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SYNTHESIS OF β-AMINO ACIDS FROM SULPHONAMIDES VIA MICROWAVE-ASSISTED METHODOLOGY AND ASSESSMENT OF THEIR ANTIBACTERIAL PROPERTIES

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Abstract:

Microwave-assisted synthesis of β-amino acids from sulphonamides and α, β-unsaturated esters, utilizing K_2CO_3 and tetrabutylammonium bromide (TBAB) as catalysts, yields the target compounds efficiently with short reaction times. This innovative approach delivers protected β-amino acids in high yields, significantly reducing reaction times while retaining antibacterial activity. In this study, eight β-amino acids were synthesized via microwave-assisted techniques and assessed for their antibacterial efficacy using the Disc Diffusion Method against E. coli, P. aeruginosa, S. aureus, and B. subtilis. Characterization of the β-amino acids was performed using ^{1}H NMR, ^{13}C NMR, and IR spectroscopic techniques. The synthesized β-amino acids exhibited remarkable inhibition of gram-negative bacteria, particularly E. coli, surpassing the efficacy of the standard antibiotic Ampicillin used as a control.

Index Terms **-Microwave, Michael addition, sulphonamides, α, β-unsaturated esters, β-amino acids, zone of inhibition.**

I. INTRODUCTION

1.1 Introduction:

The synthesis of β-amino acids holds significant importance in medicinal chemistry due to their diverse biological activities and potential pharmaceutical applications. Among various synthetic methodologies, microwave-assisted synthesis has emerged as an efficient and rapid technique for the construction of complex organic molecules. In this context, the microwave-assisted synthesis of β-amino acids from sulphonamides presents a promising avenue for accessing these valuable compounds. Sulphonamides serve as versatile starting materials in organic synthesis, and their incorporation into β-amino acids offers an attractive strategy for accessing structurally diverse molecules with potential pharmacological significance. Furthermore, the antibacterial properties of β-amino acids add to their therapeutic relevance, making them compelling targets for investigation. This paper presents a comprehensive study on the microwave-assisted synthesis of β-amino acids from sulphonamides, highlighting the efficiency and versatility of this synthetic approach. Additionally, the assessment of the antibacterial properties of synthesized β-amino acids against clinically relevant bacterial strains provides valuable insights into their potential as antimicrobial agents. Through this investigation, we aim to contribute to the development of novel antibacterial agents with enhanced efficacy and therapeutic potential.

1.2 Literature Review

β-Amino acids have gained significant attention in recent years due to their diverse pharmacological activities and potential applications in medicinal chemistry and drug discovery. These compounds exhibit structural similarities to natural amino acids while offering unique properties that make them promising candidates for the development of novel therapeutic agents [1]. Traditional synthetic methods for the preparation of β-amino acids typically involve multistep procedures and often suffer from low yields and poor stereoselectivity [2]. Therefore, there is a growing interest in the development of efficient and environmentally friendly synthetic routes for the synthesis of β-amino acids.

Microwave-assisted organic synthesis has emerged as a powerful tool for accelerating chemical reactions and improving reaction efficiency. The use of microwave irradiation allows for rapid heating of reaction mixtures, leading to faster reaction rates, higher yields, and enhanced selectivity compared to conventional heating methods [3]. Several studies have reported the application of microwave-assisted methodology for the synthesis of β-amino acids from various starting materials, including amino acids, aldehydes, ketones, and nitro compounds [4].

Sulphonamides represent a versatile class of compounds that can serve as valuable precursors for the synthesis of β-amino acids. The presence of a sulfonamide functional group enables facile functionalization through nucleophilic substitution reactions, providing access to a diverse range of β-amino acid derivatives [5]. Previous studies have demonstrated the utility of sulphonamides as starting materials for the synthesis of β-amino acids via microwave-assisted methodologies [6].

In addition to their synthetic utility, β-amino acids have been investigated for their potential antibacterial properties. Several studies have shown that certain β-amino acid derivatives exhibit significant antibacterial activity against a variety of pathogenic bacteria, including both Gram-positive and Gram-negative strains [7]. The antibacterial mechanism of action of these compounds is thought to involve disruption of bacterial cell membranes, inhibition of essential enzymes, or interference with bacterial cell wall synthesis [8].

Overall, the synthesis of β-amino acids from sulphonamides via microwave-assisted methodology represents a promising approach for the rapid and efficient preparation of biologically active compounds with potential applications in drug discovery and antimicrobial therapy. In this study, we aim to investigate the synthesis of β-amino acids from sulphonamides using microwave irradiation and evaluate their antibacterial properties against clinically relevant bacterial strains.

