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Microwave assisted one-pot synthetic route to imidazo[1,2-*a*]pyrimidine derivatives of imidazo/triazole clubbed pyrazole and their pharmacological screening†

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An efficient synthesis of imidazo[1,2-*a*]pyrimidine derivatives of pyrazole in excellent yield over a short reaction time based on a microwave-assisted, one-pot three-component condensation reaction of pyrazole aldehyde clubbed with imidazole **4** and triazole **5** nuclei, (substituted-phenyl/hetero-aryl)ethanones **6(a–g)**, and 2-amino benzimidazole **7** in the presence of the strong base KOH is described. All the compounds were screened for their preliminary *in vitro* antimicrobial, antituberculosis and antimalarial activities against a panel of pathogenic strains. The majority of the compounds exhibited excellent inhibitory action against *S. typhi*, *S. pneumoniae*, *B. subtilis*, and *C. tetani*. Some of the compounds showed good antifungal activity and moderate antituberculosis activity as compared to first line drugs. Two of the compounds **8b** and **9b** exhibited excellent antimalarial activity against *P. falciparum* strains.

1. Introduction

The synthesis of nitrogen-containing fused heterocycles or heterocyclic motifs containing multi-structures in one molecule has attracted much attention in the last couple of decades.[1] Since small and simple heterocyclic scaffolds often exhibit amazing multifaceted pharmacological properties, medicinal chemists have been trying to constitute the most imperative module of new active molecules for biological applications by annulating different heterocycles such as indoles, pyrroles, pyrimidines, imidazoles, pyrazoles and pyridines using various synthetic strategies[2] as it increases the range of potential applications.

On the other hand, fused imidazole fragments and imidazo-[1,2-*a*]-fused pyrimidine derivatives are important structural moieties, which have been found to be used as core templates for the synthesis of many drugs prescribed for a lot of pathologies and natural products (Fig. 1). The synthesis of many imidazo-[1,2-*a*] pyrimidines and pyridine fragments has attracted considerable attention from the pharmaceutical industry and these compounds are widely recognized because of their important biological activities and interesting therapeutic properties,[5] including anti-bacterial,[6] anti-inflammatory, antifungal, anti-microbial, antituberculosis, and anticancer activities, and also used as potent p38MAP kinase inhibitors, *etc.* Previously, imidazo[1,2-*a*]pyrimidines have been prepared using different synthetic approaches.[7]

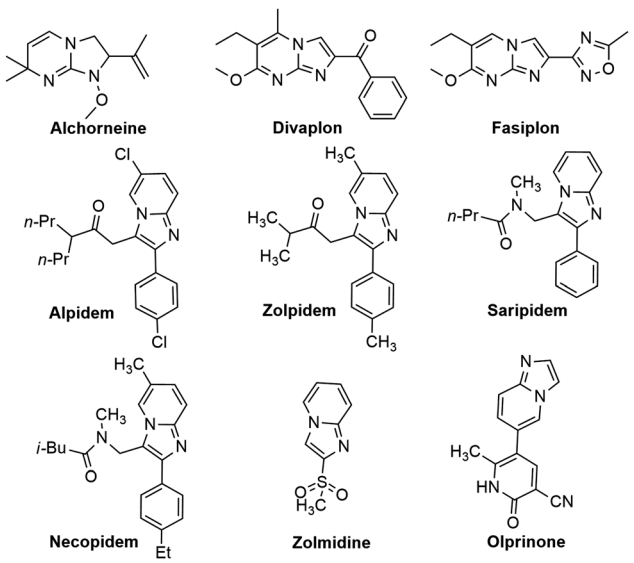

Fig. 1 Alkaloids and drugs containing similar imidazo[1,2-*a*]pyrimidine/pyridine derivatives.

Therefore, the development of efficient and practical synthetic routes to generate imidazo[1,2-*a*]pyrimidine units for the synthesis of natural and biologically potent compounds is in great demand. Typically, the synthesis of imidazo[1,2-*a*] heterocycles requires long

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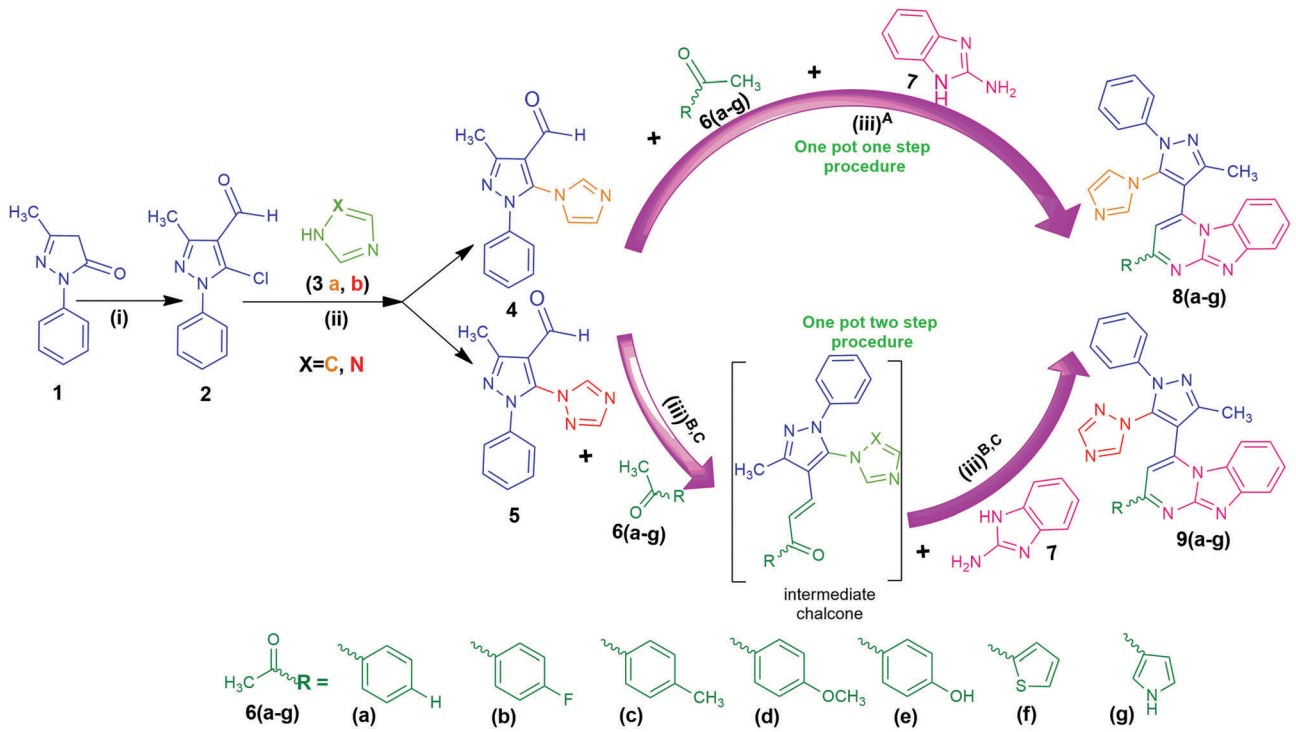
reaction times and high temperatures *via* a conventional route, hence limiting the accessibility of these biologically important structures. One of the ways to achieve this goal is the development of cascade reactions in addition to replacing the oil bath with a microwave reactor and opening a new window in microwave-assisted organic synthesis (MAOS), which allow the sequential transformations of two or more reactants in the same reaction vessel, leading to performing the reactions in spectacularly abbreviated time, minimizing the number of laboratory operations and generation of waste chemicals, and increasing the yields under much cleaner and easier reaction conditions. It has also gained the attention of chemists over the last decade as a very proficient tool. It has been applied to accelerate the course of many organic reactions due to its unique advantages such as higher selectivity, lower quantities of side products and high yields and is recognized as a "green" technology in the field of organic synthesis.[4]

Secondly, in support of this technology, multi-component reactions (MCRs) offer excellent atom economy and are readily applicable for the preparation of extensive libraries of drug-like heterocycles.[3] One-pot methods involving multi-component condensation using different reagents and catalysts are popular in synthetic organic chemistry for the synthesis of heterocyclic compounds where speed is of the essence due to their reduced reaction times, simplicity of product isolation and higher yields.

