Arch Pharm Res Vol 26, No 8, 686-696, 2003

Archives of Pharmacal Research
http://apr.psk.or.kr

Synthesis and Biological Investigations of New Thiazolidinone and Oxadiazoline Coumarin Derivatives

Omaima Mohamed Abd Elhafez, Ezz El Din Ahmed Mohamed El Khrisy, Farid Badria[1], and Alaa El Din Mohamed Fathy

Chemistry of Natural and Microbial Products Department, National Research Centre, Dokki, Cairo and [1]Pharmacognosy Department, Faculty of Pharmacy, Mansoura University

(Received January 28, 2003)

Ethyl (coumarin-4-oxy)acetate **1** was prepared through the reaction of 4-hydroxycoumarin with ethyl bromoacetate. Compound **1** was allowed to react with hydrazine hydrate to produce coumarin-4-oxyacetic hydrazide **2**. The synthesis of *N*-(arylidene and alkylidene)-coumarin-4-oxyacetic hydrazones **3-20** was performed. The preparation of 2-substituted-3-[(coumarin-4-oxy) acetamido]thiazolidinones **21-26** and 2-[(coumarin-4-oxy)methyl]-4-acetyl-5-substituted-Δ^2-1,3,4-oxadiazolines **27-33** was performed by the reaction of the hydrazones **3**, **4**, **7**, **9**, **12**, **14** with mercaptoacetic acid and the hydrazones **3**, **4**, **5**, **7**, **12**, **15**, **16** with acetic anhydride, respectively. The antiviral activities, cytotoxicities and structure-activity relationship (SAR) towards different microorganisms of the prepared compounds were studied.

Key words: 4-Hydroxycoumarin, Ethyl (coumarin-4-oxy)-acetate, Coumarin-4-oxyacetic hydrazide, Hydrazones, Thiazolidinones, Oxadiazolines, Antiviral, Cytotoxicity, Structure-activity relationship

INTRODUCTION

Many biological properties for coumarins have been reported such as antimicrobial (Nagesam *et al.*, 1989; Rao *et al.*, 1986; Singh *et al.*, 1989), diuretic (Selleri *et al.*, 1966), vasodilatory (Erbring *et al.*, 1967; Smith, *et al.*, 1957), anticoagulant (Arora *et al.*, 1963; Chemielewska *et al.*, 1958) and antitumor activities (Dalla Via *et al.*, 1999; El-Subbagh *et al.*, 2000; Matsumoto *et al.*, 2000; Seo *et al.*, 2000; Valenti *et al.*, 1997; Weber *et al.*, 1998). Moreover, N=CH, thiazolidinone and oxadiazoline groups possess biological activities such as antimicrobial (Kamdar *et al.*, 1987; Meher *et al.*, 1981; Choubey *et al.*, 1970; Fathy *et al.*, 1990) and anticancer (Popp, 1961; Abdel-Hamid *et al.*, 1993). Therefore, our aim in this work was to prepare some new hydrazone, thiazolidinone and oxadiazoline derivatives using the biologically active 4-hydroxycoumarin moiety as a starting compound (Abbas *et al.*, 1994; Sagitullin *et al.*, 1959). We also studied the antiviral activities, cytotoxicities and structure-activity relationship (SAR) towards different microorganisms of the newly prepared compounds.

Correspondence to: Dr. Omaima Mohamed Abd Elhafez, Chemistry of Natural and Microbial Products Department, National Research Centre, Dokki, Cairo
E-mail: mohamed_serry@hotmail.com

MATERIALS AND METHODS

The stated melting points are uncorrected and were performed on electrothermal capillary melting point apparatus Stuart Scientific Melting Point SMP1. Elemental microanalysis was obtained by the Microanalytical Laboratory Services, Faculty of Science, Cairo University and National Research Centre, Cairo, Egypt. All reactions were followed up by TLC (aluminium sheets) using chloroform-methanol (9:1 v/v) as eluent and sprayed with iodine-potassium iodide reagent. IR spectra were performed on a Beckman infrared spectrophotometer PU 9712 using KBr disks, at the National Research Centre. The ^{1}H-NMR spectra were determined on a Jeol-EX 270 MHz spectrometer and a Varian mercury 300 MHz spectrometer using TMS as an internal standard. The Mass spectra were recorded on GCMS-QP 1000 EX, Schimadzu spectrometer, Japan E.I. 70 ev.

Ethyl (coumarin-4-oxy)acetate (1)

A mixture of 4-hydroxycoumarin (16.2 g, 0.1 mol), anhydrous potassium carbonate (13.8 g, 0.1 mol) and ethyl bromoacetate (11.1 mL, 0.1 mol) in dry acetone (200 mL) was refluxed for 10 h. The reaction mixture was filtered while hot and the residue washed with boiling acetone (20

mL). The solvent was evaporated under reduced pressure and the residue was poured on ice/water (400 mL) The solid obtained (23.1 g) was filtered off and crystallized to give white needles of ethyl (coumarin-4-oxy)acetate (**1**) (Table I).

Coumarin-4-oxyacetic hydrazide (2)

Hydrazine hydrate (1.1 mL, 0.022 mol) in absolute ethanol (5 mL) was added to a well stirred suspension of ethyl (coumarin-4-oxy)acetate (**1**) (4.96 g, 0.02 mol) in absolute ethanol (25 mL). After 7 h stirring at room temperature, the reaction mixture was cooled for 1 h in an ice box, filtered off and washed with ethanol to give a white precipitate of coumarin-4-oxyacetic hydrazide (**2**) (4.2 g) which was crystallized to give colourless plates (Table I).

Table I. Physical and analytical data of the prepared compounds (**1-33**)

Comp. No	m.p.°C Solvent	Yield %	Formula (M. wt)	Analysis Calcd / found				
				C%	H%	N%	Cl%	S%
1	97 EtOH	93	$C_{13}H_{12}O_5$ (248.23)	62.90 63.20	4.87 4.40			
2	227 MeOH/H_2O	89	$C_{11}H_{10}N_2O_4$ (234.21)	56.41 56.80	4.30 4.00	11.96 11.90		
3	256 benzene	73	$C_{18}H_{14}N_2O_4$ (322.32)	67.08 66.80	4.38 4.00	8.69 8.30		
4	269 benzene	85	$C_{20}H_{19}N_3O_4$ (365.39)	65.74 65.30	5.24 5.40	11.50 11.10		
5	286 AcOH/H_2O	87	$C_{19}H_{16}N_2O_5$ (352.35)	64.77 64.80	4.58 4.80	7.95 7.70		
6	247 MeOH	78	$C_{21}H_{20}N_2O_7$ (412.40)	61.16 61.40	4.89 4.30	6.79 6.30		
7	283 DMF/H_2O	88	$C_{18}H_{13}N_2O_4Cl$ (356.76)	60.60 60.50	3.67 4.00	7.85 7.70	9.94 9.30	
8	295 DMF/H_2O	95	$C_{18}H_{13}N_2O_4Cl$ (356.76)	60.60 61.00	3.67 4.00	7.85 7.90	9.94 10.00	
9	318 AcOH	61	$C_{18}H_{13}N_3O_6$ (367.32)	58.86 58.40	3.57 3.90	11.44 11.70		
10	287 AcOH	55	$C_{18}H_{13}N_3O_6$ (367.32)	58.86 58.40	3.57 3.80	11.44 11.80		
11	279 MeOH	90	$C_{18}H_{14}N_2O_5$ (338.32)	63.90 63.50	4.17 4.20	8.28 8.00		
12	235 benzene	79	$C_{16}H_{12}N_2O_5$ (312.28)	61.54 61.90	3.87 4.00	8.97 9.00		
13	274 benzene	69	$C_{16}H_{12}N_2O_4S$ (328.34)	58.53 58.90	3.68 4.40	8.53 8.30		9.76 9.10
14	265 benzene	95	$C_{17}H_{13}N_3O_4$ (323.31)	63.16 63.00	4.05 4.40	13.00 13.00		
15	237 benzene	98	$C_{20}H_{16}N_2O_4$ (348.36)	68.96 68.70	4.63 4.30	8.04 8.10		
16	193 EtOH	72	$C_{13}H_{12}N_2O_4$ (260.25)	60.00 60.20	4.65 4.60	10.76 10.30		
17	182 EtOH	60	$C_{15}H_{16}N_2O_4$ (288.30)	62.49 62.90	5.59 6.00	9.72 9.30		
18	286 AcOH/H_2O	51	$C_{19}H_{24}N_2O_4$ (344.41)	66.26 66.70	7.02 6.60	8.13 7.70		
19	127 EtOH/H_2O	52	$C_{21}H_{26}N_2O_4$ (370.45)	68.09 68.50	7.07 7.50	7.56 7.30		
20	204 H_2O	58	$C_{17}H_{20}N_2O_9$ (396.35)	51.52 51.90	5.09 5.50	7.07 7.10		

