

Supporting information

Unveiling the anticancer potential of a new ciprofloxacin-chalcone hybrid as an inhibitor of topoisomerases I & II and apoptotic inducer

Doaa Mohamed Elroby Ali^{1†}, Hossameldin A. Aziz^{2†}, Stefan Bräse^{3*}, Areej Al Bahir⁴, Abdullah Alkhamash⁵, Gamal El-Din A. Abuo-Rahma^{6,7}, Ali M. Elshamsy⁷, Hamada Hashem^{*8}, Walid M. Abdelmagid⁹

¹Department of Biochemistry, Faculty of Pharmacy, Sohag University, Sohag, 82524, Egypt.

²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, New Valley University, New Valley, 72511, Egypt.

³Institute of Biological and Chemical Systems – Functional Molecular Systems (IBCS-FMS), Karlsruhe Institute of Technology (KIT), Kaiserstrasse 12, 76131 Karlsruhe

⁴Chemistry Department, Faculty of Science, King Khalid University, Abha 64734, Saudi Arabia

⁵Department of Pharmacology, College of Pharmacy, Shaqra University, Shaqra, 11961, Saudi Arabia

⁶Department of Medicinal Chemistry, Faculty of Pharmacy, Minia University, Minia 61519, Egypt.

⁷Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Deraia University, New Minia City, 61768, Egypt.

⁸Pharmaceutical Chemistry Department, Faculty of Pharmacy, Sohag University, Sohag, 82524, Egypt

⁹Medicinal Chemistry and Drug Discovery Research Centre, Swenam College, 210-6125 Sussex Avenue, Burnaby, BC, V5H 4G1, Canada

[†]Both authors contributed equally to this work.

*Correspondence: hamada.hashem@pharm.sohag.edu.eg (H.H.); stefan.braese@kit.edu (S.B.)

