RESEARCH ARTICLE

View Article Online
View Journal | View Issue Cite this: *Med. Chem. Commun.*,
2018, 9, 1142Identification of potent catalytic inhibitors of
human DNA topoisomerase II by structure-based
virtual screening†‡Guoqiang Dong,§^a Ying Wu,§^a Ying Sun,§^b Na Liu,^a Shanchao Wu,^a
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Accepted 18th May 2018

DOI: 10.1039/c8md00219c

rsc.li/medchemcomm

Human DNA topoisomerase II (Top2) is a promising target for cancer treatment. To overcome the limitations of known Top2 inhibitors, novel Top2 catalytic inhibitors with new scaffolds were identified by structure-based virtual screening. In particular, compound **8** showed good *in vitro* antiproliferative activity with a broad spectrum. Top2-mediated cleavage assay and molecular modeling rationalized the mode of action. The new Top2 inhibitors are considered as good starting points for further hit-to-lead optimization in anticancer drug discovery.

Introduction

DNA topoisomerases are essential enzymes that regulate cellular processes such as replication, transcription, recombination, and chromatin and chromosomal segregation by altering DNA topology.^{1,2} DNA topoisomerases can be classified into two types, topoisomerase I (Top1) and topoisomerase II (Top2).³ They catalyze the breakage and religation of DNA, maintaining the topological changes of DNA and various DNA metabolic processes. Top1 cleaves DNA by forming a transiently single-strand break and allows the broken strand to rotate around another single strand to achieve relaxation.^{4,5} In contrast, Top2 introduces double-strand breaks, creating a DNA-linked protein gate and passing one duplex through another.⁶ Due to their important role in cell viability, topoisomerases have been considered as the most important targets for cancer chemotherapy.^{1,3,7,8}

Top2 inhibitors are generally divided into two broad classes: Top2 poisons and Top2 catalytic inhibitors.⁷ Top2 poisons can stabilize and increase the levels of Top2-DNA cleavable complexes thereby disrupting the normal catalytic cycle. Top2 catalytic inhibitors interfere with a single step of the enzyme catalytic cycle.^{5,6} Several Top2 poisons are widely used in clinical cancer treatment, such as doxorubicin, etoposide

(Eto), mitoxantrone, dexrazoxane (ICRF-187), aclarubicin (Fig. 1), amsacrine and ellipticine.⁹ However, their therapeutic application is hampered by several limitations, such as cardiotoxicity, drug resistance, significant side effects and chromosomal translocation which can trigger secondary malignancies.^{10–12} Top2 catalytic inhibitors have the advantages of modulating the cytotoxic effects of Top2 poisons and overcoming multidrug resistance (MDR).^{13,14} Several catalytic inhibitors have been used in clinics. For example, dexrazoxane is approved as a cardiac protective agent to prevent anthracycline-mediated cardiotoxicity and aclarubicin is used as an antineoplastic agent with less cardiotoxicity than doxorubicin.^{7,15,16}

Top2 is an ATPase belonging to the GHKL (gyrase, HSP90, histidine kinase, MutL) superfamily.¹⁷ It uses the energy derived from ATP hydrolysis to trigger conformational changes

Fig. 1 Chemical structures of human DNA Top2 inhibitors.

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c8md00219c

‡ The authors declare no competing interests.

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for the completion of the catalytic cycle.⁵ Therefore, blocking the ATP-binding site with an ATP competitive inhibitor is an efficient strategy to prevent ATP hydrolysis and inhibit the Top2 activity.⁷ Actually, ATP binding site (ATPase) binders represent an important class of Top2 catalytic inhibitors. Recently, several classes of ATP competitor inhibitors were identified as novel catalytic Top2 inhibitors, such as purine analogues,^{18,19} imidazoles,¹³ thiosemicarbazone and xanthenes.^{20,21} Until now, none of these inhibitors have progressed into clinical development due to their poor antineoplastic activity and unsatisfactory bioavailability. Thus, it is highly desirable to identify new Top2 catalytic inhibitors with novel chemical scaffolds. Herein, novel Top2 catalytic inhibitors which showed potent *in vitro* anti-proliferative activity were identified by structure-based virtual screening (SBVS).

