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Synthesis, characterization and biological screening of novel 5-imidazopyrazole incorporated fused pyran motifs under microwave irradiation†

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A novel combinatorial library of fused pyran derivatives has been designed and synthesized under microwave irradiation by one-pot three-component cyclocondensation reaction of 5-(1*H*-imidazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde with two active methylene compounds and enolizable ketones/phenols in the presence of piperidine as a basic catalyst. All the newly synthesized compounds have been characterized by elemental analysis and various spectroscopic methods. All the final motifs have been screened for their preliminary *in vitro* antibacterial activity against a panel of pathogenic strains of bacteria and fungi, preliminary *in vitro* antituberculosis activity against *Mycobacterium tuberculosis* H37Rv and also for their antimalarial activity against *Plasmodium falciparum*.

1. Introduction

Malaria is a mosquito-borne infectious disease of humans and other animals caused by protists of the genus *Plasmodium*. The WHO estimates that the mortality rate due to malaria is comparable to roughly 2000 deaths every day.[1,2] *Plasmodium falciparum* is the parasite responsible for most malaria cases (80%), which often proves harmless. The malaria burden is concentrated in 14 endemic countries, which account for an estimated 80% of malaria deaths.[3] Likewise, the occurrence of multidrug-resistant *M. tuberculosis* strains is one of the chief causes of the revival of tuberculosis observed in the world since the mid-1980s.[4] Multidrug-resistant TB and extensively drug-resistant TB are inflexible to handle with any of the usually accessible drugs. This warrants the need for the latest component of drugs to counter successfully the danger posed by these strains.[5] It is a disgusting revelation that MDR-TB is present in almost all countries as per the recent survey by the WHO.[6] The WHO has declared tuberculosis to be a 'global emergency' and estimates that about 30 million people will be infected by mycobacteria tuberculosis within the next 20 years.[7] All the above facts disclose that there is an urgent need for expansion of new drugs with divergent and unique structure and with a mechanism of action possibly different from that of existing drugs. In this regard, it was thought that it is worth synthesizing various novel compounds which may have combined applications as antimalarial, antituberculosis and antimicrobial agents.

Pyrazole and its derivatives are an important class of heterocycles. Pyrazole derivatives possess a broad spectrum of pharmacological activities such as anticancer,[8] antibacterial,[9] antiviral,[10] analgesic[11] and anti-inflammatory[12] activities. Meanwhile, imidazole derivatives are known to be associated with various biological properties, such as anti-inflammatory,[13] analgesic, anti-convulsant,[14] antitubercular,[15] antimicrobial,[16] anticancer and anti-Parkinson[17] activities. The fused pyran ring is an important heterocyclic core constitution and prominent structural motif found in numerous pharmaceutically active compounds. Among the significant pharmacophores responsible for antimicrobial and antituberculosis activity, the fused pyran scaffold is still considered a viable lead structure for the synthesis of more efficacious and broad spectrum antimicrobial and antituberculosis agents. Fused pyran derivatives represent an important class of compounds which hold high activity profile due to their wide range of biological activities such as spasmolytic, diuretic, anticoagulant, anticancer, antianaphylactic,[18] antimicrobial,[19] antiviral,[20] anticancer,[21] antimalarial,[22] anti-HIV, anti-tuberculosis, anti-inflammatory and anti-fungal agents.[23]

In modern era, the multi-component reactions are extremely significant because of their broad range of applications in pharmaceutical chemistry for manufacture of the diversified structural scaffolds and combinatorial libraries for drug discovery.[24] Multicomponent reactions (MCRs) are enormously convergent, producing a remarkably high increase of molecular complexity in just one step.[25] Moreover, microwave irradiation

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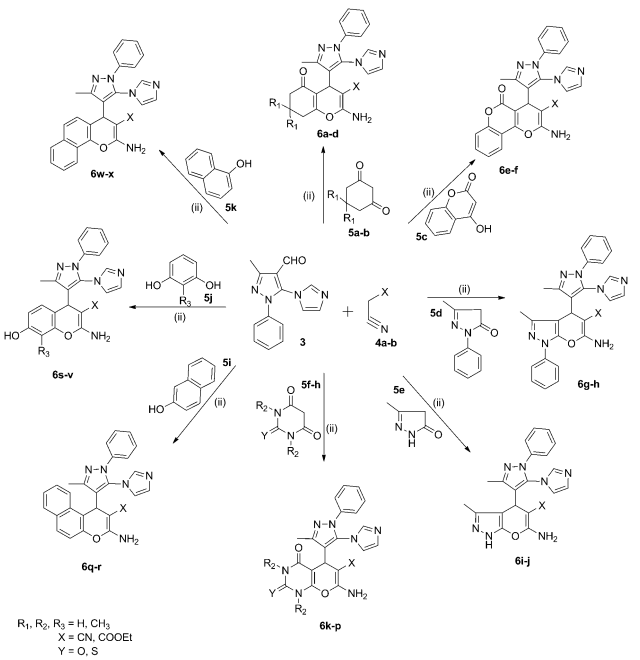
has also become a useful tool in the synthesis of organic molecules. The conventional procedures do not give satisfactory results with regard to operational simplicity, effectiveness and yield.[26] In recent years, microwave irradiation has been reputable not only to considerably accelerate many organic reactions, but also to improve yields and selectivity. In context of the above biological consequences, we thought of synthesizing some fused pyran scaffolds by using various heterocyclic intermediates and studying their antimalarial, antitubercular and antimicrobial activities. We constructed pyrazole and imidazole motifs in one molecule which may play an essential role as significant building blocks in the final synthesized compounds (**6a–x**). In continuation of our research work,[27] we report herein on the utility of 5-(1*H*-imidazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde as the building block for the synthesis of a functionalized fused pyran scaffold. The disparity in the nature and size of substituents in the structures was thought to be of interest representing variable electronic, lipophilic, and steric surroundings that would influence the anticipated pharmacological activities.

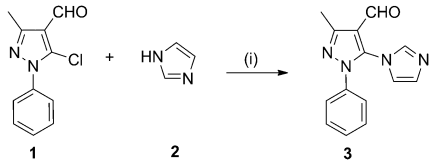
2. Results and discussion

2.1. Chemistry

The synthetic approach adopted to obtain the targeted 5-imidazopyrazole incorporated fused pyran derivatives is summarized in Scheme 1. The starting material 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **1** was prepared according to Vilsmeier–Haack reaction of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one.[28] The final aldehyde 5-(1*H*-imidazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **3** was prepared by nucleophilic displacement



Scheme 1 Synthesis of the substituted 4-(5-(1*H*-imidazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-4*H*-pyran-2-amine **6a–x**. (ii) Ethanol, piperidine, MW, 2–4 min, 80 °C.



Scheme 2 Synthesis of 5-(1*H*-imidazol-1-yl)-3-methyl-1-phenyl-1*H* pyrazole-4-carbaldehyde **3** (i) DMF, K_2CO_3, Reflux 2 h.

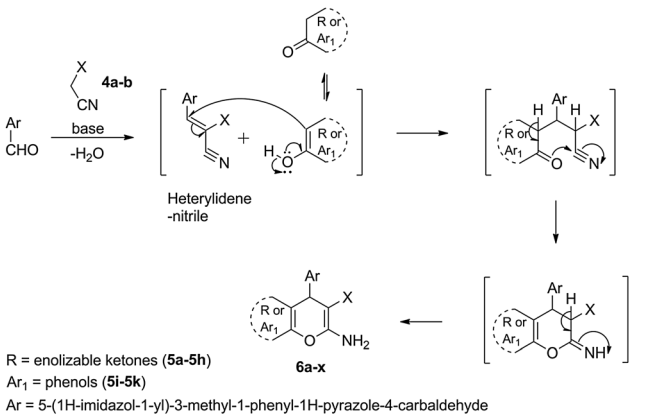


Fig. 1 Plausible mechanistic pathway for the synthesis of fused pyran derivatives (**6a–x**).

of the chloro group at C5 in 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **1** with secondary amine of imidazole **2** in refluxing DMF using anhydrous potassium carbonate as base (Scheme 2).

Subsequently, 4-(5-(1*H*-imidazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-4*H*-pyran-2-amine derivatives **6a–x** have been synthesized. Thus one-pot three-component cyclocondensation reaction of 5-(1*H*-imidazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **3**, malononitrile **4a** or ethylcayno acetate **4b** and compounds **5a–k** was performed in a microwave oven in the presence of piperidine as a catalyst to give moderate to good yield (66–86%) (Scheme 1). The reaction mechanism (Fig. 1) occurred *via* the *in situ* initial formation of the heterylidenenitrile, containing the electron-poor C=C double bond, by the Knoevenagel condensation between 5-(1*H*-imidazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **3** and malononitrile **4a** or ethylcayno acetate **4b** by loss of water molecules. Finally, Michael addition of **5a–k** to the initially formed unsaturated nitrile, *i.e.* nucleophilic attack of hydroxyl moiety to the cyano moiety, afforded cyclized pyran derivatives **6a–x**.

