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Synthesis and Anticancer Activity of Amide Derivatives of 1,2-Isoxazole Combined 1,2,4-Thiadiazole

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Abstract—A series of amide derivatives of 1,2-isoxazole combined with 1,2,4-thiadiazole are synthesized **11a–11j**. Their chemical structures are confirmed by ^{1}H and ^{13}C NMR, and mass spectra. The products are tested for their anticancer activity against four types of human cancer cell lines, including MCF-7 (breast), A549 (lung), Colo-205 (colon), and A2780 (ovarian). Etoposide is used as a positive control. Most of the compounds show good anticancer activity. The compounds **11b, 11c, 11d, 11e, 11g,** and **11j** demonstrate more potent activity than etoposide.

Keywords: Luminespib, Cefozopram, isoxazole, thiadiazole, anticancer activity

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INTRODUCTION

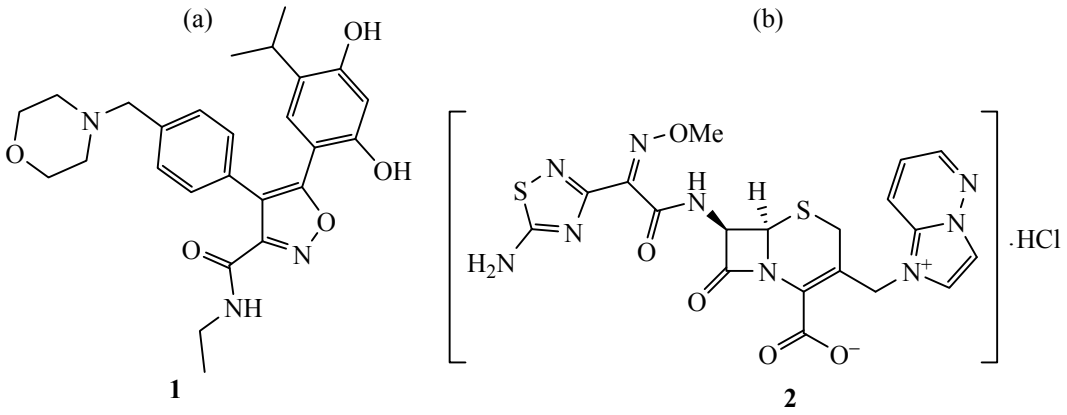
Different types of heterocyclic derivatives are used efficiently in anticancer chemotherapy [1–14]. Isoxazole derivatives are used extensively as agrochemicals and in medicine [15–18] due to a broad spectrum of activity, including anticancer [19], antifungal [20], anti-inflammatory [21], and antimicrobial [22]. Among these, Luminespib (**1**, NVP-AUY922) (see figure) is an FDA approved anticancer drug candidate. Thiadiazole derivatives are important functional components of molecules of many natural compounds [23] and drugs, for example, such as antibiotic Cefozopram (**2**) (see figure) which is used for treatment of CNS [24].

Based on the above information accumulated for isoxazole and thiadiazole and in continuation of our studies of heterocyclic compounds, we designed and synthesized a series of amide derivatives of 1,2-isoxazole combined with 1,2,4-thiadiazole **11a**–**11j**. Their structures were confirmed by ^{1}H and ^{13}C NMR and mass spectra. The compounds were tested for anticancer activity against four human cancer cell lines.

RESULTS AND DISCUSSION

Synthetic approach to the target compounds **11a**–**11j** (Scheme 1) started with introduction of compound **3** in the Claisen-Schmidt reaction with 4-cyano-benzaldehyde **4** which led to pure chalcone **5** with good yield. The following reaction of the intermediate **5** with 4-nitrobenzothioamide **6** in presence of $AlCl_3$ gave the product of cycloaddition **7**, which reacted with hydroxylamine hydrochloride to give isoxazole derivative **8**. The following reduction of compound **8** with Zn-dust in acetic acid with formation of amine **9**, and reaction of the latter with aromatic chloroanhydrides **10a**–**10j** led to the title compounds **11a**–**11j**.

