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DNA Binding, Antiviral Activities and Cytotoxicity of New Furochromone and Benzofuran Derivatives

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Bromination of visnagin (**1**) afforded 9-bromovisnagin (**2**) which on its alkaline hydrolysis afforded the 3-acetyl benzofuran derivative (**3**). The condensation of (**3**) with hydrazine hydrate, phenylhydrazine and/or hydroxylamine hydrochloride afforded the corresponding pyrazole derivatives (**4a**, **b**) and isoxazole derivative (**4c**). On the other hand, when compound **3** was condensed with some aromatic aldehydes, this yielded corresponding α, β-unsaturated keto derivatives (**5a-e**). Furthermore, when **1** was subjected to chlorosulfonation, the visnagin-sulfonylchloride derivative **6** was afforded, which on amidation using morpholine, a sulonamido derivative (**7**) was obtained. Alkaline hydrolysis of the latter compound yielded 7-N-morpholinosulsamidobenzofuran (**8**) which was condensed with some aromatic aldehydes to yield the corresponding chalcone compounds (**9a-e**). Demethylation of visnagin afforded norvisnagin (**10**). The reaction of **10** with ethylbromoacetate in dry acetone yielded the ester benzopyran derivative (**11**) which reacted with hydrazine hydrate to afford the corresponding hydrazide derivative (**12**) and this was condensed with 3,4,5-trimethoxybenzaldehyde to give the corresponding hydrazone (**13**). A thaizolidinone derivative (**14**) was obtained by condensation of (**13**) with thioglycolic acid. Chloromethylation of norvisnagin afforded a 4-chloromethyl derivative (**15**) which reacted with different primary and secondary amines to yield the corresponding ethylamino derivative (**16a, b**). Moreover, mannich bases (**16a, b**) and (**17a-c**) were obtained by reacting norvisnagin with different primary and secondary amines in the presence of formalin but benzoylation of (**16a**, **b**) and (**17a-c**) afforded 4-oxybenzoyl derivative (**18a-e**). The prepared compounds were tested for their interaction with DNA; bromovisnagin **2** showed the highest affinity and compounds **6**, **15**, **8a**, **> 14**, **> 16b**, **17a**, and **16a** showed moderate activity in decreasing potency. Moreover, compound **2** also was the most active as antiviral agent toward HS-I virus and compounds **6**, **7**, **15**, **14**, **16a**, and **18a** were found to be moderately active. CD_{50} of the active compounds were also measured.

Key words: Visnagin, Norvisnagin, Pyrazolines, Isoxazolines, DNA affinity, Antiviral, Cytotoxicity

INTRODUCTION

Furochromones of *Ammi visnaga* such as visnagin have been known to exhibit many biological and physiological activities (Duarte et al., 1999, 2000; Ragab et al., 2007; Lee et al., 2010). Further, it is well known that some benzofuran derivatives show antiviral, antitumor (Rida et al., 2006; Galal et al., 2009), and DNA binding activities. In addition, some groups as pyrazole (Park et al., 2005; Bouabdallah et al., 2006; Tan et al., 2006), isoxazole (Kochetkov and Sokolov, 1963; Rescifina et al., 2006), chalcones (Dimmock et al., 1999; El-Subbagh et al., 2000; Kamal et al., 2008), Mannich bases (Shivarama Holla et al., 2003; Wenzel et al., 2010), sulfonamides (Scozzafava et al., 2003; García-Giménez et al., 2009) and thiazolidinones (Abd Elhafez et al., 2003; Rao et al., 2004; Sriram et al., 2005; Terzioğlu et al., 2006; Wu et al., 2006; Balzarini

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et al., 2007; Mijatovic et al., 2008; Lv et al., 2010) also exhibited different antiviral, antitumor, and DNA binding activities.

The mechanism of several known antitumor agents involves the interaction with DNA. Examples include alkylating agents (e.g. chlorabucil, cyclophosphamide, Mephalan, streptozocin), antitumor antibiotics (e.g. bleomycin, dexorubacin, mithramycin), and various other substances (e.g. cisplatin, chloroquine) (El-Sherbeny et al., 1995; El-Bendary and Badria, 2000; El-Subbagh et al., 2000). Based on the interaction of small molecular weight compounds with DNA, some short term procedures have been devised that are applicable for the discovery and evaluation of naturally occurring and synthetic compounds that function by this mechanism. All the previous findings prompted us to synthesize new derivatives of visnagin with the aforementioned functional groups: pyrazoles, isoxazoles, chalcones, Mannich bases, sulfonamides, and thiazolidinones to study their antiviral against herpes simplex type I virus as well as their cytotoxic and DNA binding activities.

MATERIALS AND METHODS

Melting points were determined with a Electrothermal IA 9000 apparatus and were uncorrected. Elemental microanalysis was performed on an Elementar, Vario EL, at the Microanalytical Center, National Research Center. The infrared spectra were recorded on a Nexus 670 FT-IR FT-Raman spectrometer using potassium bromide discs, at National Research Center. The proton nuclear magnetic resonance spectra were determined on a Varian mercury 300 MHz spectrometer, using tetramethylsilane (TMS) as an internal standard, at Faculty of Science, Cairo University and National Research Center. ^{13}Carbon nuclear magnetic resonance spectra were determined using ^{1}D-^{13}C NMR Bruker Jeol-EX 125 MHz spectrometer using TMS as an internal standard. The mass spectra were performed on Jeol JMS-AX500 mass spectrometer at National Research Center. The reactions were followed by thin layer chromatography (silica gel, aluminum sheets 60 F254, Merck) using benzene-ethyl acetate (8:2 v/v) as eluent and sprayed with iodine-potassium iodide reagent.

