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Homotaurine and Curcumin Analogues as Potential Anti-Amyloidogenic Agents

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Abstract: Currently, there is neither a cure for Alzheimer's disease (AD) nor a way to stop the progressive death of neuronal cells associated with this devastating aliment. To date, there are only medications that temporarily slow its progression, and do not interfere with its pathogenesis. One of the hallmarks of AD is the presence of amyloid-beta plaques derived from the metabolism of the amyloid precursor protein, via the cleavage by beta followed by gamma secretase. Homotaurine, a naturally occurring small molecule found in some seaweeds, and curcumin, a phenolic antioxidant found in *Curcuma longa*, have been extensively studied as potential compounds to prevent/reverse plaque formation. In this study, libraries of chalcones and extended chalcones based on curcumin, as well as aminopropylsulfonamides inspired by homotaurine, were synthesized. Using fluorescence spectroscopic analysis with Thioflavin T, the anti-aggregation effect on $A\beta_{42}$ was determined. A select number of newly synthesized chalcones and extended chalcone analogs were revealed to be potential anti-amyloidogenic agents. These were further evaluated with regard to their neurotoxicity/neuroprotection. The extended chalcone analogs that displayed the most anti-aggregation effect on $A\beta_{42}$ were further analyzed in an MTT assay. Although none of the compounds alone displayed any neurotoxicity, none were able to provide neuroprotection against $A\beta_{42}$.

Keywords: Alzheimer's disease; anti-amyloidogenic; aminopropylsulfonamides; extended chalcones

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1. Introduction

Alzheimer's disease (AD), the most prominent form of dementia, is an irreversible multifaceted, progressive brain disorder that displays slow cognitive decline [1]. The etiology of AD is not fully understood, however, studies have demonstrated that AD is related to low levels of acetylcholine [2], aggregates of amyloid-beta peptide (Aβ) [3], hyperphosphorylation of tau proteins [4], and oxidative stress [5]. The amyloid cascade hypothesis suggested that a sequence of abnormalities in the cleavage of the amyloid precursor protein (APP) [6] leads to the production and accumulation of insoluble Aβ peptides, with the most common isoforms being 40 ($A\beta_{40}$) or 42 ($A\beta_{42}$) amino acids in length [7]. The Aβ peptides can aggregate into oligomers, forming insoluble fibrils, ultimately leading to plaques. These plaques interfere in neuronal signaling which disrupts brain cell functions. The result is a loss of neuronal synapses, progressive decline in neurotransmitter activity, inflammation, and neuronal cell death. The amyloid cascade hypothesis suggests that the Aβ plaques initiate mitochondrial oxidative stress and promote hyperphosphorylation of tau proteins, resulting in neurotoxicity [8]. Amyloid inhibitor therapies have been attempted to reduce Aβ peptide production, either via: α-secretase stimulation, inhibition of γ-secretase, and/or inhibition of β-secretase [9]. Earlier this year, aducanumab, a monoclonal antibody developed by Biogen which targets the Aβ aggregates, was approved by the FDA and became the first drug to treat AD [10].

Homotaurine (Figure 1) is a natural product found in marine red algae and has been chemically synthesized [11]. It was clinically tested through Phase 3, as a potential anti-amyloidogenic agent for AD [12] Unfortunately, it failed to show statistically significant superiority over the placebo [12]. Curcumin (Figure 1), an orange-yellow polyphenol found in turmeric, underwent clinical study in 2008. A six-month randomized, placebo-controlled, double-blind, clinical trial failed to show health benefits [13]. It was suggested that curcumin may act on AD by Aβ disaggregation, anti-inflammation, and/or antioxidation [13]. In vitro studies revealed that curcumin inhibited $A\beta_{40}$ aggregation and prevented $A\beta_{42}$ oligomer formation at concentrations between 0.1 and 1.0 μM but may have BBB permeability issues [14]. We thus aimed to design a series of sulfonamides and chalcone derivates based upon the structures of homotaurine and curcumin, respectively, and determined their activity as possible anti-amyloidogenic agents.

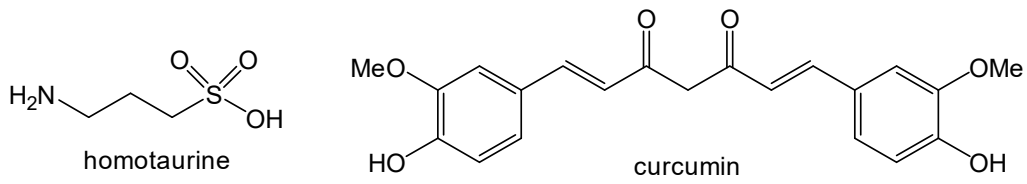
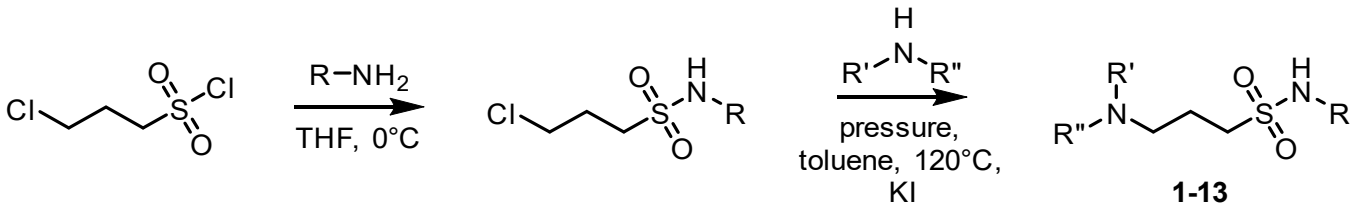


Figure 1. Chemical structures of homotaurine and curcumin.

2. Results and Discussion

2.1. Homotaurine-Based Analogues

We hypothesized that homotaurine's lack of clinical success may have been related to the highly anionic nature and subsequent low logP. The low logP was attributed to possessing both sulfonic acid and amine moieties, thus hindering membrane permeability. Thus, we looked at replacing this moiety with a weak acid, specifically sulfonamide. We synthesized sulfonamide derivatives from 3-chloropropanesulfonyl chloride (Scheme 1) whereby several primary and secondary amines could be employed. It was crucial that we utilized a primary amine in the first step to ensure the presence of an acidic proton in the final sulfonamide product. The reaction of sulfonyl chloride with various amines was carried out in THF at 0 °C, as this was a highly exothermic reaction. In the second step, it was determined that the addition of KI facilitated the nucleophilic attack on the alkyl chloride, reducing the reaction time from >96 to 48 h. We avoided using water to allow for easier purification of this potential zwitterion via flash chromatography. The final chemical yields ranged from 9 to 71%, primarily driven by sterics of the amine.



Scheme 1. Synthesis of sulfonamide derivative compound (**1**–**13**).

The in vitro anti-aggregation of the amyloid-beta (**1**–**42**) peptide was conducted with all compounds initially at 100 μM and incubated at 37 °C, in the presence of Thioflavin T (200 μM) over 120 min. Homotaurine itself did not significantly affect the aggregation, while our positive control (Phenol red) showed a significant decrease over 2 h (Figure 2). Unfortunately, none of the sulfonamide derivatives showed any noticeable effect on the aggregation of the Aβ peptide. In fact, the kinetic curves of all synthesized sulfonamides were similar to the homotaurine and, at the same time, similar to the peptide without the inhibitor (Figure 2), thus, the IC_{50} values could only be estimated as >100 μM (Table 1).

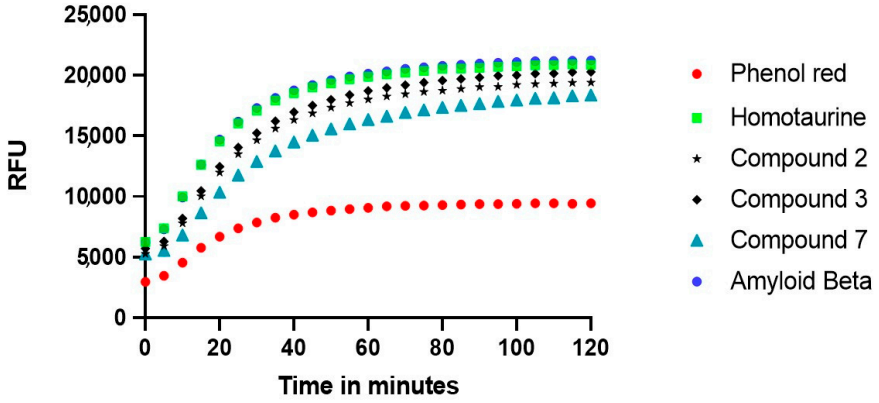
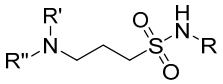
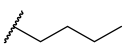
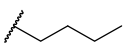
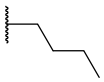
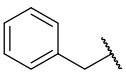
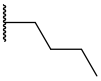


Figure 2. ThT fluorescence assay. Select sulfonamide derivatives (**2**, **3**, **7**) at 100 μM with Phenol red (100 μM) as a positive control. Fluorescence was recorded every 5 min for 120 min.

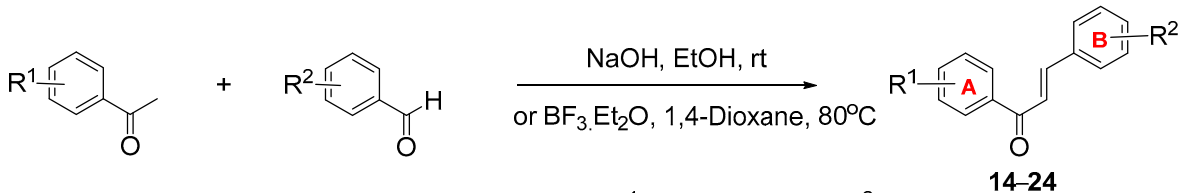
Table 1. Library and IC_{50} values of homotaurine derivatives.



Cmpd #	IC_{50}	R	R′	R″
1	>100 μM			H
2	>100 μM			
3	>100 μM			
4	>100 μM			
5	>100 μM			
6	>100 μM			
7	>100 μM			
8	>100 μM			
9	>100 μM			
10	>100 μM			
11	>100 μM			H
12	>100 μM			
13	>100 μM			

2.2. Curcumin-Based Analogues

With knowledge that curcumin has been reported to display anti-amyloidogenic activity [14], we synthesized nine chalcones (**14–22**) in addition to two hybrid chalcone-sulfonamide derivatives (**23**, **24**). Most of the chalcones were synthesized via a classic condensation coupling of an acetophenone and a benzaldehyde (Scheme 2). This one-step synthesis yielded our products without the need for further purification. Due to the fact that the final products were not soluble in the cold water/ethanol solution, while the starting materials were, simple filtration and washing were utilized. A few chalcones (**21–24**) were synthesized by using boron trifluoride etherate ($BF_3.Et_2O$) as a condensing agent in the reaction. This $BF_3.Et_2O$-assisted method produces higher chemical yields, requiring shorter reaction times, with minimal side reactions [15].



