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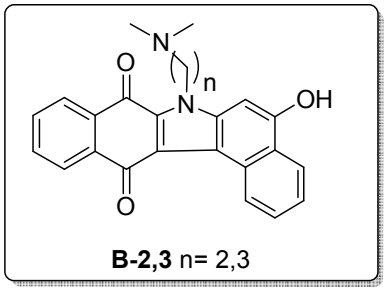

B-2,3 n= 2,3

Cmpd.	IC_{50} (μ M)				
	HCT116	**PC3**	**U87MG**	**HepG2**	**SK-OV-3**
B-2	0.7064	0.3889	**0.1694**	0.8443	1.010
B-3	0.9956	0.6119	**0.3249**	1.974	0.7829

Compounds **B-2** and **B-3** exhibit the most potent activity against U87MG cell growth with IC_{50} of 169 nM and 325 nM, respectively.

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Design, synthesis and antitumor activity of non-camptothecin topoisomerase I inhibitors

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State Key Laboratory of Natural and Biomimetic Drugs; Department of Chemical Biology, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China

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ABSTRACT

Three groups of non-camptothecin compounds with four to five fused rings have been designed and synthesized. Their in vitro anti-proliferative activity has been evaluated with five different cancer cell lines (HCT116, PC3, U87MG, HepG2, SK-OV-3). Compound **B-2** and **B-3** showed the most potent cell growth inhibition with IC_{50} of 169 nM and 325 nM against U87MG cell line correspondingly.

DNA topoisomerases (Top) are well documented targets for anti-cancer drug development. [1] There are two sub family of Top: type I (Top1) and type II (Top2) depending on whether they cleave single or double strands of DNA.[2] Top1 can relax supercoiled DNA by breaking and re-connecting single-strand to control DNA replication.[2] These agents reversibly block Top1-mediated cleavage of DNA complex, leading to DNA strand breaks, which eventually lead to the cell cycle arrest and activation of apoptosis[2] Top1 inhibitors are broadly used in clinic as chemotherapeutic agents.

Camptothecin(CPT)[3] was the first small molecule identified as a Top1 inhibitor. Efforts to lower its toxicity and pharmacokinetic profiles led to the development of two CPT derivatives, topotecan and irinotecan with improved water solubility.[45] Both compounds are current chemotherapeutic agents in clinic for ovarian cancer and colorectal cancer treatment. However, CPTs derivatives have several limitations including chemical instability[6] and subsequent resistance.[7] To overcome the main drawbacks of CPTs, several classes of non-CPT scaffold Top1 inhibitors were developed as new anti-cancer drug candidates.[5,8,9]

As our ongoing investigations in the development of Top inhibitors, we have recently studied the *in vitro* and *in vivo* anti-cancer activity of novel triazolonaphthalimide derivatives.[10] In this study, we fused the structure characteristics of triazolonaphthalimideand CPT to design and synthesize three series of CPT mimics(**Fig.1**), which were expected to show Top I inhibitory activities.

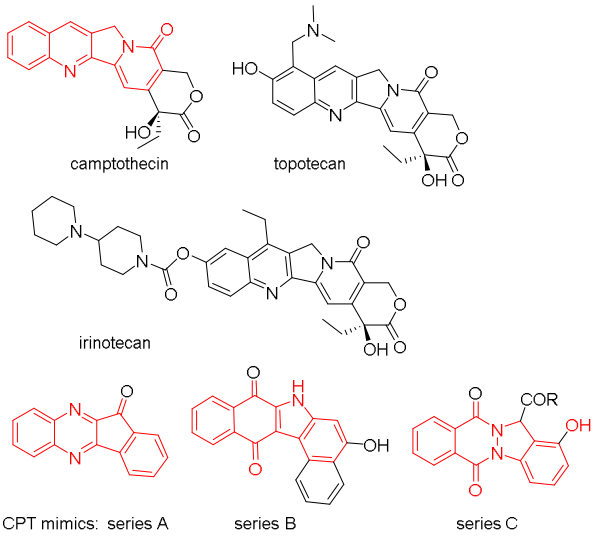


Figure 1. Structures of camptothecin, topotecan, irinotecan and designed CPT mimics

These compounds share the [6,6,5,6] fused aromatic structures that have been reported to be critical for the anti-cancer activities[5,8,9]. In addition, several parental core scaffolds including ninhydrin, [10] 1,4-

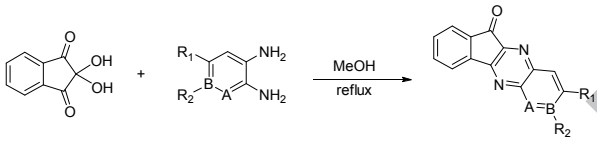
*Corresponding Author: Tel.: 86-10-82801714;
E-mail addresses: xbmeng@bjmu.edu.cn

naphthoquinone, [11] 2-hydroxy-1,4- naphthoquinone [12] as well as indazolo[1,2- b]phthalazine [13] structures were all proved to be important for the anti-cancer activity.