2.2. Pharmacology

2.2.1. *In vitro* antimicrobial activity. The antibacterial activity of all synthesized compounds **6a–x** was screened against three *Gram positive bacteria* (*Bacillus subtilis* MTCC 441, *Clostridium tetani* MTCC 449, *Streptococcus pneumoniae* MTCC 1936) and three *Gram negative bacteria* (*Escherichia coli* MTCC 443, *Salmonella typhi* MTCC 98, *Vibrio cholerae* MTCC 3906) by using ampicillin, norfloxacin and ciprofloxacin as the standard antibacterial drugs. Antifungal activity was screened

Table 1 *In vitro* antimicrobial activity (MIC, μg mL^{-1}) of compounds **6a–x**

	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
Comp.	1936	441	449	443	98	3906	227	3008
6a	200	**250**	200	250	250	200	>1000	200
6b	125	**200**	250	200	250	200	>1000	250
6c	200	**200**	250	100	250	250	1000	500
6d	**100**	200	250	200	**100**	100	1000	500
6e	200	**250**	500	250	**100**	250	**500**	250
6f	**100**	100	200	250	**100**	200	**500**	200
6g	**100**	250	100	200	250	200	>1000	>1000
6h	**100**	250	125	250	200	200	**250**	500
6i	250	**200**	250	100	125	**62.5**	500	1000
6j	250	**250**	200	100	250	200	1000	500
6k	500	500	**100**	200	200	250	**500**	500
6l	250	500	**200**	125	250	200	1000	200
6m	200	**250**	250	200	**100**	200	**500**	500
6n	**100**	100	200	62.5	100	200	1000	500
6o	125	**200**	200	200	200	**62.5**	>1000	200
6p	200	**250**	500	**100**	250	100	>1000	250
6q	125	**125**	250	200	250	100	**200**	1000
6r	**100**	200	125	125	250	125	1000	500
6s	**100**	250	100	62.5	100	200	**500**	1000
6t	250	**200**	250	200	**100**	200	1000	500
6u	500	500	500	**100**	250	250	>1000	1000
6v	**100**	500	500	250	500	500	**500**	1000
6w	**100**	125	125	250	250	250	**250**	>1000
6x	250	**100**	200	250	125	200	**500**	1000
A	100	250	250	100	100	100	n.t.[a]	n.t.
B	10	100	50	10	10	10	n.t.	n.t.
C	25	50	100	25	25	25	n.t.	n.t.
D	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	100	100
E	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: Norfloxacin, C: Ciprofloxacin, D: Nystatin, E: Griseofulvin,[a] n.t.: not tested.

against two fungal species (*Candida albicans* MTCC 3008 and *Aspergillus fumigatus* MTCC 227) where nystatin and griseofulvin were used as the standard antifungal drugs. The minimal inhibitory concentration (MIC) of all compounds **6a–x** was determined by the broth microdilution method according to National Committee for Clinical Laboratory Standards (NCCLS).[29] The inoculum concentration of the test strain was adjusted to 108 CFU (colony-forming units) per mL by comparing the sample turbidity. Mueller Hinton broth was used as nutrient medium to grow and dilute the drug suspension for the test. DMSO was used as the diluent to get the desired concentration of compounds to test upon the standard bacterial strains. The obtained results are presented in Table 1.

2.2.2. *In vitro* antituberculosis activity

All the synthesized compounds **6a–x** were evaluated for their *in vitro* antituberculosis activity against the *Mycobacterium tuberculosis* H37Rv strain. Primary screening of all the newly synthesized compounds was conducted at 250 μg mL^{-1} and 100 μg mL^{-1} by using Lowensteine–Jensen medium as described by Rattan.[30] Rifampicin and Isoniazid were used as the standard drugs. The results of antituberculosis screening data are shown in Table 2.

Table 2 *In vitro* antituberculosis activity (% inhibition) of compounds **6a–x** against *M. tuberculosis* H37Rv (at concentrations 250 μg mL^{-1} and 100 μg mL^{-1})

	% Inhibition			% Inhibition	
Comp.	250 μg mL^{-1}	100 μg mL^{-1}	Comp.	250 μg mL^{-1}	100 μg mL^{-1}
6a	16	14	**6n**	**84**	71
6b	24	17	**6o**	**95**	**92**
6c	11	5	**6p**	42	31
6d	75	47	**6q**	71	61
6e	48	37	**6r**	36	25
6f	20	17	**6s**	78	68
6g	36	24	**6t**	**84**	77
6h	**92**	**92**	**6u**	33	29
6i	74	45	**6v**	12	7
6j	32	28	**6w**	8	4
6k	51	47	**6x**	71	66
6l	77	61	**Rifampicin**	98	98
6m	37	30	**Isoniazid**	99	99

2.2.3. *In vitro* antimalarial activity

All newly fused pyran derivatives **6a–x** were evaluated for their antimalarial activity against *P. falciparum* strain. Chloroquine and quinine were used as the reference drugs. The results of the pharmacological screening are expressed as the drug concentration resulting in 50% inhibition (IC_{50}) of parasite growth. The obtained results are presented in Table 3.

2.3. Analytical results

The structures of all the newly synthesized compounds were confirmed by ^{1}H NMR, FT-IR, ^{13}C NMR, mass and elemental analysis. The ^{1}H NMR spectra of compounds **6a–x** exhibited the presence of the –CH (C4 proton of pyran ring) as a sharp singlet around δ 4.25–5.14 ppm and one more singlet arising at δ 2.15–2.80 ppm due to methyl proton (–CH_3) present in the pyrazole ring. The aromatic protons resonate as multiplets at around δ 6.31–7.93 ppm. The IR spectrum of compounds **6a–x** exhibited characteristic absorption bands around 2185–2191 cm^{-1} and 1680–1729 cm^{-1} which may be attributed to the presence of nitrile C$\equiv$N stretching and ester carbonyl (odd and even number of compounds at C3 position in pyran ring respectively). A strong absorption band was observed in the range of 1365–1382 cm^{-1} due to the CH_3 stretching. While the characteristic absorption band in the range 3478–3301 cm^{-1} may be attributed to asymmetric and

Table 3 *In vitro* antimalarial activity of compounds **6a–x**

Comp.	IC_{50} (μg mL^{-1})	Comp.	IC_{50} (μg mL^{-1})
6a	1.55	**6n**	**0.049**
6b	**0.067**	**6o**	1.54
6c	**0.088**	**6p**	0.97
6d	1.47	**6q**	0.32
6e	0.42	**6r**	1.69
6f	0.78	**6s**	0.38
6g	**0.034**	**6t**	**0.089**
6h	**0.062**	**6u**	0.52
6i	0.57	**6v**	**0.061**
6j	0.82	**6w**	0.54
6k	1.36	**6x**	1.040
6l	1.88	**Chloroquine**	0.020
6m	**0.082**	**Quinine**	0.268

symmetric stretching of NH_2. The characteristic absorption band of C–O–C ether linkage is found at around 1214–1250 cm^{-1}, which supports the formation of pyran derivatives. Similarly in ^{13}C NMR spectra, the signals at around δ 55.1–59.4 ppm and 74.3–76.6 ppm are assigned to carbon attached to carbonitrile and the ester group respectively. One more signal arising at δ 12.5–13.3 ppm may be due to the methyl proton ($-CH_3$) present in the pyrazole ring. The mass spectrum of all the compounds showed a molecular ion peak at M^+ corresponding to its molecular weight, which confirmed its chemical structure.

