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RESEARCH ARTICLE-CHEMISTRY

Design, Synthesis, and Biological Evaluation of 1,2,4-Thiadiazole-1,2,4-Triazole Derivatives Bearing Amide Functionality as Anticancer Agents

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Abstract
A novel library of amide functionality having 1,2,4-thiadiazole-1,2,4-triazole (**8a–j**) analogs was designed, synthesized, and structures were characterized by ^{1}H NMR, ^{13}C NMR, and mass (ESI–MS) spectral data. Further, all compounds were evaluated for their anticancer activities against four different cancer cell lines including breast cancer (MCF-7, MDA MB-231), lung cancer (A549), and prostate cancer (DU-145) by MTT reduction assay method, and etoposide acts as a standard drug. The results confirmed that majority of the synthesized compounds showed moderate to potent anticancer activities aligned with four cell lines. Among the synthesized compounds, **8b, 8c, 8d, 8e, 8g** and **8i** displayed more potent activity along with inhibitory concentration values ranging from 0.10 ± 0.084 to 11.5 ± 6.49 μM than the standard IC_{50} values, which ranges from 1.91 ± 0.84 to 3.08 ± 0.135 μM, respectively.

Keywords Letrozole · 3,5-Bis(pyridin-3-yl)-1,2,4-thiadiazole · 1,2,4-Triazole · 1,2,4-Thiadiazole · Anticancer activity

1 Introduction

Cancer is very dangerous disease with uncontrolled growth and rapid spreading of abnormal cells [1]. Several external and internal factors are caused to abnormal growth of cell lines and induced the different cancers [2–7]. Currently, three types of treatment are available for cancer disease including chemotherapy, radiotherapy, and surgery [8]. The standard treatment for cancer patients is chemotherapy, in which different chemotherapeutic agents are used to kill the cancer cells without any harmful effective on normal kidney cells [9–13].

Nitrogen atoms contain heterocyclic ring moieties that are present both in natural products and in synthetic derivatives and exhibited potent anticancer activities against different human cancer cell lines [14–32]. Three nitrogen atoms containing heterocyclic ring such as 1,2,4-triazoles play an important critical role in the structural elucidation of various natural products [33] and are able to form hydrogen bonding with suitable targets leading to improving of pharmacokinetics, pharmacological, and toxicological properties [34, 35]. These 1,2,4-triazole derivatives are associated with different pharmaceutical activities such as anticancer [36], antibacterial [37], antitubercular [38], antifungal [39], antiviral [40], analgesic [41], anti-inflammatory [42], and tubulin inhibitors [43]. Letrozole (**1**, Fig. 1) [44, 45] is a triazole structural unit containing aromatase inhibitor and is used for cancer treatment.

Similarly, 1,2,4-thiadiazoles are considered as most significant subclass of bioactive five-membered organic compounds for medicinal chemistry [46] and showed a remarkable biological activities such as cyclooxygenase inhibitors [47], human leukemia [48], antibacterial [49], antiulcerative [50], antihypertensive [51], cathepsin B inhibitors [52], anticonvulsant [53], antidiabetic [54], anti-inflammatory [47], and allosteric modulators [55]. One of the anticancer drug scaffolds like 3,5-bis(pyridin-3-yl)-1,2,4-thiadiazole (**2**) is inhibitor of aromatase and used for treatment of various types of cancers [56, 57].

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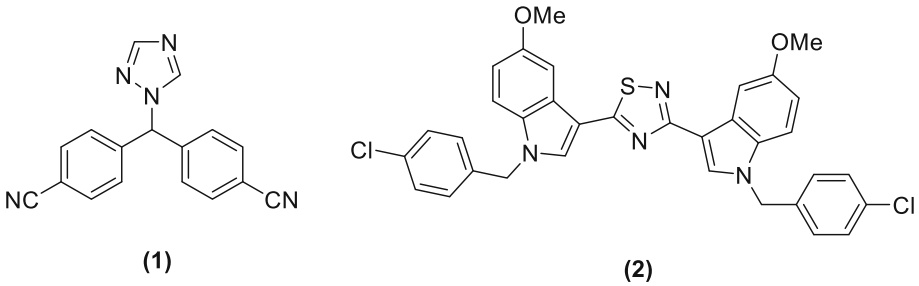


Fig. 1 Structures of letrozole (**1**) and 3,5-bis(pyridine-3-yl)-1,2,4-thiadiazole (**2**)

Previously, we reported the synthesis of a library of novel 2-(4-arylsubstituted-1*H*-1,2,3-triazol-1-yl)-*N*-{4-[2-(thiazol-2-yl)benzo[*d*]thiazol-6-yl]phenyl}acetamide derivatives and screened their anticancer activities against MCF-7, A549, Colo-205, and A2780 cell lines with etoposide as standard drug. The anticancer target compounds reported by us and the literature reveal hazardous solvent usage, harsh reaction conditions, and longer reaction sequences. To overcome the above drawbacks and inspired by special features of both 1,2,4-triazole and 1,2,4-oxadiaxole, we have to design and synthesize new amide functionality bearing 1,2,4-thiadiazole-1,2,4-triazole derivatives (**8a–j**). These derivatives were examined for their anticancer activities against four different human cancer cell lines like breast cancer (MCF-7, MDA MB-231), lung cancer (A549), and prostate cancer (DU-145). These derivatives may act as drug lead molecules in cancer chemotherapy.

