Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 4451–4454

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# Synthesis of New 3-Arylisoquinolinamines: Effect on Topoisomerase I Inhibition and Cytotoxicity

Won-Jea Cho,* Sun Young Min, Thanh Nguyen Le and Tae Sung Kim

*College of Pharmacy, Chonnam National University, Yong-Bong dong, Buk-gu, Kwangju 500-757, South Korea*

Received 24 July 2003; revised 30 August 2003; accepted 4 September 2003

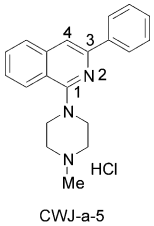
**Abstract**—To investigate the structure–activity relationships of 3-arylisoquinolines, diverse substituted 3-aryisoquinolinamines were synthesized and tested in vitro antitumor activity against four tumor cell lines. Some of the compounds showed potent topoisomerase I inhibitory activity. Docking study of **7d** with topoisomerase I–DNA complex was also performed.


## Introduction

Eukaryotic DNA topoisomerase I (top I) is an essential enzyme that act to relax supercoiled DNA during the transcription, replication and mitosis.[1] Intracellular levels of top I are elevated in a number of human solid tumors, relative to the respective normal tissues, suggesting that controlling the top I level is important to treat cancer.[2] Top I poisons show their antitumor activities by stabilizing the cleavable ternary complex consisting of top I enzyme, DNA, and drug.[3] Thus, top I is a promising target for the development of new cancer chemotherapeutics against a number of solid tumors. Camptothecin is a representative top I inhibitor and its derivatives, topotecan and irinotecan, have been launched as clinically used drugs.[4] Several non-camptothecin top I inhibitors have been reported over the last decade. These include benzo[*c*]phenanthridines,[5] indenoisoquinolines,[6] indolocarbazones,[7] saintopin,[8] and benzophenazines.[9] The X-ray crystal structure of human top I covalently joined to double-stranded DNA base pair and bound to the topotecan was also revealed.[10,11]

We recently reported the synthesis and biological evaluation of 3-arylisoquinolines related to the lead compound (CWJ-a-5) with the 3-D-QSAR study.[12] Most of these compounds were shown to be highly cytotoxic against several kinds of human tumor cell lines. From this investigation we found the amide carbonyl group of isoquinolin-1-one played an important role for exhibiting the cytotoxicities. When the carbonyl group was transformed to amines, water solubility was increased with retaining the activities. Among the synthesized compounds, CWJ-a-5 was tested in vivo assay using BDF1 mice (P388 leukemia) and resulted in 160 T/C% with low toxicity.[13] The high oral bioavailability as well as promising pharmacokinetic data of CWJ-a-5 served as useful information in future clinical studies of related compounds[14] (Fig. 1).

Our research was next focused on introducing the various amines on C-1 position replacing *N*-methylpiperazine in 3-arylisoquinolines. For further investigation of isoquinolinamines on the antitumor cytotoxicities with top I inhibition activities, a systematic synthesis was performed to get interesting results. Based on the X-ray structure of ternary top I–DNA complex with topotecan, a docking study was also carried out.




CWJ-a-5

**Figure 1.** The structure of 1-(4-methylpiperazinyl)-3-phenylisoquinoline hydrochloride (CWJ-a-5).

*Corresponding author. Tel.: +82-625-302-933; fax: +82-625-302-911; e-mail: wjcho@chonnam.ac.kr

