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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Novel S-triazine accommodated 5-benzylidino-4-thiazolidinones: synthesis and in vitro biological evaluations

Aniruddhasinh M. Rana · Pooja Trivedi · Kishor R. Desai · Smita Jauhari

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Abstract In order to explore various pharmacological effects associated with S-triazine accommodated 5-benzylidino-4-thiazolidinones, a series of compounds based on 5-benzylidene-2-[4-(4,6-bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-thiazolidin-4-one were synthesized. Variation in the functional group at 5-benzylidine ring of thiazolidinone moiety led to a set of compounds bearing S-triazine accommodated with 4-thiazolidinones. Structures of the synthesized final compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR spectroscopy, ESI mass spectrometry, and elemental analysis. Synthesized compounds were screened against some bacterial and fungal strains using Kirby-Bauer disk diffusion technique and serial broth dilution technique. Synthesized compounds were screened to find out antitubercular activity against *Mycobacterium tuberculosis* H37RV by L. J. MIC method and BACTEC MGIT method. Relations between structure and its biological activities have been discussed.

Keywords S-triazine · 5-Benzylidino-4-thiazolidinone · SAR study · Antituberculosis activity · Antimicrobial activity

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A. M. Rana · P. Trivedi · S. Jauhari (✉)
Applied Chemistry Department, S.V. National Institute of Technology, Surat 395007, Gujarat, India
e-mail: smita.jauhari11@gmail.com

K. R. Desai
Department of Chemistry, Uka-Tarsadiya University, Maliba Campus, Bardoli, Gujarat, India

Introduction

Tuberculosis (TB) is the leading infectious cause of the death in the world today. According to the Global Tuberculosis Report, 2012, by WHO, about 8.7 million new cases of TB (13 % co-infected with HIV) were registered in 2011 (Global Tuberculosis Report, 2012, WHO). According to FDA, the synergy of the TB with HIV infections as well as the emergence of multidrug resistance TB (MDR-TB) and extensively drug resistance TB (XDR-TB) pose a threatening global challenge. Additionally, brewing public health crises has been obsessed by two major key factors, the emergence of resistance in pathogenic species and the recession amount of new potential drugs coming out through the pharmaceutical companies, with very low amount of new drugs approval since 2003 (FDA report, 2012). Additionally, the increasing public awareness in search for a new class of biologically active agents to address resistance provides motivation for the innovation of these new types of potential analogs (Tenover, 2006). Thus, the need for new antimicrobial and antimycobacterial agents requires an innovative, reliable, and efficient method that elevates a breakthrough in the identification of new biologically active agents.

Moreover, thiazolidinones have been considered as privileged structural fragments in pharmaceutical chemistry due to their broad spectrum of biological activities and affinity toward various bio targets. 4-thiazolidinones have been reported as novel inhibitors of the bacterial enzyme Mur B, which is precursor acting during synthesis of peptidoglycan (Andres *et al*., 2000), where peptidoglycan is an essential component of the cell wall of the both gram-positive and gram-negative bacteria. Also, 4-thiazolidinones have good affinity toward various bio targets such as JSP-1 (JNK Stimulating Phosphatase-1) (Cutshall *et al*.,

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2005), Tumor necrosis factor-α (TNF-α) (Carter *et al*., 2001), Cyclo oxygenase-2 (COX-2) (Charlier and Mishaux, 2003), and L-alaligase (Sim *et al*., 2002); thus, showing potency as anticancer-antidiabetic, antitumor, anti-inflammatory, and antimicrobial agents. S-triazine analogs have gained substantial attention due to their broad spectrum of biological activities such as antimicrobial (Gavade *et al*., 2012), antitumor (Arya and Dandia, 2007), antitubercular (Sunduru *et al*., 2010), and antimalarial (Kumar *et al*., 2008, 2009).

The combination of two biologically active substructures into a single molecular frame to achieve drugs has also received considerable attention (Hutchinson, 2004). So, with the hope of good biological activity and as a part of our ongoing research (Rana *et al*., 2012, 2013a, b; Desai and Desai, 2005, 2006; Patel *et al*., 2006), in synthesizing new antimicrobial and antituberculosis agents, a series of compounds have been synthesized in such a way so that final compounds contain both trisubstituted S-triazine and 5-benzylidino-4-thiazolidinones in a single molecular frame. In this work, the structural variations were selected by introducing different benzylidine substituents at the fifth position of the 4-thiazolidinone moiety. The purpose of this investigation is to find out the effect of different benzylidine substituents on various biological activities, as they have been exploited as bioactive arms on heterocyclic scaffolds (Gududuru *et al*., 2004, Saleh *et al*., 2010, Bruno *et al*., 2002).

Results and discussion

Chemistry

5-Benzylidene-2-[4-(4,6-bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-thiazolidin-4-one based compounds were synthesized by known synthetic approaches like nucleophilic substitution, chloro acetylation, cyclization, and Knoevenagel condensation as illustrated in Figs. 1, 2, 3, 4. The starting material, S-triazine, on nucleophilic substitution reaction twice with dimethyl amine as a nucleophile yields intermediate-3 by following the reported procedure earlier (Saleh *et al*., 2010). By following the same scenario, the third –Cl of S-triazine was replaced by para phenylene diamine to yield intermediate-4 using 1,4-dioxane as a solvent along with triethyl amine in a catalytic amount. Intermediate-4 was confirmed by the $-NH_2$ stretching frequency observed at 3315.74 cm^{-1} in its IR spectrum. N^6-(4-aminophenyl)-N^2,N^2,N^4,N^4-tetramethyl-1,3,5-triazine-2,4,6-triamine (intermediate-4) on chloro acetylation reaction with chloroacetic acid and sodium acetate yielded intermediate-5. Formation of the intermediate-5 was confirmed by disappearance of the characteristic $-NH_2$ stretching frequencies and manifestation of C=O stretching frequencies in IR spectrum. Intermediate-5 on hetero cyclization reaction with ammonium isothiocyanate in ethanolic medium yielded a new structural entity i.e., intermediate-6 having two substructures S-triazine and thiazolidine-4-one in a single molecular frame. The proposed mechanism for the cyclo condensation step to yield intermediate-6 is presented in Fig. 2. The structure of the intermediate-6 was confirmed by ^{1}H NMR spectrum by considering singlet of –NH lactam proton of thiazolidinone ring in the downfield region at 8.69 δ ppm. Intermediate-6 on Knoevenagel condensation reaction with substituted benzaldehydes derivatives, in the presence of piperidine, yielded final compounds. Same condensation reaction was also carried out in acidic condition using glacial acetic acid solvent along with sodium acetate. Reactions of various benzaldehydes with intermediate-6, in both medium, suggested that reaction in basic condition is more preferable than acidic one considering yield and reaction time. Thus, for synthesis of rest of final compounds only basic conditions were preferred (Table 1).

Formation of the *Z* isomer of the synthesized compounds was confirmed by ^{1}H NMR spectral analysis, since the exocyclic methine proton was observed in the downfield region comparatively, due to deshielding effect of the adjacent carbonyl group. Thus, by considering downfield singlet observed in the region of 7.72–8.11 δ ppm, it was concluded that only *Z* isomers of final compounds formed rather than the *E* isomer which is expected to be resonated in comparatively upfield region (Rana *et al*., 2012, Bruno *et al*., 2002) (Figs. 4, 5). Additionally, it was observed that *E* isomer was never obtained, also under different experimental conditions (i.e., performing the Knoevenagel condensation with piperidine in ethanolic medium or with sodium acetate in glacial acetic acid). ^{1}H NMR spectrum of compound 8a revealed a downfield singlet at 8.69 δ ppm accounting for a –NH lactam proton of the 4-thiazolidinone ring; moreover, proton of –NH attached with triazine moiety was observed at 8.34 δ ppm. Protons of two $-N(C\underline{H}_3)_2$ groups were observed in the range of 3.05–3.30 δ ppm. ^{13}C NMR spectrum of compound **8a** revealed a peak at 165.12 δ ppm accounting for a C=O of a thiazolidinone ring and a peak at 133.98 δ ppm for a carbon of exocyclic methine (i.e. C=$\underline{C}$H). Aromatic carbons of S-triazine ring were observed in the downfield region at 163.61 δ ppm, and rest all aromatic carbons were observed in the range of 119.16–128.78 δ ppm. Mass spectra of final compound *8a* showed M+1 peak at 461.38 m/z as a most stable fragment with intensity of 100 % (Fig. 6).

Pharmacology

Synthesized final compounds were assayed for in vitro antimicrobial activity by serial broth dilution technique to

