European Journal of Medicinal Chemistry 55 (2012) 432–438

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

A series of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles as potent cytotoxic agents

Dalip Kumar [a,*], N. Maruthi Kumar [a], Brett Noel [b], Kavita Shah [b,**]

[a] Department of Chemistry, Birla Institute of Technology and Science, Pilani, Rajasthan 333 031, India
[b] Department of Chemistry and Purdue Cancer Center, Purdue University, 560 Oval Drive, West Lafayette, IN 47907, USA

ARTICLE INFO

Article history:
Received 4 March 2012
Received in revised form
7 June 2012
Accepted 22 June 2012
Available online 3 July 2012

Keywords:
2-Arylamino-1,3,4-thiadiazoles
Indolyl heterocycles
Cytotoxic agents
1,3,4-Thiadiazoles

ABSTRACT

A series of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles **6a**–**v** were prepared and studied for their anticancer activity against selected human cancer cell lines. The reaction of indolylhydrazides **3a**–**h** with a variety of aryl isothiocyanates **4** afforded the key intermediate thiosemicarbazides **5a**–**v**, which upon treatment with acetyl chloride produced the 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles **6a**–**v** in good yields. Most of the synthesized compounds showed selective cytotoxicity towards human breast cancer cell line (MDA-MB-231). Of the synthesized 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles, compound **6f** is the most potent towards tested cancer cell lines (IC_{50} = 0.15–1.18 μM).

1. Introduction

The indole scaffold represents one of the most important structural units prevalent in various naturally occurring and bioactive compounds. Recently, there has been an increasing interest in the indole derivatives to identify new drug candidates, particularly possessing five-membered heterocycles [1,2]. Many natural and synthetic bioactive indolyl heterocycles have demonstrated the critical role of indole scaffold in their biological activities [3,4]. Indolylazoles (**1a**) such as 5-(3′-indolyl)oxazoles (Labradorins 1 & 2) and indolylthiazoles are known for their cytotoxic activities against NCI–H 460 (lung-NSC) human cancer cell line [5]. Marine indole alkaloids, Meridianins (**1b**) and their synthetic analogues have shown prominent anticancer activities against various cancer cell lines with best IC_{50} values against MCF-7 breast cancer cell line (0.25 μg/mL) [6]. A diverse variety of indolylazoles with five/six-membered heterocyclic ring were also evaluated for their anticancer activities [5,7–10].

1,3,4-Thiadiazoles are an important class of heterocyclic compounds with various biological activities such as antiviral [11], antimicrobial [12], fungicidal [13], antiinflammatory [14], antihypertensive [15], antituberculosis [16], antileishmanial [17], antidepressant [18] and anticancer [19]. Some of the marketed drugs with 1,3,4-thiadiazole scaffold such as Acetazolamide, Methazolamide and Globucid are well-known for their therapeutic applications [20]. 1,3,4-Thiadiazoles, bioisosters of oxadiazoles and thiazoles, are known to have interesting electro-optical properties and also act as corrosion and oxidation inhibitors, complexation reagents for dyes and metal ions. Furthermore, widely explored 2-aminothiadiazoles (ATDA) are in clinical trials for the treatment of patients with different tumours [21,22]. The 2-(4-fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (FABT) **1c** is a promising anticancer compound for malignant tumours of nervous system. FABT has the ability to decrease the proliferation of cancer cells and their metastasis [23,24] (Fig. 1). Naturally occurring antimitotic agents Colchicine (**1d**), Combretastatin A4 (**1e**) and their synthetic analogues have been extensively studied for cancer chemotherapy, however, lacks anticancer efficacy *in-vivo* at its maximum tolerated dose (MTD) [25–27]. In analogy to these natural products, several synthetic Combretastatin analogues bearing the trimethoxyphenyl moiety were also screened for their anticancer properties [28–31]. Recently, we have reported series of indolylazoles including 4-(3′-indolyl)oxazoles, 5-(3′-indolyl)-1,3,4-oxadiazoles, 5-(3′-indolyl)-1,3,4-thiadiazoles and indolyl-1,2,4-triazoles as potential anticancer agents against various human cancer cell lines [8,32–37]. In continuation of our efforts to identify indole-based potent anticancer agents, in this study we synthesized a variety of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles **6a**–**v** by combining bio-active indole and 2-arylamino-1,3,4-thiadiazole scaffolds into

* Corresponding author. Tel.: +91 1596 245073x279.
** Corresponding author. Tel.: +1 765 496 9470.
E-mail addresses: dalipk@bits-pilani.ac.in (D. Kumar), shah23@purdue.edu (K. Shah).

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2012.06.047

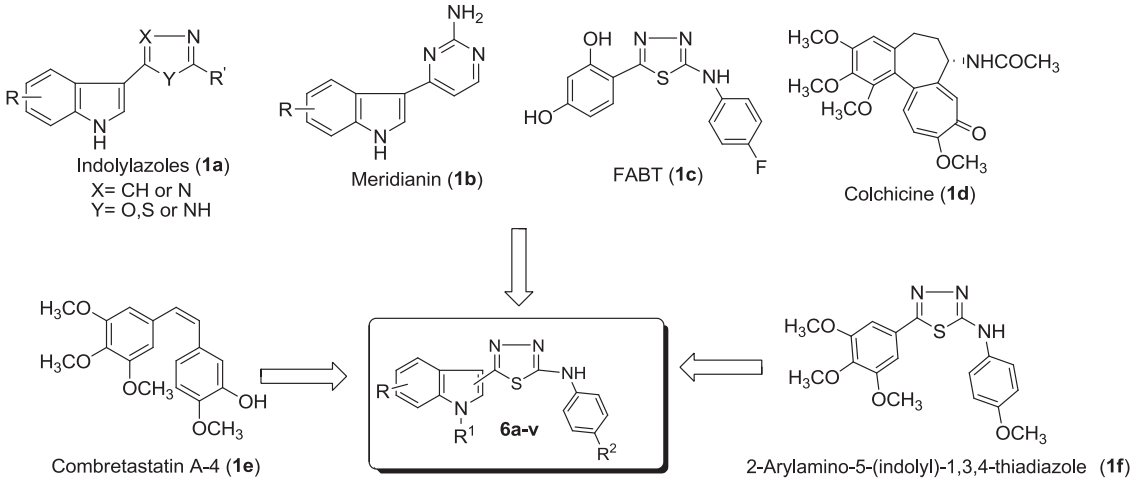


Fig. 1. Rational approach to design 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles **6a**–**v**.

single small molecules and tested their anticancer activities against a selected panel of human cancer cell lines (Fig. 1).

2. Results and discussion

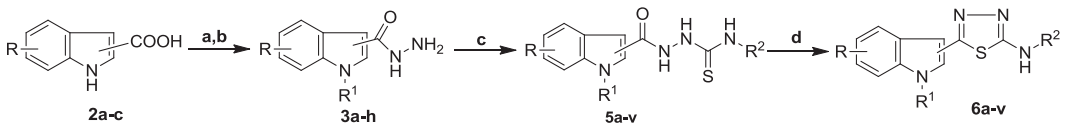
2.1. Chemistry

General synthetic methods for the construction of 1,3,4-thiadiazoles involve the use of diacylhydrazide precursors under various reaction conditions. Symmetrical 2,5-disubstituted-1,3,4-thiadiazoles were prepared by the condensation reaction of aryl aldehydes, hydrazine hydrate and sulphur in ethanol under microwave irradiation [38]. A robust protocol for the solid phase synthesis of 5-alkyl/aryl-2-alkylamino-1,3,4-thiadiazoles was described from resin bound thiosemicarbazides [39]. Oruc et al. prepared 1,3,4-thiadiazoles in four steps utilizing acyl halides and aryl isothiocyanates [16]. The 1,3,4-thiadiazoles were also achieved from the reaction of 1,3,4-oxadiazoles with thiourea [40]. Recently, Kumar et al. reported one-pot convenient synthesis of 1,3,4-thiadiazoles **1f** by reacting aryl aldehydes, hydrazine hydrate and aryl isothiocyanates and studied their anticancer activities [32]. Rostamizadeh et al. utilizes ionic liquid or thiourea mediated oxidation of *in situ* generated thiosemicarbazones for the one-pot synthesis of 1,3,4-thiadiazoles [41,42].

