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# Comparative Antimicrobial screening study on Triazine Derivatives

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## ABSTRACT

A series of Novel Triazine has been synthesized and screened for antibacterial activities. The compound having methoxy and methyl substitutions are exhibits signification antibacterial activity against Bacillus substilis, Escherichia coli, S. aureus and Pseudomonas aeruginosa.

**KEYWORDS:** Triazine; Antimicrobial, antibacterial activities.

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## INTRODUCTION

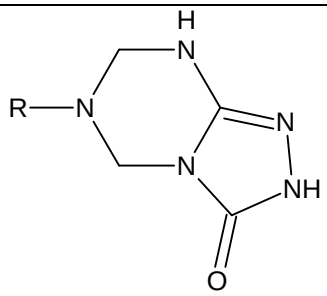
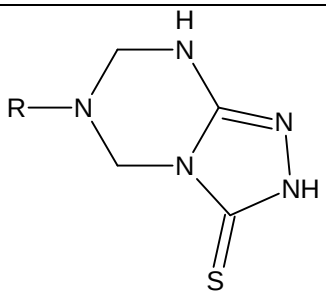
Antimicrobial drugs are the greatest contribution of the present century to therapeutics. Their advent changed the outlook of Physician about the 'power drug' can have on disease. They have one of the few curative drugs; their importance is magnified in developing countries where infective diseases predominate. Drug in this class differ from other in that they are design to inhibit/ kill the infecting organisms & to have no/ minimal effect on recipient . Many natural and synthetic compounds containing heterocyclic rings such as Triazine like s-triazine and endogenous polyfunctional heterocyclic compounds, to exhibit biological[1] activity in mammals. Triazine are known to possess a wide range of pharmacological activities like antibacterial[1], antifungal, anti-HIV[2], anticonvulsant, antiviral, anticancer[3]. Triazine bases can act as inhibitors of human a-thrombin. Some of the new triazine bases, reported as potential biologically active compounds. It was identified in animals as a major component of the endogenous. Triazine is a versatile lead molecule for potential bioactive compounds and its derivatives were reported to possess antimicrobial activity. Triazine derivatives are an important class of organic heterocycles because of their potential bioactivity[11]. The pharmacological activity of s-triazine varies from a range of actions in the brain and to be protective against certain types of infections.

## EXPERIMENTAL

The entire compound produced by following standard synthetic methods in synthetic chemistry laboratory and analytical screening done on calibrated and validated equipments and/or instruments.

All the compounds are synthesized by using Microwave assisted organic synthesis (MAOS) technique, which offers simple, clean, fast, efficient, and economic for the synthesis of a large number of organic molecules. For analytical characterization pre-coated Thin-layer chromatography (TLC) plates were used to confirm purity, Fourier-transform infrared spectroscopy (FTIR), Nuclear magnetic resonance (NMR) and Mass spectroscopic instrument were used for structural characterization of synthesized compounds. Melting points was carried out on calibrated digital apparatus.

**Table: 1**

| Compound |  | Compound |  |
|---|---|---|---|
| RA1 | - C6H5 | RB1 | - C6H5 |
| RA2 | - 4Cl C6H4 | RB2 | - 4Cl C6H4 |
| RA3 | - 4F C6H4 | RB3 | - 4F C6H4 |
| RA4 | - 4CH3 C6H4 | RB4 | - 4CH3 C6H4 |
| RA5 | - 4OCH3 C6H4 | RB5 | - 4OCH3 C6H4 |
| RA6 | - 4NO2 C6H4 | RB6 | - 4NO2 C6H4 |
| RA7 | - 2Cl C6H4 | RB7 | - 2Cl C6H4 |
| RA8 | - 2F C6H4 | RB8 | - 2F C6H4 |
| RA9 | - 2CH3 C6H4 | RB9 | - 2CH3 C6H4 |
| RA10 | - 2OCH3 C6H4 | RB10 | - 2OCH3 C6H4 |
| RA11 | - 2NO2 C6H4 | RB11 | - 2NO2 C6H4 |

## METHODS

### *Antimicrobial activity*

***Chemical*** All chemicals and solvents were procured from commercial sources and purified and sterilized using standard procedures.