14: R^1=R^2=H
15: R^1=4-OMe, R^2=H
16: R^1=4-Cl, R^2=4-NMe_2
17: R^1=4-Me, R^2=4-NMe_2
18: R^1=3-CF_3, R^2=4-NMe_2
19: R^1=4-Bromophenyl, R^2=4-NMe_2
20: R^1=H, R^2=4-NMe_2
21: R^1=3-OH, R^2=3-CN
22: R^1=4-OH, R^2=3-CN
23: R^1=4-$SO_2N(C_3H_7)_2$, R^2=3-CN
24: R^1=4-$SO_2N(C_5H_{11})_2$, R^2=3-CN

Scheme 2. Synthesis of chalcone derivatives (**14–24**).

We again tested each of the chalcones in the same ThT assay, initially at 100 μM for 120 min. Modest activity for the majority of the chalcones possessing a 4-dimethylamino group on ring B was observed, with a kinetic curve being similar to Phenol red (Figure 3). The chalcone-sulfonamide hybrid compounds (**23**,**24**) again displayed a lack of activity towards Aβ aggregation. Therefore, we abandoned any further homotaurine analogs.

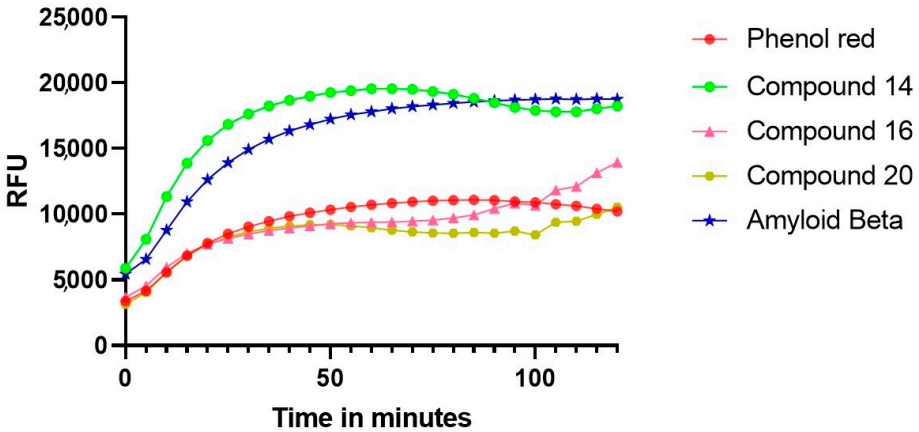
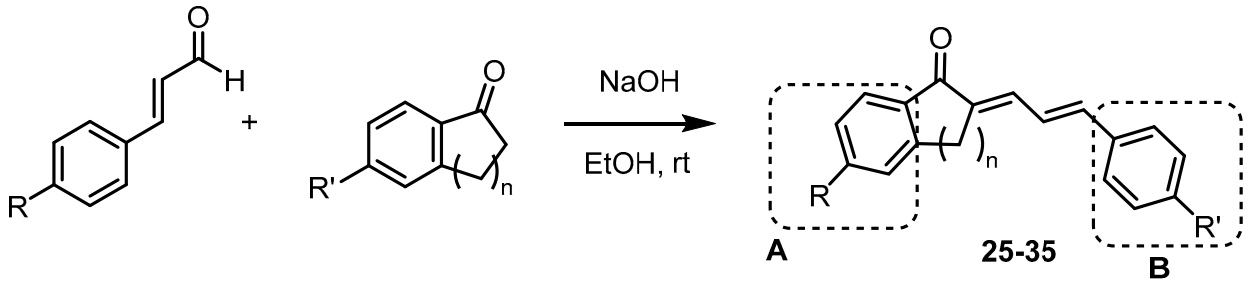


Figure 3. Select ThT fluorescence assay of chalcone compounds 14, 16, and 20 at 100 μM. Phenol red 100 μM is a positive control. Fluorescence was recorded every 5 min for 120 min.

Curcumin's extended conjugation (specifically in its enol tautomeric form) inspired us to extend our chalcone derivatives. We synthesized a diversity of extended chalcones with different substitution patterns as well as the inclusion of some with fused ring systems (**31–33**) via the condensation of an acetophenone and a cinnamaldehyde (Scheme 3). A noticeable improvement in anti-amyloidogenic profiles for those possessing a 4-dimethylamino group on ring B was observed (Figure 4).



Scheme 3. Synthesis of extended chalcone derivatives (**25–35**).

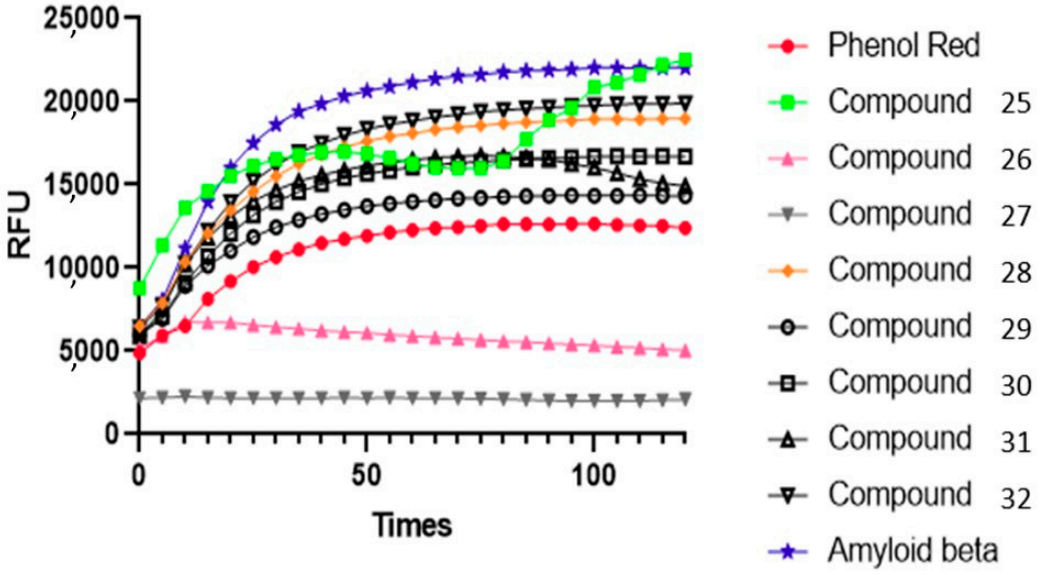
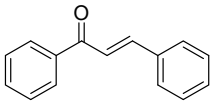
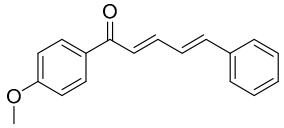
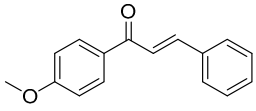
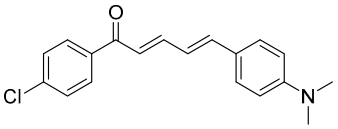
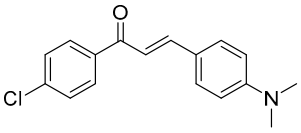
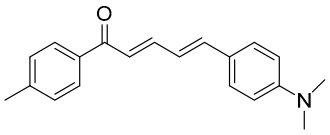
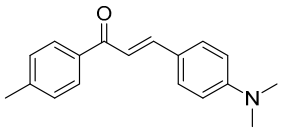
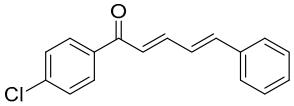
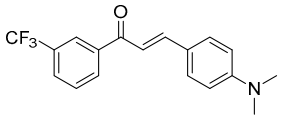
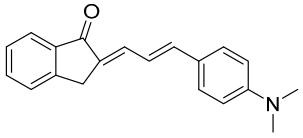
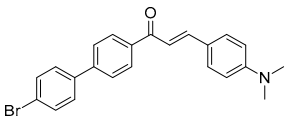
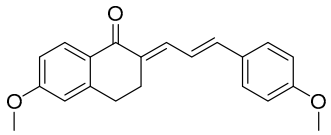
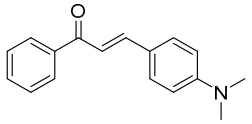
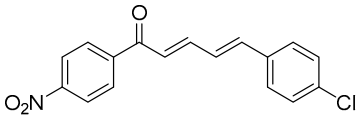
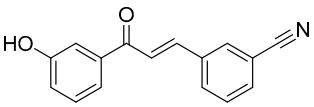
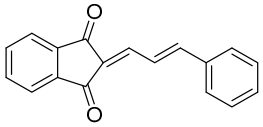
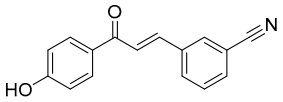
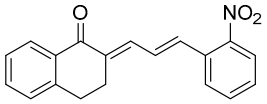
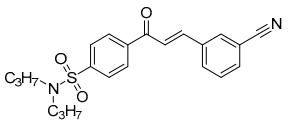
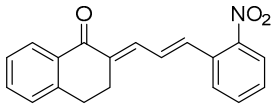
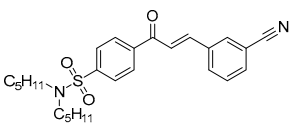
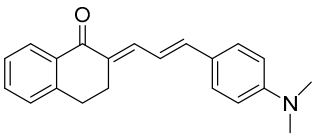


Figure 4. ThT fluorescence assay. Select extended chalcone compounds (**25–32**) at 100 μM with Phenol red (100 μM) as a positive control. Fluorescence was recorded every 5 min for 120 min.

