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Original article

Ultrasound-assisted one-pot four-component synthesis of novel 2-amino-3-cyanopyridine derivatives bearing 5-imidazopyrazole scaffold and their biological broadcast

Piyush N. Kalaria*, Shailesh P. Satasia, Jemin R. Avalani, Dipak K. Raval

Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar 388 120, Gujarat, India

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ABSTRACT

An alternative and environmentally caring way for the synthesis of novel 2-amino-3-cyanopyridine derivatives bearing 5-imidazopyrazole nucleus is reported by one-pot four-component cyclo-condensation reaction of substituted 5-(1*H*-imidazol/4-methyl-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**3a**–**b**), malononitrile (**4**), ammonium acetate (**5**) and aromatic (**6a**–**f**)/heterocyclic methyl ketones (**7a**–**d**) under ultrasonic irradiation. The newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against a panel of pathogenic stains of bacteria and fungi, *in vitro* antitubercular activity against *Mycobacterium tuberculosis* H37Rv stain and *in vitro* antioxidant activity by ferric-reducing antioxidant power method. Compounds **8e**, **8h**, **8l**, **9c**, **9g** and **9h** exhibited excellent antibacterial activity and compounds **3a**, **8k**, **9a** and **9b**showed moderate antituberculosis activity as compared with the first line drugs. Majority of the compounds showed excellent antioxidant activity. This approaches claimed to be an environment friendly protocol as it afforded numerous advantages i.e. excellent yields, cleaner reaction profile and shorter reaction time.

1. Introduction

Mycobacterium tuberculosis is a deadly pathogen and contributory agent of tuberculosis (TB) which remains a chief reason of death worldwide [1–3]. Almost one third of the world's population is contaminated with TB bacilli and each year approximately 8 million people are added in the list suffering from active TB and 2 million die as a result [4]. HIV-positive patients are more likely to be infected with TB. The co-infection with HIV is considered accountable for these serious circumstances. The difficulty in treating drug-resistant TB, such as multidrug- resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) also contributes to the increased morbidity and mortality. There has been remarkable raise in the frequency of bacterial and fungal infections over the past few decades. These organisms possess the ability to withstand attack by currently available antimicrobial drugs. The uncontrolled rise in drug resistant pathogens have threatened lives [5]. Innovation of novel drug molecules for the action of complete mycoses is one of the most imperative challenges in infectious disease research. The investigation of new antimicrobial drugs is an evergreen area characterized by energetic search with the intention of conquering episode of abundant drug resistance. To establish preeminent ways to build up efficient therapy, the urgency for the need of novel, unique and persuasive antimicrobial and antitubercular agents attracts immediate attention.

Synthesis of the pyrazole ring system and its derivatives occupy an important place in the realm of synthetic organic chemistry, due to their pharmaceutical activities such as, anticancer [6], antibacterial [7], analgesic [8], antiviral [9] and anti-inflammatory [10] activities. Imidazole derivatives are known to be allied with diverse biological properties, such as antimicrobial [11], antitubercular [12], anti-inflammatory [13], anti-Parkinson [14], analgesic, anti-convulsant and anticancer activities [15]. In recent years, pyridine derivatives represent an imperative class of compounds which possesses high activity profile due to their therapeutic and pharmacological properties [16–18]. Pyridine ring is an essential part for the anticancer and anti-inflammatory agents [19,20]. The biologically active compounds having carbon–nitrogen bond especially the natural and synthetic compounds with cyanopyridine moiety are proved to display significant antifungal [21], antibacterial [22] and anticancer [23] activities.2-Amino-3-cyanopyridine motifs are known to have numerous biological activities, such as anti-microbial [24], anti-inflammatory [25],

* Corresponding author.
E-mail address: piyush_kalaria@yahoo.com (P.N. Kalaria).

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cardiotonic [26], anti-parkinsonism properties [27] and potent inhibitor of HIV-1 integrase [28].

Literature survey revealed that a number of 2-amino-3-cyanopyridine derivatives have been synthesized using a variety of aldehydes [25,26,29–31]. 5-(1*H*-imidazol/4-methyl-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehydes has not been reported so far for the purpose. Thus, with a view to obtain biologically more potent heterocyclic systems, our aim was focused on the prologue of chemical diversity in the molecular framework to synthesize pharmacologically interesting compounds of different composition by green protocol.

Ultrasonic-assisted organic synthesis (UAOS) offers a resourceful and too easy pathway for a large variety of scaffolds. The significant features of the ultrasound approach are formation of pure products in prominent yields, improved rate of reaction, easier handling and also considered as a processing aid in terms of energy conservation compared with conventional methods [32,33].

The single-pot synthesis proceeds over shorter reaction time without the need for isolation of the intermediates [34]. Hence, as a part of programme directed towards environmentally friendly methodologies for the preparation of heterocyclic compound [35–41], herein we report a simple, cost-effective, green and expeditious method for the synthesis of novel 2-amino-3-cyanopyridine derivatives bearing 5-imidazopyrazole moieties under ultrasound irradiation and investigation on their antimicrobial, antituberculosis and antioxidant activities (Scheme 1).