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.09.001

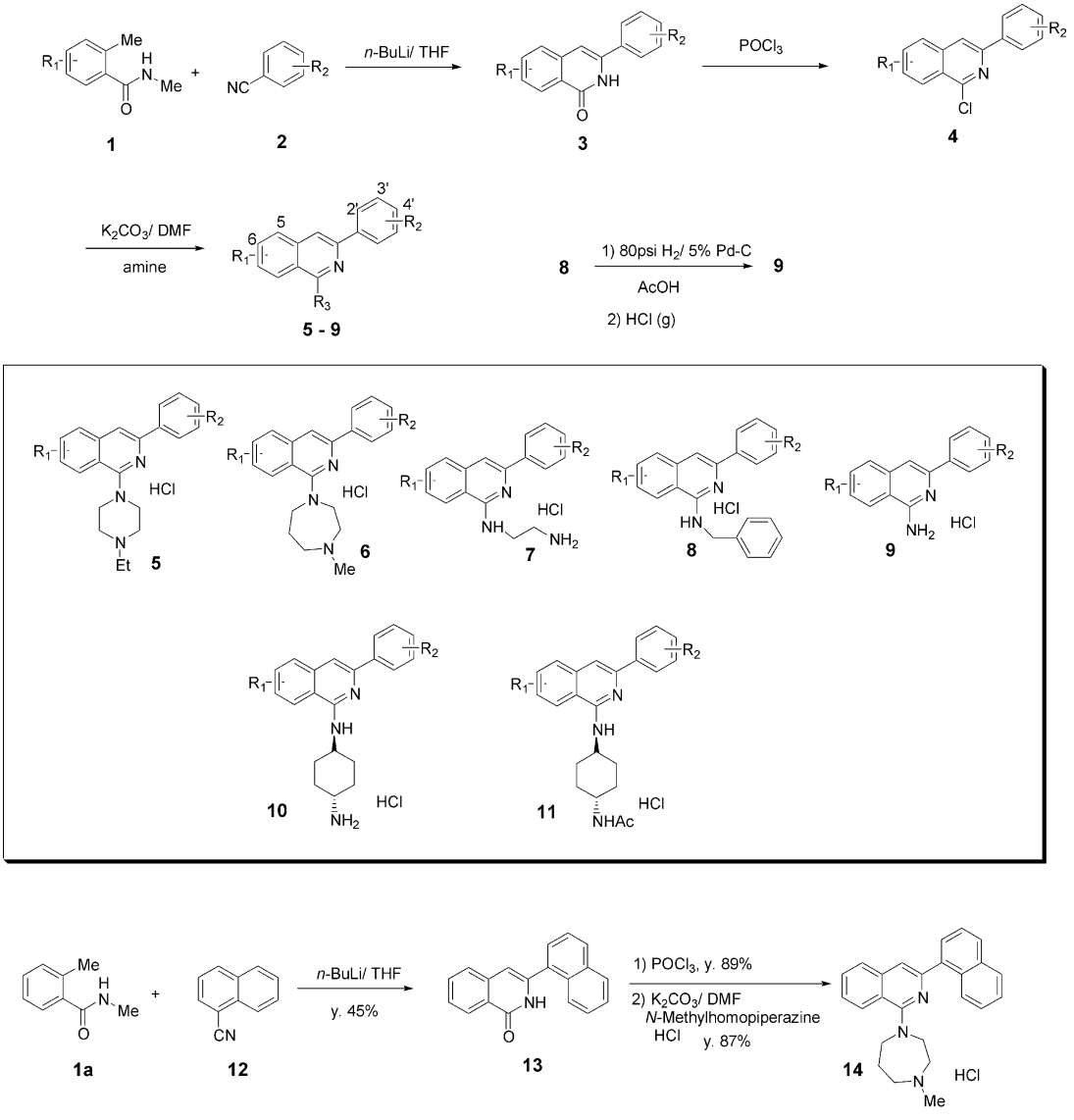
## Synthesis

The coupling reaction of *N*-methyl-*o*-tuluamide (**1**) with benzonitrile (**2**) was accomplished via dilithio species using *n*-BuLi in moderate yield. The imine chloride (**4**) derived from the corresponding amide by treating $POCl_3$ was reacted with various amines in refluxing DMF to provide the 1-substituted isoquinolines in good yield. For the preparation of primary amines (**9**), the benzylamines (**8**) was performed to a catalytic hydrogenation reaction with 5% Pd/C on 80 psi hydrogen atmosphere to afford the corresponding amines in moderate yield. 3-Naphthylisoquinoline (**14**) was also obtained efficiently using the above method as shown in Scheme 1.

