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Synthesis and Antimicrobial Activity of some new Benzocoumarin Amino Acid Derivative

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ABSTRACT

A series of new benzocoumarin amino acid derivatives were synthesized with the aim of evaluating their antimicrobial activity. Their chemical structures were confirmed by ^{1}H-NMR and mass spectrometry. The antimicrobial activity was screened on two types of bacteria *S. aureus* and *E. coli* besides, two types of fungi *Alternari brassicicola* and *Fusarium oxysporum.* Out of the eleven tested compounds, two derivatives benzo [5,6] coumarin-3-carboxylic acid 1 and Benzo [5,6] coumarine-3-carboxyl DL-threonine methyl ester 4c exhibited good activity at 1000 ppm against *Gram*-positive bacteria *S. aureus* especially compound 1 which has an activity greater than that of the reference drug (Gentamicin ®).
Keywords: Amino acids, Antibacterial activity, Benzocoumarin.

INTRODUCTION

The synthesis of Coumarins and their derivatives has attracted considerable attention from organic and medicinal chemists for many years as a large number of natural products contain this heterocyclic nucleus (Monga, 2012). They are widely used as additives in food, perfumes, cosmetics, pharmaceuticals, optical brighteners, dispersed fluorescent and laser dyes (Kennedy and Thrones, 1997). These observations promoted us to undertake systematic study of the coupling of [5,6]benzocoumarin-3-carboxylic acid with various amino acids. The synthetic routes of the prepared derivatives are shown in schemes **1**. The structures of the newly synthesized derivatives were elucidated by ^{1}H-NMR and in some cases by mass spectrometry.

MATERIAL AND METHODS

Materials and reagents

The boiling point range of the petroleum ether used was 40-60 ^{o}C. Thin layer chromatography (TLC) was carried out on silica gel 60 F_{254} plastic plates (E. Merck, layer thickness 0.2 mm) in the following solvent systems, S_1: petroleum ether/ethyl acetate (3 : 1); S_2: petroleum ether/ethyl acetate (1 : 1); S_3: petroleum ether/ethyl acetate (1 : 2); S_4: methanol/chloroform (1:10), S_5: n-hexane/ethyl acetate (15:1). The spots on thin layer plates were detected by UV lamp. Melting points were determined on a Buchi 510 melting-point apparatus and the values are uncorrected. ^{1}H-NMR spectra were measured on Bruker (300 MHz) and TMS was used as internal standard and Mass spectra were measured on a GC-MSQP 1000EX Schimadzu at microanalytical laboratory, Cairo University, Cairo, Egypt.

Chemical method

Benzo[5,6]coumarine-3-carboxyl β-alanine methyl ester (2)

Yellow crystals (0.95 g, 67%), R_f = 0.49(S_2), m.p. 170 ^{o}C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 9.68 (s, 1H, CH pyranone ring), 9.18-9.22 (brs, 1H, NH), 8.51-7.50 (m, 6H, ArH), 8.14-7.50 (m, 5H, ArH) , 3.81-3.77 (m, 2H, $NHCH_2$), 3.75 (s, 3H, OCH_3), 2.73-2.69 (m, 2H, CH_2CO). m/z 325.

Benzo[5,6]coumarine-3-carboxyl DL-phenylalanine methyl ester (4a)

Faint yellow crystals (1.4 g, 80%), R_f = 0.39(S_1), m.p. 94^{o}C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 9.62 (s, 1H, CH pyranone ring), 9.30 (d, 1H, *J* = 7.2 Hz, NH), 8.41 (d, 1H, *J* = 8.4 Hz, ArH), 8.12 (d, 1H, *J* = 9.0 Hz, ArH), 7.95 (d, 1H, *J* = 8.1 Hz, ArH), 7.75-7.64 (m, 2H, ArH), 7.51 (d, 1H, *J* = 9.0 Hz, ArH), 7.32-7.25 (m, 5H, ArH), 5.04-5.02 (m, 1H, NCH), 3.77 (s, 3H, OCH_3), 3.29-3.21 (m, 2H, CH_2-ph).

Benzo[5,6]coumarine-3-carboxyl L-methionine methyl ester (4b)

Brown crystals (1.09 g, 65%), R_f = 0. 35(S_1), m.p. 118-121^{o}C . ^{1}H-NMR (300 MHz,$CDCl_3$): δ = 9.68 (s, 1H, CH pyranone ring), 9.36 (d, 1H, NH), 8.44 (d ,1H, *J* = 8.4 Hz, ArH), 8.15 (d, 1H, *J* = 9.0 Hz, ArH), 7.94-7.64 (m, 3H, ArH), 7.54 (d, 1H, *J* = 9.0 Hz, ArH), 5.10-4.91 (m, 1H, NCH), 3.81 (s, 3H, OCH_3), 3.01-3.04 (m, 2H, SCH_2), 2.64-2.61 (m, 2H, CH_2), 2.16 (s, 3H, SCH_3). m/z 385.