Our initial attempts to synthesize 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles **6** using reported protocols [32] involved oxidative cyclization of the corresponding thiosemicarbazone obtained via the reaction of aryl thiosemicarbazide with indole-3-carboxaldehyde. The cyclization of thiosemicarbazone **5a** using reagents like $FeCl_3$, $ZnCl_2$, iodobenzene diacetate, ferric ammonium sulphate and chloramines-T was failed to give the desired 2-arylamino-5-indolyl-1,3,4-thiadiazole **6a**. In an alternative route to synthesize desired indolyl-1,3,4-thiadiazoles **6a**–**v** involved the initial preparation of indolylhydrazides **3a**–**h** from the corresponding indole carboxylic acids **2a** as illustrated in the Scheme 1. The reaction of indolylhydrazides **3a**–**h** and aryl isothiocyanates **4** in ethanol afforded the intermediate thiosemicarbazides **5a**–**v** in good yields. Finally, the oxidative cyclization of **5a**–**v** was accomplished in presence of acetyl chloride to afford **6a**–**v** in good yields (Scheme 1). Optimized reaction conditions were used to synthesize a series of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles **6a**–**v** from indolylhydrazides **3a**–**h** and aryl isothiocyanates **4**. All the synthesized compounds were well characterized by their IR, Mass and NMR (^1H & ^{13}C) spectral data.

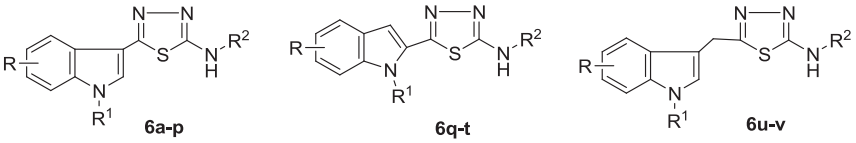
3. *In-vitro* anticancer activity

All the synthesized 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles **6a**–**v** were screened against prostate (DU145 and LnCaP), breast (MCF-7 and MDA-MB-231), cervical (HeLa) and ovarian (Ovcar-3) human cancer cell lines (Table 1). Most of the compounds exhibited high selectivity towards breast cancer cell line (MBA-MB-231) with IC_{50} values of less than 1 μM. Activity results proved that substituents at C-2 and C-5 positions of the 1,3,4-thiadiazole ring play a crucial role in imparting the anticancer activity. Incorporation of an indole ring at 5-position of 1,3,4-thiadiazole led to significant improvement in anticancer activity when compared with an aryl ring as reported in our previous studies on 2-arylamino-5-aryl-1,3,4-thiadiazoles [32]. For example, the compound **6a** (IC_{50} = 0.9 μM) showed 100-fold improved cytotoxicity when compared with the previously reported 2-phenylamino-5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadizole (IC_{50} = 122.4 μM) against MDA-MB-231 breast cancer cell line [32]. The compound **6a** exhibited sixty fold selective cytotoxicity against tested breast cancer cell line, MDA-MB-231 (IC_{50} = 0.9 μM) over MCF-7 (IC_{50} = 55.58 μM) and showed moderate activity against Ovcar-3, LnCap and HeLa cancer cell lines ($<IC_{50}$ = 10 μM). The para-substitutions (CH_3, Cl, OCH_3 and CF_3) on C-2 arylamino moiety led to compounds **6b**–**e** without any significant improvement in cytotoxicity, except the compounds **6c** and **6e** possessing electron withdrawing groups (Cl and CF_3) exhibited slight increase in cytotoxicity against LnCap (IC_{50} = 3.14 and 5.24 μM) and MDA-MB-



Scheme 1. Reagents and conditions: (a) (i) EtOH/H^+; (ii) R^1X/NaH; (b) $NH_2NH_2.H_2O$, EtOH, reflux; (c) R^2NCS (4), EtOH, 60 °C; (d) CH_3COCl, 20 °C.

Table 1
In-vitro cytotoxicity data of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles 6a–v against selected human cancer cell lines.



Compound	*R*	R^1	R^2	IC_{50} (μM)					
				MCF-7	LnCap	MDA-MB-231	DU145	HeLa	Ovcar-3
6a	H	H	C_6H_5	55.58	7.57	0.91	19.70	8.41	3.49
6b	H	H	4-$CH_3C_6H_4$	21.10	7.37	0.82	31.97	27.14	3.25
6c	H	H	4-ClC_6H_4	10.19	3.14	0.55	22.88	10.08	4.05
6d	H	H	4-$OCH_3C_6H_4$	8.79	10.65	0.94	22.17	19.44	3.58
6e	H	H	4-$CF_3C_6H_4$	16.50	5.24	0.51	20.95	21.58	3.70
6f	H	H	3,4,5-$(OCH_3)_3C_6H_2$	0.91	0.15	0.44	22.35	3.64	1.18
6g	H	H	$CH_2C_6H_5$	34.56	10.51	0.62	63.92	>100	3.81
6h	H	CH_3	C_6H_5	11.77	6.63	0.47	21.63	47.88	3.85
6i	H	CH_3	4-ClC_6H_4	12.81	8.31	0.44	19.44	10.60	3.96
6j	H	4-$ClC_6H_4CH_2$	C_6H_5	9.13	18.32	0.99	18.04	30.80	3.55
6k	H	4-$ClC_6H_4CH_2$	3,4,5-$(OCH_3)_3C_6H_2$	10.59	7.53	0.81	24.37	40.00	3.69
6l	H	4-$OCH_3C_6H_4CH_2$	4-$OCH_3C_6H_4$	8.88	11.45	0.90	21.16	72.87	3.68
6m	H	4-$OCH_3C_6H_4CH_2$	3,4,5-$(OCH_3)_3C_6H_2$	23.15	12.38	0.90	90.33	50.73	3.54
6n	5-OCH_3	H	4-$OCH_3C_6H_4$	21.68	7.58	0.58	16.11	24.36	3.43
6o	6-OCH_3	H	4-$OCH_3C_6H_4$	23.38	2.16	0.93	19.81	63.16	3.56
6p	6-OCH_3	H	3,4,5-$(OCH_3)_3C_6H_2$	13.37	3.69	0.64	18.95	32.34	3.46
6q	H	H	4-ClC_6H_4	7.55	11.38	0.64	16.42	21.79	3.30
6r	H	H	4-$OCH_3C_6H_4$	8.04	2.93	0.23	28.83	14.39	3.10
6s	H	H	3,4,5-$(OCH_3)_3C_6H_2$	6.48	8.67	0.25	42.02	9.74	2.00
6t	H	H	$CH_2C_6H_5$	9.92	5.92	0.46	53.75	86.57	3.75
6u	H	H	4-$OCH_3C_6H_4$	12.72	2.30	5.94	27.74	>100	3.82
6v	H	H	3,4,5-$(OCH_3)_3C_6H_2$	>100	5.91	1.34	24.46	>100	3.29
Doxorubicin				8.62	2.76	19.01	10.86	20.93	10.53

