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ARTICLE

Synthesis and evaluation of novel imidazo[4,5-c]pyridine derivatives as antimycobacterial agents against *Mycobacterium tuberculosis*

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The current study involves the synthesis of novel imidazo[4,5-c]pyridine derivatives (IPD) contain amide/urea/sulfonamide. The synthesized compounds were evaluated for *in vitro* and *in vivo* antimycobacterial activities against *Mycobacterium tuberculosis.* The pharmacological activities were determined by the objective to better understand their structure-activity relationship (SAR) for their *in vitro* antimycobacterial activity against *M. tuberculosis.* Some synthesized compounds showed significant activity against *M. tuberculosis* by the agar dilution method. Among forty-one compound screened, compounds **21, 22** and **23** were found to be the most active compounds against *M. tuberculosis*. In the *in vivo* animal model, **21**, **22** and **23** decreased the bacterial load in lung and spleen tissues at the dose of 50 mg/kg body weight.

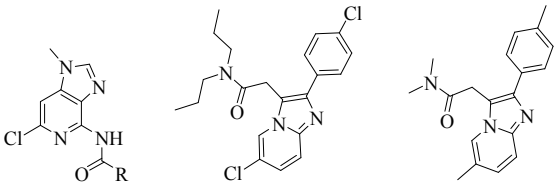
Introduction

Mycobacterium tuberculosis is a pathogenic bacteria and the causative agent of most cases of tuberculosis (TB). TB is one of the most prevalent diseases and is responsible for the deaths of about one billion people during the last two centuries.[1] The statistics show that around 32% of the world's population are infected with *M. tuberculosis*, the main causal agent of TB and today more people die from tuberculosis than ever before.[2] According to estimates from the World Health Organization (WHO), TB is a frequently fatal infectious disease that causes more than 1.4 million deaths annually and there were an estimated 8.7 million new cases of TB every year.[3]

TB is an airborne infectious disease that often remains in its latent form, it is very different from that for other bacterial infections. The organism has a long generation time and a capacity for dormancy when its low metabolic activity makes it a difficult therapeutic target.[4] In addition, *M. tuberculosis* may be located in pulmonary cavities, empyemapus, or solid caseous material, where penetration of antibiotics is difficult or the pH is sufficiently low to inhibit the activity of most antibiotics.[5] Despite the fact that the present chemotherapy has a cure rate of up to 95%, because of poor patient compliance arising from prolonged therapy, the disease has been spreading at a steady rate. This leads to the evolution of multidrug-resistant (MDR-TB) and extensively drug-resistant TB causing a major impediment in treating the disease.[6]

The resurgence in TB is alarming due to the development of pathogenic synergy with HIV.[7] The currently employed first-line drugs, isoniazid, rifampin, ethambutol, and pyrazinamide for the initial 2 months and rifampin and isoniazid for an additional 4 months. The need for such lengthy treatment is large because the drugs are relatively ineffective against the persistent form of the disease[8] and second-line agents such as kanamycin, p-aminosalicylic acid, ciprofloxacin or cycloserine[9] also suffer from associated side-effects and poor efficacy in eradicating dormant pathogens. In the last 50 years, only a few drugs have been approved by the Food and Drug Administration (FDA) to treat TB, which reflects the inherent difficulties in the discovery and clinical testing of new agents.[10] Hence, the discovery of fast-acting effective newer drugs to effectively cure TB, including multidrug resistant tuberculosis, is imperative.

Drugs such as necopidem, saripidem, alpidem, zolpidem, and olprinone contain nitrogen-containing bicyclic, condensed-imidazopyridines as bioactive scaffolds[11-13] and ironically bears strong structural similarity to the imidazopyridine antitubercular agents (Fig. 1). With its bioisosterimidazo ring (Fig. 1). Further, the 3D structural similarity and Low r.m.s.d. (root mean square distance) value suggested the good 3D structural similarity between the designed molecule and zolpidem. This prompted us to synthesize a series of imidazo[4,5-c]pyridine derivatives and check their antimycobacterial potential we discovered to have outstanding in vitro potency against both replicating Mtb $H_{37}Rv$



IPD 11-50 Alpidem Zolpidem

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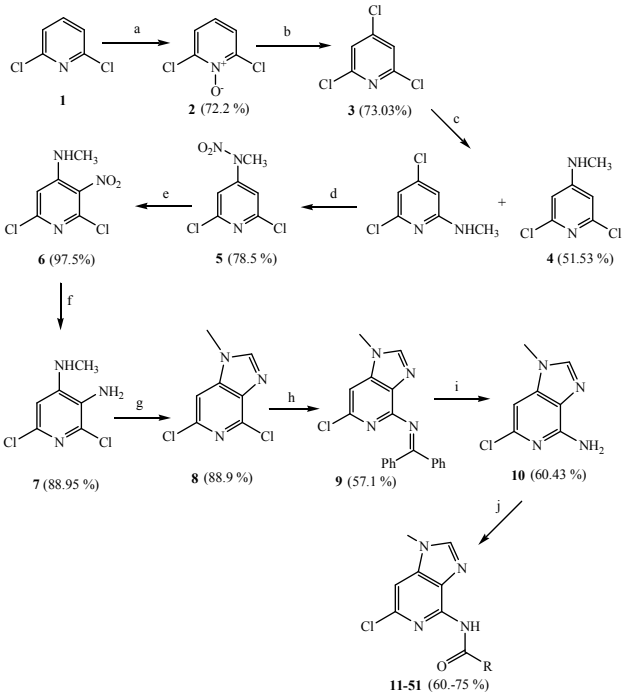
Fig. 1 Structures of IPD, Alpidemand Zolpidem

Results and discussion

A general route for the synthesis of the title compounds imidazo[4,5-c]pyridine derivatives (**11-51**) is depicted in Scheme 1. A variety of combinatorial approaches has described by which pharmacophoric groups were attached to such relatively rigid scaffold. The synthesis begins with chlorination of 2,6-dichloropyridine using trifluoroacetic acid, hydrogen peroxide, and $POCl_3$. The Chloro - amine coupling was done using methylamine and ethanol solvent.[14] The nitration was done using fuming nitric acid and sulfuric acid. The reduction was carried using iron powder, ammonium chloride, and methanol; water solvent. Construction of imidazole ring was made by triethylorthoformate and ethanol at refluxed temperature. The carbon-nitrogen bond was done by Buchwald coupling using benzophenone imine, xantphos, cesium carbonate Tris(dibenzylideneacetone)dipalladium(0) and dioxane solvent at refluxed temperature. Target key intermediate was accomplished by cleavage of imine bond[15] with 1.5N HCl and followed by basification with NaOH solution to pH-10. The aim of the tenth step was introduced by acid chloride, isocyanate and sulfonyl chloride to lead desired final compounds. This was furnished by normal nucleophilic addition / elimination reaction[16,17] from acid chlorides/ isocyanate / sulfonyl chloride, with good yield. The formation of each compound was confirmed by ^{1}H NMR, ^{13}C NMR and MS spectra.

It has been observed that in 6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-amine scaffold, the amine group is a more reactive site. However, no such increase of reactivity was required in the present reaction system and the final compounds were done workup and purified by column chromatography to get pure products

The absence of NH_2 absorption band at 1620 cm^{-1} in the IR spectra confirmed that the synthesized compounds were obtained *via* condensation. The appearance of a strong absorption band at around 3435 cm^{-1} is the stretching vibration due to the presence of -NH band in the synthesized compounds. The proton spectral data agree with respect to the number of protons and their chemical shifts with the proposed structures. The proton spectral data of the intermediate, *N*-substituted imidazo[4,5-c]pyridine **10** shows resonance at δ 6..63 ppm (s, 2H, NH_2). In all the synthesized compounds, the above resonance disappeared and additional resonances assigned to the –NH–C=O (s, 11.12–10.92 ppm), –NH–C=O (s, 11.23 and 9.39 ppm) and–NH–S=O (s, 11.90–11.05 ppm) were observed, which confirmed the condensation between the amino group and carbonyl group of isocyanate and sulfonyl compounds, respectively. The chemical formula, physical data and yield of all the synthesized compounds are given in Table 1.



Scheme 1 Reagents and conditions: a) trifluoroacetic acid, 30 % hydrogen peroxide, 90 °C, 12 h; b) $POCl_3$, reflux, 12 h; c) methylamine, ethanol, 12 h, 40 °C; d) sulfuric acid, fuming nitric acid, 1h, -10 °C – 0 °C; e) sulfuric acid, RT, 12 h; f) iron power, ammonium chloride, methanol-water, 3 h, 90 °C; g) triethyl orthoformate, ethanol, 36 h, 105 °C; h) benzophenone imine xantphos, cesium carbonate, Tris(dibenzylidene acetone) dipalladium(0), dioxane, 18 h, 110 °C; i) 1.5 N HCl, NaOH, RT , 6 h; j) sodium hydride, acid chloride/isocyanate/sulfonyl chloride, DMF, RT, 18 h.

