IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Design, Synthesis and Characterization of Novel Substituted Amide Derivatives for Antibacterial Activity and Development of a Proficient Synthesis Method

DEVI HIMANI*1

1 Assistant Professor (Pharmaceutical Chemistry), Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India.

BHATT DR. MANVI 2

2 Assistant Professor (Pharmacy Practice), Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India.

SAXENA PAYAL3

3 Assistant Professor (Pharmacology), Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India.

ABSTRACT

Excellent and broad bio-spectrum of amide derivatives has generated an attractive platform for synthetic chemists to introduce structural modification in its nucleus by conventional access to new approaches. A new sequences of various amide derivatives have been design and synthesized. The methodology is proficient and simple to perform, giving the desired amides with excellent yield. An innovative series of four derivatives of amide were synthesized by the condensation of different compound like acetanilide, aniline, sulphanilamide and urea with benzoyl chloride in presence of sodium hydroxide as a catalyst.

The reactions steps and the purity of the products were crisscross by using Thin Layer Chromatography (TLC). The chemical structure of final compounds were characterized and inveterate by measuring their melting points, elemental analysis, IR and 1H-NMR.

The *in-vitro* antibacterial activity is done by using disc diffusion methods. The compounds were confirmed at different concentration from 0.02-0.10mg/ml in order to plaid the percentage inhibition of compounds. From the result compounds A1 and A2 presented highly significant activity against gram positive bacteria in comparison to standard sulphanilamide.

A preliminary study of antimicrobial activity was performed in which antibacterial activity was done in two strains of bacterial activity was done. The results showed that compounds A1 and A2 have significant antibacterial activity compared with standard drugs and the other tested compounds have moderate to good activity.

(Keywords: Antimicrobial, amide, antibacterial activity**)**

1.INTRODUCTION OF ANTIMICROBIAL

An antimicrobial is a substance that either kills microorganisms or inhibits their growth. These antimicrobial agents are often categorized based on their effectiveness against specific types of microorganisms, with antibiotics targeting bacteria and antifungals addressing fungi.

Additionally, antimicrobial agents can be classified based on their mode of action. Those that kill microbes are referred to as microbicidal, while those that primarily inhibit their growth are termed biostatic. The use of antimicrobial medications to treat infections is known as antimicrobial chemotherapy, while their preventive use to avoid infections is called antimicrobial prophylaxis.

Antimicrobial agents exhibit disinfectant properties, capable of killing a broad range of microbes on inanimate surfaces. This property is valuable in preventing the spread of diseases. Antiseptics, on the other hand, are designed for use on living tissues, helping to reduce infections during surgical procedures. Antibiotics, originally described as substances derived from living microorganisms, now also include synthetic antimicrobials like sulphonamides and fluoroquinolones. The term "antibiotic" is commonly used to denote substances with antibacterial activity. Antibacterial agents can be further classified into bactericidal agents, which kill bacteria, and bacteriostatic agents, which primarily slow down or halt bacterial growth in specific areas.[4]

1.1.Amide

Amides have nitrogen atom which is openly attached to a carbon atom in a carbonyl group.[6]

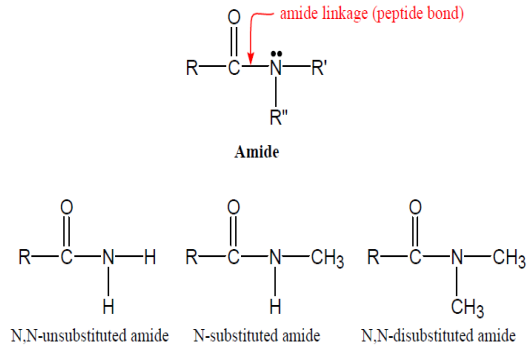


Figure 1

1.2.SAR of Amide:

Substituents on the Nitrogen Atom: The nature and size of substituents attached to the nitrogen atom in the amide group can influence the molecule's properties, including its biological activity.

Substituents on the Carbonyl Carbon: Modifying the substituents attached to the carbonyl carbon can impact the electronic and steric properties of the amide, affecting its reactivity and interactions with biological targets.

Steric Effects: The size and shape of the amide group, as well as the overall three-dimensional arrangement of the molecule, play a crucial role. Bulky substituents may hinder binding to a target site or enhance selectivity.

Electron Density and Resonance: The electron density of the amide bond and the presence of resonance structures can influence the stability and reactivity of the compound.

Conformational Flexibility: The ability of the amide-containing molecule to adopt different conformations can impact its interaction with biological targets. Rigid or flexible structures may be more suitable depending on the target.

Hydrogen Bonding: Amides are capable of forming hydrogen bonds, and the strength and nature of these interactions can be crucial for biological activity. Modifying hydrogen-bonding capabilities can alter a compound's pharmacological profile.

