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Convenient synthesis of indeno[1,2-*c*]isoquinolines as constrained forms of 3-arylisoquinolines and docking study of a topoisomerase I inhibitor into DNA–topoisomerase I complex

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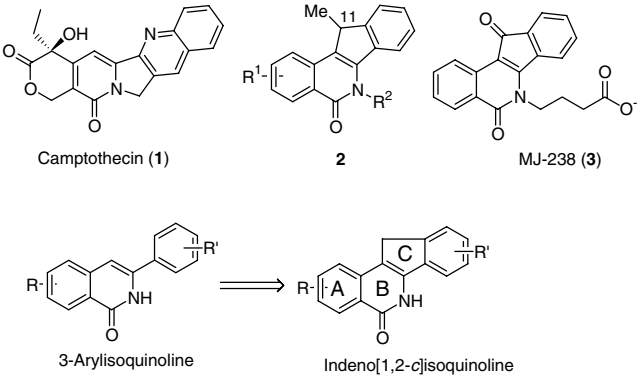
Abstract—11-Hydroxyindeno[1,2-*c*]isoquinolines **12a–c** were prepared as constrained forms of 3-arylisoquinolines through an intramolecular cyclization reaction. Among the synthesized compounds, the 11-^{t}butoxy analog **15l** displayed potent in vitro cytotoxicity against four different tumor cell lines as well as topoisomerase 1 inhibitory activity. A FlexX docking study was performed to explain the topoisomerase 1 activity of **15l**.

Topoisomerase 1 (top 1) inhibitors have emerged as promising anticancer drugs since topotecan and irinotecan were launched.[1] Both drugs are camptothecin (CPT) derivatives, which were developed considering physicochemical properties of camptothecin (**1**) such as water solubility and stability. The critical drawbacks of camptothecin analogs can be summarized as follows.[2,3] These drugs must be infused for long periods for cancer treatment as they reverse the CPT-trapped cleavage complexes within minutes and their inactive decomposed carboxylates are in equilibrium with active lactone forms under physiological conditions. Moreover, these analogs can cause resistance because the drugs are also substrates for the efflux transporters.[4] Therefore, the development of novel non-camptothecin top 1 inhibitors has been actively pursued.[5–7]

As a part of our ongoing effort to develop isoquinoline antitumor agents, we designed indenoisoquinolines as constrained forms of 3-arylisoquinolines as shown in Figure 1. Generally, constrained structures are considered to have little conformational entropy compared to flexible forms and can be more efficiently fitted into the active site of a receptor.[8] 11-Methylindenoisoquinoline analogs of **2** that bear several substituents on aromatic ring A and on the nitrogen atom have previously been synthesized and have top 1 activities; their cytotoxicities have also been tested.[9]



Camptothecin (**1**) **2** MJ-238 (**3**)

Figure 1. Structure of camptothecin and constrained form of 3-arylisoquinoline to indeno[1,2-*c*]isoquinoline.

Keywords: Indenoisoquinoline; Topoisomerase 1; Docking study; Antitumor agents; Synthesis; Cytotoxicity.
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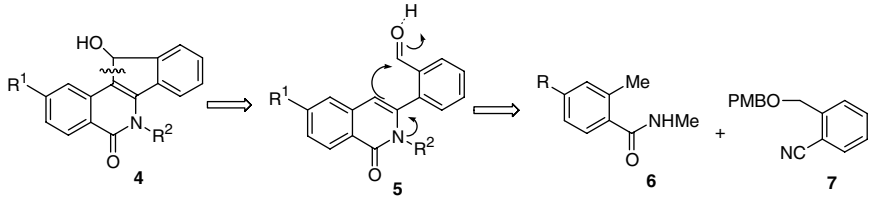
Although 11-methylindenoisoquinolines **2** have weak top 1 inhibitory activity, their potent cytotoxicities against tumor cell lines led us to explore the structure–activity relationships of indenoisoquinolines. Next, our research focused on introducing an oxygen functionality at C 11 because the carbonyl group was known to be essential for H-bonding with Arg 364 of top 1.[10] Moreover, modification of the carbonyl group to another group, such as hydroxy, reduction of carbonyl group or its replacement with an alkoxy group would provide detailed information of the structure–activity relationship of indeno[1,2-*c*]isoquinolines in the binding pocket.

