Research Article

SYNTHESIS AND EVALUATION OF IODINATED PHENYTOIN FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT

5,5-bis(4'-iodophenyl)imidazolidine-2,4-dione was prepared either by direct iodination of 5,5-diphenyl imidazolidine-2,4-dione (phenytoin) or through the iodination of phenytoin intermediates (benzoin and benzil) and follow-up the standard procedure used for conversion of them to phenytoin. The targeted compound was evaluated and compared with the antimicrobial activity of phenytoin.

Keywords: Phenytoin (5,5-diphenyl imidazolidine-2,4-dione), 5,5-bis(4'-iodophenyl)imidazolidine-2,4-dione, N-3-acetylcarboxy-5,5-bis(4'-iodophenyl)imidazolidine-2,4-dione, antibacterial activity

INTRODUCTION

Phenytoin sodium became available as an anticonvulsant drug and was found to be useful in the treatment of grand mal, partial epilepsy and status epilepticus¹. Phenytoin sodium is effective in the treatment of arrhythmia due to digitalis intoxication it decreases the automaticity and contractility of the heart with slowing of his bundle conduction². In 1958 shapiro found out that oral phenytoin sodium pretreatment improved pain and accelerates healing of surgically created gingival wound in patients with periodontal disease³. However phenytoin sodium was applied topically when used in the healing of burns⁴ diabetic gangrenous ulcer on the wound surfaces, later on in the treatment of leprosy ulcers⁵⁻⁷. Previous work on using phenytoin topically in wound healing revealed less growth of *E. coli*, *S. aureus* and *K. pneumonia* in the treated wound area compared to the control⁴. For this the antibacterial activity of phenytoin sodium was tested in vitro against *Proteus mirabilis*, *Aeromonas hydrophila* and *Klebsiella pneumonia*. The drug proved to be effective antibacterial agent⁸. In view of the above facts and as continuation of our program of identification of new candidates that may be valuable in design and synthesis of new active leads⁹ we report in the present work the synthesis and antimicrobial activity of 5,5-bis(4'-iodophenyl)imidazolidine-2,4-dione (iodinated phenytoin).

MATERIALS AND METHODS

Here we have chosen the disk diffusion method to determine the antimicrobial susceptibility of phenytoin and compare it with the antimicrobial susceptibility of iodinated phenytoin. The disk diffusion susceptibility method is a simple, practical and has been well-standardized. Therefore, it is noted as the method of choice in average laboratories in even advanced countries. The test was performed by preparing absorbent paper discs. Each disc was impregnated in 10 µL of each tested chemical species, then left to dry at room temperature. This step was repeated to ensure full saturation of the paper

disc. Four bacterial strains were used in the experiment; *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis* and *Klebsiella pneumoniae*. Bacterial inoculums of 2×10^8 CFU/ ml were prepared of each of the mentioned species. Each prepared bacterial solution was applied on the surface of a large (150 mm diameter) Muller-Hinton agar plate. Absorbent paper discs of each chemical substance were placed aseptically on the center of each inoculated agar plate. Plates were incubated at 37°C for 24 hours. Then results have been observed on each agar plate for both chemicals.

RESULTS AND DISCUSSION

Due to poor electrophilic strength of iodine, direct iodination of aromatic compound with iodine is difficult and requires the presence of an activated agents in order to produce a strongly electrophilic I^+ species. Several iodination procedures was carried out by using molecular iodine together with a strong oxidizing agent such as nitric acid, sulfuric acid, iodic acid, sulfur trioxide or hydrogen peroxide in order to generate a better electrophile by oxidation of molecular iodine. Synthesis of bis(4-chlorophenyl)-, bis(4-bromophenyl)imidazolidine-2,4-dione were reported¹⁰ having several derivatives located at the N-1 and N-3 position and all of them were in a very low yield. The only iodinated 5,5'-diphenylhydantoin (phenytoin) was reported and marketed as mephentyoin¹¹, the parent molecules was phenytoin which iodinated with N-iodosuccinimide (NIS) in trifluorosulphonic acid in only 17 % yield. Direct iodination of diphenylhydantoin with iodine monochloride ICl was tried in dichloromethane with the existence of hydrogen chloride captured reagent zinc oxide (ZnO), effectively took place to give 70 % yield 5,5-bis(4'-iodophenyl)imidazolidine-2,4-dione. Further attempt was carried out to obtain a better yield for the targeted compound 3, by iodination of the basic intermediates (benzoin or benzil) used in the most straight forward condition for the synthesis of 5,5'-diphenylhydantoin^{10,12}, or phenytoin 1 (Scheme 1).

