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One-pot synthesis and evaluation of novel 3-Aryl-6-ethoxycarbonyl-4-hydroxy-2H-pyran-2-one as potent cytotoxic agents

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A series of new open chain analogs of phelligridin J are synthesized by clean one-pot approach. These compounds were evaluated for their *in vitro* cytotoxicity against normal and breast cancer cell lines. All the compounds exhibited potent cytotoxic activity in lower micro molar range. Compound **5o** exhibited maximum cytotoxic activity with IC_{50} values of 12.49 and 13.76, while compound **5a** showed two-fold selectivity *viz.* IC_{50} values of 21.80 to 43.40 μM against breast cancer (MCF7) and normal fibroblast (NIH3T3) cell lines, respectively.

Introduction

Many successful drug discovery programs have emerged from natural products.[1] The imperative pharmacological properties and limited supply from nature ascribed importance to natural products and their simple laboratory synthesis. Poor physical and pharmacodynamic properties, complex structures of natural products are bottle neck of rapid progress in drug discovery. Analogizing of natural products is often used not only to address above problems but also as a tool to understand molecular mode of action and biological problems from a chemical biologist's point of view.[1] Hence, efforts have been made by medicinal chemists to find highly efficient and simple methods to synthesize natural products and their analogues.[2]

4-Hydroxy pyran-2-one is a scaffold **1** found in important class of natural products which possesses wide range of biological activities. Scaffold **1** is the building block of styrylpyrones from medicinal fungi *viz.* hispidin, hypholomins, fasciculins, phelligridins, inoscavins, interfungins, inonoblins, davallialactones, phellinins, meshinokobnol and squarrosidine (Fig. 1). This class of compounds exhibit anti-oxidant, cytotoxic, anti-inflammatory, anti-diabetic, anti-platelet aggregation, anti-viral and anti-dementia activities.[3] Most significantly, scaffold **1** has shown anti-HIV activity in a class of compounds which are nonpeptidic HIV-protease inhibitors.[4] Anticoagulants typified by 4-hydroxycoumarin class of drugs which are of immense

therapeutic value also have inbuilt scaffold **1**.[5] Although, 4-hydroxy pyran-2-one are different form pulvinic acids which are antioxidants,[6] these compounds in their structure bear similar bonds as scaffold **1** (Fig. 1). We have employed a one-pot approach to synthesize novel open chain analogues of Phelligridin J as 3-aryl-6-ethoxycarbonyl-4-hydroxy-2H-pyran-2-one embedded with scaffold **1**.

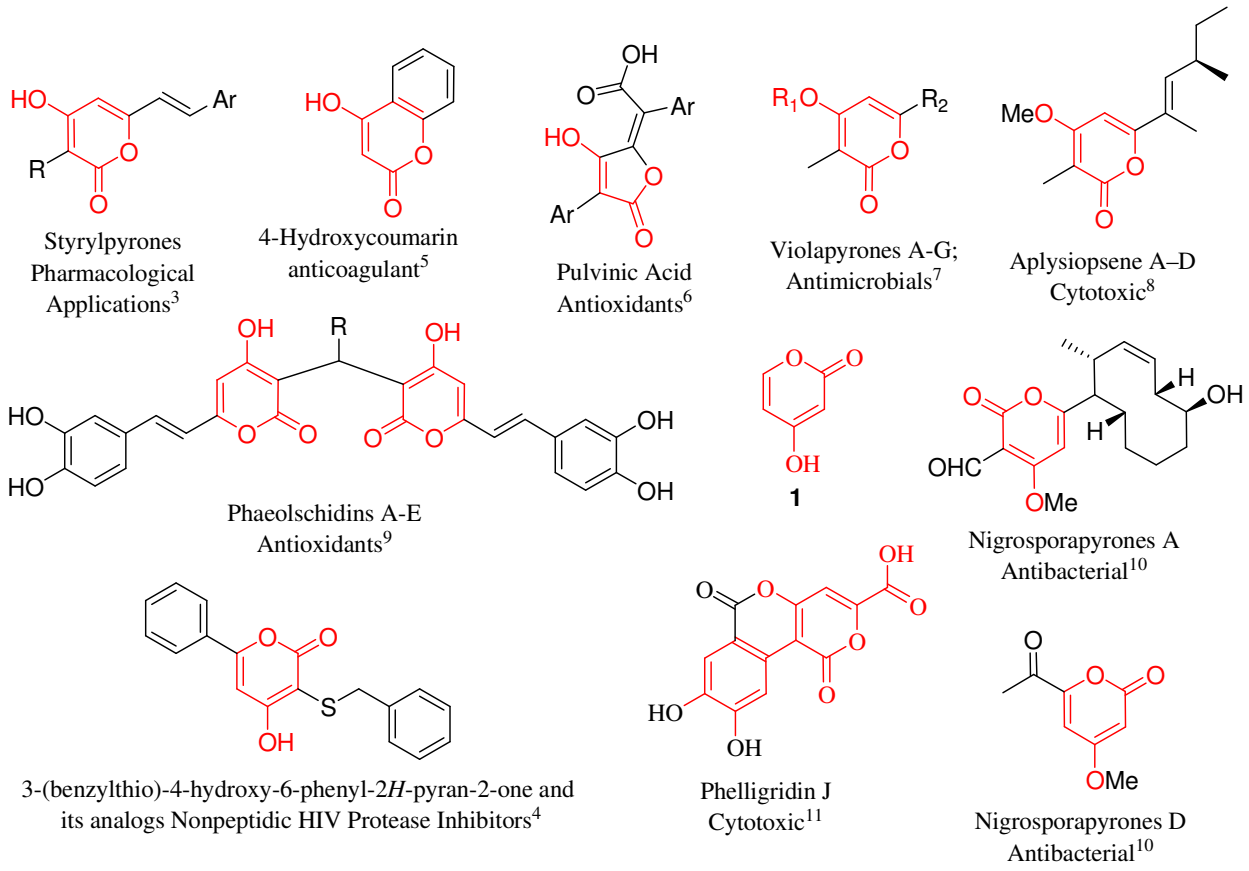
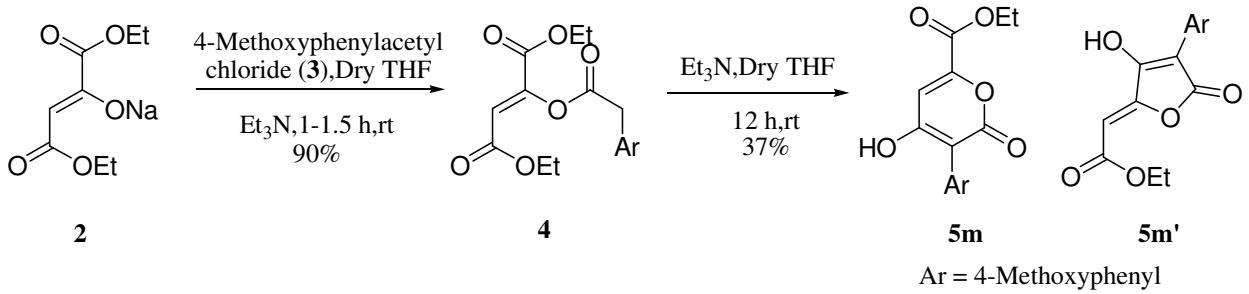