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1. Chemistry

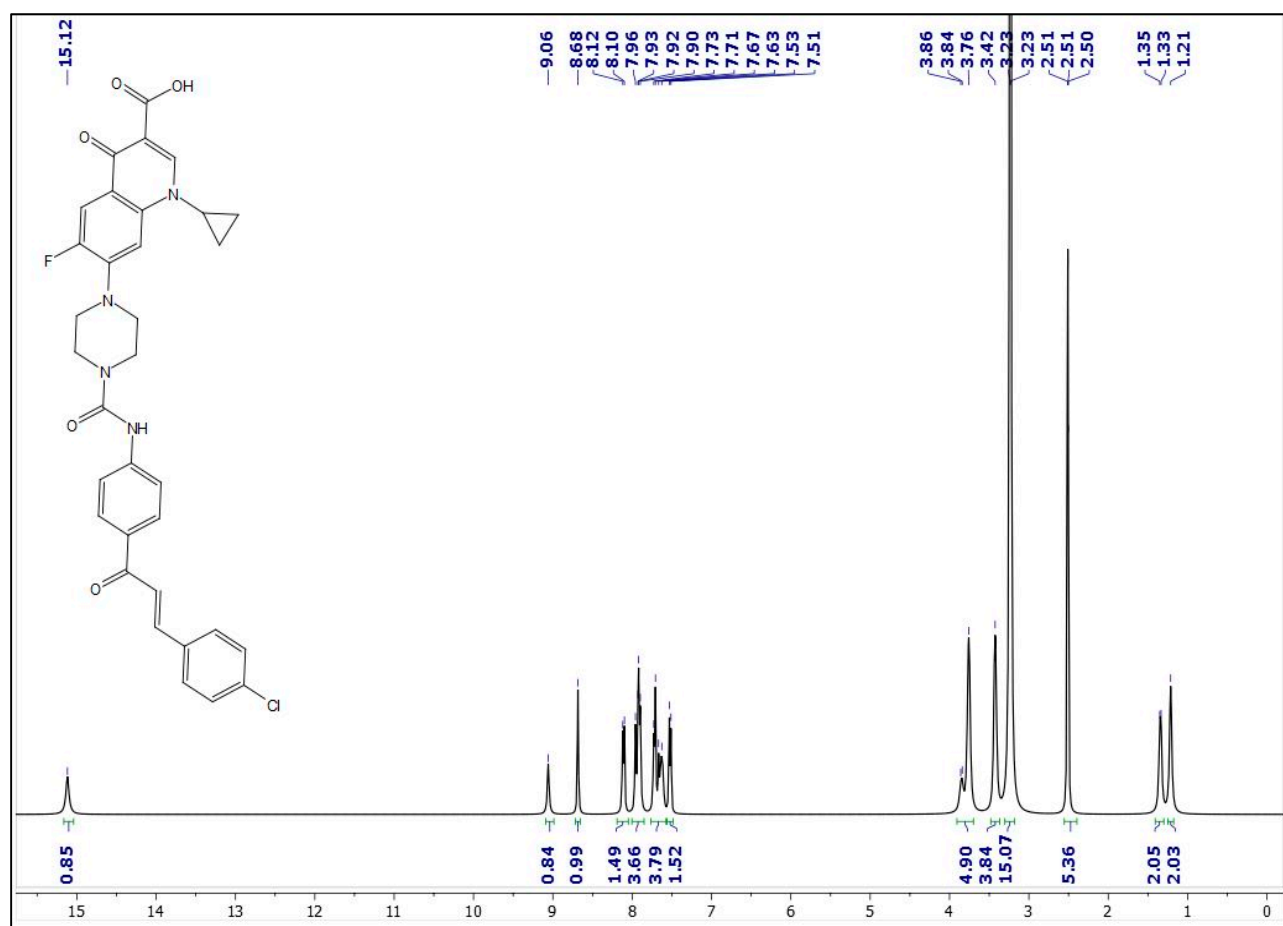


Figure S1: ¹H NMR spectrum of compound CP-derivative (400 MHz, CDCl₃)

