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Original article

Design, synthesis and evaluation of small molecule imidazo[2,1-*b*][1,3,4]thiadiazoles as inhibitors of transforming growth factor-β type-I receptor kinase (ALK5)

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ABSTRACT

A new series of imidazo[2,1-*b*][1,3,4]thiadiazoles **5**(**a**–**g**), **6**(**a**–**g**), **9**(**a**–**i**) and **12**(**a**–**h**) were synthesized as transforming growth factor-β (TGF-β) type I receptor (also known as activin receptor-like kinase 5 or ALK5) inhibitors. These compounds were evaluated for their ALK5 inhibitory activity in an enzyme assay and their TGF-β -induced Smad2/3 phosphorylation inhibitory activity in a cell-based assay. Compound **6d**, 2-(5-((2-cyclopropyl-6-(4-fluorophenyl) imidazo [2,1-b][1,3,4]thiadiazol-5-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid, shows prominent ALK5 inhibition (IC_{50} = 0.0012 μM) and elective inhibition (91%) against the P38αkinase at10 μM. The binding mode of compound **6d** by XP docking studies shows that it fits well into the active site cavity of ALK5 by forming broad and tight interactions. Lipinski's rule and in silico ADME pharmacokinetic parameters are within the acceptable range defined for human use thereby indicating their potential as a drug-like molecules.

1. Introduction

Transforming growth factor-beta (TGF-β) is a ubiquitous cytokine that affects various biological processes such as regulation of cell proliferation, immune responses, growth, differentiation, angiogenesis, and apoptosis of different cell types [1]. TGF-β1 transduces signals through two highly conserved single transmembrane serine/threonine kinases, the type I and type II TGF-β receptors (TβR-I and TβR-II, respectively) [2]. TβR-II activates TβR-I upon formation of the ligand–receptor complex by hyperphosphorylating serine/threonine residues in the GS region of the TβR-I or activin-like kinase (ALK5), which creates a binding site for Smad proteins. The activated TβR-I in turn phosphorylates Smad2/Smad3 proteins at the C-terminal SSXS-motif thereby causing dissociation from the receptor and heteromeric complex formation with the Smad4 [3–5]. Smad complexes translocate to the nucleus, assemble with specific DNA-binding co-factors and co-modulators to finally activate transcription of an extracellular matrix component, and inhibitors of matrix-degrading proteases [6]. Therefore, it becomes evident that inhibition of ALK5 phosphorylation of Smad2/Smad3 could reduce TGF-β1-induced excessive accumulation of the extracellular matrix. Small molecules inhibitors of TGF-βR1 offer an attractive way to regulate the TGF-β pathway and can consequently find applications in the treatment of various diseases, especially, cancer [7]. Our on-going interest in the design and synthesis of novel anti-cancer agents [8–13], and recent reports by Hoelzemann and collaborators [14] suggesting the imidazo[2,1-*b*][1,3,4]thiadiazoles scaffold as a template to the design of inhibitors of ALK5; inspired us to synthesize and *in vitro* evaluated imidazo[2,1-*b*][1,3,4]thiadiazoles **5**(**a**–**g**), **6**(**a**–**g**), **9**(**a**–**i**) and **12**(**a**–**h**) for the ALK5 inhibitory activity in an enzyme assay and their TGF-β

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-induced Smad2/3 phosphorylation inhibitory activity in a cell-based assay.

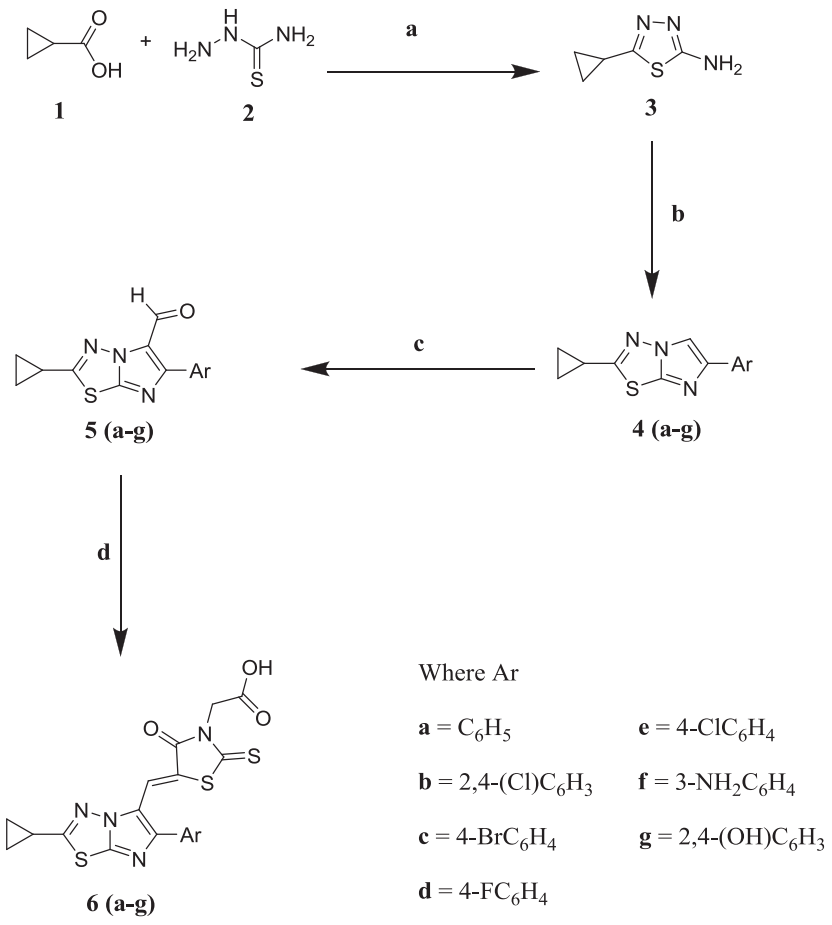
2. Chemistry

The synthetic route of the compound **5**(**a**–**g**), **6**(**a**–**g**), **9**(**a**–**i**) and **12**(**a**–**h**) is outlined in Schemes 1–3 respectively. 2-amino-5-substituted-1,3,4-thiadiazole **3**, **8** and **11** was obtained by direct cyclization of benzoic acid **1**, substituted benzoic acid **7**, **10**, and thiosemicarbazide **2** in the presence of phosphorus oxychloride, the latter refluxed with substituted α-haloaryl ketones in dry ethanol with drop of dimethyl formamide to yield the imidazo[2,1-*b*][1,3,4]-thiadiazoles **4**(**a**–**g**), **9**(**a**–**i**) and **12**(**a**–**h**) in good yield as per the procedure reported by Gadad et al. [15] It is well established that this reaction proceeds via the intermediate iminothiadiazole [15], which undergoes dehydrocyclisation to form the desired fused heterocycle under reflux temperature spontaneously. The ring nitrogen of the imino form of thiadiazole is involved preferably on the nucleophilic displacement of bromine of the α-bromoketones forming an intermediate. It undergoes further cyclodehydration on heating with a suitable medium like ethanol, DMF to afford imidazothiadiazoles in good yields. The cyclodehydration involves intramolecular nucleophilic addition of the 2-amino group to carbonyl functions of the intermediate followed by the elimination of water. 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 groups impart less nucleophilic character to the nitrogen at 4th position of the 1,3,4-thiadiazole.

In the next step, imidazo[2,1-*b*][1,3,4]-thiadiazoles **4**(**a**–**g**) were subjected to Vilsmeier–Hack reaction to afford 2-cyclopropyl-6-substituted phenylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde **5**(**a**–**g**). The obtained imidazo[2,1-b][1,3,4]thiadiazoles-5-carbaldehydes **5**(**a**–**g**) were subjected to Knoevenagel condensation [16,17] using 2-(4-oxo-2-thioxothiazolidin-3-yl)acetic acid (rhodanine acetic acid) in the presence of the catalytic amount of piperidine acetate to afford the 2-(5-((2-cyclopropyl-6-substituted phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid **6**(**a**–**g**). The physical and elemental data of all the synthesized compounds is shown in Tables 1 and 2.

3. Results and discussion

To evaluate whether these compounds **5**(**a**–**g**), **6**(**a**–**g**), **9**(**a**–**i**) and **12**(**a**–**h**) could inhibit ALK5, an enzyme based kinase assay and TGF-β-induced Smad2/3 phosphorylation inhibitory activity in a cell-based assay was performed (Table 3). The ALK5 inhibitory



Reagents: (**a**) $POCl_3$ (**b**) substituted phenacyl bromides, Dry EtOH (**c**) DMF, $POCl_3$ (Vilsmeier-Haack reagent) (**d**) 2-(4-oxo-2-thioxothiazolidin-3-yl)acetic acid, piperidine, CH_3COOH, toluene

Scheme 1. Synthesis of title compounds 5(a-g) and 6(a-g).

