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Synthesis and *in vitro* antimicrobial evaluation of amine substituted *s*-triazine based thiazolidinone/chalcone hybrids

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ABSTRACT

In an attempt to control multidrug resistant dilemma, a library of *s*-triazine based on two novel series of thiazolidinone (4a-e) and chalcone (7a-e) derivatives were designed and synthesized with simple and efficient etiquette. The newly synthesized compounds were studied for efficacy against several bacteria (*Staphylococcus Aureus*, *Bacillus Cereus*, *Pseudomonas Aeruginosa*, *Klebsiella Pneumoniae*,) and fungi (*Candida Albicans*, *Aspergillus Clavatus*) using the broth dilution technique. Compound 7d was the best bioactive desired antibacterial analogue with less MIC value against different tested strains. Results of bioassay study revealed the future hope of the potent drug-candidate based on *s*-triazine scaffold. All the final synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, mass and elemental analysis.

***Keywords*:** Antibacterial; Antifungal; Chalcone; *s*-Triazine; 4-thiazolidinone

1. INTRODUCTION

s-Triazine or 1,3,5-triazine has played a pivotal role in novel drug discovery for modulating physical and biological properties of the molecule [1]. Owing to wide range of biological applications, *s*-triazine nucleus has received an immense attention among chemists through fertile source of pharmacological activities such as antibacterial [2,3], antimalarial [4], antiprotozoal [5], antifungal [6], anticancer [7], antimychobacterial [8], and antiviral [9]. In addition to this several *s*-triazine derivatives bearing p-amino benzonitrile [10] and 2,4,6-trimethyl aniline [11] moiety have been found to possess an enhanced antimicrobial profile and improved antitubercular [12] and profound anticancer activity [13] as well. Moreover, 4-thiazolidinone bearing *s*-triazine analogues are emerged as potential bioactive molecules [14]. *s*-Triazie based chalcone conjugated also found the interesting hybrid for potential biological effects [15]. Consequences of such potential effects of triazine and an imperative need in search of new chemical entities lead us to synthesize some biologically efficient molecules.

Multidrug resistant (MDR) Strain, a rapid development of pathogens causing a severe resistance towards currently available standard drugs, poses the frightening threat by increasing severe opportunistic microbial infections in past decades [16-18]. Such resistant organisms were due for dramatic and alarming increase in microbial infections which results

in pressing problem worldwide. In view of the above dilemma, recent scenario highlights the urgent need to develop new agents with specific activity with increased potency to sustain a pool of new bioactive entities. Therefore, design and synthesis of new compounds likely to be unaffected by existing resistance mechanisms is an area of immense significance for medicinal chemists [19,20].

As it is proved to be a worth the synthesis of *s*-triazine bearing bioactive moieties, our group herein presented the facile route for the synthesis of novel *s*-triazine compounds and screened for antibacterial and antifungal activity. This novel molecule has also been structurally designed based on the potent biological agents (Figure 1). Eventually, compound 7d was found more active anti microbial agents than the rest against the tested strains.

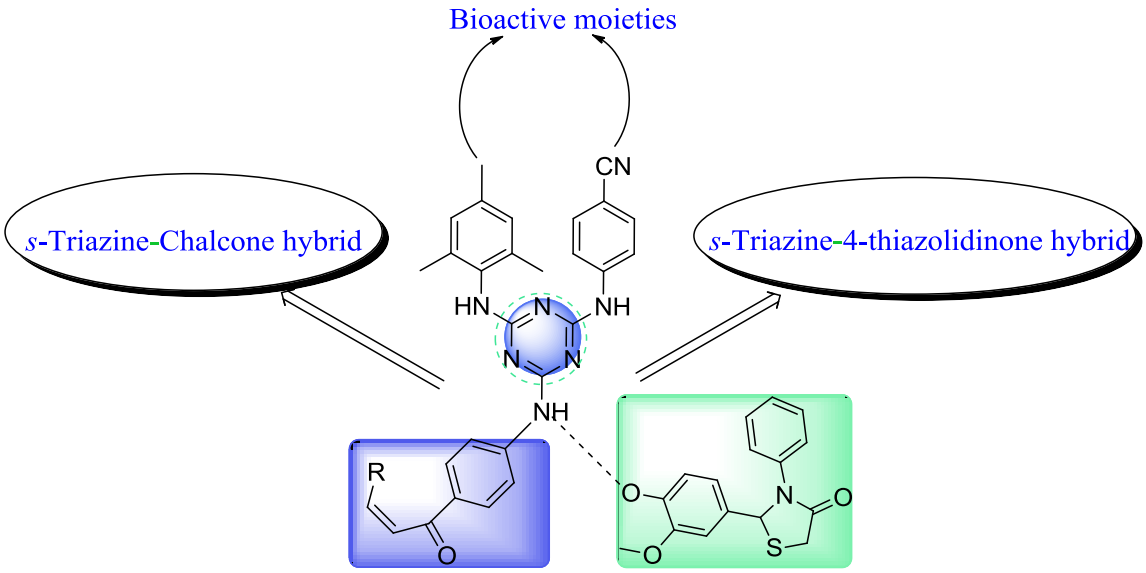


Figure 1. Design of the Title *s*-triazine based hybrids.

