



STRUCTURAL
CHEMISTRY

Volume 76 (2020)

Supporting information for article:

**Revisiting the absolute chirality and polymorphism of (–)-Istanbulin
A**

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S1. Diffraction studies

S1.1. Additional comments regarding crystallization and crystal forms

With the aim to explore polymorphic transformations or findings between crystal forms I and II of (–)-Istanbulin A, different crystallization procedures using several solvents and conditions were followed (pure Ethyl Acetate, CH₂Cl₂, CHCl₃, ethyl ether, acetone, hexane:Ethyl Acetate mixtures (25:75, 50:50 and 60:30), using slow evaporation of the solvent strategy at RT and at c.a. 10°C). All these experiments gave place to the orthorhombic phase, confirmed by lattice parameters determination and space group suggestions during single crystal XRD experiments performed using the pre-experiment routine implemented in CrysAlis PRO program. ~~On the other hand,~~ Figure S1 shows the powder pattern of the pure polycrystalline sample after purification obtained directly from the chromatography column. Results show an excellent agreement with the simulated powder pattern using single crystal data confirming that it corresponds to the polymorph II. ~~Single crystals of polymorph I reported by López-Rodríguez et al. were obtained directly after purifications procedures and no further crystallization was followed (López-Rodríguez et al., 2009).~~

Table S1 Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($\text{\AA}^2 \times 10^3$) for **1**. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{ij} tensor.

Atom	x	y	z	U(eq)
O1	6911(4)	4941(2)	6809(2)	76.2(9)
O2	-672(6)	2921(3)	4683(2)	89.2(11)
O3	801(4)	4206(2)	5374.7(16)	60.9(7)
O4	533(4)	5349(2)	6563.8(19)	57.2(7)
C1	5925(5)	4803(3)	7454(3)	56.2(9)
C2	6567(6)	4879(3)	8394(3)	68.9(12)
C3	5881(6)	3991(3)	8979(3)	65.2(11)
C4	3857(5)	3792(3)	8860(2)	55.9(9)
C5	3387(5)	3593(3)	7870(2)	47.7(8)
C6	1317(5)	3437(3)	7760(2)	52.4(9)
C7	761(5)	3488(3)	6807(2)	48.0(8)
C8	1355(5)	4427(3)	6286(2)	49.4(8)

C9	3376(5)	4562(3)	6347(3)	54.3(9)
C10	3935(5)	4585(3)	7327(2)	49.5(8)
C11	-59(5)	2823(3)	6267(3)	53.6(9)
C12	-66(6)	3273(3)	5368(3)	62.3(10)
C13	-868(7)	1778(3)	6428(3)	72.0(12)
C14	4369(7)	2640(3)	7501(3)	62.3(10)
C15	3228(8)	2937(4)	9496(3)	79.5(14)

Table S2 Anisotropic Displacement Parameters ($\text{\AA}^2 \times 10^3$) for **1**. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+\dots]$.

Atom	U11	U22	U33	U23	U13	U12
O1	51.2(16)	82(2)	95(2)	2.2(17)	12.9(16)	-6.6(15)
O2	127(3)	85(2)	55.6(17)	-7.6(16)	-25.2(19)	-15(2)
O3	75.8(19)	65.0(16)	42.0(13)	8.4(11)	-3.9(13)	-8.9(14)
O4	48.9(15)	54.3(14)	68.3(17)	-2.3(12)	0.1(13)	1.0(12)
C1	43(2)	46.2(17)	79(3)	-5.1(17)	3(2)	1.2(16)
C2	49(2)	67(2)	91(3)	-12(2)	-13(2)	-4.4(19)
C3	64(3)	63(2)	69(3)	-6.5(19)	-14(2)	4(2)
C4	57(2)	60(2)	50(2)	-4.4(16)	-5.3(17)	1.6(17)
C5	49(2)	46.6(17)	47.2(18)	-1.6(14)	1.5(15)	-0.9(15)
C6	54(2)	61(2)	42.1(17)	1.6(16)	1.1(16)	-7.6(16)
C7	44.3(18)	54.2(18)	45.5(18)	2.8(14)	1.6(15)	-4.8(16)
C8	53(2)	51.6(18)	43.9(17)	1.8(15)	2.8(15)	-2.7(15)
C9	51(2)	56(2)	56(2)	7.2(17)	9.3(16)	0.5(17)
C10	44(2)	47.9(17)	56.2(19)	-3.0(15)	4.8(16)	2.1(14)
C11	56(2)	52(2)	52(2)	2.5(16)	-3.4(17)	-4.7(16)
C12	77(3)	59(2)	51(2)	-2.7(17)	-9(2)	-2(2)

C13	80(3)	57(2)	80(3)	4.6(19)	-13(3)	-15(2)
C14	76(3)	51(2)	60(2)	-4.5(17)	0(2)	5.5(19)
C15	90(4)	95(3)	54(2)	9(2)	-7(2)	-11(3)

Table S3 Bond Lengths for **1**.

Atom Atom Length/Å			Atom Atom Length/Å		
O1	C1	1.221(5)	C5	C6	1.547(5)
O2	C12	1.207(5)	C5	C10	1.568(5)
O3	C8	1.452(4)	C5	C14	1.530(5)
O3	C12	1.362(5)	C6	C7	1.485(5)
O4	C8	1.397(4)	C7	C8	1.505(5)
C1	C2	1.488(6)	C7	C11	1.324(5)
C1	C10	1.505(5)	C8	C9	1.502(5)
C2	C3	1.527(6)	C9	C10	1.524(5)
C3	C4	1.523(6)	C11	C12	1.465(5)
C4	C5	1.542(5)	C11	C13	1.493(5)
C4	C15	1.529(6)			

Table S4 Bond Angles for **1**.

Atom Atom Atom Angle/°				Atom Atom Atom Angle/°			
C12	O3	C8	108.2(3)	C11	C7	C8	109.7(3)
O1	C1	C2	123.3(4)	O3	C8	C7	104.3(3)
O1	C1	C10	120.5(4)	O3	C8	C9	111.0(3)
C2	C1	C10	116.2(3)	O4	C8	O3	108.9(3)
C1	C2	C3	112.9(3)	O4	C8	C7	113.8(3)

C4	C3	C2	112.5(4)	O4	C8	C9	108.3(3)
C3	C4	C5	111.1(3)	C9	C8	C7	110.5(3)
C3	C4	C15	110.2(4)	C8	C9	C10	109.2(3)
C15	C4	C5	114.2(3)	C1	C10	C5	109.8(3)
C4	C5	C6	110.2(3)	C1	C10	C9	112.8(3)
C4	C5	C10	107.7(3)	C9	C10	C5	114.5(3)
C6	C5	C10	107.7(3)	C7	C11	C12	107.8(3)
C14	C5	C4	112.0(3)	C7	C11	C13	131.9(4)
C14	C5	C6	108.8(3)	C12	C11	C13	120.3(4)
C14	C5	C10	110.2(3)	O2	C12	O3	120.8(4)
C7	C6	C5	111.6(3)	O2	C12	C11	129.3(4)
C6	C7	C8	116.9(3)	O3	C12	C11	109.9(3)
C11	C7	C6	133.1(3)				

