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One-pot synthesis and anticancer studies of 2-arylamino-5-aryl-1,3,4-thiadiazoles

Dalip Kumar [a,*], Buchi Reddy Vaddula [a], Kuei-Hua Chang [b], Kavita Shah [b,*]

[a] Department of Chemistry, Birla Institute of Technology and Science, Pilani 333031, Rajasthan, India
[b] Department of Chemistry and Purdue Cancer Center, Purdue University, 560 Oval Drive, West Lafayette, IN 47907, USA

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ABSTRACT

A series of 2-arylamino-5-aryl-1,3,4-thiadiazoles **1a–j** were synthesized and screened for their anticancer activity against various human cancer cell lines. The novel one-pot synthesis of 1,3,4-thiadiazoles was achieved by refluxing aryl aldehydes, hydrazine hydrate, and aryl isothiocyanates in methanol followed by oxidative cyclization with ferric ammonium sulfate. The compounds **1g–j** with trimethoxyphenyl at the C-5 position displayed extremely potent anticancer activity with at least twofold selectivity (IC_{50}: 4.3–9.2 μM). The nature of substituent on the C-2 arylamino ring may be critical in opting for the selectivity towards a particular cancer cell.

1,3,4-Thiadiazoles are five-membered ring systems that have gained prominence by exhibiting a wide variety of biological activities as well as producing useful intermediates in several organic preparations.[1–7] They have interesting pharmacophores that display a broad spectrum biological activity. The lower toxicity and in vivo stability of thiadiazole nucleus is attributed to its aromaticity.[8] Thiadiazoles have exhibited potential antiglaucoma,[9] antiinflammatory,[1] antitumor,[7] antiulcer,[10] antibacterial,[11] antiviral,[12] analgesic,[13] antiepileptic,[5] antifungal,[11] and radioprotective activities.[14] The marketed drugs, acetazolamide, methazolamide, globucid, etc. showcase their therapeutic potential. Thiadiazoles, bioisosters of thiazoles and oxadiazoles, are known to have interesting electro-optical properties[15] and also act as corrosion and oxidation inhibitors,[16] complexation reagents for dyes and metal ions.[17–20]

The 1,3,4-thiadiazoles, their isomeric forms and bioisosters are extensively investigated for their anticancer activity due to their therapeutic potential.[7,21–23] In this direction, our research group has successfully explored various heterocycles to develop a potential anticancer compound.[24–27] 2-Amino-1,3,4-thiadiazole, 2-ethylamino-1,3,4-thiadiazole, 2,2′-(methylenediamino)bis-1,3,4-thiadiazole and their N-substituted derivatives were found to exhibit very good anticancer activity but suffered from adverse side-effects.[28–30] 2-(4-Fluorophenylamino)-5-(2,4-dihydroxy-phenyl)-1,3,4-thiadiazole (FABT), a promising anticancer compound for treating malign tumors of the nervous system, inhibits proliferation by decreasing cell division and inhibiting metastasis.[22,31] It was shown that the one of the important structural unit in several known natural antimitotic agents such as Combretastatin A-4, Colchicine, Podophyllotoxin, and Steganacin, which bind at the Colchicine site on tubulin, is a trimethoxyaryl moiety (Fig. 1).[32,33] The incorporation of crucial structural features of anticancer compounds (3,4,5-trimethoxyphenyl and 4-hydroxy-3-methoxyphenyl moieties) into 1,3,4-thiadiazoles may lead to a potent anticancer compound (Fig. 1).

