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Synthesis and *In Vitro* Characterization of Novel Allosteric Modulators of the α7 Nicotinic Acetylcholine Receptors

Master’s Thesis Defense

by

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ABBREVIATIONS:

ACh – Acetylcholine
nAChRs – Nicotinic acetylcholine receptors
ABD – ACh binding domain
AChBP – Acetylcholine binding protein
AD - Alzheimer’s disease
PD – Parkinson’s disease
ADHD - Attention-deficit/hyperactivity disorders
NAM – Negative allosteric modulator
PAM – Positive allosteric modulator
ago-PAM – allosteric agonist as well as a PAM
TQS – 4-Naphthalene-1-yl-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide
4BP-TQS – 4-(4-bromophenyl)-3a,4,5,9b tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide
α-BTX – α-Bungarotoxin
MLA – Methyllycaconitine
LBD – Ligand binding domain
AChBP - Acetylcholine binding protein
5-HI - 5-Hydroxy indole
EC₅₀ – Measure of potency
h- Hour
RT – Room temperature
5-HT – 5- Hydroxytryptamine
DPPA – Diphenylphosphoryl azide
TEA – Triethyl amine
THF – Tetrahydrofuran
TBAB – Tetrabutylammonium bromide
ACN – Acetonitrile
EtOAc – Ethyl acetate
DCM – Dichloro methane
InCl₃ – Indium trichloride
mg – Milligram
mmol – Millimoles
equiv – equivalents

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The α7 nicotinic acetylcholine receptors (nAChRs) belong to the Cys-loop superfamily of neurotransmitter-gated ion channels and have been implicated in a number of neurological disorders due to their presence in pre-synaptic, post-synaptic and peripheral locations. As a result, the α7 nAChRs play a key role in the pathology of several cognitive disorders such as Alzheimer’s disease (AD), Parkinson’s disease (PD), attention-deficit hyperactivity disorders (ADHD), and more recently, in neuropathic pain and inflammation. Alpha7 receptors are acted upon by the endogenous neurotransmitter acetylcholine (ACh), which binds to these receptors at the orthosteric site and propagates various signaling cascades. Alpha7 receptors can also be modulated from allosteric sites - binding sites distinct from the orthosteric site. Recent research efforts are being focused on targeting allosteric sites since α7 nAChRs tend to get desensitized upon prolonged exposure to the agonists. Agonists also tend to disrupt the endogenous cholinergic tone. Therefore allosteric modulation is an alternative approach for targeted therapy since these modulators either potentiate (positive allosteric modulators, PAMs) or decrease (negative allosteric modulators, NAMs) agonist-evoked responses from a distinct binding site, without disrupting the endogenous cholinergic tone. PAMs can be further classified into Type I and Type II depending on their desensitization kinetics. Although both Type I and Type II PAMs enhance agonist-evoked responses, only Type II PAMs are known to drastically decrease the desensitization kinetics of the α7 nAChRs. 4BP-TQS (4-(4-bromophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide), a recently discovered Type II PAM, has unique properties: it acts as an individual allosteric agonist, in addition to being a potent PAM (ago-PAM). To date, it is the only ago-PAM of α7 receptors known to signal independently of acetylcholine. Hence, 4BP-TQS could be used as a significant tool for the treatment of...
neurodegenerative diseases associated with a depletion of cholinergic tone (AD, for instance). It could also be used to reactivate receptors that have otherwise been desensitized by the use of agonists. This highlights the need to further characterize its binding site. A recent study, with contributions from our lab, had identified key that aromatic amino acid residues Trp-55 and Tyr-93, present at the interface of the subunits at the orthosteric complimentary binding site, are required for the activity of PAMs. Using site directed mutagenesis studies, another study had identified a key amino acid, M253L, located in the TM2 subunit of α7 receptors as crucial for the binding and activity of PAMs. The same study had shown that at maximal concentration, 4BP-TQS produces a 45-fold larger agonist response as compared to that of ACh, with an 8-fold lower EC\textsubscript{50}.

Consequently, further optimization of the key molecular features of 4BP-TQS would be beneficial in understanding its ago-PAM activity. Earlier studies from our lab had established the stereochemical requirements for ago-PAM activity at α7 receptors. We had not only developed a method for the expeditious synthesis of 4BP-TQS, but we had also separated the enantiomers of the pure, racemic cis-diastereomer of 4BP-TQS. Our findings suggest that the (+)-enantiomer, GAT107, with 3aR4S9bS absolute stereochemistry is the biologically active enantiomer. In an effort to further characterize the binding site of ago-PAMs, our lab had made a focused library of compounds, with modifications to the southern region of 4BP-TQS, which seemed crucial for binding. One particular modification, which replaced the 4-bromo-phenyl ring with a NHBoc group, GAT141, was found to generate a 4-fold increase in ago-PAM activity as compared to 4BP-TQS. This threw light over the fact that there could be a potential secondary binding site (NHBoc moiety) that enhances the ability of GAT141 to bind to the receptors and elicit ago-PAM activity. Therefore, the goal of my thesis is to explore the stereo-electronic properties of this newly
discovered secondary site present at the southern tail of GAT141. To determine if the NHBoc moiety is crucial for binding, we have 1) modified the positioning of the heteroatoms to change the electronics, 2) added bulky substituents to change the angle of rotation and the room available for binding at the binding pocket and 3) removed the heteroatoms and preserved the carbon chain. Thirteen novel final analogs of GAT141 -- incorporating these modifications at the southern tail -- had been synthesized and are currently being evaluated for allosteric activity at the α7 nAChRs in electrophysiology experiments. We have recently received preliminary data on three final analogs, out of which GAT186 (compound 12) and GAT187 (compound 11) turned out to be active ago-PAMs, reconfirming the possibility of a potential secondary binding site at the α7 nAChRs. We are still awaiting in depth electrophysiological data summary on these three compounds (and the remaining ones) from our collaborator.

**SPECIFIC AIMS**

1. To make analogs of GAT141 with southern tail modifications and explore stereo-electronic requirements for having ago-PAM activity at the α7 nAChR

2. To evaluate the electrophysiological activity of these analogs and establish SAR at the α7 nAChR
INTRODUCTION

Overview: nicotinic acetylcholine receptors (nAChRs)

The pentameric nAChRs belong to a superfamily of Cys-loop, ligand-gated ion channels that include GABA, glycine and 5-HT3 serotonin receptors. About 17 different subunits (α1-α10, β1-β4, γ, δ, and ε) coassemble to form a diverse range of nAChR subtypes with different pharmacological and biophysical properties. nAChRs regulate the effects of the endogenous neurotransmitter ACh, which in turn plays an important role in activating various signaling pathways that aid in the release of dopamine, glutamate, GABA (γ-aminobutyric acid), and serotonin. Due to their presence in dendritic, somal, axonal, presynaptic and postsynaptic locations, nAChRs play a major role in synaptic plasticity and are thus involved in learning, cognition and memory, attention and development. Consequently, nAChRs have been implicated in a number of neurological disorders such as schizophrenia, Alzheimer’s disease (AD), addiction and neuropathic pain, emerging as new drug targets for therapy.

Structural features of nAChRs

The basic structure of the nAChRs was obtained from high resolution electron microscopy images of the protein extract from the electric organ of Torpedo Marmorata. The 4 Å resolution images obtained from the pseudo-crystalline structures had revealed that neuronal nAChRs are made up of five subunits that are transmembrane in nature and surround a central, water-filled cation pore. nAChRs distributed in the central and peripheral nervous system form αβ receptor combinations from α2-α6 and β2-β4 subunits. α7-α9 are capable of forming homomeric subunits, where as the α10 subunit forms a heteromer with α9. The α8 subunit has only been identified in
avian tissue. Of all the nAChR subtypes, α7 is the simplest and the most abundant subtype present in the mammalian brain. All subtypes differ in the genes that encode them. So far, 16 genes encoding for different subunits have been identified in mammalian and human genomes. It is believed that these genes have been derived from a common ancestor and have been highly conserved throughout evolution in the simplest to the most complex of organisms. It has also been shown that nAChRs share sequence homology with the bacterial ionotropic channels and can hence be used as prototypes for 3D structural elucidation.

**Figure 1:** Molecular structure of the nAChR
The molecular features of the neuronal nAChRs are homologous to the muscle nAChR\textsuperscript{24}. Each subunit has a long, extracellular N-terminal domain, four transmembrane units (TM1-TM4), and a short C-terminal domain\textsuperscript{1}. Both N and C-terminal domains face the extracellular space while TM2 of each subunit lines the central cation-pore\textsuperscript{1}. The agonist (ACh) binding pockets are located at the interface between two adjacent subunits\textsuperscript{24} (α-epsilon subunits), with the α-subunit being the main component of the binding site\textsuperscript{2}. Current knowledge of the ACh binding domain (ABD) came from studies of the crystal structure of the Ach binding protein (AChBP), which was extracted from the glial cells in mollusks and was found to quench ACh in the synapse (Lymnea)\textsuperscript{1,2}. AChBP is a homopentamer and binds ACh with high affinity. It forms crystals in the presence of ligands such as nicotine or carbamylcholine, providing an important insight into the ligand-protein interactions i.e., ligands bind at the junction between two adjacent subunits and interact with specific amino acids\textsuperscript{25}. Two adjacent cysteine residues in loop-C of the α-subunit are thought to participate in ligand binding\textsuperscript{2}. These cysteines are highly mobile and adjust loop-C in a contracted form in the presence of agonists and in an extended form in the presence of antagonists\textsuperscript{26}.

**Genes encoding different nAChR subunits**

nAChRs can be either be homopentameric (formed by 5 identical subunits) and heteropentameric (formed from combination of different subunits). Further divisions amongst subunits include α and β subunits; α subunits have adjacent cysteine residues at position 192 and 193 and are thought to participate in ligand binding\textsuperscript{2}. Genes encoding α, β, γ, δ, and ε subunits are termed CHRNA, CHRNB, CHRNG, CHRND, and CHRNE respectively\textsuperscript{2}. These genes are localized on different regions on the chromosome, as presented in the 2013 Pharmacology and Therapeutics
review by Hurst and colleagues. Homomeric nAChRs are the simplest of all subtypes -- α7 receptors being prime examples. With their high calcium permeability and fast desensitization kinetics, they have been implicated in numerous physiological functions. Heteromeric receptors on the other hand are composed of two or more different subunits and tend to have diverse pharmacological profiles. One example of the heteropentameric receptor is the α4β2 receptor, which is formed from the coassembly of two α4 and three β2 subunits, known for its high affinity for nicotine. Both α4β2 and α7 receptors are involved in cognitive processes. However, α4β2 receptors are challenging as targets for drug design because the pharmacology at these receptors depends on the ratio of α and β subunits present. The ratio of the subunits in turn determines the extent of agonist sensitivity and calcium permeability. No such challenges are posed at the α7 nAChR because of its homomeric nature and identical chemistry at the ligand binding pockets.

**Biophysical properties of the nAChRs**

In the mammalian brain, nAChRs are mainly located at the presynaptic, somal, axonal, and dendritic locations and modulate the release of other neurotransmitters by initiating action potentials. Somal, axonal and dendritic neurotransmitters help spread the action potential by increasing neuronal excitability. nAChRs are also present in synaptic and non-synaptic locations where they influence the resting potential of the neurons, and alter the space constant of the cell membrane. But overall, synaptic and non-synaptic transmission is influenced by release and volume transmission of ACh, which activates and desensitizes the nAChRs.

