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Supplementary Data

Iridoid esters from *Valeriana pavonii* Poepp. & Endl. as $GABA_A$ modulators: Structural insights in their binding mode and structure-activity relationship

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SUPPLEMENTARY DATA

1. Identification and structural elucidation, compound 1 of *V. pavonii.*

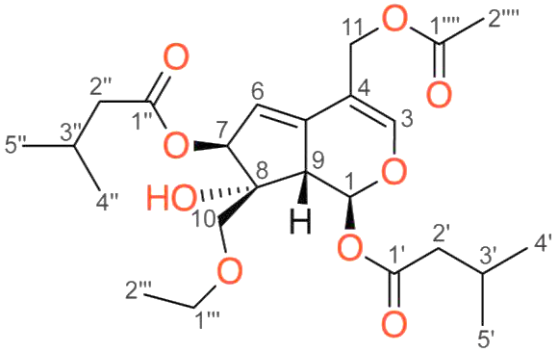

Figure S1. Structure of compound **1**.

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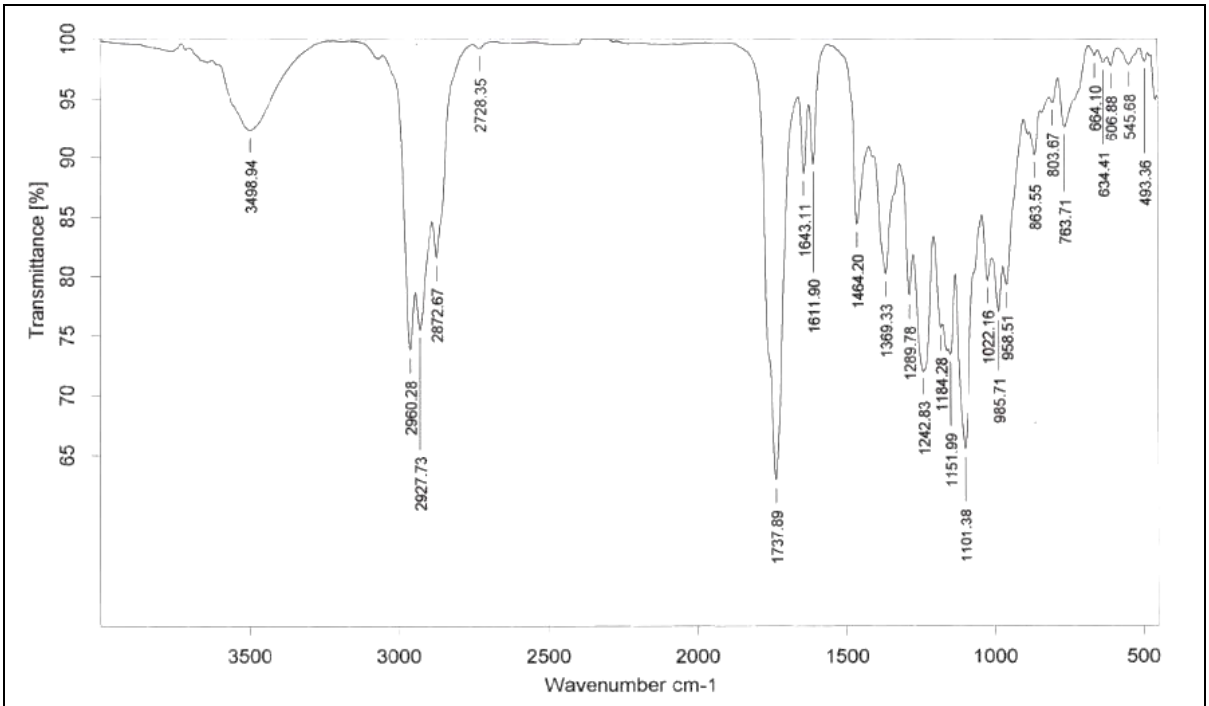
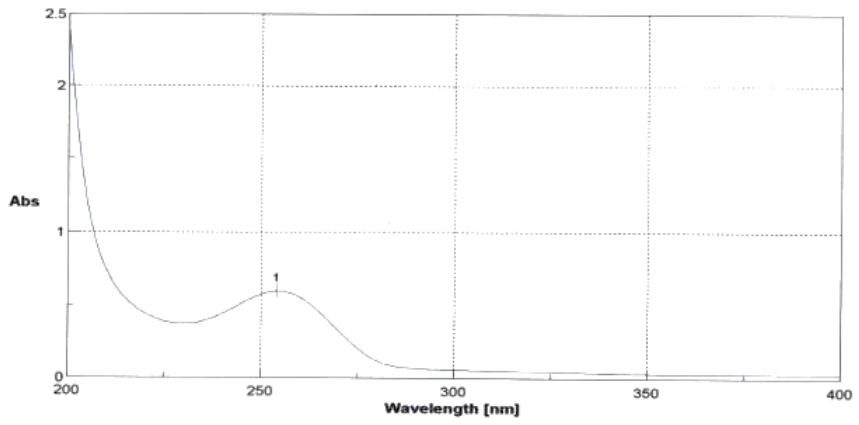


Figure S2. IR spectrum, compound **1** of *V. pavonii.*



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Response	Fast
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Data pitch	1nm
Scanning speed	40nm/min
Sample ID	153
No. of cycle	1
Sample name	
Operator	Jesús Trujillo Vázquez
Comment	

