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A series of α7 nicotinic acetylcholine receptor allosteric modulators with close chemical similarity but diverse pharmacological properties

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Running title: Allosteric modulation of the α7 nicotinic receptor

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Abbreviations: nAChR, nicotinic acetylcholine receptor; **2BP-TQS**, 4-(2-bromophenyl)-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide; **2N-TQS**, 4-(naphthalen-2-yl)-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide; **3BP-TQS**, 4-(3-bromophenyl)-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide; **3IP-TQS**, 4-(3-iodophenyl)-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide; **3,4BP-TQS**, 4-(3,4-dibromophenyl)-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide; **4BP-TQS**, 4-(4-bromophenyl)-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide; **4CP-TQS**, 4-(4-chloroophenyl)-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide; **4FP-TQS**, 4-(4-fluorophenyl)-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide; **4HP-TQS**, 4-(4-hydroxyphenyl)-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide; **4IP-TQS**, 4-(4-iodoophenyl)-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide; **4MP-TQS**, 4-p-tolyl-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide; **4TF-TQS**, 4-(4-(trifluoromethyl)phenyl)-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide; **P-**

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TQS, 4-phenyl-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide; **TQS**, 4-(napthalen-1-yl)-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulphonamide

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Abstract

Acetylcholine activates nicotinic acetylcholine receptors (nAChRs) by binding to an extracellular site located at the interface of two adjacent subunits. In contrast, recent studies have provided evidence that positive allosteric modulators (PAMs) such as TQS and allosteric agonists such as 4BP-TQS interact at an intra-subunit transmembrane site. Here, we describe the synthesis and pharmacological characterization of a series of chemically related allosteric modulators of the α7 nAChR. Minimal changes in the chemical structure of these compounds have been found to exert profound effects on their pharmacological properties. For example, compounds containing a bromine atom at either the *ortho* or *meta* position on the phenyl ring (2BP-TQS and 3BP-TQS), rather than the *para* position (4BP-TQS), display no allosteric agonist activity but retain PAM activity on α7 nAChRs, demonstrating the importance of the location of the halogen atom upon pharmacological properties. Replacement of the bromine atom in 4BP-TQS with either a chlorine or iodine atom (4CP-TQS and 4IP-TQS) results in compounds with pharmacological properties characteristic of allosteric agonists but which display differences in activation rates, inactivation rates and in levels of desensitization. In contrast, replacement of the bromine atom in 4BP-TQS with a fluorine atom (4FP-TQS) generated a compound that lacks allosteric agonist activity but which acts a potentiator of responses to acetylcholine. In addition, 4FP-TQS was found to act as an antagonist of responses evoked by allosteric agonists such as 4BP-TQS. These findings provide evidence of the pharmacological diversity of compounds interacting with the allosteric transmembrane site on α7 nAChRs.

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Introduction

Nicotinic acetylcholine receptors (nAChRs) are transmembrane receptors for the neurotransmitter acetylcholine. They are members of structurally related family of 'Cys-loop' ligand-gated ion channels that also includes receptors for neurotransmitters such as 5-hydroxytryptamine (5-HT; serotonin), γ-amino butyric acid (GABA) and glycine (Lester et al., 2004). In common with other members of the Cys-loop family of receptors, nAChRs are pentameric complexes in which the conventional 'orthosteric' agonist binding site is located at an extracellular location at the interface of two subunits (Arias, 2000; Unwin, 2005).

Nicotinic receptors comprise a diverse family of receptors. In vertebrates, seventeen subunits have been identified that assemble into a large number of receptor subtypes with distinct subunit composition (Millar and Gotti, 2009). In addition to the well characterized nAChRs expressed at the mammalian neuromuscular junction, a diverse family of neuronal nAChRs are expressed in the mammalian central and peripheral nervous system (Millar and Gotti, 2009). Muscle nAChRs and most neuronal nAChRs are heteromeric complexes of more than one type of subunit. In addition, some neuronal nAChR subunits, such as α7, are able to form functional homomeric nAChRs (Couturier et al., 1990).

A considerable amount of effort has been devoted to the characterization of compounds (including agonists and competitive antagonists) that interact with the conventional orthosteric binding site of nAChRs (Jensen et al., 2005; Arneric et al., 2007; Haydar and Dunlop, 2010). In addition, studies have begun to explore the pharmacological diversity of compounds acting at allosteric binding sites (Bertrand and Gopalakrishnan, 2007; Haydar and Dunlop, 2010; Williams et al., 2011). For example, recent studies have revealed that allosteric modulators of α7 nAChRs have cognitive enhancing effects that may potentially have use in the treatment of neurological and psychiatric disorders such as Alzheimer's disease and schizophrenia (Ng et al., 2007; Timmermann et al., 2007; Haydar and Dunlop, 2010).

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The term positive allosteric modulator (PAM) has been used to describe compounds that act at a site that is distinct from the orthosteric agonist binding site (Bertrand and Gopalakrishnan, 2007). Typically, such compounds lack agonist activity themselves but potentiate responses evoked by conventional agonists such as acetylcholine. Two types of α7-selective PAMs (type I and type II) have been identified (Bertrand and Gopalakrishnan, 2007). Both types potentiate peak agonist-induced responses but they have different effects on the rate of agonist-induced receptor desensitization. Type I PAMs have little or no effect on the rapid rate of desensitization that is characteristic of α7 nAChRs, whereas type II PAMs cause a dramatic slowing of receptor desensitization. Recently, evidence has emerged to demonstrate that compounds acting at an allosteric site can activate α7 nAChRs in the absence of conventional agonists. Such compounds have been described as nAChR allosteric agonists (Gill et al., 2011), to distinguish them from agonists that act at the classical extracellular orthosteric site. In many respects, such compounds are analogous to the allosteric agonists (sometimes referred as ago-allosteric compounds) that have been described for G-protein coupled receptors (Langmead and Christopoulos, 2006; Schwartz and Holst, 2006). It is probable that nAChRs contain a variety of distinct allosteric binding sites (Taly et al., 2009) but recent studies have provided evidence that one such site is located in an intra-subunit cavity located between the four α-helical transmembrane domains of a single α7 subunit (Young et al., 2008; Gill et al., 2011). In addition, there is evidence that this is a site at which allosteric agonists, as well as both type I and type II potentiators can act (Young et al., 2008; Collins et al., 2011; Gill et al., 2011).

Here, we have examined the pharmacological properties of a series of compounds (Fig. 1), all with close chemical similarity to two previously described α7-selective allosteric modulators: TQS, a PAM displaying no agonist activity (Grønlien et al., 2007; Gill et al., 2011) and 4BP-TQS a potent non-desensitizing allosteric agonist (Gill et al., 2011). We have found that small changes to the structure of these compounds (for example, changing the

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position or nature of a single halogen atom) can result in dramatic changes in pharmacological properties. These pharmacologic effects include the loss of allosteric agonist activity (whilst retaining PAM activity) and more subtle changes in allosteric agonist activation rates, inactivation rate and desensitization rates.

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Materials and Methods

Chemical synthesis

TQS compounds were prepared by $InCl_3$-catalysed reaction of sulfanilamide, cyclopentadiene and the corresponding substituted benzaldehyde according to methods described previously (Becker et al., 2004). In all cases, the cis-cis diastereoisomer was obtained as shown in Figure 1. Details concerning the synthesis of these compounds is provided (Supplemental Figure 1).

Subunit cDNAs and plasmid expression vectors. All experiments were performed with human α7 nAChRs. Human wild type and mutant (M253L) nAChR α7 subunit cDNA constructs in plasmid pSP64GL have been described previously (Broadbent et al., 2006; Gill et al., 2011).

***Xenopus* oocyte electrophysiology.** *Xenopus laevis* oocytes were isolated and defolliculated as described previously (Young et al., 2007) by treatment with collagenase (2 mg/ml; Worthington) in calcium-free Barth's solution containing 88 mM NaCl, 2.4 mM $NaHCO_3$, 1 mM KCl, 0.82 mM $MgSO_4$ and 15 mM Hepes pH 7.6. To express human α7 nAChRs, *in vitro* transcribed cRNA was injected into the oocyte cytoplasm. *In vitro* transcription of cRNA was carried out using mMESSAGE mMACHINE SP6 transcription kit (Ambion, Huntington, UK). Oocytes were injected with 6-12 ng cRNA per oocyte in a volume of 32.2 nl using a Drummond variable volume microinjector. After injection, oocytes were incubated at 18ºC in a calcium-containing Barth's solution (composition, as above, but with 0.77 mM $CaCl_2$) supplemented with antibiotics (100 units/ml penicillin, 100 µg/ml streptomycin, 4 µg/ml kanamycin and 50 µg/ml tetracycline). Experiments were performed on oocytes after 3-5 days of incubation. Oocytes were placed in a recording chamber and continuously perfused with a saline solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 10 mM Hepes, pH 7.3) with a flow rate of approximately 15 ml/min. Two electrode voltage-clamp

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recordings were performed (with the oocyte membrane potential held at -60mV), as described previously (Young et al., 2007) using a Warner Instruments OC-725C amplifier (Harvard Apparatus, Edenbridge, UK), PowerLab 8SP and Chart 5 software (AD Instruments, Oxford, UK). Agonists and PAMs were applied to oocytes using a BPS-8 solenoid valve solution exchange system (ALA Scientific Inc., Westbury, NY). For multiple comparisons, statistical significance was determined by ANOVA with Tukey's post-hoc test. Student's unpaired *t*-tests were used for pairwise comparisons.

All 'TQS' compounds were dissolved in dimethyl sulfoxide (to generate a 100 mM stock solution) prior to dilution into saline recording solution. Due to their limited solubility in aqueous solution, the maximum concentration that could be tested was 100 μM for all compounds. A problem we have encountered is that all of these compounds have a tendency to stick to plastic tubing and to plastic apparatus used for oocyte perfusion. This was found to be a particular problem with silicone tubing. We have found that the problem could be reduced somewhat by using polyurethane tubing (e.g. type 502109-15; World Precision Instruments Inc, Sarasota, USA) rather than silicone tubing but it was still necessary to rinse all perfusion tubing (and also the Perspex recording chamber) with ethanol after each use. In situations where solenoid pinch valves were used, a small section of silicone tubing was used (e.g. TUB1942; Scientific Laboratory Supplies, Nottingham, UK). However, because of problems in washing compounds off the silicone tubing, all silicone tubing was replaced after each change of TQS compound in the perfusion system.

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Results

Agonist activation of α7 nAChRs

A series of compounds with close chemical similarity to TQS (a PAM of α7 nAChRs) and 4BP-TQS (an allosteric agonist of α7 nAChRs) have been synthesized (Fig. 1). The pharmacological properties of these compounds have been examined by two-electrode voltage-clamp recordings using *Xenopus* oocytes expressing recombinant α7 nAChRs.

Initial studies were focused upon a series of compounds containing different halogen atoms at the 4-position of the phenyl ring (Fig. 1). As has been shown previously for 4BP-TQS (Gill et al., 2011), compounds containing either a chlorine or an iodine atom at this position (4CP-TQS and 4IP-TQS, respectively) were found to have potent agonist activity on α7 nAChRs (Fig. 2A). In contrast, replacement of the bromine atom with a fluorine atom at the 4-position (4FP-TQS) resulted in the complete loss of agonist activity (Fig. 2A). The EC_{50} values determined for 4BP-TQS, 4CP-TQS and 4IP-TQS were not significantly different from one another (Table 1). However, as has been reported previously for 4BP-TQS (Gill et al., 2011), agonist concentrations of these compounds causing half-maximal activation are significantly lower than that of acetylcholine (Table 1).