2. EXPERIMENTAL

2. 1. Material and methods

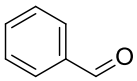
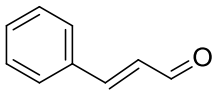
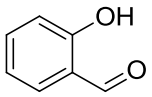
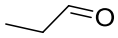
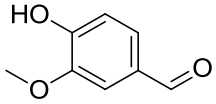
All the chemicals and solvents used for the synthesis work acquired from commercial sources were of analytical grade, and were used without further purification. 2,4,6-Trichloro-1,3,5-triazine, 2,4,6-trimethyl aniline and 4-amino-benzonitrile was procured from Sigma Aldrich Chemicals Pvt. Ltd., Mumbai, India. Substituted amine and aldehyde were purchased from Spectrochem Pvt. Ltd., Mumbai, India. Vanillin, 4-amino acetophenone and TLC plates were obtained from Merck, Germany. Evaporation of solvents was carried out on a rotary evaporator under reduced pressure or using a high-vacuum pump. Melting points were determined by using open capillary tubes and are uncorrected. TLC was checked on E-Merck pre-coated 60 F254 plates and the spots were rendered visible by exposing to UV light or iodine. IR spectrums were recorded on BRUKER TENSOR series FT-IR and SHIMADZU HYPER IR using KBr pellets. NMR spectra were recorded by 400 MHz BRUKER AVANCE instrument using TMS as internal standard (Chemical Shift in δ, ppm) and DMSO-*d6* as a solvent. Spectra were taken with a resonant frequency of 400 MHz for ^{1}H and 100 MHz for

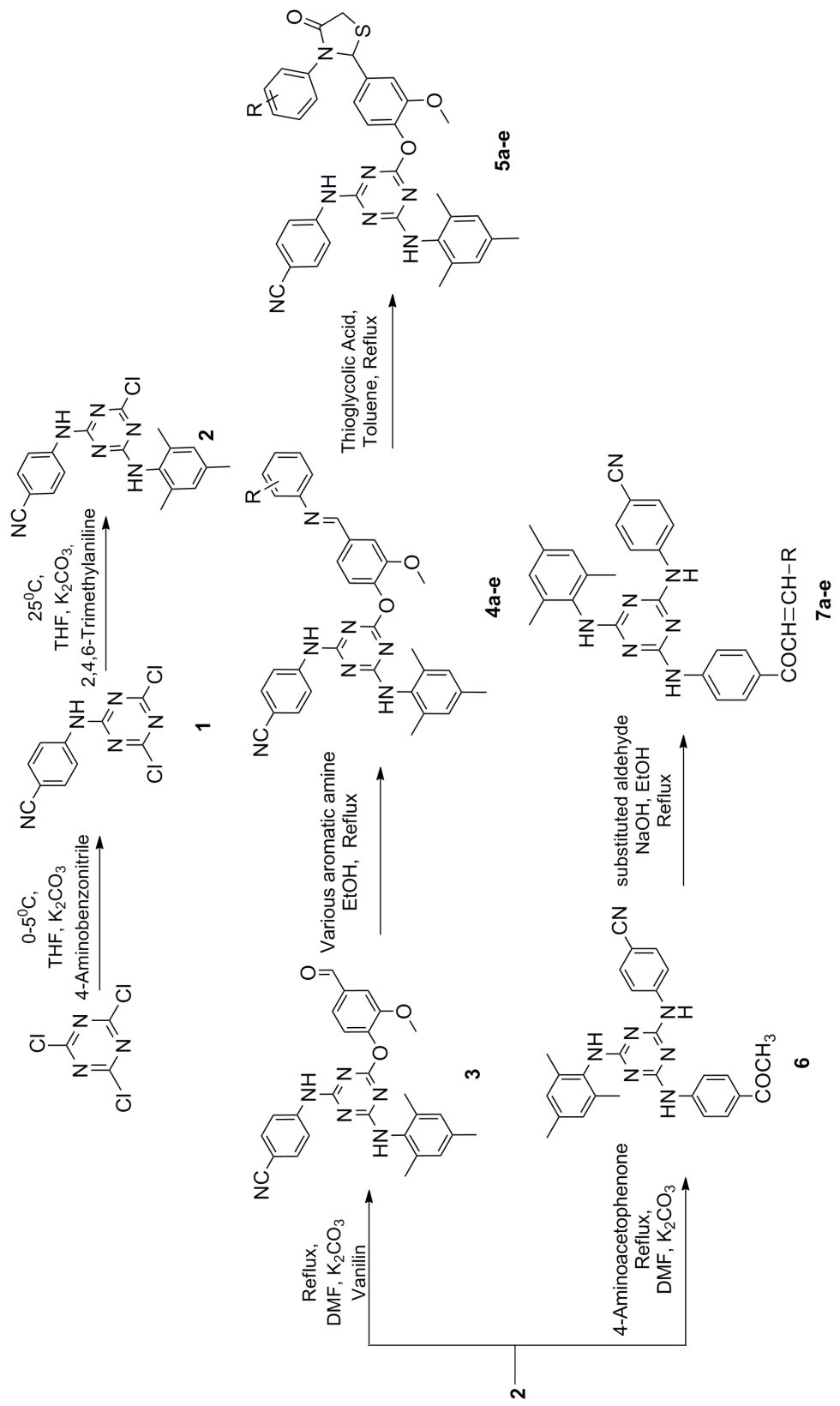
^{13}C NMR. The splitting patterns are designated as follows; s, singlet; d, doublet; dd, doublet of doublets; and m, multiplet. Elemental analysis was done on "Haraeus Rapid Analyser". The mass spectra were recorded on JOEL SX-102 (EI) model with 60 eV ionizing energy.

2. 2. Chemistry

Scheme 1 outlines the synthetic pathway used to obtain compounds (5a-e) and (7a-e). The first step comprises formation of 4-((4,6-dichloro-1,3,5-triazin-2-yl)amino)benzonitrile (1) in very good Yield by the nucleophilic displacement of one chlorine atom of s-triazine ring by 4-amino-benzonitrile at 0-5 °C [10]. The synthesis of disubstituted s-triazine intermediate (2) was achieved by the reaction between compound (1) and 2,4,6-trimethyl aniline in the presence of potassium carbonate at 40-45 °C.