Table 1 Chemical structure and yield of the compounds (11-51)

Compound	R	Yield (%)
11	C_6H_5CO	75
12	2-$H_3CC_6H_4CO$	63
13	3-$H_3CC_6H_4CO$	67
14	4-$H_3CC_6H_4CO$	64
15	2- ClC_6H_4CO	61
16	3- ClC_6H_4CO	62
17	4- ClC_6H_4CO	65
18	2- FC_6H_4CO	66
19	3- FC_6H_4CO	63
20	4- FC_6H_4CO	64
21	2- $F_3CC_6H_4CO$	62
22	3- $F_3CC_6H_4CO$	63
23	4- $F_3CC_6H_4CO$	63
24	3- NCC_6H_4CO	66
25	4- NCC_6H_4CO	66
26	C_6H_5NCO	73
27	$C_6H_4CH_2NCO$	65
28	2-$H_3CC_6H_4NCO$	68
29	3-$H_3CC_6H_4NCO$	65
30	4-$H_3CC_6H_4NCO$	67
31	2- ClC_6H_4NCO	66

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32	3- ClC_6H_4NCO	64
33	4- ClC_6H_4NCO	62
34	2- FC_6H_4NCO	70
35	3- FC_6H_4NCO	72
36	4- FC_6H_4NCO	71
37	3- NCC_6H_4NCO	66
38	4- NCC_6H_4NCO	70
39	$C_6H_5SO_2$	71
40	$C_6H_4CH_2SO_2$	66
41	2-$H_3CC_6H_4SO_2$	64
42	3-$H_3CC_6H_4SO_2$	62
43	2-$ClC_6H_4SO_2$	60
44	3-$ClC_6H_4SO_2$	61
45	4-$ClC_6H_4SO_2$	63
46	3-$BrC_6H_4SO_2$	60
47	2-$FC_6H_4SO_2$	67
48	3-$FC_6H_4SO_2$	65
49	4-$FC_6H_4SO_2$	63
50	3-$NCC_6H_4SO_2$	60
51	4-$NCC_6H_4SO_2$	66

The compounds were screened for their *in vitro* antimycobacterial activity against *M. tuberculosis* by the agar dilution method[18] for the determination of MIC in triplicates. The MIC is defined as the minimum concentration of compound required to inhibit 99% of bacterial growth, and the MIC values of the synthesized compounds along with the standard drugs for comparison are presented in Table 2. As evident from the Table 2, as do many compounds belonging to sulfonamide series show indeed moderate antimycobacterial activity (MIC >25-6.25 µM), in sharp contrast to amide series, wherein most others displayed good activity, suggesting that electronegative atom like fluorine (lipophilic group) in all amide, urea, and sulfonamide, is a key structural element for antimycobacterial activity.

It was also gratifying to note that many compounds of the amide, urea, and sulfonamide respectively, showed enhanced activity against *M. tuberculosis* with MIC ranging from 0.25 to >25.00 μM. Eight compounds, **18, 19, 20, 34, 35, 36, 47** and **48** were more potent than the standard drug ethambutol (MIC: 7.64 μM), while three compounds, **21, 22** and **23** were more potent than the standard drug isoniazid (MIC: 0.36 μM), Compounds **21, 22** and **23** with a MIC value of 0.25 μM, were found to be the most potent in the library, being 1.4, 18.8 and 30.6 times more active than isoniazid, ciprofloxacin, and ethambutol, respectively. However, all the compounds were less active than rifampicin (MIC: 0.12 μM).

Table 2 *In vitro* antimycobacterial activity of the Compounds (11-51) against MTB H37Rv

Compound	MTB[a] (MIC) (μM)
11	>25
12	>25
13	>25
14	>25
15	25±0.13
16	25±0.17
17	12.5±0.08
18	6.25±0.12
19	6.25±0.26
20	6.25±0.19
21	0.25±0.04
22	0.25±0.16
23	0.25±0.11
24	25±0.34
25	25±0.08
26	25±0.15
27	25±0.17
28	25±0.24
29	25±0.29
30	12.5±0.03
31	12.5±0.18
32	12.5±0.13
33	25±0.21
34	6.5±0.07
35	6.5±0.14
36	6.5±0.26
37	>25
38	>25
39	>25
40	>25
41	>25
42	25±0.32
43	25±0.21
44	12.5±0.29
45	25±0.17
46	12.5±0.14
47	6.5±0.11
48	6.5±0.05
49	12.5±0.18
50	>25
51	>25
Rifampicin	0.12±0.03
Isoniazid	0.36±0.12
Ciprofloxacin	4.71±0.07
Ethambutol	7.64±0.15

[a] *mycobacterium tuberculosis* H37Rv

To improve antimycobacterial potency and explore the structure-activity relationships, the authors has synthesized a series of 40 new *N*-substituted imidazo[4,5-c]pyridine derivatives having different groups at phenyl ring. However, the authors have performed the biological activity on three series of compounds like amide, urea, and sulfonamide in that, the results demonstrated that the antimycobacterial activity was in the order: amide >urea > sulfonamide. On the basis of the above, six compounds in the amide series (**18, 19, 20, 21, 22** and **23**), three in series urea (**34, 35** and **36**), and two in series sulfonamide (**47** and **48**) were more active against *M. tuberculosis*. Among these compounds from the series amide, urea, and sulfonamide, compounds **21, 22** and **23** (MIC: 0.25 µM) were found to be more active. This was due to

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amide bond the electron-withdrawing nature of the carbonyl group where the lone pair of electrons on the nitrogen is delocalized by resonance, and in urea the more electronegative oxygen atom pulls electrons away from the carbon forming a greater electron density around the oxygen, giving the oxygen a partial negative charge and forming a polar bond. In the sulfonamide, the electron-withdrawing nature of the sulfur group where the lone pair of electrons on the nitrogen is delocalized by resonance.

Further, to increase structure-activity relationship, the authors have introduced the different substituents at different positions of aryl rings like simple phenyl ring, electron donating methyl group, moderated electronegative atom chlorine, strong electronegative fluorine and trifluoromethyl groups and electron withdrawing cyano groups were examined. The results demonstrated that the simple phenyl ring bearing compounds **11, 26, 27, 39** and **40** were less active (MIC:>25-25 μM). The electron donating (hydrophobic) methyl group at different positions ortho, meta and para on the phenyl ring bearing compounds **12, 13, 14, 28, 29, 30, 41** and **42** showed moderate activity (MIC: >25-12.5 μM). Similarly, the moderate electronegative chlorine atom at different positions ortho, meta and para on the phenyl ring bearing compounds **16, 17, 31, 32, 33, 43, 44** and **45** showed good activity (MIC: 25-12.5 μM). In this series, the more electronegative element (lipophilic groups) fluorine and trifluoromethyl groups at different positions ortho, meta and para on the phenyl ring bearing compounds **18, 19, 20, 34, 35, 36, 47, 48** and **21, 22, 23** display maximum potent antimycobacterial activity (MIC: 12.5-6.5 μM) (MIC: 0.25 μM).[19-21] The influence of electron withdrawing group (cyano group) at different positions on the phenyl ring bearing compounds **24, 25, 37, 38, 50** and **51,** markedly decreased the antimycobacterial activity (MIC: >25-25 μM).

Synthesis and antimycobacterial activity of certain novel imidazo[4,5-c]pyridine derivatives have been carried out. Some of the synthesized compounds showed significant activity against *M. tuberculosis*. Further studies on the synthesis and examination of structure-activity relationships of a wide range of heterocyclic compounds with various substituents at phenyl ring and *N* atom of the imidazopyridine moiety suggest that the more electronegative (lipophilic groups) group like fluorine (**18, 19, 20, 34, 35, 36, 47** and **48**) and trifluoromethyl groups (**21, 22** and **23**) on phenyl ring compounds increases the activity to display good *in vitro* antimycobacterial activity against *M. tuberculosis*, among which three compounds (**21, 22** and **23**) were more potent than the standard first-line drugs isoniazid, ciprofloxacin, and ethambutol. And, the result shows that the antimycobacterial activity was in the order: amide >urea > sulfonamide. In comparison with the antibiotics commonly used in the therapy, test compounds were found significantly potent.

Table 3 Cytotoxicity of 21, 22, 23 and isoniazid in a mammalian Vero cell line

Compound	IC_{50} (μM)
21	169.19
22	183.35
23	162.07
Isoniazid	>455.78

Some compounds were further examined for toxicity (IC_{50}) in a mammalian Vero cell line.[22] The IC_{50} values of compounds **21, 22** and **23** were found to be 169.07, 183.35 and 162.07 μM, respectively (Table 3). In this study, interpretation of the *in vitro* assay (MIC) data assisted in the selection of compounds for further testing *in vivo*. In this regard, three compounds (**21, 22** and **23**) along with isoniazid (INH) as reference compound were selected for *in vivo* antimycobacterial efficacy against *M. tuberculosis* at a dose of 50 mg/kg (Table 4) in CD-1 mice.[23] Compound **23** decreased the bacterial load in lung and spleen tissues with 2.47 and 3.68 log 10 reductions, while compound **21** (2.43 and 3.56) and **22** (1.81 and 2.62) showed, log 10 reductions in lungs and spleen, respectively. All these compounds (**21, 22** and **23**) were considered to be promising in reducing bacterial count in lung and spleen tissues. As can be seen in Table 4, the compounds **21** and **23** demonstrated efficacy comparable to INH in mice lungs, while **22** was less efficacious. Generally, the compounds were more effective in lungs. When compared with isoniazid at the same dose level, **23** decreased the bacterial load with 0.28 and 0.50 log10 protections in lung and spleen tissues, respectively, while that of compound **21** with 0.24 and 0.62 and compound **22** with 0.38 and 1.56. These data together suggest that imidazopyridine derivatives may represent promising lead candidates against TB that are worthy of further investigation.

