DESIGN, SYNTHESIS, CHARECTERIZATION AND ANTIBACTERIAL ACTIVITY OF 2, 3 DICHLORO NITROBENZENE

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Abstract

Nitrobenzene derivatives are organic compounds that have been widely synthesized and used in chemical industries such as the polymer industry, lumber preservatives, textile industry, pesticides, and warlike weapons industry. The rapid growth of nitrobenzene derivatives in the industry requires research into the effects of toxicity in the environment. The synthesis of nitrobenzene and carried out by using Nitration means Electrophilic aromatic substitution reaction. PASS (prediction of activity spectra for biologically active substance models were useful in understanding how chemical structure relates to the Pharmacological activity of probably active molecules. The Synthesized derivatives are to be confirmed by using characterization physical method and IR spectra to confirm the structure of the synthesized compounds. These synthesized Derivative are performed for the Antibacterial Activity

Keywords

Nitrobenzene, Synthesis, Drug Discovery and Development, Antibacterial activity

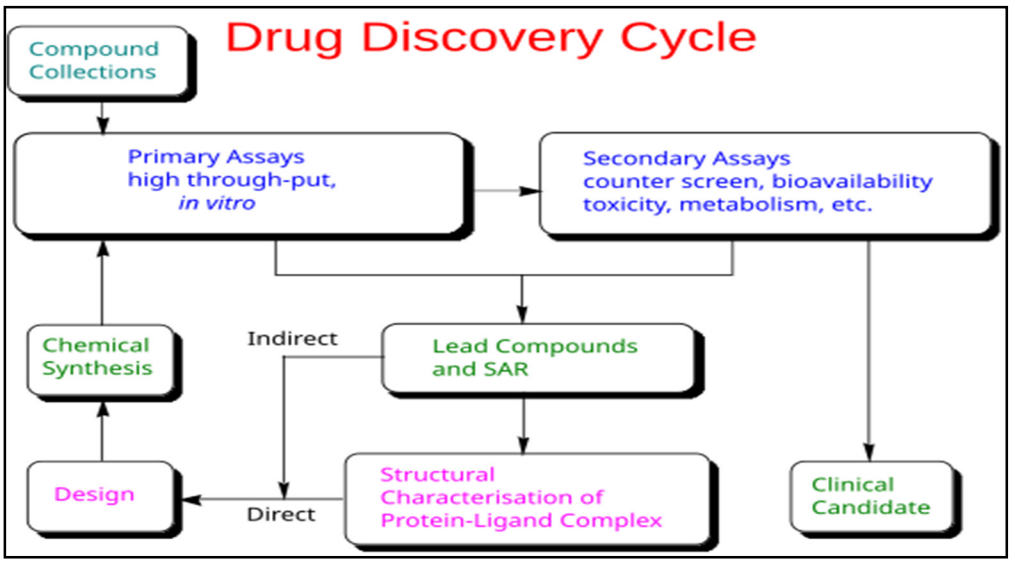
INTRODUCTION

Medicinal chemistry

The discipline of medicinal chemistry leads to the discovery and development of new agent for treating diseases. Most of the activity is directed to new natural or synthetic organic compounds. Medicinal chemistry deals with the discovery, development, identification and interpretation of the mode of action of biologically active compounds Add the molecular level. It is also concerned with the study, identification and synthesis off the metabolic products of drug and related compounds. It involves isolation off compound from the natural or synthesis off the new molecules, Investigation of the relationships between the structure of natural synthetic compound And their biological activities, Elucidation of their interactions with receptors of various kinds, Including enzyme and DNA, The determination of their adsorption, transport, Distributary properties and studies of the metabolic transformation of this chemicals into other chemical and their excretion.It deals with the discovery and design of new therapeutic chemicals and their development into useful medicines and the forefront of innovation, Blending of synthetic chemistry, molecular modeling, And pharmacological studies to discover design new drugs and to investigation their interaction at the molecular, cellular and animal level. Medicinal chemist has a greater role in development of numerous organic compounds suitable for treatment of illness and maintenance and field of human beings.