No.	nm	Abs
1	254	0.59612

Figure S3. UV spectrum, compound **1** of *V. pavonii.*

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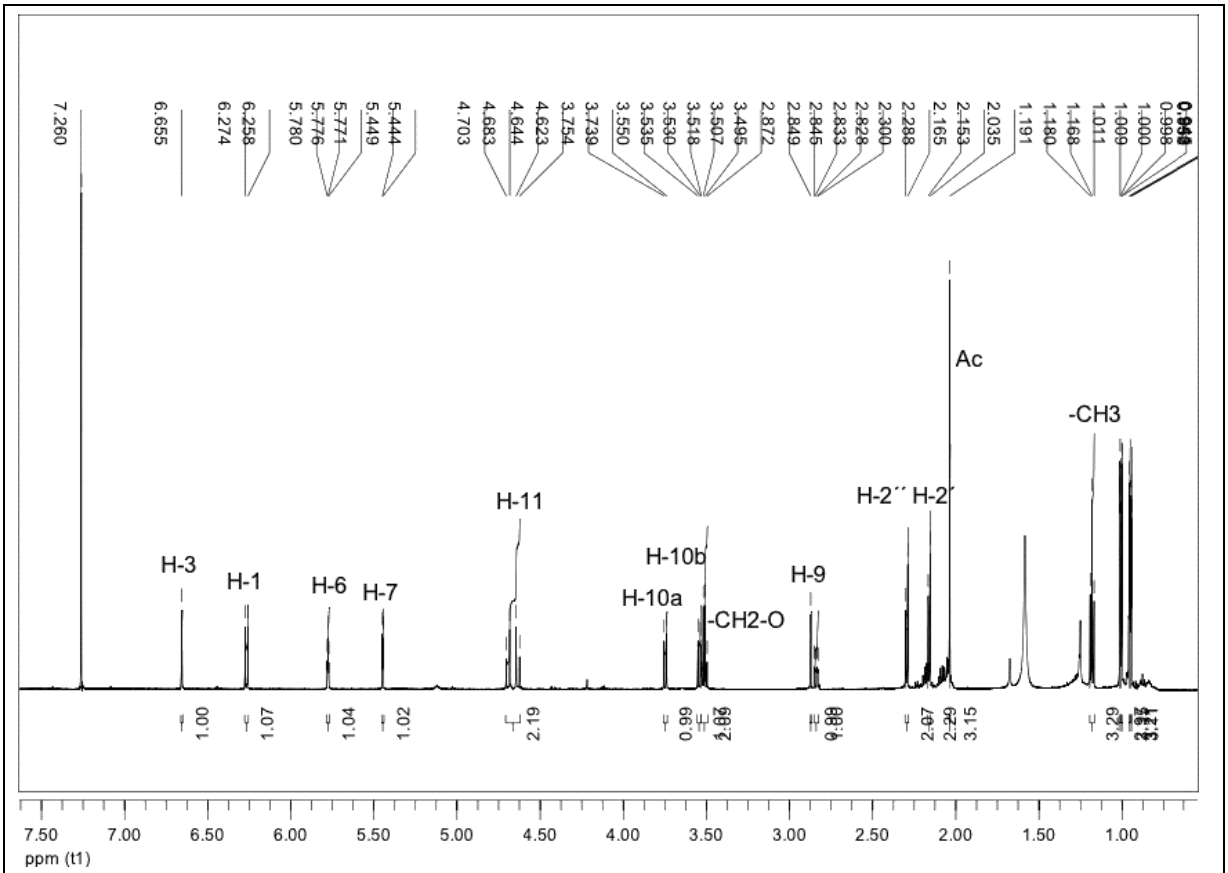

Figure S4. NMR spectrum ^{1}H (600 MHz, $CDCl_3$), compound **1** of *V. pavonii.*

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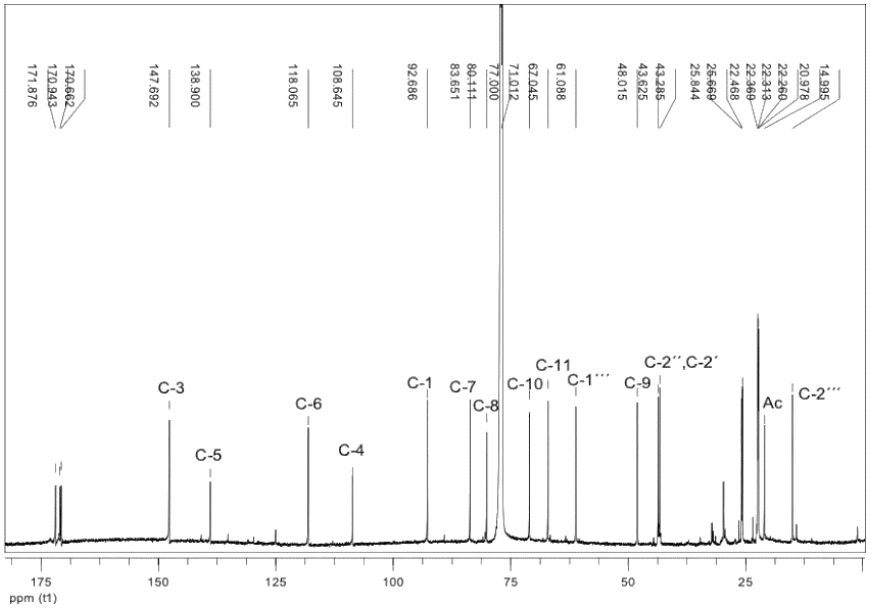

Figure S5. NMR spectrum ^{13}C (150 MHz, $CDCl_3$), compound **1** of *V. pavonii.*

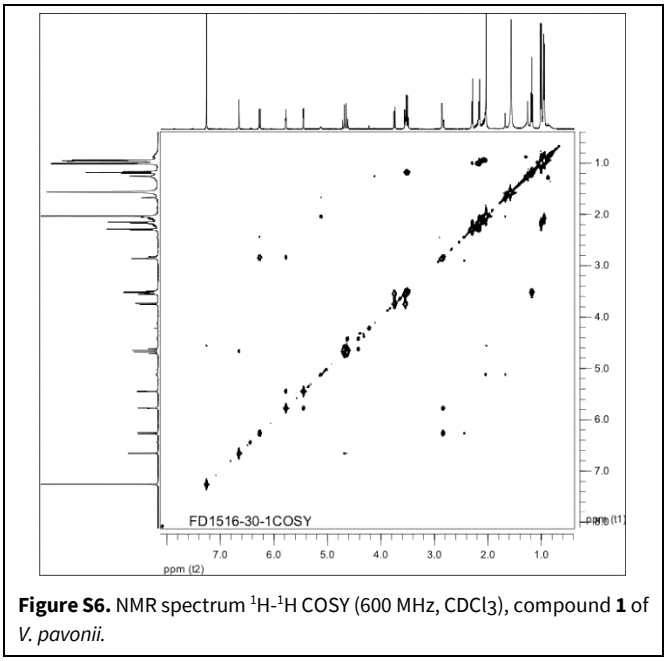

Figure S6. NMR spectrum ^{1}H-^{1}H COSY (600 MHz, $CDCl_3$), compound **1** of *V. pavonii.*

