Supporting Information for

Studies of (–)-Pironetin Binding to α-Tubulin: Conformation, Docking and Molecular Dynamics

Angel E. Bañuelos-Hernández,[†] José Alberto Mendoza-Espinoza,[†]

Rogelio Pereda-Miranda,[‡] and Carlos M. Cerda-García-Rojas*,[†]

[†]*Departamento de Química y Programa de Posgrado en Farmacología, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, A. P. 14-470, México, D. F. 07000 Mexico*

[‡]*Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Circuito Exterior Ciudad Universitaria, México, D. F. 04510 Mexico*

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Table S1. DFT relative Gibbs free energies, population, theoretical ^{1}H-^{1}H coupling constants, and comparison with the experimental values of pironetin.

Conformer	ΔG[a]	P[b]	$J_{3,4}$[c]	$J_{4,5}$[c]	$J_{5,16proR}$[c]	$J_{5,16proS}$[c]	$J_{16proR,16proS}$[c]	$J_{5,6}$[c]	$J_{6,7proR}$[c]	$J_{6,7proS}$[c]	J_{7gem}[c]
1-1	0.000	29.090	9.96	6.38	10.98	4.14	−13.59	3.51	0.89	9.95	−13.45
1-2	0.675	9.317	9.97	6.38	10.98	4.16	−13.59	3.53	0.81	9.91	−13.54
1-3	0.753	8.162	9.96	6.38	11.01	4.07	−13.60	3.57	0.74	9.68	−13.61
1-4	0.793	7.627	9.96	6.37	11.00	4.01	−13.57	3.51	0.83	9.89	−13.45
1-5	1.111	4.458	9.96	6.38	10.98	4.20	−13.58	3.49	0.93	10.04	−13.41
1-6	1.219	3.716	9.92	6.28	4.20	3.55	−15.32	4.41	0.95	10.08	−13.67
1-7	1.263	3.454	9.91	6.29	4.23	3.50	−15.27	4.34	1.09	10.28	−13.49
1-8	1.273	3.396	10.27	5.45	9.97	3.93	−12.96	2.36	0.81	8.54	−12.77
1-9	1.520	2.237	10.01	6.36	10.91	4.05	−13.72	3.45	3.45	10.04	−15.40
1-10	1.709	1.627	9.69	6.68	0.29	7.85	−15.83	3.39	1.13	10.52	−13.14
1-11	1.723	1.589	9.96	6.37	11.05	3.90	−13.58	3.58	0.78	9.85	−13.46
1-12	1.739	1.546	9.96	2.12	4.12	12.45	−14.43	6.66	1.20	10.66	−14.09
1-13	1.753	1.509	9.96	6.36	10.96	4.28	−13.60	3.53	1.09	10.25	−13.91
1-14	1.775	1.454	9.87	6.70	10.48	4.65	−12.76	3.41	10.66	2.53	−14.53
1-15	1.790	1.417	9.96	6.38	10.99	4.02	−13.62	3.54	0.75	9.71	−13.57
1-16	1.895	1.188	10.08	2.07	11.47	1.74	−14.20	6.77	1.15	10.55	−14.12
1-17	1.953	1.077	9.95	6.39	11.00	4.17	−13.61	3.51	0.95	10.05	−13.44
1-18	1.962	1.060	9.93	2.14	4.14	12.44	−14.44	6.58	0.96	10.35	−14.27
1-19	2.007	0.982	9.95	6.37	10.98	4.19	−13.60	3.46	1.21	10.40	−13.79
1-20	2.056	0.906	9.94	2.12	4.14	12.46	−14.45	6.63	1.21	10.69	−14.05
1-21	2.083	0.865	9.86	6.73	10.44	4.72	−12.73	3.35	10.65	2.51	−14.31
1-22	2.164	0.755	9.93	6.28	4.17	3.57	−15.30	4.38	0.98	10.19	−13.58
1-23	2.172	0.744	9.88	6.70	10.45	4.52	−12.75	3.43	10.65	2.65	−14.47
1-24	2.223	0.682	9.91	6.26	4.20	3.55	−15.30	4.39	1.06	10.20	−13.61
1-25	2.231	0.674	9.88	6.71	10.43	4.53	−12.73	3.39	10.73	2.86	−14.30
1-26	2.245	0.658	9.69	6.69	0.32	7.99	−15.81	3.36	0.97	10.35	−13.28
1-27	2.295	0.605	9.88	6.71	10.41	4.61	−12.74	3.37	10.76	2.90	−14.33

[a] Relative Gibbs free energies in kcal/mol calculated at the B3PW91/DGTZVP level, absolute G for the global minimum is –654,901.593 kcal/mol. [b] Obtained from ΔG values at 298 K and 1 atm. [c] Theoretical coupling constants in Hz calculated at the B3PW91/DGTZVP level using the optimized structures. [d] Boltzmann-averaged using the equation $\Sigma_i J^i \times P^i$, where J^i is the coupling constant value for each conformer and P^i is the population for the ith conformation. The averaged coupling constants were scaled with factors: $f_{H(sp3)-H(sp3)}$= 0.910; $f_{H(sp3)-H(sp2)}$= 0.929 and $f_{H(sp3)-H(sp3)}$= 0.977. [e] In Hz obtained by non-linear fit of the experimental ^{1}H NMR spectrum to the simulated spectrum.

Table S1 (*continued*). DFT relative Gibbs free energies, population, theoretical ^{1}H-^{1}H coupling constants, and comparison with the experimental values of pironetin.

Conformer	ΔG[a]	P[b]	$J_{3,4}$[c]	$J_{4,5}$[c]	$J_{5,16proR}$[c]	$J_{5,16proS}$[c]	$J_{16proR,16proS}$[c]	$J_{5,6}$[c]	$J_{6,7proR}$[c]	$J_{6,7proS}$[c]	J_{7gem}[c]
1-28	2.318	0.582	9.92	6.27	4.15	3.59	−15.28	4.42	1.05	10.24	−13.47
1-29	2.319	0.581	9.95	6.38	10.99	4.14	−13.59	3.52	0.86	9.93	−13.67
1-30	2.327	0.572	9.67	6.69	0.28	7.83	−13.36	3.35	1.17	10.52	−13.28
1-31	2.385	0.519	9.93	2.13	4.14	12.44	−14.42	6.66	1.15	10.61	−14.29
1-32	2.389	0.516	9.68	6.69	0.31	7.93	−15.82	3.32	1.10	10.49	−13.16
1-33	2.412	0.497	10.09	2.06	11.55	1.82	−14.14	6.73	1.40	10.78	−13.83
1-34	2.418	0.491	9.87	6.70	10.43	4.42	−12.77	3.33	10.74	2.87	−14.31
1-35	2.454	0.463	9.68	6.68	0.32	8.01	−15.83	3.33	1.10	10.51	−13.16
1-36	2.456	0.461	9.95	6.38	10.99	4.04	−13.63	3.49	0.84	9.85	−13.43
1-37	2.527	0.409	9.91	6.27	4.18	3.58	−15.30	4.38	1.08	10.26	−13.45
1-38	2.556	0.389	9.96	6.37	11.01	4.09	−13.61	3.55	0.76	9.73	−13.63
1-39	2.629	0.344	9.99	6.35	10.88	4.29	−13.68	3.48	0.88	10.31	−15.43
1-40	2.631	0.343	9.86	6.70	10.48	4.17	−12.76	3.18	10.79	3.06	−14.46
1-41	2.656	0.329	9.87	6.71	10.45	4.21	−12.78	3.19	10.77	3.00	−14.38
1-42	2.712	0.299	9.96	6.38	10.98	4.17	−13.60	3.51	0.88	9.97	−13.46
1-43	2.716	0.297	10.09	2.06	11.52	1.78	−14.18	6.73	1.34	10.75	−13.89
1-44	2.737	0.287	10.09	2.07	11.54	1.77	−14.21	6.73	1.37	10.84	−13.97
1-45	2.815	0.251	9.93	2.12	4.13	12.45	−14.44	6.65	1.20	10.68	−14.04
1-46	2.853	0.236	9.87	6.72	10.45	4.68	−12.76	3.42	10.66	2.64	−14.21
1-47	2.927	0.208	10.08	2.07	11.48	1.75	−14.16	6.75	1.28	10.62	−14.02
1-48	2.961	0.197	9.94	6.79	4.16	12.47	−14.44	6.65	1.14	10.69	−14.16
1-49	2.977	0.191	10.00	6.36	10.88	4.11	−13.74	3.45	0.51	9.85	−15.46
1-50	2.981	0.190	9.81	6.57	4.96	3.15	−15.81	4.31	10.54	2.04	−14.33
1-51	2.992	0.186	10.00	6.36	10.91	4.14	−13.69	3.44	0.93	10.24	−15.35
1-52	2.998	0.185	9.96	6.39	11.00	4.05	−13.61	3.50	0.83	9.87	−13.52
1-53	3.000	0.184	9.93	6.27	4.04	3.71	−15.24	4.47	0.87	10.01	−13.50
		$J_{calculated}$[d]	**9.95**	**6.08**	**9.54**	**4.54**	**−13.82**	**3.77**	**1.55**	**9.54**	**−13.62**
		$J_{experimental}$[e]	**9.80**	**6.00**	**9.70**	**4.70**	**−13.60**	**3.50**	**2.50**	**9.90**	**−13.70**

[a] Relative energies in kcal/mol calculated at the B3PW91/DGTZVP level, absolute G for the global minimum is –654,901.593 kcal/mol. [b] Obtained from ΔG values at 298 K and 1 atm. [c] Theoretical coupling constants in Hz calculated at the B3PW91/DGTZVP level using the optimized structures. [d] Boltzmann-averaged using the equation $\Sigma_i J^i \times P^i$, where J^i is the coupling constant value for each conformer and P^i is the population for the ith conformation. The averaged coupling constants were scaled with factors: $f_{H(sp3)\text{-}H(sp3)}$= 0.910; $f_{H(sp3)\text{-}H(sp2)}$= 0.929 and $f_{H(sp3)\text{-}H(sp3)}$= 0.977. [e] In Hz obtained by non-linear fit of the experimental ^{1}H NMR spectrum to the simulated spectrum.

Table S1 (*continued*). DFT relative Gibbs free energies, population, theoretical ^{1}H-^{1}H coupling constants, and comparison with the experimental values of pironetin.

