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# Synthesis and Biological Activities of Topoisomerase I Inhibitors, 6-*N*-amino analogues of NB-506

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**Abstract:** 6-*N*-Amino analogues of NB-506 [6-*N*-formylamino-12,13-dihydro-1,11-dihydroxy-13-(β-D-glucopyranosyl)-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione] (**3b**) were synthesized and tested with respect to topoisomerase inhibition, cytotoxicity and anticancer effects. Among them, a 1,3-dihydroxypropane analogue (J-109,404, **5t**) showed more than ten times more potent anticancer activity in MKN-45 human stomach cancer cells implanted in mice than NB-506. 

*Keywords*: Anticancer agents; Topoisomerase; Indolocarbazole; Substituent effects

DNA topoisomerase I is one of the important enzymes for cellular processes such as DNA replication, RNA transcription and recombination. The key role of the enzyme is to change DNA topology by catalyzing a single-stranded breakage of the phosphodiester bond and subsequent religation of the nicked strand in DNA.[1] Recently, Morham *et al.*[2] reported that topoisomerase I is essential for cell growth in mammalian cells by targeted disruption of the mouse topoisomerase I gene. Moreover, Husain *et al.*[3] reported that level of topoisomerase I is much higher in advanced-stage human colon cancer cells than those in normal. Thus, it is quite possible that inhibitors to topoisomerase I could be a potent anticancer drug. Indeed, several clinical trials showed that topoisomerase I inhibitors such as irinotecan and topotecan are promising anticancer agents.

In our previous effort to discover topoisomerase I inhibitors, we found BE-13793C (**1**)[4] in the culture broth of *Streptverticillium* species. After various modifications of BE-13793C, a 13-*N*-β-glucopyranoside of **1**, ED-110 (**2**)[5], showed greatly improved topoisomerase inhibition and cytotoxicity; however, its anticancer activity could not be evaluated precisely because of its poor aqueous solubility. To improve the solubility of ED-110, an amino functional group was added at the 6-*N* position. The compound **3a** showed more potent topoisomerase I inhibition than that of ED-110, but its solubility was also insufficient for use in *in vivo* studies. As previously reported, a formylated analogue of **3a**, NB-506 (**3b**)[6], which has better solubility than that of ED-110 showed not only potent *in vitro* cytotoxicity against various cell lines but also a wide chemotherapeutic spectrum against several human tumor xenografts in mice. These results suggested that modification at the 6-*N*-amino position of compound **3a** might provide better anticancer drugs than NB-506.

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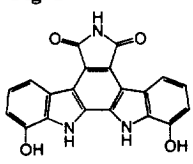
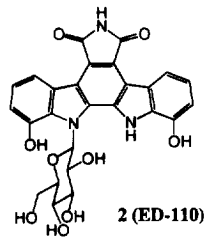
In this paper we describe the topoisomerase I inhibition, cytotoxicity and anti-tumor activity of a series of 6-*N*-amino analogues of compound **3a**.

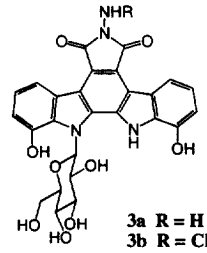
**Fig. 1**



**1 (BE-13793C)**



**2 (ED-110)**



**3a R = H**
**3b R = CHO (NB-506)**

## Chemistry

The 6-*N*-amino analogues **5** were obtained through two synthetic routes (Scheme 1) starting from the common intermediate **4**[7] which was prepared by the method reported previously by us.

One route is a direct conversion of **4** with appropriate hydrazine derivatives generally in DMF at 80 °C. The other route is a coupling reaction of compound **3a**, obtained by treating **4** with hydrazine in DMF at 80 °C, with acid anhydrides, sulfonic acid anhydrides, isocyanates or alkylhalides in THF or DMF at room temperature or at 80 °C,or reductive amination of **3a**. The synthetic yields and reaction conditions in preparation of the compounds **5** were summarized in Table 1

