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Design, Synthesis, and Anticancer Activity of Bis-isoxazole Incorporated Benzothiazole Derivatives

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Abstract—A novel series of bis-isoxazole incorporated benzothiazole derivatives has been designed and synthesized. Molecular structures of the compounds have been confirmed by ^{1}H and ^{13}C NMR, and mass spectra. All products have been tested for their in vitro anticancer activity against breast MCF-7 and MDA MB-231, lungs A549, and prostate DU-145 cancer cell lines using the MTT assay and etoposide as a standard drug. Most of the compounds have demonstrated good to moderate activity, and some of those have exhibited more potent activity than etoposide.

Keywords: benzothiazole, isoxazole, anticancer activity

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INTRODUCTION

Heterocyclic compounds, and specifically for the current study benzothiazoles, demonstrate a broad range of biological activities including anticancer [1], antimicrobial [2], antiviral [3], analgesic [4], antimalarial [5], fungicidal [6], antihelmintic [7], antitubercular [8], and many more. For example, molecules of compounds NSC-710305 (**1**, Fig. 1) and violatinctamine (**2**, Fig. 1) [9] contain benzothiazole moieties in their structures. Compound **1** demonstrated anticancer activity and has been processed to phase-1 clinical trial [10].

Similarly, 1,2-isoxazoles demonstrated antitumor [11], anti-oxidant [12], antifungal [13], and a range of other activities. One of the anticancer drug candidates Luminespib (**3**, NVP-AUY922, Fig. 1) contains a synthetic 1,2-isoxazole scaffold [14].

In view of the above and in continuation of the ongoing studies, we have designed and synthesized a new series of bis-isoxazole incorporated benzothiazole derivatives (**14a**–**14j**) and confirmed their structures by ^{1}H and ^{13}C NMR, and mass spectra. The products were screened for their anticancer activity towards four human cancer cell lines.

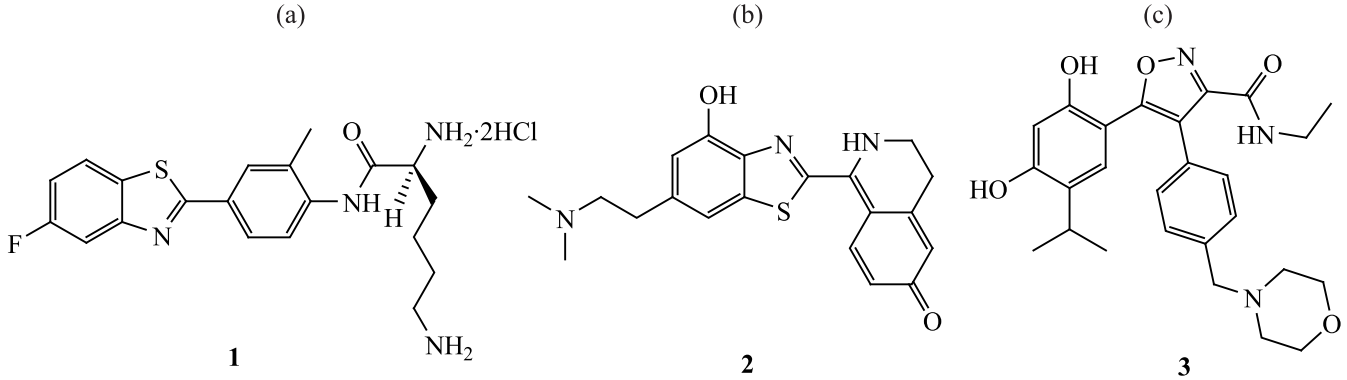


Fig. 1. Structures of anticancer drugs: (a) NSC-710305, **1**, (b) Violatinctamine, **2**, and (c) NVP-AUY922, **3**.

Table 1. In vitro cytotoxicity of the synthesized compounds **14a**–**14j** (IC_{50}, μM)[a]

Compound	MCF-7	A549	DU-145	MDA MB-231
14a	3.45±2.67	4.11±2.10	ND	13.4±6.45
14b	0.45±0.059	0.10±0.012	1.22±0.72	0.11±0.087
14c	1.29±0.94	1.98±0.33	1.56±0.27	2.34±1.23
14d	7.10±4.56	3.77±2.88	9.94±5.34	2.86±1.66
14e	12.4±6.20	5.69±3.14	4.71±2.18	Not determined
14f	15.3±7.34	Not determined	Not determined	5.04±3.77
14g	0.041±0.0078	0.023±0.0056	0.65±0.064	0.07±0.0091
14h	0.32±0.061	0.95±0.033	0.49±0.077	0.05±0.0039
14i	0.76±0.083	0.33±0.099	0.85±0.018	0.90±0.083
14j	1.52±0.56	1.61±0.48	2.08±1.89	0.18±0.066
Etoposide	2.11 ± 0.024	3.08 ± 0.135	1.97 ± 0.45	1.91 ± 0.84

[a] Each data is presented as mean ±S.D value.

RESULTS AND DISCUSSION

Synthesis of new series of bis-isoxazole incorporated benzothiazole derivatives **14a**–**14j** is outlined in Scheme 1. The 3,4,5-trimethoxyphenyl oxime (**4**) was reacted with 1-ethynyl-4-nitrobenzene (**5**) in presence aq NaOCl in anhydrous media of CH_2Cl_2 at room temperature to give pure isoxazole derivative **6**.

Reduction of the nitro group in the intermediate **6** by Pd/C, H_2 in ethanol yielded pure amino intermediate **7**. Its reaction with 4-isothiocyanatophenol (**8**) in ethanol led to pure intermediate **9**, cyclization of which in the presence of [bbim][Br_3] ionic liquid at 70°C afforded pure benzothiazole intermediate **10**. Reaction of compound **10** with propargyl bromide **11** in presence of K_2CO_3 in anhydrous DMF resulted in formation of the intermediate **12**, following cyclization of which with different types of aryl oximes (**4**, **13a**–**13i**) in presence of aq. NaOCl and TEA gave the corresponding pure target compounds **14a**–**14j**.

