Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry
Bioorganic & Medicinal Chemistry 14 (2006) 1229–1235

Solid phase combinatorial synthesis of benzothiazoles and evaluation of topoisomerase II inhibitory activity

Suk-June Choi,[a] Hyen Joo Park,[a] Sang Kook Lee,[a] Sang Woong Kim,[b] Gyoonhee Han[c] and Hea-Young Park Choo[a,*]

[a]*Ewha Womans University, School of Pharmacy, Seoul 120-750, Republic of Korea*
[b]*Leadgenex, Inc. Venture Town Dasan, 1687-2 Shinil Dong, Daedeok Gu, Deajon 306-230, Republic of Korea*
[c]*Yonsei University, Department of Biotechnology, Seoul 120-749, Republic of Korea*

Received 24 June 2005; accepted 21 September 2005
Available online 18 October 2005

Abstract—To investigate one possible mechanism of action of the cytotoxic activity of benzothiazoles, we synthesized 2-(substituted-phenyl)benzothiazoles and evaluated their ability to inhibit topoisomerase II activities. Solid phase combinatorial method using trityl resin was employed and benzothiazole derivatives with various substitution on 2′-, 3′-, or 4′-position of phenyl group were obtained in ca. 30 mg scale (7–96% yield). Most of the compounds synthesized exhibited topoisomerase II inhibitory activity at 100 μM. 2-(3-Amino-4-methylphenyl)benzothiazole showed high activity (IC_{50} = 71.7 μM), comparable to etoposide (IC_{50} = 78.4 μM).

1. Introduction

Simple 2-(4-aminophenyl)benzothiazoles display potent and selective antitumor activity against breast, ovarian, colon, and renal cell lines, but their mechanism of action has not yet to be elucidated. These compounds are active only against certain human cancer cell lines such as breast MCF-7, MDA 468, renal TK 10, and ovarian IGROV1.[1] The biological profile is unlike any known anticancer agents, and is the focus of extensive study. The activities were considered to be related to the metabolism, since the sensitive cell lines efficiently retained and metabolized 2-(4-aminophenyl)benzothiazoles to acetylated and 6-hydroxylated derivatives.[2–4] In addition to 2-(4-aminophenyl)benzothiazoles, 4-methoxy-2-(4-trifluoromethyl/*t*-butyl phenyl)benzothiazoles were reported effective against B-12 melanoma and A-NK cells.[5]

DNA topoisomerases are ubiquitous enzymes that control and modify the topological states of DNA. They are considered to play important roles in replication, recombination, transcription, chromosome condensation, and the maintenance of genome stability by catalyzing the passage of individual DNA strands (topoisomerase I) or double helices (topoisomerase II) through one another. In accordance, topoisomerase activities are activated in cancer cell growths, and thus are good targets for antineoplastic drugs. Many antitumor drugs such as camptothecin family target topoisomerase I (topo I), while doxorubicin and etoposide are representative topoisomerase II (topo II) inhibitors.[6] Many antitumor topo II inhibitors act as a result of interactions with both the enzyme and DNA, for example, hypericin interacts with DNA at the N_7 sites of purine residues.[7,8] The decatenation assay is specific for 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.[9] The removal of these KDNA by the enzyme can be seen in agarose gels.

In this study, to investigate one possible mechanism of action of the cytotoxic activity of benzothiazoles, we synthesized 2-(substituted-phenyl)benzothiazoles and evaluated their cytotoxicity and ability to inhibit topo II activities. The reported method by Mourtas et al. was employed for the preparation of these compounds.[10]

Keywords: Benzothiazoles; Solid phase synthesis; Topoisomerase II inhibitory activity; Cytotoxicity.
* Corresponding author. Tel.: +82 2 3277 3042; fax: +82 3277 2851; e-mail: hypark@ewha.ac.kr

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.09.051

2. Results

2.1. Solid phase synthesis of benzothiazoles

The procedure developed by Mourtas and co-workers was very useful for the synthesis of various benzothiazoles (Scheme 1). The yield was 7–96% after column chromatography. This method had difficulties in preparation of compounds in large quantities, but was very effective for the synthesis of diverse compounds having various functional groups in small scale (30 mg scale). Compounds with alkyl, alkoxy, halogen, CF_3, amino, nitro, and ethylthio phenyl/pyridine were prepared. However, the reaction of solid bound-2-aminobenzothiol with 1-pyrrolidine carbonyl chloride or benzo[*b*]thiophene-2-carbonyl chloride was not successful. The structure and yield of the synthesized compounds are given in Table 1.

2.2. Cytotoxicity

The in vitro cytotoxic activities were evaluated using SRB assay method.[11] The following human solid tumor cell lines were used: A549 (lung cancer), Col2 (colon cancer), SNU-638 (stomach cancer), HT1080 (fibrosarcoma cancer), and HL-60 (myeloid leukemia). The IC_{50} values evaluated were compared with those of ellipticine, doxorubicin, and etoposide.

