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Design, docking, and synthesis of novel indeno[1,2-*c*]isoquinolines for the development of antitumor agents as topoisomerase I inhibitors

Won-Jea Cho,[a,*] Quynh Manh Le,[a] Hue Thi My Van,[a] Kwang Youl Lee,[a] Bok Yun Kang,[a] Eung-Seok Lee,[b] Sang Kook Lee[c] and Youngjoo Kwon[c]

[a]*College of Pharmacy and Research Institute of Drug Development, Chonnam National University, Kwangju 500-757, Republic of Korea*
[b]*College of Pharmacy, Yeungnam University, Kyongsan 712-749, Republic of Korea*
[c]*College of Pharmacy, Ewha Womans University, Seoul 120-750, Republic of Korea*

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Abstract—An intramolecular radical cyclization reaction of 4-bromo-3-arylisoquinolines **11a**–**c** allowed the efficient synthesis of 11-methylindenoisoquinolines **2a**–**c**. 5-(2-Aminoethylamino)indeno[1,2-*c*]isoquinolin-11-one **4** was also prepared in the convenient manner. The synthesized compounds were tested in vitro for cytotoxicity and DNA-topoisomerase 1 (top 1) inhibitory activity. The dramatic enhancement of top 1 inhibitory activity of **4** was explained by a docking study using the FlexX program.

DNA-topoisomerase 1 (top 1) is considered an important enzyme to relax supercoiled DNA for transcription, replication, and mitosis.[1] Elevation of the top 1 levels in solid tumors compared to normal tissue has made top 1 a promising target for antineoplastic drugs.[2] The X-ray crystal structures of DNA-top 1 have been revealed with ligands such as topotecan, indenoisoquinoline, and indolocarbazole molecules.[3] Interestingly, the binding pockets of all of these compounds are almost identical despite their structural diversity. Furthermore, the detailed information on the binding sites has made structure-based drug design possible.

Irinotecan and topotecan are camptothecin derivatives, and they are the only approved drugs for cancer treatment.[4] Although these two drugs were developed as top 1 inhibitors, new compounds need to be found because of the instability and short infusion times for maximum activity of these drugs.[5]

Recently, we reported not only the syntheses of isoquinoline alkaloids, but also quantitative structure–activity relationship studies of 3-arylisoquinolines that showed top 1 inhibition and cytotoxicity.[6–9] The current investigation was undertaken to explore indenoisoquinolines that are considered constrained forms of 3-arylisoquinolines as depicted in Figure 1.

11-Methylindenoisoquinoline **2b** was chosen as a first target compound because of the potent cytotoxicity of **1**[6] and the ethylenediamine moiety was scheduled to introduce on 5 position of compound **4** due to the strong top 1 activity of **3**.[7]

Generally, a rigid molecule has little conformational entropy but is to fit optimally into the receptor. We planned to add one carbon atom between the B and C rings of 3-arylisoquinolines to produce indenoisoquinolines. Although the Cushman group have reported profoundly valuable researches on indenoisoquinolines,[10] 11-methyl or 5-ethylenediamino substituted compounds have not yet been reported.

The 3-arylisoquinolines **7a**–**c** were synthesized by the previously reported lithiated toluamide-benzonitrile cycloaddition method.[11] *N*-methyl-*o*-toluamide **5a**–**b**

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* Corresponding author. Tel.: +62 530 2933; fax: +62 530 2911; e-mail: wjcho@jnu.ac.kr

