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The scope of thallium nitrate oxidative cyclization of chalcones; synthesis and evaluation of isoflavone and aurone analogs for their inhibitory activity against interleukin-5

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ABSTRACT

The oxidative cyclization of 2′-hydroxy-6′-cyclohexylmethoxychalcones **5** using thallium (III) nitrate (TTN) in alcoholic solvents produced isoflavones **2** and (or) aurones **3** depending on the electronic nature of *p*-substituents on ring B. Chalcones with strong electron donating substituents (OH, OCH_3) were exclusively converted to isoflavones **2**. Chalcone with weak electron donating substituents (CH_2CH_3) was transformed into isoflavone **2** and the aurone **3** in approximate ratio 1:1. Chalcones with hydrogen or electron withdrawing substituents (Cl, CHO, $COOCH_3$, and NO_2) formed aurones **3**. Synthesized isoflavones **2** and aurones **3** were evaluated for their inhibitory activity against interleukin-5. Among them, 5-(cyclohexylmethoxy)-3-(3,4,5-trimethoxyphenyl)-4*H*-chromen-4-one (**2h**, >100% inhibition at 50 μM, IC_{50} = 6.1 μM) gave most potent activity. All the aurones **3** were inactive.

1. Introduction

Isoflavones are the naturally abundant compounds with remarkably diverse biological properties. They have been reported for antioxidant[1,2] antitumor,[3,4] anticataracts,[5] anti-inflammatory[6] and antifertility[7] activity. As isoflavones show various pharmacological properties, these compounds have been important target for the research.

Recently, a number of isoflavones have also been reported as inhibitors of interleukin-5 (IL-5),[8,9] which is a proven target for finding new therapeutics for eosinophilia-associated allergic inflammation.[10,11] Naturally occurring isoflavone, sophoricoside (**1**, 92.1% inhibition at 50 μM, IC_{50} = 1.4 μM, Fig. 1), show selective inhibitory activity against the bioactivity of IL-5.[12] Thus, we studied the structure–activity relationship (SAR) of isoflavonoid analogs.[8,9] In order to define the more detail structure–activity relationship of isoflavone analogs, various substitutions on ring B of isoflavone are required. However, their preparation has been hampered due to limitations of available synthetic methods.

The oxidative cyclization of chalcones by the treatment with the thallium (III) nitrate (TTN) has been considered as a standard procedure for the preparation of isoflavones **2**.[13–15] However, this reaction shows a great deal of diversity to form the different products such as **2**, **3** and **4** (Fig. 1).[13,16,17] Some findings indicate that the reaction pathway of cyclic oxidation depends on the nature of substituents present at the starting chalcones. They observed that 2′-hydroxychalcones substituted with chloro or nitro group at ring A on reaction with TTN gave aurone **3** through the corresponding (α-methoxybenzyl) coumaranones.[17,18] On contrary to

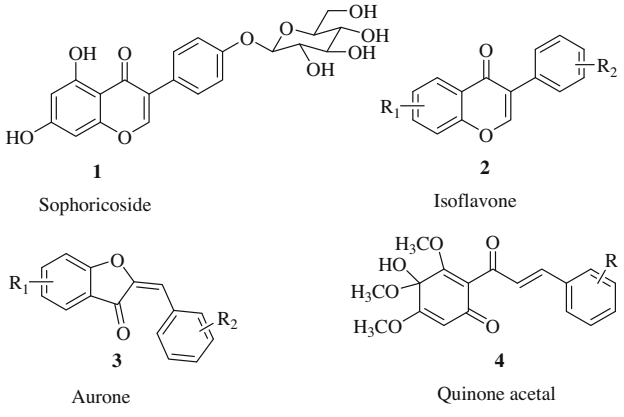

Figure 1. Structures of sophoricoside **1**, isoflavone **2**, aurone **3** and quinone acetal **4**.

