

# Khellinoflavanone, a Semisynthetic Derivative of Khellin, Overcomes Benzo[a]pyrene Toxicity in Human Normal and Cancer Cells that Express CYP1A1

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## ABSTRACT.

Cytochrome P450 family 1 (CYP1) enzymes catalyze the metabolic activation of environmental procarcinogens such as benzo[a]pyrene, B[a]P, into carcinogens, which initiate the process of carcinogenesis. Thus, stopping the metabolic activation of procarcinogens can possibly prevent the onset of cancer. Several natural products have been reported to show unique ability in inhibiting CYP1 enzymes. We found that khellin, a naturally occurring furanochromone from *Ammi visnaga*, inhibits CYP1A1 enzyme with an IC<sub>50</sub> value of 4.02 μM in CYP1A1-overexpressing human HEK293 suspension cells. To further explore this natural product for discovery of more potent and selective CYP1A1 inhibitors, two sets of semisynthetic derivatives were prepared. Treatment of khellin with alkali results in opening of a pyrone ring, yielding khellinone (**2**). Claisen-Schmidt condensation of khellinone (**2**) with various aldehydes in presence of potassium hydroxide, at room temperature, provide a series of furanochalcones **3a-v** (khellinochalcones). Treatment of khellinone (**2**) with aryl aldehydes in presence of piperidine, under reflux, affords the flavanone series of compounds **4a-p** (khellinoflavanones). The khellinoflavanone **4l** potently inhibited CYP1A1 with IC<sub>50</sub> value of 140 nM in live cells, with 170-fold selectivity over CYP1B1 (IC<sub>50</sub> for CYP1B1 = 23.8 μM). Compound **4l** at 3 x IC<sub>50</sub> concentration for inhibition of CYP1A1, completely protected HEK293 cells from CYP1A1-mediated B[a]P toxicity. Lung cancer cells, A549 (p53<sup>+</sup>) and Calu-1 (p53-null), blocked in growth at the S-phase by B[a]P were restored into the cell cycle by compound **4l**. The results presented herein strongly indicate the potential of these khellin derivatives for further development as cancer chemopreventive agents.

**KEYWORDS.** Khellin; cytochrome P50 family 1 enzymes; CYP1A1; khellinone; flavanones; polycyclic aromatic hydrocarbons; cancer chemoprevention.

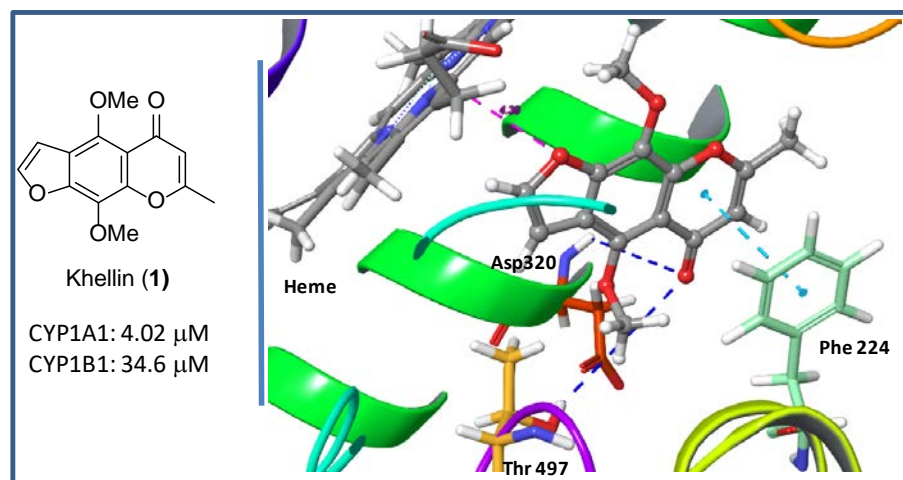
## INTRODUCTION

*Ammi visnaga* (commonly known as 'Khella'; Family: Apiaceae) is a widespread flowering plant native to Europe, Asia, and North Africa, and has many therapeutic properties.<sup>1</sup> The khella seeds are being used in traditional medicine for respiratory conditions including bronchitis, asthma, and cough.<sup>2</sup> The seeds of this plant contain a  $\gamma$ -pyrone, furanochromone khellin as a major constituent, which is a vasodialator, and antispasmodic.<sup>1a, 3</sup> Inspired from the vasodialatory and antispasmodic property of khellin, Roger Altounyan, an Anglo-Armenian physician discovered cromoyln sodium, a chromone class of antiasthma drug.<sup>4</sup> Khellin has also been extensively studied for its cardioactive properties, and is being used for angina pectoris therapy.<sup>5</sup> Based on the cardioactive property of khellin, the Labaz company from Belgium, have discovered anti-arrhythmic drug amiodarone<sup>6</sup> via medicinal chemistry on benzofuran scaffold. Other known pharmacological activities of khellin and its derivatives includes melanin-inhibition,<sup>1d, 7</sup> anti-inflammatory,<sup>8</sup> anticancer,<sup>9</sup> and cancer chemopreventive activity.<sup>10</sup>

Many natural compounds and their derivatives modulate the expression and activities of cytochrome P450 family 1 (CYP1) enzymes which are known to bioactivate procarcinogens.<sup>11</sup> The cytochrome P450 proteins comprise a large family of enzymes that can catalyze the oxidative metabolism of a variety of xenobiotic chemicals including toxic compounds, drugs and chemical carcinogens as well as endogenous compounds. As a part of our continuing efforts to discover CYP1A1 inhibitors as cancer chemopreventive agents;<sup>12</sup> the furanochromone khellin (**1**) was identified as a selective CYP1A1 inhibitor (CYP1A1 IC<sub>50</sub> = 4.02  $\mu$ M, CYP1B1 IC<sub>50</sub> = 34.6  $\mu$ M) via screening of institutional natural products repository (Figure 1).<sup>10</sup>

To further explore this natural product for fine-tuning its potency and selectivity profile, semisynthetic modifications were carried out. Literature reports indicated that semisynthetic modifications of khellin have been performed earlier,<sup>13</sup> however, never been explored as cancer chemopreventive agents. Herein, we report synthesis of two series of khellin derivatives (khellinochalcones and khellinoflavanones) and their biological evaluation as CYP1A1 inhibitors and possible cancer chemopreventive agents.

The in-vivo studies<sup>14</sup> have demonstrated that CYP1A1 is involved in benzo[a]pyrene, B[a]P-induced carcinogenesis in mice. These studies have also shown that polycyclic aromatic hydrocarbon (PAH)-treated mice overexpresses CYP1A1 in lungs and liver. Therefore, herein we also studied the ability of khellin derivatives to protect lung cancer cells and normal cells from B[a]P-induced toxicity.

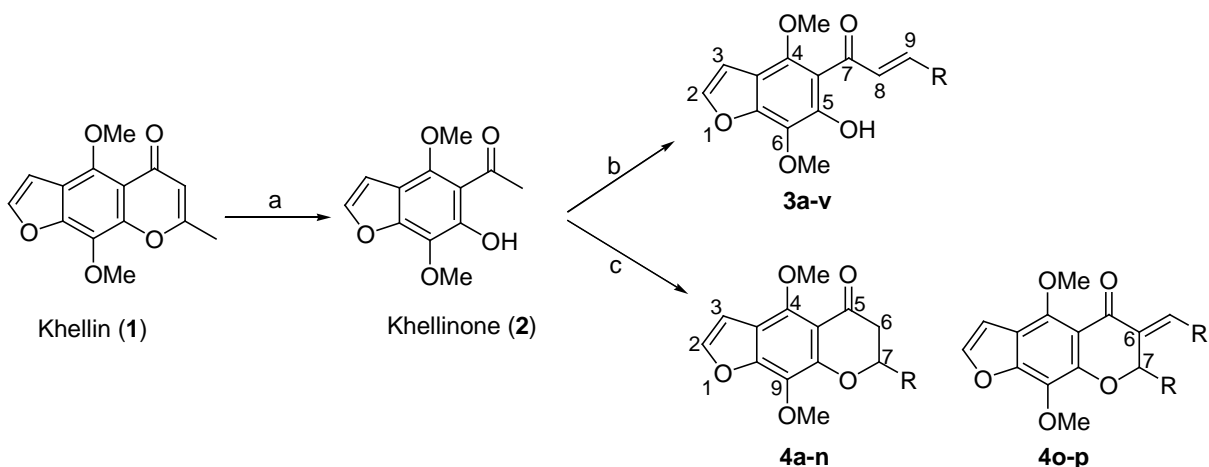


**Figure 1.** Structure of khellin (1), its CYP1A1, CYP1B1 inhibitory activity, and interactions with CYP1A1 active site. The H-bonding is shown by dark blue dotted lines, whereas  $\pi$ - $\pi$  interactions by light-blue dotted lines.

## RESULTS AND DISCUSSION

**Synthesis of khellin derivatives.** The khellin was identified as one of the 'hit' (active) from the natural products repository screening,<sup>10</sup> in the search of new leads for cancer chemoprevention. However, the level of activity was in a micromolar range ( $\text{IC}_{50}$ , 4.02  $\mu\text{M}$ ); therefore it was necessary to modify the structure of khellin to modulate its ability to inhibit CYP1A1. Based on the structural features of khellin, it was decided to convert khellin to khellinone followed by its condensation with aryl aldehydes. The presence of acetyl group in khellinone provides an easiest handle for chemical modifications.

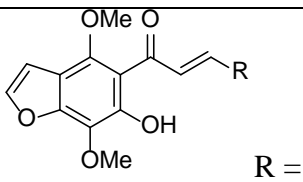
Khellin (**1**) was treated with potassium hydroxide in ethanol, resulting in formation of khellinone (**2**).<sup>15</sup> Two series of compounds were prepared from khellinone (**2**);<sup>16</sup> (a) chalcones by the Claisen-Schmidt condensation of khellinone with different substituted aryl aldehydes; and (b) furanoflavanones by treatment of khellinone with aryl aldehydes in presence of piperidine. The khellinone (**2**) was treated with aryl aldehydes under alkaline condition (KOH), at room temperature, to obtain khellinochalcones **3a-v** (Figure 2, path b). Interestingly, with slight modifications in the reaction conditions (base and reaction temperature), the another series of products were formed. Khellinone (**2**), when it was reacted with aryl aldehydes in ethanol with catalytic amounts of piperidine (instead of KOH), under reflux conditions, it yielded furanoflavanones **4a-p** (khellinoflavanones) (Figure 2 path c). Mechanistically, the flavanones were formed via cyclization of chalcones. All synthesized compounds were characterized based on their spectroscopic properties. The coupling constant of newly formed bond (C8-C9) in chalcones was found to be 16 Hz, which confirmed the *trans*-orientation of the double bond. The <sup>1</sup>H NMR spectrum of flavanone **4b** showed the signals of H-7 at  $\delta$  5.45 ppm and H-6 at  $\delta$  3.04 and 2.94 ppm confirming the formation of flavanone skeleton. Further, the <sup>13</sup>C NMR and DEPT135 spectrum confirmed the appearance of extra -CH<sub>2</sub> at  $\delta$  45.0 (C-6), and a -CH at  $\delta$  78.4 (C-7). Interestingly, when khellinone (**2**) was treated with thiophene-2-carboxaldehyde, it underwent double aldehyde coupling, producing product **4p**. The <sup>1</sup>H NMR of **4p** did not show presence of signal for CH<sub>2</sub>, however, the extra proton appeared at  $\delta$  7.13 ppm indicating the presence of an additional olefinic proton.



**Figure 2.** Synthesis of khellin derivatives **3a-v** and **4a-p**. Reagents and conditions (a) KOH (1M), ethanol, 80 °C, 12 h, 61%; (b) RCHO (R = aryl or heteroaryl), KOH (1 M), methanol, 0 °C to rt, 12-14 h, 40-85%; (c) RCHO (R = aryl or heteroaryl), piperidine, ethanol, reflux at 70 °C, 12 h, 51-68%. The 'R' groups of different derivatives **3a-v** and **4a-p** are shown in Table 1 and 3.

**The pyridyl-furanochalcone derivative 3g is a pan-inhibitor of CYP1 family enzymes.** A series of furanochalcones **3a-v** were tested for inhibition of CYP1A1 in yeast microsomes (Sacchrosomes™) and human live HEK293 cells, overexpressing CYP1A1, at 10 μM (Table 1).<sup>10, 12</sup> The parent natural product khellin (**1**) showed 88% and 81% inhibition of CYP1A1, at 10 μM, in the two assays. Several chalcone derivatives showed higher inhibition of CYP1A1 in both assays. The furanochalcone **3a**, bearing a simple phenyl ring (as R group) showed 92% and 97% inhibition of CYP1A1 in the microsomal and live cells assays. This result was expected, as chalcones have been reported to possess potent CYP1A1 inhibition activity.<sup>12a</sup> The most potent derivative among the series of chalcones was the compound **3g**, which bears a pyridin-3yl moiety as R group. This compound showed 97% and 99% inhibition of CYP1A1 in Sacchrosomes™ and in live human cells, respectively. The results of *in-vitro* CYP1A1 inhibition by furanochalcones in Sacchrosomes™ and HEK293 cells, at 10 μM concentration, are shown in Table 1.

**Table 1.** *In-vitro* CYP1A1 inhibition by furanochalcones in Sacchrosomes™ and HEK293 cells, overexpressing CYP1A1, at 10 μM concentration.