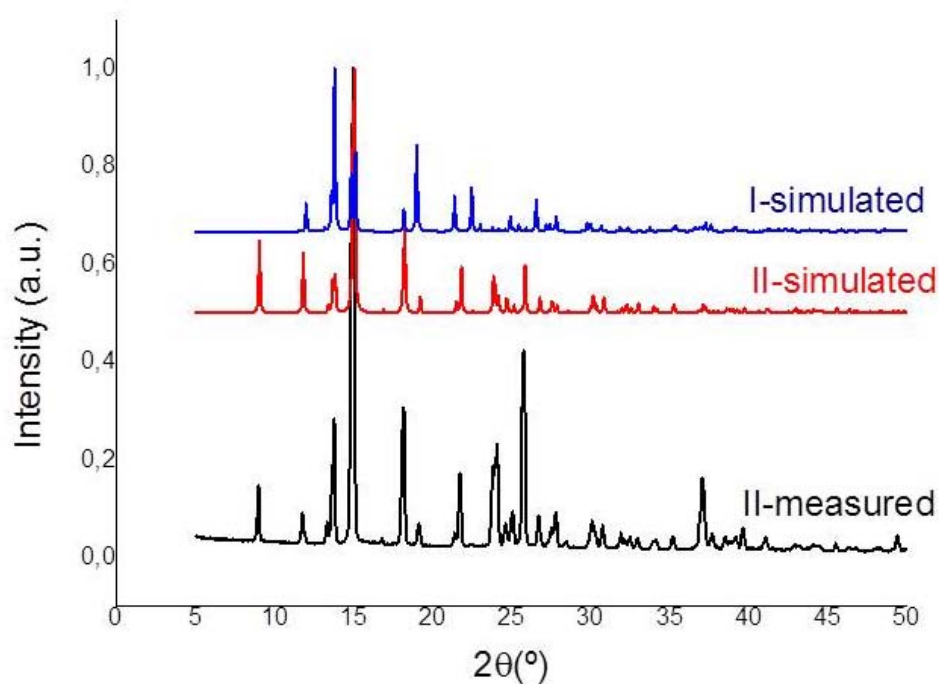


Figure S1 Powder X-ray diffraction patterns of **1**, (-)-Istanbulin A form II, experimental (black) and simulated from single crystal X-ray diffraction data (red). In blue the simulated diffraction patterns from single crystal X-ray diffraction data for (-)-Istanbulin A, is also shown.

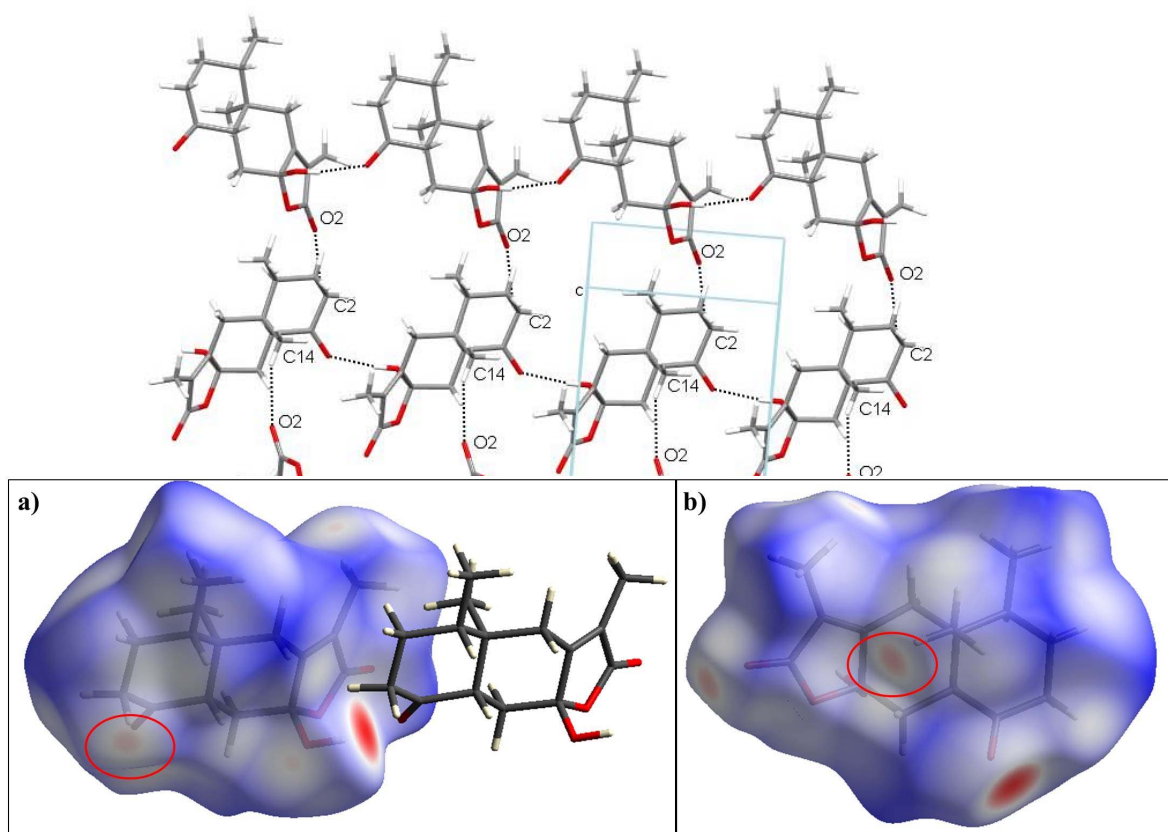


Figure S2 3D supramolecular arrangement for **1**

S2. Additional comments regarding Hirshfeld surface analysis

The Hirshfeld surface analysis is a valuable method for the analysis of intermolecular contacts that offers a whole-of-the-molecule approach. In order to visualize the intermolecular interactions in the crystal of compound **1**, a Hirshfeld surface analysis (Hirshfeld, 1977; Spackman & Jayatilaka, 2009) was carried out using CrystalExplorer17.5 (Turner *et al.*, 2017). Three-dimensional molecular Hirshfeld surfaces were generated using a 'high standard' surface resolution colour-mapped over the normalized contact distance. The red, white and blue regions indicate contacts with distances shorter, longer and equal to the van der Waals radii (Figure S3).

Figure S3 Hirshfeld surface (HS) plots. In a) front view including a neighbour molecule, light-red dot indicates C2–H2A···O2 interaction and intensive red dot indicates the O4–H4···O1 contact. In b) back view, light-red dot corresponds to the C14–H14C···O2 interaction.

