Bioorganic & Medicinal Chemistry Letters 19 (2009) 2551–2554

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structural modification of 3-arylisoquinolines to isoindolo[2,1-*b*]isoquinolinones for the development of novel topoisomerase 1 inhibitors with molecular docking study

Hue Thi My Van, Won-Jea Cho *

College of Pharmacy and Research Institute of Drug Development, Chonnam National University, Gwangju 500-757, Republic of Korea

ARTICLE INFO

Article history:
Received 29 January 2009
Revised 4 March 2009
Accepted 7 March 2009
Available online 16 March 2009

Keywords:
Isoindolo[2,1-*b*]isoquinoline
Synthesis
Topoisomerase 1 inhibitor
Docking study
Cytotoxicity

ABSTRACT

Isoindolo[2,1-*b*]isoquinolinones **9a**–**i** were designed and synthesized as constrained forms of 3-arylisoquinolines through an intramolecular cyclization reaction. Among the synthesized compounds, **9d** exhibited potent topoisomerase 1 inhibitory activity with cytotoxicities against three different tumor cell lines. A Surflex-dock docking study was performed to clarify the topoisomerase 1 inhibitory activity of **9d**.

Camptothecin (CPT) **1** was the representative compound identified as a topoisomerase 1 (top 1) inhibitor.[1,2] Topotecan and irinotecan have been used clinically as CPT analogues[3,4] and these two drugs have a hydrolysis susceptible lactone ring incorporated within their structures. Ring-cleavage of the CPT lactone moiety yields an inactive structure that has high affinity for human serum albumin. Moreover, the fact that these drugs are substrates for efflux transporters associated with resistance has prompted further development of novel top 1 inhibitors.[5–7]

The binding mode of a drug to its receptor site is governed by subtle electronic or stereo factors, and these two functions play a critical role in the bioactive conformation of the drug molecule.[8] This conformation is the one that a drug molecule adopts when it binds to the target, and this interaction is greatly influenced by entropic factors that are related to the structural flexibility of the molecule.

In view of these considerations, we designed the constrained molecules to mimic the bioactive conformation as the basis for designing potent top 1 inhibitors.[9,10] As evident from its structure, the free rotation of the 3-aryl ring could conceivably be constrained to indeno[1,2-*c*]isoquinolinone **3** via route A ring formation of 3-arylisoquinoline **2**. The synthesis, top 1 activity, and cytotoxicity of **3** were already reported.[9] We then focused on isoindolo[2,1-*b*]isoquinolin-5-one **4**, which could be synthesized through route B as shown in Figure 1. Because isoindolo[2,1-*b*]isoquinolin-5-ones have not yet been studied, we investigated their

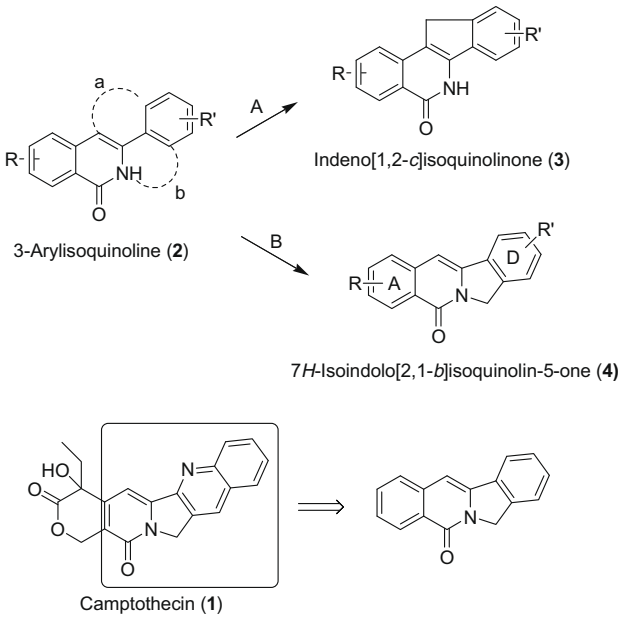

Figure 1. Structure of CPT **1** and constrained form of 3-arylisoquinoline to isoindolo[2,1-*b*]isoquinolin-5-one **4**.