Table 4 *In vivo* activity data of 21, 22, 23 and isoniazid against MTB ATCC 35801 in mice

Compound	Lungs (log CFU ± SEM)	Spleen (log CFU ± SEM)
Control	7.92 ± 0.13	8.97 ± 0.10
21 (50 mg/kg)	5.49 ± 0.01	5.41 ± 0.18
22 (50 mg/kg)	6.11 ± 0.17	6.35 ± 0.13
23 (50 mg/kg)	5.45 ± 0.09	5.29 ± 0.16
Isoniazid (25 mg/kg)	5.73 ± 0.03	4.79 ± 0.18

Conclusions

The compounds (**21, 22** and **23**) containing trifluoro methyl group showed potent activity similar to that of standard drugs acting. The antioxidant activity of synthesized molecules was evaluated by DPPH method. The compounds with electron donating moiety (methyl) seem to give good results. The compounds **21, 22** and **23** were more potent than the standard first-line drugs isoniazid, ciprofloxacin, and ethambutol. In comparison with the antibiotics commonly used in the therapy, these data together suggest that imidazopyridine derivatives may represent promising lead candidates against TB that are worthy of further investigation. Additional studies are planned to further assess the *in vivo* efficacy of compounds **21, 22** and **23** in the standard mouse model of TB. The synthesized compounds were screened *in vitro* for their antimicrobial activities and showed variable activities.

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Experimental

General: Melting points were determined in one end open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra were recorded on Bruker Avance-400 MHz NMR instrument using TMS as an internal solvent and DMSO-d_6/$CDCl_3$ as a solvent. Chemical shift is given in part per million (δ-scale) and coupling constant is given in Hertz. Mass spectra were recorded on Perkin–Elmer LC-MS PE Sciex API/65 Spectrophotometer. IR spectra were recorded using KBr on 8400S Shimadzu Fourier Transform Spectrophotometer ($_{max}$ in cm^{-1}). Column chromatography was performed using Merck 7734 silica gel (60-120 mesh) and Merck made pre-coated TLC plates were used. Elemental analysis (C, H, and N) was undertaken with Perkin-Elmer model 240C analyzer.

Synthesis

N-oxide 2,6-dichloropyridine (2). 2,6-Dichloropyridine (50 g, 0.34 mol), trifluoroacetic acid (TFA) (300 mL, 3.38 mol) and hydrogen peroxide 30% in water (75 mL) were mixed slowly, then it was stirred at 90 °C for 12 h. After completion of the reaction (TLC), TFA was removed under vacuum and the reaction mixture was neutralized with the Na_2CO_3 solution, extracted with CH_2Cl_2 (3 x 500 mL) and dried over anhydrous Na_2SO_4. The resulting precipitate was concentrated *in vacuo* to give the crude product which was recrystallized from hexane to get a cream colored pure solid. Cream color solid; (40 g, 72.2 %); ^1H NMR (400 MHz, $CDCl_3$): δ=7.12 (t, J = 8.00 Hz, 1H), 7.45 (d, J = 8.40 Hz, 2H ppm; MS: m/z 163.99 (M^+), 165 (M+1); Anal. calcd for $C_5H_3Cl_2NO$: C, 36.62; H, 1.84; N, 8.54, Found: C, 36.64; H, 1.82; N, 8.56.

2,4,6-trichloropyridine (3)

Mixture of *N*–oxide-2,6-dichloropyridine (40 g, 0.24 mol) and $POCl_3$ (79.57 mL, 0.85 mol), were refluxed for 12 h. After completion of the reaction (TLC), $POCl_3$ was removed under vacuum and the reaction mixture was neutralized with Na_2CO_3 solution, extracted with CH_2Cl_2 (3 x 400 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give crude product as black liquid. Light black liquid; (32.5g, 73.03%); ^1H NMR (400 MHz, $CDCl_3$): δ=7.28 (d, J = 7.60 Hz, 2H) ppm; MS: m/z 182.4 (M^+), 183.4 (M+1); Anal. calcd for $C_5H_2Cl_3N$: C, 32.92; H, 1.10; N, 7.68, Found: C, 32.94; H, 1.12; N, 7.70.

2,6-dichloro-N-methylpyridine-4-amine (4)

Mixture of 2,4,6-trichloropyridine (32.5 g, 0.18 mol) and methylamine (130 mL, 30 % solution) in ethanol (33 mL) were heated on a oil bath with stirring at 40 °C for 12 h. After completion of the reaction (TLC), reaction mixture was filtered. The crude product was recrystallized from diethyl ether to get white colored solid. White solid; (16.25 g, 51.53 %); ^1H NMR 400 MHz, [D_6]DMSO: δ=7.34 (d, J = 4.80 Hz, 1H), 6.53 (s, 2H), 2.73 (d, J = 4.80 Hz, 3H) ppm; MS: m/z 177 (M^+), 178 (M+1); Anal. calcd for $C_6H_6Cl_2N_2$: C, 40.71; H, 3.42; N, 15.82, Found: C, 40.72; H, 3.44; N, 15.81.

2,6-dichloro-N-methyl-N-nitro pyridine-4-amine (5). A mixture of 2,6-dichloro-*N*-methylpyridine-4-amine (16.25 g, 0.09 mol) in sulfuric acid (40.6 mL) was cooled to -10 °C then fuming nitric acid was added slowly, Then it was stirred at 0 °C for 1h. After completion of the reaction (TLC), the reaction mixture was poured into crushed ice , extracted with CH_2Cl_2 (3 x 160 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the product which was recrystallized from hexane to get yellow colored solid. Yellow solid; (16 g, 78.5 %); ^1H NMR (400 MHz, $CDCl_3$): δ=7.29 (d, J = 8.20 Hz, 2H), 3.75 (s, 3H) ppm; MS: m/z 222 (M^+), 223 (M+1); Anal. calcd for $C_6H_5Cl_2N_3O_2$: C, 32.46; H, 2.27; N, 18.93, Found: C, 32.41; H, 2.20; N, 18.98.

2,6-dichloro-N-methyl-3-nitro pyridine-4-amine (6)

A mixture of 2,6-dichloro-*N*-methyl-*N*-nitropyridine-4-amine (16 g, 0.07 mol) in sulfuric acid (40 mL) was stirred at room temperature for 12 h. Completion of the reaction was checked by TLC, then the reaction mixture was poured in to crushed ice, extracted with CH_2Cl_2 (3 x 160 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to get the yellow colored solid. Yellow solid; (15.6 g, 97.5%); ^1H NMR 400 MHz, [D_6]DMSO: δ=7.76 (d, J = 4.00 Hz, 1H), 6.99 (s, 1H), 2.84 (d, J = 4.80 Hz, 3H); MS: m/z 222.1 (M^+), 223 (M+1); Anal. calcd for $C_6H_5Cl_2N_3O_2$: C, 32.46; H, 2.27; N, 18.93, Found: C, 32.40; H, 2.21; N, 18.95.

2,6-dichloro-N-4-methylpyridine-3,4-diamine (7). A mixture of 2,6-dichloro-*N*-methyl-3-nitropyridine-4-amine (15.6 g, 0.07 mol), iron powder (23.54 g, 0.4215 mol), ammonium chloride (30.06 g, 0.56 mol) and methanol; water (78 mL) was heated on a oil bath with stirring at 90 °C for 3 h. After completion of the reaction (TLC), the reaction mixture was filtered through celite, extracted with CH_2Cl_2 (3 x 150 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the brown colored solid. Brown solid; (12 g, 88.95 %); ^1H NMR 400 MHz, [D_6] DMSO: δ=6.32 (t, J = 4.00 Hz, 2H), 4.90 (s, 2H), 2.78 (d, J = 4.80 Hz, 3H)), MS: m/z 192 (M^+), 193 (M+1); Anal. calcd for $C_6H_7Cl_2N_3$: C, 37.52; H, 3.67; N, 21.88, Found: C, 37.48; H, 3.61; N, 21.82.

4,6-dichloro-1-methyl-1H-imidazo [4,5-c]pyridine (8). Mixture of 2,6-dichloro-*N*-4-methylpyridine-3,4-diamine (12 g, 0.06 mol) and triethylorthoformate (48 mL) in ethanol (60 mL) were heated on a oil bath with stirring at 105 °C for 36 h. After completion of the reaction (TLC), the reaction mixture was concentrated *in vacuo* to give the crude product which was recrystallized from ethyl acetate: hexane mixture (50:50) to get brown colored solid. Brown solid; (10.5 g, 88.9 %); ^1H NMR 400 MHz, [D_6]DMSO: δ=8.48 (s, 1H), 7.92 (s, 1H), 3.87 (s, 3H); MS: m/z 202 (M^+), 203 (M+1); Anal. calcd for $C_7H_5Cl_2N_3$: C, 41.61; H, 2.49; N, 20.80, Found: C, 41.58; H, 2.45; N, 20.86.

