SYNTHESIS OF A NEW SERIES OF AMINO ACID DERIVATIVES OF SUBSTITIUTED COUMARIN AND ITS ANTIMICROBIAL ACTIVITY APPLICATIONS

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Abstract

A series of new substituted coumarin amino acid derivatives have been synthesized, characterized and screened for their (*In Vitro*) antimicrobial activity against Gram-positive and Gram-negative bacteria as well as for their antifungal activity and (*In Vivo*) application against plant pathogenic fungus, *Fusarium oxysporium* the causal agent of beans (*Phaseolus vulgaris .L.*), plant wilt disease Interestingly, our results show available antimicrobial response of the newly substituted coumarins in two main aspects firstly, (*in vitro*) the inhibition potential and the types of the effective coumarins compounds tested were more upon the filamentous plant pathogenic fungi than on both Gram positive and Gram-negative bacteria tested ,this may give an indication that the mechanism of the inhibition that exerted upon filamentous fungi as Eukaryotic representative class is completely different from that upon bacteria as Prokaryotic representative class .Secondary,.(*In Vivo*), some of the newly substituted coumarins can successfully inhibit the growth of plant pathogenic fungus as *Fusarium oxysporium* in a different degrees.

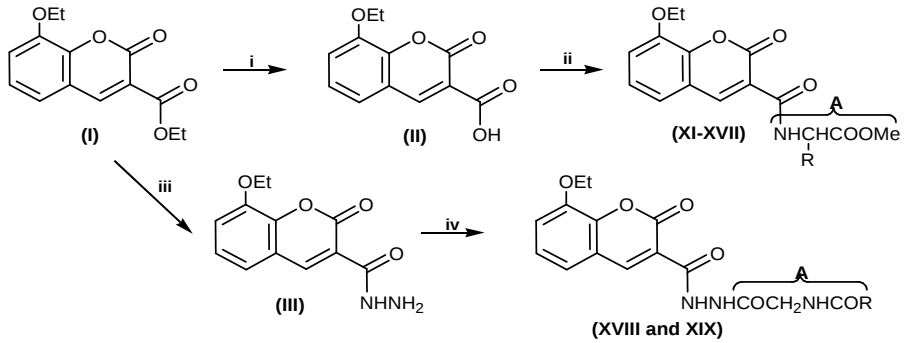
Keywords: coumarin, amino acids, antimicrobial activity, plant pathogenic fungi , fusarium wilt disease .Beans Plants (*Phaseolus. Vulgaris L.*) .

Introduction

Naturally occurring coumarins have exhibited applications including platelet aggregation[(1,2)], cytotoxic activity[(3)], enzyme inhibition[(4,5)], antiviral[(6)], antibacterial and antifungal activities[(7-11)]. New coumarin derivatives have been isolated from plants with an ever increasing variety of uses[(12-14)]. More recently, specific studies looking at the effects of coumarins as a cytochrome P-450 inhibitor which is a carcinogen metabolizing enzyme[(15)]. Coumarins have also been found to have beneficial effects on malaria[(16)]. Based on the biological importance of several coumarins [(17,18)] and in continuation of our work on the structure- activitry corelation in a variety of amino acid derivatives [(19-22)], we undertook the synthesis of a new series of coumarin and benzocoumarin derivatives incorporating with amino acid moieties to evaluate the antimicrobial activity.