All the synthesized compounds exhibited less cytotoxicity than ellipticine or doxorubicin, clinically used agents for the treatment of solid tumor. Only compounds **4** and **18**, with the high topo II inhibitory activity, showed a significant cytotoxicity on HL-60 human leukemia cell line, comparable to higher than that of doxorubicin. In addition, relatively cytotoxic compounds such as **4**, **18**, and **17** have an amino at the 3′- or 4′-position of phenyl ring. However, their nitro analogs (**23**, **24**, **25**, and **11**) and dimethylamino analog (**2**) showed weak activities. Fluoro-containing compounds, including 3′-trifluoromethoxy (**8**) and 3′-difluoromethylthio (**16**) substituted analogs, also showed weak cytotoxic activities.

In the literature, the structure–activity relationship analysis on 2-(4-aminophenyl)benzothiazoles reported that the benzothiazole nucleus was essential for potent cytotoxicity, and the substitution at 3′-position of the phenyl ring with alkyl or halogen groups increased the cytotoxicity of antitumor benzothiazoles.[4]

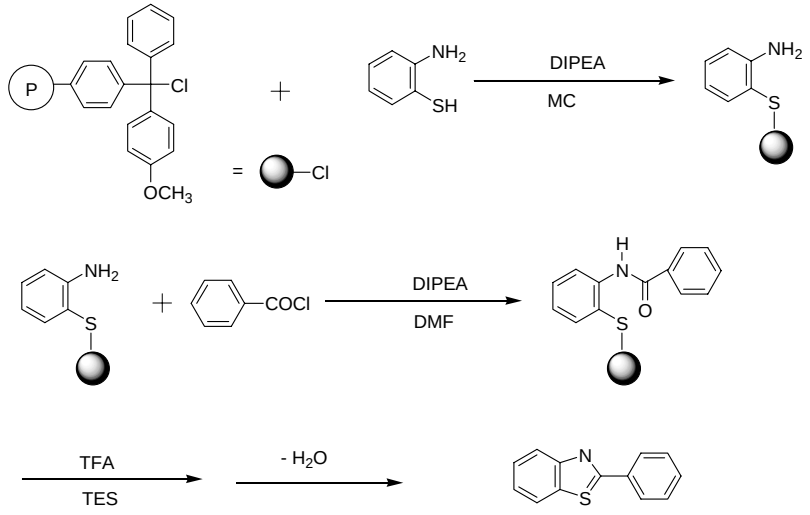
2.3. Topo II inhibition

A plasmid cleavage assay was used to investigate the compounds on human DNA. The reference drug, etoposide, is a potent inhibitor stabilizing specifically DNA–topo II covalent complexes.[12] All the synthesized compounds showed topo II inhibitory activity (9–89%) at 100 μM as shown in Table 3. Especially, the cytotoxic compounds, **4** and **18**, showed relatively high topo II inhibitory activity. The IC_{50} values of **4** and **18** were 71.7 and 107.8 μM, respectively, and were comparable to etoposide (IC_{50} = 78.4 μM). The electrophotogram with the inhibitory effects of test compounds are shown in Figure 1.

Recently, some fused heterocyclic compounds such as benzoxazole, benzimidazole, benzothiazole, and oxazolopyridine derivatives are reported to have eukaryotic topo II inhibitor activities.[13] Especially, 2-phenoxymethylbenzothiazole was reported to have very high topo II inhibitor activity.

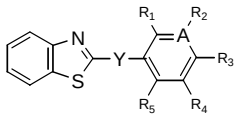
3. Discussion

We designed and synthesized a series of 2-(4-aminophenyl)benzothiazole, based on the antitumor activity of benzothiazoles. Cytotoxic effects of the compounds were measured by the SRB protein-staining method using cultured human lung (A549), colon (Col2), stomach (SNU-638), fibrosarcoma (HT1080), and myeloid leukemia (HL-60) cancer cells. As shown in Table 2, the compounds tested exhibited moderate cytotoxic activity, and compounds **4** had a potential selective cytotoxicity



Scheme 1. Solid-phase synthesis of benzothiazoles.[10]

Table 1. The structure and yield of benzothiazoles



Compound	A	Y	R_1	R_2	R_3	R_4	R_5	Yield (%)
1	N	—	SC_2H_5	H	H	H	H	92
2	C	—	H	H	$N(CH_3)_2$	H	H	18
3	C	–CH=CH–	H	Cl	H	H	H	13
4	C	—	H	NH_2	CH_3	H	H	40
5	C	—	H	H	CH_3	H	H	11
6	C	—	H	H	*t*-Butyl	H	H	15
7	C	—	H	CH_2Cl	H	H	H	12
8	C	—	H	OCF_3	H	H	H	13
9	C	—	H	OCH_3	OCH_3	H	H	8
10	C	—	H	H	*n*-Heptyl	H	H	20
11	C	—	H	H	NO_2	H	H	40
12	C	—	H	OCH_3	OCH_3	H	H	13
13	C	—	OCH_3	H	H	H	OCH_3	7
14	C	—	OC_2H_5	H	H	H	H	15
15	C	—	F	H	H	CF_3	H	10
16	C	—	H	$SCHF_2$	H	H	H	9
17	C	—	Cl	H	NH_2	H	H	42[a]
18	C	—	Cl	H	H	NH_2	H	19[a]
19	C	—	H	Cl	H	Cl	H	86
20	C	—	H	Cl	Cl	H	H	48
21	C	—	$SCHF_2$	H	H	H	H	50
22	C	—	H	H	CN	H	H	75
23	C	—	Cl	H	H	NO_2	H	20
24	C	—	Cl	H	NO_2	H	H	40
25	C	—	H	NO_2	CH_3	H	H	96