Bioisosterism: Replacement of certain atoms or functional groups within the amide structure with bioisosteres can lead to compounds with similar biological activity.

2. MATERIAL AND METHOD

2.1. CHEMICAL SYNTHESIS

The amide derivatives were synthesized by different procedures. The reactions were supervised by thin layer chromatographic methods using suitable solvents and purification of compound was carried out

2.1.1. Synthesis of N-phenyl benzamide (Benzanilide) compound (A1)

In a dry beaker with a mechanical stirrer, 1 ml of the corresponding aniline and 1.5 ml of benzoyl chloride were combined. Subsequently, a sodium hydroxide solution (15 ml, 10% concentration) was introduced as a catalyst into the mixture. The stirred mixture was maintained at room temperature for approximately 20 minutes. Following this, the stirring mixture underwent filtration and was collected in a separate beaker. The solution was then neutralized using an appropriate solvent and washed with water. The resulting product was dried and subjected to recrystallization using methylated spirit. Purification of the compound was achieved through a TLC (thin-layer chromatography) method, employing a suitable solvent like n-butanol:benzene in a ratio of 9:1.

2.1.2. Synthesis of compound (A2)

In a dry beaker with a mechanical stirrer, 4 ml of the corresponding sulphonamide (5%) and 6 ml of benzoyl chloride were combined. Subsequently, a sodium hydroxide solution (15 ml, 10% concentration) was introduced as a catalyst into the mixture. The stirred mixture was maintained at room temperature for approximately 20 minutes. Following this, the stirring mixture underwent filtration and was collected in a separate beaker. The solution was then neutralized using an appropriate solvent and washed with water. The resulting product was dried and subjected to recrystallization using methylated spirit. Purification of the compound was achieved through a TLC (thin-layer chromatography) method, employing a suitable solvent like n-butanol:benzene in a ratio of 9:1.

2.1.3. Synthesis of compound (A3)

In a dry beaker with a mechanical stirrer, 6 ml of the corresponding acetanilide (12%) and 10 ml of benzoyl chloride were combined. Subsequently, a sodium hydroxide solution (15 ml, 10% concentration) was introduced as a catalyst into the mixture. The stirred mixture was maintained at room temperature for approximately 20 minutes. Following this, the stirring mixture underwent filtration and was collected in a separate beaker. The solution was then neutralized using an appropriate solvent and washed with water. The resulting product was dried and subjected to recrystallization using methylated spirit. Purification of the compound was achieved through a TLC (thin-layer chromatography) method, employing a suitable solvent like n-hexane:benzene in a ratio of 3:3.

2.1.4. Synthesis of compound (A4)

In a dry beaker with a mechanical stirrer, 8 ml of the corresponding urea (12%) and 10 ml of benzoyl chloride were combined. Subsequently, a sodium hydroxide solution (15 ml, 10% concentration) was added to the mixture as a catalyst. Following the addition of the catalyst solution, the mixture was stirred for approximately 20 minutes at room temperature. The stirred mixture was then filtered and collected in a separate beaker. Subsequently, the solution was neutralized using an appropriate solvent and washed with water. The resulting product underwent drying and recrystallization with methylated spirit. The compound's purification involved employing a TLC (thin-layer chromatography) method, using a suitable solvent such as n-hexane:benzene in a ratio of 3:3.

3. Method of characterization and identification

3.1. Melting point

Procedure

Firstly close one end of the thick capillary tube by using igniting over the flame and insert pinch amount of the dry sample to be perceived. After that place a mercury thermometer (0-360oC) in the holder. And then place compound fill capillary tube in the holder of the melting apparatus. After that switch on the melting point apparatus and adjust the energy knob at moderated level and on the visualize light to check the status of the capillary tube in the holder of the apparatus. And finally noted down the temperature on the

thermometer scale when the sample was started to melt. And then take the mean of both the temperature and find out the actual melting point of the compound.

3.2.Solubility
The solubility are the important physiochemical parameter of the compound to know the solubilize property of compound with suitable solvents. The solubility of any material is usually achieved the equilibrium after the solution are saturated. The compounds are liquefied in different solvent and monitor the solubility of the compounds with suitable solvent.

3.3.Optical rotation
Rudolph polarimetry Apparatus was used to determine all rotations reported in this work. The rotation checked at 589 lambda at room temperature.
Apparatus – Optical rotation of any compound is measured with an apparatus such as polarimeter. The zero point in the polarimeter can be unwavering with the empty tube but closed for liquid sample and filled with the definite solvent for solution of solid substance. A polarimeter displays accuracy to 0.050 of angular rotation of the sample and accomplished of being read with the same precession of the sample.