In lieu with other ligand-gated ion channels, nAChRs are acted upon by an external signaling molecule (ACh for instance), which activates the receptors and begins a signaling cascade that mobilizes ions into and out of the cell. At presynaptic nAChRs, the endogenous
agonists permit the release of neurotransmitters such as ACh, dopamine (DA), serotonin, norepinephrine, GABA and glutamate. This release at presynaptic sites initiates a calcium signal that further enhances neurotransmitter release. Influx of Ca\(^{2+}\) through the central pore of the receptors not only depolarizes the cell membrane, but also triggers a calcium-induced calcium release from the intracellular space. Calcium influx serves to enhance neuronal excitability and indirectly activates other signal cascades – with glutamergic transmission being one of the most important ones.

Once released from the presynaptic neurons, endogenous agonists such as ACh, bind to the orthosteric site of the receptor and change its conformational state. After binding to the orthosteric site, ACh modifies the rate constants between three transitional states of the receptor: open, closed and desensitized. Although the mechanisms linking ligand binding to channel opening have not yet been characterized, it is known that the binding of ACh stabilizes the receptor in an open conformation. Then, the ACh dissociates, and the receptors become deactivated. On the other hand, it is also likely that the receptors become desensitized, acquiring a different conformation. Desensitization occurs when the receptors are not open or closed, but are still bound by the agonist, and do not conduct any signals. In this state, the receptor has high affinity for the agonist yet remains inactive. Desensitization is defined mathematically by various rate constants and is measured as a degree of inhibition by agonist (IC\(_{50}\) values). These rate constants can also be manipulated by allosteric molecules, or entities that do not necessarily bind to the orthosteric sites. The rate constants are also dependent upon the type of agonist activating the receptor, and the subunit composition of the receptor.
**Alpha7 nAChRs – fundamental aspects**

Unlike the muscle type and peripheral nAChRs, the α7 nAChRs are mainly localized in the cortex and hippocampal regions of the brain. These brain regions are involved in cognition, and cholinergic transmission (both muscarinic and nicotinic). Therefore, neuronal nAChRs such as the α7 receptors have great potential for therapy of cognitive disorders. The homopentameric α7 receptors, first identified in chicken, have been in the limelight for their unique properties such as: genomic structure and location, high calcium permeability, and fast kinetics\(^{35}\). α7 nAChRs are encoded by the CHRNA7 gene, which is located on chromosome 15q14\(^2\). The CHRNA7 gene is closest to the ancestral genes that had evolved millions of years ago, and is believed to have preserved its calcium permeability features all through evolution\(^2\). The homomeric nature of these receptors means that both the principle binding site and the complimentary binding site are made up of the same subunit. Due to this simplicity, nAChRs can be used in the determination of structure-function relationships. For instance, making a single alteration in an amino acid in the channel protein can alter the entire receptor function. The reason for the high calcium permeability of the nAChRs is due to the presence of charged residues on the inner lip of central pore and polar residues on the outer lip\(^1\). Similarly, the cation selectivity of the channel is due to the presence of highly conserved amino acids in the inner part of the ion pore\(^1,2\).

**Alpha7 nAChRs receptors as targets for cognitive disorders**

There is great interest in targeting nAChRs for neurological disorders but over the past four decades only a couple of drugs had been approved for use\(^2\): 1) the nAChR antagonist, mecamylamine for hypertension and 2) the α4β2 partial agonist, Varenicline for nicotine dependence. The main reason many drug candidates do not advance into the market is because of
narrow therapeutic index – either due to lack of efficacy and a potential to cause gastrointestinal and neurological side effects\(^2\). However, over the past decade several agonists, antagonists and allosteric modulators have been developed as potential therapeutic agents for the treatment of cognitive and peripheral disorders.

Decline of nicotinic endogenous tone has been observed in impairments such as AD, dementia, down syndrome, autism, and PD\(^1\). Studies with mutant mice have also implicated nAChRs role in anxiety, depression and pain\(^1\). In AD, there is a loss of cholinergic neurons as the disease progresses. In addition, it is speculated that Aβ\(_{1-42}\) peptides bind to α7 receptors with high affinity,\(^3\) implicating that α7 receptors may play a causative role in the pathology of AD. For ADHD, the treatments being prescribed as of yet are psychostimulants, however increased tobacco use among ADHD patients had thrown light over the fact that nicotinic transmission may be involved in the disease mechanism\(^6\). However, a 12-week phase 2 clinical trial using the α7 agonist TC-5619 had a negative outcome, indicating that the α7 receptors may not be involved in the pathology of ADHD\(^2\). Similarly, it has been observed that there is a decreased incidence of PD in cigarette smokers\(^4\). Hence the use of tobacco products in patients with cognitive disorders such as PD, Schizophrenia and ADHD had led researchers to hypothesize that nicotine in tobacco might induce neuroprotective properties by modulating dopaminergic function\(^5\).

**Agonists and antagonists of neuronal nAChRs**

Nicotinic agonists in general have shown to improve learning and memory in animal models\(^2\). Although acute and chronic nicotine administration had improved the working memory in humans and non-human primates\(^3\), nicotinic agents have found to improve only certain kinds of memory – for example radial maze studies done on rodents indicate that nicotinic mechanisms
improved working memory, not reference memory or response latency. In fact, effective nicotinic influences occurred only when the subject was learning impaired or performing a difficult task\textsuperscript{38}.

Despite the fact, nicotinic agonists have been used in therapy for cognitive disorders, acting at the orthosteric site of the receptors, and stabilizing their open conformations. A full agonist will open the channels to a 100\% open probability ($P_{\text{open}}$) at saturated concentrations, whereas partial agonists evoke less total current at maximal concentrations\textsuperscript{2}. For example, ACh and glycine act as full agonists at their respective receptors\textsuperscript{39}, whereas partial agonists such as Varenicline bind at the same site but are less effective at evoking conformational changes\textsuperscript{40,41}. Epibatidine, a naturally occurring alkaloid isolated from the skin of Ecuadorian frog *E. Tricoloris*, is a potent, non-selective nAChR agonist\textsuperscript{42}. TC-1698 was modeled after the scaffold of naturally occurring ligands such as nicotine and epibatidine and was found to exhibit neuroprotective effects via JAK-2/PI-3K cascade\textsuperscript{43}. GTS-21, with its cytoprotective and cognitive enhancement features in animal models, was shown to be one of the earliest potent partial agonist at $\alpha_7$ receptors in rats\textsuperscript{44}. AR-R 17779 is a full agonist selective for the human $\alpha_7$ nAChRs\textsuperscript{45}. Being more potent than nicotine, it has shown to improve cognition and lower anxiety in rat models\textsuperscript{46}. ABT-594, a 3-pyridyl ether, is active in neuropathic pain models, being 40-100 fold more potent than morphine, and found to be a full agonist at $\alpha_4\beta_2$, $\alpha_7$, and $\alpha\beta\delta\gamma$ subtypes\textsuperscript{47}. Although scientists have been successful at making agonists, nicotinic receptors have the tendency to get desensitized rapidly in their presence\textsuperscript{12}. In addition, they might be capable of disrupting endogenous tone\textsuperscript{13}, and hence their utility as clinical candidates might be questionable.
Antagonists on the other hand compete with agonists for the same orthosteric binding site and prevent channel function. Most antagonists of nAChRs are mainly derived from plant sources. Two examples include: α-Bungarotoxin and methyllycaconitine (MLA). α-Bungarotoxin was obtained from snake venom and it acts as an antagonist at multiple nAChRs. MLA a toxic plant alkaloid, is specific for α7 receptors. Additionally, multiple α-connotoxins such as Iml, MII, PnIA, BuIA and ArIB have also shown antagonistic activity at several nAChR subtypes.

**Allosteric modulation at nAChRs**

As mentioned above, nicotinic agonists have poor selectivity and rapid desensitization kinetics, impeding the advancement of targeted therapy. A quest for alternative methods began when endogenous peptides such as Lynx1, with similar folding pattern to α-Bungarotoxin, had shown to modulate the activity of neuronal nAChRs. Consequently, this began a search for endogenous allosteric modulators. nAChRs themselves have been considered to be allosteric proteins since they acquire conformational changes after ligand binding. When the nAChRs transition from an open, to resting to desensitized states, there are energy barriers that need to be overcome. Ligands that bind to a site that is distinct from the orthosteric binding site, and change
the free energy between different conformational states of the receptor are known as allosteric modulators\textsuperscript{1,2} Allosteric modulators that enhance agonist activity at the nAChRs are known as positive allosteric modulators (PAMs) and those that inhibit agonist activity are known as negative allosteric modulators (NAMs). The earliest example of a PAM is Ivermectin, an antiparasitic agent that modulates α7 nAChRs\textsuperscript{52}. 5-hydroxy indole (5-HI) allosterically increases ACh responses at α7 receptors without affecting the response time course\textsuperscript{53}. Galantamine, used for the treatment of AD, has been reported to act as a PAM at various nAChRs\textsuperscript{2,17} 17-β-estradiol modulates the human α4β2 receptors by directly interacting with binding elements on the receptor\textsuperscript{54}. Phosphorylation of the intracellular region between TM3 and TM4, and dephosphorylation of the α7 receptor by genistein are other forms of allosteric modulation\textsuperscript{55,56}.

Overall, allosteric modulators have become promising targets for alternative therapy for two main reasons: 1) they increase or decrease ACh-induced responses without potentially causing a permanent alteration to the functionality of the receptors\textsuperscript{1,2} and 2) since they bind at sites distinct from the orthosteric ligands, they do not produce any harmful side effects and therefore provide a safer, alternative route for therapy\textsuperscript{1}. Akin to benzodiazepines as modulators of GABA\textsubscript{A} receptors, allosteric modulators of nAChRs are expected to provide promising applications for targeted therapy\textsuperscript{2}.

**Classification of nAChR PAMs**

PAMs decrease the energy barrier between the closed and open states of the receptor\textsuperscript{2} and can be subdivided into two classes: Type I and Type II. Type I PAMs enhance agonist-evoked responses but do not affect the rate of desensitization of the receptors\textsuperscript{57}. Type II PAMs, on the other hand, not only potentiate agonist-evoked responses but also dramatically slow down the
rate of desensitization\textsuperscript{58, 59}. Both classes of PAMs are efficacious in the animal model of cognition, but only Type II PAMs have been efficacious in neuropathic pain models\textsuperscript{60}. PAMs with properties intermediate to Type I and Type II PAMs have also been identified\textsuperscript{61, 62}. Examples of intermediate PAMs include SB-206553\textsuperscript{62} and JNJ1930942. The latter is an allosteric modulator at \(\alpha_7\) receptor; it increases agonist evoked peak currents (but not in the absence of the agonist), while decreasing the rate of desensitization\textsuperscript{61}.