Table I. continued

Comp. No	m.p.°C Solvent	Yield %	Formula (M. wt)	Analysis Calcd / found				
				C%	H%	N%	Cl%	S%
21	258 DMF/H_2O	57	$C_{20}H_{16}N_2O_5S$ (396.42)	60.60 60.90	4.07 4.50	7.07 6.70		8.09 8.30
22	294 dec. DMF	48	$C_{22}H_{21}N_3O_5S$ (439.49)	60.12 60.10	4.82 4.60	9.56 9.20		7.29 7.00
23	148 AcOH	51	$C_{20}H_{15}N_2O_5SCl$ (430.86)	55.75 56.00	3.51 3.50	6.50 6.60		7.44 7.20
24	128 AcOH/H_2O	59	$C_{20}H_{15}N_3O_7S$ (441.41)	54.42 54.70	3.43 3.40	9.52 9.10		7.26 7.60
25	312 AcOH	56	$C_{18}H_{14}N_2O_6S$ (386.38)	55.95 55.60	3.65 3.30	7.25 7.30		8.30 8.30
26	305 DMF	45	$C_{19}H_{15}N_3O_5S$ (397.40)	57.43 57.10	3.80 4.20	10.57 10.50		8.07 7.80
27	220 EtOH	47	$C_{20}H_{16}N_2O_5$ (364.36)	65.93 65.40	4.43 4.60	7.69 7.90		
28	110 MeOH/H_2O	40	$C_{22}H_{21}N_3O_5$ (407.43)	64.86 64.40	5.19 5.60	10.31 9.90		
29	198 MeOH	55	$C_{21}H_{18}N_2O_6$ (394.38)	63.96 63.60	4.60 4.80	7.10 6.70		
30	148 EtOH	62	$C_{20}H_{15}N_2O_5Cl$ (398.80)	60.24 59.80	3.79 3.10	7.02 7.40		
31	234 EtOH	50	$C_{18}H_{14}N_2O_6$ (354.32)	61.02 60.90	3.98 3.50	7.91 7.70		
32	144 EtOH/H_2O	50	$C_{22}H_{18}N_2O_5$ (390.39)	67.69 67.50	4.65 4.40	7.18 7.20		
33	168 EtOH	39	$C_{15}H_{14}N_2O_5$ (302.29)	59.60 59.20	4.67 4.20	9.27 9.10		

***N*-Arylidene coumarin-4-oxyacetic hydrazones (3-15)**

A solution of the appropriate aromatic and heterocyclic aldehydes, namely, benzaldehyde, 4-dimethylaminobenzaldehyde, 4-methoxybenzaldehyde, 3,4,5-trimethoxybenzaldehyde, 4-chlorobenzaldehyde, 2-chlorobenzaldehyde, 4-nitrobenzaldehyde, 2-nitrobenz-aldehyde, salicylaldehyde, furfural, thiophen-2-carboxaldehyde, nicotinaldehyde and/or cinnamaldehyde, (0.011 mol) in methanol (5 mL) was added dropwise to a well-stirred solution of compound **2** (2,34 g, 0.01 mol) in a boiling methanol-water mixture (50:6 v/v). The reaction mixture was refluxed for 1-5 h while the stirring was continued, then concentrated and cooled. The solid product was filtered off, washed with dilute methanol and crystallized from the proper solvent (Table I).

***N*-Alkylidene coumarin-4-oxyacetic hydrazones (16-20)**

A mixture of coumarin-4-oxyacetic hydrazide (**2**) (1.17 g, 0.005 mol) and the appropriate aliphatic aldehydes, namely, acetaldehyde, butyraldehyde, oct-aldehyde, citronellal and/or D-mannose, (0.006 mole) in a methanol/water mixture (25:3 v/v) in the presence of a few drops of acetic acid was refluxed for 6-8 h while stirring, then concentrated and cooled. The solid obtained was filtered off, washed with ether and crystallized from the proper solvent (Table I).

2-Substituted-3-[(coumarin-4-oxy)acetamido]thiazolidin-4-ones (21-26)

To a well-stirred suspension of the appropriate hydrazone derivatives, namely, *N*-benzylidenecoumarin-4-oxyacetic hydrazone (**3**), *N*-(4-dimethylaminobenzylidene)coumarin-4-oxyacetic hydrazone (**4**), *N*-(4-chlorobenzylidene)coumarin-4-oxyacetic hydrazone (**7**), *N*-(4-nitrobenzylidene)coumarin-4-oxyacetic hydrazone (**9**), *N*-(2-furylidene)coumarin-4-oxyacetic hydrazone (**12**) and/or *N*-(nicotinylidene)coumarin-4-oxyacetic hydrazone (**14**), (0.001 mol) in dry benzene (50 mL), mercaptoacetic acid (0.42 mL, 0.006 mol) in dry benzene (5 mL) was added. The reaction mixture was refluxed for 15-18 h and the solvent was evaporated under reduced pressure. The residue was triturated with boiling water (100 mL), left overnight, filtered off, washed with water, dried and crystallized from the proper solvent (Table I).

2-[(Coumarin-4-oxy)methyl]-4-acetyl-5-substituted-Δ^2-1,3,4-oxadiazolines (27-33)

The appropriate hydrazones, namely, *N*-benzylidene coumarin-4-oxyacetic hydrazone (**3**), *N*-(4-dimethylaminobenzylidene) coumarin-4-oxyacetic hydrazone (**4**), *N*-(4-methoxybenzylidene)coumarin-4-oxyacetic hydrazone (**5**), *N*-(4-chlorobenzylidene)coumarin-4-oxyacetic hydrazone (**7**), *N*-(2-furylidene)coumarin-4-oxyacetic hydrazone (**12**), *N*-cinnamylidenecoumarin-4-oxyacetic hydrazone (**15**) and/or *N*-ethylidene coumarin-4-oxyacetic hydrazone (**16**), (0.001 mol) were refluxed with acetic anhydride (15 mL) for 8-10 h. Unreacted acetic anhydride was removed and the residue was solidified by trituration with ether. The solid obtained was filtered off, washed with ether and crystallized from the proper solvent (Table I).

Biological activity

Structure-activity relationship (SAR)

Some of the newly synthesized compounds have been tested for their antimicrobial activities against the following various groups of microorganisms (Eukaryotes and Prokaryotes): Bacteria (Gram +ve bacilli: Bacillus subtilis, Gram +ve cocci: Staphylococcus aureus, Gram ve bacilli: Escherichia coli, Gram ve pathogenic bacilli: Salmonella typhi, Pseudomonas spp. and Proteus spp.), Yeast (Non pathogenic yeast: Saccharomyces cerevisiae, Pathogenic yeast: Candida albicans) and Filamentous fungi (Fungi belonging to Aspergilli: Aspergillus niger and Aspergillus terreus). The screening of these compounds was performed according to the agar diffusion method (Egorov, 1985) as detailed below:

Sample preparation

Samples of 200 mg were dissolved in 2 mL DMSO (Dimethyl Sulfhoxide) and warmed to 60°C to increase their solubility in DMSO.