Scheme 1

Experiment

Chemistry

2-hydroxy-1,2-di(4-iodophenyl)ethanone (1)

1 g of benzoin in RBF 100 ml dissolved in 20 ml of thanol with warming to speed up the reaction. 1.2 g of NaI added to the reaction mixture and stirred until solution become homogenous, place it into ice bath and cool it to 0°C. Add quickly 9.2 ml of 6 % NaOCl and stirring vigorously to completely mix the content. Color changes from clear mixture to dark red brown to lighter shade of yellow till pale yellow the reaction is completed. Leave the reaction settled for 10 minutes, add 10 ml of sodium thiosulfate with stirring, after that the mixture was carefully acidified with 10 % HCl filter wash and dry the ppt.

1,2-bis(4-iodophenyl)ethane-1,2-dione (2)

Method A

By applying the same procedure used for 1, using sodium iodide to afford the in 89 % yield (reported¹⁰ 70 %). Its m.p. was found to be 92°C.

Method B

By applying the standard procedure used for conversion of benzoin to benzyl. Place 2.0 g of benzoin in a 125-ml round bottom flask with 10 ml of glacial acetic acid and 5 ml concentrated nitric acid. Heat the reaction mixture in a water bath at 85-95°C for 15 minutes. Cool the reaction mixture and add 40 ml of water with some ice. The filtered product re crystallized from methanol in 78 % yield.

5,5-bis(4'-iodophenyl)imidazolidine-2,4-dione (3)

Method A: by direct iodination of using iodine monochloride

Phenytoin 0.018 mole was dissolved in 22.5 ml of glacial acetic acid in a 250 ml beaker. To this was added with stirring a solution of 6.2 g (0.038 mole) of iodine monochloride in 16.5 ml of glacial acetic acid; then 72.5 ml of water was added. A yellow precipitate appears. The

reaction mixture was gradually heated with stirring on a hot plate to 80° and kept for 20 minutes. At the end of the reaction the mixture becomes rather difficult to stir because of the voluminous precipitate. After cooling to room temperature, the precipitate was filtered and washed with acetic acid and then with water. The solid 7.5 g was dissolved in 10 ml of warm acetone and filtered. To the filtrate 40 ml of water is slowly added with shaking. The fine, flocculent precipitate is filtered by suction, washed with water, and dried to give 3.

Method B: by applying the standard procedure¹¹ for the preparation of phenytoin to give compound 3 in 60 % yields.

N-3-acetylcarboxy-5,5-bis(4'-iodophenyl)imidazolidine-2,4-dione (4)

0.05 mole of iodinated diphenylhydantoin 3 and 3.3 g of KOH were mixed in 150 ml of ethanol and refluxed for 10 minutes, then an (0.05 mole) of ethyl bromoacetate in 50 ml of water was added and the mixture was stirred at room temperature for 16 h, after the reaction was completed ethanol and water were removed and 50 ml of acetone were added KCl was removed by filtration to give oil solidified on standing to give the targeted compound (91 %) yield. its m.p was found to be 288-291°C (literature¹⁰ 293°C).

Biology

Antimicrobial susceptibility testing

With the introduction of a variety of antimicrobials, it became necessary to perform the antimicrobial susceptibility test as a routine. Appropriate antimicrobial drug use has unquestionable benefit, but physicians and public frequently use these agents inappropriately. Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. As resistance develops to "first-line" antibiotics, therapy with new, broad spectrum, more expensive antibiotics increases, but it might be followed by development of resistance to the new class of drugs¹³.

Antimicrobial susceptibility testing (AST) may be the single most important activity performed in the clinical microbiology laboratory. AST results are often used to dictate specific management for individual patients, to drive empiric antimicrobial therapy, to detect resistance in individual bacterial isolates and to investigate new emerging antimicrobial substances¹⁴. Antimicrobial susceptibility testing methods include broth/ agar dilution method, or automated instrument methods that use commercially marketed materials and devices. Manual methods that provide flexibility and possible cost saving include the disk diffusion method and gradient diffusion method. All methods provide qualitative assessments using the categories susceptible, intermediate, or resistance. Results were observed of disc diffusion method for both chemical species

and for each bacterial strain after 24 hours incubation at 37°C. For the standard phenytoin, results showed no zone of inhibition to any of the four bacterial species. However, for iodinated phenytoin, results showed areas of no bacterial growth for all bacterial species. Diameters of the zone of inhibition of each strain were measured to determine the susceptibility of that strain to iodinated phenytoin (susceptible, intermediate, resistance). The zones of inhibition diameters were recorded as follows; agar plate containing *Staphylococcus aureus* Figure 1A showed a zone of inhibition of 18 – 20 mm. Both *Escherichia coli* and *Klitsiella pneumoniae* Figure 1B and C have showed zone of inhibition of 17 mm each. The zone of inhibition of agar plate containing *Proteus mirabilis* Figure 1D was 11 – 13 mm.

Figure 1: The zone of inhibitions of four chosen bacterial strains by the disc diffusion method

Figure 2a: The disc diffusion method using phenytoin

Figure 2b: The disc diffusion method using iodinated phenytoin

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