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Synthesis and evaluation of some novel furocoumarin derivatives for radical scavenging profile and cytotoxic studies

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ABSTRACT

The synthesis and biological evaluation of some novel angular furocoumarins was aimed at creating a new molecular frame work. Considering the side effect profile of linear furocoumarins, six new and unexplored angular furocoumarins i.e. 4-methyl-9-(substituted phenyl)-2H-furo[2,3-h]chromen-2-ones have been synthesized by reacting different para substituted acyl bromides with 4-methyl-7-hydroxy coumarin followed by cyclization in acidic medium. All these compounds were characterized by physical and spectral data. These compounds were screened for free radical scavenging activity by DPPH method and preliminary cytotoxic activity by using Ehrlich Ascites Carcinoma cells and Tryptan blue dye exclusion method. Among the compounds tested for radical scavenging and cytotoxic studies, 4b and 4c showed appreciable results when compared with the standard drugs ascorbic acid and 5-fluorouracil respectively.

Keywords: Angular Furocoumarins, Di-adducts, DPPH Radical scavenging activity, Tryphan blue dye & EAC cell lines.

INTRODUCTION

The chemical structure of furanocoumarins consists of a furan ring fused with coumarin. The furan may be fused in different ways producing several isomers. The compounds that form the core structure of the two most common isomers are psoralen and angelicin. Derivatives of these two core structures are referred to respectively as linear and angular furanocoumarins.

The mechanism of action[1] of these compounds is based on their ability to form photoadducts with DNA and other cellular components such as RNA, proteins, and several components of the membrane such as phospholipases A2 and C, Ca-dependent and cAMPdependent protein-kinase[2], epidermal growth factor. Furocoumarins intercalate between base pairs of DNA and after ultraviolet-A irradiation, give [2+2] cycloadducts[3]. On the one hand, the ability of these compounds to form monoadducts appears to be the main reason for their therapeutic activity, because they suppress the transmission of information and therefore the DNA replication. On the other hand, the formation of cross link is associated with the more negative aspects such as cutaneous phototoxicity or carcinogenicity[4]. For this reason it is understandable why the main aim of recent works has been related with the search for monofunctional furocoumarins with a good ability to interact with DNA.

Literature reveals furocoumarins possesses various activities like anti-fungicidal[5], insecticidal[6], insect antifeedant[7], Anti-HIV, Anticancer[8], Vasorelaxant[9], Photodynamic activities[10].

