A NEW STRATEGY TO IMPROVE THE METABOLIC STABILITY OF LACTONE:

Discovery of (20*S*,21*S*)-21-Fluorocamptothecins as Novel, Hydrolytically Stable Topoisomerase I Inhibitors

Miao, Z. et al., *J. Med. Chem.* **2013**, *56*, 7902-7910.

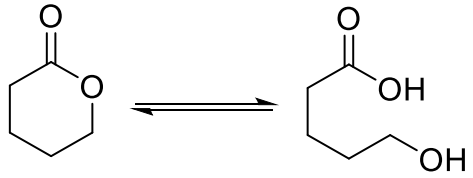
Current Literature

11/9/2013

Celeste Alverez

BACKGROUND

- Lactones are readily hydrolyzed in the plasma to the open acid form



- Metabolism by CYP450 also common
  - More common on the lactone than the hydrolyzed acids
  - Potentially leads to further deactivation of therapeutic agent
- Hydrolysis can be beneficial or negative to activity
  - Statins: acid form is active
  - Camptothecins: lactone is active

Yi, L. Anal. Chem. **2005**, *77*, 6655.
Fujino, H.; Kojima, J. Drug Metabolism and Transporter Profiles of Statins. In *Focus on Statin Research*, Wong, B.A., Ed; Nova Science Publishers: New York, 2006, p. 137.

CAMPTOTHECIN

- Discovered in 1966 by M.E. Wall and M.C. Wani
- From a screen of 1000 plant extracts only those from the bark and stem *Camptotheca acuminata* had high antitumor activity against CA-755 (adenocarcinoma cell line)
- Camptothecin was found to be the most active component
  - Showed prolongation of life in leukemic mice at doses as low as 0.5 mg/kg
  - Active in solid tumors (Walker 256 cells, Yoshida sarcoma)
- Target is topoisomerase I
- Made it into early clinical trials
  - Terminated due to low solubility, low metabolic stability, and high hepatotoxicty

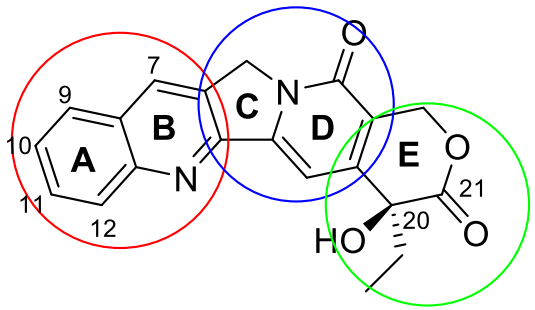
Wall, M. E. *Cancer Res.* **1995**, *55*, 753.
Miao, Z. *J. Med. Chem.* **2013**, *56*, 7902.

CAMPTOTHECIN

- Numerous total syntheses, semi-syntheses, and formal syntheses
  - Allowing for analog development
- SAR:

A B rings
- Substitution at 7, 9, 10, 11 increase potency and metabolic stability
- Hexaxcylic core (between 10-11 or 7-9) enhance potency
- Substitution at 12 not tolerated

C D rings
- Changes decrease potency



E ring
- Ring expansion leads to enhanced potency
- Carbonyl thought to be essential (reduction to alcohol = loss of activity)

ANALOGS

- Irinotecan:
  - FDA approved for colon, pancreatic, and ovarian cancers
- Topotecan:
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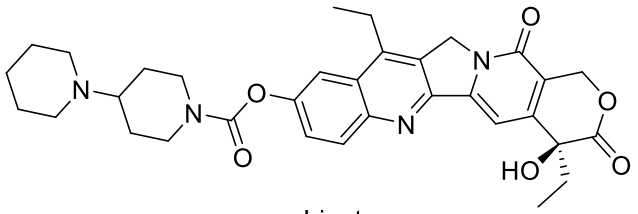
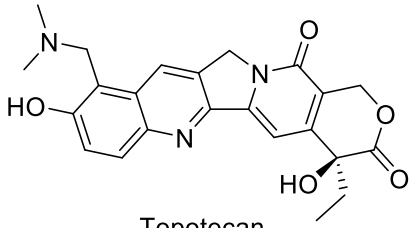


Irinotecan



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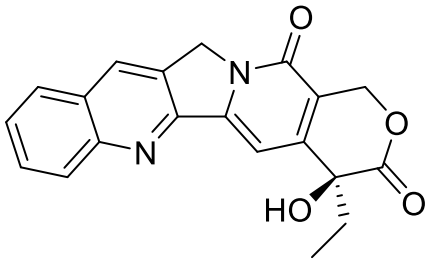

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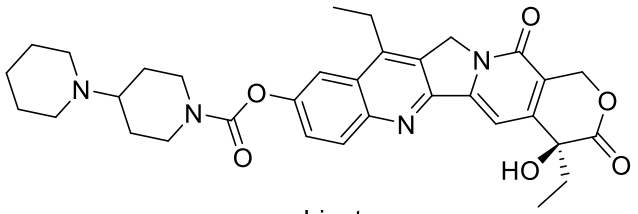

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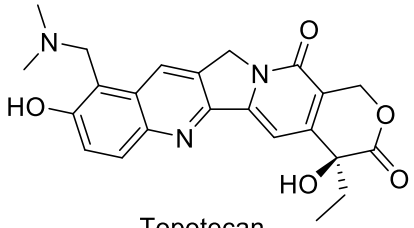