[a] The yield after Pd-C/H_2 reduction.

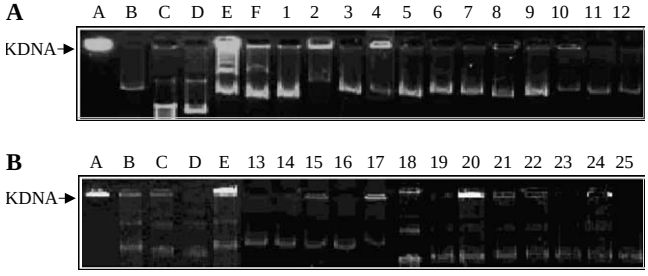


Figure 1. Effects of the synthesized analogs on the decatenation of KDNA by topoisomerase II. (A) Lane A, KDNA without enzyme (catenated form); lane B, KDNA with 1 U topoisomerase II (decatenated form); lane C, decatenated KDNA marker; lane D, linear KDNA marker; lane E, etoposide, 200 μM; lane F, etoposide, 100 μM; lanes 1–12, KDNA with 1 U topoisomerase II in the presence of compounds **1**–**12** at a concentration of 100 μM, respectively. (B) Lane A, KDNA without enzyme (catenated form); lane B, KDNA with 1 U topoisomerase II (decatenated form); lane C, decatenated KDNA marker; lane D, linear KDNA marker; lane E, etoposide, 200 μM; lanes 13–25, KDNA with 1 U topoisomerase II in the presence of compounds **13**–**25** at a concentration of 100 μM, respectively.

against myeloid leukemic cancer cells (IC_{50} = 0.02 μM) compared to other cancer cell lines.

Topo II is an important nuclear enzyme controlling DNA topology through catalysis of a transient breakage of double-stranded DNA in an ATP-dependent fashion, allowing for the passage of double-stranded DNA followed by a resealing of the DNA.[9] Relaxation of DNA supercoils by topo II is considered crucial to its role in DNA replication and transcription. Furthermore, topo II plays a critical role in chromosome condensation and separation during mitosis, and in the attachment of DNA loops to the nuclear matrix and chromosomal scaffold.[14]

Based on this information, we evaluated the benzothiazoles for the catalytic activity of topo II. With respect to topo II, the decatenation of KDNA was induced by topo II. In this case, compounds **4** and **20** showed the strongest inhibitory activity with the IC_{50} of 71.7 and 70.5 μM, respectively, and were comparable with the antitumor agent etoposide with IC_{50} of 78.4 μM. Compounds **18** and **2** caused moderate inhibition of 70% and 54% at 100 μM, respectively, but others showed weak inhibition (Table 3). No considerable functional groups were taken into consideration for topo II inhibitory activity among the tested compounds. Cytotoxic compounds **4** and **18** possess amino substitution and showed high topo II activity. However, another amino-containing cytotoxic compound **17** showed low topo II activity.

In view of the inhibitory activity of topo II by benzothiazoles, the intercalating potential of an individual

Table 2. Effects of benzothiazole derivatives on cytotoxicity in human cancer cells[a]

Compound	Cytotoxicity[a] (IC_{50}, μM)[b]				
	A549	SNU-638	Col2	HT1080	HL-60
1	>50	>50	>50	>50	>50
2	>50	43.49	>50	>50	46.36
3	>50	>50	>50	>50	>50
4	>50	>50	>50	>50	0.02
5	>50	43.79	>50	>50	>50
6	>50	>50	33.30	>50	>50
7	>50	>50	>50	>50	>50
8	23.54	13.09	27.19	>50	17.56
9	>50	>50	>50	>50	>50
10	>50	48.02	>50	>50	>50
11	19.73	>50	>50	21.39	>50
12	>50	>50	>50	>50	>50
13	>50	>50	>50	>50	>50
14	>50	31.48	36.54	>50	>50
15	>50	>50	>50	>50	>50
16	>50	28.53	40.48	>50	>50
17	>50	29.95	47.12	33.94	38.68
18	>50	>50	>50	>50	0.05
19	>50	>50	>50	>50	>50
20	>50	>50	>50	49.78	>50
21	>50	>50	>50	>50	>50
22	>50	>50	>50	>50	>50
23	>50	>50	>50	>50	>50
24	>50	>50	27.91	>50	>50
25	21.72	>50	33.11	>50	>50
Ellipticine	1.15	2.87	1.86	1.34	3.42
Doxorubicin	0.15	0.063	0.093	0.064	0.041
Etoposide	2.44	6.19	1.24	020	0.14

[a] Cytotoxicity was measured as described previously.[11]
[b] The IC_{50} values were determined from triplicate tests.

