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Novel Chalcone Derivatives as Potent Nrf2 Activators in Mice and Human Lung Epithelial Cells

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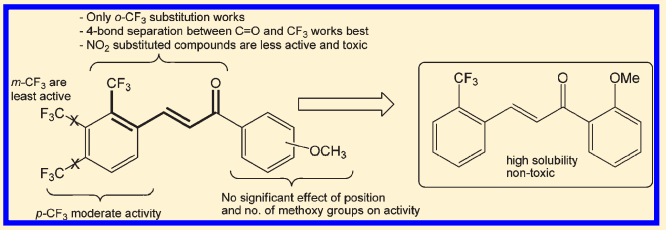
ABSTRACT: Nrf2-mediated activation of antioxidant response element is a central part of molecular mechanisms governing the protective function of phase II detoxification and antioxidant enzymes against carcinogenesis, oxidative stress, and inflammation. Nrf2 is sequestered in the cytoplasm by its repressor, Keap1. We have designed and synthesized novel chalcone derivatives as Nrf2 activators. The potency of these compounds was measured by the expression of Nrf2 dependent antioxidant genes GCLM, NQO1, and HO1 in human lung epithelial cells, while the cytotoxicity was analyzed using MTT assay. In vivo potency of identified lead compounds to activate Nrf2 was evaluated using a mouse model. Our studies showed 2-trifluoromethyl-2′-methoxychalone (**2b**) to be a potent activator of Nrf2, both in vitro and in mice. Additional experiments showed that the activation of Nrf2 by this compound is independent of reactive oxygen species or redox changes. We have discussed a quantitative structure−activity relationship and proposed a possible mechanism of Nrf2 activation.



INTRODUCTION

Nuclear factor erythroid 2 p45-related factor 2 (Nrf2) is a basic-leucine zipper (b-ZIP) transcription factor present in the cytoplasm of normal cells. Upon activation in response to inflammatory stimulus and environmental toxicant, oxidative, and electrophilic stress, Nrf2 detaches from its cytosolic inhibitor, Kelch-like ECH-associated protein 1 (Keap1), and translocates to the nucleus and binds to the antioxidant response element (ARE) of target genes along with other binding partners leading to their transcriptional induction.[1−4] The Keap1−Nrf2 system is the major regulatory pathway of cytoprotective gene expression against oxidative and/or electrophilic stresses. Keap1 acts as a stress sensor protein in this system. While Keap1 constitutively suppresses Nrf2 activity under unstressed conditions, oxidants or electrophiles provoke the repression of Keap1 activity, inducing the Nrf2 activation.[5−7] In addition to Keap1, the activation of different protein kinases has been shown to activate Nrf2.[8−12] The Nrf2-regulated genes include almost all of the relevant antioxidants and cytoprotective genes such as heme oxygenase-1 (HO-1), NAD(P)H/quinone oxidoreductase 1 (NQO1), glutamate−cysteine ligase modifier subunit (GCLM), γ-glutamyl cysteine synthase, glutathione peroxidase (GPx), and several members of the glutathione *S*-transferase family[6,13−18] that express an ARE in their promoter.[19] Small molecules that activate Nrf2 signaling are being investigated as potential anticancer or anti-inflammatory agents. A wide variety of dietary and synthetic compounds that function as potent inducers of ARE-regulated gene expression have been shown to exert chemopreventive activities, e.g., sulforaphane,[4,20−22] dithiolethione,[23−25] curcumin,[26] and caffeic acid phenethyl ester (CAPE).[26] It is notable that both curcumin and CAPE bear an α,β-unsaturated ketone moiety and can therefore act as Michael acceptors that are able to modify cysteine thiols present in Keap1.

Chalcones or 1,2-diphenyl-2-propen-1-ones are Michael acceptors and constitute an important group of natural products belonging to the flavonoid family.[27,28] They have been reported to possess many biological properties including anticancer,[29,30] antimalarial,[31,32] anti-inflammatory,[33−35] antileishmanial,[33−35] antituberclulosis,[36] nitric oxide inhibition,[37,38] antimitotic,[39] analgesic, antipyretic, antioxidant,[40−43] antibacterial, anti-HIV,[44] antifungal,[45] and antiprotozoal activities.[46−48] They are also reported to be gastric protectant,[49] antimutagenic, and antitumorogenic.[50−52] Natural and synthetic chalcones have been reported to possess strong antiproliferative effects in primary and established ovarian cancer cells[53] and in gastric cancer cells.[52] Chalcones contain two aromatic rings separated by α,β-unsaturated ketone, and this unique structure is responsible for various activities of these molecules.[27] It is well-known that the α,β unsaturated carbonyl entity in chalcones is a soft electrophile and would attract soft nucleophiles like thiols rather than hard nucleophiles like amino and hydroxyl groups. Chalcones are

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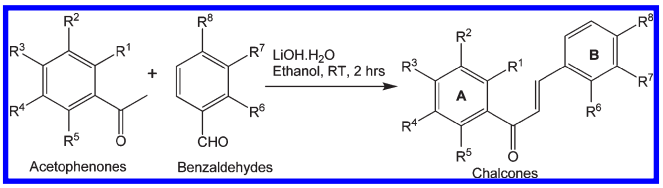
Table 1. Synthesis of Chalcone Derivatives by Claisen−Schmidt Condensation and Their Nrf2 Induction Activity



		substituents on ring A					substituents on ring B			relative fold change[a]	
entry	chalcones	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	GCLM	NQO1
1	**1a**	H	H	H	H	H	H	H	H	0.7	1.8
2	**1b**	OMe	H	H	H	H	H	H	H	3.6	3.2
3	**1c**	H	OMe	H	H	H	H	H	H	1.5	2.4
4	**1d**	H	H	OMe	H	H	H	H	H	1.9	2.8
5	**1e**	OMe	H	OMe	H	H	H	H	H	2.7	2.8
6	**1f**	OMe	H	H	H	OMe	H	H	H	2.5	1.9
7	**1g**	OMe	H	H	OMe	H	H	H	H	3.0	2.6
8	**1h**	H	OMe	OMe	H	H	H	H	H	2.3	2.5
9	**1i**	H	OMe	H	OMe	H	H	H	H	3.0	2.4
10	**1j**	H	OMe	OMe	OMe	H	H	H	H	0.7	0.7
11	**1k**	OMe	OMe	OMe	H	H	H	H	H	2.4	2.4
12	**1l**	OMe	H	OMe	H	OMe	H	H	H	3.1	1.8
13	**2a**	H	H	H	H	H	CF_3	H	H	5.0	5.3
14	**2b**	OMe	H	H	H	H	CF_3	H	H	4.5	4.6
15	**2c**	H	OMe	H	H	H	CF_3	H	H	5.6	4.3
16	**2d**	H	H	OMe	H	H	CF_3	H	H	5.4	4.6
17	**2e**	OMe	H	OMe	H	H	CF_3	H	H	5.4	4.5
18	**2f**	OMe	H	H	H	OMe	CF_3	H	H	5.7	5.3
19	**2g**	OMe	H	H	OMe	H	CF_3	H	H	4.4	2.7
20	**2h**	H	OMe	OMe	H	H	CF_3	H	H	4.3	2.7
21	**2i**	H	OMe	H	OMe	H	CF_3	H	H	4.0	3.7
22	**2j**	H	OMe	OMe	OMe	H	CF_3	H	H	3.0	3.1
23	**2k**	OMe	OMe	OMe	H	H	CF_3	H	H	5.4	4.1
24	**2l**	OMe	H	OMe	H	OMe	CF_3	H	H	4.4	4.3
25	**3a**	H	H	H	H	H	H	CF_3	H	3.1	2.8
26	**3b**	OMe	H	H	H	H	H	CF_3	H	3.9	2.9
27	**3c**	H	OMe	H	H	H	H	CF_3	H	4.6	3.5
28	**3d**	H	H	OMe	H	H	H	CF_3	H	2.7	2.8
29	**3e**	OMe	H	OMe	H	H	H	CF_3	H	4.6	4.0
30	**3f**	OMe	H	H	H	OMe	H	CF_3	H	1.8	0.6
31	**3g**	OMe	H	H	OMe	H	H	CF_3	H	1.8	0.8
32	**3h**	H	OMe	OMe	H	H	H	CF_3	H	4.1	2.9
33	**3i**	H	OMe	H	OMe	H	H	CF_3	H	3.8	3.9
34	**3j**	H	OMe	OMe	OMe	H	H	CF_3	H	1.7	0.8
35	**3k**	OMe	OMe	OMe	H	H	H	CF_3	H	3.6	3.2
36	**3l**	OMe	H	OMe	H	OMe	H	CF_3	H	1.4	0.7
37	**4a**	H	H	H	H	H	H	H	CF_3	3.1	2.9
38	**4b**	OMe	H	H	H	H	H	H	CF_3	4.3	5.4
39	**4c**	H	OMe	H	H	H	H	H	CF_3	2.9	3.1
40	**4d**	H	H	OMe	H	H	H	H	CF_3	3.5	4.6
41	**4e**	OMe	H	OMe	H	H	H	H	CF_3	5.0	4.8
42	**4f**	OMe	H	H	H	OMe	H	H	CF_3	1.4	0.6
43	**4g**	OMe	H	H	OMe	H	H	H	CF_3	4.1	4.0
44	**4h**	H	OMe	OMe	H	H	H	H	CF_3	3.5	2.7

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Table 1. Continued

		substituents on ring A					substituents on ring B			relative fold change[a]	
entry	chalcones	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	GCLM	NQO1
45	**4i**	H	OMe	OMe	OMe	H	H	H	CF_3	4.1	3.7
46	**4j**	OMe	OMe	OMe	H	H	H	H	CF_3	4.1	3.9
47	**4k**	OMe	H	OMe	H	OMe	H	H	CF_3	2.4	2.4
48	**5a**	H	H	H	H	H	NO_2	H	H	2.1	1.6
49	**5b**	OMe	H	H	H	H	NO_2	H	H	1.9	1.4
50	**5c**	H	OMe		H	H	NO_2	H	H	2.9	2.7
51	**5d**	H	H	OMe	H	H	NO_2	H	H	4.6	4.3
52	**5e**	OMe	H	OMe	H	H	NO_2	H	H	2.7	3.3
53	**5f**	OMe	H	H	H	OMe	NO_2	H	H	4.1	3.7
54	**5g**	OMe	H	H	OMe	H	NO_2	H	H	1.7	0.7
55	**5h**	H	OMe	OMe	H	H	NO_2	H	H	2.3	2.7
56	**5i**	H	OMe	H	OMe	H	NO_2	H	H	3.4	4.1
57	**5j**	H	OMe	OMe	OMe	H	NO_2	H	H	1.2	0.7
58	**5k**	OMe	OMe	OMe	H	H	NO_2	H	H	2.9	3.3
59	**5l**	OMe	H	OMe	H	OMe	NO_2	H	H	2.6	2.0
	DMSO									1.0	1.0
	sulforaphane									2.7	3.6

[a] Data presented are representative of three independent experiments. Values shown are mean of quadruplicate wells.

unlikely to react with the amino and hydroxyl groups on nucleic acids and thus would unlikely induce mutagenicity and carcinogenicity commonly associated with alkylating agents used in cancer chemotherapy.[28]

The remarkable biological potential of chalcones is due to their possible interactions with various proteins related to cell apoptosis and proliferation.[54,55] A number of recent studies have indicated that the anti-inflammatory effect of chalcones is due to the inhibition of the NF-κB pathway, which is mediated by IκB degradation and the phosphorylation of c-Jun N-terminal kinase (JNK) and c-Jun.[56−58] It has been reported that the electrophilic α,β-unsaturated carbonyl moiety on a chalcone resulted in the activation of the Nrf2/ARE pathway and the induction of phase II detoxifying enzyme expression.[59,60] This moiety acts as an electrophile and reacts with free sulfhydryl groups of thioredoxin and cysteine residues in proteins.[58,59,61] It is also reported that electrophilic phytochemicals could give rise to thiyl radicals, which could also interact with sulfhydryl residues of intracellular targets, including Nrf2.[62] These studies demonstrate that the endogenous electrophilic activity, through its α,β-unsaturated carbonyl moiety, is involved in the antioxidant and anti-inflammatory properties of chalcone. In the present study, we synthesized novel chalcone derivatives and tested their Nrf2 activating activity in human bronchial epithelial cells. Furthermore, to draw a structure−activity relationship, a possible mechanism for Nrf2 activation is proposed.

