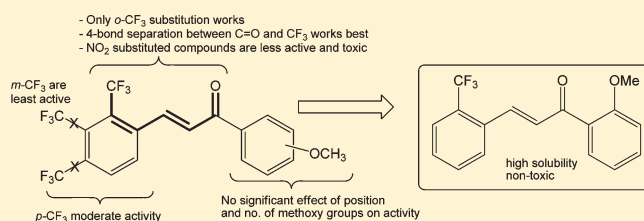


Novel Chalcone Derivatives as Potent Nrf2 Activators in Mice and Human Lung Epithelial Cells

Vineet Kumar,^{†,||} Sarvesh Kumar,^{†,||} Mohammad Hassan,[†] Hailong Wu,[‡] Rajesh K. Thimmulappa,[‡] Amit Kumar,[§] Sunil K. Sharma,[§] Virinder S. Parmar,[§] Shyam Biswal,^{*,‡} and Sanjay V. Malhotra^{*,†}[†]Laboratory of Synthetic Chemistry, SAIC Frederick, Inc., National Cancer Institute at Frederick, Frederick, Maryland 21702, United States[‡]Department of Environmental Health Sciences, Johns Hopkins School of Public Health, Johns Hopkins University, Baltimore, Maryland 21205, United States[§]Bioorganic Laboratory, Department of Chemistry, University of Delhi, Delhi 110007, India

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'-methoxychalcone (**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

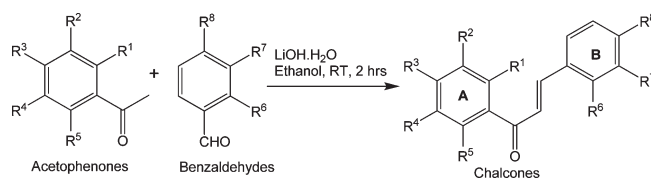
gene expression have been shown to exert chemopreventive activities, e.g., sulforaphane,^{4,20–22} dithiolethione,^{23–25} curcumin,²⁶ and caffeic acid phenethyl ester (CAPE).²⁶ It is notable that both curcumin and CAPE bear an α,β -unsaturated ketone moiety and can therefore act as Michael acceptors 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} antituberculosis,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 cells⁵³ 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

Received: February 28, 2011

Published: May 03, 2011

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



entry	chalcones	substituents on ring A					substituents on ring B			relative fold change ^a	
		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

Table 1. Continued

entry	chalcones	substituents on ring A					substituents on ring B			relative fold change ^a	
		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- κ B pathway, which is mediated by I κ B 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 thyl 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. Chalcones 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 NaNO₃/natural phosphate.⁶⁹ They can also be synthesized by acid-catalyzed aldol condensations, e.g.,

AlCl₃,⁷⁰ BF₃,⁷¹ dry HCl,⁷² Zn(bpy)(OAc)₂,⁷³ Cp₂ZrH₂/NiCl₂,⁷⁴ and RuCl₃ (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·H₂O 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·H₂O. 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, ¹³C 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 μ M) for 16 h and analyzed the expression of GCLM and NQO1 by

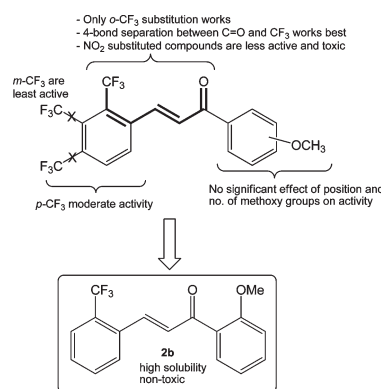
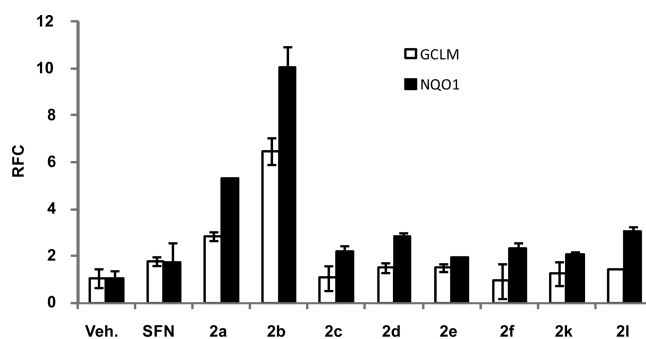
Table 2. List of Positive Leads from Primary Screening and Their Effect on Cell Viability

entry	compd	relative fold change ^a		% cell viability
		GCLM	NQO1	
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

^aData 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–I** without any substitution on ring B were not active. The activity of similar derivatives with trifluoromethyl (CF₃) substitution on ring B enhanced the activity dramatically. The position of CF₃ substitution was also crucial for the activity and cytotoxicity of these compounds. In general, the chalcone derivatives with CF₃ 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 CF₃-substituted chalcones were noncytotoxic. This shows that four-bond separation between carbonyl and CF₃ is crucial for the induction activity. Interestingly, with nitro (NO₂) 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 administered 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**

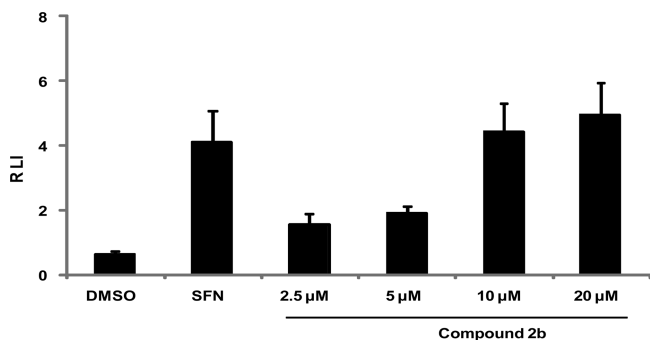


Figure 3. Levels of NQO1-ARE luciferase activity after treatment with compound **2b**. NQO1-ARE luciferase activity was measured by using stably transfected Beas-2B cells after treatment with compound **2b** or sulforaphane (SFN) or dimethylsulfoxide (DMSO). The exposure to compound **2b** resulted in 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 \pm SD of quadruplicate wells ($P \leq 0.05$).