Conformer	ΔG[a]	P[b]	$J_{7proR,8}$[c]	$J_{7proS,8}$[c]	$J_{8,9}$[c]	$J_{9,10}$[c]	$J_{10,11}$[c]	$J_{11,12proR}$[c]	$J_{11,12proS}$[c]	J_{12gem}[c]
1-1	0.000	29.090	9.98	1.16	1.09	0.99	9.76	10.31	2.10	−13.73
1-2	0.675	9.317	9.50	0.85	1.54	1.97	9.06	10.78	4.07	−13.08
1-3	0.753	8.162	9.89	1.01	1.05	0.27	4.12	11.21	1.90	−13.97
1-4	0.793	7.627	9.79	1.07	1.48	1.67	9.75	4.45	3.04	−15.68
1-5	1.111	4.458	9.88	1.14	1.38	1.61	9.76	4.66	2.94	−15.26
1-6	1.219	3.716	9.88	0.98	1.04	0.28	4.18	11.27	1.95	−14.01
1-7	1.263	3.454	10.06	1.24	1.10	1.04	9.76	10.36	2.13	−13.69
1-8	1.273	3.396	9.14	1.19	1.06	0.40	3.22	10.02	2.16	−12.81
1-9	1.520	2.237	2.92	9.67	1.64	2.13	9.25	11.23	3.09	−14.25
1-10	1.709	1.627	9.77	1.05	1.41	1.85	9.29	11.06	3.01	−13.45
1-11	1.723	1.589	9.69	1.02	1.43	1.45	9.38	0.88	9.16	−16.33
1-12	1.739	1.546	9.80	0.96	1.16	1.12	9.78	10.32	2.00	−13.61
1-13	1.753	1.509	10.34	1.40	1.37	9.41	2.08	10.80	2.18	−13.50
1-14	1.775	1.454	10.58	1.54	0.97	0.21	3.43	11.08	2.20	−13.37
1-15	1.790	1.417	10.19	1.00	2.71	9.70	0.23	11.76	2.05	−13.64
1-16	1.895	1.188	9.70	0.82	1.07	0.30	4.19	11.33	1.95	−13.98
1-17	1.953	1.077	10.14	1.28	0.97	0.70	9.59	9.76	0.90	−19.32
1-18	1.962	1.060	9.73	0.85	1.13	0.26	4.11	11.21	1.88	−13.37
1-19	2.007	0.982	10.33	1.50	1.28	9.54	1.83	2.66	10.64	−15.22
1-20	2.056	0.906	9.78	0.98	1.51	1.61	9.82	4.61	2.98	−15.33
1-21	2.083	0.865	10.23	1.44	1.40	1.74	9.19	11.19	3.31	−13.82
1-22	2.164	0.755	9.71	0.97	1.49	1.93	9.04	10.76	4.23	−13.06
1-23	2.172	0.744	10.44	1.36	1.00	0.20	3.34	10.97	2.30	−13.05
1-24	2.223	0.682	10.26	1.26	1.01	0.19	3.57	11.16	2.18	−13.62
1-25	2.231	0.674	9.86	1.07	1.59	1.75	9.72	4.31	3.12	−15.32
1-26	2.245	0.658	9.48	0.84	1.60	2.01	9.11	10.76	4.10	−13.08
1-27	2.295	0.605	9.85	1.05	1.59	1.88	9.19	10.86	3.97	−13.43

[a] Relative energies in kcal/mol calculated at the B3PW91/DGTZVP level, absolute G for the global minimum is –654,901.593 kcal/mol. [b] Obtained from ΔG values at 298 K and 1 atm. [c] Theoretical coupling constants in Hz calculated at the B3PW91/DGTZVP level using the optimized structures. [d] Boltzmann-averaged using the equation $\Sigma_i J^i \times P^i$, where J^i is the coupling constant value for each conformer and P^i is the population for the ith conformation. The averaged coupling constants were scaled with factors: $f_{H(sp3)\text{-}H(sp3)}$= 0.910; $f_{H(sp3)\text{-}H(sp2)}$= 0.929 and $f_{H(sp3)\text{-}H(sp3)}$= 0.977. [e] In Hz obtained by non-linear fit of the experimental ^{1}H NMR spectrum to the simulated spectrum.

Table S1 (*continued*). DFT relative Gibbs free energies, population, theoretical ^{1}H-^{1}H coupling constants, and comparison with the experimental values of pironetin.

Conformer	ΔG[a]	P[b]	$J_{7proR,8}$[c]	$J_{7proS,8}$[c]	$J_{8,9}$[c]	$J_{9,10}$[c]	$J_{10,11}$[c]	$J_{11,12proR}$[c]	$J_{11,12proS}$[c]	J_{12gem}[c]
1-28	2.318	0.582	9.83	1.10	1.46	1.71	9.70	4.50	10.84	−15.73
1-29	2.319	0.581	9.92	1.00	1.39	0.25	1.98	2.51	10.83	−15.35
1-30	2.327	0.572	10.36	1.38	0.93	0.19	3.63	11.18	2.12	−13.66
1-31	2.385	0.519	9.63	0.77	1.08	0.25	3.98	11.40	2.13	−14.02
1-32	2.389	0.516	9.87	1.15	1.38	1.61	9.76	4.46	3.03	−15.68
1-33	2.412	0.497	9.87	1.06	1.49	1.65	9.76	4.54	3.05	−15.36
1-34	2.418	0.491	9.63	0.92	1.62	1.71	9.80	4.44	3.07	−14.98
1-35	2.454	0.463	9.81	1.08	1.40	1.61	9.79	4.63	2.96	−15.31
1-36	2.456	0.461	10.28	1.07	2.60	9.72	1.40	11.03	2.44	−13.72
1-37	2.527	0.409	9.87	1.12	1.39	1.64	9.79	4.66	2.95	−15.18
1-38	2.556	0.389	9.92	0.98	1.30	0.21	2.41	10.96	2.06	−13.35
1-39	2.629	0.344	2.02	9.37	1.49	1.81	9.85	4.30	3.13	−14.97
1-40	2.631	0.343	10.02	0.75	2.64	9.93	0.21	3.35	11.72	−14.76
1-41	2.656	0.329	9.96	0.74	2.70	9.79	0.23	11.65	1.88	−13.68
1-42	2.712	0.299	10.13	1.25	0.97	0.94	7.22	3.45	10.23	−13.92
1-43	2.716	0.297	9.79	1.01	1.51	1.67	9.76	4.51	2.98	−15.75
1-44	2.737	0.287	9.58	0.81	1.54	1.95	9.08	10.74	4.17	−13.09
1-45	2.815	0.251	9.77	0.99	1.53	1.69	9.76	4.56	2.94	−15.82
1-46	2.853	0.236	10.16	1.44	1.39	1.38	9.37	0.51	8.51	−16.24
1-47	2.927	0.208	10.14	1.13	1.04	0.20	4.09	11.29	2.05	−13.54
1-48	2.961	0.197	9.53	0.78	1.56	1.96	9.09	10.76	4.06	−13.10
1-49	2.977	0.191	3.31	9.78	1.83	2.01	9.82	4.54	3.05	−14.43
1-50	2.981	0.190	10.55	1.73	0.96	0.20	3.45	11.03	2.09	−13.29
1-51	2.992	0.186	1.40	9.10	0.99	0.90	9.49	10.04	2.33	−13.85
1-52	2.998	0.185	10.41	1.02	3.47	9.90	1.42	3.57	11.22	−15.90
1-53	3.000	0.184	9.76	1.08	1.49	1.39	9.42	0.87	10.02	−16.40
		$J_{calculated}$[d]	**9.67**	**1.35**	**1.28**	**1.57**	**7.83**	**9.20**	**2.91**	**−14.09**
		$J_{experimental}$[e]	**9.70**	**2.70**	**1.70**	**4.00**	**6.60**	**8.00**	**4.30**	**−13.50**

[a] Relative energies in kcal/mol calculated at the B3PW91/DGTZVP level, absolute G for the global minimum is –654,901.593 kcal/mol. [b] Obtained from ΔG values at 298 K and 1 atm. [c] Theoretical coupling constants in Hz calculated at the B3PW91/DGTZVP level using the optimized structures. [d] Boltzmann-averaged using the equation $\Sigma_i J^i \times P^i$, where J^i is the coupling constant value for each conformer and P^i is the population for the ith conformation. The averaged coupling constants were scaled with factors: $f_{H(sp3)\text{-}H(sp3)}$= 0.910; $f_{H(sp3)\text{-}H(sp2)}$= 0.929 and $f_{H(sp3)\text{-}H(sp3)}$= 0.977. [e] In Hz obtained by non-linear fit of the experimental ^{1}H NMR spectrum to the simulated spectrum.

Table S1 (*continued*). DFT relative Gibbs free energies, population, theoretical ^{1}H-^{1}H coupling constants, and comparison with the experimental values of pironetin.

Conformer	ΔG	P_i	$J_{12proR,13}$	$J_{12proS,13}$	$J_{13,14}$	Conformer	ΔG	P_i	$J_{12proR,13}$	$J_{12proS,13}$	$J_{13,14}$
1-1	0.000	29.090	10.21	3.69	14.71	**1**-28	2.318	0.582	11.07	3.35	14.80
1-2	0.675	9.317	4.73	9.68	14.81	**1**-29	2.319	0.581	3.05	10.49	14.88
1-3	0.753	8.162	9.89	3.45	14.63	**1**-30	2.327	0.572	3.98	10.77	14.57
1-4	0.793	7.627	11.06	3.36	14.80	**1**-31	2.385	0.519	13.92	3.30	14.64
1-5	1.111	4.458	3.96	11.02	14.51	**1**-32	2.389	0.516	11.07	3.38	14.80
1-6	1.219	3.716	9.86	3.43	14.63	**1**-33	2.412	0.497	3.85	11.07	14.53
1-7	1.263	3.454	10.22	3.73	14.71	**1**-34	2.418	0.491	3.93	10.93	14.52
1-8	1.273	3.396	3.95	9.37	15.10	**1**-35	2.454	0.463	3.90	11.05	14.50
1-9	1.520	2.237	10.37	3.60	14.67	**1**-36	2.456	0.461	10.03	3.73	14.62
1-10	1.709	1.627	10.19	3.77	14.73	**1**-37	2.527	0.409	4.08	10.99	14.52
1-11	1.723	1.589	3.04	10.26	14.98	**1**-38	2.556	0.389	10.42	3.64	14.62
1-12	1.739	1.546	10.19	3.84	14.72	**1**-39	2.629	0.344	10.82	3.49	15.09
1-13	1.753	1.509	10.47	3.69	14.61	**1**-40	2.631	0.343	3.66	10.43	14.78
1-14	1.775	1.454	9.97	3.73	14.63	**1**-41	2.656	0.329	9.83	3.70	14.62
1-15	1.790	1.417	9.88	3.65	14.63	**1**-42	2.712	0.299	10.82	4.43	14.60
1-16	1.895	1.188	9.83	3.37	14.63	**1**-43	2.716	0.297	11.09	3.40	14.79
1-17	1.953	1.077	5.11	3.13	15.63	**1**-44	2.737	0.287	4.65	9.71	14.91
1-18	1.962	1.060	4.22	10.71	14.58	**1**-45	2.815	0.251	11.09	3.34	14.79
1-19	2.007	0.982	3.04	10.52	14.91	**1**-46	2.853	0.236	3.27	10.28	14.90
1-20	2.056	0.906	3.98	11.03	14.53	**1**-47	2.927	0.208	4.27	10.72	14.57
1-21	2.083	0.865	10.26	3.57	14.64	**1**-48	2.961	0.197	4.63	9.70	14.90
1-22	2.164	0.755	4.77	9.68	14.81	**1**-49	2.977	0.191	4.35	10.79	14.59
1-23	2.172	0.744	4.17	10.66	14.61	**1**-50	2.981	0.190	9.94	3.77	14.63
1-24	2.223	0.682	4.08	10.73	14.56	**1**-51	2.992	0.186	3.65	10.45	14.77
1-25	2.231	0.674	10.92	3.41	14.83	**1**-52	2.998	0.185	2.82	10.45	15.06
1-26	2.245	0.658	4.73	9.70	14.81	**1**-53	3.000	0.184	3.02	10.23	14.98
1-27	2.295	0.605	4.30	10.01	14.68						
								$J_{calculated}$ [d]	**8.39**	**5.58**	**14.73**
								$J_{experimental}$ [e]	**7.30**	**6.40**	**14.80**

[a] Relative energies in kcal/mol calculated at the B3PW91/DGTZVP level, absolute *G* for the global minimum is –654,901.593 kcal/mol. [b] Obtained from ΔG values at 298 K and 1 atm. [c] Theoretical coupling constants in Hz calculated at the B3PW91/DGTZVP level using the optimized structures. [d] Boltzmann-averaged using the equation $\Sigma_i J^i \times P^i$, where J^i is the coupling constant value for each conformer and P^i is the population for the *i*th conformation. The averaged coupling constants were scaled with factors: $f_{H(sp3)\text{-}H(sp3)}$= 0.910; $f_{H(sp3)\text{-}H(sp2)}$= 0.929 and $f_{H(sp3)\text{-}H(sp3)}$= 0.977. [e] In Hz obtained by non-linear fit of the experimental ^{1}H NMR spectrum to the simulated spectrum.