Biological activity of the products. The newly synthesized bis-isoxazole incorporated benzothiazole derivatives **14a**–**14j** were tested for their in vitro anticancer activity against breast MCF-7 and MDA MB-231, lungs A549, and prostate DU-145 cancer cell lines by using the MTT assay and etoposide as the standard drug (Table 1). All the compounds exhibited low to moderate activity. Among those the products **14b**, **14c**, **14g**–**14j** were determined to be more potent than etoposide. The structure-activity relationship (SAR) analysis indicated that the compound with 3,4,5-trimethoxyphenyl ring (**14b**) exhibited high anticancer activity on all cell lines, whereas the one with 2,4-dimethoxyphenyl ring (**14c**) displayed somewhat lower activity, and the 2,3-dimethoxyphenyl substituted analogue (**14d**) was characterized by very poor activity. The compounds **14e** and **14f** with 3-bromophenyl and 4-nitrophenyl rings exhibited moderate activity. The compound **14g** containing 4-pyridyl heterocyclic ring demonstrated excellent activity on all cell lines. Replacement of 4-pyridyl ring with 2-pyrrolyl ring resulted in slightly lower activity of compound **14h.** Compound **14i** containing 2-thiophenyl ring exhibited lower activity than **14h**. 5-Bromothiophenyl substituent in derivative **14j** caused its slightly decreased activity than that of **14i.**

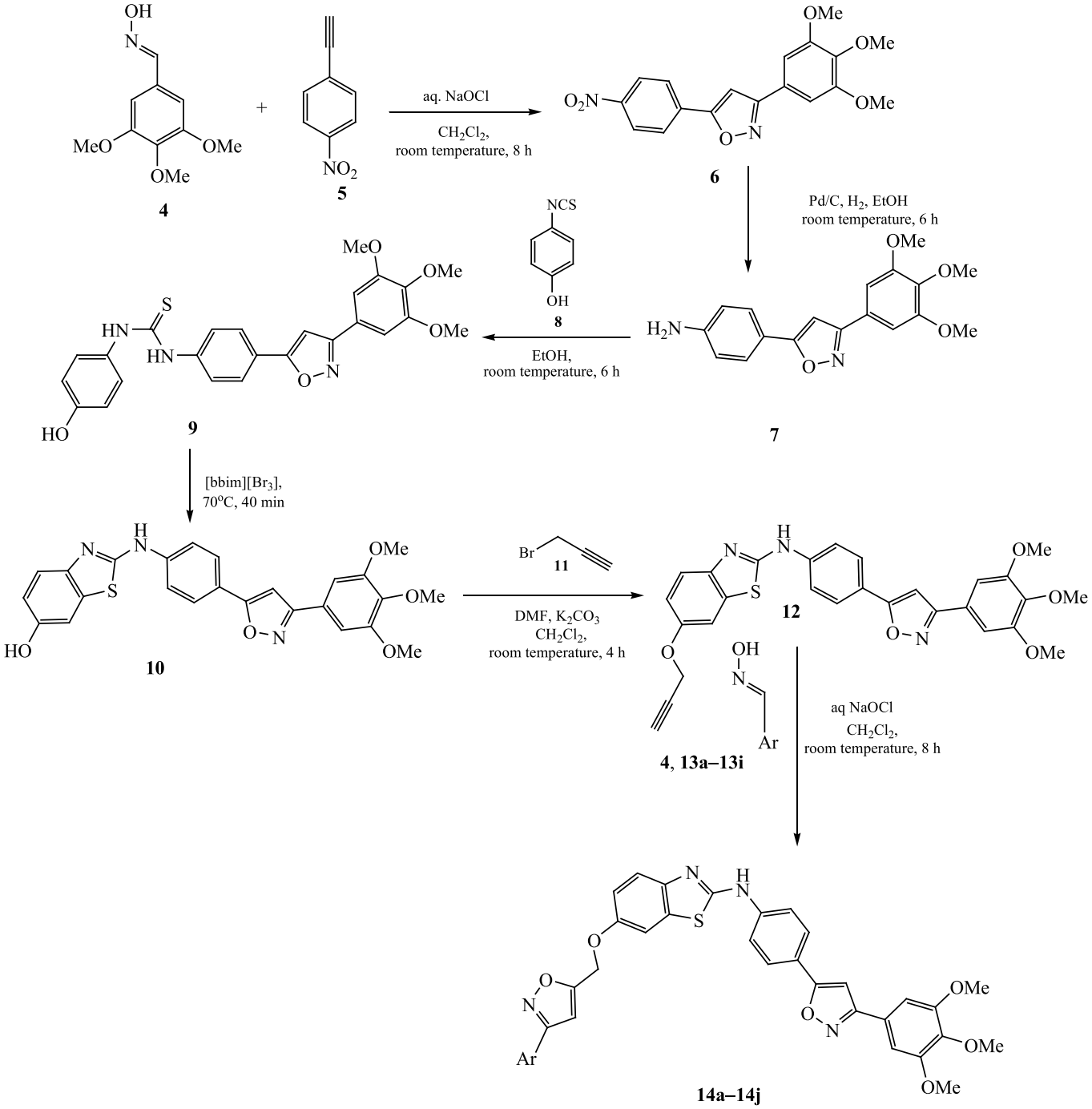
Molecular docking studies. Molecular docking studies were carried out for the synthesized compounds **14a**–**14j** to identify the molecular interactions between anticancer target human topoisomerase II beta in complex with DNA ligands bound with inhibitor etoposide (PDB ID:3QX3) by using AutoDock4.2.4.

All the docked ligands demonstrated interactions with Glu477, Asp479, Arg503, Met555, and Gln778 of Topo-II enzyme and DT9, DC8, DA12, and DG13 of DNA nucleotide residues (Table 2).

Binding affinity of the docked compounds was expressed as negative energy in kcal/mol (dock score). The ligands with more negative value of dock score had higher affinity with Topo-II and DNA binding. As an example, pyridinyl substituted compound **14g** formed H-bond with Gln778 (2.71 Å) and Arg503 (3.01 Å) residues, as well as hydrophobic and electrostatic interactions with Arg503 residue (Table 2, Fig. 2).

