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Design, Synthesis, and Anticancer Activity of 1,2,3-Triazole Likned Thiazole-1,2-isoxazole Derivatives

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Abstract—A series of novel 1,2,3-triazole linked thiazole-1,2-isoxazole derivatives has been designed, synthesized and characterized by ^{1}H and ^{13}C NMR, and mass spectral analysis. The compounds have been tested for their anticancer activity towards MCF-7 (breast cancer), A549 (lung cancer), Colo-205 (colon cancer), and A2780 (ovarian cancer) by using the MTT method using etoposide as the reference. Most of the tested compounds demonstrate good to moderate activity against all cell lines. The compounds **14b, 14e, 14g,** and **14h** are characterized by inhibitory activity stronger than that of etoposide.

Keywords: triazole, thiazole, isoxazole, MTT assay, anticancer activity

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INTRODUCTION

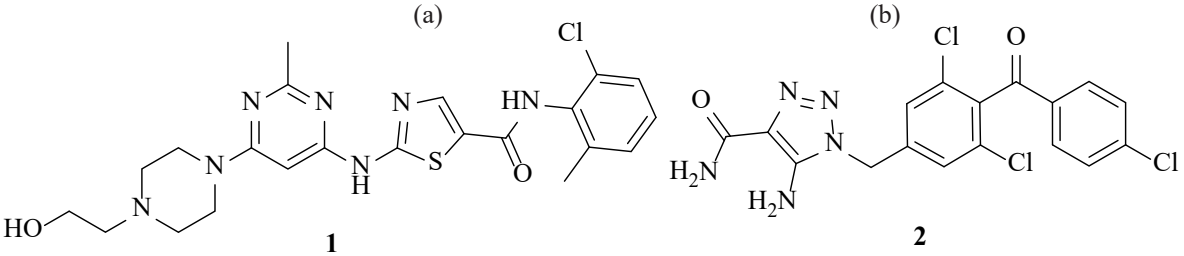
Thiazoles are characterized by a wide variety of biological activities including antitumour [1], antioxidant [2], antiviral [3], and many more. Thiazole makes a structural block of an US-FDA approved anticancer drug candidate Dasatinib (**1**, see the figure), used for treatment of acute lymphoblastic leukemia [4].

Similarly, 1,2,3-triazole derivatives exhibited numerous biological properties including anticancer [5], antifungal [6], antitubercular [7], and anti-HIV [8]. Triazole cycle is a fragment of molecular structure of the anticancer drug Carboxyamido (**2,** CAI) [9-11].

In view of the above as well as our recent studies [12], we have designed and synthesized novel 1,2,3-triazole linked thiazole-1,2-isoxazole derivatives **14a**–**14j** and characterized their structures by NMR and Mass spectral data. The synthesized compounds were tested for anticancer activity against a panel of human cancer cell lines.

