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# Synthesis of 1-/2-substituted-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-diones and evaluation of their cytotoxicity and topoisomerase II inhibition

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**Abstract**—Studies on antitumor heterocyclic quinones containing nitrogens revealed that the number and position of nitrogens on the heterocyclic ring have significance on cytotoxicity of quinones. In our continuous effort to find more cytotoxic quinone compounds, we designed triazolophthalazine analogues in order to introduce more nitrogens on the heterocyclic quinones. 1-/2-Substituted-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-diones were synthesized by 1,3-dipolar addition of phthalazine-5,8-dione and 4-methoxybenzyl azide by modification of previously reported method. The cytotoxicity of the synthesized compounds was evaluated by a SRB (sulforhodamine B) assay against nine types of human cancer cell lines and inhibition against topoisomerase II (Topo II) of them was assessed by a decatenation assay. Most of the synthesized compounds showed considerably higher cytotoxicity than that of doxorubicin. Also, topoisomerase II inhibitory activity of the tested compounds was higher than that of etoposide and $IC_{50}$ values of the compounds were 19.4–64.5 μM.


## 1. Introduction

Quinones have been widely known to have various physiological activities as antimicrobial and anticancer agents. Especially, heterocyclic quinones have occupied large classes of antitumor agents. Although the toxicity mechanism of these compounds is still to be elucidated, their mechanisms have been proposed as redox cycling and alkylation.[1] The study on quinone reductases has focused on DT-diaphrase or NAD(P)H:quinone oxidoreductase 1 (NQO1). Streptonigrin, a quinone antitumor, is metabolized by NQO1,[2] and the compound is regarded to lead to Topo II-mediated DNA cleavage.[3] DNA topoisomerases are nuclear enzymes regulating the topological state of DNA by breaking and rejoining of DNA strands. Topo II breaks both strands of the duplex. A DNA intercalator has cytotoxic activity by stabilizing the ternary DNA intercalator–Topo complex.[4] The precise mechanism of ellipticine, a natural antitumor alkaloid has not yet been fully understood but was suggested including DNA intercalation and inhibition of topo II activity. Also, the antitumor activity of anthracyclines, such as doxorubicin, mainly resulted from an inhibition of mammalian topo II.[5,6] The topoisomerase is involved in the control of the shape of DNA. The DNA intercalators have cytotoxocity by inhibiting topoisomerase. The representative DNA intercalators are acridines, alkaloids, anthracyclines, anthracenediones, arylaminoalcohols, coumarins, indoles, phenanthridines, quinolines and quinoxalines.[7] In our previous reports, the heterocyclic quinones, tricycles such as imidazoquinoxalines **1**, pyrazinoquinoxalines **2**,[8] imidazoquinolines **3**[9] and imidazophthalazines **4**[10] were synthesized and their cytotoxicity was evaluated (Fig. 1).

Shaikh et al. emphasized that the number or position of nitrogen atoms in the heterocyclic quinone compound plays an important role in an anticancer activity and that DNA intercalation activity increases in the order of naphthalene, quinoline and diazanaphthalene.[11] Quinone structure constituted with 3 or 4 planar fused

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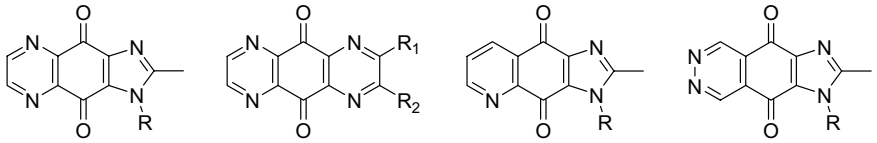



**Figure 1.** Structures of the heterocyclic quinones, tricycles developed in our laboratory.

cycles including two or more nitrogen atoms and conjugated carbonyl groups in *para* is required as DNA intercalator. The planarity of heterocycles is susceptible to intercalate into DNA helix, which leads to the extension of π electron stacking effect and the stability of the DNA-intercalator complex. The nitrogen atoms and carbonyl groups presented in heterocyclic compounds also increase the stability of the complex through forming hydrogen bonds with DNA. Based on such reasons the number or position of nitrogen atoms in the heterocyclic compounds plays an important role in exhibiting an anticancer activity.[12,13]

In this paper, we report the synthesis, cytotoxicity, and inhibitory activity against Topo II of 1-/2-substituted-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-diones **8**, **9** (Scheme 1). We suggest that the coplanar tricyclic heteroquinones possessing five nitrogen atoms can be novel DNA-intercalating heterocyclics.