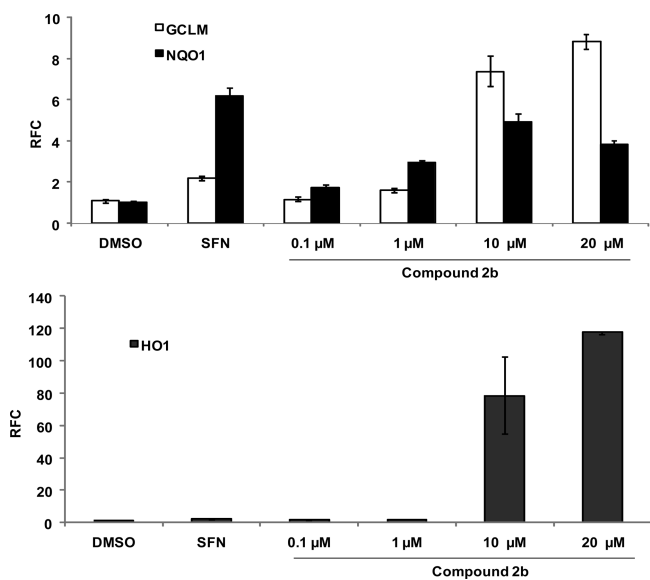


Figure 4. Expression of Nrf2-regulated genes after treatment with compound **2b**. Human bronchial epithelial cells (Beas-2B) were treated with compound **2b** at the indicated concentrations for 16–20 h. The expression of Nrf2-regulated genes GCLM, HO1, and NQO1 was analyzed in the tissues by qRT-PCR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

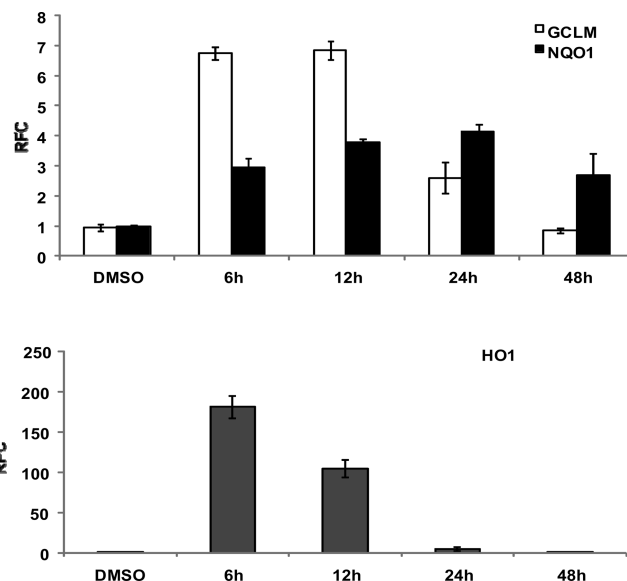


Figure 5. Time-dependent increase in Nrf2-regulated genes after treatment with compound **2b**. Human bronchial epithelial cells (Beas-2B) were treated with compound **2b** (10 μ M) at various time points. The expression of Nrf2-regulated genes GCLM, HO1, and NQO1 was analyzed in the tissues by qRT-PCR.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

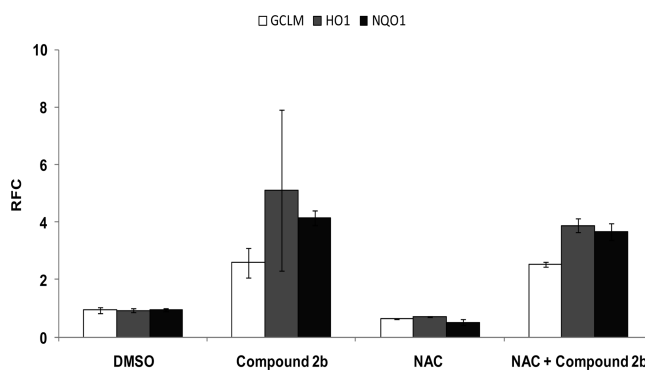


Figure 6. Activation of Nrf2 genes by compound **2b** is independent of ROS generation. Human bronchial epithelial cells (Beas-2B) were treated with compound **2b** (10 μ M) in the presence of an antioxidant, N-acetylcysteine (10 mM, NAC). Cells were harvested 24 h after the treatment, and the Nrf2-driven 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 \pm SD of triplicate wells ($P \leq 0.05$).

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 μ M) 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 \sim 5- and 10-fold increase in the expression of GCLM and NQO1, respectively, at the highest concentration (20 μ M) with no cytotoxicity. Interestingly, we

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 Nrf2-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 ¹H (400 MHz), ¹³C (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 \times 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 \times 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. ¹H 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). ¹³C 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 ($[\text{C}_{15}\text{H}_{12}\text{O} + \text{H}]^+$ calcd 209.0961). Purity 100.00% (t_{R} 7.39 min).