Previous studies have reported that, whereas activation of α7 nAChRs by acetylcholine results in rapidly desensitizing responses (Couturier et al., 1990), activation by the allosteric agonist 4BP-TQS results in largely non-desensitising responses (Gill et al., 2011). Here we have examined longer applications of 4BP-TQS and have detected very slow desensitization that occurs over a period of many minutes (Fig. 2B). By examining similarly long applications of 4CP-TQS and 4IP-TQS we have observed a progressive slowing of the rate of receptor desensitization as the size of the halogen atom increases (Fig. 2B & Table 1). In addition, the rate at which receptors return to their resting state after agonist activation differs between the three halogen-containing agonists (Fig. 2C & Table 1). A further difference between

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activation by acetylcholine and by 4CP-TQS, 4BP-TQS or 4IP-TQS is the slower activation rate that is observed with the latter compounds (Fig. 2B, 2C & Table 1).

Differences in the activation rates prompted us to examine the consequence of co-application of acetylcholine with allosteric agonists (Fig. 3). Interestingly, co-application of these compounds with acetylcholine resulted in receptor activation that can be resolved into two components (Fig. 3). An initial desensitizing response is seen, which is typical of activation by acetylcholine. This is followed by a secondary response, that displays slower activation and slower desensitization, and which is typical of activation by 4CP-TQS, 4BP-TQS and 4IP-TQS.

To examine further the relative influence of the size or the chemical properties of groups attached at the 4-position of the phenyl ring, several additional compounds were synthesized and characterized. Insertion of a methyl or trifluoromethyl group at the 4-position of the phenyl ring (4MP-TQS and 4TF-TQS) generated compounds that retained agonist activity (Figs 2 & 4), whereas inclusion of a hydrogen atom or hydroxyl group at this position (P-TQS and 4HP-TQS) resulted in complete loss of agonist activity (Fig. 4). These findings support the conclusion that a relatively large group attached at the 4-position of the phenyl ring is required for agonist activity. In addition, a further compound (2N-TQS) was synthesized in which the naphthyl group was attached in a different orientation to that in TQS (Fig. 1). Whereas no agonist activity is observed with TQS (Gill et al., 2011), 2N-TQS displayed clear agonist activity (Fig. 4). Taken together, these findings provide strong evidence that a relatively large group is required at the 4-position of the phenyl ring to confer agonist activity. We also examined the importance of the position at which halogen atoms were attached on the phenyl ring by synthesis and characterization of compounds in which bromine was attached at either the *ortho* or *meta* position (2BP-TQS and 3BP-TQS, respectively; Fig. 1) or in which iodine was attached at the meta position (3IP-TQS; Fig. 1). In contrast to the potent agonist activity observed with 4BP-TQS, no agonist activation was detected with either 2BP-

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TQS, 3BP-TQS or 3IP-TQS (Fig. 4), indicating that the position, as well as the size, of groups attached to the phenyl ring is critical in determining agonist activity. In contrast to the potent agonist activity observed with 4BP-TQS, no agonist activation was detected with a compound containing a bromine atom at both the *meta* and *para* positions on the phenyl ring (3,4BP-TQS). Taken together, it is clear that minor changes to the structure of these compounds have substantial influence on pharmacological properties.

We have reported previously (Gill et al., 2011) that at high concentrations of 4BP-TQS (above ~ 30 μM) an increase in agonist response is observed after agonist application ceases. It seems likely that this is a consequence of receptor/channel-blocking activity and is a feature that is also observed with high concentrations of orthosteric agonists such as acetylcholine, where it has been described as a 'hump current' (Liu et al., 2008). Amongst the agonists examined in the present study we have seen a wide variation in the extent of this phenomenon. As is illustrated in Fig. 4, a maximal concentration of 4TF-TQS (100 μM) produced a very large hump current (252 ± 78 % of the agonist response), whereas a maximal concentration of 2N-TQS (100 μM) produced a relatively small hump current (2.6 ± 1.9 % of the agonist response). It is possible that these differences reflect diffent abilities of these compounds to block the receptor, perhaps by interacting at a site other than the allosteric agonist site.

Positive allosteric modulation of α7 nAChRs

It is clear that alterations at the 4-position of the benzene ring have a dramatic effect on agonist effects of this series of compounds. It is also of interest that several of the compounds tested had no agonist activity, despite close chemical similarity. For each of the compounds that lacked agonist activity (2BP-TQS, 3BP-TQS, 3IP-TQS, 3,4BP-TQS, 4FP-TQS, 4HP-TQS and P-TQS) we examined whether they were able to act as PAMs. In all cases, these compounds potentiated acetylcholine-evoked responses (Fig. 5), as has been reported previously for TQS (Grønlien et al., 2007; Gill et al., 2011).

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Previous studies have demonstrated that TQS acts as a classical 'type II' PAM, causing potentiation of acetylcholine-evoked responses and a dramatic loss of receptor desensitization (Grønlien et al., 2007; Gill et al., 2011). Interestingly, just as we had observed differences in rates of desensitisation of α7 nAChRs after activation by 4BP-TQS, 4CP-TQS and 4IP-TQS, marked differences were apparent in the rate of desensitization after potentiation of acetylcholine-evoked responses with different PAMs (Fig. 5B & Table 2).

Influence of the transmembrane M253L mutation

It is well established that acetylcholine activates nAChRs by binding to an extracellular site. In contrast, recent studies have proposed that α7-selective PAMs such as TQS, and allosteric agonists such as 4BP-TQS, act via a transmembrane binding site (Young et al., 2008; Collins et al., 2011; Gill et al., 2011). One of the lines of evidence supporting this proposal is that potentiation by TQS, and agonist activation by 4BP-TQS, is completely abolished on α7 receptors containing the transmembrane mutation M253L (Gill et al., 2011). In contrast, M253L has been shown to have no significant effect on activation by the conventional orthosteric agonist acetylcholine (Young et al., 2008; Gill et al., 2011). The effect of M253L was examined on agonist activation by 4CP-TQS, 4IP-TQS and 4MP-TQS and was found to cause complete loss of agonist activation. In addition, the effect of M253L was examined on allosteric potentiation by 2BP-TQS, 3BP-TQS, 3IP-TQS, 3,4BP-TQS, 4FP-TQS, 4HP-TQS and P-TQS and was found to cause complete loss of PAM activity of all of these compounds. These findings support the conclusion the all of the TQS-related compounds examined in this study act by a similar mechanism of action. The simplest explanation being that they all act via a shared allosteric binding site, as has been proposed previously for TQS and 4BP-TQS (Gill et al., 2011).

Antagonism by 4FP-TQS of 4BP-TQS evoked responses

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As has been described above, the replacement of a single bromine atom with a fluorine atom converts the allosteric agonist 4BP-TQS into a PAM that lacks agonist activity (4FP-TQS). Based on previous studies with α7-selective allosteric modulators (Young et al., 2008; Gill et al., 2011), it seems reasonable to hypothesize that 4BP-TQS and 4FP-TQS might bind competitively at a common allosteric site. If this assumption is correct, we would predict that 4FP-TQS would act as an antagonist if co-applied with 4BP-TQS. We have tested this hypothesis by applying an EC_{50} concentration of 4BP-TQS (10 μM) and then co-applying 4BP-TQS with a range of concentrations of 4FP-TQS (Fig. 6). As predicted, the co-application of 4FP-TQS resulted in a dose-dependent inhibition of responses evoked by 4BP-TQS, with an IC_{50} concentration of 4.4 ± 1.3 μM (Fig. 6A & B). In contrast, co-application of 4FP-TQS with an EC_{50} concentration of acetylcholine results in a dose-dependent potentiation of responses evoked by acetylcholine, with an EC_{50} concentration of 23 ± 8.1 μM (Fig. 6C). These findings suggest that 4BP-TQS and 4FP-TQS bind to a common site, which is distinct from that of acetylcholine.

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Discussion

There is extensive evidence demonstrating that conventional nAChR agonists such as acetylcholine bind to an extracellular 'orthosteric' site located at the interface between two subunits (Arias, 2000; Karlin, 2002; Sine, 2002). Typically, heteromeric nAChRs contain two or three potential orthosteric agonist binding sites. In the case of the well-characterised nAChR subtype from *Torpedo* electric organ, there are two acetylcholine binding sites: located at the α/δ and the α/γ subunit interfaces (Blount and Merlie, 1989; Sine and Claudio, 1991). In contrast, in homomeric nAChRs, such as α7, there are five potential binding sites for agonists such as acetylcholine or for other ligands that interact with the orthosteric binding site ligands, such as competitive antagonists (Palma et al., 1996).

More recently, activation of nAChRs by allosteric agonists has been described and has been proposed to occur by the interaction with a transmembrane binding site located within an intrasubunit cavity (Gill et al., 2011). This allosteric site has also been proposed as being the binding site for a series of α7 PAMs (Young et al., 2008; Collins et al., 2011). In addition, photoaffinity labelling studies, conducted with nAChRs purified from *Torpedo* electric organ, have provided further evidence that a variety of ligands (including volatile anaesthetics) interact with a transmembrane modulatory site (Ziebell et al., 2004; Garcia et al., 2007). Indeed, there is increasing evidence that transmembrane sites are important modulatory sites in a range of Cys-loop neurotransmitter receptors, including those gated by GABA and glycine (Ye et al., 1998; Hosie et al., 2006).

In previous studies (Gill et al., 2011), one of the most obvious differences between agonist activation of α7 by acetylcholine and by the allosteric agonist 4BP-TQS was the marked difference in rates of agonist-induced receptor desensitization. Here we have examined a series of compounds, all chemically related to 4BP-TQS. Significant differences were observed in the rates of receptor desensitization when the bromine atom of 4BP-TQS was

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replaced by either a chlorine or iodine atom (Fig. 2B), however, in all cases, levels of desensitization caused by these compounds were much slower than the very rapid desensitization that is characteristic of α7 nAChRs when activated by acetylcholine (Couturier et al., 1990). Rates of desensitization, rates of activation and also rates of recovery after removal of agonist, were found to increase as the size of the halogen atom decreased (Fig. 2C). However, introduction of the smallest halogen atom (fluorine) at this position (4FP-TQS) resulted in a complete loss of agonist activity (Fig. 4), as did the introduction of a hydrogen atom (P-TQS) or a hydroxyl group (4HP-TQS). However, in all cases, PAM activity was retained (Fig. 5). It appears, therefore, that relatively minor changes to the structure of these compounds can have a profound influence upon their pharmacological properties. It is possible that these differences can be explained entirely by steric effects. For example, the slower activation rate observed with 4IP-TQS than with either 4BP-TQS or 4CP-TQS may be a consequence of reduced accessibility to its binding site.

Although it is possible that the differences in size between fluorine and the other three halogens could explain the differences in agonist activity observed, differences in chemical properties may also be significant. For example, halogens differ in their electrostatic surface potential. Indeed, such differences have been suggested to be responsible for the ability of organic compounds containing chlorine, bromine or iodine (but not those containing fluorine) to form halogen bonds (Auffinger et al., 2004; Politzer et al., 2007). However, the fact that agonist activity was seen when the halogen atom was replaced by a methyl group (4MP-TQS) or a tri-fluoromethyl group (4TF-TQS), but not when replaced with a hydrogen atom (P-TQS) or a hydroxyl group (4HP-TQS) argues that the size of the group attached to the 4-position of the phenyl ring may be more important than its chemical or electrostatic properties in determining allosteric agonist activity.