6-chloro-1-methyl-N-(diphenylmethylene)-1H-imidazo[4,5-c] pyridine-4-amine (9)

A mixture of 4,6-dichloro-1-methyl-1H-imidazo[4,5-c]pyridine (10.5 g, 0.34 mol), benzophenone imine (15.1 g, 0.08 mol) xanthphose (1.60 g, 0.002 mol), cesium carbonate (36.22 g ,0.11 mol) and Tris(dibenzylideneacetone) dipalladium(0) (3.19 g, 0.005 mol) in dioxane (110 mL) was degassed using argon, Then it was stirred at 110 °C for 18 h. After completion of the reaction (TLC), dioxane was removed under vacuum, extracted with ethyl acetate (3 x 100 mL) .dried over Na_2SO_4 and concentrated *in vacuo* to give the crude product, which was purified by column chromatography on silica employing ethyl acetate – hexane (1:4 v/v) as an eluent to obtain pure 6-chloro-1-methyl-*N*-(diphenylmethylene)-1H-imidazo[4,5-c]pyridine-4-amine brown colored solid. Brown solid; (11g, 57.1 %); ^1H NMR 400 MHz, [D_6]DMSO: δ=8.16 (s, 1H), 7.52-7.71 (m, 5H), 7.36 (s, 1H), 7.15-7.24 (m, 5H), 3.74 (s, 3H); MS: m/z 346.81 (M^+), 348 (M+1); Anal. calcd for $C_{20}H_{15}ClN_4$: C, 69.26; H, 4.36; N, 16.15, Found: C, 69.20; H, 4.31; N, 16.11.

6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-amine (10)

A mixture of 6-chloro-1-methyl-*N*-(diphenylmethylene)-1H-imidazo[4,5-c] pyridine-4-amine (11 g, 0.03 mol) in 1.5 N HCl (100 mL) was stirred at room temperature for 6 h. After completion of the reaction (TLC), the reaction mixture was neutralized with NaOH solution to pH-10, extracted with CH_2Cl_2 (3 x 100 mL), dried over

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Na_2SO_4 and concentrated *in vacuo* to give the crude product. The formed product was purified by column chromatography on silica employing methanol–CH_2Cl_2 (1:4 v/v) as an eluent to obtain pure 6-chloro-1-methyl- -1H-imidazo[4,5-c]pyridine-4-amine colorless solid. White solid; (3.5 g, 60.43 %); ^{1}H NMR 400 MHz, [D_6]DMSO: δ=8.02 (s, 1H), 6.84 (s, 1H), 6.64 (s, 2H), 3.72 (s, 3H); MS: m/z 182.6 (M^+), 183.6 (M+1); Anal. calcd for $C_7H_7ClN_4$ C, 46.04; H, 3.86; N, 30.68, Found, C, 46.08; H, 3.90; N, 30.72.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl) amide (11-25)

A mixture of sodium hydride (26.28 mg, 1.09 mmol), 6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-amine (100 mg, 0.54 mmol), acid chloride (0.65 mmol) in dimethylformamide (6 mL), was stirred at room temperature for 18 h. After completion of the reaction (TLC), it was quenched with saturated NH_4Cl solution, extracted with ethyl acetate (3 x 4 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give the crude product, which was purified by column chromatography on silica employing $CHCl_3$/MeOH (100:0!90:10) as an eluent to obtain pure solid.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)benzamide ***(11)*** White solid; (118 mg, 75 %): *R*f = 0.77 ($CHCl_3$/MeOH, 9:1); mp:166-168°C; ^{1}H NMR 400 MHz, [D_6]DMSO: δ=10.90 (s, 1H), 8.32 (s, 1H), 8.04 (d, J = 1.48 Hz, 2H), 7.76 (s, 1H), 7.62 (d, J = 7.40 Hz, 1H), 7.55 (t, J = 7.24 Hz, 2H), 3.87 (s, 3H) ppm; IR (KBr): ῡ = 3415, 1655 cm^{-1}; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=164.46, 161.28, 142.92, 140.73, 140.71, 134.12, 132.21, 128.94, 128.92, 127.14, 127.12, 125.54, 94.42, 30.85 ppm; MS: m/z 286.7 (M^+), 287.7 (M+1); Anal. calcd for $C_{14}H_{11}ClN_4O$ C, 58.65; H, 3.87; N, 19.54, found, C, 58.61; H, 3.82; N, 19.50.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-2-methylbenzamide ***(12).*** White solid; (115 mg, 63 %): *R*f = 0.79 ($CHCl_3$/MeOH, 9:1); mp: 169-171 °C ; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=10.72 (s, 1H), 8.33 (s, 1H), 7.72 (s, 1H), 7.52 (d, J = 7.32 Hz, 1H), 7.39 (t, J = 7.12 Hz, 1H), 7.26-7.28 (m, 2H), 3.86 (s, 3H), 2.48 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=164.51, 161.21, 142.96, 140.74, 140.72, 137.19, 135.19, 132.26, 128.96, 128.94, 126.12, 125.56, 94.41, 30.85, 24.38 ppm; IR (KBr): ῡ = 3418, 1660 cm^{-1}; MS: m/z 300.7 (M^+), 302 (M+1); Anal. calcd for $C_{15}H_{13}ClN_4O$ C, 59.91; H, 4.36; N, 18.63, found, C, 59.96; H, 4.32; N, 18.59.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-methyl benzamide ***(13).*** White solid; (110 mg, 67 %): *R*f = 0.76 ($CHCl_3$/MeOH, 9:1); mp: 171-173 °C ; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=10.82 (s, 1H), 8.32 (s, 1H), 7.83-7.83 (m, 2H), 7.75 (s, 1H), 7.43 (d, J = 5.92 Hz, 2H), 3.07 (s, 3H), 2.41 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=164.48, 161.19, 142.92, 140.75, 140.73, 137.16, 135.14, 132.21, 128.92, 128.90, 126.15, 125.52, 94.46, 30.81, 24.02 ppm; IR (KBr): ῡ = 3421, 1663 cm^{-1}; MS: m/z 300.7 (M^+), 302(M+1); Anal. calcd for $C_{15}H_{13}ClN_4O$ C, 59.91; H, 4.36; N, 18.63, found, C, 59.96; H, 4.32; N, 18.59.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-4-methyl benzamide ***(14).*** White solid; (106 mg, 64 %): *R*f = 0.77 ($CHCl_3$/MeOH, 9:1); mp: 168-170 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=10.80 (s, 1H), 8.31 (s, 1H), 7.95 (d, J = 8.12 Hz, 2H), 7.74 (s, 1H), 7.35 (d, J = 8.08 Hz, 2H), 3.89 (s, 3H), 2.41 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=164.51, 161.26, 142.91, 140.75, 140.74, 137.08, 135.14, 132.19, 128.88, 128.86, 126.18, 125.51, 94.41, 30.83, 24.10 ppm; IR (KBr): ῡ = 3419, 1662 cm^{-1}; MS: m/z 300.7 (M^+), 302 (M+1); Anal. calcd for $C_{15}H_{13}ClN_4O$ C, 59.91; H, 4.36; N, 18.63, found, C, 59.96; H, 4.32; N, 18.59.

2-chloro-N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl) benzamide ***(15).*** White solid; (108 mg, 61 %): *R*f = 0.61 ($CHCl_3$/MeOH, 9:1); mp: 183-185 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.00 (s, 1H), 8.33 (s, 1H), 7.69 (d, J = 2.00 Hz, 2H), 7.42-7.58 (m, 4H), 3.84 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=164.61, 161.51, 142.98, 140.89, 140.88, 134.42, 132.41, 132.23, 129.98, 128.87, 127.30, 126.32, 94.51, 30.86 ppm; IR (KBr): ῡ = 3424, 1669 cm^{-1}; MS: m/z 321.1 (M^+), 322 (M+1); Anal. calcd for $C_{14}H_{10}Cl_2N_4O$ C, 52.36; H, 3.14; N, 17.45, found, C, 52.32; H, 3.12; N, 17.41.

3-chloro-N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)benzamide ***(16).*** White solid; (109 mg, 62 %): *R*f = 0.64 ($CHCl_3$/MeOH, 9:1); mp: 181-183 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.06 (s, 1H), 8.33 (s, 1H), 7.56-8.07 (m, 6H), 3.86 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=164.66, 161.59, 142.96, 140.87, 140.86, 135.31, 134.47, 132.41, 129.94, 128.82, 126.31, 125.67, 94.52, 30.83 ppm; IR (KBr): ῡ = 3428, 1671 cm^{-1}; MS: m/z 321.1 (M^+), 322 (M+1); Anal. calcd for $C_{14}H_{10}Cl_2N_4O$ C, 52.36; H, 3.14; N, 17.45, found, C, 52.32; H, 3.12; N, 17.41.

