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Design Synthesis Characterization Of Some Novel Chromane Based Analogues And Its Antimicrobial Evaluation

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Abstract

Because of its diverse variety of pharmacological effects, chromatane (4H-benzopyran) is a privileged scaffold in medicinal chemistry. In order to increase antibacterial activity, new chromane-based analogues were created and synthesized for this investigation. The produced compounds' antibacterial ability was assessed against a range of bacterial and fungal strains, and their structural characteristics were assessed through the use of spectroscopic techniques. Significant antibacterial activity was demonstrated by a number of compounds, suggesting that they could be excellent leads for more research. Numerous new compounds based on chromane were created and demonstrated encouraging antibacterial properties. Changes in structure have a major impact on potency. These results serve as a foundation for additional chromane analogue research and optimization as possible antibacterial agents.

Keyword: *Chromane, Coumarin, antimicrobial, synthesis and characterization*

INTRODUCTION

Public health is seriously threatened by the global increase in antimicrobial resistance (AMR), which makes the urgent search and development of novel treatment agents with enhanced efficacy and safety profiles necessary [1]. Because resistant bacteria strains are evolving so quickly, traditional antibiotics are losing their effectiveness. In order to meet this increasing challenge, medicinal chemistry has made the investigation of novel chemical scaffolds and molecular frameworks a primary priority [2, 3]. A unique class of heterocyclic compounds, chromane (4H-benzo[b]pyran) and its derivatives are recognized for a variety of biological actions, such as antibacterial, antioxidant, anti-inflammatory, anticancer, antidiabetic, and neuroprotective properties [4, 5]. The chromane scaffold's structural adaptability makes it a desirable template for the creation of new bioactive compounds because it permits a variety of chemical alterations [6]. The chromane core's biological significance is further shown by the fact that it is found in a number of naturally occurring flavonoids and tocopherols (derivatives of vitamin E) [7, 8].

Numerous research conducted over the past few decades have demonstrated the potential of compounds based on chromane as strong antibacterial agents [9]. The biological activity of these compounds has been demonstrated to be considerably impacted by strategic substitutions at several locations on the chromane ring, including C-2, C-3, C-6, and C-7 [10]. It has been very successful to enhance antibacterial characteristics by incorporating pharmacophores including sulfonamides, triazoles, amides, and heterocyclic moieties into the chromane framework [11, 12].

The present study aims to design and synthesize a novel series of chromane-based analogues by structural modifications to improve antibacterial activity [13, 14]. NMR, FT-IR, and mass spectrometry were employed as spectroscopic and analytical methods to characterize and confirm the structures of the produced compounds. The antibacterial efficacy of the compounds was assessed by standard in vitro assays against several bacterial strains, encompassing both Gram-positive bacteria and Gram-negative bacteria, in addition to fungal species [15]. This study's findings aim to aid in the creation of new antimicrobial medicines that can address resistant microbial diseases and offer valuable insights into the structure-activity correlations (SAR) of chromane derivatives [16].

MATERIAL AND METHODS:

MATERIAL:

The chemicals used for this study were purchased from nearby vendors. The compounds infrared spectra were noted. Compounds' 1H NMR is recorded, and the Agar diffusion method is used to evaluate produced compounds. There are three distinct forms of agar diffusion techniques: the Agar cup method, the Paper disc method, and the Agar ditch method. This experiment employed the Agar cup method. The reference medicine employed was tobramycin.

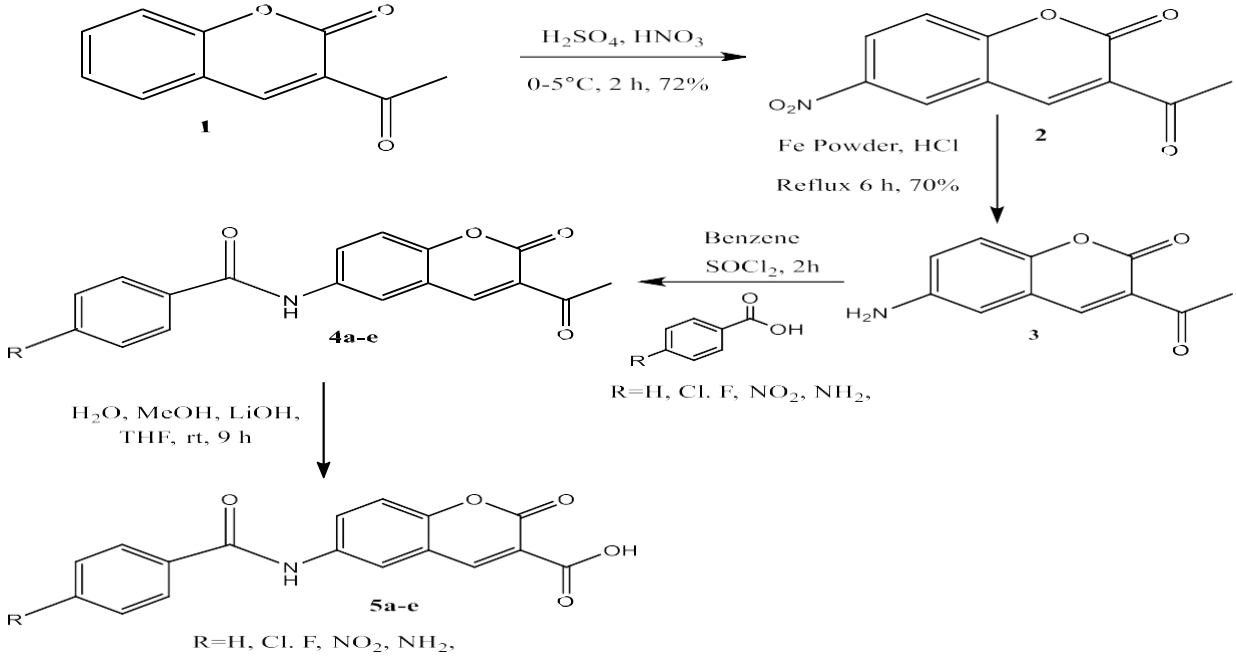
Step 1: Compound 2: Synthesis of 3-acetyl-6-nitro coumarin