Of particular note was the much faster activation rate observed with acetylcholine than with any of the allosteric agonists examined. This difference in activation rates prompted us to

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examine the consequence of co-applying acetylcholine with allosteric agonists (Fig. 3). Interestingly, co-application of acetylcholine with an allosteric agonist resulted in receptor activation that could be resolved into two components (Fig. 3). An initial desensitizing response was seen, which is typical of activation by acetylcholine, and was followed by a secondary response displaying slower activation and slower desensitization. This secondary response is typical of activation by allosteric agonists (Gill et al., 2011). These two components of the agonist response are, presumably, a consequence of the ability of acetylcholine to access its extracellular orthosteric binding site more rapidly than allosteric agonists are able to access their binding sites. This is further evidence for a difference in the mechanism of action of these two classes of agonist. In previous studies (Gill et al., 2011), we have shown that the response to a submaximal concentration of 4BP-TQS is greatly potentiated by the subsequent co-application of acetylcholine, indicating that 4BP-TQS may be more potent as a positive allosteric modulator than as an allosteric agonist. Similar experiments conducted with other allosteric agonists described in the present study suggest that this is a common phenomenon for this series of compounds.

In addition to the size of the halogen atom being important in determining pharmacological properties, the position of the halogen is also critical. This was illustrated by the finding that changing the location of the bromine from the *para* position to either the *ortho* or *meta* positions resulted in loss of agonist activity. Similarly, no agonist activity was observed for 3,4BP-TQS, indicating that the allosteric agonist properties of 4BP-TQS can also be lost by the addition of a second halogen atom to the phenyl ring. Taken together, these findings illustrate that the arrangement of groups attached to the phenyl ring is critical in conferring allosteric agonist properties on these compounds.

As has been described in the Methods, studies conducted with the TQS series of compounds pose some technical problems, due in particular to low solubility and a tendency to stick to plastic tubing and apparatus. This has complicated construction of dose responses curves.

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However, in all cases examined (Table 1), activation by allosteric agonists resulted in significantly steeper dose-response curves (and significantly greater Hill coefficients) than observed with acetylcholine. This was particularly apparent for 4CP-TQS, which produced particularly steep and highly reproducible dose-response curves. It is possible that the Hill coefficient estimated for the other allosteric agonists (Table 1) may be an underestimate, due to problems associated with their slow association rates and difficulty in defining the maximum response with the maximum concentrations that could be used.

Several of the compounds examined in this study lacked allosteric agonist activity but caused dramatic potentiation of responses evoked by acetylcholine. Such effects are typical of a range of compounds that have been described as nAChR PAMs. Relatively small changes in chemical structure result in clear pharmacological differences. For example, as has been reported previously (Grønlien et al., 2007; Gill et al., 2011), minimal desensitization is observed with TQS (Fig. 5B). In contrast, progressively faster rates of desensitization were observed with the other PAMs examined (Fig. 5B). The terms ‘type I’ and ‘type II’ have been used extensively to describe PAMs acting on α7 nAChR that either have no effect on receptor desensitization (type I) or cause a loss of desensitization (type II). However, it appears that this system of classification may be an oversimplification (Dunlop et al., 2009; Malysz et al., 2009; Williams et al., 2011) and data obtained in this study support this conclusion. Indeed, it has been suggested that α7 PAMs may have a continuous spectrum of effects ranging from those with minimal effects on desensitization to those that cause complete loss (Dinklo et al., 2011).

We have also demonstrated that α7 nAChR PAMs such as 4FP-TQS can act as antagonists of responses evoked by allosteric agonists such as 4BP-TQS (Fig. 6). The simplest explanation for this observation is that these two chemically similar allosteric modulators interact at a common site. The binding of a PAM such as 4FP-TQS to its allosteric site can have two opposing effects: allosteric potentiation of responses to orthosteric agonists such

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as acetylcholine and antagonism of responses to allosteric agonists such as 4BP-TQS. The former is presumably a consequence of 4FP-TQS and acetylcholine binding to different sites, and the latter a consequence of 4FP-TQS and 4BP-TQS binding competitively to a common site. Interestingly, in cases where this was examined, the range of EC_{50} values determined for the allosteric agonists (Table 1) was not significantly different to that of the related compounds with only PAM activity (Table 2). This would argue that, in addition to evidence that allosteric agonists and PAMs may be acting at a common site, they appear to do so with broadly similar apparent affinities.

Work described here and elsewhere supports the conclusion that allosteric agonists and PAMs of α7 nAChRs can bind to common transmembrane site (Young et al., 2008; Collins et al., 2011; Gill et al., 2011). This is supported by evidence that the effects of both allosteric agonists and PAMs can be blocked completely by a single point mutation (M253L) located in the transmembrane region; a mutation that has no significant effect on agonist activation by acetylcholine (Young et al., 2008). Presumably, both allosteric agonists and PAMs are able to reduce the energy barrier for transitions between open and closed states of the receptor. The difference between the two classes of allosteric modulator being that those compounds that lack agonist activity are able to stabilize the open conformation of the receptor efficiently only in the presence an orthosteric agonist such as acetylcholine.

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Authorship Contributions

Participated in research design: Gill, Dhankher, Sheppard, Sher and Millar

Conducted experiments: Gill and Dhankher

Contributed new reagents: Dhankher and Sheppard

Performed data analysis: Gill, Dhankher, Sheppard, Sher and Millar

Wrote or contributed to writing the manuscript: Gill, Dhankher, Sheppard, Sher and Millar

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Footnotes

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Figure Legends

Figure 1 Chemical structure of α7 nAChR allosteric modulators examined in the present study.

Figure 2 Pharmacological properties of agonists on α7 nAChRs expressed in *Xenopus* oocytes. A, Dose response data are presented for a range of concentrations of acetylcholine (filled circles), 4BP-TQS (open circles), 4CP-TQS (open triangles), 4FP-TQS (crosses), 4IP-TQS (open squares) and 4MP-TQS (open diamonds). Data are means ± SEM of at least three independent experiments. B, Representative recordings obtained in the continued presence of a maximum concentration of agonist, illustrating activation and desensitization rates. C, Representative recordings of agonists (at a maximum concentration) that were applied only until the peak response is reached, thereby illustrating activation rates and rates of recovery after agonist wash-off. Responses have been normalised to their peak response.

Figure 3 Agonist activation of α7 nAChRs. Application of acetylcholine (3 mM) results in receptor activation, followed by rapid desensitization (*Left)*. Co-application of acetylcholine (3 mM) with 4IP-TQS (100 μM) results in a two-component response; an initial desensitizing acetylcholine response followed by a secondary response with a slower activation which is a typical of an allosteric agonist such as 4IP-TQS (*Right*). Agonist applications are indicated by horizontal lines. Data shown for 4IP-TQS is typical of data obtained with all allosteric agonists examined.

Figure 4 Agonist activity of TQS derivatives on α7 nAChRs. A, Agonist responses observed with a maximum concentration of each compound (100 μM) are normalized to responses with a maximum concentration of acetylcholine (3 mM). Data are means ± SEM of 7-23 independent experiments (see Table 1). B, Representative recordings obtained in the continued presence of a maximum concentration of 2N-TQS and 4TF-TQS, illustrating

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activation and desensitization rates. See Fig. 2 for representative responses for other agonists. C, Representative recordings of 2N-TQS and 4TF-TQS (at a maximum concentration) that were applied only until the peak response is reached, thereby illustrating activation rates and rates of recovery after agonist wash-off. Responses have been normalised to their peak response.

Figure 5 Positive allosteric modulation of α7 nAChRs. A, Bar Graphs illustrate levels of potentiation caused by a maximum concentration of each compound (100 μM). Potentiation of agonist-evoked responses were determined with a submaximal (EC_{50}) concentration of acetylcholine (100 μM). B, Representative recordings are shown, illustrating the differences in activation and desensitization rates for various PAMs. The PAM (100 μM) was pre-applied for 5 s and then co-applied with a submaximal (EC_{50}) concentration of acetylcholine (100 μM). Traces are normalized to the same peak response. Data are means ± SEM of 3-13 independent experiments (see Table 2).

Figure 6 Antagonism by 4FP-TQS of 4BP-TQS evoked responses on α7 nAChRs. A, Representative trace showing activation of wild-type α7 nAChRs by 4BP-TQS (10 μM) followed by the co-application of 4FP-TQS (100 μM). Applications of allosteric modulators are indicated by horizontal lines. B, Dose-response data illustrating the ability of 4FP-TQS to inhibit responses evoked by a submaximal (EC_{50}) concentration of 4BP-TQS (10 μM). C, Dose-response data are presented for a range of concentrations of 4FP-TQS on responses evoked by a submaximal (EC_{50}) concentration of acetylcholine with either wild-type α7 nAChRs (filled circles) or α7 nAChRs containing the M253L mutation (open circles). Data are means ± SEM of at least three independent experiments, each from different oocytes.

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TABLE 1
Pharmacological and kinetic properties of allosteric agonists

Agonist	EC_{50}[†] (μM)	n_H[††]	I_{max}[†††]	Desensitization rate (s)[‡]	Activation rate (s)[‡‡]	Inactivation rate (s)[‡‡‡]
Acetylcholine	128 ± 12	1.3 ± 0.2	1	0.10 ± 0.01	0.49 ± 0.03	ND
4CP-TQS	10 ± 0.3	3.9 ± 0.2	11 ± 2.9	29 ± 10	19 ± 1.8	17 ± 1.6
4BP-TQS	17 ± 3.4	2.3 ± 0.4	38 ± 6.3	95 ± 36	30 ± 5.9	30 ± 3.1
4IP-TQS	18 ± 4.3	1.9 ± 0.1	8.5 ± 1.3	2496 ± 1275	71 ± 11	73 ± 8.0
4MP-TQS	27 ± 3.1	2.2 ± 0.2	9.2 ± 1.4	1047 ± 648	58 ± 5.0	56 ± 9.7
2N-TQS	-	-	2.0 ± 0.4	153 ± 20	72 ± 10	28 ± 2.2
4TF-TQS	-	-	0.94 ± 0.14	44 ± 4.0	11 ± 0.37	36 ± 6.9

Data are means of 3-23 independent experiments, ± SEM.

[†] EC_{50} values of all compounds were significantly different to acetylcholine (ANOVA; $P < 0.001$).

[††] For all compounds examined, Hill coefficients were significantly different to acetylcholine in pairwise comparisons (*t*-test; $P < 0.05$).

[†††] Maximal current, normalized to a maximal (3 mM) acetylcholine response.

[‡] Time (s) for response to decline from peak to half of the peak response in the continuous presence of agonist. All compounds were significantly different to acetylcholine by ANOVA ($P < 0.05$) or by pairwise comparisons to acetylcholine (*t*-test; $P < 0.01$).

[‡‡] Time (s) from the start of agonist application to peak response. All compounds except 4CP-TQS were significantly different to acetylcholine by ANOVA ($P < 0.05$). In pairwise comparisons, all compounds were significantly different to acetylcholine (*t*-test; $P < 0.001$).

[‡‡‡] Time (s) for recovery to half of the peak response after removal of agonist (this parameter could not be determined for acetylcholine, due to rapid rate of desensitization).

