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Original research paper

Synthesis and biological evaluation of novel 1,3,4-thiadiazole derivatives as possible anticancer agents

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The synthesis of new *N*-(5-substituted-1,3,4-thiadiazol-2-yl)-2-[(5-(substituted amino)-1,3,4-thiadiazol-2-yl)thio]acetamide derivatives and investigation of their anticancer activities were the aims of this work. All the new compounds' structures were elucidated by elemental analyses, IR, ^{1}H NMR, ^{13}C NMR and MS spectral data. Anticancer activity studies of the compounds were evaluated against MCF-7 and A549 tumor cell lines. In addition, with the purpose of determining the selectivity of cytotoxic activities, the most active compound was screened against a noncancer NIH3T3 cell line (mouse embryonic fibroblast cells). Among the tested compounds, compound **4y** (*N*-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-((5-(*p*-tolylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide), showed promising cytotoxic activity against MCF7 cancer cell with an IC_{50} value of 0.084 ± 0.020 mmol L^{-1} and against A549 cancer cell with IC_{50} value of 0.034 ± 0.008 mmol L^{-1}, compared with cisplatin. The aromatase inhibitory activity was evaluated for compound **4y** on MCF-7 cell line showing promising activity with IC_{50} of 0.062 ± 0.004 mmol L^{-1}.

Keywords: 1,3,4-thiadiazole, anticancer activity, aromatase inhibitory activity, MTT assay

Cancer remains a major cause of death worldwide despite multiple approaches used in prevention and therapy. After lung cancer, breast cancer is the second leading cause of cancer death among women. About one million women in the world are diagnosed with breast cancer every year. Moreover, according to World Conference on Breast Cancer, around 400,000 women die from this disease each year (1, 2).

The investigations for breast cancer suggest that many tumors occur and develop through estrogen-dependent mechanisms (3). Accordingly, it can be assumed that estrogen deprivation may prevent the formation of these cancers or may result in the regression of occurred tumors. The target of this suppression is the inhibition of the aromatase enzyme, responsible for the cyclization and structuring of estrogen (4).

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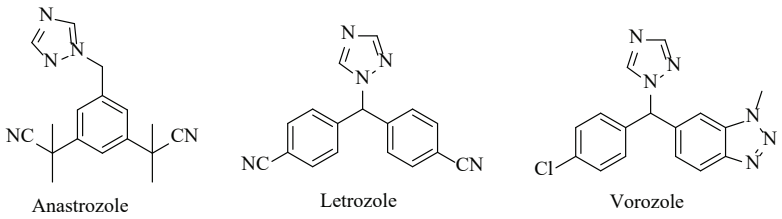


Fig. 1. The third generation of aromatase inhibitors.

Aromatase is a very promising target for the endocrine treatment of estrogen-dependent diseases. In this sense, attention has been focused on the discovery of aromatase inhibitors able to control the progression of hormone-sensitive breast cancer in women, and decrease the circulating levels of estrogens (5, 6). According to their chronological order of clinical development, these inhibitors can be divided into three classes. The third-generation aromatase inhibitors (non-steroid aromatase inhibitors) are used as first-line therapy for the treatment of breast cancer in both early and advanced tumors as shown in Fig. 1. They are more selective and/or potent compared with first- and second-generation agents (7).

The strongest interaction with aromatase is a coordinative bond between the lone electron pair of an aromatic heterocyclic nitrogen atom and the heme iron of the enzyme (8). Compounds containing heterocycles and more than one nitrogen also exert inhibitory activity towards aromatase (9).

Thiadiazole has been studied extensively for more than one hundred years due to its outstanding therapeutic applications. Thiadiazole is a 5-membered planar aromatic motif comprising a sulfur atom, which improves the liposolubility of thiadiazole derivatives and hence their pharmacokinetics (10). From the literature survey, it was noticed that 1,3,4-thiadiazole derivatives possess many pharmacological activities, such as antiviral (11), antibacterial (12), antileishmanial (13), antitubercular (14), antimycobacterial (15), anticonvulsant (16) and analgesic activity (17). Different mechanisms of the antitumor activity of 1,3,4-thiadiazoles have been reported, including specific inhibition of epidermal growth factor receptor (EGFR) kinase (18), carbonic anhydrase (19), phosphodiesterase-7 (PDE7) (20), tyrosine kinase (21) and aromatase (22). In the view of the aforesaid, we designed and synthesized two thiadiazole-ring systems.

EXPERIMENTAL

Materials and methods

All of the chemicals used in the study were purchased either from Merck (Merck KGaA, Germany) or Sigma-Aldrich (USA) and used without further purification.

The purity of the compounds was checked by classical TLC on silica gel 60 F254 (Merck).

Melting points of the synthesized compounds were determined using an MP90 series automatic melting point determination system (Mettler-Toledo, USA) and were presented as uncorrected.

^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 by a Bruker digital FT-NMR spectrometer (Bruker Bioscience, USA) at 300 MHz and 75 MHz, resp. (splitting patterns in

the NMR spectra were designated as follows: s – singlet, d – doublet, t – triplet, m – multiplet; coupling constants (*J*) were reported in hertz). M+1 peaks were determined by the Shimadzu LC/MS ITTOF system (Shimadzu, Japan).

Syntheses

General procedure for N-*substituted-hydrazinecarbothioamides (**1a**-**j**).* – A mixture of suitable isothiocyanate derivative (0.02 mol) and hydrazine hydrate (0.04 mol) was stirred in ethanol (99 %) (30 mL) for 4 h at 80 °C. After completion of the reaction, the precipitated product was filtered and washed with cold-ethanol.

*General procedure for 5-(substituted-amino)-1,3,4-thiadiazole-2-thiols (**2a**-**j**).* – Carbon disulfide (0.019 mol) was added into a solution of compound **1a-j** (0.018 mol) in EtOH (99 %) in the presence of sodium hydroxide (0.019 mol) and then the mixture was refluxed for 8 h. Afterward, the solution was cooled and acidified to pH 4–5 with hydrochloric acid and crystallized from ethanol.

