

Supporting Information

Article

Crystal Structures, Molecular Docking and In Vitro Investigations of Two 4-Substituted 2-(5,5-dimethyl-3-styrylcyclohex-2-enylidene)malononitrile Derivatives as Potential Topoisomerase II Inhibitors

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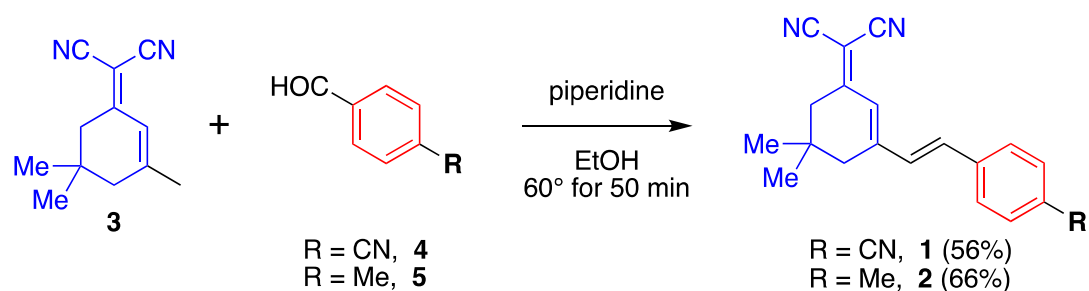


Figure S1. General scheme for the synthesis of compounds **1** and **2**.

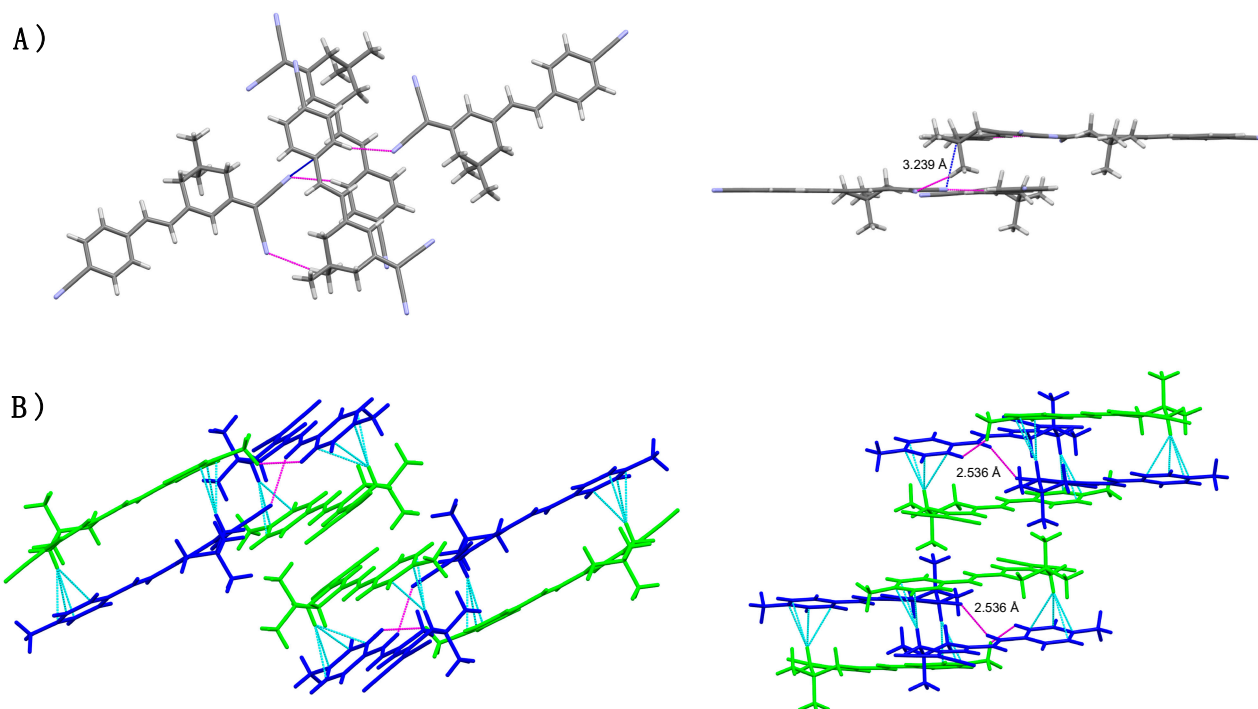


Figure S2. Selected intermolecular interactions in compounds **1** and **2**. The crystals packing is shown in two different directions of about 90° around *c* axes (panel A, for **1**) and about 180° around *c* axes (panel B, for **2**). The crystal structure of the major occupied disordered part of compound **1** is shown as capped sticks coloured by element, while the crystal structure of compound **2** is presented in capped sticks coloured by symmetry equivalence. The respective non-classical C-H...N interactions are shown as dashed magenta lines, while the σ ...N and σ ... π interactions in **1** and **2** are shown in blue and cyan dashed lines, respectively.

