ISSN 1070-3632, Russian Journal of General Chemistry, 2019, Vol. 89, No. 4, pp. 770–779. © Pleiades Publishing, Ltd., 2019.

Design and Synthesis of 1,3,4-Thiadiazole Derivatives as Novel Anticancer and Antitubercular Agents[1]

D. Chandra Sekhar[a,b], D. V. Venkata Rao[a], A. Tejeswara Rao[c], U. Lav Kumar[b], and Anjali Jha[a]*

[a] *Department of Chemistry, GIS, GITAM University, Rushikonda, Visakhapatnam, Andhra Pradesh, 530045 India*
**e-mail: anjalimanishjha@yahoo.com*

[b] *Shilpa Medicare, API, R and D Unit, Modavalasa, Vizianagaram, Andhra Pradesh, 531162 India*

[c] *Centre for Chemical Sciences and Technology, Department of Chemistry, Institute of Science and Technology, Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Hyderabad, Telangana, 500085 India*

Received December 31, 2017; revised October 12, 2018; accepted April 2, 2019

Abstract—A series of novel 5-phenyl-substituted 1,3,4-thiadiazole-2-amines were designed, synthesized, and screened for their antitumor and antitubercular activities. The target compounds were synthesized starting from isocyanates and acid hydrazides by conventional and microwave-assisted protocols. The structures of the products were confirmed by ^{1}H NMR, ^{13}C NMR, high-resolution mass spectrometry, and IR spectroscopy and elemental analysis. Some of the synthesized compounds showed significant *invitro* antitumor activities against breast cancer and normal human cell lines. Among them, *N*-benzyl-5-(4-fluorophenyl)-, *N*-benzyl-5-(4-nitrophenyl)-, and 5-phenyl-*N*-(*p*-tolyl)-1,3,4-thiadiazole-2-amines demonstrated higher inhibitory activities against the MDA-MB-231 cell line than the cisplatin control (IC_{50} 3.3 μM). *N*-Benzyl-5-(4-methoxyphenyl)-, 5-phenyl-*N*-{[4-(trifluoromethyl)phenyl]methyl}-, *N*-benzyl-5-(4-fluorophenyl)-, and *N*-benzyl-5-(4-nitrophenyl)-1,3,4-thiadiazole-2-amines exhibited high inhibitory activities against the HEK293T cell line (IC_{50} 52.63, 42.67, 34.71, and 33.74 μM, respectively), which were higher compared to the cisplatin control. In antitubercular activity testing against *mycobacterium smegmatis* MC155, 5-phenyl-*N*-{[4-(trifluoromethyl)-phenyl]methyl}-1,3,4-thiadiazole-2-amine proved to be a more potent agent (MIC 26.46 μg/mL) compared to the Isoniazid control (12 μg/mL). Potential bioactivities of the synthesized compounds were computed using Molinspiration and Molsoft software tools.

Keywords: 1,3,4-thiadiazoles, anticancer activity, antitubercular activity, Molinspiration, Molsoft

DOI: 10.1134/S1070363219040224

INTRODUCTION

Heterocyclic compounds are commonly used scaffolds for pharmacophores for the design of potent and selective drugs. 1,3,4-Thiadiazole represent a key motif in heterocyclic chemistry and a privileged structure in medicinal chemistry due to its broad-range pharmacological activity [1, 2]. Despite the progress in medicine over the past century, cancer and tuberculosis (TB) still remain the leading causes of death in the world. This makes critically important to continue search for new anticancer and anti-TB drug candidates. Thiadiazole is a 5-membered planar aromatic motif comprising a sulfur atom, which improves the lipid solubility of thiadiazole derivatives and hence their pharmacokinetics [3]. The two-electron donor nitrogen system (N=C–S) and hydrogen-binding domain enhance the ability of thiadiazole molecules for binding with receptors [4, 5].

1,3,4-Thiadiazoles have been reported to exhibit antifungal [6], antibacterial [7], inflammatory [8], anti anxiety [9], and anti-tubercular activities [10]. Different mechanisms of the antitumor activity of 1,3,4-thiadiazoleshave been reported, including specific inhibition of DNA and RNA syntheses without appreciable impact on protein synthesis [11], inhibition of carbonic anhydrase [12], phosphodiesterase-7 (PDE7) [13], and histonedeacetylase [14], or adenosine A3 receptor antagonism [15]. The list of thiadiazole-containing clinically used drugs or drug candidates includes Acetazolamide, Zibotentan, Sulfamethizole, Methazolamide, Megazol, and Globucid. In view of the aforesaid, we designed (Fig. 1, Scheme 1) and synthesized novel thiadiazole molecules and performed their anticancer and anti-TB activity testing.

[1] Supporting materials are available from authors.

RESULTS AND DISCUSSION

Synthesis and structure. Novel 1,3,4-thiadiazole derivatives **4a**–**4m** were synthesized by the reaction of substituted isocyanates **1** with acid hydrazides **2** by conventional (Scheme 1, path *a*) and microwave-assisted protocols (Scheme 1, path *b*). The conventional synthesis involved two stages: (1) the reaction of compounds **1** with compounds **2** in the presence of triethyl amine (TEA) in dry THF to obtain intermediate derivatives **3** and (2) the reaction of intermediates **3** with TEA and *p*-TsCl in *N*-methyl-2-pyrrolidone (NMP) under heating to obtain the target 1,3,4-thiadiazole derivatives **4a**–**4m** (Scheme 1). The microwave-assisted synthesis involved a one-pot reaction of compound **1** with compound **2** in presence of TEA, Dry THF, P-TsCl, NMP to obtained desired products **4a**–**4m** with excellent yields. Under non-classical reaction condition (MWI), the reaction rates were faster than classical heating conditions [16, 17]. For example, synthesis of **4b** was obtained in 4.5 h by traditional methods whereas under M_w condition, it was obtained in 5 min (**4b,** Table 1). The structures of all synthesized compounds were assigned on the basis of the IR, mass, ^{1}H NMR, ^{13}C NMR spectral data. For the compound **4b**,on the ^{1}H NMR timescale, disappearance of labile protons ($NHNH_2$) signals at δ 8.45 and 4.55 ppm for NH, CH_2 protons as a triplet, doublet and appearance of a signal at δ 7.83–7.25 ppm for aromatic hydrogens asserted **4b**. The resonance