Similarly, bromothiophene substituted compound **14j** demonstrated H-bond interactions with Glu777(2.73), DG10(2.09), DT9(2.82), DA12(2.41), and electrostatic interactions with Arg503, DC8, DT9, and hydrophobic interactions with DC8, DA12, DG10, Arg503 active residues (Table 2, Fig. 3).

Scheme 1. Synthetic approach to the target bis-isoxazole incorporated benzothiazole derivatives **14a**–**14j**.



Ar = 3,4,5-trimethoxyphenyl (**4**, **14b**), phenyl (**13a**, **14a**), 2,4-dimethoxyphenyl (**13b**, **14c**), 2,3-dimethoxyphenyl (**13c**, **14d**), 3-bromophenyl (**13d**, **14e**), 4-nitrophenyl (**13e**, **14f**), pyridine-4-yl (**13f**, **14g**), pyrrole-2-yl (**13g**, **14h**), thiophene-2-yl (**13h**, **14i**), 5-bromothiophene-2-yl (**13i**, **14j**).

All ligands demonstrated the same interactions with Topo-II protein and DNA nucleotides and accordingly the compounds had potential of inhibiting the Topo-II protein and intercalating with the base pairs of DNA nucleotides.

EXPERIMENTAL

All chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA) and Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA,

Table 2. Molecular docking data for the synthesized compounds against the DNA topoisomerase II beta in complex with DNA (PDB ID: 3QX3)

Compound	Dock score	LE	Ki, pM	Interactions		
				H-bond (distance, Å)	elecrostatic	hydrophobic
14a	–9.99	–0.23	4769	Lys505(3.54)	Glu477, DC8	DC8, His775, DG7, Lys505
14b	–11.78	–0.34	2721	DC8 (3.14), DT9 (3.99), Met555 (3.14)	DC8, DT9	Ala481, Arg503, Leu484, DA12
14c	–8.87	–0.28	2940	Glu477 (3.09), Gln778 (2.25), DT9 (3.84)	Asp503, DC8	Leu484, DA12, DG13
14d	–11.64	–0.23	2840	DT9 (3.68), Asp848 (2.96), Gln778 (3.64)	DT9	Ala772, DT9, DC8, Arg503
14e	–12.27	–0.28	189.78	Arg503 (3.05), DT9 (3.43), DG10 (3.36), Gln778 (3.74)	Asp557, DT9	DA12, His774, DC8, DG13
14f	–12.92	–0.26	338.78	Ala481 (3.13), Arg503 (3.03), Gln778 (3.02)	Arg503, Glu477, Asp557, DT9	DC8, DT9, Met555, Leu484
14g	–12.44	–0.27	764.14	Arg503 (3.01), Gln778 (2.71), Pro501 (2.88), DC8 (3.28)	Arg503	Phe500, DC8, DA12, DG13, DC8, Arg503
14h	–11.91	–0.26	1850	Ser480 (2.65), Asp557 (3.55), Tyr643 (3.30)	Glu477	DA12, DG13, DT9, Leu484
14i	–12.71	–0.28	481.83	DT9 (3.34), DC8 (2.88), Met555 (2.99), Gln778 (2.90)	Glu477, Asp557, DT9	Ala481, DA12, Met555, Leu484, DT9
14j	–12.97	–0.28	313.5	Glu777 (2.73), DG10 (2.09), DT9 (2.82), DA12 (2.41)	Arg503, DC8, DT9	DC8, DA12, DG10, Arg503
Etoposide	–13.54	–0.20	158.36	Asp479 (2.51), Gln778 (3.11), DC8 (3.21), DG13 (2.71), Gly478 (3.02), DT9 (3.30)	Arg503, Met782	DG13, DA12, Met782

USA), and used without further purification. Reactions were monitored by TLC, performed on silica gel plates covered by 60 F-254 and visualized under UV light or by iodine indicator. Melting points were determined with an electro thermal melting point apparatus and are uncorrected.

^{1}H and ^{13}C NMR spectra were measured on a BRUKER NMR 300 MHz and 400 MHz spectrometers using TMS as the internal standard. ESI spectra were measured on a Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector.

3-(3,4,5-Trimethoxyphenyl)-5-(4-nitrophenyl)-isoxazole (6). The compound **4** (33.7 g, 0.158 mmol) and 1-ethynyl-4-nitrobenzene **5** (15 g, 0.101 mmol) were mixed with dichloromethane (80 mL), and then cooled down to 0°C. Aqueous NaOCl (11 mL, 0.158 mmol) was added drop wise to the above mixture for over 30 min, and then stirred vigorously for 8 h at room temperature. The layers were separated, and the aqueous layer was extracted with dichloromethane. The combined organic layers were dried with Na_2SO_4 and concentrated. The crude product was purified by column chromatography with ethyl acetate/hexanes (4 : 6) to afford pure compound **6**, yield 66%. ^{1}H NMR spectrum, δ, ppm: 3.71 s (3H), 3.92 s (6H), 7.23 s (2H), 7.91 s (1H), 8.21 d (2H, J = 8.7 Hz), 8.42 d (2H, J = 8.7 Hz). MS (ESI): 357 $[M + H]^+$. Found, %: C 60.62; H 4.49; N 7.91. $C_{18}H_{16}N_2O_6$. Calculated, %: C 60.67; H 4.53; N 7.86.