Drug discovery

Drug discovery is process of designing and synthesizing new compound and evaluating to assess drug safety and efficacy in humans. The traditional way to discover new drugs has been to screen a large number of synthetic chemical compound or natural product or desirable effects. Modification of lead compounds are often carried out to improve activity, Reduce side effects and to improve performance.

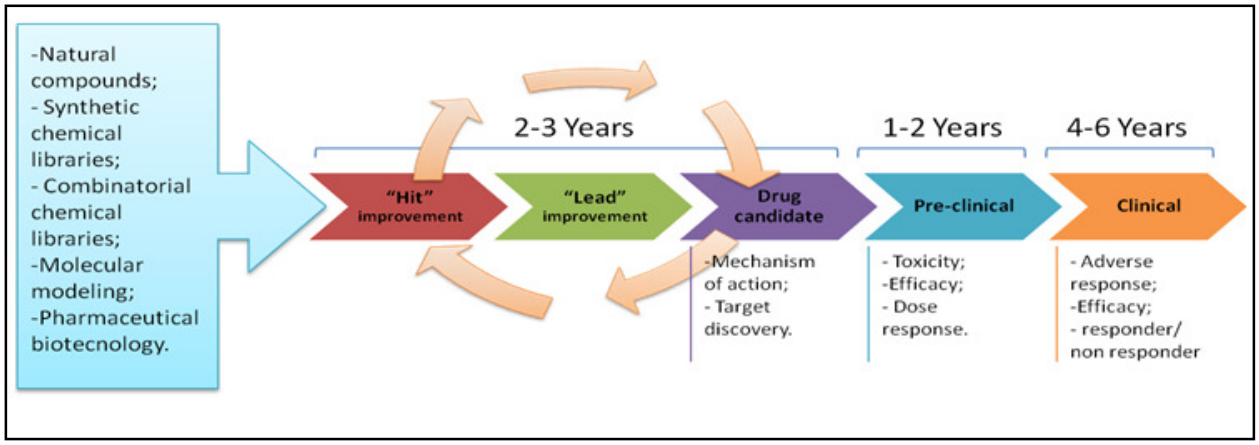


Synthesis

Chemical synthesis is the preparation of a compound, usually an organic compound which repaired or synthesized by performing various chemical reactions using an In expensive starting material and changing its molecular structure, By reaction with other chemicals. The best chemical synthesis are those that use cheap starting materials which requires only a few steps, And have a good output of product based on amounts of starting chemicals.

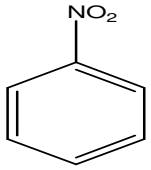
Importance of heterocycles in medicinal chemistry

Heterocycles are organic compounds containing at least one atom of carbon and at least one element other than carbon such as sulfur, Oxygen or nitrogen with a ring structure. It is one of the vital classes of organic compounds which are used to mainly biological fields due to its activity in multiple illness. Today there are lot of heterocyclic Compounds are known, Day by day due to their synthetic utility. Heterocycles have been found for key structural in medicinal chemistry and also they are frequently found in large percent in bio-molecules such as enzymes Natural products and biological active compound including antifungal, anti-inflammatory, antibacterial, anti oxidant, anti-convulsant, anti allergic, enzyme inhibitors, anti-diabetic, anti HIV, anti cancer activity.

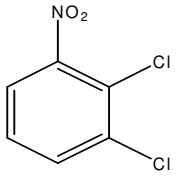


Nitrobenzene is an organic compound with chemical formula $C_6H_5NO_2$. It is colourless to pale yellow liquid with a characteristic's almond like odour. Nitrobenzene and its derivatives (NBDs) are highly toxic compounds that have been released into the environment by anthropogenic activities. Many bacteria and fungi have been well-characterized for their ability to degrade NBDs. The biochemical and molecular characterization of the microbial degradation of NBDs has also been studied. In this review, we have summarized the toxicity and degradation profiles of nitrobenzene, monochloronitrobenzenes, polynitrobenzenes, and pentachloronitrobenzene. This review will increase our current understanding of toxicity and microbial degradation of NBDs.Nitro substituent on the benzene ring (nitrobenzene derivatives) constitutes an organic compound that has been widely synthesized and used in the chemical industry.

