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CHEMICAL AND BIOLOGICAL STUDIES OF SOME NATURALLY OCCURRING FUROCOUMARINS

[1*]Sally S. El-Nakkady, [1]Hanaa F.Roaiah, [1]Weam S.El-Serwy, [1]Fatma Bassyouni, [2]A.M.Soliman.

[1]Chemistry of Natural and Microbial Products Department, National Research Centre, El-Tahrir Street, Dokki, Giza, Cairo, Egypt, 12311.

[2]Therapeutical Chemistry Department, National Research Centre, El-Tahrir Street, Dokki, Giza, Cairo, Egypt, 12311.

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***Correspondence for Author**
Dr. Sally S. El-Nakkady
Chemistry of Natural and Microbial Products Department, National Research Centre, El-Tahrir Street,Dokki, Giza, Cairo, Egypt, 12311.

ABSTRACT

Furocoumarins and their related compounds are a plentiful source for drugs that are candidates in relation to their safety and efficiency. The present investigation is concerned with the synthesis, chemical study and characterization of new furocoumarin derivatives with the objective of discovering novel and potent anticancer agents. This challenging goal prompted us to diversify the reactions in order to obtain end products with different functional groups. Friedel-Craft's acetylation of **I**,**II**,**III**,**IV**,**V** and **VI** namely Xanthotoxin, 4-methoxy-5-hydroxyl psoralen, 4,5-dimethoxy psoralen, 4,9-dimethoxy-5-hydroxy psoralen, 4,5,9-trimethoxy psoralen, and imperatorin yielded the acetyl compounds (**VII**, **VIII**, **IX**, **X**, **XI**,**XII**, **XIII** and **XIV**) respectively. Aldol condensation of (**VII**) with aromatic and/ or hetero aldehydes formed chalcones (**XV**) and (**XVI**). Bromination of xanthotoxin **I** in acetic acid yielded the bromo derivative (**XVII**), which reacted with alkaline dimethyl sulfate, aluminum chloride, acetic anhydride, hydrazine hydrate, malononitrile, ethyl cyanoacetate and sodium ethoxide to give (**XVIII**, **XIX**, **XX, XXI**, **XXII**, **XXIII** and **XXIV**) respectively. The bromo acrylic acid benzofuran (**XXIV**) reacted with malononitrile and or ethyl cyanoacetateto to give (**XXII**) and (**XXIII**) respectively. The Structures of the synthesized compounds were established on the basis of elemental analysis, *IR*, *^{1}H NMR*, Mass and spectral data. The anticancer activity of some of the synthesized compounds was evaluated against liver cancer cell lines HEPG2 that induced a moderate growth inhibition in a dose-dependent manner

against HEPG2 when compared to 5-FU and DOX chemotherapy. It can be deduced from the results that compound (**XVII**) was the highest compound in activity when compared to the reference anticancer drug (5-FU and DOX).

KEYWORDS: Acetylation, Bromination, Cytotoxicity, Furocoumarins, Liver cancer cell line.

INTRODUCTION

Linear furocoumarins known as psoralens are naturally occurring, and are known to be of great pharmacological interest.[1,2] Their skin photosensitizing effect[3] is utilized in leucodermic vitiligo therapy[4,5] and psoriasis treatment.[6] Some furocoumarins exert a higher anti-proliferative activity.[7] They were introduced clinically to treat T-cell lymphoma, cell cycle progression[8] and other autoimmune diseases[9] such as in the treatment of demyelinating diseases particularly in multiple sclerosis.[10] Their effects on chromosomes was extensively studied[11], as well as their anti-coagulating properties.[12] Some furocoumarins possess antifungal[13] and antibacterial properties.[14] Furocoumarins are selective coronary vaso dilators[15], they are also used in cardiovascular diseases and acts as spasmolytic agents.[15] Thus, the synthesis and biological evaluation of some novel furocoumarins aimed at creating a new molecular frame work and are building blocks for the synthesis of other heterocycles with diversified activities. In this article, synthesis and characterization of new furocoumarin heterocyclic compounds containing different functional groups in the main chain was reported.

MATERIALS AND METHODS

In view of these observations and as a continuation of our previous work in heterocyclic chemistry, we have synthesized some new furocoumarin compounds, and tested them for selected biological activity as anticancer agents.

I) Antitumor activity: The tested compounds (**I**, **VII**, **XIV**, **XVII**, **XXI**, **XXII**, and **XXIII**) were soluble in DMSO at concentrations high enough to allow cell experiments, the in *vitro* biological activity of these compounds was evaluated by their growth-inhibitory potency in liver HEPG2 cancer cell lines. The cytotoxic potency of tested compounds (**I**, **VII**, **XIV**, **XVII**, **XXI**, **XXII** and **XXIII**) were studied in comparison to the known anticancer drugs 5-Flurouracil (5-FU) and Doxorubicin (DOX).

II) The biochemical effects: The biological effects of the tested compounds (**I**, **VII, X**, **XIV**, **XVI**, **XVII**, **XVIII**, **XIX**, **XXI**, **XXII** and **XXIII**) on some enzymes such as aspartate, alanine aminotransferases (AST and ALT), alkaline phosphatase (ALP), as well as their effects on albumin, globulins, creatinine, total lipids, cholesterol, triglycerides and bilirubin in serum of mice were investigated.

RESULTS AND DISCUSSION

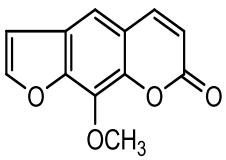
Chemistry

Furocoumarins are widely distributed in many plant species included the in the families of *Umbeliferae, Rutaceae, Leguminosae, and Mimosae*. They can be synthesized when the furan ring is built on a suitably substituted coumarin molecule in 12 different ways and each of the resulting compounds can become the parent of a family of derivatives[16], such as (psoralen, xanthotoxin, bergapten, imperatorin, isopimpinillin and angelicin). The present investigation deals with the acetylation and bromination of furocoumarins mainly xanthotoxin, 5-OH-isopimpinellin, 5-OH bergapten as well as their methoxyl derivatives.

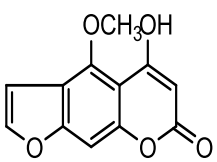
Friedel-Craft's acetylation of furocoumarins is typically an electrophilic substitution into the aromatic ring with the use of a Lewis base. The distinctive feature of synthesis with aluminum chloride is that it catalyzes not only the acylation but also the DE methylation of benzene homologues.[17]

In this work, acetylation is sometimes preceded by demethylation according to the condition of the reaction.

Xanthotoxin (9-methoxy psoralen) **I**, 5-hydroxy bergapten (4-methoxy-5-hydroxy psoralen) **II**, 5-methoxybergapten(4,5-dimethoxy psoralen) **III**, 5-hydroxy-isopimpinellin(4,9-dimethoxy-5-hydroxy-psoralen) **IV**, 5-methoxy isopimpinellin(4,5,9-trimethoxy–psoralen) **V** and imperatorin(9-(3-methyl-2-butenyloxy psoralen) **VI** were the starting materials for the Friedel-Craft's acylation.



