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COMMUNICATION

Green synthesis and pharmacological screening of polyhydroquinoline derivatives bearing a fluorinated 5-aryloxypyrazole nucleus†

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A novel series of polyhydroquinoline scaffolds **8a–p** has been designed and synthesized under ultrasonic irradiation by a one-pot three-component cyclocondensation reaction of 3-methyl-5-substituted aryloxy-1-phenyl-1*H*-pyrazole-4-carbaldehydes **3a–d** with malononitrile **7** and various enhydrazinoketones **6a–e** in the presence of piperidine as basic catalyst. All the synthesized compounds have been characterized by various spectroscopic techniques and elemental analysis. All the synthesized compounds were evaluated for their *in vitro* antibacterial activity against a panel of pathogenic strains of bacteria and fungi, *in vitro* antitubercular activity against *Mycobacterium tuberculosis* H37Rv strain and also for their *in vitro* antimalarial activity against *Plasmodium falciparum*. Compounds **8c**, **8i**, **8j**, **8l** and **8m** exhibited excellent antimalarial potency. The cytotoxicity of the synthesized compounds was tested using a bioassay of *S. pombe* cells at the cellular level. Compounds **8i**, **8j**, **8k** and **8l** were found to have maximum toxicity, while compounds **8e**, **8m**, **8c** were found to be less cytotoxic. Some of them displayed luminous antibacterial activity and reasonable antituberculosis activity as compared with the first line drugs.

1. Introduction

Malaria and tuberculosis (TB) are the most disturbing infectious diseases in the world, due to their high mortality and morbidity. The protozoan parasite *Plasmodium falciparum* and the pathogen *Mycobacterium tuberculosis* (MTB) are respectively responsible for their occurrence. It has been estimated that around 500 million people get infected in subtropical and tropical countries by malaria and 2.5 million deaths occur annually.[1–3] The World Health Organization has declared TB to

be a 'global emergency' and a recent estimation by WHO showed that within the next 20 years around 30 million people will be infected by *M. tuberculosis*.[4,5] The recurrence of TB infection has been connected to co-infection with the human immunodeficiency virus (HIV),[6] the appearance of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *M. tuberculosis* strains that are resistant to the existing therapies.[7] Thus to overcome the threat of MDR-TB and XDR-TB, there is an imperative need for the development of new drugs with divergent and unique structures and possibly with a different mechanism of action from that of the existing drugs.[8]

Multi-component reactions (MCRs) are the most efficient and powerful methods in the context of modern drug discovery for the preparation of bioactive heterocyclic compounds because of their atom economy, high yields of the products and simple experimentation.[9,10] Ultrasound irradiation is also a superior technique in green synthetic approach, which is being used to accelerate organic reactions. It is considered as a processing aid in terms of energy conservation compared with conventional methods as it provides uniform and noncontact heating.[11,12] The significant features of the ultrasound approach are improved rate of reaction, easier handling and formation of pure products in prominent yields.

Fluorine substituted quinolones (Fig. 1) such as norfloxacin, ofloxacin, ciprofloxacin, temafloxacin, defloxacin, sparfloxacin, delafloxacin, lomefloxacin *etc.* are used clinically as they possess effective antibacterial potency.[13] The substitution of fluorine in to a potential drug molecule can extend not only pharmacokinetic properties but also enhances the properties of pharmacodynamics, toxicology and efficacy of drugs.[14]

Pyrazole and its derivatives are the key structural motifs in heterocyclic chemistry and occupy a significant position in biological and medicinal chemistry. They exhibited a broad spectrum of pharmacological activities such as such as anticancer,[15] antibacterial,[16] antiviral,[17] analgesic,[18] anti-inflammatory,[19] antimalarial, antituberculosis and antifungal activities.[20] Polyhydroquinoline derivatives have experienced tremendous upsurge in the modern era. They exhibited diverse therapeutic

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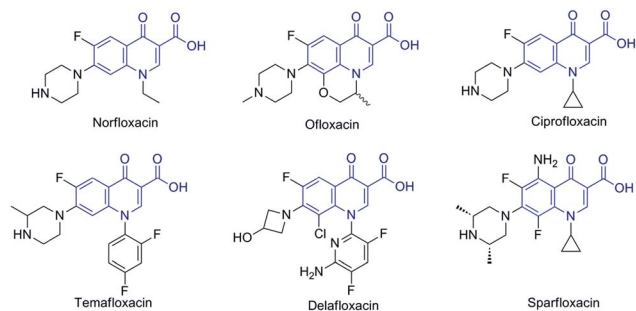
Fig. 1 Fluorine substituted quinolone drugs.

and pharmacological importance, such as calcium channel blockers, hepatoprotective agents, vasodilators, antiathero-sclerotic agents, bronchodilators, geroprotective agents, anti-tumor agents, antidiabetic agents, *etc.*[21–25] Substituted 1,4-dihydropyridines are also well known as calcium channel blockers (CCB) which are often used to treat orderly cardiovas-cular diseases including hypertension.[26–28]

Lichitsky and co-workers reported the conventional synthesis of fused N-substituted 1,4-dihydropyridines by react-ing cyclic enhydrazinoketones and arylidene derivatives of malononitrile in two step without biological evaluation. They employed simple aromatic aldehydes and only two substituted phenyl hydrazine hydrochlorides for the synthesis.[29] In the context of our interest we designed and synthesized fluorinated 5-(substituted aryloxy)-pyrazole nucleus based on poly-hydroquinoline scaffold Fig. 2. In current work, we have concentrated on following points: (a) synthesis of target compounds by single step one-pot three-component cyclo-condensation reaction employing conventional as well as ultrasonic irradiation method (b) use of heteroaromatic alde-hydes (3-methyl-5-substituted-aryloxy-1-phenyl-1*H*-pyrazole-4-carbaldehydes) and variously substituted phenyl hydrazine hydrochlorides (4-F, 4-Br, 4-Cl, 4-OMe) in synthesizing the target molecules (c) biological evaluation including antibacte-rial, antimalarial and antituberculosis studies and cytotoxicity.

In continuation of our efforts to synthesize some novel heterocyclic motifs with biological interest,[30–37] herein we report for the synthesis and biological screening of novel polyhydroquinoline derivatives bearing a fluorinated 5-
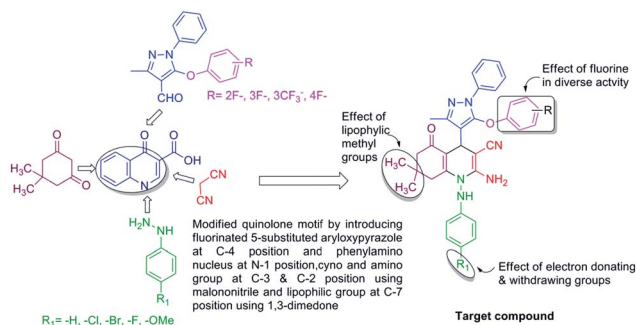
aryloxypyrazole nucleus using ultrasonic irradiation. In the context of biological importance, we intended to develop novel approach for structural diversity of heterocycles incorporating polyhydroquinoline scaffold. The modifications made on qui-nolone core for probing antibacterial, antimalarial and antitu-bercular activity includes; (A) fluorinated 5-substituted aryloxypyrazole at C-4 position (B) 4-substituted phenylamino nucleus at N-1 position and (C) methyl group on C-7 position to validate lipophilicity of the target molecules.