Generally, preparation of 1,3,4-thiadiazoles involve use of diacylhydrazide precursors under different reaction conditions. Symmetrical 2,5-disubstituted-1,3,4-thiadiazoles are prepared by condensation of aryl aldehydes, hydrazine, and sulfur in ethanol under microwave irradiation.[34] The general routes for the preparation of various 1,3,4-thiadiazoles involve either synthesis of acyl thiohydrazides and then cyclization or thionation of acyl hydrazides followed by oxidative cyclization of thiosemicarbazones. Gierczyk and Zalas reported the four step synthesis of 2,5-disubstituted-1,3,4-thiadiazoles from pentafluorophenyl esters.[35] Also a robust protocol for the solid phase synthesis of 5-alkyl/aryl-2-alkylamino-1,3,4-thiadiazoles was described from resin bound thiosemicarbazide.[36] Oruc et al., prepared 1,3,4-thiadiazoles in four steps utilizing acyl halides and aryl isothiocyanates.[37] The 1,3,4-thiadiazoles were also achieved in good yields from the reaction of 1,3,4-oxadiazoles with thiourea.[38] Recently reported one-pot convenient synthesis of 1,3,4-thiadiazoles by

* Corresponding authors. Tel.: +91 1596 245073 279; fax: +91 1596 244183 (D.K.); tel.: +1 765 496 9470 (K.S.).
E-mail addresses: dalipk@bits-pilani.ac.in (D. Kumar), shah23@purdue.edu (K. Shah).

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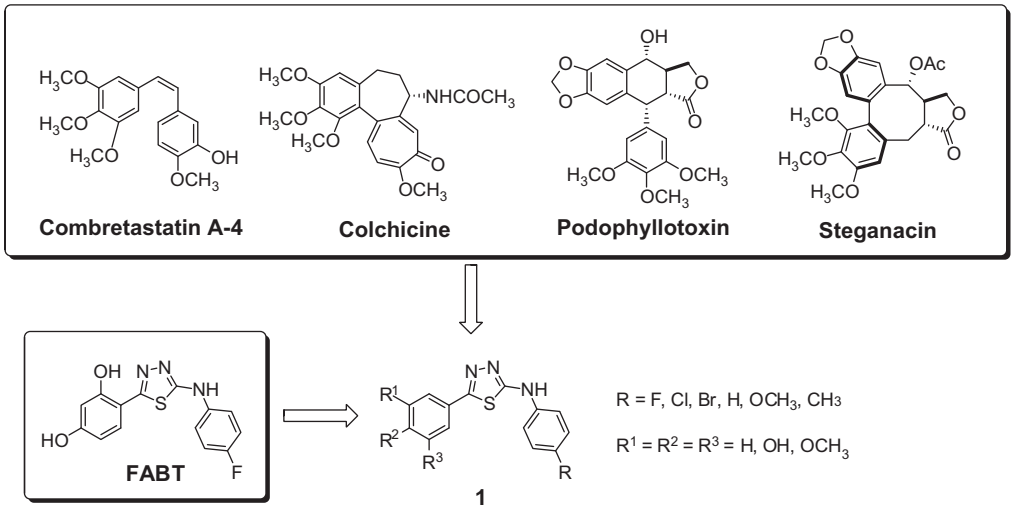
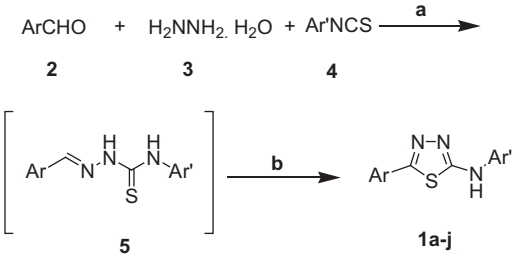


Figure 1. Rational for the design of 2-arylamino-5-aryl-1,3,4-thiadiazoles **1**.

Rostamizadeh et al., utilizes ionic liquid/thiourea mediated oxidation of in situ generated thiosemicarbazones.[39] In general, most of the reported protocols are time consuming, laborious and require multi synthetic steps.

