INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN BIOLOGY AND MEDICINE
ISSN: 2455-944X
https://darshanpublishers.com/ijcrbm/ijcrbmindex.html
Volume 7, Issue 1 - 2022

Original Research Article

DOI: http://dx.doi.org/10.22192/ijcrbm.2022.07.01.004

Synthesis, Characterization and Biological evaluation of Novel 2,4,6-trisubstituted-1,3,5-triazine derivatives

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Abstract

In present work different 1,3,5-triazine derivative were synthesized. Synthesis process based on nucleophilic substitution reaction as chloro group of 2,4,6-tricholro-1,3,5-triazine was substituted stepwise at different temperature by various amines. Since the report regarding this compound suggest a trisubstituted 2,4,6-triamino-1,3,5-triazine posses a good bioactive moiety. Melting points of the synthesized compounds was taken in open capillary tubes and was uncorrected. Thin layer chromatography was preformed using silica gel coated plates. Spots were visualized through the iodine chamber and the R_f value was calculated. All the synthesized compounds were found to be freely soluble in DMF, DMSO, Ethanol. The Infra Red spectroscopy was performed with KBr on Perkin FT-IR instrument. Presence of stretching in the range 3290 cm^{-1} to 3440 cm^{-1} indicates the presence of NH functional group. Stretching between 1590 cm^{-1} to 1670 cm^{-1} indicates the presence of C=C. Stretching range between 2230 to 2270 implies the presence of C=N. and 1030$cm^{-1}$to 1180 cm^{-1} indicates that bending of C-N functional groups. ^{1}HNucler Magnetic Resonance spectroscopy was recorded on Bruker Avance II 400 MHz NMR Spectrometer. ^{1}HNMR the chemical shifts were reported as parts per million downfield from tetramethylsilane, solvent used as Dimethylsulfoxide. Mass spectroscopy was performed on LC-MSD-Tranp-SL 2010A shimadzu using dimethylsulfoxide as solvent. Antimicrobial and antifungal activities were measured by cup plate method. All the synthesized compounds were subjected to biological evaluation for antimicrobial and antifungal activities. Minimum inhibitory concentration value of the compounds against the bacteria stains reveals that compound 3b and 3e have better activity against all bacteria stains as compared to that of standard.

Antifungal screening revealed that the test compounds showed antifungal activity against *Aspergillus niger*. Minimum inhibitory concentration value of the compounds determined, compound 3d and 3e have good antifungal activity.

Keywords: 1,3,5-triazine, Spectroscopy, antimicrobial, antifungal, bioactive moiety

Introduction

The discipline of medicinal chemistry is devoted to the discovery and development of new agents for treating diseases[1]. Most of this activity is directed to new natural or synthetic organic compounds. Inorganic compounds continue to be important in therapy, e.g., trace elements in nutritional therapy, antacids and radiopharmaceuticals, but organic molecules with increasingly specific pharmacological activities are clearly dominant. Development of organic compounds has grown beyond traditional synthetic methods. It deals with many aspects of organic medicinal how they are discovered, how they act and how they developed into clinical agents. The process of establishing a new pharmaceutical is exceedingly complex and involves the talents of people from a variety of disciplines, including chemistry, biochemistry, molecular biology, physiology, pharmacology, pharmaceutics and medicine. Medicinal chemistry[2], itself, is concerned mainly with the organic, analytical and biochemical aspects of this

process, but the chemist must interact productively with those in other disciplines. Thus, the medicinal chemistry occupies a strategic position at the interface of chemistry and biology.

To provide an understanding of the principles of medicinal chemistry, it is necessary to consider the physicochemical properties used to develop new pharmacologically active compounds and their mechanisms of action, the drug's metabolism including possible biological activities of the metabolites, the importance of stereochemistry in drug design and the methods used to determine what "space" a drug occupies.

The discipline of medicinal chemistry and development of new agents for treating diseases. Most of the activity is directed to new natural or synthetic organic compound. Inorganic compound continue to be important in therapy e.g. Trace elements. Medicinal chemistry is the science that deals with the discovery and design of new therapeutic chemicals and their development into useful medicines. Medicinal chemistry had its beginning when chemist, pharmacist and physician isolated and purified active principle of plant and animal tissue and later from microorganism and their fermented products. During the later decades of the 20th century the traditional dividing line between biological, chemical and physical science were erased and new border line investigation such molecular biology, molecular pharmacology, biomedicine and others begin to capture the interest of medicinal scientists. Medicinal chemistry which had organic chemistry, biology and some area of physics extended new root into these emerging topics.