2. Chemistry

The synthesis of targeted 5-(variously fluorinated aryloxy)-pyrazole incorporated polyhydroquinoline derivatives are 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.[38] 3-Methyl-5-substituted aryloxy-1-phenyl-1*H*-pyrazole-4-carbaldehydes **3a–d** were prepared by refluxing compound **1** and substituted phenols **2a–d** in pres-ence of anhydrous K_2CO_3 as basic catalyst in DMF as solvent. The required enhydrazinoketones **6a–e** were prepared by the reaction of β-diketone dimedone **4** with 4-substituted phenyl hydrazine hydrochlorides **5a–e** under aqueous condition. The targeted compounds fluorinated pyrazole incorporated poly-hydroquinoline derivatives **8a–p** were synthesized by cyclo-condensation reaction of 3-methyl-5-substituted aryloxy-1-phenyl-1*H*-pyrazole-4-carbaldehydes **3a–d**, substituted enhy-drazinoketones **6a–e** and malononitrile **7** in absolute ethanol using piperidine as the basic catalyst by conventional and ultrasonic irradiation methods (Scheme 1 and Table 1).

A plausible mechanism for the reaction is outlined in Scheme 2. The reaction occurs *via in situ* initial formation of the heterylidenenitrile, containing the electron-poor C═C double

Fig. 2 Present study.
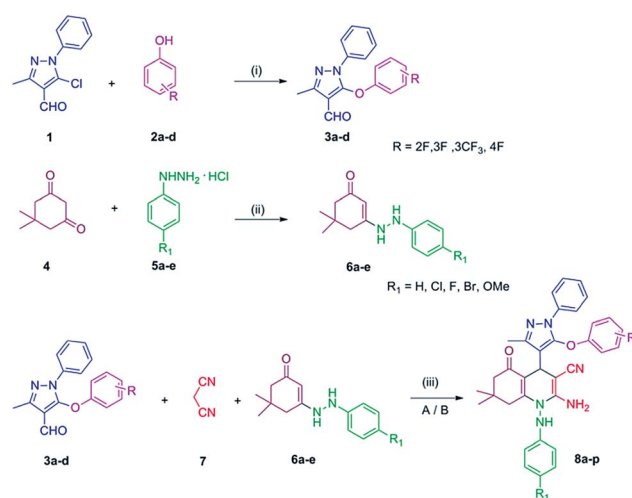
Scheme 1 Synthesis of 2-amino-7,7-dimethyl-4-(3-methyl-5-substituted aryloxy-1-phenyl-1*H*-pyrazol-4-yl)-5-oxo-1-(phenyl-amino)-1,4,5,6,7,8-hexahydro quinoline-3-carbonitrile **8a–p** (i) DMF, K_2CO_3, reflux 2 h. (ii) H_2O stirring RT, 6 h. (iii) (A) Ethanol, piperidine, reflux 1.5–2.5 h (iii) (B) ethanol, piperidine RT 15–30 min.

Table 1 Reaction parameters under conventional method and sonication (**8a–p**)

Entry	R	R_1	Conventional method		Ultrasonic method	
			Time (h)	Yield^a (%)	Time (min)	Yield^a (%)
8a	2-F	4-Br	2.5	79	25	88
8b	2-F	4-F	2.0	78	20	91
8c	2-F	4-Cl	2.5	64	30	79
8d	2-F	–H	2.5	76	20	87
8e	4-F	–H	1.5	73	15	84
8f	2-F	4-OMe	1.5	76	15	88
8g	3-F	–H	2.0	65	15	76
8h	3-CF$_3$	–H	2.5	73	20	85
8i	3-CF$_3$	4-OMe	2.0	76	15	83
8j	3-CF$_3$	4-Br	2.5	75	25	87
8k	3-CF$_3$	4-F	2.0	61	20	74
8l	3-F	4-OMe	1.5	71	20	81
8m	3-F	4-Br	2.0	77	20	86
8n	3-F	4-F	2.5	79	25	89
8o	4-F	4-Br	2.0	62	30	78
8p	4-F	4-F	1.5	68	20	80

^{a} Isolated.

bond, from the Knoevenagel condensation between 3-methyl-5-substituted aryloxy-1-phenyl-1*H*-pyrazole-4-carbaldehydes **3a–d** and malononitrile **7** by loss of water molecule, Michael addition of enhydrazinoketones **6a–e** to the ylidenic bond to form an acyclic intermediate which undergoes cyclization by nucleophilic attack of the –NH group on the electron deficient cyano carbon, followed by tautomerisation to the final products **8a–p**.

3. Pharmacology

3.1. *In vitro* antimicrobial activity

The antimicrobial activity of the newly synthesized compounds **8a–p** was carried out by broth micro dilution method according to National Committee for Clinical Laboratory Standards (NCCLS).[39] Antibacterial activity was screened against three Gram positive (*Bacillus subtilis* MTCC 441, *Clostridium tetani* MTCC 449, and *Streptococcus pneumoniae* MTCC 1936) and three Gram negative (*Salmonella typhi* MTCC 98, *Escherichia coli*

Ar$_1$ = 4-Substituted phenyl ring
Ar = 3-methyl-5-Substituted aryloxy-1-phenyl-1*H*-pyrazole-4-carbaldehydes

Scheme 2 Plausible mechanistic pathway for the synthesis of polyhydroquinoline.

MTCC 443, and *Vibrio cholerae* MTCC 3906) bacteria by using ampicillin, ciprofloxacin, norfloxacin and chloramphenicol as the standard antibacterial drugs. Antifungal activity was screened against two fungal species (*Aspergillus fumigatus* MTCC 3008 and *Candida albicans* MTCC 227) where griseofulvin and nystatin were used as the standard antifungal drugs. The strains employed for the activity were procured from the Institute of Microbial Technology, Chandigarh (MTCC-Micro Type Culture Collection). 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 result of antimicrobial screening data is shown in Table 2.

3.2. *In vitro* antituberculosis activity

A primary *in vitro* antituberculosis activity of the newly synthesized compounds **8a–p** was conducted at 250 μg mL^{-1} against *Mycobacterium tuberculosis* H37Rv strain by using Lowenstein–Jensen medium as described by Rattan.[40] The obtained results are presented in Table 3 in form of % inhibition. Isoniazid and rifampicin were used as the standard drugs.

Table 2 *In vitro* antimicrobial activity of polyhydroquinoline derivatives **8a–p** (MICs, μg mL^{-1})^a

	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
8a	200	500	250	200	250	500	1000	500
8b	500	500	125	125	500	250	1000	1000
8c	100	250	125	250	100	250	1000	500
8d	125	62.5	500	100	250	200	500	500
8e	500	200	200	250	250	250	500	1000
8f	125	250	250	250	200	100	>1000	500
8g	200	100	250	250	200	250	1000	500
8h	250	200	100	500	250	125	1000	1000
8i	62.5	250	125	200	100	100	250	100
8j	100	62.5	100	62.5	200	250	250	500
8k	125	100	500	250	250	250	1000	1000
8l	500	200	500	100	200	62.5	1000	100
8m	500	500	250	200	500	200	500	1000
8n	125	100	200	250	500	250	200	500
8o	200	200	250	200	100	250	1000	>1000
8p	250	500	250	100	500	200	1000	>1000
A	100	250	250	100	100	100	n. t.^{b}	n. t.
B	10	100	50	10	10	10	n. t.	n. t.
C	50	50	50	50	50	50	n. t.	n. t.
D	25	50	100	25	25	25	n. t.	n. t.
E	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.	100	100
F	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.	500	100

^{a} 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: chloramphenicol, D: ciprofloxacin. ^{b} n.t.: not tested.
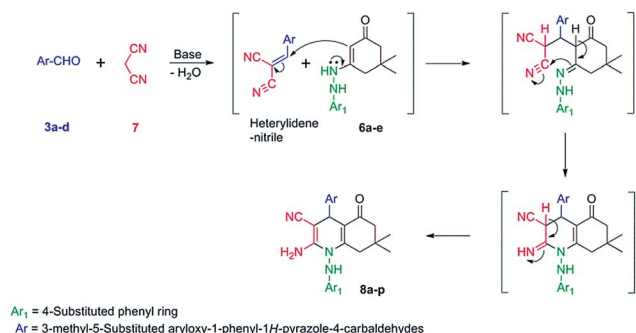
Table 3 *In vitro* antituberculosis activity (% inhibition) of poly-hydroquinoline derivatives **8a–p** against *M. tuberculosis* H37Rv (at concentration 250 μg mL^{-1})

Comp.	% Inhibition	Comp.	% Inhibition
8a	26	**8j**	21
8b	16	**8k**	**95**
8c	52	**8l**	**88**
8d	48	**8m**	**91**
8e	**94**	**8n**	20
8f	63	**8o**	36
8g	14	**8p**	**89**
8h	25	**Rifampicin**	98
8i	30	**Isoniazid**	99

3.3. *In vitro* antimalarial activity

In vitro antimalarial activity of the newly synthesized compounds **8a–p** against *P. falciparum* strain was performed using quinine and chloroquine as the reference compounds. The consequences of the antimalarial screening are expressed as the drug concentration resulting in 50% inhibition (IC$_{50}$) of parasite growth and are listed in Table 4.

