European Journal of Medicinal Chemistry 45 (2010) 4664–4668

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Short communication

Synthesis and anticancer activity of 5-(3-indolyl)-1,3,4-thiadiazoles

Dalip Kumar [a,*], N. Maruthi Kumar [a], Kuei-Hua Chang [b], Kavita Shah [b,**]

[a] Chemistry Group, 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 29 April 2010
Received in revised form
13 July 2010
Accepted 13 July 2010
Available online 21 July 2010

Keywords:
1,3,4-Thiadiazole
Indolyl heterocycles
Anticancer agents
Indolyl azoles

ABSTRACT

A series of 5-(3-indolyl)-2-substituted-1,3,4-thiadiazoles **5a**–**m** were synthesized and their cytotoxicity analyzed against six human cancer cell lines. The reaction of indole-3-carboxylic acid **3** with aryl or heteroaryl hydrazides afforded the *N,N′*-diacylhydrazines **4**, which upon treatment with Lawesson's reagent resulted in the formation of indolyl-1,3,4-thiadiazoles **5a**–**m** in good yields. Indolyl-1,3,4-thiadiazole **5m** with 4-benzyloxy-3-methoxyphenyl and 5-bromo indolyl substituents is the most active in suppressing the growth of cancer cells (IC_{50} 1.5 μM, PaCa2). The compounds **5b, 5e** and **5h** bearing C-2 substituent as benzyl, 3,4-dimethoxyphenyl and 4-benzyloxy-3-methoxyphenyl, respectively, have shown significant cytotoxicity against multiple cancer cell lines. Introduction of 4-dimethylamino (**5d** and **5k**) and 3,4,5-trimethoxy (**5l**) groups in the C-2 phenyl ring induced selectivity against MCF7 and MDA-MB-231 cancer cell lines (compounds **5d, 5k** and **5l**).

1. Introduction

Recent drug discovery efforts are highly focused towards design and synthesis of small molecules as anticancer agents [1]. A wide range of heterocyclic ring systems has been studied for the development of novel chemical entities as a lead molecule in the drug discovery paradigm. Thiadiazoles are one of the privileged structural fragments in medicinal chemistry having broad spectrum of pharmacological activities [2–4]. Particularly, 1,3,4-thiadiazoles are much explored for their broad spectrum of biological activities including antiinflammatory [5], antihypertensive [6], antibacterial [7], anticonvulsant, antimicrobial [8], antidepressants [9], antileishmanial [10] and anticancer [11]. Furthermore, widely explored 2-aminothiadiazoles are in clinical trials for the treatment of patients with different tumors [12].

Among the important heterocycles, many of the natural and synthetic indole-based heterocycles with diverse mechanism of action have been reported as lead anticancer molecules [13–15]. Various indolylazole and bisindolylazoles are known for their anticancer activities. Camalexin (indolylthiazole) **1a** which is a phytoalexin was detected and isolated from the leaves of Cruciferae *Camelina sativa* infected with *Alternaria brassicae*. Analogues of **1a** were evaluated for their cytotoxic activity against human breast tumor cell lines [16]. A thiazolyl indolequinone, BE 10988 **1b** (Fig. 1), isolated from culture broths of *Streptomyces* strain, is known to increase DNA-topoisomerase complex formation and displayed significant anticancer activities [17]. Recently, we have reported 4-(3-indolyl)oxazoles **1c** [18] and 5-(3-indolyl)-1,3,4-oxadiazoles **1d** [19] as potential anticancer agents against many types of human cancer cell lines.

Encouraging activities of indolylazoles prompted us to investigate new analogues with further modification of five-membered heterocyclic ring and indolyl moiety in order to optimize the structure-activity relationship (SAR) leading to potent and selective anticancer agents. In this paper we report a facile synthesis of diverse 5-(3-indolyl)-2-substituted-1,3,4-thiadiazoles and their anticancer activities against various human cancer cell lines.

The common method for the preparation of 1,3,4-thiadiazoles involves the reaction of aldehydes, hydrazine hydrate and elemental sulfur under conventional and microwave conditions [20]. Thionation of dibenzoylhydrazines using Lawesson's reagent or phosphorus pentasulfide followed by cyclization and dehydrosulfurization is reported to produce 1,3,4-thiadiazoles in good yields [21]. Varma et al. reported microwave accelerated solvent-free synthesis of 1,3,4-thiadiazoles from the reaction of acid hydrazides and triethylorthoalkanates in presence of phosphorus pentasulfide in alumina [22].