Table S2. Cartesian coordinates for the global minimum energy conformer of pironetin (conformer **1**-1, representative of the *L*-type family). Sum of electronic and thermal free energies (DFT B3PW91/DGTZVP) = –654,901.593 kcal/mol (# of imaginary frequencies = 0).

Atom	X	Y	Z
C	3.944777	-0.311060	0.261945
C	2.518850	-0.327706	-0.290925
O	2.087119	1.010447	-0.659484
C	2.900437	1.750071	-1.452698
O	2.480971	2.763419	-1.966265
C	4.298825	1.297781	-1.577376
C	4.808175	0.379222	-0.754190
C	1.456450	-0.879102	0.643605
C	4.085240	0.319430	1.660895
C	5.513144	0.318991	2.197935
C	0.151762	-1.183479	-0.085924
C	-1.013251	-1.505132	0.873936
C	-2.382586	-1.482060	0.160644
C	-2.869396	-0.083553	-0.269108
C	-3.225021	0.775715	0.961957
C	-3.473957	2.216797	0.636384
C	-4.590427	2.888016	0.910575
C	-4.824297	4.333156	0.607920
C	-4.062650	-0.163721	-1.219452
O	-2.307559	-2.359249	-0.983770
C	-3.257300	-3.410117	-1.009172
C	-0.811873	-2.822907	1.623812
O	0.424143	-2.252264	-0.988379
H	4.265450	-1.358833	0.330596
H	2.519615	-0.930172	-1.205789
H	4.895026	1.828367	-2.309368
H	5.865320	0.133577	-0.799497
H	1.273356	-0.162442	1.449263
H	1.837551	-1.803041	1.089540
H	3.700447	1.343696	1.627416
H	3.443693	-0.233778	2.353474
H	5.931012	-0.692485	2.220429
H	6.176978	0.942014	1.593094
H	5.541571	0.709339	3.218094
H	-0.117618	-0.284960	-0.656745
H	-1.030967	-0.696962	1.613213
H	-3.129830	-1.894515	0.854223
H	-2.051260	0.413773	-0.804061
H	-2.407794	0.729104	1.690746
H	-4.107340	0.350755	1.456317
H	-2.652696	2.741936	0.147143
H	-5.409656	2.359688	1.398719
H	-5.029196	4.899864	1.522810
H	-3.960465	4.785433	0.115679
H	-5.695423	4.465229	-0.043091
H	-4.920473	-0.645384	-0.736547
H	-4.378377	0.839365	-1.513460
H	-3.814644	-0.722520	-2.122499
H	-3.158052	-4.065582	-0.135534
H	-3.063879	-3.993501	-1.911056
H	-4.282847	-3.027412	-1.052456
H	-1.632279	-2.996914	2.325991
H	0.115363	-2.819769	2.201222
H	-0.762671	-3.667580	0.933936
H	-0.438726	-2.507159	-1.353209

Table S3. Cartesian coordinates for the global minimum energy conformer of pironetin (conformer **1**-13, representative of the *M*-type family). Sum of electronic and thermal free energies (DFT B3PW91/DGTZVP) = –654,899.84 kcal/mol (# of imaginary frequencies = 0).

Atom	X	Y	Z
C	-4.305949	-0.860608	0.378908
C	-2.996768	-0.075136	0.474754
O	-2.978177	1.030114	-0.466805
C	-4.039448	1.874626	-0.480998
O	-3.957917	2.933698	-1.062047
C	-5.275894	1.400454	0.168237
C	-5.429624	0.123948	0.525429
C	-1.731538	-0.877880	0.231883
C	-4.453593	-1.718174	-0.893155
C	-5.767129	-2.490474	-0.968491
C	-0.469781	-0.116346	0.623175
C	0.826000	-0.822177	0.178145
C	2.039039	0.109754	0.369611
C	3.396358	-0.513766	-0.003507
C	4.547804	0.449922	0.344568
C	5.903800	-0.181859	0.272063
C	6.912340	0.238537	-0.488783
C	8.270187	-0.384143	-0.545957
C	3.459530	-0.945892	-1.467146
O	1.826646	1.288619	-0.418665
C	1.799272	2.507463	0.291519
C	1.011383	-2.173703	0.867631
O	-0.528858	0.056870	2.049269
H	-4.328560	-1.536953	1.243636
H	-2.939742	0.357570	1.479709
H	-6.070168	2.132778	0.243621
H	-6.383948	-0.222552	0.911553
H	-1.669859	-1.160318	-0.822637
H	-1.796638	-1.797021	0.822714
H	-4.351764	-1.071484	-1.770495
H	-3.621061	-2.427211	-0.928269
H	-6.630064	-1.823093	-1.035806
H	-5.784842	-3.134024	-1.851329
H	-5.905792	-3.130895	-0.091707
H	-0.503821	0.859315	0.129437
H	0.725219	-0.979203	-0.902507
H	2.094862	0.391942	1.432721
H	3.522741	-1.399147	0.631413
H	4.390313	0.818733	1.367999
H	4.508927	1.321186	-0.317082
H	6.059958	-1.053553	0.909902
H	6.754172	1.107795	-1.127151
H	9.046081	0.332673	-0.255567
H	8.344170	-1.250003	0.116162
H	8.512793	-0.711470	-1.562894
H	2.741022	-1.737799	-1.692855
H	3.246741	-0.099049	-2.124503
H	4.455517	-1.322162	-1.710927
H	2.731001	2.693165	0.839600
H	0.960636	2.556467	1.001984
H	1.659834	3.301791	-0.443731
H	0.129626	-2.805234	0.739907
H	1.171757	-2.049557	1.941633
H	1.857256	-2.725377	0.454361
H	0.004153	0.821013	2.289785

Table S4. Cartesian coordinates and simulated B-factors for the M-loop of α-tubulin in water.

Atom no.	Type	Residue	Chain	Residue no.	X	Y	Z	B-Factor
2475	N	ILE	A	274	78.944	28.614	63.887	51.11
2476	H	ILE	A	274	79.794	28.654	63.357	56.48
2477	CA	ILE	A	274	78.584	27.264	64.317	70.47
2478	CB	ILE	A	274	79.784	26.334	64.127	111.12
2479	CG1	ILE	A	274	79.504	24.874	64.507	168.05
2480	CG2	ILE	A	274	81.094	26.874	64.707	172.19
2481	CD	ILE	A	274	78.874	24.194	63.287	214.06
2482	C	ILE	A	274	78.074	27.194	65.757	59.38
2483	O	ILE	A	274	78.604	27.924	66.607	62.49
2484	N	SER	A	275	77.044	26.384	65.917	54.39
2485	H	SER	A	275	76.684	25.904	65.117	63.71
2486	CA	SER	A	275	76.534	25.994	67.247	46.30
2487	CB	SER	A	275	75.224	25.214	67.167	47.75
2488	OG	SER	A	275	75.344	23.994	66.427	77.17
2489	HG	SER	A	275	74.734	23.304	66.827	126.19
2490	C	SER	A	275	77.434	25.224	68.217	46.30
2491	O	SER	A	275	78.394	24.544	67.857	57.85
2492	N	ALA	A	276	77.234	25.674	69.447	46.87
2493	H	ALA	A	276	76.524	26.374	69.537	49.21
2494	CA	ALA	A	276	78.144	25.404	70.567	59.97
2495	CB	ALA	A	276	78.304	26.754	71.257	77.58
2496	C	ALA	A	276	77.874	24.254	71.547	59.70
2497	O	ALA	A	276	78.264	24.174	72.707	87.66
2498	N	GLU	A	277	77.034	23.324	71.117	49.84
2499	H	GLU	A	277	76.224	23.514	70.557	46.29
2500	CA	GLU	A	277	77.034	21.994	71.747	66.55
2501	CB	GLU	A	277	75.774	21.784	72.587	87.19
2502	CG	GLU	A	277	75.584	22.934	73.587	127.42
2503	CD	GLU	A	277	74.364	23.814	73.367	137.27
2504	OE1	GLU	A	277	74.354	25.034	73.667	200.94
2505	OE2	GLU	A	277	73.304	23.284	72.977	177.59
2506	C	GLU	A	277	77.074	20.874	70.707	72.73
2507	O	GLU	A	277	77.604	19.794	70.997	98.00
2508	N	LYS	A	278	76.354	20.894	69.587	68.82
2509	H	LYS	A	278	75.824	21.634	69.187	68.04
2510	CA	LYS	A	278	76.184	19.754	68.667	85.81
2511	CB	LYS	A	278	75.124	20.144	67.637	96.77
2512	CG	LYS	A	278	73.674	20.124	68.127	143.27
2513	CD	LYS	A	278	72.894	20.644	66.917	194.78
2514	CE	LYS	A	278	71.414	20.844	67.257	297.91
2515	NZ	LYS	A	278	70.684	21.724	66.337	377.53
2516	HZ1	LYS	A	278	70.774	22.694	66.557	433.79
2517	HZ2	LYS	A	278	71.034	21.494	65.427	426.29
2518	HZ3	LYS	A	278	69.714	21.484	66.277	429.48
2519	C	LYS	A	278	77.444	19.144	68.047	92.51
2520	O	LYS	A	278	77.574	17.944	68.277	112.10
2521	N	ALA	A	279	78.254	19.944	67.347	91.20
2522	H	ALA	A	279	77.984	20.874	67.107	93.24
2523	CA	ALA	A	279	79.484	19.324	66.847	102.87
2524	CB	ALA	A	279	79.044	18.754	65.497	130.84
2525	C	ALA	A	279	80.674	20.294	66.767	85.63
2526	O	ALA	A	279	80.564	21.474	66.437	88.07
2527	N	TYR	A	280	81.694	19.874	67.517	83.46
2528	H	TYR	A	280	81.854	18.894	67.627	99.32
2529	CA	TYR	A	280	82.844	20.704	67.907	74.10
2530	CB	TYR	A	280	82.924	20.584	69.427	75.19
2531	CG	TYR	A	280	82.224	21.774	70.087	70.60
2532	CD1	TYR	A	280	81.124	21.604	70.917	90.55
2533	HD1	TYR	A	280	80.484	20.754	70.687	153.42
2534	CD2	TYR	A	280	82.814	23.024	69.907	115.44
2535	HD2	TYR	A	280	83.614	23.104	69.177	196.24
2536	CE1	TYR	A	280	80.784	22.654	71.767	94.67
2537	HE1	TYR	A	280	80.094	22.544	72.597	159.15
2538	CE2	TYR	A	280	82.424	24.094	70.697	121.83
2539	HE2	TYR	A	280	82.694	25.114	70.417	209.54
2540	CZ	TYR	A	280	81.474	23.864	71.677	81.04
2541	OH	TYR	A	280	81.474	24.644	72.787	96.39