Nitrobenzene derivatives have been used as depolymerizing agents in the polymer industry to lower molecular weight. Nitrobenzene derivatives have also been shown to be good as a lumber preservative. The textile industry uses nitrobenzene derivatives- in the manufacturing and processing of textiles, particularly in the wet processing of textiles. Modern pesticides typically contain a nitrobenzene derivative as their active ingredient, which protects plants and crops. Nitrobenzene-based explosives, which are often low in sensitivity and great in performance, have been utilized in warlike weapons as one sort of explosive. Nitrobenzene is used as a precursor to aniline, which is a key intermediate in the production of dyes, drugs, and other chemicals. It is also used in the manufacture of lubricants, pesticides, and pharmaceuticals. Handling and storage of nitrobenzene require proper safety precautions.



NITROBENZENE



2,3 DICHLORO NITROBENZENE

APPLICATION

Nitrobenzene is a versatile chemical compound with various applications across different industries. Some of its key applications include:

1. Industrial Applications

- Aniline production: Nitrobenzene is a precursor to aniline, a key intermediate in the production of polyurethane foams, dyes, and pharmaceuticals.
- Dyes and pigments: Nitrobenzene is used in the synthesis of various dyes and pigments, such as azo dyes and vat dyes.
- Pharmaceuticals: Nitrobenzene is used as an intermediate in the production of certain pharmaceuticals, such as acetaminophen and benzodiazepines.

2. Laboratory Applications

- Solvent: Nitrobenzene is used as a solvent in various laboratory applications, including chromatography and spectroscopy.
- Reagent: Nitrobenzene is used as a reagent in various chemical reactions, such as the synthesis of organic compounds.

EXPERIMENTAL WORK:

Preparation of Nitrobenzene Derivative
Materials:
- Benzene
- Concentrated nitric acid(HNO3)
- Barium chloride (BaCl2)
- Concentrated Sulphuric acid (H2SO4)
- Nitrobenzene (C6H5NO2)
- Round-bottam flask
- Condenser,Separating funnel
- Ice bath, Beaker
- Magnetic stirrer, Burette, Filter

Procedure:

Step 1

1. Preparation of Nitrating Mixture: In a round-bottom flask, mix concentrated nitric acid and sulfuric acid in a specific ratio (typically 1:1 or 1:2).
2. Cooling the Mixture: Cool the nitrating mixture in an ice bath to a temperature range of 0-5°C.
3. Addition of Benzene: Slowly add benzene to the cooled nitrating mixture while stirring.
4. Nitration Reaction: Allow the mixture to react for a specified period, typically 30 minutes to 1 hour, while maintaining the temperature below 5°C.
5. Quenching the Reaction: Pour the reaction mixture into a large volume of ice-cold water to quench the reaction.
6. Separation and Purification: Separate the nitrobenzene layer from the aqueous layer using a separating funnel. Wash the nitrobenzene layer with water and dry it over anhydrous calcium chloride or magnesium sulfate.
7. Product Collection: Collect the purified nitrobenzene.

Step 2

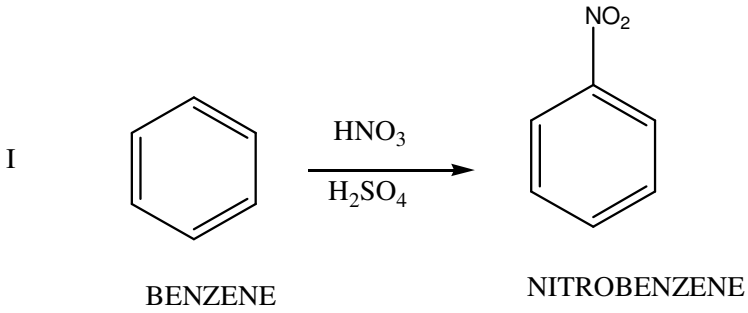
1. Initial Preparation: In a beaker, add 15 mL of BaCl2 solution
2. Addition of Sulphuric Acid: Slowly add 25 mL of sulphuric acid to the BaCl2 solution using a burette, while maintaining the mixture at a low temperature using an ice bath. Stir the mixture gently during the addition.
3. Addition of Nitrobenzene: With continued stirring using a magnetic stirrer, slowly add 19 mL of nitrobenzene to the acid mixture.
4. Stirring and Cooling: Continue stirring the mixture for a sufficient period to ensure thorough mixing and reaction.
5. Filtration: Filter the resulting mixture to isolate the product.
6. Product Formation: A pale yellow precipitate should form, indicating the successful preparation of the nitrobenzene derivative.

