Indian Journal of Chemistry
Vol. 61, March 2022, pp. 285-292

Design and synthesis of novel *s*-triazine based coumarin, quinoline, morpholine and isoniazid derivatives and their antitubercular and antimicrobial evaluation

Jyotindra Mahyavanshi*, Vijay Shrivastava, Shraddha Patel & Jayesh Pandya
Department of Chemistry, Sankalchand Patel University,Visnagar 384 315, India
E-mail: mjyotindra44@gmail.com

Received 4 May 2020; accepted (revised) 26 October 2021

This study presents the synthesis of novel *s*-triazine derivatives. The synthetic route to final *s*-triazines consists of two nucleophilic substitution reactions of 4-hydroxy benzonitrile and 8-hydroxyquinoline or 8-hydroxy-7-methyl coumarin or isoniazid or morpholine with 2,4,6-trichloro-1,3,5-triazine resulting in 2,4-disubstituted-6-chloro-1,3,5-triazine derivatives to introduce the various amines functionality. The structures of the compounds have been elucidated with the aid of IR, ^{1}H and ^{13}C NMR, mass spectroscopy and elemental analysis. The title compounds have then been investigated for their *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv strain by using BACTEC MGIT and Lowenstein-Jensen MIC method and antimicrobial evaluation.

Keywords: *s*-Triazine, antimycobacterial activity, coumarin, quinoline, isoniazide, morpholine

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis.* After AIDS, it is the world's second common cause of death by infectious diseases[1]. According to the World Health Organization (WHO), 2 million people die every year and at least 9 million are getting infected, which provides a pool for the development of new active form of tuberculosis[2]. In addition, the emergence of multi drug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB) as a consequence of lengthy treat- ment, makes patient compliance difficult. The term

MDR-TB is used to describe strains that are resistant to two or more of the five first-line anti-TB drugs (isoniazid, rifampicin, pyrazinamide, ethambutol and streptomycin)[3,4]. MDR-TB takes longer to treat with second- line drugs (DOT-Plus), which are more expensive and have more side effects. XDR-TB will develop when these second-line drugs are mismanaged and therefore also become ineffective[5,6]. This development of drug resistance in the population has increased concern that TB may once again become an incurable disease in developing countries; the prevalence of XDR-TB is increasing as a consequence of poor financial resources6and thus provides a strong motivation for the development of effective and affordable antitubercular agents.

During the last few years, the potential of *s*-triazine derivatives in agrochemical and medicinal properties have been subjected to investigation. Literature survey reveals that substituted *s*-triazine derivatives are associated with a number of pronounced biological activities[7-9]. *s*-Triazine is a six-membered heterocyclic ring, with three nitrogens situated at 1st, 3rd and 5th positions. *s*-Triazine ring is an important pharmacophore, and its coupling with other rings could furnish new biologically active compounds (Figure 1). These derivatives exhibit various types of biological properties, such as anti HIV, anti tuberculosis, anticancer, antimycobacterial, antifungal, and antibacterial[10–13].

1,3,5-Triazine derivatives are implicated in a variety of biological applications, such as antimicrobial[14,15], antiprotozoal[16] anticancer[17] antimalarial[18], and antiviral[19] activities. Recently several *s*-triazine derivatives bearing morpholine moieties are characterized by an enhanced antibacterial profile and improved pharmaco kinetic properties as antitubercular activity and as a consequence of large therapeutic potential of such bio labile analogues, it was rationalized to synthesize newer structural elements potentially endowed with antibacterial and antifungal activities.

Results and Discussion

Compounds 5**a–u** were synthesized according to Figure 2. The disubstituted *s*-triazine intermediate (**1-4**) was obtained in very good yield by the reaction