2.4. Biological results

2.4.1. Antibacterial activity. Upon investigation of antimicrobial screening data (Table 1), it has been observed that the majority of the compounds showed excellent activity against *gram positive bacteria B. subtilis* and *C. tetani* as compared to ampicillin (MIC = 250 μg mL^{-1}). Compounds **6d** (R = cyclohexane-1, 3-dione, X = COOEt), **6f** (R = 4-hydrohxy coumarin, X = COOEt), **6g** (R = 3-methyl-1-phenyl pyrazol-5-one, X═CN), **6h** (R = 3-methyl-1-phenyl pyrazol-5-one, X = COOEt), **6n** (R = *N*-methyl barbituric acid, X = COOEt), **6r** (Ar_1 = β-napthol, X = COOEt), **6s** (Ar_1 = resorcinol, X = CN), **6v** (Ar_1 = 2-methyl resorcinol, X = COOEt) and Ar_1 (Ar = α-napthol, X = CN) against *S. pneumoniae* showed the same potency as compared to ampicillin (MIC = 250 μg mL^{-1}). Whereas compounds **6f** (R = 4-hydroxy coumarin, X = COOEt), **6n** (R = *N*-methyl barbituric acid, X = COOEt) and **6x** (Ar_1 = α-napthol, X = COOEt) showed equal activity against *B. subtilis* as compared to norfloxacin (MIC = 100 μg mL^{-1}). The compounds **6g** (R = 3-methyl-1-phenyl pyrazol-5-one, X = CN), **6k** (R = thiobarbituric acid, X = CN) and **6s** (Ar_1 = resorcinol, X = CN) showed same potency against *C. tetani* as compared to ciprofloxacin (MIC = 100 μg mL^{-1}). Whereas in inhibiting *gram negative bacteria*, the compounds **6i** (R = 3-methyl-1*H*-pyrazol-5(4*H*)-one, X = CN), **6o** (R = barbituric acid, X = CN) and **6n** (R = *N*-methyl barbituric acid, X = COOEt), **6s** (Ar_1 = resorcinol, X = CN) showed brilliant activity *i.e.* 62.5 μg mL^{-1} against *V. cholera* and *E. coli* as compared to ampicillin (MIC = 100 μg mL^{-1}). Compounds **6c** (R = cyclohexane-1, 3-dione, X = CN), **6i** (R = 3-methyl-1*H*-pyrazol-5(4*H*)-one, X = CN), **6j** (R = 3-methyl-1*H*-pyrazol-5(4*H*)-one, X = COOEt) and **6u** (Ar_1 = 2-methyl resorcinol, X = CN) against *E. coli* showed equal activity as compared to ampicillin (MIC = 100 μg mL^{-1}), while compounds **6d** (R = cyclohexane-1, 3-dione, X = COOEt), **6e** (R = 4-hydroxy coumarin, X = CN), **6f** (R = 4-hydroxy coumarin, X = COOEt), **6m** (R = *N*-methyl barbituric acid, X = CN), **6n** (R = *N*-methyl barbituric acid, X = COOEt), **6s** (Ar_1 = resorcinol, X = CN) and **6t** (Ar_1 = resorcinol, X = COOEt) against *S. typhi* as well as compounds **6d** (R = cyclohexane-1, 3-dione, X = COOEt), **6p** (R = barbituric acid, X = COOEt) and **6q** (Ar_1 = β-napthol, X = CN) against *V. cholerae* showed same potency as compared to ampicillin (MIC = 250 μg mL^{-1}).

2.4.2. Antituberculosis activity. The cheering results from the antibacterial activity encouraged us to go for preliminary screening of all synthesized compounds for their *in vitro* antituberculosis activity. Antituberculosis screening of all fused pyran derivatives **6a–x** was conducted at two concentrations *i.e.* 250 μg mL^{-1} and 100 μg mL^{-1} against *M. tuberculosis* H37Rv strain (Table 2). At the commencement of this study in the preliminary screening, compound **6o** (R = barbituric acid, X = CN) displayed better activity and showed 95% inhibition at 250 μg mL^{-1} concentration and 92% inhibition at 100 μg mL^{-1} concentration. Compound **6h** (R = 3-methyl-1-phenyl pyrazol-5-one, X = COOEt) was found to possess excellent activity at both the concentrations *i.e.* 92% at 250 μg mL^{-1} and 100 μg mL^{-1}. While compounds **6n** (R = *N*-methyl barbituric acid, X = COOEt) and **6t** (Ar = resorcinol, X = COOEt) are moderately active against *M. tuberculosis* H37Rv. All other compounds showed poor inhibition of *M. tuberculosis* growth. From the above results, it can be concluded that compounds **6o** (R = barbituric acid, X = CN) and **6h** (R = 3-methyl-1-phenyl pyrazol-5-one, X = COOEt) may become a new class of antitubercular agents in future.

2.4.3. Antimalarial activity. All the compounds **6a–x** were evaluated for their antimalarial activity against the *P. falciparum* strain. All experiments were performed in duplicate and the mean value of IC_{50} is given in Table 3. Compounds **6b** (R = dimedone, X = COOEt), **6c** (R = cyclohexane-1, 3-dione, X = CN), **6g** (R = 3-methyl-1-phenyl pyrazol-5-one, X = CN), **6h** (R = 3-methyl-1-phenyl pyrazol-5-one, X = COOEt), **6m** (R = *N*-methyl barbituric acid, X = CN), **6n** (R = *N*-methyl barbituric acid, X = COOEt), **6t** (Ar_1 = resorcinol, X = COOEt) and **6v** (Ar_1 = 2-methyl resorcinol, X = COOEt) were found to have IC_{50} in the range of 0.034 to 0.089 for the *P. falciparum* strain. These compounds showed principal activity against *P. falciparum* strain as compared to quinine IC_{50} 0.268. Whereas compound **6g** (R = 3-methyl-1-phenyl pyrazol-5-one, X = CN) was found to possess moderate activity *i.e.* IC_{50} 0.034 aligned with chloroquine. All other compounds were found to be not as much active as chloroquine and quinine against *P. falciparum* strain.

2.4.4. Structure–activity relationship (SAR). The structure–activity relationship study (Fig. 2) revealed that various heterocyclic motifs fused at positions 5 and 6 in pyran were responsible for a broad range of antimicrobial, antituberculosis and antimalarial activities. The different nature of the fused motifs has led to significant variations in all three activities. Compound **6i** made up of 3-methyl-1*H*-pyrazol-5-one showed maximum activity against *V. cholerae* and *B. subtilis* while compound **6n** containing *N*-methyl barbituric acid showed highest activity against *E. coli*, *B. subtilis* and *C. tetani* and also increased antimalarial activity against *P. falciparum*. Compound **6o** bearing barbituric acid illustrated maximum antibacterial activity against *V. cholera*, *B. subtilis* and *C. tetani* as well as increased antituberculosis activity against *M. tuberculosis* H37Rv strain. Compound **6s** containing resorcinol showed highest inhibition against bacterial strain *E. coli* and *C. tetani* and also showed highest inhibition against fungal strain *C. albicans*. Compounds **6h** and **6g** containing 3-methyl-1-phenyl pyrazol-5-one showed excellent antimalarial activity against *P. falciparum* and only **6h** showed good antituberculosis activity against *M. tuberculosis* H37Rv strain. It can be concluded that carbonitrile and ester groups present at the third position in the pyran ring may be considered responsible for variation in activity. Both the

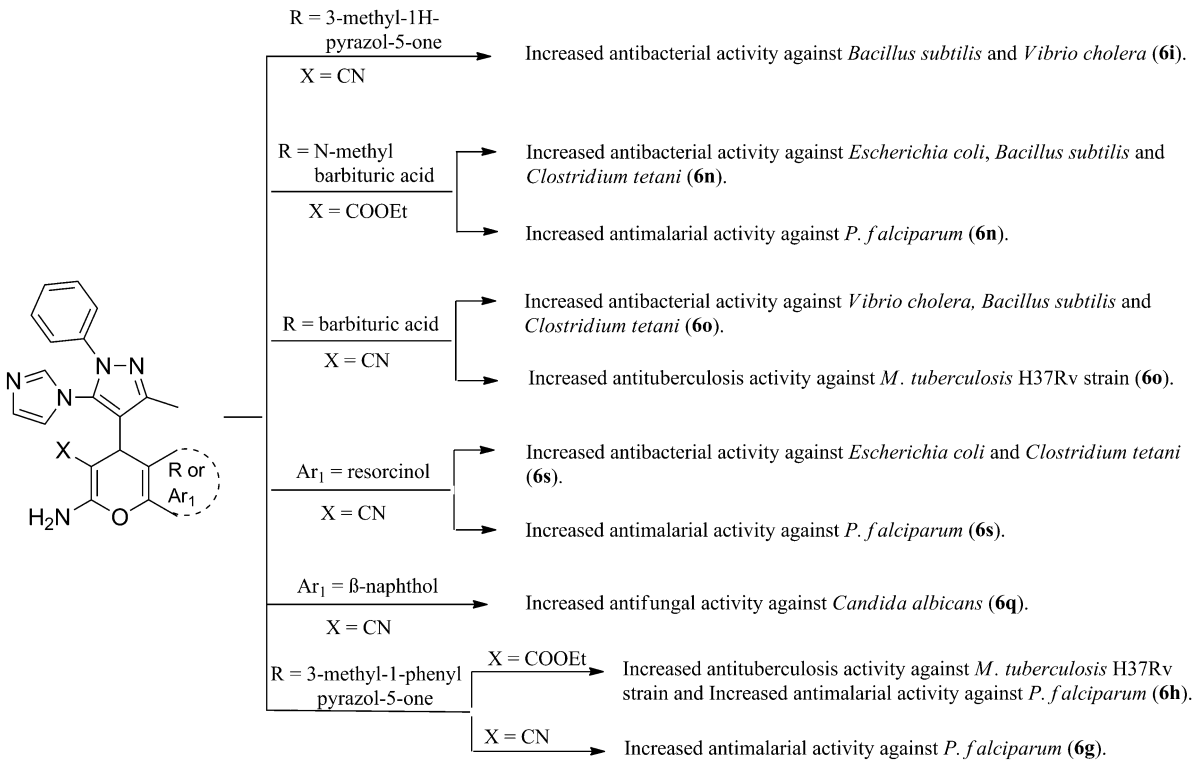


Fig. 2 Structure–activity relationship for antimicrobial, antituberculosis and antimalarial activity of the synthesized compound **6a–x**.

groups are electron withdrawing by the resonance effect even though they showed different activity. Compounds **6n** and **6m** contain *N*-methyl barbituric acid. Among them compound **6n** containing the carbonitrile group showed superior antibacterial activity against *E. coli* and increased antimalarial activity against *P. falciparum*. Compound **6m** having an ester group showed poor activity against *E. coli* and *P. falciparum*. Compounds **6g** and **6h** bear 3-methyl-1-phenyl pyrazol-5-one, among them compound **6h** containing an ester group showed good antituberculosis activity. On the other hand, both compounds possess better antimalarial activity. This may be attributed to the hydrophobicity and size of the group. Pyrazolone and barbituric acid are found as the intermediates in many standard drugs because of their wide range of biological activities.