Entry		% inhibition of CYP1A1 at 10 μM (Mean ± SD) <sup>b</sup>	
		Sacchrosomes™	HEK293 cells
<b>1</b>	-	88.3 ± 4.3	81.3 ± 2.1
<b>2</b>	-	47.5 ± 3.3	81.1 ± 3.3
<b>3a</b>	- Ph	91.6 ± 3.5	96.6 ± 4.5

<b>3b</b>	- Ph (4-Br)	26.8 ± 2.3	57.7 ± 1.7
<b>3c</b>	- Ph (4-Cl)	77.5 ± 2.8	85.7 ± 2.1
<b>3d</b>	- Ph (3-Br,4-F)	83.1 ± 3.3	82.3 ± 2.1
<b>3e</b>	- Ph (4-F)	92.4 ± 3.6	89.5 ± 2.2
<b>3f</b>	- Ph (2,4-di-F)	23.9 ± 2.2	85.4 ± 2.8
<b>3g</b>	- pyridin-3-yl	97.2 ± 3.8	99.2 ± 3.3
<b>3h</b>	- Ph (4-OMe)	28.9 ± 2.3	55.7 ± 1.8
<b>3i</b>	- Ph (2,6-di-Cl)	19.6 ± 1.8	58.8 ± 1.8
<b>3j</b>	- Ph (2-OEt,5-Br)	64.3 ± 2.8	31.7 ± 1.5
<b>3k</b>	- Ph (2-Cl)	23.6 ± 2.1	19.5 ± 0.8
<b>3l</b>	- Ph (2,3-di-OMe)	15.2 ± 1.5	21.6 ± 0.9
<b>3m</b>	- Ph (2,4-di-OMe)	10.2 ± 1.1	22.9 ± 0.9
<b>3n</b>	- Ph (penta-F)	21.9 ± 1.8	71.6 ± 2.4
<b>3o</b>	- Ph (3-Br,4-OMe)	37.0 ± 2.4	74.0 ± 2.1
<b>3p</b>	- Ph (2,4,5-tri-OMe)	92.2 ± 3.5	96.8 ± 3.9
<b>3q</b>	- Ph (3,4-methylene-dioxy)	48.4 ± 2.7	-1.1 ± 2.1
<b>3r</b>	- thiophen-3-yl	95.1 ± 3.6	79.4 ± 2.8
<b>3s</b>	- Ph (2,4-di-Cl)	80.2 ± 3.3	71.4 ± 2.5
<b>3t</b>	- Ph (2-F)	49.7 ± 2.6	-2.1 ± 2.1
<b>3u</b>	- naphthalen-2-yl	41.1 ± 2.6	0.2 ± 2.6
<b>3v</b>	- anthracen-2-yl	43.4 ± 2.6	-1.1 ± 3.5
<b>ANF<sup>a</sup></b>	-	97.1 ± 2.6	30.2 ± 10.8

<sup>a</sup>ANF ( $\alpha$ -naphthoflavone) was used as a positive control. <sup>b</sup> The percentage inhibition values represent mean and standard deviations ( $\pm$  SD) of results obtained from two independent experiments.

The IC<sub>50</sub> values were then determined for selected furanochalcones in a panel of enzymes belonging to cytochrome P450 sub-families 1, 2, and 3. The results are shown in Table 2. Chalcones **3e** (bearing 4-fluoro-phenyl as R group) and **3p** (bearing 2,4,5-tri-OMe phenyl as R group) displayed highly selective inhibition of CYP1A1 with IC<sub>50</sub> value of 0.32 and 0.30  $\mu$ M, respectively. These two compounds do not inhibit the other two enzymes from CYP1 sub-family, i.e. CYP1A2 or CYP1B1 (IC<sub>50</sub> > 10  $\mu$ M). Further, they do not inhibit the CYP2 and CYP3 sub-family of enzymes commonly involved in drug

metabolism (Table 2). The pyridyl furanochalcone **3g** showed inhibition of Sacchrosomes™ containing CYP1A1, CYP1A2 and CYP1B1 with IC<sub>50</sub> values of 0.34, 0.17 and 0.66 μM, respectively; whereas no inhibition of CYP2 and CYP3 sub-family of enzymes was observed (IC<sub>50</sub> > 20 μM). The compound **3g** showed IC<sub>50</sub> values of 0.47 and 0.27 μM for CYP1A1 and CYP1B1 inhibition in live human cell assays.

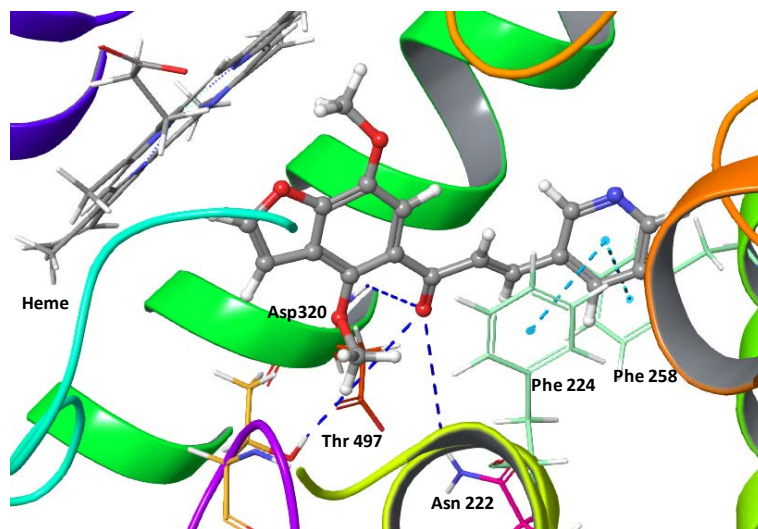
**Table 2.** IC<sub>50</sub> values of selected furanochalcones in a panel of CYP enzymes in Sacchrosomes™

Entry	IC <sub>50</sub> in μM (Sacchrosomes™) <sup>a</sup>			
	1A1	1A2	1B1	2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4
<b>3e</b>	0.32 ± 0.04	>10	>10	>20
<b>3g</b>	0.34 ± 0.03	0.17 ± 0.02	0.66 ± 0.04	>20
<b>3p</b>	0.30 ± 0.05	>20	>20	>20
<b>3r</b>	0.28 ± 0.03	4.2 ± 0.8	0.78 ± 0.04	>20

<sup>a</sup>The IC<sub>50</sub> values represent average (± standard deviations) of results obtained from three independent experiments.

The *in-silico* docking studies have shown that the chalcone **3g** displays H-bonding and π-π interactions with the active site residues of CYP1A1. The carbonyl oxygen of the chalcone **3g** showed three-way H-bonding interactions with Asp 320, Thr 497 and Asn 222 residues, whereas the pyridine ring displays π-π stacking with Phe 224 and Phe 258 residues. The furan ring of the chalcone **3g** has been oriented towards the heme of the CYP1A1 enzyme (Figure 3), as it was observed in case of khellin (Figure 1).





**Figure 3.** The interactions of chalcone **3g** with CYP1A1 (PDB ID: 4I8V) active site. The H-bonding is shown by dark blue dotted lines, whereas  $\pi$ - $\pi$  interactions by light-blue dotted lines.

**The furanoflavanone derivative 4l is a potent and selective inhibitor of CYP1A1 enzyme.** A series of furanoflavanones **4a-p** were tested for inhibition of CYP1A1 enzyme borne on yeast microsomes (Sacchrosomes™) and CYP1A1-overexpressing HEK293 cells, at 10  $\mu$ M concentration (Table 3). Several flavanone derivatives showed >85% inhibition of CYP1A1 enzyme in Sacchrosomes™ as well as in live human cells overexpressing CYP1A1. The compound **4a**, bearing a simple phenyl as R group, has shown 97% and 87% inhibition of CYP1A1 in Sacchrosomes™ and in live human cells, respectively. The 3-pyridyl substituted flavanone (compound **4e**) also showed potent inhibition of CYP1A1 enzyme in both assay systems.

**Table 3.** *In-vitro* CYP1A1 inhibition by furanoflavanones in Sacchrosomes™ and HEK293 suspension cells, overexpressing CYP1A1, at 10  $\mu$ M concentrations.<sup>a</sup>

$\begin{array}{c} \text{OMe} \quad \text{O} \\   \quad    \\ \text{---} \end{array}$	$\begin{array}{c} \text{OMe} \quad \text{O} \\   \quad    \\ \text{---} \end{array}$	% inhibition of CYP1A1 at 10 $\mu$ M
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Entry	R =	(Mean $\pm$ SD) <sup>b</sup>	
		Sacchrosomes™	HEK293 cells
<b>4a</b>	- Ph	97.0 $\pm$ 2.4	87.4 $\pm$ 3.4
<b>4b</b>	- Ph (2-Br)	97.0 $\pm$ 3.1	98.5 $\pm$ 2.1
<b>4c</b>	- Ph (3,4-methylene-dioxy)	78.1 $\pm$ 1.4	74.1 $\pm$ 3.4
<b>4d</b>	- Ph (5-Br, 2-OMe)	87.8 $\pm$ 3.9	72.6 $\pm$ 5.2
<b>4e</b>	- pyridin-3-yl	96.8 $\pm$ 3.2	87.0 $\pm$ 2.5
<b>4f</b>	- thiophen-3-yl	79.9 $\pm$ 0.4	44.3 $\pm$ 4.7
<b>4g</b>	- Ph (4-NO <sub>2</sub> )	48.1 $\pm$ 2.9	-1.5 $\pm$ 2.9
<b>4h</b>	- Ph (2-Cl)	87.1 $\pm$ 0.9	56.9 $\pm$ 6.2
<b>4i</b>	- Ph (2,4-di-Cl)	94.3 $\pm$ 2.3	72.6 $\pm$ 3.5
<b>4j</b>	- naphthalen-2-yl	71.7 $\pm$ 4.2	72.2 $\pm$ 2.7
<b>4k</b>	- Ph (2,6-di-Cl)	75.6 $\pm$ 1.2	54.1 $\pm$ 2.4
<b>4l</b>	- Ph (3-Br,4-F)	96.2 $\pm$ 1.8	89.4 $\pm$ 1.3
<b>4m</b>	- Ph (2-F)	97.1 $\pm$ 1.4	77.8 $\pm$ 3.1
<b>4n</b>	- Ph (4-F)	94.2 $\pm$ 1.5	79.5 $\pm$ 2.3
<b>4o</b>	- Ph	60.7 $\pm$ 0.5	0.4 $\pm$ 2.4
<b>4p</b>	-thiophene-3-yl	95.8 $\pm$ 4.2	68.5 $\pm$ 2.9
<b>ANF</b>	-	97.1 $\pm$ 2.6	30.2 $\pm$ 10.8

<sup>a</sup>The IC<sub>50</sub> values represent average ( $\pm$  standard deviation) of results obtained from three independent experiments.

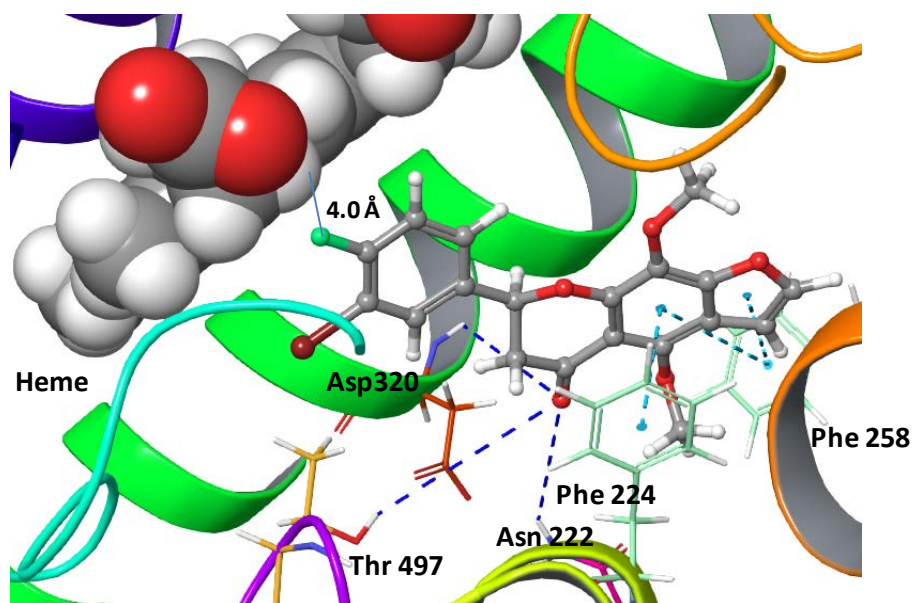
Next, the IC<sub>50</sub> values were determined for three best flavanones, in live human cell assays expressing specific CYP enzymes. Compound **4a** was found to be a dual inhibitor of CYP1A1 and CYP1B1 with slight selectivity towards CYP1B1. However, the compounds **4e** and **4l** were selective CYP1A1 inhibitors, showing 9.2 and 170-fold selectivity over CYP1B1 (Table 4). These compounds does not inhibit CYP1A2, CYP2D6 and CYP3A4 (IC<sub>50</sub> > 10  $\mu$ M). The excellent selectivity profile of flavanone **4l** against CYP2D6, CYP3A4 and also within the CYP family 1 enzymes, indicated its potential for further exploration.

**Table 4.** IC<sub>50</sub> values of selected furanochalcones for CYP1A1, CYP1A2, CYP1B1, CYP2D6 and CYP3A4 inhibition in live human HEK293 cells

Entry	IC <sub>50</sub> in $\mu$ M (live cells) <sup>a</sup>				
	CYP1A1	CYP1A2	CYP1B1	CYP2D6	CYP3A4
<b>4a</b>	1.84 $\pm$ 0.14	>10	0.566 $\pm$ 0.08	>10	>10
<b>4e</b>	0.45 $\pm$ 0.06	>10	4.16 $\pm$ 0.25	>10	>10
<b>4l</b>	0.14 $\pm$ 0.04	>10	23.8 $\pm$ 1.2	>10	>10

<sup>a</sup>The IC<sub>50</sub> values represent average ( $\pm$  standard deviation) of results obtained from three independent experiments.

