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Molecular design, synthesis and docking study of benz[*b*]oxepines and 12-oxobenzo[*c*]phenanthridinones as topoisomerase 1 inhibitors

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ABSTRACT

Benz[*b*]oxepines **4a–g** and 12-oxobenzo[*c*]phenanthridines **5a–d** were designed and synthesized as constrained forms of 3-arylisoquinolines through an intramolecular radical cyclization reaction. Radical cyclization of *O*-vinyl compounds preferentially led to the 7-*endo-trig* cyclization pathway to the benz[*b*]oxepines and 12-oxobenzo[*c*]phenanthridines through 6-*exo-trig* path as minor products. Among the synthesized compounds, benz[*b*]oxepine derivative **4e** exhibited potent in vitro cytotoxicity against three different tumor cell lines, as well as topoisomerase 1 inhibitory activity. A Surflex–Dock docking study was performed to clarify the topoisomerase 1 activity of **4e**.

Since topotecan and irinotecan were launched as anti-neoplastic drugs, topoisomerase 1 (topo 1) has been considered a promising target in the development of anticancer drugs.[1] The cytotoxicities of these compounds come from their abilities to inhibit the religation process of the topo 1 reaction, thereby resulting in the accumulation of DNA–drug–topo 1 ternary complex.[2] Both drugs are camptothecin (CPT) derivatives that were developed considering the physicochemical properties of CPT **1**. However, they have critical problems: (i) these drugs must be infused for long periods for cancer treatment as they reverse the CPT-trapped cleavage complexes within minutes; (ii) their inactive decomposed carboxylates are in equilibrium with active lactone forms under physiological conditions; and (iii) these analogs can cause resistance because the drugs are also substrates for efflux transporters.[3] So far, a number of trials to develop novel non-CPT topo 1 inhibitors have been actively pursued.[4–7]

The 3D structure determination of drug–DNA–topo 1 complex provided valuable information of the drug binding mode in the active site and a computer-aided molecular modeling study was utilized to develop novel topo 1 inhibitors.[8]

We have investigated not only the syntheses of isoquinoline alkaloids such as protoberberines and benzo[*c*]phenanthridines, but also QSAR studies of 3-arylisoquinolines that showed topo 1 inhibition and cytotoxicity.[9–12]

Recently, we reported the synthesis and biological evaluation of indenoisoquinolines **3** as constrained forms of 3-arylisoquinolines **2** as shown in Figure 1.[9,12] Rigid structures are commonly considered to have little conformational entropy compared to flexible structures and can be more efficiently fitted into the active site of a receptor. In the previous study, we found that some indenoisoquinoline analogs show potent topo 1 inhibitory activities and cytotoxicities.

Our research next focused on synthesizing new six- or seven-membered heterocyclic rings containing the 3-arylisoquinoline skeleton. Because benz[*b*]oxepine **4** and 12-oxobenzo[*c*]phenanthridines **5** have not yet been synthesized, we investigated their syntheses and biological evaluation.

As depicted in Scheme 1, the benz[*b*]oxepine **4** and 12-oxobenzo[*c*]phenanthridinone **5** could be constructed through intramolecular radical cyclization of compound **6**. The endo type (A) or exo type (B) pathway would yield the benz[*b*]oxepine **4** as well as 12-oxobenzo[*c*]phenanthridinone **5**, respectively. *O*-vinyl compound **6** could be synthesized via the toluamide-benzonitrile cycloaddition reaction from **7** and **8.**

The previously reported lithiated toluamide-benzonitrile cycloaddition method was used to synthesize the 3-arylisoquinolines **9a–c**.[13] *N*-Diethyl-*o*-toluamides **7a–c** were treated with *n*-BuLi to give the anions, which were then reacted with benzonitrile **8** to af-