(*E*)-1-(2-Methoxyphenyl)-3-phenylprop-2-en-1-one (**1b**). It was obtained as a yellow oil in 71% yield. ¹H 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). ¹³C 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 ($[\text{C}_{16}\text{H}_{14}\text{O}_2 + \text{H}]^+$ 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. ¹H 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). ¹³C 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 ($[\text{C}_{16}\text{H}_{14}\text{O}_2 + \text{H}]^+$ 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. ¹H 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). ¹³C 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 ($[\text{C}_{16}\text{H}_{14}\text{O}_2 + \text{H}]^+$ 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. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C 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 ($[\text{C}_{17}\text{H}_{16}\text{O}_3 + \text{H}]^+$ 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. ¹H 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). ¹³C 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 ($[\text{C}_{17}\text{H}_{16}\text{O}_3 + \text{H}]^+$ 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. $^1\text{H NMR}$ (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). $^{13}\text{C NMR}$ (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 ($[\text{C}_{17}\text{H}_{16}\text{O}_3 + \text{H}]^+$ 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. $^1\text{H NMR}$ (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). $^{13}\text{C NMR}$ (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 ($[\text{C}_{17}\text{H}_{16}\text{O}_3 + \text{H}]^+$ 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. $^1\text{H NMR}$ (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). $^{13}\text{C NMR}$ (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 ($[\text{C}_{17}\text{H}_{16}\text{O}_3 + \text{H}]^+$ 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. $^1\text{H NMR}$ (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). $^{13}\text{C NMR}$ (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 ($[\text{C}_{18}\text{H}_{18}\text{O}_4 + \text{H}]^+$ 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. $^1\text{H NMR}$ (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). $^{13}\text{C NMR}$ (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 ($[\text{C}_{18}\text{H}_{18}\text{O}_4 + \text{H}]^+$ 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. $^1\text{H NMR}$ (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). $^{13}\text{C NMR}$ (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 ($[\text{C}_{18}\text{H}_{18}\text{O}_4 + \text{H}]^+$ 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. $^1\text{H NMR}$ (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). $^{13}\text{C NMR}$ (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 ($[\text{C}_{16}\text{H}_{11}\text{F}_3\text{O} + \text{H}]^+$ 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. $^1\text{H NMR}$ (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). $^{13}\text{C NMR}$ (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 ($[\text{C}_{17}\text{H}_{13}\text{F}_3\text{O}_2 + \text{H}]^+$ 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. $^1\text{H NMR}$ (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). $^1\text{H NMR}$ (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). $^{13}\text{C NMR}$ (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 ($[\text{C}_{17}\text{H}_{13}\text{F}_3\text{O}_2 + \text{H}]^+$ 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. $^1\text{H NMR}$ (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). $^{13}\text{C NMR}$ (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 ($[\text{C}_{17}\text{H}_{13}\text{F}_3\text{O}_2 + \text{H}]^+$ 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. $^1\text{H NMR}$ (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). $^{13}\text{C NMR}$ (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 ($[\text{C}_{18}\text{H}_{15}\text{F}_3\text{O}_3 + \text{H}]^+$ 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. $^1\text{H NMR}$ (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). $^{13}\text{C NMR}$ (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 ($[\text{C}_{18}\text{H}_{15}\text{F}_3\text{O}_3 + \text{H}]^+$ 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. $^1\text{H NMR}$ (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). $^{13}\text{C NMR}$ (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 ($[\text{C}_{18}\text{H}_{15}\text{F}_3\text{O}_3 + \text{H}]^+$ 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. $^1\text{H NMR}$

(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 ($[\text{C}_{18}\text{H}_{15}\text{F}_3\text{O}_3 + \text{H}]^+$ 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. ^1H 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). ^1H 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 ($[\text{C}_{18}\text{H}_{15}\text{F}_3\text{O}_3 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{19}\text{H}_{17}\text{F}_3\text{O}_4 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{19}\text{H}_{17}\text{F}_3\text{O}_4 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{19}\text{H}_{17}\text{F}_3\text{O}_4 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{16}\text{H}_{11}\text{F}_3\text{O} + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{17}\text{H}_{13}\text{F}_3\text{O}_2 + \text{H}]^+$ 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. ^1H 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 = 7.8 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 ($[\text{C}_{17}\text{H}_{13}\text{F}_3\text{O}_2 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{17}\text{H}_{13}\text{F}_3\text{O}_2 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{18}\text{H}_{15}\text{F}_3\text{O}_3 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{18}\text{H}_{15}\text{F}_3\text{O}_3 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{18}\text{H}_{15}\text{F}_3\text{O}_3 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{18}\text{H}_{15}\text{F}_3\text{O}_3 + \text{H}]^+$ calcd 337.1046). Purity 98.47% ($t_{\text{R}} = 8.03$ 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. ^1H 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 ($[\text{C}_{18}\text{H}_{15}\text{F}_3\text{O}_3 + \text{H}]^+$ calcd 337.1046). Purity 100.00% ($t_{\text{R}} = 9.55$ 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. ^1H 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 ($[\text{C}_{19}\text{H}_{17}\text{F}_3\text{O}_4 + \text{H}]^+$ calcd 367.1152). Purity 96.17% ($t_{\text{R}} = 8.61$ 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. ^1H 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 ($[\text{C}_{19}\text{H}_{17}\text{F}_3\text{O}_4 + \text{H}]^+$ calcd 367.1152). Purity 97.99% ($t_{\text{R}} = 8.83$ 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. ^1H 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 ($[\text{C}_{19}\text{H}_{17}\text{F}_3\text{O}_4 + \text{H}]^+$ calcd 367.1152). Purity 100.00% ($t_{\text{R}} = 7.86$ min).