***Preparation:*** MacConkey agar, Soybean casein digest medium and normal saline solution were sterilized in autoclave at 15 lbs pressure (121$^{o}$C) for 15 mins. Petri plates, disc of whatman paper, cotton swabs were sterilized in oven at 160$^{o}$C for 2 hrs.

***Dilution of the compounds:*** All the synthesized compounds of triazine derivatives dissolved in dimethylsulfoxide (DMSO) so as to get concentration of 30 µg/ml. Amoxicillin were separately dissolved in dimethylsulfoxide (DMSO) so as to get concentration of 30 µg/ml and it used as standard drug for antibacterial & antifungal screening respectively.

***Preparation of Slants:*** *MacConkey agar slant* prepared as 206 mg of MacConkey agar was dissolved in 4ml of distilled water, boiled and then poured it in the test tube and the test tube was

plugged with cotton and then sterilized in autoclave at 15 lbs pressure (121°C) for 15 min. After the sterilization the tubes containing the MacConkey agar were kept in inclined position for ½ hrs. Then on the solid surface of these slants the pure culture of the test bacteria i.e. *Klebsiella penumoniae* and *Escherichia coli* were streaked in aseptic condition and then incubated at 37°C for 24 hrs. and *Nutrient Agar medium slant* prepared as 112 mg of Nutrient agar medium and 100 mg of agar agar powder was dissolved in 4ml of distilled water boiled and then poured it in the test tube and the test tube was plugged with cotton and then sterilized in autoclave at 15 lbs pressure (121°C) for 15mins. After sterilization the tube containing Nutrient Agar medium were kept in inclined position for 30 mins. Then on solid surface of these slants pure culture of test bacteria i.e. *Bacillus Subtilis, S. aureus, Staphylococcus aureus* and *Pseudomonas aeruginosa* were streaked in aseptic condition and then incubated at 37°C for 24 hrs.; *Saboraud's agar slant prepared as* 250 mg of Saboraud's agar was dissolved in 4ml of distilled water, boiled and then poured it in the test tube and the test tube was plugged with cotton and then sterilized in autoclave at 15 lbs pressure (121°C) for 15 min.

Preparation of suspension of test bacteria (standardized inoculum): By using the 24 hrs old growth of the test bacteria from the slant, suspension of the bacteria were made separately in sterile normal saline solution (0.85% NaCl in distilled water) in aseptic condition, to get moderate turbidity. The turbidity of each suspension was compared and adjusted with the turbidity of the solution resulting by mixing 0.5 ml of 1.175% of barium chloride and 99.5 ml of 0.36N, $H_2SO_4$ acid.

Antimicrobial sensitivity test have been carried out by using disc-diffusion method, performed in nutrient agar for bacterial.

***Preparation of culture media for antibacterial sensitivity test:*** Soybean casein digest agar was prepared by weighing 3 gms of soybean casein digest medium and 2.5 gms of agar agar powder in 100 ml of distilled water. Then it was sterilized in autoclave at 15 lbs pressure (121°C) for 15 mins. After sterilization the media was cooled up to 45°C and then it was poured in sterile petri plates in aseptic condition. Approximately 20-25 ml of media was poured in each plate. Then the media from the plate was allowed to get solidified.

*Inoculation of suspension of bacteria on culture media:* Sterile, non-toxic cotton swab were dipped into the standardized inoculum (turbidity as adjusted as to obtained confluent growth on the Petri plate) and then the entire agar surface of the plate was streaked with the swab three times, turning the plate at 60° angle between streaking. Then the streaked inoculum was allowed to dry for 5-15 mins with lid in place.

Sterile paper disc made by punching whatman (No.41) paper were dipped separately in to the solutions containing synthesized drug (50 µg/ml of DMSO) and standard drug amoxicillin (30 µg/ml of DMSO) in aseptic condition with help of sterile forcep and were then placed on the surface of inoculated culture media after which the plates were kept in refrigeration for 30 mins for the diffusion of the drug from the paper disc in to the culture media. After 30 mins the plates were incubated at 37ºC.