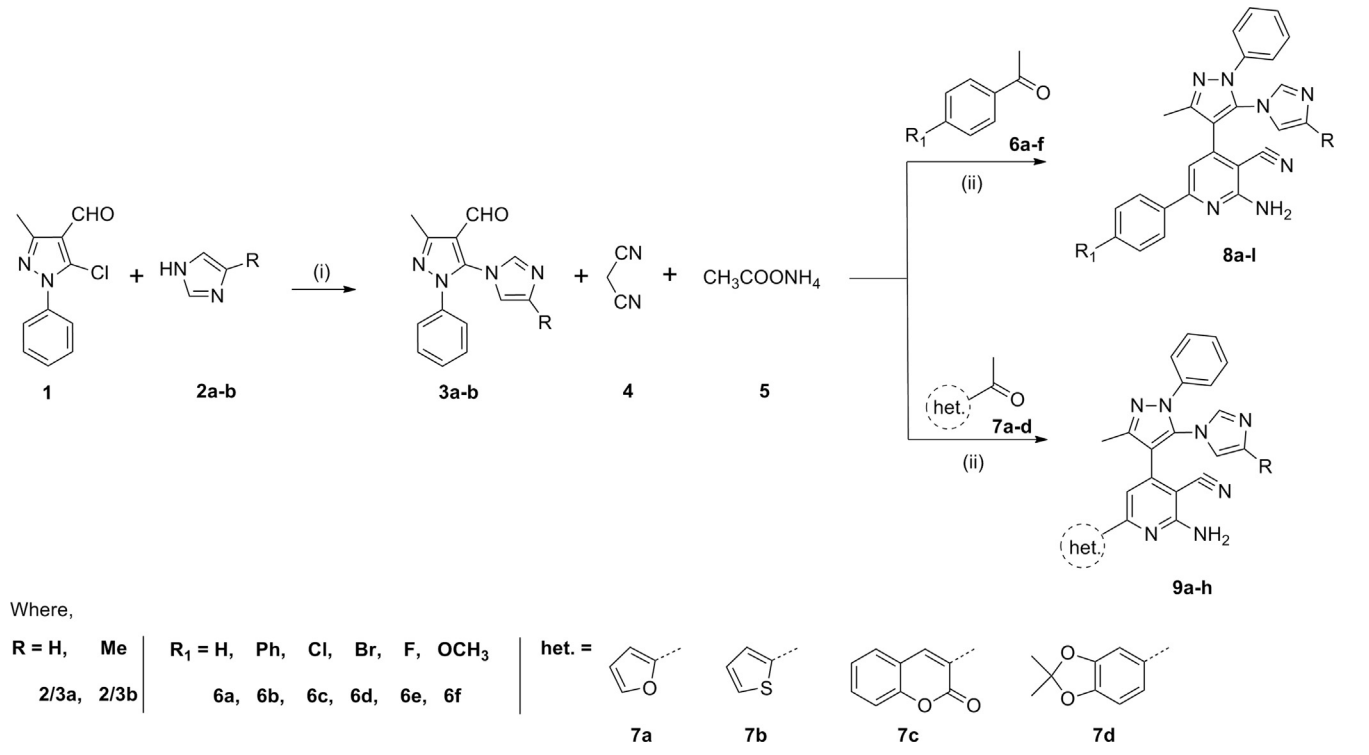
2. Chemistry

The synthetic approach adopted to acquire the targeted 4-(5-(1*H*-imidazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2-aminonicotinonitrile derivatives **8a**–**l** and **9a**–**h** is summarized in Scheme 1. The starting material 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde **1** was prepared according to Vilsmeier–Haack reaction of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one [42]. The final aldehydes 5-(1*H*-imidazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **3a**–**b** were prepared by nucleophilic displacement of chloro group at C5 in 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **1** with secondary amine of imidazole **2a**–**b**in refluxing DMF using anhydrous potassium carbonate as a base. The targeted compounds substituted 4-(5-(1*H*-imidazol/4-metyl-1-yl)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2-aminonicotinonitrile **8a**–**l** and **9a**–**h** were prepared in moderate to good yield (64–84%) by the reaction of substituted 5-(1*H*-imidazol/4-methyl-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **3a**–**b**, malononitrile **4**, ammonium acetate **5** and aromatic/heterocyclic methyl ketone **6a**–**f**/**7a**–**d** in absolute alcohol by one-pot four-component cyclocondensation reaction (Scheme 1 , Table 1). The formation of compounds **8a**–**l** and **9a**–**h** took place *via* imine formed from ketone and ammonium acetate. Imine reacted with alkylidene malononitrile formed from Knoevenagel condensation of aldehyde and malononitrile, followed by cycloaddition, isomerization and aromatization to give the targeted compounds **8a**–**l** and **9a**–**h**. The mechanism of compound **6c** is illustrated in Scheme 2.

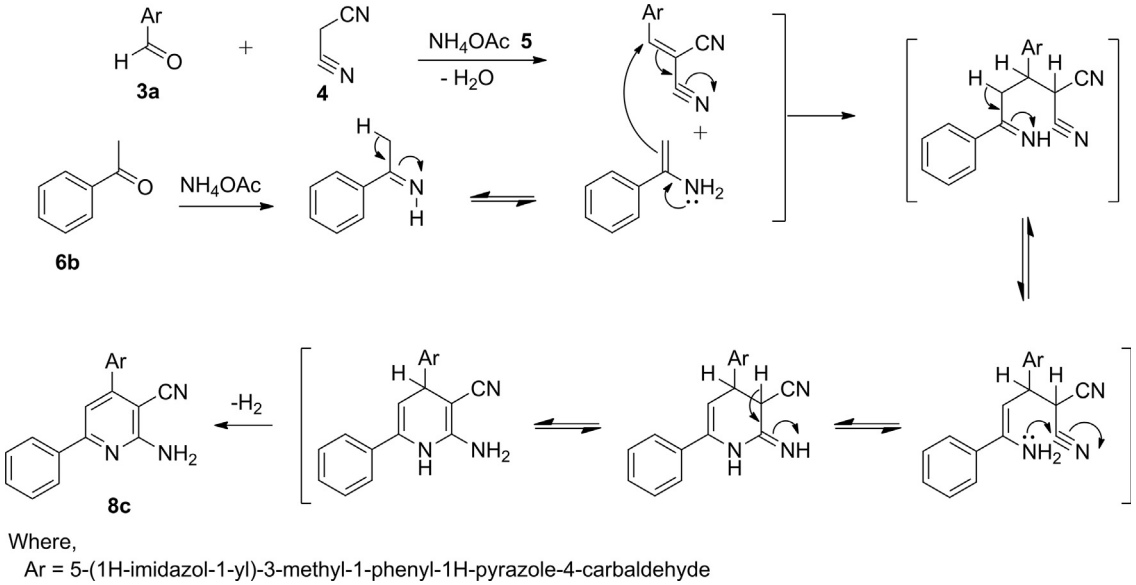
3. Pharmacology

3.1. Antimicrobial activity

The minimal inhibitory concentration (MIC) of all the synthesized compounds **8a**–**l** and **9a**–**h** was determined by broth microdilution method according to National Committee for Clinical Laboratory Standards (NCCLS) [43]. Antibacterial activity was screened against three Gram positive (*Streptococcus pneumoniae* MTCC 1936, *Clostridium tetani* MTCC 449 and *Bacillus subtilis* MTCC 441) and three Gram negative (*Escherichia coli* MTCC 443, *Vibrio cholerae* MTCC 3906 and *Salmonella typhi* MTCC 98) bacteria by



Scheme 1. Synthesis of the substituted 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-aminonicotinonitrile **8a**–**l** and **9a**–**h**. (i) DMF, K_2CO_3, Reflux 2 h (ii) Ethanol, ultrasound irradiation, room temperature **Or** Ethanol, Reflux 1.5–3 h.



Scheme 2. Plausible mechanistic pathway for the synthesis of compound **8c**.

using ampicillin as the standard antibacterial drugs. Antifungal activity was screened against two fungal species (*Candida albicans* MTCC 227 and *Aspergillus fumigats* MTCC 3008) where griseofulvin was used as the standard antifungal agents. All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh and tested against above mentioned known drugs. The results of antimicrobial screening are summarized in Table 2.

3.2. Antituberculosis activity

In vitro antituberculosis activity of all newly synthesized compounds **8a**–**l** and **9a**–**h** was conducted at 250 μg/mL against *M. tuberculosis* H37Rv stain by using Lowensteine–Jensen medium as described by Rattan [44].The observed results are presented in Table 3 in the form of %inhibition. Rifampicin and Isoniazid were used as the standard antitubercular drugs.

3.3. Antioxidant activity

In vitro antioxidant activity of the newly synthesized compounds **8a**–**l** and **9a**–**h** was carried out by using ascorbic acid as the standard antioxidant and the results are summarized in Table 5. The antioxidant potential of the compounds was estimated as their power to reduce the TPTZ-Fe (III) complex to TPTZ-Fe (II) complex. Ferric reducing antioxidant power (FRAP) was measured by modified method of Benzie and Strain [45].The absorbance of intensive blue colour [Fe (II)–TPTZ] complex was measured at 593 nm. The results are expressed as ascorbic equivalent (mmol/100 g compound).

4. Result and discussion

4.1. Analytical results

The identity of all the synthesized compounds was confirmed by FT-IR, ^{1}H NMR, mass spectroscopy and elemental analysis. IR spectra of the compounds showed absorption in the range of 3413–3312 cm^{-1} due to symmetric and asymmetric –NH stretching of an amine. The C–H rocking in $-CH_3$was observed around 1376–1368 cm^{-1}. The strong absorption band observed in the range of 2212–2179 cm^{-1}could be attributed to the presence of –C≡N group. The C–H stretching in methyl group was observed around 3015–2951 cm^{-1}. The ^{1}H NMR spectra of the targeted compounds **8a**–**l** and **9a**–**h** showed the absence of the aldehydic proton, moreover a sharp singlet due to methyl proton appeared around δ 2.47–2.55 ppm. The aromatic protons resonate as multiplet at around δ 6.93–8.72 ppm. The mass spectrum of all the compounds showed molecular ion peak at M^+ corresponding to their molecular weights, which confirmed the respective chemical structures. The obtained elemental analysis values are in good agreement with theoretical data.

4.2. Biological results

4.2.1. In vitro antimicrobial assay

The antibacterial screening of compounds **3a**, **3b** and their pyridine derivatives **8a**–**l** and **9a**–**h** (Table 2) indicated that compound **8h** exhibited outstanding inhibitory effect i.e. 62.5 μg/mL against *B. subtilis* as compared to ampicillin i.e. 250 μg/mL and norfloxacin i.e. 100 μg/mL respectively. Compounds **3b**, **8a**, **8b**, **8g**, **8i**, **8k**, **9a**, **9c** and **9f** showed excellent activity against gram positive bacteria *B. subtilis* as that of the ampicillin i.e. 250 μg/mL. Among them, compounds **3b**, **8b**, **8k**, and **9a** showed comparable activity i.e. 100 μg/mL against *B. subtilis* as compared tonorfloxacin. While compounds **3a**, **8d**, **8l** and **9h** were found to be equipotent against *B. subtilis* as compared to ampicillin i.e. 250 μg/mL. Against *C. tetani*, compounds **3b**, **8g**, **8h**, **8k**, **9a**, **9c**, and **9f** were found to be more potent than ampicillin i.e. 250 μg/mL. Among them, compound 8hwas found to be equipotent to ciprofloxacin i.e. 100 μg/mL. The compounds **3a**, **8a**, **8c**, **8d**, **8j** and **8l** showed comparable activity against *C. tetani* as compared to ampicillin i.e. 250 μg/mL. Against *S. pneumoniae*, compounds **8e**, **8j**, **8l**, **9a** and **9c**–**9f** were found to have identical activity as compared to ampicillin i.e. 100 μg/mL. In case of inhibiting gram negative bacteria, the compounds **9g**, **9h** and **9a**, **9c** showed superior activity i.e. 62.5 μg/mL against *S. typhi* and *V. cholera* relative to ampicillin i.e. 100 μg/mL respectively and compound **8e** also illustrated marvellous activity i.e. 62.5 μg/mL against *E. coli* as compared to ampicillin. Compounds **3a**, **8c**, **8g**, **9g** and **9a** showed the same inhibitory effects as that of the standard ampicillin i.e. 100 μg/mL against *E. coli* and *S. typhi* respectively.