2 Experimental

2.1 General Conditions

All the solvents, salts, reagents, and fine chemicals were purchased from Sigma-Aldrich and Alfa Aesar companies. These chemical items were used without further purification. ^{1}H and ^{13}C NMR spectra were recorded with 400 MHz and 300 MHz frequency Gemini Varian-VXR-unity instruments. Chemical shifts (δ) were noted in ppm toward downfield with respect to tetramethylsilane as internal standard. ESI spectra were recorded at 3.98 kV capillary voltages with micro-mass, Quattro LC instrument using ESI + software. Melting points were noted with the help of electrothermal melting point apparatus.

2.2 Synthesis

2.2.1 5-(3,4,5-Trimethoxyphenyl)-3-*p*-Tolyl-1*H*-1,2,4-Triazole (5)

To a dried 250-mL round-bottom flask were added 4-methylbenzonitrile (**4**) (4.7 mL, 0.039 mol), 3,4,5-trimethoxy benzamidine (**3**) (14 g, 0.066 mol), Cs_2CO_3 (43 g, 0.316 mol), CuBr (312 mg, 0.00217 mol, 5 mol%), and DMSO (80 mL). The reaction mixture was stirred under atmospheric air at 120 °C for 24 h. After cooling to atmospheric temperature, the reaction mixture was extracted with ethyl acetate solvent ($3 \times 15 = 45$ mL) and successively washed with 5% aqueous $NaHCO_3$ ($3 \times 10 = 30$ mL) and brine (10 mL). The organic layer was dried on $MgSO_4$ and concentrated under reduced pressure. The crude residue was purified with silica gel through column chromatography by using 2:8 ratio ethyl acetate/hexane solvent mixture to afford pure yellow color compound **5** as 13.8 g with 64% yield. Mp: 166–168 °C. ^{1}HNMR (400 MHz, DMSO-d_6): δ 2.43 (*s*, 3H, –CH_3), 3.72 (*s*, 3H, –OCH_3), 3.89 (*s*, 6H, 2-OCH_3), 7.73 (*d*, 2H, $J = 8.2$ Hz), 7.89 (*s*, 2H), 8.29 (*d*, 2H, $J = 8.2$ Hz), 10.32 (brs, 1H, –NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.8, 56.2, 60.9, 107.3, 124.5, 126.9, 128.6, 133.3, 141.6, 141.9, 155.8, 162.4, 169.1; MS (ESI): *m*/*z* 326 $[M + H]^+$.

2.2.2 5-(3,4,5-Trimethoxyphenyl)-3-(4-(3-(3,4,5-Trimethoxyphenyl)-1,2,4-Thiadiazol-5-yl)Phenyl)-1*H*-1,2,4-Triazole (6)

A mixture of 5-(3,4,5-trimethoxyphenyl)-3-*p*-tolyl-1*H*-1,2,4-triazole (**5**) (12 g, 0.037 mol), 3,4,5-trimethoxy benzamidine (**3**) (15.5 g, 0.0738 mol), sulfur S (5.9 g, 0.185 mol), potassium phosphate tribasic trihydrate (29.5 g, 0.111 mol), and DMSO (100 mL) was discharged in 200 mL of dried round-bottom flask under air. The reaction vessel was stirred at 130 °C for 12 h. After cooling to 27 °C, the solvent was removed under reduced pressure. The crude residue was purified on silica gel through column chromatography with 3:7 ratio of ethyl acetate/hexane solvent mixture to afford the

desired yellow color product **6** as 14.6 g with 71% yield. Mp: 196–198 °C; ^{1}HNMR (400 MHz, DMSO-d_6): δ 3.72 (*s*, 3H, –OCH_3), 3.79 (*s*, 6H, 2-OCH_3), 3.89 (*s*, 6H, 2-OCH_3), 3.92 (*s*, 3H, –OCH_3), 7.78 (*s*, 2H), 7.89 (*d*, 2H, $J = 8.4$ Hz), 8.10 (*s*, 2H), 8.49 (*d*, 2H, $J = 8.4$ Hz), 10.34 (brs, 1H, –NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 56.3, 56.6, 60.8, 106.6, 108.7, 124.2, 124.6, 128.9, 130.1, 133.6, 136.5, 142.8, 144.1, 154.2, 155.1, 162.4, 168.9, 171.4, 184.2; MS (ESI): *m/z* 562 $[M + H]^+$.