RESULTS AND DISCUSSION

Chemistry. Chaclones can be readily synthesized by the base-catalyzed Claisen−Schmidt condensation of an aldehyde and ketone in a polar solvent like ethanol or methanol. The traditional synthesis of chalcones involves the use of strong bases such as NaOH,[31,35,39,63] KOH,[16,64] $Ba(OH)_2$,[65,66] hydrotalcites,[67] and LiHMDS,[68] calcined $NaNO_3$/natural phosphate.[69] They can also be synthesized by acid-catalyzed aldol condensations, e.g., $AlCl_3$,[70] BF_3,[71] dry HCl,[72] $Zn(bpy)(OAc)_2$,[73] Cp_2ZrH_2/$NiCl_2$,[74] and $RuCl_3$ (for cyclic and acyclic ketones).[75] Suzuki coupling has also been employed for the synthesis of chalcone derivatives.[76] Several disadvantages of these procedures include long reaction time, high reaction temperature, complex reaction conditions, and the use of expensive and noncommercial reagents. Recently, an efficient and facile synthesis of chalcones by condensation of aldehydes and ketones has been reported using $LiOH\cdot H_2O$ as a dual activation catalyst under mild conditions.[77] We employed similar conditions for the synthesis of all chalcone derivatives used in the present study. In short, the appropriately substituted acetophenone was dissolved in ethanol followed by addition of a catalytic amount of $LiOH\cdot H_2O$. The reaction mixture was stirred at room temperature for 15 min, and the desired substituted benzaldehyde was added. The reaction was carried out at room temperature until completion, and the corresponding chalcone derivative (**1a**−**5l**) was isolated by crystallization or by silica gel flash chromatography (Table 1). All chalcone derivatives were characterized by ^1H, ^{13}C NMR, and HRMS. To the best of our knowledge, compounds **2b**, **2f**, **2i**, **2k**, **2l**, **3b**, **3c**, **3f**, **3i**, **3k**, **5f**, **5g**, and **5i** have never been reported before, whereas compounds **2e**, **2g**, **2h**, **3g**, and **3h** are commercially available as per the SciFinder search; however, there is no report on these compounds in mice and human lung epithelial cells.

Biology. *Potency of Chalcone Derivatives To Activate the Expression of Nrf2-Regulated Cytoprotective Genes in Human Lung Epithelial Cells.* To investigate the potency of chalcone derivatives to activate Nrf2, we measured the expression of antioxidant genes GCLM and NADPH-NQO1, two well characterized transcriptional targets of Nrf2, as surrogate markers. Previously, we and others have shown that oxidants or small molecule activators of Nrf2 increase GCLM and NQO1 in cells or tissues of wild-type but not in Nrf2-deficient mice.[78] In this study, to screen novel Nrf2 activators, we treated normal human bronchial epithelial cells (Beas-2B) with chalcone derivatives (10 μM) for 16 h and analyzed the expression of GCLM and NQO1 by

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Table 2. List of Positive Leads from Primary Screening and Their Effect on Cell Viability

		relative fold change[a]		
entry	compd	GCLM	NQO1	% cell viability
1	DMSO	1	1	100
2	sulforaphane	2.7	3.6	97.3
3	**2a**	5	5.3	144.8
4	**2b**	4.5	4.6	100.9
5	**2c**	5.6	4.3	96.6
6	**2d**	5.4	4.6	96
7	**2e**	5.4	4.5	105
8	**2f**	5.7	5.3	101.1
9	**2i**	4	3.7	87.1
10	**2k**	5.4	4.1	101.6
11	**2l**	4.4	4.3	145.3
12	**3c**	4.6	3.5	95.3
13	**3i**	3.8	3.9	108.2
14	**4b**	4.3	5.4	91
15	**4d**	3.5	4.6	104.1
16	**4e**	5	4.8	92.6
17	**4g**	4.1	4	91.6
18	**4j**	4.1	3.7	100.1
19	**5d**	4.6	4.3	93.2
20	**5f**	4.1	3.7	75.8
21	**5i**	3.4	4.1	90.9

[a] Data presented are representative of three independent experiments. Values shown are mean of quadruplicate wells.

quantitative RT-PCR (qRT-PCR). We included sulforaphane, a well-known potent activator of Nrf2, as a positive control. We identified 59 chalcone derivatives that induce the expression of GCLM and NQO1 (Table 1). Concurrent with the gene expression analysis, we analyzed the cytotoxicity of the chalcone derivatives using the MTT assay. A total of 20 chalcones showed a higher induction of Nrf2-regulated transcriptional targets than the positive control, i.e., sulforaphane (Table 2).

Preliminary Structure−Activity Relationship. The structure−activity relationship analysis showed that the chalcone derivatives **1a**−**l** without any substitution on ring B were not active. The activity of similar derivatives with trifluoromethyl (CF_3) substitution on ring B enhanced the activity dramatically. The position of CF_3 substitution was also crucial for the activity and cytotoxicity of these compounds. In general, the chalcone derivatives with CF_3 substitution at ortho position on ring B were the most active compounds (entries 13−24, Table 1), followed by para (entries 37−47, Table 1) and meta (entries 25−36, Table 1) substitution. Also, the cytotoxicity data show that the ortho CF_3-substituted chalcones were noncytotoxic. This shows that four-bond separation between carbonyl and CF_3 is crucial for the induction activity. Interestingly, with nitro (NO_2) substitution at the ortho position on ring B, the activity decreased and the toxicity increased significantly (entries 48−59 of Table 1 and entries 19−21 of Table 2). A brief description of SAR is presented in Figure 1. Thus, on the basis of these data, we selected only those chalcone derivatives that showed >4-fold induction of GCLM and NQO1 genes and >95% cell viability. On the basis of these stringent criteria, of the 20 compounds that showed potency to increase Nrf2 activity, we selected compounds **2a**−**f**, **2k**, and **2l** for further analysis.

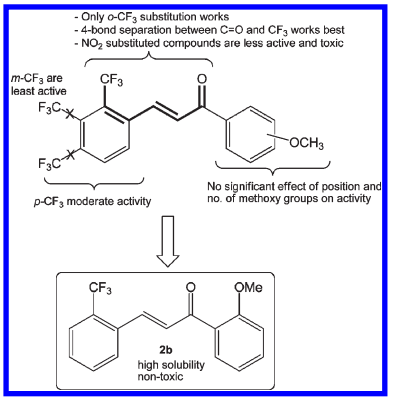

Figure 1. Structure−activity relationship of chalcone derivatives.

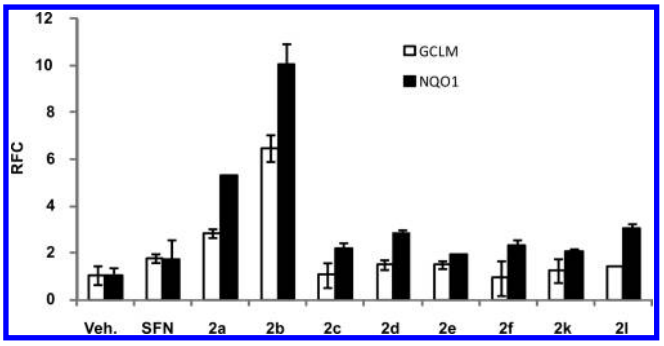

Figure 2. Expression of Nrf2-regulated genes in small intestine after treatment with chalcone derivatives. Mice ($n = 4$) were fed with vehicle (DCP (10% DMSO + 10% Cremophor + 80% phosphate buffered saline)) or chalcone derivatives or sulforaphane (50 mg/kg body weight) by gavage, and the small intestines were harvested 24 h later. The expression of Nrf2-regulated genes GCLM and NQO1 was analyzed in the tissues by qRT-PCR as a surrogate marker of Nrf2 activity. β-Actin was used for normalization. Data are representative of three independent experiments ($P \leq 0.05$).

In Vivo Potency of Identified Lead Compounds To Activate Nrf2 Using Mouse Models. Next, we evaluated the potency of the eight lead chalcones identified in the in vitro screening to activate the Nrf2 pathway in mouse models. First, we tested various formulations to dissolve the compounds, and the DCP (10% DMSO + 10% Cremophor EL + 80% phosphate buffered saline) formulation offered the maximum solubility for delivery of these compounds by oral route. Mice (C57BL/6) were administrated with a single dose of vehicle or test compound(s) or sulforaphane as the positive control at a dose of 50 mg/kg body weight by gavage, and small intestines were harvested 24 h later. The expression of Nrf2-regulated genes GCLM and NQO1 was analyzed in the tissue by qRT-PCR. All eight lead compounds increased the expression of GCLM and NQO1 in the small intestine. However, **2b** (Figure 1) was the most potent inducer of Nrf2 activity (Figure 2). The expression of GCLM and NQO1 in the small intestine of mice treated with **2b** was 6-fold and 10-fold higher compared to vehicle, respectively. Similarly, the expression of GCLM and NQO1 in the small intestine treated with **2b** was 3-fold and 5-fold higher compared to sulforaphane, respectively. Taken together, we selected **2b** as the most potent activator of Nrf2 for further studies.

*Nrf2 Is Essential for Induction of Antioxidant Genes by Compound **2b**.* We further characterized Nrf2 induction by **2b**

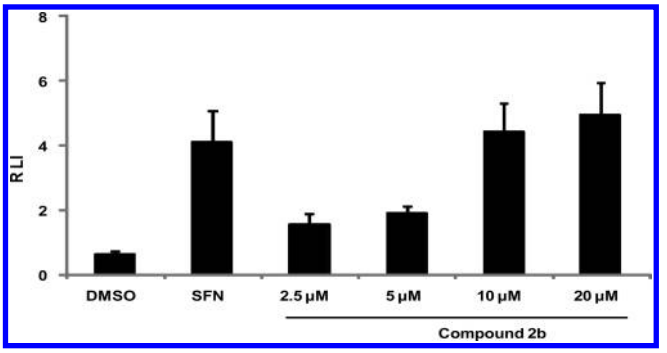

Figure 3. Levels of NQO1-ARE luciferase activity after treatment with compound **2b**. NQO1-ARE luciferase activity was measured by using stably transfected Beas-2B cells after treatment with compound **2b** or sulforaphane (SFN) or dimethylsulfoxide (DMSO). The exposure to compound **2b** resulted in a significant concentration-dependent increase in luciferase activity as relative luminescence intensity (RLI). Data are representative of three independent experiments. Values shown are the mean ± SD of quadruplicate wells ($P \leq 0.05$).

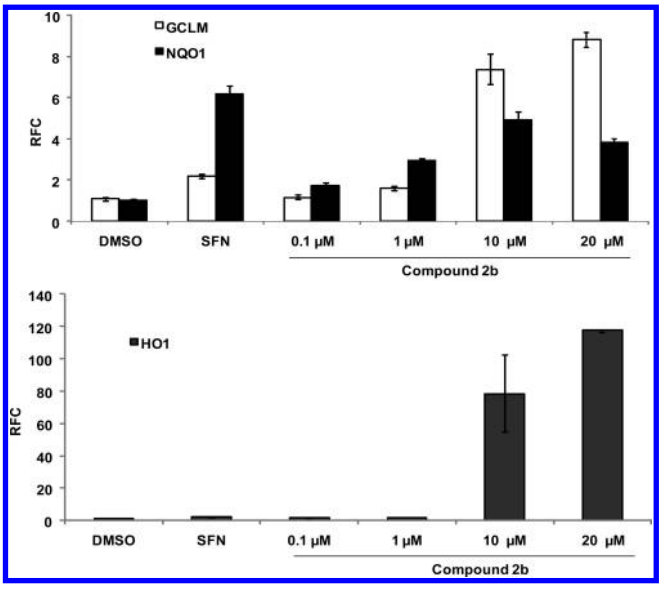

Figure 4. Expression of Nrf2-regulated genes after treatment with compound **2b**. Human bronchial epithelial cells (Beas-2B) were treated with compound **2b** at the indicated concentrations for 16−20 h. The expression of Nrf2-regulated genes GCLM, HO1, and NQO1 was analyzed in the tissues by qRT-PCR as a surrogate marker of Nrf2 activity. β-Actin was used for normalization. Data are representative of three independent experiments. Values shown are mean ± SD of triplicate wells ($P \leq 0.05$).