2. Biology

2.1. Screening of the anticancer activity at 10 μ M

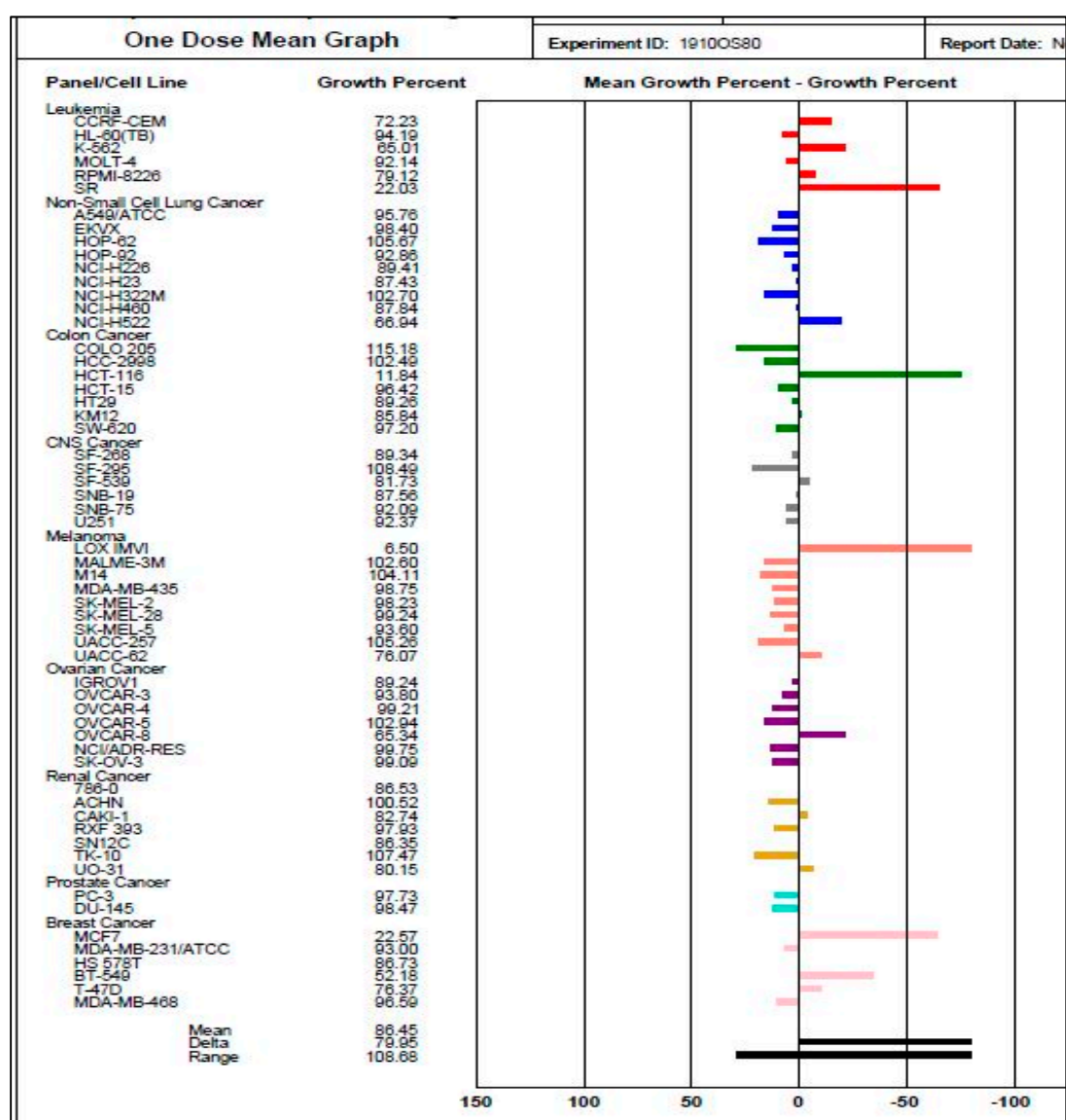


Figure S2: In vitro screening of the anticancer activity at single dose of 10 μ M

2.2. *Cell viability Assay*

Table S1. Cytotoxicity results of CP-derivative in comparison to Staurosporine as a positive control against HCT-116 cancer cell line.

Ser	Sample		cytotoxicity IC ₅₀ uM		
	Code	M.W g/mol	HCT116	LOX IMVI	WI-38
1	CP-derivative		4.98±0.27	1.26 ± 0.02	15.96± 0.72
2	Staurosporine		8.29±0.39	1.63 ± 0.04	13.34 ± 0.54

2.3. *Annexin V assay and cell cycle analysis using flowcytometry*

Table S2. Cell cycle phase distribution of untreated cells and cells treated with the IC₅₀ concentration of CP-derivative for 24 h.

ser	Sample data		Results DNA content				
	code	IC ₅₀ μ M	%G0-G1	%S	%G2/M	%Pre-G1	Comment
1	CP-derivative		55.16	42.06	1.78	39.21	Cell growth arrest@ G2/M
2	control		51.36	39.42	9.22	1.99	

Table S3. The apoptosis and necrosis assay of colon HCT-116 induced by DMSO (control, A), and CP-derivative (IC₅₀, 4.98 μ M, B).

s	code	Conc	Apoptosis			Necrosis
			Total	Early	Late	
1	CP-derivative		39.21	1.56	23.53	14.12
2	control		1.99	0.73	0.12	1.14