2542	HH	TYR	A	280	82.124	25.404	72.657	89.42
2543	C	TYR	A	280	84.164	20.284	67.267	77.18
2544	O	TYR	A	280	84.724	19.194	67.397	90.41
2545	N	HIS	A	281	84.844	21.284	66.717	76.08
2546	H	HIS	A	281	84.274	22.004	66.317	82.03
2547	CA	HIS	A	281	86.294	21.364	66.497	81.42
2548	CB	HIS	A	281	86.664	21.654	65.037	87.62
2549	CG	HIS	A	281	88.114	21.544	64.547	139.90
2550	ND1	HIS	A	281	89.254	21.894	65.137	182.70
2551	CD2	HIS	A	281	88.504	20.834	63.497	220.63
2552	CE1	HIS	A	281	90.284	21.354	64.497	276.54
2553	NE2	HIS	A	281	89.824	20.714	63.427	308.51
2554	HE2	HIS	A	281	90.314	20.304	62.647	421.37
2555	C	HIS	A	281	87.244	22.224	67.327	77.47
2556	O	HIS	A	281	88.004	21.664	68.117	89.16
2557	N	GLU	A	282	86.924	23.514	67.437	81.26
2558	H	GLU	A	282	86.064	23.814	67.047	96.46
2559	CA	GLU	A	282	87.524	24.454	68.387	85.10
2560	CB	GLU	A	282	87.494	23.974	69.847	111.72
2561	CG	GLU	A	282	86.094	23.794	70.447	126.40
2562	CD	GLU	A	282	86.144	23.234	71.867	148.22
2563	OE1	GLU	A	282	85.374	23.694	72.737	196.57
2564	OE2	GLU	A	282	86.864	22.214	71.967	186.42
2565	C	GLU	A	282	88.944	24.824	67.947	75.33
2566	O	GLU	A	282	89.244	25.994	67.677	73.79
2567	N	GLN	A	283	89.864	23.914	68.257	76.42
2568	H	GLN	A	283	89.624	23.154	68.857	86.47
2569	CA	GLN	A	283	91.284	24.084	67.927	72.67
2570	CB	GLN	A	283	92.064	23.024	68.707	90.32
2571	CG	GLN	A	283	92.574	23.514	70.057	109.65
2572	CD	GLN	A	283	93.434	24.784	70.037	142.19
2573	OE1	GLN	A	283	93.254	25.674	69.207	196.65
2574	NE2	GLN	A	283	94.224	25.024	71.067	175.26
2575	1HE2	GLN	A	283	94.224	24.314	71.777	188.08
2576	2HE2	GLN	A	283	94.814	25.834	71.167	226.61
2577	C	GLN	A	283	91.564	24.144	66.427	63.78
2578	O	GLN	A	283	92.424	23.414	65.947	78.40
2579	N	LEU	A	284	91.104	25.234	65.817	55.20
2580	H	LEU	A	284	90.484	25.854	66.297	60.14
2581	CA	LEU	A	284	91.244	25.554	64.387	53.66
2582	CB	LEU	A	284	90.074	26.444	63.947	63.24
2583	CG	LEU	A	284	88.684	25.824	64.097	70.59
2584	CD1	LEU	A	284	87.724	27.014	64.127	101.19
2585	CD2	LEU	A	284	88.384	24.984	62.847	124.73
2586	C	LEU	A	284	92.404	26.514	64.137	44.49
2587	O	LEU	A	284	92.614	27.454	64.907	47.56

Table S5. Cartesian coordinates and simulated B-factors for the M-loop of α-tubulin interacting with pironetin in water.

Atom no.	Type	Residue	Chain	Residue no.	X	Y	Z	B-Factor
2475	N	ILE	A	274	93.357	31.216	46.069	63.86
2476	H	ILE	A	274	94.327	31.406	46.159	68.62
2477	CA	ILE	A	274	92.827	29.966	46.629	51.84
2478	CB	ILE	A	274	94.127	29.166	46.769	43.55
2479	CG1	ILE	A	274	93.997	27.746	47.329	57.01
2480	CG2	ILE	A	274	95.187	29.876	47.619	83.04
2481	CD	ILE	A	274	93.597	27.456	48.779	86.41
2482	C	ILE	A	274	91.987	30.096	47.909	51.74
2483	O	ILE	A	274	92.217	31.066	48.629	77.19
2484	N	SER	A	275	90.897	29.346	47.859	54.48
2485	H	SER	A	275	90.597	29.056	46.949	74.77
2486	CA	SER	A	275	89.897	29.336	48.939	58.11
2487	CB	SER	A	275	88.537	29.116	48.269	79.80
2488	OG	SER	A	275	88.277	27.846	47.659	65.11
2489	HG	SER	A	275	87.277	27.806	47.579	71.34
2490	C	SER	A	275	90.197	28.256	49.979	52.44
2491	O	SER	A	275	90.617	27.146	49.659	48.32
2492	N	ALA	A	276	89.797	28.606	51.199	93.45
2493	H	ALA	A	276	89.197	29.406	51.279	124.98
2494	CA	ALA	A	276	90.037	27.856	52.439	138.81
2495	CB	ALA	A	276	89.747	28.816	53.599	244.51
2496	C	ALA	A	276	89.337	26.506	52.539	147.33
2497	O	ALA	A	276	89.917	25.506	52.949	180.23
2498	N	GLU	A	277	88.047	26.416	52.219	148.47
2499	H	GLU	A	277	87.477	27.206	51.969	142.66
2500	CA	GLU	A	277	87.307	25.156	52.279	195.98
2501	CB	GLU	A	277	85.797	25.246	52.079	233.15
2502	CG	GLU	A	277	85.207	25.806	53.379	432.17
2503	CD	GLU	A	277	85.267	24.866	54.579	549.10
2504	OE1	GLU	A	277	84.237	24.156	54.649	637.46
2505	OE2	GLU	A	277	86.357	24.716	55.169	667.45
2506	C	GLU	A	277	87.787	24.126	51.249	183.62
2507	O	GLU	A	277	87.907	22.946	51.569	265.86
2508	N	LYS	A	278	87.877	24.526	49.979	148.63
2509	H	LYS	A	278	87.887	25.486	49.689	150.99
2510	CA	LYS	A	278	88.127	23.566	48.899	170.98
2511	CB	LYS	A	278	87.417	24.076	47.639	136.68
2512	CG	LYS	A	278	85.887	24.026	47.749	191.69
2513	CD	LYS	A	278	85.107	24.526	46.529	210.11
2514	CE	LYS	A	278	83.617	24.516	46.849	324.04
2515	NZ	LYS	A	278	82.957	24.926	45.599	402.84
2516	HZ1	LYS	A	278	83.227	24.286	44.889	452.43
2517	HZ2	LYS	A	278	81.967	24.816	45.689	461.71
2518	HZ3	LYS	A	278	83.047	25.896	45.379	458.61
2519	C	LYS	A	278	89.507	22.916	48.789	232.07
2520	O	LYS	A	278	89.907	22.236	49.729	225.11
2521	N	ALA	A	279	90.157	23.076	47.629	347.94
2522	H	ALA	A	279	90.007	23.936	47.149	393.60
2523	CA	ALA	A	279	91.427	22.476	47.189	478.79
2524	CB	ALA	A	279	91.367	22.516	45.669	715.76
2525	C	ALA	A	279	92.627	23.266	47.719	394.45
2526	O	ALA	A	279	92.567	24.496	47.719	357.06
2527	N	TYR	A	280	93.747	22.576	47.959	418.56
2528	H	TYR	A	280	93.747	21.606	47.729	514.00
2529	CA	TYR	A	280	95.017	23.196	48.359	365.93
2530	CB	TYR	A	280	95.227	23.066	49.869	354.56
2531	CG	TYR	A	280	94.387	24.026	50.719	305.45
2532	CD1	TYR	A	280	93.177	23.706	51.329	329.65
2533	HD1	TYR	A	280	92.837	22.676	51.259	390.01
2534	CD2	TYR	A	280	95.157	25.046	51.269	338.44
2535	HD2	TYR	A	280	96.207	25.106	51.019	409.69
2536	CE1	TYR	A	280	92.727	24.396	52.449	368.07
2537	HE1	TYR	A	280	91.827	24.066	52.979	462.30
2538	CE2	TYR	A	280	94.657	25.786	52.339	369.93
2539	HE2	TYR	A	280	95.057	26.736	52.699	461.03
2540	CZ	TYR	A	280	93.477	25.426	52.989	374.85
2541	OH	TYR	A	280	93.067	26.006	54.149	482.70

2542	HH	TYR	A	280	93.727	26.736	54.329	559.32
2543	C	TYR	A	280	96.227	22.496	47.719	345.45
2544	O	TYR	A	280	96.377	21.276	47.769	352.25
2545	N	HIS	A	281	97.127	23.326	47.219	341.60
2546	H	HIS	A	281	96.867	24.296	47.169	359.76
2547	CA	HIS	A	281	98.447	22.996	46.659	334.58
2548	CB	HIS	A	281	98.417	23.306	45.159	377.87
2549	CG	HIS	A	281	99.797	23.316	44.509	371.83
2550	ND1	HIS	A	281	100.297	24.386	43.889	314.02
2551	CD2	HIS	A	281	100.687	22.336	44.359	531.21
2552	CE1	HIS	A	281	101.407	24.046	43.239	427.98
2553	NE2	HIS	A	281	101.637	22.766	43.529	561.22
2554	HE2	HIS	A	281	102.487	22.296	43.289	722.03
2555	C	HIS	A	281	99.547	23.586	47.549	304.77
2556	O	HIS	A	281	100.447	22.816	47.909	314.14
2557	N	GLU	A	282	99.407	24.856	47.909	297.11
2558	H	GLU	A	282	98.697	25.356	47.409	323.39
2559	CA	GLU	A	282	100.197	25.636	48.879	281.16
2560	CB	GLU	A	282	100.347	25.016	50.259	367.09
2561	CG	GLU	A	282	99.047	25.236	51.039	369.54
2562	CD	GLU	A	282	99.267	24.726	52.469	507.72
2563	OE1	GLU	A	282	99.777	25.476	53.339	608.01
2564	OE2	GLU	A	282	98.837	23.606	52.789	616.37
2565	C	GLU	A	282	101.597	26.076	48.449	192.59
2566	O	GLU	A	282	101.817	27.266	48.669	179.53
2567	N	GLN	A	283	102.367	25.086	48.009	184.40
2568	H	GLN	A	283	102.167	24.106	48.039	234.45
2569	CA	GLN	A	283	103.777	25.446	47.769	154.73
2570	CB	GLN	A	283	104.627	24.196	47.959	269.05
2571	CG	GLN	A	283	104.617	23.766	49.419	343.93
2572	CD	GLN	A	283	105.337	24.646	50.449	513.83
2573	OE1	GLN	A	283	105.677	25.796	50.199	623.01
2574	NE2	GLN	A	283	105.507	24.026	51.619	673.43
2575	1HE2	GLN	A	283	105.117	23.126	51.809	673.78
2576	2HE2	GLN	A	283	105.847	24.496	52.429	868.75
2577	C	GLN	A	283	103.927	26.056	46.379	113.67
2578	O	GLN	A	283	103.697	25.386	45.369	129.09
2579	N	LEU	A	284	104.067	27.376	46.389	108.01
2580	H	LEU	A	284	104.197	27.926	47.209	155.56
2581	CA	LEU	A	284	104.117	28.266	45.219	80.61
2582	CB	LEU	A	284	102.797	29.036	45.189	97.93
2583	CG	LEU	A	284	101.457	28.306	45.229	119.39
2584	CD1	LEU	A	284	100.427	29.426	45.419	194.39
2585	CD2	LEU	A	284	101.177	27.596	43.899	156.14
2586	C	LEU	A	284	105.267	29.266	45.389	66.26
2587	O	LEU	A	284	105.337	30.166	46.219	83.20