4-[3-(3,4,5-Trimethoxyphenyl)isoxazol-5-yl]benzenamine (7). Compound 6 (22 g, 0.0617 mmol) was dissolved in ethanol and mixed with 10% Pd/C (656 mg 0.00617 mmol). The reaction mixture was stirred under the atmosphere of H_2 (1 atm) at room temperature for 6 h. After completion of reaction (TLC), the catalyst was filtered off over celite and the filtrate was concentrated in vacuo to provide the product **7** (yield 91%) which was used further without purification. ^{1}H NMR spectrum, δ, ppm: 3.71 s (3H), 3.92 s (6H), 7.23 s (2H), 7.31 s (2H), 7.89 s (1H), 8.15 d (2H, J = 8.4 Hz), 8.29 d (2H, J =

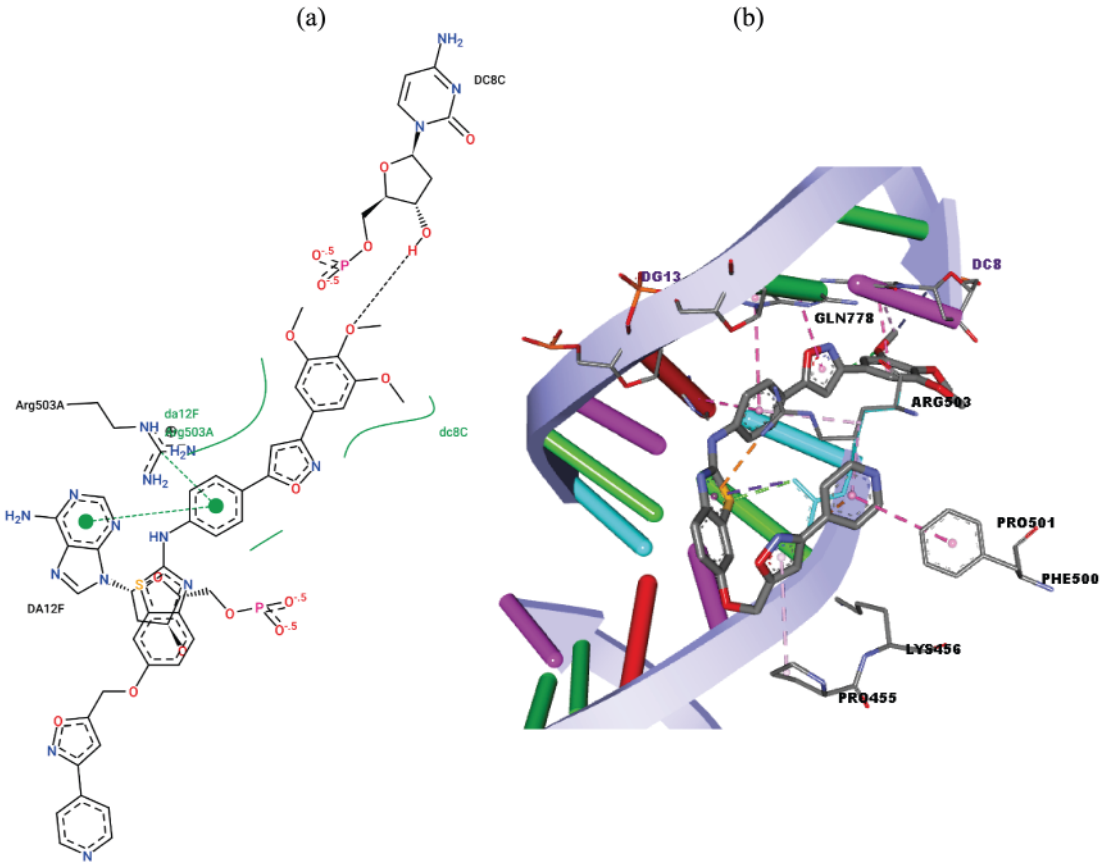


Fig. 2. (a) 2D and (b) 3D interactions of compound **14g** with DNA Topoisomerase II beta in complex with DNA (PDB ID: 3QX3).

8.4 Hz). MS (ESI): 327 $[M + H]^+$. Found, %: C 66.21; H 5.52; N 8.63. $C_{18}H_{18}N_2O_4$. Calculated, %: C 66.25; H 5.56; N 8.58.

1-(4-Hydroxyphenyl)-3-{4-[3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl]phenyl}thiourea (9). To a solution of compound **7** (17 g, 0.0521 mmol) in absolute ethanol (5 mL), 4-isothiocyanatophenol (**8**) (8.45 mL, 0.0782 mmol) was added at 0°C, and stirring of the mixture was continued for 6 h at room temperature. The formed solid was filtered off and washed thoroughly with ethanol to afford the pure compound **9**, yield 87%. ^{1}H NMR spectrum, δ, ppm: 3.71 s (3H), 3.92 s (6H), 7.23 s (2H), 7.34 d (2H, J = 7.56 Hz), 7.65 d (2H, J = 7.56 Hz), 7.93 s (1H), 8.13 d (2H, J = 8.1 Hz), 8.25 d (2H, J = 8.1 Hz), 9.41 s (1H), 10.32 bs (1H), 11.40 s (1H). MS (ESI): 478 $[M + H]^+$. Found, %: C 62.84; H 4.82; N 8.84. $C_{25}H_{23}N_3O_5S$. Calculated, %: C 62.88; H 4.85; N 8.80.

2-{4-[3-(3,4,5-Trimethoxyphenyl)isoxazol-5-yl]-phenylamino}benzo[d]thiazol-6-ol (10). Compound **9** (20 g, 0.0419 mol) was added to 1,3-di-*n*-butylimidazolium bromide ([bbim][Br]) (11 g, 0.0419 mmol), and the reaction mixture was stirred at 70°C for 40 min. After completion of the reaction (TLC), chloroform was added to the mixture and the product was separated as crude residue. The mixture was filtered and the residue was recrystallized from ethanol to afford pure compound **10**, yield 87%. ^{1}H NMR spectrum, δ, ppm: 3.72 s (3H), 3.93 s (6H), 7.24 s (2H), 7.41 s (1H), 7.48 d (1H, J = 8.6 Hz), 7.62 d (1H, J = 8.6 Hz), 7.95 s (1H), 8.16 d (2H, J = 8.3 Hz), 8.27 d (2H, J = 8.3 Hz), 10.36 bs (1H), 12.54 s (1H). MS (ESI): 476 $[M + H]^+$. Found, %: C 63.11; H 4.42; N 8.89. $C_{25}H_{21}N_3O_5S$. Calculated, %: C 63.15; H 4.45; N 8.84.