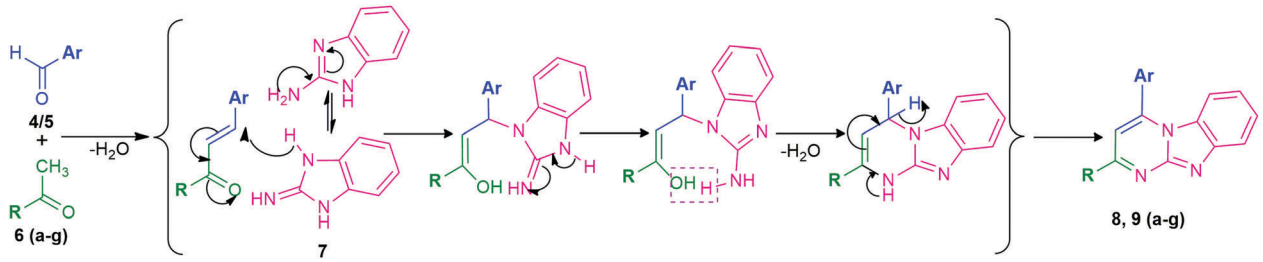
Consequently, in view of the remarkable aforementioned pharmacological activities of imidazo[1,2-*a*]pyrimidine derivatives and in continuation of our enduring research on the design and development of new methodologies for the synthesis of biologically potent heterocycles,[8] herein we present an efficient microwave-induced one-pot route for the synthesis of imidazo[1,2-*a*]pyrimidine derivatives of pyrazole fused to imidazole and triazole scaffolds triggered by eco-friendly base KOH with the belief that the amalgamation of separate pharmacophoric groups with analogous activity into a single nucleus may produce novel heterocycles with fascinating antimicrobial activity.

2. Chemistry

The required 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **2** was prepared *via* the Vilsmeier–Haack reaction according to a previously reported procedure.[9] The substituted imidazo/1,2,4-triazole pyrazolyl aldehydes **4** and **5** were prepared by a nucleophilic displacement of the chloro group of starting material **2** with secondary amines **3a** and **b**, respectively, using anhydrous potassium carbonate in refluxing DMF solvent. Further, we commenced this project by examining known reported synthetic methods for the preparation of imidazo[1,2-*a*]pyrimidines (Scheme 1). One such similar type of method involves a two-step procedure using the conventional heating method beginning with chalcone followed by cyclocondensation with 1,2,4-triazol-3amine, which provides the dihydro-triazolo-pyrimidine analog, which can be oxidized using either *N*-bromosuccinimide (NBS)[10] or Br_2 to give 1,2,4-triazolo[1,5-*a*]pyrimidines.[11] On the other hand, the other method involves condensation reactions employing high temperatures (*e.g.* >220 °C in ethylene glycol,[12] 190 °C in



Scheme 1 Synthesis of pyrazole based imidazo[1,2-*a*]pyrimidine derivatives **8(a–g)** and **9(a–g)**. (i) DMF, $POCl_3$, reflux 2 h. (ii) DMF, K_2CO_3, 80 °C, reflux 2 h. (iii) Microwave method: A (one pot, one-step procedure) – EtOH : H_2O equimixture, KOH, 340 W/15–20 min; B and C (one pot, two-step procedure): (i) H_2O : EtOH/KOH equimixture, r.t., 10 min; B(ii) 340 W, 10–15 min; C(ii) 28 h reflux.



Scheme 2 Plausible mechanistic pathway for the imidazo[1,2-*a*]pyrimidine derivative compounds.

1-methyl-2-pyrrolidinone[13]), neat at 160 °C[14] or refluxing in *t*-BuOH in the presence of the strong base *t*-BuOK,[15] or refluxing in DMF in the presence of triethylamine as a catalyst in an open vessel at 120 °C,[16] which is indeed the most common scenario, and none of the methods involve a one-pot method. By employing our own independent investigations in this area sequentially (one-pot three-component condensation, microwave irradiation protocol (340 W, 10–15 min, equimixture EtOH : H_2O, KOH)), the reaction can be efficiently tuned toward the exclusive formation of imidazo[1,2-*a*]pyrimidine derivatives **8(a–g)** and **9(a–g)** in good yield *via* method B. Transformations according to methods A, B and C (Scheme 2 and Table 1) were in the 68–78%, 87–93% and 70–81% ranges, respectively.

The formation of compounds **8(a–g)** and **9(a–g)** may proceed in two steps: (i) the initial *in situ* formation of an intermediate Michael adduct is accomplished according to the Claisen–Schmidt condensation of 1-(4-substituted-phenyl/hetero-aryl)ethanones **6(a–g)** with substituted imidazo/1,2,4-triazole pyrazolyl aldehydes **4/5** at room temperature and (ii) then the formed intermediate Michael adduct undergoes sequential [3+3] cycloaddition with 2-amino benzimidazole **7** followed by the removal of hydrogen to furnish the final aromatized products (Scheme 2). The products obtained according to method A (Scheme 2 and Table 1) were in the 68–78% range, and it appears that this unusual three-component one-step reaction may lead to a significant number of side products as all three components are added at once under microwave exposure, thus increasing the possibility of side reactions. In order to obtain a pure product, it required chromatographic purification and thus did not lend itself a well high-throughput methodology. On the basis of our mechanistic assumption, an alternative one-pot, two-step procedure is elaborated (Table 1, methods B and C).

Table 1 Synthesis and substituent patterns for the synthesized compounds **8(a–g)** and **9(a–g)**

Building blocks				Building blocks			
Code	X	R	Yield (%)	Code	X	R	Yield (%)
8a	C	C_6H_5	72^A 93^B 81^C	**9a**	N	C_6H_5	78^A 92^B 83^C
8b	C	4-FC_6H_4	70^A 87^B 81^C	**9b**	N	4-FC_6H_4	72^A 90^B 80^C
8c	C	4-MeC_6H_4	75^A 85^B 78^C	**9c**	N	4-MeC_6H_4	75^A 92^B 81^C
8d	C	4-$OMeC_6H_4$	75^A 92^B 80^C	**9d**	N	4-$OMeC_6H_4$	71^A 88^B 82^C
8e	C	4-OHC_6H_4	68^A 91^B 79^C	**9e**	N	4-OHC_6H_4	74^A 90^B 82^C
8f	C	SC_4H_3	60^A 80^B 72^C	**9f**	N	SC_4H_3	67^A 82^B 75^C
8g	C	NC_4H_4	62^A 78^B 68^C	**9g**	N	NC_4H_4	65^A 81^B 73^C

Microwave assisted: method A – one pot, one-step procedure, method B – one pot, two-step procedure. Conventional method: method C – one pot, two-step procedure.

This protocol involved the initial *in situ* formation of Michael adducts at room temperature, with the total consumption of both starting material compounds **6(a–g)** and **4/5** as evidenced by TLC, followed by the addition of appropriate 2-aminobenimidazole, which resulted in quantitative yields in both experiments. Although, in terms of reaction efficiency and facileness, efforts have been made towards reducing the reaction time, both the microwave-assisted heteroannulation experiments proved to be very convenient in comparison to method C. The identities of the synthesized compounds **8(a–g)** and **9(a–g)** were determined using ^{1}H NMR, ^{13}C NMR, and FT-IR spectral data and elemental analysis, and the molecular weights of the compounds were confirmed by mass spectrometry. In the ^{1}H NMR spectra of compounds **8(a–g)** and **9(a–g)**, a singlet peak of the methyl proton of pyrazole appeared at around δ 2.258 ppm, while the aromatic protons resonate as multiplets at δ 6.76–8.12 ppm. There were no amine proton peaks detected, which shows that the cyclisation involved the aromatization of complete compounds. A peak was observed at around δ 13.07 ppm for the methane carbon of pyrazole in the ^{13}C NMR spectra of compounds **8(a–g)** and **9(a–g)**. In FT-IR spectra, compounds **8(a–g)** and **9(a–g)** exhibited an absorption band at around 1589–1651 cm^{-1} for (–C=N) stretching.