231 (IC_{50} = 0.55 and 0.51 μM) when compared to compound **6a** (MCF-7 activity). Our earlier studies with indolyl-1,2,4-triazoles and indolyl chalcones delivered potent compounds consisting a 3,4,5-trimethoxyphenyl moiety. On similar grounds the compound **6f** was synthesized with enhanced cytotoxicity against all the tested cancer cell lines at sub-micromolar concentrations (LnCap (IC_{50} = 0.15 μM) and MCF-7 (IC_{50} = 0.91 μM), MDA-MB-231 (IC_{50} = 0.44 μM) and Ovcar-3 (IC_{50} = 1.18 μM)). Benzylamino group at C-2 position (compound **6g**) has no impact on cytotoxicity. *N*-Methylation of indole ring (compounds **6h**–**i**) was found to be beneficial for the anticancer activity when compared with their parent compounds (**6a** and **6c**). In particular, *N*-methylindole analogues **6h** and **6i** exhibited improved cytotoxicity with IC_{50} values of 0.47 and 0.44 μM against MDA-MB-231 breast cancer cell line. However, introduction of bulkier groups (N-1) such as *p*-chlorobenzyl and *p*-methoxyphenyl (compounds **6j**–**m**) is detrimental for the activity against tested cancer cell lines. Introduction of a methoxy group at position-5/6 in the indole ring of **6d** led to compounds **6n** and **6o** with improved cytotoxicity (IC_{50} = 7.58 and 2.16 μM, LnCap) and selectivity. While retaining important trimethoxyphenyl moiety, inclusion of a methoxy substituent at position-6 of indole ring led to compound **6p** with reduced antiproliferative activity. Shifting the connectivity of 1,3,4-thiadiazole with indole ring from C-3 to C-2 position provided the compounds **6q**–**t** with improved cytotoxicity. The compound **6r** found to be more cytotoxic (compounds **6r** vs **6d)** against LnCap and MDA-MB-231 cancer cells with IC_{50} values of 2.93 and 0.23 μM, respectively. The compounds **6s** and **6t** with 3,4,5-trimethoxyphenylamino and benzylamino substituent induced selectivity against MDA-MB-231 (IC_{50} = 0.25 and 0.46 μM) cancer cells when compared with their regioisomers (compounds **6f** and **6g)**. Further, introduction of a methylene unit between indole and 1,3,4-thiadiazole rings (compounds **6u** and **6v**) showed no appreciable improvement in the anticancer activity, particularly resulted in poor cytotoxicity against HeLa cancer cell line (IC_{50} > 100 μM). The compound **6v** with 3,4,5-trimethoxyphenylamino substituent induced selective cytotoxicity against MDA-MB-231 (IC_{50} = 1.34 μM) cancer cells. The synthesized indolylthiadiazoles **6a**–**v** was found to be more cytotoxic against tested cancer cell lines than the control Doxorubicin.

4. Conclusion

We have synthesized a series of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles **6a**–**v** which displayed a potent cytotoxicity against various tested cancer cell lines. Compound **6f** with 3,4,5-trimethoxyphenylamino substituent showed high activity profile against multiple cancer cell lines, particularly promising results against prostate cancer cell line (IC_{50} = 0.15 μM, LnCaP). Most of the 2-arylamino-5-(indolyl)-1,3,4-thiadaizoles **6a**–**v** exhibited good cytotoxic (IC_{50} < 1 μM) selectivity against breast cancer cell line (MDA-MB-231). The anticancer activity results revealed that 2-arylamino-5-(indolyl)-1,3,4-thiadaizoles are more potent than our previously reported 2-aryl-5-(indolyl)-1,3,4-thiadiazoles [33]. The studies to improve cytotoxicity and identify the cellular targets of 2-arylamino-5-(indolyl)-1,3,4-thiadaizoles **6a**–**v** are in progress.

5. Experimental

5.1. Physical measurements

All the laboratory grade reagents were obtained commercially. The reaction was monitored by thin layer chromatography, which was performed on Merck pre-coated plates (silica gel. 60 F_{254}, 0.25 mm) and was visualized by fluorescence quenching under UV light (254 nm). Recrystallization was done by commercially available absolute ethanol. Solvents were evaporated using Büchi rotary evaporator. Melting points were determined with

electrothermal capillary melting point apparatus (E-Z melting). ^{1}H NMR spectra were recorded on a Bruker Advance II (400 MHz) spectrometer. The coupling constant (*J*) values are in Hz. Mass spectra were measured on a 'Hewlett–Packard' model HP GS/MS 5890/5972.

5.2. General procedure for the synthesis of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles **6a–v**

5.2.1. Preparation of indole-2(3)-carboxylic acid and indole-3-acetic acid (**2**)

Indole-3-carboxylic acid, 5-methoxyindole-3-carboxylic acid and 6-methoxyindole-3-carboxylic acid were prepared according to the reported protocol [43]. Indole-2-carboxylic acid and indole-3-acetic acid were procured form commercial sources.

*5.2.1.1. General procedure for the synthesis of indole-2(3)-carbohydrazides and indole-3-acetohydrazide (**3a–h**).* To a solution of indole carboxylic acid **2** (1 mmol) in ethanol (20 mL) was added catalytic amount of concentrated sulphuric acid (0.2 mL) and allowed to reflux for 20 h. After completion of the reaction, ethanol was removed and the residue was extracted with ethyl acetate (30 mL) and washed with saturated sodium bicarbonate solution (25 mL). Organic layer was dried over sodium sulphate and evaporated to give corresponding ester in good yields (85–95%). The solution of an appropriate ester (1 mmol) and hydrazine hydrate (2 mmol) in ethanol (15 mL) was refluxed for 4 h. Reaction mixture was cooled and the solid obtained was filtered and recrystallized from ethanol to obtain pure hydrazides **3a–c**, **3g** and **3h**.

*5.2.1.2. General procedure for the synthesis of N-alkylated indole-3-carbohydrazides (**3d–f**).* To a solution of ethyl ester (10 mmol) of indole-3-carboxylic acid **2a** in tetrahydrofuran (20 mL) was slowly added 60% sodium hydride (15 mmol) and stirred the reaction mixture at 10 °C for 15 min. To this suspension, an appropriate alkyl halide (11 mmol) was added in one portion and continued to stir the contents at room temperature for 4 h. The reaction mixture was carefully poured into ice-cold water and extracted with ethyl acetate (2 × 50 mL). Combined organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford pure *N*-alkylated indole ester which was treated with hydrazine hydrate to obtain *N*-alkylated indole-3-carbohydrazides **3d–f**.

*5.2.1.3. Indole-3-carbohydrazide (**3a**).* Yield 85%; White solid, mp. 232–234 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.31 (s, 1H), 9.14 (s, 1H), 8.17 (d, J = 7.10 Hz, 1H), 7.95 (d, J = 2.80 Hz, 1H), 7.41 (d, J = 8.10 Hz, 1H), 7.20–6.99 (m, 2H), 3.95 (s, 2H). IR (KBr, ν cm^{-1}): 3256, 3109, 1660, 1607, 1583, 1523, 1433, 1240, 736. MS (ESI) *m/z* calcd for $C_9H_9N_3O$ $(M)^+$ 175.1, obsd 175.2.

*5.2.1.4. 5-Methoxyindole-3-carbohydrazide (**3b**).* Yield 90%; White solid, mp. 178–179 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.68 (s, 1H), 9.64 (s, 1H), 7.61 (s, 1H), 7.49 (s, 1H), 7.40 (d, J = 8.00 Hz, 2H), 4.18 (s, 2H), 3.86 (s, 3H). IR (KBr, ν cm^{-1}): 3340, 3290, 3050, 2920, 1646, 1605, 1545, 778, 724. MS (ESI) *m/z* calcd for $C_{10}H_{11}N_3O_2$ $(M + H)^+$ 206.1, obsd 206.2.