***N*-{4-[3-(3,4,5-Trimethoxyphenyl)isoxazol-5-yl]-phenyl}-6-(prop-2-ynyloxy)benzo[d]thiazol-2-amine (12).** Compound **10** (16 g, 0.0336 mmol) was dissolved in dry *N*,*N*-dimethyl formamide (50 mL), and K_2CO_3 (9.3 g, 0.0673 mmol) was added to the mixture which was stirred for 15 min at room temperature. Propargyl bromide **11** (2.6 mL, 0.0336 mmol) was slowly added drop wise to the above mixture over a period of 15 min and stirring was continued for 4 h. The reaction was quenched with water and extracted with ethyl acetate (3×20 mL). The combined extracts were washed with water (3×25 mL)

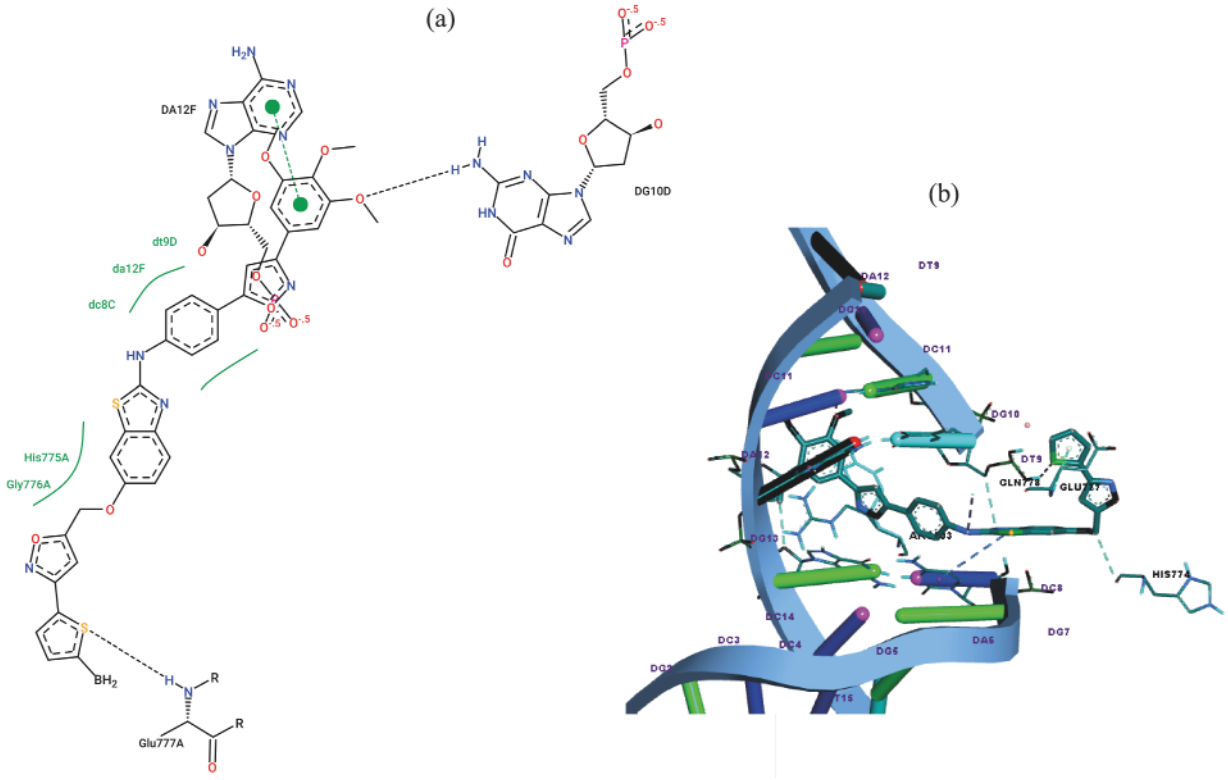


Fig. 3. (a) 2D and (b) 3D interactions of **14j** with DNA topoisomerase II beta in complex with DNA (PDB ID: 3QX3).

and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered, evaporated under reduced pressure, and purified by silica gel chromatography with ethyl acetate/hexane (1 : 1) to afford pure intermediate **12**, yield 80%. ^{1}H NMR spectrum, δ, ppm: 3.10 s (1H), 3.72 s (3H), 3.93 s (6H), 4.90 s (2H), 7.24 s (2H), 7.43 s (1H), 7.50 d (1H, J = 8.6 Hz), 7.63 d (1H, J = 8.6 Hz), 7.95 s (1H), 8.15 d (2H, J = 8.4 Hz), 8.26 d (2H, J = 8.4 Hz), 12.55 s (1H). MS (ESI): 514 $[M + H]^+$. Found, %: C 65.44; H 4.47; N 8.23. $C_{28}H_{23}N_3O_5S$. Calculated, %: C 65.48; H 4.51; N 8.18.

6-[(3-Phenylisoxazol-5-yl)methoxy]-*N*-{4-[3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl]phenyl}benzo[*d*]-thiazol-2-amine (14a). Benzaldoxime (**13a**) (0.17 mL, 15.5 mmol) and compound **12** (500 mg, 9.7 mmol) were dissolved in dichloromethane (10 mL), and the solution was cooled to 0°C. Aqueous NaOCl (0.1 mL, 15.5 mmol) was added dropwise over 30 min, and the reaction mixture was stirred vigorously for 8 h at room temperature. The layers were separated, and the aqueous layer was extracted with dichloromethane. The combined organic layers were dried with Na_2SO_4 and concentrated. The crude product was purified by column chromatography with ethyl acetate/hexanes (6 : 4) to afford pure compound **14a.** Yield 50%, mp 212–214°C NMR spectrum, δ, ppm: 3.72 s (3H), 3.92 s (6H), 5.12 s (2H), 7.23 s (2H), 7.39–7.56 m (5H), 7.64 d (1H, J = 8.7 Hz), 7.71 d (2H, J = 7.65 Hz), 7.81 s (1H), 7.96 s (1H), 8.15 d (2H, J = 8.5 Hz), 8.27 d (2H, J = 8.5 Hz), 12.56 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.4, 61.8, 65.3, 96.3, 96.9, 107.3, 108.5, 115.3, 119.6, 120.5, 124.5, 125.6, 126.2, 126.8, 127.5, 128.2, 130.4, 134.5, 137.3, 142.5, 149.6, 154.7, 155.4, 157.6, 160.5, 164.7, 165.8, 171.8. MS (ESI): 633 $[M + H]^+$. Found, %: C 66.40; H 4.41; N 8.90. $C_{35}H_{28}N_4O_6S$. Calculated, %: C 66.44; H 4.46; N 8.86.