## 2. Results and discussion

### 2.1. Chemistry

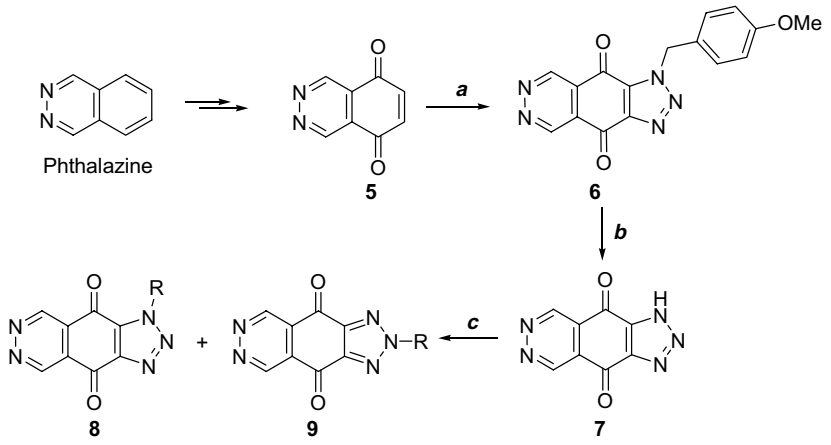
Previously reported[10] synthetic method of 1*H*-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-dione derivatives included monoamination, acylation, amination and diazotization of 6,7-dichlorophthalazine-5,8-dione, which had been prepared from phthalazine. However, this scheme comprises too many complicated steps and inevitably utilizes ammonia gas for direct amination of halide. Therefore, we employed 1,3-dipolar addition reaction of naphthoquinone for the synthesis of triazolophthalazinedione. Synthesis of 1-/2-substituted-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-diones **8**, **9** has been achieved by the modified reaction of reported method[14] starting from phthalazine-5,8-dione **5**[15] and 4-methoxybenzyl azide.[16] The 1-(4-methoxybenzyl)-1H-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-dione **6** was produced by 1,3-dipolar addition of the 4-methoxybenzyl azide to the phthalazine-5,8-dione.[17] The 4-methoxybenzyl group was removed by trifluoroacetic acid. The N-alkylation of the triazolo-phthalazine-dione by alkylhalides gave both isomers **8** and **9**. Generally, the alkylation activity decreased with increase in the alkyl chain length. Also the alkylating agents with strong electron-withdrawing group, such as trifluoroethyliodide, gave very poor yield. Two geometric isomers were obtained, and generally 2-substituted isomers were obtained in higher yield than 1-substituted isomer. The two isomers were separated by column chromatography (hexane/EtOAc = 1:2) (Scheme 1).