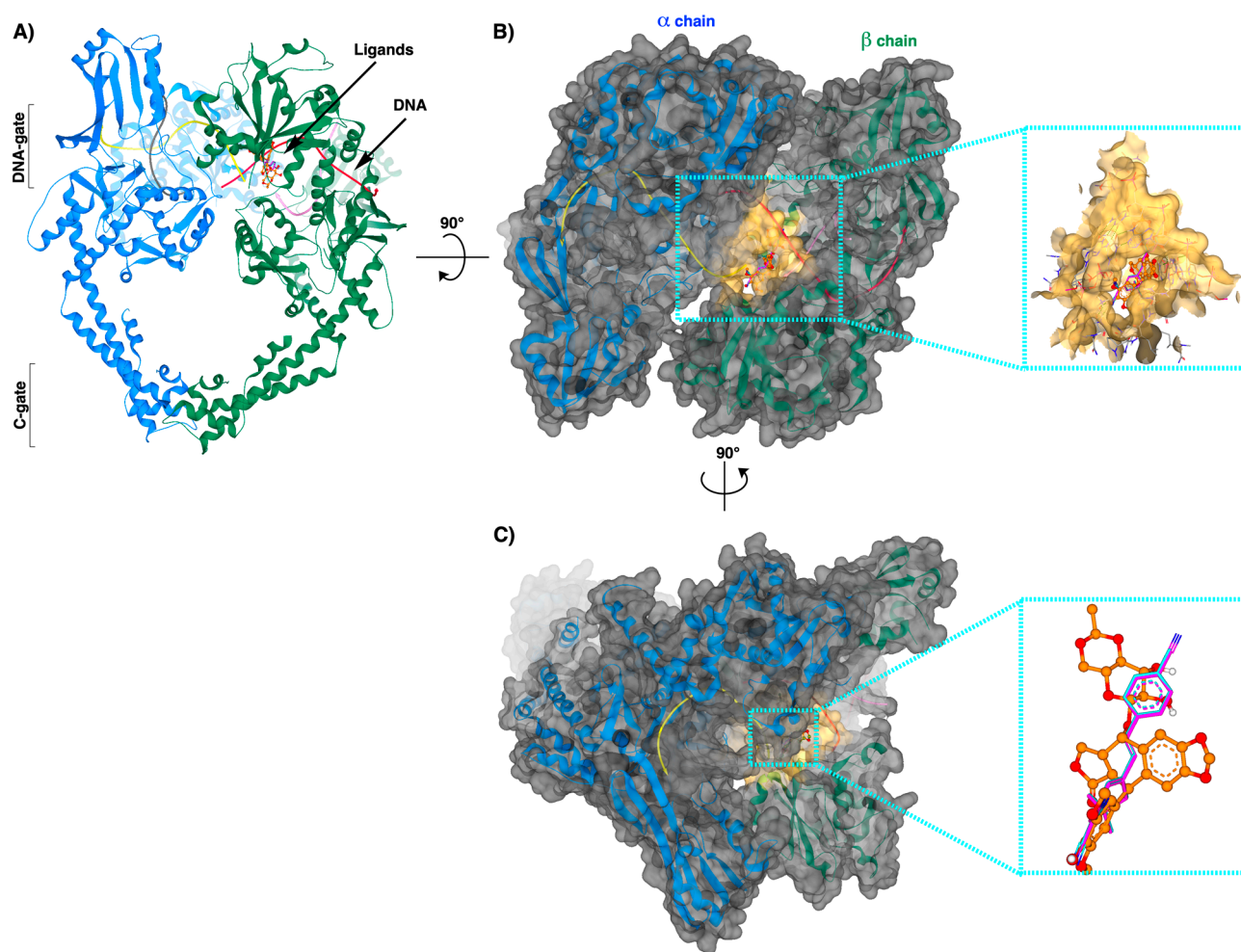


Figure S3. Representation of the hTOP2 β -DNA cleavage complex bonded to the investigated ligands. A) Orthogonal views of the overall structure of the hTOP2 β core in complex with DNA, the anticancer drug etoposide (PDB ID: 3QX3, res. 2.16 Å), and docked ligands (compounds **1** and **2**), superposed within the active site of chain β in hTOP2 β . The hTOP2 β monomers - α and β chain, are represented with blue and green ribbons, respectively, while the DNA chains are shown in grey (chain C), red (chain D), magenta (chain E), and yellow (chain F). The respective DNA- and C-gate are indicated. The ligands are shown as follows: etoposide in orange sticks and balls, compounds **1** and **2** in magenta and cyan sticks, respectively. B) Transparent surface representation of hTOP2 β core (rotated at 90° around the x axes) in complex with DNA and all superposed ligands within the active site of chain β in hTOP2 β (coloured in goldenrod transparent surface). The amino acid residues of the hTOP2 β core active site are shown as wire-frame. C) Transparent surface representation of hTOP2 β core rotated at 90° around the y axes with superposed binding modes of the ligands (shown and coloured as indicated above).