Scheme for Synthesis:

STEP 1

Nitric Acid Is Treated With Concentrated Sulphuric Acid Formation of Electrophile And Nucleophile. Then Attack Of Electrophile on Aromatic Benzene Ring to Form Carbocation or Carbonium Ion. In This Step Resonance Stabilization. After Loss of Proton Formation of Nitrobenzene.

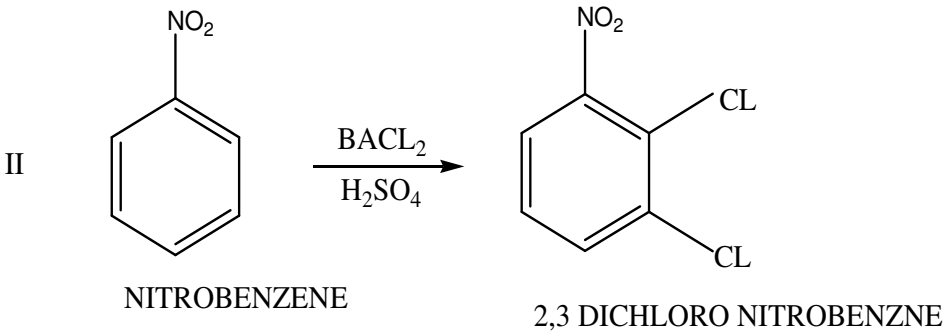
PROPOSED REACTION



STEP 2

These Nitrobenzene is treated with the barium chloride and concentrated Sulfuric acid it gives 2,3 dichloro nitrobenzene.