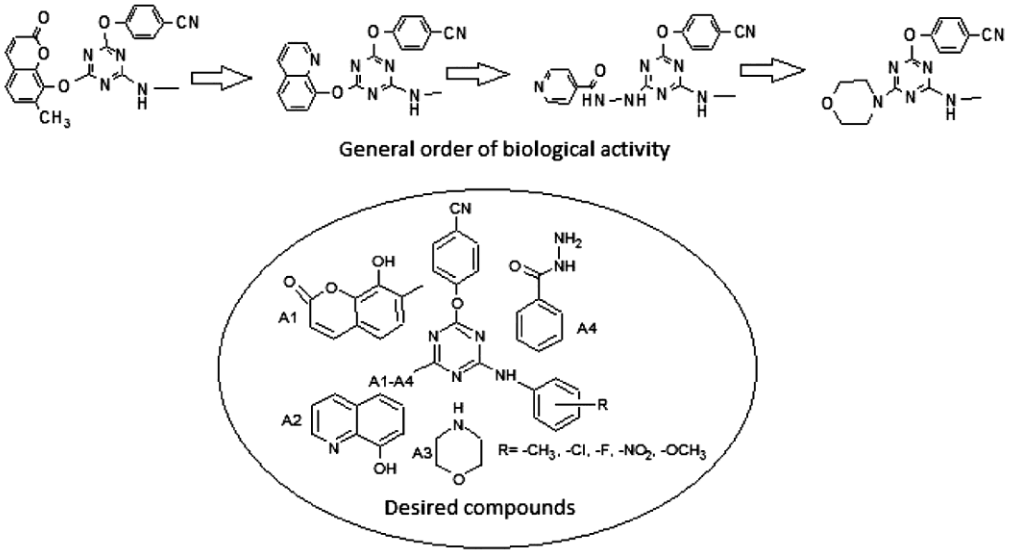

Figure 1 — Strategy for the design of the compounds

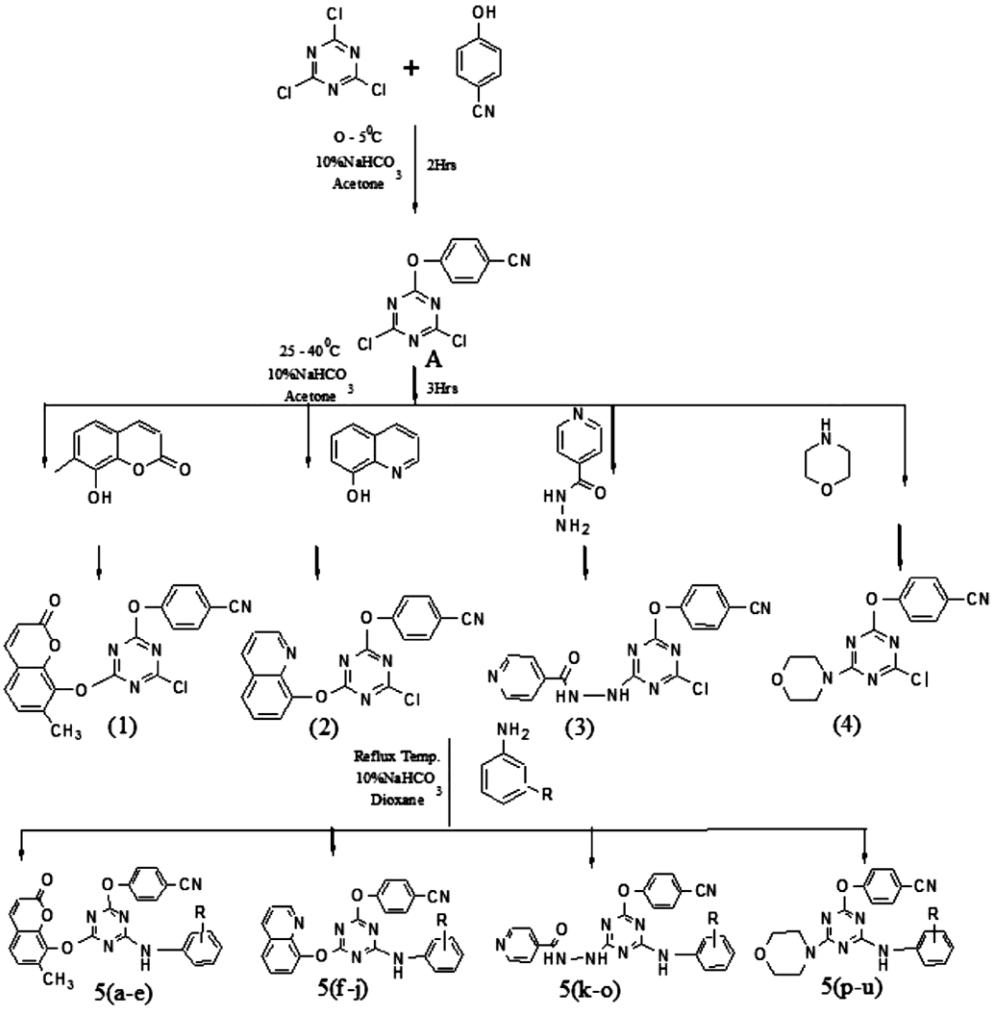

Figure 2 — Synthetic procedure for the synthesis of compounds 5(a-u)

between 4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]ben zonitrile (**A**) and 8-hydroxy-7-methyl coumarin, 8-hydroxy quinoline, isoniazid and morpholine in the presence of 10% $NaHCO_3$ at 45–50°C. Nucleophilic substitution of one chlorine atom of *s*-triazine ring produced **A** in good yield from 2,4,6-trichloro-1,3,5-triazine and 4-hydroxy benzonitrile. Condensation of **1**, **2**, **3** and **4** with appropriate amine substituents (Figure 3) in 1,4-dioxane at 70–80°C provided the target compounds **5a–u**. A C_3N_3 stretching in the *s*-triazine ring was observed at 810–820 cm^{-1}. Compound **1** displayed an absorption band at 2,218–2,225 cm^{-1} confirming the presence of a –CN group, Moreover, a characteristic band appeared at 1,248–1,260 cm^{-1} corresponding to the C–O–C linkage, while disappearance of the OH peak at 3,606–3,632 cm^{-1} belonging to the 8-hydroxyquinoline and 8-hydroxy-7-methyl coumarin, gave correction to the formation of intermediate. The absence of a C–Cl stretching band at 700–760 cm^{-1} confirmed the formation of the final products by the condensation of amines to *s*-triazine ring as all the chlorine atoms of *s*-triazine ring were substituted by 4-hydroxy benzonitrile, 8-hydroxy-7-methyl coumarin or 8-hydroxy quinoline or isoniazid or morpholine and amine. The synthesis of **5a–u** was confirmed on the basis of NMR spectra, IR and mass spectra. The morpholine proton assigned a signal at δ 3.34–3.72 integrating eight proton atoms, some of the proton atoms corresponding to the isoniazid and quinoline nuclei resonated at δ 7.70–8.60 region, the –NH group at δ 9.70–9.95 ppm. ^{13}C NMR spectral assigned signals in the range δ 171–173, 168–170 and 165–167 attributed to the carbon atoms of *s*-triazine ring from which the chlorine atoms were replaced by 4-hydroxy benzonitrile, 8-hydroxy quinoline and amine. The carbon atoms nearer to the nitrogen hetero atom in the quinoline ring integrated at δ 155–160 ppm. The carbon atom corresponding to the cyano functional group (-CN) and the carbon atom of the amino benzonitrile ring to which the cyano group is attached were found to reveal the peaks at around δ 105 and 96–98 respectively.