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these studies, Thakkar and co-workers[19,20] claimed that the TTN oxidation of 2′-hydroxy-5′-methoxychalcones gave only thermodynamically stable *Z*-isomers of aurones **3** regardless of the presence of electron donating or withdrawing groups attached at *p*-position of ring B or substitutions on the starting chalcones. However, some researchers observed that TTN oxidation of a 2′-hydroxy-4′,5′,6′-trioxygenated chalcones in methanol gave quinone acetal **4** instead of isoflavones **2** and aurones **3** by the oxidation of ring A (Fig. 1).[16,21] In our previous work,[8,9] we have observed the formation of isoflavone analogs of **1** from chalcones with *p*-methoxymethoxy substituents using TTN oxidative cyclization for investigating their inhibitory activity against IL-5. In this regard, we examine the scope of this reaction based on the substituents of ring B of 2′-hydroxy-6′-cyclohexylmethoxychalcones as starting materials and the inhibitory activity of the synthesized compounds against IL-5.

2. Results and discussion

2.1. Synthesis and characterization of isoflavones 2 and aurones 3

In continuation of our previous work[8,9] on the modification of ring B of isoflavones **2**, we have synthesized a series of new isoflavone and aurone analogs starting from chalcones **5** prepared according to the literature.[22,23] Sequential treatments of compounds **5** with thallium (III) nitrate in methanol solvent at room temperature for 24 h and then heating at 65 °C followed by addition of hydrochloric acid in one pot (oxidative cyclization) afforded the corresponding isoflavones **2** or (and) aurones **3** (Scheme 1). This conversion has been known as a key step for the classical preparation of isoflavone.[24,25] This oxidative cyclization with **5b** provided **2a**, which was treated with methoxymethyl chloride to get 5-(cyclohexylmethoxy)-3-(4-(methoxymethoxy)phenyl)-4*H*-chromen-4-one **(2b)** with 80.5% yield as indicated in Table 1. The reaction with 2′-hydroxyl-6′-cyclohexylmethoxy-4′′-methoxychalcone (**5c**) only gave the desired isoflavone **2c**. Similarly, the preparation of other isoflavone derivatives with substituents on ring B such as 3,4-$(OCH_3)_2$ (**2d**), 2,4-$(OCH_3)_2$ (**2e**), 3-OCH_3, 4-CH_2Ph (**2f**) and 3,4,5-$(OCH_3)_3$ **(2h)** has been done. In these reactions, we observed only the formation of isoflavone analogs. However, presence of ethyl substituent at the *p*-position of 2′-hydroxy-6′-cyclohexylmethoxychalcone **5** led to formation of approximately 1:1 mixture of isoflavone **2i** and aurone **3i**. This result supported a previous finding of Cummins et al.[26] in which they observed that oxidative cyclization of 2′-hydroxy-6′-methoxychalcone yielded 4-methoxyaurone and 5-methoxyisoflavone (1:1). Further, on decreasing the electron density around ring B of 2′-hydroxy-6′-cyclohexylmethoxychalcone by introduction of H, Cl, CHO, $COOCH_3$ and NO_2 group we ended up having respective aurone derivatives **3j–n**. The ^1H NMR of the aurone derivatives shows that this oxidative cyclization procedure yields only *Z*-geometrical isomer, as reported in literatures.[20,27] These results are contradictory to another study of Thakkar and Cushman,[20] which showed only aurone formation regardless the presence of electron donating or withdrawing groups attached at the *p*-position of ring B. Interestingly, the chalcone **5o** substituted with carboxylic group did not undergo any reaction in this condition.

Table 1
The results of oxidative cyclization of chalcones **5**

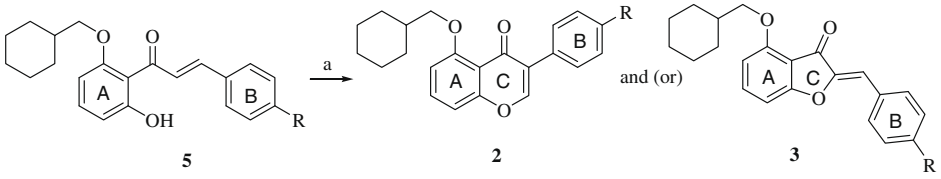
Starting material		Yield (%)		
Chalcone **5**	R	Chalcone **5** % (recovered)	Isoflavone **2** % (compd No.)	Aurone **3** % (compd No.)
5a	OH	0	61.5 (**2a**)[9]	0 (**3a**)
5b	OCH_2OCH_3	—	80.5 (**2b**)[a]	—
5c	OCH_3	0	48.6 (**2c**)	0 (**3c**)
5d	3,4-$(OCH_3)_2$	0	49.7 (**2d**)	0 (**3d**)
5e	2,4-$(OCH_3)_2$	0	44.3 (**2e**)	0 (**3e**)
5f	3-OCH_3, 4-OCH_2Ph	0	53.4 (**2f**)	0 (**3f**)
5g	3-OCH_3, 4-OH	—	98.1 (**2g**) [b]	—
5h	3,4,5-$(OCH_3)_3$	0	48.2 (**2h**)	0 (**3h**)
5i	CH_2CH_3	35.7	24.3 (**2i**)	21.7 (**3i**)
5j	H	38.2	0 (**2j**)	44.8 (**3j**)
5k	Cl	44.0	0 (**2k**)	38.7 (**3k**)
5l	CHO	40.1	0 (**2l**)	32.7 (**3l**)
5m	$COOCH_3$	50.1	0 (**2m**)	42.7 (**3m**)
5n	NO_2	0	0 (**2n**)	74.5 (**3n**)
5o	COOH	95.4	0	0