The compounds **14b**–**14j** were synthesized according to the method developed for **14a** using the appropriate benzaldoximes **4**, **13b**–**13i**.

6-{[3-(3,4,5-Trimethoxyphenyl)isoxazol-5-yl]-methoxy}-*N*-{4-[3-(3,4,5-trimethoxyphenyl) isoxazol-5-yl]phenyl}benzo[d]thiazol-2-amine (14b). Yield 52%, mp 222–224°C NMR spectrum, δ, ppm: 3.72 s (3H), 3.86 s (3H), 3.92 s (6H), 3.96 s (6H), 5.09 s (2H), 7.24 s (2H), 7.43 s (1H), 7.52–7.60 m (3H), 7.64 d (1H, J = 8.5 Hz), 7.80 s (1H), 7.95 s (1H), 8.14 d (2H, J = 8.4 Hz), 8.26 d (2H, J = 8.4 Hz), 12.54 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.5, 58.3, 61.7, 62.4, 65.4, 96.4, 96.8, 106.5, 107.4, 107.7, 115.6, 119.6, 120.4, 126.4, 127.6, 128.7,

130.5, 134.2, 137.6, 142.3, 149.6, 154.3, 155.5, 157.3, 158.1, 159.5, 164.6, 165.7, 171.8. MS (ESI): 723 [M + H]$^+$. Found, %: C 63.11; H 4.70; N 7.80. $C_{38}H_{34}N_4O_9S$. Calculated, %: C 63.15; H 4.74; N 7.75.

6-{[3-(2,4-Dimethoxyphenyl)isoxazol-5-yl]methoxy}-*N*-{4-[3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl]-phenyl}benzo[d]thiazol-2-amine (14c). Yield 47%, mp 227–229°C NMR spectrum, δ, ppm: 3.70 s (3H), 3.72 s (3H), 3.86 s (3H), 3.92 s (6H), 4.97 s (2H), 6.85 s (1H), 7.09 d (1H, J = 7.34 Hz), 7.24 s (2H), 7.43 s (1H), 7.55 d (1H, J = 8.3 Hz), 7.61–7.68 m (2H), 7.81 s (1H), 7.95 s (1H), 8.15 d (2H, J = 8.5 Hz), 8.26 d (2H, J = 8.5 Hz), 12.55 s (1H). ^{13}C NMR spectrum, δ, ppm: 56.4, 57.6, 58.5, 61.7, 65.7, 96.4, 97.6, 101.7, 107.4, 107.8, 109.5, 110.3, 115.4, 119.6, 120.3, 126.4, 126.7, 127.5, 130.5, 134.2, 137.5, 142.3, 149.5, 154.3, 157.6, 158.2, 160.4, 162.4, 164.5, 164.7, 165.6, 171.7. MS (ESI): 693 [M + H]$^+$. Found, %: C 64.10; H 4.62; N 8.14. $C_{37}H_{32}N_4O_8S$. Calculated, %: C 64.15; H 4.66; N 8.09.

6-{[3-(2,3-Dimethoxyphenyl)isoxazol-5-yl]methoxy}-*N*-{4-[3-(3,4,5-trimethoxyphenyl) isoxazol-5-yl]-phenyl}benzo[*d*]thiazol-2-amine (14d). Yield 51%, mp 225–227°C NMR spectrum, δ, ppm: 3.71 s (3H), 3.73 s (3H), 3.87 s (3H), 3.92 s (6H), 4.97 s (2H), 6.86–6.90 m (1H), 7.12 t (1H), 7.23 s (2H), 7.44 s (1H), 7.56 d (1H, J = 8.2 Hz), 7.60–7.69 m (2H), 7.82 s (1H), 7.96 s (1H), 8.15 d (2H, J = 8.3 Hz), 8.27 d (2H, J = 8.3 Hz), 12.56 s (1H). ^{13}C NMR spectrum, δ, ppm: 56.4, 57.7, 61.5, 61.8, 65.7, 96.4, 97.5, 107.3, 107.8, 115.4, 118.2, 119.5, 120.2, 120.6, 121.5, 123.4, 126.5, 127.6, 130.4, 134.2, 137.2, 142.4, 147.5, 149.7, 154.3, 154.7, 157.4, 157.8, 162.4, 164.7, 165.3, 171.8. MS (ESI): 693 [M + H]$^+$. Found, %: C 64.11; H 4.63; N 8.14. $C_{37}H_{32}N_4O_8S$. Calculated, %: C 64.15; H 4.66; N 8.09.

6-{[3-(3-Bromophenyl)isoxazol-5-yl]methoxy}-*N*-{4-[3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl]phenyl}-benzo[*d*]thiazol-2-amine (14e). Yield 58%, mp 229–231 NMR spectrum, δ, ppm: 3.72 s (3H), 3.92 s (6H), 5.08 s (2H), 7.24 s (2H), 7.39–7.51 m (3H), 7.56 d (1H, J = 8.3 Hz), 7.59–7.70 m (2H), 7.84 s (1H), 7.96 s (1H), 8.10 s (1H), 8.15 d (2H, J = 8.5 Hz), 8.27 d (2H, J = 8.5 Hz), 12.56 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.4, 61.7, 65.8, 96.4, 97.2, 107.3, 107.7, 115.3, 119.5, 120.5, 125.4, 126.4, 127.5, 128.2, 130.4, 131.3, 133.4, 133.6, 134.7, 137.4, 142.5, 149.7, 154.6, 155.6, 157.4, 160.5, 164.5, 165.3, 171.8. MS (ESI): 713 [M + H]$^+$. Found, %: C 59.04; H 3.77; N 7.92. $C_{35}H_{27}N_4O_6BrS$. Calculated, %: C 59.08; H 3.82; N 7.87.