Table 1. Physical and analytical data of final synthesized *s*-triazinyi thiazolidinone (5a-e) and chalcone (7a-e) derivatives.

Entry	R	Mol. Formula	M.Wt.	% Yield	M. P. (°C)	Elemental Analysis					
						Calcd.			Found		
						%C	%H	%N	%C	%H	%N
5a	H	$C_{35}H_{31}N_7O_3S$	629.73	66	211-212	66.75	4.96	15.57	66.56	4.97	15.53
5b	2-NO_2	$C_{35}H_{30}N_8O_5S$	674.73	67	176-177	62.30	4.48	16.61	62.39	4.47	16.57
5c	4-Cl	$C_{35}H_{30}ClN_7O_3S$	664.18	67	198-201	63.29	4.55	14.76	63.42	4.54	14.73
5d	4-F	$C_{35}H_{30}FN_7O_3S$	647.72	66	208-209	64.90	4.67	15.14	64.72	4.66	15.10
5e	4-CH_3	$C_{36}H_{33}N_7O_3S$	643.76	61	204-206	67.17	5.17	15.23	67.01	5.16	15.19
7a		$C_{34}H_{29}N_7O$	551.64	66	135-138	74.03	5.30	17.77	73.96	5.31	17.73
7b		$C_{36}H_{31}N_7O$	577.68	67	162-164	74.85	5.41	16.97	74.68	5.39	17.01
7c		$C_{34}H_{29}N_7O_2$	567.64	67	132-134	71.94	5.15	17.27	72.13	5.16	17.22
7d		$C_{30}H_{29}N_7O$	503.60	65	163-165	71.55	5.80	19.47	71.35	5.79	19.42
7e		$C_{35}H_{31}N_7O_3$	597.67	67	210-211	70.34	5.23	16.40	70.54	5.22	16.36



Scheme 1. Synthetic route for the synthesis of *s*-triazine based thiazolidinone (5a-j) and chalcone (7a-e) derivatives.

The third chlorine was then replaced by refluxing (2) with vanillin moiety in DMF to obtain 4-((4-(4-formyl-2-methoxyphenoxy)-6-(mesitylamino)-1,3,5-triazin-2-yl)amino) benzonitrile (3). The aldehyde group present in intermediate (3) was condensed with aromatic amines using ethanol, thus formed Schiff bases which were cyclized via thioglycolic acid to form final thiazolidinone derivatives (5a-e).

Besides this, intermediate (2) was further refluxed with 4-aminoacetophenone moiety using DMF as solvent and potassium carbonate as base that produced 4-((4-((4-acetylphenyl)amino)-6-(mesitylamino)-1,3,5-triazin-2-yl)amino)benzonitrile (6) analogue. Eventually, compound (6) was condensed with substituted aldehyde to give desired final chalcone (7a-e) derivatives. The accuracy of the synthesis of (5a-e) and (5a-e) compounds was confirmed on the basis of ^{1}H NMR, ^{13}C NMR and Mass spectra and the purity was ascertained by elemental analysis. Physical and analytical data of compounds (5a-e) and (7a-e) are elaborated in Table 1.

3. RESULTS AND DISCUSSION

3. 1. Synthetic route

Synthesis of 4-((4,6-dichloro-1,3,5-triazin-2-yl)amino)benzonitrile (1)

To a solution of cyanuric chloride (10 g, 0.054 mol) in dry THF (150 ml) at 0-5 °C were slowly added K_2CO_3 (14.9 g, 0.108 mole) and 4-aminobenzonitrile (6.41 g, 0.054 mole). The solution was stirred for 4 hrs 0-5 °C. After completion of reaction based on TLC monitoring Toluene:Acetone (7:3), 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 (1) [10].

Yield- 79 %; mp 247-250 °C; IR (ν_{max} cm^{-1}): 3277 (N–H, secondary), 2228 (C≡N), 833 (C_3N_3, *s*-triazine), 802 (C-Cl); ^{1}H NMR (400 MHz, DMSO-*d6*, δ ppm): 8.61 (s, 1H, –NH 4-amino benzonitrile), 7.86–7.78 (m, 4H, Ar–H aromatic proton); ^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm): 171.72, 166.61, 165.35, 140.76, 132.17, 125.38, 122.61, 120.68, 119.32, 100.82; ESI-MS (m/z): 265.94 (M^+).

Synthesis of 4-((4-chloro-6-(mesitylamino)-1,3,5-triazin-2-yl)amino)benzonitrile (2)

A mixture of 4-((4,6-dichloro-1,3,5-triazin-2-yl)amino)benzonitrile (1) (5 g, 0.018 mole) and 2,4,6-trimethyl aniline (2.54 g, 0.018 mole) was stirred in dry THF at room temperature. To this solution K_2CO_3 (5.18 g, 0.037 mole) was slowly added and stirring was continued for 4-5 hrs and monitored by TLC Hexane:Ethyl acetate (9:1). After the completion of the reaction, it was treated with crushed ice, neutralized by dilute HCl. The precipitates thus obtained was filtered, dried and recrystallized from THF to afford desired compound (2).