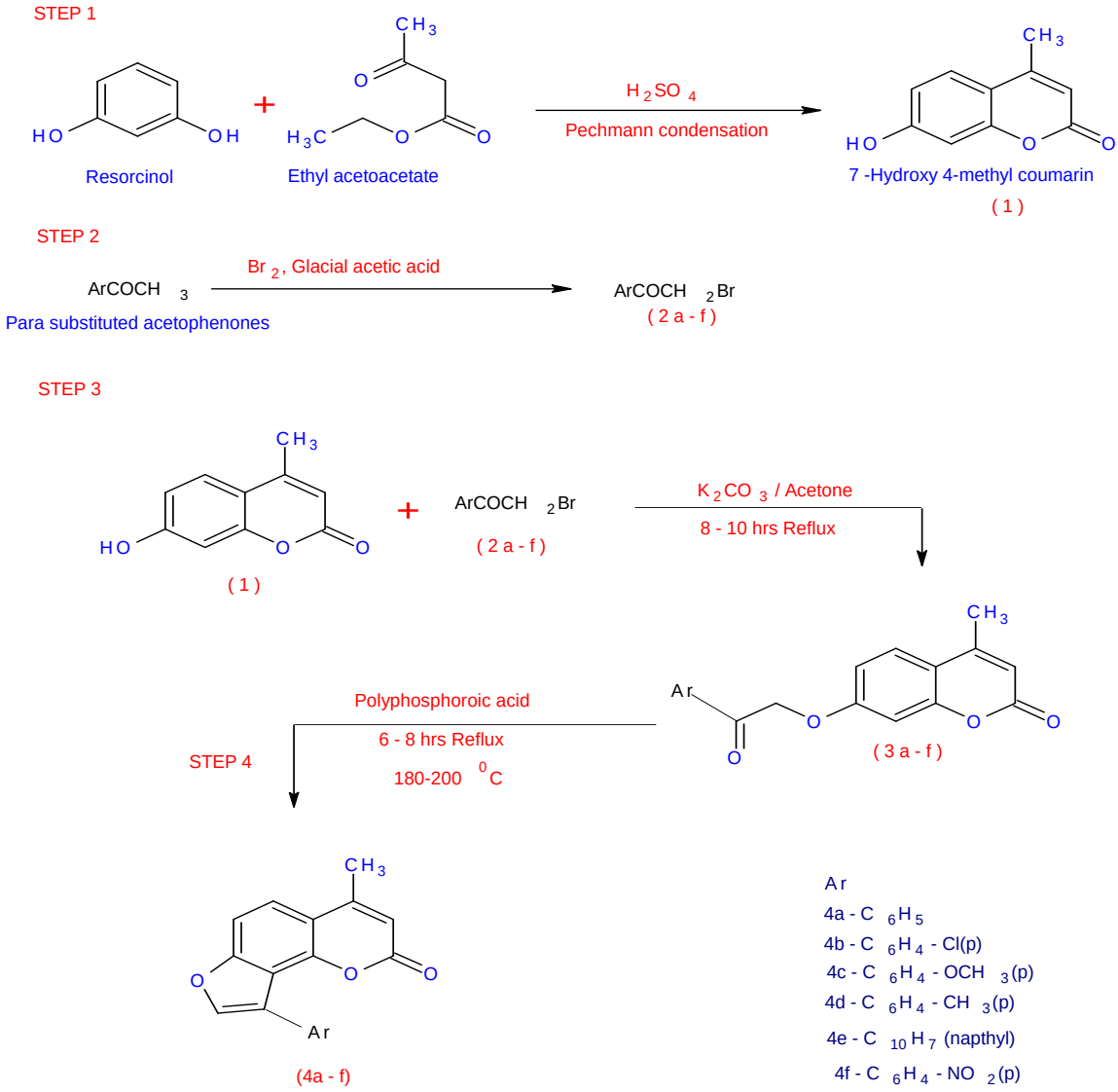
The retrospective look at furocoumarins reveals undoubtly that besides providing the effective treatment for skin ailments, they unleashed a revolution in photo chemotherapy and substantiating the concept of photobiology. These linear furocoumarins, in presence of UV intercalates with the cellular components and forms mono and di adducts specially with nucleosides. In order to minimize the di adduct formation and thus the side effects, the study aimed at synthesizing some novel angular furocoumarins which are expected to have cytotoxic activity devoid of serious side effects.

EXPERIMENTAL SECTION

All the chemicals and solvents used were of synthetic grade obtained from Sd Fine chemicals Ltd.,(Mumbai, India), E. Merck . Completion of the reactions was monitored from time to time by analytical thin layer chromatography (TLC) using E-Merck silica gel 60 F_{254} 0.25 mm precoated aluminium plates. Visualization was accomplished with UV light (254 nm) and iodine chamber.

Melting points were determined on open capillary tubes in ANALAB melting point apparatus and were uncorrected. All the IR spectra were recorded on SCHIMADZU FT-IR spectrophotometer by using 1 % potassium bromide discs. Mass spectra and NMR data of the compounds were recorded on Mass spectrometer (Aligent 1100 series; EI/ES-MS) and Brucker AC-400(400 MHz) at Indian Institute of Chemical Technology, Hyderabad and Hetero labs, Hyderabad.

In the present scheme, the angular furocoumarins were synthesized by in following steps.
1. Condensation of different phenacyl bromides (α-halo ketones) with 4-methyl-7-hydroxy coumarin.
2. Cyclization of oxoethers in presence of polyphosphoric acid as cyclizing agent.
Finally, these synthesized compounds are planned to screen for and also for anti oxidant and preliminary cytotoxic studies.



