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Synthesis and anticancer evaluation of novel 2-cyclopropylimidazo[2,1-*b*][1,3,4]-thiadiazole derivatives

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ABSTRACT

A series of 2,5,6-trisubstituted imidazo[2,1-*b*][1,3,4]-thiadiazole derivatives **4**(**a**–**k**) have been prepared by reaction of 2-amino-5-cyclopropyl-1,3,4-thiadiazole and an appropriate phenacyl bromide. Further 5-bromo **5**(**a**–**k**) and 5-thiocyanato **6**(**a**–**k**) derivatives were synthesized in order to study the effect of these substituents on antitumor activity. Structures of these compounds were established by IR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopy. Seven compounds were granted NSC code at National Cancer Institute (NCI), USA for anticancer activity at a single high dose (10^{-5} M) in full NCI 60 cell panel. Among the compounds tested, 5-bromo-6-(4-chlorophenyl)-2-cyclopropylimidazo[2,1-*b*][1,3,4]thiadiazole **5b** (**NSC D-96022/1**) was found to be the most active candidate of the series at five dose level screening with degree of selectivity toward Leukemic cancer cell line.

1. Introduction

Cancer is a class of diseases in which cell, or a group of cells display uncontrolled growth, invasion, and sometimes metastasis. It affects people at all ages with the risk of most types increasing with age. It caused about 13% (7.6 million) of all human deaths in 2007. Levamisole (**I**) an anthelmintic agent was found to be an immuno-stimulant by Rebnoux in 1972. It appears to be most effective in patients with small tumor burdens and it acts by stimulating the responsiveness of lymphocytes to tumor antigen [1]. An early report on 2-amino-1,3,4-thiadiazole derivatives deals with the activity of these compounds against several transplanted animal tumors is available [2]. Gadad et al., in 1999 reported the antitumor effects of imidazo[2,1-*b*][1,3,4]-thiadiazoles [3]. Nalan et al., in 2003 have reported some hydrazone derivatives of 2,6-dimethylimidazo[2,1-*b*][1,3,4]-thiadiazole-5-carbohydrazide as anticancer agents against ovarian cancer cell line OVCAR [4]. Andrew et al., in 2000 have studied on some imidazo[2,1-*b*]-thiazole guanyl hydrazones which were active against various cancer cell lines [5]. Ibrahim in 2009 prepared 4-(3-substituted(1,2,4)triazolo(3,4-*b*) [1,3,4]thiadiazole-6-yl) aniline derivatives as a novel class of potential antitumor agents [6]. Consequently, a large number of imidazo thiadiazole derivatives have been reported to possess diverse pharmacological properties such as antitubercular [7], antibacterial [8], antifungal [9], anticonvulsant, analgesic [10], antisecretory [11], anti-inflammatory [12], cardiotonic [13], diuretic [14] and herbicidal [15] activities. In addition, the imidazo[2,1-*b*] thiazole derivatives of the Levamisole have been reported as potential antitumor agents (**II**) [16]. Later antitumor activity of 5-formyl-6-aryl imidazo [21-*b*][1,3,4]thiadiazole sulfonamides (**III**) were also reported [3].

In view of the above facts and in continuation of our search for novel anticancer agents [17–25] in the present study a new series of 2-cyclopropylimidazo[2,1-*b*][1,3,4]thiadiazoles and their 5-bromo and 5-cyanato derivatives have been synthesized and screened in vitro at NCI (National Cancer Institute))-USA (Fig. 1).

2. Chemistry

The synthetic route of the compound **5**(**a**–**k**) and **6**(**a**–**k**) is outlined in Scheme 1. 2-Amino-5-cyclopropyl-1,3,4-thiadiazole **3** was prepared by refluxing cyclopropane carbonyl chloride **1** and

