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Synthesis and antibacterial activity of novel N-Mannich bases of 1,2,4-Triazines

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ABSTRACT

Triazines are a widely studied class of compounds having various pharmacological activities. In this work, Mannich bases of 1,2,4-triaizine were synthesized and evaluated for antibacterial action. The synthesis was achieved in two simple steps involving formation of traizine nucleus and subsequent Mannich base formatiom. The compound TZM5 & TZM6 showed the highest antimicrobial activities against all of the tested organisms (bacteria). In contrast, compound TZM1 showed lower antimicrobial activity against all tested microorganisms.

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Introduction

Triazines are a widely studied class of compounds having various pharmacological activities.[1-7] The literature studies revealed that Mannich bases are very reactive and can be easily converted to other compounds, for example, reduced to form physiologically active amino alcohols. Mannich bases are known to possess potent activities like anti-inflammatory, anticancer, antifilarial, antibacterial, antifungal, anticonvulsant, anthelmintic, antitubercular, analgesic, anti-HIV, antimalarial, antipsychotic, antiviral activities and so forth.[8] Along with biological activities Mannich bases are also known for their uses in detergent additives, resins, polymers, surface active agents, and so forth.[3]

Prodrugs of Mannich bases of various active compounds have been prepared to overcome the limitations. Mannich bases (optically pure chiral) of 2-naphthol are employed for catalysis (ligand accelerated and metal mediated) of the enantio-selective carbon-carbon bond formation. Mannich bases and their derivatives are intermediates for the synthesis of bioactive molecules. Mannich reaction is widely used for the construction of nitrogen containing compounds. Mannich bases have gained importance due to their application in antibacterial activity and other applications are in agrochemicals such as plant growth regulators.

Considering the biological activities of these two classes, it was envisaged that preparing Mannich bases of Triazine heterocyclic would be helpful in enhancing the pharmacological potential of the compounds.

Material and Methods

All the reagents and chemical used were of synthetic grade and used as obtained. Melting point were determined using open capillary technique and are reported uncorrected.

The scheme for synthesis of Triazines was adapted from report by Musatov et al.[9] The synthesis of mannich bases was accomplished according to the scheme depicted at figure 1.

Synthesis of 3-Amino-5,6-diphenyl-1,2,4-triazine

A weighed quantity of aminoguanidine bicarbonate (13.61g, 0.1 mol) was added to a solution of dibenzoyl(21.02g, 0.1 mol) in 50 ml of n-BuOH with intensive stirring. The reaction mixture heated under reflux for 6 h, cooled to room temperature and maintained at 5 ^{o}C for 24 h. Crystals of triazine precipitated out, dried up and washed with mixture diethylether-hexane (1:1). The product was dried under vacuum and stored in desiccator for further use.

General method for synthesis of Mannich bases

Ketone (Substituted acetophenones) (0.05 mol), paraformaldehyde (3 g, 0.1 mol), triazine (0.05 mol), 0.1 ml conc. HCl and 2-propanol (15–25 ml) were refluxed with stirring for 4 h. In less than an hour the reactants had dissolved and sometimes the Mannich base hydrochloride started to separate from the reaction mixture as a solid after about 2 h when a small volume of 2-propanol was employed. After the reaction mixture had been kept in a freezer overnight, the crystals were filtered off, washed with acetone (or diethyl ether) and purified by recrystallization from ethanol.

3-(5,6-diphenyl-1,2,4-triazin-3-ylamino)-1-phenylpropan-1-one [TZM1]

Color – Pale Yellow; Yield – 68%; 1H NMR: 7.4 (CH-Benzene), 7.3 (CH-Benzene), 7.2 (CH-Benzene), 7.8 (CH-Benzene); IR (KBr); 3500-3100 cm^{-1} (pri and sec. amine), 1700-1500 cm^{-1} (c=c aro.), 3200-3000cm^{-1} (CH Aro.), 1500cm^{-1} (NH), 1300-1100 cm^{-1}(CN-amine)

3-(5,6-diphenyl-1,2,4-triazin-3-ylamino)-1-(4-hydroxyphenyl)propan-1-one [TZM2]

Color – Pale Yellow; Yield – 70%; 1H NMR: 3.7 (Aro.NH2), 2.5 (CH), 7.4 (CH-Benzene), 7.2(CH-Benzene), 7.3 (CH-Benzene), 0.86 (CH3); IR KBr: 3500-3100 cm^{-1} (1° and 2° amine), 1700-1500 cm^{-1} (c=c Ar), 3200-3000cm-1 (CH Ar), 1500cm^{-1} (NH), 1300-1100 cm $^{-1}$ (CN-amine)