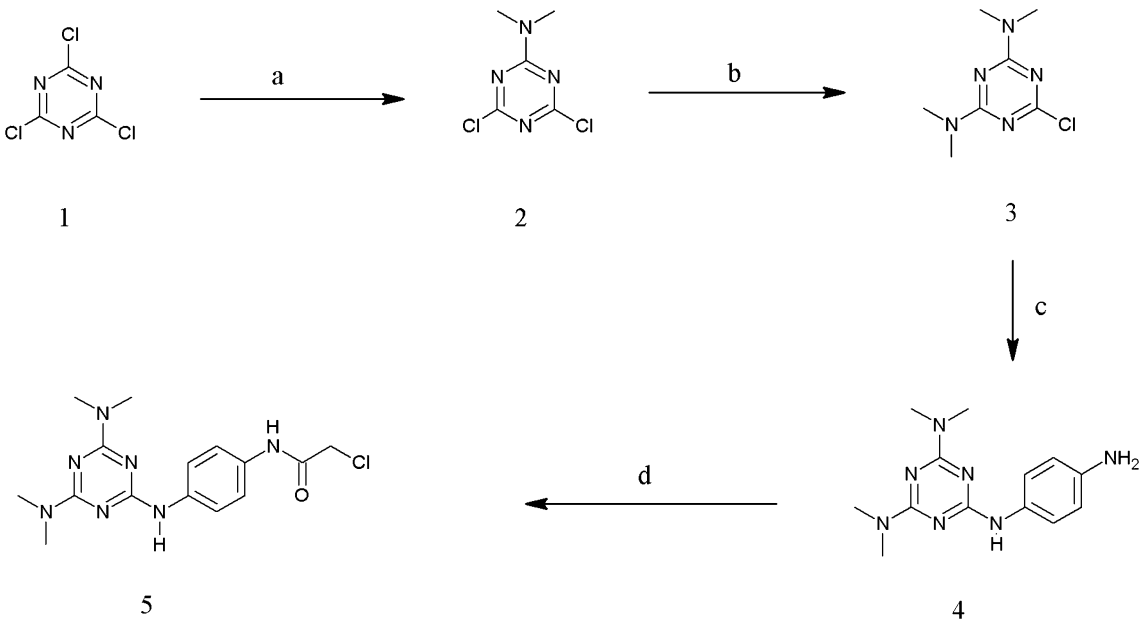


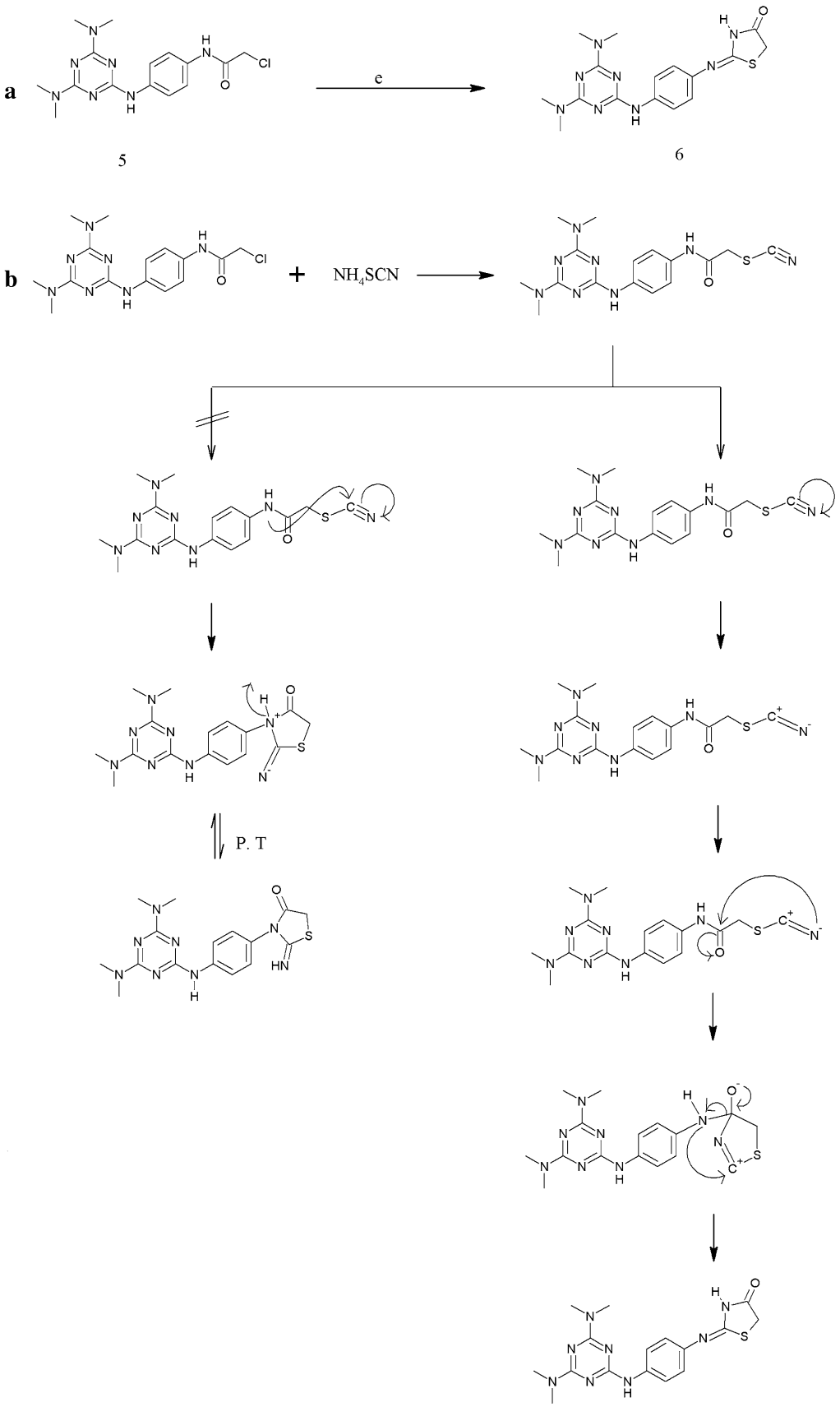
Fig. 1 Schematic diagram for the synthesis of intermediate-5; where **a** $(CH_3)_2NH$, acetone, K_2CO_3, 0–5 °C, 2 h, **b** $(CH_3)_2NH$, acetone, K_2CO_3, 35 °C, 8 h, **c** para phenylene diamine, 1,4-dioxane, Et_3N, reflux, 7 h, **d** chloroacetyl, chloride, acetone, reflux, 6 h

investigate MIC values and bared to find out zone of inhibition by Kirby-Bauer disk diffusion technique against the following various groups of microorganisms (Eukaryotes and Prokaryotes): Bacteria (Gram-positive bacilli: *B. subtilis,* Gram-positive cocci: *S. aureus,* and Gram-negative bacilli: *E. coli* and *P. aeruginosa*), and Fungi (Yeast strain: *C. albicans* and Filamentous fungi: *A. niger*) (Fig 3).

Antibacterial evaluation

The results of the antibacterial screening of the synthesized compounds against gram-positive and gram-negative bacterial strains are reported in Table 2 (serial broth dilution technique for MIC value) and Table 3 (Kirby-Bauer technique to determine zone of inhibition), and compared with the standard drugs. The impact of the substituent at the 5-benzylidine ring on biological activity has been reported. By unification of the results obtained from both the techniques (MIC determination and Zone of inhibition study), the activity differences between the compounds bearing the same MIC values were measured. In general, it was observed that the final compounds with halo substituent (–Cl, –F) at the 5-benzylidine ring (**8b**, **8c**, **8d**, **8e**, **8f**) showed improved activity (MIC $\leq$40 μg ml^{-1}) against strain *E. coli* with exception of compound 8g in comparison with the unsubstituted final compound **8a** (MIC, 80 μg ml^{-1}). Compounds with electron-withdrawing groups (–Br, $–NO_2$, and $–CF_3$) at 5-benzylidine ring were found inert in contribution for activity against *E. coli* and showed potency at 80 μg ml^{-1} of MIC. Final compound with electron-releasing $–OCH_3$ group at *para* position (**8j**) showed good potency at 20 μg ml^{-1} of MIC against *E. coli*, in comparison with the standard drugs and parent unsubstituted compound **8a** (MIC, 80 μg ml^{-1}). Final compound with electron-withdrawing chloro group at *ortho* position (**8d**) was found to be the most active and showed potency at 20 μg ml^{-1} of MIC against Gram-negative strain *P. aeruginosa*. Focusing to the activity results of compounds **8m** and **8o**, it was remarked that the presence of electron-withdrawing groups ($–CF_3$ and $–NO_2$) at *para* position of 5-benzylidine ring decreased inhibition potency against *P. aeruginosa*, and also other electron-withdrawing –Cl and –Br groups at *para* position were found to be inert in activity contribution. Electron-releasing $–CH_3$ group at *para* and *meta* positions of the 5-benzylidine ring (**8h** and **8i**) was found to be responsible for loss of activity against *P. aeruginosa,* and showed potency at 100 μg ml^{-1} of MIC.

From the obtained activity results against gram-positive strain *S. aureus*, it was marked that the presence of electron-withdrawing –Cl substituent at *ortho*, *meta*, or *para* positions (**8b**, **8c**, **8d**, and **8e**) decreased the inhibition potency in comparison with the unsubstituted parent compound **8a** (MIC, 60 μg ml^{-1}) while contradictory to that electron-withdrawing $–NO_2$ group at *para* and *meta* positions (**8m** and **8n**) increased the inhibition potency and showed activity at 20 and 40 μg ml^{-1} of MIC, respectively. In general, it was marked that for better inhibition against *S. aureus*, the presence of electron-withdrawing -F, –Br, and $–NO_2$ groups are more favorable than chloro


a
e
5
6
b
+
NH4SCN
P. T

◀**Fig. 2** **a**, Schematic diagram for the synthesis of intermediate-6, where **e** NH_4SCN, ethanol, reflux, 6 h. **b** Proposed mechanism for the synthesis of intermediate-6

group containing compounds. These series of compounds showed maximum inhibition potency against gram-positive bacterial strain *B. subtilis* than other tested bacterial strains