### 2.2. Cytotoxicity by SRB assay

The in vitro cytotoxic activities were evaluated by SRB assay method. The various human tumor cell lines (lung; A549, ovarian; SK-OV-3, melanoma; SK-MEL-2, CNS; XF498, colon; HCT15, stomach; SNU-638, fibro sarcoma; HT1080 and myeloid leukemic; HL-60) were used. The cell growth inhibitory potential was determined as described.[18] The inhibitory activities were presented as micromolar concentrations of the compounds that cause 50% inhibition per unit of enzyme ($IC_{50}$) under the assay conditions and compared with that of doxorubicin and etoposide, the clinically used agents for the treatment of solid tumors (Table 1). In general, the cytotoxicity of most 2-substituted-triazolophthalazine-4,9-diones (**9a**–**9f**) was superior to that of doxorubicin and showed higher activity than the corresponding 1-substituted-triazolophthalazine-diones (**8a**–**8f**) against all kinds of cancer cell lines. Especially, 2-methyl-substituted compound **9a** showed significantly high cytotoxicity against almost all cell lines, and its cytotoxicity against human ovarian cell line (SK-OV-3) was 100 times of doxorubicin. 1-*iso*-Butyl-triazolophthalazinedione (**8f**) showed low cytotoxicity among the synthesized compounds. This might be caused by the reduced hydrogen-bonding of carbonyl oxygen in the cells, which is caused by the bulky isobutyl group. The *iso*-propyl derivatives (**8e** and **9e**) represented similar or predominant cytotoxicity to the other derivatives. However, cytotoxicity of compound **6** having bulky methoxybenzyl group as 1-substituent was weaker than that of corresponding alkyl derivatives. Also, non-substituted triazolo-phthalazine-dione **7** did not show any activity against all tumor cell lines.

### 2.3. Inhibitory activity for topoisomerase II

To investigate the cytotoxic mechanism of tested compounds, inhibitory activity for topo II, essential enzymes for DNA metabolism was evaluated. The decatenation assay[19,20] is specific in measuring topo II activity because it is based on the conversion of catenated DNA to its decatenated form, which requires DNA double strand breakage followed by strand rotation and ligation activities uniquely done by topo II. The removal of these KDNA by the enzyme can be seen in agarose gels. Based on this information, effects of the synthesized compounds for the catalytic activity of topo II were evaluated. The decatenation of KDNA was induced by topo II. The results of topo II inhibition of the tested compounds and etoposide[21] are shown in Table 2. Also, the electrophotogram on the inhibitory activity of compounds is presented in Figure 2. When these compounds




***a***. *4-MeOPhCH₂N₃/EtOAc*, ***b***. *TFA*, ***c***. *RI, K₂CO₃/ DMF*

| No | R | No | R |
|---|---|---|---|
| **8a** | $CH_3$ | **8d** | $CH_2CH_2CH_2CH_3$ |
| **9a** | $CH_3$ | **9d** | $CH_2CH_2CH_2CH_3$ |
| **8b** | $CH_2CH_3$ | **8e** | $CH(CH_3)_2$ |
| **9b** | $CH_2CH_3$ | **9e** | $CH(CH_3)_2$ |
| **8c** | $CH_2CH_2CH_3$ | **8f** | $CH_2CH(CH_3)_2$ |
| **9c** | $CH_2CH_2CH_3$ | **9f** | $CH_2CH(CH_3)_2$ |

**Scheme 1.** Synthesis of 1-/2-substituted-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-diones.

**Table 1.** Cytotoxicity of 1-/2-substituted-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-diones against human tumor cell lines

