



Synthesis Characterisation and Antimicrobial Activity of Substituted Benzilic Acids

R. Sudha¹, Charles C.Kanakam², Nithya.G¹.

¹Department of Chemistry, School of Engineering, Vels University, Pallavaram, Chennai-600117, Tamil Nadu, India.

²Department of Chemistry, Formerly Presidency College, University of Madras, Tamil Nadu, India.

Abstract: The different substituents in the benzilic acid and its derivatives strongly influence the biological activity. A series of substituted benzilic acid like (Methyl benzilate, 4,4'-dibromo benzilic acid, 2,2'-dichloro benzilic acid and 2'-chloro-4-methoxy-3-nitro benzilic acid) were synthesised and their antimicrobial activity were studied by disc diffusion method. The antimicrobial results reveal that among the synthesized compounds 2,2'-dichloro benzilic acid, 2'-chloro-4-methoxy-3-nitro benzilic acid showed excellent antibacterial activity. Some benzilic acid derivatives already characterised to evoke a particular biological activity. The structure of synthesized compounds has been established on the basis of their spectral (FT-IR, ¹HNMR, ¹³CNMR Mass analysis) data. The purity of the compounds was confirmed by TLC.

Key words: Synthesis, Characterisation, Antibacterial activity, Disc diffusion method.

Introduction

Benzilic acid and its derivatives are of wide interest because of their diverse biological activity and clinical applications, and are remarkably effective compounds both with respect to their inhibitory activity and their favourable selectivity ratio. Antimicrobial drugs have caused a dramatic change not only of the treatment of infectious diseases but of a fate of mankind. The resistance of bacteria against antimicrobial agents has become a widespread medical problem. There are various problems arising with the use of antimicrobials such as local tissue irritation, interference with wound healing process, hypersensitivity reaction, and narrow antimicrobial spectrum. Moreover, the toxic effects produced by many antibiotics must not be forgotten. Resistance to number of antimicrobial agents among a variety of clinically significant bacteria is becoming increasingly important. So, the increasing clinical importance of drug resistant microbial pathogens has additional urgency in microbiological research. The extraordinary progress represented by the arrival of antibiotics has changed the medical prognosis of minor and major infections ¹. Bacterial resistance continues to develop and pose a significant threat both in hospitals and more recently in the community ². A relevant report on resistant antibacterial agents for human medicine is provided by World Health Organization. The panel agreed that the list of critically important antibacterial agents should be updated regularly as new information becomes available, including data on resistance patterns, new and emerging diseases and the development of new drugs³. The current interest in the development of new antimicrobial agents can be partially ascribed both to the increasing emergence of bacterial resistance to antibiotic therapy and to newly emerging pathogens. From the literature survey, it had been found that derivative of benzilic acid has anti acetylcholine activity and anti histamine activity⁴. Accordingly, the development of new antibacterial agents that could overcome the

resistance problem has become the subject of an ongoing research⁵⁻¹⁰. Much research has been carried out with the aim to discover the therapeutic values of benzoic acid derivatives. The variation in the substituent of the benzoic acid reveals that it has been proposed to analyse the anti proliferative activity with different *in vitro* models. The antimicrobial activity¹¹ of chemical compound is influenced by physical and biological characteristics¹². It has been well established that physiological activity is a function of the chemical structure of compound¹³⁻¹⁴.

Materials and Methods

Materials

The homogeneity of all the newly synthesized compounds was routinely checked by thin layer chromatography (TLC) on silica gel G plates. All chemicals and solvents were obtained from Sigma Aldrich and s.d.fine, India, AR. The infrared spectra of the compounds were recorded using KBr pellet in FT-IR/ATR spectrophotometer 450-4000 cm^{-1} manufactured by Perkin-Elmer. The mass spectra were recorded on GC-MS spectrometer - JOEL GC Mate. The ¹H NMR and ¹³C NMR was recorded on Bruker FTNMR 500 MHz.

Synthesis of Methyl benzoate

Methyl benzoate was prepared from 1g of benzoic acid with catalytic amount of Conc. H_2SO_4 and 5 mL of methanol allowed to reflux for 20 minutes. The reaction mixture was cooled to room temperature and neutralized with NaHCO_3 , the white coloured precipitate was filtered, washed with water and dried over vacuum for an hour, 95% yield.

