European Journal of Medicinal Chemistry 95 (2015) 49–63

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Synthesis and biological evaluation of new imidazo[2,1-b][1,3,4] thiadiazole-benzimidazole derivatives

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ARTICLE INFO

Article history:
Received 11 November 2014
Received in revised form
11 March 2015
Accepted 12 March 2015
Available online 14 March 2015

Keywords:
Imidazo[2,1-b][1,3,4]thiadiazole
Benzimidazole
Mycobacterium tuberculosis
Antibacterial agent
Antioxidant activity
Cytotoxicity

ABSTRACT

In this report, we describe the synthesis and biological evaluation of a new series of 2-(imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzimidazole derivatives (**5a-ac**). The molecules were analyzed by ^{1}H NMR, ^{13}C NMR, mass spectral and elemental data. The structure of one of the pre-final compounds, 6-(4-methoxyphenyl)-2-(4-methylphenyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**4d**) and that of a target compound, 2-[2-methyl-6-(4-methyl phenyl) imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl]-1*H*-benzimidazole (**5aa**) were confirmed by single crystal XRD studies. All the target compounds were screened for *in vitro* anti-tuberculosis activity against *Mycobacterium tuberculosis H37Rv strain*. Seven (**5c**, **5d**, **5l**, **5p**, **5r**, **5z** and **5aa**) out of twenty nine compounds showed potent anti-tubercular activity with a MIC of 3.125 μg/mL. A p-substituted phenyl group (p-tolyl or p-chlorophenyl) in the imidazo[2,1-*b*][1,3,4]thiadiazole ring and/or a chloro group in the benzimidazole ring enhance anti-tuberculosis activity whereas a nitro group in the benzimidazole ring reduces the activity. In the antibacterial screening, compounds **5i**, **5w** and **5ac** showed promising activity against the tested bacterial strains. Further, antifungal and antioxidant activities of these molecules were also investigated. In the cytotoxicity study, the active antitubercular compounds exhibited very low toxicity against a normal cell line.

1. Introduction

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis (Mtb)*. It is an instant disease and is the most single infectious killer disease after HIV/AIDS. According to the report released from the world health organization (WHO) in 2013 [1], 8.6 million people fell sick with TB and 1.3 million died from TB. Over 95% of TB deaths occur in low and middle income countries. In 2012, an estimated 5,30,000 children became ill with TB and 74,000 HIV negative children died of TB. In most of the cases, TB does not show any serious symptoms because the bacteria can live in an inactive form in the body. However, if immune system disorders cause abnormally low resistance, such as in people with HIV, TB bacteria can become active. Some of the available anti-tuberculosis drugs such as isoniazid and rifampin have a vital effect in the treatment of TB over past decades. However, the global dominance of the extensively drug-resistant tuberculosis (XDRTB) and multidrug resistant tuberculosis (MDRTB) made these traditional anti-TB agents with limited efficacy. Many active molecules were developed in the last four decades to treat multidrug resistant tuberculosis. But only one molecule, Bedaquiline, was approved by U.S. Food and Drug Administration [2]. Thus there is an emergent need to develop more effective drugs to treat TB. In this direction, a variety of heterocyclic compounds are being synthesized to check the possible potency of the molecules to act against TB [3–6].

The imidazo[2,1-b][1,3,4]thiadiazole moiety is an important class of heterocyclic compounds [7] in medicinal chemistry

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http://dx.doi.org/10.1016/j.ejmech.2015.03.024
0223-5234/

research. There are several reports available in the literature describing the imidazo[2,1-b][1,3,4]thiadiazole derivatives for their various biological activities. The most relevant and recent studies revealed that these molecules exhibit antimicrobial [8–10], anti-inflammatory [11], anticonvulsant [12,13], antituberculosis [14–16], anticancer [17–19] and anti hyperlipidemic [20] activities. In fact, 1,3,4-thiadiazole core is found in several marketed drugs such as Acetazolamide (I) and Methazolamide (II) (which are carbonic anhydrase inhibitors for treatment of glaucoma), and sulfamethizole (III), Cefazedone (IV), Cefazolin (V), Ceftezole (VI) (which are used as antibacterial drugs) (Fig. 1). A few imidazo[2,1-b][1,3,4] thiadiazole derivatives carrying other active heterocyclic pharmacophores particularly at position-5 (XI, XII) have been found to possess good activity against *Mtb* $H_{37}Rv$ strain [21–23]. For instance, Alegaon et al. reported the synthesis and antitubercular evaluation of a series of imidazo[2,1-b][1,3,4]thiadiazoles carrying different heterocyclic moieties at position-5 (XIII) [21]. On the other hand, benzimidazole and its derivatives are finding great importance in medicinal chemistry research due to their important biological actions as well as their synthetic applications [24]. Benzimidazole is a core structural moiety found in some of the important drugs like albendazole (VII), mebendazole (VIII), thiabendazole (IX), rabeprazole (X) etc (Fig. 1). A few recent reports demonstrated the promising antitubercular activity of benzimidazole derivatives. For example, a series of pyrido[1,2-a]benzimidazole based molecules (XIV) exhibit excellent bactericidal activity, with several of them having MIC_{MABA} values lower than 1 μg/mL [25]. Further, Stanley et al. identified a novel benzimidazole compound, N-(2,4-dichlorobenzyl)-1-propyl-1*H*-benzo[d]imidazol-5-amine (XV), which targets mycobacterial membrane protein large 3 (MmpL3) [26]. Also, a library of novel trisubstituted benzimidazoles were developed through rational drug design by Kumar et al. and some of these compounds (XVI, XVII) exhibited promising MIC values in the range of 0.5–6.1 μg/mL against *Mtb* H37Rv strain [27]. Thus, in view of the promising bactericidal activity exhibited by the imidazo[2,1-b][1,3,4]thiadiazole and benzimidazole systems (Fig. 2), we envisaged to amalgamate these two structural units in a single molecular frame and to explore the effects of this structural amalgamation towards their antitubercular activity. Hence we synthesized a series of imidazo[2,1-b][1,3,4]thiadiazole containing benzimidazole derivatives and evaluated their antitubercular, antibacterial, antifungal and antioxidant activities.

2. Results and discussion

2.1. Chemistry

The twenty nine new 2-subsituted-6-arylimidazo[2,1-b][1,3,4] thiadiazole derivatives (**5a-ac**) were synthesized according to the synthetic route presented in Scheme 1. One of the key intermediates, 5-methyl-1,3,4-thiadiazol-2-amine (**2a**) was synthesized by treating acetyl chloride with thiosemicarbazide using the reported procedure [28] with a modification of the work up method to get a better yield (80% as against the reported yield of 23%) of the product (without column chromatography purification). Other intermediate compounds, 5-aromatic-1,3,4-thiadiazole-2-amines (**2b-f**) were synthesized by treating the corresponding aromatic acid with thiosemicarbazide in the presence of phosphorous oxychloride [29]. The 2-subsituted-6-arylimidazo [2,1-b][1,3,4]thiadiazole derivatives (**3a**–**m**) were synthesized by the reaction between 5-substituted-1,3,4-thiadiazol-2-amine (**2a-f**) and the corresponding substituted α-halo aryl ketone under heating conditions. The formation of compounds **3a-m** was confirmed by ^1H NMR, ^{13}C NMR and mass spectral analysis. For instance, the ^1H NMR spectrum of compound **3b** showed a singlet with one proton at δ 7.92 ppm, which indicates the presence of imidazole ring proton (C_5–H). Further, the spectrum displayed singlets at δ 2.38 and 2.71 ppm representing the methyl protons on phenyl and 1,3,4-thiadiazole rings respectively. Also, its mass spectrum showed a molecular ion peak at *m*/*z* 230.0, which corresponds to M+1 peak of the molecule.

In the next step, imidazo[2,1-b][1,3,4]thiadiazoles **(3a**–**m)** were subjected to Vilsmeier–Haack formylation reaction to afford 2-substituted-6-arylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehydes **(4a**–**m)**, the structures of which were confirmed by spectral analysis. In the ^1H NMR spectrum of **4b**, the singlet peak due to imidazole ring proton (C_5–H) disappeared whereas a new singlet appeared at δ 10 ppm confirming the presence of aldehyde (-CHO) group. Also, its mass spectrum showed a molecular ion peak at *m*/*z* 257.9, which corresponds to M+1 peak of the molecule. The ^1H NMR spectrum of **4d** shows two singlets at 2.5 and 3.9 ppm which indicates the presence of methyl and methoxy groups in the molecule respectively, along with other characteristic signals. Single crystals of **4d** were analyzed by single crystal X-ray diffractometer to confirm its three dimensional structure. Finally, the

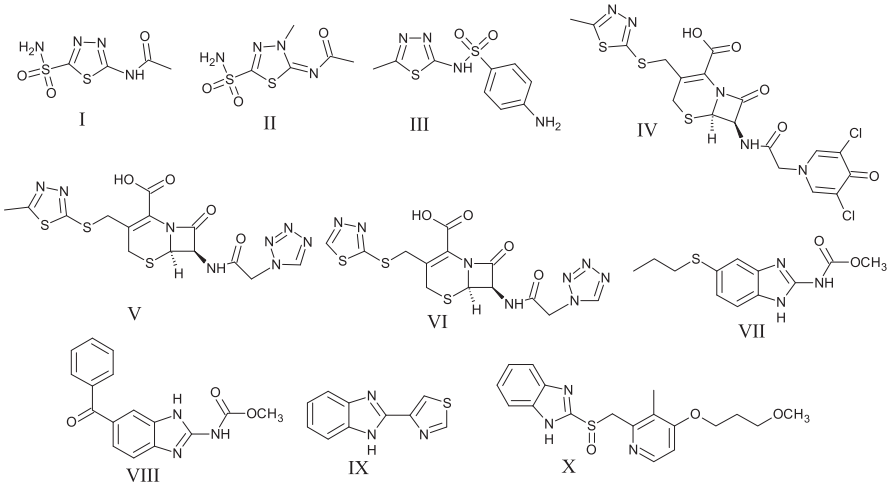


Fig. 1. Representative thiadiazole and benzimidazole based drug molecules (I–X).

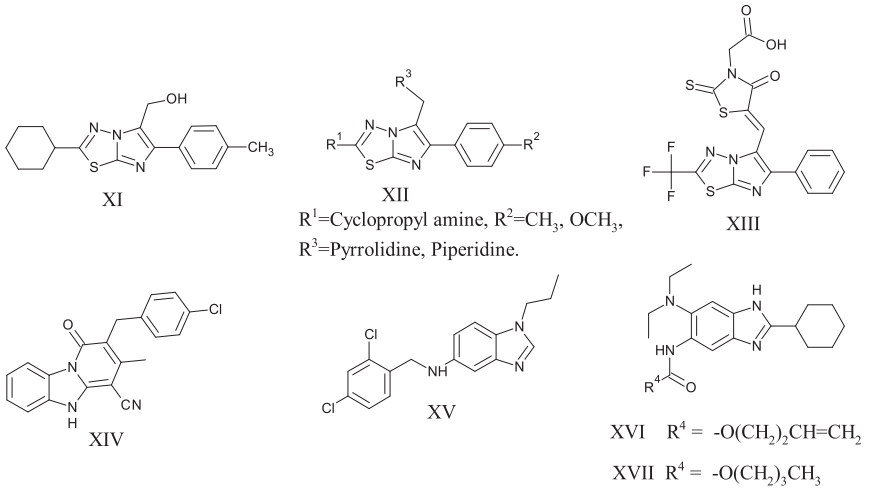
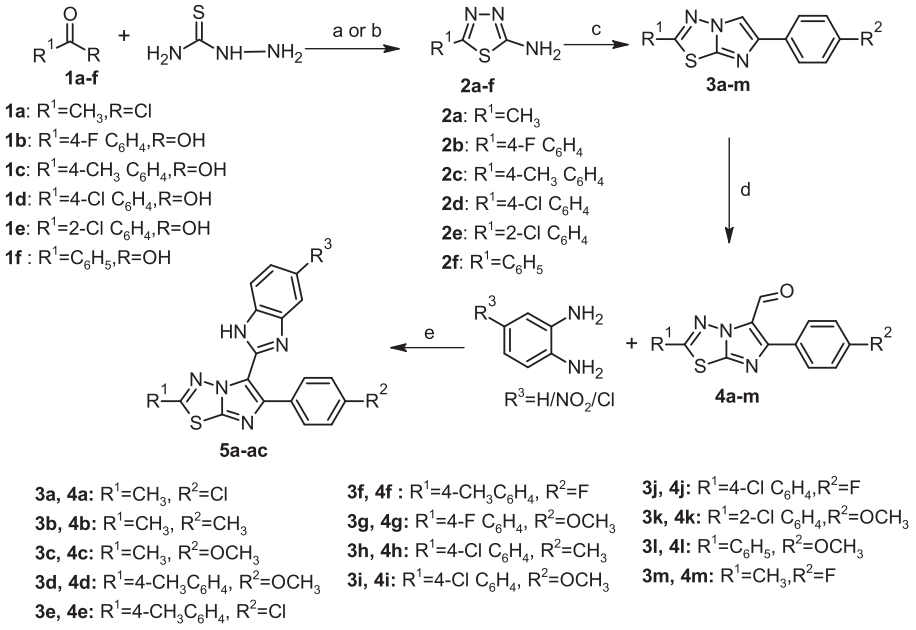


Fig. 2. Imidazo [2,1-b][1,3,4]thiadiazole and benzimidazole based antitubercular agents.



Scheme 1. Synthesis of imidazo[2,1-b][1,3,4]thiadiazole-benzimidazole derivatives. Reaction condition a: Acetyl chloride, 0 °C - RT, 4 h; b: Substituted aromatic acid, $POCl_3$, 75 °C, 30 min; c: Phenacyl bromide, ethanol, 80–85 °C, 24 h; d: DMF, $POCl_3$, 60 °C, 6 h; e: sodium meta bisulfite, DMF, 120 °C, 3 h.

target compounds, 2-(2-substituted-6-phenylimidazo[2,1-b][1,3,4] thiadiazol-5-yl)-1*H*-benzimidazoles (**5a-ac**), were synthesized by treating compounds **4a**–**m** with different substituted o-phenylenediamines in the presence sodium meta bisulfite ($Na_2S_2O_5$) under heating conditions using DMF as the solvent [30,31]. The structural features and the physical constant of compounds **5a-ac** are given in Table 1. The chemical structure of the target compounds was confirmed by spectral analysis and elemental data. The ^{1}H NMR spectrum of **5aa** showed a broad singlet at δ 12.8 ppm due to NH proton of the benzimidazole ring. Further, singlets at δ 2.9 and δ 2.3 ppm indicate the methyl protons of the 1,3,4-thiadiazole and phenyl rings respectively. In addition, the spectrum displayed two doublets at δ 7.18 and 7.8 ppm, multiplet in the region of δ 7.65 ppm and double of doublet in the region of δ 7.26 ppm due to aromatic (phenyl) protons. Also, its mass spectrum showed a molecular ion peak at *m*/*z* 346.0, which corresponds to M+1 peak of the molecule and is in agreement with its molecular formula $C_{19}H_{15}N_5S$. The three dimensional structure of **5aa** was evidenced by single crystal X-ray diffraction study. The spectral and elemental analysis data of all target compounds are given in the experimental part.