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TABLE 2

Pharmacological and kinetic properties of positive allosteric modulators

PAM	EC_{50} (μM)	n_H	Fold potentiation[†]	Desensitization rate (s)[††]	Activation rate (s)[†††]
TQS	6.2 ± 0.6	1.4 ± 0.3	28 ± 6.2	>1000	16 ± 2.6
4FP-TQS	23 ± 8.1	2.2 ± 1.9	33 ± 6.2	12 ± 2.1	2.8 ± 0.6
2BP-TQS	-	-	12 ± 2.7	13 ± 3.8	2.0 ± 0.2
3BP-TQS	-	-	8.6 ± 0.5	8.1 ± 0.3	1.5 ± 0.1
3,4BP-TQS	-	-	30 ± 4.6	10 ± 1.1	1.8 ± 0.2
3IP-TQS	-	-	21 ± 2.3	6.3 ± 0.1	4.0 ± 0.4
4HP-TQS	-	-	59 ± 15	13 ± 2.2	7.9 ± 1.0
P-TQS	-	-	11 ± 4.0	36 ± 5.0	5.5 ± 0.9

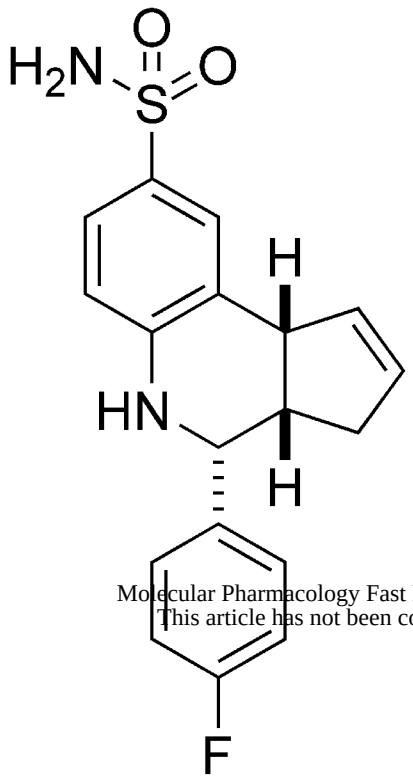
Data are means of 3-13 independent experiments, ± SEM.

[†] Fold potentiation of a submaximal (EC_{50}) concentration of acetylcholine (100 μM).

[††] Time (s) for response to decline from peak to half of the peak response in the continuous presence of agonist. All compounds were significantly different to TQS (ANOVA; ($P < 0.001$).

[†††] Time (s) from the start of agonist application to peak response. All compounds were significantly different to TQS (ANOVA; $P < 0.001$).

Figure 1



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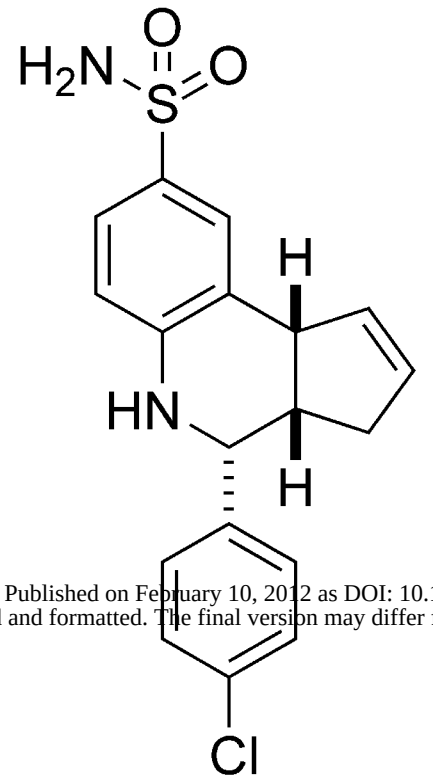
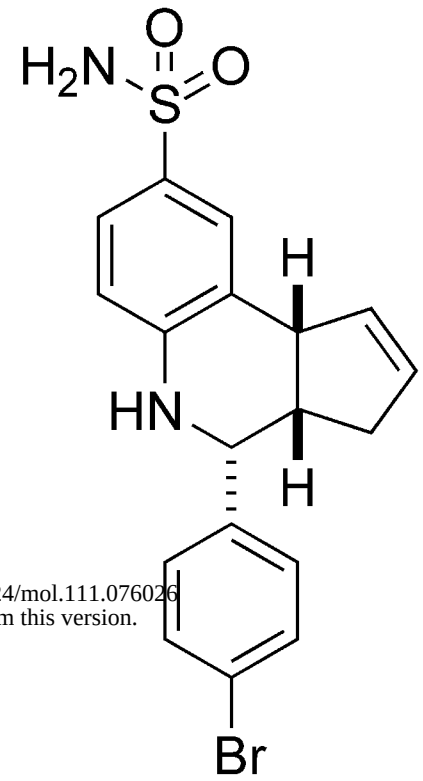
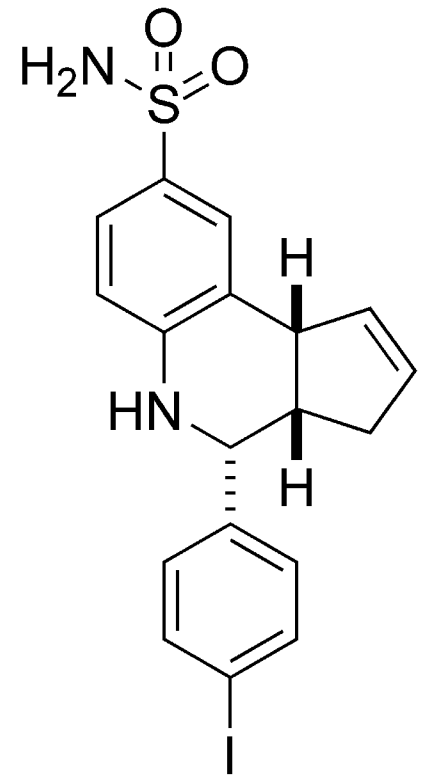