6-{[3-(4-Nitrophenyl)isoxazol-5-yl]methoxy}-*N*-{4-[3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl]phenyl}-benzo[*d*]thiazol-2-amine (14f). Yield 60%, mp 230–232°C NMR spectrum, δ, ppm: 3.72 s (3H), 3.92 s (6H), 5.13 s (2H), 7.24 s (2H), 7.44 s (1H), 7.56 d (1H, J = 8.4 Hz), 7.66 d (1H, J = 8.4 Hz), 7.86 s (1H), 7.96 s (1H), 8.15 d (2H, J = 8.5 Hz), 8.22 d (2H, J = 7.67 Hz), 8.27 d (2H, J = 8.5 Hz), 8.34 d (2H, J = 7.67 Hz), 12.56 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.6, 61.8, 65.6, 96.4, 97.6, 107.6, 108.3, 115.4, 119.5, 120.7, 126.4, 127.3, 127.8, 128.6, 130.5, 131.3, 134.7, 137.6, 142.4, 149.6, 150.4, 154.6, 155.5, 157.4, 160.6, 164.5, 165.8, 171.8. MS (ESI): 678 [M + H]$^+$. Found, %: C 62.99; H 3.97; N 10.37. $C_{35}H_{27}N_5O_8S$. Calculated, %: C 62.03; H 4.02; N 10.33.

6-{[3-(Pyridin-4-yl)isoxazol-5-yl]methoxy}-*N*-{4-[3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl]phenyl}-benzo[*d*]thiazol-2-amine (14g). Yield 56%, mp 255–257°C NMR spectrum, δ, ppm: 3.72 s (3H), 3.92 s (6H), 5.15 s (2H), 7.24 s (2H), 7.45 s (1H), 7.56 d (1H, J = 8.5 Hz), 7.65 d (1H, J = 8.5 Hz), 7.87 s (1H), 7.96 s (1H), 8.15 d (2H, J = 8.6 Hz), 8.27 d (2H, J = 8.6 Hz), 8.52 d (2H, J = 8.01 Hz), 8.78 d (2H, J = 8.01 Hz), 12.56 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.4, 61.8, 65.7, 96.4, 97.8, 107.5, 108.3, 115.3, 119.6, 120.5, 121.8, 126.5, 127.4, 130.5, 134.6, 137.3, 138.5, 142.6, 149.7, 152.4, 154.6, 155.7, 157.4, 160.5, 164.5, 165.8, 171.8. MS (ESI): 634 [M + H]$^+$. Found, %: C 64.39; H 4.25; N 11.10. $C_{34}H_{27}N_5O_6S$. Calculated, %: C 64.44; H 4.29; N 11.05.

6-{[3-(1*H*-Pyrrol-2-yl)isoxazol-5-yl]methoxy}-*N*-{4-[3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl]phenyl}-benzo[*d*]thiazol-2-amine (14h). Yield 60%, mp 258–260°C NMR spectrum, δ, ppm: 3.72 s (3H), 3.92 s (6H), 5.10 s (2H), 7.24 s (2H), 7.32 d (1H, J = 8.10 Hz), 7.39 t (1H), 7.44 s (1H), 7.52–7.59 m (2H), 7.66 d (1H, J = 8.4 Hz), 7.86 s (1H), 7.96 s (1H), 8.15 d (2H, J = 8.6 Hz), 8.27 d (2H, J = 8.6 Hz), 12.56 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.6, 61.8, 65.7, 94.5, 96.7, 107.3, 107.8, 113.4, 115.4, 116.7, 119.5, 120.4, 122.3, 126.5, 127.3, 129.5, 130.4, 134.2, 137.5, 142.4, 149.6, 150.3, 154.5, 157.3, 157.8, 164.5, 165.2, 171.7. MS (ESI): 622 [M + H]$^+$. Found, %: C 63.72; H 4.33; N 11.32. $C_{33}H_{27}N_5O_6S$. Calculated, %: C 63.76; H 4.38; N 11.27.

6-{[3-(Thiophen-2-yl)isoxazol-5-yl]methoxy}-*N*-{4-[3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl]phenyl}-benzo[d]thiazol-2-amine (14i). Yield 61%, mp 263–265°C NMR spectrum, δ, ppm: 3.72 s (3H), 3.92 s (6H), 5.11 s (2H), 7.24 s (2H), 7.32 t (1H), 7.38 d (1H, J = 8.3 Hz), 7.44 s (1H), 7.54–7.59 m (2H), 7.66 d (1H, J = 8.4 Hz), 7.86 s (1H), 7.96 s (1H), 8.15 d (2H, J = 8.6 Hz),

8.27 d (2H, J = 8.6 Hz), 12.56 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.5, 61.8, 65.7, 96.4, 97.3, 107.4, 107.8, 115.6, 119.4, 120.4, 125.2, 126.4, 127.5, 128.2, 130.2, 130.8, 134.5, 137.6, 139.6, 142.4, 149.6, 151.5, 154.3, 157.3, 157.6, 164.5, 165.2, 171.8. MS (ESI): 639 [M + H]$^+$. Found, %: C 62.01; H 4.05; N 8.82. $C_{33}H_{26}N_4O_6S_2$. Calculated, %: C 62.05; H 4.10; N 8.77.

