Preliminary Cytotoxic Study of Some Novel Furo-2-quinolone Compounds

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Abstract

In this research, new series of Furo-2-quinolone [FQ] compounds have been synthesized. These novel [FQ] compounds were prepared from coumarin derivatives (Furocoumarins: psoralen and isopsoralen).Identifications of these FQ compounds were performed by using infrared spectrum (I.R), Ultraviolet spectrum (U.V) and Nuclear Magnetic Resonance spectrum (H^1-NMR) besides some physical data. The cytotoxic screening involves ;using HEP-2 cell line which gave differential responses against tested compounds : 4,6 Dimethyl furo[2, 3-g] coumarin **(C_1)**, 1-(2`, 4`, Dimethoxy benzylideneimino)-2,6-dimethyl Furo [2, 3-g] quinoline-2-one **(C_3)** and the angular psoralen of the same derivative with the chemical name; 1-(2`,4`-Dimethoxybenzylidenimine)-4,8,9-trimethyl furo[3,2-h] quinoline-2-one **(C_{3A})**.These preliminary studies using different cell lines in addition to full cytotoxic screening may facilitate generation of better structural activity relationship and then shed the light for new lead promising anticancer compounds.

Keyword: Coumarin, Angelicin, HEP-2 Cell line, cytotoxicity,

الخلاصة

تم في هذا البحث، تحضير سلسلة جديدة من مركبات فيورو-2-كينولون[FQ] هذه المركبات الحديثة حضرت من مشتقات الكومارين (فيورو كومارين: بسورالين وايزوبسورالين). تشخيص هذه المركبات تم باستخدام طيف الاشعة تحت الحمراء (IR) وطيف الاشعة فوق البنفسجية (UV) والرنين النووي المغناطيسي بالإضافة إلى بعض الصفات الفيزياوية. تضمنت دراسة السمية للخلايا باستخدام خط خلايا HEP-2 والتي أعطت استجابات مختلفة ضد المركبات قيد الاختبار وهي:4 ,6 داي مثايل فيورو [2,3-جي] كومارين **(C_1)** و 1-(2' ,4' داي ميثوكسي بنزايلدين- ايمينو) -6,2- داي ميثايل –فيورو [3,2- g] كينولين – 2-أون(**C3**) بالإضافة إلى 1-(2',4'- داي ميثوكسي بنزابلدين- ايمنيو)- 9,8,4- ثلاثي ميثايل فيورو [2,3-H] كينولون -2- اون **(C3A)**. هذه الدراسة الأولية وباستخدام خطوط لخلايا سرطانية ممتنوعة بالإضافة إلى مسح ضد سمي شامل لها قد يسرع ولادة لعلاقة وظيفية بالتركيب الكيماوي لها ومن ثم إزاحة الضوء لمركبات قيادية ذات فعالية ضد سرطانية متوقعة وواعدة.

Introduction

Furoquinoline-2-one(I) compounds are considered as a main derivatives of furocoumarines(II)(1). Coumarin(III) is being the main nucleus of these compounds which classified into linear furocoumarin ; psoralen(II) and angular furocoumarin ; angelicin(IV) [Fig.1]. The investigation of coumarin compounds revealed that a wide spectrum of medicinal plant extracts that were in use as early as 1000 A. D. contain a high content of coumarins. Subsequent analysis of scientific literature revealed numerous reports on the antiproliferalive and antitumor activities of a variety of coumarin compounds(2-5), and have demonstrated activity against several types of animal tumors(6-10). These compounds have also been reported in clinical trials to demonstrate activity against prostate cancer, malignant melanoma and metastic renal cell carcinoma(11, 12). Recently, series of Furo-2-quinolone compounds [Fig. 2] were synthesized(13) and identified by using infrared (FT/IR), Ultraviolet (U.V.) and Nuclear Magnetic Resonance Spectroscopy (1HNMR) besides some physical data .