Fig. 1 Compounds containing 4-hydroxy pyran-2-one structural motif

Results and discussion

In our preliminary investigations, diethyl oxalacetate sodium salt **2** was *O*-acylated using 4-methoxyphenyl acetyl chloride **3** in the presence of triethylamine to give **4** as novel intermediate in 90 % yield. Dieckmann cyclisation of compound **4** using triethylamine as a base gave a single product in 37 % yield which we predicted as a six membered ring compound **5m** (Scheme 1) on the basis of Baldwin's "Enolate rules for Ring Closure".[12] As the compound **4** can cyclise either to 5 (**5m'**) or 6 (**5m**) membered ring, the above selectivity can be explained by applying Baldwin's rule (Fig. 2), which emphasizes a delicate balance between formation of Dieckmann

like 5 and 6-membered pyrones i.e. 6-(enolendo)-exo-trig reactions are favored over 5-(enolendo)-exo-trig cyclisations. As cost in angle strain to achieve planar transition state apparently overweighs the stability that would come from kinetically controlled 5-memberd cyclisation as compared to 6-memberd cyclisation; thus favoring **5m** over **5m'** in our case.



Scheme 1 Stepwise synthesis of compound **5m**.

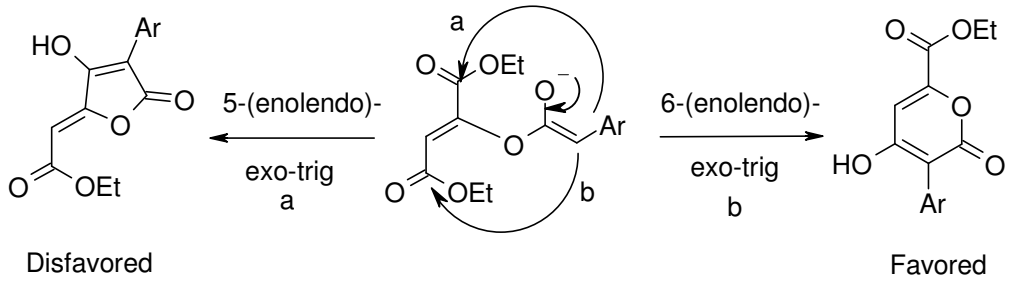
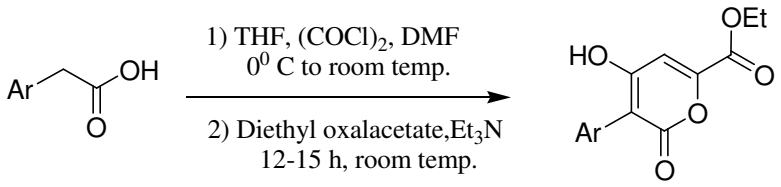


Fig. 2 Baldwin's Enolate rules for Ring Closure.