[a] The reaction was started with **5b** to give **2a** in 61.5% yield, which was then converted to **2c** by the treatment with methoxymethyl chloride in 80.5% yield.
[b] A product **2g** was obtained from **2f** by H_2/Pd-reduction.

The major products obtained from these reactions were separated by flash column chromatography and evaluated for their IL-5 inhibitory activity. The yield of above obtained compounds was moderate as shown in Table 1. Thus, the electronic nature of *p*-substituents on ring B of chalcone determines the product in this oxidative cyclization of 2′-hydroxy-6′-cyclohexylmethoxychalcones **5** using thallium (III) nitrate (TTN) in alcoholic solvent. From the above study it can be concluded that the strong electron donating substituents (OCH_2OCH_3, OCH_3) led to exclusive formation of isoflavones **2**, while the weak electron donating substituent (CH_2CH_3) proceeded to the formation of isoflavones **2** and the aurones **3** in approximate ratio 1:1. Only aurones **3** are produced with hydrogen or electron withdrawing substituents (Cl, CHO, $COOCH_3$, NO_2) of **5**.

2.2. Pharmacology

The measurement of inhibitory activity of IL-5 of these analogs was performed with the comparison of cell proliferation of Y16 cell with/without the samples.[12] An Y16 cell line originated from murine early B cell is proliferated in the presence of IL-5, which was used as the parameter of IL-5 bioactivity. Non-radioactive procedures that measure cell metabolism as an index of proliferation have now become popular. 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H538 tetrazolium sodium salt (WST-1)



Scheme 1. Synthesis of isoflavones **2** or (and) aurones **3**. Reagents and conditions: (a) TTN, methanol, overnight at room temperature; hydrochloric acid, 50 °C, 5 h. Substituents R and the ratio of formation of **2** and (or) **3** are listed in Table 1.

was used to form soluble formazan product on exposure to the dehydrogenase activity in metabolizing cells. Proliferation of Y16 cells in the presence of IL-5 was specifically blocked by treatment of polyclonal antibody against IL-5 or monoclonal antibody against IL-5Ra. Sample and IL-5 were simultaneously added to the Y16 cells, and the bioassay system can identify the IL-5 antagonist and its signal inhibitor. Data for % inhibition at 50 μM and IC_{50} values are mean values from three separate experiments as shown in Table 2.

2.3. Structure–activity relationship of 2 and 3 for the inhibition of IL-5

Previously we have synthesized a number of isoflavones for their IL-5 inhibitory activity (Table 2) and found that 4-hydroxyl on the ring B and benzyloxy (or cyclohexylmethoxy) group on the ring A play an important role in modulating inhibitory activity of IL-5.[8,9] The isoflavone **2a** (91.7% inhibition at 50 μM, IC_{50} = 5.8 μM) gave best inhibitory activity against IL-5.[9] This indicates that ring B of isoflavone analogs needs a strong electron donating group with hydrogen bonding acceptor property. For the confirmation of our point here, we studied the effect of electron donating and electron withdrawing groups on their IL-5 inhibitory activity. For that we first introduced methoxy group at *p*-position of ring B as in **2c** (15.4% inhibition at 50 μM, IC_{50} >30 μM). Surprisingly, the compound **2c** was very weak as compared to **2a** (Table 2). This implies that the small size of hydrogen bonding group like hydroxyl as well as a strong electron donating group at this position might be important factor in the activity. In continuation, the other derivatives such as **2d** (30.6% inhibition at 50 μM, IC_{50} >30 μM), **2e** (15.6% inhibition at 50 μM, IC_{50} >30 μM), **2f** (99.8% inhibition at 50 μM, IC_{50} = 17.4 μM), **2g** (15.7% inhibition at 50 μM, IC_{50} >30 μM) and **2h** (>100% inhibition at 50 μM, IC_{50} = 6.1 μM) were also tested for their inhibitory activity against IL-5. Among them compounds **2f** and **2h** gave good inhibition against IL-5. Especially, the compound **2h** exhibited best potency which is almost comparable to **2a**. For more insight of SAR we also introduced some other electron donating groups such as $-OCH_2OCH_3$ group in **2b** (10.5% inhibition at 50 μM, IC_{50} >30 μM) and $-CH_2CH_3$ group in **2i** (9.7% inhibition at 50 μM, IC_{50} >30 μM) at *p*-position of ring B. These substitutions did not turn up with any inhibition at all as shown in Table 2. This enforces our point again that the importance of electron density or strong electron donating group on phenyl ring is vital for the activity.

