



Synthesis, Characterization and Antibacterial Activity of 4- (Phenyl Sulfonyl) Aminoacetophenone

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ABSTRACT

The synthesis of 4-(phenylsulfonyl) aminoacetophenone which involve the base catalyzed sulfonylation of 4-aminoacetophenone using benzene sulfonyl chloride is reported. The chemical structure of the synthesized compound was elucidated using FT-IR, UV, ¹HNMR and ¹³CNMR characterization. The compound was screened for its antibacterial activities against gram positive and gram negative bacterial strains namely: *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*. The antibacterial activity was determined by measuring inhibition zone diameter. It was found that the compound has potent antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with IZD of 18 mm and 17 mm respectively.

Keywords: Antibacterial, Characterization, sulphonamides, Synthesis, 4-phenylsulphonyl) minoacetophenone

INTRODUCTION

The wellbeing of modern society is threatened by myriads of problems. One of these pressing problems is the emergence of new diseases and increasing microbial resistance to existing antibiotics. This has prompted renewed interest in the search for chemical agents that can serve as possible sources of new therapeutic agents (Ajani *et al.*, 2015). Sulfonamides are a group of organic compounds with the general molecular formula $RSO_2NR^1R^2$ where R may be alkyl, aryl or hetero aryl groups and R¹ and R² may be hydrogen, alkyl or aryl groups. Sulfonamides are the first drugs largely used as preventive and chemotherapeutic agents against various infectious diseases (Ajani *et al.*, 2015). They are one of the classes of bioactive compounds which have been employed as anti-diabetic (Berredjem *et al.*, 2015), antioxidant (Saeedi *et al.*, 2014), anticancer (El-Sayed *et al.*, 2011), antihypertensive (Bhagwat *et al.*, 2014) antibacterial (Rathod *et al.*, 2012), analgesic (Zebardast *et al.*, 2009) among other uses. Sulfonamides are still being widely used in the clinics because of their easy availability and relative low cost in comparison to other antibiotics. To date, more than twenty thousand sulfonamide derivatives have been synthesized and characterized (Giotello *et al.*, 2013). These syntheses have resulted in the discovery of new compounds with varying pharmacological properties (Kolaczek *et al.*, 2014). The relative importance of these sulfonamides has made it imperative that efficient methods for their syntheses be explored. Although, many synthetic methods have been reported for the synthesis of

sulfonamides (Thomas *et al.*, 2011), in spite of their potential utility, many of these methods involved various draw backs such as the use of expensive or less easily available reagents, vigorous reaction conditions and difficulties in the isolation of the pure products (Bahrami *et al.*, 2009). It is therefore important to find milder and shorter routes for the syntheses of these sulfonamides which are clean and cheap while at the same time giving high product yield. The reduction in the number of steps in the synthesis will lead to a reduction in the amount of reagents and solvents used and hence a reduction in the amount of waste generated. The synthetic method reported by (Xiaohu *et al.*, 2006) which made use of water as solvent for the reaction with modification was utilized in this synthesis.

MATERIALS AND METHODS

Analyses

Nuclear Magnetic Resonance (NMR) analysis of the compound was carried out using a JEOL-LA-400MHz NMR spectrophotometer, where CDCl₃ was used as internal standard. Fourier Transform Infrared Spectroscopy (FT-IR) analysis was carried out using 8400S INFRARED Spectrophotometer, by employing KBr discs. UV-Visible analysis was carried out on a HELIOSα UV-Visible Spectrophotometer v4.24. The evaluation of the antimicrobial activity of the compound was carried out at the Microbiology Advanced Laboratory, Department of Biological Sciences, Federal University of Agriculture, Makurdi.

Synthesis of 4-(Phenyl Sulfonyl) aminoacetophenone .