2.2. Single crystal X-ray crystallography studies

Single crystals of **4d** and **5aa** were grown from a solvent mixture of methanol and chloroform (1:1) by the slow evaporation of the solvent mixture at room temperature. The compounds (**4d** and **5aa**) crystallized in the monoclinic system with P 2_1/n space group. The

Table 1
Physical properties and structural features of target compounds (**5a-5ac**).

Entry	Product	R^1	R^2	R^3	Yield (%)	m.p (°C)
1	**5a**	4-CH_3·C_6H_4	OCH_3	H	78	247–248
2	**5b**	4-CH_3·C_6H_4	OCH_3	NO_2	73	243–244
3	**5c**	4-CH_3·C_6H_4	OCH_3	Cl	76	218–219
4	**5d**	4-Cl·C_6H_4	CH_3	H	74	269–270
5	**5e**	4-Cl·C_6H_4	CH_3	NO_2	72	226–227
6	**5f**	4-Cl·C_6H_4	CH_3	Cl	76	259–260
7	**5g**	2-Cl·C_6H_4	OCH_3	NO_2	78	235–236
8	**5h**	C_6H_5	OCH_3	NO_2	74	259–260
9	**5i**	4-CH_3·C_6H_4	F	H	77	243–244
10	**5j**	4-CH_3·C_6H_4	F	NO_2	74	266–267
11	**5k**	4-Cl·C_6H_4	OCH_3	H	72	264–265
12	**5l**	4-Cl·C_6H_4	F	H	79	258–259
13	**5m**	4-F·C_6H_4	OCH_3	H	77	236–237
14	**5n**	4-F·C_6H_4	OCH_3	NO_2	78	211–212
15	**5o**	4-F·C_6H_4	OCH_3	Cl	74	201–202
16	**5p**	4-CH_3·C_6H_4	Cl	H	78	222–223
17	**5q**	4-CH_3·C_6H_4	Cl	NO_2	76	208–209
18	**5r**	4-CH_3·C_6H_4	Cl	Cl	79	268–269
19	**5s**	CH_3	OCH_3	H	82	179–180
20	**5t**	CH_3	OCH_3	NO_2	86	189–190
21	**5u**	CH_3	F	H	80	186–187
22	**5v**	CH_3	F	NO_2	82	192–193
23	**5w**	CH_3	F	Cl	79	170–171
24	**5x**	CH_3	Cl	H	86	179–180
25	**5y**	CH_3	Cl	NO_2	87	181–182
26	**5z**	CH_3	Cl	Cl	78	180–181
27	**5aa**	CH_3	CH_3	H	88	173–174
28	**5ab**	CH_3	CH_3	NO_2	84	202–203
29	**5ac**	CH_3	CH_3	Cl	82	193–194

crystal structure of the compounds is shown in Fig. 3. The crystal data and measurement details for compounds **4d** and **5aa** are given in Table 2.

2.3. In vitro antimycobacterial activity

The synthesized imidazo[2,1-b][1,3,4]thiadiazole-benzimidazole hybrids (**5a-5ac**) were screened against *M. tuberculosis* H37Rv (ATCC27294) by agar dilution method to evaluate their antimycobacterial activity in terms of minimum inhibitory concentration (MIC) values. The MIC is defined as the minimum concentration of a compound required to completely inhibit the bacterial growth. The MIC values in μg/mL of **5a-5ac** along with those of standard drugs for comparison are presented in Fig. 4, which shows that the values are in the range 3.125–50.0 μg/mL. It is evident that among the twenty nine compounds, seven compounds namely **5c**, **5d**, **5l**, **5p**, **5r**, **5z** and **5aa** showed potent anti-tubercular activity with MIC of 3.125 μg/mL and are equipotent with the standard Ethambutol. Four other compounds (**5e**, **5j**, **5s** and **5u**) showed promising activity against *M. tuberculosis* with MIC of 6.25 μg/mL.

The nature of the substituents on the imidazo[2,1-b][1,3,4]thiadiazole (R^1/R^2) and benzimidazole (R^3) rings was found to affect the activity of these compounds. The presence of a nitro group on the benzimidazole ring substantially decreased the anti-TB activity as can be seen in case of compounds **5g**, **5h**, **5v**, **5y** and **5ab** which showed an MIC value of 50 μg/mL. Whereas compounds with unsubstituted or chlorosubstituted benzimidazole rings showed excellent to moderate activity. Though two compounds (**5z** and **5aa**) with a methyl substitution at position-2 of the imidazo[2,1-b][1,3,4]thiadiazole ring exhibited significant activity, replacing the methyl group with a p-substituted phenyl group (p-tolyl or p-chlorophenyl) was found to be effective in enhancing anti-TB activity. This is evident from the fact that compounds with a nitrobenzimidazole substitution also have shown moderate activity when a methyl group is replaced with a p-substituted phenyl group. For instance, compounds **5e** and **5j** showed improved activity (MIC = 6.25 μg/mL) when compared to the activity of compounds **5v** and **5ab** (MIC = 50 μg/mL) respectively. This information on structure–activity relationship explored in the present study could be helpful in further structural modification and development of new imidazo[2,1-b][1,3,4]thiadiazole-benzimidazole hybrids as potent antitubercular agents.

2.4. Antibacterial activity

The *in vitro* antibacterial activity of synthesized compounds **5a-5ac** were determined using Muller Hinton Agar method [32,33]. These compounds were screened against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. The compounds were dissolved in DMSO and appropriate dilutions were made (1 mg/mL and 0.5 mg/mL). Streptomycin was taken as the standard drug for the screening. The result showed that among the tested compounds, **5i** exhibit good activity against all the tested bacterial strains at concentrations of 1 and 0.5 mg/mL, whereas compounds **5w** and **5ac** are moderately active against all these microbial strains. The results of the antibacterial screening are summarized in Table 3, where in, the figures represent the zone of inhibition.

2.5. Antifungal activity

The synthesized compounds **5a-5ac** were screened also for their antifungal activity against *Aspergillus flavus*, *Chrysosporium keratinophilum and Candida albicans*. These compounds were dissolved in DMSO and antifungal activity was determined using the well plate method [34,35] at concentrations of 1 and 0.5 mg/mL and the results are tabulated in Table 4. Fluconazole was taken as the standard drug. It is evident from Table 4 that none of the compounds show any significant activity against the tested fungal strains. Only two compounds **5w** and **5x**, both containing a 4-chlorophenyl substitution on the imidazo[2,1-b][1,3,4]thiadiazole ring and a chloro substitution on the benzimidazole ring, exhibited moderate activity. These *in vitro* screening results show that the new imidazo[2,1-b][1,3,4]thiadiazole-benzimidazole hybrids (**5a-5ac**) are very selective against *M. tuberculosis* H37Rv strain, 19 compounds of the series exhibited MIC of 12.5 μg/mL or less.

2.6. Antioxidant studies: DPPH radical scavenging assay

The free radical scavenging activity of test samples **5a**–**ac** was measured using the DPPH method [36]. Butylated hydroxytoluene (BHT) was used as the standard. Among the tested compounds, **5r and 5z** showed significant DPPH scavenging activity (>65%) at 100 μg concentration (Fig. 5).

2.7. In vitro cytotoxicity studies

The *in vitro* cytotoxicity of the active compounds (MIC ≤6.25 μg/mL against *M. tuberculosis*) were evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [37] against normal Vero cell line. The graphical representation of the cell growth inhibition of the compounds at a concentration of 62.5 μg/mL is shown in Fig. 6. Some of the most potent antitubercular compounds **5r**, **5s** and **5aa** exhibited very low toxicity of 7.4, 7.1 and 5.03% respectively. Also, none of the other active compounds are toxic to the normal cells thus proving the lack of general cellular toxicity.

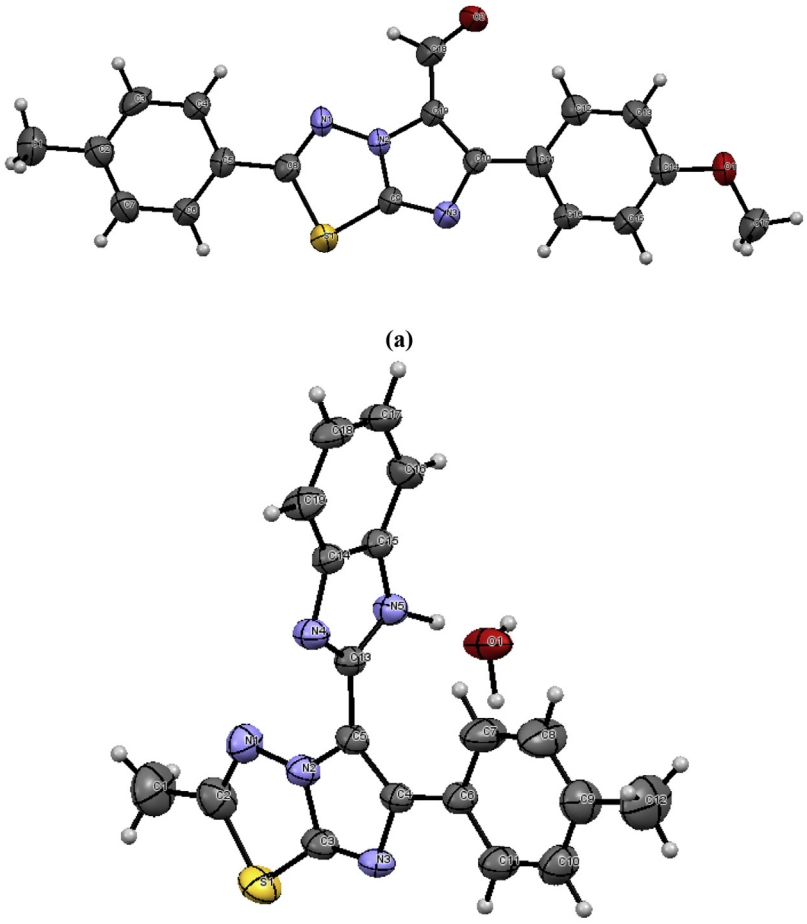


Fig. 3. ORTEP diagram showing the X-ray crystal structure of compounds a) **4d** and b) **5aa**.

3. Conclusions

A series of twenty nine 2-(imidazo [2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzimidazole derivatives were designed and synthesized. These compounds were characterized by ^{1}H NMR, ^{13}C NMR, mass spectroscopic techniques and elemental analysis. The compounds were screened for their anti-tubercular activity against *M. tuberculosis* H37Rv by Agar dilution method. Among the tested compounds, seven compounds (**5c**, **5d**, **5l**, **5p**, **5r**, **5z** and **5aa**) exhibited significant activity against the growth of *M. tuberculosis* with MIC of 3.125 μg/mL and a moderate activity with MIC of 6.25 μg/mL was observed for compounds **5u**, **5e**, **5s** and **5j**. The structure–activity relationship reveals that various substituents on the imidazo[2,1-b][1,3,4]thiadiazole and the benzimidazole rings affect significantly the anti-TB activity of these compounds. Compounds with a chloro substituted or unsubstituted benzimidazole ring exhibit better activity when compared with the activity of nitro substituted derivatives. Further, a phenyl group with a p-methyl/p-chloro substituent at position-2 of the imidazo[2,1-b][1,3,4]thiadiazole ring enhanced the activity. Hence, these compounds with promising anti-TB activity could serve as promising lead molecules for further generation of potent antitubercular agents. Further, these compounds were screened also for their antibacterial, antifungal and antioxidant activity. Among the tested compounds, **5i** showed good activity and compounds **5w** and **5ac** showed moderate activity against the tested bacterial strains. Compounds **5w** and **5x** showed moderate activity against the fungal strains. The antioxidant property of compound **5z** is comparable with that of the standard BHT. In conclusion, the new imidazo[2,1-b][1,3,4]thiadiazole-benzimidazole hybrids (**5a-5ac**) show remarkable selectivity against *M. tuberculosis* H37Rv strain and interestingly, most

Table 2
Crystal data and measurement details for compounds **4d** and **5aa**.

Parameters	Crystal data of 4d	Crystal data of 5aa
Empirical formula	$C_{19}H_{15}N_3O_2S$	$C_{19}H_{15}N_5S{\cdot}H_2O$
Formula weight	349.41	363.45
Crystal system	Monoclinic	Monoclinic
Crystal dimension	0.5 mm × 0.24 mm × 0.14 mm	0.42 mm × 0.22 mm × 0.10 mm
Space group	P 2_1/n	P 2_1/n
a (Å)	7.1761(7)	7.1846(5)
b (Å)	19.468(17)	22.9252(13)
c (Å)	12.022(11)	11.0542(6)
Volume (Å^3)	1611.2(3)	1820.72(19)
Angle α, β, γ	90, 106.408(6), 90	90, 90.087(3), 90
Z	4	4
F_{000}	728	760
μ (mm^{-1})	0.219	0.196
Temperature (T)	296k	296k
Radiation wavelength (Å)	0.71073	0.71073
Radiation type	Mo Kα	Mo Kα
Radiation source	Mo	Mo
CCDC number	1,032,180	1,032,193

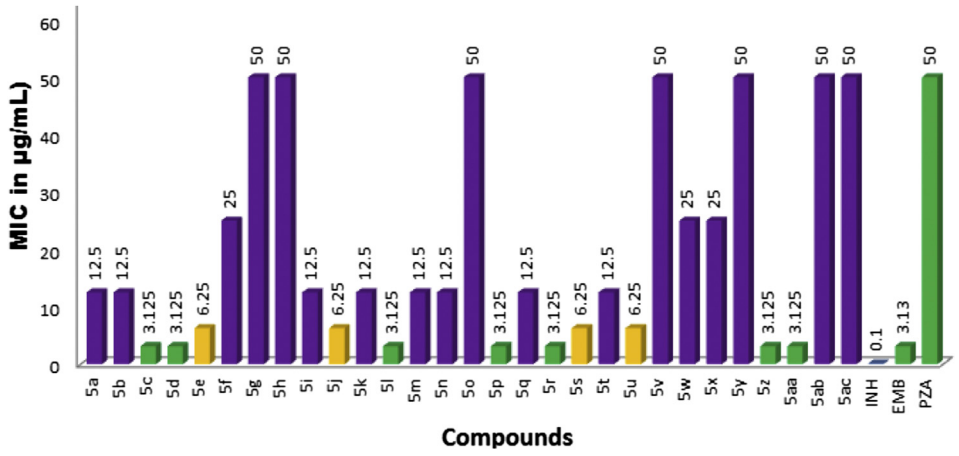


Fig. 4. Antitubercular activity of **5a-5ac** against *Mycobacterium tuberculosis* H37RV (INH: Isoniazid; EMB: Ethambutol; PZA: Pyrazinamide).