A solution of 3-acetyl coumarin (0.188 g) was formulated in concentrated sulfuric acid (1.10 mL) and subsequently cooled to 0 °C. The amalgamation was agitated for 15 minutes under these parameters. A nitrating mixture, consisting of 0.2 mL of concentrated sulfuric acid and 0.06 mL of nitric acid, was thereafter added gradually while preserving the temperature between 0 and 5 °C. The reaction was permitted to continue with constant stirring for 2 hours at 5 °C. Following completion, the mixture was transferred into 25 mL of crushed ice water. The resultant yellow precipitate was obtained using filtration, rinsed with cold water, and air-dried. Th

e crude product was purified using silica gel column chromatography employing a 1:1 (v/v) combination of petroleum ether and benzene as the mobile phase, resulting in a 79% yield of 3-acetyl-6-nitro coumarin (Compound 2) [17, 18].

Step 2: Compound 3: Synthesis of 3-acetyl-6-amino coumarin

A refluxing combination of compound 2 (0.233 g), fine iron powder (0.296 g), concentrated hydrochloric acid (1.11 mL), and ethanol (10 mL) was prepared. The effect was sustained under reflux for six hours. Upon cooling to ambient temperature, the resultant solid was subjected to filtration, extensively rinsed with water, and subsequently dried. The crude product underwent recrystallization from ethanol, yielding pure 3-acetyl-6-amino coumarin (Compound 3) with a 72% yield [19, 20].



Scheme 1: Synthesis chromane derivatives

Step 3: Compounds 4a–e: Synthesis of Coumarin–Benzoic Acid Adducts

General Method for the Synthesis of Coumarin-Benzamide Derivatives

Add thionyl chloride (0.141 g) and benzene (1.5 mL) to a stirred mixture of 3-acetyl-6-aminocoumarin

(0.203 g) and substituted benzoic acid. The reaction mixture refluxed for two hours. After completion, the reaction mixture was placed in 25 mL crushed ice water. The precipitate was separated, filtered, dissolved in 1.25 mL 2N NaOH, and filtered again. After acidification with 2N HCl, the crude product was filtered via a Büchner funnel, washed with additional cold water, and concentrated under decreased pressure. The benzamide derivative was obtained by recrystallizing the crude solid from ethanol [21, 22].

Step 4: Compounds 5a–e: Hydrolysis of Coumarin–Benzamide Derivatives

General Procedure for the Synthesis of Coumarin-3-Carboxylic Acids

Each coumarin–benzamide derivative (4a–e) was solubilized in 1.5 mL of water, methanol, and THF. After adding $LiOH.H_2O$ the reaction mixture was stirred at room temperature for 6 hours. After completion, the reaction mixture was acidified with 3M HCl to 2.0 pH and extracted with ethyl acetate. First, the organic layer was dehydrated with anhydrous Na_2SO_4, then filtered and concentrated under reduced pressure. To obtain the carboxylic acid derivative, silica gel column chromatography was used to purify the residue in hexane and ethyl acetate (8:2 v/v) [23, 24].

Evaluation of Antimicrobial Activity of synthesized compounds:

Selection of Bacteria:

The bacteria were chosen for an in vitro investigation.

