Multifunctional Donepezil Analogues as Cholinesterase and BACE1 Inhibitors

Keith D. Green, Marina Y. Fosso and Sylvie Garneau-Tsodikova *

Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536-0596, USA; kgr234@uky.edu (K.D.G.); marina.fosso@uky.edu (M.Y.F.)
* Correspondence: sylviegtsodikova@uky.edu

Academic Editors: Diego Muñoz-Torrero and Michael Decker
Received: 25 October 2018; Accepted: 7 December 2018; Published: 8 December 2018

Abstract: A series of 22 donepezil analogues were synthesized through alkylation/benzylation and compared to donepezil and its 6-O-desmethyl adduct. All the compounds were found to be potent inhibitors of both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), two enzymes responsible for the hydrolysis of the neurotransmitter acetylcholine in Alzheimer’s disease patient brains. Many of them displayed lower inhibitory concentrations of EeAChE ($IC_{50} = 0.016 \pm 0.001 \mu M$ to $0.23 \pm 0.03 \mu M$) and EfBChE ($IC_{50} = 0.11 \pm 0.01 \mu M$ to $1.3 \pm 0.2 \mu M$) than donepezil. One of the better compounds was tested against HsAChE and was found to be even more active than donepezil and inhibited HsAChE better than EeAChE. The analogues with the aromatic substituents were generally more potent than the ones with aliphatic substituents. Five of the analogues also inhibited the action of $\beta$-secretase (BACE1) enzyme.

Keywords: Alzheimer’s disease; acetylcholinesterase; butyrylcholinesterase; $\beta$-secretase; inhibitors

1. Introduction

Alzheimer’s disease (AD) is a neurodegenerative disorder that is characterized by memory loss and cognitive deficits. It is the most common form of dementia among older adults and the sixth leading cause of death in the United States [1]. In 2018, the World Health Organization (WHO) reported that there have been more than 2 million deaths associated to AD and other dementias in 2016, and this number has doubled since 2000 [2]. In the United States alone, more than 5 million people are currently living with AD, and this number is expected to triple by 2050. Unfortunately, there is currently no cure for AD, which contributes to the deadly nature of this disease.

Despite all the research efforts invested, the specific cause(s) of AD remain(s) unclear [3]. Several molecular mechanisms of AD have been proposed, including the $\beta$-amyloid cascade, oxidative stress, metal imbalance, and cholinergic hypothesis [4]. The latter appears to be the most efficient therapeutic avenue in providing temporary relief of AD symptoms. Indeed, five drugs have been approved by the United States Food and Drug Administration (FDA) for the symptomatic treatment of AD, four of which are acetylcholinesterase (AChE) inhibitors: rivastigmine, galantamine, donepezil, and tacrine. These drugs prevent the action of cholinesterases (ChEs), which are responsible for the hydrolysis of the neurotransmitter acetylcholine (ACh), thereby increasing the levels of ACh in the brain and improving the cholinergic functions in AD patients [5,6]. In addition to AChE, another type of enzyme involved in the hydrolysis of the neurotransmitter ACh is butyrylcholinesterase (BChE). The activity and expression of BChE have been suggested to increase throughout the progression of AD, indicating that BChE may play an important role in the late stage of AD [7]. Therefore, inhibition of AChE and BChE remains a potential therapeutic target for AD treatment. However, targeting ChEs alone is definitely not sufficient.
Another hallmark of AD pathology is the accumulation of amyloid-β (Aβ) plaques on the brain [8]. These plaques are composed of Aβ peptides that result from the cleavage of the transmembrane amyloid precursor protein (APP) by secretases to form Aβ monomers that will aggregate to toxic fibrils [9]. β-secretase (BACE1) is an aspartyl protease that cleaves APP near the membrane surface, and it has been targeted for the development of potential therapies against AD [10].

Due to the multifactorial nature of AD, the development of compounds that could target different pathological features of the disease appears to be a viable research area. We previously reported on the synthesis and biological evaluation of a number of multifunctional molecules derived from tacrine and chalcones that are capable of targeting ChEs and Aβ [11–15]. Since donepezil is the most commonly prescribed medication for AD [4], and several other studies have focused on this drug to generate multifunctional compounds targeting various hallmarks of AD, including BACE1 [16–19], we decided to generate multi-targeted analogues derived from donepezil that would inhibit ChEs and β-secretase.

2. Results and Discussion

2.1. Chemistry

The synthetic route utilized for the synthesis of donepezil analogues is outlined in Scheme 1. Starting from ferulic acid (1), hydrogenation in the presence of Pd/C, followed by cyclization in the presence of methanesulfonic acid (MsOH) produced ketone 3 with 67% yield [20,21]. Attempts to react the ketone 3 with the aldehyde 5 through aldol condensation were met with little success. To overcome this shortcoming, the free hydroxyl group in compound 3 was protected with a TBDMS group to yield the corresponding ketone 4 with 90% yield. This was then successfully condensed with the aldehyde 5 in the presence of KOH, to yield the α,β-unsaturated ketone 6 with 65% yield. Selective reduction of the double bond in the presence of a ketone and a benzyl group was achieved through a controlled poisoning of the palladium catalyst with thioanisole to give the 6-O-desmethyl donepezil adduct 7 with 94% yield. The latter bears a free hydroxyl group that was reacted with the corresponding alkyl or benzyl halides to yield 22 donepezil analogues (8a–v) with 32–95% yields.
2.2. Cholinesterase Inhibition

To evaluate the potential cholinesterase (ChE) inhibitory activity of donepezil, 6-O-desmethyl donepezil 7, and the 22 newly synthesized donepezil analogues 8a–v, their IC50 values were determined against AChE from Electrophorus electricus (EeAChE) (Table 1 and Figures S82–S83) and EfBChE from equine serum (Equus ferus) (Table 1 and Figures S84–S85) using the well-established Ellman method [22].

Table 1. Inhibition (IC50 values in µM, with standard error) of the activity of EeAChE (from Electrophorus electricus) and BChE (from Equus ferus) by donepezil and its analogues 7 and 8a–v, and the selectivity index (SI) for each inhibitor based on IC50 values.

<table>
<thead>
<tr>
<th>Cpd</th>
<th>EeAChE</th>
<th>EfBChE</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donepezil HCl</td>
<td>0.12 ± 0.01</td>
<td>2.0 ± 0.1</td>
<td>17</td>
</tr>
<tr>
<td>7</td>
<td>0.41 ± 0.05</td>
<td>4.3 ± 0.4</td>
<td>11</td>
</tr>
<tr>
<td>8a</td>
<td>0.054 ± 0.003</td>
<td>0.57 ± 0.04</td>
<td>11</td>
</tr>
<tr>
<td>8b</td>
<td>0.021 ± 0.003</td>
<td>0.48 ± 0.03</td>
<td>23</td>
</tr>
<tr>
<td>8c</td>
<td>0.14 ± 0.02</td>
<td>2.1 ± 0.3</td>
<td>15</td>
</tr>
<tr>
<td>8d</td>
<td>0.059 ± 0.004</td>
<td>1.3 ± 0.1</td>
<td>22</td>
</tr>
<tr>
<td>8e</td>
<td>0.044 ± 0.003</td>
<td>1.3 ± 0.2</td>
<td>30</td>
</tr>
<tr>
<td>8f</td>
<td>0.061 ± 0.007</td>
<td>1.3 ± 0.2</td>
<td>21</td>
</tr>
<tr>
<td>8g</td>
<td>0.79 ± 0.28</td>
<td>5.2 ± 1.6</td>
<td>6.6</td>
</tr>
<tr>
<td>8h</td>
<td>0.13 ± 0.01</td>
<td>0.70 ± 0.05</td>
<td>5.4</td>
</tr>
<tr>
<td>8i</td>
<td>0.23 ± 0.03</td>
<td>1.0 ± 0.2</td>
<td>4.3</td>
</tr>
<tr>
<td>8j</td>
<td>0.13 ± 0.01</td>
<td>0.67 ± 0.17</td>
<td>5.2</td>
</tr>
<tr>
<td>8k</td>
<td>0.13 ± 0.02</td>
<td>0.46 ± 0.06</td>
<td>3.5</td>
</tr>
<tr>
<td>8l</td>
<td>0.071 ± 0.015</td>
<td>0.72 ± 0.10</td>
<td>10</td>
</tr>
<tr>
<td>8m</td>
<td>0.081 ± 0.005</td>
<td>0.57 ± 0.10</td>
<td>7.0</td>
</tr>
<tr>
<td>8n</td>
<td>0.16 ± 0.02</td>
<td>0.96 ± 0.15</td>
<td>6.0</td>
</tr>
<tr>
<td>8o</td>
<td>0.12 ± 0.02</td>
<td>0.76 ± 0.12</td>
<td>6.3</td>
</tr>
<tr>
<td>8p</td>
<td>0.032 ± 0.010</td>
<td>0.25 ± 0.08</td>
<td>7.8</td>
</tr>
<tr>
<td>8q</td>
<td>0.11 ± 0.01</td>
<td>0.48 ± 0.08</td>
<td>4.4</td>
</tr>
<tr>
<td>8r</td>
<td>0.090 ± 0.009</td>
<td>0.60 ± 0.15</td>
<td>6.7</td>
</tr>
<tr>
<td>8s</td>
<td>0.016 ± 0.001</td>
<td>0.44 ± 0.05</td>
<td>28</td>
</tr>
<tr>
<td>8t</td>
<td>0.054 ± 0.007</td>
<td>0.37 ± 0.05</td>
<td>6.9</td>
</tr>
<tr>
<td>8u</td>
<td>0.027 ± 0.004</td>
<td>0.20 ± 0.03</td>
<td>7.4</td>
</tr>
<tr>
<td>8v</td>
<td>0.17 ± 0.02</td>
<td>0.11 ± 0.01</td>
<td>0.69</td>
</tr>
</tbody>
</table>

2.2.1. AChE Inhibition

When comparing the 6-O-desmethyl donepezil 7 (R = H; IC50 = 0.41 ± 0.05 µM) with donepezil (R = Me; IC50 = 0.12 ± 0.01 µM) and its analogues 8a–f and 8h–v (R = various alkyl and benzylic groups; IC50 = 0.016 ± 0.001 µM to 0.23 ± 0.03 µM), it becomes evident that 6-O-alkylation/benzylation enhances EeAChE inhibition, with the only exception being 8g (IC50 = 0.79 ± 0.28 µM), which bears a hydrophobic 1-bromododecyl group. Other analogues were equal to or even better than donepezil at inhibiting the action of EeAChE in vitro. With similar IC50 values, compound 8c (R = n-propyl; IC50 = 0.14 ± 0.02 µM) was as potent as donepezil (R = Me; IC50 = 0.12 ± 0.01 µM). However, substituting the terminal methyl in the R group of compound 8c (R = n-propyl; IC50 = 0.14 ± 0.02
μM) by a terminal amine in compound 8b (R = H₂NCH₂CH₂; IC₅₀ = 0.021 ± 0.003 μM) drastically increased the potency. Indeed, a 6-fold reduction of the IC₅₀ value of donepezil was observed. The amine group may form hydrogen bonds with Tyr70, Asp72, and Gln74 residues near the PAS [11].

Cancellation of this hydrogen bonding by replacing the terminal amine in the R group of compound 8b (R = H₂NCH₂CH₂; IC₅₀ = 0.021 ± 0.003 μM) with a chlorine atom in 8d (R = ClCH₂CH₂; IC₅₀ = 0.059 ± 0.004 μM) or a bromine atom in 8e (R = BrCH₂CH₂; IC₅₀ = 0.044 ± 0.003 μM) resulted in an increase in the IC₅₀ values, which only represented a 2- or 3-fold enhanced potency when compared to donepezil, respectively. Elongation of the R group also appeared to worsen the IC₅₀ values. Indeed, adding an extra methylene to compound 8b (R = H₂NCH₂CH₂; IC₅₀ = 0.021 ± 0.003 μM) gives compound 8a (R = H₂NCH₂CH₂CH₂; IC₅₀ = 0.054 ± 0.003 μM), while the addition of two methylene groups to compound 8e (R = BrCH₂CH₂; IC₅₀ = 0.044 ± 0.003 μM) gives compound 8f (R = BrCH₂CH₂CH₂CH₂; IC₅₀ = 0.061 ± 0.007 μM). Nevertheless, all these analogues remained better inhibitors of EcAChE than donepezil.

Replacing the alkyl group in donepezil (R = Me; IC₅₀ = 0.12 ± 0.01 μM) by an aromatic group in compound 8h (R = Bn; IC₅₀ = 0.13 ± 0.01 μM) did not affect the IC₅₀ value. Likewise, additional substitutions at the para-position of the benzyl group resulted in IC₅₀ values that were similar to that of donepezil. Indeed, compounds 8i (R = 4-BrBn; IC₅₀ = 0.23 ± 0.03 μM), 8j (R = 4-OMeBn; IC₅₀ = 0.13 ± 0.01 μM), 8k (R = 4-NO₂Bn; IC₅₀ = 0.13 ± 0.02 μM), 8l (R = 4-BrBn; IC₅₀ = 0.071 ± 0.015 μM), and 8m (R = 4-FBn; IC₅₀ = 0.081 ± 0.005 μM) displayed IC₅₀ values that were still within 1- to 2-fold of that of donepezil (R = Me; IC₅₀ = 0.12 ± 0.01 μM). Similarly, when the fluoro group was moved from the para-position in compound 8m (R = 4-FBn; IC₅₀ = 0.081 ± 0.005 μM) to the meta- or ortho-positions in compounds 8n (R = 3-FBn; IC₅₀ = 0.16 ± 0.02 μM) and 8o (R = 2-FBn; IC₅₀ = 0.12 ± 0.02 μM), respectively, the potency of these analogues was comparable to donepezil (R = Me; IC₅₀ = 0.12 ± 0.01 μM). However, replacing the fluoro group in 8o (R = 2-FBn; IC₅₀ = 0.12 ± 0.02 μM) by a CF₃ group in 8p (R = 2-CF₃Bn; IC₅₀ = 0.032 ± 0.010 μM) improved the IC₅₀ by 4-fold. This may suggest that enhanced electron-withdrawing effect on the aromatic ring may improve the potency of the analogue.