*General procedure for 2-chloro-*N-*(5-substituted-1,3,4-thiadiazol-2-yl)acetamides (**3a**,**b**).* – A solution of 5-(substituted-amino)-1,3,4-thiadiazole-2-thiols (**2a**-**j**) (0.06 mol) in tetrahydrofuran was cooled in an ice bath in the presence of triethylamine (0.07 mol, 10.2 mL), and chloroacetyl chloride (0.07 mol, 5.8 mL) was added dropwise with stirring. After completion of the reaction, the solvent was evaporated under reduced pressure, the product was washed with water, dried and recrystallized from ethanol.

General procedure for N-*(5-substituted-1,3,4-thiadiazol-2-yl)-2-[(5-(substituted-amino)--1,3,4-thiadiazol-2-yl)thio]acetamides (**4a**-**y**).* In order to obtained the target compounds, compounds **3a**,**b** (2.5 mmol) and compounds **2a**-**j** (2.5 mmol) were reacted at room temperature in acetone. After completion of the reaction, the solvent was evaporated under reduced pressure, product was washed with water, dried and recrystallized from ethanol.

The synthesis pathway of the target compounds **4a**-**y** is given in Scheme 1.

Physicochemical data of compounds **4a**-**y** are given in Table I. The final compounds were purified and their structures were characterized by spectroscopic methods (FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy and elemental analyses). Spectral data of compounds **4a-y** are given in Table II.

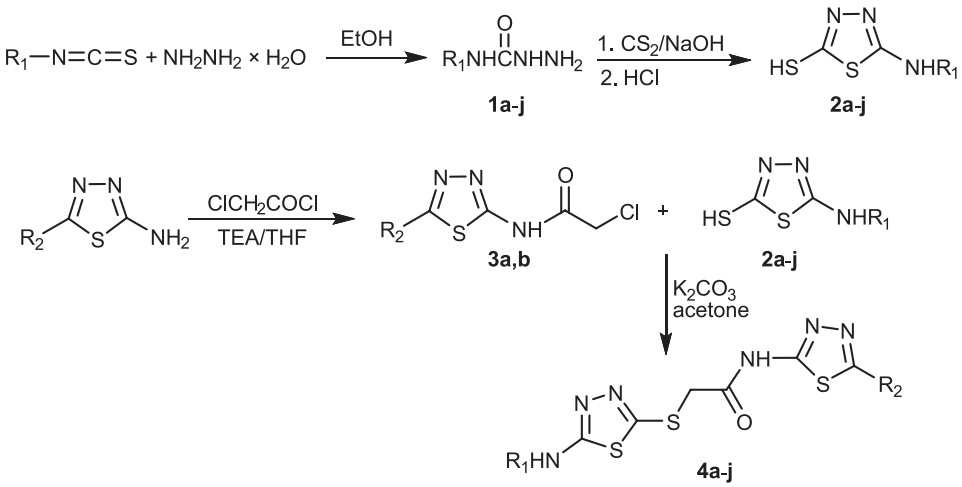
Cytotoxicity test

MTT assay, based on the ability of metabolically active cells to convert the pale yellow MTT to a spectrophotometrically measured blue formazan salt, is one of the most favoured cytotoxicity tests (23). All of the synthesized compounds at various concentrations (1, 0.316, 0.1, 0.0316, 0.01, 0.00316, 0.001 and 0.000316 mmol L^{-1}) were assayed for anticancer activity in cancer cell lines A549 and MCF-7 (ATCC, USA) using MTT method (24, 25). Cisplatin (Sigma Aldrich, USA) was used as a reference drug. The cytotoxic properties of the most active compounds (**4j**, **4v** and **4y**) were evaluated using NIH3T3 cell line (ATCC) (23, 24).

Aromatase inhibition assay

This assay was carried out according to the kit procedure [Aromatase (CYP19A) Inhibitor screening kit (fluorometric), BioVision, Boai NKY Medical Holdings Ltd., China]. The

compounds were dissolved in DMSO and added to the assay in at least 8 concentrations ranging from 1000 to 7.81 μmol L^{-1}. Recombinant human aromatase stock was prepared by reconstitution with 1 mL of aromatase assay buffer. The content was mixed thoroughly by vortexing to obtain a homogeneous solution which was transferred to a 15-mL conical tube. The volume was brought to 2450 μL with aromatase assay buffer and 50 μL of NADPH production system (100X) was added for a final total volume of 2.5 mL. Letrozole (Biovision) was used as a positive inhibition control. For solvent control, a small aliquot of aromatase assay buffer containing the organic solvent used to dissolve the test compounds was used. Reaction wells containing test compounds and corresponding no inhibitor-containing controls (which may also serve as solvent controls), as well as a background control



Compd.	R_1	R_2	Compd.	R_1	R_2
4a	Methyl	Methyl	**4l**	Methyl	Ethyl
4b	Ethyl	Methyl	**4m**	Ethyl	Ethyl
4c	Methoxyethyl	Methyl	**4n**	Methoxyethyl	Ethyl
4d	Propyl	Methyl	**4o**	Propyl	Ethyl
4e	Isopropyl	Methyl	**4p**	Isopropyl	Ethyl
4f	Allyl	Methyl	**4r**	Allyl	Ethyl
4g	Butyl	Methyl	**4s**	Butyl	Ethyl
4h	Isobutyl	Methyl	**4t**	Isobutyl	Ethyl
4i	Cyclohexyl	Methyl	**4u**	Cyclohexyl	Ethyl
4j	Phenyl	Methyl	**4v**	Phenyl	Ethyl
4k	4-Methylphenyl	Methyl	**4y**	4-Methylphenyl	Ethyl

Scheme 1.