4-Methoxy-6-hydroxy-5-(3-methylpyrazol-5-yl)-7-bromobenzofuran derivatives (4a, b)

To a solution of 9-bromovisnagin (**2**) (0.3 g, 1 mmol) in 15 mL absolute ethanol, hydrazine hydrate or phenyl hydrazine (3 mmol) was added. The reaction mixture was refluxed for 2 h and then diluted with 15 mL of water. After cooling, the formed solid product was filtered off, air dried and crystallized (Table I).

4-Methoxy-6-hydroxy-5-(3-methylisoxazol-5-yl)-7-bromo benzofuran (4c)

A mixture of 9-bromovisnagin (0.3 g, 1 mmol) in 20 mL anhydrous pyridine and hydroxylamine hydrochloride (0.4 g, 6 mmol) was refluxed for 4 h. The reaction mixture was cooled and then acidified with dilute hydrochloric acid (10 mL) and the formed solid was filtered off, washed well with water, air dried and crystallized (Table I).

Chalcone derivatives (5a-e)

A solution of 7-bromovisnaginone (0.5 g, 1.8 mmol) in 10 mL absolute ethanol, one gram sodium hydroxide dissolved in 3 mL water and the appropriate aromatic aldehydes (1.8 mmol) namely, benzaldehyde, 4-chlorobenzaldehyde, 4-methoxybenzaldehyde, thiophenaldehyde, and buteraldehyde, was stirred for 1 h at room temperature and left overnight. After acidification of the reaction mixture with dilute hydrochloric acid, the formed solid was filtered off, washed with water, air dried and crystallized (Table I).

4-Methoxy-7-methyl-5-oxo-5H-7-methylfuro (3,2-g) (1) benzopyran-9-sulfonyl chloride (6)

Visnagin (**1**) (2.3 g, 10 mmol) was added portion-wise to monochlorosulfonic acid (3.4 mL, 5 mmol) within a period of 1 h while stirring at room temperature. After complete addition of visnagin, the reaction mixture was stirred 1 more h and then poured on ice-water. The solid formed was filtered off, washed with water, air dried and crystallized to give the title compound (El-Gamal et al., 1987) (Table I).

4-Methoxy-7-methyl-5-oxo-5H-furo (3,2-g)-9-N-morpholino-sulfonamido (1) benzopyran (7)

A mixture of visnagin-9-sulfonyl chloride (**6**) (3.28 g, 10 mmol) in 15 mL dioxin and morpholine (0.87 mL, 10 mmol) and a few drops triethylamine was refluxed for 2 h. The deposited product after cooling was filtered off, air dried to give the title compound (Mandour et al., 1994) (Table I).

4-Methoxy-5-acetyl-6-hydroxy-7-N-7-methyl-5-oxo-5H-furo (3,2-g)-9-N-morpholinosulfonamido benzofuran (8)

A solution of visnagin-9-morphilinosulfonamide (**7**) (0.38 g, 1 mmol) in 15% aqueous potassium hydroxide was refluxed for 1 h. The reaction mixture was cooled and acidified with dilute hydrochloric acid. The formed product was filtered, washed with water, air dried

Table I. Physical and analytical data of the prepared compounds

Comp. No.	M.P. (°C) (Solvent)	yield %	Mol. For (M.wt)	analysis calc/found %					
				C	H	N	S	Br	Cl
4a	208-10	57	$C_{13}H_{11}BrN_2O_3$	48.30	3.40	8.67		24.73	
	(ethanol)		(323.14)	48.56	3.63	8.69		24.69	
4b	235-37	61	$C_{19}H_{15}BrN_2O_3$	57.14	3.75	7.01		20.01	
	(ethanol)		(399.24)	57.02	3.62	6.92		20.09	
4c	189-90	50	$C_{13}H_{10}BrNO_4$	48.15	3.09	4.32		24.65	
	(ethanol)		(324.13)	48.37	3.28	4.31		24.92	
5a	158-60	90	$C_{18}H_{13}BrO_4$	57.91	3.4			21.41	
	(ethanol)		(373.2)	57.96	3.69			20.99	
5b	190-192	62	$C_{18}H_{12}BrClO_4$	53.01	2.95			19.60	8.70
	(methanol)		(407.64)	52.91	2.89			19.54	8.69
5c	175-177	70	$C_{19}H_{15}BrO_5$	56.58	3.72			19.82	
	(ethanol)		(403.22)	56.28	3.69			19.76	
5d	147-149	95	$C_{16}H_{11}BrO_4S$	50.66	2.90		8.46	21.07	
	(ethanol)		(379.23)	50.83	2.83		8.54	21.12	
5e	379-381	50	$C_{15}H_{15}BrO_4$	53.10	4.43			23.56	
	(methanol)		(339.18)	52.90	4.39			23.44	
6	187-89	30	$C_{13}H_9ClO_6S$						
	(methylen chloride)		(328.72)						
7	258-260	60	$C_{17}H_{17}NO_7S$						
	(ethanol)		379.38						
8	170-171	65	$C_{15}H_{17}NO_7S$						
	(ethanol)		(355.36)						
9a	123-125	58	$C_{22}H_{20}ClNO_7S$	55.29	4.19	2.93	6.71		7.42
	(methanol)		(477.91)	55.11	4.03	2.90	6.77		7.64
9b	179-80	60	$C_{23}H_{23}O_8S$	59.26	5.35	5.76	6.77		
	(methanol)		(473.5)	59.23	5.28	5.71	6.87		
9c	203-5	43	$C_{24}H_{26}N_2O_7S$	59.26	5.35	5.76	6.59		
	(ethanol)		(486.54)	59.23	5.28	5.71	6.76		
10	156-8	85	$C_{12}H_8O_4$						
	(glacial acetic acid)		(216.19)						
11	138-41	80	$C_{16}H_{14}O$	63.57	4.64				
	(ethanol)		(302.28)	63.49	4.72				
12	247-9	87	$C_{14}H_{12}N_2O_5$	58.33	4.16	9.70			
	(ethanol)		(288.26)	58.09	4.51	9.66			
13	237-40	53	$C_{24}H_{22}N_2O_8$	61.80	4.72	6.01			
	(ethanol)		(466.44)	61.69	4.43	6.23			
14	171-3	70	$C_{26}H_{24}N_2O_9S$	57.77	4.44	5.18	5.93		
	(ethanol)		(540.54)	57.94	4.66	5.49	5.87		
15	278-80	95	$C_{14}H_{11}ClO_4$						
	(chloroform)		(278.69)						
16a	262-5	65	$C_{14}H_{13}NO_4$	64.86	5.02	5.41			
	(ethanol)		(259.26)	64.89	5.08	5.36			
16b	286-8	61	$C_{18}H_{14}N_2O_4$	67.08	4.35	8.70			
	(ethanol)		(322.31)	67.09	4.33	8.69			
17a	165-7	85	$C_{18}H_{19}NO_4$						
	(ethanol)		(313.35)						
17b	184-6	65	$C_{17}H_{17}NO_5$						
	(ethanol)		(315.32)						
17c	157-9	66	$C_{17}H_{19}NO_4$						
	(ethanol)		301.34						
18a	188-90	75	$C_{21}H_{17}NO_5$	69.42	4.68	3.86			
	(ethanol)		(363.36)	69.39	4.66	3.89			
18b	312-14	82	$C_{25}H_{18}N_2O_5$	70.42	4.22	6.57			
	(ethanol)		(426.42)	70.38	4.13	6.49			
18c	318-20	81	$C_{25}H_{23}NO_5$	71.94	5.52	3.36			
	(ethanol)		(417.45)	71.89	5.53	3.39			
18d	320-22	75	$C_{24}H_{21}NO_6$	68.74	5.01	3.34			
	(ethanol)		(419.43)	68.69	5.03	3.29			
18e	290-2	62	$C_{24}H_{23}NO_5$	71.11	5.68	3.46			
	(ethanol)		(405.44)	71.09	5.63	3.39			