**Scheme 1**

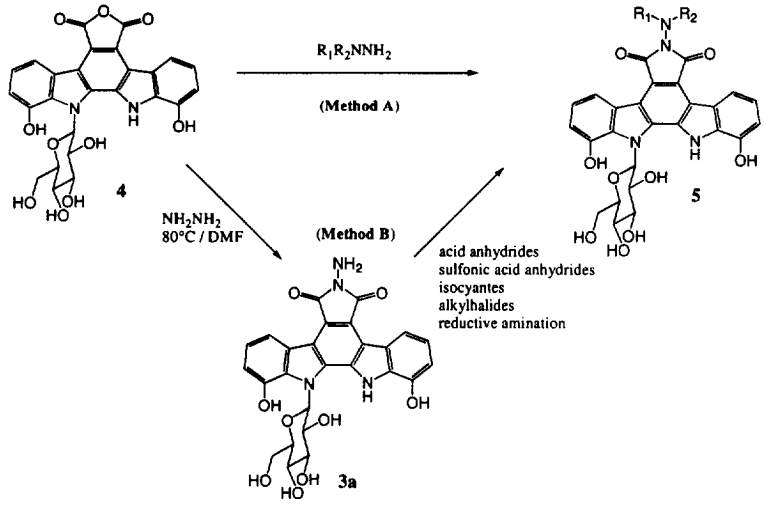


**Table 1**

| | $R_1$ | $R_2$ | Method | Reagent | Solvent | Reaction Temperature (°C) | Yield (%) |
|---|---|---|---|---|---|---|---|
| **5a** | COMe | H | B | $Ac_2O$ | AcOH | 90 | 78 |
| **5b** | COEt | H | B | $(CH_2CH_2CO)_2O$ | THF | room temp. | 55 |
| **5c** | COPh | H | B | BzCl | THF | room temp. | 80 |
| **5d** | CO-2-Py | H | A | $NH_2NHCO$-2-Py | DMF | 80 | 98 |
| **5e** | $COCH_2CN$ | H | A | $NH_2NHCOCH_2CN$ | DMF | 80 | 88 |
| **5f** | $COCH_2OH$ | H | A | $NH_2NHCOCH_2OH$ | DMF | 80 | 85 |
| **5g** | $COCH_2NMe_2$ | H | B | $HOOCCH_2NMe_2$, DCC | $CH_2Cl_2$ | room temp. | 31 |
| **5h** | $SO_2CH_3$ | H | B | $Ms_2O$ | THF | room temp. | 90 |
| **5i** | $SO_2Ph$(4-Me) | H | B | $p$-$Ts_2O$ | THF | room temp. | 48 |
| **5j** | $CONH_2$ | H | A | $NH_2NHCONH_2$ | MeOH | reflux | 82 |
| **5k** | CONHPh | H | B | NCOPh | THF | room temp. | 60 |
| **5l** | Me | Me | B | MeI | DMF | room temp. | 52 |
| **5m** | Me | H | B | 1) HCHO, 2) $H_2$/Pd-C | MeOH | room temp. | 25 |
| **5n** | Et | H | A | $NH_2NHEt$ | DMF | 80 | 62 |
| **5o** | nPr | H | A | $NH_2NHnPr$ | DMF | 80 | 64 |
| **5p** | $CH_2Ph$(3-OH) | H | A | $NH_2NHCH_2Ph$(3-OH) | DMF | 80 | 91 |
| **5q** | $CH_2CH_2CN$ | H | A | $NH_2NHCH_2CH_2CN$ | DMF | 80 | 73 |
| **5r** | $CH_2CH_2OH$ | H | A | $NH_2NHCH_2CH_2OH$ | DMF | 80 | 93 |
| **5s** | $CH_2CH(OH)CH_2OH$ | H | A | $NH_2NHCH_2CH(OH)CH_2OH$ | DMF | 80 | 90 |
| **5t** | $CH(CH_2OH)_2$ | H | A | $NH_2NHCH(CH_2OH)_2$ | DMF | 80 | 85 |
| **5u** | Ph(4-COOH) | H | A | $NH_2NHPh$(4-COOH) | DMF | 80 | 81 |
| **5v** | $CH_2COOH$ | H | B | 1) OHCCOOH, 2) $H_2$/Pd-C | MeOH | room temp. | 88 |