S3. Spectroscopic characterization additional information and supplementary figures and tables

S3.1. General experimental characterization techniques and procedures

All 1D and 2D NMR spectra (COSY, HSQC and HMBC) were recorded in a CDCl₃ solution on a Bruker Advance II 500 (¹H to 500.13 MHz; ¹³C to 125.77 MHz). δ are expressed in ppm relative to Me₄Si as internal standard, *J* in Hz. The processing software was Topspin 2.1 (Bruker). IR spectra were measured on a Nicolet Avatar 320 FTIR spectrometer with a Spectra Tech cell for KBr pellets. UV–visible spectra were recorded using a Hewlett-Packard 8453 diode array spectrometer in 10 mm optical path quartz cuvettes. HR-ESI-MS (high resolution electrospray mass spectrometry) studies were performed using an ESI-TOF Xevo G2S Q-TOF (Waters Corp.) spectrometer. For the analysis, compound **1** was dissolved in a mixture of (MeOH:H₂O (80:20 v/v) working at positive mode. The optical rotation was measured with a Perkin Elmer series 343 polarimeter, in a bucket of 1 decimetre of length.

S3.2. HR-ESI-MS (high resolution electrospray mass spectrometry)

IS1_MeOH_Dil 1:10000_nueva

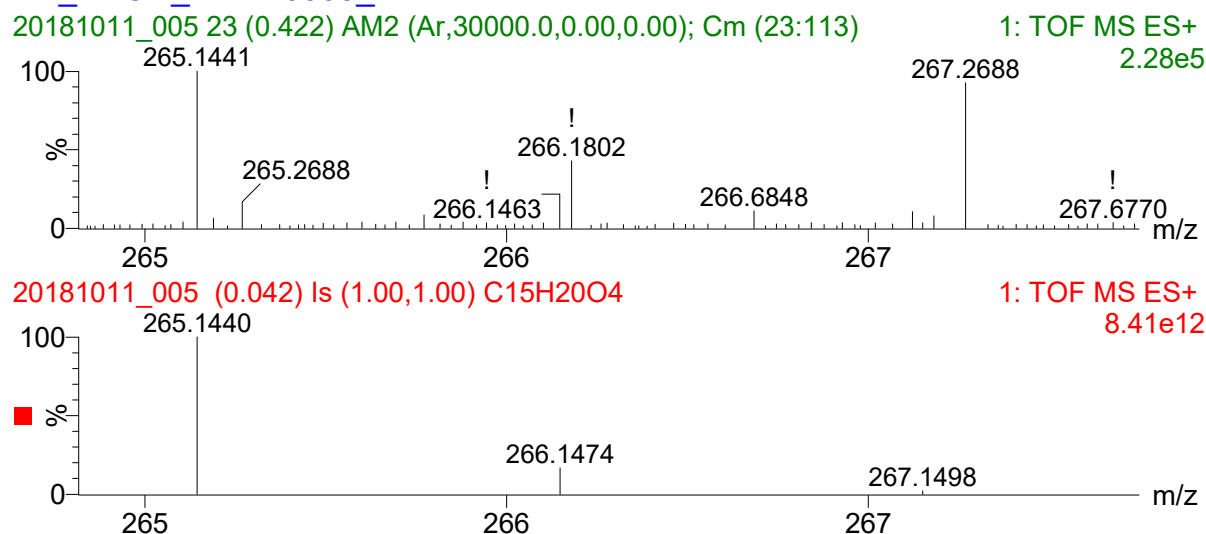
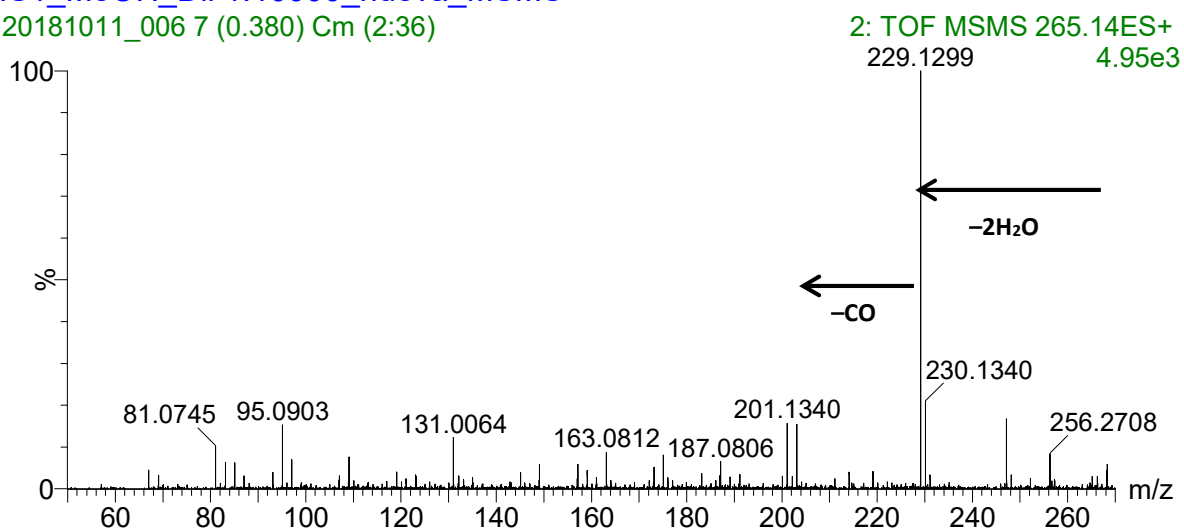


Figure S4 ESI-MS spectra of compound **1**, experimental (top) and calculated (bottom) for the region associated with [M+H]⁺.

IS1_MeOH_Dil 1:10000_nueva_MSMS

20181011_006 7 (0.380) Cm (2:36)

**Figure S5** HR ESI-MSMS spectrum of m/z 265.14.**S3.3. IR and UV spectroscopy**

The IR and UV spectra supported the presence of the α - β -unsaturated- γ -lactone (1757 and 1691 cm^{-1} , 219 nm), a six-membered ring ketone (1710 cm^{-1} -shoulder-, 281) and an hydroxyl group (3280 cm^{-1}) (Figures S6 and S7)