Table 3
Antibacterial activity of target compounds (**5a-5ac**) against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa and Salmonella typhi*.

Compound	*Escherichia coli*		*Staphylococcus aureus*		*Pseudomonas aeruginosa*		*Salmonella typhi*	
Cocn. in mg/ml	1	0.5	1	0.5	1	0.5	1	0.5
Control	0		0		0		0	
SM	18.6 ± 0.2	14.3 ± 0.1	16.8 ± 0.2	12.5 ± 0.2	16.7 ± 0.2	13.6 ± 0.2	17.2 ± 0.2	15.7 ± 0.2
5a	–	–	–	–	–	–	–	–
5b	–	–	–	–	–	–	–	–
5c	04 ± 0.1	02 ± 0.2	06 ± 0.1	03 ± 0.2	07 ± 0.1	03 ± 0.2	3 ± 0.2	01 ± 0.1
5d	05 ± 0.2	03 ± 0.1	04 ± 0.1	01 ± 0.1	04 ± 0.2	01 ± 0.1	05 ± 0.4	03 ± 0.5
5e	–	–	–	–	–	–	–	–
5f	04 ± 0.1	02 ± 0.4	03 ± 0.1	03 ± 0.1	04 ± 0.1	03 ± 0.1	03 ± 0.1	02 ± 0.2
5g	–	–	–	–	–	–	–	–
5h	04 ± 0.4	03 ± 0.3	04 ± 0.2	02 ± 0.1	05 ± 0.1	03 ± 0.2	06 ± 0.1	03 ± 0.2
5i	13 ± 0.5	09 ± 0.5	14 ± 0.1	11 ± 0.2	13 ± 0.1	10 ± 0.2	15 ± 0.3	13 ± 0.2
5j	04 ± 0.5	02 ± 0.1	04 ± 0.1	02 ± 0.2	03 ± 0.2	02 ± 0.2	04 ± 0.1	01 ± 0.2
5k	03 ± 0.2	01 ± 0.1	03 ± 0.1	02 ± 0.2	02 ± 0.2	01 ± 0.2	03 ± 0.2	01 ± 0.1
5l	05 ± 0.1	03 ± 0.2	07 ± 0.1	03 ± 0.2	08 ± 0.1	05 ± 0.2	03 ± 0.3	01 ± 0.1
5m	–	–	–	–	–	–	–	–
5n	05 ± 0.3	03 ± 0.4	05 ± 0.1	03 ± 0.2	06 ± 0.1	03 ± 0.2	04 ± 0.1	03 ± 0.2
5o	05 ± 0.2	01 ± 0.1	04 ± 0.1	01 ± 0.1	02 ± 0.1	01 ± 0.1	03 ± 0.5	0
5p	04 ± 0.2	02 ± 0.2	03 ± 0.1	02 ± 0.1	06 ± 0.1	03 ± 0.2	04 ± 0.2	02 ± 0.2
5q	–	–	–	–	–	–	–	–
5r	04 ± 0.1	02 ± 0.2	05 ± 0.2	03 ± 0.1	03 ± 0.1	02 ± 0.1	–	–
5s	–	–	–	–	–	–	–	–
5t	–	–	–	–	–	–	–	–
5u	04 ± 0.1	03 ± 0.1	02 ± 0.1	01 ± 0.2	03 ± 0.2	02 ± 0.3	02 ± 0.1	01 ± 0.5
5v	04 ± 0.3	02 ± 0.4	03 ± 0.1	02 ± 0.2	01 ± 0.1	03 ± 0.2	02 ± 0.1	01 ± 0.2
5w	11 ± 0.2	08 ± 0.2	09 ± 0.4	06 ± 0.6	13 ± 0.1	10 ± 0.2	10 ± 0.1	07 ± 0.2
5x	04 ± 0.2	03 ± 0.1	04 ± 0.2	03 ± 0.3	05 ± 0.2	03 ± 0.1	03 ± 0.1	01 ± 0.1
5y	06 ± 0.1	04 ± 0.2	09 ± 0.1	07 ± 0.2	08 ± 0.1	05 ± 0.2	06 ± 0.3	04 ± 0.5
5z	04 ± 0.1	02 ± 0.4	03 ± 0.2	02 ± 0.1	04 ± 0.1	01 ± 0.1	04 ± 0.2	02 ± 0.2
5aa	05 ± 0.1	02 ± 0.2	05 ± 0.2	03 ± 0.1	03 ± 0.1	02 ± 0.1	06 ± 0.3	–
5ab	–	–	–	–	–	–	–	–
5acc	08 ± 0.1	05 ± 0.2	10 ± 0.1	07 ± 0.2	09 ± 0.1	06 ± 0.2	08 ± 0.1	07 ± 0.2

SM: Streptomycin (anti-bacterial standard); –: inhibition not detected; control: dimethylsulfoxide.

derivatives (19 compounds) of the series exhibited MIC of 12.5 µg/mL or less. Further, the cytotoxicity study revealed that none of the active molecules are toxic to the Vero cell line thus proving the lack of general cellular toxicity.

4. Experimental

4.1. Materials and instruments

The required chemicals and solvents were procured from Sigma Aldrich (Germany), Merck (India) and Spectrochem Chemicals Pvt. Ltd. All the solvents were distilled and dried before usage. The progress of the reaction was monitored by TLC using pre coated aluminum sheets with 60 F254 silica gel (Merck KGaA). Melting point of the synthesized compounds was recorded by a Stuart SMP3 melting point apparatus. Infrared spectra of the compounds were recorded on a Jasco FTIR 4200 spectrometer. ^{1}H NMR spectra of the intermediates and final compounds were recorded using Bruker 400 MHz NMR spectrometers using TMS as internal standard. ^{13}C NMR spectra of the compounds were recorded using a Bruker 100 MHz NMR spectrometer. Elemental analysis was done using a Thermo electron corporation EA-112 series C, H, N, S analyzer. Mass

Table 4
Antifungal activity of target compounds (**5a-5ac**) against *Aspergillus flavus*, *Chrysosporium keratinophilum* and *Candida albicans*.

Compound	*Aspergillus flavus*		*Chrysosporium keratinophilum*		*Candida albicans*	
Conc. in mg/ml	1	0.5	1	0.5	1	0.5
Control	0		0		0	
FZ	14 ± 0.2	10 ± 0.1	16 ± 0.2	14 ± 0.2	23 ± 0.2	20 ± 0.2
5a	–	–	–	–	–	–
5b	–	–	–	–	–	–
5c	03 ± 0.1	02 ± 0.1	04 ± 0.2	02 ± 0.1	03 ± 0.1	02 ± 0.1
5d	05 ± 0.1	03 ± 0.1	05 ± 0.1	02 ± 0.1	04 ± 0.2	02 ± 0.1
5e	–	–	–	–	–	–
5f	03 ± 0.2	02 ± 0.1	03 ± 0.1	02 ± 0.1	04 ± 0.1	03 ± 0.1
5g	–	–	–	–	–	–
5h	03 ± 0.2	01 ± 0.1	04 ± 0.2	02 ± 0.2	03 ± 0.1	–
5i	06 ± 0.2	04 ± 0.1	05 ± 0.2	03 ± 0.2	06 ± 0.1	04 ± 0.2
5j	05 ± 0.1	02 ± 0.1	04 ± 0.2	02 ± 0.1	05 ± 0.1	02 ± 0.2
5k	–	–	–	–	–	–
5l	04 ± 0.1	02 ± 0.1	04 ± 0.1	03 ± 0.2	03 ± 0.2	01 ± 0.3
5m	–	–	–	–	–	–
5n	05 ± 0.1	03 ± 0.2	04 ± 0.1	02 ± 0.2	05 ± 0.1	02 ± 0.1
5o	04 ± 0.2	02 ± 0.2	04 ± 0.1	01 ± 0.1	05 ± 0.1	02 ± 0.2
5p	–	–	–	–	–	–
5q	–	–	–	–	–	–
5r	05 ± 0.1	02 ± 0.2	03 ± 0.2	01 ± 0.1	06 ± 0.1	04 ± 0.2
5s	–	–	–	–	–	–
5t	02 ± 0.1	01 ± 0.1	03 ± 0.1	02 ± 0.2	02 ± 0.1	01 ± 0.2
5u	03 ± 0.1	02 ± 0.2	04 ± 0.1	03 ± 0.2	02 ± 0.1	01 ± 0.2
5v	–	–	–	–	–	–
5w	07 ± 0.1	05 ± 0.2	08 ± 0.2	06 ± 0.1	09 ± 0.2	07 ± 0.2
5x	06 ± 0.1	05 ± 0.1	07 ± 0.2	06 ± 0.1	06 ± 0.1	04 ± 0.1
5y	–	–	–	–	–	–
5z	03 ± 0.1	01 ± 0.2	05 ± 0.1	03 ± 0.2	04 ± 0.1	02 ± 0.2
5aa	–	–	–	–	–	–
5ab	–	–	–	–	–	–
5ac	03 ± 0.1	01 ± 0.2	02 ± 0.1	–	04 ± 0.1	01 ± 0.2

FZ: Flucanazole (anti-fungal standard); –: inhibition not detected; control: dimethylsulfoxide.

spectra were recorded using a Waters micro mass Q-Tofmicro spectrometer with an ESI source. X-ray intensity data for compounds **4d** and **5aa** was collected at room temperature using a Bruker smart Apex Duo single crystal–ray diffractometer equipped with dual system (compact copper micro-porous source plus molybdenum scaled tube source) CCD detector. Monochromatic Mo-Kα radiation (λ = 0.71073 Å) was used for the measurement.

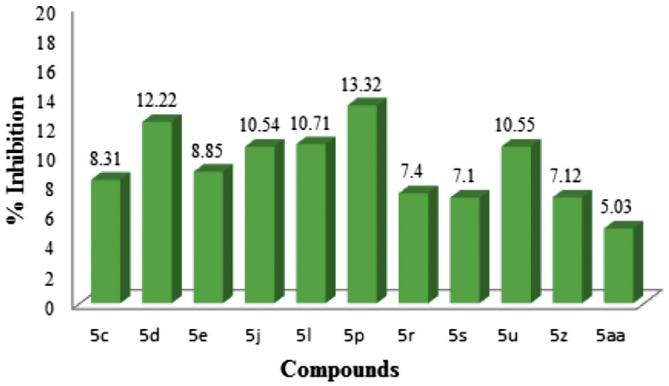


Fig. 6. Growth inhibition activity of active compounds (at a concentration of 62.5 μg/mL) against VERO cell line.

4.2. Synthesis

4.2.1. Synthesis of 5-amino-2-methyl-1, 3, 4-thiadiazole (**2a**)

To the thiosemicarbazide (15.0 g, 164.58 mmol), acetyl chloride (28.54 mL, 329.16 mmol) was added slowly and the mixture was stirred for 4 h at RT. To the reaction mixture, ice cold water was added and the solid obtained was filtered off. The solid was then added to ice cold water. To this slurry, a solution of 50% NaOH was added till the pH of the solution becomes basic. The solid obtained was filtered off and dried under vacuum to get the compound (**2a**) as white solid. Yield: 14.4 g, 76%; m.p: 269–270 °C; FTIR (ATR, cm^{-1}): 3288, 3105, 2925, 1614, 689; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 2.18 (s, 3H, CH_3), 13.05 (br s, 2H, NH_2); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 10.9, 148.8, 165.7; ESI-MS (*m*/*z*) 116.02 (M + H) +; calculated for $C_3H_5N_3S$; C, 31.9; H, 4.38; N, 36.49; S, 27.84. Found: C, 31.89; H, 4.41; N, 36.42; S, 27.44.

4.2.2. General procedure for the synthesis of 5-subsituted-1,3,4-thiadiazole-2-yl amine (**2b-f**)

*4.2.2.1. 5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-amine (**2b**).* A mixture of 4-fluoro benzoic acid (5.0 g, 35.70 mmol), thiosemicarbazide (3.25 g, 35.70 mmol) and $POCl_3$ (9.25 ml) was heated to 75 °C and maintained same temperature for 30 min under

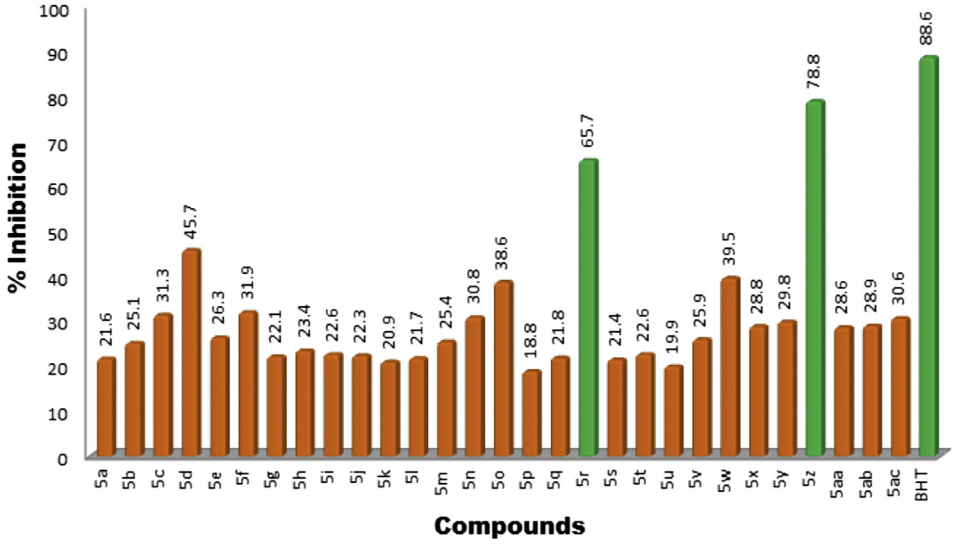


Fig. 5. Antioxidant activity of compounds **5a-ac**.

stirring. The reaction mixture cooled to room temperature, water (55 ml) was added and refluxed for 4 h. After cooling, the mixture was basified with 50% NaOH to pH 8 by the drop wise addition under stirring. The obtained solid was filtered and recrystallized from ethanol to give the target compound **2b** as a colorless solid. Yield: 5.69 g, 82%; m.p: 230–231 °C; FTIR (ATR, cm^{-1}): 3346, 3251, 2934, 1633, 1063, 683; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 7.29–7.33 (t, J = 8.77 Hz, 2H, Ar–H), 7.41 (s, 2H), 7.79–7.83 (dd, J = 5.46 Hz, 8.59 Hz, 2H, Ar–H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 118.13, 129.43, 130.21, 154.32, 157.28, 168.10; **ESI-MS**: m/z 196.4 [M+H] +; calculated for $C_8H_6FN_3S$; C, 49.22; H, 3.10; N, 21.52; S, 16.43. Found: C, 49.20; H, 3.11; N, 21.52; S, 16.44.