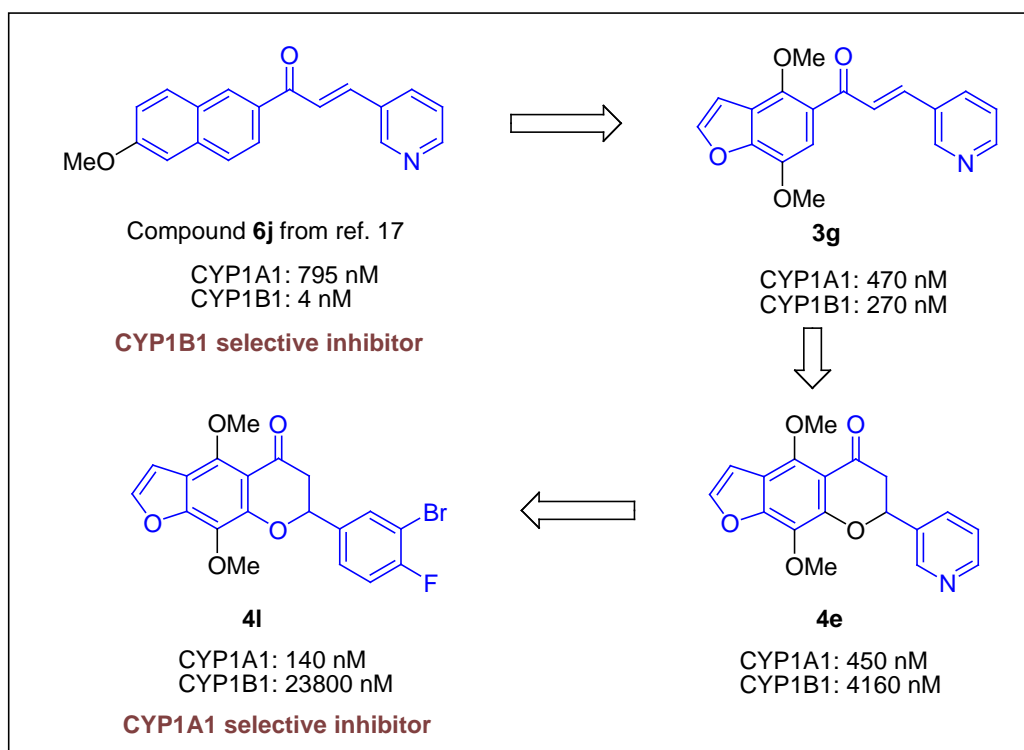
The *in-silico* docking studies of flavanone **4l** with CYP1A1 have shown that it display interactions similar to chalcone **3g**; however, the molecule was found to be flipped by 180° inside the active site. The furan ring of chalcone **3g** was oriented towards the heme, whereas it is away from heme in case of flavanone **4l**. However, despite this flip, the three-way H-bonding interaction of carbonyl oxygen with Asp 320, Thr 497 and Asn 222 residues was common in both the inhibitors. The flavanone **4l** also showed two  $\pi$ - $\pi$  stacking interactions with Phe 224 and Phe 258 residues. The interactions of **4l** with CYP1A1 are shown in Figure 4.



**Figure 4.** The interactions of flavanone **4I** with CYP1A1 (PDB ID: 4I8V) active site.

On comparison of the CYP1A1 and CYP1B1 inhibition data of naphthyl pyridochalcones published earlier by us,<sup>17</sup> with the data of khellinochalcones and khellinoflavanones, an interesting structure-activity relationship was observed. The 3-pyridyl naphthyl chalcone<sup>17</sup> (structure shown in Figure 5) has been reported as potent and selective CYP1B1 inhibitor with an IC<sub>50</sub> value of 4 nM, showing selectivity over CYP1A1. The replacement of naphthyl ring with 4,7-dimethoxybenzofuran has resulted in improved CYP1A1 inhibitory activity (Figure 5). The 4,7-dimethoxybenzofuran ring bearing 3-pyridyl chalcone **3g** showed CYP1A1 inhibition with IC<sub>50</sub> value of 470 nM. Compound **3g** inhibited CYP1B1 in live human cell assay with an IC<sub>50</sub> of 270 nM, indicating slight selectivity towards CYP1B1. Furthermore,<sup>17</sup> when the chalcone skeleton was cyclized in the form of a flavanone (**3g** versus **4e**), there was a huge gain in CYP1A1 selectivity. This indicated that flavanones have better selectivity towards CYP1A1, compared with chalcones. Interestingly, when the pyridyl ring of flavanone **4e** was replaced with substituted phenyl, the potent and selective CYP1A1 inhibitor **4I** was identified. The compound **4I** bearing 3-bromo-4-fluorophenyl ring instead of 3-pyridyl (of **4e**), inhibited CYP1A1 with IC<sub>50</sub> value of

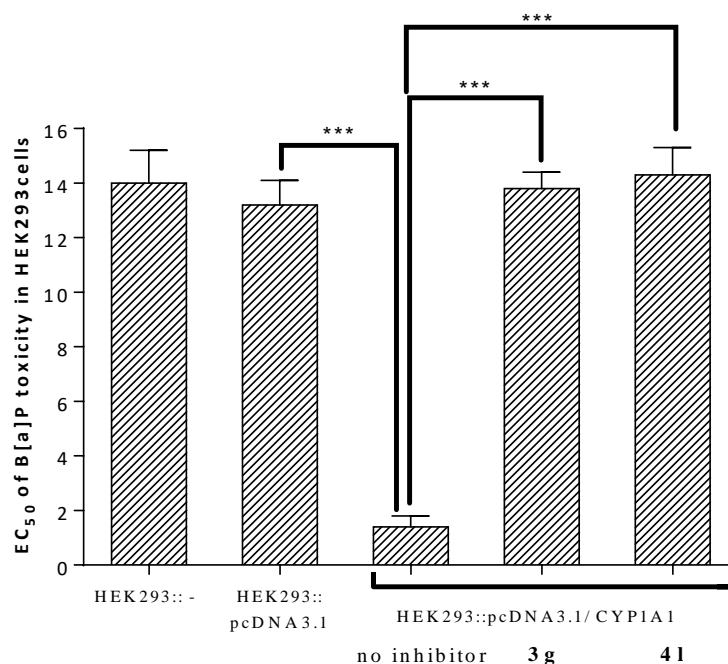
140 nM, and 170-fold selectivity over CYP1B1 ( $IC_{50}$  for CYP1B1 = 23.8  $\mu$ M). The CYP1A1 and CYP1B1 activities of chalcones and flavanones, indicating selectivity within these two enzymes, is depicted in Figure 5.



**Figure 5.** Structure-activity relationship between chalcones and flavanones.

**Furanochalcone 3g and furanoflavanone 4l protect CYP1A1-overexpressing normal human cells from CYP1A1 mediated B[a]P toxicity.** CYP1A1 is an aryl hydroxylase which provides metabolic activation of polycyclic aromatic hydrocarbons (PAHs) and polyhalogenated aromatic hydrocarbons (PHAHs). It is mainly expressed in the extrahepatic tissue like lung, placenta, skin etc.<sup>18</sup> The CYP1A1 enzyme is known to metabolically activate benzo[a]pyrene to its carcinogenic form.<sup>18-19</sup> Therefore, to investigate the potential of identified CYP1A1 inhibitors to stop the process of metabolic activation, thereby protecting normal cells from carcinogenesis, the B[a]P toxicity in normal HEK293 cells and HEK293 cells,<sup>20</sup> overexpressing CYP1A1, was studied.<sup>10, 12</sup> The  $EC_{50}$  of B[a]P in normal HEK293 cells was found to be 14  $\mu$ M, whereas in CYP1A1 overexpressing cells, it was highly toxic

(EC<sub>50</sub> 1.4 μM), indicating that CYP1A1 metabolizes B[a]P to its toxic form. The co-treatment of these CYP1A1 overexpressing cells with CYP1A1 inhibitors **3g** or **4l**, at their 3 x IC<sub>50</sub> concentrations, resulted in nullifying of B[a]P toxicity. The cells were completely protected from B[a]P toxicity as indicated by restoration of the B[a]P EC<sub>50</sub> to normal (i.e. 14 μM) (Figure 6).

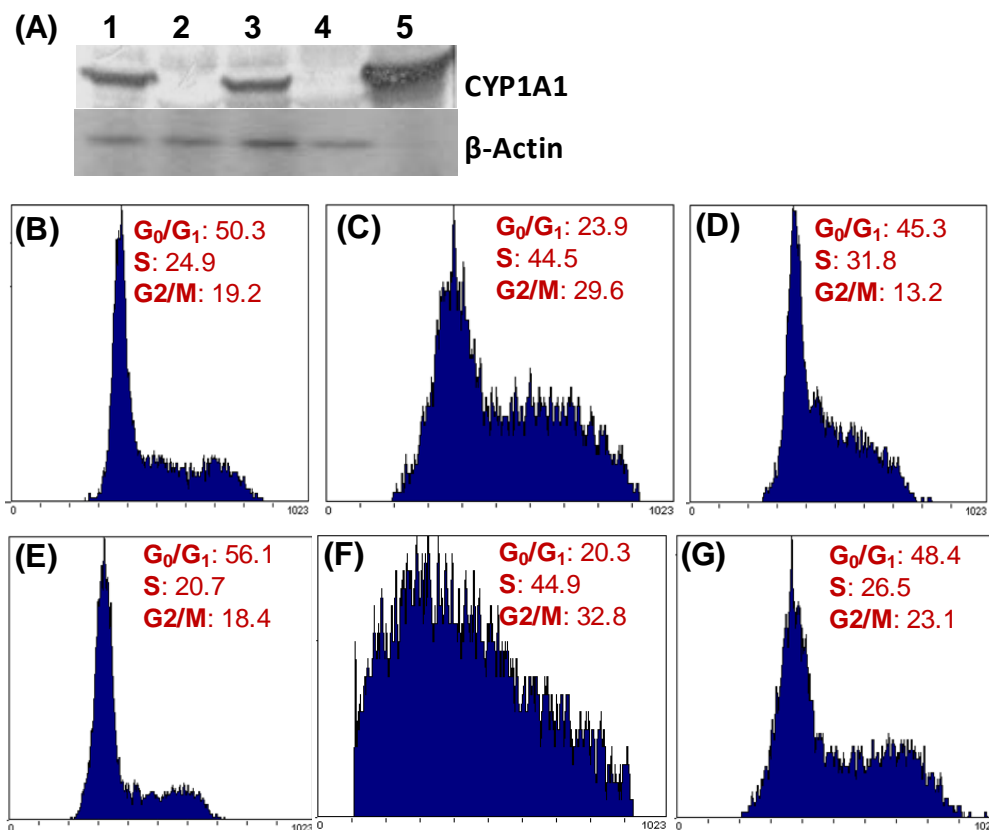


**Figure 6.** Protection of CYP1A1-overexpressing HEK293 cells from B[a]P toxicity. B[a]P was used in the concentration range of 0.05 μM – 100 μM, in the presence of 3 x IC<sub>50</sub> values of the compounds **3g** or **4l** (as determined in the human cell assay where cells expressed CYP1A1 when they were grown in suspension). The cell viability was determined by MTT assay. The cell viability determination using sulforhodamine B (SRB) assay<sup>21</sup> provided similar results to the MTT assay. EC<sub>50</sub> values are presented in μM concentrations, and represents the average of three independent experiments (\*\*\*P < 0.001).

**Furanoflavanone 4l prevents B[a]P-mediated S-phase block in human lung cancer cells, A549 and Calu-1.** The human lung cancer cell lines A549<sup>22</sup> and Calu-1<sup>23</sup> are known to express CYP1A1 enzyme. Furthermore, the in vivo studies in mice model<sup>14</sup> have shown that PAH-treated mice overexpress CYP1A1 in lung tissues; therefore lung cancer cell lines were chosen for this study. The effect of CYP1A1 inhibitors on the cell cycle, in the presence of B[a]P, was investigated by first pre-treating A549 (ATCC CCL-185) and Calu-1 (ATCC HTB-54) lung cancer cells (which express

CYP1A1; Figure 7A) with the most potent CYP1A1 inhibitor, compound **4I**, and then exposing cells to B[a]P. The endogenous CYP1A1 is expected to metabolize B[a]P to its diol-epoxide (BPDE) and corresponding *O*-quinone, products which are known to intercalate DNA. DNA-intercalation should block cells primarily at the S phase of the cell division cycle. It has been suggested that DNA-adduct formation cause DNA mutations that lead to the onset of cancer.<sup>24</sup>

Upon exposure of asynchronous A549 and Calu-1 lung cancer cells (Figures 7B and E) to a 15  $\mu$ M concentration of B[a]P for 3 h and then releasing cells into fresh medium for 57 h, the cells did face a block at the S phase of the cell cycle (Figures 7C and F). This would be expected if the CYP1A1 protein (Figure 7A), expressed within A549 and Calu-1 cells, were to be a functional enzyme. However, when cells were at first pre-incubated for 20 h with the CYP1A1-specific inhibitor, compounds **4I**, at its 3x IC<sub>50</sub> concentrations, and then treated with a 15  $\mu$ M concentration of B[a]P for 3 h, followed by release into fresh medium, the cells were preponderantly released from the block (Figures 7D and G). This would imply that CYP1A1-mediated metabolism of B[a]P in A549 and Calu-1 cells has been prevented by compound **4I**, demonstrating the efficacy of the compound in preventing B[a]P-mediated toxicity in lung cancer cells.



**Figure 7.** Western blot and FACS analyses of A549 and Calu-1 lung cancer cells. (A) Expression of CYP1A1, in 5  $\mu$ g of total protein, isolated from A549 (lane 1), Calu-1 cells (lane 3), normal HEK293 cells (lane 2; as negative control) and normal WI-38 cells (lane 4; as negative control) was confirmed by Western blotting; CYP1A1-bearing Sacchrosomes™ (1pmol) were taken as positive control (lane 5). The CYP1A1-specific antibody, used for confirming CYP1A1 expression in the upper panel, was from ProteinTech(#13241-1-A); beta-actin was used as a loading control and the blot (lower panel) was probed with an antibody from Sigma (# A2228). (B) and (E): FACS analyses of untreated A549 (B) and Calu-1 cells (E) growing asynchronously. FACS analyses of A549 cells, treated with B[a]P, in the absence of compound **4I** (C) and in the presence of compound **4I** (D). FACS analyses of Calu-1 cells, treated with B[a]P, in the absence of compound **4I** (F) and in the presence of compound **4I** (G). The percentage of cells in G<sub>0</sub>/G<sub>1</sub>, S and G<sub>2</sub>/M phases of the cell division cycle is shown in the upper right-hand corner of each of the FACS analyses.



## CONCLUSION

In summary, we have identified that the semisynthetic modifications on the natural product khellin has resulted in significant improvement in its CYP1A1 inhibitory activity. The furanochalcone derivative **3g** and furanoflavanone derivative **4l** displayed potent inhibition of CYP1A1 with IC<sub>50</sub> values of 470 and 140 nM, respectively. These inhibitors completely protected normal human cells from the toxicity of procarcinogen B[a]P via inhibition of its metabolic activation. Moreover, compound **4l** also prevented manifestation of B[a]P-mediated S-phase block (i.e. DNA-intercalation that occurs at S-phase upon B[a]P treatment) in lung cancer cells. Thus, the leads identified herein have potential for further investigation as cancer chemopreventive agents.