to give the title compound (Mandour et al., 1994) (Table I).

Chalcones derivatives (9a-c)

To a solution of visnaginone-7-N-morpholinosulfonamido (**8**) (0.71 g, 2 mmol) in 5 mL ethanol, 0.5 g sodium hydroxide in 3 mL water and (2 mmol) of the chosen aldehydes, namely, *p*-methoxybenzaldehyde, *p*-chlorobenzaldehyde, and *p*-N,Ndimethylaminobenzaldehyde was added. The reaction mixture was stirred for 1 h at room temperature and left overnight, then acidified with dilute hydrochloric acid. The precipitate formed was filtered off, air dried and crystallized (Table I).

4-Hydroxy-7-methyl-5-oxo-5H-furo (3,2-g) (1) benzopyran (norvisnagin) (10)

To a solution of visnagin (**1**) (1 g, 4.3 mmol) in 15 mL water, 15 mL concentrated hydrochloric acid was added. The reaction mixture was refluxed for 1 h where chromone dissolved and after 20 min, a precipitate began to form and the amount increased gradually. After cooling, the precipitate was filtered off and air dried to give the title compound (Schönberg and Aziz, 1953) (Table I).

4-Ethyl-oxyacetate-7-methyl-5-oxo-5H-furo (3,2-g) benzopyran (11)

To a solution of norvisnagin (**10**) (0.2 g, 1 mmol) and anhydrous potassium carbonate (0.28 g, 2 mmol) in 20 mL dry acetone, ethylbromoacetate (0.12 mL, 1.1 mmol) was added. The reaction mixture was refluxed while stirring for 8 h. The reaction mixture was filtered while hot and the residue was washed with 20 mL hot dry acetone. The filtrate was evaporated till dryness and the residue was triturated with 20 mL diethylether. The obtained solid was filtered off to give **11** (Table I).

4-Oxyacetic acid hydrazide-7-methyl-5-oxo-5-H-furo-(3,2-g) benzopyran (12)

To a solution of 98% hydrazine (0.1 mL, 2 mmol) in 20 mL absolute ethanol, a solution of the ester (**11**) (0.3 g, 1 mmol in 20 mL ethanol) was added dropwise while stirring. The reaction mixture was warmed in a water bath (60-70°C) for 6 h. The reaction mixture was evaporated *in vacuo* to 1/3 of its volume and the formed precipitate was filtered off, air dried and recrystallized (Table I).

4-Oxyacetic acid (3,4,5-trimethoxyphenylhydrazono)-7-methyl-5-oxo-furo (3,2-g) benzopyran (13)

A mixture of hydrazide (**12**) (0.23 g, 1 mmol) and 3,4,5-trimethoxybenzaldehyde (1 mmol) in 20 mL absolute ethanol containing a few drops of acetic acid was refluxed for 5-7 h. The reaction mixture was evaporated to 1/3 of its volume and the formed solid was filtered off, air dried to obtain the title compound (Table I).

4-Oxyacetamido-N-[3(3-thiazolidinyl-4-oxo-2(3,4,5-trimethoxyphenyl]-7-methyl-5-oxo-5H-furo-(3,2-g) benzopyran (14)

A mixture of the hydrazone (**13**) (0.5 g, 1 mmol) and mercaptoacetic acid (0.2 mL, 2 mmol) in 15 mL dry benzene was refluxed for 13 h and the reaction progress was checked by TLC. The reaction mixture was triturated with boiling water followed by diethyl ether (Table I).

4-Methoxy-5-oxo-7-methyl-9-chloromethylfuro (3,2-g) benzopyran (15)

Norvisnagin (**10**) (2 g, 9 mmol) was dissolved in 15 mL glacial acetic acid by gentle heating. To this solution, 25 mL concentrated hydrochloric acid and 10 mL of 40% formalin were added. The mixture was allowed to stand for 30 min at room temperature. The formed product was filtered off, washed with water and air dried (Hishmat et al., 1971) (Table I).