I



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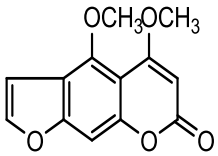
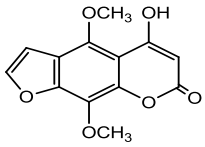
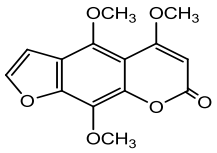


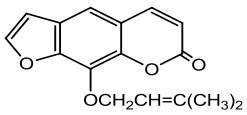
III



IV

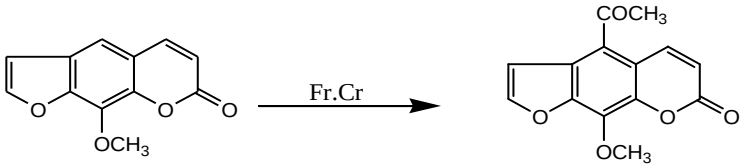


V



VI

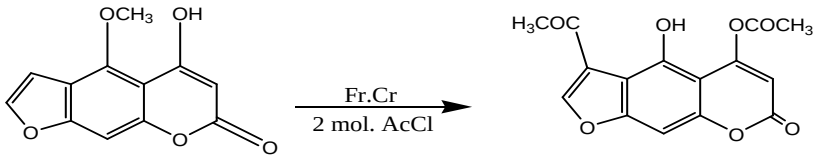
Acylation of xanthotoxin (9-methoxy psoralen) **I** occurs according to the Friedel Craft reaction in the aromatic nucleus where electrophilic substitution is most likely favored at C-4 to yield (**VII**).



I VII

The *[1]H-NMR* of 4-acetyl-9-methoxy psoralen **VII** revealed the appearance of a singlet at σ2.77 ppm (3H) corresponding to the acetyl group at C-4.

In the acetylation of 4-methoxy-5-hydroxy psoralen (5-OH bergapten) **II** using 2 moles of acetyl chloride to give (**VIII**), acetylation occurs first at C-3 and the hydroxyl group attached to C-5 followed by demethylation of the methoxyl group at C-4 to give 3-acetyl-4-hydroxy-5-acetoxy psoralen **VIII**.

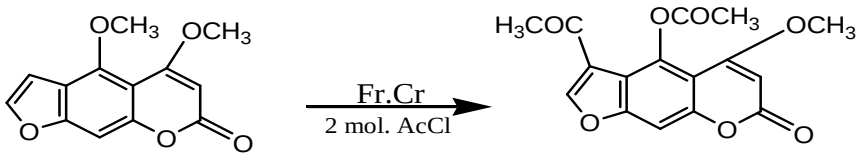


II VIII

This is confirmed by the spectral data, where the *[1]HNMR* of (**VIII**) revealed the absence of the methoxy group affirming demethylation. The presence of a singlet at σ 7.92 ppm (2H) corresponds to the furan proton at C-2 and the proton at C-9. The singlet at δ 6.9ppm (1H) represents the hydrogen at C-6. The two singlets at δ 2.83 and 2.75 ppm (3H) correspond to the acetoxy group at C-5 and the acetyl group at C-3 respectively. Its mass spectrum showed a molecular ion peak M^+ at m/e 302.

The acetylation of 4,5-dimethoxy psoralen (5-methoxy bergapten) **III** using 2 moles of acetyl chloride gave (**IX**) and proceeded in a different manner than with **II**, first acetylation of the furan moiety at C-3, followed by demethylation of the methoxy group at C-4 and

spontaneous acetylation of the intermediate 3-acetyl-4-hydroxy-5-methoxy psoralen, position 4 being the most favorable for acetylation as demethylation at C-4 seems to be more facile.

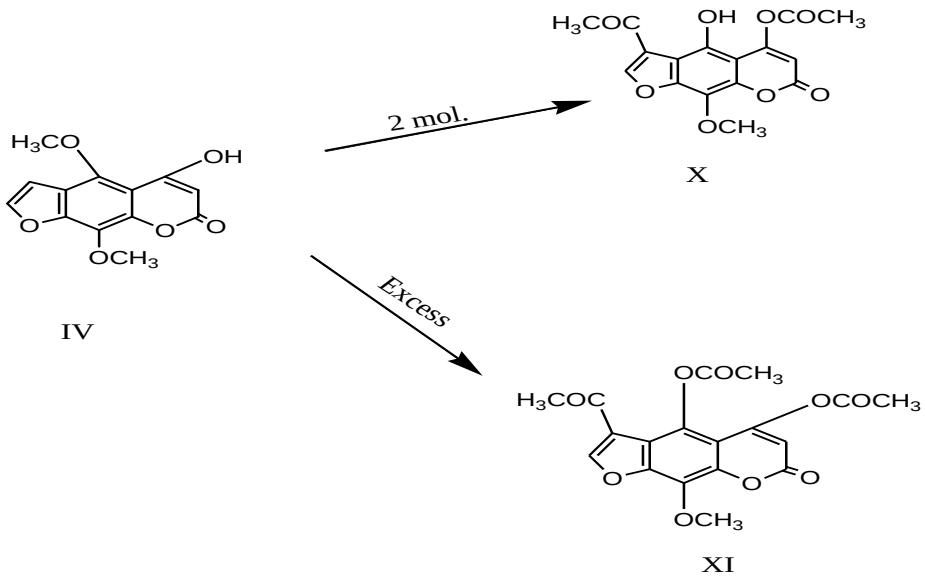


III IX

The[1]*HNMR* of 3-acetyl-4-acetoxy-5-methoxy psoralen **IX** confirmed the above-mentioned interpretation of the acetylation process of (**III**). The absence of the Methoxy group at C-4 and the appearance of two singlets at σ 2.75, 2.55 ppm (3H) each representing the acetoxy and acetyl groups at C-4 and C-3 respectively.

Its mass spectrum showed a molecular ion peak M^+ m/e at 316.

Acetylation of 4, 9-dimethoxy-5-hydroxy psoralen (5-OH isopimpenilin) **IV** gave either a diacetyl or triacetyl derivative (**X** and **XI**) depending on the molar ratio of the acetyl chloride used. Acetylation of the furan moiety at C-3 occurs in both cases, however, using excess acetyl chloride leads to acetylation at C-4 and C-5 to give 3- acetyl-4,5-diacetoxy-9-methoxy psoralen **XI**. On the other hand, when two moles of acetyl chloride were used C-5 only was acetylated in addition to C-3 to produce 3-acetyl-4-hydroxy-5-acetoxy-9-methoxy psoralen **X**.