In order to improve these reaction conditions so as to make it more efficient and user-friendly, we turned to "One-pot approach" in which we can generate arylacetyl chloride *in situ* followed by triethylamine catalyzed cascade of reactions *viz.* acylation, intramolecular nucleophilic substitution and enolization to give 3-aryl-6-ethoxycarbonyl-4-hydroxy-2H-pyran-2-one. When 4-mentoxyphenyl acetic acid was subjected to the above reaction sequence, compound **5m** was isolated in 45% yield over four steps one-pot process. To investigate the scope of this methodology a variety of phenylacetic acids were used. The result showed that various phenylacetic acids were smoothly converted to corresponding novel open chain analogues of Phelligridin J (**5a-o)** in overall good yields (Table 1).

Table 1 One-pot reaction of Arylacetic acid and diethyl oxalacetate sodium salt.



6 **5**

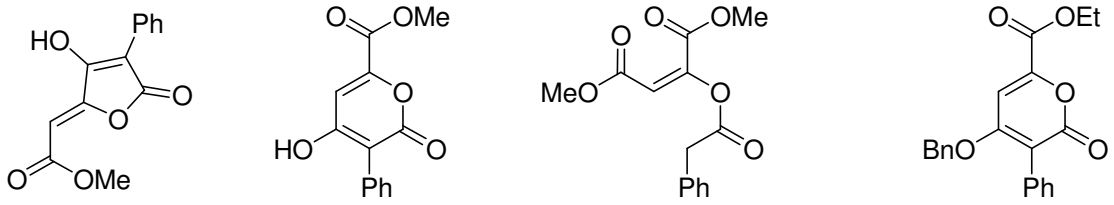
Sr.No	Ar	Compound no.	% Yield[a]
1	Phenyl-	5a	61
2	2-Fluoro phenyl-	5b	42
3	4-Fluoro phenyl-	5c	45
4	2-Chloro phenyl-	5d	38
5	2,4-Dichloro phenyl-	5e	21
6	4-Chloro phenyl-	5f	30
7	4-Bromo phenyl-	5g	57
8	2-Methy phenyl-	5h	42
9	3-Methy phenyl-	5i	48
10	4-Methy phenyl	5j	51
11	2-Methoxy phenyl-	5k	39
12	3-Methoxy phenyl-	5l	40
13	4-Methoxy phenyl-	5m	45
14	2-Methylbenzoate-	5n	30
15	2-Naphthyl-	5o	36

[a] Isolated yields for the four steps one-pot reaction.

Using the present protocol we have reduced the reaction time, improved yields and simplified the four step reaction procedure into a one-pot paradigm. Our method also offers use of readily available starting materials, reagents and provides simple product isolation by non-chromatographic method such as recrystallization.

Despite the fact that **5a** is not only the backbone but also can be a precursor for a variety of biologically active natural products but literature speaks only in vague terms about its synthesis. Although the formation of **8** has been described previously from dimethyl oxalacetate sodium salt.[13] However, Robert Ramage and coworker have elucidated that its reported structure is misassigned as **7** and have suggested an alternate structure **8** for the product from cyclisation

of **9**.[14] But juxtaposition of structural constraint of **8** and **9** did not unambiguously establish its structure (Fig. 3).

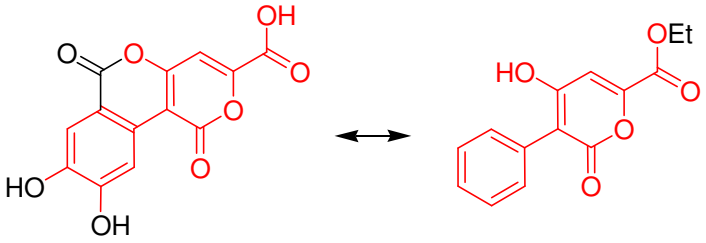


7 **8** **9** **10**

Fig. 3 Chemical structures of compounds **7-10**.

In order to unequivocally determine the identity of our cyclisation product, alkylation of **5a** was done using benzyl bromide to get **10** and NMR data confirms it and hence confirming structure of **5a** as **5a**.[15] These results confirm that our one-pot procedure gives exclusively 6 membered products.

Table 2 Cytotoxic activity of open chain phelligridin J analogs.



Phelligridin J Cytotoxic[11] **5a-5o**

Compound	IC_{50} for MCF-7 (μM)[a]	IC_{50} for NIH3T3 (μM)[a]
5a	21.80	43.40
5b	13.64	19.60
5c	13.89	18.14
5d	14.64	15.76
5e	14.52	16.25
5f	14.45	17.98
5g	14.37	15.43
5h	16.09	18.40
5i	13.16	16.45

5j	14.82	14.75
5k	19.31	16.07
5l	16.87	17.42
5m	21.48	21.10
5n	17.94	15.98
5o	12.49	13.76
Cisplatin	15.00	50.00

[a] Data represent mean values for three independent determinations.