Yield- 67 %; mp 187-190 °C; IR (ν_{max} cm^{-1}): 3327 (N–H, secondary), 2180 (C≡N), 1485 (-CH_3), 821 (C_3N_3, *s*-triazine), 785 (C-Cl); ^{1}H NMR (400 MHz, DMSO–*d6*, δ ppm): 7.47 (d, *J* = 7.5 Hz, 2H), 7.14 (d, *J* = 7.1 Hz, 2H), 6.77 (s, 2H), 6.12 (s, 1H), 4.76 (s, 1H), 2.24 (s, 3H), 2.14 (s, 6H); ^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm): 163.23, 161.90, 160.68, 143.61, 143.10, 134.43, 132.05, 131.48, 130.37, 121.66, 119.12, 100.32, 21.81, 19.13; ESI-MS (m/z): 365.83 (M^+).

Synthesis of 4-((4-(4-formyl-2-methoxyphenoxy)-6-(mesitylamino)-1,3,5-triazin-2-yl) amino)benzonitrile (3)

A mixture of 4-((4-chloro-6-(mesitylamino)-1,3,5-triazin-2-yl)amino)benzonitrile (2) (5 g, 0.013 mole) and 4-Hydroxy-3-methoxybenzaldehyde (2 g, 0.013 mole) was refluxed in dry DMF for 3-4 hrs. The pH was adjusted to neutral by K_2CO_3 (3.78 g, 0.027 mole). Progress of reaction was mointored by TLC Toluene:Acetone (9:1). After the completion of reaction, it was poured into crushed ice. The product thus obtained was filtered, dried and recrystallized from methanol to afford desired compound (3).

Yield- 53 %; mp 164-167 °C; IR (ν_{max} cm^{-1}): 3487 (N–H, secondary), 2245 (C≡N), 1712 (C=O), 1497 (-CH_3), 1084 (C-O-C), 897 (C_3N_3, *s*-triazine); ^{1}H NMR (400 MHz, DMSO-*d6*, δ ppm): 10.03 (s, 1H), 7.41 (d, *J* = 7.2 Hz, 2H), 7.44 – 7.32 (m, 2H), 7.16 (d, *J* = 6.7 Hz, 2H), 7.07 – 6.96 (m, 3H), 5.97 (s, 1H), 4.94 (s, 1H), 3.79 (s, 3H), 2.36 (s, 3H), 2.30 (s, 6H); ^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm): 190.67, 170.43, 161.48, 161.13, 152.57, 144.59, 143.66, 143.10, 137.47, 134.43, 132.05, 131.48, 130.37, 125.20, 121.66, 119.74, 119.12, 114.10, 100.32, 56.79, 21.81, 17.24; ESI-MS (m/z): 481.53 (M^+).

General method for the preparation of 4-((4-(mesitylamino)-6-(2-methoxy-4-((substituted phenylimino)methyl)phenoxy)-1,3,5-triazin-2-yl)amino)benzonitrile (4a-e)

A solution of 0.01 mol of 4-((4-(4-formyl-2-methoxyphenoxy)-6-(mesitylamino)-1,3,5-triazin-2 yl)amino)benzonitrile (3) and equimolar amount of appropriate substituted amine in 60 mL of EtOH was heated under reflux for 1-2 hr. Progress of reaction was observed by TLC Hexane:

General method for the preparation of 4-((4-(mesitylamino)-6-(2-methoxy-4-(4-oxo-3-substituted phenylthiazolidin-2-yl)phenoxy)-1,3,5-triazin-2-yl)amino)benzonitrile (5a-e)

A mixture of (4a-e) (0.01 mol) and thioglycolic acid (0.01 mol) was refluxed for 12-18 hrs in dry toluene (100 ml) using a Dean–Stark water separator. Progress of reaction was monitored by TLC Toluene:Acetone (4:6). Excess toluene was evaporated in vacuo. The resulting residue was triturated with saturated $NaHCO_3$ solution until CO_2 evolution ceased. The solid was washed with water, dried and recrystallized from ethanol to afford final respective compounds (5a-e).

Spectral data of compounds (5a-e)

4-((4-(Mesitylamino)-6-(2-methoxy-4-(4-oxo-3-phenylthiazolidin-2-yl)phenoxy)-1,3,5-triazin-2-yl)amino)benzonitrile (5a)

IR (ν_{max} cm^{-1}): 3319 (N-H, secondary), 3020 (C–H, aromatic), 2920 (C–H, aliphatic), 2224 (C≡N), 1685 (C=O), 1309 (C-N), 1259 (C-O-C, aryl), 1030 (-O-CH_3), 806 (C-N, *s*-triazine), 692 (C-S, stretch); ^{1}H NMR (400 MHz, DMSO–*d6*, δ ppm): 7.41-7.11 (m, 9H, Ar-H), 6.98 (s, 2H, Ar–H), 6.93-6.82 (m, 3H, Ar–H), 6.44 (s, 1H), 6.38 (s, 1H, -NH), 4.45 (s. 1H, -NH), 3.72 (s, 3H, -OCH_3), 3.49 (s, 2H, -CH_2), 2.38 (s, 3H, -CH_3), 2.18 (s, 6H, -CH_3); ^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm): 170.30, 164.77, 162.00, 160.58, 150.16, 145.62, 143.06, 140.18, 138.79, 137.10, 135.59, 134.08, 132.72, 131.49, 130.56, 127.20, 125.48, 123.95, 120.09, 119.97, 118.29, 114.00, 100.65, 70.18, 56.75, 35.11, 21.49, 18.95; ESI-MS (m/z): 630.49 (M^+).