Biological evaluation. *In vitro cytotoxicity.* The synthesized compounds **11a**–**11j** were screened for their anticancer activity against four human cancer cell lines such as MCF-7 (breast), A549 (lung), Colo-205 (colon), and A2780 (ovarian) by the MTT assay (see the table). Etoposide was used as a positive control. The products **11b**, **11c**, **11d**, **11e**, **11g**, and **11j** displayed higher activity than etoposide. The com-



1 **2**

Structures of (a) Luminespib and (b) Cefozopram.

pounds were examined for structure-activity relationship (SAR). The compound **11b** containing 3,4,5-trimethoxy substituents on the phenyl ring demonstrated high activity with IC_{50} values MCF-7 = 0.24±0.089 μM, A549 = 0.18±0.023 μM, Colo-205 = 0.11±0.05 μM, and A2780 = 0.55±0.072 μM, respectively. Compound **11c** with 3,5-dimethoxy substituents displayed lower activity (MCF-7 = 0.39±0.033, A549 = 1.33±0.45, Colo-205 = 0.93±0.065, and A2780 = 1.37±0.35 μM) than **11b**. The compound **11d** containing one 4-methoxy substituent exhibited the activity lower than the above two analogues. Replacement of 4-methoxy group by 4-chloro substituent on the phenyl ring **11e** resulted in its poorest activity. The compound **11g** with the 4-nitro group exhibited the highest activity. The compound **11h** with 3,5-dinitro substitution exhibited very poor activity. Compounds **11f** and **11i** with the 4-cyano substituent was of moderate activity.

EXPERIMENTAL

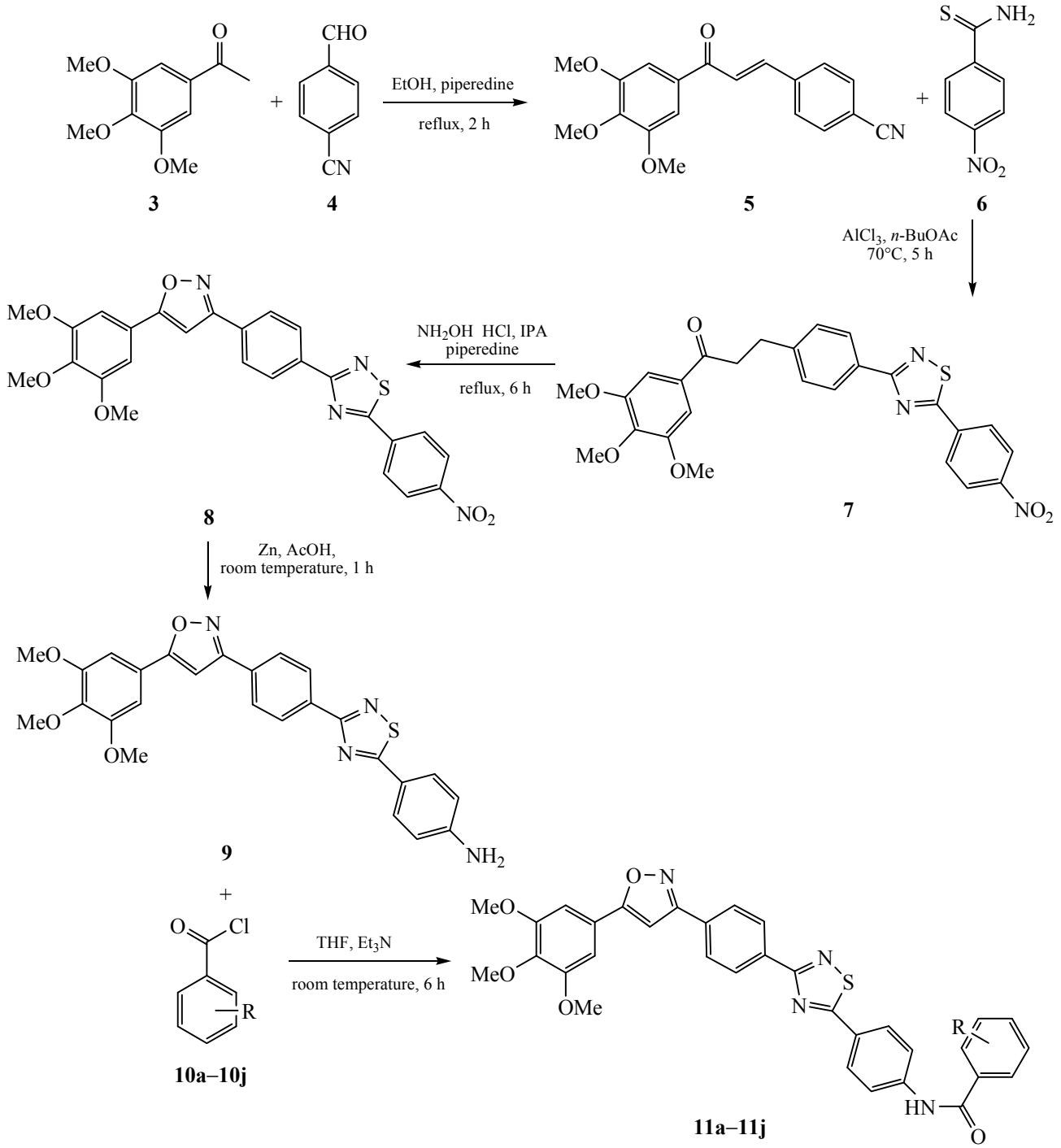
All chemicals and reagents were obtained from Aldrich (Sigma-Aldrich, St. Louis, MO, USA) and Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA), and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and visualized by UV light or iodine indicator. ^{1}H and ^{13}C NMR spectra were measured on a Gemini Varian-VXR-unity (300 MHz) spectrometer using DMSO-d_6 as a solvent ($CDCl_3$ for 5) and TMS as the internal standard. ESI spectra were recorded on a Micro mass,