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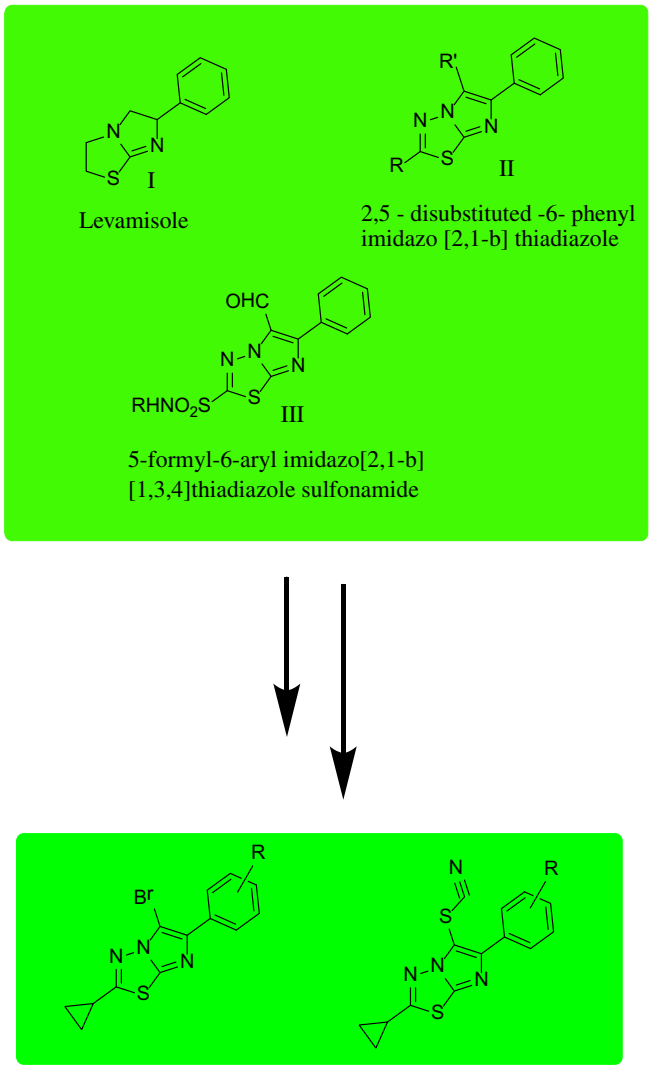
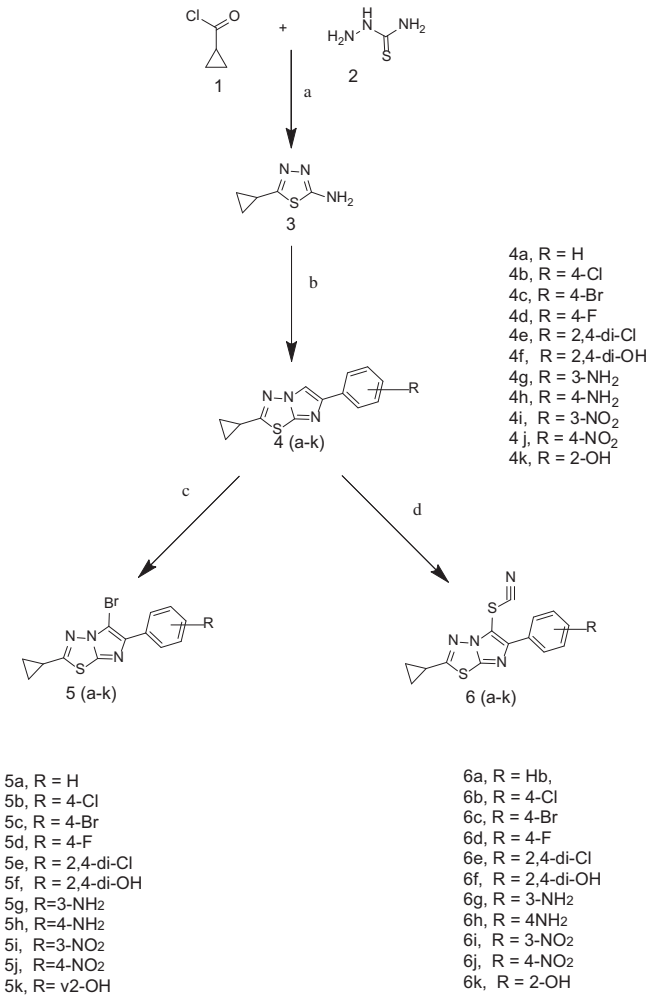


Fig. 1. Reported and proposed antitumor imidazo[2,1-*b*][1,3,4]thiadiazole derivatives.



Scheme 1. Reagent and conditions: a) $POCl_3$, reflux b) substituted phenacyl bromide, alcohol, reflux c) Br_2, GAA d) KSCN, Br_2, GAA.

thiosemicarbazide **2** in $POCl_3$. The 2-cyclopropyl-6-substituted phenylimidazo[2,1-*b*][1,3,4]thiadiazole derivatives **4**(**a**–**k**) reported in Scheme 1 were prepared by reaction of 2-amino-5-cyclopropyl-1,3,4-thiadiazole **3** with the appropriate phenacyl bromide, and neutralization with cold aqueous sodium carbonate gave the free base in 40–60% yield. It is well established that this reaction proceeds via the intermediate iminothiadiazole [8], which undergoes dehydrocyclization to form the desired fused heterocycle under reflux temperature spontaneously. The electronic and steric factors at 5th position of 2-amino-5-substituted-1,3,4-thiadiazole are crucial in determining the course of its reaction with substituted α-haloaryl ketones. The strongly electronegative group impart less nucleophilic character to nitrogen at 4th position of the 1,3,4-thiadiazole. The various phenacyl bromides were prepared by bromination of the corresponding ketones in glacial acetic acid. The substituted imidazo[2,1-*b*][1,3,4] thiadiazole derivatives **4**(**a**–**k**) thus obtained were subjected to electrophilic substitution reaction at the 5 position with bromine in the presence of sodium acetate in acetic acid to obtain the 5-bromo derivatives **5**(**a**–**k**) in good yield. Introduction of thiocyanate functional group at the 5 position was carried out by reaction between imidazo[2,1-*b*][1,3,4]-thiadiazoles **4**(**a**–**k**) and potassium thiocyanate in glacial acetic acid by drop wise addition of bromine in glacial acetic acid to get **6**(**a**–**k**) in good yield.

The formation of 2-aminothiadiazole **3** by the reaction between cyclopropane carbonyl chloride and thiosemicarbazide was confirmed by IR spectra, which showed the presence of amino ($-NH_2$) band ~3200 and the absence of carbonyl stretching of carboxylic acid ~1700–1600. Structures of imidazo thiadiazole derivatives **4**(**a**–**k**) were established by the absence of ($-NH_2$) band ~3200 in IR spectra and appearance of imidazole proton (H-5) around δ 8 ppm in the ^{1}H NMR spectra. The formation of title compound **5**(**a**–**k**) and **6**(**a**–**k**) was confirmed by the absence of signal for imidazole proton (H-5) in ^{1}H NMR spectra and presence of bromine (Br) band around 600 cm^{-1} for **5**(**a**–**k**) and SCN band around 2100 cm^{-1} for **6**(**a**–**k**). The mass spectra of these compounds further confirmed the assigned structure.

3. Pharmacology

3.1. In vitro cancer screen at NCI-USA

The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10^{-5} M. The output from the single dose screen is reported as a mean graph and is available for analysis by the COMPARE program. Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five concentration levels. The human tumor cell lines of the cancer screening panel are grown

in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 μL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line are fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 μl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 μl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed *in situ* by the gentle addition of 50 μl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μl) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma (tris(hydroxymethyl)aminomethane) base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

$[(Ti - Tz)/(C - Tz)] \times 100$ for concentrations for which $Ti \geq Tz$

$[(Ti - Tz)/Tz] \times 100$ for concentrations for which $Ti < Tz$.

Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50% (GI_{50}) is calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from $Ti = Tz$. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(Ti-Tz)/Tz] \times 100 = -50$. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested [26–28].

