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Authors	Suman Rani, Nisha Kamra, Sumit Thakral, Devinder Kumar, Ajeet Singh, Payare L. Sangwan and Shashank K. Singh
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ORCID® iDs	Devinder Kumar - https://orcid.org/0000-0002-1371-0680

Design and synthesis of highly oxygenated furo[3,2-c]pyran-4-ones and furo[3,2-c]chromen-4-ones scaffold as potential anticancer and antimicrobial agent

Suman Rani[a], Nisha Kamra[a], Sumit Thakral[a], Devinder Kumar[a*], Ajeet Singh[b], Payare L. Sangwan[b], Shashank K. Singh[b]

[a]Department of Chemistry, Guru Jambheshwar University of Science and Technology, Hisar-125001, Haryana, India

[b]CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu-180001, India

*Corresponding Author
Prof. Devinder Kumar

dk_ic@yahoo.com
Phone No. +91-1662-263358

ABSTRACT

Synthesis of a number of highly oxygenated furo[3,2-c]pyran-4-one (**4**, **5**) and furo[3,2-c]chromen-4-one (**8**, **9**) has been accomplished by a simple one pot reaction from easily available versatile starting materials - dehydroacetic acid and 3-acetyl-4-hydroxycoumarin. All the synthesized molecules were characterized utilizing various spectroscopic techniques and screened for anticancer activity (*in vitro*) against three Colon (HCT-116, SW-620, HT-24), Lung (A-549), Prostate-(PC-3), Breast-(MCF-7) cell lines. Compounds **5a**, **9d**, **9f** showed good activity against breast MCF-7 cancer cell line having IC_{50} values 6.9, 2.8, 5.3 µM, respectively. Out of these compound **9d** showed better activity against prostate PC-3 cell line with IC_{50} value 3.8 µM. The synthesized compounds were also studied for potential antibacterial activity (*in vitro*) using different strains of bacteria (*Bacillus subtilis* and *Staphylococcus aureus* -Gram-positive, and *Escherichia coli*- Gram negative) as well as fungal strains (*Aspergillus niger* and *Candida albicans*) using Norfloxacin and Fluconazole as antibacterial and antifungal standard drugs, respectively. The outcome of the antimicrobial screening study showed that compound **9f** exhibited promising activity against *S. aureus* and *B. subtilis* while **5h** showed excellent and **5i** and **9b** showed better activity against *E. coli*. The compounds **5c**-**5e** displayed excellent activity against *C. albicans* and *A. niger* than Fluconazole.

Keywords: 3-Acetyl-4-hydroxycoumarin, anticancer, antimicrobial, dehydroacetic acid, furo[3,2-c]pyrone, furo[3,2-c]chromen-4-one

Introduction

The development of innovative anticancer remedies with minimum toxicity and considerable activity is continuously explored area of anticancer research and among these, naturally derived agents grew substantial consideration because of their appreciable antitumor activity. Naturally occurring molecules possessing furo[3,2-c]pyran-4-one skeleton display different biological activity e.g. Neo-tanshinlactone, a steroid isolated from *Tanshen* is a good cytotoxic agent against human breast cancerous cell lines (MCF-7 and ZR-75-1) [1-4]. Niveulone, a terpenoid compound isolated from fungus i.e. *Dasyscyphus niveus*, reported cytotoxic having lesser cytotoxic effect to human cell lines [5]. Inoscavin A and Phelligridin F, possessing a 2,3-dihydrofuro[3,2-c]pyran-4-one skeleton, are natural products isolated from a fungus and exhibited free radical scavengers with cytotoxic activities [6,7]. Niveulone, containing a 2,3-dihydrofuro[3,2-c]pyran-4-one moiety that is linked to a terpenoid part in a heterocyclic spiro compound isolated from *Dasyscyphus niveusis* weakly cytotoxic towards human cell lines [8]. The phellifuropyranone, 2-(3,4-dihydroxyphenyl)-6(20-(3,4-dihydroxyphenyl)-E-ethenyl)furo[3,2-c]pyran4-one, has antiproliferative activity against mouse melanoma cells and human lung cancer cells [9]. Coumestrol, coumestan [10], medicagol, wedelolactone [11], plicadin [12], psoralidin [13] are other naturally occurring and therapeutics molecules possessing furo[3,2-c]pyran-4-one skeleton. The structure of selected naturally occurring and bioactive molecules possessing furo[3,2-c]pyrone and furo[3,2-c]coumarin are shown in Figure 1.

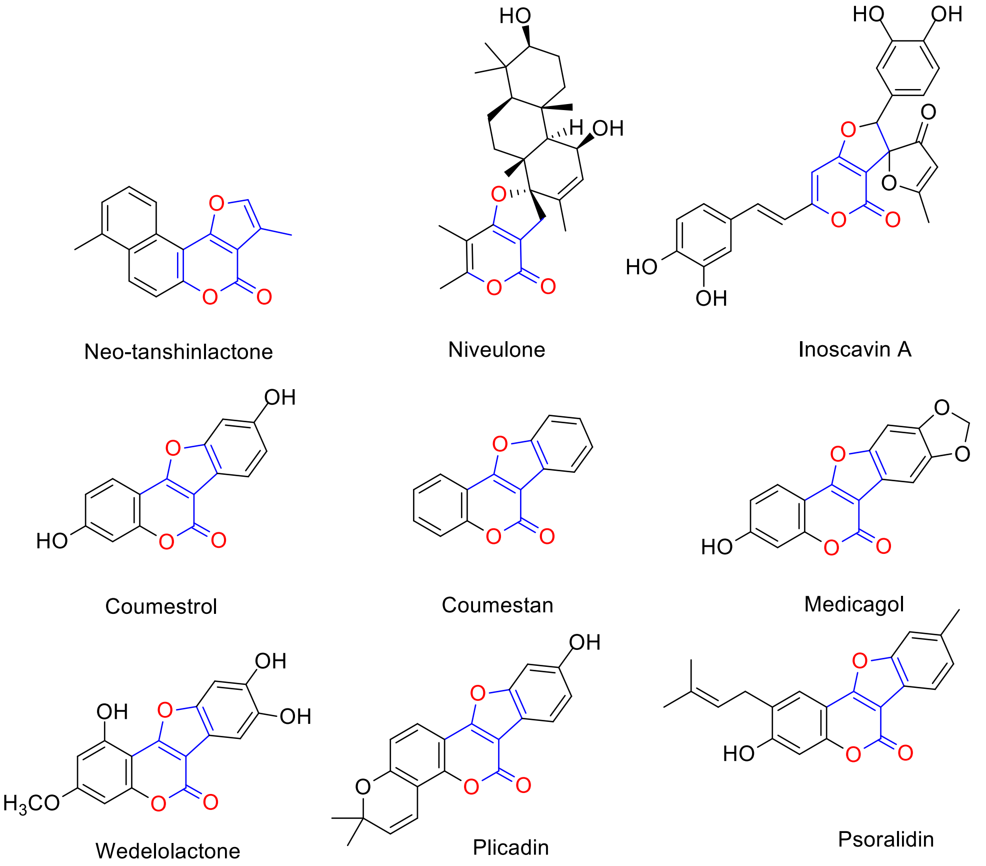
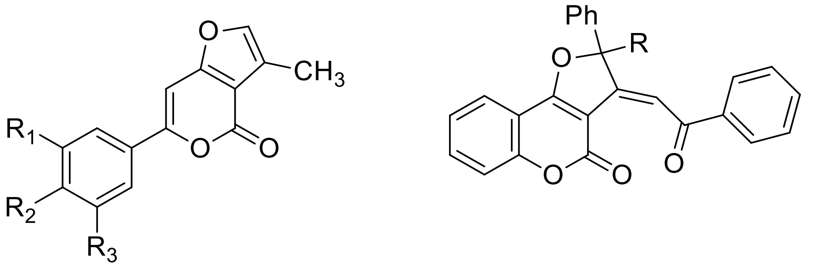