Table 1
Reaction parameters under sonication and by conventional method (**8a**–**l** and **9a**–**h**).

Entry	R	R_1/Het.	Sonication method[a]		Conventional method[b]	
			Time (min)	Yield[c] (%)	Time (h)	Yield[c] (%)
8a	H	H	60	84	2.0	78
8b	CH_3	H	45	85	1.5	79
8c	H	Ph	75	93	2.5	84
8d	CH_3	Ph	60	92	2.5	81
8e	H	Cl	45	81	2.0	71
8f	CH_3	Cl	60	80	2.5	69
8g	H	Br	30	82	2.5	72
8h	CH_3	Br	30	84	2.5	76
8i	H	F	45	81	2.0	73
8j	CH_3	F	30	83	2.0	72
8k	H	OCH_3	30	91	1.5	79
8l	CH_3	OCH_3	30	88	1.5	75
9a	H	2-furyl	75	82	3.0	71
9b	CH_3	2-furyl	60	83	2.5	68
9c	H	2-thienyl	90	79	3.0	64
9d	CH_3	2-thienyl	75	78	3.0	67
9e	H	3-coumaryl	90	81	3.0	71
9f	CH_3	3-coumaryl	90	85	3.0	73
9g	H	4-(3',4'-Methylene dioxy)phenyl	45	91	1.5	77
9h	CH_3	4-(3',4'-Methylene dioxy)phenyl	45	93	1.5	77

[a] Reaction condition: Reaction of aldehydes, malononitrile, aromatic/heterocyclic methyl ketones and ammonium acetate in ethanol at room temperature under ultrasound irradiation.
[b] Reaction condition: Reaction of aldehydes, malononitrile, aromatic/heterocyclic methyl ketones and ammonium acetate in ethanol under reflux condition.
[c] Yields of isolated products.

Against *V. cholera*, compounds **8d**, **8j**, **9a** and **9d** showed comparable activity i.e. 100 μg/mL as compared to ampicillin.

The antifungal screening data (Table 2) revealed that against *C. albicans*, compounds **3b**, **9f** and **9g** were found to have significant activity i.e. 250 μg/mL when compared with griseofulvin i.e. 500 μg/mL and compounds **8c**, **8d**, **8g**, **8l**, **9a** and **9e** showed same potency as that of griseofulvin. None of the compounds were observed to be active against *A. fumigatus*.

Table 2
In vitro antimicrobial activity (MICs, μg/mL) of compounds (**3a**–**3b**, **8a**–**8l**, **9a**–**9h**).

Entry	*Gram positive bacteria*			*Gram negative bacteria*			*Fungi*	
	S.P.	B.S.	C.T.	E.C.	S.T.	V.C.	C.A.	A.F.
	MTCC	MTCC	MTCC	MTCC	MTCC	MTCC	MTCC	MTCC
	1936	441	449	443	98	3906	227	3008
3a	500	250	250	100	125	250	>1000	>1000
3b	500	**100**	**150**	250	200	250	**250**	500
8a	250	**200**	250	250	200	250	1000	1000
8b	500	**100**	500	200	250	200	>1000	>1000
8c	500	500	250	100	125	500	500	500
8d	125	250	250	250	200	100	500	500
8e	100	500	500	**62.5**	200	500	1000	500
8f	200	500	500	200	250	250	1000	500
8g	200	**200**	**200**	100	200	200	500	500
8h	250	**62.5**	**100**	500	500	250	1000	>1000
8i	200	**200**	500	500	500	200	1000	1000
8j	100	500	250	500	250	100	>1000	1000
8k	125	**100**	**200**	125	200	200	>1000	1000
8l	100	250	250	125	250	**62.5**	500	1000
9a	100	**100**	**150**	250	100	100	500	>1000
9b	250	500	500	250	200	200	>1000	>1000
9c	100	**200**	**200**	500	500	**62.5**	>1000	500
9d	100	500	500	250	250	100	>1000	>1000
9e	100	500	500	200	500	125	500	1000
9f	100	**200**	**200**	250	250	125	**250**	500
9g	250	500	500	100	**62.5**	200	**250**	500
9h	500	250	500	125	**62.5**	500	1000	1000
A	100	250	250	100	100	100	n.t.[a]	n.t.
B	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	500	100

S.P.: *Streptococcus pneumoniae*, B.S.: *Bacillus subtilis*, C.T.: *Clostridium tetani*, E.C.: *Escherichia coli*, S.T.: *Salmonella typhi*, V.C.: *Vibrio cholerae*, C.A.: *Candida albicans*, A.F.: *Aspergillus fumigatus*, MTCC: Microbial Type Culture Collection. A: Ampicillin, B: Griseofulvin.
[a] n.t.: not tested.

4.2.2. *In vitro* antituberculosis assay

The encouraging results from the antimicrobial activity impelled us to set out for the preliminary screening of the synthesized compounds for their *in vitro* antituberculosis activity against *M. tuberculosis* H37Rv bacteria (Table 3). The antituberculosis broadcast data revealed that all the tested compounds showed moderate inhibitory effect. The conclusion of the antitubercular result exposed that, compounds **3a**, **8k**, **9a** and **9b** found to have moderate activity (i.e. 91%, 94%, 96% and 92% respectively at 250 μg/mL) against *M. tuberculosis* H37Rv. While compounds **8b**, **8h** and **9g** are fairly active i.e. 84% against *M. tuberculosis* H37Rv. Remaining all other compounds showed poor inhibition of *M. tuberculosis* growth. The compounds **3a**, **8k**, **9a** and **9b** which showed higher inhibition against *M. tuberculosis* H37Rv, they are further screened for their MICs. Among them compound **9a** MIC = 25 μg/mL found to possess highest potency against *M. tuberculosis* with 96% inhibition

Table 3
In vitro antituberculosis activity (% inhibition) of compounds (**3a**–**3b**, **8a**–**8l**, **9a**–**9h**) against *M. tuberculosis* H37Rv (at concentration 250 μg/mL).

Entry	% Inhibition	Entry	% Inhibition
3a	**91**	**8k**	**94**
3b	65	8l	56
8a	25	**9a**	**96**
8b	**84**	**9b**	**92**
8c	45	9c	41
8d	23	9d	23
8e	16	9e	61
8f	22	9f	45
8g	37	**9g**	**84**
8h	**84**	9h	78
8i	15	**Rifampicin**	98
8j	62	**Isoniazid**	99

Table 4
In vitro antituberculosis activity of title compounds exhibiting higher % inhibition against *M. tuberculosis* H37Rv (MICs, μg/mL).

Entry	% Inhibition	MIC, μg/mL
3a	91	100
8k	94	62.5
9a	96	25
9b	92	100
Rifampicin	98	0.15
Isoniazid	99	0.20

(Table 4). Compounds **8k** and **3a**, **9b** MIC = 62.5 μg/mL and MIC = 100 μg/mL exhibited better inhibition of 94% and 91%, 92% respectively. From the above results, it can be accomplished that, compound **9a** may prove themselves as an innovative category of antitubercular agents in future.

4.2.3. In vitro antioxidant assay

The ferric reducing antioxidant power (FRAP) of all the title compounds were determined. The FRAP assay of more than half of the compounds (**8a**–**8f**, **8i**, **8k**, **9a** and **9d**–**9f**) was found to range from 401.22 to 497.10 mmol/100 g. This indicated that the compounds are excellent antioxidant. The compounds **8g** and **8h** illustrated pitiable antioxidant power, while remaining all other compounds (**3a**, **3b**, **8j**, **8l**, **9b**, **9c**, **9g** and **9h**) exhibited moderate antioxidant activity (Table 5).