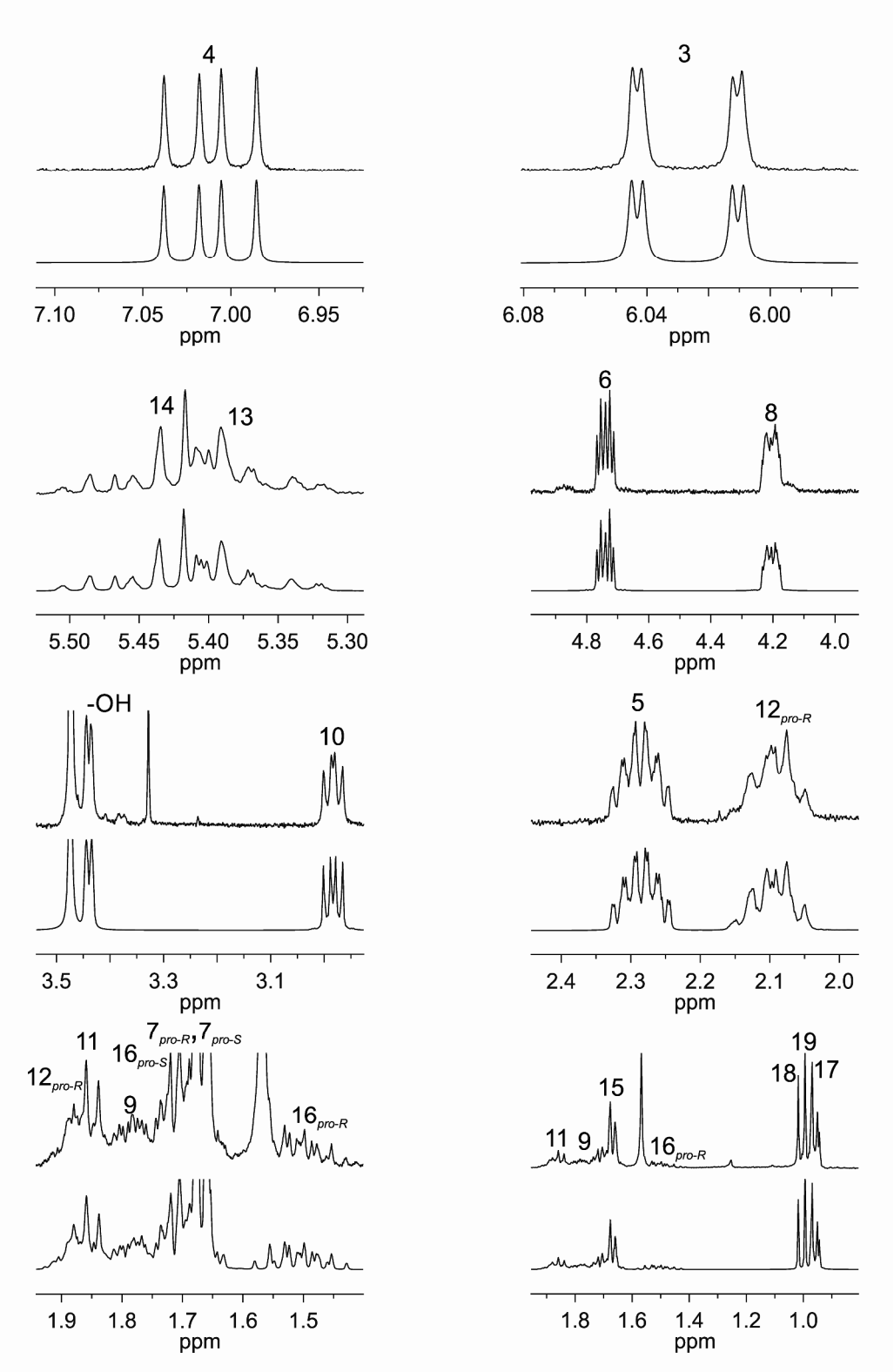

Figure S1. Comparison between the complete experimental ^{1}H NMR spectrum of pironetin in $CDCl_3$ at 300 MHz (upper traces) and the simulated spectrum (lower traces) obtained with RMS of 0.37 Hz.

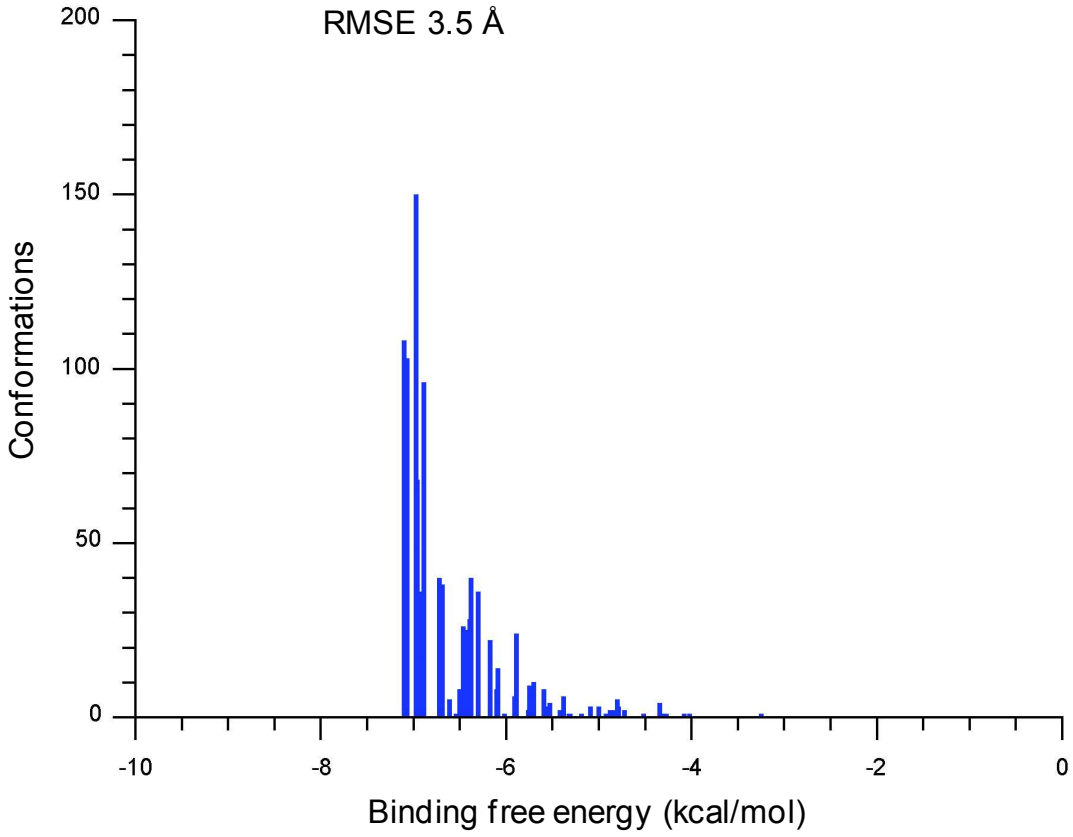

Figure S2. Conformational clustering histogram for the pironetin-tubulin complexes obtained after 1000 independent simulations.

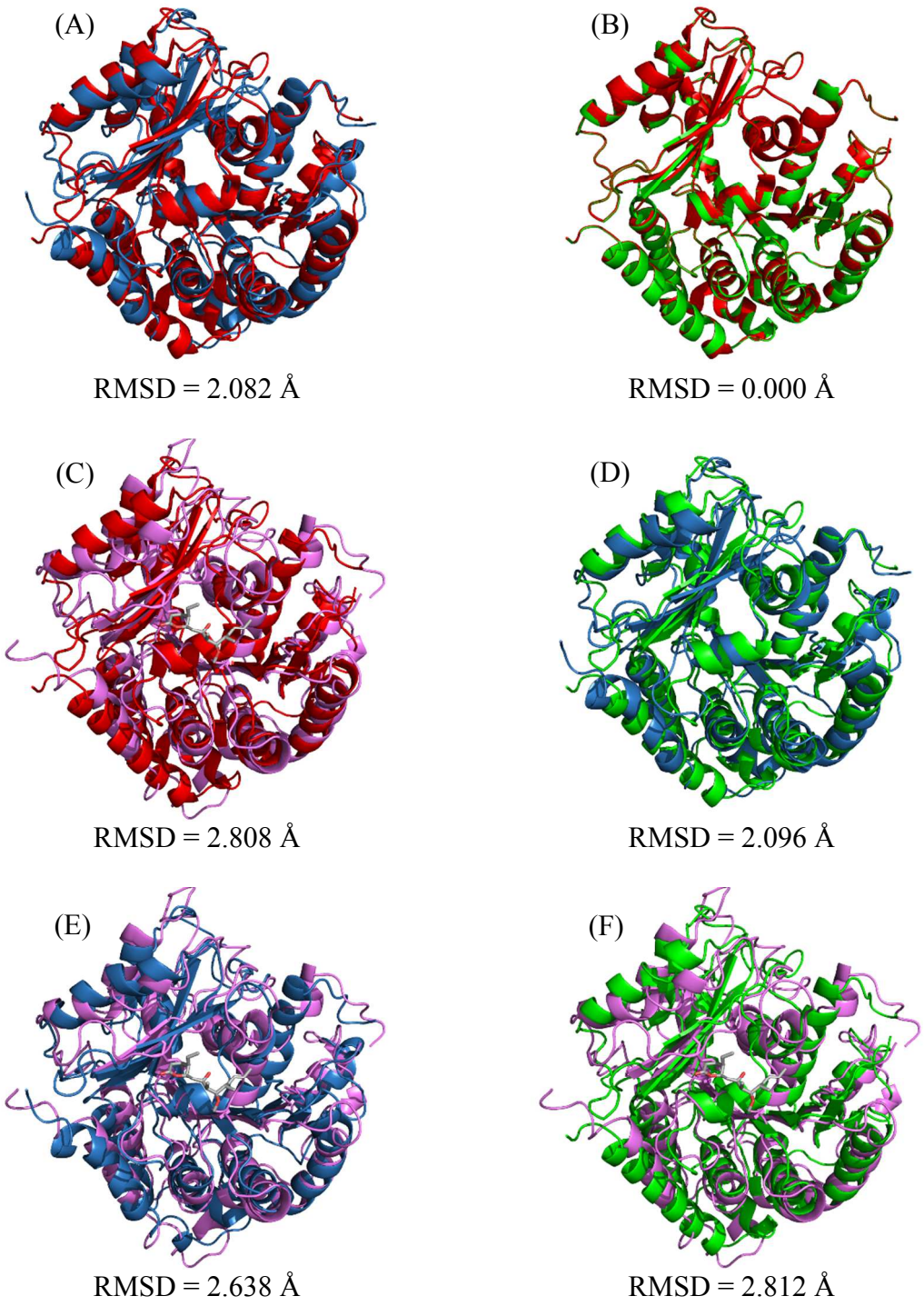

Figure S3. Superimposed structures and RMSD values of α-tubulin models. (A) The crystallographic model (red) and the minimized model without the ligand (blue). (B) The crystallographic model (red) and the protein model after docking (green). (C) The crystallographic model (red) and the minimized model with pironetin bonded to αLys352 (violet). (D) The minimized model without the ligand (blue) and the protein model after docking (green). (E) The minimized model without the ligand (blue) and the minimized model with pironetin bonded to αLys352 (violet). (F) The protein model after docking (green) and the minimized model with pironetin bonded to αLys352 (violet).