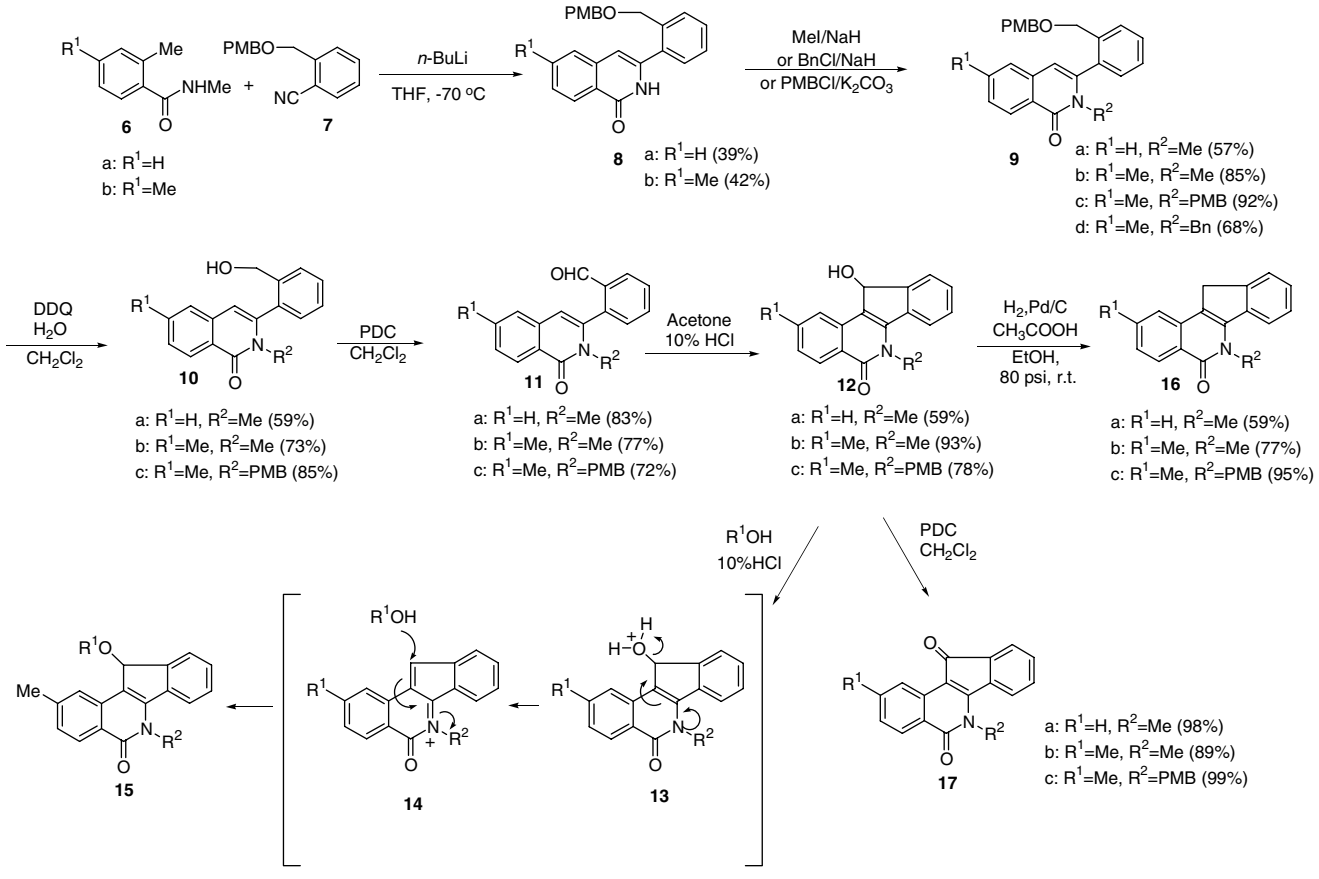
The C ring of indeno[1,2-*c*]isoquinoline **4** could be constructed through intramolecular enamide aldehyde cyclization of compound **5**. 3-Arylisoquinoline **5** could be synthesized via toluamide-benzonitrile cycloaddition reaction from **6** and **7** as depicted in Schemes 1 and 2.[11]