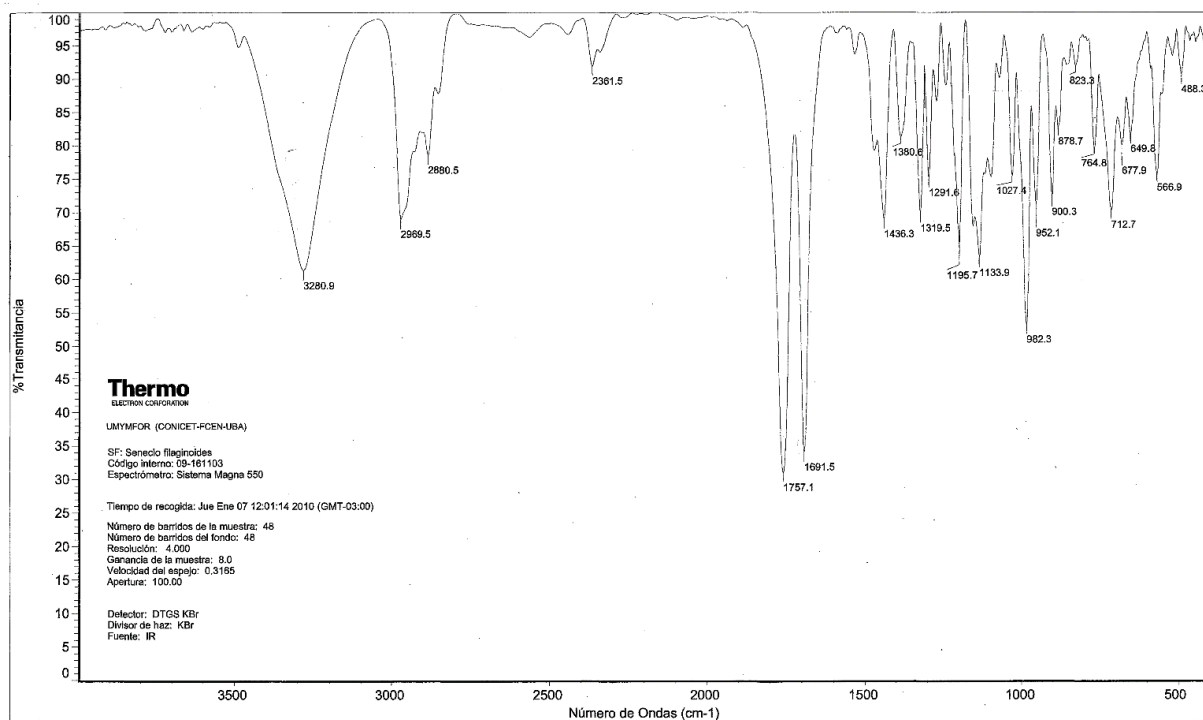
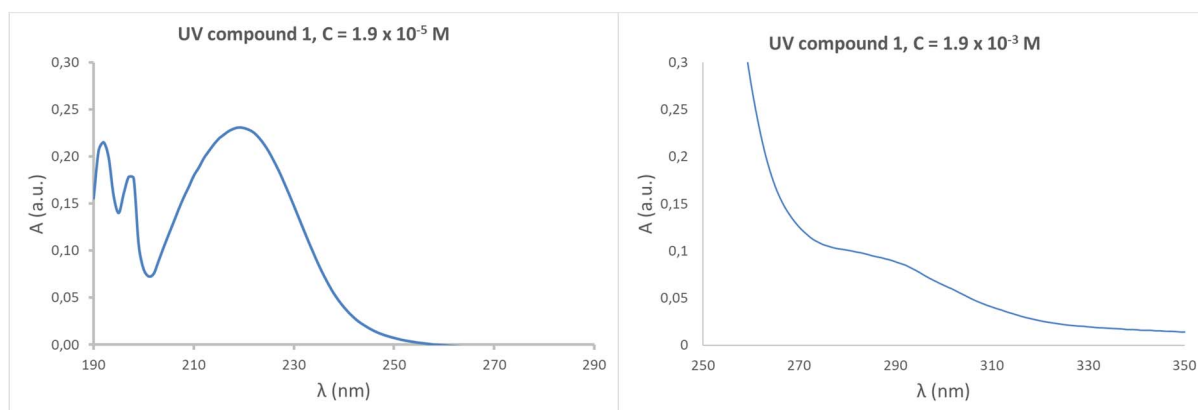


Figure S6 FTIR spectrum of compound **1** (KBr pellet).**Figure S7** UV spectrum of compound **1** (dichloromethane solution) at different concentrations.

S3.4. NMR characterization

NMR data agreed with proposed formula since 15 carbon and 19 hydrogen resonances were observed in the ^{13}C and ^1H NMR experiments, respectively (Figure 1, Table S1 and Figures S4 and S5). The complete assignment of all protons and carbon atoms were performed based on COSY, HSQC, NOESY and HMBC studies (Table S5 and Figures S8-S12). The ^{13}C NMR spectrum showed two carbonyl groups, C1 at 210 ppm and C-12 at 172 ppm, corresponding to keto and ester functionalities, respectively and two further signals in the chemical shift range of sp^2 carbons (C-1, C-7, C-11 and C-12), associated to an unsaturated lactone. ^{13}C NMR information accounted also for six degrees of unsaturation and explained the connections between two cyclohexane rings and the lactone as well as, the presence of the two carbonyl groups as mentioned. Then, signals at δ 8.08, 11.45 and 14.78 ppm agreed with the presence of three methyl groups. The four methylenes and two methines were finally assigned with the information obtained from HSQC and COSY experiments. The ^1H NMR spectrum confirmed the three methyl groups at δ 1.02 (3H, d, $J = 7$ Hz), 0.54 (3H, s) and 1.80 (3H, s). Based on the NMR studies the systematic name for compound **1** was assigned as: 9a-hydroxy-3,4a,5-trimethyl-4a,6,7,8a,9,9a-hexahydro-4H,5H-naphtho[2,3-b]-furan-2,8-dione.

Table S5 NMR spectral data for compound **1**: δ values, CDCl_3 , ^{13}C NMR at 125 MHz, ^1H MR at 500 MHz and HMBC correlations.

C	$\delta^{13}\text{C}$	Type of C	$\delta^1\text{H}(J)$	HMBC ($^1\text{H} \rightarrow ^{13}\text{C}$)
1	210.24	C	-	C-3,C-10
2	41.09	CH_2	2.39(1H,dd,2,14) 2.47(1H,m)	C-3,C-4,C-10
3	31.02	CH_2	1.65(1H,ddd,5,13,27) 1.95(1H,m)	C-4,C-15
4	42.02	CH	2.08(1H,m)	C-3,C-10,C-14,C-15
5	44.73	C	-	C-1,C-3,C-4,C-6,C-10,C-14
6	36.89	CH_2	2.62(1H,da,13) 2.32(1H,da,13.5)	C-4,C-10,C-11,C-14
7	157.73	C	-	C-6, C-13
8	103.47	C	-	C-6,C-13

9	33.69	CH ₂	1.74(1H,dd,13,14) 2.37(1H,dd,3.5,14)	C-1,C-10
10	54.20	CH	2.82(1H,dd,3.5,13)	C-1,C-4,C-6, C-14
11	123.90	C	-	C-6,C-13
12	172.32	C	-	C-13
13	8.08	CH ₃	1.80(3H,s)	C-11, C-12
14	11.45	CH ₃	0.54(3H,s)	C-6,C-10
15	14.78	CH ₃	1.02(3H,d,7)	C-3,C-4

