Studies on synthesis and characterization of bio-active 1,3,5-triazine derivatives

Himanshu D. Patel1, Keshav C. Patel2, Kalpesh M. Mehta1, Paresh S. Patel3 and Lina A. Patel1

1Department of Chemistry, Silvassa Institute of Higher Learning, A Government Aided College of Arts, Commerce and Science, DNHUSS, Silvassa – Naroli – 396235 (U. T. of D & NH) India
2Department of Chemistry, Veer Narmad South Gujarat University, Udhna-Magdalla Road, Surat – 395007, Gujarat, India.
3Department of Chemistry, Narmada College of Science and Commerce, Zadeshwer, Bharuch - 392011, Gujarat, India.

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ABSTRACT
Series of compounds containing s-triazine derivatives were synthesized. The synthetic strategy utilizes cyanuric chloride as a starting material to obtain the several Schiff’s base A(1-5), azetidinone B(1-5) and thiazolidinone C(1-5) viz. condensations and cyclizations. All newly synthesized compounds have been characterized by physical processes (melting points, Thin layer chromatography, elemental analysis, IR and NMR spectra) and have been tested for their antimicrobial activity against gram (+)ve and gram (-)ve bacteria and also on different stains of fungi in which some of these derivatives exhibited potential antibacterial and antifungal activity.

Introduction
Among a wide variety of heterocyclic that have been explored for developing pharmaceutical important molecules such as 1,3,5-triazine, Schiff’s base, azetidinone, thiazolidinone have played an important role in medicinal chemistry. As a reagent in organic synthesis, 1,3,5-triazine is used as the equivalent of hydrogen cyanide (HCN). One application is in the Gattermann reaction, used to attach the formyl group to aromatic substrates. Several derivatives of s-triazine show antimicrobial, antibiotic, and anti-HIV activity. They are used as a potential hormone receptor antagonists, anti-tryposomal drugs, anti-malarial activity, antitumor activity, Cyclin Dependent Kinase (CDK) inhibitor and photosynthesis inhibiting activity. In this entire work deals with synthesis and characterization of bio-active 1,3,5-triazine derivatives having the route shown in experimental section.

Experimental
All the melting points reported are uncorrected and were recorded using an electro thermal melting point apparatus. Thin layer chromatography was performed on Merck precoated 60 F254 plates. Ethyl acetate : Benzene (7.5 : 2.5) was used as solvent for the TLC and the spots were rendered visible by exposing to UV light. IR spectra were recorded on Shimadzu FT-IR (Japan) instrument. 1H NMR spectra were recorded on Bruker Avance II NMR spectrometer. Chemical shifts (δ ppm) reported are referred to internal reference TMS. Elemental analyses were obtained using a Carlo-Erba CHNS-O EA 1108 elemental analyzer. 1,3,5-triazine was received from Atul limited, Valsad (Gujarat) India. Synthetic route are described in Scheme-I and Scheme-II.

Synthesis of 2,4-dichloro-6-(4-methylphenoxo)-1,3,5-triazine (I)
To a stirred solution of 1,3,5-triazine (0.01mole, 1.81gm) in acetone (25 ml) at 0-5 °C, the solution of 4 - cresol (0.01mole, 1.09gm) in acetone (5 ml) was added and pH was maintained neutral by the addition of 10 % sodium carbonate solution. The stirring was continued at 0-5 °C for 4 hrs. After the completion of reaction, mixture was poured on ice cold water. The solid product obtained was filtered and dried. The crude product was purified by crystallization from acetone to give 2,4-dichloro-6-(4-methylphenoxo)-1,3,5-triazine.

Synthesis of 6-(4-methylphenoxo)-4-[4-(4-methoxyphenyl)piperazine-1-yl]-2-(chloro)-1,3,5-triazine (II)
To a stirred solution of 2,4-dichloro-6-(4-methylphenoxo)-1,3,5-triazine (I) (0.01mole, 2.56gm) in acetone (10 ml) at 30-35 °C, the solution of 4-methoxy N-phenyl piperazine (0.01mole, 1.92gm) in acetone (5 ml) was added drop wise maintaining the temperature 35°C. The pH was adjusted neutral by the addition of 10 % sodium bicarbonate solution. The temperature was gradually raised to 45°C during 2 hrs. After the completion of reaction, the resultant content was poured into ice cold water. The solid product obtained was filtered and dried to give 6-(4-methylphenoxo)-4-[4-(4-methoxyphenyl)piperazine-1-yl]-2-(chloro)-1,3,5-triazine (II).
Synthesis of 2-hydraziny1-6-(4-(4-methoxyphenyl)piperazin-1-yl)-4-(p-tolyl oxy)-1,3,5-triazine (I)