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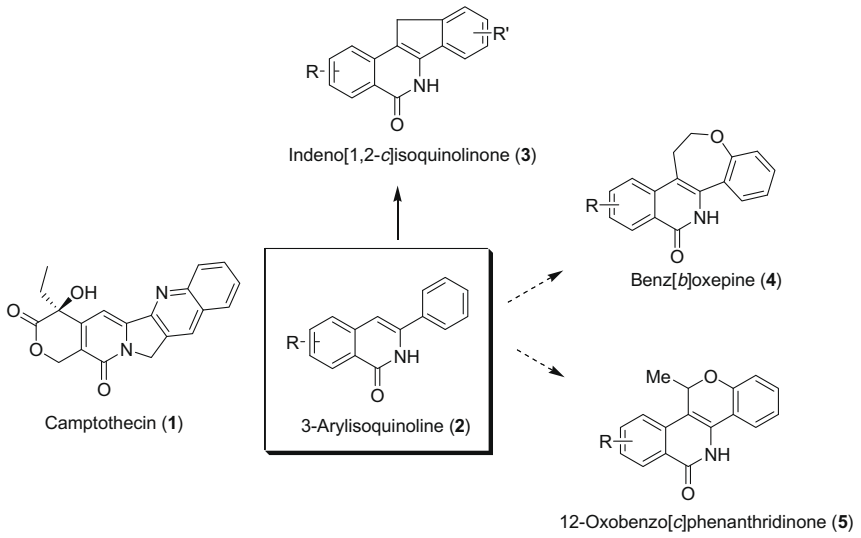
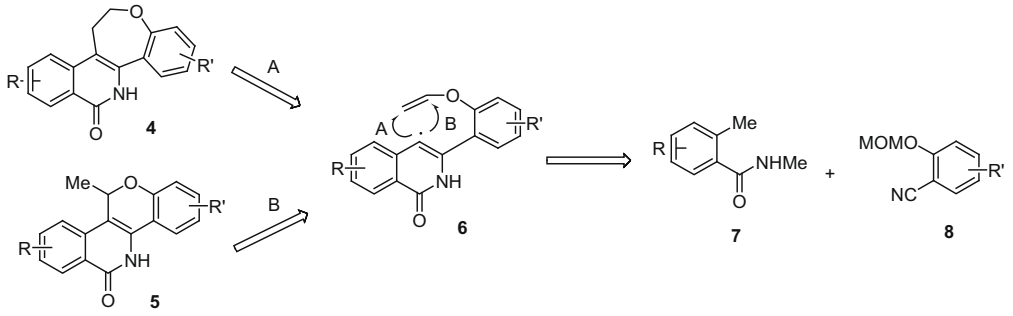


Figure 1. Structure of CPT (**1**) and constrained form of 3-arylisoquinoline to benz[*b*]oxepine (**4**) and 12-oxobenzo[*c*]phenanthridinone (**5**).



Scheme 1. Retrosynthesis of benz[*b*]oxepine (**4**) and 12-oxobenzo[*c*]phenanthridinone (**5**).