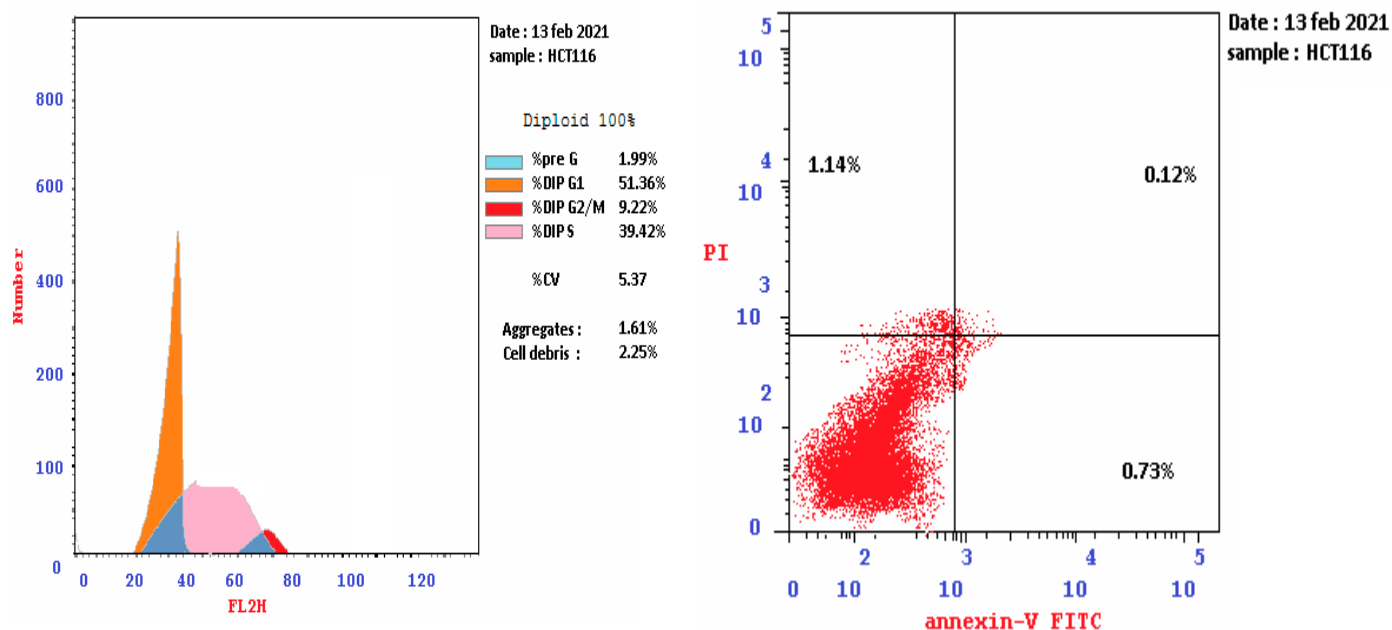


Figure S3. Cell cycle analysis and apoptosis of untreated HCT-116.