4-Hydroxy-5-oxo-7-methyl-9-methylaminomethyl (16a) and 9-methylamino (2-pyridyl) (16b) furo (3,2-g) benzopyrans

To a solution of (**15**) (0.26 g, 1 mmol) in 15 mL dry benzene, 1 mmol of the appropriate amines namely, ethylamine or 2-aminopyridine, was added. The reaction mixture was refluxed for 2 h. The reaction mixture was concentrated to half its volume. The formed product was filtered off and air dried (Table I).

Mannich bases (16a, b and 17a-c)

To a solution of norvisnagin (**10**) (0.2 g, 1 mmol) in 15 mL dry ethanol, 1 mmol of the appropriate amines namely, ethylamine, 2-aminopyridine, diethylamine, piperidine or morpholine and 0.2 mL of 40% formalin were added. The reaction mixture was refluxed for 1-2 h. The reaction mixture was concentrated to half its volume and left to cool. The formed product was filtered off, air dried and crystallized to obtain the Mannich bases **16a**, **b** and **17a-c** (Abdel-Alim and Aboulezz, 1986) (Table I).

4-Benzoyloxo-5-oxo-7-methyl-9-methylfuro (3,2-g) benzopyran derivatives (18a-e)

To a solution of 1 mmol of compounds **16a**, **b** and/or **17a-c** in 10 mL benzene, benzoyl chloride (0.23 mL, 2 mmol) and 0.5 g anhydrous potassium carbonate were

added. The reaction mixture was refluxed for 4-5 h, filtered while hot and the filtrate was evaporated until dry. Two drops of water and ethanol were added to remove any inorganic matter, the formed product was filtered off and air dried (Table I).

DNA affinity

DNA binding assay (DNA/compound using RP-TLC)

TLC plates (RP-18 F254; 0.25 mm; Merck) were redeveloped with MeOH-H_2O (8:2). Test compounds were then applied (5 mg/mL in MeOH) at the origin, followed by addition of DNA (1 mg/mL in H_2O and MeOH solution) at the same position on the origin. The plates were then developed with the same solvent system and the position of DNA was determined by spraying with anisaldehyde reagent which yields a blue color on reaction with DNA and the intensity of the color is proportional to the quantity of DNA added to the plate. Ethididium bromide was used as a positive control.

Colorimetric assay for compounds that bind to DNA

DNA/methyl green (20 ng Sigma) was suspended in 100 mL of 0.05 M Tris-HCl buffer, pH 7.5 containing 7.5 mM $MgHSO_4$ and stirred at 37°C with a magnetic stirrer for 24 h. Unless otherwise indicated, samples to be tested were dissolved in EtOH in Eppendorff tubes. Solvent was removed under vacuum and 200 μL of DNA-methyl green solution was added to each tube. The absorption maxima for DNA/methyl green complex is 642.5-645 nm. Samples were incubated in the dark at ambient temperature. After 24 h, the final absorbance of samples was determined. Readings were corrected for initial absorbance and normalized as a percentage of the untreated DNA-methyl green complex absorbance value. IC_{50} values were determined for each compound as shown in Table II.

Table II. Activity of compounds in methyl green/DNA displacement assay*

DNA active compounds	DNA/methyl green IC_{50}, μg/mL
2	28 ± 3
6	40 ± 1
15	41 ± 3
7	47 ± 1
17a	72 ± 2
14	68 ± 2
16a	70 ± 1
16b	84 ± 2
18a	51 ± 3
Remaining compounds	< 85

*Values represent the concentration (means ± S.D., n = 3 to 5 separate determinations) required to decrease the initial absorbance of the DNA/methyl green solution by 50%.

Antiviral and cytotoxic assays

Sample preparation

Samples were prepared for assay by dissolving in 50 mL of DMSO and diluting the aliquots into sterile culture medium at 0.4 mg/mL. These solutions were diluted again to 0.02 mg/mL in sterile medium and the two solutions were used as stocks to test samples at 100, 50, 20, 10, 5, 2, and 1 mg/mL in microtiter plates.

Virus assay and culture

The compounds were tested for antiviral activity against Herpes simplex type I (HS-I) grown in Vero African green monkey Kidney cells.

Herpes simplex type I (HS-I) was the gift of Dr. R. G. Hughes, Roswell Park Memorial Institute, Buffalo, NY. Virus stocks were prepared as aliquots in culture medium from Vero cells infected at multiplicity 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 assayed in triplicate on Vero monolayers in the wells of microtiter plates. Viral suspensions that gave about 30 plaques per well were stored at 4°C until use. Vero African green monkey kidney cells were purchased from Viromed Laboratories, Minnetonka, MN and grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) calf serum (Hyclone Laboratories), 60 mg/mL Penicillin G and 100 mg/mL streptomycin sulfate maintained at 37°C in a humidified atmosphere containing about 15% (v/v) CO_2 in air. All media components were obtained from Sigma Chemical Co., St. Louis, MO, unless otherwise indicated. Vero stocks were maintained at 34°C in cultured flasks filled with medium supplemented with 1% (v/v) calf serum. Subcultures for virus titration or antiviral screening were grown in microtiter plates (Falcon Microtest III 96-wells trays, Becton Dickinson Labware) by suspending Vero cells in medium following trypsin-EDTA treatment, diluting in medium containing 10% calf serum to 2×10^4 cells per in 200 mL culture, aliquoting into each well and cultured until confluency.