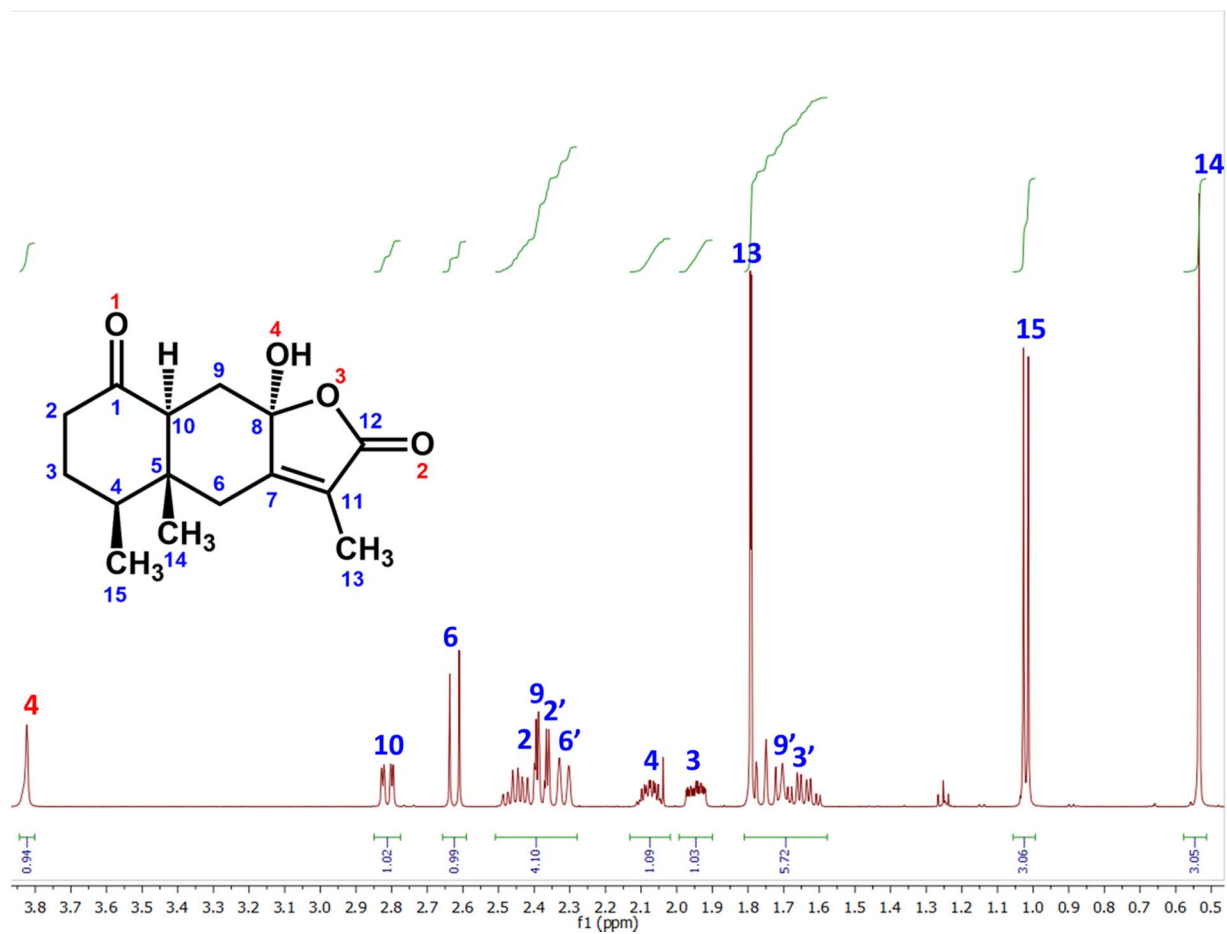


Figure S8 ¹H NMR (500 MHz, CDCl₃) spectrum of compound 1.

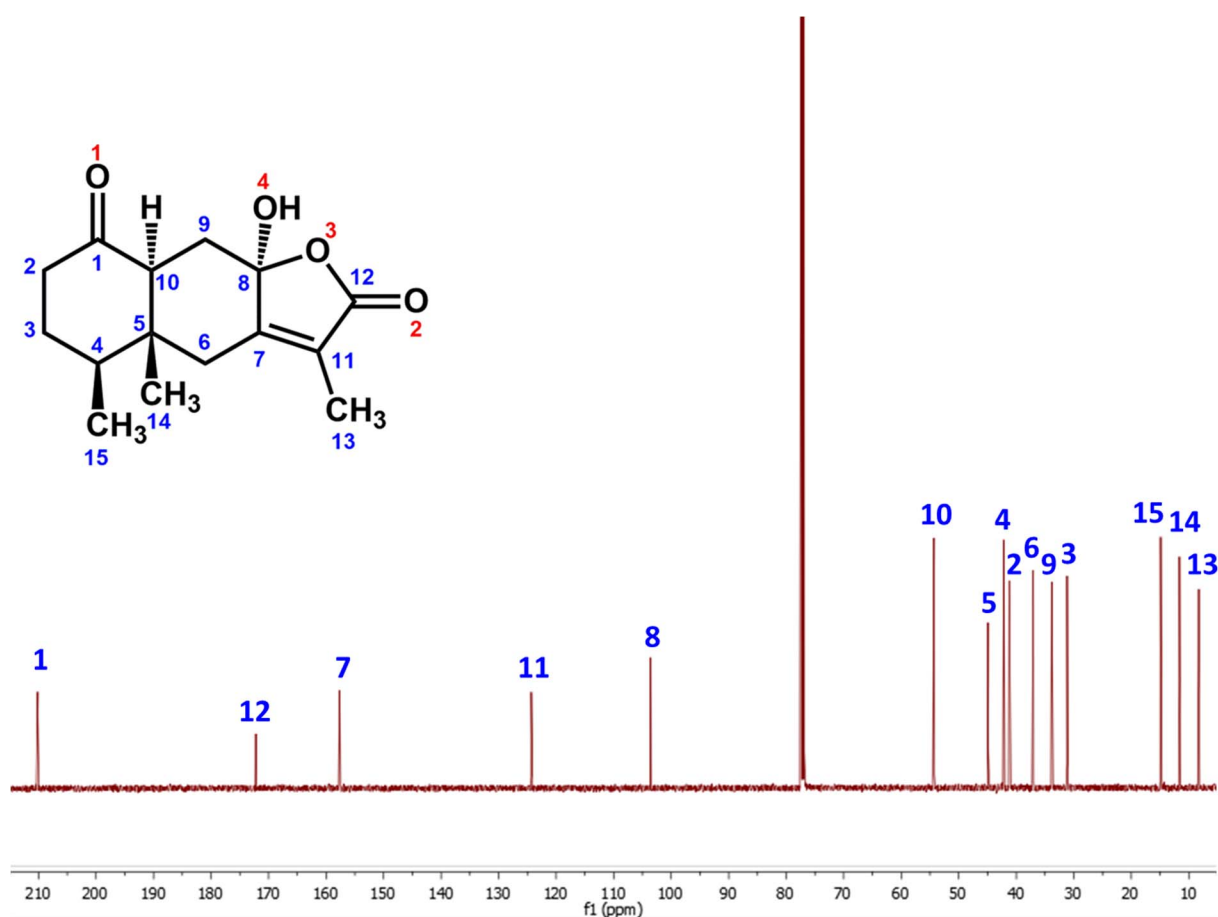


Figure S9 ^{13}C NMR (125 MHz, CDCl_3) spectrum of compound 1.