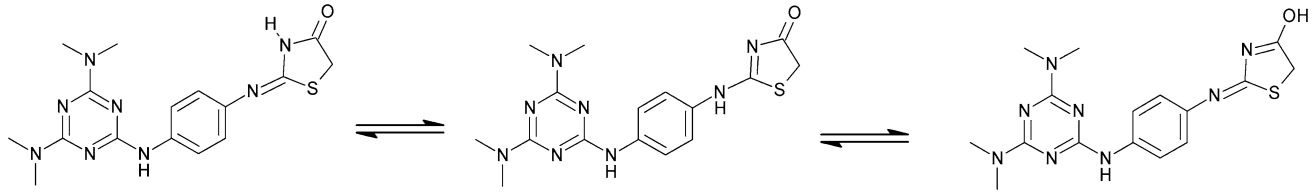


Fig. 3 Possible tautomerism of intermediate-6

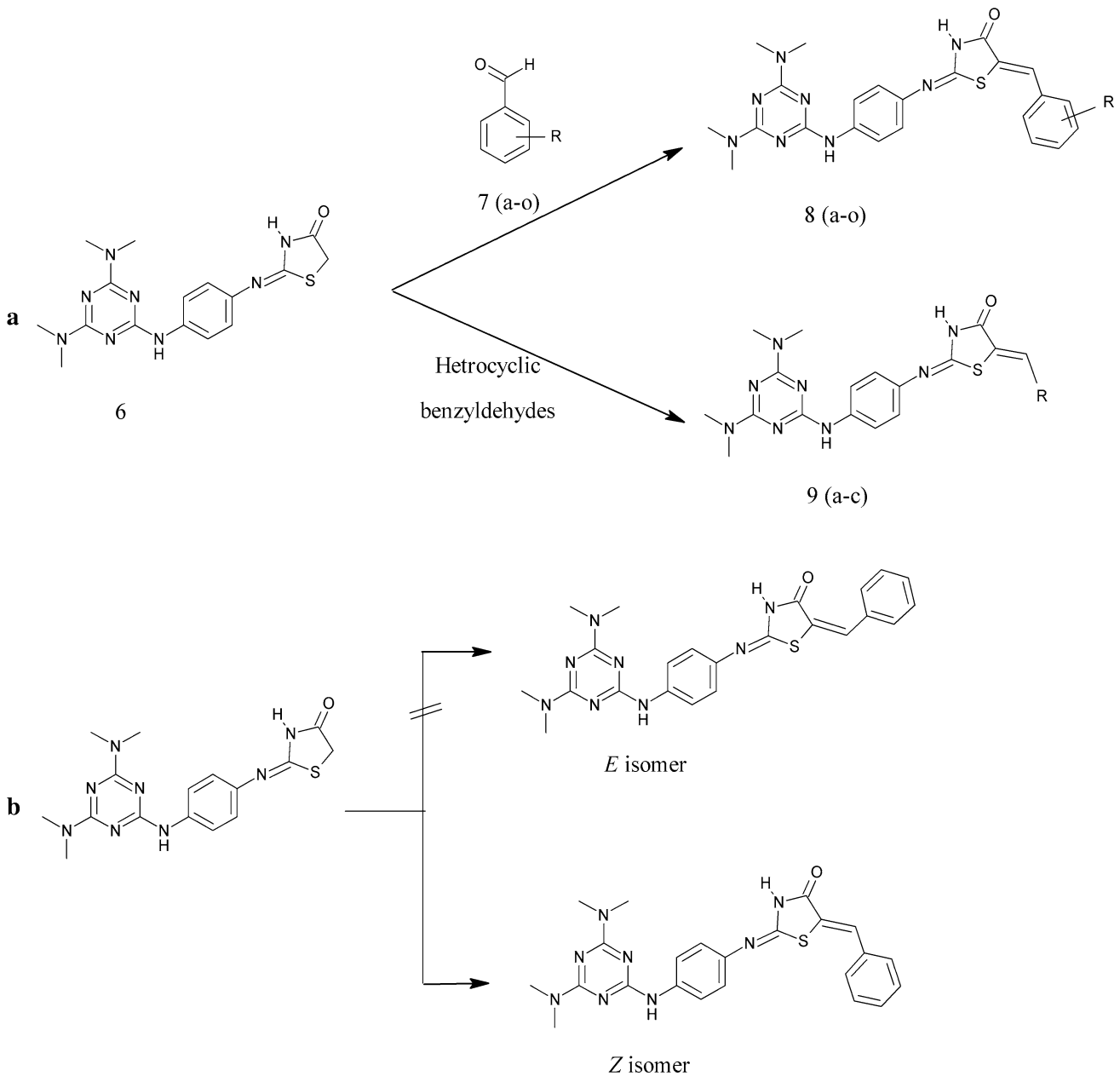


Fig. 4 **a** Schematic diagram for the synthesis of final compounds; where **f**:ethanol, piperidine, **8(a–o)** R = **8a**: H, **8b**: 4-CI, **8c**: 3-CI, **8d**: 2-CI, **8e**: 2,4-CI, **8f**: 3-F, **8g**: 4-Br, 8 h:4-CH_3, **8i**: 3-CH_3, **8j**: 4-OCH_3, **8k**: 3-OCH_3, **8l**: 3,4-OCH_3, **8m**: 4-NO_2, **8n**: 3-NO_2, **8o**: 4-CF_3; **9(a–c)** R = **9a**: 2-pyrrole, **9b**: 2-indole, **9c**: 3- thiopene. **b** **E–Z** isomerism of final compounds

Table 1 Physical and analytical data of synthesized compounds

Entry	Mol. formula	M.W	M.P. (°C)	Elemental analysis					
				Found (%)			Calculated (%)		
				C	H	N	C	H	N
8a	$C_{23}H_{24}N_8OS$	460.55	253–254	60.04	5.27	24.34	59.98	5.25	24.33
8b	$C_{23}H_{23}ClN_8OS$	495.00	212–213	55.83	4.71	22.65	55.81	4.68	22.64
8c	$C_{23}H_{23}ClN_8OS$	495.00	217–218	55.86	4.70	22.66	55.81	4.68	22.64
8d	$C_{23}H_{23}ClN_8OS$	495.00	223–225	55.84	4.75	22.68	55.81	4.68	22.64
8e	$C_{23}H_{22}Cl_2N_8OS$	529.44	238–240	52.25	4.24	21.19	52.18	4.19	21.16
8f	$C_{23}H_{23}FN_8OS$	478.55	>270	57.77	4.87	23.47	57.73	4.84	23.42
8g	$C_{23}H_{23}BrN_8OS$	539.45	209–210	51.20	4.29	20.75	51.21	4.30	20.77
8h	$C_{24}H_{26}N_8OS$	478.58	215–216	60.79	5.54	23.62	60.74	5.52	23.61
8i	$C_{24}H_{26}N_8OS$	478.58	221–222	60.81	5.55	23.63	60.74	5.52	23.61
8j	$C_{24}H_{26}N_8O_2S$	490.58	230–231	58.78	5.38	22.85	58.76	5.34	22.84
8k	$C_{24}H_{26}N_8O_2S$	490.58	230–231	58.79	5.37	22.85	58.76	5.34	22.84
8l	$C_{25}H_{28}N_8O_3S$	520.61	263–264	57.72	5.45	21.55	57.68	5.42	21.52
8m	$C_{23}H_{23}N_9O_3S$	505.55	215–216	54.66	4.60	24.95	54.64	4.59	24.94
8n	$C_{23}H_{23}N_9O_3S$	505.55	264–265	54.68	4.62	24.96	54.64	4.59	24.94
8o	$C_{24}H_{23}F_3N_8OS$	528.55	>270	54.58	4.43	21.22	54.54	4.39	21.20
9a	$C_{21}H_{23}N_9OS$	449.53	253–254	56.14	5.17	28.05	56.11	5.16	28.04
9b	$C_{25}H_{25}N_9OS$	499.59	259–260	60.14	5.07	25.28	60.10	5.04	25.23
9c	$C_{21}H_{22}N_8OS_2$	466.58	267–268	54.11	4.74	24.03	54.06	4.75	24.02

i.e., *E. coli*, *P. aeruginosa*, and *S. aureus*. Compounds **8d**, **8g**, and **8m** showed very good inhibition potency (MIC $\leq$20 μg ml^{-1}) against strain *B. subtilis* in comparison with the unsubstituted parent compound (**8a**) and the reference drugs. Compounds with electron-releasing groups at the 5-benzylidine ring showed good to moderate inhibition potency ranging from 40 to 60 μg ml^{-1} of MIC. It was observed that for good activity against strain *B. subtilis*, the final compounds with electron-withdrawing groups are more preferable than final compounds with electron-releasing groups.

To check the contribution of the heterocyclic scaffolds (pyrrole, indole and thiophene) at the 5th position of thiazolidinone moiety in activity, compounds **9a**, **9b** and **9c** were screened against the same bacterial strains. Here, for the inhibition of *E. coli*, compound **9a** showed twofold better activity (MIC, 40 μg ml^{-1}) than **9b** and **9c** (MIC, 80 μg ml^{-1}). For inhibition of gram-negative strain *P. aeruginosa*, both **9a** and **9b** showed good potency at 40 μg ml^{-1} of MIC while compound **9c** was found inactive up to 150 μg ml^{-1} concentrations. For inhibition of strain *S. aureus*, compound **9c** showed twofold better activity than compounds **9a** and **9b**. Compound **9b** showed very good inhibition potency at 20 μg ml^{-1} of MIC against strain *B. subtilis* in comparison with the reference drugs Ampicillin and Carbenicillin.

Antifungal evaluation

Antifungal screening results of synthesized compounds are reported in Table 2 (broth dilution technique for MIC determination) and Table 3 (Kirby-bauer technique for Zone of inhibition determination), where obtained results were compared with the reference drugs. In general it was observed that, compounds showed very poor anti-Candida activity (MIC, $\geq$100 μg ml^{-1}) when they were substituted by electron-withdrawing groups (–Cl, –F, –Br, $–NO_2$ and $–CF_3$) at the 5-benzylidine ring. Final compounds with electron-releasing groups ($–CH_3$ and $–OCH_3$) at 5-benzylidine ring, showed moderate to poor anti-Candida activity (MIC, 60–150 μg ml^{-1}). Compounds **8h** and **8j** having electron-withdrawing $–CH_3$ and $–OCH_3$ groups at *para* position showed poor to moderate potency at >150 and 80 μg ml^{-1} of MIC respectively, and when the same substituent were present at the *meta* position of the 5-benzylidine ring, there is improvement in the anti-Candida activity, leading to the conclusion that electron-releasing $–CH_3$ and $–OCH_3$ groups at *meta* position of final compounds favors anti-Candida activity than compounds with these groups at *para* position. Somewhat same scenario was observed for the inhibition of fungal strain *A. niger*, where comparatively compounds with electron-releasing groups showed better activity than the rest compounds having electron-withdrawing groups. Final