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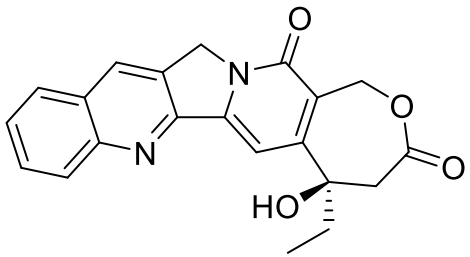

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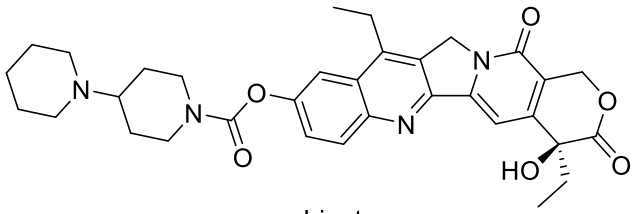

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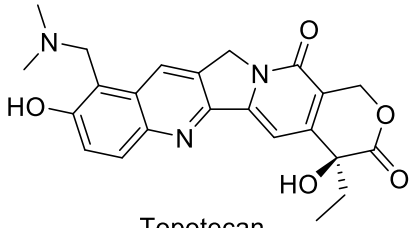

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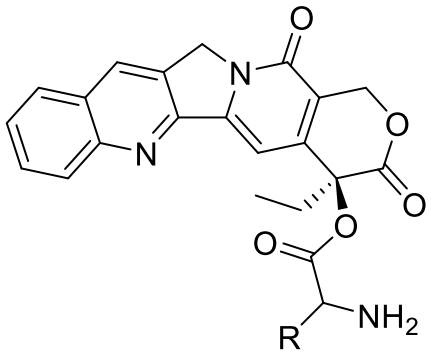

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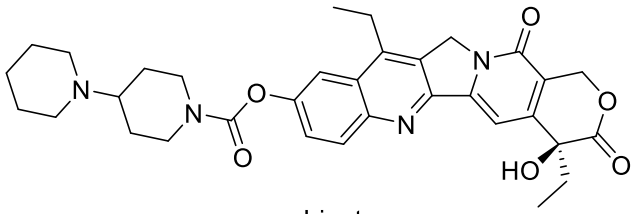

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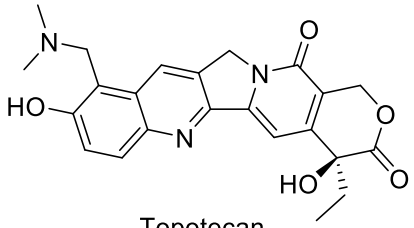

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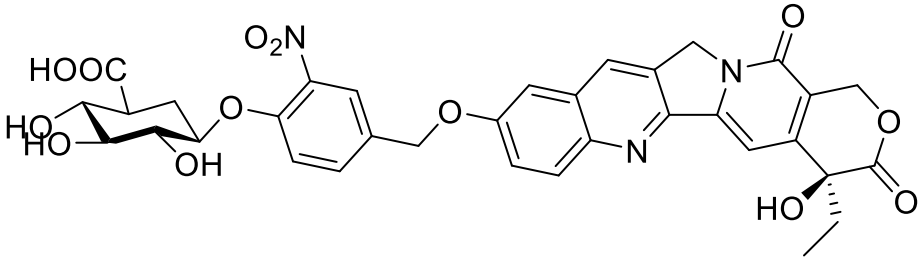

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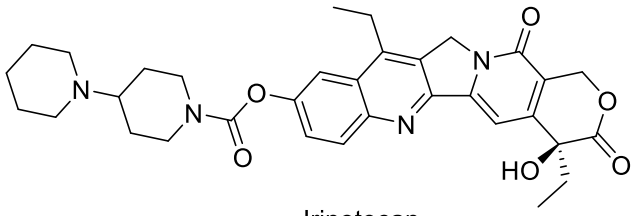

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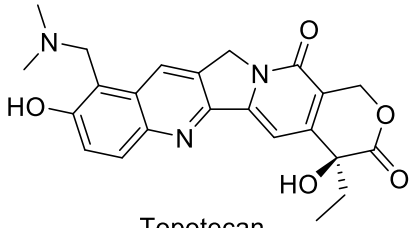

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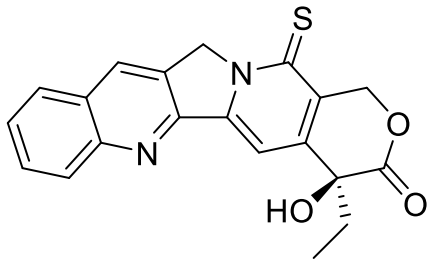

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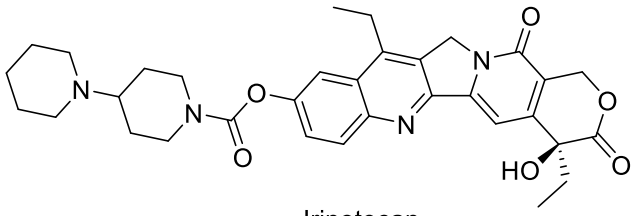