Table S1. Hydrogen bonds and hydrophobic interaction of ligands with hTOP2 β .

Compounds	Surrounding amino acid residues	Surrounding DNA bases	Hydrophobic interaction ¹	H-bonds ¹
Compound 1	LYS456; GLU477; GLY478; ASP479;	G7; C8; T9; G10; C11; A12;	C8; T9; G10; A12; G13	–
	SER480; PRO501; LEU502;	G13; C14		
	ARG503; GLY504; LYS505;		GLY478; ARG503; GLY504;	ASP479
	GLN778; MET782		MET782	
Compound 2	LYS456; GLU477; GLY478; ASP479;	G7; C8; T9; G10; C11; A12;	C8; T9; G10; A12; G13	–
	SER480; PRO501; LEU502;	G13; C14		
	ARG503; GLY504; LYS505;		GLY478; ARG503; GLY504;	ASP479
	GLN778; MET782		MET782	
Etoposide	LYS456; GLU477; GLY478; ASP479;	G7; C8; T9; G10; C11; A12;	C8; T9; G10; A12; G13	G13
	SER480; PRO501; LEU502;	G13; C14		
	ARG503; GLY504; LYS505; ILE565;		GLU477; GLY478; ARG503;	ASP479 (x2)
	GLN778; ALA779; MET782;		GLY504; GLN778; MET782;	HOH241
	ALA817; SER818; PRO819;		PRO819	HOH364
	ARG820			HOH1355

¹ The common hydrophobic and H-bond interactions are indicated in bold.

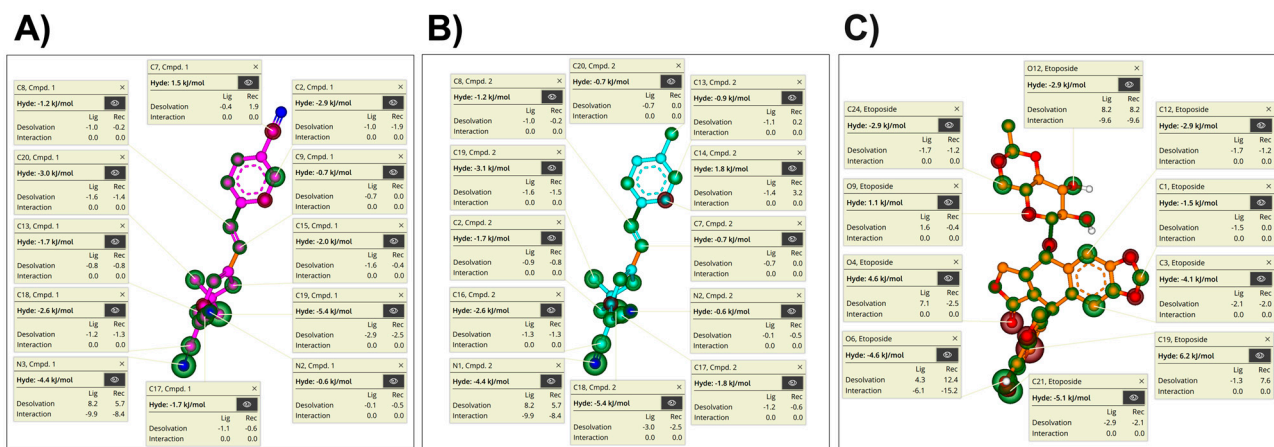


Figure S5. Complete HYDE visual assessment of compound 1 (A), compound 2 (B), and etoposide (C) obtained from their best docking poses within the binding pocket of hTOP2 β (cf. Fig. S3). The partial desolvation (lipophilic) and interaction (H-bonding) effects of all non-hydrogen (heavy atoms) of the ligand (Lig) and the receptor (Rec) that contributed to the overall binding affinity (the free energy of Gibbs $\Delta G = \Delta H - T\Delta S$, kJ/mol) of both compounds are colored using HYDE coronas: green = good, red = bad for affinity. Both compounds are shown as ball-and-stick model with the carbon atoms in off-white. Please consider that the atom numbering is given as generated from SeeSAR v.13 (www.biosolveit.de/seesar).

