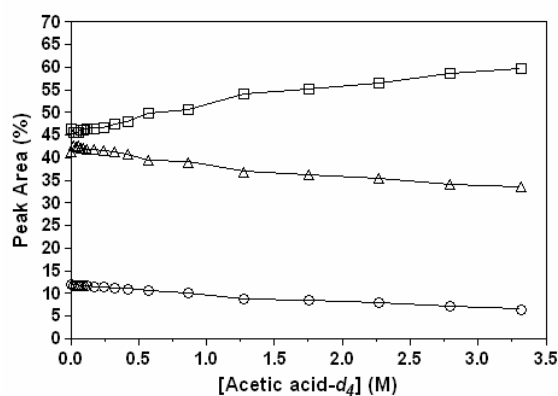


Figure S4. A ^1H NMR titration plot showing how the isomeric distribution of warfarin in CDCl_3 changes upon the addition of an increasing concentration of acetic acid- d_4 at 298K. Studying the methyl proton resonances in this solvent (1.72 ppm for the major cyclic hemiketal, 1.68 ppm for the minor cyclic hemiketal and 2.29 ppm for the open side chain isomer), a change in their corresponding peaks areas was observed when adding an increasing concentration of acetic acid- d_4 (0.0175 – 3.313 M) from 46.5% to 59.8% for the major cyclic hemiketal (\square), 41.3% to 33.3% for the minor cyclic hemiketal (Δ) and finally 12.2% to 6.6% for the open side chain isomer (\circ).