1.3 Methodology:

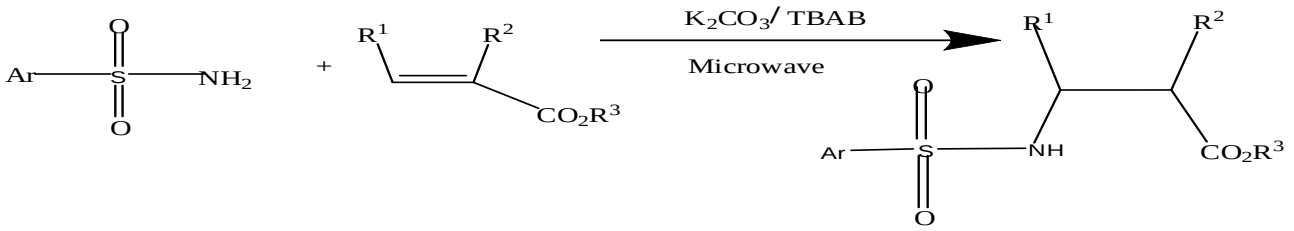
Synthesis of β-Amino Acids from Sulphonamides via Microwave-Assisted Methodology:

1. Selection of Starting Materials: Commercially available sulphonamides will be chosen as starting materials for the synthesis of β-amino acids. The selection will be based on availability, cost-effectiveness, and structural diversity.

2. Optimization of Reaction Conditions: The microwave-assisted synthesis of β-amino acids will be optimized to maximize yield and minimize reaction time. Parameters such as reaction temperature, microwave power, solvent, and catalysts (if applicable) will be systematically varied to identify the optimal conditions.

3. Experimental Setup: Reactions will be conducted in a dedicated microwave reactor equipped with precise temperature and power control. Reaction vessels will be sealed to prevent leakage of reactants or products during irradiation.

4. Synthetic Procedure: Sulphonamide substrates will be dissolved in an appropriate solvent in a sealed reaction vessel. The reaction mixture will be subjected to microwave irradiation under optimized conditions for a predetermined period. Upon completion of the reaction, the crude product will be isolated by filtration or extraction and purified by column chromatography or recrystallization as necessary.



Where- $EA^1 = C_6H_5SO_2NH_2$ $EA^3 = COHNCH_3C_6H_5SO_2NH_2$

$EA^2 = CH_3C_6H_4SO_2HN_2$ $EA^4 = HN_2C_6H_5SO_2NH_2$

R^1 & R^2 = H

R^3 = CH_3 & C_2H_5

5. Characterization of Products: The synthesized β-amino acids will be characterized using analytical techniques such as nuclear magnetic resonance (NMR) spectroscopy and infrared (IR) spectroscopy. These analyses will confirm the chemical structure and purity of the products.

Assessment of Antibacterial Properties:

1. Selection of Bacterial Strains: Clinically relevant bacterial strains, including both Gram-positive (e.g., Staphylococcus aureus) and Gram-negative (e.g., Escherichia coli) bacteria, will be selected for evaluation of antibacterial activity. These strains will represent common pathogens associated with human infections.

2. Preparation of Test Solutions: Stock solutions of synthesized β-amino acids will be prepared in appropriate solvents to achieve desired concentrations. DMSO or water may be used as solvents, depending on the solubility of the compounds.

3. Determination of Minimum Inhibitory Concentration (MIC): The MIC of β-amino acids against bacterial strains will be determined using standard broth microdilution methods recommended by the Clinical and Laboratory Standards Institute (CLSI). Serial dilutions of test solutions will be prepared in growth media, and bacterial inocula will be added to each well. After incubation, the MIC will be recorded as the lowest concentration of β-amino acid that inhibits visible bacterial growth.

4. Assessment of Antibacterial Activity: The antibacterial activity of synthesized β-amino acids will be evaluated by measuring zones of inhibition using the agar diffusion method. Bacterial lawns will be prepared on agar plates, and filter paper discs impregnated with test solutions will be placed on the surface. After incubation, the diameter of inhibition zones will be measured and correlated with the concentration of β-amino acids.