Cultivation medium

Nutrient Agar was used for the determination of the antimicrobial activities of different compounds. The medium was composed of (g/L): Peptone, 5.0; Beef extract, 3.0 and Agar 20.0. The pH was adjusted to 7.0 before autoclaving.

Medium inoculation and cultivation condition

The nutrient agar medium was mixed with microorganisms at 50°C and poured immediately in petri-dishes of 150 mm diameter. Since the thickness of the agar medium plays a critical role in the inhibition zone diameter, the amount of medium per petri-dish was 50 mL to keep the same thickness of agar layer in all petri-dishes. After agar solidification, small wheels of 5 mm diameter were made in the petri dishes by means of a Cork porer. Samples of 75 L (0.1 mg/(L) were added in each wheel. The petri-dishes were stored for 1 h at 5°C to allow the diffusion of chemical compounds under study in agar medium prior to cell growth. Thereafter, the petri dishes were transferred to an incubator adjusted to 37°C and the diameter of the inhibition zones was recorded after 24 h in the case of bacteria. On the other hand, for determination of antifungal activities (for both yeast and filamentous fungi), the inoculated dishes were cultivated in an incubator at 28°C for 48 h.

Antiviral and cytotoxic activities

Sample preparation

Antiviral and cytotoxicity assays were carried out essentially according to the reported method (El-Bendary *et al.*, 2000; El-Sherbeny *et al.*, 1996; El-Sherbeny *et al.*, 1995; El-Subbagh *et al.*, 1994; El-Subbagh *et al.*, 1995). Samples were prepared for assay by dissolving in 50 µL of DMSO and diluting aliquots into sterile culture medium at 0.4 mg/mL. These solutions were subdiluted to 0.02 mg/mL in sterile medium and the two solutions were used as stocks to test samples for subdilution at 100, 50, 20, 10, 5, 2 and 1 µg/mL in triplicate in the wells of the microtiter plates. Higher concentrations of some tested compounds were required to determine the cytotoxic concentrations (CD_{50}).

Virus used in assay

The compounds were tested for antiviral activity against Herpes simplex type 1 (HS-1) grown on Vero African green monkey kidney cells.

Cultures

Herpes simplex type 1 (HS-1) was the gift of Dr. R. G. Hughes, Roswell Park Memorial Institute, Buffalo, NY. Virus stocks were prepared as aliquots of culture medium from Vero cells infected at a concentration of 1 virion per 10 cells and cultured for 3 days. They were stored at 80 °C. Working stocks were prepared by titering virus by serial dilution in culture medium and assaying in triplicate on Vero monolayers in the wells of the microtiter trays. Virus suspensions that gave about 30 plaques per culture well were stored at 4°C until used. Vero African green monkey kidney cells were purchased from Viromed Laboratories, Minnetonka, MN, and were grown in Dulbeccos modified Eagles medium supplemented with 10% (v/v) calf serum (HyClone Laboratories, Ogden, UT), 60 µg/mL Penicillin G and 100 µg/mL streptomycin sulfate. Cells and viruses were cultured at 37°C in a humidified atmosphere containing about 15% (v/v) CO_2 in air. All medium components were obtained from Sigma Chemical Co., St. Louis, MO, unless otherwise indicated. Vero stocks were maintained at 34°C in culture flasks filled with medium supplemented with 1% (v/v) calf serum.

Subcultures for virus titration or antiviral screening were grown in the wells of the microtiter trays (Falcon Microtest III 96-wells trays, Becton Dickinson Labware, Lincolin Park, NJ) by suspending Vero cells in medium following trypsin-EDTA treatment, counting the suspension with a hemocytometer, diluting in medium containing 10% (v/v) calf serum to 2×10^4 cells per 200 µL culture, aliquoting into each well of a tray and culturing until confluent.

Procedure

Microtiter trays with confluent monolayer cultures of Vero cells were inverted, the medium was shaken out, and replaced with serial dilutions of sterile samples in triplicate in 100 µL of medium followed by titered virus in 100 µL medium containing 10% (v/v) calf serum in each well. In each tray, the last row of wells was reserved for controls that were untreated with compounds or virus. The trays were cultured for 66 hours. The trays were inverted onto a pad of paper towels, the remaining cells rinsed carefully with medium, and fixed with 3.7% (v/v) formaldehyde in saline for at least 20 minutes. The fixed cells were rinsed with water, stained with 0.5% crystal violet in 20% aqueous ethanol for 30 minutes, rinsed with water, and examined visually. Antiviral activity was identified as confluent, relatively unaltered monolayers of stained Vero cells treated with HS-1. Antiviral activity was estimated as the lowest concentration that caused the indicated percent reduction in the number of virus plaques. Cytotoxicity was estimated as the concentration that caused approximately 50% loss of the monolayer present around the plaques caused by HS-1.

Results of Structure-activity relationship (SAR) of newly prepared compounds towards different groups of microorganisms

The compounds under study were tested against the following various types of bacteria: 2 G+ve bacteria (*Bacillus subtillis* and *Staphylococcus aureus*, which represent bacilli and cocci, respectively) and 4 G-ve bacteria (Pseudomonas spp., Proteus spp., Salmonella typhi and *Escherichia coli*; all are bacilli). The anti fungal activities of these compounds were tested using 2 yeast strains (Saccharomyces cerevisiae and Candida albicans, which represent non-pathogenic and pathogenic yeast, respectively) and 2 filamentous fungi (Aspergillus niger and Aspergillus terreus). As all compounds were dissolved in warm DMSO, the first step was to ensure that DMSO has no antimicrobial activities against the test microorganisms. As shown in Table II, DMSO showed no inhibition zone in all petri dishes inoculated by different microorganisms. Therefore, the presence of inhibition zone (the antimicrobial activities) of these compounds did not interfere with the solvent used (DMSO). The results in Table II show the inhibition zone in mm which represents the antimicrobial activities of different compounds. Coumarin had no effect on G+ve bacteria but showed antimicrobial activity against two G-ve bacteria (Pseudomonas spp. and Proteues spp.). On the other hand, coumarin inhibited the growth of all Eukaryotic cells used (Yeast and Filamentous fungi) to different extents. The starting compound of this study was 4-hydroxycoumarin, which showed antimicrobial activities against G+ve bacteria (Staphylococcus aureus), G-ve bacteria (Pseudomonas spp. and Proteus spp.), and yeast and fungi (Saccharomyces cerevisiae, Aspergillus niger and Aspergillus terreus). In general, the antimicrobial activities of the ethyl ester, carbohydrazide of 4-hydroxycoumarin (**1,2**) and hydrazones (**3-20**) were shifted in the case of G+ve bacteria from Staph. aureus to *B. subtilis* and in the case of G-ve bacteria from *Pseudomonas* spp. and *Proteus* spp. to *S. typhi* and *E. coli*, respectively. On the other hand, these compounds showed no antimicrobial activities against all eukaryotic microbes (yeast and filamentous fungi). The loss of the antimicrobial activities of these compounds against eukaryotic cells may be because of the increased molecular weight of the compound. The eukaryotic cell is characterized by more complex cell wall structure than the bacterial cell. Both yeast and filamentous fungi have a chitinized cell wall structure which decreases the permeability of the tested chemicals and therefore, becomes less effective against cell components. Therefore, we attributed the loss of these compounds activities to the reduced permeability compared to the start (4-hydroxycoumarin). However, the *o*-substituents of compounds **8, 10,** and **11** decreased the antimicrobial activities of these compounds towards the tested microorganisms compared to its *p*-substituents of the same compounds. With some exceptions, the compounds **21-33** were less active against the microorganisms than their start compounds (hydrazones). The decreased antimicrobial activity of these compounds may due to their cyclization.

