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

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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.
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-dihydropyro[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-dihydropyro[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.
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]chromene-4-one (Figure 2) also reported 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].

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

**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 straightforward 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 scaffold 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 economical, 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]pyrones and 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%   |
Acetonitrile  |  Reflux  |  3h  |  97%

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.

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 $\delta$ 3.90 ppm due to methoxy group of at position-4 of aroyl ring and one doublet at $\delta$ 6.43 ppm due to 1H of pyran-4-one and phenyl ring protons displayed doublet having $J$ value 6.88 Hz at $\delta$ 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 $\delta$ 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](image)

The compound 9a showed two singlet at $\delta$ 3.81, 3.88 ppm due to methoxy group of styryl group and one singlet at $\delta$ 6.85 ppm due to 2H and one singlet at $\delta$ 7.17 ppm due to 1H were attributed to 3”'-H and 6”'-H and 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 $\delta$ 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 $\delta$ 7.55 ppm due to 3'-H and 5'-H with $J$ value 8.9 Hz and doublet at $\delta$ 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.
hydrogen-hydrogen correlation was observed in coumarin H₉ with H₈, H₈ with H₇, and H₇ with H₆ and aroyl hydrogens H₂/H₆' with H₉/H₅', and H₆' with α proton and H₃/H₅' with H₄'. The carbon-hydrogen correlations were ascertained by analyzing the HSQC spectrum that the position of carbon signals at δ 55.89, 56.64 (OCH₃), 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 ¹H and ¹³CNMR with correlations of compound 9a are demonstrated in Figure 6. The assignment of each hydrogen and carbon was established by interpreting the 2DNR (COSY, HSQC).

HRMS also confirmed structures of the compound 4c and 9a. For compound 4c, HRMS: m/z (M⁺) calcd. for C₁₇H₁₄O₅: 298.0841 and found: 299.0958 (M+H)⁺. For compound 9a, HRMS: m/z (M⁺) calculated for C₂₈H₂₀O₆: 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** ¹H 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   | 0   |
| 8b  | 0  | 8   | 21  | 46  | 0   | 0   | 0   |
| 8c  | 18 | 0   | 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   | 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 A549 cell 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\textsubscript{50} values of selected synthesized compounds at different concentrations

| Tissue | Lung | Prostate | Breast | Colon | Colon | Colon |
|--------|------|----------|--------|-------|-------|-------|
| Cell Lines | A-549 | PC-3 | MCF-7 | HT-29 | HCT-116 | SW-620 |
| Code | IC\textsubscript{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 styryl group resulted in increased activity against *E. coli*.

c) Substitution of hydrogen by methyl, methoxy groups in aroyl group resulted 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]chromene-4-ones were synthesized through simple and straightforward 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-hydroxycoumarin. 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 $\alpha$-bromoketone (2, 1 mmol) and potassium carbonate (K$_2$CO$_3$) (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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