Discussion

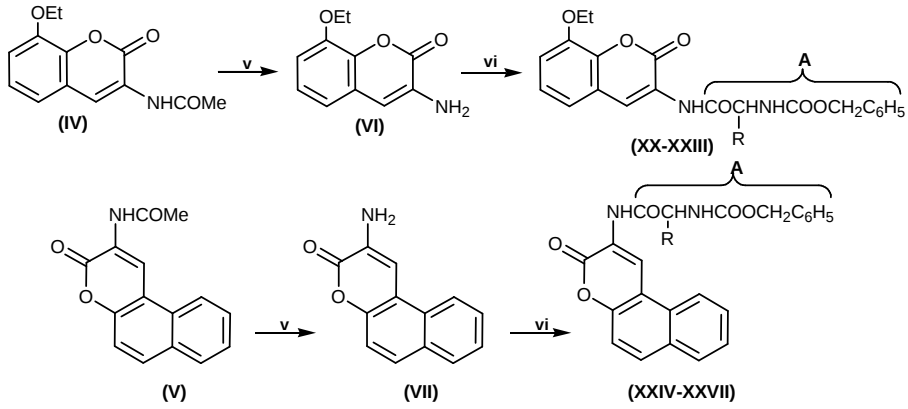
Thus, condensation of 3-ethylsalicylaldehyde with diethyl malonate in ethanolic piperidine solution under Knoevenagel reaction[23] afforded ethyl 8-ethoxy-coumarin-3-carboxylate (I) which on alkaline hydrolysis produced its free carboxylic acid derivative (II), while its reaction with ethanolic hydrazine hydrate afforded the corresponding hydrazide compound (III). By using the mixed carbonic anhydride method procedures[24], 8-ethoxycoumarin-3-carbonylamino acid methyl ester derivatives (XI-XVII)and N^1-(8-ethoxycoumarin-3-carbonyl)-N^2-(*N*-substituted glycyl)hydrazines (XVIII,XIX) were prepared, isolated, and purified through the reaction of (II) or (III) with different amino acid methyl ester or *N*-(acetyl- or benzoyl)glycine respectively (Scheme 1).



Scheme 1

Reagents:
i = 10% Ethanolic sodium hydroxide solution
ii = Amino acid ester hydrochloride / - 10 ℃
iii = Hydrazine hydrate / 0℃
iv = Acetyl or benzoylglycine / - 10℃

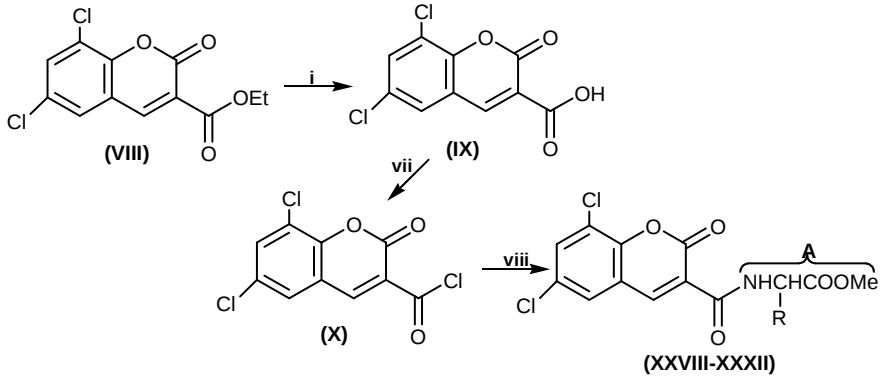
Condensation of 3-ethylsalicylaldehyde or 2-hydroxy-1-naphthaldehyde with glycine in acetic acid in presence of fused sodium acetate furnished the 3-acetamido-8-ethoxy-coumarin or 3-acetamido-5,6-benzocoumarin (IV or V) respectively. Acid hydrolysis of these products yielded the corresponding free 3-amino derivatives (VI or VII) which when coupled with *N*-benzyloxycarbonyl(Cbz)amino acids under the conditions of the mixed carbonic anhydride method, the following 3-(*N*-Cbz-amino-acyl)amino-8-ethoxy-coumarin or 3-(*N*-Cbz-aminoacyl)amino-5,6-benzocoumarins XX-XXIII or XXIV-XXVII respectively) were obtained (Scheme 2).