Attempts to spread out the electron-withdrawing effect throughout the aromatic ring led to compounds 8q (R = 2,4-diF-Bn; IC₅₀ = 0.11 ± 0.01 μM), 8r (R = 2,5-diF-Bn; IC₅₀ = 0.090 ± 0.009 μM), 8s (R = 2,6-diF-Bn; IC₅₀ = 0.016 ± 0.001 μM), and 8t (R = 4-Br-2-F-Bn; IC₅₀ = 0.054 ± 0.007 μM), with two electron-withdrawing groups, and compounds 8u (R = 2,4,6-triF-Bn; IC₅₀ = 0.027 ± 0.004 μM) and 8v (R = 2,3,4,5-pentaF-Bn; IC₅₀ = 0.17 ± 0.02 μM), with three and five electron-withdrawing groups, respectively. It thus appears that both ortho-positions on the benzyl group are very sensitive to the presence of electron-withdrawing groups, since 8s (R = 2,6-diF-Bn; IC₅₀ = 0.016 ± 0.001 μM) and 8u (R = 2,4,6-triF-Bn; IC₅₀ = 0.027 ± 0.004 μM) were eight and five times more potent than donepezil, respectively.

In order to confirm that the data obtained with EcAChE would also apply to HsAChE (from Homo sapiens), we tested donepezil along with a compound that displayed better inhibition than donepezil, 8t. We found that both donepezil and compound 8t inhibited HsAChE better than the EcAChE (Table 2). Compound 8t (R = 4-Br-2-F-Bn; IC₅₀ = 0.0018 ± 0.0006 μM) inhibited HsAChE 18-fold better than donepezil (R = Me; IC₅₀ = 0.032 ± 0.011 μM). In the case of EcAChE, compound 8t (R = 4-Br-2-F-Bn; IC₅₀ = 0.054 ± 0.007 μM) had an IC₅₀ value that was 2.2-fold better than donepezil (R = Me; IC₅₀ = 0.12 ± 0.01 μM). These data would suggest that our inhibitors are well suited for working with HsAChE.

**Table 2.** Inhibition (IC₅₀ values in μM) of the activity of HsAChE by donepezil and its analogue 8t.

<table>
<thead>
<tr>
<th>Cpd</th>
<th>IC₅₀ (μM)</th>
<th>SI a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donepezil HCl</td>
<td>0.032 ± 0.011</td>
<td>3.8</td>
</tr>
<tr>
<td>8t</td>
<td>0.0018 ± 0.0006</td>
<td>30</td>
</tr>
</tbody>
</table>

* Selectivity index of EcAChE versus HsAChE, based on IC₅₀ values.
2.2.2. E/BChE Inhibition

As expected, donepezil analogues 8a–v were less effective against E/BChE than EcAChE. Indeed, donepezil is highly selective for EcAChE over E/BChE [23], and as a result, it is expected for its analogues to behave similarly. However, when compared to donepezil (R = Me; IC_{50} = 2.0 ± 0.1 μM), all but compounds 8c (R = CH_{2}CH_{2}CH_{2}; IC_{50} = 2.1 ± 0.3 μM) and 8g (IC_{50} = 5.2 ± 1.6 μM) appeared to be more effective at inhibiting the action of BChE. The presence of a terminal amine in compounds 8a (R = H_{2}NCH_{2}CH_{2}CH_{2}; IC_{50} = 0.57 ± 0.04 μM) and 8b (R = H_{2}NCH_{2}CH_{2}; IC_{50} = 0.48 ± 0.03 μM) still drastically increased their potency by 4-fold when compared to donepezil. Substitution of the amine group in 8p (R = H_{2}NCH_{2}CH_{2}; IC_{50} = 0.48 ± 0.03 μM) with a chlorine atom in 8d (R = ClCH_{2}CH_{2}; IC_{50} = 1.3 ± 0.1 μM) or a bromine atom in 8e (R = BrCH_{2}CH_{2}; IC_{50} = 1.3 ± 0.2 μM) resulted again in an increase in the IC_{50} values, which only represented a 2-fold enhanced potency when compared to donepezil. Elongation of the R group did not have much effect, as the IC_{50} value of 8f (R = BrCH_{2}CH_{2}CH_{2}CH_{2}; IC_{50} = 1.3 ± 0.2 μM) still remained within 2-fold that of donepezil.

A greater improvement of the IC_{50} values was more noticeable when the alkyl group in donepezil (R = Me; IC_{50} = 2.0 ± 0.1 μM) was replaced by an aromatic group. Compound 8h (R = Bn; IC_{50} = 0.70 ± 0.05 μM) was 3-fold more potent than donepezil. Substitutions at the para-position of the benzyl group also contributed to reducing the IC_{50} values. Indeed, compounds 8i (R = 4-Me-Bn; IC_{50} = 1.0 ± 0.2 μM), 8j (R = 4-OMe-Bn; IC_{50} = 0.67 ± 0.17 μM), 8k (R = 4-NO_{2}Bn; IC_{50} = 0.46 ± 0.06 μM), 8l (R = 4-Br-Bn; IC_{50} = 0.72 ± 0.10 μM), and 8m (R = 4-F-Bn; IC_{50} = 0.57 ± 0.10 μM) displayed inhibitory efficacies of 2- to 4-fold better than donepezil (R = Me; IC_{50} = 2.0 ± 0.1 μM). Similarly, when the fluoro group was moved from the para-position in compound 8n (R = 4-F-Bn; IC_{50} = 0.57 ± 0.10 μM) to the meta- or ortho-positions in compounds 8o (R = 3-F-Bn; IC_{50} = 0.96 ± 0.15 μM) and 8o (R = 2-F-Bn; IC_{50} = 0.76 ± 0.12 μM), respectively, the potency of these analogues was reduced by 1- to 2-fold. Replacing the fluoro group in 8o (R = 2-F-Bn; IC_{50} = 0.76 ± 0.12 μM) by a CF_{3} group in 8p (R = 2-CF_{3}Bn; IC_{50} = 0.25 ± 0.08 μM) once again improved the IC_{50} by 8-fold. This is in agreement with the trend observed in EcAChE inhibition. Indeed, the additional electron-withdrawing effect on the aromatic ring still appeared to increase the potency of the analogue. Compounds 8q (R = 2,4-diF-Bn; IC_{50} = 0.48 ± 0.08 μM), 8r (R = 2,5-diF-Bn; IC_{50} = 0.60 ± 0.15 μM), 8s (R = 2,6-diF-Bn; IC_{50} = 0.44 ± 0.05 μM), and 8t (R = 4-Br-2-F-Bn; IC_{50} = 0.37 ± 0.05 μM), with two electron-withdrawing groups, and compounds 8u (R = 2,4,6-triF-Bn; IC_{50} = 0.20 ± 0.03 μM) and 8v (R = 2,3,4,5-pentaF-Bn; IC_{50} = 0.11 ± 0.01 μM), with three and five electron-withdrawing groups, respectively, were all better E/BChE inhibitors than donepezil (R = Me; IC_{50} = 2.0 ± 0.1 μM). Compound 8q (R = 2,4-diF-Bn; IC_{50} = 0.48 ± 0.08 μM) was 4-fold better than donepezil, while 8r (R = 2,5-diF-Bn; IC_{50} = 0.60 ± 0.15 μM) and 8s (R = 2,6-diF-Bn; IC_{50} = 0.44 ± 0.05 μM) were 3- and 5-fold better, respectively. Replacing the fluorine atom at the para-position in 8q (R = 2,4-diF-Bn; IC_{50} = 0.48 ± 0.08 μM) with a bromine atom in 8t (R = 4-Br-2-F-Bn; IC_{50} = 0.37 ± 0.05 μM) did not impart a noticeable change. Compounds 8u (R = 2,4,6-triF-Bn; IC_{50} = 0.20 ± 0.03 μM) and 8v (R = 2,3,4,5-pentaF-Bn; IC_{50} = 0.11 ± 0.01 μM), with three and five electron-withdrawing groups, respectively, were 10- and 18-fold better than donepezil. The active site gorge of BChE is less constrained than that of AChE, allowing BChE to better accommodate bulky inhibitors [24]. This supports our observations that additional substitution on the aromatic ring increased the potency of the donepezil analogues against E/BChE more than against EcAChE.

We also calculated the selectivity index (SI) to understand the utility of the compounds. For all but one compound, 8v, EcAChE was inhibited 3.5- to 30-fold better than E/BChE. Interestingly, compound 8v was 1.5-fold more selective for E/BChE. Clearly the donepezil analogues are better suited for inhibiting EcAChE. We also looked at the selectivity of the inhibitors for EcAChE versus HsAChE. We observed that donepezil was 3-fold more selective for HsAChE. Perhaps more interesting, compound 8t was 30-fold more selective for HsAChE over EcAChE.
2.3. BACE1 Inhibition

It has previously been reported that donepezil has some BACE1 inhibitory activity [19]. Keeping this in mind we decided to test these compounds for BACE1 inhibitory activity (Table 3). Unlike with AChE and BChE, in general, donepezil analogues 8a–v were not better than the parent donepezil at inhibiting the action of BACE1 in vitro, with the exception of 8c, 8e, 8f, and 8l, which were in the low micromolar range. Indeed, 8c (R = CH$_3$CH$_2$CH$_2$; IC$_{50}$ = 6.1 ± 0.1 µM), 8e (R = BrCH$_2$CH$_2$; IC$_{50}$ = 7.9 ± 0.9 µM), 8f (R = BrCH$_2$CH$_2$CH$_2$CH$_2$; IC$_{50}$ = 7.9 ± 2.4 µM), and 8l (R = 4-Br-Bn; IC$_{50}$ = 3.4 ± 0.1 µM) were within 5-fold of the IC$_{50}$ values of donepezil. This suggests that our analogues are more selective in targeting the ChEs, but they do still target BACE1. As a control for the BACE1 inhibition assays, we used BACE inhibitor IV. Our inhibitors were poorer inhibitors than BACE inhibitor IV (IC$_{50}$ = 0.63 ± 0.18 nM). While BACE inhibitor IV is better, it was designed to be very specific for that one target. However, with an illness such as Alzheimer’s disease, which has many facets and contributing factors, having multifunctional inhibitors that display activity against BACE1 and ChEs is beneficial.

Table 3. Inhibition (IC50 values in µM, unless otherwise noted) of the activity of BACE1 by donepezil and its analogues 7, and 8a–v. BACE inhibitor IV was used as a control.

<table>
<thead>
<tr>
<th>Cpd</th>
<th>IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donepezil HCl</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>7</td>
<td>~ –</td>
</tr>
<tr>
<td>8a</td>
<td>95 ± 12</td>
</tr>
<tr>
<td>8b</td>
<td>~100</td>
</tr>
<tr>
<td>8c</td>
<td>6.1 ± 0.1</td>
</tr>
<tr>
<td>8d</td>
<td>~100</td>
</tr>
<tr>
<td>8e</td>
<td>7.9 ± 0.9</td>
</tr>
<tr>
<td>8f</td>
<td>7.9 ± 2.4</td>
</tr>
<tr>
<td>8g</td>
<td>~ –</td>
</tr>
<tr>
<td>8h</td>
<td>58 ± 1</td>
</tr>
<tr>
<td>8i</td>
<td>58 ± 2</td>
</tr>
<tr>
<td>8j</td>
<td>~ –</td>
</tr>
<tr>
<td>8k</td>
<td>~ –</td>
</tr>
<tr>
<td>8l</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>8m</td>
<td>~100</td>
</tr>
<tr>
<td>8n</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>8o</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>8p</td>
<td>34 ± 9</td>
</tr>
<tr>
<td>8q</td>
<td>37 ± 5</td>
</tr>
<tr>
<td>8r</td>
<td>~100</td>
</tr>
<tr>
<td>8s</td>
<td>30 ± 8</td>
</tr>
<tr>
<td>8t</td>
<td>169 ± 2</td>
</tr>
<tr>
<td>8u</td>
<td>91 ± 14</td>
</tr>
<tr>
<td>8v</td>
<td>29 ± 6</td>
</tr>
<tr>
<td>BACE1 inhibitor IV</td>
<td>0.63 ± 0.18 nM</td>
</tr>
</tbody>
</table>
2.4. BACE1 Modeling

To aid in the understanding of donepezil and its analogues’ inhibitory activity of BACE1, we used SwissDock to perform some modeling studies. Figure 1 shows the crystal structure (PDB# 4FM7 [25], with a published inhibitor of BACE1 (published IC50 value = 0.1 µM). This inhibitor shares the vicinyl dioxygen-substituted phenyl ring found in donepezil. Based on the results of the modeling, the aromatic ring of donepezil aligns with that of the inhibitor originally co-crystallized with BACE1 (Figure 1A,B). When looking at the docking of donepezil (Figure 1C), it is apparent that it binds in a similar location to the reported co-crystallized inhibitor (Figure 1B), albeit not as tightly as apparent by the IC50 values, which are 10-fold different. When examining the docking of compound 8l (Figure 1D), it is slightly twisted, likely due to the bulky 4-bromobenzyl substitution. This slight torsion could explain the roughly doubled IC50 value of compound 8l when compared to that of donepezil. Based on the modeling, there is also room for more optimization at this location, reasoning that modifications of donepezil have the potential to yield better inhibitors than the parent compound if modified correctly.

![Figure 1](image-url). Molecular docking showing the overlay of donepezil (green) and compound 8l (navy blue) with the known BACE1 inhibitor (gray) crystallized with BACE1 (PDB# 4FM7 [25]), shown as ribbons. Panel A shows the three compounds in the active site of BACE1. Panels B–D show the zoomed-in view of the known inhibitor (B), donepezil (C), and compound 8l (D).