*5.2.1.5. 6-Methoxyindole-3-carbohydrazide (**3c**).* Yield 90%; White solid, mp 213–214 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.72 (s, 1H), 9.58 (s, 1H), 7.64 (s, 1H), 7.53 (s, 1H), 7.38 (d, J = 8.00 Hz, 2H), 4.18 (s, 2H), 3.83 (s, 3H). IR (KBr, ν cm^{-1}): 3345, 3265, 2935, 1653, 1610, 1543, 736, 720. MS (ESI) *m/z* calcd for $C_{10}H_{11}N_3O_2$ $(M)^+$ 205.1, obsd 205.1.

*5.2.1.6. 1-Methyl-indole-3-carbohydrazide (**3d**).* Yield 80%; White solid, mp 149–150 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 9.18 (s, 1H), 8.14 (d, J = 7.80 Hz, 1H), 7.92 (d, J = 7.80 Hz, 1H), 7.37 (d, J = 8.10 Hz, 1H), 7.20–6.99 (m, 2H), 4.03 (s, 2H), 3.65 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): δ 160.88, 153.05, 141.03, 136.82, 130.96, 129.00, 125.32 124.60, 122.62, 121.58, 120.90, 117.21, 110.46, 105.69, 33.07. IR (KBr, ν cm^{-1}): 3212, 3098, 1658, 1604, 1582, 1520, 1465, 1235, 740. MS (ESI) *m/z* calcd for $C_{10}H_{11}N_3O$ $(M)^+$ 189.1, obsd 189.1.

*5.2.1.7. 1-(4-Chlorobenzyl)-indole-3-carbohydrazide (**3e**).* Yield 75%; White solid, mp 173–174 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 9.08 (s, 1H), 8.19 (d, J = 8.0 Hz, 1H), 7.98 (s, 1H), 7.37 (d, J = 6.2 Hz, 1H), 7.15 (d, J = 7.0 Hz, 4H), 6.83 (d, J = 7.45 Hz, 2H), 5.28 (s, 2H), 4.43 (s, 2H). IR (KBr, ν cm^{-1}): 3117, 3098, 1648, 1601, 1576, 1518, 1456, 1228, 764. MS (ESI) *m/z* calcd for $C_{16}H_{14}ClN_3O$ $(M + H)^+$ 300.0, obsd 300.0.

*5.2.1.8. 1-(4-Methoxybenzyl)-indole-3-carbohydrazide (**3f**).* Yield 75%; White solid, mp 193–194 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): δ 8.20 (d, J = 7.3 Hz, 1H), 7.83 (s, 1H), 7.33–7.23 (m, 6H), 7.06–7.09 (m, 2H), 5.30 (s, 2H), 4.45 (s, 2H), 3.91 (s, 3H). IR (KBr, ν cm^{-1}): 3112, 3098, 1650, 1602, 1580, 1521, 1448, 1223, 750. MS (ESI) *m/z* calcd for $C_{17}H_{17}N_3O_2$ $(M + H)^+$ 296.1, obsd 296.1.

*5.2.1.9. Indole-2-carbohydrazide (**3g**).* Yield 90%; White solid, mp. 247–248 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): δ 11.70 (s, 1H), 9.85 (s, 1H), 7.80–7.10 (m, 5H), 4.2 (s, 2H). IR (KBr, ν cm^{-1}): 3356 3217, 3080, 2900, 1640, 1615, 1550, 780, 740. MS (ESI) *m/z* calcd for $C_9H_9N_3O$ $(M + H)^+$ 176.1, obsd 176.2.

*5.2.1.10. Indole-3-acetohydrazide (**3h**).* Yield 85%; Off-white solid, mp. 145–146 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): δ 11.0 (s, 1H), 9.65 (s, 1H), 7.59 (dd, J = 7.8 Hz, J = 1.6 Hz, 1H), 7.30 (d, J = 7.8 Hz, 1H), 7.09 (d, J = 7.8 Hz, 1H), 6.98 (d, J = 7.8 Hz, 2H), 4.45 (s, 2H), 3.40 (s, 2H). IR (KBr, ν cm^{-1}): 3317, 1649, 1623, 1235, 1063, 812. MS (ESI) *m/z* calcd for $C_{10}H_{11}N_3O$ $(M + H)^+$ 190.1, obsd 190.1.

5.2.2. General synthesis of 1-(indole-3-carbonyl)-4-arylthiosemicarbazides (**5**)

Indole-2(3)-carbohydrazide **3** (10 mmol) and aryl isothiocyanate **4** (10 mmol) were taken into round bottom flask containing 10 mL of ethanol and stirred the reaction mixture at 60 °C for 2–3 h. The solid was filtered and dried well to obtain thiosemicarbazides **5** in good yields (80–95%). The products were sufficiently pure and taken as such for further reaction.

5.2.3. General procedure for the synthesis of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles (**6**)

The thiosemicarbazide **5** (10 mmol) was charged into 10 mL round bottom flask containing 4 mL of freshly distilled acetyl chloride. The reaction mixture was stirred at 20 °C temperature till the completion of the reaction as monitored by thin layer chromatography. The contents were poured onto crushed ice and neutralized with aqueous ammonia solution. Solid obtained was filtered and recrystallized from ethanol to get desired arylamino-(indolyl)-1,3,4-thiadiazoles **6** in good yields.

*5.2.3.1. 5-(1H-Indol-3-yl)-N-phenyl-1,3,4-thiadiazol-2-amine (**6a**).* Yield 65%; White solid, mp. 210–212 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.52 (s, 1H), 10.16 (s, 1H), 8.18 (d, J = 7.1 Hz, 1H), 7.94 (s, 1H), 7.65–7.59 (m, 3H), 7.30–7.24 (m, 3H), 7.20–7.11 (m, 2H). IR (KBr, ν cm^{-1}): 3377, 3250, 1612, 1570, 1431, 1124, 746, 675. MS (ESI) *m/z* obsd for $C_{16}H_{12}N_4S$: 117.9, 176.0, 260.7, 292.2 $(M)^+$, 331.0 $(M + K)$.

*5.2.3.2. 5-(1H-Indol-3-yl)-N-p-tolyl-1,3,4-thiadiazol-2-amine (**6b**).* Yield 70%; Off-white solid, mp. 205–206 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.54 (s, 1H), 10.07 (s, 1H), 8.20 (s, 1H), 7.75 (d, J = 8.1 Hz, 1H), 7.56 (d, J = 8.2 Hz, 1H), 7.46 (d, J = 8.0 Hz, 1H), 7.39–7.26 (m, 2H), 7.21–7.07 (m, 3H), 2.30 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 161.22, 153.14, 141.01, 136.82, 130.95, 128.77, 124.61, 122.52, 121.38, 120.97, 120.73, 117.21, 109.99, 106.07, 32.33. IR (KBr, ν cm^{-1}): 3236, 3122, 1610, 1534, 1498, 1247, 747, 685. MS (ESI) *m/z* obsd for $C_{17}H_{14}N_4S$: 307.0 (M + H)$^+$, 308.0 (M+2).