Table 3. Effects of benzothiazoles (100 μM) on topo II activities

Compound	Decatenation activity[a] (topo II % inhibition)
1	41
2	54
3	39
4	89
5	30
6	32
7	34
8	28
9	32
10	31
11	23
12	20
13	21
14	21
15	27
16	24
17	37
18	70
19	16
20	84
21	28
22	26
23	14
24	34
25	9
Etoposide	58

[a] Decantation assay for topo II catalytic activity was done by the protocol by TopoGen, Inc.

compound, because of the planar structure of compounds, might be one of the factors for the catalytic activity, and further study is needed to clarify the additional factors. No obvious correlation was observed between the cytotoxicity of the individual compound and the inhibitory activity of DNA decatenation by topo II, in vitro. However, it is not plausible to exclude the possibility that differences in compound-uptake into cells exist, or that compounds are metabolized or sequestered in different ways, or that other mechanisms of actions might be included.

In summary, new structural modifications of benzothiazoles were suggested for cytotoxic agents against human cancer cells, some compounds inhibited the catalytic activity of topo II, but further study for the mechanism of action by the compounds might be needed.

4. Conclusion

When a series of 2-(4-aminophenyl)benzothiazole were synthesized and topo II inhibitory activity was evaluated, most of compounds showed moderate inhibition at 100 μM. The compound **4** had the strongest inhibitory activity with IC_{50} of 71.7 μM, which were comparable with the antitumor agent etoposide with IC_{50} of 78.4 μM. Also the compound **4** showed a potential selective cytotoxicity against myeloid leukemic cancer cells (IC_{50} = 0.02 μM) compared to other cancer cells.

5. Experimental

5.1. Materials and methods

All melting points were taken in Pyrex capillaries using electrothermal digital melting point apparatus (Büchi). The IR spectra were recorded on a FT-Infrared spectrometer (Bio-Rad Co., USA) using KBr pellet. ^{1}H NMR spectra were recorded on a 400 MHz Varian FT-NMR spectrometer using trimethylsilane as an internal standard. Samples were dissolved in acetone-d_6. Mass spectra were obtained on a Tandem Mass spectrometer JMS-HX110/110A (Jeol).

5.2. General procedure for solid phase synthesis

After 0.3 g 4-methoxytrityl resin (1.8 mmol/g) with an equimolar amount of 2-aminothiophenol and 0.7 equiv diisopropylethylamine (DIPEA) was reacted in dichloromethane (DCM) for 0.5–4 h at rt, the resin was washed and filtered with the mixture of DCM/methanol/DIPEA (85:10:5). The resin was swelled in DMF. Then, threefold equivalents of benzoyl chloride and DIPEA were added, the mixture was shaken for 3 h at rt, and the mixture was washed and filtered with DCM and DMF, alternatively. Washed resin on the filter paper was filtrated with 7 × 6 ml of 1.1% TFA in DCM/TES (95:5). The filtrates of seven treatments were concentrated in vacuum to an oily residue and dissolved

in 6–10 ml of DMF:methanol (9:1) which contained 0.1–0.2 mmole DTT. After 3 h of standing at rt, the mixture was extracted with ethyl acetate, washed with water, dried and concentrated in vacuum, and purified by the column chromatography (hexane:ethyl acetate = 3:1).

5.2.1. 2-(2-(Ethylthio)pyridin-3-yl)benzo[*d*]thiazole (1). Yellow oil, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.59 (1H, dd, J = 1.2 and 8.0 Hz), 8.26 (1H, dd, J = 1.2 and 8.0 Hz), 8.06–8.15 (2H, m), 7.58 (1H, dt, J = 1.2 and 8.4 Hz), 7.50 (1H, dt, J = 1.2 and 8.4 Hz), 7.29 (1H, d, J = 8.0 Hz). FABHRMS(NBA-CsI) m/z 273.0522 (M^+Cs, $C_{14}H_{13}N_2S_2$ requires 273.0520).

5.2.2. 4-(Benzo[*d*]thiazol-2-yl)-*N*,*N*-dimethylbenzenamine (2). Dark brown oil, ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.93–8.00 (3H, m), 7.91 (1H, dd, J = 1.2 and 8.0 Hz), 7.46 (1H, dt, J = 1.2 and 8.0 Hz), 7.35 (1H, dt, J = 1.2 and 8.0 Hz), 6.83–6.87 (2H, m), 3.18 (3H, s), 2.78 (3H, s). FABHRMS(NBA-CsI) m/z 255.0961 (M^+Cs, $C_{15}H_{14}N_2S$ requires 255.0956).