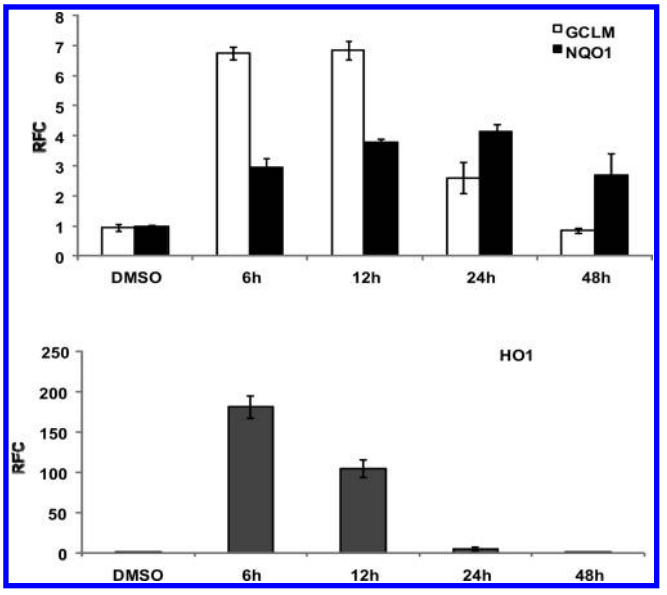

Figure 5. Time-dependent increase in Nrf2-regulated genes after treatment with compound **2b**. Human bronchial epithelial cells (Beas-2B) were treated with compound **2b** (10 μM) at various time points. The expression of Nrf2-regulated genes GCLM, HO1, and NQO1 was analyzed in the tissues by qRT-PCR. β-Actin was used for normalization. Data are representative of three independent experiments. Values shown are mean ± SD of triplicate wells ($P \leq 0.05$).

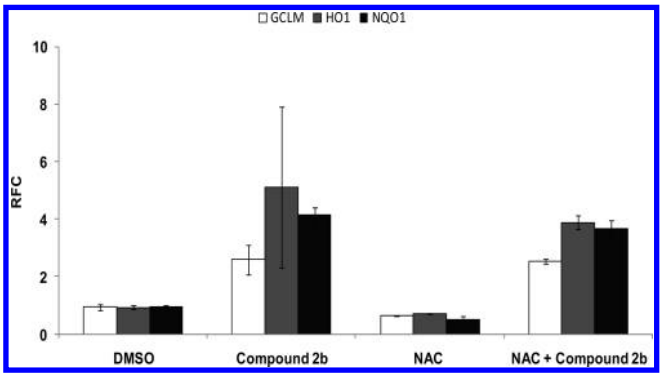

Figure 6. Activation of Nrf2 genes by compound **2b** is independent of ROS generation. Human bronchial epithelial cells (Beas-2B) were treated with compound **2b** (10 μM) in the presence of an antioxidant, *N*-acetylcystiene (10 mM, NAC). Cells were harvested 24 h after the treatment, and the Nrf2-driven expression of NQO1, HO-1, and GCLM was quantified. β-Actin was used for normalization. Data are representative of three independent experiments. Values shown are the mean ± SD of triplicate wells ($P \leq 0.05$).

using cell-based assays. Nrf2 increases the expression of NQO1 and GCLM by binding to the ARE present in the promoter region of these genes.[79] We determined whether the ARE mediates the transcriptional regulation of NQO1 by **2b**. We measured the expression of the luciferase gene under the control of NQO1-ARE sequence using stably transfected Beas-2B cells treated with **2b**. The exposure to compound **2b** resulted in a significant concentration-dependent increase in luciferase activity as measured by the chemiluminescence-based assay (Figure 3). These results implicate the ARE element in the induction of NQO1 gene by compound **2b**. The transcriptional activation of antioxidant genes through an ARE is largely dependent upon Nrf2, suggesting that **2b** up-regulates antioxidant genes via Nrf2 activation.

Concentration and Time-Course Studies. Next, we examined the concentration-dependent effect of **2b** on the mRNA levels of Nrf2-driven antioxidant genes GCLM, NQO1, and HO1. We measured the expression of these genes at 24 h after treatment with various concentrations (2.5, 5, 10, 20 μM) of **2b** in Beas-2B cells. As shown in Figure 5, compound **2b** significantly increased the Nrf2-regulated gene expression in a concentration-dependent manner. There was ∼5- and 10-fold increase in the expression of GCLM and NQO1, respectively, at the highest concentration (20 μM) with no cytotoxicity. Interestingly, we

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found a dramatic concentration-dependent activation of Nrf2 genes. At 10 μM **2b**, the expression of HO-1 was 6-fold higher compared to that of sulforaphane (Figure 4).

For the time-course studies, we measured the expression of antioxidant genes (GCLM, NQO1, and HO-1) at 6, 12, 24, and 48 h after treatment with **2b** (10 μM) in Beas-2B cells. The time-course studies showed the highest induction of GCLM (~7-fold) and HO-1 (~150-fold) at 6 h after treatment with **2b** (Figure 5). The expression of GCLM and HO-1 decreased at 6 h after treatment with **2b** and was comparable to vehicle by 48 h. The expression of NQO1 was highest at 24 h and remained significantly elevated even at 48 h after treatment with compound **2b** compared to vehicle. Taken together, these results suggest that **2b** is a potent activator of Nrf2-regulated antioxidant defenses.

*Activation of Nrf2 by Compound **2b** Is Independent of ROS Generation.* The activation of Nrf2 by various electrophiles and compounds that are Michael acceptors is attributed to changes in ROS production and or redox environment and or direct cysteine modification in Keap1.[80,81] We examined whether **2b** activates Nrf2 by generating ROS or redox changes. Beas-2B cells were co-incubated with compound **2b** and with or without *N*-acetylcysteine (NAC, 10 mM), and the expression of GCLM, NQO1, and HO1 was measured 24 h later. We found that **2b** potentially increases the expression of Nr2-regulated antioxidant genes in the presence of NAC (Figure 6). NAC alone showed no induction of Nrf2-regulated genes. Taken together, these results suggest that the activation of Nrf2 by **2b** is independent of ROS or redox changes. Further studies are required to determine whether compound **2b** activates Nrf2 by direct thiol modification of Keap1.

CONCLUSIONS

We have identified a novel chalcone **2b** as a potent activator of Nrf2 signaling pathway after screening a series of chalcone derivatives using cell-based and mouse models. Further studies are needed to evaluate and develop chalcone **2b** as a potential drug for the treatment of inflammatory disorders.

EXPERIMENTAL SECTION

Chemistry. *General Methods.* TLCs were run on precoated Merck silica gel 60F254 plates and observed under UV light. The products were isolated and purified by crystallization or using a Teledyne ISCO Rf flash chromatography system with hexanes and ethyl acetate as eluents. The ^{1}H (400 MHz), ^{13}C (101 MHz), gCOSY, and gHSQC NMR spectra were taken on a Varian 400-MR spectrophotometer using TMS as an internal standard. Chemical shifts (δ) are expressed in ppm. Coupling constants (J) are expressed in Hz, and splitting patterns are described as follows: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; dd = doublet of doublets; dt = doublet of triplets; td = triplet of doublets; ddd = doublet of doublet of doublets. For the verification of the product and purity analysis, the LC−MS results were taken on an Agilent 1200 series system with an Agilent 6210 time-of-flight (TOF) mass detector using Agilent Eclipse XDB-C-18 column (5 mm, 4.6 mm × 150 mm) using a flow rate of 0.9 mL/min and solvent system water (with 0.1% formic acid)/acetonitrile (ACN) (gradient, 50% ACN at 0 min, 80% ACN at 7 min, 80% ACN at 10 min, and 50% ACN at 15 min). All synthesized compounds were >95% pure (exact purity is given in the subsequent sections with the characterization data). All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and were used without further purification.

General Procedure for Synthesis of Chalcones. In a 14 mL vial, the substituted acetophenone (1.25 mmol) and lithium hydroxide monohydrate (0.25 mmol) were dissolved in ethanol (5 mL), and the mixture was stirred at room temperature for 10 min followed by addition of substituted benzaldehyde (1.27 mmol). The reaction mixture was then stirred at room temperature and monitored by TLC using 25% ethyl acetate/hexanes as the solvent system. The reaction was quenched after 2 h by pouring into 50 mL of stirring ice cold water. If the product precipitated out after quenching with cold water, it was filtered off and crystallized with hot ethanol. In some examples, a sticky mass was observed in the aqueous solution after quenching. In those cases, the product was extracted by ethyl acetate (3 × 50 mL), dried over sodium sulfate, and concentrated under vacuum. The crude product was purified by flash chromatography using ethyl acetate/hexanes as the solvent system in increasing order of polarity.

*(E)-2,3-Diphenylprop-2-en-1-one (**1a**).* It was obtained as a light yellow solid in 80% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.16−8.14 (m, 1H), 8.13 (t, J = 1.71, 1.71 Hz, 1H), 7.92 (d, J = 15.67 Hz, 1H), 7.89−7.85 (m, 2H), 7.74 (d, J = 15.68 Hz, 1H), 7.69−7.63 (m, 1H), 7.59−7.53 (m, 2H), 7.47−7.42 (m, 3H). ^{13}C NMR (101 MHz, DMSO) δ 189.67, 144.48, 137.99, 135.08, 133.60, 131.09, 129.37, 129.34, 129.24, 128.96, 122.51. LC−MS (ESI-TOF): m/z 209.0963 ($[C_{15}H_{12}O + H]^+$ calcd 209.0961). Purity 100.00% (rt 7.39 min).

*(E)-1-(2-Methoxyphenyl)-3-phenylprop-2-en-1-one (**1b**).* It was obtained as a yellow oil in 71% yield. ^{1}H NMR (400 MHz, DMSO) δ 7.76−7.67 (m, 2H), 7.56−7.46 (m, 3H), 7.44−7.37 (m, 4H), 7.18 (d, J = 7.9 Hz, 1H), 7.05 (td, J = 7.5, 0.9 Hz, 1H), 3.85 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 192.60, 158.17, 142.97, 135.01, 133.53, 130.94, 129.98, 129.45, 129.23, 128.97, 127.41, 121.01, 112.79, 56.27. LC−MS (ESI-TOF): m/z 239.1072 ($[C_{16}H_{14}O_2 + H]^+$ calcd 239.1067). Purity 98.02% (t_R = 7.21 min).

*(E)-1-(3-Methoxyphenyl)-3-phenylprop-2-en-1-one (**1c**).* It was obtained as a yellow oil in 54% yield. ^{1}H NMR (400 MHz, DMSO) δ 7.95−7.85 (m, 3H), 7.78−7.70 (m, 2H), 7.62−7.58 (m, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.47−7.42 (m, 3H), 7.23 (ddd, J = 8.2, 2.6, 0.8 Hz, 1H), 3.84 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 189.36, 159.99, 144.57, 139.42, 135.07, 131.12, 130.40, 129.42, 129.36, 122.51, 121.52, 119.69, 113.41, 55.83. LC−MS (ESI-TOF): m/z 239.1071 ($[C_{16}H_{14}O_2 + H]^+$ calcd 239.1067). Purity 98.52% (t_R = 7.84 min).

