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SYNTHESIS OF NEW COUMARIN - 7 - DERIVATIVES WITH EXPECTED ANTIMICROBIAL ACTIVITY.

By

Mandour, A. H.

Natural Products Department, National Research Centre, Cairo, Egypt.

ABSTRACT

New heterocyclic derivatives of 7-hydroxy-6-methoxycoumarin (1) have been synthesized. The heterocyclic thiazolidone, oxadiazole, triazole and thiadiazole derivatives were synthesized by the cyclo-condensation of hydrazide (III). The antimicrobial activity of the new compounds was estimated.

INTRODUCTION

Several coumarin derivatives possess pesticidal, fungicidal, anticoagulant and bacterial activities[1-9]. 7-0-Ether of 7-hydroxy-coumarin and substituted hydrazides possess various biological activity[10-15]. Moreover, oxadiazoles, triazoles and thiadiazoles are known to possess bacteriocidal activities[16-23].

In view of the biological properties of hydrazides and various nitrogen heterocyclic compounds, it was of interest to synthesize various coumarin

derivatives having the above moieties incorporated in it starting with the naturally occurring scopoletin (6-methoxy-7-hydroxycoumarin).

DISCUSSION

Scopoletin (6 - methoxy - 7 - hydroxycoumarin) (I) is a naturally *occurring* compound[24] *occurring* in roots of Scopolin Japonica Maxim. It was synthesized by Crosby[25] and Desai[26].

The reaction of 6-methoxy-7-hydroxycoumarin (I) with ethyl chloroacetate was carried out in the presence of anhydrous potassium carbonate in dry acetone giving rise to ethyl (6 - methoxy - 7 - coumarinyloxy) acetate (II). Treatment of (II) with hydrazine hydrate in ethanol led to the formation of 6 - methoxy - 7 - coumarinyloxy acetic acid hydrazide (III) (Scheme 1).

The IR spectrum of compound (II) showed characteristic absorption bands (cm^{-1}) at 1710 (CO - lactone), 1740 (CO - ester), and 1190 (C-O-C). Also the IR spectrum of (III) showed bands (cm^{-1}) at 1720 (CO - lactone), 1690 (CO - amide), 3290 (NH), 3300 & 3340 (NH_2) and 1190 (C-O-C) beside, the other characteristic bands.

Condensation of the acid hydrazide (III) with different aromatic aldehydes namely, benzaldehyde, p - methoxybenzaldehyde, p - chloro - benzaldehyde or p - nitrobenzaldehyde in ethanol and few drops of glacial acetic acid gave the correspnding Schiff's bases (IV_{a-d}), respectively (Scheme 1). The structure of the obtained compounds was confirmed by

their correct elemental analysis, IR and ^1H - NMR spectra. The physical and analytical data are illustrated in Table (1).

The IR spectrum of compounds (IV_{a-d}) showed absorption bands (cm^{-1}) at 1720 - 1730 (CO - lactone), 1680 - 1690 (CO - amide), 1180 - 1190 (C - O - C) and 1570 - 1590 (C = N).

The ^1H - NMR spectrum (DMSO - d_6) of compound (IV_a) revealed signals (δ = ppm) at 8.6 (1H, s, CH = N), 7.5 - 7.6 (7H, m, aromatic), 7.8 and 7.9 (2H, dd, coumarin H - 3 and H - 4), 4.2 (3H, s, OCH_3) and 5.2 (2H, s, O - CH_2).

Cyclocondensation of the Schiff's bases (IV_{a-d}) with mercaptoacetic acid in dioxane afforded the subsituted - 1, 3 - thiazolidone derivatives (V_{a-d}), respectively (Scheme 1).

The IR spectra of compounds (V_{a-d}) showed absorption bands (cm^{-1}) at 1720 - 1740 (CO - lcatone), 1670 - 1690 (CO - amide), 1650 - 1660 (CO - thiazalidone), 990 - 1000 (C - S - C), 1170 - 1190 (C - O - C) and 3450 - 3470 (NH).

The ^1H - NMR spectrum (DMSO - d_6) of compound (V_c) revealed signals (δ = ppm) at 5.9 (1H, s, thiazolidone), 5.0 (2H, s, thiazolidone), 5.3 (2H, s, O - CH_2), 4.2 (3H, s, OCH_3), 7.2 - 7.4 (6H, m, ArH) and 7.6 & 7.8 (2H, dd, H - 3 and H - 4 coumarin).