## EXPERIMENTAL SECTION

**General.** The commercially available khellin was purchased from Sigma Aldrich (CAS: 82-02-0, Lot# BCBJ3836U). NMR spectra were recorded on Bruker-Avance DPX FT-NMR 500 and 400 MHz instruments. Chemical shift values for protons/ carbons are reported in parts per million (ppm) downfield from tetramethylsilane. ESI-MS and HRMS spectra were recorded on Agilent 1100 LC-Q-TOF and HRMS-6540-UHD machines. IR spectra were recorded on Perkin-Elmer IR spectrophotometer. Melting points were recorded on digital melting point apparatus.

The purity of all derivatives was determined using HPLC analysis. The sample solution of compounds of concentration 0.5 mg/ml prepared in acetonitrile and filtered through 0.45 μ membrane filter injected (15 μl) in the HPLC system (Waters) using Chromolith C-18e column (Merck, 50 X 4.6 mm). The binary mobile phase consisted of A (water) and B (acetonitrile) was used. The isocratic elution was used as follows: 80 min run time with 90% acetonitrile and 10% water. The flow rate was 1 mL/minutes and the results were analyzed using UV-PDA detector.

**Synthesis of khellinone (2).** The mixture of khellin (900 mg, 3.46 mmol) and potassium hydroxide (1 M) in ethanol was refluxed at 90 °C over a period of 12-14 h. After completion of the reaction, ethanol

was evaporated over vacuo rotavapor and the obtained residue was partitioned in between dichloromethane and water. The dichloromethane layer was washed with brine solution and was dried over anhydrous sodium sulphate. The solvent was evaporated over vacuo rotavapor and the obtained crude product was purified using silica gel column chromatography (5-10% ethyl acetate in hexane) to get khellinone (**2**) as a yellow powder. The purchased khellin along with khellinone were characterized using spectral analysis.

*Khellin (1)*: white crystals; HPLC:  $t_R = 4.6$  min (99% purity); m.p. 155-156 °C; IR (CHCl<sub>3</sub>):  $\nu_{\max}$  3436, 3160, 3137, 2989, 2931, 2960, 2830, 1619, 1586, 1471, 1444, 1424, 1364, 1380, 1300, 1265 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.51 (d,  $J = 2.2$  Hz, 1H, CH), 6.91 (d,  $J = 4.0$  Hz, 1H, CH), 4.15 (s, 3H, OMe), 4.05 (s, 3H, OMe), 2.73 (s, 3H, Me); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 206.2 (C=O), 153.5 (C-7a), 152.3 (C-6), 151.6 (C-3), 143.8 (OCH=CH), 128.8 (C-7), 110.8 (C-3a), 110.5 (C-5), 106.7 (OCH=CH), 61.0 (OMe), 60.9 (OMe), 33.2 (Me); HR-ESIMS:  $m/z$  237.0759 [M+H]<sup>+</sup> calcd for C<sub>12</sub>H<sub>12</sub>O<sub>5</sub> + H<sup>+</sup>(237.0757).<sup>25</sup>

*1-(6-Hydroxy-4,7-dimethoxy-benzofuran-5-yl) (2)*: yellow crystals; HPLC:  $t_R = 4.6$  min (99% purity); yield: 61%; m.p. 169-170 °C; IR (CHCl<sub>3</sub>):  $\nu_{\max}$  3436, 3160, 3137, 2989, 2931, 2960, 2830, 1619, 1586, 1471, 1444, 1424, 1364, 1380, 1300, 1265 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.51 (d,  $J = 2.2$  Hz, 1H, CH), 6.91 (d,  $J = 4.0$  Hz, 1H, CH), 4.15 (s, 3H, OMe), 4.05 (s, 3H, OMe), 2.73 (s, 3H, Me); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 206.2 (C=O), 153.5 (C-7a), 152.3(C-6), 151.6 (C-3), 143.8 (OCH=CH), 128.8 (C-7), 110.8 (C-3a), 110.5 (C-5), 106.7 (OCH=CH), 61.0 (OMe), 60.9 (OMe), 33.2 (Me); HR-ESIMS:  $m/z$  237.0759 [M+H]<sup>+</sup> calcd for C<sub>12</sub>H<sub>12</sub>O<sub>5</sub> + H<sup>+</sup>(237.0757).<sup>15</sup>

**Synthesis of furanochalcones 3a-v.** Khellinone (80 mg, 0.338 mmol) was reacted with different aromatic aldehydes (1.2 equiv.) in the presence of catalytic amount of 1 M of KOH in 50 ml methanol at a temperature of 0 °C to 1 °C over a period of 12-14 h. After completion of the reaction, methanol was evaporated over vacuo rotavapor and the obtained residue was partitioned in between dichloromethane and water. The dichloromethane layer was washed with brine solution and was dried over anhydrous

sodium sulphate. The solvent was evaporated over vacuo rotavapor and the obtained crude product was purified using silica gel column chromatography (5-25%) to get furanochalcones **3a-v** in 40-85% yield.

*(1-(6-Hydroxy-4,7-dimethoxy-benzofuran-5-yl)-3-phenyl-propenone)* (**3a**): yellow orange solid; HPLC:  $t_R = 4.7$  min (99% purity); yield: 41%; m.p. 123-126 °C; IR (CHCl<sub>3</sub>):  $\nu_{max}$  3860, 3791, 3697, 3436, 3060, 2930, 2850, 1630, 1606, 1559, 1494, 1446, 1360 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.88 (d,  $J = 2.2$  Hz, 2H, CH), 7.64-7.67 (m, 2H), 7.53 (d,  $J = 2.2$  Hz, 1H, OCH=CH), 7.42-7.45 (m, 3H), 6.89 (d,  $J = 4.0$  Hz, 1H, OCH=CH), 4.09 (s, 3H, OMe), 4.05 (s, 3H, OMe); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 194.7 (C=O), 153.2 (C-9a), 151.9 (C-6), 150.7 (C-4), 144.1(CH=CH), 143.5 (OCH=CH), 135.1, 130.4, 129.6, 129.0, 128.5, 127.0, 112.8, 111.9, 105.2 (OCH=CH), 62.0, 61.0; HR-ESIMS:  $m/z$  325.1078 [M+H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>16</sub>O<sub>5</sub> + H<sup>+</sup> (325.1075).<sup>16</sup>

*3-(4-Bromo-phenyl)-1-(6-hydroxy-4,7-dimethoxy-benzofuran-5-yl)-popenone)* (**3b**): white solid; HPLC:  $t_R = 4.5$  min (100% purity); yield: 52%; m.p. 135-137 °C; IR (CHCl<sub>3</sub>):  $\nu_{max}$  3400, 2919, 2850, 1682, 1613, 1544, 1414, 1389, 1435, 1349, 1279, 112, 1012 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.82 (d,  $J = 12.0$  Hz, 2H, CH), 7.58 (d,  $J = 8.0$  Hz, 1H, CH), 7.53 (d,  $J = 4.0$  Hz, 1H, OCH=CH), 7.40 (d,  $J = 12.0$  Hz, 2H, CH), 6.89 (d,  $J = 4.0$  Hz, 1H, OCH=CH), 4.09 (s, 3H, OMe), 4.04 (s, 3H, OMe); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 194.4 (C=O), 153.1 (C-6), 152.1 (C-9a), 150.7 (C-4), 144.3 (OCH=CH), 141.4, 137.3, 133.1, 130.9, 130.5, 127.2, 123.1, 112.7, 111.7, 106.2 (OCH=CH), 62.0, 61.0; HR-ESIMS  $m/z$  403.0175 [M+H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>15</sub>BrO<sub>5</sub> + H<sup>+</sup> (403.0175).

*3-(4-Chlorophenyl)-1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)prop-2-en-1-one* (**3c**): orange crystals; HPLC:  $t_R = 49.6$  min (90% purity); yield: 48%; m.p. 152-154 °C; IR (CHCl<sub>3</sub>):  $\nu_{max}$  3448, 3053, 2928, 2868, 2304, 1730, 1656, 1619, 1585, 1462, 1386, 1327, 1313, 12679, 1262, 1210, 1149, 1039 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.98 (d,  $J = 4.0$  Hz, 2H, CH), 7.52 (d,  $J = 4.0$  Hz, 1H, OCH=CH), 7.40 (d,  $J = 8.0$  Hz, 2H, CH), 7.19 (m, 2H, CH), 6.88 (d,  $J = 4.0$  Hz, 1H, OCH=CH), 4.09 (s, 3H, OMe), 4.05 (s, 3H, OMe); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 194.5 (C=O), 153.2 (C-6), 152.0 (C-9a), 150.7 (C-4), 144.2 (OCH=CH), 141.9, 136.3, 133.6, 129.6, 129.3, 127.5, 127.4 112.7,

111.8, 106.2 (OCH=CH), 62.0, 61.0; HR-ESIMS:  $m/z$  359.0677 [M+H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>15</sub>ClO<sub>5</sub> + H<sup>+</sup> (359.0608).<sup>26</sup>

*3-(3-Bromo-4-fluorophenyl)-1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)prop-2-en-1-one* (**3d**): white solid; HPLC:  $t_R$  = 5.0 min (97% purity); yield: 44%; m.p.186-188 °C; IR (CHCl<sub>3</sub>):  $\nu_{\max}$  3400, 2921, 2850, 1630, 1557, 1494, 1463, 1442, 1417, 1382, 1359, 1332, 1299, 1269, 1151, 1091 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.85 (m, 1H, CH), 7.76 (d,  $J$  = 8.0 Hz, 2H, CH), 7.54 (t,  $J$  = 4.0 Hz, 2H, CH), 7.12 (t,  $J$  = 12.0 Hz, 1H, CH), 6.89 (d,  $J$  = 2.2 Hz, 1H, OCH=CH), 4.09 (s, 3H, OMe), 4.05 (s, 3H, OMe); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 194.5 (C=O), 165.3 (d, <sup>1</sup> $J_{CF}$  = 251 Hz), 162.8, 153.1, 151.9, 150.6, 144.2 (OCH=CH), 142.2, 131.4, 130.4 (d, <sup>3</sup> $J_{CF}$  = 8.0 Hz), 130.3, 129.6, 126.7, 116.2 (<sup>2</sup> $J_{CF}$  = 22.0 Hz), 112.8, 111.9, 106.1 (OCH=CH), 62.0, 61.0; HR-ESIMS:  $m/z$  423.0060 [M+H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>14</sub>BrFO<sub>5</sub>+ H<sup>+</sup> (423.0060).

*3-(4-Fluoro-phenyl)-1-(6-hydroxy-4,7-dimethoxy-benzofuran-5-yl)-propenone* (**3e**): cream colored oil; HPLC:  $t_R$  = 48.4 min (98% purity); yield: 40%; m.p.129-130 °C; IR (CHCl<sub>3</sub>):  $\nu_{\max}$  3400, 2922, 2851, 1628, 1601, 1556, 1544, 1510, 1461, 1443, 1413, 1360, 1297, 1299, 1266, 1151, 1091 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.82 (s, 2H, CH), 7.64 (dd,  $J$  = 8.0, 5.5 Hz, 2H, CH), 7.53 (d,  $J$  = 2.2 Hz, 1H, OCH=CH), 7.12 (t,  $J$  = 8.6 Hz, 2H, CH), 6.88 (d,  $J$  = 2.3 Hz, 1H, OCH=CH), 4.09 (s, 3H, OMe), 4.05 (s, 3H, OMe); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 194.5 (C=O), 165.0 (d, <sup>1</sup> $J_{CF}$  = 251.2 Hz), 163.0, 153.1, 151.9, 150.7, 144.2 (OCH=CH), 142.2, 131.4, 130.4 (d, <sup>3</sup> $J_{CF}$  = 8.75 Hz), 126.7 (d, <sup>4</sup> $J_{CF}$  = 3.0 Hz), 116.2 (d, <sup>2</sup> $J_{CF}$  = 22.5 Hz), 112.8, 111.8, 106.2 (OCH=CH), 62.0, 61.0; HR-ESIMS:  $m/z$  365.0838 [M+Na]<sup>+</sup> calcd for C<sub>19</sub>H<sub>15</sub>FNaO<sub>5</sub> (365.0801).<sup>27</sup>

*3-(2,4-Difluoro-phenyl)-1-(6-hydroxy-4,7-dimethoxy-benzofuran-5-yl)-propenone* (**3f**): white solid; HPLC:  $t_R$  = 42.4 min (98% purity); yield: 40%; m.p. 154-156°C; IR (CHCl<sub>3</sub>):  $\nu_{\max}$  3400, 2919, 2850, 1633, 1618, 1588, 1562, 1542, 1464, 1438, 1412, 1377, 1357, 1286, 1211, 1153, 1119 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.83 (d,  $J$  = 16.0 Hz, 1H, CH), 7.72 (d,  $J$  = 12.0 Hz, 1H, CH), 7.54 (d,  $J$  = 2.2 Hz, 1H, OCH=CH), 7.14 (d,  $J$  = 4.0 Hz, 2H, CH), 6.89 (d,  $J$  = 4.0 Hz, 1H, CH), 6.86 (d,  $J$  = 4.0 Hz, 1H, OCH=CH), 4.09 (s, 3H, OMe), 4.07 (s, 3H, OMe); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 194.2

(C=O), 164.8, 162.0, 153.2, 152.2, 150.8, 144.3 (OCH=CH), 140.2, 138.5, 129.5, 112.5, 111.6, 111.0, 110.9, 110.7, 105.4, 105.2 (OCH=CH), 61.9, 61.0; HR-ESIMS:  $m/z$  361.0912 [M+H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>14</sub>F<sub>2</sub>O<sub>5</sub> + H<sup>+</sup> (361.0882).

*1-(6-Hydroxy-4,7-dimethoxybenzofuran-5-yl)-3-(pyridin-3-yl)prop-2-en-1-one* (**3g**): yellow orange crystals; HPLC:  $t_R$  = 49.6 min (95%); yield: 85%; m.p. 112-114 °C; IR (CHCl<sub>3</sub>): 3400, 2919, 2850, 1633, 1618, 1588, 1562, 1542, 1464, 1438, 1412, 1377, 1357, 1286, 1211, 1153, 1119 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 8.88 (s, 1H, CH), 8.63 (d,  $J$  = 4.0 Hz, 1H, CH), 7.94 (m, 2H, CH), 7.81 (d,  $J$  = 16.0 Hz, 1H, CH), 7.54 (d,  $J$  = 2.3 Hz, 1H, OCH=CH), 7.37 (dd,  $J$  = 8.0, 4.9 Hz, 1H, CH), 6.89 (d,  $J$  = 2.3 Hz, 1H, OCH=CH), 4.09 (s, 3H, OMe), 4.06 (s, 3H, OMe); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 194.2 (C=O), 153.2, 152.2, 150.9, 149.8, 144.3, 139.3, 134.8, 131.0, 128.9, 123.8, 112.5, 112.5, 111.6, 111.5, 106.3, 61.8, 61.1; HR-ESIMS:  $m/z$  326.1033 [M+H]<sup>+</sup> calcd for C<sub>18</sub>H<sub>15</sub>NO<sub>5</sub> + H<sup>+</sup> (326.1023).