The majority of our active compounds are either pyrazolone or barbituric acid derivatives. These two biologically active intermediates could be considered responsible for the observed activity. The strongly active compounds of the series with reference to the standard drugs are shown in Fig. 3.

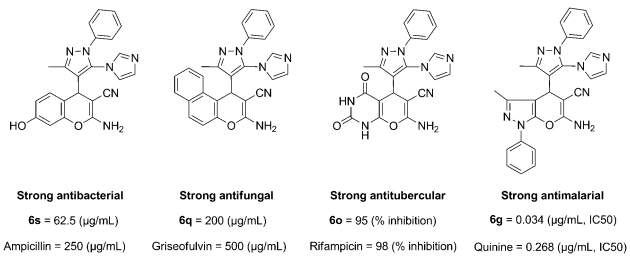


Fig. 3 Strong antibacterial, antifungal, antitubercular and antimalarial compounds from the series.

3. Conclusion

Novel fused pyran derivatives bearing the 5-imidazopyrazole nucleus have been synthesized by one-pot multicomponent reaction under microwave irradiation. All the new compounds were examined for their antimicrobial, antimalarial and antituberculosis activities with the hope of discovering new structure leads helping as potent antimicrobial, antimalarial and antituberculosis agents. It can be concluded that compounds **6i**, **6n**, **6o** and **6s** were found to be the most proficient members of the series. The majority of the compounds were found to be active against *C. tetani* and *B. subtilis.* As for antifungal activity, compounds **6h**, **6q** and **6w** have shown excellent activity against *C. albicans* as compared to griseofulvin. Compounds **6h**, **6n**, **6o** and **6t** exhibited good antituberculosis activities. While as for antimalarial activity, one third of the compounds have shown excellent activity against strains of *P. falciparum* as compared to quinine. Imidazole and pyrazole nuclei, which are present in the synthesized compounds, are expected to be responsible for the biological activity.

4. Experimental section

4.1. Chemistry

All the reagents were obtained commercially and used without further purification. Solvents used were of analytical grade.

Melting points were taken in melting point apparatus μThermoCal$_{10}$ (Analab Scientific Pvt. Ltd, India) and are uncorrected. Thin-layer chromatography (TLC, on aluminum plates coated with silica gel 60 F254, 0.25 mm thickness, Merck) was used for monitoring the progress of all reactions. Mass spectra were recorded on Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan) purchased under PURSE programme of DST at Sardar Patel University, Vallabh Vidyanagar. The IR spectra were recorded on Shimadzu FTIR 8401 spectrophotometer using potassium bromide pellets in the range 4000–400 cm^{-1} and frequencies of only characteristic peaks are expressed in cm^{-1}. The elemental analysis was carried out by using Perkin-Elmer 2400 series-II elemental analyzer (Perkin-Elmer, USA) at Sophisticated Instrumentation Centre for Applied Research & Training (SICART), Vallabh Vidyanagar and all compounds are within ±0.4% of the theoretical compositions. All the reactions were carried out at atmospheric pressure using a multimode microwave reactor (Microwave Synthesis System, Model: Cata-R, Catalyst™ Systems, Pune-India). Microwaves are generated by magnetron at a frequency of 2450 MHz having an adjustable output power levels (*i.e.* 1 to 10 levels from 140 to 700 Watts) and with an individual sensor for temperature control through attachment of reflux condenser with constant stirring. The temperature was monitored with an external flexible probe. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d$_6$ on a Bruker Avance 400F (MHz) spectrometer using the solvent peak as internal standard at 400 and 100 MHz, respectively. Chemical shifts are reported in parts per million (ppm).

4.1.1. General procedure for the synthesis of 5-(1*H*-imidazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (3). 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **1** (1.1 gm, 5 mmol), appropriate imidazole **2** (0.51 gm, 7.5 mmol) and anhydrous potassium carbonate (0.6 gm, 10 mmol) in dimethylformamide (5 mL) were charged in a 50 mL round bottom flask equipped with a mechanical stirrer and a 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 followed by neutralization with 1 N HCl until pH 7. The separated precipitates of 5-(1*H*-imidazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **3** were filtered, thoroughly washed with water, dried, and recrystallized from ethanol.

Yield 79%; m.p. 203–205 °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$^+$).

4.1.2. General procedure for the synthesis of 4-(5-(1*H*-imidazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-4*H*-pyran-2-amine derivatives (6a–x). 5-(1*H*-imidazol-1-yl)-3-methyl-1-phenyl-1*H*-pyrazole-4 carbald ehyde **3** (10 mmol), malononitrile **4a** or ethylcaynoacetate **4b** (10 mmol) and phenol **5i–k**/enolizable ketone **5a–h** (10 mmol) were thoroughly mixed in ethanol (10 mL) with catalytic amounts of piperidine (2–3 drops). The reaction mixture was irradiated in microwave oven at 350 W (50% of output power) for 2–4 min. The reaction mixture attained 80 °C temperature at this power level. After the completion of reaction, monitored by TLC (ethyl acetate : hexane = 1 : 1) the reaction mixture was cooled to room temperature. The solid separated was filtered, washed with ethanol (10 mL), dried and recrystallized from chloroform to get the pure solid sample **6a–x**. The physicochemical and spectroscopic characterization data of the prepared compounds are given below.

*4.1.2.1. 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**6a**).* Yield 84%; m.p. 228–230 °C; IR (KBr, ν_{max}, cm^{-1}): 3476 & 3362 (*asym.* & *sym.* stretching of –NH$_2$), 2188 (C≡N stretching), 1365 (–CH$_3$ stretching), 1250 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.09 (s, 3H, CH$_3$), 1.10 (s, 3H, CH$_3$), 2.22 (d, *J* = 17.6 Hz, 2H, CH$_2$), 2.31 (s, 3H, CH$_3$), 2.40 (d, *J* = 18 Hz, 1H, CH$_2$), 4.25 (s, 1H, CH), 4.84 (s, 2H, NH$_2$), 7.09–7.32 (m, 7H, Ar–H), 7.75 (s, 1H, imidazole); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 13.3 (CH$_3$), 27.8, 28.4 (2C, CH$_3$), 28.9 (C$_4$), 32.4 (C(CH$_3$)$_2$), 40.6, 48.8 (2(CH$_2$)$_2$), 59.4 (C–CN), 108.2, 120.0, 122.1, 122.5, 122.9, 126.9, 129.8, 129.9, 130.0, 132.4, 134.3, 134.5, 137.9, 148.1, (14C, Ar–C), 195.7 (C=O); ESI-MS (*m*/*z*): 441.0 (M$^+$); Anal. Calcd (%) for C$_{25}$H$_{24}$N$_6$O$_2$: C, 68.17; H, 5.49; N, 19.08. Found: C, 67.12; H, 5.42; N, 19.20.

*4.1.2.2. Ethyl 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carboxylate (**6b**).* Yield 76%; m.p. 222–224 °C; IR (KBr, ν_{max}, cm^{-1}): 3470 & 3350 (*asym.* & *sym.* str. of –NH$_2$), 1721 (C=O, ester str.), 1375 (–CH$_3$ str.), 1230 (C–O–C ether stretching); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 0.81 (s, 3H, CH$_3$), 0.86 (s, 3H, CH$_3$), 0.95 (t, *J* = 7.2, 3H, CH$_3$), 1.70 (d, *J* = 17.6 Hz, 1H, CH2), 1.94 (d, *J* = 17.2 Hz, 1H, CH2), 2.14 (s, 2H, CH$_2$), 2.41 (s, 3H, CH$_3$) 3.44 (q, *J* = 7.2, 2H, CH$_2$), 4.30 (s, 1H, CH), 4.97 (s, 2H, NH$_2$) 7.44–7.73 (m, 7H, Ar–H), 7.85 (s, 1H, imidazole); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 13.1 (CH$_3$), 27.9, 28.4, 15.6 (3C, CH$_3$), 28.6 (C$_4$), 32.6 (C(CH$_3$)$_2$), 40.6, 48.6 (2(CH$_2$)$_2$), 58.5 (C, OCH$_2$), 76.1 (C–COOEt), 108.5, 120.5, 122.5, 123.0, 123.2, 127.2, 129.5, 129.9, 130.2, 132.9, 134.1, 134.5, 138.2, 147.6 (14C, Ar–C), 160.5, 195.9 (2C, C=O); ESI-MS (*m*/*z*): 488.1 (M$^+$); Anal. Calcd (%) for C$_{27}$H$_{29}$N$_5$O$_4$: C, 66.51; H, 6.00; N, 14.36. Found: C, 66.40; H, 6.08; N, 14.35.