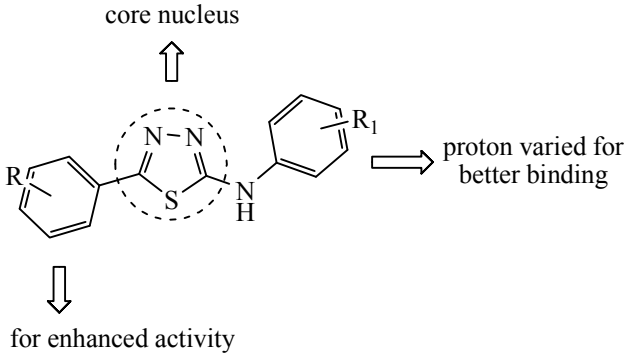
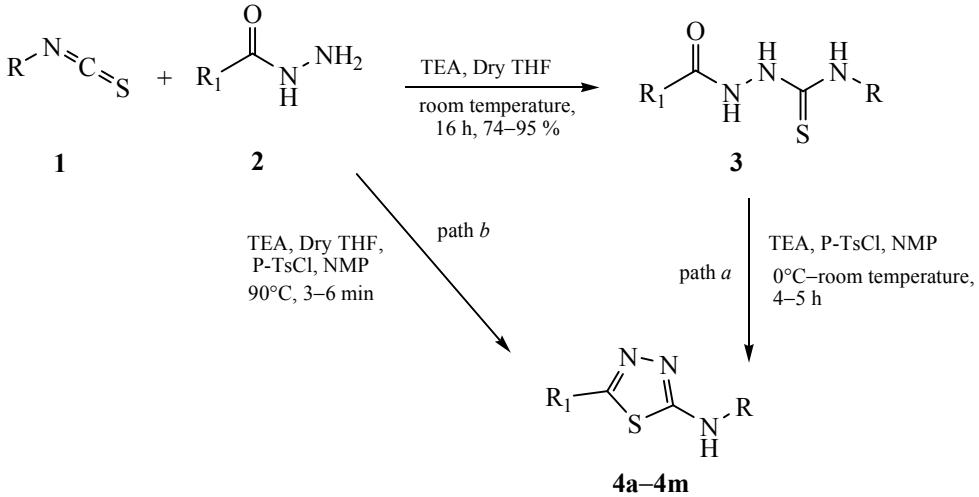


Fig. 1. Design strategy for the synthesis of the target compounds.

signals in the ^{13}C NMR spectra at δ 168.92 (C^2), 162.18 (C^5) ppm validated the construction of 1,3,4-thiadiazole core. The IR spectrum of **4b** displayed characteristic absorption bands at ν_{max} 3370 cm^{-1} (NH str), 3058 cm^{-1} (Ar=CH str), 1399 cm^{-1} (C=N), and 1218 cm^{-1} (C–S–C) clearly evidenced the presence of cyclic C–S–C linkage and N–N=C in the thiadiazole frame work. And finally the molecular weight of the **4b** is *m/z* = 284 [*M* – 1].

Biological activity testing . The in vitro cytotoxicity of compounds **4a**–**4m** was assessed by employing the MTT colorimetric assay as per ATCC protocol at 25 μM against a panel of cancer cell lines, namely, human breast adenocarcinoma (MDAMB231) and normal cell lines (HEK293T). Cisplatin was used

Scheme 1. Design strategy for the synthesis of the target compounds.



R = $PhCH_2$, R^1 = Ph (**a**); R = $PhCH_2$, R^1 = *p*-FC_6H_4 (**b**); R = $PhCH_2$, R^1 = *p*-$NO_2C_6H_4$ (**c**); R = $PhCH_2$, R^1 = *p*-ClC_6H_4 (**d**); R = $PhCH_2$, R^1 = *p*-$MeOC_6H_4$ (**e**); R = $PhCH_2$, R^1 = *p*-MeC_6H_4Ph (**f**); R = *p*-$MeOC_6H_4$, R^1 = Ph (**g**); R = *p*-$CF_3C_6H_4$, R^1 = Ph (**h**); R = *p*-FC_6H_4, R^1 = Ph (**i**); R = *p*-$ClOC_6H_4$, R^1 = Ph (**j**); R = *p*-MeC_6H_4, R^1 = Ph (**k**); R = *p*-$NO_2C_6H_4$, R^1 = Ph (**l**); R = *p*-$MeOC_6H_4$, R^1 = Ph (**m**).

Table 1. Synthesis times and yields of compounds **4a**–**4m** by two protocols

Comp. no	Microwave-assisted synthesis: min/yield, %	Conventional synthesis: h/yield, %
4a	6.0/87	4.0/68
4b	5.0/79	5.0/82
4c	4.0/80	4.5/60
4d	4.5/82	4.0/69
4e	5.5/72	5.0/62
4f	5.0/73	5.0/60
4g	4.0/70	5.0/53
4h	4.0/68	4.0/65
4i	4.5/66	4.0/44
4j	4.0/72	4.5/58
4k	5.0/63	5.0/55
4l	4.5/74	4.0/62
4m	5.0/68	4.5/53

as standard, and the results are summarized in Table 2. Compounds **4k, 4b,** and **4c** (% inhibition 30.29, 22.44, and 21.84 respectively) exhibited the highest anticancer activity against MDAMB231 cells. Among the test compounds, **4e** (52.63 μM) displayed promising activity, and products **4h**, **4b,** and **4c** showed moderate to good activity against HEK293T cells with percentage inhibitions at 25 μM of 42.67, 34.71, and 33.74 respectively. Homologation of **4a** to **4f** in turn to **4l** and then to **4g** decreased the activity