In the next set of experiment, aurones **3i** (3.8% inhibition at 50 μM, IC_{50} >30 μM), **3j** (3.1% inhibition at 50 μM, IC_{50} >30 μM), **3k** (12.2% inhibition at 50 μM, IC_{50} >30 μM), **3l** (8.0% inhibition at 50 μM, $IC_{50} \geqslant 30$ μM), **3m** (3.5% inhibition at 50 μM, $IC_{50} \geqslant 30$ μM) and **3n** (5.7% inhibition at 50 μM, $IC_{50} \geqslant 30$ μM) were also studied for their activity against IL-5. These did not show any notable inhibitory activity against IL-5. These results indicate the importance of isoflavone ring with electron donating group.

Table 2
Inhibitory activity against IL-5 of **2a–i** and **3i–n**

Compound No.	R	% Inhibition (at 50 μM)	IC_{50}[b] (μM)
2a[a]	4-OH	91.7	5.8
2b	4-OCH_2OCH_3	10.5	>30
2c	4-OCH_3	15.4	>30
2d	3,4-$(OCH_3)_2$	30.6	>30
2e	2,4-$(OCH_3)_2$	15.6	>30
2f	3-OCH_3,4-OCH_2Ph	99.8	17.4
2g	3-OCH_3, 4-OH	15.7	>30
2h	3,4,5-$(OCH_3)_3$	>100	6.1
2i	4-CH_2CH_3	9.7	>30
3i	4-CH_2CH_3	3.8	>30
3j	4-H	3.1	>30
3k	4-Cl	12.7	>30
3l	4-CHO	8.0	>30
3m	4-$COOCH_3$	3.5	>30
3n	4-NO_2	5.7	>30
Budesonide		70.3	26.2

[a] Analytical data of compound **2a** is consistent with Ref. 9.
[b] IC_{50} values are taken as a mean from three experiments.

3. Conclusion

The novel isoflavone derivatives were synthesized by oxidative cyclization of substituted 2′-hydroxy-6′-cyclohexylmethoxychalcones using thallium (III) nitrate and tested them for their IL-5 inhibitory activity. This reaction proceeded to isoflavone **2** and (or) aurone **3** depending on the electronic nature of *p*-substituents on ring B of chalcone. The strong electron donating groups at *p*-position of ring B gives only isoflavones **2**, the weak electron donating group leads to the mixture and the electron withdrawing groups end with aurones **3**. The isoflavones gave inhibitory activity towards IL-5 while the aurones did not. The structural requirement for the isoflavone possessing the inhibitory activity against IL-5 could be summarized as (1) importance of hydrophobic group such as cyclohexylmethoxy at C-5 position of ring A, (2) requirement of ring B with high electron density groups and (3) planar nature of chromen-4-one ring.

4. Materials and methods

4.1. Chemistry

Melting points (mp) were determined on Electro thermal 1A 9100 MK2 apparatus and were uncorrected. All commercial chemicals were used as obtained and all solvents were purified by the standard procedures prior to use. Thin layer chromatography was performed on E Merck Silica Gel GF-254 precoated plates and the identification was done with UV light and colorization with spray 10% phosphomolybdic acid followed by heating. Flash column chromatography was performed with E Merck Silica Gel (230–400 mesh). A FT-IR spectrum was recorded with Nicolet—380 models. NMR spectra were measured against the peak of tetramethylsilane by Varain Unity Inova 400 NMR (400 MHz) spectrometers. High resolution mass spectrum (HRMS) was recorded on API2000 mass spectrometer (PE Sciex, Toronto, Canada).