Benzo[5,6]coumarine-3-carboxyl DL-threonine methyl ester (4c)

Brown crystals (1.06 g, 68%), R_f = 0.39 (S_2), m.p. 158^{o}C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 9.62 (s, 1H, CH pyranone ring), 9.54 (d, 1H, *J* = 8.1 Hz, NH), 8.39 (d, 1H, *J* = 8.4 Hz ,ArH), 8.10 (d, 1H, *J* = 9.0 Hz, ArH), 7.93 (d, 1H, *J* = 8.1 Hz, ArH) , 7.77-7.58 (m, 2H, ArH), 7.49 (d, 1H, *J* = 9.0 Hz, ArH), 4.85-4.81 (m, 1H, NCH), 4.51-4.46 (m, 1H, OCH), 3.83 (s, 3H, OCH_3), 2.6-2.8 (brs, 1H, OH), 1.35-1.23 (m, 3H, CH_3). m/z 355.

Benzo[5,6]coumarine-3-carboxyl L-serine methyl ester (4d)

Yellow crystals (1.05 g, 70%), R_f = 0.23(S_2), m.p. 120 ^{o}C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 9.68 (s, 1H, CH pyranone ring), 9.68-9.66 (m, 2H, NH, CH pyranone), 8.44 (d, 1H, *J* = 8.4 Hz, ArH) , 8.15 (d, 1H, *J* = 9.0 Hz, ArH) , 7.96 (m, 1H, *J* = 7.8 Hz, ArH), 7.80-7.75 (m, 1H, ArH), 7.67-7.62 (m, 1H, ArH), 7.54 (d, 1H, *J* = 9.0 Hz, ArH), 4.94-4.89 (m, 1H, NCH), 4.13-4.11 (m, 2H, OCH_2), 3.86 (s, 3H, OCH_3). m/z 341

Benzo[5,6]coumarine-3-carboxyl L-tyrosiene methyl ester (4e)

Yellowish brown (1.09 g, 60%), R_f =0.44(S_2), m.p. 122-125^{o}C. ^{1}H-NMR (300 MHz,$CDCl_3$): δ = 9.59 (s, 1H, CH pyranone ring), 9.31 (d, 1H, *J* = 7.8 Hz, NH), 8.37 (d, 1H, *J* = 8.4 Hz, ArH), 8.09 (d, 1H, *J* = 9.0 Hz

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,ArH), 7.92 (d ,1H, *J* = 7.8 Hz ,ArH), 7.74 (d, 1H, *J* = 8.4 Hz, ArH), 7.64 (d, 1H, *J* = 7.2 Hz, ArH), 7.48 (d, 1H, *J* = 9.0 Hz, ArH), 7.14-7.11 (m, 2H , ArH), 6.82-6.79 (m, 2H, ArH), 5.01-5.00 (m, 1H, NCH), 3.77 (s, 3H, OCH_3), 3.23-3.08 (m, 2H, CH_2-ph). m/z 417

Biological activity

Bacterial strains

Clinical isolates of *Gram*-positive bacteria (*S. aureus*) and *Gram*-negative bacteria (*E. coli*) were used to evaluate the antibacterial activity of the synthesized compounds.

Fungal strains

Clinical isolates of fungi *Alternari brassicicola* and *Fusarium oxysporum* were used to evaluate the antifungal activity of the synthesized compounds.

Tested compounds

The tested compounds were dissolved in dimethyl formamide (DMF) to make a concentration of 10^3 µg/ml (1000 ppm), DMF was used as control.

Reference therapeutic drugs

Three antibiotic drugs were used in this work:

i) Floxacin (disc 10µg)
ii) Gentamicin (disc 10µg)
iii) Ciprofloxacin (disc 5µg)

Preparation of inoculums

The bacterial inoculum was prepared by transferring 24 hrs old culture grown on nutrient agar slant to 10 ml sterile distilled water. Fungal inoculum was prepared by transferring 7 days old spores grown on PDA slant to 10 ml sterile distilled water. Each slant was shaken vigorously for one minute, so one milliliter of these suspensions was used for the incubation of the antibiograms.

Evaluation of antibacterial activity

The antibacterial activity was determined by the agar diffusion technique (Carrod and Grady, 1972), the medium was of the following composition (g/L); peptone (50 g), beef extract (3 g), sodium chloride (8 g), agar no. 2 (12 g), distilled water (1L) and PH=7.3±0.2. Bacterial suspensions from activity growing cultures were prepared using sterile distilled water.