* Corresponding author. Tel.: +82 62 530 2933; fax: +82 62 530 2911.
E-mail address: wjcho@chonnam.ac.kr (W.-J. Cho).

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doi:10.1016/j.bmcl.2009.03.042

synthesis and evaluated them biologically. Moreover, the structural similarity between isoindolo[2,1-*b*]isoquinolin-5-one and CPT prompted us to investigate these compounds.

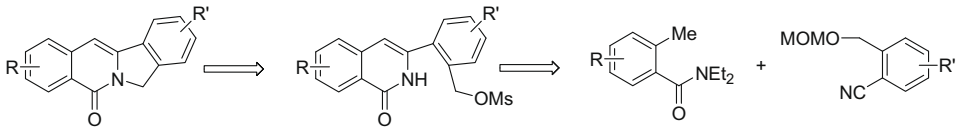
Since the 3D structure determination of drug-DNA–top 1 complex has been reported, computer-aided molecular modeling studies afford valuable information of drug binding mode in the active site of DNA–top 1.[11] We investigated not only the syntheses of isoquinoline alkaloids such as protoberberines and benzo[*c*]phenanthridines, but also conducted molecular modeling studies of indeno[1,2-*c*]isoquinoline analogs that showed top 1 inhibition and cytotoxicity.

As depicted in Scheme 1, the C ring of isoindolo[2,1-*b*]isoquinolin-5-one could be constructed by an intramolecular S_N2 reaction. The mesyl compound could be synthesized via toluamide–benzonitrile cycloaddition reaction.

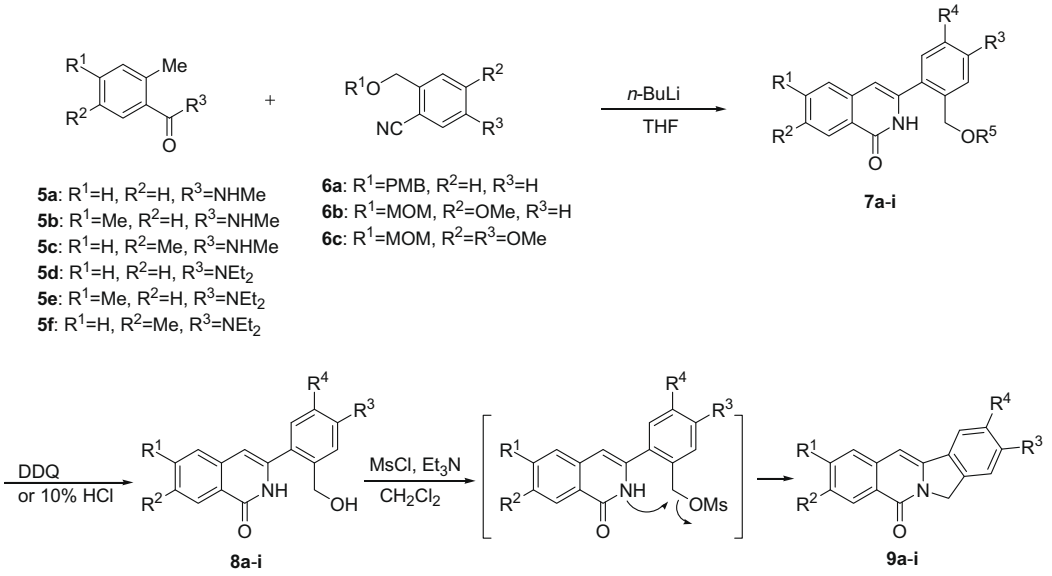
The previously reported lithiated toluamide–benzonitrile cycloaddition method was used to synthesize 3-arylisoquinolines **7a–i**.[12] *N*-Methyl-*o*-toluamides **5a–c** or *N,N*-diethyl-*o*-toluamides **5d–f** were treated with *n*-BuLi to give the anions, which were then reacted with benzonitrile **6a–c** to afford the corresponding 3-arylisoquinolines **7a–i** in moderate yields. Treatment of **7a–c** with DDQ provided the corresponding hydroxymethyl compounds **8a–c**. Deprotection of MOM-substituted compounds **7d–i** was conducted with 10% HCl to give the desired products. Hydroxymethyl compounds **8a–i** were reacted with MsCl/Et_3N in methylene chloride to provide the desired isoindolo[2,1-*b*]isoquinolines **9a–i** in moderate yields as shown in Scheme 2.[13]