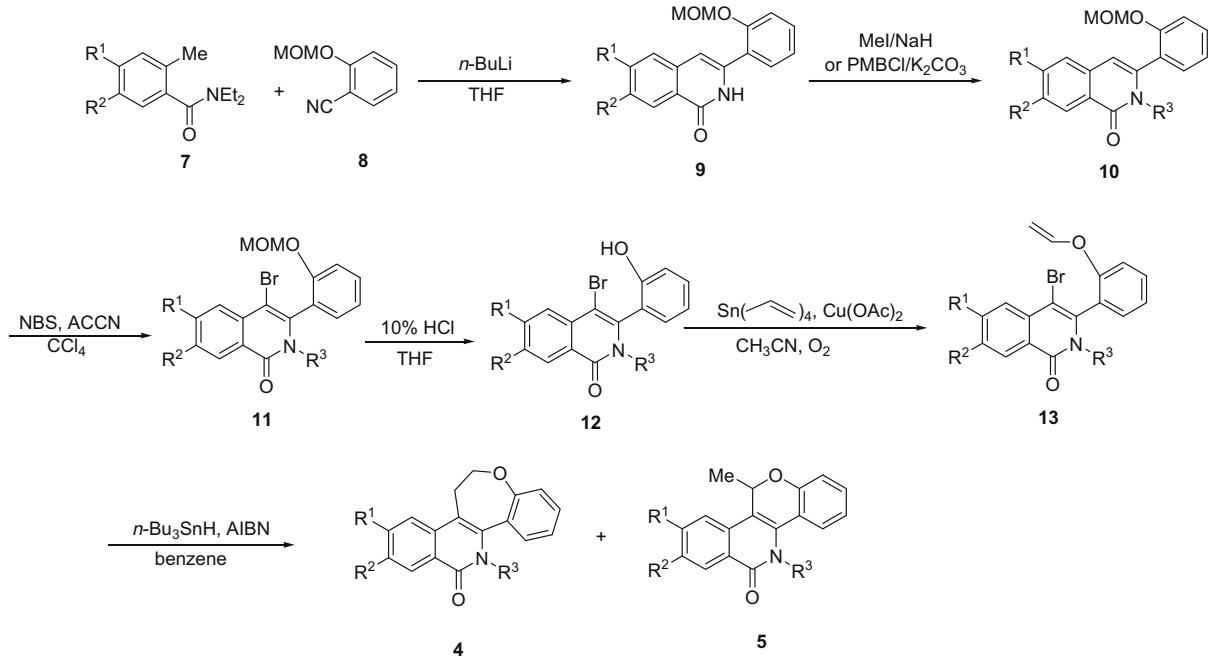
ford the 3-arylisoquinolines **9a–c** in moderate yield. Treatment of **9a–c** with alkyl halides such as MeI or PMBCl in the presence of NaH or K_2CO_3 provided the corresponding N-alkylated compounds 10a–**g** in good yield. Regioselective bromination of **10a–g** on C-4 was accomplished by treatment with *N*-bromosuccinimide and ACCN to yield the desired compounds **11a–g** in good yield.[14] Deprotection of the methoxymethyl group of **11a–g** was achieved by treatment with 10% HCl in THF to give the phenols **12a–g,** which were then reacted with tetravinyl tin and $Cu(OAc)_2$ in the presence of O_2 to afford the desired *O*-vinyl compounds **13a–g**.[15] Radical cyclization reaction of **13a**–**g** with *n*-Bu_3SnH with AIBN in benzene provided the desired benz[*b*]oxepine **4a–g** and 12-oxobenzo[*c*]phenanthridinone **5a–d** in 56–86% yield.[16,17] The ratio of products depended on the substitution pattern of the starting materials **13a–g**. The yields of the final products were as follows: **4a**:**5a** (56:0), **4b**:**5b** (86:0), **4c**:**5c** (61:0), **4d**:**5d** (38:27), **4e**:**5e** (45:31), **4f**:**5f** (40:25), **4g**:**5g** (35:27). Interestingly, PMB-substituted isoquinolines **13a–c** furnished exclusively 7-*endo-trig* pathway products, benz[*b*]oxepines (Scheme 2).

The in vitro cytotoxicity experiments were performed using the synthesized compounds against three human tumor cell lines comprising of A 549 (lung cancer), HL60 (leukemia), and HeLa (cervical cancer) cells in sulforhodamine B (SRB) assays.[18] The topo 1 inhibitory activity assay was carried out using the supercoiled DNA unwinding method.[19]

3-Arylisoquinoline **9a,b** displayed stronger cytotoxicities than N-alkylated compounds **10a–13f** against three tumor cell lines as shown in Table 1. In general, potent cytotoxic activities were observed for the non substituted 3-arylisoquinolines **9a,b** at C2 position relative to their *N*-Me or *N*-PMB compounds. The next worth mentioning is the discrepancy between the cytotoxicity of **9a** (0.62 μM), **9b** (0.16 μM) and their 0% topo 1 inhibition activity. This result is not entirely without precedence, and it is noticeable that the cytotoxicity of these molecules are not related to their capability to inhibit topo 1, but must be dependent upon different unknown biological target.