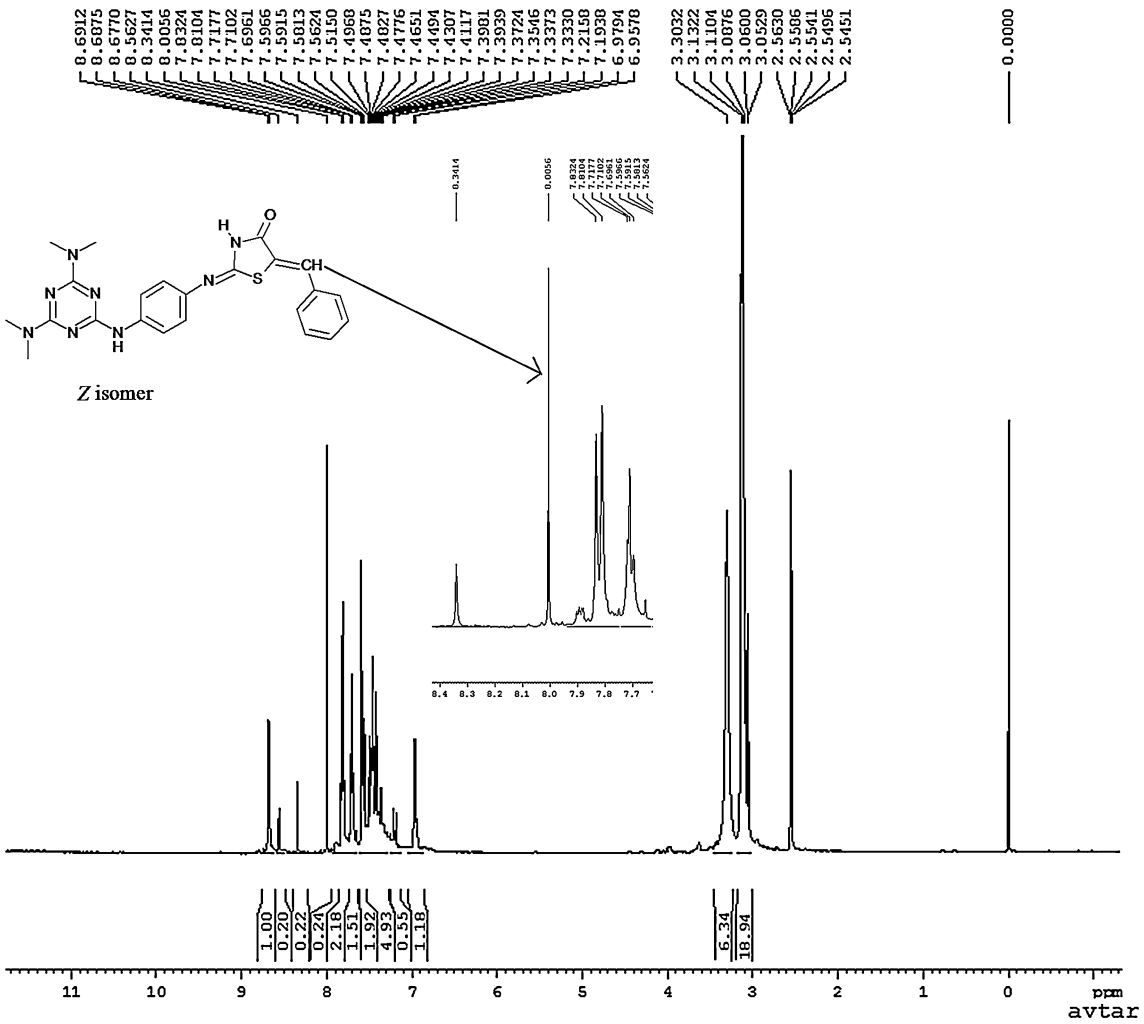


Fig. 5 ^{1}H-NMR spectrum of synthesized compound **8a**

compound with electron-releasing –CH_3 and –OCH_3 groups at *para* position showed potency at 100 and 40 μg ml^{-1} of MIC, respectively, while final compounds with same groups at *meta* position showed improvement in activity against *A. niger* (60 and 20 μg ml^{-1} of MIC, respectively). In general, it was concluded that –OCH_3 group at *meta*, *para*, or *meta-para* positions showed very good antifungal activity against *A. niger* (MIC, 20 or 40 μg ml^{-1}). Compounds with heterocyclic moiety at the 5th position of 4-thiazolidinone (**9a**, **9b**, and **9c**) showed deprived antifungal activity against both of the fungal strains *C. albicans* and *A. niger*. Among all final synthesized compounds, electron-releasing –OCH_3 group bearing compounds (**8j**, **8k**, and **8l**) only showed good antifungal activity against both the tested fungal strains.

Antituberculosis evaluation

In vitro antituberculosis activities of the compounds **8**(**a**–**o**) and **9**(**a**–**c**) were assessed against *M. tuberculosis* H37RV using BACTEC method at single concentration, i.e., 32 μg ml^{-1}. The activity results are presented in Table 4. Screening results of compounds **8g**, **8j**, and **8m** showed 99 % or higher inhibition at 32 μg ml^{-1} of MIC, so based on that the rest of the final compounds were exposed for secondary screening by L. J. MIC method at the selected concentrations 250, 125, 62.5, and 32 μg ml^{-1}. Compounds **8g**, **8j**, and **8m** showed highest inhibition potency at 32 μg ml^{-1} of MIC. It was observed that the analogs that having electron-withdrawing substituents (–Cl, –Br, and –NO_2) at the 5-benzylidine ring presented comparatively good antituberculosis activity than the rest ones with exceptions of compound **8f**, that having –F group at *meta* position and found inactive up to 250 μg ml^{-1}. Compounds with electron-releasing –OCH_3 substituent were found more active than the analogs having –CH_3 substituent against *M. tuberculosis*. Final analogs that having electron-releasing –CH_3 group at *meta* and *para* positions were found inactive up to 250 μg ml^{-1}. Compounds with heterocyclic scaffolds (**9a**, **9b**, and **9c**) at the 5th position

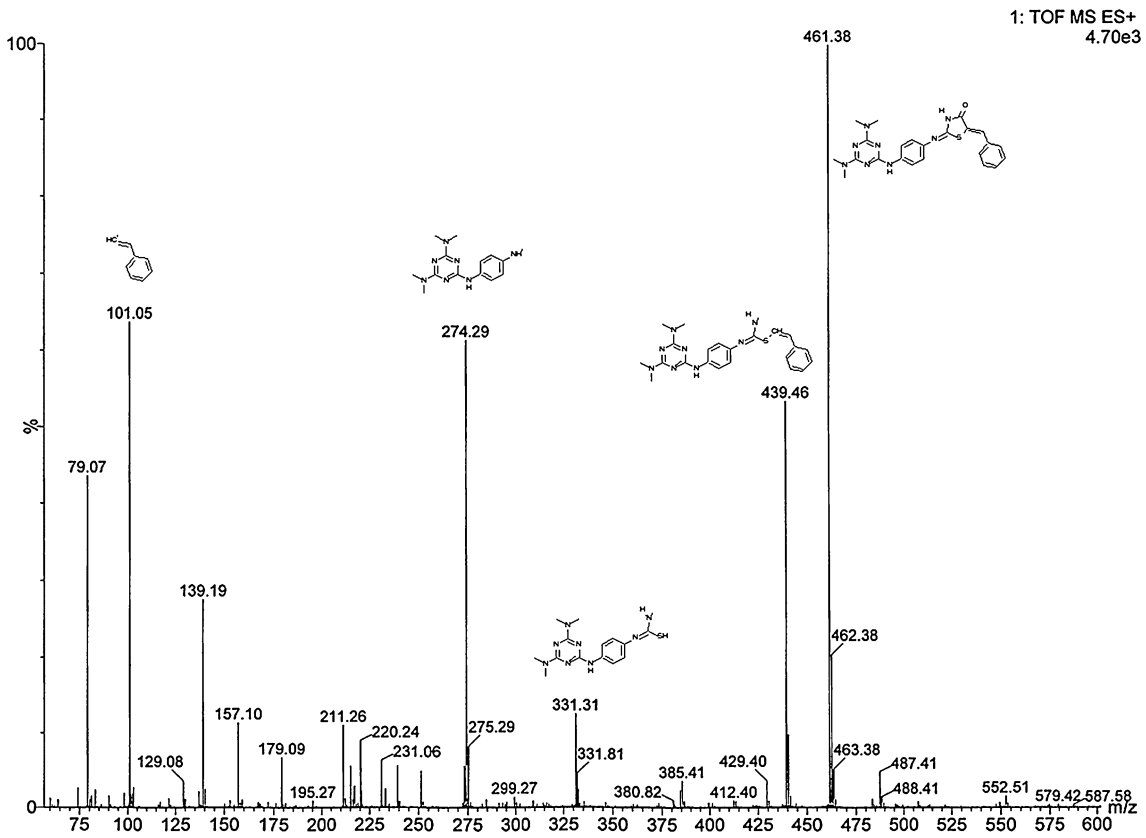


Fig. 6 Mass spectrum of compound **8a** with possible mass fragments

of thiazolidinone were found inactive up to 250 μg ml^{-1} concentration against *M. tuberculosis*. The above said statement provides hint that for better antimycobacterial activity of these series of compounds, benzylidine substituents are better than heterocyclic once at the 5th position of thiazolidinone.

Experimental methods

Materials and instrumentation

All the reagents and solvents were of analytical reagent grade and were used without further purification. Solvents were purchased commercially from Labort Chemicals Pvt Ltd., Surat. 2-chloro benzaldehyde, 3-chlorobenzaldehyde, 4-chlorobenzaldehyde, 2,4-dichloro benzaldehyde, 4-methyl benzaldehyde, and 3-methyl benzaldehyde were gifted by Benzo Chem industries Pvt Ltd., Jalgaon.

The melting points were determined in open capillaries on a Veego (Model: VMP-D, Veego Instrument Corporation, Mumbai, India) electronic apparatus and are uncorrected. To monitor the reactions as well as to establish the identity and purity of reactants and products, thin-layer chromatography was performed on E. Merck Silica gel 0.50-mm plate, and spots were visualized under UV radiation. FT-IR spectra (4,000–400 cm^{-1}) were recorded on Shimadzu spectrophotometer (Model: 8400-S, Shimadzu India Pvt Ltd., Mumbai, India) using KBr disk. ^{1}H NMR and ^{13}C NMR were performed at SAIF, Chandigarh, (Bruker Advance-II Spectrophotometer, 400 MHz) using DMSO as a solvent and TMS as an internal reference (Chemical shifts are in δ ppm). Mass analysis was performed at SAIF, Chandigarh using ESI-TOF technique.