*4.1.2.3. 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**6c**).* Yield 81%; m.p. 217–219 °C; IR (KBr, ν_{max}, cm^{-1}): 3429 & 3335 (*asym.* & *sym.* str. of –NH$_2$), 2189 (C≡N str.), 1370 (–CH$_3$ str.), 1225 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.84 (s, 3H, CH$_3$), 2.00–2.39 (m, 6H, 3CH$_2$), 4.35 (s, 1H, CH), 5.39 (s, 2H, NH$_2$), 7.34–7.73 (m, 7H, Ar–H), 7.92 (s, 1H, imidazole); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 13.3 (CH$_3$), 21.3 (CH$_2$), 28.5 (C$_4$), 30.4, 38.2 (2(CH$_2$)$_2$), 59.2 (C–CN), 108.3, 120.3, 122.4, 122.9, 123.5, 127.6, 129.5, 130.0, 130.7, 132.4, 134.4, 134.6, 138.4, 147.9 (14C, Ar–C), 195.7 (C=O); ESI-MS (*m*/*z*): 413.1 (M$^+$); Anal. Calcd (%) for C$_{23}$H$_{20}$N$_6$O$_2$: C, C, 66.98; H, 4.89; N, 20.38. Found: C, 67.13; H, 4.72; N, 20.47.

*4.1.2.4. Ethyl 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carboxylate (**6d**).* Yield 75%; m.p. 220–222 °C; IR (KBr, ν_{max}, cm^{-1}): 3430 & 3321

(*asym.* & *sym.* str. of –NH_2), 1700 (C═O, ester str.), 1369 (–CH_3 str.), 1219 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.08 (t, J = 17.2, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.31–2.48 (m, 6H, 3CH_2), 3.87 (q, J = 13.2, 2H, CH_2), 4.58 (s, 1H, CH), 5.59 (s, 2H, NH_2), 6.94–7.42 (m, 8H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.2 (CH_3), 21.4 (CH_2), 28.8 (C_4), 30.6, 38.0 (2(CH_2)$_2$), 58.7 (C, OCH_2), 75.9 (C–COOEt), 108.4, 120.4, 122.2, 122.7, 123.7, 126.5, 128.9, 130.1, 131.5, 132.3, 134.3, 134.5, 138.3, 147.2 (14C, Ar–C), 160.4, 195.8 (2C, C═O); ESI-MS (m/z): 460.0 (M^+); Anal. Calcd (%) for $C_{25}H_{25}N_5O_4$: C, 65.35; H, 5.48; N, 15.24. Found: C, 65.32; H, 5.48; N, 15.29.

*4.1.2.5. 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (**6e**).* Yield 86%; m.p. 266–268 °C; IR (KBr, ν_{max}, cm^{-1}): 3410 & 3360 (*asym.* & *sym.* str. of –NH_2), 2185 (C≡N str.), 1375 (–CH_3 str.), 1236 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 3H, CH_3), 4.39 (s, 1H, CH), 6.92–7.78 (m, 14H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.6 (CH_3), 26.8 (C_4), 55.9 (C–CN), 95.8 (C_5), 101.4, 101.9, 103.4, 111.1, 113.1, 117.1, 117.9, 119.8, 123.0, 125.1, 128.1, 129.6, 133.4, 137.9, 147.7, 152.6, 154.0, 157.6, 158.5 (20C, Ar–C), 160.2 (C═O); ESI-MS (m/z): 463.0 (M^+); Anal. Calcd (%) for $C_{26}H_{18}N_6O_3$: C, 67.53; H, 3.92; N, 18.17. Found: C, 67.58; H, 3.99; N, 18.12.

*4.1.2.6. Ethyl 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-5-oxo-4,5-dihydropyrano[3,2-c] chromene-3-carboxylate (**6f**).* Yield 78%; m.p. 257–259 °C; IR (KBr, ν_{max}, cm^{-1}): 3422 & 3352 (*asym.* & *sym.* str. of –NH_2), 1694 (C═O, ester str.), 1372 (–CH_3 str.), 1232 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.03 (t, J = 7.6, 3H, CH_3), 2.65 (s, 3H, CH_3), 3.92 (q, J = 7.2, 2H, CH_2), 4.38 (s, 1H, CH), 6.93–7.81 (m, 14H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.5, 15.5 (2C, CH_3), 26.8 (C_4), 58.5 (C, OCH_2), 75.2 (C–COOEt), 94.8 (C_5), 101.9, 102.2, 102.9, 111.5, 113.1, 117.4, 118.4, 119.7, 122.7, 124.7, 128.2, 129.6, 133.4, 137.8, 146.2, 152.0, 153.4, 157.8, 158.7 (20C, Ar–C), 160.2, 176.1 (2C, C═O); ESI-MS (m/z): 510.0 (M^+); Anal. Calcd (%) for $C_{28}H_{23}N_5O_5$: C, 66.00; H, 4.55; N, 13.75. Found: C, 66.18; H, 4.69; N, 13.62.

*4.1.2.7. 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-6-amino-3-methyl-1-phenyl-1,4-dihydropyrano [2,3-c]pyrazole-5-carbonitrile (**6g**).* Yield 76%; m.p. 278–280 °C; IR (KBr, ν_{max}, cm^{-1}): 3450 & 3322 (*asym.* & *sym.* str. of –NH_2), 2190 (C≡N str.), 1379 (–CH_3 str.), 1221 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.94 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 4.58 (s, 1H, CH), 6.96–7.67 (m, 15H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.6, 12.9 (2C, CH_3), 26.4 (C_4), 56.6 (C–CN), 96.2 (C_5), 120.7, 120.8, 121.7, 123.0, 123.2, 126.8, 128.1, 129.6, 129.7, 133.2, 137.8, 138.0, 138.9, 144.3, 145.4, 147.4, 159.8 (17C, Ar–C); ESI-MS (m/z): 475.0 (M^+); Anal. Calcd (%) for $C_{27}H_{22}N_8O$: C, 68.34; H, 4.67; N, 23.61. Found: C, 68.30; H, 4.57; N, 23.54.

*4.1.2.8. Ethyl 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-6-amino-3-methyl-1-phenyl-1,4-dihydropyrano [2,3-c]-pyrazole-5-carboxylate (**6h**).* Yield 72%; m.p. 262–264 °C; IR (KBr, ν_{max}, cm^{-1}): 3452 & 3310 (*asym.* & *sym.* str. of –NH_2), 1710 (C═O, ester str.), 1374 (–CH_3 str.), 1225 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.06 (t, J = 6.8, 3H, CH_3), 1.92 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 4.09 (q, J = 13.6, 2H, CH_2), 4.71 (s, 1H, CH), 6.96–7.88 (m, 15H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.6, 12.9, 15.9 (3C, CH_3), 26.5 (C_4), 58.9 (C, OCH_2), 75.8 (C–COOEt), 95.8 (C_5), 120.9, 121.4, 121.6, 123.2, 124.0, 126.5, 128.2, 129.4, 130.2, 133.3, 138.0, 138.6, 139.4, 143.9, 145.5, 147.1 (16C, Ar–C), 160.1 (C, C═O); ESI-MS (m/z): 522.2 (M^+); Anal. Calcd (%) for $C_{29}H_{27}N_7O_3$: C, 66.78; H, 5.22; N, 18.80. Found: C, 66.81; H, 5.29; N, 18.62.

*4.1.2.9. 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-6-amino-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (**6i**).* Yield 73%; m.p. 218–220 °C; IR (KBr, ν_{max}, cm^{-1}): 3402 & 3336 (*asym.* & *sym.* str. of –NH_2), 2191 (C≡N str.), 1376 (–CH_3 str.), 1235 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.94 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 4.67 (s, 1H, CH), 6.76–7.43 (m, 9H, Ar–H + NH_2), 7.82 (s, 1H, imidazole), 11.96 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 10.3, 12.8 (2C, CH_3), 24.5 (C_4), 55.6 (C–CN), 98.1 (C_5), 120.8, 121.3, 123.2, 127.8, 129.6, 130.1, 132.5, 134.1, 135.6, 137.6, 145.1, 147.2, 153.0, 155.0, 161.9 (15C, Ar–C); ESI-MS (m/z): 398.9 (M^+); Anal. Calcd (%) for $C_{21}H_{18}N_8O$: C, 63.31; H, 4.55; N, 28.12. Found: C, 63.29; H, 4.43; N, 27.95.