2.2.3 (5-(3,4,5-Trimethoxyphenyl)-3-(4-(3-(3,4,5-Trimethoxyphenyl)-1,2,4-Thiadiazol-5-yl)Phenyl)-1*H*-1,2,4-Triazol-1-yl)(Phenyl)Methanone (8a)

The compound 5-(3,4,5-trimethoxyphenyl)-3-(4-(3-(3,4,5-trimethoxyphenyl)-1,2,4-thiadiazol-5-yl)phenyl)-1*H*-1,2,4-triazole (**6**) (500 mg, 0.891 mol) was dissolved in 40 mL of dry acetonitrile, followed by addition of benzoyl chloride (**7a**) (0.1 mL, 0.891 mmol) and Cs_2CO_3 (580 mg, 1.78 mol). The reaction mixture allowed for stirring at room temperature over a time period of 12 h. After completion of reaction, the reaction mass was washed with 3 mL water and diluted with dichloromethane ($3 \times 3 = 9$ mL). It was dried on anhydrous Na_2SO_4. The crude residue was purified with silica gel through column chromatography by using 1:1 ratio ethyl acetate/hexane solvent mixture and then afforded pure yellow color compound **8a** in 310.8 mg, 53% yield. Mp: 178–180 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.72 (*s*, 3H, –OCH_3), 3.79 (*s*, 6H, 2-OCH_3), 3.89 (*s*, 6H, 2-OCH_3), 3.92 (*s*, 3H, –OCH_3), 7.58–7.62 (*m*, 1H), 7.67–7.73 (*m*, 2H), 7.79 (*s*, 2H), 7.93 (*d*, 2H, $J = 8.5$ Hz), 8.15 (*d*, 2H, $J = 7.6$ Hz), 8.48 (*s*, 2H), 8.66 (*d*, 2H, $J = 8.5$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 57.6, 58.5, 61.4, 61.8, 106.7, 109.5, 116.4, 125.7, 129.3, 129.7, 132.2, 132.5, 134.3, 135.7, 136.3, 137.6, 142.4, 144.5, 153.4, 154.6, 155.4, 164.5, 168.7, 169.4, 176.7; MS (ESI): *m/z* 666 $[M + H]^+$.

2.2.4 (3,4,5-Trimethoxyphenyl)(5-(3,4,5-Trimethoxyphenyl)-3-(4-(3-(3,4,5-Trimethoxyphenyl)-1,2,4-Thiadiazol-5-yl)Phenyl)-1*H*-1,2,4-Triazol-1-yl)Methanone (8b)

This compound **8b** was synthesized by the same method involved in the synthesis of **8a**, employing **6** (500 mg, 0.891 mol) with 3,4,5-trimethoxybenzoyl chloride (**7b**) (206 mg, 0.891 mmol), Cs_2CO_3 (580 mg, 1.78 mol), and the crude residue was purified with silica gel through column chromatography by using 1:1 ratio ethyl acetate/hexane solvent mixture and then afforded pure yellow color compound **8b**, 338.4 mg in 50% yield. Mp: 200–202 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.65 (*s*, 3H, –OCH_3), 3.72 (*s*, 3H, –OCH_3), 3.80 (*s*, 6H, 2-OCH_3), 3.89 (*s*, 6H, 2-OCH_3), 3.92 (*s*, 3H, –OCH_3), 3.95 (*s*, 6H, 2-OCH_3), 7.68 (*s*, 2H), 7.79 (*s*, 2H), 7.92 (*d*, 2H, $J = 8.4$ Hz), 8.47 (*s*, 2H), 8.67 (*d*, 2H, $J = 8.4$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 57.4, 57.8, 58.5, 61.4, 61.7, 62.4, 106.7, 107.8, 109.3, 116.5, 125.6, 131.5, 132.4, 133.7, 134.5, 137.2, 142.9, 144.7, 145.8, 153.6, 154.3, 155.8, 157.9, 162.5, 168.5, 169.4, 176.8; MS (ESI): *m/z* 756 $[M + H]^+$.

2.2.5 (3,5-Dimethoxyphenyl)(5-(3,4,5-Trimethoxyphenyl)-3-(4-(3-(3,4,5-Trimethoxyphenyl)-1,2,4-Thiadiazol-5-yl)Phenyl)-1*H*-1,2,4-Triazol-1-yl)Methanone (8c)