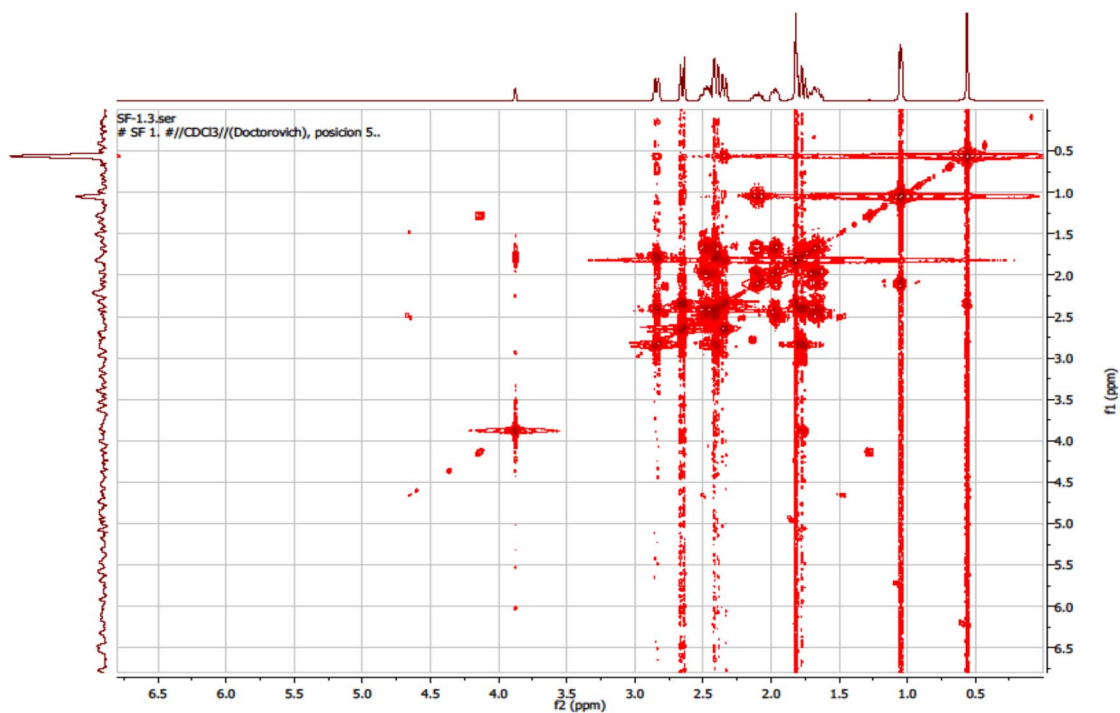


Figure S10 ^1H - ^1H COSY spectrum (CDCl_3) of compound 1.