(*E*)-1-Phenyl-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (**4a**). It was obtained as a white solid in 59% yield. ^1H 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 ($[\text{C}_{16}\text{H}_{11}\text{F}_3\text{O} + \text{H}]^+$ calcd 277.0835). Purity 100.00% ($t_{\text{R}} = 9.35$ 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. ^1H 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 ($[\text{C}_{17}\text{H}_{13}\text{F}_3\text{O}_2 + \text{H}]^+$ calcd 307.0940). Purity 94.00% ($t_{\text{R}} = 9.09$ 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. ^1H 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 ($[\text{C}_{17}\text{H}_{13}\text{F}_3\text{O}_2 + \text{H}]^+$ calcd 307.0940). Purity 100.00% ($t_{\text{R}} = 9.52$ 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. ^1H 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 ($[\text{C}_{17}\text{H}_{13}\text{F}_3\text{O}_2 + \text{H}]^+$ calcd 307.0940). Purity 100.00% ($t_{\text{R}} = 9.13$ 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. ^1H 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 ($[\text{C}_{18}\text{H}_{15}\text{F}_3\text{O}_3 + \text{H}]^+$ calcd 337.1046). Purity 96.35% ($t_{\text{R}} = 9.13$ 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. ^1H 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 ($[\text{C}_{18}\text{H}_{15}\text{F}_3\text{O}_3 + \text{H}]^+$ calcd 337.1046). Purity 100.00% ($t_{\text{R}} = 7.97$ 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. ^1H 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). ^1H 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 ($[\text{C}_{18}\text{H}_{15}\text{F}_3\text{O}_3 + \text{H}]^+$ calcd 337.1046). Purity 96.42% ($t_{\text{R}} = 9.16$ 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. ^1H 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 ($[\text{C}_{18}\text{H}_{15}\text{F}_3\text{O}_3 + \text{H}]^+$ calcd 337.1046). Purity 95.94% ($t_{\text{R}} = 8.19$ 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. ^1H 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 ($[\text{C}_{19}\text{H}_{17}\text{F}_3\text{O}_4 + \text{H}]^+$ calcd 367.1152). Purity 96.17% ($t_{\text{R}} = 8.78$ 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. ^1H 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). ^1H 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 ($[\text{C}_{19}\text{H}_{17}\text{F}_3\text{O}_4 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{19}\text{H}_{17}\text{F}_3\text{O}_4 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{15}\text{H}_{11}\text{NO}_3 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{16}\text{H}_{13}\text{NO}_4 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{16}\text{H}_{13}\text{NO}_4 + \text{H}]^+$ 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. ^1H 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). ^1H 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 ($[\text{C}_{16}\text{H}_{13}\text{NO}_4 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{17}\text{H}_{15}\text{NO}_5 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{17}\text{H}_{15}\text{NO}_5 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{17}\text{H}_{15}\text{NO}_5 + \text{H}]^+$ 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. ^1H 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). ^1H 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 ($[\text{C}_{17}\text{H}_{15}\text{NO}_5 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{17}\text{H}_{15}\text{NO}_5 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{18}\text{H}_{17}\text{NO}_6 + \text{H}]^+$ 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. ^1H 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 ($[\text{C}_{18}\text{H}_{17}\text{NO}_6 + \text{H}]^+$ 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. ^1H NMR (400 MHz, DMSO) δ = 8.03 (dd, J = 8.1 Hz, 1.0 Hz, 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 ($[\text{C}_{18}\text{H}_{17}\text{NO}_6 + \text{H}]^+$ 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 genetin. 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 methylthiazolyl-diphenyl-tetrazolium 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 $\mu\text{g}/\text{mL}$. 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 \pm SE and analyzed by student's *t* test. Differences were considered significant at $P \leq 0.05$.

AUTHOR INFORMATION

Corresponding Author

*For S.B.: phone, 410-502-1946; fax, 410-955-1946; e-mail, sbiswal@jhsp.edu. For S.V.M.: phone, +1-301-846-5151; fax, 1-301-846-5206; e-mail, malhotrasa@mail.nih.gov.

Author Contributions

^{||}These authors contributed equally.

ACKNOWLEDGMENT

V.K., M.H., and S.V.M. thank the National Cancer Institute (NCI) Developmental Therapeutics Program. This project has been funded in whole or in part with funds from the NCI, National Institutes of Health, Grant HSN26120080001E. This work was also supported by National Institutes of Health Grant HL081205 (S.B.), National Heart, Lung, and Blood Institute Specialized Centers of Clinically Oriented Research Grant P50HL084945, the Flight Attendant Medical Research Institute (S.B. and R.K.T.), National Cancer Institute Grants 5P50CA058184 and 5R01CA140492, NIH Phase 2 grant 1U01HL105569, and National Institute on Environmental Health Sciences Grants P50ES015903, P01 ES018176, and ES03819 (S.B.). V.S.P. and S. KS. thank the University of Delhi and the Department of Scientific and Industrial Research (DSIR, New Delhi) for financial support.

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

REFERENCES

- (1) Kensler, T. W.; Wakabayash, N.; Biswal, S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu. Rev. Pharmacol. Toxicol.* **2007**, *47*, 89–116.
- (2) Rangasamy, T.; Guo, J.; Mitzner, W. A.; Roman, J.; Singh, A.; Fryer, A. D.; Yamamoto, M.; Kensler, T. W.; Tuder, R. M.; Georas, S. N.; Biswal, S. Disruption of Nrf2 enhances susceptibility to severe airway inflammation and asthma in mice. *J. Exp. Med.* **2005**, *202*, 47–59.
- (3) Sussan, T. E.; Rangasamy, T.; Blake, D. J.; Malhotra, D.; El-Haddad, H.; Bedja, D.; Yates, M. S.; Kombairaju, P.; Yamamoto, M.; Liby, K. T.; Sporn, M. B.; Gabrielson, K. L.; Champion, H. C.; Tuder, R. M.; Kensler, T. W.; Biswal, S. Targeting Nrf2 with the triterpenoid CDDO-imidazolide attenuates cigarette smoke-induced emphysema and cardiac dysfunction in mice. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 250–255.
- (4) Thimmulappa, R. K.; Mai, K. H.; Srisuma, S.; Kensler, T. W.; Yamamoto, M.; Biswal, S. Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. *Cancer Res.* **2002**, *62*, 5196–5203.
- (5) Misra, V.; Lee, H.; Singh, A.; Huang, K.; Thimmulappa, R. K.; Mitzner, W.; Biswal, S.; Tankersley, C. G. Global expression profiles from C57BL/6J and DBA/2J mouse lungs to determine aging-related genes. *Physiol. Genomics* **2007**, *31*, 429–440.
- (6) Surh, Y. J.; Kundu, J. K.; Na, H. K. Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. *Planta Med.* **2008**, *74*, 1526–1539.
- (7) Singh, A.; Boldin-Adamsky, S.; Thimmulappa, R. K.; Rath, S. K.; Ashush, H.; Coulter, J.; Blackford, A.; Goodman, S. N.; Bunz, F.; Watson, W. H.; Gabrielson, E.; Feinstein, E.; Biswal, S. RNAi-mediated silencing of nuclear factor erythroid-2-related factor 2 gene expression in non-small cell lung cancer inhibits tumor growth and increases efficacy of chemotherapy. *Cancer Res.* **2008**, *68*, 7975–7984.
- (8) Niture, S. K.; Jain, A. K.; Jaiswal, A. K. Antioxidant-induced modification of Nrf2 cysteine 151 and PKC-delta-mediated phosphorylation of Nrf2 serine 40 are both required for stabilization and nuclear translocation of Nrf2 and increased drug resistance. *J. Cell Sci.* **2009**, *122*, 4452–4464.