Figure 1 Natural products containing furo[3,2-c]pyran-4-one and furo[3,2-c]chromen-4-one skeleton.

The synthetic derivative, 6-phenyl-4H-furo[3,2-c]pyran-4-one have been reported for potent growth inhibition against the SK-BR-3 breast cancer cell line [14]. Similarly, the synthetic derivative, furo[3,2-c]chrome-4-one (Figure 2) also reprted for exhibition of very decent anticancer activity against HCT–15 cell line (colon cancer) by cell growth inhibition [15]. Besides anticancer activity, there are diversity of furo[3,2-c]pyrones and furo[3,2-c]chromen-4-ones which have attracted the significant attention world over due to wide spectrum of remarkable biological properties e.g. antimicrobial [16] , anti-inflammatory [17] , anticoagulant [18] , insect antifeedant [19] and insecticidal [20].



$R_1 = R_2 = R_3 = OCH_3$
$R_1 = R_3 = OCH_3$; $R_2 = H$

R= OCH_2CH_3, $N(CH_2CH_2)_2$

Figure 2 Structure of the effective anticancer furo[3,2-c]pyran-4-one equivalents.

Further, there is growing interest in the development of synthetic strategy for furo[3,2-c]pyran-4-ones and furo[3,2-c]chromen-4-ones. Many methods have been successfully advanced for the synthesis of furo[3,2-c]pyran-4-ones and furo[3,2-c]chromen-4-ones utilizing different typical procedures [21-27]. Therefore, herein we report a simple and straight forward synthesis of furo[3,2-c]pyran-4-ones and furo[3,2-c]chromen-4-ones from readily available versatile materials, dehydroacetic acid and 4-hydroxycoumarin, respectively. In continuation of previous work [28], we planned to synthesized further furo[3,2-c]pyran-4-ones and furo[3,2-c]chromen-4-ones using different methods and synthesized compounds were characterized using different spectroscopic technique and screened for cytotoxicity as well as antimicrobial activities to identify the potent molecules.

RESULTS AND DISCUSSION

Chemistry

The designing of highly oxygenated furo[3,2-c]pyran-4-ones and furo[3,2-c]chromen-4-ones was based on the literature report of significant anticancer activity of the four molecules of same scaffolod as shown in Fig. 3. The protocol for the synthesis of furo[3,2-c]pyran-4-one derivatives (**4a-4c, 5a-5l**) is very ecomonical, simple and versatile which is outlined in Scheme **1**. Dehydroacetic acid (DHAA) and 3-acetyl-4-hydroxycoumarin are easily and economically available starting material for synthesis of highly oxygenated molecules having basic furo[3,2-c]pyran-4-one structural unit as well as variety of aroyl and styryl moiety with methyl and methoxy substituents. Different α,β-unsaturated carbonyl compounds of DHAA (chalcones) were prepared by condensation of different aryl aldehyde in methanol in presence of base piperidine. DHAA (**1**) and its chalcone (**2**) were reacted separately with α-bromoketones to obtain the desired furo[3,2-c]pyran-4-ones scaffold containing aroyl and styryl moiety. The conditions were optimized in order to get the excellent yield in less reaction time by using dry acetone under reflux, dichloromethane and water in presence of phase transfer catalyst at room temperature and finally in acetonitrile under reflux (Table 1). The reaction was efficiently completed producing excellent yield of furo[3,2-c]pyran-4-ones (**4a-4c, 5a-5l**) in acetonitrile under reflux in presence of potassium carbonate (Scheme 1).