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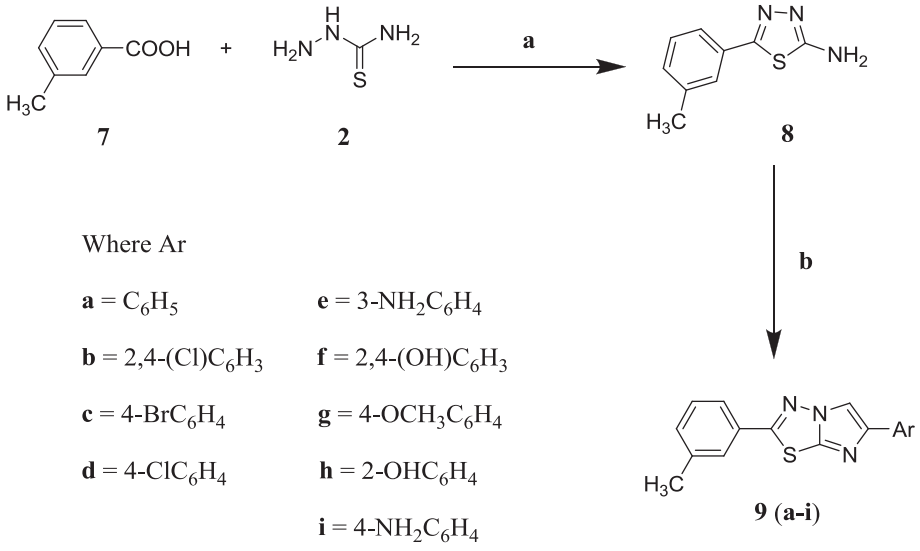
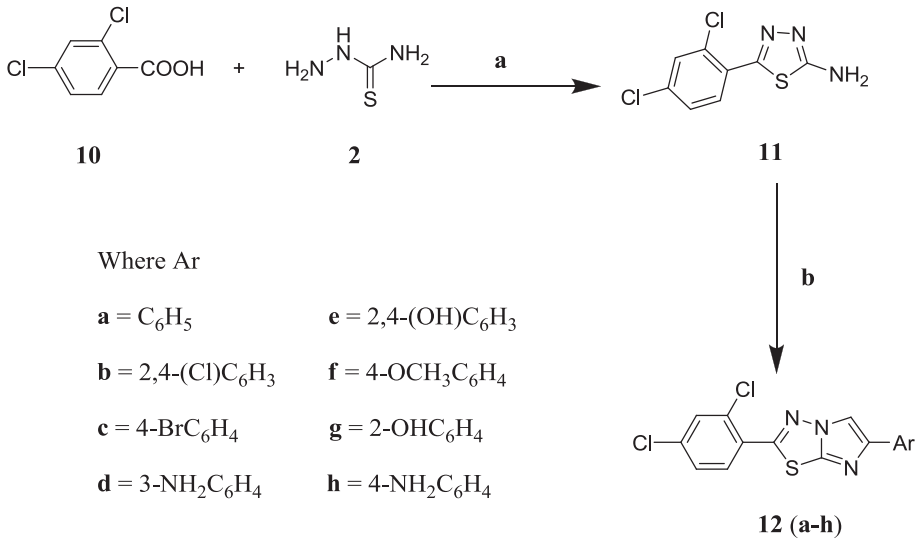


Reagents: (**a**) $POCl_3$ (**b**) Substituted phenacyl bromide, Dry EtOH

Scheme 2. Synthesis of title compounds 9(a-i).



Reagents: (**a**) $POCl_3$ (**b**) Substituted phenacyl bromide, Dry EtOH

Scheme 3. Synthesis of title compounds 12(a-h).

activity of imidazo[2,1-*b*][1,3,4]thiadiazoles having substitution at 2, 5 and 6 position was compared. In case of compound **5(a–g)** and **6(a–g)** having cyclopropyl substitution at C-2 position; it is seen that the rhodanine acetic acid derivatives are more potent as compared to formyl derivatives at C-5, it is obvious by comparing **6d** (IC_{50} = 0.0012 μM) and **5d** (IC_{50} = 0.019 μM). Further structure activity relationship at C-6 position of imidazo[2,1-*b*][1,3,4]thiadiazoles indicates that presence of electron withdrawing group on phenyl ring is more crucial for ALK5 inhibition e.g. **5d** (IC_{50} = 0.019 μM) and **6d** (IC_{50} = 0.0012 μM). Comparison of **5b** (IC_{50} = 0.064 μM) and **5d** (IC_{50} = 0.019 μM); **6b** (IC_{50} = 0.03 μM) and **6d** (IC_{50} = 0.0012 μM) reveals that at C-6 position more than one electron withdrawing group has diminishing effect on the activity. Compound **5d**, the most potent analogue, was evaluated for electivity using a diverse kinase panel and it was found to be selective toward the P 38α kinase with significant percentage of inhibition (91%) at10 μM as shown in Table 4.

The docking studies in the X-ray crystal structure of ALK5 (PDB code: 1RW8) have revealed that the central imidazo[2,1-*b*][1,3,4] thiadiazole ring and its substituent form several interactions with amino acid residues in the binding pocket, facilitating binding of the ligand deep into the active site (Table 5). The sulfur atom of

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Table 1
Physicochemical properties of the synthesized compounds **5** (**a**–**g**), **6** (**a**–**g**), **9** (**a**–**i**) and **12** (**a**–**h**).

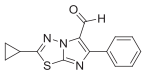
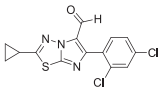
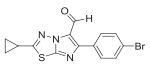
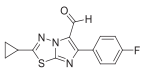
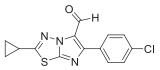
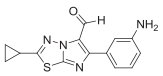
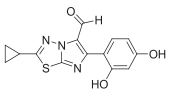
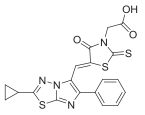
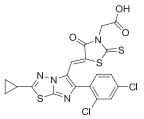
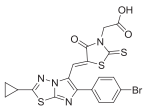
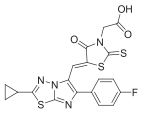
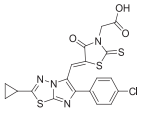
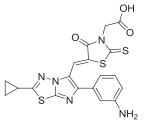
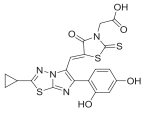
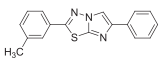
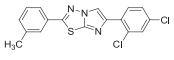
Sr. No.	Compound	Molecular formula	Molecular weight	Yield %	Melting point (°C)	Solvent system	R_f (cm)
5a		$C_{14}H_{11}N_3OS$	269.3216	70	112–115	B:A (9:1)	0.68
5b		$C_{14}H_9Cl_2N_3OS$	338.2118	65	154–158	B:A (9:1)	0.71
5c		$C_{14}H_{10}BrN_3OS$	348.2177	69	159–162	B:A (9:1)	0.79
5d		$C_{14}H_{10}FN_3OS$	287.3121	68	124–126	B:A (9:1)	0.71
5e		$C_{14}H_{10}ClN_3OS$	303.7667	70	137–140	B:A (9:1)	0.70
5f		$C_{14}H_{12}N_4OS$	284.3363	67	143–146	B:A (9:1)	0.69
5g		$C_{14}H_{11}N_3O_3S$	301.3204	68	197–199	B:A (9:1)	0.75
6a		$C_{19}H_{14}N_4O_3S_3$	442.5345	77	232–235	T:E:F (5:4:1)	0.64
6b		$C_{19}H_{12}Cl_2N_4O_3S_3$	511.4246	69	222–224	T:E:F (5:4:1)	0.66
6c		$C_{19}H_{13}BrN_4O_3S_3$	521.4305	78	213–215	T:E:F (5:4:1)	0.59
6d		$C_{19}H_{13}FN_4O_3S_3$	460.5249	76	209–212	T:E:F (5:4:1)	0.61
6e		$C_{19}H_{13}ClN_4O_3S_3$	476.9795	76	190–192	T:E:F (5:4:1)	0.63
6f		$C_{19}H_{15}N_5O_3S_3$	457.5491	68	168–171	T:E:F (5:4:1)	0.76
6g		$C_{19}H_{14}N_4O_5S_3$	474.5333	58	156–159	T:E:F (5:4:1)	0.73
9a		$C_{17}H_{13}N_3S$	291.3702	70	138–140	$CHCl_3$:CH_3OH (9:1)	0.55
9b		$C_{17}H_{11}Cl_2N_3S$	360.2603	58	151–154	$CHCl_3$:CH_3OH (9:1)	0.58

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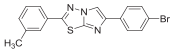
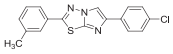
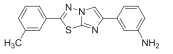
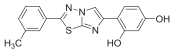
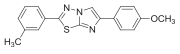
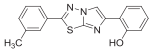
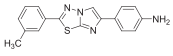
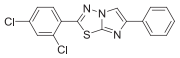
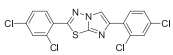
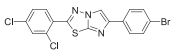
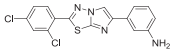
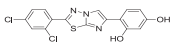
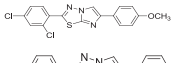
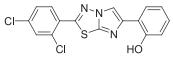
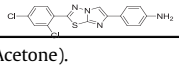
Table 1 (*continued*)

Sr. No.	Compound	Molecular formula	Molecular weight	Yield %	Melting point (°C)	Solvent system	R_f (cm)
9c		$C_{17}H_{12}BrN_3S$	370.2663	68	170–173	$CHCl_3$:CH_3OH (9:1)	0.67
9d		$C_{17}H_{12}ClN_3S$	325.8153	69	146–149	$CHCl_3$:CH_3OH (9:1)	0.61
9e		$C_{17}H_{14}N_4S$	306.3849	78	156–159	$CHCl_3$:CH_3OH (9:1)	0.54
9f		$C_{17}H_{13}N_3O_2S$	323.3690	69	134–137	$CHCl_3$:CH_3OH (9:1)	0.67
9g		$C_{18}H_{15}N_3OS$	321.3962	65	112–115	$CHCl_3$:CH_3OH (9:1)	0.51
9h		$C_{17}H_{13}N_3OS$	307.3696	64	122–124	$CHCl_3$:CH_3OH (9:1)	0.60
9i		$C_{17}H_{14}N_4S$	306.3849	67	148–151	$CHCl_3$:CH_3OH (9:1)	0.59
12a		$C_{16}H_9Cl_2N_3S$	346.2338	70	172–174	$CHCl_3$:CH_3OH (9:1)	0.55
12b		$C_{16}H_7Cl_4N_3S$	415.1239	58	196–198	$CHCl_3$:CH_3OH (9:1)	0.54
12c		$C_{16}H_8BrCl_2N_3S$	425.1298	68	147–150	$CHCl_3$:CH_3OH (9:1)	0.62
12d		$C_{16}H_{10}Cl_2N_4S$	361.2484	78	178–181	$CHCl_3$:CH_3OH (9:1)	0.58
12e		$C_{16}H_9Cl_2N_3O_2S$	378.2326	69	170–172	$CHCl_3$:CH_3OH (9:1)	0.61
12f		$C_{17}H_{11}Cl_2N_3OS$	376.2597	65	152–154	$CHCl_3$:CH_3OH (9:1)	0.57
12g		$C_{16}H_9Cl_2N_3OS$	362.2332	64	162–164	$CHCl_3$:CH_3OH (9:1)	0.75
12h		$C_{16}H_{10}Cl_2N_4S$	361.2484	67	157–159	$CHCl_3$:CH_3OH (9:1)	0.63