Herein, we designed and synthesized three series of coplanar tetracyclic or pentacylic aromatic compounds which mimic the molecular shape of CPTs as shown in red(**Fig. 1**).

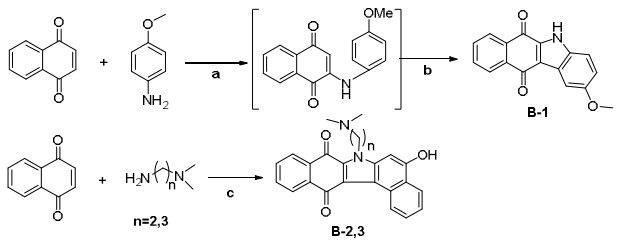
To synthesize target compounds, various synthetic routes have been proposed and carried out. The procedure to synthesize series A compounds was based on the reaction of ninhydrin and substituted ortho-phenylenediamine in methanol under reflux,[15] which was described in **Table 1**. Due to the unsymmetrical substituent groups of ortho-phenylenediamine derivatives, the reaction produces two regioisomers. Some structures of them, including **A-2** to **A-4**, and **A-9**, have been reported in the literature.[16, 17, 18] While other compounds like**A-5** to **A-8** have not been reported. Therefore, we isolated each regioisomer via silica gel chromatography. Compounds **A-10** and **A-11** showed similar melting point to previous report.[19] Michael addition of anisidine to naphthoquinone catalyzed by zinc triflate was carried out in refluxing toluene, then the product was cyclized to form tetracyclic aromatic compound **B-2**,[20] The third type reaction was adopted from previously published literature, which went on a series of tandem reactions to get compound **B-3** and **B-4**.[21]

Table 1. Synthesis of Series A compounds:Indanones derivatives



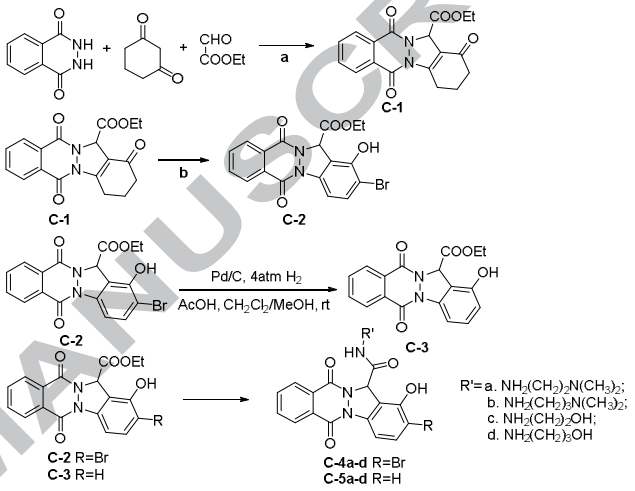
NO.	A	B	R_1	R_2	yeild(%)	note
A-1	C	C	H	H	92	
A-2	C	C	CH_3	H	80	
A-3	C	C	CH_3	CH_3	61	
A-4	C	C	CH_3	F	60	
A-5	C	C	CF_3 or H	H or CF_3	17	a
A-6	C	C	H or CF_3	CF_3 or H	25	b
A-7	C	C	OMe or H	H or OMe	11	a
A-8	C	C	H or OMe	OMe or H	11	b
A-9	C	C			73	
A-10	N or C	C or N	H	H	36	a
A-11	C or N	N or C	H	H	21	b

a: the upper substance in TLC(MeOH: CH_2Cl_2=10:1)
b: the lower substance in TLC(MeOH: CH_2Cl_2=10:1)



Scheme 1. Synthesis of naphthoquinone-based compounds. (a) toluene, $Zn(OTf)_2$, rt. (b) $Pd(TFA)_2$, CH_3COOH, reflux (c) EtOH, rt.