PROPOSED REACTION



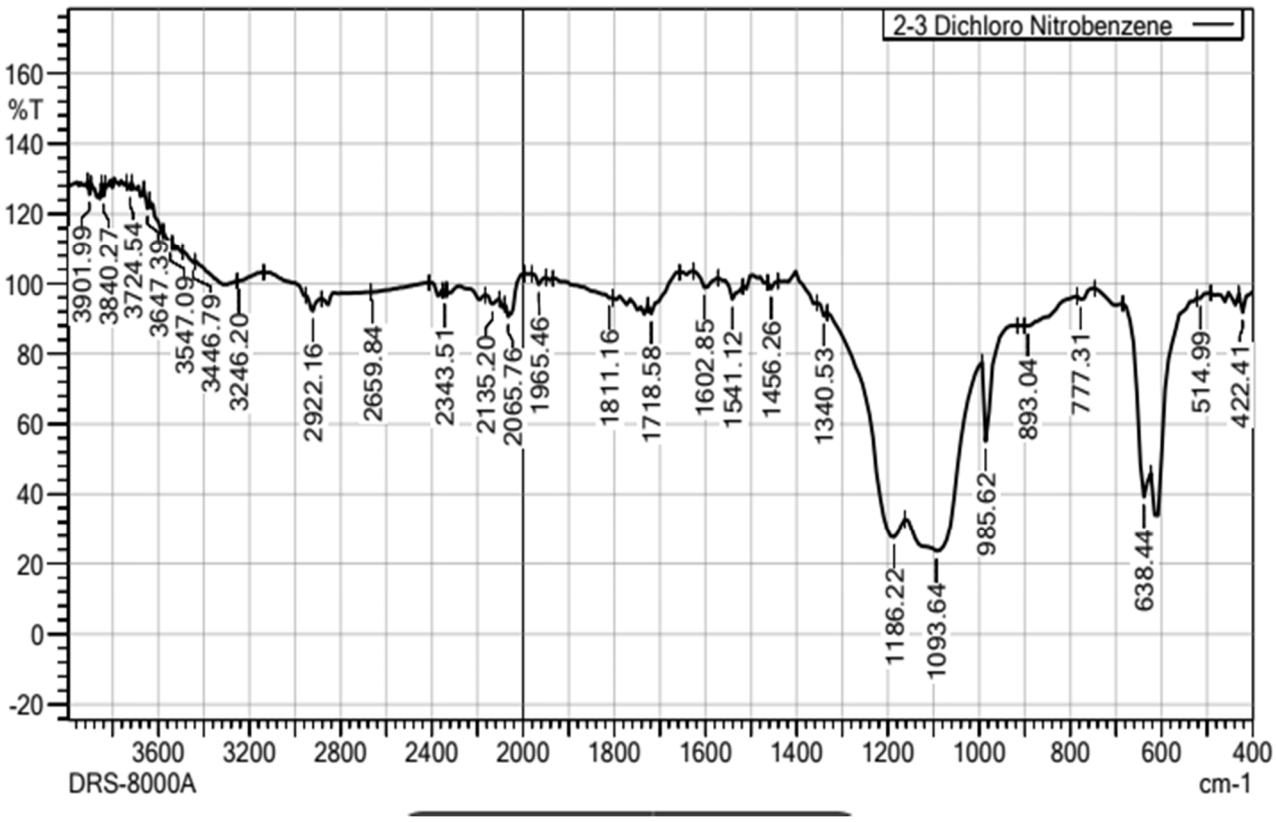
RESULT AND DISCUSSION

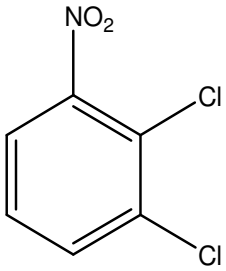
1. Preliminary Test and Physical Examination
2. Structural elucidation using Infra-Red spectroscopy

1. Preliminary Test and Physical Examination

Test	Observation	Interference
State	solid	Generally high molecular weight usually having more than 6 carbon atom chain . Eg-amides and amine higher hydrocarbon may be present .
colour	Pale yellow	Nitro compounds ex. Nitrobenzene, dinitrobenzene ,nitrophenol, quinones, iodoform
odour	Fishy	Aromatic amine
	Bitter almond	Nitrobenzene
Ignition Test		
Flame test	Sooty flame	Aromatic compound
Test for Halogens		
Silver Nitrate Test Stock solution + dil. HNO_3- heat, boil and reduce to half the volume + a few drops of $AgNO_3$	White ppt, which freely dissolves in 2 ml of ammonia solution	Chlorine present
	Pale yellow ppt ,which is diff.to dissolve in 2 ml of ammonia solution	Bromine absent
	Yellow ppt ,which is insoluble in 2ml of ammonia solution	Iodine absent
Test for nitro compounds		
Sub+NaOH solution -boil	No smell of NH_3	Amide absent
Sub + NaOH solution +$CHCl_3$ Boil continuously for some time	No smell of carbylamines	Anilide absent
Sub + conc. HCl + Tin pieces. Wait till reaction ceases then boil till layer disappears . Cool ,filter,dilute and ice cold $NaNO_2$ solution	Clear solution -further add ice cold β- naphthol in NaOH in solution dropwise .Red/orange dye obtained	Nitro compound present
Test for Nitrogen		
Prussian blue test Stock solution +$FeSO_4$ solu.-boil and cool. H_2SO_4	Green or blue colour	Nitrogen present
	No green or blue colour	Nitrogen absent

2. Structural elucidation using Infra-Red spectroscopy





2,3 DICHLORO NITROBENZENE

Sr.No.	Functional Groups	2,3 Dichloro Nitrobenzene range
1.	C-H	3246
2.	C=C	1718
3.	NO_2	1186
4.	C-Cl	638
5.	Ar-Ring	3449

MELTING POINT:

Melting point was found to be 83-85°C

ANTIMICROBIAL EVALUATION

Antibacterial Activity:

The antibacterial activity of newly synthesized compounds was evaluated against gram positive bacteria i.e. staphylococcus aureus and gram negative bacteria i.e. Escherichia coli. Ciprofloxacin was used as a standard drug.