*5.2.3.3. N-(4-Chlorophenyl)-5-(1H-indol-3-yl)-1,3,4-thiadiazol-2-amine (**6c**).* Yield 58%; White solid, mp. 232–234 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.59 (s, 1H), 10.42 (s, 1H), 8.17 (d, J = 7.4 Hz, 1H), 8.09 (d, J = 7.2 Hz, 1H), 8.05 (s, 1H), 7.71 (d, J = 8.9 Hz, 2H), 7.46 (d, J = 7.5 Hz, 2H), 7.22–7.10 (m, 2H). IR (KBr, ν cm^{-1}): 3329, 3253, 1622, 1546, 1496, 1116, 745, 669. ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.46, 165.79, 159.39, 140.78, 138.89, 136.15, 135.38, 135.06, 134.58, 132.80, 129.23, 128.46, 126.64, 124.98, 122.82, 122.28, 121.29, 121.12, 117.23, 110.12, 107.40, 106.88, 49.20. MS (ESI) *m/z* obsd for $C_{16}H_{11}ClN_4S$ 327.0 (M + H)$^+$, 328.0 (M+2)$^+$, 349.3 (M + Na)$^+$. MALDI-TOF *m/z* calcd for $C_{16}H_{11}ClN_4S$ 327.0471 (M + H)$^+$, obsd 327.0470.

*5.2.3.4. 5-(1H-Indol-3-yl)-N-(4-methoxyphenyl)-1,3,4-thiadiazol-2-amine (**6d**).* Yield 65%; White solid, mp. 200–201 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.60 (s, 1H), 10.05 (s, 1H), 8.16 (t, J = 8.6 Hz, 1H), 7.79 (dd, J = 8.0, 2.7 Hz, 1H), 7.57 (d, J = 8.9 Hz, 2H), 7.52–7.45 (m, 1H), 7.25–7.16 (m, 2H), 6.90–6.87 (m, 2H), 3.84 (s, 3H). IR (KBr, ν cm^{-1}): 3319, 3182, 1616, 1583, 1450, 1247, 1180, 819, 750. MS (ESI) *m/z* obsd for $C_{17}H_{14}N_4OS$: 148.1, 206, 279.1, 317.1, 323.0 (M + H)$^+$, 345.0 (M + Na).

*5.2.3.5. 5-(1H-Indol-3-yl)-N-(4-(trifluoromethyl)phenyl)-1,3,4-thiadiazol-2-amine (**6e**).* Yield 72%; White solid, mp. 239–240 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.64 (s, 1H), 10.65 (s, 1H), 8.21 (d, J = 7.3 Hz, 1H), 8.10 (s, 1H), 7.89 (d, J = 8.4 Hz, 2H), 7.60 (d, J = 8.6 Hz, 2H), 7.48 (d, J = 7.5 Hz, 1H), 7.23–7.18 (m, 2H). IR (KBr, ν cm^{-1}): 3396, 3194, 1614, 1547, 1419, 1247, 1196, 840, 748, 671. ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 160.54, 154.49, 143.98, 138.22, 136.44, 126.66, 125.73, 124.22, 122.37, 121.84, 121.57, 120.60, 120.19, 116.86, 116.56, 111.80, 106.67. MS (ESI) *m/z* obsd for $C_{17}H_{11}F_3N_4S$: 149.0, 186, 346.0, 361.0 (M + H)$^+$.

*5.2.3.6. 5-(1H-Indol-3-yl)-N-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-amine (**6f**).* Yield 75%; White solid, mp. 223–224 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.55 (s, 1H), 10.16 (s, 1H), 8.18 (d, 7.5 Hz, 1H), 8.01 (s, 1H), 7.75 (d, J = 2.8 Hz, 1H), 7.53–7.41 (m, 1H), 7.23–7.15 (m, 2H), 7.04 (d, J = 2.2 Hz, 1H), 3.86 (s, 6H), 3.72 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): δ 161.25, 158.19, 152.68, 137.57, 136.38, 135.31, 132.17, 130.69, 127.50, 124.34, 122.38, 120.89, 111.93, 106.53, 99.52, 94.88, 59.92, 55.63. IR (KBr, ν cm^{-1}): 3313, 32,113, 1608, 1550, 1433, 1238, 1146, 827, 746, 621. MS (ESI) *m/z* obsd for $C_{19}H_{18}N_4O_3S$: 149.0, 353.2, 367.1, 383.0 (M + H)$^+$, 405.0 (M + Na)$^+$ MALDI-TOF *m/z* calcd for $C_{19}H_{18}N_4O_3S$ 383.1178 (M + H)$^+$, obsd 383.1176.

*5.2.3.7. N-Benzyl-5-(1H-indol-3-yl)-1,3,4-thiadiazol-2-amine (**6g**).* Yield 62%; Off-white solid, .150–151 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.59 (s, 1H), 10.01 (s, 1H), 8.10 (d, J = 7.7 Hz, 1H), 7.74 (d, J = 2.5 Hz, 1H), 7.48–7.42 (m, 3H), 7.36–7.28 (m, 3H), 7.19–7.14 (m, 2H) 4.64 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): δ 165.93, 161.60, 152.27, 138.07, 136.37, 128.44, 127.65, 127.27, 124.04, 122.64, 120.73, 120.52, 112.01, 106.28, 48.29. IR (KBr, ν cm^{-1}): 3221, 3140, 1645, 1583, 1429, 1244, 1135, 745, 696. MS (ESI) *m/z* obsd for $C_{17}H_{14}N_4S$: 279.4, 307.0 (M + H)$^+$, 324.0 (M + NH$_4$)$^+$, 329.8 (M + Na)$^+$, 345.6 (M + K)$^+$.

*5.2.3.8. 5-(1-Methyl-1H-indol-3-yl)-N-phenyl-1,3,4-thiadiazol-2-amine (**6h**).* Yield 75%; White solid, mp. 217–218 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 10.25 (s, 1H), 8.18–8.24 (m, 3H), 7.92 (s, 1H), 7.75–7.57 (m, 2H), 7.40 (d, J = 8.0 Hz, 1H), 7.34–7.28 (m, 3H), 3.88 (s, 3H). IR (KBr, ν cm^{-1}): 3194, 1624, 1546, 1456, 1246, 747, 653. ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): δ 160.88, 153.05, 141.03, 136.82, 130.96, 129.00, 125.32 124.60, 122.62, 121.58, 120.90, 117.21, 110.46, 105.69, 33.07. MS (ESI) *m/z* obsd for $C_{17}H_{14}N_4S$: 307.0 (M + H)$^+$. MALDI-TOF *m/z* calcd for $C_{17}H_{14}N_4S$ 307.1018 (M + H)$^+$, obsd 307.1013.

*5.2.3.9. N-(4-Chlorophenyl)-5-(1-methyl-1H-indol-3-yl)-1,3,4-thiadiazol-2-amine (**6i**).* Yield 72%; White solid, mp 188–189 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 10.23 (s, 1H), 8.20 (d, J = 7.9 Hz, 1H), 7.90 (s, 1H), 7.75–7.57 (m, 3H), 7.44 (d, J = 8.2 Hz, 1H), 7.37–7.20 (m, 3H), 3.89 (s, 3H). IR (KBr, ν cm^{-1}): 3255, 1616, 1514, 1428, 1219, 815, 736, 667. ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 163.92, 160.85, 157.87, 139.61, 136.93, 130.65, 129.77, 128.54, 125.17, 124.41, 122.61, 120.77, 118.78, 110.16, 105.95, 99.07, 33.06. MS (ESI) *m/z* obsd for $C_{17}H_{13}ClN_4S$: 149.0214.1, 325.0, 341.0 (M + H)$^+$, 342.0 (M+2)$^+$, 363.0 (M + Na)$^+$.