Table S2. Structure exploration of compounds **1** and **2** using Inspirator module in SeeSAR. The compounds predicted to be the most active ones in each series are indicated in bold, while the modifications within the respective core structure are presented in orange.

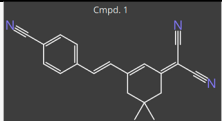
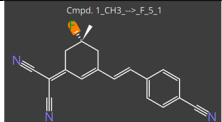
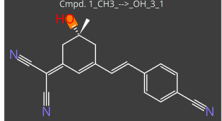
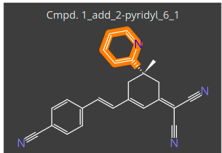
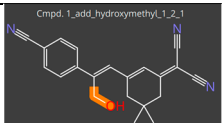
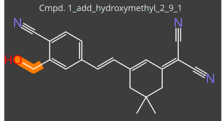
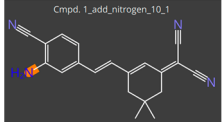
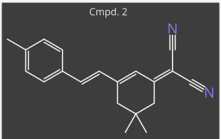
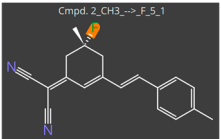
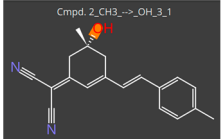
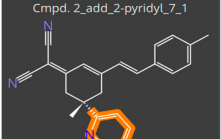
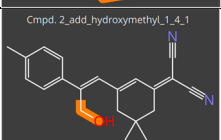
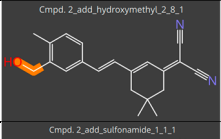
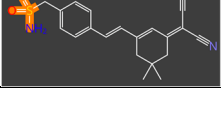
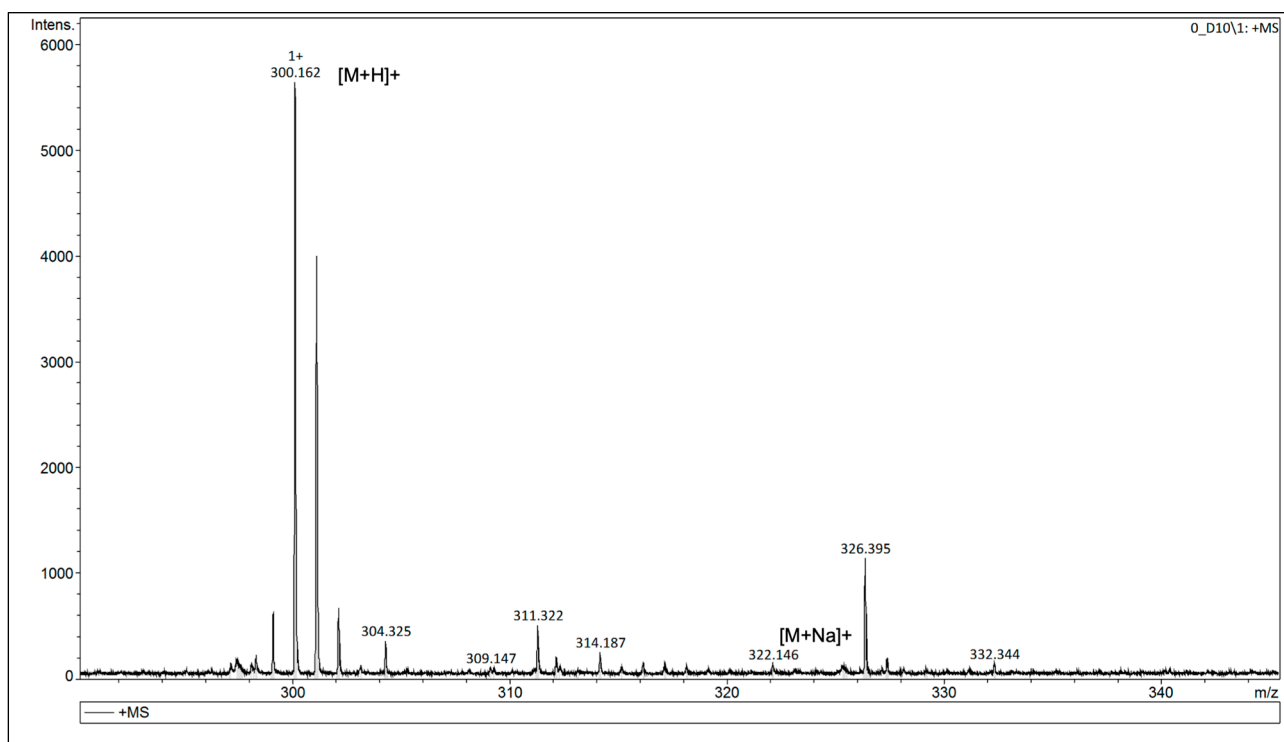
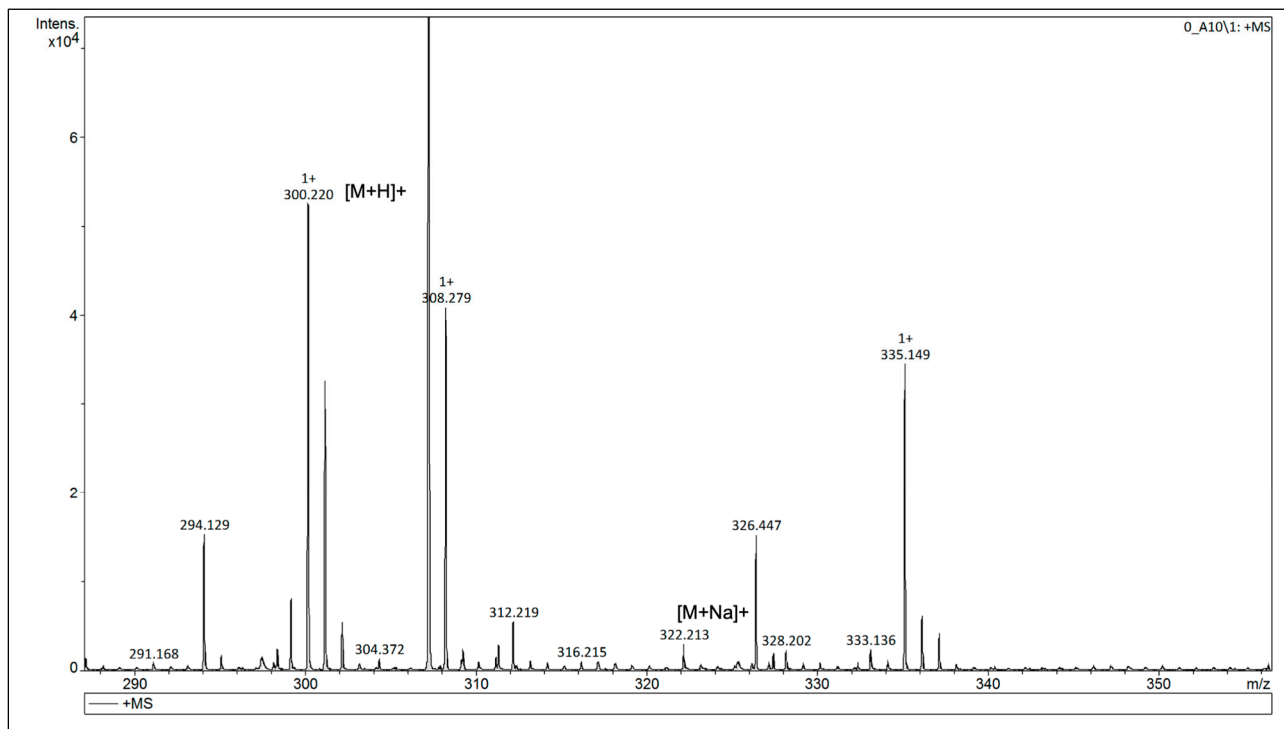