4-chloro-N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)benzamide ***(17).*** White solid; (114 mg, 65 %): *R*f = 0.66 ($CHCl_3$/MeOH, 9:1); mp: 184-186 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.01 (s, 1H), 8.32 (s, 1H), 8.05 (t, J = 6.64 Hz, 2H), 7.76 (d, J = 0.84 Hz, 1H), 7.63 (t, J = 6.64 Hz, 2H), 3.87 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=164.62, 161.55, 142.99, 140.87, 140.85, 137.34, 133.41, 129.96, 129.94, 128.67, 128.65, 126.31, 94.52, 30.83 ppm; IR (KBr): ῡ = 3423, 1668 cm^{-1}; MS: m/z 321.1 (M^+), 322 (M+1); Anal. calcd for $C_{14}H_{10}Cl_2N_4O$ C, 52.36; H, 3.14; N, 17.45, found, C, 52.32; H, 3.12; N, 17.41.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-2-fluoro benzamide ***(18).*** White solid; (110 mg, 66 %): *R*f = 0.53 ($CHCl_3$/MeOH, 9:1); mp: 189-191 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=10.83 (s, 1H), 8.33 (d, J = 9.40 Hz, 1H), 7.71 (t, J = 5.08 Hz, 2H), 7.53-7.61 (m, 1H), 7.32 (t, J = 8.64 Hz, 2H), 3.85 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=164.72, 161.71, 157.82, 142.96, 140.85, 140.83, 134.12, 129.98, 126.30, 125.23, 124.87, 116.08, 94.51, 30.86 ppm; IR (KBr): ῡ = 3433, 1672 cm^{-1}; MS: m/z 304.7 (M^+), 306 (M+1); Anal. calcd for $C_{14}H_{10}ClFN_4O$ C, 55.18; H, 3.31; N, 18.39, found, C, 55.14; H, 3.35; N, 18.34.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-fluoro benzamide ***(19).*** White solid; (105 mg, 63 %): *R*f = 0.51 ($CHCl_3$/MeOH, 9:1); mp: 188-190 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.01 (s, 1H), 8.32 (s, 1H), 7.87-7.89 (m, 1H), 7.80-7.83 (m, 1H), 7.76 (s, 1H), 7.57-7.62 (m, 1H), 7.46-7.50 (m, 1H), 3.86 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=164.76, 162.76, 161.74, 142.95, 140.82, 140.81, 135.31, 130.47, 126.35, 123.23, 118.92, 112.56, 94.56, 30.81 ppm; IR (KBr): ῡ = 3435, 1675 cm^{-1}; MS: m/z 304.7 (M^+), 306 (M+1); Anal. calcd for $C_{14}H_{10}ClFN_4O$ C, 55.18; H, 3.31; N, 18.39, found, C, 55.14; H, 3.35; N, 18.34.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-4-fluoro benzamide ***(20).*** White solid; (107 mg, 64 %): *R*f = 0.57 ($CHCl_3$/MeOH, 9:1); mp: 190-192 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=10.93 (s, 1H), 8.30 (s, 1H), 8.08-8.12 (m, 2H), 7.74 (s, 1H), 7.35-7.39 (m, 2H), 3.85 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=164.77, 164.70, 161.75, 142.99, 140.87, 140.85, 129.84, 129.23, 129.21, 126.31, 116.87, 116.85, 94.52, 30.85 ppm; IR (KBr): ῡ =3431, 1671 cm^{-1}; MS: m/z 304.7 (M^+), 306 (M+1); Anal. calcd for $C_{14}H_{10}ClFN_4O$ C, 55.18; H, 3.31; N, 18.39, Found, C, 55.14; H, 3.35; N, 18.34.

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N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-2-(trifluoro methyl)benzamide ***(21).*** White solid; (120 mg, 62 %): *R*f = 0.42 ($CHCl_3$/MeOH, 9:1); mp: 195-197 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.12 (s, 1H), 8.34 (s, 1H), 7.84 (d, J = 7.64 Hz, 1H), 7.66-7.78 (m, 4H), 3.86 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=164.82, 161.74, 142.86, 140.78, 140.76, 132.61, 132.53, 131.42, 127.98, 126.87, 126.36, 125.30, 117.89, 94.56, 30.82 ppm; IR (KBr): ῦ = 3438, 1678 cm^{-1}; MS: m/z 354.7 (M^+), 356 (M+1); Anal. calcd for $C_{15}H_{10}ClF_3N_4O$ C, 50.79; H, 2.84; N, 15.79, found, C, 50.72; H, 2.81; N, 15.75.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-(trifluoro methyl)benzamide ***(22).*** White solid; (122 mg, 63 %): *R*f = 0.45 ($CHCl_3$/MeOH, 9:1); mp: 194-196 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.23 (s, 1H), 8.33 (t, J = 7.60 Hz, 3H), 8.00 (d, J = 7.24 Hz, 1H), 7.79 (t, J = 11.16 Hz, 2H), 3.86 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=164.85, 161.77, 142.82, 140.77, 140.75, 134.81, 132.03, 131.22, 129.87, 128.24, 126.41, 124.30, 124.32, 94.52, 30.85 ppm; IR (KBr): ῦ = 3435, 1675 cm^{-1}; MS: m/z 354.7 (M^+), 356 (M+1); Anal. calcd for $C_{15}H_{10}ClF_3N_4O$: C 50.79, H 2.84, N 15.79, found: C 50.72, H 2.81, N 15.75.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-4-(trifluoro methyl)benzamide ***(23).*** White solid; (122 mg, 63 %): *R*f = 0.41 ($CHCl_3$/MeOH, 9:1); mp: 197-199 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.23 (s, 1H), 8.34 (s, 1H), 8.06 (t, J = 6.68 Hz, 2H), 7.76 (d, J = 2.86Hz, 1H), 7.65 (t, J = 6.68 Hz, 2H), 3.87 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=164.89, 161.78, 142.85, 140.79, 140.77, 140.75, 134.98, 130.26, 130.24, 127.65, 127.62, 126.41, 124.38, 94.52, 30.85 ppm; IR (KBr): ῦ = 3435, 1675 cm^{-1}; MS: m/z 354.7 (M^+), 356 (M+1); Anal. calcd for $C_{15}H_{10}ClF_3N_4O$ C, 50.79; H, 2.84; N, 15.79, found, C, 50.76; H, 2.85; N, 15.76.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-cyano benzamide ***(24).*** White solid; (112 mg, 66 %): *R*f = 0.35 ($CHCl_3$/MeOH, 9:1); mp: 204-206 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.16 (s, 1H), 8.44 (s, 1H), 8.31 (t, J = 7.92 Hz, 2H), 8.10 (d, J = 7.64 Hz, 1H), 7.76 (t, J = 8.64 Hz, 2H), 3.86 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=164.66, 161.59, 142.94, 140.87, 140.86, 135.81, 134.97, 131.41, 129.94, 129.96, 128.81, 126.35, 116.21, 94.52, 30.83 ppm; IR (KBr): ῦ = 3439, 1682 cm^{-1}; MS: m/z 311.7 (M^+), 312.7 (M+1); Anal. calcd for $C_{15}H_{10}ClN_5O$ C, 57.79; H, 3.23; N, 22.47, found, C, 57.75; H, 3.21; N, 22.45.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-4-cyano benzamide ***(25).*** White solid; (112 mg, 66 %): *R*f = 0.31 ($CHCl_3$/MeOH, 9:1); mp: 206-208 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.18 (s, 1H), 8.47 (s, 1H), 8.31 (t, J = 7.92 Hz, 2H), 8.10 (d, J = 7.64 Hz, 1H), 7.76 (t, J = 8.64 Hz, 2H), 3.82 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=164.68, 161.55, 142.96, 140.84, 140.82, 138.40, 132.98, 132.96, 128.94, 128.92, 126.28, 126.32, 115.96, 94.55, 30.88 ppm; IR (KBr): ῦ = 3439, 1682 cm^{-1}; MS: m/z 311.7 (M^+), 312.7 (M+1); Anal. calcd for $C_{15}H_{10}ClN_5O$ C, 57.79; H, 3.23; N, 22.47, found, C, 57.72; H, 3.20; N, 22.41.

1-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-urea (26-38)

A mixture of sodium hydride (26.28 mg 1.09 mmol), 6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-amine (100 mg, 0.54 mmol) and isocyanate (0.65 mmol) in dimethyl formamide (6 mL), was stirred at room temperature for 18 h. After completion of the reaction (TLC) , it was quenched with saturated NH_4Cl solution, extracted with ethyl acetate (3 x 4 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give the crude product which was purified by column chromatography on silica employing $CHCl_3$/MeOH (100:0!90:10) as an eluent to obtain pure solid.