Anticancer activity of the synthesized compounds **11a**–**11j** (IC_{50} μM)[a]

Compound	MCF-7[b]	A549[c]	Colo-205[d]	A2780[e]
11a	2.090±1.87	3.410±1.930	Not active	4.55±2.330
11b	0.240±0.089	0.180±0.023	0.11±0.050	0.55±0.072
11c	0.390±0.033	1.330±0.450	0.93±0.065	1.37±0.350
11d	1.990±0.540	1.830±0.560	1.44±0.880	2.43±1.880
11e	2.440±1.900	1.760±0.190	3.65±1.980	Not active
11f	4.110±2.400	9.670±5.100	Not active	8.34±5.090
11g	0.034±0.004	0.011±0.001	1.23±0.480	0.33±0.022
11h	2.170±1.230	2.880±1.990	7.33±4.100	5.60±4.300
11i	10.40±6.330	3.190±2.150	13.2±7.230	6.23±5.770
11j	1.460±0.320	1.670±0.450	1.42±0.360	Not active
Etoposide	2.110±0.024	3.080±0.135	0.13±0.017	1.31±0.270

[a] Each data is represented as mean ±S.D. of different experiments performed in triplicates. [b] (MCF-7) human breast cancer cell line. [c] (A549) human lung cancer cell line. [d] (Colo-205) human colon cancer cell line. [e] (A2780) human ovarian cancer cell line.

Scheme 1. Synthesis of amide derivatives of 1,2-isoxazole combined with 1,2,4-thiadiazole.



R = H (**10a, 11a**), 3,4,5-trimethoxy (**10b, 11b**), 3,5-dimethoxy (**10c, 11c**), 4-methoxy (**10d, 11d**), 4-cloro (**10e, 11e**), 4-bromo (**10f, 11f**), 4-nitro (**10g, 11g**), 3,5-dinitro (**10h, 11h**), 4-cyano, (**10i, 11i**), 4-methyl (**10j, 11j**).

Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. Melting points were determined on an electrothermal melting point apparatus, and are uncorrected.