## Biological Results and Discussion

The cytotoxicity experiment of the synthesized compounds was performed in vitro against four human tumor cell lines such as A 549 (lung), SKOV-3 (ovarian), SK-MEL-2 (melanoma), and HCT 15 (colon) using sulforhodamine B (SRB) assay.[15] The top I inhibitory activity was carried out by the supercoiled DNA unwinding assay method.[16] The $IC_{50}$ cytotoxicity values obtained with cell lines are summarized in Table 1 and the top I inhibitory results are shown in Figure 2.

*N*-Ethylpiperazine compound **5** proved to be less or slightly more active than the lead compound (CWJ-a-5), depending on the cell lines tested. However, the *N*-methylhomopiperazine compound **6f** (0.44 μM, A 549), primary amine **9b** (0.14 μM, SK-MEL-2) and naphthalene compound **14** (0.52 μM, A 549) displayed submicromolar cytotoxicity concentrations. Both of these compounds possess 6-methyl and 2′-methyl substituents on aromatic rings. Previous work on our laboratory has found that alkyl groups on aromatic rings of 3-arylisoquinolines enhanced the cytotoxic activities and molecular modeling results also supported the hydrophobic favoring effect.[12] Weak cytotoxicity was observed for ethylenediamine analogues **7** (>300 μM) although they are potent top I inhibitors as shown in Figure 2. In this




**Scheme 1.** The synthesis of 3-arylisoquinolinamines.

**Table 1.** Synthetic yield of 3-arylisoquinolines, $IC_{50}$ cytotoxicity (μ mole) and topoisomerase I activity

| No. | Compd | R1 | R2 | Yield[a] | A549 | SK-OV-3 | SK-MEL-2 | HCT15 | Top activity[b] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | **5a** | H | H | 87 | 55.35 | 68.91 | 95.73 | 7.22 | + |
| 2 | **5b** | 6-Me | H | 78 | 18.16 | 41.80 | 11.92 | 2.42 | + + |
| 3 | **5c** | 6-Me | 2′-Me | 88 | 20.98 | 61.96 | 12.85 | 8.65 | + + |
| 4 | **5d** | 6-Me | 4′-Me | 77 | 26.94 | 63.37 | 14.40 | 8.07 | + |
| 5 | **5e** | 6-Me | 2′-Cl | 79 | 13.36 | 194.91 | 5.58 | 5.58 | + + + |
| 6 | **6a** | H | H | 90 | 10.84 | 17.01 | 8.38 | 11.09 | + + + |
| 7 | **6b** | H | 3′-Me | 86 | 8.57 | 8.22 | 8.85 | 9.20 | + |
| 8 | **6c** | H | 4′-Me | 89 | 1.22 | 9.34 | 6.18 | 7.84 | + + |
| 9 | **6d** | H | 4′-Cl | 91 | 11.77 | 19.96 | 8.55 | 9.78 | + + |
| 10 | **6e** | H | 4′-Br | 78 | 6.04 | 3.85 | 4.12 | 5.26 | + + |
| 11 | **6f** | 6-Me | 2′-Me | 79 | 0.44 | 0.70 | 0.99 | 1.20 | + + |
| 12 | **7a** | 6-Me | H | 57 | > 300 | > 300 | > 300 | > 300 | + + + |
| 13 | **7b** | 6-Me | 2′-Me | 67 | > 300 | > 300 | > 300 | > 300 | + |
| 14 | **7c** | 6-Me | 2′-Cl | 82 | > 300 | > 300 | > 300 | > 300 | + |
| 15 | **7d** | 6-Me | 4′-Me | 62 | > 300 | > 300 | > 300 | > 300 | + + + |
| 16 | **8a** | H | H | 67 | 176.75 | 101.84 | 82.97 | 87.36 | + |
| 17 | **8b** | H | 3′-Me | 89 | 102.93 | 167.74 | 85.05 | 107.65 | + |
| 18 | **8c** | 6-Me | 3′-Me | 88 | 170.94 | 145.82 | 88.73 | 111.37 | + |
| 19 | **8d** | H | 4′-Me | 78 | 168.12 | 192.86 | 133.28 | 171.43 | nt[c] |
| 20 | **8e** | 6-Me | H | 72 | 122.11 | 156.05 | 94.37 | 64.22 | nt |
| 21 | **9a** | H | H | 46 | 2.73 | 2.03 | 1.48 | 1.87 | nt |
| 22 | **9b** | 6-Me | 2′-Me | 67 | 0.17 | 0.21 | 0.14 | 0.17 | + + |
| 23 | **9c** | H | 2′-Me | 57 | 3.14 | 1.81 | 2.11 | 1.92 | nt |
| 24 | **10a** | H | H | 46 | 7.95 | 4.36 | 7.70 | 7.24 | + + |
| 25 | **10b** | H | 2′-Me | 48 | 10.24 | 9.17 | 6.45 | 7.65 | + |
| 26 | **11** | H | H | 90 | 145.30 | 186.50 | 183.44 | 252.57 | + |
| 27 | **14** | — | — | 87 | 0.52 | 3.64 | 1.98 | 1.93 | + |
| 28 | CWJ-a-5 | — | — | — | 13.56 | 13.71 | 14.53 | 13.94 | nt |
| 29 | Doxorubicin | — | — | — | 0.97 | 1.17 | 4.78 | 1.67 | nt |
| 30 | Camptothecin | — | — | — | — | — | — | — | + + |