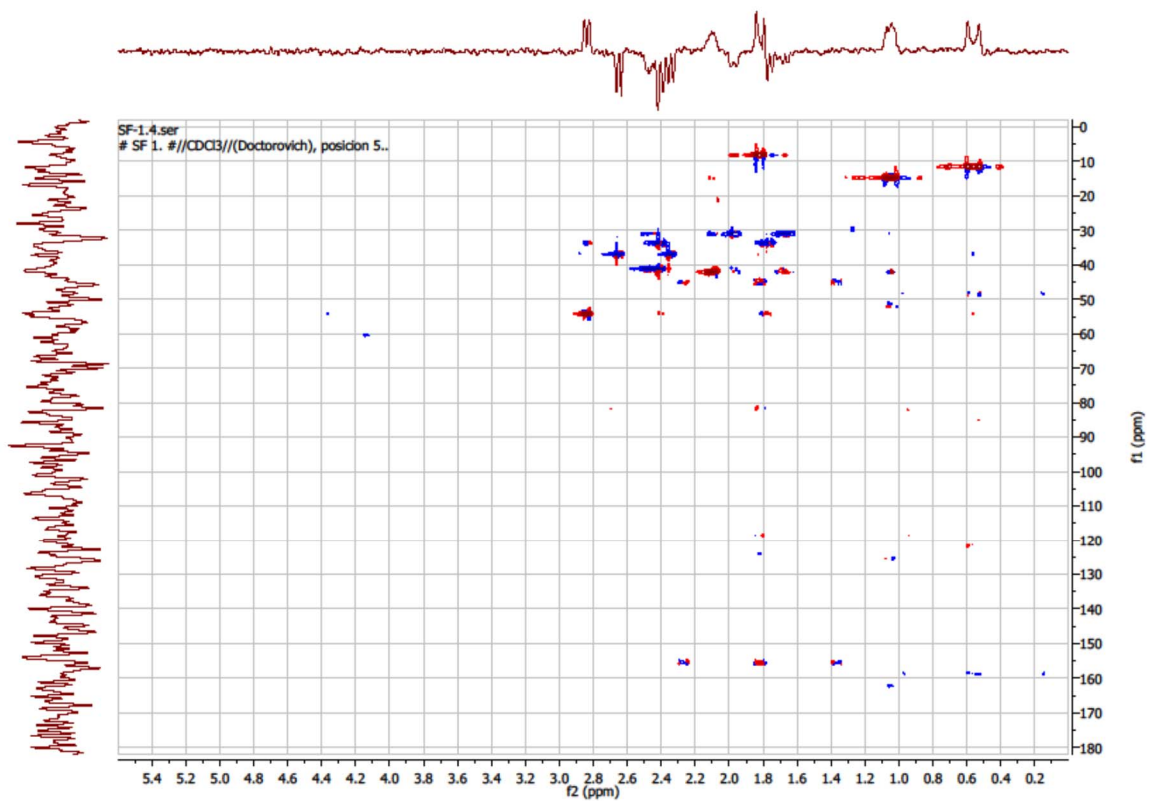


Figure S11 HSQC spectrum (CDCl₃) of compound 1

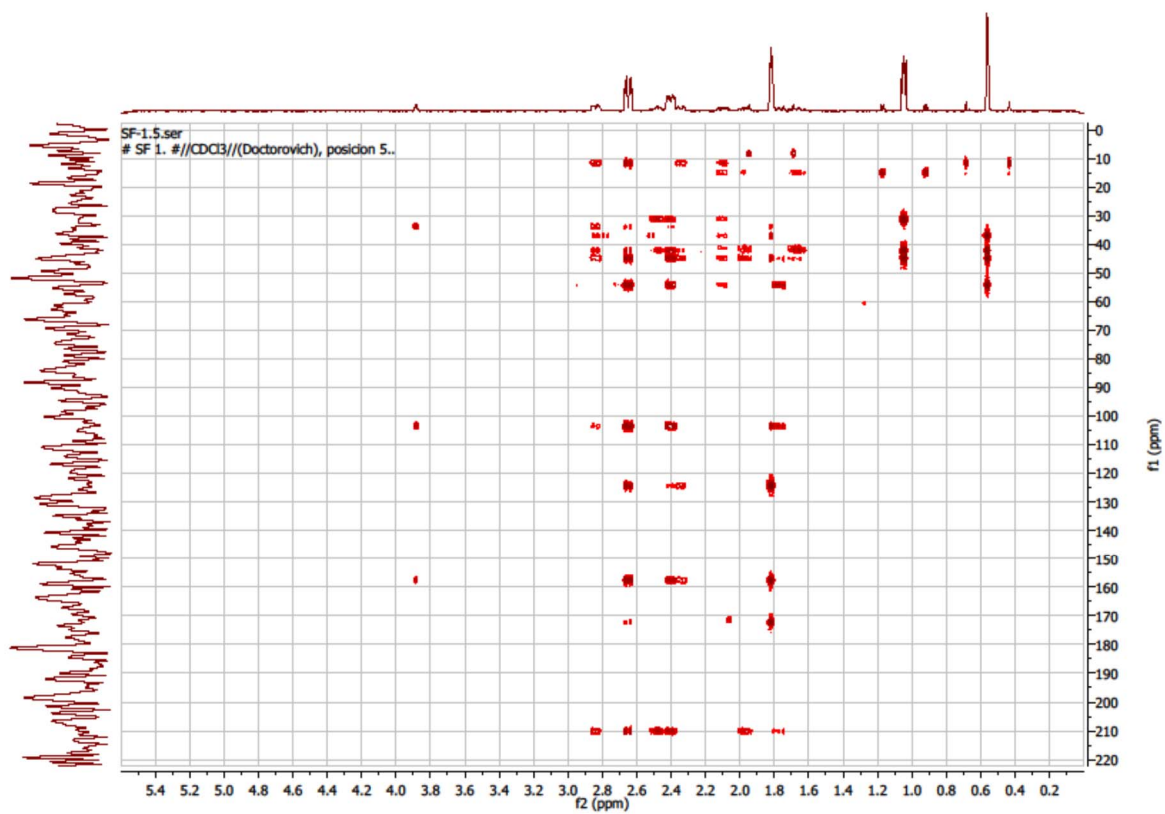


Figure S12 HMBC spectrum (CDCl₃) of compound 1.

S4. Comparative structural studies of Istanbulins

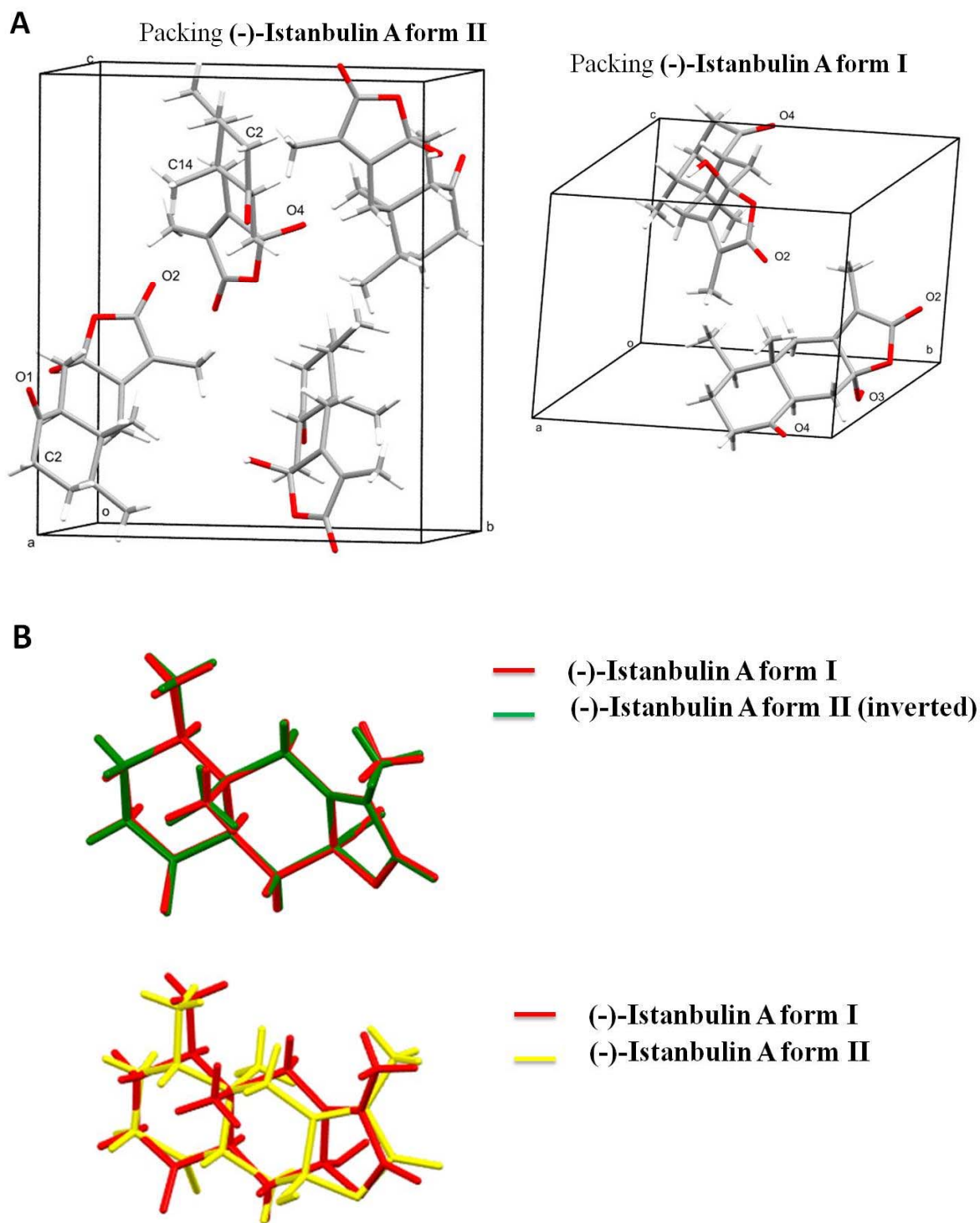


Figure S13 A) Unit cell comparison of (-)-Istanbulin A form II and (-)-Istanbulin A form I. B) Molecular comparison of both Istanbulins.