Synthesis of 4, 4'-dibromo benzoic acid

4, 4' dibromo benzoic acid was prepared from (3 g) of 4-bromo benzaldehyde in 30 mL of ethanol with the presence of catalyst 0.15g KCN. To 1.0 g of 4, 4'-di bromo benzoic acid, 10.0 mL of concentrated nitric acid was added slowly and allowed to reflux for 30 minutes or until no more NO_2 gas is apparent. Add 30 mL of water to the reaction mixture cool to room temperature and wash the yellow coloured substance using water. The yield is about 28%. To 0.8 g of 4, 4'-di bromo benzoic acid, 2.0 mL of 95% ethanol was added and the mixture is heated with constant stirring until the benzoic acid was completely dissolved. To this mixture 2.5 mL of an aqueous potassium hydroxide was added dropwise. The reaction mixture was stirred and refluxed well for 20 minutes. After completion of reaction, mixture was cooled in an ice-water bath for an additional 15 minutes, potassium benzoate was formed. This solution was cooled and poured into crushed ice containing 6 mL of 1 M HCl precipitate was filtered, washed with water and dried to afford 4, 4'-dibromo benzoic acid yield about 44%. The pH of the reaction mixture should be maintained at 2.

Synthesis of 2, 2'-dichloro benzoic acid

2, 2'-dichloro benzoic acid was prepared from (2 g) of 2-chloro benzaldehyde in 25 mL of ethanol with the presence of catalyst 0.2g KCN. To 1.0 g of 2, 2'-dichloro benzoic acid 10.0 mL of concentrated nitric acid was added slowly and allowed to reflux for 30 minutes or until no more NO_2 gas is apparent. Add 30 mL of water to the reaction mixture cool to room temperature and wash the yellow coloured substance using water, yield about 50%. About 1.0 g of benzoic acid, 3.0 mL of 95% ethanol was added and the mixture is heated with constant stirring until the benzoic acid was completely dissolved. To this mixture 2.5 mL of aqueous potassium hydroxide was added dropwise. The reaction mixture was stirred and refluxed well for 20 minutes. After completion of reaction, mixture was cooled in an ice-water bath, potassium benzoate was formed. This solution was cooled and poured into crushed ice containing 6 mL of 1 M HCl precipitate was filtered, washed with water and dried to afford 2, 2'-dichloro benzoic acid yield about 50%. The pH of the reaction mixture should be maintained at 2.

Synthesis of 2'-chloro-4-methoxy-3-nitro benzoic acid

2'-chloro-4-methoxy benzoic acid was prepared from (1g) of 2-chloro benzaldehyde and 1g of 4-methoxy benzaldehyde in 30 mL of ethanol with the presence of catalyst 0.2 g KCN. To 1.0 g of 2'-chloro-4-methoxy benzoic acid, 15.0 mL of concentrated nitric acid was added slowly and allowed to reflux for 30 minutes or until no more NO_2 gas is apparent. Add about 30 mL of water to the reaction mixture cool to room temperature and wash the yellow coloured substance (2'-chloro-4-methoxy-3-nitro benzoic acid) using water, yield 95%. About 0.95 g

of benzil was taken in 4.5 mL of 95% ethanol was added and the mixture is heated with constant stirring until the benzil was completely dissolved. To this mixture 2.5 mL of aqueous potassium hydroxide was added dropwise. The reaction mixture was stirred and refluxed well for 25 minutes. After completion of reaction, mixture was cooled in an ice-water bath, potassium benzilate was formed. This solution was cooled and poured into crushed ice containing 5 mL of 1 M HCl precipitate was filtered, washed with water and dried to afford 2'-chloro-4-methoxy-3-nitro benzilic acid yield about 53%. The pH of the reaction mixture should be maintained at 2.

Spectroscopic data of the synthesized compounds

Methyl benzilate

The IR of methyl benzilate showed O-H at 3469 cm^{-1} , C=O at 1710 cm^{-1} , C-O at 1283 cm^{-1} and an aromatic at 935 and 912 cm^{-1} , ester at 1730 cm^{-1} . The molecular mass of the compound is identified by mass spectral analysis and it was found to be m/e value 242. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ ppm: 7.36-7.38 (m, 10H, aromatic proton), 3.85(s, 3H, COOCH_3): $^{13}\text{CNMR}$ δ ppm (CDCl_3) 144.2, 129.2, 126.2, 127.4, 52.3.