4-[(*E*)-3-(3,4,5-Trimethoxyphenyl)-3-oxoprop-1-enyl]benzonitrile (5). 3,4,5-Trimethoxyacetophenone **3** (20 g, 0.0951 mmol) was dissolved in 50 mL of ethanol, followed by addition of 4-cyanobenzaldehyde

4 (12.5 g, 0.0951 mmol) and 3 drops of piperdine base. The reaction mixture was refluxed for 2 h. After cooling the reaction mixture down, water (20 mL) was added slowly to it. The crystalline precipitate was filtered off and purified by column chromatography using ethyl acetate–hexane (1 : 1) as an eluent to afford pure compound **5**. Yield 73%. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.96 s (6H), 7.28 s (2H), 7.55 d (1H, J = 15.5 Hz), 7.68 d (2H, J = 7.23 Hz), 7.73 d (2H, J = 7.23 Hz), 7.78 d (1H, J = 15.5 Hz). MS (ESI): 325 $[M + H]^+$.

(*E*)-1-(3,4,5-Trimethoxyphenyl)-3-{4-[5-(4-nitrophenyl)-1,2,4-thiadiazol-3-yl]phenyl}prop-2-en-1-one (7). 4-[(*E*)-3-(3,4,5-Trimethoxyphenyl)-3-oxoprop-1-enyl]benzonitrile (**5**) (20 g, 0.0617 mmol) and $AlCl_3$ (8.2 g, 0.0617 mmol) were mixed in *n*-butyl acetate (50 mL), and then, upon stirring at 70°C, 4-nitrobenzothioamide **6** (3.9 mL, 0.030 mmol) was added dropwise. The mixture was stirred at 70°C for 5 h. After cooled down to room temperature and addition of 0.3 mL of water the reaction mixture was stirred at room temperature for 24 h. The organic and aqueous layers were separated, and the aqueous layer was extracted with ethyl acetate (3×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography on silica gel, using ethyl acetate–petroleum ether (1 : 1) as an eluent to afford pure compound **7**. Yield 70%. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.96 s (6H), 7.28 s (2H), 7.57 d (1H, J = 15.6 Hz), 7.70 d (2H, J = 7.25 Hz), 7.75 d (2H, J = 7.25 Hz), 7.78 d (1H, J = 15.6 Hz), 7.83 d (2H, J = 7.27 Hz), 8.10 d (2H, J = 7.27 Hz). MS (ESI): 505 $[M + H]^+$.

3-{4-[5-(3,4,5-Trimethoxyphenyl)isoxazol-3-yl]-phenyl}-5-(4-nitrophenyl)-1,2,4-thiadia zole (8). A mixture of compound 7 (20 g, 0.039 mmol) with hydroxylamine hydrochloride (8.2 g, 0.117 mmol) was dissolved in 50 mL of 2-propanol, then 3 mL of pyridine were added and the reaction mixture was stirred upon refluxing for 6 h. After completion of reaction, according to TLC, the solvent was evaporated under reduced pressure. The precipitated product was washed with water (3×20 mL) and purified by column chromatography using ethyl acetate–hexane (7 : 3) as an eluent to afford pure compound **8**. Yield 70%. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.96 s (6H), 6.66 s (1H), 7.27 s (2H), 7.71 d (2H, J = 7.26 Hz), 7.77 d (2H, J = 7.26 Hz), 7.82 d (2H, J = 7.28 Hz), 8.11 d (2H, J = 7.28 Hz). MS (ESI): 518 $[M + H]^+$.

4-{3-(4-[5-(3,4,5-Trimethoxyphenyl)isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}benzenamine (9). To a solution of (*E*)-1-(3,4,5-trimethoxyphenyl)-3-{4-[5-(4-nitrophenyl)-1,2,4-thiadiazol-3-yl]phenyl}prop-2-en-1-one (**8**) (13 g, 0.0251 mmol) in acetic acid (40 mL) was added zinc powder (2.3 g, 0.0751 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the process, according to TLC, the reaction mixture was filtered (Celite), and the filtrate was evaporated to dryness giving pure compound **9**. Yield 76%. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.96 s (6H), 5.51 br.s (2H), 6.66 s (1H), 7.28 s (2H), 7.70 d (2H, J = 7.25 Hz), 7.76 d (2H, J = 7.25 Hz), 7.80 d (2H, J = 7.27 Hz), 8.09 d (2H, J = 7.27 Hz). MS (ESI): 488 $[M + H]^+$.