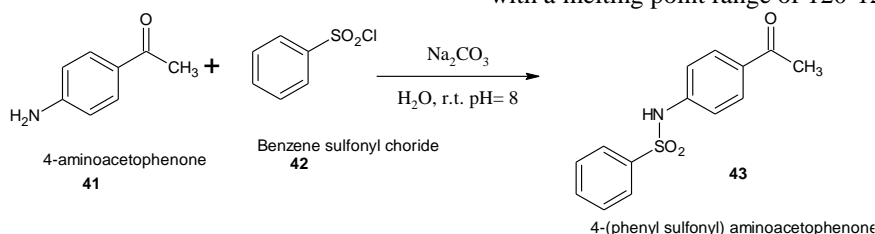
4-Aminoacetophenone (2.5 g, 0.02 mol) was added to a conical flask containing benzenesulfonyl chloride (3.5 g , 0.02 mol,) and a magnetic stirring bar. The mixture was stirred with an electromagnetic stirrer. Aqueous solution of Na₂CO₃ (25 mL, 2 M) was added in portions to the reaction mixture and the set-up was placed in a fume chamber. The pH of the reaction mixture was strictly monitored and maintained at pH 9 by adding portions of Na₂CO₃ solution at regular intervals. The medium was made alkaline to enable the complete dissolution of the starting material and help in the removal of hydrogen chloride being formed. The reaction progress was monitored by withdrawing an aliquot and spotting on a TLC plate. The TLC plate was developed in a tank which has propanol as eluent. At the end of the reaction the pH was adjusted to 2 by adding few drops of concentrated HCl to the reaction mixture. The pH was adjusted to neutralize the alkaline medium and decrease the solubility of the product in the reaction medium and enable its collection. The precipitates formed were collected, filtered and washed with distilled water before recrystallizing from ethanol to obtain a white solid product which melted at 120-122° C. The spectra analyses of the compound gave following; UV – visible (EtOH) λ_{max}; 301nm. IR (KBr) ν_{max} (cm⁻¹); 3742, 3086, 743, 1681, 1519, 1458, 1334, 1265, 1149 and 1080. ¹H NMR (400 MHz, dCDCl₃, δ): 10.87(s, 1H, aromatic amine), 7.85 (m, 2H aromatic), 7.84(d, 1H, aromatic), 7.63(d, 2H, aromatic), 7.58(m, 2H, aromatic), 7.2m (d, 1H, aromatic), 3.2(s, 3H, methyl). ¹³CNMR (dCDCl₃, δ): 196.6(C=O), 142(C=C-N), 139.76(C=C-S), 133.71, 132.47, 130.27, 129.92, 127.00, 118.44(C=C), 26.60(methyl carbon).

Evaluation of antimicrobial activities

The antimicrobial assay was conducted using agar-well diffusion method (Perez *et al.*, 1995). 20 mg of the synthesized compound was dissolved in 1 mL of DMSO (1DMSO dimethyl sulfoxide). A single colony of each test isolate was suspended in 2 mL sterile nutrient broth. The suspension of each isolate was adjusted to 0.5 McFarland turbidity standards (corresponding to approximately 10⁸Cfu/mL) and used to inoculate the surface of the iso-sensitive agar and the excess fluid drained into discarded pot containing a disinfectant. The inoculated agar surface was allowed to dry and the plates appropriately labeled. Using a cork borer of 6 mm in diameter wells were bored in the inoculated iso-sensitive agar. With a micro pipette, 50 µl of the test compound was delivered into each well. Plates were left on the bench for 30 min to allow the compound to diffuse into the agar. Thereafter, the plates were incubated at 37°C for 24 h. After incubation the plates were observed for inhibition zones around the wells. The diameters of the zones were measured with meter rule to the nearest whole millimeter.

RESULTS AND DISCUSSION

At the commencement of the reaction sulfonyl chloride was seen floating on top of the reaction mixture. This gradually disappeared in the course of time to yield a homogenous mixture. The addition of Na₂CO₃ solution created an alkaline environment and also made the removal of hydrogen chloride formed easier. The reaction progress was monitored by spotting on TLC plate. The completion of the reaction was noticed by the change in pH value from basic to acidic due to the formation of HCl. Acidification at the end of the reaction resulted in the formation of precipitates which were collected by filtration. The crude product was recrystallized from hot ethanol to afford a white solid product in 2.6 g (70%) yield with a melting point range of 120-122°C.



Scheme 1: Synthesis of 4-(Phenyl sulfonyl)

The UV –Visible spectrum of the compound showed an absorption peak at 310 nm which indicates the presence of conjugation. The FT-IR spectrum of 4-(phenyl sulfonyl) aminoacetophenone showed stretching vibration of some functional groups with their intensities and absorption peaks. In the FT-IR spectrum the band at 3742 cm⁻¹ is due to N-H stretching of the amines

while the absorption band at 3086 cm⁻¹ is due to the C-H stretching and vibration of the aromatics. The band at 1743 cm⁻¹ is due to C=O stretching of the carbonyl and the absorption bands at 1681, 1519 and 1458 cm⁻¹ are due to C=C stretching vibration of the aromatics. The bands at 1334 and 1265 cm⁻¹ are due to C-N stretching of aromatic amines. The band at 1149 cm⁻¹ is due to the N-S=O stretching

and the band at 1080 cm⁻¹ is due to the S=O group of the sulfonamides.