**Type I PAMs**

![Figure 3. First generation PAMs](image)

NS-1738\textsuperscript{63} is a classic example of a Type I PAM. As an analog from the biarylurea series, it improved recognition memory in rat, in addition to reversing learning impairment caused by the muscarinic antagonist scopolamine in the rat Morris water maze behavioral model\textsuperscript{52}. 5-HI is another Type I PAM shown to enhance agonist peak currents but is non-selective, requiring high concentrations to produce an effect\textsuperscript{53}. On the other hand, Compound 6\textsuperscript{52}, \(\text{N-}(4\text{-chlorophenyl})\text{-alpha-}[[(4\text{-chloro-phenyl})\text{amino}]\text{methylen}e]3\text{-methyl-5-isoxazoleacetamide}\) was designed after a class of compounds that modulated the GABA\textsubscript{A} receptors\textsuperscript{64}. It was one of the earliest Type I PAMs to exhibit efficacy across various cognitive domains. Although nowhere as potent as 5-HI or NS-
1738, the acetylcholine esterase inhibitor, galantamine, was recently resolved at the atomic level with AChBP was found to bind at a location distinct from the ACh binding site\textsuperscript{52}. LY-2087101 is another Type I PAM, yet not selective for $\alpha_7$ nAChRs\textsuperscript{57}.

**Type II PAMs**

Continued exposure to agonist desensitizes the receptors and reduces peak currents to a non-detectable level. Application of a well-characterized Type II PAM such as PNU-120596 had increased the amplitude of the currents by several fold\textsuperscript{65}. Although PNU-120596 and NS-1738 are both urea derivatives, NS-1738's marginal effects on desensitization kinetics suggest that these two molecules have different mechanisms/sites of action\textsuperscript{52}. Another class of Type II PAMs belonging to the tetrahydroquinoline series have been efficacious in treating cognitive disorders. 4-naphthalene-1-yl-3a,4,5,9b- tetrahydro-3-H-cyclopenta[c]quinoline-8-sulfonic acid amide (TQS), has not only increased peak currents but has also slowed desensitization\textsuperscript{58}. Its analog, 4BP-TQS, increases peak currents in the absence of a potentiator at the ligand binding domain, making it a potent ago-PAM\textsuperscript{15, 66}. Both TQS and 4BP-TQS produced a leftward shift in the $EC_{50}$ values of various agonists in concentration response studies\textsuperscript{15, 58}. However, only 4BP-TQS is the only molecule to date that has shown to evoke agonist peak currents in the absence of endogenous agonist, making it the most potent ligand at the $\alpha_7$ nAChRs.

![Figure 4. Structure of a Type II PAM (PNU-120596) and a NAM (HDMP)](image-url)
Alpha7 NAMs

Unlike PAMs, NAMs have not been studied extensively, and have mostly been used as pharmacological tools to study analgesia. By function, NAMs are effectors that increase the energy barrier between open and desensitized states, thereby inhibiting α7 nAChR function. Examples of NAMs include 1,2,3,3a,4,8b-hexahydro-2-benzyl-6-N,N-dimethylamino-1-methylindenob[1,2-b]pyrrole (HDMP), a novel compound that exhibited antagonist activity both in vitro and in vivo at α7 nAChRs (IC$_{50}$ = 0.07 uM). It was also shown that HDMP did not exhibit binding affinity for α7 receptors, suggesting a non-competitive mechanism of action. In vivo, it reversed nicotine induced analgesia in the tail flick but not the hotplate model (AD$_{50}$ = 0.008 mg/kg). MD-354 (m-chlorophenylguanidin; m-CPG) is another small molecule NAM of the α7 receptors that was also shown to inhibit antinociceptive properties of (-)nicotine.

Significance of the Type II PAM, 4BP-TQS

![Figure 5. Structures of PAMs, TQS, 4-BPTQS, and GAT141](image)

Structurally derived from the TQS, 4BP-TQS was found to be an agonist in addition to being a PAM. In the absence of agonist, 4BP-TQS dramatically slowed down the rate of desensitization.
as compared to ACh\textsuperscript{15}. Agonist dose response data comparing ACh and 4BP-TQS (nH = 2.3 ± 0.4) showed that curve with 4BP-TQS was significantly steeper than that of ACh (nH = 1.3 ± 0.2)\textsuperscript{15}. Moreover, agonist responses generated by a maximal concentration of 4BP-TQS were 45-fold larger than those generated by ACh\textsuperscript{15}. MLA, a reversible competitive antagonist of ACh, acts as a noncompetitive antagonist of 4BP-TQS\textsuperscript{15}. This was attributed to the fact that ACh and 4BP-TQS exert their actions through different binding sites and are hence acted upon differently by MLA\textsuperscript{15}. These findings suggest that 4BP-TQS binds at a site distinct from the ACh binding site and activates nAChRs through a different mechanism.

![Figure 6](image)

\textit{Figure 6}\textsuperscript{15}. Comparison of peak currents of ACh and 4BP-TQS – desensitization effect

The same study had generated a series of α7 and 5-HT\textsubscript{3} receptor chimeras to investigate whether 4BP-TQS binds at the previously identified allosteric binding site of α7 PAMs. The results showed that 4BP-TQS had no agonist activity when tested with the chimeras suggesting that allosteric potentiation is dependent on the α7 transmembrane region\textsuperscript{15}. On the other hand, site directed mutagenesis studies demonstrated that a single point mutation of the amino acid M253L in TM2 region had completely cut off agonist and modulatory effects of 4BP-TQS\textsuperscript{15}. The same mutation did not have any effect on ACh-evoked responses, giving further support to the idea that ACh and 4BP-TQS have different sites of action\textsuperscript{15}. In conclusion, this study demonstrated that 4BP-
TQS is a more efficacious agonist of α7 receptors than ACh (45-fold larger maximal response and 8-fold lower EC\textsubscript{50}) and could have a therapeutic advantage over other Type II PAMs in treating cognitive disorders and neuropathic pain.

**Type II PAMs in-house**

Realizing the unique pharmacological profile and ago-PAM potential of 4BP-TQS, an expedited method for its synthesis had been developed in house. In addition, several analogs of this compound were also synthesized to characterize the binding site. The previously developed three-component Povarov cyclization (Diels-Alder reaction), which was used to synthesize TQS, gave a yield of 37% over a period of 24 hours\textsuperscript{69}. However, the in-house expeditious methodology using microwave irradiation (15 min, 100 °C) gave up to 70% yield for 4BP-TQS and was reproducible in gram quantities\textsuperscript{70}. The reaction time was not only significantly reduced but also the yields were improved. In addition to developing a synthetic methodology, our lab had also characterized the stereochemistry of 4BP-TQS. The 4BP-TQS scaffold has three chiral centers, with the cis-diastereomer being the major product and the biologically active isomer\textsuperscript{91}. In the cis configuration, the cyclopentene ring on the quinoline scaffold and the southern phenyl ring face the same direction, pointing up and away from the plane of the molecule. The coupling constant between the protons 3a and 4 (\(J=3.5\) Hz) is indicative of cis isomerism\textsuperscript{71}. 


Further exploration of the binding site of 4BP-TQS led to the discovery of GAT141 (Figure 5), which turned out to have excellent ago-PAM activity. In fact, there was a 4-fold increase in ago-PAM activity as compared to 4BP-TQS. This highlights the fact that there could be a secondary binding site that is improving the binding affinity of GAT141 at the α7 receptors. Therefore, mapping this secondary binding site would prove to be beneficial for: 1) investigating if the positioning of the heteroatoms and the chain length play an important role in binding to the receptors, and 2) imparting the selectivity, potency and better physicochemical properties at the binding site of α7 nACHRs.

**RATIONALE FOR DESIGN**

A previous study had summarized the pharmacological properties of a series of chemically related analogs of 4BP-TQS. Their findings suggested that changing the position of the bromo group from the para position on the southern bromo-phenyl to meta and ortho positions had muted the ago-PAM activity of 4BP-TQS but retained PAM characteristics, suggesting that
location of the halogen atom on the para position of the bromophenyl ring is important for imparting allosteric agonist activity\textsuperscript{69}. In addition, increasing the size of the halogen atom – for instance, going from bromo- to iodo- had manifested differences in activation rates, inactivation rates and desensitization rates of the receptors\textsuperscript{69}. Therefore, although the para-position on the bromophenyl ring is critical for activity, having high molecular weight substituents such as iodine instead of bromine was not of great consequence. In contrast to the nature of this finding, GAT141 which has a relatively large substituent at the para position of the bromophenyl ring – a carbamate group and a tert-butyl group – was found to be four times more potent than 4BP-TQS at evoking allosteric agonist activity. This highlights the fact that there could be an additional binding pocket at the \(\alpha7\) receptors that is well suited for the bulky NHBoc moiety.

Figure 8. A map of the NHBoc moiety in GAT141
In order to characterize the stereo-electronic requirements for binding at this secondary binding site, A, B and C and D were replaced with various chemical substituents that are summarized in the table below (Table 1).

<table>
<thead>
<tr>
<th>Compound</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAT 180</td>
<td>-COOH</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GAT 181</td>
<td>-NH</td>
<td>-C=O</td>
<td>-O</td>
<td>-CH₃</td>
</tr>
<tr>
<td>GAT 182</td>
<td>-NH</td>
<td>-C=O</td>
<td>-O</td>
<td>-(CH₂)CH₃</td>
</tr>
<tr>
<td>GAT 183</td>
<td>-NH</td>
<td>-C=O</td>
<td>-O</td>
<td>Cyclohexyl</td>
</tr>
<tr>
<td>GAT 184</td>
<td>-NH</td>
<td>-C=O</td>
<td>-O</td>
<td>1-Adamantyl</td>
</tr>
<tr>
<td>GAT 185</td>
<td>-NH</td>
<td>-C=O</td>
<td>-O</td>
<td>2-Adamantyl</td>
</tr>
<tr>
<td>GAT 186</td>
<td>-NH</td>
<td>-C=O</td>
<td>-CH₂</td>
<td>tert-butyl</td>
</tr>
<tr>
<td>GAT 187</td>
<td>-O</td>
<td>-C=O</td>
<td>-NH</td>
<td>tert-butyl</td>
</tr>
<tr>
<td>GAT 188</td>
<td>-CH₂</td>
<td>-CH₂</td>
<td>-CH₂</td>
<td>tert-butyl</td>
</tr>
<tr>
<td>GAT 190</td>
<td>-NH</td>
<td>-C=O</td>
<td>-O</td>
<td>-CH(CH₃)₂</td>
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<td>GAT 191</td>
<td>-O</td>
<td>-C=O</td>
<td>-CH₂</td>
<td>tert-butyl</td>
</tr>
<tr>
<td>GAT 192</td>
<td>-C=O</td>
<td>-O</td>
<td>-CH₂</td>
<td>tert-butyl</td>
</tr>
<tr>
<td>GAT 193</td>
<td>-CH₂</td>
<td>-CH₂</td>
<td>-CH₂</td>
<td>-Br</td>
</tr>
</tbody>
</table>

Table 1. Summary of final analogs of GAT141 with respective stereo-electronic modifications

Conserving the chain length of GAT141, we had added different alkyl groups at position “D”, such as methyl, ethyl and isopropyl to replace the tert-butyl group. We have also added cyclic, bulky substituents such as cyclohexyl-, and 1 and 2-adamantyl groups to determine if the binding
pocket can tolerate variability in size at the tert-butyl moiety. This would also change the stereochemistry and the angle of rotation of the chain, which could help further elucidate the spatial requirements for binding. Various functional groups, such as the ones seen in GAT187 (which has a amide functional group instead of a carbamate) and GAT 191,192 (which have ester functional groups) and have also been made to determine the effect of changing the positions of the heteroatoms. GAT180, with just a carboxylic acid moiety in the “A” position was synthesized as a precursor to GAT191 and 192, in order to test the effect of the absence of nitrogen and the rest of the carbon chain. Moreover, GAT187 is a unique analog in which the positions of the heteroatoms are oriented exactly opposite of the way they are oriented in the original GAT141, making it a “reverse carbamate” compound. GAT193 and GAT188, on the other hand, are characterized by the complete absence of heteroatoms – activity data from these molecules would determine the role of the heteroatoms in binding.

