Effects of Structural and Electronic Characteristics of Chalcones on the Activation of Peroxisome Proliferator-Activated Receptor Gamma

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Chalcones share some structural similarities with GW-1929, a highly-selective and potent agonist for peroxisome proliferator-activated receptor-gamma (PPARγ). In this study, we tested 53 structurally diverse chalcones to identify characteristics essential for PPARγ activation in a GAL4-based transactivation assay. This screen identified several novel chalcone agonists of PPARγ. Our results indicate that chalcones with an electron rich group or sterically large groups such as naphthyl on the carbonyl side tend to activate PPARγ. The absence of any strict structural or electronic requirements suggests that the flexibility of the PPARγ ligand binding pocket may allow binding of diverse chalcones with some preference for a slightly larger electron-rich group on the carbonyl side. We predict that further structure–activity relationship studies on chalcones with naphthalene or electron-rich groups near the carbonyl moiety will lead to the development of more potent PPARγ agonists.

Key words: naphthyl-chalcone; peroxisome proliferator-activated receptor gamma; GAL4 assay

Peroxisome proliferator-activated receptor-gamma (PPARγ) is an important pharmacological target for treatment of hyperglycemia, dyslipidemia, inflammation, atherosclerosis, and several other conditions.1) Thiazolidinediones are among the best-known PPARγ specific ligands and are often prescribed for management of hyperglycemia in type 2 diabetes mellitus. One of the underlying causes of hyperglycemia is increased gluconeogenesis resulting from accumulation of triglycerides in liver (steatosis). Thiazolidinediones attenuate liver steatosis by promoting differentiation of pre-adipocytes into adipocytes, which improves vascular lipid profile. Activation of PPARγ in adipocytes also leads to increased expression of adiponectin and attenuated expression of pro-inflammatory genes.1,2) All of these events increase insulin sensitivity, improve dyslipidemia, and could attenuate inflammation and atherosclerosis.3) These drugs are also beneficial in management of non-alcoholic steatohepatitis and possibly Metabolic Syndrome (Syndrome X).3) Nevertheless, thiazolidinediones are also known to increase the risk of bone fracture, myocardial infarction, and cancer.4–6) One possible solution is to develop partial PPARγ agonists, selective PPARγ modulators, or less potent agonists that are selective for PPAR isoforms.2) For example, GQ-16 has a maximal activation that is lower than rosiglitazone’s EC₅₀ for PPARγ, yet it promotes insulin sensitization.7) However, GQ-16 and several other selective PPARγ modulators have not progressed further than preclinical or phase II trials.2) This necessitates the development of additional pharmacophores that activate PPARγ.

One of the most potent and selective PPARγ agonists is GW-1929 (1), which has an EC₅₀ of approximately 0.01 μM.8) A structural analysis of GW-1929 reveals a benzophenone core with some structural similarities to chalcones. Previous studies have found that chalcones with hydroxyl and methoxy groups present in certain positions had more potent or similar PPARγ agonistic activity compared with the reference dose of troglitazone and 15-deoxy-Δ²,14-prostaglandin J₂ (PGJ₂).9) However, the chosen chalcone library consisted mostly of small molecules, and the vast majority contained electron-rich groups. Because of those limitations we have chosen to further investigate chalcones as potential PPARγ agonists. Chalcones are relatively easy to synthesize in the laboratory from a wide variety of commercially available and affordable starting materials, which makes them advantageous when preparing a diverse library of potential PPARγ ligands. Here we report on the observed effects of using a varied set of chalcones to probe for additional steric and electronic requirements for ligand-dependent activation of PPARγ.

