Journal Pre-proofs

Synthesis and Biological Evaluation of Novel Indole-Pyrazoline Hybrid Derivatives as Potential Topoisomerase 1 Inhibitors

Bing Shu, Qian Yu, De-xuan Hu, Tong Che, Shang-shi Zhang, Ding Li

PII: S0960-894X(19)30907-2
DOI: https://doi.org/10.1016/j.bmcl.2019.126925
Reference: BMCL 126925

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 27 October 2019
Revised Date: 15 December 2019
Accepted Date: 17 December 2019

Please cite this article as: Shu, B., Yu, Q., Hu, D-x., Che, T., Zhang, S-s., Li, D., Synthesis and Biological Evaluation of Novel Indole-Pyrazoline Hybrid Derivatives as Potential Topoisomerase 1 Inhibitors, *Bioorganic & Medicinal Chemistry Letters* (2019), doi: https://doi.org/10.1016/j.bmcl.2019.126925

Synthesis and Biological Evaluation of Novel Indole-Pyrazoline Hybrid Derivatives as Potential Topoisomerase 1 Inhibitors

Bing Shu[a]*, Qian Yu[a, b], De-xuan Hu[b], Tong Che[c], Shang-shi Zhang[c], and Ding Li[b]*

[a]*School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, PR China.*

[b]*School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou University City, 132 Waihuan East Road, Guangzhou 510006, P. R. China.*

[c]*Center for Drug Research and Development, Guangdong Pharmaceutical University, Guangzhou 510006, PR China.*

**Corresponding author E-mail address: shubing@gdpu.edu.cn (B. Shu); liding@mail.sysu.edu.cn (D. Li).*

Abstract:

A series of novel indole-pyrazoline hybrid derivatives were designed, synthesized, and evaluated for topoisomerase 1 (Top1) inhibitory activity. Top1-mediated relaxation assays showed that our synthesized compounds had variable Top1 inhibitory activity. Among these compounds, 3-(5-(naphthalen-1-yl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)-1-(phenylsulfonyl)-1*H*-indole (**6n**) was found to be a strong Top1 inhibitor with better inhibitory activity than CPT and hit compounds. Our further experiments rationalized the mode of action for this new type of inhibitors, which showed no significant binding to supercoiled DNA.

Key words: topoisomerase 1, indole-pyrazoline hybrid derivatives, CPT, supercoiled DNA

DNA topoisomerase 1 (Top1) is overexpressed among various cancer cell lines and widely considered as an essential nuclear enzyme to regulate the cellular DNA-topological processes such as DNA replication, transcription, chromatin assembly, recombination, and chromosome segregation, by relaxing supercoiled DNA to alter DNA topology.[1-2] The active site tyrosine OH group (Tyr723 in human Top1) could act as a nucleophile to attack DNA phosphodiester backbone at 3′-end of the cutting strand to produce a single strand break, and subsequently attached to the broken DNA, resulting in the formation of a binary enzyme-DNA covalent complex (Top1 CC). Top1 inhibitors, such as Camptothecin (Fig. 1), bound to the interface of Top1 CC, resulting in stable Top1 CC to prevent further religation of the broken DNA. When replication and transcription machineries encounter the trapped Top1 CC, DNA damage was generated, triggering cell death.[3-5] Hence the ability of inhibiting the function of Top1, or trapping Top1 CC could be some effective strategies for inducing DNA damage, which could eventually induce cancer cell apoptosis.[6]

On the base of mechanisms for molecular actions, compounds targeting Top1 or Top1 CC could be classified into two categories: one is Top1 poison and the another is Top1 catalytic inhibitor.[6] Top1 poisons are able to trap Top1 CC and prevent further religation of the DNA single-strand breaks, resulting in DNA strand breaks accumulation and DNA damage. Top1 catalytic inhibitors could inhibit catalytic DNA cleavage reaction and prevent formation of Top1-DNA covalent complex, which are different from Top1 poisons.[7] The first known Top1 poison is Camptothecin (**CPT, Fig. 1**). To date, two structurally modified derivatives, topotecan and irinotecan have been approved by FDA for cancer treatment, and other derivatives such as Belotecan and 10-hydroxy Camptothecin, are in clinical trials (**Fig. 1**).[8-10] In addition, several non-Camptothecin Top1 poisons have been reported and studied in preclinical or clinical trials, including indolocarbazole (BMS-250749), dibenzonaphthyridinones (ARC-111 and Genz-644282), indenoisoquinolines indotecan (LMP400), indimitecan (LMP776) and MJ-III-65 LMP744, (**Fig. 1**).[11-12] Unfortunately, some camptothecins suffered from disadvantages such as poor chemical stability, drug resistance, gastrointestinal toxicity or significant side effects. Therefore, further discovery and

development of novel Top1 inhibitors are required.

