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 a 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], antiinflammatory [50], antihypertensive [51], cathepsin B inhibitors [52], anti-convulsant [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].
Previously, we reported the synthesis of a library of novel 2-(4-arylsubstituted-1H-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-oxadiazole, 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. 1H and 13C 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-1H-1,2,4-Triazole

To a dried 250-mL round-bottom flask were added 4-methylbenzonitrile (4) (4.7 mL, 0.039 mol), 3,4,5-trimethoxybenzamidine (3) (14 g, 0.066 mol), Cs2CO3 (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 × 15 = 45 mL) and successively washed with 5% aqueous NaHCO3 (3 × 10 = 30 mL) and brine (10 mL). The organic layer was dried on MgSO4 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. 1HNMR (400 MHz, DMSO-d6): δ 2.43 (s, 3 H, –CH3), 3.72 (s, 3 H, –OCH3), 3.89 (s, 6H, 2-OCH3), 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); 13C NMR (75 MHz, DMSO-d6): 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]+.
desired yellow color product 6 as 14.6 g with 71% yield. Mp: 196–198 °C; ³¹H NMR (400 MHz, DMSO-d₆): δ 3.72 (s, 3H, –OCH₃), 3.79 (s, 6H, 2-OCH₃), 3.89 (s, 6H, 2-OCH₃), 3.92 (s, 3H, –OCH₃), 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); ¹³C NMR (75 MHz, DMSO-d₆): 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-triazole (trimethoxyphenyl)-1,2,4-thiadiazol-5-yl)phenyl)-1H-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)-1H-1,2,4-Triazol-1-yl)(Phenyl)Methanone (8a) was prepared following the method described for the preparation of the compound 8a, employing 6 (500 mg, 0.891 mol) with 3,5-dimethoxybenzoyl chloride (7c) (179 mg, 0.891 mol), Cs₂CO₃ (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 8a in 310.8 mg, 53% yield. Mp: 178–180 °C, ¹¹H NMR (300 MHz, DMSO-d₆): δ 3.72 (s, 3H, –OCH₃), 3.79 (s, 6H, 2-OCH₃), 3.89 (s, 6H, 2-OCH₃), 3.92 (s, 3H, –OCH₃), 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); ¹³C NMR (75 MHz, DMSO-d₆): 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 726 [M + H]⁺.

2.2.4 (3,4,5-Trimethoxyphenyl)(5-(3,4,5-Trimethoxyphenyl)-1,2,4-Thiadiazol-5-yl)(Phenyl)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), Cs₂CO₃ (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, ¹¹H NMR (300 MHz, DMSO-d₆): δ 3.65 (s, 3H, –OCH₃), 3.72 (s, 3H, –OCH₃), 3.80 (s, 6H, 2-OCH₃), 3.89 (s, 6H, 2-OCH₃), 3.92 (s, 3H, –OCH₃), 7.89 (d, 2H, J = 8.4 Hz), 8.47 (s, 2H), 8.67 (d, 2H, J = 8.4 Hz); ¹³C NMR (75 MHz, DMSO-d₆): 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-(3,4,5-
Trimethoxyphenoxyl)-1,2,4-Thiadiazol-5-yl)Phenyl)-1H-
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), Cs₂CO₃ (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. ¹H NMR (300 MHz, DMSO-d₆): δ 3.72 (s, 3H, –OCH₃), 3.83 (s, 6H, 2-OCH₃), 3.89 (s, 6H, 2-OCH₃), 3.93 (s, 3H, –OCH₃), 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); ¹³C NMR (75 MHz, DMSO-d₆): 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-(3,4,5-
Trimethoxyphenoxyl)-1,2,4-Thiadiazol-5-yl)Phenyl)-1H-
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), Cs₂CO₃ (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. ¹H NMR (300 MHz, DMSO-d₆): δ 3.73 (s, 3H, –OCH₃), 3.84 (s, 6H, 2-OCH₃), 3.89 (s, 6H, 2-OCH₃), 3.95 (s, 3H, –OCH₃), 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); ¹³C NMR (75 MHz, DMSO-d₆): 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-Trimethoxyphenoxyl)-1,2,4-Thiadiazol-5-
yl)Phenyl)-1H-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 (7g) (0.11 mL, 0.891 mol), Cs₂CO₃ (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. ¹H NMR (300 MHz, DMSO-d₆): δ 3.73 (s, 3H, –OCH₃), 3.82 (s, 6H, 2-OCH₃), 3.89 (s, 6H, 2-OCH₃), 3.93 (s, 3H, –OCH₃), 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); ¹³C NMR (75 MHz, DMSO-d₆): 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]⁺.
2.2.12 (5-(3,4,5-Trimethoxyphenyl)-3-(4-(3,4,5-
Trimethoxyphenyl)-1H-1,2,4-Thiadiazol-5-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), Cs2CO3 (580 mg, 1.78 mmol), and the crude
residue was purified with silica gel through column chro-
matography 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. 1H NMR
(300 MHz, DMSO-d6): δ 2.45 (s, 3H, –CH3), 3.73 (s, 3H,
–OCH3), 3.81 (s, 6H, 2-OCH3), 3.89 (s, 6H, 2-OCH3), 3.92
(s, 3H, –OCH3), 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); 13C NMR (75 MHz, DMSO-d6):
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, 144.8, 146.6,
153.2, 154.6, 155.8, 164.5, 168.4, 169.7, 176.9; MS (ESI):