Table 2. Cytotoxicities, lipophilicities, and antimycobacterial activities of compounds **4a**–**4m**

Comp. no.	Cytotoxicity (% inhibition at 25 μM)		Lipophilicity (log *P*/*c*log *P*)	Anti-TB activity (MIC, μM)
	MDAMB231 cell line	HEK293T cell line		*M. smegmatis* MC 155 strain
4a	–3.30	15.06	4.79/3.92	>100
4b	22.44	34.71	4.95/4.07	36.05
4c	21.84	33.74	0.00/3.67	45.23
4d	11.64	20.14	5.35/4.64	>100
4e	8.04	52.63	4.67/3.98	45.09
4f	–12.27	20.30	5.28/4.42	>100
4g	–0.93	9.30	4.67/3.84	28.78
4h	5.59	42.67	5.71/4.80	26.46
4i	18.20	22.61	5.19/4.61	36.15
4j	20.10	17.45	5.59/5.18	>100
4k	30.29	24.58	5.51/4.95	78.72
4l	15.06	–5.40	0.00/4.26	>100
4m	5.18	2.70	4.90/4.38	>100
Cisplatin	3.30	2.80	0.00/–2.50	Not determined
Isoniazid	Not determined	Not determined	–0.6/–0.66	12

profile for both cell lines. For the series **4b** (R^1 = *p*-FC_6H_4), **4c** (R^1 = *p*-$NO_2C_6H_4$), **4h** (R = *p*-$CF_3C_6H_4$), the activity against the lung cancer cell line increases with decreasing –I effect of the substituent. Hence, it is believed that the synthesized compounds may act as effective anticancer agents. The synthesized derivatives were also tested for cytotoxicity using normal human cells HEK293T and were found to exhibit mediocre safety profiles. Among the test cell lines, HEK293T cells were more sensitive to all the test compounds than MDAMB231 cells. As an example, Fig. 2 presents the anticancer activity profile for compound **4k**.

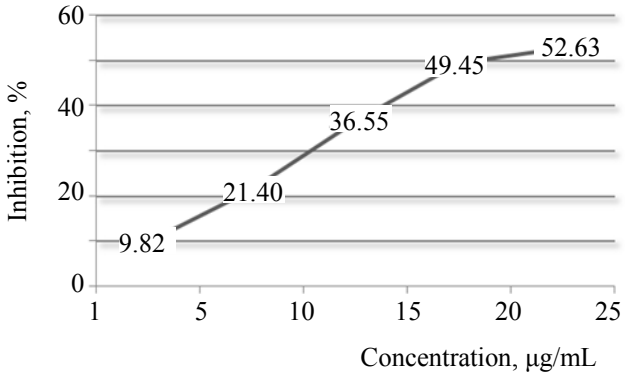


Fig. 2. Dose–response curve for the most active compound **4k** in the HEK293T cell line.

Additionally, the synthesized compounds were screened for antimycobacterial capacity against *M. smegmatis*, and the resulting data are summarized in Table 2. As seen from the data in the table, some compounds from the series showed promising antimycobacterial properties. Compounds **4h, 4g** and **4b** exhibited the highest antimycobacterial activity with MIC values of 26.46, 28.78, and 36.05 μM, respectively. Compounds **4e, 4c,** and **4k** showed a moderate anti-mycobacterial activity with MIC values of 45.09, 45.13, and 78.72 μM, respectively. Compounds **4a, 4d, 4f, 4j,** and **4l** did not show any growth inhibition even at the highest concentration tested.

Absorption, polar surface area, druglikeness, and pharmacokinetic properties. High oral bioavailability is an important factor for the development of bioactive molecules as therapeutic agents. Good intestinal absorption, reduced molecular flexibility, low polar surface area, or total hydrogen bond count (some of donors and some of accepters) are important predictors of this property [18]. Physicochemical characteristics play a vital role in the generation and determination of the bioactivity of any compound. Molecular properties, such as membrane permeability and bioavailability, are always confederate with some basic molecular descriptors such as partition coefficient (log *P*), molecular weight (M_w), or numbers of hydrogen bond acceptors and donors in the molecule [19]. The rule states that most molecules with good membrane permeability have log $P \leq 5$, $M_w \leq 500$, number of hydrogen bond acceptors ≤ 10, and number of hydrogen bond donors ≤ 5. This rule is widely used as a filter for drug-like properties. In the present consideration, the percentage absorption (ABS) was calculated by the equation ABS = 109–0.345·PSA [20]. Hydrogen bonding capacity was also recognized as an important parameter for describing drug permeability [21]. A poor permeation or absorption is more likely, when the compound has more than five H-bond donors and more than ten H-bond acceptors. In the present study, 2,5-substituted-1,3,4-thiadiazoles have a number of H-bond acceptors (≤ 10) and a lower number of hydrogen bond donors (≤ 5) (Table 3). The number of rotatable bonds relates to conformational flexibility and is essential for binding acceptors or passages. To meet the oral bioavailability criteria, the number of rotatable bonds should be less than 10. The title compounds in general possess high number of rotatable bonds (0–5) and therefore, exhibit large conformational flexibility [22].