*4.2.2.2. 5-(4-Methylphenyl)-1,3,4-thiadiazol-2-amine (**2c**).* Compound **2c** was synthesized by following the above procedure with 4-methyl benzoic acid (5.0 g, 36.76 mmol), thiosemicarbazide (3.35 g, 36.76 mmol) and $POCl_3$ (9.25 ml). White solid; yield: 6.31 g, 90%; m.p: 213–214 °C; FTIR (ATR, cm^{-1}): 3278, 3103, 2955, 1611, 690; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 2.33 (s, 3H), 7.25–7.27 (d, J = 8.00 Hz, 2H, Ar–H), 7.36 (s, 2H), 7.63–7.65 (d, J = 8.12 Hz, 2H, Ar–H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 21.35, 126.72, 128.80, 130.11, 139.76, 156.95, 168.65; **ESI-MS**: m/z 192.2 [M+H]+. calculated for $C_9H_9N_3S$; C, 56.22; H, 4.74; N, 21.97; S, 16.77. Found: C, 56.20; H, 4.74; N, 21.90; S, 16.74.

*4.2.2.3. 5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-amine (**2d**).* The above procedure was followed for 4-chloro benzoic acid (5.0 g, 32.05 mmol), thiosemicarbazide (2.92 g, 32.05 mmol) and $POCl_3$ (9.25 ml) to afford compound **2d** as White solid. Yield: 5.20 g, 77%; m.p: 210–211 °C; FTIR (ATR, cm^{-1}): 3258, 3093, 1633, 751, 684; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 7.47 (s, 2H), 7.52–7.54 (d, J = 6.55 Hz, 2H, Ar–H), 7.76–7.78 (d, J = 6.24 Hz, 2H, Ar–H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm):128.38, 129.63, 130.34, 134.43, 155.59, 168.30; ESI-MS: m/z 212.6 [M+H]+. calculated for $C_8H_6ClN_3S$; C, 45.39; H, 2.86; N, 19.85; S, 15.15. Found: C, 45.30; H, 2.88; N, 19.90; S, 15.14.

*4.2.2.4. 5-(2-Chlorophenyl)-1, 3, 4-thiadiazol-2-amine (**2e**).* Compound **2e** was synthesized by following the above procedure for the 2-chloro benzoic acid (5.0 g, 32.05 mmol), thiosemicarbazide (2.92 g, 32.05 mmol) and $POCl_3$ (9.25 ml). Yield: 5.41 g, 80% (white solid); m.p: 220–221 °C; FTIR (ATR, cm^{-1}): 3269, 3098, 1636, 748, 694; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 7.97–8.01 (m, 2H, Ar–H), 7.57–7.60 (m, 1H, Ar–H), 7.44–7.47 (m, 1H, Ar–H), 7.42 (s, 2H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 126.50, 128.45, 129.32, 130.21, 132.01, 136.81, 155.70, 168.41; **ESI-MS**: m/z 212.10 [M+H] +. calculated for $C_8H_6ClN_3S$; C, 45.39; H, 2.86; N, 19.85; S, 15.15. Found: C, 45.31; H, 2.89; N, 19.85; S, 15.16.

*4.2.2.5. 5-Phenyl-1, 3, 4-thiadiazol-2-amine (**2f**).* Compound **2f** was synthesized by following the above procedure for benzoic acid (5.0 g, 40.32 mmol), thiosemicarbazide (3.67 g, 40.321 mmol) and $POCl_3$ (9.3 ml). Yield: 5.56 g, 78.0% (white solid); m.p:224–225 °C; FTIR (ATR, cm−1): FTIR (ATR, cm^{-1}): 3288, 3100, 1611, 690; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 7.40 (s, 2H), 7.43–7.49 (dd, J = 6.38 Hz, 14.00 Hz, 2H, Ar–H), 7.74–7.76 (d, J = 6.94 Hz, 2H, Ar–H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 126.71, 128.70, 129.98, 138.56, 156.94, 168.45; **ESI-MS**: m/z 178.3 [M+H]+. calculated for $C_8H_7N_3S$; C,54.22; H, 3.98; N, 23.71; S, 18.09. Found: C, 54.21; H, 3.90; N, 23.80; S, 18.10.

*4.2.3. General procedure for the synthesis of 2-subsituted-6-arylimidazo[2,1-b][1,3,4] thiadiazoles (**3a-m**)*

*4.2.3.1. 6-(4-Chlorophenyl)-2-methyl imidazo[2,1-b][1,3,4]thiadiazole (**3a**).* A mixture of **2a** (2 g, 17.39 mmol) and 4-chloro phenacyl bromide (4.032 g, 17.39 mmol) was refluxed in dry ethanol (20 mL) for 24 h. The excess of solvent was removed under reduced pressure and the solid hydrobromide salt was suspended in water, and neutralized by the aqueous sodium carbonate solution to get free base. It was then filtered, washed with water, dried, and recrystallized from ethanol to get compound **3a** as light yellow solid. Yield: 3.4 g, 80%; m.p: 184–185 °C; FTIR (ATR, cm^{-1}): FTIR (ATR, cm−1): 3066, 2938, 1592, 1504, 1466, 843, 741, 682; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.72 (s, 3H), 7.37 (dd, J = 2.0, 6.8 Hz, 2H, Ar–H),7.74 (dd, J = 1.8, 6.6 Hz, 2H, Ar–H), 7.94 (s, 1H, H-5 imidazole), **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 17.86, 102.29, 126.24, 128.87, 132.48, 1 33.09, 144.93, 145.86, 159.59; **ESI-MS** (m/z) 249.9 (M + H)+; calculated for $C_{11}H_8ClN_3S$; C, 52.91; H, 3.23; N, 16.83; S, 12.84. Found: C, 52.98; H, 3.28; N, 16.92; S, 12.54.

Compounds **3b-m** were synthesized by following the above procedure by reacting 2 g of 5-subsituted-1,3,4-thiadiazole-2-yl amine with same equivalent of corresponding phenacyl bromide derivative. The structural characterization data for compounds **3b-m** are given below.

*4.2.3.2. 2-Methyl-6-(4-methylphenyl)imidazo[2,1-b][1,3,4]thiadiazole (**3b**).* Yellow solid, yield: 3.26 g, 82.0%; m.p: 211–212 °C; FTIR (ATR, cm^{-1}): 3067, 2924, 1592, 1504, 1477, 842, 668; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 2.37(s, CH_3), 2.71 (s, CH_3), 7.21 (d, J = 7.9 Hz, 2H, Ar–H),7.70 (d, J = 7.9 Hz, 2H, Ar–H) 7.92 (s, H-5 imidazole, 1H), **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 17.82, 21.26, 108.76, 124.94, 129.41, 131.14, 137.27, 145.53, 146.17, 159.14; **ESI-MS** m/z 230.0 [M+H]+; calculated for $C_{12}H_{11}N_3S$; C, 62.86; H, 4.84; N, 18.33; S, 13.98. Found: C, 62.98; H, 4.78; N, 18.40; S, 14.02.

*4.2.3.3. 6-(4-Methoxyphenyl)-2-methylimidazo[2,1-b][1,3,4]thiadiazole (**3c**).* Yellow solid, yield: 3.44 g, 81%; m.p:192–193 °C; FTIR (ATR, cm^{-1}): 3043, 2935, 1540, 1462, 1239, 1023, 830, 670; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.70 (s, CH_3), 3.84 (s, OCH_3), 6.95–6.97 (m, 2H, ArH),7.73 (d, J = 8.8 Hz, 2H, ArH),7.871 (s, H-5 imidazole, 1H), **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 17.52, 54.60, 109.77, 125.65, 128.42, 131.15, 132.78, 145.23, 146.20, 159.40; **ESI-MS** m/z 245.9 [M+H]+; calculated for $C_{12}H_{11}N_3OS$; C, 58.76; H, 4.52; N, 17.13; S, 13.07. Found: C, 58.86; H, 4.60; N, 17.12; S, 13.14.

*4.2.3.4. 6-(4-Methoxyphenyl)-2-(4-methylphenyl)imidazo[2,1-b][1,3,4]thiadiazole (**3d**).* Yellow solid, yield: 2.62 g, 78.0%; m.p:177–178 °C; FTIR (ATR, cm^{-1}): 3042, 2934, 1540, 1461, 1234, 1023, 830, 671; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.43 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 6.96 (d, J = 8.8 Hz, 2H, ArH),7.29 (d, J = 22.82 Hz, 2H, Ar–H), 7.75–7.78 (m, 4H, Ar–H), 7.94 (s, imidazole-H, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 21.57, 55.34, 108.35, 114.17, 126.37, 126.61, 126.72, 127.60, 129.95, 142.18, 144.97, 146.39, 159.26, 161.28; **ESI-MS** m/z 322.0 [M+H]+; calculated for $C_{18}H_{15}N_3OS$; C, 67.27; H, 4.70; N, 13.07; S, 9.98. Found: C, 67.15; H, 4.75; N, 13.10; S, 10.01.

*4.2.3.5. 6-(4-Chlorophenyl)-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole (**3e**).* Off white solid, yield: 2.72 g, 80%; m.p: 234–235 °C; FTIR (ATR, cm^{-1}): 3068, 2924, 1592, 1511, 1474, 840, 729, 670; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.45 (s, CH_3), 7.38 (m, 2H, Ar–H), 7.74 (dd, J = 2.0, 6.8 Hz, 2H, Ar–H), 7.96 (s, 1H, imidazole-H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 17.82, 102.30, 126.30, 128.78, 128.87, 129.21, 132.48, 133.10, 141.21, 144.89, 158.54; **ESI-MS** m/z 326.0 [M+H]+; calculated for $C_{17}H_{12}ClN_3S$; C, 62.67; H, 3.71; N, 12.90; S, 9.84. Found: C, 62.65; H, 3.75; N, 12.85; S, 9.86.

*4.2.3.6. 6-(4-Fluorophenyl)-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole (**3f**).* White solid, yield: 2.55 g, 79.0%; m.p: 228–230 °C; FTIR (ATR, cm^{-1}): 3067, 2924, 1539, 1464, 1062, 827, 671; **^{1}H NMR** (400 MHz,

$CDCl_3$) δ (ppm): 2.42 (s, 3H, CH_3),7.431 (d, J = 8.4 Hz, 2H, Ar–H), 7.58–7.60 (m, 2H, Ar–H), 7.95 (d, J = 7.9 Hz, Ar–H), 8.12–8.15 (m, 2H, Ar–H),7.98 (s, 1H, imidazole-H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 21.57, 109.02, 115.61, 115.82, 126.72, 126.79, 126.87, 127.42, 130.02, 142.46, 142.49, 145.20, 161.25; **ESI-MS** *m/z* 310.1 [M+H]+; calculated for $C_{17}H_{12}FN_3S$; C, 66.00; H, 3.91; N, 13.58; S, 10.36. Found: C, 66.12; H, 3.95; N, 13.54; S, 10.41.

*4.2.3.7. 2-(4-Fluorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazole (**3g**).* Light yellow solid, yield: 2.47 g, 75%; m.p: 203–204 °C; FTIR (ATR, cm^{-1}): 3063, 2935, 1539, 1463, 1243, 1066, 1022, 828, 671; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 3.81 (s, 3H, OCH_3), 7.22–7.30 (m, 4H, ArH), 7.64–7.68 (m, 2H, ArH), 7.85 (d, J = 7.4 Hz, 2H, Ar–H), 7.92 (s, imidazole-H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 54.78, 109.77, 115.61, 125.65, 126.38, 128.45, 130.97, 131.15, 132.80, 145.33, 146.21, 157.28, 168.10; **ESI-MS** *m/z* 326.10 [M+H]+; calculated for $C_{17}H_{12}FN_3OS$; C, 62.76; H, 3.72; N, 12.91; S, 9.86. Found: C, 62.66; H, 3.75; N, 12.94; S, 9.81.

*4.2.3.8. 2-(4-Chlorophenyl)-6-p-tolylimidazo[2,1-b][1,3,4]thiadiazole (**3h**).* Yellow solid, yield: 2.40 g,78.2%; m.p: 239–240 °C; FTIR (ATR, cm^{-1}): 3087, 2919, 1593, 1513, 1460, 828, 761, 668; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.34 (s, 3H, CH_3), 7.21 (d, J = 7.6 Hz, 2H, Ar–H), 7.30 (m, 2H, Ar–H), 7.70–7.66 (m, 4H, Ar–H), 7.94 (s, imidazole-H, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 21.26, 108.78, 124.89, 129.42, 128.89, 130.56, 131.14, 136.61, 137.27, 138.42, 145.54, 146.27, 160.21; **ESI-MS** (*m/z*) 326.04 (M + H)+; calculated for $C_{17}H_{12}ClN_3S$; C, 62.67; H, 3.71; N, 12.90; S, 9.84. Found: C, 62.66; H, 3.73; N, 12.84; S, 9.83.

*4.2.3.9. 2-(4-Chlorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazole **(3i)**.* Yellow solid, yield: 2.54 g,79.0%; m.p: 159–160 °C; FTIR (ATR, cm^{-1}): 3067, 2934, 1539, 1462, 1239, 1023, 828, 758, 671; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 3.81 (s, 3H, CH_3), 7.23–7.33 (m, 4H, Ar–H), 7.66–7.75 (m, 2H, Ar–H),7.96 (d, J = 7.9 Hz, 2H, Ar–H),7.93 (s, imidazole-H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 54.80, 108.77, 125.75, 128.42, 128.94, 129.10, 130.61, 131.85, 132.78, 136.56, 143.65, 150.15, 159.40; **ESI-MS** (*m/z*) 342.0 (M + H)+; calculated for $C_{17}H_{12}ClN_3OS$; C, 59.73; H, 3.54; N, 12.29; S, 9.38. Found: C, 59.76; H, 3.54; N, 12.32; S, 9.33.