This compound **8c** was synthesized by the same method involved in the synthesis of **8a**, employing **6** (500 mg, 0.891 mol) with 3,5-dimethoxybenzoyl chloride (**7c**) (179 mg, 0.891 mol), Cs_2CO_3 (580 mg, 1.78 mol), and the crude residue was purified with silica gel through column chromatography by using 1:1 ratio ethyl acetate/hexane solvent mixture and then afforded pure yellow color compound **8c**, 320.6 mg in 50% yield. Mp: 190–192 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.67 (*s*, 3H, –OCH_3), 3.72 (*s*, 3H, –OCH_3), 3.80 (*s*, 6H, 2-OCH_3), 3.89 (*s*, 6H, 2-OCH_3), 3.92 (*s*, 3H, –OCH_3), 7.24 (*s*, 1H), 7.34 (*s*, 2H), 7.79 (*s*, 2H), 7.93 (*d*, 2H, $J = 8.5$ Hz), 8.47 (*s*, 2H), 8.66 (*d*, 2H, $J = 8.5$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 56.7, 57.8, 58.5, 61.5, 62.4, 106.5, 108.2, 109.7, 116.4, 120.5, 125.5, 132.4, 133.6, 133.9, 134.6, 137.4, 142.3, 144.8, 153.2, 154.6, 155.8, 162.3, 166.8, 168.2, 169.6, 176.8; MS (ESI): *m/z* 726 $[M + H]^+$.

2.2.6 (4-Methoxyphenyl)(5-(3,4,5-Trimethoxyphenyl)-3-(4-(3-(3,4,5-Trimethoxyphenyl)-1,2,4-Thiadiazol-5-yl)Phenyl)-1*H*-1,2,4-Triazol-1-yl)Methanone (8d)

This compound **8d** was prepared following the method described for the preparation of the compound **8a**, employing **6** (500 mg, 0.891 mol) with 4-methoxybenzoyl chloride (**7d**) (0.12 mL, 0.891 mol), Cs_2CO_3 (580 mg, 1.78 mol), and the crude residue was purified with silica gel through column chromatography by using 1:1 ratio ethyl acetate/hexane solvent mixture and then afforded afford pure yellow color compound **8d**, 312.8 mg in 51% yield. Mp: 195–197 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.72 (*s*, 3H, –OCH_3), 3.76 (*s*, 3H, –OCH_3), 3.80 (*s*, 6H, 2-OCH_3), 3.89 (*s*, 6H, 2-OCH_3), 3.92 (*s*, 3H, –OCH_3), 7.79 (*s*, 2H), 7.91–8.07 (*m*, 4H), 8.17 (*d*, 2H, $J = 7.9$ Hz), 8.47 (*s*, 2H), 8.67 (*d*, 2H, $J = 8.6$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 56.7, 57.6, 58.7, 61.4, 62.5, 106.5, 109.2, 114.7, 116.8, 125.4, 130.2, 131.4, 132.6, 133.8, 134.6, 137.4, 142.3, 144.6, 153.7, 154.5, 155.8, 164.2, 166.8, 168.4, 169.7, 176.7; MS (ESI): *m/z* 696 $[M + H]^+$.

2.2.7 (5-(3,4,5-Trimethoxyphenyl)-3-(4-(3-(3,4,5-Trimethoxyphenyl)-1,2,4-Thiadiazol-5-yl)Phenyl)-1*H*-1,2,4-Triazol-1-yl)(4-Nitrophenyl)Methanone (8e)

This compound **8e** was synthesized by the same method involved in the synthesis of **8a**, employing **6** (500 mg, 0.891 mol) with 4-nitrobenzoyl chloride (**7e**) (165 mg, 0.891 mol), Cs_2CO_3 (580 mg, 1.78 mol), and the crude residue was purified with silica gel through column chromatography by using 1:1 ratio ethyl acetate/hexane solvent mixture and then afforded pure yellow color compound **8e**, 385.4 mg in 61% yield. Mp: 230–232 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.72 (*s*, 3H, –OCH_3), 3.83 (*s*, 6H, 2-OCH_3), 3.89 (*s*, 6H, 2-OCH_3), 3.93 (*s*, 3H, –OCH_3), 7.80 (*s*, 2H), 7.94 (*d*, 2H, J = 8.7 Hz), 8.30 (*d*, 2H, J = 8.1 Hz), 8.40 (*d*, 2H, J = 8.1 Hz), 8.49 (*s*, 2H), 8.68 (*d*, 2H, J = 8.7 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 57.6, 58.7, 61.4, 62.7, 106.4, 109.7, 116.8, 125.3, 126.5, 131.2, 132.6, 133.5, 134.8, 137.6, 141.3, 142.6, 44.5, 153.4, 154.6, 154.9, 155.6, 164.5, 168.4, 169.7, 176.8; MS (ESI): *m*/*z* 711 $[M + H]^+$.