B:A (Benzene:Acetone).
T:E:F (Toluene:Ethyl acetate:Formic acid).
$CHCl_3$:CH_3OH (Chloroform:Methanol).

imidazo[2,1-*b*][1,3,4]thiadiazole of **5** (**a**–**g**), **6** (**a**–**g**), **9** (**a**–**i**) and **12** (**a**–**h**) forms hydrogen bonding via sulfur atom with His-283 in the hinge region of the kinase respectively, originally a binding pocket for the adenine ring of ATP, and it is major interaction of inhibitor with the binding site [18,19]. The imidazo[2,1-*b*][1,3,4]thiadiazole ring occupies the hydrophobic pocket containing Ser280, which is critical for the selectivity of the ALK5 [20]. The fluorine atom of compound **5d**, **6d** and N-3 of imidazo[2,1-*b*][1,3,4]thiadiazoles of remaining compound forms water mediated hydrogen bond with Asp-351 and Tyr-249 which is one of key interactions observed in the X-ray structures of ALK5 complexed with other inhibitors as shown in Fig. 1 [3]. It was reported that Asp351 is usually located close to bound inhibitors in the inactivated conformation of ALK5 [18]. The H-bonding between imidazo[2,1-*b*][1,3,4]thiadiazoles with Asp351 may contribute to hold the loop containing Asp351 and stabilize inactive conformation of ALK5. The present docking model demonstrates a possible broad and tight interaction of **6d** with the ALK5 active site ranging from His-283, Asp351 and Tyr-249, and this may be related to the excellent activity of compound **6d**. Conclusively, the binding mode of **6d** generated by docking studies supports the strong and selective activity of this compound and provides the insights for further modification to develop analogs with more desirable biological activity.

As a part of our study, the compliance of compounds to Lipinski's rule of five was evaluated. As discussed by Lipinski, molecular properties are closely related to the oral bioavailability of a drug. The QikProp 3.2 was used to analyze drug likeness (Lipinski's Rule of Five) and in silico ADME evaluation; the results are given in Table 6 and it was found that all the synthesized compounds comply with these rules with the exception of **6b** and **6c**, which did not comply for molecular weight and QPlogP O/W; showing violation for Lipinski's Rule of Five. In addition, it is well known that numerous drug candidates have failed during clinical tests because of problems related to ADME (absorption, distribution, metabolism and excretion) properties. The results for ADME prediction is shown in Table 6. Human Intestinal Absorption (HIA) and Caco-2 (QPPCaco) permeability are good indicators of drug absorbance in the intestine and Caco-2 monolayer penetration, respectively. Human Intestinal Absorption data are the sum of bioavailability and absorption evaluated from the ratio of excretion or cumulative excretion in urine, bile and feces [21]. The predicted percentages of intestinal absorption are excellent for all of the compounds tested, with values above 59–100% in all cases. The compounds present

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Table 2
Elemental analysis of **5** (**a**–**g**), **6** (**a**–**g**), **9** (**a**–**i**) and **12** (**a**–**h**).

Compound code	Compound	Elemental analysis (%)					
		Calculated			Found		
		C	H	N	C	H	N
5a	$C_{14}H_{11}N_3OS$	62.43	4.12	15.60	62.45	4.10	15.63
5b	$C14H_9Cl_2N_3OS$	49.72	2.68	12.42	49.74	2.68	12.43
5c	$C_{14}H_{10}BrN_3OS$	48.29	2.89	12.07	48.31	2.91	12.07
5d	$C_{14}H_{10}FN_3OS$	58.53	3.51	14.63	58.56	3.54	14.66
5e	$C_{14}H_{10}ClN_3OS$	55.35	3.32	13.83	55.38	3.32	13.85
5f	$C_{14}H_{12}N_4OS$	59.14	4.25	19.70	59.16	4.223	19.68
5g	$C_{14}H_{11}N_3O_3S$	55.80	3.68	13.95	55.78	3.70	13.97
6a	$C_{19}H_{14}N_4O_3S_3$	51.57	3.19	12.64	51.59	3.21	12.68
6b	$C_{19}H_{12}Cl_2N_4O_3S_3$	44.62	2.37	10.96	44.64	2.39	10.94
6c	$C_{19}H_{13}BrN_4O_3S_3$	43.76	2.51	10.74	43.74	2.53	10.77
6d	$C_{19}H_{13}FN_4O_3S_3$	49.55	2.85	12.17	49.57	2.87	12.19
6e	$C_{19}H_{13}ClN_4O_3S_3$	47.84;	2.75	11.75	47.84;	2.77	11.76
6f	$C_{19}H_{15}N_5O_3S_3$	49.88	3.30	15.31	49.90	3.32	15.33
6g	$C_{19}H_{14}N_4O_5S_3$	48.09	2.97	11.81	48.11	2.95	11.79
9a	$C_{17}H_{13}N_3S$	70.08	4.50	14.42	70.06	4.50	14.44
9b	$C_{17}H_{11}Cl_2N_3S$	56.68	3.08	11.66	56.70	3.06	11.68
9c	$C_{17}H_{12}BrN_3S$	55.14	3.27	11.35	55.16	3.29	11.37
9d	$C_{17}H_{12}ClN_3S$	62.67	3.71	12.90	62.69	3.73	12.93
9e	$C_{17}H_{14}N_4S$	66.64	4.61	18.29	66.66	4.63	18.31
9f	$C_{17}H_{13}N_3O_2S$	63.14	4.05	12.99	63.16	4.07	12.99
9g	$C_{18}H_{15}N_3OS$	67.27	4.70	13.07	67.29	4.73	13.05
9h	$C_{17}H_{13}N_3OS$	66.43	4.26	13.67	66.45	4.29	13.65
9i	$C_{17}H_{14}N_4S$	66.64	4.61	18.29	66.66	4.63	18.31
12a	$C_{16}H_9Cl_2N_3S$	55.50	2.62	12.14	55.53	2.65	12.17
12b	$C_{16}H_7Cl_4N_3S$	46.29	1.70	10.12	46.32	1.67	10.15
12c	$C_{16}H_8BrCl_2N_3S$	45.20	1.90	9.88	45.20	1.92	9.85
12d	$C_{16}H_{10}Cl_2N_4S$	53.20	2.79	15.51	53.23	2.77	15.53
12e	$C_{16}H_9Cl_2N_3O_2S$	50.81	2.40	11.11	50.84	2.43	11.14
12f	$C_{17}H_{11}Cl_2N_3OS$	54.27	2.95	11.17	54.30	2.94	11.16
12g	$C_{16}H_9Cl_2N_3OS$	53.05	2.50	11.60	53.04	2.51	11.62
12h	$C_{16}H_{10}Cl_2N_4S$	53.20	2.79	15.51	53.22	2.79	15.53

good permeability values in Caco-2 (QPPCaco) cells, ranging from 57.285 to 4759.477. Hence, theoretically, all of these compounds should present good passive oral absorption except compound **6f** and **6g**. The partition coefficient (QPlogPo/w) and water solubility (QPlogS), critical for estimation of absorption and distribution of drugs within the body ranged between 1.416 and 5.145 and −3.79 to −6.229. Cell permeability (QPPCaco), a key factor governing drug metabolism and its access to biological membranes, ranged from 57.285 to 4759.477. We similarly studied number of violations of Jorgensen's rule of three. The three rules are: QPlogS > −5.7, QPCaco > 22 nm/s, Primary Metabolites < 7. Compounds with fewer (and preferably no) violations of these rules are more likely to be orally available. All the compounds are following this rule except **6b**, **6f** and **6g**; showing best candidate for oral bioavailability. All these pharmacokinetic parameters are within the acceptable range defined for human use (see Table 6 footnote), thereby indicating their potential as a drug-like molecules.

4. Conclusion

In conclusion, a new series of imidazo[2,1-*b*][1,3,4]thiadiazoles **5**(**a**–**g**), **6**(**a**–**g**), **9**(**a**–**i**) and **12**(**a**–**h**) were synthesized. To evaluate the ALK5 inhibitory activity of imidazo[2,1-*b*][1,3,4]thiadiazoles an enzyme based kinase assay and TGF-β-induced Smad2/3 phosphorylation inhibitory activity in a cell-based assay was performed. Among them, compound **6d** shows highest ALK5 inhibition $IC_{50} = 0.0012$ μM and it was found to be selective toward the P 38α kinase with significant percentage of inhibition (91%) at10 μM. The docking studies revealed that the sulfur atom of imidazo[2,1-*b*][1,3,4]thiadiazole of **5**(**a**–**g**), **6**(**a**–**g**), **9**(**a**–**i**) and **12**(**a**–**h**) forms hydrogen bonding via sulfur atom with His-283 in the hinge region

Table 3
Inhibitory profile of **5** (**a**–**g**), **6** (**a**–**g**), **9** (**a**–**i**) and **12** (**a**–**h**).