4.2.4. Structure–activity relationship (SAR)

The outcome of biological evaluation revealed that the activity was significantly affected by replacement of −H with lipophilic group $-CH_3$ at R position in imidazole ring. The antibacterial potency against *E. coli* was found to increase by more than three times (**8e** and **8f**). In the same way, it also raised antibacterial activity by three times towards *Bacillus. subtilis* in **8g** and **8h**. Decrease in antibacterial potency by one third folds against *V. cholera* was observed by the replacement of $-CH_3$ with −H (**9c** and **9d**). In contrast, replacement of lipophilic group $-CH_3$ with −H improved antifungal activity by four times against *C. albicans* (**9g** and **9h**). The same replacement was found to decrease antifungal activity by two times against *C. albicans* (**9e** and **9f**). The antituberculosis activity was also affected by substituting $-CH_3$ with −H. Presence of $-CH_3$ group as R in compounds **8b** and **8h** exhibited increased antitubercular activity, while compounds **8a** and **8g** having −H showed lower antitubercular activity. In disparity, compound **9a** containing −H and **9b** containing $-CH_3$ illustrated good antitubercular activity. In case of antioxidant activity, majority of the compounds showed terrific activity. The effect of substituting −H by $-CH_3$ was found to increase antioxidant activity also by two times i.e. **9c** and **9d** whereas the presence of −H in place of $-CH_3$ decreased antioxidant activity by two times i.e. **8k** and **8l**.

The activity was also considerably affected by various heterocyclic and substituted aromatic motifs present at six positions in pyridine ring (Fig. 1). Compounds **8e** and **8h** exhibited strong inhibitory effects against *E. coli* and *B. subtilis*. This tremendous inhibition could be attributed to the strong electron negativity of −Cl and −Br atoms. The activity is observed to decrease by two times and four times against *E. coli* and *B. subtillis* respectively (**8k** and **8l**) on replacement of electronegative groups with electron donating $-OCH_3$ group. The presence of electron donating $-OCH_3$ group raised the antibacterial potency against *V. cholerae*; while electron negative groups were observed to be responsible for depleting the activity. 2-thienyl group (**7b**) enhanced antibacterial activity towards *V. cholerae* (**9c**), whereas other three heterocyclic groups (**7a**, **7c**, **7d**) depleted the potency. 4-(3',4'-methylenedioxy)-phenyl group (**7d**) elevated the antibacterial potency against *S. typhi* (**9g**, **9h**) whereas **7a**, **7b** and **7c** groups caused remarkable reduction in activity.

It is interesting to point out that the compounds **9a** and **9b** carrying 2-furyl group showed reasonable antituberculosis potency towards H37RV. Electron donating $-OCH_3$ group inhibited the antituberculosis potency. Electronegative groups also did not promote the antitubercular activity. In case of antioxidant activity, the compounds with electron donating phenyl group possessed superior inhibition than the compounds with electron withdrawing and other heterocyclic groups. The compounds which showed highest potency in all activities are recognized in Fig. 2.

5. Conclusion

The aim of the present study was to design and synthesize novel 5-imidazopyrazole incorporated 2-amino-3-cyano pyridine derivatives through a facile one-pot multicomponent reaction by green method and to test for their *in vitro* antimicrobial, antituberculosis and antioxidant activity. The spectral data supported the structures of all newly synthesized compounds. This synthetic strategy allows the assembly of relatively complicated nitrogen containing heterocyclic system as well as the introduction of diverse aromatic and heterocyclic substitutions into position-6 of pyridine ring. Compounds **8e**, **8h**, **8l**, **9c**, **9g** and **9h** showed significant antibacterial potency and compounds **3b**, **9f** and **9g** displayed promising antifungal activity. Compounds **3a**, **8k**, **9a** and **9b** displayed moderate inhibition against antitubercular activity as compared to standard drugs. Majority of the compounds displayed good antioxidant activity. The present library model can be further explored to design the new class of antimicrobial, antitubercular and antioxidant agents under ultrasound irradiation.

6. Experimental section

6.1. Chemistry

All the reagents were obtained commercially and used without further purification. Ultrasonication was performed in D-Compact ultrasonic cleaner with a frequency of 50 kHz and power of 250 W. The reaction flask was suspended at the center of ultrasonic bath so as surface of the reactants remained slightly lower than the level of water in the bath. All reactions were monitored by thin-layer chromatography (TLC, on aluminium plates coated with silica gel60F_{254}, 0.25 mm thickness, Merck) carried on fluorescent coated plates and detection of the components was made by exposure to iodine vapours or UV light. Melting points were taken in melting point apparatus μThermoCal$_{10}$ (Analab Scientific Pvt. Ltd, India) and are uncorrected. ^{1}H Nuclear Magnetic Resonance spectra were recorded in DMSO-d_6 on a Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using residual solvent signal as an internal standard at 400 MHz. Chemical shifts are reported in parts per million (ppm). Splitting patterns were designated as follows: s, singlet; d, doublet; dd, doublet of doublet and m, multiplet. Mass spectra were recorded on Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan) at Sardar Patel University (PURSE programme of DST), Vallabh Vidyanagar. The IR spectra were recorded in KBr on a Perkin–Elmer Spectrum GX FT-IR Spectrophotometer (Perkin–Elmer, USA) and only the characteristic peaks are reported in cm^{-1}. Elemental analysis (% C, H, N) was carried out on Perkin Elmer 2400 CHN elemental analyzer at Sophisticated Instrumentation Centre for Applied Research & Training (SICART), Vallabh Vidyanagar. All compounds are within ±0.4% of the theoretical compositions. Yields are not optimized. Ampicillin,

Table 5
In vitro antioxidant activity of the compounds (**3a**–**3b**, **8a**–**8l**, **9a**–**9h**).

Entry	OD (593 nm)	FRAP value[a]	Entry	OD (593 nm)	FRAP value[a]
3a	1.221	242.88	**8k**	**2.012**	**400.22**
3b	1.072	213.24	8l	0.899	178.82
8a	**2.147**	**427.08**	**9a**	**2.294**	**456.32**
8b	**2.020**	**401.81**	9b	1.997	397.24
8c	**2.499**	**497.10**	9c	1.033	205.48
8d	**2.493**	**495.90**	**9d**	**2.123**	**422.30**
8e	**2.465**	**490.33**	**9e**	**2.017**	**401.22**
8f	**2.035**	**404.80**	**9f**	**2.179**	**433.44**
8g	0.340	67.63	9g	1.665	331.20
8h	0.355	70.61	9h	1.935	384.91
8i	**2.037**	**405.20**	A.A.	2.501	
8j	1.974	392.66			

A.A. = Ascorbic acid, Concentration of compounds used = 200 mg/mL, Concentration of standard (A.A.) = 176 mg/mL.
[a] A.A. mm/100 g sample.

ciprofloxacin, norfloxacin, chloramphenicol, griseofulvin, nystatin, isoniazid and rifampicin were purchased from the local market.

6.2. General procedure for the synthesis of substituted 5-(1H-imidazol/4methyl-1-yl)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (*3a*–*b*)

5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **1** (1.1 g, 5 mmol), imidazole **2a**/4-methyl imidazole **2b**(7.5 mmol) and anhydrous potassium carbonate (0.6 g, 10 mmol) in dimethylformamide (5 mL) were charged in a 50 mL round bottom flask equipped with mechanical stirrer and condenser. The reaction mixture was refluxed for 2 h and the progress of the reaction was monitored by TLC. After the completion of reaction (as evidenced by TLC), the reaction mixture was cooled to room temperature and then poured into ice cold water (50 mL) with continuous stirring

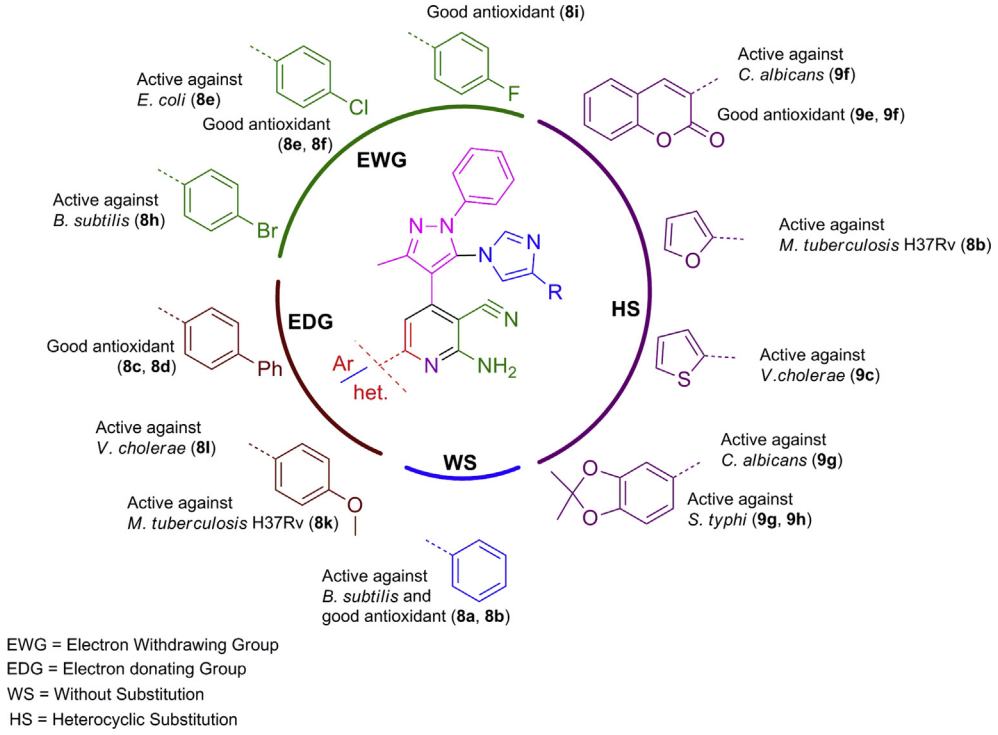


Fig. 1. Structure–activity relationship for antimicrobial, antituberculosis and antioxidant activity of the synthesized compounds.