Results of antiviral and cytotoxic activities

The prepared compounds were tested with an improved plaque-reduction assay at the University of Minnesota for their possible antiviral and cytotoxic activities. Plaque reduction assays typically use a monolayer of cultured host cells which are allowed to bind the virus, and are then overlaid with a layer of medium thickened with agar or another thickener which makes plaque formation possible by preventing mixing due to currents in the medium. Samples to be tested for antiviral activity are either incorporated into the thickened layer or absorbed in a paper disc laid on the thickened layer. The thickened layer can cause several types of technical problems. Shier (Hufford *et al.*,1991) modified this approach to allow the production of acceptable Herpes simplex type I (HS-1) plaques

Table II. Antimicrobial activites of newly prepared compounds on different microorganisms

Comp. No.	G+ve Bacteria		G-ve Bacteria				Yeast		Filamentous fungi	
	Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas spp.*	*Proteus spp.*	*Salmonella typhi*	*Escherichia coli*	*Saccharomyces cerevisiae*	*Candidia albicans*	*Aspergillus niger*	*Aspergillus terreus*
	(mm) Diameter of inhibition zone									
DMSO	–	–	–	–	–	–	–	–	–	–
Coumarin	–	–	1.1	1.2	–	–	1.8	1.0	1.7	1.6
4-Hydroxycoumarin	–	1.5	1.5	2.2	–	–	0.9	–	1.1	0.8
1	1.5	–	–	–	1.4	1.1	–	–	–	–
2	1.3	–	–	–	1.2	-	–	–	–	–
3	1.3	–	–	–	1.5	1.1	–	–	–	–
4	1.3	–	–	–	1.4	-	–	–	–	–
5	1.4	–	–	–	1.6	1.2	–	–	–	–
6	1.3	–	–	–	1.5	1.2	–	–	–	–
7	1.3	–	–	–	1.6	1.2	–	–	–	–
8	1.1	–	–	–	–	-	–	–	–	–
9	1.4	–	–	–	1.6	1.2	–	–	–	–
10	–	–	–	–	–	-	–	–	–	–
11	1.2	–	–	–	–	-	–	–	–	–
12	1.3	–	–	–	–	1.0	–	–	–	–
13	1.1	–	–	–	–	–	–	–	–	–
14	-	–	–	–	–	–	–	–	–	–
15	1.3	–	–	–	–	–	–	–	–	–
16	1.3	–	–	–	1.3	–	–	–	–	–
17	1.3	–	–	–	1.5	–	–	–	–	–
18	–	–	–	–	1.4	–	–	–	–	–
19	–	–	–	–	1.5	–	–	–	–	–
20	–	–	–	–	-	–	–	–	–	–
21	–	–	–	–	-	–	–	1.1	–	–
22	–	–	–	–	-	–	–	–	–	–
23	1.5	–	–	1.3	-	1.4	1.7	–	1.4	–
24	–	–	–	–	1.2	–	–	1.0	–	–
25	–	–	–	–	-	–	–	-	–	–
26	–	–	–	–	-	–	–	0.9	–	–
27	–	1.1	–	1.6	1.5	–	–	-	–	–
28	–	–	–	–	-	–	–	-	–	–
29	1.1	–	–	1.1	1.3	–	–	-	–	–
30	1.3	–	–	1.2	–	1.1	–	1.2	–	–
31	–	–	–	–	–	–	–	-	–	–
32	1.4	–	–	–	2.0	1.5	–	-	–	–
33	1.3	–	–	–	1.8	-	–	-	–	–

without the use of thickening. In his assay, Shier reduced the assay size to fit the wells of 96-well microtiter trays, and he also used serial dilutions of samples in parallel wells in a tray which allows an estimation of end-point concentrations for antiviral agents. Shiers modifications reduced the sample size and the costs, and eliminated the potential for interference by thickening agents. At the same time this assay retains the ability to estimate the cytotoxicity which is reflected as loss of the cell monolayer in which the plaques are normally formed. The modified assay was used to examine our isolated compounds. Aphidicolin was used as a positive control. The compounds

were tested against (HS-1) grown on Vero African green monkey kidney cells. Each compound exhibited some cytotoxicity. In general, compounds **23**, **27**, and **29** showed moderate activity. Compound **23** had the highest activity among all the compounds in this studyand was able to reduce the number of plaques by 30% at a concentration of 0.12 mg/mL. Compounds **1**, **2**, **3**, **4**, **5**, **6**, **7**, **9**, **16**, **17**, **24**, and **33** exhibited weak antiviral activity, as shown in Table III. On the other hand, compounds **8**, **10**, **11**, **13**, **15**, **18**, **19**, **20**, **21**, **22**, **25**, **26**, **31**, and **32** didnt show any reduction in the number of plaques at the same concentration (0.12 mg/mL). The carbohydrazide (**2**) reduced the number of plaques by 20% at 0.2 mg/mL while the hydrazones **5**, **9**, and **17** and the *N*-acetyloxadiazoline derivative **33** reduced the number of plaques by 19%, 15%, 13% and 12%, respectively, at the same concentration (0.2 mg/mL).

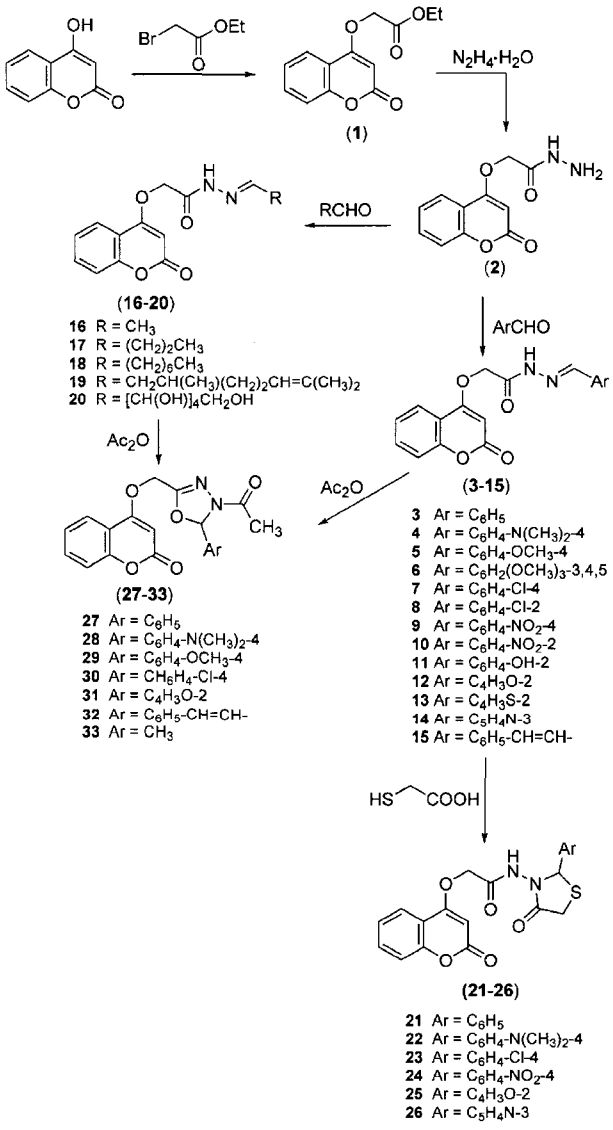
From this study, CD_{50} of compounds **2**, **3**, **5**, **7**, **9**, **17**, **23**, **27**, **29**, and **33** was in a range of 0.02-0.04 mg/mL; i.e. good cytotoxic activity. These compounds may be used as potential anticancer agents.