Results and Discussion

To monitor the activation of PPARγ by chalcones, the PPARγ ligand binding domain (LBD) was fused with the yeast GAL4 DNA binding domain (DBD) and expressed in HEK293 cells along with a GAL4 responsive Firefly luciferase reporter. Transfected cells were treated in quadruplicate with a 10 μM dose of test compound, and luciferase expression was quantified 24 h later using a luminometer. The chalcones were classified into three categories based on the substratients at the carbonyl and alkene ends. Series A (Table 1) consists of molecules containing the basic chalcone skeleton with simple electron-rich or electron-poor substituents on one or both benzene rings; series B (Table 2) contains the naphthyl moiety at the carbonyl end; and series C (Table 3) is comprised of chalcone-like compounds with heteroatom-containing groups (furan, thiophene, pyrrole, pyridine) at one end. Two of the compounds listed as part of series C have different structural features. Compound 52 contained an additional double bond.
from cinnamaldehyde) and compound 54 (Fig. 1) was the unexpected result of a double Claisen–Schmidt condensation reaction. This wide variety of steric and electronic components has allowed for a detailed structure–activity study.

The results for all the chalcones and chalcone-like compounds synthesized and tested in the GAL4-PPARγ transactivation assay are presented in Fig. 2. Here, we have included GW-1929 (1), which has an EC50 of approximately 0.01 µM in GAL4-transactivation assays, as the positive control at a 0.1 µM dose in our GAL4-PPARγ assay.8) The parent chalcone (2), which failed to activate PPARγ, served as the “baseline.” Chalcones which activated PPARγ by a fold-change greater than 3 standard deviations (3σ) over dimethylsulfoxide (DMSO) (vehicle control) were classified as activators. According to this criterion, 23 of the 52 compounds tested (not including 1, 2) can be considered activators. We further differentiated (arbitrarily) between weak (1.51–1.70), moderate (1.71–2.00), and strong (2.01–3.2) activators. Eight compounds can be classified as strong activators and of these, five compounds (3, 41, 42, 21, 53; in order of activation) were found to give better results than 0.1 µM GW-1929 (1). It is possible that these moderate–strong chalcone activators can yield beneficial effects similar to those observed for other partial agonists of PPARγ such as GQ-16.7)

Analysis of the three individual series shows that 16 out of the 39 compounds from series A are activators (>1.5-fold activation compared to the DMSO vehicle control). Two of these compounds (3, 21) showed higher PPARγ activation than 0.1 µM GW-1929 (1). In fact, chalcone 3 showed the best response of all the compounds that we tested. All but one of the compounds from series B were activators (>1.5-fold activation) and 2 compounds (41, 42) showed better activation than 0.1 µM GW-1929 (1). Three out of the nine compounds from series C were activators but only 53 was a better activator than 0.1 µM GW-1929 (1).
Our initial analysis did not reveal any obvious structural trends in chalcone-mediated PPARγ activation, but we nonetheless made a number of interesting observations. When the moiety on the carbonyl side of the molecule is an electron-rich group (aromatic ring with electron-rich substituent attached or naphthalene), the molecule is typically an activator. About half of the compounds (14 out of 27) with electron-rich substituents were activators and the three best compounds (21, 42, 43) in this particular set were all better than 0.1 \( \mu \)M GW-1929 (1). Interestingly, four of the five naphthalene compounds (series B) were part of this group suggesting a special feature inherent to this functionality. It is also noteworthy that compounds containing the thiophene, furan, or pyridine moiety (series C) failed to activate the PPARγ LBD. Comparing this set of compounds to those that have an electron-poor group (aromatic ring with electron-withdrawing substituent) attached to the carbonyl side demonstrated the importance of the electron-rich character of this particular group. Of the 19 compounds with electron-poor groups, only five were considered activators and none were strong activators.

A similar analysis of the group attached to the alkene moiety suggests that the activity of the chalcones and chalcone-like compounds is not influenced as much by this side of the molecule. The set of compounds with an electron-rich group (30 compounds total; 12 activators; three compounds (3, 41, 53) in the overall top five) is similar to the set of compounds with an electron-poor group (19 compounds total; nine activators; two compounds (21, 42) in the overall top five).