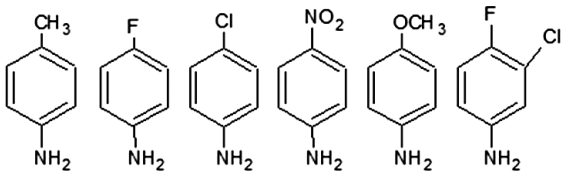

Figure 3 — Various amines

In vitro antituberculosis results observed for final analogues (**5a–u**) from BACTEC MGIT method (Table I) indicated that final *s*-triazine, **5c** bearing electron withdrawing chlorine groups incorporated into the 4th positions of the phenyl ring of amine base, **5n** with electron-releasing methoxy groups to the 4th positions of the amine moiety, **5p** bearing mono-methyl amine; **5s** bearing nitro group attached to the *para* position of phenyl ring of amine base; and **5t** with methoxy groups introduced into the *para* positions of the phenyl ring of amine base, condensed to *s*-triazine nucleus exhibited excellent inhibition (99%) against mycobacterial strain. These compounds were considered to be the most potent analogues among all the final compounds studied. The primary BACTEC MGIT bioassay results obtained have driven us to examine the potency (MIC) of the remaining compounds against *M. tuberculosis* H37Rv. In the secondary biological screening (L. J. agar dilution method), it was observed that final *s*-triazinyl compound **5h**, involving insertion of amine moiety containing isoniazid, **5q** and **5k**, incorporating monohalo (fluoro)-substituted phenyl ring of amine entity bridged to *s*-triazine core, displayed good inhibition effect, *i.e*., half-fold activity than the most active analogues tested. Compounds **5c** and **5u**, incorporating fluorine and chlorine group to the phenyl ring of amine base condensed to nucleus, demonstrated good activity against *M. tuberculosis* H37Rv. Final morpholine bearing compound **5s** displayed moderate inhibition of *M. tuberculosis* H37RV at the MIC level of 100mg/mL, whereas some derivatives were found to be inactive even at a higher concentration of 100mg/mL.

The antimicrobial bio assay results presented in Table II revealed that, generally, all the tested compounds were found more active towards gram-positive bacteria than against gram-negative bacteria. Final *s*-triazinyl compounds **5d**, **5s** and **5t** showed potent activity against gram-positive strain *S. aureus*. Compounds **5n**, **5p**, **5q**, **5r** and **5u** were found to possess half-fold activity against *S. aureus* as compared to the most active analogues tested against the same strain. Final *s*-triazinyl analogues **5s** and **5t** displayed excellent inhibitory profile against gram-positive *B. cereus* along with half- fold comparative activity of compounds **5h**, **5p**, **5q** and **5u** against the same bacterial strain. Compounds **5h**, **5t** and **5u** were found to contribute promising activity along with similar inhibitory concentration level of compound **5n**

toward gram-negative strain *E. coli*, while compound**5i** demonstrated inhibitory concentration level against *E. coli*. Compounds **5p**, **5r** and **5s** appeared with diminished activity against gram-negative *P. aeruginosa*, where the half-fold activity was observed for compounds **5h**, **5n** and **5u** against the same bacteria. All the remaining final *s*-triazine derivatives were found to demonstrate good to poor activity profiles at minimum inhibitory concentration levels ranging from 25 to 100 μg/mL, whereas some final deriva- tives were found to be inactive even at a higher concentration of100μg/mL.

Table I — *In vitro* antituberculosis activity

Compd	R	LogP	BactecMgit method		L. J. Mic method	
			MIC (mg/mL)	% inhibition	MIC (mg/mL)	% inhibition
5a	4-CH3	4.78	>6.25	–	250	96
5b	4-F	4.43	>6.25	–	25	97
5c	4-Cl	5.00	>6.25	–	50	98
5d	4- NO2	4.04	>6.25	–	100	95
5e	4-CH3	3.00	>6.25	–	12.5	99
5f	4-F	2.66	>6.25	–	250	96
5g	4-Cl	3.23	>6.25	–	62.5	97
5h	4-NO2	2.28	>6.25	–	100	96
5i	4-OCH3	3.43	>6.25	–	62.5	95
5j	4-CH3	5.66	>6.25	–	250	97
5k	4-F	5.32	>6.25	–	50	98
5l	4-Cl	5.89	>6.25	–	100	96
5m	4-NO2	4.94	>6.25	–	12.5	99
5n	4- OCH3	5.09	>6.25	–	25	99
5o	3-F, 4-Cl	6.04	>6.25	99	12.5	99
5p	4-CH3	4.31	>6.25	–	250	96
5q	4-F	3.96	>6.25	–	12.5	99
5r	4- Cl	4.53	>6.25	–	100	95
5s	3-NO2	3.58	>6.25	–	250	98
5t	4 -OCH3	3.74	>6.25	–	62.5	97
5u	3-Cl,4-F	4.68	>6.25	–	12.5	99
Isoniazid	0.2	99%				
Rifampicin	0.25	99%				
Ethambutol	3.12	99%				
Pyrazinamide	6.25	99%				

Table II — Antibacterial and Antifungal Activity

Compd	Minimum Inhibitory Concentration (MIC) in μg/mL					
	Gram Positive Bacteria		Gram Negative Bacteria		Fungi	
	S. aureus ATCC No.5923	*B. cereus* ATCC No.29212	*E. coli* ATCC No.25922	*P. aeruginosa* ATCC No.27853	*C. albicans* ATCC No.11651	*A. niger* ATCC No. 11394
5a	125	250	125	250	125	250
5b	15.62	250	62.5	31.5	125	125
5c	15.62	250	31.25	31.5	62.5	31.25
5d	15.62	125	62.5	62.5	125	62.5
5e	125	250	31.25	250	250	125
5f	250	250	250	500	250	250
5g	62.5	125	250	125	125	250
5h	15.62	125	62.5	31.5	62.5	62.5
5i	31.5	62.5	125	250	250	125
5j	31.5	62.5	31.5	31.5	62.5	125
Fluconazole	–	–	–	–	125	62.5