3.2. Pharmacological (in vitro anticancer activity)

The tumor growth inhibition properties of the selected seven compounds **5b**–**d**, **5f**, **6b**, **6f** and **6h** with the NCI codes **NSC D-96022/1**, **NSC D-96019/1**, **D-96021/1**, **D-96155/1**, **D-96023/1**, **D-96154/1** and **D-96024/1** respectively among the synthesized compounds **5**(**a**–**k**) and **6**(**a**–**k**) were screened on human tumor cell lines at 10^{-5} M at the NCI, NIH, Bethesda, Maryland, USA, under the drug discovery program of the NCI. Among the compounds tested, compound **5b** (**NSC D-96022/1**) was further screened for 5-log dose molar range as it has shown prominent cell growth inhibition at 10^{-5} M concentration against variety of cell lines.

3.2.1. Primary single high dose (10^{-5} M) full NCI 60 cell panel in vitro assay

All the selected compounds submitted to National Cancer Institute (NCI) for in vitro anticancer assay were evaluated for their anticancer activity. Primary in vitro one dose anticancer assay was performed in full NCI 60 cell panel representing leukemia, melanoma and cancers of lung, colon brain breast, ovary, kidney and prostate in accordance with the protocol of the NCI, USA. The compounds were added at a single concentration (10^{-5} M) and the culture was incubated for 48 h. End point determinations were made with a protein binding dye, Sulforhodamine B. Results for each compound were reported as a mean graph of the percent growth of the treated cells when compared to the untreated control cells. There after obtaining the results for one dose assay, analysis of historical Development Therapeutics Programme (DTP) was performed and compound **5b** (**NSC D-96022/1**) which satisfied pre-determined threshold inhibition criteria was selected for NCI full panel 5 dose assay.

3.2.2. In vitro 5 dose full NCI 60 cell panel assay and discussion

All the cell lines (about 60), representing nine tumor subpanels, were incubated at five different concentrations (0.01, 0.1, 1, 10 & 100 μM). The outcomes were used to create log concentration vs % growth inhibition curves and three response parameters (GI50, TGI and LC50) were calculated for each cell line. The GI50 value (growth inhibitory activity) corresponds to the concentration of the compound causing 50% decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compound resulting in total growth inhibition and LC_{50} value (cytotoxic activity) is the concentration of the compound causing net 50% loss of initial cells at the end of the incubation period of 48 h. Compound under investigation **5b** (**NSC D-96022/1**) exhibited significant anticancer activity against most of the tested cell lines representing nine different subpanels with GI_{50} values between "1.79–43.4 μM". With regard to the sensitivity against some individual cell lines (Table 1) the compound showed high activity against Leukemia K-562, Colon Cancer HCT-15, Melanoma SK-MEL and Prostate Cancer PC-3 with GI_{50} 1.79, 2.02, 2.17, and 2.22 μM respectively. On the other hand compound showed least activity against Non-small cell lung cancer: HOP-62 and NCI-H322M; CNS Cancer: SF-268 and SNB-19; Melanoma: SK-MEL-28; Ovarian Cancer: IGROV 1, OVCAR-5 and SK-OV-3; Renal Cancer: 786, A-498, SN-12C and UO-31; Prostate Cancer: DU-145. Obtained data revealed an obvious sensitivity profile toward Leukemic subpanel (GI_{50} value ranging from 1.79 to 5.66 μM), least for K-562 and maximum for CCRF-CEM cell line. The criterion for selectivity of a compound depends upon the ratio obtained by dividing the full panel MID (the average sensitivity of all cell lines toward the test agent) by their individual subpanel MID (the average sensitivity of all cell lines of a particular subpanel toward the test agent). Ratios between 3 and 6 refer to moderate selectivity; ratios greater than 6 indicate high selectivity toward the corresponding cell line, while compounds not meeting either of these criteria rated non-selective [28]. As per this criterion, compound in the study was found to be moderate selective toward Leukemic cancer subpanel only with selectivity ratio of 3.36, whereas it was found to be non-selective against remaining cell panel (Table 1).

Table 1
NCI in vitro testing result of compound **5b** (**NSC D-96022/1**) at five dose level in μM.

Panel	Cell Line	GI_{50}			TGI	LC_{50}
		Concentration per cell line	Subpanel MID[b]	Selectivity ratio (MID[a]:MID[b])		
Leukemia			2.98	3.36		
	CCRF-CEM	3.66			>100	>100
	HL-60(TB)	3.34			>100	>100
	K-562	1.79			>100	>100
	MOLT-4	2.97			>100	>100
	RPMI-8226	3.22			>100	>100
	SR	2.90			>100	>100
Non-Small Cell Lung Cancer			13.37	0.75		
	A549/ATCC	7.42			>100	>100
	EKVX	5.22			>100	>100
	HOP-62	38.1			>100	>100
	HOP- 92	5.59			37.9	>100
	NCI-H226	4.99			>100	>100
	NCI-H23	5.10			>100	>100
	NCI-H322M	43.4			>100	>100
	NCI-H460	5.25			>100	>100
	NCI-H522	5.31			55.4	>100
Colon Cancer			5.31	1.888		
	COLO 205	7.23			>100	>100
	HCC-2998	8.19			>100	>100
	HCT-116	2.96			>100	>100
	HCT-15	2.02			>100	>100
	HT 29	6.12			>100	>100
	KM 12	3.77			>100	>100
	SW-620	6.88			>100	>100
CNS Cancer			10.535	0.9759		
	SF-268	19.3			>100	>100
	SF-295	5.04			>100	>100
	SF-539	6.78			>100	>100
	SNB-19	15.6			>100	>100
	SNB-75	12.2			51.9	>100
	U251	4.29			>100	>100
Melanoma			9.89	1.0139		
	LOX IMVI	9.20			>100	>100
	MALME-3M	8.79			>100	>100
	M14	7.70			>100	>100
	MDA-MB-435	6.08			>100	>100
	SK-MEL-2	11.0			59.3	>100
	SK-MEL-28	35.6			>100	>100
	SK-MEL-5	2.17			>100	>100
	UACC-257	4.14			>100	>100
	UACC-62	4.36			>100	>100
Ovarian Cancer			15.92	0.6458		
	IGROV1	17.3			>100	>100
	OVCAR-3	4.63			>100	>100
	OVCAR-4	12.7			82.1	>100
	OVCAR-5	31.9			>100	>100
	OVCAR-8	5.76			>100	>100
	NCI/ADR-RES	4.18			>100	>100
	SK-OV-3	35.0			>100	>100
Renal Cancer			13.54	0.7406		
	786–0	13.6			>100	>100
	A-498	30.8			>100	>100
	ACHN	7.07			>100	>100
	CAKI-1	9.69			>100	>100
	RXF-393	2.93			14.8	>100
	SN-12C	14.3			>100	>100
	UO-31	16.4			>100	>100
Prostate Cancer			10.71	0.9363		
	PC-3	2.22			>100	>100
	DU-145	19.2			>100	>100
Breast Cancer			5.256	1.90		
	MCF7	8.18			>100	>100
	MDA-MB-231/ATCC	6.59			>100	>100
	BT -549	4.07			>100	>100
	T-47D	4.49			73.5	>100
	MDA-MB-468	2.95			>100	>100
MID[a]		10.0282			69.5	>100

