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Microwave Synthesis of Iodo Coumarin Derivatives and Antimicrobial Activities of the Products

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Abstract: ***A series of iodo coumarin derivatives have been synthesized under microwave irradiation and the structures of newly synthesized compounds were confirmed on the basis of IR and CO-IR data. The synthesized compounds were tested for their in vitro antimicrobial activity in a disc diffusion technique and streak plate isolation method against four strains of bacteria and three fungal strains. The tested compounds have shown different activity in terms of growth inhibition of microorganism.***

Keywords: Iodo coumarin derivatives, Microwave irradiation, Disc diffusion technique, Streak plate isolation method, antibacterial activity, and antifungal activity.

1. Introduction

Coumarin compounds are a class of lactones structurally constructed by a benzene ring fused to α-pyrone ring, and essentially possess - conjugated system with rich electron and good charge-transport properties. The simplicity and versatility of the coumarin scaffold make it an interesting starting-point for a wide range of applications. There are coumarins as perfumes, cosmetics, and industrial additives. Some of its derivatives have been used as aroma enhancers in tobaccos and certain alcoholic drinks. But their most relevant role is described in natural products, organic chemistry, and medicinal chemistry. Moreover, a lot of coumarin compounds as medicinal candidates for drugs with strong pharmacological activity, low toxicity and side effects, fewer drug resistance, high bioavailability, broad spectrum, better curative effects, etc., to treat various types of diseases are being actively studied Several efforts have been made mainly in developing coumarin-based anticoagulant, antioxidant antimicrobial (anti-viral, antifungal, and anti-parasitic anticancer, anti-diabetic, analgesic, anti-neurodegenerative, and anti-inflammatory agents). A convenient approach to the microwave synthesis of iodo coumarin derivatives is presented here. Due to the importance of iodo-substituted organic compounds as synthos or valuable precursors in organic synthesis, in addition to their use as radioactively-labeled markers in medical diagnosis, the introduction of iodine into organic molecules has received significant attention among the scientific community. Since molecular iodine itself is poorly reactive, substantial efforts have been invested in the development of efficient, selective and mild methods for direct introduction of iodine into organic compounds. Iodo-organic derivatives have been widely used as diagnostic imaging drugs (such as diatrizoate meglumine, diatrizoic acid,iodipamide, iodixanol, iohexol, iomeprol and iopamidol) and asamebicides In view of the important biological properties of the iodo coumarin derivatives medical agents, we planned to synthesize some new iodo coumarin derivatives bearing side chains with different structures, as such derivatives could possess interesting and useful biological properties.

2. Materials and Methods

Solvents and reagents used for the synthesis were of reagent grade and were purified by standard methods. Melting points were determined by using a (Joshibha) Model melting point approach and are uncorrected IR spectra were recorded on Brucker Model. The reaction progress and purity of all prepared compounds was followed by TLC in the system benzene/ethyl acetate and benzene/pet ether visualizing spots with UV lamp and iodine vapour. All microwave reactions were carried out in a microwave oven (IFB Model 179 MIS).Laminar airflow Cabit (Kemi), autoclave (Osworld,"Autoclave Steam Sterilizer" JRIC-39) and incubator (Genuine) were used for microbial activity.

3. Microwave aided synthesis of iodo coumarins

1. Synthesis of iodo 4- hydroxycoumarin (1A)
A mixture of 4-hydroxy coumarin (100mg), zeolite (76 mg), iodine (154mg) and THF (5ml) on irradiation in a microwave oven, changed dark brown after 5 minutes, After 7 minutes, TLC showed a brown coloured spot under iodine. The spot due to reactant vanished completely after 12 minutes. At the end of 12 minutes, the mixture was treated with methanol the product was obtained as a semisolid. The yield and melting point could not be recorded, as the compound was very hygroscopic in nature. CO-IR of reactant and product was recorded.

2. Synthesis of iodo 7-hydroxy-4-methyl coumarin (2A)
A mixture of 7-hydroxy-4-methyl coumarin (100mg), zeolite (76 mg), iodine (154mg) and THF (5ml) on irradiation in a microwave oven, changed dark brown after 5 minutes, After 7 minutes, TLC showed a brown coloured spot under iodine. The spot due to reactant vanished completely after 37 minutes. At the end of 37 minutes, the mixture was treated with methanol the product was obtained as a semisolid. The yield and melting point could not be recorded, as the compound was very hygroscopic in nature. CO-IR of reactant and product was recorded.