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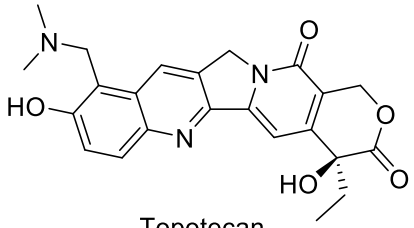

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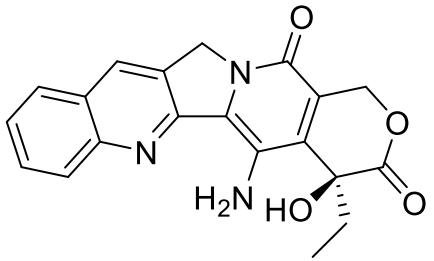

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BIOISOSTERE

- Groups or substituents that are physically or chemically similar which result in similar biological activity
- Goal of use is to reduced toxicity, increase potency, altering physical properties, reduce metabolism, ect
- Classic: based on valencies; atoms, ions, and molecules with the same number of valent electrons

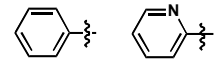
1) Monovalent atoms or groups
D and H
F and H
NH_2 and OH
RSH and ROH
F, OH, NH_2 and CH_3
Cl, Br, SH and OH

2) Divalent atoms or groups
C=C, C=N, C=O, C=S
$-CH_2-$, -NH-, -O- , -S-

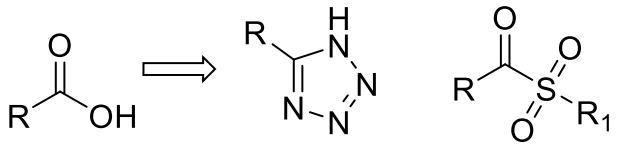
3) Trivalent atoms or groups
-CH=, -N=

4) Tetrasubstituted atoms
R_4C, R_4Si, R_4N^+

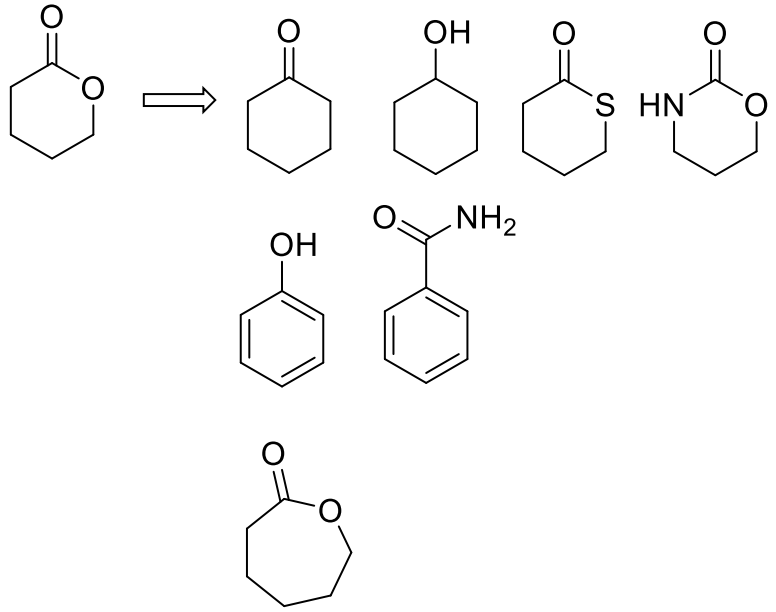
5) Ring equivalent



- Non-classical: structurally distinct, have steric and electronic properties



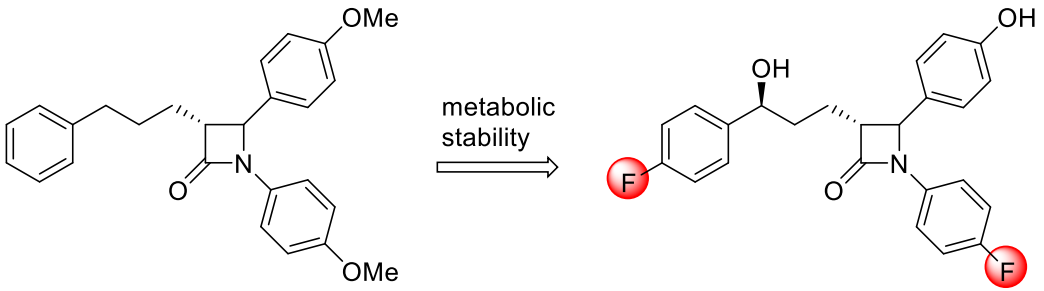
LACTONE BIOISOSTERES



- More stable, potential new metabolism problems
- Potential for improvement

FLUORINATION

- H → F is one of the most common isostere substitutions:
  - About 20% of all drugs are fluorinated
- Fluorine is of similar small size
- Can modulate basicity/acidity
- Increases lipophilicity
- Increases metabolic stability