*1-(6-Hydroxy-4,7-dimethoxybenzofuran-5-yl)-3-(4-methoxyphenyl)prop-2-en-1-one* (**3h**): yellow crystals; HPLC:  $t_R$  = 5.3 min (100% purity); yield: 52%; m.p. 148-149 °C IR (CHCl<sub>3</sub>):  $\nu_{max}$  3435, 2923, 2851, 1630, 1606, 1564, 1543, 1456, 1438, 1422, 1404, 1358, 1306, 1293, 1250, 1153, 1119 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.84 (d,  $J$  = 16.0 Hz, 2H, CH), 7.62 (d,  $J$  = 4.0 Hz, 2H, CH), 7.53 (d,  $J$  = 2.3 Hz, 1H, OCH=CH), 7.30 (d,  $J$  = 12.0 Hz, 1H, CH), 6.95 (d,  $J$  = 4.0 Hz, 2H, CH), 6.89 (d,  $J$  = 12.0 Hz, 1H, CH), 6.88 (d,  $J$  = 4.0 Hz, 1H, OCH=CH), 4.09 (s, 3H, OMe), 4.04 (s, 3H, OMe), 3.87 (s, 3H, OMe); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 194.6 (C=O), 161.7, 153.2, 151.7, 150.6, 144.1 (OCH=CH), 143.8, 130.3, 127.8, 124.5, 114.5, 113.4, 112.9, 112.0, 105.1 (OCH=CH), 62.0, 61.0, 55.4; HR-ESIMS:  $m/z$  355.1171 [M+H]<sup>+</sup> calcd for C<sub>20</sub>H<sub>18</sub>O<sub>6</sub> + H<sup>+</sup> (354.1103).<sup>27</sup>

*3-(2,6-Dichlorophenyl)-1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)prop-2-en-1-one* (**3i**): orange yellow crystals; HPLC:  $t_R$  = 5.3 min (100% purity); yield: 57%; m.p. 298-299 °C; IR (CHCl<sub>3</sub>):  $\nu_{max}$  3399, 3161, 3090, 2951, 2921, 2851, 1640, 1613, 1577, 1472, 1441, 1427, 1378, 1357, 1328, 1301, 1275, 1242, 1213, 1185, 1145 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.98 (d,  $J$  = 8.0 Hz, 2H, CH), 7.52 (d,  $J$  = 2.3 Hz, 1H, OCH=CH), 7.40 (d,  $J$  = 8.0 Hz, 2H, CH), 7.23 (d,  $J$  = 8.0 Hz, 1H, CH),

6.88 (d,  $J = 4.0$  Hz, 1H, OCH=CH), 4.09 (s, 3H, OMe), 4.07 (s, 3H, OMe);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 194.4 (C=O), 153.4, 152.2, 151.1, 143.9 (OCH=CH), 141.7, 136.3, 135.3, 134.7, 132.6, 129.9, 129.3, 128.8, 112.3, 111.5, 105.3 (OCH=CH), 61.9 (OMe), 61.1 (OMe); HR-ESIMS:  $m/z$  393.0287  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{19}\text{H}_{14}\text{Cl}_2\text{O}_5 + \text{H}^+$  (393.0291).

*3-(5-Bromo-2-ethoxyphenyl)-1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)prop-2-en-1-one* (**3j**): orange powder; HPLC:  $t_{\text{R}} = 5.1$  min (100% purity); yield: 55%; m.p. 260-261 °C; IR ( $\text{CHCl}_3$ ):  $\nu_{\text{max}}$  3399, 3161, 3090, 2951, 2921, 2851, 1640, 1613, 1577, 1472, 1441, 1427, 1378, 1357, 1328  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 8.12 (d,  $J = 16.0$  Hz, 1H, CH), 7.90 (d,  $J = 16.0$  Hz, 1H, CH), 7.73 (d,  $J = 4.0$  Hz, 1H, CH), 7.53 (d,  $J = 2.2$  Hz, 1H, OCH=CH), 7.43 (d,  $J = 4.0$  Hz, 1H, CH), 6.88 (d,  $J = 2.2$  Hz, 1H, OCH=CH), 6.82 (d,  $J = 8.0$  Hz, 1H, CH), 4.13 (m, 2H,  $\text{CH}_2$ ), 4.09 (s, 3H, OMe), 4.04 (s, 3H, OMe), 1.50 (t,  $J = 7.0$  Hz, 3H, Me);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 194.7 (C=O), 157.1, 157.0, 153.2, 151.9, 150.8, 144.2 (OCH=CH), 137.2, 134.0, 130.9, 128.1, 126.1, 113.9, 112.8, 112.8, 111.9, 106.2 (OCH=CH), 64.4, 62.0, 61.0, 14.7; HR-ESIMS:  $m/z$  447.0433  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{21}\text{H}_{19}\text{BrO}_6 + \text{H}^+$  (447.0437).

*3-(2-Chlorophenyl)-1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)prop-2-en-1-one* (**3k**): yellow powder; HPLC:  $t_{\text{R}} = 5.1$  min (100% purity); yield: 55%; m.p. 150-151 °C; IR ( $\text{CHCl}_3$ ):  $\nu_{\text{max}}$  3435, 2922, 2851, 2650, 2342, 1693, 1628, 1591, 1571, 1469, 1439, 1408, 1363, 1316  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 8.23 (d,  $J = 16.0$  Hz, 1H, CH), 7.86 (d,  $J = 8.0$  Hz, 1H, CH), 7.74 (d,  $J = 2.2$  Hz, 1H, CH), 7.53 (d,  $J = 2.3$  Hz, 1H, OCH=CH), 7.33 (dd,  $J = 2.3, 4.0$  Hz, 2H, CH), 6.88 (d,  $J = 2.3$  Hz, 1H, OCH=CH), 4.09 (s, 3H, OMe), 4.05 (s, 3H, OMe);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 194.4 (C=O), 153.1, 152.1, 150.7, 144.3 (OCH=CH), 141.1, 137.3, 133.1, 130.9, 130.5, 129.5, 128.3, 127.2, 123.1, 112.7, 111.7, 105.2, 62.0, 61.0; HR-ESIMS:  $m/z$  359.0680  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{19}\text{H}_{15}\text{ClO}_5 + \text{H}^+$  (359.0680).<sup>26</sup>

*3-(4,7-dimethoxy-6-hydroxybenzofuran-5-yl)-1-(2-3-dimethoxyphenyl)-3-oxopropene* (**3l**): HPLC:  $t_{\text{R}} = 4.7$  min (100% purity); yield: 57%; m.p. 260-262 °C; IR ( $\text{CHCl}_3$ ):  $\nu_{\text{max}}$  3400, 2923, 2851, 1627, 1561, 1511, 1463, 1439, 1383, 1360, 1301, 1264, 1064, 1022  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 7.83

(d,  $J = 16.0$  Hz, 2H, CH), 7.53 (d,  $J = 4.0$  Hz, 1H, OCH=CH), 7.25 (d,  $J = 2.2$  Hz, 2H, CH), 7.16 (d,  $J = 4.0$  Hz, 1H, CH), 6.91 (d,  $J = 12.0$  Hz, 1H, CH), 6.87 (d,  $J = 4.0$  Hz, 1H, OCH=CH), 4.09 (s, 3H, OMe), 4.03 (s, 3H, OMe), 3.95 (s, 3H, OMe), 3.93 (s, 3H, OMe);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 195.6 (C=O), 163.8, 161.1, 154.0, 152.2, 151.3, 144.7, 140.3, 131.4, 130.2, 125.3, 125.2, 118.0, 113.6, 112.9, 106.2, 105.8, 62.7, 61.7, 56.2, 56.2; HR-ESIMS:  $m/z$  385.1278  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{21}\text{H}_{20}\text{O}_7 + \text{H}^+$  (385.1281).

*3-(4,7-Dimethoxy-6-hydroxybenzofuran-5-yl)-1-(2-4-dimethoxyphenyl)-3-oxopropene (3m)*: colorless oil; HPLC:  $t_{\text{R}} = 4.7$  min (100% purity); yield: 55%; m.p. 264-266 °C; IR ( $\text{CHCl}_3$ ):  $\nu_{\text{max}}$  3400, 2923, 2851, 1627, 1561, 1511, 1463, 1439, 1383, 1360, 1301, 1264, 1064, 1022  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 8.21 (d,  $J = 16.0$  Hz, 1H, CH), 7.93 (d,  $J = 16.0$  Hz, 1H, CH), 7.61 (d,  $J = 8.0$  Hz, 1H, CH), 7.51 (d,  $J = 2.2$  Hz, 1H, OCH=CH), 6.87 (s, 1H, CH), 6.53 (d,  $J = 4.0$  Hz, 1H, OCH=CH), 6.48 (s, 1H, CH), 4.09 (s, 3H, OMe), 3.91 (s, 3H, OMe), 3.87 (s, 3H, OMe);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 194.9 (C=O), 163.2, 160.5, 153.3, 151.5, 150.6, 144.0, 139.6, 130.7, 129.4, 124.6, 117.3, 112.9, 112.2, 105.5, 105.2, 98.4, 62.0, 61.0, 55.5, 55.5; HR-ESIMS:  $m/z$  385.1276  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{21}\text{H}_{20}\text{O}_7 + \text{H}^+$  (385.1281).<sup>26</sup>

*1-(6-Hydroxy-4,7-dimethoxybenzofuran-5-yl)-3-(perfluorophenyl)prop-2-en-1-one (3n)*: white solid; HPLC:  $t_{\text{R}} = 5.0$  min (92% purity); yield: 50%; m.p. 112-114 °C; IR ( $\text{CHCl}_3$ ):  $\nu_{\text{max}}$  3400, 2924, 2853, 1726, 1656, 1500, 1462, 1385, 1280, 1209, 1151, 1053, 1021  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 8.12 (d,  $J = 16.0$  Hz, 1H, CH), 7.81 (d,  $J = 16.0$  Hz, 1H, CH), 7.53 (d,  $J = 4.0$  Hz, 1H, OCH=CH), 6.91 (d,  $J = 4.0$  Hz, 1H, OCH=CH), 4.17 (s, 3H, OMe), 4.11 (s, 3H, OMe);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 194.3 (C=O), 153.5, 152.2, 151.0, 144.9, 144.1 (OCH=CH), 133.0, 132.9, 129.3, 127.2, 112.1, 111.3, 108.6, 105.1 (OCH=CH), 61.3, 61.0; HR-ESIMS:  $m/z$  415.0603  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{19}\text{H}_{11}\text{F}_5\text{O}_5 + \text{H}^+$  (415.0599).

*1-(6-Hydroxy-4,7-dimethoxybenzofuran-5-yl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (3o)*: reddish orange crystals; HPLC:  $t_{\text{R}} = 8.7$  min (100 % purity); yield: 50%; m.p. 131-133 °C; IR ( $\text{CHCl}_3$ ):  $\nu_{\text{max}}$  3434, 2930, 2867, 1724, 1656, 1517, 1463, 1385, 1263, 1210, 1159, 1084, 1024  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR

(400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 8.29 (d,  $J = 16.0$  Hz, 1H, CH), 7.92 (d,  $J = 16.0$  Hz, 1H, CH), 7.59 (s, 1H, OCH=CH), 7.22 (m, 1H, CH), 6.94 (s, OCH=CH), 6.60 (s, 1H, CH), 4.16-3.97 (m, 15H, OMe); <sup>13</sup>C NMR: (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 194.7, 154.8, 153.2, 152.7, 151.5, 150.5, 144.0, 143.3, 139.3, 129.6, 124.4, 115.7, 113.0, 112.2, 111.2, 105.1, 96.7, 62.0 (OMe), 61.0 (OMe), 56.5 (OMe), 56.3 (OMe), 56.1 (OMe); HR-ESIMS:  $m/z$  415.1390 [M+H]<sup>+</sup> calcd for C<sub>22</sub>H<sub>21</sub>O<sub>8</sub> + H<sup>+</sup> (415.1390).

*3-(3-Bromo-4-methoxyphenyl)-1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)prop-2-en-1-one* (**3p**): orange crystals; HPLC:  $t_R = 5.4$  min (100% purity); yield: 52%; m.p. 261-263 °C; IR (CHCl<sub>3</sub>):  $\nu_{max}$  3454, 2927, 2866, 1730, 1654, 1590, 1464, 1386, 1365, 1326, 1312, 1279, 1102, 1084, 1048 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 8.11 (d,  $J = 16.0$  Hz, 1H, CH), 7.86 (d,  $J = 16.0$  Hz, 1H, CH), 7.73 (s, 1H, CH), 7.52 (s, 1H, CH), 6.88 (s, 1H, CH), 6.84 (d,  $J = 8.0$  Hz, 1H, CH), 4.09 (s, 3H, OMe), 4.06 (s, 3H, OMe), 3.91 (s, 3H, OMe); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 194.8 (C=O), 157.7, 153.2, 151.9, 150.7, 144.2, 137.1, 134.0, 134.0, 130.9, 128.3, 128.3, 126.1, 113.0, 112.7, 105.2, 62.0 (OMe), 61.0 (OMe), 55.8; HR-ESIMS:  $m/z$  432.0300 [M+H]<sup>+</sup> calcd for C<sub>20</sub>H<sub>17</sub>BrO<sub>6</sub> + H<sup>+</sup> (432.0281).