1-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridin-4-yl)-3-phenylurea ***(26).*** White solid; (120 mg, 73 %): *R*f = 0.72 ($CHCl_3$/MeOH, 9:1); mp:213-215 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.30 (s, 1H), 9.43 (s, 1H), 8.34 (s, 1H), 7.54 (d, J = 7.32 Hz, 3H), 7.36 (t, J = 8.12 Hz, 2H), 7.06 (t, J = 7.40 Hz, 1H), 3.84 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.72, 156.36, 142.91, 140.76, 140.75, 136.06, 129.56, 129.54, 126.59, 125.41, 122.62, 122.61, 94.51, 30.72 ppm; IR (KBr): ῦ = 3426, 1662 cm^{-1}; MS: m/z 301.7 (M^+), 303 (M+1); Anal.calcd for $C_{14}H_{12}ClN_5O$ C, 55.73; H, 4.01; N, 23.21, found, C, 55.72; H, 4.03; N, 23.19.

1-benzyl-3-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)urea ***(27).*** White solid; (112 mg, 65 %): *R*f = 0.76 ($CHCl_3$/MeOH, 9:1); mp: 222-224 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.30 (s, 1H), 9.31 (s, 1H), 8.99 (s, 1H), 8.30 (s, 1H), 7.46 (s, 1H), 7.37 (d, J = 3.72 Hz, 3H), 7.27-7.27 (m, 1H), 4.51 (d, J = 5.72 Hz, 2H), 3.83 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.76, 156.42, 142.93, 140.78, 140.77, 140.24, 129.16, 129.14, 128.46, 128.46, 127.61, 126.54, 94.59, 47.82, 30.75 ppm; IR (KBr): ῦ = 3423, 1658 cm^{-1}; MS:m/z 315.7 (M^+), 317 (M+1); Anal. calcd for $C_{15}H_{14}ClN_5O$ C, 57.06; H, 4.47; N, 22.18, found, C, 57.02; H, 4.44; N, 22.15.

1-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-o-tolylurea ***(28).*** White solid; (118 mg, 68 %); *R*f = 0.79 ($CHCl_3$/MeOH, 9:1); mp: 218-220 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.24 (s, 1H), 9.39 (s, 1H), 8.36 (s, 1H), 7.56 (s, 1H), 7.39 (s, 1H), 7.22-7.24 (m, 3H), 6.89 (d, J = 8.04 Hz, 1H), 3.85 (s, 3H), 2.32 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.81, 156.62, 142.86, 140.63, 140.60, 135.06, 135.02, 129.56, 126.54, 126.62, 124.52, 122.32, 94.51, 30.72, 24.48 ppm; IR (KBr): ῦ = 3420, 1659 cm^{-1}; MS: m/z 315.7 (M^+), 317 (M+1); Anal. calcd for $C_{15}H_{14}ClN_5O$ C, 57.06; H, 4.47; N, 22.18, found, C, 57.02; H, 4.41; N, 22.13.

1-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-m-tolylurea ***(29).*** White solid; (113 mg, 65 %); *R*f = 0.73 ($CHCl_3$/MeOH, 9:1); mp: 219-221 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.23 (s, 1H), 9.39 (s, 1H), 8.34 (s, 1H), 7.54 (s, 1H), 7.38 (s, 1H), 7.22-7.23 (m, 2H), 6.88 (d, J = 7.52 Hz, 1H), 3.84 (s, 3H), 2.31 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.86, 156.59, 142.96, 140.78, 140.76, 138.98, 135.86, 129.56, 126.68, 124.54, 124.52, 120.32, 94.65, 30.67, 24.16 ppm; IR (KBr): ῦ = 3428, 1668 cm^{-1}; MS: m/z 315.7 (M^+), 317 (M+1); Anal. calcd for $C_{15}H_{14}ClN_5O$ C, 57.06; H, 4.47; N, 22.18, found, C, 57.02; H, 4.41; N, 22.13.

1-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-p-tolylurea ***(30).*** White solid; (115 mg, 67 %); *R*f = 0.77 ($CHCl_3$/MeOH, 9:1); mp: 223-225 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.23 (s, 1H), 9.35 (s, 1H), 8.33 (s, 1H), 7.53 (s, 1H), 7.43 (d, J = 8.36 Hz, 2H), 7.24 (d, J = 9.12 Hz, 2H), 3.84 (s, 3H), 2.27 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.78, 156.67, 142.92, 140.67, 140.65, 135.26, 134.86, 129.78, 129.75, 126.56, 121.66, 121.62, 94.56, 30.81, 24.32 ppm; IR (KBr): ῦ = 3433, 1672 cm^{-1}; MS: m/z 315.7 (M^+), 317 (M+1); Anal. calcd for $C_{15}H_{14}ClN_5O$ C, 57.06; H, 4.47; N, 22.18, found, C, 57.02; H, 4.41; N, 22.13.

1-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-(2-chlorophenyl)urea ***(31).*** White solid; (122 mg, 66 %); *R*f = 0.63 ($CHCl_3$/MeOH, 9:1); mp: 227-229 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.66 (s, 1H), 9.64 (s, 1H), 8.35-8.39 (m, 2H), 7.51-7.56 (m, 2H), 7.33-7.37 (m, 1H), 7.07-7.12 (m, 1H), 3.84 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.86, 156.65, 142.81, 140.76, 140.74, 135.12, 134.09, 129.22, 126.19, 126.17, 126.65, 124.08, 94.62,

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30.76 ppm; IR (KBr): ṽ = 3431, 1669 cm^{-1}; MS: m/z 335.1 (M^+), 336.1 (M+1); Anal. calcd for $C_{14}H_{11}Cl_2N_5O$ C, 50.02; H, 3.30; N, 20.83, found, C, 50.06; H, 3.33; N, 20.78.

1-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-(3-chloro phenyl)urea ***(32).*** White solid; (117 mg, 64 %); *R*f = 0.65 ($CHCl_3$/MeOH, 9:1); mp: 225-227 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.33 (s, 1H), 9.63 (s, 1H), 8.34 (s, 1H), 7.81 (t, J = 2.00 Hz, 1H), 7.55 (s, 1H), 7.30-7.40 (m, 2H), 7.10-7.13 (m, 1H), 3.84 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.72, 156.71, 142.85, 140.82, 140.80, 140.79, 134.66, 130.22, 126.73, 125.70, 121.69, 121.65, 94.68, 30.69 ppm; IR (KBr): ṽ = 3429, 1663cm^{-1}; MS: m/z 335.1 (M^+), 336.1 (M+1); Anal. calcd for $C_{14}H_{11}Cl_2N_5O$ C, 50.02; H, 3.30; N, 20.83, Found, C, 50.06; H, 3.33; N, 20.78.

1-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-(4-chloro phenyl)urea ***(33).*** White solid; (114 mg, 62 %); *R*f = 0.69 ($CHCl_3$/MeOH, 9:1); mp: 228-230 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.32 (s, 1H), 9.56 (s, 1H), 8.34 (s, 1H), 7.56 (t, J = 4.16 Hz, 3H), 7.41 (d, J = 8.68 Hz, 2H), 3.84 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.79, 156.69, 142.91, 140.79, 140.75, 138.88, 129.88, 129.85, 129.82, 126.75, 124.24, 124.21, 94.59, 30.71 ppm; IR (KBr): ṽ = 3434, 1668 cm^{-1}; MS: m/z 335.1 (M^+), 336.1 (M+1); Anal. calcd for $C_{14}H_{11}Cl_2N_5O$ C, 50.02; H, 3.30; N, 20.83, Found, C, 50.06; H, 3.33; N, 20.78.

1-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-(2-fluoro phenyl)urea ***(34).*** White solid; (119 mg, 70 %); *R*f = 0.57 ($CHCl_3$/MeOH, 9:1); mp: 232-234 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.70 (s, 1H), 9.70 (s, 1H), 8.35 (s, 1H), 8.28-8.28 (m, 1H), 7.55 (s, 1H), 7.31 (q, J = 8.28 Hz, 1H), 7.19 (t, J = 7.72 Hz, 1H), 7.05-7.07 (m, 1H), 3.85 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.83, 162.81, 156.58, 142.88, 140.77, 140.74, 126.65, 125.63, 124.09, 124.06, 121.18, 121.15, 94.67, 30.81 ppm; IR (KBr): ṽ = 3432, 1664 cm^{-1}; MS: m/z 319.7 (M^+), 321 (M+1); Anal. calcd for $C_{14}H_{11}ClFN_5O$ C, 52.59; H, 3.47; N, 21.90, Found, C, 52.54; H, 3.43; N, 21.86.

1-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-(3-fluorophenyl)ure ***(35).*** White solid; (126 mg, 72 %); *R*f = 0.59 ($CHCl_3$/MeOH, 9:1); mp: 235-237 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.40 (s, 1H), 9.60 (s, 1H), 8.34 (s, 1H), 7.57-7.58 (m, 1H), 7.39 (q, J = 7.08 Hz, 1H), 7.16 (d, J = 8.08 Hz, 1H), 6.86-6.87 (m, 1H), 3.84 (s, 1H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.79, 162.75, 156.77, 142.91, 140.88, 140.83, 140.79, 130.85, 126.74, 120.74, 120.71, 120.68, 94.68, 30.69 ppm; IR (KBr): ṽ = 3428, 1664 cm^{-1}; MS: m/z 319.7 (M^+), 321 (M+1); Anal. calcd for $C_{14}H_{11}ClFN_5O$ C, 52.59; H, 3.47; N, 21.90, Found, C, 52.55; H, 3.44; N, 21.81.