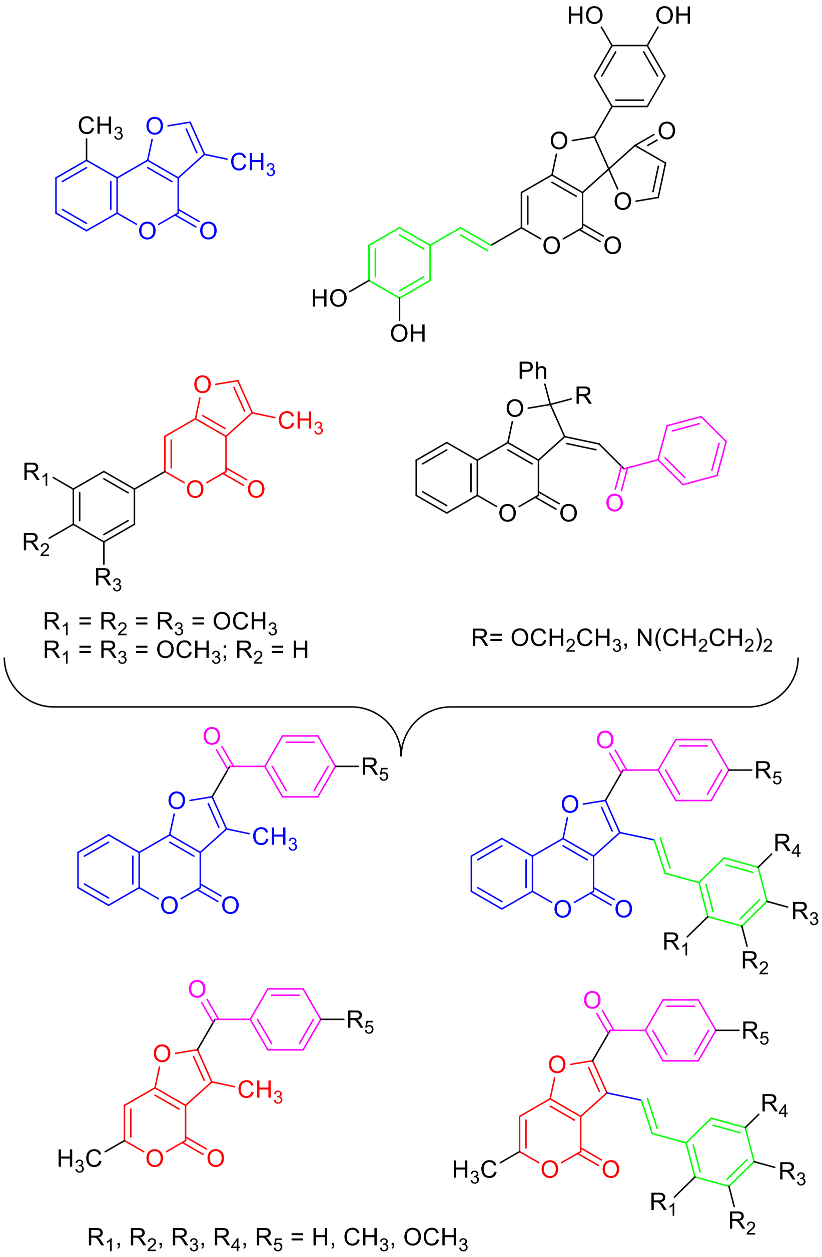
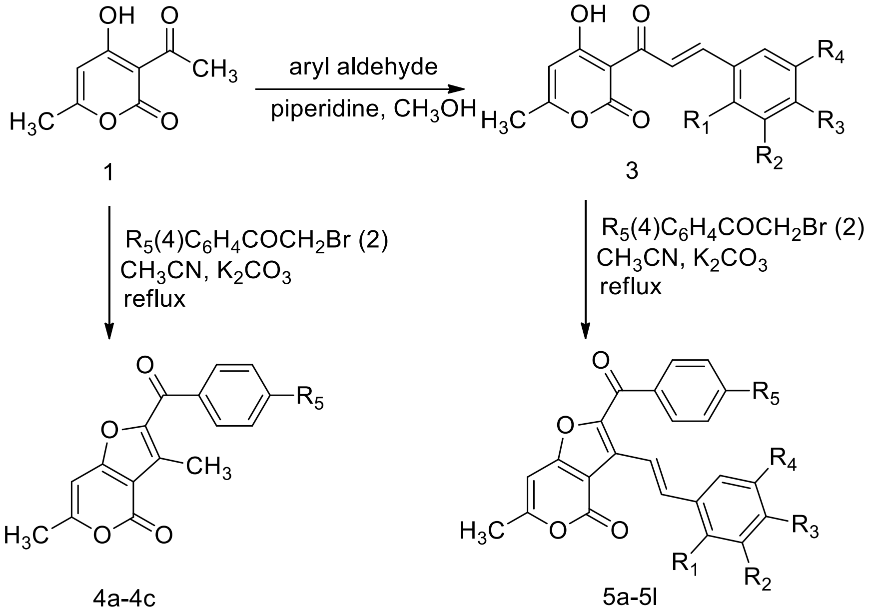


Figure 3 Designing of furo[3,2-c]pyran-4-ones and furo[3,2-c]chromen-4-ones.

Table 1. Reaction conditions for the synthesis of furo[3,2-c]pyronesand furo[3,2-c]chromen-4-ones.

Entry	Solvent	Reaction condition	Time	Yield
1.	dry acetone	Reflux	36h	80%
2.	dichloromethane and water	stirring with PTC	3h	75%

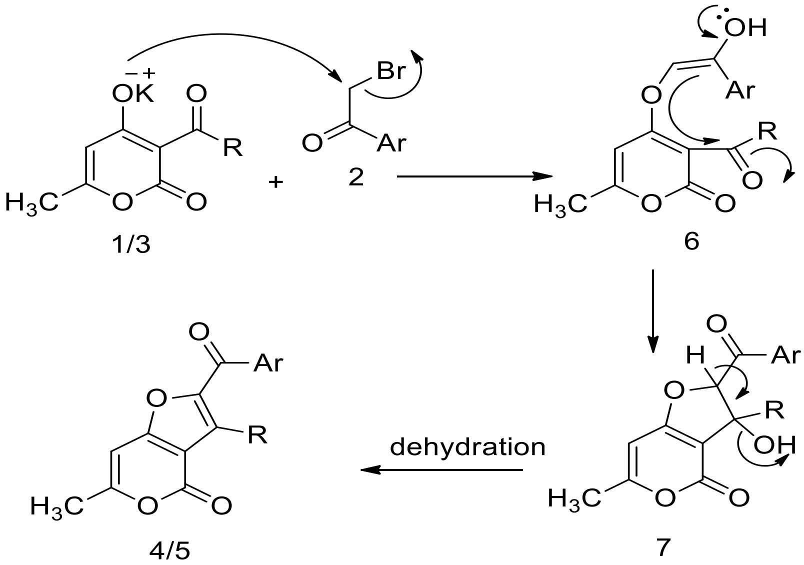
3.	Acetonitrile	Reflux	3h	97%



4a; R_5=H; 4b; R_5=CH_3 4c; R_5=OCH_3
5a; R_1=OCH_3, R_2=H, R_3=H, R_4=OCH_3, R_5=H 5b; R_1=OCH_3, R_2=H, R_3=H, R_4=OCH_3, R_5=CH_3
5c; R_1=OCH_3, R_2=H, R_3=H, R_4=OCH_3, R_5=OCH_3 5d; R_1=OCH_3, R_2=H, R_3=OCH_3, R_4=H R_5=H
5e; R_1=OCH_3, R_2=H, R_3=OCH_3, R_4=H R_5=CH_3 5f; R_1=OCH_3, R_2=H, R_3=OCH_3, R_4=H R_5=OCH_3
5g; R_1=OCH_3, R_2=OCH_3, R_3=H, R_4=H, R_5=H 5h; R_1=OCH_3, R_2=OCH_3, R_3=H, R_4=H, R_5=CH_3
5i; R_1=OCH_3, R_2=OCH_3, R_3=H, R_4=H, R_5=OCH_3 5j; R_1=H, R_2=OCH_3, R_3=OCH_3, R_4=OCH_3 R_5=H
5k; R_1=H, R_2=OCH_3, R_3=OCH_3, R_4=OCH_3 R_5=CH_3 5l; R_1=H, R_2=OCH_3, R_3=OCH_3, R_4=OCH_3 R_5=OCH_3

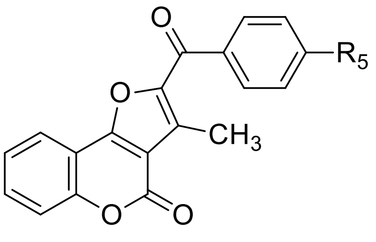
Scheme 1 Synthesis of furo[3,2-c]pyran-4-ones (**4a-4c, 5a-5l**).