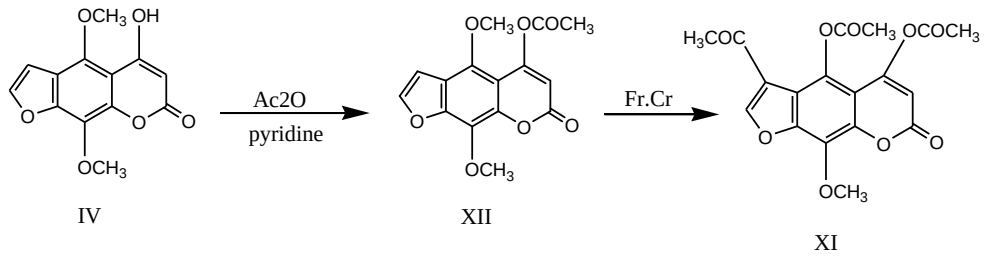


IV X XI

The [1]*HNMR* of (**X**) revealed the presence of two singlets at δ 2.85 and 2.73 ppm (3H) each correspond to the acetoxy group and an acetyl group at C-5 and C-3 respectively. Its *IR* spectrum showed a broad absorption band at 3700 cm^{-1} corresponding to OH group at C-4.

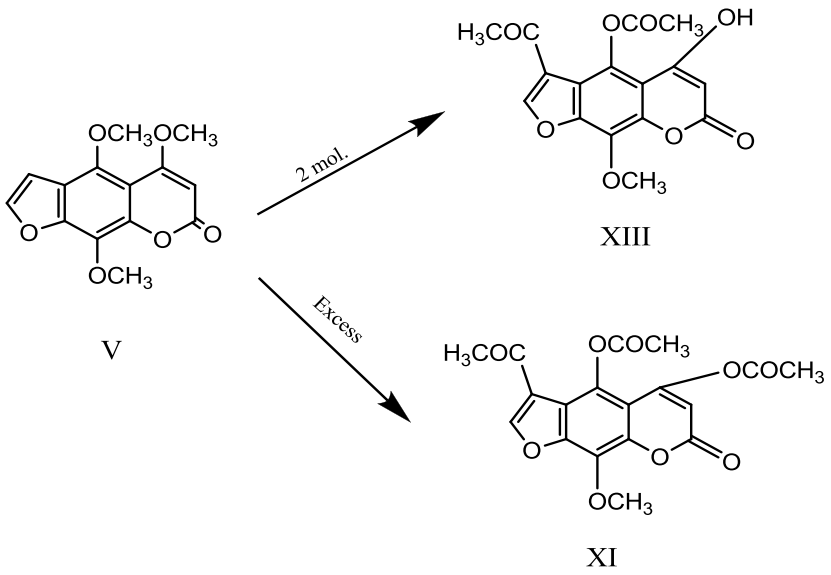
The mass spectrum of (**X**) revealed a molecular ion M+ m/e at 332, which is the molecular weight of the diacetyl product.

In order to confirm the structure of the triacetyl product (**XI**), 4, 9-dimethoxy-5acetoxy psoralen**XII** was synthesized using 5-hydroxy isopimpinillin **IV** with acetic anhydride and pyridine. (**XII**) Was subjected to the Friedel-Craft reaction using acetyl chloride and aluminum chloride. The product obtained was identical to (**XI**).



IV XII XI

When two moles of acetyl chloride were used for the acetylation of 4, 5, 9 trimethoxy-psoralen **V** using either nitrobenzene or/ carbon disulfide as a solvent, product (**XIII**) was formed. It gave a green coloration with ferric chloride indicating the presence of a free hydroxyl group at C-5.[18] This means that demethylation occurred at C-5. Also the presence of one methoxyl group only in the *^{1}HNMR* of (**XI**) is an evidence of the demethylation of the methoxy group at C-4 followed by spontaneous acetylation. On the other hand, when an excess of the reagents was used, 3-acetyl 4,5 di-acetoxy psoralen **XI** was obtained.



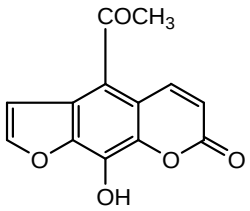
V XIII XI

The *^{1}HNMR* spectrum of 3-acetyl-4-acetoxy-5-hydroxy -9-methoxy psoralen **XIII** revealed the presence of a singlet at δ 7.85 ppm (1H) representing the proton at C-2, a singlet at δ 7.35

ppm (1H) for the hydrogen at position C6, a singlet at δ 4.08 ppm (s,3H, OCH_3) at C- 9, two singlets at δ 2.65 and 2.79 ppm (3H) each characteristic of the acetoxy at C-4 and the acetyl at C-3 respectively Its *IR* spectrum revealed the presence of an (OH) group at 3600 cm^{-1}, (C=O) at 1735 cm^{-1}.

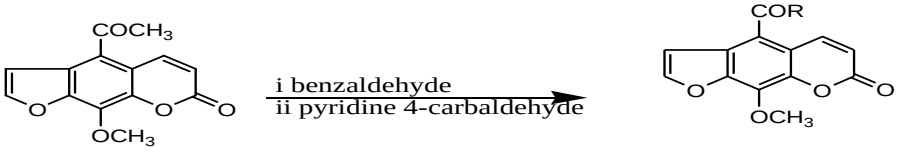
The mechanism seems to proceed according to the following: Normal Friedel–Craft acetylation occurring at position 3. Demethylation at C-4 occurred prior to acetylation; it seems that the more liable site for acetylation is C-4 as it is demethylated first. This was confirmed by the isolation of (**VIII**) and (**X**) when two moles of acetyl chloride were used. It is of interest to note that the intermediate (**XI**) was isolated when only two moles of aluminum chloride were used, thus proving the preferential demethylation at C-4 first and leaving the methoxyl group at C-5. However, when excess of aluminum chloride was used demethylation occurred also at C-5 to give (**XIII**). Excess of both reagents led to demethylation of the methoxy group at C-5 followed by acetylation thus forming the triacetyl derivative (**XI**).

Acetylation of 9-(3-methyl-2- butenyloxy) psoralen **VI** gave 4-acetyl-9-hydroxy psoralen **XIV**. Dealkylation of the alkyl group attached to C-9 occurs due to the dealkylating effects of the aluminum chloride, although demethylation has never occurred at position C-9.



XIV

The mass spectrum of (**XIV**) revealed a molecular ion peak at M^+ m/e 244. The base beak is at 202 which are the 9-hydroxy psoralen. 4-Acetyl-9-methoxy psoralen (acetyl xanthotoxin) **VII** formed chalcones by the condensation of the aromatic and / or hetero aldehydes (benzaldehyde or pyridine-4-carbaldehyde) in the presence of sodium hydroxide solution as Aldol condensation reaction.



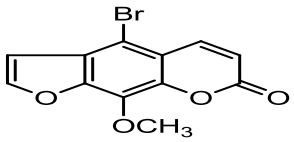
VII

XV, R =CHC_6H_5

XV1 , R= $CH\text{-}CHC_6H_5$

The *[1]HNMR* spectrum of 4-cinnamoyl-9-methoxy psoralen **XV** showed a singlet at δ 4.4 ppm (s,3H,OCH3) of the methoxyl group at C-9, a multiplet between δ 7.6-7.4 ppm (7 H) corresponds to the 5 aromatic and 2 vinylic protons, two doublets between δ 6.4- 8.2 ppm (*J= 2.2*) of the furan protons at C-6 and C-5, and two doublets between δ 7.6-6.9 ppm of the furan protons each (*J=2.2*).The mass spectrum showed molecular ion peak M^+ at 346. The base peak is 131 and represents the cinnamoyl residue at C-4.