(containing no fluorogenic aromatase substrate), were prepared. The plate was incubated for at least 10 min at 37 °C to allow test ligands to interact with aromatase. After incubation, 30 μL of the aromatase substrate/$NADP^+$ mixture was added to each well. Immediately (within 1 min), the fluorescence at ex/em = 488/527 nm was measured.

RESULTS AND DISCUSSION

Chemistry

The compounds **4a**-**y** were synthesized as outlined in Scheme 1. Firstly, suitable isothiocyanate and excess of hydrazine hydrate were reacted in order to obtain *N*-substituted hydrazinecarbothioamides (**1a**-**j**). Secondly, the reaction of compounds **1a-j** and carbondisulfide afforded 5-(substituted-amino)-1,3,4-thiadiazole-2-thiols (**2a**-**j**). The acetylation of thiadiazoles was performed using chloroacetyl chloride. Finally, 5-(substituted-amino)--1,3,4-thiadiazole-2-thiols (**2a**-**j**) and 2-chloro-*N*-(5-substituted-1,3,4-thiadiazol-2-yl)acetamides (**3a**,**b)** were reacted in order to synthesize target compounds **4a**-**y**.

Stretching absorption of NH groups was observed at 3285–3303 cm^{-1} as expected. Carbonyl (C=O) group gave characteristic stretching absorption in the region of 1652–1666 cm^{-1}. The stretching absorption at about 2916–2995 cm^{-1} was recorded for an aliphatic C-H bond. The stretching absorption belonging to 1,4-disubstituted benzene was detected at 844–850 cm^{-1} for compounds **4k** (*N*-(5-methyl-1,3,4-thiadiazol-2-yl)-2-((5-(*p*-tolylamino)--1,3,4-thiadiazol-2-yl)thio)acetamide) and **4y** (*N*-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-((5-(*p*--tolylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide).

In the ^1H NMR spectrum, the protons of the methyl (-CH_3) substituents were observed as a singlet peak between 2.60–2.62 ppm for compounds **4a**-**k**. The protons of the ethyl (-C_2H_5) were observed as a triplet (CH_3) and quartet (-CH_2) peak between 1.28–1.33 and 2.98–2.99 ppm, resp., for compounds **4l**-**y**. Methyl protons of compound **4y** (*N*-(5-ethyl--1,3,4-thiadiazol-2-yl)-2-((5-(*p*-tolylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide) were found at 2.24 ppm as a singlet. The protons of the ethyl group in compound **4y** were observed at 1.28 ppm as a triplet and 2.98 ppm as a quartet. Moreover, methylene protons of compound **4y** had singlet peak at 4.21 ppm. Disubstituted benzene protons of compound **4y** had doublet peaks at 7.13 ppm (J = 8.3 Hz) and 7.43 ppm (J = 8.5 Hz). In the ^{13}C NMR spectrum, aliphatic peaks belonging to substituents were observed between 11.81 ppm and 70.38 ppm. Aromatic carbons were identified between 116.72 and 175.17 ppm. Carbonyl carbon gave a peak between 166.82 and 175.17 ppm. The HRMS spectra of compounds were found to be in full agreement with their molecular formula.

Cytotoxicity assay

As shown in Table III, compounds **4j** (*N*-(5-methyl-1,3,4-thiadiazol-2-yl)-2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide), **4v** (*N*-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-((5--(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide) and **4y** (*N*-(5-ethyl-1,3,4-thiadiazol-2--yl)-2-((5-(*p*-tolylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide) were identified as the most active compounds. These compounds were tested against noncancer NIH3T3 cells, in order to measure the selectivity towards cancer cells. The IC_{50} values of the compounds against cell lines are presented in Table III.

Table I. Chemical names, molecular formula, chemical analysis, yields and melting points of new compounds ***4a-y***

Compd.	Chemical name	Molecular formula	Chemical analysis Calcd./found	Yield (%)	M. p. (°C)
4a	*N*-(5-methyl-1,3,4-thiadiazol-2-yl)-2-((5-(methylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide	$C_8H_{10}N_6OS_3$	C 31.77, H 3.33, N 27.79 C 31.72, H 3.32, N 27.82	78	251.4–252.3
4b	2-((5-(ethylamino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)acetamide	$C_9H_{12}N_6OS_3$	C 34.16, H 3.82, N 26.56 C 34.18, H 3.81, N 26.64	80	262.2–263.0
4c	2-((5-((2-methoxyethyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)acetamide	$C_{10}H_{14}N_6O_2S_3$	C 34.67, H 4.07, N 24.26 C 34.74, H 4.08, N 24.32	77	243.1–244.4
4d	*N*-(5-methyl-1,3,4-thiadiazol-2-yl)-2-((5-(propylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide	$C_{10}H_{14}N_6OS_3$	C 36.35, H 4.27, N 25.43 C 36.37, H 4.26, N 25.48	85	222.4–224.5
4e	2-((5-(isopropylamino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)acetamide	$C_{10}H_{14}N_6OS_3$	C 36.35, H 4.27, N 25.43 C 36.42, H 4.26, N 25.46	75	261.3–262.7
4f	2-((5-(allylamino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)acetamide	$C_{10}H_{12}N_6OS_3$	C 36.57, H 3.68, N 25.59 C 36.66, H 3.67, N 25.61	81	253.3–255.5
4g	2-((5-(butylamino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)acetamide	$C_{11}H_{16}N_6OS_3$	C 38.35, H 4.68, N 24.40 C 38.41, H 4.67, N 24.33	76	251.4–252.9
4h	2-((5-(isobutylamino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)acetamide	$C_{11}H_{16}N_6OS_3$	C 38.35, H 4.68, N 24.40 C 38.43, H 4.69, N 24.43	83	262.0–263.6
4ı	2-((5-(cyclohexylamino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)acetamide	$C_{13}H_{18}N_6OS_3$	C 42.14, H 4.90, N 22.68 C 42.24, H 4.88, N 22.72	79	268.7–269.9
4j	*N*-(5-methyl-1,3,4-thiadiazol-2-yl)-2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide	$C_{13}H_{12}N_6OS_3$	C 42.84, H 3.32, N 23.06 C 42.92, H 3.32, N 23.12	80	262.9–264.5
4k	*N*-(5-methyl-1,3,4-thiadiazol-2-yl)-2-((5-(*p*-tolylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide	$C_{14}H_{14}N_6OS_3$	C 44.43, H 3.73, N 22.20 C 44.52, H 3.72, N 22.24	82	261.1–263.4