3.4.pH-
The pH of different compounds was determined by Eutech pH meter.
1. Switch on the pH meter, rinse the electrode with de-ionized water or distilled water before use.
2. Firstly maintain pH at neutral by using distilled water, dip the electrode on distilled and maintain the neutral pH.
3. Observe the pH of the compound and allow time for the reading to stabilize.
4. Record the observed pH values of the compounds.

3.5.Spectroscopy Method:

3.5.1 Ultraviolet spectroscopy:
Firstly switch on the main power of the instrument and allow to stabilize for 15 min. After that put the mode selector at % T position in the instruments and adjust zero % transmittance with the help of controller. And then remove the cuvette and wash with blank solution after that rinse with sample and then filled with sample solution. And clean the outer surface of the reference cuvette with tissue paper for protect the contamination of any foreign particles the affected the absorbance of the sample solution. And then placed the tube in the holder and close the compartment, Then measured the wavelength of sample solution.

3.5.2 Chromatographic parameters
Thin layer chromatography
In the process of thin layer chromatography ascending TLC was run on silica gel with pre-coated aluminum sheets for checking the purity of the sample as well as monitoring the improvement of the run sample on the solvent. And then final sample was exposed with UV light.

Finally chromatogram was checked by the following solvent systems such as:
1. n-Hexane: Ethyl acetate (8:2)

2. Chloroform: Ethanol (4:6)

Retention factor = Distance travel by solute/Distance travel by solvent

PROCEDURE
Step 1: Prepare the developing container
The TLC developing chamber can be specially designed chamber with a jar and a beaker attached with watch glass. Firstly pour the solvent into the chamber depth less than 0.5cm and then add the TLC plate into the chamber and cover with watch glass and allow to stand for run the solvent on to the sample for finding the retention factor of the sample.
Step 2: Prepare the TLC plate
Basically the dimension of the TLC plate is 5 cm x 20 cm sheets used. Large sheet cut horizontally into 5 cm length with different width. After that using a pencil to draw a line through the plate at the 0.5 cm mark.
Step 3: Spot the TLC plate

Than about 1 mg of sample dissolve in 1 ml of methanol and dip the microcap into the sample solution and then gradually touch the end of it onto the TLC plate.

Step 4: Develop the plate

Finally place the plate into the developing chamber, cover with watch glass and allow to stand without disturbing. The solvent will rise up the TLC plate by capillary action and then remove the plate from the beaker and immediately mark the solvent with a pencil and finally allow the plate to dry.

Step 5: Visualize the spots

And then hold a UV lamp over the dry plate and circle if any spots you can see onto the plate.

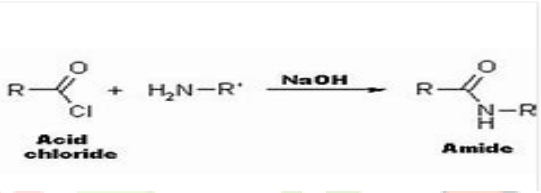
3.6 Antibacterial Activity of compound

The antibacterial activity was carried out by disc diffusion method. In this technique the Whatman No.1 filter paper with sterile disc of 5 mm diameter, saturated with the test compounds (10 µg/ml of ethanol) beside with standard were placed onto the nutrient agar plate at 37oC for 24 hrs in BOD incubator. After that the embarrassment around dried permeated disc was measured after completion 24 hrs. The bacterial activity was mainly classified as highly active (dia = > 15 mm), moderate active (dia =10-15 mm) and partially active (dia = 5-10mm) with different diameter.

4. RESULT

After the studies, we developed a new synthetic methodology for the synthesis of amide derivatives. In the starting reaction material aniline was reacted with benzoyl chloride to give the corresponding amide group, In the Schotten Boumann mechanism was took place in the presence of sodium hydroxide as a catalyst.