To see the effect of both groups (carbonyl and alkene sides) we compared (i) compounds that had electron-rich groups on both sides, (ii) compounds with electron-poor groups on both sides, and (iii) compounds with mixed groups. The best results are obtained when compounds have at least one electron-rich group on either the carbonyl or the alkene side. The compounds that have electron-rich groups on both sides (22 compounds total) contained 11 activators, three of which (3, 41, 53) were better than 0.1 \( \mu \)M GW-1929 (1). The set of compounds with mixed groups (22 compounds total) had ten activators with two (21, 42) better than the positive control (1). The best performers in the mixed set were the compounds with electron-rich groups attached to the carbonyl side.

Next, we did an analysis of individual groups to see if using any specific activating group would give a significant advantage. No such requirement was observed. The compounds that yielded greater than two-fold activation included neutral or electron-rich groups on the carbonyl side of the molecule: hydrogen (3, 36, 53), naphthalene (41, 42), methyl (8, 21), or methoxy (27, 32, 35) groups. Interestingly, almost every one of these ten compounds had a different group on the alkene side: methoxy (3, 36), n-butoxy (8, 27), trifluoromethyl (21), hydrogen (32), nitro (35), methyl (41), chloro (42), and pyrrole (53). This observation again suggests that the nature of the alkene side of the molecule is less important than the group or substituent present on the carbonyl side.

Conceptually, our observation that compounds with a variety of steric and electronic features can activate PPARγ is supported by the evidence that the LBDs of PPARγ and other nuclear receptors are highly flexible and often change their structure to bind to their ligand.10–12 For example, rosiglitazone stabilizes the PPARγ LBD into a single conformation, whereas partial agonists may alter the conformational dynamics of the receptor by binding in multiple orientations.12 Furthermore, a recent study by Kumari and co-workers in which computer-aided virtual screening was used to identify novel chalcones as candidate PPARγ agonists also lends support to our observations.13 Several of the top hits in that study were chalcones with high molecular weight, including some with naphthalene-like moieties. According to our data, a wide variety of chalcones are PPARγ agonists but not all of them have high molecular weights. The active site of PPARγ exhibits a fairly large cavity that could easily house our chalcones with the carbonyl group lined up to hydrogen bond with Ser 289.14 The fact that most of the naphthalene-containing chalcones are among the top activators suggests that the cavity is well-suited to accommodate that type of functionality.14 Furthermore, identification of PPARγ agonists in all three series raises the possibility that the mixed chalcones with electron poor groups on the carbonyl side bind less stably to the PPARγ LBD in a “flipped” orientation such that the electron-rich and carbonyl moieties still interact with the functionally important residues but over longer distances, and the alkene side is positioned in the fairly large space towards Glu 343 and Phe 264.

Fig. 2. Identification of a Variety of Chalcones as PPARγ Activators

GAL4-PPAR γ fusion construct or empty GAL4-DBD control vector were co-electroporated with the GAL4 9xUAS luciferase reporter into HEK293 cells. The cells were treated in quadruplicate for 24h with vehicle (0.1% DMSO), 0.1 \( \mu \)M GW-1929 (a highly-selective and potent PPARγ agonist with an EC_{50} of 0.01 \( \mu \)M),10 or 10 \( \mu \)M of the indicated chalcone. Luciferase measurements were normalized to DMSO controls, and then fold-activities for GAL4-PPARγ samples were normalized to the average fold-activities for GAL4-DDB samples for each treatment. Data shown are the mean of 2 or more quadruplicate measurements±S.E.M.
All of the chalcones tested in this study are much smaller than the known PPARγ agonists such as thiazolidinediones, GQ-16 and GW-1929. Thus, we conclude that the flexibility of the LBD allows binding of a fairly diverse group of chalcones. The size of the tested chalcones also allows this study to be considered as an optimization of a “fragment” that binds to a small portion of the PPARγ LBD. There is a growing body of literature that supports the notion that these types of fragment hits can be successfully assembled with other fragment hits to generate a lead compound.