Procedures

Microtiter trays with confluent monolayer cultures with Vero cells were inverted, the medium was shaken out and replaced with serial dilutions of sterile extracts in triplicate in 100 μL medium followed by titered virus in 100 μL medium containing 10% (v/v)

calf serum. In each tray, the last row of wells was reserved for controls that were not treated with compounds or virus. The trays were cultured for 66 h. The trays were inverted onto a pad of paper towels; the remaining cells were rinsed carefully with medium and fixed with 3.7% (v/v) formaldehyde in saline for at least 20 min. The fixed cells were rinsed with water and examined visually. Antiviral activity was identified as confluent, relatively unaltered monolayers of stained Vero cells treated with HS-I. Cytotoxicity was estimated as the concentration that caused approximately 50% loss of the monolayer around the plaques caused by HS-I.

The prepared compounds were tested at the University of Minnesota for their possible antiviral activity against herpes simplex type I (HS-I) grown on Vero African green monkey kidney cells. An improved plaque-reduction assay was used to test the compounds using aphidicolin as the positive control (Table II). Plaque reduction assays typically use a monolayer of cultured host cells which are allowed to bind virus, then overlapped with a layer of a medium thickened with agar or another thicker 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 thickened agar or absorbed on a paper disc laid on the thickened layer. The thickened layer can cause several types of technical problems including toxicity of the thickener to virus or host cells and absorption or other types of interference with unknown antiviral agents being tested. Cytotoxicity was estimated as the loss of cell monolayers in which the plaques are normally formed (Table III).

Statistical analysis

The data are expressed as means ± S.E.M. Statistical analysis was carried out using a One-way Analysis of Variance "ANOVA" followed by Tukey-Kramer multiple comparison test. Statistical analysis was carried out using InStat 2 Computer Program (Graphpad software INC V2.04).

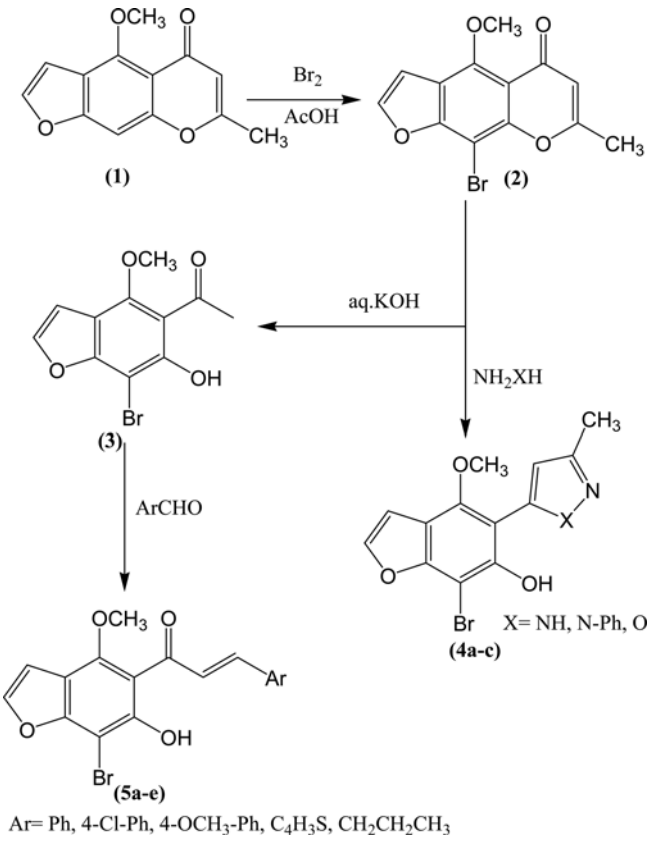
Table III. Antiviral and cytotoxicity for the tested compounds

Compound	% Reduction in plaque	Minimal antiviral conc. (mg/mL)	Cytotoxicity CD_{50} (mg/mL)
Aphidicolin	100	0.005	0.2
2	100	0.01	0.08
6	73	0.01	0.10
15	67	0.01	0.19
7	69	0.01	0.17
17a	none	0.01	0.15
14	59	0.01	0.19
16a	none	0.01	0.20
16b	55	0.01	0.20
18a	64	0.01	0.20