[a]Chemical yield represents the final step for the preparation of each compounds.
[b]Activity of the compounds is expressed semi-quantitatively as follows: +; weak activity, + +; similar activity as the camptothecin; + + +; greater activity than the camptothecin.
[c]nt, not tested.

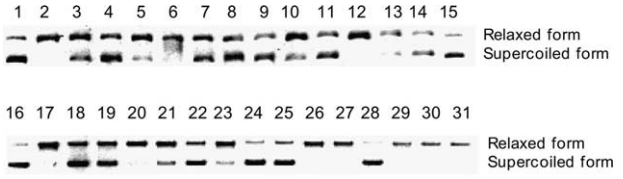



**Figure 2.** Topoisomerase I inhibitory activities of the compounds: Lane 1,16: pBR322 DNA; lane 2, 17: pBR322 DNA + top I; lane 3, 18: pBR322 DNA + top I + camptothecin (0.1 mg/mL); lane 19: pBR322 + top I + camptothecin (0.01 mg/mL); the others follows the lane number (prepared compound number, 0.1 mg/mL), 4 (**6a**), 5 (**14**), 6 (**6b**), 7 (**6c**), 8 (**6d**), 9 (**6e**), 10 (**6f**), 11 (**10a**), 12 (**11**), 13 (**10b**), 14 (**9b**), 20 (**5a**), 21 (**5b**), 22 (**5c**), 23 (**5d**), 24 (**5e**), 25 (**7a**), 26 (**7b**), 27 (**7c**), 28 (**7d**), 29 (**8a**), 30 (**8b**), 31 (**8c**). Thicker band of the supercoiled form implies more potent inhibitory activity of the compound. The semi-quantitative comparison of the inhibitory activities was exhibited in Table 1. Detailed assay procedure is also described in ref 19.

series of isoquinolinamines, top I inhibitory activity did not correlate particularly well with cytotoxicity, at least not in any quantitative sense, since the most potent cytotoxic compound **9b** was not the most top I inhibitor. This is not the unusual result because cell membrane penetration ability to reach the target affects the cytotoxicity. Benzylamines **8a–e** were all less potent than the CWJ-a-5. The acetylated amine **11** showed dramatically decreased cytotoxicity compared to **10a**. Interestingly, amine **9b** exhibited very potent cytotoxicity against daunorubicin-resistant AML-2 subline (0.0027 μg/mL) and doxorubicin-resistant subline DX-100 (0.0056 μg/mL) as shown in Table 2.