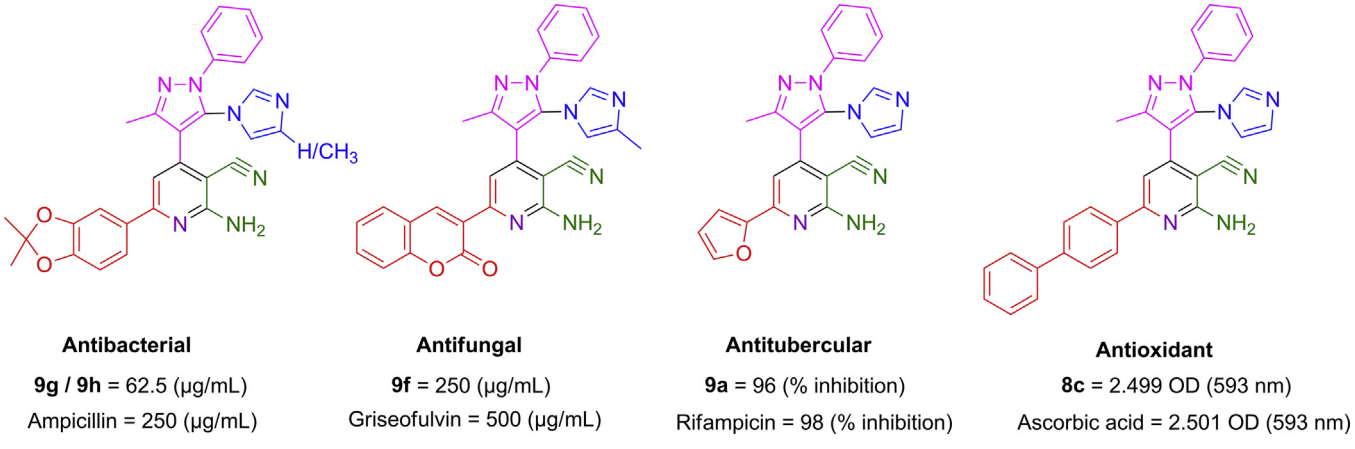


Fig. 2. Most active compounds of the series.

followed by neutralization with 1 N HCl until pH 7. The separated precipitates of substituted 5-(1*H*-imidazol/4-methyl-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4 carbaldehyde **3a**–**b** were filtered, thoroughly washed with water, dried, and recrystallized from hot ethanol.

*6.2.1. 5-(1H-Imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (**3a**)*

Yield 79%; m.p. 204 °C; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.59 (s, 3H, CH$_3$), 7.07–7.46 (m, 7H, Ar–H), 7.94 (s, 1H, imidazole), 9.74 (s, 1H, CHO); ESI-MS (*m*/*z*): = 252.0 (M$^+$)

*6.2.2. 3-Methyl-5-(4-methyl-1H-imidazol-1-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde (**3b**)*

Yield 82%; m.p. 215 °C; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 2.05 (s, 3H, CH$_3$), 2.52 (s, 3H, CH$_3$), 7.10–7.58 (m, 7H, Ar–H), 7.97 (s, 1H, imidazole), 9.59 (s, 1H, CHO); ESI-MS (*m*/*z*): = 267.0 (M$^+$)

*6.3. General procedure for the synthesis of substituted 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-aminonicotinonitrile (**8a**–**l**, **9a**–**h**) under conventional conditions*

The substituted 5-(1H-imidazol/4-methyl-1-yl)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde **3a**–**b** (5 mmol), malononitrile **4** (5 mmol), aromatic/heterocyclic methyl ketone **6a**–**f**/**7a**–**d** (5 mmol), ammonium acetate **5** (40 mmol) and absolute alcohol (10 mL) were charged in a 50 mL round bottom flask. The reaction mixture was refluxed for 1.5–3 h. Progress of the reaction was monitored by the TLC. After the completion of reaction, the reaction mixture was cooled to room temperature and stirred for 0.5 h. The resulting solid was collected by filtration and washed well with absolute alcohol to obtain the pure solid sample of product **8a**–**l** and **9a**–**h**.

*6.4. General procedure for the synthesis of substituted 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-aminonicotinonitrile (**8a**–**l**, **9a**–**h**) under ultrasound irradiation*

The substituted 5-(1H-imidazol/4-methyl-1-yl)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde **3a**–**b** (5 mmol), malononitrile **4** (5 mmol), aromatic/heterocyclic methyl ketone **6a**–**f**/**7a**–**d** (5 mmol), ammonium acetate **5** (40 mmol) and absolute alcohol (10 mL) were charged in a 50 mL round bottom flask. The reaction mixture was irradiated by ultrasound at room temperature for required minutes. Progress of the reaction was monitored by the TLC. The resulting solid was collected by filtration and washed well with absolute alcohol to obtain the pure solid sample of product **8a**–**l** and **9a**–**h**.

*6.4.1. 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-6-phenylnicotinonitrile (**8a**)*

m.p. 221–222 °C; IR (KBr, ν_{max}, cm^{-1}): 3435 & 3356 (asym. & sym. stretching of –NH$_2$), 2951 (Ar C–H), 2189 (C≡N stretching), 1376 (–CH$_3$ stretching); ^{1}H NMR δ ppm (DMSO-d$_6$): δ 2.11 (s, 3H, CH$_3$), 7.14–8.22 (m, 16H, Ar–H + NH$_2$); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 13.2 (CH$_3$), 89.7 (C–CN), 110.3, 116.5, 116.9, 117.7, 123.2, 124.2, 125.2, 127.5, 128.2, 128.4, 128.5, 128.9, 129.1, 130.2, 131.5, 139.4, 140.3, 140.8, 147.4, 156.3, 159.2, 161.2 (22C, Ar–C); ESI-MS (*m*/*z*): 418.2 (M$^+$); Anal. Calcd. (%) for C$_{25}$H$_{19}$N$_7$: C, 71.93; H, 4.59; N, 23.49. Found: C, 71.80; H, 4.61; N, 23.66.

*6.4.2. 2-Amino-4-(3-methyl-5-(4-methyl-1H-imidazol-1-yl)-1-phenyl-1H-pyrazol-4-yl)-6-phenylnicotinonitrile (**8b**)*

m.p. 223–224 °C; IR (KBr, ν_{max}, cm^{-1}): 3459 & 3335 (asym. & sym. stretching of –NH$_2$), 2992 (Ar C–H), 2179 (C≡N stretching), 1371 (–CH$_3$ stretching); ^{1}H NMR δ ppm (DMSO-d$_6$): δ 2.13 (s, 3H, CH$_3$), 2.56 (s, 3H, CH$_3$), 7.26–8.23 (m, 15H, Ar–H + NH$_2$); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 12.8 (CH$_3$), 13.4 (CH$_3$), 88.6 (C–CN), 109.1, 115.5, 116.2, 116.9, 123.3, 124.2, 125.4, 127.9, 128.5, 128.8, 129.5, 130.2, 131.1, 131.9, 140.4, 141.3, 141.9, 145.3, 155.2, 159.2, 160.9 (21C, Ar–C); ESI-MS (*m*/*z*): 432.2 (M$^+$); Anal. Calcd. (%) for C$_{26}$H$_{21}$N$_7$: C, 72.37; H, 4.91; N, 22.72. Found: C, 72.19; H, 5.05; N, 22.79.