The in vitro cytotoxicity experiments were performed with the synthesized compounds against three human tumor cell lines, including A 549 (lung), HCT15 (colon), and OV-3 (ovarian), using sulforhodamine B (SRB) assays.[14] The top 1 inhibitory activity assays were conducted using the supercoiled DNA unwinding method.[15] Stock solutions of the compounds (20 mM) were prepared in DMSO. The DNA–top 1 activity was determined by assessing the relaxation of supercoiled DNA pBR322. A mixture of 200 ng of plasmid pBR322 DNA and 2 units of calf thymus DNA–top 1 (Fermentas, USA) was incubated without and with the prepared compounds at 37 °C for 30 min in relaxation buffer [35 mM Tris–HCl (pH 8.0), 72 mM KCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 2 mM spermidine, 0.01% bovine serum albumin]. The reaction (final volume of 20 μL) was terminated by adding 5 μL of stop solution containing 10% SDS, 0.2% bromophenol blue, 0.2% xylene cyanol and 30% glycerol. DNA samples were then electrophoresed on 1% agarose gel at 15 V for 6 h with TAE running buffer. Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/mL). DNA bands were visualized by transillumination with UV light and were quantitated using AlphaImager™ (Alpha Innotech Corporation).

From the biological data presented in Table 1, general features are clear from the comparison of 3-arylisoquinolines. PMB-protected 3-arylisoquinoline **7a–c** displayed stronger cytotoxicites against the three tumor cell lines than MOM-protected compounds **7d–i**. The hydroxyl methyl-substituted compounds **8a–i** exhibited stronger cytotoxicity than the protected compounds **7a–i** (0.21–22.10 μg/mL). Isoindolo[2,1-*b*]isoquinolin-5-ones exerted potent cytotoxicities (3.70–49.09 μg/mL). Unexpectedly, some isoindoloisoquinolines **9e–i** exhibited poor top 1 inhibitory activity as depicted in Figure 2. The methyl substituted compounds **9b** and **9c** on A ring showed less top 1 activity than unsubstituted ones and this could be explained by the steric interaction between methyl group with side chain of Thr 718. The semi-quantitative assay was conducted to show the relative top 1 potency of the compounds. Compounds **9a** and **9d** had similar potency (++++) compared to the reference CPT (++++). However, compounds **9b–c** exhibited less potent inhibitory activity than CPT (Fig. 3).



Scheme 1. Retrosynthesis of isoindolo[2,1-*b*]isoquinolin-5-one **4**.



Scheme 2. The synthesis of isoindolo[2,1-*b*]isoquinoline analogs.

Table 1
Chemical yield, cytotoxicity (μg/mL and top 1 inhibitory activity of the compounds