2.1. Optimization of the reaction conditions

To optimize the reaction conditions for the formation of the target compound **8a**, a mixture of respective 5-(1*H*-imidazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **4**, acetophenone **6a**, and 2-aminobenzimidazole **7** in a 1 : 1 : 1.5 ratio was

Table 2 Optimization of the reaction conditions for **8a** by employing the most suitable method B

Entry	Base (equiv.)	Solvent	Yield **8a**[a] (%)
1	No base	No solvent	<05
2	No base	EtOH	<10
3	KOH, 1.0	EtOH	61
4	KOH, 1.0	H_2O	58
5	KOH, 1.0	EtOH : H_2O (1 : 1)	78
6	**KOH, 1.5**	EtOH : H_2O (1 : 1)	**93**
7	KOH, 2.0	EtOH : H_2O (1 : 1)	83
8	NaOH, 1.5	EtOH : H_2O (1 : 1)	71
9	K_2CO_3, 1.5	EtOH : H_2O (1 : 1)	43
10	*t*-BuOK, 1.5	Glycol	43[b]
11	TEA, 1.5	*t*-BuOH	67
12	DMAP, 1.5	EtOH	23
13	Piperidine, 1.5	*t*-BuOH	54[b]

[a] Isolated yield. [b] TLC indicated a complicated reaction in which significant amounts of side products were observed.

subjected to microwave irradiation by employing method B, and the results of these comparative experiments are summarized in Table 2. Initially, we sought to screen bases and their equivalents including green solvents used in the reaction (entries 1–13) to determine the one that can give the best results. It was found that with alkaline enhancement of the reaction system the yield of product **8a** improved (Table 2, entries 3–13).

No significant reaction was observed under either set of conditions (entries 1 and 2). With this encouraging result, we sought to screen inorganic (entries 3–11) and organic bases (entries 12 and 13) used in the reaction, and found that the inorganic bases (entries 9–11) gave modest yields, and bases such as KOH (entry 6) and NaOH (entry 8) are more efficient with almost quantitative yields. Similarly good yields were detected when TEA was used as a base (entry 11).

In contrast, weaker bases such as K_2CO_3 (entry 9) and organic bases such as DMAP and piperidine (entries 12 and 13) gave decreased yields. We also studied the effect of the equiv. of the base used, and found that 1 equiv. of KOH gave a comparatively lower yield – *i.e.*, the reaction was still incomplete (entries 3–5). When 1.5 equiv. of KOH was used (entry 6), it demonstrated superior activity and gave the best yield (93%). A further increase in the equiv. of KOH (entry 7) led to a decrease in the reaction yield due to the multifarious reaction mixture. To find the most appropriate solvent for the reaction, various green solvents (glycol, *t*-BuOH, EtOH, H_2O) and an EtOH + H_2O mixture were used. Among the different reaction media, the use of glycol and *t*-BuOH solvents in contrast to EtOH led to a significant decrease in the yield. However, it was in fact pleasing to note that there was a significant increase in the yield of **8a** with the choice of solvent as water (entry 4) and ethanol (entry 3) as well, with a negative aspect that the desired product obtained was a sticky solid in the former and a lower ratio than the expected one in the latter case. Hence, the appropriate reaction conditions were restrained to the selection of an eco-friendly and safe solvent medium – EtOH : H_2O (1 : 1) (entries 5–8). Thus, during these studies, we ascertained that the best results were obtained using this system. Employing the optimized conditions, we next studied the scope of the reaction using the same aldehyde **4** and 2-aminobenzimidazole **7** as starting materials. Under the optimized conditions, a variety of structurally diverse 4-substituted phenyl/heteroaryl ketones were investigated and a series of new imidazo[1,2-*a*]pyrimidine derivatives of substituted pyrazole with the 4-substituted phenyl/heteroaryl group residing in the 6-position of the fused imidazo-pyrimidine nucleus were obtained in good yields. The results signified that aromatic ketones bearing either electron-withdrawing or electron-donating functional groups, such as fluoro, methyl, methoxy, or hydroxyl, gave consistently good yields. Moreover, the heterocyclic aryl ketone gave compounds in modest yields (Table 1).

3. Pharmacology

3.1. *In vitro* antimicrobial activity

The *in vitro* anti-microbial activity was investigated against 24 h old cultures of three Gram-positive, Gram-negative bacteria and three fungi by the disc diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS).[17] Mueller–Hinton broth was used as the nutrient medium to grow and dilute the compound suspension for the test bacteria and Sabouraud Dextrose broth was used for fungal nutrition. The compounds were tested at 1000 ppm in DMF solution. The strains used for the activity were procured from MTCC (Microbial Type Culture Collection), Institute of Microbial Technology, Chandigarh. The titled final **8(a–g)** and **9(a–g)** compounds were tested for their anti-bacterial activity against Gram-negative bacteria such as *Escherichia coli* (MTCC 443), *Salmonella typhi* (MTCC 98) and *Vibrio cholerae* (MTCC 3906) and Gram-positive bacteria such as *Streptococcus pneumonia* (MTCC 1936), *Bacillus subtilis* (MTCC 441) and *Clostridium tetani* (MTCC 449), and their anti-fungal activity against *Candida albicans* (MTCC 227), *Trichophyton rubrum* (MTCC 297) and *Aspergillus niger* (MTCC 282). Ampicillin, ciprofloxacin, norfloxacin, nystatin, and griseofulvin were used as standard drugs for comparison of anti-bacterial and anti-fungal activities, respectively. Discretion was evidenced by measuring the diameter of the inhibition zone at the end of 24 h for bacteria at 35 °C and 48 h for fungi at 28 °C. The results are summarized in Table 3.

3.2. *In vitro* antituberculosis activity

The motivating results obtained by antimicrobial screening prompted us to execute the preliminary screening of the title synthesized compounds **8(a–g)** and **9(a–g)** for their *in vitro* antituberculosis activity against the *Mycobacterium tuberculosis* H37Rv strain. Primary screening of all the synthesized compounds was conducted at a concentration of 250 μg mL^{-1} using Löwenstein–Jensen medium as described by Rattan.[18] Rifampicin and isoniazid were used as the standard drugs for comparison, and the results are summarized in Table 4.

3.3. *In vitro* antimalarial activity

All the synthesized compounds were screened for their *in vitro* antimalarial activity against chloroquine and quinine sensitive strains of *P. falciparum*. All experiments were performed in duplicate, and the IC_{50} mean values are given in Table 5.