We have developed an expeditious one-pot synthesis of 1,3,4-thiadiazoles **1** which involve the reaction of aryl aldehydes **2**, hydrazine hydrate **3**, and aryl isothiocyanates **4** followed by oxidative cyclization of in situ generated thiosemicarbazones with ferric ammonium sulfate (FAS) (Scheme 1). Our initial efforts to prepare 1,3,4-thiadiazoles **1** involved one-pot sequential reaction of 4-hydroxy-3-methoxybenzaldehyde **2a**, hydrazine hydrate **3**, and phenyl isothiocyanate **4a** in methanol. The reaction of equimolar quantities of 4-hydroxy-3-methoxybenzaldehyde **2a** and hydrazine hydrate **3** in methanol at 80 °C resulted in the intermediate benzylidene hydrazine which was further reacted with phenyl isothiocyanate **4a** to afford thiosemicarbazone **5a**. Further, we have attempted to prepare thiosemicarbazone **5a** in one-pot by the simultaneous reaction of 4-hydroxy-3-methoxybenzaldehyde **2a**, hydrazine hydrate **3**, and phenyl isothiocyanate **4a** in methanol. To our delight, we found that the thiosemicarbazone **5a** was obtained in 90% yields. It was isolated by simple filtration and subjected to oxidative cyclization. Taking a cue from the literature reports, the cyclization of **5a** was attempted using various reagents (Table 1). Of the screened reagents, FAS smoothly produced the desired 1,3,4-thiadiazole in good yields.

The overall one-pot protocol was established by the reaction of equimolar quantities of 4-hydroxy-3-methoxybenzaldehyde **2a**, hydrazine hydrate **3**, and phenyl isothiocyanate **4a** in 25 mL methanol at 80 °C for 1 h. Upon completion of the reaction, as indicated by TLC, the solid product was allowed to settle down. The excess solvent was decanted and added 3 equiv of finely ground FAS to the reaction pot and heated at 80 °C for further 1 h to obtain the 1,3,4-thiadiazole **1a** in 85% yield.[40] The formation of the thiadiazole **1a** was confirmed by its ^{1}H NMR and MS data.[41–43] The ^{1}H NMR spectrum of **1a** evidenced a characteristic singlet at δ 9.57 due to C–2N–H and the mass spectrum shows the $[M+H]^+$ peak at m/z 300.1 which is in agreement with the calculated value.



Scheme 1. One-pot synthesis of 1,3,4-thiadiazoles **1a–j**. Reagents and conditions: (a) methanol, 80 °C, 1 h; (b) FAS, 80 °C, methanol, 1 h.

Table 1
Conditions investigated for the conversion of thiosemicarbazone **5a** to 1,3,4-thiadiazole **1a**

S. No.	Reagent	Solvent	Temp. (°C)	Time (h)	Yield (%)
1	$FeCl_3$ (10% soln)	C_2H_5OH	80	4	10
2	$FeCl_3$ (4 mmol)	H_2O	90	4	0
3	FAS (1 mmol)	CH_3OH	80	3	40
4	FAS (3 mmol)	CH_3OH	80	1	85
5	FAS (6 mmol)	CH_3OH	80	1	75
6	$ZnCl_2$ (excess)	CH_3OH	80	24	0
7	AcOH (excess)	—	120	24	0
8	IBD (1 mmol)[a]	CH_2Cl_2	0 to rt	4	0
9	HTIB (1 mmol)[a]	CH_2Cl_2	0 to rt	4	0
10	$HgCl_2$ (1.1 mmol)	CH_3CN	rt	10	0

[a] IBD: iodobenzenediacetate; HTIB: [Hydroxy(tosyloxy)iodo]benzene.