In general compounds **5**, **6** and **7** that contain ethylenediamine units show relatively potent top I inhibitory activity.

Docking study was carried out using Sybyl 6.7 (Tripos, Inc.)[17] on a Silicon Graphics Indigo 2 workstation. Construction of protein–ligand was based on X-ray structure of ternary top I-DNA-topotecan complex (PDB entry 1K4T).[12] Ligand was manually docked into the active site of topotecan binding region. The ethylenediamine group of compound **7d** was considered to have strong hydrogen bonding with carboxyl group of Asp 533 (2.221 Å), phosphoester group of Guanine 12 (1.490 Å) and amide carbonyl moiety of Gln 633 (2.739 Å) as shown in Figure 3. On the other hand, primary amines **9b** did not have good interaction to this binding

**Table 2.** Cytotoxicity (μg/mL) of **9b** against daunorubin and doxorubicin resistant cells[18]

| Compd | AML-2[a] | | |
|---|---|---|---|
| | WT[b] | D100[c] | DX-100[d] |
| **9b** | 0.0078 | 0.0027 | 0.0056 |
| Doxorubicin | 0.01 | 1.93 | 3.20 |
| Daunorubicin | 0.001 | 0.52 | 0.43 |

[a]AML-2 means acute myelogenous leukaemia.
[b]WT: wild type AML-2 leukemia.
[c]D100: daunorubicin (100 nM) resistant AML-2.
[d]DX-100: doxorubicin (100 ng/mL) resistant AML-2.

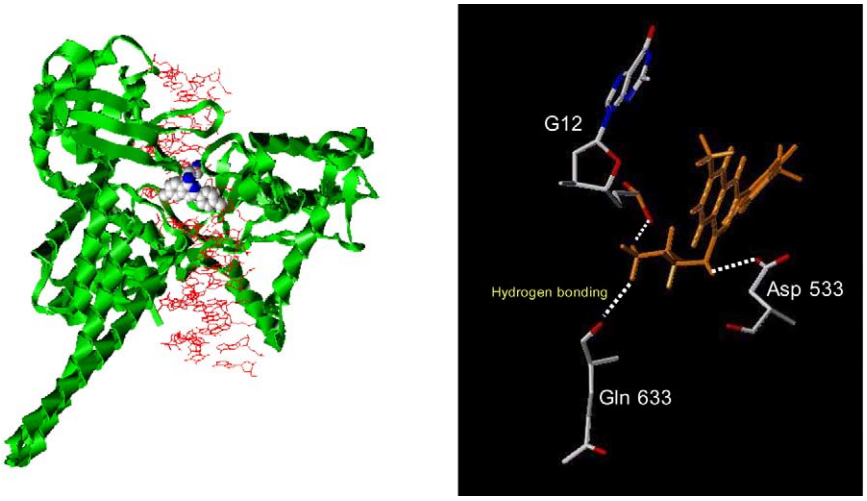



**Figure 3.** Hypothetical docking model of **7d** with topoisomerase I–DNA complex.

site due to the lacks of the length of chain to reach the protein functional group.

In summary, amine substituents at the C-1 position of 3-arylisoquinolines provide novel, potent and water soluble top I inhibitors with significant antitumor cytotoxicity. The selective cytotoxicity of **9b** against doxorubicin- and daunorubicin-resistant tumor cells is highly significant. Finally, these compounds represent a promising new class of non-camptothecin top I inhibitors worthy of a further study.

## Acknowledgements

The financial support from Korea Research Foundation (KRF-2002-015-EP0142) of Korea is appreciated.