General method of synthesis of amide derivatives 11a–11j. The compound 9 (500 mg, 0.0010 mmol) was dissolved in 10 mL of dry THF, and 0.0010 mmol of one of benzoyl chlorides **10a**–**10j** and 0.002 mmol of Et_3N were added. The reaction mixture was stirred at room temperature for 6 h, till completion of the process (TLC), then it was washed with water and extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4. The crude product was purified by column chromatography using ethyl acetate–hexane (1 : 1) as an eluent to obtain the corresponding pure compound **11a**–**11j**.

***N*-(4-{3-(4-[5-(3,4,5-Trimethoxyphenyl)isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}phenyl)benzamide (11a).** Yield 51%, mp 300–302°C. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.96 s (6H), 6.66 s (1H), 7.28 s (2H), 7.52 t (1H), 7.56–7.66 m (2H), 7.70 d (2H, J = 7.24 Hz), 7.75 d (2H, J = 7.24 Hz), 7.78–7.88 m (4H), 8.07 d (2H, J = 7.26 Hz), 8.57 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.4, 61.8, 96.4, 106.3, 123.8, 126.5, 128.3, 129.4, 129.7, 130.4, 131.2, 132.7, 133.4, 134.7, 140.5, 145.3, 156.7, 158.6, 159.5, 160.7, 168.4, 170.4. MS (ESI): 592 $[M + H]^+$.

3,4,5-Trimethoxy-*N*-(4-{3-(4-[5-(3,4,5-trimethoxyphenyl)isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}-phenyl)benzamide (11b). Yield 47%, mp 317–319°C. ^{1}H NMR spectrum, δ, ppm: 3.87 s (6H), 3.90 s (3H), 3.93 s (3H), 3.96 s (6H), 6.65 s (1H), 7.27 s (2H), 7.32 s (2H), 7.69 d (2H, J = 7.23 Hz), 7.76 d (2H, J = 7.23 Hz), 7.80 d (2H, J = 7.25 Hz), 8.08 d (2H, J = 7.25 Hz), 8.56 s (1H). ^{13}C NMR spectrum, δ, ppm: 56.5, 57.8, 61.2, 62.5, 96.3, 106.4, 107.8, 123.4, 126.5, 127.6, 129.8, 130.4, 130.8, 131.5, 133.6, 134.6, 139.6, 143.2, 145.6, 156.3, 157.8, 158.2, 160.5, 163.2, 169.3, 170.6. MS (ESI): 682 $[M + H]^+$.

3,5-Dimethoxy-*N*-(4-{3-(4-[5-(3,4,5-trimethoxyphenyl)isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}phenyl)benzamide (11c). Yield 62%, mp 312–314°C. ^{1}H NMR spectrum, δ, ppm: 3.77 s (6H), 3.92 s (3H), 3.95 s (6H), 6.66 s (1H), 7.27 s (2H), 7.30 s (2H), 7.68 d (2H, *J* = 7.24 Hz), 7.77 d (2H, *J* = 7.24 Hz), 7.81 d (2H, *J* = 7.26 Hz), 8.09 d (2H, *J* = 7.26 Hz), 8.57 s (1H). ^{13}C NMR spectrum, δ, ppm: 56.4, 57.3, 61.9, 96.4, 106.3, 107.4, 118.4, 123.4, 126.7, 127.4, 129.6, 130.4, 130.8, 133.5, 134.5, 135.2, 139.6, 145.6, 156.4, 158.6, 160.4, 161.5, 164.7, 169.5, 170.7. MS (ESI): 652 $[M + H]^+$.