4.   Results and discussion

4.1.   Optimization of reaction conditions for the target compounds

The reactions leading to the desired products were performed using conventional method (Path A in Scheme 1) as well as ultrasonic irradiation (Path B in Scheme 1). It was observed that, all the reactions by conventional route consumed very long time (1.5–2.5 h) for completion with relatively lower yields (61–79%). The same transformations could be successfully accomplished under ultrasound irradiation in comparatively shorter duration (15–30 min) with moderate to excellent yields (Table 1). The differences in yield and reaction time as compared to conventional method may be ascribed to cavitation in irradiated reaction mixture enhancing the mass transfer which allows chemical reactions to occur at the faster rate.[41] Thus ultrasonic irradiation method was found to be more advantageous for the synthesis of novel polyhydroquinoline derivatives bearing a fluorinated 5-substituted aryloxypyrazole nucleus.

Table 4 *In vitro* antimalarial activity of polyhydroquinoline derivatives **8a–p**

Comp.	IC$_{50}$ (μg mL^{-1})	Comp.	IC$_{50}$ (μg mL^{-1})
8a	0.58	**8j**	**0.073**
8b	0.95	**8k**	1.54
8c	**0.090**	**8l**	**0.067**
8d	1.45	**8m**	**0.088**
8e	1.24	**8n**	1.47
8f	1.56	**8o**	0.42
8g	2.10	**8p**	0.78
8h	0.59	**Chloroquine**	0.020
8i	**0.042**	**Quinine**	0.268

4.2.   Analytical results

The structures of the newly synthesized compounds were confirmed by ^{1}H NMR, FT-IR, mass spectrometry and elemental analysis. The IR spectrum of compounds **8a–p** exhibited characteristic absorption band in the range 1263–1224 cm^{-1}. This can be attributed to the presence of ether linkage. The carbonyl group stretching frequency was observed at 1665–1645 cm^{-1}. The absorption band in the range of 2188–2210 cm^{-1} observed for all the compounds are due to –C≡N stretching. The strong absorption band was also observed in the range of 1375–1364 cm^{-1} due to –CH$_3$ rocking. The characteristic absorption band in the range 3486–3321 cm^{-1} may be attributed to asymmetric & symmetric stretching of –NH$_2$. The ^{1}H NMR spectra of compounds **8a–p** exhibited the presence of the –CH proton (C$_4$–H of polyhydroquinoline ring) as a sharp singlet around δ 4.56–4.53 ppm and a broad singlet at δ 8.70–8.22 ppm arising due to –NH proton. Multiplets in the range between δ 7.53–5.78 ppm appeared for amine and aromatic protons. The mass spectrum of all the compounds showed molecular ion peak at (M$^+$) corresponding to their respective molecular weights, which confirmed the chemical structures.

4.3.   Biological section

4.3.1.   *In vitro* antibacterial activity. The antibacterial screening of the tested compounds **8a–p** showed moderate to excellent inhibitory activity (Table 2). It has been observed that against *S. pneumonia*, compound **8i** (R = 3-CF$_3$, R$_1$ = 4-OCH$_3$) was found to be more potent *i.e.* 62.5 μg mL^{-1} as compared to ampicillin *i.e.* 100 μg mL^{-1}. While compounds **8c** (R = 2-F, R$_1$ = 4-Cl) and **8j** (R = 3-CF$_3$, R$_1$ = 4-Br) showed comparable activity to that of ampicillin. Against *B. subtilis*, compounds **8d** (R = 2-F, R$_1$ = 4-H) and **8j** (R = 3-CF$_3$, R$_1$ = 4-Br) showed maximum potency *i.e.* 62.5 μg mL^{-1} as compared to ampicillin *i.e.* 250 μg mL^{-1} as well as norfloxacin *i.e.* 100 μg mL^{-1}. Compounds **8c** (R = 2-F, R$_1$ = 4-Cl), **8f** (R = 2-F, R$_1$ = 4-OCH$_3$), **8i** (R = 3-CF$_3$, R$_1$ = 4-OCH$_3$) MIC = 250 μg mL^{-1} showed equivalent potency to that of ampicillin. Compounds **8e** (R = 4-F, R$_1$ = 4-H), **8h** (R = 3-CF$_3$, R$_1$ = 4-H), **8l** (R = 3-F, R$_1$ = 4-OCH$_3$) and **8o** (R = 4-F, R$_1$ = 4-Br) *i.e.* 200 μg mL^{-1} were found to be more effective as compared to ampicillin MIC = 250 μg mL^{-1}. Against *C. tetani*, compounds **8a** (R = 2-F, R$_1$ = 4-Br), **8f** (R = 2-F, R$_1$ = 4-OCH$_3$), **8m** (R = 3-F, R$_1$ = 4-Br), **8o** (R = 4-F, R$_1$ = 4-Br), **8p** (R = 4-F, R$_1$ = 4-F), and **8g** (R = 3-F, R$_1$ = 4-H), MIC = 250 μg mL^{-1} showed same influence as that of ampicillin. Compounds **8h** (R = 3-CF$_3$, R$_1$ = 4-H), **8j** (R = 3-CF$_3$, R$_1$ = 4-Br) MIC = 100 μg mL^{-1} and compound **8b** (R = 2-F, R$_1$ = 4-F), **8c** (R = 2-F, R$_1$ = 4-Cl), **8i** (R = 3-CF$_3$, R$_1$ = 4-OCH$_3$) MIC = 125 μg mL^{-1} were found to more active than ampicillin MIC = 250 μg mL^{-1}.

In case of inhibiting Gram negative bacteria, compounds **8j** (R = 3-CF$_3$, R$_1$ = 4-Br) and **8l** (R = 3-F, R$_1$ = 4-OCH$_3$) MIC = 62.5 μg mL^{-1} were found to have higher potency against *E. coli* and *V. cholera* respectively as compared to ampicillin. Compounds **8c** (R = 2-F, R1 = 4-Cl), **8i** (R = 3-CF$_3$, R$_1$ = 4-OCH$_3$), **8o** (R = 4-F, R$_1$ = 4-Br) and compounds **8f** (R = 2-F, R$_1$ = 4-OCH$_3$), **8i** (R = 3-CF$_3$, R$_1$ = 4-OCH$_3$) also exhibited the similar

Table 5 The percentage viability of different chemically synthesized compounds was listed in table

Conc.	8C	8M	8K	8L	8I	8J	8E	DMSO	Untreated cell
—	—	—	—	—	—	—	—	97	98
2.5	94	95	76	78	82	66	85		
5	90	91	74	74	80	62	76		
7.5	84	90	72	64	76	60	73		
10	78	88	63	51	71	58	70		
12.5	75	85	57	55	64	56	63		

activity as that of ampicillin *i.e.* 100 μg mL^{-1} respectively against *S. typhi* and *V. cholera* (Table 2).