In 2005, the X-ray crystal structure of the indenoisoquinoline analog (MJ238)-DNA–top 1 ternary complex was revealed.[12] Interestingly, in this paper, the camptothecin analog (topotecan), the indenoisoquinoline derivative (MJ238) (**3**), and the indolocarbazole analog bound to the same binding sites, despite the structural difference of these compounds.[12] Disclosure of the detailed binding pocket in the cleavage site of the top 1–DNA complex enabled researchers to do computational investigations such as docking studies and virtual screening using databases to find novel ligands.

The previously reported lithiated toluamide-benzonitrile cycloaddition method was used to synthesize the 3-arylisoquinolines **8a**, **b**. *N*-Methyl-*o*-toluamides **6a**, **b** were treated with *n*-BuLi to give the anions, which were then reacted with benzonitrile **7** to afford the 3-arylisoquinolines **8a**, **b** in 39% and 42% yield,



Scheme 1. Retrosynthesis of indenoisoquinoline.



Scheme 2. The synthesis of indeno[1,2-*c*]isoquinolines.

respectively.[13,14] Careful treatment of alkyl halides such as MeI, BnCl, and PMBCl with **8a**, **b** in the presence of NaH or K_2CO_3 produced the corresponding N-alkylated compounds **9a–d** in 57–92% yield. Deprotection of the benzyl group on the hydroxymethyl on **9a–d** was achieved by treatment of DDQ in methylene chloride. Interestingly, under these reaction conditions, the PMB group attached to the amide nitrogen on **9c** was retained. PDC oxidation of **10a–c** provided the corresponding aldehydes **11a–c**, which were then treated with 10% HCl in acetone to give the desired cyclization adducts **12a–c** in 59–93% yield. Interestingly, when the alcohols **12a–c** were reacted with various alcohols in the presence of 10% HCl, the corresponding alkoxy compounds **15a–m** were obtained in good yield. This result could be explained by the successive reactions: dehydration of **13** in the acidic condition and consecutive nucleophilic attack of alcohols at C-11 position to provide C-11 alkoxy compounds **15a–m**. The catalytic hydrogenation of **12a–c** with 5% Pd/C under 80 psi hydrogen gas in EtOH afforded **16a–c** in 59–95% yield. The hydroxyl group at C-11 was oxidized by PDC in methylene chloride to provide the corresponding 11-keto indenoisoquinolines **17a–c** in excellent yield.

The in vitro cytotoxicity experiments of the synthesized compounds were performed against four human tumor cell lines including A 549 (lung), SKOV-3 (ovarian), SK-MEL-2 (melanoma), and HCT 15 (colon) using sulforhodamine B (SRB) assays.[15] The top 1 inhibitory activity assays were carried out using the supercoiled DNA unwinding method. Five hundred nanograms of supercoiled pBR 322 DNA was incubated with 1 U top 1 in the absence or presence of camptothecin or the synthesized compounds for 30 min at 37 °C. The reaction mixtures were analyzed on 1% agarose gel followed by ethidium bromide staining.[16]

In Table 1, the IC_{50} cytotoxicity values obtained with cell lines and the relative potencies of the compounds are expressed semi-quantitatively as follows: +, weak activity; +++, lower activity than 0.1 μM camptothecin; ++++, similar or greater activity than 0.1 μM camptothecin.