RESULTS AND DISCUSSION

The parent compound 4-hydroxycoumarin was allowed to react with ethyl bromoacetate in the presence of anhydrous potassium carbonate to give ethyl (coumarin-4-oxy) acetate (**1**). The ester (**1**) reacted with an equimolecular amount of hydrazine hydrate at room temperature to produce coumarin-4-oxyacetic hydrazide (**2**). The carbohydrazide (**2**) was allowed to condense with some aromatic and heterocyclic aldehydes, namely, benzaldehyde, 4-dimethylaminobenzaldehyde, 4-methoxybenzaldehyde, 3,4,5-trimethoxybenzaldehyde, 4-chlorobenzaldehyde, 2-chlorobenzaldehyde, 4-nitrobenzaldehyde, 2-nitrobenzaldehyde, salicylaldehyde, furfural, thiophen-2-carboxaldehyde, nicotinaldehyde and cinnamaldehyde, to produce the corresponding *N*-arylidene coumarin-4-oxyacetic hydrazones (**3-15**). Also, the carbohydrazide (**2**) was condensed with some aliphatic aldehydes, namely, acetaldehyde, butyraldehyde, octaldehyde, citronellal and D-mannose, to give the corresponding *N*-alkylidene coumarin-4-oxyacetic hydrazones (**16-20**). The hydrazones (**3**, **4**, **7**, **9**, **12**, **14**)

Table III. Results of Antiviral/Cytotoxicity testing

Compound No	% Reduction in number of plaques	Minimum antiviral Conc. (mg/mL)	Cytotoxicity (CD_{50}) mg/mL
Aphidicolin (standard)	100	0.005	0.20
a-Moderately Active Compounds			
23	30	0.12	0.02
27	28	0.14	0.02
29	29	0.13	0.03
b-Weakly Active Compounds			
1	20	0.21	0.01
2	20	0.20	0.03
3	18	0.21	0.02
4	19	0.22	0.01
5	19	0.20	0.03
6	19	0.21	0.01
7	16	0.23	0.03
9	15	0.20	0.02
16	15	0.24	0.01
17	13	0.20	0.02
24	12	0.23	0.01
33	12	0.20	0.03



Scheme 1. Synthesis of thiazolidinone and oxadiazoline

were allowed to react with mercaptoacetic acid to produce the corresponding 2-substituted-3-[(coumarin-4-oxy) acetamido]thiazolidin-4-ones (**21-26**). On the other hand, the hydrazones (**3, 4, 5, 7, 12, 15, 16**) were refluxed with acetic anhydride to give the corresponding 2-[(coumarin-4-oxy)methyl]-4-acetyl-5-substituted-Δ^2-1,3,4-oxadiazolines (**27-33**) according to Scheme I. The IR, ^{1}H NMR and MS spectra of all the prepared compounds (1-33) are shown in Table IV.

ACKNOWLEDGEMENT

The authors would like to express their thanks to Dr. Hisham El-Anshasy, Researcher, Genetic Engineering and Biotechnology Research Institute, Mubarak City of Scientific Research and Technology Application, for his

Table IV. IR, ^{1}H NMR and MS spectra of the prepared compounds (**1-33**)

Comp. No	IR spectra (KBr, ν cm^{-1})	^{1}H-NMR spectra (DMSO-d_6, δ ppm)	m/z (rel. int. %)
1	(C=O ester) 1755, (C=O α-pyrone) 1703, (C-O) 1215	7.85 (1H, d, H-8), 7.65 (1H, t, H-5), 7.40 (2H, t, H-6 and H-7), 5.90 (1H, s, H-3), 5.10 (2H, s, OCH_2), 4.20 (2H, q, CH_2) and1.25 (3H, t, CH_3).	M^+ 248 (100), $(M+1)^+$ 249.1 (39), 220 (14), 203 (7) , 175 (14), 162 (16).
2	(NH acid hydrazide) 3302, 3203, (C=O α-pyrone)1685, (C=O acid hydrazide) 1650, (C-O) 1250	9.50 (1H, s, NH), 8.05 (1H, d, H-8), 7.65 (1H, t, H-5), 7.40 (2H, t, H-6 and H-7), 5.85 (1H, s, H-3), 4.75 (2H, s, OCH_2) and 4.40 (2H, s, NH_2).	M^+ 234.1 (100) , $(M+1)^+$ 235. 1 (28), 217 (19) , 202.9 (9) , 174.9 (6) , 162 (7) .
3	(N-H) 3195, (C=O α-pyrone) 1720, (C=O amide) 1687, (C=N) 1628	11.75 (1H, s, NH), 8.30, 8.00 (1H, 2s, N=CH), 7.95-7.45 (9H, aromatic protons), 5.90 (1H, s, H-3) and 5.45, 4.95 (2H, 2s, OCH_2).	M^+ 322 (28), (M + 1)$^+$ 323 (10), 264 (15), 235 (3), 219 (12), 177 (100), 162 (8), 161.1 (13).
4	(N-H)3200, (C=O α-pyrone) 1724, (C=O amide) 1684, (C=N) 1625	11.50, 11.40 (1H, 2s, NH), 8.20, 7.90 (1H, 2s, N=CH), 7.95-6.75 (8H, aromatic protons), 5.90, 5.85 (1H, 2s, H-3), 5.45, 4.95 (2H, 2s, OCH_2) and 3.00 [6H, s, $N(CH_3)_2$].	M^+ 365.1 (100), (M + 1)$^+$ 366.1 (23), 306.3 (3), 234.3 (1), 218.3 (1), 190.1 (2), 175.3 (4), 162.2 (4),160.1 (2).
5	(N-H)3190, (C=O α-pyrone) 1720, (C=O amide) 1682, (C=N) 1626	11.65 (1H, s, NH), 8.25, 8.00 (1H, 2s, N=CH), 7.90-7.00 (8H, aromatic protons), 5.90 (1H, s, H-3), 5.45, 4.95 (2H, 2s, OCH_2) and 3.85 (3H, s, OCH_3).	M^+ 352.1 (100), (M + 1)$^+$ 353.1 (37), 294.2 (17), 266.2 (4), 234.1 (5), 218.2 (2), 191.1 (2), 177.1 (17), 162 (2), 161 (1).
6	(N-H)3210, (C=O α-pyrone) 1715, (C=O amide) 1684, (C=N) 1624	11.80, 11.65 (1H, 2s, NH), 8.25, 7.95 (1H, 2s, N=CH), 7.90-7.00 (6H, aromatic protons), 5.90, 5.85 (1H, 2s, H-3), 5.50, 5.00 (2H, 2s,OCH_2), 3.90 (3H, d, OCH_3), 3.80 (3H, s, OCH_3) and 3.70 (3H, s, OCH_3).	M^+ 412.1 (100), (M + 1)$^+$ 413.1 (37), 354.1 (3), 251.1(7), 237 (4), 235 (2), 221(3), 219 (1), 209.1 (10),193 (53), 178 (37), 177 (13), 163 (8).
7	(N-H)3195, (C=O α-pyrone) 1714, (C=O amide) 1685, (C=N) 1626, (C-Cl) 764	11.80 (1H, s, NH), 8.30, 8.00 (1H, 2s, N=CH), 7.95-7.40 (8H, aromatic protons), 5.90 (1H, s, H-3) and 5.45, 4.95 (2H, 2s, OCH_2).	M^+ 356, 358 (87, 21), (M + 1)$^+$ 357.1 (31), 296.8, 298.9 (7,2), 263.2 (2), 234.2 (3), 219 (8), 195, 197.1 (18, 6), 176.6 (100),162.2 (16), 161.4 (20).
8	(N-H)3205, (C=O α-pyrone) 1716, (C=O amide) 1691, (C=N) 1628, (C-Cl) 754	11.95 (1H, s, NH), 8.70, 8.40 (1H, 2s, N=CH), 8.10-7.45 (8H, aromatic protons), 5.95 (1H, s, H-3) and 5.50, 5.00 (2H, 2s, OCH_2).	M^+ 357, 359.1 (14, 8), (M + 1)$^+$ 358 (5), 264.1 (4), 218.1 (18),195, 197.1 (28, 8), 176.9 (100), 162.2 (5),161.5 (1).
9	(N-H)3200, (C=O α-pyrone)1707, (C=O amide) 1686, (C=N) 1626, (N=O) 1518, 1342	12.05, 12.00 (1H, 2s, NH), 8.45, 8.15 (1H, 2s, N=CH), 8.30-7.45 (8H, aromatic protons), 5.95, 5.90 (1H, 2s, H-3) and 5.55, 5.05 (2H, 2s, OCH_2).	M^+ 367.2 (4), $(M+1)^+$ 368.1 (77), 263 (24), 234 (3), 217 (3), 206 (35), 177 (100), 163 (31), 160 (9).
10	(N-H)3210, (C=O α-pyrone) 1716, (C=O amide) 1692, (C=N) 1626, (N=O) 1522,1340	12.05 (1H, s, NH), 8.70, 8.40 (1H, 2s, N=CH), 8.15-7.45 (8H, aromatic protons), 5.95 (1H, s, H-3) and 5.50, 5.00 (2H, 2s, OCH_2).	M^+ 367.4 (7), $(M+1)^+$ 368.1 (25), 263.1 (3), 232.1 (9), 217.1 (4), 206 (12), 177 (100), 162 (16), 160 (2).
11	(N-H)3195, (C=O α-pyrone) 1718, (C=O amide) 1684, (C=N) 1624, (O-H) 3430	11.90, 11.70 (1H, 2s, NH), 10.95, 10.00 (1H, 2s, OH), 8.55, 8.35 (1H, 2s, N=CH), 8.00-6.90 (8H, aromatic protons), 5.90, 5.85 (1H, 2s, H-3) and 5.45, 5.00 (2H, 2s, OCH_2).	M^+ 338 (100), $(M+1)^+$ 339.1 (92), 280 (5), 218.1 (6), 176.9 (70), 163 (65), 160 (11).
12	(N-H)3190, (C=O α-pyrone) 1728, (C=O amide) 1685, (C=N) 1628	11.70, 11.60 (1H, 2s, NH), 8.20, 7.90 (1H, 2s, N=CH), 7.95-7.40 (4H, coumarin protons), 6.95, 6.65 (3H, furan protons), 5.95, 5.85 (1H, 2s, H-3) and 5.40, 4.95 (2H, 2s, OCH_2).	M^+ 312.1 (31), $(M+1)^+$ 313 (100), 254 (7), 225 (6), 218 (1), 202.9 (11), 177 (41), 162.1 (2), 151 (34), 110 (37).