As the new compounds are open chain analogues of Phelligridin J[11] which is cytotoxic, we expected similar type of cytotoxic activity from these compounds. For this purpose we screened all compounds for their *in vitro* cytotoxicity against MCF-7 human tumor cell line and NIH3T3 normal mouse fibroblast cell line using MTT colorimetric assay. Cisplatin was included as a control and results are summarized in Table 2. All compounds were found to be highly cytotoxic. Compound **5a** with simple phenyl group showed IC_{50} values of 21.80 and 43.40 μM against both the cell lines. Introduction of either electron withdrawing or donating group in phenyl ring resulted in an overall improvement of cytotoxicity. Thus methoxy carbonyl substituent at 2 position (**5n**) showed IC_{50} values of 17.94 and 15.98 μM, while compounds with halogen substitutes (**5b-g**) showed further enhancement in cytotoxic activity (IC_{50} = 13.64 – 19.60 μM). On the other hand, analogues (**5h-m**) with electron donating groups like methyl or methoxy also exhibited increased efficiency (IC_{50} = 13.16 – 21.48 μM). In this series methyl group was found to be more effective than methoxy group. Similarly, methyl or methoxy group at meta position in benzene ring have pronounced effect on activity against MCF-7 cell line as compared to their ortho or para counterparts. The most promising cytotoxic activity (IC_{50} = 12.49 and 13.76 μM) was observed in compound **5o** with 2-Naphthyl group. Most of the compounds showed some selectivity towards MCF-7 cell line as compared to NIH3T3 cell line. Interestingly, compound **5a** showed two fold selectivity between normal and tumor cell line (Fig. 4).

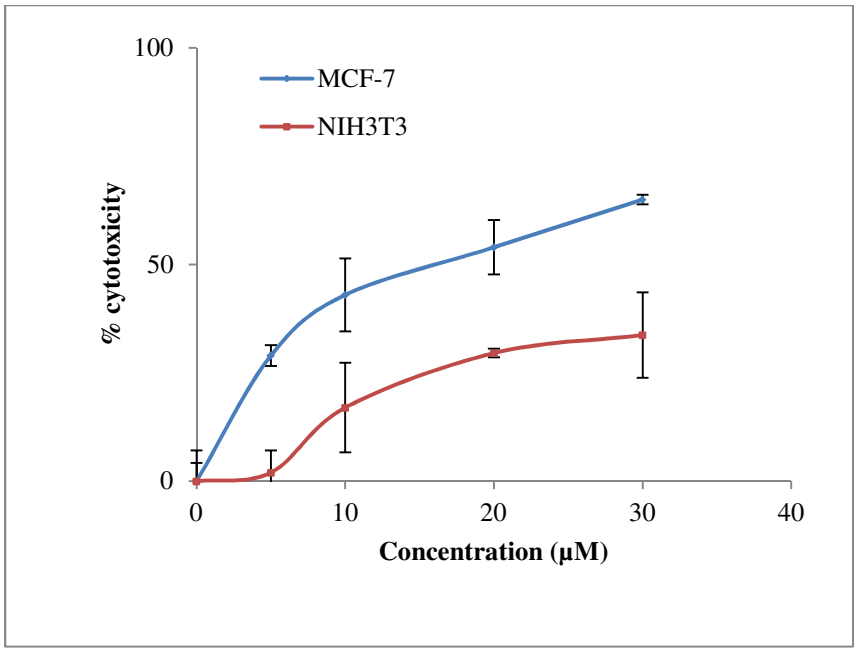


Fig. 4 Dose response curve of **5a**.

Conclusions

We have demonstrated that novel open chain analogues of Phelligridin J are easily accessible via present four steps, one-pot model. Our methodology is highly regioselective and offers a number of advantages: firstly, it allows simple and highly efficient synthesis of multiple functionalized pyran ring structures which are of chemical and pharmaceutical interest. Secondly, it mimics the metal catalyzed cross coupled synthesis of these molecules. Thirdly, it is time efficient, more user-friendly and has wide scope.

These open chain analogs of phelligridin J were also found to be cytotoxic against human breast cancer (MCF-7) and normal fibroblast cells (NIH3T3), compound **5o** was found to be the most potent with IC_{50} values of 12.39 and 13.76 µM respectively. Compound **5a** showed two-fold selectivity against MCF-7 and NIH3T3 cell lines with IC_{50} values of 21.8 and 43.4 µM respectively. Further studies are in progress to expand the scope of this protocol.

Experimental

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance II 400 NMR spectrometer at 400 MHz & 100 MHz respectively. Chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane (TMS) for all recorded NMR spectra. IR spectra were

recorded on Perkin Elmer Spectrum RX-IFTIR spectrometer. High-resolution mass spectra (HRMS) were performed with a QTOF Micromass Mass Spectrometer by electro spray ionization mode. All air- or moisture-sensitive reactions were conducted under nitrogen atmosphere. Starting materials and reagents used in reactions were obtained commercially from Avra synthesis Pvt. Ltd., Spectrochem, Alfa aesar, and were used without purification, unless otherwise indicated. The purity of all compounds was confirmed by ^{1}H, ^{13}C NMR and HRMS.