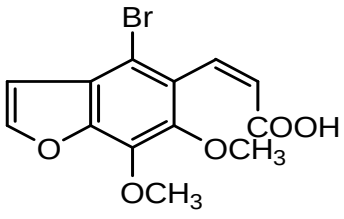
The, β- unsaturated ketone (**XVI**) was obtained in a similar manner. The *[1]-HNMR* of **XVI** revealed two doublets between δ 8.4 and 8.1ppm (*J=10*) which correspond to the protons at position C-6, C-5. The two doublets at δ 7.7 and 6.6 ppm (*J= 2.2*) correspond to the furan protons for C-3 and C-2, two doublets between δ 8.4-6.9 ppm revealed the protons at positions 2\,6\,3\ and 5\ of the pyridine moiety respectively, two doublets at δ 7.4-7.3 ppm of the olefinic protons (*J=16*), and a singlet at δ 4.3 ppm (3H,OCH_3). Its mass spectrum showed a molecular ion peak M^+ at m/e 347, the base peak at 132 which correspond to the chalcone moiety. As reported, the bromination of xanthotoxin **I** in chloroform resulted in the formation of a mixture of the mono and tri-bromo derivatives.[19] In order to obtain the mono derivative we used one or two moles of bromine (an equivalent amount) in acetic acid to form the 4-bromo derivative, 4-bromo-9-methoxy-*7H-furo* [3, 2-g] chromen-7-one **XVII**.



XVII

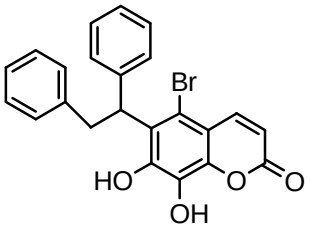
The *[1]HNMR* of (**XVII**) revealed the presence of two doublets at δ 7.06 and 7.02 ppm *(J=2.2)* representing the protons at C-2, C-3 of furan respectively. Two doublets at δ 8.4 and 6.5 ppm (*J=10*) representing H-6 and H-5 respectively and a singlet at δ 4.2 corresponding to C-9 (s, 3H, OCH3). Its mass spectrum showed molecular ions peak M+ at m/e 295, 293.

The reaction of compound (**XVII**) with alkaline dimethyl sulfate resulted in opening of the coumarin ring to give (z)-3-(4-bromo-6, 7-dimethoxy benzofuran-5-yl) acrylic acid **XVIII**.



XVIII

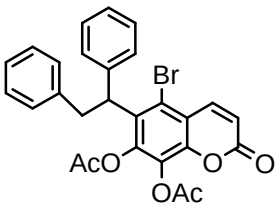
^{1}*HMNR* spectrum of (**XVIII**) revealed the presence of two doublets at δ 8.1 and 6.7 ppm corresponding to the furan protons at C-2, C-3 respectively. A doublet of doublet at δ 7.4-7.3 ppm (*J=10)* of the olefinic protons C-6 and C-5 respectively, and two singlets at δ 4.1- 3.9 ppm representing (6H), 2 methoxy groups C-7and C-6 respectively. Its *IR* spectrum showed the presence of a broad band absorption at 3400-3300 cm^{-1} (OH of COOH), CH=CH at 3020 cm^{-1} and C=O of acid at 1732 cm^{-1} Dealkylation of compound (**XVII**) with aluminum chloride in benzene resulted in opening of the furan ring to yield 5-bromo-7, 8-dihydroxy-6-(1, 2-diphenylethyl) *2H*-chromen-2-one **XIX**.



XIX

The^{1} *HNMR* spectrum of (**XIX**) revealed the presence of two broad signals at δ10, 9.9 ppm (2 OH), the absence of the methoxy group at C-9, and a multiplet between δ 7.1-7.4 ppm representing the aromatic protons and a doublet at δ 3.5 ppm (CH_2). Its *IR* spectrum showed broad absorption bands at 3400 -3196 cm^{-1} (2OH) and 2967 cm^{-1} (CH=CH). Its mass spectrum showed a molecular ion M^{+} at 436.

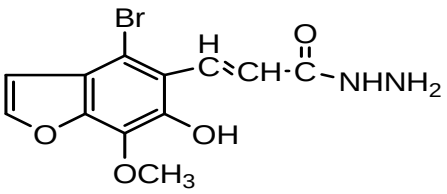
Treatment of the dihydroxy compound (**XIX**) with acetic anhydride and pyridine gave 5-bromo-7, 8-diacetoxy-6-(1, 2-diphenylethyl)-*2H*-chromen-2-one **XX**.



XX

The ^{1}*HNMR* spectrum of compound (**XX**) indicated the disappearance of (OH) signals and their replacement by two singlets at δ 2.8- 2.7 ppm (2-$OCOH_3$). Its *IR* spectrum revealed absorption bands at 3000 cm^{-1} of (CH_3), 2952 cm^{-1} of (CH_2), 1783 cm^{-1}(ester) and 1720 cm^{-1}(lactone).

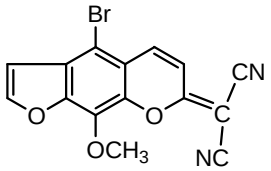
Reaction of hydrazine hydrate with compound (**XVII**) led to the opening of the lactone ring to give (*E)*-3-(4-bromo-6-hydroxy-7-methoxybenzofuran-5-yl) acrylohydrazide **XXI**.



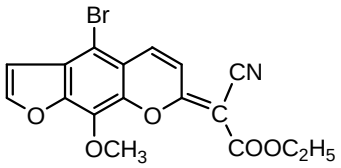
XXI

The *IR* spectrum of (**XXI**) showed absorption bands at 3845, 3742, 3679 cm^{-1} of (NH, NH_2, and OH groups), and 1626 cm^{-1} of (amide). Its mass spectrum showed molecular ion M^+ at 327.

The reaction of compound (**XVII**) with malononitrile and/or ethyl cyanoacetate gave 2-(4-bromo-9-methoxy-7H-furo [3, 2-g] chromen-7-ylidene) malononitrile and (2Z)-ethyl 2-(4-bromo-9-methoxy-7H-furo[3,2-g]chromen-7-ylidene)-2-cyanoacetate **XXII** and **XIII** respectively.