3. Materials and Methods

3.1. General Information

All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA), Alfa Aesar (Ward Hill, MA, USA), and AK scientific (Union, CA, USA), and used without further purification. Chemical reactions were monitored by thin layer chromatography (TLC) using Merck (Darmstadt, Germany), Silica gel 60 F250 plates. Visualization was achieved using UV (model UVGL-58, UVP, Upland, CA, USA) light and a ceric molybdate stain (5 g (NH4)2Ce(NO3)6, 120 g (NH4)6Mo7O24 4H2O, 80 mL H2SO4, 720 mL H2O). 1H and 13C-NMR spectra were recorded at 400 and 100 MHz, respectively, on a Varian 400 MHz spectrometer (Varian, Palo Alto, CA, USA), using the indicated deuterated solvents.
Chemical shifts (δ) are given in parts per million (ppm). Coupling constants (J) are given in Hertz (Hz), and conventional abbreviations used for signal shape are as follows: s = singlet; d = doublet; t = triplet; m = multiplet; dd = doublet of doublets; ddd = doublet of doublet of doublets; br s = broad singlet; dt = doublet of triplets. High-resolution mass spectra were recorded on an AB SCIEX Triple TOF 5600 System (AB SCIEX, Framingham, MA, USA). The purity of the compound was further confirmed to be ≥95% by RP-HPLC (model 1260 Infinity, Agilent, Santa Clara, CA, USA) by using the following method: Flow rate = 0.5 mL/min; λ = 254 nm; column = Vydac 201SP™ C18, 250 × 4.6 mm, 90A 5 µm; eluents: A = H₂O + 0.1% TFA, B = MeCN; gradient profile: starting from 5% B, increasing from 5% to 100% B over 17 min, holding at 100% for 5 min, decreasing from 100% to 5% over 3 min. Prior to each injection, the HPLC column was equilibrated for 5 min with 5% B.

3.2. Synthesis of Compounds 2–8v

3.2.1. 3-(4-Hydroxy-3-methoxyphenyl)propanoic acid (2)

A catalytic amount of 10% Pd/C (0.43 g) was added to a solution of ferulic acid (1, 6.0 g, 30.9 mmol) in degassed EtOAc (100 mL). The reaction flask was then sealed with a rubber septum and freed of air. The reaction mixture was stirred at room temperature (RT) overnight under H₂ atmosphere. Upon completion, the reaction mixture was filtered through a bed of celite, and concentrated to afford the known compound 2 [26] (6.1 g, quant.) as an off-white solid: ¹H-NMR (400 MHz, CDCl₃, which matches the literature [26], Figure S1) δ 10.50 (very br s, 1H, CO₂H), 6.82 (d, J = 7.6 Hz, 1H, aromatic), 6.69 (s, 1H, aromatic), 6.68 (d, J = 7.6 Hz, 1H, aromatic), 5.60 (very br s, 1H, OH), 3.85 (s, 3H, PhOCH₃), 2.87 (t, J = 7.2 Hz, 2H, PhCH₂CH₂CO₂H), 2.64 (t, J = 7.2 Hz, 2H, PhCH₂CH₃CO₂H).

3.2.2. 6-Hydroxy-5-methoxy-2,3-dihydroinden-1-one (3)

A solution of compound 2 (6.3 g, 32.1 mmol) in methanesulfonic acid (50 mL) was refluxed at 120 °C for 1 h. After cooling to RT, the reaction mixture was poured into ice-water, stirred for 5 min, and filtered to afford a crude dark brown solid, which was recrystallized from EtOH to afford the known compound 3 [20] (3.8 g, 67%) as a yellow solid: ¹H-NMR (400 MHz, (CD₂)₂SO, which matches the lit. [20], Figure S2) δ 9.38 (s, 1H, O H), 7.03 (s, 1H, aromatic), 6.89 (s, 1H, aromatic), 5.60 (very br s, 1H, OH), 3.85 (s, 3H, PhOCH₃), 2.92 (t, J = 5.6 Hz, 2H, CH₂CH₂C=O), 2.49 (t, J = 5.6 Hz, 2H, CH₂CH₃C=O).

3.2.3. 6-[tert-Butyl(dimethyl)silyloxy]-5-methoxy-2,3-dihydroinden-1-one (4)

TBDMSCI (3.2 g, 21.3 mmol) was added to a solution of compound 3 (1.9 g, 10.7 mmol), DMAP (0.5 g, 4.3 mmol) and Et₃N (3.0 mL, 21.3 mmol) in freshly distilled CH₂Cl₂ (100 mL). The reaction mixture was stirred at RT overnight before being quenched with H₂O (100 mL). The organic layer was separated, washed with H₂O (2 × 100 mL) and brine (100 mL), dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure to afford a crude dark brown solid, which was purified by flash column chromatography (SiO₂ gel, pure hexanes to hexanes:EtOAc/3:1) to yield a brown solid, which was further triturated in hexanes to give compound 4 (2.8 g, 90%) as a white solid: ¹H-NMR (400 MHz, CDCl₃, Figure S3) δ 7.17 (s, 1H, aromatic), 6.84 (s, 1H, aromatic), 3.87 (s, 3H, PhOCH₃), 3.02 (app. t, J = 5.6 Hz, 2H, CH₂CH₂C=O), 2.64 (app. t, J = 5.6 Hz, 2H, CH₂CH₂C=O), 0.98 (s, 9H, Si(CH₃)₃), 0.14 (s, 6H, Si(CH₃)₂); ¹³C-NMR (100 MHz, CDCl₃, Figure S4) δ 205.7 (C=O), 157.5 (C), 150.9 (C), 145.2 (C), 130.0 (C), 114.1 (CH), 107.8 (CH), 55.6 (CH₃), 36.6 (CH₂), 25.62 (CH₃, three carbons), 25.56 (CH₂), 18.4 (C), −4.7 (CH₃, two carbons); m/z calcd. for C₁₆H₂₅O₂Si⁺ [M + H⁺]⁺ 293.1567; found 293.1563.

3.2.4. (E)-2-[(1-Benzylpiperidin-4-yl)methylene]-6-hydroxy-5-methoxy-2,3-dihydroinden-1-one (6)

To a solution of compound 4 (1.00 g, 3.42 mmol) and N-benzylpiperidine-4-carboxaldehyde (5, 0.68 mL, 3.42 mmol) in EtOH (10 mL) was added KOH (0.5 g), and the mixture was refluxed at 65 °C. After 1 h, the reaction was analyzed by TLC (CH₂Cl₂:MeOH/19:1, Rf 0.30 in CH₂Cl₂:MeOH/19:1).
The reaction mixture was concentrated under reduced pressure to give a crude yellow solid, which was re-dissolved in H2O (10 mL). 1 N aqueous HCl was then slowly added until pH 5 to yield a yellow precipitate, which was recrystallized in MeCN to afford compound 6 (0.81 g, 65%) as a yellow solid:

\[ ^1 \text{H-NMR} (400 \text{ MHz, CDCl}_3, \text{Figure S5}) \delta 7.32–7.24 (m, 6H, aromatic), 6.87 (s, 1H, aromatic), 6.63 (d, J = 10.0 Hz, 1H, C=CH), 5.70 (br s, 1H, \text{OH}), 3.98 (s, 3H, OCH_3), 3.56 (s, 2H), 3.51 (s, 2H), 2.91 (d, J = 11.6 Hz, 2H), 2.30 (m, 1H), 2.04 (t, J = 11.6 Hz, 2H), 1.70–1.60 (m, 4H); 13C-NMR (100 MHz, CDCl_3, Figure S6) \delta 192.6 (C=O), 152.6 (C), 145.8 (C), 143.4 (C), 139.9 (C), 138.2 (C), 135.5 (CH), 132.5 (CH), 129.2 (CH, two carbons), 128.2 (CH, two carbons), 127.0 (C), 108.7 (CH), 106.8 (CH), 63.5 (CH_2), 56.2 (CH_3), 53.1 (CH_2, two carbons), 37.2 (CH_2), 31.2 (CH_2, two carbons), 29.5 (CH); m/z calcd. for C_{23}H_{26}NO_3^+ [M + H]^+ 364.1907; found 364.1909.

3.2.5. 2-[(1-Benzylpiperidin-4-yl)methyl]-6-hydroxy-5-methoxy-2,3-dihydroinden-1-one (7)

To a solution of compound 6 (101 mg, 0.28 mmol) in degassed THF (2.5 mL), 10% Pd/C was added (wet support, Sigma 520829-10G, 10 mg). The reaction flask was then sealed with a rubber septum and freed of air. Thioanisole (14.2 \times 10^{-7} \text{ mL}, obtained using 5 \muL of a stock solution comprising 14.2 \muL of thioanisole in 50 mL of anhydrous THF) was added, and the reaction mixture was stirred at RT overnight. The reaction mixture was then diluted with H_2O and extracted with EtOAc (3 \times 15 mL). The combined organic layers were washed with H_2O, filtered, and concentrated under reduced pressure. The crude product obtained was purified by column chromatography (SiO_2, hexanes:EtOAc/5:1) to yield the known compound 7 (96 mg, 94%) as a yellow solid:

\[ ^1 \text{H-NMR} (400 \text{ MHz, CDCl}_3, \text{Figure S7}) \delta 7.30–7.20 (m, 6H, aromatic), 6.82 (s, 1H, aromatic), 3.96 (s, 3H, OCH_3), 3.49 (s, 2H, NCH_2Ph), 3.20 (dd, J_1 = 18.0 Hz, J_2 = 7.6 Hz, 1H), 2.87 (m, 2H), 2.66 (dt, J_1 = 13.6 Hz, J_2 = 3.6 Hz, 2H), 1.98–1.82 (m, 3H), 1.72–1.63 (m, 2H), 1.48 (m, 1H), 1.39–1.24 (m, 3H); 13C-NMR (100 MHz, CDCl_3, Figure S8) \delta 192.6 (C=O), 152.9 (C), 147.6 (C), 145.8 (C), 138.3 (C), 130.0 (C), 129.3 (CH, two carbons), 128.1 (CH, two carbons), 126.9 (CH), 108.1 (CH), 106.9 (CH), 63.4 (CH_2), 56.2 (CH_3), 53.7 (CH_2, two carbons), 45.3 (CH), 38.7 (CH_2), 34.4 (CH_2), 33.4 (CH_2), 32.9 (CH_2), 31.7 (CH); m/z calcd. for C_{23}H_{26}NO_3^+ [M + H]^+ 366.2064; found 366.2065. The purity of the compound was further confirmed by RP-HPLC: R_f = 17.17 min (96%; Figure S9).

3.2.6. tert-Butyl N-(3-chloropropyl)carbamate (Boc-protected 3-chloropropylamine)

A solution of NaHCO_3 (5.9 g, 70.8 mmol) in H_2O (15 mL) was slowly added to a mixture of 3-chloropropylamine hydrochloride (1.0 g, 7.69 mmol), Boc_2O (3.0 g, 13.8 mmol) and 1,4-dioxane (10 mL). The resulting mixture was stirred at 60 °C for 3 h. The reaction mixture was then diluted with H_2O, and extracted with EtOAc (3 \times). The combined organic layers were washed with H_2O (3 \times) and brine (3 \times), dried over anhydrous MgSO_4, filtered, and concentrated under reduced pressure. The crude product obtained was purified by column chromatography (SiO_2 gel, hexanes:EtOAc/5:1; R_f 0.31 in hexanes:EtOAc/5:1) to yield the known compound tert-butyl N-(3-chloropropyl)carbamate [27] (0.55 g, 36%) as a colorless oil: 1H-NMR (400 MHz, CDCl_3, which matches the lit. [27], Figure S10) \delta 4.65 (br s, 1H, NH), 3.56 (t, J = 6.4 Hz, 2H, C\text{ICH}_2\text{CH}_2), 3.26 (q, J = 6.4 Hz, 2H, CH_2\text{CH}_2\text{NH\text{Boc}}), 1.94 (p, J = 6.4 Hz, 2H, CH_2\text{CH}_2\text{CH}_2), 1.42 (s, 9H, C(\text{CH}_3)_3).

3.2.7. 2-[(1-Benzylpiperidin-4-yl)methyl]-6-[[3-tert-butyl-N-propylcarbamate]oxy]-5-methoxy-2,3-dihydroinden-1-one (Boc-protected compound 8a)

A solution of compound 7 (215 mg, 0.59 mmol), tert-butyl N-(3-chloropropyl)carbamate (228 mg, 1.18 mmol), C\text{O}_2\text{CO}_2\text{Me} (575 mg, 1.76 mmol), and TBAI (109 mg, 0.29 mmol) in anhydrous DMF (5 mL) was stirred at RT overnight. The reaction mixture was then diluted with H_2O, and extracted with EtOAc (3 \times). The combined organic layers were washed with H_2O (3 \times) and brine (3 \times), dried over anhydrous MgSO_4, filtered, and concentrated under reduced pressure. The crude product obtained was purified by column chromatography (SiO_2 gel, pure CH_2Cl_2 to CH_2Cl_2-MeOH/19:1; R_f 0.55 in CH_2Cl_2-MeOH/9:1) to yield 2-[(1-benzylpiperidin-4-yl)methyl]-6-[[3-tert-butyl-N-propylcarbamate]oxy]-5-methoxy-2,3-dihydroinden-1-one (276 mg, 90%) as a white foam: 1H-NMR (400 MHz, CDCl_3, Figure S11) \delta 7.30–7.20 (m, 5H, aromatic), 7.11 (s, 1H, aromatic), 6.82 (s, 1H, aromatic), 5.52 (very br t, 1H, NH), 4.08 (t, J = 5.6 Hz, 2H, OCH_2CH_2),
3.93 (s, 3H, OCH₃), 3.49 (s, 2H, NCH₃-Ph), 3.34 (q, J = 5.2 Hz, 2H, CH₂CH₂NHBoc), 3.20 (dd, J₁ = 17.6 Hz, J₂ = 8.0 Hz, 1H), 2.88 (m, 2H), 2.67 (m, 2H), 2.02–1.85 (m, 5H), 1.72–1.62 (m, 2H), 1.44 (s, 9H, C(CH₃)₃), 1.36–1.02 (m, 4H); 13C-NMR (100 MHz, CDCl₃, Figure S12) δ 207.7 (C=O), 156.0 (CH), 155.6 (C=O), 148.9 (C), 148.4 (C), 138.3 (C), 129.2 (CH, two carbons), 128.1 (CH, three carbons), 126.9 (C), 107.4 (CH), 105.3 (CH), 78.9 (C), 68.3 (CH₂), 63.4 (CH₂), 56.0 (CH₃), 53.74 (CH₂), 53.72 (CH₂), 45.4 (CH), 39.1 (CH₂), 38.7 (CH₂), 34.4 (CH₂), 33.4 (CH₂), 33.0 (CH₂), 31.7 (CH), 29.0 (CH₂), 28.5 (CH₃, three carbons); m/z calcd. for C₃₁H₄₅N₂O₅⁺ [M + H⁺] 523.3166; found 523.3131.