1-(4-chlorophenyl)-3-(5,6-diphenyl-1,2,4-triazin-3-ylamino)propan-1-one [TZM3]

Color – Pale Yellow; Yield – 75%; 1H NMR: 7.4 (CH-Benzene), 7.3 (CH-Benzene), 7.2 (CH-Benzene), 2.6 (CH3).; IR (KBr): 3500-3100 cm^{-1} (pri and sec. amine), 1700-1500 cm^{-1} (c=c aro.), 3200-3000cm^{-1} (CH Aro.), 1500cm^{-1} (NH), 1300 -1100 cm^{-1}(CN-amine), 3600-3200 cm^{-1}(OH)

1-(4-bromophenyl)-3-(5,6-diphenyl-1,2,4-triazin-3-ylamino)propan-1-one [TZM4]

Color – Dark Yellow; Yield – 58%; 1H NMR: 7.4 (CH-Benzene), 7.3 (CH-Benzene), 7.2 (CH-Benzene), 3.5 (Ar C-NH), 6.9 (CH-Benzene), 2.5 (CH), 3.5 (CH3); IR (KBr): 3500-3100 cm^{-1}(pri and sec. amine), 1700-1500 cm^{-1}(c=c aro.), 3200-3000cm^{-1} (CH Aro.), 1500cm^{-1} (NH), 1300 -1100 cm^{-1} (CN-amine)

1-(4-aminophenyl)-3-(5,6-diphenyl-1,2,4-triazin-3-ylamino)propan-1-one [TZM5]

Color – Yellowish brown; Yield –66%; 1H NMR: 7.4 (CH-Benzene), 7.3 (CH-Benzene), 7.2 (CH-Benzene), 3.7 (Ar CNH), 2.5 (CH3), 7.0 (CH-Benzene), 7.1 (CH-Benzene); IR (KBr): 3500-3100 cm^{-1}(pri and sec. amine), 1700-1500 cm^{-1} (c=c aro.), 3200-3000cm^{-1} (CH Aro.), 1500cm^{-1} (NH), 1300-1100 cm^{-1}(CN-amine), 800 cm^{-1}(cl)

3-(5,6-diphenyl-1,2,4-triazin-3-ylamino)-1-(4-nitrophenyl)propan-1-one [TZM6]

Color – Dark Yellow; Yield – 52%; 1H NMR: 7.4 (CH-Benzene), 7.3 (CH-Benzene), 7.2 (CH-Benzene), 3.7 (Ar CNH), 2.5 (CH3), 7.0 (CH-Benzene), 7.1 (CH-Benzene); IR (KBr): 3500-3100 cm^{-1} (pri and sec. amine), 1700-1500 cm^{-1} (c=c aro.), 3200-3000cm^{-1} (CH Aro.), 1500cm^{-1} (NH), 1300-1100 cm^{-1} (CN-amine), 800 cm-1 (cl)

Antimicrobial activity

The microorganisms used in this study as test organisms comprising of clinical isolates of three bacteria *Escherichia coli* -M.T.C.C. 3261, *Bacillus Subtilis*- M.T.C.C.1134, *Pseudomonas aeruginosa* -M.T.C.C. 647. The typed cultures of bacteria were sub-cultured on Nutrient agar and stored at 4^{o}C until required for study.

The antimicrobial activities of the synthesized compounds were determined by filter paper disc and agar well diffusion methods.[10]

Preparation of test solutions

The synthesized compounds were accurately weighed and dissolved in DMSO to prepare appropriate dilution to get required concentrations of 150, 250 and 350 µg/ml. They were kept under refrigerated condition unless they were used for the experiment.

Preparation of dried filter paper discs

Whatman filter paper (No:1) was used to prepare discs approximately 6 mm in diameter, which are placed in hot air for sterilization. After sterilization, the discs were loaded with dif-

ferent concentration of broad spectrum antibiotic gentamicin and the test solution of different concentrations.

Inoculum Preparation

Inoculum of *Escherichia coli, Pseudomonas aeruginosa, and Bacillus subtilis* were prepared in nutrient broth medium, same as for broth dilution method and kept for incubation at 35°Cfor 8 hrs.