*(E)-1-(4-Methoxyphenyl)-3-phenylprop-2-en-1-one (**1d**).* It was obtained as a white solid in 76% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.16 (d, J = 9.0 Hz, 2H), 7.94 (d, J = 15.6 Hz, 1H), 7.90−7.83 (m, 2H), 7.69 (d, J = 15.6 Hz, 1H), 7.44 (dd, J = 5.1, 1.9 Hz, 3H), 7.07 (d, J = 9.0 Hz, 2H), 3.85 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 187.79, 163.68, 143.60, 135.24, 131.39, 130.88, 130.85, 129.33, 129.24, 122.43, 114.48, 56.03. LC−MS (ESI-TOF): m/z 239.1068 ($[C_{16}H_{14}O_2 + H]^+$ calcd 239.1067). Purity 100.00% (t_R = 7.36 min).

*(E)-3-Phenyl-1-(2,4-dimethoxyphenyl)prop-2-en-1-one (**1e**).* It was obtained as a yellow oil in 40% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.76 (d, J = 8.6 Hz, 1H), 7.68 (d, J = 15.8 Hz, 1H), 7.60 (dd, J = 7.3, 1.8 Hz, 2H), 7.52 (d, J = 15.8 Hz, 1H), 7.43−7.34 (m, 3H), 6.57 (dd, J = 8.6, 2.2 Hz, 1H), 6.50 (d, J = 2.1 Hz, 1H), 3.91 (s, 3H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 189.73, 164.44, 160.71, 141.62, 135.32, 132.51, 130.65, 129.43, 128.78, 127.52, 121.79, 106.46, 99.07, 56.42, 56.06. LC−MS (ESI-TOF): m/z 269.1171 ($[C_{17}H_{16}O_3 + H]^+$ calcd 269.1172). Purity 96.00% (t_R = 7.25 min).

*(E)-3-Phenyl-1-(2,6-dimethoxyphenyl)prop-2-en-1-one (**1f**).* It was obtained as a white solid in 60% yield. ^{1}H NMR (400 MHz, DMSO) δ = 7.72−7.58 (m, 2H), 7.45−7.31 (m, 4H), 7.17 (d, J = 16.2 Hz, 1H), 6.97 (d, J = 16.2 Hz, 1H), 6.74 (d, J = 8.4 Hz, 2H), 3.70 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ = 194.53, 157.29, 144.91, 134.61, 131.35, 131.11, 129.43, 129.04, 129.01, 118.25, 104.86, 56.24. LC−MS (ESI-TOF): m/z 269.1175 ($[C_{17}H_{16}O_3 + H]^+$ calcd 269.1172). Purity 100.00% (t_R = 6.19 min).

*(E)-3-Phenyl-1-(2,5-dimethoxyphenyl)prop-2-en-1-one (**1g**).* It was obtained as a yellow oil in 62% yield. ^{1}H NMR (400 MHz, DMSO) δ = 7.76−7.67 (m, 2H), 7.50 (d, J = 16.0 Hz, 1H), 7.43 (d, J = 2.7 Hz, 3H), 7.40 (d, J = 12.2 Hz, 1H), 7.14−7.09 (m, 2H), 7.03 (dd, J = 2.6, 0.8 Hz, 1H), 3.80 (s, 3H), 3.73 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 192.17, 153.47, 152.36, 143.16, 135.00, 130.98, 129.67, 129.46, 128.99, 127.25, 119.02, 114.36, 114.33, 56.80, 56.00. LC−MS (ESI-TOF): m/z 269.1170 ($[C_{17}H_{16}O_3 + H]^+$ calcd 269.1172). Purity 98.59% (t_R = 7.31 min).

*(E)-3-Phenyl-1-(3,4-dimethoxyphenyl)prop-2-en-1-one (**1h**).* It was obtained as a yellow oil in 62% yield. ^{1}H NMR (400 MHz, DMSO) δ 7.95 (d, J = 15.6 Hz, 1H), 7.93−7.85 (m, 3H), 7.70 (d, J = 15.6 Hz, 1H), 7.60 (d, J = 2.0 Hz, 1H), 7.44 (dd, J = 1.9, 5.1 Hz, 3H), 7.09 (d, J = 8.5 Hz, 1H), 3.86 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 187.77, 153.67, 149.23, 143.53, 135.25, 130.89, 130.87, 129.32, 129.27, 123.87, 122.36, 111.31, 111.11, 56.23, 56.02. LC−MS (ESI-TOF): m/z 269.1173 ($[C_{17}H_{16}O_3 + H]^+$ calcd 269.1172). Purity 97.87% (t_R = 6.33 min).

*(E)-3-Phenyl-1-(3,5-dimethoxyphenyl)prop-2-en-1-one (**1i**).* It was obtained as a yellow oil in 66% yield. ^{1}H NMR (400 MHz, DMSO) δ 7.94−7.86 (m, 3H), 7.73 (d, J = 15.6 Hz, 1H), 7.44 (dd, J = 2.6, 3.8 Hz, 3H), 7.25 (d, J = 2.3 Hz, 2H), 6.78 (t, J = 2.3 Hz, 1H), 3.82 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ 189.16, 161.14, 144.70, 140.05, 135.06, 131.12, 129.48, 129.33, 122.46, 106.71, 105.53, 56.01. LC−MS (ESI-TOF): m/z 269.1176 ($[C_{17}H_{16}O_3 + H]^+$ calcd 269.1172). Purity 100.00% (t_R = 8.09 min).

*(E)-3-Phenyl-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**1j**).* It was obtained as a white solid in 68% yield. ^{1}H NMR (400 MHz, DMSO) δ 7.99−7.85 (m, 3H), 7.73 (d, J = 15.5 Hz, 1H), 7.52−7.36 (m, 5H), 3.89 (s, 6H), 3.75 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 188.34, 153.37, 144.31, 142.44, 135.15, 133.39, 131.03, 129.42, 129.32, 122.35, 106.62, 60.64, 56.67. LC−MS (ESI-TOF): m/z 299.1284 ($[C_{18}H_{18}O_4 + H]^+$ calcd 299.1278). Purity 100.00% (t_R = 6.97 min).

*(E)-3-Phenyl-1-(2,3,4-trimethoxyphenyl)prop-2-en-1-one (**1k**).* It was obtained as a white solid in 66% yield. ^{1}H NMR (400 MHz, DMSO) δ = 7.73 (dd, J = 6.8, 2.8 Hz, 2H), 7.54 (d, J = 15.9 Hz, 1H), 7.48−7.39 (m, 4H), 7.37 (d, J = 8.7 Hz, 1H), 6.93 (d, J = 8.8 Hz, 1H), 3.86 (s, 3H), 3.83 (s, 3H), 3.77 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 190.50, 157.16, 153.34, 142.86, 142.07, 135.08, 130.88, 129.47, 128.89, 126.98, 126.58, 125.60, 108.34, 62.16, 60.96, 56.54. LC−MS (ESI-TOF): m/z 299.1275 ($[C_{18}H_{18}O_4 + H]^+$ calcd 299.1278). Purity 100.00% (t_R = 7.19 min).

*(E)-3-Phenyl-1-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (**1l**).* It was obtained as a yellow oil in 71% yield. ^{1}H NMR (400 MHz, DMSO) δ = 7.64 (dd, J = 6.6, 3.1 Hz, 2H), 7.39 (dd, J = 5.1, 1.8 Hz, 3H), 7.19 (d, J = 16.1 Hz, 1H), 6.94 (d, J = 16.1 Hz, 1H), 6.30 (s, 2H), 3.82 (s, 3H), 3.70 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ = 193.70, 162.41, 158.53, 143.97, 134.81, 130.90, 129.46, 129.40, 128.91, 111.45, 91.53, 56.26, 55.91. LC−MS (ESI-TOF): m/z 299.1276 ($[C_{18}H_{18}O_4 + H]^+$ calcd 299.1278). Purity 100.00% (t_R = 6.23 min).

*(E)-1-Phenyl-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (**2a**).* It was obtained as a yellow solid in 64% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.35 (d, J = 7.8 Hz, 1H), 8.25−8.15 (m, 2H), 8.05 (d, J = 15.3 Hz, 1H), 7.98 (dd, J = 15.5 Hz, 2.0 Hz, 1H), 7.89−7.76 (m, 2H), 7.76−7.65 (m, 2H), 7.61 (t, J = 7.7 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ = 189.33, 138.24 (d, J = 1.8 Hz), 137.47, 134.01, 133.42, 133.22 (d, J = 1.5 Hz), 130.99, 129.32, 129.26, 129.16, 127.96 (q, J = 29.2 Hz), 126.64, 126.62 (q, J = 6.0 Hz), 124.61 (q, J = 274.5 Hz). LC−MS (ESI-TOF): m/z 277.0833 ($[C_{16}H_{11}F_3O + H]^+$ calcd 277.0835). Purity 100.00% (t_R = 6.97 min).

*(E)-1-(2-Methoxyphenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (**2b**).* It was obtained as a yellow oil in 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.90−7.82 (m, 1H), 7.71 (d, J = 7.8 Hz, 1H), 7.63 (d, J = 7.8 Hz, 1H), 7.55 (dd, J = 7.6 Hz, 1.8, 1H), 7.50 (t, J = 7.6 Hz, 1H), 7.45−7.36 (m, 2H), 7.22 (d, J = 15.7 Hz, 1H), 6.97 (td, J = 7.5 Hz, 0.8 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 3.82 (s, 4H). ^{13}C NMR (101 MHz, DMSO) δ = 192.21, 158.40, 136.83 (d, J = 2.1 Hz), 134.05, 133.58, 133.29 (d, J = 1.6 Hz), 131.30, 130.82, 130.15, 128.79, 128.58, 127.81 (q, J = 29.2 Hz), 126.68 (q, J = 5.2 Hz), 124.55 (q, J = 274.5 Hz), 121.07, 112.76, 56.31. LC−MS (ESI-TOF): m/z 304.0940 ($[C_{17}H_{13}F_3O_2 + H]^+$ calcd 307.0940). Purity 96.40% (t_R = 8.69 min).

*(E)-1-(3-Methoxyphenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (**2c**).* It was obtained as a yellow solid in 26% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.35 (d, J = 7.9 Hz, 1H), 8.06−7.94 (m, 2H), 7.88−7.76 (m, 3H), 7.72−7.63 (m, 2H), 7.52 (t, J = 7.9 Hz, 1H), 7.28 (ddd, J = 8.2 Hz, 2.7 Hz, 0.8 Hz, 1H), 3.87 (s, 3H). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.13 (d, J = 15.6 Hz, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.74 (d, J = 7.8 Hz, 1H), 7.60 (dd, J = 13.7 Hz, 7.1 Hz, 2H), 7.55−7.47 (m, 2H), 7.41 (dd, J = 15.9 Hz, 8.5 Hz, 2H), 7.15 (dd, J = 8.2 Hz, 1.9 Hz, 1H), 3.89 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 189.12, 160.04, 138.92, 138.33 (d, J = 2.2 Hz), 133.41, 133.21 (d, J = 1.7 Hz), 131.00, 130.47, 129.32, 127.96 (d, J = 29.2 Hz), 126.71, 126.62 (d, J = 6. 0 Hz), 124.62 (d, J = 273.5 Hz), 121.69, 120.04, 113.64, 55.86. LC−MS (ESI-TOF): m/z 304.0945 ($[C_{17}H_{13}F_3O_2 + H]^+$ calcd 307.0940). Purity 100.00% (t_R = 9.13 min).

*(E)-1-(4-Methoxyphenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (**2d**).* It was obtained as a yellow solid in 37% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.34 (d, J = 7.9 Hz, 1H), 8.24−8.17 (m, 2H), 8.04 (d, J = 15.3 Hz, 1H), 7.95 (dd, J = 15.4 Hz, 2.2, 1H), 7.87−7.76 (m, 2H), 7.67 (t, J = 7.6 Hz, 1H), 7.16−7.07 (m, 2H), 3.89 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 187.42, 164.00, 137.46 (d, J = 2.2 Hz), 133.45 (d, J = 1.6 Hz), 133.38, 131.63, 130.78, 130.40, 129.21, 127.87 (d, J = 29.2 Hz), 126.67, 126.58 (d, J = 6.0 Hz), 124.64 (d, J = 274.5 Hz), 114.58, 56.08. LC−MS (ESI-TOF): m/z 304.0944 ($[C_{17}H_{13}F_3O_2 + H]^+$ calcd 307.0940). Purity 100.00% (t_R = 8.75 min).