4.1 Reaction mechanism of Schotten Boumann reaction :



Scheme No. 1

4.2 Physicochemical properties

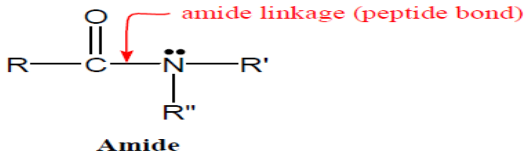
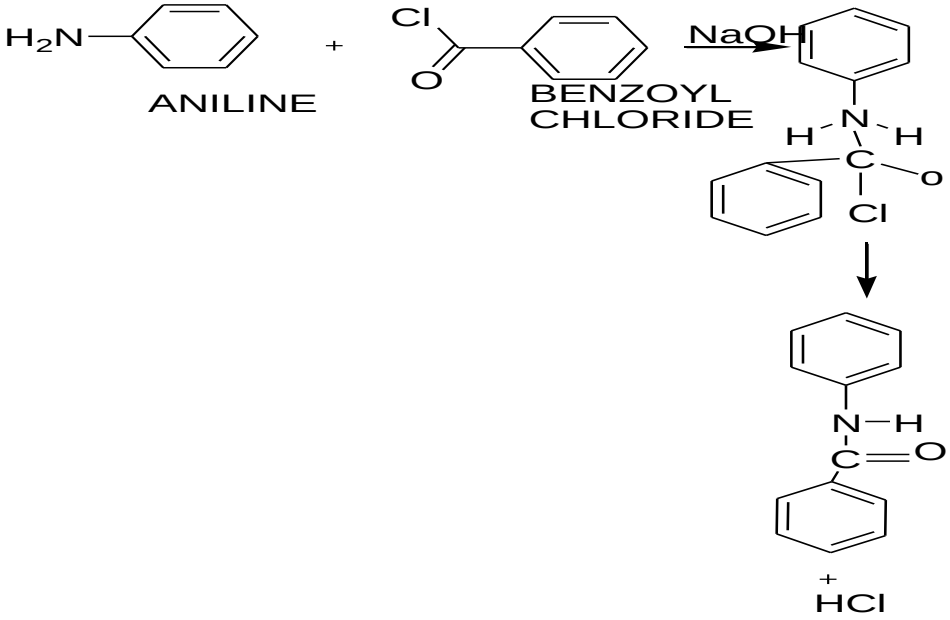