*4.1.2.10. Ethyl 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-6-amino-3-methyl-1,4-dihydropyrano[2,3-c] pyrazole-5-carboxylate (**6j**).* Yield 69%; m.p. 224–226 °C; IR (KBr, ν_{max}, cm^{-1}): 3420 & 3351 (*asym.* & *sym.* str. of –NH_2), 1699 (C═O, ester str.), 1371 (–CH_3 str.), 1229 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.05 (t, J = 8, 3H, CH_3), 1.92 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 4.09 (q, J = 6.8, 2H, CH_2), 4.71 (s, 1H, CH), 6.96–7.70 (m, 10H, Ar–H + NH_2), 12.0 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 10.4, 12.8, 15.0 (3C, CH_3), 24.3 (C_4), 59.0 (C, OCH_2), 74.3 (C–COOEt), 98.3 (C_5), 121.1, 121.6, 122.8, 127.7, 129.6, 129.9, 132.0, 133.8, 135.6, 138.3, 145.2, 147.2, 152.7, 154.9, 162.4 (15C, Ar–C), 169.1 (C═O); ESI-MS (m/z): 446.3 (M^+); Anal. Calcd (%) for $C_{21}H_{18}N_7O_3$: C, 62.01; H, 5.20; N, 22.01. Found: C, 61.89; H, 5.26; N, 21.96.

*4.1.2.11. 5-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-7-amino-4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (**6k**).* Yield 81%; m.p. 242–244 °C; IR (KBr, ν_{max}, cm^{-1}): 3478 & 3370 (*asym.* & *sym.* str. of –NH_2), 2188 (C≡N str.), 1382 (–CH_3 str.), 1216 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.31 (s, 3H, CH_3), 4.26 (s, 1H, CH), 6.95–7.40 (m, 9H, Ar–H + NH_2), 7.86 (s, 1H, imidazole), 9.50 (s, 1H, NH), 11.88 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.5 (CH_3), 28.5 (C_4), 56.6 (C–CN), 87.1 (C_5), 119.4, 119.9, 122.3, 123.0, 127.9, 130.0, 133.1, 138.2, 147.4, 150.3, 151.5 (11C, Ar–C), 158.4 (C, C═O); ESI-MS (m/z): 445.0 (M^+); Anal. Calcd (%) for $C_{21}H_{16}N_8O_2S$: C, 56.75; H, 3.63; N, 25.21. Found: C, 56.76; H, 3.72; N, 25.04.

*4.1.2.12. Ethyl 5-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-7-amino-4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carboxylate (**6l**).* Yield 74%; m.p. 250–252 °C; IR (KBr, ν_{max}, cm^{-1}): 3449 & 3310 (*asym.* & *sym.* str. of –NH_2), 1706 (C═O, ester str.), 1375 (–CH_3 str.), 1232 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.11 (t, J = 13.2, 3H, CH_3), 2.35 (s, 3H,

CH_3), 3.84 (q, J = 13.2, 2H, CH_2), 4.58 (s, 1H, CH), 6.13 (s, 2H, NH_2), 6.89–7.67 (m, 7H, Ar–H), 7.78 (s, 1H, imidazole), 9.51 (s, 1H, NH), 11.98 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.5, 16.6 (2C, CH_3), 28.8 (C_4), 58.5 (C, OCH_2), 77.3 (C–COOEt), 110.8, 122.3, 122.5, 127.6, 129.5, 131.7, 132.7, 138.2, 141.0, 145.9, 147.0, 157.9 (12C, Ar–C), 159.7, 161.5, 170.0 (3C, C═O); ESI-MS (m/z): 491.9 (M^+); Anal. Calcd (%) for $C_{23}H_{21}N_7O_4S$: C, 56.20; H, 4.31; N, 19.95. Found: C, 56.06; H, 4.21; N, 20.10.

*4.1.2.13. 5-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-7-amino-1,3-dimethyl-2,4-dioxo-2,3,4,5-tetrahy dro-1H-pyrano[2,3-d]-pyrimidine-6-carbonitrile (**6m**).* Yield 78%; m.p. 224–226 °C; IR (KBr, ν_{max}, cm^{-1}): 3461 & 3351 (*asym.* & *sym.* str. of –NH_2), 2186 (C≡N str.), 1369 (–CH_3 str.), 1228 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.29 (s, 3H, CH_3), 3.13 (s, 3H, CH_3), 3.22 (s, 3H, CH_3), 4.28 (s, 1H, CH), 7.06–7.37 (m, 9H, Ar–H + NH_2), 7.70 (s, 1H, imidazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.6 (CH_3), 26.4, 26.6 (2C, 2N–CH_3), 28.3 (C_4), 56.6 (C–CN), 86.7 (C_5), 119.0, 119.6, 122.3, 122.8, 128.0, 129.7, 132.9, 137.9, 147.5, 150.2, 151.2 (11C, Ar–C), 158.1, 161.0 (2C, C═O) ESI-MS (m/z): 456.8 (M^+); Anal. Calcd (%) for $C_{23}H_{20}N_8O_3$: C, 60.52; H, 4.42; N, 24.55. Found: C, 60.43; H, 4.41; N, 24.41.

*4.1.2.14. Ethyl 5-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-7-amino-1,3-dimethyl-2,4-dioxo-2,3,4,5-tetrahy dro-1H-pyrano[2,3-d]pyrimidine-6-carboxylate (**6n**).* Yield 88%; m.p. 219–221 °C; IR (KBr, ν_{max}, cm^{-1}): 3470 & 3353 (*asym.* & *sym.* stretching of –NH_2), 1722 (C═O, ester stretching), 1381 (–CH_3 stretching), 1240 (C–O–C ether stretching); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.00 (t, J = 6.8, 3H, CH_3), 2.80–2.90 (m, 9H, $(CH_3)_3$), 3.87 (q, J = 8.4, 2H, CH_2), 4.41 (s, 1H, CH), 6.23 (s, 2H, NH_2) 7.06–7.60 (m, 7H, Ar–H), 7.72 (s, 1H, imidazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.4, 16.6 (2C, CH_3), 26.9, 27.5 (2C, $(N–CH_3)_2$) 29.0 (C_4), 58.4 (C, OCH_2), 77.6 (C–COOEt), 111.1, 122.2, 122.5, 127.4, 129.3, 132.1, 132.5, 137.9, 140.7, 145.7, 148.2, 158.3 (12C, Ar–C), 160.0, 161.6, 169.7 (3C, C═O); ESI-MS (m/z): 504.2 (M^+); Anal. Calcd (%) for $C_{25}H_{25}N_7O_5$: C, 59.63; H, 5.00; N, 19.47. Found: C, 59.55; H, 4.86; N, 19.55.

*4.1.2.15. 5-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-7-amino-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyra no[2,3-d]pyrimidine-6-carbonitrile (**6o**).* Yield 80%; m.p. 263–265 °C; IR (KBr, ν_{max}, cm^{-1}): 3480 & 3369 (*asym.* & *sym.* stretching of –NH_2), 2191 (C≡N stretching), 1369 (–CH_3 stretching), 1214 (C–O–C ether stretching); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.16 (s, 3H, CH_3), 4.31 (s, 1H, CH), 6.98–7.94 (m, 12H, Ar–H + NH_2 + 2NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.4 (CH_3), 28.4 (C_4), 56.9 (C–CN), 86.8 (C_5), 119.2, 119.8, 122.3, 123.2, 128.3, 129.8, 133.3, 138.0, 147.2, 150.0, 151.3 (11C, Ar–C), 158.2 (C, C═O); ESI-MS (m/z): 428.9 (M^+); Anal. Calcd (%) for $C_{21}H_{16}N_8O_3$: C, 58.88; H, 3.76; N, 26.16. Found: C, 58.76; H, 3.59; N, 25.95.

*4.1.2.16. Ethyl 5-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-7-amino-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyra no[2,3-d]-pyrimidine-6-carboxylate (**6p**).* Yield 73%; m.p. 270–272 °C; IR (KBr, ν_{max}, cm^{-1}): 3399 & 3339 (*asym.* & *sym.* stretching of –NH_2), 1729 (C═O, ester stretching), 1369 (–CH_3 stretching), 1226 (C–O–C ether stretching); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.02 (t, J = 6.8, 3H, CH_3), 2.25 (s, 3H, CH_3), 3.85 (q, J = 8.4, 2H, CH_2), 4.34 (s, 1H, CH), 6.86–7.89 (m, 12H, Ar–H + NH_2 + 2NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.3, 16.8 (2C, CH_3), 28.6 (C_4), 58.8 (C, OCH_2), 77.3 (C–COOEt), 111.3, 122.5, 123.0, 127.1, 129.5, 132.0, 132.4, 138.2, 141.7, 146.3, 147.0, 157.9 (12C, Ar–C), 159.6, 161.4, 169.7 (3C, C═O); ESI-MS (m/z): 475.8 (M^+); Anal. Calcd (%) for $C_{23}H_{21}N_7O_5$: C, 58.10; H, 4.45; N, 20.62. Found: C, 58.01; H, 4.29; N, 20.49.