Preparation of nutrient agar medium

1.	Peptic digest of animal tissue	5gm
2.	Sodium chloride	5gm
3.	Beef extract	1.50gm
4.	Yeast extract	1.50gn
5.	Agar	15gm
6.	Distilled water	1000ml

All the ingredients were dissolved in distilled water, adjust the ph to 8.0-8.4 with 5M NaOH solution and boil for 10-15 min. filtered the solution. Adjust the ph of the medium by 7.4±0.2 by the addition of dil. HCL. Sterilized the medium in autoclave for 15min. At 121°C.

Preparation of test solution:

The solution of the various Nitrobenzene derivatives in concentration of 100ug was prepared in DMSO.

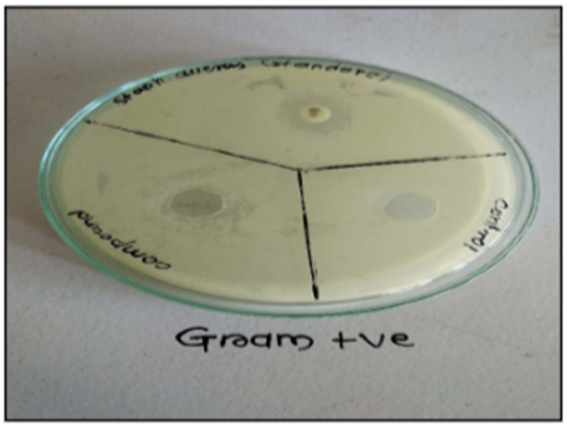
Preparation of standard solution:

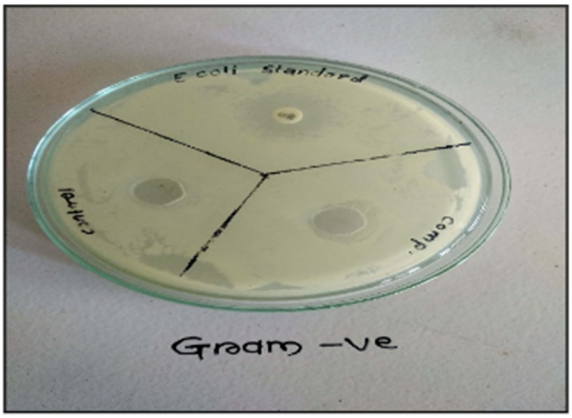
Weigh 10mg of standard drug and diluted to 10ml to from 1000ug/ml of stock solution from this stock solution, we took 1ml and diluted to 10ml to form 100ug/ml of standard solution.

Procedure:

Inoculate previously liquified sodium appropriate to assay with quantity of suspension of the microorganisms. Add the suspension to the medium add temperature 40 to 50°C and immediately pour the inoculated Medium into Petri dishes to give a depth of three to four minute ensures that the layer of the medium are uniform in thickness by placing the dishes on the level of surface. Made few cavities on the surface of medium. Pour the solution off non concentration of the standard preparation and taste preparation to cavities by means of micropipette in a sterile condition. Leave the dishes standing for one to four hours in refrigerator as appropriate as a period of pre incubation diffusion to minimize the effects of variation in time between the

applications of different solution. Incubate them for about 24 hours at the temperature indicated accurately measured the diameter of zone of inhibition.





Sr.No	Compound	Zone of inhibition in (mm)	
		Staphylococcus aureus	**Escherichia coli**
1	2,3dichloro Nitrobenzene	11	09
2	Chloramphenicol	14	15

Data of antimicrobial activity of synthesized compound

CONCLUSION:

2, 3 Dichloro Nitrobenzene derivatives were Design and synthesized. The synthesized 2, 3 Dichloro Nitrobenzene derivatives to show mild antimicrobial activity with Chloramphenicol used as an standard. While not exhibiting the potency of the standard drugs used in the study, the compounds showed a discernible inhibitory effect against the tested microorganisms. This suggests that the 2, 3 Dichloro Nitrobenzene scaffold holds promise for further development as a potential source of new antimicrobial agents. Future research should focus on structural modifications aimed at enhancing the activity and selectivity of these compounds.

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