Results and discussion

Procedure of virtual screening

The X-ray crystallographic structure of the ATPase domain of Top2 α in complex with ADPNP (PDB: 1ZXN) is available in the Protein Data Bank. ADPNP was extracted and subsequently docked into ATPase using GOLD version 5.0 which is a powerful docking program for proteins containing metal ions.²² GoldScore, ChemSore, ASP and ChemPLP were used to evaluate the binding mode. The best conformation was chosen. The docking reliability was evaluated through a comparison of the root-mean-square deviation (RMSD) values between the positions of heavy atoms of the ligand in the calculated and experimental structures found in the docked positions of the ligand and the experimental ones. As a result, ChemPLP seemed to be the best scoring function which gave the best results with the lowest average RMSD value (RMSD = 0.70 Å, Fig. 2A and S1†), and thus it was adopted in the subsequent virtual screening for ATPase of Top2.

Fig. 2 (A) Matrix of RMSD values obtained by the docking studies. (B) Flowchart of the virtual screening procedure.

ChemPLP is an empirical scoring function based on ant colony optimization (ACO), which treats neutral and repulsive contacts with a piecewise linear potential (PLP). It has been found to give the highest success rates for both pose prediction and virtual screening experiments against diverse validation test sets and is therefore the default scoring function in GOLD 5.0.^{23,24} The overall virtual screening protocol is summarized in Fig. 2B.

A total of 287 000 compounds in the SPECS database (www.specs.com) were first filtered by “Lipinski’s Rule of Five” to make the retrieved compounds more drug-like and then screened through molecular docking (cutoff >85). In the next step, the top ranked 600 molecules were visually analyzed and selected on the basis of the scores and interactions with Mg²⁺ ions and other key residues in the active site. In order to achieve good chemical diversity, the resulting 200 candidates were clustered and filtered by similarity/substructure search. Consequently, 37 compounds were selected and purchased from SPECS (Table 1 and Fig. 3).

Table 1 Physicochemical properties of the compounds selected by virtual screening

	MW	H-Acceptor	H-Donor	Rotatable bond	log <i>P</i> ^a
1	439.5	9	3	8	1.59
2	442.46	6	0	7	3.50
3	404.81	7	1	8	0.23
4	429.39	8	1	7	2.56
5	440.46	7	2	8	4.54
6	439.47	7	0	6	3.52
7	358.39	2	1	8	4.79
8	491.34	5	1	6	4.76
9	421.45	6	2	8	3.28
10	413.48	8	2	6	2.60
11	477.52	7	2	8	3.59
12	464.59	5	0	8	4.12
13	479.38	5	1	9	3.83
14	401.42	7	2	6	1.88
15	484.57	7	4	7	2.91
16	386.47	6	3	8	3.62
17	420.92	6	3	8	4.70
18	400.50	6	3	8	4.11
19	348.36	7	1	5	2.12
20	385.47	4	1	6	4.16
21	438.91	6	4	10	2.08
22	438.55	5	0	10	3.91
23	366.42	5	1	8	4.09
24	431.29	5	1	7	4.49
25	354.43	6	1	8	3.45
26	420.45	8	2	5	1.95
27	402.43	5	1	3	4.37
28	358.39	2	1	8	4.89
29	429.43	6	1	8	3.62
30	384.42	6	1	7	2.29
31	485.58	8	2	9	3.60
32	410.52	6	1	7	4.82
33	429.39	8	1	7	2.56
34	467.50	7	3	9	3.20
35	372.42	7	2	8	3.61
36	460.56	6	1	8	3.24
37	376.44	4	1	6	4.27

^a log *P* values were calculated using the Discovery Studio 3.0 software package.

Fig. 3 Chemical structures of the purchased compounds by virtual screening.