Molecular polar surface area (PSA) is a very useful parameter for the prediction of drug transport properties and PSA is a sum of surfaces of polar atoms (usually oxygen, nitrogen and attached hydrogen) in a molecule. Topological polar surface area (TPSA) was determined by the fragment-based method of Ertl and co-workers PSA has been used for characterizing drug absorption, including abdominal absorption, bioavailability, $CaCO_2$ permeability, and blood brain barrier permeability. PSA and volume is inversely proportional to percentage of absorption (%ABS) Table 4. Number of rotatable bonds (nrotb) is a simple topological parameter that measures molecular flexibility. Rotatable bond is defined as any single non ring bond, bounded to non terminal atom and it has been shown to be a very good descriptor of oral bioavailability of drug. The drug likeness score was a consolidate effect of physicochemical properties, pharmacokinetics and pharmaco dynamics of a compound and is represented by a numerical value computed by MolSoft (MolSoft, 2007). The drug likeness score was calculated by considering Milog P

Table 3. Molinspiration bioactivity scores for compounds **4a**–**4m**

Compound	GPCRL	ICM	KI	NRL	PI	EI	n_{ON}	n_{OHNH}	$N_{violations}$
4a	–0.47	–0.69	–0.06	–0.42	–0.60	–0.13	3	1	0
4b	–0.39	–0.68	–0.02	–0.33	–0.55	–0.14	3	1	0
4c	–0.49	–0.67	–0.13	0.38	–0.57	0.24	6	1	0
4d	–0.41	–0.67	–0.04	–0.38	–0.58	–0.16	3	1	0
4e	–0.41	–0.73	–0.03	–0.32	–0.53	–0.17	4	1	0
4f	–0.46	–0.75	–0.07	–0.40	–0.60	–0.19	4	1	0
4g	–0.57	–0.84	–0.10	–0.55	–0.73	0.28	4	1	0
4h	–0.21	–0.48	0.12	–0.07	–0.32	0.10	3	1	0
4i	–0.57	–0.79	–0.06	–0.59	–0.78	0.27	3	1	0
4j	–0.59	–0.78	–0.13	–0.64	–0.80	0.29	3	1	1
4k	–0.64	–0.88	–0.17	–0.65	–0.82	0.33	3	1	1
4l	–0.63	–0.76	–0.20	–0.59	–0.74	0.33	6	1	0
4m	–0.57	–0.84	–0.10	–0.55	–0.73	0.28	4	1	0
Isoniazid	–1.39	–1.45	–1.05	–2.33	–1.23	–0.66	4	3	0

(Logarithm of the octanol/water partition coefficient), molecular weight, number of heavy atoms, number of hydrogen donor, number of hydrogen acceptor and number of violation, number of rotatable bonds, volume. The maximum drug-likeness score (–0.21) was found to be **4b** in Table 4.The bioactivity scores of the isolated compounds are compared with standard drug on the basis of GPCR ligand (GPCRL), ion channel modulator (ICM), nuclear receptor legend (NRL), kinase inhibitor (KI), protease inhibitor (PI), enzyme inhibitor (EI) in Table 3.

EXPERIMENTAL

All reactions were carried out under argon in oven dried glassware with magnetic stirring. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. All solvents were reagent grade. Unless otherwise noted, organic extracts were dried with anhydrous Na_2SO_4, filtered through a fitted glass funnel, and concentrated on a rotary evaporator (20–30 Torr). Commercial chemicals were treated as follows: DMF was distilled over CaH_2 and degassed (freeze and thaw) three times prior to use; THF, ether, and hexane were distilled over Na/benzophenone. Flash chromatography was performed on silica gel (200–300 mesh), using the mobile phases indicated. The spots were observed by exposure to iodine vapours, UV light or *p*-anisaldehyde stain solution. Elemental analysis was performed on a Carlo Erba 1108 analyzer. The ^{1}H NMR spectra were measured on a Bruker Advance spectrometer at 400.1 and 100.6 MHz for ^{1}H for ^{13}C respectively, in $CDCl_3$ or DMSO-d_6 against internal TMS. Chemical shifts are given in ppm (δ) and referenced to the residual proton resonances of the solvents (^{1}H NMR: TMS at 0.00 ppm, $CDCl_3$ at 7.26 ppm, DMSO at 2.50 ppm; ^{13}C NMR: $CDCl_3$ at 77.16 ppm, DMSO at 40.00 ppm).

General procedure for synthesis of substituted hydrazine carbothioamides (3a–3m). Aromatic iso thiocyanates **1** (2.40 mmol) was added to a stirred solution of aromatic substituted hydrazides **2** (2 mmol) and triethylamine (2 mmol) in 10 mL of THF. The reaction mixture was stirred at room temperature, and then the solvents were removed on a rotary evaporator. The residue was triturated with diethyl ether–ethyl acetate (95 : 5) to afford 95% of the desired thiosemicarbazide.

General procedure for synthesis of 2,5-substituted-1,3,4-thiadiazoles (4a–4m). *a. Conventional method.* *p*-Toluene sulfonyl chloride (0.60 mmol) was added to a stirred solution of substituted thiosemicarbazide **3**

Table 4. Calculated percentage absorptions, polar surface areas, Lipinski parameters, and drug-likeness model scores for compounds **4a**–**4m**

Compound	Absorption, %	*V*	TPSA	NROTB	HBA	HBD	milog *P*	M_W	Drug-likeness model score
4a	95.55	238.46	37.81	4	3	1	3.83	267.36	–0.77
4b	95.55	243.40	37.81	4	3	1	3.99	285.35	–0.21
4c	47.70	261.80	83.64	5	5	1	3.79	312.35	–0.50
4d	95.55	252.00	37.81	4	3	1	4.51	301.80	0.02
4e	74.27	264.01	47.05	5	4	1	3.89	297.38	–0.35
4f	95.55	255.03	37.81	4	3	1	4.28	281.38	–0.55
4g	92.76	247.21	47.05	4	4	1	4.62	283.36	–0.74
4h	95.55	269.76	37.81	5	3	1	4.72	335.35	–0.93
4i	95.55	226.59	37.81	3	3	1	4.73	271.32	–0.52
4j	95.55	235.20	37.81	3	3	1	5.24	287.77	–0.27
4k	95.55	238.22	37.81	3	3	1	5.01	267.36	–1.01
4l	80.14	245.00	83.64	4	5	1	4.52	298.33	–1.17
4m	92.76	247.21	47.05	4	4	1	4.62	283.36	–0.74
Isoniazid	85.53	122.56	68.01	1	3	3	–9.70	137.14	0.51
Cisplatin	90.50	245.26	53.60	0	2	4	–0.69	298.96	–1.12