The antifungal bioassay results summarized in Table II revealed that final *s*-triazine derivatives **5q** and **5s** displayed excellent antigrowth activity against *A. niger*, which was found equivalent to the standard drug tested. Compounds **5d**, **5t**, and **5u**, appeared with half-fold inhibitory action against the same fungi. Compounds **5d**, **5r**, and **5s** appeared with strong inhibition of *C. albicans.* Compounds **5c**, **5t**, **5u** and **5q** indicated half-fold activity compared to the most active analogues toward *C. albicans.* Remaining final compounds were found to contribute good to poor activities against all the mentioned fungal strains at the concentration levels ranging from 25 to 100μg/mL, whereas some final derivatives were found to be inactive even at a higher concentration of100μg/mL.

Experimental Section

Materials

2,4,6-Trichloro-1,3,5-triazine and 8-hydroxyquinoline were purchased from Sigma Aldrich Chemicals Pvt. Ltd., Mumbai, India. 4-hydroxy benzonitrile was purchased from Merck India Ltd. Acetone, tetrahydrofuran and 1,4-dioxane of HPLC grade were purchased from Rankem, Surat, India. The TLC plates (silica gel 60 F254) were obtained from Merck, Germany.

Methods

All the melting points were recorded on cintex melting point apparatus and are uncorrected. IR spectra in KBr were recorded on Shimadzu FTIR spectro photometer in cm^{-1}, ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker DRX-400 MHz NMR instrument chemical shifts are reported in using TMS as internal standard on the δ scale. Mass spectra of compounds were recorded on Agilent 1100 series mass spectrometer.

Synthesis

Synthesis of 4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy] benzonitrile (A)

To a stirred solution of cyanuric chloride (0.1mol) in acetone (100 mL) at 0-5°C, the solution of 4-hydroxy Benzonitrile (0.1 mole) in acetone (90 mL) was added drop wise in two hours. During the reaction 10% $NaHCO_3$ was added to maintain the reaction mixture neutral. The progress of reaction was monitored by TLC using acetone: toluene (2:8) as eluent. After the completion of the reaction, the stirring stopped and the solution treated with crushed ice. The product obtained was filtered and dried. The crude product was purified and recrystallized from alcohol. The yield was 85% having melting point 255°C and FTIR (KBr) 2223 cm^{-1}, yield:92%.

Synthesis of disubstituted derivatives of *s*-triazine, 1-4

To a stirred solution of 4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]benzonitrile **1** in acetone at RT the solution of morpholine or 8-hydroxy-4-methyl coumarin, isoniazid, 8-hydroxy quinoline in acetone was slowly added in 2 hours and the temperature was raised to 45°C during 2 hours. 10% solution of $NaHCO_3$ was added to maintain the reaction mixture neutral and further maintained for 2 hours and the progress of reaction was monitored by TLC using Acetone:Toulene (2:8). After completion of reaction, the solution was poured into ice cold water, the solid product which was obtained after filtration was dried and recrystallized from absolute alcohol to give the title compound. The yield was 75% having melting point 266°C and FTIR (KBr): 2223, 1255 cm^{-1} (C-O-C). Yield: 84%.

General procedure for the synthesis of compounds 5a–u

To a solution of disubstituted derivatives (0.01 mol in 1,4-dioxane 20 mL) added appropriate different substituted aryl amines derivatives and the reaction mixture was refluxed for 6-10 hrs. 10% $NaHCO_3$ was used for neutralization of the reaction. Thin Layer Chromatography was used for monitoring the progress of reaction using toluene:acetone (95:5 v/v) as eluent. After the completion of the reaction, the mixture was poured into crushed ice and neutralized by diluted HCl. The precipitate thus obtained was filtered off, dried, and recrystallized from THF to give the final compounds, **5a–u**.

4-({4-[(4-Methylphenyl)amino]-6-[(2-oxo-4a,8a-dihydro-2*H*-chromen-8-yl)oxy]-1,3,5-triazin-2-yl}oxy) benzonitrile, 5a: IR (*v* max in cm^{-1}): 2237 (-CN str), 1241 (C-O-Cstr), 3310 (NH) and 1320 (CN), 3079 (Aromatic CH str), 1319 (CH_3, C–H bend.); ^{1}H NMR: δ 10.11 (s, 1H, CONH, D_2O exchangeable), 4.07 (s, 1H, NH), 9.19 (s, 1H, NH–Ar), 7.94–8.74 (m, 12H, Ar–H), 7.23-7.43 (m, 4H, Ar-H); ^{13}C NMR: δ ppm: 104.9 (1C, CN), 119.1–147.9 (Ar–C), 161.3, 171.5, 178.7 (C=N of *s*-triazine); MS: *m/z* 479.59.

4-({4-[(4-Fluorophenyl)amino]-6-[(2-oxo-4a,8a-dihydro-2*H*-chromen-8-yl)oxy]-1,3,5-triazin-2-yl}oxy)benzonitrile, 5b: IR (*v* max in cm^{-1}): 2233 (-CN str), 1240(C-O-Cstr), 3329 (NH) and 1317

(CN), 3089 (Aromatic CH str), 827 (C–Cl str); ^{1}H NMR: δ 10.18 (s, 1H, CONH, D_2O exchangeable), 4.27 (s, 1H, NH), 9.29 (s, 1H, NH–Ar), 7.74–8.92 (m, 12H, Ar–H).7.23-7.43(m, 4H, Ar-H); ^{13}C NMR: δ 103.2 (1C,CN),111.7–147.9 (Ar–C), 164.7, 169.5, 177.8 (C=N of *s*-triazine); MS: *m/z* 483.45.