One ml of bacterial suspensions was transferred to sterile petri dishes, and then the media was poured on to these plates, evenly distributed and finally allowed to solidify. Cups (6 mm in diameter) were made by punching in to the agar surface with a sterile cork borer and scooping out the punched part of the agar. In to each of these cups, 0.04 ml (40 µg) of each tested compound solution and control were added by using a micropipette besides antibiotic discs were used as a reference in separate plates. DMF was used as a control (solvent) and did not possess any inhibition zone. The plates were incubated at 37^{o}C for 24 hrs. The activity was determined by measuring the zone inhibition in mm.

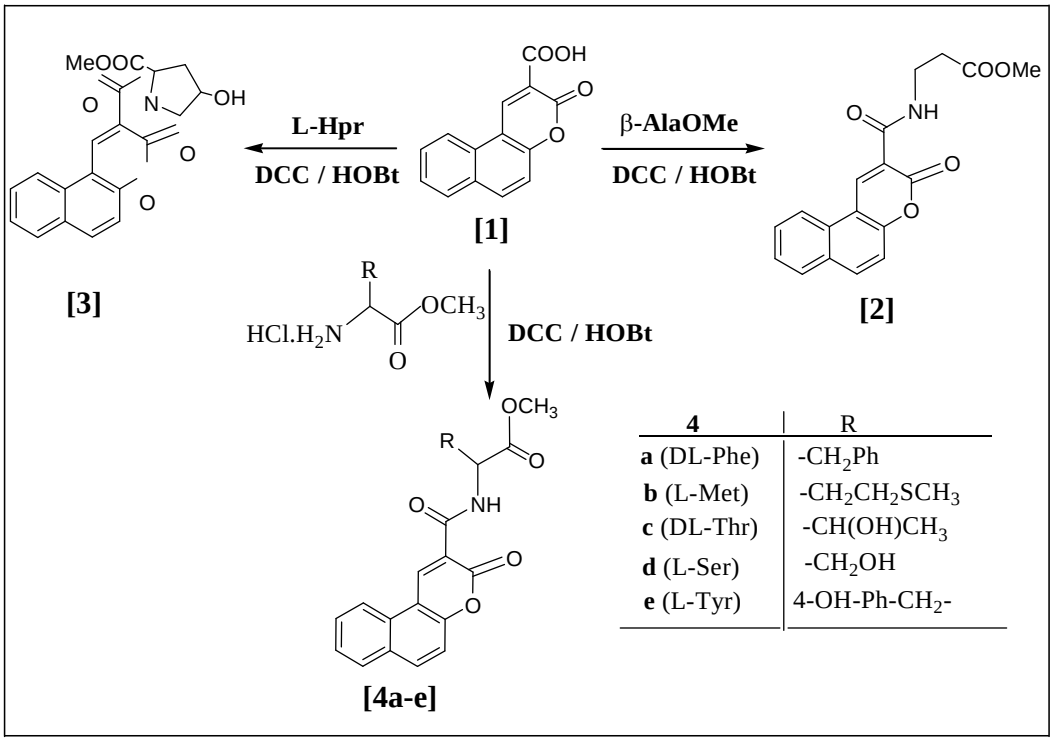
Evaluation of antifungal activity

The antifungal activity was determined by the agar diffusion technique (Carrod and Grady, 1972), the medium was of the following composition (g/L); sucrose (30 g), sodium nitrate (3 g), magnesium sulfate (0.5 g), potassium chloride (0.5 g), dipotassium phosphate (1.0 g) agar (15 g), distilled water (1L) and PH=5.5-6. Fungal suspensions from activity growing cultures were prepared using sterile distilled water. One ml of fungal suspensions was transferred to sterile petri dishes, and then the media was poured on to these plates, evenly distributed and finally allowed to solidify. Cups (6 mm in diameter) were made by punching in to the agar surface with a sterile cork borer and scooping out the punched part of the agar. In to each of these cups, 0.04 ml (40 µg) of each tested compound solution and control was added by using a micropipette. DMF was used as a control (solvent) and did not possess any inhibition zone. The plates were incubated at 37 ^{o}C for 7 days. The activity was determined by measuring the zone inhibition in mm.