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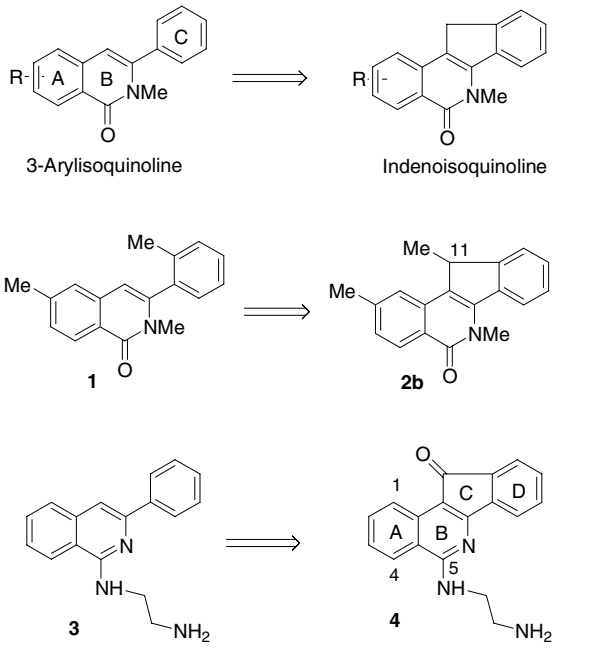


Figure 1. The constrained structure of 3-arylisoquinolines to indeno[1,2-*c*]isoquinolines.

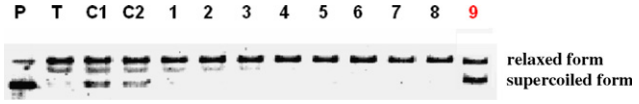


Figure 2. Top 1 inhibitory activities of the compounds. lane P: pBR322; lane T: pBR322 + topoisomerase I; lane C1: pBR322 + topoisomerase I + camptothecin (0.1 mg/ml); lane C2: pBR322 + topoisomerase I + camptothecin (0.01 mg/ml); lanes 1–9 (prepared compound number, 0.1 mg/ml): 1(**10b**), 2(**11b**), 3(**11c**), 4(**9b**), 5(**9c**), 6(**2a**), 7(**2b**), 8(**2c**), and 9(**4**).

was treated with *n*-BuLi to give the anions, which were then reacted with benzonitrile **6**[12] to afford the 3-arylisoquinolines. The obtained compounds were treated with MeI/60% NaH or PMBCl/K_2CO_3 to give *N*-alkylated compounds **7a**–**c** without detecting *O*-alkylated ones. Selective bromination of **7a**–**c** was accomplished with NBS/ACCN in CCl_4 to provide **8a**–**c** in good yield. *P*-Methoxybenzyl groups were removed from **8a**–**c** by DDQ oxidation to give the allyl alcohols **9a**–**c**, which were then oxidized with PDC to afford the aldehydes **10a**–**c** in excellent yield. In this reaction, *p*-methoxybenzyl group on the *N* of the amide group was not affected by DDQ oxidation. Wittig reaction of aldehydes **10a**–**c** with Ph_3PCH_3Br in the presence of *n*-BuLi was carried out to give the styrenes **11a**–**c** in good yield (62–95%). Styrenes **11a**–**c** were treated with *n*-Bu_3SnH in the presence of azobiscyclohexanecarbonitrile (ACCN) to provide the desired 6,11-dihydro-5*H*-indeno[1,2-*c*]isoquinolin-5-ones **2a**–**c** in 55–88% yield. This result could be explained by Baldwin's rule that the 5-*exo*-trig pathway is favored over 6-*endo*-trig in a general way during ring closure reactions.[13] Commercially available 6-oxabenzo[*a*]fluorine-5,11-dione **12** was efficiently converted to indenoisoquinoline **13** by treatment with NH_4OH; **13** was then reacted with $POCl_3$ followed by substitution reaction with ethylenediamine to give the desired 5-(2-aminoethylamino)indeno[1,2-*c*]isoquinolin-11-one **4** in good yield (Scheme 1).