m/z 680 [M + H]+.

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) under-
goes cyclization reaction with 4-methylbenzonitrile (4) in the
presence of CuBr catalyst, Cs2CO3 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 (K3PO4·3H2O) 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 Cs2CO3 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 deriva-
tives 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 deriva-
tives 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 IC50 (μM) values in Table 1. The results indicated
that most of the synthesized compounds exhibited moder-
ate to excellent anticancer activity aligned with four cell
lines. Among the library of examined compounds, com-
ounds 8b, 8c, 8d, 8e, 8g, and 8i displayed more potent
activity with IC50 values ranging from 0.10 ± 0.084 to
11.5 ± 6.49 μM and standard showed IC50 value range as
1.91 ± 0.84 μM to 3.08 ± 0.135 μM. Further, all these com-
ounds 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 IC50 val-
ues 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 IC50 values of
MCF-7 = 1.12 ± 0.64 μM; A549 = 1.79 ± 0.59 μM; DU145

2.3 MTT Assay

Individual wells microtiter plate from a 96-well tissue culture
was inoculated with 100 μL of complete medium contain-
ing 1 × 104 cells. These microtiter plates were incubated at
a temperature of 37 °C in 5% CO2-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 for-
mazan crystals were dissolved in 100 μL extraction buffer.
The OD value was read with multimode Varios Instrument,
Themo Scientific microplate reader at 570 nm. The
% of DMSO-d6 in the medium should not exceed 0.25% at
any time. Each of the data of the IC50 values were repre-
sented as mean of ±SD values that means each experiment
was performed three times.
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 –OCH₃ group at 3,4,5 positions on phenyl ring displayed

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

**Table 1** In vitro cytotoxicity of newly target compounds 8a–j with IC₅₀ 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.
excellent potent anticancer activities against four specified cancer cell lines. The decrease in anticancer activity would be observed with two –OCH3 groups at 3, 5 positions and one –OCH3 group at 4th position. The presence of strong-withdrawing group –NO2 at 3, 5 positions on phenyl ring displayed very less anticancer activity against specified cancer cell lines, when compared to one –NO2 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 IC50 (μM) values. The results indicated that most of the synthesized compounds exhibited moderate to excellent potent anticancer activities against four specified cancer cell lines, when compared to one –NO2 group at 4th position. Among them, compounds 8b, 8c, 8d, 8e, 8g, and 8i displayed more potent activity with IC50 values ranging from 0.10±0.084 to 11.5±6.49 μM and standard showed IC50 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.