Table 3 % Yields of the synthesized compounds **8(a–g)** and **9(a–g)** and their *in vitro* antimicrobial activity (MIC, μg mL^{-1})

Entry	Gram-negative bacteria			Gram-positive bacteria			Fungi		
	E.C. MTCC 443	S.T. MTCC 98	V.C. MTCC 3906	S.P. MTCC 1936	B.S. MTCC 441	C.T. MTCC 449	C.A. MTCC 227	T.R. MTCC 297	A.N. MTCC 282
4	200	200	200	100	200	**62.5**	1000	>1000	1000
5	250	100	250	**62.5**	100	200	500	1000	>1000
8a	100	200	100	**62.5**	100	250	>1000	1000	1000
8b	100	**62.5**	100	100	100	**62.5**	500	**500**	**100**
8c	250	100	200	200	**125**	100	500	>1000	1000
8d	250	250	200	100	250	250	>1000	**500**	500
8e	100	250	100	**62.5**	100	200	>1000	**500**	1000
8f	**62.5**	125	100	100	**62.5**	250	**250**	>1000	500
8g	250	100	**62.5**	100	100	100	1000	>1000	>1000
9a	200	100	125	100	250	250	500	1000	1000
9b	100	**62.5**	100	**62.5**	**125**	**62.5**	**100**	**500**	500
9c	200	250	250	200	250	250	1000	>1000	>1000
9d	100	100	200	200	**62.5**	250	1000	1000	>1000
9e	250	**62.5**	100	100	200	200	>1000	**500**	**100**
9f	100	100	125	125	200	100	500	**500**	1000
9g	250	250	200	200	100	100	1000	>1000	>1000
Ampicillin	**100**	**100**	**100**	**100**	**250**	**250**	—	—	—
Ciprofloxacin	**25**	**25**	**25**	**50**	**50**	**100**	—	—	—
Norfloxacin	**10**	**10**	**10**	**10**	**100**	**50**	—	—	—
Nystatin	—	—	—	—	—	—	**100**	**500**	**100**
Greseofulvin	—	—	—	—	—	—	**500**	**500**	**100**

Boldfaced values indicate the active compounds; E.C., *Escherichia coli*; S.T., *Salmonella typhi*; V.C., *Vibrio cholerae*; S.P., *Streptococcus pneumoniae*; B.S., *Bacillus subtilis*; C.T., *Clostridium tetani*; C.A., *Candida albicans*; T.R., *Trichophyton rubrum*; A.F.: *Aspergillus niger*; MTCC, microbial-type culture collection; '—' indicates not tested.

Table 4 *In vitro* antituberculosis activity (% inhibition) of compounds against *M. tuberculosis* H37Rv (at a concentration of 250 μg mL^{-1})

Entry	% Inhibition	Entry	% Inhibition
4	62	**9a**	70
5	69	**9b**	**94**
8a	61	**9c**	62
8b	**91**	**9d**	**85**
8c	78	**9e**	**90**
8d	64	**9f**	74
8e	**83**	**9g**	73
8f	65	Rifampicin	98
8g	61	Isoniazid	99

Table 5 *In vitro* antimalarial activity of the compounds

Entry	IC_{50} (μg mL^{-1})	Entry	IC_{50} (μg mL^{-1})
4	1.24	**9a**	1.45
5	1.32	**9b**	**0.041**
8a	**0.051**	**9c**	1.50
8b	**0.030**	**9d**	1.45
8c	1.84	**9e**	**0.054**
8d	1.52	**9f**	0.83
8e	1.19	**9g**	**0.092**
8f	1.75	Chloroquine	0.020
8g	**0.079**	Quinine	0.268

4. Results and discussion

4.1. Biological results

4.1.1. *In vitro* antibacterial activity. Upon reviewing the antimicrobial screening data (Table 3), it has been observed that a majority of the compounds showed excellent potential against *S. typhi*, *S. pneumonia*, *B. subtilis* and *C. tetani* compared to the standard drug ampicillin. In comparison to the standard drug ampicillin (MIC 100 μg mL^{-1}) against Gram-negative bacteria, against *E. coli* compound **8f**, against *S. typhi* compounds **8b**, **9b**, and **9e** and against *V. cholera* compound **8g** were found to have outstanding activity (MIC 62.5 μg mL^{-1}); whereas compounds **8a**, **8b**, **8e**, **9b**, **9d**, and **9f** against *E. coli*, compounds **5**, **8c**, **9a**, **9d**, and **9f** against *S. typhi* and compounds **8a**, **8b**, **8e**, **8f**, **9b**, and **9e** against *V. cholerae* showed significant equipotency (MIC 100 μg mL^{-1}) to the standard drug ampicillin (MIC 100 μg mL^{-1}). In inhibiting Gram-positive bacteria, against *S. pneumoniae* compounds **5**, **8a**, **8e**, and **9b** (MIC 62.5 μg mL^{-1}) showed excellent potential and compounds **4**, **8b**, **8d**, **8f**, **8g**, **9a**, and **9e** (MIC 100 μg mL^{-1}) showed equipotency to the standard drug ampicillin (MIC 100 μg mL^{-1}); against *B. subtilis* compounds **8f** and **9d** (MIC 62.5 μg mL^{-1}) and **8c** and **9b** (MIC 125 μg mL^{-1}) showed outstanding activity in comparison to the standard drugs ampicillin (MIC 250 μg mL^{-1}) and norfloxacin (MIC 100 μg mL^{-1}), while compounds **5**, **8a**, **8b**, **8e**, **8g**, and **9g** (MIC 100 μg mL^{-1}) showed equivalent results to norfloxacin (MIC 100 μg mL^{-1}) and excellent potency compared to ampicillin (MIC 250 μg mL^{-1}). Similarly, compounds **4**, **8b**, and **9d** (MIC 62.5 μg mL^{-1}) against *C. tetani* showed excellent activity compared to the standard drugs ampicillin (MIC 2500 μg mL^{-1}) and ciprofloxacin (MIC 100 μg mL^{-1}). Compounds **8c**, **8g**, **9f**, and **9g** (MIC 100 μg mL^{-1}) exhibited greater potency than ampicillin (MIC 250 μg mL^{-1}) and equipotency to ciprofloxacin (MIC 100 μg mL^{-1}).

4.1.2. *In vitro* antifungal activity. *In vitro* antifungal screening data (Table 3) demonstrated that, against *C. albicans*, compound **9b** (MIC 100 μg mL^{-1}) exhibited equivalent activity to nystatin (MIC 100 μg mL^{-1}), and along with it, **8f** (MIC 250 μg mL^{-1}) exhibited fabulous activity compared to griseofulvin (MIC 500 μg mL^{-1}), whereas compounds **5**, **8b**, **8c**, **9a**,

and **9f** (MIC 500 μg mL^{-1}) were of comparable potential to griseofulvin (MIC 500 μg mL^{-1}). Against *T. rubrum*, compounds **8b**, **8d**, **8e**, **9b**, **9e**, and **9f** (MIC 500 μg mL^{-1}) were found to be equally active as nystatin and griseofulvin (MIC 500 μg mL^{-1}). Compounds **8b** and **9e** (MIC 100 μg mL^{-1}) were equivalent to both antifungal standard drugs (MIC 100 μg mL^{-1}).

4.1.3. *In vitro* antitubercular activity. Encouraged by the antimicrobial screening results, we screened for the antitubercular activity of the target compounds **8(a–g)** and **9(a–g)**. Compounds **8b**, **9b** and **9e** were found to possess the highest potency with 91, 94 and 90% inhibition, respectively. On the other hand, two compounds **8e** and **9d** are moderately active against *M. tuberculosis* H37Rv. All other compounds showed poor inhibition of *M. tuberculosis* growth.

4.1.4. *In vitro* antimalarial activity. Compounds **8a**, **8b**, **8g**, **9b**, **9e** and **9g** showed principal activity against the *P. falciparum* strain in comparison to quinine (IC_{50} 0.268), as their IC_{50} values were in the 0.030–0.092 range. While compound **8b** was found to possess moderate activity (*i.e.* IC_{50} 0.030) aligned with chloroquine, the remaining compounds showed less antimalarial activity against the *P. falciparum* strain in comparison to chloroquine and quinine drugs. From the above results, it can be concluded that in this set of synthesized heterocyclics, compounds **8b**, **9b** and **9e** may become a promising new class of antimicrobial, antitubercular and antimalarial agents in the future.