[a] MID = Average sensitivity of all cell line in μM.
[b] MID = Average sensitivity of all cell line of a particular subpanel in μM.

4. Experimental

All chemicals and solvents were supplied by Merck, S.D. Fine Chemical Limited, Mumbai. All the solvents were distilled and dried before use. The reactions were monitored with the help of thin-layer chromatography using pre-coated aluminum sheets with GF254 silica gel, 0.2 mm layer thickness (E. Merck). Melting points of the synthesized compounds were recorded on the Veego (VMP-MP) melting point apparatus. IR spectrum was acquired on a Shimadzu Infra Red Spectrometer, (model FTIR-8400S). Both ^{1}H NMR (DMSO) and ^{13}C NMR (DMSO) spectra of the synthesized compounds were performed with Bruker Avance-II 400 NMR Spectrometer operating at 400 MHz in SAIF, Punjab University (Chandigarh). Chemical shifts were measured relative to internal standard TMS (δ: 0). Chemical shifts are reported in δ scale (ppm). Mass spectra of the synthesized compounds were recorded at MAT 120 in SAIF, Punjab University.

4.1. Synthesis of 5-cyclopropyl-1,3,4-thiadiazol-2-amine (**3**)

An equimolar mixture of cyclopropane carbonyl chloride (0.01 M) **1** and thiosemicarbazide (0.1 M) **2** was refluxed in the presence of phosphorous oxychloride (5 mL) for 4 h. After 4 h the mixture was cooled and diluted with water (10 mL). Then the mixture was filtered and filtrate was neutralized with potassium hydroxide solution. The precipitate was filtered off and recrystallized from ethanol.

Yield 63%; mp 214–216 °C; IR (KBr) ν_{max} 3277.4, 3113.5, 2832.8, 1631.2, 1545 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 0.93–2.17 (m, 5H, cyclopropyl), 6.32 (s, 2H, NH_2); ^{13}C NMR (DMSO-d_6) δ ppm: 166.6, 158.2, 13.1, 10.4.

4.2. Synthesis of 2-cyclopropyl-6-substituted phenylimidazo[2,1-b][1,3,4]thiadiazole **4**(**a**–**k**)

A mixture of equimolar quantities of 5-cyclopropyl-1,3,4-thiadiazol-2-amine (0.01 mol) **3** and substituted phenacyl bromides (0.01 mol) was refluxed in dry ethanol for 16 h. The excess of solvent was distilled off and the solid hydrobromide that separated was collected by filtration, suspended in water and neutralized by aqueous sodium carbonate solution to get free base **4**(**a**–**k**). It was filtered, washed with water, dried and recrystallized from ethanol.

4.2.1. 2-Cyclopropyl-6-phenylimidazo[2,1-b][1,3,4]thiadiazole (**4a**)

Yield 68%; mp 220–222 °C; IR (KBr) ν_{max} 3137.1, 3067.7, 1687.2, 1439.5 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.19–2.31 (m, 5H, cyclopropyl), 7.26–7.28 (m, 5H, Ar–H), 7.92 (s, 1H, C-5-H, imidazole); ^{13}C NMR (DMSO-d_6) δ ppm: 166.2, 145.8, 137.8, 134.8, 30.6, 128.6, 122.5, 13.1, 10.4; Mass (EI) m/z, 242.4557 (M + 1).

4.2.2. 6-(4-Chlorophenyl)-2-cyclopropylimidazo[2,1-b][1,3,4]thiadiazole (**4b**)

Yield 62%; mp 230–234 °C; IR (KBr) ν_{max} 3131.2, 3056.7, 1666.4, 1456.8, 648.4 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.25–2.41 (m, 5H, cyclopropyl), 7.18–7.80 (m, 4H, Ar–H), 7.96 (s, 1H, C-5-H, imidazole); ^{13}C NMR (DMSO-d_6) δ ppm: 166.7, 145.1, 137.1, 135.2, 132.3, 129.5, 128.4, 122.8, 13.2, 10.5; HRMS (EI) m/z calcd for $C_{13}H_{10}ClN_3S$: 275.0284; found: 275.0289.

4.2.3. 6-(4-Bromophenyl)-2-cyclopropylimidazo[2,1-b][1,3,4]thiadiazole (**4c**)

Yield 66%; mp 260–264 °C; IR (KBr) ν_{max} 3129.8, 3042.6, 1685.4, 1461.8, 562.4 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.13–2.26 (m, 5H, cyclopropyl), 7.25–7.68 (m, 4H, Ar–H), 7.90 (s, 1H, C-5-H, imidazole); ^{13}C NMR (DMSO-d_6) δ ppm: 166.8, 145.4, 137.2, 133.2, 132.1, 129.3, 124.7, 122.7, 13.8, 10.2; HRMS (EI) m/z calcd for $C_{13}H_{10}BrN_3S$: 318.9779; found: 318.9784.