Synthesis of diethyl 2-(2-(4-methoxyphenyl) acetoyloxy) fumarate (4)

To a dry solution THF (30 mL) of diethyl oxalacetate sodium salt **2** (2.2 g, 10.4682 mmol) and triethylamine (1.46 mL, 10.4682 mmol) was added **3** (1.68 mL, 10.9916 mmol) at 0 °C; the resulting mixture was stirred at 0 °C to room temperature for 1.0-1.5 h. After completion of reaction (TCL check) THF was removed on vaccuo. Water (50 mL) was added to reaction mixture and product was extracted with ethyl acetate (3 x 25 mL). The combine organic extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by a column chromatography using 5 % ethyl acetate in n-hexane as an eluent to afford compound **4** as colorless oil in 90 % yield.

Colorless oil, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (m, 2H, Ar-H), 6.90 (m, 2H, Ar-H), 6.69 (s, 1H, C3-H), 4.23 (m, 4H, -OCH_2), 3.85 (s, 2H, -OCH_2Ph), 3.80 (s, 3H, -OCH_3), 1.27 (m, 6H,-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0, 162.8, 161.1, 158.9, 146.7, 130.6, 124.8, 117.3, 114.0, 62.5, 61.2, 55.3, 39.6, 14.1, 13.9; LCMS (ES-API) m/z: 337.0 $(M+H)^+$.

Synthesis of 6-Ethoxycarbonyl-4-hydroxy-3-(4-methoxyphenyl)-2H-pyran-2-one (5m)

Compound **8** (0.5 g, 1.488 mmol) was dissolved in dry THF (5 mL) to it triethylamine (0.2 ml, 1.488 mmol) was added and reaction was stirred for further 12 h at room temperature. After completion of solvent was removed on vaccuo and water was added to reaction mixture which was extracted with ethyl acetate (3 x 15 mL). The organic extract was dried over anhydrous Na_2SO_4, concentrated under reduced pressure. Crude product was purified by column chromatography using 90 % ethyl acetate in n-hexane as an eluent to afford **5m** as a yellow solid in 37 % yield.

One-pot procedure for synthesis of 3-Aryl-6-ethoxycarbonyl-4-hydroxy-2H-pyran-2-one (5a-o)

To a freshly dried THF solution (10 mL/mmol) of arylacetic acid (2 g., 1.0 eq.) and oxalyl chloride (1.0 eq.), was added a catalytic amount of DMF at 0 °C under nitrogen atmosphere; after 1.0 to 1.5 h of 0 °C to room temperature stirring, added a solution of diethyl oxalacetate sodium salt (1.0 eq.) in dry THF followed by addition of triethylamine (2.0 eq.). Reaction mixture was stirred for further 12-15 h at room temperature. After completion of reaction (TLC check) THF was removed under vaccuo, water (50 mL) was added to reaction mixture, after 15 min of stirring at room temperature reaction mixture was filtered off to get crude product which was recrystallized from ethyl acetate to offer yellow colored products.

6-Ethoxycarbonyl-4-hydroxy-3-phenyl-2H-pyran-2-one (5a)

Yellow solid, IR νmax (cm-1) 3510, 3267, 1759, 1745,, 1684, 1577, 1226, 1029, 847, 741; ^{1}H NMR (400 MHz, DMSO-d6) δ 7.50 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.28 (t, *J* = 7.6 Hz, 2H, Ar-H), 7.08 (t, *J* = 7.3 Hz, 1H, Ar-H), 6.21 (s, 1H, C3-H), 4.16 (q, *J* = 7.0 Hz, 2H, -OCH2), 1.27 (t, *J* = 7.1 Hz, 3H, -CH3); ^{13}C NMR (100 MHz, DMSO-d6) δ 167.0, 164.4, 162.7, 148.6, 136.2, 128.2, 128.1, 124.7, 98.3, 97.9, 58.3, 14.4; HRMS (ESI): *m/z* calculated for $C_{14}H_{11}O_5$ $[M-H]^-$: 259.0601, found: 259.0608.

6-Ethoxycarbonyl-4-hydroxy-3-(2-fluorophenyl)-2H-pyran-2-one (5b)

Yellow solid, IR νmax (cm-1) 3581, 3180, 1761, 1693, 1596, 1452, 1235, 1028, 933, 742; ^{1}H NMR (400 MHz, DMSO-d6) δ 7.90 (m, 1H, Ar-H), 7.08 (m, 3H, Ar-H), 6.38 (s, 1H, C3-H), 4.17 (q, *J* = 7.1 Hz, 2H, -COCH2), 1.28 (t, *J* = 7.0 Hz, 3H, -CH3); ^{13}C NMR (100 MHz, DMSO-d6) δ 166.6, 164.2, 162.7, 159.9, 157.4, 150.4, 129.7, 129.7, 125.9, 125.8, 124.1, 124.0, 123.9, 123.9, 114.7, 114.5, 97.6, 88.4, 88.3, 58.2, 14.4. HRMS (TOF MS ES+): *m/z* calculated for $C_{14}H_{11}O_5FNa$ $[M + Na]^+$: 301.0488, found: 301.0489.