*4.2.3.10. 2-(4-Chlorophenyl)-6-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazole **(3j)**.* White solid, yield: 2.52 g, 81.2%; m.p: 225–226 °C; FTIR (ATR, cm^{-1}): 3066, 2925, 1594, 1503, 1474, 1087, 839, 729, 670; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 7.45 (d, J = 8.3 Hz, 2H, Ar–H), 7.66–7.70 (m, 4H, Ar–H), 8.09 (d, J = 8.0 Hz, 2H, Ar–H), 7.96 (1H, s, imidazole-H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 108.26, 115.34, 118.21, 128.56, 130.10, 131.21, 137.21, 149.45, 154.33, 161.45, 162.44, 164.21; **ESI-MS** (*m/z*) 330.0 (M + H)+; calculated for $C_{16}H_9ClFN_3S$; C, 58.27; H, 2.75; N, 12.74; S, 9.72. Found: C, 58.26; H, 2.76; N, 12.72; S, 9.73.

*4.2.3.11. 2-(2-Chlorophenyl) -6- (4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazole (**3k**).* Off white solid, yield: 2.30 g, 75.0%; m.p: 213–214 °C; FTIR (ATR, cm^{-1}): 3068, 2921, 1594, 1503, 1476, 1245, 1024, 840, 752, 670; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 3.81 (s, 3H, OCH_3), 7.02 (d, J = 8.3 Hz, 2H, Ar–H),7.62 (d, J = 6.6 Hz, 1H, Ar–H), 7.67 (s, 1H, Ar–H), 7.71 (d, J = 7.5 Hz, 1H, Ar–H),7.84 (d, J = 8.7 Hz, 1H, Ar–H), 8.67 (d, J = 8.3 Hz, 2H, Ar–H), 7.97 (s, 1H, imidazole-H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 54.80, 108.77, 115.21, 125.75, 128.42, 128.94, 129.10, 130.61, 131.85, 132.51, 132.82, 136.46, 143.65, 150.25, 160.40; **ESI-MS** (*m/z*) 342.0 (M + H)+; calculated for $C_{17}H_{13}N_3OS$; C, 59.73; H, 3.54; N, 12.29; S, 9.38. Found: C, 59.69; H, 3.56; N, 12.26; S, 9.40.

*4.2.3.12. 6-(4-Methoxyphenyl)-2-phenylimidazo[2,1-b][1,3,4]thiadiazole (**3l**).* White solid, yield: 2.84 g, 82.0%; m.p: 219–220 °C; FTIR (ATR, cm^{-1}): 3066, 2924, 1593, 1503, 1465, 1244, 1028, 840, 668; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 3.79 (s, 3H, OCH_3), 7.02 (d, J = 8.3 Hz, 2H, Ar–H), 7.32–7.42 (m, 3H, Ar–H), 7.43–7.49 (m, 2H), 7.61 (d, J = 8.8 Hz, 2H) 7.87 (s, 1H, imidazole-H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 54.60, 108.27, 124.65, 128.22, 128.84, 129.10, 130.61, 131.21, 131.75, 132.78, 142.55, 150.15, 158.40; **ESI-MS** (*m/z*) 308.10 (M + H)+; calculated for $C_{17}H_{13}N_3OS$; C, 66.43; H, 4.26; N, 13.67; S, 10.43. Found: C, 66.45; H, 4.24; N, 13.66; S, 10.42.

*4.2.3.13. 6-(4-Fluorophenyl)-2-methylimidazo[2,1-b][1,3,4]thiadiazole (**3m**).* Light yellow solid, yield: 2.56 g, 79.0%; m.p:179–180 °C; FTIR (ATR, cm^{-1}): 3068, 2926, 1597, 1538, 1470, 1064, 834, 670; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.73 (s, 3H),7.38 (m, 2H, Ar–H),7.741 (d, J = 7.6 Hz, 2H, Ar–H), 7.96 (s, 1H, H-5 imidazole); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 17.86, 102.29, 126.24, 128.87, 132.48, 133.09, 144.93, 145.86, 159.59; **ESI-MS** (*m/z*) 234.1 (M + H)+; calculated for $C_{11}H_8FN_3S$; C, 56.64; H, 3.46; N, 18.01; S, 13.75. Found: C, 56.66; H, 3.44; N, 18.06; S, 13.72.

*4.2.4. General procedure for the preparation of 2-subsituted-6-arylimidazo [2,1-b][1,3,4]-thiadiazole-5-carbaldehydes (**4 a-m**)*

*4.2.4.1. 6-(4-Chlorophenyl)-2-methylimidazo [2,1-b][1,3,4]thiadiazole-5-carbaldehyde(**4a**).* Vilsmeier-Haack salt was synthesized by adding $POCl_3$ (1.49 mL, 16.06 mmol) drop-wise to a dry RB containing DMF (1.23 mL, 16.06 mmol) maintaining temperature at 0–5 °C under N_2 atmosphere. Later, a solution of **3a** (2.0 g, 8.032 mmol) in 20 mL of DMF was added to the resulting complex at a stretch. The resulting solution was stirred at 60 °C for about 6 h. The container was cooled to room temperature and quenched to ice cold water while stirring. The solid obtained was filtered, washed with excess of water and then purified by column chromatographic technique using ethyl acetate and hexane (3:7) system to give compound **4a** as white solid. Yield:1.9 g, 85%, m.p: 174–175 °C; FTIR (ATR, cm^{-1}): 3066, 2924, 1679, 1592, 1504, 1479, 843, 728, 671; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.85 (3H, CH_3), 7.48 (dd, J = 1.7, 6.6 Hz, 2H, Ar–H),7.82 (d, J = 8.3 Hz, 2H, Ar–H),10.35 (s, CHO, 1H), **^{13}CNMR** (100 MHz, $CDCl_3$) δ(ppm): 18.09, 124.04, 129.09, 130.28, 130.76, 136.05, 151.20, 154.51, 162.28, 177.18; **ESI-MS** *m/z* 277.9 [M+H]+;. calculated for $C_{12}H_8ClN_3OS$; C, 51.90; H, 2.90; N, 15.13; S, 11.55. Found: C, 51.86; H, 2.88; N, 15.15; S, 11.44.

Compounds **4b-m** were synthesized by following the above procedure and structural characterization data for the compounds are given below.

*4.2.4.2. 2-Methyl-6-(4-methylphenyl)imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (**4b**).* Light yellow solid, yield: 1.84 g, 82.2%; m.p:145–146 °C; FTIR (ATR, cm^{-1}): 3068, 2924, 1678, 1592, 1504, 1478, 842, 670; **^{1}H NMR** (400 MHz, $CDCl_3$) δ(ppm): 2.48(s, CH_3), 2.84 (s, CH_3), 7.28 (d, J = 7.9 Hz, 2H, Ar–H),7.71 (d, J = 7.9 Hz, 2H, Ar–H) 10.00 (s, CHO, 1H),**^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 18.08, 21.41, 123.98, 129.06, 129.44, 129.63, 140.10, 151.45, 156.76, 161.85, 177.61; **ESI-MS**: *m/z* 257.9 [M+H]+; calculated for $C_{13}H_{11}N_3OS$; C, 60.68; H, 4.31; N, 16.33; S, 12.46. Found: C, 60.66; H, 4.31; N, 16.35; S, 12.44.

*4.2.4.3. 6-(4-Methoxyphenyl)-2-methylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (**4c**).* Off white solid, yield: 1.89 g, 85%; m.p:161–162 °C; FTIR (ATR, cm^{-1}): 3044, 2935, 1679, 1540, 1462, 1238, 1024, 830, 671; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.84 (s,

3H, CH_3), 3.88 (s, 3H, OCH_3), 7.02–7.04 (m, 2H, Ar–H),7.7–7.8 (m, 2H, Ar–H), 9.99 (s, 1H, CHO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 17.82, 55.60, 109.97, 125.95, 128.62, 131.25, 133.1, 145.83, 146.60, 159.70, 177.41; **ESI-MS**: *m/z* 274.0 [M+H]+; calculated for $C_{13}H_{11}N_3O_2S$; C, 57.13; H, 4.06; N, 15.37; S, 11.73. Found: C, 57.16; H, 4.01; N, 15.35; S, 11.74.

*4.2.4.4. 6-(4-Methoxyphenyl)-2-(4-methylphenyl)imidazo[2,1-b][1,3,4]thiadiazole-5- carbaldehyde (**4d**).* Light yellow solid, yield: 1.73 g, 80%; m.p: 195–196 °C; FTIR (ATR, cm^{-1}): 3043, 2935, 1679, 1540, 1462, 1238, 1024, 830, 671; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.45 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 7.03–7.05 (m, 2H, Ar–H), 7.33 (d, J = 7.9 Hz, 2H, Ar–H),7.85–7.91 (m, 4H, Ar–H), 10.10 (s, 1H, CHO), **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 21.65, 55.44, 114.28, 123.71, 124.91, 126.82, 127.14, 130.06, 130.55, 143.06, 150.45, 156.02, 161.10, 163.85, 177.44; **ESI-MS**: *m/z* [M+H]+ 350.0; calculated for $C_{19}H_{15}N_3O_2S$; C, 65.31; H, 4.33; N, 12.03; S, 9.18. Found: C, 65.28; H, 4.32; N, 12.05; S, 9.20.

*4.2.4.5. 6-(4-Chlorophenyl)-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (**4e**).* Light brown solid, yield: 1.69 g, 78%; m.p: 209–210 °C; FTIR (ATR, cm^{-1}): 3068, 2924, 1679, 1592, 1504, 1476, 840, 729, 669; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.48 (s, 3H, CH_3),7.39 (m, 2H, Ar–H), 7.05–7.08 (m, 2H, Ar–H), 7.50 (d, J = 7.5 Hz, 2H, Ar–H),7.75 (d, J = 8.72 Hz 2H, Ar–H),10.18 (s, 1H,CHO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 17.86, 115.21, 116.21, 127.30, 128.18, 128.97, 129.21, 131.15, 132.68, 134.10, 142.31, 145.39, 159.54, 162.51, 177.83; **ESI-MS** *m/z* 354.0 [M+H]+; calculated for $C_{18}H_{12}ClN_3OS$; C, 61.10; H, 3.42; N, 11.88; S, 9.06. Found: C, 61.12; H, 3.42; N, 11.90; S, 9.09.

*4.2.4.6. 6-(4-Fluorophenyl)-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (**4f**).* White solid, yield: 1.78 g, 82.0%; m.p: 193–194 °C; FTIR (ATR, cm^{-1}): 3066, 2925, 1678, 1539, 1462, 1061, 827, 671; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.46 (s, 3H, CH_3), 7.53 (m, 2H, Ar–H), 7.59 (d, J = 8.4 Hz, 2H, ArH), 7.98 (d, J = 7.8 Hz, 2H, ArH), 8.14–8.17 (m, 2H, ArH), 10.25 (s, CHO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 21.64, 115.76, 115.98, 126.70, 127.15, 130.11, 130.97, 131.05, 143.26, 150.11, 154.25, 162.61, 164.34, 165.10, 177.23; **ESI-MS** *m/z* 338.1 [M+H]+; calculated for $C_{18}H_{12}FN_3OS$; C, 64.08; H, 3.59; N, 12.46; S, 9.50. Found: C, 64.12; H, 3.52; N, 12.45; S, 9.56.

*4.2.4.7. 2-(4-Fluorophenyl)-6-(4-methoxyphenyl) imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (**4g**).* Light yellow solid, yield: 1.78 g, 82.0%; m.p: 189–190 °C; FTIR (ATR, cm^{-1}): 3043, 2935, 1677, 1539, 1463, 1244, 1062, 1023, 828, 671; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 3.82 (s, 3H, OCH_3), 7.26–7.30 (m, 4H, ArH), 7.66–7.70 (m, 2H, ArH), 7.87 (d, J = 7.9 Hz, 2H, Ar–H), 10.21 (s, 1H, CHO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 54.88, 115.71, 116.21, 125.95, 126.38, 129.45, 130.97, 131.75, 132.98, 145.33, 146.31, 157.28, 162.61, 168.10, 177.34; **ESI-MS** *m/z* 354.0 [M+H]+; calculated for $C_{18}H_{12}FN_3O_2S$; C, 61.18; H, 3.42; N, 11.89; S, 9.07. Found: C, 61.22; H, 3.39; N, 11.89; S, 9.06.

*4.2.4.8. 2-(4-Chlorophenyl)-6-p-tolylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (**4h**).* Light yellow solid, yield: 1.69 g, 78.0%; m.p: 207–208 °C; FTIR (ATR, cm^{-1}): 3087, 2918, 1678, 1592, 1512, 1459, 828, 760, 668; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.35 (s, 3H, CH_3), 7.21 (m, 2H, Ar–H), 7.32 (m, 2H, Ar–H), 7.68–7.72 (m, 4H, Ar–H), 10.10 (s, 1H, CHO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 21.36, 116.26, 124.89, 128.62, 128.99, 130.76, 131.24, 136.71, 137.27, 138.62, 145.74, 147.27, 160.21, 177.21; **ESI-MS** (*m/z*) 354.0 (M + H)+; calculated for $C_{18}H_{12}ClN_3OS$; C, 61.10; H, 3.42; N, 11.88; S, 9.06. Found: C, 61.25; H, 3.42; N, 11.91; S, 9.16.

*4.2.4.9. 2-(4-Chlorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (**4i**).* White solid, yield:1.71 g, 79.2%; m.p: 196–197 °C; FTIR (ATR, cm^{-1}): 3043, 2935, 1679, 1539, 1462, 1239, 1023, 828, 758, 671; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 3.821 (s, 3H, CH_3), 7.25–7.34 (m, 4H, Ar–H), 7.68–7.78 (m, 2H, Ar–H), 7.98 (d, J = 7.4 Hz, 2H, Ar–H), 10.22 (s, 1H, CHO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 54.84, 116.25, 126.15, 128.52, 128.84, 129.25, 130.81, 131.95, 133.81, 136.66, 143.15, 150.25, 160.21, 177.21; **ESI-MS** (*m/z*) 370.1 (M + H)+; calculated for $C_{18}H_{12}ClN_3O_2S$; C, 58.46; H, 3.27; N, 11.36; S, 8.67. Found: C, 58.45; H, 3.25; N, 11.35; S, 8.66.