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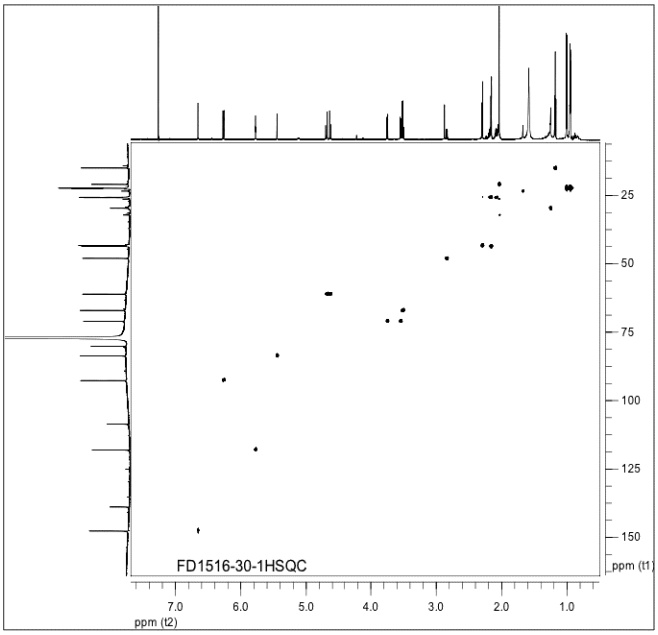

Figure S7. HSQC spectrum (600 MHz, $CDCl_3$), compound **1** of *V. pavonii.*

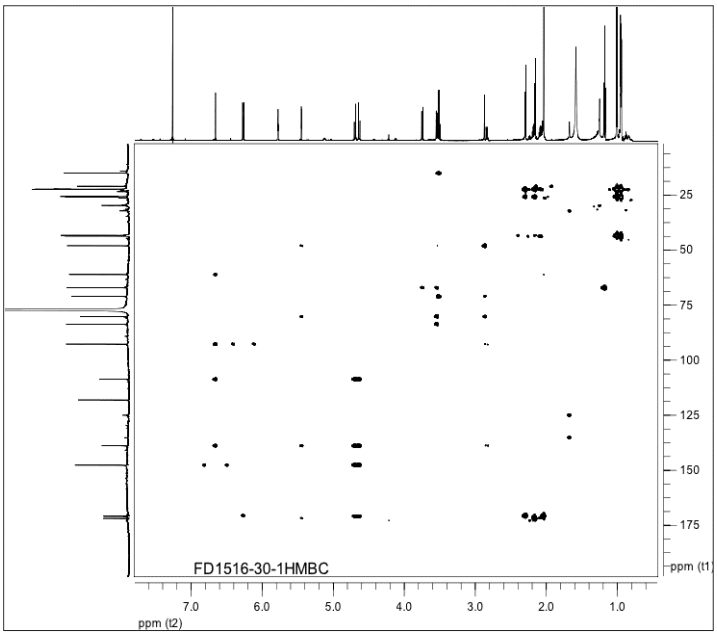

Figure S8. HMBC spectrum (600 MHz, $CDCl_3$), compound **1** of *V. pavonii.*

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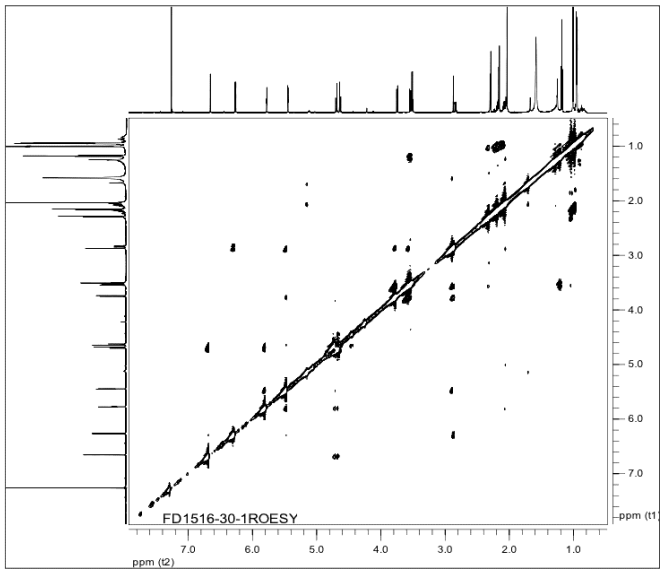

Figure S9. NMR spectrum ^{1}H-^{1}H ROESY (600 MHz, $CDCl_3$), compound **1** of *V. pavonii.*

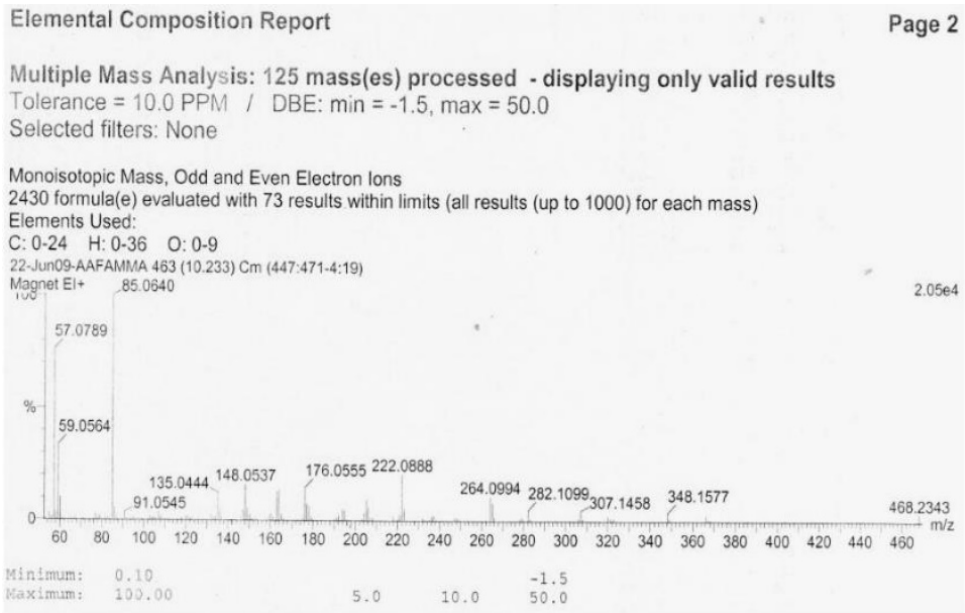

Figure S10. Mass spectrum (EI), compound **1** of *V. pavonii.*

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Supplementary Data

Table S1. ^{1}H (600 MHz) and ^{13}C-NMR (150 MHz) spectroscopic data for compound **1** (in $CDCl_3$) and valerjatadoid A.