4-({4-[(4-Chlorophenyl)amino]-6-[(2-oxo-4a,8a-dihydro-2*H*-chromen-8-yl)oxy]-1,3,5-triazin-2-yl}oxy)benzonitrile, 5c: IR (*v* max in cm^{-1}): 2243 (-CN str), 1233(C-O-Cstr), 3329 (NH) and 1327 (CN), 3079 (Aromatic CH str), 845 (C–Cl str). ^{1}H NMR: δ 10.15 (s, 1H, CONH, D_2O exchangeable), 4.28 (s, 1H, NH), 9.89 (s, 1H, NH–Ar), 7.74–8.92 (m, 12H, Ar–H).7.23-7.43(m, 4H, Ar-H); ^{13}C NMR: δ 102.9 (1C,CN),112.7–145.9 (Ar–C), 163.7, 167.9, 178.7 (C=N of *s*-triazine); MS: *m/z* 499.8.

4-({4-[(4-Nitrophenyl)amino]-6-[(2-oxo-4a,8a-dihydro-2*H*-chromen-8-yl)oxy]-1,3,5-triazin-2-yl}oxy)benzonitrile, 5d: IR (*v* max in cm^{-1}): 2223 (-CN str), 1242 (C-O- C str), 3340 (NH) and 1320 (CN), 1519, 1377 (NO_2), 3077 (Aromatic CH str), 1517, 1371 (NO2, N=O str.). ^{1}H NMR: δ 10.15 (s, ^{1}H, CONH, D_2O exchangeable), 4.14 (s, 1H, NH), 9.25 (s, 1H, NH–Ar), 7.07–8.29 (m, 12H, Ar–H). 7.23-7.43 (m, 4H, Ar-H); ^{13}C NMR: δ 104.9 (1C, CN), 111.7–147.7 (Ar–C), 164.7, 168.7, 176.9 (C=N of *s*-triazine); MS: *m/z* 510.50.

N'-[4-({4-[(4-Methylphenyl)amino]-6-(4-cyanophenoxy)-1,3,5-triazin-2-yl] benzohydrazide, 5e: IR (*v* max in cm^{-1}):2227 (-CN str), 1245(C-O-C str), 1675 ([C=O of amide, C=O str), 3320 (NH) and 1320 (CN), 3089 (Aromatic CH str), 1309 (CH3, C–H bend.). ^{1}H NMR: δ 10.18 (s, 1H, CONH,D_2O exchangeable), 4.09 (s, 1H, NH), 9.21 (s, 1H, NH–Ar), 7.94–8.74 (m,12H,Ar–H), 7.23-7.43 (m,4H,Ar-H); ^{13}C NMR: δ 104.9(1C,CN),119.1–147.9(Ar–C), 160.2, 171.7, 179.9 (C=N of *s*-triazine),164.2(CO); MS: *m/z* 438.47.

N'-[4-({4-[(4-Fluorophenyl)amino]-6-(4-cyanophenoxy)-1,3,5-triazin-2-yl]benzohydrazide, 5f: IR (*v* max in cm^{-1}): 2223 (-CN str), 1242(C-O-Cstr), 1677 ([C=O of amide, C=O str), 3319 (NH) and 1327 (CN), 3099 (Aromatic CH str), 837 (C–Cl str). ^{1}H NMR: δ 10.19 (s, 1H, CONH, D_2O exchange- able), 4.27 (s, 1H, NH), 9.29 (s, 1H, NH–Ar), 7.74–8.92 (m, 12H, Ar–H). 7.23-7.43 (m, 4H, Ar-H); ^{13}C NMR: δ 103.2 (1C, CN), 111.7–147.9 (Ar–C), 163.7,169.9, 176.7 (C=N of *s*-triazine),163.9 (CO); MS: *m/z* 442.41.

N'-[4-({4-[(4-Chlorophenyl)amino]-6-(4-cyanophenoxy)-1,3,5-triazin-2-yl]benzohydrazide, 5g: IR (*v* max in cm^{-1}): 2243 (-CN str), 1233(C-O-Cstr), 1667 ([C=O of amide, C=O str), 3329 (NH) and 1327 (CN), 3089 (Aromatic CH str), 847 (C–Cl str). ^{1}H NMR: δ 10.19 (s, 1H, CONH, D_2O exchangeable), 4.37 (s,1H,NH), 9.29 (s,1H,NH–Ar), 7.74–8.92 (m, 12H, Ar–H). 7.23-7.43 (m, 4H, Ar-H); ^{13}C NMR: δ 102.9 (1C, CN), 112.7–145.9 (Ar–C),164.7,166.9,177.7(C=N of *s*-triazine),164.9(CO); MS: *m/z* 458.70.

N'-[4-({4-[(4-Nitrophenyl)amino]-6-(4-cyanophenoxy)-1,3,5-triazin-2-yl]benzohydrazide, 5h: IR (*v* max in cm^{-1}): 2223 (-CN str), 1242(C-O-C str), 1670 ([C=O of amide, C=O str), 3340 (NH) and 1320 (CN), 1519, 1377 (NO2), 3077 (Aromatic CH str), 1517, 1371 (NO_2, N=O str.). ^{1}H NMR: δ 10.15 (s, 1H, CONH, D_2O exchangeable), 4.14 (s, 1H, NH), 9.25 (s, 1H, NH–Ar), 7.07–8.29 (m, 12H, Ar–H). 7.23-7.43 (m, 4H, Ar-H); ^{13}C NMR: δ 104.9 (1C, CN), 111.7–147.7(Ar–C),164.7,168.7,176.9(C=Nof *s*-triazine), 164.77 (CO); MS: *m/z* 469.33.