*3-(Benzo[d][1,3]dioxol-5-yl)-1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)prop-2-en-1-one* (**3q**): orange crystals; HPLC:  $t_R = 5.6$  min (92% purity); yield: 53%; m.p. 248-251 °C; IR (CHCl<sub>3</sub>):  $\nu_{max}$  3743, 3385, 3130, 2850, 1729, 1627, 1565, 1542, 1489, 1470, 1446, 1353, 1300, 1255 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.78 (d,  $J = 16.0$  Hz, 2H, CH), 7.53 (d,  $J = 4.0$  Hz, 1H, OCH=CH), 7.15 (m, 2H, CH), 6.88 (d,  $J = 2.2$  Hz, 1H, OCH=CH), 6.87 (d,  $J = 8.0$  Hz, 1H, CH), 6.04 (s, 2H, CH<sub>2</sub>), 4.09 (s, 3H, OMe), 4.04 (s, 3H, OMe); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 194.4 (C=O), 153.2, 151.7, 150.6, 149.9, 148.4, 144.1, 143.8, 143.6, 129.6, 125.4, 124.9, 112.8, 111.9, 108.7, 106.6, 105.2, 101.6, 62.0 (OMe), 61.0 (OMe); HR-ESIMS:  $m/z$  369.0968 [M+H]<sup>+</sup> calcd for C<sub>20</sub>H<sub>17</sub>O<sub>7</sub> + H<sup>+</sup> (369.0954).<sup>26</sup>

*1-(6-Hydroxy-4,7-dimethoxybenzofuran-5-yl)-3-(thiophen-3-yl)prop-2-en-1-one* (**3r**): orange crystals; HPLC:  $t_R = 3.9$  min (85% purity); yield: 42%; m.p. 139-141 °C; IR (CHCl<sub>3</sub>):  $\nu_{max}$  3584, 3136, 2922, 2850, 1626, 1586, 1561, 1543, 1470, 1442, 1364, 1416, 1297, 1131 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.89 (d,  $J = 16.0$  Hz, 1H, CH), 7.73 (d,  $J = 16.0$  Hz, 1H, CH), 7.62 (s, CH, 1H), 7.52 (s, 1H, CH), 7.40 (d,  $J = 8.0$  Hz, 2H, CH), 6.87 (s, CH, 1H), 4.09 (s, 3H, OMe), 4.03 (s, 3H, OMe); <sup>13</sup>C NMR



(100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 194.8 (C=O), 153.1, 151.8, 150.6, 144.1, 138.5, 137.2, 129.6, 129.1, 127.0, 126.6, 125.3, 112.8, 111.9, 105.1, 62.0 (OMe), 61.0 (OMe); HR-ESIMS:  $m/z$  331.0619 [M+H]<sup>+</sup> calcd for C<sub>17</sub>H<sub>15</sub>O<sub>5</sub>S + H<sup>+</sup> (331.0634).

*3-(2,4-Dichlorophenyl)-1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)prop-2-en-1-one* (**3s**): orange crystals; HPLC:  $t_R$  = 5.0 min (95% purity); yield: 42%; m.p. 148-149 °C; IR (CHCl<sub>3</sub>):  $\nu_{\max}$  3399, 3161, 3090, 2951, 2921, 2851, 1640, 1613, 1577, 1472, 1441, 1427, 1378, 1357, 1328, 1301, 1275, 1242, 1213, 1185, 1145 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 8.14 (d,  $J$  = 16.0 Hz, 1H, CH), 7.83 (d,  $J$  = 16.0 Hz, 1H, CH), 7.67 (d,  $J$  = 12.0 Hz, 1H, CH), 7.53 (d,  $J$  = 4.0 Hz, 1H, OCH=CH), 7.48 (d,  $J$  = 3.0 Hz, 1H, CH), 7.31 (m, 1H, CH), 6.88 (d,  $J$  = 2.2 Hz, 1H, OCH=CH), 4.09 (s, 3H, OMe), 4.03 (s, 3H, OMe); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 194.2 (C=O), 153.2, 152.1, 150.7, 144.3, 137.5, 136.3, 136.1, 132.0, 130.1, 129.8, 129.5, 128.4, 127.6, 112.6, 111.7, 105.2, 61.9, 61.0; HR-ESIMS:  $m/z$  393.0275 [M+H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>14</sub>Cl<sub>2</sub>O<sub>5</sub> + H<sup>+</sup> (393.0291).

*1-(6-Hydroxy-4,7-dimethoxybenzofuran-5-yl)-3-(naphthalen-2-yl)prop-2-en-1-one* (**3t**): orange crystals; HPLC:  $t_R$  = 7.1 min (99% purity); yield: 42%; m.p. 158-159 °C; IR (CHCl<sub>3</sub>):  $\nu_{\max}$  3584, 3136, 2922, 2850, 1626, 1586, 1561, 1543, 1470, 1442, 1379, 1297, 1149 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 8.03 (m, 3H, CH), 7.86 (m, 4H, CH), 7.54 (d,  $J$  = 2.2 Hz, 1H, OCH=CH), 7.53 (m, 3H, CH), 6.90 (d,  $J$  = 2.2 Hz, 1H, OCH=CH), 4.10 (s, 3H, OMe), 4.07 (s, 3H, OMe); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 194.6 (C=O), 153.5, 153.2, 150.7, 144.2, 143.7, 134.3, 133.4, 132.7, 130.7, 128.7, 128.6, 127.8, 127.4, 127.1, 126.8, 123.7, 112.9, 112.0, 110.6, 105.2, 62.1, 60.5; HR-ESIMS:  $m/z$  375.1194 [M+H]<sup>+</sup> calcd for C<sub>23</sub>H<sub>18</sub>O<sub>5</sub> + H<sup>+</sup> (375.1127).<sup>26</sup>

*3-(2-Fluorophenyl)-1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)prop-2-en-1-one* (**3u**): orange crystals; HPLC:  $t_R$  = 5.0 min (95% purity); yield: 52%; m.p. 139-141 °C; IR (CHCl<sub>3</sub>):  $\nu_{\max}$  3400, 2922, 2851, 1628, 1601, 1556, 1544, 1510, 1461, 1443, 1413, 1360, 1297, 1299, 1266, 1151 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.96 (q,  $J$  = 15.8 Hz, 2H, CH), 7.64 (t,  $J$  = 7.1 Hz, 1H, CH), 7.53 (d,  $J$  = 2.2 Hz, 1H, OCH=CH), 7.38 (m, 1H, CH), 7.17 (m, 2H, CH), 6.89 (d,  $J$  = 4.0 Hz, 1H, OCH=CH); <sup>13</sup>C

NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 194.8 (C=O), 162.8 (d,  $^1J_{CF}$  = 246 Hz), 160.7, 153.3, 152.1, 150.9, 144.1, 136.0, 131.8 (d,  $^3J_{CF}$  = 8.75 Hz), 129.8 (d,  $^4J_{CF}$  = 3.7 Hz), 129.6 (d,  $^3J_{CF}$  = 6.0 Hz), 124.5 (d,  $^4J_{CF}$  = 3.7 Hz), 123.3, 116.4 (d,  $^2J_{CF}$  = 21.2 Hz), 112.5, 111.7, 105.3, 61.8, 61.0; HR-ESIMS:  $m/z$  432.0300 [M+H]<sup>+</sup> calcd for C<sub>20</sub>H<sub>17</sub>BrO<sub>6</sub> + H<sup>+</sup> (432.0281).<sup>26</sup>

*3-(Anthracen-2-yl)-1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)prop-2-en-1-one* (**3v**): orange crystals; HPLC:  $t_R$  = 7.4 min (100% purity); yield: 52%; m.p. 156-158 °C; IR (CHCl<sub>3</sub>):  $\nu_{max}$  3419, 2920, 2850, 2103, 1632, 1569, 1442, 1408, 1359, 1215, 1146, 1065 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 8.82 (d,  $J$  = 15.8 Hz, 1H, CH), 8.49 (s, 1H, CH), 8.40 (d,  $J$  = 8.4 Hz, 2H, CH), 8.05 (d,  $J$  = 7.4 Hz, 2H, CH), 7.76 (d,  $J$  = 15.8 Hz, 1H, CH), 7.53 (m, 5H, CH), 6.87 (d,  $J$  = 2.2 Hz, 1H, OCH=CH), 4.11 (s, 3H, OMe), 4.07 (s, 3H, OMe); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 194.3 (C=O), 153.3, 152.3, 150.9, 144.0, 140.2, 135.9, 131.3, 130.3, 129.7, 129.2, 128.9, 128.9, 128.4, 128.4, 128.3, 126.3, 126.3, 125.4, 126.4, 111.7, 111.5, 105.5, 61.4, 61.1; HR-ESIMS:  $m/z$  425.1374 [M+H]<sup>+</sup> calcd for C<sub>27</sub>H<sub>20</sub>O<sub>5</sub> + H<sup>+</sup> (425.1383).

**Synthesis of furanoflavonones 4a-p.** Khellinone (100 mg, 0.423 mmol) was treated with different aldehydes (1.2 equiv.) in the presence of catalytic amount of piperidine in ethanol (2 ml) at 70 °C for 12 hrs. The solvent was evaporated on rotary evaporator and the remaining mixture was diluted with water and dichloromethane. The organic layer was separated, dried over anhydrous sodium sulphate and concentrated to dryness on rotary evaporator. The obtained crude product was purified using silica gel column chromatography (5-40%) to yield furanoflavonones **4a-p** in 51-68% yield.

*4,9-Dimethoxy-7-phenyl-6,7-dihydro-5H-furo[3,2-g]chromen-5-one* (**4a**): yellow powder; HPLC:  $t_R$  = 4.1 min (100% purity); yield: 65%; m.p. 148-150 °C; IR (CHCl<sub>3</sub>):  $\nu_{max}$  3444, 3021, 2932, 2851, 2347, 2072, 1682, 1614, 1542, 1477, 1435, 1351, 1278, 1163, 1066, 1029 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.52 (d,  $J$  = 2.2 Hz, 1H, OCH=CH), 7.50 (m, 2H, CH), 6.93 (d,  $J$  = 2.2 Hz, 1H, OCH=CH), 5.49 (dd,  $J$  = 12.3, 3.0 Hz, 1H, CH), 4.08 (s, 3H, OMe), 4.06 (s, 3H, OMe), 3.09 (dd,  $J$  = 16.7, 12.4 Hz, 1H), 2.94 (dd,  $J$  = 16.7, 3.1 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 190.7 (C=O), 152.0 (C-9a),

151.4 (C-4), 150.0 (C-8a), 144.4 (OCH=CH), 138.8 (C-1'), 130.0 (C-9), 128.8 (Ar-3', Ar-5'), 128.5 (Ar-4), 126.0 (Ar-2', Ar-6'), 115.6 (C-4a), 111.4 (C-3a), 105.6 (OCH=CH), 79.3 (C-7), 61.5 (OMe), 61.4 (OMe), 46.1 (C-6); HR-ESIMS:  $m/z$  325.1072 [M+H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>16</sub>O<sub>5</sub> + H<sup>+</sup> (325.1070).

*7-(2-Bromophenyl)-4,9-dimethoxy-6,7-dihydro-5H-furo[3,2-g]chromen-5-one (4b)*: yellow powder; HPLC:  $t_R$  = 4.3 min (100% purity); yield: 63%; m.p. 146-148 °C; IR (CHCl<sub>3</sub>):  $\nu_{\max}$  3708, 3418, 2922, 2850, 2400, 2095, 1659, 1644, 1477, 1435, 1349, 1302 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.68 (d,  $J$  = 1.4 Hz, 1H, CH), 7.53 (d,  $J$  = 2.3 Hz, 1H, OCH=CH), 7.51 (m, 1H, CH), 7.42 (d,  $J$  = 7.8 Hz, 1H, CH), 7.29 (d,  $J$  = 8.0 Hz, 1H, CH), 6.94 (d,  $J$  = 2.3 Hz, 1H, OCH=CH), 5.45 (dd,  $J$  = 12.2, 3.2 Hz, 1H, CH), 4.08 (s, 3H, OMe), 4.06 (s, 3H, OMe), 3.04 (dd,  $J$  = 12.2, 1.3 Hz, 1H), 2.94 (dd,  $J$  = 6.7, 3.3 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 190.7 (C=O), 152.0 (C-9a), 151.0 (C-4), 150.0 (C-8a), 144.5 (OCH=CH), 141.0 (C-1'), 131.7 (C-3'), 130.4 (C-4'), 130.0 (C-9), 129.1 (C-5'), 124.6 (C-6'), 122.9 (C-2'), 115.7 (C-4a), 111.2 (C-3a), 106.7 (OCH=CH), 78.4 (C-7), 61.4 (OMe), 61.4 (OMe), 46.0 (C-6); HR-ESIMS:  $m/z$  403.0170 [M+H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>15</sub>BrO<sub>5</sub> + H<sup>+</sup> (403.1750).

*7-(Benzo[d][1,3]dioxol-5-yl)-4,9-dimethoxy-6,7-dihydro-5H-furo[3,2-g]chromen-5-one (4c)*: yellow powder; HPLC:  $t_R$  = 3.5 min (100% purity); yield: 60%; m.p. 150-154 °C; IR (CHCl<sub>3</sub>):  $\nu_{\max}$  3743, 3584, 3415, 2922, 2850, 1681, 1614, 1542, 1504, 1491, 1477, 1445, 1390, 1349, 1300, 1277 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.52 (d,  $J$  = 2.2 Hz, 1H, OCH=CH), 7.02 (d,  $J$  = 1.4 Hz, 1H, CH), 6.94 (d,  $J$  = 2.3 Hz, 1H, OCH=CH), 5.99 (s, 2H, CH<sub>2</sub>), 5.39 (dd,  $J$  = 12.2, 3.2 Hz, 1H, CH), 4.07 (s, 3H, OMe), 4.04 (s, 3H, OMe), 3.06 (dd,  $J$  = 16.7, 12.3 Hz, 1H), 2.89 (dd,  $J$  = 16.7, 3.1 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 190.7 (C=O), 152.0 (C-9a), 151.3 (C-4), 150.0 (C-8a), 148.0 (C-3'), 147.8 (C-4'), 144.4 (OCH=CH), 132.6 (C-9), 129.9 (C-1'), 119.9 (C-6'), 115.5 (C-4a), 111.2 (C-3a), 108.3 (C-5'), 106 (C-2'), 105.6 (OCH=CH), 101.3 (CH<sub>2</sub>), 79.2 (C-7), 61.5 (OMe), 61.4 (OMe), 46.1 (C-6); HR-ESIMS:  $m/z$  369.0968 [M+H]<sup>+</sup> calcd for C<sub>20</sub>H<sub>16</sub>O<sub>7</sub> + H<sup>+</sup> (369.0968).