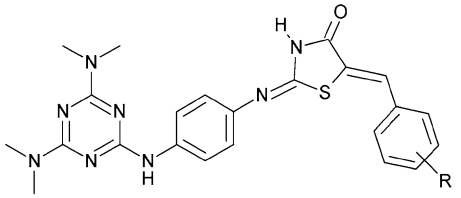
General procedure for the synthesis of 5-benzylidene-2-[4-(4,6-bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-thiazolidin-4-one based compounds

S-triazine was treated with dimethyl amine by acetone solvent and K_2CO_3 by maintaining the temperature 0–5 °C to form intermediate-2 as reported earlier (Saleh *et al.*, 2010). By following the same procedure and dimethyl amine reactant, second –Cl was also replaced by maintaining the reaction temperature 35–40 °C to form intermediate-3.

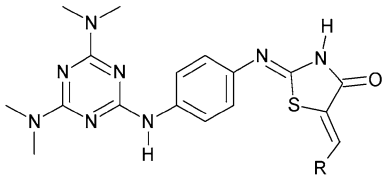
*Synthesis of N^6-(4-aminophenyl)-N^2,N^2,N^4,N^4-tetramethyl-1,3,5-triazine-2,4,6-triamine (**4**)*

Intermediate-3 was dissolved in 1,4-dioxane along with para phenylene diamine (1.1 eq.) and triethyl amine in a

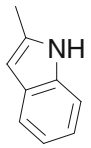
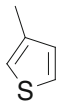
Table 2 In vitro antibacterial and antifungal activity of the newly synthesized compounds (MIC determination by serial broth dilution technique)



8 (a-o)



9 (a-c)

Entry	R	MIC in μg/ml					
		Gram-negative strains		Gram-positive strains		Fungal strains	
		E.c	*P.a*	*S.a*	*B.s*	*C.a*	*A.n*
8a	H	80	80	60	60	>150	>150
8b	4-Cl	40	80	150	80	>150	100
8c	3-Cl	40	60	100	40	100	>150
8d	2-Cl	10	20	80	20	150	60
8e	2,4-Cl	40	80	150	100	>150	80
8f	3-F	40	80	80	80	>150	150
8g	4-Br	80	>150	40	10	100	60
8h	4-CH_3	60	100	40	40	>150	100
8i	3-CH_3	100	100	60	40	100	60
8j	4-OCH_3	20	40	80	40	80	40
8k	3-OCH_3	60	80	40	40	60	20
8l	3,4-OCH_3	100	80	40	40	80	40
8m	4-NO_2	100	80	20	20	>150	100
8n	3-NO_2	80	40	40	40	>150	>150
8o	4-CF_3	80	>150	100	>150	>150	>150
9a		40	40	80	40	>150	150
9b		80	40	80	20	100	>150
9c		80	>150	40	40	150	100
NC	DMSO	–	–	–	–	–	–
PC_1	Ciprofloxacin	10	10	5	5	–	–
PC_2	Ampicillin	40	>150	40	60	–	–
PC_3	Carbenicillin	80	40	>150	40	–	–
PC_4	Flucanazole	–	–	–	–	5	5
PC_5	Ketaconazole	–	–	–	–	20	>150
PC_6	Itraconazole	–	–	–	–	>150	100

E.c, *Escherichi coli*; *P.a*, *Pseudomonas aeruginosa*; *S.a Staphylococcus aureus*; *B.s Bacillus subtilis*; *C.a Candida albicans*; *A.n Aspergillus niger*, *NC* negative control, *PC* positive control

catalytic amount, and reaction mixture was refluxed for about 6–8 h. The status of the reaction was monitored by TLC using solvent system chloroform: methanol (4.8:0.2). On complete conversion, the reaction mass was treated with crushed ice to separate the product. Obtained brown solid product was filtered, washed with distilled water, dried, and recrystallized by methanol.

M.P: 229–230 °C; ^{1}H NMR (400 MHz, DMSO(d_6), δ ppm): 8.73 (1H, s, –NH exchangeable with D_2O), 6.97–7.52 (4H, m, Ar–H), 4.61 (2H, s, –NH_2), 3.03–3.31 (12H, s, 2N(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO (d_6), δ ppm): 163.61 (Ar–C, S-triazine ring), 127.14–120.11 (Ar–C), 35.59–35.57 (N–(CH_3)$_2$); IR (KBr, cm^{-1}): 3315.74 (–NH str., 1° amine), 1508.94 (ArC–H, str.), 1396.28 (C–N str.)

*Synthesis of N-[4-(4,6-bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenyl]-2-chloro-acetamide (**5**)*

Intermediate-4 was dissolved in dry acetone along with chloroacetyl chloride (1.1 eq.) and K_2CO_3 (1.3 eq.) maintaining the temperature 0–5 °C during addition. After complete addition, the reaction mixture was refluxed for about 6–8 h. The status of the reaction was monitored by TLC using solvent system chloroform: methanol (4.8:0.2). After complete conversion, the reaction mass was treated with crushed ice to separate the product. Obtained brown solid product was treated with saturated $NaHCO_3$ solution followed by washing with distilled water, dried, and recrystallized by acetone.

M. P: 215–216 °C; ^{1}H NMR (400 MHz, DMSO(d_6), δ ppm): 9.32 (1H, s, –NH–C=O), 8.75 (1H, s, –NH exchangeable with D_2O), 6.97–7.61 (4H, m, Ar–H), 4.13 (2H, s, –CH_2), 3.07–3.30 (12H, s, 2N(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO(d_6), δ ppm): 163.08 (C=O), 161.58 (Ar–C, S-triazine ring), 125.55–119.92 (Ar–C), 35.60–35.56 (N–(CH_3)$_2$); IR (KBr, cm^{-1}): 3437.86 (–NH str., 2° amine), 1693.20 (–C=O str.)

*Synthesis of 2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-thiazolidin-4-one (**6**)*

Intermediate-5 was dissolved in ethanol, and ammonium thiocyanate (1.1 eq.) in ethanol was added gradually. Reaction mixture was refluxed for about 6–8 h. The status of the reaction was monitored by TLC using solvent system chloroform: methanol (4.8:0.2). After complete conversion, the reaction mixture was treated with crushed ice to separate the product. Obtained brown solid product was filtered, washed with distilled water, dried, and recrystallized by using ethanol.

M.P: 200–201 °C; ^{1}H NMR (400 MHz, DMSO(d_6), δ ppm): 8.73 (1H, s, –NH exchangeable with D_2O), 8.68 (1H, s, –NH exchangeable with D_2O), 6.94–7.59 (4H, m, Ar–H), 3.82 (2H, s, –CH_2, 4-thiazolidinone), 3.06–3.28 (12H, s, N(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO(d_6), δ ppm): 163.84 (C=O), 163.18 (C=O), 161.63 (Ar–C, S-triazine ring), 126.39–119.78 (Ar–C), 35.77–35.48 (N–(CH_3)$_2$), 32.28 (–CH_2, 4-thiazolidinone); IR (KBr, cm^{-1}): 3440.19 (–NH str., 2° amine), 1704.22 (C=O str.), 1396.72 (C–N str.).

*General procedure for synthesis of 5-Benzylidene-2-[4-(4,6-bis-dimethylamino-[1,3,5]triazin-2-ylamino)-PHENYLIMINO]-thiazolidin-4-one based compounds (**8a–o** and **9a–c**)*

Intermediate-6 was dissolved in ethanol along with substituted benzaldehydes (**7a–o**) (1.1 eq.) and piperidine (1.3 eq.) as a catalyst. Reaction mixture was refluxed for about 6–8 h. The status of the reaction was monitored by TLC using solvent system chloroform: methanol (4.8:0.2). After complete conversion, the reaction mass was treated with crushed ice to separate the product. Obtained green solid product was filtered, washed with distilled water, dried, and recrystallized by glacial acetic acid.

Same above-mentioned knoevenagel condensation can also be carried out in acidic condition. First, the intermediate-6 was dissolved in glacial acetic acid along with different benzaldehyde derivatives (1.1 eq.) and sodium acetate (2.1 eq.). Reaction mass was refluxed for about 10–12 h. The status of the reaction was monitored by TLC using solvent system chloroform: methanol (4.8:0.2). After complete conversion, the reaction mass was treated with crushed ice to separate the product. Obtained green solid product was filtered, washed with distilled water, dried, and recrystallized by glacial acetic acid.

*5-Benzylidene-2-[4-(4,6-bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-thiazolidin-4-one (**8a**)* ^{1}H NMR (400 MHz, DMSO(d_6), δ ppm): 8.69 (1H, s, –NH exchangeable with D_2O), 8.34 (1H, s, –NH exchangeable with D_2O), 8.005 (1H, s, exocyclic=CH), 6.97–7.83 (9H, m, Ar–H), 3.05–3.30 (12H, s, N(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO(d_6), δ ppm): 165.12 (C=O), 163.61 (Ar–C, S-triazine ring), 133.98 (=CH), 129.33 (C=CH, C_5 of thiazolidinone), 129.26 (C=N, thiazolidinone), 128.78–119.16 (Ar–C), 35.63–35.56 (N–(CH_3)$_2$); IR (KBr, cm^{-1}): 3433.86 (–NH str., 2° amine), 1501.29 (ArC–H str.), 1396.72 (C–N str.); TOF–MS (*m*/*z*): 461.38 $[M+H]^+$, 439.46, 331.31, 274.29, 101.05.

