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Synthesis, characterization and pharmacological activities of 2-[4-cyano-(3-trifluoromethyl)phenyl amino)] -4-(4-quinoline/coumarin-4-yloxy)-6-(fluoropiperazinyl)-*s*-triazines

Rahul V. Patel [a,*], Premlata Kumari [a], Dhanji. P. Rajani [b], Kishor H. Chikhalia [c]

[a] *Department of Applied Chemistry, S.V. National Institute of Technology, Surat 395007, Gujarat, India*
[b] *Microcare Laboratory, Surat 395001, Gujarat, India*
[c] *Department of Chemistry, School of Science, Gujarat University, Ahmedabad 380009, Gujarat, India*

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ABSTRACT

A series of 2-[4-cyano-(3-trifluoromethyl)phenyl amino)]-4-(4-quinoline/coumarin-4-yloxy)-6-(fluoropiperazinyl)-*s*-triazines has been synthesized by a simple and efficient synthetic protocol. The antimicrobial activity of the compounds was studied against several bacteria (*Staphylococcus aureus* MTCC 96, *Bacillus cereus* MTCC 619, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 741, *Klebsiella pneumoniae* MTCC 109, *Salmonella typhi* MTCC 733, *Proteus vulgaris* MTCC 1771, *Shigella flexneria* MTCC 1457) and fungi (*Aspergillus niger* MTCC 282, *Aspergillus fumigatus* MTCC 343, *Aspergillus clavatus* MTCC 1323, *Candida albicans* MTCC 183) using paper disc diffusion technique and agar streak dilution method. Newly synthesized compounds were also tested for their *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv using BACTEC MGIT and Lowenstein–Jensen MIC method.

1. Introduction

Fluorine has played a pivotal role in novel drug discovery for modulating physical and biological properties of the molecule. Due to its higher electonegativity, incorporation of fluorine atom(s) within the molecule can enhance their biopotency, bioavailability, metabolic stability and lipophilicity. Trifluoromethylation is one of the most significant strategies to improve pharmacological activities of the molecule due to its high lipophilicity, thereby enhancing *in vivo* uptake and transport of the candidate [1].

The treatment of opportunistic microbial infections has become an important and challenging problem due to the emergence of multiple-drug-resistant organisms [2–6]. Hence, there is an urgent need to develop new classes of agents likely to be unaffected by existing resistance mechanisms. As a part of our ongoing studies to establish new *s*-triazine candidates with improved biological profiles [7–9], here in we report the synthesis and pharmacological activities of novel 2-[4-cyano-(3-trifluoromethyl)phenyl amino)]-4-(4-quinoline/coumarin-4-yloxy)-6-(fluoropiperazinyl)-*s*-triazines. 1,3,5-Triazine analogues have gathered an immense attention among chemists due to their wide range of biological activities such as antimicrobial [10,11], antiprotozoal [12], anticancer [13], antimalarial [14] and antiviral [15] activity. The literature survey revealed that *s*-triazinyl analogues with insertion of *N*-fluorophenyl piperazine bases have been reported to demonstrate a wide range of pharmacological activities [16–22]. In addition, quinolones such as ciprofloxacin, ofloxacin, lomefloxacin, and enoxacin are established synthetic antibacterial agents [23] and are widely prescribed for the treatment of infections in humans. The presence of 4-hydroxy-chromen-2-one is also found to enhance the various biological activities [24–26]. It is worth to mention that the intermediate 4-amino-2-trifluoromethyl benzonitrile is a structural unit of anti cancer agent bicalutamide, used in the present study to provide essential F-atom(s) to the basic core [27]. Prompted by these observations it was contemplated to synthesize a novel series of *s*-triazine analogues bearing fluorinated moieties to identify new candidates that may be

* Corresponding author. Tel.: +91 9712755525.
E-mail addresses: rahul.svnit11@gmail.com (R.V. Patel), premlatakumari@gmail.com (P. Kumari), microcaresurat@yahoo.co.in (Dhanji. P. Rajani), chikhalia_kh@yahoo.com (K.H. Chikhalia).

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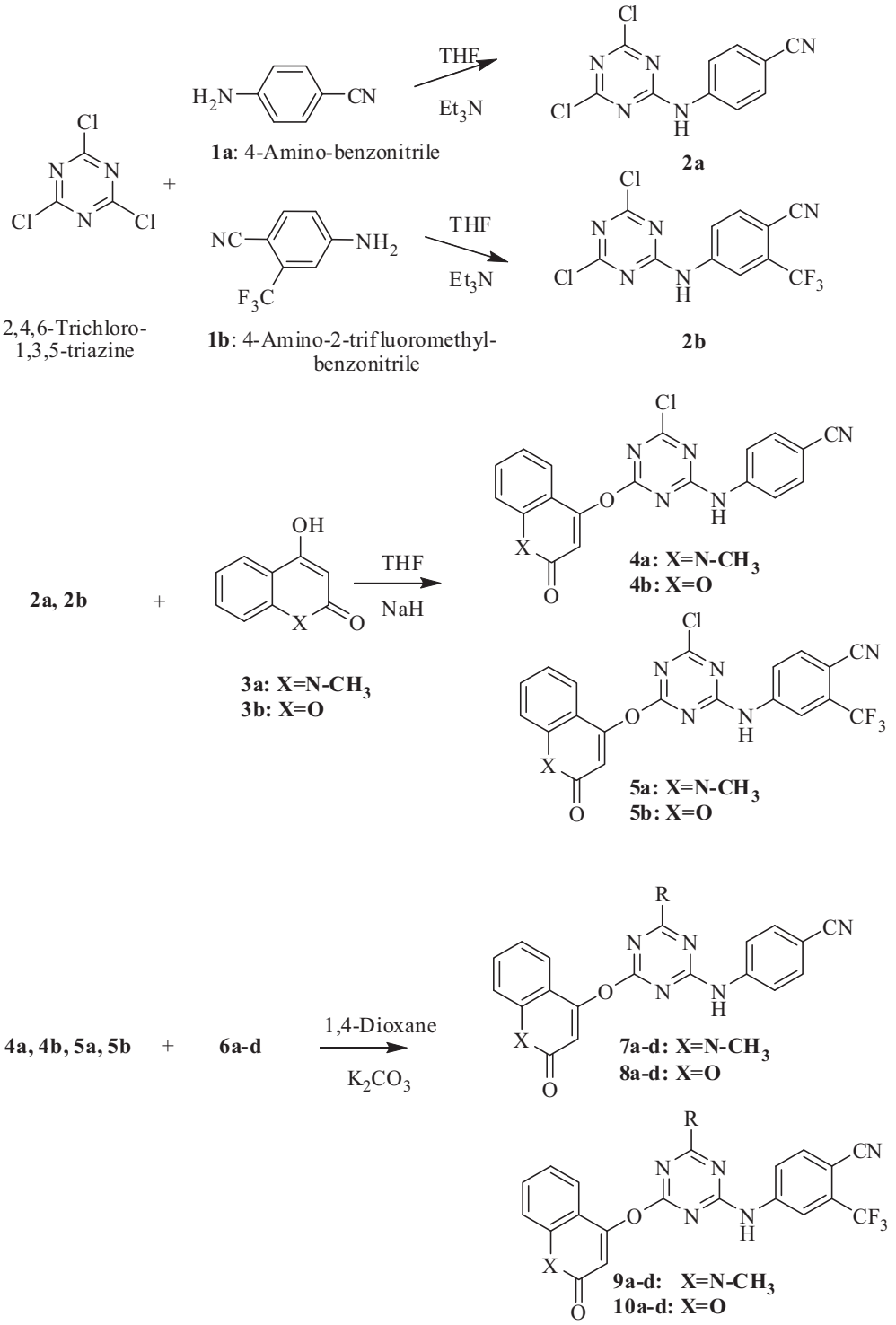
value in designing new potent derivatives endowed with various biological activities (Schemes 1 and 2).

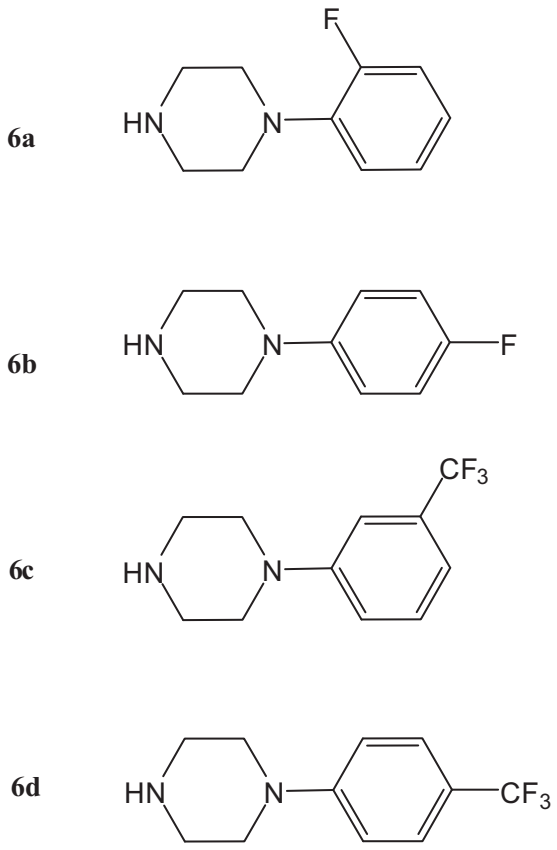
2. Results and discussion

2.1. Chemistry

The first step comprises formation of intermediate 4-(4,6-dichloro-1,3,5-triazin-2-ylamino)-benzonitrile (**2a**) and 4-(4,6-dichloro-1,3,5-triazin-2-ylamino)-2-trifluoromethyl-benzonitrile (**2b**) in good yield by the nucleophilic displacement of one chlorine atom of *s*-triazine ring by 4-amino-benzonitrile and 4-amino-2-trifluoromethyl benzonitrile using triethyl amine. The synthesis of disubstituted *s*-triazine intermediates **4a**, **4b**, **5a** and **5b** was achieved in good yields by the reaction between **2a** and **2b** with 4-hydroxy-1-methyl-2(1*H*)-quinolone and 4-hydroxy-chromen-2-one, respectively, in the presence of 60% NaH at 45–50 °C. Subsequent coupling of the so formed intermediates with the



Scheme 1. Synthesis of final *s*-triazinyl fluoro-piperazines.