N'-[4-({4-[(4-Methoxyphenyl)amino]-6-(4- cyanophenoxy)-1,3,5-triazin-2-yl]benzohydrazide, 5i: IR(*v*max in cm^{-1}): 2233(-CN str),1242(C-O-C str), 1690 (C=O of amide, C=O str), 3325 (NH) and 1329 (CN), 3092 (Aromatic CH str), 2829 (OCH_3); ^{1}H NMR: δ 10.24 (s, ^{1}H, CONH, D_2O exchangeable), 3.77 (s, 3H, OCH_3), 4.18 (s,1H, NH), 9.11 (s,1H, NH–Ar), 7.72–8.94 (m, 12H, Ar–H).7.23-7.43(m, 4H, Ar-H); ^{13}C NMR: δ 104.8 (1C,CN),60.9(OCH_3),118.1–149.1(Ar–C),162.1,172.2,177.7(C=N of *s*-triazine), 164.9 (CO); MS: *m/z* 445.44.

4-{[4-({4-[(4-Methylphenyl)amino]-6-(quinolin-8-yloxy)-1,3,5-triazin-2-yl]oxy}benzonitrile, 5j: IR (*v* max in cm^{-1}): 3414 (-NH str), 2918 (C-H str), 2225 (CN str), 1259 (C-O-C str), 1475 (-CH_2 str), 814(*s*-triazine C-N str). ^{1}H NMR: δ 8.91-7.90(1H,d,quinoline), 2.50(3H,s,-CH_3),6.71-6.87(4H, m, Ar-H),7.48-7.23(4H, m, Ar-H),7.62-7.50(4H,m,Ar-H),7.70-7.64 (2H, m, Ar-H), 9.55(1H,s,-NH); ^{13}C NMR: δ 104.9 (1C, CN), 119.1–147.9 (Ar–C), 160.2,171.7,179.9 (C=N of *s*-triazine), 22.9 (-CH_3); MS: *m/z* 448.49.

4-{[4-({4-[(4-Fluorophenyl)amino]-6-(quinolin-8-yloxy)-1,3,5-triazin-2-yl]oxy}benzonitrile, 5k: IR (*v* max in cm^{-1}): 3321 (-NH str), 2818 (C-H str), 2245 (CN str), 1241(C-O-C str), 816 (*s*-triazine C-N str), 1015-1400 (C-F str). ^{1}H NMR: δ 8.81-7.70 (1H, d, quinoline), 6.81-6.87 (4H, m, Ar-H),7.48-

7.23(4H,m,Ar-H),7.66-7.50 (4H,m,Ar-H),7.77-7.63 (2H, m, Ar-H), 9.55 (1H, s, NH), ^{13}C NMR: δ 104.9(1C, CN) 118.1–149.1 (Ar–C), 162.1, 172.2,177.7(C=N of *s*-triazine); MS: *m/z* 452.42.

4-{[4-({4-[(4-Chlorophenyl) amino]-6-(quinolin-8-yloxy)-1,3,5-triazin-2-yl]oxy}benzonitrile, 5l: IR (*v* max in cm^{-1}): 3369 (-NH str), 2866 (C-H str), 2220-2225 (CN str),1239 (C-O-C str), 806 (*s*-triazine C-Nstr.),611-800(CClstr). ^{1}H NMR: δ 8.90-7.90 (1H, d, quinoline), 6.69-6.88 (4H, m, Ar-H),7.44-7.26(4H,m,Ar-H),7.65-7.51(4H,m,Ar-H),7.71-7.66 (2H, m, Ar-H), 9.85 (1H, s, -NH); ^{13}C NMR: δ 104.9(1C, CN), 111.7–147.9 (Ar–C), 163.7, 169.9,176.7(C=N of *s*-triazine); MS: *m/z* 468.98.

4-{[4-({4-[(4-Nitrophenyl)amino]-6-(quinolin-8-yloxy)-1,3,5-triazin-2-yl]oxy}benzonitrile, 5m: IR (*v* max in cm^{-1}): 3316 (-NH str), 2866 (C-H str), 2220-2225(CN str),1239(C-O-C str),1470(-CH_2 str),806 (*s*-triazine C-N str), 611-800 (CCl str). ^{1}H NMR: δ 8.90-7.90 (1H,d, quinoline), 6.69-6.88 (4H,m,Ar-H), 7.44-7.26 (4H,m, Ar-H), 7.65-7.51 (4H, m, Ar-H), 7.71-7.66 (2H, m, Ar-H), 9.85 (1H, s, NH); ^{13}C NMR: δ 104.9(1C,CN),113.6–145.7(Ar–C),162.5, 168.7, 175.4 (C=N of *s*-triazine); MS: *m/z* 479.4.

4-{[4-({4-[(4-Methoxyphenyl) amino]-6-(quinolin-8-yloxy)-1,3,5-triazin-2-yl]oxy} benzonitrile, 5n: IR (Max in cm^{-1}): 3436 (-NH str), 2814 (C-H str), 2220-2228(CN str),1239(C-O-C str),1468(-CH_2 str),808 (*s*-triazine C-N str.), 1082-1150(C-Ostr); ^{1}HNMR: δ 8.91-7.90 (1H, d, quinoline), 3.55 (3H, s, CH_3), 6.68-6.86 (4H, m, Ar-H), 7.38-7.33 (4H,m,Ar-H),7.64-7.55(4H,m,Ar-H),7.71-7.65(2H,m,Ar-H),9.57(1H,s,-NH); ^{13}C NMR: δ 104.9(1C,CN), 60.9 (OCH_3), 118.1–149.1 (Ar–C), 162.1,172.2,177.7 (C=N of *s*-triazine); MS: *m/z* 464.42.