#	Name	2D molecule	SMILES	MW (Da)	LogP	TPSA [Å ²]	K _i HYDE (μM)	Torsion quality	Intra clash type	Inter clash type
1	Cmpd. 1		<chem>N#Cc1ccc(cc1)/C=C/C2=CC(=C(C#N)C#N)CC(C2)(C)C</chem>	299.3753	4.662	71.4	2.263–224.9	green	green	green
1_1	Cmpd. 1_CH3-->_F_5_1		<chem>F[C@]1(CC(=C(C#N)C#N)C=C/C=C/c2ccc(C#N)cc2)C1)C</chem>	303.3386	4.364	71.4	1.393–138.5	green	green	green
1_2	Cmpd. 1_CH3-->_OH_3_1		<chem>O[C@]1(CC(=C(C#N)C#N)C=C/C=C/c2ccc(C#N)cc2)C1)C</chem>	301.3475	3.386	91.6	11.1–1101	green	green	green
1_3	Cmpd. 1_add_2-pyridyl_6_1		<chem>N#Cc1ccc(cc1)/C=C/C2=CC(=C(C#N)C#N)C[C@@](c3ncccc3)(C2)C</chem>	362.4342	4.988	84.3	2.902–288.4	green	green	green
1_4	Cmpd. 1_add_hydroxymethyl_1_2_1		<chem>OC/C/c1ccc(C#N)cc1)=C\C2=C(C(=C(C#N)C#N)CC(C2)(C)C</chem>	329.4011	4.024	91.6	1.593–158.3	green	green	green
1_5	Cmpd. 1_add_hydroxymethyl_2_9_1		<chem>OCc1c(C#N)ccc(c1)/C=C/C2=CC(=C(C#N)C#N)CC(C2)(C)C</chem>	329.4011	4.154	91.6	5.712–567.6	green	green	green
1_6	Cmpd. 1_add_nitrogen_10_1		<chem>N#Cc1c(N)cc(cc1)/C=C/C2=CC(=C(C#N)C#N)CC(C2)(C)C</chem>	314.3902	4.244	97.4	0.353–35.1	green	green	green