CHEMISTRY AND SCHEMES

Inspired by the ago-PAM activity data (in house) of GAT141, all chemistry efforts were focused on modifying the southern region of the NHBocTQS scaffold. For six of the 13 final compounds synthesized, the intermediate carbamate-aldehydes were rearrangement products (Scheme 1). These intermediates were then coupled to the tetrahydro-3H-cyclopenta[c]quinoline scaffold using the three-component Povarov reaction (Scheme 8).
The carbamate intermediates were synthesized by Curtius rearrangement\textsuperscript{73}. Out of the six carbamates synthesized, 2\textsubscript{a}, 2\textsubscript{b} and 2\textsubscript{c} were synthesized using respective alcohol as a solvent, whereas 2\textsubscript{d}, 2\textsubscript{e} and 2\textsubscript{f} were synthesized using alcohol in stoichiometric amount (1.1 equiv), and toluene as a solvent since the alcohols were either high boiling or solids.

Compound 2\textsubscript{g}, ("Reverse Carbamate"), was synthesized from 4-hydroxybenzaldehyde and tert-butyl isocyanate following Scheme 2\textsuperscript{74}, which yielded 44\% of the desired product.
The cis-diastereomer of 4-nitroTQS, or compound 18 was synthesized from 4-nitrobenzaldehyde using the microwave procedure for Povarov cyclization (Scheme 8), with a 71% yield. 4-aminoTQS, compound 19, on the other hand, was synthesized when the nitro group on 18 was selectively reduced with saturated ammonium chloride solution and zinc. The cis-diastereomer was separated out after column chromatography, with a 55% yield.
(4,4-dimethylpentyl) benzene 22 was the product of a C-C bond coupling between 3-bromopropyl benzene (20) and tert-butyl magnesium chloride (21) in the presence of 10 mol% CuI, 20 mol% TMEDA and 1 equiv. lithium methoxide taken in THF\textsuperscript{75}. The reaction gave quantitative yield at 0 °C, in about 2-3 hours, whereas in the originally reported publication, the reaction took about 24 hours for completion\textsuperscript{75}. Formylation of 22 with the reagent hexamethylenetetramine in trifluoroacetic acid at 80 °C for 12-15 hours\textsuperscript{76} successfully yielded the aldehyde 24, 4-(4,4-dimethylpentyl)benzaldehyde, as a colorless oil (60% yield).

Formylation of (20) was carried out by a method using dichloro(methoxy)methane (25) and titanium tetrachloride as reagents\textsuperscript{77}. This method yielded 59% of the product, 4-(3-bromopropyl)benzaldehyde, 26 as a liquid. The previous method of formylation using hexamethylenetetramine was not a successful substitute for the synthesis of 26 due to low yields and mixture of ortho para products.
Neopentyl 4-formylbenzoate 30 was synthesized from 4-formylbenzoic acid (1), neopentyl bromide (29), and sodium bicarbonate in DMSO with tetrabutyl ammonium bromide (TBAB) as a phase transfer catalyst. The reaction was carried out under microwave irradiation to yield the product with a 34% yield after chromatography.

Molecule (27), 3,3-dimethylbutanoic acid, was converted to 3,3-dimethylbutanoyl chloride 28 in
presence of thionyl chloride and chloroform and the resultant 28 was then added to a mixture of 4-hydroxybenzaldehyde in the presence of TEA\textsuperscript{78}. This procedure slightly varies from the referred procedure in the use of chloroform instead of THF.

As mentioned earlier, all final TQS analogs were synthesized using the three-component Povarov cyclization. Cyclopentadiene (3 equiv) was added to a mixture of the aldehyde (1 equiv), 4-aminosulfonamide (1 equiv), and indium trichloride (0.3 equiv), in acetonitrile (25 ml) and either stirred at room temperature for 24 hours\textsuperscript{79,69} or heated at 100 °C in the microwave for 15 minutes\textsuperscript{71} (Compounds 24 and 32).

Of the 13 final analogs of GAT141 produced, 11 were synthesized under room temperature conditions, and two analogs were prepared under microwave conditions (Scheme 8). Compounds with electron withdrawing substituents in the southern region (acid and esters) produced diastereoselective final products with higher yields, whereas compounds with electron donating substituents (alkyl groups and bulky cyclic groups) produced lower yields (Table 1).
All purified cis-diastereomers (>96% pure) of the final compounds are currently being evaluated in electrophysiology experiments in *Xenopus* oocytes.

<table>
<thead>
<tr>
<th>Aldehyde</th>
<th>Reaction conditions</th>
<th>Final Product (Yield)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>RT, 24 hours</td>
<td>(5), 52%</td>
</tr>
<tr>
<td>2b</td>
<td>RT, 24 hours</td>
<td>(6), 20.3%</td>
</tr>
<tr>
<td>2c</td>
<td>RT, 24 hours</td>
<td>(7), 19.4%</td>
</tr>
<tr>
<td>2d</td>
<td>RT, 24 hours</td>
<td>(8), 42.3%</td>
</tr>
<tr>
<td>2e</td>
<td>RT, 24 hours</td>
<td>(9), 32%</td>
</tr>
<tr>
<td>2f</td>
<td>RT, 24 hours</td>
<td>(10), 25.8%</td>
</tr>
<tr>
<td>2g</td>
<td>RT, 24 hours</td>
<td>(11), 19.7%</td>
</tr>
<tr>
<td>*19</td>
<td>*RT, 12-15 hours</td>
<td>*(12), 58.6%</td>
</tr>
<tr>
<td>24</td>
<td>MW, 100 °C, 15 min</td>
<td>(13), 9.6%</td>
</tr>
<tr>
<td>26</td>
<td>RT, 24 hours</td>
<td>(14), 26.9%</td>
</tr>
<tr>
<td>30</td>
<td>RT, 24 hours</td>
<td>(15), 62.3%</td>
</tr>
<tr>
<td>31</td>
<td>RT, 24 hours</td>
<td>(16), 78.4%</td>
</tr>
<tr>
<td>32 (R₂ = COOH)</td>
<td>MW, 100 °C, 15 min</td>
<td>(17), 56.1%</td>
</tr>
</tbody>
</table>

Table 2. List of final NHBocTQS analogs, their intermediates, yields and reaction conditions.

*See Scheme 9 for the synthesis of 12.*
Compound 12 ("Amide TQS") was the only final product synthesized by a protocol other than Povarov reaction. It was much more efficient to couple the acid chloride 28, (see Scheme 7) with the 4-aminoTQS (19) scaffold in the presence of pyridine. This reaction yielded the cis-diastereomer by precipitation from DCM after aqueous workup (59% yield).

**RESULTS AND DISCUSSION**

Thirteen final analogs of GAT141 (NHBocTQS) were successfully synthesized using the three-component Povarov reaction. Reaction between the respective aldehydes (synthesized using Curtius Rearrangement and other methods), aniline, cyclopentadiene and the Lewis acid, indium trichloride (InCl₃) yielded the final products in a one-pot fashion. The methodology for the synthesis of the TQS scaffold was previously established. In a 24-hour room temperature
reaction, TQS was synthesized with a yield of 22%, using 20 mol% InCl$_3$ as catalyst\textsuperscript{69}. However our lab had reported the microwave-accelerated synthesis (15 min, at 100° C) of 4BP-TQS with better yields (70%)\textsuperscript{70}. The in-house synthesis had not only significantly reduced the time required to synthesize 4BP-TQS, but it was also reproducible in gram quantities\textsuperscript{70}. InCl$_3$ was considered an ideal Lewis acid for these Povarov cyclizations because not only is it well tolerated by oxygen and nitrogen containing compounds, but it also enhances regio- and diastereo-selectivity\textsuperscript{81}. All analogs had three chiral centers, with the cis-diastereomer being the major product and the biologically active isomer\textsuperscript{70}. In the cis configuration, the cyclopentene ring on the quinoline scaffold and the southern phenyl ring face the same direction, and the coupling constant between the protons 3a and 4 ($J=3.5$ Hz) confirms cis isomerism\textsuperscript{71}. Most final compounds were synthesized at RT conditions as opposed to MW conditions because the carbamates were found to be sensitive to microwave-based thermal degradation. Also, it was noticed that the final yields were higher in the case of electron withdrawing substituents on the southern phenyl ring. Conclusively, all purified final compounds with cis configuration are being tested for biological activity in Xenopus oocytes (genetically modified to express human α7 nAChRs) using two-electrode voltage clamp analysis\textsuperscript{72}.

As of yet, we have only received preliminary data for three final compounds. These data do not include raw figures or graphs, but rather a general summary of their respective PAM and ago-PAM activities. GAT180, compound (17), with an acid group on the southern phenyl ring was found to be an inactive PAM and ago-PAM. On the other hand, GAT186, the “amide TQS” compound (12) and GAT187, the “reverse carbamate” compound (11) were found to be very potent ago-PAMs at 10 μM and 100 μM concentrations. In addition, their PAM activity was tested at 1 μM and 30 μM concentrations and they were both found to be very potent PAMs. Changing the
electronics - i.e., switching the position of the two oxygen atoms (11) and removing one oxygen atom (12) seems to correspond to better ago-PAM activity. Based on these two results, it can be said that the structural backbone of the parent molecule, GAT141 is necessary for ago-PAM activity at the α7 nAChRs (although the same electronics are not entirely required). Moreover, the activity of these two compounds reaffirms the existence of the secondary binding site that was discovered after characterizing SAR of the parent molecule, GAT141.

**SUMMARY:**

In conclusion, southern tail modifications of GAT141 were successfully made using one-pot Povarov cyclization after synthesizing respective intermediates via Curtius Rearrangement and other methods. We are still awaiting electrophysiological data from our collaborators, and so far we have received tentative preliminary data on three of our thirteen final compounds. The availability of the electrophysiology data might guide further SAR, aiding in better characterization of the secondary binding site of ago-PAMs at the α7 nAChRs.

**EXPERIMENTAL:**

**Chemistry**

All commercial chemicals and solvents were purchased from Sigma-Aldrich Inc. and AlfaAesar, and unless otherwise specified they were used without further purification. Biotage Initiator microwave system was used for the synthesis of intermediates and a couple of final TQS analogs. The progress of the reaction was monitored by thin layer chromatography (TLC) using commercially prepared silica gel 60 F254 glass-backed plates. All compounds were visualized under ultraviolet (UV) light. NMR spectra and other 2D spectra were recorded in DMSO-d6, unless
otherwise stated, on a Varian 500 MHz. Chemical shifts are recorded in parts per million (δ) relative to internal tetramethylsilane (TMS). Multiplicities are reported in hertz (Hz). LCMS analysis was performed using a Waters Alliance reverse-phase HPLC (electrospray ionization).