Scheme 2

Reagents:
v = Glacial acetic acid / 50% sulfuric acid solution
vi = N-Cbz-amino acid / THF / - 10°C

Finally, 6,8-dichlorocoumarin-3-carboxylic acid (IX) was synthesized via the alkaline hydrolysis of ethyl 6,8-dichlorocoumarin-3-carboxylate (VIII) which can be obtained from the condensation reaction of 3,5-dichloroslicylaldehyde with diethyl malonate. Interaction of 6,8-dichlorocoumarin-3-carbonylchloride (X) with several amino acid ester hydrochlorides previously treated with triethylamine gave the corresponding 6,8-dichlorocoumarin-3-carbonylamino acid methyl ester derivatives (XXVIII-XXXII) (Scheme 3).



Scheme 3

Reagents:
i = 10% Ethanolic sodium hydroxide solution
vii = Thionyl chloride
vi = Amino acid ester hydrochloride / THF / TEA

The structure of these derivatives was proved by elemental analysis, tlc studies and spectral data listed in table 1

Table 1: Spectral data of the prepared derivatives :

Compd. No.	**IR (ν, cm^{-1})** **^{1}H-NMR (δ, ppm) and mass spectrum m/e(intensity %)**
I	IR: 3087,1601(CH and C=C, aro), 2977(ali.CH),1703 (CO, coumarin), 1674 (C=O), 1758 (-C=O, ester), 1211 (C=O, str.) MS: 262(M^+,75), 234(46), 217(22), 188(100), 160(21),134(19), 105(19),77(15)
II	IR:3401(OH), 3056(CH, aro), 2987(CH,ali), 1720 (C=O, acid) ,1702 (CO, coumarin), 1676 (C=O) MS: 234(M^+,94), 206(60), 188(59), 160(26) , 134(100), 105(22), 77(25)
III	IR:3328,3269(NH_2), 1704 (CO, coumarin), 1671 (C=O), 1639(CO, amide), 1601 (C=C, aro.). MS: 248(M^+,42), 217(100), 189(34), 161(5), 105(10), 77(8)
IV	IR:3334(NH), 3016,1604(CH, and C=C aro),2954(CH,ali),1720(CO), 1692(C=O).
V	IR:3347(NH), 3028,1601(CH, and C=C aro),2971(CH,ali),1723(CO),1696(C=O) MS: 253(M^+,34), 211(100), 183(45), 154(15), 127(23), 77(6)
VI	IR:3404,3361(NH_2),3082,1599(CH,andC=C aro),2969(CH,ali),1731(CO),1694(C=O). MS: 205(M^+,100), 177(70),149(37), 122(55), 93(7),76(3)
VII	IR:3328,3339(NH_2), 3046,1607(CH, and C=C aro),2963, 2879(CH,ali), 1724(CO), 1696(C=O). MS: 211(M^+,100), 183(49), 154(15), 127(18),77(6)
VIII	IR:1764(COO),1708 (CO) MS: 286(M^+,46), 258(6), 241(60), 214(100),186(28), 157(28),123(4),87(15)
IX	IR:3473(broadOH),3037,1602(CH,andC=C,aro),1728(C=O, acid),1712(CO),1693(C=O) MS: 214(M^+-CO_2 ,90), 186(100), 123(64), 87(17)
XI	IR:3302(NH), 3033,1602(CH, and C=C aro),2955(CH,ali), 1716(CO), 1685,1576 (amide I