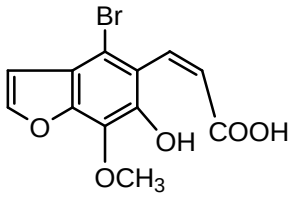
XXII



XXIII

The ^{1}*NMR* of (**XXII**) showed two doublets at δ 8.2 and 6.7 ppm (*J=2.2*)(furan) , two doublets at δ 7.0 and 8.3 ppm representing protons attached to C6 and C5, one singlet at δ 3.9 (3H OCH_3).The *IR* spectrum of compound (**XXII**) showed absorption bands at 2130 cm^{-1} (C≡N) and 1725 cm^{-1} (lactone) The mass spectrum showed molecular ion peaks M^+ at m/e 327,328. Its mass spectrum showed molecular ion peak M^+ at 295.of the bromo xanthotoxin. The ^{1}*HNMR* spectrum of compound (**XXIII**) revealed the appearance of signals at δ 7.7, 7.6 ppm of the furan ring, a signals at 7.3,7.1 ppm of (=NH), and at δ 4.2 ppm of (s,3H,OCH3). The *IR* spectrum of compound (**XXIII**) revealed the presence of absorption bands at - 3435 cm^{-1} (OH,broad) ,2924 cm^{-1} (NH) and 2364 cm^{-1} (C≡N).

Prolonged heating of compound (**XVII**) with sodium ethoxide resulted in coumarin ring opening to give (z)-3-(4-bromo-6-hydroxy-7-methoxybenzofuran-5-yl) acrylic acid **XXIV**.

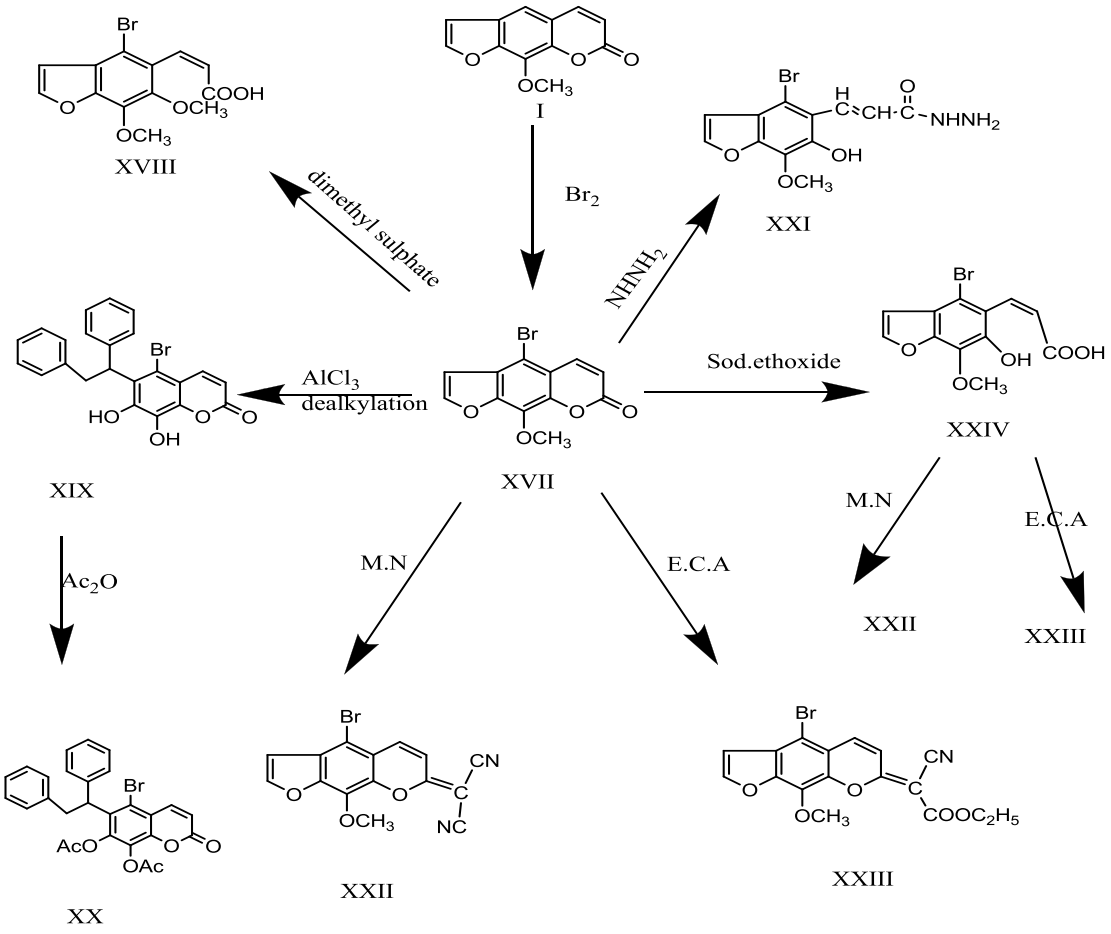


XXIV

The *IR* spectrum of compound (**XXIV**) revealed the presence of broad absorption bands at 3432- 3435 cm-^{1} (OH,COOH) , 2857 cm-^{1} (CH2) and 1740 cm-^{1} (C=O).

When compound (**XXIV**) reacted with malononitrile and /or ethyl cyanoacetate in the presence of triethylamine, it gave rise to the same compounds (**XXII)** and (**XXIII**) which proves that the intermediate (**XVII**) must have formed and reacted with the malononitrile and /or ethyl cyanoacetate to give the compounds previously identified as(XXII) and (XXIII) respectively.

The newly prepared compounds were compared to the previously prepared ones through mixed melting points and comparative infra-red.



I - Evaluation of Biological Activity

The anti-tumor activities of compounds (**I**, **VII**, **XIV**, **XVII**, **XXII**, and **XXIII**) were evaluated with HEPG2 (Human Liver Cancer Cell lines) by the **SRB** method *in vitro*[25], using 5-Flurouracil and Doxorubicin as the positive controls.

***In-vitro* measurement of potential cytotoxicity by SRB assay**

Cells were plated in 96-multi-well plate (10^4 cells/well) for 24 h before treatment with the compound (s) to allow attachment of cells to the wall of the plate. Different concentrations of the compounds under test (0, 1, 2.5, 5, 10 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound (s) for 48 h at 37^{o}C and in an atmosphere of 5% CO_2. Cultures were then fixed with trichloroacetic acid and stained for 30 minutes with 0.4% (wt/vol) sulforhodamine B (SRB) dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and protein-bound dye was extracted with 10 mM unbuffered Tris base [tris (hydroxymethyl) aminomethane] for determination of optical density in a computer-interfaced, 96-well micro titer plate reader. The SRB assay results were linear with the number of cells and with values for cellular protein measured by both the Lowry and Bradford assays at densities ranging from sparse sub confluence to multilayered supra confluence. The signal-to-noise ratio at 564 nm was approximately 1.5 with 1,000 cells per well. The relation between surviving fraction and drug concentration is plotted to get the survival curve of both cancer cell lines after the specified compound.

II- In-vivo Biochemical Analysis

Animals

Male albino mice weighing 18-20 g were used in the present study. Mice were divided into three main groups as follows.

Group (1): Untreated or control group (5 mice).

Group (2): divided into two subgroups (5 mice for each subgroup) and treated with 5-FU or DOX as reference anticancer drugs.