6-Ethoxycarbonyl-4-hydroxy-3-(4-fluorophenyl)-2H-pyran-2-one (5c)

Yellow solid, IR ν_{max} (cm^{-1}) 3580, 3170, 1756, 1678, 1597, 1226, 1162, 996, 854; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.50 (m, 2H, Ar-H), 7.05 (m, 2H, Ar-H), 6.18 (s, 1H, C3-H), 4.17 (q, *J* = 7.1 Hz, 2H, -$COCH_2$), 1.28 (t, *J* = 7.1 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.1,

164.5, 162.7, 161.0, 158.5, 148.1, 132.6, 132.6, 129.8, 129.7, 114.9, 114.7, 98.1, 97.4, 58.4, 14.4; HRMS (TOF MS ES+): *m/z* calculated for $C_{14}H_{11}O_5FNa$ $[M + Na]^+$: 301.0488, found: 301.0479.

6-Ethoxycarbonyl-4-hydroxy-3-(2-chlorophenyl)-2H-pyran-2-one (5d)

Yellow solid, IR νmax (cm-1) 3558, 3480, 3119, 1758, 1689, 1560, 1441, 1239, 1027, 764; ^{1}H NMR (400 MHz, DMSO-d6) δ 7.93 (q, *J* = 8.0 Hz and 1.5 Hz, 1H, Ar-H), 7.37 (q, *J* = 8.0 Hz and 1.2 Hz, 1H, Ar-H), 7.26 (m, 1H, Ar-H), 7.08 (m, 1H, Ar-H), 6.60 (s, 1H, C3-H), 4.18 (q, *J* = 7.0 Hz, 2H, -COCH2), 1.30 (t, *J* = 7.1 Hz, 3H, -CH3); ^{13}C NMR (100 MHz, DMSO-d6) δ 166.5, 164.3, 163.0, 150.2, 133.8, 131.2, 130.1, 129.1, 126.8, 126.1, 97.9, 93.6, 58.4, 14.4; HRMS (TOF MS ES+): *m/z* calculated for $C_{14}H_{11}O_5NaCl$ $[M + Na]^+$: 317.0193, found: 317.0190.

6-Ethoxycarbonyl-4-hydroxy-3-(2,4-dichlorophenyl)-2H-pyran-2-one (5e)

Yellow solid, IR ν_{max} (cm^{-1}) 3488, 3116, 1761, 1736, 1680, 1599, 1450, 1239, 1035, 862; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.52 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.36 (q, *J* = 8.7 Hz and 2.2 Hz, 1H, Ar-H), 6.55 (s, 1H, C3-H), 4.15 (q, *J* = 7.1 Hz, 2H, -$COCH_2$), 1.27 (t, *J* = 7.0 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.1, 164.1, 162.9, 151.5, 133.0, 131.5, 130.8, 129.0, 128.6, 127.2, 97.2, 91.7, 58.3, 14.5; HRMS (TOF MS ES+): *m/z* calculated for $C_{14}H_{11}O_5Cl_2$ $[M + H]^+$: 328.9984, found: 328.9986.

6-Ethoxycarbonyl-4-hydroxy-3-(4-chlorophenyl)-2H-pyran-2-one (5f)

Yellow solid, IR ν_{max} (cm^{-1}) 3581, 3167, 1756, 1675, 1593,1228,1166, 1027,996, 759; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.51 (m, 2H, Ar-H), 7.28 (m, 2H, Ar-H), 6.18 (s, 1H, C3-H), 4.19 (q, *J* = 7.1 Hz, 2H, -$COCH_2$), 1.29 (t, *J* = 7.0 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.6, 164.3, 162.7, 149.3, 135.1, 129.6, 129.1, 128.2, 97.9, 97.1, 58.4, 14.4; HRMS (ESI): *m/z* calculated for $C_{14}H_{10}O_5Cl$ $[M-H]^-$: 293.0211, found: 293.0221.

6-Ethoxycarbonyl-4-hydroxy-3-(4-bromophenyl)-2H-pyran-2-one (5g)

Yellow solid, IR νmax (cm-1) 3578, 3164, 1753, 1677, 1593, 1440, 1226, 1076, 996, 857; ^{1}H NMR (400 MHz, DMSO-d6) δ 7.45 (s, 4H, Ar-H), 6.18 (s, 1H, C3-H), 4.16 (s, 2H, -COCH2), 1.26 (s, 3H, -CH3); ^{13}C NMR (100 MHz, DMSO-d6) δ 166.5, 164.2, 162.5, 149.9, 135.6, 131.2,

130.0, 117.3, 97.3, 96.5, 58.2, 14.5; HRMS (TOF MS ES+): *m/z* calculated for $C_{14}H_{11}O_5NaBr$ $[M + Na]^+$: 360.9688, found: 360.9672.