2. Chemistry

Synthesis of 5-(3-indolyl)thiadiazoles **5a**–**m** was achieved following a convenient three-step procedure starting from

* Corresponding author. Tel.: +91 1596 245073 279.
** 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 © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.07.023

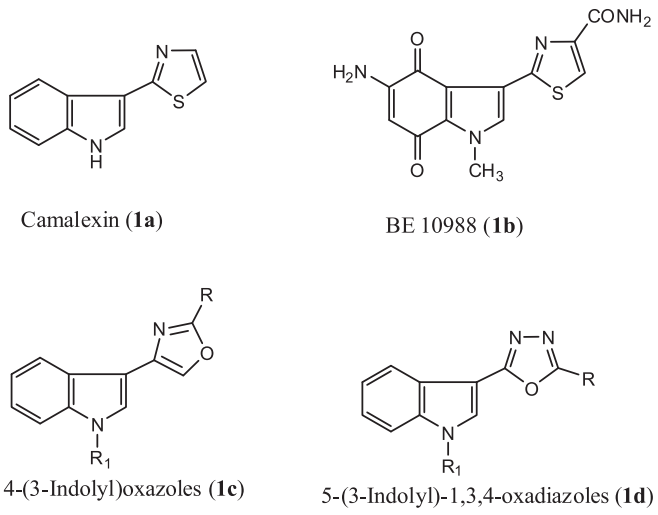


Fig. 1. Structures of some 5-(3-Indolyl)azoles.

commercially available indole **2** as outlined in the Scheme 1. The indole-3-carboxylic acids **3** were prepared from the reaction of **2** with trifluoroacetic anhydride followed by hydrolysis with sodium hydroxide. Reaction of **3** with arylhydrazides in the presence of versatile coupling reagents 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and 1-hydroxybenzotriazole in dry tetrahydrofuran afforded diacylhydrazines **4**. Thionation of diacylhydrazine **4** with Lawesson's reagent followed by oxidative cyclization in dry tetrahydrofuran led to the indolyl-1,3,4-thiadiazoles **5** in good yields. The reaction of **4** with Lawesson's reagent was also screened in different solvents including toluene, dioxane and xylene but afforded less yields when compared to tetrahydrofuran. Further, the reaction of **4** with Lawesson's reagent under solvent-free conditions using microwave irradiation gave a mixture of by-products along with desired indolyl-1,3,4-thiadiazole. All the synthesized 5-(3-indolyl)-2-substituted-1,3,4-thiadiazoles **5a**–**m** were characterized by their NMR and MS data.

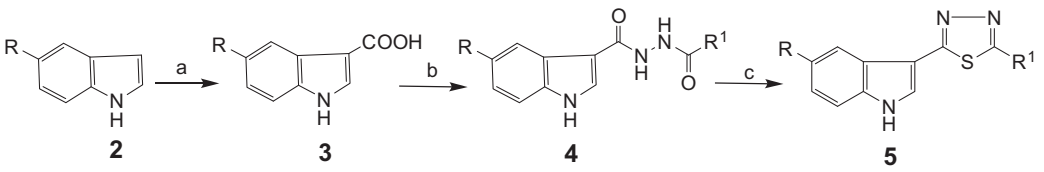
3. Anticancer activity

A series of diverse 5-(3-indolyl)-2-substituted-1,3,4-thiadiazoles **5a**–**m** were screened against prostate (PC3, DU145 and LnCaP), breast (MCF7 and MDA-MB-231) and pancreatic (PaCa2) cancer cell lines. These cell lines were cultured in RPMI 1640 medium containing 10% fetal bovine serum. Cells were seeded in 96-well microtiter plate, at a target cell density of 5000–10,000 cells per well. After 12 h, test compounds were added at different concentrations ranging from 100 nM to 1 mM and the cells were further incubated for 48 h. The anticancer activity was determined for each cell line using formazan dye (MTT) conversion assay.