Group (3): divided into ten subgroups (5 mice for each subgroup) and treated with the selected furocoumarin derivatives.

a-The experiment was done in the National Research Center animal house in wide cages and well aerated rooms. Animals were fed on special diets containing proteins, carbohydrates, vitamins and minerals and drank clean tap water.

b- Blood collection from the animals was done with heparinized canula and they were left alive.

Treatment

Group (1): each mouse was given a single intraperitoneal injection of 0.1ml DMSO.

Group (2): each mouse was given a single intraperitoneal injection of 0.1ml containing 12 mg/kg body weight[26] 5-FU or DOX dissolved in sterile water.

Group (3): each mouse was given a single intra peritoneal injection of 0.1ml containing 12 mg/kg body weight of biochemically tested compounds (**I**, **VII**, **X**, **XIV**, **XVII**, **XVIII**, **XIX**, **XXII** and **XXIII** respectively) dissolved in DMSO. Blood was collected after 7 days from all mice groups.

The biochemical effects of the selected furocoumarin derivatives on some liver enzymes such as aspartate and alanine aminotransferases (AST and ALT) and alkaline phosphatase (ALP) , were done using blood auto analyzer (Olympus AV 400, Japan).[27] Moreover, albumin[28] globulins[29], creatinine[30], total lipids[31], cholesterol[32], triglycerides[33] and bilirubin[34] levels in serum of mice were evaluated in comparison to 5-FU and DOX.

Statistical analysis of the results was performed using Chi-square values (SPSS computer program).

RESULTS

In the present work we report the synthesis and preliminary biological activity screening of several furocoumarin dervatives. Preliminary screening of the selected compounds showed that all selected compounds exhibited a mild or moderate growth inhibition activity on the tested cell line between 1-100 µg/ml concentrations in comparison to the known anticancer drugs: 5-Flurouracil and Doxorubicin. Table (1) indicated the cytotoxic activity of the tested compounds (**I**, **VII**, **XIV**, **XVII**, **XXI**, **XXII** and **XXIII**) against liver HEPG2 cancer cell lines in comparison to the traditional anticancer drugs 5-FU and DOX. It can be deduced from the results that compound **XVII** was the most active and induced moderate growth inhibition, in a dose-dependent manner against HEPG2 when compared to 5-FU and DOX (IC_{50} equal 10.5 µg/ml, while 5-FU and DOX were 5 and 3.56 µg/ml).

Effect of antitumor compounds on the biochemical parameters

Data obtained in Table (2) presents the liver enzymatic activities (ALT, AST and ALP) in serum of control and treated groups of mice. The results showed that the values recorded for AST and ALT were significantly higher ($P < 0.001$) with 5-Fu and DOX treated groups of mice than the control. On the other hand, treatment of the tested compounds, caused inverse effects, where some values recorded for AST and ALT were non-significant (n.s.) or slightly higher ($P < 0.01$) in comparison to control. Moreover, the recorded data showed that ALP activities were significantly increased ($P < 0.001$) with the treatment of 5-Fu and DOX, while there were no significant changes in ALP activities upon treatment with some of the new compounds.

Data listed in Table (3) demonstrates the comparison between the levels of total lipids, cholesterol, triglycerides and bilirubin in serum of treated mice and the control group. It can be deduced from the present data that 5-FU and DOX caused a significant increase in the level of these parameters while treatment with the selected compounds showed moderate or no significant changes.

Table (4) represents a comparison between the levels of albumin, globulins and creatinine in serum of control and treated groups of mice. It is clear from the results in the table that there was a slight increase in the level of albumin and creatinine and globulins in the 5-FU and DOX treated groups of mice while there were moderate or non-significant changes in the other treated groups.

DISCUSSION

Cytotoxic drugs remain the main stay of cancer chemotherapy and are being administered with novel ways of therapy such as inhibitors of signals.[35] It is therefore important to iscover novel cytotoxic agents with spectra of activity and toxicity that differ from those current agents.[36] It is well known that chemotherapy aims to destroy the cancer cells with various types of chemicals.[37] The substances used are supposed to target mainly cancer cells, and doses are calculated to minimize the collateral damage to surrounding tissues, which nevertheless occur[38] This kind of treatment increases the entropy of the organism, suppresses the immune system, and forms a toxic cell environment which may destroy surrounding healthy cells. So it is important to minimize curing doses to the least amount possible as well as trying to minimize the side effects of these drugs. Considering, this novel derivatives of furocoumarin possessing a broader spectrum of antitumor activity and fewer toxic side effects

than 5-Fu and DOX have been sought. The antitumor activities of such compounds were assessed against HEPG2 cancer cell line in comparison to the traditional anticancer drugs 5-Fu and DOX. Regarding the antitumor activity study, some of the selected compounds showed moderate antitumor activity in comparison to 5-FU and DOX as shown by compound **XVII**. Moreover, study of the induced biochemical parameters of the tested compounds in mice showed insignificant differences relative to the control group, which indicates a moderate margin of safety for the selected compounds. Comparable to 5-FU and DOX, a dose augmentation of compound **XVII** searching for possible higher potency, seems, consequently, realizable without undesirable implications. Furthermore, the selected compounds have important potential advantages over 5-FU and DOX because of their lower toxicity and their ability to induce lower biochemical parameters.

EXPERIMENTAL

Preparation of 4-methoxy-5-hydroxy psoralen (II) and 4, 9-dimethoxy -5-hydroxy psoralen[14] IV

A mixture of 5 g of visnaginone) ⁄ (khellinone) suspended in 20 g of diethyl carbonate was added to freshly prepared powdered sodium metal (5g). The mixture was shaken vigorously and warmed for 1 hr. at 100^0C. The solid mixture decomposed with cold water (120) ml and extracted with ether. The aqueous layer was acidified with dilute hydrochloric acid; the solid obtained was filtered, washed with water and crystallized from: acetone. m.p 245^0C (as reported). Yield 80%., methanol. m.p 195^0C; yields 78 %.

Preparation of 4, 5-dimethoxy psoralen III and 4, 5, 9-trimethoxy psoralen V

A mixture of 2g(8.6 mmol) of (**II**), 8 g (5.9 mmol) of anhydrous potassium carbonate and 12 ml of dimethyl sulfate in 200 ml of acetone were refluxed for 12 hrs. The reaction mixture was then filtered while hot. The inorganic residue was repeatedly washed with hot acetone. The solvent was evaporated under reduced pressure and the solid was crystallized from methanol m.p 191^0C, yield 70%⁄methanol m.p 211^0C, yield 69%.

Preparation of 4, 9-dimethoxy -5-acetoxy psoralen XII

One g(3.8 mmol) of (**IV**) was suspended in 5 ml of pyridine, 5 ml acetic anhydride were then added .The reaction mixture was well stirred, left for 1hr .The solid that precipitate was filtered, washed with benzene and crystallized from benzene, m.p 175 ^{0}C; yield 90 %.

Using nitro benzene as a solvent

The furocoumarin (1.0 mmol) was dissolved in 20 ml of nitro benzene, (2 m. mol) or excess of acetyl chloride was added cautiously. The reaction mixture was stirred, (2 mmol) or excess of anhydrous aluminum chloride was added over a period of 25 min. Then left for 48 hrs, decomposed with dilute hydrochloric acid, and the nitro benzene was steam distilled. The solid that remained was filtered, dried, and crystallized from benzene.