All chalcones (**14–24**) and extended chalcones (**25–35**) were initially screened at 100 μM. Active compounds were defined as those displaying a kinetic curve, similar to or superior to Phenol red. These were further tested to obtain IC_{50} values (determined by linear regression parameter), again utilizing the ThT assay [16–18]. Encouragingly, four chalcones (**16–18**, and **20**) inhibited Aβ peptide aggregation with IC_{50} values in the micromolar range (Table 2). Moreover, five extended chalcones (**26**,**27**,**29**,**34**,**35**) also displayed noticeable anti-amyloidogenic activity (Table 2)—the most potent being compound **26** from the extended chalcone library with an IC_{50} value of 2.43 μM. Again, generally the most active compounds possessed the 4-dimethylamino on ring B. Overall, it was clear that the extended chalcones displayed superior activity over the simple chalcones with the same substituent pattern on the aromatic rings. For example, **20** had an IC_{50} value of 40.2 μM, compared to the extended chalcone counterpart (i.e., **34**) of 18.7 μM. This was also observed with **17** compared to **27** (6.9 vs. 3.4 μM, respectively) and **16** compared to **26** (66.2 vs. 2.4 μM, respectively). Other electron-donating (OMe) or -withdrawing groups (CN, Cl, or NO_2) on ring B for either the chalcone or extended chalcone showed no activity. Conversely, both electron-donating and -withdrawing groups (Cl, Me, CF_3) or fused rings (**29** and **35**) on ring A displayed good anti-amyloidogenic activity as long as the 4-dimethylamino on ring B was present.

Table 2. IC_{50} values of (left) chalcone and chalcone-homotaurine hybrids and (right) extended chalcones. Data reported as triplicates.

Cmpd #	IC_{50}	Structure	Cmpd #	IC_{50}	Structure
14	>100 μM		**25**	>100 μM	
15	>100 μM		**26**	2.4 μM	
16	65.2 μM		**27**	3.4 μM	
17	6.9 μM		**28**	>100 μM	
18	33.6 μM		**29**	17.40 μM	
19	>100 μM		**30**	>100 μM	
20	40.2 μM		**31**	>100 μM	
21	>100 μM		**32**	>100 μM	
22	>100 μM		**33**	>100 μM	
23	>100 μM		**34**	18.68 μM	
24	>100 μM		**35**	13.47 μM	

In addition, we wanted to determine whether the compounds showing low IC_{50} values, (**26**, **27**, and **34**) exhibited any neuroprotection using a neuronal cell line; specifically, neuroblastoma SH-SY5Y cells. The cells were obtained from the neuroblastoma cell line SK-N-SH and maintained in DMEM:F12 media. Of note, a plethora of cytotoxicity assays have been reported; however, these are not necessarily useful in these studies for a variety of reasons. For example, the lactate dehydrogenase (LDH) release assay was attempted [19]; unfortunately, we did not obtain reportable data from this study. This was due to the fact that the LDH release assay assesses necrotic cell death, whereas amyloid beta causes apoptotic cell death in SH-SY5Y cells. We next performed a CellTiter-Glo assay [20], which determines the number of viable cells by quantifying the amount of ATP. This assay did not produce positive data since ATP is required for amyloid-beta-induced apoptosis. The MTS assay [21] also did not yield any significant data. Ultimately, the cell viability/cell cytotoxicity-based 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed according to the previous literature (Figure 5) [22]. The cell viability of SH-SY5Y cells exposed to compounds **26**, **27**, and **34** were measured at their IC_{50} values. All three compounds displayed no significant changes in cell viability at these concentrations. Conversely, the addition of $A\beta_{42}$ led to a ~40% decrease in cell viability, highlighting its known neuro-cytotoxicity. Unfortunately, the co-administration of compounds **26**, **27**, or **34** at their IC_{50} concentration did not demonstrate significant decreases in $A\beta_{42}$-induced cytotoxicity in SH-SY5Y cells.

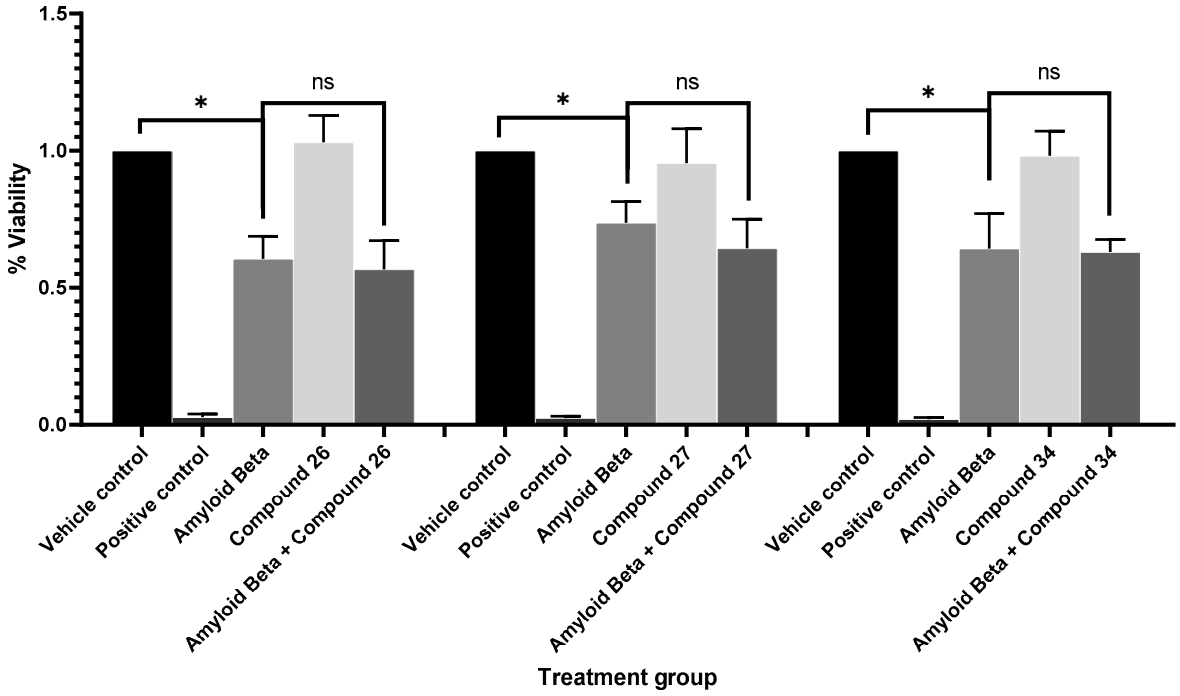


Figure 5. Amyloid beta alone significantly decreases cell viability in SH-SY5Y cells with compounds **26, 27,** and **34** (at IC_{50} concentrations), providing no significant neuroprotection. Cell viability was measured as an MTT reduction, and data ± SEM (n = 3) were normalized as % vehicle control (black column). Asterisk (*) indicates significant difference from vehicle controls as determined by one-way ANOVA followed by Tukey's *post hoc* test ($p < 0.05$); ns, not significant.

3. Conclusions

Unfortunately, the synthesized homotaurine-inspired library displayed no anti-amyloid genic activity even at very high concentrations. However, some of our curcumin-based analogous, whether chalcones or extended chalcones, did display activity against Aβ aggregation. Those with an electron-donating group, specifically 4-dimethylamino on the B side, displayed the greatest activity. Currently, it is unclear whether electron-withdrawing

or -donating groups on the A side increases activity. When neurotoxicity studies were performed on the three extended chalcones with the greatest activity, no notable neuroprotection was observed. Future studies will focus upon ascertaining what modifications are necessary to translate positive ThT assay outcomes into cell viability results.

4. Materials and Methods

All chemicals were purchased from Millipore Sigma and used without further purification. All synthesized compounds were purified using flash column chromatography. ^{1}H-NMR and ^{13}C-NMR were recorded at 300 MHz on a Varian instrument using VnmrJ version 4.2A. NMR spectroscopy for compounds 21–24 was performed on a Bruker AVANCE III 400 MHz spectrometer. For in vitro studies, a fluorometric assay was performed in 96 non-binding microplates from Greiner Bio-One with a clear bottom on a Synergy Bio-tek HTS plate reader.

4.1. Preparation and Characterization of Homotaurine and Curcumin Analogues

1. *N*-(isobutyl)-3-(isobutylamino)propane-1-sulfonamide

3-chloropropanesulfonyl chloride (0.89 g, 5.03 mmol) was added to a round-bottom flask at 0 °C and dissolved in THF (3 mL) under argon, whereby isobutyl amine (4 mL, 0.04 mol) was added dropwise. The mixture was stirred for 20 min, after which time THF and the excess of amine were evaporated under reduced pressure. The remaining residue was dissolved in dichloromethane (10 mL) and washed with 1 M hydrochloric acid (10 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated. ^{1}H-NMR was run on this intermediate to confirm complete conversion and used without further purification. The resulting liquid product was transferred to a pressure vessel and dissolved in toluene (4 mL). Potassium iodide (10 mg, 0.06 mmol) and isobutyl amine (0.89 mL, 8.95 mmol) were added and the mixture was heated to 130 °C for 48 h. The resulting yellow liquid was filtered and the excess of toluene was evaporated. The resulting mixture was purified using flash column chromatography (gradient elution-ethyl acetate: hexane with an increase in ethyl acetate from 66% to 100%) to obtain a yellow liquid (0.52 g, 42%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (d, *J* = 6.7 Hz, 6H), 0.95 (d, *J* = 6.7 Hz, 6H), 1.76 (m, 2H), 1.98 (quint, *J* = 6.5 Hz, 1H), 2.40 (d, *J* = 6.8 Hz, 2H), 2.73 (t, *J* = 6.4 Hz, 2H), 2.91 (d, *J* = 6.8 Hz, 2H), 3.11 (t, *J* = 7.3 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d) δ 54.91, 50.25, 48.82, 46.53, 28.86, 26.25, 21.18, 20.68, 20.40.

2. *N*-(isobutyl)-3-(diethylamino)propane-1-sulfonamide

3-chloropropanesulfonyl chloride (0.58 g, 3.27 mmol) was added to a round-bottom flask at 0 °C and dissolved in THF (3 mL) under argon, whereby isobutyl amine (4 mL, 0.04 mol) was added dropwise. The mixture was stirred for 20 min, after which time THF and the excess of amine were evaporated under reduced pressure. The remaining residue was dissolved in dichloromethane (10 mL) and washed with 1 M hydrochloric acid (10 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated. ^{1}H-NMR was run on this intermediate to confirm complete conversion and used without further purification. The resulting liquid product was transferred to a pressure vessel and dissolved in toluene (4 mL). Potassium iodide (10 mg, 0.06 mmol) and diethyl amine (0.42 mL, 5.7 mmol) were added and the mixture was heated to 130 °C for 48 h. The resulting yellow liquid was filtered and the excess of toluene was evaporated. The resulting mixture was purified using flash column chromatography (gradient elution-ethyl acetate: hexane with an increase in ethyl acetate from 66% to 100%) to obtain a yellow liquid (0.15 g, 18%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.95 (d, *J* = 6.7 Hz, 6H), 1.00 (t, *J* = 7.2 Hz, 6H), 1.78 (m, 1H), 1.94 (quint, *J* = 7.4 Hz, 2H), δ 2.52 (m, 6H), δ 2.90 (t, *J* = 5.2 Hz, 2H), 3.07 (t, *J* = 7.6 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 51.14, 50.72, 50.54, 46.48, 29.00, 21.54, 19.89, 11.42.