In summary, a wide variety of chalcones can activate PPARγ to varying levels. Our analysis revealed two trends. First, several of the more potent PPARγ activators possessed an electron-rich group on the carbonyl side. Second, naphthalene and the anisyl (methoxybenzene) groups appeared several times among the top activators. However, our screen of 53 materials by 1H-NMR clearly suggested the formation of the 1H-chalcone as shown by the coupling constant ($\delta = 3.3$). The size of the tested chalcones also allows this study to be considered as an optimization of a “fragment” that binds to a small portion of the PPARγ LBD. This poses a significant challenge to the development of simple chalcones as novel PPARγ ligands. Nonetheless, our observations suggest that development of larger, complex chalcones or chalcone-like compounds with an electron rich group or sterically large groups such as naphthyl on the carbonyl side should yield better PPARγ partial agonists.

### Experimental

**Chemistry**

GW1929 (I) was obtained from Sigma-Aldrich and dissolved in DMSO to a stock concentration of 10 mM. (E)-Chalcone (2) was obtained from Alfa Aesar and used as received. The remaining chalcones were prepared via a Claisen-Schmidt condensation using commercially available substituted aldehydes and ketones. Analysis of the purified materials by 1H-NMR clearly suggested the formation of the (E)-chalcone as shown by the coupling constant ($\delta = 15$ Hz) for the 2 doublets representing the double bond. 1H (400 MHz) and 13C (101 MHz) NMR spectra were obtained with a Bruker Avance 400 spectrometer. Chemical shifts ($\delta$) are reported in ppm relative to tetramethylsilane (TMS) as the internal standard. The mass of the compounds was determined by electron impact (EI) mass spectrometry using a Hewlett Packard 5917 quadrupole mass analyzer connected to a Hewlett Packard 5890 Series II gas chromatograph.

**General Procedure for Preparation of Chalcones**

A 100-mL round bottom flask was charged with the appropriate aldehyde (5.0 mmol) and ketone (5.3 mmol). The contents were stirred and 6 mL of 95% ethanol was added to the flask. Slowly, sodium hydroxide (15 M, 0.7 mL) was added drop-wise and the reaction was continued until the reaction mixture solidified. Afterwards, 20 mL of ice water was added and the mixture was stirred until a suspension formed. The crude product was washed, collected by suction filtration and then recrystallized from an appropriate solvent. Characterization of known compounds was done by 1H- and 13C-NMR, EI-MS, and melting point (uncorrected).

### 1H-NMR

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<th>Compound</th>
<th>δ (ppm)</th>
<th>J (Hz)</th>
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<tr>
<td>(2E)-1-(4-Ethoxyphenyl)-3-(3-methylphenyl)prop-2-en-1-one</td>
<td>8.10 (d, J = 15.6 Hz, 1H), 7.88 (d, J = 6.2 Hz, 2H), 7.62 (dd, J = 7.6, 12 Hz, 1H), 7.61 (d, J = 7.6 Hz, 1H), 7.55 (d, J = 7.2 Hz, 1H), 7.48 (dd, J = 7.2, 6.8 Hz, 2H), 7.37 (ddd, J = 8.8, 7.8, 2.0 Hz, 1H), 6.98 (dd, J = 7.6, 7.4 Hz, 1H), 6.93 (d, J = 8.4 Hz, 1H)</td>
<td>3.90 (s, 3H)</td>
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### 13C-NMR

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<th>Compound</th>
<th>δ (ppm)</th>
<th>J (Hz)</th>
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<tbody>
<tr>
<td>(2E)-1-(4-Ethoxyphenyl)-3-(3-methylphenyl)prop-2-en-1-one</td>
<td>130.27, 127.26, 119.07, 115.69 (d, J = 21.9 Hz), 134.88, 134.85, 130.95 (d, J = 9.2 Hz), 128.53, 120.62, 115.69 (d, J = 21.9 Hz), 135.64, 33.37, 22.34, 13.92.</td>
<td>282.1 (M⁺); Calcd for C13H18O2, 282.351</td>
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### Recrystallization