*(E)-1-(2,4-Dimethoxyphenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (**2e**).* It was obtained as a yellow solid in 50% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.07 (d, J = 7.6 Hz, 1H), 7.79 (dt, J = 14.8 Hz, 7.9 Hz, 3H), 7.69−7.60 (m, 3H), 6.71 (d, J = 2.3 Hz, 1H), 6.67 (dd, J = 8.6 Hz, 2.3 Hz, 1H), 3.92 (s, 3H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 189.18, 164.88, 161.01, 135.47 (d, J = 2.0 Hz), 133.64 (d, J = 1.3 Hz), 133.59, 132.74, 131.55, 130.55, 128.69, 127.74 (d, J = 29.2 Hz), 126.64 (d, J = 6.0 Hz), 124.62 (d, J = 274.5 Hz). 121.23, 106.69, 99.04, 56.50, 56.13. LC−MS (ESI-TOF): m/z 337.1050 ($[C_{18}H_{15}F_3O_3 + H]^+$ calcd 337.1046). Purity 100.00% (t_R = 8.71 min).

*(E)-1-(2,6-Dimethoxyphenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (**2f**).* It was obtained as a white solid in 67% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.07 (s, 1H), 8.01 (d, J = 7.9 Hz, 1H), 7.74 (d, J = 7.8 Hz, 1H), 7.61 (t, J = 7.8 Hz, 1H), 7.38 (t, J = 8.4 Hz, 1H), 7.30 (d, J = 16.3 Hz, 1H), 7.14 (d, J = 16.2 Hz, 1H), 6.74 (d, J = 8.5 Hz, 2H), 3.70 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ 194.45, 157.36, 142.84, 135.92, 132.43, 131.45, 130.69, 130.41, 130.26 (q, J = 31.1 Hz), 127.17 (q, J = 3.6 Hz), 125.92 (q, J = 3.8 Hz), 124.38 (q, J = 272.5 Hz), 118.22, 104.90, 56.27. LC−MS (ESI-TOF): m/z 337.1045 ($[C_{18}H_{15}F_3O_3 + H]^+$ calcd 337.1046). Purity 100.00% (t_R = 7.68 min).

*(E)-1-(2,5-Dimethoxyphenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (**2g**).* It was obtained as a yellow solid in 80% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.09 (d, J = 7.9 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.80−7.72 (m, 2H), 7.65 (t, J = 7.6 Hz, 1H), 7.51 (d, J = 15.7 Hz, 1H), 7.16 (d, J = 1.8 Hz, 2H), 7.09 (t, J = 1.8 Hz, 1H), 3.83 (s, 3H), 3.76 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 191.72, 153.50, 152.64, 136.95 (d, J = 2.1 Hz), 133.59, 133.28 (d, J = 1.7 Hz), 131.14, 130.86, 128.96, 128.79, 127.82 (q, J = 29.2 Hz), 126.69 (q, J = 16.6 Hz), 124.56 (d, J = 273.5 Hz), 119.72, 114.41, 114.33, 56.80, 56.04. LC−MS (ESI-TOF): m/z 337.1049 ($[C_{18}H_{15}F_3O_3 + H]^+$ calcd 337.1046). Purity 100.00% (t_R = 8.76 min).

*(E)-1-(3,4-Dimethoxyphenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (**2h**).* It was obtained as a yellow solid in 72% yield. ^{1}H NMR

(400 MHz, DMSO) δ = 8.33 (d, J = 7.8 Hz, 1H), 8.04 (d, J = 15.3 Hz, 1H), 7.96 (dd, J = 6.2 Hz, 2.1 Hz, 1H), 7.94 (d, J = 2.0 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.80 (t, J = 7.6 Hz, 1H), 7.67 (t, J = 7.7 Hz, 1H), 7.63 (d, J = 2.0 Hz, 1H), 7.13 (d, J = 8.5 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 187.44, 154.02, 149.31, 137.42 (d, J = 2.0 Hz), 133.49 (d, J = 1.0 Hz), 133.37, 130.76, 130.44, 129.26, 127.85 (q, J = 29.2 Hz), 126.66, 126.58 (q, J = 5.2 Hz), 124.65 (q, J = 273.5 Hz), 124.22, 111.40, 111.28, 56.28, 56.07. LC−MS (ESI-TOF): m/z 337.1048 ($[C_{18}H_{15}F_3O_3 + H]^+$ calcd 337.1046). Purity 100.00% (t_R = 7.77 min).

*(E)-1-(3,5-Dimethoxyphenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (**2i**).* It was obtained as a yellow oil in 72% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.36 (d, J = 7.9 Hz, 1H), 7.98 (s, 2H), 7.85 (d, J = 7.8 Hz, 1H), 7.80 (t, J = 7.6 Hz, 1H), 7.68 (t, J = 7.6 Hz, 1H), 7.30 (d, J = 2.2 Hz, 2H), 6.83 (t, J = 2.2 Hz, 1H), 3.85 (s, 6H). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.12 (d, J = 13.9 Hz, 1H), 7.82 (d, J = 7.8 Hz, 1H), 7.73 (d, J = 7.8 Hz, 1H), 7.61 (t, J = 7.6 Hz, 1H), 7.51 (t, J = 7.7 Hz, 1H), 7.35 (d, J = 15.6 Hz, 1H), 7.14 (d, J = 2.2 Hz, 2H), 6.69 (t, J = 2.1 Hz, 1H), 3.86 (s, 7H). ^{13}C NMR (101 MHz, DMSO) δ = 188.97, 161.20, 139.53, 138.45 (d, J = 2.2 Hz), 133.39, 133.18 (d, J = 1.6 Hz), 131.00, 129.40, 127.96 (q, J = 29.2 Hz), 126.67, 129.60 (q, J = 5.2 Hz), 124.62 (q, J = 273.5 Hz), 106.93, 105.88, 56.05. LC−MS (ESI-TOF): m/z 337.1047 ($[C_{18}H_{15}F_3O_3 + H]^+$ calcd 337.1046). Purity 100.00% (t_R = 9.37 min).

*(E)-1-(3,4,5-Trimethoxyphenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (**2j**).* It was obtained as a yellow solid in 80% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.31 (d, J = 7.8 Hz, 1H), 8.02 (d, J = 15.2 Hz, 1H), 7.95 (dd, J = 15.4 Hz, 2.1 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 7.7 Hz, 1H), 7.66 (t, J = 7.6 Hz, 1H), 7.44 (s, 2H), 3.88 (s, 6H), 3.76 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 188.07, 153.41, 142.77, 138.14 (d, J = 2.0), 133.37, 132.86, 130.91, 129.40, 127.77 (q, J = 29.2 Hz), 126.61, 126.61 (q, J = 5.0 Hz), 124.63 (q, J = 274.5 Hz), 106.86, 60.66, 56.68. LC−MS (ESI-TOF): m/z 367.1160 ($[C_{19}H_{17}F_3O_4 + H]^+$ calcd 367.1152). Purity 100.00% (t_R = 8.38 min).

*(E)-1-(2,3,4-Trimethoxyphenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (**2k**).* It was obtained as a yellow solid in 58% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.10 (d, J = 7.7 Hz, 1H), 7.80 (dt, J = 22.4, 7.7 Hz, 3H), 7.66 (t, J = 7.6 Hz, 1H), 7.56 (d, J = 15.5 Hz, 1H), 7.46 (d, J = 8.8 Hz, 1H), 6.97 (d, J = 8.9 Hz, 1H), 3.89 (s, 3H), 3.86 (s, 3H), 3.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 189.74, 157.66, 153.66, 142.08, 136.44 (d, J = 2.1 Hz), 133.60, 133.46 (d, J = 2.0 Hz), 131.02, 130.73, 128.71, 127.78 (d, J = 29.2 Hz), 126.66 (d, J = 5.0 Hz), 125.95 (d, J = 7.0 Hz), 124.59 (d, J = 274.5 Hz), 108.48, 62.21, 60.98, 56.60. LC−MS (ESI-TOF): m/z 367.1152 ($[C_{19}H_{17}F_3O_4 + H]^+$ calcd 367.1152). Purity 96.17% (t_R = 8.70 min).

*(E)-1-(2,4,6-Trimethoxyphenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (**2l**).* It was obtained as a yellow solid in 72% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.04 (d, J = 7.8 Hz, 1H), 7.77 (d, J = 7.7 Hz, 1H), 7.71 (t, J = 7.5 Hz, 1H), 7.60 (t, J = 7.6 Hz, 1H), 7.48 (dd, J = 15.9, 2.2 Hz, 1H), 7.01 (d, J = 15.8 Hz, 1H), 6.30 (s, 2H), 3.82 (s, 3H), 3.70 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ = 193.37, 162.78, 162.78, 158.71, 138.33 (d, J = 2.2 Hz), 133.51, 133.07, 130.80, 128.83, 127.58 (q, J = 29.2 Hz), 126.61 (q, J = 6.0 Hz), 124.46 (q, J = 274.5 Hz), 110.73, 91.34, 56.23, 55.96. LC−MS (ESI-TOF): m/z 367.1157 ($[C_{19}H_{17}F_3O_4 + H]^+$ calcd 367.1152). Purity 96.17% (t_R = 7.58 min).

*(E)-1-Phenyl-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (**3a**).* It was obtained as a white solid in 68% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.36 (s, 1H), 8.26−8.18 (m, 3H), 8.15 (d, J = 15.7 Hz, 1H), 7.89−7.78 (m, 2H), 7.75−7.67 (m, 2H), 7.64−7.57 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 189.51, 142.61, 137.72, 136.28, 133.83, 133.36, 130.38, 130.26 (q, J = 28.1 Hz), 127.14 (q, J = 3.8 Hz), 125.62 (q, J = 3.7 Hz), 124.15 (q, J = 272.5 Hz), 124.38. LC−MS (ESI-TOF): m/z 277.0840 ($[C_{16}H_{11}F_3O + H]^+$ calcd 277.0835). Purity 100.00% (t_R = 9.20 min).

*(E)-1-(2-Methoxyphenyl)-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (**3b**).* It was obtained as a yellow oil in 45% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.57 (t, J = 1.9 Hz, 1H), 8.29−8.19 (m, 2H), 7.73 (t, J = 8.0 Hz, 1H), 7.69−7.61 (m, 2H), 7.61−7.57 (m, 1H), 7.57−7.52 (m, 1H), 7.22 (d, J = 7.9 Hz, 1H), 7.08 (td, J = 7.5, 0.9 Hz, 1H), 3.89 (s, 3H). LC−MS (ESI-TOF): m/z 304.0941 ($[C_{17}H_{13}F_3O_2 + H]^+$ calcd 307.0940). Purity 96.00% (t_R = 8.88 min).

*(E)-1-(3-Methoxyphenyl)-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (**3c**).* It was obtained as a white solid in 21% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.35 (s, 1H), 8.21 (d, J = 7.8 Hz, 1H), 8.11 (d, J = 15.7 Hz, 1H), 7.87−7.77 (m, 3H), 7.70 (t, J = 7.78 Hz, 1H), 7.66 (dd, J = 1.6, 2.5 Hz, 1H), 7.52 (t, J = 7.94 Hz, 1H), 7.27 (ddd, J = 0.8, 2.6, 8.2 Hz, 1H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 189.28, 160.03, 142.68, 139.18, 136.27, 133.32, 130.41, 130.36, 130.25 (q, J = 31.2 Hz), 127.15 (q, J = 3.7 Hz), 125.72 (q, J = 3.7 Hz), 124.50 (q, J = 272.5 Hz), 124.46, 121.70, 119.76, 113.68, 55.85. LC−MS (ESI-TOF): m/z 304.0945 ($[C_{17}H_{13}F_3O_2 + H]^+$ calcd 307.0940). Purity 100.00% (t_R = 9.35 min).