Take a compound 6-(4-methoxyphenyl)piperazine-1-yl)-2-(chloro)-1,3,5-triazine (0.01mole, 4.23gm) and hydrazine hydrate (0.05mole, 2.50gm) in alcohol (25 ml) was refluxed in a water bath. The temperature was gradually raised to 80-90 °C during 3 hrs. The pH was adjusted neutral by the addition of 10 % sodium bicarbonate solution. After the completion of reaction, the refluxed content was added to cold water. The solid product obtained was filtered and dried. The crude product was purified by crystallization from absolute alcohol.

Synthesis of 2-(2-substituted benzyldenehydrazinyl)-6-(4-(4-methoxyphenyl)piperazin-1-yl)-4-(p-tolyl oxy)-1,3,5-triazine (A1 to A3)

A mixture of 2-(methylphenyloxy)-6-(4-(4-methoxyphenyl)piperazine-1-yl)-4-(hydrox)1,3,5-triazine (III) (0.01mole, 4.23gm) and substituted benzyldahyde (0.01mole) was dissolved in absolute ethanol (25 ml) and add few drops of glacial acetic acid. The reaction mixture was refluxed for 8 hrs in a water bath then cooled and poured into ice cold water, solid product obtained was filtered and dried.

2-(2-(4-chloro)benzyldenehydrazinyl)-6-(4-(4-methoxyphenyl)piperazin-1-yl)-4-(p-tolyl oxy)-1,3,5-triazine (A1)

Yield 82%; m.p. 165-167 °C; Anal. Calcd. for C29H28O2N3Cl (511.57 gm/mole): C, 56.30; H, 4.24; N, 15.39. IR (KBr, cm-1): 231 (s, 3H, -CH-Cl), 3.81 (s, 3H, -OCH3), 2.31 (s, 3H, -OCH3), 3.10 (s, 1H, -OCH3), 3.78 (s, 3H, -OCH3), 3.98 (s, 4H, -N-CH2), 6.75-7.75 (m, 12H, Ar-H, Ar-H), 8.43 (s, 1H, -NH), 10.55 (s, 1H, -NH).

2-(2-(4-hydroxy)benzyldenehydrazinyl)-6-(4-(4-methoxyphenyl)piperazin-1-yl)-4-(p-tolyl oxy)-1,3,5-triazine (A2)

Yield 79%; m.p. 165-167 °C; Anal. Calcd. for C29H28O2N3Cl (511.57 gm/mole): C, 56.30; H, 4.24; N, 15.39. IR (KBr, cm-1): 5.65 (s, 1H, -OH), 6.70-7.80 (m, 11H, Ar-H), 8.35 (s, 1H, -N-CH), 10.62 (s, 1H, -NH).

2-(2-(4-methoxyphenyl)piperazin-1-yl)-4-(p-tolyl oxy)-1,3,5-triazine (A3)

Yield 77%; m.p. 164-166 °C; Anal. Calcd. for C29H28O2N3Cl (525.60 gm/mole): C, 66.27; H, 5.94; N, 18.65. Found: C, 66.39; H, 5.87; N, 18.58. IR (KBr, cm-1): 820, 1369, 1445 (C-O-C), 1582, 1615, 2978, 3151, 3212. 1H NMR (400 MHz, DMSO-de6, δ ppm): 2.39 (s, 3H, -CH3), 3.19 (t, 4H, -CH2-N), 3.70 (s, 3H, -OCH3), 3.95 (t, 4H, -NCH2), 4.02 (s, 3H, -OCH3), 6.75-7.80 (m, 12H, Ar-H), 8.40 (s, 1H, -N-CH), 10.58 (s, 1H, -NH).

2-(2-(4,4-dichloro)benzyldenehydrazinyl)-6-(4-(4-methoxyphenyl)piperazin-1-yl)-4-(p-tolyl oxy)-1,3,5-triazine (A4)

Yield 85%; m.p. 180-182 °C; Anal. Calcd. for C29H28O2N3Cl (546.64 gm/mole): C, 59.58; H, 4.82; N, 17.37. Found: C, 59.66; H, 4.91; N, 17.49. IR (KBr, cm-1): 727 (C-Cl), 812, 1370, 1450, 1570, 1609, 2975, 3140, 3230. 1H NMR (400 MHz, DMSO-de6, δ ppm): 2.40 (s, 3H, -CH3), 3.10 (t, 4H, -NCH2), 3.75 (s, 3H, -OCH3), 3.90 (t, 4H, -NCH2), 6.70-7.80 (m, 11H, Ar-H), 8.35 (s, 1H, -NCH2), 10.62 (s, 1H, -NH).