	and II). MS: 305(M^+,22), 246(22), 217(100), 189(32), 160(3), 105(7), 77(2) ^{1}H-NMR:9.19(br,1H,NH),8.80(s,1H,H-4),7.28-7.08(m,3H,Ar-H), 4.18(s,2H,CH_2CO), 4.15(q,2H,CH_2), 3.17(s,3H,OMe), 1.38(t,3H,CH_3)
XII	IR:3321(NH), 3017(CH, aro),2977(CH,ali),1715(CO), 1682, 1582(amide I and II) MS: 319(M^+,4), 260(60), 217(100), 189(18),160(3), 105(7), 77(3) ^{1}H-NMR:9.17(br,1H,NH),8.76(s,1H,H-4),7.30-7.10(m,3H,Ar-H), 4.87(q,1H,CHCO), 4.18 (q,4H,CH_2), 3.67(s,3H,OMe), 1.36 (t,3H,CH_3)
XIV	IR: 3324(NH), 1711(CO),17061572(amide I and II). MS: 361(M^+,2), 302(56), 217(100), 189(26), 160(9), 105(13),77(9) ^{1}H-NMR:9.12(br,1H,NH), 8.78(s,1H,H-4), 7.20-7.05 (m,3H,Ar-H), 4.63(2d,1H,CH,) , 3.68(s,3H,OMe), 1.45 (t,3H,CH_3), 0.91,0.85 (2d,6H,2CH_3)
XV	IR:3305(NH), 1725(COO),1709(amide I) ^{1}H-NMR:9.21(br,1H,NH),7.25-7.15(m,8H,Ar-H),4.74(t,1H,CHCO),4.13(q,2H,OCH_2), 3.71(s,3H,OMe),1.5(d,2H,CH_2), 1.47 (t,3H,CH_3).
XVIII	IR:3311(NH), 3029,1601(CH, and C=C aro),2989,2867(CH,ali), 1713(CO), 1699(C=O) ,1679 (amide II) MS: 347(M^+,9), 304(11), 288(17, 275(100),217(84)
XIX	MS: 408(M^+,7), 188(70), 217(100), 105(12)
XX	IR:3400,3331(NH),1703(CO),1700,1691(C=O) MS: 396(M^+,8), 295(31), 232(19), 205(27), 177(24), 107(30), 91(100), 79(3)
XXI	IR:3413,3339(NH), 1710(CO),1698(CO) MS: 408(M^+-2,8), 305(31),205(79), 177(87), 149(24), 122(30), 108(100), 79(3)
XXIII	IR:3342,3327(NH), 3060,1604(CH, and C=C aro),2981(CH,ali),1712(CO), 1682(C=O)
XXIV	IR:3334,3310(NH), 1723(CO),1696(C=O) MS: 311(M^+-CH_2Ph, 64), 211(100),183(41), 154(10), 127(19), 77(4)
XXVII	IR:3336,3317(NH), 1716(CO), 1707,1698(CO)
XXVIII	MS: 329(M^+,11), 270(45), 241(100), 157(21), 87(9)
XXIX	MS: 343(M^+,1), 284(79), 241(100), 213(4), 157(18), 87(5)
XXX	IR:3349(NH), 3008,1598(CH, and C=C aro),2972,2881(CH,ali),1711(CO), 1711,1587(amide I and II) MS: 371(M^+,2), 312(100), 241(90), 213(5)157(22) , 87(6)
XXXII	IR:3323(NH), 3027,1605(CH, and C=C aro),2992,2861(CH,ali),1714(CO), 1689,1576(amide I and II). MS: 419(M^+,1), 360(6), 241(60), 213((3),162(100), 131(15), 91(9)

Experimental

Melting points were uncorrected and measured on electric melting point apparatus SMPI. The infrared, IR, spectra (ν_{max}, $cm^{-1)}$ were taken in KBr discs using FTIR-2000 instrument. The Nuclear Magnetic Resonance, ^{1}H-NMR spectra were recorded in DMSO-d_6 at 300 MHz on a Varian Gemini NMR spectrometer using TMS as an internal standard. The mass spectra were obtained on GC-MS Aglient

5973N mass spectrometer. Elemental analyses were carried out at Microanalytical Unit, Faculty of Science, Cairo University. All data were listed in Table 2.

Synthaesis of ethyl 8-ethoxy or 6,8-dichlorocoumarin-3-carboxylate derivatives (I or VIII).

A mixture of 3-ethoxy- or 3,5-dichloro-2-hydroxybenzaldehyde (0.01 mol), diethyl malonate (0.011 mol) in abs.ethanol was refluxed for 2 hrs in presence of few drops of piperidine. The reaction mixture was allowed to cool, the solid product was filtered , and then recrystallized from ethanol.