Compound 1			**Valerjatadoid A***	
Position	**1_H**	**13_C**	**1_H**	**13_C**
1	6.24 (d, J =10.0, 1H)	92.5	6.29 (d, J=10.0, 1H)	92.7
3	6.67 (s, 1H)	148.0	6.68 (s, 1H)	147.7
4		108.7		108.7
5		139.0		139.0
6	5.77 (t, J=2.6, 1H)	117.5	5.80 (s, 1H)	118.1
7	5.44 (d, J=2.8Hz, 1H)	83.6	5.47 (d, J=2.8, 1H)	83.7
8		80.1		80.2
9	2.84 (dd, J=2.5, 2.5, 1H)	48.0	2.86 (dd, J=10.0, 2.6, 1H)	48.1
10	3.54, 3.74 (2 d, J=9.1, 2H)	71.01	3.57, 3.77 (2 d, J=9.1, 2H)	71.1
11	4.63, 4.69 (2 d, J=12.3, 2H)	61.1	4.66, 4.72 (d, J=12.4, 2H)	61.1
R^1 – 1´		170.6		170.7
2´	2.29 (d, J=7.6, 2H)	43.2	2.31–2.32 (m, 2H)	43.3
3´	2.16 (m, 1H)	25.6	2.09–2.13 (m, 1H)	25.7
4´	0.99 (d, J=1.2, 3H)	22.3	1.02 (d, J=1.3, 3H)	22.3
5´	1.01 (d, J=1.2, 3H)	22.4	1.03 (d, J=1.3, 3H)	22.3
R^7 – 1´´		171.8		170.9
2´´	2.15 (d, J=7.3, 2H)	43.6	2.18–2.19 (m, 2H)	43.7
3´´	2.07 (m, 1H)	25.8	2.09–2.13 (m, 1H)	25.8
4´´	0.94 (d, J=2.3, 3H)	22.2	0.97 (d, J=2.3, 3H)	22.4
5´´	0.95 (d, J=2.3, 3H)	22.3	0.98 (d, J=2.3, 3H)	22.5
R^{10} – 1´´´	3.51 (q, J=7.0, 2H)	67.0	3.53, 3.55 (2 d, J=7.0, 2H)	67.1
2´´´	1.18 (t, J=7.0, 3H)	15.0	1.20 (t, J=7.0, 3H)	15.0
R^{11} – 1´´´´		170.9		171.9
2´´´´	2.03 (s, 3H)	20.9	2.06 (s, 3H)	20.9

* ^{1}H-NMR (at 600 MHz in $CDCl_3$); ^{13}C-NMR (at 150 MHz). (Yang et al., 2015). δ in ppm, J in Hz.

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Supplementary Data

2. Identification and structural elucidation, compound 2 of *V. pavonii.*

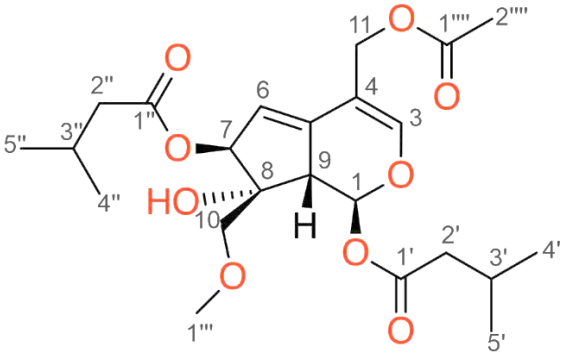

Figure S11. Structure of compound **2**.