6-{[3-(5-Bromothiophen-2-yl)isoxazol-5-yl]-methoxy}-*N*-{4-[3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl]phenyl}benzo[d]thiazol-2-amine (14j). Yield 59%, mp 270–272°C NMR spectrum, δ, ppm: 3.72 s (3H), 3.92 s (6H), 5.13 s (2H), 7.24 s (2H), 7.42–7.50 m (2H), 7.56 d (1H, J = 7.95 Hz), 7.66 d (1H, J = 8.5 Hz), 7.69 d (1H, J = 8.2 Hz), 7.87 s (1H), 7.96 s (1H), 8.15 d (2H, J = 8.7 Hz), 8.27 d (2H, J = 8.7 Hz), 12.56 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.6, 61.8, 65.8, 96.5, 97.2, 107.5, 107.9, 115.2, 115.7, 119.5, 120.7, 126.4, 127.4, 130.3, 130.7, 131.5, 134.5, 137.3, 138.6, 141.4, 149.7, 152.4, 154.5, 157.3, 157.8, 164.5, 165.6, 171.9. MS (ESI): 719 [M + H]$^+$. Found, %: C 55.19; H 3.47; N 7.86. $C_{33}H_{25}N_4O_6S_2Br$. Calculated, %: C 55.23; H 3.51; N 7.81.

MTT assay. Individual wells of a 96-well tissue culture micro titer plate were inoculated with 100 μL of complete medium containing 1×10^4 cells. The plates were incubated at 37°C in a humidified 5% CO_2 incubator for 18 h prior to the experiment. After medium removal, 100 μL of fresh medium containing the test compounds and etoposide at concentrations 0.5, 1 and 2 μM were added to each well and incubated at 37°C for 24 h. Then the medium was discarded and replaced with 10 μL MTT dye. Plates were incubated at 37°C for 2 h. The resulting formazan crystals were solubilized in 100 μL extraction buffer. The optical density was recorded at 570 nm with a micro plate reader (Multi-mode Varioskan Instrument-Themo Scientific). Percentage of DMSO in the medium never exceeded 0.25%.

Molecular docking. The DNA ligand structures were drawn using the build panel and prepared using Maestro. Energy minimization was carried out using OPLS-2005 forcefield. For docking, grid parameter file (.gpf) and docking parameter files (.dpf) were written using MGL Tools-1.5.6. Receptor grids were generated using 86×68×96 Grid points in xyz with grid spacing of 0.441 Å with grid centre, x = 30.911, y = 101.462, and z = 41.536. Grid box was generated by considering active site residues. Map types were generated using Autogrid 4.2. Docking was carried out with a number of runs: 50, population size: 150, number of evaluations: 2,500,000, and number of generations: 27,000, using Autodock 4.2. Analysis of docking results was done using MGL Tools-1.5.6. Top scoring molecule in the largest cluster was analysed for its interactions with the protein. The 2D interactions were generated from Proteinplus online pose viewer. The 3D interactions were obtained with Discovery studio Visualizer. The synthesized compounds were docked into the X-ray crystal structure of human topoisomerase IIbeta domain (PDB ID: 3QX3) for considering the possible target mechanism of action.

CONCLUSIONS

In summary, we have designed and synthesized a novel series of bis-isoxazole incorporated benzothiazole derivatives and tested those in vitro against human cancer cell lines such as breast MCF-7 and MDA MB-231, lungs A549, and prostate DU-145 using the MTT assay and etoposide as a standard drug. Most of the compounds have demonstrated good to moderate activity, and some of those have exhibited more potent activity than etoposide. According to molecular docking, the compounds can be characterized by high free energy of binding interactions with human topoisomerase II beta in complex with DNA.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

REFERENCES

1. Huang, S.T., Hsei, I.J., and Chen, C., *Bioorg. Med. Chem.*, 2006, vol. 14, p. 6106. https://doi.org/10.1016/j.bmc.2006.05.007
2. Singh, M., Singh, S.K., Gangwar, M., Nath, G., and Singh, S.K., *RSC Adv*., 2014, vol. 4, p. 19013. https://doi.org/10.1039/C4RA02649G
3. Akhtar, T., Hameed, S., Al-Masoudi, N., Loddo, R., and Colla, P., *Acta Pharm*., 2008, vol. 58, p. 135. https://doi.org/10.2478/v10007-008-0007-2
4. Siddiqui, N., Alam, M., and Siddiqui, A.A., *Asian J. Chem*., 2004, vol. 16, p. 1005.
5. Burger, A., and Sawhey, S.N., *J. Med. Chem*., 1968, vol. 11, p. 270. https://doi.org/10.1021/jm00308a018
6. Singh, S.P. and Segal, S., *Ind. J. Chem. B*, 1988, vol. 27, p. 941.

7. Suresh, C.H., Rao, J.V., Jayaveera, K.N., and Subudhi, S.K., *Int. J. Pharma*., 2013, vol. 2, p. 257.
8. Palmer, F.J., Trigg, R.B., and Warrington, J.V., *J. Med. Chem*., 1971, vol. 14, p. 248. https://doi.org/10.1021/jm00285a022
9. Chill, L., Rudi, A., Benayahu, Y., and Kashman, Y., *Tetrahedron Lett*., 2004, vol. 45, p. 7925. https://doi.org/10.1016/j.tetlet.2004.08.137
10. Hutchinson, I., Bradshaw, T.D., Matthews, C.S., Stevens, M.F., and Westwell, A. D., *Bioorg. Med. Chem. Lett.*, 2003, vol. 13, p. 471. https://doi.org/10.1016/S0960-894X(02)00930-7
11. Poma, P., Notarbartolo, M., Labbozzetta, M., Maurici, A., Carina, V., Alaimo, A., Rizzi, M., Simoni, D., and D'Alessandro, N., *Int. J. Mol. Med*., 2007, vol. 20, p. 329. https://doi.org/10.3892/ijmm.20.3.329
12. Musad, E.A., Mohamed, R., Ali Saeed, B., Vishwanath, B.S., and Lokanatha Rai, K.M., *Bioorganic Med. Chem. Lett*., 2011, vol. 21, p. 3536. https://doi.org/10.1016/j.bmcl.2011.04.142
13. Sreenatha, N.R., Lakshminarayana, B.N., Kumar, S.M., Prasad, T.N.M., Kiran, D.K.S., Vijayshankar, S., and Byrappa, K., *Chem. Data Collections*., 2017, vols. 11–12, p. 131. https://doi.org/10.1016/j.cdc.2017.09.001
14. Jensen, M.R., Schoepfer, J., and Radimerski, T., *Breast Cancer Res*., 2008, vol. 10, p. R33. https://doi.org/10.1186/bcr1996