Table IV. Continued

Comp. No	IR spectra (KBr, ν cm^{-1})	^{1}H-NMR spectra (DMSO-d_6, δ ppm)	m/z (rel. int. %)
13	(N-H)3180, (C=O α-pyrone) 1720, (C=O amide) 1685, (C=N) 1626	11.90, 11.70 (1H, 2s, NH), 8.50, 8.20 (1H, 2s, N=CH), 7.95-7.10 (7H, coumarin and thiophene protons), 5.85, 5.75 (1H, 2s, H-3) and 5.35, 4.95 (2H, 2s, OCH_2).	M^+ 328 (16), $(M+1)^+$ 329 (20), 270 (4), 219 (4), 203 (14), 176.9 (100), 167 (13), 162 (6), 126.1 (30), 110.1 (11), 96 (18).
14	(N-H)3195, (C=O α-pyrone) 1716, (C=O amide) 1689, (C=N) 1626	11.95, 11.90 (1H, 2s, NH), 8.95-7.45 (8H, coumarin and pyridine protons), 8.40, 8.05 (1H, 2s, N=CH), 5.95, 5.90 (1H, 2s, H-3) and 5.50, 5.00 (2H, 2s, OCH_2).	M^+ 323.1 (12), $(M + 1)^+$ 324.2 (27), 265.1 (21), 246 (55), 234.1 (14), 220.1 (8), 177 (100), 162 (29), 161 (15).
15	(N-H)3185, (C=O α-pyrone) 1724, (C=O amide) 1682, (C=N) 1628,(C=C) 1608	11.70, 11.60 (1H, 2s, NH), 8.05-6.90 (12H, N=CH, aromatic and vinylic protons), 5.90, 5.80 (1H, 2s, H-3) and 5.40, 4.95 (2H, 2s, OCH_2).	M^+ 348.1 (3), $(M + 1)^+$ 349.2 (2), 289.1 (2), 235.1 (2), 219 (4), 188 (15), 177 (61), 162 (8), 145 (36), 129 (99), 115 (100).
16	(N-H) 3199,(C-H aliphatic) 2966, (C=O α-pyrone) 1720, (C=Oamide) 1684, (C=N) 1628	11.45, 11.35 (1H, 2s, NH), 7.90-7.40 (4H, coumarin protons), 6.20 (1H, q, N=CH), 5.85, 5.75 (1H, 2s, H-3), 5.30, 4.90 (2H, 2s, OCH_2) and 1.85 (3H, d, CH_3).	M^+ 260 (8), $(M+1)^+$ 261 (100), 245 (18), 203 (7), 188 (6), 176.9 (25), 174.9 (10), 162.9 (36).
17	(N-H) 3247,(C-H aliphatic) 2958, (C=O α-pyrone) 1732, (C=Oamide) 1697, (C=N) 1626	11.35, 11.25 (1H, 2s, NH), 7.95-7.30 (4H, coumarin protons), 5.90-5.75 (2H, N=CH and H-3), 5.25, 4.85 (2H, 2s, OCH_2) and 2.10-0.90 (7H, aliphatic protons).	M^+ 288.2 (100), $(M+1)^+$ 289.2 (71), 257.1 (61), 245 (47), 230.1 (54), 203 (31), 188 (64), 176 (69), 163 (91), 162 (62), 126 (85).
18	(N-H) 3201,(C-H aliphatic) 2955, (C=O α-pyrone) 1726, (C=Oamide) 1685, (C=N) 1626	11.45, 11.35 (1H, 2s, NH), 8.05-7.40 (4H, coumarin protons), 5.90-5.75 (2H, N=CH and H-3), 5.25, 4.95 (2H, 2s, OCH_2) and 2.20-0.85 (15H, aliphatic protons).	M^+ 344.2 (8), $(M + 1)^+$ 345.2 (18), 260.1 (51), 245.1 (12), 235.2 (86), 220.2 (100), 203 (31), 188 (34), 175.1 (64), 162 (54), 109.1 (77), 95.1 (78), 81.1 (73). 54.9 (89).
19	(N-H) 3207,(C-H aliphatic) 2964, (C=O α-pyrone) 1734, (C=Oamide) 1682, (C=N) 1628, (C=C) 1608	11.35, 11.25 (1H, 2s, NH), 7.90-7.40 (4H, coumarin protons), 5.90-5.75 (2H, N=CH and H-3), 5.25, 4.90 (2H, 2s, OCH_2), 5.10 (1H, t, $CH=C(CH_3)_2$) and 2.30-0.90 (16H, aliphatic protons).	M^+ 370.3 (48), $(M + 1)^+$ 371.3 (66), 355.2 (5), 301.2 (5), 288.1 (12), 260.1 (8), 235.1 (13), 220.1 (12), 208.2 (19), 203.1 (6), 193.1 (12), 188 (7), 176.1 (11), 165.1 (14), 163 (100), 152.1 (34), 136.1 (84), 109.1 (24).
20	(N-H) 3200,(C-H aliphatic) 2954, (C=O α-pyrone) 1714, (C=Oamide) 1685, (C=N) 1658, (O-H) 3480-3200	11.50, 11.40 (1H, 2s, NH), 8.05-7.40 (4H, coumarin protons), 5.90-5.75 (2H, N=CH and H-3), 5.25, 4.90 (2H, 2s, OCH_2), 5.15 (1H, OH of CH_2OH), 4.50-4.25 (4H, 4 OH of 4 CHOH) and 4.00-3.25 (6H, aliphatic protons).	M^+ 395.9 (0.41), $(M + 1)^+$ 397.3 (0.46), 277.1 (2), 261.1 (2), 245.1 (7), 234 (19), 219.1 (100), 202 (7), 178 (14), 176 (11), 163 (26), 162.1 (11).
21	(N-H) 3190, (C=O α-pyrone) 1720, (C=O thiazolidinone) 1680, (C=O amide) 1616	10.65 (1H, s, NH), 8.05-7.35 (9H, aromatic protons), 5.90 (1H, s, H-3), 5.70 (1H, s, CH of thiazolidinone), 4.85 (2H, s, OCH_2) and 3.95, 3.80 (2H, dd, CH_2 of thiazolidinone).	M^+ 396.4 (13), $(M+1)^+$ 397.1 (100), 323.1 (9), 235.1 (4), 219.1 (4), 178 (50), 177 (20), 161.1 (6).
22	(N-H) 3201, (C=O α-pyrone) 1724, (C=O thiazolidinone) 1685, (C=O amide) 1618	10.45 (1H, s, NH), 8.15-7.40 (8H, aromatic protons), 5.90 (1H, s, H-3), 5.60 (1H, s, CH of thiazolidinone), 4.95 (2H, s, OCH_2), 4.00, 3.80 (2H, dd, CH_2 of thiazolidinone) and 3.00 (6H, s, $N(CH_3)_2$).	M^+ 439.4 (5), $(M+1)^+$ 440.4 (8), 365.1 (25), 321.2 (11), 234.2 (19), 220 (45), 219 (23), 176 (63), 161.9 (100).
23	(N-H) 3170, (C=O α-pyrone) 1734, (C=O thiazolidinone) 1670, (C=O amide) 1618, (C-Cl) 769	10.60 (1H, s, NH), 8.15-7.35 (8H, aromatic protons), 5.85 (1H, s, H-3), 5.70 (1H, s, CH of thiazolidinone), 4.85 (2H, s, OCH_2) and 4.00, 3.80 (2H, dd, CH_2 of thiazolidinone).	M^+ 431.1, 433.2 (6, 2), $(M+1)^+$ 432.2 (3), 357.1, 358.9 (6, 2), 232.4 (4), 217 (12), 212, 213.9 (100, 39), 177 (66), 163 (20).
24	(N-H) 3200, (C=O α-pyrone) 1735, (C=O thiazolidinone) 1689, (C=O amide) 1622, (N=O) 1522, 1344	10.70 (1H, s, NH), 8.20-7.35 (8H, aromatic protons), 6.00 (1H, s, H-3), 5.65 (1H, s, CH of thiazolidinone), 4.90 (2H, s, OCH_2) and 4.00, 3.80 (2H, dd, CH_2 of thiazolidinone).	M^+ 441.2 (13), 368.2 (19), 350 (16), 223.1 (58), 219 (20), 203 (21), 177 (100), 163 (38).
25	(N-H) 3201, (C=O α-pyrone) 1724, (C=O thiazolidinone) 1685, (C=O amide) 1618	10.45 (1H, s, NH), 8.05-7.40 (4H, coumarin protons), 6.95, 6.65 (3H, furan protons), 5.90 (1H, s, H-3), 5.65 (1H, s, CH of thiazolidinone), 4.95 (2H, s, OCH_2) and 3.95, 3.80 (2H, dd, CH_2 of thiazolidinone).	M^+ 386.3 (1), $(M+1)^+$ 387.1 (4), 319.1 (2), 313.1 (4), 234 (17), 220 (60), 176 (39), 167.9 (75), 162.9 (100).
26	(N-H) 3205, (C=O α-pyrone) 1724, (C=O thiazolidinone) 1685, (C=O amide)1620	10.45 (1H, s, NH), 8.95-7.40 (8H, pyridine and coumarin protons), 5.90 (1H, s, H-3), 5.60 (1H, s, CH of thiazolidinone), 4.95 (2H, s, OCH_2) and 4.00, 3.80 (2H, dd, CH_2 of thiazolidinone).	M^+ 397.1 (3), $(M+1)^+$ 398 (1), 339.1 (4), 324.1 (2), 220.1 (100), 179.1 (91), 177 (28), 162 (13).