Top2-mediated supercoiled pBR322 relaxation assays

Top2-mediated supercoiled pBR322 relaxation assays were performed to investigate the inhibitory activity of the purchased compounds with their concentrations ranging from 50 to 100 μM . Top2 (TG2000H-1, TopoGEN, Inc.) and pBR322 plasmid DNA (#3050, TaKaRa Biotechnology (Dalian) Co., Ltd.) were used and Eto was the positive control. As shown in Fig. 4, 14 hits were found to be active against Top2 at 100 μM . Compounds 2, 8, 15, 17, 26 and 27 were more active than Eto at 100 μM . The Top2 inhibitory activity of the 14 hits was further tested at a lower concentration (50 μM). Compounds 2, 8 and 26 showed potent Top2 inhibitory activity. In contrast, Eto was almost inactive at 50 μM . Compounds 2, 8, 15, 17, 26 and 27 were further tested at eight different concentrations (1000, 500, 200, 100, 80, 40, 20, and 5 μM), respectively. The IC_{50} values were calculated using Eto as the positive drug (Table 2). The results revealed that compounds 2, 8, 26, and 27 were more potent than Eto with IC_{50} values in the range of 16 μM to 76 μM .

Top2-mediated DNA cleavage assay

To investigate whether these potent hits act as Top2 poisons or catalytic inhibitors, Top2-mediated cleavage assay was

Fig. 4 Gel electrophoresis of Top2-mediated DNA relaxation assay for hits from virtual screening. (A–D) Inhibition of Top2 relaxation activity at 100 μM . (E) Inhibition of Top2 relaxation activity at 50 μM . Lane 1, pBR322 DNA; lane 2, pBR322 DNA + Top2; lane 3, pBR322 DNA + Top2 + Eto; other lanes, DNA + Top2 + hits.

Table 2 Top2 inhibitory activity and *in vitro* antiproliferative potency of the compounds obtained from virtual screening (IC₅₀, μ M)

Compd	Top2	A549	HCT116	ZR-75-30
1	n.t. ^a	n.a. ^b	n.a.	n.a.
2	76 $\pm$ 8.2	48 $\pm$ 3.2	n.a.	n.a.
3	n.t.	n.a.	n.a.	n.a.
4	n.t.	n.a.	n.a.	n.a.
5	n.t.	53 $\pm$ 4.8	46 $\pm$ 3.9	n.a.
6	n.t.	n.a.	n.a.	n.a.
7	n.t.	n.a.	n.a.	n.a.
8	43 $\pm$ 5.6	6.8 $\pm$ 0.52	1.8 $\pm$ 0.09	1.5 $\pm$ 0.02
9	n.t.	n.a.	n.a.	n.a.
10	n.t.	n.a.	n.a.	n.a.
11	n.t.	57 $\pm$ 2.6	11 $\pm$ 1.0	10 $\pm$ 2.0
12	n.t.	n.a.	n.a.	n.a.
13	n.t.	n.a.	n.a.	n.a.
14	n.t.	n.a.	n.a.	n.a.
15	349 $\pm$ 28	n.a.	n.a.	62 $\pm$ 3.6
16	n.t.	n.a.	98 $\pm$ 8.3	57 $\pm$ 4.5
17	461 $\pm$ 39	n.a.	n.a.	n.a.
18	n.t.	93 $\pm$ 6.3	57 $\pm$ 3.4	26 $\pm$ 1.5
19	n.t.	n.a.	n.a.	n.a.
20	n.t.	1.2 $\pm$ 0.08	1.0 $\pm$ 0.05	1.2 $\pm$ 0.06
21	n.t.	n.a.	n.a.	n.a.
22	n.t.	n.a.	n.a.	n.a.
23	n.t.	n.a.	n.a.	n.a.
24	n.t.	79 $\pm$ 3.5	37 $\pm$ 2.0	19 $\pm$ 3.6
25	n.t.	n.a.	n.a.	n.a.
26	16 $\pm$ 2.3	38 $\pm$ 2.9	78 $\pm$ 5.6	n.a.
27	41 $\pm$ 5.0	n.a.	n.a.	n.a.
28	n.t.	n.a.	n.a.	n.a.
29	n.t.	n.a.	54 $\pm$ 4.8	58 $\pm$ 4.1
30	n.t.	n.a.	n.a.	n.a.
31	n.t.	n.a.	n.a.	n.a.
32	n.t.	n.a.	32 $\pm$ 2.1	15 $\pm$ 1.6
33	n.t.	n.a.	n.a.	n.a.
34	n.t.	n.a.	n.a.	n.a.
35	n.t.	n.a.	n.a.	n.a.
36	n.t.	n.a.	n.a.	n.a.
37	n.t.	37 $\pm$ 1.9	10 $\pm$ 1.0	14 $\pm$ 1.3
Eto ^c	184 $\pm$ 20	68 $\pm$ 5.5	1.1 $\pm$ 0.08	1.5 $\pm$ 0.07