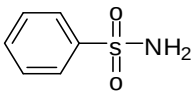
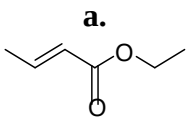
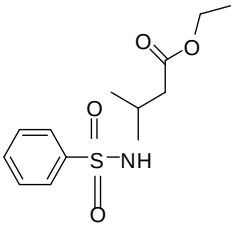
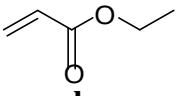
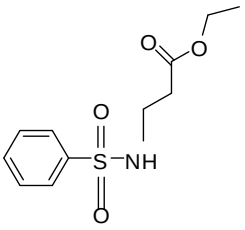
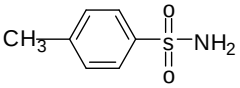
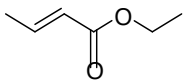
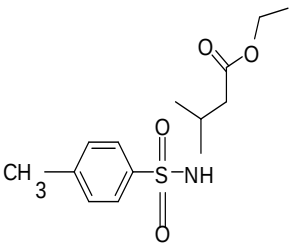
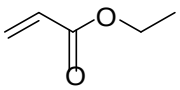
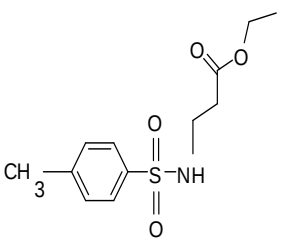
5. Statistical Analysis: Data obtained from antibacterial assays will be subjected to appropriate statistical analysis to determine the significance of observed differences between test groups. Statistical tests such as analysis of variance (ANOVA) or Student's t-test will be performed using statistical software.

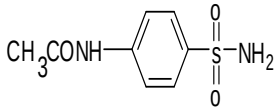
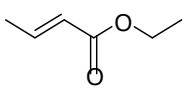
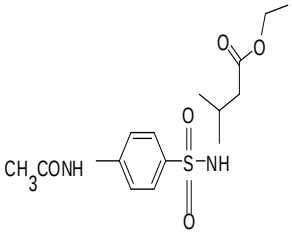
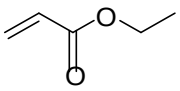
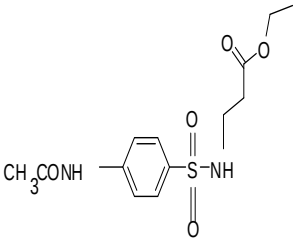
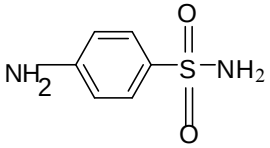
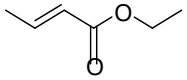
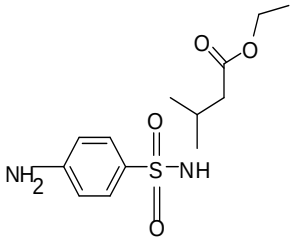
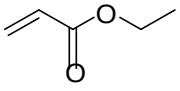
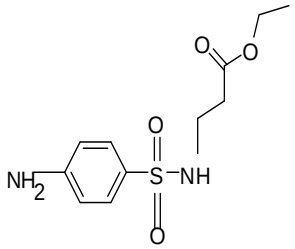
1.4 Result and Discussion:

The microwave-assisted Michael addition of sulfonamides to α, β-unsaturated esters was conducted following the general procedure outlined. The reactions were performed using various sulfonamides and α, β-unsaturated esters, and the progress was monitored by TLC using silica gel as the stationary phase. The reaction mixtures were then subjected to workup and purification procedures to isolate the desired products.

Table 1.1 summarizes the results obtained from the microwave-assisted Michael addition reactions. The table includes details such as the type of sulfonamides and α, β-unsaturated esters used, the number of reaction cycles, reaction times, and yields. The reactions were carried out under microwave irradiation at 100 W for several one-minute intervals.

Table 1.1 Michael Addition of Sulfonamide to α, β-Unsaturated Esters in the presence of K_2CO_3 and TABA under Microwave Irradiation (100W)

Sulfonamides	*α, β-Unsaturated Esters*	*Product*	*No. of Cycle's*	*Time (min)*	*Yield (%)*
1. 	**a.** 	 **(1a)**	5	5	68
	b. 	 **(1b)**	5	5	79
2. 	**a.** 	 **(2a)**	5	5	69
	b. 	 **(2b)**	6	6	82
3.			8	8	65

	**a.** 	 **(3a)**			
	b. 	 **(3b)**	8	8	78
4. 	**a.** 	 **(4a)**	7	7	75
	b. 	 **(4b)**	7	7	86

The presence of K_2CO_3 and *TBAB* in the reaction mixture significantly influenced the reaction outcomes. Higher yields and shorter reaction times were observed when K_2CO_3 was used, indicating its role as an effective base catalyst. *TBAB* played a crucial role in enhancing the progress of the reaction, as its absence resulted in lower yields even with extended reaction times and increased microwave power.