No.	Compd	R^1	R^2	R^3	R^4	R^5	Yield	A549	HCT15	OV-3	Top 1[a]
1	**7a**	H	H	H	H	PMB	44	26.56	17.39	24.66	nt[b]
2	**7b**	Me	H	H	H	PMB	38	7.44	13.27	2.37	nt
3	**7c**	H	Me	H	H	PMB	52	3.33	4.15	4.76	nt
4	**7d**	H	H	OMe	H	MOM	41	32.98	21.84	17.06	nt
5	**7e**	H	H	OMe	OMe	MOM	58	16.04	24.70	12.68	nt
6	**7f**	Me	H	OMe	H	MOM	43	80.22	29.26	31.26	nt
7	**7g**	Me	H	OMe	OMe	MOM	77	43.65	34.55	34.54	nt
8	**7h**	H	Me	OMe	H	MOM	42	66.52	40.05	23.08	nt
9	**7i**	H	Me	OMe	OMe	MOM	47	25.02	31.13	28.22	nt
10	**8a**	H	H	H	H	—	89	6.89	3.88	21.98	nt
11	**8b**	Me	H	H	H	—	47	3.66	3.34	8.22	nt
12	**8c**	H	Me	H	H	—	59	12.78	23.11	0.22	nt
13	**8e**	H	H	OMe	H	—	45	3.65	0.21	0.37	nt
14	**8e**	H	H	OMe	OMe	—	23	7.32	19.68	12.58	nt
15	**8f**	Me	H	OMe	H	—	30	19.43	4.28	14.59	nt
16	**8g**	Me	H	OMe	OMe	—	12	22.10	7.17	7.80	nt
17	**8h**	H	Me	OMe	H	—	50	10.11	7.39	9.69	nt
18	**8i**	H	Me	OMe	OMe	—	11	15.15	22.45	0.67	nt
19	**9a**	H	H	H	H	—	51	3.70	43.93	14.84	++++
20	**9b**	Me	H	H	H	—	38	15.94	46.35	44.12	++
21	**9c**	H	Me	H	H	—	40	41.44	35.27	48.92	++
22	**9d**	H	H	OMe	H	—	58	9.25	7.29	12.01	++++
23	**9e**	H	H	OMe	OMe	—	35	36.75	9.12	12.69	–
24	**9f**	Me	H	OMe	H	—	32	5.95	8.78	5.52	+
25	**9g**	Me	H	OMe	OMe	—	52	49.09	34.98	36.15	–
26	**9h**	H	Me	OMe	H	—	49	45.12	34.33	15.34	+
27	**9i**	H	Me	OMe	OMe	—	36	24.13	6.56	7.23	+
28	CPT							0.072	0.089	0.035	++++

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

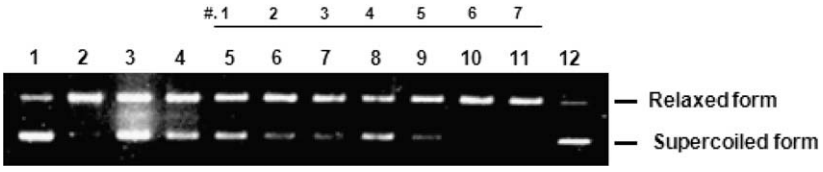


Figure 2. Top 1 inhibitory activities of the compounds. The DNA–top 1 activity was determined by assessing the relaxation of supercoiled DNA pBR322. The upper band is relaxed form and the lower is supercoiled DNA. Lane 1, 12: pBR322 only, Lane 2: pBR322 + Top 1, Lane 3: pBR322 + Top 1 + CPT (100 μg/mL), Lane 4: pBR322 + Top 1 + CPT (100 μg/mL), Lane 5–11, compound (100 μg/mL) + pBR322 + Top 1, 5 (**9a**), 6 (**9b**), 7 (**9c**), 8 (**9d**), 9 (**9f**), 10 (**9e**), 11 (**9g**).