Cytotoxicity experiments with the synthesized compounds were performed by sulforhodamine B (SRB) for A549 (lung), Col2 (colon), SNU-638 (stomach), HT1080 (fibro sarcoma) cell lines and MTT assay for HL-60 (myeloid leukemic) cells.[14]

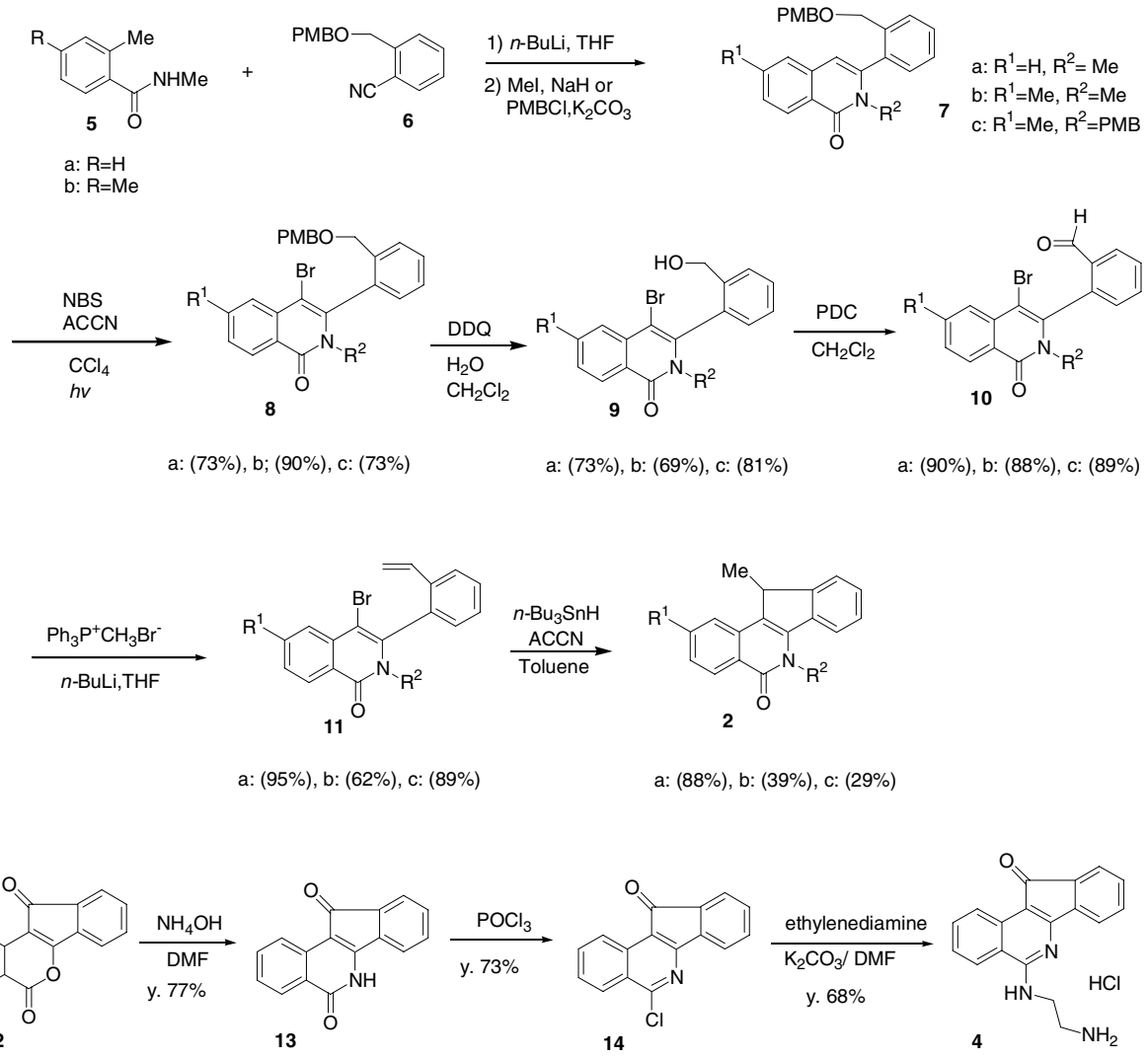
To determine top 1 catalytic activity, assays were performed with supercoiled pBR32 DNA as the substrate according to protocol.[7] Semi-quantitative comparisons of the inhibitory activities are shown in Table 1.