SCHEME

Step 1: Synthesis of 7- hydroxy -4- methyl coumarin:

0.18 mol of resorcinol was dissolved in 0.18 mol. of ethyl acetoacetate and the mixture was cooled to below 15˚C. While the solution was cool , 100 ml of conc. H_2SO_4 was added drop by drop over a period of ½ an hour and was brought to room temperature and ice-cold water was added to it with stirring. The precipitate obtained was filtered and was recrystallized from methanol /ethanol as creamy coloured needles.

Step 2: Synthesis of phenacyl bromides:

To 0.05 mol acetophenone / substituted acetophenones dissolved in 20 ml of glacial acetic acid, 0.05 mol of bromine in acetic acid was added slowly and with constant stirring in cool conditions. After adding all the bromine, the reaction mixture was heated on water bath to give

off all the hydrobromic acid. The colour of the solution lightened to straw yellow. Then this solution was brought to room temperature and was added to ice-cold water. The precipitate of brominated compound commences to form. The precipitate obtained was filtered and recrystallized from ethanol.

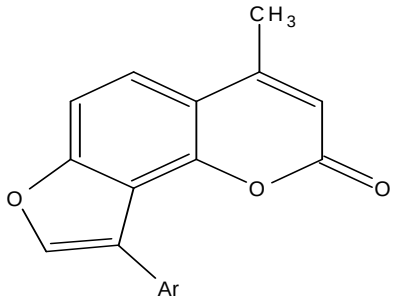
Step 3: Synthesis of 4-methyl-7-[2-(4-methylphenyl)-2-oxoethoxy]-2*H*-chromen-2-ones:
0.0088 mol of 7-hydroxy-4-methylcoumarin (1), 0.019 mol of substituted phenacyl bromides (2a-f) and potassium carbonate (1 g) in dry acetone (50 ml) were taken in a RB flask and allowed to undergo reflux for about 8-10 hrs. The progress of the reaction was monitored by TLC (Solvent system: ethyl acetate: n-Hexane: 6:4). After the reaction was complete, the mixture was cooled; the solid obtained was filtered off and washed with fresh acetone. The solvent was evaporated from combined filtrate and washings under reduced pressure and the residue was collected to give (3a-f).

Step 4: Synthesis of 4-methyl-9-(4-methylphenyl)-2*H*-furo [2,3-*h*]chromen-2-ones:
0.01 mol of above compound were taken in a double necked RB flask. One neck was attached with condenser and the other was used to add 5-10 ml of freshly prepared polyphosphoric acid. Then the mixture was allowed to reflux in oil bath at 180-200^{0}C for 6-8 hrs. The temperature is allowed to rise slowly for the reaction to happen. The progress of the reaction was monitored by using TLC. After a single green fluorescent spot is appeared in TLC, the reaction was stopped and the reaction mixture was brought to room temperature. Then this mixture was poured into crushed ice and excess polyphosphoric acid was neutralized with 10% $NaHCO_3$. The precipitate of furocoumarin commences to form.

RESULTS AND DISCUSSION

The compounds were synthesized according to the scheme and interpreted by Physical, chemical and analytical data as shown in Table-1 and Table-2.

Table 1: Physical data of some final compounds



Cmpd	Ar	IUPAC Name	M.P (^{0}C)	R_f	Yield (%)
4b	C_6H_4-Cl(p)	9-(4-chlorophenyl)-4-methyl-2*H*-furo[2,3-*h*]chromen-2-one	196-198	0.75	70
4c	C_6H_4-OCH_3(p)	9-(4-methoxyphenyl)-4-methyl-2*H*-furo[2,3-*h*]chromen-2-one	175-177	0.70	75