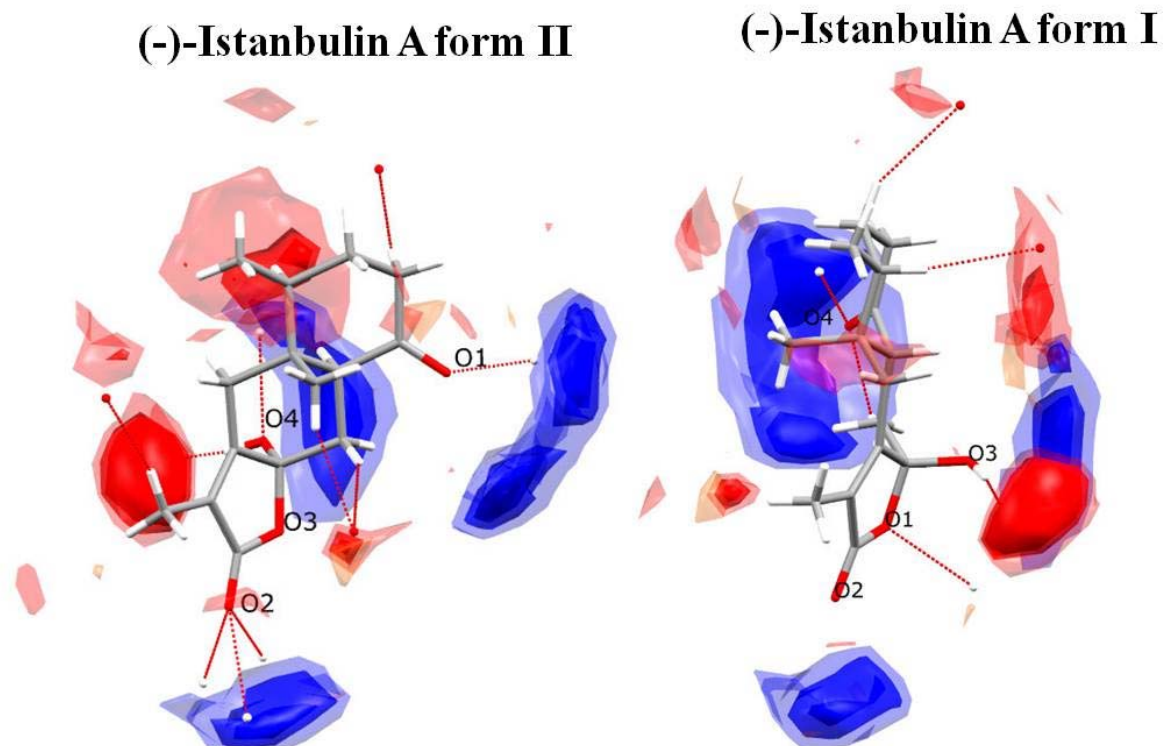


Figure S14 Full Interaction Maps (FIM) generated with Mercury Program (Wood et al, 2013) calculations, left (-)-Istanbulin A form II and right (-)-Istanbulin A form I.

Table S6 Comparative structural information for form I and II of (-)-Istanbulin A.

	(-)-Istanbulin A, form I	(-)-Istanbulin A, form II
Reference	López-Rodríguez <i>et al.</i> , 2009	This work
ID Code	BUFJUI	
Chemical formula	C ₁₅ H ₂₀ O ₄	C ₁₅ H ₂₀ O ₄
M_r	264.31	264.31
Crystal system, space group	Monoclinic, $P2_1$	Orthorhombic, $P2_12_12_1$
a, b, c (Å)	7.432 (4), 13.010 (6), 8.161 (6)	7.3660 (4), 12.8932 (6), 14.9661 (7)
α, β, γ (°)	90, 115.47 (4), 90	90, 90, 90
V (Å ³)	712.4 (8)	1421.35 (11)
Z	2	4

Radiation type	Mo $K\alpha$	Cu $K\alpha$
Absolute configuration	--	4 <i>S</i> ,5 <i>R</i> ,8 <i>R</i> ,10 <i>S</i>

Table S7 Short contacts distances (Å) and angles (°) developed in (–)-Istanbulin A form I; D: donor, A: acceptor

D	H	A	d(D-H)	d(H-A)	d(D-A)	D-H-A
O3	H3	O4	0.92 (4)	1.84(4)	2.749(3)	164 (4)
C4	H4	O1	0.98 (3)	2.58 (3)	3.540 (4)	169 (2)
C3	H3B	O4	0.97	2.64	3.534	154

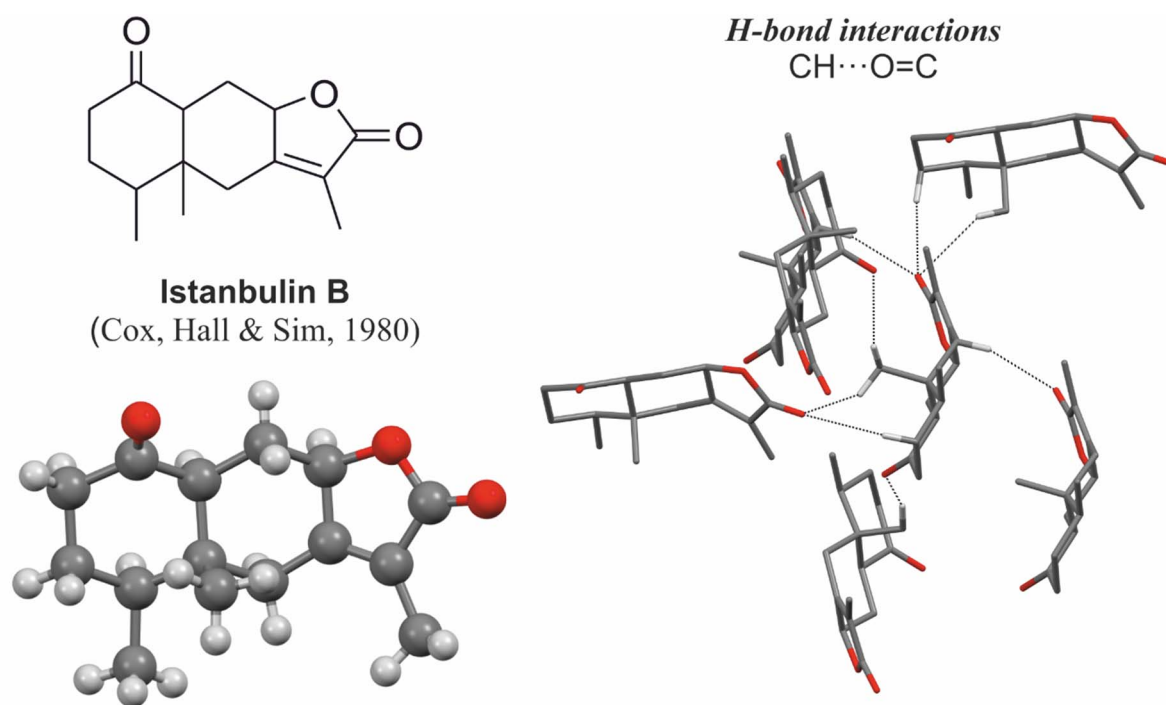


Figure S15 Molecular and supramolecular structure of Istanbulin B