*4.2.4.10. 2-(4-Chlorophenyl)-6-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (**4j**).* Off white solid, yield: 1.66 g, 76.6%; m.p: 189–190 °C; FTIR (ATR, cm^{-1}): 3068, 2921, 1676, 1594, 1503, 1476, 1088, 839, 729, 668; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 7.48 (d, J = 8.6 Hz, 2H, Ar–H), 7.72–7.68 (m, 4H, Ar–H), 8.12 (d, J = 8.3 Hz, 2H, Ar–H), 10.20 (s, 1H, CHO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm):115.84, 116.06, 128.39, 129.80, 131.00, 131.09, 138.74, 150.15, 154.73, 162.67, 162.82, 165.17, 177.18; **ESI-MS** (*m/z*) 358.1 (M + H)+; calculated for $C_{17}H_9ClFN_3OS$; C, 57.07; H, 2.54; N, 11.74; S, 8.96. Found: C, 57.15; H, 2.52; N, 11.71; S, 8.96.

*4.2.4.11. 2-(2-Chlorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (**4k**).* Off white solid, yield: 1.69 g, 78.2%; m.p: 178–180 °C; FTIR (ATR, cm^{-1}): 3068, 2921, 1676, 1594, 1503, 1244, 1028, 1476, 839, 752, 671; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm):3.81 (s, 3H, OCH_3),7.026 (d, J = 8.7 Hz, 2H, Ar–H), 7.623 (d, J = 6.6 Hz, 1H, Ar–H),7.67 (s, 1H, Ar–H) 7.72 (d, J = 8.4 Hz, 1H, Ar–H),7.84 (d, J = 8.5 Hz, 1H, Ar–H), 8.70 (d, J = 8.3 Hz, 2H, Ar–H), 10.28 (s, 1H, CHO); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 54.80, 115.21, 116.81, 126.35, 128.42, 128.94, 129.10, 130.81, 131.85, 132.51, 133.12, 137.16, 143.85, 151.25, 162.40, 177.38; **ESI-MS** (*m/z*) 370.1 (M + H)+; calculated for $C_{18}H_{12}ClN_3O_2S$; C, 58.46; H, 3.27; N, 11.36; S, 8.67. Found: C, 58.44; H, 3.30; N, 11.41; S, 8.68.

*4.2.4.12. 6-(4-Methoxyphenyl)-2-phenylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (**4l**).* White solid, yield: 1.63 g, 75.0%; m.p: 179–180 °C; FTIR (ATR, cm^{-1}): 3067, 2925, 1678, 1594, 1503, 1464, 1244, 1028, 1476, 839, 668; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 3.81 (s, 3H, OCH_3), 7.10 (d, J = 8.3 Hz, 2H, Ar–H), 7.32–7.42 (m, 3H, Ar–H), 7.45–7.52 (m, 2H, ArH), 7.63 (d, J = 8.8 Hz, 2H),10.18 (s, 1H, CHO); **^{13}CNMR** (100 MHz, $CDCl_3$) δ(ppm): **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 54.60, 116.21, 125.15, 127.32, 128.94, 129.10, 130.81, 131.31, 131.95, 133.16, 142.55, 150.15, 160.10, 177.31; **ESI-MS** (*m/z*) 336.10 (M + H)+; calculated for $C_{18}H_{13}N_3O_2S$; C, 64.46; H, 3.91; N, 12.53; S, 9.56. Found: C, 64.50; H, 3.90; N, 12.46; S, 9.58.

*4.2.4.13. 6-(4-Fluorophenyl)-2-methylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (**4m**).* Light yellow solid, yield: 1.81 g, 81.1%; m.p:169–170 °C; FTIR (ATR, cm^{-1}): 3068, 2926, 1678, 1597, 1538, 1470, 1064, 834, 670; **^{1}H NMR** (400 MHz, $CDCl_3$) δ(ppm): 2.76 (s, 3H, CH_3), 7.40 (m, 2H, Ar–H),7.761 (d, J = 8.6 Hz, 2H, Ar–H),10.25 (s, CHO, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 17.82, 115.62, 116.09, 128.32, 130.17, 130.94, 146.20, 159.23, 163.10, 177.21; **ESI-MS** *m/z* 262.0 [M+H]+; calculated for $C_{12}H_8FN_3OS$; C, 55.16; H, 3.09; N, 16.08; S, 12.27. Found: C, 55.20; H, 3.10; N, 16.06; S, 12.21.

*4.2.5. General procedure and spectral data for the synthesis of target molecules **5a-ac***

*4.2.5.1. 2-(6-(4-Methoxy phenyl)-2-p-tolyl imidazo[2,1-b][1,3,4] thiadiazol-5-yl)-1H-benzo[d]imidazole (**5a**).* To a stirred solution of o-phenylenediamine (0.046 g, 0.429 mmol) in DMF (3 mL) under nitrogen atmosphere, compound **4d** (0.15 g, 0.0429 mmol) was added and stirred for 5 min after that sodium met bisulfite (0.122 g, 0.644 mmol) was added. The reaction mixture was then heated to 120 °C and maintained same temperature for 3 h. Reaction was monitored with TLC and once starting material was completely consumed the solvent was removed by under vacuum. The solid obtained was dissolved in ethyl acetate (10 mL) and washed with water (10 mL). The aqueous layer was extracted with ethyl acetate (3 × 10 mL). The organic extracts were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The crude product was purified by flash column chromatography eluting with methanol/chloroform (1:9) to give **5a** as Off white solid. FTIR (ATR, cm^{-1}): 3352, 3077, 2938, 1603, 1563, 1466, 1244, 1026, 827, 691; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 2.41 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 7.30 (dd, J = 3.0, 6.1 Hz, 2H, Ar–H), 7.43 (d, J = 8.3 Hz, 2H, Ar–H), 7.48 (d, J = 8.5 Hz, 2H, Ar–H), 7.70–7.72 (m, 2H, Ar–H), 7.96 (d, J = 8.3 Hz, 2H, Ar–H), 8.12 (d, J = 8.6 Hz, 2H, Ar–H), 11.6–14.2 (s, 1H, NH); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 21.60, 55.52, 115.70, 123.21, 127.02, 128.80, 129.86, 132.77, 133.26, 143.23, 144.43, 145.83, 160.7, 163.21; **ESI-MS** (m/z) 438.1 (M + H)+; calculated for $C_{25}H_{19}N_5OS$; C, 68.63; H, 4.38; N, 16.01; S, 7.33. Found: C, 68.54; H, 4.40; N, 16.10; S, 7.32.

Compounds **5b-ac** were synthesized by following the above procedure by reacting 0.15 g of imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (**4a-m**) with same equivalent of corresponding substituted o-phenylenediamine.

*4.2.5.2. 2-(6-(4-Methoxyphenyl)-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-5-nitro -1H-benzo[d]imidazole(**5b**).* Yellow solid. FTIR (ATR, cm^{-1}): 3348, 3073, 2934, 1604, 1517, 1457, 1244, 1024, 836, 681; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 2.42 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 7.01 (d, J = 8.5 Hz, 2H, Ar–H), 7.45 (2H, d, J = 7.8 Hz, ArH), 7.86 (d, J = 8.9 Hz, 1H, Ar–H),8.00–8.20 (m, 4H, Ar–H), 8.612 (d, J = 1.8 Hz, 1H, Ar–H), 13.10–13.29 (s, 1H, NH); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 21.69, 55.82, 114.24, 115.12, 123.39, 126.51, 127.19, 127.28, 127.46, 130.74, 142.90, 143.08, 143.63, 146.90, 146.09, 159.72, 162.54; **ESI-MS** (m/z) 483.1 (M + H)+; calculated for $C_{25}H_{18}N_6O_3S$; C, 62.23; H, 3.76; N, 17.42; S, 6.65. Found: C, 62.25; H, 3.78; N, 17.40; S, 6.67.

*4.2.5.3. 5-Chloro-2-(6-(4-methoxyphenyl)-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1H-benzo[d]imidazole(**5c**).* Green solid. FTIR (ATR, cm^{-1}): 3353, 3044, 2935, 1540, 1462, 1238, 1024, 828, 751, 691; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 2.41 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 6.98 (d, J = 8.8 Hz, 2H, Ar–H), 7.30 (dd, J = 1.7, 8.3 Hz, 1H, Ar–H), 7.43 (d, J = 8.3 Hz, 2H, Ar–H), 7.71 (d, J = 8.8 Hz, 1H, Ar–H),7.76 (s, 1H, Ar–H), 7.96 (d, J = 8.3 Hz, 2H, Ar–H), 8.01 (d, J = 8.8 Hz, 2H, Ar–H), 12.4–13.6 (s, NH, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 21.59, 55.62, 114.14, 114.22, 123.19, 126.31, 127.09, 127.28, 127.46, 129.54, 130.54, 143.08, 143.53, 145.60, 146.09, 159.82, 162.64; **ESI-MS** (m/z) 472.0 (M + H)+; calculated for $C_{25}H_{18}ClN_5OS$; C, 63.62; H, 3.84; N, 14.84; S,6.79. Found: C, 63.52; H, 3.82; N, 14.86; S, 6.74.

*4.2.5.4. 2-(2-(4-Chlorophenyl)-6-p-tolylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1H-benzo[d]imidazole (**5d**).* Light yellow solid. FTIR (ATR, cm^{-1}): 3353, 3072, 2936, 1596, 1518, 1459, 828, 756, 693; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 2.34 (s, 3H, CH_3),7.21 (d, J = 7.6 Hz, 2H, Ar–H),7.30 (m, 2H, Ar–H), 7.70–7.68 (m, 4H, Ar–H), 7.908 (d, J = 7.6 Hz, 2H, Ar–H), 8.06 (d, J = 8.0 Hz, 2H, Ar–H), 12.88 (1H, br, NH), **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 21.33, 115.11, 123.06, 127.96, 128.70, 129.29, 129.34, 130.12, 131.04, 137.35, 138.11, 141.78, 145.66, 146.02, 161.44; **ESI-MS** (m/z) 442.0 (M + H)+; calculated for $C_{24}H_{16}ClN_5S$; C, 65.23; H, 3.65; N, 15.85; S,7.26. Found: C, 65.25; H, 3.67; N, 15.83; S, 7.24.

*4.2.5.5. 2-(2-(4-Chlorophenyl)-6-p-tolylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-5-nitro-1H-benzo[d]imidazole (**5e**).* Yellow solid. FTIR (ATR, cm^{-1}): 3348, 3072, 2934, 1596, 1517, 1462, 827, 754, 682; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 2.34 (3H, s, CH_3), 7.21 (m, 2H, ArH), 7.30 (m, 2H, ArH), 7.70–7.66 (m, 4H, ArH),8.18 (1H, d, J = 8.5 Hz), 8.26 (d, J = 7.6 Hz, 1H, ArH), 8.55 (s, 1H, ArH), 12.91 (s, 1H, NH); **^{13}C NMR** (100 MHz, DMSO-d_6): 21.33, 110.5, 116.2, 118.4, 128.70, 129.29, 130.34, 130.72, 131.04, 137.35, 138.11, 139.8, 141.78, 142.8, 145.86, 145.2, 146.12, 161.54; **ESI-MS** (m/z) 487.0 (M + H)+; calculated for $C_{24}H_{15}ClN_6O_2S$; C, 59.20; H, 3.10; N, 17.26; S,6.59. Found: C, 69.10; H, 3.10; N, 17.20; S, 6.48.

*4.2.5.6. 5-Chloro-2-(2-(4-chlorophenyl)-6-p-tolylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1H-benzo[d]imidazole (**5f**).* Green solid. FTIR (ATR, cm^{-1}): 3351, 3084, 2936, 1598, 1463, 827, 756, 693; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 2.34 (s, 3H, CH_3), 7.21 (m, 2H, 4H), 7.27 (d, J = 8.5 Hz, 1H, Ar–H), 7.30 (m, 2H, Ar–H), 7.64 (d, J = 7.4 Hz, 1H, Ar–H), 7.66–7.70 (m, 4H, Ar–H), 7.72 (s, 1H, Ar–H),12.91 (s, 1H, NH); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 21.33, 115.8, 116.2, 124.1, 128.70, 129.20,129.29,130.34, 130.72, 131.04, 137.35, 138.11, 140.3, 141.78, 145.86, 145.2, 146.12, 161.54;**ESI-MS** (m/z) 476.0 (M + H)+; calculated for $C_{24}H_{15}Cl_2N_5S$; C, 60.51; H, 3.17; N, 14.88; S,6.73. Found: C, 60.55; H, 3.18; N, 14.90; S, 6.70.

*4.2.5.7. 2-(2-(2-Chlorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-5-nitro-1H-benzo[d]imidazole (**5g**).* Light yellow solid. FTIR (ATR, cm^{-1}): 3351, 3068, 2921, 1594, 1517, 1503, 1463, 1244, 1028, 838, 752, 681; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 3.81 (s, 3H, OCH_3), 7.02 (d, J = 8.3 Hz, 2H, Ar–H), 7.62 (d, J = 6.6 Hz, 1H, Ar–H), 7.67 (s, 1H, Ar–H), 7.70 (d, J = 7.5 Hz, 1H, Ar–H),7.84 (d, J = 8.7 Hz, 1H, Ar–H), 8.07 (d, J = 8.3 Hz, 2H, Ar–H), 8.18 (d, J = 8.4 Hz, 1H, Ar–H), 8.268 (d, J = 7.5 Hz, 1H, Ar–H), 8.589 (s, 1H, Ar–H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 159.72, 18.17, 55.82, 114.24, 115.12,127.0, 129.0, 130.6, 132.5, 131.6, 134.02, 142.90, 143.08, 143.63, 146.90, 146.09, 159.72, 159.72; **ESI-MS** (m/z) 503.0 (M + H)+; calculated for $C_{24}H_{15}ClN_6O_3S$; C, 57.32; H, 3.01; N, 16.71; S,6.38. Found: C, 57.34; H, 3.08; N, 16.75; S, 6.35.

*4.2.5.8. 2-(6-(4-Methoxyphenyl)-2-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-5-nitro-1H-benzo[d]imidazole (**5h**).* Light yellow solid. FTIR (ATR, cm^{-1}): 3351, 3078, 2935, 1563, 1519, 1466, 1248, 1028, 833, 683; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 3.78 (s, 3H, OCH_3), 7.104 (d, J = 8.8 Hz, 2H, Ar–H), 7.34–7.45 (m, 3H, Ar–H), 7.58–7.60 (m, 2H, Ar–H), 7.913 (d, J = 8.4 Hz, 2H, Ar–H), 8.18 (d, J = 8.5 Hz, 1H, Ar–H), 8.26 (d, J = 7.6 Hz, 1H, Ar–H),8.55 (s, 1H, Ar–H),12.91 (s, 1H, NH); **^{13}C NMR** (100 MHz, CDCl3) δ (ppm): 55.59, 114.62, 115.44, 115.65, 123.18, 126.37, 129.43, 126.91, 132.45, 144.12, 142.70, 146.20, 160.74, 162.32; **ESI-MS** (m/z) 469.1 (M + H)+; calculated for $C_{14}H_{16}N_6O_3S$; C, 61.53; H, 3.44; N, 17.94; S,6.84. Found: C, 61.60; H, 3.45; N, 17.96; S, 6.85.