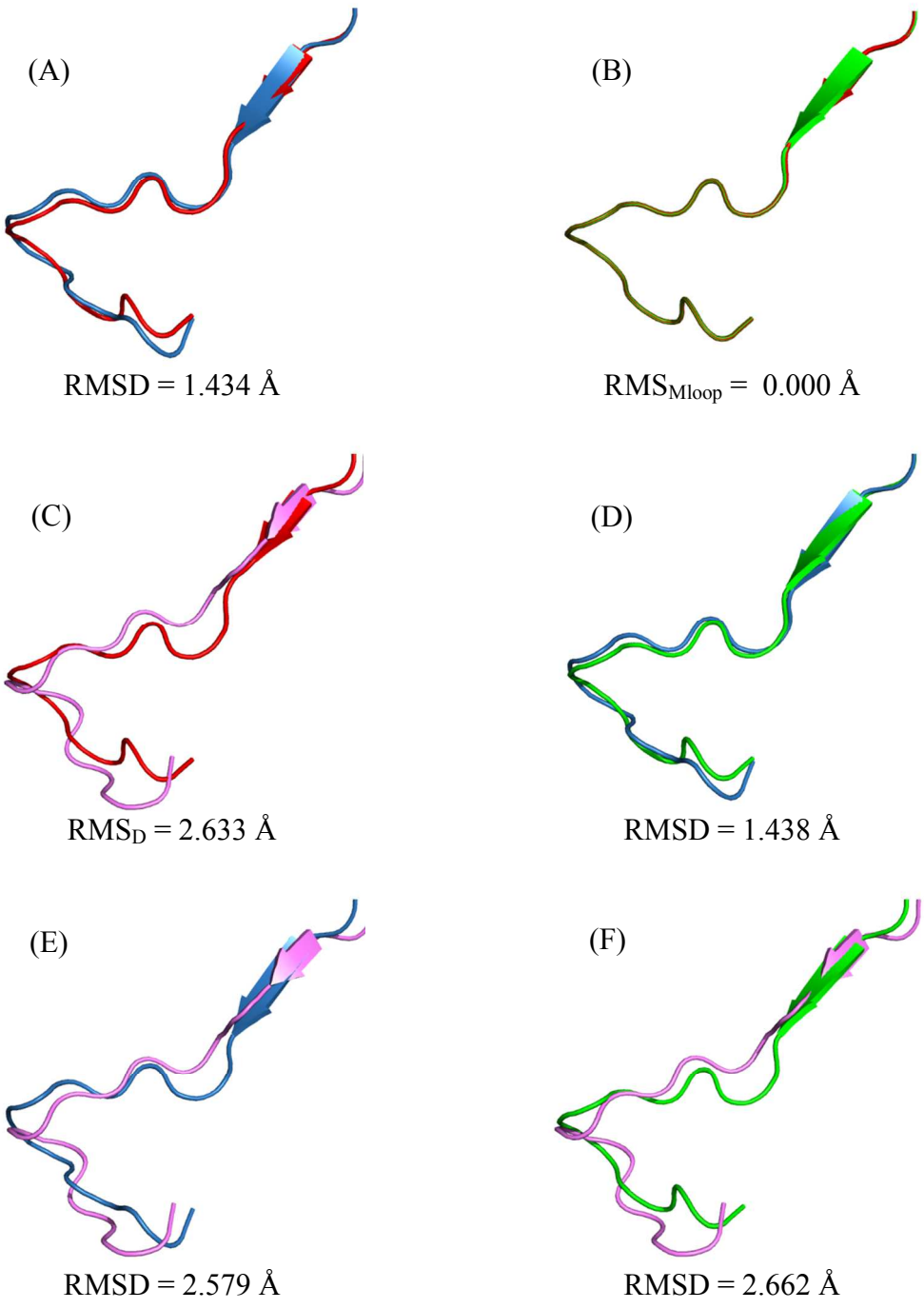

Figure S4. Superimposed structures and RMSD values for α-tubulin M-loop. (A) The crystallographic model (red) and the minimized model without the ligand (blue). (B) The crystallographic model (red) and the M-loop after docking (green). (C) The crystallographic model (red) and the minimized model with pironetin bonded to αLys352 (violet). (D) The minimized model without the ligand (blue) and the M-loop after docking (green). (E) The minimized model without the ligand (blue) and the minimized model with pironetin bonded to αLys352 (violet). (F) The M-loop after docking (green) and the minimized model with pironetin bonded to αLys352 (violet).

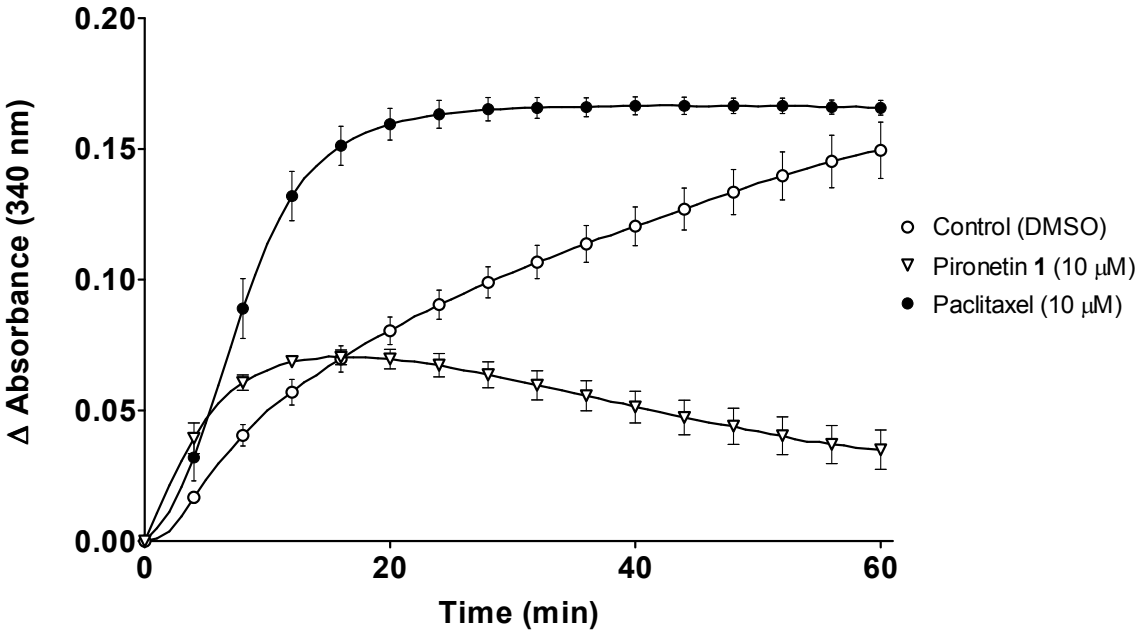

Figure S5. Inhibition of tubulin polymerization by pironetin in comparison with the control and paclitaxel.

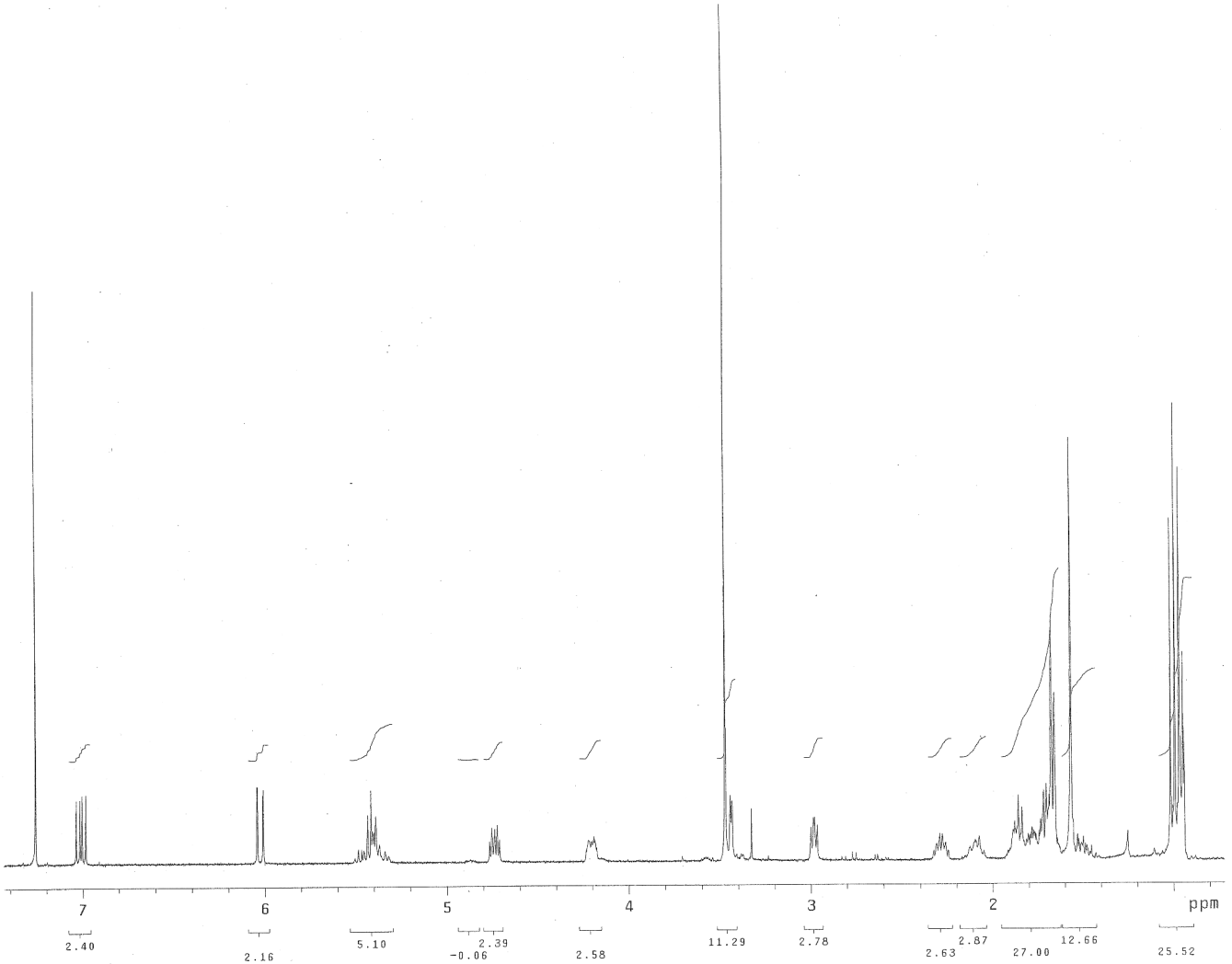

Figure S6. ^{1}H NMR spectrum of pironetin (**1**) in $CDCl_3$ (300 MHz).