4, 4'-dibromo benzilic acid

The IR of 4,4'-dibromo benzilic acid showed O-H at 3469 cm^{-1} , carboxylic acid at 2852 cm^{-1} , C=O at 1701 cm^{-1} , C-O at 1255 cm^{-1} and an aromatic at 947 and 927 cm^{-1} . The molecular mass of the compound is identified by mass spectral analysis and it was found to be m/e value 384. $^1\text{H-NMR}$ (500 MHz, DMSO-d_6) δ ppm: aromatic proton 7.90 (d, 1H), 7.3 (d, 1H), 13.0 (s, 1H, COOH) 3.6 (s, 1H, OH): $^{13}\text{CNMR}$ δ ppm (125 MHz, DMSO-d_6) 166.9, 138.2, 131.5, 130.1, 129.2, 120.0, 83.0

2, 2'- dichloro benzilic acid

The IR of 2,2' dichloro benzilic acid showed O-H at 3428 cm^{-1} , carboxylic acid at 2649 cm^{-1} , C=O at 1685 cm^{-1} , C-O at 1267 cm^{-1} and an aromatic at 976 and 957 cm^{-1} . The molecular mass of the compound is identified by mass spectral analysis and it was found to be m/e value 297. $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ ppm: aromatic protons 7.47-7.49 (m, 1H), 7.34-7.37 (m, 2H), 7.27-7.30 (m, 1H): $^{13}\text{CNMR}$ δ ppm (CDCl_3) 173.7, 136.3, 133.5, 131.8, 130.2, 129.9, 126.9, 82.4

2'-chloro-4-methoxy-3-nitro benzilic acid

The IR of 2'-chloro-4-methoxy-3-nitro benzilic acid showed O-H at 3419 cm^{-1} , carboxylic acid at 2648 cm^{-1} , C=O at 1690 cm^{-1} , C-O at 1284 cm^{-1} and an aromatic at 955 and 915 cm^{-1} , NO_2 at 1537 cm^{-1} . The molecular mass of the compound is identified by mass spectral analysis and it was found to be m/e value 337. $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ ppm: aromatic protons, 8.40 (s, 1H), 8.30 (d, 1H), 7.83 (d, 1H), 7.48-7.51 (m, 2H), 7.37-7.47 (m, 2H), 4.92 (s, 3H, $-\text{OCH}_3$) $^{13}\text{CNMR}$ δ ppm (125 MHz, CD_3OD) 176.5, 155.7, 143.0, 140.0, 137.9, 130.0, 130.4, 126.4, 113.4, 77.4, 56.1

Method

Disc diffusion method

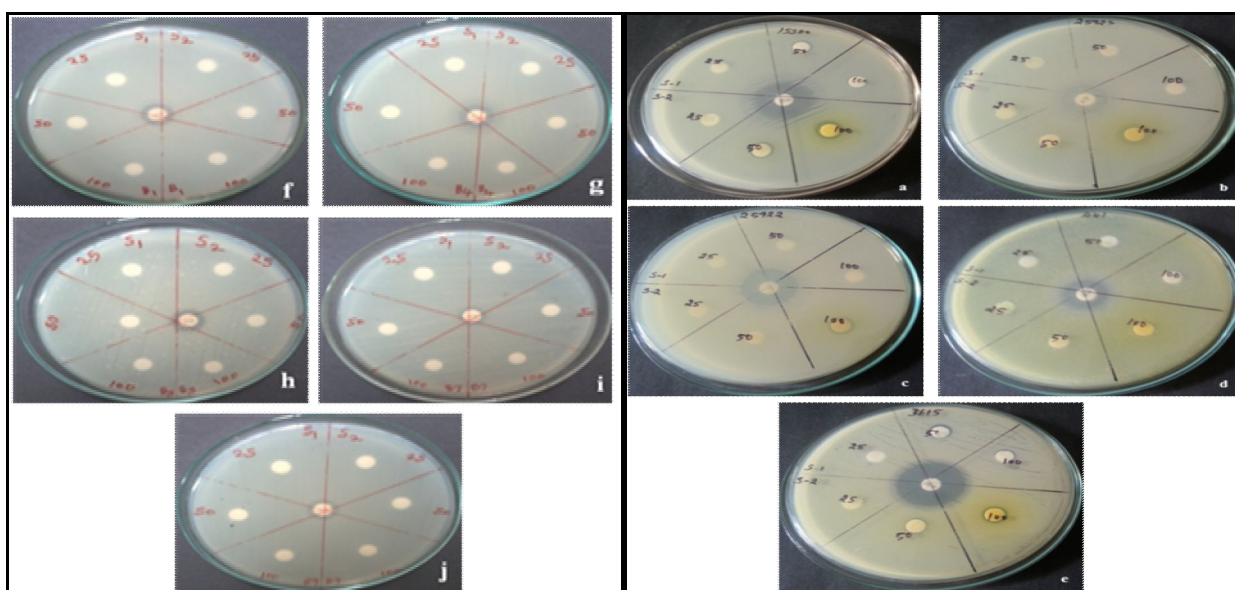
Antibacterial activity was carried out using disc diffusion method (Murray et al., 1995). Petri plates were prepared with 20 ml of sterile MHA (Hi-media, Mumbai). The test culture (100 μl of suspension containing 108 CFU/ml bacteria) were swabbed on the top of the solidified media and allowed to dry for 10 mins. Three different concentrations of the compounds (25, 50 and 100 $\mu\text{g}/\text{disc}$) were loaded on a sterile disc and placed on the surface of the medium and left for 30 mins at room temperature for compound diffusion¹⁵. Streptomycin (10 $\mu\text{g}/\text{disc}$) was used as a positive control. These plates were incubated for 24 hrs at 37 °C. Zone of inhibition was recorded in millimetres. The compounds were screened for antibacterial activity against human pathogens such as Gram positive bacterial *Bacillus subtilis* (ATCC 441), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermitis* (MTCC 3615) and Gram negative bacteria *E. coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC15380) using Disc diffusion method.