4-{[4-({4-[(3-Chloror-4-fluorophenyl)amino]-6-(quinolin-8-yloxy)-1,3,5-triazin-2-yl]oxy}benzonitrile, 5o: IR (*v*max in cm^{-1}): 3436 (-NH str), 2814 (C-H str),2220-2228 (C-N str),1239 (C-O-C str),1468 (-CH_2str), 808 (*s*-triazine C-N str.), 1082-1150 (C-O str); ^{1}H NMR: δ 8.91-7.90 (1H, d, quinoline), 3.55 (3H, s, CH_3), 6.68-6.86 (4H, m, Ar-H), 7.38-7.33 (4H,m,Ar-H),7.64-7.55(4H,m,Ar-H),7.71-7.65(2H,m,Ar-H),9.57(1H,s,-NH); ^{13}C NMR: δ 104.9(1C,CN),110.8–144.9(Ar–C), 165.7, 168.9, 178.7 (C=N of *s*-triazine); MS: *m/z* 486.8.

4-({4-[(4-Methylphenyl)amino]-6-(morpholin-4-yl)-1,3,5-triazin-2-yl}oxy)benzonitrile, 5p: IR (*v*max in cm^{-1}): 3416 (-NH str), 2918 (C-H str), 2220-2225 (C-N str),1255 (C-O-C str),1475 (-CH_2 str),806 (*s*-triazine C-N str); ^{1}H NMR: δ 3.34-3.70 (8H, m, morpholine), 2.50 (3H,s,-CH_3), 6.71-6.87(2H, d, Ar-H), 7.28-7.43 (2H, d, Ar-H), 7.92 (2H, d, Ar-H), 9.55 (1H, s, -NH); ^{13}C NMR: δ 104.9(1C, CN), 22.9 (1C, CH_3), 118.1–149.1 (Ar–C),162.1,172.2, 177.7 (C=N of *s*-triazine), 58.9, 71.4 (4C, morpholine); MS: *m/z* 390.9.

4-**({4-[(4-Fluorophenyl)amino]-6-(morpholin-4-yl)-1,3,5-triazin-2-yl}oxy) benzonitrile, 5q**: IR (*v*max in cm^{-1}): 3426 (-NH str), 2928 (C-H str), 2220-2225 (C-N str),1255(C-O-C str),1475 (-CH_2str), 806 (*s*-triazine C-N str); ^{1}H NMR: δ 3.34-3.70 (8H, m, morpholine), 2.52 (3H,s,-CH_3),6.70-6.86(2H, d, Ar-H), 7.27-7.41 (2H, d, Ar-H), 7.92 (2H, d, Ar-H), 9.45 (1H, s, -NH), ^{13}C NMR: δ 104.9(1C, CN), 21.9 (1C, CH_3), 117.1–148.2 (Ar–C),162.1,171.3, 176.5 (C=N of *s*-triazine), 57.9, 72.4 (4C, morpholine); MS: *m/z* 392.28.

4-({4-[(4-Chlorophenyl)amino]-6-(morpholin-4-yl)-1,3,5-triazin-2-yl}oxy)benzonitrile, 5r: IR (*v*max in cm^{-1}): 3449 (-NH str), 2977 (C-H str), 2220-2225(C-N str),1255(C-O-C str),1474(-CH_2 str),804 (*s*-triazine C-N str). ^{1}H NMR: δ 3.34-3.70 (8H, m, morpholine), 2.53(3H, s,-CH_3), 6.71-6.87 (2H,d, Ar-H), 7.28-7.43 (2H, d, Ar-H), 7.92 (2H, d, Ar-H), 9.55 (1H, s, -NH), ^{13}C NMR: δ 104.9 (1C, CN),22.9 (1C, CH_3), 118.1–149.1 (Ar–C), 162.1,172.2,177.7 (C=N of *s*-triazine), 58.9, 71.4 (4C, morpholine); MS: *m/z* 408.82.

4-({4-[(4-Nitrophenyl)amino]-6-(morpholin-4-yl)-1,3,5-triazin-2-yl}oxy)benzonitrile 5s: IR (*v*max in cm^{-1}): 3416 (-NH str), 2918 (C-H str), 2220-2225(C-N str),1255(C-O-C str),1475(-CH_2str), 806 (*s*-triazine C-N str); ^{1}H NMR: δ 3.34-3.70 (8H, m, morpholine), 2.50 (3H, s, -CH_3), 6.71-6.87 (2H, d, Ar-H), 7.28-7.43 (2H, d, Ar-H), 7.92 (2H, d, Ar-H), 9.50 (1H, s, -NH); ^{13}C NMR: δ 104.8 (1C, CN), 22.8 (1C, CH_3), 118.1–147.1 (Ar–C), 161.1, 174.7, 178.7 (C=N of *s*-triazine), 59.9, 73.4 (4C, morpholine); MS: *m/z* 419.39.

4-({4-[(4-Methoxyphenyl)amino]-6-(morpholin-4-yl)-1,3,5-triazin-2-yl}oxy)benzonitrile, 5t: IR (*v*max in cm^{-1}): 3422 (-NH str), 2918 (C-H str),2220-2225(C-N str),1255(C-O-Cstr),1475(-CH_2str), 806 (*s*-triazine C-N str). ^{1}H NMR: δ 3.34-3.70 (8H, m, morpholine), 2.50 (3H,s,-CH_3),6.71-6.87(2H, d, Ar-H), 7.28-7.33 (2H, d, Ar-H), 7.82 (2H, d, Ar-H), 9.49 (1H, s, -NH); ^{13}C NMR: δ 103.8(1C, CN), 21.9 (1C, CH_3), 118.1–149.1 (Ar–C),161.2,174.3, 176.5 (C=N of *s*-triazine), 57.8, 70.3 (4C, morpholine); MS: *m/z* 404.41.

