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A STUDY OF SYNTHESIS AND CHARACTERIZATION OF COUMARIN ANALOGS: EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES

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ABSTRACT
Due to their wide range of biological activity, heterocyclic motifs have been extensively used in the drug discovery and development process. In particular, the pharmacological and pharmaceutical communities have focused on coumarins and their derivatives for their wide variety of therapeutic effects. Antibacterial, antifungal, antiinflammatory, antioxidant, anticancer, and anti-HIV properties have been documented for a variety of coumarin analogs. Food, fragrance, cosmetics, drugs, and optical brighteners all make use of coumarins in some capacity. Researchers have recently shown that changing the structure of compounds containing a coumarin moiety results in molecules with variable quantitative and qualitative biological activity. There is a close relationship between the kind and location of the structural substituents and the pharmacological, biochemical, and therapeutic activities of coumarin derivatives. For instance, the lipophilicity has been improved by adding halogens, especially bromo, fluoro, and fluoroalkyl substituents in the coumarin moiety. Furthermore, lipophilicity is a crucial physicochemical characteristic in antibacterial drug development since it is directly connected to the permeability through a lipid coat of bacteria. The antibacterial potential was also shown to be affected by the amount and location of substitutions in the coumarin ring.

KEYWORDS: Coumarin Analogs, Antimicrobial, Antioxidant Activities, wide range, biological activity

Introduction

The research of novel antibacterial drugs is gaining momentum in response to the emergence of bacterial strains that are resistant to many existing therapies. Inactivate, exclude, or prevent the inhibitory or deadly mechanism of antimicrobial drugs; this is what we mean when we talk about microorganisms that have evolved antimicrobial resistance. It is still a huge difficulty for scientists to find novel structural leads that might be useful in developing new, effective, and widely effective antimicrobial drugs. In light of these results, coumarin analogs with a 4-fluoro phenyl oxadiazole moiety were recently identified as a potent antibacterial agent. Antioxidants may prevent the oxidative stress generated by free radicals from damaging the cellular organelles. Hydrogen peroxide, the superoxide anion, and the hydroxyl radical are all examples of free radicals. Exogenous chemicals, stress, and the modern food system all contribute to the production of highly reactive free radicals. These free radicals may oxidize biomolecules, leading to cancer, heart disease, and high blood pressure. Catalase, superoxide dismutase, and the peroxidaseglutathione system are examples of endogenous defense mechanisms that typically scavenge most of the free radicals produced during metabolism. As a result of these findings,

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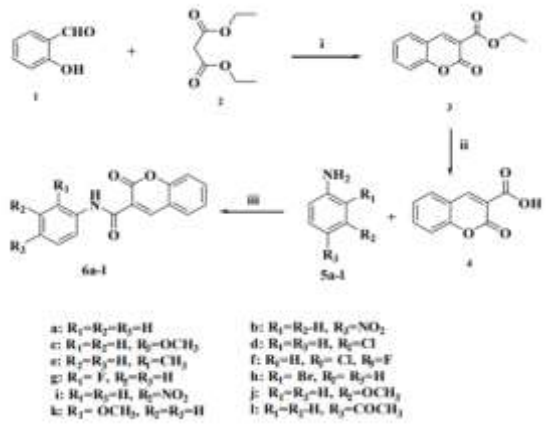
several hetrocyclic compounds have been created for use as antioxidants. Examples of effective antioxidants are coumarin analogs with 4-methyl phenyl moieties. We prepared new phenylamide coumarin analogs bearing different substituent and anticipated to possess potent antimicrobial and antioxidant activities because of the high level of bio activity displayed by coumarin heterocyclic analogs and because of our ongoing work on heterocycles having potent biological activities.

Chemistry: Plan of the synthesis

The compounds 6a-l of the title were synthesized using the synthetic process indicated in scheme 1. Salicylaldehydes (1) and diethyl malonate (2) were reacted in a knoevenagel condensation to produce ethyl 2-oxo-2H-chromene-3-carboxylates (3). The hydrolysis of component 3 produced coumarin-3-carboxylic acid (4). Compound 4 was able to be converted into the desired end compounds 6a-l when it was treated with substituted anilines (5a-l) in the presence of lutidine and o-(benzotriazol1-yl)- N,N,N',N'-tetramethyl aminium tetrafluoroborate (TBTU) as a coupling agent. Elements and spectra validated the identity of all produced substances.