*4.2.5.9. 2-[6-(4-Fluorophenyl)-2-(4-methylphenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl]-1H-benzimidazole (**5i**).* Yellow solid. FTIR (ATR, cm^{-1}): 3353, 3077, 2934, 1602, 1538, 1464, 1061, 827, 688; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 2.50 (s, 3H, CH_3), 7.23–7.33 (m,

4H, Ar–H), 7.431 (d, J = 8.4 Hz, 2H, Ar–H), 7.66–7.76 (m, 2H, Ar–H),7.96 (d, J = 7.9 Hz,2H,Ar–H),8.12–8.15 (m, 2H, Ar–H), 12.82 (s, 1H, NH); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 21.59, 115.70, 123.55, 127.05, 127.50, 130.12, 130.47, 141.81, 143.13, 145.58, 161.25, 162.85, 163.69; **ESI-MS** (m/z) 426.0 (M + H)+; calculated for $C_{24}H_{16}FN_5S$; C, 67.75; H, 3.79; N, 16.46; S,7.54. Found: C, 67.70; H, 3.81; N, 16.50; S, 7.52.

*4.2.5.10. 2-(6-(4-Fluorophenyl)-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-5-nitro-1H-benzo[d]imidazole (**5j**).* Light yellow solid. FTIR (ATR, cm^{-1}): 3351, 3068, 2926, 1597, 1538, 1475, 1064, 834, 687; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 2.42 (s, 3H, CH_3), 7.29 (t, J = 17.5 Hz, 2H, Ar–H), 7.39–7.46 (m, 2H, Ar–H), 7.83–07.91 (m, 2H, Ar–H), 8.301 (d, J = 10.1 Hz, 2H, Ar–H), 8.20 (m, 2H, Ar–H), 8.60 (s, 1H, Ar–H), 13.29 (s, 1H, NH); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 21.62, 114.58, 115.44, 115.65, 126.91, 127.02, 127.63, 130.21, 130.53, 130.95, 143.31, 161.45, 163.90; **ESI-MS** (m/z) 468.9 (M–H)+; calculated for $C_{24}H_{15}FN_6O_2S$; C, 61.27; H, 3.21; N, 17.86; S,6.82. Found: C, 61.25; H, 3.23; N, 17.82; S, 6.80.

*4.2.5.11. 2-[2-(4-Chlorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl]-1H-benzimidazole(**5k**).* Light yellow solid. FTIR (ATR, cm^{-1}): 3350, 3077, 2937, 1608, 1538, 1437, 1028, 826, 751, 689; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 3.783 (s, 3H, CH_3),6.983 (d, J = 8.8 Hz, 2H, Ar–H), 7.2–7.4 (m, 2H, Ar–H), 7.694 (d, J = 8.8 Hz, 4H, Ar–H), 8.02–8.10 (m, 4H, Ar–H), 12.77 (s, NH, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 55.63, 114.8, 126.35, 123.06, 128.73, 129.27, 129.41, 130.12, 137.30,141.78, 145.50, 145.66, 159.80, 161.18; **ESI-MS** (m/z) 458.0 (M + H)+; calculated for $C_{24}H_{16}ClN_5S$; C, 62.95; H, 3.52; N, 15.29; S,7.00. Found: C, 62.90; H, 3.53; N, 15.50; S, 6.90.

*4.2.5.12. 2-[2-(4-Chlorophenyl)-6-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl]-1H-benzimidazole (**5l**).* Light yellow solid. FTIR (ATR, cm^{-1}): 3351, 3068, 2936, 1616, 1537, 1467, 1068, 837, 748, 693; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 7.24–7.29 (m, 4H, Ar–H), 7.70 (d, J = 8.4 Hz, 4H, Ar–H), 8.10–8.17 (m, 4H, Ar–H), 12,83 (s, 1H, NH); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 115.50, 115.72, 123.12, 128.63, 129.33, 130.10, 130.20, 130.28, 137.43, 144.80, 145.71, 161.30, 161.68, 163.78; **ESI-MS** (m/z) 446.0 (M + H)+; calculated for $C_{23}H_{13}ClFN_5S$; C, 61.95; H, 2.94; N, 15.71; S,7.19. Found: C, 61.90; H, 2.96; N, 15.65; S, 7.20.

*4.2.5.13. 2-(2-(4-Fluorophenyl)-6- (4-methoxyphenyl) imidazo [2,1-b][1,3,4] thiadiazol-5-yl)-1H-benzo[d] imidazole (**5m**).* Off white solid. FTIR (ATR, cm^{-1}): 3349, 3073, 2937, 1611, 1540, 1467, 1244, 1061, 1028, 827, 686; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 3.79 (s, 3H, OCH_3),6.97 (d, J = 8.4 Hz, 2H, Ar–H), 7.3–7.5 (m, 2H, Ar–H), 7.69–7.72 (m, 4H, Ar–H), 8.04–8.08 (m, 4H, Ar–H), 12.76 (s, 1H, NH); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 55.73, 114.7, 126.45, 123.16, 127.63, 129.37, 129.21, 130.32, 142.98, 145.70,145.66, 159.80, 161.18, 165.20; **ESI-MS** (m/z) 442.1 (M + H)+; calculated for $C_{24}H_{16}CFN_5S$; C, 65.29; H, 3.65; N, 15.86; S,7.26. Found: C, 64.90; H, 3.68; N, 15.60; S, 7.20.

*4.2.5.14. 2-(2-(4-Fluorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-5-nitro-1H-benzo[d]imidazole (**5n**).* Yellow solid. FTIR (ATR, cm^{-1}): 3350, 3077, 2936, 1603, 1518, 1464, 1244, 1071, 1028, 831, 691; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 3.81 (s, 3H, OCH_3),7.02 (d, J = 8.8 Hz, 2H, Ar–H),7.46 (d, J = 7.4 Hz, 2H, Ar–H), 7.86 (d, J = 8.8 Hz, 2H, Ar–H), 8.00–8.20 (m, 4H, Ar–H), 8.61 (s, 1H, Ar–H), 13.10–13.293 (s, 1H, NH); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 55.73, 114.7, 115.32, 123.19, 126.45, 123.16, 127.63, 129.37, 129.21, 130.32, 134.31, 142.98, 145.70, 145.66, 159.80, 161.18, 165.20; **ESI-MS** (m/z) 487.1 (M + H) +; calculated for $C_{24}H_{15}FN_6O_3S$; C, 59.25; H, 3.11; N, 17.28; S, 6.59. Found: C, 59.22; H, 3.12; N, 17.26; S, 6.62.

*4.2.5.15. 5-Chloro-2-(2-(4-fluorophenyl)-6-(4-methoxy phenyl) imidazo [2,1-b][1,3,4] thiadiazol-5-yl)-1H-benzo[d]imidazole (**5o**).* Light brown solid. FTIR (ATR, cm^{-1}): 3351, 3078, 2937, 1601, 1538, 1463, 1068, 1024, 1028, 751, 686; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 3.84 (s, 3H, OCH_3), 6.99 (d, J = 8.4 Hz, 2H, Ar–H), 7.30 (dd, J = 1.7, 8.3 Hz, 1H, Ar–H), 7.43 (m, 2H, Ar–H),7.71 (d, J = 8.8 Hz, 1H, Ar–H),7.76 (s, 1H, Ar–H), 7.96 (d, J = 8.3 Hz, 2H, Ar–H), 8.01 (m, 2H, Ar–H), 12.4–13.6 (s, 1H, br); **^{13}C NMR** (100 MHz, DMSO-d_6): 55.42, 114.24, 114.32, 123.29, 126.61, 127.19, 127.56, 129.94, 131.44, 143.08, 143.53, 145.70, 146.09, 159.92, 162.64, 165.20; **ESI-MS** (m/z) 476.1 (M + H)+; calculated for $C_{24}H_{15}ClFN_5OS$; C, 60.57; H, 3.18; N, 14.72; S,6.74. Found: C, 60.48; H, 3.20; N, 14.68; S, 3.20.

*4.2.5.16. 2-(6-(4-Chlorophenyl)-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1H-benzo [d] imidazole (**5p**).* Light yellow solid. FTIR (ATR, cm^{-1}): 3348, 3068, 2924, 1592, 1534, 1476, 840, 727, 689; **^{1}H NMR** (400 MHz, DMSO-d_6) δ(ppm): 2.41 (s, 3H, CH_3), 7.30 (dd, J = 3.0, 6.1 Hz, 2H, Ar–H), 7.40 (d, J = 8.3 Hz, 2H, Ar–H),7.48 (d, J = 8.7 Hz, 2H, Ar–H), 7.70–7.72 (m, 2H, Ar–H), 7.96 (d, J = 8.3 Hz, 2H, Ar–H), 8.10 (d, J = 8.3 Hz, 2H, Ar–H), 11.6–14.2 (s, 1H, NH); **^{13}C NMR** (100 MHz, DMSO-d_6) δ(ppm): 21.60, 115.60, 123.21, 127.02, 127.55, 128.80, 129.76, 130.57, 132.77, 133.26, 143.23, 144.43, 145.83, 163.21; **ESI-MS** (m/z) 442.0 (M + H)+; calculated for $C_{24}H_{16}ClN_5S$; C, 65.23; H, 3.65; N, 18.44; S, 7.26. Found: C, 65.18; H, 3.68; N, 18.46; S, 7.28.

*4.2.5.17. 2-(6-(4-Chlorophenyl)-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-5-nitro-1H-benzo[d]imidazole (**5q**).* Yellow solid. FTIR (ATR, cm^{-1}): 3351, 3068, 2924, 1592, 1534, 1518, 1476, 837, 748, 686; **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 2.41 (s, 3H, CH_3),7.43 (d, J = 7.9 Hz, 2H, Ar–H), 7.49 (d, J = 8.3 Hz, 2H, Ar–H), 7.72 (d, J = 7.5 Hz, 1H, Ar–H), 7.98 (d, J = 7.9 Hz, 2H, Ar–H), 8.10 (d, J = 8.4 Hz, 2H, Ar–H), 8.26 (d, J = 7.5 Hz, 1H, Ar–H), 8.58 (s, 1H, Ar–H), 12.6–14.2 (s, 1H, NH); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 21.39, 112.64, 113.32, 123.19, 126.31, 127.29, 127.38, 127.86, 130.44, 134.31, 142.6, 143.18, 143.33, 145.30, 146.29, 160.32; **ESI-MS** (m/z) 487.0 (M + H)+; calculated for $C_{24}H_{15}ClN_6O_2S$; C, 59.20; H, 3.10; N, 17.26; S, 6.59. Found: C, 59.22; H, 3.12; N, 17.26; S, 6.60.

*4.2.5.18. 5-Chloro-2-(6-(4-chlorophenyl)-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1H-benzo[d]imidazole (**5r**).* Light yellow solid. FTIR (ATR, cm^{-1}): 3351, 3073, 2935, 1598, 1540, 1464, 827, 693; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 2.416 (s, 3H, CH_3),7.31 (d, J = 8.4 Hz, 1H, Ar–H), 7.43 (d, J = 7.9 Hz, 2H, Ar–H), 7.49 (d, J = 8.3 Hz, 2H, Ar–H), 7.72 (d, J = 7.9 Hz, 1H, Ar–H), 7.77 (s, 1H, Ar–H), 7.98 (d, J = 7.9 Hz, 2H, Ar–H), 8.10 (d, J = 8.4 Hz, 2H, Ar–H), 12.6–13.1 (s, 1H, NH); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 21.49, 112.14, 113.12, 123.09, 126.21, 127.19, 127.38, 127.56, 129.44, 130.44, 134.31, 143.08, 143.43, 145.50, 146.19, 160.82; **ESI-MS** (m/z) 476.0 (M + H)+; calculated for $C_{24}H_{15}ClN_6O_2S$; C, 59.20; H, 3.10; N, 17.26; S, 6.59. Found: C, 59.22; H, 3.12; N, 17.26; S, 6.60.

*4.2.5.19. 2-(6-(4-Methoxyphenyl)-2-methylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1H-benzo [d]imidazole (**5s**).* White solid. FTIR (ATR, cm^{-1}): 3273, 3071, 2965, 1592, 1494, 1437, 1248, 1028, 835, 691; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 2.78 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 6.94 (d, J = 8.8 Hz, 2H, Ar–H),7.25 (m, 2H, Ar–H),7.58 (d, J = 7 Hz, 1H, Ar–H), 7.70 (d, J = 7.4 Hz, 1H, Ar–H), 7.91 (d, J = 8.8 Hz,

2H, Ar–H),12.73 (s, 1H, NH) **^{13}C NMR** (100 MHz, DMSO-d_6): 18.07, 55.60, 114.19, 114.33, 119.69, 126.56, 129.10, 142.23, 144.99, 146.12, 159.61, 161.93; **ESI-MS** (*m*/*z*) 362.0 (M + H)+; calculated for $C_{19}H_{15}N_5OS$; C, 63.14; H, 4.18; N, 19.38; S,8.87. Found: C, 63.10; H, 4.20; N, 19.40; S, 8.85.

*4.2.5.20. 2-(6-(4-Methoxyphenyl)-2-methylimidazo[2,1-b][1,3,4] thiadiazol-5-yl)-5-nitro-1H-benzo[d]imidazole(**5t**).* Yellow solid. FTIR (ATR, cm^{-1}): 3273, 3068, 2965, 1604, 1518, 1497, 1436, 1246, 1024, 836, 687; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 2.78 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3),7.913 (d, J = 8.8 Hz, 2H, Ar–H),7.58–7.60 (m, 2H, Ar–H), 8.18 (d, J = 8.5 Hz, 1H, Ar–H), 8.26 (d, J = 7.6 Hz, 1H, Ar–H), 8.55 (s, 1H, Ar–H),12.91 (s, 1H, NH); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 18.22, 55.60, 114.52, 123.08, 127.37, 129.43, 132.35, 144.12, 142.8, 146.20, 160.7, 162.42; **ESI-MS** (*m*/*z*) 407.0 (M + H)+; calculated for $C_{19}H_{14}N_6O_3S$; C, 56.15; H, 3.47; N, 20.68; S,7.89. Found: C, 56.10; H, 3.45; N, 20.70; S, 7.85.