6-Ethoxycarbonyl-4-hydroxy-3-(*o*-tolyl)-2H-pyran-2-one (5h)

Yellow solid, IR ν_{max} (cm^{-1}) 3442, 1740, 1686, 1580,1232, 1182, 1022, 826, 741; ^1H NMR (400 MHz, DMSO-d_6) δ 7.71 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.14 (t, *J* = 7.1 Hz, 2H, Ar-H), 7.00 (t, *J* = 7.4 Hz, 1H, Ar-H), 6.34 (s, 1H, C3-H), 4.15 (q, *J* = 7.0 Hz, 2H, -$COCH_2$), 2.28 (s, 3H, Ar-CH_3), 1.28 (t, *J* = 7.0 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.2, 164.4, 162.6, 148.5, 134.6, 134.4, 129.7, 128.8, 125.5, 124.8, 97.8, 95.3, 58.2, 20.1, 14.5; HRMS (TOF MS ES+): *m/z* calculated for $C_{15}H_{14}O_5K$ $[M + K]^+$: 313.0478, found: 313.0488.

6-Ethoxycarbonyl-4-hydroxy-3-(*m*-tolyl)-2H-pyran-2-one (5i)

Yellow solid, IR ν_{max} (cm^{-1}) 3505, 3269, 1743, 1686, 1586, 1447, 1225, 1026, 903, 760; ^1H NMR (400 MHz, DMSO-d_6) δ 7.31 (d, *J* = 8.64 Hz, 2H, Ar-H), 7.13 (t, *J* = 7.52 Hz, 1H, Ar-H), 6.87 (d, *J* = 7.52 Hz, 2H, Ar-H), 6.17 (s, 1H, C3-H), 4.18 (q, *J* = 7.1 Hz, 2H, -$COCH_2$), 2.28 (s, 3H, Ar-CH_3), 1.29 (t, *J* = 7.0 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.0, 164.4, 162.4, 148.5, 137.0, 136.1, 128.8, 128.0, 125.5, 125.5, 98.5, 98.1, 58.2, 21.2, 14.5; HRMS (TOF MS ES+): *m/z* calculated for $C_{15}H_{14}O_5Na$ $[M + Na]^+$: 297.0739, found: 297.0734.

6-Ethoxycarbonyl-4-hydroxy-3-(*p*-tolyl)-2H-pyran-2-one (5*J*)

Yellow solid, IR ν_{max} (cm^{-1}) 3507, 3270, 1743, 1684, 1577, 1449, 1225, 1028, 844, 762; ^1H NMR (400 MHz, DMSO-d_6) δ 7.39 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.06 (d, *J* = 8.1 Hz, 2H, Ar-H), 6.16 (s, 1H, C3-H), 4.18 (q, *J* = 7.1 Hz, 2H, -$COCH_2$), 2.26 (s, 3H, Ar-CH_3), 1.29 (t, *J* = 7.1 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.1, 164.4, 162.4, 148.1, 133.8, 133.5, 128.8, 128.1, 98.1, 97.7, 58.2, 20.7, 14.5; HRMS (ESI): *m/z* calculated for $C_{15}H_{13}O_5$ $[M-H]^-$: 273.0757, found: 273.0767.

6-Ethoxycarbonyl-4-hydroxy-3-(2-methoxyphenyl)-2H-pyran-2-one (5k)

Yellow solid, IR ν_{max} (cm^{-1}) 3536, 3297, 1758, 1686, 1582,1442, 1245, 1032, 860, 733; ^1H NMR (400 MHz, DMSO-d_6) δ 7.83 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.07 (m, 1H, Ar-H), 6.88 (t, *J* = 7.52 Hz, 2H, Ar-H), 6.61 (s, 1H, C3-H), 4.21 (q, *J* = 7.04 Hz, 2H, -$COCH_2$), 3.82 (s, 3H,-OCH_3), 1.34 (t, *J* = 7.08 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.2, 164.4, 162.6, 155.5, 148.3,

129.3, 125.9, 124.9, 120.2, 110.5, 98.1, 91.9, 58.1, 55.3, 14.4; HRMS (TOF MS ES+): *m/z* calculated for $C_{15}H_{14}O_6K$ $[M + K]^+$:329.0427, found: 329.0424.

6-Ethoxycarbonyl-4-hydroxy-3-(3-methoxyphenyl)-2H-pyran-2-one (5l)

Yellow solid, IR ν_{max} (cm^{-1}) 3561, 3450, 1749, 1683, 1572, 1444, 1217, 1026, 874, 690; ^1H NMR (400 MHz, DMSO-d_6) δ 7.17 (t, *J* = 8.2 Hz, 1H, Ar-H), 7.07 (t, *J* = 1.5 Hz, 2H, Ar-H), 6.64 (m, 1H, Ar-H), 6.18 (s, 1H, C3-H), 4.17 (q, *J* = 7.1 Hz, 2H, $-OCH_2$), 3.38 (s, 3H, $-OCH_3$), 1.28 (t, *J* = 7.1 Hz, 3H, $-CH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.8, 164.3, 162.4, 159.2, 149.2, 137.6, 129.0, 120.9, 113.6, 110.2, 97.7, 97.4, 58.1, 54.7, 14.5; HRMS (ESI): *m/z* calculated for $C_{15}H_{13}O_6$ $[M-H]^-$: 289.0707, found: 289.0717.