*(E)-1-(4-Methoxyphenyl)-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (**3d**).* It was obtained as a white solid in 59% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.33 (s, 1H), 8.24−8.20 (m, 2H), 8.18 (d, J = 7.8 Hz, 1H), 8.13 (d, J = 15.7 Hz, 1H), 7.82−7.74 (m, 2H), 7.69 (t, J = 7.8 Hz, 1H), 7.14−7.07 (m, 2H), 3.88 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 187.69, 163.85, 141.74, 136.45, 133.23, 131.57, 130.67, 130.34, 130.24 (q, J = 32.7 Hz), 126.93 (q, J = 3.8 Hz), 125.49 (q, J = 3.7 Hz), 124.52 (q, J = 272.5 Hz), 124.45, 114.49, 56.05. LC−MS (ESI-TOF): m/z 304.0951 ($[C_{17}H_{13}F_3O_2 + H]^+$ calcd 307.0940). Purity 100.00% (t_R = 8.99 min).

*(E)-1-(2,4-Dimethoxyphenyl)-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (**3e**).* It was obtained as a white solid in 52% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.11−8.03 (m, 2H), 7.77 (d, J = 7.8 Hz, 1H), 7.72−7.58 (m, 4H), 6.70 (d, J = 2.3 Hz, 1H), 6.66 (dd, J = 2.3, 8.6 Hz, 1H), 3.90 (s, 3H), 3.86 (s, 3H). ^{13}C NMR (101 MHz, D_2O) δ 189.57, 164.52, 160.77, 139.58, 136.52, 132.49, 131.97, 130.39, 130.18 (q, J = 31.2 Hz), 129.38, 126.69 (q, J = 3.7 Hz), 125.52 (q, J = 3.8 Hz, H), 124.37 (q, J = 272.5 Hz), 121.55, 106.44, 99.03, 56.38, 56.02. LC−MS (ESI-TOF): m/z 337.1044 ($[C_{18}H_{15}F_3O_3 + H]^+$ calcd 337.1046). Purity 100.00% (t_R = 8.93 min).

*(E)-1-(2,6-Dimethoxyphenyl)-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (**3f**).* It was obtained as a light yellow solid in 72% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.07 (d, J = 7.8 Hz, 1H), 7.76 (d, J = 7.8, 1H), 7.71 (dd, J = 11.4 Hz, 4.0 Hz, 1H), 7.61 (t, J = 7.6 Hz, 1H), 7.45 (d, J = 2.1 Hz, 1H), 7.40 (dd, J = 11.1 Hz, 5.7 Hz, 1H), 7.02 (d, J = 15.9 Hz, 1H), 6.75 (d, J = 8.4 Hz, 2H), 3.70 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ = 194.52, 157.33, 139.62 (d, J = 2.2 Hz), 133.52, 132.81 (d, J = 1.6 Hz), 132.57, 131.77, 131.02, 128.90, 127.59 (q, J = 30.2 Hz), 126.64 (q, J = 5.7 Hz), 124.38 (q, J = 274.5 Hz), 117.44, 104.69, 56.22. LC−MS (ESI-TOF): m/z 337.1050 ($[C_{18}H_{15}F_3O_3 + H]^+$ calcd 337.1046). Purity 98.65% (t_R = 7.83 min).

*(E)-1-(2,5-Dimethoxyphenyl)-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (**3g**).* It was obtained as a yellow oil in 71% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.12 (s, 1H), 8.08 (d, J = 7.8 Hz, 1H), 7.79 (d, J = 7.8 Hz, 1H), 7.67 (t, J = 7.8 Hz, 1H), 7.62 (d, J = 16.1 Hz, 1H), 7.55 (dd, J = 0.9, 16.0 Hz, 1H), 7.18−7.12 (m, 2H), 7.09−7.03 (m, 1H), 3.82 (d, J = 0.8 Hz, 3H), 3.76 (d, J = 0.9 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 192.15, 153.49, 152.47, 141.17, 136.27, 132.29, 130.48, 130.27 (q, J = 31.2 Hz), 129.50, 129.14, 127.06 (q, J = 3.7 Hz), 125.83 (q, J = 3.3 Hz), 124.42 (d, J = 272.5 Hz), 119.20, 114.42, 114.37, 56.84, 56.04. LC−MS (ESI-TOF): m/z 337.1048 ($[C_{18}H_{15}F_3O_3 + H]^+$ calcd 337.1046). Purity 96.60% (t_R = 8.96 min).

*(E)-1-(3,4-Dimethoxyphenyl)-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (**3h**).* It was obtained as a white solid in 64% yield. ^{1}H NMR (400 MHz, d_2o) δ 8.11 (dd, J = 11.7, 8.7 Hz, 3H), 7.98−7.91 (m, 1H), 7.79 (dd, J = 19.6, 12.0 Hz, 3H), 7.63 (d, J = 1.9 Hz, 1H), 7.13 (d, J = 8.5 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, d_2o) δ 187.59, 153.86, 149.24, 141.43, 139.26 (d, J = 1.3 Hz), 130.59, 130.23 (d, J = 31.2 Hz), 129.74, 126.02 (q, J = 3.7 Hz), 125.06, 124.45 (d, J = 272.5 Hz),

124.08, 111.29, 111.13, 56.21, 56.00. LC−MS (ESI-TOF): m/z 337.1041 ($[C_{18}H_{15}F_3O_3 + H]^+$ calcd 337.1046). Purity 98.47% (t_R = 8.03 min).

*(E)-1-(3,5-Dimethoxyphenyl)-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (**3i**).* It was obtained as a light yellow solid in 72% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.34 (s, 1H), 8.22 (d, J = 7.8 Hz, 1H), 8.08 (d, J = 15.7 Hz, 1H), 7.88−7.77 (m, 2H), 7.70 (t, J = 7.8 Hz, 1H), 7.32 (d, J = 2.3 Hz, 2H), 6.83 (t, J = 2.2 Hz, 1H), 3.85 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ 189.08, 161.18, 142.83, 139.77, 136.24, 133.33, 130.33, 130.24 (q, J = 32.2 Hz), 127.17 (q, J = 3.6 Hz), 125.85 (q, J = 3.7 Hz), 124.50 (q, J = 273.5 Hz), 124.36, 106.98, 105.52, 56.03. LC−MS (ESI-TOF): m/z 337.1049 ($[C_{18}H_{15}F_3O_3 + H]^+$ calcd 337.1046). Purity 100.00% (t_R = 9.55 min).

*(E)-1-(3,4,5-Trimethoxyphenyl)-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (**3j**).* It was obtained as a white solid in 60% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.28−8.19 (m, 2H), 8.06 (d, J = 15.7 Hz, 1H), 7.86−7.75 (m, 2H), 7.69 (t, J = 7.8 Hz, 1H), 7.44 (s, 2H), 3.89 (s, 6H), 3.76 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 188.34, 153.39, 142.69, 142.48, 136.33, 133.16, 132.95, 130.33, 130.24 (q, J = 32.2 Hz), 127.13 (q, J = 3.7 Hz), 126.02 (q, J = 3.7 Hz), 124.49 (q, J = 272.5 Hz), 124.34, 106.90, 60.66, 56.75. LC−MS (ESI-TOF): m/z 367.1155 ($[C_{19}H_{17}F_3O_4 + H]^+$ calcd 367.1152). Purity 96.17% (t_R = 8.61 min).

*(E)-1-(2,3,4-Trimethoxyphenyl)-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (**3k**).* It was obtained as a yellow oil in 46% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.13 (s, 1H), 8.09 (d, J = 7.8 Hz, 1H), 7.78 (d, J = 7.7 Hz, 1H), 7.71−7.56 (m, 3H), 7.42 (d, J = 8.7 Hz, 1H), 6.96 (d, J = 8.9 Hz, 1H), 3.89 (s, 3H), 3.85 (s, 3H), 3.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 190.46, 157.30, 153.41, 142.11, 140.87, 136.36, 132.23, 130.49, 130.27 (q, J = 31.2 Hz), 128.92, 126.96 (q, J = 3.7 Hz), 126.42, 125.69, 125.66 (q, J = 5.0 Hz), 124.43 (q, J = 272.5 Hz), 108.33, 62.14, 60.97, 56.57. LC−MS (ESI-TOF): m/z 367.1157 ($[C_{19}H_{17}F_3O_4 + H]^+$ calcd 367.1152). Purity 97.99% (t_R = 8.83 min).

*(E)-1-(2,4,6-Trimethoxyphenyl)-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (**3l**).* It was obtained as a light yellow solid in 69% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.05 (s, 1H), 8.00 (d, J = 7.8 Hz, 1H), 7.73 (d, J = 7.8 Hz, 1H), 7.61 (t, J = 7.8 Hz, 1H), 7.32 (d, J = 16.2 Hz, 1H), 7.11 (d, J = 16.2 Hz, 1H), 6.30 (s, 2H), 3.82 (s, 3H), 3.70 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ 193.58, 162.54, 158.63, 141.94, 136.11, 132.29, 130.39, 130.24 (q, J = 30.2 Hz), 127.00 (d, J = 4.0 Hz), 125.81 (d, J = 4.0 Hz), 124.40 (q, J = 272.5 Hz), 111.35, 91.55, 56.27, 55.93. LC−MS (ESI-TOF): m/z 367.1154 ($[C_{19}H_{17}F_3O_4 + H]^+$ calcd 367.1152). Purity 100.00% (t_R = 7.86 min).

*(E)-1-Phenyl-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (**4a**).* It was obtained as a white solid in 59% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.20−8.11 (m, 3H), 8.08 (d, J = 15.7 Hz, 2H), 7.83−7.75 (m, 3H), 7.72−7.65 (m, 1H), 7.62−7.54 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 189.53, 142.43, 139.13 (d, J = 1.3 Hz), 137.68, 133.87, 130.49 (q, J = 32.2 Hz), 129.91, 129.30, 129.11, 126.14 (q, J = 3.8 Hz), 125.13, 124.44 (q, J = 272.5 Hz). LC−MS (ESI-TOF): m/z 277.0834 ($[C_{16}H_{11}F_3O + H]^+$ calcd 277.0835). Purity 100.00% (t_R = 9.35 min).

*(E)-1-(2-Methoxyphenyl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (**4b**).* It was obtained as a yellow oil in 79% yield. ^{1}H NMR (400 MHz, DMSO) δ 7.97 (d, J = 8.1 Hz, 2H), 7.79 (d, J = 8.2 Hz, 2H), 7.72 (d, J = 8.2 Hz, 1H), 7.61−7.48 (m, 6H), 7.22 (d, J = 8.0 Hz, 1H), 7.08 (td, J = 7.5, 0.9 Hz, 1H), 3.88 (s, 3H), 3.86 (s, 2H). LC−MS (ESI-TOF): m/z 304.0948 ($[C_{17}H_{13}F_3O_2 + H]^+$ calcd 307.0940). Purity 94.00% (t_R = 9.09 min).

*(E)-1-(3-Methoxyphenyl)-4-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (**4c**).* It was obtained as a white solid in 61% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.14 (d, J = 8.1 Hz, 2H), 8.07 (d, J = 15.7 Hz, 1H), 7.85−7.80 (m, 3H), 7.79 (d, J = 4.3 Hz, 1H), 7.67−7.62 (m, 1H), 7.52 (t, J = 7.9 Hz, 1H), 7.27 (dd, J = 8.2, 2.6 Hz, 1H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 189.29, 160.04, 142.49, 139.13, 13.50 (d, J = 31.2 Hz), 130.45, 129.95, 126.12 (q, J = 3.7 Hz), 124.5 (d, J = 272.46 Hz), 125.20, 121.65, 119.90, 113.58, 55.86. LC−MS (ESI-TOF): m/z 304.0943 ($[C_{17}H_{13}F_3O_2 + H]^+$ calcd 307.0940). Purity 100.00% (t_R = 9.52 min).