4.1.1. General procedure[24,25] for the preparation of compounds 2b–i and 3i–n

To a 2% solution of chalcone **5** in methanol was added 2 equiv of thallium nitrate trihydrate. The resulting solution was stirred at room temperature for one day. After concentration to about its half volume, two equivalents of hydrochloric acid (2 M) was added, with the resulting mixture stirred at 65 °C for 5 h. After removal of the insoluble material by filtration, the filtrate was concentrated under vacuum at room temperature. The crude product was dissolved in chloroform, and then washed three times with water. The organic layer was dried with anhydrous sodium sulfate, and then concentrated under vacuum. The crude product was purified by flash column chromatography to give isoflavone **2** and (or) aurone **3**.

4.1.1.1. 5-(Cyclohexylmethoxy)-3-[4-(methoxymethoxy)phenyl]-4*H*-chromen-4-one (2b). The reaction was started with **5b** to give **2a** in 61.5% yield, which was then converted to **2b** by the treatment with methoxymethyl chloride[8]; yellow solid; yield 80.5%; mp 105–106 °C; IR (neat) 2950, 1650, 1510 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.10–1.14 (m, 2H), 1.18–1.29 (m, 3H), 1.67–1.76 (m, 3H), 1.95–2.03 (m, 3H), 3.48 (s, 3H), 3.86 (d, J = 6.4 Hz, 2H), 5.20 (s, 2H), 6.78 (d,

J = 8.4 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 1H), 7.09 (d, *J* = 8.4 Hz, 2H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.78 (s, 1H). HRMS calcd for $C_{24}H_{26}O_5$ *m*/*z* 394.1780, found 394.1774.

4.1.1.2. 5-(Cyclohexylmethoxy)-3-(4-methoxyphenyl)-4*H*-chromen-4-one (2c). The reaction was started with **5c**; white solid; yield 48.6%; mp 105–106 °C; IR (neat) 2950, 1650, 1510 cm^{-1}, ^1H NMR ($CDCl_3$): δ 1.05–1.10 (m, 2H), 1.18–1.35 (m, 3H), 1.67–1.76 (m, 3H), 1.95–2.03 (m, 3H), 3.80 (s, 3H), 3.86 (d, *J* = 6.4 Hz, 2H), 6.78 (d, *J* = 8.4 Hz, 1H), 6.93–6.99 (m, 3H), 7.44–7.52 (m, 3H), 7.78 (s, 1H). HRMS calcd for $C_{23}H_{24}O_4$ *m*/*z* 364.1675, found 364.1671.

4.1.1.3. 5-(Cyclohexylmethoxy)-3-(3,4-dimethoxyphenyl)-4*H*-chromen-4-one (2d). The reaction was started with **5d**; yellow solid; yield 49.7%; mp 112–114 °C; IR (neat) 2922, 2850, 1645, 1601, 1502 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.08–2.04 (m, 11H), 3.81 (d, *J* = 6.0 Hz, 2H), 3.87 (s, 6H), 6.68 (d, *J* = 8.4 Hz, 1H), 6.87 (d, *J* = 8.4 Hz, 1H), 6.91–7.22 (m, 2H), 7.23 (s, 1H), 7.20–7.33 (m, 2H), 7.33 (t, *J* = 8.4 Hz, 1H), 7.81 (s, 1H). HRMS calcd for $C_{24}H_{26}O_5$ *m*/*z* 394.1780, found 394.1773.

4.1.1.4. 5-(Cyclohexylmethoxy)-3-(2,5-dimethoxyphenyl)-4*H*-chromen-4-one (2e). The reaction was started with **5e**; yellow solid; yield 44.3%; mp 132–134 °C; IR (neat) 2927, 2851, 1642, 1593, 1507 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.07–2.01 (m, 11H), 3.73 (s, 3H), 3.77 (s, 3H), 3.84 (d, *J* = 6.0 Hz, 2H), 6.77 (d, *J* = 8.4 Hz, 1H), 6.87–6.92 (m, 3H), 6.97 (d, *J* = 8.4 Hz, 1H), 7.47 (t, *J* = 8.4 Hz, 1H), 7.51 (s, 1H). HRMS calcd for $C_{24}H_{26}O_5$ *m*/*z* 394.1780, found 394.1774.