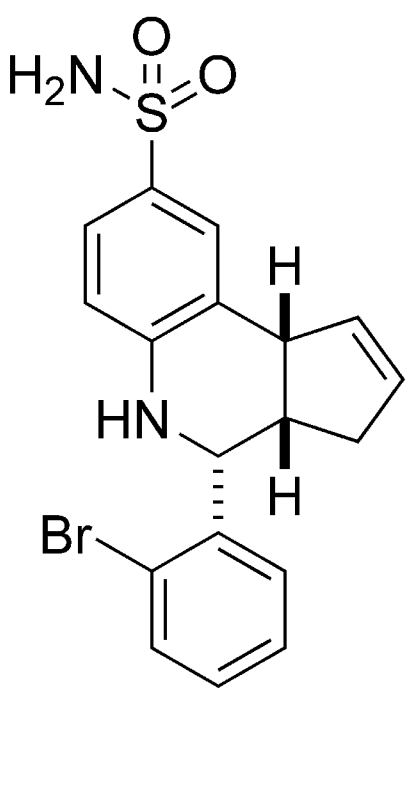
4CP-TQS



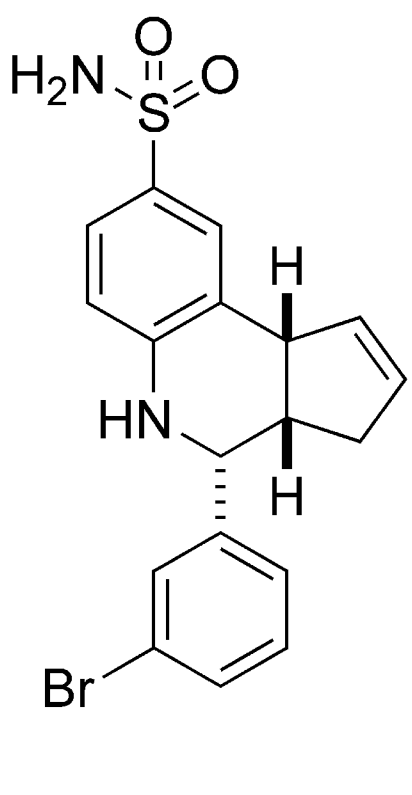
4BP-TQS



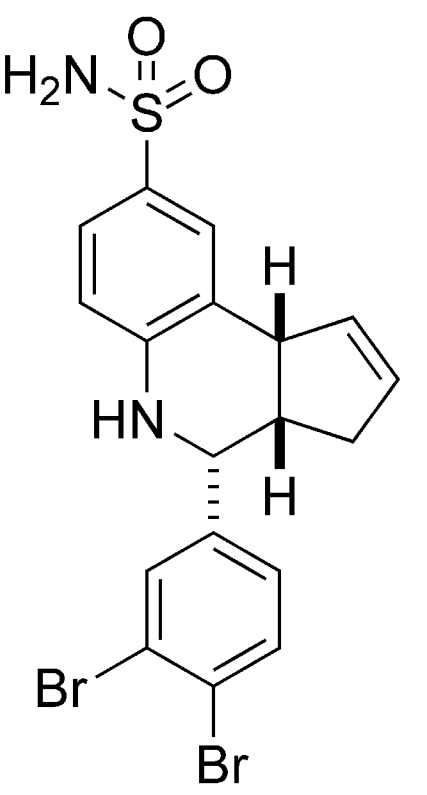
4IP-TQS



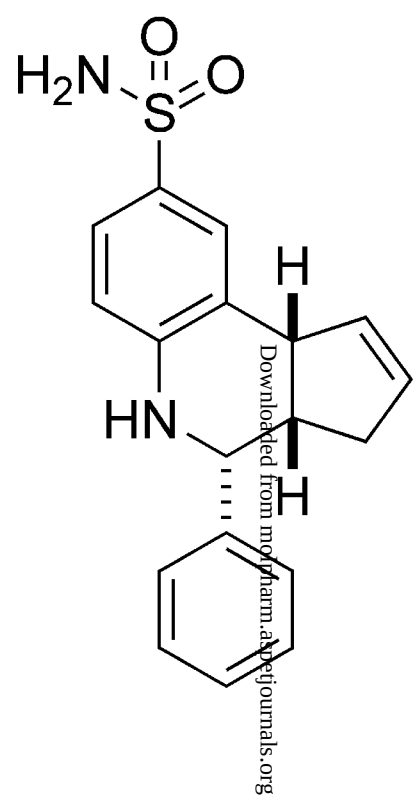
2BP-TQS



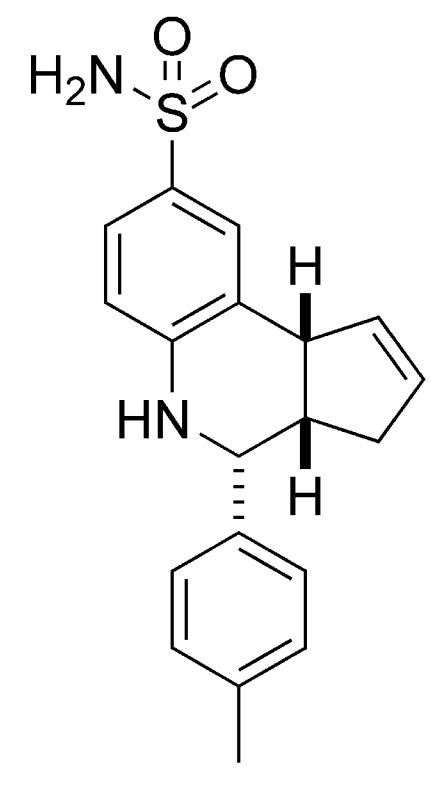
3BP-TQS



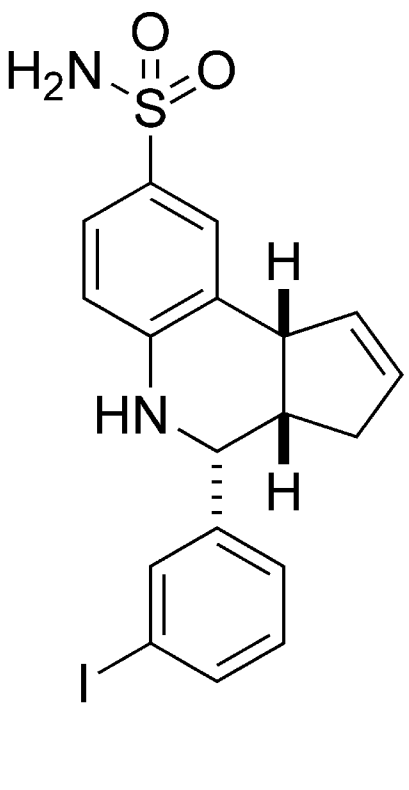
3,4BP-TQS



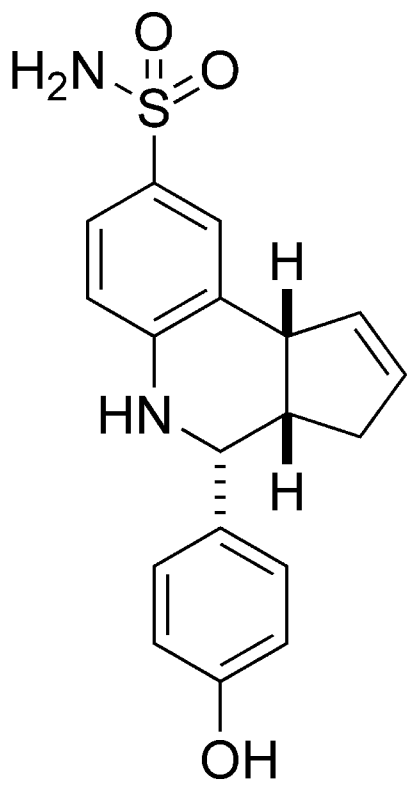
P-TQS



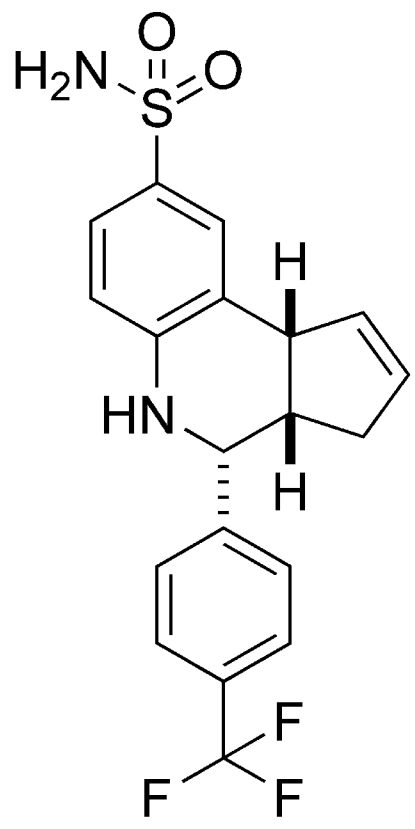
4MP-TQS



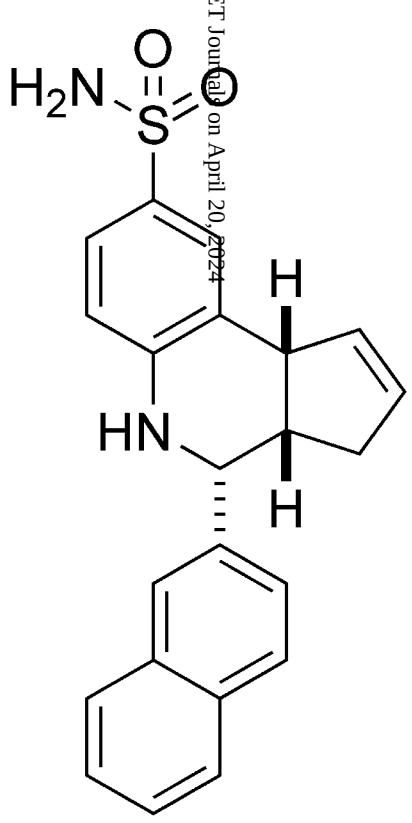
3IP-TQS



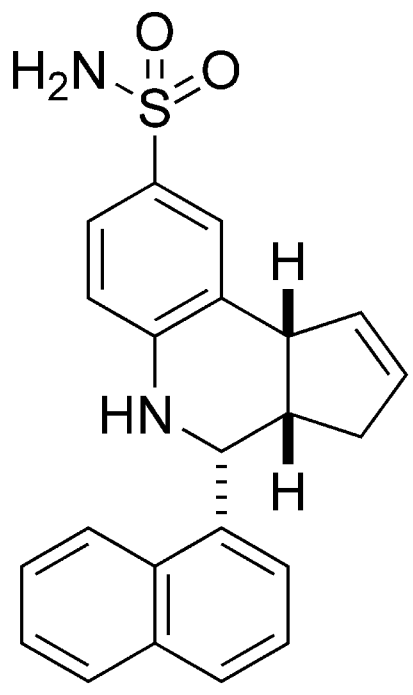
4HP-TQS



4TF-TQS



2N-TQS



TQS

Figure 2

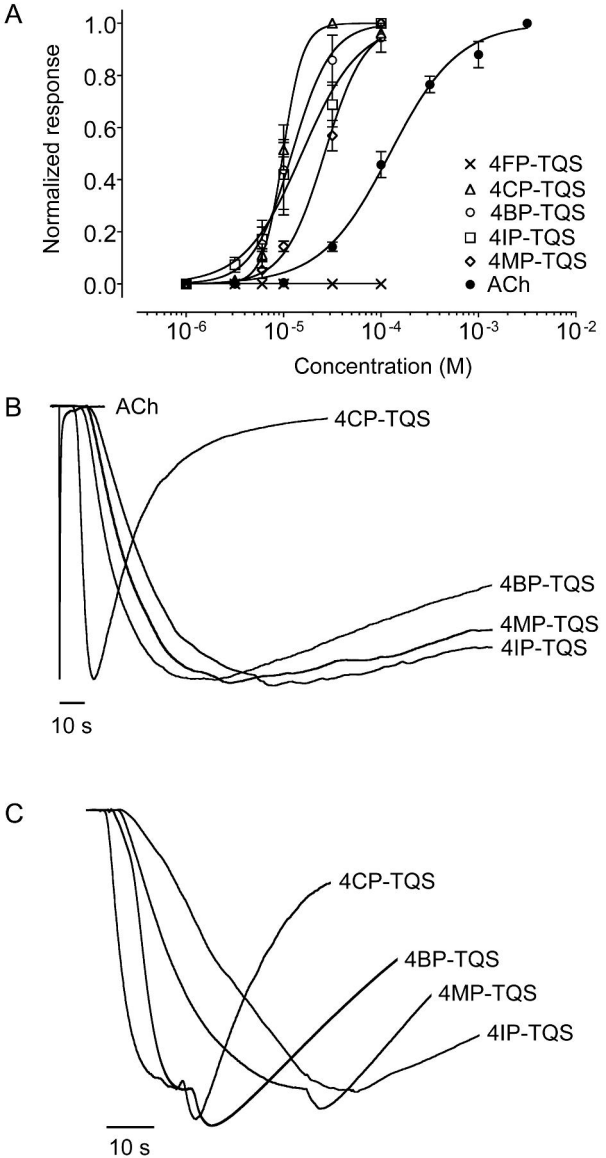

A
Normalized response
1.0
0.8
0.6
0.4
0.2
0.0
4FP-TQS
4CP-TQS
4BP-TQS
4IP-TQS
4MP-TQS
ACh
10-6
10-5
10-4
10-3
10-2
Concentration (M)
B
ACh
4CP-TQS
4BP-TQS
4MP-TQS
4IP-TQS
10 s
C
4CP-TQS
4BP-TQS
4MP-TQS
4IP-TQS
10 s