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This previous literature survey urged us to direct this paper towards preliminary cytotoxic study of the "building block" (4,6 Dimethyl furo[2, 3-g] coumarin (C1) , one of linear psoralen ; 1-(2`, 4`, Dimethoxy benzylideneimino)-2-6-dimethyl Furo [2, 3-g] quinoline-2-one (C3) and the angular psoralen of the same derivative with the chemical name; 1-(2`,4`-Dimethoxybenzylidenimine)-4,8,9-trimethyl furo[3,2-h] quinoline-2-one (C3A)These three selected lead compounds were screened against human larynx epidermoid carcinoma (HEP-2) cell line. The preliminary results indicate that HEP-2 cell differs in its sensitivity towards these compounds.

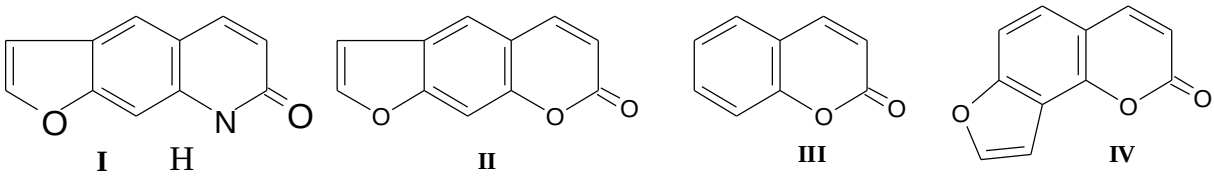


Figure (1)

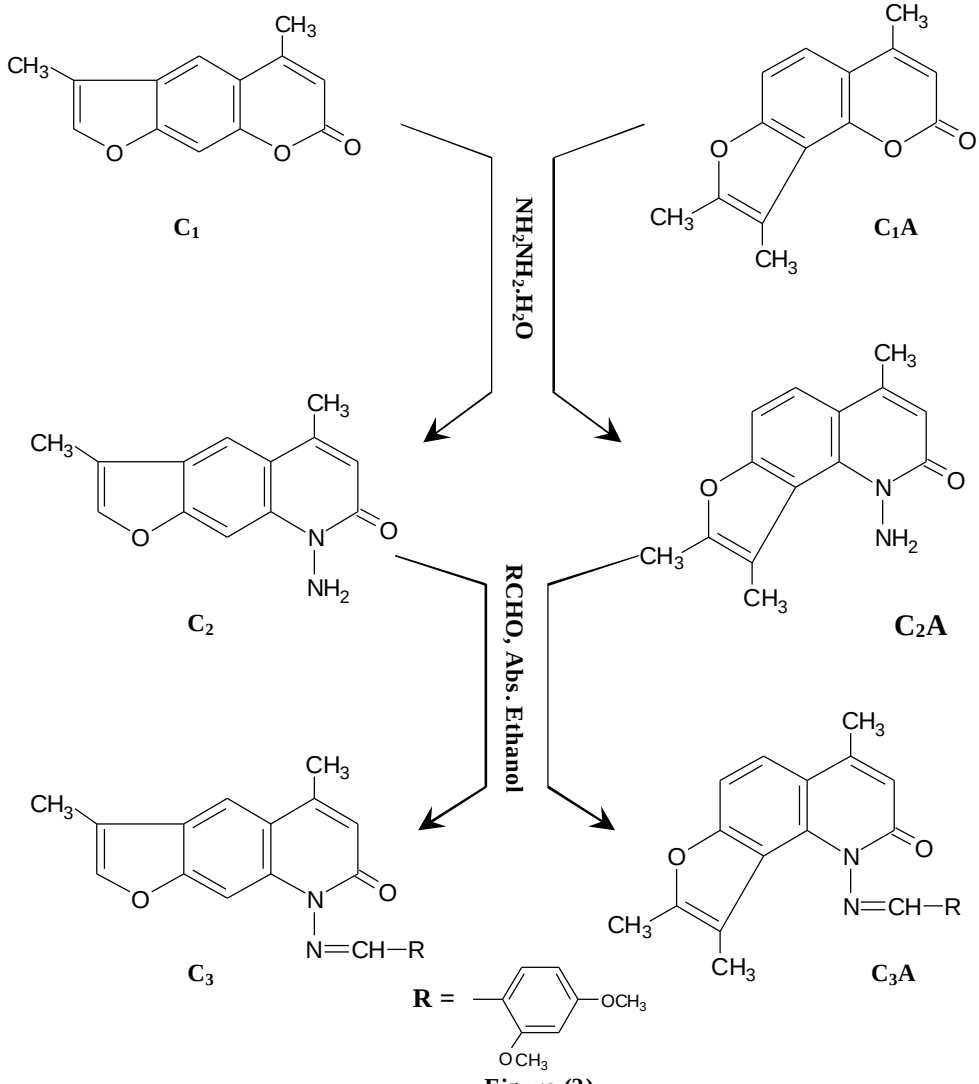


Figure (2)