4-((4-(Mesitylamino)-6-(2-methoxy-4-(3-(2-nitrophenyl)-4-oxothiazolidin-2-yl)phenoxy)-1,3,5-triazin-2-yl)amino)benzonitrile (5b)

IR (ν_{max} cm^{-1}): 3425 (N-H, secondary), 3012 (C–H, aromatic), 2957 (C–H, aliphatic), 2232 (C≡N), 1698 (C=O), 1547 (-NO_2), 1323 (C-N), 1196 (C-O-C, aryl), 1030 (-O-CH_3), 833 (C-N, *s*-triazine), 678 (C-S, stretch); ^{1}H NMR (400 MHz, DMSO-*d6*, δ ppm): 7.74 (m, 1H), 7.70 (d, *J* = 7.3, 1.4 Hz, 1H), 7.57 – 7.48 (m, 3H), 7.32 (dd, *J* = 6.8 Hz, 1H), 7.15 (d, *J* = 5.6 Hz, 2H), 7.01 (s, 2H), 6.96 – 6.91 (m, 2H), 6.80 (dd, *J* = 7.1, 1H), 6.60 (s, 1H), 6.03 (s, 1H), 4.52 (s, 1H), 3.78 (s, 3H), 3.53 (q, *J* = 5.5 Hz, 2H), 2.37 (s, 3H), 2.32 (s, 6H); ^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm): 172.06, 169.94, 161.48, 161.13, 150.77, 145.12, 143.66, 143.10, 142.57, 137.90, 135.51, 134.43, 132.05, 131.48, 130.37, 128.33, 127.24, 125.72, 121.66, 120.11, 119.12, 115.72, 115.20, 100.32, 67.45, 56.79, 35.65, 21.81, 18.27; ESI-MS (m/z): 675.75 (M^+).

4-((4-(4-(3-(4-Chlorophenyl)-4-oxothiazolidin-2-yl)-2-methoxyphenoxy)-6-(mesitylamino)-1,3,5-triazin-2-yl)amino)benzonitrile (5c)

IR (ν_{max} cm^{-1}): 3345 (N-H, secondary), 3004 (C–H, aromatic), 2945 (C–H, aliphatic), 2234 (C≡N), 1701 (C=O), 1315 (C-N), 1275 (C-O-C, aryl), 1085 (-O-CH_3), 814 (C-N, *s*-triazine), 620 (C-S, stretch); ^{1}H NMR (400 MHz, DMSO–*d6*, δ ppm): 7.56 (d, *J* = 7.0 Hz, 2H), 7.42 (d, *J* = 7.1 Hz, 2H), 7.26 (dd, *J* = 5.4, 4H), 7.00 – 6.91 (m, 2H), 6.81 (s, 2H), 6.77 (t, *J* = 4.4 Hz, 1H), 6.53 (s, 1H), 6.44 (s, 1H), 4.13 (s, 1H), 3.61 (s, 3H), 3.48 (q, *J* = 6.7 Hz, 2H), 2.31 (s, 3H), 2.25 (s, 6H); ^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm): 173.34, 164.94, 162.48, 160.13, 152.77, 148.12, 145.66, 141.10, 138.90, 136.78, 133.43, 131.26, 131.05, 130.48, 130.37, 129.62, 124.81, 121.66, 120.57, 119.23, 115.56, 115.42, 100.87, 67.01, 56.75, 35.34, 21.24, 17.57; ESI-MS (m/z): 665.35 (M^+).

4-((4-(4-(3-(4-Fluorophenyl)-4-oxothiazolidin-2-yl)-2-methoxyphenoxy)-6-(mesitylamino)-1,3,5-triazin-2-yl)amino)benzonitrile (5d)

IR (ν_{max} cm^{-1}): 3312 (N-H, secondary), 3075 (C–H, aromatic), 2935 (C–H, aliphatic), 2215 (C≡N), 1773 (C=O), 1385 (C-N), 1215 (C-O-C, aryl), 1068 (-O-CH_3), 868 (C-N, *s*-triazine), 664 (C-S, stretch); ^{1}H NMR (400 MHz, DMSO-*d6*, δ ppm): 7.54 (d, *J* = 7.4 Hz, 2H), 7.24 – 7.20 (m, 2H), 7.18 (d, *J* = 7.2 Hz, 2H), 7.11 (t, *J* = 7.7 Hz, 2H), 7.05 – 6.99 (m, 3H), 6.96 (d, *J* = 6.4 Hz, 1H), 6.91 (dd, *J* = 6.8 Hz, 1H), 6.53 (s, 1H), 5.76 (s, 1H), 4.51 (s, 1H), 3.84 (s, 3H), 3.52 (q, *J* = 5.7 Hz, 2H), 2.37 (s, 3H), 2.31 (s, 6H); ^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm): 174.24, 169.75, 164.35, 162.75, 160.37, 154.78, 144.78, 142.45, 141.04, 137.67, 134.43, 133.37, 132.11, 131.87, 130.34, 126.82, 121.04, 120.78, 119.64, 116.72, 115.20, 115.32, 100.67, 67.64, 56.25, 35.64, 21.14, 16.54; ESI-MS (m/z): 648.86 (M^+).