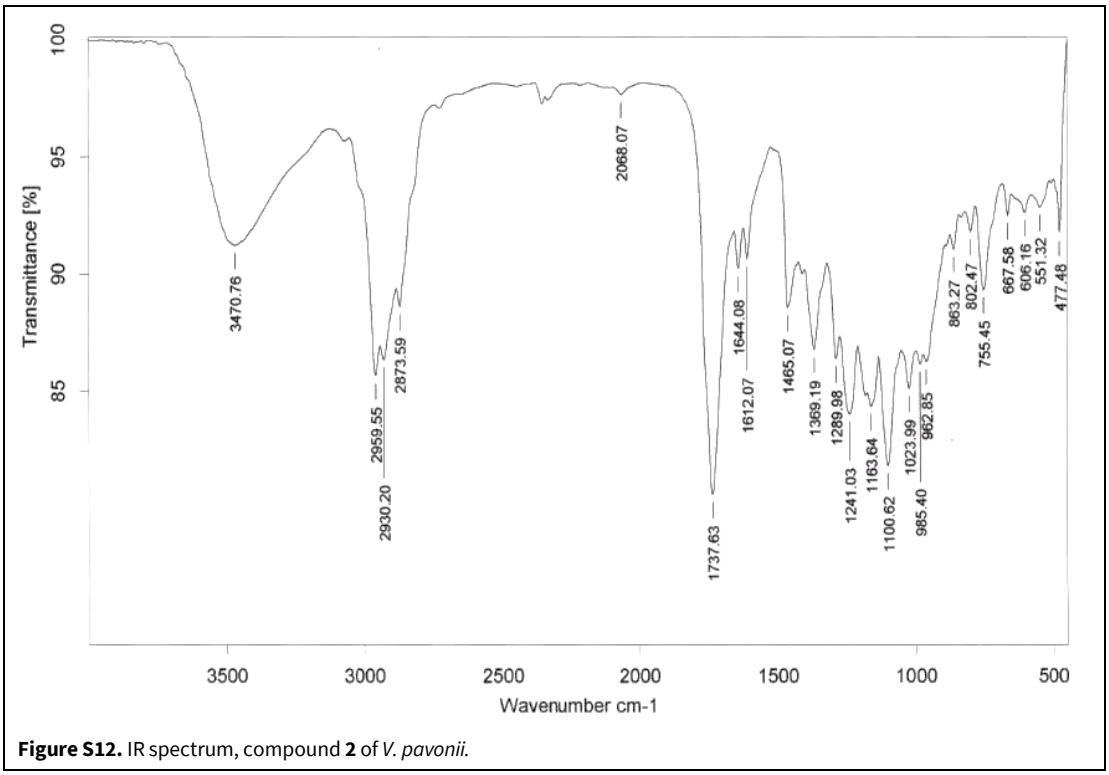

Figure S12. IR spectrum, compound **2** of *V. pavonii.*

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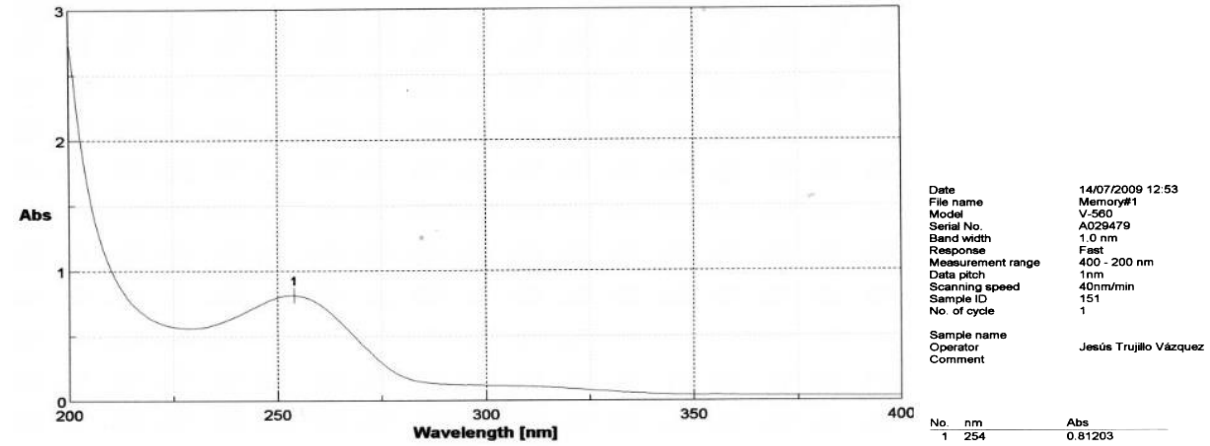

Figure S13. UV spectrum, compound **2** of *V. pavonii.*

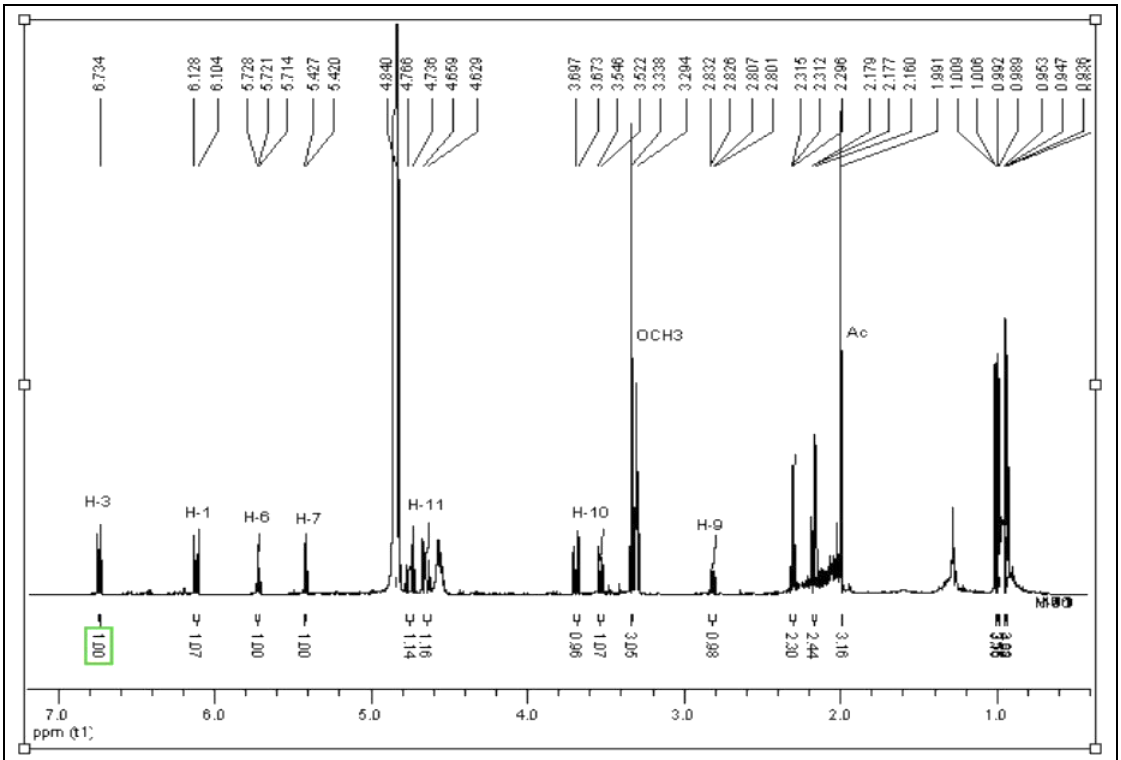

Figure S14. NMR spectrum ^{1}H (400 MHz, MeOD), compound **2** of *V. pavonii*.

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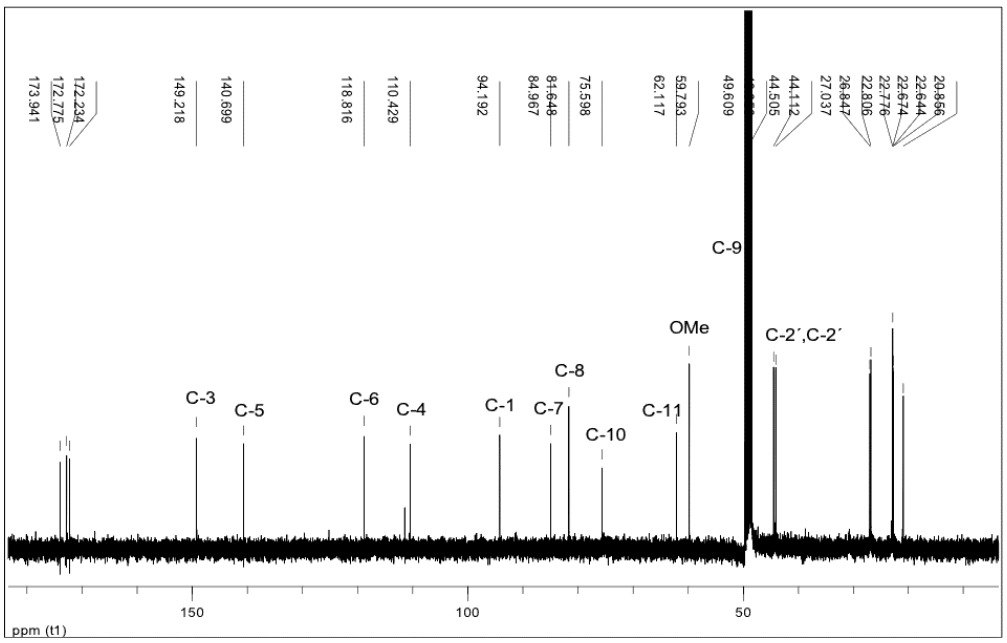

Figure S15. NMR spectrum ^{13}C (100 MHz, MeOD), compound **2** of *V. pavonii*.