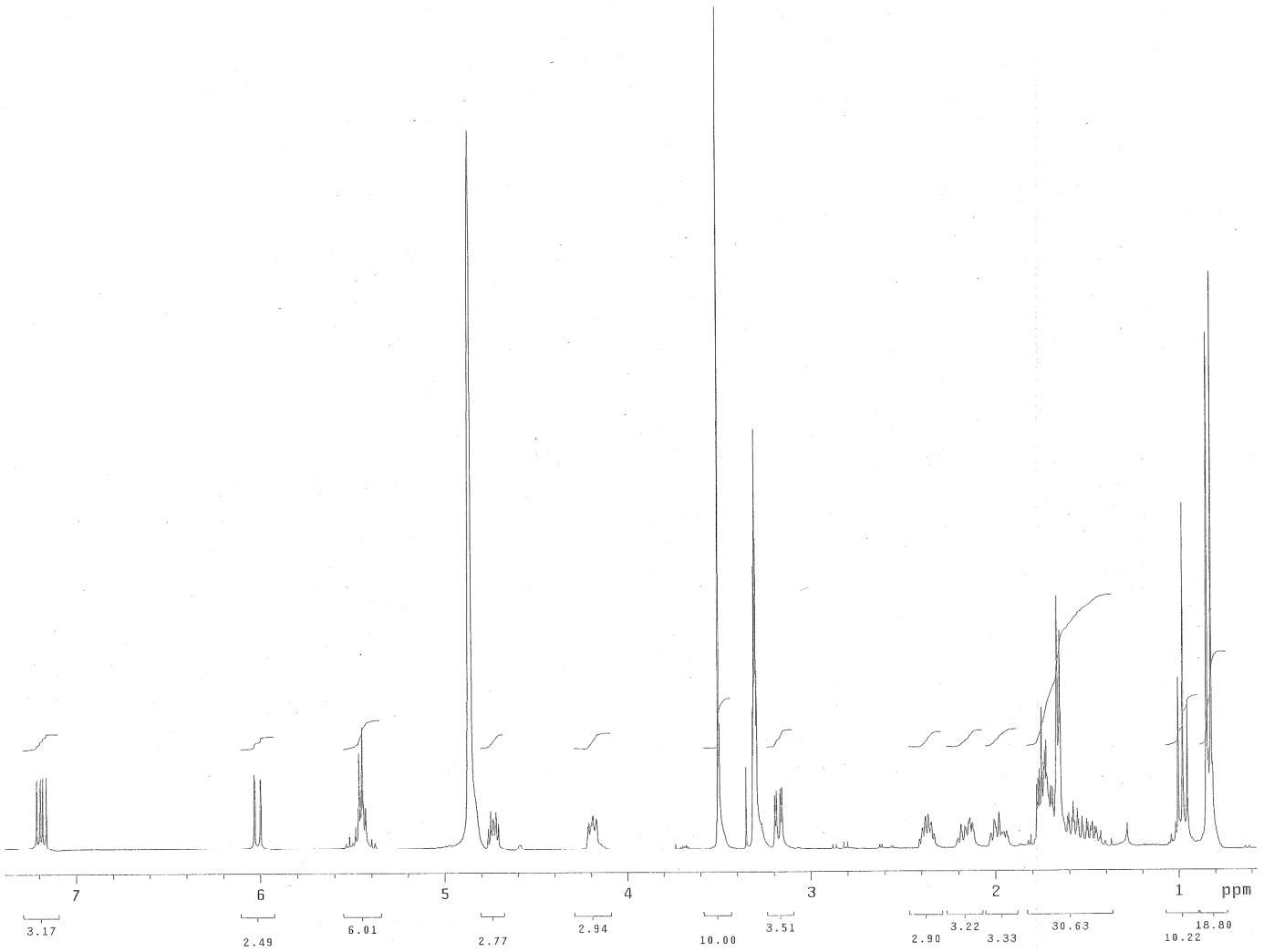

Figure S7. ^{1}H NMR spectrum of pironetin (**1**) in CD_3OD (300 MHz).

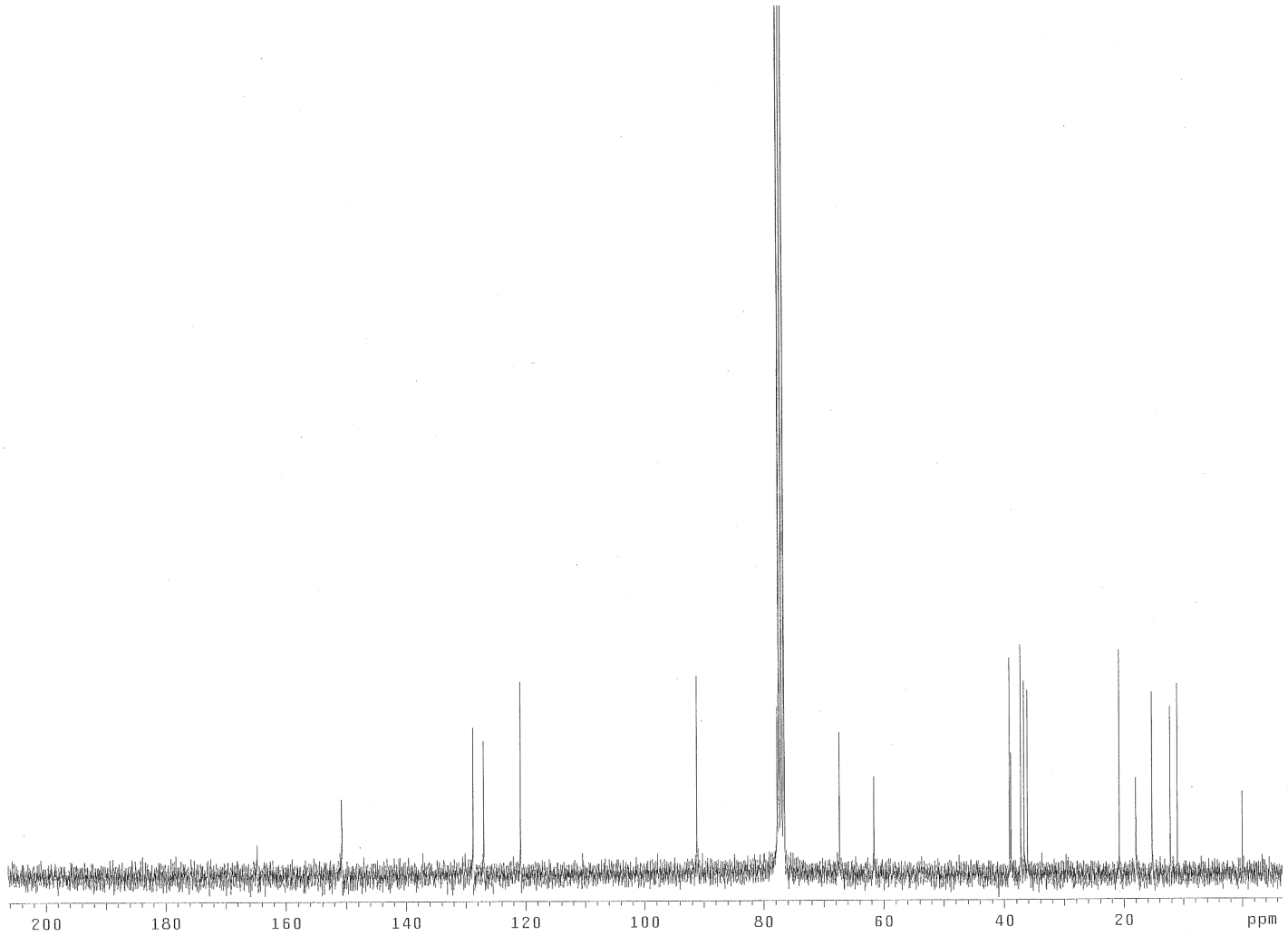

Figure S8.[13]C NMR spectrum of pironetin (**1**) in $CDCl_3$ (75.4 MHz).

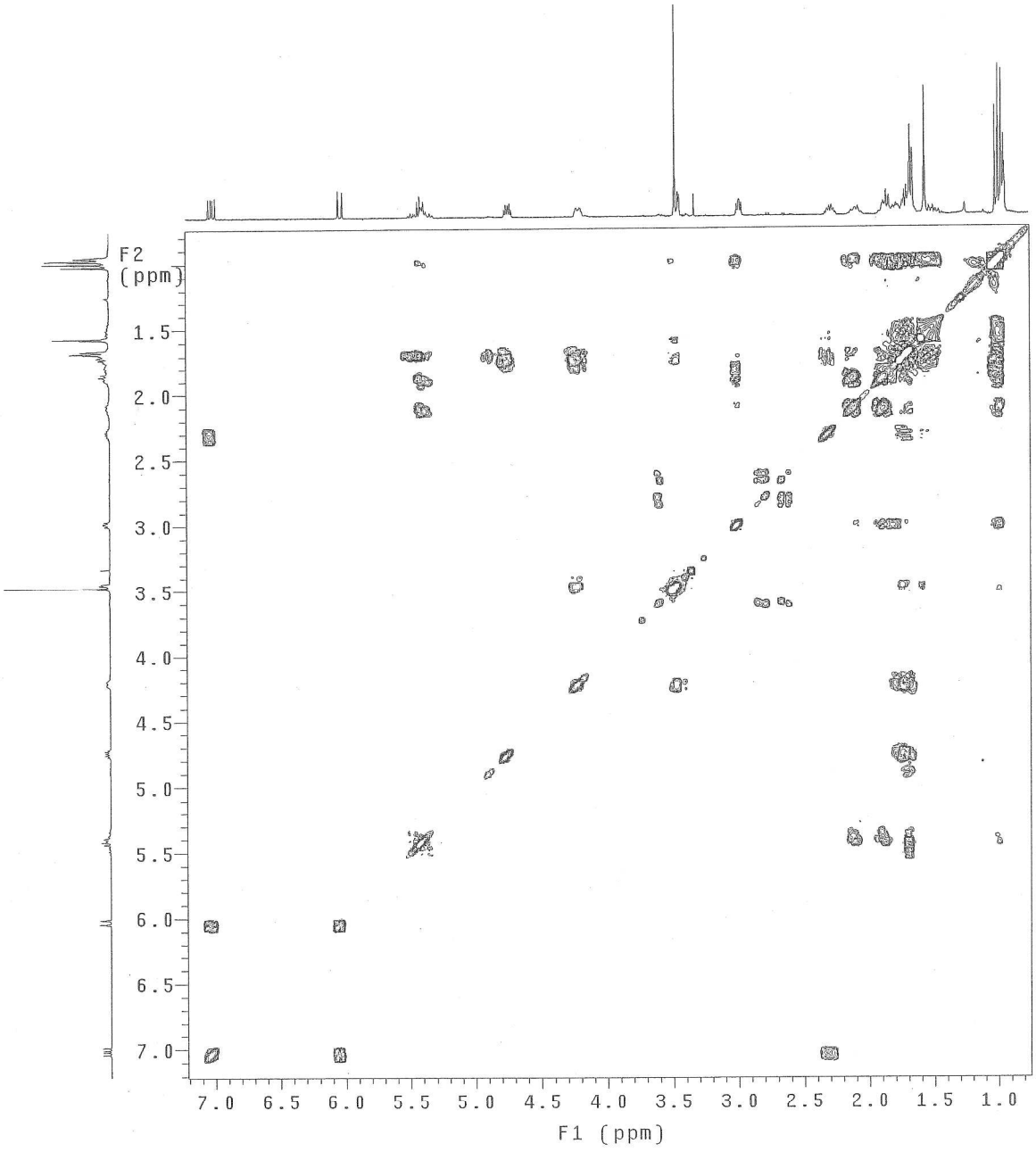

Figure S9. COSY spectrum of pironetin (**1**) in $CDCl_3$.