Figure 3

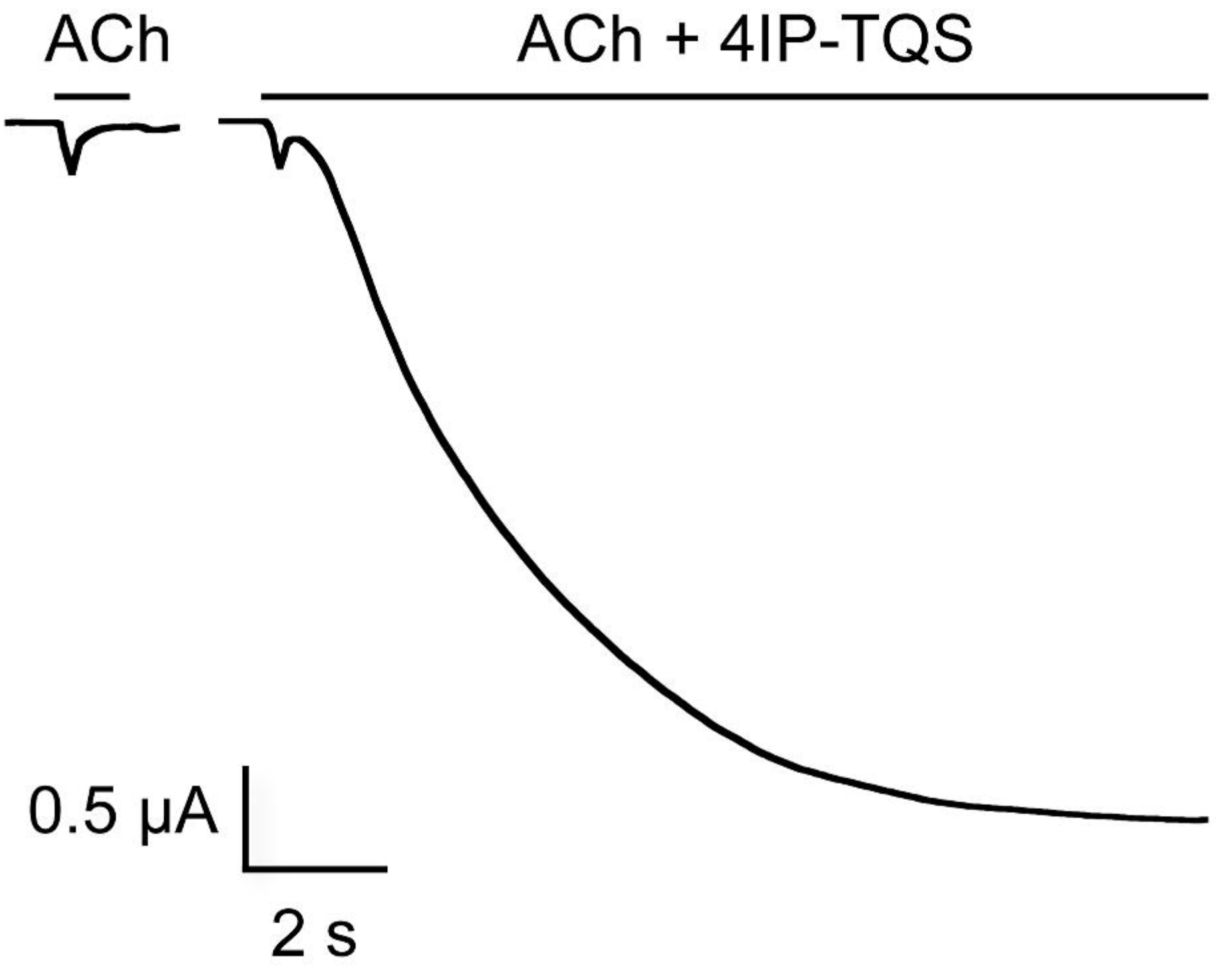


Figure 4

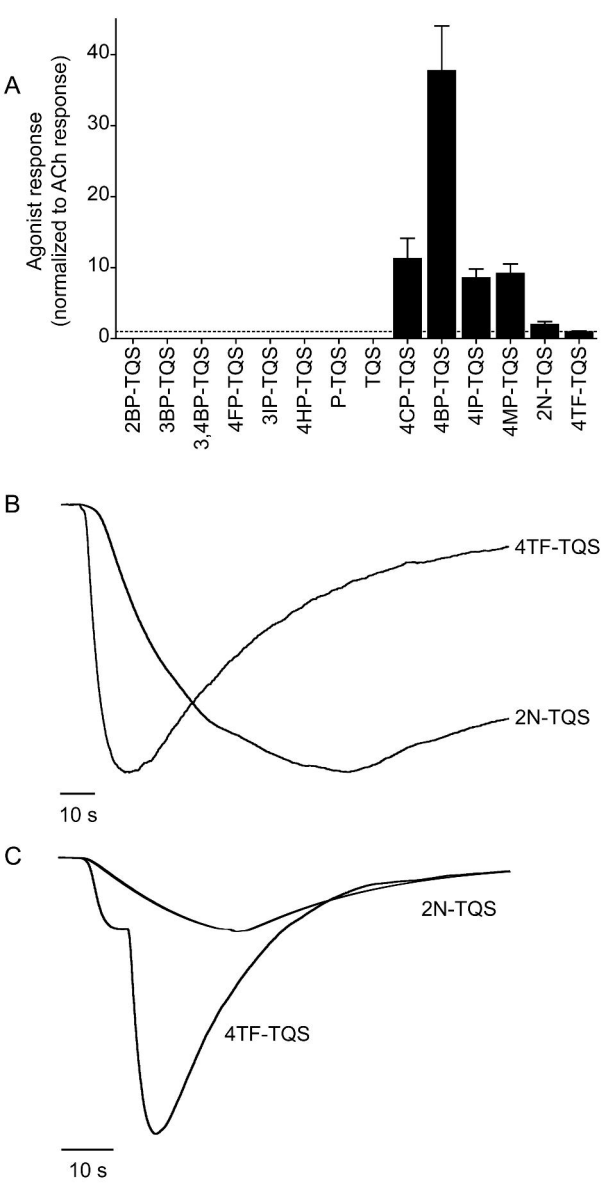

A
Agonist response
(normalized to ACh response)
40
30
20
10
0
2BP-TQS
3BP-TQS
3,4BP-TQS
4FP-TQS
3IP-TQS
4HP-TQS
P-TQS
TQS
4CP-TQS
4BP-TQS
4IP-TQS
4MP-TQS
2N-TQS
4TF-TQS
B
4TF-TQS
2N-TQS
10 s
C
2N-TQS
4TF-TQS
10 s

Figure 5

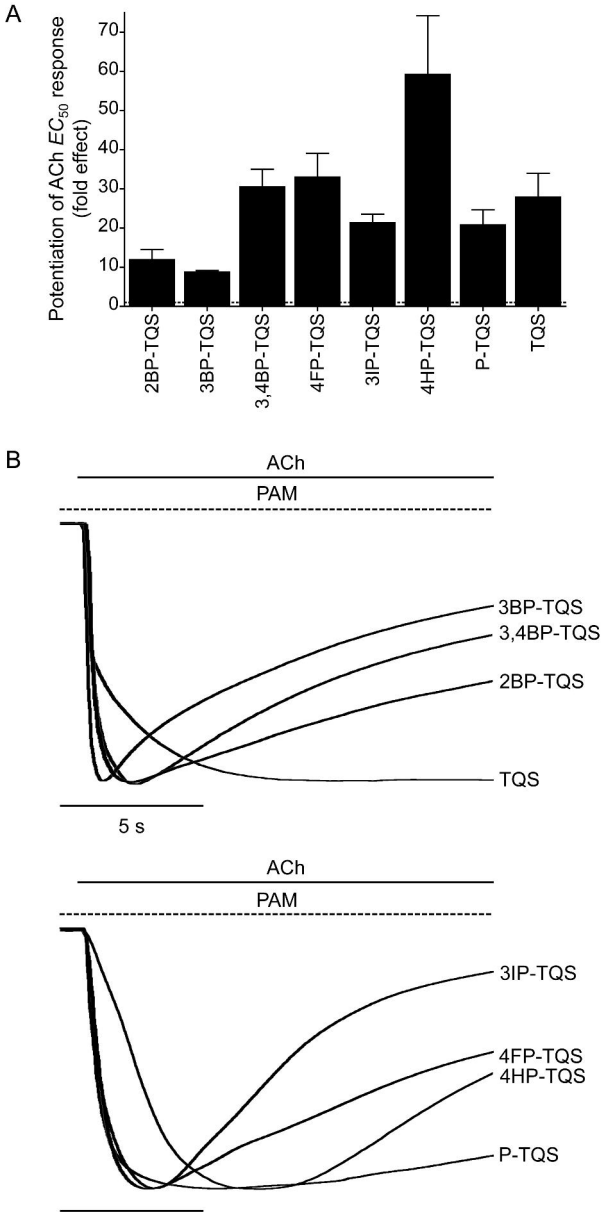
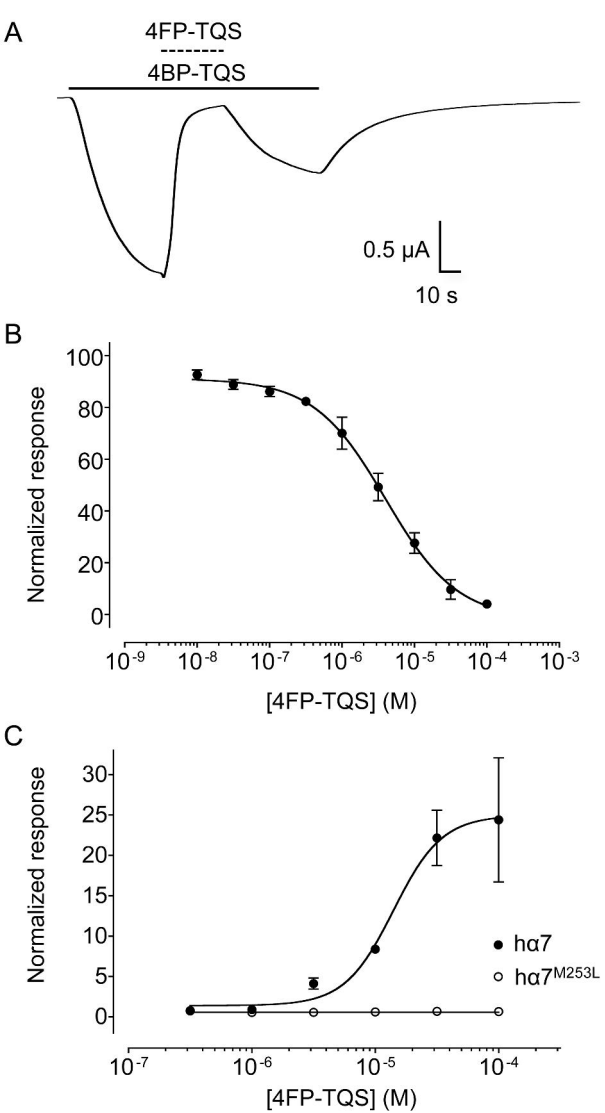

A
Potentiation of ACh EC_{50} response (fold effect)
0
10
20
30
40
50
60
70
2BP-TQS
3BP-TQS
3,4BP-TQS
4FP-TQS
3IP-TQS
4HP-TQS
P-TQS
TQS
B
ACh
PAM
3BP-TQS
3,4BP-TQS
2BP-TQS
TQS
5 s
ACh
PAM
3IP-TQS
4FP-TQS
4HP-TQS
P-TQS
5 s

Figure 6


A
4FP-TQS
4BP-TQS
0.5 µA
10 s
B
Normalized response
100
80
60
40
20
0
10-9
10-8
10-7
10-6
10-5
10-4
10-3
[4FP-TQS] (M)
C
Normalized response
30
25
20
15
10
5
0
10-7
10-6
10-5
10-4
[4FP-TQS] (M)
hα7
hα7M253L

Supplemental data

A series of α7 nicotinic acetylcholine receptor allosteric modulators with close chemical similarity but diverse pharmacological properties

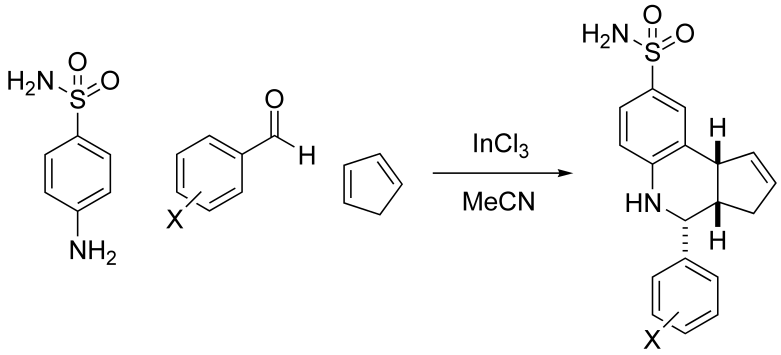
JasKiran K. Gill, Persis Dhankher, Tom D. Sheppard, Emanuele Sher and Neil S. Millar

Molecular Pharmacology

Cyclopentaquinoline Synthetic Procedures

General protocol

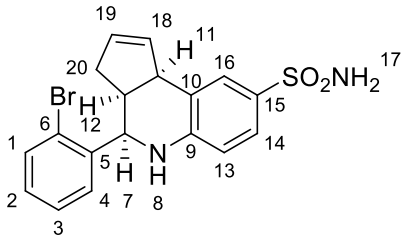
Indium trichloride (0.2 mmol) was added to a solution of aldehyde (1 mmol), sulphanilamide (1 mmol) and cyclopentadiene (3 mmol) in acetonitrile (3.2 mL). The reaction mixture was stirred for 24 hrs at room temperature. Aqueous (0.1 M) Na_2CO_3 (3 mL) was added and the aqueous mixture was extracted with chloroform (3×3 mL), washed with water (3×3 mL) and brine (9 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by chromatography and/or recrystallisation.



2BP-TQS

4-(-2-bromophenyl)-3a,4,5,9b-tetrahydro-3*H*-cyclopena[c]quinoline-8-sulphonamide

Purified by recrystallisation from isopropanol/petrol to give the title compound as a pale yellow powder (523 mg, 60%).