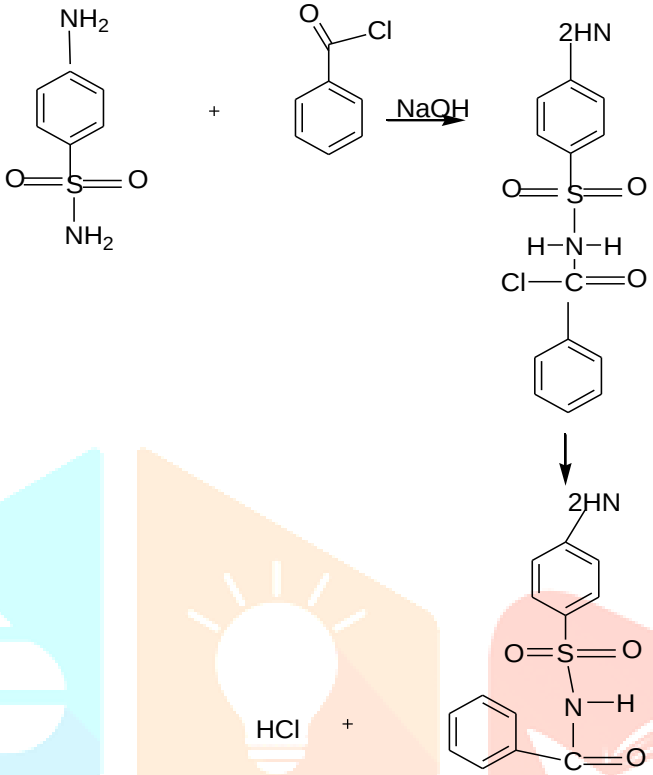
Figure No.2

4.3 Synthesis of amide derivative from aniline and benzoyl chloride



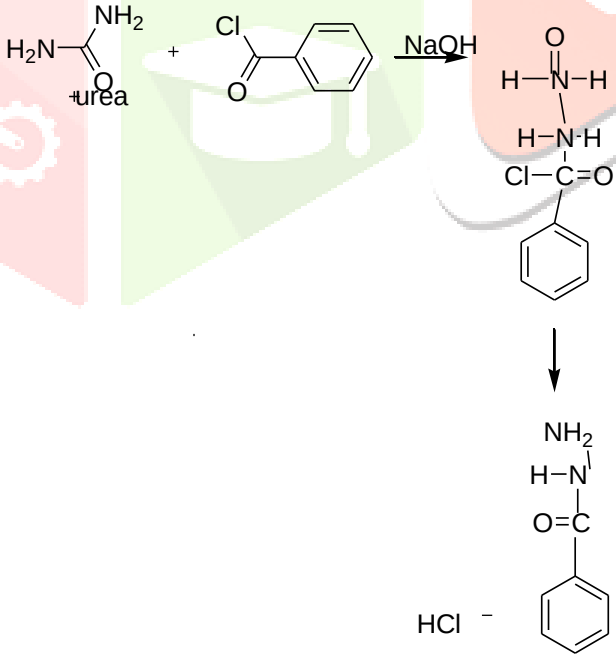
Scheme No. 2

4.4 Synthesis of amide derivative from sulphonilamide and benzoyl chloride



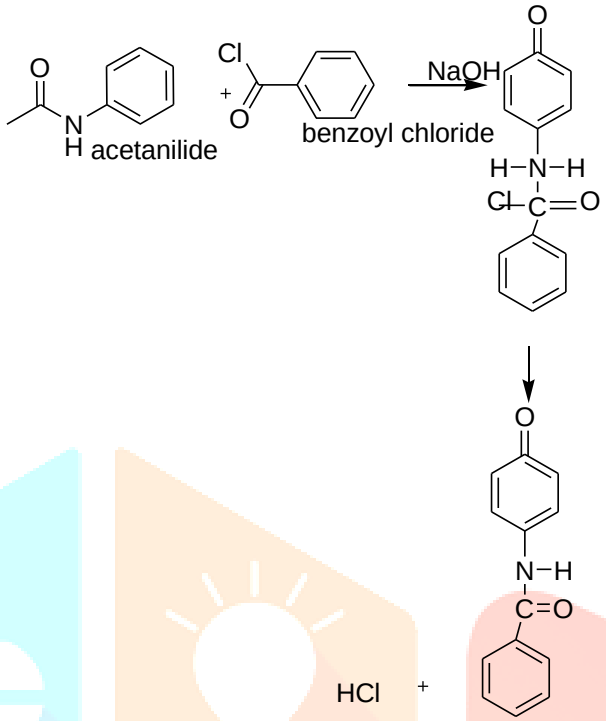
Scheme No. 3

4.5 Synthesis of amide derivative from urea and benzoyl chloride



Scheme No. 4

4.6 Synthesis of amide derivative from acetinalide and benzoyl chloride



Scheme No. 5

4.7 Physicochemical properties of various amide derivatives (A1-A4)

Table 4.7.1.: Physicochemical properties of various amide derivatives (A1-A4)

S.No.	COMPOUND	APPEARANCE	PRACTICAL YIELD
1.	A1	Off White powder	87%
2.	A2	White crystals	92%
3.	A3	White crystals	73%
4.	A4	White crystals	69%

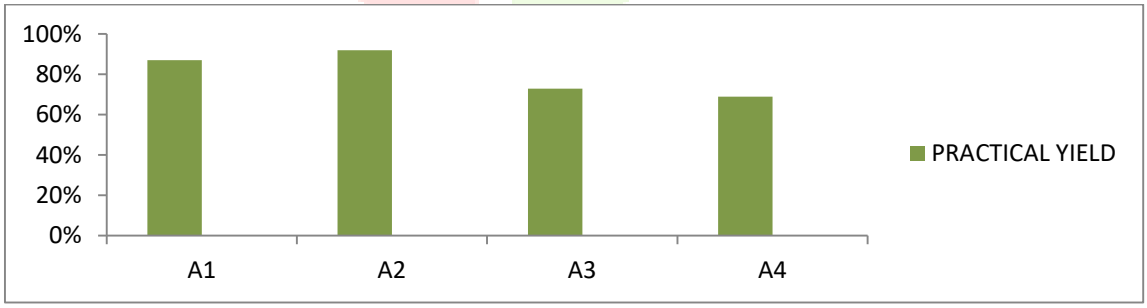


Figure No.3

4.8 MELTING POINT PARAMETER

Table 4.8.1. : Melting point of various amide derivatives (A1-A4)

S.NO.	COMPOUND	MELTING POINTS
1.	A1	(42) – (58)
2.	A2	135 – 138.5
3.	A3	78 – 81
4.	A4	128 – 134

4.9 SOLUBILITY

Table 4.9.1. : Solubility of various amide derivatives (A1-A4)

S.NO.	COMPOUND	SOLUBILITY
1.	A1	Soluble in Ethyle acetate, Hot ethanol and Cyclohexane
2.	A2	Hot ethanol, Ethyl acetate and slightly soluble in ethanol

3.	A3	Soluble in ethanol and hexane
4.	A4	Soluble in ethyle acetate, cyclohexane and slightly soluble in water

4.10 OPTICAL ROTATION

Table 4.10.1. : Optical rotation of various amide derivatives (A1-A4)