*4.2.5.21. 2-(6-(4-Fluorophenyl)-2-methylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1H-benzo [d] imidazole (**5u**).* White solid. FTIR (ATR, cm^{-1}): 3273, 3074, 2964, 1597, 1538, 1437, 1062, 834, 687; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 2.804 (s, 3H, CH_3),7.22 (d, 2H, ArH, J = 8.8 Hz), 7.26–7.28 (m, 2H, Ar–H), 7.67(dd, J = 5.6, 3.2 Hz, 2H, Ar–H), 8.04 (dd, J = 8.4, 6.0 Hz, 2H, Ar–H), 12.82 (s, 1H, NH); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 18.10, 115.09, 115.52, 115.74, 122.99, 129.87, 129.95, 130.52, 130.55, 141.87, 144.04, 146.42, 161.17, 163.61; **ESI-MS** (*m*/*z*) 350.08 (M + H)+; calculated for $C_{18}H_{12}FN_5S$; C, 61.88; H, 3.46; N, 20.04; S, 9.18. Found: C, 61.76; H, 3.48; N, 21.06; S, 9.16.

*4.2.5.22. 2-(6-(4-Fluorophenyl)-2-methyl imidazo [2,1-b][1,3,4]thiadiazol-5-yl)-5-nitro-1H-benzo[d]imidazole **(5v)**.* Yellow solid. FTIR (ATR, cm^{-1}): 3347, 3077, 2935, 1603, 1518, 1464, 1092, 831, 690; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 2.82 (s, 3H, CH_3),7.68 (dd, J = 2.0, 6.8 Hz, 2H, Ar–H), 7.70 (d, J = 7.6 Hz, 1H, Ar–H), 8.06 (dd, J = 3.2, 5.6 Hz, 2H, Ar–H),8.26 (d, J = 7.6 Hz, 1H, Ar–H), 8.58 (s, 1H, Ar–H), 12.89 (s, 1H, NH); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 18.15, 115.19, 123.19, 129.87, 129.97, 130.52, 138.55, 141.77, 144.04, 146.82, 161.27, 163.61; **ESI-MS** (*m*/*z*) 395.1 (M + H)+; calculated for $C_{18}H_{11}FN_6O_2S$; C, 54.82; H, 2.81; N, 21.31; S,8.13. Found: C, 54.80; H, 2.83; N, 21.32; S, 8.11.

*4.2.5.23. 5-Chloro-2-(6-(4-fluorophenyl)-2-methylimidazo[2,1-b] [1,3,4]thiadiazol-5-yl)-1H-benzo[d]imidazole **(5w)**.* FTIR (ATR, cm^{-1}): 3352, 3071, 2937, 1603, 1478, 1437, 1071, 837, 752, 690; Light green solid. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 2.80 (s, 3H, CH_3), 7.28 (dd, J = 1.8, 8.8 Hz, 1H, Ar–H), 7.45 (d, J = 8.3 Hz, 2H, Ar–H), 7.64 (d, J = 8.7 Hz, 1H, Ar–H), 7.72 (s, 1H, Ar–H), 8.09 (d, J = 8.0 Hz, 2H, Ar–H), 12.83 (s, 1H, NH); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 18.25, 115.0, 115.7, 123.62, 127.48, 129.81, 121.63, 131.29, 132.89, 133.27, 138.90, 142.1, 143.44, 144.28, 158.9, 162.97; **ESI-MS** (*m*/*z*) 383.9 (M + H) +; calculated for $C_{18}H_{11}ClFN_5S$; C, 56.33; H, 2.89; N, 18.25; S, 8.35. Found: C, 56.31; H, 2.90; N, 18.19; S, 8.31.

*4.2.5.24. 2-(6-(4-Chlorophenyl)-2-methylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1H-benzo[d] imidazole **(5x)**.* Off white solid. FTIR (ATR, cm^{-1}): 3272, 3070, 2965, 1590, 1495, 1436, 835, 751, 690; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 2.81 (s, 3H, CH_3), 7.23 (d, J = 8.8 Hz, 2H, Ar–H), 7.28–7.30 (m, 2H, Ar–H), 7.69 (dd, J = 1.8, 8.8 Hz, 2H, Ar–H), 8.06 (dd, J = 6.0, 8.4 Hz, 2H, Ar–H), 12.83 (s, 1H, NH); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 18.20, 115.09, 123.29, 129.89, 129.95, 130.53, 130.75, 134.2, 141.77, 144.14, 146.32, 161.13; **ESI-MS** (*m*/*z*) 366.08 (M + H)+; calculated for $C_{18}H_{12}ClN_5S$; C, 59.09; H, 3.31; N, 19.14; S, 8.76. Found: C, 59.06; H, 3.33; N, 19.12; S, 8.74.

*4.2.5.25. 2-(6-(4-Chlorophenyl)-2-methylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-5-nitro-1H-benzo[d]imidazole (**5y**).* Yellow solid. FTIR (ATR, cm^{-1}): 3278, 3070, 2968, 1591, 1496, 1434, 834, 758, 689; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 2.83 (s, 3H, CH_3),7.68 (dd, J = 1.9, 7.6 Hz, 2H, Ar–H),7.70 (d, J = 7.5 Hz, 1H, Ar–H), 8.08 (dd, J = 1.8, 8.8 Hz, 2H, Ar–H), 8.26 (d, J = 7.6 Hz, 1H, Ar–H), 8.58 (s, 1H, Ar–H), 12.85 (s, 1H, NH); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm): 18.16, 115.96, 123.42, 127.58, 129.21, 121.93, 130.83, 132.89, 133.27, 145.14, 146.28, 162.96; **ESI-MS** (*m*/*z*) 411.0 (M + H)+; calculated for $C_{18}H_{11}ClN_6O_2S$; C, 52.62; H, 2.70; N, 20.46; S, 7.80. Found: C, 52.55; H, 2.71; N, 20.48; S, 7.82.

*4.2.5.26. 5-Chloro-2-(6-(4-chlorophenyl)-2-methylimidazo[2,1-b] [1,3,4]thiadiazol-5-yl)-1H-benzo[d]imidazole(**5z**).* Light green solid. FTIR (ATR, cm^{-1}): 3275, 3069, 2965, 1592, 1497, 837, 753, 690; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 2.81 (s, 3H, OCH_3), 7.29 (dd, J = 8.7, 1.7 Hz, 1H, ArH), 7.46 (d, J = 8.3 Hz, 2H, ArH), 7.67 (d, J = 8.3 Hz, 1H, ArH), 7.73 (s, 1H, ArH), 8.00 (d, J = 8.3 Hz, 2H, Ar–H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 18.15, 114.96, 123.32, 127.38, 128.77, 129.20, 129.73, 130.89, 132.79, 133.17, 143.14, 144.18, 146.87, 162.94; **ESI-MS** (*m*/*z*) 400.0 (M + H)+; calculated for $C_{18}H_{11}Cl_2N_5S$; C, 54.01; H, 2.77; N, 17.50; S, 8.01. Found: C, 54.02; H, 2.80; N, 17.52; S, 8.04.

*4.2.5.27. 2-(2-Methyl-6-p-tolylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1H-benzo[d]imidazole (**5aa**).* Off white solid. FTIR (ATR, cm^{-1}): 3273, 3069, 2964, 1590, 1497, 1437, 838, 751, 691; **^{1}H NMR** (400 MHz, DMSO-d_6) δ(ppm): 2.30 (s, 3H, CH_3), 2.89 (s, 3H, CH_3), 7.18 (d, J = 8.4 Hz, 2H, Ar–H),7.26 (dd, J = 3.1, 6.2 Hz, 2H, Ar–H),7.65 (m, 2H, Ar–H),7.80 (d, J = 8.4 Hz, 2H, Ar–H),12.76 (s, 1H, NH); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 18.07, 21.29, 114.82, 122.88, 127.67, 129.33, 131.25, 137.78, 142.09, 145.09, 146.22, 162.15; **ESI-MS** (*m*/*z*) 346.0 (M + H)+; calculated for $C_{19}H_{15}N_5S$; C, 66.07; H, 4.38; N, 20.27; S, 9.28. Found: C, 66.12; H, 4.40; N, 20.25; S, 9.25.

*4.2.5.28. 2-(2-Methyl-6-p-tolyl imidazo [2, 1-b][1,3,4] thiadiazol-5-yl)-5- nitro- 1H-benzo[d]imidazole (**5ab**).* Yellow solid. FTIR (ATR, cm^{-1}): 3271, 3068, 2924, 1598, 1518, 1438, 842, 688; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ (ppm): 2.30 (s, 3H, CH_3), 2.78 (s, 3H, CH_3), 7.26 (dd, J = 3.1, 6.2 Hz, 1H, Ar–H), 7.29 (d, J = 8.7 Hz, 1H, Ar–H), 7.65 (m, 2H, Ar–H), 8.12 (d, J = 7.4 Hz, 1H, Ar–H), 8.26 (d, J = 8.4 Hz, 1H, Ar–H), 8.68 (s, 1H, Ar–H), 12.90 (s, 1H, NH); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 18.12, 21.30, 114.62, 122.98, 127.27, 129.43, 131.35, 137.78, 145.12, 142.8, 146.30, 162.32; **ESI-MS** (*m*/*z*) 391.08 (M + H)+; calculated for $C_{19}H_{14}N_6O_2S$; C, 58.45; H, 3.61; N, 21.53; S, 8.21. Found: C, 58.29; H, 3.62; N, 21.55; S, 8.23.

*4.2.5.29. 5-Chloro-2-(2-methyl-6-p-tolylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1H-benzo[d] imidazole **(5ac)**.* Light green solid. FTIR (ATR, cm^{-1}): 3273, 3070, 2965, 1590, 1498, 1435, 835, 751, 691; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ(ppm): 2.30 (s, 3H, CH_3), 2.79 (s, 3H, CH_3), 7.01 (d, J = 8.8 Hz, 2H, Ar–H), 7.49 (d, J = 8.3 Hz, 2H, Ar–H), 8.10 (d, J = 8.4 Hz, 1H, Ar–H), 8.26 (d, J = 7.6 Hz, 1H, Ar–H), 8.58 (s, 1H, Ar–H), 12.94 (s, 1H, NH); **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 18.16, 21.35, 114.98, 122.42, 127.48, 129.61, 121.93, 130.89, 132.79, 133.17, 143.14, 144.18,151.2, 152.3, 162.94; **ESI-MS** (*m*/*z*) 380.0 (M + H)+; calculated for $C_{19}H_{14}ClN_5S$; C, 66.07; H, 3.71; N, 18.44; S, 8.44. Found: C, 66.06; H, 3.70; N, 18.46; S, 8.40.

4.3. Antitubercular studies

Two-fold serial dilutions of each test compound/drug were prepared and incorporated into Middlebrook 7H11 agar medium with oleic acid, albumin, dextrose, and catalase (OADC) growth supplement to get final concentrations of 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 μg/mL. Inoculum of MTB H37Rv ATCC 27294/XDR-TB was prepared from fresh Middlebrook 7H11 agar slants with OADC (Difco) growth supplement adjusted to 1 mg/mL (wet weight) in Tween 80 (0.05%) saline diluted to 10^{-2} to give a concentration of ~10^7 cfu/mL. Five microliters of this bacterial suspension was spotted onto 7H11 agar tubes containing different concentrations of the drug as discussed above. The tubes were incubated at 37 °C, and final readings (as MIC in mg/mL) were determined after 28 days. This method is similar to that recommended by the National Committee for Clinical Laboratory Standards for the determination of MIC in triplicate.

4.4. Antibacterial studies

All bacterial strains were maintained on nutrient agar medium at 37 °C. The cultures were inoculated in fresh 10 mL Nutrient Broth to yield an initial suspension of approximately 10–100 cfu/mL. All broths were then incubated statically at 37 °C for 18–24 h. Susceptibility of the test organism to the organic compound was determined by well plate technique. The bacterial suspensions were serially diluted in saline and 0.1 ml from the appropriate dilution was spread on nutrient agar. The wells were dug in each petri plate by sterilized cork borer. The compounds were dissolved in DMSO and appropriate dilutions were made (1 mg/mL and 0.5 mg/mL). Each experiment was carried out in triplicate. The same procedure was repeated for all microorganisms. After the inoculation of organism and compound, the petri plates were incubated for 18 h at 37 °C. The diameter of zone of inhibition was measured and the values for DMSO solvent were subtracted to get the actual values.

4.5. Antifungal studies

The compounds were dissolved in DMSO and antimicrobial activity was determined at concentration of 1 and 0.5 mg/mL. The required amounts of each fungal strain were removed from the stock and suspended in 5 mL of distilled water with 2 drops of Tween 80. This suspension was uniformly spread on petri plates containing Potato dextrose agar media using sterile swabs. After adding the test samples into the wells, the plates were incubated at 25 °C for 3 days. The plates were then examined for the presence of zones of inhibition and the results were recorded.

4.6. Antioxidant studies

Free radical-scavenging capacities of the compounds **5a**–**ac** were determined using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). An aliquot of 100 μg concentrations of synthesized compounds in methanol was added to 3 ml of 0.004% w/v DPPH radical solution and each test tube was made up to final volume of 4 ml. BHA was used as a reference standard and dissolved in methanol to get the same concentration as that of synthesized compounds. Each mixture was vortexed for a few seconds and allowed to stand in the dark for 10 min at ambient temperature. The absorbance of each reaction mixture was measured at 517 nm against a blank of methanol using a UV–visible spectrometer (Shimadzu UV-1800, Japan). The level of DPPH radical scanning activity was calculated as:

$$\%\text{Scavenging Activity} = \frac{\text{Absorbance of the control} - \text{Absorbance of the test sample}}{\text{Absorbance of the control}} \times 100$$

4.7. In vitro cytotoxicity studies

Vero (African green monkey kidney) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in MEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 μg/ml) and amphotericin B (5 μg/ml) in an humidified atmosphere of 5% CO_2 at 37 °C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India). For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with MEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out MTT assay. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 × 10^5 cells/ml using MEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 μL of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37 °C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 μl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37 °C in 5% CO_2 atmosphere. The supernatant was removed and 100 μL of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm.

$$\%\,\text{Growth Inhibition} = 100 - \left(\frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100\right)$$

Acknowledgments

Authors are thankful to NITK, Surathkal for providing the research facilities and to Dr. Reddy's institute of life sciences, HCU campus for providing analytical facilities.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.03.024.

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