Synthesis of 8-ethoxy- or 6,8-dichlorocoumarin-3-carboxylic acid derivatives (II or IX)

A mixture of the ester compound (I or VIII, 0.05 mol), 10% sodium hydroxide solution was refluxed for 1 h. After cooling, the reaction solution was acidified to (pH=5) with dil. HCl. The solid product was filtered, washed with cold water, dried and then recrystallized from ethanol.

Synthesis of 6,8-dichlorocoumarin-3-carbonyl chloride (X).

A suspension of 6,8-dichlorocoumarin-3-carboxylic acid (IX, 0.004 mol) in 15 ml of pure thionyl chloride was heated under reflux for 2.5 hrs. The excess of thionyl chloride was removed under reduced pressure. The crude oil product was taken up in 20 ml of dry benzene, filtered, cooled and then mixed with pet.ether 40-60 to give the desired product.

Synthesis of 8-ethoxy-3-acetamidocoumarin (IV) or 3-acetamido-5,6-benzocoumarin (V)

A mixture of 3-ethoxy-2-hydroxybenzaldehyde or 2-hydroxy-1-naphthaldehyde (0.01 mol), glycine (0.05 mol), fused sodium acetate (0.013 mol) in acetic anhydride was heated in an oil bath at 140ºC for 1 h. , and at 160 ºC for additional 1 hr. The reaction mixture was allowed to cool and poured into crushed ice, and then kept overnight . The solid separated washed with water, dil. NaOH solution, few ml. of hot methanol and then recrystallized from acetic acid .

Synthesis of 8-ethoxy-3-aminocoumarin(VI)or 3-amino-5,6-benzocoumarin (VII)

The acetamido compound (IV or V, 1 g) was suspended in a mixture of glacial acetic acid (15 ml) and 50% sulfuric acid (15 ml) , and then heated at 50-60ºC for 30-45 min. The clear reaction solution was poured into 50 ml cold water, cooled and

then neutralized by sodium bicarbonate solution. The solid product was filtered, washed with water ,dried and then recrystallized from the proper solvent.

Synthesis of 8-ethoxycoumarin-3-carbonyl hydrazide (III)

A mixture of ethyl 8-ethoxycoumarin-3-carboxylate (I, 0.002 mol) dissolved in ethanol and hydrazine hydrate (0.006 mol) was stirred at 0ºC for 2 hrs. The product was filtered off, washed with cold ethanol and then recrystallized from hot ethanol.

Synthaesis of 8-ethoxycoumarin-3-carbonylamino acid methyl ester (XI-XVII), N^1-(8-ethoxycoumarin-3-carbonyl)-N^2-(acylglycyl)-hydrazine (XVIII and XIX) or 3-(N-Cbz-aminoacyl)amino-5,6-benzocoumarin derivatives(XXIV-XXVIII).

In an ice bath at -10ºC , *N*-methylmorpholine (0.0022 mol) and isobutylchloroformate (0.0011 mol) were added to a solution of 8-ethoxycoumarin-3-carboxylic acid or its hydrazide derivative (II or III) Cbz-amino acid (0.0011 mol) in THF. The reaction mixture was stirred for 20 min. then the amino acid methyl ester hydrochloride , *N*-acylglycine or 3-amino-5,6-benzocoumarin (VII) (0.0013 mol) was added respectively .The mixture was stirred for additional 1 hr at 0ºC , left overnight at room temperature, and then evaporated under reduced pressure. The residual oil material was taken in a separating funnel with a mixture of 100 ml of ethylacetate and 50 ml of water , the water layer was excluded while the organic layer washed with saturated sodium bicarbonate solution followed by water and then filtered over anhydrous sodium sulfate. The solvent was evaporated and the residual product was recrystallized from the proper solvent.**Synthaesis of 8-ethoxy-3-(N-Cbz-aminoacyl)aminocoumarin derivatives (XX-XXIII).**