5.2.3. 2-(2-Chlorostyryl)benzo[*d*]thiazole (3). Pale yellow solid, mp 116–118 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.31–8.35 (2H, m), 8.21 (1H, d, J = 8.4 Hz), 8.15 (1H, dd, J = 0.8 and 8.0 Hz), 8.11 (1H, dd, J = 0.8 and 8.0 Hz), 7.97–8.00 (2H, m), 7.94 (1H, d, J = 8.4 Hz), 7.61 (1H, dt, J = 1.2 and 8.0 Hz), 7.53 (1H, dt, J = 1.2 and 8.0 Hz). FABHRMS(NBA-CsI) m/z 272.0302 (M^+Cs, $C_{15}H_{11}ClNS$ requires 272.0301).

5.2.4. 5-(Benzo[*d*]thiazol-2-yl)-2-methylbenzenamine (4). White solid, mp 72–74 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.30 (1H, dd, J = 0.8 and 8.0 Hz), 7.97 (1H, dd, J = 0.8 and 8.0 Hz), 7.48–7.52 (2H, m), 7.41 (1H, dt, J = 1.2 and 8.0 Hz), 7.30 (1H, dd, J = 1.6 and 7.6 Hz), 7.13 (1H, d, J = 7.6 Hz). FABHRMS(NBA-CsI) m/z 241.0798 (M^+Cs, $C_{14}H_{13}N_2S$ requires 241.0799).

5.2.5. 2-*p*-Tolylbenzo[*d*]thiazole (5). White solid, mp 62–66 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.07 (1H, dd, J = 0.8 and 8.4 Hz), 8.00–8.05 (3H, m), 7.54 (1H, dt, J = 1.2 and 8.0 Hz), 7.44 (1H, dt, J = 1.2 and 8.0 Hz), 2.43 (3H, s). FABHRMS(NBA-CsNa) m/z 248.0505 (M^+Cs, $C_{14}H_{11}NSNa$ requires 248.0510).

5.2.6. 2-(4-*tert*-Butylphenyl)benzo[*d*]thiazole (6). White solid, mp 104–105 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.05–8.09 (3H, m), 8.03 (1H, dd, J = 1.2 and 8.8 Hz), 7.60–7.64 (2H, m), 7.54 (1H, dt, J = 1.2 and 7.2 Hz), 7.44 (1H, dt, J = 1.2 and 7.2 Hz), 1.39 (9H s). FABHRMS(NBA-CsI) m/z 268.1155 (M^+Cs, $C_{17}H_{18}NS$ requires 268.1160).

5.2.7. 2-(3-(Chloromethyl)phenyl)benzo[*d*]thiazole (7). Pale yellow solid, mp 82–83 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.94 (1H, dd J = 1.2 and 7.6 Hz), 7.92–7.88 (2H, m), 7.41 (1H, dt, J = 1.2 and 8.0 Hz), 7.31 (1H, dt, J = 1.2 and 8.0 Hz), 7.29–7.24 (2H, m), 2.30 (2H, s). FABHRMS(NBA-CsI) m/z 260.0305 (M^+Cs, $C_{14}H_{11}ClNS$ requires 260.0301).

5.2.8. 2-(3-(Trifluoromethoxy)phenyl)benzo[*d*]thiazole (8). Pale yellow solid, mp 99–102 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.27–8.31 (2H, m), 8.13 (1H, dd, J = 1.2 and 8.0 Hz), 8.08 (1H, dd, J = 1.2 and 8.0 Hz), 7.53–7.61 (3H, m), 7.49 (1H, dt, J = 1.2 and 8.0 Hz). FAB-HRMS(NBA-CsI) m/z 296.0357 (M^+Cs, $C_{14}H_9F_3NOS$ requires 296.0357).

5.2.9. 2-(3,4-Dimethoxyphenyl)benzo[*d*]thiazole (9). Pale yellow solid, mp 132–133 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.91(1H, dd, J = 1.2 and 8.4 Hz), 7.87 (1H, dd, J = 1.2 and 8.4 Hz), 7.40 (1H, d, J = 2.0 Hz), 7.30 (1H, dd, J = 2.0 and 8.4 Hz), 7.39 (1H, dt, J = 1.2 and 8.4 Hz), 7.29 (1H, dt, J = 1.2 and 8.4 Hz), 6.99 (1H, d, J = 8.4 Hz), 3.82 (3H, s), 3.78 (3H, s). FAB-HRMS(NBA-CsI) m/z 271.9800 (M^+Cs, $C_{15}H_{13}NO_2S$ requires 271.9803).

5.2.10. 2-(4-Heptylphenyl)benzo[*d*]thiazole (10). White solid, mp 46–48 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.06 (1H, d, J = 8.0 Hz), 7.90–8.02 (2H, m), 7.90 (1H, d, J = 8.0 Hz), 7.48 (1H, dt, J = 1.6 and 8.4 Hz), 7.37 (1H, dt, J = 1.6 and 8.4 Hz), 7.29–7.32 (2H, m), 2.68 (2H, t, J = 8.0 Hz), 1.61–1.70 (2H, m), 1.25–1.35 (10H, m), 0.89 (3H, t, J = 5.8 Hz). FABHRMS(NBA-CsI) m/z 310.1625 (M^+Cs, $C_{20}H_{24}NS$ requires 310.1629).