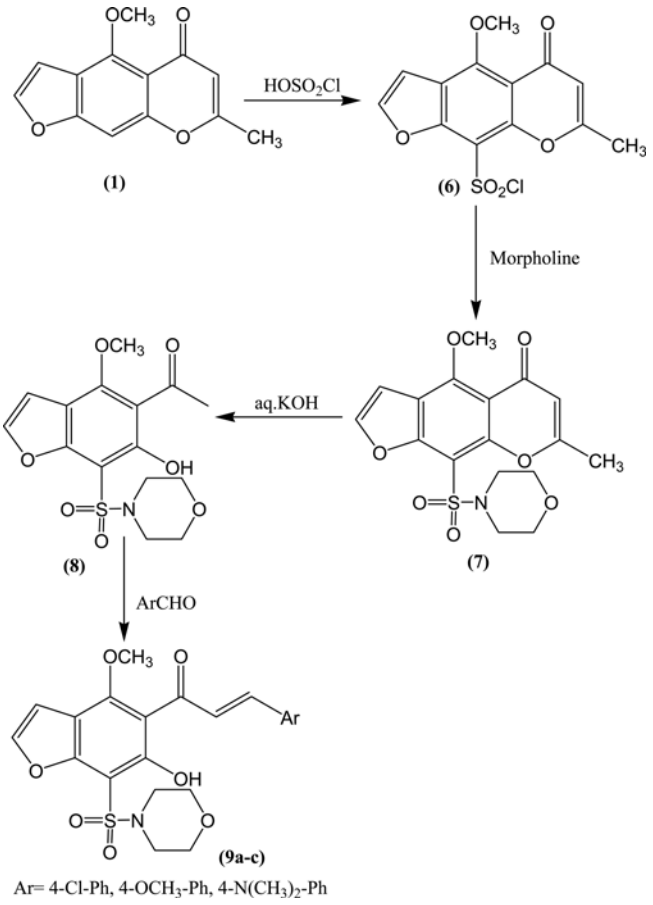
RESULTS AND DISCUSSION

Chemistry

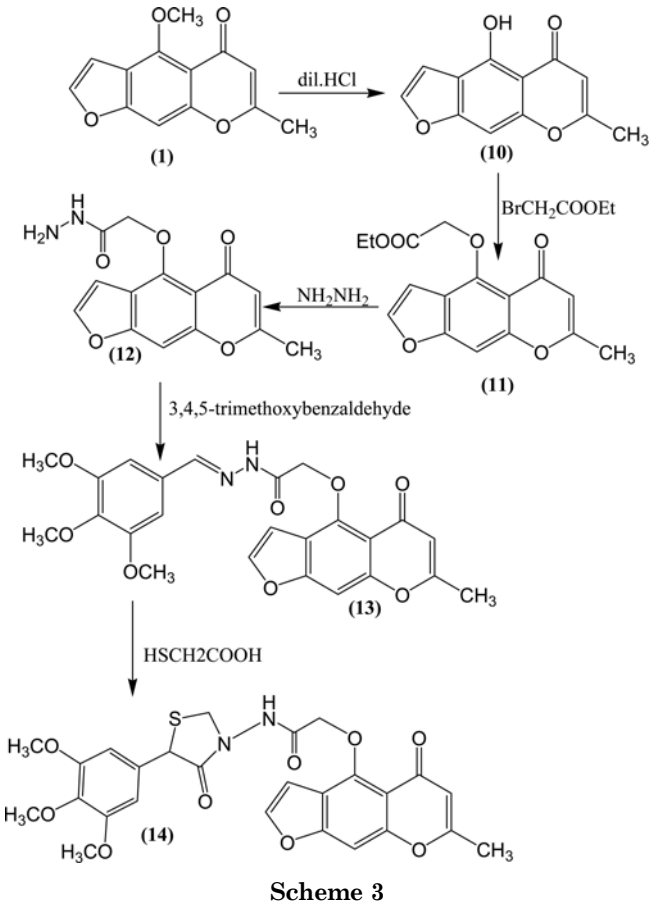
We described here a convenient approach for the preparation of bromovisnagin (**2**) which was obtained by bromination of visnagin (**1**) using bromine in acetic acid (Starkowsky, 1959). Condensation of **2** with hydrazine hydrate, phenylhydrazine and/or hydroxylamine hydrochloride afforded the corresponding pyrazole derivatives and isoxazole derivatives **4a**, **4b**, and **4c**, respectively. On the other hand, 4-methoxy-5-acetyl-6-hydroxy-7-bromobenzofuran **3** (Starkowsky, 1959) which was prepared by alkaline hydrolysis of **2** was condensed with some aromatic aldehydes namely, benzaldehyde, 4-chlorobenzaldehyde, 4-methoxybenzaldehyde, thiophealdehyde, and buteraldehyde to yield the corresponding α, β-unsaturated keto derivatives (**5a-e**) (Scheme 1). Chlorosulfonation of visnagin **1** using chlorosulfonic acid at room temperature without solvent yielded visnagin-9-sulfonylchloride (**6**) which



Scheme 1



Scheme 2



Scheme 3

on amidation using morpholine in dioxane yielded the 9-sulfonamido derivative (**7**). Alkaline hydrolysis of the later compound by 3% aqueous potassium hydroxide yielded 4-methoxy-5-acetyl-6-hydroxy-7-N-morpholino-sulfonamido benzofuran (**8**) which was condensed with some aromatic aldehydes namely, 4-chlorobenzaldehyde, 4-methoxybenzaldehyde, and dimethyl aminobenzaldehyde in alkaline medium to yield the corresponding α, β-unsaturated keto derivatives (**9a-c**) (Scheme 2).

Demethylation of (**1**) using hydrochloric acid afforded 4-demethyl-visnagin (norvisnagin) (**10**) which reacted with ethylbromoacetate in dry acetone in the presence of anhydrous potassium carbonate to yield 4-ethoxy-5-oxo-2- methylfuro[3,2-g] benzopyran (**11**). The later compound reacted with hydrazine hydrate to give the corresponding 4-oxyacetohydrazide derivative (**12**). The later compound was further condensed with 3,4,5-trimethoxybenzaldehyde to give the hydrazone derivative (**13**) which on its condensation with mecaptoacetic acid yielded thiazolidinone derivative (**14**) (Scheme 3).

Chloromethylation of demethylvisnagin (**10**) afforded 9-chloromethylnorvisnagin (**15**). **16a**, **b** were obtained either by the effect of primary amines such as methyl-amine and 2-aminopyridine on **15** or by the effect of primary amines in the presence of formaldehyde on norvisnagin (**10**). The Mannich bases (**17a-c**) were obtained by the treatment of **10** with the secondary amines, diethylamine, piperidine, and morpholine in the presence of formaldehyde. Benzoylation of Mannich bases (**16a**, **b**) and (**17a-c**) by benzoyl chloride in benzene afforded the corresponding benzoyloxy derivatives (**18a-e**) (Table IV).

Pharmacological screening

DNA binding affinity

The compounds were tested for DNA affinity and it was found that bromovisnagin (**2**) showed high affinity to. Compounds **6**, **7**, **15**, **18a**, **14**, **16b**, **17a**, and **16a** showed moderate activity in decreasing order. The remaining compounds showed weak activity (Table II).