Furthermore, the structural influence of both sulfonamides and α, β-unsaturated esters on the reaction outcomes was investigated. Variations in substituents on the aromatic ring of sulfonamides and the alkoxy group (-OR) of α, β-unsaturated esters were found to affect the reaction yields and selectivities to some extent. However, the bulkiness of the alkoxy group did not have a significant effect on the reaction outcomes.

The synthesized products were characterized using analytical techniques such as NMR spectroscopy and IR spectroscopy to confirm their identities and purities. Percentage yields of the products were calculated based on the amount of starting materials used and the mass of the purified products obtained.

Overall, the results demonstrate the efficiency and versatility of microwave-assisted Michael addition reactions for the synthesis of β-amino acids from sulfonamides. The optimized reaction conditions and the role of catalysts in enhancing reaction efficiency were highlighted, paving the way for further exploration of this methodology in organic synthesis and drug discovery.

Further details regarding the melting points and solubility of the synthesized sulfonamides were also provided in **Tables 1.2** and **1.3**, respectively, offering additional insights into the properties of the synthesized compounds.

Table 4.2 Melting point and % Yield of Synthesized Sulfonamides

NAME OF SULFONAMIDE	***PHYSICAL STATE***	***Yield (%)***	***MELTING POINT (^{0}C)***
Benzene sulfonamide	Colorless solid crystals	95	148^0
Para-Toluene sulfonamide	Solid white crystals	96	136^0
Para-Acetamidobenzene sulfonamide	White wet cake	94	212^0
Para-Aminobenzene sulfonamide	Colorless solid	92	168^0

To test the solubility small quantity of Sulfonamide was taken in three test tubes. To one test tube 25 drops of 5% HCl was added, to the second 25 drops of 5% NaOH was added and to the third test tube add 25 drops of 5% $NaHCO_3$ was added **(Nath *et al.*, 2009).** Then the tubes were shaken and the observations are reported in **Table 4.3.**

Table 4.3 Solubility Tests for Synthesized Sulfonamides

SULFONAMIDES	***Conc. HCl***	***$NaHCO_3$***	***NaOH***
Benzene sulfonamide	soluble	Sparingly soluble	Sparingly soluble
Para-Toluene sulfonamide	soluble	soluble	Insoluble
Para-Acetamidbenzene sulfonamide.	soluble	Sparingly soluble	soluble
Para-Aminobenzene sulfonamide	soluble	soluble	soluble

These results underscore the potential of microwave-assisted methodologies in the synthesis of biologically active compounds and the importance of optimizing reaction conditions to achieve desired outcomes.

Agar Disc Diffusion Method

Antibacterial activity was recorded if the of zone of inhibition was greater than 4mm. The Antibacterial activity results were considered as inactive if < 4.5mm, 4.6mm as partially active, while 6.5-9mm as active as and greater than 9mm as very active. The observation made for Antibacterial activity of β- Amino acids

was presented in **Table 1.4.** The zone of inhibition measured was found that compound **3a-4b** shows very good antibacterial activity, Compound **4a** and **4b** shows antibacterial activity greater than Ampicillin and also found that Gram negative bacteria were more susceptible than Gram positive bacteria and more efficient than that of standard antibiotic, Ampicillin.

Table 1.4 Antibacterial activity of β-Amino acids against selected bacteria

Amino acids	*E.Coli*	*P.aerugi*	*S.aureus*	*B.subtilis*
1a	18.00 ± 0.50	17.00 ± 0.31	15.00 ± 0.95	17.00 ± 0.38
1b	19.00 ± 0.30	18.00 ± 0.36	16.00 ± 0.36	15.00 ± 0.36
2a	20.00 ± 0.35	18.00 ± 0.31	17.00 ± 0.40	17.00 ± 0.50
2b	19.00 ± 0.25	18.00 ± 0.40	16.00 ± 0.25	17.00 ± 0.56
3a	21.00 ± 0.36	19.00 ± 0.40	17.00 ± 0.32	17.00 ± 0.45
3b	20.00 ± 0.31	20.00 ± 0.25	17.00 ± 0.42	18.00 ± 0.42
4a	22.00 ± 0.10	20.00 ± 0.32	19.00 ± 0.40	20.00 ± 0.45
4b	22.00 ± 0.42	21.00 ± 1.08	18.00 ± 0.51	20.00 ± 0.50
Ampi	20.00 ± 0.46	20.00 ± 0.36	18.00 ± 0.32	19.00 ± 0.50