RESULTS AND DISCUSSION

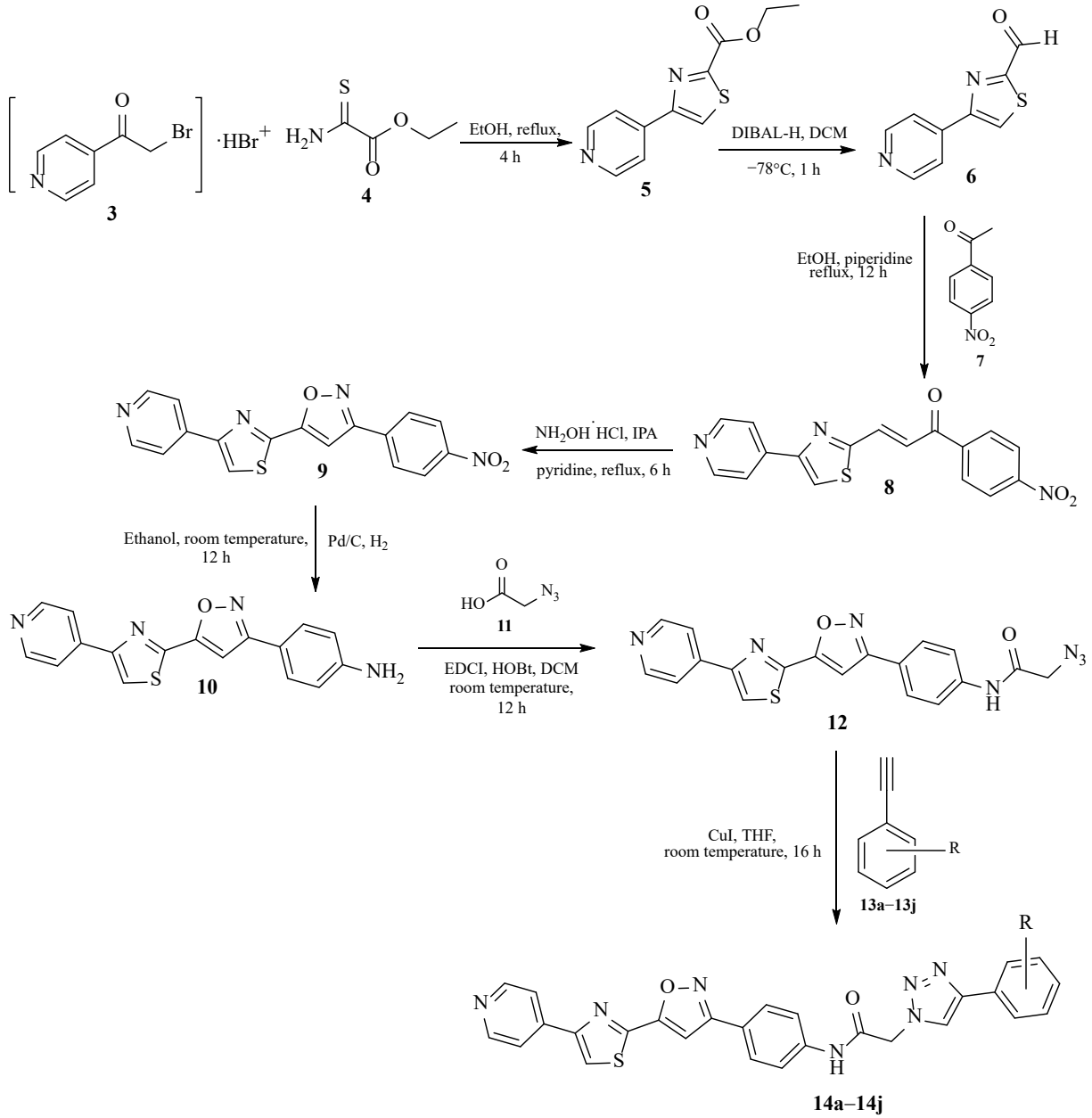
The synthetic approach to 1,2,3-triazole linked thiazole-1,2-isoxazole derivatives **14a**–**14j** is outlined in Scheme 1. Reaction of 2-bromo-1-(pyridin-4-yl)ethanone hydrobromide (**3**) with ethyl thiocarbamoylformate (**4**) gave ethyl 4-(pyridin-4-yl)thiazole-2-carboxylate (**5**). Its following reduction in presence of DBAL-H afforded the aldehyde **6**. The Claisen-Schmidt condensation of the intermediate **6** with 1-(4-nitrophenyl)ethanone (**7**) in



1 **2**

Structures of Dasatinib (a) **1** and (b) CAI **2**.

Scheme 1. Synthesis of the target 1,2,3-triazole linked thiazole-1,2-isoxazole derivatives.