Series C compounds were prepared via a three-component reaction. Compound **C-1** was synthesized from chemical blocks including 2, 3-dihydrophthalazine-1,4-dione, cyclohexane-1,3-dione, and ethyl 2-oxoacetate via a previous reported reaction.[22] To further improve the conjugation of these compounds, we further oxidized compound **C-1** to afford **C-2** with bromide substitution on the benzene ring.[23] The bromide atom could be easily removed by catalytic hydrogenation in the presence of 2eq AcOH to afford **C-3**. Ester **C-2** or **C-3** could react with primary amine to afford the corresponding amide **C-4** and **C-5** (**Scheme 2**).[24]



Scheme 2. Synthesis of indazolo[1,2-b]phthalazine derivatives. (a) H_2SO_4, EtOH/H_2O, 80°C. (b) Br_2, CH_3CN, 80 °C. (c) a,b: EtOH, 80°C; c,d: n-butanol,120°C.

The cytotoxicity of these compounds against five cancer cell lines including HCT-116 (colorectal carcinoma), PC-3 (prostate carcinoma), U87MG (brain tumor), HepG2 (liver cancer), SK-OV-3 (ovarian cancer) were determined with the *in vitro* cell proliferation assay (**Table 2**). For series A and C compounds, most of them shows moderate inhibitory activity except for **A-11 that** significantly inhibited HCT-116, PC3 and HepG2 cell proliferation at 10μM. In addition, compound **B-1** with similar scaffold also show better activity against these cell lines. But to our surprise, compounds **B-2** and **B-3** display potent inhibitory activity to almost all these cancer cell lines. Later literature search revealed that compounds with similar structures have been reported. [25, 26]

Table 2. Cytotoxicity of compounds series A, B, and C (10 μM)[a]

Compd	Inhibition rate%				
	HCT116	PC3	U87MG	HepG2	SK-OV-3
A-1	-1.83%	-8.39%	-14.46%	53.19%	10.09%
A-2	6.32%	-8.92%	-13.91%	6.44%	11.20%
A-4	22.68%	2.65%	-13.22%	50.45%	9.60%
A-5	12.21%	-1.15%	-7.67%	42.85%	-0.82%
A-6	17.21%	15.94%	-15.57%	43.82%	14.17%
A-7	16.94%	-1.15%	-7.74%	6.56%	-7.19%
A-8	8.68%	-4.44%	-14.45%	14.70%	8.07%
A-9	7.32%	11.75%	-11.50%	2.82%	-8.16%
A-10	1.49%	36.17%	-23.26%	15.18%	2.89%
A-11	**71.92%**	**52.66%**	20.83%	**55.42%**	13.15%
B-1	**76.15%**	8.56%	49.00%	26.59%	**57.57%**
B-2	**92.03%**	**66.46%**	**78.78%**	**88.15%**	**82.42%**
B-3	**91.23%**	**59.57%**	**86.04%**	**87.96%**	**77.43%**
C-1	3.50%	11.10%	1.50%	12.00%	-4.89%
C-2	3.30%	0.80%	4.10%	2.54%	-3.43%
C-3	0.40%	4.90%	1.90%	11.25%	-3.97%
C-4a	-5.80%	14.30%	0.10%	3.89%	-6.16%
C-4b	-5.70%	6.40%	-2.50%	-7.99%	-4.10%
C-4c	3.70%	6.50%	0.40%	15.06%	-4.34%
C-4d	5.40%	5.30%	-4.70%	-8.92%	-4.25%
C-5a	-4.20%	4.00%	1.10%	-2.82%	-1.46%
C-5b	-4.50%	11.10%	-4.70%	-8.92%	-4.01%
C-5c	-0.60%	5.00%	-0.60%	-0.87%	-7.14%
C-5d	-3.80%	7.60%	2.40%	-3.15%	-5.15%
C-6	7.50%	5.20%	3.60%	9.65%	-2.88%

a. Mean inhibitory rate of the triplicate experiment.

Based on the screening results, we further examined the IC_{50} values of compound **A-11**, **B-2** and **B-3**(**Table 3**). Compounds **B-2** and **B-3** showed IC_{50} values in sub-micromolar level. Both of the compounds share similar side amine chains which are considered to be the key moiety when interacting with negatively charged DNA double strands to fully exert their biological effects.