Inoculation of Test Plates

A sterile cotton swab (Hi media, readily prepared sterile swabs) was dipped into the turbid culture suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.

The dried surface of a Mueller-Hinton agar plate was inoculated by swabbing the swab over the entire sterile agar surface. This procedure was repeated by swabbing two more times, rotating the plate approximately 60°C each time to ensure an even distribution of Inoculum. As a final step, the rim of the agar was swabbed. The lid may be left aside for 3 to 5 minutes, but not more than15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

Application of Discs to Inoculated Agar Plates

Previously prepared paper discs were dispensed onto the surface of the inoculated agar plate. Each disc was pressed down firmly to ensure complete contact with the agar surface. The discs were placed on the medium suitably apart and the plates were incubated at 5°C for 1 hr to permit good diffusion and then transferred to incubator at 37°C for 24 hrs. After completion of 24hrs, the plates were inverted and placed in an incubator set to 37°C for 24 hrs.

Results and Discussion

Synthesis was carried out by utilizing the desired scheme. Five derivative were synthesized and characterized by using TLC, IR, NMR spectroscopy. The compounds were found to be freely soluble in chloroform and acetone whereas insoluble in water.

The MICs (ug mL^{-1}) of tested compounds against bacteria are shown in table. All synthesized compounds exhibit moderate to excellent inhibitory effect with MIC values against three strains of bacteria (gram negative and gram positive).

The 1, 2, 4- triazine compounds are a representative class of heterocyclic compounds with a wide variety of interesting properties which are used in medicine and agriculture. It has been associated with diverse pharmacological activities such as hypertension and inhibition of platelets, anti-leukemic, anti-inflammatory and potent neuroprotective agents. The 1, 2, 4 – triazine moiety is a structural element in antimalarial, anticancer, antifungal, anticonvulsant, antibacterial and antiviral compounds containing a 1, 2, 4 – triazine nucleus reported to possess pesticidal, neuro-pharmacological, analgesic and antidepressant properties. Also, 1, 2, 4, - triazine exhibiting various types of pharmacological activity and are useful for the treatment and / or prevention of various disease. All the synthesized compounds exhibited the IR peak for C=C aromatic, CH aromatic and amine. NMR spectra revealed benzene (CH) signal and aromatic C=NH and ethylene.

The quantitative antibacterial results are reported in terms of minimum inhibitory concen-

trations (MICs). Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of antimicrobial that will inhibit the visible growth of microorganism after overnight incubation. The tube dilution test is the standard method for determining levels of resistance to an antibiotic. Serial dilutions of the antibiotic are made in a liquid medium which is inoculated with a standardized number of organisms and incubated for a prescribed time. The lowest concentration (highest dilution) of antibiotic preventing appearance of turbidity is considered to be the minimal inhibitory concentration (MIC). Although the tube dilution test is fairly precise, the test is laborious because serial dilutions of the antibiotic must be made and only one isolate can be tested in each series of dilutions.

Antimicrobial activity of the synthesized compounds against three tested microorganisms was determined. As presented in table, all compound showed different dose dependent antimicrobial effect against the different organisms, recording the highest activity against bacillus subtilis. Compound TZM5 & TZM6 showed the highest antimicrobial activities against all of the tested organisms (bacteria). In contrast, compound TZM1 showed lower antimicrobial activity against all tested microorganisms.

The result revealed that most of the tested compounds showed antibacterial activities with varying magnitudes. In this study the different tested compounds had a various inhibitory effects on the growth of the different tested microorganisms. This difference might be due to different structure of chemical substances and the efficiency of side chains in the reaction with other compounds. The presence of polar functionality as side chain increases the antibacterial activities against gram positive as compare to gram negative whereas presence of a nonpolar benzene ring in the side chain imparts activity against both gram positive and gram negative. Substitution of electron withdrawing groups on the ring increase gram positive activity. Based upon the result, it will also be necessary to optimize by the substituting a series of electron withdrawing group on aromatic ring and selectively modifying the 1,2,4- triazine nucleus.

Conclusion

In the effort to develop new potential molecule for the betterment of the health condition, a few of novel substituted Mannich base of 1,2,4-triazines for antimicrobial potency were synthesized and characterized. All the compounds were evaluated for antibacterial activity. The antibacterial studies of these compounds indicated that compounds were found to be showing good activity against some bacteria compared to standard antibiotic drugs. The substitution of hydroxyl group and presence of halo groups emerged as active in antibacterial screening.