**Experimental procedure for the synthesis of car bamates:**

(2a). *Methyl (4-formylphenyl) carbamate*: A mixture of 4-formylbenzoic acid (200 mg, 1.33 mmol, 1 equiv), diphenylphosphoryl azide (403 mg, 1.47 mmol, 1.1 equiv) and triethyl amine (202 mg, 2 mmol, 1.5 equiv) in methanol (10 ml) was refluxed with stirring for 12-15 hours. The solvent was evaporated, and the mixture was quenched with saturated NH₄Cl solution (10 ml) and extracted with ethyl acetate (3 x 20 ml). The organic extracts were combined, washed with brine (10 ml) and dried over Na₂SO₄. The residue was concentrated under reduced pressure, and purified by chromatography (EtOAc/Hexane =0/100 → 20/80) to yield a white solid (60 mg, 25.1%). Rf = 0.45 (EtOAc/Hexane = 20/80) ¹H NMR (500 MHz, CDCl₃) δ 10.1 (s, 1H), 8.21 (d, J = 8.5 Hz, 2H), 7.96 (d, J = 9 Hz, 2H), 3.97 (s, 3H), 1.25 (s, 1H).

(2b). *Ethyl (4-formylphenyl) carbamate*: A mixture of 4-formylbenzoic acid (2 g, 13.3 mmol, 1 equiv), diphenylphosphoryl azide (4.04 g, 14.7 mmol, 1.1 equiv) and triethyl amine (2.02 g, 20 mmol, 1.5 equiv) in ethanol (20 ml) was refluxed with stirring for 12-15 hours. The solvent was evaporated, and the mixture was quenched with saturated NH₄Cl solution (20 ml) and extracted with ethyl acetate (3 x 30 ml). The organic layers were combined, washed with brine (20 ml) and dried over Na₂SO₄. The residue was concentrated under reduced pressure, and purified by chromatography (EtOAc/Hexane 0/100 → 20/80) to yield a white solid (700 mg, 27.2%). Rf =
(2c). Isopropyl (4-formylphenyl) carbamate: A mixture of 4-formylbenzoic acid (2 g, 13.3 mmol, 1 equiv), diphenylphosphoryl azide (4.04 g, 14.7 mmol, 1.1 equiv) and triethyl amine (2.02 g, 20 mmol, 1.5 equiv) in isopropyl alcohol (20 ml) was refluxed with stirring for 12-15 hours. The solvent was evaporated, and the mixture was quenched with saturated NH₄Cl solution (20 ml) and extracted with ethyl acetate (3 x 30 ml). The organic layers were combined, washed with brine (20 ml) and dried over Na₂SO₄. The residue was concentrated under reduced pressure, and purified by chromatography (EtOAc/Hexane 0/100 → 20/80) to yield a white solid (1 g, 36.2%). Rf = 0.56 (EtOAc/Hexane = 20/80) ¹H NMR (500 MHz, CDCl₃) δ 9.91 (s, 1H), 7.84 (d, J = 9 Hz, 2H), 7.55 (d, J = 8.5 Hz, 2H), 6.79 (br s, 1H) 5.05 (septet, J = 6.5 Hz, 1H), 1.32 (d, J = 6.5 Hz, 6H)

(2d). Cyclohexyl (4-formylphenyl) carbamate: A mixture of 4-formylbenzoic acid (400 mg, 2.67 mmol, 1 equiv), diphenylphosphoryl azide (807 mg, 2.93 mmol, 1.1 equiv), triethyl amine (404 mg, 4 mmol, 1.5 equiv) and cyclohexanol (294 mg, 2.93 mmol, 1.1 equiv) in toluene (20 ml) was refluxed with stirring for 12-15 hours. The solvent was evaporated, and the mixture was quenched with saturated NH₄Cl solution (10 ml) and extracted with ethyl acetate (3 x 25 ml). The organic layers were combined, washed with brine (10 ml) and dried over Na₂SO₄. The residue was concentrated under reduced pressure, and purified by chromatography (EtOAc/Hexane 0/100 → 20/80) to yield a white solid (320 mg, 48.5%). Rf = 0.40 (EtOAc/Hexane = 20/80) ¹H NMR (500 MHz, CDCl₃) δ 9.90 (s, 1H), 7.84 (d, J = 8.5 Hz, 2H), 7.56 (d, J = 8 Hz, 2H), 6.84 (br s, 1H) 4.79 (septet, J = 5 Hz, 1H), 1.99-1.22 (comp, 10H)
(2e). 1-adamantyl (4-formylphenyl) carbamate: A mixture of 4-formylbenzoic acid (300 mg, 2 mmol, 1 equiv), diphenylphosphoryl azide (605 mg, 2.2 mmol, 1.1 equiv), triethyl amine (303 mg, 3 mmol, 1.5 equiv) and 1-adamantanol (335 mg, 2.2 mmol, 1.1 equiv) in toluene (20 ml) was refluxed with stirring for 24 hours. The solvent was evaporated, and the mixture was quenched with saturated NH₄Cl solution (10 ml) and extracted with ethyl acetate (3 x 20 ml). The organic layers were combined, washed with brine (10 ml) and dried over Na₂SO₄. The residue was concentrated under reduced pressure, and purified by chromatography (EtOAc/Hexane 0/100 → 20/80) to yield a solid (870 mg, quantitative yield). Rf = 0.40 (EtOAc/Hexane = 20/80) ¹H NMR (500 MHz, CDCl₃) δ 9.89 (s, 1H), 7.82 (d, J = 9 Hz, 2H), 7.53 (d, J = 9 Hz, 2H), 6.74 (br s, 1H) 2.24-2.15 (comp, 9H) 1.73-1.67 (comp, 6H)

(2f). 2-adamantyl (4-formylphenyl) carbamate: A mixture of 4-formylbenzoic acid (400 mg, 2.67 mmol, 1 equiv), diphenylphosphoryl azide (807 mg, 2.93 mmol, 1.1 equiv), triethylamine (404 mg, 4 mmol, 1.5 equiv) and 2-adamantanol (447 mg, 2.93 mmol, 1.1 equiv) in toluene (20 ml) was refluxed with stirring for 24 hours. The solvent was evaporated, and the mixture was quenched with saturated NH₄Cl solution (10 ml) and extracted with ethyl acetate (3 x 20 ml). The organic layers were combined, washed with brine (10 ml) and dried over Na₂SO₄. The residue was concentrated under reduced pressure, and purified by chromatography (EtOAc/Hexane 0/100 → 20/80) to yield a white solid (692 mg, 86.7%). Rf = 0.40 (EtOAc/Hexane = 20/80) ¹H NMR (500 MHz, CDCl₃) δ 9.89 (s, 1H), 7.85 (d, J = 8.5 Hz, 2H), 7.59 (d, J = 8.5 Hz, 2H), 6.98 (br s, 1H) 4.98-4.94 (m, 1H) 2.14-1.56 (comp, 14H)
(2g) 4-formylphenyl tert-butylcarbamate: Tert-butyl isocyanate (1.83 g, 18.45 mmol, 1.5 equiv) was added to a mixture of 4-hydroxybenzaldehyde (1.5 g, 12.3 mmol, 1 equiv) and triethylamine (1.49 g, 14.76 mmol, 1.2 equiv) in dichloromethane and stirred at room temperature for 5 hours. Dichloromethane was removed; the reaction mixture was quenched with saturated NH₄Cl (25 ml) and extracted with ethyl acetate (3 x 35 ml). The organic extracts were combined, concentrated and purified by column chromatography to yield an off-white solid (1.2 g, 44.4%). Rf = 0.37 (EtOAc/Hexane = 20/80) ¹H NMR (500 MHz, DMSO) δ 9.97 (s, 1H), 7.93 (d, J = 8.5 Hz, 2H), 7.78 (br s, 1H) 7.32 (d, J = 8.5 Hz, 2H), 1.30 (s, 9H).

(18). 4-(4-nitrophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide:
Cyclopentadiene (1.97 g, 29.8 mmol, 3 equiv) was added to a microwave vial containing a mixture of the aldehyde (1.5 g, 9.93 mmol, 1 equiv), 4-aminosulfonamide (1.71 g, 9.93 mmol, 1 equiv), and indium trichloride (659 mg, 2.98 mmol, 0.3 equiv), in acetonitrile (10 ml) and heated to 100 °C for 20 minutes. After cooling down, the reaction mixture was quenched with aqueous sodium carbonate (20%, 15 ml), and extracted with dichloromethane (3 x 35 ml). The organic extracts were consolidated, dried over sodium sulfate, and concentrated under reduced pressure. Washing the residue with dichloromethane (3 x 10 ml), yielded a brown precipitate that was isolated by filtration (2.6 g, 70.5%). Rf = 0.39 (EtOAc/Hexane = 40/60, 2 runs) ¹H NMR (500 MHz, DMSO) δ 8.28 (d, J = 9Hz, 2H) 7.74 (d, J = 9Hz, 2H), 7.46 (d, J = 2Hz, 1H), 7.36 (dd, J = 8.5 Hz, 3.5 Hz, 1H), 6.99 (s, 2H), 6.82 (d, J = 8.5 Hz, 1H), 6.53 (s, 1H), 5.94-5.88 (m, 1H), 5.62 (d, J = 5 Hz, 1H), 4.81 (d, J = 3 Hz, 1H), 4.11 (d, J = 9 Hz, 1H), 3.05-2.96 (m, 1H), 2.37-2.28 (m, 1H), 1.60 (dd, J = 15 Hz, 8.5 Hz, 1H)
(19). **4-(4-aminophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide:** Saturated NH₄Cl and H₂O (1:1, 50 ml) and zinc granules (1-1.5 g) were added to a suspension of Compound (18) (1.5 g, 4.039 mmol, 1 equiv) in acetone (80 ml) and refluxed at 70°C overnight. Acetone was evaporated and the reaction mixture was worked up with water (20 ml) and ethyl acetate (3 x 30 ml). The ethyl acetate layers were combined, concentrated and purified by column chromatography (EtOAc/Hexane 40/60 → 70/30) to yield a yellow solid (763 g, 55.3%). R_f = 0.45 (EtOAc/Hexane = 20/80) R_f = 0.35 (EtOAc/Hexane = 50/50, 2 runs) ¹H NMR (500 MHz, DMSO) δ 7.40 (d, J = 2Hz, 1H) 7.30 (dd, J = 8.5 Hz, 2.5 Hz, 1H), 7.07 (d, J = 8.5 Hz, 2H), 6.93 (s, 2H), 6.77 (d, J = 9 Hz, 1H), 6.56 (d, J = 8.5 Hz, 2H), 6.18 (s, 1H), 5.90-5.84 (m, 1H), 5.63 (d, J = 4.5 Hz, 1H), 4.98 (s, 2H), 4.44 (d, J = 3 Hz, 1H), 4.01 (br d, 7.5 Hz, 1H) 2.83 (br q, J = 8.5, 1H), 2.44-2.34 (m, 1H), 1.72 (dd, J = 15 Hz, 8.5 Hz, 1H)

(28). **3,3-dimethylbutanoyl chloride:** 3,3-dimethylbutanoic acid (300 mg, 2.58 mmol, 1 equiv) and thionyl chloride (399 mg, 3.36 mmol, 1.3 equiv) were refluxed in chloroform (10 ml, 99.8% Anh) with stirring at 65°C for 3-4 hours, and used directly in the preparation of compound 12.