On the other hand, treatment of the acid hydrazide (III) with phenyl isothiocyanate or tolyl isothiocyanate in ethanol and in the presence of few drops of glacial acetic acid gave the corresponding (6 - methoxy - 7 -

coumarinyloxy) acetic acid - N - (phenyl or p - tolyl) hydrazine carbothiamides VI_a and VI_b, respectively (Scheme 2).

Cyclocondensation of (VI_a) and / or (VI_b) with iodine solution in sodium hydroxide led to the formation of 2 - arylamino - 5 - substituted - 1, 3, 4 - oxadiazole derivatives (VII_a) and (VII_b), respectively (Scheme 2).

The IR spectra of $VII_{a,b}$ showed absorption bands (cm^{-1}) at 1710 & 1730 (CO - lactone), 3400 and 3390 (NH), 1170 and 1190 (C - O - C), and 1560 and 1570 (C = N).

The ^1H - NMR spectrum (DMSO - d_6) of compound VII_a revealed signals (δ = ppm) at 5.1 (2H, s, O - CH_2), 4.3 (3H, s, OCH_3), 7.4 - 7.5 (7H, m, ArH) and 7.6 and 7.8 (2H, dd, H - 3 and H - 4 coumarin).

Also, cyclocondensation of the carbothiamide VI_a and / or VI_b using 4N sodium hydroxide gave the corresponding 1, 2, 4 - triazole - 3 - thiones $VIII_a$ and $VIII_b$, respectively (Scheme 2).

The IR spectra of compounds $VIII_{a,b}$ showed absorption bands (cm^{-1}) at 1720 - 1725 (CO - lactone), 1250 & 1240 (C - O - C), 2420 & 2430 (SH), 1570 & 1560 (C = N), 3300 & 3330 (NH) and 1160 and 1150 (C = S). The bands 3300 (NH) and 1160 (C = S) showed the predominance of the thiol form confirmed the tautomeric mixture.

The ^1H - NMR spectrum of compound $VIII_b$ (DMSO - d_6) revealed signals (δ = ppm) at 2.3 (3H, s, CH_3 - tolyl), 4.9 (2H, s, O - CH_2), 4.1 (3H, s, OCH_3), 7.2 - 7.3 (4H, m, ArH), 6.7 and 6.9 (2H, s, H - 5, H - 8, coumarin) and 7.5 and 7.6 (2H, dd, H - 3, H - 4 coumarin).

Moreover, the cyclocondensation of the carbothiamide VI_a and / or VI_b using orthophosphoric acid gave the corresponding 2 - arylamino - 5 - subsituted 1, 3, 4 - thiadiazole derivatives (IX_a) and (IX_b), respectively (Scheme 2).

The IR spectra of compounds IX_a and IX_b showed absorption bands (cm^{-1}) at 1730 - 1735 (CO - lactone), 1250 - 1260 (C - O - C), 1550 & 1570 (C = N), 1100 and 1090 (C - S - C) and 3340 - 3390 (NH).

The ^1H - NMR spectrum of compound (IX_a) (DMSO - d_6) revealed signals (δ = ppm) at 5.2 (2H, s, O - CH_2), 4.3 (3H, s, OCH_3), 7.2 - 7.5 (7H, m, ArH) and 7.6 & 7.8 (2H, dd, H - 3, H - 4 coumarin).

EXPERIMENTAL

Melting points were taken in open capillary tubes on Stuart Scientific Melting Point SMPI (U. K.). The compounds were checked by TLC. IR spectra were recorded on Karl Zeis IMR 16 spectophotometer and Mattson 1000, FTIR spectrophotometer. The ^1H - NMR spectra were determined on Jeol EX - 270 MH_2 spectrometer using TMS as internal standard.

1) Ethyl (6 - methoxy - 7 - coumarinyloxy) acetate (II) :-

To a mixture of 6 - methoxy - 7 - hydroxycoumarin[1] (0.01 mol) in 100 ml dry acetone and 1.4 g anhydrous potassium carbonate, (0.012 mol) of ethyl chloroacetate was added. The reaction mixture was refluxed on water bath for 16 h then filtered while hot. The solvent was evaporated to 1 / 3 of its volume and the found solid after cooling was collected and

crystallized from chloroform - methanol to give the title compound in about 70% yield.