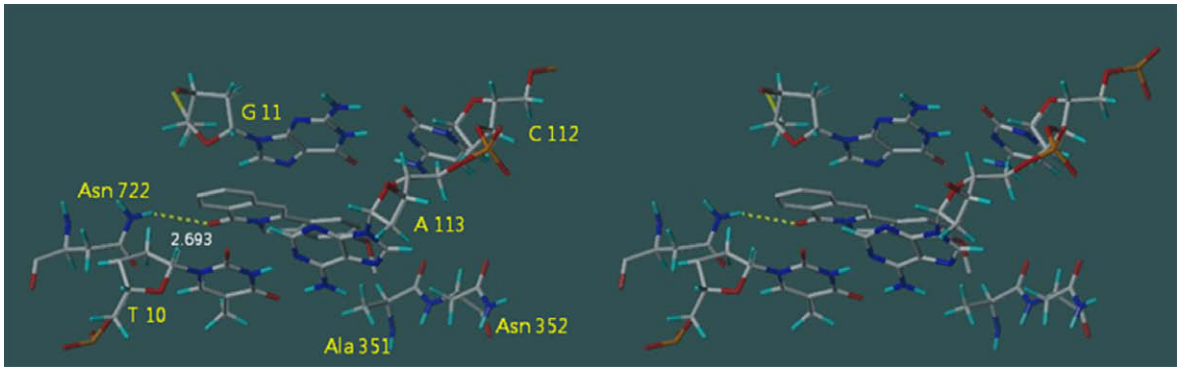


Figure 3. Wall-eyed viewing docked model of compound **9d**.

In many cases, the top 1 inhibitory activity does not correlate well with cytotoxicity and these results can not be explained by low aqueous solubility or poor membrane permeability.

To understand the binding mode of action of the most potent top 1 inhibitor **9d**, we performed a docking study using Surflex-Dock in Sybyl version 8.05 by Tripos Associates, operating under Red Hat Linux 4.0 with an IBM computer (Intel Pentium 4, 2.8 GHz CPU, 1 GB memory).

Surflex-Dock docks ligands automatically into a receptor's ligand binding site using a protomol-based method and an empirically derived scoring function.[16] The protomol is a unique and important element of the docking algorithm and means a computational representation of putative 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 procedure, the function of Surflex-Dock has re-

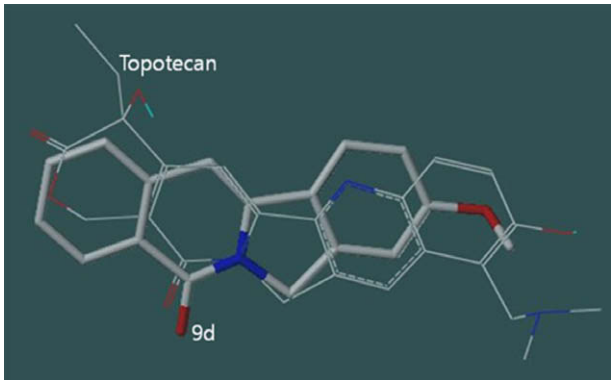


Figure 4. Superimposition of compound **9d** with Topotecan.

cently been improved by incorporating a base portion matching algorithm that allows prepositioning of a fragment of the ligand being docked in the binding site. The fragment is allowed to transfer from its original position to a certain point during pose optimization. This is significant when the position of the base portion is not completely set. Ligand docking with the base fragment matching characteristic is proposed to yield docking and scoring of ligands constrained to match an exact binding motif.

The structure of the inhibitor **9d** 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.[11] The phosphoester bond of G12 in 1SC7 was rebuilt, and the SH of G11 on the scissile strand was changed to OH. After running Surflex-Dock, 10 docked conformers were displayed in a molecular spread sheet to rank the scores. We selected the best total score (4.85) conformer and speculated regarding the detailed binding patterns in the cavity. The resulting docking model revealed a similar binding mode as the indenoisoquinoline model. In our model, the isoquinoline ring intercalated between the −1 and +1 bases, parallel to the plane of the base pairs, and the amide carbonyl group had H-bond to Asn 722, which is considered an important amino acid that interacts with the ligand in the DNA–top 1 active site. In our model, the isoindoloisoquinoline ring worked as a DNA intercalator as a blocker of the rewinding step of the phosphoester. The binding geometry of CPT in the DNA–top 1 complex was investigated by an ab initio quantum mechanics calculation to give the fact that the π–π stacking interactions, DNA intercalating forces was much more important than hydrogen bonding of the ligand to the surrounding amino acid residues of the protein, or to the base pairs.[17] Our molecular docking study proved the importance of DNA intercalation of **9d** and it was clarified by the superimposition of **9d** with Topotecan as shown in Figure 4.