The optimized protocol was extended to other aryl isothiocyanates and aryl aldehydes. The reaction of 4-hydroxy-3-methoxybenzaldehyde **2a** with hydrazine hydrate **3** and aryl isothiocyanates **4b–e** afforded **1b–e** in good yields (75–85%). All aryl isothiocyanates **4a–e** were almost equally reactive to generate the 1,3,4-thiadiazoles **1a–e**. The deactivated aldehyde, 3,4,5-trimethoxybenzaldehyde **2c**, also reacted as efficiently as 4-hydroxy-3-methoxybenzaldehyde **2a** affording products **1g–j** in good yields (70–80%). Employing 3-cyclopentyloxy-4-methoxybenzaldehyde **2b** with phenyl isothiocyanate **4a** resulted in **1f** with relatively low yields (55%) which may be due to the cleavage of cyclopentyl group during oxidative cyclization.

The synthesized series of diverse 2-arylamino-5-aryl-1,3,4-thiadiazoles **1a–j** were screened against prostate (PC3, DU145, and LnCaP), breast (MCF7 and MDA-MB-231), and pancreatic (PaCa2) cancer cell lines. All compounds decreased cell viability as determined by colorimetric MTT assay, with IC_{50} values ranging from micromolar to greater than 1 mM (Table 2).[44] More importantly, a few compounds were highly potent and exhibited specificity towards one cell type relative to others.

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

Entry	Ar	Ar′	LnCap	DU145	PC3	MCF7	MDA-MB-231	PaCa2
1a	4-OH-3-$OCH_3C_6H_3$	C_6H_5	205.5	57.6	30	55.3	39	58.5
1b	4-OH-3-$OCH_3C_6H_3$	4-$CH_3C_6H_4$	544.6	655.6	>1000	175.2	479.8	449.6
1c	4-OH-3-$OCH_3C_6H_3$	4-ClC_6H_4	204.2	435.4	634.7	204.7	386.4	49.1
1d	4-OH-3-$OCH_3C_6H_3$	4-$NO_2C_6H_4$	62.3	>1000	>1000	>1000	>1000	>1000
1e	4-OH-3-$OCH_3C_6H_3$	4-$OCH_3C_6H_4$	>1000	>1000	>1000	>1000	>1000	>1000
1f	3-OC_5H_9-4-$OCH_3C_6H_3$	C_6H_5	111.8	177.1	96.9	84.7	433.9	33.6
1g	3,4,5-$(OCH_3)_3C_6H_2$	C_6H_5	92.8	283.9	81.8	**9.2**	122.4	25.9
1h	3,4,5-$(OCH_3)_3C_6H_2$	4-$NO_2C_6H_4$	66.5	99.7	71.4	**6.6**	13.8	242.6
1i	3,4,5-$(OCH_3)_3C_6H_2$	4-$CH_3C_6H_4$	102.6	130.7	**20.2**	**10.2**	32.1	**5.8**
1j	3,4,5-$(OCH_3)_3C_6H_2$	4-$OCH_3C_6H_4$	29.5	33.3	**14.5**	**11.1**	25.2	**4.3**
Doxorubicin			**6.3**	**12.3**	124	66.2	**6.8**	25.3

IC_{50} values of about 20 μM are indicated in bold face; OC_5H_9: cyclopentyloxy
[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.