A mixture of equimolar amounts of 8-ethoxy-3-aminocoumarin (VI) and N-Cbz-amino acid (0.001 mol) in THF was stirred at 0ºC and *N*,*N*-dicyclohexylcarbodiimide (DCC, 0.0011 mol) was added with continuous stirring for 3-4 hrs. The precipitated dicyclohexylurea (DCU) was filtered off and the solvent was removed in vaccuo. The residual product was dissolved in ethylacetate containing few drops of glacial acetic acid and left overnight and filtered again. The solvent was re-evaporated and the residual crude product was recrystallized two times from the proper solvent.

Synthaesis of 6,8-dichlorocoumarin-3-carbonylamino acid methyl ester derivatives (XXVIII-XXXII).

Amino acid methyl ester hydrochloride (0.0011 mol) was suspended in 20 ml of THF containing triethylamine (0.0024 mol) with stirring for 45 min. at room temperature. The triethylamine hydrochloride was removed and the filtrate was

added to a solution of 6,8-dichlorocoumarin-3-carbonyl chloride (X, 0.001 mol) in THF. The reaction mixture was continued stirred for additional 3hrs and then left overnight .The precipitated triethylaminehydrochloride was removed again and the filtrate was evaporated till dryness and the residual oil was recrystallized from the proper solvent.

Table 2: The physical data of derivatives (I-XXXII)

Compd .No	A	.Cryst *.solv	.M.P C°	% Yield	Mol. Formula M.wt	** Elemental Analysis		
						%C	%H	%N
I	---	A	110-112	92	$C_{14}H_{14}O_5$ 262	64.12 64.10	5.34 5.24	---
II	---	A	176-178	76	$C_{12}H_{10}O_5$ 234	61.54 61.67	4.27 4.12	---
III	---	A	162-164	72	$C_{12}H_{12}N_2O_4$ 248	58.06 57.78	4.84 4.99	11.29 11.45
IV	---	B	232-234	32	$C_{13}H_{13}NO_4$ 247	63.16 63.12	5.26 5.43	5.66 5.79
V	---	B	239	51	$C_{15}H_{11}NO_3$ 253	71.14 71.35	4.35 4.13	5.53 5.24
VI	---	C	142-144	46	$C_{11}H_{11}NO_3$ 205	64.39 64.71	5.36 5.61	6.83 7.11
VII	---	A	155-157	43	$C_{13}H_9NO_2$ 211	73.93 73.76	4.26 4.02	6.63 6.61
VIII	---	A	112-114	85	$C_{12}H_8Cl_2O_4$ 287	50.17 50.36	2.79 2.62	---
IX	---	A	190-192	71	$C_{10}H_4Cl_2O_4$ 259	46.33 46.51	1.54 1.70	---
X	---	D	119	74	$C_{10}H_3Cl_3O_3$ 277.5	43.24 43.51	1.08 1.20	---
XI	Gly.OMe	E	192-193	70	$C_{15}H_{15}NO_6$ 305	59.01 59.23	4.92 4.66	4.59 4.76
XII	L-Ala.OMe	F	154	65	$C_{16}H_{17}NO_6$ 319	60.18 60.34	5.33 5.21	4.39 4.45
XIII	L-Val.OMe	E	131-132	63	$C_{18}H_{21}NO_6$ 347	62.24 62.02	6.05 6.31	4.03 3.87
XIV	L-Leu.OMe	A	111-113	61	$C_{19}H_{23}NO_6$ 361	63.16 63.27	6.37 6.59	3.88 3.96
XV	L-Phe.OMe	A	160-161	73	$C_{22}H_{21}NO_6$ 395	66.83 67.07	5.31 5.22	3.54 3.48
XVI	L-Ser.OMe	F	167	58	$C_{16}H_{17}NO_7$ 335	57.31 57.63	5.07 5.23	4.18 4.04
XVII	L-Thr.OMe	E	120	55	$C_{17}H_{19}NO_7$ 349	58.45 58.60	5.44 5.32	4.01 4.32
XVIII	*N*-acetyl-Gly	F	176	64	$C_{16}H_{17}N_3O_6$ 347	55.33 55.30	4.90 4.76	12.10 11.97