4.1.5. Structure–activity relationship (SAR). The structural activity relationship analysis (Fig. 2) demonstrated that the imidazole and triazole nuclei at the fifth position of the pyrazole ring in connotation with the different natures of groups at the para position of the phenyl ring and various heterocyclic motifs **6(a–g)** at the sixth position of the pyrimidine ring are prone to incongruity in biological activities. The imidazole nucleus is responsible for improved antibacterial activity against *C. tetani* (**4**) but in combination with various substituted phenyl and heterocycle containing rings demonstrated increased activity against all pathogens in comparison to triazole. In combination compounds with benzylic substitutions (–F, –OH) were found highly active against *S. pneumoniae*, *S. typhi* and *C. tetani* (**8b** and **8e**) and showed excellent antifungal activity against fungal strains *T. rubrum* (**8b** and **8e**) and *A. niger* (**8b**), these substitutions also enhanced antimalarial and antitubercular activities. Secondly, compounds with –OMe and –Me substituents were found to be reluctant towards all the strains in the former case; **8c** was only active against *B. subtilis* in the former case, while the one without any substituent showed inhibition against *S. pneumoniae* and malarial strains. Further, in combination with heteroatom-containing motifs **6f** and **g** increased antibacterial activity against *E. coli*, *B. subtilis* (**8f**), and *V. cholerae* (**8g**) was shown; increased antifungal activity against *C. albicans* and *T. rubrum* (**8f**); and antimalarial activities against *P. falciparum* strain (**8g**). The triazole ring increases the antibacterial activity against *S. pneumoniae* (**5**), but in combination with the phenyl ring having an electron withdrawing group (–F) exhibits increased antimicrobial potency against *C. tetani*, *S. pneumoniae*, and *C. albicans*, and also amplifies the antitubercular and antimalarial activities (**9b**). Further analysis shows that electron releasing groups (–Me, –OMe) decrease the potency towards fungal strains: the latter showed higher inhibition against *B. subtilis* (**9d**) and also increased antitubercular activity, but the compound with the former substituent was poorly active against all pathogens. The compound with the –OH group replacement (**9e**) exhibited excellent pronounced antimalarial, antitubercular, and antimicrobial activities against *P. falciparum*, *Mycobacterium tuberculosis* H37Rv, *S. typhi* and *T. rubrum* strains, respectively; while the compounds without any phenyl ring substitution were dubious against any strains. On the other hand, in association with sulphur and nitrogen containing heterocycles, compound **5** demonstrated enhanced antifungal activity against *T. rubrum* and *A. niger* (**9f**) in

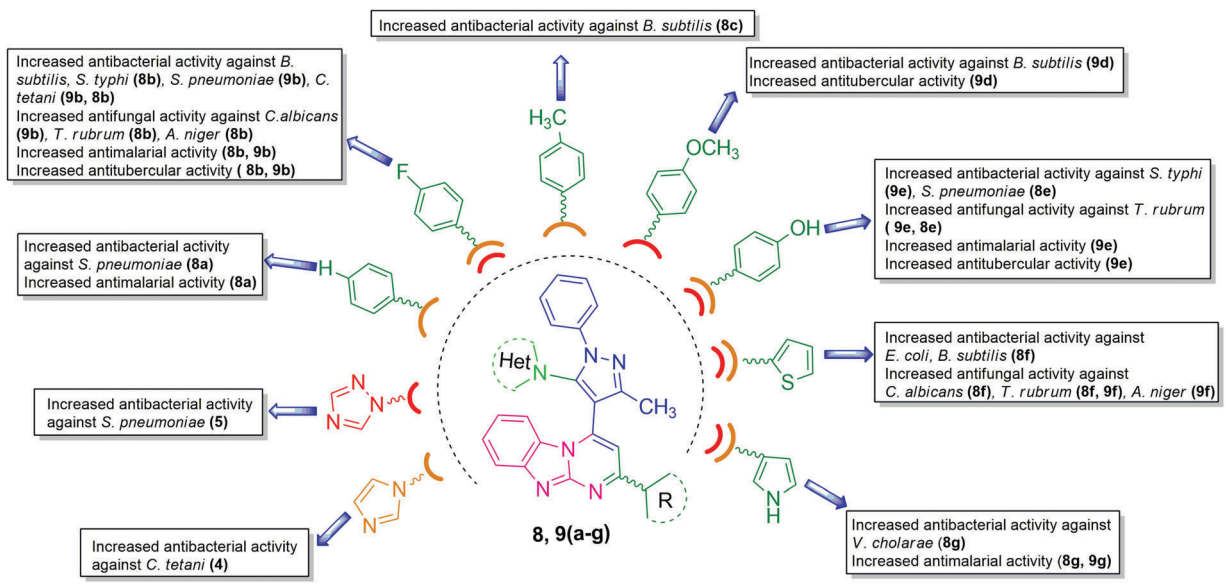


Fig. 2 Structure–activity relationships for the antimicrobial, antimalarial and antituberculosis activities of the synthesized compounds **8(a–g)** and **9(a–g)**.

the former case and increased antimalarial activity (**9g**) in the latter case and was poorly active against bacterial strains. Thus, appraising the activity data, it is noteworthy that the various pyrimidine target compounds with the imidazole nucleus were found to be much more pharmacologically active than those with the triazole nucleus. The overall activity of the target compounds depends not only on the nature of the nucleus attached at the fifth position of the pyrazole ring but also on the peripheral substituents appended through the phenyl ring and heteroaromatic moieties, and positional changes.

5. Conclusion

In summary, we have reported a proficient, facile, mild green environmentally benign synthetic protocol for the synthesis of imidazo[1,2-*a*]pyrimidine derivatives of pyrazole clubbed with the imidazole and triazole nuclei in good yield based on a microwave-assisted, one-pot three-component condensation reaction for the first time. The key step in the suggested mechanistic pathway involves the base-mediated formation of α,β-unsaturated ketone generated *in situ* followed by cyclization with 2-amino benzimidazole to afford the final targeted product through an easy way. All the synthesized compounds were examined for their various biological activities with the hope of discovering new structural classes of agents with antimicrobial, antimalarial and antitubercular activities, which can be of use for further meticulous pre-clinical explorations. A majority of compounds have emerged as effective inhibitory agents against *S. typhi*, *S. pneumoniae*, *B. subtilis*, and *C. tetani.* Compounds **8f** and **9b** showed outstanding activity against *C. albicans*, while compounds **8b** and **9e** showed against *A. niger* as compared to griseofulvin and nystatin. Compounds **8b**, **9b**, **9e** and **9d** exhibited the best antimalarial and antituberculosis activities. The fluoro, hydroxyl, and thiophene groups and the imidazole nucleus in comparison to that of the triazole nucleus to some extent in the imidazopyrimidine derivative of pyrazole compounds are considered to be responsible for the biological activity.

6. Experimental

6.1. Chemistry

All reactions were performed with commercially available reagents, which were used without further purification. Phenylhydrazine, potassium hydroxide and acetophenones were purchased from S.D. Fine Chem Ltd, Vadodara, Gujarat, India. 2-Aminobenzimidazole was purchased from Sigma-Aldrich. Solvents were purchased from Spectrochem and were purified and dried before being used. The microwave-assisted reactions are conducted in a "RAGA's Modified Electromagnetic Microwave System," wherein microwaves are generated by a magnetron at a frequency of 2450 MHz having adjustable output power levels, *i.e.*, 10 levels from 140 to 700 W and with an individual sensor (a fiber-optic sensor) for temperature control attached to a reflux condenser with constant stirring (thus avoiding the risk of high pressure development). Thin-layer chromatography (TLC, on aluminum plates precoated with silica gel, 60F, 0.25 mm thickness) (Merck, Darmstadt, Germany) was used for monitoring the progress of all reactions, purity, and homogeneity of the synthesized compounds (eluent ethyl acetate : hexane 7 : 3). The melting points of all the titled compounds were determined by an open-tube capillary method and are uncorrected. UV radiation and/or iodine were used as the visualizing agents. Mass spectra were recorded on a Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan) purchased under the PURSE program of DST at Sardar Patel University, Vallabh Vidyanagar. The IR spectra were recorded using KBr on a Perkin-Elmer Spectrum GX FT-IR Spectrophotometer (Perkin-Elmer, USA) and only the characteristic peaks are reported at 254 cm^{-1}. Elemental analysis (% C, H, and N) was carried out using a Perkin-Elmer 2400 series-II elemental analyzer (Perkin-Elmer, USA) and all the compounds are within ±0.4% of theory specified. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solvent on a Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd, Switzerland) using the residual solvent signal as an internal standard at 400 MHz and 100 MHz respectively. Chemical shifts are reported in parts per million (ppm).