References

1. Jemal, A.; Bray, F.; Center, M.M.; Ferlay, J.; Ward, E.; Forman, D.: Global cancer statistics. CA Cancer J. Clin. 61, 69–90 (2011)
2. Park, S.K.; Cho, L.Y.; Yang, J.I.; Park, B.; Chang, S.H.; Lee, K.S.; Kim, H.; Yoo, K.Y.; Lee, C.T.: Lung cancer risk and cigarette smoking, lung tuberculosis according to histologic type and gender in a population based case-control study. Lung Cancer 68, 20–26 (2010)
3. Meffert, M.K.; Chang, J.M.; Wiltgen, B.J.; Fanselow, M.S.; Baltimore, D.: NF-kappa B functions in synaptic signalling and behaviour. Nat. Neurosci. 6, 1072–1078 (2003)
4. Clemons, M.R.: Free radicals in chemical carcinogenesis. Klin. Wochenschr. 69, 1123–1134 (1991)
5. Mantovani, A.; Allavena, P.; Sica, A.; Balkwill, F.: Cancer-related inflammation. Nature 454, 436–444 (2008)
6. Clayton, P.E.; Banerjee, I.; Murray, P.G.; Renehan, A.G.: Growth hormone, the insulin-like growth factor axis, insulin and cancer risk. Nat. Rev. Endocrinol. 7, 11–24 (2011)
7. Porta, C.; Riboldi, E.; Sica, A.: Mechanisms linking pathogens-associated inflammation and cancer. Cancer Lett. 305, 250–262 (2011)
8. Khan, F.A.; Akhtar, S.S.; Sheikh, M.K.: Cancer treatment—objectives and quality of life issues. Malays. J. Med. Sci. 12, 3–5 (2005)
9. Menta, E.; Palumbo, M.: Novel antineoplastic agents. Exp. Opin. Ther. Patents 7, 1401–1426 (1997)
10. Nussbaumer, S.; Bonnabry, P.; Veuthey, J.-L.; Fleury-Souverain, S.: Analysis of anticancer drugs: a review. Talanta 85, 2265–2289 (2011)
11. Rebbuci, M.; Michiels, C.: Molecular aspects of cancer cell resistance to chemotherapy. Biochem. Pharmacol. 85, 1219–1226 (2013)
12. Holohan, C.; Van Schaeybroeck, S.; Longley, D.B.; Johnston, P.G.: Cancer drug resistance: an evolving paradigm. Nat. Rev. Cancer 13, 714–726 (2013)
13. Rosa, R.; Monte Leone, F.; Zambrano, N.; Bianco, R.: In vitro and in vivo models for analysis of resistance to anticancer molecular therapies. Curr. Med. Chem. 21, 1595–1606 (2014)
14. Reddy, N.B.; Burra, V.R.; Ravindranath, L.K.; Sreenivasulu, R.; Kumar, V.N.: Synthesis and biological evaluation of benzoxazole fused combretastatin derivatives as anticancer agents. Monatsh. Chem. 147, 593–598 (2016)
15. Reddy, N.B.; Burra, V.R.; Ravindranath, L.K.; Kumar, V.N.; Sreenivasulu, R.; Sadanandam, P.: Synthesis and biological evaluation of benzoimidazole fused edelfosine derivatives as anticancer agents. Monatsh. Chem. 147, 599–604 (2016)
16. Hatti, I.; Sreenivasulu, R.; Jadav, S.S.; Jayaprakash, V.; Kumar, C.G.; Raju, R.R.: Synthesis, cytotoxic activity and docking studies of new 4-aza podophyllotoxin derivatives. Med. Chem. Res. 24, 3305–3313 (2015)