4.1.1.5. 3-(4-(Benzyloxy)-3-methoxyphenyl)-5-(cyclohexylmethoxy)-4*H*-chromen-4-one (2f). The reaction was started with **5f**; yellow solid; yield 53.4%; mp 128–130 °C; IR (neat) 2922, 2854, 1647, 1596, 1497, 1459 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.08–1.99 (m, 11H), 3.82 (d, *J* = 6.0 Hz, 2H), 3.92 (s, 3H), 5.19 (s, 2H), 6.78 (d, *J* = 8.0 Hz, 1H), 6.90–6.99 (m, 2H), 7.16 (s, 1H), 7.28–7.53 (m, 8H), 7.77 (s, 1H). HRMS calcd for $C_{30}H_{30}O_5$ *m*/*z* 470.2093, found 470.2088.

4.1.1.6. 5-(Cyclohexylmethoxy)-3-(4-hydroxy-3-methoxyphenyl)-4*H*-chromen-4-one (2g). The reaction was started with **2f** by catalytic hydrogenolysis (H_2, Pd–C); yellow solid; yield 98.1%; mp 72–75 °C; IR (neat) 2957, 2850, 1700, 1619, 1527, 1437 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.87–2.00 (m, 11H), 3.82 (d, *J* = 6.0 Hz, 2H), 3.92 (s, 3H), 6.48 (d, *J* = 8.4 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 6.86 (m, 3H), 7.49 (t, *J* = 8.4 Hz, 1H), 7.78 (s, 1H). HRMS calcd for $C_{23}H_{24}O_5$ *m*/*z* 380.1624, found 380.1620.

4.1.1.7. 5-(Cyclohexylmethoxy)-3-(3,4,5-trimethoxyphenyl)-4*H*-chromen-4-one (2h). The reaction was started with **5h**; yellow solid; yield 48.2%; mp 89–90 °C; IR (neat) 2926, 2857, 1642, 1594, 1503, 1453 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85–2.17 (m, 11H), 3.85 (d, *J* = 4.8 Hz, 2H), 3.88 (s, 9H), 6.73 (s, 2H), 6.81 (d, *J* = 8.4 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 7.52 (t, *J* = 8.4 Hz, 1H), 7.81 (s, 1H). HRMS calcd for $C_{25}H_{28}O_6$ *m*/*z* 424.1886, found 424.1881.

4.1.1.8. 5-(Cyclohexylmethoxy)-3-(4-ethylphenyl)-4*H*-chromen-4-one (2i) and (*Z*)-4-(cyclohexylmethoxy)-2-(4-ethylbenzylidene)benzofuran-3(2*H*)-one (3i). The reaction was started with **5i**. Compound (**2i**): yellow solid; yield 24.3%; mp 118–120 °C; IR (neat) 2920, 2852, 1747, 1645 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.095 (t, *J* = 3.4 Hz, 3H), 1.17–2.00 (m, 1H), 2.62 (q, *J* = 7.6 Hz, 2H), 3.87 (d, *J* = 6.4 Hz, 2H), 6.75 (d, *J* = 8.0 Hz, 1H), 6.90 (d, *J* = 8.8 Hz, 1H), 7.12–1.24 (m, 4H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.47 (t, *J* = 8.4 Hz, 1H). HRMS calcd for $C_{24}H_{26}O_3$ *m*/*z* 362.1882, found 362.1875. Compound (**3i**): yellow solid; yield 21.7%; starting chalcone recovered 35.7%; mp 120–124 °C; IR (neat) 3200, 2850, 1700, 1600 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.09–1.37 (m, 8H), 1.69–1.79 (m, 3H), 1.93–1.96 (m, 3H), 2.69 (q, *J* = 7.6 Hz, 2H), 3.94 (d, *J* = 6.0 Hz, 2H), 6.58 (d, *J* = 8.4 Hz, 1H), 6.78 (s, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 2H), 7.51 (t, *J* = 8.4 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 2H). HRMS calcd for $C_{24}H_{26}O_3$ *m*/*z* 362.1882, found 362.1876.