The probable mechanism involves the nucleophilic attack of potassium salt of DHAA (**1**) or its chalcone (**3**) on α-bromoketones thus resulting in intermediate ether **6** with the elimination of hydrogen bromide. The intermediate ether **6** so formed *in situ* is cyclized to produce the intermediate **7** which on dehydration leads to the formation of desired furo[3,2-c]pyran-4-ones (**4**/**5**) (Scheme 2).

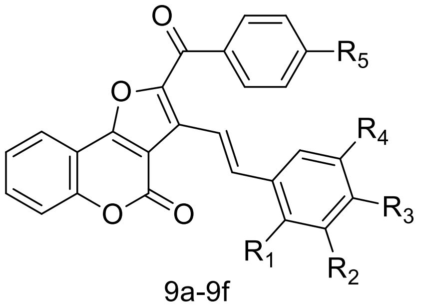


Scheme 2 Probable mechanism for the synthesis of furo[3,2-c]pyran-4-ones.

In a similar manner, 3-acetyl-4-hydroxycoumarin and its chalcone were reacted separately with α-bromoketones to obtain the desired products (**8a-8c, 9a-9f**) in acetonitrile under reflux in presence of potassium carbonate in excellent yield (Fig.4). All the products were characterized by IR, NMR (1H & 13C) and mass spectral data interpretation.



8a-8c



9a-9f

8a; R_5=H
8b; R_5=CH_3
8c; R_5=OCH_3

9a; R_1=OCH_3, R_2=H, R_3=H, R_4=OCH_3, R_5=H
9b; R_1=OCH_3, R_2=H, R_3=H, R_4=OCH_3, R_5=CH_3
9c; R_1=OCH_3, R_2=H, R_3=H, R_4=OCH_3, R_5=OCH_3
9d; R_1=H, R_2=OCH_3, R_3=OCH_3, R_4=OCH_3 R_5=H
9e; R_1=H, R_2=OCH_3, R_3=OCH_3, R_4=OCH_3 R_5=CH_3
9f R_1=H, R_2=OCH_3, R_3=OCH_3, R_4=OCH_3 R_5=OCH_3

Figure 4 Synthesis of furo[3,2-c]chromen-4-ones compounds **8a-8c** and **9a-9f**.

The compounds **4c** and **9a** exhibited two moderate absorption bands. In compound **4c** the band at 1746 cm^{-1} was due to stretching vibrations of lactone carbonyl group and the band at 1629 cm^{-1} was due to aroyl carbonyl group while in compound **9a,** the band at 1753 cm^{-1} was due to stretching vibrations of lactone carbonyl group and the band at 1600 cm^{-1} was due to aroyl carbonyl group.

In ^{1}H NMR spectrum, compound **4c** showed one singlet at δ 3.90 ppm due to methoxy group of at position-4 of aroyl ring and one doublet at δ 6.43 ppm due to 1H of pyran-4-one and phenyl ring protons displayed doublet having *J* value 6.88 Hz at δ 7.99 and 6.99 ppm due to 2'-H/6'-H and 3'-H/5'-H, respectively. In ^{13}C NMR spectrum, the peak at 182.41 ppm was assigned to carbonyl carbon and at 95.61 ppm was due pyran-4-one carbon and methoxy group assigned at 55.53 ppm. COSY exhibited the connectivity of hydrogen atoms through intervening bond where the aryol group hydrogens $H_{3'}$/$H_{5'}$ correlated with $H_{2'}$/$H_{6'}$and H_6 with H_7. HSQC spectrum of **4c** provided the carbon hydrogen correlation at δ 10.82 (6-CH_3), 20.53 (3-CH_3), 55.53 (OCH_3), 95.61 (C-7), 113.78 (C-3', C-5'), 131.89(C-2',C-6'). The ^{1}H and ^{13}CNMR data with hydrogen-hydrogen correlations of compound **4c** are demonstrated in Figure 5.