Scheme 2. Where **6a–d**, **R** = piperazine bases coupled to compounds **4a**, **4b**, **5a** and **5b**.

desired fluoro-piperazines under basic conditions in 1,4-dioxane solvent at 70–80 °C produced the corresponding 2-[4-cyano-(3-trifluoromethyl)phenyl amino)]-4-(4-quinoline/coumarin-4-yloxy) -6-(fluoropiperazinyl)-*s*-triazines. Compounds **2a** and **2b** displayed absorption bands at 2223 cm^{-1} confirming the presence of a cyano group and strong bands at 3288 cm^{-1} and 3282 cm^{-1} appearing due to the presence of an –NH functional group. A C_3N_3 stretching frequency in *s*-triazine ring was observed at 827–839 cm^{-1} in the IR spectra of final compounds. Moreover, absorption bands ranging from 1249 to 1259 cm^{-1} corresponded with the C–O–C linkage formed between *s*-triazine ring and quinoline or coumarin moieties by disappearing stretching peak at 3610 cm^{-1} of O–H gave correction to the formation of intermediates **4a**, **4b**, **5a** and **5b**. The absence of absorption of C–Cl in the region 700–760 cm^{-1} indicated the formation of final compounds. In the ^{1}H NMR spectra, the synthesis of aimed compounds was confirmed on the basis of the fact that the proton atoms of piperazine ring appeared as two doublets at δ, 3.41–3.55 and δ, 3.79–3.89. Signal varying from δ values 9.14 to 9.28 ppm attributable to an –NH group. The ^{1}H NMR spectra of **7b** displayed two doublets at 8.13 and 7.56 ppm attributed to the C-8 and C-5 protons, respectively of the quinoline ring, whereas, another two signals observed at 7.73 and 7.49 ppm in the form of triplate assigned due to the C-7 and C-6 protons of the quinoline ring, respectively. A sharp singlet at 7.41 ppm was due the C-3 proton of the quinoline ring for **7b**. The protons corresponding to the methyl group appeared as singlet at 3.69 ppm. The ^{1}H NMR spectra of the compound **10b** showed a singlet at 9.15 ppm of –NH group, set of doublet of doublets at 8.06 ppm due to the C-5 proton of the coumarin ring system as well as a triplet at 7.46 ppm attributable to the C-6 proton of the coumarin ring. Proton atoms corresponding to the C-7 and C-8 carbon atoms found to resonate in the form of multiplets in the range either at 7.55–7.59 or at 7.29–7.33 ppm in the ^{1}H NMR of **10b**. ^{13}C NMR spectra of the compound **8c** displayed signals at 178.7 ppm and 165.9 ppm assigned due to the C-6 and C-4 carbon atoms of triazinyl ring. Another two signals observed at 165.6 and 165.1 appeared due to either C-2 atom of triazine ring or C=O belonging to coumarin nucleus. Two peaks observed at 126.3 and 131.1 ppm indicated the presence of trifluoromethyl functional group in the benzonitrile moiety of **8c**, while cyano group indicated the corresponding signals at 105.1 ppm and 98.6 ppm. The carbon atoms of the piperazine ring gave signals at 50.2 ppm and 44.9 ppm in the ^{13}C NMR spectra of **8c**. Final analogues with single fluorine substituted piperazine bases gave a peak in the region 150–152 ppm corresponding to the carbon atom at which fluorine atom is introduced. The determined values of ^{19}F NMR chemical shifts being in compliance with the reported literature [28]. ^{19}F NMR spectra obtained for compounds **9a** and **9b** indicated the confirmation of the presence of single fluorine atom to the *ortho* (2) and *para* (4) position of the phenyl ring attached to the amino nitrogen of piperazine base by giving the corresponding resonating peaks at −120.60 ppm and −118.09 ppm, respectively. Whereas, the trifluoromethyl group of amino-benzonitrile moiety found to reveal a singlet at −64.02 and −63.89 ppm in the ^{19}F NMR spectra of compounds **10c** and **10d**, respectively. Additionally, trifluoromethyl group present at the *meta* (3) and *para* (4) position of the phenyl ring of piperazine base found to reveal singlet peaks at −63.60 ppm and −65.73 ppm, respectively. Assignments of the structure of final scaffolds are based on correct elemental analysis which was found to be within ±0.2% limit (Table 1).

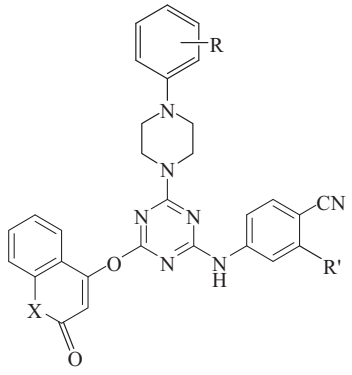
2.2. Pharmacology

Investigation on antibacterial screening data (Table 2) showed some of the compounds were active against all the mentioned bacteria. Final *s*-triazinyl compounds **9c** and **9d** have the highest ability (MIC, 6.25 μg/mL and 27 mm of zone of inhibition) to inhibit *Staphylococcus aureus*. Compound **7d** exhibited similar minimum inhibitory concentration against *S. aureus* with quite reduced inhibition zone (26 mm). Final analogues **7d** and **9b** showed higher effectiveness (26 mm of zone of inhibition) at 12.5 μg/mL against *Bacillus cereus* with similar MIC of compound **9c** (inhibition zone: 25 mm). The best activity (MIC, 12.5 μg/mL) was observed with compound **7a** against *Escherichia coli*, whereas, MIC values exhibited by **7b** and **8b** were equal to that of **7a** with a slight enhancement in the inhibition zones (25 mm). Compounds **7b**, **9b** and **10c** exhibited the greatest activity against *Pseudomonas aeruginosa* at 6.25 μg/mL of MIC. Besides, compound **9d** was found to have similar inhibitory effect in terms of MIC against *P. aeruginosa* with slight reduced inhibitory zone (23 mm). Inhibition of *Klebsiella pneumoniae* was also noted for *s*-triazine derivative **10d** at 25 μg/mL (23 mm of zone of inhibition). Compounds **9a** and **9c** had an effective action (MIC, 25 μg/mL) against *Salmonella typhi* along with similar MIC profile of compound **9d** (inhibition zone: 23 mm). Compound **9d** was superior in inhibiting the growth (25 mm of zone of inhibition) of *Proteus vulgaris* (MIC, 12.5 μg/mL). Strong inhibitory effect was shown by final derivatives **7d** and **9c** at MIC, 12.5 μg/mL (26 mm of zone of inhibition) against *Shigella flexneria* along with similar efficacy of compounds **7c** and **9a** in terms of MIC (MIC, 12.5 μg/mL) against the same bacteria with slight reduced diameter of inhibition zone (25 mm). All the remaining final *s*-triazine derivatives exerted good to moderate activity.

Table 1
Physical and analytical data of newly synthesized compounds.

Entry	Yield (%)	m.p. (°C)	Mol. formula	Elemental analysis					
				Found%			Cal.%		
				C	H	N	C	H	N
7a	79	247–249	$C_{30}H_{25}FN_8O_2$	65.52	4.47	20.61	65.68	4.59	20.43
7b	84	269–271	$C_{30}H_{25}FN_8O_2$	65.56	4.39	20.31	65.68	4.59	20.43
7c	72	280–282	$C_{31}H_{25}F_3N_8O_2$	62.38	4.08	18.53	62.20	4.21	18.72
7d	80	>300	$C_{31}H_{25}F_3N_8O_2$	61.98	4.13	18.61	62.20	4.21	18.72
8a	86	247–250	$C_{29}H_{22}FN_7O_3$	64.87	4.27	18.51	65.04	4.14	18.31
8b	85	288–290	$C_{29}H_{22}FN_7O_3$	64.89	3.99	18.22	65.04	4.14	18.31
8c	85	280–282	$C_{30}H_{22}F_3N_7O_3$	61.33	4.01	16.53	61.54	3.79	16.74
8d	80	>300	$C_{30}H_{22}F_3N_7O_3$	61.42	3.63	16.64	61.54	3.79	16.74
9a	79	219–222	$C_{31}H_{24}F_4N_8O_2$	60.21	3.78	18.02	60.39	3.92	18.17
9b	85	250–253	$C_{31}H_{24}F_4N_8O_2$	61.24	4.07	18.36	60.39	3.92	18.17
9c	72	258–260	$C_{32}H_{24}F_6N_8O_2$	57.55	3.48	16.60	57.66	3.63	16.81
9d	80	260–261	$C_{32}H_{24}F_6N_8O_2$	57.49	3.42	17.94	57.66	3.63	16.81
10a	86	219–223	$C_{30}H_{21}F_4N_7O_3$	59.89	3.58	16.10	59.70	3.51	16.25
10b	85	250–254	$C_{30}H_{21}F_4N_7O_3$	61.83	3.66	16.41	59.70	3.51	16.25
10c	85	258–261	$C_{31}H_{21}F_6N_7O_3$	57.19	3.48	14.81	56.97	3.24	15.00
10d	82	260–262	$C_{31}H_{21}F_6N_7O_3$	56.89	3.33	15.09	56.97	3.24	15.00

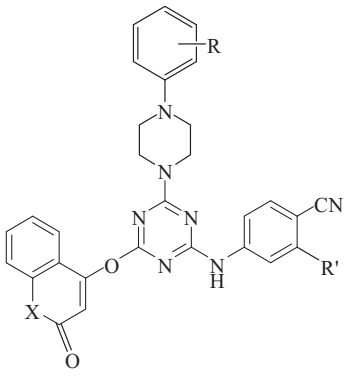
Table 2
In vitro antimicrobial activity.