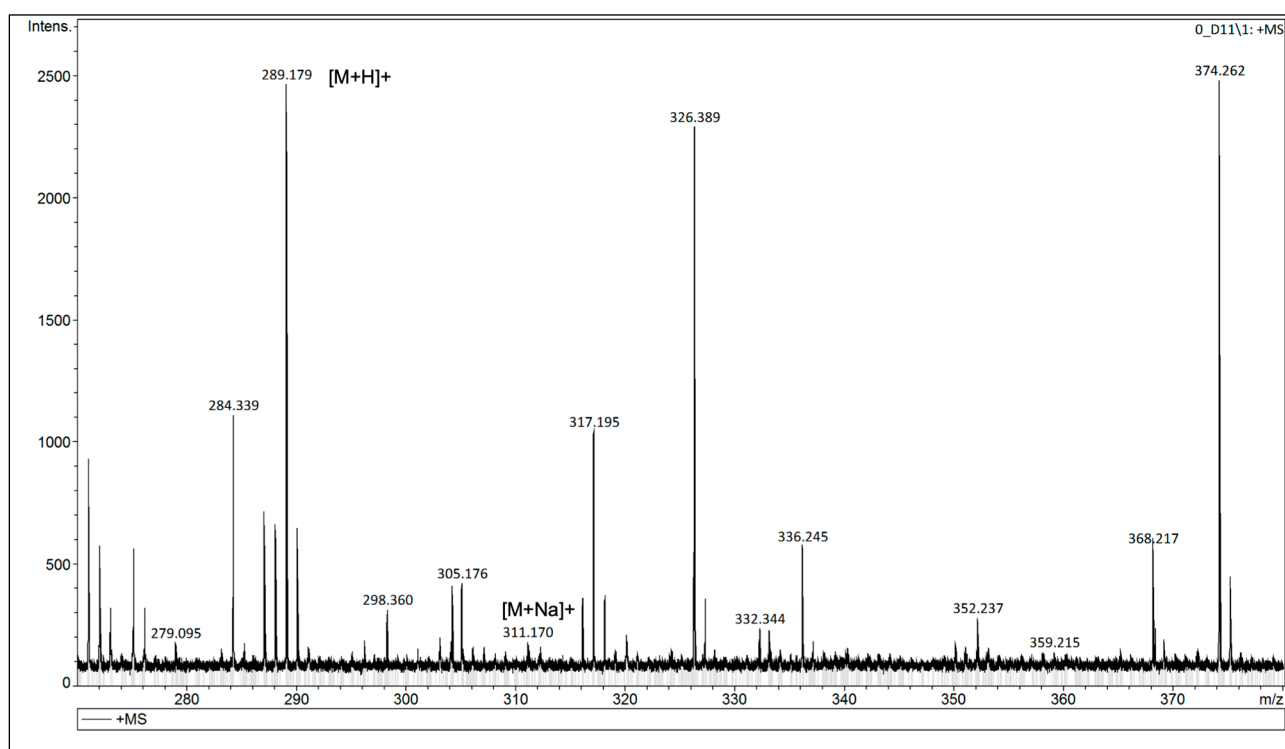
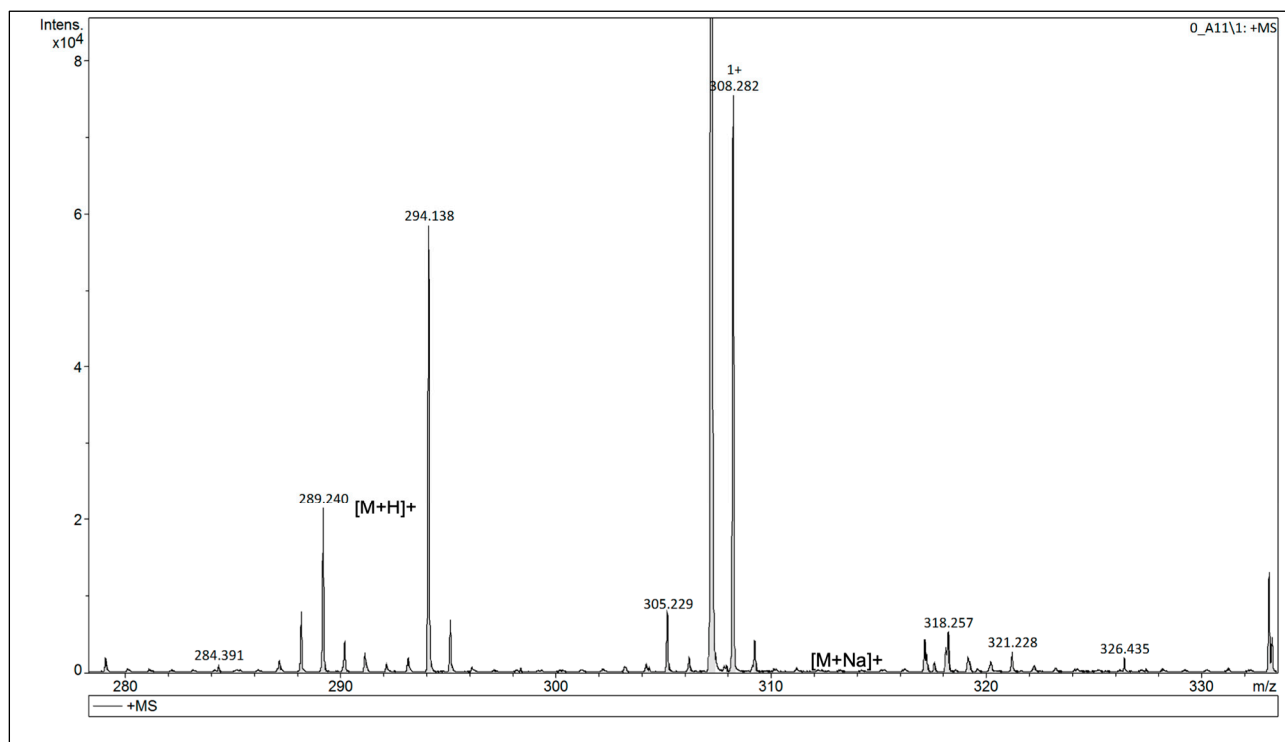
Table S2 (continued).