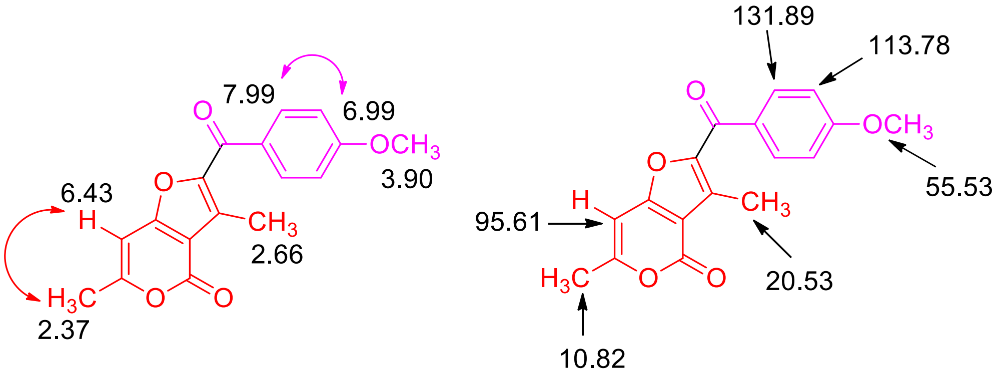


Figure 5 ^{1}H and ^{13}C NMR with hydrogen correlation of compound **4c**

The compound **9a** showed two singlet at δ 3.81, 3.88 ppm due to methoxy group of styryl group and one singlet at δ 6.85 ppm due to 2H and one singlet at δ 7.17 ppm due to 1H were attributed to 3''-H and 6''-Hand 4''-H, respectively. The peaks of a and b protons of styryl group resonated at 7.93 (d) and 8.75 (d), respectively with *J* value of 16.0 Hz thereby confirming the E geometry of double bond. Coumarin protons exhibited four peaks at δ 7.59-7.65 (m), 7.48 (d), 7.37 (m) and 7.90 (dd) ppm due to 8-H, 6-H, 7-H and 9-H, respectively. The aroyl group attached with furan ring displayed doublet at δ 7.55 ppm due to 3'-H and 5'-H with *J* value 8.9 Hz and doublet at δ 8.00 ppm due to 2'-H and 6'-H. In ^{13}C NMR spectrum, compound **9a** gave different peaks at 184.17 ppm was assigned for carbonyl carbon and 55.89, 56.64 ppm were due to two methoxy carbon. In DEPT-135, there were fourteen signals due to CH and CH_3; four peaks at 132.70, 124.75, 121.94 and 117.32 ppm for coumarin carbon and two peaks at 129.56 and 128.49 ppm for aroylmoiety. The connectivity of hydrogen atoms through intervening bond was established by COSY. The hydrogen-hydrogen correlation was observed in coumarin H_9 with H_8, H_8 with H_7, and H_7 with H_6 and aroyl hydrogens $H_{2'}/H_{6'}$ with $H_{3'}/H_{5'}$ and $H_{6'}$ with α proton and $H_{3'}/H_{5'}$ with $H_{4'}$. The carbon-hydrogen correlations were ascertained by analyzing the HSQC spectrum that the position of carbon signals at δ 55.89, 56.64 (OCH_3), 112.17 (C-4”), 112.84 (C-3”), 115.97 (C-6”), 116.70 (C-α), 117.32 (C-6), 121.94 (C-9), 124.75 (C-8), 128.49 (C-3’,C-5’), 129.56 (C-2’,C-6’), 132.54 (C-4’), 132.70 (C-7), 136.28 (C-β) ppm. The ^{1}H and ^{13}CNMR with correlations of compound **9a** are demonstrated in Figure 6. The assignment of each hydrogen and carbon was established by interpreting the 2DNMR (COSY, HSQC).

HRMS also confirmed structures of the compound **4c** and **9a**. For compound **4c**, HRMS: m/z (M^+) calcd. for $C_{17}H_{14}O_5$: 298.0841 and found: 299.0958 $(M+H)^+$. For compound **9a**, HRMS: m/z (M^+) calculated for $C_{28}H_{20}O_6$: 452.1260 and found: $(M+H)^+$ 453.1354. The data of all other synthesized compounds were also analyzed and found to be in consonance with the structure assigned.

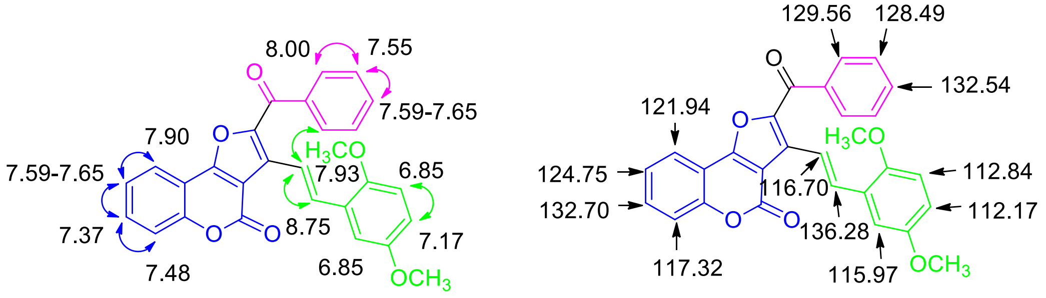


Figure 6 ^{1}H and ^{13} CNMR with hydrogen correlation of compound **9a.**

Pharmacology/Biology

Anticancer activity

All the synthesized furo[3,2-c]pyran-4-one (**4**,**5**) and furo[3,2-c]chromen-4-ones (**8**, **9**) compounds were studied for anti-cancer potential by screening cytotoxicity through % growth inhibition using SRB assay [29]. All the compounds were screened for % growth inhibition at 50 µM concentration against six different human cancer cell lines. The cytotoxicity were performed using three different Colon cancer cell lines (HCT-116, SW620, HT-24), Lung A549, Prostate PC-3, Breast MCF-7 human cancerous cells by SRB assay. Paclitaxel was taken as positive control. Results are summarized in Table 2. On analysis of results from the Table 2, it was found that the furo[3,2-c]chromen-4-one **9b, 9d-9f** displayed significant growth inhibition effects against six cancer cell lines and **9f** showed 100 % growth inhibition effect against colon HCT-116. The furo[3,2-c]pyran-4-one **5a** showed noteworthy growth inhibition effects against four cancer cell lines examined except colon PC-3 and SW620, whereas **5b, 5g** and **5l** showed noteworthy growth inhibition effects against four cancer cell lines examined except colon SW620 and colon HT29. It has also been noted that **5c** exhibited 51 and 57 % growth inhibition effects against prostate PC-3 and breast MCF-7, respectively. Beside this, the results of compounds are also represented graphically (Figure 7). Those compounds which demonstrated >75% growth inhibition at 50 µM were further taken up for determination of their IC_{50} by screening at six different concentrations i.e. 1.0, 2.5, 5.0, 7.5, 10 and 50 µM. IC_{50} values of these compounds which were determined against all above cell lines and summarized in the Table 3. Graphical representation of the IC_{50} value indicated that furo[3,2-c]chromen-4-one **9d** showed good cytotoxicity at low concentration against PC-3 (Prostate) and MCF-7 (Breast) cell lines having IC_{50} value 3.8 and 2.8 µM, respectively using positive control drug paclitaxel.