4-Methoxy-*N*-(4-{3-(4-[5-(3,4,5-trimethoxyphenyl)-isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}phenyl)-benzamide (11d). Yield 59%, mp 307–309°C. ^{1}H NMR spectrum, δ, ppm: 3.79 s (3H), 3.93 s (3H), 3.95 s (6H), 6.65 s (1H), 7.27 s (2H), 7.55 d (2H, *J* = 7.20 Hz), 7.69 d (2H, *J* = 7.25 Hz), 7.73–7.80 m (4H), 7.84 d (2H, *J* = 7.27 Hz), 8.09 d (2H, *J* = 7.27 Hz), 8.57 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.4, 58.3, 61.8, 96.4, 106.3, 115.7, 123.4, 126.5, 128.6, 129.5, 130.4, 130.7, 131.6, 132.4, 133.6, 134.2, 140.7, 145.6, 156.3, 158.7, 160.3, 161.5, 164.6, 169.6, 170.8. MS (ESI): 622 $[M + H]^+$.

4-Chloro-*N*-(4-{3-(4-[5-(3,4,5-trimethoxyphenyl)-isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}phenyl)-benzamide (11e). Yield 70%, mp 288–290°C. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.95 s (6H), 6.66 s (1H), 7.27 s (2H), 7.63 d (2H, *J* = 7.30 Hz), 7.68 d (2H, *J* = 7.26 Hz), 7.70–7.81 m (4H), 7.86 d (2H, *J* = 7.28 Hz), 8.10 d (2H, *J* = 7.28 Hz), 8.58 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.6, 61.8, 96.4, 106.4, 123.7, 126.4, 127.6, 129.7, 130.6, 131.3, 132.7, 133.4, 134.2, 134.7, 135.2, 140.3, 140.7, 145.4, 156.4, 158.3, 160.5, 161.6, 169.6, 170.7. MS (ESI): 626 $[M + H]^+$.

4-Bromo-*N*-(4-{3-(4-[5-(3,4,5-trimethoxyphenyl)-isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}phenyl)-benzamide (11f). Yield 66%, mp 276–278°C. ^{1}H NMR spectrum, δ, ppm: 3.92 s (3H), 3.96 s (6H), 6.67 s (1H), 7.26 s (2H), 7.65 d (2H, *J* = 7.31 Hz), 7.69 d (2H, *J* = 7.27 Hz), 7.72–7.83 m (4H), 7.87 d (2H, *J* = 7.29 Hz), 8.10 d (2H, *J* = 7.29 Hz), 8.58 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.3, 61.8, 96.5, 106.4, 123.4, 124.6, 126.5, 129.5, 130.3, 130.8, 131.4, 131.7, 133.4, 134.7, 135.4, 135.8, 140.4, 145.6, 156.3, 158.3, 160.4, 162.8, 168.9, 170.7. MS (ESI): 671 $[M + H]^+$.

***N*-(4-{3-(4-[5-(3,4,5-Trimethoxyphenyl)isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl]phenyl)-4-nitro-benzamide (11g).** Yield 73%, mp 283–285°C. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.96 s (6H), 6.67 s (1H), 7.27 s (2H), 7.69 d (2H, *J* = 7.28 Hz), 7.73 d (2H, *J* = 7.28 Hz), 7.84–7.95 m (4H), 8.11 d (2H, *J* = 7.30 Hz), 8.20 d (2H, *J* = 7.32 Hz), 8.59 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.4, 61.8, 96.7, 106.4, 123.4, 125.7, 126.5, 127.5, 129.7, 130.3, 130.8, 131.4, 133.6, 134.6, 136.5, 140.4, 145.6, 151.3, 156.7, 158.4, 160.5, 161.7, 169.5, 170.7. MS (ESI): 637 $[M + H]^+$.