4.3.2. *In vitro* antifungal activity. The antifungal screening data from Table 2 revealed that against *C. albicans*, compound **8n** (R = 3-F, R$_1$ = 4-F) *i.e.* 200 μg mL^{-1}, compounds **8i** (R = 3-CF$_3$, R$_1$ = 4-OCH$_3$) and **8j** (R = 3-CF$_3$, R$_1$ = 4-Br) *i.e.* 250 μg mL^{-1} were found to have significant activity as compared to griseo-fulvin. Compounds **8i** (R = 3-CF$_3$, R$_1$ = 4-OCH$_3$) and **8l** (R = 3-F, R$_1$ = 4-OCH$_3$) exhibited equivalent potency *i.e.* 100 μg mL^{-1} against *A. fumigates*. From the above results, it can be concluded that compound **8j** showed the potential to become new class of antimicrobial agent in future.

4.3.3. *In vitro* antituberculosis activity. Antituberculosis screening of the novel compounds **8a–p** was conducted at 250 μg mL^{-1} concentrations against *Mycobacterium tuberculosis* H37Rv strain. Compounds **8e** (R = 4-F, R$_1$ = 4-H), **8k** (R = 3-CF$_3$, R$_1$ = 4-F) and **8m** (R = 3-F, R$_1$ = 4-Br) were found to possess brilliant activity (*i.e.* 94%, 95% and 91% at 250 μg mL^{-1}) against *M. tuberculosis* H37Rv. The compounds **8l** (R = 3-F, R$_1$ = 4-OCH$_3$) and **8p** (R = 4-F, R$_1$ = 4 F) are moderately active and remaining all other compounds showed poor inhibition against *M. tuberculosis* H37Rv growth (Table 3).

4.3.4. *In vitro* antimalarial activity. The newly synthesized compounds **8a–p** were evaluated for their antimalarial screening against chloroquine and quinine sensitive strain of *P. falciparum*. All the experiments were performed in duplicate and a mean value of IC$_{50}$ is mentioned in Table 4. The compounds **8c** (R = 2-F, R$_1$ = 4-Cl), **8i** (R = 3-CF$_3$, R$_1$ = 4-OCH$_3$), **8j** (R = 3-CF$_3$, R$_1$ = 4-Br), **8l** (R = 3-F, R$_1$ = 4-OCH$_3$) and **8m** (R = 3-F, R$_1$ = 4-Br) were found to have IC$_{50}$ in the range of 0.042 to 0.090 upon *P. falciparum* strain. Above compounds displayed fabulous activity against *P. falciparum* strain as compared to quinine IC$_{50}$ 0.268.
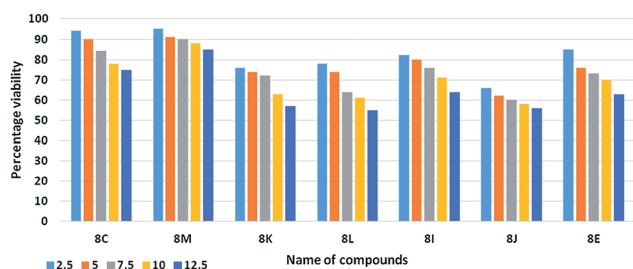

Fig. 3 Effect of synthesized compounds on viability of *S. Pombe* at different concentrations.

4.3.5. Cytotoxicity. Cytotoxicity of synthesized compounds was tested using bioassay of *S. pombe* cells at the cellular level. From the result, cell death caused by toxicity of the synthesized compounds could be easily monitored by vital staining (Table 5). The toxicity was found to vary with the type of substituent present and concentrations of the synthesized compounds. It has been observed that cytotoxicity was found to increase with concentration of drugs. Compounds **8i**, **8j**, **8k** and **8l** were found to have maximum toxicity, while compounds **8e**, **8m**, **8c** were found to be less cytotoxic (Fig. 3). After 17 h of the treatment, many of the *S. pombe* cells were killed due to toxic nature of the compound.

4.4. Structure–activity relationship (SAR)

The results of the biological screening showed that the activity was significantly affected by introducing fluorinated 5-substituted aryloxypyrazole at C-4 position and phenylamino nucleus at N-1 position on polyhydroquinoline scaffold (Fig. 4).

We perceived that the methoxy group existing at R$_1$ position in N-phenyl moiety at N-1 in polyhydroquinoline and –CF$_3$ group at *meta* position of aryloxy ring in pyrazole nucleus exhibited excellent antimalarial activity against *P. falciparum* strain as compared to quinine as well as improved antibacterial activity against *S. pneumonia*. Without any substitution at R$_1$ position and fluoro group at *ortho* position of aryloxy ring revealed highest activity against *B. subtilis.* The fluoro group prevailing at R$_1$ positions and –CF$_3$ group at *meta* position of aryloxy ring displayed superior antituberculosis activity against *M. tuberculosis* H37Rv. The replacement of electron donating methoxy group with electron withdrawing chloro groups at R$_1$ position increased antimalarial activity. The bromo group present at R$_1$ position and –CF$_3$ group at *meta* position in aryloxy ring exhibited brilliant activity against *B. subtilis* and improved antimalarial activity. The bromo, fluoro and methoxy groups present at R$_1$ position and –CF$_3$ and –F groups at *meta*
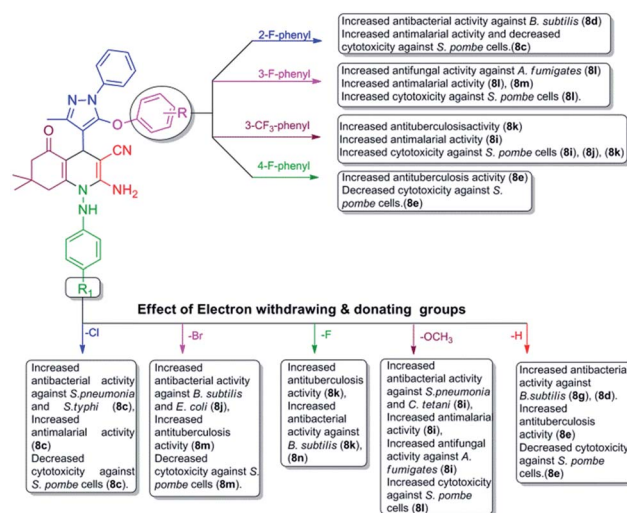

Fig. 4 Structure–activity relationships for antimicrobial, antituberculosis, antimalarial and cytotoxicity activity of the synthesized compounds **8a–p**.

position in aryloxy ring demonstrate higher cytotoxicity against *S. pombe* cells at a cellular level. But the chloro and bromo groups existing at R$_1$ positions and –F group at *ortho* and *meta* positions of aryloxy ring displayed inferior cytotoxicity against *S. pombe* cells at a cellular level. It can be concluded that electron donating and withdrawing groups at R$_1$ position and –CF$_3$ group at *meta* position of aryloxy ring are observed to be chiefly responsible for deviation in biological potency.

5. Conclusion

We designed and synthesized some novel polyhydroquinoline derivatives bearing a fluorinated 5-substituted aryloxypyrazole nucleus advantageously using ultrasonic irradiation and examined their antimicrobial, antimalarial and antituberculosis activities. The results indicated that majority of the compounds were found to be most active against *B. subtilis* and *C. tetani*. Amongst the tested compounds, **8e**, **8k**, and **8m** showed prominent antituberculosis activities. The Compounds **8c**, **8i**, **8l** and **8m** displayed superior antimalarial activity. Finally compound **8i** could be recognized as the most biologically active member within the synthesized series showing an interesting dual antimalarial and antibacterial profile. The SAR results revealed that the presence of electron donating and withdrawing groups at R$_1$ position and –CF$_3$ group at *meta* position of aryloxy ring played effective role in boasting the prepared polyhydroquinoline derivatives as potent antimicrobial, antimalarial and antitubercular agents. The chloro and bromo groups existing at R$_1$ positions and –F group at *ortho* and *meta* positions of aryloxy ring displayed inferior cytotoxicity against *S. pombe* cells at a cellular level. Consequently, polyhydroquinoline scaffold represents a class that needs further investigation with the hope of discovering new antimicrobial and antimalarial agents.