| Compound | $IC_{50}$ (μM) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A549 | SK-OV-3 | SK-MEL-2 | XF498 | HCT15 | SNU-638 | HT1080 | HL-60 |
| Doxorubicin | 0.022 | 0.041 | 0.019 | 0.044 | 0.251 | 0.052 | 0.022 | 0.044 |
| Etoposide | 1.16 | 4.28 | 5.72 | 3.48 | 2.80 | 0.23 | 0.25 | 0.37 |
| **6** | 0.054 | 0.012 | 0.041 | 0.118 | 0.059 | 0.257 | 0.330 | 0.130 |
| **7** | 9.02 | 7.64 | >10.00 | >10.00 | >10.00 | >5 | >5 | >5 |
| **8a** | 0.004 | 0.003 | 0.008 | 0.035 | 0.103 | 0.241 | 0.073 | 0.082 |
| **9a** | 0.002 | 0.0004 | 0.002 | 0.004 | 0.020 | 0.054 | 0.0078 | 0.124 |
| **8b** | 0.006 | 0.003 | 0.008 | 0.034 | 0.039 | 0.126 | 0.068 | 0.056 |
| **9b** | 0.018 | 0.003 | 0.004 | 0.017 | 0.018 | 0.046 | 0.204 | 0.090 |
| **8c** | 0.026 | 0.014 | 0.020 | 0.085 | 0.176 | 0.212 | 0.075 | 0.083 |
| **9c** | 0.019 | 0.009 | 0.009 | 0.018 | 0.018 | 0.043 | 0.033 | 0.157 |
| **8d** | 0.081 | 0.021 | 0.023 | 0.181 | 0.177 | 0.155 | 0.099 | 0.089 |
| **9d** | 0.018 | 0.017 | 0.015 | 0.019 | 0.018 | 0.050 | 0.030 | 0.151 |
| **8e** | 0.032 | 0.049 | 0.016 | 0.049 | 0.033 | 0.057 | 0.114 | 0.050 |
| **9e** | 0.009 | 0.031 | 0.007 | 0.014 | 0.014 | 0.011 | 0.055 | 0.018 |
| **8f** | 0.11 | 0.19 | 0.066 | 0.14 | 0.11 | 0.069 | 0.077 | 0.075 |
| **9f** | 0.019 | 0.043 | 0.015 | 0.019 | 0.029 | 0.014 | 0.085 | 0.038 |

A549, human lung tumor cell line; SK-OV-3, human ovarian tumor cell line; SK-MEL-2, human melanoma tumor cell line; XF498, human CNS tumor cell line; HCT15, human colon tumor cell line; SNU-638, human stomach tumor cell line; HT1080, human fibro sarcoma tumor cell line; HL-60, human myeloid leukemic tumor cell line.

were tested at a concentration of 50 μM, most of the compounds showed topo II inhibition activity. $IC_{50}$ values of the compounds were 19.4–64.5 μM. As a result of these tests, all tested 1/2-substituted-1*H*-[1,2,3]triazolo [4,5-*g*]phthalazine-4,9-diones were considered as potent topo II inhibitors higher than etoposide. Although the

**Table 2.** Inhibitory activity of 1-/2-substituted-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-diones against on topoisomerase II

| Compound | Decatation activity (for Topo II) $IC_{50}$ (μM) | Compound | Decatation activity (for Topo II) $IC_{50}$ (μM) |
|---|---|---|---|
| **6** | 28.6 | **8d** | 43.2 |
| **7** | 52.8 | **9d** | 64.8 |
| **8a** | 39.0 | **8e** | 33.3 |
| **9a** | 55.6 | **9e** | 54.5 |
| **8b** | 55.6 | **8f** | 29.8 |
| **9b** | 61.5 | **9f** | 19.4 |
| **8c** | 49.0 | Etoposide | 92.9 |
| **9c** | 44.4 | | |

Decatenation assay for topo II catalytic activity was done as described in Section 4.

correlation of the cytotoxicity and the inhibitory activity of DNA decatenation by topo II is not clear, DNA topoisomerase related to DNA intercalation is considered as an important target for inhibition of human cancer cells.[22]

## 3. Conclusion

1-/2-Substituted-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-diones, the coplanar tricyclic heteroquinones with five nitrogen atoms, were synthesized and evaluation for their cytotoxic activity using SRB assay was performed in vitro. In general, most triazolophthalazines showed great cytotoxic effects against human cancer cell lines and 2-substituted-triazolophthalazine-4,9-diones exhibited higher cytotoxicity against all kinds of cancer cell lines than the corresponding 1-substituted-triazolophthalazine-diones. The cytotoxic effects of most 2-substituted-triazolophthalazine-4,9-diones were superior to that of doxorubicin. At a concentration of 50 μM, most compounds showed good topo II inhibition activity and $IC_{50}$ values of the compounds were 19.4–64.5 μM. As a result of these tests, the 1/2-substituted-1*H*-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-diones were considered as a topo II inhibitor with potential cytotoxic effect.