Table IV. Continued

Comp No	IR spectra (KBr, ν cm^{-1})	^{1}H-NMR spectra (DMSO-d_6, δ ppm)	m/z (rel. int. %)
27	(C=O α-pyrone) 1714, (C=O amide) 1688, (C=N) 1624	8.50 (1H, s, CH of oxadiazoline), 8.05-7.05 (9H, aromatic protons), 5.90 (1H, s, H-3), 5.40 (2H, s, OCH_2) and 2.40 (3H, s, $COCH_3$).	M^+ 364 not detected, $(M + 1)^+$ 365 (0.1), 322.1 (1), 289.2 (4), 276 (12), 234.1 (14), 220.1 (18), 202.1 (34), 176 (42), 162(88), 120 (100), 92.1 (66).
28	(C=O α-pyrone) 1718, (C=O amide) 1685, (C=N) 1624	8.50 (1H, s, CH of oxadiazoline), 8.00-7.00 (8H, aromatic protons),5.90 (1H, s, H-3), 5.35 (2H, s, OCH_2), 3.00 (6H, s, $2CH_3$) and 2.40 (3H, s, $COCH_3$).	M^+ 407.2 (6), $(M + 1)^+$ 408.2 (3), 393.2 (22), 379.2 (13), 365.1 (9), 351.1 (11), 276 (25), 261.1 (10), 234.1 (26),220.1 (50), 203 (41), 176.1 (78), 162 (78), 132.1 (79), 120 (100).
29	(C=O α-pyrone) 1716, (C=O amide) 1708, (C=N) 1628	8.50 (1H, s, CH of oxadiazoline), 8.00-7.05 (8H, aromatic protons), 6.05 (1H, s, H-3), 5.45 (2H, s, OCH_2), 3.80 (3H, s, OCH_3) and 2.40 (3H, s, $COCH_3$).	M^+ 394.1 (49), $(M + 1)^+$ 395.1 (25), 352.1 (49), 276 (26), 233.1 (62), 203 (15), 177 (47), 163 (64), 150 (100), 120 (85).
30	(C=O α-pyrone) 1714, (C=O amide) 1685, (C=N) 1626, (C-Cl) 760	8.50 (1H, s, CH of oxadiazoline), 8.05-7.05 (8H, aromatic protons), 5.90 (1H, s, H-3), 5.35 (2H, s, OCH_2) and 2.40 (3H, s, $COCH_3$).	M^+ 399.1, 401.1 (22, 8), $(M + 1)^+$ 400.1 (9), 356, 358.1 (11, 3), 203 (26), 175 (50), 163 (20), 154, 156 (17, 5), 124, 126 (36, 12), 92.1 (100).
31	(C=O α-pyrone) 1705, (C=O amide) 1655, (C=N) 1616	8.50 (1H, s, CH of oxadiazoline), 7.85-7.40 (4H, coumarin protons), 6.95, 6.65 (3H, furan protons), 5.90 (1H, s, H-3), 5.40 (2H, s, OCH_2) and2.40 (3H, s, $COCH_3$).	M^+ 354.1 (0.2), $(M + 1)^+$ 355.1 (1), 312.1 (0.5), 276 (1), 234 (3), 220 (100), 203 (4), 192 (37), 176 (10), 163 (14).
32	(C=O α-pyrone) 1720, (C=O amide) 1685, (C=N) 1628, (C=C) 1510	7.95-6.95 (11H, aromatic and vinylic protons), 6.00 (1H, d, CH of oxadiazoline), 5.85 (1H, s, H-3), 5.40 (2H, s, OCH_2) and 2.40 (3H, s, $COCH_3$).	M^+ 390.1 (2), $(M + 1)^+$ 391.1 (13), 348.1 (99.9), 276 (9), 271 (29), 234 (4), 229.1 (13), 203 (20), 175 (29), 163 (91), 144 (100), 115.1 (86).
33	(C=O α-pyrone) 1718, (C=O amide) 1707, (C=N) 1624	7.85-7.35 (4H, coumarin protons), 5.80 (1H, s, H-3), 5.35 (2H, s, OCH_2), 3.75 (1H, q, CH of oxadiazoline), 2.30 (3H, s, $COCH_3$) and 1.85 (3H, d, CH_3).	M^+ 302.3 (0.04), $(M + 1)^+$ 303.3 (0.07), 286.1 (0.34), 276.2 (0.07), 259.2 (0.03), 234.1 (100), 220.1 (21), 203.1 (6), 175.1 (9), 162 (3).

help in the study of the structure-activity relationship (SAR) towards different groups of organisms of the newly prepared compounds of this study.