Table 3.IC_{50} values of selected compounds over five cancer cell lines.[a]

Compound	IC50 (μM)				
	HCT116	PC3	U87MG	HepG2	SK-OV-3
A-11	44.57	4.377	/	33.80	/
B-2	0.7064	0.3889	**0.1694**	0.8443	1.010
B-3	0.9956	0.6119	**0.3249**	1.974	0.7829

a. MTT assays were used for evaluation, and values were expressed as mean IC_{50} of the triplicate experiment

We also performed Top I inhibitory assay to examine if compound **B-2** and **B-3** target Top I. The result is shown in **Figure 2**. Compounds **B-2** and **B-3** significantly inhibit the process in which supercoiled DNA strand transform into its relaxed state. While due to the poor solubility of CPT, the Top I Inhibitory activity of positive control CPT is actually less active than the new derivatives.

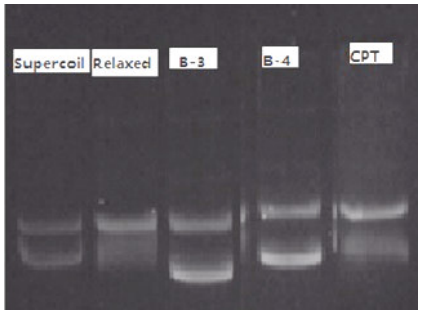


Figure 2. Top I inhibitory evaluation of compounds **B-2** and **B-3** (50μM, respectively) and **CPT** (saturated in 0.1% DMSO in water)

Docking study was also conducted to help us get a full image of the interaction between B-2 and Topo I. The docking result shows that carbonyl group can form hydrogen bond with ASN722, and the hydroxyl group can form hydrogen bond with ARG364 and TGP11 from up and down simultaneously, thus the planar structure of B3 can be steadily embedded into the topotecan binding pocket of topoisomerase I.

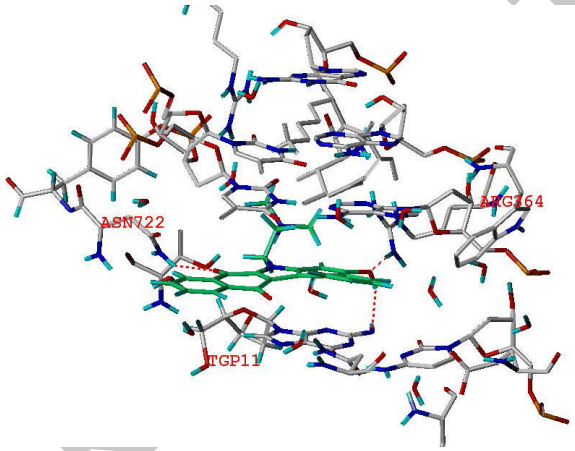


Figure 3. Docking study between compound B-2 and Topo I

In conclusion, we have synthesized three series of coplanar aromatic compounds and evaluated their cytotoxicity against five different cancer cell lines. We also tested identified compounds for their Top I inhibitory activity. Both **B-2** and **B-3** show potent anti-proliferative effects by inhibiting Top I. **B-2** and **B-3** would be promising non-CPT structure anticancer agents which merit further investigation.

Acknowledgments

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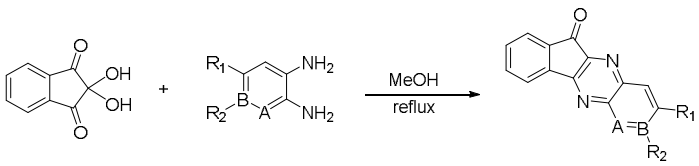
Table 1. Synthesis of Series A compounds: Indanones derivatives



NO.	A	B	R_1	R_2	Yield (%)	note
A-1	C	C	H	H	92	
A-1	C	C	CH_3	H	80	
A-3	C	C	CH_3	CH_3	61	
A-4	C	C	CH_3	F	60	
A-5	C	C	CF_3 or H	H or CF_3	17	a
A-6	C	C	H or CF_3	CF_3 or H	25	b
A-7	C	C	OMe or H	H or OMe	11	a
A-8	C	C	H or OMe	OMe or H	11	b
A-9	C	C			73	a
A-10	N or C	C or N	H	H	36	b
A-11	C or N	N or C	H	H	21	a

a：the upper substance in TLC (MeOH: CH_2Cl_2=10:1)
b : the lower substance in TLC (MeOH: CH_2Cl_2=10:1)