4. Results and discussion

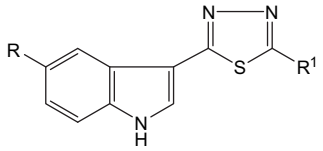
The activity results mentioned in Table 1 show the cytotoxic effects of 5-(3-indolyl)-2-substituted-1,3,4-thiadiazoles **5a**–**m** against various cancer cell lines. Most of the compounds showed significant cytotoxic effect. The structure-activity relationship (SAR) study revealed that the substitution at C-2 position of the 1,3,4-thiadiazole ring plays an important role in imparting the cytotoxic activity to the compound. The compound **5a** possessing a phenyl ring at C-2 position was found to be selectively cytotoxic against PaCa2 cell line (IC_{50} 41.7 μM). Replacement of phenyl ring with benzyl moiety led to the compound **5b** with increased cytotoxicity against all cancer cell lines, the IC_{50} values are nearly uniform against LnCaP, MCF7 and PaCa2 cell lines and below 30 μM in PC3, DU145 and MDA-MB-231 cell lines. Next, we investigated the effect of electron with-drawing and electron-donating groups by introducing chloro and 4-dimethylamino substituents at the para position of C-2 aryl ring. It was observed that compound **5c** with *p*-chlorophenyl is moderately active against four cancer cell lines (<100 μM), whereas, compound **5d** with 4-(dimethylamino)phenyl showed an apparent increase in activity against three cancer cell lines LnCaP (23 μM), DU145 (35.6 μM) and MCF7 (12.3 μM).

Introducing 3,4-dimethoxyphenyl group at C-2 resulted in compound **5e** with significantly increased activity against all the cancer cell lines especially against MCF7 with an IC_{50} value of 6.8 μM. Interestingly, a 3,4-methylenedioxy moiety (compound **5g**) in the C-2 aryl ring drastically reduces the activity. Introduction of third methoxy group on phenyl ring (compound **5f**) also reduced the activity but enhanced the selectivity against PaCa2 cell line (IC_{50} 14.2 μM). Increasing the size of 4-methoxy group by replacing it with 4-benzyloxy group (compound **5h**) led to substantial gain in activity and selectivity against MCF7 (IC_{50} 6.5 μM) and DU145 (IC_{50} 9.1 μM) cell lines. Compound with C-2 pyridyl substituent (compound **5j**) displayed improved activity and selectivity against PC3 cell line (IC_{50} < 50 μM) when compared with **5a**. Shifting the position of heteroatom in the ring led to 4-pyridyl derivative **5i** with further improved activity. The compound **5k** with C-2 4-dimethylaminophenyl and 5-bromoindole exhibited reduced activity except selective cytotoxicity against MDA-MB-231 cell line with an IC_{50} value of 12.7 μM. Replacing 4-dimethylaminophenyl of **5k** with trimethoxyphenyl resulted in **5l** with reduced activity, whereas 4-benzyloxy-3-methoxyphenyl group led to compound **5m** with significant improved activity and selectivity against PaCa2 (IC_{50} 1.5 μM) cancer cell line. The comparison of activity results of 5-(3-indolyl)-1,3,4-thiadiazoles with that of 4-(3-indolyl)oxazoles [18], 5-(3-indolyl)-1,3,4-oxadiazoles [19] and 5-(3-indolyl)-1,2,4-triazoles [23] shows that the five-membered heterocyclic ring plays a vital role in the cytotoxicity and selectivity of indolyl azoles towards cancer cells. The most potent compounds in various indolyl azoles bearing a common C-5 indole moiety have different substituents at C-2 position of five-membered heterocyclic ring. In general, the anticancer activity of 5-(3-indolyl)-2-substituted-1,3,4-thiadiazoles **5** is better than that of 4-(3-indolyl)oxazoles [18],



Scheme 1. Synthesis of indolyl-1,3,4-thiadiazoles (**5**). Reagents and conditions: (a) (i) $(CF_3CO)_2O$, DMF; (ii) Aqueous NaOH, reflux; (b) EDCI, HOBt, $R^1CONHNH_2$, THF, rt; (c) Lawesson's reagent, THF, reflux.