4-((4-(Mesitylamino)-6-(2-methoxy-4-(4-oxo-3-(p-tolyl)thiazolidin-2-yl)phenoxy)-1,3,5-triazin-2-yl)amino)benzonitrile (5e)

IR (ν_{max} cm^{-1}): 3334 (N-H, secondary), 3057 (C–H, aromatic), 2964 (C–H, aliphatic), 2258 (C≡N), 1734 (C=O), 1335 (C-N), 1253 (C-O-C, aryl), 1086 (-O-CH_3), 813 (C-N, *s*-triazine), 664 (C-S, stretch); ^{1}H NMR (400 MHz, DMSO-*d6*, δ ppm): 7.53 (d, *J* = 7.1 Hz, 2H), 7.25 – 7.19 (m, 4H), 7.15 (d, *J* = 7.0 Hz, 2H), 7.01 (s, 2H), 6.93 (dd, *J* = 5.4 Hz, 2H), 6.80 (dd, *J* = 6.5, 1H), 6.54 (s, 1H), 6.02 (s, 1H), 4.51 (s, 1H), 3.78 (s, 3H), 3.50 (q, *J* = 5.4 Hz, 2H), 2.37 (s, 3H), 2.32 (d, *J* = 5.1 Hz, 9H); ^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm): 175.42, 168.24, 164.64, 163.47, 150.12, 145.04, 143.46, 143.34, 137.90, 136.67, 134.74,

134.64, 132.32, 131.67, 130.57, 130.15, 122.05, 121.75, 120.22, 119.86, 115.45, 115.35, 100.23, 68.24, 55.35, 35.68, 20.24, 20.13, 17.35; ESI-MS (m/z): 644.65 (M^+).

Synthesis of 4-((4-((4-acetylphenyl)amino)-6-(mesitylamino)-1,3,5-triazin-2-yl)amino) benzonitrile (6)

A mixture of 4-((4-chloro-6-(mesitylamino)-1,3,5-triazin-2-yl)amino)benzonitrile (**2**) (5 g, 0.013 mole) and 4-amino acetophenone (1.85 g, 0.013 mole) was refluxed in dry DMF for 3-4 hrs. The pH was adjusted to neutral by K_2CO_3 (3.78 g, 0.027 mole). Progress of reaction was observed by TLC Toluene:Acetone (6:4). After the completion of reaction, it was poured into crushed ice. The product thus obtained was filtered, dried and recrystallized from methanol to afford desired compound (6).

Yield- 75 %; mp 204-207 °C; IR (ν_{max} cm^{-1}): 3364 (N–H, secondary), 2245 (C≡N), 1687 (C=O), 1457 (-CH_3), 887 (C_3N_3, *s*-triazine); ^{1}H NMR (400 MHz, DMSO-*d6*, δ ppm): 7.79 (d, *J* = 7.4 Hz, 2H), 7.52 (d, *J* = 7.1 Hz, 2H), 7.16 (dd, *J* = 7.5 Hz, 4H), 6.98 (s, 2H), 6.34 (s, 1H), 6.22 (s, 1H), 4.89 (s, 1H), 2.52 (s, 3H), 2.36 (s, 3H), 2.30 (s, 6H); ^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm): 197.14, 161.80, 161.42, 143.66, 143.10, 141.25, 134.43, 132.06, 131.48, 130.37, 130.28, 121.66, 119.12, 116.39, 100.32, 27.79, 21.81, 18.93; ESI-MS (m/z): 464.42 (M^+).

General method for the preparation of 4-((4-(mesitylamino)-6-((4-(3-substituted acryloyl)phenyl)amino)-1,3,5-triazin-2-yl)amino)benzonitrile (7a-e)

0.01 mol of 4-((4-((4-acetylphenyl)amino)-6-(mesitylamino)-1,3,5-triazin-2-yl)amino) benzonitrile (6) and 0.013 mole of various substituted aldehyde were slowly added in 60 mL of MeOH. 10 % 1 M NaOH was then added and the reaction was stirred for 12-20 hrs at room temperature. Progress of reaction was followed by TLC MeOH: Acetone (6:4). After completion, the pH of the solution was adjusted to 2 with HCl solution. The precipitate thus obtained was filtered off, washed with water and recrystallized with boiling EtOH to get respective compounds (7a-e).

Spectral data of compounds (7a-e)

4-((4-(Mesitylamino)-6-((4-(3-phenylacryloyl)phenyl)amino)-1,3,5-triazin-2-yl)amino) benzonitrile (7a)

IR (ν_{max} cm^{-1}): 3370 (N-H, secondary), 3024 (C–H, aromatic), 2930 (C–H, aliphatic), 2222 (C≡N), 1651 (C=O), 1593 (-CH=CH-), 1309 (C-N), 850 (C-N, *s*-triazine); ^{1}H NMR (400 MHz, DMSO-*d6*, δ ppm): 8.12 (d, 1H, -CH), 7.95-7.69 (m, 4H, Ar-H), 7.53 (d, 1H, -CH), 7.40-7.19 (m, 9H, Ar-H), 7.00 (s, 2H, Ar-H), 6.54 (s, 1H, -NH), 6.33 (s, 1H, -NH), 4.62 (s, 1H, -NH), 2.38 (s, 3H, -CH_3), 2.21 (s, 6H, -CH_3); ^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm): 190.55, 163.49, 162.02, 161.66, 145.20, 143.18, 142.06, 140.33, 137.29, 135.00, 134.56, 133.80, 132.74, 131.53, 130.22, 129.61, 128.98, 126.75, 123.65, 120.49, 118.14, 117.26, 100.80, 21.10, 18.33; ESI-MS (m/z): 552.34 (M^+).