**From aq. ethanol:** Yellow crystals, 70% yield, mp 79.5–80.2°C. 1H-NMR (400 MHz, CDCl3) δ: 8.11–7.99 (m, 2H), 7.78 (d, J = 15.6 Hz, 1H), 7.62–7.54 (m, 2H), 7.37 (d, J = 15.6 Hz, 1H), 7.20–7.11 (m, 2H), 6.96–6.87 (m, 2H), 4.00 (t, J = 6.5 Hz, 2H), 1.78 (ddt, J = 8.9, 7.9, 6.4 Hz, 2H), 1.60–1.43 (m, 2H), 0.98 (t, J = 7.4 Hz, 3H). 13C-NMR (101 MHz, CDCl3) δ: 188.88, 165.46 (d, $J_{CF} = 254$ Hz), 161.43, 145.03, 134.88, 134.85, 130.95 (d, $J_{CF} = 9.2$ Hz), 128.53, 120.62, 115.69 (d, $J_{CF} = 21.9$ Hz), 135.64, 33.37, 22.34, 13.92. EI-MS m/z: 282.1 (M⁺); Calcd for C13H18O2, 282.351.

**From ethanol:** Yellow crystals, 54% yield, mp 83.0–84.0°C. 1H-NMR (400 MHz, CDCl3) δ: 8.10 (d, J = 15.5 Hz, 1H), 8.07–8.01 (m, 2H), 7.69 (dd, J = 7.8, 1.5 Hz, 1H), 7.47 (d, J = 15.5 Hz, 1H), 7.35–7.27 (m, 1H), 7.23 (dd, J = 7.9, 1.8 Hz, 2H), 7.01–6.93 (m, 2H), 4.13 (q, J = 7.0 Hz, 2H), 1.46 (t, J = 7.0 Hz, 3H). 13C-NMR (101 MHz, CDCl3) δ: 188.70, 163.82, 145.11, 138.24, 130.71, 130.67, 128.71, 126.49, 114.79, 63.78, 19.90, 14.70. EI-MS m/z: 266.0 (M⁺); Calcd for C13H18O2, 266.1307.

### Recrystallization

**From aq. ethanol:** Yellow crystals, 70% yield, mp 79.5–80.2°C. 1H-NMR (400 MHz, CDCl3) δ: 8.11–7.99 (m, 2H), 7.78 (d, J = 15.6 Hz, 1H), 7.62–7.54 (m, 2H), 7.37 (d, J = 15.6 Hz, 1H), 7.20–7.11 (m, 2H), 6.96–6.87 (m, 2H), 4.00 (t, J = 6.5 Hz, 2H), 1.78 (ddt, J = 8.9, 7.9, 6.4 Hz, 2H), 1.60–1.43 (m, 2H), 0.98 (t, J = 7.4 Hz, 3H). 13C-NMR (101 MHz, CDCl3) δ: 188.88, 165.46 (d, $J_{CF} = 254$ Hz), 161.43, 145.03, 134.88, 134.85, 130.95 (d, $J_{CF} = 9.2$ Hz), 128.53, 120.62, 115.69 (d, $J_{CF} = 21.9$ Hz), 135.64, 33.37, 22.34, 13.92. EI-MS m/z: 282.1 (M⁺); Calcd for C13H18O2, 282.351.
(2E)-3-(4-Chlorophenyl)-1-(3-methoxyphenyl)prop-2-en-1-one (14)[2]: 
1H-NMR (400 MHz, CDCl 3) δ: 8.44 (dd, J = 2.2, 1.7 Hz, 1H), 8.46 (dd, J = 8.2, 2.3, 1.1 Hz, 1H), 8.35 (dd, J = 7.8, 1.7 Hz, 1H), 7.85 (dd, J = 15.6 Hz, 1H), 7.73 (t, J = 8.0 Hz, 1H), 7.66–7.58 (m, 2H), 7.51 (d, J = 15.7 Hz, 1H), 7.47–7.40 (m, 2H).