3. *N*-(*t*-butyl)-3-(diethylamino)propane-1-sulfonamide

3-chloropropanesulfonyl chloride (1.52 g, 8.57 mmol) was added to a round-bottom flask at 0 °C and dissolved in THF (3 mL) under argon, whereby *t*-butyl amine (4 mL,

0.04 mol) was added dropwise. The mixture was stirred for 20 min, after which time THF and the excess of amine were evaporated under reduced pressure. The remaining residue was dissolved in dichloromethane (10 mL) and washed with 1 M hydrochloric acid (10 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated. ^{1}H-NMR was run on this intermediate to confirm complete conversion and used without further purification. The resulting liquid product was transferred to a pressure vessel and dissolved in toluene (4 mL). Potassium iodide (10 mg, 0.06 mmol) and diethyl amine (2 mL, 19.2 mmol) were added and the mixture was heated to 130 °C for 48 h. The resulting yellow liquid was filtered and the excess of toluene was evaporated. The resulting mixture was purified using flash column chromatography (gradient elution-ethyl acetate: hexane with an increase in ethyl acetate from 66% to 100%) to obtain a yellow liquid (1.00 g, 46%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.98 (t, *J* = 7.15 Hz, 6H), 1.36 (s, 9H), 1.92 (quint, *J* = 7.2 Hz, 2H), 2.5 (m, 6H), 3.06 (t, *J* = 7.7 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 54.47, 54.40, 51.05, 46.56, 30.34, 22.00, 11.61.

4. *N*-butyl-3-(diethylamino)propane-1-sulfonamide

3-chloropropanesulfonyl chloride (1.46 g, 8.22 mmol) was added to a round-bottom flask at 0 °C and dissolved in THF (3 mL) under argon, whereby n-butyl amine (4 mL, 0.04 mol) was added dropwise. The mixture was stirred for 20 min, after which time THF and the excess of amine were evaporated under reduced pressure. The remaining residue was dissolved in dichloromethane (10 mL) and washed with 1 M hydrochloric acid (10 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated. ^{1}H-NMR was run on this intermediate to confirm complete conversion and used without further purification. The resulting liquid product was transferred to a pressure vessel and dissolved in toluene (4 mL). Potassium iodide (10 mg, 0.06 mmol) and diethyl amine (0.42 mL, 5.7 mmol) were added and the mixture was heated to 130 °C for 48 hrs. The resulting yellow liquid was filtered and the excess of toluene was evaporated. The resulting mixture was purified using flash column chromatography (gradient elution-ethyl acetate: hexane with an increase in ethyl acetate from 66% to 100%) to obtain a yellow liquid (1.46 g, 71%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.91 (t, *J* = 7.4 Hz, 3H), 0.98 (t, *J* = 7.1 Hz 6H), 1.36 (m, 2H), 1.52 (quint, *J* = 7.8 Hz, 2H), δ 1.92 (quint, *J* = 7.2 Hz, 2H), 2.50 (m, 6H), 3.06 (m, 4H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 51.23, 50.84, 46.46, 42.93, 32.41, 21.63, 19.78, 13.63, 11.46.

5. *N*-isopropyl-3-(diethylamino)propane-1-sulfonamide

3-chloropropanesulfonyl chloride (0.59 g, 3.33 mmol) was added to a round-bottom flask at 0 °C and dissolved in THF (3 mL) under argon, whereby isopropyl amine (4 mL, 0.05 mol) was added dropwise. The mixture was stirred for 20 min, after which time THF and the excess of amine were evaporated under reduced pressure. The remaining residue was dissolved in dichloromethane (10 mL) and washed with 1 M hydrochloric acid (10 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated. ^{1}H-NMR was run on this intermediate to confirm complete conversion and used without further purification. The resulting liquid product was transferred to a pressure vessel and dissolved in toluene (4 mL). Potassium iodide (10 mg, 0.06 mmol) and diethyl amine (1 mL, 9.6 mmol) were added and the mixture was heated to 130 °C for 48 h. The resulting yellow liquid was filtered and the excess of toluene was evaporated. The resulting mixture was purified using flash column chromatography (gradient elution-ethyl acetate: hexane with an increase in ethyl acetate from 66% to 100%) to obtain a yellow liquid (0.55 g, 70%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.02 (d, *J* = 7.4 Hz, 6H), δ 2.04 (quint, *J* = 7.2 Hz, 2H), 2.93 (t, *J* = 7.6 Hz, 2H), 3.35 (m, 1H), 3.5 (m, 4H).

6. *N*-isobutyl-3-(dipropylamino)propane-1-sulfonamide

3-chloropropanesulfonyl chloride (1.30 g, 7.32 mmol) was added to a round-bottom flask at 0 °C and dissolved in THF (3 mL) under argon, whereby isobutyl amine (4 mL, 0.04 mol) was added dropwise. The mixture was stirred for 20 min, after which time THF and the excess of amine were evaporated under reduced pressure. The remaining

residue was dissolved in dichloromethane (10 mL) and washed with 1 M hydrochloric acid (10 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated. ^{1}H-NMR was run on this intermediate to confirm complete conversion and used without further purification. The resulting liquid product was transferred to a pressure vessel and dissolved in toluene (4 mL). Potassium iodide (10 mg, 0.06 mmol) and dipropyl amine (2 mL, 14.62 mmol) were added and the mixture was heated to 130 °C for 48 h. The resulting yellow liquid was filtered and the excess of toluene was evaporated. The resulting mixture was purified using flash column chromatography (gradient elution-ethyl acetate: hexane with an increase in ethyl acetate from 66% to 100%) to obtain a yellow liquid (0.41 g, 20%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.86 (t, *J* = 7.3 Hz, 6H), 0.95 (d, *J* = 6.7 Hz, 6H), 1.42 (sextet, *J* = 7.4 Hz, 4H), 1.79 (m, 1H), 1.92 (m, 2H), 2.35 (t, *J* = 7.4 Hz, 4H), 2.51 (t, *J* = 6.5 Hz, 2H), 2.92 (t, *J* = 6.3 Hz, 2H), 3.08 (t, *J* = 7.6 Hz, 2H). Yield: 70%. ^{13}C-NMR (75 MHz, $CDCl_3$) δ 55.83, 52.41, 50.84, 50.61, 29.01, 21.84, 20.10, 19.88, 11.91.

7. *N*-isopropyl-3-(dipropylamino)propane-1-sulfonamide

3-chloropropanesulfonyl chloride (1.01 g, 5.70 mmol) was added to a round-bottom flask at 0 °C and dissolved in THF (3 mL) under argon, whereby isopropyl amine (4 mL, 0.05 mol) was added dropwise. The mixture was stirred for 20 min, after which time THF and the excess of amine were evaporated under reduced pressure. The remaining residue was dissolved in dichloromethane (10 mL) and washed with 1 M hydrochloric acid (10 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated. ^{1}H-NMR was run on this intermediate to confirm complete conversion and used without further purification. The resulting liquid product was transferred to a pressure vessel and dissolved in toluene (4 mL). Potassium iodide (10 mg, 0.06 mmol) and dipropyl amine (2 mL, 14.62 mmol) were added and the mixture was heated to 130 °C for 48 hrs. The resulting yellow liquid was filtered and the excess of toluene was evaporated. The resulting mixture was purified using flash column chromatography (gradient elution-ethyl acetate: hexane with an increase in ethyl acetate from 66% to 100%) to obtain a yellow liquid (0.22 g, 14%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.82 (t, *J* = 7.4 Hz, 6H), 1.19 (d, *J* = 6.5 Hz, 6H), 1.39 (m, 4H), 1.88 (quint, *J* = 6.8 Hz, 2H), 2.32 (t, *J* = 7.5 Hz, 4H), 2.47 (t, *J* = 6.4 Hz, 2H), 3.04 (t, *J* = 7.8 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 55.68, 52.16, 51.94, 46.00, 24.19, 21.73, 19.99, 11.83, 11.81, 11.79.

8. *N*-butyl-3-(dipropylamino)propane-1-sulfonamide

3-chloropropanesulfonyl chloride (1.31 g, 7.39 mmol) was added to a round-bottom flask at 0 °C and dissolved in THF (3 mL) under argon, whereby n-butyl amine (4 mL, 0.04 mol) was added dropwise. The mixture was stirred for 20 min, after which time THF and the excess of amine were evaporated under reduced pressure. The remaining residue was dissolved in dichloromethane (10 mL) and washed with 1 M hydrochloric acid (10 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated. ^{1}H-NMR was run on this intermediate to confirm complete conversion and used without further purification. The resulting liquid product was transferred to a pressure vessel and dissolved in toluene (4 mL). Potassium iodide (10 mg, 0.06 mmol) and dipropyl amine (2 mL, 14.62 mmol) were added and the mixture was heated to 130 °C for 48 hrs. The resulting yellow liquid was filtered and the excess of toluene was evaporated. The resulting mixture was purified using flash column chromatography (gradient elution-ethyl acetate: hexane with an increase in ethyl acetate from 66% to 100%) to obtain a yellow liquid (0.19 g, 10%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.85 (t, *J* = 7.3 Hz, 6H), 0.91 (t, *J* = 7.3 Hz, 3H), 1.40 (m, 6H), 1.53 (quint, *J* = 7.6 Hz, 2H), 1.92 (quint, *J* = 7.5 Hz, 2H), 2.36 (t, *J* = 7.5 Hz, 4H), 2.52 (t, *J* = 6.5 Hz, 2H), 3.07 (m, 4H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 55.64, 52.27, 50.68, 42.98, 32.39, 21.67, 19.89, 19.79, 13.64, 11.90.