*6.4.3. 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-6-([1,1'-biphenyl]-4-yl)-2-aminonicotinonitrile (**8c**)*

m.p. 231–232 °C; IR (KBr, ν_{max}, cm^{-1}): 3420 & 3318 (asym. & sym. stretching of –NH$_2$), 3006 (Ar C–H), 2201 (C≡N stretching), 1375 (–CH$_3$ stretching); ^{1}H NMR δ ppm (DMSO-d$_6$): δ 2.30 (s, 3H, CH$_3$), 7.11–8.06 (m, 20H, Ar–H + NH$_2$); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 12.6 (CH$_3$), 89.7 (C–CN), 107.4, 115.2, 116.1, 122.6, 123.5, 123.9, 124.4, 125.1, 126.2, 126.8, 127.5, 127.9, 128.0, 128.2, 128.7, 128.9, 129.5, 132.4, 137.7, 138.6, 139.0, 140.6, 141.1, 141.7, 146.9, 155.5, 160.2, 162.5 (28C, Ar–C); ESI-MS (*m*/*z*): 494.4 (M$^+$); Anal. Calcd. (%) for C$_{31}$H$_{23}$N$_7$: C, 75.44; H, 4.70; N, 19.87. Found: C, 75.31; H, 4.83; N, 19.81.

*6.4.4. 6-([1,1'-Biphenyl]-4-yl)-2-amino-4-(3-methyl-5-(4-methyl-1H-imidazol-1-yl)-1-phenyl-1H-pyrazol-4-yl) nicotinonitrile (**8d**)*

m.p. 233–234 °C; IR (KBr, ν_{max}, cm^{-1}): 3449 & 3339 (asym. & sym. stretching of –NH$_2$), 3015 (Ar C–H), 2209 (C≡N stretching), 1370 (–CH$_3$ stretching); ^{1}H NMR δ ppm (DMSO-d$_6$): δ 2.11 (s, 3H, CH$_3$), 2.50 (s, 3H, CH$_3$), 7.09–7.72 (m, 19H, Ar–H + NH$_2$); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 12.6 (CH$_3$), 13.9 (CH$_3$), 88.4 (C–CN), 106.3, 114.0, 115.6, 122.1, 122.9, 123.4, 125.4, 125.9, 126.4, 127.0, 127.9, 128.0, 128.2, 128.5, 129.0, 131.4, 133.6, 137.0, 139.5, 139.9, 141.0.6, 142.0, 142.7, 145.8, 153.4, 157.2, 160.2 (27C, Ar–C); ESI-MS (*m*/*z*): 508.5 (M$^+$); Anal. Calcd. (%) for C$_{32}$H$_{25}$N$_7$: C, 75.72; H, 4.96; N, 19.32. Found: C, 75.59; H, 5.06; N, 19.43.

*6.4.5. 4-(5-(1H-Imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-6-(4-chlorophenyl)nicotinonitrile (**8e**)*

m.p. 254–255 °C; IR (KBr, ν_{max}, cm^{-1}); 3422 & 3336 (asym. & sym. stretching of –NH$_2$), 2999 (Ar C–H), 2192 (C≡N stretching), 1372 (–CH$_3$ stretching); ^{1}H NMR δ ppm (DMSO-d$_6$): δ 2.20 (s, 3H, CH$_3$), 7.13–7.82 (m, 15H, Ar–H + NH$_2$); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 12.8 (CH$_3$), 89.5 (C–CN), 108.2, 115.4, 116.9, 117.2, 118.9, 123.4, 126.6, 127.5, 128.2, 128.9, 129.5, 129.8, 130.2, 131.2, 136.5, 139.1, 140.0, 142.7, 147.5, 155.8, 160.2, 161.4 (22C, Ar–C); ESI-MS (*m*/*z*): 452.8 (M$^+$), 453.6 (M+2); Anal. Calcd. (%) for C$_{25}$H$_{18}$ClN$_7$: C, 66.44; H, 4.01; N, 21.70. Found: C, 66.29; H, 3.91; N, 21.64.

*6.4.6. 2-Amino-6-(4-chlorophenyl)-4-(3-methyl-5-(4-methyl-1H-imidazol-1-yl)-1-phenyl-1H-pyrazol-4-yl)nicotinonitrile (**8f**)*

m.p. 260–261 °C; IR (KBr, ν_{max}, cm^{-1}); 3449 & 3354 (asym. & sym. stretching of –NH$_2$), 3012 (Ar C–H), 2199 (C≡N stretching), 1373 (–CH$_3$ stretching); ^{1}H NMR δ ppm (DMSO-d$_6$): δ 2.10 (s, 3H, CH$_3$), 2.50 (s, 3H, CH$_3$), 6.98–7.93 (m, 14H, Ar–H + NH$_2$); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 12.9 (CH$_3$), 14.0 (CH$_3$), 89.7 (C–CN), 107.4, 114.1, 115.9, 116.1, 117.8, 123.5, 126.6, 127.0, 128.1, 128.5, 128.9, 129.5, 131.2, 135.4, 138.9, 140.6, 141.7, 146.4, 153.2, 158.2, 160.9 (21C, Ar–C); ESI-MS (*m*/*z*): 466.7 (M$^+$), 467.7 (M+2); Anal. Calcd. (%) for C$_{26}$H$_{20}$ClN$_7$: C, 67.02; H, 4.33; N, 21.04. Found: C, 66.89; H, 4.23; N, 21.21.

*6.4.7. 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-6-(4-bromophenyl)nicotinonitrile (**8g**)*

m.p. 269–270 °C; IR (KBr, ν_{max}, cm^{-1}); 3460 & 3345 (asym. & sym. stretching of –NH$_2$), 3001 (Ar C–H), 2212 (C≡N stretching), 1376 (–CH$_3$ stretching): ^{1}H NMR δ ppm (DMSO-d$_6$): δ 2.08 (s, 3H, CH$_3$), 7.27–8.24 (m, 15H, Ar–H + NH$_2$); ^{13}C NMR (100 MHz, DMSO-

d_6) δ: 12.1 (CH_3), 88.9 (C–CN), 107.4, 115.4, 115.9, 116.4, 117.8, 122.3, 125.6, 127.5, 128.1, 128.8, 129.9, 130.0, 130.8, 132.4, 135.5, 138.9, 141.1, 142.9, 148.9, 156.7, 159.6, 162.3 (22C, Ar–C); ESI-MS (*m*/*z*): 497.10 (M^+), 497.9 (M+2); Anal. Calcd. (%) for $C_{25}H_{18}BrN_7$: C, 60.49; H, 3.66; N, 19.75. Found: C, 60.57; H, 3.65; N, 19.65.

*6.4.8. 2-Amino-6-(4-bromophenyl)-4-(3-methyl-5-(4-methyl-1H-imidazol-1-yl)-1-phenyl-1H-pyrazol-4-yl)nicotinonitrile (**8h**)*

m.p. 276–277 °C; IR (KBr, ν_{max}, cm^{-1}); 3430 & 3337 (asym. & sym. stretching of $-NH_2$), 2993 (Ar C–H), 2203 (C≡N stretching), 1368 ($-CH_3$ stretching); ^{1}H NMR δ ppm (DMSO-d_6): δ 2.10 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 7.31–8.19 (m, 14H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.0 (CH_3), 13.9 (CH_3), 89.9 (C–CN), 107.5, 113.5, 114.7, 115.1, 117.8, 123.5, 125.6, 127.2, 128.0, 128.6, 129.0, 129.9, 130.4, 134.3, 137.2, 139.5, 142.9, 145.8, 155.2, 159.2, 161.2 (21C, Ar–C); ESI-MS (*m*/*z*): 511.2 (M^+), 512.1 (M+2); Anal. Calcd. (%) for $C_{26}H_{20}BrN_7$: C, 61.18; H, 3.95; N, 19.21. Found: C, 61.11; H, 4.05; N, 19.37.

*6.4.9. 4-(5-(1H-Imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-6-(4-fluorophenyl) nicotinonitrile (**8i**)*

m.p. 243–244 °C; IR (KBr, ν_{max}, cm^{-1}); 3449 & 3312 (asym. & sym. stretching of $-NH_2$), 2989 (Ar C–H), 2189 (C≡N stretching), 1373 ($-CH_3$ stretching); ^{1}H NMR δ ppm (DMSO-d_6): δ 2.09 (s, 3H, CH_3), 7.08–7.82 (m, 15H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.1 (CH_3), 90.0 (C–CN), 109.4, 116.3, 116.9, 118.0, 118.9, 124.4, 125.6, 127.5, 128.0, 128.8, 129.9, 130.8, 130.9, 132.2, 137.9, 140.1, 141.0, 142.7, 148.5, 156.8, 161.9, 163.3 (22C, Ar–C); ESI-MS (*m*/*z*): 436.2 (M^+); Anal. Calcd. (%) for $C_{25}H_{18}FN_7$: C, 68.95; H, 4.17; N, 22.52. Found: C, 69.09; H, 4.29; N, 22.36.