The anticancer activity profiles of the screened compounds indicate that the substitution on the C-5 aryl ring plays a great role in furnishing the activity. The compounds **1a–e** with 4-OH-3-OCH_3–C_6H_3 and **1f** with 3-OC_5H_9-4-OCH_3–C_6H_3 at C-5 position were found to be less active than the corresponding compounds **1g–j** with 3,4,5-$(OCH_3)_3C_6H_2$. The variation of C-2 arylamino group seems to have little effect on the activity of 1,3,4-thiadiazoles. As it was hypothesized, the trimethoxyphenyl substitution might be playing a crucial role by means of binding to the Colchicine site on tubulin. Our earlier studies on 3,5-disubstituted-1,2,4-oxadiazoles delivered a very potent compound (IC_{50} = 10 nM) with 3-OC_5H_9-4-OCH_3–C_6H_3 and piperdin-4-yl.[45] The compound **1f** designed following similar pattern resulted in moderate activity. The compounds **1g–j** with trimethoxyphenyl moiety exhibited very good activity (IC_{50}: 4.3–20.2 μM). The 1,3,4-thiadiazole **1g** having phenylamino group at the 2-position showed greater potency towards breast cancer cell line (MCF7, IC_{50} = 9.2 μM) with at least threefold selectivity compared to other tested cells. Introduction of electron-withdrawing group in the C-2 arylamino unit (compound **1h**) is beneficial for the activity against breast cancer cell lines (MCF7, IC_{50} = 6.6 μM; MDA-MB-231, IC_{50} = 13.8 μM), whereas electron-donating group such as CH_3 or OCH_3 (compounds **1i** and **1j**) resulted in a significant increase in cytotoxic potency except against MCF7 cell line. In particular, compounds **1g** and **1h** were found to be selectively cytotoxic in MCF7 cells. The 4-methoxy analogue **1j** possessed potent anticancer activity against all the tested cell lines while simultaneously exhibiting at least threefold selectivity towards PaCa2. The compounds **1a–e** have exhibited moderate to poor anticancer activity (though some of these have achieved good selectivity) indicating that 4-OH-3-$OCH_3C_6H_3$ group at 5-position is detrimental for the activity. It earmarks the prominence of trimethoxyphenyl substitution at 5-position of 1,3,4-thiadiazole. Doxorubicin was used as a control, which showed higher cytotoxicity towards LnCAP, DU145, and MDA-MB-231 cell lines.

In conclusion, the one-pot reaction of aryl aldehyde, hydrazine hydrate and aryl isothiocyanate led to the expeditious synthesis of 1,3,4-thiadiazoles **1a–j** in good yields. The advantages of the protocol include simple reaction workup, easily available starting materials and convenient isolation. The compounds **1g–j** exhibited very good anticancer activity with good selectivity indicating the importance of 3,4,5-trimethoxyphenyl at C-5 position. The detailed SAR and molecular target study of these 1,3,4-thiadiazoles are in progress and will be reported in due course.