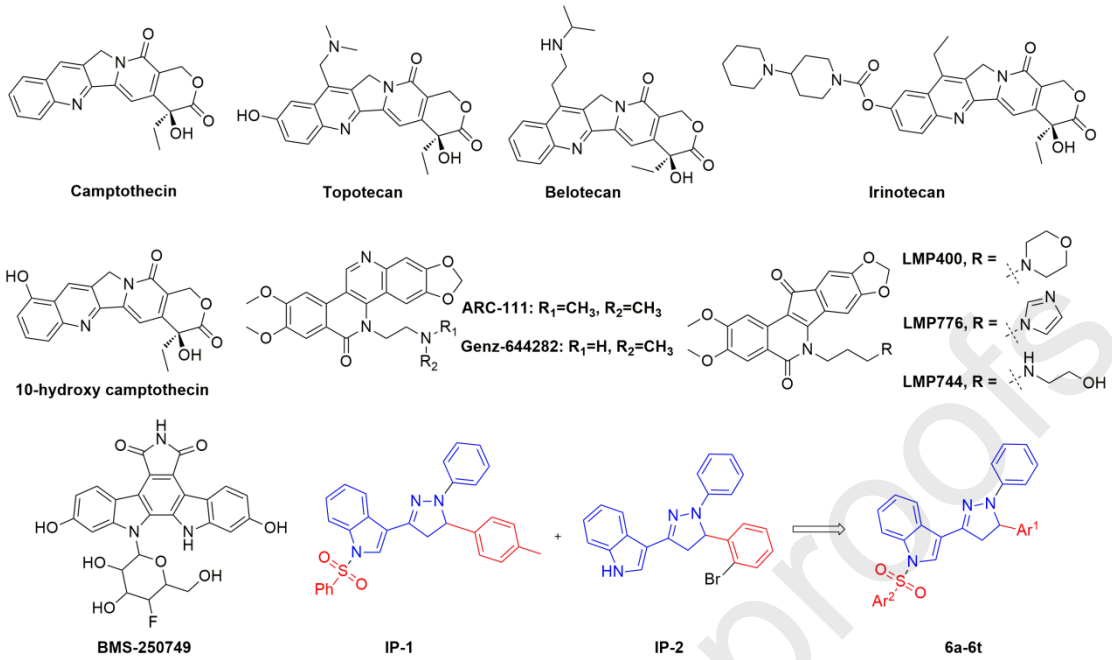
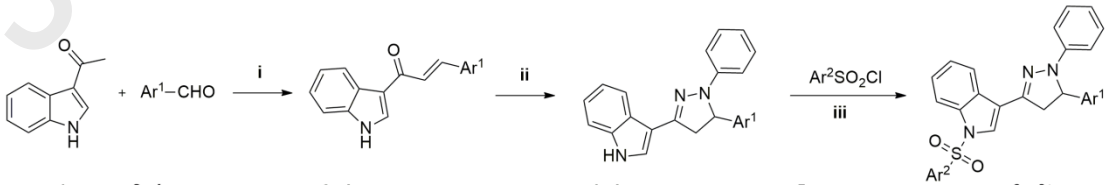


Fig.1 Chemical structures of Camptothecin, Topotecan, Belotecan, Irinotecan, 10-hydroxy camptothecin, ARC-111, Genz-644282, LMP400, LMP776, LMP744, BMS-250749, hit compound **IP-1**, **IP-2**, and designed compounds **6a-6t.**