Scheme 1: Synthesis of 2-oxo-2H-chromene-3-carboxylic acid phenylamide analogs

(i). Piperidine/Ethanol, Reflux; (ii). Aq.NaOH/Ethanol, Reflux; (iii). TBTU/Lutidine, dry DCM, Stirring.

Synthesis of 2-oxo-2H-chromene-3-carboxylic acid phenylamide analogs (6a-l):

Compound 4 (0.0037 mol) was added to dry dichloromethane (15 mL) containing lutidine (1.2 vol.), and then amino benzene (5a, 0.0037 mol) was added, all while stirring at 25-30 °C for 30 minutes. The reaction mixture was cooled to a temperature of 5 degrees Celsius or below, and TBTU (0.0037 mol) was added over a period of 30 minutes. The reaction was stirred overnight while TLC with a chloroform:methanol (9:1) eluent observed the progress of the reaction. After adding 20 mL of dichloromethane and three 20 mL washes of 1.5 N hydrochloric acid, the reaction mixture was ready for use. Good yields of 2-oxo-2Hchromene-3-carboxylic acid phenylamide (6a) were obtained by washing the organic layer with water (3 × 25 mL), drying it over anhydrous sodium sulphate, concentrating it to a syrupy liquid, and recrystallizing it twice from diethyl ether. Compounds 4 and 5b-l served as the building blocks for the synthesis of compounds 6b-d.

Biology

Antimicrobial activity

Disc diffusion and minimum inhibitory concentration (MIC) tests were used to evaluate the antibacterial activity of compounds 6a-l. Antibiotic ampicillin from Sigma (Mumbai) and antifungal ketoconazole from Himedia (Mumbai) were used as positive controls.

Tested microbes

Streptococcus pyogenes (SP), Staphylococcus aureus (SA), and Bacillus subtilis (BS) were the gram-positive

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bacteria employed in the studies. Salmonella typhimurium (ST), Klebsiella pneumonia (KP), and Escherichia coli (EC) were some of the gramnegative bacteria found. Aspergillus niger (AN), Aspergillus flavus (AF), and Candida albicans (CA) were also employed in the tests. All cultures were procured from the Manasagangotri, Mysuru, and Department of Microbiology.

Preparation of inoculums

After cultivating bacteria for 24 hours in Mueller Hinton Broth (MHA, Himedia), inoculums were made and incubated at 37 degrees Celsius. Sterile MHB was used to dilute the cell suspensions to a concentration of around 104 CFU/mL. After incubating the filamentous fungus at 28 degrees Celsius for 10 days, the spores were collected in sterile double-distilled water and then homogenized.

Disc diffusion assay

The disc diffusion technique was used to test for antibacterial activity. Sterile MHA in the volume of 20 mL was poured onto petri dishes. After allowing the solidified medium to cure for 10 minutes, the test cultures were swabbed and placed on top. The discs were spun at 1000 g for the testing. Compound diffusion occurred over the course of 30 minutes at room temperature after the loaded discs were put on the surface of the medium. The relevant solvent was used to make a negative control. As a positive control, we employed a combination of ampicillin and ketoconazole (10 g/disc). Incubation times for the bacterial and fungal plates were 24 hours at 37 ^{0} C and 48 hours at 28 ^{0} C, respectively. The experiment was conducted three times, each time recording the size of the inhibitory zone in millimeters.

Minimum Inhibitory Concentration (MIC)

Compounds 6a-l were synthesized, and their minimal inhibitory concentrations (MICs) were determined using the serial dilution technique, which is the gold standard for testing against bacteria and filamentous fungus. Each well contributed 100 L of inoculum. As positive controls, we tested with antifungal drugs like ketoconazole and fluconazole, and antibacterial drugs like streptomycin and ciprofloxacin. The plates were incubated for 48-72 hours at 28 degrees Celsius for the fungus, and for 24 hours at 37 degrees Celsius for the bacteria. Fungi's minimal inhibitory concentration (MIC) was determined as the lowest extract concentration at which no fungal growth was seen during incubation. In addition, sterile MHA plates for bacteria were used to incubate 5 ml of testing broth at the appropriate temperatures. The minimal inhibitory concentration (MIC) of a chemical against bacteria was calculated as the lowest concentration at which visible growth of test cultures was inhibited on an agar plate.