2.2.8 (5-(3,4,5-Trimethoxyphenyl)-3-(4-(3-(3,4,5-Trimethoxyphenyl)-1,2,4-Thiadiazol-5-yl)Phenyl)-1*H*-1,2,4-Triazol-1-yl)(3,5-Dinitrophenyl)Methanone (8f)

This compound **8f** was synthesized by the same method involved in the synthesis of **8a**, employing **6** (500 mg, 0.891 mol) with 3,5-dinitrobenzoyl chloride (**7f**) (205 mg, 0.891 mol), Cs_2CO_3 (580 mg, 1.78 mol), and the crude residue was purified with silica gel through column chromatography by using 1:1 ratio ethyl acetate/hexane solvent mixture and then afforded pure yellow color compound **8f**, 410.5 mg in 61% yield. Mp: 254–256 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.73 (*s*, 3H, –OCH_3), 3.84 (*s*, 6H, 2-OCH_3), 3.89 (*s*, 6H, 2-OCH_3), 3.95 (*s*, 3H, –OCH_3), 7.80 (*s*, 2H), 7.94 (*d*, 2H, J = 8.8 Hz), 8.52 (*s*, 2H), 8.68 (*d*, 2H, J = 8.8 Hz), 8.92 (*s*, 2H), 9.14 (*s*, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 57.6, 58.4, 61.5, 62.7, 106.4, 109.7, 116.6, 125.4, 126.7, 128.5, 132.4, 133.6, 134.5, 135.7, 137.4, 142.3, 144.5, 148.6, 153.4, 154.6, 155.7, 157.6, 168.4, 169.7, 176.8; MS (ESI): *m*/*z* 756 $[M + H]^+$.

2.2.9 (4-Chlorophenyl)(5-(3,4,5-Trimethoxyphenyl)-3-(4-(3-(3,4,5-Trimethoxyphenyl)-1,2,4-Thiadiazol-5-yl)Phenyl)-1*H*-1,2,4-Triazol-1-yl)Methanone (8g)

This compound **8g** was synthesized by the same method involved in the synthesis of **8a**, employing **6** (500 mg, 0.891 mol) with 4-chlorobenzoyl chloride (**7 g**) (0.11 mL, 0.891 mol), Cs_2CO_3 (580 mg, 1.78 mol), and the crude residue was purified with silica gel through column chromatography by using 1:1 ratio ethyl acetate/hexane solvent mixture and then afforded pure yellow color compound **8g**, 348.7 mg in 56% yield. Mp: 233–235 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.73 (*s*, 3H, –OCH_3), 3.82 (*s*, 6H, 2-OCH_3), 3.89 (*s*, 6H, 2-OCH_3), 3.93 (*s*, 3H, –OCH_3), 7.73 (*d*, 2H, J = 8.02 Hz), 7.80 (*s*, 2H), 7.94 (*d*, 2H, J = 8.7 Hz), 8.19 (*d*, 2H, J= 8.02 Hz), 8.50 (*s*, 2H), 8.68 (*d*, 2H, J = 8.7 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 57.6, 58.7, 61.5, 62.8, 106.5, 109.8, 116.5, 125.4, 130.5, 132.5, 133.2, 134.7, 135.2, 135.7, 137.5, 142.3, 142.6, 144.5, 153.4, 154.6, 155.8, 164.3, 168.3, 169.7, 176.8; MS (ESI): *m*/*z* 700 $[M + H]^+$.

2.2.10 (4-Bromophenyl)(5-(3,4,5-Trimethoxyphenyl)-3-(4-(3-(3,4,5-Trimethoxyphenyl)-1,2,4-Thiadiazol-5-yl)Phenyl)-1*H*-1,2,4-Triazol-1-yl)Methanone (8h)

This compound **8h** was synthesized by the same method involved in the synthesis of **8a**, employing **6** (500 mg, 0.891 mol) with 4-bromobenzoyl chloride (**7h**) (196 mg, 0.891 mol), Cs_2CO_3 (580 mg, 1.78 mol), and the crude residue was purified with silica gel through column chromatography by using 1:1 ratio ethyl acetate/hexane solvent mixture and then afforded pure yellow color compound **8h**, 336.2 mg in 51% yield. Mp: 241–243 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.73 (*s*, 3H, –OCH_3), 3.82 (*s*, 6H, 2-OCH_3), 3.89 (*s*, 6H, 2-OCH_3), 3.93 (*s*, 3H, –OCH_3), 7.78 (*d*, 2H, J= 8.00 Hz), 7.80 (*s*, 2H), 7.94 (*d*, 2H, J = 8.6 Hz), 8.15 (*d*, 2H, J= 8.00 Hz), 8.51 (*s*, 2H), 8.68 (*d*, 2H, J = 8.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 57.5, 58.7, 61.4, 62.7, 106.8, 109.8, 116.4, 125.6, 126.3, 130.2, 132.4, 133.2, 134.5, 134.7, 135.6, 137.6, 142.4, 144.5, 153.2, 154.6, 155.8, 164.5, 168.4, 169.8, 177.1; MS (ESI): *m*/*z* 746 $[M + H]^+$.