## Results and Discussion

As shown in Table 2, replacement of the substituents at the 6-*N*-amino position did not affect selectivity towards topoisomerase II and EGF receptor kinase (EGFRK), while analogues with aryl substituents such as compounds **5c**, **5d**, **5i**, **5k**, **5p** and **5u** showed moderate inhibition of protein kinase C (PKC). Most of the analogues such as the alkyl, acyl, sulfonyl and carbamoyl derivatives showed potent activity for topoisomerase I-mediated DNA cleavage. However, the activity of a di-substituted analogue, compound **5l**, was extremely low. This result suggests that a proton at the 6-*N*-amino position might be necessary for inhibitory activity. In the acyl analogues, arylcarbonyl(**5c**) and heteroarylcarbonyl(**5d**) substituents gave more potent activity for topoisomerase I-mediated DNA cleavage than alkylcarbonyl substituents, while the alkyl, sulfonyl and carbamoyl analogues were not the case. In the alkyl analogues, substitution of cyano, hydroxyl and aryl group at the alkyl side chain increased in the potency. The inhibitory activity against topoisomerase I tested using the enzyme assay (Topo-I cleavage, $EC_{50}$) and the cellular assay ($K^+$/SDS, $EC_{200}$) systems did not always correlate, probably because of differences in penetration into the cells. Strangely enough, a correlation between the inhibitory activity against topoisomerase I ($K^+$/SDS $EC_{200}$) and cytotoxicity in P388 cells also was not always found (for example, 5h), probably because of other mechanisms for showing cytotoxicity. Several derivatives soluble in the vehicle (up to 26.6 % (*v/w*) aqueous Polyethylene Glycol-400) were tested with respect to anticancer efficacy in mice.

**Table 2** *In vitro* activity of various analogues

| | $R_1$ | $R_2$ | Topo-I[a] Cleavage $EC_{50}$ (μM) | Topo-II[a] Cleavage $EC_{50}$ (μM) | $K^+$/SDS[b] (P388/S) $EC_{200}$ (μM) | CTX[c] P388/S $IC_{50}$ (μM) | CTX[c] MKN-45 $IC_{50}$ (μM) | EGFRK[d] $IC_{50}$ (μM) | PKC[e] $IC_{50}$ (μM) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | (BE-13793C) | – | >3.0 | >50 | 3.0 | 2.0 | NT[f] | 29 | 40 |
| 2 | (ED-110) | – | 3.0 | >50 | 2.0 | 0.044 | 0.54 | 90 | 3.8 |
| 3a | H | H | 0.27 | >50 | 0.50 | 0.040 | 0.13 | >200 | >200 |
| 3b | CHO (NB-506) | H | 0.70 | >50 | 1.4 | 0.075 | 0.31 | >200 | 200 |
| 5a | COMe | H | 1.0 | >50 | 2.0 | 0.15 | 0.26 | >200 | 130 |
| 5b | COEt | H | 0.48 | >50 | 6.0 | 0.045 | 0.19 | >200 | 36 |
| 5c | COPh | H | 0.052 | NT | 10 | 0.012 | 0.048 | >200 | 35 |
| 5d | CO-2-Py | H | 0.090 | >50 | >10 | 0.47 | 2.5 | >200 | 41 |
| 5e | $COCH_2CN$ | H | 0.55 | >50 | 20 | 0.49 | 1.9 | >200 | 65 |
| 5f | $COCH_2OH$ | H | 2.8 | >50 | 5.0 | 0.24 | 0.66 | >200 | >200 |
| 5g | $COCH_2NMe_2$ | H | 0.17 | NT | >10 | 0.26 | 0.46 | >200 | >200 |
| 5h | $SO_2CH_3$ | H | 0.58 | >50 | >10 | 0.060 | 0.27 | >200 | >200 |
| 5i | $SO_2Ph$(4-Me) | H | 0.27 | >50 | >10 | NT[f] | NT[f] | >200 | 12 |
| 5j | $CONH_2$ | H | 0.36 | >50 | 2.8 | 0.035 | 0.27 | >200 | >200 |
| 5k | CONHPh | H | 0.25 | NT | >10 | 0.25 | 0.25 | 95 | 6.8 |
| 5l | Me | Me | >10 | >50 | >10 | 1.6 | >10 | >200 | 59 |
| 5m | Me | H | 0.35 | >50 | 2.5 | 0.075 | 0.13 | 100 | 55 |
| 5n | Et | H | 3.5 | >50 | 1.2 | 0.018 | 0.059 | >200 | >200 |
| 5o | nPr | H | 1.2 | >50 | 6.3 | 0.055 | 0.21 | 59 | 65 |
| 5p | $CH_2Ph$(3-OH) | H | 0.30 | >50 | 0.26 | 0.0070 | 0.012 | >200 | 13 |
| 5q | $CH_2CH_2CN$ | H | 0.070 | >50 | 0.90 | 0.0060 | 0.020 | >200 | >200 |
| 5r | $CH_2CH_2OH$ | H | 0.43 | >50 | 0.30 | 0.0060 | 0.0030 | >200 | >200 |
| 5s | $CH_2CH(OH)CH_2OH$ | H | 1.0 | NT | 0.70 | 0.029 | 0.079 | 180 | >200 |
| 5t | $CH(CH_2OH)_2$ | H | 0.58 | >50 | 0.45 | 0.017 | 0.13 | >200 | >200 |
| 5u | Ph(4-COOH) | H | 0.16 | >50 | >50 | 6.5 | >10 | 90 | 23 |
| 5v | $CH_2COOH$ | H | 9.5 | >50 | >10 | 0.80 | 2.9 | >200 | 110 |