Lead compound

ED_{50} (rat) = 2.0 mg/kg

Ezetimib

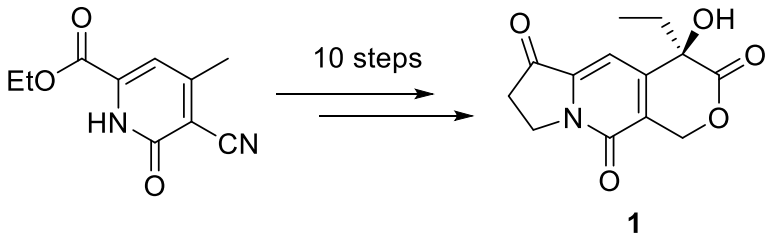
ED_{50} (rat) = 0.03 mg/kg

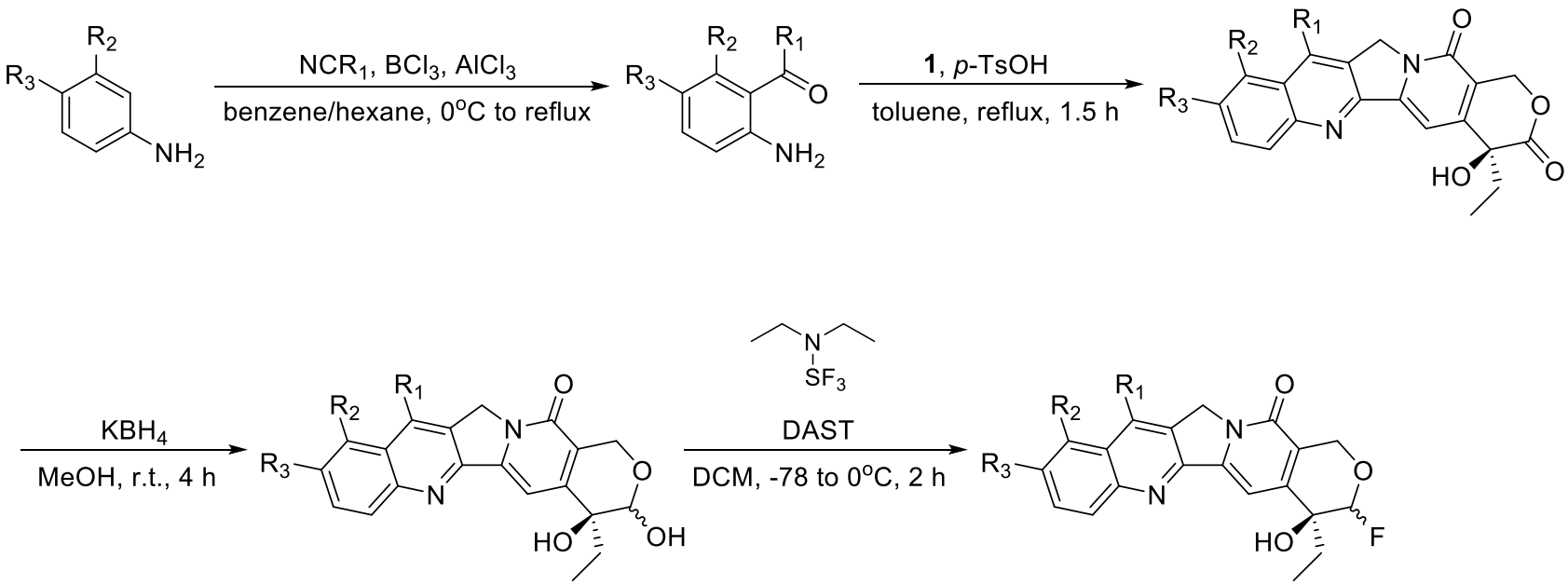
α-FLUORO ETHER AS A ISOSTERE

- C-F bond electrostatic properties are similar to a C=O
- C-F can form protein-ligand interactions similarly to carbonyl or differently – potentially enhancing potency
  - Similar dipole interactions – possibly H-F bonding
  - More lipophilic and can enhance hydrophobic interactions
- Fluorination will affect physiochemical properties
- Readily obtained isostere from carbonyl parent

- Obtain SAR around C-21 position

SYNTHESIS





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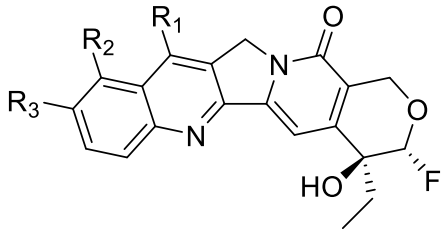
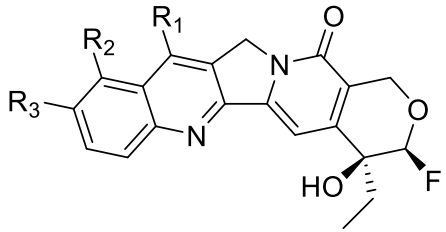