Development of organic compounds had grown beyond traditional synthetic method. It not includes the exciting new field of biotechnology using the cell's biochemistry to synthesis new compounds. Medicinal chemistry has developed in recent years from a random process of searching hundreds of compounds for biologically active agents to one based on a more rational, mechanistic approach. Nature is still an excellent source of new drugs or more commonly of precursors to drugs. Typically, when a natural product is found to be active, it is chemically modified to improve its properties. The elements in nutritional therapy antacid and radiopharmaceuticals, but organic molecule with increasing specific pharmacological activity are clearly dominant. Drug designing is a multi-disciplinary activity involving chemists, biologists, biochemists, pharmacologists and many others. The chemist's role is central in inventing new compounds, which exert a beneficial effect. However once a lead for a new active drug has been established, its effective toxological studies undertaken to demonstrate its safety before clinical trials can commence.

Drug design is the inventive process of finding new medications based on the knowledge of the biological target. The drug is most commonly an organicsmall molecule which activates or inhibits the function of a biomolecule such as a protein which in turn results in a therapeutic benefit to the patient. In the most basic sense, drug design involves finding small molecules that are complementary in shape and charge to the biomolecular target to which they interact. Drug design frequently but not necessarily relies on computer modeling techniques. This type of modeling often referred to as Computer-Aided Drug Design (CADD).

Materials and Methods

STEP-1: Synthesis of 2,4-Dichloro-6-substituted amino-1,3,5- triazine (1)

2,4,6-trichloro-1,3,5-triazine (0.1mole) was dissolved in acetone (100ml) and cooled to 0^{0}c. A solution of secondary amine (0.1mole) was dissolved in acetone (100ml) and added with stirring to the above solution contents were stirred for 4 hr at 0-5^{0}c. Sodium carbonate solution was added to neutralized hydrochloric acid evolved during the reaction and finally the content was poured into crushed ice the precipitate compound was filtered, washed, dried and recrystallized from ethanol.

M.P.-195^{o}C , R_f- 0.66 , % Yield -90% , IR(KBr); N-H str.- 3410, N-H bending-1338, C-H str.- 2965, C-H bending -1282, C=C str.-1566, C=N str.-1374, C-N str.-1178, Cl-770.

STEP-2: Synthesis of 2-Chloro-4-substituted amino-6-substituted amino-1,3,5-triazine. (2)