9. *N*-(*t*-butyl)-3-(dipropylamino)propane-1-sulfonamide

3-chloropropanesulfonyl chloride (1.33 g, 7.52 mmol) was added to a round-bottom flask at 0 °C and dissolved in THF (3 mL) under argon, whereby *t*-butyl amine (4 mL,

0.04 mol) was added dropwise. The mixture was stirred for 20 min, after which time THF and the excess of amine were evaporated under reduced pressure. The remaining residue was dissolved in dichloromethane (10 mL) and washed with 1 M hydrochloric acid (10 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated. ^{1}H-NMR was run on this intermediate to confirm complete conversion and used without further purification. The resulting liquid product was transferred to a pressure vessel and dissolved in toluene (4 mL). Potassium iodide (10 mg, 0.06 mmol) and dipropyl amine (2 mL, 14.62 mmol) were added and the mixture was heated to 130 °C for 48 h. The resulting yellow liquid was filtered and the excess of toluene was evaporated. The resulting mixture was purified using flash column chromatography (gradient elution-ethyl acetate: hexane with an increase in ethyl acetate from 66% to 100%) to obtain a yellow liquid (0.74 g, 35%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.86 (t, *J* = 7.3 Hz, 6H), 1.41 (m, 13H), 1.92 (quint, *J* = 6.8 Hz, 2H), 2.35 (t, *J* = 7.5 Hz, 4H), 2.50 (t, *J* = 6.8 Hz, 2H), 3.10 (t, *J* = 7.8 Hz, 2H), 4.32 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 55.86, 54.44, 54.40, 52.31, 30.33, 22.14, 20.17, 11.91.

10. *N*-isopropyl-3-(butyl(ethyl)amino)propane-1-sulfonamide

3-chloropropanesulfonyl chloride (1.43 g, 8.07 mmol) was added to a round-bottom flask at 0 °C and dissolved in THF (3 mL) under argon, whereby isopropyl amine (4 mL, 0.05 mol) was added dropwise. The mixture was stirred for 20 min, after which time THF and the excess of amine were evaporated under reduced pressure. The remaining residue was dissolved in dichloromethane (10 mL) and washed with 1 M hydrochloric acid (10 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated. ^{1}H-NMR was run on this intermediate to confirm complete conversion and used without further purification. The resulting liquid product was transferred to a pressure vessel and dissolved in toluene (4 mL). Potassium iodide (10 mg, 0.06 mmol) and N-ethylbutyl amine (2 mL, 16.75 mmol) were added and the mixture was heated to 130 °C for 48 h. The resulting yellow liquid was filtered and the excess of toluene was evaporated. The resulting mixture was purified using flash column chromatography (gradient elution-ethyl acetate:hexane with an increase in ethyl acetate from 66% to 100%) to obtain a yellow liquid (0.22 g, 10%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.92 (t, *J* = 7.5 Hz, 3H), 1.04 (t, *J* = 7.0 Hz, 3H), 1.24 (d, *J* = 6.5 Hz, 6H), 1.29 (m, 2H), 1.44 (m, 2H), 1.99 (quint, *J* = 7.2 Hz, 2H), 2.48 (t, *J* = 7.8 Hz, 2H), 2.60 (m, 4H), 3.08 (t, *J* = 7.5 Hz, 2H), δ 3.64 (quint, *J* = 6.5 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 52.70, 52.03, 51.46, 47.01, 46.18, 28.49, 24.34, 21.46, 20.61, 14.02, 11.09.

11. *N*-benzyl-3-(*t*-butylamino)propane-1-sulfonamide

3-chloropropanesulfonyl chloride (1.22 g, 6.89 mmol) was added to a round-bottom flask at 0 °C and dissolved in THF (3 mL) under argon, whereby benzyl amine (4 mL, 0.03 mol) was added dropwise. The mixture was stirred for 20 min, after which time THF and the excess of amine were evaporated under reduced pressure. The remaining residue was dissolved in dichloromethane (10 mL) and washed with 1 M hydrochloric acid (10 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated. ^{1}H-NMR was run on this intermediate to confirm complete conversion and used without further purification. The resulting liquid product was transferred to a pressure vessel and dissolved in toluene (4 mL). Potassium iodide (10 mg, 0.06 mmol) and *t*-butyl amine (2 mL, 19.03 mmol) were added and the mixture was heated to 130 °C for 48 hrs. The resulting yellow liquid was filtered and the excess of toluene was evaporated. The resulting mixture was purified using flash column chromatography (gradient elution-ethyl acetate:hexane with an increase in ethyl acetate from 66% to 100%) to obtain a yellow liquid (1.07 g, 54%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.01 (s, 9H), 1.92 (quint, *J* = 6.8 Hz, 2H), 2.63 (t, *J* = 6.2 Hz, 2H), 3.07 (t, *J* = 6.2 Hz, 2H), 3.69 (s, 1H), 4.28 (d, *J* = 7.8 Hz, 2H), 7.32 (m, 5H).

12. *N*-isopropyl-3-(butyl(methyl)amino)propane-1-sulfonamide

3-chloropropanesulfonyl chloride (0.88 g, 4.96 mmol) was added to a round-bottom flask at 0 °C and dissolved in THF (3 mL) under argon, whereby isopropyl amine (4 mL, 0.05 mol) was added dropwise. The mixture was stirred for 20 min, after which time THF

and the excess of amine were evaporated under reduced pressure. The remaining residue was dissolved in dichloromethane (10 mL) and washed with 1 M hydrochloric acid (10 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated. ^{1}H-NMR was run on this intermediate to confirm complete conversion and used without further purification. The resulting liquid product was transferred to a pressure vessel and dissolved in toluene (4 mL). Potassium iodide (10 mg, 0.06 mmol) and N-methylbutyl amine (1.6 mL, 12.66 mmol) were added and the mixture was heated to 130 °C for 48 h. The resulting yellow liquid was filtered and the excess of toluene was evaporated. The resulting mixture was purified using flash column chromatography (gradient elution-ethyl acetate:hexane with an increase in ethyl acetate from 66% to 100%) to obtain a yellow liquid (0.16 g, 9%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.89 (t, *J* = 7.1 Hz, 3H), 1.21 (d, *J* = 6.5 Hz, 6H), 1.28 (m, 2H), 1.41 (m, 2H), 1.94 (m, 2H), 2.18 (s, 3H), 2.31 (t, *J* = 7.4 Hz, 2H), 2.42 (t, *J* = 6.6 Hz, 2H), 3.06 (t, *J* = 7.6 Hz, 2H), 3.61 (quint, *J* = 6.4 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 57.34, 55.86, 52.18, 46.07, 41.79, 29.25, 24.36, 21.74, 20.62, 14.06.

13. *N*-isopropyl-3-morpholinopropane-1-sulfonamide

3-chloropropanesulfonyl chloride (1.44 g, 8.15 mmol) was added to a round-bottom flask at 0 °C and dissolved in THF (3 mL) under argon, whereby isopropyl amine (4 mL, 0.05 mol) was added dropwise. The mixture was stirred for 20 min, after which time THF and the excess of amine were evaporated under reduced pressure. The remaining residue was dissolved in dichloromethane (10 mL) and washed with 1 M hydrochloric acid (10 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated. ^{1}H-NMR was run on this intermediate to confirm complete conversion and used without further purification. The resulting liquid product was transferred to a pressure vessel and dissolved in toluene (4 mL). Potassium iodide (10 mg, 0.06 mmol) and morpholine (1 mL, 11.49 mmol) were added and the mixture was heated to 130 °C for 48 hrs. The resulting yellow liquid was filtered and the excess of toluene was evaporated. The resulting mixture was purified using flash column chromatography (gradient elution-ethyl acetate: hexane with an increase in ethyl acetate from 66% to 100%) to obtain a yellow liquid (0.16 g, 32%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.20 (d, *J* = 6.7 Hz, 6H), 1.95 (quint, *J* = 7.5 Hz, 2H), 2.41 (m, 6H), 3.06 (t, *J* = 7.7 Hz, 2H), 3.59 (m, *J* = 6.7 Hz, 1H), 3.67 (t, *J* = 4.6 Hz, 4H), 4.67 (d, *J* = 7.6 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 66.84, 56.76, 53.41, 51.90, 46.12, 24.34, 20.87.

14. *(E)*-Chalcone

A solution of acetophenone (1.05 g, 8.17 mmol) in absolute ethanol (10 mL) was added to an aqueous solution of 10% NaOH (30 mL) at 0 °C. The mixture was stirred for 15 min, after which time benzaldehyde (1.0347 g, 9.75 mmol) was added. The reaction mixture was then stirred at room temperature for 24 h. The precipitated product was vacuum-filtered and washed with small portions of water/ethanol to yield the desired chalcone as a white solid (1.52 g, 75%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.42 (m, 3H), 7.54 (m, 4H), 7.65 (m, 2H), 7.82 (d, *J* = 15.7 Hz, 1H), 8.02 (d, *J* = 9 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 190.59, 144.88, 138.20, 134.87, 132.82, 130.58, 128.98, 128.65, 128.52, 128.47, 122.06. Melting point: 52.6–55.3 °C.

15. (*E*)-1-(4-methoxyphenyl)-3-phenylprop-2-en-1-one

A solution of 1-(4-methoxyphenyl)ethan-1-one (1.02 g, 6.82 mmol) in absolute ethanol (10 mL) was added to an aqueous solution of 10% NaOH (30 mL) at 0 °C. The mixture was stirred for 15 min, after which time benzaldehyde (1.0324 g, 9.72 mmol) was added. The reaction mixture was then stirred at room temperature for 24 h. The precipitated product was vacuum-filtered and washed with small portions of water/ethanol to yield the desired chalcone as a white solid (1.87 g, 81%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.89 (s, 3H), 6.98 (d, *J* = 8.9 Hz, 2H), 7.42 (m, 3H), 7.55 (d, *J* = 15.7 Hz, 1H), 7.64 (m, 2H), 7.80 (d, *J* = 15.7 Hz, 1H), 8.05 (d, *J* = 8.9 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 188.74, 163.43, 143.99, 135.07, 130.83, 130.35, 128.93, 128.37, 125.76, 121.86, 113.85, 55.52. Melting point: 104.0–107.4 °C.