2) 6-Methoxy-7- coumarinyloxy acetic acid hydrazide (III) :-

To a solution of compound II (0.005 mol) in 50 ml absolute ethanol, 0.005 mol (98 %) of hydrazine hydrate was added. The reaction mixture was left for 2 days at room temperatuer. The formed solid material was filtered off, air dried and crystallized from ethanol to give the title compound in a yield about 55 %.

3) N-(substituted-benzylidene) (6-methoxy-7-coumarinyloxy) acetic acid hydrazone (IV_{a-d}) :-

A solution of the hydrazide (III, 0.001 mol) in 20 ml ethanol was warmed, cooled and to it the appropriate aromatic aldehyde (0.001 mol) and a few drops of glacial acetic acid were added. The mixtrure was refluxed for 8 h and the separated solid, after cooling, was filtered off, washed with cold ethanol and crystallized from glacial acetic acid to give the title compounds in yield about 50 - 60 %.

4) 7-[(2-substituted-phenyl-4-oxo-3-thiazolidinyl carbomoyl) methoxy]-6-methoxycoumarin (V_{a-d}) :-

A mixture of IV_{a-d} (0.001 mol) and thioglycolic acid (0.01 mol) in dioxane (15 ml) was refluxed for 7 - 9 h. The solvent was distilled off under reduced pressure and the reaction mixture was cooled and extracted with 5% sodium bicarbonate solution. The bicarbonate extract was acidified with diluted hydrochloric acid and the resulting solid was filtered off, washed

with water and crystallized from dimethyl formamide to give the title compounds in yield about 35 - 40 %.

5) 7-[(2-Methoxy-2-oxo-2H-1-benzopyran-7-yl) oxy] acetic acid-2-[(4-substituted phenyl)-amino) thioxomethyl] hydrazide (VI_{a-d}) :-

A solution of phenyl or tolyl isothiocyanate (0.001 mol) and hydrazide (II, 0.001 mol) in 20 ml absolute ethanol and few drops of glacial acetic acid was refluxed for 7 h. The reaction mixture was cooled, filtered and then the solvent was removed under vacuum. The yielded product was crystallized from dioxane to give the title compounds in yield 40 and 45 %.

6) 2-Arylamino-5-[(6-methoxy-2-oxo-2H-1-benzopyran-7-yl) methoxy]-1, 3, 4-oxadiazole (VII_a) and (VII_b) :-