2.2.11 4-[(5-(3,4,5-Trimethoxyphenyl)-3-4-[3-(3,4,5-Trimethoxyphenyl)-1,2,4-Thiadiazol-5-yl]Phenyl-1*H*-1,2,4-Triazol-1-yl)Carbonyl]Benzonitrile (8i)

This compound **8i** was synthesized by the same method involved in the synthesis of **8a**, employing **6** (500 mg, 0.891 mol) with 4-cyanobenzoyl chloride (**7i**) (148 mg, 0.891 mol), Cs_2CO_3 (580 mg, 1.78 mol), and the crude residue was purified with silica gel through column chromatography by using 1:1 ratio ethyl acetate/hexane solvent mixture and then afforded pure yellow color compound **8i**, 156.8 mg in 26% yield. Mp: 247–249 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.73 (*s*, 3H, –OCH_3), 3.82 (*s*, 6H, 2-OCH_3), 3.89 (*s*, 6H, 2-OCH_3), 3.93 (*s*, 3H, –OCH_3), 7.81 (*s*, 2H), 7.94 (*d*, 2H, J = 8.6 Hz), 8.21 (*d*, 2H, J = 8.04 Hz), 8.39 (*d*, 2H, J= 8.04 Hz), 8.53 (*s*, 2H), 8.68 (*d*, 2H, J = 8.6 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 57.4, 58.6, 61.6,

62.7, 106.4, 109.7, 114.6, 116.7, 119.4, 125.4, 131.2, 132.5, 133.6, 134.2, 135.8, 137.3, 138.6, 142.5, 144.7, 153.4, 154.7, 155.6, 164.2, 168.6, 169.7, 177.3; MS (ESI): *m/z* 691 $[M + H]^+$.

2.2.12 (5-(3,4,5-Trimethoxyphenyl)-3-(4-(3-(3,4,5-Trimethoxyphenyl)-1,2,4-Thiadiazol-5-yl)Phenyl)-1*H*-1,2,4-Triazol-1-yl)(*p*-Tolyl)Methanone (8j)

This compound **8j** was synthesized by the same method involved in the synthesis of **8a**, employing **6** (500 mg, 0.891 mol) with 4-methylbenzoyl chloride (**7j**) (0.8 mL, 0.891 mmol), Cs_2CO_3 (580 mg, 1.78 mmol), and the crude residue was purified with silica gel through column chromatography by using 1:1 ratio ethyl acetate/hexane solvent mixture and then afforded pure yellow color compound **8j**, 362.4 mg in 60% yield. Mp: 186–188 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.45 (*s*, 3H, $-CH_3$), 3.73 (*s*, 3H, $-OCH_3$), 3.81 (*s*, 6H, 2-OCH_3), 3.89 (*s*, 6H, 2-OCH_3), 3.92 (*s*, 3H, $-OCH_3$), 7.68 (*d*, 2H, $J = 7.9$ Hz), 7.81 (*s*, 2H), 7.94 (*d*, 2H, $J = 8.6$ Hz), 8.18 (*d*, 2H, $J = 7.9$ Hz), 8.49 (*s*, 2H), 8.68 (*d*, 2H, $J = 8.6$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 24.8, 57.6, 58.3, 61.4, 62.6, 106.7, 109.5, 116.3, 125.7, 129.4, 130.2, 132.5, 133.6, 134.4, 135.3, 137.5, 142.4, 144.7, 146.6, 153.2, 154.6, 155.8, 164.5, 168.4, 169.7, 176.9; MS (ESI): *m/z* 680 $[M + H]^+$.

2.3 MTT Assay

Individual wells microtiter plate from a 96-well tissue culture was inoculated with 100 μL of complete medium containing 1×10^4 cells. These microtiter plates were incubated at a temperature of 37 °C in 5% CO_2-humidified incubator over a time period of 18 h prior to the experiment. After the removal of medium, a fresh medium of 100 μL containing both the test compounds and standard drug and etoposide at a variable concentrations of 0.5, 1, 2, and 4 μM was added to each well and incubated over 24-h time period at 37 °C temperature. Now, this medium was removed and replaced by 10 μL MTT assay dye. Again, the plates were allowed for incubation at a temperature of 37 °C over 2-h time period. The obtained formazan crystals were dissolved in 100 μL extraction buffer. The OD value was read with multimode Varioskan Instrument, Themo Scientific microplate reader at 570 nm. The % of DMSO-d_6 in the medium should not exceed 0.25% at any time. Each of the data of the IC_{50} values were represented as mean of ± SD values that means each experiment was performed three times.