*7-(5-Bromo-2-methoxyphenyl)-4,9-dimethoxy-6,7-dihydro-5H-furo[3,2-g]chromen-5-one (4d)*: yellow powder; HPLC:  $t_R$  = 4.4 min (100% purity); yield: 66%; m.p. 178-180 °C; IR (CHCl<sub>3</sub>):  $\nu_{\max}$

3743, 3584, 3415, 2922, 2850, 1681, 1614, 1542, 1504, 1491, 1477, 1445, 1390, 1349, 1300, 1277  $\text{cm}^{-1}$ ;  
 $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 7.79 (d,  $J = 2.0$  Hz, 1H, CH), 7.53 (d,  $J = 2.3$  Hz, 1H,  $\text{OCH}=\text{CH}$ ),  
7.43 (dd,  $J = 8.7, 2.5$  Hz, 1H, CH), 6.94 (d,  $J = 2.3$  Hz, 1H,  $\text{OCH}=\text{CH}$ ), 6.79 (d,  $J = 8.8$  Hz, 1H, CH),  
5.73 (dd,  $J = 12.9, 2.7$  Hz, 1H, CH), 4.09 (s, 3H, OMe), 4.07 (s, 3H, OMe), 3.82 (OMe), 2.99 (dd,  $J =$   
16.8, 2.9 Hz, 1H), 2.83 (dd,  $J = 16.8, 12.9$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 190.8  
(C=O), 154.8 (C-2'), 151.9 (C-9a), 151.6 (C-4), 150.1 (C-8a), 144.4 ( $\text{OCH}=\text{CH}$ ), 131.8 (C-4'), 130.1  
(C-9), 129.8 (C-1'), 129.6 (C-6'), 115.8 (C-4a), 113.3 (C-3a), 112.2 (C-3'), 111.4 (C-5'), 105.6  
( $\text{OCH}=\text{CH}$ ), 74.2 (C-7), 61.5 (OMe), 61.4 (OMe), 45.1 (C-6); HR-ESIMS:  $m/z$  433.0274  $[\text{M}+\text{H}]^+$  calcd  
for  $\text{C}_{20}\text{H}_{17}\text{O}_7 + \text{H}^+$  (433.0281).

*4,9-Dimethoxy-7-(pyridin-3-yl)-6,7-dihydro-5H-furo[3,2-g]chromen-5-one (4e)*: yellow powder;  
HPLC:  $t_R = 3.3$  min (100% purity); yield: 65%; m.p. 149-151  $^\circ\text{C}$ ; IR ( $\text{CHCl}_3$ ):  $\nu_{\text{max}}$  3584, 3380, 3124,  
3074, 2937, 2850, 1668, 1599, 1479, 1547, 1437, 1344, 1392, 1285, 1078  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  
 $\text{CDCl}_3$ ):  $\delta$  (ppm) 8.78 (s, 1H, CH), 8.65 (d,  $J = 4.6$  Hz, 1H, CH), 7.86 (d,  $J = 7.9$  Hz, 1H, CH), 7.54 (d,  
 $J = 2.1$  Hz, 1H,  $\text{OCH}=\text{CH}$ ), 7.38 (dd,  $J = 7.8, 4.8$  Hz, 1H, CH), 6.95 (d,  $J = 2.1$  Hz, 1H,  $\text{OCH}=\text{CH}$ ),  
5.54 (dd,  $J = 12.3, 3.1$  Hz, 1H, CH), 4.09 (s, 3H, OMe), 4.06 (s, 3H, OMe), 3.10 (dd,  $J = 16.6, 12.4$  Hz,  
1H), 2.97 (dd,  $J = 16.7, 3.2$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 190.8 (C=O), 152.0 (C-9a),  
150.9 (C-4), 150.1 (C-8a), 150.0 (C-6'), 147.8 (C-4'), 144.5 ( $\text{OCH}=\text{CH}$ ), 134.3 (C-9), 133.6 (C-2'),  
130.0 (C-1'), 123.6 (C-3'), 115.8 (C-4a), 111.2 (C-3a), 106.6 ( $\text{OCH}=\text{CH}$ ), 77.3 (C-7), 61.4 (OMe), 61.3  
(OMe), 45.6 (C-6); HR-ESIMS:  $m/z$  326.1021  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{18}\text{H}_{16}\text{NO}_5 + \text{H}^+$  (325.1023).

*4,9-Dimethoxy-7-(thiophen-3-yl)-6,7-dihydro-5H-furo[3,2-g]chromen-5-one (4f)*: yellow powder;  
HPLC:  $t_R = 5.3$  min (89% purity); yield: 66%; m.p. 159-161  $^\circ\text{C}$ ; IR ( $\text{CHCl}_3$ ):  $\nu_{\text{max}}$  3428, 1927, 2851,  
2079, 1673, 1651, 1620, 1477, 1542, 1435, 1348, 1300, 1216  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$   
(ppm) 7.52 (d,  $J = 2.2$  Hz, 1H,  $\text{OCH}=\text{CH}$ ), 7.37 (m, 2H, CH), 7.21 (dd,  $J = 4.9, 1.3$  Hz, 1H, CH), 6.92  
(d,  $J = 2.3$  Hz, 1H,  $\text{OCH}=\text{CH}$ ), 5.60 (dd,  $J = 10.3, 4.1$  Hz, 1H, CH), 4.07 (s, 3H, OMe), 4.06 (s, 3H,  
OMe), 3.12 (dd,  $J = 16.7, 10.3$  Hz, 1H), 3.04 (dd,  $J = 16.7, 4.1$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):

$\delta$  (ppm) 190.5 (C=O), 152.0 (C-9a), 151.0 (C-4), 150.0 (C-8a), 144.4 (OCH=CH), 139.8 (C-1'), 128.8 (C-9), 126.7 (C-3'), 125.8 (C-2'), 122.6 (C-5'), 115.5 (C-4a), 111.4 (C-3a), 106.7 (OCH=CH), 75.4 (C-7), 61.4 (OMe), 61.4 (OMe), 45.1 (C-6); HR-ESIMS:  $m/z$  331.0643 [M+H]<sup>+</sup> calcd for C<sub>17</sub>H<sub>14</sub>O<sub>5</sub>S+ H<sup>+</sup> (331.0634).

*4,9-Dimethoxy-7-(4-nitrophenyl)-6,7-dihydro-5H-furo[3,2-g]chromen-5-one* (**4g**): yellow powder; HPLC:  $t_R$  = 3.6 min (100% purity); yield: 61%; m.p. 180-181 °C; IR (CHCl<sub>3</sub>):  $\nu_{max}$  3745, 3383, 2850, 2918, 1734, 1702, 1604, 1519, 1503, 1467, 1345, 1273, 1213, 1149, 1066 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 8.10 (d,  $J$  = 8.7 Hz, 2H, CH), 7.56-7.49 (dd,  $J$  = 7.6, 5.5 Hz, 2H, CH), 6.91 (d,  $J$  = 2.3 Hz, 1H, OCH=CH), 4.88 (dd,  $J$  = 6.5, 4.4 Hz, 1H, CH), 4.12 (s, 3H, OMe), 4.06 (s, 3H, OMe), 3.47 (dd,  $J$  = 16.2, 4.0 Hz, 1H), 3.04 (dd,  $J$  = 14.6, 6.6 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 196.5 (C=O), 159.6 (C-9a), 154.7 (C-4), 147.1 (C-1'), 146.9 (C-4'), 144.5 (OCH=CH), 143.1 (C-8a), 130.6 (C-2', C-6'), 127.7 (C-9), 123.5 (C-3', C-5'), 113.8 (C-4a), 108.3 (C-3a), 106.1 (OCH=CH), 85.4 (C-7), 61.2 (OMe), 60.8 (OMe), 37.1 (C-6); HR-ESIMS:  $m/z$  370.0933 [M+H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>15</sub>NO<sub>7</sub>+ H<sup>+</sup> (370.0931).

*7-(2-Chlorophenyl)-4,9-dimethoxy-6,7-dihydro-5H-furo[3,2-g]chromen-5-one* (**4h**): yellow powder; HPLC:  $t_R$  = 4.4 min (100% purity); yield: 65%; m.p. 142-143 °C; IR (CHCl<sub>3</sub>):  $\nu_{max}$  3625, 3584, 3441, 2934, 2849, 2079, 1682, 1651, 1542, 1435, 1391, 1303, 1278 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.67 (t,  $J$  = 7.3 Hz, 1H, CH), 7.54 (d,  $J$  = 2.1 Hz, 1H, OCH=CH), 7.40-7.34 (m, 1H, CH), 7.25 (dd,  $J$  = 12.2, 4.4 Hz, 1H, CH), 7.15-7.08 (m, 1H, CH), 6.95 (d,  $J$  = 2.1 Hz, 1H, OCH=CH), 5.77 (dd,  $J$  = 12.5, 3.0 Hz, 1H), 4.12 (s, 3H, OMe), 4.06 (s, 3H, OMe), 3.07 (dd,  $J$  = 16.7, 12.6 Hz, 1H), 2.96 (dd,  $J$  = 16.8, 3.2 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 190.3 (C=O), 152.0 (C-9a), 151.4 (C-4), 150.2 (C-8a), 144.5 (OCH=CH), 136.7 (C-1'), 131.7 (C-2'), 130.0 (C-9), 129.7 (C-3'), 129.5 (C-4'), 127.4 (C-6'), 127.1 (C-5'), 115.7 (C-4a), 111.3 (C-3a), 105.7 (OCH=CH), 77.5 (C-7), 61.5 (OMe), 61.5 (OMe), 44.9 (C-6); HR-ESIMS:  $m/z$  359.0670 [M+H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>15</sub>ClO<sub>5</sub>+ H<sup>+</sup> (359.0675).

(2,4-Dichlorophenyl)-4,9-dimethoxy-6,7-dihydro-5H-furo[3,2-g]chromen-5-one (**4i**): yellow powder; HPLC:  $t_R = 4.5$  min (100% purity); yield: 64%; m.p. 123-125 °C; IR (CHCl<sub>3</sub>):  $\nu_{\max}$  3584, 3354, 3019, 2930, 2850, 1683, 1614, 1543, 1479, 1485, 1348, 1278, 1165, 1130, 1102, 1018 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.79 (d,  $J = 8.4$  Hz, 1H, CH), 7.55 (d,  $J = 2.1$  Hz, 1H, OCH=CH), 7.44 (m, 1H, CH), 7.38 (dd,  $J = 8.4, 2.1$  Hz, 1H, CH), 6.96 (d,  $J = 2.3$  Hz, 1H, OCH=CH), 5.78 (dd,  $J = 13.0, 2.8$  Hz, 1H, CH), 4.11 (s, 3H, OMe), 4.05 (s, 3H, OMe), 3.03 (dd,  $J = 16.8, 2.9$  Hz, 1H), 2.86 (dd,  $J = 16.8, 13.0$  Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 189.9 (C=O), 152.0 (C-9a), 151.1 (C-4), 150.2 (C-8a), 144.6 (OCH=CH), 135.3 (C-9), 134.8 (C-1'), 132.3 (C-4'), 130.1 (C-2'), 129.5 (C-3'), 128.1 (C-6'), 127.8 (C-5'), 115.8 (C-4a), 111.1 (C-3a), 105.7 (OCH=CH), 76.0 (C-7), 61.5 (OMe), 61.5 (OMe), 44.8 (C-6); HR-ESIMS:  $m/z$  393.0282 [M+H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>15</sub>Cl<sub>2</sub>O<sub>5</sub> + H<sup>+</sup> (393.0291).

4,9-Dimethoxy-7-(naphthalen-2-yl)-6,7-dihydro-5H-furo[3,2-g]chromen-5-one (**4j**): yellow powder; HPLC:  $t_R = 5.2$  min (100% purity); yield: 60%; m.p. 143-145 °C; IR (CHCl<sub>3</sub>):  $\nu_{\max}$  3418, 2925, 2852, 2085, 1680, 1616, 1545, 1497, 1436, 1390, 1302, 1249, 1126, 1018 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.79 (m, 4H, CH), 7.63 (d,  $J = 8.5$  Hz, 1H, OCH=CH), 7.52 (m, 3H, CH), 6.94 (d,  $J = 2.2$  Hz, 1H, OCH=CH), 5.66 (dd,  $J = 16.7, 12.2$  Hz, 1H, CH), 4.09 (s, 3H, OMe), 4.07 (s, 3H, OMe), 3.20 (dd,  $J = 16.7, 12.2$  Hz, 1H), 3.04 (dd,  $J = 16.7, 3.1$  Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 190.7 (C=O), 152.1 (C-9a), 151.4 (C-4), 150.1 (C-8a), 144.4 (OCH=CH), 136.1 (C-9), 133.8 (C-8'), 133.1 (C-3'), 130.0 (C-1'), 128.7 (C-9'), 128.2 (C-10'), 127.7 (C-4', C-7'), 115.6 (C-3a), 111.4 (C-4a), 106.7 (OCH=CH), 79.4 (C-7), 61.5 (OMe), 61.4 (OMe), 46.0 (C-6), HR-ESIMS:  $m/z$  375.1230 [M+H]<sup>+</sup> calcd for C<sub>23</sub>H<sub>18</sub>O<sub>5</sub> + H<sup>+</sup> (375.1227).

7-(2,6-Dichlorophenyl)-4,9-dimethoxy-6,7-dihydro-5H-furo[3,2-g]chromen-5-one (**4k**): yellow powder; HPLC:  $t_R = 4.4$  min (100% purity); yield: 65%; m.p. 143-145 °C; IR (CHCl<sub>3</sub>):  $\nu_{\max}$  3444, 3011, 2936, 2848, 2111, 1682, 1614, 1581, 1564, 1477, 1434, 1376, 1348, 1277, 1247, 1211 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.53 (d,  $J = 2.2$  Hz, 1H, OCH=CH), 7.39 (d,  $J = 8.0$  Hz, 2H, CH), 7.28 (m, 1H, CH), 6.95 (d,  $J = 2.2$  Hz, 1H, OCH=CH), 6.25 (dd,  $J = 14.4, 3.1$  Hz, 1H, CH), 4.11 (s,

3H, OMe), 4.03 (s, 3H, OMe), 3.77 (dd,  $J = 16.8, 14.5$  Hz, 1H), 2.69 (dd,  $J = 16.8, 3.1$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 190.1 (C=O), 152.3 (C-9a), 151.5 (C-4), 150.3 (C-8a), 144.5 (OCH=CH), 135.1 (C-9), 134.8 (C-1'), 132.5 (C-2', C-6'), 130.2 (C-3'), 129.7 (C-4'), 128.9 (C-5'), 115.4 (C-3a), 111.0 (C-4a), 105.7 (OCH=CH), 75.9 (C-7), 61.9 (OMe), 61.5 (OMe), 41.0 (C-6); HR-ESIMS:  $m/z$  393.0331  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{19}\text{H}_{14}\text{Cl}_2\text{O}_5 + \text{H}^+$  (393.0291).