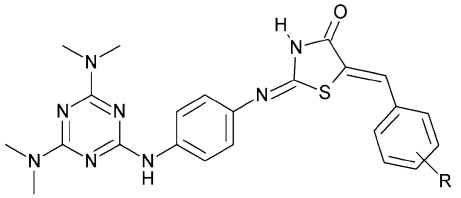
*2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-5-(4-chloro-benzylidene)-thiazolidin-4-one (**8b**)* ^{1}H NMR (400 MHz, DMSO(d_6), δ ppm): 8.71 (1H, s, -NH exchangeable with D_2O), 8.61 (1H, s, –NH exchangeable with D_2O), 7.92 (1H, s, exocyclic=CH), 6.93–7.88 (8H, m, Ar–H), 3.02–3.28 (12H, s, N(CH_3)$_2$); ^{13}C

Table: 3 In vitro antibacterial and antifungal activity (Determination of Zone of inhibition by Kirby-Bauer disk diffusion technique)

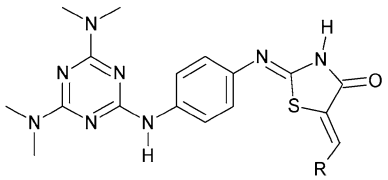
Entry	Zone of Inhibition study (Kirby-Bauer disk diffusion technique)																	
	E.c			***P.a***			***S.a***			***B.s***			***C.a***			***A.n***		
	IC_Z ($\mu g\ ml^{-1}$)	100 $\mu g\ ml^{-1}$ ZD (mm)	150 $\mu g\ ml^{-1}$	IC_Z $\mu g\ ml^{-1}$	100 $\mu g\ ml^{-1}$ ZD (mm)	150 $\mu g\ ml^{-1}$	IC_Z $\mu g\ ml^{-1}$	100 $\mu g\ ml^{-1}$ ZD (mm)	150 $\mu g\ ml^{-1}$	IC_Z $\mu g\ ml^{-1}$	100 $\mu g\ ml^{-1}$ ZD (mm)	150 $\mu g\ ml^{-1}$	IC_Z $\mu g\ ml^{-1}$	100 $\mu g\ ml^{-1}$ ZD (mm)	150 $\mu g\ ml^{-1}$	IC_Z $\mu g\ ml^{-1}$	100 $\mu g\ ml^{-1}$ ZD (mm)	150 $\mu g\ ml^{-1}$
8a	100	12	15	80	13	17	80	12	18	60	14	18	>150	–	–	>150	–	–
8b	60	14	19	80	12	15	150	–	11	100	11	12	>150	–	–	100	11	13
8c	60	13	16	60	14	18	150	–	12	40	15	19	100	12	14	>150	–	–
8d	20	15	20	20	16	21	80	11	14	40	13	16	150	–	11	80	12	14
8e	40	14	17	100	11	13	150	–	12	150	–	11	>150	–	–	100	11	12
8f	60	13	14	80	12	15	100	11	13	80	12	15	>150	–	–	>150	–	–
8g	100	11	13	>150	–	–	40	14	20	20	16	22	100	11	15	80	13	15
8h	60	12	15	100	11	14	60	13	15	40	14	17	>150	–	–	150	–	11
8i	150	–	11	150	–	11	60	13	16	60	12	16	100	11	12	80	12	15
8j	20	16	21	40	15	20	100	12	16	40	15	17	100	12	15	60	13	16
8k	80	12	16	80	13	17	40	14	17	60	13	15	60	12	16	40	13	17
8l	150	–	11	80	11	13	60	12	14	40	15	20	100	11	13	60	15	20
8m	150	–	12	100	12	15	20	17	23	40	16	21	>150	–	–	150	–	11
8n	80	11	13	40	15	17	60	12	14	40	13	16	>150	–	–	>150	–	–
8o	100	12	13	>150	–	–	100	11	13	>150	–	–	>150	–	–	>150	–	–
9a	60	14	16	40	14	16	100	11	14	40	15	17	>150	–	–	150	–	11
9b	80	13	15	40	16	19	100	12	15	40	15	18	150	–	11	>150	–	–
9c	80	12	14	>150	–	–	40	14	17	40	14	19	>150	–	–	100	11	12
NC	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PC_1	10	–	NT	10	–	NT	05	–	NT	05	–	NT	–	–	–	–	–	–
PC_2	40	–	NT	>150	–	NT	40	–	NT	60	–	NT	–	–	–	–	–	–
PC_3	80	–	NT	60	–	NT	>150	–	NT	80	–	NT	–	–	–	–	–	–
PC_4	–	–	–	–	–	–	–	–	–	–	–	–	5	–	NT	5	–	NT
PC_5	–	–	–	–	–	–	–	–	–	–	–	–	20	–	NT	>150	–	NT
PC_6	–	–	–	–	–	–	–	–	–	–	–	–	>150	–	NT	100	13	NT

IC_Z initial concentration (maximum dilution) for appearance of zone, *ZD* zone diameter in mm, *NC* negative control (DMSO effect on microbes), *PC* positive control (standard reference drugs), *PC_1* Ciprofloxacin, *PC_2* ampicillin, *PC_3* carbenicillin, *PC_4* flucanazole, *PC_5* ketaconazole, *PC_6* itraconazole, *NT* not tested

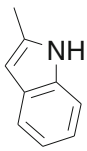
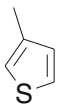
Table: 4 Antituberculosis activity of the newly synthesized compounds



8 (a-o)



9 (a-c)

Entry	R=	BACTEC MGIT method		L. J MIC method	
		MIC (μg ml^{-1})	% Inhibition	MIC (μg ml^{-1})	% Inhibition
8a	H	>32	–	>250	–
8b	4-Cl	>32	–	125	96
8c	3-Cl	>32	–	62.5	98
8d	2-Cl	>32	–	125	94
8e	2,4-Cl	>32	–	250	98
8f	3-F	>32	–	>250	–
8g	4-Br	32	99	32	99
8 h	4-CH_3	>32	–	>250	–
8i	3-CH_3	>32	–	>250	–
8j	4-OCH_3	32	99	32	99
8k	3-OCH_3	>32	–	250	95
8l	3,4-OCH_3	>32	–	150	95
8m	4-NO_2	32	99	32	99
8n	3-NO_2	>32	–	125	98
8o	4-CF_3	>32	–	>250	–
9a		>32	–	>250	–
9b		>32	–	>250	–
9c		>32	–	>250	–
PC_1	Isoniazide	0.5	99		
PC_2	Rifampicin	6.25	99		
PC_3	Ethambutol	20	99		
PC_4	Pyrazinamide	32	99		

PC positive control, i.e., standard drugs

NMR (100 MHz, DMSO(d_6), δ ppm): 165.23 (C=O), 163.52 (Ar–C, S-triazine ring), 134.59 (Ar–C–Cl), 133.49 (=CH), 128.97 (C=CH, C_5 of thiazolidinone), 129.67 (C=N, thiazolidinone), 128.81–119.63 (Ar–C), 35.59–35.51 (N–(CH_3)$_2$); IR (KBr, cm^{-1}): 3430.21(–NH str., 2° amine), 1389 (C–N str.), 694.26 (C–Cl str.); TOF–MS (*m/z*): 496.15 ($[M+H]^+$ for ^{35}Cl), 498.08 ($[M+H]^+$ for ^{37}Cl), 273.46, 138.29.

*2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-5-(3-chloro-benzylidene)-thiazolidin-4-one (**8c**)* ^1H NMR (400 MHz, DMSO(d_6), δ ppm): 8.70 (1H, s, –NH exchangeable with D_2O), 8.58 (1H, s, –NH exchangeable with D_2O), 7.95 (1H, s, exocyclic=CH), 6.89–7.80 (8H, m, Ar–H), 3.06–3.21 (12H, s, N(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO(d_6), δ ppm): 165.59 (C=O), 163.

48 (Ar–C, S-triazine ring), 134.66 (Ar–C–Cl), 132.87 (=CH), 129.09 (C=CH, C_5 of thiazolidinone), 129.32 (C=N, 4-thiazolidinone), 128.90–119.67 (Ar–C), 35.66–35.52 (N–(CH_3)$_2$); IR (KBr, cm^{-1}): 3428.64 (–NH str., 2° amine), 1512.59 (ArC–H str.), 699.28 (C–Cl str.); TOF–MS (*m/z*): 496.48 ($[M+H]^+$ for ^{35}Cl), 498.43 ($[M+H]^+$ for ^{37}Cl), 273.51, 138.32.

*2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-5-(2-chloro-benzylidene)-thiazolidin-4-one (**8d**)* ^1H NMR (400 MHz, DMSO(d_6), δ ppm): 8.69 (1H, s, –NH exchangeable with D_2O), 8.53 (1H, s, –NH exchangeable with D_2O), 8.00 (1H, s, exocyclic=CH), 6.80–7.83 (8H, m, Ar–H), 3.09–3.19 (12H, s, N(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO(d_6), δ ppm): 165.92 (C=O), 163.61 (Ar–C, S-triazine ring), 135.02 (Ar–C–Cl), 132.99 (=CH), 128.95 (C=CH, C_5 of thiazolidinone), 129.24 (C=N, 4-thiazolidinone), 126.76–120.37 (Ar–C), 35.77–35.28 (N–(CH_3)$_2$); IR (KBr, cm^{-1}): 3430.22 (–NH str., 2° amine), 701.13 (C–Cl str.); TOF–MS (*m/z*): 496.52 (For ^{35}Cl, $[M+H]^+$), 498.47 ($[M+H]^+$ for ^{37}Cl), 273.50, 138.31.

*2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-5-(2,4-dichloro-benzylidene)-thiazolidin-4-one (**8e**)* ^1H NMR (400 MHz, DMSO(d_6), δ ppm): 8.63 (1H, s, –NH exchangeable with D_2O), 8.58 (1H, s, –NH exchangeable with D_2O), 8.00 (1H, s, exocyclic=CH), 6.80–7.83 (7H, m, Ar–H), 3.11–3.18 (12H, s, N(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO(d_6), δ ppm): 166.03 (C=O), 163.74 (Ar–C, S-triazine ring), 135.33 (Ar–C–Cl), 132.85 (=CH), 128.81 (C=CH, C_5 of thiazolidinone), 129.37 (C=N, 4-thiazolidinone), 126.76–120.43 (Ar–C), 35.75–35.11 (N–(CH_3)$_2$); IR (KBr, cm^{-1}): 3435.18 (–NH str., 2° amine), 705.08 (C–Cl str.); TOF–MS (*m/z*):530.82 ($[M+H]^+$ for ^{35}Cl), 532.78 ($[M+H]^+$ for ^{35}Cl and ^{37}Cl), 534.97 ($[M+H]^+$ for ^{37}Cl), 274.46, 173.09.

*2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-5-(3-fluoro-benzylidene)-thiazolidin-4-one (**8f**)* ^1H NMR (400 MHz, DMSO(d_6), δ ppm): 8.75 (1H, s, –NH exchangeable with D_2O), 8.43 (1H, s, –NH exchangeable with D_2O), 7.89 (1H, s, exocyclic=CH), 6.95–7.79 (8H, m, Ar–H), 3.11–3.28 (12H, s, N(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO(d_6), δ ppm): 165.11 (C=O), 163.45 (Ar–C, S-triazine ring), 157.22 (C–F), 133.76 (=CH), 128.64 (C=CH, C_5 of thiazolidinone), 129.67 (C=N, 4-thiazolidinone), 128.81–119.23 (Ar–C), 35.71–35.50 (N–(CH_3)$_2$); IR (KBr, cm^{-1}): 3440.81 (-NH str., 2° amine),1511.36 (ArC–H str.), 1289.37 (C–F str.); TOF–MS (*m/z*): 480.11 $[M+H]^+$, 122.82.