4.2.4. 2-Cyclopropyl-6-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**4d**)

Yield 63%; mp 242–246 °C; IR (KBr) ν_{max} 3136.2, 3032.6, 1681.4, 1462.8, 1212.4 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.35–2.31 (m, 5H, cyclopropyl), 7.20–7.90 (m, 4H, Ar–H), 7.94 (s, 1H, C-5-H, imidazole); ^{13}C NMR (DMSO-d_6) δ ppm: 166.6, 160.2, 145.2, 137.3, 130.2, 129.2, 124.3, 116.4, 13.7, 10.3; HRMS (EI) m/z calcd for $C_{13}H_{10}FN_3S$: 259.0579; found: 259.0583.

4.2.5. 2-Cyclopropyl-6-(2,4-dichlorophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**4e**)

Yield 57%; mp 238–242 °C; IR (KBr) ν_{max} 3131.5, 3014.8, 1685.8, 1458.6, 641.6 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.15–2.45 (m, 5H, cyclopropyl), 7.31–7.85 (m, 3H, Ar–H), 7.96 (s, 1H, C-5-H, imidazole); ^{13}C NMR (DMSO-d_6) δ ppm: 166.6, 145.4, 137.2, 136.2, 134.2, 132.1, 130.2, 129.3, 128.4, 122.6, 13.2, 10.7; HRMS (EI) m/z calcd for $C_{13}H_9Cl_2N_3S$: 308.9894; found: 308.9898.

4.2.6. 4-(2-Cyclopropyl imidazo[2,1-b][1,3,4]thiadiazol-6-yl) benzene-1,3-diol (**4f**)

Yield 72%; mp 264–266 °C; IR (KBr) ν_{max} 3410.4, 3139.8, 3018.4, 1671.6, 1462.6 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.21–2.21 (m, 5H, cyclopropyl), 6.12 (s, 2H, OH), 7.14–7.81 (m, 3H, Ar–H), 7.91 (s, 1H, C-5-H, imidazole); ^{13}C NMR (DMSO-d_6) δ ppm: 166.2, 162.3, 158.2, 145.8, 137.8, 135.2, 123.2, 113.2, 112.8, 111.2, 13.8, 10.4; HRMS (EI) m/z calcd for $C_{13}H_{11}N_3O_2S$: 273.0572; found: 273.0576.

4.2.7. 3-(2-Cyclopropylimidazo[2,1-b][1,3,4]thiadiazol-6-yl)aniline (**4g**)

Yield 81%; mp 221–224 °C; IR (KBr) ν_{max} 3241.6, 3121.8, 3061.3, 1671.4, 1461.5 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.21–2.01 (m, 5H, cyclopropyl), 6.42 (s, 2H, NH_2), 7.13–7.76 (m, 4H, Ar–H), 7.89 (s, 1H, C-5-H, imidazole); ^{13}C NMR (DMSO-d_6) δ ppm: 166.8, 151.2, 145.4, 137.3, 135.3, 132.7, 123.4, 120.4, 118.2, 116.2, 13.8, 10.1; HRMS (EI) m/z calcd for $C_{13}H_{12}N_4S$: 256.0783; found: 256.0787.

4.2.8. 4-(2-Cyclopropylimidazo[2,1-b][1,3,4]thiadiazol-6-yl)aniline (**4h**)

Yield 77%; mp 228–232 °C; IR (KBr) ν_{max} 3256.6, 3119.6, 3058.6, 1678.2, 1459.2 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.31–2.45 (m, 5H, cyclopropyl), 6.39 (s, 2H, NH_2), 7.17–7.86 (m, 4H, Ar–H), 7.96 (s, 1H, C-5-H, imidazole); ^{13}C NMR (DMSO-d_6) δ ppm: 166.6, 148.8, 145.2, 137.1, 130.2, 125.3, 124.2, 116.4, 13.2, 10.6; HRMS (EI) m/z calcd for $C_{13}H_{12}N_4S$: 256.0783; found: 256.0786.

4.2.9. 2-Cyclopropyl-6-(3-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**4i**)

Yield 52%; mp 251–253 °C; IR (KBr) ν_{max} 3121.6, 3046.8, 1664.8, 1546.8, 1451.8, 1356.8 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.21–2.35 (m, 5H, cyclopropyl), 7.11–7.81 (m, 4H, Ar–H), 7.90 (s, 1H, C-5-H, imidazole); ^{13}C NMR (DMSO-d_6) δ ppm: 166.2, 149.2, 145.4, 137.1, 135.6, 134.2, 132.4, 125.6, 123.6, 122.8, 13.7, 10.2; HRMS (EI) m/z calcd for $C_{13}H_{10}N_4O_2S$: 286.0524; found: 286.0528.

4.2.10. 2-Cyclopropyl-6-(4-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**4j**)

Yield 78%; mp 268–272 °C; IR (KBr) ν_{max} 3146.6, 3061.2, 1681.2, 1561.8, 1461.9, 1335.2 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.14–2.38

(m, 5H, cyclopropyl), 7.01–7.61 (m, 4H, Ar–H), 7.98 (s, 1H, C-5-H, imidazole); ^{13}C NMR (DMSO-d_6) δ ppm: 166.6, 151.2, 145.3, 137.4, 126.4, 125.8, 124.2, 13.2, 10.4; HRMS (EI) *m/z* calcd for $C_{13}H_{10}N_4O_2S$: 286.0524; found: 286.0529.