Among the various cytotoxic functions of indenoisoquinolines, the carbonyl group at C11 position is known to contribute to hydrogen bonding with Arg 364 of top 1. As shown in Table 1, the cytotoxic activities of indenoisoquinoline analogs are highly influenced by the alkoxy analogs, rather than by ketone or hydroxyl derivatives. 11-Hydroxy analogs **12b–c** did not exhibit significant cytotoxicities against the four tumor cell lines. These results were not surprising due to the fact that the hydroxyl group does not work well as a hydrogen-bonding donor with Arg 364 of top 1. Compounds **16a–c** also did not display potent cytotoxicity. Compound **16c** showed low potency (8.9 μmol) against the HCT 15 cell line. Unexpectedly, 11-keto analogs **17a–c** exhibited weak cytotoxicity (14–30 μmol) or even worse activity than **16a–c**. Furthermore, these compounds did not have any top 1–DNA inhibitory activity. These results could not be explained by their low aqueous solubility or poor membrane permeability. However, dramatic enhancement of cytotoxicity and top 1 inhibitory activity was observed when the hydroxyl groups were transformed to alkoxy analogs, especially compounds **15g–m**. These compounds contain *p*-methoxybenzyl group at C6 nitrogen and homologous alkoxy

Table 1. Synthetic yield, IC_{50} cytotoxicity (μM), and top 1 activity of compounds

No.	Compound	R^1	R^2	A549	HCT15	OV-3	MEL-2	Top 1[a]
1	**12b**	Me	Me	300.47	20.88	60.11	110.97	–
2	**12c**	Me	PMB	100.41	20.25	70.99	180.72	–
3	**15a**	Me	Me	130.47	20.86	60.06	70.26	–
4	**15b**	Et	Me	90.03	20.61	70.94	40.85	–
5	**15c**	^{n}Pr	Me	40.60	10.89	70.68	80.34	–
6	**15d**	^{n}Bu	Me	110.24	10.19	30.48	20.91	–
7	**15e**	^{i}Bu	Me	28.15	27.39	38.37	10.52	–
8	**15f**	^{n}Pt	Me	16.19	22.01	13.33	26.50	–
9	**15g**	Me	PMB	10.25	1.11	1.36	15.16	+
10	**15h**	Et	PMB	6.71	1.93	5.89	3.60	+
11	**15i**	^{n}Pr	PMB	3.45	1.42	5.76	6.26	+++
12	**15j**	^{i}Pr	PMB	6.22	0.91	1.21	2.43	+++
13	**15k**	^{n}Bu	PMB	3.24	4.40	2.01	3.37	–
14	**15l**	^{i}Bu	PMB	1.87	9.92	1.63	2.07	+++++
15	**15m**	^{n}Pt	PMB	11.21	5.59	5.70	11.25	–
16	**16a**	H	Me	30.09	20.50	30.56	130.31	–
17	**16b**	Me	Me	130.18	20.14	260.79	80.26	–
18	**16c**	Me	PMB	50.98	8.9	23.2	31.9	–
19	**17a**	H	Me	23.51	30.55	80.95	90.20	–
20	**17b**	Me	Me	20.05	14.34	16.29	17.14	–
21	**17c**	Me	PMB	155.30	171.36	102.22	157.54	–
22	CPT			0.067	0.080	0.024	0.075	+++
23	Doxo rubicin			0.97	1.67	1.17	4.78	

[a] Activity is expressed semi-quantitatively as follows: –, very weak activity; +, weak activity; +++, similar activity to camptothecin; +++++, stronger activity than camptothecin.

Figure 2. Top 1 inhibitory activities of the compounds. Lane P, pBR322; lane T, pBR322 + topoisomerase 1; lane C, pBR322 + topoisomerase 1 + camptothecin (0.01 mg/ml); lanes 1–10 (prepared compound number, 0.1 mg/ml): 1 (**15 g**), 2 (**15 d**), 3 (**15h**), 4 (**15e**), 5 (**15f**), 6 (**15k**), 7 (**15m**), 8 (**15i**), 9 (**15j**), and 10 (**15l**).

groups from methoxy to ^{n}pentoxy at the C11 position. The isobutoxy compound **15l** exhibited the most potent top 1 activity as well as strong cytotoxicity (1.63–9.92 μmol) against all four tumor cell lines. Interestingly, compounds **15g–m**, which contain *p*-methoxybenzyl group at C4 nitrogen, showed more potent cytotoxicities than the *N*-methyl substituted compounds **15a–f**. Top 1 inhibitory activity of the compounds is depicted in Figure 2. The semi-quantitative assay was carried out to show the relative top 1 potency of the compounds. Compounds **15i** and **15j** had the same potency as the reference camptothecin. However, compound **15l** exhibited much more potent inhibition activity than camptothecin. In many cases, the top 1 activity does not correlate well with cytotoxicities. However, compound **15l** showed potent cytotoxicity and potent top 1 activity.