Using carbon disulphide as solvent

The furocoumarin (1 mmol) was dissolved in carbon disulphide (25 ml anhydrous aluminum chloride (2 mmol) or excess and acetyl chloride (2m mol) or excess were then added to the solution drop wisely. The reaction mixture was then refluxed for 1 hr. and left to cool. The carbon disulphide was decanted and the reaction mixture was decomposed with dilute hydrochloric acid .The solid mixture was filtered and crystallized from benzene.

4-Acetyl-9-methoxy psoralen VII: m.p. 175^{0}C, yield 60%.

3-Acetyl-4-hydroxy-5-acetoxy psoralen VIII: m.p. 250 ^{0}C, yield 70 %.

3-Acetyl-4-acetoxy-5-methoxy psoralen IX: m.p.170^{0}C, yield 65%.

3-Acetyl-4-hydroxy-5-acetoxy-9-methoxy psoralen X: m.p. 211^{0}C, yield 65%.

3-Acetoxy-4, 5-diacetoxy-9-methoxy psoralen XI: m.p. 280^{0}C, yield 67%.

3-Acetyl-4-acetoxy-5-hydroxy-9-methoxy psoralen XIII: m.p. 225 ^{0}C, yield 61%.

4-Acetyl-9-hydroxy psoralen XIV: m.p.220^{0}C, yield 59%.

Preparation of chalcones XV and XVI

General procedure

Two g of 4-acetyl- 9-methoxy psoralen **VII** was dissolved in 50 ml of ethanol, 10 ml sodium hydroxide solution (10 %) was added followed by 1.1 g of the aldehyde, namely benzaldehyde and/ or pyridine-4- carbaldehyde. The reaction mixture was shaken vigorously for two hrs then left at room temperature for 48 hrs. It was then neutralized with dilute hydrochloric acid, filtered, washed with water and dried. **XV** was obtained as pale yellow crystals from methanol, m.p 200 0C.; yield 65%. **XVI** was obtained as yellowish brown crystals from benzene, m.p 170 0C.; yield 61%.

Preparation of 4-bromo-9-methoxy -*7H*-furo [3, 2-g] chromen-7-on XVII

Bromine (0.286 ml) was added drop wisely to a solution of xanthotoxin **I** (0.260 mol) in acetic acid (40 ml). The solution was stirred for 2 hrs at room temperature, the reaction

mixture was poured onto water and treated with 5% sodium bisulfate solution to remove the excess bromine. The solid so obtained was filtered, washed with water and crystallized from ethanol. m.p, 256 ^{0}C, yield: 60 %.

Prerаration of (Z)-3-(4-bromo-6, 7-dimethoxybenzofuran-5-yl)acrylic acid XVIII

One g (2.7mmol) of (**VII**) was dissolved in 50 ml acetone. Dimethyl sulfate (1ml) was added, followed by 50 ml of 20% potassium hydroxide. The solution was refluxed for 2 hrs, then acidified and the acetone removed in vacuum. The product was collected and crystallized from dilute methanol. m.p; 210 ^{0}C, yield: 65 %.

Preparation of 5-bromo-7, 8-dihydroxy-6-(1,2-diphenylethyl)-*2H* chromen-2-one XIX

One g (3 mmol) of (**XVII**) was refluxed with 2g (5 mmol) of aluminum chloride in 100 ml benzene for 30 min. The benzene was decanted and evaporated to dryness, the residue was acidified with dilute hydrochloric acid and crystallized from ethanol .m.p; 195^{0}C, yield: 61 %

Preparation of 5-bromo-7, 8-diacetoxy-6-(1, 2-diphenylethyl)-*2H*-chromen-2- one XX

2g (4.5 mmol) of (**XIX**) was refluxed in acetic anhydride and pyridine for two hrs. The solution was poured into water and the product was collected and recrystallized from ethanol m.p; 198 ^{0}C, yield: 59%.

Preparation of (E)-3-(4-bromo-6-hydroxy-7-methoxybenzofuran-5-yl) acrylohydrizide XXI

Two g (5.5 m mol) of bromo derivative **VII** and hydrazine hydrate (1.4 g ,3 moles) were refluxed for 24 hrs in 100 ml of 95% ethanol .The solution was then diluted with water and cooled ,the product was collected and recrystallized from ethanol. m.p; 269^{0}C, yield: 70 %.

Preparation of 2-(4-bromo-9-methoxy-7H-furo [3, 2-g] chromen-7-ylidene) malononitrile and (2Z)-ethyl 2-(4-bromo-9-methoxy-7H-furo[3,2-g]chromen-7-ylidene)-2-cyanoacetate XXII and XIII respectively

A mixture of (0.01 mole) of (**XVII**) and\ or (0.01 mole) of malononitrile, ethyl cyanoacetate in 20 ml absolute ethanol and few drops of trimetylamine\ piperidine was heated under reflux for 3 hrs , the solid obtained was filtered , dried and crystallized from ethanol**.**

XII: m.p; 236 ^{0}C., yield: 65%. **XIII:** m.p; 270 ^{0}C., yield: 60%.

Preparation of (Z)-3-(4-bromo-6-hydroxy-7-methoxybenzofuran-5-yl) acrylic acid XXIV

0.2 g (0.55 mmol) of (**VII**) was refluxed with an alcoholic solution of sodium ethoxide in 20 ml absolute (2 g sodium in 20 ml absolute alcohol) for 6 hrs. The solution turned yellow and then turbid due to the separation of a colorless solid. The mixture was evaporated to dryness in vacuum and the residue was dissolved in little distilled water, concentrated to remove most of the alcohol, then slightly acidified with hydrochloric acid and cooled upon which a crystalline solid deposited. It was then filtered off, washed with water to remove the inorganic salts, dried, and recrystallized from ethyl acetate as colorless crystals. m.p; 256 ^{0}C, yield: 60 %

Table 1: Effect of some selected newly synthesized furocoumarin compounds on liver carcinoma cell line (HEPG2).

Compound	IC_{50}
5-Flurouracil	5µg/ml
Doxorubicin	3.56µg.ml
I	35.3 µg/ml
VII	28.7 µg/ml
XIV	24.0 µg/ml
XVII	10.5µg /ml
XXI	31.0 µg/ml
XXII	31.8 µg/ml
XXIII	23.7 µg/ml

IC50: dose of the compound which reduces survival to 50%.

Table 2: Biochemical treatment effects with 5-FU, Dox. and furocoumarin derivatives on serum ALT, AST and ALP in mice.