(0.5 mmol) and triethylamine (1.1 mmol) in 4 mL of NMP. The reaction mixture was stirred at room temperature for 2 h and extracted with DCM (15 mL) and distilled water (10 mL), after which the aqueous layer was removed. The aqueous layer was back-extracted with DCM (3 × 10 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to leave the desired crude product, which was purified by column chromatography on silica gel (hexane/ethyl acetate) to obtain 2,5-substituted-1,3,4-thiadiazole derivative **4** (yield 44–82%).

b. Microwave-assisted method. A mixture of compound isothiocyanate **1** (1.0 equiv), aromatic substituted hydrazide **2** (0.8 equiv), and trimethyl amine (0.8 equiv) in THF (5 vol %) was loaded into a closed vessel and subjected to MW irradiation at 65°C for 18 min. Completion of the reaction to form product **3** was monitored by TLC monitoring. After that thiosemicarbazide, tri ethylamine, and NMP were added to the reaction mixture, and it was MW-irradiated at 90°C for 3–6 min. The organic solvent was then distilled off, and the residue was treated with DCM (2×20 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under reduced pressure. The obtained crude product was recrystallized from methanol to obtain 2,5-substituted-1,3,4-thiadiazole derivative **4** (yield 66–87%).

***N*-Benzyl-5-phenyl-1,3,4-thiadiazole-2-amine (4a).** Brown solid, mp 180–181°C. IR spectrum, ν, cm^{-1}: 3438 (NH), 3070 (Ar=CH), 2958, 2868 (CH), 1597, 1555, 1488 (ArC=C), 1392 (C=N), 1217 (CSC). ^1H NMR spectrum, δ, ppm: 7.81–7.77 m (2H, ArH), 7.41–7.31 m (8H, ArH), 5.72 br.s (1H, NH), 4.60 d (2H, *J* = 4 Hz, CH_2). ^{13}C NMR spectrum, δ_C, ppm: 169.46 (C^5), 158.38 (C^2), 136.97 (ArC), 130.98 (ArC), 129.85 (ArC), 128.90 (ArC), 128.89 (ArC), 128.06 (ArC), 127.81 (ArC), 126.92 (ArC), 50.75 (CH_2). Found, %: C 67.78; H 4.96; N 15.91; S 12.16. $C_{15}H_{13}N_3S$. Calculated, %: C 67.39; H 4.90; N 15.72; S 11.99. $[M]^+$ 266.

***N*-Benzyl-5-(4-fluorophenyl)-1,3,4-thiadiazole-2-amine (4b).** White solid, mp 173–175°C. IR spectrum,

ν, cm^{-1}: 3370 (NH), 3058 (Ar=CH), 2932, 2867 (CH), 1593, 1514 (ArC=C), 1399 (C=N), 1218 (CSC), 979 (C–F). ^{1}H NMR spectrum, δ, ppm: 8.45 t (1H, *J* = 8 Hz, NH), 7.83–7.75 m (2H, ArH), 7.41–7.25 m (7H, ArH), 4.55 d (2H, *J* = 4 Hz, CH_2). ^{13}C NMR spectrum, δ_C, ppm: 168.92 (C^5), 162.18 ($^1J_{CF}$ = 248.5 Hz), 155.61 (C^2), 139.02 (ArC), 119.91 ($^3J_{CF}$ = 9.1 Hz), 128.92, 128.05 (ArC), 127.91 ($^4J_{CF}$ = 3.0 Hz), 127.67 (ArC), 116.70 ($^2J_{CF}$ = 22.2 Hz), 48.57 (CH_2). Found, %: C 63.84; H 4.92; F 6.78; N 14.98; S 11.46. $C_{15}H_{12}N_3SF$. Calculated, %: C 63.14; H 4.24; F 6.66; N 14.73; S 11.24. $[M]^+$ 284.

***N*-Benzyl-5-(4-nitrophenyl)-1,3,4-thiadiazole-2-amine (4c).** Yellow solid, mp 198–200°C.IR spectrum, ν, cm^{-1}: 3420 (NH), 3062 (Ar=CH), 2985, 2856 (CH), 1592, 1551, 1487 (ArC=C), 1345 (C=N), 1205 (C–S–C). ^{1}H NMR spectrum, δ, ppm: 8.78 t (1H, *J* = 4 Hz, NH), 8.30 d (2H,*J* = 4 Hz, 2H, ArH), 8.03–8.01 d (2H, *J* = 8 Hz, ArH), 7.42–7.29 m (5H, ArH), 4.60 d (2H, *J* = 4 Hz, CH_2).^{13}C NMR spectrum, δ_C, ppm: 170.19 (C^5), 154.19 (C^2), 147.97 (ArC), 138.67 (ArC), 137.11 (ArC), 128.93 (ArC), 128.08 (ArC), 127.79 (ArC), 127.64 (ArC), 124.92 (ArC), 48.64 (CH_2). Found, %: C 57.70; H 3.90; N 18.14; S 10.30. $C_{15}H_{12}O_2N_4S$. Calculated, %: C 57.68; H 3.87; N 17.94; S 10.27. $[M]^+$ 310.