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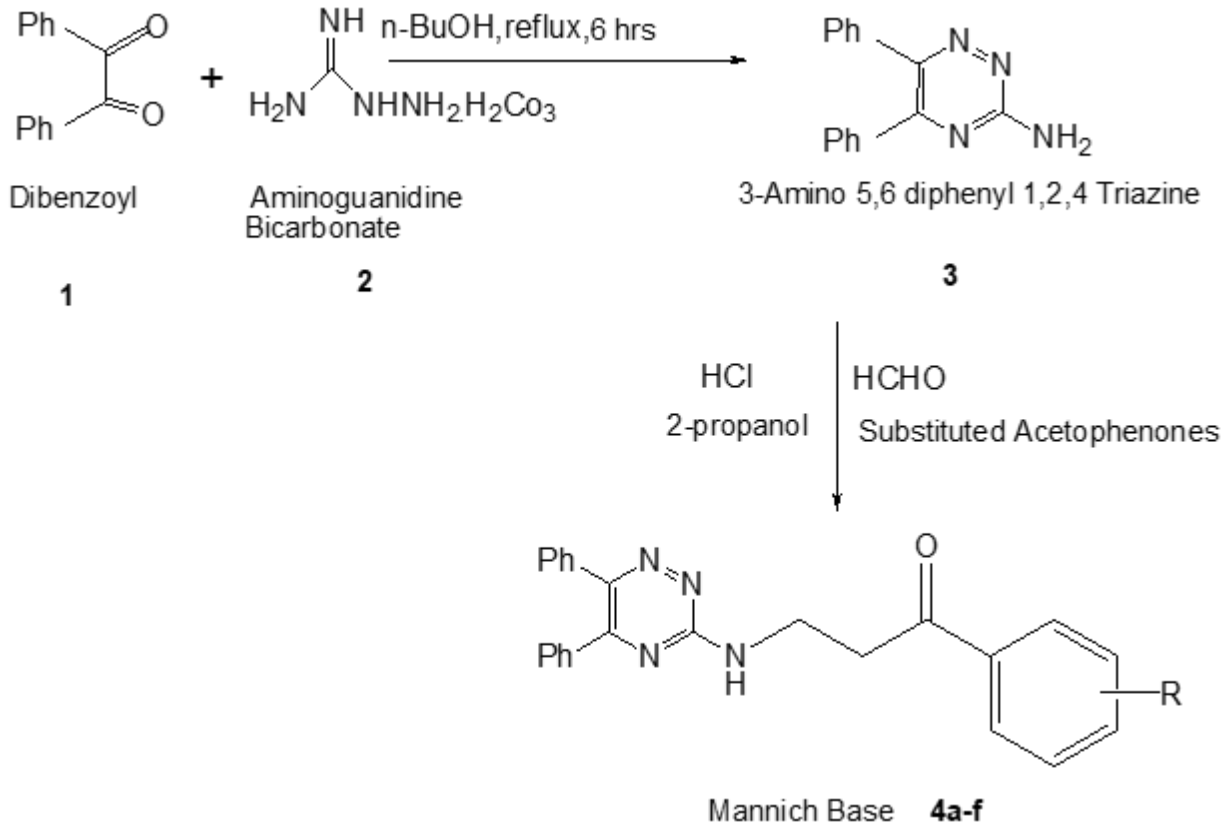

Figure 1 Scheme of the synthesis of Mannich base Triazine Derivatives.

Table 2 Antibacterial activity of the synthetic compound (Zone of inhibition in mm)

for Compounds TZM1 to TZM3

Pathogen	**TZM1 (µg/ml)**			**TZM2 (µg/ml)**			**TZM3 (µg/ml)**		
	150	**250**	**350**	**150**	**250**	**350**	**150**	**250**	**350**
E.coli	12	14	16	13	15	16	11	12	11
B. subtilis	11	12	15	14	15	17	12	12	12
P.auregenosa	12	13	15	15	17	18	13	14	15

Table 3 Antibacterial activity of the synthetic compound (Zone of inhibition in mm) for Compounds TZM4 to TZM6

Pathogen	TZM4 (µg/ml)			TZM5 (µg/ml)			TZM6 (µg/ml)		
	150	**250**	**350**	**150**	**250**	**350**	**150**	**250**	**350**
E.coli	12	14	16	17	18	20	17	18	20
B. subtilis	11	12	15	17	18	20	17	18	20
P.auregenosa	12	13	15	15	16	17	17	18	19