*6.4.10. 2-Amino-6-(4-fluorophenyl)-4-(3-methyl-5-(4-methyl-1H-imidazol-1-yl)-1-phenyl-1H-pyrazol-4-yl) nicotinonitrile (**8j**)*

m.p. 239–240 °C; IR (KBr, ν_{max}, cm^{-1}); 3451 & 3341 (asym. & sym. stretching of $-NH_2$), 3004 (Ar C–H), 2211 (C≡N stretching), 1373 ($-CH_3$ stretching); ^{1}H NMR δ ppm (DMSO-d_6): δ 2.08 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 6.93–8.06 (m, 14H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.2 (CH_3), 14.1 (CH_3), 88.9 (C–CN), 107.3, 112.5, 113.7, 114.1, 117.8, 122.9, 124.5, 126.2, 127.9, 128.5, 129.3, 130.1, 130.9, 133.1, 136.1, 138.4, 141.5, 144.8, 156.2, 159.9, 162.9 (21C, Ar–C); ESI-MS (*m*/*z*): 450.4 (M^+); Anal. Calcd. (%) for $C_{26}H_{20}FN_7$: C, 69.48; H, 4.48; N, 21.81. Found: C, 69.64; H, 4.54; N, 21.74.

*6.4.11. 4-(5-(1H-Imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2amino-6-(4-methoxyphenyl)nicotinonitrile (**8k**)*

m.p. 212–213 °C; IR (KBr, ν_{max}, cm^{-1}); 3423 & 3313 (asym. & sym. stretching of $-NH_2$), 3003 (Ar C–H), 2184 (C≡N stretching), 1375 ($-CH_3$ stretching); ^{1}H NMR δ ppm (DMSO-d_6): δ 2.09 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 7.32–8.16 (m, 15H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.5 (CH_3), 53.5 (OCH_3), 89.0 (C–CN), 109.1, 111.4, 112.9, 115.6, 116.9, 122.3, 126.6, 127.9, 128.0, 128.5, 128.7, 128.9, 129.8, 131.2, 132.8, 136.9, 139.5, 140.7, 147.9, 156.0, 161.2, 162.4 (22C, Ar–C); ESI-MS (*m*/*z*): 448.2 (M^+); Anal. Calcd. (%) for $C_{26}H_{21}N_7O$: C, 69.78; H, 4.73; N, 21.91. Found: C, 69.91; H, 4.82; N, 22.04.

*6.4.12. 2-Amino-6-(4-methoxyphenyl)-4-(3-methyl-5-(4-methyl-1H-imidazol-1-yl)-1-phenyl-1H-pyrazol-4-yl) nicotinonitrile (**8l**)*

m.p. 234–235 °C; IR (KBr, ν_{max}, cm^{-1}); 3447 & 3342 (asym. & sym. stretching of $-NH_2$), 2985 (Ar C–H), 2200 (C≡N stretching), 1372 ($-CH_3$ stretching); ^{1}H NMR δ ppm (DMSO-d_6): δ 2.04 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 7.24–8.18 (m, 14H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.8 (CH_3), 54.6 (OCH_3), 88.9 (C–CN), 108.1, 111.4, 112.9, 114.7, 115.8, 121.3, 125.9, 126.0, 127.6, 128.1, 128.6, 129.3, 130.9, 133.4, 136.9, 139.6, 141.8, 145.6, 155.0, 162.1, 162.6 (21C, Ar–C); ESI-MS (*m*/*z*): 462.3 (M^+); Anal. Calcd. (%) for $C_{27}H_{23}N_7O$: C, 70.27; H, 5.02; N, 21.24. Found: C, 70.42; H, 4.99; N, 21.31.

*6.4.13. 4-(5-(1H-Imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-6-(furan-2-yl) nicotinonitrile (**9a**)*

m.p. 211–212 °C; IR (KBr, ν_{max}, cm^{-1}); 3462 & 3330 (asym. & sym. stretching of $-NH_2$), 2970 (Ar C–H), 2212 (C≡N stretching), 1375 ($-CH_3$ stretching); ^{1}H NMR δ ppm (DMSO-d_6): δ 2.21 (s, 3H, CH_3), 7.47–8.19 (m, 14H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.9 (CH_3), 90.0 (C–CN), 99.8, 107.5, 114.3, 115.6, 116.0, 117.2, 122.4, 125.6, 127.9, 128.3, 128.7, 128.9, 129.6, 138.4, 141.2, 142.6, 147.5, 149.7, 151.5, 162.3 (20C, Ar–C); ESI-MS (*m*/*z*): 408.1 (M^+); Anal. Calcd. (%) for $C_{23}H_{17}N_7O$: C, 67.80; H, 4.21; N, 24.06. Found: C, 67.90; H, 4.31; N, 23.96.

*6.4.14. 2-Amino-6-(furan-2-yl)-4-(3-methyl-5-(4-methyl-1H-imidazol-1-yl)-1-phenyl-1H-pyrazol-4-yl) nicotinonitrile (**9b**)*

m.p. 218–219 °C; IR (KBr, ν_{max}, cm^{-1}); 3423 & 3349 (asym. & sym. stretching of $-NH_2$), 3012 (Ar C–H), 2190 (C≡N stretching), 1369 ($-CH_3$ stretching); ^{1}H NMR δ ppm (DMSO-d_6): δ 2.21 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 7.22–7.98 (m, 13H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.9 (CH_3), 13.8 (CH_3), 89.2 (C–CN), 99.1, 106.1, 113.2, 114.5, 117.1, 117.9, 123.4, 126.1, 126.9, 128.5, 128.9, 129.4, 130.5, 137.1, 140.1, 143.4, 145.6, 151.9, 161.8 (19C, Ar–C); ESI-MS (*m*/*z*): 422.2 (M^+); Anal. Calcd. (%) for $C_{24}H_{19}N_7O$: C, 68.40; H, 4.54; N, 23.26. Found: C, 68.42; H, 4.54; N, 23.36.

*6.4.15. 4-(5-(1H-Imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-6-(thiophen-2-yl) nicotinonitrile (**9c**)*

m.p. 246–247 °C; IR (KBr, ν_{max}, cm^{-1}); 3441 & 3317 (asym. & sym. stretching of $-NH_2$), 3001 (Ar C–H), 2210 (C≡N stretching), 1372 ($-CH_3$ stretching); ^{1}H NMR δ ppm (DMSO-d_6): δ 2.22 (s, 3H, CH_3), 7.09–8.07 (m, 14H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.5 (CH_3), 88.9 (C–CN), 100.1, 106.4, 114.5, 115.3, 116.9, 117.6, 122.5, 125.9, 128.0, 128.4, 128.8, 129.3, 129.8, 138.5, 141.2, 143.5, 144.6, 148.4, 150.6, 161.9 (20C, Ar–C); ESI-MS (*m*/*z*): 424.5 (M^+); Anal. Calcd. (%) for $C_{23}H_{17}N_7S$: C, 65.23; H, 4.05; N, 23.15. Found: C, 65.17; H, 3.97; N, 23.34.

*6.4.16. 2-Amino-4-(3-methyl-5-(4-methyl-1H-imidazol-1-yl)-1-phenyl-1H-pyrazol-4-yl)6-(thiophen-2-yl)nicotinonitrile (**9d**)*

m.p. 252–253 °C; IR (KBr, ν_{max}, cm^{-1}); 3456 & 3342 (asym. & sym. stretching of $-NH_2$), 2981 (Ar C–H), 2181 (C≡N stretching), 1376 ($-CH_3$ stretching); ^{1}H NMR δ ppm (DMSO-d_6): δ 2.16 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 6.94–7.94 (m, 13H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.1 (CH_3), 13.9 (CH_3), 89.3 (C–CN), 100.5, 104.2, 112.2, 113.9, 116.1, 116.9, 123.4, 125.1, 126.8, 127.3, 129.1, 129.8, 130.4, 136.8, 140.1, 143.9, 146.4, 150.2, 160.7 (19C, Ar–C); ESI-MS (*m*/*z*): 438.3 (M^+); Anal. Calcd. (%) for $C_{24}H_{19}N_7S$: C, 65.88; H, 4.38; N, 22.41. Found: C, 66.02; H, 4.29; N, 22.62.