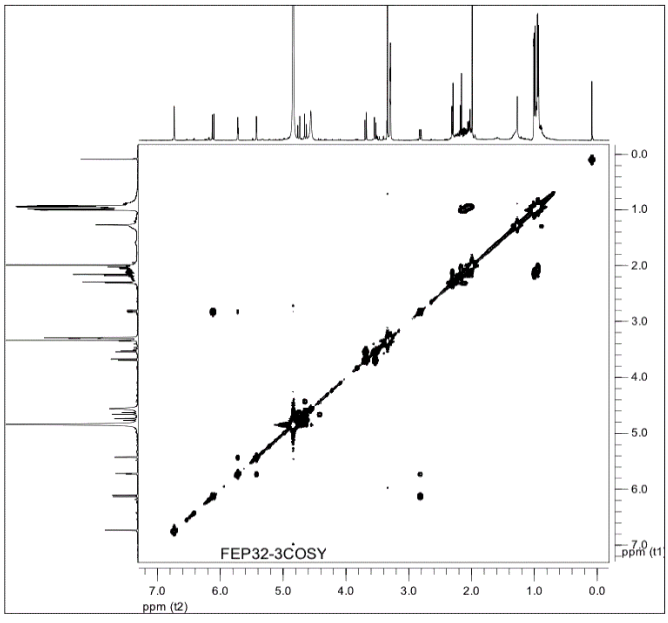

Figure S16. NMR spectrum ^{1}H-^{1}H COSY (400 MHz, MeOD), compound **2** of *V. pavonii.*

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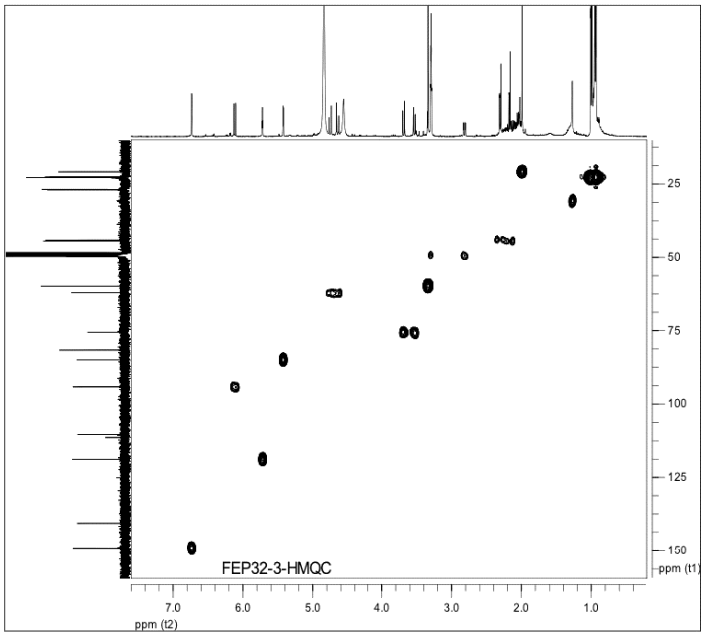

Figure S17. NMR spectrum HMQC (400 MHz, MeOD), compound **2** de *V. pavonii.*

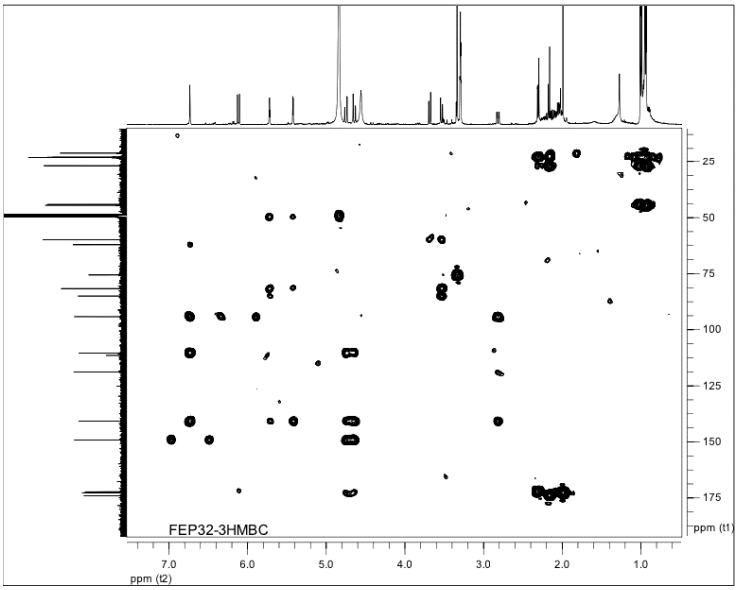

Figure S18. NMR spectrum HMBC (400 MHz, MeOD), compound **2** of *V. pavonii.*

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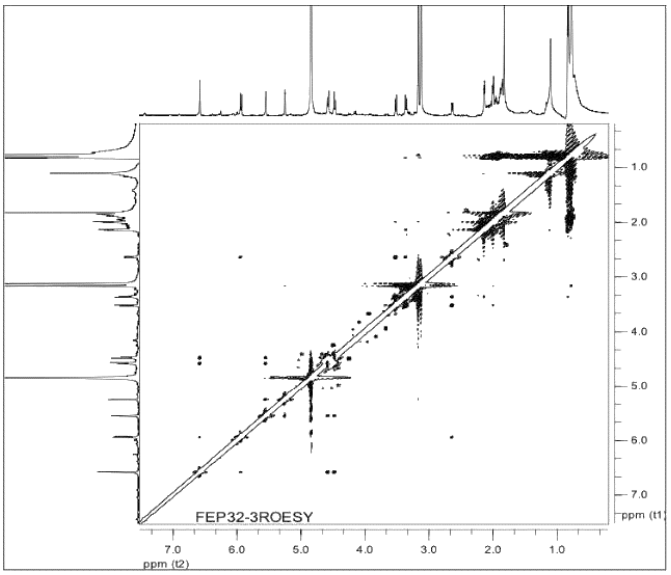

Figure S19. NMR spectrum ^{1}H-^{1}H ROESY (400 MHz, MeOD), compound **2** of *V. pavonii.*