(2E)-3-(4-Fluorophenyl)-1-(3-nitrophenyl)prop-2-en-1-one (15): Recrystallized from chloroform. Brown crystals, 66% yield, mp 165.0–167.5°C. 1H-NMR (400 MHz, CDCl 3) δ: 8.84 (dd, J = 2.3, 1.7 Hz, 1H), 8.45 (dd, J = 8.2, 2.3, 1.1 Hz, 1H), 8.35 (dd, J = 7.7, 1.7 Hz, 1H), 7.87 (d, J = 15.6 Hz, 1H), 7.77–7.63 (m, 3H), 7.47 (d, J = 15.7 Hz, 1H), 7.20–7.10 (m, 2H).

13C-NMR (101 MHz, CDCl 3) δ: 187.94, 164.61 (d, J = 19.0 Hz), 145.59, 139.59, 134.22, 130.89 (d, J = 8.7 Hz), 130.77 (d, J = 15.3 Hz), 130.11, 127.27, 123.39, 120.50 (d, J = 12.2 Hz), 116.51 (d, J = 22.1 Hz). EI-MS m/z: 271.0 (M+) Caled for C19H19ClO, 298.8066.

(2E)-3-(4-Tert-Butyloxyphenyl)-1-(4-chlorophenyl)prop-2-en-1-one (16)[2]: Recrystallized from ethanol. Yellow crystals, 51% yield, mp 114.0–116.0°C. 1H-NMR (400 MHz, DMSO-d 6) δ: 8.00–7.91 (m, 2H), 7.79 (d, J = 15.6 Hz, 1H), 7.61–7.52 (m, 2H), 7.51–7.44 (m, 2H), 7.38 (d, J = 15.6 Hz, 1H), 7.07–6.99 (m, 2H), 1.40 (s, 9H). 13C-NMR (101 MHz, CDCl 3) δ: 189.48, 154.62, 145.51, 139.22, 136.82, 132.10, 130.03, 129.6, 128.55, 126.14, 120.88, 35.13, 31.30. EI-MS m/z: 299.0 (M+) Caled for C21H21ClO, 314.8059.

(2E)-1-(4-Chlorophenyl)-3-(isopropyl)prop-2-en-1-one (26)[2]: Recrystallized from ethanol. White crystals, 45% yield, mp 107.9–110.0°C. 1H-NMR (400 MHz, CDCl 3) δ: 8.00–7.92 (m, 2H), 7.81 (d, J = 15.7 Hz, 1H), 7.62–7.55 (m, 2H), 7.51–7.40 (m, 3H), 7.32–7.27 (m, 2H), 2.95 (p, J = 6.9 Hz, 1H), 1.28 (d, J = 6.9 Hz, 6H).

(2E)-3-(4-Butyloxyphenyl)-1-(3-methoxyphenyl)prop-2-en-1-one (17)[2]: Recrystallized from ethanol. Yellow crystals, 51% yield, mp 114.0–116.0°C. 1H-NMR (400 MHz, CDCl 3) δ: 7.79 (d, J = 15.6 Hz, 1H), 7.63–7.56 (m, 3H), 7.54 (d, J = 2.6, 1.5 Hz, 1H), 7.44–7.35 (m, 2H), 7.12 (ddd, J = 8.2, 2.7, 0.9 Hz, 1H), 6.96–6.88 (m, 2H), 4.01 (t, J = 6.5 Hz, 2H), 3.88 (s, 3H), 1.79 (ddt, J = 9.0, 7.9, 6.3 Hz, 2H), 1.59–1.42 (m, 2H), 0.99 (t, J = 7.4 Hz, 3H). 13C-NMR (101 MHz, CDCl 3) δ: 190.47, 161.50, 160.02, 145.00, 140.12, 130.39, 129.64, 127.53, 121.10, 119.81, 119.19, 115.08, 112.97, 68.04, 55.64, 31.35, 19.37, 13.98. EI-MS m/z: 310.0 (M+) Caled for C18H20O3, 313.0869.