Entry	R	R′	X	Zone of inhibition [mm (MIC in μg/mL)]							
				Gram-positive bacteria[a]		Gram-negative bacteria[b]					
				S.a.	*B.c.*	*E.c.*	*P.a.*	*K.p.*	*S.t.*	*P.v.*	*S.f.*
7a	2-F	H	N-CH_3	21 (100)	22 (50)	**26 (12.5)**	20 (50)	<10 (100)	20 (100)	21 (100)	19 (100)
7b	4-F	H	N-CH_3	20 (100)	21 (100)	**25 (12.5)**	**24 (6.25)**	17 (100)	22 (50)	19 (100)	17 (100)
7c	3-CF_3	H	N-CH_3	22 (50)	21 (100)	22 (50)	19 (100)	19 (100)	**23 (25)**	24 (25)	**25 (12.5)**
7d	4-CF_3	H	N-CH_3	**26 (6.25)**	**26 (12.5)**	23 (50)	22 (12.5)	20 (100)	22 (50)	22 (50)	**26 (12.5)**
8a	2-F	H	O	17 (100)	18 (100)	24 (25)	19 (100)	17 (100)	16 (100)	<10 (100)	18 (100)
8b	4-F	H	O	18 (100)	18 (100)	**25 (12.5)**	21 (25)	17 (100)	19 (100)	15 (100)	20 (100)
8c	3-CF_3	H	O	21 (100)	20 (100)	22 (50)	20 (50)	<10 (100)	19 (100)	18 (100)	22 (50)
8d	4-CF_3	H	O	23 (25)	19 (100)	21 (100)	17 (100)	<10 (100)	20 (100)	20 (100)	21 (100)
9a	2-F	CF_3	N-CH_3	22 (50)	24 (25)	22 (50)	20 (50)	22 (50)	**24 (25)**	22 (50)	**25 (12.5)**
9b	4-F	CF_3	N-CH_3	20 (100)	**26 (12.5)**	22 (50)	**24 (6.25)**	18 (100)	22 (50)	21 (100)	22 (50)
9c	3-CF_3	CF_3	N-CH_3	**27 (6.25)**	**25 (12.5)**	20 (100)	21 (25)	22 (50)	**24 (25)**	23 (50)	**26 (12.5)**
9d	4-CF_3	CF_3	N-CH_3	**27 (6.25)**	24 (25)	21 (100)	**23 (6.25)**	21 (100)	**23 (25)**	**25 (12.5)**	25 (25)
10a	2-F	CF_3	O	20 (100)	21 (100)	19 (100)	20 (50)	<10 (100)	19 (100)	18 (100)	21 (100)
10b	4-F	CF_3	O	19 (100)	18 (100)	17 (100)	19 (100)	20 (100)	20 (100)	18 (100)	20 (100)
10c	3-CF_3	CF_3	O	23 (25)	22 (50)	22 (50)	**24 (6.25)**	22 (50)	20 (100)	21 (100)	23 (50)
10d	4-CF_3	CF_3	O	25 (12.5)	23 (25)	19 (100)	21 (25)	**23 (25)**	21 (50)	23 (50)	22 (50)
Cip.				30 (≤3.12)	31 (≤3.12)	32 (≤3.12)	33 (≤3.12)	33 (≤3.12)	30 (≤3.12)	31 (≤3.12)	32 (≤3.12)
DMSO				–	–	–	–	–	–	–	–

Cip. – ciprofloxacin.
Bold values refer to the higher activities in terms of lowest MICs.
[a] *S.a.* – *Staphylococcus aureus* and *B.c.* – *Bacillus cereus*.
[b] *E.c.* – *Escherichia coli*, *P.a.* – *Pseudomonas aeruginosa*, *K.p.* – *Klebsiella pneumoniae*, *S.t.* – *Salmonella typhi*, *P.v.* – *Proteus vulgaris*, and *S.f.* – *Shigella flexneria*.

Table 3
In vitro antifungal activity.



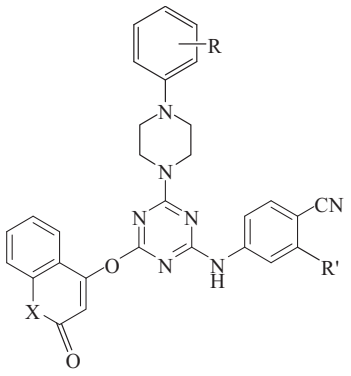
Entry	R	R′	X	Zone of inhibition [mm (MIC in μg/mL)] Fungal strains[a]			
				A.n.	*A.f.*	*A.c.*	*C.a.*
7a	2-F	H	N-CH_3	13 (100)	<10 (100)	18 (100)	13 (100)
7b	4-F	H	N-CH_3	17 (100)	<10 (100)	22 (50)	14 (100)
7c	3-CF_3	H	N-CH_3	22 (50)	18 (100)	**23 (25)**	**24 (25)**
7d	4-CF_3	H	N-CH_3	23 (25)	21 (50)	20 (100)	**24 (25)**
8a	2-F	H	O	<10 (100)	<10 (100)	15 (100)	12 (100)
8b	4-F	H	O	14 (100)	<10 (100)	16 (100)	16 (100)
8c	3-CF_3	H	O	17 (100)	14 (100)	19 (100)	22 (50)
8d	4-CF_3	H	O	18 (100)	16 (100)	19 (100)	16 (100)
9a	2-F	CF_3	N-CH_3	20 (100)	16 (100)	**24 (25)**	20 (100)
9b	4-F	CF_3	N-CH_3	19 (100)	**22 (25)**	22 (50)	22 (50)
9c	3-CF_3	CF_3	N-CH_3	**25 (12.5)**	21 (50)	**24 (25)**	**24 (25)**
9d	4-CF_3	CF_3	N-CH_3	**24 (12.5)**	**23 (25)**	22 (50)	22 (50)
10a	2-F	CF_3	O	19 (100)	<10 (100)	20 (100)	16 (100)
10b	4-F	CF_3	O	20 (100)	16 (100)	17 (100)	16 (100)
10c	3-CF_3	CF_3	O	23 (25)	18 (100)	16 (100)	**23 (25)**
10d	4-CF_3	CF_3	O	22 (50)	19 (100)	**24 (25)**	22 (50)
Kit.				30 (≤3.12)	29 (≤3.12)	31 (≤3.12)	33 (≤3.12)
DMSO				–	–	–	–

Kit. – ketoconazole.
Bold values refer to the higher activities in terms of lowest MICs.
[a] *A.n.* – *Aspergillus niger*, *A.f.* – *Aspergillus fumigates*, *A.c.* – *Aspergillus clavatus*, and *C.a.* – *Candida albicans*.

The antifungal results data (Table 3) revealed that, the synthesized compounds showed variable degree of inhibition against the tested fungi. Final *s*-triazine derivative **9c** displayed antigrowth activity (MIC, 12.5 μg/mL) against *Aspergillus niger*. Compound **9d** displayed activity same as **9c** in terms of MIC against the same fungi with one mm of reduced growth inhibitory diameter (24 mm). A significant inhibition was also observed for compounds **9d** against *Aspergillus fumigatus* at 25 μg/mL of MIC (23 mm of zone of inhibition). Similar minimum inhibitory concentration level (25 μg/mL) observed against the same fungi for compound **9b** with slightly reduced inhibition zone (22 mm). Compounds **9a**, **9c** and **10d** act as the most potent inhibitors of the growth of *Aspergillus clavatus* fungi (MIC, 25 μg/mL), while compound **7c** was found to display similar MIC of 25 μg/mL and quite reduced inhibition zone (23 mm). Compounds **7c**, **7d** and **9c** possessed the highest antifungal activity against *Candida albicans* at 25 μg/mL (24 mm of zone of inhibition) as well as compound **10c** with similar MIC and one mm of lesser inhibition zone (23 mm). All the remaining final *s*-triazine derivatives exerted good to moderate activity (Table 4).

In vitro antimycobacterial activities of compounds **5a–u** were assessed against *Mycobacterium tuberculosis* H37Rv strain. The preliminary results observed from BACTEC MGIT method indicated that compounds **7d**, **9c** and **9d** exhibited highest inhibition (99%) at a constant concentration level (6.25 μg/mL) against *M. tuberculosis* H37Rv. The primary BACTEC MGIT bioassay results have driven us to examine the real potency (MIC) of the title compounds against *M. tuberculosis* H37Rv. The secondary biological screening was performed using Lowenstein–Jensen MIC method and it is worthwhile to note that compounds **7d** and **9c** were the compounds displaying inhibition of *M. tuberculosis* H37Rv completely (99%) at the MIC of 3.12 μg/mL. These compound were considered to display even better efficacy, than the standard drug, pyrazinamide, whereas the MIC of compound **9d** was found (6.25 μg/mL) as same as BACTEC MGIT test. Compound **9b** appeared with good inhibition effect in the term of MIC at 12.5 μg/mL, while Compound **9a** demonstrated 25 μg/mL of MIC against *M. tuberculosis* H37Rv. Final *s*-triazine derivative **7c** diminishes good activity at MIC level of 50 μg/mL, whereas, compound **7b** indicated inhibition of *M. tuberculosis* H37Rv at 62.5 μg/mL. Final compounds **10c** as well as **10d** were able to produce moderate inhibitory activity against *M. tuberculosis* $H37R_V$ at the MIC level of 100 μg/mL. All the remaining derivatives were found to exert higher MIC at 200–1000 μg/mL.

Table 4
In vitro antimycobacterial activity.