R = H (**13a**, **14a**), 3,4,5-trimethoxy (**13b**, **14b**), 3,5-dimethoxy (**13c**, **14c**), 4-methoxy (**13d**, **14d**), 4-chloro (**13e**, **14e**), 4-bromo (**13f**, **14f**), 4-nitro (**13g**, **14g**), 3,5-dinitro (**13h**, **14h**), 4-methyl (**13i**, **14i**), 3,5-dimethyl (**13j**, **14j**).

presence of piperidine base led to chalcone **8,** which was subjected to cyclization with hydroxyl amine hydrochloride in pyridine with formation of isoxazole intermediate **9**. Reduction of compound **9** with $Pd/C,H_2$ led to the amino intermediate **10**, further coupling of which with 2-azidoacetic acid **11** in presence of EDCI, HOBt afforded azide **12**. Its following 1,3-dipolar cycloaddition with various substituted aryl alkynes **13a**–**13j** catalysed by CuI gave the corresponding target products **14a**–**14j**.

Biological tests. In vitro *cytotoxicity.* The newly prepared compounds **14a**–**14j** were tested in vitro against four human cancer cell lines, including MCF-7 (breast cancer), A549 (lung cancer), Colo-205 (colo cancer), and A2780 (ovarian cancer) by employing the MTT method

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

Compound	MCF-7	A549	Colo-205	A2780
14a	2.66±1.87	Not determined	2.88±1.23	Not determined
14b	1.55±0.83	1.95±0.32	0.38±0.063	1.10±0.72
14c	3.17±2.13	2.43±1.64	2.11±1.22	Not determined
14d	4.56±2.88	10.3±6.45	12.9±6.11	7.82±4.55
14e	1.66±0.19	1.87±0.22	0.93±0.061	1.34±0.89
14f	8.11±4.67	Not determined	Not determined	5.77±3.17
14g	0.83±0.018	0.19±0.093	0.11±0.045	1.23±0.77
14h	0.083±0.005	0.01±0.009	0.014±0.002	0.77±0.017
14i	9.61±5.44	2.04±1.09	18.5±7.12	Not determined
14j	16.4±4.33	10.22±7.56	6.32±3.90	19.5±5.13
Etoposide	2.11± 0.024	3.08± 0.135	0.13± 0.017	1.31± 0.27

[a] MCF-7: human breast cancer cell line. A549: human lung cancer cell line. Colo-205: human colon cancer cell line. A2780: human ovarian cancer cell line.

(see the table). Etoposide was used as a reference. The results indicated most of the tested products as highly to moderately active against all considered cell lines with IC_{50} values ranging from 0.01±0.009 to 19.5±5.13 μM, IC_{50} values for etoposide ranged within 0.13± 0.017 to 3.08±0.135 μM. The compounds **14b**, **14e**, **14g**, and **14h** demonstrated higher anticancer activity than etoposide against four cell lines. The compound **14h** exhibited the most promising activity. According to the preliminary structure-activity relationships (SARs) analysis of the compounds, presence of the electron-donating group (3,4,5-trimethoxyphenyl) (**14b**) resulted in its high anticancer activity. Presence of two 3,5-dimethoxyphenyl substituent in compound **14c** induced its deceased activity against four cell lines in comparison with **14b**. Moving along the same line, compound **14d** with the 4-methoxy substituent demonstrated even lower activity than **14c**. Replacement of the 4-methoxy group by 4-chloro (electron-withdrawing) substituent (**14e**) resulted in slightly improved activity of the product against four cell lines. The compound **14g** containing the strong electron-withdrawing group (4-nitro) on the phenyl ring was characterized by excellent activity. The compound **14h** with 3,5-dinitro substituents on the phenyl ring exhibited the highest anticancer activity against all cell lines.

EXPERIMENTAL

All chemicals and reagents were obtained from Sigma–Aldrich and Lancaster (Alfa Aesar) companies, and used without further purification. Reactions progress was monitored by TLC, performed on silica gel (60 F-254) coated glass plates, and visualized under UV light or by iodine indicator. ^1H and ^{13}C NMR spectra were measured on a BRUKER NMR (300 MHz, 400 MHz) spectrometer. 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. Melting points were determined on an electro thermal melting point apparatus, and are uncorrected.