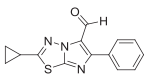
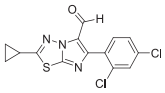
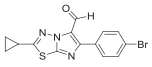
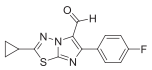
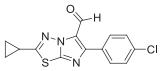
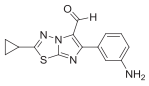
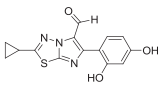
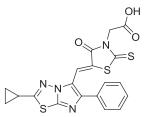
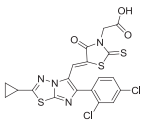
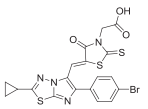
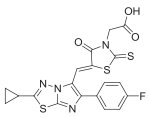
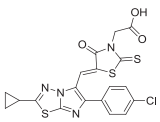
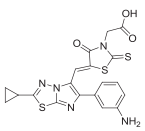
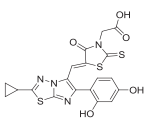
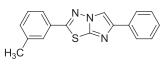
Compound code	Compound	IC_{50} (μM)	
		ALK5[a]	Smad2/3[a]
5a		0.081	0.529
5b		0.064	0.537
5c		0.03	0.223
5d		0.019	0.268
5e		0.025	0.693
5f		0.112	0.486
5g		0.123	0.257
6a		0.04	0.126
6b		0.03	0.133
6c		0.015	0.014
6d		0.0012	0.0209
6e		0.018	0.093
6f		0.042	0.358
6g		0.047	0.104
9a		0.214	1.691

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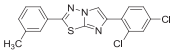
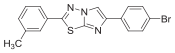
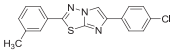
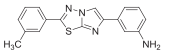
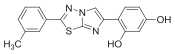
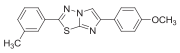
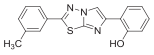
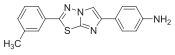
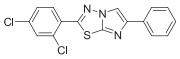
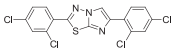
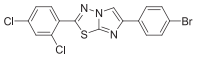
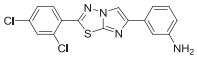
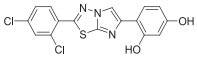
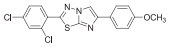
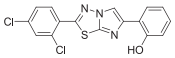
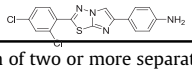
Table 3 (*continued*)

Compound code	Compound	IC_{50} (μM)	
		ALK5[a]	Smad2/3[a]
9b		0.452	7.82
9c		0.11	0.539
9d		0.107	0.519
9e		>10	>10
9f		>10	>10
9g		3.548	>10
9h		3.98	>10
9i		1.25	6.67
12a		1.778	>10
12b		0.04	0.050
12c		0.019	0.118
12d		1.023	5.98
12e		1.17	6.589
12f		0.38	0.268
12g		0.724	0.429
12h		0.823	>10

[a] Values are the mean of two or more separate experiment.

of the kinase which is a major interaction for ALK5 inhibition. The imidazo[2,1-*b*][1,3,4]thiadiazole ring occupies the hydrophobic pocket containing Ser280, which is critical for the selectivity of the ALK5. Lipinski's rule and in silico ADME pharmacokinetic parameters are within the acceptable range defined for human use thereby indicating their potential as drug-like molecules. The overall outcome of this model revealed that: (i) the imidazo[2,1-*b*][1,3,4]thiadiazole ring is a satisfactory backbone for ALK5 inhibition; (ii) presence of electron withdrawing group on phenyl ring at C-6 of imidazo[2,1-*b*][1,3,4]thiadiazole is more crucial for ALK5 inhibition; (iii) rhodanine acetic acid derivatives at C-5 of imidazo[2,1-*b*][1,3,4]thiadiazole are more potent as compared to the other derivatives. Lipinski's rule and in silico ADME pharmacokinetic parameters are within the acceptable range defined for human use thereby indicating their potential as drug-like molecules. These encouraging results of biological screening of the tested compounds could offer an excellent framework in this field that may lead to discovery of potent ALK5 inhibitors. Finally, it is conceivable that further derivatization of such compounds will be of interest with the hope to get more selective and potent ALK5 inhibitors.

5. 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 GF_{254} 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.

5.1. 5-Cyclopropyl-1,3,4-thiadiazol-2-amine (3)

It is synthesized as per the procedure reported [10].

5.2. 2-Cyclopropyl-6-substituted phenylimidazo[2,1-b][1,3,4] thiadiazole 4(a–g)

It is synthesized as per the procedure reported [10].

5.3. General procedure for the synthesis of 2-cyclopropyl-6-substituted phenylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde 5(a–g)

The Vilsmeir–Haack reagent was prepared by adding phosphorus oxychloride (3 mL) to dimethyl-formamide (20 mL) at 0 °C with stirring. Then, compound 2-cyclopropyl-6-substituted phenylimidazo[2,1-*b*][1,3,4]thiadiazole **4**(**a**–**g**) (0.01 mol) was added to the reagent and stirred at 0 °C for 30 min. The mixture was further stirred at room temperature for 2 h and then at 60 °C for an additional 2 h. The reaction mixture was then poured onto sodium carbonate solution and stirred at 90 °C for 2 h. After cooling, the mixture was diluted with water and extracted with chloroform. The chloroform layer was washed with water, dried over anhydrous sodium sulfate and the residue obtained after removal of the solvent was crystallized from chloroform/methanol.

5.3.1. 2-Cyclopropyl-6-phenylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (5a)

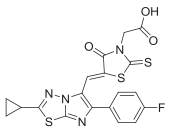
This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3054.00 (Ar C–H stretch), 2939.21 (Ali. C–H stretch) and 1724.15 [(C=O) aldehyde]; ^{1}H NMR (DMSO-d_6) δ ppm): 10.00 (s, 1H, CHO), 7.26–7.84 (m, 5H, Ar–H), 1.51–2.42 (m, 5H, cyclopropyl); ^{13}C NMR (DMSO-d_6) δ ppm: 186.32, 166.74,157.83, 143.53, 134.53, 133.62, 129.52, 128.63, 127.84,13.12, 10.84; HRMS (EI) *m*/*z* calcd for $C_{14}H_{11}N_3OS$: 269.0623; found: 269.0628.

5.3.2. 2-Cyclopropyl-6-(2,4-dichlorophenyl)imidazo[2,1-b][1,3,4] thiadiazole-5-carbaldehyde (5b)

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3059.50 (Ar C–H stretch), 2920.42 (Ali. C–H stretch), 1718.59 (C=O), 721 (C–Cl); ^{1}H NMR (DMSO-d_6) δ ppm): 9.97 (s, 1H, CHO), 7.46–8.41 (m, 3H, Ar–H) and 1.13–2.50 (m, 5H, cycloproyl); ^{13}C NMR (DMSO-d_6) δ ppm: 186.74,

Please cite this article in press as: H.M. Patel, et al., Design, synthesis and evaluation of small molecule imidazo[2,1-*b*][1,3,4]thiadiazoles as inhibitors of transforming growth factor-β type-I receptor kinase (ALK5), European Journal of Medicinal Chemistry (2014), http://dx.doi.org/10.1016/j.ejmech.2014.09.002

Table 4
Selectivity of compound **6d** against a panel of kinases.[a]

Compound	
AMPK	35%
CDK2/A	29%
CHK1	21%
CK1	37%
CK2	11%
CSK	32%
DYRK 1A	23%
ERK2	24%
GSK 3b	21%
MKK 1A	36%
MKK 1	19%
MKK 2	24%
MSK 1	10%
NEK2A	16%
NEK 6	18%
KDR	42%
P 38α	91%
P 38β2	41%
P 38βγ	39%
P 38βδ	35%
PBK	12%
PDK 1	48%
PK A	34%
PK B	21%

[a] Values are % inhibition at 10 μM using 100 μM ATP.

166.42,157.83, 143.12, 134.64, 134.74, 132.12, 130.23, 129.64, 128.73, 126.12, 13.84, 10.63; HRMS (EI) *m*/*z* calcd for $C_{14}H_9Cl_2N_3OS$: 336.9843; found: 336.9847.

5.3.3. 6-(4-Bromophenyl)-2-cyclopropylimidazo[2,1-b][1,3,4] thiadiazole-5-carbaldehyde ***(5c)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3072.93 (Ar C–H stretch), 2920.97 (Ali. C–H stretch) 1722.74 [(C=O) aldehyde], 621 (C–Br); ^{1}H NMR (DMSO-d_6) δ ppm): 9.97 (s, 1H, CHO), 7.55–7.85 (m, 4H, Ar–H), 1.14–2.50 (m, 5H, cylopropyl); ^{13}C NMR (DMSO-d_6) δ ppm: 186.24, 166.53,157.43, 143.84, 134.12, 132.12, 128.24, 126.34, 124.45, 13.12, 10.36; HRMS (EI) *m*/*z* calcd for $C_{14}H_{10}BrN_3OS$: 346.9728; found: 346.9732.

5.3.4. 2-Cyclopropyl-6-(4-fluorophenyl)imidazo[2,1-b][1,3,4] thiadiazole-5-carbaldehyde ***(5d)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3060.36 (Ar C–H stretch), 2924.12 (Ali. C–H stretch), 1720.52 [(C=O) aldehyde], 1210 (C–F); ^{1}H NMR (DMSO-d_6) δ ppm): 10.02 (s, 1H, CHO), 7.16–7.92 (m, 4H, Ar–H), 1.16–2.44 (m, 5H, cylopropyl); ^{13}C NMR (DMSO-d_6) δ ppm: 186.12, 166.83, 160.82, 157.73, 143.62, 134.12, 129.62, 128.12, 121.82, 13.62, 10.34; HRMS (EI) *m*/*z* calcd for $C_{14}H_{10}FN_3OS$: 287.0529; found: 287.0533.