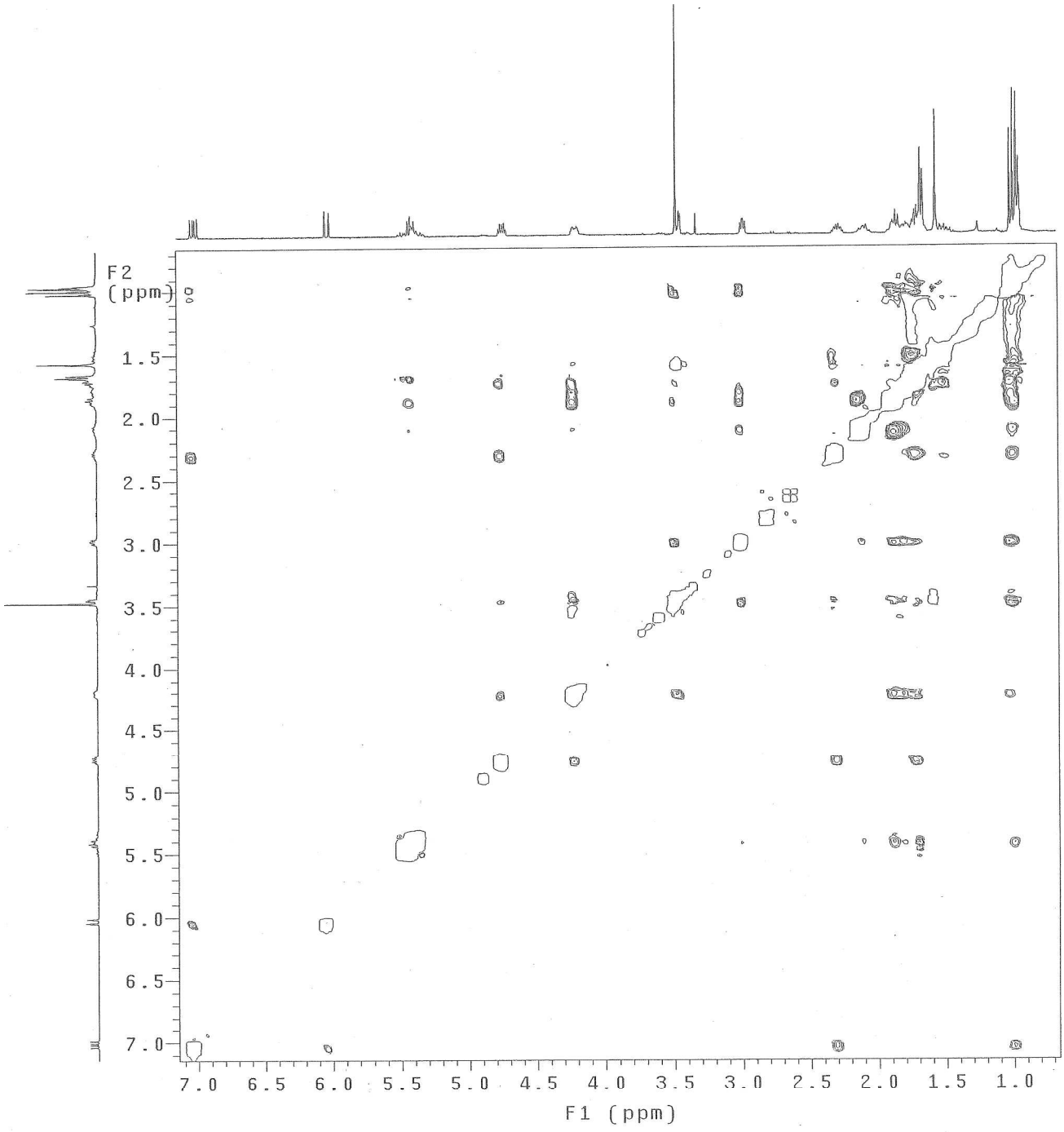

Figure S10. NOESY spectrum of pironetin (**1**) in $CDCl_3$.

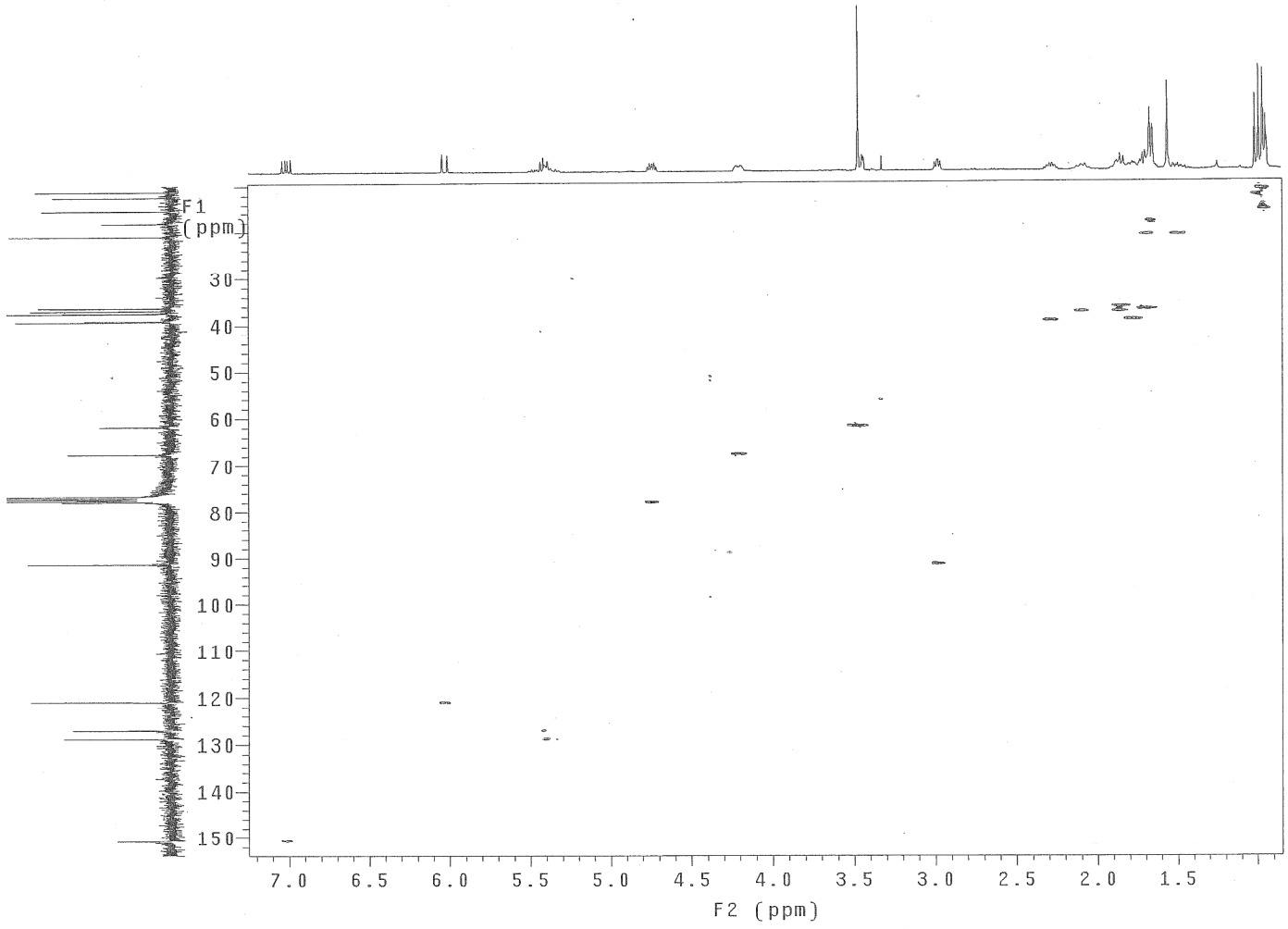

Figure S11. *g*HSQC spectrum of pironetin (**1**) in $CDCl_3$.

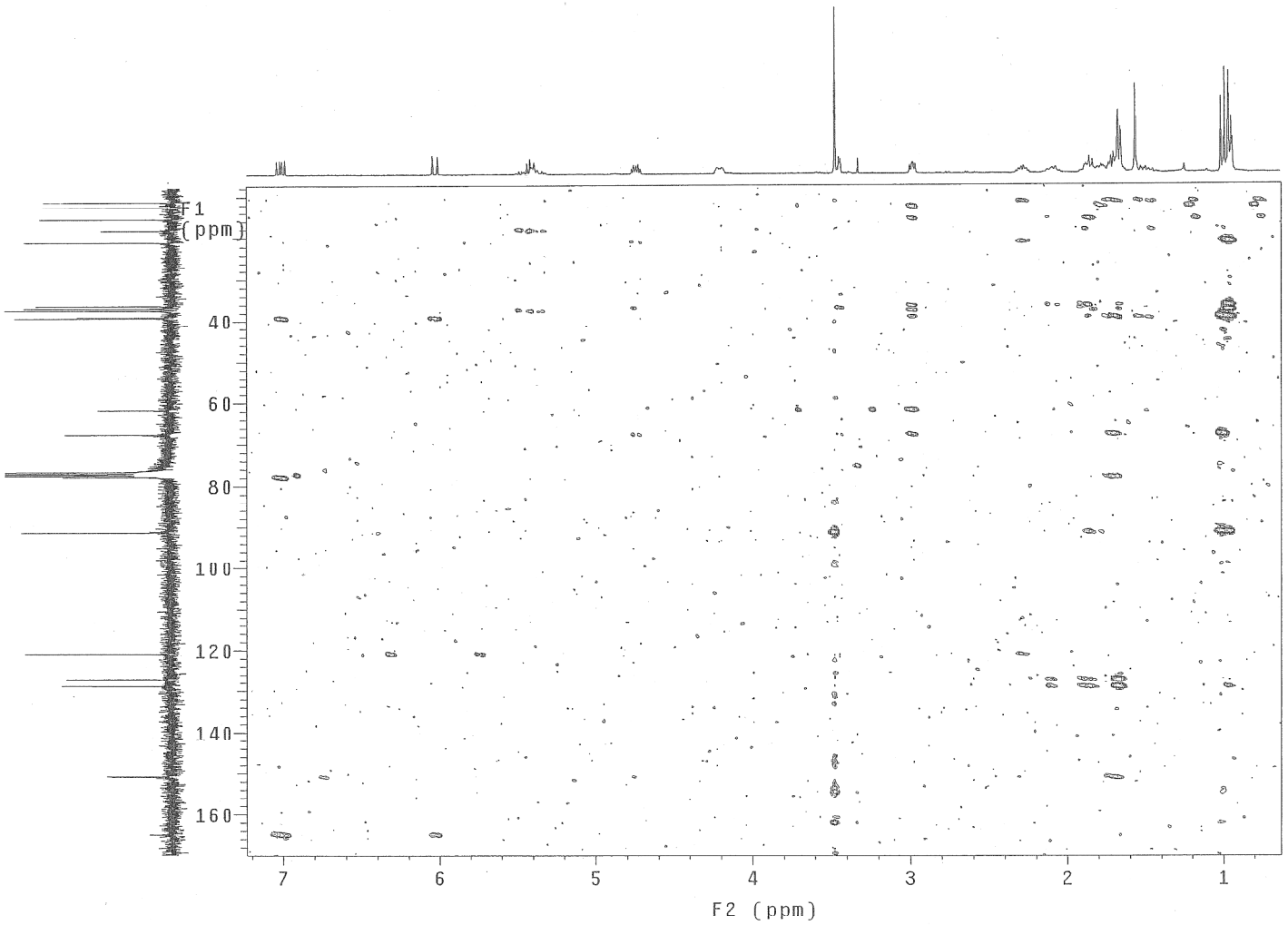

Figure S12. *g*HMBC spectrum of pironetin (**1**) in $CDCl_3$.