^a n.t. = not tested. ^b n.a. = not active. ^c Eto = etoposide.

further performed. Supercoiled pBR322 DNA was treated with an excess amount of Top2 in the presence of the tested compounds, and then Top2 was hydrolyzed by proteinase K followed by phenol extraction to remove the compounds. As shown in Fig. 5, Eto (Top2 poisons) could stabilize the Top2-DNA cleavage complex and led to the formation of a new DNA band corresponding to linear plasmid DNA. In compari-

Fig. 5 Top2-mediated DNA cleavage gel assay of test compounds using supercoiled pBR322. Lane 1, supercoiled plasmid DNA (pBR322); lane 2, DNA + Top2; lane 3, DNA + Top2 + etoposide (100 μ M); lane 4, DNA + Top2 + compound **8** (100 μ M). The positions of nicked DNA (Nck), linear DNA (Lin), relaxed DNA (Rel) and supercoiled DNA (SC) are indicated.

son with Eto, no linear plasmid DNA was detected in the case of compound **8**, which suggested that these compounds did not trap double-strand cleavage complexes. Thus, it could be confirmed that compound **8** acted as a Top2 catalytic inhibitor rather than a Top2 poison.

In vitro antiproliferative activity

Three human cancer cell lines (*i.e.*, A549 lung cancer cells, HCT116 colon cancer cells and ZR-75-30 breast cancer cells, provided by the China State Institute of Pharmaceutical Industry) were chosen for testing the *in vitro* antiproliferative activity of the purchased compounds using the standard MTT assay.²⁵ Eto was used as the reference drug. Generally, the antiproliferative activity correlated well with the Top2 inhibitory activity. As depicted in Table 2, most of the compounds without Top2 inhibitory activity did not exhibit *in vitro* antiproliferative activity. Several hits (2, 15, 26 and 28) were found to have moderate to good antiproliferative potency. Compounds **8** and **20** showed good *in vitro* antiproliferative activity with a broad spectrum. Compound **8** was active against all the three human tumor cell lines with IC₅₀ values in the range of 1.5–6.8 μ M. It was 10-fold more active than Eto against the A549 cell line (6.8 μ M *versus* 68 μ M). Compound **20** showed the best antiproliferative activity with IC₅₀ values ranging from 1.0 μ M to 1.2 μ M and was more active than Eto against all the three cell lines. However, compound **20** displayed only moderate inhibitory activity against Top2 at 100 μ M, indicating a different mechanism of action against the cancer cells tested.

Docking study of compound **8**

In order to obtain an accurate binding mode of the hits, compound **8** was docked onto ATPase using the protocol described earlier. As shown in Fig. 6, compound **8** was well-fitted into the ATP-binding domain of Top2. Cation- π interaction was observed between the Mg²⁺ cation and the phenyl group. The carboxy group was engaged with a hydrogen bonding network with Tyr165, Gly166 and Gln376. The carbonyl group in pyrazolone formed two hydrogen bonds with the backbone NH of Ser149 and side chain NH₂ of Asn150, respectively.

Fig. 6 Docking of compound **8** in ATPase (PDB ID 1ZXN). The figure was generated using PyMol (<http://www.pymol.org/>).

Conclusions

On the basis of the crystal structures of ATPase, SBVS was applied to discover novel Top2 catalytic inhibitors. From the 37 compounds selected by virtual screening, a total of 14 compounds were found to be active (hit rate: 38%). Two hits (8 and 20) showed good *in vitro* antiproliferative activity with a broad spectrum. Compound 8 was proven to be a Top2 catalytic inhibitor, which did not stabilize the Top2-DNA cleaved complex. Molecular docking and molecular dynamic simulations predicted that compound 8 might bind at the ATP binding site. It was considered as a good starting point for the development of new antitumor lead compounds targeting Top2.

Conflicts of interest

The authors declare no competing interests.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant 81725020), the Shanghai Education Development Foundation and the Shanghai Municipal Education Commission (Chenguang Program, Grant 15CG42).

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