Unexpectedly, 12-oxobenzo[*c*]phenanthridine analogs **5a–d** exhibited weak cytotoxicity with the exception of dimethoxy-substituted compound **5d** (8.54–13.76 μM). Furthermore, most of these compounds, except **5b** (30.4%) and **5d** (5.3%), had no topo 1 inhibitory activity. These results could not be explained by their low aqueous solubility or poor membrane permeability. However, dramatic enhancement of topo 1 inhibitory activity was observed in benz[*b*]oxepines **4d** (98.2%) and **4e** (94.5%). Moreover, benz[*b*]oxepine compounds showed relatively potent cytotoxicities and topo 1 inhibitory activities compared to 12-oxobenzo[*c*]phenanthridines. Among the benz[*b*]oxepines, the *N*-PMB-substituted compounds **4e**–**g** exhibited greater cytotoxicities than the *N*-Me-substituted compounds **4a**–**d**. Interestingly, compound **4e**, which contains the PMB group at C2 nitrogen, showed more potent cytotoxicities than the *N*-methyl substituted compound **4a**.

The topo 1 inhibitory activity of the compounds is depicted in Figure 2. A quantitative assay was carried out to assess the relative topo 1 inhibitory potency of the compounds. Compound **11d** had half the potency (40.7%) of the reference CPT (86.2%). However, compounds **4d** and **4e** exhibited much more potent inhibitory



Scheme 2. The synthesis of benz[*b*]oxepine and 12-oxobenzo[*c*]phenanthridine analogs. (a) R^1 = H, R^2 = H, R^3 = Me; (b) R^1 = Me, R^2 = H, R^3 = Me; (c) R^1 = H, R^2 = Me, R^3 = Me; (d) R^1 = OMe, R^2 = OMe, R^3 = Me; (e) R^1 = H, R^2 = H, R^3 = PMB; (f) R^1 = Me, R^2 = H, R^3 = PMB; (g) R^1 = H, R^2 = Me, R^3 = PMB.

Table 1
Cytotoxicity (IC_{50}, μM) and topo 1 inhibitory activity (% inhibition) of the compounds

No	Compd	R^1	R^2	R^3	A549	HL60	HELA	Topo 1
1	**4a**	H	H	Me	66.76	37.59	19.66	—[a]
2	**4b**	Me	H	Me	61.32	33.57	41.57	—
3	**4c**	H	Me	Me	30.33	14.12	8.76	11.9
4	**4d**	OMe	OMe	Me	72.98	34.82	38.06	98.2
5	**4e**	H	H	PMB	11.04	4.49	4.78	94.5
6	**4f**	Me	H	PMB	>100	9.26	10.26	—
7	**4g**	H	Me	PMB	4.56	4.40	4.54	—
8	**5a**	H	H	Me	65.52	30.05	40.08	—
9	**5b**	Me	H	Me	46.00	29.13	28.32	30.4
10	**5c**	H	Me	Me	66.89	nt[b]	21.88	—
11	**5d**	OMe	OMe	Me	13.76	8.54	9.02	5.3
12	**9a**	H	H	H	23.6	0.62	0.62	—
13	**9b**	Me	H	H	>100	0.16	0.17	—
14	**10a**	H	H	Me	>100	59.48	64.58	—
15	**10b**	Me	H	Me	99.76	14.28	28.58	—
16	**11c**	H	Me	Me	82.00	17.17	17.81	—
17	**11d**	OMe	OMe	Me	>100	37.79	99.28	40.7
18	**12e**	H	H	PMB	71.25	31.59	36.78	—
19	**12f**	Me	H	PMB	53.01	24.45	20.37	—
20	**13e**	H	H	PMB	>100	59.47	36.47	—
21	**13f**	Me	H	PMB	67.76	29.17	14.77	—
22		CPT			0.058	0.089	0.072	86.2

[a] No inhibition activity.
[b] Not tested.

activity than CPT. In many cases, the topo 1 inhibitory activity did not correlate well with the cytotoxicity. However, compound **4e** showed potent cytotoxicity and potent topo 1 inhibitory activity.