Given the X-ray crystallographic structure of top 1–DNA complex with indenoisoquinoline (MJ238), docking studies of indenoisoquinolines into the active site have been considered more convincing than that of molecules to non-clarified binding sites. To understand the binding mode of action of the most potent top1 inhibitor **15l**, we performed a docking study using FlexX in the Sybyl 7.2.5 version by Tripos Associates, operating under Red Hat Linux 4.0 with an IBM computer (Intel Pentium 4, 2.8 GHz CPU, 1GB memory).

FlexX docking into the DNA–top 1 active site cavity consisted of three steps: (1) defining the active site; (2) constructing the ligand structure and, if needed, building a ligand database for multi-ligand docking process; (3) defining the receptor description file (RDF). FlexX was developed as a new technique for structure-based drug design. Fragments of the ligand are automatically placed into the active site using a new algorithmic approach based on a pattern recognition technique called pose clustering. Placement of the ligand is scored based on protein–ligand interactions. Finally, the binding energy is estimated, and placements are ranked.

The structure of the inhibitor **15l** was drawn into the Sybyl package with standard bond lengths and angles and minimized using the conjugate gradient method until the gradient was 0.001 kcal/mol with the Tripos force field. The Gasteiger–Huckel charge, with a distance-dependent dielectric function, was applied for the minimization process. We chose the 1SC7 (PDB code) structure in Protein Data Bank and the structure was refined as follows. The phosphoester bond of G12 in 1SC7 was rebuilt and the SH of G11 on the scissile strand was changed to OH. After the active site was defined with a 6.5 Å radius, DNA nucleotides such as G12, G11, T10, and T9 on the scissile strand and C112, A113, and A114 on the non-scissile strand were selected as heteroatoms for the RDF file. Docking simulations were carried out using FlexX Single Receptor mode with a Mol2 file molecule as a Ligand Source. After running FlexX, 30 docked conformers were displayed in a molecular spread sheet to rank the scores. We selected the best total score conformer (−19.188) and speculated regarding the detailed binding patterns in the cavity. The resulting docking model revealed a very different binding mode compared to the former 11-methylindenoisoquinoline model.[9] In our model, the benzene ring of *p*-methoxybenzyl group intercalated between the −1 and +1 bases, parallel to the plane of the base pairs, and the indenoisoquinoline skeleton, which was positioned between the −1 and +1 bases in the 11-methylindenoisoquinoline model, was placed in the cavity between the DNA and the top 1 residues, Ala 351, Asn 352, and Lys 425, perpendicular to the DNA base pairs as depicted in Figure 3. The oxygen of the *p*-methoxybenzyl group was H-bonded to Arg 364, which is considered an essential amino acid that interacts with the ligand in the DNA–top 1 active site.