The proton NMR spectrum of compound **3** showed absorption peak at 3.2 δ which can be ascribed to the three methyl protons that are equivalent. The absorption in aromatic region represent the protons attached to the aromatic ring and the band at 10.87 δ showed the amine proton attached to an aromatic moiety. In the ¹³CNMR spectrum peak at 196.60 δ is due to the carbonyl carbon while the peak at 142.68 δ is due an aromatic carbon bonded to the nitrogen atom. The absorption peaks of the other aromatic carbon showed that they are not all equivalent. The methyl carbon showed absorption in the single bond region at 26.60 δ showing that it is singly bonded to the carbonyl carbon.

Antimicrobial Assay

The antimicrobial assay was done using agar well diffusion method to measure the zone of

inhibition exhibited by the compound. The zone of inhibition is a qualitative means to measure the ability of chemical agent to inhibit the growth of a control organism. Zone of inhibition is the area on an agar plate where the growth of a control organism is prevented by a chemical agent. The value of inhibition zone diameter (IZD) obtained helps to show whether the microbe is resistant or susceptible to the chemical agent applied. The IZD of 10 mm or less show that the microbe is resistant while IZD of 16 mm and above shows that the microbe is susceptible (Johnson and Case, 1995). The organisms used were *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*. The choice of these organisms was because they are associated with the gastro intestinal tract damage in both man and animals. The result of the inhibition zone diameter for the synthesized compound together with that of the standard drug Ciprofloxacin for microbes is presented on Table 1.

Table 1: Result of the Zone of inhibition of 4-(phenyl sulfonyl) aminoacetophenone and Ciprofloxacin.

Test organism	IZD	Ciprofloxacin IZD (mm)
<i>Staphylococcus aureus</i>	18.00	32
<i>Escherichia coli</i>	11.00	16
<i>Salmonella typhi</i>	14.00	26
<i>Pseudomonas aeruginosa</i>	17.00	28

The result in Table 1 shows that 4-(phenylsulfonyl)aminoacetophenone has activity against the tested microbes. The highest inhibition zone diameter (IZD) of 18 mm for *Staphylococcus aureus* showed that the microbe is susceptible to the synthesized compound. However, the standard drug Ciprofloxacin with IZD of 32 mm for the same microbe showed higher activity. The synthesized compound showed the least activity against *Escherichia coli* with IZD value of 11 mm.

CONCLUSION

The synthesis and characterization of 4-(phenylsulfonyl)aminoacetophenone was successfully undertaken. The result of the antimicrobial screening revealed that the synthesized compound has potential for application/use as structural template in the design and development of new antimicrobial agent for *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