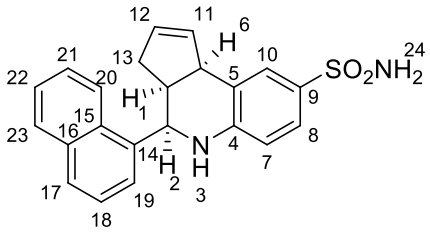


mp 204-206 °C; ν_{max} 3396.3 (N-H), 3353.9 (N-H), 3259 (N-H), 2927.9 (N-H), 1599.3 (aromatic), 1587.6 (aromatic), 1316 (SO_2-N), 1156.9 (S=O), 1090.7 (S=O), 665 (C-Br) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.62 (d, 1H, *J* = 1.3 Hz, H-16), 7.59 (dd, 1H, *J* = 8.0, 1.3 Hz, H-1), 7.57 (dd, 1H, *J* = 8.0, 1.1 Hz, H-4), 7.53 (dd, 1H, *J* = 8.0, 1.3 Hz, H-14), 7.37 (td, 1H, *J* = 8.0, 1.1 Hz, H-2), 7.18 (td, 1H, *J* = 8.0, 1.3 Hz, H-3), 6.67 (d, 1H, *J* = 8.0 Hz, H-13), 5.91-5.89 (m, 1H, H-18), 5.69-5.67 (m, 1H, H-19), 5.06 (d, 1H, *J* = 2.9 Hz, H-7), 4.76 (s, 2H, N*H*$_2$), 4.14 (d, 1H, *J* = 7.0 Hz, H-11), 4.10 (s, 1H, N*H*), 3.29 (dtd, 1H, *J* = 9.9, 7.0, 2.9 Hz, H-12), 2.53 (dtd, 1H, *J* = 16.1, 9.9, 2.9 Hz, H-15), 1.75 (br dd, 1H, *J* = 16.1, 7.0 Hz, H-15); δ_C (150 MHz, $CDCl_3$) 149.7 (C_q), 140.2 (C_q), 133.5 (CH), 133.3 (CH), 131.2 (C_q), 131.0 (CH), 129.2 (CH), 128.2(CH), 127.9 (CH), 127.8 (CH), 126.1 (C_q), 125.4 (CH), 123.1 (C_q), 115.9 (CH), 56.5 (CH), 45.6 (CH), 41.9 (CH), 31.5 (CH_2).

2N-TQS

4-(naphthalen-1-yl)-3a,4,5,9b-tetrahydro-1H-cyclopenta[c]quinoline-8-sulphonamide

Purified by recrystallisation from isopropanol/petrol to give the title compound as a pale yellow solid (216 mg, 22%).

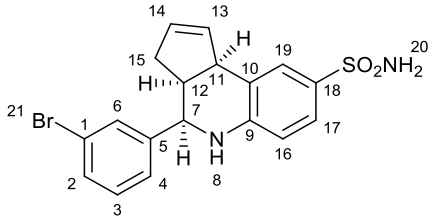


mp 203-205 °C; ν_{max} 3344 (N-H), 3268 (N-H), 2930 (N-H), 1598 (aromatic), 1496 (aromatic), 1321-1288 (SO_2-N), 1197 (S=O), 1127 (SO_2–N), 1091 (S=O) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 8.09 (d, 1H, *J* = 8.4 Hz, H-20), 7.92 (d, 1H, *J* = 8.1 Hz, H-23), 7.84 (d, 1H, *J* = 8.4 Hz, H-17), 7.73 (d, 1H, *J* = 7.1 Hz, H-19), 7.66 (d, 1H, *J* = 1.9 Hz, H-10), 7.58-7.51 (m, 4H, H-22, H-21,H-18, H-8), 6.73 (d, 1H, *J* = 8.2 Hz, H-7), 5.90-5.88 (m, 1H, H-11), 5.67-5.64 (m, 1H, H-12), 5.52 (d, 1H, *J* = 1.56 Hz, H-2), 4.66 (s, 2H, N*H*$_2$), 4.25 (d, 1H, *J* = 8.83 Hz, H-6), 4.22 (s, 1H, N*H*), 3.34 (dtd, 1H, *J* = 9.4, 7.3, 3.3 Hz, H-12), 2.58 (ddd, 1H, *J* = 16.7, 9.6, 2.4 Hz, H-15), 1.65 (br dd, 1H, *J* = 16.2, 5.1 Hz, H-15); δ_C (150 MHz, $CDCl_3$) 150.3 (C_q), 137.2 (C_q), 133.9 (C_q), 133.4 (CH), 131.2 (CH), 130.9 (C_q), 130.4 (C_q), 129.3 (CH), 128.2 (d, 5.4 Hz, CH), 126.5 (CH), 126.3 (C_q), 122.7 (CH), 122.3 (CH), 115.9 (CH), 53.3 (CH), 45.9 (CH), 43.7 (CH), 32.0 (CH_2).

3BP-TQS

4-(3-Bromophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulphonamide.

Purified by recrystallisation from isopropanol/petrol to give the title compound as a green/yellow solid (178 mg, 49%).

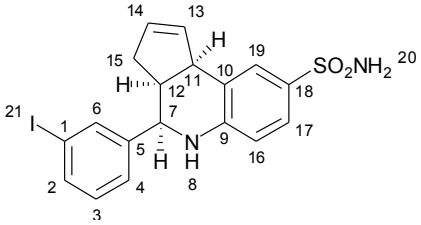


mp 139-142 °C; ν_{max} 3260-3252 (N-H), 2921-2900 (N-H), 1597 (aromatic), 1307-1288 (SO_2-N), 1187 (S=O), 1151-1128 (SO_2-N), 1090 (S=O), 711 (C-Br) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.60 (d, 1H, *J* = 2.0 Hz, H-19), 7.57 (t, 1H, *J* =1.6 Hz, H-6), 7.53 (dd, 1H, *J* = 8.5, 2.0 Hz, H-17), 7.45 (br d, 1H, *J* = 7.7 Hz, H-2), 7.34 (br d, 1H, *J* = 7.7 Hz, H-4), 7.26 (t, 1H, *J* = 7.7 Hz, H-3), 6.67 (d, 1H, *J* = 8.5 Hz, H-16), 5.90-5.88 (m, 1H, H-13), 5.69-5.68 (m, 1H, H-14), 4.70 (s, 2H, NH_2), 4.68 (d, 1H, *J* = 3.1 Hz, H-7), 4.19 (br s, 1H, -NH), 4.10 (d, 1H, *J* = 8.1 Hz, H-11), 3.01 (dtd, 1H, *J* = 9.4, 8.1, 3.1 Hz, H-12), 2.52 (ddd, 1H, *J* = 16.3, 9.4, 2.3 Hz, H-15), 1.83 (br dd, 1H, *J* = 16.3, 8.1 Hz, H-15); δ_C (150 MHz, $CDCl_3$) 149.3 (C_q), 144.1 (C_q), 133.4 (CH), 131.1 (C_q), 131.0 (CH), 130.9 (CH), 130.4 (CH), 129.5 (CH), 128.2 (CH), 125.8 (C_q), 125.5 (CH), 125.2 (CH), 123.0 (C_q), 115.8 (CH), 57.0 (CH), 45.72 (CH), 45.71 (CH), 31.5 (CH_2). HRMS (EI) *m/e* calcd. for $C_{18}H_{17}O_2N_2SBr$ 405.02724, found 405.02674.

3IP-TQS

4-(3-Iodophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide.

Purified by recrystallisation from isopropanol : petrol to give the title compound as a pale brown powder (34 mg, 7.5%).

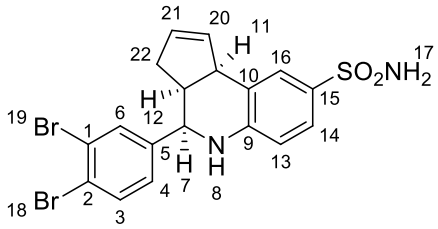


$R_{f\ =}$ 0.34 (3 : 2 EtOAc : Petrol); mp 177-179°C; IR ν_{max} 3338-3258 (N-H), 2911-2901 (N-H), 1600 (aromatic), 1308-1288 (SO_2-N), 1191 (S=O), 1170-1132 (SO_2-N), 1092 (S=O), 700 (C-I) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.77 (br t, 1H, *J* = 1.4 Hz, H-6), 7.66 (br dt, 1H, *J* = 7.9, 1.7 Hz, H-2), 7.60 (br d, 1H, *J* = 1.7 Hz, H-19), 7.53 (dd, 1H, *J* = 8.5, 1.7 Hz, H-17), 7.37 (br d, 1H, *J* = 7.8 Hz, H-4), 7.13 (t, 1H, *J* = 7.8 Hz, H-3), 6.67 (d, 1H, *J* = 8.5 Hz, H-16), 5.90-5.88 (m, 1H, H-13), 5.70-5.67 (m, 1H, H-14), 4.65 (d, 1H, *J* = 3.2, H-7), 4.63 (s, 2H, NH_2), 4.17 (br s, 1H, -NH), 4.10 (d, 1H, *J* = 8.3 Hz, H-11), 3.00 (dtd, 1H, *J* = 9.4, 8.3, 3.2 Hz, H-12), 2.52 (ddd, 1H, *J* = 16.3, 9.4, 2.3 Hz, H-15), 1.84 (br dd, 1H, *J* = 16.3, 8.3, Hz, H-15); δ_C (150 MHz, $CDCl_3$) 149.4 (C_q), 144.2 (C_q), 136.9 (CH), 135.4 (CH), 133.4 (CH), 131.1 (C_q), 131.0 (CH), 130.6 (CH), 128.2 (CH), 125.8 (CH), 125.7 (C_q), 125.5 (CH), 115.8 (CH), 94.9 (C_q), 56.9 (CH), 45.7 (2 × CH), 31.5 (CH_2). HRMS (EI) *m/e* calcd. for $C_{18}H_{17}O_2N_2SI$ 452.00499, found 452.00563.

3,4BP-TQS

4-(3,4-Dibromophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulphonamide

Purified by recrystallisation from isopropanol/petrol to give title compound as a light brown powder (180 mg, 37%).

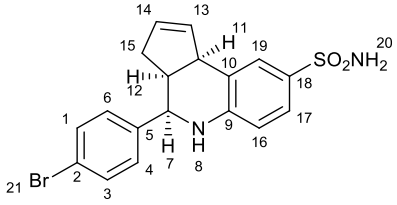


m.p. 143-145˚C; ν_{max} 3361 (N-H), 3255 (N-H), 2929 (N-H), 1598 (aromatic), 1495 (SO_2-N), 1313 (SO_2-N), 1307-1289 (O=S=O), 1151-1127 (O=S=O), 109.8 (S=O), 665 (C-Br) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.69 (d, 1H, *J* = 1.7 Hz, H-6), 7.63 (d, 1H, *J* = 8.2 Hz, H-3), 7.60 (d, 1H, *J* = 1.6 Hz, H-16), 7.53 (dd, 1H, *J* = 8.5, 1.6 Hz, H-14), 7.22 (dd, 1H, *J* = 8.2, 1.7 Hz, H-4), 6.68 (d, 1H, *J* = 8.5 Hz, H-13), 5.90-5.88 (m, 1H, H-20), 5.69 (br d, 1H, *J* = 4.8 Hz, H-21), 4.67 (d, 1H, *J* = 5.4 Hz, H-7), 4.66 (s, 2H, NH_2), 4.14 (br s, 1H, N*H*), 4.10 (br d, 1H, *J* = 8.7 Hz, H-11), 2.98 (m, 1H, H-12), 2.50 (m, 1H, H-22), 1.83 (m, 1H, H-22); δ_C (150 MHz, $CDCl_3$) 149.1 (C_q), 142.9 (C_q), 134.1 (CH), 133.4 (CH), 131.6 (CH), 131.5 (C_q), 131.0 (CH), 128.2 (CH), 126.7 (CH), 125.7 (C_q), 125.5 (CH), 125.3 (C_q), 123.9 (C_q), 115.9 (CH), 56.6 (CH), 45.6 (CH), 45.5 (CH), 31.5 (CH_2). HRMS (EI) *m/e* calcd. for $C_{18}H_{16}O_2N_2SBr_2$ 481.92938, found 481.93005.