Table 1
In vitro cytotoxicity data of 5-(3-indolyl)-2-substituted-1,3,4-thiadiazoles **5a**–**m**[a] against selected human cancer cell lines IC_{50} (μM).[b]



Compound	R^1	R	LnCaP	DU145	PC3	MCF7	MDA-MB-231	PaCa2
5a	C_6H_5	H	264.9	261.5	366.7	280.5	445.7	41.7
5b	$CH_2C_6H_5$	H	**13.9**	**17**	29	**13.5**	27.8	**13**
5c	4-ClC_6H_4	H	53.1	79.6	99.2	149.5	54.7	>1000
5d	4-*N*,*N*′$(CH_3)_2C_6H_4$	H	23	35.6	704.9	**12.3**	124.1	188.1
5e	3,4-$(OCH_3)_2C_6H_3$	H	21	**10.7**	**11.9**	**6.8**	24	**13.8**
5f	3,4,5-$(CH_3O)_3C_6H_2$	H	40	168.5	29.3	144.9	54.5	**14.2**
5g	3,4-$(OCH_2O)C_6H_3$	H	799	162	157.9	>1000	>1000	231.1
5h	4-BnO-3-$OCH_3C_6H_3$	H	**19.2**	**9.1**	22.3	**6.5**	26.2	28.9
5i	3-pyridyl	H	151.7	170	37.2	91.6	96.3	>1000
5j	4-pyridyl	H	95	77	39.2	67	53.8	451.3
5k	4-*N*,*N*′$(CH_3)_2C_6H_4$	Br	31.3	22	26.6	28.7	**12.7**	130.5
5l	3,4,5-$(CH_3O)_3C_6H_2$	Br	66.4	58.4	95.4	594.7	**19.1**	>1000
5m	4-BnO-3-$OCH_3C_6H_3$	Br	**8.9**	**3.6**	**7.5**	**8.3**	**6.2**	**1.5**

Bold values show IC_{50} of less than 20 μM.
[a] These experiments were conducted in triplicates at three independent times.
[b] IC_{50} values were obtained using a dose response curve by nonlinear regression using a curve fitting program, GraphPad Prism 5.0.

whereas, comparable to 5-(3-indolyl)-1,3,4-oxadiazoles [19] and 5-(3-indolyl)-1,2,4-triazoles [23].

5. Conclusion

In summary, we have synthesized a novel series of 5-(3′-indolyl)-2-substituted-1,3,4-thiadiazoles which have displayed variable extent of cytotoxic activity against various cancer cell lines. The cytotoxicity results of **5a**–**m** demonstrated the importance of substituents such as 4-benzyloxy-3-methoxyphenyl, 5-bromoindole and 4-(dimethylamino)phenyl at C-2 and C-5 positions of 1,3,4-thiadiazoles. Compound **5m** with 4-benzyloxy-3-methoxyphenyl at C-2 position and 5-bromoindole at C-5 position is the most potent compound of this series. Further structure-activity studies are required to clearly elucidate the role of heterocyclic linker in indolyl azoles and identify their molecular targets.

6. Experimental

6.1. Physical measurements

All the laboratory grade reagents were obtained commercially. The reaction was monitored by thin layer chromatography, which was performed on Merck precoated plates (silica gel. 60 F_{254}, 0.25 mm) and was visualized by fluorescence quenching under UV light (254 nm). Column chromatography was performed using 100–200 mesh silica gel and appropriate mixture of hexane and ethyl acetate for elution. The solvents were evaporated using Buchi 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 obtained on a 'Hewlett–Packard' HP GS/MS 5890/5972.

6.1.1. Preparation of indole-3-carboxylic acid (**3**)

Indole-3-carboxylic acid and 5-bromoindole-3-carboxylic acid were prepared by the reported methods [24].

6.1.2. General procedure for the synthesis of 1,2-diacylhydrazines (**4**)

A mixture of indole-3-carboxylic acid **3** (1.61 g, 10 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.91 g, 10 mmol) and 1-hydroxy-benzotriazole (1.35 g, 10 mmol) in dry tetrahydrofuran (10 mL) was stirred at room temperature for 15 min. To this reaction mixture, appropriate arylhydrazide (1 mmol) was added and continued stirring at room temperature for 6 h. The reaction contents were concentrated at reduced pressure, the solid 1,2-diacylhydrazine **4** was filtered off, washed with water and dried to use as such for the next step.