4.1.1.9. (*Z*)-2-(Benzylidene)-4-(cyclohexylmethoxy)benzofuran-3(2*H*)-one (3j). The reaction was started with **5j**; yellow solid; yield 44.8%; starting chalcone recovered 38.2%; mp 125–126 °C; IR (neat) 3100, 2950, 1700, 1600 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.08–1.96 (m, 11H), 3.94 (d, *J* = 6.0 Hz, 2H), 6.58 (d, *J* = 8.4 Hz, 1H), 6.78 (s, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 7.35–7.39 (m, 1H), 7.42–7.45 (m, 2H), 7.52 (t, *J* = 8.4 Hz, 1H), 7.88–7.90 (m, 2H). HRMS calcd for $C_{22}H_{22}O_3$ *m*/*z* 334.1569, found 334.1562.

4.1.1.10. (*Z*)-2-(4-Chlorobenzylidene)-4-(cyclohexylmethoxy) benzofuran-3(2*H*)-one (3k). The reaction was started with **5k**; white solid; yield 38.7%; starting chalcone recovered 44.0%; mp 179–180 °C; IR (neat) 3100, 2850, 1710, 1600 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.08–1.95 (m, 11H), 3.95 (d, *J* = 6.0 Hz, 2H), 6.60 (d, *J* = 8.4 Hz, 1H), 6.71 (s, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.53 (t, *J* = 8.4 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 2H). HRMS calcd for $C_{22}H_{21}ClO_3$ *m*/*z* 368.1179, found 368.1174.

4.1.1.11. (*Z*)-4-{[4-(Cyclohexylmethoxy)-3-oxobenzofuran-2(3*H*)-ylidene]methyl}benzaldehyde (3l). The reaction was started with **5l**; yellow solid; yield 32.7%; starting chalcone recovered 40.1%; mp 163–165 °C; IR (neat) 3100, 2950, 1700, 1600 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.08–1.96 (m, 11H), 3.96 (d, *J* = 6.0 Hz, 2H), 6.63 (d, *J* = 8.4 Hz, 1H), 6.78 (s, 1H), 6.87 (d, *J* = 8.4 Hz, 1H), 7.57 (t, *J* = 8.4 Hz, 1H), 7.94 (d, *J* = 8.4 Hz, 2H), 8.04 (d, *J* = 8.4 Hz, 2H), 10.05 (s, 1H). HRMS calcd for $C_{23}H_{22}O_4$ *m*/*z* 362.1518, found 362.1515.

4.1.1.12. Methyl(*Z*)-4-[4-(cyclohexylmethoxy)-3-oxobenzofuran-2(3*H*)-ylidene]methylbenzoate (3m). The reaction was started with **5m**; yellow solid; yield 42.7%; starting chalcone recovered 50.1%; mp 152–153 °C; IR (neat) 3100, 2920, 1700, 1600 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.09–1.12 (m, 2H), 1.25–1.34 (m, 3H), 1.73–1.80 (m, 3H), 1.93–1.95 (m, 3H), 3.94 (s, 3H), 3.96 (m, 2H), 6.61 (d, *J* = 8.4 Hz, 1H), 6.76 (s, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 7.54 (t, *J* = 8.4 Hz, 1H), 7.93 (d, *J* = 8.4 Hz, 2H), 8.08 (d, *J* = 8.4 Hz, 2H). HRMS calcd for $C_{24}H_{24}O_5$ *m*/*z* 392.1624, found 392.1620.

4.1.1.13. (*Z*)-4-(Cyclohexylmethoxy)-2-(4-nitrobenzylidene)benzofuran-3(2*H*)-one (3n). The reaction was started with **5n**; yellow solid; yield 74.5%; mp 171–173 °C; IR (neat) 3150, 2950, 1700, 1650 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.06–1.38 (m, 5H), 1.62–1.80 (m, 3H), 1.92–1.95 (m, 3H), 3.95 (d, *J* = 6.0 Hz, 2H), 6.63 (d, *J* = 8.4 Hz, 1H), 6.74 (s, 1H), 6.86 (d, *J* = 8.4 Hz, 1H), 7.57 (t, *J* = 8.4 Hz, 1H), 8.01 (d, *J* = 8.8 Hz, 2H), 8.26 (d, *J* = 8.8 Hz, 2H). HRMS calcd for $C_{22}H_{21}NO_5$ *m*/*z* 379.1420, found 379.1414.

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