17. Sreenivasulu, R.; Reddy, K.T.; Sujitha, P.; Ganesh, C.; Raju, R.R.: Synthesis, antiangiogenic and apoptosis induction potential activities of novel bis(indolyl)hydrazide-hydrazide derivatives. Bioorg. Med. Chem. 27, 1043–1055 (2019)
18. Agarwal, M.; Singh, V.; Sharma, S.C.; Sharma, P.; Ansari, MdY.; Jadav, S.S.; Yasmin, S.; Sreenivasulu, R.; Hassan, MdH: Design and synthesis of new 2,5-disubstituted 1,3,4-oxadiazole analogues as anticancer agents. Med. Chem. Res. 25, 2289–2303 (2016)
19. Spandana, Z.; Sreenivasulu, R.; Rekha, T.M.; Rao, M.V.B.: Novel 1,3,4-oxadiazole fused thiadiazole derivatives: synthesis and study of anticancer activities. Lett. Drug Des. Discov. 16, 656–662 (2019)
20. Madhavi, S.; Sreenivasulu, R.; Raju, R.R.: Synthesis and biological evaluation of oxadiazole incorporated ellipticine derivatives as anticancer agents. Monatsh. Chem. 148, 933–938 (2017)
21. Sreenivasulu, R.; Durgesh, R.; Jadav, S.S.; Sujitha, P.; Kumar, C.G.; Raju, R.R.: Synthesis, anticancer evaluation and molecular docking studies of bis(indolyl)triazinones, nortopsentin analogues. Chem. Pap. 72, 1369–1378 (2018)
22. Subramanyam, M.; Sreenivasulu, R.; Rambabu, G.; Rao, M.V.B.; Rao, K.P.: Synthesis, biological evaluation and docking studies of 1,3,4-oxadiazole fused benzothiazole derivatives for anticancer drugs. Lett. Drug Des. Discov. 15, 1299–1307 (2018)
23. Ahsan, M.J.; Choudhary, K.; Jadav, S.S.; Yasmin, S.; Ansari, M.Y.; Sreenivasulu, R.: Synthesis, antiproliferative activity and molecular docking studies of curcumin analogues bearing pyrazole ring. Med. Chem. Res. 24, 4166–4180 (2015)
24. Madhavi, S.; Sreenivasulu, R.; Jyotsna, Y.; Raju, R.R.: Synthesis of chalcone incorporated quinazoline derivatives as anticancer agents. Saudi Pharm. J. 25, 275–279 (2017)
25. Hatti, I.; Sreenivasulu, R.; Jadav, S.S.; Ahsan, M.J.; Raju, R.R.: Synthesis and biological evaluation of 1,3,4-oxadiazole linked bis indole derivatives as anticancer agents. Monatsh. Chem. 146, 1699–1705 (2015)
26. Durgesh, R.; Sreenivasulu, R.; Pinapati, S.R.; Raju, R.R.: Synthesis and anticancer evaluation of indazole-aryl hydrazone-hydrazone derivatives. J. Ind. Chem. Soc. 95, 433–438 (2018)
27. Spandana, Z.; Sreenivasulu, R.; Rao, M.V.B.: Design, synthesis and anticancer evaluation of carbazole fused aminopyrimidine derivatives. Lett. Org. Chem. 16, 662–667 (2019)
28. Madhavi, S.; Sreenivasulu, R.; Ansari, MdY.; Ahsan, M.J.; Raju, R.R.: Synthesis, biological evaluation and molecular docking studies of pyridine incorporated chalcone derivatives as anticancer agents. Lett. Org. Chem. 13, 682–692 (2016)