4.2.11. 2-(2-Cyclopropyl imidazo[2,1-b][1,3,4]thiadiazol-6-yl) phenol (**4k**)

Yield 73%; mp 212–216 °C; IR (KBr) ν_{max} 3301.2, 3149.6, 3063.4, 1683.2, 1449.6 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.10–2.61 (m, 5H, cyclopropyl), 5.61 (s, 1H, OH), 7.09–7.82 (m, 4H, Ar–H), 7.99 (s, 1H, C-5-H, imidazole); ^{13}C NMR (DMSO-d_6) δ ppm: 166.2, 154.2, 145.4, 137.8, 132.6, 131.3, 124.6, 121.6, 120.3, 118.5, 13.5, 10.6; HRMS (EI) *m/z* calcd for $C_{13}H_{11}N_3OS$: 257.0623; found: 257.0627.

4.3. Synthesis of 5-bromo-2-cyclopropyl-6-substituted phenylimidazo[2,1-b][1,3,4]thiadiazole **5**(**a**–**k**)

To a well stirred solution of **4**(**a**–**k**) (0.01 mol) in glacial acetic acid (5 ml) and anhydrous sodium acetate (0.02 mol) was added bromine (0.01 mol) drop wise with stirring at room temperature. After the addition, stirring was continued for 2 h. The reaction mixture was poured on ice cold water and basified with ammonia solution. The separated solid was collected, washed with water, dried and purified by column chromatography.

4.3.1. 5-Bromo-2-cyclopropyl-6-phenylimidazo[2,1-b][1,3,4] thiadiazole (**5a**)

Yield 52%; mp 234–236 °C; IR (KBr) ν_{max} 3136.4, 3002.3, 1684.4, 1476.2, 582.6 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.19–2.31 (m, 5H, cyclopropyl), 7.26–7.82 (m, 5H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.9, 145.9, 137.4.,134.0, 128.7, 127.4, 125.0, 99.9, 13.1, 10.4; Mass (EI) *m/z*, 322.3154 (M + 2).

4.3.2. 5-Bromo-6-(4-chlorophenyl)-2-cyclopropylimidazo[2,1-b] [1,3,4]thiadiazole (**5b**)

Yield 56%; mp 250–252 °C; IR (KBr) ν_{max} 3141.3, 3004.8, 1681.8, 1431.8, 602.5 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.20–2.23 (m, 5H, cyclopropyl), 7.26–7.96 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.2, 149.7, 138.3, 134.5, 133.2, 131.2, 130.2, 96.2, 13.2, 10.4; HRMS (EI) *m/z* calcd for $C_{13}H_9BrClN_3S$: 352.9389; found: 352.9392.

4.3.3. 5-Bromo-6-(4-bromophenyl)-2-cyclopropylimidazo[2,1-b] [1,3,4]thiadiazole (**5c**)

Yield 46%; mp 278–281 °C; IR (KBr) ν_{max} 3139.3, 3010.8, 1669.8, 1429.6, 613.6 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.17–2.21 (m, 5H, cyclopropyl), 7.28–7.98 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.3, 150.2, 139.2, 138.6, 134.8, 130.2, 123.4, 96.5, 13.7, 10.4; HRMS (EI) *m/z* calcd for $C_{13}H_9Br_2N_3S$: 396.8884; found: 396.8888.

4.3.4. 5-Bromo-2-cyclopropyl-6-(4-fluorophenyl)imidazo[2,1-b] [1,3,4]thiadiazole (**5d**)

Yield 48%; mp 254–256 °C; IR (KBr) ν_{max} 3141.6, 3012.6, 1671.6, 1431.8, 1021.8, 615.4 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.19–2.39 (m, 5H, cyclopropyl), 7.31–8.01 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.3, 164.2, 147.2, 139.2, 138.6, 132.8, 116.3, 96.8, 13.2, 10.4; HRMS (EI) *m/z* calcd for $C_{13}H_9BrFN_3S$: 336.9685; found: 336.9689.

4.3.5. 5-Bromo-2-cyclopropyl-6-(2,4-dichlorophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**5e**)

Yield 56%; mp 276–278 °C; IR (KBr) ν_{max} 3129.6, 3018.2, 1671.8, 1434.8, 685.8, 503.6 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.21–2.29 (m, 5H, cyclopropyl), 7.31–7.92 (m, 3H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.2, 139.2, 138.2, 136.5, 134.8, 132.8, 130.2, 128.7, 126.3, 96.2, 13.2, 10.3; HRMS (EI) *m/z* calcd for $C_{13}H_8BrCl_2N_3S$: 386.8999; found: 386.8996.

4.3.6. 4-(5-Bromo-2-cyclopropylimidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzene-1,3-diol (**5f**)

Yield 45%; mp 272–274 °C; IR (KBr) ν_{max} 3301.3, 3131.6, 3001.7, 1659.8, 1462.8, 512.8 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.18–2.24 (m, 5H, cyclopropyl), 6.01 (s, 2H, OH), 7.32–7.96 (m, 3H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.2, 162.3, 158.2, 139.8, 138.3, 135.2, 115.2, 112.2, 111.2, 96.1, 13.8, 10.6; HRMS (EI) *m/z* calcd for $C_{13}H_{10}BrN_3O_2S$: 350.9677; found: 350.9681.

4.3.7. 3-(5-Bromo-2-cyclopropyl imidazo[2,1-b][1,3,4]thiadiazol-6-yl)aniline (**5g**)

Yield 55%; mp 232–236 °C; IR (KBr) ν_{max} 3241.8, 3119.7, 3004.8, 1661.2, 1465.8, 561.2 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.16–2.21 (m, 5H, cyclopropyl), 6.91 (s, 2H, NH_2), 7.32–7.91 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.2, 150.2, 139.2, 138.2, 135.4, 132.8, 120.2, 119.2, 116.4, 96.4, 13.2, 10.1; HRMS (EI) *m/z* calcd for $C_{13}H_{11}BrN_4S$: 333.9888; found: 333.9892.

4.3.8. 4-(5-Bromo-2-cyclopropyl imidazo[2,1-b][1,3,4]thiadiazol-6-yl)aniline (**5h**)

Yield 52%; mp 214–216 °C; IR (KBr) ν_{max} 3256.2, 3141.2, 3012.8, 1646.8, 1456.2, 591.8 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.15–2.18 (m, 5H, cyclopropyl), 6.85 (s, 2H, NH_2), 7.35–7.98 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.4, 148.2, 141.2, 138.2, 136.2, 130.2, 115.4, 13.4, 10.2; HRMS (EI) *m/z* calcd for $C_{13}H_{11}BrN_4S$: 333.9888; found: 333.9891.