Experimental

Chemistry

General Method

Melting points were uncorrected & determined by using electrothermal 9300. The identification of the prepared compounds were done by using FT/IR spectrophotometer tensor 27 as KBr disc. Ultraviolet by schimadzu-UV visible UV. 1650 PC. Nuclear Magnetic Resonance spectrum were carried out by using Bruker-Avance 400 MHz / France.

Synthesis

1-Amino-4,6-dimethyl furo[2, 3-g] quinoline-2-one[14] (C_2).

1-Amino-4,8,9-trimethylfuro[3,2-h] quinoline-2-one[13](C_{2A}).

Dissolve (0.02 M) of Furocoumarin in (30 ml) of dry pyridine with continuous stirring and gentile heating, then cool the mixture and add to it (0.025M, 1-3 ml) of aqueous hydrazine drop wise, reflux for 6 hours. Cool the mixture until formation of precipitate, filter and wash many times with cold water and recrystallized from ethanol. Yield 42%, the melting point is (C_2= 240 - 241 ^oC) & (C_{2A} 166-169^oC).

		C=O	C-N	N-N	N-H	U.V. EtOH nm.
IR cm^{-1} KBr	C_2	1654	1252	1184	3107	300
	C_{2A}	1672	1244	1173	3188	304

		CH_3(s)	CH=	NH_2	Phenyl
^{1}HNMR ppm/$CDCl_3$	C_2	2.2, 2.4	6.3 , 6.5	5.3	7.2 , 7.4
	C_{2A}	2.2, 2.4, 2.5	6.5	5.8	7.0 - 7.4 complex

1-(2`,4`-Dimethoxy benzylidene imino)-4,6-dimethyl furo[2, 3-g] quinoline-2-one (C_3).

1-(2`, 4`-Dimethoxy benzylidene imino)-4, 8, 9-trimethyl furo[2,3-h] quinoline-2-one (C_{3A}).

Dissolve an equimolar (0.003 M) of **C2** or **$C2_A$** with 2, 4-dimethoxy benzaldehyde in 50ml of absolute ethanol, reflux the reaction mixture for 1 hr. cool and filter. The precipitate is washed with cold ethanol and recrystallized by methanol. M.Pt. (202-204^oC, pale brown) and (90-93^oC, yellow) for $C_3$and C_{3A} respectively

		C=O	C=N	N-N	U.V/ EtOH nm.
I.R. /cm^{-1} KBr	C_3	1675	1575	1180	296 nm
	C_{3A}	1673	1562	1182	304 nm

		CH_3 (S)	CH= (S)	OCH_3 (S)	CH=N (S)	Phenyl
^{1}H NMR ppm/$CDCl_3$	C_3	2.22, 2.42	6.3,6.5	3.74, 3.81	8.1	7.2 ,7.4 (S) (S)
	C_{3A}	2.22, 2.42 ,2.5	6.3, 6.5	3.78, 3.82	8.21	7.0 -7.4 complex

II.Cytotoxic screening

II.Material

Bovine serum (BS), Phosphate buffered saline (PBS) PH 7.2, HEP-2 cell line and all other solutions and media for cytotoxicity study were kindly provided by the Iraqi Center for Cancer and Medical Genetics Research / Baghdad.

to the cell sheet and the flask was rocked gently. After approximately 30 seconds most of the trypsin was poured off and the cells incubated at 30°C until they had detached from the flask. Afterwards, 200 l of cells in growth medium were added to each well of a sterile 96-well microtitration plate. The plates were incubated at 37°C in 5% CO_2 humidified atmosphere incubator, the medium was removed and serial dilutions of the compound (500, 250, 125, 62.5) g/m) under assay in SFM were added to the well. The cell line was exposed to cisplatin (EBEWE, Austria Europe) as a reference (positive control); where the negative control were the cells treated with SFM only. The cells in each well were washed with PBS exposed to diluted formalin exposed to crystal violet dye then washed and left to dry[18]. Reading the result was accomplished using a multi-well plate reader at 492 nm.