5.3.5. 6-(4-Chlorophenyl)-2-cyclopropylimidazo[2,1-b][1,3,4] thiadiazole-5-carbaldehyde ***(5e)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3063.93 (Ar C–H stretch), 2920.77 (Ali. C–H stretch) and 1718.02 [(C=O) aldehyde], 731 (C–Cl); ^{1}H NMR (DMSO-d_6) δ ppm): 9.98 (s, 1H, CHO), 7.46–7.79 (m, 4H, Ar–H), 1.13–2.54 (m, 5H, cylopropyl); ^{13}C NMR (DMSO-d_6) δ ppm: 186.12, 166.83,157.82, 143.23, 134.74, 133.63, 131.83, 130.82,

Table 5
Glide docking results based on glide dock score, glide energy, glide emodel and hydrogen bonding interaction of synthesized compounds **5**(**a**–**g**), **6** (**a**–**g**), **9** (**a**–**i**) and **12** (**a**–**h**).

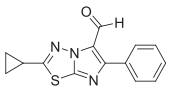
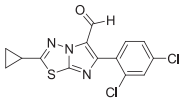
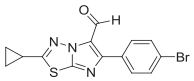
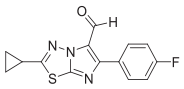
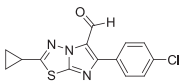
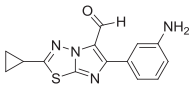
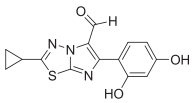
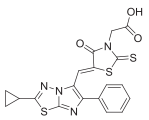
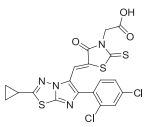
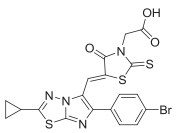
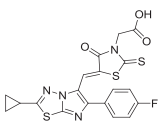
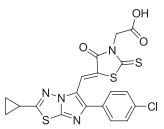
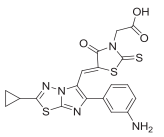
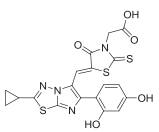
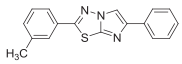
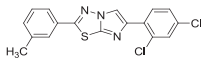
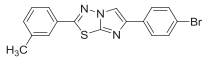
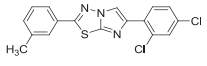
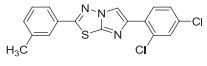
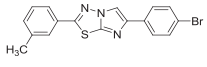
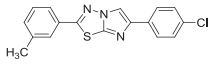
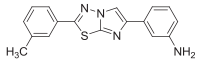
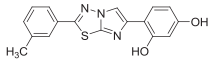
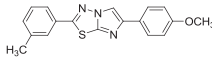
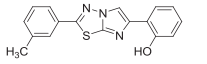
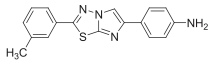
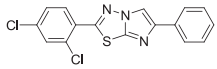
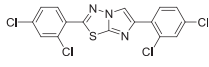
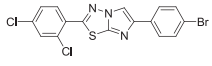
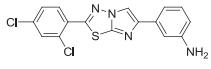
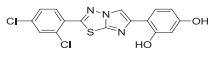
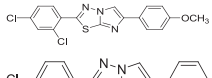
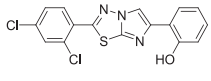
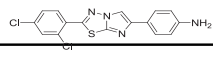
Sr. No.	Compounds	Docking score	Glide energy (kcal/mol)	Glide emodel
5a		−7.311282	−42.178455	−60.375544
5b		−7.626115	−44.974126	−63.757605
5c		−7.308838	−45.354199	−63.704139
5d		−7.50468	−42.650333	−60.342029
5e		−8.54196	−44.61315	−63.130529
5f		−7.666718	−44.495791	−63.405014
5g		−6.007193	−46.855993	−67.139747
6a		−6.831427	−45.11235	−62.625068
6b		−7.452996	−45.783689	−42.820554
6c		−9.856962	−48.93824	−61.945298
6d		−10.238062	−58.242453	−70.046054
6e		−9.616341	−49.454991	−66.552017
6f		−6.571304	−48.061152	−61.449938
6g		−6.913584	−52.962205	−75.295121

Table 5 (*continued*)

Sr. No.	Compounds	Docking score	Glide energy (kcal/mol)	Glide emodel
9a		−6.436358	−38.453996	−51.320462
9b		−6.876485	−41.066372	−59.100521
9c		−6.517072	−39.232817	−51.944223
9b		−6.876485	−41.066372	−59.100521
9b		−6.876485	−41.066372	−59.100521
9c		−6.517072	−39.232817	−51.944223
9d		−6.927513	−40.685597	−53.246391
9e		−7.159149	−40.847965	−56.083704
9f		−7.163349	−43.078769	−67.151507
9g		−6.038678	−42.56103	−56.704833
9h		−6.588913	−45.4578	−57.506909
9i		−6.249185	−37.768197	−51.745678
12a		−6.299645	−43.771403	−54.995851
12b		−6.723885	−44.609527	−60.087791
12c		−7.051427	−42.216975	−58.205195
12d		−7.18204	−45.93587	−60.077071
12e		−7.248774	−45.32108	−61.212514
12f		−5.352337	−43.902908	−59.632729
12g		−5.274743	−44.391567	−62.866919
12h		−5.449304	−42.293621	−59.041235

128.91, 13.12, 10.01; HRMS (EI) *m*/*z* calcd for $C_{14}H_{10}ClN_3OS$: 303.0233; found: 303.0237.

*5.3.6. 6-(3-Aminophenyl)-2-cyclopropylimidazo[2,1-b][1,3,4] thiadiazole-5-carbaldehyde **(5f)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3346.05, 3267.37 (N—H stretch), 3105.15 (Ar C—H stretch), 2910.37 (Ali. C—H stretch), 1711.81 [(C═O) aldehyde]; ^1H NMR (DMSO-d_6) δ ppm): 9.97 (s, 1H, CHO), 7.46–7.99 (m, 4H, Ar—H), 4.85 (s, 2H, NH_2), 1.13–2.53 (m, 5H, cylopropyl); ^{13}C NMR (DMSO-d_6) δ ppm: 186.23, 166.73,157.12, 146.63, 143.12, 134.63, 132.12, 129.32, 123.83, 120.62, 118.63, 12.53, 10.01; HRMS (EI) *m*/*z* calcd for $C_{14}H_{12}N_4OS$: 284.0732; found: 284.0736.

*5.3.7. 2-Cyclopropyl-6-(2,4-dihydroxyphenyl)imidazo[2,1-b][1,3,4] thiadiazole-5-carbaldehyde **(5g)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3415.36 (O—H stretch), 3023.88 (Ar C—H stretch), 2902.81(Ali. C—H stretch), 1722.59 [(C═O) aldehyde]; ^1H NMR (DMSO-d_6) δ ppm): 10.19 (s, 1H, CHO), 7.24–7.80 (m, 3H, Ar—H), 5.49 (s, 1H, OH), 5.20 (s, 1H, OH) and 1.10–2.22 (m, 5H, cylopropyl); ^{13}C NMR (DMSO-d_6) δ ppm: 186.12, 166.63, 158.73, 157.12, 156.73, 143.63, 134.23, 130.74, 121.23, 114.73, 110.73, 13.22, 10.12; HRMS (EI) *m*/*z* calcd for $C_{14}H_{11}N_3O_3S$: 301.0521; found: 301.0526.

*5.4. General procedure for the synthesis of 2-(5-((2-cyclopropyl-6-substituted phenylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)-4-oxo-2 thioxothiazolidin-3-yl)acetic acid **6(a–g)***

A mixture of 2-cyclopropyl-6-substituted imidazo[2,1-*b*][1,3,4] thiadiazole-5-carbaldehyde **5**(**a**–**g**) (0.001 mol), piperidine (0.001 mol) and rhodanine acetic acid (0.001 mol) in toluene (50 mL) was heated under reflux with azeotropic removal of water for 16–30 h. The mixture was cooled to 5 °C, filtration gave crude compound **6(a–g)**, which was crystallized from ethanol.

*5.4.1. 2-(5-((2-cyclopropyl-6-phenylimidazo[2,1-b][1,3,4] thiadiazol-5-yl)methylene)-4-oxo-2 thioxothiazolidin-3-yl)acetic acid **(6a)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3057.02 (Ar C—H stretch), 2893.10 (Ali. C—H stretch), 2510.10 [(O—H stretch) acid], 1720.46 [(C═O) acid]; ^1H NMR (DMSO-d_6) δ ppm): 9.06 (s, 1H, OH), 8.13 (s, 1H, CH), 7.46–7.66 (m, 5H, Ar—H), 4.64 (s, 2H, CH_2), 1.34–1.62 (m, 5H, cyclopropyl); ^{13}C NMR (DMSO-d_6) δ ppm: 192.71, 166.20, 164.73, 144.62, 134.73, 132.23, 131.43, 130.34, 129.78, 128.24, 124.89, 120.23, 64.78, 44.23, 13.12, 10.73; HRMS (EI) *m*/*z* calcd for $C_{19}H_{14}N_4O_3S_3$: 442.0228; found: 442.0232.

*5.4.2. 2-(5-((2-cyclopropyl-6-(2,4-dichlorophenyl)imidazo[2,1-b] [1,3,4]thiadiazol-5-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid **(6b)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3063.95 (Ar C—H stretch), 2849.92 (Ali. C—H stretch), 2519.92 [(O—H stretch) acid], 1718.12 [(C═O) acid], 701 (C—Cl); ^1H NMR (DMSO-d_6) δ ppm): 9.24 (s, 1H, OH), 8.22 (s, 1H, CH), 7.24–7.89 (m, 3H, Ar—H), 4.49 (s, 2H, CH_2), 1.10–2.22 (m, 5H, cylopropyl); ^{13}C NMR (DMSO-d_6) δ ppm: 192.24, 166.53, 165.74, 145.74, 138.34, 136.73, 134.21, 132.73, 130.56, 128.24, 127.89, 126.35, 122.67, 120.56, 65.89, 44.56, 13.72, 10.11; HRMS (EI) *m*/*z* calcd for $C_{19}H_{12}Cl_2N_4O_3S_3$: 509.9449; found: 509.9454.