- (9) Cheng, S. E.; Lee, I. T.; Lin, C. C.; Kou, Y. R.; Yang, C. M. Cigarette smoke particle-phase extract induces HO-1 expression in human tracheal smooth muscle cells: role of the c-Src/NADPH oxidase/MAPK/Nrf2 signaling pathway. *Free Radical Biol. Med.* **2010**, *48*, 1410–1422.
- (10) Nioi, P.; McMahon, M.; Itoh, K.; Yamamoto, M.; Hayes, J. D. Identification of a novel Nrf2-regulated antioxidant response element (ARE) in the mouse NAD(P)H:quinone oxidoreductase 1 gene: reassessment of the ARE consensus sequence. *Biochem. J.* **2003**, *374*, 337–348.
- (11) Chartoumpakis, D.; Ziros, P. G.; Psyrogiannis, A.; Kyriazopoulou, V.; Papavassiliou, A. G.; Habeos, I. G. Simvastatin lowers reactive oxygen species level by Nrf2 activation via PI3K/Akt pathway. *Biochem. Biophys. Res. Commun.* **2010**, *396*, 463–466.
- (12) Cullinan, S. B.; Diehl, J. A. PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress. *J. Biol. Chem.* **2004**, *279*, 20108–20117.
- (13) Giudice, A.; Arra, C.; Turco, M. C. Review of Molecular Mechanisms Involved in the Activation of the Nrf2-ARE Signaling Pathway by Chemopreventive Agents. In *Transcription Factors: Methods and Protocols*; Higgins, P. J., Ed.; Humana Press: New York, 2010; Vol. 647, pp 37–74.
- (14) Wan, J. X.; Diaz-Sanchez, D. Antioxidant enzyme induction: a new protective approach against the adverse effects of diesel exhaust particles. *Inhalation Toxicol.* **2007**, *19*, 177–182.
- (15) Singh, A.; Rangasamy, T.; Thimmulappa, R. K.; Lee, H.; Osburn, W. O.; Brigelius-Flohe, R.; Kensler, T. W.; Yamamoto, M.; Biswal, S. Glutathione peroxidase 2, the major cigarette smoke-inducible isoform of GPX in lungs, is regulated by Nrf2. *Am. J. Respir. Cell Mol. Biol.* **2006**, *35*, 639–650.
- (16) Lin, H. J.; Zhou, H. Y.; Dai, A. H.; Huang, H. F.; Lin, J. H.; Frankl, H. D.; Lee, E. R.; Haile, R. W. Glutathione transferase GSTT1, broccoli, and prevalence of colorectal adenomas. *Pharmacogenetics* **2002**, *12*, 175–179.
- (17) Kirsch, M.; De Groot, H. NAD(P)H, a directly operating antioxidant? *FASEB J.* **2001**, *15*, 1569–1574.
- (18) Lee, J. S.; Surh, Y. J. Nrf2 as a novel molecular target for chemoprevention. *Cancer Lett.* **2005**, *224*, 171–184.
- (19) Nguyen, T.; Sherratt, P. J.; Pickett, C. B. Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. *Annu. Rev. Pharmacol. Toxicol.* **2003**, *43*, 233–260.
- (20) Dinkova-Kostova, A. T.; Holtzclaw, W. D.; Cole, R. N.; Itoh, K.; Wakabayashi, N.; Katoh, Y.; Yamamoto, M.; Talalay, P. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11908–11913.
- (21) Yu, R.; Lei, W.; Mandelkar, S.; Weber, M. J.; Der, C. J.; Wu, J.; Kong, A. N. T. Role of a mitogen-activated protein kinase pathway in the induction of phase II detoxifying enzymes by chemicals. *J. Biol. Chem.* **1999**, *274*, 27545–27552.
- (22) McMahon, M.; Itoh, K.; Yamamoto, M.; Hayes, J. D. Keap1-dependent proteasomal degradation of transcription factor Nrf2 contributes to the negative regulation of antioxidant response element-driven gene expression. *J. Biol. Chem.* **2003**, *278*, 21592–21600.
- (23) Kwak, M. K.; Itoh, K.; Yamamoto, M.; Kensler, T. W. Enhanced expression of the transcription factor Nrf2 by cancer chemopreventive agents: role of antioxidant response element-like sequences in the nrf2 promoter. *Mol. Cell. Biol.* **2002**, *22*, 2883–2892.
- (24) Kwak, M. K.; Wakabayashi, N.; Itoh, K.; Motohashi, H.; Yamamoto, M.; Kensler, T. W. Modulation of gene expression by cancer chemopreventive dithiolethiones through the Keap1-Nrf2 pathway: identification of novel gene clusters for cell survival. *J. Biol. Chem.* **2003**, *278*, 8135–8145.
- (25) Kwak, M.-K.; Egner, P. A.; Dolan, P. M.; Ramos-Gomez, M.; Groopman, J. D.; Itoh, K.; Yamamoto, M.; Kensler, T. W. Role of phase 2 enzyme induction in chemoprotection by dithiolethiones. *Mutat. Res.* **2001**, 305–315.
- (26) Balogun, E.; Hoque, M.; Gong, P. F.; Killeen, E.; Green, C. J.; Foresti, R.; Alam, J.; Motterlini, R. Curcumin activates the haem oxygenase-1 gene via regulation of Nrf2 and the antioxidant-responsive element. *Biochem. J.* **2003**, *371*, 887–895.