7-(3-Bromo-4-fluorophenyl)-4,9-dimethoxy-6,7-dihydro-5H-furo[3,2-g]chromen-5-one (**4l**): yellow powder; HPLC:  $t_{\text{R}} = 4.1$  min (95% purity); yield: 68%; m.p. 140-143 °C; IR ( $\text{CHCl}_3$ ):  $\nu_{\text{max}}$  3429, 2933, 2850, 2091, 1681, 1614, 1544, 1498, 1478, 1435, 1417, 1391, 1349, 1278, 1249, 1067  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 7.73 (dd,  $J = 6.4, 2.0$  Hz, 1H, CH), 7.54 (d,  $J = 2.2$  Hz, 1H, OCH=CH), 7.44-7.39 (m, 1H, CH), 7.18 (t,  $J = 8.4$  Hz, 1H, CH), 6.95 (d,  $J = 2.2$  Hz, 1H, OCH=CH), 5.43 (dd,  $J = 12.1, 3.1$  Hz, 1H, CH), 4.08 (s, 3H, OMe), 4.06 (s, 3H, OMe), 3.03 (dd,  $J = 16.7, 12.1$  Hz, 1H), 2.92 (dd,  $J = 16.7, 3.3$  Hz, 1H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 189.9 (C=O), 160.0 (d,  $^1J_{\text{CF}} = 246$  Hz, C-4'), 158.0 (C-9a), 152.4 (C-4), 150.8 (C-8a), 150.1 (C-9), 144.6 (OCH=CH), 136.2 (d,  $^4J_{\text{CF}} = 3.7$  Hz, CH-1'), 131.4 (C-2'), 129.9 (C-3a), 126.8 (d,  $^3J_{\text{CF}} = 7.2$  Hz, CH-6'), 116.9 (CH-5'), 111.1 (C-4a), 109.6 (d,  $^2J_{\text{CF}} = 21.2$  Hz, C-3'), 106.7 (OCH=CH), 77.9 (C-7), 61.4 (OMe), 61.4 (OMe), 45.9 (C-6); HR-ESIMS:  $m/z$  423.0060  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{19}\text{H}_{14}\text{BrFO}_5 + \text{H}^+$  (423.0060).

7-(2-Fluorophenyl)-4,9-dimethoxy-6,7-dihydro-5H-furo[3,2-g]chromen-5-one (**4m**): yellow powder; HPLC:  $t_{\text{R}} = 3.7$  min (93% purity); yield: 64%; m.p. 142-145 °C; IR ( $\text{CHCl}_3$ ):  $\nu_{\text{max}}$  3584, 3360, 3126, 3070, 2938, 2843, 1687, 1682, 1615, 1543, 1505, 1478, 1459, 1436, 1349, 1302, 1278, 1252, 1233, 1165, 1130, 1067  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 7.67 (t,  $J = 7.3$  Hz, 1H, CH), 7.54 (d,  $J = 2.1$  Hz, 1H, OCH=CH), 7.37 (dd,  $J = 13.3, 6.0$  Hz, 1H, CH), 7.25 (dd, 1H,  $J = 12.2, 4.4$  Hz, CH), 7.15-7.08 (m, 1H, CH), 6.95 (d,  $J = 2.1$  Hz, 1H, OCH=CH), 5.77 (dd,  $J = 12.5, 3.0$  Hz, 1H, CH), 4.10 (s, 3H, OMe), 4.05 (s, 3H, OMe), 3.07 (dd,  $J = 16.7, 12.6$  Hz, 1H), 2.96 (dd,  $J = 16.8, 3.2$  Hz, 1H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 190.4 (C=O), 160.7 (d,  $^1J_{\text{CF}} = 246$  Hz, C-2'), 158.7 (C-9a), 152.1 (C-4), 151.4 (C-8a), 150.2 (C-9), 144.5 (OCH=CH), 130.2 (d,  $^3J_{\text{CF}} = 8.7$  Hz, CH-4'), 130.0 (C-3a), 127.4

(CH-6'), 126.2 (CH-1'), 124.6 (d,  $^4J_{CF} = 3.7$  Hz CH-5'), 115.8 (d,  $^2J_{CF} = 21.2$  Hz, CH-3'), 111.1 (C-4a), 105.7 (OCH=CH), 73.8 (CH-7), 61.5 (OMe), 61.5 (OMe), 45.1 (C-6); HR-ESIMS:  $m/z$  343.0990 [M+H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>15</sub>FO<sub>5</sub>+ H<sup>+</sup> (343.0976).

*7-(4-Fluorophenyl)-4,9-dimethoxy-6,7-dihydro-5H-furo[3,2-g]chromen-5-one (4n)*: yellow powder; HPLC:  $t_R = 4.4$  min (100% purity); yield: 61%; m.p. 142-145 °C; IR (CHCl<sub>3</sub>):  $\nu_{max}$  3744, 3397, 2920, 2351, 1683, 1575, 1512, 1477, 1414, 1348, 1301, 1226, 1126, 1065 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.53 (d,  $J = 2.2$  Hz, 1H, OCH=CH), 7.49 (dd,  $J = 8.5, 5.4$  Hz, 2H, CH), 7.12 (t,  $J = 8.6$  Hz, 1H, CH), 6.94 (d,  $J = 2.2$  Hz, 1H, OCH=CH), 5.47 (dd,  $J = 12.3, 2.9$  Hz, 1H, CH), 4.09 (s, 3H, OMe), 4.05 (s, 3H, OMe), 3.07 (dd,  $J = 16.7, 12.3$  Hz, 1H), 2.93 (dd,  $J = 16.7, 3.2$  Hz, 1H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 190.5 (C=O), 163.7 (d,  $^1J_{CF} = 246$  Hz, C-4'), 161.7 (C-9a), 152.0 (C-4), 151.2 (C-8a), 150.0 (C-9), 144.5 (OCH=CH), 134.6 (CH-1'), 127.9 (d,  $^3J_{CF} = 8.7$  Hz, C-2', C-6'), 115.8 (d,  $^2J_{CF} = 20.0$  Hz, C-3', C-5'), 115.6 (C-3a), 111.2 (C-4a), 105.6 (OCH=CH), 78.6 (C-7), 61.4 (OMe), 61.4 (OMe), 45.9 (C-6); HR-ESIMS:  $m/z$  343.0990 [M+H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>15</sub>FO<sub>5</sub>+H<sup>+</sup> (343.0976).

*(E)-6-Benzylidene-4,9-dimethoxy-7-phenyl-6,7-dihydro-5H-furo[3,2-g]chromen-5-one (4o)*: yellow powder; HPLC:  $t_R = 3.7$  min (87% purity); yield: 51%; m.p. 159-161 °C; IR (CHCl<sub>3</sub>):  $\nu_{max}$  3585, 3415, 2922, 2850, 2094, 1641, 1619, 1475, 1437, 1348, 1277, 1126, cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 8.09 (s, 1H, CH), 7.98 (d,  $J = 2.1$  Hz, 1H, CH), 7.50 (m, 5H, CH), 7.44 (t,  $J = 6.9$  Hz, 4H), 7.38 (d,  $J = 7.1$  Hz, 1H, CH), 7.18 (d,  $J = 2.1$  Hz, 1H, CH), 6.76 (s, 1H, CH), 4.00 (s, 3H, OMe), 3.94 (s, 3H, OMe); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 180.0, 151.2, 149.8, 147.6, 145.4, 138.0, 137.1, 133.3, 132.5, 130.1, 129.9, 129.8, 129.0, 128.9, 127.2, 115.3, 111.7, 105.7, 77.2, 61.1, 60.8; HR-ESIMS:  $m/z$  413.1383 [M+H]<sup>+</sup> calcd for C<sub>26</sub>H<sub>20</sub>O<sub>5</sub>+ H<sup>+</sup> (413.1383).

*(Z)-4,9-Dimethoxy-6-(thiophen-2-ylmethylene)-7-(thiophen-3-yl)-6,7-dihydro-5H-furo[3,2-g]chromen-5-one (4p)*: yellow powder; HPLC:  $t_R = 4.6$  min (95% purity); yield: 55%; m.p. 148-150 °C; IR (CHCl<sub>3</sub>):  $\nu_{max}$  3429, 2933, 2850, 2091, 1681, 1614, 1544, 1498, 1478, 1435, 1417, 1391, 1349, 1278, 1249, 1067 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.94-7.91 (m, 2H, CH), 7.86 (d,  $J = 1.9$



Hz, 1H, CH), 7.67 (dd,  $J = 4.9, 2.9$  Hz, 1H, CH), 7.53 (d,  $J = 2.9$  Hz, 1H, OCH=CH), 7.38-7.34 (m, 1H, CH), 7.24 (d,  $J = 5.0$  Hz, 1H, CH), 7.18 (d,  $J = 5.0$  Hz, 1H, CH), 7.13 (d,  $J = 2.2$  Hz, 1H, 1H), 6.83 (s, 1H, CH), 3.96 (s, 3H, OMe), 3.86 (s, 3H, OMe);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 180.8 (C=O), 151.6, 150.2, 148.4, 144.1, 139.1, 135.8, 132.1, 131.0, 130.8, 129.1, 128.6, 127.0, 126.8, 126.7, 124.7, 116.5, 112.7, 105.5, 75.1, 61.5, 61.4; HR-ESIMS:  $m/z$  425.0495  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{22}\text{H}_{16}\text{O}_5\text{S}_2+\text{H}^+$  (425.0511).

**CYP screening and protection from B[a]P toxicity.** The activity of test compounds for inhibition of CYP enzymes was investigated using Sacchrosomes<sup>TM</sup> and recombinant human HEK293 cells using protocols, as described in our earlier publications.<sup>10, 12</sup> The protection from B[a]P toxicity in CYP1A1-expressing normal adherent HEK293 human cells was studied as per protocols described in our earlier publications.<sup>10, 12</sup>

For  $\text{IC}_{50}$  determination for CYP inhibition, the enzyme inhibition was plotted using sigmoidal curve (4 parameter variable slope equation) and half-inhibitory concentration ( $\text{IC}_{50}$ ) values were analyzed statistically using GraphPad Prism Software (Version 6.0). The  $\text{IC}_{50}$  values of CYP inhibitors were determined by fitting a non-linear curve of uninhibited fraction with incubation concentration of the inhibitors, using the Hill equation. The  $\text{EC}_{50}$  values for B[a]P toxicity were determined by plotting dose-response curve of cell viability *versus* log concentration of B[a]P or B[a]P + test compound.  $\text{EC}_{50}$  values were obtained using GraphPad Prism Software (Version 6.0), indicating the concentration of B[a]P required for 50% reduction in cell viability.

**Profiling of khellinochalcones on a panel of 12 humanized CYP450 enzymes.**  $\text{IC}_{50}$  values of furanochalcone **3g** were obtained by kinetic microplate assay using 15 minutes reaction time. Recombinant human P450s from a *Saccharomyces cerevisiae* microsome expression system supplied from CYP Design was used. Each 100  $\mu\text{l}$  reaction was performed in 100 mM potassium phosphate buffer, pH 7.4 and with a NADPH regenerating system (final well concentrations 0.04 units of glucose-6-phosphate dehydrogenase, 1.3 mM  $\text{NADP}^+$ , 3.3 mM glucose-6-phosphate, 3.3 mM  $\text{MgCl}_2$  and 50  $\mu\text{M}$

tribasic sodium citrate). Test samples were serially diluted in 10% DMSO in decreasing final well concentrations from 20  $\mu$ M downwards/ 0.5 % DMSO. CYP2E1 is sensitive to solvents and so test samples were diluted in 1% DMSO in decreasing final well concentrations from 10  $\mu$ M downwards/ 0.5 % DMSO. The assay was initiated by adding substrate and the NADPH regenerating system. P450 content and substrate concentrations were pre-optimised.<sup>12</sup>

**In vitro cell proliferation assays and flow cytometric analyses.** They were performed as described earlier.<sup>28</sup> Control cells (i.e. in the absence of any compound) and cells (i.e. treated with compound) cells were harvested after treatment with trypsin. Cells were fixed in chilled (-20°C) 70% ethanol for an hour, after washing once with PBS. Fixed cells were centrifuged at room temperature. Pellets were re-suspended in PBS, in the presence of DNase-free ribonuclease (0.5 mg/mL; Sigma-Aldrich # R-5503) before staining with a propidium iodide solution (50  $\mu$ g/mL; Sigma-Aldrich, # P-4170) for an hour (in the dark) at 4 °C. Cell cycle analysis was performed on the Beckman Coulter (Epics Altra) fluorescence-activated cell sorter (Beckman Coulter UK Ltd). To exclude cell doublets or cell clumps, all events that represent single cells were gated. Cytograms of the fluorescence peak of propidium iodide were plotted against the integrated fluorescence/linear signal. Data points on a straight line, within a single gate, were isolated and the gated data was employed for plotting a histogram that represents a complete cell cycle. The total number of events did not exceed 200 events per second. Data acquisition was stopped after collection of around 10,000 events.

**In silico docking with CYP1A1.** The docking of identified CYP1A1 inhibitors with the crystal structure of CYP1A1 (PDB ID: 4I8V)<sup>18</sup> was performed using the protocol as described earlier.<sup>10, 12</sup>

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S.B. Bharate and B. Chaudhuri designed, executed and coordinated this whole study; R. Sharma performed synthesis of compounds; I.S. Williams, L. Gatchie and V.R. Sonawane performed all CYP screening; S.B. Bharate and B. Chaudhuri contributed to manuscript writing.

## **Funding**

This work was supported by CSIR 12<sup>th</sup> five year plan project (BSC-0205) and CYP Design Ltd.

**Notes.** The authors declare no competing financial interest.

**ACKNOWLEDGEMENTS.** Authors thank analytical department, IIIM for analytical support.

## **ASSOCIATED CONTENT.**

The Supporting Information is available free of charge via the Internet at <http://pubs.acs.org>.

- <sup>1</sup>H, <sup>13</sup>C, DEPT NMR, data scans of all compounds

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