29. Sreenivasulu, R.; Sujitha, P.; Gadad, S.S.; Ahsan, M.J.; Kumar, C.G.; Raju, R.R.: Synthesis, antioxidant activity and molecular docking studies of indole-indazolyl hydrazide–hydrazide derivatives. Monatsh. Chem. 148, 305–314 (2017)

30. Reddy, K.T.; Sreenivasulu, R.; Raju, R.R.: Synthesis and biological evaluation of 1,2,4-oxadiazole linked imidazopyrazine derivatives as anticancer agents. J. Ind. Chem. Soc. 96, 1085–1090 (2019)

31. Murthy, I.S.; Sreenivasulu, R.; Alluraiah, G.; Raju, R.R.: Design, synthesis and anticancer evaluation of 1,2,3-triazole linked 1,2-oxazolopyrazinimidazo[4,5-b]pyridine derivatives. Russ. J. Gen. Chem. 89, 1718–1723 (2019)

32. Kala, P.; Sharif, S.K.; Krishna, C.H.M.; Ramachandran, D.; Design, synthesis and anticancer evaluation of 1,2,4-oxadiazole functionalyzed quinoline derivatives. Med. Chem. Res. 2019. https://doi.org/10.1007/s00044-019-02467-6

33. Asami, T.; Min, Y.K.; Nagata, N.; Amagishi, K.Y.; Takatsuto, S.; Fujioka, S.; Murofushi, N.; Yamaguchi, Y.; Ishioda, Y.: Characterization of brassinazole, a triazole-type brassinosteroid biosynthesis inhibitor. Plant Physiol. 123, 93–100 (2000)

34. Kaur, P.; Chawla, A.: Hepatoprotective activity of Inula cappa DC. Aqueous extract against carbon tetrachloride induced hepatotoxicity in Wistar rats. Int. Res. J. Pharm. 8, 14–19 (2017)

35. Kapron, B.; Luszczki, J.J.; Plaziska, A.; Siwek, A.; Karcz, T.; Gryszow, K.; Wodzinska, J.; Cielecka, J.; Ciesla, M.; Szalast, K.; Marciniak, S.; Paczkowska, M.; Ćielecka, J.; Ciesla, L.M.; Plech, T.: Development of the 1,2,4-triazole-based anti-convulsant drug candidates acting on the voltage-gated sodium channels. Insights from in vivo, in vitro, and in silico studies. Eur. J. Pharm. Sci. 129, 42–57 (2019)

36. Holla, B.S.; Poojary, K.N.; Rao, B.S.; Shivanda, M.: New bisaminomercapto triazoles and bis-triazolothiadiazoles as possible anticancer agents. Eur. J. Med. Chem. 37, 511–517 (2002)

37. Eswaran, S.; Adhikari, A.V.; Shetty, N.S.: Synthesis and antimicrobial activities of novel quinoline derivatives carrying 1,2,4-triazole moiety. Eur. J. Med. Chem. 44, 4637–4647 (2009)

38. Zhang, S.; Xu, Z.; Gao, C.; Ren, Q.C.; Chang, L.; Lv, Z.S.; Feng, L.S.: Triazole derivatives and their anti-tubercular activity. Eur. J. Med. Chem. 138, 501–513 (2017)

39. Jin, R.Y.; Zeng, C.Y.; Liang, X.H.; Sun, X.H.; Liu, Y.F.; Wang, Y.Y.; Zhou, S.: Design, synthesis, biological activities and DFT calculation of novel 1,2,4-triazole Schiff base derivatives. Bioorg. Med. Chem. 25, 4637–4647 (2009)

40. Wittine, K.; Babic, M.S.; Makuc, D.; Plavec, J.; Pavelic, S.; Sedic, M.; Pavelic, K.; Leysen, P.; Neys, J.; Balzarini, J.; Mintas, M.: Novel 1,2,4-triazole and imidazole derivatives of l-ascorbic and imino-ascorbic acid: synthesis, anti-HCV and antitumor activity evaluations. Bioorg. Med. Chem. 20, 3675–3685 (2012)

41. Mathew, V.; Keshavya, J.; Vaidya, V.P.; Giles, D.: Studies on synthesis and pharmacological activities of 3,6-disubstituted-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles and their dihydro analogues. Eur. J. Med. Chem. 42, 823–840 (2007)

42. El Shehry, M.F.; Abu-Hashem, A.A.; El-Telbani, E.M.: Synthesis of 3-(2-(4-chlorophenoxo) imethyl)-1,2,4-triazolo(thiadiazoles and thiadiazines) as anti-inflammatory and molluscicidal agents. Eur. J. Med. Chem. 45, 1906–1911 (2010)

43. Zhang, Q.; Peng, Y.; Wang, X.L.; Keenan, S.M.; Arora, S.; Welsh, W.J.: Highly potent triazole-based tubulin polymerization inhibitors. J. Med. Chem. 50, 749–754 (2007)

44. Maiti, A.; Reddy, P.V.N.; Sturdy, M.; Marler, L.; Pegan, S.D.; Mescar, A.D.; Pezzuto, J.M.; Cushman, M.: Synthesis of casimiroin and optimization of its quinone reductase 2 and aromatase inhibitory activities. J. Med. Chem. 2009, 1873–1884 (2009)