The in vitro antibacterial and antifungal activity was carried out against 24 hours old culture of four bacteria and one fungus. The bacteria used were
*Bacillus substilis, Escherichia coli, S. aureus, Pseudomonas aeruginosa*. Serial dilution method was used for determining minimum inhibitory concentration (MIC). Nutrient broth was used as growth medium for bacteria and Sabouraund's medium was used for growth of fungus. Dimethylsulfoxide (DMSO) was used as solvent. The result obtained was compared with standard drug Amoxicillin for antibacterial activity. The result of MIC value was summarized in Table 2A and Table 2B.

**Table 2A: In Vitro antimicrobial activity of the compounds (RA1-RA11)**

| Compound | MIC values (50ug/ml) | | | |
|---|---|---|---|---|
| | Gram-positive organisms | | Gram-negative organisms | |
| | *B. subtilis* | *S. aureus* | *E-Coil* | *P.areginosa* |
| RA1 | ++ | - | - | ++ |
| RA2 | - | +++ | - | - |
| RA3 | + | ++ | + | - |
| RA4 | - | + | - | ++ |
| RA5 | +++ | + | +++ | ++ |
| RA6 | ++ | ++ | + | - |
| RA7 | + | +++ | - | + |
| RA8 | - | - | ++ | - |
| RA9 | +++ | ++ | - | ++ |
| RA10 | - | + | ++ | - |
| RA11 | ++ | - | + | - |
| Control (DMSO) | - | - | - | - |
| Standard | +++ | +++ | +++ | +++ |

Table 2B: In Vitro antimicrobial activity of the compounds (RB1-RB11)

| Compound | MIC values (50ug/ml) | | | |
|---|---|---|---|---|
| | Gram-positive organisms | | Gram-negative organisms | |
| | *B. subtilis* | *S. aureus* | *E-Coil* | *P.areginosa* |
| RB1 | + | ++ | + | ++ |
| RB2 | - | - | ++ | - |
| RB3 | ++ | ++ | + | ++ |
| RB4 | ++ | + | - | - |
| RB5 | +++ | + | ++ | +++ |
| RB6 | +++ | +++ | - | - |
| RB7 | + | - | +++ | + |
| RB8 | - | ++ | + | + |
| RB9 | ++ | - | +++ | - |
| RB10 | +++ | - | + | ++ |
| RB11 | - | +++ | - | - |
| Control (DMSO) | - | - | - | - |
| Standard | +++ | +++ | +++ | +++ |

Where, Comp.– Synthesized compound; B. sub – Bacillus substilis; E. coli – Escherichia coli; S. aureus - Staphylococcus aureus; P.aur. - Pseudomonas aeruginosa

Key to symbols: Highly active: +++ (inhibition zone > 18 mm); Moderately active: ++ (inhibition zone 12-18 mm); Slightly active: + (inhibition zone 6 - 12 mm) and Inactive: - (inhibition zone < 6 mm)

## RESULTS AND DISCUSSION

Amoxicillin is used as standard drug for the comparing the antibacterial activity of triazine derivatives. Clotrimazole was used as standard for the comparison of antifungal activity of triazine derivatives.

All the compounds screened for antibacterial activity and they showed an result as tabulated in Table No. 3 against the gram positive species Bacillus substilis, S. aureus of bacteria used for screening, which is comparable with standard used. In case of gram negative species i.e. S. aureus, Escherichia

coli, Pseudomonas aeruginosa all the synthesized compounds screened and showed varies activities compared with standard as tabulated in Table No. 3.

**Table No. 3 : Antibacterial Activity result of compounds**

| Activity | Bacillus substilis | S. aureus | Escherichia coli | Pseudomonas aeruginosa |
|---|---|---|---|---|
| High activity | RA5<br>RA9 | RA2<br>RA7 | RA5 | - |
| | RB5<br>RB6<br>RB10 | RB6<br>RB11 | RB7<br>RB9 | RB5 |

## CONCLUSION

All synthesized compounds were screened for Antimicrobial activity. It can be concluded from the above study that among all the synthesized compounds are compared.

The compounds having meta substitution with methoxy and ortho substituted methyl group showing promising activity against Bacillus substilis, Escherichia coli and Pseudomonas aeruginosa, whereas Nitro substituted compound is also showing high activity against Bacillus substilis and Escherichia coli. However, chloro substituted derivatives are also exhibited better antibacterial activities.

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