16. (*E*)-1-(4-chlorophenyl)-3-(4-(dimethylamino) phenyl) prop-2-en-1-one

A solution of 1-(4-chlorophenyl)ethan-1-one (1.24 g, 8.03 mmol) in absolute ethanol (10 mL) was added to an aqueous solution of 10% NaOH (30 mL) at 0 °C. The mixture was stirred for 15 min, after which time 4-(dimethylamino)benzaldehyde (1.0524 g, 7.05 mmol) was added. The reaction mixture was then stirred at room temperature for 24 h. The precipitated product was vacuum-filtered and washed with small portions of water/ethanol to yield the desired chalcone as an orange solid (1.59 g, 79%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.05 (s, 6H), 6.69 (d, *J* = 8.9 Hz, 2H), 7.29 (d, *J* = 15.4 Hz, 1H), 7.45 (d, *J* = 8.6 Hz, 2H), 7.5 (d, *J* = 8.9 Hz, 2H), 7.80 (d, *J* = 15.4 Hz, 1H), 7.95 (d, *J* = 8.6 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 188.74, 163.43, 143.99, 135.07, 130.83, 130.35, 128.93, 128.37, 125.76, 121.86, 113.85, 55.52. Anal. Calcd for C17H16ClNO: C, 71.45; H, 5.64; Cl, 12.41; N, 4.90. Found: C, 71.44; H, 5.68; Cl, 12.21; N, 4.98. Melting point: 137.9–141.9 °C.

17. (*E*)-3-(4-(dimethylamino)phenyl)-1-(p-tolyl)prop-2-en-1-one

A solution of 1-(p-tolyl)ethan-1-one (1.37 g, 10.2 mmol) in absolute ethanol (10 mL) was added to an aqueous solution of 10% NaOH (30 mL) at 0°C. The mixture was stirred for 15 min, after which time 4-(dimethylamino)benzaldehyde (1.1548 g, 7.74 mmol) was added. The reaction mixture was then stirred at room temperature for 24 h. The precipitated product was vacuum-filtered and washed with small portions of water/ethanol to yield the desired chalcone as an orange solid (1.44 g, 70%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.42 (s, 3H), 3.04 (s, 6H), 6.77 (d, *J* = 7.8 Hz, 2H), 7.28 (d, *J* = 7.8 Hz, 2H), 7.35 (d, *J* = 15.5 Hz, 1H), 7.55 (d, *J* = 8.3 Hz, 2H), 7.78 (d, *J* = 15.5 Hz, 1H), 7.92 (d, *J* = 7.6 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 190.20, 145.38, 142.89, 136.40, 130.35, 129.17, 128.46, 116.94, 111.87, 40.21, 21.66. Anal. Calcd for C18H19NO: C, 81.47; H, 7.22; N, 5.28. Found: C, 81.70; H, 7.17; N, 5.26. Melting point: 118.6–120.9 °C.

18. (*E*)-3-(4-(dimethylamino)phenyl)-1-(3-(trifluoromethyl)phenyl)prop-2-en-1-one

A solution of 1-(3-(trifluoromethyl) phenyl)ethan-1-one (1.08 g, 5.74 mmol) in absolute ethanol (10 mL) was added to an aqueous solution of 10% NaOH (30 mL) at 0 °C. The mixture was stirred for 15 min, after which time 4-(dimethylamino)benzaldehyde (1.0043 g, 6.73 mmol) was added. The reaction mixture was then stirred at room temperature for 24 h. The precipitated product was vacuum-filtered and washed with small portions of water/ethanol to yield the desired chalcone as an orange solid (1.42 g, 66%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.06 (s, 6H), 6.70 (d, *J* = 8.9 Hz, 2H), 7.30 (d, *J* = 15.4 Hz, 1H), 7.61 (m, 4H), 7.83 (m, 2H), 8.20 (m, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 189.05, 152.29, 147.10, 139.67, 131.46, 130.75, 129.08, 128.55, 128.50, 125.12, 125.07, 122.19, 115.75, 111.78, 40.12. Anal. Calcd for C18H16F3NO: C, 67.70; H, 5.05; F, 17.85; N, 4.39. Found: C, 66.94; H, 4.96; F, 17.87; N, 4.22. Melting point: 85.2–86.4 °C.

19. (*E*)-1-(4′-bromo-[1,1′-biphenyl]-4-yl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one

A solution of 1-(4′-bromo-[1,1′-biphenyl]-4-yl)ethan-1-one (1.03 g, 3.74 mmol) in absolute ethanol (10 mL) was added to an aqueous solution of 10% NaOH (30 mL) at 0 °C. The mixture was stirred for 15 min, after which time 4-(dimethylamino)benzaldehyde (1.1178 g, 7.49 mmol) was added. The reaction mixture was then stirred at room temperature for 24 h. The precipitated product was vacuum-filtered and washed with small portions of water/ethanol to yield the desired chalcone as a yellow solid (2.25 g, 74%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.06 (s, 6H), 6.70 (d, *J* = 7.5 Hz, 2H), 7.36 (d, *J* = 15.4 Hz, 1H), 7.59 (m, 8H), 7.84 (d, *J* = 15.3 Hz, 1H), 8.09 (d, *J* = 6.9 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 189.91, 152.08, 145.97, 143.54, 139.06, 138.12, 132.05, 130.50, 129.02, 128.83, 126.93, 122.59, 122.42, 116.65, 111.82, 40.15. Anal. Calcd for C23H20BrNO: C, 67.99; H, 4.96; Br, 19.67; N, 3.45. Found: C, 67.68; H, 5.05; Br, 19.46; N, 3.39. Melting point: 183.2–187.6 °C.

20. (*E*)-3-(4-(dimethylamino)phenyl)-1-phenylprop-2-en-1-one

A solution of acetophenone (1.08 g, 9.01 mmol) in absolute ethanol (10 mL) was added to an aqueous solution of 10% NaOH (30 mL) at 0 °C. The mixture was stirred for 15 min, after which time 4-(dimethylamino)benzaldehyde (0.9784 g, 6.55 mmol) was added. The reaction mixture was then stirred at room temperature for 24 h. The precipitated product

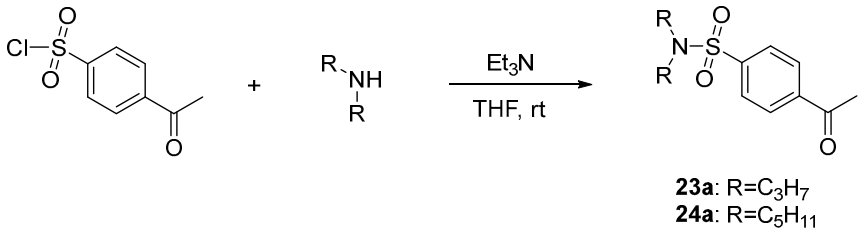
was vacuum-filtered and washed with small portions of water/ethanol to yield the desired chalcone as an orange solid (1.33 g, 81%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.05 (s, 6H), 6.70 (d, *J* = 8.8 Hz, 2H), 7.33 (d, *J* = 15.5 Hz, 1H) 7.52 (m, 5H), 7.79 (d, *J* = 15.5 Hz, 1H), 8.00 (d, *J* = 6.9 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 190.72, 152.02, 145.89, 139.08, 139.06, 130.44, 128.47, 128.32, 122.61, 116.87, 111.82, 40.16. Anal. Calcd for C17H17NO: C, 81.24; H, 6.82; N, 5.57. Found: C, 80.81; H, 6.82; N, 5.50. Melting point: 106.9–110.3 °C.

21. (*E*)-3-(3-(3-hydroxyphenyl)-3-oxoprop-1-en-1-yl)benzonitrile

Boron trifluoride etherate (48% BF_3, 781 mg, 5.5 mmol) was added to a stirred solution of 3′-hydroxyacetophenone (150 mg, 1.1 mmol) and 3-cyanobenzaldehyde (289 mg, 2.2 mmol) in 1,4-dioxane (10 mL), and the reaction mixture was heated at 80 °C for 14–24 h. After cooling, the resultant solution was partitioned with EtOAc, washed with 10% HCl (aq), distilled water, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified using column chromatography (*n*-hexane:EtOAc = 3:1, 1:1) to obtain **21** as a solid (114 mg, 41%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.84 (s, 1H), 8.50 (s, 1H), 8.19 (d, *J* = 7.8 Hz, 1H), 8.04 (d, *J* = 15.8 Hz, 1H), 7.90 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.73 (dt, *J* = 15.7 Hz, 1H), 7.70–7.63 (m, 2H), 7.51–7.48 (m, 1H), 7.39 (t, *J* = 7.9 Hz, 1H), 7.08 (ddd, *J* = 8.1, 2.6, 1.0 Hz, 1H).

22. (*E*)-3-(3-(4-hydroxyphenyl)-3-oxoprop-1-en-1-yl)benzonitrile

The procedure applied to the synthesis of **21** was used with boron trifluoride etherate (48% BF_3, 781 mg, 5.5 mmol), 4-hydroxy acetophenone (150 mg, 1.1 mmol) and 3-cyanobenzaldehyde (289 mg, 2.2 mmol) to obtain **22** as a yellow solid (92 mg, 34%) (Scheme 4). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.48 (s, 1H), 8.48 (s, 1H), 8.17 (d, *J* = 8.0 Hz, 1H), 8.13–8.06 (m, *3*H), 7.88 (dt, *J* = 8.0, 1.6 Hz, 1H), 7.69 (d, *J* = 15.6 Hz, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 6.93–6.89 (m, 2H).



Scheme 4. Synthesis of 4 substituted acetophenones (**23a** and **24a**).

23. (23a) 4-Acetyl-N,N-dipropylbenzenesulfonamide

A mixture of 4-acetylbenzenesulfonyl chloride (300 mg, 1.37 mmol), dipropylamine (151 mg, 1.50 mmol), and triethylamine (277 mg, 2.74 mmol) in anhydrous THF (10 mL) was stirred at room temperature overnight. Water was added, and the reaction mixture was extracted with EtOAc (3 times). The combined organic layer was washed with brine (100 mL), dried over Na_2SO_4, and filtered. The removal of solvent in vacuo presented as yellow oil (154 mg, 40%).

(23) (*E*)-4-(3-(3-cyanophenyl)acryloyl)-N,N-dipropylbenzenesulfonamide

The procedure applied to the synthesis of **21** was used with boron trifluoride etherate (48% BF_3, 325 mg, 2.29 mmol), **23a** (130 mg, 0.46 mmol), and 3-cyanobenzaldehyde (120 mg, 0.92 mmol) to obtain **23** as an ivory fluffy solid (81 mg, 45%) after purification by column chromatography (*n*-hexane:EtOAc = 10:1, 5:1). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.52 (s, 1H), 8.35 (d, *J* = 8.6 Hz, 2H), 8.22 (d, *J* = 8.0 Hz, 1H), 8.13 (d, *J* = 15.7 Hz, 1H), 7.99 (d, *J* = 8.5 Hz, 2H), 7.92 (d, *J* = 7.8 Hz, 1H), 7.82 (d, *J* = 15.7 Hz, 1H), 7.69 (t, *J* = 7.7 Hz, 1H), 3.09 (d, *J* = 7.6 Hz, 4H), 1.49 (sextet, *J* = 7.6 Hz, 4H), 0.82 (t, *J* = 7.6 Hz, 6H).