Gram-positive bacteria	*Staphylococcus aureus*
	Bacillus subtilis
Gram-negative bacteria	*Escherichia coli*
	Pseudomonas aeruginosa

Preparing the culture medium:

Agar (2.0 gm), peptone (1.0 gm), sodium chloride (0.5 gm), and meat extracts (0.3 gm) are all utilized in the process of making the culture medium. Together, these components make up the culture medium. Once all of the components, with the exception of agar, had been dissolved in one hundred milliliters of distilled water and the pH of the medium had been adjusted to 7.6, agar was added to it and dispensed in 25-milliliter portions in test tubes that were maintained in separate locations from one another. Following the completion of a twenty-minute sterilization process at a temperature of 121.5 degrees Celsius, the test tubes were then plugged with cotton wool [25-30].

Testing for antibacterial susceptibility involved inoculating nutrient agar broth with 0.5 ml of 24 h old culture medium, stirring the mixture thoroughly, and then putting it onto a sterilized Petri plate (25 ml each). After letting the poured mixture settle for a while, a sterile cup borer was used to pierce the agar surface to create the "cups." A previously sanitized micropipette was used to add the test solution into these "cups." The plates were noticed. All of the synthetic compounds' antibacterial activity was examined in vitro at a concentration of 1000 ppm, and the results are displayed in Tables 1 and 2 as a percentage (%) of inhibition [26, 29-34].

After a period of five days, the percentage of inhibition for bacteria was determined by applying the formula that was provided.

$$\textbf{Percentage of Inhibition} = 100\,[1 - X/Y]$$

Where,

X = Area of colony in control plate.

Y = Area of colony in test plate.

The percentage (%) data are shown in table 1 and 2 as follows;

(+) = Small clearing zone (<50%), slightly active,

(++) = Medium clearing zone (51-55%), moderately active,

(+++) = Large clearing zone (56-60%), highly active and,

(++++) = Very large clearing zone (>60%), very high activity.

RESULT AND DISCUSSION:

Mass spectrometry (MS), 1H NMR, and infrared spectroscopy were used to confirm the synthesis of coumarin-based derivatives. The suggested structures and the spectral data agreed. All of the produced

compounds' structures are coherently confirmed by the IR, 1H NMR, and MS data. Successful substitutions at the 6-position and modifications at the 3-position of the coumarin core are supported by distinctive signals for functional groups (acetyl, amide, nitro, amino, halogens, and carboxylic acid). The success of the synthetic process and the impact of substituents on spectral attributes are both demonstrated by the spectral consistency throughout each series.

Evaluation of Antimicrobial Activity of Synthesized compounds:

The compounds tested for antibacterial activity show the percentage (%) of the zone of inhibition of bacterial growth of both Gram-positive and Gram-negative bacteria. The antibacterial qualities of four compounds from Series 1 (4a–e) and four from Series 2 (5a–e) were tested against Gram-positive bacterial strains *B. subtilis* and *S. aureus*, as well as Gram-negative bacterial strains *P. aeruginosa* and *E. Coli*. Compound 4d in Series 1 demonstrated promising activity against the E. coli and the S. aureus bacterial strains. Compound 5d in Series 2 shown promising efficacy against *B. subtilis* and *P. aeruginosa* bacterial strains.

Table 1: % Zone of Inhibition synthesized Compounds 4a-e

Compounds	Zone of Inhibition			
	Gram Positive Bacteria		Gram Negative Bacteria	
	Staphylococcus Aureus	***Bacillus Subtilis***	***E Coli***	***Pseudomonas aeruginosa***
4a	++	+	++	+
4b	+	++	++	++
4c	+	+++	++	+++
4d	+++	++	+++	++
4e	++	++	++	++
Tobramycin	+++	++++	++++	++++

Table 2: % Zone of Inhibition synthesized Compounds 5a-e

Compounds	Zone of Inhibition			
	Gram Positive		Gram Negative	
	Staphylococcus Aureus	***Bacillus subtilis***	***E Coli***	***Pseudomonas aeruginosa***
5a	++	++	++	++
5b	+	+	++	++
5c	++++	++	++++	+++
5d	+++	++++	+++	++++
5e	+++	++	++	++
Tobramycin	+++	++++	++++	++++

CONCLUSION:

All of the structures of the compounds that were synthesized have been confirmed in a consistent manner by the IR, 1H NMR, and MS investigations. Distinct signals for functional groups (acetyl, amide, nitro, amino, halogens, and carboxylic acid) provide support for successful substitutions at the 6-position and modifications at the 3-position of the coumarin core. These signals are necessary for the successful substitutions and modifications. The spectral consistency that is present throughout each series is evidence of both the efficacy of the synthetic process and the influence that substituents have on the spectral characteristics of the compounds. N-(3-acetyl-coumarin-6-yl) is a formula. Despite the fact that -4-nitrobenzamide, which is the molecule 6-(4-nitrobenzamido)-2-oxo-2H-chromene-3-carboxylic acid, demonstrates the strongest antibacterial activity, additional research is necessary to discover the chemical mechanism.

DECLARATIONS:

Consent for publication:
All the authors approved the manuscript for publication.
Competing interests:
All authors declare no competing interests.
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