## References and Notes

1. (a) Wang, J. C. *Annu. Rev. Biochem.* **1996**, *65*, 635. (b) Liu, L. F. *Annu. Rev. Biochem.* **1989**, *58*, 351.
2. Chen, A. Y.; Liu, L. F. *Annu. Rev. Pharmacol. Toxicol.* **1994**, *34*, 191.
3. Pommier, Y.; Pourquier, P.; Fan, Y.; Strumberg, D. *Biochim. Biophys. Acta* **1998**, *1400*, 83.
4. Rajendra, R.; Gounder, M. K.; Saleem, A.; Schellens, J. H. M.; Ross, D. D.; Bates, S. E.; Sinko, P.; Rubin, E. H. *Cancer Res.* **2003**, *63*, 3228, and references cited therein.
5. (a) Makhey, D.; Gatto, B.; Yu, C.; Liu, A.; Liu, L. F.; LaVoie, E. *J. Med. Chem. Res.* **1995**, *5*, 1. (b) Janin, Y. L.; Croisy, A.; Rious, J.-L.; Bisagni, E. *J. Med. Chem.* **1993**, *36*, 3686. (c) Singh, S. K.; Ruchelman, A. L.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *J. Med. Chem.* **2003**, *46*, 2254.
6. (a) Jayaraman, M.; Fox, B. M.; Hollingshead, M.; Kohlhagen, G.; Pommier, Y.; Cushman, M. *J. Med. Chem.* **2002**, *45*, 242. (b) Fox, B. M.; Xiao, X.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Staker, L. S.; Cushman, M. *J. Med. Chem.* **2003**, *46*, 3275.
7. Yamashita, Y.; Fujii, N.; Murakaya, C.; Ashizawa, T.; Okabe, M.; Nakano, H. *Biochemistry* **1992**, *31*, 12069.
8. Yamashita, Y.; Kawada, S.; Fujii, N.; Nakano, H. *Biochemistry* **1991**, *30*, 5838.
9. Vicker, N.; Burgess, L.; Chuckowree, I. S.; Dodd, R.; Fokes, A. J.; Hardick, D.; Hancox, T. C.; Miller, W.; Milton, J.; Sohal, S.; Wang, S.; Wren, S. P.; Charlton, P. A.; Dangerfield, W.; Liddle, C.; Mistry, P.; Stewart, A. J.; Denny, W. A. *J. Med. Chem.* **2002**, *45*, 721.
10. Redinbo, M. R.; Stewart, L.; Kuhn, P.; Champoux, J. J.; Hol, W. G. J. *Science* **1998**, *279*, 1504.
11. Staker, B. L.; Hjerrild, K.; Feese, M. D.; Behnke, C. A.; Burgin, A. B., Jr.; Stewart, L. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15387.
12. Cho, W.-J.; Kim, E.-K.; Park, I. Y.; Jeong, E. Y.; Kim, T. S.; Le, T. N.; Kim, D.-D.; Lee, E.-S. *Bioorg. Med. Chem.* **2002**, *10*, 2953.
13. Cho, W.-J.; Park, M.-J.; Chung, B.-H.; Lee, C.-O. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 41.
14. Kim, K. E.; Cho, W.-J.; Chang, S. J.; Yong, C. S.; Lee, C. H.; Kim, D. D. *Int. J. Pharm.* **2001**, *217*, 101.
15. (a) Rubinstein, L. V.; Shoemaker, R. H.; Paull, K. D.; Simon, R. M.; Tosini, S.; Skehan, P.; Scudiero, D. A.; Monks, A.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1113. (b) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
16. Fukuda, M.; Nishio, K.; Kanzawa, F.; Ogasawara, H.; Ishida, T.; Arioka, H.; Bojanowski, K.; Oka, M.; Saijo, N. *Cancer Res.* **1996**, *576*, 789.
17. The Sybyl program (Version 6.7) was supplied by Tripos Associates, 1699 South Hanley Road, Suite 303, St. Louis, MO 63144, USA.
18. Kim, H.-S.; Lee, T.-B.; Choi, C.-H. *Biochem. Biophys. Res. Commun.* **2001**, *281*, 109.
19. 500 ng supercoiled pBR 322 DNA was incubated with 1 unit topoisomerase I in the absence or presence of camptothecin or the compounds for 30 min at 37 °C. The reaction mixtures were analyzed on 1% agarose gel followed by ethidium bromide staining.