45. Stroesser, D.M.; Turner, S.D.; McNamara, J.; Stocker, P.; Miller, V.P.; Crespi, C.L.; Patien, C.J.: A high-throughput screen to identify inhibitors of aromatase (CYP19). Anal. Biochem. 284, 427–430 (2000)

46. Kumar, D.; Kumar, N.-M.; Chang, K.-H.; Shah, K.: Synthesis and anticancer activity of 5-(3-indolyl)-1,3,4-thiadiazoles. Eur. J. Med. Chem. 45, 4664–4668 (2010)

47. Unangst, P.C.; Shrum, G.P.; Connor, D.T.; Dyer, R.D.; Schrier, D.J.: Novel 1,2,4-oxadiazoles and 1,2,4-thiadiazoles as dual 5-lipoxygenase and cyclooxygenase inhibitors. J. Med. Chem. 35, 3691–3698 (1992)

48. Romagnoli, R.; Baraldi, P.G.; Cartion, M.D.; Cruz-Lopez, O.; Preti, D.; Tabrizi, M.A.; Fruttarolo, F.; Hellmann, P.; Bermejo, J.; Estevez, F.: Hybrid molecules containing benzoo[4,5]imidazo[1,2-d][1,2,4]thiadiazole and a-furoxamcarbonyl moiety as potent apoptosis inducers on human myeloid leukemia cells. Bioorg. Med. Chem. Lett. 17, 2844–2848 (2007)

49. Harai, R.; Sakamoto, K.; Hisamichi, N.; Nagano, N.: Structure–activity relationships of cephalexoporins having a (dimethyl isoxazolidino nio) vinyl moiety at their 3-position. J. Antibiot. 49, 1162–1171 (1996)

50. Kharimian, K.; Tam, T.F.; Leung-Long, R.C.; Li, W.: Thiazole compounds useful as inhibitors of hb/kb atpase. PCT Int. Appl. WO9951584A1 (1999).

51. Kohara, Y.; Kubo, K.; Imamiya, E.; Wada, T.; Inada, Y.; Naka, T.: Synthesis and angiotensin II receptor antagonistic activities of benzinimidazol derivatives bearing acidic heterocycles as novel tetrazole biososteres. J. Med. Chem. 39, 5228–5235 (1996)

52. Leung-Long, R.; Wodzinska, J.; Li, W.; Lowrie, J.; Kukreja, R.; Desilets, D.; Kariiman, K.; Tam, T.F.: 1,2,4-thiadiazole: a novel cathepsin B inhibitor. Bioorg. Med. Chem. 11, 5529–5537 (2003)

53. Castro, A.; Castano, T.; Encinas, A.; Porcal, W.; Gil, C.: Advances in the synthesis and recent therapeutic applications of 1,2,4-thiadiazole heterocycles. Bioorg. Med. Chem. 14, 1644–1652 (2006)

54. Johnstone, C.; Mckjercher, D.; Pike, K.G.; Waring, M.J.: Hetaryaryl benzamide derivatives for use as Glk activators in the treatment of diabetes. PCT Int. Appl. WO2005121110A1 (2005).

55. van den Nieuwendijk, A.M.C.H.; Pietra, D.; Heitman, L.; Goblyos, A.; Izzerman, A.P.: Synthesis and biological evaluation of 2,3,5-substituted [1,2,4]thiazoles as allosteric modulators of adenosine receptors. J. Med. Chem. 47, 663–672 (2004)

56. Mayhoub, A.S.; Marler, L.; Kondratyuk, T.P.; Park, E.J.; Pezzuto, J.M.; Cushman, M.C.: Optimizing thiazole analogues of resveratrol versus three chemopreventive targets. Bioorg. Med. Chem. 20, 510–520 (2012)

57. Kumar, D.; Kumar, N.M.; Chang, K.H.; Gupta, R.; Shah, K.: Synthesis and in vitro anticancer activity of 3,5-bis(indolyl)-1,2,4-thiadiazoles. Bioorg. Med. Chem. Lett. 21, 5897–5900 (2011)