Entry	R	R′	X	BACTEC MGIT method		L. J. MIC method	
				MIC (μg/mL)	% Inhibition	MIC (μg/mL)	% Inhibition
7a	2-F	H	N-CH_3	>6.25	–	200	95
7b	4-F	H	N-CH_3	>6.25	–	62.5	97
7c	3-CF_3	H	N-CH_3	>6.25	–	50	98
7d	4-CF_3	H	N-CH_3	6.25	99	**3.12**	99
8a	2-F	H	O	>6.25	–	500	96
8b	4-F	H	O	>6.25	–	500	95
8c	3-CF_3	H	O	>6.25	–	250	97
8d	4-CF_3	H	O	>6.25	–	1000	95
9a	2-F	CF_3	N-CH_3	>6.25	–	25	98
9b	4-F	CF_3	N-CH_3	>6.25	–	12.5	97
9c	3-CF_3	CF_3	N-CH_3	6.25	99	**3.12**	99
9d	4-CF_3	CF_3	N-CH_3	6.25	99	6.25	99
10a	2-F	CF_3	O	>6.25	–	500	96
10b	4-F	CF_3	O	>6.25	–	500	95
10c	3-CF_3	CF_3	O	>6.25	–	100	96
10d	4-CF_3	CF_3	O	>6.25	–	100	95
Isoniazid				0.20	99		
Refampicin				0.25	99		
Ethambutol				3.12	99		
Pyrazinamide				6.25	99		

Bold values refer to the higher activities in terms of lowest MICs.

3. Conclusion

In conclusion, it is clear that the antimicrobial activity of the title compounds is strongly bound to the nature of the piperazine substituent inserted at C-6 position of *s*-triazine ring, together with the fluorinated substituent linked to C-2 position of the *s*-triazine core. Bioassay results revealed that seven compounds (**7b**, **7c**, **7d**, **9a**, **9b**, **9c**, and **9d)** out of the 16 studied displayed variable *in vitro* antibacterial, antifungal and antimycobacterial inhibitory effects. The presence of 4-hydroxy-1-methyl-2(1*H*)-quinolone ring in the title compounds significantly increase the *in vitro* biological potency of the resultant compounds compared to that of with 4-hydroxy-chromen-2-one. Furthermore, final derivatives with piperazine bases containing trifluoromethyl functional group were found more active than that of containing single fluorine atom substitution. In addition, the greater biological profiles were observed for the compounds containing fluorinated amino-benzonitrile component incorporated to the nucleus than non-fluorinated amino-benzonitrile insertion. Therefore, it is concluded that there exists ample scope for further study in this class of compounds in order to discover varied biological profiles such as anticancer activity or anti-HIV activity. The study is currently under investigation and the results will be published in due course.

4. Experimental

4.1. Chemistry

4.1.1. General experimental procedures

2,4,6-Trichloro-1,3,5-triazine, 4-hydroxy-1-methyl-2(1*H*)-quinolone and 4-trifluoromethyl phenyl piperazine were purchased from Sigma Aldrich Chemicals Pvt. Ltd., Mumbai, India. 4-Hydroxy-chromen-2-one was a gift from Ami Organics Pvt. Ltd., Sachin, Surat, India. 4-Amino-benzonitrile was a gift from Yashashvi Rasayan Pvt. Ltd., Maroli, India and 4-amino-2-trifluoromrthyl-benzonitrile was a gift from Ramdev Chemicals Pvt. Ltd., Boisar, India. 3-Trifluoromethylphenyl piperazine, 2-fluorophenyl piperazine and 4-fluorophenyl piperazine were gifts from Dr. Prem's Molecules Pvt. Ltd., Vadodara, India. Acetone, Tetrahydrofuran and 1,4-dioxane of HPLC grade were purchased from Rankem, Surat, India. The TLC plates (silica gel 60 F254 grade) were obtained from Merck, Germany.

Melting points were determined in open capillaries on a Veego electronic apparatus VMP-D (Veego Instrument Corporation, Mumbai, India) and are uncorrected. IR spectra (4000–400 cm^{-1}) of synthesized compounds were recorded on a Shimadzu 8400-S FT-IR spectrophotometer (Shimadzu India Pvt. Ltd., Mumbai, India) using KBr pellets. Thin layer chromatography

was performed on object glass slides (2 cm × 7.5 cm) coated with silica gel-G and spots were visualized under UV irradiation. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400 MHz model spectrometer (Varian India Pvt. Ltd., Mumbai, India) using DMSO as a solvent and TMS as internal standard with ^{1}H resonant frequency of 400 MHz and ^{13}C resonant frequency of 100 MHz. ^{19}F NMR spectra were obtained on the same spectrometer using $CDCl_3$ as a solvent and $CFCl_3$ as an external standard with ^{19}F resonant frequency of 400 MHz. The ^{1}H NMR, ^{13}C NMR and ^{19}F NMR chemical shifts were reported as parts per million (ppm) downfield from TMS (Me_4Si) and $CFCl_3$ and were performed at centre for excellence, Vapi, India. The splitting patterns are designated as follows; s, singlet; br s, broad singlet; d, doublet; m, multiplet. Elemental analyses (C, H, N) were performed using a Heraeus Carlo Erba 1180 CHN analyzer (Hanau, Germany).

4-(4,6-Dichloro-1,3,5-triazin-2-ylamino)-benzonitrile (**2a**) was synthesized according to the reported literature [29].

4.1.2. General procedure for the synthesis of 4-(4,6-dichloro-1,3,5-triazin-2-ylamino)-2-trifluoromethyl-benzonitrile (2b)

To a stirred solution of 2,4,6-trichloro-1,3,5-triazine (10 g, 0.054 mol) in anhydrous THF (150 mL) was added 4-amino-2-trifluoromethyl-benzonitrile **1b** (10.09 g in THF, 0.054 mol) drop wise at 0–5 °C. The resulting reaction mixture was stirred at this temperature for 3 h, then triethyl amine (5.48 g, 0.054 mol) was added in the reaction mixture and stirring was continued for another 6 h. Progress of the reaction was monitored by TLC using toluene:acetone (9:1) as eluent. The resulted reaction mixture was then treated with crushed ice, followed by neutralization by dilute HCl and then filtered, dried and recrystallized from acetone to afford **2b**. Yield: 90%, m.p. 259–261 °C (dec.), FT-IR (KBr, cm^{-1}): 3282 (–NH), 2223 (CN).

4.1.3. General procedure for the synthesis of 4-(4-chloro-6-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)-1,3,5-triazin-2-ylamino)benzonitrile (4a) and 4-(4-chloro-6-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)-1,3,5-triazin-2-ylamino)-2-trifluoromethyl-benzonitrile (5a)

To a stirred solution of 4-hydroxy-1-methyl-2(1*H*)-quinolone (**3a**) (8 g, 0.045 mol) in anhydrous THF (150 mL) was added 60% NaH (1.08 g, 0.055 mol) at room temperature for 1 h and **2a** (12.15 g, 0.045 mol) and **2b** (15.03 g, 0.045 mol) was added into the reaction mixture. Stirring was continued for another 8 h (**2a**) and 12 h (**2b**) at 45 °C. Progress of the reaction was monitored by TLC using toluene:acetone (7:3) as eluent. After the completion of the reaction, it was treated with crushed ice, filtered and dried by THF to afford **4a** and **5a**, **4a**: Yield: 80%, m.p. 277–229 °C (dec.). IR (KBr, cm^{-1}): ν 2219 (CN), 1256 (C–O–C); **5a**: Yield: 83%, m.p. 229–231 °C (dec.). IR (KBr, cm^{-1}): ν 2222 (CN), 1259 (C–O–C).

4.1.4. General procedure for the synthesis of 4-[4-chloro-6-(quinazolin-4-yloxy)-1,3,5-triazine-2-ylamino]-benzonitrile (4b) and 4-[4-chloro-6-(quinazolin-4-yloxy)-1,3,5-triazine-2-ylamino]-2-trifluoromethyl-benzonitrile (5b)

To a stirred solution of 4-hydroxy-chromen-2-one (**3b**) (8 g, 0.049 mol) in anhydrous THF (150 mL) was added 60% NaH (1.11 g, 0.049 mol) at room temperature for 1 h and **2a** (13.03 g, 0.049 mol) and **2b** (16.36 g, 0.049 mol) was added into the reaction mixture. Stirring was continued for another 6 h (**2a**) and 8 h (**2b**) at 45 °C. Progress of the reaction was monitored by TLC using toluene: acetone (97:3, v/v) as eluent. After the completion of the reaction, it was treated with crushed ice, filtered and dried by THF to afford **4b** and **5b** [30]. **4b**: Yield: 74%, m.p. 285–287 °C (dec.). IR (KBr, cm^{-1}): ν 2218 (CN), 1249 (C–O–C). **5b**: Yield: 80%, m.p. 209–213 °C (dec.). IR (KBr, cm^{-1}): ν 2223 (CN), 1253 (C–O–C).

4.1.5. General procedure for synthesis of compounds (7a–d, 8a–d, 9a–d, and 10a–d)

To a solution of **4a**, **4b**, **5a** and **5b** (0.01 mol) in 1,4-dioxane (20 mL), the respective substituted piperazine derivative (**6a–d**) was added and the reaction mixture was refluxed for 16–22 h. Potassium carbonate was used for the neutralization of the reaction mixture. Progress of the reaction was monitored by TLC using toluene:acetone (8:2) as eluent. After the completion of the reaction, it was treated with crushed ice, neutralized by dilute HCl. The precipitates thus obtained was filtered off, dried and recrystallized from THF to afford desired compounds **7a–d**, **8a–d**, **9a–d** and **10a–d**.