24. (24a) 4-Acetyl-N,N-dipentylbenzenesulfonamide

The procedure applied to the synthesis of **23a** was used with 4-acetylbenzenesulfonyl chloride (300 mg, 1.37 mmol), diamylamine (235 mg, 1.50 mmol), and triethylamine (277 mg, 2.74 mmol) to obtain **24a** as yellow oil (187 mg, 40%).

(24) (*E*)-4-(3-(3-cyanophenyl)acryloyl)-N,N-dipentylbenzenesulfonamide

The procedure applied to the synthesis of **21** was used with boron trifluoride etherate (48% BF_3, 271 mg, 1.91 mmol), **24a** (130 mg, 0.38 mmol), and 3-cyanobenzaldehyde (100 mg, 0.77 mmol) to obtain **24** as an off-white crystal (37 mg, 21%) after purification by column chromatography (*n*-hexane:EtOAc = 10:1). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.51 (s, 1H), 8.35 (d, *J* = 8.6 Hz, 2H), 8.22 (d, *J* = 7.6 Hz, 1H), 8.13 (d, *J* = 15.7 Hz, 1H), 7.97 (d, *J* = 8.8 Hz, 2H), 7.92 (dt, *J* = 7.7, 1.2 Hz, 1H), 7.81 (d, *J* = 15.7 Hz, 1H), 7.69 (t, *J* = 7.7 Hz, 1H), 3.11 (t, *J* = 7.6 Hz, 4H), 1.46 (quintet, *J* = 7.6 Hz, 4H), 1.29–1.18 (m, 8 H), 0.84 (t, *J* = 7.2 Hz, 6H).

25. (2*E*,4*E*)-1-(4-methoxyphenyl)-5-phenylpenta-2,4-dien-1-one

4′-methoxyacetophenone (200 mg, 1.33 mmol) and *trans*-cinnamaldehyde (0.168 mL, 1.33 mmol) were dissolved in absolute ethanol (10 mL) at room temperature. To this stirring solution, 6M NaOH (1 mL) was added dropwise. Precipitate formed instantaneously and the mixture was stirred for 15 min at room temperature. A few chips of ice were added, and the reaction mixture was cooled in an ice bath for 15 min. The precipitate was vacuum-filtered and washed with small portions of cold water/ethanol solution to yield the desired chalcone as a bright yellow powder (321 mg, 91%). ^{1}H-NMR (300 MHz, Chloroform-*d*) δ 8.00 (d, *J* = 8.0 Hz, 2H), 7.69–7.53 (m, 1H), 7.49 (d, *J* = 8.0 Hz, 2H), 7.42–7.27 (m, 3H), 7.11 (d, *J* = 14.5 Hz, 1H), 7.04–6.88 (m, 4H), 3.86 (s, 3H). ^{13}C-NMR (75 MHz, Chloroform-*d*) δ 188.66, 163.33, 144.04, 141.43, 136.17, 131.06, 130.70, 129.12, 128.84, 127.25, 127.04, 125.20, 113.81, 55.48. Melting point: 73–76 °C.

26. (2*E*,4*E*)-1-(4-chlorophenyl)-5-(4-(dimethylamino)phenyl)penta-2,4-dien-1-one

4′-chloroacetophenone (0.250 mL, 1.92 mmol), and 4-(dimethylamino)cinnamaldehyde (305 mg, 1.75 mmol) were dissolved in absolute ethanol (15 mL) and THF (1 mL) at 50 °C. The solution was slowly cooled to room temperature, and 6M NaOH (1 mL) was added dropwise during this time. Precipitate slowly formed, and the reaction mixture was stirred for 1 h at room temperature. A few chips of ice were added, and the reaction mixture was cooled in an ice bath for 15 min. The precipitate was vacuum-filtered and washed with small portions of cold water/ethanol solution to yield the desired chalcone as an orange powder (486 mg, 81%). ^{1}H-NMR (300 MHz, Chloroform-*d*) δ 7.91 (d, *J* = 8.5 Hz, 2H), 7.63 (dd, *J* = 14.7, 10.9 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 2H), 7.40 (d, *J* = 8.9 Hz, 3H), 7.04–6.77 (m, 3H), 6.68 (d, *J* = 8.9 Hz, 2H), 3.02 (s, 6H). ^{13}C-NMR (75 MHz, Chloroform-*d*) δ 189.07, 151.14, 146.96, 143.74, 138.55, 137.00, 129.70, 129.05, 128.76, 123.96, 122.17, 121.82, 111.96, 40.22. Melting point: 161–164 °C.

27. (2*E*,4*E*)-5-(4-(dimethylamino)phenyl)-1-(p-tolyl)penta-2,4-dien-1-one

4′-methylacetophenone (0.250 mL, 1.88 mmol) and 4-(dimethylamino)cinnamaldehyde (300 mg, 1.71 mmol) were dissolved in absolute ethanol (15 mL) and THF (1 mL) at 50 °C. The solution was slowly cooled to room temperature, and 6M NaOH (1 mL) was added dropwise during this time. Precipitate slowly formed, and the reaction mixture was stirred for 1 h at room temperature. A few chips of ice were added, and the reaction mixture was cooled in an ice bath for 15 min. The precipitate was vacuum-filtered and washed with small portions of cold water/ethanol solution to yield the desired chalcone as a bright red powder (351 mg, 71%). ^{1}H-NMR (300 MHz, Chloroform-*d*) δ 7.89 (d, *J* = 8.2 Hz, 2H), 7.63 (dd, *J* = 14.8, 10.6 Hz, 1H), 7.40 (d, *J* = 8.9 Hz, 2H), 7.28 (d, *J* = 8.1 Hz, 2H), 7.00–6.87 (m, 3H), 6.68 (d, *J* = 8.9 Hz, 2H), 3.02 (s, 6H), 2.42 (s, 3H). ^{13}C-NMR (75 MHz, Chloroform-*d*) δ 190.06, 151.03, 145.96, 143.01, 142.81, 136.08, 129.18, 128.85, 128.42, 124.22, 122.64, 122.51, 112.00, 40.23, 21.66. Melting point: 158–160 °C.

28. (2*E*,4*E*)-1-(4-chlorophenyl)-5-phenylpenta-2,4-dien-1-one

To a stirring solution of 4′-chloroacetophenone (0.250 mL, 1.92 mmol) and *trans*-cinnamaldehyde (0.250 mL, 1.99 mmol), in absolute ethanol (10 mL), 6 M NaOH (1 mL)

was added dropwise at room temperature. Precipitate formed instantaneously and the reaction mixture was stirred for an additional 15 min at room temperature. A few chips of ice were added, and the reaction mixture was cooled in an ice bath for 15 min. The precipitate was vacuum-filtered and washed with small portions of cold water/ethanol solution to yield the desired chalcone as a yellow green powder (445 mg, 95%). ^{1}H-NMR (300 MHz, Chloroform-*d*) δ 7.92 (d, *J* = 8.6 Hz, 2H), 7.69–7.55 (m, 1H), 7.54–7.43 (m, 4H), 7.37 (m, 3H), 7.10–6.91 (m, 3H). ^{13}C-NMR (75 MHz, Chloroform-*d*) δ 189.09, 145.34, 142.43, 139.06, 136.48, 135.96, 129.79, 129.78, 129.38, 128.89, 127.35, 126.74, 124.74. Melting point: 138–139 °C.

29. (E)-2-((E)-3-(4-(dimethylamino)phenyl)allylidene)-2,3-dihydro-1H-inden-1-one

1-indanone (200 mg, 1.51 mmol) and 4-(dimethylamino)cinnamaldehyde (240 mg, 1.38 mmol) were dissolved in absolute ethanol (15 mL) and THF (1 mL) at 50 °C. The solution was slowly cooled to room temperature, and 6M NaOH (1 mL) was added dropwise during this time. Precipitate slowly formed, and the reaction mixture was stirred for 1 h at room temperature. A few chips of ice were added, and the reaction mixture was cooled in an ice bath for 15 min. The precipitate was vacuum-filtered and washed with small portions of cold water/ethanol solution to yield the desired chalcone as an orange powder (327 mg, 82%). ^{1}H-NMR (300 MHz, Chloroform-*d*) δ 7.87 (d, *J* = 7.6 Hz, 1H), 7.56 (m, 2H), 7.48–7.35 (m, 4H), 6.99 (d, *J* = 15.3 Hz, 1H), 6.84 (dd, *J* = 15.2, 11.4 Hz,10H), 6.68 (d, *J* = 8.8 Hz, 2H), 3.82 (s, 2H), 3.02 (s, 6H). ^{13}C-NMR (75 MHz, Chloroform-*d*) δ 193.63, 151.06, 148.85, 143.14, 139.74, 134.96, 133.89, 133.35, 128.93, 127.34, 126.12, 124.43, 123.96, 119.81, 111.99, 40.22, 30.54. Melting point: 168–170 °C.

30. (E)-6-methoxy-2-((E)-3-(4-methoxyphenyl)allylidene)-3,4-dihydronaphthalen-1(2H)-one

6-methoxy-1-tetralone (250 mg, 1.42 mmol) and 4-methoxycinnamaldehyde (230 mg, 1.42 mmol) were dissolved in absolute ethanol (10 mL) at room temperature and 6M NaOH (1 mL) was added dropwise. Precipitate slowly formed, and the reaction mixture was stirred for 30 min at room temperature. A few chips of ice were added, and the reaction mixture was cooled in an ice bath for 15 min. The precipitate was vacuum-filtered and washed with small portions of cold water/ethanol solution to yield the desired chalcone as a bright yellow powder (248 mg, 55%). ^{1}H-NMR (300 MHz, Chloroform-*d*) δ 8.09 (d, *J* = 8.7 Hz, 1H), 7.52 (d, J = 8.6 Hz, 1H), 7.46 (d, *J* = 8.6 Hz, 2H), 7.10–6.79 (m, 5H), 6.72 (s, 1H), 3.86 (s, 3H), 3.83 (s, 3H), 2.98 (s, 4H). ^{13}C-NMR (75 MHz, Chloroform-*d*) δ 186.26, 163.28, 160.21, 145.84, 140.28, 135.83, 133.36, 130.52, 129.55, 128.57, 127.45, 121.49, 114.23, 113.15, 112.33, 55.44, 55.36, 29.19, 25.99. Melting point: 139–140 °C.