In conclusion, we accomplished concise synthesis of various isoindolo[2,1-*b*]isoquinolin-5-one as analogs of constrained 3-arylisoquinolines structures. An intramolecular cycloaddition reaction was employed to efficiently generate isoindolo[2,1-*b*]isoquinolin-5-one. 3-Arylisoquinoline synthetic intermediates exerted relatively strong cytotoxicities and most of the newly synthesized isoindolo[2,1-*b*]isoquinolines exhibited potent top 1 inhibitory activity with potent cytotoxicities. In particular, **9a** and **9d** had potent top 1 activity as well as potent cytotoxicity against three different tumor cell lines. For explanation of the top 1 activity of **9d**, molecular docking studies were carried out with the Surflex–Dock program to give the reasonable binding mode of the compound into the binding sites of DNA and top 1. In further studies of the other constrained structures of 3-arylisoquinolines, diverse structural modifications are currently being investigated through synthesis with computer modeling and the results will be given in due course.

Acknowledgment

This work was supported by Korea Research Foundation grant (KRF-2006-312-E00171).

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13. *Synthetic experimental of the representative compounds*; **7d**: A solution of *N,N*-diethylbenzamide **5d** (1.68 g, 8.8 mmol) and benzonitrile **6b** (1.52 g, 7.3 mmol) in dry THF (20 mL) were added dropwise to a solution of *n*-butyllithium (6 mL of 2.5 M in hexane, 15 mmol) in THF (20 mL) at −70 °C, and then the reaction mixture was stirred at the same temperature for 6 h. The reaction was quenched with water, extracted with ethyl acetate and dried over sodium sulfate. After removal of the solvent, the residue was purified by column chromatography with *n*-hexane–ethyl acetate (1:1) to afford compound **7d** as yellow oil (985 mg, 41%). IR (cm^{-1}): 3447 (NH), 1655 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ: 9.79 (s, 1H), 8.40 (d, 1H), 7.67 (m, 1H), 7.56 (m, 1H), 7.48 (m, 2H), 7.03 (m, 1H), 6.97 (m, 1H), 6.52 (s, 1H), 4.80 (s, 2H), 4.56 (s, 2H), 3.87 (s, 3H), 3.43 (s, 3H). EIMS *m/z* (%) 325 (M^+, 100). **9a**: To a solution of compound **8a** (50 mg, 0.2 mmol) in CH_2Cl_2 was added triethylamine (46 mg, 0.4 mmol) followed by methanesulfonylchloride (101 mg, 1.0 mmol) at 0 °C. The reaction mixture was warmed-up to room temperature and stirred overnight. The reaction was quenched with water and extracted with CH_2Cl_2. The organic layers were washed with water, brine, and dried over sodium sulfate. After removing the solvent, the residue was purified by column chromatography with *n*-hexane–ethyl acetate to afford the isoindoloisoquinoline **9a** as yellow solid (24 mg, 51 %). Mp: 151.7–154.1 °C. IR (cm^{-1}): 1657 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.48 (d, J = 8.1 Hz, 1H), 7.75–7.43 (m, 7H), 6.98 (s, 1H), 5.15 (s, 2H). EIMS *m/z* (%) 233 (M^+, 100). **9b**; mp: 157.8–160.2 °C. IR (cm^{-1}): 1657 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.38 (d, J = 8.1 Hz, 1H), 7.80 (m, 1H), 7.57 (m, 1H), 7.48 (m, 2H), 7.42 (s, 1H), 7.31 (d, 1H), 6.97 (s, 1H), 5.19 (s, 2H), 2.50 (s, 3H). EIMS *m/z* (%) 247 (M^+, 92). **9c**; mp: 155.2–158.8 °C. IR (cm^{-1}): 1657 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.30 (s, 1H), 7.79 (m, 1H), 7.57–7.45 (m, 5H), 7.02 (s, 1H), 5.20 (s, 2H), 2.51 (s, 3H). EIMS *m/z* (%) 247 (M^+, 100).
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