(2E)-3-(4-Tert-Butyloxyphenyl)-1-(3-methoxyphenyl)prop-2-en-1-one (18)[2]: Recrystallized from ethanol. Orange crystals, 12% yield, mp 59.6–60.7°C. 1H-NMR (400 MHz, CDCl 3) δ: 8.40–8.31 (m, 2H), 8.18–8.09 (m, 2H), 7.82 (d, J = 15.6 Hz, 1H), 7.65–7.56 (m, 2H), 7.35 (d, J = 15.6 Hz, 1H), 6.99–6.90 (m, 2H), 4.02 (t, J = 6.5 Hz, 2H), 1.87–1.72 (m, 2H), 1.56–1.44 (m, 2H), 0.99 (t, J = 7.4 Hz, 3H).

(2E)-3-(4-Tert-Butyloxyphenyl)-1-(3-methoxyphenyl)prop-2-en-1-one (19)[2]: Recrystallized from ethanol. Orange crystals, 12% yield, mp 59.6–60.7°C. 1H-NMR (400 MHz, CDCl 3) δ: 8.40–8.31 (m, 2H), 8.18–8.09 (m, 2H), 7.82 (d, J = 15.6 Hz, 1H), 7.63–7.55 (m, 2H), 7.38 (d, J = 15.6 Hz, 1H), 7.09–7.01 (m, 2H), 1.42 (s, 9H). 13C-NMR (101 MHz, CDCl 3) δ: 189.17, 187.94, 164.61 (d, J = 19.0 Hz), 145.59, 139.59, 134.22, 130.89 (d, J = 8.7 Hz), 130.77 (d, J = 15.3 Hz), 130.11, 127.27, 123.39, 120.50 (d, J = 12.2 Hz), 116.51 (d, J = 22.1 Hz). EI-MS m/z: 271.0 (M+) Caled for C19H19ClO, 298.8066.
C19H19NO4, 325.5284.

\[\text{1H-NMR (400 MHz, CDCl}_3\text{)}\]

\[\delta 123.70, 119.88, 79.80, 29.08.\]

\[\text{EI-MS } m/z: 299.0 \text{ (M+)}; \text{ Calcd for C}_{19}\text{H}_{19}\text{ClO}, 298.8065.\]

\[\text{13C-NMR (101 MHz, CDCl}_3\text{)}\]

\[\delta 159.03, 150.12, 146.77, 143.51, 130.01, 129.48, 129.00, 123.97, 123.70, 119.88, 79.80, 29.08. \text{ EI-MS } m/z: 257.0 \text{ (M+)}; \text{ Calcd for C}_{16}\text{H}_{15}\text{NO}_2\text{Cl}, 256.7268.\]

\[\text{1H-NMR (400 MHz, CDCl}_3\text{)}: \delta 7.87–7.75 \text{ (m, 3H)}, 7.67 \text{ (d, J=15.5 Hz, 1H), 7.72–7.05 \text{ (m, 2H)}, 7.04–6.97 \text{ (m, 2H)}, 4.04 \text{ (t, J=6.5 Hz, 2H)}, 3.87 \text{ (s, 3H), 1.78–1.65 \text{ (m, 2H)}, 1.52–1.37 \text{ (m, 2H)}, 0.94 \text{ (t, J=7.4 Hz, 3H).}\]

\[\text{31C-NMR (101 MHz, CDCl}_3\text{)}\]