Compd.	Chemical name	Molecular formula	Chemical analysis Calcd./found	Yield (%)	M. p. (°C)
4l	*N*-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-((5-(methylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide	$C_9H_{12}N_6OS_3$	C 34.16, H 3.82, N 26.56 C 34.26, H 3.81, N 26.62	77	226.0–228.9
4m	2-((5-(ethylamino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(5-ethyl-1,3,4-thiadiazol-2-yl)acetamide	$C_{10}H_{14}N_6OS_3$	C 36.35, H 4.27, N 25.43 C 36.44, H 4.26, N 25.52	85	239.9–241.7
4n	2-((5-((2-methoxyethyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(5-ethyl-1,3,4-thiadiazol-2-yl)acetamide	$C_{11}H_{16}N_6O_2S_3$	C 36.65, H 4.47, N 23.31 C 36.73, H 4.48, N 23.36	81	216.3–218.7
4o	*N*-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-((5-(propylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide	$C_{11}H_{16}N_6OS_3$	C 38.35, H 4.68, N 24.40 C 38.43, H 4.69, N 24.43	85	233.0–234.8
4p	2-((5-(isopropylamino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(5-ethyl-1,3,4-thiadiazol-2-yl)acetamide	$C_{11}H_{16}N_6OS_3$	C 38.35, H 4.68, N 24.40 C 38.46, H 4.68, N 24.45	78	226.3–228.9
4r	2-((5-(allylamino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(5-ethyl-1,3,4-thiadiazol-2-yl)acetamide	$C_{11}H_{14}N_6OS_3$	C 38.58, H 4.12, N 24.54 C 38.66, H 4.11, N 24.59	84	236.2–238.0
4s	2-((5-(butylamino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(5-ethyl-1,3,4-thiadiazol-2-yl)acetamide	$C_{12}H_{18}N_6OS_3$	C 40.20, H 5.06, N 23.44 C 40.32, H 5.07, N 23.50	77	237.9–239.6
4t	2-((5-(isobutylamino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(5-ethyl-1,3,4-thiadiazol-2-yl)acetamide	$C_{12}H_{18}N_6OS_3$	C 40.20, H 5.06, N 23.44 C 40.31, H 5.05, N 23.48	85	220.5–222.8
4u	2-((5-(cyclohexylamino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(5-ethyl-1,3,4-thiadiazol-2-yl)acetamide	$C_{14}H_{20}N_6OS_3$	C 43.73, H 5.24, N 21.85 C 43.81, H 5.25, N 21.92	82	236.2–238.7
4v	*N*-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide	$C_{14}H_{14}N_6OS_3$	C 44.43, H 3.73, N 22.20 C 44.52, H 3.72, N 22.14	76	232.1–234.8
4y	*N*-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-((5-(*p*-tolylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide	$C_{15}H_{16}N_6OS_3$	C 45.90, H 4.11, N 21.41 C 45.82, H 4.10, N 21.43	72	228.1–230.5