Biochemical Parameters Mice Groups	Alanine amino transferase Mean ± SD ALT (IU/ml)	Aspartate amino transferase Mean ± SD AST (IU/ml)	Alkaline phosphatase Mean ± SD ALP (k.k./dl)
Control	43.5 ± 2.03	108.32 ± 4.19	18.70 ± 1.10
5-FU P<	51.47 ± 9.02 0.001	130.431 ± 8.92 0.001	25.485 + 6.03 0.001
Doxorubcin P<	59.26 ± 12.03 0.001	147.226 ± 16.34 0.001	30.317 ± 5.14 0.001
I P<	80.7±19.09 0.001	162.17±34.5 0.001	38.58±12.61 0.001
VII P<	38.9 ± 8.9 n.s.	123.9 ± 11.4 0.01	18.83 ± 6.29 n.s.
X P<	39.56±6.7 n.s.	112.54±12.7 0.01	19.94±4.35 n.s.
XIV P<	46.21 ± 4.17 n.s.	107.81 ± 4.25 n.s.	21.94 ± 3.4 0.01
XVI P<	53.7 ± 10.08 0.001	142.3 ± 29.7 0.001	45.42 ± 10.41 0.001
XVII P<	81.34± 27.3 0.001	151.52 ± 45.6 0.001	43.7 ± 8.36 0.001
XVIII P<	33.42 ± 7.05 n.s	60.5±9.7 0.001	156.22±20.1 0.001
XIX P<	60.5±9.7 0.001	156.22±20.1 0.001	44.26±7.01 0.001
XXI P<	68.34 ± 11.9 0.001	146.4 ± 28.1 0.001	36.9 ± 9.8 0.001
XXII P<	50.81±12.01 0.01	119±9.56 0.01	22.07±3.42 0.01

XXIII P<	73.09±14.2 0.001	140.09±31.01 0.001	30.41±9.22 0.001

Data are expressed as Mean+S.D

P> 0.05 insignificant, P< 0.01: significant, P< 0.001: highly significant, n.s.: non-significant.

Table 3: Biochemical treatment effects with 5-FU, Dox. and furocoumarin derivatives on serum total lipids, cholesterol, triglycerides and bilirubin in mice.

Biochemical Parameters Mice Groups	Total Lipids mg/dl	Cholestrol mg/dl	Triglycerides mg/dl	Bilirubin mg/dl
Control	323.41 ± 27.1	94.32 ± 13.5	108.7 ± 16.8	0.63± 0.04
5-FU P<	378.2±31.4 0.001	105.9±11.7 0.001	126.5±19.4 0.001	0.75± 0.10 0.001
Doxorubcin P<	366.7 ± 6.10 0.001	109.3 ± 14.2 0.001	137.8 ± 17.10 0.001	0.81± 0.19 0.001
I P<	363.6 ± 29.3 0.001	116.4 ± 8.3 0.01	98.4 ± 10.6 n.s.	0.96 ± 0.8 0.001
VII P<	329.34 ± 19.7 n.s.	95.3 ± 9.4 n.s.	119.7 ± 18.8 0.01	0.65 ± 0.9 n.s.
X P<	331.63± 17.5 n.s.	96.4± 10.5 n.s.	118.6± 19.70 0.01	0.68± 0.11 n.s.
XIV P<	317.4 ± 30.7 n.s.	93.24 ± 19.53 n.s.	116.23 ± 20.5 n.s.	0.51± 0.08 0.01
XVI P<	372.8 ± 37.6 0.001	110.3 ± 17.8 0.01	93.6 ± 9.5 n.s.	1.08 ± 0.7 0.001
XVII P<	382.09 ± 23.6 0.001	111.76 ± 34.3 0.01	172.9 ± 36.3 0.001	0.84 ± 0.4 0.01
XVIII P<	328.3 ± 13.7 n.s.	96.8 ± 17.2 n.s.	112.3 ± 10.6 n.s.	0.67 ± 0.01 n.s.
XIX	374.9± 36.6 0.001	123.3± 26.9 0.001	132.8± 26.3 0.001	0.99± 0.07 0.001
XXI	326.3± 18.7 n.s.	95.6± 14.9 n.s.	113.7± 8.6 n.s.	0.61± 0.04 n.s.

XXII P<	364.19±23.8 0.001	105.6± 17.4 0.01	119.8± 19.3 0.01	0.76± 0.15 0.01
XXIII P<	371.23± 26.7 0.001	110.9± 31.2 0.01	152.6± 34.5 0.001	0.77± 0.2 0.01

Data are expressed as Mean + S.D.

P> 0.05 insignificant, P< 0.01: significant, P< 0.001: highly significant, n.s.: non-significant.

Table 4: Biochemical treatment effects with 5-FU, Dox. and furocoumarin derivatives on serum albumin, globulin, creatinine in mice.

Biochemical Parameters Mice Groups	**Albumin mg/dl**	**Globulin mg/dl**	**A / G ratio**	**Creatinine mg/dl**
Control	5.63 ±0.51	4.32 ± 0.9	1.3	0.69±0.03
5-FU P<	6.49±0.92 0.01	5.75±0.8 0.01	1.13 0.01	0.81± 0.06 0.01
Doxorubcin P<	6.37 ± 0.85 0.01	5.91 ± 0.63 0.01	1.078 0.01	0.78± 0.04 0.01
I P<	7.1 ± 0.31 0.01	7.62 ± 0.76 0.01	1.003 0.001	0.78 ± 0.03 0.01
VII P<	7.2 ± 0.61 0.01	6.62 ± 0.86 0.01	1.006 0.001	0.8 ± 0.1 0.01
X P<	5.97± 0.34 n.s.	4.09± 0.63 n.s.	1.46 n.s.	0.65± 0.09 n.s.
XIV P<	5.92 ± 0.82 n.s.	5.12 ± 0.9 n.s.	1.15 n.s.	0.73± 0.04 n.s.
XVI P<	6.87 ± 0.49 0.01	6.86 ± 0.8 0.01	1.02 0.001	1.7 ± 0.43 0.001
XVII P<	11.43 ± 1.48 0.001	8.97 ± 0.9 0.001	1.13 0.01	0.76 ± 0.25 n.s.
XVIII P<	5.62 ± 0.68 n.s.	4.68 ± 1.06 n.s.	1.12 n.s.	0.68 ± 0.08 n.s.
XIX	6.47 ± 0.46 0.01	6.42 ± 0.7 0.01	1.001 0.001	0.77 ± 0.05 0.01

XXI	5.92 ± 0.81 n.s.	4.72 ± 0.91 n.s.	1.15 n.s.	0.84 ± 0.6 0.01
XXII P<	7.73± 0.52 0.01	6.25± 0.82 0.01	1.23 0.01	0.84± 0.06 0.01
XXIII P<	10.22± 1.35 0.001	8.96± 0.91 0.001	1.14 0.01	0.72± 0.21 n.s.

Data are expressed as Mean + S.D.

P> 0.05 insignificant, P< 0.01: significant, P< 0.001: highly significant, n.s.: non-significant.

CONCLUSION

In this study, we have identified furocoumarin derivatives as a novel class of compounds related to anti-proliferative activity. The lead compound **XVII** was the more potent in the biological assay employed (e.g.: improved growth inhibition potential as compared to the reference anticancer drugs). These experimental findings may provide support for the use of these novel compounds as new weapons in the fight against different types of cancer.

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