S.NO.	COMPOUND	OBSERVATION
1.	A1	0.254
2.	A2	0.263
3.	A3	0.258
4.	A4	0.250

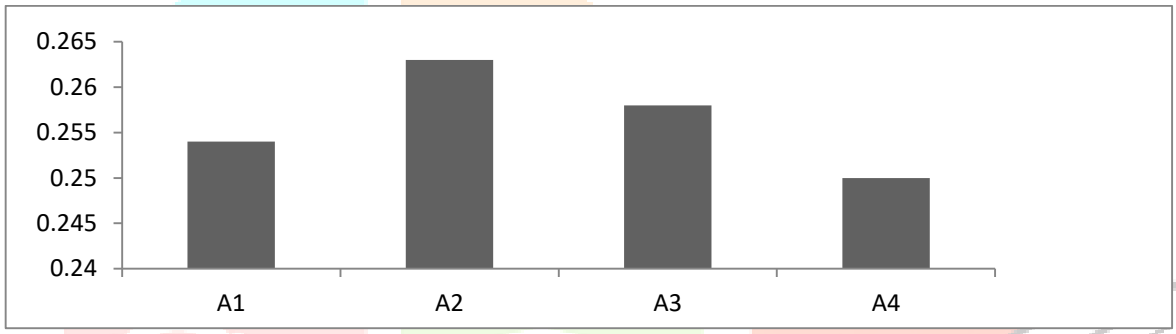


Figure No.4

4.11 pH

Table 4.11.1. : pH properties of various amide derivatives (A1-A4)

S.NO.	COMPOUND	OBSERVATION
1.	A1	8.72
2.	A2	9.41
3.	A3	6.79
4.	A4	7.72

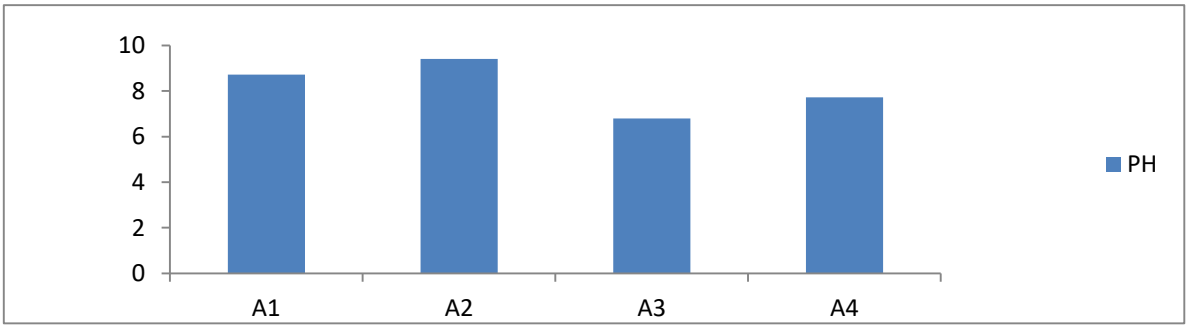


Figure No. 5

4.12. ULTRAVIOLET SPECTROSCOPY

Ultraviolet spectroscopy was done in Elico Double Beam Spectrophotometer SL 210. The solvent system used was ethanol.

Table 4.12..1. : wave length of amide derivatives (A1-A5)

S. No.	COMPOUND	SOLVENT	λmax(nm)
1.	A1	Ethanol	233
2.	A2	Ethanol	254
3.	A3	Ethanol	272

4.	A4	Ethanol	291

4.13 THIN LAYER CHROMATOGRAPHY:

The TLC of synthesized amide derivative was done by using solvent such as n-hexane: ethyl acetate (8:2) solvent system and visualizes by using uv radiation.

S.No.	COMPOUND	RETENTION FACTOR
1.	A1	0.71
2.	A2	0.84
3.	A3	0.92
4.	A4	0.82

4.14. The characteristic of 1H NMR data and interpretation of synthesized amide derivative

The amide derivatives were synthesized and characterized with the help by 1H NMR spectra. 1H NMR was done at 400 MHz using DMSO as a solvent. In the synthesize amide derivatives A1 and A2 have more reactivity as compare to A3 and A4.

4.15IR Spectra of synthesized amide derivatives :

After the synthesis of amide derivative with different substitution of amide derivative with different functional group, the IR technique may used to check which functional group may be present in the synthesized compound.

Different synthesized amide derivative may have different functional group determine by IR technique.