*4.1.2.17. 1-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-amino-1H-benzo [*f*]chromene-2-carbonitrile (**6q**).* Yield 81%; m.p. 238–240 °C; IR (KBr, ν_{max}, cm^{-1}): 3389 & 3328 (*asym.* & *sym.* stretching of –NH_2), 2190 (C≡N stretching), 1382 (–CH_3 stretching), 1238 (C–O–C ether stretching); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.16 (s, 3H, CH_3), 5.14 (s, 1H, CH), 6.98–7.93 (m, 16H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.6 (CH_3), 28.2 (C_4), 55.5 (C–CN), 112.6, 116.9, 117.0, 120.0, 121.5, 121.7, 122.9, 123.0, 123.4, 125.4, 127.8, 128.1, 129.1, 129.2, 129.6, 130.2, 130.3, 131.1, 132.4, 137.8, 147.1, 160.2 (22C, Ar–C); ESI-MS (m/z): 445.0 (M^+); Anal. Calcd (%) for $C_{27}H_{20}N_6O$: C, 72.96; H, 4.54; N, 18.91. Found: C, 73.12; H, 4.63; N, 18.77.

*4.1.2.18. Ethyl 1-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-amino-1H-benzo [*f*]chromene-2-carboxylate (**6r**).* Yield 71%; m.p. 227–229 °C; IR (KBr, ν_{max}, cm^{-1}): 3395 & 3331 (*asym.* & *sym.* stretching of –NH_2), 1712 (C═O, ester stretching), 1382 (–CH_3 stretching), 1225 (C–O–C ether stretching); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.00 (t, J = 8, 3H, CH_3), 2.18 (s, 3H, CH_3), 4.10 (q, J = 7.6, 2H, CH_2), 5.11 (s, 1H, CH), 7.06–7.46 (m, 15H, Ar–H + NH_2), 7.96 (s, 1H, imidazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.8, 17.2 (2C, CH_3), 28.3 (C_4), 59.0 (C, OCH_2), 75.4 (C–COOEt), 111.9, 117.0, 117.5, 120.1, 121.9, 122.4, 123.1, 123.9, 124.4, 125.4, 127.9, 128.5, 128.9, 129.7, 130.2, 130.3, 131.3, 131.8, 132.0, 138.2, 146.9, 159.9 (22C, Ar–C), 169.0 (C═O); ESI-MS (m/z): 492.0 (M^+); Anal. Calcd (%) for $C_{29}H_{25}N_5O_3$: C, 70.83; H, 5.13; N, 14.25. Found: C, 70.70; H, 4.99; N, 14.22.

*4.1.2.19. 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-7-hydroxy-4H-chromene-3-carbonitrile (**6s**).* Yield 79%; m.p. 230–232 °C; IR (KBr, ν_{max}, cm^{-1}): 3413 & 3301 (*asym.* & *sym.* stretching of –NH_2), 2190 (C≡N stretching), 1375 (–CH_3 stretching), 1229 (C–O–C ether stretching); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.16 (s, 3H, CH_3), 4.66 (s, 1H, CH), 6.34–7.58 (m, 13H, Ar–H + NH_2), 9.64 (s, b, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.7 (CH_3), 28.4 (C_4), 59.0 (C–CN), 102.2, 112.9, 114.0, 122.0, 122.7, 123.1, 127.7, 129.6, 129.9, 130.4, 132.1, 138.2, 147.0, 149.3, 157.4, 161.2, 168.6 (17C, Ar–C); ESI-MS (m/z): 410.9 (M^+); Anal. Calcd (%) for $C_{23}H_{18}N_6O_2$: C, 67.31; H, 4.42; N, 20.48. Found: C, 67.22; H, 4.29; N, 20.31.

*4.1.2.20. Ethyl 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-7-hydroxy-4H-chromene-3-carboxylate (**6t**).* Yield 74%; m.p. 246–248 °C; IR (KBr, ν_{max}, cm^{-1}): 3422 & 3306 (*asym.* & *sym.* stretching of –NH_2), 1680 (C═O, ester stretching), 1382 (–CH_3 stretching), 1217 (C–O–C ether

stretching); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.01 (t, J = 6.8, 3H, CH_3), 2.17 (s, 3H, CH_3), 4.00 (q, J = 8, 2H, CH_2), 4.69 (s, 1H, CH), 7.14–7.72 (m, 13H, Ar–H + NH_2), 9.64 (s, b, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 12.7, 15.6 (2C, CH_3), 28.2 (C_4), 58.5 (C, OCH_2), 75.5 (C–COOEt), 102.3, 113.2, 114.2, 122.1, 123.1, 123.8, 127.8, 129.6, 130.2, 130.5, 131.9, 138.6, 147.1, 149.0, 157.8, 161.5, 168.8 (17C, Ar–C), 169.5 (C═O); ESI-MS (m/z): 458.0 (M^+); Anal. Calcd (%) for $C_{25}H_{23}N_5O_4$: C, 65.63; H, 5.07; N, 15.31. Found: C, 65.49; H, 4.89; N, 15.12.

*4.1.2.21. 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-7-hydroxy-8-methyl-4H-chromene-3-carbonitrile (**6u**).* Yield 78%; m.p. 241–243 °C; IR (KBr, ν_{max}, cm^{-1}): 3400 & 3321 (*asym.* & *sym.* stretching of –NH_2), 2189 (C≡N stretching), 1381 (–CH_3 stretching), 1232 (C–O–C ether stretching); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.98 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 4.31 (s, 1H, CH), 6.57–7.38 (m, 11H, Ar–H + NH_2), 7.69 (1H, imidazole), 9.57 (s, b, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 8.0, 12.6 (2C, CH_3), 28.3 (C_4), 59.2 (C–CN), 102.1, 113.0, 114.1, 122.3, 122.9, 123.1, 127.7, 129.6, 129.9, 130.1, 132.0, 138.5, 147.3, 149.4, 157.6, 161.4, 168.7 (17C, Ar–C); ESI-MS (m/z): 424.8 (M^+); Anal. Calcd (%) for $C_{24}H_{20}N_6O_2$: C, 67.91; H, 4.75; N, 19.80. Found: C, 68.12; H, 4.63; N, 19.59.

*4.1.2.22. Ethyl 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-7-hydroxy-8-methyl-4H-chromene-3-carboxylate (**6v**).* Yield 68%; m.p. 226–228 °C; IR (KBr, ν_{max}, cm^{-1}): 3391 & 3330 (*asym.* & *sym.* stretching of –NH_2), 1721 (C═O, ester stretching), 1382 (–CH_3 stretching), 1222 (C–O–C ether stretching); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.07 (t, J = 6.8, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 4.14 (q, J = 10.8, 2H, CH_2), 4.64 (s, 1H, CH), 6.55–7.82 (m, 12H, Ar–H + NH_2), 9.48 (s, b, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 8.3, 12.5, 15.6 (3C, CH_3), 28.5 (C_4), 58.4 (C, OCH_2), 75.5 (C–COOEt), 102.0, 113.1, 113.9, 122.2, 123.2, 123.7, 128.8, 129.9, 130.4, 130.8, 132.4, 138.5, 147.2, 149.2, 158.2, 161.4, 168.6 (17C, Ar–C), 169.5 (C═O); ESI-MS (m/z): 471.9 (M^+); Anal. Calcd (%) for $C_{26}H_{23}N_5O_4$: C, 66.23; H, 5.34; N, 14.85. Found: C, 66.09; H, 5.19; N, 14.73.

*4.1.2.23. 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-4H-benzo[h]chromene-3-carbonitrile (**6w**).* Yield 74%; m.p. 244–246 °C; IR (KBr, ν_{max}, cm^{-1}): 3451 & 3327 (*asym.* & *sym.* stretching of –NH_2), 2188 (C≡N stretching), 1374 (–CH_3 stretching), 1225 (C–O–C ether stretching); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.97 (s, 3H, CH_3), 4.62 (s, 1H, CH), 7.05–8.20 (m, 14H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 12.8 (CH_3), 28.5 (C_4), 55.1 (C–CN), 112.3, 116.4, 117.2, 120.0, 121.4, 121.9, 123.2, 123.4, 123.5, 125.0, 128.0, 128.1, 128.9, 129.6, 130.0, 130.2, 130.7, 131.1, 132.4, 138.0, 147.3, 159.9 (22C, Ar–C); ESI-MS (m/z): 445.2 (M^+); Anal. Calcd (%) for $C_{27}H_{20}N_6O$: C, 72.96; H, 4.54; N, 18.91. Found: C, 73.12; H, 4.21; N, 19.01.