Ethyl 4-(pyridin-4-yl)thiazole-2-carboxylate (5). A mixture of 4-bromoacetylpyridine hydrobromide **3** (10 g, 0.0533 mmol) with ethyl thiooxamate **4** (4.4 mL, 0.0355 mmol) in ethanol (40 mL) was refluxed upon stirring for 4 h. The reaction mixture was concentrated under reduced pressure, and water was added to the residue. pH Of the mixture was adjusted to 10 with 8 N NaOH aqueous solution at 0°C and extracted with chloroform. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford compound **5** as brown solid, yield 75%. ^1H NMR spectrum, δ, ppm: 1.48 t (3H), 4.53 q (2H), 7.85 d.d (2H, J = 4.4, 1.6 Hz), 7.98 s (1H), 8.71 d.d (2H, J = 4.4, 1.6 Hz). MS (ESI): m/z: 235 $[M + H]^+$.

4-(Pyridin-4-yl)thiazole-2-carbaldehyde (6). To a solution of ethyl 4-(pyridin-4-yl)thiazole-2-carboxylate **5** (7 g, 0.0299 mmol) in anhydrous THF (30 mL) was added DIBAL-H (9.5 mL, 0.5M, in THF–hexane, 0.0478 mmol) dropwise at –78°C, then the reaction mixture was stirred at the same temperature for 1 h. The reaction mixture was hydrolyzed with 1 N aq HCl (5 mL), and the prod-

uct was extracted with diethyl ether (10 mL). The ether layer was dried over anhydrous magnesium sulphate to afford the crude product which was purified by column chromatography with ethyl acetate/hexane (2:8) to afford pure compound **6,** yield 89%. ^{1}H NMR spectrum, δ, ppm: 7.96 d.d (2H, J = 4.5, 1.8 Hz), 8.10 s (1H), 8.80 d.d (2H, J = 4.5, 1.8 Hz), 9.23 s (1H). MS (ESI): *m/z*: 191 $[M + H]^+$.

(*E*)-1-(4-Nitrophenyl)-3-[4-(pyridin-4-yl)thiazol-2-yl]prop-2-en-1-one (8). To 4-(pyridin-4-yl)thiazole-2-carbaldehyde **6** (4.5 g, 0.0236 mmol) dissolved in 20 mL of ethanol, were added 4-nitroacetophenone **7** (3.9 g, 0.0236 mmol) and 3 drops of piperidine. The reaction mixture was refluxed for 12 h. After completion of reaction, cold water (20 mL) was added slowly. The crystalline precipitate was filtered off and purified by recrystallization from ethanol to afford pure compound **8**, yield 84%. ^{1}H NMR spectrum, δ, ppm: 7.66 d (2H, J = 7.8 Hz), 7.86 d.d (2H, J = 4.7, 1.9 Hz), 7.90 d (1H, J = 15.1 Hz), 7.95 d (1H, J = 15.1 Hz), 8.05 s (1H), 8.14 d (2H, J = 7.8 Hz), 8.78 d.d (2H, J = 4.7, 1.9 Hz). MS (ESI): *m/z*: 338 $[M + H]^+$.

4-{2-[3-(4-Nitrophenyl)isoxazol-5-yl]thiazol-4-yl}-pyridine (9). A mixture of compound **8** (6.3 g, 0.0187 mmol) with hydroxylamine hydrochloride (2 g, 0.0280 mmol) was dissolved in 20 mL of 2-propanol, then 2 mL of pyridine were added, and the reaction mixture was refluxed upon stirring for 6 h. After completion of the process (TLC), the solvent was evaporated under reduced pressure. The precipitated product was washed with water (3×20 mL), and the crude product was purified by column chromatography with ethyl acetate/hexane (3 : 7) to afford pure compound **9,** yield 80%. ^{1}H NMR spectrum, δ, ppm: 7.73 s (1H), 7.87 d.d (2H, J = 4.8, 1.7 Hz), 7.92 d (2H, J = 8.1 Hz), 8.10 s (1H, thio), 8.23 d (2H, J = 8.1 Hz), 8.79 d.d (2H, J = 4.8, 1.7 Hz). MS (ESI): *m/z*: 351 $[M + H]^+$.