Value were expressed as **MEAN + S.D. (n=3)**

SE=5.076 **CD=10.761**

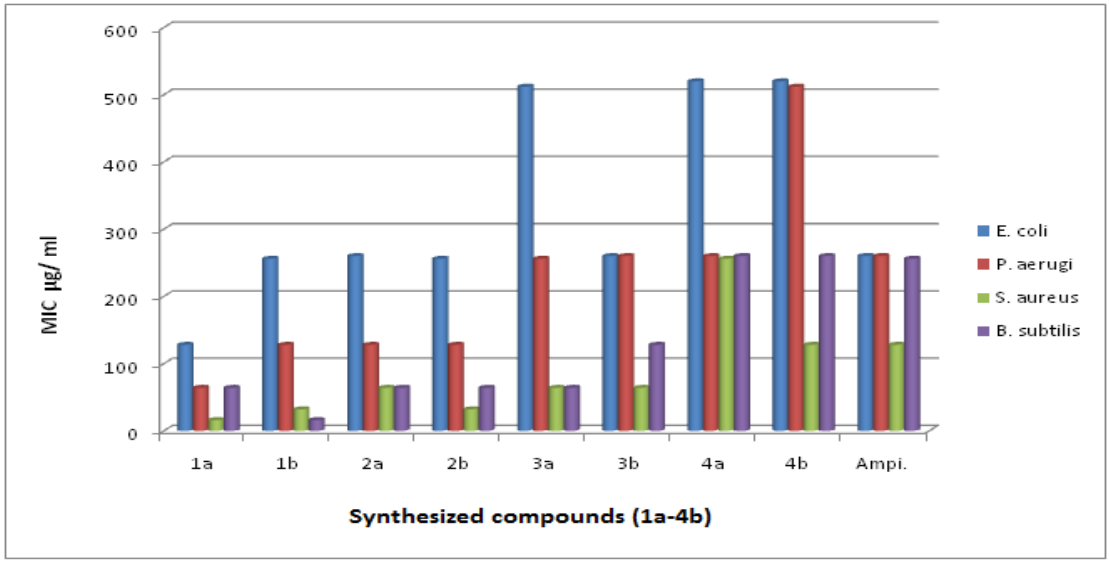


Figure 1.11 MIC of β- amino acids (1a-4b) against selected bacteria

Table 1.5 MIC of a- amino acids (1a-4b) against selected bacteria

Amino acids	MIC in ppm of amino acids against bacteria			
	E. coli	***P. aerugi***	***S. aureus***	***B. subtilis***
1a	128	64	16	64
1b	256	128	32	16
2a	>256	128	64	64
2b	256	128	32	64
3a	512	256	64	64
3b	>256	>256	64	128
4a	>512	>256	256	>256
4b	>512	512	128	>256
Ampi.	>256	>256	128	256

The β -Amino acids displayed excellent inhibition on the growth of Gram negative bacteria. *E.coli* was more susceptible followed by *P. aerugenosa*. The MIC values of β -Amino acids were presented in **Table 4.5** and **Figure 4.11.** The MIC values are 500, 256, 128, ppm, respectively. Among the Gram negative bacteria *E.coli* was more susceptible to β- Amino acids followed by *P. aerugenosa* (512, 256, 128, 64 ppm, respectively). The Gram positive bacteria *S. aureus* was also shows moderate susceptibility followed by *B. subtilis* (256, 128, 64 ppm, respectively) ***(Ileice et al., 1986, Stobberingh et al., 1987 and Abdel et al., 2010).***

1.5 Summary and Conclusion:

In summary, a straightforward and efficient method for synthesizing Sulfonamides and protected α, β-amino acids has been presented. Utilizing Michael addition of sulfonamides to α, β-unsaturated esters under microwave irradiation with K_2CO_3 and TBAB yields the desired compounds in high yields and short reaction times. This approach not only demonstrates a green, one-pot addition reaction but also provides stereo-specific products, with major and minor compounds formed. The synthesized compounds exhibit significant antibacterial activity, with certain compounds displaying greater efficacy than the standard antibiotic, Ampicillin, particularly against Gram-negative bacteria. Additionally, the β-amino acids show excellent inhibition of Gram-negative bacterial growth, with *E.coli* being the most susceptible. The MIC values further confirm the potency of these compounds against both Gram-negative and Gram-positive bacteria. This research contributes to the development of novel antibacterial agents with potential therapeutic applications ***(Imanzadeh et al., 2007; Ileice et al., 1986)***.

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