Elemental Composition Report Page 1

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Selected filters: None

Monoisotopic Mass, Odd and Even Electron Ions
3119 formula(e) evaluated with 73 results within limits (all results (up to 1000) for each mass)
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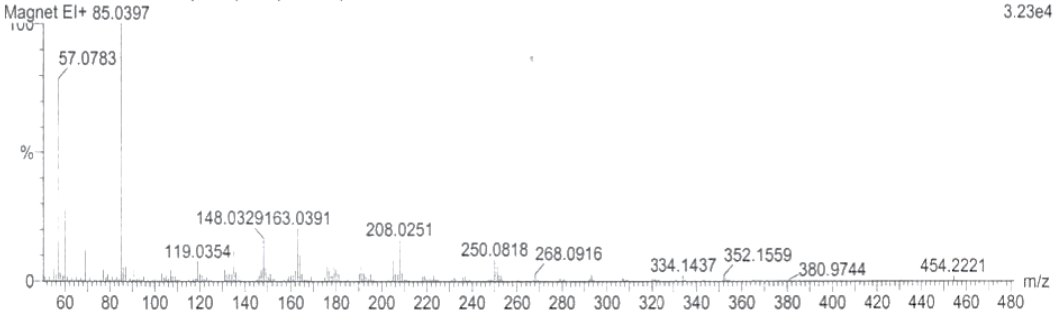

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Maximum: 100.00 5.0 10.0 50.0

Figure S20. Mass spectrum (EI), compound **2** of *V. pavonii.*

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Table S2. ^{1}H (400 MHz) and ^{13}C-NMR (100 MHz) spectroscopic data for compound **2** (in MeOD) and valeriandoid F.

Compound 2			**Valeriandoid F ***	
Position	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**
1	6.11 (d, J=10.1, 1H)	94.1	6.16 (d, J=10.0, 1H)	92.4
3	6.73 (s, 1H)	149.2	6.58 (s, 1H)	147.5
4		110.4		108.3
5		140.7		138.6
6	5.72 (t, J=2.7, 1H)	118.8	5.68 (dd, J=2.6, 2.3, 1H)	117.7
7	5.42 (d, J=2.7, 1H)	85.0	5.37 (d, J=2.6, 1H)	83.3
8		81.6		79.9
9	2.81 (dd, J= 2.7, 2.7, 1H)	49.6	2.78 (dd, J=10.0, 2.3, 1H)	47.9
10	3.68, 3.53 (2d, J=9.6, 2H)	75.6	3.63, 3.45 (2d, J=9.2, 2H)	73.4
11	4.74, 4.64 (2d, J=12.3, 12.2, 2H)	62.1	4.62, 4.55 (2d, J=12.3, 2H)	60.8
R^{1} – 1´		172.2		170.4
2´	2.30 (d, J=7.6, 2H)	44.1	2.23 (m, 2H)	42.9
3´	2.17 (m, 1H)	26.8	2.11 (m, 1H)	25.3
4´	1.00 (d, J=1.1, 3H)	22.8	0.93 (d, J=6.6, 3H)	22.1
5´	0.99 (d, J= 1.1, 3H)	22.7	0.93 (d=6.6, 3H)	22.1
R^{7} – 1´´		173.9		171.7
2´´	2.16 (d, J=6.6, 2H)	44.5	2.09 (m, 2H)	43.3
3´´	2.02 (m, 1H)	27.0	1.99 (m, 1H)	25.5
4´´	0.95 (d, J=2.2, 3H)	22.6	0.87 (d, J=6.7, 3H)	22.0
5´´	0.93 (d, J=2.1, 3H)	22.6	0.87 (d, J=6.7, 3H)	22.0
R^{10} – 1´´´	3.33 (s, 3H)	59.8	3.29 (s, 3H)	59.0
R^{11} – 1´´´´		172.7		170.7
2´´´´	1.99 (s, 3H)	20.8	1.95 (s, 3H)	20.6

* ^{1}H-NMR (at 400 MHz in $CDCl_3$); ^{13}C-NMR (at 100 MHz). (Xu et al., 2012). δ in ppm, J in Hz.

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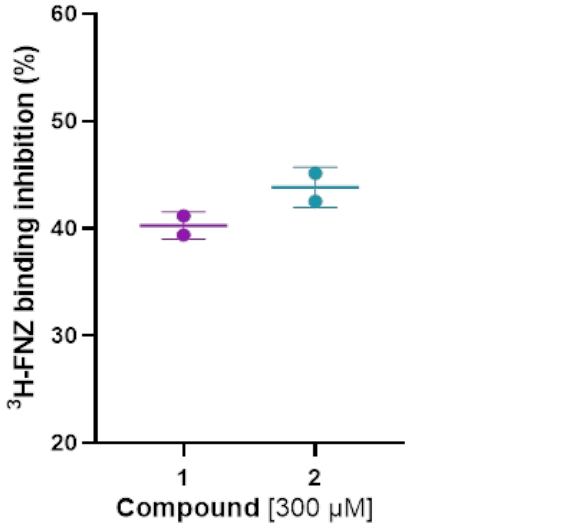

Figure S21. Capacity of the compounds (at 300 µM) to inhibit the binding of ^{3}H-FNZ to the BDZ-bs of the $GABA_A$ receptor from rat cerebral cortex synaptosomal membranes.

Values are expressed as means ± S.E.M.

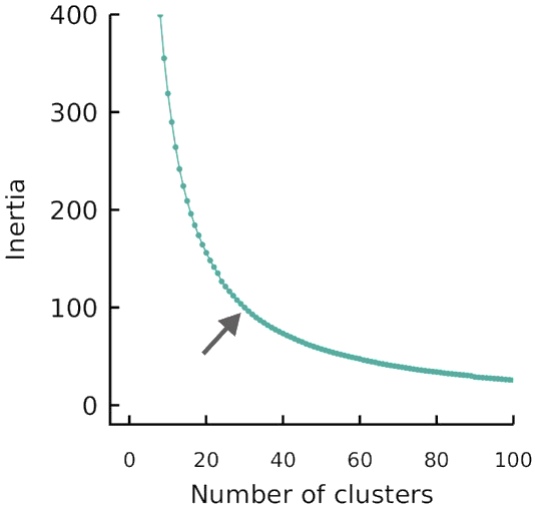

Figure S22. Variation of inertia as a function of the number of clusters.

The inertia was calculated as the sum of the RMSD of the structures at their centroid between each cluster; then the value was averaged over the number of clusters.