2-(2-(3,4-dihydroxy)benzyldenehydrazinyl)-6-(4-(4-methoxyphenyl)piperazin-1-yl)-4-(p-tolyl oxy)-1,3,5-triazine (A5)

Yield 80%; m.p. 103-105 °C; Anal. Calcd. for C29H28O2N3Cl (527.57 gm/mole): C, 63.74; H, 5.54; N, 18.58. Found: C, 63.68; H, 5.47; N, 18.49. IR (KBr, cm-1): 816, 1372, 1443, 1575, 1615, 2980, 3145, 3222, 3270 (O-H). 1H NMR (400 MHz, DMSO-de6, δ ppm): 2.40 (s, 3H, -CH3), 3.10 (t, 4H, -NCH2), 3.75 (s, 3H, -OCH3), 3.90 (t, 4H, -NCH2), 5.65 (s, 1H, -OH), 6.70-7.80 (m, 11H, Ar-H), 8.35 (s, 1H, -NCH3), 10.62 (s, 1H, -NH).
3-chloro-1-(6-(4-(4-methoxyphenyl)piperazin-1-yl)-4-(p-tolyl oxy)-1,3,5-triazin-2-ylamino)-4-(3,4-dihydroxyphenyl)azetidin-2-one (B$_2$)  
Yield 75%; m. p. 218–220 °C; Anal. Calcd. for C$_{30}$H$_{30}$O$_3$N$_2$Cl (604.05 gm/mole): C, 59.65; H, 5.10; N, 16.23. Found: C, 56.59; H, 5.18; N, 16.03. IR (KBr, cm$^{-1}$): 832, 1341, 1456, 1525, 1732 (-C=O Azetidinone), 3015, 3253, 3270 (O-H). $^1$H NMR (400 MHz, DMSO-d$_6$, $\delta$ ppm): 2.51 (s, 3H, -CH$_3$), 3.05 (t, 4H, -N-CH$_2$), 3.53 (s, 3H, -OCH$_3$), 3.89 (t, 4H, -N-CH$_2$), 4.71 (s, 1H, -CH-Cl), 5.15 (s, 1H, -OH), 5.45 (s, 1H, -OH), 5.90 (s,1H, -N-CH), 6.70-7.84 (m, 11H, Ar-H), 9.65 (s, 1H, -NH).

Synthesis of 3-(6-(4-(4-methoxyphenyl)piperazin-1-yl)-4-(p-tolyl)oxy)-1,3,5-triazin-2-ylamino)-2-(substitutedphenyl) thiazolidin-4-one (C$_1$ to C$_3$)  
To a solution of 2-(2-substituted benzylidenehydrazinyl)-6-(4-(4-methoxyphenyl)piperazin-1-yl)-4-(p-tolyl)oxy)-1,3,5-triazine (A$_1$ to A$_3$) (0.01mole) and 1gm of anhydrous zinc chloride as a catalyst in dry benzene (15 ml), thioglycolic acid (0.02mole, 1.84gm) was added by dropping funnel with stirring at constant temperature and refluxed it for 8-9 hrs, then cooled it and poured in sodium bicarbonate solution (10%) to get neutralized. The solid thus obtained was recrystallized from alcohol to yield target compounds (C$_1$ to C$_3$).

3-(6-(4-(4-methoxyphenyl)piperazin-1-yl)-4-(p-tolyl)oxy)-1,3,5-triazin-2-ylamino)-2-(4-chlorophenyl)thiazolidin-4-one (C$_1$)  
Yield 73%; m. p. 179-181 °C; Anal. Calcd. for C$_{30}$H$_{28}$O$_3$N$_2$Cl (604.12 gm/mole): C, 59.64; H, 5.01; N, 16.23. Found: C, 56.72; H, 5.13; N, 16.35. IR (KBr, cm$^{-1}$): 690, 750 (C=Cl), 813, 1300, 1380, 1510, 1698, 3050, 3250. $^1$H NMR (400 MHz, DMSO-d$_6$, $\delta$ ppm): 2.31 (s, 3H, -CH$_3$), 2.75 (s, 2H, -S-CH$_3$), 3.10 (t, 4H, -N-CH$_2$), 3.75 (s, 3H, -OCH$_3$), 3.92 (t, 4H, -N-CH$_2$), 5.90 (s,1H, -N-CH), 6.80-7.50 (m, 12H, Ar-H), 11.01 (s, 1H, -NH).