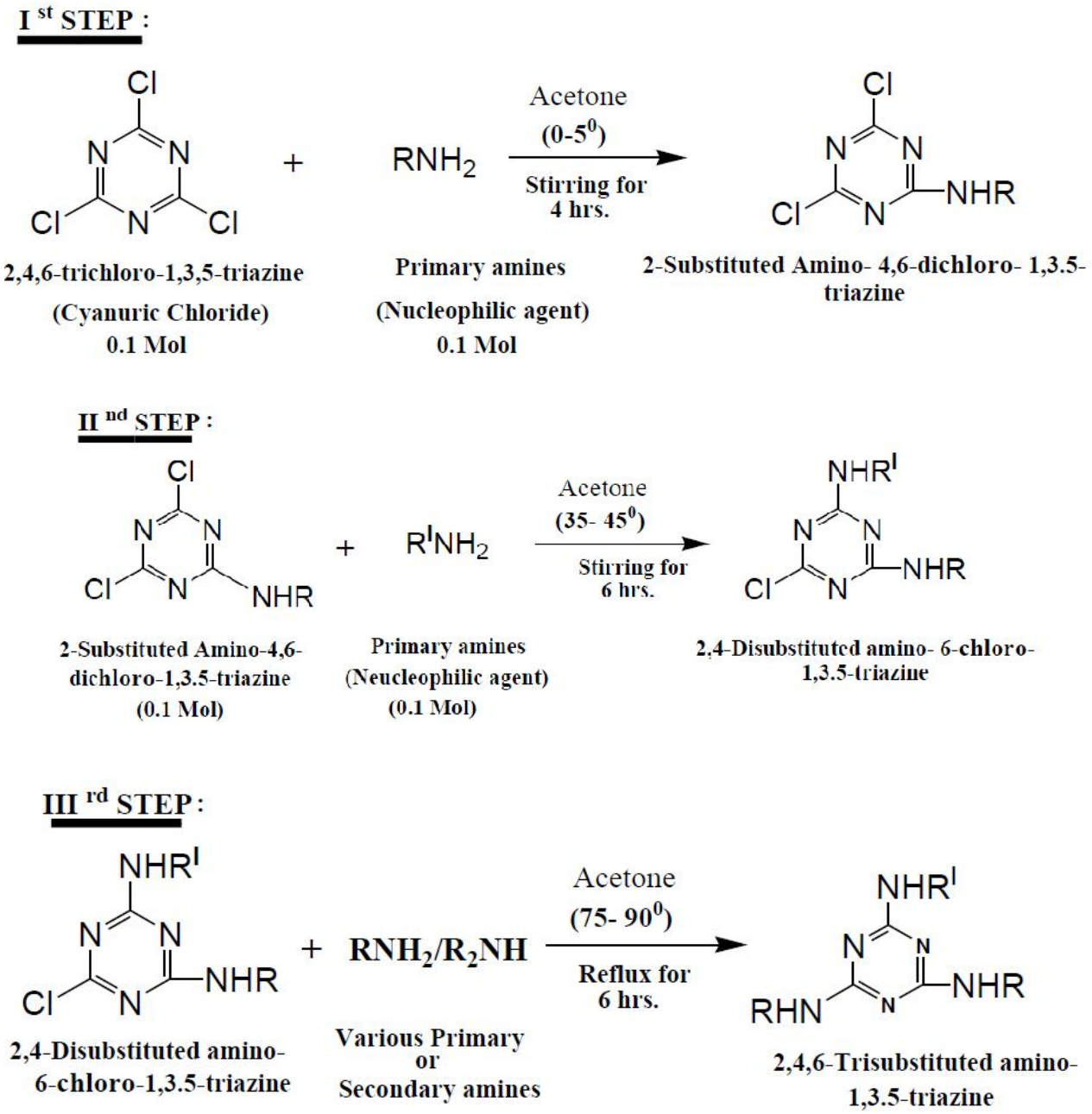
A solution of Compound-I (0.1mol) in acetone (100ml) was added to secondary amine (0.1mol) in acetone (100ml) with constant stirring. The contents were stirred for 6hr at 35-45^{0}c. Then it was neutralized with Sodium carbonate solution and poured into crushed ice. The product was filtered, washed, dried and recrystallized from ethanol.

M.P.-220°C , R_f- 0.60 , % Yield -80 % , IR(KBr); N-H str.- 3430, N-H bending-1375, C-H str.- 2950, C-H bending -1335, C=C str.-1567, C=N str.-1452, C-N str.-1175, Cl-747.

STEP-3: Synthesis of 2,4,6-substituted amino-1,3,5-triazine. (3a-3f)

Compound-II (0.01mol) was dissolved in acetone (50ml). Then it was added to various amines (0.01ml) in acetone (50ml).Then it was refluxed for 6 hr at 55-90^{0}c. After cooling it was poured into ice water and neutralized with Sodium carbonate solution to get the product. Then it was filtered, washed, dried and recrystallized from ethanol.

General scheme for synthesis of 1,3,5-triazine derivative



Biological evaluation

All the synthesized compounds were screened for biological evaluation

- Antibacterial
- Anti fungal activity

Antibacterial and anti fungal Evaluation:

The antimicrobial activities of the compounds were evaluated by using Cup Plate method

Antibacterial Activity

In present study the following bacteria were used.

A. *Escherichia coli (Gram -ve)*
B. *Pseudomonas aeruginosa (Gram-ve)*
C. *Klebsiella pneumoniae (Gram+ve)*
D. *Staphylococcus aureus (Gram+ve)*

In present study the cup-plate method was used to evaluate the *In-vitro* antimicrobial activity of the synthesized compounds. The standard antibiotic selected for study of the antibacterial activity was ciprofloxacin.