3. Synthesis of iodo 4, 7-dihyoxycoumarin (3A)
A mixture of 4, 7-dihyoxycoumarin (100mg), zeolite (76 mg), iodine (154mg) and THF (5ml) on irradiation in a microwave oven, changed dark brown after 5 minutes, After 7 minutes, TLC showed a brown coloured spot under iodine. The spot due to reactant vanished completely after 13minutes. At the end of 13 minutes, the mixture was treated with methanol the product was obtained as a semisolid. The yield and melting point could not be recorded, as the compound was very hygroscopic in nature. CO-IR of reactant and product was recorded.

4. Synthesis of iodo 4-methyl-7, 8-dihydroxycoumarin (4A)
A mixture of 4-methyl-7, 8-dihydroxycoumarin (100mg), zeolite (76 mg), iodine (154mg) and THF (5ml) on irradiation in a microwave oven, changed dark brown after 5 minutes, After 7 minutes, TLC showed a brown coloured spot under iodine. The spot due to reactant vanished completely after 50minutes. At the end of 50 minutes, the mixture was treated with methanol the product was obtained as a semisolid. The yield and melting point could not be recorded, as the compound was very hygroscopic in nature. CO-IR of reactant and product was recorded.

5. Synthesis of iodo 4, 7-dimethylcoumarin (5A)
A mixture of 4, 7-dimethylcoumarin (100mg), zeolite (76 mg), iodine (154mg) and THF (5ml) on irradiation in a microwave oven, changed dark brown after 5 minutes, After 7 minutes, TLC showed a brown coloured spot under iodine. The spot due to reactant vanished completely after 45minutes. At the end of 45 minutes, the mixture was treated with methanol the product was obtained as a semisolid. The yield and melting point could not be recorded, as the compound was very hygroscopic in nature. CO-IR of reactant and product was recorded.

6. Synthesis of iodo 7-hydroxycoumarin (6A)
A mixture of 7-hydroxycoumarin (100mg), zeolite (76 mg), iodine (154mg) and THF (5ml) on irradiation in a microwave oven, changed dark brown after 5 minutes, After 7 minutes, TLC showed a brown coloured spot under iodine. The spot due to reactant vanished completely after 18 minutes. At the end of 18 minutes, the mixture was treated with methanol the product was obtained as a semisolid. The yield and melting point could not be recorded, as the compound was very hygroscopic in nature. CO-IR of reactant and product was recorded.

4. Antimicrobial Activity of Compounds

The synthesized iodo coumarin derivatives were tested for their antifungal and antibacterial activity and activity was compared.

5. Preparation of Culture Media for Antibacterial Antifungal Studies

Preparation of Nutrient agar medium
Three grams of beef extract, 50g of peptone of NaCl and 15g of agar were taken in a beaker and then distilled water (1000ml) was added. The mixture was boiled and mixed thoroughly with a glass rod. After complete dissolution of agar the medium it was dispensed into several conical flask of 250ml volume. The conical flasks were closed with cotton plug and rapped with aluminum foil. It was there auto claved for 15 minutes at 121˚C and 15 psi. After autoclaving, the medicine was used for culturing different micro organism.

Preparation of Sabouard Dextrose Agar. (SDA medium)
65g of SDA were suspended in 100ml distilled water. It was heated to boiling to dissolve the medium completely and the sterilized by autoclaving at 15 ibs, pressure (121°C) for 15min.

6. Antimicrobial Testing

Disc method for determination of zone of inhibition of antibacterial
Paper discs of 4mm in diameter and glass Petri plates of 90mm in diameter were used throughout the experiment. Paper discs were sterilized in an autoclave and dried at 100°C in oven. Then the disc was soaked with test chemicals at the rate of 50g per disc for antibacterial analysis. One drop of bacterial suspension was taken in sterile Petri dish and then approximately 20ml of sterilized and melted NA (~45°C) was poured into the plate, and then mixed thoroughly. The paper discs after soaking with test chemicals were placed at the center of the inoculated pour plate. A control plate was also maintained in each case with alcohol. Firstly, the plates were kept for 4hrs at low temperature (4°C) the test chemicals diffused from disc to the surrounding medium by this time. The plates were then incubated at (35± 2) °C for growth of test organisms and were observed at 24 hours intervals for two days. The activity was expressed in terms of inhibition zone diameter in mm. Each experiment was repeated three times. The standard antibiotic, streptomycin and gentamycin was used as a positive control and compared with test chemicals under identical condition. The antimicrobial activities of the compounds were recorded.