Given the X-ray crystallographic structure of topo 1-DNA complex with indenoisoquinoline, docking studies of indenoisoquinolines in the active site have been more convincing than those of molecules to non-clarified binding sites.[8] To understand the binding mode of action of the most potent topo 1 inhibitor **4e**, we performed a docking study using Surflex–Dock in Sybyl version 8.02 (Tripos Associates) operating under Red Hat Linux 4.0 on an IBM computer (Intel Pentium 4, 2.8 GHz CPU, 1 GB memory).[20]

Surflex–Dock docks ligands automatically into a receptor's ligand binding site using a protomol-based method and an empirically derived scoring function. The protomol is a unique and important factor of the docking algorithm and is a computational representation of assumed ligands that interact with the binding site. Surflex–Dock's scoring function contains hydrophobic, polar, repulsive, entropic, and solvation terms. In addition to the automated docking process, the function in Surflex–Dock has recently been improved by incorporating a base portion matching algorithm that allows a fragment of the ligand to be prepositioned as it docks in the binding site. The fragment is allowed to convey from its original position to a certain point during pose optimization. This is noteworthy when the position of the base portion is not completely set. Ligand docking with the base fragment-matching characteristic is anticipated to yield docking and scoring of ligands constrained to match an exact binding motif.

The structure of the inhibitor **4e** was drawn into the Sybyl package with standard bond lengths and angles and minimized using the conjugate gradient method. The Gasteiger–Huckel charge, with a distance-dependent dielectric function, was applied for the minimization process. We chose the 1SC7 (PDB code) structure from the Protein Data Bank and the structure was polished as follows. The phosphoester bond of G12 in 1SC7 was rebuilt and the SH of G11 on the scissile strand was altered to OH. After running Surflex–Dock, the scores of 10 docked conformers were ranked in a molecular spread sheet. We selected the best total score conformer (5.89) and speculated regarding the detailed binding patterns in the cavity. The resulting docking model revealed a binding mode similar to the indenoisoquinoline model. In our model, the oxepine ring intercalated between the −1 and +1 bases, parallel to the plane of the base pairs as depicted in Figure 3. The oxygen of the oxepine ring had an H-bond with the amino group of A 113, which is considered an essential nucleotide that interacts with the ligand in the DNA–top 1 active site. In our model, the isoquinoline ring and seven-membered oxepine ring worked as DNA intercalator as well as blocker of the religation step of the phosphoester. In this docking study, we observed that the seven-membered oxepine ring

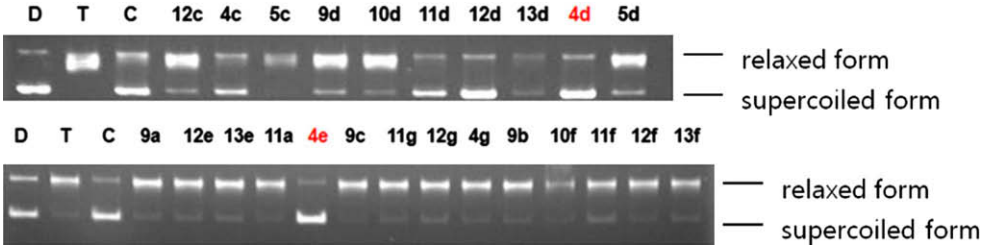


Figure 2. Topo 1 inhibitory activities of compounds. Compounds were examined at a final concentration of 100 μM. Lane D: pBR322 only, Lane T: pBR322 + Topo 1, Lane C: pBR322 + Topo 1 + CPT, Lane 1–10 or 1–14: pBR322 + Topo 1 + compounds.

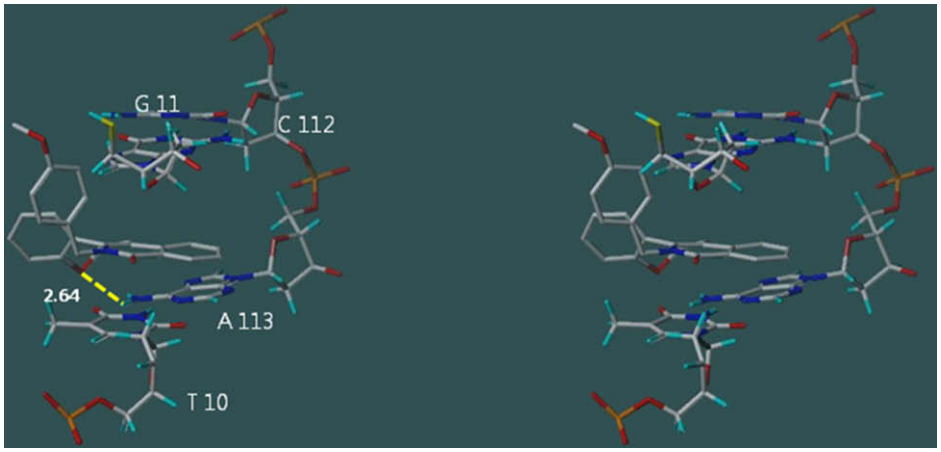


Figure 3. Wall-eyed viewing docked model of compound **4e**.

was positioned as a DNA intercalator, and the PMB group was located in the cavity between DNA and topo 1.