3.2.8. 6-[(3-Aminopropyl)oxy]-2-[(1-benzylpiperidin-4-yl)methyl]-5-methoxy-2,3-dihydroinden-1-one (8a)

A solution of the 2-[(1-benzylpiperidin-4-yl)methyl]-6-[(3-tert-butyloxy)carbamate]oxy]-5-methoxy-2,3-dihydroinden-1-one (100 mg, 0.19 mmol) in CH₂Cl₂ (2 mL) was treated with TFA (1 mL) and allowed to stir at RT for 5 min. The reaction was then quenched by addition of saturated aqueous NaHCO₃ and the resulting mixture was extracted with CH₂Cl₂ (3 ×). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The crude product obtained was purified by column chromatography (SiO₂ gel, pure CH₂Cl₂ to CH₂Cl₂:MeOH/19:1; Rₜ 0.12 in CH₂Cl₂:MeOH/9:1) to yield compound 8a (53 mg, 65%) as a white solid: ¹H-NMR (400 MHz, CDCl₃, Figure S13) δ 7.28–7.18 (m, 5H, aromatic), 7.13 (s, 1H, aromatic), 6.81 (s, 1H, aromatic), 4.09 (t, J = 6.4 Hz, 2H, OCH₂CH₂), 3.90 (s, 3H, OCH₃), 3.47 (s, 2H, NCH₂Ph), 3.19 (dd, J₁ = 17.6 Hz, J₂ = 8.0 Hz, 1H), 2.84–2.90 (m, 4H), 2.67 (m, 2H), 1.98–2.04 (m, 7H), 1.72–1.58 (m, 2H), 1.45 (m, 1H), 1.38–1.23 (m, 3H); 13C-NMR (100 MHz, CDCl₃, Figure S14) δ 207.8 (C=O), 155.7 (C), 148.73 (C), 148.66 (C), 138.4 (C), 129.2 (CH, three carbons), 128.1 (CH, two carbons), 126.9 (C), 107.5 (CH), 105.6 (CH), 67.4 (CH₂), 63.4 (CH₂), 56.2 (CH₃), 53.76 (CH₂), 53.74 (CH₂), 45.4 (CH), 39.4 (CH₂), 38.7 (CH₂), 34.7 (CH₂), 33.3 (CH₂), 33.0 (CH₂), 32.5 (CH₂), 31.8 (CH); m/z calcd. for C₂₆H₃₅N₂O₅⁺ [M + H⁺] 423.2642; found 423.2656. The purity of the compound was further confirmed by RP-HPLC: Rₜ = 15.86 min (96%; Figure S15).

3.2.9. tert-Butyl N-[(2-chloroethyl)carbamate]

A solution of NaHCO₃ (6.7 g, 79.3 mmol) in H₂O (15 mL) was slowly added to a mixture of 2-chloroethylamine hydrochloride (1.0 g, 8.6 mmol), Boc₂O (3.4 g, 15.5 mmol) and 1,4-dioxane (10 mL) at 0 °C. The resulting mixture was allowed to warm to RT and was stirred overnight. The reaction mixture was then diluted with H₂O and extracted with CH₂Cl₂ (3 ×). The combined organic layers were washed with H₂O (3 ×) and brine (3 ×), dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The crude product obtained was purified by column chromatography (SiO₂ gel, Hexanes:EtOAc/9:1; Rₜ 0.55 in hexanes:EtOAc/4:1) to yield the known compound tert-butyloxycarbamate [28] (1.25 g, 83%) as a colorless oil: ¹H-NMR (400 MHz, CDCl₃, which matches the lit. [28], Figure S16) δ 4.94 (br s, 1H, NH₂), 3.57 (t, J = 6.0 Hz, 2H, CH₂CH₂OCH₂), 3.44 (q, J = 6.0 Hz, 2H, CH₂CH₂NHBoc), 1.47 (s, 9H, C(CH₃)₃).

3.2.10. 2-[(1-Benzylpiperidin-4-yl)methyl]-6-[(3-tert-butyloxy)-N-ethylcarbamate]oxy]-5-methoxy-2,3-dihydroinden-1-one (Boc-protected compound 8b).

A solution of compound 7 (216 mg, 1.20 mmol), Cs₂CO₃ (196 mg, 0.60 mmol), and TBAI (56 mg, 0.15 mmol) in anhydrous DMF (5 mL) was stirred at RT overnight. The reaction mixture was then diluted with H₂O and extracted with EtOAc (3 ×). The combined organic layers were washed with H₂O (3 ×) and brine (3 ×), dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The crude product obtained was purified by column chromatography (SiO₂ gel, pure CH₂Cl₂ to CH₂Cl₂:MeOH/19:1; Rₜ 0.48 in CH₂Cl₂:MeOH/9:1) to yield 2-[(1-benzylpiperidin-4-yl)methyl]-6-[(3-tert-butyloxy)carbamate]oxy]-5-methoxy-2,3-dihydroinden-1-one (93 mg, 61%) as a pale yellow solid: ¹H-NMR (400 MHz, CDCl₃, Figure S17) δ 7.34–7.20 (m, 5H, aromatic), 7.14 (s, 1H, aromatic), 6.83 (s, 1H, aromatic), 5.05 (m, 1H, NH trope), 4.05 (br t, 2H, OCH₂CH₂), 3.92 (s, 3H, OCH₃), 3.53 (m, 7H), 2.85 (m, 2H), 2.62 (m, 2H), 2.20 (m, 2H), 1.98–1.84 (m, 7H), 1.72–1.58 (m, 2H), 1.45 (m, 1H), 1.38–1.23 (m, 3H); 13C-NMR (100 MHz, CDCl₃, Figure S14) δ 207.8 (C=O), 155.7 (C), 148.73 (C), 148.66 (C), 138.4 (C), 129.2 (CH, three carbons), 128.1 (CH, two carbons), 126.9 (C), 107.5 (CH), 105.6 (CH), 67.4 (CH₂), 63.4 (CH₂), 56.2 (CH₃), 53.76 (CH₂), 53.74 (CH₂), 45.4 (CH), 39.4 (CH₂), 38.7 (CH₂), 34.7 (CH₂), 33.3 (CH₂), 33.0 (CH₂), 32.5 (CH₂), 31.8 (CH); m/z calcd. for C₂₆H₃₅N₂O₅⁺ [M + H⁺] 423.2642; found 423.2656. The purity of the compound was further confirmed by RP-HPLC: Rₜ = 15.86 min (96%; Figure S15).
4H, NCH$_3$Ph, CH$_3$CH$_2$NHBOC), 3.21 (dd, $J_1 = 17.2$ Hz, $J_2 = 8.4$ Hz, 1H), 2.90 (m, 2H), 2.67 (m, 2H), 1.99 (m, 2H), 1.88 (m, 1H), 1.69 (m, 2H), 1.43 (m, 10H), 1.37–1.23 (m, 3H); 13C-NMR (100 MHz, CDCl$_3$, Figure S18) δ 207.6 (C=O), 155.8 (C and C=O), 149.2 (C), 148.3 (C), 138.3 (C), 129.3 (CH, two carbons), 128.1 (CH, two carbons), 56.1 (CH$_3$), 53.7 (CH$_2$, two carbons), 34.4 (CH$_2$), 33.9 (CH$_2$), 32.9 (CH$_2$), 31.7 (CH), 28.4 (CH$_3$, three carbons); $m/z$ calcd. for C$_{30}$H$_{41}$N$_2$O$_5$$^+$ [M + H]$^+$ 509.3010; found 509.3025.

3.2.11. 6-[(3-Aminoethyl)oxy]-2-[(1-benzylpiperidin-4-yl)methyl]-5-methoxy-2,3-dihydroinden-1-one (8b)

A solution of 2-[(1-benzylpiperidin-4-yl)methyl]-6-[(3-tert-butyl-N-ethylcarbamate)oxy]-5-methoxy-2,3-dihydroinden-1-one (83 mg, 0.16 mmol) in CH$_2$Cl$_2$ (1 mL) was treated with TFA (1 mL) and allowed to stir at RT overnight. The reaction mixture was then diluted with H$_2$O and extracted with CH$_2$Cl$_2$. The combined organic layers were washed with brine, dried over anhydrous MgSO$_4$, filtered, and concentrated under reduced pressure to yield compound 8b (62 mg, 93%) as a white solid: 1H-NMR (400 MHz, CDCl$_3$, Figure S19) δ 7.32–7.20 (m, 5H, aromatic), 7.15 (s, 1H, aromatic), 6.83 (s, 1H, aromatic), 4.03 (t, $J = 5.2$ Hz, 2H, OCH$_2$CH$_2$), 3.92 (s, 3H, OCH$_3$), 3.57 (s, 2H, NCH$_2$Ph), 3.21 (dd, $J_1 = 17.6$ Hz, $J_2 = 8.0$ Hz, 1H), 3.11 (t, $J = 5.2$ Hz, 2H, 1H), 2.67 (m, 2H), 2.03 (m, 2H), 1.89 (m, 1H), 1.71 (m, 2H), 1.52 (m, 2H), 1.44–1.23 (m, 4H); $^{13}$C-NMR (100 MHz, CDCl$_3$, Figure S20) δ 207.7 (C=O), 155.8 (C), 148.9 (C), 148.6 (C), 138.1 (C), 129.3 (CH, four carbons), 129.2 (CH), 128.1 (CH, two carbons), 127.0 (C), 107.6 (CH), 106.0 (CH), 71.1 (CH$_2$), 63.3 (CH$_2$), 56.1 (CH$_3$), 53.68 (CH$_3$), 53.65 (CH$_2$), 45.4 (CH), 41.2 (CH$_2$), 38.6 (CH$_2$), 34.3 (CH$_2$), 33.3 (CH$_2$), 32.8 (CH$_2$), 31.7 (CH); $m/z$ calcd. for C$_{26}$H$_{33}$N$_2$O$_4$$^+$ [M + H]$^+$ 409.2486; found 409.2496. The purity of the compound was further confirmed by RP-HPLC: $R_t = 15.74$ min (95%; Figure S21).

3.2.12. 2-[(1-Benzylpiperidin-4-yl)methyl]-5-methoxy-6-propoxy-2,3-dihydroinden-1-one (8c).

A solution of compound 7 (50 mg, 0.14 mmol) and K$_2$CO$_3$ (95 mg, 0.68 mmol) in anhydrous DMF (5 mL) was treated with 1-bromopropane (0.06 mL, 0.68 mmol), and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H$_2$O, and extracted with EtOAc (3×). The combined organic layers were washed with H$_2$O (3×) and brine (3×), dried over anhydrous MgSO$_4$, filtered, and concentrated under reduced pressure. The crude product obtained was purified by column chromatography (SiO$_2$ gel, pure CH$_2$Cl$_2$ to CH$_2$Cl$_2$:MeOH:19:1; $R_t$ 0.38 in CH$_2$Cl$_2$:MeOH:19:1) to yield compound 8c (53 mg, 95%) as an off-white solid: 1H-NMR (400 MHz, CDCl$_3$, Figure S22) δ 7.32–7.20 (m, 5H, aromatic), 7.13 (s, 1H, aromatic), 6.82 (s, 1H, aromatic), 3.98 (t, $J = 6.8$ Hz, 2H, CH$_3$CH$_2$CH$_2$OAr), 3.92 (s, 3H, OCH$_3$), 3.57 (s, 2H, NCH$_2$Ph), 3.20 (dd, $J_1 = 17.6$ Hz, $J_2 = 8.4$ Hz, 1H), 2.90 (m, 2H), 2.66 (dt, $J_1 = 14.0$ Hz, $J_2 = 3.6$ Hz, 2H), 1.98 (m, 2H), 1.90 (m, 1H), 1.85 (sextet, $J = 7.2$ Hz, 2H, CH$_3$CH$_2$CH$_2$OAr), 1.73–1.64 (m, 2H), 1.49 (m, 1H), 1.40–1.24 (m, 3H), 1.02 (t, $J = 7.2$ Hz, 3H, CH$_3$CH$_2$CH$_2$OAr); $^{13}$C-NMR (100 MHz, CDCl$_3$, Figure S23) δ 207.8 (C=O), 155.8 (C), 148.9 (C), 148.5 (C), 129.3 (CH, two carbons), 129.2 (C), 128.2 (CH, three carbons), 127.1 (C), 107.5 (CH), 105.5 (CH$_2$), 63.2 (CH$_2$), 56.2 (CH$_3$), 53.7 (CH$_2$), 53.6 (CH$_2$), 45.4 (CH), 38.6 (CH$_2$), 34.3 (CH$_2$), 33.3 (CH$_2$), 32.7 (CH$_2$), 31.6 (CH), 22.2 (CH$_2$), 10.3 (CH$_3$); $m/z$ calcd. for C$_{26}$H$_{34}$N$_2$O$_4$$^+$ [M + H]$^+$ 408.2533; found 408.2524. The purity of the compound was further confirmed by RP-HPLC: $R_t = 19.30$ min (96%; Figure S24).

3.2.13. 2-[(1-Benzylpiperidin-4-yl)methyl]-6-[(chloroethyl)oxy]-5-methoxy-2,3-dihydroinden-1-one (8d)

A solution of compound 7 (50 mg, 0.14 mmol) and K$_2$CO$_3$ (189 mg, 1.37 mmol) in anhydrous DMF (5 mL) was treated with 1,2-dichloroethane (0.11 mL, 1.37 mmol), and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H$_2$O, and extracted with
CH₂Cl₂ (3 ×). The combined organic layers were washed with H₂O (3 ×) and brine (3 ×), dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The crude product obtained was purified by column chromatography (SiO₂ gel, pure CH₂Cl₂ to CH₂Cl₂:MeOH/19:1; Rf 0.38 in CH₂Cl₂:MeOH/19:1) to yield compound 8d (50 mg, 85%) as a brown oil. ¹H-NMR (400 MHz, CDCl₃, Figure S25) δ 7.30–7.20 (m, 5H, aromatic), 7.14 (s, 1H, aromatic), 6.84 (s, 1H, aromatic), 4.25 (t, J = 6.0 Hz, 2H), 3.91 (s, 3H, OCH₃), 3.82 (t, J = 6.0 Hz, 2H), 3.49 (s, 2H, NCH₂Ph), 3.20 (dd, J₁ = 17.6 Hz, J₂ = 8.4 Hz, 1H), 2.88 (m, 2H), 2.66 (dt, J₁ = 14.4 Hz, J₂ = 2.8 Hz, 2H), 1.99–1.93 (m, 2H), 1.92–1.85 (m, 1H), 1.72–1.63 (m, 2H), 1.47 (m, 1H), 1.40–1.23 (m, 3H); ¹³C-NMR (100 MHz, CDCl₃, Figure S26) δ 207.6 (C=O), 155.9 (C), 149.5 (C), 148.0 (C), 138.1 (C), 129.3 (C, two carbons), 129.2 (CH, two carbons), 128.1 (C), 108.0 (CH), 106.6 (CH), 69.0 (CH₂), 63.3 (CH₂), 56.2 (CH₃), 53.68 (CH₂), 53.65 (CH₂), 45.4 (CH), 41.4 (CH₂), 38.6 (CH₂), 34.3 (CH₂), 33.3 (CH₂), 32.8 (CH₂), 31.7 (CH); m/z calcd. for C₂₅H₂₃BrNO₂⁺ [M + H]⁺ 472.1482; found 472.1477. The purity of the compound was further confirmed by RP-HPLC: Rf = 19.10 min (97%; Figure S27).

3.2.14. 2-[[1-Benzylpiperidin-4-yl]methyl]-6-[[bromomethyl]oxy]-5-methoxy-2,3-dihydroinden-1-one (8e)

A solution of compound 7 (100 mg, 0.27 mmol) and K₂CO₃ (380 mg, 2.74 mmol) in anhydrous DMF (5 mL) was treated with 1,2-dibromoethane (0.24 mL, 2.74 mmol) and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H₂O, and extracted with CH₂Cl₂ (3 ×). The combined organic layers were washed with H₂O (3 ×) and brine (3 ×), dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The crude product obtained was purified by column chromatography (SiO₂ gel, pure CH₂Cl₂ to CH₂Cl₂:MeOH/19:1; Rf 0.49 in CH₂Cl₂:MeOH/19:1) to yield compound 8e (70 mg, 54%) as an off-white solid. ¹H-NMR (400 MHz, CDCl₃, Figure S28) δ 7.30–7.20 (m, 5H, aromatic), 7.15 (s, 1H, aromatic), 6.85 (s, 1H, aromatic), 4.32 (t, J = 6.4 Hz, 2H), 3.93 (s, 3H, OCH₃), 3.65 (t, J = 6.4 Hz, 2H), 3.49 (s, 2H, NCH₂Ph), 3.21 (dd, J₁ = 17.6 Hz, J₂ = 8.0 Hz, 1H), 2.88 (m, 2H), 2.67 (dt, J₁ = 14.0 Hz, J₂ = 2.8 Hz, 2H), 1.98–1.86 (m, 3H), 1.72–1.62 (m, 2H), 1.47 (m, 1H), 1.40–1.24 (m, 3H); ¹³C-NMR (100 MHz, CDCl₃, Figure S29) δ 207.6 (C=O), 155.9 (C), 149.5 (C), 147.8 (C), 138.4 (C), 129.2 (CH, 3 carbons), 128.1 (CH, 2 carbons), 126.9 (C), 108.0 (CH), 106.6 (CH), 68.8 (CH₂), 63.4 (CH₂), 56.3 (CH₃), 53.75 (CH₂), 53.72 (CH₂), 45.4 (CH), 38.7 (CH₂), 34.4 (CH₂), 33.4 (CH₂), 33.0 (CH₂), 31.8 (CH), 28.4 (CH₂); m/z calcd. for C₂₅H₂₃BrNO₂⁺ [M + H]⁺ 472.1482; found 472.1477. The purity of the compound was further confirmed by RP-HPLC: Rf = 19.10 min (97%; Figure S30).

3.2.15. 2-[[1-Benzylpiperidin-4-yl]methyl]-6-[[bromobutyl]oxy]-5-methoxy-2,3-dihydroinden-1-one (8f)

A solution of compound 7 (50 mg, 0.14 mmol) and K₂CO₃ (189 mg, 1.37 mmol) in anhydrous DMF (5 mL) was treated with 1,4-dibromobutane (0.16 mL, 1.37 mmol), and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H₂O, and extracted with CH₂Cl₂ (3 ×). The combined organic layers were washed with H₂O (3 ×) and brine (3 ×), dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The crude product obtained was purified by column chromatography (SiO₂ gel, pure CH₂Cl₂ to CH₂Cl₂:MeOH/19:1; Rf 0.38 in CH₂Cl₂:MeOH/19:1) to yield compound 8f (63 mg, 93%) as an off-white solid. ¹H-NMR (400 MHz, CDCl₃, Figure S31) δ 7.32–7.22 (m, 5H, aromatic), 7.13 (s, 1H, aromatic), 6.83 (s, 1H, aromatic), 4.04 (t, J = 6.4 Hz, 2H), 3.92 (s, 3H, OCH₃), 3.52 (s, 2H, NCH₂Ph), 3.48 (t, J = 6.4 Hz, 2H), 3.20 (dd, J₁ = 17.6 Hz, J₂ = 8.0 Hz, 1H), 2.90 (m, 2H), 2.67 (m, 2H), 2.10–1.93 (m, 6H), 1.92–1.85 (m, 1H), 1.73–1.64 (m, 2H), 1.48 (m, 1H), 1.40–1.23 (m, 3H); ¹³C-NMR (100 MHz, CDCl₃, Figure S32) δ 207.8 (C=O), 155.8 (C), 148.8 (C), 148.6 (C), 129.4 (CH, two carbons), 129.2 (C), 128.2 (CH, three carbons), 127.2 (C), 107.5 (CH), 105.6 (CH), 68.0 (CH₂), 63.2 (CH₂), 56.2 (CH₃), 53.6 (CH₂, two carbons), 45.3 (CH), 38.6 (CH₂), 34.2 (CH₂), 33.3 (CH₂, two carbons), 32.6 (CH₂), 31.5 (CH), 29.4 (CH₂), 27.6 (CH₂); m/z calcd. for
C_{27}H_{35}BrNO_{3}^{+} [M + H]^{+} 500.1795; found 500.1794. The purity of the compound was further confirmed by RP-HPLC: R_{t} = 20.22 min (95%; Figure S33).

3.2.16. 2-[[1-Benzylpiperidin-4-yl)methyl]]-6-[[bromododecyl]oxy]-5-methoxy-2,3-dihydroinden-1-one (8g)

A solution of compound 7 (100 mg, 0.27 mmol) and K_{2}CO_{3} (38 mg, 0.27 mmol) in anhydrous DMF (5 mL) was treated with 1,12-dibromododecane (900 mg, 2.74 mmol) and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H_{2}O, and extracted with CH_{2}Cl_{2} (3×). The combined organic layers were washed with H_{2}O (3×) and brine (3×), dried over anhydrous MgSO_{4}, filtered, and concentrated under reduced pressure. The crude product obtained was purified by column chromatography (SiO_{2} gel, pure CH_{2}Cl_{2} to CH_{2}Cl_{2}:MeOH/19:1; R_{f} 0.49 in CH_{2}Cl_{2}:MeOH/19:1) to yield compound 8g (53 mg, 32%) as an off-white solid: 1^H-NMR (400 MHz, CDCl_{3}, Figure S34) δ 7.32–7.22 (m, 5H, aromatic), 7.13 (s, 1H, aromatic), 6.82 (s, 1H, aromatic), 3.99 (t, J = 6.8 Hz, 2H), 3.92 (s, 3H, OCH_{3}), 3.80 (d, J = 6.8 Hz, 2H), 3.20 (dd, J_{1} = 17.6 Hz, J_{2} = 8.0 Hz, 1H), 2.91 (m, 2H), 2.66 (dt, J_{1} = 14.0 Hz, J_{2} = 3.6 Hz, 2H), 1.99 (m, 2H), 1.92–1.78 (m, 5H), 1.78–1.62 (m, 3H), 1.44–1.38 (m, 6H), 1.36–1.22 (m, 13H); 13C-NMR (100 MHz, CDCl_{3}, Figure S35) δ 207.8 (C=O), 155.8 (C), 148.5 (C), 129.3 (CH, two carbons), 129.2 (C), 128.2 (CH, three carbons), 127.1 (C), 107.4 (CH), 105.5 (CH), 69.1 (CH_{2}), 63.2 (CH_{2}), 56.2 (CH_{3}), 53.6 (CH_{2}, two carbons), 45.4 (CH), 38.6 (CH), 34.3 (CH_{2}), 34.1 (CH_{2}), 33.3 (CH_{2}), 32.8 (CH_{2}), 32.6 (CH_{2}), 31.6 (CH), 29.5 (CH_{2}, two carbons), 29.4 (CH_{2}), 29.3 (CH_{2}), 28.9 (CH_{2}), 28.7 (CH_{2}), 28.1 (CH_{2}), 26.9 (CH_{2}), 25.9 (CH_{2}); m/z calc. for C_{35}H_{38}BrNO_{3}^{+} [M + H]^{+} 612.3047; found 612.3045. The purity of the compound was further confirmed by RP-HPLC: R_{t} = 25.00 min (96%; Figure S36).

3.2.17. 6-[[Benzyl]oxy-2-[[1-benzylpiperidin-4-yl)methyl]-5-methoxy-2,3-dihydroinden-1-one (8h)

A solution of compound 7 (50 mg, 0.14 mmol) and K_{2}CO_{3} (38 mg, 0.27 mmol) in anhydrous DMF (5 mL) was treated with benzyl bromide (20 µL, 0.16 mmol), and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H_{2}O, and extracted with EtOAc (3×). The combined organic layers were washed with H_{2}O (3×) and brine (3×), dried over anhydrous MgSO_{4}, and filtered. After standing at RT overnight, white solids precipitated out, which were filtered off. The filtrate was further concentrated under reduced pressure, and purified by column chromatography (SiO_{2} gel, pure CH_{2}Cl_{2} to CH_{2}Cl_{2}:MeOH/19:1; R_{f} 0.37 in CH_{2}Cl_{2}:MeOH/19:1) to yield compound 8h (51 mg, 82%) as an off-white solid: 1^H-NMR (400 MHz, CDCl_{3}, Figure S37) δ 7.42 (d, J = 7.6 Hz, 2H, aromatic), 7.35 (t, J = 7.6 Hz, 2H, aromatic), 7.31–7.28 (m, 5H, aromatic), 7.24 (s, 1H, aromatic), 7.19 (s, 1H, aromatic), 6.85 (s, 1H, aromatic), 5.13 (s, 2H, OCH=Ph), 3.93 (s, 3H, OCH_{3}), 3.51 (s, 2H, NCH=Ph), 3.20 (dd, J_{1} = 17.6 Hz, J_{2} = 8.4 Hz, 1H), 2.89 (m, 2H), 2.63 (dt, J_{1} = 14.0 Hz, J_{2} = 4.0 Hz, 2H), 1.97 (m, 2H), 1.88 (m, 1H), 1.73–1.64 (m, 2H), 1.48 (m, 1H), 1.40–1.23 (m, 3H); 13C-NMR (100 MHz, CDCl_{3}, Figure S38) δ 207.6 (C=O), 156.0 (C), 148.9 (C), 148.5 (C), 136.3 (C), 129.4 (CH, two carbons), 129.2 (C), 128.6 (CH, two carbons), 128.2 (CH, two carbons), 128.0 (CH, two carbons), 127.4 (CH, two carbons), 127.1 (C), 107.6 (CH), 106.4 (CH), 70.8 (CH_{2}), 63.2 (CH_{2}), 56.2 (CH_{3}), 53.6 (CH_{2}, two carbons), 45.3 (CH), 38.6 (CH_{2}), 34.3 (CH_{2}), 33.4 (CH_{2}), 32.7 (CH_{2}), 31.6 (CH); m/z calc. for C_{33}H_{34}NO_{3}^{+} [M + H]^{+} 456.2533; found 456.2528. The purity of the compound was further confirmed by RP-HPLC: R_{t} = 20.05 min (96%; Figure S39).

3.2.18. 2-[[1-Benzylpiperidin-4-yl)methyl]-5-methoxy-6-[[4-methylbenzyl]oxy]-2,3-dihydroinden-1-one (8i)

A solution of compound 7 (50 mg, 0.14 mmol) and K_{2}CO_{3} (38 mg, 0.27 mmol) in anhydrous DMF (5 mL) was treated with 4-methylbenzyl bromide (30 mg, 0.16 mmol), and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H_{2}O, and extracted with CH_{2}Cl_{2} (3×). The combined organic layers were washed with H_{2}O (3×) and brine (3×), dried over anhydrous MgSO_{4}, and filtered. After standing at RT overnight, the white solids precipitated out, which were
filtered off. The filtrate was further concentrated under reduced pressure, and purified by column chromatography (SiO₂ gel, pure CH₂Cl₂ to CH₂Cl₂:MeOH/19:1; Rf 0.35 in CH₂Cl₂:MeOH/19:1) to yield compound 8i (37 mg, 58%) as an off-white solid: ¹H-NMR (400 MHz, CDCl₃, Figure S40) δ 7.31 (d, J = 8.0 Hz, 2H, aromatic), 7.31–7.29 (m, 4H, aromatic), 7.24 (s, 1H, aromatic), 7.18 (s, 1H, aromatic), 7.15 (d, J = 8.0 Hz, 2H, aromatic), 6.83 (s, 1H, aromatic), 5.09 (s, 2H, OCH₂Ph), 3.92 (s, 3H, OCH₃), 3.51 (s, 2H, NCH₂Ph), 3.20 (dd, J₁ = 17.6 Hz, J₂ = 8.4 Hz, 1H), 2.89 (m, 2H), 2.63 (dt, J₁ = 13.6 Hz, J₂ = 3.6 Hz, 2H), 2.32 (s, 3H, CH₃Ph), 1.97 (m, 2H), 1.91–1.84 (m, 1H), 1.72–1.64 (m, 2H), 1.47 (m, 1H), 1.40–1.23 (m, 3H); ¹³C-NMR (100 MHz, CDCl₃, Figure S41) δ 207.6 (C=O), 156.0 (C), 148.9 (C), 148.5 (C), 137.8 (C), 133.3 (C), 129.0 (CH, two carbons), 129.25 (CH, two carbons), 129.17 (C), 128.2 (CH, three carbons), 127.5 (CH, two carbons), 127.0 (C), 107.6 (CH), 106.4 (CH), 70.7 (CH₂), 63.3 (CH₂), 56.2 (CH₂), 53.7 (CH₂, 2 carbons), 45.4 (CH), 38.7 (CH₂), 34.3 (CH₂), 33.4 (CH₂), 32.8 (CH₂), 31.7 (CH), 21.2 (CH₃); m/z calcd. for C₃₁H₃₆NO⁺ [M + H]+ 470.2690; found 470.2681. The purity of the compound was further confirmed by RP-HPLC: Rᵣ = 20.65 min (95%; Figure S42).

3.2.19. 2-[[1-Benzylpiperidin-4-yl]methyl]-5-methoxy-6-[[4-methoxybenzyl]oxy-2,3-dihydroinden-1-one (8j)

A solution of compound 7 (50 mg, 0.14 mmol) and K₂CO₃ (189 mg, 1.37 mmol) in anhydrous DMF (5 mL) was treated with 4-methoxybenzyl chloride (0.19 mL, 1.37 mmol), and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H₂O, and extracted with CH₂Cl₂ (3 ×). The combined organic layers were washed with H₂O (3 ×) and brine (3 ×), dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The crude product obtained was purified by column chromatography (SiO₂ gel, pure CH₂Cl₂ to CH₂Cl₂:MeOH/19:1; Rf 0.30 in CH₂Cl₂:MeOH/19:1) to yield compound 8j (24 mg, 36%) as an off-white solid: ¹H-NMR (400 MHz, CDCl₃, Figure S43) δ 7.34 (d, J = 8.8 Hz, 2H, aromatic), 7.33–7.26 (m, 5H, aromatic), 7.19 (s, 1H, aromatic), 6.87 (d, J = 8.8 Hz, 2H, aromatic), 6.83 (s, 1H, aromatic), 5.05 (s, 2H, OCH₂Ph), 3.91 (s, 3H, OCH₃), 3.78 (s, 3H, OCH₃), 3.56 (s, 2H, NCH₂Ph), 3.20 (dd, J₁ = 17.6 Hz, J₂ = 8.4 Hz, 1H), 2.95 (m, 2H), 2.65 (dt, J₁ = 13.6 Hz, J₂ = 3.6 Hz, 2H), 2.08–1.98 (m, 2H), 1.92–1.82 (m, 1H), 1.76–1.64 (m, 2H), 1.53 (m, 1H), 1.40–1.23 (m, 3H); ¹³C-NMR (100 MHz, CDCl₃, Figure S44) δ 207.6 (C=O), 159.5 (C), 156.0 (C), 148.9 (C), 148.5 (C), 137.2 (C), 129.5 (CH, two carbons), 129.2 (CH, two carbons), 129.1 (C), 128.4 (CH), 128.2 (CH, two carbons), 127.3 (C), 114.0 (CH, 2 carbons), 107.6 (CH), 106.5 (CH), 70.6 (CH₂), 63.1 (CH₂), 56.2 (CH₃), 53.6 (CH₂), 53.5 (CH₂), 45.3 (CH), 38.6 (CH₂), 34.1 (CH₂), 33.4 (CH₂), 32.4 (CH₂), 31.4 (CH); m/z calcd. for C₃₁H₃₆NO⁺ [M + H]+ 486.2639; found 486.2635. The purity of the compound was further confirmed by RP-HPLC: Rᵣ = 19.93 min (95%; Figure S45).

3.2.20. 2-[[1-Benzylpiperidin-4-yl]methyl]-5-methoxy-6-[[4-nitrobenzyl]oxy-2,3-dihydroinden-1-one (8k)

A solution of compound 7 (50 mg, 0.14 mmol) and K₂CO₃ (38 mg, 0.27 mmol) in anhydrous DMF (5 mL) was treated with 4-nitrobenzyl bromide (35 mg, 0.16 mmol), and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H₂O, and extracted with EtOAc (3 ×). The combined organic layers were washed with H₂O (3 ×) and brine (3 ×), dried over anhydrous MgSO₄, and filtered. After standing at RT overnight, white solids precipitated out, which were filtered off. The filtrate was further concentrated under reduced pressure and purified by column chromatography (SiO₂ gel, pure CH₂Cl₂ to CH₂Cl₂:MeOH/19:1; Rf 0.37 in CH₂Cl₂:MeOH/19:1) to yield compound 8k (44 mg, 65%) as a brown foam: ¹H-NMR (400 MHz, CDCl₃, Figure S46) δ 8.22 (d, J = 8.4 Hz, 2H, aromatic), 7.60 (d, J = 8.4 Hz, 2H, aromatic), 7.31–7.28 (m, 4H, aromatic), 7.24 (s, 1H, aromatic), 7.14 (s, 1H, aromatic), 6.88 (s, 1H, aromatic), 5.23 (s, 2H, OCH₂Ph), 3.97 (s, 3H, OCH₃), 3.50 (s, 2H, NCH₂Ph), 3.22 (dd, J₁ = 17.6 Hz, J₂ = 8.0 Hz, 1H), 2.89 (m, 2H), 2.68 (m, 2H), 1.96 (m, 2H), 1.87 (m, 1H), 1.72–1.63 (m, 2H), 1.47 (m, 1H), 1.36–1.23 (m, 3H); ¹³C-NMR (100 MHz, CDCl₃, Figure S47) δ 207.5 (C=O), 155.9 (C), 149.5 (C), 147.8 (C), 147.6 (C), 143.8 (C), 129.3 (CH), 129.2 (CH), 128.2 (CH, two carbons), 127.5 (CH, three carbons), 127.0 (C), 123.9 (CH, two carbons +
C), 107.9 (CH), 106.6 (CH), 69.5 (CH2), 63.3 (CH2), 56.3 (CH3), 53.7 (CH2, two carbons), 45.4 (CH), 38.6 (CH2), 34.3 (CH2), 33.4 (CH2), 32.8 (CH2), 31.6 (CH); m/z calcd. for C30H33BrNO3+: [M + H]+ 501.2384; found 501.2385. The purity of the compound was further confirmed by RP-HPLC: Rf = 19.98 min (96%; Figure S48).

3.2.21. 2-[(1-Benzylpiperidin-4-yl)methyl]-6-[(4-bromobenzyl)oxy]-5-methoxy-2,3-dihydroinden-1-one (8l)

A solution of compound 7 (50 mg, 0.14 mmol) and K2CO3 (38 mg, 0.27 mmol) in anhydrous DMF (5 mL) was treated with 4-bromobenzyl bromide (41 mg, 0.16 mmol) and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H2O, and extracted with EtOAc (3 ×). The combined organic layers were washed with H2O (3 ×) and brine (3 ×), dried over anhydrous MgSO4, and filtered. After standing at RT overnight, white solids precipitated out, which were filtered off. The filtrate was further concentrated under reduced pressure and purified by column chromatography (SiO2 gel, pure CH2Cl2 to CH2Cl2:MeOH/19:1; Rf 0.37 in CH2Cl2:MeOH/19:1) to yield compound 8l (61 mg, 84%) as an off-white solid: 1H-NMR (400 MHz, CDCl3, Figure S49) δ 7.47 (d, J = 8.4 Hz, 2H, aromatic), 7.30 (d, J = 8.4 Hz, 2H, aromatic), 7.31–7.28 (m, 5H, aromatic), 7.24 (s, 1H, aromatic), 7.14 (s, 1H, aromatic), 6.85 (s, 1H, aromatic), 5.07 (s, 2H, OCH2Ph), 3.94 (s, 3H, OCH3), 3.51 (s, 2H, NCH2Ph), 3.21 (dd, J1 = 17.6 Hz, J2 = 8.4 Hz, 1H), 2.89 (m, 2H), 2.67 (dt, J1 = 14.4 Hz, J2 = 4.0 Hz, 2H), 1.96 (m, 2H), 1.72–1.63 (m, 2H), 1.47 (m, 1H), 1.40–1.23 (m, 3H), 13C-NMR (100 MHz, CDCl3, Figure S50) δ 207.6 (C=O), 155.9 (C), 149.1 (C), 148.2 (C), 135.4 (C), 131.7 (CH, two carbons + C), 129.4 (CH), 129.2 (CH), 129.0 (CH, three carbons), 128.2 (CH, two carbons), 127.1 (C), 122.0 (C), 107.7 (CH), 106.5 (CH), 70.1 (CH2), 56.2 (CH3), 53.6 (CH2, two carbons), 45.3 (CH), 38.6 (CH2), 34.2 (CH2), 33.4 (CH2), 32.7 (CH2), 31.6 (CH); m/z calcd. for C30H33BrNO3+: [M + H]+ 534.1638; found 534.1650. The purity of the compound was further confirmed by RP-HPLC: Rf = 21.05 min (96%; Figure S51).

3.2.22. 2-[(1-Benzylpiperidin-4-yl)methyl]-6-[(4-fluorobenzyl)oxy]-5-methoxy-2,3-dihydroinden-1-one (8m)

A solution of compound 7 (50 mg, 0.14 mmol) and K2CO3 (38 mg, 0.27 mmol) in anhydrous DMF (5 mL) was treated with 4-fluorobenzyl bromide (41 mg, 0.16 mmol), and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H2O, and extracted with EtOAc (3 ×). The combined organic layers were washed with H2O (3 ×) and brine (3 ×), dried over anhydrous MgSO4, and filtered. After standing at RT overnight, white solids precipitated out, which were filtered off. The filtrate was further concentrated under reduced pressure and purified by column chromatography (SiO2 gel, pure CH2Cl2 to CH2Cl2:MeOH/19:1; Rf 0.31 in CH2Cl2:MeOH/19:1) to yield compound 8m (56 mg, 86%) as an off-white solid: 1H-NMR (400 MHz, CDCl3, Figure S52) δ 7.40 (dd, J1 = 8.8 Hz, J2 = 5.6 Hz, 2H, aromatic), 7.30 (m, 4H, aromatic), 7.24 (s, 1H, aromatic), 7.18 (s, 1H, aromatic), 7.04 (t, J = 8.8 Hz, 2H, aromatic), 6.85 (s, 1H, aromatic), 5.08 (s, 2H, OCH2Ph), 3.93 (3H, OCH3), 3.51 (s, 2H, NCH2Ph), 3.21 (dd, J1 = 17.6 Hz, J2 = 8.4 Hz, 1H), 2.90 (m, 2H), 2.67 (dt, J1 = 14.0 Hz, J2 = 3.6 Hz, 2H), 1.98 (m, 2H), 1.88 (m, 1H), 1.69 (m, 2H), 1.49 (m, 1H), 1.36–1.23 (m, 3H), 13C-NMR (100 MHz, CDCl3, Figure S53) δ 207.6 (C=O), 163.8–161.3 (d, J1C,F = 245.2 Hz, C, one carbon), 159.9 (C), 149.1 (C), 148.3 (C), 138.1 (C), 132.14–132.11 (d, J1C,F = 3.8 Hz, C, one carbon), 129.4–129.27 (d, J1C,F = 8.4 Hz, CH, two carbons), 129.28 (CH, two carbons), 129.2 (CH), 128.2 (CH, two carbons), 127.0 (C), 115.6–115.4 (d, J1C,F = 21.2 Hz, CH, two carbons), 107.7 (CH), 106.4 (CH), 70.1 (CH2), 63.3 (CH2), 56.2 (CH3), 53.70 (CH2), 53.68 (CH2), 45.4 (CH), 38.7 (CH2), 34.3 (CH2), 33.3 (CH2), 32.8 (CH2), 31.7 (CH); m/z calcd. for C30H33FNO3+: [M + H]+ 474.2439; found 474.2442. The purity of the compound was further confirmed by RP-HPLC: Rf = 20.14 min (96%; Figure S54).
3.2.23. 2-[(1-Benzylpiperidin-4-yl)methyl]-6-[(3-fluorobenzyl)oxy-5-methoxy-2,3-dihydroinden-1-one (8n)

A solution of compound 7 (50 mg, 0.14 mmol) and K$_2$CO$_3$ (38 mg, 0.27 mmol) in anhydrous DMF (5 mL) was treated with 3-fluorobenzyl bromide (20 µL, 0.16 mmol), and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H$_2$O, and extracted with EtOAc (3×). The combined organic layers were washed with H$_2$O (3×) and brine (3×), dried over anhydrous MgSO$_4$, and filtered. After standing at RT overnight, the white solids precipitated out, which were filtered off. The filtrate was further concentrated under reduced pressure, and purified by column chromatography (SiO$_2$ gel, pure CH$_2$Cl$_2$ to CH$_2$Cl$_2$:MeOH/19:1; R$_f$ 0.31 in CH$_2$Cl$_2$:MeOH/19:1) to yield compound 8n (57 mg, 88%) as an off-white solid: $^1$H-NMR (400 MHz, CDCl$_3$, Figure S55) δ 7.34–7.26 (m, 5H, aromatic), 7.24 (s, 1H, aromatic), 7.18–7.12 (m, 3H, aromatic), 6.98 (td, $J$ = 8.4 Hz, $J_2$ = 2.4 Hz, 1H, aromatic), 6.86 (s, 1H, aromatic), 5.12 (s, 2H, OCH$_2$Ph), 3.95 (s, 3H, OCH$_3$), 3.54 (s, 2H, NCH$_2$Ph), 3.21 (dd, $J_1$ = 17.6 Hz, $J_2$ = 8.0 Hz, 1H), 2.92 (m, 2H), 2.67 (dt, $J_1$ = 14.4 Hz, $J_2$ = 4.4 Hz, 2H), 2.0 (m, 2H), 1.87 (m, 1H), 1.73–1.66 (m, 2H), 1.50 (m, 1H), 1.40–1.26 (m, 3H); $^{13}$C-NMR (100 MHz, CDCl$_3$, Figure S56) δ 207.6 (C=O), 164.2–161.8 (d, $^1$J$_{CF}$ = 245.2 Hz, C, 1 carbon), 159.5 (C), 149.2 (C), 148.2 (C), 139.0–138.9 (d, $^1$J$_{CF}$ = 7.6 Hz, C, one carbon), 137.4 (C), 130.2–130.1 (d, $^1$J$_{CF}$ = 7.6 Hz, CH, one carbon), 129.4 (CH, two carbons), 129.1 (CH), 128.2 (CH, two carbons), 127.2 (C), 122.7–122.6 (d, $^2$J$_{CF}$ = 3.1 Hz, CH, one carbon), 115.0–114.8 (d, $^2$J$_{CF}$ = 20.5 Hz, CH, one carbon), 114.2–114.0 (d, $^2$J$_{CF}$ = 22.0 Hz, CH, one carbon), 107.7 (CH), 106.4 (CH), 69.9 (CH$_2$), 63.1 (CH$_2$), 56.2 (CH$_3$), 53.6 (CH$_2$), 45.3 (CH), 38.6 (CH$_2$), 34.2 (CH$_2$), 33.4 (CH$_2$), 32.5 (CH$_2$), 31.5 (CH); m/z calcd. for C$_{30}$H$_{33}$FNO$_3$ $^+$ [M + H]$^+$ 474.2439; found 474.2426. The purity of the compound was further confirmed by RP-HPLC: R$_t$ = 20.20 min (96%; Figure S57).

3.2.24. 2-[(1-Benzylpiperidin-4-yl)methyl]-6-[(2-fluorobenzyl)oxy-5-methoxy-2,3-dihydroinden-1-one (8o)

A solution of compound 7 (50 mg, 0.14 mmol) and K$_2$CO$_3$ (38 mg, 0.27 mmol) in anhydrous DMF (5 mL) was treated with 2-fluorobenzyl bromide (20 µL, 0.16 mmol), and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H$_2$O, and extracted with EtOAc (3×). The combined organic layers were washed with H$_2$O (3×) and brine (3×), dried over anhydrous MgSO$_4$, and filtered. After standing at RT overnight, the white solids precipitated out, which were filtered off. The filtrate was further concentrated under reduced pressure, and purified by column chromatography (SiO$_2$ gel, pure CH$_2$Cl$_2$ to CH$_2$Cl$_2$:MeOH/19:1; R$_f$ 0.31 in CH$_2$Cl$_2$:MeOH/19:1) to yield compound 8o (56 mg, 86%) as an off-white solid: $^1$H-NMR (400 MHz, CDCl$_3$, Figure S58) δ 7.49 (t, $J$ = 7.6 Hz, 1H, aromatic), 7.31–7.25 (m, 5H, aromatic), 7.24 (s, 1H, aromatic), 7.23 (d, $J$ = 7.6 Hz, 1H, aromatic), 7.12 (t, $J$ = 7.6 Hz, 1H, aromatic), 7.06 (t, $J$ = 8.4 Hz, 1H, aromatic), 6.85 (s, 1H, aromatic), 5.18 (s, 2H, OCH$_2$Ph), 3.93 (s, 3H, OCH$_3$), 3.52 (s, 2H, NCH$_2$Ph), 3.21 (dd, $J_1$ = 17.6 Hz, $J_2$ = 8.0 Hz, 1H), 2.90 (m, 2H), 2.67 (m, 2H), 1.98 (m, 2H), 1.88 (m, 1H), 1.73–1.65 (m, 2H), 1.49 (m, 1H), 1.36–1.23 (m, 3H); $^{13}$C-NMR (100 MHz, CDCl$_3$, Figure S59) δ 207.6 (C=O), 161.7–159.2 (d, $^1$J$_{CF}$ = 245.9 Hz, C, one carbon), 156.0 (C), 149.2 (C), 148.3 (C), 129.8 (CH), 129.7 (CH), 129.57–129.53 (d, $^3$J$_{CF}$ = 3.8 Hz, CH, one carbon), 129.3 (C, two carbons), 129.2 (CH), 128.2 (CH, two carbons), 127.1 (C), 124.23–124.20 (d, $^3$J$_{CF}$ = 3.8 Hz, CH, one carbon), 123.66–123.51 (d, $^3$J$_{CF}$ = 14.4 Hz, CH, one carbon), 115.5–115.3 (d, $^3$J$_{CF}$ = 20.5 Hz, CH, one carbon), 107.7 (CH), 106.6 (CH), 64.80–64.75 (d, $^3$J$_{CF}$ = 4.5 Hz, CH$_2$, one carbon), 63.2 (CH$_2$), 56.2 (CH$_3$), 53.6 (CH$_2$, two carbons), 45.4 (CH), 38.6 (CH$_2$), 34.3 (CH$_2$), 33.4 (CH$_2$), 32.7 (CH$_2$), 31.6 (CH); m/z calcd. for C$_{30}$H$_{33}$FNO$_3$ $^+$ [M + H]$^+$ 474.2439; found 474.2429. The purity of the compound was further confirmed by RP-HPLC: R$_t$ = 20.06 min (96%; Figure S60).

3.2.25. 2-[(1-Benzylpiperidin-4-yl)methyl]-6-[(2-trifluoromethylbenzyl)oxy-5-methoxy-2,3-dihydroinden-1-one (8p)

A solution of compound 7 (50 mg, 0.14 mmol) and K$_2$CO$_3$ (38 mg, 0.27 mmol) in anhydrous DMF (5 mL) was treated with 2-trifluoromethylbenzyl bromide (25 µL, 0.16 mmol) and the resulting mixture
was stirred at RT overnight. The reaction mixture was then diluted with H$_2$O, and extracted with EtOAc (3×). The combined organic layers were washed with H$_2$O (3×) and brine (3×), dried over anhydrous MgSO$_4$, and filtered. The filtrate was concentrated under reduced pressure, and purified by column chromatography (SiO$_2$ gel, pure CH$_2$Cl$_2$ to CH$_2$Cl$_2$:MeOH/19:1; R$_f$ 0.41 in CH$_2$Cl$_2$:MeOH/19:1) to yield compound 8p (65 mg, 90%) as an off-white solid: $^1$H-NMR (400 MHz, CDCl$_3$, Figure S61) δ 7.75 (d, $J = 7.6$ Hz, 1H, aromatic), 7.67 (d, $J = 8.0$ Hz, 1H, aromatic), 7.54 (t, $J = 8.0$ Hz, 1H, aromatic), 7.39 (t, $J = 7.6$ Hz, 1H, aromatic), 7.31–7.27 (m, 4H, aromatic), 7.24 (m, 1H, aromatic), 7.18 (s, 1H, aromatic), 6.88 (s, 1H, aromatic), 5.31 (s, 2H, OCH$_2$Ph), 3.96 (s, 3H, OCH$_3$), 3.51 (s, 2H, NCH$_2$Ph), 3.22 (dd, $J_1 = 17.6$ Hz, $J_2 = 8.0$ Hz, 1H), 2.90 (m, 2H), 2.68 (dt, $J_1 = 14.4$ Hz, $J_2 = 3.2$ Hz, 2H), 1.97 (m, 2H), 1.88 (m, 1H), 1.69 (m, 2H), 1.50 (m, 1H), 1.40–1.24 (m, 3H); 13C-NMR (100 MHz, CDCl$_3$, Figure S62) δ 207.6, 156.0, 149.4, 148.2, 138.1, 135.0, 135.0, 132.1, 129.3 (two carbons), 129.2, 128.4, 128.2 (two carbons), 127.8, 127.5, 127.2, 127.0, 126.01, 125.95, 125.8, 125.6, 125.6, 122.9, 107.8, 106.6, 67.04, 67.01, 63.3, 56.2, 53.71, 53.68, 45.4, 38.7, 34.3, 33.4, 32.8, 31.7; m/z calcd. for C$_{31}$H$_{33}$F$_3$NO$_3^+$ [M + H]$^+$ 524.2407; found 524.2401. The purity of the compound was further confirmed by RP-HPLC: R$_t$ = 20.90 min (96%; Figure S63).

3.2.26. 2-[(1-Benzylpiperidin-4-yl)methyl]-6-[2,4-difluorobenzoyl]oxy-5-methoxy-2,3-dihydroinden-1-one (8q)

A solution of compound 7 (50 mg, 0.14 mmol) and K$_2$CO$_3$ (38 mg, 0.27 mmol) in anhydrous DMF (5 mL) was treated with 2,4-difluorobenzyl bromide (21 µL, 0.16 mmol) and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H$_2$O, and extracted with EtOAc (3×). The combined organic layers were washed with H$_2$O (3×) and brine (3×), dried over anhydrous MgSO$_4$, and filtered. The filtrate was concentrated under reduced pressure, and purified by column chromatography (SiO$_2$ gel, pure CH$_2$Cl$_2$ to CH$_2$Cl$_2$:MeOH/19:1; R$_f$ 0.17 in CH$_2$Cl$_2$:MeOH/19:1) to yield compound 8q (57 mg, 85%) as an off-white solid: $^1$H-NMR (400 MHz, CDCl$_3$, Figure S64) δ 7.46 (dd, $J_1 = 14.8$ Hz, $J_2 = 8.4$ Hz, 1H, aromatic), 7.31–7.27 (m, 4H, aromatic), 7.24 (s, 1H, aromatic), 7.22 (s, 1H, aromatic), 6.88–6.79 (m, 3H, aromatic), 5.12 (s, 2H, OCH$_2$Ph), 3.93 (s, 3H, OCH$_3$), 3.52 (s, 2H, NCH$_2$Ph), 3.22 (dd, $J_1 = 17.6$ Hz, $J_2 = 8.0$ Hz, 1H), 2.90 (m, 2H), 2.67 (dt, $J_1 = 14.4$ Hz, $J_2 = 3.2$ Hz, 2H), 1.98 (m, 2H), 1.89 (m, 1H), 1.69 (m, 2H), 1.49 (m, 1H), 1.40–1.26 (m, 3H); 13C-NMR (100 MHz, CDCl$_3$, Figure S65) δ 207.6, 164.0, 164.0, 161.9, 161.8, 161.7, 161.6, 159.4, 159.3, 156.0, 149.3, 148.2, 137.9, 130.81, 130.75, 130.72, 130.66, 129.3 (two carbons), 129.2, 128.2 (two carbons), 127.0, 119.7, 119.6, 119.54, 119.50, 111.55, 111.51, 111.34, 111.30, 107.7, 106.6, 104.2, 103.9, 103.7, 64.34, 64.30, 63.3, 56.2, 53.7, 53.6, 45.4, 38.6, 34.3, 33.4, 32.8, 31.6; m/z calcd. for C$_{30}$H$_{32}$F$_2$NO$_3^+$ [M + H]$^+$ 492.2345; found 492.2353. The purity of the compound was further confirmed by RP-HPLC: R$_t$ = 20.25 min (95%; Figure S66).

3.2.27. 2-[(1-Benzylpiperidin-4-yl)methyl]-6-[(2,5-difluorobenzoyl]oxy-5-methoxy-2,3-dihydroinden-1-one (8r)

A solution of compound 7 (50 mg, 0.14 mmol) and K$_2$CO$_3$ (38 mg, 0.27 mmol) in anhydrous DMF (5 mL) was treated with 2,5-difluorobenzyl bromide (21 µL, 0.16 mmol), and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H$_2$O, and extracted with EtOAc (3×). The combined organic layers were washed with H$_2$O (3×) and brine (3×), dried over anhydrous MgSO$_4$, and filtered. The filtrate was concentrated under reduced pressure and purified by column chromatography (SiO$_2$ gel, pure CH$_2$Cl$_2$ to CH$_2$Cl$_2$:MeOH/19:1; R$_f$ 0.17 in CH$_2$Cl$_2$:MeOH/19:1) to yield compound 8r (67 mg, 85%) as an off-white solid: $^1$H-NMR (400 MHz, CDCl$_3$, Figure S67) δ 7.32–7.28 (m, 4H, aromatic), 7.24 (m, 2H, aromatic), 7.19 (s, 1H, aromatic), 7.02 (td, $J_1 = 8.8$ Hz, $J_2 = 4.0$ Hz, 1H, aromatic), 6.97–6.91 (m, 1H, aromatic), 6.87 (s, 1H, aromatic), 5.16 (s, 2H, OCH$_2$Ph), 3.95 (s, 3H, OCH$_3$), 3.54 (s, 2H, NCH$_2$Ph), 3.22 (dd, $J_1 = 17.6$ Hz, $J_2 = 8.0$ Hz, 1H), 2.92 (m, 2H), 2.68 (dt, $J_1 = 14.0$ Hz, $J_2 = 3.6$ Hz, 2H), 2.05 (m, 2H), 1.88 (m, 1H), 1.70 (m, 2H), 1.51 (m, 1H), 1.40–1.24 (m, 3H); 13C-NMR (100 MHz, CDCl$_3$, Figure S68) δ 207.5, 160.02, 160.00, 157.62, 157.60, 157.21, 157.19, 155.9, 154.79, 154.77, 149.4, 148.0, 137.7, 129.4 (two carbons), 129.2, 128.2 (two carbons), 127.1, 125.6, 125.5, 125.4, 125.3, 116.6,
116.5, 116.3, 116.2, 116.0, 115.9, 115.8, 115.7, 115.6, 115.43, 115.38, 107.8, 106.5, 64.2, 64.1, 63.2, 62.6, 56.2, 55.6, 53.60, 53.57, 45.3, 38.6, 34.2, 32.4, 31.6; m/z calcd. for C$_{30}$H$_{32}$F$_2$NO$_3$$^+$ [M + H]$^+$ 492.2345; found 492.2350. Purity of the compound was further confirmed by RP-HPLC: R$_t$ = 20.28 min (96%); Figure S69).

3.2.28. 2-[(1-Benzylpiperidin-4-yl)methyl]-6-[(2,6-difluorobenzyl)oxy]-5-methoxy-2,3-dihydroinden-1-one (8s)

A solution of compound 7 (50 mg, 0.14 mmol) and K$_2$CO$_3$ (38 mg, 0.27 mmol) in anhydrous DMF (5 mL) was treated with 2,6-difluorobenzyl bromide (34 mg, 0.16 mmol), and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H$_2$O, and extracted with EtOAc (3×). The combined organic layers were washed with H$_2$O (3×) and brine (3×), dried over anhydrous MgSO$_4$, and filtered. The filtrate was concentrated under reduced pressure and purified by column chromatography (SiO$_2$ gel, pure CH$_2$Cl$_2$ to CH$_2$Cl$_2$:MeOH/19:1; R$_f$ 0.34 in CH$_2$Cl$_2$:MeOH/19:1) to yield compound 8s (62 mg, 93%) as an off-white solid: $^1$H-NMR (400 MHz, CDCl$_3$, Figure S70) δ 7.32–7.27 (m, 6H, aromatic), 7.24 (m, 1H, aromatic), 6.90 (t, $J$ = 8.0 Hz, 2H, aromatic), 6.84 (s, 1H, aromatic), 5.14 (s, 2H, OCH$_2$Ph), 3.88 (s, 3H, OCH$_3$), 3.53 (s, 2H, NCH$_2$Ph), 3.22 (dd, $J_1 = 17.6$ Hz, $J_2 = 8.0$ Hz, 1H), 2.91 (m, 2H), 2.68 (dt, $J_1 = 14.4$ Hz, $J_2 = 4.4$ Hz, 2H), 2.00 (m, 2H), 1.89 (m, 1H), 1.70 (m, 2H), 1.50 (m, 1H), 1.40–1.24 (m, 3H); 13C-NMR (100 MHz, CDCl$_3$, Figure S71) δ 207.6, 163.3, 163.2, 160.8, 160.7, 156.2, 149.5, 148.4, 137.7, 131.0, 130.9, 130.8, 129.4 (two carbons), 129.2, 128.2 (two carbons), 127.1, 112.4, 112.2, 112.0, 111.6, 111.5, 111.4, 111.3, 107.8, 107.3, 63.2, 59.19, 59.15, 59.11, 56.2, 53.61, 53.58, 45.4, 38.6, 34.2, 33.4, 32.7, 31.6; m/z calcd. for C$_{30}$H$_{32}$F$_2$NO$_3$$^+$ [M + H]$^+$ 492.2345; found 492.2352. The purity of the compound was further confirmed by RP-HPLC: R$_t$ = 19.99 min (96%); Figure S72).

3.2.29. 2-[(1-Benzylpiperidin-4-yl)methyl]-6-[(4-bromo-2-fluorobenzyl)oxy]-5-methoxy-2,3-dihydroinden-1-one (8t)

A solution of compound 7 (50 mg, 0.14 mmol) and K$_2$CO$_3$ (38 mg, 0.27 mmol) in anhydrous DMF (5 mL) was treated with 4-bromo-2-fluorobenzyl bromide (44 mg, 0.16 mmol), and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H$_2$O, and extracted with EtOAc (3×). The combined organic layers were washed with H$_2$O (3×) and brine (3×), dried over anhydrous MgSO$_4$, and filtered. The filtrate was concentrated under reduced pressure and purified by column chromatography (SiO$_2$ gel, pure CH$_2$Cl$_2$ to CH$_2$Cl$_2$:MeOH/19:1; R$_f$ 0.56 in CH$_2$Cl$_2$:MeOH/19:1) to yield compound 8t (69 mg, 91%) as an off-white solid: $^1$H-NMR (400 MHz, CDCl$_3$, Figure S73) δ 7.37 (t, $J = 7.6$ Hz, 1H, aromatic), 7.31–7.26 (m, 6H, aromatic), 7.24 (m, 1H, aromatic), 6.86 (s, 1H, aromatic), 5.12 (s, 2H, OCH$_2$Ph), 3.93 (s, 3H, OCH$_3$), 3.51 (s, 2H, NCH$_2$Ph), 3.22 (dd, $J_1 = 17.6$ Hz, $J_2 = 8.0$ Hz, 1H), 2.90 (m, 2H), 2.68 (dt, $J_1 = 14.4$ Hz, $J_2 = 3.2$ Hz, 2H), 1.98 (m, 2H), 1.88 (m, 1H), 1.69 (m, 2H), 1.48 (m, 1H), 1.40–1.24 (m, 3H); 13C-NMR (100 MHz, CDCl$_3$, Figure S74) δ 207.5, 161.4, 158.8, 155.9, 149.4, 148.0, 138.1, 130.63, 130.58, 129.3 (two carbons), 129.2, 128.1 (two carbons), 127.64, 127.60, 127.0, 123.0, 122.8, 122.2, 122.1, 119.2, 119.0, 107.8, 106.6, 64.3, 64.2, 63.3, 56.2, 53.70, 53.68, 45.4, 38.6, 34.3, 33.4, 32.9, 31.7; m/z calcd. for C$_{30}$H$_{32}$BrFNO$_3$$^+$ [M + H]$^+$ 552.1544; found 552.1546. The purity of the compound was further confirmed by RP-HPLC: R$_t$ = 21.25 min (96%); Figure S75).

3.2.30. 2-[(1-Benzylpiperidin-4-yl)methyl]-6-[(2,4,6-trifluorobenzyl)oxy]-5-methoxy-2,3-dihydroinden-1-one (8u)

A solution of compound 7 (50 mg, 0.14 mmol) and K$_2$CO$_3$ (38 mg, 0.27 mmol) in anhydrous DMF (5 mL) was treated with 2,4,6-trifluorobenzyl bromide (22 µL, 0.16 mmol) and the resulting mixture was stirred at RT overnight. The reaction mixture was then diluted with H$_2$O, and extracted with EtOAc (3×). The combined organic layers were washed with H$_2$O (3×) and brine (3×), dried over anhydrous MgSO$_4$, and filtered. After standing at RT overnight, white solids precipitated out, which were filtered off. The filtrate was further concentrated under reduced pressure and purified by column chromatography (SiO$_2$ gel, pure CH$_2$Cl$_2$ to CH$_2$Cl$_2$:MeOH/19:1; R$_f$ 0.31 in CH$_2$Cl$_2$:MeOH/19:1) to yield compound 8u (65 mg, 93%) as an off-white solid: $^1$H-NMR (400 MHz, CDCl$_3$, Figure S76)
\[ \delta 7.32-7.29 \text{ (m, 5H, aromatic)}, 7.24 \text{ (s, 1H, aromatic)}, 6.84 \text{ (s, 1H, aromatic)}, 6.68 \text{ (t, } J = 8.4 \text{ Hz, 2H, aromatic)}, 5.08 \text{ (s, 2H, OCH}_2\text{Ph)}, 5.08 \text{ (s, 3H, OCH}_3\text{)}, 3.54 \text{ (s, 2H, NH}_2\text{Ph)}, 3.22 \text{ (dd, } J_1 = 17.6 \text{ Hz, } J_2 = 8.0 \text{ Hz, 1H)}, 2.92 \text{ (m, 2H)}, 2.68 \text{ (dt, } J_1 = 14.4 \text{ Hz, } J_2 = 3.2 \text{ Hz, 2H)}, 2.02 \text{ (m, 2H)}, 1.89 \text{ (m, 1H)}, 1.70 \text{ (m, 2H)}, 1.51 \text{ (m, 1H)}, 1.40–1.26 \text{ (m, 3H)}; 13\text{C-NMR (100 MHz, CDCl}_3\text{, Figure S77)} \delta 207.6, 164.6, 164.5, 164.3, 163.7, 163.6, 163.5, 163.4, 162.1, 162.0, 161.8, 161.1, 161.04, 160.9, 152.2, 149.6, 148.2, 137.7, 129.4 (two carbons), 129.2, 128.2 (two carbons), 127.1, 108.92, 108.87, 108.72, 108.67, 108.53, 108.48, 107.9, 107.4, 100.69, 100.67, 100.5, 100.44, 100.42, 100.39, 100.35, 100.2, 100.1, 63.2, 58.80, 58.76, 58.7, 56.2, 53.63, 53.59, 45.4, 38.6, 34.2, 33.4, 32.7, 31.6; m/z calcld. for C\text{30}H\text{31}F\text{5}NO\text{3}\text{+} [M + H]\text{+} 510.2251; found 510.2255. The purity of the compound was further confirmed by RP-HPLC: Rt = 20.30 min (95%; Figure S78).

3.3. In Vitro Cholinesterase (ChE) Inhibition Assays

Experiments were performed as previously described [11,13]. Briefly, donepezil analogues (102 pm to 200 μM) were dissolved in sodium phosphate buffer ((100 μL), 0.1 M, pH 8.0) (Buffer A) and subjected to a 5-fold serial dilution. ChE (either EfAChE or EfBChE) was added to the solution of inhibitors (50 μL, containing 0.08 U/mL ChE (final concentration for both EfAChE and EfBChE) in Buffer A. The mixture of inhibitor and enzyme was incubated for 10 min before initiation with DTNB (50 μL, 0.25 mM final concentration) and acetylthiocholine (acyethylthiocholine for EfAChE and butryrylthiocholine for EfBChE) (0.5 mM final concentration) in phosphate buffer. The reaction was monitored at 412 nm taking measurements every 30 s for 10 min using a Spectra Max M5 plate reader (Molecular Devices, San Jose, CA, USA) at 25 °C. Data was corrected with the negative control (no acetylcholine), and normalized to the positive control (no inhibitor) using the initial rates (first 5 min). All assays were performed in duplicate or triplicate. HsAChE was treated in the same manner with the following exceptions: the final concentration of HsAChE was 0.16 μg/mL (0.16 U/mL), and reactions were performed at 37 °C. The data was fitted to a sigmoidal curve, and IC\text{50} values were calculated using Sigmaplot 14.0 (Systat Software, San Jose, CA, USA). The IC\text{50} curves for EfAChE and EfBChE inhibition are presented in Table 1 and Figures S82 and S83 (for EfAChE) and Figures S84 and S85 (for EfBChE). The IC\text{50} curves for HsAChE inhibition are presented in Table 2 and Figure S86.
3.4. BACE1 Inhibition

Inhibition of BACE1 was tested using the commercial kit (cat CS0010-1KT, Millipore-Sigma, St. Louis, MO, USA) following the directions accompanying the kit. All compounds were tested in duplicate at a single concentration (200 µM) in order to confirm any activity. All compounds that showed BACE1 inhibitory activity were then tested in a concentration-dependent manner. Dilutions were originally performed in DMSO, and 2 µL added to the reaction in order to account for any moderation of activity from the vehicle. Fluorescent measurements were taken after 2 h. The resulting rates were normalized to the reaction without inhibitor. In order to get an appropriate sigmoidal fit, two additional points (400 and 1000 µM) were added to the data when needed. Since the activity of the enzyme was already negligible at 200 µM, these points aid the sigmoidal nature of the curve fit. These data are presented in Table 3 and Figure S87.

3.5. Molecular Docking of Donepezil and Compound 8l with BACE1

To further validate the biochemical results obtained against BACE1, we modeled donepezil and compound 8l using a known crystal structure of BACE1 with an inhibitor, sharing the vicinyl dioxygen substitution of donepezil as a model (PDB# 4FM7 [25]). Swiss Dock [29,30] was used to identify the potential binding sites of donepezil or compound 8l with the crystal structure. Once docking calculations were completed, Chimera [31] was used to compare the potential binding sites with that of the known inhibitors. The closest alignments were selected, and they are presented in Figure 1.

4. Conclusions

We have synthesized 22 new donepezil analogues, 8a–v, and evaluated their biochemical capabilities, along with that of the parent donepezil and its 6-O-desmethyl adduct 7. Without exception, these compounds were all able to inhibit the action of EeAChE and EfBChE in the low-to-sub-micromolar ranges. Compound 8t, one of the better inhibitors of EeAChE and EfBChE was also a very efficient inhibitor of HsAChE showing the highest preference for this medically relevant enzyme. Attachment of an alkyl/aromatic group at the 6-O-position of the indanone ring also seems to enhance their efficacy. While their inhibitory capabilities were greater against EeAChE than EfBChE, the donepezil analogues 8h–v with aromatic substituents displayed a much improved potency when compared to donepezil against EfBChE than EeAChE. The analogues 8a–g with alkyl substituents showed proportional change with respect to donepezil against both EeAChE and EfBChE. The donepezil analogues 8c, 8e, 8f, and 8l also displayed potent BACE1 inhibitory activities, and thus appeared to be multifunctional compounds for the treatment of Alzheimer’s disease.

Supplementary Materials: The Supplementary Materials include 1H and 13C-NMR spectra for the molecules synthesized, as well as HPLC traces of compounds tested for activity (Figures S1–S81). The IC50 curves for the inhibition of EeAChE, HsAChE, EfBChE, and BACE1 are also provided (Figures S82–S87). The SwissDock modeling is also provided (Figure S88). These materials are available free of charge via the internet.

Author Contributions: M.Y.F. synthesized all the compounds and conducted the EeAChE and EfBChE inhibition assays; K.D.G. performed the HsAChE and BACE1 assays; K.D.G., M.Y.F., and S.G.-T. analyzed the data and wrote the paper.

Funding: This work was supported by startup funds (to S.G.-T.) from the College of Pharmacy at the University of Kentucky. Molecular graphics and analyses were performed with UCSF Chimera, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from NIH P41-GM103311.

Conflicts of Interest: The authors declare no conflict of interest.
Abbreviations

Aβ  amyloid-β
APP  amyloid precursor protein
BACE  β-secretase
ChE  cholinesterase
EeAChE  acetylcholinesterase (from Electrophorus electricus)
EFBChE  butyrylcholinesterase (from Equus ferus)
HsAChE  acetylcholinesterase (from Homo sapiens)
IC50  half maximal inhibitory concentration
KOH  potassium hydroxide
MsOH  methanesulfonic acid
TBDMS  tert-butyldimethylsilyl

References


**Sample Availability:** Samples of the compounds synthesized are available from the authors.