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40. *General procedure for the synthesis of 1,3,4-Thiadiazoles* **1**: A mixture of aryl aldehyde **2** (3.0 mmol), hydrazine hydrate **3** (3.0 mmol) and aryl isothiocyanate **4** (3.0 mmol) was refluxed in 25 mL methanol for 1 h. Upon completion of the reaction, as indicated by TLC, the reaction mixture was cooled to room temperature and allowed the solid product to settle down. The excess solvent was decanted and added finely ground FAS (9.0 mmol) to the reaction pot and heated at 80 °C for further 1 h. The reaction mixture was then filtered, washed with hot methanol and concentrated the filtrate on a rotary evaporator to obtain crude 1,3,4-thiadiazole **1** which was further recrystallized from methanol/ethanol.
41. *Spectral data of the selected 1,3,4-thiadiazoles* (**1**): *2-(3-Methoxy-4-hydroxyphenyl)-5-(phenylamino)-1,3,4-thiadiazole* (**1a**): **%**yield: 85. Mp: 246–247 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.98 (s, 1H), 9.57 (s, 1H), 7.58–7.48 (m, 3H), 7.40–7.32 (m, 2H), 6.80 (dd, J = 8.2, 2.0 Hz, 1H), 6.73 (dd, J = 9.8, 5.1 Hz, 2H), 3.50 (s, 3H). MS(ESI): m/z [M+H]$^+$ calcd for $C_{15}H_{13}N_3O_2S$: 300.1; found: 300.1; 2-(3-Methoxy-4-hydroxyphenyl)-5-(4-methylphenylamino)-1,3,4-thiadiazole (**1b**): **%**yield: 82. Mp: 248–249 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.57 (s, 1H), 9.61 (s, 1H), 7.32–7.23 (m, 4H), 6.78–6.73 (m, 3H), 3.53 (s, 3H), 2.38 (s, 3H). MS (ESI): m/z [M+H]$^+$ calcd for $C_{16}H_{15}N_3O_2S$: 314.1; found: 314.1; *2-(3-Methoxy-4-hydroxyphenyl)-5-(4-chloro-phenylamino)-1,3,4-thiadiazole* (**1c**): %yield: 78. Mp: 268–270 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.03 (s, 1H), 9.60 (s, 1H), 7.60 (d, J = 8.6 Hz, 2H), 7.41 (d, J = 8.6 Hz, 2H), 6.81 (s, 1H), 6.79–6.72 (m, 2H), 3.57 (s, 3H). MS(ESI): m/z [M+H]$^+$ calcd for $C_{15}H_{12}ClN_3O_2S$: 334.0; found: 334.0; *2-(3-Methoxy-4-hydroxyphenyl)-5-(4-nitrophenylamino)-1,3,4-thiadiazole* (**1d**): **%**yield: 76. Mp: 280–284 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.14 (s, 1H), 9.62 (s, 1H), 8.42–8.33 (m, 2H), 7.74–7.66 (m, 2H), 6.86 (s, 1H), 6.73 (s, 2H), 3.57 (s, 3H). MS(ESI): m/z [M+H]$^+$ calcd for $C_{15}H_{12}N_4O_4S$: 345.0; found: 345.0; *2-(3-Methoxy-4-hydroxyphenyl)-5-(4-methoxyphenyl amino)-1,3,4-thiadiazole* (**1e**): %yield: 80. Mp: 228–230 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.93 (s, 1H), 9.56 (s, 1H), 7.29–7.24 (m, 2H), 7.09–7.02 (m, 2H), 6.83–6.77 (m, 2H), 6.72 (d, J = 8.1 Hz, 1H), 3.82 (s, 4H), 3.55 (s, 3H). HRMS (ESI): m/z [M+H]$^+$ calcd for $C_{16}H_{15}N_3O_3S$: 330.1; found: 330.1; *2-(3-Cyclopentyloxy-4-methoxyphenyl)-5-(phenyl amino)-1,3,4-thiadiazole* (**1f**): %yield: 55. Mp: 223–225 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.53–7.47 (m, 4H), 7.36–7.31 (m, 2H), 6.98 (d, J = 8 Hz, 1H) 6.78 (d, J = 8 Hz, 1H), 6.71 (s, 1H), 4.35 (s, 1H), 3.81 (s, 3H), 1.70–1.50 (m, 8H). MS (ESI): m/z [M+H]$^+$ calcd for $C_{20}H_{22}N_3O_2S$: 368.1433; found: 368.1604; 2-(3,4,5-Trimethoxyphenyl)-5-(phenylamino)-1,3,4-thiadiazole (**1g**): %yield: 78. Mp: 228–230 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.66 (s, 1H), 7.57–7.55 (m, 2H), 7.41 (m, 2H), 7.17 (s, 1H), 7.05 (s, 2H), 3.92(s, 6H), 3.86 (s, 3H). MS (ESI): m/z [M+H]$^+$ calcd for $C_{17}H_{17}N_3O_3S$: 344.1069; found: 344.1816; 2-(3,4,5-Trimethoxyphenyl)-5-(4-methoxyphenyl amino)-1,3,4-thiadiazole (**1j**): %yield: 81. Mp: 191–193 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.64 (s, 1H), 7.48 (d, J = 8.80 Hz, 2H), 7.03 (s, 2H), 6.92 (d, J = 8.80 Hz, 2H), 3.91 (s, 6H), 3.85 (s, 3H), 3.81 (s, 3H). MS (ESI): m/z [M+H]$^+$ calcd for $C_{18}H_{19}N_3O_4S$: 374.1175; found: 374.1354.
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44. 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-dimethylthiazol-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 lysed in 100 mL dimethylsulfoxide, and the absorbance was measured at 570 nm using Tecan Spectrafluor Plus.
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