Antiviral effect and cytotoxicity

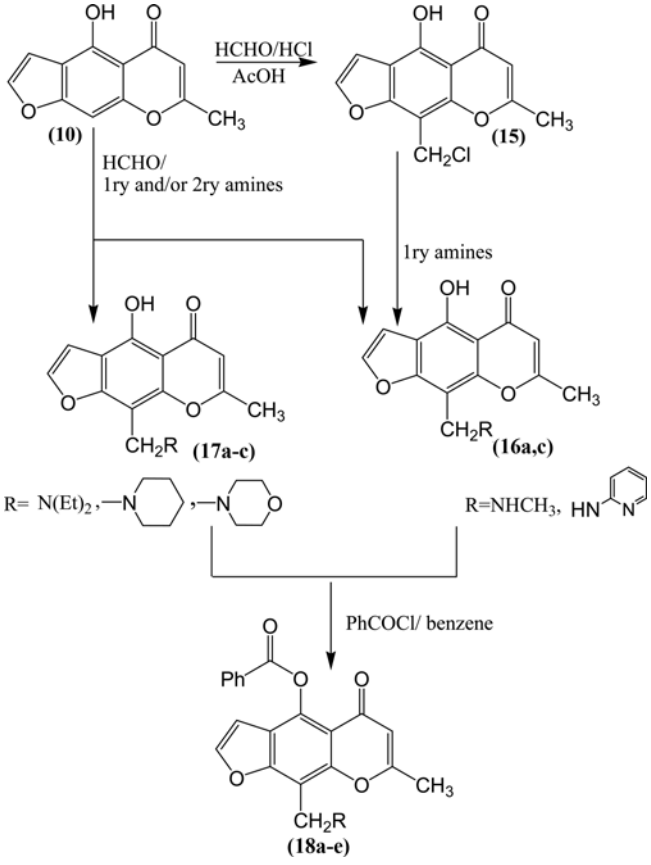
The prepared compounds were tested for their possible antiviral activity against herpes simplex type I (HS-I) grown on Vero African green monkey kidney cells. An improved plaque-reduction assay was used to

Table IV. IR, ^{1}H-NMR and MS spectra of the new compounds

Comp. No.	IR spectra (KBr, n cm^{-1})	^{1}H-NMR spectra (DMSO-d_6, δ ppm)	*m/z* (rel. int. %)
4a	3351 (OH), 3134 (NH), 1626 (C=N)	2.25 (3H, s, CH_3 pyrazole), 4.0 (3H, s, OCH_3), 6.8 (1H, s, H-4× pyrazole), 7.35 (1H, d, H-3), 8.0 (1H, d, H-2), 13.15 (1H, s, NH) and 13.35 (1H-s, OH)	322/324 (87%)
4b	3367 (OH), 1624 (C=N)	2.45 (3H, s, CH_3 pyrazole), 4.1 (3H, s, OCH_3), 6.9 (1H, s, H-4× pyrazole), 7.1-7.3 (5H, m, Ar-H), 7.32 (1H, d, H-3), 7.99 (1H, d, H-2), and 13.25 (1H-s, OH)	399/401 (85%)
4c	3415 (OH), 1622 (C=N)	2.25 (3H, s, CH3 pyrazole), 4.0 (3H, s, OCH_3), 6.5 (1H, s, H-4×), 7.35 (1H, d, H-3), 8.0 (1H, d, H-2), and 10.25 (1H-s, OH)	323/325 (98%)
5a	3413 (OH), 1666 (C=0)	4.1 (3H, s, OCH_3), 6.2, 6.5 (2H, dd, CH=CH×), 7.4 (5H, m, phenyl), 7.7 (1H, d, H-3 furan), 8.0 (1H-d, H-2 furan) and 11.2 (1H, s, OH)	372/374 (84%)
5b	3413 (OH), 1668 (C=0)	4.1 (3H, s, OCH_3), 6.2, 6.5 (2H, dd, CH=CH×), 7.2-7.4 (4H, m, phenyl), 7.6 (1H, d, H-3 furan), 8.0 (1H-d, H-2 furan) and 11.1 (1H, s, OH)	407.5/409.5 (70%)
5c	3413 (OH), 1668 (C=0)	3.99 (6 H, s, OCH_3), 6.5, 6.7 (2H, dd, CH=CH), 6.98-7.26 (4H, m, phenyl), 7.2 (1H, d, H-3 furan), 7.82 (1H-d, H-2 furan) and 11.4 (1H, s, OH)	402/404 (41%)
5d	3423 (OH), 1671 (C=0)	4.15 (3H, s, OCH_3), 7.1 (2H, dd, CH=CH), 7.4 (1H, d, H-3), 7.6 (3H, m, thiophen), 8.0 (1H, d, H-2) and 11.4 (1H, s, OH)	379/381 (50%)
5e	3421 (OH), 1628 (C=0)	1.1 (3H, t, CH_3), 1.41 (2H, m, CH_2), 1.99 (2H, t, CH_2), 4.14 (3H, s, OCH_3), 6.9, 7.1 (2H, dd, CH=CH), 7.3 (1H, d, H-3 furan), 7.9 (1H, d, H-2 furan) and 11.4 (1H, s, OH)	339/341 (55%)
9a	3375 (OH), 1668 (C=0), 1111, 1333 (SO2N)	^{1}H-NMR (DMSO-d_6, δ, ppm); 3.1 (4H, t, 2CH_2N system of morpholine), 3.67 (4H, t, 2CH_2O system of morpholine), 4.11 (3H, s, OCH_3), 6.5, 6.6 (2H, dd, CH=CH×), 7.2-7.4 (4H, m, phenyl), 7.89 (1H, d, H-3 furan), 8.00 (1H-d, H-2 furan) and 10.8 (1H, s, OH)	477/479 (4.25:1.53%)
9b	3375 (OH), 1668 (C=0), 1111, 1333 (SO2N)	3.2 (4H, t, 2CH_2N system of morpholine), 3.67 (4H, t, 2CH_2O system of morpholine), 4.21 (6H, s, OCH3), 6.5, 6.6 (2H, dd, CH=CH×), 7.2-7.4 (4H, m, phenyl), 7.99 (1H, d, H-3 furan), 8.00 (1H-d, H-2 furan) and 10.5 (1H, s, OH)	473 (2.02%)
9c	3428 (OH), 1658 (C=0), 1111, 1385 (SO2N)	3.2 (4H, t, 2CH_2N system of morpholine), 3.4 (6H, s, N(CH_3)$_2$), 3.7 (4H, t, 2CH_2O system of morpholine), 4.25 (3H, s, OCH_3), 6.4, 6.5 (2H, dd, CH=CH×), 7.3 (4H, m, phenyl), 8.0 (1H, d, H-3), 8.25 (1H-d, H-2) and 10.2 (1H, s, OH)	486 (0.07%)
11	1755, 1663 (C=0), 1619 (C=C)	ppm1.2 (3H, t, CH_3), 2.3 (3H, s, CH_3), 4.2 (2H, q, CH_2), 4.85 (2H, s, CH_2), 6.1 (1H, s, H-6), 7.1 (1H, s, H-9), 7.6 (1H, d, H-3), 8.1 (1H-d, H-2)	303 (89.5%)
12	3122 (NH), 3227, 3334 (NH2), 1676, 1604 (C=0), 1541 (C=C)	2.3 (3H, s, CH_3), 4.4 (2H, s, NH_2), 5.0 (2H, s, CH_2), 6.2 (1H, s, H-6), 7.4 (1H, s, H-9), 7.55 (1H, d, H-3), 8.1 (1H, d, H-2), 10.4 (1H, s, NH)	288 (15.8%)
13	3202 (NH), 1697, 1650 (C=0), 1604 (C=N), 1537 (C=C)	2.1 (3H, s, CH_3), 3.2 (2H, s, OCH_2), 3.8 (3H, s, OCH_3), 4.15 (3H, s, 2OCH_3), 7.1 (1H, s, H-6), 7.4 (1H, s, H-9), 7.6 (1H, d, H-3), 8.1 (1H-d, H-2), 8.45 (1H, s, CH=N) and 11.25 (1H, s, NH)	467 (7.9%)
14	3186 (NH), 1729, 1689, 1651 (C=0), 1604 (C=C)	2.0 (3H, s, CH_3), 3.8 (2H, s, OCH_2), 3.8 (9H, s, OCH_3), 4.0, 4.3 (2H, dd, CH_2 thiazolidinone), 4.70 (1H, s, CH_2 thaizolidinone), 6.3 (2H, s, phenyl), 7.2 (1H, s, H-6), 7.4 (1H, s, H-9), 7.6 (1H, d, H-3), 8.1 (1H-d, H-2), and 11.25 (1H, s, NH)	541 (7.3%)
16a	3418 (OH), 3133 (NH), 1621 (C=0)	1.7 (3H, s, CH_3), 2.47 (3H, S, N-CH_3), 2.9 (2H, s, CH_2), 6.13 (1H, s, H-6), 7.6 (1H, d, H-3), 8.1 (1H-d, H-2), 11.3 (1H, s, NH), 13.15 (1H, s, OH)	259 (11%)
16b	3424 (OH), 3133 (NH), 1676 (C=0)	1.7 (3H, s, CH_3), 2.7 (2H, s, CH_2), 7.2-8.0 (7H, m, aromatic protons), 11.3 (1H, s, NH), 13.15 (1H, s, OH)	322 (1.8%)
18a	1721, 1662 (C=0), 1631 (C=C)	1.51 (6H, m, CH_2 piperdine), 1.7 (3H, s, CH_3), 2.75 (4H, t, 2CH_2N system of piperdine), 3.70 (2H, s, CH_2), 6.13 (1H, s, H-6), 7.3-8.14 (5H, m, phenyl), 8.0 (1H, d, H-3), 8.25 (1H-d, H-2)	417 (22%)
18b	1721, 1662 (C=0), 1631 (C=C)	1.7 (3H, s, CH_3), 3.0 (4H, t, 2CH_2N system of morpholine), 3.67 (4H, t, 2CH_2O system of morpholine), 3.70 (2H, s, CH_2), 6.13 (1H, s, H-6), 7.3-8.14 (5H, m, phenyl), 8.0 (1H, d, H-3), 8.25 (1H-d, H-2)	419 (20%)