4-({4-[(3-Chloro-4-fluorophenyl)amino]-6-(morpholin-4-yl)-1,3,5-triazin-2- yl}oxy)benzonitrile, 5u: IR (*v*max in cm^{-1}): 3416 (-NH str), 2918 (C-H str),2220-2225(C-N str),1255(C-O-C str),1475(-CH_2str), 806 (*s*-triazine C-N str); ^{1}H NMR: δ 3.34-3.70 (8H, m, morpholine), 2.50 (3H,s,-CH_3),6.71-6.87(2H, d, Ar-H), 7.28-7.43 (2H, d, Ar-H), 7.92 (2H, d, Ar-H), 9.55 (1H, s, -NH); ^{13}C NMR: δ 104.9(1C, CN), 22.9 (1C, CH_3), 118.1–149.1 (Ar–C),162.1,172.2, 177.7 (C=N of *s*-triazine), 58.9, 71.4 (4C, morpholine); MS: *m/z* 426.83.

Biological activity

Methods of *in vitro* evaluation of biological activities.

Antimycobacterial activity

The preliminary antimycobacterial assessment for the final synthesized compounds was carried out using BACTEC MGIT method and the secondary antimycobacterial screening for test compounds was obtained for *M. tuberculosis* H37Rv, by means of L.J. MIC method (Table I).

Antimicrobial activity

The synthesized *s*-triazinyl derivatives **5a–u** were examined for their antimicrobial activities against several bacteria. Gram positive (*S. aureus*, ATCC- 5923, *B. cereus* ATCC-29212) gram negative (*E. coli* ATCC 25922, *P.aeruginosa* ATCC27853), and fungi (*A.niger* ATCC 11651 and *C. albicans* ATCC 11394) species using paper disk-diffusion technique and MIC of the compound was determined by agar streak dilution method (Table II).

Conclusions

In this article, we have presented the initial efforts made toward the discovery of novel, potentially activecoumarin, quinoline, isoniazid and morpholine based *s*-triazine derivatives. Owing to thepresence of three pharmacologically active nuclei in one single molecule, compounds have potent antimicrobial and antituberculosis effect. From the bioassay it is clear that the introduction of appropriate substituent on the *s*-triazine ring would lead to the more active antimicrobial derivatives. It can be stated that the variation of antimicrobial activity may be associated with the nature of tested microorganisms and is due to the chemical structure of the tested compounds. In the present study, higher potency has been observed for the final compounds bearing amine derivatives with electron withdrawing groups like chlorine and fluorine atom(s) and electron-releasing methyl and methoxy functional group(s). These privileged structures with their enhanced bioactivities represent an ideal source of core scaffolds for the design of molecules with ability to target various pathogenic microbes for further drug dis- covery process.

Acknowledgments

The authors are thankful to Director, CSIR-CDRI, Lucknow, RSIC, Chandigarh and CSIR-NCL, Pune for providing spectral and analytical data of the compounds. They are also thankful to the Microcare Laboratory, Surat (Gujarat) for the biological activity.

References

1 Ducati R G, Ruffino-Netto A, Basso L A & Santos D S, *Memórias do Instituto Oswaldo Cruz*, 101 (2006) 697
2 WHO report 2019. Available from: http://www.who.int/tb/publications/.
3 Bastian I & Colebunders R, *Drugs*, 58 (1999) 633.
4 Barry C E, Slayden R A, Sampson A E & Lee R E, *BiochemPharmacol*, 59 (2000) 221
5 WHO report 2007, Available from: http://www.who.int/tb/publications/cds_tb_ 2003_313/en/index.htmlandhttp://www.who. int/tb.xdr/faqs/en/.
6 Joshi R, Reingold A L, Menzies D & Pai M, *PLoS Medicine*, e494 (2006).
7 RavalJ P & Desai K R, *E-J Chem*, 1 (2004) 211.
8 Kumar A, Srivastava K, Kumar S R, Puri S K &Chauhan P M S, *Bioorg Med Chem Lett*, 18 (2008) 6530.
9 Singh B K, Mishra M, Saxena N, Yadav G P, Maulik P R, Sahoo M K, Gaur R L, Murthy P K & Tripathi R P, *Eur J Med Chem*, 43 (2008) 2717.
10 Solankee A, Kapadia K, Ciric A, C M, Doytchinova I S & Geronikaki A, *Eur J Med Chem*, 45 (2010) 510.
11 Xiong Y, Chen F, Balzarini J, Clercq E & Pannecouque C, *Eur J Med Chem*, 43 (2008) 1230.
12 Sunduru N, Gupta L, Chaturvedi V, Dwivedi R, Sinha S & Chauhan P M S, *Eur J Med Chem*, 45 (2010) 3335.
13 Kaswala P B, Chikhalia K H, Shah N K, Patel D P, Patel D H & Mudaliar G V, *ARKIVOC*, 11 (2009) 326.
14 Chikhalia K H & Patel M J, *J Enzyme Inhib Med Chem*, 24 (2009) 960.
15 Srinivas K, Srinivas U, Bhanuprakash K, Harakishore K, Murthy U S N & Rao V J, *Eur J Med Chem*, 41 (2006) 1240.
16 Baliani A, Bueno G J, Stewart M L, Yardley V, Brun R, Barrett M P & Gilbert I H, *J Med Chem*, 48 (2005) 5570.
17 Menicagli R, Samaritani S, Signore G, Vaglini F & Via L D, 47 (2004) 4649.
18 Melato S, Prosperi D, Coghi P, Basilico N & Monti D, *Chem Med Chem*, 3 (2008) 873.
19 Mahajan D H, Pannecouque C, Clercq E D & Chikhalia K H, *Arch Pharm*, 342 (2009) 281.