- (27) Go, M. L.; Wu, X.; Liu, X. L. Chalcones: an update on cytotoxic and chemoprotective properties. *Curr. Med. Chem.* **2005**, *12*, 483–499.
- (28) Dimmock, J. R.; Elias, D. W.; Beazely, M. A.; Kandepu, N. M. Bioactivities of chalcones. *Curr. Med. Chem.* **1999**, *6*, 1125–1149.
- (29) Xia, Y.; Yang, Z. Y.; Xia, P.; Bastow, K. F.; Nakanishi, Y.; Lee, K. H. Antitumor agents. Part 202: Novel 2'-amino chalcones: design, synthesis and biological evaluation. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 699–701.
- (30) Bois, F.; Beney, C.; Boumendjel, A.; Mariotte, A. M.; Conseil, G.; Di Pietro, A. Halogenated chalcones with high-affinity binding to P-glycoprotein: potential modulators of multidrug resistance. *J. Med. Chem.* **1998**, *41*, 4161–4164.
- (31) Liu, M.; Wilairat, P.; Go, M. L. Antimalarial alkoxylated and hydroxylated chalcones: structure–activity relationship analysis. *J. Med. Chem.* **2001**, *44*, 4443–4452.
- (32) Dominguez, J. N.; Charris, J. E.; Lobo, G.; de Dominguez, N. G.; Moreno, M. M.; Riggione, F.; Sanchez, E.; Olson, J.; Rosenthal, P. J. Synthesis of quinolinyl chalcones and evaluation of their antimalarial activity. *Eur. J. Med. Chem.* **2001**, *36*, 555–560.
- (33) Hsieh, H. K.; Lee, T. H.; Wang, J. P.; Wang, J. J.; Lin, C. N. Synthesis and anti-inflammatory effect of chalcones and related compounds. *Pharm. Res.* **1998**, *15*, 39–46.
- (34) Hsieh, H. K.; Tsao, L. T.; Wang, J. P.; Lin, C. N. Synthesis and anti-inflammatory effect of chalcones. *J. Pharm. Pharmacol.* **2000**, *52*, 163–171.
- (35) Herencia, F.; Ferrandiz, M. L.; Ubeda, A.; Dominguez, J. N.; Charris, J. E.; Lobo, G. M.; Alcaraz, M. J. Synthesis and anti-inflammatory activity of chalcone derivatives. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1169–1174.
- (36) Lin, Y. M.; Zhou, Y. S.; Flavin, M. T.; Zhou, L. M.; Nie, W. G.; Chen, F. C. Chalcones and flavonoids as anti-tuberculosis agents. *Bioorg. Med. Chem.* **2002**, *10*, 2795–2802.
- (37) Rojas, J.; Paya, M.; Dominguez, J. N.; Ferrandiz, M. L. The synthesis and effect of fluorinated chalcone derivatives on nitric oxide production. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1951–1954.
- (38) Herencia, F.; Ferrandiz, M. L.; Ubeda, A.; Guillen, I.; Dominguez, J. N.; Charris, J. E.; Lobo, G. M.; Alcaraz, M. J. 4-dimethylamino-3',4'-dimethoxychalcone downregulates iNOS expression and exerts anti-inflammatory effects. *Free Radical Biol. Med.* **2001**, *30*, 43–50.
- (39) Ducki, S.; Forrest, R.; Hadfield, J. A.; Kendall, A.; Lawrence, N. J.; McGown, A. T.; Rennison, D. Potent antimitotic and cell growth inhibitory properties of substituted chalcones. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1051–1056.
- (40) Maria, K.; Dimitra, H. L.; Maria, G. Synthesis and anti-inflammatory activity of chalcones and related Mannich bases. *Med. Chem.* **2008**, *4*, 586–596.
- (41) Viana, G. S. B.; Bandeira, M. A. M.; Matos, F. J. A. Analgesic and anti-inflammatory effects of chalcones isolated from *Myracrodruon urundeuva* Allemão. *Phytomedicine* **2003**, *10*, 189–195.
- (42) Cheng, J. H.; Hung, C. F.; Yang, S. C.; Wang, J. P.; Won, S. J.; Lin, C. N. Synthesis and cytotoxic, anti-inflammatory, and anti-oxidant activities of 2',5'-dialkoxylchalcones as cancer chemopreventive agents. *Bioorg. Med. Chem.* **2008**, *16*, 7270–7276.
- (43) Heidari, M. R.; Foroumadi, A.; Amirabadi, A.; Samzadeh-Kermani, A.; Azimzadeh, B. S.; Eskandarizadeh, A. Evaluation of Anti-Inflammatory and Analgesic Activity of a Novel Rigid 3,4-Dihydroxy Chalcone in Mice. In *Natural Compounds and Their Role in Apoptotic Cell Signaling Pathways*; Diederich, M., Ed.; Wiley-Interscience: Hoboken, NJ, 2009; pp 399–406.
- (44) Cheenpracha, S.; Karalai, C.; Ponglimanont, C.; Subhadhirasakul, S.; Tewtrakul, S. Anti-HIV-1 protease activity of compounds from *Boesenbergia pandurata*. *Bioorg. Med. Chem.* **2006**, *14*, 1710–1714.
- (45) Svetaz, L.; Tapia, A.; Lopez, S. N.; Furlan, R. L. E.; Petenatti, E.; Pioli, R.; Schmeda-Hirschmann, G.; Zacchino, S. A. Antifungal chalcones and new caffeic acid esters from *Zuccagnia punctata* acting against soybean infecting fungi. *J. Agric. Food. Chem.* **2004**, *52*, 3297–3300.