3 Results and Discussion

3.1 Chemistry

The synthesis of 1,2,4-thiadiazole-1,2,4-triazole derivatives bearing amide functionality **(8a–j)** is shown in Scheme 1. Starting material 3,4,5-trimethoxybenzamidine (**3**) undergoes cyclization reaction with 4-methylbenzonitrile (**4**) in the presence of CuBr catalyst, Cs_2CO_3 base in DMSO solvent at 120 °C temperature over 24 h to afford triazole intermediate **5**. The ESI–MS peak at *m/z* 326 $[M + H]^+$ confirmed the structure of compound **5**. The triazole compound **5** reacted with 3,4,5-trimethoxybenzamidine (**3**) in the presence of potassium phosphate tribasic trihydrate ($K_3PO_4 \cdot 3H_2O$) base, and sulfur in DMSO solvent was heated at 130 °C for 12 h to afford pure 1,2,4-thiadiazole intermediate **6**. The ESI–MS peak at *m/z* 562 $[M + H]^+$ confirmed the structure of compound **6**. Then, this intermediate **5** was coupled with substituted aromatic acid chlorides (**7a–j**) in the presence of Cs_2CO_3 base in anhydrous acetonitrile solvent at room temperature for 12 h to afford the 1,2,4-thiadiazole-1,2,4-triazole derivatives **8a–j**. The ESI–MS peak at *m/z* 666 $[M + H]^+$ confirmed the structure of compound **8a**.

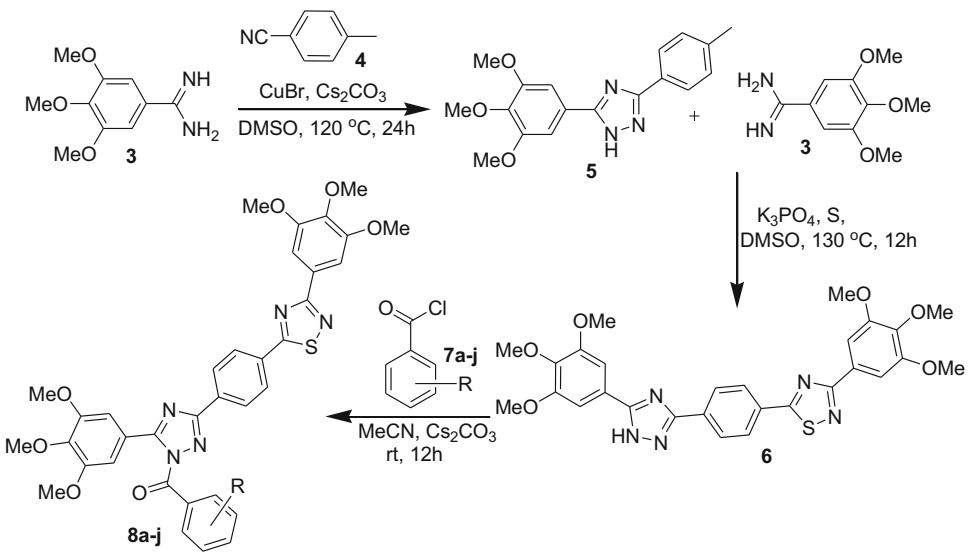
The new library of 1,2,4-thiadiazole-1,2,4-triazole derivatives having amide functionality (8a–j).

3.2 Biological Evaluation

3.2.1 In Vitro Cytotoxicity

The new library of 1,2,4-thiadiazole-1,2,4-triazole derivatives having amide functionality **(8a–j),** was examined for their anticancer activity toward a pane of four different human cancer cell lines such as breast cancer (MCF-7, MDA MB-231), lung cancer (A549), and prostate cancer (DU-145) by MTT assay and compared with the standard reference etoposide. The obtained results were presented as IC_{50} (μM) values in Table 1. The results indicated that most of the synthesized compounds exhibited moderate to excellent anticancer activity aligned with four cell lines. Among the library of examined compounds, compounds **8b, 8c, 8d, 8e, 8g**, and **8i** displayed more potent activity with IC_{50} values ranging from 0.10 ± 0.084 to 11.5 ± 6.49 μM and standard showed IC_{50} value range as 1.91 ± 0.84 μM to 3.08 ± 0.135 μM. Further, all these compounds were investigated for structure–activity relationship (SARs) studies. Compound **8b** with electron-donating group (3,4,5-trimethoxy) showed highest anticancer activity toward MCF-7, A549, DU-145, and MDA MB-231 with IC_{50} values of 0.10 ± 0.084 μM, 0.17 ± 0.032 μM, 0.83 ± 0.091 μM, and 0.28 ± 0.017 μM, respectively, where slight decrease in activity was observed for **8c** and **8d** with IC_{50} values of MCF-7 = 1.12 ± 0.64 μM; A549 = 1.79 ± 0.59 μM; DU145

Scheme 1 Synthesis of amide functionality bearing 1,2,4-thiadiazole-1,2,4-triazole derivatives



8a; R = H
8b; R = 3,4,5-trimethoxy
8c; R = 3,5-dimethoxy
8d; R = 4-methoxy
8e; R = 4-nitro
8f; R = 3,5-dinitro
8g; R = 4-chloro
8h; R = 4-bromo
8i; R = 4-cyano
8j; R = 4-methyl