***N*-(4-{3-(4-[5-(3,4,5-Trimethoxyphenyl)isoxazol-3-yl)phenyl)-1,2,4-thiadiazol-5-yl)phenyl)-3,5-dinitro-benzamide (11h).** Yield 71%, mp 309–311°C. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.96 s (6H), 6.68 s (1H), 7.27 s (2H), 7.70 d (2H, *J* = 7.28 Hz), 7.74 d (2H, *J* = 7.28 Hz), 7.83 d (2H, *J* = 7.31 Hz), 8.12 d (2H, *J* = 7.31 Hz), 8.30 s (1H), 8.36 s (2H), 8.59 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.4, 61.9, 96.7, 106.8, 123.5, 124.7, 126.7, 128.4, 129.6, 130.7, 131.3, 131.7, 133.4, 134.5, 135.2, 136.5, 145.6, 148.3, 156.5, 158.6, 159.3, 160.4, 169.8, 171.8. MS (ESI): 682 $[M + H]^+$.

4-Cyano-*N*-(4-{3-(4-[5-(3,4,5-trimethoxyphenyl)-isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}phenyl)-benzamide (11i). Yield 82%, mp 269–271°C. ^{1}H NMR spectrum, δ, ppm: 3.93 s (3H), 3.96 s (6H), 6.68 s (1H), 7.27 s (2H), 7.68 d (2H, *J* = 7.26 Hz), 7.72 d (2H, *J* = 7.26 Hz), 7.81–7.94 m (4H), 8.10 d (2H, *J* = 7.28 Hz), 8.18 d (2H, *J* = 7.27 Hz), 8.57 s (1H). ^{13}C NMR spectrum, δ, ppm: 57.6, 61.8, 96.5, 106.8, 114.5, 119.6, 123.5, 126.8, 129.7, 130.3, 130.7, 131.4, 131.9, 133.5, 134.5, 135.7, 139.4, 140.3, 145.5, 156.4, 158.6, 160.7, 163.4, 169.8, 170.8. MS (ESI): 617 $[M + H]^+$.

***N*-(4-{3-(4-[5-(3,4,5-Trimethoxyphenyl)isoxazol-3-yl]phenyl)-1,2,4-thiadiazol-5-yl}phenyl)-4-methyl-benzamide (11j).** Yield 54%, mp 265–267°C. ^{1}H NMR spectrum, δ, ppm: 2.43 s (3H), 3.93 s (3H), 3.96 s (6H), 6.66 s (1H), 7.24 s (2H), 7.46 d (2H, *J* = 7.19 Hz), 7.55 d (2H, *J* = 7.19 Hz), 7.68 d (2H, *J* = 7.23 Hz), 7.72 d (2H, *J* = 7.23 Hz), 7.80 d (2H, *J* = 7.26 Hz), 8.09 d (2H, *J* = 7.26 Hz), 8.56 s (1H). ^{13}C NMR spectrum, δ, ppm: 24.8, 57.6, 61.8, 96.4, 106.8, 123.4, 126.5, 128.5, 129.6, 130.4, 131.3, 131.7, 132.4, 133.5, 134.2, 135.4, 140.6, 143.5, 145.6, 156.7, 158.6, 160.4, 163.6, 169.7, 170.9. MS (ESI): 606 $[M + H]^+$.

MTT assay. Individual wells of a 96-well tissue culture microtiter 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 different

concentrations (0.5, 1, 2 μM) were added to each well and incubated at 37°C for 24 h. 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. Optical density (O.D.) was measured at 570 nm with a micro plate reader (Multi-mode Varioskan Instrument-Themo Scientific). The percentage of DMSO in the medium never exceeded 0.25%.

CONCLUSIONS

A number of amide derivatives of 1,2-isoxazole combined with 1,2,4-thiadiazole **11a**–**11j** is synthesized. All the compounds are tested for their anticancer activity against four types of human cancer cell lines including MCF-7 (breast), A549 (lung), Colo-205 (colon) and A2780 (ovarian). Most of the compounds demonstrate significant anticancer activity, and **11b**, **11c**, **11d**, **11e**, **11g**, and **11j** exhibit more potent activity than etoposide. The compound **11g** demonstrates the superior activity.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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