## 4. Experimental

### 4.1. Materials

**4.1.1. Synthesis.** All melting points were taken in Pyrex capillaries using electrothermal digital melting point apparatus (Büchi). $^1$H NMR spectra were recorded on a 400 MHz Varian FT-NMR spectrometer using tetramethylsilane as an internal standard. Samples were dissolved in $CDCl_3$ or DMSO-$d_6$. Mass spectra were obtained on the Mass spectrometer JMS-700 (Jeol, Japan) at the Korea Basic Science Institute (Seoul). Most of the reagents were purchased from Sigma–Aldrich Chemical Company.

**4.1.2. Cytotoxicity and topoisomerase inhibitory assay.** Trichloroacetic acid (TCA) and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (St. Louis, MO). Minimal essential medium with Eagles' salt (MEME), fetal bovine serum (FBS), non-essential amino acid solution (10 mM, 100×), trypsin–EDTA solution (1×), and antibiotic–antimycotic solution were from Invitrogen (Grand Island, NY). Topo II and assay kits were purchased from Topogen, Inc. (Columbus, OH, USA). Stock solutions of all test agents were dissolved in DMSO for topo II decatenation assay.

### 4.2. Methods

#### 4.2.1. Synthesis

**4.2.1.1. 1-(4-Methoxybenzyl)-1*H*-[1,2,3]triazolo[4,5-*g*]-phthalazine-4,9-dione (6).** A solution of phthalazine-5,8-dione (1.00 g, 6.20 mmol) and 4-methoxybenzyl azide (338 mg, 2.07 mmol) in EtOAc (60.0 mL) was refluxed overnight. The solvent was evaporated off, and the residue was purified by column chromatography under ethyl acetate to give **6** (415 mg, 62%; based on azide) as a brown solid: mp: >300 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.04 (d, $J$ = 1.2 Hz, 1H), 9.94 (d, $J$ = 1.2 Hz, 1H), 7.47 (d, $J$ = 8.8 Hz, 2H), 6.87 (d, $J$ = 8.8 Hz, 2H), 5.96 (s, 2H), 3.78 (s, 3H); $^{13}$C NMR ($CDCl_3$) $\delta$ 175.81, 174.79, 160.66, 147.28 (2C), 146.32 (2C), 130.53 (2C), 125.34, 124.96, 124.83, 114.75 (2C), 55.55, 54.26; HR-FABMS Calcd for $C_{16}H_{12}N_5O_3$ $(M+H)^+$: 322.0940. Found: 322.0943.

**4.2.1.2. 1*H*-[1,2,3]Triazolo[4,5,-*g*]phthalazine-4,9-dione (7).** A solution of the 1-(4-methoxybenzyl)-1*H*-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-dione (415 mg, 1.29 mmol) in $CF_3CO_2H$ (30.0 mL) was refluxed for 2 days and evaporated to dryness under reduced pressure. One molar aqueous NaOH (100 mL) was added to the residue, and the aqueous layer was washed with EtOAc. The aqueous phase was acidified to pH 1 with HCl, and the product was extracted into EtOAc. Evaporation of the dried ($MgSO_4$) extract gave **7** (150 mg, 58%) as a pale brown solid: mp: >300 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.89 (s, 2H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 177.37 (2C),

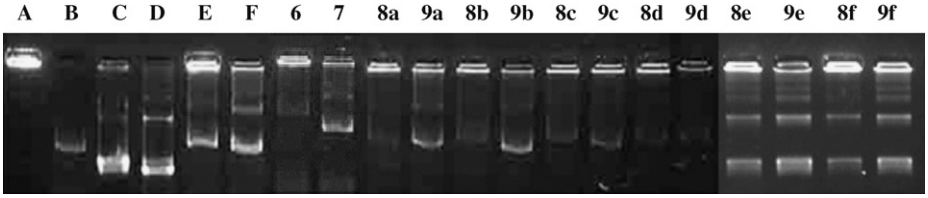



**Figure 2.** Effects of 1-/2-substituted-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-diones on the decatenation of KDNA by topoisomerase II. Lane A; KDNA without enzyme (catenated form); lane B; KDNA with 1 unit of topoisomerase II (decatenated form); lane C; decatenated KDNA marker; lane D; linear KDNA marker; lane E; etoposide, 200 μM; lane F; etoposide, 100 μM; lane 8a–9f; KDNA with 1 U of topoisomerase II in the presence of the prepared compounds at a concentration of 50 μM, respectively.