4-{5-[4-(Pyridin-4-yl)thiazol-2-yl]isoxazol-3-yl}-benzenamine (10). To a solution of compound **9** (5 g, 0.0143 mmol) in 15 mL of ethanol were added 200 mg of 10% palladium on activated carbon. The reaction mixture was stirred at room temperature for 12 h under the atmosphere of hydrogen, then filtered, and the solvent was evaporated. The crude product was purified by column chromatography with ethyl acetate–hexane (6 : 4) to obtain pure compound **10**, yield 89%. ^{1}H NMR spectrum, δ, ppm: 6.92 br. s (2H), 7.38 d (2H, J = 7.5 Hz), 7.60 s (1H), 7.85 d.d (2H, J = 4.6, 1.5 Hz), 7.89 d (2H, J = 7.5 Hz), 8.08 s (1H, thio), 8.75 d.d (2H, J = 4.6, 1.5 Hz). MS (ESI): *m/z*: 321 $[M + H]^+$.

2-Azido-*N*-(4-{5-[4-(pyridin-4-yl)thiazol-2-yl]-isoxazol-3-yl}phenyl)acetamide (12). Compounds **10** (3.8 g, 0.0119 mmol), EDCI (2.8 g, 0.0178 mmol) and HOBt (911 mg, 0.00595 mmol) were dissolved in 20 mL of anhydrous dichloromethane and stirred at 0°C for 1 h. 2-Azidoacetic acid (**11**) (1.1 mL, 0.0143 mmol) was then added to the mixture and stirred at room temperature for 12 h. After completion of the reaction, the mixture was washed with saturated sodium bicarbonate solution. The organic layer was evaporated under reduced pressure to afford pure compound **12**, yield 81%. ^{1}H NMR spectrum, δ, ppm: 4.93 s (2H), 7.56 d (2H, J = 7.7 Hz), 7.65 s (1H), 7.87 d.d (2H, J = 4.7, 1.6 Hz), 8.10 s (1H), 8.15 d (2H, J = 7.7 Hz), 8.77 d.d (2H, J = 4.7, 1.6 Hz), 12.23 br. s (1H). MS (ESI): *m/z*: 404 $[M + H]^+$.

2-(4-Phenyl-1*H*-1,2,3-triazol-1-yl)-*N*-{4-(5-[4-(pyridin-4-yl)thiazol-2-yl]isoxazol-3-yl)phenyl}acetamide (14a). Azide **12** (200 mg, 4.9 mmol) and 1-ethynylbenzene (**13a**) (0.33 mL, 4.9 mmol) were dissolved in anhydrous THF (20 mL), and CuI (466 mg, 5 mol %, 2.45 mmol) was added to it. The mixture was stirred vigorously at room temperature for 16 h. Upon completion of the process (THF), the solvent was evaporated in vacuum, and the crude product was re-dissolved in ethyl acetate (3×30 mL). The organic phase was washed with water and dried over with Na_2SO_4. After removal of the solvent in vacuum, the crude product was purified by column chromatography with ethyl acetate–hexane (6 : 4) to obtained pure compound **14a**. Yield 57%, mp 244–246°C. ^{1}H NMR spectrum, δ, ppm: 7.55–7.69 m (6H), 7.76 d (2H, J = 8.2 Hz), 7.87 d.d (2H, J = 4.7, 1.6 Hz), 7.94 d (2H, J = 8.2 Hz), 8.10 s (1H), 8.34 s (1H), 8.76 d.d (2H, J = 4.7, 1.6 Hz), 12.28 br. s (1H). ^{13}C NMR spectrum, δ, ppm: 52.5, 104.6, 116.4, 121.5, 122.3, 123.5, 126.8, 128.3, 128.7, 129.5, 129.8, 131.4, 139.2, 140.4, 142.3, 143.6, 150.5, 152.4, 159.7, 160.5, 170.5. MS (ESI): *m/z*: 506 $[M + H]^+$.