*5.2.3.10. 5-(1-(4-Chlorobenzyl)-1H-indol-3-yl)-N-phenyl-1,3,4-thiadiazol-2-amine (**6j**).* Yield 70%; White solid, mp 159–160 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 9.98 (s, 1H), 8.24–8.21 (m, 2H), 7.97 (s, 1H), 7.54–7.39 (m, 3H), 7.46–7.10 (m, 8H), 5.42 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.46, 165.79, 159.39, 140.78, 138.89, 136.15, 135.38, 135.06, 134.58, 132.80, 129.23, 128.46, 126.64, 124.98, 122.82, 122.28, 121.29, 121.12, 117.23, 110.12, 107.40, 106.88, 49.20. IR (KBr, ν cm^{-1}): 3192, 2943, 1608, 1527, 1431, 1246, 738. MS (ESI) *m/z* obsd for $C_{23}H_{17}ClN_4S$: 118, 242, 326, 417.0 (M + H)$^+$, 418.1 (M+2), MALDI-TOF *m/z* calcd for $C_{23}H_{17}ClN_4S$ 417.0941 (M + H)$^+$, obsd 417.0943.

*5.2.3.11. 5-(1-(4-Chlorobenzyl)-1H-indol-3-yl)-N-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-amine (**6k**).* Yield 65%; White solid, mp 238–239 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 10.41 (s, 1H), 8.23 (d, J = 8.1 Hz, 1H), 7.57 (s, 1H), 7.31–7.29 (m, 5H), 7.09 (d, J = 8.4 Hz, 2H), 6.72 (s, 2H), 5.33 (s, 2H), 3.89 (s, 6H), 3.85 (s, 3H). IR (KBr, ν cm^{-1}): 3124, 2937, 1597, 1537, 1429, 1234, 1182, 819, 769, 738. MS (ESI) *m/z* obsd for $C_{26}H_{23}ClN_4O_3S$: 464.0, 475.7, 491.8507.3 (M + H)$^+$, 508.0 (M+2), 529.1 (M + Na)$^+$.

*5.2.3.12. 5-(1-(4-Methoxybenzyl)-1H-indol-3-yl)-N-(4-methoxyphenyl)-1,3,4-thiadiazol-2-amine (**6l**).* Yield 55%; White solid, mp 174–175 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 10.33 (s, 1H), 8.22 (d, J = 8.8 Hz, 1H), 7.54 (s, 1H), 7.35 (d, J = 8.8 Hz, 3H), 7.29–7.26 (m, 2H), 7.13 (d, J = 8.5 Hz, 2H), 6.93 (d, J = 8.5 Hz, 2H), 6.85 (d, J = 8.6 Hz, 2H), 5.26 (s, 2H), 3.82 (s, 3H), 3.78 (s, 3H). IR (KBr, ν cm^{-1}): 3215, 2856, 1616, 1587, 1497, 1246, 809, 746, 685. MS (ESI) *m/z* obsd for $C_{25}H_{22}N_4O_2S$: 415.0, 427.8, 443.3 (M + H)$^+$, 465.7 (M + Na)$^+$.

*5.2.3.13. 5-(1-(4-Methoxybenzyl)-1H-indol-3-yl)-N-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-amine (**6m**).* Yield 65%; Pale yellow solid, mp. 189–190 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 10.99 (s, 1H), 8.23 (d, J = 7.4 Hz, 1H), 7.57 (d, J = 8.5 Hz, 1H), 7.37 (d, J = 8.3 Hz, 1H), 7.29–7.26 (m, 2H), 7.13 (d, J = 8.7 Hz, 2H), 6.92–6.82 (m, 2H), 6.77 (s, 2H), 5.28 (s, 2H), 3.90 (s, 6H), 3.86 (s, 3H), 3.78 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 161.69, 158.80, 152.92, 152.45, 137.04, 136.00, 134.84, 132.37, 132.10, 129.28, 128.73, 128.32, 124.86, 124.75, 122.60, 120.61, 116.35, 113.74, 110.44, 106.46, 99.80, 95.34, 60.17, 55.62, 55.55, 54.89, 49.07. IR (KBr, ν cm^{-1}): 3263, 2937, 1608, 1583,

1249, 831, 744, 707. MS (ESI) *m/z* obsd for $C_{27}H_{26}N_4O_4S$: 488.1, 474.0, 503.2 $(M + H)^+$, 525.2 $(M + Na)^+$.

*5.2.3.14. 5-(5-Methoxy-1H-indol-3-yl)-N-(4-methoxyphenyl)-1,3,4-thiadiazol-2-amine (**6n**).* Yield 50%; White solid, mp. 196–197 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.29 (s, 1H), 9.84 (s, 1H), 7.89 (s, 1H), 7.74 (d, J = 2.1 Hz, 1H), 7.60 (dd, J = 9.3, 5.8 Hz, 2H), 7.36–7.30 (m, 2H), 6.85 (d, J = 8.0 Hz, 2H), 3.86 (s, 3H), 3.79 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 162.85, 152.31, 149.97, 137.22, 136.35, 132.54, 127.87, 127.61, 123.48, 122.85, 121.25, 119.31, 111.94, 102.98, 95.62, 94.97, 59.47, 55.65. IR (KBr, ν cm^{-1}): 3415, 3219, 1619, 1581, 1485, 1257, 1185, 825, 794, 685. MS (ESI) *m/z* obsd for $C_{18}H_{16}N_4O_2S$: 149.0, 204.0, 337.1, 353.0 $(M + H)^+$, 375.0 $(M + Na)^+$, 391.0 $(M + K)^+$.

*5.2.3.15. 5-(6-Methoxy-1H-indol-3-yl)-N-(4-methoxyphenyl)-1,3,4-thiadiazol-2-amine (**6o**).* Yield 55%; Off-white solid, mp 182–183 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.19 (s, 1H), 9.86 (s, 1H), 8.08 (s, 1H), 7.95 (d, J = 8.8 Hz, 1H), 7.57 (d, J = 8.1 Hz, 2H), 6.95 (d, J = 8.7 Hz, 2H), 6.84 (d, J = 8.0 Hz, 2H), 3.84 (s, 3H), 3.78 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 161.92, 156.32, 154.28, 152.88, 137.06, 134.64, 125.07, 121.39, 120.89, 118.99, 118.44, 114.54, 114.09, 110.63, 106.96, 94.60, 57.56, 54.76. IR (KBr, ν cm^{-1}): 3240, 3185, 1614, 1579, 1454, 1246, 1165, 817, 746, 673. MS (ESI) *m/z* obsd for $C_{18}H_{16}N_4O_2S$: 149.0, 204.0, 353.0 $(M + H)^+$, 375.0 $(M + Na)^+$.

*5.2.3.16. 5-(6-Methoxy-1H-indol-3-yl)-N-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-amine (**6p**).* Yield 60%; Light brown solid, mp. 201–202 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.29 (s, 1H), 10.04 (s, 1H), 8.06 (d, J = 8.7 Hz, 1H), 7.99 (s, 1H), 7.61 (d, J = 2.7 Hz, 1H), 7.02 (s, 2H), 6.81 (dd, J = 8.7, 2.3 Hz, 1H), 3.86 (s, 6H), 3.84 (s, 3H), 3.72 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 161.42, 158.19, 152.85, 137.22, 136.23, 132.11, 126.55, 124.14, 122.41, 120.59, 112.16, 106.91, 100.68, 99.88, 95.34, 94.72, 60.08, 56.08, 55.64, 55.59. IR (KBr, ν cm^{-1}): 3329, 3215, 1614, 1577, 1455, 1240, 833, 773, 709. MS (ESI) *m/z* obsd for $C_{20}H_{20}N_4O_4S$: 149, 204.0, 356.1, 383.1, 413.0 $(M + H)^+$.