7a; R = H
7b; R = 3,4,5-trimethoxy
7c; R = 3,5-dimethoxy
7d; R = 4-methoxy
7e; R = 4-nitro
7f; R = 3,5-dinitro
7g; R = 4-chloro
7h; R = 4-bromo
7i; R = 4-cyano
7j; R = 4-methyl

Table 1 In vitro cytotoxicity of newly target compounds **8a–j** with IC_{50} in μM

Compound	MCF-7	A549	DU-145	MDA MB-231
8a	3.57 ± 2.81	2.98 ± 1.76	ND	4.11 ± 2.30
8b	0.10 ± 0.084	0.17 ± 0.032	0.83 ± 0.091	0.28 ± 0.017
8c	1.12 ± 0.64	1.79 ± 0.59	1.98 ± 0.22	2.33 ± 1.52
8d	1.44 ± 0.17	2.10 ± 1.44	2.76 ± 1.88	2.35 ± 1.51
8e	0.23 ± 0.014	1.64 ± 0.53	0.19 ± 0.011	1.55 ± 0.63
8f	5.66 ± 2.38	ND	7.23 ± 4.52	ND
8g	1.02 ± 0.65	1.69 ± 0.13	2.13 ± 1.98	2.15 ± 1.08
8h	7.28 ± 3.67	ND	8.22 ± 4.33	10.7 ± 5.26
8i	1.27 ± 0.92	1.90 ± 0.46	0.60 ± 0.014	1.59 ± 0.37
8j	5.94 ± 3.26	11.5 ± 6.49	9.37 ± 6.21	ND
Etoposide	2.11 ± 0.024	3.08 ± 0.135	1.97 ± 0.45	1.91 ± 0.84

ND Not determined
MCF-7: human breast cancer cell line. A549: human lung cancer cell line. DU-145: human prostate cancer cell line. MDA MB-231: human breast cancer cell line

= 1.98 ± 0.22 μM, MDA MB-231 = 2.33 ± 1.52 μM, and MCF-7 = 1.44 ± 0.17 μM; A549 = 2.10 ± 1.44 μM; DU145 = 2.76 ± 1.88 μM, MDA MB-231 = 2.35 ± 1.51 μM, when compared with **8b** compound. The replacement of 4-methoxy group with 4-nitro (**8e**) showed improved anticancer activity against four cell lines (MCF-7 = 0.23 ± 0.014 μM; A549 = 1.64 ± 0.53 μM; DU145 = 0.19 ± 0.011 μM, MDA MB-231 = 1.55 ± 0.63 μM) compared with **8d.** Replacement of 4-nitro substituent with 4-chloro and then the compound **8g** showed acceptable activity (MCF-7 = 1.02 ± 0.65 μM; A549 = 1.69 ± 0.13 μM; DU145 = 2.13 ± 1.98 μM, MDA MB-231 = 2.15 ± 1.08 μM). When 3,5-dinitro group was introduced, 4-bromo substituents on the phenyl ring resulted compounds, namely **8f** and **8h**, were displayed very poor activity on all cell lines. Interestingly, compound **8i** with 4-cyano electron-withdrawing group showed better anticancer activity (MCF-7 = 1.27 ± 0.92 μM; A549 = 1.90 ± 0.46 μM; DU145 = 0.60 ± 0.014 μM, MDA MB-231 = 1.59 ± 0.37 μM) than **8g.** Compound **8j** with weak electron-donating group on the phenyl ring demonstrated moderate activity.

From the structure–activity relationship studies, it can be concluded that the presence of three electron-donating $-OCH_3$ group at 3,4,5 positions on phenyl ring displayed

excellent potent anticancer activities against four specified cancer cell lines. The decrease in anticancer activity would be observed with two $–OCH_3$ groups at 3,5 positions and one $–OCH_3$ group at 4th position. The presence of strong-withdrawing group $–NO_2$ at 3, 5 positions on phenyl ring displayed very less anticancer activity against specified cancer cell lines, when compared to one $–NO_2$ group at 4th position. In this series, cytotoxicity effect decreases from the electron-donating group to electron-withdrawing group derivatives.

4 Conclusion

The new library of 1,2,4-thiadiazole-1,2,4-triazole derivatives having amide functionality **(8a–j)** was designed, synthesized, and examined for their anticancer activities against four different human cancer cell lines including breast cancer (MCF-7, MDA MB-231), lung cancer (A549), and prostate cancer (DU-145) by making use of MTT assay. Here, Etoposide acts as standard drug, and the obtained results were presented as IC_{50} (μM) values. The results indicated that most of the synthesized compounds exhibited moderate to excellent anticancer activity aligned with four cell lines. Among them, compounds **8b, 8c, 8d, 8e, 8g**, and **8i** displayed more potent activity with IC_{50} values ranging from 0.10 ± 0.084 to 11.5 ± 6.49 μM and standard showed IC_{50} value ranges from 1.91 ± 0.84 μM to 3.08 ± 0.135 μM. These derivatives may act as drug lead molecules in cancer chemotherapy.

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