Please cite this article in press as: H.M. Patel, et al., Design, synthesis and evaluation of small molecule imidazo[2,1-*b*][1,3,4]thiadiazoles as inhibitors of transforming growth factor-β type-I receptor kinase (ALK5), European Journal of Medicinal Chemistry (2014), http://dx.doi.org/10.1016/j.ejmech.2014.09.002

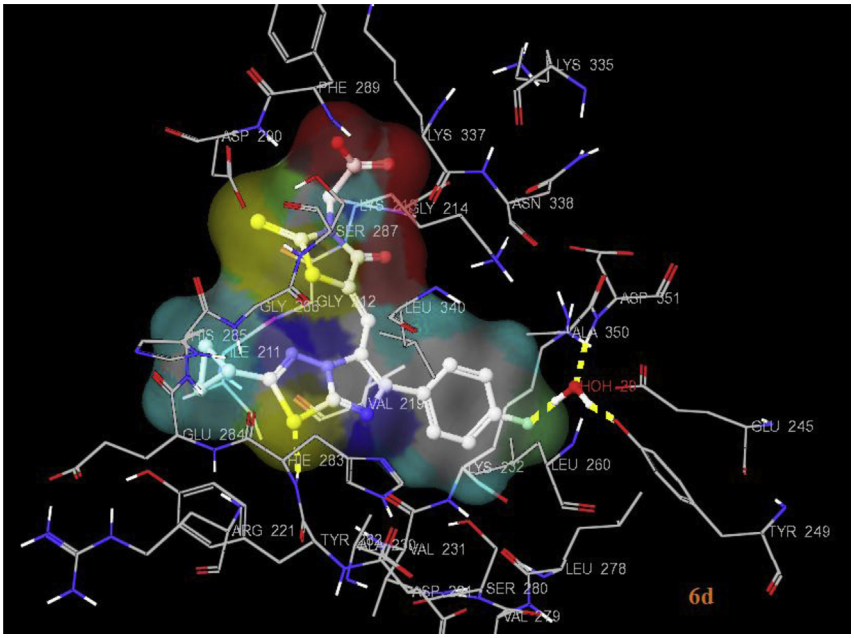


Fig. 1. Binding mode of **6d** in the X-ray crystal structure of ALK5 (PDB code: 1RW8). Compound **6d** shows hydrogen bond interaction with sulfur atom of imidazo[2,1-*b*][1,3,4] thiadiazoles hydrogen atom of amino acid backbone of Hie-283 and fluoro functional of imidazo[2,1-*b*][1,3,4]thiadiazoles with water-mediated hydrogen bond with the side chains of Tyr-249 and backbone of Asp-351.

*5.4.3. 2-(5-((6-(4-Bromophenyl)-2-cyclopropylimidazo[2,1-b] [1,3,4]thiadiazol-5-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid **(6c)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3065.95 (Ar C–H stretch), 2859.25 (Ali. C–H stretch), 2529.92 [(O–H stretch) acid], 1724.82 [(C=O) acid], 632 (C–Br); ^{1}H NMR (DMSO-d_6) δ ppm): 8.96 (s, 1H, OH), 8.19 (s, 1H, CH), 7.56–7.70 (m, 4H, Ar–H), 4.52 (s, 2H, CH_2), 1.22–2.56 (m, 5H, cylopropyl); ^{13}C NMR (DMSO-d_6) δ ppm: 193.63, 169.12, 167.63, 147.63, 138.73, 135.73, 133.83, 132.34, 128.78, 126.56, 124.34, 120.78, 64.67, 48.45, 13.34, 10.46; HRMS (EI) *m*/*z* calcd for $C_{19}H_{13}BrN_4O_3S_3$: 519.9333; found: 519.9338.

*5.4.4. 2-(5-((6-(4-Bromophenyl)-2-cyclopropylimidazo[2,1-b] [1,3,4]thiadiazol-5-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid **(6d)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3062.92 (Ar C–H stretch), 2962.33(Ali. C–H stretch), 2530.35 [(O–H stretch) acid], 1698.59 [(C=O) acid], 1052 (C–F); ^{1}H NMR (DMSO-d_6) δ ppm): 9.52 (s, 1H, OH), 8.31 (s, 1H, CH), 7.16–7.92 (m, 4H, Ar–H), 4.45 (s, 2H, CH_2), 1. 16–2.44 (m, 5H, cylopropyl); ^{13}C NMR (DMSO-d_6) δ ppm: 193.12, 169.63, 165.82, 160.12, 148.22, 138.73, 132.12, 130.32, 129.44, 122.45, 121.23, 118.12, 66.78, 49.12, 13.12, 10.23; HRMS (EI) *m*/*z* calcd for $C_{19}H_{13}FN_4O_3S_3$: 460.0134; found: 460.0140.

*5.4.5. 2-(5-((6-(4-Chlorophenyl)-2-cyclopropylimidazo[2,1-b] [1,3,4]thiadiazol-5-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid **(6e)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3057.02 (Ar C–H stretch), 2893.10 (Ali. C–H stretch), 2667.36 [(O–H stretch) acid], 1714.91 [(C=O) acid], 692 (C–Cl); ^{1}H NMR (DMSO-d_6) δ ppm) 8.95 (s, 1H, OH), 8.37 (s, 1H, CH), 7.35–7.81 (m, 4H,Ar–H), 4.42 (s, 2H, CH_2), 1.11–1.69 (m, 5H, cylopropyl); ^{13}C NMR (DMSO-d_6) δ ppm: 192.23, 169.73, 165.64, 148.24, 135.78, 133.78, 132.83, 131.24, 130.78, 129.46, 125.89, 121.36, 66.78, 48.12, 13.78, 10.12; HRMS (EI) *m*/*z* calcd for $C_{19}H_{13}ClN_4O_3S_3$: 475.9838; found: 475.9842.

*5.4.6. 2-(5-((6-(3-Aminophenyl)-2-cyclopropylimidazo[2,1-b] [1,3,4]thiadiazol-5-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid **(6f)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3268.12, 3234.27 (–NH–), 3065.95 (Ar C–H stretch), 2859.25 (Ali. C–H stretch), 2529.92 [(O–H stretch) acid], 1724.82 [(C=O) acid]; ^{1}H NMR (DMSO-d_6) δ ppm): 9.11 (s, 1H, OH), 8.18 (s, 1H, CH), 7.56–7.70 (m, 4H, Ar–H), 4.88 (s, 2H, NH_2), 4.58 (s, 2H, CH_2), 1.18–2.59 (m, 5H, cylopropyl); ^{13}C NMR (DMSO-d_6) δ ppm: 195.12, 166.56, 164.34, 144.89, 142.34, 138.78, 134.34, 130.12, 130.34, 128.92, 126.24, 121.45, 118.24, 116.89, 61.38, 42.71, 13.22, 10.12; HRMS (EI) *m*/*z* calcd for $C_{19}H_{15}N_5O_3S_3$: 457.0337; found: 457.0342.

*5.4.7. 2-(5-((2-cyclopropyl-6-(2,4-dihydroxyphenyl)imidazo[2,1-b] [1,3,4]thiadiazol-5-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid **(6g)***

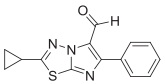
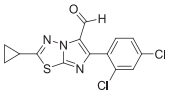
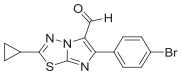
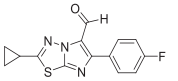
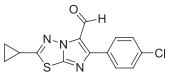
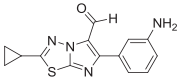
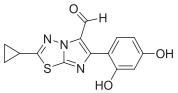
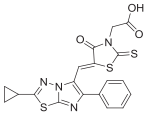
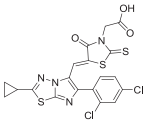
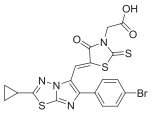
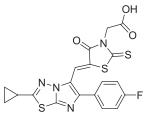
This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3451.78 (O–H stretch), 3084.89 (Ar C–H stretch), 2953.89(Ali. C–H stretch), 1722.59 [(C=O) acid]; ^{1}H NMR (DMSO-d_6) δ ppm): 10.28 (s, 1H, OH), 8.18 (s, 1H, CH), 7.24–7.80 (m, 3H, Ar–H), 5.49 (s, 1H, OH), 5.36 (s, 1H, OH), 4.21 (s, 2H, CH_2), 1.12–2.23 (m, 5H, cylopropyl); ^{13}C NMR (DMSO-d_6) δ ppm: 193.34, 169.88, 164.56, 160.34, 154.23, 148.68, 135.99, 132.78, 130.34, 128.67, 122.34, 116.89, 112.34, 110.56, 62.88, 42.71, 13.12, 10.76; HRMS (EI) *m*/*z* calcd for $C_{19}H_{14}N_4O_5S_3$: 474.0126; found: 474.0130.

*5.5. 5-m-Tolyl-1,3,4-thiadiazol-2-amine **(8)***

It is synthesized as per the reported procedure [10].

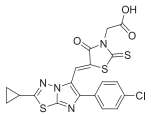
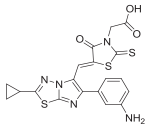
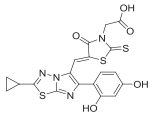
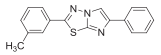
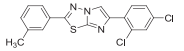
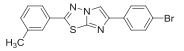
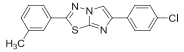
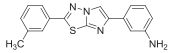
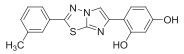
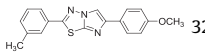
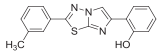
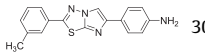
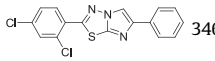
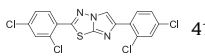
Please cite this article in press as: H.M. Patel, et al., Design, synthesis and evaluation of small molecule imidazo[2,1-*b*][1,3,4]thiadiazoles as inhibitors of transforming growth factor-β type-I receptor kinase (ALK5), European Journal of Medicinal Chemistry (2014), http://dx.doi.org/10.1016/j.ejmech.2014.09.002

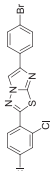
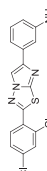
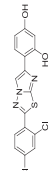
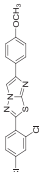
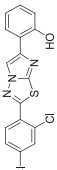
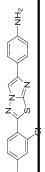
Table 6
Lipinski's rule of five for drug likeliness and in silico ADME properties of synthesized compounds **5** (**a**–**g**), **6** (**a**–**g**), **9** (**a**–**i**) and **12** (**a**–**h**) by QikProp.