5.2.11. 2-(4-Nitrophenyl)benzo[*d*]thiazole (11). Pale yellow solid, mp 225–227 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.35–8.38 (2H, m), 8.27–8.30 (2H, m), 8.14 (1H, d, J = 8.0 Hz), 7.97 (1H, d, J = 8.0 Hz), 7.56 (1H, dt, J = 0.8 and 8.0 Hz), 7.47 (1H, dd, J = 0.8 and 8.0 Hz). FABHRMS(NBA-CsI) m/z 257.0385 (M^+Cs, $C_{13}H_9N_2O_2S$ requires 257.0385).

5.2.12. 2-(3,4,5-Trimethoxyphenyl)benzo[*d*]thiazole (12). White solid, mp 141–144 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.93 (1H, dd, J = 0.8 and 8.0 Hz), 7.89 (1H, dd, J = 0.8 and 8.0 Hz), 7.41 (1H, dt, J = 1.2 and 8.0 Hz), 7.31 (1H, dt, J = 1.2 and 8.0 Hz), 7.27–7.28 (2H, m), 3.84 (6H, s), 3.70 (3H, s). FABHRMS(NBA-CsI) m/z 302.0855 (M^+Cs, $C_{16}H_{16}NO_3S$ requires 302.0851).

5.2.13. 2-(2,6-Dimethoxyphenyl)benzo[*d*]thiazole (13). Pale yellow solid, mp 117–119 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.06 (1H, dd, J = 0.8 and 8.0 Hz), 8.03 (1H, dd, J = 0.8 and 8.0 Hz), 7.52 (1H, dt, J = 1.2 and 8.0 Hz), 7.42–7.49 (2H, m), 6.79–6.82 (2H, m), 3.77 (6H, s). FABHRMS(NBA-CsI) m/z 272.0746 (M^+Cs, $C_{15}H_{14}NO_2S$ requires 272.0745).

5.2.14. 2-(2-Ethoxyphenyl)benzo[*d*]thiazole (14). Brown solid, mp 58–61 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.54 (1H, dd, J = 1.6 and 8.0 Hz), 8.09 (1H, dd, J = 1.2 and 8.0 Hz), 7.94 (1H, dd, J = 1.2 and 8.0 Hz), 7.49 (1H, dt, J = 1.2 and 8.0 Hz), 7.45 (1H, dt, J = 1.2 and 8.0 Hz), 7.38 (1H, dt, J = 1.2 and 8.0 Hz), 7.12 (1H, dt, J = 1.2 and 8.0 Hz), 7.05 (1H, dd, J = 1.6 and 8.0 Hz), 4.31 (2H, q, J = 7.2 Hz), 1.66 (3H, t, J = 7.2 Hz). FABHRMS(NBA-CsI) m/z 256.0797 (M^+Cs, $C_{15}H_{14}NOS$ requires 256.0796).

5.2.15. 2-(2-Fluoro-5-(trifluoromethyl)phenyl)benzo[*d*]-thiazole (15). Brown solid, mp 101–104 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.79 (1H, dd, J = 2.4 and 6.4 Hz), 8.16 (1H, d, J = 8.4 Hz), 7.97 (1H, d, J = 8.4 Hz), 7.72–7.76 (1H, m), 7.56 (1H, dt, J = 1.2 and 8.4 Hz), 7.46 (1H, dt, J = 1.2 and 8.4 Hz), 7.38 (1H, dd, J = 6.4 and 9.2 Hz). FABHRMS(NBA-CsI) m/z 298.0316 (M^+Cs, $C_{14}H_8F_4NS$ requires 298.0314).

5.2.16. 2-(3-(Difluoromethylthio)phenyl)benzo[*d*]thiazole (16). White solid, mp 74–76 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.37–8.39 (1H, m), 8.21 (1H, dd, J = 0.8 and 8.0 Hz), 8.12 (1H, dd, J = 0.8 and 8.0 Hz), 8.09 (1H, dd, J = 0.8 and 8.0 Hz), 7.80 (1H, d, J = 8.0 Hz), 7.68 (1H, t, J = 8.0 Hz), 7.58 (1H, dt, J = 1.2 and 8.0 Hz), 7.50 (1H, dt, J = 1.2 and 8.0 Hz), 7.41 (1H, s). FABHRMS(NBA-CsI) m/z 294.0223 (M^+Cs, $C_{14}H_{10}F_2NS_2$ requires 294.0223).