4.2. Characterization data of synthesized compounds (6a–e, 7a–e, 8a–e, 9a–e)

4.2.1. 4-[4-[4-(2-Fluoro-phenyl)-piperazin-1-yl]-6-(1-methyl-2-oxo-1,2-dihydro-quinolin-4-yloxy)-[1,3,5]triazin-2-ylamino]-benzonitrile (7a)

Dark yellow solid; IR (KBr, cm^{-1}): ν 3290 (–NH), 3069–3080 (–CH Str.), 2223 (CN), 1255 (C–O–C), 831 (*s*-triazine C–N str.), 743 (C–F). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 9.21 (s, 1H, –N<u>H</u>, *s*-triazine to amino-benzonitrile linkage), 8.08 (d, J = 7.3 Hz, 1H, C_8 proton of quinoline), 7.67 (t, J = 7.5 Hz, 1H, C_7 proton of quinoline), 7.59 (d, J = 8.1 Hz, 1H, C_5 proton of quinoline), 7.51 (t, J = 7.9 Hz, 1H, C_6 proton of quinoline), 7.40 (s, 1H, C_3 proton of quinoline), 7.31–6.88 (8H, m, Ar-H), 3.89 (4H, br s, piperazine), 3.73 (s, 3H, N-C<u>H</u>$_3$), 3.51 (4H, br s, piperazine). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 178.2 (1C, C-6, *s*-triazine, <u>C</u>–N at piperazine linkage), 168.7 (1C, C-4, *s*-triazine, <u>C</u>–O–C at quinoline linkage), 164.5, 163.5 (2C, 1C at C-2, *s*-triazine, <u>C</u>–NH at benzonitrile moiety and 1C of C=O), 151.2 (1C, <u>C</u>–F), 147.3–118.1 (16C, Ar. C), 105.1 (1C, <u>C</u>≡N), 96.5 (1C, –<u>C</u>–C≡N), 49.5, 46.3 (4C, piperazine ring carbon atoms), 30.2 (1C, N-CH_3). ^{19}F NMR (400 MHz, $CDCl_3$): δ −120.39 (1F, s, 2-F).

4.2.2. 4-[4-[4-(4-Fluoro-phenyl)-piperazin-1-yl]-6-(1-methyl-2-oxo-1,2-dihydro-quinolin-4-yloxy)-[1,3,5]triazin-2-ylamino]-benzonitrile (7b)

Dark yellow solid; IR (KBr, cm^{-1}): ν 3268 (–NH), 3058–3073 (–CH Str.), 2224 (CN), 1258 (C–O–C), 829 (*s*-triazine C–N str.), 758 (C–F). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 9.19 (s, 1H, –N<u>H</u>, *s*-triazine to amino-benzonitrile linkage), 8.13 (d, J = 7.7 Hz, 1H, C_8 proton of quinoline), 7.73 (t, J = 7.8 Hz, 1H, C_7 proton of quinoline), 7.56 (d, J = 8.3 Hz, 1H, C_5 proton of quinoline), 7.49 (t, J = 7.5 Hz, 1H, C_6 proton of quinoline), 7.41 (s, 1H, C_3 proton of quinoline), 7.24–6.83 (8H, m, Ar-H), 3.86 (4H, br s, piperazine), 3.69 (s, 3H, N-C<u>H</u>$_3$), 3.45 (4H, br s, piperazine). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 177.7 (1C, C-6, *s*-triazine, <u>C</u>–N at piperazine linkage), 167.4 (1C, C-4, *s*-triazine, <u>C</u>–O–C at quinoline linkage), 165.1, 163.7 (2C, 1C at C-2, *s*-triazine, <u>C</u>–NH at benzonitrile moiety and 1C of C=O), 150.8 (1C, <u>C</u>–F), 145.7–117.9 (16C, Ar. C), 106.3 (1C, <u>C</u>≡N), 98.5 (1C, –<u>C</u>–C≡N), 47.7, 43.3 (4C, piperazine ring carbon atoms), 29.8 (1C, N-CH_3). ^{19}F NMR (400 MHz, $CDCl_3$): δ −117.82 (1F, s, 4-F).

4.2.3. 4-{4-(1-Methyl-2-oxo-1,2-dihydro-quinolin-4-yloxy)-6-[4-(3-trifluoromethyl-phenyl)-piperazin-1-yl]-[1,3,5]triazin-2-ylamino}-benzonitrile (7c)

Brown solid; IR (KBr, cm^{-1}): ν 3288 (–NH), 3060–3068 (–CH Str.), 2223 (CN), 1257 (C–O–C), 830 (*s*-triazine C–N str.), 752 (C–F); ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 9.15 (s, 1H, –N<u>H</u>, *s*-triazine to amino-benzonitrile linkage), 8.17 (d, J = 7.6 Hz, 1H, C_8 proton of quinoline), 7.62 (t, J = 7.5 Hz, 1H, C_7 proton of quinoline), 7.51 (d, J = 8.3 Hz, 1H, C_5 proton of quinoline), 7.44 (t, J = 7.7 Hz, 1H, C_6 proton of quinoline), 7.38 (s, 1H, C_3 proton of quinoline), 7.29–6.91 (8H, m, Ar-H), 3.81 (4H, br s, piperazine), 3.77 (s, 3H, N-C<u>H</u>$_3$), 3.46 (4H, br s, piperazine). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 176.4 (1C,

C-6, *s*-triazine, C–N at piperazine linkage), 166.2 (1C, C-4, *s*-triazine, C–O–C at quinoline linkage), 164.6, 164.1 (2C, 1C at C-2, *s*-triazine, C–NH at benzonitrile moiety and 1C of C=O), 145.9–116.4 (20C, Ar. C including C–CF_3 at 131.4 and CF_3 at 125.9), 104.9 (1C, C≡N), 97.8 (1C, –C–C≡N), 48.3, 45.2 (4C, piperazine ring carbon atoms), 29.3 (1C, N-CH_3). ^{19}F NMR (400 MHz, $CDCl_3$): δ −63.95 (3F, s, 3-CF_3).

4.2.4. 4-{4-(1-Methyl-2-oxo-1,2-dihydro-quinolin-4-yloxy)-6-[4-(4-trifluoromethyl-phenyl)-piperazin-1-yl]-[1,3,5]triazin-2-ylamino}-benzonitrile (7d)

Dark brown solid; IR (KBr, cm^{-1}): ν 3279 (–NH), 3066–3074 (–CH Str.), 2224 (CN), 1253 (C–O–C), 831 (*s*-triazine C–N str.), 760 (C–F). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 9.24 (s, 1H, –NH, *s*-triazine to amino-benzonitrile linkage), 8.14 (d, J = 7.4 Hz, 1H, C_8 proton of quinoline), 7.78 (t, J = 7.9 Hz, 1H, C_7 proton of quinoline), 7.55 (d, J = 8.5 Hz, 1H, C_5 proton of quinoline), 7.50 (t, J = 7.2 Hz, 1H, C_6 proton of quinoline), 7.41 (s, 1H, C_3 proton of quinoline), 7.33–6.86 (8H, m, Ar-H), 3.84 (4H, br s, piperazine), 3.71 (s, 3H, N-CH_3), 3.42 (4H, br s, piperazine). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 178.2 (1C, C-6, *s*-triazine, C–N at piperazine linkage), 165.7 (1C, C-4, *s*-triazine, C–O–C at quinoline linkage), 164.2, 163.6 (2C, 1C at C-2, *s*-triazine, C–NH at benzonitrile moiety and 1C of C=O), 146.3–117.1 (20C, Ar. C including C–CF_3 at 130.3 and CF_3 at 125.5), 105.7 (1C, C≡N), 98.1 (1C, –C–C≡N), 51.4, 42.8 (4C, piperazine ring carbon atoms), 31.6 (1C, N-CH_3). ^{19}F NMR (400 MHz, $CDCl_3$): δ −66.62 (3F, s, 4-CF_3).

4.2.5. 4-[4-[4-(2-Fluoro-phenyl)-piperazin-1-yl]-6-(2-oxo-2H-chromen-4-yloxy)-[1,3,5]triazin-2-ylamino]-benzonitrile (8a)

Yellowish white solid; IR (KBr, cm^{-1}): ν 3282 (–NH), 3078–3085 (–CH Str.), 2223 (CN), 1254 (C–O–C), 831 (*s*-triazine C–N str.), 744 (C–F). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 9.19 (s, 1H, –NH, *s*-triazine to amino-benzonitrile linkage), 7.99 (dd, J = 1.8, 1.1 Hz, 1H, C_5 proton of coumarin), 7.59–7.62 (m, 1H, coumarin), 7.53 (t, J = 8.6 Hz, 1H, C_6 proton of coumarin), 7.33–38 (m, 1H, coumarin), 7.27–6.89 (9H, m, Ar-H), 3.86 (4H, br s, piperazine), 3.49 (4H, br s, piperazine). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 176.5 (1C, C-6, *s*-triazine, C–N at piperazine linkage), 166.7 (1C, C-4, *s*-triazine, C–O–C at quinoline linkage), 166.2, 164.6 (2C, 1C at C-2, *s*-triazine, C–NH at benzonitrile moiety and 1C of C=O), 150.8, 153.4 (2C, 1C of C–F and 1C of C-9, coumarin), 145.3–120.5 (17C, Ar. C), 105.3 (1C, C≡N), 97.7 (1C, –C–C≡N), 48.4, 44.8 (4C, piperazine ring carbon atoms). ^{19}F NMR (400 MHz, $CDCl_3$): δ −121.45 (1F, s, 2-F).

4.2.6. 4-[4-[4-(4-Fluoro-phenyl)-piperazin-1-yl]-6-(2-oxo-2H-chromen-4-yloxy)-[1,3,5]triazin-2-ylamino]-benzonitrile (8b)

Yellowish white solid; IR (KBr, cm^{-1}): ν 3281 (–NH), 3070–3081 (–CH Str.), 2223 (CN), 1256 (C–O–C), 830 (*s*-triazine C–N str.), 747 (C–F). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 9.20 (s, 1H, –NH, *s*-triazine to amino-benzonitrile linkage), 8.02 (dd, J = 1.8, 1.3 Hz, 1H, C_5 proton of coumarin), 7.51–7.54 (m, 1H, coumarin), 7.48 (t, J = 8.3 Hz, 1H, C_6 proton of coumarin), 7.32–35 (m, 1H, coumarin), 7.27–6.91 (9H, m, Ar-H), 3.81 (4H, br s, piperazine), 3.44 (4H, br s, piperazine). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 177.3 (1C, C-6, *s*-triazine, C–N at piperazine linkage), 167.2 (1C, C-4, *s*-triazine, C–O–C at quinoline linkage), 165.5, 164.3 (2C, 1C at C-2, *s*-triazine, C–NH at benzonitrile moiety and 1C of C=O), 151.1, 153.8 (2C, 1C of C–F and 1C of C-9, coumarin), 148.4–118.8 (17C, Ar. C), 104.9 (1C, C≡N), 98.5 (1C, –C–C≡N), 49.2, 45.1 (4C, piperazine ring carbon atoms). ^{19}F NMR (400 MHz, $CDCl_3$): δ −118.38 (1F, s, 4-F).