146.76 (4C), 126.91 (2C); HR-FABMS Calcd for $C_8H_4N_5O_2$ $(M+H)^+$: 202.0365. Found: 202.0362.

**4.2.1.3. General procedure for the preparation of 1-substituted-1*H*-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-diones (8a–8f) and 2-substituted-2*H*-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-diones (9a–9f).** Corresponding alkyl halide (3 equiv) was added to a solution of 1*H*-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-dione (1 equiv) in DMF (0.23 M), and the reaction mixture was stirred for 5 h at room temperature. The reaction mixture was passed through a short pad of Celite (EtOAc) and the filtrate was concentrated to dryness. Chromatography of the residue with hexane/EtOAc (1:2) gave 1/2-substituted-1/2*H*-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-diones.

**4.2.1.4. 1-Methyl-1*H*-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-dione (8a) and 2-methyl-2*H*-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-dione (9a).** Compounds **8a** and **9a** (**8a**; 27 mg and **9a**; 15 mg, 56%) were obtained from **7** (70.0 mg, 0.35 mmol) as a apricot solid **8a** and a pale brown solid **9a**: **8a**; mp: >300 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.07 (d, $J$ = 1.2 Hz, 1H), 9.96 (d, $J$ = 1.2 Hz, 1H), 4.55 (s, 3H); $^{13}$C NMR ($CDCl_3$) $\delta$ 175.72, 175.08, 147.37 (2C), 146.24 (2C), 124.91 (2C), 37.74; HR-FABMS Calcd for $C_9H_6O_2N_5$ $(M+H)^+$: 216.0521. Found: 216.0522. **9a**; mp: >300 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.03 (s, 2H), 4.54 (s, 3H); $^{13}$C NMR ($CDCl_3$) $\delta$ 176.18 (2C), 147.06 (4C), 125.46 (2C), 44.19; HR-FABMS Calcd for $C_9H_6O_2N_5$ $(M+H)^+$: 216.0521. Found: 216.0517.

**4.2.1.5. 1-Ethyl-1*H*-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-dione (8b) and 2-ethyl-2*H*-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-dione (9b).** Compounds **8b** and **9b** (**8b**; 24 mg and **9b**; 33 mg, 52%) were obtained from **7** (100 mg, 0.50 mmol) as a pale brown solid **8b** and a yellow solid **9b**: **8b**; mp: >300 °C; $^1$H NMR ($CDCl_3$) $\delta$ 9.98 (d, $J$ = 1.2 Hz, 1H), 9.89 (d, $J$ = 1.2 Hz, 1H), 4.87 (q, $J$ = 7.2 Hz, 2H), 1.62 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR ($CDCl_3$) $\delta$ 175.86, 174.88, 147.32 (2C), 146.32 (2C), 124.98, 124.87, 46.87, 15.41; HR-FABMS Calcd for $C_{10}H_8O_2N_5$ $(M+H)^+$: 230.0678. Found: 230.0683. **9b**; mp: >300 °C; $^1$H NMR ($CDCl_3$) $\delta$ 9.96 (s, 2H), 4.73 (q, $J$ = 7.2 Hz, 2H), 1.69 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR ($CDCl_3$) $\delta$ 176.32 (2C), 147.07 (4C), 125.51 (2C), 53.14, 14.83; HR-FABMS Calcd for $C_{10}H_8O_2N_5$ $(M+H)^+$: 230.0678. Found: 230.0676.