5a: R_1= R_2= R_3= H

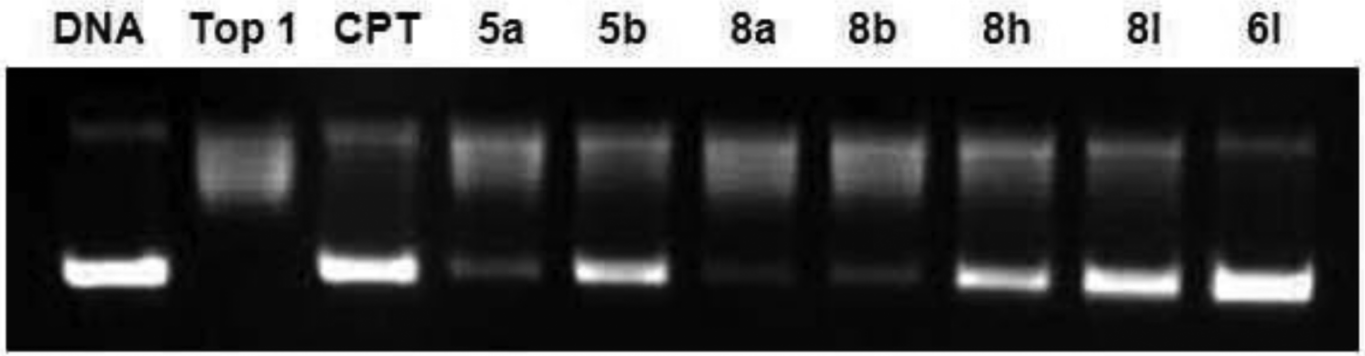


5b: R_1= R_2= R_3= H

8a: R_1= H, R_2= NO_2, R_3= H
8b: R_1= H, R_2= H, R_3= OMe
8c: R_1= Et, R_2= H, R_3= OMe

8d: R_1= Me, R_2= H, R_3= H
8e: R_1= Et, R_2= H, R_3= H
8f: R_1= *n*-Pr, R_2= H, R_3= H
8g: R_1= *i*-Pr, R_2= H, R_3= H
8h: R_1= *n*-Bu, R_2= H, R_3= H
8i: R_1= cyclopropyl, R_2= H, R_3= H
8j: R_1= cyclobutyl, R_2= H, R_3= H
8k: R_1= cyclopentyl, R_2= H, R_3= H
8l: R_1= cyclohexyl, R_2= H, R_3= H

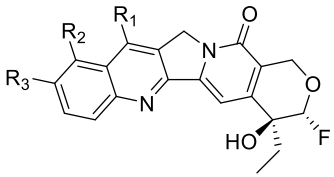
RESULTS



RESULTS

Table 1. In Vitro Antitumor Activity of 21-Fluorocamptothecin Diastereoisomers against Three Cancer Cell Lines (IC_{50}, μM)

compd	A549	MDA-MB-435	HCT116
5a	46.21	>100	50.91
5b	9.95	58.33	6.35
CPT	0.65	0.45	0.07



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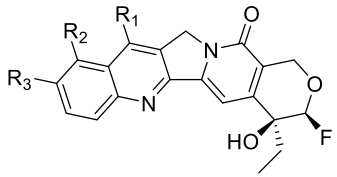

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8f: R_1= *n*-Pr, R_2= H, R_3= H
8g: R_1= *i*-Pr, R_2= H, R_3= H
8h: R_1= *n*-Bu, R_2= H, R_3= H
8i: R_1= cyclopropyl, R_2= H, R_3= H
8j: R_1= cyclobutyl, R_2= H, R_3= H
8k: R_1= cyclopentyl, R_2= H, R_3= H
8l: R_1= cyclohexyl, R_2= H, R_3= H

Table 2. In Vitro Antitumor Activity of (20*S*,21*S*)-Fluorocamptothecins against Three Cancer Cells (IC_{50}, μM)

compd	A549	MDA-MB-435	HCT116
8a	11.35	53.02	1.02
8b	41.72	>100	6.15
8c	6.35	12.67	1.45
8d	4.23	8.66	1.46
8e	12.74	10.89	8.53
8f	2.54	2.32	0.82
8g	>100	41.14	65.71
8h	17.70	3.53	0.27
8i	15.46	9.41	3.39
8j	1.00	15.79	95.39
8k	4.14	11.80	19.80
8l	0.71	0.41	0.07
CPT	1.05	<0.001	0.38