The compounds **14b**–**14j** were synthesized according to the above method presented for **14a** from the corresponding intermediates **13b**–**13j**.

2-[4-(3,4,5-Trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl]-*N*-(4-{5-[4-(pyridin-4-yl)thiazol-2-yl]isoxazol-3-yl}-phenyl)acetamide (14b). Yield 45%, mp 276-278°C. ^{1}H NMR spectrum, δ, ppm: 3.89 s (3H), 3.92 s (6H), 5.03 s (2H), 7.42 s (2H), 7.68 s (1H), 7.75 d (2H, J = 8.05 Hz), 7.88 d.d (2H, J = 4.8, 1.6 Hz), 7.92 d (2H, J = 8.05 Hz), 8.11 s (1H), 8.32 s (1H), 8.75 d.d (2H, J = 4.8, 1.6 Hz), 12.26 br. s (1H). ^{13}C NMR spectrum, δ, ppm: 52.4, 57.8,

61.6, 104.5, 110.5, 116.7, 121.4, 122.7, 123.6, 127.6, 128.3, 129.4, 139.2, 140.3, 142.6, 144.9, 145.7, 150.3, 152.4, 155.6, 159.3, 160.4, 170.6. MS (ESI): *m/z*: 596 $[M + H]^+$.

2-[4-(3,5-Dimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl]-*N*-(4-{5-[4-(pyridin-4-yl)thiazol-2-yl]isoxazol-3-yl}-phenyl)acetamide (14c). Yield 53%, mp 288–290°C. ^{1}H NMR spectrum, δ, ppm: 3.78 s (6H), 4.98 s (2H), 6.89 s (1H), 7.33 s (2H), 7.67 s (1H), 7.72 d (2H, *J* = 8.08 Hz), 7.88 d.d (2H, *J* = 4.8, 1.6 Hz), 7.91 d (2H, *J* = 8.08 Hz), 8.11 s (1H), 8.33 s (1H), 8.76 d.d (2H, *J* = 4.8, 1.6 Hz), 12.29 (br. s, 1H). ^{13}C NMR spectrum, δ, ppm: 52.6, 57.8, 99.6, 104.5, 110.3, 116.7, 121.4, 122.5, 123.7, 128.2, 129.1, 132.4, 139.7, 140.2, 142.4, 145.6, 150.3, 152.6, 159.2, 160.6, 163.5, 170.6. MS (ESI): *m/z*: 566 $[M + H]^+$.

2-[4-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-1-yl]-*N*-(4-{5-[4-(pyridin-4-yl)thiazol-2-yl]isoxazol-3-yl}-phenyl)acetamide (14d). Yield 53%, mp 268–270°C. ^{1}H NMR spectrum, δ, ppm: 3.79 s (3H), 5.09 s (2H), 7.14 d (2H, *J* = 7.5 Hz), 7.66 s (1H), 7.70 d (2H, *J* = 8.10 Hz), 7.88 d.d (2H, *J* = 4.8, 1.6 Hz), 7.92 d (2H, *J* = 8.10 Hz), 8.11 s (1H), 8.34 s (1H), 8.76 d.d (2H, *J* = 4.8, 1.6 Hz), 12.32 br. s (1H). ^{13}C NMR spectrum, δ, ppm: 52.7, 57.8, 104.6, 115.6, 116.8, 121.5, 122.3, 123.7, 124.2, 127.6, 128.7, 129.2, 139.3, 140.4, 142.3, 143.8, 150.4, 152.4, 159.6, 160.5, 160.9, 170.6. MS (ESI): *m/z*: 536 $[M + H]^+$.