*(E)-1-(4-Methoxyphenyl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (**4d**).* It was obtained as a white solid in 56% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.23−8.01 (m, 5H), 7.76 (dd, J = 21.4, 11.6 Hz, 3H), 7.08 (d, J = 8.2 Hz, 2H), 3.86 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 187.70, 163.89, 141.58, 139.30, 131.55, 130.60, 130.32 (d, J = 32.2 Hz), 129.78, 126.11 (dd, J = 3.8 Hz), 125.18, 124.5 (d, J = 272.5 Hz), 114.55, 56.06. LC−MS (ESI-TOF): m/z 304.0940 ($[C_{17}H_{13}F_3O_2 + H]^+$ calcd 307.0940). Purity 100.00% (t_R = 9.13 min).

*(E)-1-(2,4-Dimethoxyphenyl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (**4e**).* It was obtained as an off-white solid in 40% yield. ^{1}H NMR (400 MHz, DMSO) δ 7.92 (d, J = 7.4 Hz, 2H), 7.76 (d, J = 7.5 Hz, 2H), 7.70−7.50 (m, 3H), 6.74−6.57 (m, 2H), 3.89 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 189.45, 164.74, 160.94, 139.41, 132.65, 130.12) q, J = 32.2 Hz), 130.10, 129.33, 126.21 (dd, J = 3.5 Hz), 124.50 (d, J = 272.5 Hz), 121.48, 106.62, 99.07, 56.48, 56.10. LC−MS (ESI-TOF): m/z 337.1046 ($[C_{18}H_{15}F_3O_3 + H]^+$ calcd 337.1046). Purity 96.35% (t_R = 9.13 min).

*(E)-1-(2,6-Dimethoxyphenyl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (**4f**).* It was obtained as a light yellow solid in 64% yield. ^{1}H NMR (400 MHz, DMSO) δ 7.92 (d, J = 8.3 Hz, 2H), 7.75 (d, J = 8.2 Hz, 2H), 7.41 (t, J = 8.4 Hz, 1H), 7.29 (d, J = 16.2 Hz, 1H), 7.13 (d, J = 16.2 Hz, 1H), 6.77 (d, J = 8.5 Hz, 2H), 3.73 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ 194.25, 157.40, 142.53, 138.76 (d, J = 1.4 Hz), 131.59, 131.29, 130.55 (q, J = 31.2 Hz), 126.15 (q, J = 3.8 Hz), 124.42 (q, J = 272.5 Hz), 118.12, 104.93, 56.29. LC−MS (ESI-TOF): m/z 337.1047 ($[C_{18}H_{15}F_3O_3 + H]^+$ calcd 337.1046). Purity 100.00% (t_R = 7.97 min).

*(E)-1-(2,5-Dimethoxyphenyl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (**4g**).* It was obtained as a yellow oil in 45% yield. ^{1}H NMR (400 MHz, DMSO) δ 7.95 (d, J = 8.1 Hz, 2H), 7.77 (d, J = 8.2 Hz, 2H), 7.59 (d, J = 16.0 Hz, 1H), 7.54 (d, J = 16.0 Hz, 1H), 7.17−7.12 (m, 2H), 7.08−7.04 (m, 1H), 3.82 (s, 3H), 3.74 (s, 3H). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (dd, J = 17.3, 8.7 Hz, 5H), 7.51 (d, J = 15.9 Hz, 1H), 7.23 (d, J = 3.1 Hz, 1H), 7.06 (dd, J = 9.0, 3.2 Hz, 1H), 6.96 (d, J = 9.0 Hz, 1H), 3.88 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 191.85, 153.50, 152.62, 140.78, 139.13 (d, J = 1.4 Hz), 130.37 (d, J = 32.2 Hz), 129.72, 129.54, 129.28, 126.22 (q, J = 3.7 Hz), 124.46 (d, J = 272.5 Hz), 119.51, 114.44, 114.37, 56.83, 56.01. LC−MS (ESI-TOF): m/z 337.1048 ($[C_{18}H_{15}F_3O_3 + H]^+$ calcd 337.1046). Purity 96.42% (t_R = 9.16 min).

*(E)-1-(3,4-Dimethoxyphenyl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (**4h**).* It was obtained as a white solid in 64% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.31 (s, 1H), 8.20 (d, J = 7.8 Hz, 1H), 8.12 (d, J = 15.6 Hz, 1H), 7.99 (dd, J = 2.0, 8.5 Hz, 1H), 7.83−7.75 (m, 2H), 7.70 (t, J = 7.8 Hz, 1H), 7.62 (d, J = 2.0 Hz, 1H), 7.12 (d, J = 8.5 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 187.70, 153.90, 149.31, 141.68, 136.46, 133.10, 130.72, 130.33, 130.24 (d, J = 32.2 Hz), 126.93 (q, J = 3.6 Hz), 125.64 (q, J = 3.6 Hz), 124.52 (d, J = 272.4 Hz), 124.39, 124.24, 111.30, 111.21, 56.27, 56.09. LC−MS (ESI-TOF): m/z 337.1042 ($[C_{18}H_{15}F_3O_3 + H]^+$ calcd 337.1046). Purity 95.94% (t_R = 8.19 min).

*(E)-1-(3,4,5-Trimethoxyphenyl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (**4i**).* It was obtained as a white solid in 77% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.11 (d, J = 8.2 Hz, 2H), 8.06 (d, J = 15.6 Hz, 1H), 7.83−7.74 (m, 3H), 7.43 (s, 2H), 3.89 (s, 6H), 3.76 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 188.24, 153.40, 142.69, 142.27, 139.19 (d, J = 1.3 Hz), 133.08, 130.44 (q, J = 31.2 Hz), 129.95, 126.08 (q, J = 3.7 Hz), 125.04, 124.50 (q, J = 271.4 Hz), 106.80, 60.65, 56.70. LC−MS (ESI-TOF): m/z 367.1158 ($[C_{19}H_{17}F_3O_4 + H]^+$ calcd 367.1152). Purity 96.17% (t_R = 8.78 min).

*(E)-1-(2,3,4-Methoxyphenyl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (**4j**).* It was obtained as a white solid in 63% yield. ^{1}H NMR

(400 MHz, DMSO) δ 7.95 (d, J = 6.8 Hz, 2H), 7.77 (d, J = 7.0 Hz, 2H), 7.58 (s, 2H), 7.40 (d, J = 8.3 Hz, 1H), 6.94 (d, J = 8.6 Hz, 1H), 3.86 (s, 3H), 3.83 (s, 3H), 3.77 (s, 3H). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (dd, J = 23.7, 8.2 Hz, 5H), 7.59 (d, J = 15.9 Hz, 1H), 7.53 (d, J = 8.8 Hz, 1H), 6.78 (d, J = 8.8 Hz, 1H), 3.94 (s, 4H), 3.92 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ 190.22, 157.48, 153.53, 142.08, 140.54, 139.21, 130.30 (q, J = 32.2 Hz), 126.26, 126.23, 125.79, 124.48 (q, J = 271.5 Hz), 108.41, 62.17, 60.97, 56.58. LC−MS (ESI-TOF): m/z 367.1158 ($[C_{19}H_{17}F_3O_4 + H]^+$ calcd 367.1152). Purity 100.00% (t_R = 9.05 min).

(E)-1-(2,4,6-Methoxyphenyl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (**4k**). It was obtained as a yellow oil in 86% yield. ^{1}H NMR (400 MHz, DMSO) δ 7.91 (d, J = 8.2 Hz, 2H), 7.75 (d, J = 8.3 Hz, 2H), 7.31 (d, J = 16.2 Hz, 1H), 7.11 (d, J = 16.2 Hz, 1H), 6.32 (s, 2H), 3.84 (s, 3H), 3.73 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ 193.27, 162.66, 158.73, 141.53, 138.97 (d, J = 1.4 Hz), 130.33 (q, J = 32.2 Hz), 126.14 (q, J = 3.8 Hz), 124.45 (q, J = 271.4 Hz), 111.28, 91.57, 91.40, 56.29, 55.94. LC−MS (ESI-TOF): m/z 367.1152 ($[C_{19}H_{17}F_3O_4 + H]^+$ calcd 367.1152). Purity 96.54% (t_R = 8.03 min).

(E)-1-Phenyl-3-(2-nitrophenyl)prop-2-en-1-one (**5a**). It was obtained as an off-white solid in 46% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.24−8.20 (m, 1H), 8.20−8.15 (m, 2H), 8.11 (dd, J = 8.1, 1.2 Hz, 1H), 8.00 (d, J = 15.5 Hz, 1H), 7.91 (d, J = 15.5 Hz, 1H), 7.88−7.81 (m, 1H), 7.76−7.68 (m, 2H), 7.60 (t, J = 7.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ = 189.52, 149.24, 139.03, 137.48, 134.22, 133.98, 131.53, 130.19, 129.98, 129.33, 129.18, 126.83, 125.16. LC−MS (ESI-TOF): m/z 254.0819 ($[C_{15}H_{11}NO_3 + H]^+$ calcd 254.0812). Purity 100.00% (t_R = 6.68 min).

(E)-1-(2-Methoxyphenyl)-3-(2-nitrophenyl)prop-2-en-1-one (**5b**). It was obtained as a light yellow solid in 49% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.09 (dd, J = 8.2, 1.2 Hz, 1H), 7.99 (dd, J = 7.9, 1.2 Hz, 1H), 7.84−7.76 (m, 2H), 7.73−7.66 (m, 1H), 7.61−7.57 (m, 1H), 7.55 (dd, J = 7.7, 1.7 Hz, 1H), 7.42 (d, J = 15.7 Hz, 1H), 7.22 (d, J = 8.0 Hz, 1H), 7.08 (td, J = 7.5, 0.9 Hz, 1H), 3.90 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 192.08, 158.46, 149.07, 137.47, 134.36, 134.07, 131.33, 130.24, 130.22, 129.62, 128.56, 125.20, 121.06, 112.82, 56.32. LC−MS (ESI-TOF): m/z 284.0916 ($[C_{16}H_{13}NO_4 + H]^+$ calcd 284.0917). Purity 97.28% (t_R = 6.52 min).

(E)-1-(3-Methoxyphenyl)-3-(2-nitrophenyl)prop-2-en-1-one (**5c**). It was obtained as an off-white solid in 21% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.18 (d, J = 7.7 Hz, 1H), 8.08 (d, J = 8.0 Hz, 1H), 7.97 (d, J = 15.5 Hz, 1H), 7.83 (dd, J = 17.6, 11.4 Hz, 2H), 7.75 (d, J = 7.5 Hz, 1H), 7.69 (t, J = 7.6 Hz, 1H), 7.61 (s, 1H), 7.50 (t, J = 7.9 Hz, 1H), 7.25 (d, J = 6.4 Hz, 1H), 3.84 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 189.36, 160.02, 149.23, 139.13, 138.91, 134.21, 131.53, 130.50, 130.16, 130.02, 126.91, 125.14, 121.70, 120.06, 113.60, 55.87. LC−MS (ESI-TOF): m/z 284.0918 ($[C_{16}H_{13}NO_4 + H]^+$ calcd 284.0917). Purity 100.00% (t_R = 6.91 min).

(E)-1-(4-Methoxyphenyl)-3-(2-nitrophenyl)prop-2-en-1-one (**5d**). It was obtained as an off-white solid in 32% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.17 (t, J = 7.9 Hz, 3H), 8.07 (d, J = 8.1 Hz, 1H), 7.97−7.85 (m, 2H), 7.81 (t, J = 7.6 Hz, 1H), 7.68 (t, J = 7.7 Hz, 1H), 7.09 (d, J = 8.4 Hz, 2H), 3.86 (s, 3H). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.15−8.00 (m, 3H), 7.74 (d, J = 7.6 Hz, 1H), 7.68 (t, J = 7.6 Hz, 1H), 7.56 (t, J = 7.1 Hz, 1H), 7.32 (d, J = 15.6 Hz, 1H), 7.00 (d, J = 8.9 Hz, 2H), 3.90 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 187.58, 163.98, 149.23, 138.00, 134.15, 131.63, 131.36, 130.38, 130.26, 129.91, 126.89, 125.09, 114.59, 56.08. LC−MS (ESI-TOF): m/z 284.0919 ($[C_{16}H_{13}NO_4 + H]^+$ calcd 284.0917). Purity 100.00% (t_R = 6.55 min).