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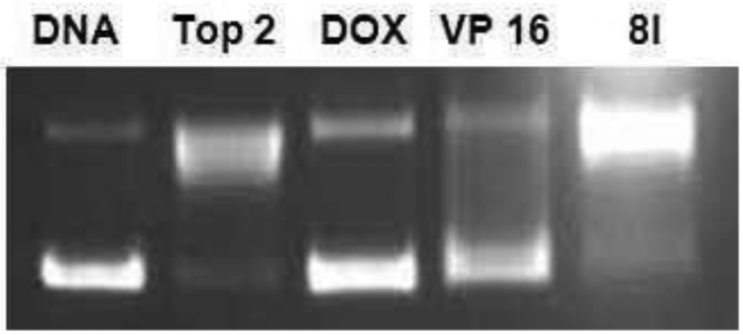
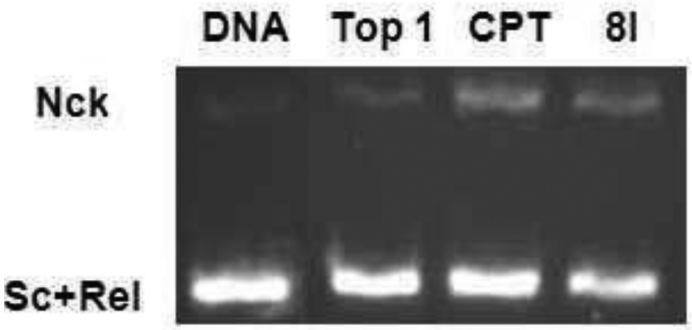
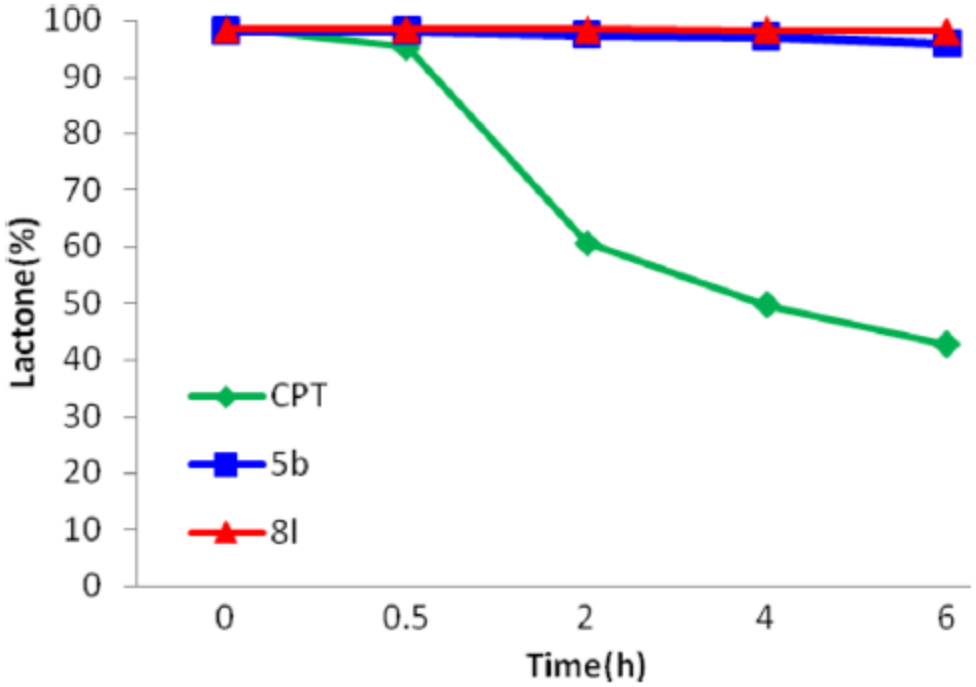
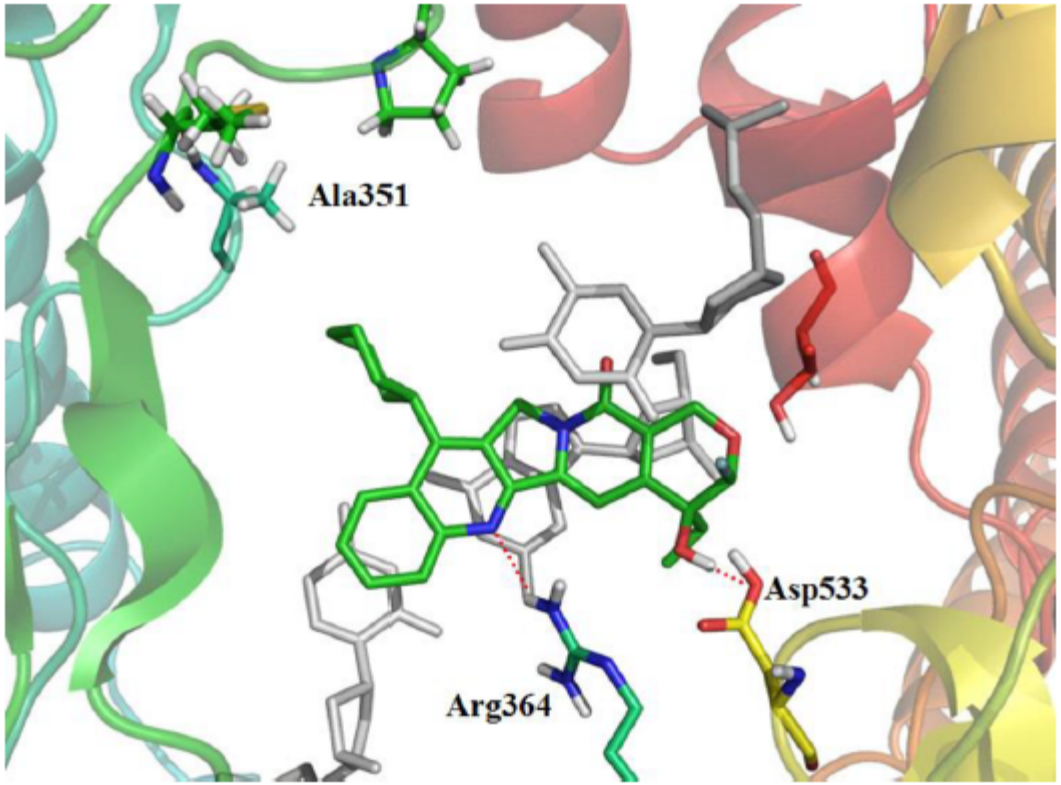