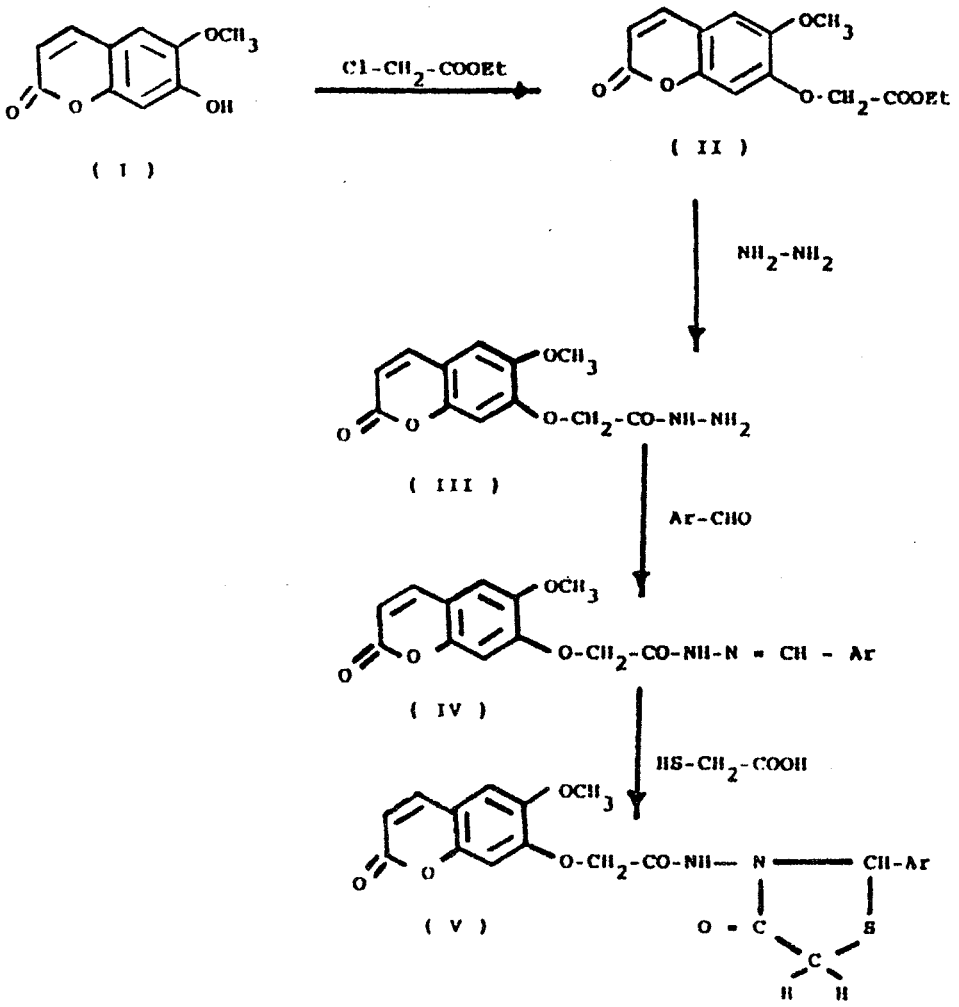
To the carbothiamide (VI, 0.001 mol) dissolved in minimum quantity of ethanol was added 6N sodium hydroxide (15 ml). A 5 % solution of iodine in potassium iodide was then added dropwise and the reaction mixture was kept at room temperature. The addition of iodine solution was continued till the colour of iodine persisted and the reaction mixture was refluxed for 5 h on a water bath (70 - 80 °C). On cooling, the separated solid was filtered off, washed with water, air dried and crystallized from methanol-chloroform to give the title compounds in yield 30 and 35 %.

7) 5-[(6-Methoxy-2-oxo-2H-1-benzopyran-7-yl) methoxy]-2, 4-dihydro-4-subs. phenyl-3H-1, 2, 4-triazole-3-thiones ($VIII_a$) and ($VIII_b$) :-

A mixture of the carbothiamide (VI, 0.001 mol) in 4N sodium hydroxide (20 ml) was refluxed for 4 h. The reaction mixture was cooled and then neutralized with diluted hydrochloric acid. The formed solid was filtered off, washed with water, air dried and crystallized from chloroform to give the title compounds in yield about 35 %.

8) 2-Arylamino-5-[(6-methoxy-2-oxo-2H-1-benzopyran-7-yl) methoxy]-1, 3, 4-thiadiazoles (IX_a) and (IX_b) :-