3-(6-(4-(4-methoxyphenyl)piperazin-1-yl)-4-(p-tolyl)oxy)-1,3,5-triazin-2-ylamino)-2-(4-hydroxyphenyl)thiazolidin-4-one (C$_2$)  
Yield 76%; m. p. 185-187 °C; Anal. Calcd. for C$_{30}$H$_{28}$O$_3$N$_2$S (604.12 gm/mole): C, 61.52; H, 5.34; N, 16.74. Found: C, 61.63; H, 5.42; N, 16.66. IR (KBr, cm$^{-1}$): 692, 815, 1305, 1378, 1515, 1690, 3050, 3250, 3275 (O-H). $^1$H NMR (400 MHz, DMSO-d$_6$, $\delta$ ppm): 2.28 (s, 3H, -CH$_3$), 2.70 (s, 2H, -S-CH$_3$), 3.15 (t, 4H, -N-CH$_2$), 3.80 (s, 3H, -OCH$_3$), 3.95 (t, 4H, -N-CH$_2$), 5.40 (s, 1H, -OH), 5.95 (s,1H, -N-CH), 6.70-7.40 (m, 12H, Ar-H), 11.10 (s, 1H, -NH).

3-(6-(4-(4-methoxyphenyl)piperazin-1-yl)-4-(p-tolyl)oxy)-1,3,5-triazin-2-ylamino)-2-(4-methoxyphenyl)thiazolidin-4-one (C$_3$)  
Yield 81%; m. p. 124-126 °C; Anal. Calcd. for C$_{30}$H$_{28}$O$_3$N$_2$S (599.70 gm/mole): C, 61.98; H, 5.70; N, 16.32. Found: C, 61.89; H, 5.83; N, 16.46. IR (KBr, cm$^{-1}$): 710, 825, 1320, 1380, 1525, 1695, 3040, 3205. $^1$H NMR (400 MHz, DMSO-d$_6$, $\delta$ ppm): 2.32 (s, 3H, -CH$_3$), 2.71 (s, 2H, -S-CH$_3$), 3.20 (t, 4H, -N-CH$_2$), 3.60 (s, 3H, -OCH$_3$), 3.75 (s, 3H, -OCH$_3$), 3.99 (t, 4H, -N-CH$_2$), 5.90 (s,1H, -N-CH), 6.70-7.50 (m, 12H, Ar-H), 11.05 (s, 1H, -NH).

Scheme - I

3-(6-(4-(4-methoxyphenyl)piperazin-1-yl)-4-(p-tolyl)oxy)-1,3,5-triazin-2-ylamino)-2-(2,4-dichlorophenyl)thiazolidin-4-one (C$_4$)  
Yield 72%; m. p. 114-116 °C; Anal. Calcd. for C$_{30}$H$_{28}$O$_3$N$_2$Cl (638.56 gm/mole): C, 56.43; H, 4.58; N, 15.35. Found: C, 56.54; H, 4.66; N, 15.26. IR (KBr, cm$^{-1}$): 685, 760 (C=Cl), 825, 1305, 1375, 1520, 1695, 3055, 3245. $^1$H NMR (400 MHz, DMSO-d$_6$, $\delta$ ppm): 2.34 (s, 3H, -CH$_3$), 2.65 (s, 2H, -S-CH$_2$), 3.03 (t, 4H, -N-CH$_2$), 3.70 (s, 3H, -OCH$_3$), 3.95 (t, 4H, -N-CH$_2$), 5.85 (s,1H, -N-CH), 6.85-7.55 (m, 11H, Ar-H), 11.05 (s, 1H, -NH).
Results and Discussion

The synthetic route of the compounds (A, B & C) is outlined in schemes I & II respectively. The synthesis of 2-(2-substituted benzylidenehydrazinyl)-6-(4-(4-methoxyphenyl)piperazin-1-yl)-4-(4-fluoro-3,4-dihydroxy) triazine (A₁ to A₃) as shown in Scheme-I was carried out from p-cresol and cyanuric chloride. The compound-I and N-phenyl piperazine was refluxed to give compound-II which on refluxed with hydrazine hydrate in alcohol to give compound-III. The compound-III was refluxed with different substituted benzaldehyde to give (A₁ to A₃). The compound (B₁ to B₃) was synthesized by the reaction of (A₁ to A₃) with chloroacetyl chloride in presence of base. The Compound (C₁ to C₅) was synthesized by thioglycolic acid and compound (A₁ to A₅) as shown in scheme-II.