*6.4.17. 4-(5-(1H-Imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)2-amino-6-(2-oxo-2H-chromen-3-yl)nicotinonitrile (**9e**)*

m.p. 205–206 °C; IR (KBr, ν_{max}, cm^{-1}); 3421 & 3333 (asym. & sym. stretching of $-NH_2$), 3012 (Ar C–H), 2291 (C≡N stretching), 1370 ($-CH_3$ stretching); ^{1}H NMR δ ppm (DMSO-d_6): δ 2.09 (s, 3H, CH_3), 7.16–8.06 (m, 16H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.7 (CH_3), 88.5 (C–CN), 104.2, 115.6, 116.0, 116.4, 116.9, 121.4, 122.9, 124.1, 126.8, 127.5, 127.9, 128.3, 128.7, 128.9, 129.5, 130.9, 131.3, 137.9, 140.2, 141.4, 145.5, 152.6, 156.9, 161.9 (24C, Ar–C); ESI-MS (*m*/*z*): 486.4 (M^+); Anal. Calcd. (%) for $C_{28}H_{19}N_7O_2$: C, 69.27; H, 3.94; N, 20.20. Found: C, 69.41; H, 4.10; N, 20.03.

*6.4.18. 2-Amino-4-(3-methyl-5-(4-methyl-1H-imidazol-1-yl)-1-phenyl-1H-pyrazol-4-yl)-6-(2-oxo-2H-chromen-3-yl)nicotinonitrile (**9f**)*

m.p. 208–210 °C; IR (KBr, ν_{max}, cm^{-1}); 3413 & 3312 (asym. & sym. stretching of $-NH_2$), 3005 (Ar C–H), 2199 (C≡N stretching), 1373 ($-CH_3$ stretching); ^1H NMR δ ppm (DMSO-d_6): δ 2.12 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 7.28–7.73 (m, 15H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.9 (CH_3), 88.4 (C–CN), 105.1, 114.9, 115.4, 116.0, 116.8, 122.0, 123.3, 124.1, 125.9, 127.2, 127.8, 128.3, 128.4, 128.8, 129.0, 131.2, 132.4, 136.3, 140.5, 141.9, 145.5, 156.1, 162.8 (23C, Ar–C); ESI-MS (*m/z*): 500.2 (M^+); Anal. Calcd. (%) for $C_{29}H_{21}N_7O_2$: C, 69.73; H, 4.24; N, 19.63. Found: C, 69.83; H, 4. 25; N, 19.53.

*6.4.19. 4-(5-(1H-Imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-6-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl) nicotinonitrile (**9g**)*

m.p. 229–230 °C; IR (KBr, ν_{max}, cm^{-1}); 3449 & 3329 (asym. & sym. stretching of $-NH_2$), 2973 (Ar C–H), 2212 (C≡N stretching), 1374 ($-CH_3$ stretching); ^1H NMR δ ppm (DMSO-d_6): δ 1.67 (s, 6H, $2CH_3$), 2.17 (s, 3H, CH_3), 7.33–8.19 (m, 14H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.7 (CH_3), 23.7, 23.8 (2C, CH_3), 89.6 (C–CN), 108.1, 111.3, 115.9, 116.4, 117.2, 119.4, 122.5, 123.6, 127.3, 128.0, 128.4, 128.8, 129.0, 129.5, 130.4, 138.5, 140.4, 141.9, 142.9, 147.8, 155.8, 161.5, 162.9 (23C, Ar–C); ESI-MS (*m/z*): 490.1 (M^+); Anal. Calcd. (%) for $C_{28}H_{23}N_7O_2$: C, 68.70; H, 4.74; N, 20.03. Found: C, 68.83; H, 4.68; N, 19.93.

*6.4.20. 2-Amino-6-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)-4-(3-methyl-5-(4-methyl-1H-imidazol-1-yl)-1-phenyl-1H-pyrazol-4-yl) nicotinonitrile (**9h**)*

m.p. 235–236 °C; IR (KBr, ν_{max}, cm^{-1}); 3430 & 3321 (asym. & sym. stretching of $-NH_2$), 3000 (Ar C–H), 2197 (C≡N stretching), 1368 ($-CH_3$ stretching); ^1H NMR δ ppm (DMSO-d_6): δ 1.66 (s, 6H, $2CH_3$), 2.18 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 7.32–8.18 (m, 13H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.7 (CH_3), 13.9 (CH_3), 23.9, 24.0 (2C, CH_3), 88.9 (C–CN), 107.2, 110.4, 114.8, 115.3, 116.1, 119.4, 122.8, 123.5, 126.8, 127.4, 128.3, 128.6, 128.9, 129.3, 130.4, 137.1, 140.7, 142.0, 143.5, 146.9, 154.7, 160.6, 161.7 (22C, Ar–C); ESI-MS (*m/z*): 504.4 (M^+); Anal. Calcd. (%) for $C_{29}H_{25}N_7O_2$: C, 69.17; H, 5.00; N, 19.47. Found: C, 69.35; H, 5.13; N, 19.29.

7. Biological evaluation

7.1. In vitro antimicrobial assay

Mueller–Hinton broth was used as nutrient medium to grow and dilute the compound suspension for the test bacteria. 2% DMSO in water was used as the diluent to get the desired concentration of compounds to test upon standard bacterial stains. Sabouraud Dextrose broth was used for fungal nutrition. Inoculum size for test stain was adjusted to 10^8 CFU mL^{-1} by comparing the turbidity. Serial dilutions were prepared in primary and secondary screening. Each synthesized compound and the standard drugs were diluted obtaining 2000 μg/mL concentration as a stock solution. The drugs which were found to be active in primary screening (i.e. 500, 250 and 200 μg/mL concentrations) were further screened in their second set of dilution at 100, 50, 25 and 12.5 μg/mL concentration against all microorganisms. 10 μL suspensions were further inoculated on appropriate media and growth was noted after 24 and 48 h. The tubes were then put overnight for incubation at 37 °C. The control tube containing no antibiotic was instantaneously subcultured by spreading a loopful consistently over an area of plate of medium fitting for the growth of the test organism. The maximum dilution preventing appearance of turbidity after spot subculture was considered as minimal inhibitory concentration (MIC, μ/L). The tubes showing no visible growth (same as the control tube) were subcultured and incubated overnight at 37 °C. The amount of growth from the control tube before incubation was compared. In this study Ampicillin was used as the standard antibacterial drugs while Griseofulvin was used as standard antifungal drugs. The outcomes are summarized in Table 2.

7.2. In vitro antituberculosis assay

Drug susceptibility and determination of antituberculosis activity was carried out by adding 250 μg/mL dilution of each compound to Lowensteine–Jensen medium and then media was uncontaminated by inspissations method. A culture of *M. tuberculosis* H37Rv growing on Lowensteine–Jensen medium was harvested in 0.85% saline in bijou bottle. The stock solutions of the title compounds were prepared in DMSO i.e. 250 μg/mL. These tubes were then incubated at 37 °C for 1 day followed by streaking of *M. tuberculosis* H37Rv (5×10^{-4} bacilli per tube). The growth of bacilli was seen after two weeks, three weeks and finally after four weeks of incubation. The tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* H37Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of the tested compound. The standard drugs isoniazid and rifampicin were used for comparison purpose. The results are summarized in Table 3.

7.3. In vitro antioxidant assay

FRAP assay measure a reduction power of all the synthesized compounds, converting ferric tripyridyl triazine (Fe (III)-TPTZ) complex into a blue colour ferrous tripyridyl triazine (Fe (II)-TPTZ) complex at 593 nm [45]. Fe(II)-TPTZ(2,4,6-tripyridyl-s-triazine) reagent was prepared by mixing a 10.0 mL TPTZ solution (0.155 g TPTZ was dissolved in 100 mL 40 mM HCl), 10 mL $FeCl_3 \cdot 6H_2O$ solution (0.324 g $FeCl_3$ was dissolved in 100 mL distilled water) and 100 mL acetate buffer (0.187 g sodium acetate and 1.6 mL acetic acid dissolved in double distilled water to make 100 mL) at pH 3.6. A mixture of 200.0 mL sample solution and 3 mL of Fe(II)TPTZ reagent was incubated at 37 °C for 25 min. The absorbance of colour complex Fe(II)TPTZ was measured at 593 nm using ascorbic acid as standard. The results were expressed as ascorbic equivalent (mmol/100 g compound) summarized in Table 5.

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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.2014.06.071.

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