Criteria →		Lipinski's rule of five (drug likeliness)					In silico ADME by QikProp, Schordinger 9.0						
Sr. No.	Compounds	Molecular weight	QPlogP O/W[a]	H-bond donor	H-bond acceptor	Violation of Lipinski's rule	QPlogS[b]	QPlogHERG[c]	QPPCaco[d]	QPMDCK[e]	QPlogKhsa[f]	% Human oral absorption[g]	Violation of rule of three
5a		269.32	2.515	0	4.5	0	−3.79	−5.048	1079.516	930.248	−0.093	95.959	0
5b		338.211	3.484	0	4.5	0	−5.196	−4.888	1104.443	4874.467	0.16	100	0
5c		348.216	3.1	0	4.5	0	−4.679	−5.019	1079.604	2469.754	0.061	100	0
5d		287.311	2.755	0	4.5	0	−4.166	−4.933	1079.63	1683.594	−0.046	100	0
5e		303.765	3.021	0	4.5	0	−4.558	−4.985	1079.567	2296.875	0.035	100	0
5f		284.335	1.844	1.5	5.5	0	−4.033	−4.923	288.321	223.274	−0.055	81.769	0
5g		301.319	1.416	2	6	0	−3.852	−4.828	137.021	99.914	−0.148	73.478	0
6a		442.525	4.271	1	7	0	−5.505	−4.167	62.11	161.587	0.284	84.047	0
6b		511.415	5.145	1	7	2	−7.654	−3.814	61.245	753.14	0.501	63.138	1
6c		521.421	4.775	1	7	1	−5.167	−3.94	57.285	368.913	0.416	73.41	0
6d		460.515	4.537	1	7	0	−5.644	−3.949	65.409	312.627	0.335	86.009	0

(*continued on next page*)

Table 6 (*continued*)

Criteria →		Lipinski's rule of five (drug likeliness)					In silico ADME by QikProp, Schordinger 9.0						
Sr. No.	Compounds	Molecular weight	QPlogP O/W[a]	H-bond donor	H-bond acceptor	Violation of Lipinski's rule	QPlogS[b]	QPlogHERG[c]	QPPCaco[d]	QPMDCK[e]	QPlogKhsa[f]	% Human oral absorption[g]	Violation of rule of three
6e		476.97	4.794	1	7	0	−5.211	−3.978	65.771	428.269	0.408	87.555	0
6f		457.539	3.412	2.5	8	0	−6.229	−3.934	17.497	41.41	0.117	69.17	2
6g		474.523	2.937	3	8.5	0	−6.078	−3.936	7.517	16.756	0.002	59.824	2
9a		291.37	4.673	0	2.5	0	−5.316	−6.216	4409.718	4228.445	0.724	100	0
9b		360.26	4.675	0	2.5	0	−5.701	−6.101	4759.477	10,000	0.974	100	0
9c		370.266	4.273	0	2.5	0	−5.287	−6.173	4409.407	10,000	0.878	100	0
9d		325.815	4.194	0	2.5	0	−5.045	−6.14	4409.216	10,000	0.852	100	0
9e		306.384	3.787	1.5	3.5	0	−5.563	−6.086	1175.906	1013.259	0.537	100	0
9f		323.369	3.34	2	4	0	−5.294	−6.003	595.04	485.343	0.407	96.161	0
9g		321.396	4.746	0	3.25	0	−5.623	−6.121	4408.952	4227.273	0.699	100	0
9h		307.369	4.109	1	3.25	0	−5.621	−6.128	1964.13	1764.445	0.596	100	0
9i		306.384	3.786	1.5	3.5	0	−5.564	−6.085	1171.015	1008.692	0.538	100	0
12a		346.233	4.956	0	2.5	0	−5.535	−6.147	4114.758	10,000	0.802	100	0
12b		415.123	4.958	0	2.5	0	−5.078	−6.03	4435.807	10,000	1.052	100	0

12c		425.129	4.876	0	2.5	0	−5.532	−6.101	4114.375	10.000	0.956	100	0
12d		361.248	4.382	1.5	3.5	0	−5.352	−6.017	1097.224	4627.844	0.608	100	0
12e		378.232	3.933	2	4	0	−5.078	−5.934	555.153	2216.848	0.475	100	0
12f		376.259	4.349	0	3.25	0	−5.186	−6.051	4112.901	10.000	0.777	100	0
12g		362.233	4.706	1	3.25	0	−5.286	−6.06	1832.321	8058.4	0.669	100	0
12h		361.248	4.381	1.5	3.5	0	−5.352	−6.015	1092.577	4606.875	0.609	100	0

[a] Predicted octanol/water partition co-efficient log *p* (acceptable range: −2.0–6.5).
[b] Predicted aqueous solubility in mol/L (acceptable range: −6.5 to 0.5).
[c] Predicted IC_{50} value for blockage of HERG K+ channels (concern below −5.0).
[d] Predicted Caco-2 cell permeability in nm/s (acceptable range: <25 is poor and >500 is great).
[e] Predicted apparent MDCK cell permeability in nm/s.
[f] Prediction of binding to human serum albumin.
[g] Percentage of human oral absorption (<25% is poor and >80% is high).

5.6. General procedure for the synthesis of 6-substituted phenyl-2-m-tolylimidazo[2,1-b][1,3,4]thiadiazole **9(a–i)**

A mixture of equimolar quantities of 5-m-tolyl-1,3,4-thiadiazol-2-amine **8** (0.01 mol) and substituted phenacyl bromides (0.01 mol) was refluxed in dry ethanol (50 mL) for 24 h. The excess of solvent was distilled off and the solid hydrobromide salt that separated out was collected by filtration, suspended in water and neutralized by sodium carbonate to get free base **9**(**a**–**i**). The product was filtered, washed with water, dried and crystallized from carbon tetrachloride.

5.6.1. 5 6-Phenyl-2-m-tolylimidazo[2,1-b][1,3,4]thiadiazole **(9a)**

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3063.52 (Ar C–H stretch), 2924.25 (Ali. C–H stretch); ^{1}H NMR (DMSO-d_6) δ ppm): 8.25 (s, 1H, C-5-H, imidazoles), 7.24–7.81 (m, 9H, Ar–H), 2.37 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 145.21, 142.34, 140.23, 138.57, 133.85, 133.72, 131.45, 130.23, 130.01, 129.24, 128.12, 127.78, 126.12, 124.78, 23.66; HRMS (EI) *m*/*z* calcd for $C_{17}H_{13}N_3S$: 291.0830; found: 291.0834.

5.6.2. 6 6-(2,4-Dichlorophenyl)-2-m-tolylimidazo[2,1-b][1,3,4] thiadiazole **(9b)**

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3021.87 (Ar C–H stretch), 2945.94 (Ali. C–H stretch), 734.78 (C–Cl); ^{1}H NMR (DMSO-d_6) δ ppm): 8.41 (s, 1H, C-5-H, imidazoles), 6.88–8.36 (m, 7H, Ar–H), 2.28 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 145.56, 143.89, 140.33, 138.67, 137.56, 134.78, 132.46, 131.23, 130.12, 130.01, 129.24, 129.00, 128.01, 127.42, 126.12, 122.34, 23.86; HRMS (EI) *m*/*z* calcd for $C_{17}H_{11}Cl_2N_3S$: 359.0051; found: 359.0055.

5.6.3. 6-(4-Bromophenyl)-2-m-tolylimidazo[2,1-b][1,3,4] thiadiazole **(9c)**

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3032.85 (Ar C–H stretch), 2987.24 (Ali. C–H stretch), 623.23 (C–Br); ^{1}H NMR (DMSO-d_6) δ ppm): 8.41 (s, 1H, C-5-H, imidazoles), 7.20–7.84 (m, 8H, Ar–H), 2.36 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 145.45, 142.68, 140.12, 138.28, 136.94, 132.81, 131.20, 130.24, 129.91, 129.12, 128.23, 126.12, 124.78, 122.34, 24.34; HRMS (EI) *m*/*z* calcd for $C_{17}H_{12}BrN_3S$: 368.9935; found: 368.9939.

5.6.4. 6-(4-Chlorophenyl)-2-m-tolylimidazo[2,1-b][1,3,4] thiadiazole **(9d)**

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3057.78 (Ar C–H stretch), 2956.78 (Ali. C–H stretch), 714.68 (C–Cl); ^{1}H NMR (DMSO-d_6) δ ppm): 8.33 (s, 1H, C-5-H, imidazoles), 7.35–8.08 (m, 8H, Ar–H), 2.41 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 145.46, 142.68, 141.42, 138.24, 136.78, 135.12, 132.24, 131.14, 130.23, 129.13, 129.12, 128.12, 126.92, 124.13, 23.34,; HRMS (EI) *m*/*z* calcd for $C_{17}H_{12}ClN_3S$: 325.0440; found: 325.0444.

5.6.5. 3-(2-m-Tolylimidazo[2,1-b][1,3,4]thiadiazol-6-yl)aniline **(9e)**

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3334.42 and 3312.43 (NH-stretch), 3083.37 (Ar C–H stretch), 2932.89 (Ali. C–H stretch); ^{1}H NMR (DMSO-d_6) δ ppm): 8.03 (s, 1H, C-5-H, imidazoles), 6.56–7.72 (m, 8H, Ar–H), 4.54 (s, 2H,NH_2), 2.33 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 149.46, 146.32, 142.12, 140.82, 138.42, 136.82, 134.46, 132.21, 131.46, 130.23, 129.12, 128.24, 126.68, 124.12, 121.78, 118.36, 23.28; HRMS (EI) *m*/*z* calcd for $C_{17}H_{14}N_4S$: 306.0939; found: 306.0935.