31. (2*E*,4*E*)-5-(4-chlorophenyl)-1-(4-nitrophenyl)penta-2,4-dien-1-one

4′-nitroacetophenone (161 mg, 0.972 mmol) and 4-chlorocinnamaldehyde (162 mg, 0.972 mmol) were dissolved in absolute ethanol (15 mL) at 50 °C. The solution was slowly cooled to room temperature, and 6 M NaOH (1 mL) was added dropwise during this time. Precipitate formed instantaneously, and the reaction mixture was stirred at room temperature for 15 min. A few chips of ice were added, and the reaction mixture was cooled in an ice bath for 15 min. The precipitate was vacuum-filtered and washed with small portions of cold water/ethanol solution to yield the desired chalcone as a yellow green powder (252 mg, 83%). ^{1}H-NMR (300 MHz, Chloroform-*d*) δ 8.34 (d, *J* = 8.9 Hz, 2H), 8.10 (d, *J* = 9.0 Hz, 2H), 7.62 (ddd, *J* = 14.9, 6.5, 3.9 Hz, 1H), 7.45 (d, *J* = 8.7 Hz, 2H), 7.36 (d, *J* = 8.7 Hz, 2H), 7.11–6.97 (m, 3H). ^{13}C-NMR (75 MHz, Chloroform-*d*) δ 188.77, 149.97, 146.29, 142.93, 141.99, 135.44, 134.22, 129.29, 129.20, 128.60, 126.95, 124.81, 123.87. Melting point: 122–123 °C.

32. (*E*)-2-((*E*)-3-(4-(dimethylamino)phenyl)allylidene)-2,3-dihydro-1H-inden-1-one

1,3-indandione (200 mg, 1.37 mmol) and *trans*-cinnamaldehyde (0.190 mL, 1.51 mmol) were dissolved in absolute ethanol (15 mL) at room temperature, and 6 M NaOH (1 mL) was added dropwise. The reaction mixture was stirred at room temperature overnight, then cooled in an ice bath for 15 min. The precipitate was vacuum-filtered and washed

with small portions of cold water/ethanol solution to yield the desired chalcone as a yellow powder (90 mg, 25%). ^{1}H-NMR (300 MHz, Chloroform-*d*) δ 8.45 (dd, *J* = 15.5, 12.0 Hz, 1H), 8.03–7.91 (m, 2H), 7.84–7.75 (m, 2H), 7.72–7.59 (m, 3H), 7.43 (m, 3H), 7.34 (d, *J* = 15.5 Hz, 1H). ^{13}C-NMR (75 MHz, Chloroform-*d*) δ 151.16, 144.68, 142.14, 135.51, 135.15, 135.03, 130.94, 129.05, 128.70, 123.62, 123.14, 122.97. Melting point: 151–152 °C.

33. (*E*)-2-((*E*)-3-(2-nitrophenyl)allylidene)-3,4-dihydronaphthalen-1(2H)-one

1-tetralone (0.210 mL, 1.58 mmol) and 2-nitrocinnamaldehyde (250 mg, 1.41 mmol) were dissolved in absolute ethanol (20 mL) and THF (2 mL) at 50 °C. The solution was slowly cooled to room temperature, and 6 M NaOH (1 mL) was added dropwise during this time. Precipitate formed instantaneously and the reaction mixture was stirred for 15 min at room temperature. A few chips of ice were added, and the reaction mixture was cooled in an ice bath for 15 min. The precipitate was vacuum-filtered and washed with small portions of cold water/ethanol solution to yield the desired chalcone as a yellow powder (260 mg, 60%). ^{1}H-NMR (300 MHz, Chloroform-*d*) δ 7.86 (d, *J* = 7.8 Hz, 1H), 7.76 (d, *J* = 8.1 Hz, 1H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.44 (t, *J* = 7.9 Hz, 1H), 7.35–7.21 (m, 4H), 7.15 (t, *J* = 7.5 Hz, 1H), 7.08 (d, *J* = 7.6 Hz, 1H), 6.95 (dd, *J* = 15.1, 11.7 Hz, 1H), 2.83 (s, 6H). ^{13}C-NMR (75 MHz, Chloroform-*d*) δ 186.93, 147.71, 143.29, 136.61, 134.59, 134.40, 133.25, 133.21, 133.16, 131.87, 128.95, 128.30, 128.22, 127.96, 127.83, 126.90, 124.69, 28.46, 26.02. Melting point: 188–189 °C.

34. (2*E*,4*E*)-5-(4-(dimethylamino)phenyl)-1-phenylpenta-2,4-dien-1-one

Acetophenone (0.200 mL, 1.71 mmol) and 4-(dimethylamino)cinnamaldehyde (275 mg, 1.56 mmol) were dissolved in absolute ethanol (10 mL) and THF (1 mL) at 50 °C. The solution was slowly cooled to room temperature, and 6 M NaOH (1 mL) was added dropwise during this time. Precipitate slowly formed, and the reaction mixture was stirred for 1 h at room temperature. A few chips of ice were added, and the reaction mixture was cooled in an ice bath for 15 min. The precipitate was vacuum-filtered and washed with small portions of cold water/ethanol solution to yield the desired chalcone as a red flaky solid (327 mg, 76%). ^{1}H-NMR (300 MHz, Chloroform-*d*) δ 7.98 (d, *J* = 6.8 Hz, 2H), 7.64 (dd, *J* = 14.8, 10.6 Hz, 1H), 7.58–7.44 (m, 3H), 7.40 (d, *J* = 8.9 Hz, 2H), 7.04–6.78 (m, 3H), 6.68 (d, *J* = 8.9 Hz, 2H), 3.01 (s, 6H). ^{13}C-NMR (75 MHz, Chloroform-*d*) δ 190.55, 151.09, 146.45, 143.18, 138.71, 132.28, 128.94, 128.48, 128.29, 124.13, 122.58, 122.41, 112.00, 40.22. Melting point: 153–154 °C.

35. (*E*)-2-((*E*)-3-(4-(dimethylamino) phenyl)allylidene)-3,4-dihydronaphthalen-1(2H)-one

1-tetralone (0.200 mL, 1.50 mmol) and 4-(dimethylamino)cinnamaldehyde (240 mg, 1.36 mmol) were dissolved in absolute ethanol (15 mL) and THF (1 mL) at 50 °C. The solution was slowly cooled to room temperature, and 6 M NaOH (1 mL) was added dropwise during this time. Precipitate slowly formed, and the reaction mixture was stirred for 1 h at room temperature. A few chips of ice were added, and the reaction mixture was cooled in an ice bath for 15 min. The precipitate was vacuum-filtered and washed with small portions of cold water/ethanol solution to yield the desired chalcone as a red powder (191 mg, 46%). ^{1}H-NMR (300 MHz, Chloroform-*d*) δ 8.11 (d, *J* = 7.7 Hz, 1H), 7.60 (dd, *J* = 8.1, 2.3 Hz, 1H), 7.44 (m, 3H), 7.35 (t, *J* = 7.5 Hz, 1H), 7.25 (d, *J* = 6.5 Hz, 1H), 7.05–6.85 (m, 2H), 6.68 (d, *J* = 8.7 Hz, 3H), 3.02 (s, 6H), 3.00 (s, 4H). ^{13}C-NMR (75 MHz, Chloroform-*d*) δ 187.25, 150.88, 143.36, 142.10, 137.61, 134.16, 132.68, 131.51, 128.72, 128.06, 127.97, 126.86, 124.77, 119.12, 112.04, 40.26, 28.79, 25.77. Melting point: 145–147 °C softening, 275–285 °C melting.

4.2. In Vitro Assay

To test the anti-aggregation effects of the different compounds, the SensoLyte Thioflavin T β-Amyloid (**1**–**42**) Aggregation kit was purchased, and assays were performed as described in the literature [16–18].

4.2.1. Thioflavin T (ThT) Fluorescence Assay

Samples of ThT (final concentration of 200 μM) and amyloid-beta (**1**–**42**) peptide (final concentration of 35 μM) were incubated at 37 °C in a black μClear bottom 96-well plate. The ThT fluorescence intensity of each sample was immediately measured every 5 min for 120 min with 440⁄485 nm excitation/emission filters and with 15 s shaking between reads to facilitate aggregation. An inhibitor control contained $A\beta_{42}$ and an aggregation inhibitor was supplied (either Morin or Phenol Red) at a final concentration of 100 μM. Positive control contained $A\beta_{42}$ without inhibitor. The vehicle control contained the assay buffer and DMSO, of concentrations that did not exceed 1%. The tested compound wells contained $A\beta_{42}$ peptide and either the homotaurine, chalcone, or homotaurine/chalcone derivatives at various concentrations. All the wells were brought to 100 μL as a final volume [16–18].

4.2.2. In Vitro Cell Viability Assay

Cell Culture and Exposure

SH-SY5Y cells (CRL-2266, ATCC, Manassas, VA) were maintained in Dulbecco's Modified Eagle's Medium and Ham's F-12 Medium (DMEM:F12) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin and incubated at 37 °C, 5% CO_2, and 90% humidity. For the MTT assay, cells were subcultured using trypsin-ethylenediaminetetraacetic acid (EDTA) (0.25%) solution into 96-well plates at a density of 2×10^4 cells/well and allowed to adhere for 24 h. Following the removal of growth media, compounds (IC_{50}), $A\beta_{42}$ at a final concentration of 20 μM, compound + $A\beta_{42}$, along with positive (500 μM H_2O_2) and vehicle controls were added to separate wells in triplicate. Cells were then incubated for 48 h.

Failures

We evaluated LDH release [19], CellTiter Glo [20], and MTS assays [21]. These did not produce any useful results.

MTT Assay and Cell Viability

The MTT [22] assay was used as a measure of cell viability with metabolically active cells reducing the MTT reagent into insoluble formazan. Briefly, following the removal of media, 100 μL of 0.5 mg/mL MTT reagent was added to each well, and the plate was incubated at 37 °C, 5% CO_2, and 90% humidity for 3 h. At the end of this incubation, the MTT reagent was removed, and 100 μL of DMSO was added to dissolve the insoluble formazan. Following a 1 h incubation with DMSO at 37 °C, 5% CO_2, and 90% humidity, the plate was read at 570 nm.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism 9 (GraphPad, LaJolla, CA). Data were compared by one-way ANOVA followed by Tukey's post hoc test to compare the differences between all treatment groups ($p < 0.05$ considered significant). The results are expressed as the mean ± S.E.M. of multiple experiments where n represents the number of individual cell passages.

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