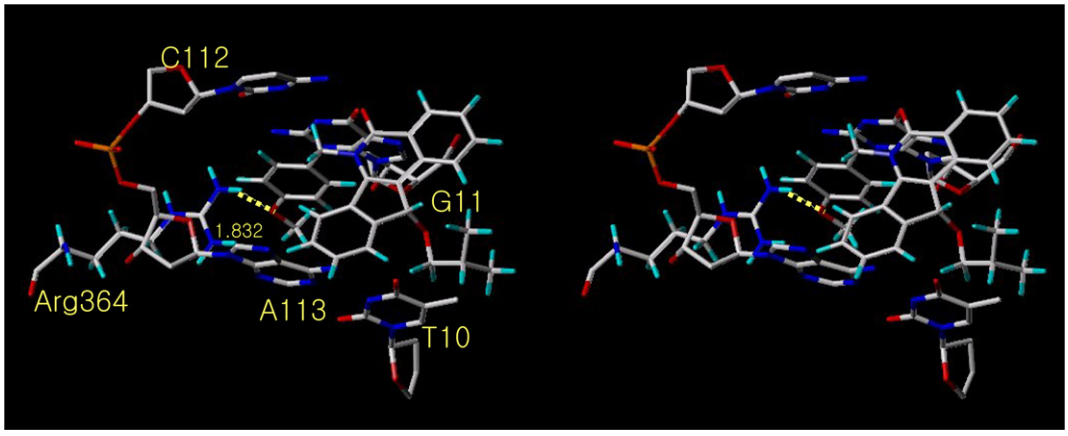


Figure 3. Wall-eyed viewing docked model of compound **15l**.

In our model, the *p*-methoxybenzyl group worked as a DNA intercalator and as a blocker of the religation step of the phosphoester. From this docking study, we observed that the indenoisoquinoline ring could be positioned in the active site, not as a DNA intercalator, and the other aromatic ring could replace it.

In conclusion, we prepared various indeno[1,2-*c*]isoquinoline analogs as constrained 3-arylisoquinoline structures. An intramolecular cycloaddition reaction was employed to efficiently generate 11-hydroxyindenoisoquinolines. In order to investigate the structure–activity relationships, the 11-hydroxy group of the compounds was modified to another group such as a ketone, dihydro or alkoxy group. The cytotoxic activity of these analogs was then measured in various cancer cells. The alkoxy derivatives displayed higher cytotoxicity and top 1 inhibitory activity than the 11-hydroxy and 11-keto compounds. Although the reason for these higher cytotoxicities and top 1 activity is presently not clear, the top 1 activity could be explained by a docking study using FlexX in the Sybyl program. To this end, we are currently investigating the structure–activity relationships of diverse substituted indenoisoquinolines, and the results will be reported in due course.

Acknowledgment

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14. All synthesized compounds were fully characterized by spectroscopy. Selected data for some compounds: compound **12a**; mp: 217–219 °C. IR (cm^{-1}): 3338, 1621 ^1H NMR (DMSO-d_6): δ 8.22 (d, J = 8.1, 1H), 7.72–7.37 (m, 7H), 5.80 (d, J = 7.4, 1H), 5.50 (d, J = 8.3, 1H), 3.94 (s, 3H). EIMS *m*/*z* (%) 263 (M^+, 100). Compound **12b**; mp: 235–237 °C. ^1H NMR ($CDCl_3$): δ 8.13 (d, J = 8.2, 1H), 7.94–7.28 (m, 6H), 5.80 (d, J = 8.5, 1H), 5.52 (d, J = 8.4, 1H), 3.95 (s, 3H), 2.46 (s, 3H). EIMS *m*/*z* (%) 277 (M^+, 86). Compound **12c**; mp: 226–228 °C. ^1H NMR (DMSO-d_6): δ 8.18 (d, J = 8.2, 1H), 7.89 (s, 1H), 7.64–6.85 (m, 9H), 5.82 (d, J = 8.6, 1H), 5.69 (s, 2H), 5.55 (d, J = 8.5, 1H), 3.69 (s, 3H), 3.35 (s, 3H). EIMS *m*/*z* (%) 383 (M^+, 64). Compound **15l**; mp: 175–176 °C. ^1H NMR ($CDCl_3$): δ 8.37 (d, J = 8.3, 1H), 7.89–6.82 (m, 10H), 5.80–5.70 (m, 2H), 5.73 (s, 1H), 3.74 (s, 3H), 2.92–2.87 (m, 1H), 2.79–2.74 (m, 1H), 2.53 (s, 3H), 1.81–1.76 (m, 1H), 0.86–0.83 (m, 6H). EIMS *m*/*z* (%) 439 (M^+, 100). Compound **17a**; mp: 218- 221 °C. ^1H NMR ($CDCl_3$): δ 8.58 (d, J = 8.0, 1H), 8.28–7.35 (m, 7H), 4.00 (s, 3H). EIMS *m*/*z* (%) 261 (M^+, 75).
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