*5.2.3.17. N-(4-Chlorophenyl)-5-(1H-indol-2-yl)-1,3,4-thiadiazol-2-amine (**6q**).* Yield 65%; White sold, mp. 238–239 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.57 (s, 1H), 10.39 (s, 1H), 7.65 (d, J = 8.7 Hz, 2H), 7.50 (d, J = 7.9 Hz, 1H), 7.48–7.39 (m, 3H), 7.11 (t, J = 7.6 Hz, 1H), 6.98 (t, J = 7.4 Hz, 1H), 6.75 (s, 1H). IR (KBr, ν cm^{-1}): 3342, 3199, 1624, 1535, 1458, 1230, 798, 734, 648. MS (ESI) *m/z* obsd for $C_{16}H_{11}ClN_4S$: 149, 189.1, 215.1, 327.0 $(M + H)^+$.

*5.2.3.18. 5-(1H-Indol-2-yl)-N-(4-methoxyphenyl)-1,3,4-thiadiazol-2-amine (**6r**).* Yield 74%; Pale yellow solid, mp 206 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.54 (s, 1H), 10.10 (s, 1H), 7.56–7.48 (m, 3H), 7.21–7.16 (m, 2H), 7.09–7.05 (m, 1H), 6.89 (d, J = 7.2 Hz, 2H), 6.77 (s, 1H), 3.79 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 164.23, 159.55, 154.61, 149.92, 136.77, 133.87, 128.24, 127.45, 122.62, 120.37, 119.61, 118.62, 113.98, 112.00, 103.02, 99.47, 55.05. IR (KBr, ν cm^{-1}): 3388, 3317, 1606, 1539, 1413, 1234, 833, 795, 676. MS (ESI) *m/z* obsd for $C_{17}H_{14}N_4OS$: 291.6, 308.8, 323.4 $(M + H)^+$, 345.1 $(M + Na)^+$.

*5.2.3.19. 5-(1H-Indol-2-yl)-N-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-amine (**6s**).* Yield 78%; White solid, mp. 232–233 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.63 (s, 1H), 10.25 (s, 1H), 7.56 (d, J = 7.9 Hz, 2H), 7.46 (d, J = 7.9 Hz, 2H), 7.18 (d, J = 2.4 Hz, 2H), 6.81 (s, 1H), 3.87 (s, 6H), 3.75 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): δ 163.58, 159.57, 152.89, 151.19, 150.27, 137.15, 136.70, 132.68, 128.22, 127.58, 123.33, 120.61, 119.86, 111.80, 103.51, 95.62, 59.93, 55.64. IR (KBr, ν cm^{-1}): 3369, 3215, 1604, 1587, 1462, 1238, 1186, 833, 750, 713. MS (ESI) *m/z* obsd for $C_{19}H_{18}N_4O_3S$: 368.8, 383.4 $(M + H)^+$, 405.1 $(M + Na)^+$, obsd MALDI-TOF *m/z* calcd for $C_{19}H_{18}N_4O_3S$ 383.1178 $(M + H)^+$, obsd 383.1173.

*5.2.3.20. N-Benzyl-5-(1H-indol-2-yl)-1,3,4-thiadiazol-2-amine (**6t**).* Yield 60%; White solid, mp. 175–176 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.52 (s, 1H), 8.17 (s, 1H), 7.52 (d, J = 7.9 Hz, 1H), 7.45–7.40 (m, 3H), 7.36 (t, J = 7.2 Hz, 2H), 7.27 (d, J = 7.2 Hz, 1H), 7.14 (t, J = 7.6 Hz, 1H), 7.02 (t, J = 7.2 Hz, 1H), 6.69 (d, J = 1.6 Hz, 1H), 4.60 (d, J = 7.2 Hz, 2H). IR (KBr, ν cm^{-1}): 3364, 3271, 1610, 1543, 1358, 1246, 745, 685. MS (ESI) *m/z* obsd for $C_{17}H_{14}N_4S$: 307.3 $(M + H)^+$, 329.5 $(M + Na)^+$.

*5.2.3.21. 5-((1H-Indol-3-yl)methyl)-N-(4-methoxyphenyl)-1,3,4-thiadiazol-2-amine (**6u**).* Yield 65%; White solid, mp. 193–194 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 10.71 (s, 1H), 9.72 (s, 1H), 7.50 (d, J = 7.9 Hz, 1H), 7.45 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 8.1 Hz, 1H), 7.21 (d, J = 2.4 Hz, 1H), 7.09 (t, J = 8.0 Hz, 1H), 6.99 (t, J = 7.4 Hz, 1H), 6.82 (d, J = 8.8 Hz, 2H), 4.28 (s, 2H), 3.74 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): δ 164.92, 160.00, 154.25, 136.33, 134.29, 126.52, 123.72, 121.21, 119.03, 118.76, 114.28, 111.51, 110.59, 55.26, 26.08. IR (KBr, ν cm^{-1}): 3344, 3199, 1624, 1566, 1436, 1230, 1126, 829, 734, 648. MS (ESI) *m/z* obsd for $C_{18}H_{16}N_4OS$: 337.1 $(M + H)^+$, 359.0 $(M + Na)^+$, 375.0 $(M + K)$.

*5.2.3.22. 5-((1H-Indol-3-yl)methyl)-N-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-amine (**6v**).* Yield 65%; Off-white solid, mp. 235 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 10.81 (s, 1H), 9.88 (s, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.21 (d, J = 2.4 Hz, 1H), 7.09 (t, J = 7.1 Hz, 1H), 6.99 (t, J = 7.8 Hz, 1H), 6.92 (s, 2H), 4.35 (s, 2H), 3.79 (s, 6H), 3.68 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): δ 164.30, 160.46, 152.99, 137.17, 136.37, 132.22, 130.60, 126.62, 123.80, 121.27, 118.66, 118.30, 111.75, 110.39, 95.52, 60.02, 55.54, 26.08. IR (KBr, ν cm^{-1}): 3383, 3221, 1616, 1585, 1421, 1240, 1165, 844, 746, 624. MS (ESI) *m/z* obsd for $C_{20}H_{20}N_4O_3S$: 382.9, 397.3 $(M + H)^+$, 419.1 $(M + Na)^+$, 435.1 $(M + K)^+$

5.3. MTT assay

Six human cancer cell lines (LnCaP, DU145, MCF-7, MDA-MB-231, HeLa and Ovcar-3) were cultured in RPMI-1640 media supplemented with 10% heat inactivated foetal bovine serum and 1% penicillin/streptomycin. They were seeded in 96-well plates at a density of 4×10^3 cells per well for 12 h. Cells were incubated with various concentrations of the compounds ranging from 10 nM to 1 mM. After 48 h, MTT (3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetrazoliumbromide) was added to the final concentration of 0.2 mg/ml and incubated for 30 min. The cells were washed twice with PBS and lyses in 100 μL dimethylsulfoxide, and the absorbance was measured at 570 nm using Tecan Spectrafluor Plus.

Acknowledgements

The authors thank CSIR, New Delhi for the financial support and SAIF, Panjab University for providing analytical support. NMK also acknowledge the CSIR, New Delhi for the award of SRF.