4.3.9. 5-Bromo-2-cyclopropyl-6-(3-nitrophenyl)imidazo[2,1-b] [1,3,4]thiadiazole (**5i**)

Yield 49%; mp 260–264 °C; IR (KBr) ν_{max} 3139.6, 3014.6, 1651.4, 1539.8, 1461.8, 1338.2, 588.2 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.17–2.23 (m, 5H, cyclopropyl),7.74–8.36 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.8, 150.2, 141.2, 138.2, 135.8, 132.8, 125.2, 124.8, 96.8, 13.2, 10.1; HRMS (EI) *m/z* calcd for $C_{13}H_9BrN_4O_2S$: 363.9630; found: 363.9634.

4.3.10. 5-Bromo-2-cyclopropyl-6-(4-nitrophenyl)imidazo[2,1-b] [1,3,4]thiadiazole (**5j**)

Yield 41%; mp 254–256 °C; IR (KBr) ν_{max} 3135.7, 3016.7, 1656.2, 1534.8, 1406.1, 1341.6, 601.4 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.11–2.10 (m, 5H, cyclopropyl), 7.64–8.16 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.2, 158.2, 149.2, 138.2, 136.2, 128.2, 126.2, 96.8, 13.2, 10.6; HRMS (EI) *m/z* calcd for $C_{13}H_9BrN_4O_2S$: 363.9630; found: 363.9635.

4.3.11. 2-(5-Bromo-2-cyclopropylimidazo[2,1-b][1,3,4]thiadiazol-6-yl)phenol (**5k**)

Yield 56%; mp 236–238 °C; IR (KBr) ν_{max} 3301.2, 3131.2, 3010.2, 1641.6, 1441.6, 610.2 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 1.12–2.12 (m, 5H, cyclopropyl),6.12 (s, 1H, OH), 7.38–8.15 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.2, 158.2, 139.2, 138.2, 132.6, 130.2, 122.4, 122.4, 118.3, 96.2, 13.8, 10.2; HRMS (EI) *m/z* calcd for $C_{13}H_{10}BrN_3OS$: 334.9728; found: 334.9732.

4.4. Synthesis of 2-cyclopropyl-6-substituted phenyl-5-thiocyanatoimidazo[2,1-b][1,3,4]thiadiazole **6**(**a**–**k**)

To a well stirred solution of **4**(**a**–**k**) (0.01 mol) in glacial acetic acid (5 ml) and potassium thiocyanate (0.02 mol) was added bromine (0.01 mol) in glacial acetic acid, drop wise with stirring at room temperature. Then stirring was continued for 1 h at 20–25 °C and then at room temperature for 30 min. The reaction mixture was poured into ice water. The separated solid was collected, washed with water, dried and recrystallized from ethanol.

*4.4.1. 2-Cyclopropyl-6-phenyl-5-thiocyanatoimidazo[2,1-b][1,3,4] thiadiazole (**6a**)*

Yield 46%; mp 230–232 °C; IR (KBr) ν_{max} 3136.2, 3002.7, 2178.6, 1684.8, 1405.8 cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 1.17–2.27 (m, 5H, cyclopropyl), 7.25–7.88 (m, 5H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.4, 140.2, 138.6, 135.6, 130.2, 128.6, 126.8, 122.6, 112.6, 13.2, 10.4; HRMS (EI) *m/z* calcd for $C_{14}H_{10}N_4S_2$: 298.0347; found: 298.0351.

*4.4.2. 6-(4-Chlorophenyl)-2-cyclopropyl-5-thiocyanatoimidazo [2,1-b][1,3,4]thiadiazole (**6b**)*

Yield 58%; mp 240–242 °C; IR (KBr) ν_{max} 3131.2, 3004.6, 2156.8, 1671.2, 1441.8, 618.6 cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 1.13–2.14 (m, 5H, cyclopropyl), 7.46–8.13 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.6, 140.1, 138.1, 136.2, 132.8, 131.8, 128.8, 122.6, 114.6, 13.2, 10.2; HRMS (EI) *m/z* calcd for $C_{14}H_9ClN_4S_2$: 331.9957; found: 331.9961.

*4.4.3. 6-(4-Bromophenyl)-2-cyclopropyl-5-thiocyanatoimidazo [2,1-b][1,3,4]thiadiazole (**6c**)*

Yield 52%; mp 286–288 °C; IR (KBr) ν_{max} 3139.2, 2922.6, 2157.8, 1693.4, 1464.2, 503.2 cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 1.15–2.27 (m, 5H, cyclopropyl), 7.26–7.68 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.4, 140.2, 138.6, 134.4, 132.8, 130.2, 122.6, 114.8, 13.2, 10.6; HRMS (EI) *m/z* calcd for $C_{14}H_9BrN_4S_2$: 375.9452; found: 375.9456.

*4.4.4. 2-Cyclopropyl-6-(4-fluorophenyl)-5-thiocyanatoimidazo [2,1-b][1,3,4]thiadiazole (**6d**)*

Yield 54%; mp 262–264 °C; IR (KBr) ν_{max} 3141.2, 2941.6, 2149.2, 1681.2, 1461.8, 1208.6 cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 1.15–2.16 (m, 5H, cyclopropyl), 7.49–8.31 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.4, 161.6, 140.2, 138.6, 130.7, 128.6, 122.6, 118.3, 114.8, 13.5, 10.2; HRMS (EI) *m/z* calcd for $C_{14}H_9FN_4S_2$: 316.0253; found: 316.0257.