As expected, indenoisoquinolines **2a**–**c** exhibited more potent cytotoxicity against these five cell lines than 3-arylisoquinolines **8a**–**c**, **9b**–**c**, **10b**–**c**, and **11b**–**c** (Table 1). Indenoisoquinolines **2a**–**c** displayed 1.70–8.5 μM activity against the HL 60 cell line, and its activity ranged from 2.58 to 9.0 μM against the other 4 cell lines. All 4-bromo-3-arylisoquinolines **8a**–**11c** exhibited very weak cytotoxicities. Moreover, none of these compounds had strong top 1 inhibitory activity, including the indenoisoquinolines **2a**–**c**. On the other hand, 5-(2-aminoethylamino)indeno[1,2-*c*]isoquinolin-11-one **4** exhibited potent top 1 inhibitory activity and cytotoxicity (Fig. 2).

In the isoquinolinamine series such as **2a**–**c** and **4**, we found that top 1 inhibitory activity did not correlate particularly well with cytotoxicity. This discrepancy indicates that top 1 may not be the sole biological target for these compounds. Similar results could be found in other reports[15] and explained by the ability of the compounds to penetrate the cell membrane and reach the target as well as distribution differences within the cell.

Therefore, we used the FlexX docking program, a molecular modeling tool, to determine the top 1 inhibitory activity of **4**. Computational calculations were performed by Sybyl molecular modeling software, version 7.2.5, supplied by Tripos Associates, operating under Red Hat Linux 4.0 with an IBM computer (Intel Pentium 4, 2.8G CPU, 1G memory).

The X-ray crystallographic structure of the indenoisoquinoline (MJ238)-DNA-top 1 complex[3] (PDB:1SC7) in Protein Data Bank was selected for the docking study. The bond to the disconnected phosphoester of G12 in 1SC7 was reconstructed, and the SH of G11 on the scissile strand was changed to OH. The position and orientation of the ligand should be considered to determine whether it could protect the religation of the G11 hydroxyl group to the PTR723-phosphoester bond or not. For this, we defined the receptor descriptor file based on indenoisoquinoline molecule (MJ238) intercalated in the ternary complex. The active site was defined with a 6.5 Å radius based on indenoisoquinoline. DNA nucleotides including G12, G11(+1), T10(−1), T9 on the scissile strand and C112, A113, A114 on the non-scissile strand were selected as heteroatoms for RDF file preparation. After running FlexX, 30 docked conformers were displayed as a molecular spread sheet ranking the scores.



Scheme 1. The synthesis of indenoisoquinolines.

Table 1. IC_{50} Cytotoxicity (μM) and top 1 activity of the compounds

No	Compound	R^1	R^2	A549	Col2	SNU-638	HL60	HT1080	Top 1[a]
1	**8a**	H	Me	77.64	>100	>100	76.7	>100	–
2	**8c**	Me	PMB	>100	>100	>100	>100	>100	–
3	**9b**	Me	Me	25.06	99.3	18.7	>100	70.2	–
4	**9c**	Me	PMB	43.37	23.4	16.4	>100	31.7	–
5	**10a**	H	Me	60.67	67.4	16.4	6.0	11.4	–
6	**10b**	Me	Me	37.58	33.8	22.0	17.6	46.5	++
7	**10c**	Me	PMB	18.46	69.8	19.8	>100	17.8	–
8	**11b**	Me	Me	49.35	61.1	14.0	18.3	38.6	++
9	**11c**	Me	PMB	84.27	16.8	14.5	>100	42.2	–
10	**2a**	H	Me	2.58	4.90	7.40	1.70	5.80	–
11	**2b**	Me	Me	2.98	7.4	9.0	7.1	5.9	–
12	**2c**	Me	PMB	3.84	4.40	6.60	8.50	4.20	–
13	**13**	–	–	>100	>100	>100	>100	40.1	+
14	**4**	–	–	0.85	1.43	1.68	10.4	9.6	++++
15	Ellipticine	–	–	1.9	2.3	2.2	5.8	4.3	nt[b]
16	Camptothecin	–	–	0.069	0.045	0.098	0.018	0.080	++++

[a] Activity is expressed semi-quantitatively as follows: –, very weak activity; ++, weak activity; ++++, similar activity as camptothecin.
[b] nt, not tested.