*2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-5-(4-bromo-benzylidene)-thiazolidin-4-one (**8g**)* ^1H NMR (400 MHz, DMSO (d_6), δ ppm): 8.77 (1H, s, –NH exchangeable with D_2O), 8.63 (1H, s, –NH exchangeable with D_2O), 8.08 (1H, s, exocyclic=CH), 7.03-7.83 (8H, m, Ar–H), 3.15–3.29 (12H, s, N(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO (d_6), δ ppm): 164.82 (C=O), 163.46 (Ar–C, S-triazine ring), 133.81 (=CH), 129.13 (C=CH, C_5 of 4-thiazolidinone), 129.46 (C=N, 4-thiazolidinone), 128.93–119.83 (Ar–C), 35.60–35.56 (N–(CH_3)$_2$); TOF–MS (*m/z*): 540.27 ($[M+1]^+$ for ^{79}Br), 542.19 ($[M+H]^+$ for ^{81}Br), 184.97.

*2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-5-(4-methyl-benzylidene)-thiazolidin-4-one (**8h**)* ^1H NMR (400 MHz, DMSO(d_6), δ ppm): 8.71 (1H, s, –NH exchangeable with D_2O), 8.33 (1H, s, –NH exchangeable with D_2O), 8.01 (1H, s, exocyclic=CH), 6.82–7.75 (8H, m, Ar–H), 3.12–3.28 (12H, s, N(CH_3)$_2$), 2.45 (3H, s, –CH_3); ^{13}C NMR (100 MHz, DMSO(d_6), δ ppm): 165.19 (C=O), 163.55 (Ar–C, S-triazine ring), 134.02 (=CH), 130.29 (C=CH, C_5 of thiazolidinone), 128.32 (C=N, thiazolidinone), 127.70–120.26 (Ar–C), 35.65–35.46 (N–(CH_3)$_2$ TOF–MS (*m/z*): 476.64 $[M+H]^+$, 118.33.

*2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-5-(3-methyl-benzylidene)-thiazolidin-4-one (**8i**)* ^1H NMR (400 MHz, DMSO(d_6), δ ppm): 8.75 (1H, s, –NH exchangeable with D_2O), 8.37 (1H, s, –NH exchangeable with D_2O), 8.05 (1H, s, exocyclic=CH), 6.72-7.81 (8H, m, Ar–H), 3.13–3.32 (12H, s, N(CH_3)$_2$), 2.42 (3H, s, –CH_3); ^{13}C NMR (100 MHz, DMSO(d_6), δ ppm): 165.38 (C=O), 163.49 (Ar–C, S-triazine ring), 134.15 (=CH), 130.49 (C=CH, C_5 of thiazolidinone), 128.43 (C=N, thiazolidinone), 127.59–120.30 (Ar–C), 35.62–35.13 (N–(CH_3)$_2$); TOF–MS (*m/z*): 475.95 $[M+H]^+$, 118.32.

*2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-5-(4-methoxy-benzylidene)-thiazolidin-4-one (**8j**)* ^1H NMR (400 MHz, DMSO(d_6), δ ppm): 8.87 (1H, s, –NH exchangeable with D_2O), 8.71 (1H, s, –NH exchangeable with D_2O), 8.00 (1H, s, exocyclic=CH), 6.89–7.62 (8H, m, Ar–H), 3.54 (3H, s, –OCH_3), 3.11–3.28 (12H, s, N(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO (d_6), δ ppm): 165.09 (C=O), 163.61 (Ar–C, S-triazine ring), 154.36 (C–OCH_3), 134.05 (=CH), 129.39 (C=CH, C_5 of thiazolidinone), 129.32 (C=N, 4-thiazolidinone), 128.78–119.20 (Ar–C), 56.82 (–OCH_3), 35.68–35.41 (N–(CH_3)$_2$); IR (KBr, cm^{-1}): 3438.47 (–NH str., 2° amine), 1505.39 (ArC–H str.); TOF–MS (*m/z*): 491.11 $[M+H]^+$, 134.44.

*2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-5-(3,-methoxy-benzylidene)-thiazolidin-4-one (**8k**)* ^1H NMR (400 MHz, DMSO(d_6), δ ppm): 8.92 (1H, s, –NH exchangeable with D_2O), 8.68 (1H, s,

–NH exchangeable with D_2O), 8.03 (1H, s, exocyclic=CH), 6.93–7.48 (8H, m, Ar–H), 3.50 (3H, s, $-OCH_3$), 3.15–3.33 (12H, s, $N(CH_3)_2$); ^{13}C NMR (100 MHz, DMSO (d_6), δ ppm): 164.92 (C=O), 163.34 (Ar–C, S-triazine ring), 155.67 (C–OCH_3), 139.88 (=CH), 129.62 (C=CH, C_5 of thiazolidinone), 129.17 (C=N, 4-thiazolidinone), 128.08–119.44 (Ar–C), 56.90 ($-OCH_3$), 35.73–35.55 ($N-(CH_3)_2$); IR (KBr, cm^{-1}): 3450.67 (–NH str., 2° amine), 1506.28 (ArC–H str.); TOF–MS (*m/z*): 492.01 $[M+H]^+$, 134.37.

2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-5-(3,4-dimethoxy-benzylidene)-thiazolidin-4-one (***8l***) ^1H NMR (400 MHz, DMSO(d_6), δ ppm): 8.85 (1H, s, –NH exchangeable with D_2O), 8.70 (1H, s, –NH exchangeable with D_2O), 7.93 (1H, s, exocyclic=CH), 6.91–7.60 (7H, m, Ar–H), 3.51 (6H, s, $-OCH_3$), 3.09–3.23 (12H, s, $2N(CH_3)_2$); ^{13}C NMR (100 MHz, DMSO (d_6), δ ppm): 165.13 (C=O), 163.58 (Ar–C, S-triazine ring), 154.36 (C–OCH_3), 153.95 (C–OCH_3), 134.10 (=CH), 129.44 (C=CH, C_5 of thiazolidinone), 129.30 (C=N, thiazolidinone), 128.86–119.19 (Ar–C), 56.82 ($-OCH_3$), 56.85 ($-OCH_3$), 35.68-35.41 ($N-(CH_3)_2$); IR (KBr, cm^{-1}): 3419.79 (–NH str., 2° amine), 1507.54 (ArC–H str.); TOF–MS (*m/z*): 521.49 $[M+H]^+$, 165.22.

2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-5-(4-nitro-benzylidene)-thiazolidin-4-one (***8m***) ^1H NMR (400 MHz, DMSO(d_6), δ ppm): 8.87 (1H, s, Ar–H, adjacent to $-NO_2$ group), 8.70 (1H, s, –NH exchangeable with D_2O), 8.68 (1H, s, –NH exchangeable with D_2O), 8.02 (1H, s, exocyclic=CH), 6.91–7.73 (8H, m, Ar–H), 3.17–3.25 (12H, s, $N(CH_3)_2$); ^{13}C NMR (100 MHz, DMSO (d_6), δ ppm): 164.98 (C=O), 163.77 (Ar–C, S-triazine ring), 141.96 (Ar–C–NO_2), 134.02 (=CH), 129.38 (C=CH, C_5 of thiazolidinone), 129.31 (C=N, thiazolidinone), 128.81–119.06 (9C, Ar–C), 35.55–35.49 ($N-(CH_3)_2$); IR (KBr, cm^{-1}): 3428.37 (–NH str., 2° amine); 1341.16 ($-NO_2$), 1548.56 ($-NO_2$); TOF–MS (*m/z*): 507.58 $[M+H]^+$, 149.99.

2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-5-(3-nitro-benzylidene)-thiazolidin-4-one (***8n***) ^1H NMR (400 MHz, DMSO(d_6), δ ppm): 8.85 (1H, s, Ar–H, adjacent to $-NO_2$ group), 8.70 (1H, s, –NH exchangeable with D_2O), 8.72 (1H, s, –NH exchangeable with D_2O), 8.01 (1H, s, exocyclic=CH), 6.91–7.83 (8H, m, Ar–H), 3.17–3.21 (12H, s, $N(CH_3)_2$); ^{13}C NMR (100 MHz, DMSO(d_6), δ ppm): 165.03 (C=O), 163.80 (Ar–C, S-triazine ring), 141.88 (Ar–C–NO_2), 133.73 (=CH), 129.45 (C=CH, C_5 of thiazolidinone), 129.58 (C=N, thiazolidinone), 127.51–119.12 (Ar–C), 35.43–35.59 ($N-(CH_3)_2$); IR (KBr, cm^{-1}): 3434.67 (–NH str., 2° amine), 1347.51 ($-NO_2$), 1551.27 ($-NO_2$); TOF–MS (*m/z*): 506.98 $[M+H]^+$, 149.78.

2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-5-(4-trifluoromethyl-benzylidene)-thiazolidin-4-one (***8o***) ^1H NMR (400 MHz, DMSO(d_6), δ ppm): 8.70 (1H, s, –NH exchangeable with D_2O), 8.57 (1H, s, –NH exchangeable with D_2O), 7.90 (1H, s, exocyclic=CH), 6.89–7.67 (8H, m, Ar–H), 3.10–3.28 (12H, s, $N(CH_3)_2$); ^{13}C NMR (100 MHz, DMSO(d_6), δ ppm): 165.08 (C=O), 163.38 (Ar–C, S-triazine ring), 133.76 (=CH), 132.07 (Ar–C–CF_3), 128.64 (C=CH, C_5 of thiazolidinone), 129.67 (C=N, thiazolidinone), 128.39 ($-CF_3$), 126.81-119.23 (9C, Ar–C), 35.67–35.59 ($(CH_3)_2$); IR (KBr, cm^{-1}): 3425.07 (–NH str., 2° amine), 1508.22 (ArC-H str.), 1288.06 (C–F str.); TOF–MS (*m/z*): 530.08 $[M+H]^+$.