S.NO.	COMPOUND	R1	R2
1.	A1	C6H5	C6H5
2.	A2	C6H5NH2	C6H5COO
3.	A3	NH2	C6H5
4.	A4	C6H5COO	C6H5

4.16. ANTIBATERIAL ACTIVITY

Figure 6: Preparation of agar plate for growth of bacteria

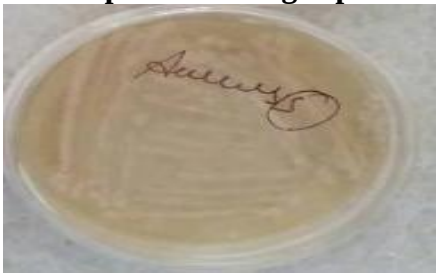


Figure 7: Growth of *E. coli* and *S. aureus* in all agar plates

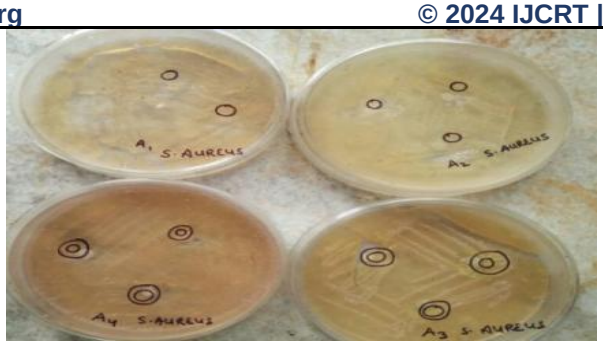


Figure 8 : Zone of inhibition in *S. Aureus*

Table 4.16.1. : Screening of the newly synthesized amide derivatives shown as antibacterial activity

S. NO.	COMPOUND NO.	GRAM +VE BACTERIA	GRAM -VE BACTERIA
1.	A1	16	20
2.	A2	18	18
3.	A3	12	16
4.	A4	10	14

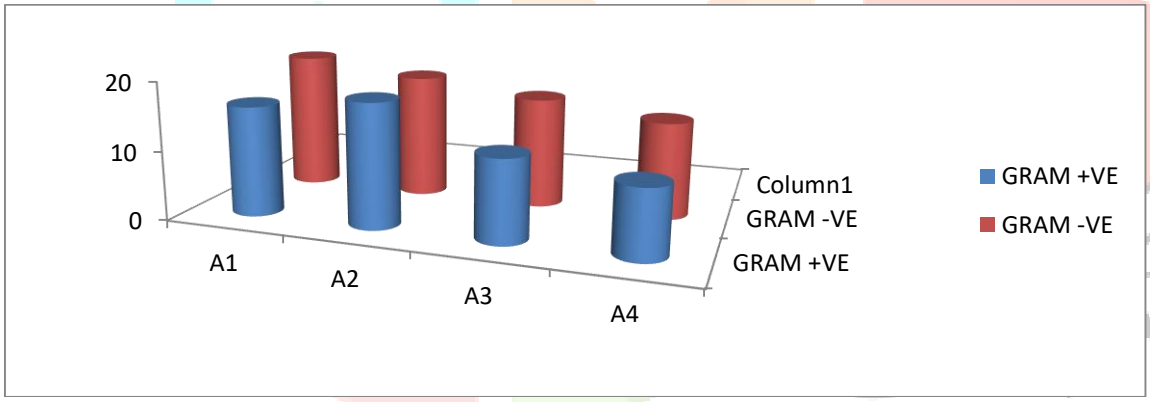


Figure No. 9

DISCUSSION

All the amide compounds synthesized were evaluated for their antibacterial and antioxidant activities through in-vitro screening. The antibacterial assessment involved using the agar disc diffusion method to measure the zone of inhibition in millimeters under specified conditions. Sulphonamide (µg/ml) served as the standard drug for evaluating antibacterial activity against E. coli and S. aureus microorganisms on nutrient agar media.

In the disc diffusion method, sterilized agar media were spread on petri plates, and 6mm diameter discs were utilized. Each synthesized amide compound (µg/ml) was individually placed on different petri plates for diffusion over a 1-hour period. DMSO served as the suitable solvent for all compounds. The plates were then incubated at 37oC for 24 hours and 28oC for 48 hours to assess antibacterial activities under varying temperature and time intervals. The zones of inhibition around the discs were measured after the completion of the incubation period to determine the activity of the synthesized compounds.

Following the synthesis of amide derivatives with diverse substitution patterns and functional groups, infrared (IR) spectroscopy was employed to identify the presence of strong electron-donating groups in A1 and A2. Subsequently, the synthesized amide derivatives were characterized using 1H NMR spectra, conducted at 400 MHz with DMSO as the solvent. Results indicated that compounds A1 and A2 exhibited higher reactivity compared to A3 and A4.

A Structure-Activity Relationship (SAR) study revealed that the introduction of different substitutions exerted varied biological activity. Specifically, electron-donating groups were strategically chosen as substitutions in the chemical structure of the targeted compounds. Compounds A1, A2, A3, and A4, each with distinct substituents, demonstrated a minimum inhibitory concentration against microorganisms such as E. coli and S. aureus, outperforming the standard used in antibacterial screening.

CONCLUSION

The aim of the current research was to design, synthesize, and evaluate the antibacterial activity of substituted amide derivatives. The research protocol was established, and all necessary documentation, including annexures, was meticulously completed. Various parameters such as physical properties (pH, solubility, optical rotation), elemental analysis, IR, NMR, TLC, and antibacterial activity were investigated.