6-Ethoxycarbonyl-4-hydroxy-3-(4-methoxyphenyl)-2H-pyran-2-one (5m)

Yellow solid, IR ν_{max} (cm^{-1}) 3507, 3246, 1753, 1737, 1683, 1260, 1223, 1028, 990, 758; ^1H NMR (400 MHz, DMSO-d_6) δ 7.56 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.87 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.60 (s, 1H, C3-H), 4.32 (q, *J* = 7.1 Hz, 2H, $-OCH_2$), 3.77 (s, 3H, $-OCH_3$), 1.33 (t, *J* = 7.1 Hz, 3H, $-CH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.8, 161.0, 158.9, 146.9, 141.3, 131.2, 126.2, 113.9, 111.6, 109.0, 60.6, 54.9, 13.9; HRMS (ESI): *m/z* calculated for $C_{15}H_{13}O_6$ $[M-H]^-$: 289.0707, found: 289.0715.

6-Ethoxycarbonyl-4-hydroxy-3-(2-(methoxycarbonyl) phenyl)-2H-pyran-2-one (5n)

Yellow solid, IR ν_{max} (cm^{-1}) 3435, 3126, 1744, 1689, 1584, 1441,1238, 1076, 970, 730; ^1H NMR (400 MHz, DMSO-d_6) δ 7.86 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.69 (d, *J* = 7.3 H z, 1H, Ar-H), 7.45 (t, *J* = 7.8 Hz, 1H, Ar-H), 7.16 (t, *J* = 7.5 Hz, 1H, Ar-H), 6.89 (s, 1H, C3-H), 4.21 (q, *J* = 7.0 Hz, 3H, $-COCH_2$), 3.83 (s, 3H, $-COCH_3$), 1.33 (t, *J* = 7.9 Hz, 2H, $-CH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.9, 166.7, 164.3, 163.0, 149.6, 135.9, 131.0, 130.2, 129.4, 128.7, 124.4, 97.8, 95.3, 58.2, 51.7, 14.4; HRMS (TOF MS ES+): *m/z* calculated for $C_{16}H_{14}O_7Na$ $[M+Na]^+$: 341.0637, found: 341.0648.

6-Ethoxycarbonyl-4-hydroxy-3-(naphthalene-2-yl)-2H-pyran-2-one (5o)

Yellow solid, IR ν_{max} (cm^{-1}) 3418, 3044, 1742, 1688, 1582, 1440, 1232, 1025, 828, 762; ^1H NMR (400 MHz, DMSO-d_6) δ 7.94 (m, 1H, Ar-H), 7.68 (m, 2H, Ar-H), 7.50 (m, 1H, Ar-H), 7.31 (m,

3H), 6.76 (s, 1H, C3-H), 4.08 (q, J = 7.1 Hz, 2H, -OCH_2), 1.19 (t, J = 7.1 Hz, 3H, -CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.2, 164.4, 162.6, 149.9, 133.4, 132.2, 131.0, 128.4, 126.6, 125.7, 125.6, 125.4, 125.2, 123.7, 97.4, 93.4, 58.2, 14.6; HRMS (ESI): *m/z* calculated for $C_{18}H_{13}O_5$ $[M-H]^-$: 309.0757, found: 309.0768.

Cytotoxicity assay

MCF-7 human breast cancer cells and NIH3T3 mouse fibroblast cells were grown in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic solution (penicillin & streptomycin) at 37 °C in humidified chamber under 5% CO_2. Cell viability was measured in 96-well plates by quantitative colorimetric assay using MTT.[16] Briefly, 10^5 cells per ml were seeded in 96-well plates for assay. The compounds were dissolved in DMSO and were diluted to the desired concentrations using plain DMEM. The compounds were treated to the cells at various concentrations ranging from 0 to 30 µM. The compound solutions were passed through 0.22 µm syringe filters for sterilization before treatment. After 24 hours, media was removed and 5 mg/mL MTT (final concentration) was added to the wells and the cells were incubated at 37 °C for another 3 h. The MTT solution was removed and the colored formazan crystals in each well were dissolved in 150 µL dimethyl sulfoxide. Absorbance at 595 nm was measured using a µQuant, Biotek Instruments microplate reader. The IC_{50} values of the compounds were calculated using ED50V10 excel add-in tool.

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One-pot synthesis and evaluation of novel 3-Aryl-6-ethoxycarbonyl-4-hydroxy-2H-pyran-2-one as potent cytotoxic agents

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A series of new open chain analogs of phelligridin J are synthesized and these compounds were found to be highly potent cytotoxic agents.