2	Cmpd. 2		<chem>N#CC(C#N)=C1C=C(/C=C/c2ccc(cc2)C)CC(C1)(C)C</chem>	288.392	5.098	47.6	2.839–282.2	green	green	green
2_1	Cmpd. 2_CH3_-->_F_5_1		<chem>F[C@]1(CC(=C(C#N)C#N)C=C(/C=C/c2ccc(cc2)C)C1)C</chem>	292.3553	4.800	47.6	1.531–152.2	green	green	green
2_2	Cmpd. 2_CH3_-->_OH_3_1		<chem>O[C@]1(CC(=C(C#N)C#N)C=C(/C=C/c2ccc(cc2)C)C1)C</chem>	290.3642	3.823	67.8	10.7–106.4	green	green	green
2_3	Cmpd. 2_add_2-pyridyl_7_1		<chem>N#CC(C#N)=C1C=C(/C=C/c2ccc(cc2)C)C[C@](c3ncccc3)(C1)C</chem>	351.4509	5.421	60.5	3.273–325.2	green	green	green
2_4	Cmpd. 2_add_hydroxymethyl_1_4_1		<chem>OC/C(/c1ccc(cc1)C)=C\C2=CC(=C(C#N)C#N)CC(C2)(C)C</chem>	318.4178	4.461	67.8	2.242–222.8	green	green	green
2_5	Cmpd. 2_add_hydroxymethyl_2_10_1		<chem>OCc1c(ccc(c1)C)/C=C/C2=CC(=C(C#N)C#N)CC(C2)(C)C</chem>	318.4178	4.591	67.8	3.429–340.7	green	green	green
2_6	Cmpd. 2_add_hydroxymethyl_2_8_1		<chem>OCc1c(ccc(c1)/C=C/C2=CC(=C(C#N)C#N)CC(C2)(C)C</chem>	318.4178	4.591	67.8	4.075–404.9	green	green	green
2_7	Cmpd. 2_add_sulfonamide_1_1_1		<chem>S(=O)(=O)(N)Cc1ccc(cc1)/C=C/C2=CC(=C(C#N)C#N)CC(C2)(C)C</chem>	367.4699	4.229	107.7	6.240–620.1	green	green	green