(22). **(4,4-dimethylpentyl) benzene:** Lithium methoxide (172 mg, 4.52 mmol, 1 equiv) and CuI (90 mg, 0.452 mmol, 0.1 equiv) were added to a 100 ml round-bottom flask. Three cycles of vacuum and argon were alternatively passed through the flask after addition. 3-bromopropyl benzene (900 mg, 4.52 mmol, 1 equiv), Tetramethylethylenediamine (TMEDA) (105 mg, 0.904 mmol, 0.2 equiv) and tert-butyl magnesium chloride (1 M in MeTHF, 4.7 ml) in THF (5ml) were then added to the flask, under argon, at 0°C. The resulting mixture was stirred at 0°C for 2-3 hours, quenched with saturated NH₄Cl solution (20 ml) and extracted with ethyl acetate (3 x 30 ml). The extracts
were combined and ethyl acetate was evaporated under reduced pressure to yield the product as a colorless liquid (1.15 g, quantitative yield). \( R_f = 0.82 \) (EtOAc/Hexane = 0/100) \(^1\)H NMR (500 MHz, CDCl\textsubscript{3}) \( \delta \) 7.31-7.14 (comp, 5H), 2.57 (t, \( J = 8.5 \) Hz, 2H), 1.62-1.54 (comp, 2H), 1.26-1.20 (comp, 2H), 0.87 (s, 9H)

(24). \((4,4\text{-dimethylpentyl})\text{benzaldehyde}\): A mixture of (4,4-dimethylpentyl) benzene (400 mg, 2.27 mmol, 1 equiv) and hexamethylenetetramine (350 mg, 2.50 mmol, 1.1 equiv) was refluxed overnight in trifluoroacetic acid (10 ml) at 80\(^\circ\) C. After cooling, the solvent was evaporated and the mixture was made alkaline with saturated NaHCO\textsubscript{3} (20 ml) and ice and extracted with dichloormethane. The extract was washed with brine, dried over sodium sulfate and purified by column chromatography (EtOAc/Hexane 0/100 \( \rightarrow \) 2/98) to yield a colorless oil (279 mg, 60.1%). \( R_f = 0.55 \) (EtOAc/Hexane = 5/95) \(^1\)H NMR (500 MHz, CDCl\textsubscript{3}) \( \delta \) 9.98 (s, 1H) 7.80 (d, \( J = 8.5 \) Hz, 2H), 7.35 (d, 8 Hz, 2H) 2.66 (t, \( J = 8 \) Hz, 2H), 1.65-1.58 (comp, 2H), 1.26-1.20 (comp, 2H), 0.87 (s, 9H)

(10). \((3\text{-bromopropyl})\text{benzaldehyde}\): Titanium tetrachloride, TiCl\textsubscript{4} (2.4 g, 12.7 mmol, 1.6 equiv) was added to a mixture of (3-bromopropyl)benzene (4 g, 20.1 mmol, 1 equiv) and dichloro(methoxy)methane (800 mg, 6.96 mmol, 0.84 equiv) at 0\(^\circ\) C in dichloormethane (12 ml). After addition, the reaction mixture was brought to room temperature and stirred overnight. Dichloromethane was evaporated and the reaction mixture was quenched with water (35 ml), washed with brine and extracted with DCM (3 x 40 ml). The extracts were concentrated under reduced pressure and purified by column chromatography to yield an off-white liquid product (2.7 g, 59.2%). \( R_f = 0.20 \) (EtOAc/Hexane = 20/80) \(^1\)H NMR (500 MHz, CDCl\textsubscript{3}) \( \delta \) 9.99 (s, 1H) 7.83 (d, \( J = 8 \) Hz, 2H), 7.38 (d, 8.5 Hz, 2H) 3.40 (t, \( J = 7 \) Hz, 2H), 2.88 (t, \( J = 7 \) Hz, 2H), 2.20 (quintet, \( J = 7 \) Hz, 2H)
(30). neopentyl 4-formylbenzoate: To a mixture of 4-formylbenzoic acid (500 mg, 3.33 mmol, 1 equiv), neopentyl bromide (554 mg, 3.67 mmol, 1.1 equiv) and NaHCO$_3$ (840 mg, 10 mmol, 3 equiv) in DMSO (1 ml), tetrabutyl ammonium bromide (20 mg) was added in catalytic quantity and the reaction mixture was heated to 80° C for 30 minutes in a microwave reactor. After cooling, the reaction mixture was worked up with water (25 ml), washed with brine and extracted with dichloromethane (3 x 30 ml). The organic extracts were combined, concentrated under reduced pressure and purified by column chromatography (EtOAc/Hexane: 0/100 → 3/97) to yield an off-white solid (250 mg, 34.1%). R$_f$ = 0.80 (EtOAc/Hexane = 20/80) $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 10.1 (s, 1H), 8.22 (d, J = 8.5 Hz, 2H), 7.97 (d, J = 8.5 Hz, 2H) 4.06 (s, 2H), 1.06 (s, 9H).

(28). 3,3-dimethylbutanoyl chloride: To a solution of chloroform (20 ml, 99.8% Anh), 3,3-dimethylbutanoic acid (1.05 g, 9.0 mmol, 1.1 equiv) and thionyl chloride (1.17 g, 9.82 mmol, 1.2 equiv) were added and refluxed with stirring at 65° C for 2 hours. This acid chloride was then directly used in the following reaction (31).

(31). 4-formylphenyl 3,3-dimethylbutanoate: The mixture from (28) was cooled to 0° C and transferred to another flask containing a solution of 4-hydroxybenaldehyde (1 g, 8.19 mmol, 1 equiv) and triethyl amine (1.65 g, 16.4 mmol, 2 equiv) in chloroform (20 ml) and stirred at room temperature for 12-15 hours. The resulting mixture cooled to 0° C, washed with brine (20 ml), extracted with chloroform (3 x 30 ml), and purified by column chromatography (EtOAc/Hexane: 0/100 → 3/97) to yield a white solid (800 mg, 44.3%). R$_f$ = 0.80 (EtOAc/Hexane = 20/80) $^1$H NMR
(500 MHz, CDCl₃) δ 9.99 (s, 1H), 7.92 (d, J = 9 Hz, 2H), 7.27 (d, J = 9 Hz, 2H) 2.47 (s, 2H), 1.15 (s, 9H).

**Povarov: General Procedure (Room Temperature Reactions):**

(5). *methyl*(4-((8-sulfamoyl-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-4-yl)phenyl)carbamate:

Cyclopentadiene (221 mg, 3.34 mmol, 3 equiv) was added to a mixture of *methyl* (4-formylphenyl)carbamate (200 mg, 1.12 mmol, 1 equiv), 4-aminosulfonamide (192 mg, 1.12 mmol, 1 equiv), and indium trichloride (74.1 mg, 0.335 mmol, 0.3 equiv), in acetonitrile (20 ml) and stirred at room temperature for 24 hours. Acetonitrile was removed; the reaction mixture was quenched with aqueous Na₂CO₃ (20%, 30 ml) and extracted with dichloromethane (3 x 30 ml). The organic layers were combined, dried over sodium sulfate, and concentrated under reduced pressure. Pure *cis*-isomer precipitated out as a white solid upon treating the residue with dichloromethane (10 ml) (231 mg, 51.9% yield). Rf = 0.57 (EtOAc/Hexane = 50/50, 2 runs) ¹H NMR (500 MHz, DMSO) δ 8.31 (s, 1H), 7.99 (d, J = 8.5 Hz, 2H) 7.61 (d, J = 8.5 Hz, 2H), 7.45 (d, J = 1.5 Hz, 1H), 7.35 (dd, J = 8.5 Hz, 2 Hz, 1H), 6.95 (s, 2H), 6.82 (d, J = 8 Hz, 1H), 6.46 (s, 1H), 5.92-5.87 (m, 1H), 5.61 (d, J = 6 Hz, 1H), 4.73 (d, J = 2.5 Hz, 1H), 4.09 (br d, J = 8.5 Hz, 1H), 3.86 (s, 2H) 2.98 (br q, J = 9.5 Hz, 1H), 2.39-2.30 (m, 1H), 1.60 (dd, J = 16.5 Hz, 8.5 Hz, 1H)

(6). *ethyl*(4-((8-sulfamoyl-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-4-yl)phenyl)carbamate:

Cyclopentadiene (308 mg, 4.66 mmol, 3 equiv) was added to a mixture of *ethyl* (4-formylphenyl)carbamate (300 mg, 1.55 mmol, 1 equiv), 4-aminosulfonamide (268 mg, 1.55 mmol,
1 equiv), and indium trichloride (103 mg, 0.466 mmol, 0.3 equiv), in acetonitrile (20 ml) and stirred at room temperature for 24 hours. Acetonitrile was removed and the reaction mixture was quenched with aqueous Na$_2$CO$_3$ (20%, 20 ml) and extracted with dichloromethane (3 x 30 ml). The organic layers were combined, dried over sodium sulfate, concentrated under reduced pressure and purified by column chromatography (EtOAc/Hexane: 20/80 → 50/50) to yield a mixture of cis and trans isomers (131 mg, 20.3%). R$_f$ = 0.60 (EtOAc/Hexane = 50/50, 2 runs)

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.59 (d, J = 1.5 Hz, 1H) 7.52 (dd, J = 8 Hz, 2.5 Hz, 1H), 7.41 (d, J = 8.5 Hz, 2H), 7.34 (d, J = 8.5 Hz, 2H), 6.65 (d, J = 8.5 Hz, 1H), 6.59 (s, 1H), 5.91-5.86 (m, 1H), 5.69 (d, J = 4.5 Hz, 1H), 4.68 (d, J = 3 Hz, 1H), 4.61 (s, 2H), 4.25 (q, J = 7 Hz, 2H), 4.19 (s, 1H), 4.10 (d, J = 8 Hz, 1H), 2.99 (qd, J = 15 Hz, 7 Hz, 2.5 Hz, 1H), 2.59-2.51 (m, 1H), 1.84 (dd, J = 13 Hz, 5.5 Hz, 1H), 1.33 (t, J = 7 Hz, 3H)

(7).

**isopropyl(4-(8-sulfamoyl-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-4-yl)phenyl)carbamate:** Cyclopentadiene (48 mg, 0.723 mmol, 3 equiv) was added to a mixture of isopropyl (4-formylphenyl)carbamate (50 mg, 0.241 mmol, 1 equiv), 4-aminosulfonamide (42 mg, 0.241 mmol, 1 equiv), and indium trichloride (16 mg, 0.0723 mmol, 0.3 equiv), in acetonitrile (10 ml) and stirred at room temperature for 24 hours. Acetonitrile was removed, and the reaction mixture was quenched with aqueous Na$_2$CO$_3$ (20%, 10 ml) and extracted with dichloromethane (3 x 15 ml). The organic layers were combined, dried over sodium sulfate, concentrated under reduced pressure and purified by column chromatography (EtOAc/Hexane: 20/80 → 50/50) to yield a mixture of cis and trans isomers (20 mg, 19.4%). R$_f$ = 0.67 (EtOAc/Hexane = 50/50, 2 runs)$^1$H NMR (500 MHz, DMSO) δ 9.55 (s, 1H) 7.46 (d, J = 9 Hz, 2H) 7.42 (d, J = 2 Hz, 1H), 7.35-7.30 (comp, 3H), 6.95 (s, 2H), 6.79 (d, J = 8.5 Hz, 1H), 6.31 (s, 1H), 5.90-5.84 (m, 1H), 5.62 (d, J = 5.5 Hz,
1H), 4.89 (septet, J = 6.5 Hz, 1H), 4.56 (d, J = 3.5 Hz, 1H), 4.05 (d, J = 8.5 Hz, 1H) 3.86 (s, 2H) 2.90 (br q, J = 8 Hz, 1H), 2.42-2.33 (m, 1H), 1.66 (dd, J = 15 Hz, 8.5 Hz, 1H), 1.26 (d, J = 6.5 Hz, 6H). Rf = 0.67 (EtOAc/Hexane = 50/50, 2 runs)