XIX	*N*-benzoyl-Gly	E	187	68	$C_{21}H_{19}N_36$ 409	61.61 61.46	4.64 4.39	10.26 10.60
XX	N-Cbz.Gly	C	168-171	68	$C_{21}H_{20}N_2O_6$ 396	63.63 63.79	5.05 5.23	7.07 6.89
XXI	N-Cbz-L-Ala	C	162	67	$C_{22}H_{22}N_2O_6$ 410	64.39 64.57	5.36 5.14	6.83 6.48
XXII	N-Cbz-L-Val	F	141-142	60	$C_{24}H_{26}N_2O_6$ 438	65.75 65.58	5.94 6.14	6.39 6.03
XXIII	N-Cbz-L-Ser	C	150-151	54	$C_{22}H_{22}N_2O_6$ 426	61.97 61.72	5.16 5.43	6.57 6.83
XXIV	N-Cbz.Gly	A	184	61	$C_{23}H_{18}N_2O_5$ 402	68.65 68.79	4.51 4.23	6.96 6.89
XXV	N-Cbz-L-Ala	A	135-137	57	$C_{24}H_{20}N_2O_5$ 416	69.23 64.57	4.80 5.04	6.73 6.48
XXVI	N-Cbz-L-Val	E	146	63	$C_{26}H_{24}N_2O_5$ 444	70.27 70.58	5.40 5.27	6.30 6.03
XXVII	N-Cbz-L-Ser	F	113-114	57	$C_{24}H_{20}N_2O_6$ 432	66.66 6672	4.63 4.43	6.48 6.53
XXVIII	Gly.OMe		221-223	68	$C_{13}H_9Cl_2NO_5$ 330	47.27 47.65	2.73 2.52	4.24 4.36
XXIX	L-Ala.OMe	F	142	65	$C_{14}H_{11}Cl_2NO_5$ 344	48.84 48.59	3.20 3.02	4.07 4.35
XXX	L-Val.OMe	A	156-157	63	$C_{16}H_{15}Cl_2NO_5$ 372	51.61 51.71	4.03 4.31	3.76 3.87
XXXI	L-Leu.OMe	A	102-103	61	$C_{17}H_{17}Cl_2NO_5$ 386	52.85 52.98	4.40 4.59	3.63 3.90
XXXII	L-Phe.OMe	F	163-165	67	$C_{20}H_{15}Cl_2NO_5$ 420	57.14 57.07	3.57 3.41	3.33 3.48

recrystallization solvent : A = Ethanol , B= Acetic acid , C=Methanol , * D=Benzene-pet.ether 40-60, E=Dioxane , F = Methanol-benzene .calculated / found**

Antimicrobial activities of the prepared compounds :

Sensitivity of microorganisms to antimicrobial compounds :

For testing antimicrobial activity of compounds, we used more than one test organisms as filamentous fungi ; *Alternaria alterna*ta , *Fusarium oxysporum* and Gram positive and gram-negative bacteria: Bacillus subtilis *(ATCC-6051), Staphylococcus aureus (ATCC-12600), and* Escherichia coli *(ATCC-11775)* to increase the range of antibiotic detection in the tested materials by using filter paper disk method[25]. A filter paper discs must be of uniform thickness and size and containing an equal and graded amount of the agent to be tested for its antimicrobial activity. The method was performed by dissolving 5mg. of the sample in one ml. of solvent solution, *N*,*N*-dimethylformamide (DMF), then a sterile filter paper discs were dipped into this solution . After absorption, the discs were dried and placed on

test organisms seeded plates to be tested for their antimicrobial activity. The inhibition zone were measured in millimeters at the end of incubation period. The data were recorded in Table 3.