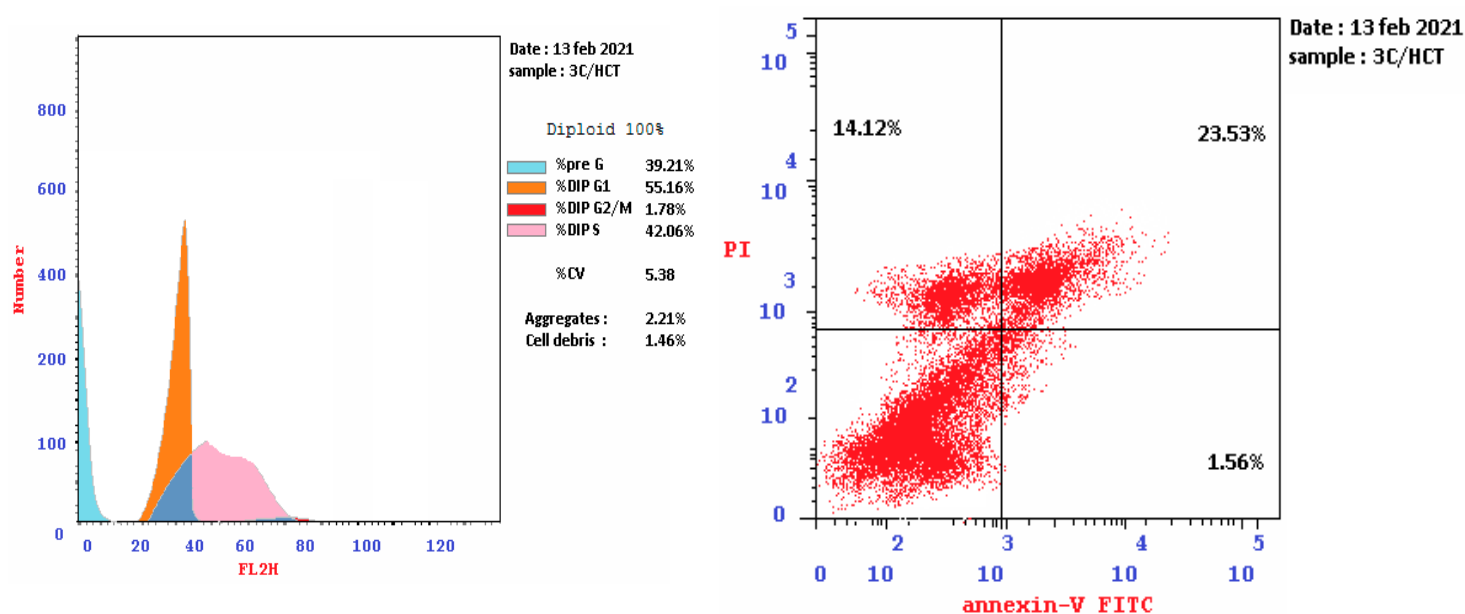


Figure S4. Cell cycle analysis and apoptosis of HCT-116 treated with IC50 of CP-derivative

2.4 Expression of Bax, Bcl-2 and Caspase 9 genes

Table S4. Expression of Bax, Bcl-2 and Caspase 9 genes

Ser	Sample			Gene Expression Fold Change			
	code	conc	Cells	Bax fld	Bcl2 fld	Casp9 fld	
	Cp-derivative		HCT116	4.614	0.320	5.573	
	control			1	1	1	

Detailed results



14 Hilly Street Mortlake NSW 2137 Australia
T + 61 2 9736 1320
F + 61 2 9736 1364
W www.corbettlifescience.com

Quantitation Report

Experiment Information

Run Name	Run 2021-02-13 (1)
Run Start	2021-02-13 02:42:26 PM
Run Finish	2021-02-13 05:11:05 PM
Operator	ERA
Notes	---
Run On Software Version	Rotor-Gene 1.7.87
Run Signature	The Run Signature is valid.
Gain Green	10.
Gain Yellow	9.33

Quantitation data

This report generated by Rotor-Gene 6000 Series Software 1.7 (Build 87)
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primers

CASP9-F 5'-TCA GTG ACG TCT GTG TTC AGG AGA-3'
 CASP9-R 5'-TTG TTG ATG ATG AGG CAG TAG CCG-3'

Bax F 5'-ATGTTTTCTGACGGCAACTTC-3'
 Bax R 5'-AGTCCAATGTCCAGCCCAT-3'

Bcl2 F 5'-ATGTGTGTGGAGACCGTCAA-3'
 Bcl2 R 5'-GCCGTACAGTTCCACAAAGG-3'

β-actin F 5'-GTGACATCCACACCCAGAGG-3'
β-actin R 5'-ACAGGATGTCAAACTGCCC-3'

Table S5. Expression of Bax, Bcl-2 and Caspase 9 genes

Sample			Bax							
Ser	code	Conc	Control cells			Test cells			FLD	
			B Actin	Bax	ΔCTC	B Actin	Bax	ΔCTE	ΔΔ CT	2 ^Δ ΔΔCT
			HC	TC	TC-HC	HE	TE	TE-HE	ΔCTE-ΔCTC	E _{amp} =1.839
1	3C		23.51	33.49	9.98	23.88	31.35	7.47	-2.51	4.614239582
2	control		23.51	33.49	9.98	23.51	33.49	9.98	0	1