***N*-Benzyl-5-(4-chlorophenyl)-1,3,4-thiadiazole-2-amine (4d).** White solid, mp 188–190°C. IR spectrum, ν, cm^{-1}: 3405 (NH), 3052 (Ar=CH), 2945, 2850 (CH), 1590, 1548 (ArC=C), 1445 (C=N), 1198 (CSC), 826 (CCl). ^{1}H NMR spectrum, δ, ppm:8.21 d (2H, *J* = 8.0 Hz, ArH), 7.61 d (2H, *J* = 8.0 Hz, ArH), 7.45–7.36 m (5H, ArH), 5.21 t (1H, *J* = 3.9 Hz, NH), 4.60 d (2H, *J* = 4.1 Hz, CH_2).^{13}C NMR spectrum, δ_C, ppm: 178.61 (C^5), 168.41 (C^2), 143.13 (ArC), 137.71 (ArC), 135.12 (ArC), 131.85 (ArC), 130.19 (ArC), 129.21 (ArC), 128.61 (ArC), 127.92 (ArC), 127.21 (ArC), 55.61 (CH_2). Found, %: C 60.45; H 4.91; Cl, 11.82; N 14.12; S 10.71. $C_{15}H_{12}ClN_3S$. Calculated, %: C 59.70; H 4.01; Cl, 11.75; N 13.92; S 10.62. $[M]^+$ 300.

***N*-benzyl-5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-amine (4e).** White solid, mp 229–230°C. IR spectrum, ν, cm^{-1}: 3440 (NH), 3052 (Ar=CH), 2976 (CH), 1585, 1541 (ArC=C), 1384 (C=N), 1218 (CSC), 1161 (COC). ^{1}H NMR spectrum, δ, ppm: 8.42 t (1H, *J* = 4 Hz, NH), 7.78–7.75 d.d (2H, *J* = 8 Hz, ArH), 7.45 d (3H, *J* = 8 Hz, ArH), 7.36 d (2H, *J* = 12 Hz, ArH), 6.85–6.83 d.d (2H, *J* = 8.5 Hz, ArH), 4.47 d (2H, *J* = 4 Hz, CH_2), 3.65 s (3H, OCH_3). ^{13}C NMR spectrum, δ_C, ppm: 173.21 (C^5), 163.89 (C^2), 157.12 (ArC), 132.19 (ArC), 130.71 (ArC), 128.10 (ArC), 126.04 (ArC), 115.78 (ArC), 56.46 (OCH_3), 42.13 (CH_2). Found, %: C 64.72; H 5.91; N 14.84; S 10.04. $C_{16}H_{15}N_3OS$. Calculated, %: C 64.62; H 5.08; N 14.13; S 10.78. $[M]^+$ 297.

***N*-benzyl-5-(*p*-tolyl)-1,3,4-thiadiazol-2-amine (4f).** White solid, mp 170–171°C. IR spectrum, ν, cm^{-1}: 3425 (NH), 3056 (Ar=CH), 2984 (CH), 1545, 1491 (ArC=C), 1331 (C=N), 1228 (C–S–C), 1136 (C–O–C). ^{1}H NMR spectrum, δ, ppm: 8.24 d (2H, *J* = 7.5 Hz, ArH), 7.45–7.40 m (3H, ArH), 7.28 d (2H, *J* = 8.2 Hz, ArH), 7.16 d (2H, *J* = 8.2 Hz, ArH), 5.42 t (1H, *J* = 4 Hz, NH), 4.35 d (2H, *J* = 4 Hz, CH_2), 2.95 s (3H, CH_3). ^{13}C NMR spectrum, δ_C, ppm: 176.18 (C^5), 154.19 (C^2), 141.26 (ArC), 134.41 (ArC), 133.15 (ArC), 131.78 (ArC), 130.52 (ArC), 130.03 (ArC), 128.18 (ArC), 125.51 (ArC), 119.84 (ArC), 35.89 (CH_2), 29.40 (CH_3). Found, %: C 68.45; H 5.40; N 14.75; S 11.91. $C_{15}H_{13}N_3S$. Calculated, %: C 68.30; H 5.37; N 14.93; S 11.40. $[M]^+$ 281.

***N*-(4-Methoxybenzyl)-5-phenyl-1,3,4-thiadiazole-2-amine (4g).** White solid, mp 227–229°C. IR spectrum, ν, cm^{-1}: 3449 (NH), 3062 (Ar=CH), 2980, 2842 (CH), 1565, 1512 (ArC=C), 1348 (C=N), 1215 (CSC), 1189 (COC). ^{1}H NMR spectrum, δ, ppm: 8.40 t (1H, *J* = 4 Hz, NH), 7.76–7.74 d.d (2H, *J* = 8 Hz, ArH), 7.47 d (3H, *J* = 8 Hz, ArH), 7.34 d (2H, *J* = 12 Hz, ArH), 6.94–6.91 d.d (2H, *J* = 8.5 Hz, ArH), 4.47 d (2H, *J* = 4 Hz, ArH), 3.74 s (3H, OCH_3). ^{13}C NMR spectrum, δ_C, ppm: 172.45 (C^5), 164.24 (C^2), 158.94 (ArC), 133.78 (ArC), 133.38 (ArC), 129.12 (ArC), 128.79 (ArC), 127.94 (ArC), 114.9 (ArC), 55.8 (OCH_3), 46.8 (CH_2).Found, %: C 64.84; H 5.78; N 14.23; S 10.94. $C_{16}H_{15}N_3OS$. Calculated, %: C 64.62; H 5.08; N 14.13; S 10.78. $[M]^+$ 296.

***N*-[4-(Trifluoromethyl)benzyl]-5-phenyl-1,3,4-thiadiazole-2-amine (4h).** White solid, mp 153–155°C. IR spectrum, ν, cm^{-1}: 3478 (NH), 3067 (Ar=CH), 2982, 2835 (CH), 1570, 1513 (ArC=C), 1345 (C=N), 1189 (CSC), 942 (C–F). ^{1}H NMR spectrum, δ, ppm: 8.57 t (1H, *J* = 4 Hz, NH), 7.75–7.72 m (4H, ArH), 7.52–7.50 d (2H, *J* = 8 Hz, ArH), 7.50–7.41 m (3H, ArH), 4.67 d (2H, *J* = 4 Hz, CH_2). ^{13}C NMR spectrum, δ_C, ppm: 168.68 (C^5), 157.13 (C^2), 144.17 (ArC), 131.28 (ArC), 130.25 (ArC), 129.57 (ArC), 128.56 (ArC), 126.93 (ArC), 128.21 ($2J_{CF}$ = 31.3 Hz), 125.72 ($^3J_{CF}$ = 3.8 Hz), 124.86 ($1J_{CF}$ = 273.7 Hz), 47.92 (CH_2). Found, %: C 57.78; H 3.92; F 17.76; N 12.46; S

9.91. $C_{16}H_{12}F_3N_3S$. Calculated, %: C 57.31; H 3.61; F 17.00; N 12.53; S 9.56. $[M]^+$ 333.