6.1.1. General procedure for the synthesis of substituted 5-(heteroaryl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehydes (4 and 5). 5-Chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **1** (1 mmol), substituted heteroaryl compound imidazole **2**/1,2,4-triazole **3** (1 mmol) and anhydrous potassium carbonate (2 mmol) in dimethylformamide (5 mL) were charged into a 100 mL round bottom flask equipped with a mechanical stirrer and condenser. The reaction mixture was refluxed and heated at 80 °C for 2 h and the progress of the reaction was monitored by TLC. After the completion of the 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 followed by neutralization with dilute protonic acid. The separated precipitates of 5-(1*H*-imidazol/1,2,4-triazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehydes **4** and **5** were filtered, thoroughly washed with water, dried, and recrystallized from ethanol.

6.1.2. General procedure for the synthesis of 4-(5-(1*H*-imidazol/1,2,4-triazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2-(4-substituted-phenyl/hetero-aryl)benzo[4,5]imidazo[1,2-*a*]pyrimidine derivatives (8a–g and 9a–g)

6.1.2.1. Microwave irradiation method

Method A (one pot, one-step). In a 50 mL round bottom flask, equimolar amounts of the corresponding 5-(1*H*-imidazol/1,2,4-triazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **4**/**5** (1.0 mmol), 1-(4-substituted-phenyl/hetero-aryl)ethanones **6a–g** (1.0 mmol) and 2-aminobenzimidazole **7** (1.5 mmol) in H_2O : EtOH (1 : 1) containing powdered potassium hydroxide (1.5 mmol) were mixed thoroughly and subjected to microwave irradiation at 340 W for 15–20 min. After the completion of the reaction (evidenced by TLC, ethyl acetate : hexane (7 : 3)), the resulting solution was cooled to room temperature. Then, an EtOH–H_2O equimixture was added to it and heated with vigorous stirring. The solid mass that separated upon cooling was filtered, washed well with a cold water : ethanol equimixture, dried and purified by chromatography using ethyl acetate : hexane (7 : 3) as eluent to obtain a pure solid sample.

Method B (one-pot, two-step). 5-(1*H*-Imidazol/1,2,4-triazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **4/5** (1.0 mmol), 1-(4-substituted-phenyl/hetero-aryl)ethanones **6a–g** (1.0 mmol) and potassium hydroxide (1.5 mmol) in a H_2O : EtOH equimixture were mixed and stirred at room temperature for 10–15 min until the formation of a precipitate. After the completion of the reaction (as evidenced by TLC), 2-aminobenzimidazole **7** (1.5 mmol) was added to the mixture, which was then subjected to microwave irradiation at 340 W for 10–15 min in a 50 mL round bottom flask. An EtOH–H_2O mixture (1 : 1) was added to the reaction mixture and heated with vigorous stirring. Then the solid mass that separated upon cooling was removed by filtration, washed well with cold EtOH–H_2O (1 : 1) and dried in air and purified by leaching in an equal volume ratio of chloroform and methanol, affording pure solid compounds.

6.1.2.2. Conventional method

Method C (one pot, two-step). 5-(1*H*-Imidazol/1,2,4-triazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **4/5** (1.0 mmol), 1-(4-substituted-phenyl/hetero-aryl)ethanones **6a–g** (1.0 mmol) and potassium hydroxide (1.5 mmol) in a H_2O : EtOH equimixture were mixed and stirred at room temperature for 10–15 min until the formation of a precipitate. Subsequently, after the completion of the reaction (as evidenced by TLC) in a 50 mL round bottom flask, 2-aminobenzimidazole **7** (1.5 mmol) was added and then the reaction mixture was allowed to stir for 28 h until chalcone was completely consumed. Then to the resulting reaction mass, water (10 mL) was added. The mixture was ultrasonically agitated for 30 min and then filtered. The solid mass that separated upon cooling was filtered, washed well with a cold water : ethanol equimixture, dried and purified by leaching in an equal volume ratio of chloroform and methanol, affording pure solid compounds.

*6.1.2.3. 4-(5-(1H-Imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-phenylbenzo[4,5]imidazo[1,2-a]pyrimidine (**8a**).* Yellow solid; m.p. 215 °C; IR (KBr, ν, cm^{-1}): 3011 (aromatic ring –CH), 1651 (–C═N), 1370 (Ar-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 3H, –C$\underline{H}_3$), 6.75–8.24 (m, 18H, Ar-$\underline{H}$), ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.0, 100.0, 107.0, 109.9, 112.6, 119.9, 120.9, 122.4, 123.1, 126.5, 127.9, 129.0, 129.8, 131.2, 131.6, 134.1, 136.2, 137.0, 137.2, 138.5, 145.6, 148.8, 151.8, 156.0, 156.4; MS (*m*/*z*) calc.: 467.52, found: 468.14 [M + 1]; anal. calc. for $C_{29}H_{21}N_7$: C, 74.50; H, 4.53; N, 20.97; found: C, 75.02; H, 4.13; N, 21.05%.

*6.1.2.4. 4-(5-(1H-Imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-(4-fluorophenyl)benzo[4,5]imidazo[1,2-a]pyrimidine (**8b**).* Orange solid; m.p. 210 °C; IR (KBr, ν, cm^{-1}): 3021 (aromatic ring –CH), 1591 (–C═N), 1367 (Ar-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 3H, –C$\underline{H}_3$), 6.75–8.23 (m, 17H, Ar-$\underline{H}$), ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.1, 106.9, 109.9, 112.5, 119.9, 120.7, 122.3, 123.1, 126.4, 127.4, 127.8, 128.9, 129.8, 131.1, 133.4, 134.1, 136.9, 137.2, 138.3, 142.3, 145.5, 148.8, 151.9, 161.0, 162.5; anal. calc. for $C_{29}H_{20}FN_7$ (485.51): C, 71.74; H, 4.15; F, 3.91; N, 20.19; found: C, 71.47; H, 4.91; N, 20.24%.

*6.1.2.5. 4-(5-(1H-Imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-(p-tolyl)benzo[4,5]imidazo[1,2-a]pyrimidine (**8c**).* Yellow solid; m.p. 208 °C; IR (KBr, ν, cm^{-1}): 2967 (aromatic ring –CH), 1589 (–C═N), 1371 (Ar-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.25 (s, 3H, C$\underline{H}_3$), 2.43 (s, 3H, ArC$\underline{H}_3$), 6.77–8.12 (m, 17H, Ar$\underline{H}$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.1, 21.6, 109.8, 112.6, 116.0, 116.3, 119.9, 120.9, 122.5, 123.0, 126.6, 127.3, 129.0, 129.8, 129.9, 130.1, 131.2, 132.4, 134.1, 136.9, 137.2, 138.7, 145.5, 148.8, 151.7, 159.8; anal. calc. for $C_{30}H_{23}N_7$ (481.55): C, 74.83; H, 4.81; N, 20.36; found: C, 75.06; H, 5.12; N, 20.62%.