In order to find small molecular ligands targeting Top1, we performed Top1-mediated relaxation assay to screen our synthesized compounds, including acridone derivatives, imidazole derivatives, ketone derivatives, quinoline derivatives, and acridine derivatives. CPT, a well-known Top1 poison, was used as a positive control. Top1-mediated relaxation assay has been previously characterized and performed to block the formation of Top1-DNA covalent complex and research the Top1 inhibitory activities of the targeted compounds.[5, 13-14] Briefly, supercoiled DNA and Top1-DNA covalent complex could produce different migration bands in agarose gel. Generally, DNA could be recognized by Top1 to attack the DNA phosphodiester backbone, and covalently attached to the end of the broken DNA with formation of a transient enzyme-DNA covalent complex (known as Top1 CC), resulting in a slower migration band. On the contrary, supercoiled DNA alone could immigrate faster.[5, 13-14] As shown in **Fig. S1-2**, we found that two indole-pyrazoline hybrids, **IP-1** and **IP-2**, (**Fig. 1**) could block the formation of Top1 CC to release supercoiled DNA at

300 μM, indicating that indole-pyrazoline hybrids could be potential hits of Top1 inhibitors. However, other compounds such as acridone derivatives (**B01-06**), imidazole derivatives (**5a-5c**), ketone derivatives (**D01-03**), quinoline derivatives (**Q01-04**), and acridine derivatives (**AD01-02**) did not show obvious Top1 inhibition ability at 300 μM concentration (**Fig. S1-2**). In order to investigate the effect for introduction of various substituents and understand the structure-activity relationship (SAR), a series of dual aryl substituted indole-pyrazoline hybrid derivatives (**6a-6t**, **Fig. 1**) were designed and synthesized. Besides, their Top1 inhibition activity, binding affinity to supercoiled DNA and molecular docking were evaluated and reported here.

The synthetic pathway for indole-pyrazoline hybrid derivatives **6a-6t** was shown in Scheme **1**. 3-Acetylindole (**1**) was used as starting material to react with substituted aldehydes (**2a-h**) through aldol condensation reaction catalyzed with NaOH in EtOH solution to give the desired intermediates 2-en-1-ones (**3a-h**), which were used directly for the next step reaction. Then, the obtained intermediates **3a-h** were reacted with phenylhydrazine hydrochloride under weak alkaline condition such as triethylamine in EtOH solution to give key compounds **4a-h**. Finally, the target indole-pyrazoline hybrid derivatives **6a-6t** were obtained through nucleophilic substitution reaction between **4a-h** and various arylsulfonyl chlorides (**5a-c**) in a solution of benzyltriethylammonium chloride (TEBAC) and sodium hydroxide in dichloromethane (**Table I**). The synthesized indole-pyrazoline hybrid derivatives were purified and their structures were determined by using ^{1}H NMR, ^{13}C NMR and mass spectra. All compounds used for subsequent biological evaluations were analyzed by using HPLC with their purity determined to be more than 95%.



1

2a-h
2a: (p-OCH_3)Ph-
2b: (3,4,5-3OCH_3)Ph-
2c: (p-Cl)Ph-
2d: Ph-
2e: (o-Br)Ph-
2f: naphthoic-1-yl-
2g: (p-isopropyl)Ph-
2h: (m-NO_2)Ph-

3a-h
3a: (p-OCH_3)Ph-
3b: (3,4,5-3OCH_3)Ph-
3c: (p-Cl)Ph-
3d: Ph-
3e: (o-Br)Ph-
3f: naphthoic-1-yl-
3g: (p-isopropyl)Ph-
3h: (m-NO_2)Ph-

4a-h
4a: (p-OCH_3)Ph-
4b: (3,4,5-3OCH_3)Ph-
4c: (p-Cl)Ph-
4d: Ph-
4e: (o-Br)Ph-
4f: naphthoic-1-yl-
4g: (p-isopropyl)Ph-
4h: (m-NO_2)Ph-

5a-c
5a: Ph-
5b: (p-CH_3)Ph-
5c: naphthoic-1-yl-

6a-6t

Scheme I. Reagents and conditions: (i) 5 °C, NaOH, EtOH; (ii) phenylhydrazine hydrochloride, TEA, EtOH, 80 °C, yield 60-90%; (iii) TEBAC, NaOH, dichloromethane, room temperature, yield 52-89%.