***N*-(4-Fluorophenyl)-5-phenyl-1,3,4-thiadiazole-2-amine (4i).** White solid, mp 247–250°C. IR spectrum, ν, cm^{-1}: 3476 (NH), 3048 (Ar=CH), 1561, 1520 (ArC=C), 1341 (C=N), 1195 (CSC), 938 (C–F). ^1H NMR spectrum, δ, ppm: 9.34 br.s (1H, NH), 7.86–7.84 m (2H, ArH), 7.47–7.25 m (5H, ArH), 7.14–7.09 m (2H, ArH. ^{13}C NMR spectrum, δ_C, ppm: 169.14 (C^5), 162.58 (C^2), 161.25 d ($^1J_{CF}$ = 248.0 Hz), 152.05 (ArC), 131.68 d ($^3J_{CF}$ = 9.4 Hz), 131.34 d ($^4J_{CF}$ = 3.1 Hz), 130.86 (ArC), 129.02 (ArC), 128.85 (ArC), 126.28 (ArC), 116.69 d ($^2J_{CF}$ = 22.6 Hz). Found, %: C 62.19; H 3.87; F 7.67; N 15.78; S 12.12. $C_{14}H_{10}FN_3S$. Calculated, %: C 61.98; H 3.72; F 7.00; N 15.49; S 11.82. $[M]^+$ 269.

***N*-(4-Chlorophenyl)-5-phenyl-1,3,4-thiadiazole-2-amine (4j).** White solid, mp 168–170°C. IR spectrum, ν, cm^{-1}: 3478 (NH), 3038 (Ar=CH), 1546, 1489 (ArC=C), 1410 (C=N), 1174 (CSC), 886 (C–Cl). ^1H NMR spectrum, δ, ppm: 8.11 d (2H, *J* = 7.8 Hz, ArH), 7.61–7.56 m (3H, ArH), 7.45 d (2H, *J* = 8.2 Hz, ArH), 7.39 d (2H, *J* = 8.2 Hz, ArH), 4.60 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 172.16 (C^5), 162.44 (C^2), 142.13 (ArC), 139.18 (ArC), 135.91 (ArC), 133.85 (ArC), 132.11 (ArC), 130.91 (ArC), 130.12 (ArC), 129.92 (ArC), 127.23 (ArC). Found, %: C 58.78; H 3.98; Cl, 12.46; N 14.78; S 11.79. $C_{15}H_{10}ClN_3S$. Calculated, %: C 58.43; H 3.50; Cl, 12.32; N 14.60; S 11.14. $[M]^+$ 270.

***N*-(*p*-Tolyl)-5-phenyl-1,3,4-thiadiazole-2-amine (4k).** White solid, mp 168–170°C. IR spectrum, ν, cm^{-1}: 3412 (NH), 3047 (Ar=CH), 2972, 2845 (CH), 1572, 1483 (ArC=C), 1340 (C=N), 1215 (C–S–C), 1148 (C–O–C). ^1H NMR spectrum, δ, ppm: 8.09 d (2H, *J* = 7.5 Hz, ArH), 7.52–7.45 m (3H, ArH), 7.38 d (2H, *J* = 8.2 Hz, ArH), 7.32 d (2H, *J* = 8.2 Hz, ArH), 4.92 s (1H, NH), 2.97 s (3H, CH_3).^{13}C NMR spectrum, δ_C, ppm: 174.12 (C^5), 155.40 (C^2), 142.15 (ArC), 134.16 (ArC), 133.85 (ArC), 131.12 (ArC), 130.98 (ArC), 130.05 (ArC), 129.91 (ArC), 126.26 (ArC), 120.32 (ArC), 28.49 (CH_3). Found, %: C 68.12; H 5.26; N 15.98; S 12.24. $C_{15}H_{13}N_3S$. Calculated, %: C 67.39; H 4.90; N 15.72; S 11.99. $[M]^+$ 266.

***N*-(4-Nitrophenyl)-5-phenyl-1,3,4-thiadiazole-2-amine (4l).** White solid, mp 189–191°C. IR spectrum, ν, cm^{-1}: 3426 (NH), 3098 (Ar=CH), 1601, 1542 (ArC=C), 1340 (C=N), 1214 (CSC). ^1H NMR spectrum, δ, ppm: 8.19 d (2H, *J* = 7.4 Hz, ArH), 8.11 d (2H, *J* = 8.2 Hz, ArH), 7.58–7.52 m (3H, ArH), 7.12 d (2H, *J* = 8.2 Hz, ArH), 4.52 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 175.23 (C^5), 159.41 (C^2), 141.13 (ArC), 138.24 (ArC), 137.91 (ArC), 134.85 (ArC), 132.16 (ArC), 130.92 (ArC), 130.34 (ArC), 129.22 (ArC), 128.23 (ArC). Found, %: C 56.68; H 3.92; N 18.92, S 10.92. $C_{14}H_{10}N_4O_2S$. Calculated, %: C 56.37; H 3.38; N 18.78, S 10.75. $[M]^+$ 297.