*4.4.5. 2-Cyclopropyl-6-(2,4-dichlorophenyl)-5-thiocyanatoimidazo [2,1-b][1,3,4]thiadiazole (**6e**)*

Yield 48%; mp 274–276 °C; IR (KBr) ν_{max} 3071.2, 2946.8, 2146.1, 1645.8, 1446.4, 681.2 cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 1.24–2.31 (m, 5H, cyclopropyl), 7.12–7.89 (m, 3H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.3, 140.2, 138.6, 136.2, 134.2, 132.8, 130.2, 128.4, 122.6, 114.2, 13.8, 10.4; HRMS (EI) *m/z* calcd for $C_{14}H_8Cl_2N_4S_2$: 365.9567; found: 365.9571.

*4.4.6. 4-(2-Cyclopropyl-5-thiocyanatoimidazo[2,1-b][1,3,4] thiadiazol-6-yl)benzene-1,3-diol (**6f**)*

Yield 49%; mp 258–265 °C; IR (KBr) ν_{max} 3301.2, 3081.6, 2941.4, 2134.6, 1681.2, 1456.8 cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 1.21–2.19 (m, 5H, cyclopropyl), 6.14 (s, 2H, OH), 7.10–7.98 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.4, 161.2, 158.3, 140.2, 138.6, 122.6, 115.8, 112.8, 111.2, 109.8, 105.4, 13.2, 10.4; HRMS (EI) *m/z* calcd for $C_{14}H_{10}N_4O_2S_2$: 330.0245; found: 330.0249.

*4.4.7. 3-(2-Cyclopropyl-5-thiocyanatoimidazo[2,1-b][1,3,4] thiadiazol-6-yl)aniline (**6g**)*

Yield 39%; mp 246–250 °C; IR (KBr) ν_{max} 3201.2, 3071.4, 2931.6, 2154.6, 1671.8, 1439.8 cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 1.18–2.18 (m, 5H, cyclopropyl), 6.46 (s, 2H, NH_2), 7.10–8.02 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.4, 152.2, 140.2, 138.6, 135.2, 132.4, 122.6, 120.4, 118.2, 116.2, 13.6, 10.2; HRMS (EI) *m/z* calcd for $C_{14}H_{11}N_5S_2$: 313.0456; found: 313.0460.

*4.4.8. 4-(2-Cyclopropyl-5-thiocyanatoimidazo[2,1-b][1,3,4] thiadiazol-6-yl)aniline (**6h**)*

Yield 43%; mp 280–284 °C; IR (KBr) ν_{max} 3081.2, 2901.6, 2121.6, 1666.1, 1484.5 cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 1.16–2.15 (m, 5H, cyclopropyl), 6.41 (s, 2H, NH_2), 7.11–8.31 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.4, 146.3, 140.2, 138.6, 130.2, 125.6, 122.6, 118.2, 114.2, 13.2, 10.1; HRMS (EI) *m/z* calcd for $C_{14}H_{11}N_5S_2$: 313.0456; found: 313.0461.

*4.4.9. 2-Cyclopropyl-6-(3-nitrophenyl)-5-thiocyanatoimidazo[2,1-b][1,3,4]thiadiazole (**6i**)*

Yield 56%; mp 266–268 °C; IR (KBr) ν_{max} 3085.2, 2978.6, 2134.8, 1646.2, 1561.2, 1461.2, 1356.6 cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 1.15–2.15 (m, 5H, cyclopropyl), 7.21–8.31 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.4, 151.2, 138.6, 136.2, 134.2, 132.2, 130.2, 125.2, 124.2, 122.4, 114.2, 13.5, 10.6; HRMS (EI) *m/z* calcd for $C_{14}H_9N_5O_2S_2$: 343.0198; found: 343.0194.

*4.4.10. 2-Cyclopropyl-6-(4-nitrophenyl)-5-thiocyanatoimidazo[2,1-b][1,3,4]thiadiazole (**6j**)*

Yield 54%; mp 256–260 °C; IR (KBr) ν_{max} 3081.7, 2923.2, 2162.8, 1616.7, 1433.2, 1556.4, 1345.2 cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 1.18–2.20 (m, 5H, cyclopropyl), 7.39–8.15 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.4, 151.2, 140.6, 136.2, 134.2, 128.2, 126.2, 122.2, 13.6, 10.2; HRMS (EI) *m/z* calcd for $C_{14}H_9N_5O_2S_2$: 343.0198; found: 343.0195.

*4.4.11. 2-(2-Cyclopropyl-5-thiocyanatoimidazo[2,1-b][1,3,4] thiadiazol-6-yl)phenol (**6k**)*

Yield 36%; mp 270–272 °C; IR (KBr) ν_{max} 3312.2, 3041.2, 2931.6, 2114.5, 1656.2, 1461.2 cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 1.16–2.18 (m, 5H, cyclopropyl), 6.01 (s, 1H, OH), 7.35–8.21 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 166.4, 158.2, 140.2, 138.6, 132.1, 123.2, 122.4, 120.4, 118.4, 114.2, 13.2, 10.4; HRMS (EI) *m/z* calcd for $C_{14}H_{10}N_4OS_2$: 314.0296; found: 314.0293.

5. Conclusion

In this paper, we report the synthesis, and anti-tumor activity of series of 2-cyclopropylimidazo[2,1-*b*][1,3,4]thiadiazoles. These compounds were prepared by the cyclodehydration process between 2-amino-5-cyclpropyl-1,3,4-thiadiazole and an appropriate phenacyl bromide. In light of the NCI-60 results, five dose selected compound 5-bromo-6-(4-chlorophenyl)-2-cyclopropylimidazo[2,1-*b*][1,3,4]thiadiazole **5b** (**NSC D-96022/1**) was found to be the most active candidate of the series against Leukemia K-562, Colon Cancer HCT-15, Melanoma SK-MEL and Prostate Cancer PC-3 with GI_{50} 1.79, 2.02, 2.17, and 2.22 μM respectively with degree of selectivity toward Leukemic cancer cell line based upon MG MID ratio (3.6). These preliminary encouraging results of biological screening of the tested compounds could offer an excellent framework in this field that may lead to discovery of potent anti-tumor agent.

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