Top1 inhibitory activity of the synthesized compounds was measured by using Top1-mediated relaxation assay with CPT as a positive control, and semiquantitatively expressed relative to CPT at 25 μM as follows: +++, more than 80% of the activity; ++, between 40% and 79% of the activity; +, less than 40% of the activity, indicating no significant interaction between ligand and Top1. After the DNA was incubated with Top1 in the absence or presence of final concentration of 25 μM compounds, the obtained mixture samples were electrophoresed, Gelred-stained and results summarized as shown in **Table 1 and Table S1**. We found that hit compounds **IP-1** and **IP-2** did not show inhibitory activity at 25 μM. Compared with the hit compounds **IP-1** and **IP-2**, some synthesized compounds displayed Top1 inhibitory activity. Among these compounds, compounds **6a**, **6d-6e** with electron-donating OCH_3 substitutive group at Ar^1, and compounds **6q, 6s** with electron-donating isopropyl substitutive group had increased Top1 inhibition activity, indicating that electron-donating group at Ar^1 is helpful to improve Top1 inhibitory activity. In comparison, compounds **6f-6g** with weak electron-withdrawing *p*-Cl substitutive group, compounds **6k-6m** with weak electron-donating *o*-Br substitutive group or compound **6t** with electron-withdrawing *m*-NO_2 substitutive group did not show Top1 inhibition activity. Compound **6n** with large aromatic group naphthalene ring at Ar^1, exhibited higher Top1 inhibition activity than CPT and hit compounds, possibly because the π-π stacking interaction between **6n** and Top1 blocked the formation of Top1-DNA complex, which indicated that π-π stacking interaction could be necessary for strong Top1 inhibitory activity. However, introduction of large aromatic groups at Ar^2 could produce unfavorable effect. For example, compounds **6c, 6g, 6j, 6m, 6p** with naphthalene at Ar^2 did not show any inhibition activity. As mentioned before, compound **6n** had the best inhibition activity to Top1, and therefore, was selected for further investigation.

Table 1

The structures and the *in vitro* Top1 inhibitory activities of the indole-pyrazoline hybrid derivatives

Comp.	Ar^1	Ar^2	Relaxation assay[a]
6a	(*p*-OCH_3)Ph-	Ph-	++
6b	(*p*-OCH_3)Ph-	(*p*-CH_3)Ph-	+
6c	(*p*-OCH_3)Ph-	naphthoic-1-yl-	+
6d	(*3,4,5*-$3OCH_3$)Ph-	Ph-	++
6e	(*3,4,5*-$3OCH_3$)Ph-	(*p*-CH_3)Ph-	++
6f	(*p*-Cl)Ph-	Ph-	+
6g	(*p*-Cl)Ph-	naphthoic-1-yl-	+
6h	Ph-	Ph-	++
6i	Ph-	(*p*-CH_3)Ph-	++
6j	Ph-	naphthoic-1-yl-	+
6k	(*o*-Br)Ph-	Ph-	+
6l	(*o*-Br)Ph-	(*p*-CH_3)Ph-	+
6m	(*o*-Br)Ph-	naphthoic-1-yl-	+
6n	naphthoic-1-yl-	Ph-	+++
6o	naphthoic-1-yl-	(*p*-CH_3)Ph-	++
6p	naphthoic-1-yl-	naphthoic-1-yl-	+
6q	(*p*-isopropyl)Ph-	Ph-	++
6r	(*p*-isopropyl)Ph-	(*p*-CH_3)Ph-	+
6s	(*p*-isopropyl)Ph-	naphthoic-1-yl-	++
6t	(*m*-NO_2)Ph-	Ph-	+
IP-1	-	-	+
IP-2	-	-	+

[a]Top1 inhibitory activity was semi-quantitatively expressed relative to CPT as follows: +++, more than 80% of the activity; ++, between 40% and 79% of the activity; +, less than 40% of the activity, indicating no significant interaction between ligand and Top1. Every experiment was repeated at least twice independently.