Cup-Plate Method Using Nutrient Agar

Materials used:

- Nutrient agar, growth culture in 18-24hrs.
- Sterile Petri dishes
- Sterile pipettes
- Sterile cotton swabs
- Sterile cork borer

Preparation of nutrient agar:

The definite volumes off peptone (0.6%), yeast extract (0.15%), dipotassium dihydrogen phosphate (0.36%) and potassium dihydrogen phosphate (0.13%) were dissolved in distilled water and pH was adjusted to 7.2. this solution was sterilized by autoclaving at 15 p.s.i. for 20 minutes.

Preparation of sub-culture:

One day prior to this testing, inoculation of the above bacterial cultures were made in the nutrient agar and incubated at 37 ^{o}C for 18-24 hrs.

Results and Discussion

In the present work different 1,3,5-triazine derivative (3a-3e) were synthesized. Synthesis process based on nucleophilic substitution reaction as chloro group of 2,4,6-tricholro-1,3,5-triazine was substituted stepwise at different temperature by various amines. The first step involve the substitution of napthylamine and the next by diethyl amine. The final chloro group in the synthesized compound-2 was replaced by various amino groups. Since the report regarding this compound suggest a trisubstituted 2,4,6-triamino-1,3,5-triazine posses a good bioactive moiety.

Melting points of the synthesized compounds was taken in open capillary tubes and was uncorrected. Thin layer chromatography was preformed using silica gel coated plates. Spots were visualized through the iodine chamber and the $R_f$value was calculated. All the synthesized compounds were found to be freely soluble in DMF, DMSO, Ethanol.

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^{1}HNucler Magnetic Resonance spectroscopy was recorded on Bruker Avance II 400 MHz NMR Spectrometer. ^{1}HNMR the chemical shifts were reported as parts per million downfield from tetramethylsilane, solvent used as Dimethylsulfoxide. All compounds showed respective downfield that supported the structure of various synthesized derivative of triazine.

Mass spectroscopy was performed on LC-MSD-Tranp-SL 2010A SHIMADZU using dimethylsulfoxide as solvent. All the compounds showed characteristic molecular ion peak and base peak that further confirms the structure of the synthesized compounds.

Antimicrobial and Antifungal Activity

Antimicrobial and antifungal activities were measured by cup plate method. All the synthesized compounds were subjected to biological evaluation for

antimicrobial and antifungal activities. The antimicrobial activity revealed that some of the test compounds showed inhibition at 100 µg/ml, 200 µg/ml and 300 µg/ml concentration. Minimum inhibitory concentration value of the compounds against the bacteria stains reveals that compound 3b and 3e have better activity against all bacteria stains as compared to that of standard.

Antifungal screening revealed that the test compounds showed antifungal activity against *Aspergillus niger*. Minimum inhibitory concentration value of the compounds determined, compound 3d and 3e have good antifungal activity.

Antibacterial Activity Data for MIC

Compound code	Minimum Inhibitory Concentration(MIC) in µg/ml			
	E. coli	*S. aureus*	*B. subtilus*	*K. pneumoinea*
3a	58	54	53	57
3b	45	47	48	51
3c	48	51	54	56
3d	57	59	55	57
3e	43	46	47	42
Standard	35	39	37	34

Antifungal Activity against *Aspergillus niger*

Compound code	Minimal Inhibitory Concentration in µg/ml
	Aspergillus niger
3a	58
3b	53
3c	55
3d	45
3e	43
DMSO	-
Standard	39

Ketoconazole 10µg/mL used as standard drug against *A.niger*.

Minimal Inhibitory Concentration (MIC) of Compounds 3a-3e in µg/ml

Concentration in µg/ml	Diameter of Zone of Inhibition in mm					
	3a	**3b**	**3c**	**3d**	**3e**	**Std**
100	**13**	**13**	**14**	**14**	**14**	**24**
200	**14**	**15**	**15**	**16**	**17**	**24**
300	**16**	**17**	**17**	**18**	**19**	**24**

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How to cite this article:
Anisha Mourya, Deepak Kumar Basedia, B. K. Dubey, Mayur R. Wadbude. (2022). Synthesis, Characterization and Biological evaluation of Novel 2,4,6-trisubstituted-1,3,5-triazine derivatives. Int. J. Curr. Res. Biol. Med. 7(1): 25-31.
DOI: http://dx.doi.org/10.22192/ijcrbm.2022.07.01.004