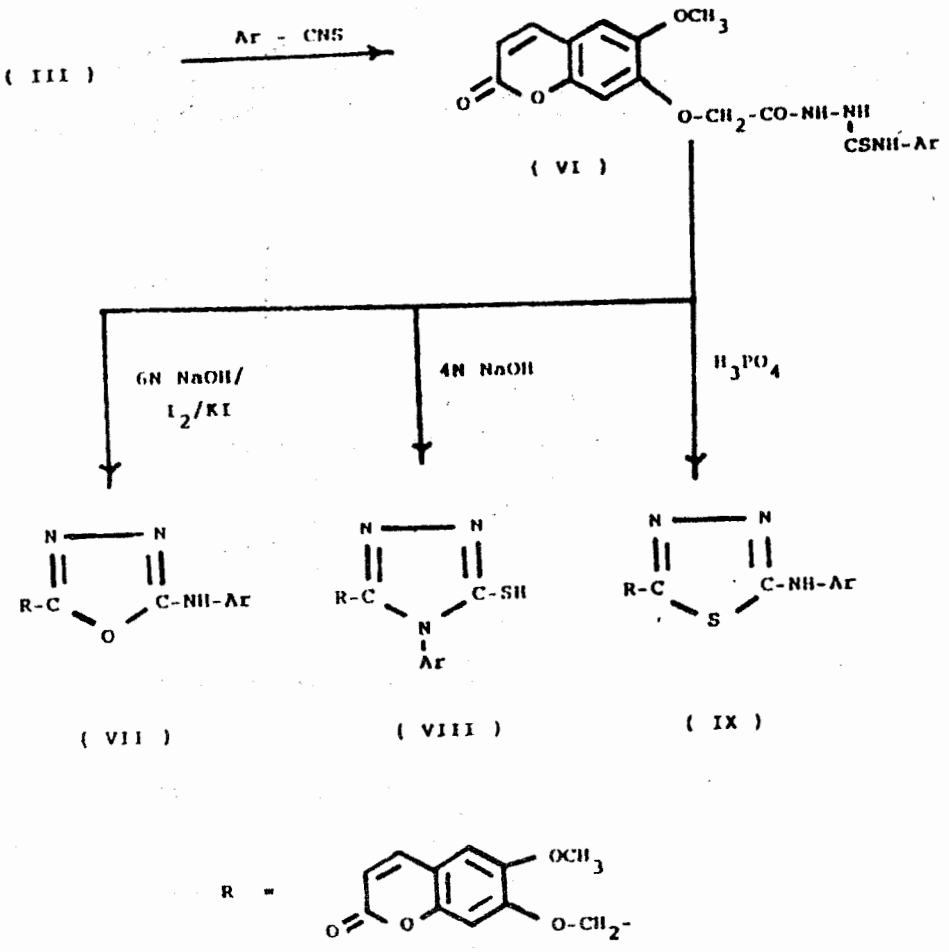
To (0.001 mol) of carbothiamide VI, was added gradually while stirring, 5 ml orthophosphoric acid and the reaction mixture was heated at 110 - 120 °C for 6 h. After cooling, the reaction mixture was neutrallized with ammonia solution. The formed solid was filtered off, washed with water, air dried and crystallized from chloroform - ethanol to give the title compounds in 30 % yield.



(I) (II) (III) (IV) (V)

IV and V a, Ar = ph
b, Ar = C_6H_4-OCH_3 (p)
c, Ar = C_6H_4-Cl (p)
d, Ar = C_6H_4-NO_2 (p)

Scheme (1)



VII, VIII and IX, a, Ar = ph

b, Ar = C_6H_4-CH_3 (p)

Scheme (2)

9) Antimicrobial Activity Test :-

The prepared compounds were tested against two local strains of G - positive and two strains of G - negative bacteria, and two strains of fungi. The antimicrobial activity was performed using the disk diffusion method[27].

From the data obtained, it was noticed that, the Schiff's bases IV_{a-d} possess moderate activity towards each of Gram - positive bacteria *(Bacellus subtilis* and *Staphylococus aureus)* and fungi (*Aspergillus flavus* and *Aspergillus niger*) and inactive towards the Gram - negative bacteria (*Escherichia coli and Proteus mirabilis*). On the other hand, the thiazolidones V_{a-d} possess moderate activity towards each of Gram - positive, Gram - negative bacteria and high activity towards fungi.

Moreover, it was noticed that the oxadiazoles $VII_{a,b}$ triazoles $VIII_{a,b}$ and thiadiazoles $IX_{a,b}$ possess moderate activity towards Gram - positive bacteria, inactive towards Gram - negative bacteria and possess high activity towards fungi.

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Table (1): The physical and analytical data of the prepared compounds.