1-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-(4-fluorophenyl)urea ***(36).*** White solid; (124 mg, 71 %); *R*f = 0.58 ($CHCl_3$/MeOH, 9:1); mp: 238-240 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.27 (s, 1H), 9.47 (s, 1H), 8.33 (s, 1H), 7.53-7.55 (m, 3H), 7.20 (q, J = 2.12 Hz, 2H), 3.84 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.88, 162.85, 156.65, 142.89, 140.79, 140.75, 130.63, 126.71, 123.24, 123.21, 119.86, 119.83, 94.71, 30.78 ppm; IR (KBr): ṽ = 3431, 1664 cm^{-1}; MS: m/z 319.7 (M^+), 321 (M+1); Anal. calcd for $C_{14}H_{11}ClFN_5O$ C, 52.59; H, 3.47; N, 21.90, Found, C, 52.55; H, 3.45; N, 21.89.

1-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-(3-cyanophenyl)urea ***(37).*** White solid; (118 mg, 66 %); *R*f = 0.35 ($CHCl_3$/MeOH, 9:1); mp: 236-238 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.48 (s, 1H), 9.73 (s, 1H), 8.34 (s, 1H), 8.08 (d, J = 1.64 Hz, 1H), 7.68-7.69 (m, 1H), 7.51-7.51 (m, 3H), 3.85 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.76, 156.59, 142.93, 140.85, 140.83, 140.81, 128.02, 127.99, 127.97, 126.62, 126.59, 120.49, 116.25, 94.51, 30.72 ppm; IR (KBr): ṽ = 3436, 1669 cm^{-1}; MS: m/z 326.74(M^+), 328 (M+1); Anal. calcd for $C_{15}H_{11}ClN_6O$ C, 55.14; H, 3.39; N, 25.72, Found, C, 55.12; H, 3.36; N, 25.71.

1-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-(3-cyanophenyl)urea ***(38).*** White solid; (126 mg, 70 %); *R*f = 0.38 ($CHCl_3$/MeOH, 9:1); mp: 239-241 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.48 (s, 1H), 9.78 (s, 1H), 8.35 (s, 1H), 7.82 (d, J = 8.68 Hz, 2H), 7.48 (d, J = 8.96 Hz, 2H), 7.57 (s, 1H), 3.86 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.81, 156.56, 142.86, 140.76, 140.73, 140.71, 134.65, 134.62, 126.65, 124.19, 124.15, 120.21, 116.52, 94.66, 30.82 ppm; IR (KBr): ṽ = 3432, 1664 cm^{-1}; MS: m/z 326.74(M^+), 328 (M+1); Anal. calcd for $C_{15}H_{11}ClN_6O$ C, 55.14; H, 3.39; N, 25.72, Found, C, 55.11; H, 3.37; N, 25.70.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl) sulfonamide (39-51). A mixture of sodium hydride (26.28 mg 1.09 mmol), 6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-amine (100 mg, 0.54 mmol) and sulfonyl chloride (0.65 mmol) in dimethyl formamide (6 mL), was stirred at room temperature for 18 h. After completion of the reaction (TLC), it was quenched with saturated NH_4Cl solution, extracted with ethyl acetate (3 x 4 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give the crude product, which was purified by column chromatography on silica employing $CHCl_3$/MeOH (100:0!90:10) as an eluent to obtain pure solid.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)benzenesulfonamide ***(39).*** White solid; (126 mg,71 %); *R*f = 0.72 ($CHCl_3$/MeOH, 9:1); mp: 227-229 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.50 (s, 1H), 8.28 (s, 1H), 8.07 (d, J = 1.48 Hz, 2H), 7.56-7.57 (m, 3H), 7.42 (s, 1H), 3.77 (s, 1H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.51, 142.96, 140.89, 140.87, 140.85, 132.41, 129.98, 129.95, 127.30, 127.27, 126.29, 94.51, 30.82 ppm; IR (KBr): ṽ = 3424, 1372 cm^{-1}; MS: m/z 322.77 (M^+), 324 (M+1); Anal. calcd for $C_{13}H_{11}ClN_4O_2S$ C, 48.37; H, 3.44; N, 17.36, Found, C, 48.32; H, 3.41; N, 17.34.

6-chloro-1-methyl-N-[(phenylsulfonyl)methyl]-1H-imidazo[4,5-c]pyridine-4-amine ***(40).*** White solid; (121 mg, 66 %); *R*f = 0.75 ($CHCl_3$/MeOH, 9:1); mp: 231-233 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.05 (s, 1H), 8.32 (s, 1H), 7.59 (s, 1H), 7.35 (s, 5H), 5.09 (s, 2H), 3.84 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.46, 142.93, 140.76, 140.74, 134.32, 129.55 129.52, 128.77, 128.73, 126.52, 126.49, 94.68, 60.61, 30.78 ppm; IR (KBr): ṽ = 3428, 1374 cm^{-1}; MS: m/z 336.8 (M^+), 338 (M+1); Anal. calcd for $C_{14}H_{13}ClN_4O_2S$ C, 49.93; H, 3.89; N, 16.64, Found, C, 49.91; H, 3.85; N, 16.62.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-2-methylbenzenesulfonamide ***(41).*** White solid; (118 mg, 64 %); *R*f = 0.78 ($CHCl_3$/MeOH, 9:1); mp: 233-235 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.80 (s, 1H), 8.28 (s, 1H), 8.10 (d, J = 7.92 Hz, 1H), 7.48 (t, J = 6.16 Hz, 1H), 7.39 (t, J = 8.36 Hz, 2H), 7.31 (d, J = 7.56 Hz, 2H), 3.77 (s, 3H), 2.62 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.65, 142.91, 140.83, 140.80, 140.78, 136.98, 129.96, 129.94, 127.53, 127.51, 126.38, 94.52, 30.84, 24.29 ppm; IR (KBr): ṽ = 3425, 1372 cm^{-1}; MS: m/z 336.8 (M^+), 338 (M+1); Anal. calcd for $C_{14}H_{13}ClN_4O_2S$ C, 49.93; H, 3.89; N, 16.64, Found, C, 49.90; H, 3.85; N, 16.62.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-methylbenzenesulfonamide ***(42).*** White solid; (115 mg, 62 %); *R*f = 0.71 ($CHCl_3$/MeOH, 9:1); mp: 235-237 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.50 (s, 1H), 8.27 (s, 1H), 7.97 (s, 1H), 7.84 (t, J = 4.52 Hz, 1H), 7.44 (q, J = 4.68 Hz, 3H), 3.77 (s, 3H), 2.39 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.59, 142.77, 140.95, 140.92,

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140.89, 137.75, 133.74, 127.51, 127.49, 127.47, 126.53, 94.64, 30.79, 24.31 ppm; IR (KBr): ῦ = 3426, 1338 cm^{-1}; MS: m/z 336.8 (M^+), 338 (M+1); Anal. calcd for $C_{14}H_{13}ClN_4O_2S$ C, 49.93; H, 3.89; N, 16.64, Found, C, 49.91; H, 3.87; N, 16.61.

2-chloro-N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)benzenesulfonamide ***(43).*** White solid; (118 mg, 60 %); *R*f = 0.63 ($CHCl_3$/MeOH, 9:1); mp: 223-225 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=8.30 (s, 1H), 8.21 (d, J = 1.28 Hz, 1H), 7.56-7.57 (m, 3H), 7.40 (s, 1H), 3.79 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.63, 142.88, 140.68, 140.65, 140.62, 133.64, 132.11, 129.56, 128.30, 128.27, 126.51, 94.63, 30.85 ppm; IR (KBr): ῦ = 3431, 1358cm^{-1}; MS: m/z 357.2 (M^+), 358 (M+1); Anal. calcd for $C_{13}H_{10}Cl_2N_4O_2S$ C, 43.71; H, 2.82; N, 15.68 , Found, C, 43.70; H, 2.81; N, 15.66.

3-chloro-N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)benzenesulfonamide ***(44).*** White solid; (120 mg, 61 %); *R*f = 0.68 ($CHCl_3$/MeOH, 9:1); mp: 226-228 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=8.31 (s, 1H), 8.17 (s, 1H), 7.99 (d, J = 7.84 Hz, 1H), 7.72-7.72 (m, 1H), 7.63 (t, J = 7.96 Hz, 1H), 7.48 (s, 1H), 3.79 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.56, 142.94, 140.98, 140.95, 141.24, 134.45, 132.67, 130.24, 126.78, 126.75, 126.74, 94.58, 30.89 ppm; IR (KBr): ῦ = 3433, 1362 cm^{-1}; MS: m/z 357.2 (M^+), 358 (M+1); Anal. calcd for $C_{13}H_{10}Cl_2N_4O_2S$ C, 43.71; H, 2.82; N, 15.68, Found, C, 43.69; H, 2.80; N, 15.65.