Results and Discussion

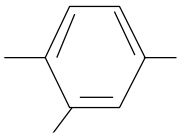
Preparation of C2 & C2A from reaction of Furocoumarin with hydrazine hydrate is a nucleophilic substitution lead to cyclic cleavage followed by recyclization with removal of one molecule of water. This reaction product shows three singlet bands at (2.2, 2.4, 2.5 ppm), due to trimethyl substituent at C4, 8, 9 respectively of C2A ,and the singlet proton of C3 appears at 6.5 ppm.The two methyl groups in C2 appears at (2.2 and 2.4 ppm) and the two singlet protons of this compound at C3 and C7 are interfere with the aromatic region at (6.3 and 6.5 ppm) respectively, due to the aromatic properties of this compound. The FT/IR spectra of the compounds C3 and C3A (Schiff bases derivatives) shows a significant C = N band between (1575 and 1562 cm-1) respectively. The 1HNMR of these compounds appears approximately not far from the values of compounds C2 and C2A..The U.V. spectra showed max between 296nm and 304 nm respectively The other spectral data of these compounds U.V., I.R. and 1HNMR are appear in the previous Tables.

Cell line synthesis for cytotoxicity study[17]

The growth medium was decanted off and the cell sheet was washed twice with PBS. Two to three ml of trypsin-versene was added

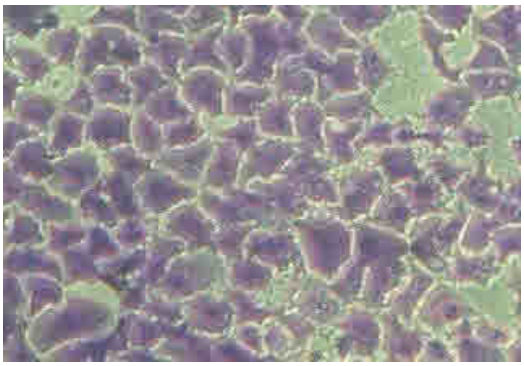
Cytotoxicity screening

Compounds C1, C3 & C3A were screened for their cytotoxicity testing. Morphological changes of HEP-2 cell upon exposure to selected compounds [Fig. 3] accompanied with reduction in size i.e. loss of contact with neighboring cells as the apoptotic cells shrank and become detached from the adjacent cells.The dose-response curve of C1, shows a dramatic positive downward curve [Fig. 4 , Table1] and highly HEP-2 sensitivity comparing with resistance cisplatin (positive control), indicate delivering of good prominent lead compound, this significant difference from cisplatin shown at lower concentrations 62.5& 125 g/10ml.A different response [Fig. 5, Table 2] could be exploited from C3 although a cross-resistance at lower doses, but a decrease in % survival appeared with higher dose which may be clearer with time-course interval higher then 48 hours. HEP-2 cell shows a gradual sensitivity with cisplatin C3A [Fig.6, Table3] and significant difference from cisplatin at $P < 0.001$ at 5 different dilution doses.From the collective dose response curves [Fig.7] C1, C3 & C3A we can conclude that insertion of

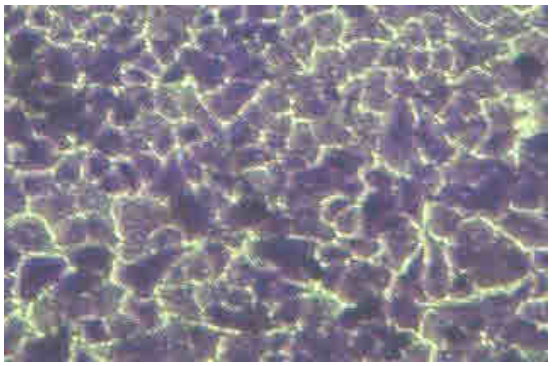
(R =  to **C3** may

obt OCH_3 n SAR yield from cytotoxic screening of all synthesized compounds may accumulate informations & shed light to new promising anti-proliferative agents.