6. Experimental section

6.1. Chemistry

All the reagents were obtained commercially and used without further purification. Solvents used were of analytical grade. Melting points (°C, uncorrected) were determined in open capillaries on μThermoCal$_{10}$ melting point apparatus (Analab Scientific Pvt. Ltd, India). Precoated silica gel plates (silica gel 0.25 mm, 60 G F 254; Merck, Germany) were used for thin layer chromatography. Electron impact Mass Spectra were recorded on Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan) purchased under PURSE programme of DST at Sardar Patel University, Vallabh Vidyanagar, India. 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 performed on Perkin-Elmer 2400 series-II elemental analyzer (Perkin-Elmer, USA) at Sophisticated Instrumentation Centre for Applied Research & Training (SICART), Vallabh Vidyanagar, India. All the compounds were found to be within ±0.4% of their theoretical values. The reaction mixtures were irradiated by ultrasound at room temperature in a D-compact ultrasonic cleaner with a frequency of 30 kHz and an output power of 250 W. The reaction flask was kept at the maximum energy area in the cleaner and the level of the reactants was kept slightly lower than the level of water in the bath. ^{1}H NMR spectra (in DMSO-d_6) were recorded on Bruker Avance 400F (MHz) NMR Spectrometer at 400 MHz using TMS as the internal standard.

6.1.1. General procedure for the synthesis of 3-methyl-5-substituted aryloxy-1-phenyl-1*H*-pyrazole-4-carbaldehydes (3a–d). 5-Chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde 1 (1 mmol), substituted phenols **2a–d** (1 mmol) and anhydrous potassium carbonate (2 mmol) in dimethylformamide (10 mL) were charged in a 100 mL round bottom flask equipped with a mechanical stirrer and a condenser. The reaction mixture was heated at 90 °C for 2 h and the progress of the reaction was monitored by TLC. After the completion of reaction confirmed by the TLC, the reaction mixture was poured in to 100 mL ice-water and filtered, washed thoroughly with water, dried and recrystallized from hot ethanol (10 mL) to obtain a white solid.

6.1.1.1 5-(2-Fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (3a). Yield 85%; m.p. 225–227 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.46 (s, 3H, –CH$_3$), 7.13–7.64 (m, 9H, Ar–H), 9.56 (s, 1H, –CHO).

6.1.1.2 5-(3-Fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (3b). Yield 78%; m.p. 210–212 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.47 (s, 3H, –CH$_3$), 6.92–7.63 (m, 9H, Ar–H), 9.61 (s, 1H, –CHO).

6.1.1.3 5-(4-Fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (3c). Yield 82%; m.p. 245–247 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.46 (s, 3H, –CH$_3$), 7.17–7.64 (m, 9H, Ar–H), 9.54 (s, 1H, –CHO).

6.1.2. General procedure for the synthesis of substituted 5,5-dimethyl-3-(2-phenylhydrazinyl)-cyclohex-2-enones (6a–e). 1,3-Dimedone **4** (10 mmol), substituted phenyl hydrazine hydrochlorides **5a–e** (10 mmol) and water (10 mL) were charged in a 100 mL round bottom flask equipped with a mechanical stirrer. The reaction mixture was stirred at room temperature for 6 h. After the completion of reaction (checked by TLC), the separated substituted enhydrazinoketones **6a–e** were filtered and washed with water to obtain the pure solid product.

6.1.3. General procedure for the conventional synthesis of 2-amino-7,7-dimethyl-4-(3-methyl-5-substituted aryloxy-1-phenyl-1*H*-pyrazol-4-yl)-5-oxo-1-(phenylamino)-1,4,5,6,7,8-hexa hydro quinoline-3-carbonitriles (8a–p). A 100 mL round bottomed flask was charged with a mixture of 3-methyl-5-substituted aryloxy-1-phenyl-1H-pyrazole-4-carbaldehydes **3a–d** (1 mmol), malononitrile **7** (1 mmol), substituted enhydrazinoketones **6a–e** (1 mmol), and catalytic amount of piperidine (2–3 drops) in ethanol (10 mL). The reaction mixture was refluxed for an appropriate time period till the completion of the reaction as indicated by TLC (Table 1). After the completion of reaction, the reaction mixture was stirred magnetically for further 10 min. After cooling the separated solid mass was collected by filtration, washed well with ethanol (10 mL) and crystallized from hot ethanol (10 mL).

6.1.3. General procedure for the synthesis of 2-amino-7,7-dimethyl-4-(3-methyl-5-substituted aryloxy-1-phenyl-1*H*-pyrazol-

4-yl)-5-oxo-1-(phenylamino)-1,4,5,6,7,8-hexahydro quinoline-3-carbonitriles (8a–p) using sonochemical method. A 100 mL round bottomed flask was charged with a mixture of 3-methyl-5-aryloxy-1-phenyl-1H-pyrazole-4-carbaldehydes **3a–d** (1 mmol), malononitrile **7** (1 mmol), substituted enhydrazinoketones **6a–e** (1 mmol) and catalytic amount of piperidine (2–3 drops) in ethanol (10 mL). The reaction flask was located in the ultrasonic bath so as to keep the level of reactants slightly lower than the level of water in bath. The reaction mixture was sonicated at room temperature for an appropriate time period till the completion of the reaction as indicated by TLC (Table 1). After the completion of reaction, the reaction mixture was stirred magnetically for further 10 min. The separated solid mass was collected by filtration, washed well with cold ethanol (10 mL) and crystallized from hot ethanol (10 mL). The physicochemical and spectroscopic characterization data of the synthesized compounds **8a–p** are given below.

6.1.3.1. 2-Amino-1-((4-bromophenyl)amino)-4-(5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (8a). Yield 88%; m.p. 215–217 °C; IR (KBr, ν_{max}, cm^{-1}): 3478 & 3336 (asym. & sym. str. of −NH$_2$), 2188 (C≡N str.), 1650 (C=O str.), 1368 (−CH$_3$ rocking), 1260 (C−O−C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.85 (s, 3H, −CH$_3$), 0.91 (s, 3H, −CH$_3$), 1.68–2.20 (m, 4H, 2 × −CH$_2$), 2.31 (s, 3H, −CH$_3$), 4.56 (s, 1H, −CH), 5.98–7.50 (m, 15H, Ar−H and −NH$_2$), 8.62 (s, 1H, −NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.93 (pyrazole−CH$_3$), 26.68, 28.46 (C(CH$_3$)$_2$), 32.31 (C$_4$), 37.65 (CH$_2$), 49.21 (CH$_2$−CO), 56.33 (C−CN), 109.98, 110.84, 111.47, 114.13, 117.40, 120.69, 120.77, 121.86, 122.14, 123.59, 125.10, 126.90, 127.15, 130.78, 131.85, 132.81, 134.55, 134.70, 135.07, 138.15, 142.86, 144.08, 152.97, 154.16, 161.18 (25C, Ar−C), 195.07 (C=O); ESI-MS (m/z): 653.1 (M$^+$), 655.1 (M + 2); anal. calcd (%) for C$_{34}$H$_{30}$BrFN$_6$O$_2$: C, 62.48; H, 4.63; N, 12.86. Found: C, 62.24; H, 4.40; N, 12.63.

6.1.3.2. 2-Amino-4-(5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-((4-fluorophenyl)amino)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (8b). Yield 91%; m.p. 202–204 °C; IR (KBr, ν_{max}, cm^{-1}): 3472 & 3340 (asym. & sym. str. of −NH$_2$), 2198 (C≡N str.), 1645 (C=O str.), 1375 (−CH$_3$ rocking), 1231 (C−O−C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (s, 3H, −CH$_3$), 0.89 (s, 3H, −CH$_3$), 1.69–2.02 (m, 4H, 2 × −CH$_2$), 2.31 (s, 3H, −CH$_3$), 4.56 (s, 1H, −CH), 597–7.50 (m, 15H, Ar−H and −NH$_2$), 8.46 (s, 1H, −NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.57 (pyrazole−CH$_3$), 26.35, 28.03 (C(CH$_3$)$_2$), 31.92 (C$_4$), 37.65 (CH$_2$), 49.35 (CH$_2$−CO), 56.67 (C−CN), 109.75, 109.93, 113.93, 114.38, 121.54, 121.88, 122.22, 129.65, 137.99, 138.17, 142.05, 143.51, 143.90, 144.03, 144.34, 144.61, 147.76, 150.16, 151.20, 152.59, 152.72, 152.88, 152.24, 154.18, 161.25 (25C, Ar−C), 195.04 (C=O); ESI-MS (m/z): 593.2 (M$^+$); anal. calcd (%) for C$_{34}$H$_{30}$F$_2$N$_6$O$_2$: C, 68.91; H, 5.10; N, 14.18. Found: C, 68.68; H, 4.83; N, 13.97.