2-[4-(4-Chlorophenyl)-1*H*-1,2,3-triazol-1-yl]-*N*-(4-{5-[4-(pyridin-4-yl)thiazol-2-yl]isoxazol-3-yl}phenyl)-acetamide (14e). Yield 70%, mp 258–260°C. ^{1}H NMR spectrum, δ, ppm: 5.24 s (2H), 7.42 d (2H, *J* = 7.7 Hz), 7.68 s (1H), 7.71–7.83 m (4H), 7.89 d.d (2H, *J* = 4.9, 1.7 Hz), 7.94 d (2H, *J* = 8.11 Hz), 8.11 s (1H), 8.36 s (1H), 8.77 d.d (2H, *J* = 4.9, 1.7 Hz), 12.39 br. s (1H). ^{13}C NMR spectrum, δ, ppm: 52.6, 104.8, 117.8, 121.5, 122.7, 123.4, 129.3, 129.7, 130.4, 131.7, 131.9, 133.4, 139.7, 140.6, 142.5, 143.8, 150.7, 152.8, 159.6, 160.7, 171.4. MS (ESI): *m/z*: 540 $[M + H]^+$.

2-[4-(4-Bromophenyl)-1*H*-1,2,3-triazol-1-yl]-*N*-(4-{5-[4-(pyridin-4-yl)thiazol-2-yl]isoxazol-3-yl}phenyl)-acetamide (14f). Yield 45%, mp 282–284°C. ^{1}H NMR spectrum, δ, ppm: 5.20 s (2H), 7.39 d (2H, *J* = 7.6 Hz), 7.67 s (1H), 7.70–7.82 m (4H), 7.88 d.d (2H, *J* = 4.6, 1.5 Hz), 7.93 d (2H, *J* = 8.10 Hz), 8.11 s (1H), 8.35 s (1H), 8.75 d.d (2H, *J* = 4.6, 1.5 Hz), 12.37 br. s (1H). ^{13}C NMR spectrum, δ, ppm: 52.7, 104.7, 117.6, 121.5, 122.6, 122.9, 123.8, 129.6, 129.8, 130.3, 130.5, 133.6, 139.6, 140.5, 142.5, 143.6, 150.7, 152.3, 159.7, 160.6, 171.5. MS (ESI): *m/z*: 586 $[M + H]^+$.

2-[4-(4-Nitrophenyl)-1*H*-1,2,3-triazol-1-yl]-*N*-(4-{5-[4-(pyridin-4-yl)thiazol-2-yl]isoxazol-3-yl}phenyl)-acetamide (14g). Yield 70%, mp 310–312°C. ^{1}H NMR spectrum, δ, ppm: 5.32 s (2H), 7.48 d (2H, *J* = 7.8 Hz), 7.69 s (1H), 7.74 d (2H, *J* = 8.12 Hz), 7.88 d.d (2H, *J* = 4.8, 1.6 Hz), 7.93 d (2H, *J* = 8.12 Hz), 8.11 s (1H), 8.37 s (1H), 8.54 d (2H, *J* = 7.8 Hz), 8.77 d.d (2H, *J* = 4.8, 1.6 Hz), 12.41 br. s (1H). ^{13}C NMR spectrum, δ, ppm: 52.7, 104.7, 118.5, 121.5, 122.7, 123.8, 126.8, 129.5, 130.2, 131.7, 135.7, 139.6, 140.2, 142.5, 143.2, 147.8, 159.2, 152.8, 159.6, 160.5, 171.7. MS (ESI): *m/z*: 551 $[M + H]^+$.

2-[4-(3,5-Dinitrophenyl)-1*H*-1,2,3-triazol-1-yl]-*N*-(4-{5-[4-(pyridin-4-yl)thiazol-2-yl]isoxazol-3-yl}-phenyl)acetamide (14h). Yield 59%, mp 325–327°C. ^{1}H NMR spectrum, δ, ppm: 5.45 s (2H), 7.72 s (1H), 7.76 d (2H, *J* = 8.16 Hz), 7.88 d.d (2H, *J* = 4.8, 1.6 Hz), 7.95 d (2H, *J* = 8.16 Hz), 8.12 s (1H), 8.41 s (1H), 8.63 s (2H), 8.77 d.d (2H, *J* = 4.8, 1.6 Hz), 9.23 s (1H), 12.53 br. s (1H). ^{13}C NMR spectrum, δ, ppm: 52.8, 104.7, 117.6, 118.7, 121.5, 122.6, 123.8, 128.5, 129.7, 133.6, 133.9, 139.3, 140.5, 142.9, 147.5, 147.9, 150.4, 152.3, 159.7, 160.8, 171.8. MS (ESI): *m/z*: 596 $[M + H]^+$.