5.2.17. 4-(Benzo[*d*]thiazol-2-yl)-3-chlorobenzenamine (17). Yellow solid, mp 220–224 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.03 (1H, d, J = 8.4 Hz), 7.91 (1H, d, J = 8.0 Hz), 7.85 (1H, dd, J = 0.8 and 8.0 Hz), 7.38 (1H, dt, J = 1.2 and 8.0 Hz), 7.28 (1H, dt, J = 1.2 and 8.0 Hz), 6.75 (1H, d, J = 2.4 Hz), 6.66 (1H, dd, J = 2.4 and 8.4 Hz). FABHRMS(NBA-CsI) m/z 261.0252 (M^+Cs, $C_{13}H_{10}ClN_2S$ requires 261.0253).

5.2.18. 3-(Benzo[*d*]thiazol-2-yl)-4-chlorobenzenamine (18). Brown solid, mp 96–98 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.03 (1H, d, J = 8.0 Hz), 7.84–7.96 (2H, m), 7.36 (1H, dt, J = 1.2 and 8.0 Hz), 7.28 (1H, dt, J = 1.2 and 8.0 Hz), 6.75 (1H, d, J = 2.0 Hz), 6.66 (1H, dd, J = 0.8 and 8.0 Hz). FABHRMS(NBA-CsI) m/z 261.0257 (M^+Cs, $C_{13}H_{10}ClN_2S$ requires 261.0253).

5.2.19. 2-(3,5-Dichlorophenyl)benzo[*d*]thiazole (19). White solid, mp 120–121 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.19 (1H, d, J = 2.4 Hz), 8.00 (1H, dd, J = 0.8 and 8.0 Hz), 7.94–7.98 (2H, m), 7.66 (1H, d, J = 8.4), 7.47 (1H, dt, J = 1.2 and 8.0 Hz), 7.38 (1H, dt, J = 1.2 and 8.0 Hz). FABHRMS(NBA-CsI) m/z 279.9756 (M^+Cs, $C_{13}H_7Cl_2NS$ requires 279.9755).

5.2.20. 2-(3,4-Dichlorophenyl)benzo[*d*]thiazole (20). Brown solid, mp 150–155 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.32 (1H, d, J = 2.4 Hz), 8.14 (1H, dd, J = 1.6 and 7.2 Hz), 8.07–8.11 (2H, m), 7.79 (1H, d, J = 8.8 Hz), 7.60 (1H, dt, J = 1.2 and 8.4 Hz), 7.51 (1H, dt, J = 1.2 and 8.4 Hz). FABHRMS(NBA-CsI) m/z 279.9753 (M^+Cs, $C_{13}H_7Cl_2NS$ requires 279.9755).

5.2.21. 2-(2-(Difluoromethylthio)phenyl)benzo[*d*]thiazole (21). White solid, mp 188–190 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.07 (1H, s), 8.00 (1H, dd, J = 0.8 and 8.0 Hz), 7.94–7.90 (2H, m), 7.87 (1H, J = 0.8 and 8.0 Hz), 7.45 (1H, dt, J = 1.2 and 8.0 Hz). FAB-HRMS(NBA-CsI) m/z 294.0225 (M^+Cs, $C_{15}H_{13}NO_2S$ requires 294.0223).

5.2.22. 4-(Benzo[*d*]thiazol-2-yl)benzonitrile (22). Pale yellow solid, mp 120–123 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.08–8.19 (2H, m), 7.87–7.90 (2H, m), 7.59 (1H, dt, J = 1.2 and 8.0 Hz), 7.51 (1H, dt, J = 1.2 and 8.0 Hz), 7.14–7.18 (2H, m). FABHRMS(NBA-CsI) m/z 237.0489 (M^+Cs, $C_{14}H_9N_2S$ requires 237.0486).

5.2.23. 2-(2-Chloro-5-nitrophenyl)benzo[*d*]thiazole (23). Pale yellow solid, mp 190–193 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 9.23 (1H, d, J = 2.8 Hz), 8.41 (1H, dd, J = 2.8 and 8.8 Hz), 8.20–8.24 (2H, m), 8.00 (1H, d, J = 8.8 Hz), 7.67 (1H, dt, J = 1.2 and 7.2 Hz), 7.59 (1H, dt, J = 1.2 and 7.2 Hz). FABHRMS(NBA-CsI) m/z 290.9993 (M^+Cs, $C_{13}H_7ClN_2O_2S$ requires 290.9995).

5.2.24. 2-(2-Chloro-4-nitrophenyl)benzo[*d*]thiazole (24). Yellow solid, mp 80–82 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.67 (1H, d, J = 8.8 Hz), 8.51 (1H, d, J = 2.0 Hz), 8.41 (1H, dd, J = 2.0 and 8.8 Hz), 8.23 (1H, dd, J = 0.8 and 8.0 Hz), 8.23 (1H, dd, J = 0.8 and 8.0 Hz), 7.67 (1H, dt, J = 0.8 and 8.4 Hz), 7.59 (1H, dt, J = 0.8 and 8.4 Hz). FABHRMS(NBA-CsI) m/z 290.9994 (M^+Cs, $C_{13}H_7ClN_2O_2S$ requires 290.9995).