- (46) Saavedra, M. J.; Borges, A.; Dias, C.; Aires, A.; Bennett, R. N.; Rosa, E. S.; Simoes, M. Antimicrobial activity of phenolics and gluconolate hydrolysis products and their synergy with streptomycin against pathogenic bacteria. *Med. Chem.* **2010**, *6*, 174–183.
- (47) Aponte, J. C.; Verastegui, M.; Malaga, E.; Zimic, M.; Quiliano, M.; Vaisberg, A. J.; Gilman, R. H.; Hammond, G. B. Synthesis, cytotoxicity, and anti-*Trypanosoma cruzi* activity of new chalcones. *J. Med. Chem.* **2008**, *51*, 6230–6234.
- (48) Lahtchev, K. L.; Batovska, D. I.; Parushev, S. P.; Ubiyovok, V. M.; Sibirny, A. A. Antifungal activity of chalcones: a mechanistic study using various yeast strains. *Eur. J. Med. Chem.* **2008**, *43*, 2220–2228.
- (49) Batovska, D. I.; Todorova, I. T. Trends in utilization of the pharmacological potential of chalcones. *Curr. Clin. Pharmacol.* **2010**, *5*, 1–29.
- (50) Torigoe, T.; Arisawa, M.; Itoh, S.; Fujii, M.; Maruyama, H. B. Anti-mutagenic chalcones: antagonizing the mutagenicity of benzo-(A)pyrene on *Salmonella-typhimurium*. *Biochem. Biophys. Res. Commun.* **1983**, *112*, 833–842.
- (51) Lee, S. A.; Ryu, H. W.; Kim, Y. M.; Choi, S.; Lee, M. J.; Kwak, T. K.; Kim, H. J.; Cho, M.; Park, K. H.; Lee, J. W. Blockade of four-transmembrane L6 family member 5 (TM4SF5)-mediated tumorigenicity in hepatocytes by a synthetic chalcone derivative. *Hepatology* **2009**, *49*, 1316–1325.
- (52) Shibata, S. Anti-tumorigenic chalcones. *Stem Cells* **1994**, *12*, 44–52.
- (53) Devincenzo, R.; Scambia, G.; Panici, P. B.; Ranelletti, F. O.; Bonanno, G.; Ercoli, A.; Dellemonache, F.; Ferrari, F.; Piantelli, M.; Mancuso, S. Effect of synthetic and naturally-occurring chalcones on ovarian cancer cell-growth-structure-activity-relationships. *Anti-Cancer Drug Des.* **1995**, *10*, 481–490.
- (54) Wei, B. L.; Teng, C. H.; Wang, J. P.; Won, S. J.; Lin, C. N. Synthetic 2',5'-dimethoxychalcones as G(2)/M arrest-mediated apoptosis-inducing agents and inhibitors of nitric oxide production in rat macrophages. *Eur. J. Med. Chem.* **2007**, *42*, 660–668.
- (55) Takahashi, T.; Takasuka, N.; Iigo, M.; Baba, M.; Nishino, H.; Tsuda, H.; Okuyama, T. Isoliquiritigenin, a flavonoid from licorice, reduces prostaglandin E-2 and nitric oxide, causes apoptosis, and suppresses aberrant crypt foci development. *Cancer Sci.* **2004**, *95*, 448–453.
- (56) Lee, J. H.; Jung, H. S.; Giang, P. M.; Jin, X.; Lee, S.; Son, P. T.; Lee, D.; Hong, Y. S.; Lee, K.; Lee, J. J. Blockade of nuclear factor-kappa B signaling pathway and anti-inflammatory activity of cardamomin, a chalcone analog from *Alpinia conchigera*. *J. Pharmacol. Exp. Ther.* **2006**, *316*, 271–278.
- (57) Ban, H. S.; Suzuki, K.; Lim, S. S.; Jung, S. H.; Lee, S.; Ji, J.; Lee, H. S.; Lee, Y. S.; Shin, K. H.; Ohuchi, K. Inhibition of lipopolysaccharide-induced expression of inducible nitric oxide synthase and tumor necrosis factor-alpha by 2'-hydroxychalcone derivatives in RAW 264.7 cells. *Biochem. Pharmacol.* **2004**, *67*, 1549–1557.
- (58) Liu, Y. C.; Hsieh, C. W.; Wu, C. C.; Wung, B. S. Chalcone inhibits the activation of NF-kappa B and STAT3 in endothelial cells via endogenous electrophile. *Life Sci.* **2007**, *80*, 1420–1430.
- (59) Foresti, R.; Hoque, M.; Monti, D.; Green, C. J.; Motterlini, R. Differential activation of heme oxygenase-1 by chalcones and rosolic acid in endothelial cells. *J. Pharmacol. Exp. Ther.* **2005**, *312*, 686–693.
- (60) Wu, C. C.; Hsieh, C. W.; Lai, P. H.; Lin, J. B.; Liu, Y. C.; Wung, B. S. Upregulation of endothelial heme oxygenase-1 expression through the activation of the JNK pathway by sublethal concentrations of acrolein. *Toxicol. Appl. Pharmacol.* **2006**, *214*, 244–252.
- (61) Shibata, T.; Yamada, T.; Ishii, T.; Kumazawa, S.; Nakamura, H.; Masutani, H.; Yodoi, J.; Uchida, K. Thioredoxin as a molecular target of cyclopentenone prostaglandins. *J. Biol. Chem.* **2003**, *278*, 26046–26054.
- (62) Heiss, E.; Herhaus, C.; Klimo, K.; Bartsch, H.; Gerhauser, C. Nuclear factor kappa B is a molecular target for sulforaphane-mediated anti-inflammatory mechanisms. *J. Biol. Chem.* **2001**, *276*, 32008–32015.
- (63) Micheli, F.; Degiorgis, F.; Feriani, A.; Paio, A.; Pozzan, A.; Zarantonello, P.; Seneci, P. A combinatorial approach to [1,5]benzothiazepine derivatives as potential antibacterial agents. *J. Comb. Chem.* **2001**, *3*, 224–228.