The structures of the synthesized compounds were characterized by analytical methods and spectral data (IR and 1H NMR). In the IR spectra, the -N=C bands were observed at 815, 1600-1670 cm⁻¹ respectively. In compound (C₁ to C₅) C-S-C bands were observed in the range of 670-720 cm⁻¹. In the 1H NMR spectra range 3.70-4.20 ppm and 9.00-11.00 ppm (singlet) peaks for all compounds confirmed the availability of -OCH₃ and -NH group respectively. In compound (B₁ to B₃)-CH-Cl peaks at 4.58-4.71 ppm (singlet). In (C₁ to C₅) -S-CH₂ peaks at 3.70-3.95 ppm (singlet).

The antimicrobial activities of the synthesized compounds are also shown in the table III. The antimicrobial activities of these compounds active against variety of bacterial stains in which some of these derivatives exhibited potential antibacterial and antifungal activity. The antimicrobial activities of the synthesized compounds are shown in the Table-I which were found moderate to good active against tested organism. Compounds A₁, A₂, B₃ & C₅ showed good activity (25µg/ml) against S.aureus compared to Gentamycin which may be due to 4-chloro, and 2,4-dichloro substituents. Compounds B₁ & C₁ showed good activity against S.pyogenes which may be due to 3-methoxy and 2,4-dichloro substituents. Compounds A₂ & C₁ showed good activity against C.albicans compared to Nystatin which may be due to 3-hydroxy and 4-chloro substituents. Compounds B₂ & C₅ showed good activity against A.clavatus which possess due to 3,4-dihydroxy and 2,4-dichloro substituents. From the antibacterial and antifungal results (table-I) indicated that rest of all compounds moderate to weak activity against these species.

Antimicrobial activity

All the synthesized compounds (A, B & C) were screened for their antimicrobial activity by agar disc diffusion method[15,16]. DMSO was used as diluents to get desired concentration of drugs to test upon standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. All the tubes not showing visible growth are subcultured and incubated overnight at 37 °C. The lowest concentration inhibit growth of the organism was recorded as the MIC. The growth, inhibition is measured and compound is applied in the method to determine

### Table-I: Antimicrobial activity of the synthesized compounds

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<th>Compound No.</th>
<th>Minimal Inhibitory Concentration (µg/ml)</th>
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<td></td>
<td><strong>Antibacterial Activity</strong></td>
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<td></td>
<td><strong>Gram (+ve) bacteria</strong></td>
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<tr>
<td><strong>S. aureus</strong></td>
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**Antibacterial Activity**

- S. aureus
- S. pyogenes
- E. coli
- P. aeruginosa

**Antifungal Activity**

- C. albicans
- A. niger
- A. clavatus

The MIC values are shown in Table-I and indicate the effectiveness of the compounds against the tested bacteria and fungi.
the activity in µg/ml concentration. All the compounds were screened against Gram (+ve) bacteria [Staphylococcus aureus (MTCC-96) and Streptococcus pyogenus (MTCC-443)] and Gram (-ve) bacteria [Escherichia coli (MTCC-442) and Pseudomonas aeruginosa (MTCC-441)]. The antifungal activity was tested against Gram (+ve) fungus [Candida albicans (MTCC-227)] and Gram (-ve) fungi [Aspergillus niger (MTCC-282) and Aspergillus clavatus (MTCC-1323)]. The results of all the synthesized compounds were compared with Gentamycin and Nystatin as a reference drugs. The results are shown in table-I.

Conclusion
The antimicrobial activity of the synthesized compounds showed that compounds containing 4-hydroxy, 4-chloro and 2,4-dichloro substituents are found active against tested organism. This fact reveals that the activity is not affected due to the electronic properties of the substituents. Compounds A₁ & A₄ Schiff’s base, B₄ azetidinone and C₄ thiazolidinone were found more active against these gram (+ve) and gram (-ve) bacterial and fungal species.

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