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APPENDIX

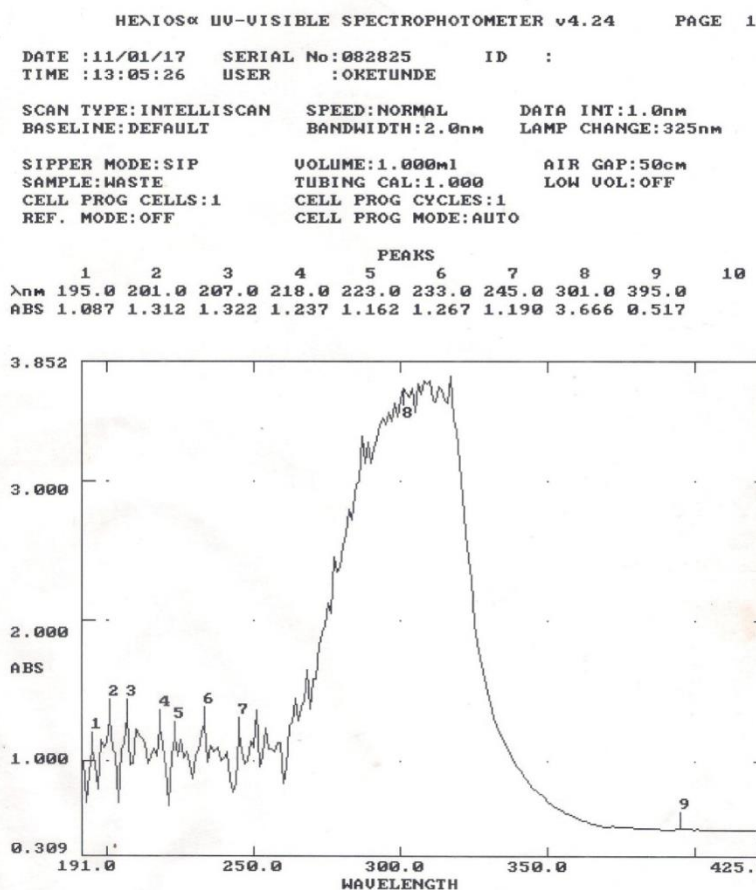


Figure 1: UV Spectrum of 4-(phenyl sulfonyl) aminoacetophenone

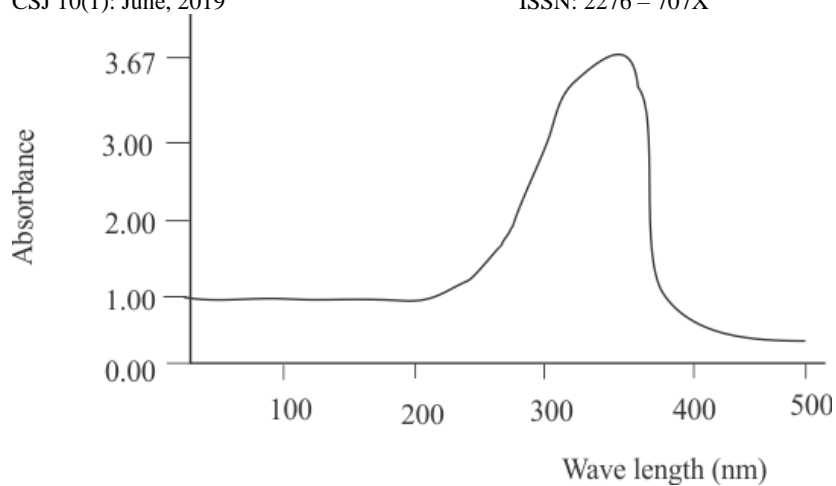


Figure 2: UV-visible spectra of 4-(phenyl sulfonyl) aminoacetophenone

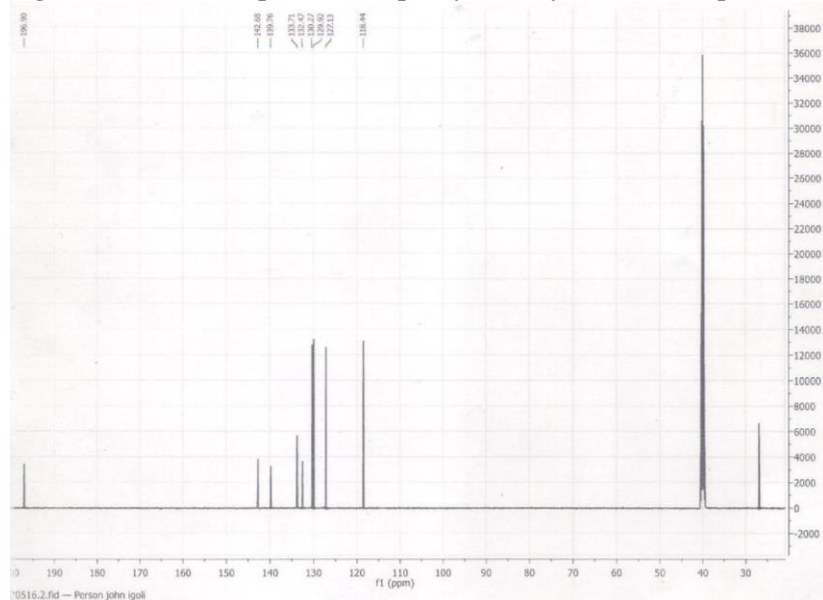


Figure 3: ¹³CNMR spectrum of 4-(phenyl sulfonyl) aminoacetophenone

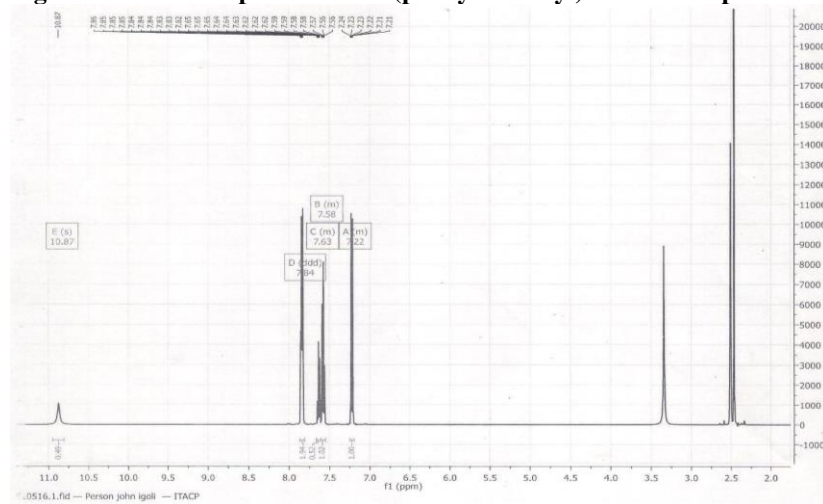


Figure 5: ¹HNMR spectrum of 4-(phenyl sulfonyl) aminoacetophenone