5.2.25. 2-(4-Methyl-3-nitrophenyl)benzo[*d*]thiazole (25). Yellow solid, mp 183–185 °C, ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.70 (1H, s), 8.32 (1H, s), 8.13 (2H, d, J = 8.0 Hz), 7.72 (1H, s), 7.61 (1H, t, J = 12.0 Hz), 7.52 (1H, t, J = 12.0 Hz). FABMS m/z 271.3 (M^++1).

5.3. Cytotoxic activity

A human lung carcinoma (A549, ATCC), colon carcinoma (Col2, University of Illinois at Chicago), stomach carcinoma (SNU-638, Korea Cell Bank at Seoul National University), fibrosarcoma cancer (HT1080), and myeloid leukemia (HL-60) cells (5×10^4 cells/ml) were treated with different concentrations of the test agent for 3 days. After treatment, cells were fixed with TCA and cell viability was measured using the sulforhodamine B (SRB) protein-staining method.[11] The result was expressed in percentage, relative to solvent-treated control incubations, and the IC_{50} values were calculated using non-linear regression analysis (percent survival versus concentration).

5.4. KDNA decatenation activity of topo II

The topo II assay was done by the protocol provided by TopoGEN, Inc. The total reaction volume of the topo 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, and 30 μg/ml bovine serum albumin (BSA)] containing 200 ng of KDNA (TopoGen), and a solution of the test drugs were added to 1 U of the human recombinant topo 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 the addition of 5 μl of stop buffer containing the loading dye (1% sarkosyl, 0.025% bromophenol blue, and 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, and 2 mM Na-EDTA, pH 8.3). Gels were stained with SYBR Green I (Molecular Probes, Eugene, OR), and

observed under UV illumination. For the quantitative determination of topo II activity, photographic negatives were densitometrically scanned using AlphaImager 2200 (AlphaEase version 5.5). The inhibition of topo II was calculated from the equation: % inhibition = [intensity of sample-treated DNA/intensity of vehicle-treated control DNA] × 100.

Acknowledgments

This work was supported by Grant No. 2000-0-215-001-3 from the Basic Research Program of the Korea Science and Engineering Foundation and M10310200002-03B4620-00200 from Korea Institute of Science & Technology Evaluation and Planning.

References and notes

1. Shi, D.-F.; Bradshaw, T. D.; Wrigley, S.; McCall, C. J.; Lelieveld, P.; Fichtner, I.; Stevens, M. F. G. *J. Med. Chem.* **1996**, *39*, 3375.
2. Chua, M.-S.; Shi, D.-F.; Wrigley, S.; Bradshaw, T. D.; Huctchison, I.; Shaw, P. N.; Barrett, D. A.; Stanley, L. A.; Stevens, M. F. G. *J. Med. Chem.* **1999**, *42*, 381.
3. Kashiyama, E.; Huctchison, I.; Chua, M.-S.; Stinson, F. S.; Phillips, L. R.; Kaur, G.; Sausville, E. A.; Bradshaw, T. D.; Westwell, A. D.; Stevens, M. F. G. *J. Med. Chem.* **1999**, *42*, 4172.
4. Huctchison, I.; Chua, M.-S.; Browne, H. L.; Trapani, V.; Bradshaw, T. D.; Westwell, A. D.; Stevens, M. F. G. *J. Med. Chem.* **2001**, *44*, 1446.
5. Goldfarb, R. H.; Ohashi, M.; Yoshino, K. U.S. Patent 6,013,659 **2000**.
6. Walker, J.; Nitiss, J. L. *Cancer Invest.* **2002**, *20*, 570.
7. Burden, D. A.; Osheroff, N. *Biochim. Biophys. Acta* **1998**, *1400*, 139.
8. Muller, S. O.; Eckert, I.; Lutz, W. K.; Stopper, H. *Mutat. Res.* **1996**, *371*, 165.
9. Pommier, Y.; Pourquier, P.; Fan, Y.; Strumberg, D. *Biochim. Biophys. Acta* **1998**, *1400*, 83.
10. Mourtas, S.; Gatos, D.; Barlos, K. *Tetrahedron Lett.* **2001**, *42*, 2201.
11. Lee, S. K.; Cui, B.; Mehta, R. R.; Kinghorn, A. D.; Pezzuto, J. M. *Chem. Biol. Interact.* **1998**, *115*, 215.
12. Joseph, B.; Facompre, M.; Da Costa, H.; Routier, S.; Merour, J.-Y.; Colson, P.; Houssier, C.; Bailly, C. *Bioorg. Med. Chem.* **2001**, *9*, 1533.
13. Pinar, A.; Yurdakul, P.; Yildiz, I.; Temiz-Arpaci, O.; Acan, L.; Aki-Sener, E.; Yalcin, I. *Biochem. Biophys. Res. Commun.* **2004**, *317*, 670.
14. Roca, J. *Trends Biochem. Sci.* **1995**, *20*, 156.