6.1.3.3. 2-Amino-1-((4-chlorophenyl)amino)-4-(5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (8c). Yield 79%; m.p. 218–220 °C; IR (KBr, ν_{max}, cm^{-1}): 3439 & 3334 (asym. & sym. str. of −NH$_2$), 2210 (C≡N str.), 1660 (C=O str.), 1371 (−CH$_3$ rocking), 1224 (C−O−C ether str.); 760 (C−Cl stretching); ^{1}H NMR (400 MHz, DMSO-d_6): ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.85 (s, 3H, −CH$_3$), 0.91 (s, 3H, −CH$_3$), 1.68–2.20 (m, 4H, 2 × −CH$_2$), 2.31 (s, 3H, −CH$_3$), 4.56 (s, 1H, −CH),

5.99–7.50 (m, 15H, Ar−H and −NH$_2$), 8.61 (s, 1H, −NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.54 (pyrazole−CH$_3$), 28.08, 28.55 (C(CH$_3$)$_2$), 31.96 (C$_4$), 37.72 (CH$_2$), 49.82 (CH$_2$−CO), 56.74 (C−CN), 109.95, 109.97, 113.86, 114.26, 121.57, 121.85, 122.19, 124.14, 129.71, 138.01, 138.26, 143.93, 144.49, 145.15, 146.10, 147.77, 147.82, 147.93, 150.18, 152.25, 152.48, 152.79, 153.10, 154.15, 161.22 (25C, Ar−C), 195.07 (C=O); ESI-MS (m/z): 610.1 (M$^+$); anal. calcd (%) for C$_{34}$H$_{30}$ClFN$_6$O$_2$: C, 67.04; H, 4.96; N, 13.80. Found: C, 66.79; H, 4.75; N, 13.53.

6.1.3.4. 2-Amino-4-(5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-7,7-dimethyl-5-oxo-1-(phenylamino)-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (8d). Yield 87%; m.p. 196–198 °C; IR (KBr, ν_{max}, cm^{-1}): 3431 & 3321 (asym. & sym. str. of −NH$_2$), 2188 (C≡N str.), 1658 (C=O str.), 1369 (−CH$_3$ rocking), 1232 (C−O−C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (s, 3H, −CH$_3$), 0.90 (s, 3H, −CH$_3$), 1.74–2.20 (m, 4H, 2 × −CH$_2$), 2.31 (s, 3H, −CH$_3$), 4.56 (s, 1H, −CH), 5.95–7.51 (m, 16H, Ar−H and −NH$_2$), 8.49 (s, 1H, −NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.62 (pyrazole−CH$_3$), 28.28, 28.49 (C(CH$_3$)$_2$), 31.95 (C$_4$), 38.01 (CH$_2$), 49.64 (CH$_2$−CO), 56.74 (C−CN), 109.78, 111.73, 112.13, 116.45, 117.58, 120.37, 120.73, 121.56, 121.86, 122.05, 122.17, 124.34, 125.32, 127.04, 129.55, 129.70, 129.85, 130.32, 144.39, 144.73, 147.05, 147.88, 152.74, 153.36, 154.09 (25C, Ar−C), 195.07 (C=O); ESI-MS (m/z): 575.2 (M$^+$); anal. calcd (%) for C$_{34}$H$_{31}$FN$_6$O$_2$: C, 71.06; H, 5.44; N, 14.62. Found: C, 70.78; H, 5.21; N, 14.36.

6.1.3.5. 2-Amino-4-(5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-7,7-dimethyl-5-oxo-1-(phenylamino)-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (8e). Yield 84%; m.p. 192–194 °C; IR (KBr, ν_{max}, cm^{-1}): 3452 & 3332 (asym. & sym. str. of −NH$_2$), 2210 (C≡N str.), 1646 (C=O str.), 1350 (−CH$_3$ rocking), 1232 (C−O−C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (s, 3H, −CH$_3$), 0.88 (s, 3H, −CH$_3$), 1.73–2.19 (m, 4H, 2 × −CH$_2$), 2.40 (s, 3H, −CH$_3$), 4.55 (s, 1H, −CH), 5.84–7.53 (m, 16H, Ar−H and −NH$_2$), 8.55 (s, 1H, −NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.62 (pyrazole−CH$_3$), 28.13, 28.37 (C(CH$_3$)$_2$), 32.11 (C$_4$), 37.79 (CH$_2$), 49.52 (CH$_2$−CO), 56.33 (C−CN), 109.55, 111.99, 112.14, 113.93, 116.91, 117.10, 120.51, 121.42, 121.93, 122.08, 126.83, 126.95, 129.57, 129.68, 129.91, 138.08, 145.08, 145.40, 147.06, 147.81, 152.57, 152.77, 153.32, 154.21, 159.55 (25C, Ar−C), 195.07 (C=O); ESI-MS (m/z): 575.1 (M$^+$); anal. calcd (%) for C$_{34}$H$_{31}$FN$_6$O$_2$: C, 71.06; H, 5.44; N, 14.62. Found: C, 70.85; H, 5.15; N, 14.36.

6.1.3.6. 2-Amino-4-(5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-((4-methoxyphenyl)amino)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (8f). Yield 88%; m.p. 240–242 °C; IR (KBr, ν_{max}, cm^{-1}): 3470 & 3339 (asym. & sym. str. of −NH$_2$), 2192 (C≡N str.), 1655 (C=O str.), 1362 (−CH$_3$ rocking), 1262 (C−O−C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.84 (s, 3H, −CH$_3$), 0.91 (s, 3H, −CH$_3$), 1.76–2.20 (m, 4H, 2 × −CH$_2$), 2.38 (s, 3H, −CH$_3$), 3.69 (s, 3H, −OCH$_3$), 4.55 (s, 1H, −CH), 5.91–7.51 (m, 15H, Ar−H and −NH$_2$), 8.22 (s, 1H, −NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.94 (pyrazole−CH$_3$), 28.19, 28.48 (C(CH$_3$)$_2$), 32.57 (C$_4$), 36.31 (CH$_2$), 49.20 (CH$_2$−CO), 51.04 (Ar−C−OCH$_3$), 55.97 (C−CN), 104.97, 106.41, 110.22, 111.14, 114.19, 117.20, 117.86, 121.71, 124.76, 125.53, 125.92, 126.85, 127.53, 129.80, 131.89, 137.89, 143.76, 145.68, 147.70, 148.46,

149.77, 152.22, 155.71, 157.23, 161.20 (25C, Ar–C), 195.05 (C=O); ESI-MS (m/z): 605.1 (M$^+$); anal. calcd (%) for $C_{35}H_{33}FN_6O_3$: C, 69.52; H, 5.50; N, 13.90. Found: C, 69.29; H, 5.26; N, 13.62.