***N*-(4-Methoxyphenyl)-5-phenyl-1,3,4-thiadiazole-2-amine (4m).** White solid, mp 227–229°C. IR spectrum, ν, cm^{-1}: 3423 (NH), 3048 (Ar=CH), 2921 (CH), 1578, 1489 (ArC=C), 1342 (C=N), 1226 (CSC), 1165 (COC). ^1H NMR spectrum, δ, ppm: 8.86 s (1H, NH), 7.83 d.d (2H, *J* = 8 Hz, ArH), 7.47–7.42 m (3H, ArH), 7.25 m (2H, ArH), 6.95 d (2H, *J* = 8.5 Hz, 2H, ArH), 3.83 s (3H, OCH_3).^{13}C NMR spectrum, δ_C, ppm: 169.32 (C^5), 159.94 (C^2), 151.12 (ArC), 130.68 (ArC), 130.14 (ArC), 129.12 (ArC), 128.56 (ArC), 127.60 (ArC), 126.44 (ArC), 114.87 (ArC), 55.78 (OCH_3). Found, %: C 60.67; H 5.02; N 14.92, S 11.92. $C_{15}H_{13}N_3OS$. Calculated, %:C, 63.58; H 4.62; N 14.83,S, 11.32. $[M]^+$ 282.

Biological activity screening. *Cytotoxicity.* The in vitro anti-cancer activity of compounds **4a**–**4m** was tested using the MTT colorimetric assay following the ATCC protocol [23,24]. The cell lines used for in vitro cytotoxicity testing included MDAMB231 derived from human lung carcinoma cells (ATCC No. CCL-185) and HEK293T derived from human embryonic kidney cells (non-cancerous; ATCC No. CRL-1573), purchased from American Type Culture Collection (Manassas, VA, USA). The breast cancer cell line A549 was maintained in DMEM medium supplemented with 10% newborn calf serum (NBCS), 1% nonessential amino acids, 0.2 % sodium bicarbonate, 1% sodium pyruvate, and 1% antibiotic mixture (10 000U of penicillin and 10 mg of streptomycin per mL). MDAMB231 and HEK293T were maintained in RPMI-1640 medium supplemented with 10% NBCS, 100 IU/mL penicillin, 100 mg/mL streptomycin, and 2 mM glutamine. Cell lines were maintained at 37°C in a humidified 5% CO_2 incubator (Thermo Scientific). Cell lines were processed by initial trypsinization to detach the adhered cells followed by centrifugation to obtain a cell pellet. In the fresh medium the pellet of a cell count using haemocytometer and take in 96-well plate added to 100 μL of medum with range from 5.000–6.000 per well. The plate was incubated overnight in CO_2 incubator for cells to adhere and regain their shape. After 24 h, cells were treated with

the test compounds (25 μM in the medium) and incubated for 48 h to assay the effect of the test compounds on different cell lines. Zero hour reading was taken with untreated cells and also control with 1% DMSO to subtract further from the 48 h reading. After 48 h incubation, cells were treated by the MTT reagent [4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] dissolved in PBS (5 mg/mL) and incubated for 3–4 h at 37 ˚C. The formazan crystals thus formed were dissolved in 100 μL of DMSO, and viability was measured at 540nm on a Spectra Max multimode reader.

Antitubercular activity. The antitubercular activity of the synthesized compounds was tested using the *M. smegmatis* MC-155strain obtained from the Tuberculosis Research Center, Indian Council of Medical Research, Chennai, India. The assay was performed in a 96-well microtiter format plate. Initially, 10 mM stock concentrations of the test compounds were diluted with DMSO to achieve 1.5 mM (2%) working concentrations and 30 μM final concentrations. The agar dilution assay was performed using Middlebrook 7H9 broth supplemented with 0.1% Tween 80 and 0.5% glycerol [25–27]. At the time of inoculation, 10% albumin dextrose and saline (ADS) was added. When the optical density of the inoculum reached ~0.4, the inoculum was diluted 1 : 1000. A 2μL aliquot of a 1.5 mM concentration was added in triplicate to each well, after which 98 μL of inoculum (1 : 1000) was added, and the cultures were incubated for 30–32 h in a shaker incubator at 37°C and 200 rpm. After the incubation period, absorbance of the inoculum in each well was observed at 600nm in a Multimode Reader, and assays were usually completed in 5–8 days. The percentage inhibition was measured as GI (growth inhibition) of test sample/GI of control) ×100%. The minimum inhibitory concentration of a molecule, that caused a reduction of daily GIrate smaller than that observed with a 1 : 1000 dilute control culture on the next day, reached a GI of at least 30. Isoniazid was used as a positive control of inhibition of *M. smegmatis*.

In silico ADME prediction. Computational studies of the synthesized compounds **4a**–**4m** were performed to predict their ADME properties. We calculated molecular volume (M_v), molecular weight (M_w), logarithm of partition coefficient (milog *P*), number of hydrogen-bond donors (n_{OHNH}), number of hydrogen-bond acceptors (n_{ON}), topological polar surface area (TPSA), number of rotatable bonds (N_{rotb}), and Lipinski's rule of five using the Molinspiration online property calculation toolkit [28]. The percentage absorption was calculated by the equation [20]:

$$ABS, \% = 109–0.345 \cdot PSA.$$

CONCLUSIONS

In summary, 2,5-substituted-1,3,4-thiadiazoles were designed and synthesized. The antitubercular and cytotoxic activities of the synthesized 1,3,4-thiadiazole derivatives were evaluated against *M. smegmatis* and breast cancer cell lines and one normal cell line. The SAR analysis showed that a smaller aromatic ring and electron-withdrawing groups favor both activities, and this paves the way for further design and chemical modification of thiadiazoles. The molecular and pharmacokinetic properties, and drug likeness model scores were calculated by Molinspiration and MolSoft. In terms of the Lipinski's rule of five, compounds **4b** and **4j** displayed the highest drug likeness (–0.21, –0.27) as potential orally bioavailable antitubercular and anticancer agents.

ACKNOWLEDGMENTS

We are grateful to our research supervisor Dr. Anjali Jha and Dr. Lav Kumar for providing the required facilities and motivation for completion of the research work. We also extend our gratitude toward Department of Chemistry, GIS, GITAM University.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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