Table II. Spectral data of new compounds ***4a-y***

Compd.	FTIR (ATR, cm^{-1})	^1H NMR (300 MHz, DMSO-d_6, δ ppm, J/Hz)	^{13}C NMR (75 MHz, DMSO-d_6, δ ppm)	HRMS (m/z): $[M+H]^+$ Calcd./found
4a	3358-3285 (N-H), 2918 (aliphatic C-H), 1666 (C=O), 1605 (C=N)	2.62 (3H, s, -CH_3), 2.84 (3H, s, -CH_3), 4.11 (2H, s, -CH_2-), 7.77 (1H, br.s., -NH), 12.67 (1H, s, -NH)	15.27 (CH_3), 31.51 (CH_3), 37.77 (CH_2), 148.66 (C-thiadiazole), 158.81 (C-thiadiazole), 160.06 (C-thiadiazole), 166.96 (C=O), 171.15 (C-thiadiazole)	303.0259 303.0329
4b	3352-3272 (N-H), 2916 (aliphatic C-H), 1662 (C=O), 1616 (C=N)	1.14 (3H, t, J = 7.2 Hz, -CH_3), 2.62 (3H, s, -CH_3), 3.25 (2H, q, J = 5.3 Hz, -CH_2-), 4.11 (2H, s, -CH_2-), 7.82 (1H, br.s., -NH), 12.66 (1H, s, -NH)	19.39 (CH_3), 20.02 (CH_3), 42.49 (-CH_2), 44.60 (CH_2), 153.25 (C-thiadiazole), 163.54 (C-thiadiazole), 164.81 (C=O), 171.70 (C-thiadiazole), 174.98 (C-thiadiazole)	317.0440 317.0307
4c	3348-3245 (N-H), 2922 (aliphatic C-H), 1658 (C=O), 1636 (C=N)	2.62 (3H, s, -CH_3), 3.26 (3H, s, -CH_3), 3.42 (2H, t, J = 4.5 Hz, -CH_2-), 3.47 (2H, t, J = 4.6 Hz, -CH_2-), 4.12 (2H, s, -CH_2-), 7.92 (1H, br.s., -NH), 12.67 (1H, s, -NH)	15.27 (CH_3), 37.67 (CH_2), 44.42 (CH_2), 58.41 (-OCH_3), 70.38 (CH_2), 148.92 (C-thiadiazole), 158.79 (C-thiadiazole), 160.06 (C-thiadiazole), 166.94 (C=O), 170.18 (C-thiadiazole).	347.0558 347.0413
4d	3334-3262 (N-H), 2975 (aliphatic C-H), 1660 (C=O), 1624 (C=N)	0.92 (3H, t, J = 7.2 Hz, -CH_3), 1.62 (2H, q, J = 7.0 Hz, -CH_2-), 3.12-3.18 (2H, m, -CH_2-), 4.13 (2H, s, -CH_2-), 7.90 (1H, br.s., -NH), 12.72 (1H, s, -NH)	11.52 (CH_3), 15.27 (CH_3), 22.19 (CH_2), 37.78 (CH_2), 46.80 (CH_2), 149.55 (C-thiadiazole), 157.63 (C-thiadiazole), 165.61 (C=O), 169.01(C-thiadiazole), 171.38 (C-thiadiazole)	331.0591 331.0464
4e	3384-3254 (N-H), 2982 (aliphatic C-H), 1662 (C=O), 1644 (C=N)	1.15 (6H, d, J = 6.5 Hz, -CH_3), 2.61 (3H, s, -CH_3), 3.69-3.80 (1H, m, -CH-), 4.11 (2H, s, -CH_2-), 7.77 (1H, br.s., -NH), 12.66 (1H, s, -NH)	15.27 (CH_3), 22.55 (CH_3), 37.70 (CH_2), 47.00 (CH_2), 148.36 (C-thiadiazole), 158.79 (C-thiadiazole), 160.06 (C-thiadiazole), 166.96 (C=O), 169.41 (C-thiadiazole)	331.0601 331.0464
4f	3384-3254 (N-H), 2982 (aliphatic C-H), 1662 (C=O), 1644 (C=N)	2.61 (3H, s, -CH_3), 3.88 (2H, t, J = 5.3 Hz, -CH_2-), 4.12 (2H, s, -CH_2-), 5.16 (2H, qd, J_1 = 1.7 Hz, J_2 = 15.5 Hz, -CH_2-), 5.81-5.93 (1H, m, -CH-), 7.99 (1H, br.s., -NH), 12.67 (1H, s, -NH)	15.27 (CH_3), 37.68 (CH_2), 47.09 (CH_2), 116.72 (CH), 134.67 (CH), 149.09 (C-thiadiazole), 158.79 (C-thiadiazole), 160.06 (C-thiadiazole), 166.93 (C=O), 170.20 (C-thiadiazole)	331.0601 331.0464

Compd.	FTIR (ATR, cm^{-1})	^1H NMR (300 MHz, DMSO-d_6, δ ppm, *J*/Hz)	^{13}C NMR (75 MHz, DMSO-d_6, δ ppm)	HRMS (*m*/*z*): $[M+H]^+$ Calcd./found
4g	3334-3282 (N-H), 2980 (aliphatic C-H), 1662 (C=O), 1636 (C=N)	0.87 (3H, t, *J* = 7.2 Hz, $-CH_3$), 1.31 (2H, q, *J* = 7.5 Hz, $-CH_2-$), 1.49 (2H, q, *J* = 7.3 Hz, $-CH_2-$), 2.61 (3H, s, $-CH_3$), 3.22 (2H, q, *J* = 5.6 Hz, $-CH_2-$), 4.10 (2H, s, $-CH_2-$), 7.82 (1H, br.s., -NH), 12.66 (1H, s, -NH)	18.82 (CH_3), 20.02 (CH_3), 22.15 (CH_2), 24.72 (CH_2), 35.73 (CH_2), 49.46 (CH_2), 153.12 (C-thiadiazole), 163.55 (C-thiadiazole), 164.80 (C=O), 171.71 (C-thiadiazole), 175.17 (C-thiadiazole)	345.0760 345.0620
4h	3388-3222 (N-H), 2977 (aliphatic C-H), 1658 (C=O), 1605 (C=N)	0.88 (6H, d, *J* = 6.7 Hz, $-CH_3$), 1.77-1.90 (1H, m, -CH-), 2.61 (3H, s, $-CH_3$), 3.05 (2H, dd, J_1 = 5.7 Hz, J_2 = 6.7 Hz, $-CH_2-$), 4.10 (2H, s, $-CH_2-$), 7.87 (1H, br.s., -NH), 12.66 (1H, s, -NH)	15.27 (CH_3), 20.49 (CH_3), 27.95 (CH), 37.72 (CH_2), 52.63 (CH_2), 148.33 (C-thiadiazole), 158.79 (C-thiadiazole), 160.05 (C-thiadiazole), 166.96 (C=O), 170.59 (C-thiadiazole)	345.0759 345.0620
4i	3340-3263 (N-H), 2992 (aliphatic C-H), 1657 (C=O), 1622 (C=N)	1.18-1.30 (6H, m, -cyclohexyl), 1.52-1.56 (1H, m, -cyclohexyl), 1.65-1.68 (2H, m, -cyclohexyl), 1.89-1.93 (2H, m, -cyclohexyl), 2.61 (3H, s, $-CH_3$), 4.10 (2H, s, $-CH_2-$), 7.78 (1H, br.s., -NH), 12.65 (1H, s, -NH)	15.27 (CH_3), 24.64 (cyclohexyl CH_2), 25.64 (cyclohexyl CH_2), 26.14 (cyclohexyl CH_2), 32.43 (cyclohexyl CH_2), 33.22 (cyclohexyl CH_2), 37.74 (CH_2), 53.91 (cyclohexyl CH), 148.26 (C-thiadiazole), 158.81 (C-thiadiazole), 160.04 (C-thiadiazole), 166.98 (C=O), 169.43 (C-thiadiazole)	371.0950 371.0777
4j	3303-3218 (N-H), 2979 (aliphatic C-H), 1654 (C=O), 1628 (C=N)	2.61 (3H, s, $-CH_3$), 4.24 (2H, s, $-CH_2-$), 6.99 (1H, t, *J* = 7.3 Hz, -phenyl), 7.33 (2H, t, *J* = 7.5 Hz, -phenyl), 7.55 (2H, d, *J* = 7.7 Hz, -phenyl), 10.41 (1H, s, -NH), 12.73 (1H, s, -NH)	15.28 (CH_3), 37.32 (CH_2), 117.86 (phenyl CH), 122.51 (phenyl CH), 129.59 (phenyl CH), 140.75 (phenyl C), 158.83 (C-thiadiazole), 160.09 (C-thiadiazole), 165.59 (C=O), 166.82 (C-thiadiazole)	365.0459 365.0307
4k	3354-3224 (N-H), 2980 (aliphatic C-H), 1660 (C=O), 1636 (C=N), 850 (1,4-disubstituted benzene)	2.24 (3H, s, $-CH_3$), 2.60 (3H, s, $-CH_3$), 4.21 (2H, s, $-CH_2-$), 7.13 (2H, d, *J* = 8.3 Hz, -phenyl), 7.43 (2H, d, *J* = 8.5 Hz, -phenyl), 10.31 (1H, s, -NH), 12.68 (1H, s, -NH)	15.32 (CH_3), 20.79 (CH_3), 37.64 (CH_2), 117.97 (phenyl CH), 129.96 (phenyl CH), 131.48 (phenyl C), 138.40 (phenyl C), (C-thiadiazole), 159.40 (C-thiadiazole), 159.83 (C-thiadiazole), 165.79 (C=O), 167.05 (C-thiadiazole)	379.0627 379.0464
4l	3308-3218 (N-H), 2980 (aliphatic C-H), 1654 (C=O), 1606 (C=N)	1.28 (3H, t, *J* = 7.5 Hz, $-CH_3$), 2.84 (3H, s, $-CH_3$), 2.98 (2H, q, *J* = 7.5 Hz, $-CH_2-$), 4.10 (2H, s, -CH2-), 7.77 (1H, br.s., -NH), 12.68 (1H, s, -NH)	14.27 (CH_3), 23.16 (CH_2), 31.51 (CH_3), 37.88 (CH_2), 148.73 (C-thiadiazole), 158.72 (C-thiadiazole), 160.10 (C-thiadiazole), 167.06 (C=O), 171.13 (C-thiadiazole)	317.0441 317.0307