6.1.3. General procedure for the synthesis of indolyl-1,3,4-thiadiazole (**5**)

A mixture of 1,2-diacylhydrazines **4** (1 mmol) and Lawesson's reagent (0.44 g, 1.1 mmol) in tetrahydrofuran (10 mL) was refluxed at 80 °C for 5 h. After completion of the reaction as monitored by TLC, the crude product was adsorbed over silica gel and purified by column chromatography using ethyl acetate: hexane (7:3; v/v) as eluent to afford pure product **5**.

*6.1.3.1. 2-1H-indol-3-yl-5-phenyl-1,3,4-thiadiazole (**5a**).* Yield 65%; mp 187–191 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ_H = 12.05 (s, 1H, NH), 8.14–8.10 (m, 1H, Ar–H), 7.94 (d, 1H, J = 2.92 Hz, Ar–H), 7.49–7.47 (m, 2H, Ar–H), 7.40–7.36 (m, 3H, Ar–H), 7.33–7.27 (m, 1H, Ar–H), 7.24–7.20 (m, 2H, Ar–H). MS (ESI) calcd for $C_{16}H_{11}N_3S$ 278.07 $(M + H)^+$ found 278.20.

*6.1.3.2. 2-Benzyl-5-1H-indol-3-yl –1,3,4-thiadiazole (**5b**).* Yield 65%; mp 189–192 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ_H = 11.75 (s, 1H, NH), 8.18–8.15 (m, 1H, Ar–H), 7.86–7.81(m, 2H, Ar–H), 7.49–7.46 (m, 1H, Ar–H), 7.39–7.33 (m, 3H, Ar–H), 7.31–7.27 (m, 1H, Ar–H), 7.25–7.17 (m, 2H, Ar–H). 4.44 (s, 2H, CH_2). MS (ESI) calcd for $C_{17}H_{13}N_3S$ 292.08 $(M + H)^+$ found 292.31.

*6.1.3.3. 2-(4-Chlorophenyl)-5-1H-indol-3-yl-1,3,4-thiadiazole (**5c**).* Yield 75%; mp 231–234 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ_H = 11.98 (s, 1H, NH), 8.32–8.22 (m, 2H, Ar–H), 8.14 (d, 1H,

J = 8.48 Hz, Ar–H), 8.03 (d, 1H, J = 8.24 Hz, Ar–H), 7.72–7.64 (m, 2H, Ar–H), 7.55 (d, 1H, J = 8.2 Hz, Ar–H), 7.30–7.27 (m, 2H, Ar–H). MS (ESI) calcd for $C_{16}H_{10}ClN_3S$ 312.02 $(M + H)^+$ found 312.0.

*6.1.3.4. 2-(4-Dimethylaminophenyl)-5-1H-indol-3-yl-1,3,4-thiadiazole (**5d**).* Yield 72%; mp 195–199 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ_H = 11.89 (s, 1H, NH), 8.23–8.20 (m, 1H, Ar–H), 7.99 (d, 2H, J = 8.8 Hz), 7.62–7.49 (m, 3H, Ar–H), 7.03–6.97 (m, 2H, Ar–H), 6.87–6.81 (m, 1H, Ar–H), 2.98 (s, 6H, NCH_3). MS (ESI) calcd for $C_{18}H_{16}N_4S$ 321.10 $(M + H)^+$ found 321.34.

*6.1.3.5. 2-(3,4-Dimethoxyphenyl)-5-1H-indol-3-yl-1,3,4-thiadiazole (**5e**).* Yield 72%; mp 173–177 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ_H = 12.08 (s, 1H, NH), 8.32–8.17 (m, 2H, Ar–H), 7.72–7.07 (m, 6H, Ar–H), 3.90 (s, 6H, OCH_3). MS (ESI) calcd for $C_{18}H_{15}N_3O_2S$ 338.08 $(M + H)^+$ found 338.20.

*6.1.3.6. 2-1H-indol-3-yl-5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazole (**5f**).* Yield 75%; mp 220–221 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ_H = 12.11 (s, 1H, NH), 8.35–8.21 (m, 2H, Ar–H), 8.14 (s, 1H, Ar–H), 7.71–6.97 (m, 4H, Ar–H), 3.90 (s, 6H, OCH_3), 3.76 (s, 3H, OCH_3). MS (ESI) calcd for $C_{19}H_{17}N_3O_3S$ 368.09 $(M + H)^+$ found 368.30.