We selected the conformer with the best total score (−33.56) and speculated the detailed binding patterns in the cavity. In our model, the ligand intercalated well between the −1 and +1 bases, parallel to the plane of the base pairs. In a previous study, camptothecin was found stacked between the pyrimidine ring of T10 and

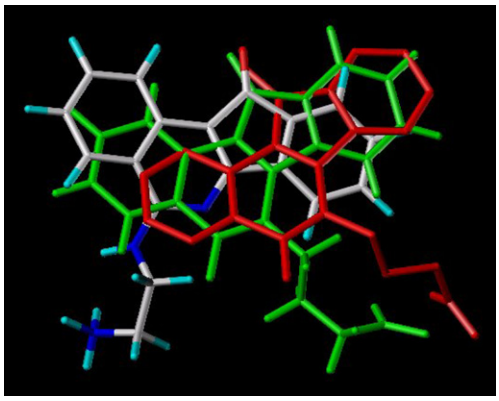


Figure 3. Superimposition of **4** (white), MJ238 of docked model (green) and MJ238 of X-ray structure (red).

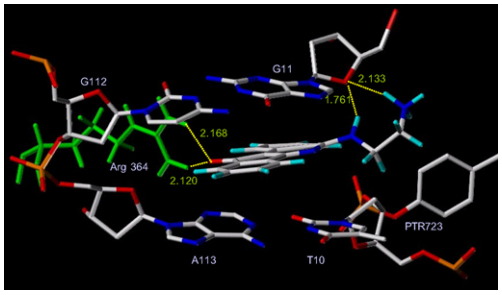
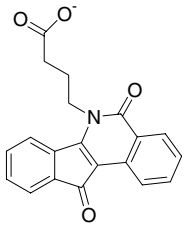


Figure 4. Docking model of **4**.



MJ-238

Figure 5. Structure of MJ-238.

the purine ring of G11 in the minor groove. To validate our docking model, the newly sketched and minimized MJ238 was first docked using the defined RDF file and then compared to the original complex structure of MJ238 in 1SC7 as depicted in Figure 3. Our docked model (green) was almost identical to MJ238 (red) between the −1 and +1 bases. The distances of carboxylates and ketones between our docked structure and MJ238 were 2.063 and 0.754 Å, respectively, and their H-bonds with Asn 352 and Arg 364 were also detected. Thus, compound **4** was docked using the same confirmed RDF file to obtain the reasonable model shown in Figure 3 (white) and Figure 4. Compound **4** intercalated at almost the same position as MJ238 between −1 and +1 bases with positioning of the D ring toward the minor groove (Fig. 5). We observed the π–π stacking interaction between the G11 purine and the B and C rings of ligand **4**, and the T10 pyrimidine and A and B rings of **4**. As shown in the X-ray complex of MJ238, the ketone group of **4** had H-bond with Arg 364. Interestingly, the ethylenediamine group of **4** had H-bond with the oxygen in the G11 ribofuranose, which could strongly block the religation process. At this stage, we should mention that the carbonyl group on the C ring is essential for H-bond with Arg 364, and the flat plane of the ligand is advantageous for intercalation with base pairs.

In summary, we have presented a novel radical cyclization that allows efficient synthesis of indenoisoquinolines. The dramatic enhancement of top 1 inhibitory activity of the synthesized compounds could be explained by the FlexX docking program. In order for ligands to show top 1 relaxation activity, they should stabilize the ternary DNA-top 1 cleavable complex through intercalations with base pairs, as well as H-bond with the enzyme in the cleavage site. The ethylenediamine group of **4** had a strong H-bond with the G11 furanose in the scissile strand. This result suggests that blocking the religation of the G11 hydroxyl group could be the main design point for novel top 1 inhibitors. Further study on the binding modes is now being carried out, the details of which will be reported in due course.

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