*4.1.2.24. Ethyl 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2-amino-4H-benzo[h]chromene-3-carboxylate (**6x**).* Yield 66%; m.p. 230–232 °C; IR (KBr, ν_{max}, cm^{-1}): 3441 & 3312 (*asym.* & *sym.* stretching of –NH_2), 1701 (C═O, ester stretching), 1380 (–CH_3 stretching), 1234 (C–O–C ether stretching); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 1.07 (t, J = 6.8, 3H, CH_3), 2.06 (s, 3H, CH_3), 4.07 (q, J = 12.8, 2H, CH_2), 4.83 (s, 1H, CH), 7.28–8.26 (m, 16H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 12.6, 17.2 (2C, CH_3), 28.1 (C_4), 59.3 (C, OCH_2), 75.0 (C–COOEt), 112.0, 117.3, 117.9, 119.9, 122.0, 122.9, 123.1, 123.9, 124.0, 125.8, 128.1, 128.5, 129.2, 129.7, 130.0, 130.9, 131.4, 131.6, 132.1, 137.9, 147.2, 159.4 (22C, Ar–C), 169.5 (C═O); ESI-MS (m/z): 491.9 (M^+); Anal. Calcd (%) for $C_{29}H_{25}N_5O_3$: C, 70.83; H, 5.13; N, 14.25. Found: C, 70.63; H, 5.02; N, 14.11.

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References

1 World Malaria Report 2011 summary (Report). World Health Organization.
2 N. B. Behrens, Malaria: an update for physicians, *Infect. Dis. Clin. North Am.*, 2012, **26**, 243–259.
3 World Malaria Report 2012 summary (Report). World Health Organization.
4 S. Ducasse-Cabanot, M. Cohen-Gonsaud, H. Marrakchi, M. Nguyen, D. Zerbib, J. Bernadou, M. Daffe, G. Labesse and A. Queémard, *Antimicrob. Agents Chemother.*, 2004, **48**, 242–249.
5 P. Ahirrao, *Mini-Rev. Med. Chem.*, 2008, **8**, 1441–1451.
6 S. Eswaran, A. V. Adhikari and R. A. Kumar, *Eur. J. Med. Chem.*, 2010, **45**, 957–966.
7 Global Tuberculosis Controlesurveillance, Planning, Financing. WHO Report (2006). http://www.who.int/tb/publications/global_report/2006/pdf/full-report_ correctedversion.pdf.
8 S. Fletcher, E. P. Keaney, C. G. Cummings, M. A. Blaskovich, M. A. Hast, M. P. Glenn, S. Y. Chang, C. J. Bucher, R. J. Floyd, W. P. Katt, M. H. Gelb, W. C. van Voorhis, L. S. Beese, S. M. Sebti and A. D. Hamilton, *J. Med. Chem.*, 2010, **53**, 6867–6888.
9 A. Tanitame, Y. Oyamada, K. Ofuji, M. Fujimoto, N. Iwai, Y. Hiyama, K. Suzuki, H. Ito, H. Terauchi, M. Kawasaki, K. Nagai, M. Wachi and J. I. Yamagishi, *J. Med. Chem.*, 2004, **47**, 3693–3696.
10 G. P. Ouyang, X. J. Cai, Z. Chen, B. A. Song, P. S. Bhadury, S. Yang, L. H. Jin, W. Xue, D. Y. Hu and S. Zeng, *J. Agric. Food Chem.*, 2008, **56**, 10160–10167.
11 A. Hall, A. Billinton, S. H. Brown, N. M. Clayton, A. Chowdhury, G. M. P. Giblin, P. Goldsmith, T. G. Hayhow, D. N. Hurst,

I. R. Kilford, A. Naylor, B. Passingham and L. Winyard, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 3392–3399.
12 B. P. Bandgar, S. S. Gawande, R. G. Bodade, N. M. Gawande and C. N. Khobragade, *Bioorg. Med. Chem.*, 2009, **17**, 8168–8173.
13 F. Suzuki, T. Kuroda, T. Tamura, S. Sato, K. Ohmori and S. Ichikawa, *J. Med. Chem.*, 1992, **35**, 2863–2870.
14 M. Pinza, Z. Farina, A. Cerri, U. Pfeiffer, M. T. Riccaboni, S. Banfi, R. Biagetti, O. Pozzi, M. Magnani and L. Dorigotti, *J. Med. Chem.*, 1993, **36**, 4214–4220.
15 J. Pandey, V. K. Tiwari, S. S. Verma, V. Chaturvedi, S. Bhatnagar, S. Sinha, A. N. Gaikwad and R. P. Tripathi, *Eur. J. Med. Chem.*, 2009, **44**, 3350–3355.
16 I. Takeuchi, M. Sugiura, K. Yamamoto, T. Ito and Y. Hamada, *Yakugaku Zasshi*, 1985, **105**, 554–561.
17 H. Miyachi, H. Kiyota and M. Segawa, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 1807–1812.
18 (*a*) L. Bonsignore, G. Loy, D. Secci and A. Calignano, *Eur. J. Med. Chem.*, 1993, **28**, 517; (*b*) S. Balalaie, M. Bararjanian and M. Sheikh-Ahmadi, *Synth. Commun.*, 2007, **37**, 1097.
19 N. J. Thumar and M. P. Patel, *ARKIVOC*, 2009, **13**, 363.
20 A. H. Shamroukh, M. E. A. Zaki, E. M. H. Morsy, F. M. Abdel-Motti and F. M. Abdel-Megeid, *Arch. Pharm. Chem. Life Sci.*, 2007, **340**, 236.
21 M. Ough, A. Lewis, E. A. Bey, J. Gao, J. M. Ritchie, W. Bornmann, D. A. Boothman, L. W. Oberley and J. J. Cullen, *Cancer Biol. Ther.*, 2005, **4**, 95.
22 (*a*) V. F. De Andrade-Neto, M. O. F. Goulart, J. F. Da silva filho, M. J. Da silva, M. D. C. F. R. Pinto, A. V. Pinto, M. G. Zalis, L. H. Carvalho and A. U. Krettli, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 1145; (*b*) E. Perez-sacau, A. Estevez-braun, A. G. Ravelo, D. G. Yapu and A. G. Turba, *Chem. Biodiversity*, 2005, **2**, 264.
23 (*a*) L. Abrunhosa, M. Costa, F. Areias, A. Venancio and F. J. Proenca, *J. Ind. Microbiol. Biotechnol.*, 2007, **34**, 787; (*b*) F. Chimenti, B. Bizzarri, A. Bolasco, D. Secci, P. Chimenti, S. Carradori, A. Granese, D. Rivanera, D. Lilli, A. Zicari, M. M. Scaltrito and F. Sisto, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 3065; (*c*) I. Kostova, *Mini-Rev. Med. Chem.*, 2006, **6**, 365; (*d*) A. Nayyar and R. Jain, *Curr. Med. Chem.*, 2005, **12**, 1873; (*e*) K. C. Fylaktakidou, D. J. Hadjipavlou-Litina, K. E. Litinas and D. N. Nicolaides, *Curr. Pharm. Des.*, 2004, **10**, 3813; (*f*) K. Asres, A. Seyoum, C. Veeresham, F. Bucar and S. Gibbons, *Phytother. Res.*, 2005, **19**, 557.
24 (*a*) R. W. Armstrong, A. P. Combs, P. A. Tempest, S. D. Brown and T. A. Keating, *Acc. Chem. Res.*, 1996, **29**, 123; (*b*) N. K. Terret, M. Gardner, D. W. Gordon, R. J. Kobylecki and J. Steel, *Tetrahedron*, 1995, **51**, 8135.
25 (*a*) J. Zhu and H. Bienaymé, *Multicomponent Reactions*, Wiley, Weinheim, 2005; (*b*) B. Jiang, S.-J. Tu, P. Kaur, W. Wever and G. J. Li, *J. Am. Chem. Soc.*, 2009, **131**, 11660.
26 M. A. Pasha and V. P. Jayshankara, *Indian J. Chem.*, 2007, **46**, 1328.
27 (*a*) J. R. Avalani, D. S. Patel and D. K. Raval, *J. Chem. Sci.*, 2012, **124**, 1091; (*b*) B. Thummar, U. P. Tarpada and D. K. Raval, *J. Heterocycl. Chem.*, DOI: 10.1002/jhet.1870; (*c*) S. P. Satasia, P. N. Kalaria and D. K. Raval, *RSC Adv.*, 2013, **3**, 3184; (*d*) J. R. Avalani, D. S. Patel and D. K. Raval, *J. Mol. Catal. B: Enzym.*, 2013, **90**, 70–75; (*e*) D. S. Patel, J. R. Avalani and D. K. Raval, *J. Braz. Chem. Soc.*, 2012, **23**, 1951–1954.
28 H. Q. Xiao, G. P. Ouyang, X. D. Sun, X. D. Yao, G. Q. Bao and C. Z. Qi, *Chin. J. Synth. Chem.*, 2005, **13**, 600–602.
29 NCCLS (National Committee for Clinical Laboratory Standards), Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement (2002), ISBN 1-56238-454-6 M100-S12 (M7).
30 A. Rattan, in *Antimicrobials in Laboratory Medicine*, ed. B. I. Churchill, Livingstone, New Delhi, 2000, pp. 85–108.