(8).  *cyclohexyl*(4-({sulfamoyl-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-4-yl})phenyl)carbamate: Cyclopentadiene (160 mg, 0.243 mmol, 3 equiv) was added to a mixture of cyclohexyl (4-formylphenyl)carbamate (200 mg, 0.809 mmol, 1 equiv), 4-aminosulfonamide (139 mg, 0.809 mmol, 1 equiv), and indium trichloride (54 mg, 0.243 mmol, 0.3 equiv), in acetonitrile (15 ml) and stirred at room temperature for 24 hours. Acetonitrile was removed, and the reaction mixture was quenched with aqueous Na$_2$CO$_3$ (20%, 10 ml) and extracted with dichloromethane (3 x 20 ml). The organic layers were combined, dried over Na$_2$SO$_4$, concentrated under reduced pressure and purified by column chromatography (EtOAc/Hexane: 20/80 → 50/50) to yield a mixture of cis and trans isomers (160 mg, 42.3%) Rf = 0.67 (EtOAc/Hexane = 50/50, 2 runs) $^1$H NMR (500 MHz, Acetone-D6) δ 8.57 (s, 1H), 7.58 (d, J = 9 Hz, 2H) 7.58 (d, J = 9 Hz, 2H), 7.54 (d, J = 2 Hz, 1H), 7.44 (dd, J = 9 Hz, 2.5 Hz, 1H), 7.39 (d, J = 8.5 Hz, 2H), 6.87 (d, J = 8 Hz, 1H), 6.19 (s, 2H), 5.94-5.89 (m, 1H), 5.65 (d, J = 4.5 Hz, 1H), 5.53 (s, 1H), 4.72-4.65 (comp, 2H) 4.12 (br d, J = 8.5 Hz, 1H) 3.07-2.99 (m, 1H), 2.57-2.49 (m, 1H), 1.95-1.22 (comp, 11H)

(9).  *adamantan-1-yl* (4-({sulfamoyl-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-4-yl})phenyl)carbamate: Cyclopentadiene (199 mg, 3.01 mmol, 3 equiv) was added to a mixture of adamantan-1-yl (4-formylphenyl)carbamate (300 mg, 1.002 mmol, 1 equiv), 4-aminosulfonamide (173 mg, 1.002 mmol, 1 equiv), and indium trichloride (67 mg, 0.3006 mmol, 0.3 equiv), in acetonitrile (15 ml) and stirred at room temperature for 24 hours. Acetonitrile was removed and
the reaction mixture was quenched with aqueous Na₂CO₃ (20%, 15 ml) extracted with dichloromethane (3 x 30 ml) and washed with brine. The organic extracts were consolidated, dried over Na₂SO₄, concentrated under reduced pressure and purified by column chromatography (EtOAc/Hexane: 20/80 → 40/60) to yield a mixture of cis and trans isomers (102.8 mg, 32.1%). Rf = 0.67 (EtOAc/Hexane = 50/50, 2 runs) ¹H NMR (500 MHz, CDCl₃) δ 7.59 (d, J = 2 Hz, 1H) 7.51 (dd, J = 8 Hz, 2 Hz, 1H), 7.38 (d, J = 9 Hz, 2H), 7.31 (d, J = 8.5 Hz, 1H) 6.64 (d, J = 8.5 Hz, 1H) 6.54 (s, 1H), 5.90-5.85 (m, 1H), 5.68 (d, J = 5 Hz, 1H), 4.70 (s, 2H), 4.65 (d, J = 3.5 Hz, 1H), 4.19 (s, 1H), 4.09 (br d, J = 10 Hz, 1H) 3.02-2.94 (m, 1H), 2.58-2.50 (m, 1H), 2.24-2.14 (comp, 9H), 1.87-1.79 (m, 1H), 1.73-1.64 (comp, 6H)

(10). adamantan-2-yl (4-(8-sulfamoyl-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-4-yl)phenyl)carbamate: Cyclopentadiene (199 mg, 3.01 mmol, 3 equiv) was added to a mixture of adamantan-2-yl (4-formylphenyl)carbamate (300 mg, 1.002 mmol, 1 equiv), 4-aminosulfonamide (173 mg, 1.002 mmol, 1 equiv), and indium trichloride (67 mg, 0.3006 mmol, 0.3 equiv), in acetonitrile (10 ml) and stirred at room temperature for 24 hours. Acetonitrile was removed; the reaction mixture was quenched with aqueous Na₂CO₃ (20%, 15 ml) extracted with dichloromethane (3 x 30 ml) and washed with brine. The organic extracts were consolidated, dried over Na₂SO₄, concentrated under reduced pressure and purified by column chromatography (EtOAc/Hexane: 20/80 → 40/60) to yield a mixture of cis and trans isomers (134.7 mg, 25.8%) Rf = 0.67 (EtOAc/Hexane = 50/50, 2 runs) ¹H NMR (500 MHz, CDCl₃) δ 7.59 (d, J = 1.5 Hz, 1H) 7.52 (dd, J = 8.5 Hz, 2.5 Hz, 1H), 7.43 (d, J = 8 Hz, 2H), 7.34 (d, J = 8 Hz, 2H), 6.68 (s, 1H) 6.65 (d, J = 8 Hz, 1H), 5.91-5.86 (m, 1H), 5.68 (d, J = 5 Hz, 1H), 4.94 (m, 1H), 4.67 (s, 2H), 4.20 (s, 1H) 4.09 (d, J = 5.5 Hz, 1H) 2.99 (qd, J = 17.5 Hz, 8.5 Hz, 3 Hz, 1H), 2.59-2.50 (m, 1H), 2.13-1.60 (comp, 15H)
(11). **4-(8-sulfamoyl-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-4-yl)phenyl tert-butylcarbamate**: Cyclopentadiene (269 mg, 4.07 mmol, 3 equiv) was added to a mixture of 4-formylphenyl tert-butylcarbamate (300 mg, 1.36 mmol, 1 equiv), 4-aminosulfonamide (234 mg, 1.36 mmol, 1 equiv), and indium trichloride (90 mg, 0.407 mmol, 0.3 equiv), in acetonitrile (20 ml) and stirred at room temperature for 24 hours. Acetonitrile was removed, and the reaction mixture was quenched with aqueous Na$_2$CO$_3$ (20%, 10 ml) and extracted with dichloromethane (3 x 20 ml). The organic layers were combined, dried over Na$_2$SO$_4$, concentrated under reduced pressure and purified by column chromatography (EtOAc/Hexane: 20/80 → 50/50) to yield a mixture of cis- and trans-isomers (118 mg, 19.7%) $R_f$ = 0.67 (EtOAc/Hexane = 50/50, 2 runs) $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.59 (d, $J$ = 1.5 Hz, 1H) 7.50 (dd, $J$ = 8 Hz, 1.5 Hz, 1H), 7.39 (d, $J$ = 8 Hz, 2H), 7.13 (d, $J$ = 8 Hz, 2H), 6.63 (d, $J$ = 8.5 Hz, 1H) 5.90-5.85 (m, 1H), 5.67 (d, $J$ = 4 Hz, 1H), 5.03 (s, 1H), 4.78 (s, 2H), 4.68 (d, $J$ = 3 Hz, 1H), 4.23 (s, 1H), 4.09 (br d, $J$ = 8.5 Hz, 1H) 2.98 (qd, $J$ = 17.5 Hz, 8.5 Hz, 2 Hz, 1H), 2.58-2.50 (m, 1H), 1.85 (dd, $J$ = 15 Hz, 7 Hz, 1.5 Hz, 1H), 1.40 (s, 9H)

(12). **3,3-dimethyl-N-(4-(8-sulfamoyl-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-4-yl)phenyl)butanamide**: 3,3-dimethylbutanoyl chloride (49 mg, 0.366 mmol, 0.83 equiv) and 4-amino TQS (150 mg, 0.439 mmol, 1 equiv) were anhydrously transferred to a flask containing dry acetone (20 ml) and pyridine (34.4 mg, 0.435 mmol, 0.99 equiv) and stirred at room temperature for 12-15 hours. After evaporating acetone, the reaction mixture was washed with saturated ammonium chloride (10 ml) and extracted with ethyl acetate (3 x 20 ml). The ethyl acetate layers were consolidated, and concentrated under reduced pressure. The product precipitated out as a white solid upon adding DCM (5-10 ml) (113 mg, 58.6%) $R_f$ = 0.37 (EtOAc/Hexane = 40/60, 3
runs) $^1$H NMR (500 MHz, DMSO) δ 9.80 (s, 1H) 7.60 (d, $J = 8.5$ Hz, 2H) 7.43 (d, $J = 1.5$ Hz, 1H), 7.36 (d, $J = 9$ Hz, 2H), 7.33 (dd, $J = 8$ Hz, 2 Hz, 1H) 6.96 (s, 2H), 6.81 (d, $J = 9$ Hz, 1H), 6.32 (s, 1H), 5.92-5.86 (m, 1H), 5.63 (d, $J = 5$ Hz, 1H), 4.58 (d, $J = 3$ Hz, 1H), 4.07 (d, $J = 9$ Hz, 1H), 2.92 (br q, $J = 7.5$ Hz, 1H), 2.44-2.35 (m, 1H), 2.20 (s, 2H), 1.68 (dd, $J = 15$ Hz, 8.5 Hz, 1H), 1.04 (s, 9H)

(14). 4-(4-(3-bromopropyl)phenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide:
Cyclopentadiene (1.91 g, 28.8 mmol, 3 equiv) was added to a mixture of 4-(3-bromopropyl)benzaldehyde (2.0 g, 9.62 mmol, 1 equiv), 4-aminosulfonamide (1.66 g, 9.62 mmol, 1 equiv), and indium trichloride (638 mg, 2.88 mmol, 0.3 equiv), in acetonitrile (50 ml) and stirred at room temperature for 24 hours. Acetonitrile was removed and the reaction mixture was quenched with aqueous Na$_2$CO$_3$ (20%, 30 ml) and extracted with dichloromethane (3 x 30 ml). The organic layers were combined, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. Pure cis-isomer precipitated out as a pink solid upon treating the residue with dichloromethane (3 x 10 ml) (1.16 g, 26.9% yield). R$_f$ = 0.60 (EtOAc/Hexane = 40/60, 2 runs) $^1$H NMR (500 MHz, DMSO) δ 7.43 (s, 1H) 7.37 (d, $J = 8.5$ Hz, 2H), 7.32 (dd, $J = 9$ Hz, 2.5 Hz, 1H), 7.24 (d, $J = 8.5$ Hz, 2H) 6.95 (s, 2H), 6.79 (d, $J = 8$ Hz, 1H), 6.34 (s, 1H), 5.91-5.85 (m, 1H), 5.62 (d, $J = 5$ Hz, 1H), 4.60 (d, $J = 3$ Hz, 1H), 4.06 (br d, $J = 8.5$ Hz, 1H), 2.92 (br q, $J = 8.5$ Hz, 1H), 2.72 (t, $J = 7$ Hz, 2H), 2.43-2.32 (m, 1H), 2.10 (quintet, $J = 7$ Hz, 2H), 1.65 (dd, $J = 16$ Hz, 9.5 Hz, 1H)