4-((4-(Mesitylamino)-6-((4-((4E)-5-phenylpenta-2,4-dienoyl)phenyl)amino)-1,3,5-triazin-2-yl)amino)benzonitrile (7b)

IR (ν_{max} cm^{-1}): 3354 (N-H, secondary), 3012 (C–H, aromatic), 2964 (C–H, aliphatic), 2234 (C≡N), 1634 (C=O), 1575 (-CH=CH-), 1334 (C-N), 857 (C-N, *s*-triazine); ^{1}H NMR (400 MHz, DMSO-*d6*, δ ppm): 7.79 (d, *J* = 7.5 Hz, 2H), 7.55 (d, *J* = 7.2 Hz, 2H), 7.51 (dd, *J*

= 6.8 Hz, 3H), 7.70 – 7.31 (m, 5H), 7.70 – 7.29 (m, 5H), 6.90 (d, *J* = 5.6 Hz, 1H), 6.90 (d, *J* = 5.5 Hz, 1H), 6.24 (s, 1H), 6.18 (s, 1H), 4.53 (s, 1H), 2.34 (d, *J* = 4.2 Hz, 9H); ^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm): 190.47, 165.35, 162.58, 161.27, 143.47, 142.21, 142.01, 140.56, 138.68, 136.25, 134.24, 132.45, 131.67, 131.25, 130.65, 129.24, 128.72, 128.33, 127.24, 125.15, 124.35, 121.53, 119.24, 117.78, 100.46, 20.45, 16.15; ESI-MS (m/z): 578.32 (M^+).

(E)-4-((4-((4-(3-(2-hydroxyphenyl)acryloyl)phenyl)amino)-6-(mesitylamino)-1,3,5-triazin-2-yl)amino)benzonitrile (7c)

IR (ν_{max} cm^{-1}): 3561 (-OH), 3435 (N-H, secondary), 3035 (C–H, aromatic), 2915 (C–H, aliphatic), 2235 (C≡N), 1614 (C=O), 1575 (-CH=CH-), 1334 (C-N), 812 (C-N, *s*-triazine); ^{1}H NMR (400 MHz, DMSO-*d6*, δ ppm): 8.96 (s, 1H), 8.17 (d, *J* = 7.4 Hz, 1H), 7.81 (d, *J* = 7.3 Hz, 2H), 7.56 (d, *J* = 7.1 Hz, 2H), 7.08 (d, *J* = 6.4, 1.4 Hz, 1H), 7.49 – 6.73 (m, 1H), 6.99 (s, 2H), 7.18 – 6.73 (m, 5H), 6.86 (td, *J* = 6.8 Hz, 1H), 6.76 (dd, *J* = 7.2 Hz, 1H), 6.24 (d, *J* = 6.4 Hz, 2H), 4.60 (s, 1H), 2.33 (d, *J* = 5.6 Hz, 9H); ^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm): 190.98, 165.24, 162.72, 160.74, 157.07, 143.66, 143.10, 141.56, 140.05, 134.43, 132.05, 131.63, 131.48, 131.33, 130.37, 128.73, 128.71, 121.83, 121.25, 120.64, 119.34, 117.24, 117.45, 100.67, 21.24, 15.57; ESI-MS (m/z): 568.24 (M^+).

(E)-4-((4-(mesitylamino)-6-((4-(pent-2-enoyl)phenyl)amino)-1,3,5-triazin-2-yl)amino) benzonitrile (7d)

IR (ν_{max} cm^{-1}): 3435 (N-H, secondary), 3005 (C–H, aromatic), 2914 (C–H, aliphatic), 2264 (C≡N), 1624 (C=O), 1557 (-CH=CH-), 1354 (C-N), 863 (C-N, *s*-triazine); ^{1}H NMR (400 MHz, DMSO-*d6*, δ ppm): 7.77 (d, *J* = 7.0 Hz, 2H), 7.54 (d, *J* = 7.3 Hz, 2H), 7.20 (d, *J* = 6.8 Hz, 4H), 7.12 (dd, *J* = 7.5 Hz, 1H), 6.98 (s, 2H), 6.87 (d, *J* = 6.4 Hz, 1H), 6.28 (s, 1H), 6.11 (s, 1H), 4.60 (s, 1H), 2.35 (s, 3H), 2.31 (s, 6H), 2.02 – 1.98 (m, 2H), 1.18 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm): 191.78, 165.80, 164.42, 160.42, 151.67, 143.66, 143.10, 141.56, 134.43, 132.05, 131.63, 131.48, 130.37, 128.73, 127.03, 121.66, 119.12, 117.54, 100.52, 26.37, 20.81, 17.23, 12.11; ESI-MS (m/z): 504.64 (M^+).

(E)-4-((4-((4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)phenyl)amino)-6-(mesitylamino)-1,3,5-triazin-2-yl)amino)benzonitrile (7e)

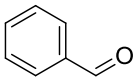
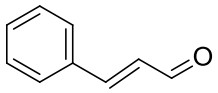
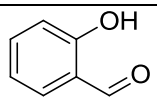
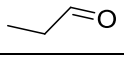
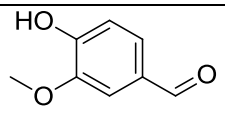
IR (ν_{max} cm^{-1}): 3564 (-OH), 3347 (N-H, secondary), 3012 (C–H, aromatic), 2945 (C–H, aliphatic), 2234 (C≡N), 1675 (C=O), 1534 (-CH=CH-), 1334 (C-N), 811 (C-N, *s*-triazine); ^{1}H NMR (400 MHz, DMSO*d6*, δ ppm): 8.57 (s, 1H), 8.10 (d, *J* = 7.3 Hz, 1H), 7.89 (s, 1H), 7.76 (d, *J* = 7.4 Hz, 2H), 7.79 – 7.30 (m, 5H), 7.20 (d, *J* = 7.0 Hz, 2H), 7.07 – 6.95 (m, 4H), 6.90 – 6.84 (m, 2H), 6.75 – 6.62 (m, 1H), 6.13 (s, 1H), 4.95 (s, 1H), 3.80 (s, 3H), 2.33 (d, *J* = 6.2 Hz, 7H); ^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm): 190.14, 161.80, 161.42, 149.57, 148.50, 144.33, 143.66, 143.10, 141.56, 134.43, 132.05, 131.63, 131.48, 130.37, 128.73, 127.58, 123.24, 123.18, 121.66, 119.12, 117.54, 115.81, 111.78, 100.24, 56.64, 21.45, 14.21; ESI-MS (m/z): 598.32 (M^+).