4.2.7. 4-{4-(2-Oxo-2H-chromen-4-yloxy)-6-[4-(3-trifluoromethyl-phenyl)-piperazin-1-yl]-[1,3,5]triazin-2-ylamino}-benzonitrile (8c)

Yellow solid; IR (KBr, cm^{-1}): ν 3280 (–NH), 3066–3075 (–CH str.), 2224 (CN), 1258 (C–O–C), 828 (*s*-triazine C–N str.), 740 (C–F). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 9.27 (s, 1H, –NH, *s*-triazine to amino-benzonitrile linkage), 8.05 (dd, J = 1.4, 1.2 Hz, 1H, C_5 proton of coumarin), 7.57–7.61 (m, 1H, coumarin), 7.49 (t, J = 8.7 Hz, 1H, C_6 proton of coumarin), 7.29–32 (m, 1H, coumarin), 7.23–6.87 (9H, m, Ar-H), 3.88 (4H, br s, piperazine), 3.53 (4H, br s, piperazine). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 178.7 (1C, C-6, *s*-triazine, C–N at piperazine linkage), 165.9 (1C, C-4, *s*-triazine, C–O–C at quinoline linkage), 165.6, 165.1 (2C, 1C at C-2, *s*-triazine, C–NH at benzonitrile moiety and 1C of C=O), 153.3 (1C of C-9, coumarin), 147.1–117.7 (19C, Ar. C including C–CF_3 at 131.1 and CF_3 at 126.3), 105.1 (1C, C≡N), 98.6 (1C, –C–C≡N), 50.2, 44.9 (4C, piperazine ring carbon atoms). ^{19}F NMR (400 MHz, $CDCl_3$): δ −63.42 (3F, s, 3-CF_3).

4.2.8. 4-{4-(2-Oxo-2H-chromen-4-yloxy)-6-[4-(4-trifluoromethyl-phenyl)-piperazin-1-yl]-[1,3,5]triazin-2-ylamino}-benzonitrile (8d)

Yellow solid; IR (KBr, cm^{-1}): ν 3284 (–NH), 3069–3078 (–CH str.), 2223 (CN), 1255 (C–O–C), 836 (*s*-triazine C–N str.), 749 (C–F). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 9.22 (s, 1H, –NH, *s*-triazine to amino-benzonitrile linkage), 8.10 (dd, J = 1.7, 1.1 Hz, 1H, C_5 proton of coumarin), 7.61–7.64 (m, 1H, coumarin), 7.45 (t, J = 8.5 Hz, 1H, C_6 proton of coumarin), 7.33–36 (m, 1H, coumarin), 7.29–6.90 (9H, m, Ar-H), 3.87 (4H, br s, piperazine), 3.48 (4H, br s, piperazine). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 175.4 (1C, C-6, *s*-triazine, C–N at piperazine linkage), 167.2 (1C, C-4, *s*-triazine, C–O–C at quinoline linkage), 165.9, 163.8 (2C, 1C at C-2, *s*-triazine, C–NH at benzonitrile moiety and 1C of C=O), 152.9 (1C of C-9, coumarin), 148.3–119.6 (19C, Ar. C including C–CF_3 at 129.7 and CF_3 at 126.1), 106.1 (1C, C≡N), 97.4 (1C, –C–C≡N), 48.6, 46.2 (4C, piperazine ring carbon atoms). ^{19}F NMR (400 MHz, $CDCl_3$): δ −65.33 (3F, s, 4-CF_3).

4.2.9. 4-[4-[4-(2-Fluoro-phenyl)-piperazin-1-yl]-6-(1-methyl-2-oxo-1,2-dihydro-quinolin-4-yloxy)-[1,3,5]triazin-2-ylamino]-2-trifluoromethyl-benzonitrile (9a)

Light yellow solid; IR (KBr, cm^{-1}): ν 3287 (–NH), 3059–3072 (–CH str.), 2223 (CN), 1258 (C–O–C), 829 (*s*-triazine C–N str.), 742 (C–F). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 9.21 (s, 1H, –NH, *s*-triazine to amino-benzonitrile linkage), 8.11 (d, J = 7.6 Hz, 1H, C_8 proton of quinoline), 7.69 (t, J = 7.5 Hz, 1H, C_7 proton of quinoline), 7.59 (d, J = 8.4 Hz, 1H, C_5 proton of quinoline), 7.56 (t, J = 7.3 Hz, 1H, C_6 proton of quinoline), 7.41 (s, 1H, C_3 proton of quinoline), 7.30–6.82 (7H, m, Ar-H), 3.81 (4H, br s, piperazine), 3.67 (s, 3H, N-CH_3), 3.44 (4H, br s, piperazine). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 175.8 (1C, C-6, *s*-triazine, C–N at piperazine linkage), 166.2 (1C, C-4, *s*-triazine, C–O–C at quinoline linkage), 164.4, 162.9 (2C, 1C at C-2, *s*-triazine, C–NH at benzonitrile moiety and 1C of C=O), 151.2 (1C, C–F), 146.9–120.1 (19C, Ar. C including C–CF_3 at 129.8 and CF_3 at 125.2), 105.5 (1C, C≡N), 96.9 (1C, –C–C≡N), 49.4, 44.1 (4C, piperazine ring carbon atoms), 30.3 (1C, N-CH_3). ^{19}F NMR (400 MHz, $CDCl_3$): δ −120.60 (F, s, 2-F), −63.01 (3F, s, CF_3 of amino-benzonitrile moiety).

4.2.10. 4-[4-[4-(4-Fluoro-phenyl)-piperazin-1-yl]-6-(1-methyl-2-oxo-1,2-dihydro-quinolin-4-yloxy)-[1,3,5]triazin-2-ylamino]-2-trifluoromethyl-benzonitrile (9b)

Yellow solid; IR (KBr, cm^{-1}): ν 3290 (–NH), 3071–3079 (–CH str.), 2221 (CN), 1256 (C–O–C), 830 (*s*-triazine C–N str.), 757 (C–F). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 9.16 (s, 1H, –NH, *s*-triazine to amino-benzonitrile linkage), 8.07 (d, J = 7.9 Hz, 1H, C_8 proton of quinoline), 7.75 (t, J = 7.6 Hz, 1H, C_7 proton of quinoline), 7.58 (d, J = 8.1 Hz, 1H, C_5 proton of quinoline), 7.53 (t, J = 7.6 Hz, 1H, C_6 proton of quinoline), 7.42 (s, 1H, C_3 proton of quinoline), 7.31–6.92 (7H, m, Ar-H), 3.88 (4H, br s, piperazine), 3.73 (s, 3H, N-CH_3), 3.52 (4H, br s, piperazine). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 178.1 (1C, C-6, *s*-triazine, C–N at piperazine linkage), 167.7 (1C, C-4, *s*-triazine, C–O–C at quinoline linkage), 165.9, 163.4 (2C, 1C at C-2, *s*-triazine, C–NH at benzonitrile

moiety and 1C of C=O), 152.0 (1C, C–F), 147.6–117.4 (19C, Ar. C including C–CF_3 at 130.1 and CF_3 at 125.4), 106.3 (1C, C≡N), 97.8 (1C, –C–C≡N), 50.4, 46.3 (4C, piperazine ring carbon atoms), 29.2 (1C, N-CH_3). ^{19}F NMR (400 MHz, $CDCl_3$): δ −118.09 (F, s, 4-F), −63.22 (3F, s, CF_3 of amino-benzonitrile moiety).

4.2.11. 4-{4-(1-Methyl-2-oxo-1,2-dihydro-quinolin-4-yloxy)-6-[4-(3-trifluoromethyl-phenyl)-piperazin-1-yl]-[1,3,5]triazin-2-ylamino}-2-trifluoromethyl-benzonitrile (9c)

Brown solid; IR (KBr, cm^{-1}): ν 3279 (–NH), 3070–3078 (–CH str.), 2225 (CN), 1256 (C–O–C), 834 (*s*-triazine C–N str.), 748 (C–F). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 9.24 (s, 1H, –NH, *s*-triazine to amino-benzonitrile linkage), 8.19 (d, *J* = 7.4 Hz, 1H, C_8 proton of quinoline), 7.64 (t, *J* = 7.9 Hz, 1H, C_7 proton of quinoline), 7.51 (d, *J* = 8.5 Hz, 1H, C_5 proton of quinoline), 7.45 (t, *J* = 7.3 Hz, 1H, C_6 proton of quinoline), 7.41 (s, 1H, C_3 proton of quinoline), 7.28–6.86 (7H, m, Ar-H), 3.86 (4H, br s, piperazine), 3.71 (s, 3H, N-CH_3), 3.48 (4H, br s, piperazine). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 177.7 (1C, C-6, *s*-triazine, C–N at piperazine linkage), 166.9 (1C, C-4, *s*-triazine, C–O–C at quinoline linkage), 166.2, 163.1 (2C, 1C at C-2, *s*-triazine, C–NH at benzonitrile moiety and 1C of C=O), 147.2–119.2 (22C, Ar. C including 2C–CF_3 at 131.2, 130.6 and 2CF_3 at 125.9, 124.9), 105.1 (1C, C≡N), 98.3 (1C, –C–C≡N), 46.4, 43.7 (4C, piperazine ring carbon atoms), 31.4 (1C, N-CH_3). ^{19}F NMR (400 MHz, $CDCl_3$): δ −63.39, −63.13 (6F, s, –CF_3 of piperazine moiety and –CF_3 of amino benzonitrile moiety).