Antioxidant activity

Compounds 6a-l were evaluated for their ability to scavenge the free radicals 1-diphenyl-2-picryl-hydrazil (DPPH), nitric oxide (NO), and hydrogen peroxide (H2O2), with ascorbic acid serving as the reference compound.

DPPH radical scavenging activity

Bleaching of the DPPH solution in methanol, which is purple in hue, served as a proxy for the compounds' hydrogen-donating or electron-donating capacities. The DPPH stable radical is used as a reagent in the spectrophotometric test. In this test, a 0.004% (w/v) methanol solution

of DPPH was mixed with 4 mL of test compounds 6al at concentrations of 25, 50, 75, 100, and 100 g/mL. The absorbance was measured at 517 nm against a blank after a 30-minute incubation at room temperature. Percentage of scavenging = [(A control _ A sample)/(A blank) x 100] was used to determine the extent to which DPPH free radical generation was inhibited (I%). In Equation (1), A control is the absorbance of the control reaction (which includes all reagents except the test chemical), and A sample is the absorbance of the sample. There were three sets of tests done.

NO scavenging activity

Using somewhat altered methodologies, we found ZERO scavenging activity. Sodium nitroprusside produced NO radicals. The test compounds 6a-l were added to 1 mL of sodium nitroprusside (10 mmol) and 1.5 mL of phosphate buffer saline (0.2 M, pH 7.4) and incubated for 2 hours and thirty minutes at 25 degrees Celsius before being treated with 1 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% naphthylethylenediamine dihydrochloride). The chromatophore's absorbance was checked at 546 nm. Using equation (1), we found that there was ZERO scavenging activity.

H_2O_2 scavenging activity

According to the described approach [40], the H2O2 scavenging activity of the test chemical was calculated. H2O2 was dissolved in phosphate buffer (pH 7.4) to create a 40 mM solution. Next, a 0.6 mL, 40 mM H2O2 solution was added to 3.4 mL of phosphate buffer containing 25, 50, 75, and 100 g/ml of test compounds 6a-l. The reaction mixture's absorbance was measured to be 230 nm. Using equation (1), we may determine what proportion of H2O2 is scavenging.

CONCLUSION

New benzophenone-thiazole analogues (10a-n) were created by chemical synthesis. drug 10h, which has methyl, fluoro, and methoxy groups connected to phenoxy, benzoyl, and the phenyl ring of thiazole, respectively, emerged as the lead drug within this series (10a-n) due to its potential antiproliferative action against two cell lines of distinct origin. In addition, ELISA and immunoblot analysis confirmed that compound 10h interacted with VEGF-A production, which may explain why this molecule had a suppressive effect on the ascites tumor. The research showed that compound 10h has the ability to inhibit VEGF-A, making it a strong tumour inhibitory. Synthesis was performed on a number of new coumarin analogs, numbered 12a through 13a, and 13a through 13f. In this series, the compound 13f with a methoxy group at the para position on the phenyl ring of thiazole, as well as another methoxy group at seventh position in coumarin ring, has shown a promising antiproliferative effect against two different cell lines of different origin in an MTT, tryphan blue, and LDH leak assay with 5-fluorouracil as the standard. As a result, it has emerged as the lead compound within this series. In addition, the anticancer properties of compound 13f were tested against the Ehrlich ascites tumor using BrdU incorporation, TUNEL, FACS, western blot, and DNA fragmentation assays, in addition to docking studies. All of these tests demonstrated that compound 13f has anticancer properties. Compound 13f has been found, according to the findings of several scientific experiments, to be

capable of inducing apoptotic cell death in cancer cells by the process of destroying the genomic DNA of these cells, consequently reducing the incidence of ascitic tumor formation in mice.

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