4-chloro-N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)benzenesulfonamide ***(45).*** White solid; (124 mg, 63 %); *R*f = 0.65 ($CHCl_3$/MeOH, 9:1); mp: 227-229 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=8.29 (s, 1H), 8.05-8.06 (m, 2H), 7.67-7.68 (m, 2H), 7.44 (s, 1H), 3.79 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.58, 142.92, 140.87, 140.85, 138.57, 138.55, 129.98, 129.95, 128.65, 128.62, 126.56, 94.72, 30.76 ppm; IR (KBr): ῦ = 3428, 1359 cm^{-1}; MS: m/z 357.2 (M^+), 358 (M+1); Anal. calcd for $C_{13}H_{10}Cl_2N_4O_2S$ C, 43.71; H, 2.82; N, 15.68, Found, C, 43.68; H, 2.79; N, 15.66.

3-bromo-N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)benzenesulfonamide ***(46).*** White solid; (132 mg, 60 %); *R*f = 0.70 ($CHCl_3$/MeOH, 9:1); mp: 232-234 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=8.31 (s, 1H), 8.03 (d, J = 7.96 Hz, 1H), 7.86 (d-d, J = 0.96 Hz, 1H), 7.58 (t, J = 7.88 Hz, 1H), 7.48 (s, 1H), 3.79 (s, 1H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.72, 142.67, 142.65, 140.77, 140.75, 135.61, 132.89, 132.85, 132.83, 126.56, 122.97, 94.68, 30.85; ppm; IR (KBr): ῦ = 3430, 1374 cm^{-1}; MS: m/z 401.67 (M^+), 403 (M+1); Anal. calcd for $C_{13}H_{10}BrClN_4O_2S$ C, 38.87; H, 2.51; N, 13.95, Found, C, 38.85; H, 2.50; N, 13.92.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-2-fluorobenzenesulfonamide ***(47).*** White solid; (125 mg,67 %); *R*f = 0.53 ($CHCl_3$/MeOH, 9:1); mp: 234-236 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=8.29 (s, 1H), 8.14 (q, J = 5.22 Hz, 2H), 7.44 (q, J = 8.88 Hz, 3H), 3.79 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.65, 160.62, 142.72, 140.64, 140.62, 133.69, 128.11, 128.08, 126.51, 126.48, 115.27, 94.60, 30.83 ppm; IR (KBr): ῦ = 3433, 1375cm^{-1}; MS: m/z 340.76 (M^+), 342 (M+1); Anal. calcd for $C_{13}H_{10}ClFN_4O_2S$ C, 45.82; H, 2.96; N, 16.44, Found, C, 45.80; H, 2.95; N, 16.42.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-fluorobenzenesulfonamide ***(48).*** White solid; (121 mg, 65 %); *R*f = 0.56 ($CHCl_3$/MeOH, 9:1); mp: 237-239 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.81 (s, 1H), 8.31 (s, 1H), 7.90 (q, J = 7.36 Hz, 2H), 7.63-7.65 (m, 1H), 7.50-7.51 (m, 1H), 7.48 (s, 1H), 3.79 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.68, 160.65, 142.57, 140.88, 140.85, 140.83, 130.25, 126.67, 122.56, 119.48, 119.45, 94.59, 30.72 ppm; IR (KBr): ῦ = 3435, 1388 cm^{-1}; MS:m/z 340.76 (M^+), 342 (M+1); Anal. calcd for $C_{13}H_{10}ClFN_4O_2S$ C, 45.82; H, 2.96; N, 16.44, Found, C, 45.81; H, 2.95; N, 16.43.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-4-fluorobenzenesulfonamide ***(49).*** White solid; (118 mg, 63 %); *R*f = 0.59 ($CHCl_3$/MeOH, 9:1); mp: 239-241 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=11.68 (s, 1H), 8.27 (s, 1H), 8.10-8.11 (m, 2H), 7.40-7.40 (m, 3H), 3.77 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.72, 162.69, 142.66, 140.78, 140.75, 135.61, 128.89, 128.85, 126.56, 119.98, 119.97, 94.68, 30.85 ppm; IR (KBr): ῦ = 3432, 1374 cm^{-1}; MS: m/z 340.76 (M^+), 342 (M+1); Anal. calcd for $C_{13}H_{10}ClFN_4O_2S$ C, 45.82; H, 2.96; N, 16.44, Found, C, 45.81; H, 2.95; N, 16.43.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-3-cyanobenzenesulfonamide ***(50).*** White solid; (115 mg, 60 %); *R*f = 0.34 ($CHCl_3$/MeOH, 9:1); mp: 229-231 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=8.49 (s, 1H), 8.29 (d, J = 6.00 Hz, 2H), 8.09 (d, J = 7.72 Hz, 1H), 7.78 (t, J = 7.76 Hz, 1H), 7.40 (s, 1H), 3.76 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.75, 142.89, 142.87, 140.83, 140.81, 136.99, 131.47, 131.45, 131.41, 126.61, 118.87, 116.98, 94.58, 30.81 ppm; IR (KBr): ῦ = 3437, 1378 cm^{-1}; MS:m/z 347.8(M^+), 349 (M+1); Anal. calcd for $C_{14}H_{10}ClN_5O_2S$ C, 48.35; H, 2.90; N, 20.14, Found, C, 48.33; H, 2.88; N, 20.11.

N-(6-chloro-1-methyl-1H-imidazo[4,5-c]pyridine-4-yl)-4-cyanobenzenesulfonamide ***(51).*** White solid; (126 mg, 66 %); *R*f = 0.36 ($CHCl_3$/MeOH, 9:1); mp: 233-235 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=8.28 (s, 1H), 8.20 (d, J = 8.28 Hz, 2H), 8.08 (d, J = 8.24 Hz, 2H), 7.42 (s, 1H), 3.78 (s, 3H) ppm; ^{13}C NMR (400 MHz, [D_6]DMSO): δ=162.59, 142.91, 142.89, 140.87, 140.85, 133.59, 133.57, 129.89, 129.86, 126.63, 118.52, 116.88, 94.64, 30.79 ppm; IR (KBr): ῦ = 3434, 1375 cm^{-1}; MS: m/z 347.8(M^+), 349 (M+1); Anal. calcd for $C_{14}H_{10}ClN_5O_2S$ C, 48.35; H, 2.90; N, 20.14, Found, C, 48.33; H, 2.88; N, 20.11.

Anti-tubercular activity

All experiments were performed in compliance with the relevant laws and institutional guidelines by Institutional Animal Ethics Committee (IAEC). The IAEC reviewed and approved all the experiments adopted. Also stated that human subject has not been used in this experiment.

All compounds were screened for their *in vitro* antimycobacterial activity against *M. tuberculosis* by an agar dilution method for the determination of MIC in duplicate. Ten-fold serial dilutions of each test compound/drug were incorporated into Middlebrook 7H11 agar medium with oleic albumin dextrose catalase (OADC) growth supplement (Drug concentration 12.5 to 0.78 μg/mL). An inoculum of *M. tuberculosis* H37Rv was prepared from fresh Middlebrook 7H11 agar slants with OADC. A 5 μL amount of the bacterial suspension was spotted in 7H11 agar tubes containing 10-fold serial dilutions of drugs per mL. The tubes were incubated at 37 °C, and final readings were recorded after 28 days. The activity of each sample was performed in triplicate. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of the compound required to give the complete inhibition of bacterial growth.

Cytotoxicity

Some compounds were further screened to assess toxicity (IC_{50}) in a mammalian Vero cell line. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay.[24]

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***In vivo* studies**

Compounds **21, 22** and **23** showed good activities in the *in vitro* study were tested for their efficacy against *M. tuberculosis* at a dose of 40 mg/kg in CD-1 mice, six per group. In this model, the mice were infected intravenously with *M. tuberculosis* (ATCC 35801). Drug treatment by intraperitoneal route began after 10 days of inoculation of the animal with microorganism and was continued for 10 days. Thirty-five days post-infection, the spleens, and right lungs were aseptically removed and ground in a tissue homogenizer, and the number of viable organisms was determined by serial 10-fold dilutions and subsequent inoculation onto 7H10 agar plates. Cultures were incubated at 37 °C in an ambient air for 4 weeks prior to counting. Bacterial counts were measured and compared with the counts from negative controls (vehicle treated) in lung and in the spleen.

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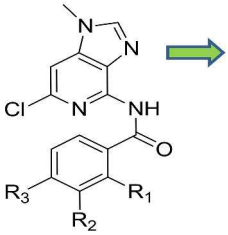

21-23

Entry	IC_{50} (µM)	Lungs (log CFU ± SEM)	Spleen (log CFU ± SEM)
Isoniazid	>455.78	5.73 ± 0.03	4.79 ± 0.18
21	169.07	5.49 ± 0.01	5.41 ± 0.18
22	183.35	5.98 ± 0.17	6.35 ± 0.13
23	162.07	5.45 ± 0.09	5.29 ± 0.16

Compound	R_1	R_2	R_3
21	CF_3	H	H
22	H	CF_3	H
23	H	H	CF_3

Novel imidazo[4,5-c]pyridine derivatives showing cytotoxicity and decreased the bacterial load in lung and spleen tissues in the *in vivo* animal model.