*6.1.3.7. 5-1H-indol-3-yl-2-(3,4-methylenedioxyphenyl)-1,3,4-thiadiazole (**5g**).* Yield 66%; mp 218–220 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ_H = 11.66 (s, 1H, NH), 8.24 (d, 2H, J = 8.32 Hz, Ar–H), 7.94–7.89 (m, 3H, Ar–H), 7.44 (d, 2H, J = 9.32 Hz, Ar–H), 6.95 (d, 1H, J = 8.08 Hz, Ar–H), 6.10 (s, 2H, CH_2). MS (ESI) calcd for $C_{17}H_{11}N_3O_2S$ 322.05 $(M + H)^+$ found 322.18.

*6.1.3.8. 2-(4-(Benzyloxy)-3-methoxyphenyl)-5-1H-indol-3-yl-1,3,4-thiadiazole (**5h**).* Yield 55%; mp 240–245 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ_H = 12.05 (s, 1H, NH), 8.30 (d, 1H, J = 2.8 Hz, Ar–H), 8.22–8.19 (m, 1H, Ar–H), 7.73–7.62 (m, 1H, Ar–H), 7.50–7.46 (m, 4H, Ar–H), 7.43–7.32 (m, 3H, Ar–H), 7.29–7.20 (m, 3H, Ar–H), 5.18 (s, 2H, CH_2), 3.79 (s, 3H, OCH_3). MS (ESI) calcd for $C_{24}H_{19}N_3O_2S$ 415.11 $(M + 2)^+$ found 415.20.

*6.1.3.9. 2-1H-indol-3-yl-5-(3-pyridyl)-1,3,4-thiadiazole (**5i**).* Yield 64%; mp 221–224 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ_H = 12.01 (s, 1H, NH), 8.56 (d, 2H, J = 8.0 Hz, Ar–H), 8.26 (d, 1H, J = 3.6 Hz, Ar–H), 8.21 (s, 1H, Ar–H), 7.94 (d, 2H, J = 5.6 Hz, Ar–H), 7.54–7.47 (m, 3H, Ar–H). MS (ESI) calcd for $C_{15}H_{10}N_4S$ 279.06 $(M + H)^+$ found 279.09.

*6.1.3.10. 2-1H-indol-3-yl-5-(4-pyridyl)-1,3,4-thiadiazole (**5j**).* Yield 65%; mp 260–263 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ_H = 12.08 (s, 1H, NH), 8.77 (d, 2H, J = 5.6 Hz, Ar–H), 8.32 (d, 1H, J = 1.2 Hz, Ar–H), 8.23 (t, 1H, J = 8.8 Hz, Ar–H), 7.94 (d, 2H, J = 5.6 Hz, Ar–H), 7.54 (t, 1H, J = 8.8 Hz, Ar–H), 7.28–7.26 (m, 2H, Ar–H). MS (ESI) calcd for $C_{15}H_{10}N_4S$ 279.06 $(M + H)^+$ found 279.10.

*6.1.3.11. 5-(5-Bromo-3-indolyl)-2-(4-dimethylaminophenyl) 1,3,4-thiadiazole (**5k**).* Yield 60%; mp 217–219 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ_H = 11.92 (s, 1H, NH), 8.38 (d, 2H, J = 8.12 Hz, Ar–H), 8.24 (d, 2H, J = 8.2 Hz, Ar–H), 7.50 (d, 1H, J = 2.0 Hz, Ar–H), 7.42–7.22 (m, 2H, Ar–H), 7.18 (s, 1H, Ar–H), 3.08 (s, 6H, CH_3). MS (ESI) calcd for $C_{18}H_{15}BrN_4S$ 401.02 $(M + 2)^+$ found 401.10.

*6.1.3.12. 2-(5-Bromo-3-indolyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazole (**5l**).* Yield 68%; mp 291–294 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ_H = 12.16 (s, 1H, NH), 8.39 (d, 1H, J = 2 Hz, Ar–H), 8.29 (d, 1H, J = 3.2 Hz, Ar–H), 7.50 (d, 1H, J = 8.8 Hz, Ar–H), 7.40–7.38 (m, 2H, Ar–H), 7.26 (s, 1H, Ar–H), 3.92 (s, 6H, 2xOCH_3), 3.74 (s, 3H, OCH_3). MS (ESI) calcd for $C_{19}H_{16}BrN_3O_3S$ 445.01 $(M)^+$ found 445.38.