Result and Discussion:

The results revealed that the activity was considerably affected by various substitutions on the aromatic ring of benzoic acid. For antibacterial activity it was observed that introduction of electron withdrawing group on benzene ring of benzoic acid showed considerable increase in antibacterial potency of the compound. It was also observed that the compound 2'-chloro-4-methoxy-3-nitro- benzoic acid containing chloro and nitro substituent showed significant potency against Gram – positive *Staphylococcus aureus* bacteria (MIC=11 mm). The same substitution showed maximum inhibition against Gram –negative bacteria like *Klebsiella pneumoniae* and *E.coli*. The compound 2, 2'-dichloro benzoic acid having two chloro moieties showed moderate potency against Gram – positive *Staphylococcus epidermitis* and Gram – negative *Klebsiella pneumoniae*. The activity is increased for the compounds 2'-chloro-4-methoxy-3-nitro benzoic acid and 2, 2'-dichloro benzoic acid and it is further enhanced by increasing the concentration of the compound dosage for all pathogens (Table 1). This result clearly reveals that amongst the compound 2, 2'-dichloro benzoic acid and 2'-chloro-4-methoxy-3-nitro benzoic acid the one which contain nitro group at meta position on substituted benzene ring showed the maximum inhibition against Gram positive bacteria due to the presence of another electron withdrawing group chloro at ortho position. The anti bacterial activity for the compound methyl benzoate and 4, 4'-dibromo benzoic acid was not observed. From the above result it was clearly evident that electro withdrawing groups showed maximum potency. The activity of the compounds were found to be dose dependent i.e., 100 µg/mL showed greater inhibition. The susceptibility of the microbes to the compound was compared with standard antibiotic streptomycin.

Table 1: Anti bacterial activity for different substituted benzoic acids

| Name of the Pathogens | Antibacterial activity - Disc diffusion method (µg) | | | | | | | | | Streptomycin (S10) |
|-----------------------------------|---|----|-----|----|----|-----|----|----|-----|--------------------|
| | Zone of Inhibition (mm) | | | | | | | | | |
| | S1, S2 | | | S3 | | | S4 | | | |
| | 25 | 50 | 100 | 25 | 50 | 100 | 25 | 50 | 100 | |
| <i>Bacillus subtilis</i> | - | - | - | - | - | - | - | - | - | 9 |
| <i>Staphylococcus aureus</i> | - | - | - | - | - | - | - | - | 11 | 20 |
| <i>Staphylococcus epidermitis</i> | - | - | - | - | 7 | 8 | - | 8 | 8 | 25 |
| <i>E.coli</i> | - | - | - | - | - | - | - | - | 10 | 20 |
| <i>Klebsiella pneumoniae</i> | - | - | - | - | 8 | 8 | - | 9 | 10 | 21 |



Antibacterial activity of compounds S1- Methyl Benzilate, S2- 4,4'-dibromo benzoic acid, S3- 2,2'-dichloro benzoic acid S4- 2'-chloro-4-methoxy-3-nitro benzoic acid by using disc diffusion method

Conclusion:

All the compounds synthesised were adequately characterised their IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and mass data. All the structures of the above compounds were in good agreement with spectral and analytical data. The synthesis, spectral studies and anti microbial activity of substituted benzoic acid has been reported. The present study revealed that the synthesized compounds having chloro substitution enhances the more antimicrobial activity compared to other compounds. This result will provide great impact on chemist and biochemist for further investigations in this field. Further improvement in the activity can possibly be achieved by slight modification in the ring substituent.

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