Table 4. Continued

Comp. No.	IR spectra (KBr, n cm^{-1})	^{1}H-NMR spectra (DMSO-d_6, δ ppm)	*m/z* (rel. int. %)
18c	1723, 1662 (C=0), 1630 (C=C)	1.1 (6H, t, $2CH_3$), 1.7 (3H, s, CH_3), 2.4 (4H, q, $2CH_2N$), 3.70 (2H, s, CH_2), 6.13 (1H, s, H-6), 7.3-8.14 (5H, m, phenyl), 8.01 (1H, d, H-3), 8.23 (1H-d, H-2)	405 (18%)
18d	3233 (NH), 1710, 1621 (C=0), 1598 (C=C)	1.7 (3H, s, CH_3), 2.4 (3H, s, N-CH_3), 3.80 (2H, s, CH_2), 6.13 (1H, s, H-6), 7.3-8.14 (5H, m, phenyl), 8.01 (1H, d, H-3), 8.23 (1H-d, H-2), 11.2 (1H, s, NH)	363 (20%)
18e	3136 (NH), 1725, 1695 (C=0), 1598 (C=C)	1.7 (3H, s, CH_3), 4.00 (2H, s, CH_2), 6.13 (1H, s, H-6), 6.65-8.11 (4H, m, pyridyl), 7.3-8.14 (5H, m, phenyl), 8.01 (1H, d, H-3), 8.23 (1H-d, H-2), 11.2 (1H, s, NH)	427 (32%)



Scheme 4

test the compounds by using aphidicolin as a positive control (Table II). Compound **2** was highly active at a concentration of 0.001 mg/mL compared with the standard (0.005 mg/mL) whereas compounds **6**, **7**, **15**, **14**, **16a**, and **18a** were moderately active towards HS-I. The remaining compounds were inactive towards the virus (Table III). CD_{50} of the active compounds towards the virus were also measured (Table III).

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