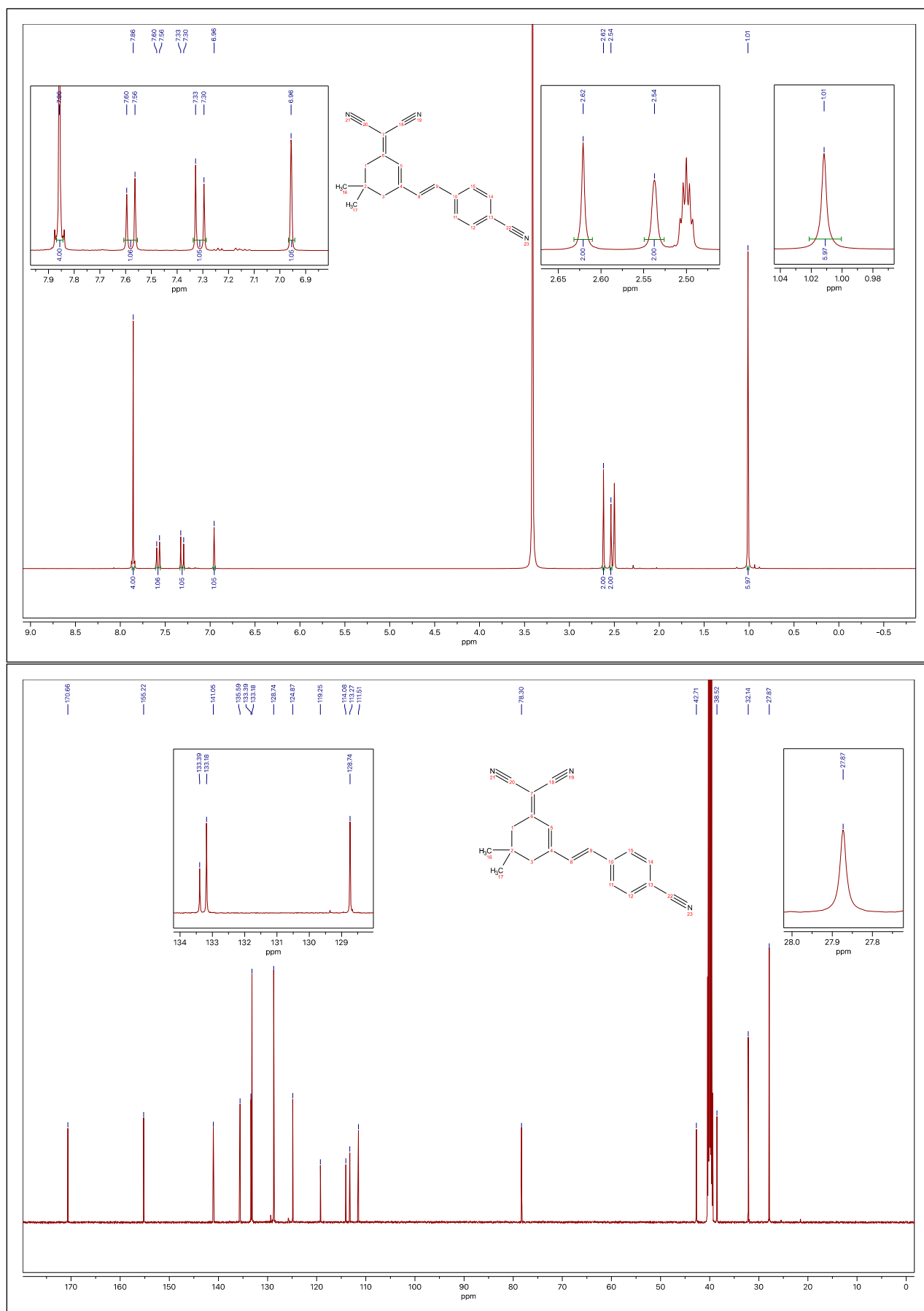
ESI-TOF-MS spectra of compound **1** measured by using α -cyano-4-hydroxycinnamic acid (top, HCCA) and sinapinic acid (bottom, SA) as a matrix. Data were analyzed using Bruker Compass DataAnalysis 4.4 software.



ESI-TOF-MS spectra of compound **2** measured by using α -cyano-4-hydroxycinnamic acid (top, HCCA) and sinapinic acid (bottom, SA) as a matrix. Data were analyzed using Bruker Compass DataAnalysis 4.4 software.



^1H NMR (top, 500 MHz) and ^{13}C NMR (bottom, 126 MHz) spectra of **compound 1** measured at 298 K in DMSO- d_6 .



^1H NMR (top, 500 MHz) and ^{13}C NMR (bottom, 126 MHz) spectra of **compound 2** measured at 298 K in $\text{DMSO-}d_6$.