- (64) Bu, X. Y.; Zhao, L. Y.; Li, Y. L. A facile synthesis of 6-C-prenylflavanones. *Synthesis* **1997**, 1246–1248.
- (65) Sinisterra, J. V.; Garciaso, A.; Cabello, J. A.; Marinas, J. M. An improved procedure for the Claisen–Schmidt reaction. *Synthesis* **1984**, 502–504.
- (66) Alcantara, A. R.; Marinas, J. M.; Sinisterra, J. V. Synthesis of 2'-hydroxychalcones and related-compounds in interfacial solid-liquid conditions. *Tetrahedron Lett.* **1987**, *28*, 1515–1518.
- (67) Climent, M. J.; Corma, A.; Iborra, S.; Velty, A. Activated hydrotalcites as catalysts for the synthesis of chalcones of pharmaceutical interest. *J. Catal.* **2004**, *221*, 474–482.
- (68) Daskiewicz, J. B.; Comte, G.; Barron, D.; Di Pietro, A.; Thomasson, F. Organolithium mediated synthesis of prenylchalcones as potential inhibitors of chemoresistance. *Tetrahedron Lett.* **1999**, *40*, 7095–7098.
- (69) Sebti, S.; Solhy, A.; Smahi, A.; Kossir, A.; Oumimoun, H. Dramatic activity enhancement of natural phosphate catalyst by lithium nitrate. An efficient synthesis of chalcones. *Catal. Commun.* **2002**, *3*, 335–339.
- (70) Calloway, N. O.; Green, L. D. Reactions in the presence of metallic halides I beta-unsaturated ketone formation as a side reaction in Friedel–Crafts acylations. *J. Am. Chem. Soc.* **1937**, *59*, 809–811.
- (71) Breslow, D. S.; Hauser, C. R. Condensations. XI. Condensations of certain active hydrogen compounds effected by boron trifluoride and aluminum chloride. *J. Am. Chem. Soc.* **1940**, *62*, 2385–2388.
- (72) Szell, T.; Sohar, I. New nitrochalcones. *Can. J. Chem.* **1969**, *47*, 1254–1258.
- (73) Irie, K.; Watanabe, K. Aldol condensations with metal(II) complex catalysts. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 1366–1371.
- (74) Nakano, T.; Irifune, S.; Umano, S.; Inada, A.; Ishii, Y.; Ogawa, M. Cross-condensation reactions of cycloalkanones with aldehydes and primary alcohols under the influence of zirconocene complexes. *J. Org. Chem.* **1987**, *52*, 2239–2244.
- (75) Iranpoor, N.; Kazemi, F. RuCl₃ catalyses aldol condensations of aldehydes and ketones. *Tetrahedron* **1998**, *54*, 9475–9480.
- (76) Eddarir, S.; Cotellet, N.; Bakkour, Y.; Rolando, C. An efficient synthesis of chalcones based on the Suzuki reaction. *Tetrahedron Lett.* **2003**, *44*, 5359–5363.
- (77) Bhagat, S.; Sharma, R.; Sawant, D. M.; Sharma, L.; Chakraborti, A. K. LiOH center dot H₂O as a novel dual activation catalyst for highly efficient and easy synthesis of 1,3-diaryl-2-propenones by Claisen–Schmidt condensation under mild conditions. *J. Mol. Catal. A: Chem.* **2006**, *244*, 20–24.
- (78) Osburn, W. O.; Yates, M. S.; Dolan, P. D.; Chen, S.; Liby, K. T.; Sporn, M. B.; Taguchi, K.; Yamamoto, M.; Kensler, T. W. Genetic or pharmacologic amplification of Nrf2 signaling inhibits acute inflammatory liver injury in mice. *Toxicol. Sci.* **2008**, *104*, 218–227.
- (79) Bloom, D.; Dhakshinamoorthy, S.; Jaiswal, A. K. Site-directed mutagenesis of cysteine to serine in the DNA binding region of Nrf2 decreases its capacity to upregulate antioxidant response element-mediated expression and antioxidant induction of NAD(P)H:quinone oxidoreductase1 gene. *Oncogene* **2002**, *21*, 2191–2200.
- (80) Nguyen, T.; Nioi, P.; Pickett, C. B. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *J. Biol. Chem.* **2009**, *284*, 13291–13295.
- (81) McMahan, M.; Lamont, D. J.; Beattie, K. A.; Hayes, J. D. Keap1 perceives stress via three sensors for the endogenous signaling molecules nitric oxide, zinc, and alkenals. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 18838–18843.
- (82) Kumar, S.; Singh, B. K.; Pandey, A. K.; Kumar, A.; Sharma, S. K.; Raj, H. G.; Prasad, A. K.; Van der Eycken, E.; Parmar, V. S.; Ghosh, B. A chromone analog inhibits TNF-alpha induced expression of cell adhesion molecules on human endothelial cells via blocking NF-kappaB activation. *Bioorg. Med. Chem.* **2007**, *15*, 2952–2962.
- (83) Singh, A.; Misra, V.; Thimmulappa, R. K.; Lee, H.; Ames, S.; Hoque, M. O.; Herman, J. G.; Baylin, S. B.; Sidransky, D.; Gabrielson, E.; Brock, M. V.; Biswal, S. Dysfunctional KEAP1-NRF2 interaction in non-small-cell lung cancer. *PLoS Med.* **2006**, *3*, 1865–1876.