considered as non essential modification to linear psoralen while angular psoralen "Angelicin" may considered as a good ***lead*** compound, and collective informations



Drug



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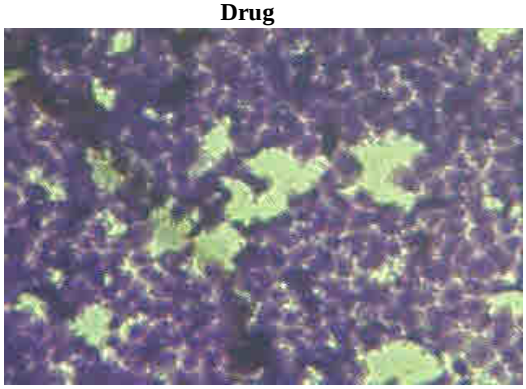
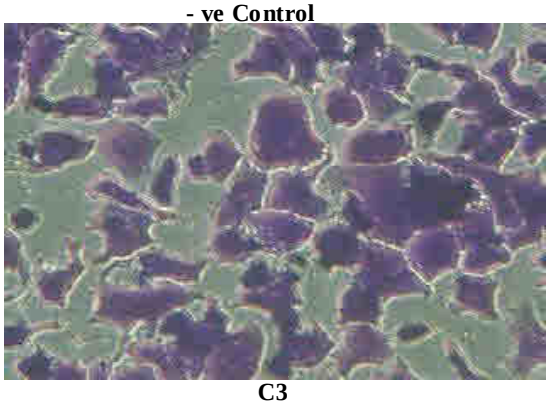
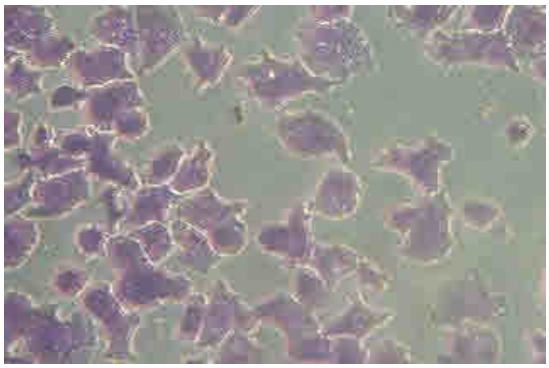


C1

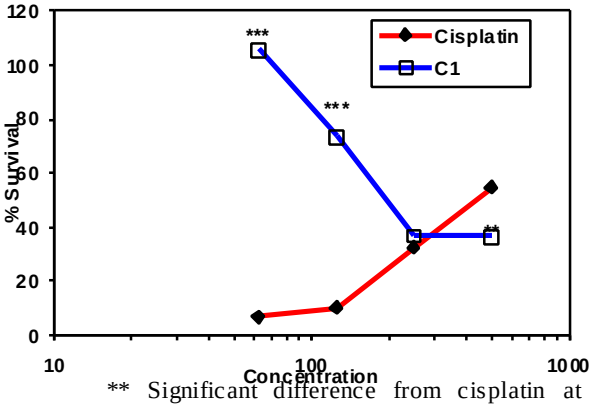


C3



C3A

Fig 3 : The morphologicl effects exerted by C1,C3, and C3A on HEP-2 , photographs were taken using a Nikon inverted light microscope (20x objective)

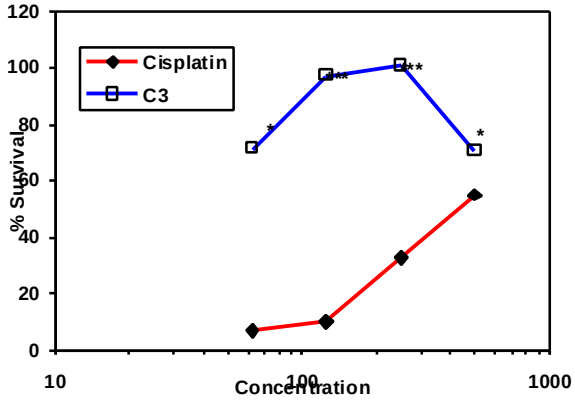


** Significant difference from cisplatin at p<0.01, *** at p<0.001

Fig 4: Graphical representation of concentration dependent effect of C1 on HEP-2 cells.