Compd.	FTIR (ATR, cm^{-1})	^1H NMR (300 MHz, DMSO-d_6, δ ppm, *J*/Hz)	^{13}C NMR (75 MHz, DMSO-d_6, δ ppm)	HRMS (*m*/*z*): $[M+H]^+$ Calcd./found
4m	3344-3262 (N-H), 2972 (aliphatic C-H), 1652 (C=O), 1632 (C=N)	1.33 (3H, t, *J* = 7.2 Hz, -CH_3), 1.29 (3H, t, *J* = 7.5 Hz, -CH_3), 2.98 (2H, q, *J* = 7.5 Hz, -CH_2-), 3.25 (2H, q, *J* = 5.3 Hz, -CH_2-), 4.10 (2H, s, -CH_2-), 7.82 (1H, br.s., -NH), 12.69 (1H, s, -NH)	14.27 (CH_3), 14.64 (CH_3), 23.16 (CH_2), 37.80 (CH_2), 39.84 (CH_2), 148.55 (C-thiadiazole), 158.63 (C-thiadiazole), 166.14 (C-thiadiazole), 167.04 (C=O), 170.21 (C-thiadiazole)	331.0601 331.0464
4n	3351-3247 (N-H), 2972 (aliphatic C-H), 1658 (C=O), 1636 (C=N)	1.28 (3H, t, *J* = 7.5 Hz, -CH_3), 2.98 (2H, q, *J* = 7.5 Hz, -CH_2-), 3.25 (3H, s, -CH_3), 3.41 (2H, t, *J* = 4.6 Hz, -CH_2-), 3.46 (2H, t, *J* = 4.4 Hz, -CH_2-), 4.10 (2H, s, -CH_2-), 7.91 (1H, br.s., -NH), 12.70 (1H, s, -NH)	14.28 (CH_3), 23.17 (CH_2), 37.81 (CH_2), 44.41 (CH_2), 58.41 (OCH_3), 70.38 (CH_2), 149.02 (C-thiadiazole), 158.78 (C-thiadiazole), 166.07 (C=O), 167.09 (C-thiadiazole), 170.16 (C-thiadiazole)	361.0745 361.0570
4o	3314-3227 (N-H), 2995 (aliphatic C-H), 1650 (C=O), 1618 (C=N)	0.88 (3H, t, *J* = 7.4 Hz, -CH_3), 1.28 (3H, t, *J* = 7.5 Hz, -CH_3), 1.54 (2H, q, *J* = 7.11 Hz, -CH_2-), 2.98 (2H, q, *J* = 7.6 Hz, -CH_2-), 3.14-3.21 (2H, m, -CH_2-), 4.10 (2H, s, -CH_2-), 7.85 (1H, br.s., -NH), 12.69 (1H, s, -NH)	11.81 (CH_3), 14.27 (CH_3), 22.19 (CH_2) 23.15 (CH_2), 37.78 (CH_2), 46.80 (CH_2), 148.42 (C-thiadiazole), 158.59 (C-thiadiazole), 166.16 (C=O), 167.04 (C-thiadiazole), 170.43 (C-thiadiazole)	345.0759 345.0620
4p	3305-3244 (N-H), 2980 (aliphatic C-H), 1654 (C=O), 1616 (C=N)	1.15 (6H, d, *J* = 6.5 Hz, -CH_3), 1.28 (3H, t, *J* = 7.5 Hz, -CH_3), 2.98 (2H, q, *J* = 7.5 Hz, -CH_2-), 3.69-3.80 (1H, m, -CH-), 4.09 (2H, s, -CH_2-), 7.77 (1H, br.s., -NH), 12.71 (1H, s, -NH)	14.29 (CH_3), 22.56 (CH_3), 23.18 (CH_2), 37.95 (CH_2), 46.99 (CH), 148.53 (C-thiadiazole), 158.99 (C-thiadiazole), 165.97 (C=O), 167.19 (C-thiadiazole) 169.38(C-thiadiazole)	345.0765 345.0620
4r	3324-3218 (N-H), 2972 (aliphatic C-H), 1657 (C=O), 1618 (C=N)	1.29 (3H, t, *J* = 7.5 Hz, -CH_3), 2.99 (2H, q, *J* = 7.5 Hz, -CH_2-), 3.88 (2H, t, *J* = 5.4 Hz, -CH_2-), 4.12 (2H, s, -CH_2-), 5.16 (2H, qd, J_1 = 1.7 Hz, J_2 = 15.6 Hz, -CH_2-), 5.81-5.94 (1H, m, -CH-), 8.00 (1H, br.s., -NH), 12.70 (1H, s, -NH)	14.27 (CH_3), 23.15 (CH_2), 37.72 (CH_2), 47.10 (CH_2), 116.72 (CH) 134.69 (CH), 149.10 (C-thiadiazole), 158.54 (C-thiadiazole), 166.18 (C=O), 166.99 (C-thiadiazole), 170.21 (C-thiadiazole)	343.0604 343.0464
4s	3348-3218 (N-H), 2980 (aliphatic C-H), 1654 (C=O), 1624 (C=N)	0.88 (3H, t, *J* = 7.3 Hz, -CH_3), 1.26-1.38 (5H, m, -CH_2-, -CH_3), 1.50 (2H, q, *J* = 7.3 Hz, -CH_2-), 2.99 (2H, q, *J* = 7.5 Hz, -CH_2-), 3.22 (2H, q, *J* = 5.5 Hz, -CH_2-), 4.11 (2H, s, -CH_2-), 7.83 (1H, br.s., -NH), 12.70 (1H, s, -NH)	14.06 (CH_3), 14.26 (CH_3), 19.97 (CH_2), 23.15 (CH_2), 30.98 (CH_2), 37.74 (CH_2), 44.71 (CH_2), 148.37 (C-thiadiazole), 158.50 (C-thiadiazole), 166.19 (C=O), 166.99 (C-thiadiazole), 170.42 (C-thiadiazole)	359.0964 359.0777