Streak plate isolation method for determination of zone of inhibition of antifungal activity
The required amount of SDA medium was taken in a conical flask separately and was sterilized in an autoclave (at120c and 15psi) for 15min.A tube of SDA was liquefied and poured into a petridish.The plate was rotated gently for uniform distribution of the medium. The inoculating loop was held at a 60°C angle in the hottest part of the Bunsen burner flame. The entire tube was heated to redness. The loop was allowed to cool for 15 to 20 seconds before it touched the culture. A small amount of the culture was removed from the tube with the sterilized inoculating loop and the microorganisms were streaked in the plate. The stock solutions were prepared by dissolving the compounds in ethanol. Inoculation process was done under aseptic condition and the spores were inoculated in the medium and incubated for 5 days. A clear zone or ring was present on SDA plate .The diameters of the zone are measured.

The compounds synthesized will be designated as 1A, 2A, 3A, 4A, 5A, 6A, for the purpose of easy reference.

7. Result

For the study 6 derivatives of iodo coumarins have been prepared and characterized by their IR spectra. Their antimicrobial activities have been assessed.

It has been found that 4-hydroxy coumarin, 7-hydroxy coumarin, 4, 7-dihydroxy coumarin got iodinated in 12- 15 minutes under microwave condition. The yield was so good. The 7-hydroxy-4 methyl coumarin, 4, 7-dimethylcoumarin, 4-methyl-7, 8-dihydroxycoumarin did not form iodo derivatives even after 45 minutes of microwave heating. This may be due to the bulky groups present in the reactant molecule. An Iodo compound was very hygroscopic in nature. So, the yield and the melting point could not be detected.

The IR spectrum of all the iodo coumarins suggested the presence of coumarin ring .Pending confirmation of the exact position of the iodo substituent it can be probably suggested that the iodo substituent may be present in the 6 or 8 position of the ring of coumarin moiety.

Anti bacterial screening was done for the various iodo coumarins against *Escherichia-coli, Staphylococcus aereus, Klebsiella pneumonia, Pseudomonas aeruginosa*.It was found from the result presented in table-1, that all the compounds exhibited good activity against all the species. That the compounds 1A, 2A, 3A, 4A, 5A, 6A can be proposed to be highly sensitive against *Escherichia-coli*. These compounds were found to inhibit more or less equally when compared with the standard drug streptomycin, which gave 7mm zone of inhibition. The inhibition of efficiency of compounds 1A, 2A, 3A, 4A, 5A, 6A was high against *Staphylococcus aereus.* These compounds were found to inhibit more or less equally when compared with the standard drug streptomycin, which gave 8 mm zone of inhibition. The compounds 1A, 2A, 3A, 4A, 5A, 6A showed better inhibition against this *Klebsiella pneumonia.* These compounds were found to inhibit more or less equally when compared with the standard drug streptomycin, which gave 11 mm zone of inhibition. The compounds 1A, 2A, 3A, 4A, 5A, 6A exhibited good sensitivity against *Pseudomonas aeruginosa*. These compounds were found to inhibit more or less equally when compared with the standard drug streptomycin, which gave 8 mm zone of inhibition.

The synthesized compounds were screened for their antifungal activity against *Candida albicans, Mucor hiemalis, Aspergillus flavus.*From the screening result presented in table -1 it can observed that all the compounds were sensitive to the fungi (*Candida albicans, Mucor hiemalis, Aspergillus flavus).*These compounds were found to inhibit more or less equally when compared to the control drug flucanozole with zone of inhibition (7mm, 8mm, 12mm).

Table 1: Zone of inhibition for antibacterial and antifungal activity studies of compounds

BACTERIA	Compounds						Control
	Zone of inhibition(mm)						
	1A	2A	3A	4A	5A	6A	Streptomycin (mm)
Escherichia-coli	8	6	7	7	7	8	7
Staphylococcus aereus	7	8	8	7	7	7	8
Klebsiella pneumoniae	7	9	6	6	8	7	11
Pseudomonas aeruginosa	7	5	7	8	9	8	8
FUNGUS	Flucanozole (mm)						
Candida albicans	5	6	6	6	6	6	7
Mucor hiemalis	4	4	6	7	6	6	8
Aspergillus flavus	6	9	7	6	6	7	12

8. Conclusion

An assessment of the antimicrobial activities of the iodo coumarin derivatives revealed exhibited maximum activity against all the bacterial and fungal species taken up for the study. This observation could be useful in carrying out further studies on the iodo derivatives particularly in clinical trials against various infections.

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