(15). neopentyl4-(8-sulfamoyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-4-yl)benzoate:
Cyclopentadiene (225 mg, 3.41 mmol, 3 equiv) was added to a mixture of neopentyl 4-formylbenzoate (250 mg, 1.14 mmol, 1 equiv), 4-aminosulfonamide (196 mg, 1.14 mmol, 1 equiv), and indium trichloride (75 mg, 0.341 mmol, 0.3 equiv), in acetonitrile (20 ml) and stirred at room
temperature for 24 hours. Acetonitrile was removed and the reaction mixture was quenched with aqueous Na$_2$CO$_3$ (20%, 20 ml) and extracted with dichloromethane (3 x 20 ml). The organic layers were combined, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. Pure cis-isomer precipitated out as a pink, crystalline solid upon treating the residue with dichloromethane (2 x 10 ml) (312 mg, 62.3% yield). R$_f$ = 0.50 (EtOAc/Hexane = 40/60, 2 runs) $^1$H NMR (500 MHz, DMSO) $\delta$ 8.01 (d, J = 8.5 Hz, 2H) 7.62 (d, J = 8.5 Hz, 2H), 7.45 (d, J = 2 Hz, 1H), 7.35 (dd, J = 8 Hz, 2H, 1H), 6.97 (s, 2H), 6.82 (d, J = 9 Hz, 1H), 6.46 (s, 1H), 5.93-5.87 (m, 1H), 5.62 (d, J = 6 Hz, 1H), 4.73 (d, J = 2.5 Hz, 1H), 4.10 (br d, J = 8 Hz, 1H), 4.02-3.96 (comp, 2H), 2.98 (br q, J = 8.5 Hz, 1H), 2.40-2.31 (m, 1H), 1.65-1.57 (m, 1H), 1.01 (s, 9H)

(16). 4-(8-sulfamoyl-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-4-yl)phenyl 3,3-dimethylbutanoate: Cyclopentadiene (225 mg, 3.41 mmol, 3 equiv) was added to a mixture of 4-formylphenyl 3,3-dimethylbutanoate (250 mg, 1.14 mmol, 1 equiv), 4-aminosulfonamide (196 mg, 1.14 mmol, 1 equiv), and indium trichloride (75.3 mg, 0.341 mmol, 0.3 equiv), in acetonitrile (20 ml) and stirred at room temperature for 24 hours. Acetonitrile was removed, and the reaction mixture was quenched with aqueous Na$_2$CO$_3$ (20%, 20 ml) and extracted with dichloromethane (3 x 20 ml). The organic layers were combined, dried over Na$_2$SO$_4$, concentrated under reduced pressure and purified by column chromatography (EtOAc/Hexane: 20/80 → 50/50) to yield the cis-isomer as a brown, crystalline solid (392 mg, 78.4% yield). R$_f$ = 0.50 (EtOAc/Hexane = 40/60, 2 runs) $^1$H NMR (500 MHz, DMSO) $\delta$ 7.49 (d, J = 8.5 Hz, 2H) 7.45 (d, J = 2 Hz, 1H), 7.34 (dd, J = 9 Hz, 2.5 Hz, 1H), 7.13 (d, J = 9 Hz, 2H, 1H), 6.97 (s, 2H), 6.81 (d, J = 9 Hz, 1H), 6.41 (s, 1H), 5.93-5.88 (m, 1H), 5.64 (d, J = 5.5 Hz, 1H), 4.66 (d, J = 3 Hz, 1H), 4.08 (br d, J = 8 Hz, 1H), 2.95 (br q, J = 8 Hz, 1H), 2.46 (s, 2H), 2.44-2.36 (m, 1H), 1.72-1.63 (m, 1H), 1.10 (s, 9H)
Povarov: (Microwave Synthesis):

(24). 4-(4-(4,4-dimethylpentyl)phenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide: Cyclopentadiene (194 mg, 2.94 mmol, 3 equiv) was added to a microwave vial containing a mixture of 4-(3,3-dimethylbutyl)benzaldehyde (200 mg, 0.979 mmol, 1 equiv), 4-aminosulfonamide (169 mg, 0.979 mmol, 1 equiv), and indium trichloride (65 g, 0.294 mmol, 0.3 equiv), in acetonitrile (5 ml) and heated to 100° C for 15 minutes. After cooling down, the reaction mixture was quenched with aqueous Na₂CO₃ (20%, 15 ml) extracted with dichloromethane (3 x 30 ml) and washed with brine. The organic extracts were consolidated, dried over Na₂SO₄, concentrated under reduced pressure and purified by column chromatography (EtOAc/Hexane: 20/80 → 40/60) to yield a mixture of cis and trans isomers (40 mg, 9.6%). Rf = 0.50 (Acetone/Hexane = 40/60, 2 runs) ¹H NMR (500 MHz, CDCl₃) δ 7.60 (d, J = 1.5 Hz, 1H) 7.52 (dd, J = 8 Hz, 2 Hz, 1H), 7.31 (d, J = 7.5 Hz, 2H), 7.21 (d, J = 8 Hz, 2H), 6.64 (d, J = 8.5 Hz, 1H), 5.91-5.86 (m, 1H), 5.71-5.66 (m, 1H), 4.69 (d, J = 3.5 Hz, 1H), 4.66 (s, 2H), 4.22 (s, 1H) 4.11 (d, J = 8.5 Hz, 1H), 3.01 (br q, J = 9 Hz, 1H), 2.59 (t, J = 7.5 Hz, 2H), 2.61-2.52 (m, 1H), 1.90-1.82 (m, 1H), 1.62-1.54 (comp, 2H), 1.27-1.20 (comp, 1H), 0.88 (s, 9H)

(13). 4-(8-sulfamoyl-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-4-yl)benzoic acid: Cyclopentadiene (264 mg, 3.99 mmol, 3 equiv) was added to a microwave vial containing a mixture of 4-formylbenzoic acid (200 g, 1.33 mmol, 1 equiv), 4-aminosulfonamide (230 mg, 1.33 mmol, 1 equiv), and indium trichloride (89 mg, 0.399 mmol, 0.3 equiv), in acetonitrile (7 ml) and heated to 100° C for 15 minutes. After cooling down, the solvent was evaporated, and the reaction
mixture was stirred in ethyl acetate. A suspension was formed, which was then filtered to yield the cis-isomer as a brown solid (277 mg, 56.1%). \( R_f = 0.05 \) (EtOAc/Hexane = 0/100, 2 runs) 1H NMR (500 MHz, DMSO) \( \delta \) 12.92 (s, 1H) 7.97 (d, \( J = 8.5 \) Hz, 2H) 7.58 (d, \( J = 8.5 \) Hz, 2H), 7.44 (d, \( J = 2 \) Hz, 1H), 7.34 (dd, \( J = 8.5 \) Hz, 1H), 6.97 (s, 2H), 6.82 (d, \( J = 8.5 \) Hz, 1H), 6.46 (s, 1H), 5.93-5.87 (m, 1H), 5.62 (d, \( J = 5 \) Hz, 1H), 4.72 (d, \( J = 3 \) Hz, 1H), 4.09 (br d, \( J = 8 \) Hz, 1H), 2.98 (br q, \( J = 8 \) Hz, 1H), 2.40-2.31 (m, 1H), 1.61 (dd, \( J = 15.5 \) Hz, 9 Hz, 1H)

**Experimental procedure for in vitro testing:** Supplementary Information, (Thakur et al, 2013)

**cDNA clones and RNA**

The human \( \alpha_7 \) nAChR receptor clone was obtained from Dr. Jon Lindstrom (University of Pennsylvania, Philadelphia, PA), and the RIC-3 clone from Dr. Millet Treinin (Hebrew University, Jerusalem, Israel) for the purpose of co-injection with \( \alpha_7 \) to improve the level and speed of receptor expression (Halevi et al, 2003). After linearization and purification of cloned cDNA's, RNA transcripts were prepared using the appropriate mMessage mMachine kit from Ambion (Austin, TX).

**Expression in *X. laevis* oocytes.**

Oocytes were obtained from mature (>9cm) female *Xenopus laevis* African frogs (Nasco, Ft. Atkinson, WI). Frogs were anesthetized in 0.7 g/L solution of ethyl 3-aminobenzoate
methanesulfonate buffered with sodium bicarbonate, and oocytes were surgically removed through an abdominal incision. Harvested oocytes were treated with 1.25 mg/ml collagenase (Worthington Biochemicals, Freehold, NJ) in calcium-free Barth’s solution (88 mM NaCl, 1 mM KCl, 2.38 mM NaHCO3, 0.82 mM MgSO4, 15 mM HEPES, 12 mg/L tetracycline, pH 7.6) for 3-4 hours to remove the follicular layer. Stage-5 oocytes were isolated and injected with 50 nl (5-20 ng) of human α7 and RIC-3 cRNA. Suitable levels of receptor expression were typically achieved 2-6 days after injection of cRNA. For experiments involving the PAM 4BP-TQS/GAT-107, where standard levels of expression result in ion currents too large to be recorded in voltage clamp, experiments were typically conducted 1-3 days after RNA injection.

**Electrophysiology.**

Two-electrode voltage clamp experiments were conducted using OpusXpress6000A (Molecular Devices, Sunnyvale, CA), an integrated system that provides automated impalement and voltage clamp of up to eight oocytes in parallel. Both the current and voltage electrodes were filled with 3 M KCl, and oocytes were clamped at a holding potential of -60 mV. Data were collected at 50 Hz and filtered at 20 Hz. The oocytes were bath-perfused with Ringer’s solution (115 mM NaCl, 10 mM HEPES, 2.5 mM KCl, 1.8 mM CaCl2, pH 7.3), and agonist solutions were delivered from a 96-well drug plate using disposable tips. Flow rates were set at 2 ml/min, with each drug or control application delivered in 12 s durations followed by 181 s washouts with Ringer’s unless noted otherwise.

**Experimental Protocols and Data Analysis.**
Responses of human α7 receptors to agonists were measured as both peak current and net charge measured over a 120 s period beginning with the compound application. Note that for data obtained in the absence of PAMs, net charge data are a more reliable measurement of response since the fast desensitization, characteristic of α7 results in the peak current being reached before agonist solution exchange is complete, giving values which do not correspond to activation produced by the ligand at the final concentration applied. In experiments involving positive allosteric modulators (PAM), the fast desensitization of α7 is eliminated, allowing peak currents is to be used as a valid measurement of the receptor-mediated responses (Wang et al., 2012; Williams et al., 2011). Oocytes received two control ACh applications prior to receiving any drug in order to establish a steady reference response, and they received one or more control ACh applications at the end of all experiments.

For experiments involving drug incubation through bath applications, all responses were normalized to the average of two ACh control responses taken immediately prior to the switch of bath solution, for each cell individually. These normalization procedures had the effect of compensating for differing levels of receptor expression among the multiple oocytes used in each experiment. Each experiment was conducted on at least four oocytes, with mean values and standard errors (S.E.M.) calculated from their normalized responses.
REFERENCES


6. Potter, A. S.; Newhouse, P. A.; Bucci, D. J. Central nicotinic cholinergic systems: A role in the cognitive dysfunction in Attention-Deficit/Hyperactivity Disorder? *Behavioural Brain Research* 2006, 175, 201-211.


34. Fisher, J. L.; Dani, J. A. Nicotinic receptors on hippocampal cultures can increase synaptic glutamate currents while decreasing the NMDA-receptor component. *Neuropharmacology* 2000, 39, 2756-69.