Compd.	FTIR (ATR, cm^{-1})	^1H NMR (300 MHz, DMSO-d_6, δ ppm, J/Hz)	^{13}C NMR (75 MHz, DMSO-d_6, δ ppm)	HRMS (m/z): $[M+H]^+$ Calcd./found
4t	3362-3241 (N-H), 2980 (aliphatic C-H), 1661 (C=O), 1628 (C=N)	0.88 (6H, d, J = 6.7 Hz, -CH_3), 1.29 (3H, t, J = 7.5 Hz, -CH_3), 1.80-1.88 (1H, m, -CH-), 2.99 (2H, q, J = 7.5 Hz, -CH_2-), 3.05 (2H, dd, J_1 = 5.7 Hz, J_2 = 6.7 Hz, -CH_2-), 4.09 (2H, s, -CH_2-), 7.87 (1H, br.s., -NH), 12.68 (1H, s, -NH)	14.28 (CH_3), 20.50 (CH_3), 23.17 (CH_2), 27.95 (CH), 37.90 (CH_2), 52.63 (CH_2), 148.45 (C-thiadiazole), 158.84 (C-thiadiazole), 166.04 (C=O), 167.14 (C-thiadiazole), 170.58 (C-thiadiazole)	359.0960 359.0777
4u	3308-3242 (N-H), 2980 (aliphatic C-H), 1654 (C=O), 1632 (C=N)	1.18-1.22 (6H, m, -cyclohexyl), 1.29 (3H, t, J = 7.5 Hz, -CH_3), 1.52-1.56 (1H, m, -cyclohexyl), 1.65-1.69 (2H, m, -cyclohexyl), 1.89-1.93 (2H, m, -cyclohexyl), 2.99 (2H, q, J = 7.5 Hz, -CH_2-), 4.09 (2H, s, -CH_2-), 7.80 (1H, br.s., -NH), 12.67 (1H, s, -NH)	14.27 (CH_3), 23.15 (CH_2), 24.65 (cyclohexyl CH_2), 25.64 (cyclohexyl CH_2), 26.42 (cyclohexyl CH_2), 32.43 (cyclohexyl CH_2), 33.14 (cyclohexyl CH_2), 37.74 (CH_2), 53.89 (cyclohexyl CH), 148.21 (C-thiadiazole), 158.46 (C-thiadiazole), 166.21 (C=O), 166.98 (C-thiadiazole), 169.45 (C-thiadiazole)	385.1102 385.0933
4v	3382-3255 (N-H), 2980 (aliphatic C-H), 1657 (C=O), 1618 (C=N)	1.28 (3H, t, J = 7.5 Hz, -CH_3), 2.99 (2H, q, J = 7.5 Hz, -CH_2-), 4.23 (2H, s, -CH_2-), 6.99 (1H, t, J = 7.4 Hz, -phenyl), 7.33 (2H, t, J = 7.5 Hz, -phenyl), 7.55 (2H, d, J = 7.7 Hz, -phenyl), 10.41 (1H, s, -NH), 12.68 (1H, s, -NH)	14.28 (CH_3), 23.19 (CH_2), 37.59 (CH_2), 117.85 (phenyl CH), 122.50 (phenyl CH), 129.58 (phenyl CH), 140.77 (phenyl C), 152.12 (C-thiadiazole), 159.06 (C-thiadiazole), 165.57 (C=O), 166.00 (C-thiadiazole), 167.05 (C-thiadiazole)	379.0624 379.0464
4y	3361-3240 (N-H), 2977 (aliphatic C-H), 1664 (C=O), 1632 (C=N), 844 (1,4-disubstituted benzene)	1.28 (3H, t, J = 7.5 Hz, -CH_3), 2.24 (3H, s, -CH_3), 2.99 (2H, q, J = 7.5 Hz, -CH_2-), 4.21 (2H, s, -CH_2-), 7.13 (2H, d, J = 8.3 Hz, -phenyl), 7.43 (2H, d, J = 8.5 Hz, -phenyl), 10.31 (1H, s, -NH), 12.68 (1H, s, -NH)	14.28 (CH_3), 20.82 (CH_3), 23.20 (CH_2), 37.67 (CH_2), 117.98 (phenyl CH), 129.96 (phenyl CH), 131.48 (phenyl C), 138.40 (phenyl C), 151.62 (C-thiadiazole), 159.13 (C-thiadiazole), 165.80 (C=O), 165.96 (C-thiadiazole) 167.09 (C-thiadiazole)	393.0787 393.0620