6.1.3.7.   2-Amino-4-(5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-7,7-dimethyl-5-oxo-1-(phenylamino)-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (8g). Yield 76%; m.p. 195–197 °C; IR (KBr, ν_{max}, cm^{-1}): 3471 & 3340 (asym. & sym. str. of –NH$_2$), 2191 (C≡N str.), 1656 (C=O str.), 1372 (–CH$_3$ rocking), 1263 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.82 (s, 3H, –CH$_3$), 0.88 (s, 3H, –CH$_3$), 1.68–2.18 (m, 4H, 2 × –CH$_2$), 2.28 (s, 3H, –CH$_3$), 4.55 (s, 1H, –CH), 5.87–7.53 (m, 16H, Ar–H and –NH$_2$), 8.56 (s, 1H, –NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.58 (pyrazole–CH$_3$), 28.44, 29.08 (C(CH$_3$)$_2$), 32.11 (C$_4$), 37.80 (CH$_2$), 49.48 (CH$_2$–CO), 56.80 (C–CN), 109.42, 109.75, 110.48, 111.46, 114.14, 120.67, 121.52, 121.91, 126.99, 129.62, 129.73, 129.89, 131.57, 138.15, 139.27, 144.31, 144.66, 147.03, 147.79, 152.60, 153.44, 154.27, 154.72, 157.64, 161.89 (25C, Ar–C), 194.92 (C=O); ESI-MS (m/z): 575.2 (M$^+$); anal. calcd (%) for $C_{34}H_{31}FN_6O_2$: C, 71.06; H, 5.44; N, 14.62. Found: C, 70.81; H, 5.21; N, 14.33.

6.1.3.8.   2-Amino-7,7-dimethyl-4-(3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)-5-oxo-1-(phenylamino)-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (8h). Yield 85%; m.p. 201–203 °C; IR (KBr, ν_{max}, cm^{-1}): 3481 & 3333 (asym. & sym. str. of –NH$_2$), 2197 (C≡N str.), 1665 (C=O str.), 1371 (–CH$_3$ rocking), 1259 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.80 (s, 3H, –CH$_3$), 0.86 (s, 3H, –CH$_3$), 1.62–2.21 (m, 4H, 2 × –CH$_2$), 2.34 (s, 3H, –CH$_3$), 4.56 (s, 1H, –CH), 5.84–7.52 (m, 16H, Ar–H and –NH$_2$), 8.56 (s, 1H, –NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.58 (pyrazole–CH$_3$), 27.97, 28.25 (C(CH$_3$)$_2$), 32.10 (C$_4$), 37.78 (CH$_2$), 49.48 (CH$_2$–CO), 56.79 (C–CN), 109.37, 111.95, 112.77, 113.85, 114.06, 119.38, 120.70, 121.73, 122.23, 127.17, 129.62, 129.92, 131.51, 131.69, 137.91, 138.05, 144.11, 146.99, 147.89, 152.60, 152.72, 152.95, 153.42, 154.32, 156.69, 156.77 (26C, Ar–C), 194.84 (C=O); ESI-MS (m/z): 625.1 (M$^+$); anal. calcd (%) for $C_{35}H_{31}F_3N_6O_2$: C, 67.30; H, 5.00; N, 13.45. Found: C, 67.06; H, 4.73; N, 13.18.

6.1.3.9.   2-Amino-1-((4-methoxyphenyl)amino)-7,7-dimethyl-4-(3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (8i). Yield 83%; m.p. 245–247 °C; IR (KBr, ν_{max}, cm^{-1}): 3472 & 3338 (asym. & sym. str. of –NH$_2$), 2197 (C≡N str.), 1664 (C=O str.), 1367 (–CH$_3$ rocking), 1254 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.80 (s, 3H, –CH$_3$), 0.86 (s, 3H, –CH$_3$), 1.63–2.23 (m, 4H, 2 × –CH$_2$), 2.41 (s, 3H, –CH$_3$), 3.73 (s, 3H, –OCH$_3$), 4.53 (s, 1H, –CH), 5.78–7.52 (m, 15H, Ar–H and –NH$_2$), 8.28 (s, 1H, –NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.60 (pyrazole–CH$_3$), 28.06, 28.25 (C(CH$_3$)$_2$), 32.17 (C$_4$), 37.79 (CH$_2$), 49.51 (CH$_2$–CO), 55.79 (Ar–C–OCH$_3$), 56.73 (C–CN), 109.27, 109.66, 113.17, 113.93, 114.16, 115.00, 115.24, 119.60, 121.72, 122.30, 127.16, 129.62, 129.91, 131.71, 137.91, 138.07, 140.50, 140.63, 144.50, 147.59, 153.11, 153.55, 153.05, 154.28, 154.47, 156.72 (26C, Ar–C), 195.11 (C=O); ESI-MS (m/z): 655.1 (M$^+$); anal. calcd (%) for $C_{36}H_{33}F_3N_6O_3$: C, 66.05; H, 5.08; N, 12.84. Found: C, 65.82; H, 4.81; N, 12.63.

6.1.3.10.   2-Amino-1-((4-bromophenyl)amino)-7,7-dimethyl-4-(3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (8j). Yield 87%; m.p. 232–234 °C; IR (KBr, ν_{max}, cm^{-1}): 3482 & 3338 (asym. & sym. str. of –NH$_2$), 2196 (C≡N str.), 1663 (C=O str.), 1366 (–CH$_3$ rocking), 1257 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.81 (s, 3H, –CH$_3$), 0.87 (s, 3H, –CH$_3$), 1.58–1.99 (m, 4H, 2 × –CH$_2$), 2.33 (s, 3H, –CH$_3$), 4.55 (s, 1H, –CH), 5.90–7.54 (m, 15H, Ar–H and –NH$_2$), 8.70 (s, 1H, –NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.52 (pyrazole–CH$_3$), 27.20, 28.13 (C(CH$_3$)$_2$), 32.12 (C$_4$), 37.74 (CH$_2$), 49.44 (CH$_2$–CO), 56.82 (C–CN), 109.57, 109.84, 111.71, 113.85, 113.95, 114.46, 119.70, 121.75, 122.28, 127.13, 129.59, 131.70, 132.38, 132.52, 137.88, 138.03, 144.26, 146.39, 147.87, 152.38, 152.71, 153.08, 153.45, 154.96, 156.64, 156.74 (26C, Ar–C), 194.87 (C=O); ESI-MS (m/z): 703.1 (M$^+$), 705.1 (M + 2); anal. calcd (%) for: $C_{35}H_{30}BrF_3N_6O_2$: C, 59.75; H, 4.30; N, 11.95. Found: C, 59.49; H, 4.05; N, 11.67.

6.1.3.11.   2-Amino-1-((4-fluorophenyl)amino)-7,7-dimethyl-4-(3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (8k). Yield 74%; m.p. 224–226 °C; IR (KBr, ν_{max}, cm^{-1}): 3486 & 3338 (asym. & sym. str. of –NH$_2$), 2193 (C≡N str.), 1662 (C=O str.), 1370 (–CH$_3$ rocking), 1263 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (s, 3H, –CH$_3$), 0.89 (s, 3H, –CH$_3$), 1.61–2.21 (m, 4H, 2 × –CH$_2$), 2.33 (s, 3H, –CH$_3$), 4.54 (s, 1H, –CH), 5.87–7.53 (m, 15H, Ar–H and –NH$_2$), 8.54 (s, 1H, –NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.63 (pyrazole–CH$_3$), 28.08, 28.23 (C(CH$_3$)$_2$), 32.09 (C$_4$), 37.88 (CH$_2$), 49.48 (CH$_2$–CO), 56.75 (C–CN), 109.50, 109.88, 114.04, 116.43, 119.26, 121.71, 122.21, 127.16, 129.58, 129.73, 131.47, 131.71, 138.08, 143.51, 144.10, 144.48, 147.96, 152.50, 152.88, 153.27, 153.71, 153.89, 156.12, 156.38, 156.47, 156.81 (26C, Ar–C), 195.07 (C=O); ESI-MS (m/z): 643.1 (M$^+$); anal. calcd (%) for: $C_{35}H_{30}F_4N_6O_2$: C, 65.41; H, 4.71; N, 13.08. Found: C, 65.17; H, 4.42; N, 12.85.