Table 2. Cytotoxic activity of synthesized compounds (**4, 5, 8 and 9**) at 50 µM concentration

Tissue	**Lung**	**Prostate**	**Colon**	**Breast**	**Colon**	**Colon**
Cell Lines	**A549**	**PC-3**	**HCT-116**	**MCF-7**	**SW620**	**HT-29**
Code	**% CYTOTOXICITY**					
4a	3	0	0	16	0	0
4b	0	0	0	12	0	0
4c	0	0	0	16	0	10
5a	**75**	59	**78**	**77**	0	**70**
5b	55	63	66	59	0	0
5c	51	46	49	57	0	0
5d	36	41	0	30	0	15
5e	18	0	16	36	0	0
5f	30	1	0	14	0	0
5g	**65**	51	**75**	**85**	39	37
5h	41	0	20	23	0	0
5i	40	27	0	32	0	0
5j	26	0	20	23	0	0
5k	41	17	0	47	0	0

5l	**77**	59	58	61	0	5
8a	0	0	0	46	0	0
8b	0	8	21	46	0	0
8c	18	0	0	0	0	0
9a	11	0	0	7	0	46
9b	**75**	42	**93**	**84**	**63**	**72**
9c	4	0	0	18	0	0
9d	**90**	**72**	**98**	**78**	**90**	**79**
9e	**87**	**75**	**96**	**83**	**94**	**80**
9f	**87**	**80**	**100**	**90**	**98**	**82**

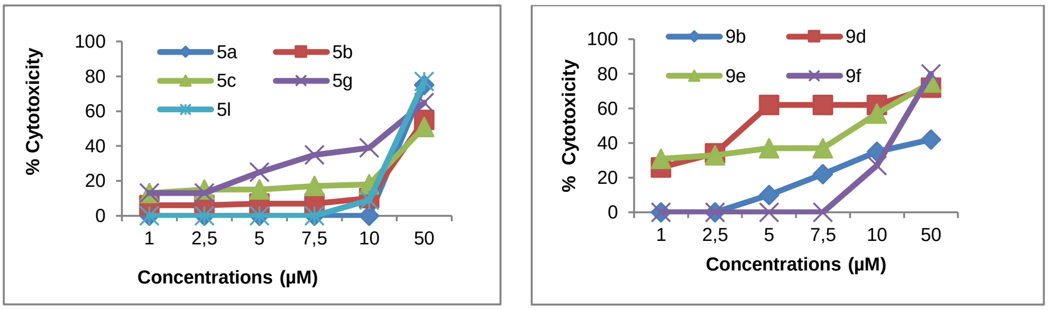


Figure 7(a) Anticancer activities (% growth inhibition) of compounds against lung A549cell lines at different concentrations.

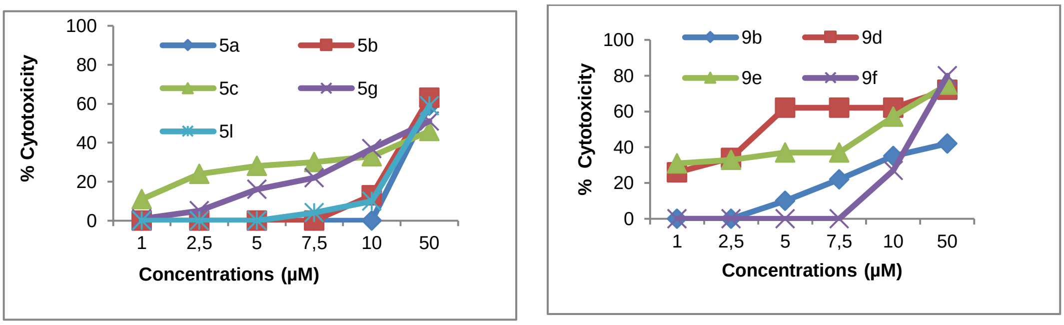


Figure 7(b) Anticancer activities (% growth inhibition) of compounds against prostate PC-3 cell lines at different concentrations.

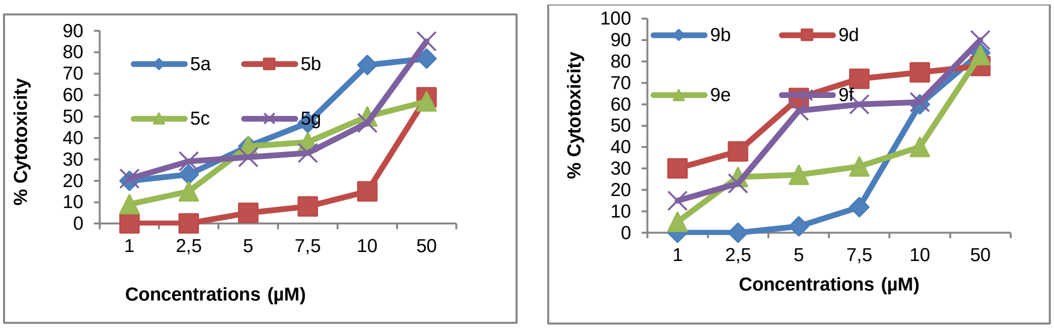


Figure 7(c) Anticancer activities (% growth inhibition) of compounds against breast MCF-7 cell lines at different concentrations.

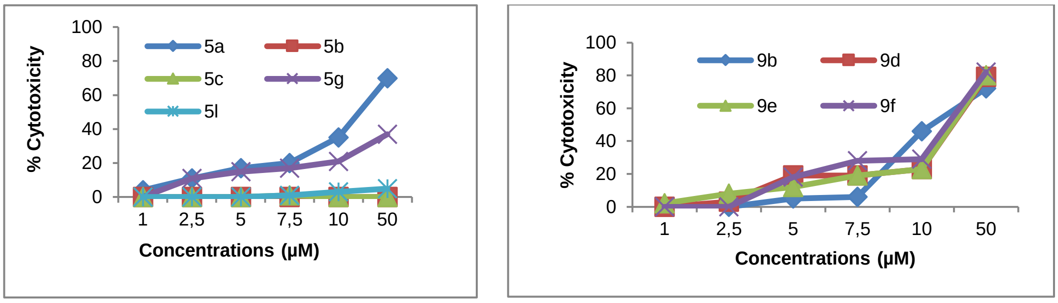


Figure 7(d) Anticancer activities (% growth inhibition) of compounds against colon HT-29 cell lines at different concentrations.

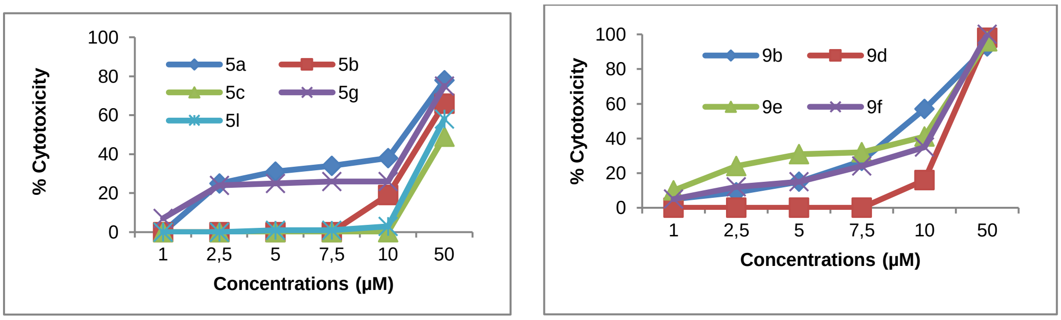


Figure 7(e) Anticancer activities (% growth inhibition) of compounds against HCT-116 (Colon) cell lines at different concentrations.