References

[1] V. Sharma, P. Kumar, D. Pathak, J. Heterocycl. Chem. 47 (2010) 491–502.
[2] A. Kamal, Y.V.V. Srikanth, M.J. Ramaiah, M.N.A. Khan, M. Kashi Reddy, M. Ashraf, A. Lavanya, S.N.C.V.L. Pushpavalli, M. Pal-Bhadra, Bioorg. Med. Chem. Lett. 22 (2012) 571–578.
[3] F.R.D.S. Alves, E.J. Barreiro, C.A.M. Fraga, Mini. Rev. Med. Chem. 9 (2009) 782–793.
[4] J.-P. Liou, N. Mahindroo, C.-W. Chang, F.-M. Guo, S.W.-H. Lee, U.-K. Tan, T.-K. Yeh, C.-C. Kuo, Y.-W. Chang, P.-H. Lu, Y.-S. Tung, K.-T. Lin, J.-Y. Chang, H.-P. Hsieh, ChemMedChem 1 (2006) 1106–1118.

[5] G.R. Pettit, J.C. Knight, D.L. Herald, R. Davenport, R.K. Pettit, B.E. Tucker, J.M. Schmidt, J. Nat. Prod. 65 (2002) 1793–1797.
[6] M.A.A. Radwan, M. El-Sherbiny, Bioorg. Med. Chem. 15 (2007) 1206–1211.
[7] N.I. Ziedan, F. Stefanelli, S. Fogli, A.D. Westwell, Eur. J. Med. Chem. 45 (2010) 4523–4530.
[8] D. Kumar, S. Sundaree, E.O. Johnson, K. Shah, Bioorg. Med. Chem. Lett. 19 (2009) 4492–4494.
[9] E. Glawischnig, Phytochemistry 68 (2007) 401–406.
[10] H. Oka, T. Yoshinari, T. Murai, K. Kawamura, F. Satoh, K. Funaishi, A. Okura, H. SUDA, M. Okanishi, Y. Shizuri, J. Antibiot. 44 (1991) 486–491.
[11] M. Kritsanida, A. Mouroutsou, P. Marakos, N. Pouli, S. Papakonstantinou-Garoufalias, C. Pannecouque, M. Witvrouw, E. De Clercq, Il Farmaco 57 (2002) 253–257.
[12] A.R. Bhat, Tazeem, A. Azam, I. Choi, F. Athar, Eur. J. Med. Chem. 46 (2011) 3158–3166.
[13] H.N. Dogan, A. Duran, S. Rollas, G. Sener, M.K. Uysal, D. Gülen, Bioorg. Med. Chem. 10 (2002) 2893–2898.
[14] R. Sharma, J. Sainy, S.C. Chaturvedi, Acta Pharma 58 (2008) 317–326.
[15] S. Turner, M. Myers, B. Gadie, S.A. Hale, A. Horsley, A.J. Nelson, R. Pape, J.F. Saville, J.C. Doxey, T.L. Berridge, J. Med. Chem. 31 (1988) 906–913.
[16] E.E. Oruç, S. Rollas, F. Kandemirli, N. Shvets, A.S. Dimoglo, J. Med. Chem. 47 (2004) 6760–6767.
[17] A. Foroumadi, S. Emami, S. Pournourmohammadi, A. Kharazmi, A. Shafiee, Eur. J. Med. Chem. 40 (2005) 1346–1350.
[18] F. Clerici, D. Pocar, M. Guido, A. Loche, V. Perlini, M. Brufani, J. Med. Chem. 44 (2001) 931–936.
[19] Q. Zeng, M.P. Bourbeau, G.E. Wohlhieter, G. Yao, H. Monenschein, J.T. Rider, M.R. Lee, S. Zhang, J. Lofgren, D. Freeman, Bioorg. Med. Chem. Lett. 20 (2010) 1652–1656.
[20] I.P. Kaur, R. Smitha, D. Aggarwal, M. Kapil, Int. J. Pharm. 248 (2002) 1–14.
[21] P.J. Elson, L.K. Kvols, S.E. Vogl, D.J. Glover, R.G. Hahn, D.L. Trump, P.P. Carbone, J.D. Earle, T.E. Davis, Invest. New Drugs 6 (1988) 97–103.
[22] J.A. Nelson, L.M. Rose, L.L. Bennett, Cancer Res. 37 (1977) 182–187.
[23] W. Rzeski, J. Matysiak, M. Kandefer-Szerszen, Bioorg. Med. Chem. 15 (2007) 3201–3207.
[24] J. Matysiak, A. Opolski, Bioorg. Med. Chem. 14 (2006) 4483–4489.
[25] Y.S. Shan, J. Zhang, Z. Liu, M. Wang, Y. Dong, Curr. Med. Chem. 18 (2011) 523–538.
[26] H. Hsieh, J. Liou, N. Mahindroo, Curr. Pharm. Des 11 (2005) 1655–1677.
[27] A. Kamal, J.S. Reddy, M.J. Ramaiah, D. Dastagiri, E.V. Bharathi, M.V. Prem Sagar, S.N.C.V.L. Pushpavalli, P. Ray, M. Pal-Bhadra, MedChemComm 1 (2010) 355–360.
[28] Y. Lu, C.-M. Li, Z. Wang, J. Chen, M.L. Mohler, W. Li, J.T. Dalton, D.D. Miller, J. Med. Chem. 54 (2011) 4678–4693.
[29] C. Peifer, R. Selig, K. Kinkel, D. Ott, F. Totzke, C. Schächtele, R. Heidenreich, M. Röcken, D. Schollmeyer, S. Laufer, J. Med. Chem. 51 (2008) 3814–3824.
[30] L. Lee, R. Davis, J. Vanderham, P. Hills, H. Mackay, T. Brown, S.L. Mooberry, M. Lee, Eur. J. Med. Chem. 43 (2008) 2011–2015.
[31] G. De Martino, G. La Regina, A. Coluccia, M.C. Edler, M.C. Barbera, A. Brancale, E. Wilcox, E. Hamel, M. Artico, R. Silvestri, J. Med. Chem. 47 (2004) 6120–6123.
[32] D. Kumar, B.R. Vaddula, K.-H. Chang, K. Shah, Bioorg. Med. Chem. Lett. 21 (2011) 2320–2323.
[33] D. Kumar, N.M. Kumar, K.-H. Chang, K. Shah, Chem. Biol. Drug Des. 77 (2011) 182–188.
[34] D. Kumar, N.M. Kumar, K.-H. Chang, R. Gupta, K. Shah, Bioorg. Med. Chem. Lett. 21 (2011) 5897–5900.
[35] D. Kumar, N.M. Kumar, K.-H. Chang, K. Shah, Eur. J. Med. Chem. 45 (2010) 4664–4668.
[36] D. Kumar, N.M. Kumar, S. Sundaree, E.O. Johnson, K. Shah, Eur. J. Med. Chem. 45 (2010) 1244–1249.
[37] D. Kumar, N.M. Kumar, K. Akamatsu, E. Kusaka, H. Harada, T. Ito, Bioorg. Med. Chem. Lett. 20 (2010) 3916–3919.
[38] M. Lebrini, F. Bentiss, M. Lagrenée, J. Heterocycl. Chem. 42 (2005) 991–994.
[39] R. Severinsen, J.P. Kilburn, J.F. Lau, Tetrahedron 61 (2005) 5565–5575.
[40] V. Padmavathi, G. Sudhakar Reddy, A. Padmaja, P. Kondaiah, S. Ali, Eur. J. Med. Chem. 44 (2009) 2106–2112.
[41] S. Rostamizadeh, R. Aryan, H. Reza Ghaieni, A. Mohammad Amani, J. Heterocycl. Chem. 47 (2010) 616–623.
[42] S. Rostamizadeh, R. Aryan, H.R. Ghaieni, A.M. Amani, Heteroatom. Chem. 19 (2008) 320–324.
[43] C. Swain, R. Baker, C. Kneen, J. Moseley, J. Saunders, E. Seward, G. Stevenson, M. Beer, J. Stanton, K. Watling, J. Med. Chem. 34 (1991) 140–151.