2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-5-(1H-pyrrol-2-ylmethylene)-thiazolidin-4-one (***9a***) ^1H NMR (400 MHz, DMSO(d_6), δ ppm): 8.69 (1H, s, –NH exchangeable with D_2O), 8.36 (1H, s, –NH exchangeable with D_2O), 7.75 (1H, s, exocyclic=CH), 7.01–7.83 (4H, m, Ar–H), 6.98 (1H, dd, –CH, pyrrole), 6.42 (1H, dd, –CH, pyrrole), 5.92 (1H, t, –CH, pyrrole), 3.08–3.29 (12H, s, $2N(CH_3)_2$); ^{13}C NMR (100 MHz, DMSO(d_6), δ ppm): 165.12 (C=O), 163.61 (Ar–C, S-triazine ring), 133.98 (=CH), 129.33 (C=CH, C_5 of thiazolidinone), 129.26 (C=N, thiazolidinone), 128.78–119.16 (Ar–C), 117.14 (pyrrole), 115.94 (pyrrole), 110.63 (pyrrole), 35.63–35.56 ($N-(CH_3)_2$); IR (KBr, cm^{-1}): 3433.86 (–NH str., 2° amine), 1509.57 (ArC–H str.); TOF–MS (*m/z*): 435.15 $[M+H]^+$.

2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-5-(1H-indol-2-ylmethylene)-thiazolidin-4-one (***9b***) ^1H NMR (400 MHz, DMSO(d_6), δ ppm): 8.65 (1H, s, –NH exchangeable with D_2O), 8.33 (1H, s, –NH exchangeable with D_2O), 7.68 (1H, s, exocyclic=CH), 7.03–7.88 (8H, m, Ar–H), 6.52 (1H, dd, –CH=CH, indole), 6.92 (1H, d, –CH=CH–N, indole), 3.06–3.22 (12H, s, $2N(CH_3)_2$); ^{13}C NMR (100 MHz, DMSO(d_6), δ ppm): 164.12 (C=O), 163.58 (Ar–C, S-triazine ring), 132.82 (=CH), 129.02 (C=CH, C_5 of thiazolidinone), 129.27 (C=N, thiazolidinone), 130.64–119.10 (Ar–C), 104.03 (CH=CH–N, indole), 118.27 (–CH=CH–N, indole), 35.59–35.28 ($N-(CH_3)_2$); IR (KBr, cm^{-1}): 3430.01 (–NH str., 2° amine),1504.11 (ArC–H str.), 1383.68 (C–N str.); TOF–MS (*m/z*): 501.66 $[M+H]^+$.

2-[4-(4,6-Bis-dimethylamino-[1,3,5]triazin-2-ylamino)-phenylimino]-5-thiophen-3-ylmethylene-thiazolidin-4-one (***9c***) ^1H NMR (400 MHz, DMSO(d_6), δ ppm): 8.71

(1H, s, –NH exchangeable with D_2O), 8.36 (1H, s, –NH exchangeable with D_2O), 7.75 (1H, s, exocyclic=CH), 7.93 (1H, dd, CH=CH–S, thiophene), 7.85 (1H, dd, CH=CH–S, thiophen), 7.07–7.61 (4H, m, Ar–H), 6.73 (1H, dd, –CH=CH–S, thiophen), 3.08–3.20 (12H, s, $N(CH_3)_2$); ^{13}C NMR (100 MHz, DMSO(d_6), δ ppm): 164.52 (C=O), 163.03 (Ar–C, S-triazine ring), 131.91 (=CH), 129.13 (C=CH, C_5 of thiazolidinone), 129.30 (C=N, thiazolidinone), 128.39 (–CH=CH–S, thiophen), 127.68–120.64 (Ar–C), 35.63–35.11 ($N–(CH_3)_2$); IR (KBr, cm^{-1}): 3432.66 (–NH str., 2° amine), 1505.37 (ArC–H str.), 1389.66 (C–N str.); TOF–MS (*m*/*z*): 468.56 $[M+H]^+$.

Methods for pharmacological evaluation

The newly synthesized final compounds **8**(**a**–**o**) and **9**(**a**–**c**) were evaluated for their in vitro antibacterial, antifungal, and antituberculosis activity. Standard strains used in this evaluation were procured from Promotech Life sciences, Bangalore. The in vitro antibacterial activity were evaluated against gram-positive bacterial strains *B. subtilis* ATCC 11774 and *S. aureus* ATCC 25923 and gram-negative bacterial strains *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 by Kirby-Bauer technique and serial broth dilution technique. Antifungal screening was carried out by following the same techniques against fungal strains *C. albicans* ATCC 66027 and *A. niger* ATCC 6275.

Kirby-Bauer disk diffusion technique

Synthesized compounds were screened by Kirby-bauer disk diffusion technique by following the reported procedure (Rana *et al*., 2013a, Murray *et al*., 1999). Here, all the compounds **8**(**a**–**o**) and **9**(**a**–**c**) were dissolved in dimethyl sulfoxide, and six dilutions (20, 40, 60, 80, 100, and 150 μg ml^{-1}) of each compound were prepared and kept in wafers to measure activity. In this biological evaluation, DMSO was used as a negative control to check the effect of DMSO on the microbes. A control disk impregnated with a dimethyl sulfoxide without any sample was also used in a same manner and did not show any inhibition. Some of the compounds were tested at higher dilution range as they showed activity below than 20 μg ml^{-1} of MIC. Ciprofloxacin, Ampicillin, and Carbenicillin were used as standard drugs during antibacterial screening; and Flucanazole, Ketaconazole, and Itraconazole were used as standard drugs for antifungal screening.

Serial broth dilution technique

MICs of the synthesized compounds were carried out by serial broth dilution technique (Rana *et al*. 2013a; Collins and Lyne, 1970, Mullen *et al*., 1988). Here, compounds **8**(**a**–**o**) and **9**(**a**–**c**) were dissolved in DMSO, and six dilutions (20, 40, 60, 80, 100, and 150 μg ml^{-1}) of each compound were prepared. Compounds that showed potency at 20 μg ml^{-1} of MIC (**8d**, **8g**, **8j**, **8k**, **8m**, and **9b**) were further exposed to screen at higher dilutions i.e., 10 and 5 μg ml^{-1} of concentration. During the screening by this method, same reference drugs were used.

BACTEC MGIT method

Primary screening of the synthesized compounds for antituberculosis activity was carried out by following the BACTEC MGIT method (Anargyros *et al*., 1990, Rana *et al*., 2013a). The mycobacteria growth indicator tube (MGIT) containing 4 ml of modified middle brook 7H9 broth base was taken along with sample compound at fixed concentration i.e., 32 μg ml^{-1}. Resulted suspension was allowed to settle for about 30 min, and the tubes were centrifuged at 3,000 rpm for 15 min. After that 10^{-7} CFU ml^{-1} of H37RV *M. tuberculosis* strain suspension prepared was added in the medium to be incubated along with 0.1 ml of egg base medium. The MGIT tubes were then mixed well, recapped, and then incubated in BACTEC MGIT instrument at 37 °C until positivity is observed. After the second day of incubation, the readings were measured daily. Positive cultures were detected usually within 10 days. For the actual results, the MGIT tubes were removed from incubator and placed on the UV light next to the positive control tube and an uninoculated tube. Bright fluorescence detected by the corresponding MGIT tube was noticed in the form of the bright orange color in the bottom of the tube along with an orange reflection on the meniscus. Compounds presenting $\geq$99 % inhibition in the primary screening were considered as the most potent compound. All the compounds were re-examined for evaluation of their actual MIC value by adopting L. J (Lowenstein and Jensen) MIC method.

L. J (Lowenstein and Jensen) MIC method

The antimycobacterial screening to evaluate MICs of the synthesized compounds was carried out against strain *M. tuberculosis* H37 RV, by L. J (Lowenstein and Jensen) MIC method (Beemer *et al*., 2004; Patel and Khan, 2011). In the present screening, stock solution having concentration of 500 μg ml^{-1} of the synthesized compounds was made and further diluted to make the solutions with concentrations 250, 125, 62.5, and 32 μg ml^{-1} in solvent DMSO. These diluted solutions along with the stock solution were added in a liquid L. J. Medium, and after that media were sterilized by following inspissations method. A

culture of *M. tuberculosis* H37 RV growing on L. J. medium was harvested in 0.85 % saline in bijou bottles. Resultant bijou bottles were then incubated at 37 °C for about 24 h followed by streaking of *M. tuberculosis* H37 RV (5×10^4 bacilli per tube). The tubes were then incubated at 37 °C. Growth of bacilli was seen after 28 days of incubation. Tubes having the compounds were compared with the control tubes. The maximum dilution of the stock solution, at which no development of the colonies occurred, was considered as the MIC concentration of the relative test compound. Strain *M. tuberculosis* H37 RV was tested with the standard drugs Isoniazide, Rifampicin, Ethambutol, and Pyrazinamide.

Conclusion

In this article, efforts have been made toward the discovery of novel and pharmacologically active compounds, owing to the presence of the two pharmacologically active nuclei i.e., S-triazine and 4-thiazolidinones in a single molecular frame. From the bioassay, it is clear that the variation by different electron-withdrawing and electron-releasing groups at the 5-benzylidine ring would let to the activity differences in the certain range. In general, it was concluded that final compounds with electron-withdrawing halo substituents showed improved inhibition potency against gram-negative strains, while the same compounds showed deprived inhibition potency against gram-positive strains. Electron-releasing $-CH_3$ and $-OCH_3$ groups containing compounds showed improved activity against tested gram-positive bacterial strains. Compounds **8d**, **8g**, **8k**, and **8m** showed good antibacterial activity, when compared with the standards. Compounds with electron-releasing $-OCH_3$ group showed improved antifungal activity. Compounds with electron-withdrawing –Br and $-NO_2$ groups and electron-releasing $-OCH_3$ group at *para* position showed improved antituberculosis activity. Thus, these newly synthesized compounds deserves further investigation in order to clarify mode of action, responsible for the activity observed, and also more extensive studies are also justified to determine additional biological parameters to have a deeper insight into structure activity relationship and QSAR studies.

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Conflict of interest The authors report no declarations of interest.

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