Sample			Bcl2							
Ser	code	Conc	Control cells			Test cells			FLD	
			B Actin	Bcl2	ΔCTC	B Actin	Bcl2	ΔCTE	ΔΔ CT	2 ^Δ ΔΔCT
			HC	TC	TC-HC	HE	TE	TE-HE	ΔCTE-ΔCTC	E _{amp} =1.839
1	3C		23.51	28.94	5.43	23.88	31.18	7.3	1.87	0.320060564
2	control		23.51	28.94	5.43	23.51	28.94	5.43	0	1

Sample			Casp9							
Ser	code	Conc	Control cells			Test cells			FLD	
			B Actin	Casp9	ΔCTC	B Actin	Casp9	ΔCTE	ΔΔ CT	2 ^Δ ΔΔCT
			HC	TC	TC-HC	HE	TE	TE-HE	ΔCTE-ΔCTC	E _{amp} =1.839
1	3C		23.51	32.91	9.4	23.88	30.46	6.58	-2.82	5.573403378
2	control		23.51	32.91	9.4	23.51	32.91	9.4	0	1

Topoisomerase I assay

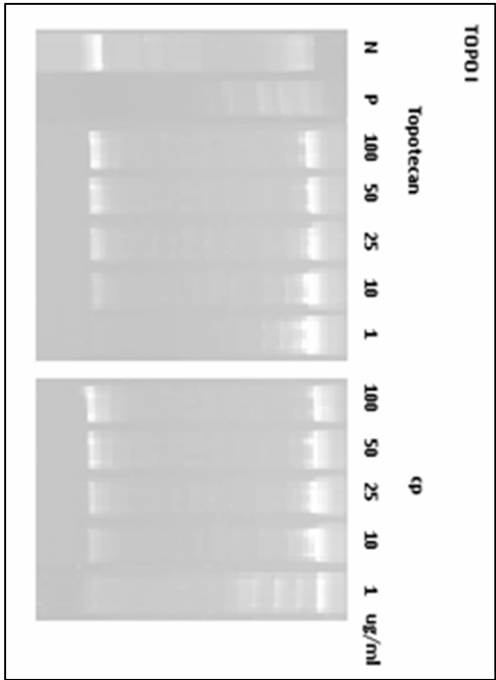
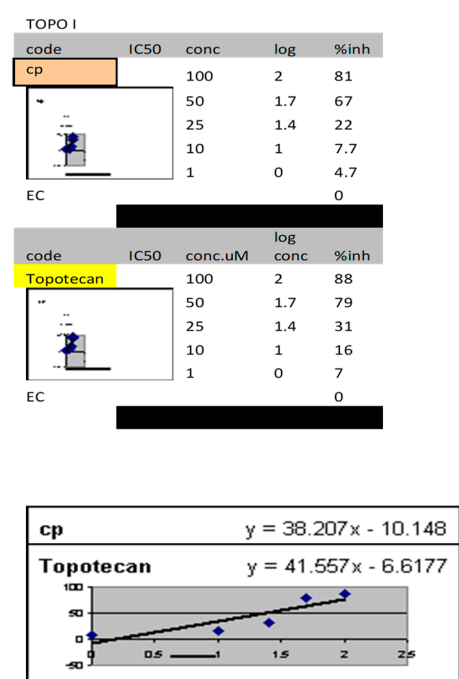


Figure S5. topoisomerase assay of CP-derivative versus topotecan

Detailed results :