RESULTS AND DISSCUSION

Chemistry

In the present study, the starting material benzo[5,6] coumarin-3-carboxylic acid **1** was prepared by Knoevenagel condensation between 2-hydroxy-1-naphthaldehyde and diethyl malonate in the presence of catalytic amount piperidine, followed by refluxing the resulting ethyl benzocoumarine-3-carboxylate ester for 2 hrs with potassium hydroxide in aqueous ethanol. [17] 3-carboxy-5,6-benzocoumarin amino acid methyl esters **2**, **3**, **4a-e** namely, of β-alanine, L-hydroxyproline, DL-phenylalanine, L-methionine, DL-threonine, L-serine and L-tyrosine have been synthesized via DCCI coupling method. The acid derivative **1 was** coupled with amino acid methyl ester hydrochloride derivatives in acetonitrile in the presence of dicyclohexyl carbodiimide, hydroxybenzotriazole and triethylamine (Scheme **1**). The chemical structure of the amino acid derivatives **2**, **3**, **4**a-e were confirmed by ^{1}H-NMR and mass spectrometery. The ^{1}H-NMR exhibit the following common data: Signals of the protons of CH of the pyranone ring appeared in the range 9.68-8.85 ppm, signals of the protons of CONH groups of the peptide bonds appeared in the range 9.66-9.18 ppm except for the L-hydroxyproline derivative, multiplet signals between 8.51-7.50 ppm are of the six aromatic protons, and singlet signal from 3.86 to 3.75 ppm for the three protons of OCH_3 of the ester groups, in addition to the other signals corresponding to protons of the individual side chains of the amino acids (see Experimental part) . The mass spectrometry data showed molecular ion peaks in agreement to the molecular weight of the corresponding compounds.



4	R
a (DL-Phe)	$-CH_2Ph$
b (L-Met)	$-CH_2CH_2SCH_3$
c (DL-Thr)	$-CH(OH)CH_3$
d (L-Ser)	$-CH_2OH$
e (L-Tyr)	4-OH-Ph-CH_2-

Scheme 1: Synthesis of different benzocoumarin amino acid derivatives.

Biological evaluation

Many coumarin derivatives exhibit antibacterial and antifungal activities, so the prepared compounds have been screened for their antimicrobial activities. The antimicrobial activities of the synthesized compounds were determined by the agar well diffusion technique (Carrod and Grady 1972).

Antibacterial activity

All the tested compounds were screened separately *in vitro* for antibacterial activity against Gram-positive bacteria *Staphylococcus aureus and* Gram-negative bacteria *Escherichia coli*. Floxacin, gentamicin and ciprofloxacin were used as a standared drugs. The plates were incubated at 37^{o}C for 24 hours. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the disc in mm (Table 1).

Antifungal activity

All the tested compounds were screened separately *in vitro* for antifungal activity against two fungi *Alternari brassicicola* and *Fusarium oxysporum*. The plates were incubated at 37^{o}C for 7 days. The antifungal activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the disc in mm. From the results only two of the synthesized compounds **1** and **4c** possess activity toward *Gram*-positive bacteria *Staphyloccus aureus*, compound **1** has IZ 15 mm more than that of gentamicin® which is 12 mm. All the synthesized compounds have no activity against *Gram*-negative bacteria *Escherichia coli* and the two types of fungi.

Table 1: The antibacterial effect of 3-substituted coumarin derivatives compared with Ciprofloxacin, Floxacin and Gentamicin.

Compound	Bacteria	
	Gram positive bacteria (*S. aureus*)	Gram negative bacteria (*E. coli*)
1	++	-
2	-	-
3	-	-
4a	-	-
4b	-	-
4c	++	-
4d	-	-
4e	-	-
Ciprofloxacin®	+++	+++
Floxacin®	+++	+++
Gentamicin®	++	++

Zone diameter of growth inhibition: (-) inactive, (+) < 10 mm, (++) 10-15 mm, (+++) >16 mm.

CONCLUSION

The article afforded an easy and facile method for preparation of benzo[5,6]coumarin-3-carboxyl amino acid ester derivatives via DCCI coupling method. In general conjugation of the benzocoumarin derivative with amino acid esters (except DL-threonine methyl ester derivative 4c) led to decrease in the antibacterial action towards *S. aureus.*

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تخليق و دراسة النشاط البيولوجى لبعض مشتقات الكومارين

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الملخص العربي

لقد تم تخليق بعض المركبات المشتقه من الكومارينات لما تتميز به الكومارينات من نشاط بيولوجى هذه المركبات تم تخليقها بدمج مركب البنزوكومارين ببعض الأحماض الأمينية. المركبات التى تم تحضيرها قمنا بدراسة النشاط البيولوجى لها باستخدام نوعين من البكتريا احداهما بكتريا موجبة الجرام والاخرى سالبة الجرام كما قمنا بدراسة تاثير نوعين من الفطريات. لقد اثبتت الدراسة وجود مركبين لهما نشاط جيد كمضاد للبكتيريا موجبة الجرام وهما مركب ٢ و ٤ج بينما بقية المركبات ليس لها نشاط. كل المركبات المخلقة ليس لها نشاط مضاد للبكتيريا سالبة الجرام أو نوعى الفطريات.