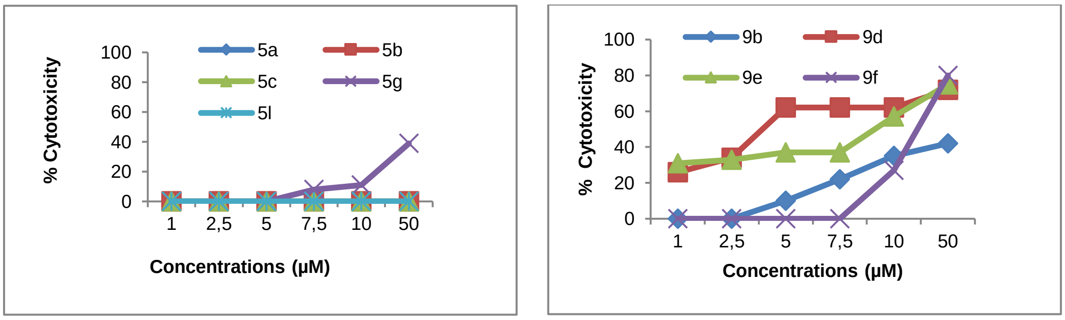


Figure 7(f) Anticancer activities (% growth inhibition) of compounds against colon SW-620 cell lines at different concentrations.

Table 3. IC_{50} values of selected synthesized compounds at different concentration

Tissue	**Lung**	**Prostate**	**Breast**	**Colon**	**Colon**	**Colon**
Cell Lines	**A-549**	**PC-3**	**MCF-7**	**HT-29**	**HCT-116**	**SW-620**
Code	**IC_{50} Value**					
5a	35.7	44.1	**6.9**	22.1	13.4	ND
5b	45.9	40.4	40.1	ND	37	ND
5c	49.18	>50	15.1	ND	>50	ND
5g	18.6	39.3	14.01	50	24.7	>50
5l	34.2	42.9	18.9	>100	44.4	ND
9b	17.8	>100	15.4	21.8	12.3	19.9
9d	14.3	**3.8**	**2.8**	23.1	27.4	10
9e	9.9	10.9	14.5	25.06	13.6	12.3
9f	30.8	28.5	**5.3**	18.1	17.2	22.16
Paclitaxel	0.1	0.063	<0.01	-	0.120	-

ND= not determined due to inactive compound

Screening of *in vitro* antimicrobial assay

All the newly synthesized furo[3,2-c]pyran-4-one (**4**, **5**) and furo[3,2-c]chromen-4-ones (**8**, **9**) were assessed for *in vitro* antibacterial evaluation using two Gram-positive bacterial strains plus one Gram-negative bacterial strain. (*S. aureus*, *B. subtilis* and*E. coli*) and antifungal activity using two fungal strains (*Aspergillus niger* and *Candida albicans*) by using serial dilution technique [30]. The Norfloxacin and Fluconazole were taken as standard reference drugs against bacterial and fungal species, respectively. Minimum inhibitory concentrations -MIC were expressed in μmol/mL. The final results of antimicrobial evaluation are summarized in **Table 4.**

Table 4 Antibacterial and antifungal in vitro studies of following compounds in MIC, µmol/mL

Compound	***S. aureus***	***B. subtilis***	***E.coli***	***C.albicans***	***A.niger***
4a	0.185	0.092	0.046	0.092	0.092
4b	0.088	0.088	0.044	0.044	0.044
4c	0.083	0.167	0.083	0.083	0.041
5a	0.059	0.119	0.119	0.029	0.059
5b	0.057	0.028	0.057	0.028	0.057
5c	**0.013**	0.055	0.055	**0.006**	0.027
5d	0.029	0.029	0.029	**0.007**	0.029
5e	0.028	0.057	0.115	0.028	**0.007**
5f	0.111	0.055	0.055	0.027	0.027
5g	0.059	0.119	0.059	0.119	0.059
5h	0.028	0.028	**0.007**	0.057	0.057
5i	0.013	**0.013**	**0.013**	0.055	0.055
5j	0.055	0.055	0.055	0.027	0.013
5k	0.054	0.054	0.027	0.027	0.108
5l	0.104	0.104	0.052	0.013	0.052
8a	0.082	0.041	0.041	0.041	0.082
8b	0.039	0.039	0.078	0.039	0.078
8c	0.037	0.037	0.074	0.074	0.037
9a	0.027	0.027	0.055	0.027	0.055
9b	**0.013**	0.026	**0.013**	0.026	0.013
9c	0.025	0.051	0.025	0.025	0.051
9d	0.025	0.025	0.050	0.025	0.051
9e	0.025	0.025	0.025	0.025	0.025
9f	**0.012**	**0.012**	0.024	0.012	0.012
Norfloxacin	0.009	0.009	0.009	-	-
Fluconazol	-	-	-	0.010	0.010

Antibacterial Activity

All the newly synthesized furo[3,2-c]pyran-4-ones (**4**, **5**) and furo[3,2-c]chromen-4-ones (**8**, **9**) displayed MIC value from 0.007 to 0.185 µmol/mL with respect to standard drug Norfloxacin having MIC value 0.009 µmol/mL. It was noticed that furo[3,2-c]chromen-4-one (**9f)** disclosed superior activity against *S. aureus* and *B. subtilis* having MIC value 0.012 µmol/mL whereas furo[3,2-c]pyran-4-one **5h** presented improved activity against *E. coli* with 0.007µmol/mL MIC value than that of the standard Norfloxacin.

Antifungal Activity

All the newly synthesized furo[3,2-c]pyran-4-ones (**4**,**5**) and furo[3,2-c]chromen-4-ones (**8**, **9**) exhibited MIC value from 0.006 to 0.115 µmol/mL with respect to standard drug fluconazole having MIC value 0.010 µmol/mL. The furo[3,2-c]pyrone **5c** and **5d** displayed virtuous activity against *C. albicans* and *A. niger* having MIC value 0.006 and 0.007 µmol/mL, respectively and furo[3,2-c]pyrone **5e** showed significant antifungal activity against *A. niger* having MIC value 0.007 µmol/mL better than that of the standards.