Table 2: Spectral data of some final compounds

Cmpd	Mol. Formula & Mol. wt	IR (KBr disc)stretching frequency in cm-1	MS (m/z)	NMR data
4b	$C_{18}H_{11}ClO_3$ 310.73	C=O(lactone):1721.36 Ar-O-C:1278.72 C=C:1612.38 C-Cl:746.40	M^+: 310	^{1}H NMR ($CDCl_3$): 6.8-7.9(m, 8H, Ar-H), 1.9(s, 3H, CH_3)
4c	$C_{19}H_{14}O_4$ 306.31	C=O(lactone):1723.40 Ar-O-C:1253.64 C=C:1612.38	M+1: 307 M+23: 329	^{1}H NMR ($CDCl_3$): 6.9-7.8(m, 8H, Ar-H), 3.5(s, 3H, OCH_3), 2.0(s,3H, CH_3)

The synthesized compounds were studied for Free radical scavenging activity and preliminary cytotoxic activities.

Free Radical Scavenging Activity:

The free radical scavenging activity of six synthesized compounds was measured by 2, 2-DiPhenyl-1-Picryl Hydrazyl (DPPH) [11, 12] using the method reported by Aquino *et. al*[13].

Cytotoxic study:

The preliminary cytotoxic activity was studied by Tryphan blue dye exclusion method [14, 15] using Ehrlich Ascites Carcinoma cells (EAC) [16].

The results corresponding to radical scavenging and cytotoxic studies are shown as percentage radical scavenging (%RSC) and percentage inhibition in the Table-3 and Figure-1 respectively.