*6.1.2.6. 4-(5-(1H-Imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-(4-methoxyphenyl)benzo[4,5]imidazo[1,2-a]pyrimidine (**8d**).* Yellow solid; m.p. 222 °C; IR (KBr, ν, cm^{-1}): 3035 (aromatic ring –CH), 1610 (–C═N), 1368 (Ar-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 3H, C$\underline{H}_3$), 3.90 (s, 3H, –OC$\underline{H}_3$), 6.75–8.20 (m, 14H, Ar$\underline{H}$), ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.1, 55.5, 106.7, 109.9, 112.5, 114.4, 119.9, 120.7, 122.2, 123.1, 125.2, 126.3, 127.4, 128.7, 129.0, 129.3, 129.6, 129.8, 131.2, 134.1, 136.9, 138.2, 145.5, 148.8, 152.0, 160.6; MS (*m*/*z*) calc.: 497.55, found: 498.23 [M + 1]; anal. calc. for $C_{30}H_{23}N_7O$: C, 72.42; H, 4.66; N, 19.71; found: C, 72.73; H, 4.45; N, 20.14%.

*6.1.2.7. 4-(4-(5-(1H-Imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)benzo[4,5]imidazo[1,2-a]pyrimidin-2-yl)phenol (**8e**).* Yellow solid; m.p. 218 °C; IR (KBr, ν, cm^{-1}): 2989 (aromatic ring –CH), 1591 (–C═N), 1373 (Ar-CH_3) 3350 (–OH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 3H, C$\underline{H}_3$), 6.75–8.08 (m, 17H, Ar$\underline{H}$), 13.52 (s, 1H, ArO$\underline{H}$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.2, 106.2, 112.6, 117.1, 119.4, 120.6, 120.6, 122.7, 123.6, 126.7, 127.6, 127.8, 129.5, 129.7, 129.9, 134.3, 136.9, 138.9, 145.1, 145.2, 146.4, 148.2, 148.9, 153.6, 155.9; anal. calc. for $C_{29}H_{21}N_7O$ (483.52): C, 72.04; H, 4.38; N, 20.28; found: C, 73.97; H, 4.63; N, 19.86%.

*6.1.2.8. 4-(5-(1H-Imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-(thiophen-2-yl)benzo[4,5]imidazo[1,2-a]pyrimidine (**8f**).* Yellow solid; m.p. 234 °C; IR (KBr, ν, cm^{-1}): 3054 (aromatic ring –CH), 1575 (–C═N), 1378 (Ar-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 3H, C$\underline{H}_3$), 6.76–8.13 (m, 16H, Ar-$\underline{H}$), ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.2, 106.7, 110.9, 112.6, 119.8, 120.7, 122.5, 123.2, 126.1, 126.4, 127.3, 128.5, 129.0, 129.5, 129.9, 132.1, 133.4, 136.9, 137.9, 142.5, 145.2, 148.9, 151.5, 153.6, 155.9; anal. calc. for $C_{27}H_{19}N_7S$: C, 68.48; H, 4.04; N, 20.70; found: C, 68.51; H, 4.25; N, 19.98%.

*6.1.2.9. 4-4-(5-(1H-Imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-(1H-pyrrol-2-yl)benzo[4,5]imidazo[1,2-a]pyrimidine (**8g**).* Orange solid; m.p. 215 °C; IR (KBr, ν, cm^{-1}): 3071 (aromatic ring –CH), 1643 (–C═N), 1370 (Ar-CH_3), ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 3H, C$\underline{H}_3$), 6.75–8.21 (m, 16H, Ar-$\underline{H}$), 9.73 (s, 1$\underline{H}$, –NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.0, 106.9, 109.9, 112.5, 119.8, 120.9, 122.4, 123.1, 126.4, 127.3, 127.9, 128.9, 129.4, 129.9, 131.1, 133.4, 134.1, 136.9, 137.3, 138.4, 142.3, 145.5, 148.6, 151.9, 161.1; MS (*m*/*z*) calc.: 456.50, found: 457.13 [M + 1]; anal. calc. for $C_{27}H_{20}N_8$: C, 71.04; H, 4.42; N, 24.55; found: C, 69.97; H, 4.13; N, 24.74%.

*6.1.2.10. 4-(3-Methyl-1-phenyl-5-(1H-1,2,4-triazol-1-yl)-1H-pyrazol-4-yl)-2-phenylbenzo[4,5]imidazo[1,2-a]pyrimidine (**9a**).* Yellow solid; m.p. 212 °C; IR (KBr, ν, cm^{-1}): 2984 (aromatic ring –CH),

1623 (–C═N), 1379 (Ar-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.28 (s, 3H, C$\underline{H}_3$), 7.19–8.27 (m, 17H, Ar$\underline{H}$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.3, 104.4, 106.9, 110.0, 112.8, 116.3, 120.8, 122.3, 123.5, 126.5, 129.3, 129.9, 130.1, 132.4, 133.4, 135.3, 136.9, 138.2, 145.6, 148.9, 151.7, 153.4, 159.8, 163.3; MS (*m*/*z*) calc.: 468.51, found: 469.02 [M + 1]; anal. calc. for $C_{28}H_{20}N_8$: C, 71.78; H, 4.30; N, 23.92; found: C, 72.01; H, 4.46; N, 23.24%.

*6.1.2.11. 2-(4-Fluorophenyl)-4-(3-methyl-1-phenyl-5-(1H-1,2,4-triazol-1-yl)-1H-pyrazol-4-yl)benzo[4,5]imidazo[1,2-a]pyrimidine (**9b**).* Orange solid; m.p. 206 °C; IR (KBr, ν, cm^{-1}): 2993 (aromatic ring –CH), 1642 (–C═N), 1372 (Ar-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.28 (s, 3H, C$\underline{H}_3$), 7.19–8.24 (m, 16H, Ar$\underline{H}$), ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.2, 104.4, 107.0, 111.1, 112.8, 116.3, 120.8, 122.4, 123.6, 126.5, 127.4, 129.9, 130.0, 132.5, 133.4, 135.4, 136.9, 138.3, 145.5, 148.9, 151.7, 153.6, 159.7, 163.3; anal. calc. for $C_{28}H_{19}FN_8$ (486.50): C, 69.13; H, 3.94; N, 23.03; found: C, 68.87; H, 4.04; N, 22.85%.

*6.1.2.12. 4-(3-Methyl-1-phenyl-5-(1H-1,2,4-triazol-1-yl)-1H-pyrazol-4-yl)-2-(p-tolyl)benzo[4,5]imidazo[1,2-a]pyrimidine (**9c**).* Yellow solid; m.p. 219 °C; IR (KBr, ν, cm^{-1}): 3087 (aromatic ring –CH), 1651 (–C═N), 1369 (Ar-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.27 (s, 3H, C$\underline{H}_3$), 2.44 (s, 3H, C$\underline{H}_3$), 7.18–8.16 (m, 16H, Ar$\underline{H}$), ppm; ^{13}C NMR (100 MHz DMSO-d_6): δ 13.2, 21.5, 101.3, 107.3, 111.3, 112.8, 120.7, 122.1, 123.6, 126.3, 127.8, 129.3, 129.8, 133.5, 136.9, 137.9, 142.2, 144.0, 145.3, 145.5, 153.6, 157.9, 158.9, 159.4, 160.9; MS (*m*/*z*) calc.: 482.54, found: 483.13 [M + 1]; anal. calc. for $C_{29}H_{22}N_8$: C, 72.18; H, 4.60; N, 23.22; found: C, 71.95; H, 4.46; N, 23.43%.

*6.1.2.13. 2-(4-Methoxyphenyl)-4-(3-methyl-1-phenyl-5-(1H-1,2,4-triazol-1-yl)-1H-pyrazol-4-yl)benzo[4,5]imidazo[1,2-a]pyrimidine (**9d**).* Yellow solid; m.p. 223 °C; IR (KBr, ν, cm^{-1}): 3064 (aromatic ring –CH), 1610 (–C═N), 1379 (Ar-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.28 (s, 3H, C$\underline{H}_3$), 3.904 (s, 3H, OC$\underline{H}_3$), 7.03–8.24 (m, 16H, Ar$\underline{H}$), ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.2, 21.5, 101.3, 107.3, 111.3, 112.8, 120.7, 122.1, 123.6, 126.3, 127.8, 129.3, 129.8, 133.5, 136.9, 137.9, 142.2, 144.0, 145.3, 145.5, 153.6, 157.9, 158.9, 159.4, 160.9; anal. calc. for $C_{29}H_{22}N_8O$ (498.54): C, 69.87; H, 4.45; N, 22.48; found: C, 69.72; H, 4.83; N, 21.97%.