In conclusion, we synthesized various benz[*b*]oxepine and 12-oxobenzo[*c*]phenanthridines as analogs of constrained 3-arylisoquinolines structures. An intramolecular radical cycloaddition reaction was employed to efficiently generate benz[*b*]oxepine and 12-oxobenzo[*c*]phenanthridines. Although 3-arylisoquinoline synthetic intermediates did not exhibit interesting biological activities, the oxepine compounds, that is, the constrained structures of 3-arylisoquinolines, exhibited more potent topo 1 inhibitory activity than CPT. Among the synthesized compounds, **4e** had potent topo 1 inhibitory activity as well as cytotoxicity against three different tumor cell lines. To further explain the topo 1 inhibitory activity of **4e**, molecular docking studies were carried out with the Surflex–Dock program to give a reasonable binding mode of the compound in the binding site of DNA and topo 1. For further study of constrained structures of 3-arylisoquinolines, we are currently investigating diverse structural modifications in the synthesis of 3-arylisoquinolines, and the results and structure–activity relationships will be reported in due course.

Acknowledgment

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17. *All synthesized compounds were fully characterized by spectroscopy*. Selected data for some compounds: **4a**; mp: 153.4–157 0 °C, IR (cm^{-1}): 1639 (C=O), ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, J = 7.8 Hz, 1H), 7.72–7.70 (m, 2H), 7.55–7.50 (m, 1H), 7.45–7.40 (m, 1H), 7.36–7.28 (m, 2H), 7.24 (dd, J = 1.1, J = 8.0 Hz, 1H), 4.58–4.53 (m, 2H), 3.55 (s, 1H), 3.15–3.08 (m, 1H), 2.53–2.41 (m, 1H). EI-MS m/z (%) 277 (M^+, 100). Compound **4b**; mp: 169.7–173.9 °C, IR (cm^{-1}): 1639 (C=O), ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45 (d, J = 8.2 Hz, 1H), 7.49 (s, 1H), 7.42 (t, J = 7.5 Hz, 1H), 7.36–7.28 (m, 3H), 7.23 (d, J = 8.1 Hz, 1H), 4.57–4.53 (m, 2H), 3.54 (s, 3H), 3.14–3.08 (m, 1H), 2.52 (s, 3H), 2.48–2.39 (m, 1H). EI-MS m/z (%) 291 (M^+, 84). Compound **5a**; mp: 173.1–176.9 ^{0}C, IR (cm^{-1}): 1648 (C=O), ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51 (d, J = 7.9 Hz, 1H), 7.71 (t, J = 8.3 Hz, 1H), 7.59 (d, J = 8.2 Hz, 1H), 7.52 (t, J = 7.6 Hz, 2H), 7.30 (t, J = 7.5 Hz, 1H), 7.11–7.06 (m, 2H), 5.80 (dd, J = 6.6, J = 13.3 Hz, 1H), 3.81 (s, 3H), 1.33 (d, J = 6.7 Hz, 3H). EI-MS m/z (%) 277 (M^+, 100). Compound **5b**; mp: 146.2–150.7 °C, IR (cm^{-1}): 1646 (C=O), ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (d, J = 8.2 Hz, 1H), 7.59 (d, J = 8.3 Hz, 1H), 7.32 (t, J = 6.9 Hz, 2H), 7.10–7.05 (m, 3H), 5.78 (dd, J = 6.6, J = 13.1 Hz, 1H), 3.79 (s, 3H), 2.52 (s, 3H), 1.33 (d, J = 6.7 Hz, 3H). EI-MS m/z (%) 291 (M^+, 88).
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