***N*-(4-{5-[4-(Pyridin-4-yl)thiazol-2-yl]isoxazol-3-yl}-phenyl)-2-(4-p-tolyl-1*H*-1,2,3-triazol-1-yl)acetamide (14i).** Yield 52%, mp 274–276°C. ^{1}H NMR spectrum, δ, ppm: 2.48 s (3H), 5.12 s (2H), 7.45–7.58 m (4H), 7.67 s (1H), 7.71 d (2H, *J* = 8.10 Hz), 7.88 d.d (2H, *J* = 4.8, 1.6 Hz), 7.91 d (2H, *J* = 8.10 Hz), 8.12 s (1H), 8.33 s (1H), 8.77 d.d (2H, *J* = 4.8, 1.6 Hz), 12.36 br. s (1H). ^{13}C NMR spectrum, δ, ppm: 24.8, 52.6, 104.6, 116.7, 121.6, 122.5, 123.2, 126.7, 127.8, 128.4, 129.7, 130.6, 138.5, 139.7, 140.4, 142.6, 143.8, 150.6, 152.4, 159.6, 160.7, 170.5. MS (ESI): *m/z*: 520 $[M + H]^+$.

2-[4-(3,5-Dimethylphenyl)-1*H*-1,2,3-triazol-1-yl]-*N*-(4-{5-[4-(pyridin-4-yl)thiazol-2-yl]isoxazol-3-yl}-phenyl)acetamide (14j). Yield 62%, mp 269–271°C. ^{1}H NMR spectrum, δ, ppm: 2.49 s (6H), 5.10 s (2H), 7.19 s (2H), 7.38 s (1H), 7.66 s (1H), 7.70 d (2H, *J* = 8.09 Hz), 7.88 d.d (2H, *J* = 4.8, 1.6 Hz), 7.90 d (2H, *J* = 8.09 Hz), 8.12 s (1H), 8.31 s (1H), 8.77 d.d (2H, *J* = 4.8, 1.6 Hz), 12.32 br. s (1H). ^{13}C NMR spectrum, δ, ppm: 26.8, 52.6, 104.6, 116.7, 121.6, 122.8, 123.5, 128.6, 129.4, 129.8, 131.5, 135.4, 139.5, 140.5, 141.3, 142.6, 146.5, 150.7, 152.6, 159.7, 160.5, 170.4. MS (ESI): *m/z*: 534 $[M + H]^+$.

MTT assay. All data are presented as mean ±S.D values. The experiments were performed in triplicates. Individual wells of a 96-well tissue culture micro titer plate were inoculated with 100 µL of complete me-

dium 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 removal of the medium, 100 μL of fresh medium containing the test compounds and etoposide (Eto) at different 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 (O.D) was recorded at 570 nm on a micro plate reader (Multi-mode Varioskan Instrument-Themo Scientific). The percentage of DMSO in the medium never exceeded 0.25%.

CONCLUSIONS

In conclusion, a series of novel 1,2,3-triazole linked thiazole-1,2-isoxazole derivatives **14a**–**14j** have been designed, synthesized and characterized by NMR and Mass spectral analysis. The compounds have been tested for their anticancer activity towards MCF-7 (breast cancer), A549 (lung cancer), Colo-205 (colon cancer), and A2780 (ovarian cancer) by the MTT method. Etoposide has been used as a reference. The accumulated results indicate that most of the tested compounds demonstrate good to moderate activity against all cell lines with IC_{50} values ranging from 0.01±0.009 to 19.5±5.13 μM (etoposide IC_{50} range from 0.13±0.017 to 3.08±0.135 μM). The compounds **14b, 14e, 14g,** and **14h** demonstrate stronger inhibitory activity than etoposide against four cell lines.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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