*6.1.3.13. 2-(4-(Benzyloxy)-5-(5-bromo-3-indolyl)-3-methoxyphenyl)-1,3,4-thiadiazole (**5m**).* Yield 75%; mp 252–254 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ_H = 12.14 (s, 1H, NH), 8.40 (d, 1H, J = 1.6 Hz, Ar–H), 8.26 (d, 1H, J = 2.8 Hz, Ar–H), 7.50 (d, 1H, J = 2.0 Hz, Ar–H), 7.50–7.46 (m, 4H, Ar–H), 7.42–7.22 (m, 4H, Ar–H), 7.20 (s, 1H, Ar–H), 5.18 (s, 2H, CH_2), 3.74 (s, 3H, OCH_3). MS (ESI) calcd for $C_{24}H_{18}BrN_3O_2S$ 493.03 $(M + 2)^+$ found 493.49.

6.2. MTT assay

Six human cancer cell lines (LnCaP, DU145, PC3, MCF7, MDA-MB-231, and PaCa2) were cultured in RPMI 1640 media supplemented with 10% heat inactivated fetal 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-diphenyltetra-zoliumbromide) was added to the final concentration of 0.2 mg/mL and incubated for 30 min. The cells were washed twice with PBS and lysed in 100 μL dimethylsulfoxide, and the absorbance was measured at 570 nm using Tecan Spectrafluor Plus.

Acknowledgements

The authors thank Department of Science and Technology, New Delhi for the financial support and SAIF, Panjab University for providing analytical support.

References

[1] (a) J.K. Buolamwini, J. Addo, S. Kamath, S. Patil, D. Mason, M. Ores, Curr. Cancer Drug Targets 5 (2005) 57–68;
(b) G. Bacher, T. Beckers, P. Emig, T. Klenner, B. Kutscher, B. Nickel, Pure Appl. Chem. 73 (2001) 1459–1464.
[2] M. Wu, Q. Sun, C. Yang, D. Chen, J. Ding, Y. Chen, L. Lin, Y. Xie, Bioorg. Med. Chem. Lett. 17 (2007) 869–873.
[3] (a) C.B. Chapleo, P.L. Myers, A.C.B. Smith, M.R. Stillings, I.F. Tulloch, D.S. Walter, J. Med. Chem. 31 (1988) 7–11;
(b) B.B. Busch, W.C. Stevens, R. Martin, P. Ordentlich, S. Zhou, D.W. Sapp, R.A. Horlick, R. Mohan, J. Med. Chem. 47 (2004) 5593–5596.
[4] L. Shen, Y. Zhang, A. Wang, E. Sieber-McMaster, X. Chen, P. Pelton, J.Z. Xu, M. Yang, P. Zhu, L. Zhou, M. Reuman, Z. Hu, R. Russell, A.C. Gibbs, H. Ross, K. Demarest, W.V. Murray, G.-H. Kuo, J. Med. Chem. 50 (2007) 3954–3963.
[5] (a) S. Schenone, C. Brullo, O. Bruno, F. Bondavalli, A. Ranise, W. Filippelli, B. Rinaldi, A. Capuano, G. Falcone, Bioorg. Med. Chem. 14 (2006) 1698–1705;
(b) A.A. Kadi, N.R. El-Brollosy, O.A. Al-Deeb, E.E. Habib, T.M. Ibrahim, A.A. El-Emam, Eur. J. Med. Chem. 42 (2007) 235–242.
[6] S. Turner, M. Myers, B. Gadie, A.J. Nelson, R. Pape, J.F. Saville, J.C. Doxey, T.L. Berridge, J. Med. Chem. 31 (1988) 902–907.
[7] (a) A. Foroumadi, S. Mansouri, Z. Kiani, A. Rahmani, Eur. J. Med. Chem. 38 (2003) 851–854;
(b) L.M. Thomasco, R.C. Gadwood, E.A. Weaver, J.M. Ochoada, C.W. Ford, G.E. Zurenko, J.C. Hamel, D. Stapert, J.K. Moerman, R.D. Schaadt, B.H. Yagi, Bioorg. Med. Chem. Lett. 13 (2003) 4193–4196.
[8] H.N. Dogan, A. Duran, S. Rollas, G. Sener, M.K. Uysal, D. Gülen, Bioorg. Med. Chem. 10 (2002) 2893–2898.
[9] M. Yusuf, R.A. Khan, B. Ahmed, Bioorg. Med. Chem. 17 (2008) 8029–8034.
[10] F. Poorrajab, S.K. Ardestani, S. Emami, M. Behrouzi-Fardmoghadam, A. Shafiee, A. Foroumadi, Eur. J. Med. Chem. 44 (2009) 1758–1762.
[11] (a) V. Padmavathi, G. Sudhakar Reddy, A. Padmaja, P. Kondaiah, S. Ali, Eur. J. Med. Chem. 44 (2009) 2106–2112;
(b) J. Matysiak, Eur. J. Med. Chem. 42 (2007) 940–947;
(c) W. Rzeski, J. Matysiak, M. Kandefer-Szerszen, Bioorg. Med. Chem. 15 (2007) 3201–3207;
(d) J. Matysiak, A. Nasulewicz, M. Pelczynska, M. Switalska, I. Jaroszewicz, A. Opolski, Eur. J. Med. Chem. 41 (2006) 475–482;
(e) J.-Y. Chou, S.-Y. Lai, S.-L. Pan, G.-M. Jow, J.-W. Chern, J.-H. Guh, Biochem. Pharmacol. 66 (2003) 115–124.
[12] (a) R. Asbury, J.A. Blessing, D. Moore, Am. J. Clin. Oncol. 19 (1996) 400–402;
(b) P.F. Engstrom, L.M. Ryan, G. Falkson, D.G. Haller, Am. J. Clin. Oncol. 14 (1991) 33–35.