(E)-1-(2,4-Dimethoxyphenyl)-3-(2-nitrophenyl)prop-2-en-1-one (**5e**). It was obtained as a yellow solid in 57% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.06 (dd, J = 8.2 Hz, 1.2 Hz, 1H), 7.94 (d, J = 7.7 Hz, 1H), 7.82−7.73 (m, 2H), 7.69−7.61 (m, 2H), 7.51 (d, J = 15.6 Hz, 1H), 6.68 (d, J = 2.2 Hz, 1H), 6.64 (dd, J = 8.6, 2.3 Hz, 1H), 3.90 (s, 4H), 3.84 (s, 4H). ^{13}C NMR (101 MHz, DMSO) δ = 189.17, 164.87, 161.02, 149.09, 136.03, 134.33, 132.76, 131.63, 131.12, 130.48, 129.55, 125.14, 121.20, 106.68, 99.03, 56.48, 56.13. LC−MS (ESI-TOF): m/z 314.1027 ($[C_{17}H_{15}NO_5 + H]^+$ calcd 314.1023). Purity 97.57% (t_R = 6.62 min).

(E)-1-(2,6-Dimethoxyphenyl)-3-(2-nitrophenyl)prop-2-en-1-one (**5f**). It was obtained as a white solid in 82% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.06 (dd, J = 8.1, 1.2 Hz, 1H), 7.98 (dd, J = 7.8, 1.3 Hz, 1H), 7.78 (ddd, J = 7.8, 1.2, 0.6 Hz, 1H), 7.70−7.64 (m, 1H), 7.52 (d, J = 16.0 Hz, 1H), 7.40 (t, J = 8.4 Hz, 1H), 6.96 (d, J = 16.0 Hz, 1H), 6.77 (d, J = 8.4 Hz, 2H), 3.75 (s, 7H). ^{13}C NMR (101 MHz, DMSO) δ = 194.37, 157.44, 148.75, 140.06, 134.33, 132.66, 131.72, 131.52, 129.86, 129.66, 125.21, 117.59, 104.80, 56.26. LC−MS (ESI-TOF): m/z 314.1024 ($[C_{17}H_{15}NO_5 + H]^+$ calcd 314.1023). Purity 100.00% (t_R = 5.74 min).

(E)-1-(2,5-Dimethoxyphenyl)-3-(2-nitrophenyl)prop-2-en-1-one (**5g**). It was obtained as a yellow solid in 49% yield. ^{1}H NMR (400 MHz, D_2O) δ = 8.09 (dd, J = 8.1, 1.1 Hz, 1H), 7.97 (d, J = 7.7 Hz, 1H), 7.85−7.76 (m, 2H), 7.73−7.65 (m, 1H), 7.41 (d, J = 15.8 Hz, 1H), 7.16 (d, J = 1.8 Hz, 2H), 7.09 (t, J = 1.8 Hz, 1H), 3.84 (s, 3H), 3.76 (s, 3H). ^{13}C NMR (101 MHz, D_2O) δ = 191.59, 153.41, 152.61, 137.66, 134.31, 131.31, 131.11, 130.19, 129.56, 128.86, 125.14, 119.67, 114.33, 114.32, 56.74, 55.96. LC−MS (ESI-TOF): m/z 314.1029 ($[C_{17}H_{15}NO_5 + H]^+$ calcd 314.1023). Purity 100.00% (t_R = 6.63 min).

(E)-1-(3,4-Dimethoxyphenyl)-3-(2-nitrophenyl)prop-2-en-1-one (**5h**). It was obtained as a yellow solid in 17% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.20 (d, J = 7.8 Hz, 1H), 8.10 (dd, J = 8.1, 1.0 Hz, 1H), 7.99−7.88 (m, 3H), 7.84 (t, J = 7.6 Hz, 1H), 7.74−7.67 (m, 1H), 7.62 (d, J = 2.0 Hz, 1H), 7.13 (d, J = 8.5 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.06− 8.12 (m, 2H), 7.74 (d, J = 7.7 Hz, 1H), 7.72−7.65 (m, 2H), 7.62 (d, J = 1.7 Hz, 1H), 7.56 (t, J = 7.2 Hz, 1H), 7.31 (d, J = 15.7 Hz, 1H), 6.95 (d, J = 8.4 Hz, 1H), 3.99 (s, 3H), 3.98 (s, 3H). ^{13}C NMR (101 MHz, D_2O) δ = 187.54, 153.92, 149.23, 149.15, 137.91, 134.06, 131.25, 130.35, 130.26, 129.89, 126.80, 125.02, 124.14, 111.33, 111.21, 56.22, 56.00. LC−MS (ESI-TOF): m/z 314.1027 ($[C_{17}H_{15}NO_5 + H]^+$ calcd 314.1023). Purity 100.00% (t_R = 5.63 min).

(E)-1-(3,5-Dimethoxyphenyl)-3-(2-nitrophenyl)prop-2-en-1-one (**5i**). It was obtained as an off-white solid in 57% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.21 (dd, J = 7.8, 1.3 Hz, 1H), 8.10 (dd, J = 8.1, 1.2 Hz, 1H), 7.98 (d, J = 15.5 Hz, 1H), 7.89−7.80 (m, 2H), 7.74−7.68 (m, 1H), 7.28 (d, J = 2.3 Hz, 2H), 6.82 (t, J = 2.3 Hz, 1H), 3.85 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ = 189.21, 161.19, 149.24, 139.53, 139.25, 134.18, 131.52, 130.15, 130.09, 126.89, 125.13, 106.91, 105.89, 56.05. LC−MS (ESI-TOF): m/z 314.1024 ($[C_{17}H_{15}NO_5 + H]^+$ calcd 314.1023). Purity 100.00% (t_R = 7.21 min).

(E)-1-(3,4,5-Trimethoxyphenyl)-3-(2-nitrophenyl)prop-2-en-1-one (**5j**). It was obtained as an off-white solid in 31% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.13 (dd, J = 27.6, 8.0 Hz, 2H), 7.97 (d, J = 15.4 Hz, 1H), 7.92−7.79 (m, 2H), 7.70 (t, J = 7.7 Hz, 1H), 7.42 (s, 2H), 3.88 (s, 6H), 3.76 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 188.38, 153.39, 149.18, 142.64, 139.01, 134.20, 132.84, 131.45, 130.35, 130.12, 126.80, 125.16, 106.80, 60.66, 56.65. LC−MS (ESI-TOF): m/z 344.1130 ($[C_{18}H_{17}NO_6 + H]^+$ calcd 344.1129). Purity 99.00% (t_R = 6.25 min).

(E)-1-(2,3,4-Trimethoxyphenyl)-3-(2-nitrophenyl)prop-2-en-1-one (**5k**). It was obtained as a light white solid in 32% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.07 (d, J = 8.0 Hz, 1H), 7.97 (d, J = 7.6 Hz, 1H), 7.85−7.75 (m, 2H), 7.67 (t, J = 7.7 Hz, 1H), 7.43 (dd, J = 12.2 Hz, 3.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 3.77 (s, 3H). LC−MS (ESI-TOF): m/z 344.1127 ($[C_{18}H_{17}NO_6 + H]^+$ calcd 344.1129). Purity 100.00% (t_R = 6.52 min).

(E)-1-(2,4,6-Trimethoxyphenyl)-3-(2-nitrophenyl)prop-2-en-1-one (**5l**). It was obtained as a yellow solid in 61% yield. ^{1}H NMR (400 MHz, DMSO) δ = 8.03 (dd, J = 8.1 Hz, 1.0, 1H), 7.93 (d, J = 7.7 Hz, 1H), 7.75 (t, J = 7.6 Hz, 1H), 7.64 (t, J = 7.8 Hz, 1H), 7.53 (d, J = 15.9 Hz, 1H), 6.92 (d, J = 15.9 Hz, 1H), 6.30 (s, 2H), 3.82 (s, 3H), 3.72 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ = 193.25, 162.76, 158.80, 148.78, 138.79, 134.30,

133.17, 131.33, 130.06, 129.61, 125.18, 110.82, 91.42, 56.26, 55.94. LC−MS (ESI-TOF): m/z 344.1141 ($[C_{18}H_{17}NO_6 + H]^+$ calcd 344.1129). Purity 100.00% (t_R = 5.77 min).

Biology. *Cell Culture and Treatment.* Human bronchial epithelial (Beas-2B) cells were cultured in DMEM/F12 (pH 7.4) supplemented with 10% (v/v) FBS, 100 mg/L gentamicin, and genetisin. Beas-2B cells were grown in 48-well plates for 24 h and then treated with a series of chalcone derivatives dissolved in DMSO for various time points. The concentration of DMSO did not exceed 0.1%. RNA was isolated, and gene expression was measured after 16 h.

Cell Viability Assay. The cytotoxicity of chalcone derivatives was analyzed by using trypan blue exclusion test and was further confirmed by colorimetric methylthiazolydiphenyltetrazolium bromide (MTT) assay as described.[82] Briefly, Beas-2B cells were treated with chalcone analogues or DMSO alone (0.1%, as vehicle) for 24 h. Four hours before the end of incubation, the medium was removed and 100 μL of MTT (5 mg/mL in serum free medium) was added to each well. The MTT was removed after 4 h. Cells were washed with PBS, and 100 μL of DMSO was added to each well to dissolve the water-insoluble MTT-formazan crystals. The absorbance was recorded at 570 nm in a plate reader (Molecular Devices, Sunnyvale, CA).

Generation of Stable Transfectants. Beas-2B cells overexpressing ARE luciferase reporter plasmid were obtained by transfecting Beas-2B cells with 3 μg of NQO1-ARE reporter plasmid and 0.3 μg of pUB6 empty vector (Invitrogen, Carlsbad, CA). Stable transfectants were selected using blasticidin at 6 μg/mL. Stable clones were expanded and screened for the expression of ARE luciferase.[83]

ARE Reporter Assay. Beas-2B cells stably expressing NQO1-ARE luciferase were seeded onto a 96-well plate at a density of 10 000 cells/well for 16 h before incubation with test compounds. Next day, cells were treated with the indicated concentrations of compound **2b**. Cells were also treated with DMSO, which was used as the solvent. The reporter activity was measured after 16 h of exposure using the luciferase assay kit (Promega, Madison, WI). The level of increase in luciferase activity reflects the degree of Nrf2 activity.[83]

Mice in Vivo Study. All experiments in mice were performed in accordance with the standards established by the U.S. Animal Welfare Acts, set forth in NIH guidelines and the Policy and Procedures Manual of the Johns Hopkins University Animal Care and Use Committee. C57BL/6 mice (male, 7 weeks) were maintained on AIN 76A diet (Harlan Tekland, Madison, WI) and water ad libitum and housed at a temperature (range, 20−23 °C) under 12 h light/dark cycles. The mice were treated with chalcone analogues (50 mg/kg body weight) or vehicle or sulforaphane as a positive control by gavage. After 24 h of treatment, the small intestines were harvested and stored at −80 °C until analysis.

RNA Extraction and Gene Expression Analysis. Total RNA was extracted from cells/tissue using Qiagen RNeasy kit (Qiagen Corporation, Valencia, CA), and reverse transcription was performed by using random hexamers and MultiScribe reverse transcriptase according to the manufacturer's recommendations (Applied Biosystems, Foster City, CA, U.S.). Quantitative real-time RT-PCR analyses of Nrf2, NQO1, HO1, and GCLM were performed by using Assay-on-Demand primers and probe sets from Applied Biosystems. Assays were performed using the ABI 7000 Taqman system (Applied Biosystems, Foster City, CA). β-Actin was used for normalization.

Statistics. The values were determined as the mean ± SE and analyzed by student's t test. Differences were considered significant at $P \leq 0.05$.

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ABBREVIATIONS USED

Nrf2, nuclear factor erythroid 2 p45-related factor 2; b-ZIP, basic-leucine zipper; Keap 1, Kelch-like ECH-associated protein 1; ARE, antioxidant response element; HO-1, heme oxygenase 1; NQO1, NAD(P)H/quinone oxidoreductase 1; GCLM, glutamate−cysteine ligase modifier subunit

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