4.2.12. 4-{4-(1-Methyl-2-oxo-1,2-dihydro-quinolin-4-yloxy)-6-[4-(4-trifluoromethyl-phenyl)-piperazin-1-yl]-[1,3,5]triazin-2-ylamino}-2-trifluoromethyl-benzonitrile (9d)

Dark brown solid; IR (KBr, cm^{-1}): ν 3282 (–NH), 3068–3076 (–CH str.), 2223 (CN), 1256 (C–O–C), 833 (*s*-triazine C–N str.), 749 (C–F). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 9.18 (s, 1H, –NH, *s*-triazine to amino-benzonitrile linkage), 8.10 (d, *J* = 7.4 Hz, 1H, C_8 proton of quinoline), 7.77 (t, *J* = 7.6 Hz, 1H, C_7 proton of quinoline), 7.59 (d, *J* = 8.6 Hz, 1H, C_5 proton of quinoline), 7.52 (t, *J* = 7.2 Hz, 1H, C_6 proton of quinoline), 7.39 (s, 1H, C_3 proton of quinoline), 7.26–6.93 (7H, m, Ar-H), 3.89 (4H, br s, piperazine), 3.77 (s, 3H, N-CH_3), 3.51 (4H, br s, piperazine). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 175.8 (1C, C-6, *s*-triazine, C–N at piperazine linkage), 167.5 (1C, C-4, *s*-triazine, C–O–C at quinoline linkage), 165.5, 163.6 (2C, 1C at C-2, *s*-triazine, C–NH at benzonitrile moiety and 1C of C=O), 144.8–116.7 (22C, Ar. C including 2C–CF_3 at 129.9, 130.4 and 2CF_3 at 125.2, 125.7), 105.9 (1C, C≡N), 95.8 (1C, –C–C≡N), 48.2, 45.6 (4C, piperazine ring carbon atoms), 30.6 (1C, N-CH_3). ^{19}F NMR (400 MHz, $CDCl_3$): δ −66.03, −63.29 (6F, s, –CF_3 of piperazine moiety and –CF_3 of amino benzonitrile moiety).

4.2.13. 4-[4-[4-(2-Fluoro-phenyl)-piperazin-1-yl]-6-(2-oxo-2H-chromen-4-yloxy)-[1,3,5]triazin-2-ylamino]-2-trifluoromethyl-benzonitrile (10a)

Yellowish white solid; IR (KBr, cm^{-1}): ν 3283 (–NH), 3067–3077 (–CH str.), 2224 (CN), 1256 (C–O–C), 830 (*s*-triazine C–N str.), 760 (C–F). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 9.16 (s, 1H, –NH, *s*-triazine to amino-benzonitrile linkage), 8.09 (dd, *J* = 1.4, 1.3 Hz, 1H, C_5 proton of coumarin), 7.52–7.55 (m, 1H, coumarin), 7.44 (t, *J* = 8.7 Hz, 1H, C_6 proton of coumarin), 7.30–34 (m, 1H, coumarin), 7.29–6.92 (8H, m, Ar-H), 3.85 (4H, br s, piperazine), 3.46 (4H, br s, piperazine). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 177.9 (1C, C-6, *s*-triazine, C–N at piperazine linkage), 166.2 (1C, C-4, *s*-triazine, C–O–C at quinoline linkage), 165.7, 164.9 (2C, 1C at C-2, *s*-triazine, C–NH at benzonitrile moiety and 1C of C=O), 151.3, 152.5 (2C, 1C of C–F and 1C of C-9, coumarin), 148.1–116.8 (18C, Ar. C), 104.8 (1C, C≡N), 96.9 (1C, –C–C≡N), 51.6, 43.9 (4C, piperazine ring carbon atoms). ^{19}F NMR (400 MHz, $CDCl_3$): δ −121.46 (F, s, 2-F), −63.08 (3F, s, CF_3 of amino-benzonitrile moiety).

4.2.14. 4-[4-[4-(4-Fluoro-phenyl)-piperazin-1-yl]-6-(2-oxo-2H-chromen-4-yloxy)-[1,3,5]triazin-2-ylamino]-2-trifluoromethyl-benzonitrile (10b)

Yellowish white solid; IR (KBr, cm^{-1}): ν 3290 (–NH), 3069–3079 (–CH str.), 2222 (CN), 1256 (C–O–C), 832 (*s*-triazine C–N str.), 745 (C–F). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 9.15 (s, 1H, –NH, *s*-triazine to amino-benzonitrile linkage), 8.06 (dd, *J* = 1.6, 1 Hz, 1H, C_5 proton of coumarin), 7.55–7.59 (m, 1H, coumarin), 7.46 (t, *J* = 8.4 Hz, 1H, C_6 proton of coumarin), 7.29–7.33 (m, 1H, coumarin), 7.25–6.83 (8H, m, Ar-H), 3.83 (4H, br s, piperazine), 3.51 (4H, br s, piperazine). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 176.5 (1C, C-6, *s*-triazine, C–N at piperazine linkage), 166.9 (1C, C-4, *s*-triazine, C–O–C at quinoline linkage), 166.3, 163.9 (2C, 1C at C-2, *s*-triazine, C–NH at benzonitrile moiety and 1C of C=O), 152.1, 153.1 (2C, 1C of C–F and 1C of C-9, coumarin), 149.4–118.2 (18C, Ar. C), 106.3 (1C, C≡N), 97.6 (1C, –C–C≡N), 50.2, 45.1 (4C, piperazine ring carbon atoms). ^{19}F NMR (400 MHz, CDCl3): δ −117.63 (F, s, 4-F), −63.31 (3F, s, CF_3 of amino-benzonitrile moiety).

4.2.15. 4-{4-(2-Oxo-2H-chromen-4-yloxy)-6-[4-(3-trifluoromethyl-phenyl)-piperazin-1-yl]-[1,3,5]triazin-2-ylamino}-2-trifluoromethyl-benzonitrile (10c)

Yellow solid; IR (KBr, cm^{-1}): ν 3288 (–NH), 3068–3076 (–CH str.), 2223 (CN), 1255 (C–O–C), 834 (*s*-triazine C–N str.), 760 (C–F). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 9.22 (s, 1H, –NH, *s*-triazine to amino-benzonitrile linkage), 7.99 (dd, *J* = 1.5, 1.1 Hz, 1H, C_5 proton of coumarin), 7.58–7.62 (m, 1H, coumarin), 7.50 (t, *J* = 8.2 Hz, 1H, C_6 proton of coumarin), 7.34–37 (m, 1H, coumarin), 7.26–6.92 (8H, m, Ar-H), 3.81 (4H, br s, piperazine), 3.43 (4H, br s, piperazine). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 176.2 (1C, C-6, *s*-triazine, C–N at piperazine linkage), 167.1 (1C, C-4, *s*-triazine, C–O–C at quinoline linkage), 166.7, 164.8 (2C, 1C at C-2, *s*-triazine, C–NH at benzonitrile moiety and 1C of C=O), 152.7 (1C of C-9, coumarin), 146.3–120.5 (22C, Ar. C including 2C–CF_3 at 130.7, 130.1 and 2CF_3 at 126.2, 125.1), 105.8 (1C, C≡N), 97.8 (1C, –C–C≡N), 47.8, 45.1 (4C, piperazine ring carbon atoms). ^{19}F NMR (400 MHz, $CDCl_3$): δ −63.60, −64.02 (6F, s, –CF_3 of piperazine moiety and –CF_3 of amino benzonitrile moiety).

4.2.16. 4-{4-(2-Oxo-2H-chromen-4-yloxy)-6-[4-(4-trifluoromethyl-phenyl)-piperazin-1-yl]-[1,3,5]triazin-2-ylamino}-2-trifluoromethyl-benzonitrile (10d)

Yellow solid; IR (KBr, cm^{-1}): ν 3279 (–NH), 3065–3077 (–CH str.), 2223 (CN), 1257 (C–O–C), 833 (*s*-triazine C–N str.), 759 (C–F). ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 9.26 (s, 1H, –NH, *s*-triazine to amino-benzonitrile linkage), 8.04 (dd, *J* = 1.6, 1.3 Hz, 1H, C_5 proton of coumarin), 7.56–7.60 (m, 1H, coumarin), 7.47 (t, *J* = 8.5 Hz, 1H, C_6 proton of coumarin), 7.35–38 (m, 1H, coumarin), 7.27–6.87 (8H, m, Ar-H), 3.86 (4H, br s, piperazine), 3.49 (4H, br s, piperazine). ^{13}C NMR (100 MHz, Me_2SO-d_6): δ 178.1 (1C, C-6, *s*-triazine, C–N at piperazine linkage), 165.6 (1C, C-4, *s*-triazine, C–O–C at quinoline linkage), 165.2, 163.6 (2C, 1C at C-2, *s*-triazine, C–NH at benzonitrile moiety and 1C of C=O), 153.3 (1C of C-9, coumarin), 147.8–117.5 (22C, Ar. C including 2C–CF_3 at 129.7, 130.4 and 2CF_3 at 125.4, 125.9), 106.4 (1C, C≡N), 99.2 (1C, –C–C≡N), 47.1, 46.6 (4C, piperazine ring carbon atoms). ^{19}F NMR (400 MHz, $CDCl_3$): δ −65.73, −63.89 (6F, s, –CF_3 of piperazine moiety and –CF_3 of amino benzonitrile moiety).