In order to further study the inhibition activity of preferred compound **6n** to Top1, a semi-quantitative Top1-mediated relaxation assay was performed.[7] As shown in **Fig. 2A-B**, **6n** had strong inhibitory activity in a dose-dependent manner. The levels of supercoiled DNA were increased to 61% and 74%, in relation to whole supercoiled DNA upon treatment with 25 and 125 μM compound **6n**, respectively. Besides, **6n** showed stronger inhibitory activity than CPT, and under the same condition only 41% and 48% supercoiled DNA remained for CPT. On the contrary, CPT showed relatively high inhibitory activity at relatively low concentrations (1 and 5 μM).

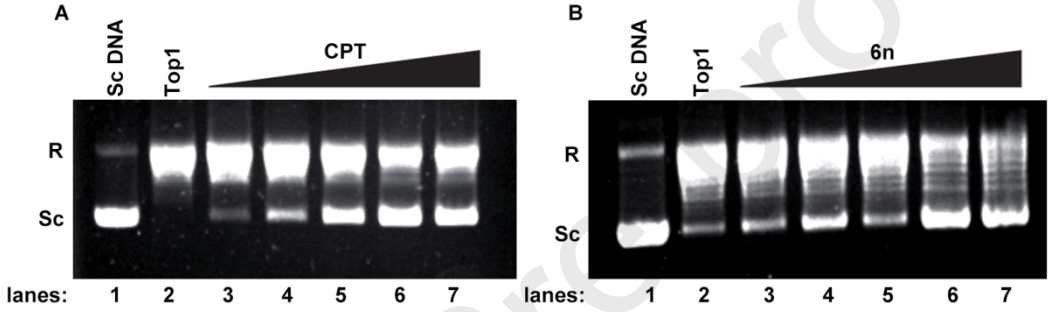


Fig.2 Semi-quantitative Top1-mediated relaxation assay. SC, supercoiled DNA; R, relaxed DNA. Lane 1, supercoiled pBR322 DNA alone; Lane 2, DNA and Top1; Lanes 3-7, DNA, Top1, and tested compound **CPT** (A) and **6n** (B) at 0.2, 1, 5, 25, 125 μM.

In order to know whether the inhibition effect was caused by affinity of the compounds to Top1 or DNA, we studied the binding affinities of the synthesized indole derivatives for supercoiled DNA. SPR assay was used as a highly efficient method to evaluate the interaction between of the ligands and targeted DNAs.[15-17] The binding constants K_D values for the binding affinity of the indole-pyrazoline hybrids to duplex DNA were determined as shown in **Table S2 and Fig. S3A-B**. Ethidium bromide (**EB**), a well-known DNA intercalator, was used as a positive control. We found that **EB** exhibited good binding affinity to duplex DNA with a K_D value of 1.2 μM (Fig. **S3B**). However, all synthesized compounds did not show significant binding at 50 μM concentration, which might indicate no specific interaction between the ligands and the DNA.

In addition, the interaction between compound **6n** and DNA was also studied by using Top1-mediated unwinding assay as reported previously.[7] **EB** was used as a positive control. As shown in **Fig. S4**, upon addition of increasing concentration of **EB,** very clear unwinding effect appeared with supercoiled pBR322 DNA as substrate. However, the representative compound **6n** had no obvious unwinding effect, which was different from the classical intercalation interaction.

Molecular docking was performed to provide a possible binding mode of **6n** with Top1-DNA complex. The ligand was docked to the ligand binding site of the Top1−DNA−ligand ternary complex (PDB ID: 1SC7). The result of **6n** with the highest score was shown in **Fig. 3**. The aromatic framework stacked with the +1 and -1 base pairs at the DNA cleavage site of Top1 CC. The sulfonyl group formed two hydrogen bonds with Lys425 (3.0 Å) and Glu356 (3.6 Å), playing important role for its Top1 inhibitory activity. In addition, Top1 has a moderate binding site with Trp416 interacting with the aromatic group substituted with the sulfonyl group, through a hydrophobic interaction. Importantly, the naphthalene ring stacked on the scissile strand bases (G and T) through π-π stacking interaction, and the indole ring stacked on the bases of non-cleaved strand (C and A). These results were consistent with our experimental data for inhibition of Top1 by **6n**.