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*5.6.6. 4-(2-m-Tolylimidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzene-1,3-diol **(9f)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3577.32 (OH stretch), 3067.45 (Ar C–H stretch), 2923.76 (Ali. C–H stretch); ^1H NMR (DMSO-d_6) δ ppm): 13.02 (s, 1H, OH), 12.45 (s, 1H, OH), 8.41 (s, 1H, C-5-H, imidazoles), 6.22–8.35 (m, 7H, Ar–H), 2.14 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 156.46, 154.88, 148.12, 142.10, 140.98, 138.52, 134.84, 132.37, 130.43, 129.18, 128.00, 126.79, 124.83, 118.31, 115.60, 112.66, 23.46; HRMS (EI) *m*/*z* calcd for $C_{17}H_{13}N_3O_2S$: 323.0728; found: 323.0724.

*5.6.7. 6-(4-Methoxyphenyl)-2-m-tolylimidazo[2,1-b][1,3,4] thiadiazole **(9g)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3023.54 (Ar C–H stretch), 2945.83 (Ali. C–H stretch); ^1H NMR (DMSO-d_6) δ ppm): 8.41 (s, 1H, C-5-H, imidazoles), 7.61–8.36 (m, 8H, Ar–H), 3.82 (s, 3H, OCH_3), 2.31 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 158.58, 148.32, 142.24, 138.68, 136.24, 133.68, 131.42, 130.56, 129.34, 128.85, 127.19, 126.38, 122.56, 120.88, 55.28, 23.46; HRMS (EI) *m*/*z* calcd for $C_{18}H_{15}N_3OS$: 321.0936; found: 321.0940.

*5.6.8. 2-(2-m-Tolylimidazo[2,1-b][1,3,4]thiadiazol-6-yl)phenol **(9h)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3578.92 (O–H stretch), 3039.38 (Ar C–H stretch), 28,235.82 (Ali. C–H stretch),; ^1H NMR (DMSO-d_6) δ ppm): 10.72 (s, 1H, OH), 8.43 (s, 1H, C-5-H, imidazoles), 7.20–8.23 (m, 8H, Ar–H), 2.34 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 156.88, 148.34, 142.56, 140.56, 138.34, 134.78, 131.34, 130.78, 130.56, 129.67, 129.56, 127.56, 122.45, 121.46, 120.68, 118.44, 23.68; HRMS (EI) *m*/*z* calcd for $C_{17}H_{13}N_3OS$: 307.0779; found: 307.0775.

*5.6.9. 4-(2-m-Tolylimidazo[2,1-b][1,3,4]thiadiazol-6-yl)aniline **(9i)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3283.34 and 3241.56 (NH-stretch), 3034.75 (Ar C–H stretch), 2923.78 (Ali. C–H stretch); ^1H NMR (DMSO-d_6) δ ppm): 8.03 (s, 1H, C-5-H, imidazoles), 6.55–7.71 (m, 8H, Ar–H), 4.42 (s, 2H,NH_2), 2.31 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 151.45, 146.89, 142.34, 140.89, 138.22, 134.84, 130.64, 129.18, 129.01, 128.34, 127.92, 126.12, 124.34, 120.22, 23.78; HRMS (EI) *m*/*z* calcd for $C_{17}H_{14}N_4S$: 306.0939; found: 306.0934.

*5.7. 5-(2,4-dichlorophenyl)-1,3,4-thiadiazol-2-amine **(11)***

It is synthesized as per the reported procedure [10].

*5.8. General procedure for the synthesis of 2-(2,4-dichlorophenyl)-6substituted phenylimidazo[2,1-b][1,3,4]thiadiazole **12(a–g)***

A mixture of equimolar quantities of 5-(2,4-dichlorophenyl)-1,3,4-thiadiazol-2-amine **11** (0.01 mol) and substituted phenacyl bromides (0.01 mol) was refluxed in dry ethanol (50 mL) for 24 h. The excess of solvent was distilled off and the solid hydrobromide salt that separated out was collected by filtration, suspended in water and neutralized by sodium carbonate to get free base **12(a–g)**. The product was filtered, washed with water, dried and crystallized from carbon tetrachloride.

*5.8.1. 2-(2,4-dichlorophenyl)-6-phenylimidazo[2,1-b][1,3,4] thiadiazole **(12a)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3054.00 (Ar C–H stretch), 2939.21 (C–H stretch), 776.12 (C–Cl); ^1H NMR (DMSO-d_6) δ ppm): 8.62 (s, 1H, C-5-H, imidazoles), 7.24–8.57 (m, 8H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 172.24, 144.73,138.27, 136.26, 136.10, 132.56, 131.60, 130.91, 130.67, 129.24, 128.37, 127.55, 126.24, 124.63; HRMS (EI) *m*/*z* calcd for $C_{16}H_9Cl_2N_3S$: 344.9894; found: 344.9899.

*5.8.2. 6-(4-Bromophenyl)-2-(2,4-dichlorophenyl)imidazo[2,1-b] [1,3,4]thiadiazole **(12b)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3032.12 (Ar C–H stretch), 2946.78 (C–H stretch), 756.83 (C–Cl), 631.48 (C–Br); ^1H NMR (DMSO-d_6) δ ppm): 8.41 (s, 1H, C-5-H, imidazoles), 7.24–8.57 (m, 7H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 172.81, 142.78, 138.56, 136.48, 134.88, 133.36, 132.28, 132.10, 130.88, 129.56, 128.44, 127.24, 124.78, 122.66; HRMS (EI) *m*/*z* calcd for $C_{16}H_8BrCl_2N_3S$: 422.8999; found: 422.8994.

*5.8.3. 3-(2-(2,4-Dichlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl)aniline **(12c)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3334.12 and 3301.46 (NH-stretch), 3021.42 (Ar C–H stretch), 2954.23 (C–H stretch), 743.54 (C–Cl); ^1H NMR (DMSO-d_6) δ ppm): 8.45 (s, 1H, C-5-H, imidazoles), 7.23–8.16 (m, 7H, Ar–H), 4.85 (s, 2H, NH_2); ^{13}C NMR (DMSO-d_6) δ ppm: 172.68, 150.24, 143.14, 138.46, 136.78, 134.34, 132.12, 131.56, 130.24, 130.12, 129.34, 128.24, 126.64, 124.68, 120.24, 118.42; HRMS (EI) *m*/*z* calcd for $C_{16}H_{10}Cl_2N_4S$: 360.0003; found: 360.0007.

*5.8.4. 4-(2-(2,4-Dichlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzene-1,3-diol **(12d)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3523.42 (OH stretch), 3067.54 (Ar C–H stretch), 2945.89 (C–H stretch), 723.23 (C–Cl); ^1H NMR (DMSO-d_6) δ ppm): 12.02 (s, 1H, OH), 11.12 (s, 1H, OH), 8.41 (s, 1H, C-5-H, imidazoles), 6.93–8.35 (m, 6H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 172.64, 158.68, 156.24, 142.24, 138.84, 136.22, 134.78, 132.56, 131.86, 130.46, 129.24, 128.23, 126.23, 122.56, 118.34, 112.78; HRMS (EI) *m*/*z* calcd for $C_{16}H_9Cl_2N_3O_2S$: 376.9793; found: 376.9798.

*5.8.5. 2-(2,4-Dichlorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-b] [1,3,4]thiadiazole **(12e)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3042.54 (Ar C–H stretch), 2978.93 (C–H stretch), 724.23 (C–Cl); ^1H NMR (DMSO-d_6) δ ppm): 8.41 (s, 1H, C-5-H, imidazoles), 7.61–8.36 (m, 7H, Ar–H), 3.82 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 171.78, 162.66, 142.78, 138.56, 136.42, 134.78, 132.16, 131.29, 130.13, 128.45, 126.14, 124.53, 122.63, 120.68, 55.34; HRMS (EI) *m*/*z* calcd for $C_{17}H_{11}Cl_2N_3OS$: 375.0000; found: 375.0005.

*5.8.6. 2-(2-(2,4-Dichlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl)phenol **(12f)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3512.12 (OH-stretch), 3076.23 (Ar C–H stretch), 2965.21 (C–H stretch), 721.54 (C–Cl); ^1H NMR (DMSO-d_6) δ ppm): 10.52 (s, 1H, OH), 8.43 (s, 1H, C-5-H, imidazoles), 7.20–8.23 (m, 7H, Ar–H); ^{13}C NMR (DMSO-d_6) δ ppm: 172.45, 158.56, 144.68, 140.24, 138.68, 136.34, 134.24, 132.11, 130.23, 129.78, 128.68, 126.66, 124.34, 122.12, 120.78, 118.48; HRMS (EI) *m*/*z* calcd for $C_{16}H_9Cl_2N_3OS$: 360.9843; found: 360.9847.

*5.8.7. 4-(2-(2,4-Dichlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl)aniline **(12g)***

This compound was prepared and purified as per the above mentioned procedure: IR (KBr, cm^{-1}): 3087.83 (Ar C–H stretch), 2939.93 (C–H stretch), 724.12 (C–Cl); ^1H NMR (DMSO-d_6) δ ppm):

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8.46 (s, 1H, C-5-H, imidazoles), 7.23–7.96 (m, 7H, Ar–H), 4.09 (s, 2H, NH_2); ^{13}C NMR (DMSO-d_6) δ ppm: 170.34, 148.78, 146.71, 140.46, 138.65, 136.24, 132.78, 130.29, 128.34, 127.43, 126.68, 124.34, 122.88, 120.56; HRMS (EI) m/z calcd for $C_{16}H_{10}Cl_2N_4S$: 360.0003; found: 360.0008.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.09.002.

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