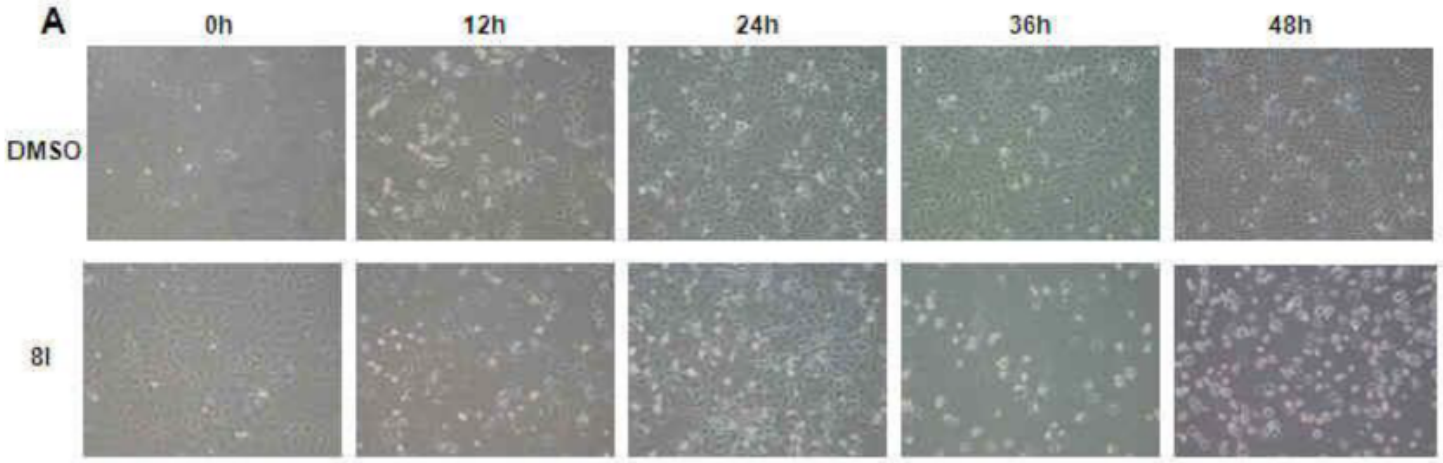
RESULTS

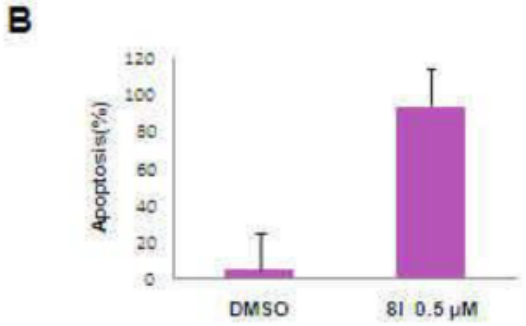


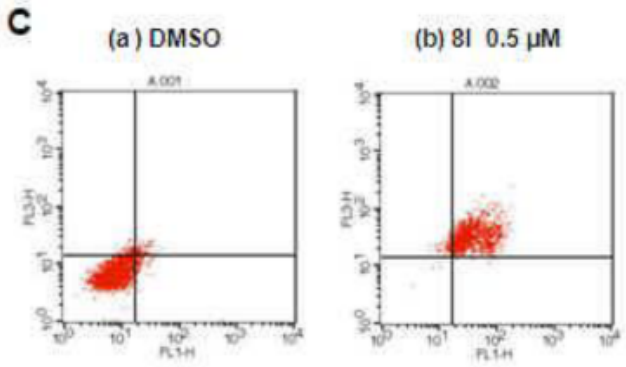
RESULTS



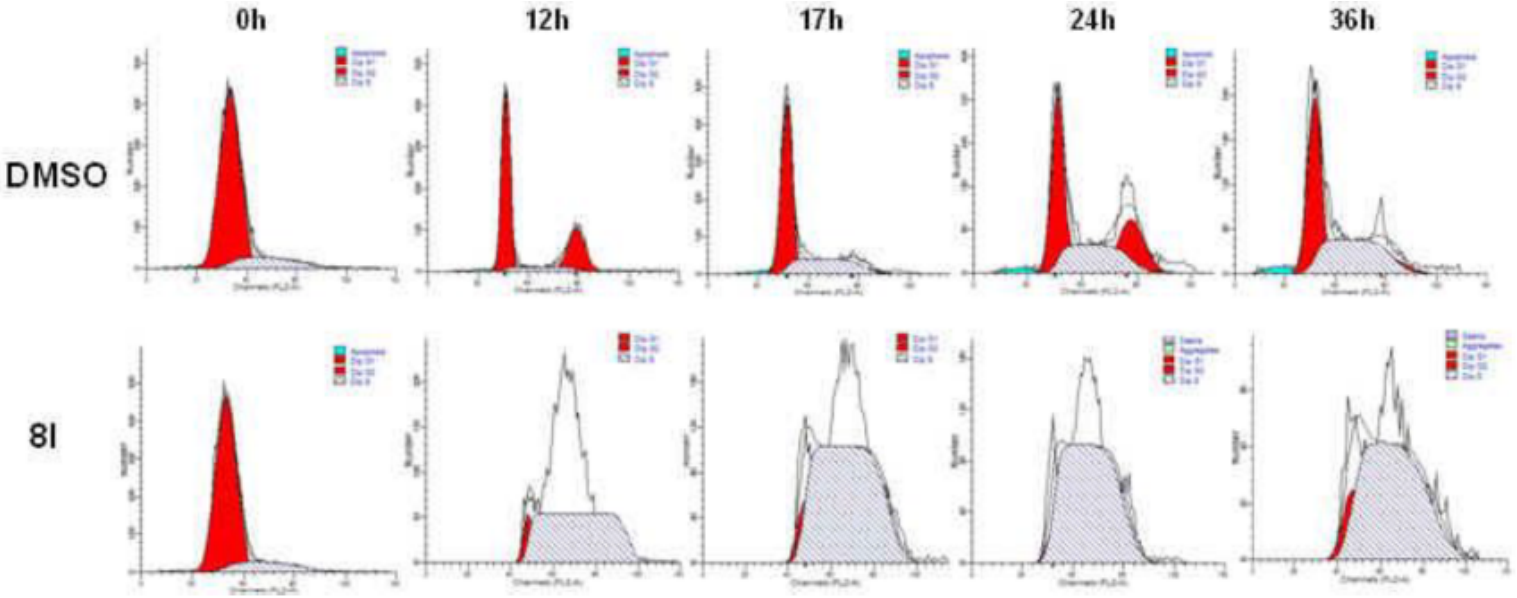
RESULTS


A
0h
12h
24h
36h
48h
DMSO
8l

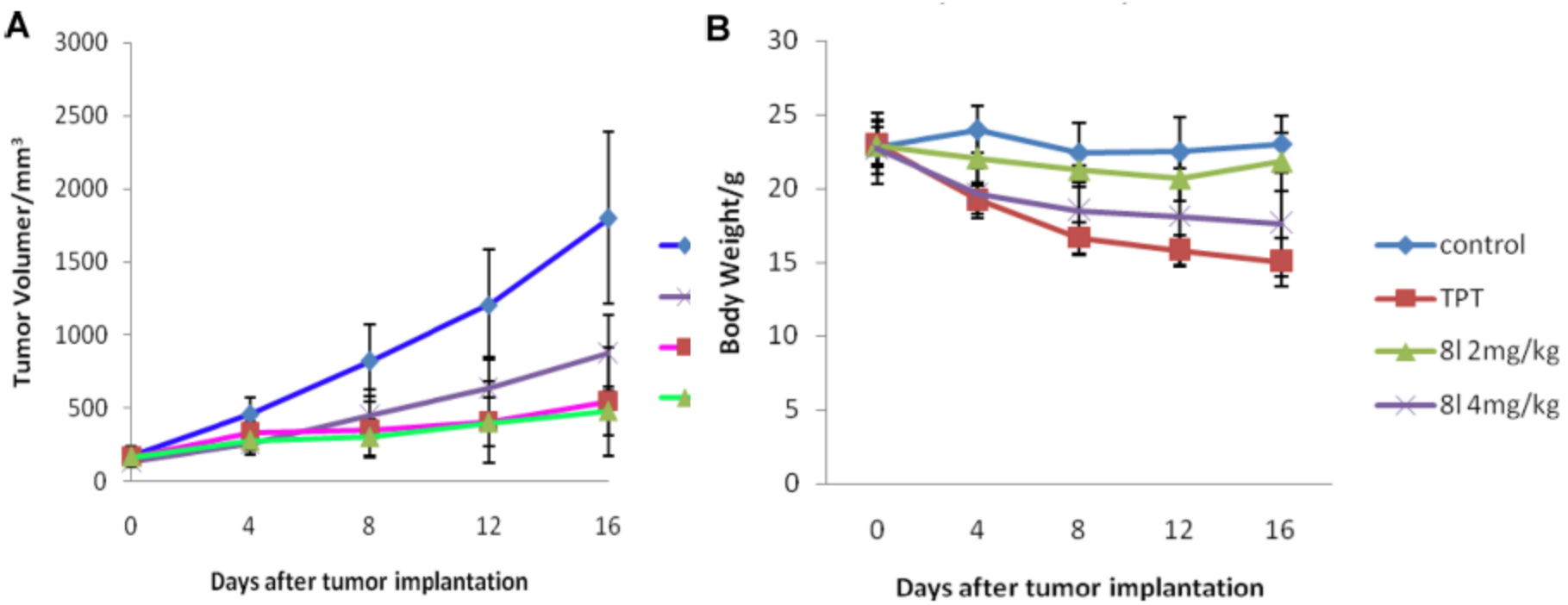

B
Apoptosis(%)
120
100
80
60
40
20
0
DMSO
8l 0.5 μM


C
(a) DMSO
(b) 8l 0.5 μM

RESULTS

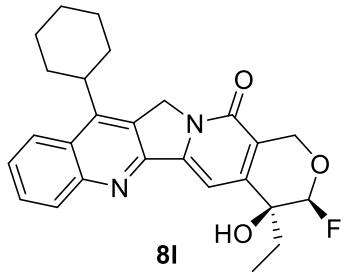


RESULTS


A
Tumor Volumer/mm³
3000
2500
2000
1500
1000
500
0
0
4
8
12
16
Days after tumor implantation
B
Body Weight/g
30
25
20
15
10
5
0
0
4
8
12
16
Days after tumor implantation
control
TPT
8l 2mg/kg
8l 4mg/kg

CONCLUSION

- Successfully designed and synthesized α-fluoro ether for first time
- Experimental and *in silico* studies show that replacement of lactone with α-fluoro ether is an effective bioisostere of lactone
- α-fluoro ethers are a more stable bioisostere for lactones
  - General use needs to be investigated
- SAR at C-21 shows that the carbonyl was not necessary for activity as previously thought
- **8l** is a new camptothecin effective analog that can be studied for further optimization



8l