\[\delta 189.37, 145.73, 139.31, 138.84, 136.72, 134.80, 131.76, 130.07, 129.24, 129.04, 125.92, 121.46, 100.13, 21.49. \text{ EI-MS } m/z: 257.0 \text{ (M+)}; \text{ Calcd for C}_{16}\text{H}_{15}\text{NO}_2\text{Cl}, 256.7268.\]

\[\text{1H-NMR (400 MHz, CDCl}_3\text{)}: \delta 8.19–8.12 \text{ (m, 2H), 7.87–7.77 \text{ (m, 3H)}, 7.67 \text{ (d, J=15.5 Hz, 1H), 7.72–7.05 \text{ (m, 2H)}, 7.04–6.97 \text{ (m, 2H)}, 4.04 \text{ (t, J=6.5 Hz, 2H)}, 3.87 \text{ (s, 3H), 1.78–1.65 \text{ (m, 2H)}, 1.52–1.37 \text{ (m, 2H)}, 0.94 \text{ (t, J=7.4 Hz, 3H).}\]

\[\text{31C-NMR (101 MHz, CDCl}_3\text{)}\]

\[\delta 189.37, 145.73, 139.31, 138.84, 136.72, 134.80, 131.76, 130.07, 129.24, 129.04, 125.92, 121.46, 100.13, 21.49. \text{ EI-MS } m/z: 257.0 \text{ (M+)}; \text{ Calcd for C}_{16}\text{H}_{15}\text{NO}_2\text{Cl}, 256.7268.\]

\[\text{1H-NMR (400 MHz, CDCl}_3\text{)}: \delta 7.87–7.75 \text{ (m, 3H)}, 7.67 \text{ (d, J=15.5 Hz, 1H), 7.72–7.05 \text{ (m, 2H)}, 7.04–6.97 \text{ (m, 2H)}, 4.04 \text{ (t, J=6.5 Hz, 2H)}, 3.87 \text{ (s, 3H), 1.78–1.65 \text{ (m, 2H)}, 1.52–1.37 \text{ (m, 2H)}, 0.94 \text{ (t, J=7.4 Hz, 3H).}\]

\[\text{31C-NMR (101 MHz, CDCl}_3\text{)}\]

\[\delta 189.37, 145.73, 139.31, 138.84, 136.72, 134.80, 131.76, 130.07, 129.24, 129.04, 125.92, 121.46, 100.13, 21.49. \text{ EI-MS } m/z: 257.0 \text{ (M+)}; \text{ Calcd for C}_{16}\text{H}_{15}\text{NO}_2\text{Cl}, 256.7268.\]
161.54, 154.67, 148.92, 145.00, 137.11, 134.80, 127.40, 118.81, 117.15, 115.05, 115.02, 73.90, 57.43, 44.34, 42.25, 41.41, 34.19, 29.89. 

251.1 (M+); Calcd for C17H17NO, 251.3229.

(2E)-3-(4-Butoxyphenyl)-1-(furan-2-yl)prop-2-en-1-one (51):
Reccrystallized from ac. ethanol. White crystals, 50% yield, mp 84.0–85.4°C. 1H-NMR (400 MHz, CDCl3) δ: 7.85 (d, J = 157.1 Hz, 1H), 7.67–7.55 (m, 3H), 7.37–7.28 (m, 2H), 6.96–6.88 (m, 2H), 6.58 (dd, J = 3.6, 1.7 Hz, 1H), 4.01 (t, J = 6.5 Hz, 2H), 1.85–1.73 (m, 2H), 1.56–1.42 (m, 2H), 0.98 (t, J = 7.4 Hz, 3H). 13C-NMR (101 MHz, CDCl3) δ: 178.32, 161.55, 154.06, 146.38, 144.07, 130.48, 127.40, 118.81, 117.15, 115.05, 112.57, 68.03, 31.34, 19.36, 13.97. EI-MS m/z: 270.0 (M+); Calcd for C17H18O3, 270.3230.