**4.2.1.6. 1-*n*-Propyl-1*H*-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-dione (8c) and 2-*n*-propyl-2*H*-[1,2,3] triazolo[4,5-*g*]phthalazine-4,9-dione (9c).** Compounds **8c** and **9c** (**8c**; 15 mg and **9c**; 23 mg, 45%) were obtained from **7** (70.0 mg, 0.35 mmol) as a brown solid **8c** and **9c**: **8c**; mp: >300 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.07 (s, 1H), 9.96 (s, 1H), 4.86 (br s, 2H), 2.04–2.12 (m, 2H), 1.03 (t, $J$ = 7.6 Hz, 3H); $^{13}$C NMR ($CDCl_3$) $\delta$ 176.19, 175.22, 147.64 (2C), 146.66 (2C), 125.33, 125.17, 53.20, 24.00, 11.41; HR-FABMS Calcd for $C_{11}H_{10}O_2N_5$ $(M+H)^+$: 244.0834. Found: 244.0831. **9c**; mp: >300 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.02 (s, 2H), 4.71 (t, $J$ = 7.2 Hz, 2H), 2.14–2.21 (m, 2H) 1.01 (t, $J$ = 7.6 Hz, 3H); $^{13}$C NMR ($CDCl_3$) $\delta$ 176.33 (2C), 147.06 (4C), 125.52 (2C), 59.39, 23.24, 11.13; HR-FABMS Calcd for $C_{11}H_{10}O_2N_5$ $(M+H)^+$: 244.0834. Found: 244.0835.

**4.2.1.7. 1-*n*-Butyl-1*H*-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-dione (8d) and 2-*n*-butyl-2*H*-[1,2,3] triazolo[4,5-*g*]phthalazine-4,9-dione (9d).** Compounds **8d** and **9d** (**8d**; 6 mg and **9d**; 20 mg, 36%) were obtained from **7** (57.0 mg, 0.28 mmol) as a orange solid **8d** and a yellow solid **9d**: **8d**; mp: >300 °C; $^1$H NMR ($CDCl_3$) 10.05 (d, $J$ = 1.2 Hz, 1H), 9.95 (d, $J$ = 1.2 Hz, 1H), 4.88 (t, $J$ = 7.6 Hz, 2H), 1.97–2.06 (m, 2H) 1.37–1.47 (m, 2H), 1.00 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR ($CDCl_3$) $\delta$ 175.87, 174.91, 147.33 (2C), 146.35 (2C), 125.01, 124.85, 51.18, 32.11, 19.80, 13.58; HR-FABMS Calcd for $C_{12}H_{12}O_2N_5$ $(M+H)^+$: 258.0991. Found: 258.0989. **9d**; mp: >300 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.03 (s, 2H), 4.75 (t, $J$ = 7.2 Hz, 2H), 2.08–2.17 (m, 2H), 1.37–1.44 (m, 2H), 0.99 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR ($CDCl_3$) $\delta$ 176.33 (2C), 147.07 (4C), 125.52 (2C), 57.62, 31.61, 19.79, 13.54; HR-FABMS Calcd for $C_{12}H_{12}O_2N_5$ $(M+H)^+$: 258.0991. Found: 258.0988.

**4.2.1.8. 1-*iso*-Propyl-1*H*-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-dione (8e) and 2-*iso*-propyl-2*H*-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-dione (9e).** Compounds **8e** and **9e** (**8e**; 6 mg and **9e**; 11 mg, 20%) were obtained from **7** (70.0 mg, 0.35 mmol) as a brown solid **8e** and **9e**: **8e**; mp: >300 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.07 (s, 1H), 9.97 (s, 1H), 5.52–5.63 (m, 1H), 1.78 (d, $J$ = 6.4 Hz, 6H); $^{13}$C NMR ($CDCl_3$) $\delta$ 176.08, 174.88, 147.27 (2C), 146.47 (2C), 125.15, 124.69, 55.44, 22.47 (2C); HR-FABMS Calcd for $C_{11}H_{10}O_2N_5$ $(M+H)^+$: 244.0834. Found: 244.0831. **9e**; mp: >300 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.04 (s, 2H), 5.13–5.25 (m, 1H), 1.76 (d, $J$ = 6.8 Hz, 6H); $^{13}$C NMR ($CDCl_3$) $\delta$ 176.44 (2C), 147.08 (4C), 125.55 (2C), 61.25, 22.52 (2C); HR-FABMS Calcd for $C_{11}H_{10}O_2N_5$ $(M+H)^+$: 244.0834. Found: 244.0834.