*Table III. IC_{50} values of compounds **4a**-**y** against cancer cell lines*

	IC_{50} (mmol L^{-1})				
Compd.	MCF-7	A549	Compd.	MCF-7	A549
4a	0.354 ± 0.261	0.119 ± 0.071	**4m**	0.806 ± 0.246	0.380 ± 0.003
4b	≥ 1	0.317 ± 0.093	**4n**	≥ 1	0.318 ± 0.062
4c	0.593 ± 0.073	0.848 ± 0.124	**4o**	≥ 1	≥ 1
4d	≥ 1	≥ 1	**4p**	≥ 1	0.276 ± 0.081
4e	≥ 1	0.356 ± 0.220	**4r**	0.174 ± 0.065	0.223 ± 0.086
4f	≥ 1	0.230 ± 0.052	**4s**	≥ 1	0.694 ± 0.068
4g	≥ 1	≥ 1	**4t**	0.133 ± 0.019	0.103 ± 0.008
4h	≥ 1	0.197 ± 0.153	**4u**	≥ 1	≥ 1
4i	≥ 1	≥ 1	**4v**	0.116 ± 0.041	0.075 ± 0.031
4j	≥ 1	0.091 ± 0.025	**4y**	0.084 ± 0.020	0.034 ± 0.008
4k	≥ 1	0.324 ± 0.011	Cisplatin	0.019 ± 0.009	0.013 ± 0.003
4l	≥ 1	0.326 ± 0.072			

Mean ± SD, $n \geq 3$.

*Table IV. IC_{50} against NIH3T3 and SI values of compounds **4j**, **4v** and **4y***

Compd.	IC_{50} (mmol L^{-1}) NIH3T3	SI
4j	1.980 ± 0.066	21.76
4v	1.764 ± 0.077	15.17
4y	1.791 ± 0.087	21.31
Cisplatin	$111.26 \pm 3.74_5$	8558.46

Mean ± SD, $n = 3$.

SAR studies

Compound **4j** and **4v**, both containing phenyl moiety, showed IC_{50} values of 0.091 and 0.075 mmol L^{-1}, resp., against A549, compared to the reference drug cisplatin (0.013 mmol L^{-1}). Additionally, compound **4y**, containing toluene moiety, showed IC_{50} values of 0.084 and 0.034 mmol L^{-1} against MCF-7 and A549, resp.

When the activity results are compared, it is seen that the introduction of an aromatic ring at the amine end of the thiadiazole increased the anticancer activity. However, the

introduction of propyl or cyclohexyl at the amine end of the thiadiazole dramatically reduced the anticancer activity.

It is crucial that an anticancer agent affects the cancer cell line with having minimal or no effect on healthy cells. For this purpose, the cytotoxic effects of the most active compounds (**4j**, **4v** and **4y**) were investigated on the NIH3T3 cell line. As seen in Table III, IC_{50} of 0.084 ± 0.020 mmol L^{-1} of compound **4y** against MCF-7 was 20-times lower than its IC_{50} of 1.790 ± 8.706 mmol L^{-1} against NIH3T3 cell line. The IC_{50} value of cisplatin was found to be greater than 1 mmol L^{-1} against NIH3T3 cell line.

The selectivity index (SI) was calculated for the effectiveness of selected compounds **4j**, **4v** and **4y**. The compounds with SI value > 3 are considered to exhibit selective inhibition towards cancer cells (26). Compounds **4j**, **4v** and **4y** exhibited good selectivity since their SI values were in the range from 15 to 21 (Table IV).

Aromatase inhibition assay

Compound **4y** which was found cytotoxic to MCF7 cancer cell line was submitted to aromatase inhibition assay. For this assay, a commercially available CYP19A inhibitor screening kit was used and letrozole was used as a positive control. Compound **4y** caused 50 % aromatase enzyme inhibition at 0.062 ± 0.004 mmol L^{-1}, whereas the respective value for letrozole was calculated as 0.020 ± 0.001 mmol L^{-1}.

CONCLUSIONS

In this study, we synthesized new *N*-(5-substituted-1,3,4-thiadiazol-2-yl)-2-[(5 (substituted--amino)-1,3,4-thiadiazol-2-yl)thio]acetamides (**4a**-**y**) and evaluated their anticancer potency. It was found that compound **4y** (*N*-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-((5-(*p*-tolylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide) showed promising activity against A549 and MCF-7 cell lines as well as promising anti-aromatase activity.

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Supplementary material available upon request.

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