REFERENCES

Abbas, S. E., El-Ansary, S. L., and Mikhael, A. N., Synthesis of some 1,3,4 -oxadiazolines and thiazolidinones of expected antibacterial activity. *Egypt. J. Pharm. Sci.,* 35(1-6), 21-30 (1994).

Abde-Hamid, A. Z. and Fathalla, O. A., Effect of some new uracil derivatives on Ehrlich ascites carcinoma cells. *Egypt. J. Pharm. Sci.,* 34(1-3), 67-80 (1993).

Arora, R. B. and Mathur, C. N., Relationship between structure and anticoagulant activity of coumarin derivatives. *Brit. J. Pharmacol.,* 20, 29-35 (1963).

Chemielewska, I. and Cieslak, J., Vitamins and antivitamin K. Tautomerism of dicoumarol. *Tetrahedron,* 4,135-146 (1958).

Choubey, V. N. and Singh, H., Synthesis of fungistatic thiazolidinones. *Bull. Chem. Soc. Japan* 43(7), 2233-2236 (1970); C.A., 73, 77120d (1970).

Dalla Via, L., Gia, O., Marciani Magno, S., Santana, L.,Teijeira, M. and Uriarte, E., New tetracyclic analogues of photochemotherapeutic drugs 5-MOP and 8-MOP: synthesis, DNA interaction, and antiproliferative activity. *J. Med. Chem.,* 42(21), 4405-4413 (1999).

Egorov, N. S., "Antibiotics: A Scientific Approach", Mir Publisher, Moscow, (1985).

El-Bendary, E. R. and Badria, F. A., Synthesis, DNA-binding and antiviral activity of certain pyrazolo[3,4 - d]pyrimidine derivatives. *Arch. Pharm. Med. Chem.,* 333(4), 99-103 (2000).

El-Sherbeny, M. A., Badria, F. A., and Kheira, S. A., Synthesis of some new[4,5-d]pyrimidine derivatives and evaluation of their antifungal, antiviral and cytotoxic activities. *Med. Chem. Res.,* 6, 28-39 (1996).

El-Sherbeny, M. A., El-Ashmawy, M. B., El-Subbagh, H. I., El-Emam, A. A., and Badria, F. A., Synthesis, antimicrobial and antiviral evaluation of certain thienopyrimidine derivatives. *Europ. J. Med. Chem.,* 30, 445-449 (1995).

El-Subbagh, H. I., Abu-Zaid, S. M., Mahran, M. A., Badria, F. A., and Al-obaid, A. M., Synthesis and biological evaluation of certain alpha, beta- unsaturated ketones and their corresponding fused pyridines as antiviral and cytotoxic agents. *J. Med. Chem.,* 43(15), 2915-2921 (2000).

El-Subbagh, H. I., El-Nagar, W. A., and Badria, F. A., Synthesis and biological testing of certain thiazole derivatives as

potential cytotoxic agents. *Sci. Pharm.,* 62, 237-245 (1994).

El-Subbagh, H. I., El-Sherbeny, M. A., Nasr, M. N., Goda, F. E., and Badria, F. A., Novel diarylsulphide derivatives as potential cytotoxic agents. *Bull. Chim. Faraceutico,* 134(2), 80-84 (1995).

Erbring, V. H., Uebel, H., and Vogel, G., Zur chemie, pharmakologie und toxikologie von visnadin. *Arzneim. Forsch.,* 17, 283-287 (1967).

Fathy, N. M., Abdel-Motti, F., and Abdel-Megeid, F. M. E., Synthesis of some thienoquinoline derivatives with expected pharmacological activity. *Egypt. J. Pharm. Sci.,* 31(1-4), 375 - 383 (1990); C.A. 114, 101767h (1991).

Hufford, C. D., Badria, F. A., Abou-Karam, M. Shier, W. T., and Rogers, R. D., Preparation, characterization and antiviral activity of microbial metabolites of stemodin. *J. Nat. Prod.,* 54(6),1543 (1991).

Kamdar, G. C., Chavda, A. C., and Parikh, A. R., Preparation and antibacterial activity of 2-phenyl-3-(2',4'- diarylamino-*s*-triazin-6-yl)-5/H/CH_3-carboxymethyl-4-thiazolidinone and 2-aryl-3-(*p*-aminosulphophenyl/2'-pyrimidylaminosulphophenyl)-*p*-carboxymethyl-4-thiazolidinones. *J. Indian Chem. Soc.,* 64 (5), 298-301 (1987).

Matsumoto, A. and Hanawalt, P. C., Histone H3 and heat shock protein GRP78 are selectively cross-linked to DNA by photoactivated gilvocarcin V in human fibroblasts. *Cancer Res.,* 60(14), 3921-3926 (2000).

Meher, S. S., Naik, S., Behera, R. K., and Nayak, A., Studies on thiazolidinones. Part XI: Synthesis and fungitoxicities of thiazolidinones, thiohydantoins and their derivatives derived from thiosemicarbazones. *J. Indian Chem. Soc.,* 58(3), 274-276 (1981).

Nagesam, M., Raju, K. M., and Raju, M.S., Synthesis and antibacterial activity of 4,6-dimethyl-3-substituted aminomethylcoumarins. *Chim. Acta Turc.,* 17(2), 255-262 (1989); C.A. 114, 61883j (1991).

Popp, F. D., Synthesis of potential anticancer agents. II. Some schiff bases. *J. Org. Chem.* 26, 1566-1568 (1961).

Rao, V. R., Rao, T. V. P., Rao, B. R., and Reddy, Y. D., Studies on coumarin derivatives. Part III. Synthesis and biological activity of some 2-[aryl/heteroarylcarbonylmethylthio]-6-methyl-8*H*-pyrano[2,3-e]benzoxazol-8-one. *Ind. J. Chem.,* sect. B, 25B(3), 332-333 (1986); C.A. 106,84453t (1987).

Sagitullin, R. S. and Kost, A. N., Reactions of the hydrazine derivatives. XXV. Action of acetic anhydride and polyphosphoric acid on acylhydrazones. *Vestnik Moskov. Univ., Ser. Mat., Mekh., Astron., Fiz. I Khim.,*14(4), 187-193 (1959); cf. C.A. 53, 21894d (1959); C.A. 54, 14553c (1960).

Selleri, R., Orzalesi, G., Caldini, O., and Spano, R., New coumarin derivatives with saluretic and choleretic activity. *Arzneim. Forsch.,* 16, 1073 (1966).

Seo, E. K., Chai, H. B., Chagwedera, T. E., Farnsworth, N. R., Cordell, G. A., Pezzuto, J. M., and Kinghorn, A D., 5-(4-Hydroxyphenthenyl)-4,7-dimethoxycoumarin, a new constituent of Monotes engleri. *Planta Med.,* 66(2), 182-184 (2000).

Singh, R., Singh, R. P., Malik, Om. P., and Makarandi, J. K., Synthesis and antifungal activity of some alkylsubstituted 4-(substituted phenoxymethyl)-2*H*-1-benzopyran-2-one. *Ind. J. Chem.,* sect. B, 28B(11), 996-998 (1989); C.A.112, 178571f (1990).

Smith, E., Hosansky, N., Bywater,W.G., and Tamelen, E.E.V., Constitution of samidin, dihydrosamidin and visnadin. *J. Am. Chem. Soc.,* 79, 3534-3540 (1957).

Valenti, P., Rampa, A., Recanatini, M., Bisi, A., Belluti, F., Da Re, P., Carrara, M., and Cima, L., Synthesis, cytotoxicity and SAR of simple geiparvarin analogues. *Anticancer Drug Des.,* 12(6), 443-451 (1997).

Weber, U. S., Steffen, B., and Siegers, C. P., Antitumor-activities of coumarin, 7-hydroxycoumarin and its glucuronide in several human tumor cell lines. *Res. Commun. Mol. Pathol. Pharmacol.*, 99(2), 193-206 (1998).