4.3. Pharmacology

4.3.1. Paper disc diffusion technique (agar streak dilution method)

The synthesized *s*-triazinyl derivatives were examined for antimicrobial activity against eight bacteria (*S. aureus* MTCC 96, *B. cereus* MTCC 619, *E. coli* MTCC 739, *P. aeruginosa* MTCC 741, *K. pneumoniae* MTCC 109, *S. typhi* MTCC 733, *P. vulgaris* MTCC 1771) and four fungi (*A. niger* MTCC 282, *A. fumigatus* MTCC 343, *A.*

clavatus MTCC 1323, *C. albicans* MTCC 183) species using paper disc diffusion technique [31]. The Mueller–Hinton agar media were sterilized (autoclaved at 120 °C for 30 min), poured at uniform depth of 5 mm and allowed to solidify. The microbial suspension (10^5 CFU/mL) (0.5 McFarland Nephelometery Standards) was streaked over the surface of media using a sterile cotton swab to ensure even growth of the organisms. The tested compounds were dissolved in dimethyl sulfoxide to give solutions of 3.12–100 μg/mL. Sterile filter paper discs measuring 6.25 mm in diameter (Whatman no. 1 filter paper), previously soaked in a known concentration of the respective test compound in dimethyl sulfoxide were placed on the solidified nutrient agar medium that had been inoculated with the respective microorganism and the plates were incubated for 24 h at 37 ± 1 °C. A control disc impregnated with an equivalent amount of dimethyl sulfoxide without any sample was also used and did not produce any inhibition. Ciprofloxacin and ketoconazole (100 μg/disc) were used as control drugs for antibacterial and antifungal activity, respectively.

MIC of the compound was determined by agar streak dilution method [32]. A stock solution of the synthesized compound (100 μg/mL) in dimethyl sulfoxide was prepared and graded quantities of the test compounds were incorporated in a specified quantity of molten sterile agar, *i.e.* nutrient agar for evaluation of antibacterial and sabouraud dextrose agar for antifungal activity, respectively. The medium containing the test compound was poured into a Petri dish at a depth of 4–5 mm and allowed to solidify under aseptic conditions. A suspension of the respective microorganism of approximately 10^5 CFU/mL was prepared and applied to plates with serially diluted compounds with concentrations in the range of 3.12–100 μg/mL in dimethyl sulfoxide and incubated at 37 ± 1 °C for 24 h (bacteria) or 48 h (fungi). The lowest concentration of the substance that prevents the development of visible growth is considered to be the MIC value.

4.3.2. BACTEC MGIT and L. J. MIC method

The preliminary antimycobacterial assessment for the final synthesized compounds was carried out using BACTEC MGIT method. The Mycobacterial Growth Indicator Tubes (MGIT) containing 4 mL of modified Middle brook 7H9 Broth Base were numbered as per the title compounds to be tasted for anti-mycobacterial efficacy by means of various concentrations prepared. The suspension was allowed to sit for 20 min and the tubes were centrifuged at 3000 rpm for 15 min. After that prepared suspension of 10^4–10^7 CFU/mL of H37 R_V *M. tuberculosis* strain was added in the medium to be incubated and 0.1 mL of egg-based medium (L. J.) was also added. The MGIT tubes were then tightly recapped, mixed well and incubated into BACTEC MGIT instrument at 37 ± 1 °C until positivity is observed. The readings were measured daily starting from the second day of incubation. Positive cultures were usually detected within 10 days. For reading the actual results the MGIT tubes were removed from incubator and placed on the UV light next to a positive control tube and an uninoculated tube (negative control). Bright fluorescence detected by the corresponding MGIT tube was noticed in the form of bright orange color in the bottom of the tube and also an orange reflection on the meniscus [33]. The primary screening was conducted at concentration of 6.25 μg/mL against *M. tuberculosis* H37 Rv in BACTEC MGIT system. Compounds demonstrating 99% inhibition in the primary screen were described as most potent compounds. All the other compounds to be tasted were re-examined for their actual MIC by using Lowenstein–Jensen MIC method. The MIC was defined as the lowest concentration inhibiting 99% of the inoculum.

The secondary antimycobacterial screening for test compounds was obtained for *M. tuberculosis* H37 Rv, by using L. J. (Lowenstein and Jensen) MIC method [34,35]. Stock solutions of primary 1000, 500, 250, and secondary 200, 100, 62.5, 50, 25, 12.5, 6.25, 3.25 μg/mL dilutions of each test compound in DMSO (dimethyl sulfoxide) were added in the liquid L. J. medium and then media were sterilized by inspissation method. A culture of *M. tuberculosis* H37 Rv growing on L. J. medium was harvested in 0.85% saline in bijou bottles. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H37 Rv (5×10^4 bacilli per tube). These tubes were then incubated at 37 ± 1 °C. Growth of bacilli was seen after 12 days, 22 days and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* H37 Rv. The concentration at which no development of colonies occurred or <20 colonies was taken as MIC concentration of test compound. The standard strain *M. tuberculosis* H37 Rv was tested with known drugs rifampicin, isoniazid, ethambutol and pyrazinamide.

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References

[1] R. Filler, R. Saha, Future Med. Chem. 1 (2009) 777–791.
[2] C. Nathan, Nature 431 (2004) 899–902.
[3] M.C. Raviglione, Tuberculosis 83 (2003) 4–14.
[4] NIAID, Available at http://www3.niaid.nih.gov/topics/tuberculosis/.
[5] TAACF, Available at http://www.taacf.org/about-TB-background.htm.
[6] World Health Organization, Global Tuberculosis Control: A Short Update to the 2009 Report, Available at http://www.who.int/tb/publications/global_report/2009/update/en/index.html.
[7] D.H. Mahajan, C. Pannecouque, E. De Clercq, K.H. Chikhalia, Arch. Pharm. Chem. Life Sci. 342 (2009) 281–290.
[8] K.H. Chikhalia, M.J. Patel, J. Enzyme Inhib. Med. Chem. 24 (2009) 960–966.
[9] D.H. Patel, K.H. Chikhalia, N.K. Shah, D.P. Patel, P.B. Kaswala, V.M. Buha, J. Enzyme Inhib. Med. Chem. 25 (2010) 121–125.
[10] C. Zhou, J. Min, L. Zhigang, Y. Anne, D. Heather, G. Tian, Bioorg. Med. Chem. Lett. 18 (2008) 1308–1311.
[11] K. Srinivas, U. Srinivas, K. Bhanuprakash, K. Harakishore, U.S.N. Murthy, R.V. Jayathirtha, Eur. J. Med. Chem. 41 (2006) 1240–1246.
[12] B. Alessandro, J.B. Gorka, L.S. Mhairi, Y. Vanessa, B. Reto, P.B. Michael, J. Med. Chem. 48 (2005) 5570–5579.
[13] M. Rita, S. Simona, S. Giovanni, V. Francesca, D.V. Lisa, J. Med. Chem. 47 (2004) 4649–4652.
[14] M. Sergio, P. Davide, C. Paolo, B. Nicoletta, M. Diego, ChemMedChem 3 (2008) 873–876.
[15] X. Yuan-Zhen, C. Fen-Er, B. Jan, D.C. Erik, P. Christophe, Eur. J. Med. Chem. 43 (2008) 1230–1236.
[16] H. Fan, Y. Chen, Z. Jiang, S. Zhang, D. Zhong, R. Ji, Y. Yang, Eur. J. Med. Chem. 43 (2008) 1706–1714.
[17] K. Manjunatha, B. Poojary, P.L. Lobo, J. Fernandes, N.S. Kumari, Eur. J. Med. Chem. 45 (2010) 5225–5233.
[18] O.A. Phillips, E.E. Udo, M.E. Abdel-Hamid, R. Varghese, Eur. J. Med. Chem. 44 (2009) 3217–3227.
[19] V. Varshney, N.N. Mishra, P.K. Shukla, D.P. Sahu, Eur. J. Med. Chem. 45 (2010) 661–666.
[20] R.S. Upadhayaya, N. Sinha, S. Jain, N. Kishore, R. Chandrab, S.K. Aroraa, Bioorg. Med. Chem. 12 (2004) 2225–2238.
[21] S. Srinivasan, R.M.B. Shafreen, P. Nithyanand, P. Manisankar, S.K. Pandian, Eur. J. Med. Chem. 45 (2010) 6101–6105.
[22] R.J. Kerns, M.J. Rybak, G.W. Kaatz, F. Vaka, R. Cha, R.G. Gruczb, V.U. Diwadkard, Bioorg. Med. Chem. Lett. 13 (2003) 2109–2112.
[23] H.J. Boehm, M. Boehringer, D. Bur, H. Gmuender, W. Huber, W. Klaus, D. Kostrewa, H. Kuehne, T. Luebbers, N. Meunier-Keller, F. Mueller, J. Med. Chem. 43 (2000) 2664–2674.
[24] N. Vukovic, S. Sukdolak, S. Solujic, N. Niciforovic, Food Chem. 120 (2010) 1011–1018.
[25] M. Mladenovic, N. Vukovic, N. Niciforovic, S. Sukdolak, S. Solujic, Molecules 14 (2009) 1495–1512.
[26] N. Vukovic, S. Sukdolak, S. Solujic, T. Milosevic, Arch. Pharm. Chem. Life Sci. 341 (2008) 491–496.
[27] P.F. Schellhammer, Expert Opin. Pharmacother. 3 (2002) 1313–1328.

[28] A. Dandia, K. Arya, M. Sati, P. Sarawgi, J. Fluorine Chem. 125 (2004) 1273–1277.
[29] R.V. Patel, P. Kumari, K.H. Chikhalia, Arch. Appl. Sci. Res. 2 (2010) 232–240.
[30] X. Yuan-Zhen, C. Fen-Er, B. Jan, D.C. Erik, P. Christophe, Chem. Biodivers. 6 (2009) 561–568.
[31] S.H. Gillespie, Medical Microbiology—Illustrated, Butterworth Heinemann Ltd., United Kingdom, 1994, pp. 234–247.
[32] P.M. Hawkey, D.A. Lewis, Medical Bacteriology—A Practical Approach, Oxford University Press, United Kingdom, 1994, pp. 181–194.
[33] P. Anargyros, D.S. Astill, I.S. Lim, J. Clin. Microbiol. 28 (1990) 1288–1291.
[34] H.D. Isenberg, Clinical Microbiology Procedures Handbook, vol. 1, American Society for Microbiology, Washington, DC, 1992.
[35] N.B. Patel, I.H. Khan, S.D. Rajani, Arch. Pharm. Chem. Life Sci. 10 (2010) 692–699.