3. 2. Biological Evaluation

All newly synthesized *s*-triazine derivatives (5a-e and 7a-e) were examined for antimicrobial activity against two gram positive bacterial strains (*Staphylococcus aureus* MTCC 96, *Bacillus cereus* MTCC 430), two gram negative bacterial strains (*Pseudomonas aeruginosa* MTCC 741, *Klebsiella pneumoniae* MTCC 109) and two fungal strains

(*Aspergillus clavatus* MTCC 1323, *Candida albicans* MTCC 183) using agar dilution method [21]. Ciprofloxacin was used as standard control drug for antibacterial activity, whereas Ketoconazole was used as standard control drug for antifungal activity.

Table 2. *In-vitro* antibacterial and antifungal activity in MIC* (µg/ml) of compounds (5a-e) and (7a-e).

Entry	R	Gram +Ve		Gram -Ve		Fungal strains	
		S. aureus	B. cereus	*P. aeruginosa*	*K. pneumoniae*	*A. clavatus*	*C. albicans*
		MTCC 96	MTCC 430	*MTCC 741*	*MTCC 109*	*MTCC 1323*	*MTCC 183*
5a	H	50	**12.5**	50	100	100	**6.25**
5b	2-NO_2	100	200	**12.5**	25	200	100
5c	4-Cl	**6.25**	200	**6.25**	**12.5**	25	200
5d	4-F	50	**12.5**	50	25	**6.25**	25
5e	4-CH_3	**12.5**	50	**6.25**	200	100	**12.5**
7a		100	**6.25**	400	25	**6.25**	50
7b		12.5	200	**6.25**	100	50	**12.5**
7c		25	100	50	**12.5**	100	100
7d		**12.5**	100	**12.5**	25	**12.5**	**6.25**
7e		**6.25**	100	400	**12.5**	25	**12.5**
Ciprofloxacin†		3.125	3.125	3.125	3.125	---	---
Ketoconazole†		---	---	---	---	3.125	3.125
DMSO (Control)		---	---	---	---	---	---

*MIC = Minimum inhibitory concentration
† Standard

3. 3. *In vitro* antibacterial activity

Table 2 shows that all the newly synthesized quinoline scaffolds were found to exhibit good to moderate activity against the specific microbial strain, among which, Compound 5c bearing 4-Chloro substituent on phenyl ring showed half-fold activity (6.25 µg/ml MIC) against Gram-positive strain *S. aureus*. Furthermore, the same analogue 5c was found the

most active analogue by showing good inhibitory activity with 6.25 and 12.5 μg/ml against *P. aeruginosa* and *K.pneumoniae* strain respectively. The unsubstituted analogue 5a prevented the growth of *B.cereus* strain at 12.5 μg/ml MIC. Compound 5e possessing electron donating methyl substituent at para position showed excellent inhibitory profile of 6.25 and 12.5 μg/ml MIC against *P. aeruginosa* and *S. aureus* strain respectively. Another analogue 5c bearing chloro substitution on the meta and para position reported to exhibit excellent growth inhibition of Gram-negative *K.pneumoniae* bacterial strain at MIC 6.25 and 12.5 μg/mL respectively. Para fluoro substituted 5d and Electron-withdrawing nitro substituted 5b derivatives exhibited very good activity against strain *B.cereus* and *P. aeruginosa* at 12.5 μg/mL of MIC. 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 derivatives were found to be inactive even at a higher concentration of 100 μg/mL.

3. 4. *In vitro* antifungal activity

Antifungal activity data (Table 2) showed that the synthesized compounds showed variable degrees of inhibition against the tested fungi *A. clavatus* and *albicans*. The growth of *A. clavatus* fungi was inhibited strongly by fluoro substituted 5d derivative at 6.25 μg/mL MIC. In case of *C. albicans* strain, unsubstituted 5a and bearing para methyl group 5e derivative displayed superior inhibitory activity at 6.25 and 12.5 μg/mL MIC, while all the other derivatives in this set of compounds exerted moderate to poor activity profiles.

4. CONCLUSIONS

In this article, we have elaborated the initial efforts made toward the sighting of novel, potentially active thiazolidinone and chalcone based two series of *s*-triazine (5a-e) and (7a-e) derivatives which were synthesized by simple and efficient method. Due to the presence of two pharmacologically active structural fragments, *viz.*, thiazolidinone and chalcone, in one single molecule, compounds have superior antimicrobial effect. From the bioassay it is clear that the introduction of appropriate halo, nitro and the methyl substituent on the phenyl ring would lead to the more active antimicrobial derivatives. Compounds were screened against wide range of pathogenic bacteria and fungal strains. Compound 7d was the best bioactive desired antibacterial derivative with less MIC value. Overall, from the bioassay results, we just conclude that findings of the present study will have a good impact on medicinal chemists to synthesize similar analogous which will show enhanced bioactivity.

Biological Screening

Antimicrobial assay:

To determine the minimum inhibitory concentration [21], a stock solution of the final synthesized compounds (100 μg/ml) was prepared in dimethyl sulfoxide and then test compounds were incorporated in a specified quantity of molten sterile agar, i.e., nutrient agar and dextrose agar for antibacterial and for antifungal screening respectively. Such medium enclosing 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 10^5 CFU/ml was prepared and added 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 temperature for 24 h (bacteria) or 48 h (fungi). Minimum concentration of the substance that prevents the development of visible growth is considered to be the MIC value.

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