*6.1.2.14. 4-(4-(3-Methyl-1-phenyl-5-(1H-1,2,4-triazol-1-yl)-1H-pyrazol-4-yl)benzo[4,5]imidazo[1,2-a]pyrimidin-2-yl)phenol (**9e**).* Yellow solid; m.p. 247 °C; IR (KBr, ν, cm^{-1}): 3017 (aromatic ring –CH), 1611 (–C═N), 1371 (Ar-CH_3), 3340 (–OH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.29 (s, 3H, C$\underline{H}_3$), 7.11–8.01 (m, 16H, Ar$\underline{H}$), 13.54 (s, 1H, O$\underline{H}$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.2, 106.3, 112.7, 117.1, 119.4, 119.3, 120.6, 122.7, 123.6, 126.7, 127.7, 127.8, 129.4, 129.9, 134.3, 136.8, 138.8, 145.1, 145.2, 146.4, 148.3, 148.9, 153.7, 155.9; anal. calc. for $C_{28}H_{20}N_8O$ (484.51): C, 69.41; H, 4.16; N, 23.13; found: C, 68.85; H, 4.24; N, 23.31%.

*6.1.2.15. 4-(3-Methyl-1-phenyl-5-(1H-1,2,4-triazol-1-yl)-1H-pyrazol-4-yl)-2-(thiophen-2-yl)benzo[4,5]imidazo[1,2-a]pyrimidine (**9f**).* Yellow solid; m.p. 227 °C; IR (KBr, ν, cm^{-1}): 2986 (aromatic ring –CH), 1643 (–C═N), 1376 (Ar-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.28 (s, 3H, C$\underline{H}_3$), 7.13–8.15 (m, 15H, Ar$\underline{H}$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.2, 106.7, 110.9, 112.6, 120.7, 122.3, 123.6, 126.2, 126.3, 127.7, 128.5, 129.1, 129.3, 129.8, 132.1, 133.4, 136.9, 137.9, 142.6, 145.3, 148.9, 151.4, 153.6, 155.9; MS (*m*/*z*) calc.: 474.54, found: 475.02 [M + 1]; anal. calc. for $C_{26}H_{18}N_8S$: C, 65.81; H, 3.82; N, 23.61; found: C, 65.45; H, 4.21; N, 23.14%.

*6.1.2.16. 4-(3-Methyl-1-phenyl-5-(1H-1,2,4-triazol-1-yl)-1H-pyrazol-4-yl)-2-(1H-pyrrol-2-yl)benzo[4,5]imidazo[1,2-a]pyrimidine (**9g**).* Orange solid; m.p. 235 °C; IR (KBr, ν, cm^{-1}): 3068 (aromatic ring –CH), 1627 (–C═N), 1378 (Ar-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.28 (s, 3H, C$\underline{H}_3$), 6.92–8.17 (m, 15H, Ar$\underline{H}$), 10.24 (s, 1H, N$\underline{H}$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.2, 106.5, 110.8, 112.6, 120.6, 122.2, 123.4, 125.9, 126.4, 127.5, 128.4, 129.2, 129.3, 129.9, 132.1, 133.4, 136.9, 137.8, 142.6, 145.3, 148.9, 151.4, 153.6, 155.9; anal. calc. for $C_{26}H_{19}N_9$: (457.49) C, 68.26; H, 4.19; N, 27.55; found: C, 67.89; H, 4.31; N, 27.54%.

6.2. Biological evaluation

6.2.1. *In vitro* evaluation of antimicrobial activity. The MICs of the synthesized compounds **8(a–g)** and **9(a–g)** were determined by a broth microdilution method.[17] Serial dilutions were prepared in primary and secondary screening. DMSO was used as a diluent to achieve the preferred concentration of compounds to test upon standard bacterial strains. The tubes were then incubated overnight. The control tube without any antibiotic was instantly subcultured (before inoculation) by dispersing a loopful evenly over a quarter of plate of medium appropriate for the growth of the test organism and put for incubation at 37 °C overnight. The MIC is defined as the lowest concentration of an antibiotic or a test sample allowing no visible growth. The MIC of the control organism was read to check the accuracy of the compound concentrations. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared. All the tubes not showing visible growth (in the same manner as the control tube described above) were subcultured and incubated overnight at 37 °C. The subcultures might show similar numbers of colonies (indicating bacteriostatic activity), a reduced number of colonies (indicating a partial or slow bactericidal activity) and no growth (if the whole inoculum was killed). Each synthesized compound was diluted to obtain 2000 μg mL^{-1} concentration as a stock solution. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. In primary screening 500, 250 and 200 μg mL^{-1} concentrations of the synthesized compounds were taken. The active synthesized compounds found in this primary screening were further tested in a second set of dilutions against all microorganisms. The compounds that were found to be active in primary screening were similarly diluted to obtain 100, 62.5, 50, 25 and 12.5 μg mL^{-1} concentrations. The highest dilution showing at least 99% inhibition is taken as the MIC.

6.2.2. *In vitro* evaluation of antituberculosis activity. The drug susceptibility and the antituberculosis activity of the test compounds against *M. tuberculosis* H37Rv were investigated by the Löwenstein–Jensen slope method[17] with a slight modification,

where 250 μg mL^{-1} dilution of each test compound was added to liquid Löwenstein–Jensen medium, and then the media were sterilized by an inspissation method. The title compounds solution of 250 mg mL^{-1} concentration was prepared in DMSO. A culture of *M. tuberculosis* H37Rv growing in Löwenstein–Jensen medium was harvested in 0.85% saline in bijou bottles. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H37Rv (5×10^4 bacilli per tube). The tubes having the compounds were compared with the control tubes where the medium alone was incubated with *M. tuberculosis* H37Rv. These tubes were then incubated at 37 °C. Growth of bacilli was seen after 12 days, 22 days and finally 28 days of incubation. The concentration at which no development of colonies occurred or there were <20 colonies was taken as the MIC concentration of the test compound. The screening results are summarized as % inhibition relative to the standard drugs isoniazid and rifampicin.

6.2.3. Trager and Jensen method for *in vitro* evaluation of antimalarial activity. All the synthesized compounds were screened for their antimalarial activity against *P. falciparum* strains. *P. falciparum* strains were cultivated by a modified method described by Trager and Jensen.[19] The *P. falciparum* strain was acquired from Shree R. B. Shah Mahavir Super-Speciality Hospital, Surat, Gujarat, India, and was used in *in vitro* tests. The compounds were dissolved in DMSO. The final concentration of DMSO used was not toxic and did not interfere with the assay. The antiparasitic effects of the compounds were measured in terms of growth inhibition percentages as described by Carvalho and Krettli.[20] For experimental purposes, the cultures were synchronized with 5% D-sorbitol when the parasites were in the ring stage.[21] The stock solutions were additionally diluted in whole medium (RPMI 1640 plus 10% human serum) to each of the used concentrations. The concentration that inhibited 50% of parasite growth (IC_{50} value) was determined by interpolation using Microcal Origin software. The parasite suspension, consisting of predominantly the ring stage, was adjusted to 1–2% parasitaemia and 2.5% haematocrit in hypoxanthine-free RPMI-1640 culture medium with 10% human plasma and was exposed to 7 concentrations of each compound for a single cycle of parasite growth of 48 h at 37 °C. A positive control with reference to antimalarial drugs at standard concentrations was used in each experiment. The standard drugs chloroquine and quinine were used as the reference antimalarial agents, blood smears were read blind and each duplicate experiment was repeated three times.

Conflicts of interest

There are no conflicts to declare.

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