[13] P. Diana, A. Carbone, P. Barraja, A. Montalbano, A. Martorana, G. Dattolo, O. Gia, L.D. Via, G. Cirrincione, Bioorg. Med. Chem. Lett. 17 (2007) 2342–2346.
[14] (a) B. Jiang, X.H. Gu, Bioorg. Med. Chem. Lett. 8 (2000) 363–371;
(b) 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) 2814–2824.
[15] A. Andreani, S. Burnelli, M. Granaiola, A. Leoni, A. Locatelli, R. Morigi, M. Rambaldi, L. Varoli, L. Landi, C. Prata, M.V. Berridge, C. Grasso, H.-H. Fiebig, G. Kelter, A.M. Burger, M.W. Kunkel, J. Med. Chem. 51 (2008) 4563–4570.
[16] C.J. Moody, J.R. Roffey, M.A. Stephens, I.J. Stratford, Anticancer Drugs 8 (1997) 489–499.
[17] (a) L. Garuti, M. Roberti, A. Pession, E. Leoncini, S. Hrelia, Bioorg. Med. Chem. Lett. 11 (2001) 3147–3149;
(b) C.J. Moody, E. Swann, J. Med. Chem. 38 (1995) 1039–1043.
[18] D. Kumar, N.M. Kumar, S. Sundaree, E.O. Johnson, K. Shah, Eur. J. Med. Chem. 45 (2010) 1244–1249.
[19] D. Kumar, S. Sundaree, E.O. Johnson, K. Shah, Bioorg. Med. Chem. Lett. 19 (2009) 4492–4494.
[20] (a) L. Mounim, B. Fouad, L. Michel, J. Heterocycl. Chem. 42 (2005) 991–994;
(b) G. Mazzone, G. Puglisi, F. Bonina, A. Corsaro, J. Heterocycl, Chem. 20 (1983) 1399–1401.
[21] (a) A.A. Kiryanov, P. Sampson, A.J. Seed, J. Org. Chem. 66 (2001) 7925–7929;
(b) B. Gierczyk, M. Zalas, Org. Prep. Proced. Int. 37 (2005) 213–222.
[22] V. Polshettiwar, R.S. Varma, Tetrahedron Lett. 49 (2008) 879–883.
[23] Unpublished results.
[24] C.J. Swain, R. Baker, C. Kneen, J. Moseley, J. Saunders, E.M. Seward, G. Stevenson, M. Beer, J. Stanton, K. Watling, J. Med. Chem. 34 (1991) 140–151.