*a*)Topoisomerase-mediated DNA cleavage assay; The cleavage reactions of topoisomerase I and II were carried out using supercoiled pBR322 plasmid DNA as reported previously.[6b] *b*) Effects on the formation of protein-DNA complex in P388 cells were investigated by the $K^+$/SDS method.[6b] *c*) Cytotoxicity (CTX) against murine leukemia cells (P388) and human stomach cancer cells (MKN-45) was measured by the colorimeric tetrazolium-formazan method and the sulforhodamine B dye-staining method, respectively.[6b] *d*) Poly($Glu_4Tyr_1$) was used as a substrate for EGF receptor kinase.[6b] *e*) The histone II-As was used as a substrate for protein kinase C.[6b] *f*) NT: not tested

As shown in Table 3, the dose of compound **5t** (J-109,404), in which the formyl group of NB-506 was replaced with a 1,3-dihydroxypropane group, was more than ten times lower for 75% growth inhibition against MKN-45 human stomach cancer cells than that of NB-506. Since the cytotoxicity to MKN-45 cells of compound 5t was almost the same as that of NB-506, the better anticancer efficacy of compound 5t than that of NB-506 would be due to good distribution into the target cancer cells. Although the compound 5t was four times more toxic than NB-506, the safety margin of this compound was three or four times better than NB-506 due to its potent anticancer activity. On the other hand, the compound **5r** with a hydroxyethyl group at the 6-*N*-amino position did not have a better anticancer effect than that of compound **5t** despite showing fifty times more potent cytotoxicity. These data suggested that not only cytotoxicity but also physicochemical properties

such as hydrophilicity might be important for showing potent anticancer activity. The anticancer effects and the toxicities in this series of compounds were almost parallel except compound **5t**.

**Table 3** Anticancer activity

| Compound[a)] | $R_1$ | $R_2$ | CTX MKN-45 $IC_{50}$ (μM) | MKN-45 $GID_{75}$[b)] mg/m$^2$ | $LD_{10}$[c)] mg/m$^2$ | Safety Margin $LD_{10}$/ $GID_{75}$[d)] |
|---|---|---|---|---|---|---|
| **3b (NB-506)** | CHO | H | 0.31 | 1152 | 1600 | 1.4 |
| **5f** | $COCH_2OH$ | H | 0.66 | 1200 | >1500 | >1.3 |
| **5n** | Et | H | 0.059 | >1500 | >1500 | ND |
| **5q** | $CH_2CH_2CN$ | H | 0.020 | 200 | 640 | 3.2 |
| **5r** | $CH_2CH_2OH$ | H | 0.0030 | 270 | 560 | 2.1 |
| **5s** | $CH_2CH(OH)CH_2OH$ | H | 0.079 | 290 | 330 | 1.1 |
| **5t (J-109,404)** | $CH(CH_2OH)_2$ | H | 0.13 | 78 | 390 | 5.0 |

*a*) Compounds were injected i.v. five times/week for 2 weeks, and treatment was initiated when tumors grew to 0.2 cm$^3$ or larger. *b*) Anticancer effect on MKN-45 human stomach cancer cells implanted s.c. into a side flank of nude mice. $GID_{75}$; approximate 75% Growth Inhibition Dose *c*) $LD_{10}$; approximate 10% Lethal Dose at the treatment schedule *d*) Safety margin: the ratio of $LD_{10}$ / $GID_{75}$

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