Topoisomerase II assay

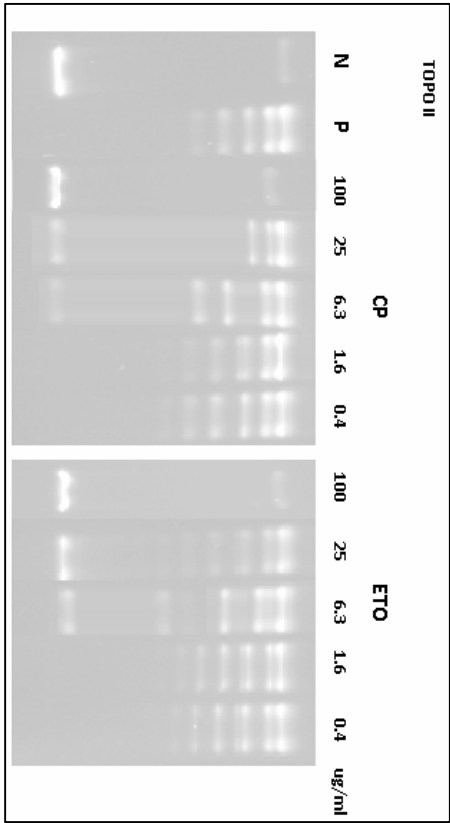


Figure S6. topoisomerase assay of CP-derivative versus topotecan

Detailed results:

TOPO II				
code	IC50	conc	log	%inh
cp		100	2	92
		50	1.7	75
		25	1.4	38
		10	1	20
		1	0	7.6
				0
EC				
code	IC50	conc	log	%inh
Etoposide		100	2	90
		50	1.7	63
		25	1.4	28
		10	1	15
		1	0	3.8
				0
EC				

Docking studies

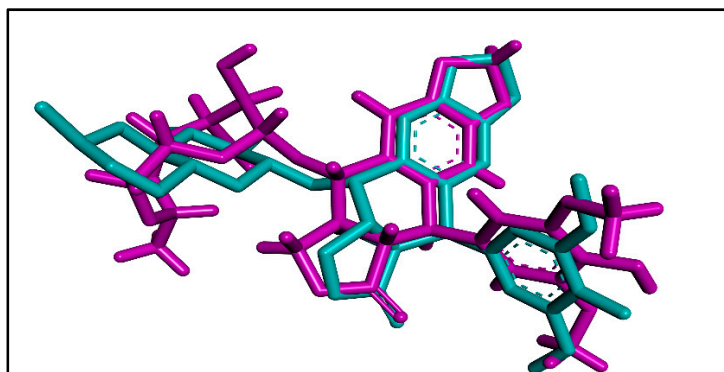


Figure S7. The superimposition of the redocked (**violet** color) and co-crystallized ligand (**blue** color) poses of etoposide.

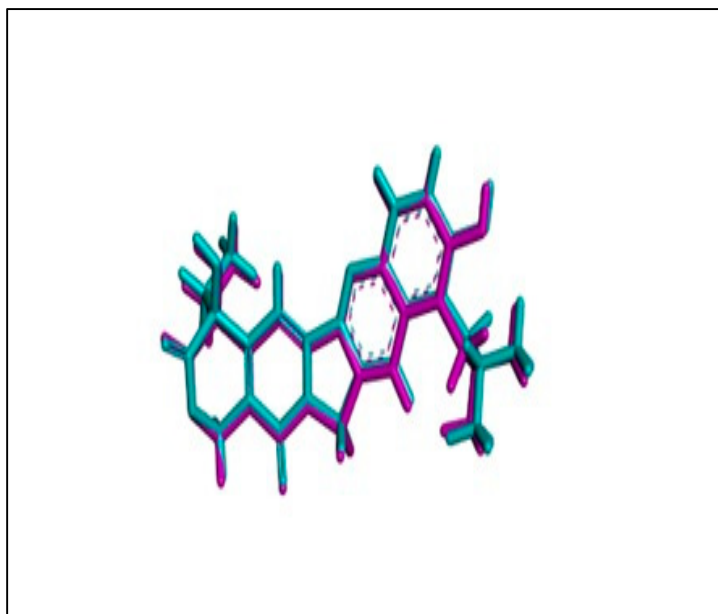


Figure S8. The superimposition of the redocked (**violet** color) and co-crystallized ligand (**blue** color) poses of Topotecan.