Table 1: The cell survival as percentage of the control for the HEP-2 cell when the cells were treated with C1.

Concentration (µg/10 ml)	% of Survival (Mean ± SD)	
	Cisplatin	C1
62.5	6.77 ± 1.27	106.01 ± 9.33
125	10.15 ± 1.88	73.79 ± 6.74
250	32.42 ± 4.29	36.83 ± 6.59
500	54.88 ± 4.60	36.96 ± 3.64

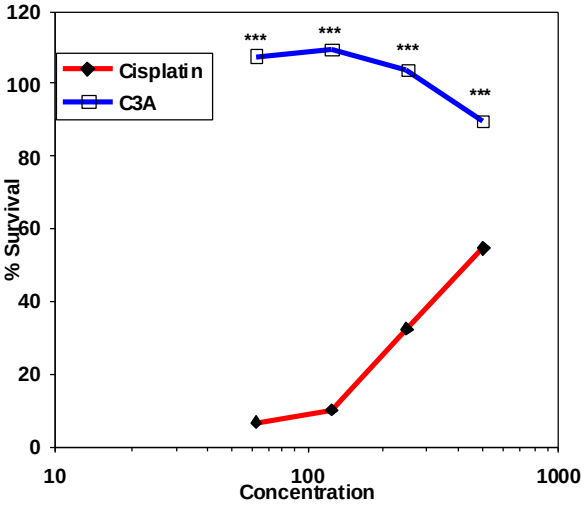


* Significant difference from cisplatin at p<0.05, *** at p<0.001

Fig 5: Graphical representation of concentration dependent effect of C3 on HEP-2 cells.

Table 2: the cell survival as percentage of the control for the HEP-2 cell when the cell were treated with C3.

Concentration (µg/10 ml)	% of Survival (Mean ± SD)	
	Cisplatin	C3
62.5	6.77 ± 1.27	71.34 ± 46.35
125	10.15 ± 1.88	97.09 ± 12.01
250	32.42 ± 4.29	100.77 ± 8.97
500	54.88 ± 4.60	70.68 ± 5.82



*** Significant difference from cisplatin at p<0.001

Fig 6 : Graphical representation of concentration dependent effect of C3A on HEP-2 cells.

Table 3: The cell survival as percentage of the control for the HEP-2 cell when the cell were treated with C3A.

Concentration (µg/10 ml)	% of Survival (Mean ± SD)	
	Cisplatin	C3A
62.5	6.77 ± 1.27	107.66 ± 10.88
125	10.15 ± 1.88	109.46 ± 5.21
250	32.42 ± 4.29	103.76 ± 4.69
500	54.88 ± 4.60	89.92 ± 6.10

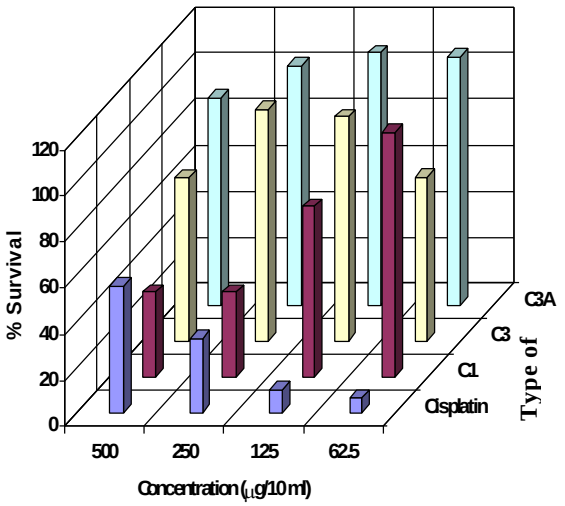


Fig 7: Three dimensional histograph of over all tested compounds (C1,C3,C3A) on HEP-2 cells.

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