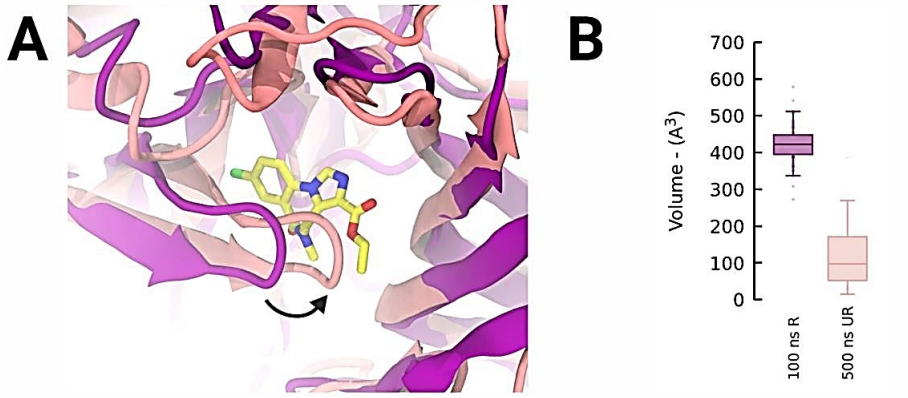

Figure S23. Binding site differences between restrained and unrestrained molecular dynamics simulations of $GABA_A$.

(A) $GABA_A$ binding site from cryo-electron microscopy structure (purple) and frame with the smallest volume from unrestrained molecular dynamics simulation (pink). **(B)** Binding site volume comparisons between unrestrained (UR) and restrained (R) molecular dynamics simulation.

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Table S3. Clusters of compounds **1** and **2** along with their binding free energy (ΔG_{Bind}).

Cluster N°	Compound 1		Cluster N°	Compound 2	
	Population	ΔG_{Bind} (kcal/mol)		Population	ΔG_{Bind} (kcal/mol)
1	337	-100.9 ± 9.37	1	342	-97.87 ± 7.94
2	166	-104.7 ± 8.86	2	135	-103.42 ± 8.62
3	87	-97.72 ± 8.22	3	133	-95.89 ± 8.21
4	27	-98.06 ± 8.66	4	25	-96.91 ± 9.34
5	19	-95.84 ± 7.22	5	24	-96.33 ± 7.64
6	19	-97.34 ± 6.35	6	22	-90.88 ± 10.35
7	15	-99.05 ± 9.57	7	20	-97.77 ± 6.2
8	14	-95.53 ± 11.07	8	19	-90.52 ± 8.31
9	13	-91.23 ± 9.92	9	17	-98.22 ± 9.04
10	13	-87.72 ± 3.76	10	16	-89.11 ± 6.55
11	12	-91.32 ± 4.09	11	15	-102.95 ± 3.97
12	11	-105.35 ± 8.22	12	15	-94.89 ± 9.14
13	10	-100.83 ± 4.44	13	15	-91.6 ± 5.43
14	10	-105.39 ± 10.01	14	11	-97.61 ± 6.47
15	9	-101.08 ± 6.05	15	9	-103.56 ± 6.95
16	9	-99.06 ± 12.2	16	7	-88.72 ± 4.31
17	9	-99.71 ± 10.99	17	5	-72.17 ± 3.23
18	8	-94.46 ± 3.61	18	5	-91.35 ± 8.22
19	8	-87.77 ± 2.19	19	5	-94.8 ± 4.66
20	7	-96.48 ± 8.93	20	4	-83.51 ± 3.12
21	7	-93.76 ± 3.28	21	4	-89.72 ± 6.29
22	4	-102.5 ± 5.98	22	4	-84.29 ± 5.66
23	4	-100.72 ± 5.04	23	3	-87.17 ± 5.27
24	3	-92.14 ± 8.07	24	3	-86.62 ± 7.34
25	3	-107.08 ± 5.94	25	3	-98.07 ± 5.36
26	3	-84.56 ± 9.42	26	3	-86.99 ± 11.52
27	2	-101.83 ± 7.4	27	2	-105.87 ± 2.5
28	2	-72.19 ± 3.1	28	2	-75.64 ± 0.99
29	2	-102.33 ± 3.55	29	2	-77.17 ± 1.3
30	2	-86.11 ± 3.83	30	2	-120.68 ± 1.61
31	2	-94.03 ± 4.58	31	1	-70.78
32	2	-100.04 ± 3.55	32	1	-79.54
33	2	-104.64 ± 8.6	33	1	-94.01

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Supplementary Data

Table S3. Clusters of compounds **1** and **2** along with their binding free energy (ΔG_{Bind}) (continued…)

Cluster Nº	Compound 1		Cluster Nº	Compound 2	
	Population	**ΔG_{Bind} (kcal/mol)**		**Population**	**ΔG_{Bind} (kcal/mol)**
34	2	-105.94 ± 2.29	34	1	-82.75
35	2	-95.99 ± 3.53	35	1	-91.09
36	2	-108.7 ± 1.51	36	1	-93.7
37	2	-92.49 ± 5.99	37	1	-100.53
38	2	-92.6 ± 0.44	38	1	-81.21
39	1	-93.53 ± 9.98	39	1	-82.74
40	1	-77.42	40	1	-79.44
41	1	-91.86	41	1	-78.86
42	1	-91.47	42	1	-86.51
43	1	-80.1	43	1	-97.65
44	1	-69.96	44	1	-77.37
45	1	-76.34	45	1	-84.98
46	1	-85.96	46	1	-89.02
47	1	-76.6	47	1	-93.65
48	1	-89.24	48	1	-72.49
49	1	-85.07	49	1	-77.57
50	1	-102.83			
51	1	-104.6			
52	1	-98.04			
53	1	-81.87			
54	1	-67.65			
Total Population		867	Total Population		891
Average Populations		16.05 ± 51.03	Average Populations		18.18 ± 54.16
Average ΔG_{Bind}		-93.53 ± 9.98	Average ΔG_{Bind}		-89.88 ± 9.98

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AUTHOR CONTRIBUTION:

Contribution	Giraldo SE	Bedoya M	Peña-Varas C	Santana PA	Bazzocchi IL	Jiménez IA	Marder M	Vergel NE	Guerrero MF	Ramírez D
Concepts or ideas	x	x			x	x	x		x	x
Design	x	x							x	x
Definition of intellectual content	x	x							x	x
Literature search	x	x								x
Experimental studies	x	x								
Data acquisition	x	x	x	x	x	x	x	x		x
Data analysis	x	x	x	x	x	x	x	x		x
Statistical analysis			x	x	x	x	x	x		
Manuscript preparation	x	x					x			x
Manuscript editing	x	x	x	x	x	x	x	x	x	x
Manuscript review	x	x	x	x	x	x	x	x	x	x

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