Structure-activity relationship

The following structure-activity relationships were established from the antimicrobial and anticancer activity data of furo[3,2-c]pyran-4-ones (**4**, **5**) and furo[3,2-c]chromen-4-ones (**8**, **9**):

Anticancer activity

a) There is enhancement in cytotoxicity of most of furo[3,2-c]pyran-4-onestofuro[3,2-c]chromen-4-ones on replacing the methyl group at position-3 with the styryl group against A549, PC-3, HCT-116 and MCF-7 cancer cell lines. The pronounced effect was noted in **5a**, **5b**, **5g** and **5l** whereas excellent effect was observed in **9b, 9d**-**9f** against all the cancer cell lines under study.

b) There is also enhancement in cytotoxicity of the compounds having methoxy group at positions-3,4,5 in styryl group and in aroyl group in **9d**-**9f** against all the cancer cell lines under study and in **5l** against A549, PC-3, HCT-116 and MCF-7 cancer cell lines.

c) The IC_{50} values of compounds **9d**, **9f** and **5a** were determined 2.8, 5.3 and 6.9 µM against breast (MCF-7) cancer cell line and **9f** is also having IC_{50} values of 3.8 µM against prostate (PC-3) cancer cell line suggesting thereby that furo[3,2-c]chromen-4-ones are more active than furo[3,2-c]pyran-4-ones.

d) The compound 9d and 9f proved to be the most potent against breast (MCF-7) cancer cell line and prostate (PC-3) cancer cell line respectively.

Antimicrobial activity

a) Substitution of hydrogen by methoxy in aroyl group and 3,4,5-trimethoxy in the styryl group in **9f** resulted in increased activity against *B. subtilis* and *S. aureus*.

b) Compound **5h** containing methyl in aroyl group and 3,4-dimethoxy in the styrylgroup resulted in increased activity against *E. coli.*

c) Substitution of hydrogen by methyl, methoxy groups in aroyl groupresulted in increased activity against most of the strains.

d) There is very slight increase in antimicrobial activity on moving from furo[3,2-c]pyran-4-ones to furo[3,2-c]chromen-4-ones.

e) There is better antimicrobial activity on changing methyl group to styryl group in most of the synthesized furo[3,2-c]pyran-4-ones to furo[3,2-c]chromen-4-ones.

CONCLUSION

A series of twenty four highly oxygenated furo[3,2-c]pyran-ones and furo[3,2-c]chrome-4-ones were synthesized through simple and straight forward procedure utilizing chalcone of easily available and versatile dehydroacetic acid/3-acetyl-4-hydroxycoumarin and α-bromoketones in acetonitrile in the presence of potassium carbonate. All the synthesized molecules were characterized utilizing various spectroscopic techniques and screened for anticancer potential (*in vitro*) against six different cell lines i.e. three Colon (HCT-116, SW620, HT-24), Lung-A549, Prostate-PC-3, Breast-MCF-7. After screening, the compounds which showed >75% growth inhibition at 50 µM were selected for determination of their IC_{50} values. The IC_{50} values of compounds **9d**, **9f** and **5a** was found 2.8, 5.3 and 6.9 µM against breast (MCF-7) cancer cell line and **9f** was having IC_{50} values of 3.8 µM against prostate (PC-3) cancer cell line suggesting thereby that furo[3,2-c]chromen-4-ones are more active than furo[3,2-c]pyran-4-ones. The synthesized compounds were also studied for antibacterial activity (in vitro) using different strains of bacteria (*Bacillus subtilis* and *Staphylococcus aureus*- Gram-positive, and *Escherichia coli*- Gram negative) as well as fungal strains (*Aspergillus niger* and *Candida albicans*) used for antifungal activity using Norfloxacin and Fluconazole as antibacterial and antifungal standard drugs, respectively. Most of the compounds exhibited better to excellent antimicrobial results. The outcome of the antimicrobial screening study indicated that compound **9f** exhibited promising activity against *S. aureus* and *B. subtilis*comparable to Norfloxacin while **5h** exhibited excellent and **5i** and **9b** showed better activity against *E. coli* with respect to reference drug. The compounds **5c-5e** displayed excellent activity against *C. albicans* and *A. niger* than Fluconazole.There is better antimicrobial activity on changing methyl group to styryl group in most of the synthesized furo[3,2-c]pyran-4-ones to furo[3,2-c]chromen-4-ones. The above study clearly demonstrate that there is a lot of further scope for carefully designing a better substitute of natural and synthetic furo[3,2-c]pyran-ones and furo[3,2-c]chrome-4-ones for evaluation of biological activities.

EXPERIMENTAL

Materials and Methods

Melting points are uncorrected and dignified in uncluttered capillaries. NMR spectrometer (Bruker Avance III) is used for ^{1}H and ^{13}C NMR spectra in $CDCl_3$ where TMS is used for an internal standard solvent. Chemical shifts values are represented in ppm (parts per million). The HSQC (Heteronuclear-single-quantum coherence), COSY (Correlation-spectroscopy) and HMBC (Heteronuclear-multiple bond correlation) spectra were scanned on NMR spectrometer. Shimadzu FTIR 8210 PC instrument is used for analysis of IR using KBr pallets and IR absorption frequency are reported in cm^{-1}. The HRMS were recorded on LC-MS/MS, SCIEX-QTOF and micOTOF-Q II mass spectrometer. Dehydroacetic acid (DHAA) was procured from Aldrich and 3-acetyl-4-hydroxycoumarin was prepared by acetylation of 4-hydroxycoumrin. Chalcones were obtained by refluxing DHAA/3-acetyl-4-hydroxycoumarin with aryl aldehyde in methanol in presence of piperidine for 5-8 hours [31, 32] .

Synthesis of highly oxygenated furo[3,2-c]pyran-4-one (4, 5, 8 and 9)

Dehydroacetic acid (1, 1 mmol) or its chalcone (4, 1 mmol) was dissolved in acetonitrile (15 mL) and refluxed for 3 hours after addition of α-bromoketone (2, 1 mmol) and potassium carbonate (K_2CO_3) (3 mmol). The reaction was monitored by TLC using hexane from petroleum-ethyl acetate (9:1). After completion, the reaction mixture was cooled to room temperature and water was added to precipitate the desired product (**4**, **5**). The precipitates were filtered, washed with water and crystallized from ethanol. Similarly, furo[3,2-c]chromen-4-ones (**8**, **9**) were synthesized using the above protocols.

Supporting Information

Supporting Information File 1: Experimental and cytotoxicity and antimicrobial assay details, compound characterization and NMR spectra.

Conflicts of Interest

The authors state that they have no conflict of interests.

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