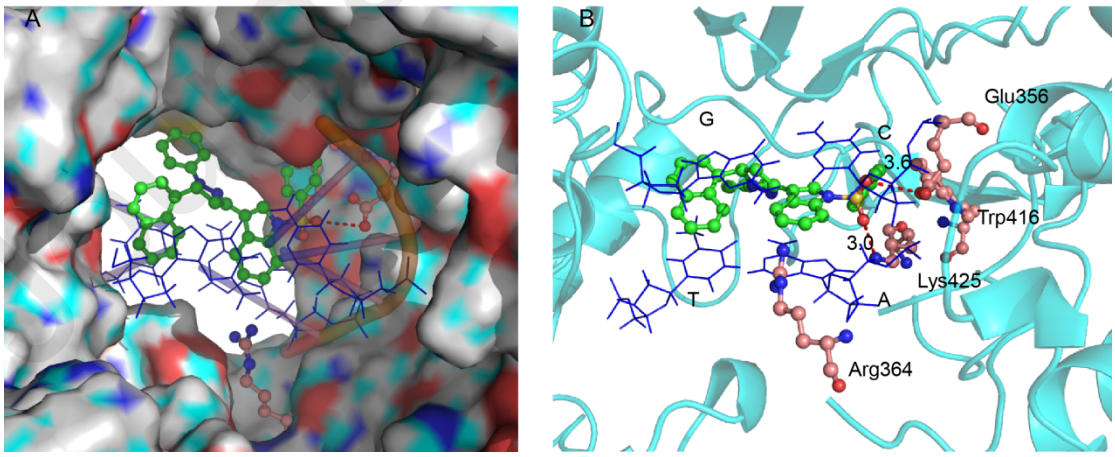


Fig. 3. Hypothetical binding mode of **6n** in the ternary Top1-DNA-drug complex (derived from PDB ID: 1SC7). The carbon atoms of **6n** are shown in green, and the base pairs are displayed in blue lines. All distances are measured from heavy atom to heavy atom.

In summary, twenty indole-pyrazoline hybrid derivatives were designed, synthesized, and characterized by using ^{1}H and ^{13}C NMR and mass spectroscopic analysis. Top1-mediated relaxation assays showed that compound **6n** could act as Top1 inhibitor with higher inhibitory activity than **CPT** and hit compounds. Besides, our studies suggested that **6n** stacked with the +1 and -1 base pairs at the DNA cleavage site of Top1 CC without significant binding interaction with supercoiled DNA. Further research about the biological activity and antitumor mechanism of these potent compounds are currently going on.

Conflict of interest and Acknowledgements

The authors declare no competing financial interests. We thank the Guangdong Medical Research Fund (Grant NO: B2019003), and Guangdong Provincial Education Department Fund (Grant NO: 51340208) for financial support of this study.

Supplementary section

Details of experimental procedures, biological assays and analytical data for novel compounds associated with this article can be found on pages S2-S17.

References

[1] Caldecott KW. Single-strand break repair and genetic disease. *Nature Reviews Genetics* **2008,** *9* (8), 619-631.

[2] Heo J, Li J, Summerlin M, Hays A, Katyal S, Mckinnon PJ, Nitiss KC, Nitiss JL, Hanakahi LA. TDP1 promotes assembly of non-homologous end joining protein complexes on DNA. *DNA Repair* **2015,** *30*, 28-37.

[3] Redinbo MR, Champoux JJ, Hol WGJ. Novel insights into catalytic mechanism from a crystal structure of human topoisomerase I in complex with DNA. *Biochemistry* **2000,** *39* (23), 6832-6840.

[4] Redinbo MR, Stewart L, Kuhn P, Champoux JJ, Hol WG. Crystal structures of human topoisomerase I in covalent and noncovalent complexes with DNA. *Science (New York, N.Y.)* **1998,** *279* (5356), 1504-1513.

[5] Zhang XR, Wang HW, Tang WL, Zhang Y, Yang H, Hu DX, Ravji A, Marchand C, Kiselev E, Ofori-Atta K, Agama K, Pommier Y, An LK. Discovery, Synthesis, and Evaluation of Oxynitidine Derivatives as Dual Inhibitors of DNA Topoisomerase IB (TOP1) and Tyrosyl-DNA Phosphodiesterase 1 (TDP1), and Potential Antitumor Agents. *J Med Chem* **2018,** *61* (22), 9908-9930.