**4.2.1.9. 1-*iso*-Butyl-1*H*-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-dione (8f) and 2-*iso*-butyl-2*H*-[1,2,3] triazolo[4,5-*g*]phthalazine-4,9-dione (9f).** Compounds **8f** and **9f** (**8f**; 9 mg and **9f**; 13 mg, 24%) were obtained from **7** (70.0 mg, 0.35 mmol) as a brown solid **8f** and **9f**: **8f**; mp: >300 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.08 (s, 1H), 9.96 (s, 1H), 4.72 (d, $J$ = 7.6 Hz, 2H), 2.34–2.46 (m, 1H), 1.03 (d, $J$ = 6.8 Hz, 6H); $^{13}$C NMR ($CDCl_3$) $\delta$ 175.90, 174.95, 147.33 (2C), 146.37 (2C), 125.04, 124.82, 58.00, 30.07, 19.91 (2C); HR-FABMS Calcd for $C_{12}H_{12}O_2N_5$ $(M+H)^+$: 258.0991. Found: 258.0988. **9f**; mp: >300 °C; $^1$H NMR ($CDCl_3$) $\delta$ 10.04 (s, 2H), 4.56 (t, $J$ = 7.6 Hz, 2H), 2.48–2.62 (m, 1H), 1.02 (d, $J$ = 6.8 Hz, 6H); $^{13}$C NMR ($CDCl_3$) $\delta$ 176.36 (2C), 147.09 (4C), 125.54 (2C), 64.72, 29.87, 19.95 (2C); HR-FABMS Calcd for $C_{12}H_{12}O_2N_5$ $(M+H)^+$: 258.0991. Found: 258.0991.

**4.2.2. In vitro antitumor activity evaluation by SRB assay[15].** The in vitro cytotoxic activities were evaluated by SRB method. Human tumor cell lines; lung; A549, ovarian; SK-OV-3, melanoma; SK-MEL-2, CNS; XF498, colon; HCT15, stomach; SNU-638, fibro sarcoma; HT1080 and myeloid leukemic; HL-60 ($5 \times 10^4$ cells/mL) was treated with different concentrations of the test agents for 3 days. After treatment, cells were fixed with TCA and cell viability was determined

with sulforhodamine B (SRB) protein staining method. The $IC_{50}$ values were calculated using non-linear regression analysis (percent survival vs concentration).

**4.2.3. Decatenation assay for topoisomerase II activity[19].** The assay was done by the protocol provided by TopoGen, Inc. The total reaction volume of the topoisomerase II-mediated cleavage reaction was fixed at 20 μL. Briefly, assay buffer [50 mM Tris–HCl, pH 8, 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM dithiothreitol, 30 μg/mL bovine serum albumin (BSA)] containing 200 ng of KDNA (TopoGEN), and a solution of the test drugs were added to one unit of the human recombinant topoisomerase II (the amount of enzyme which resulted in the complete decatenation of 200 ng of KDNA). After 10 min of incubation at 37 °C, the reaction was stopped by addition of 5 μL of stop buffer containing the loading dye (1% sarkosyl, 0.025% bromphenol blue, 5% glycerol), and then the reaction mixture was analyzed on a 1% agarose gel by running at 40 V for 3.5 h in TBE buffer (89 mM Tris, 89 mM borate, 2 mM Na–EDTA, pH 8.3). Gels were stained with SYBR Green I (Molecular Probes, Eugene, OR), and observed under UV illumination. Gels were photographed, and remaining KDNA from photographic negatives was scanned using AlphaImager 2200 (AlphaEase version 5.5). The $IC_{50}$ values were calculated using non-linear regression analysis.

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