Compd. No.	M. formula M. wt.	M.p. °C	Analysis Calcd./Found		
			C%	H%	N%
II	$C_{14}H_{14}O_6$ 378	163-65	60.43 60.50	5.03 5.00	- -
III	$C_{12}H_{12}N_2O_6$ 264	140-42	54.54 54.15	4.54 4.60	10.60 10.30
IV_a	$C_{19}H_{16}N_2O_6$ 352	210-12	64.77 64.90	4.54 4.30	7.95 7.50
IV_b	$C_{20}H_{18}N_2O_6$ 382	218-20	62.82 62.63	4.71 4.50	7.32 7.10
IV_c	$C_{19}H_{15}ClN_2O_5$ 386.5	278-80	58.99 59.30	3.88 3.10	7.24 6.90
IV_d	$C_{21}H_{15}N_3O_7$ 397	260-62	57.43 57.90	3.77 3.80	10.57 10.30
V_a	$C_{21}H_{18}N_2O_6S$ 426	110-12	59.15 59.30	4.22 4.10	6.57 6.30
V_b	$C_{22}H_{20}N_2O_7S$ 456	120-22	57.89 57.50	4.38 4.60	6.14 6.00
V_c	$C_{21}H_{17}Cl\ N_2O_6S$ 460.5	118-20	54.72 54.50	3.69 4.00	6.08 5.80
V_d	$C_{21}H_{17}N_3O_8S$ 471	230-32	53.50 53.20	3.61 3.50	8.91 8.70
VI_a	$C_{19}H_{17}N_3O_5S$ 399	180-82	57.14 57.00	4.26 4.00	10.52 10.10
VI_b	$C_{20}H_{19}N_3O_5S$ 413	215-17	58.11 57.90	4.60 4.20	10.16 10.00
VII_a	$C_{19}H_{15}N_3O_5$ 365	130-32	62.46 62.10	4.10 4.00	11.50 11.10
VII_b	$C_{20}H_{17}N_3O_5$ 375	145-47	64.00 63.80	4.53 4.30	11.20 11.00
$VIII_a$	$C_{19}H_{15}N_3O_4S$ 381	272-74	59.84 60.00	3.93 4.10	11.03 10.80
$VIII_b$	$C_{20}H_{17}N_3O_4S$ 395	213-15	60.75 61.00	4.30 4.10	10.63 11.00
IX_a	$C_{19}H_{15}N_3O_4S$ 381	215-17	59.84 59.50	3.93 3.49	11.02 11.00
IX_b	$C_{20}H_{17}N_3O_4S$ 395	235-37	60.75 60.45	4.30 4.60	10.63 10.90

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تحضير مشتقات جديدة من ٧ - كومارين المتوقع لها نشاط ضد البكتريا

عادل حامد منصور

قسم المنتجات الطبيعية – المركز القومى للبحوث – القاهرة

فى هذا البحث تم تحضير بعض المشتقات الجديدة للكومارين فى الموقع رقم ٧.

تم تفاعل ٦ – ميثوكسى – ٧ – هيدروكسى كومارين (I) مع كلورو خلات الإيثيل لينتج ٦ – ميثوكسى – ٧ – أوكسى كومارين. حمض الخليك (II) الذى يتفاعل بدوره لينتج الحامض الهيدرازينى (III).

بتكاثف المركب (III) مع بعض الألدهيدات الأروماتية تم الحصول على كواشف شيف (IV_{a-d}) التى تم حولقتها مع حامض الثيوجليكول ليعطى بدوره الحلقة الغير متجانسة الثيازوليدون المناظرة (V_{a-d}).

ومن ناحية أخرى فقد تم تفاعل الحامض الهيدرازينى (III) مع فينيل أيزوثيوسيانات أو طوليل أيزوثيوسيانات ليعطى بدوره مركبات الكاربوثيا أميد ($VI_{a,b}$).

والمركبات الأخيرة قد تم حولقتها وذلك عن طريق إستخدام محلول اليود ٥٪ مع هيدروكسيد الصوديوم ليعطى مركبات ١، ٣، ٤ – أوكساديازول ($VII_{a,b}$).

وعند معاملة المركب رقم (VI_a) أو (VI_b) بواسطة محلول ٤ عيارى هيدروكسيد الصوديوم تم الحصول على مركبات ١، ٢، ٤ – تريازول ($VIII_{a,b}$).

ومن ناحية أخرى عند معالجة المركب رقم (VI_a) أو (VI_b) بواسطة حامض الأرثوفوسفوريك تم الحصول على مركبات ١ و ٣ و ٤ – ثياديازول ($IIX_{a,b}$).

وقد تم إثبات المركبات الناتجة بواسطة الأشعة تحت الحمراء وكذلك الرنين النووى المغناطيسى والتحاليل الدقيقة.

وأخيراً تم تجربة المركبات الجديدة على بعض أنواع من البكتريا موجبة وسالبة الجرام وكذلك على نوعين من الفطريات وقد وجد أن لبعض هذه المركبات فاعلية متوسطة أوعالية ضد هذه الميكروبات.