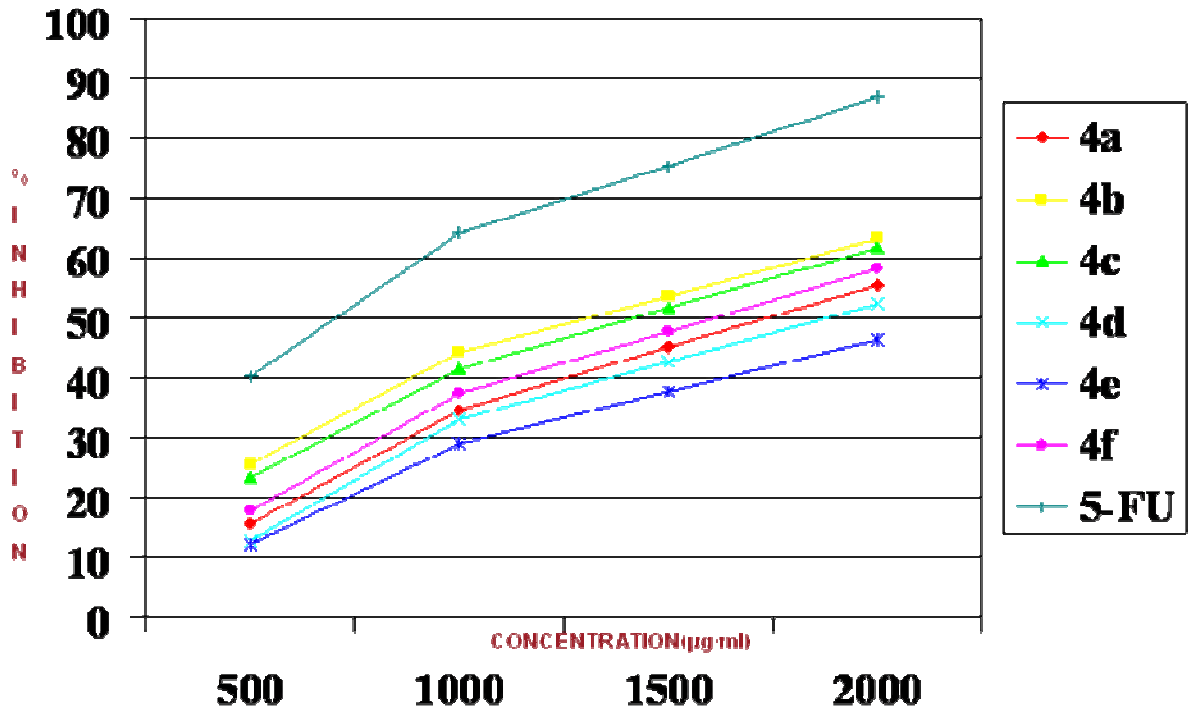


Figure-1: Graphical representation of cytotoxic results

Table 3: Percentage Radical Scavenging values of some synthesized compounds

	% RSC			
Concentration in µg/ml	**4b**	**4c**	**Concentration in µg/ml**	**% RSC of Ascorbic acid**
100	31.7	17.1	1	19.2
200	37.1	21.9	2	36.4
300	42.4	26.1	3	59.8
400	48	32.7	4	62.1
500	53.6	31.5	5	91.4
600	59	43.6	6	94.7
700	57.2	48.8	7	95.3
800	70.5	52.6	8	95.8
900	75.3	58.6	9	95.8
1000	76.7	59.6	10	95.5

CONCLUSION

The compounds 4b and 4c i.e, possessing chloro and methoxy substitutions exhibited free radical scavenging activity with IC_{50} value of 440µg/ml and 735 µg/ml respectively.The interesting thing is that compounds that exhibited radical scavenging activity i.e. 4b and 4c have also exhibited cytotoxic activity at IC_{50} of 1320 µg/ml and 1430 µg/ml respectively.

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REFERENCES

[1] Noriko Kitamura, Shigeru Kohtani, Ryoichi Nakagaki. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews.,* **2005,** 6, 168-185**.**
[2] F. Dall. Aquia, P. Martelli. *J. Photo&em. Photobiol. B: Biol.,* **1991,** *8,* 235-254
[3] Sergio Caffieri**.** *Photochem. Photobiol. Sci.,* **2002,** 1, 149–157
[4] Deidra Bethea, Brandie Fullmer, Saif Syed, Gregory Seltzer, Joseph Tiano Et. al. *Journal of Dermatological Science.,* **1999,** 19, **7**8–88**.**
[5] Soroush Sardari, Yoki Mori, Kiyoshi Horita Et. al. *Bioorganic & Medicinal Chemistry.,* **1999,** 7, 1933-1940**.**
[6] Franz Hadacek, Claudia muller Et. al. *Journal of chemical ecology.,* **1994,** 20 (8), 2035-2054.
[7] Pierre. Escoubas, Yukiharu fukushi Et. al. *Journal of chemical ecology.*, **1992,** 18(10), 1819-1831.
[8] A.M.A.G. Oliveira , M. Manuela M. Raposo, A.M.F. Oliveira-Campos. *European Journal of Medicinal Chemistry.*, **2006,** 41, 367–372**.**
[9] Manuel Campos-Toimil, Francisco Orallo, Lourdes Santana and Eugenio Uriarte. *Bioorganic & Medicinal Chemistry Letters.,* **2002,** 12, 783–786**.**
[10] Lourdes Santana, Eugenio Uriarte, Lisa Dalla Via and Ornella Gia. *Bioorganic & Medicinal Chemistry Letters.,* **2000,** 10, 135-137**.**
[11] Damiki Laloo and Alakh N Sahu. *J. Chem. Pharm. Res.,* **2011,** 3(1), 277-283

[12] Shweta Saboo, Ritesh Tapadiya, S. S. Khadabadi and U. A. Deokate. *J. Chem. Pharm. Res.*, **2010,** 2(3), 417-423
[13] Aquino et.al. *J. Natl. Prod.*, **2001,** 64, 1019.
[14] K.Vijayakumar and A. Jafar Ahamed. *J. Chem. Pharm. Res*., **2010**, 2(4), 215-224
[15] R Ian Freshney. Culture of Animal Cells, A Manual of Basic Technique, edu. **1988,** 2, 246.
[16] B. Rajkapoor, B. Jayakar. *Indian. J. Pharmacol.*, **2004,** 36(1), 38-40.