6.1.3.12.   2-Amino-4-(5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-((4-methoxyphenyl)amino)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (8l). Yield 81%; m.p. 248–250 °C; IR (KBr, ν_{max}, cm^{-1}): 3477 & 3334 (asym. & sym. str. of –NH$_2$), 2185 (C≡N str.), 1658 (C=O str.), 1365 (–CH$_3$ rocking), 1259 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.81 (s, 3H, –CH$_3$), 0.86 (s, 3H, –CH$_3$), 1.63–2.22 (m, 4H, 2 × –CH$_2$), 2.33 (s, 3H, –CH$_3$), 3.74 (s, 3H, –OCH$_3$), 4.54 (s, 1H, –CH), 5.79–7.52 (m, 15H, Ar–H and –NH$_2$), 8.37 (s, 1H, –NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.82 (pyrazole–CH$_3$), 26.78, 28.41 (C(CH$_3$)$_2$), 32.45 (C$_4$), 36.24 (CH$_2$), 49.43 (CH$_2$–CO), 51.52 (Ar–C–OCH$_3$), 56.39 (C–CN), 105.13, 106.18, 111.67, 114.53, 116.73, 117.97, 120.78, 121.86, 122.74, 123.89, 125.50, 126.70, 127.75, 130.88, 131.89, 132.71, 134.85, 134.95, 135.15, 138.75, 142.96, 144.58, 157.77, 160.93, 164.37 (25C, Ar–C), 194.47 (C=O); ESI-MS (m/z): 605.2 (M$^+$); anal. calcd (%) for: $C_{35}H_{33}FN_6O_3$: C, 69.52; H, 5.50; N, 13.90. Found: C, 69.29; H, 5.23; N, 13.62.

6.1.3.13.   2-Amino-1-((4-bromophenyl)amino)-4-(5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (8m). Yield 86%; m.p. 228–230 °C; IR (KBr, ν_{max}, cm^{-1}): 3475 & 3339 (asym. & sym. str. of –NH$_2$), 2190 (C≡N str.), 1665 (C=O str.), 1371 (–CH$_3$ rocking),

1258 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (s, 3H, CH$_3$), 0.89 (s, 3H, CH$_3$), 1.40–2.18 (m, 4H, 2 × CH$_2$), 2.33 (s, 3H, CH$_3$), 4.54 (s, 1H, CH), 6.45–7.72 (m, 15H, Ar–H and NH$_2$), 8.70 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.58 (pyrazole–CH$_3$), 28.40, 28.29 (C(CH$_3$)$_2$), 32.80 (C$_4$), 37.95 (CH$_2$), 49.51 (CH$_2$–CO), 56.33 (C–CN), 109.88, 110.28, 111.45, 111.76, 114.13, 117.40, 120.75, 121.76, 122.24, 123.49, 125.19, 126.93, 127.17, 130.88, 131.75, 132.71, 134.65, 134.79, 135.17, 138.35, 142.76, 144.88, 152.87, 154.26, 161.38 (25C, Ar–C), 195.07 (C=O); ESI-MS (m/z): 653.1 (M$^+$), 655.1 (M + 2); anal. calcd (%) for: C$_{34}$H$_{30}$BrFN$_6$O$_2$: C, 62.48; H, 4.63; N, 12.86. Found: C, 62.24; H, 4.36; N, 12.63.

6.1.3.14. 2-Amino-4-(5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-((4-fluorophenyl)amino)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (8n). Yield 89%; m.p. 204–206 °C; IR (KBr, ν_{max}, cm^{-1}): 3483 & 3340 (asym. & sym. str. of –NH$_2$), 2195 (C≡N str.), 1662 (C=O str.), 1364 (–CH$_3$ rocking), 1257 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (s, 3H, –CH$_3$), 0.90 (s, 3H, –CH$_3$), 1.36–2.19 (m, 4H, 2 × –CH$_2$), 2.33 (s, 3H, –CH$_3$), 4.54 (s, 1H, –CH), 5.90–7.53 (m, 15H, Ar–H and –NH$_2$), 8.53 (s, 1H, –NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.55 (pyrazole–CH$_3$), 28.01, 28.36 (C(CH$_3$)$_2$), 32.13 (C$_4$), 37.80 (CH$_2$), 49.48 (CH$_2$–CO), 56.83 (C–CN), 109.55, 109.91, 111.44, 113.37, 113.67, 113.92, 114.13, 116.34, 116.56, 121.53, 121.87, 122.06, 126.98, 129.60, 129.72, 131.56, 138.15, 143.51, 147.59, 152.65, 153.64, 154.77, 154.82, 157.54, 161.79 (25C, Ar–C), 194.92 (C=O); ESI-MS (m/z): 593.2 (M$^+$); anal. calcd (%) for: C$_{34}$H$_{30}$F$_2$N$_6$O$_2$: C, 68.91; H, 5.10; N, 14.18. Found: C, 68.63; H, 4.86; N, 13.97.

6.1.3.15. 2-Amino-1-((4-bromophenyl)amino)-4-(5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (8o). Yield 78%; m.p. 238–240 °C; IR (KBr, ν_{max}, cm^{-1}): 3473 & 3338 (asym. & sym. str. of –NH$_2$), 2197 (C≡N str.), 1664 (C=O str.), 1372 (–CH$_3$ rocking), 1261 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.85 (s, 3H, –CH$_3$), 0.89 (s, 3H, –CH$_3$), 1.70–2.19 (m, 4H, 2 × –CH$_2$), 2.32 (s, 3H, –CH$_3$), 4.53 (s, 1H, –CH), 5.89–7.52 (m, 15H, Ar–H and –NH$_2$), 8.68 (s, 1H, –NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.55 (pyrazole–CH$_3$), 27.68, 28.23 (C(CH$_3$)$_2$), 32.14 (C$_4$), 37.74 (CH$_2$), 49.52 (CH$_2$–CO), 56.50 (C–CN), 109.75, 110.00, 111.67, 113.80, 114.13, 116.67, 116.90, 121.47, 122.15, 123.75, 125.18, 126.85, 129.53, 129.68, 129.96, 138.27, 145.28, 145.50, 147.46, 147.71, 152.47, 152.67, 153.38, 154.48, 159.59 (25C, Ar–C), 194.95 (C=O); ESI-MS (m/z): 653.1 (M$^+$), 655.1 (M + 2); anal. calcd (%) for: C$_{34}$H$_{30}$BrFN$_6$O$_2$: C, 62.48; H, 4.63; N, 12.86. Found: C, 62.22; H, 4.40; N, 12.58.

6.1.3.16. 2-Amino-4-(5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-((4-fluorophenyl)amino)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (8p). Yield 80%; m.p. 226–228 °C; IR (KBr, ν_{max}, cm^{-1}): 3483 & 3342 (asym. & sym. str. of –NH$_2$), 2198 (C≡N str.), 1662 (C=O str.), 1367 (–CH$_3$ rocking), 1262 (C–O–C ether str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.84 (s, 3H, –CH$_3$), 0.89 (s, 3H, –CH$_3$), 1.70–2.19 (m, 4H, 2 × –CH$_2$), 2.32 (s, 3H, –CH$_3$), 4.54 (s, 1H, –CH), 5.87–7.53 (m, 15H, Ar–H and –NH$_2$), 8.52 (s, 1H, –NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.55 (pyrazole–CH$_3$), 28.15, 28.27 (C(CH$_3$)$_2$), 32.13 (C4), 37.79 (CH$_2$), 49.54 (CH$_2$–CO), 56.87 (C–CN), 109.70, 110.00, 113.36, 113.79, 113.88, 116.32, 116.59, 116.89, 117.22, 121.46, 121.87, 122.15, 126.85, 129.53, 129.68, 138.08, 143.50, 145.08, 145.38, 147.79, 152.48, 153.10, 154.50, 156.90, 159.27 (25C, Ar–C), 194.93 (C=O); ESI-MS (m/z): 593.2 (M$^+$); anal. calcd (%) for: C$_{34}$H$_{30}$F$_2$N$_6$O$_2$: C, 68.91; H, 5.10; N, 14.18. Found: C, 68.69; H, 4.84; N, 13.91.

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