The key finding of the study revealed that derivatives A1 and A2 exhibited the highest Minimum Inhibitory Concentration (MIC) against both bacterial species. This heightened activity was attributed to the presence of stronger electron-donating groups in the synthesized compounds. The results supported the conclusion that electron-donating groups have the capability to enhance electron density, rendering the compound more effective against bacterial activity. In contrast, compounds A3 and A4, synthesized with different electron-donating groups, demonstrated lower activity against bacterial agents.

REFRENCES

1. Porex Barrier (April 2009) "Antimicrobial" Merriam-Webster Online Dictionary. Archived from the original on 24 Retrieved 2009-05-02.
2. Kingston W (June 2008). "Irish contributions to the origins of antibiotics". Irish Journal of Medical Science. 177 (2): 87–92.
3. Kevin A. Boudreaux (june 1998) “introduction of amide Fundamentals of Organic Chemistry” Organic and Biochemistry for Today (Seager & Slabaugh).
4. *Sheppard, Tom D.* "Amide - Definition of amide in English by Oxford Dictionaries". *Oxford Dictionaries - English* Retrieved 15 April 2018.
5. *Valeur, Eric Bradley, Mark (2009). "Amide bond formation: beyond the myth of coupling reagents" Chem. Soc Rev 38 (2): 606–631.*
6. *Wenner, Wilhelm (1952).* "Greener Methods: Catalytic Amide Bond Formation". Retrieved 2016-09-22.
7. *Sabatini, Marco T. Boulton, Lee T. (2017-09-01)*"Amide". *The American Heritage Dictionary of the English Language (5th edition) Boston*: Houghton Mifflin Harcourt. *2014.*
8. *Valeur, Eric; Bradley, Mark (2009).*"Amide definition and meaning - Collins English Dictionary".Retrieved 15 April 2018.
9. Aoki Satosh, Nagakawa Toshiya, Konish Nobukiyo; PCT Int. Appl. (2003); Chem. Abstr., (2003).
10. Dr. Dietmar Kennepohl FCIC (Professor of Chemistry, Athabasca University) 41270 27 june 2013
11. Farmer. Prof. Steven., (Sonoma State University),Amity university Retrieved by June 2013.
12. Mr Kevin A. Boudreaux (june 1998) “ introduction of amide Fundamentals of Organic Chemistry” Organic and Biochemistry for Today (Seager & Slabaugh)
13. "Amide definition and meaning - Collins English Dictionary". Retrieved 15 April 2018.
14. "Amide" *The American Heritage Dictionary of the English Language (5th ed.). Boston:* Houghton Mifflin Harcourt. *2014.*
15. "Amide - Definition of amide in English by Oxford Dictionaries". *Oxford Dictionaries - English*. Retrieved 15 April 2018.
16. *Valeur, Eric; Bradley, Mark (2009). "Amide bond formation: beyond the myth of coupling reagents". Chem. Soc. Rev. 38*"Greener Methods: Catalytic Amide Bond Formation". Retrieved 2016-09-22.
17. *Sabatini, Marco T; Boulton, Lee T.; Sheppard, Tom D. (2017-09-01).* "Borate esters: Simple catalysts for the sustainable synthesis of complex amides" Science Advances. 3 (9):
18. *Wenner Wilhelm (1952). "Phenylacetamide". Organic Syntheses. 32: 92.*
19. *Bodroux F. (1905). Bull. Soc. Chim. France. 33: 831.*
20. *Bodroux F. (1905)*"Bodroux reaction". *Institute of Chemistry, Skopje, Macedonia.*
21. *Schulenberg J. W.; Archer, S. (1965). The Chapman Rearrangement*. Org. React. 14. pp. 1–51.

22. *Chapman, Arthur William (1925). "CCLXIX.—Imino-aryl ethers. Part III. The molecular rearrangement of N-phenylbenziminophenyl ether". Journal of the Chemical Society, Transactions.*
23. *Kleinberg J. Audrieth, L. F. (1955). March, Jerry. Advanced organic Chemistry, Reactions, mechanisms and structure (3rd ed.)..*
24. *Kleinberg J. Audrieth, L. F. (1955). "Lactamide". Organic Syntheses. 3: 516..*
25. CRC Handbook of Chemistry and Physics, 1984, 65th Edition, CRC press, pp. C-65-C-575.
26. Parkway B. 2010. The theory of pH measurement, Rosemount Analytical, Emerson Process Management, pp. 1-5.
27. Toledo M. 2013. A Guide to pH mesearment, Mettler-Toledo AG Process Analytics, pp. 5-15.
28. Determination of optical rotation and specific rotation, The International Pharmacopoeia, 2016. 6th Edition, pp. 1-2.
29. Kannan R., 2014. Basics of chromatographic Technique Center for Cellular and Medical Platform pp. 3-10.
30. Dhanarasu S., 2012. Chromatography and its Application, Intech, pp. 5-15.