Table 3: The biological activity of some newly synthesized coumarin derivatives :

Compd. No.	Gram –positive		Gram–negative	Fungi	
	BS *(ATCC-6051)*	SA *(ATCC-12600)*	EC *(ATCC-11775)*	AA *(ATCC-36378)*	FO *(ATCC-66421)*
II	+	+	-	+++	+++
III	-	-	-	+++	+
IX	++	++	-	++	-
X	-	-	+	+	++++
XI	-	-	-	+	-
XII	-	-	-	+	-
XIII	-	-	-	+	-
XIV	-	-	-	++	-
XXVIII	-	-	-	+	-
XXIX	++	++	-	-	-
XXX	+	+	-	-	-
XXXI	+	+	-	+	++
XXXII	+	+	-	-	+

1) +ve(When inhibition zone up to 8 mm)

2) ++ve (When inhibition zone was between 8–12 mm)

3) +++ve (When inhibition zone was between 12 – 15 mm)

4) ++++ve (When inhibition zone was over 15 mm)

Pathogenecity Test :

1-Fungal isolate and plant material .

A pure strain of beans plants (*phaseolus vulgaris L*) and virulent pathogenic isolate of *fusarium oxysporum* used in this study was kindly provided by the Agriculture Research Center (A.R.C) ,Ministry of agriculture , Giza -Egypt .

2-Cultivation, inoculation ,and treatment of Bean plants[26] :

Five of 14 days old Phaseolus *vulgaris* L. seedling were cultivated under green house conditions in 25 cm. diameter pots containing 2Kg. of soil , three sets of plants were used in triplicate .Two of them were inoculated with 2ml. of the pathogen spore suspension (10x 6ml -1) at the upper 10cm. of the soil , wherease the third was left without inoculation (Control). The following treatments were established :

1) Uninocula-ted plants (control). 2) Inoculated plants (diseased). 3) Inoculated plants treated with different typs of coumarins compounds (II, X, and XXXI) solution .The experiment was terminated after 8 weeks .

3- Phytopathological analysis : Disease symptoms was assessed using a scale of five classes ; 0= no symptoms, 1=Slight and few lesions, 2= moderate lesions, 3= leaves wilted, and 4= plants was completely destroyed. Disease index was calculated according to the method reported earlier[27]. we can using the formula : DI = (1n1 + 2n2 +3n3 + 4n4)100/ 4Nt, where n1 ~ n4 is the no. of plants in indicated classes and Nt is the total no. of plants.

Table 4: Effect of different treatments on wilt disease of *Phaseolus vulgaris* Caused by *Fusarium oxysporium*

Treatment	Severity Classes					Disease index	Infection %
	0	1	2	3	4		
Control (untreated plants)	18	2	0	0	0	03	10
Plants infected with *Fusarium oxysporum* (I.. P)	1	0	2	6	11	83	95
I . P + (II) Compound	15	3	2	0	0	11	25
I . P + (V) Compound	16	3	1	0	0	06	20
I . P + (XXXI) Compound	12	5	2	1	0	15	40

This study revealed that some of the synthesized compounds including 8-ethoxycoumarin-3-carboxylic acid**,** amino acid methyl ester**,** 8-ethoxycoumarin-3-carboxylic acid hydrazide, and dichloro- derivatives (I-XXXII) can successfully controlling (*In Vivo*) the fungal plant pathogens, as shown in Table (4), as *Alternaria alternate*- the causing agent of severe losses in cucumber plants[28]. Similarly, *Fusarium oxysporum* - the causal agent of beans (*Phaseolus vulgaris. L.*)- wilt disease[29]. Also ,*Fusarium oxysporum* root rot and / or wilt disease on crop plants[30,31]. Accordingly, this study may give a promissing approaches for using such compounds (*in Vivo)*, in the future as a safe tool for controlling the most deleterious plant disease pathogens.

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