[6] Pommier Y. Topoisomerase I inhibitors: camptothecins and beyond. *Nature Reviews Cancer* **2006,** *6* (10), 789-802.

[7] Yu Q, Yang H, Zhu TW, Yu LM, Chen JW, Gu LQ, Huang ZS, An LK. Synthesis, cytotoxicity and structure-activity relationship of indolizinoquinolinedione derivatives as DNA topoisomerase IB catalytic inhibitors. *Eur J Med Chem* **2018,** *152*, 195-207.

[8] Zhang X, Wang R, Zhao L, Lu N, Wang J, You Q, Li Z, Guo Q. Synthesis and biological evaluations of novel indenoisoquinolines as topoisomerase I inhibitors. *Bioorganic & medicinal chemistry letters* **2012,** *22* (2), 1276-1281.

[9] Dong G, Fang Y, Liu Y, Liu N, Wu S, Zhang W, Sheng C. Design, synthesis and evaluation of 4-substituted anthra[2,1-c][1,2,5]thiadiazole-6,11-dione derivatives as novel non-camptothecin topoisomerase I inhibitors. *Bioorganic & medicinal chemistry letters* **2017,** *27* (9), 1929-1933.

[10] Zhao Q, Xu X, Xie Z, Liu X, You Q, Guo Q, Zhong Y, Li Z. Design, synthesis and biological evaluation of 3-substituted indenoisoquinoline derivatives as topoisomerase I inhibitors. *Bioorganic & medicinal chemistry letters* **2016,** *26* (3), 1068-1072.

[11] Pommier Y, Marchand C. Interfacial inhibitors: targeting macromolecular complexes. *Nature Reviews Drug Discovery* **2012,** *11* (1), 25-36.

[12] Beck DE, Reddy PV, Lv W, Abdelmalak M, Tender GS, Lopez S, Agama K, Marchand C, Pommier Y, Cushman M. Investigation of the Structure-Activity Relationships of Aza-A-Ring Indenoisoquinoline Topoisomerase I Poisons. *J Med Chem* **2016,** *59* (8), 3840-3853.

[13] Yu LM, Hu Z, Chen Y, Ravji A, Lopez S, Plescia CB, Yu Q, Yang H, Abdelmalak M, Saha S, Agama K, Kiselev E, Marchand C, Pommier Y, An LK. Synthesis and structure-activity relationship of furoquinolinediones as inhibitors of Tyrosyl-DNA phosphodiesterase 2 (TDP2). *Eur J Med Chem* **2018,** *151*, 777-796.

[14] Tang WL, Zhang Y, Hu DX, Yang H, Yu Q, Chen JW, Agama K, Pommier Y, An LK. Synthesis and biological evaluation of 5-aminoethyl benzophenanthridone derivatives as DNA topoisomerase IB inhibitors. *Eur J Med Chem* **2019,** *178*, 81-92.

[15] Liu HY, Chen AC, Yin QK, Li Z, Huang SM, Du G, He JH, Zan LP, Wang SK, Xu YH, Tan JH, Ou TM, Li D, Gu LQ, Huang ZS. New Disubstituted Quindoline Derivatives Inhibiting Burkitt's Lymphoma Cell Proliferation by Impeding c-MYC Transcription. *J Med Chem* **2017,** *60* (13), 5438-5454.

[16] Wang YQ, Huang ZL, Chen SB, Wang CX, Shan C, Yin QK, Ou TM, Li D, Gu LQ, Tan JH, Huang ZS. Design, Synthesis, and Evaluation of New Selective NM23-H2 Binders as c-MYC Transcription Inhibitors via Disruption of the NM23-H2/G-Quadruplex Interaction. *J Med Chem* **2017,** *60* (16), 6924-6941.

[17] Shan C, Yan JW, Wang YQ, Che T, Huang ZL, Chen AC, Yao PF, Tan JH, Li D, Ou TM, Gu LQ, Huang ZS. Design, Synthesis, and Evaluation of Isaindigotone Derivatives To Downregulate c-myc Transcription via Disrupting the Interaction of NM23-H2 with G-Quadruplex. *J Med Chem* **2017,** *60* (4), 1292-1308.

Declaration of interests

☐ ☑The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: