Design, Synthesis, Biological Properties, and Molecular Modeling Investigations of Novel Tacrine Derivatives with a Combination of Acetylcholinesterase Inhibition and Cannabinoid CB₁ Receptor Antagonism


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Pyrazolines 7–10 were designed as novel CB₁ receptor antagonists, which exhibited improved turbidometric aqueous solubilities. On the basis of their extended CB₁ antagonist pharmacophore, hybrid molecules exhibiting cannabinoid CB₁ receptor antagonistic as well as acetylcholinesterase (AChE) inhibiting activities were designed. The target compounds 12, 13, 20, and 21 are based on 1 (tacrine) as the AChE inhibitor (AChEI) pharmacophore and two different CB₁ antagonist pharmacophores. The imidazole-based 20 showed high CB₁ receptor affinity (48 nM) in combination with high CB₁/CB₂ receptor subtype selectivity (>20-fold) and elicited equipotent AChE inhibitory activity as 1. Molecular modeling studies revealed the presence of a binding pocket in the AChE enzyme which nicely accommodates the CB₁ pharmacophores of the target compounds 12, 13, 20, and 21.

Introduction

Alzheimer’s disease (AD)² is a neurodegenerative disorder whose prevalence is increasing together with the life expectancy throughout the world. Cholinergic-enhancing drugs are the main current therapy. The AChEI was the first synthetic drug approved by the U.S. Food and Drug Administration for the treatment of AD (Figure 1). The tricyclic 1 is a reversible inhibitor that reduced the inactivation of acetylcholine (ACh).²

AChEIs have shown efficacy not only in AD but also in other cognitive disorders such as dementia with Lewy bodies,³ Parkinson’s disease, vascular dementia,⁶ traumatic brain injury,⁸ and cognitive impairment in multiple sclerosis.⁹ Cognitive disorders constitute also a potential therapeutic area for cannabinoid CB₁ receptor antagonists,¹⁰ since CB₁ receptor antagonists were shown to increase ACh release in certain brain areas including the cortical region and hippocampus.¹¹ On the basis of the observations that CB₁ receptor antagonists as well as AChEIs improved performance in a variety of animal memory models, Wise et al. found¹² that combined administration of subthreshold doses of the CB₁ receptor antagonist rimonabant and the AChEI donepezil, which had no discernible effects on performance when given alone, enhanced memory.

It was also found¹³ that AChEIs acting on the brain suppressed both cocaine- and morphine-induced conditioned place preference and blocked the induction and persistence of cocaine-evoked hyperlocomotion. AChEIs can thus be approached as novel and potential therapeutic agents for drug addiction. Cannabinoid CB₁ receptor antagonists have also been associated with the treatment of drug addiction and addictive behavior.

The multifactorial and complex etiology of AD has hitherto prompted several so-called “single entity—multitarget ligand” approaches,¹⁵ such as combined AChEI-histamine H3 receptor antagonists,¹⁶ dual binding site AChEIs,¹⁷ NO-donor-AChEI hybrids,¹⁸ tacrine–dihydropyridine hybrids,¹⁹ tacrine–melatonin hybrids,²⁰ dual inhibitors of monoamine oxidase and AChE,²¹ dual AChE/serotonin transporter inhibitors,²² tacrine–ferulic acid hybrids,²³ and benzofuran-based hybrid compounds.²⁴

Design

Molecular design which was based on our previous work on pyrazoline-based CB₁ receptor antagonists,²⁵,²⁶ such as 2 revealed that substitution of its amidine N-methyl group by sterically more demanding substituents would lead to compounds with retained CB₁ receptor antagonistic properties. First, this basic hypothesis is validated by the design of the target compounds 5–10 which contain either a polar group or an ionizable nitrogen atom in their amidine N substituent in order to produce novel CB₁ receptor antagonists with improved aqueous solubilities.

Second, the impact of both AChE inhibition and CB₁ receptor antagonism on cognitive deficits and drug addiction prompted the design of dual acting AChEIs/CB₁ receptor antagonists. Although the general concept of designing dual acting compounds based on 1 is not new as has been outlined above, our approach to dual acting compounds by combining the CB₁ antagonist pharmacophore with 1 is unprecedented and is of great importance because of the reported beneficial
roles of CB₁ receptor antagonists as well as AChEIs in cognitive impairment.

In our design strategy, two molecular entities (interacting with high selectivity either to the CB₁ receptor or to the AChE enzyme) were applied as the starting points, thereby combining their key structural elements to incorporate activity at both targets into a single molecule. The 3,4-diarylpyrazoline²⁵ ₂ and 1,2-diarylimidazole²⁷,²⁸ ₃ were selected as suitable CB₁ antagonistic structural elements²⁵ for this study (Figure 1), whereas ₁ was preferred as a compact AChEI moiety. It was envisioned that connecting the CB₁ antagonistic units ₂ and ₃, respectively, to ₁ by an alkylene spacer of suitable length would result in dual acting AChEIs/CB₁ receptor antagonists. The choice of ₂ and ₃ enabled us to investigate the effect of the difference in sprouting of the tail connecting the tacrine moiety.

In the course of our investigations, researchers from Esteve patented³⁰ compound ₄ and some derivatives with different alkylene spacer lengths as dual acting CB₁ receptor antagonists—μ-opioid receptor modulators. Compound ₄ was claimed by Esteve to have moderate CB₁ receptor affinity (₉ᵢ = 701 nM) and μ-opioid receptor affinity (₉ᵢ = 6543 nM). It is interesting to note that ₄ showed in vivo activities after intraperitoneal administration in CB₁ receptor as well as opioid receptor mediated models despite these observed moderate receptor affinities and its high molecular weight of 737. The reported³¹ synergistic effect between CB₁ and μ-opioid receptor modulation may have a positive impact on the observed in vivo activities of ₄.

It was also envisioned that the tacrine moiety could be amenable for derivatization with preservation of AChE inhibiting ability, based on the reported structure of tacrine derivative ₁₇ with the AChE enzyme³³ (Protein Data Bank (PDB) code 1UT6). The alkylene chain of ₁₇ points toward the outer surface into an unoccupied region. The orientation of the tacrine skeleton is the same³³ as that in the structure of AChE with ₁ based on X-ray diffraction data (PDB code 1ACJ). These observations corroborated our design hypothesis toward dual AChE/CB₁ receptor antagonists.

Results and Discussion

The CB₁ receptor has a highly lipophilic binding site, and consequently CB₁ receptor agonists as well as antagonists generally are lipophilic entities which in general exhibit low aqueous solubilities. A poor water solubility may lead to practical difficulties in pharmacological testing and may necessitate the use of solubilizing agents and complex compound formulations. The compounds ₅₋₁₀ were designed with the goal to produce CB₁ receptor antagonists with improved aqueous solubilities, due to the presence of either a polar group (₅ and ₆) or an ionizable nitrogen atom in their carboxamidine side chain (₇₋₁₀).

Compounds ₅₋₁₀ were synthesized in reasonable yields, ranging from 39% to 65%, by chlorinating the key intermediate²⁵ ₁₁ with PC₁₅₃ in chlorobenzene, followed by treatment with various amines (Scheme 1). Compound ₇ was subsequently converted to the corresponding hydrochloric acid salt by treatment with 1 M HCl in ethanol.

The pharmacological results of the target compounds ₅₋₁₀ and the reference compound ₂ are given in Table 1. The CB₁ receptor binding data of ₅₋₁₀ corroborated our hypothesis that the replacement of the amidine N-methyl group by larger and more polar groups leads to retained CB₁ receptor affinities. Compound ₇ with the N,N-dimethylaminopropyl tail showed the highest CB₁ receptor affinity (3.1 nM) in this series. In general, the compounds ₅₋₁₀ elicited high CB₁/CB₂ receptor selectivities, which is in line with the results from the reference compound ₂. It is interesting to note that the compounds ₇₋₁₀, which all have an ionizable nitrogen atom incorporated in their side chain, have approximately 10-fold lower CB₁ antagonistic potencies compared with the compounds ₂ and ₅, ₆ which are devoid of the presence of such an ionizable nitrogen atom. According to expectation, the compounds ₇₋₁₀ exhibited improved aqueous solubilities as compared to ₂ and ₅, ₆, in particular under acidic conditions (Table 1).

Our AChE/CB₁ receptor antagonist combination design considerations led to the synthesis of the series based on ₂, targeting compounds ₁₂ and ₁₃ according to Scheme 2.

![Chemical structures of the AChEI 1, CB1 receptor antagonists 2 and 3, and Esteve’s dual acting CB1 receptor antagonist—μ-opioid receptor modulator 4.](image-url)
The chlorination reaction to convert 11 into its chloromimidoyl derivative 14 was carried out under milder reaction conditions (using a phosphorus oxychloride (POCl3)/4-dimethylaminopyridine (DMAP) combination) compared to the synthesis of 5–10. Subsequent reaction with the tacrine–alkylene spacer derivatives14 15 and 16 in the presence of Hünig’s base furnished the target compounds 12 and 13 in 36% and 35% yield, respectively.

The synthesis of the 3-based series, aiming at 20 and 21, started with the hydrolysis of the ethyl ester precursor27 28 18. Hydrolysis with LiOH in a water/THF mixture led to the corresponding carboxylic acid 19 in 84% yield. Subsequent amidation with the tacrine building blocks 15 and 16 in the presence of suitable coupling reagents led to the formation of the target compounds 20 and 21 in 53% and 62% yield, respectively (Scheme 3).

The pharmacological results of the target compounds 12, 13, 20, and 21, the reference compounds 1–3, and the known CB1 receptor antagonist rimonabant35 are given in Table 2.

According to our expections, 1 was found inactive as CB1 receptor ligand but active in the AChEI assay. In addition, both rimonabant and 3 are potent CB1 receptor antagonists without significant AChE inhibiting properties. The target compounds 12, 13, 20, and 21 all showed significant CB1 receptor affinities and in general acted as CB1 receptor antagonists. The pyrazoline derivative 12 showed moderate AChE inhibiting properties, but compound 13 with a longer spacer length elicited a higher pIC50 value.

The imidazoles 20 and 21 showed also AChE inhibiting properties but in a reverse order. Compound 20 with the shorter spacer length is the more potent compound in this series, having AChE inhibitory activities comparable to those of the AChEI reference compound 1.

In order to explain these observations, molecular modeling studies were performed on both the CB1 receptor and the AChE enzyme. Several CB1 receptor homology models have been reported in the literature.25 26

In the majority of these models the loop regions spanning the helices of the G-protein-coupled receptor (GPCR) have been omitted. We have rebuilt our model using the rigid template of the recently reported human β2-adrenergic structure and taking the extracellular loop 2 into account, including the cysteine bridge which is presumed to be present in the lipids GPCR cluster. After docking of 2, all the essential features of the binding as reported earlier25 were maintained including the key interaction between Lys192 and the sulfonamide moiety of 2 and the multiple π–π stacking interactions of its pyrazoline aryl rings with a series of aromatic residues on the helices 5 and 6. The only difference with our previous model is a different orientation of the p-Cl-phenyl ring attached to the SO2 group of 2, which now points downward between the helices 3 and 6. The resulting conformation of 2

### Table 1. In Vitro Pharmacological Results of Compounds 2 and 5–10

<table>
<thead>
<tr>
<th>compd</th>
<th>$K_i$(CB1)</th>
<th>$pA_2$(CB1)</th>
<th>$K_i$(CB2)</th>
<th>aqueous solubility (μM, pH 2)</th>
<th>aqueous solubility (μM, pH 7.4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>25 ± 7</td>
<td>8.7 ± 0.3</td>
<td>&gt;1000</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>43 ± 11</td>
<td>9.0 ± 0.1</td>
<td>&gt;1000</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>31 ± 17</td>
<td>8.6 ± 0.2</td>
<td>571 ± 215</td>
<td>&lt;7</td>
<td>&lt;3</td>
</tr>
<tr>
<td>7</td>
<td>3.1 ± 0.9</td>
<td>7.8 ± 0.1</td>
<td>&gt;1000</td>
<td>38</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>19 ± 7</td>
<td>7.8 ± 0.1</td>
<td>&gt;1000</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>9</td>
<td>9.6 ± 1.4</td>
<td>7.6 ± 0.2</td>
<td>&gt;1000</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>27 ± 9</td>
<td>7.9 ± 0.2</td>
<td>&gt;1000</td>
<td>38</td>
<td>11</td>
</tr>
</tbody>
</table>

*Displacement of specific CP-55,940 binding in CHO cells stably transfected with human CB1 receptor, expressed as $K_i$ ± SEM values (nM). t[3H]Arachidonic acid release in CHO cells expressed as $pA_2$ ± SEM values. tDisplacement of specific CP-55,940 binding in CHO cells stably transfected with human CB2 receptor, expressed as $K_i$ ± SEM values (nM). The values represent the mean result based on at least three independent experiments. Turbidimetric aqueous solubility, pH 2. Estimated precipitation concentration expressed as μM. Turbidimetric aqueous solubility, pH 7.4. Estimated precipitation concentration expressed as μM.
has a higher resemblance to our earlier reported X-ray structure of the 4S-enantiomer of 2.\textsuperscript{25}

In the loop region, positioned just above the CB1 antagonist binding pocket, an additional pocket was recognized that can accommodate 1. The docking result of 12 in the homology model of the CB1 receptor is shown in Figure 2.

The protonated quinolinic nitrogen of 1 (pK\textsubscript{a} = 9.2)\textsuperscript{38} can interact with the Asp266 residue which also forms a salt bridge with Lys192. The aromatic ring of the tacrine part is postulated to stack between Phe177, Phe174, and His178. Its condensed cyclohexyl ring is enclosed by a small lipophilic pocket limited by Leu193 and Phe268. It is noted that the CB1 pharmacophore part of 12 is positioned almost exactly as in the case of 2.

The negative impact of linking a tacrine unit to the CB1 antagonist pharmacophore on the binding and activity of 12, 13, 20, and 21 is small, since they all still show CB1 receptor affinity values in the nanomolar range. The reduction in CB1 receptor affinities of the tacrine derivatives in the imidazole-based series (20 and 21) seems to be somewhat more pronounced (about 3- to 4-fold) than in the pyrazoline-based series (12 and 13) (\textsim 2-fold). This might be rationalized by the fact that in the former series one of the pharmacophoric features (the piperidinyl ring) is replaced by the linker while in the latter series the corresponding feature (the p-Cl-phenyl moiety), which is known to be important for CB1 receptor interaction, is maintained.

Table 2. In Vitro Pharmacological Results of Rimonabant, Compounds 1–3, 12–13, 20, 21

<table>
<thead>
<tr>
<th>compd</th>
<th>(K_i(\text{CB}_1))\textsuperscript{a} nM</th>
<th>p(A_2(\text{CB}_1))\textsuperscript{b}</th>
<th>(K_i(\text{CB}_2))\textsuperscript{c} nM</th>
<th>AChE inhibition (pIC\textsubscript{50})\textsuperscript{d}</th>
</tr>
</thead>
<tbody>
<tr>
<td>rimonabant·HCl</td>
<td>25 ± 15 (11.5)\textsuperscript{15}</td>
<td>8.6 ± 0.1</td>
<td>1580 ± 150 (1640)\textsuperscript{35}</td>
<td>4.6 ± 0.2</td>
</tr>
<tr>
<td>tacrine (1)</td>
<td>&gt;1000</td>
<td>nd\textsuperscript{e}</td>
<td>&gt;1000</td>
<td>6.6 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>25 ± 7</td>
<td>8.7 ± 0.3</td>
<td>&gt;1000</td>
<td>&lt; 4.5</td>
</tr>
<tr>
<td>3</td>
<td>14 ± 5</td>
<td>9.8 ± 0.2</td>
<td>&gt;1000</td>
<td>5.6 ± 0.4</td>
</tr>
<tr>
<td>12</td>
<td>42 ± 13</td>
<td>8.1 ± 0.3</td>
<td>&gt;1000</td>
<td>6.0 ± 0.3</td>
</tr>
<tr>
<td>13</td>
<td>54 ± 14</td>
<td>7.4 ± 0.2</td>
<td>&gt;1000</td>
<td>6.5 ± 0.3</td>
</tr>
<tr>
<td>20</td>
<td>48 ± 27</td>
<td>nd\textsuperscript{e}</td>
<td>&gt;1000</td>
<td>5.9 ± 0.3</td>
</tr>
<tr>
<td>21</td>
<td>88 ± 30</td>
<td>8.2\textsuperscript{f}</td>
<td>&gt;1000</td>
<td>nd = not determined.</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Displacement of specific CP-55,940 binding in CHO cells stably transfected with human CB\textsubscript{1} receptor, expressed as \(K_i\) ± SEM values (nM).

\textsuperscript{b}[\textsuperscript{3}H]Arachidonic acid release in CHO cells expressed as p\(A_2\) ± SEM values.\textsuperscript{c} Displacement of specific CP-55,940 binding in CHO cells stably transfected with human CB\textsubscript{2} receptor, expressed as \(K_i\) ± SEM values (nM). The values represent the mean result based on at least three independent experiments, unless indicated otherwise.\textsuperscript{d} AChEI; human recombinant (HEK-293 cells); photometric detection; Cerep. \textsuperscript{e}nd = not determined.\textsuperscript{f} Result based on two independent experiments (8.2 and 8.2, respectively).
The docking of 20 in AChE is depicted in Figure 3. The tacrine unit is found at the bottom of the active site gorge at exactly the same position of unlinked 1, stacking between Trp84 and Phe330 as well as bound by a hydrogen bond with the backbone carbonyl group of His440. At the outer surface, at the so-called peripheral anionic site (PAS), there is a constitution of residues that accommodate the pyrazoline-based CB1 pharmacophore surprisingly well (Figure 4).

The aromatic cluster Trp279, Phe288, Phe290, Phe330, and Phe331 can bind the aromatic rings of the CB1 antagonists by multiple π–π stacking interactions similar to the aromatic residues on helices 5 and 6 of the GPCR. The H-bonding to the sulfonamide of the 2 series and the corresponding amide of the 3 series can be realized by Glu74. The linking tail runs through the gorge following the trajectory of ACh on its way to the catalytic site.

According to the results in Table 2, the connection of the CB1 antagonist part via the n = 4 alkylene spacer to the tacrine moiety has a negative impact on the AChE inhibiting properties in the pyrazoline (2-based) series (compound 12), whereas in the imidazole (3-based) series compound 20 is equipotent compared to 1.

The distance between the anilinic nitrogen atom of 1 and the attachment nitrogen of docked 2 in the PAS is approximately 7.8 Å, while the same distance in the case of 3 is only 5.0 Å (Figure 5).

The latter can be bridged well by a C4 chain (6.3 Å) which results in 20, while in the former case the C4 chain connection, resulting in 12, will prevent an optimal fit into the AChE structure. This further illustrates that the different modes of attachment of the tacrine-spacer unit to the CB1 antagonist pharmacophore in compounds 12 and 20, respectively, have an impact on the optimal spacer lengths.29

Finally, two additional points are of interest. First, the AChEI 1 and some of its metabolites have been reported30 to elicit toxic effects. In this regard our compounds 12, 13, 20, and 21 might be expected to elicit some of the adverse effects.

Figure 2. Docking result of 12 in the homology model of the CB1 receptor. Helices 5 and 6 are not displayed for clarity.

Figure 3. Docking result of 20 (shown in purple) in the structure of AChE. The lipophilic residues of the PAS accommodating the diaryl moiety of the CB1 antagonistic part are given in orange. Some regions of the protein are not displayed for clarity.

Figure 4. Docking result of the 3,4-diarylpyrazoline-based CB1 pharmacophore in the structure of AChE.

Figure 5. Structure-based alignment of 12 and 20 showing the effect of the different sprouting of the tail from their CB1 antagonist part.
of 1. However, it is realized that \( N,N'\text{-bis}(1,2,3,4\text{-tetrahydro-}
\text{acridin-9-yl})\text{heptane-1,7-diamine (bis(7-tartrate) demonstrated}
\text{a minor toxicity in comparison with 1 and accordingly the tarcine heterodimers 12, 13, 20, and 21 would be anticipated to be considerably less toxic than 1. Second, although the more general term “antagonist” is used in this article, it is with the understanding that the majority of the reported cannabinoid CB1 receptor antagonists (10, 21, 24, 31) (including compounds such as rimonabant, 2, and 3) behave as inverse agonists” at the constitutively active \( \text{CB1 GPCR. Therefore, it can be anticipated that the compounds 5–10, 12, 13, 20, and 21 will also act as inverse agonists at the CB1 receptor.}

Conclusion

Novel hybrid molecules 12, 13, 20, 21 exhibiting AChE inhibiting activities as well as significant cannabinoid CB1 receptor antagonist properties were discovered that are based on 1 as the AChEI pharmacophore and known cannabinoid CB1 antagonist counterparts. The imidazole-based 20 showed significant CB1 receptor affinity (48 nM) in combination with high CB1/CB2 receptor subtype selectivity (>20-fold) and elicited equipotent AChE inhibitory activity compared with 1.

Experimental Section

Chemistry. \(^1\)H and \(^13\)C NMR spectra were recorded on a Varian UNO400 instrument (400 MHz) using CDCl\(_3\) as solvent with tetramethysilane as an internal standard. Chemical shifts are given in parts per million (ppm) (\( \delta \) scale) downfield from tetramethysilane. Coupling constants (J) are expressed in hertz (Hz). Thin-layer chromatography was performed on Merck precoated F254 plates, and spots were visualized with UV light. Flash chromatography was performed using silica gel 60 (0.040–0.063 mm, Merck). Column chromatography was performed using silica gel 60 (0.063–0.200 mm, Merck). Melting points were recorded on a Büchi B-545 melting point apparatus and are uncorrected. Mass spectra were recorded on a Micromass QTOF-2 instrument with MassLynx application software for acquisition and reconstruction of the data. Exact mass measurement by HRMS was done of the quasimolecular ion \([M + H]^+\). Elemental analyses were performed on a Vario EL elemental analyzer by Solvay Pharmaceuticals. Yields refer to isolated pure products and were not maximized. The purity of the target compounds 3, 5–10, 12, 13, 20, and 21 was established as ≥95%, based on combustion analysis data.

1-(4-Chlorophenyl)-2-(2-chlorophenyl)-5-ethyl-N’-[piperidin-4-yl]-1H-imidazole-4-carboxamide-\(2H\text{-}\text{H}_2\text{O} \text{(3)} \). Compound 3 was obtained from 19 according to reported procedures.\(^{27,28}\) Mp: 207–208 °C. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 1.08 (t, \( J = 7.5\) Hz, 3H), 1.39–1.48 (m, 2H), 1.69–1.81 (m, 5H), 2.86 (br s, 3H), 2.94 (q, \( J = 7.5 \) Hz, 2H), 7.09 (d, \( J = 8.7\) Hz, 2H), 7.20–7.36 (m, 6H), 7.94 (s, 1H). \(^13\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \) 13.9, 17.7, 23.4, 25.5, 57.3, 126.7, 129.7, 129.9, 130.4, 130.7, 136.1, 138.4, 139.1, 140.3, 143.2, 160.5. HRMS (C\(_{26}\)H\(_{30}\)Cl\(_2\)N\(_5\)O\(_2\)S) [M + H]\(^+\): found m/z 558.1513, calcld 558.1513. Anal. (C\(_{26}\)H\(_{30}\)Cl\(_2\)N\(_5\)O\(_2\)S) C, H, N.

1-(4-Chlorophenyl)-4-phenyl-1,5-dihydro-1H-pyrazole-1-carboxamide (8). Compound 8 was obtained from 11 and 1-methylpyriderin-4-ylamine according to the procedure for 7. Mp: 202–203 °C. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 1.38–2.17 (m, 4H), 2.49 (m, 2H), 2.69 (t, \( J = 8.6\) Hz, 2H), 7.32–7.43 (m, 5H), 7.59 (m, 6H), 7.83–8.00 (m, 1H), 7.95 (d, \( J = 8.6\) Hz, 2H), 8.05 (d, \( J = 8.6\) Hz, 2H), 8.19 (m, 1H). HRMS (C\(_{26}\)H\(_{30}\)Cl\(_2\)N\(_5\)O\(_2\)S) [M + H]\(^+\): found m/z 570.1497, calcld 570.1497. Anal. (C\(_{26}\)H\(_{30}\)Cl\(_2\)N\(_5\)O\(_2\)S) C, H, N.

1-(4-Chlorophenyl)-N’-(4-(chlorosulfonfonyl)-N-[3-(dimethylamino)-propyl]-4-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (9). Compound 9 was obtained from 11 and dimethyl(piperazin-1-yl)amine according to the procedure for 7. Mp: 164–165 °C. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 2.30 (s, 3H), 2.50 (t, \( J = 6.5\) Hz, 2H), 2.57–2.62 (m, 2H), 2.64–2.78 (m, 4H), 3.72–3.89 (m, 5H), 4.42 (t, \( J = 11.2\) Hz, 1H), 4.52–4.58 (m, 1H), 7.11 (br d, \( J = 8\) Hz, 2H), 7.23–7.34 (m, 5H), 7.36 (d, \( J = 8.6\) Hz, 2H), 7.49 (d, \( J = 8.6\) Hz, 2H), 8.73 (d, \( J = 8.8\) Hz, 2H). HRMS (C\(_{26}\)H\(_{30}\)Cl\(_2\)N\(_5\)O\(_2\)S) [M + H]\(^+\): found m/z 613.1932, calcld 613.1919. Anal. (C\(_{26}\)H\(_{30}\)Cl\(_2\)N\(_5\)O\(_2\)S) C, H, N.
3-(4-Chlorophenyl)-N-[(4-chlorophenyl)sulfonyl]-N-(4-pyridinol-1-yl)butyl-4-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (10). Compound 10 was obtained from 11 and 4-(pyridinol-1-yl)butylamine according to the procedure described for 7. Mp: 126–127 °C. 1H NMR (400 MHz, CDCl3) δ 1.56–1.65 (m, 2H), 1.65–1.75 (m, 3H), 1.75–1.81 (m, 4H), 2.44–2.54 (m, 6H), 3.59–3.67 (m, 2H), 4.12 (dd, J = 11.3 and 4.7 Hz, 1H), 4.55 (t, J = 11.5 Hz, 1H), 4.61–4.67 (m, 1H), 7.10–7.14 (m, 2H), 7.23–7.34 (m, 5H), 7.38 (d, J = 8.8 Hz, 2H), 7.50 (d, J = 8.8 Hz, 2H), 7.85 (d, J = 8.8 Hz, 2H). 13C NMR (100 MHz, CDCl3) δ 23.4, 26.2, 28.2, 44.9, 50.6, 54.2, 55.7, 57.7, 127.3, 127.4, 128.0, 128.5, 128.6, 128.7, 129.0, 129.5, 136.5, 137.1, 141.3, 151.9, 157.4. HRMS (C35H34Cl2N5O2S) [M + H]+: found m/z 598.1824, calcld 598.1810. Anal. (C35H34Cl2N5O2S) C, H, N.

4-Chloro-N-[3-(4-chlorophenyl)-4-phenyl-4,5-dihydro-1H-pyrazolyl]-[4(1,2,3,4-tetrahydroacridin-9-ylamino)butylamino]methylenibenzenesulfonamide-1H-O-2.5MeOH (12). Compound 12 was obtained from 11 and 15 according to the procedure described for 13. Mp: 125–126 °C. 1H NMR (400 MHz, CDCl3) δ 1.80–2.01 (m, 8H), 2.60–2.70 (m, 2H), 3.25–3.34 (m, 2H), 3.77–3.86 (m, 2H), 3.95–4.06 (m, 3H), 4.43 (t, J = 12 Hz, 1H), 4.67 (dd, J = 12 and 5 Hz, 1H), 6.30 (br s, 1H), 7.10 (br d, J = 8 Hz, 2H), 7.15–7.31 (m, 6H), 7.36–7.44 (m, 3H), 7.52 (d, J = 8 Hz, 2H), 7.65 (t, J = 8 Hz, 1H), 7.83 (d, J = 8 Hz, 2H), 8.20 (d, J = 8 Hz, 1H), 8.52 (d, J = 8 Hz, 1H). 13C NMR (100 MHz, CDCl3) δ 20.6, 22.0, 24.1, 27.1, 28.2, 43.9, 47.2, 50.8, 57.3, 113.1, 113.5, 120.8, 124.3, 125.1, 127.1, 127.3, 128.1, 128.3, 128.76, 128.80, 129.0, 129.5, 132.1, 133.6, 137.5, 138.8, 139.0, 143.3, 151.4, 151.6, 155.6, 157.7. HRMS (C35H35Cl2N5O) [M + H]+: found m/z 725.2255, calcld 725.2232. Anal. (C35H33Cl2N5O2S) C, H, N.

4-Chloro-N-[3-(4-chlorophenyl)-4-phenyl-4,5-dihydro-1H-pyrazolyl]-[4(1,2,3,4-tetrahydroacridin-9-ylamino)butylamino]methylenibenzenesulfonamide-1H-O-2.5MeOH (13). To a magnetically stirred solution of 1125 (1.5 g, 3.16 mmol) in dichloromethane were successively added (30 mL) DMAP (1.707 g, 13.9 mmol) and POCl3 (0.59 g, 3.85 mmol), and the resulting magnetically stirred mixture was heated at reflux temperature for 72 h. The formed precipitate was collected by filtration and washed with petroleum ether (40–60) to give 19 (4.52 g, 84% yield). 1H NMR (400 MHz, CDCl3) δ 1.09 (t, J = 7, 3H), 2.90 (q, J = 7, 2H), 3.70 (br s, 1H), 7.12 (dt, J = 8 and 2, 2H), 7.22–7.28 (m, 1H), 7.29–7.38 (m, 5H).

J = [1.0, 1.2, 3.4-Tetrahydroacridin-9-ylamino]butyl]-2-(2-chlorophenyl)-1H-imidazole-4-carboxamide-1H-O-2MeOH (21). To a magnetically stirred solution of N-[9f-[1\',2',3',4'tetrahydroacridinyl]-1,7-diaminohexane 16 (0.837 g, 3.11 mmol) in dichloromethane (40 mL) was successively added 19 (0.75 g, 2.08 mmol), HOAc (0.34 g, 2.5 mmol), and EDC (0.48 g, 2.5 mmol). The resulting mixture was stirred at room temperature for 18 h and successively washed with water (2 × 50 mL) and brine (50 mL). The organic layer was successively dried over Na2SO4 filtered, and concentrated in vacuo. The obtained crude product was purified by flash chromatography (gradient, dichloromethane/ethanol = 99:1 → dichloromethane/methanol = 90:10 (v/v)) to give pure 21 (780 mg, 62% yield). Mp: 110–111 °C. 1H NMR (400 MHz, CDCl3) δ 1.07 (t, J = 7, 3H), 1.67–1.85 (m, 4H), 1.88–1.99 (m, 4H), 2.68–2.75 (m, 2H), 2.95 (q, J = 7 Hz, 2H), 3.06–3.15 (m, 2H), 3.45–3.63 (m, 4H), 7.10 (br d, J = 8 Hz, 2H), 7.20–7.41 (m, 9H), 7.52–7.62 (m, 2H), 7.92–8.05 (m, 2H). HRMS (C35H35Cl2N5O) [M + H]+: found m/z 654.2734, calcld 654.2766. Anal. (C35H33Cl2N5O2S) C, H, N.

Turbidimetric Aqueous Solubility Assay. Four dilutions of the test compound (10 mM in DMSO) were prepared in DMSO (3, 1, 0.3, and 0.1 mM). Each test compound concentration was then further diluted 1 in 100 in buffer (typically 0.01 M phosphate buffered saline, pH 7.4, or a buffer at pH 2) so that the final DMSO concentration was 1% and the final test compound concentrations were 1, 3, 10, 30, and 100 μM. The experiment was performed at 37 °C, and seven replicate wells were designated per concentration. Following the addition of the DMSO dilution to the buffer, the plates were incubated for 2 h at 37 °C before the absorbance was measured at 620 nm. The solubility was estimated from the concentration of test compound that produced an increase in absorbance above vehicle control (i.e., 1% DMSO in buffer). Nicardipine and pyrene were included as control compounds. The solubility of nicardipine was pH dependent, whereas the solubility of pyrene was pH independent. Results were given as a calculated midrange value, based on measured lower and upper bound values.

Inhibition of ACHe in Human HEK-293 Cells.24 Test compounds were dissolved in DMSO (10 mM) and diluted to test concentrations in assay buffer. Testing was performed at a 3 log concentration range around a predetermined IC50 for the respective assay: e.g., 10, 1, 0.1, and 0.01 μM for IC50 of 0.3 μM and 300, 30, and 3 μM for IC50 of 10 nM. All determinations were performed as duplicates. The highest concentration tested for primes was 10 μM. Following incubation of test compound with an ACHe enzyme preparation (human recombinant expressed in HEK-293 cells) and the substrate acetylthiocholine (50 μM) for 30 min at 37 °C, the thio conjugate product was determined by photometry. Results were expressed as percentage of total product formed at each concentration of compound tested (duplicates). From the concentration–production inhibition curves, IC50 values were determined by nonlinear regression analysis using Hill equation curve fitting. Results were expressed as pIC50 values. Compounds with no significant affinity at concentrations of 10 μM and higher were considered inactive: pIC50 < 5.0.
Molecular Modeling. Modeling studies were performed using Schrödinger software (Maestro, version 8.5; Schrödinger LLC: New York, 2009). The cannabinoid CB₁ receptor homology model was built with Prime using the X-ray structure of the β₂-adrenergic receptor (PDB code 2RH1) as rigid template. The extracellular loop 2 was modeled with the loop prediction module. The loop was modified in order to make the Cys bridge between C257 and C264 possible via a stepwise decreasing distance constraint minimization and after building the bridge, a full minimization of the loop. The tacrine moiety was docked in the described region manually without any further modifications.

The X-ray structure of AChE was processed with the Protein Preparation Wizard in Maestro. The CB₁ receptor binding site was disclocated by adjusting the rotamer state of two lipophilic residues (Phe331 g,f,Phe330 trans g,f). The CB₁ receptor antagonist moieties of 12, 13, 20, and 21 were docked manually into this site with the tacrine part kept in the same position as that in its X-ray structure with AChE (PDB code 1ACJ).

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