4BP-TQS

4-(4-Bromophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulphonamide.

Purified by recrystallisation from isopropanol/petrol to give the title compound as a cream solid (151 mg, 37%).

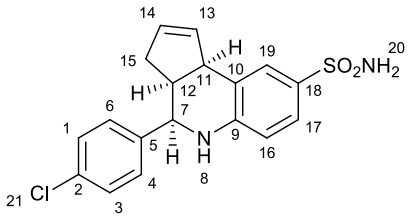


m.p. 192-194.5 ˚C; ν_{max} 3326 (N-H), 3261 (N-H), 1598 (aromatic), 1316-1293 (SO_2-N), 1184 (S=O), 1149-1125 (SO_2-N), 1086 (S=O), 722 (C-Br) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.59 (d, 1H, *J* = 2.0 Hz, H-19), 7.53 (dd, 1H, *J* = 8.4, 2.0 Hz, H-17), 7.52 (d, 2H, *J* = 8.1 Hz, H-1,3), 7.29 (d, 2H, *J* = 8.1 Hz, H-4,6), 6.67 (d, 1H, *J* = 8.4 Hz, H-16), 5.90-5.88 (m, 1H, H-13), 5.69-5.68 (m, 1H, H-14), 4.68 (d, 1H, *J* = 3.1 Hz, H-7), 4.62 (s, 2H, NH_2), 4.17 (br s, 1H, NH), 4.06 (d, 1H, *J* = 8.5 Hz, H-11), 2.98 (dtd, 1H, *J* = 9.7, 8.5, 3.1 Hz, H-12), 2.51 (ddd, 1H, *J* = 16.5, 9.7, 2.4 Hz, H-15), 1.82 (br dd, 1H, *J* = 16.5, 8.5 Hz, H-15); δ_C (150 MHz, $CDCl_3$) 149.4 (C_q), 140.8 (C_q), 133.4 (CH), 132.0 (2×CH), 131.9 (CH), 131.1 (C_q), 131.0 (CH), 128.2 (2×CH), 125.8(C_q), 125.5 (CH),121.6 (C_q), 115.8 (CH), 57.0 (CH), 45.73 (CH), 45.70 (CH), 31.5 (CH_2); HRMS (EI) *m/e* calcd. for $C_{18}H_{17}O_2N_2SBr$ 405.02724, found 405.02552.

4CP-TQS

4-(4-Chlorophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulphonamide

Purified by flash column chromatography (1:9 to 3:7 EtOAc:petrol) to give the title compound as a pale pink foam (230 mg, 63%).

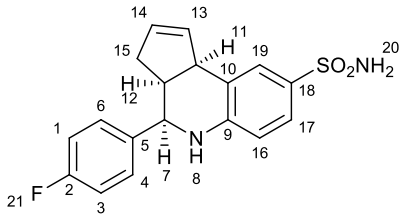


ν_{max} 3342 (N-H), 3271 (N-H), 2923 (C-H), 1597 (aromatic), 1313-1156 (SO_2-N), 1088 (S=O), 673 (C-Cl) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.60 (d, 1 H, *J* = 1.8 Hz, H-19), 7.52 (dd, 1 H, *J* = 8.4, 1.8 Hz, H-17), 7.37 (d, 2 H, *J* = 8.6 Hz, H-1,3), 7.35 (d, 2 H, *J* = 8.6 Hz, H-4,6), 6.67 (d, 1H, *J* = 8.4 Hz, H-16), 5.90-5.88 (m, 1H, H-13), 5.70-5.67 (m, 1H, H-14), 4.70 (d, 1H, *J* = 3.4 Hz, H-7), 4.63 (s, 2H, NH_2), 4.17 (br s, 1H, N*H*), 4.11 (br d, 1H, *J* = 8.7 Hz, H-11), 2.99 (dtd, 1H, *J* = 9.5, 8.7, 3.4 Hz, H-12), 2.52 (ddd, 1H, *J* = 16.1, 9.5, 2.2 Hz, H-15), 1.82 (br dd, 1H, *J* = 16.1, 8.7 Hz, H-15); δ_C (150 MHz, CDCl3) δ 149.3 (C_q), 140.1 (C_q), 133.4 (C_q), 133.3 (CH), 131.0 (C_q), 130.9 (CH) 128.9 (2×CH), 128.1 (CH), 127.7 (2×CH), 125.7 (C_q), 125.4 (CH), 115.6 (CH), 56.9 (CH), 45.7 (CH), 45.6 (CH), 31.3 (CH_2); HRMS (EI) *m/e* calcd. for $C_{18}H_{17}O_2N_2SCl$ 359.0612, found 359.0627.

4FP-TQS

4-(4-Fluorophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulphonamide

Purified by flash chromatography (Petrol to 3:7 EtOAc:petrol) and recrystallisation from isopropanol/toluene/petrol to give the title compound as a dark green amorphous solid (6 mg, 2%).



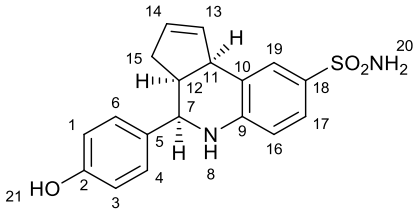
ν_{max} 3352-3226 (N-H), 2921-2851 (N-H), 1600 (aromatic), 1221.7 (C-F), 1309-1292 (SO_2-N), 1188 (S=O), 1150-1128 (SO_2-N), 1091 (S=O) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.60 (br d, 1H, *J* = 2.0 Hz, H-19), 7.55 (dd, 1H, *J* = 8.4, 2.0 Hz, H-17), 7.38 (m, 2H, H-4, 6), 7.08 (m, 2H, H-1, 3), 6.66 (d, 1H, *J* = 8.4 Hz, H-16), 5.91-5.88 (m, 1H, H-13), 5.70-5.66 (m, 1H, H-14), 4.70 (d, 1H, *J* = 3.2 Hz, H-7), 4.63 (s, 2H, NH_2), 4.18 (br s, 1H, NH), 4.11 (d, 1H, *J* = 8.7 Hz, H-11), 2.98 (dtd, 1H, *J* = 10.1, 8.7, 3.2 Hz, H-12), 2.54 (ddd, 1H, *J* = 15.8, 10.1, 2.3 Hz, H-15), 1.82 (br dd, 1H, *J* = 15.8, 8.7 Hz, H-15); δ_C (150 MHz, $CDCl_3$) 162.3 (d, C_q, *J* = 246.5 Hz), 149.6 (C_q), 137.5 (C_q), 133.5 (CH), 131.1 (CH), 131.0 (C_q), 128.2 (CH), 128.0 (d, (2 × CH), *J* = 7.7 Hz), 125.8 (C_q), 125.5 (CH), 115.7 (d, (2 × CH),

11.0 Hz), 115.6 (CH), 56.9 (CH), 45.9 (CH), 45.7 (CH), 31.5 (CH_2); HRMS (EI) *m/e* calcd. for $C_{18}H_{17}O_2N_2SF$ 344.09893, found 344.09964.

4HP-TQS

4-(4-Hydroxyphenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide.[13]

Purified by flash column chromatography using a gradient elution of 40-60% ethyl acetate in petrol to give the title compound as a pink foam (110 mg, 32%).

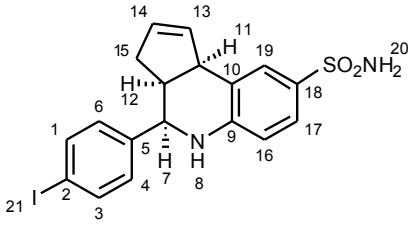


R_f 0.31(6:4 EtOAc/Petrol); mp 213-215˚C; IR υ_{max} 3389 (NH), 2923 (NH), 1598 (aromatic), 1501 (aromatic), 1307 (SO_2-N, S=O), 1287 (O-H), 1149 (SO_2-N) cm^{-1}; δ_H (600 MHz, MeOD) 7.52 (d, 1H, *J* = 1.8 Hz, H-19), 7.42 (dd, 1H, *J* = 8.5, 1.8 Hz, H-17), 7.26 (d, 2H, *J* = 8.5 Hz, H-1,3), 6.79 (d, 2H, *J* = 8.5 Hz, H-4,6), 6.75 (d, 1H, 8.5 Hz, H-16), 5.90-5.89 (m, 1H, H-13), 5.64-5.63 (m, 1H, H-14), 4.57 (d, 1H, *J* = 3.4 Hz, H-7), 4.06 (d, 1H, *J* = 8.8 Hz, H-11), 2.95 (dtd, 1H, *J* = 9.6, 8.8, 3.4 Hz, H-12), 2.51 (ddd, 1H, *J* = 16.0, 9.6, 2 Hz, H-15) 1.77 (br dd, 1H, *J* = 16.0, 8.8, H-15); δ_C (150 MHz, MeOD) 157.7 (C_q), 151.7 (C_q), 135.0 (CH), 134.4 (C_q), 132.6 (C_q), 131.4 (CH), 130.6 (CH), 128.7 (2×CH), 128.5 (CH), 126.4 (C_q), 125.6 (CH), 116.2 (2×CH), 57.8 (CH), 47.9 (CH), 47.7 (CH), 32.6 (CH_2).

4IP-TQS

4-(4-Iodophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulphonamide

Purified by recrystallisation from isopropanol/toluene/petrol to give the title compound as a brown/grey powder (68 mg, 15%).

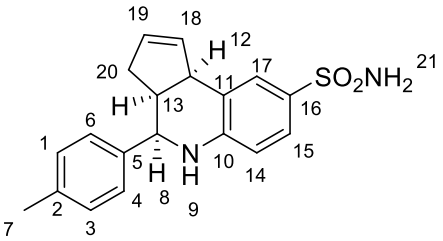


m.p. 220-222˚C; ν_{max} 3327 (N-H), 1598 (aromatic), 1149 (SO_2-N), 722 (C-I) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.72 (d, 2H, *J* = 8.5 Hz, H-1, 3), 7.59 (br s, 1H, H-19), 7.52 (dd, 1H, *J* = 8.4, 1.8 Hz, H-17), 7.16 (d, 2H, *J* = 8.5 Hz, H-4, 6), 6.65 (d, 1H, *J* = 8.4 Hz, H-16), 5.90-5.87 (m, 1H, H-13), 5.70-5.67 (br d, 1H, 4.58 Hz, H-14), 4.66 (s, 3H, NH_2, H-7), 4.16 (br s, NH), 4.10 (d, 1H, *J* = 8.1 Hz, H-11), 2.98 (dtd, 1H, *J* = 9.1, 8.1, 2.9 Hz, H-12), 2.51 (ddd, 1H, *J* = 15.7, 9.1, 1.5 Hz, H-15), 1.82 (br dd, 1H, *J* = 15.7, 8.1 Hz, H-15); δ_C (150 MHz, $CDCl_3$) 149.4 (C_q), 141.5 (C_q), 137.9 (2×CH), 133.4 (CH), 131.1 (C_q),131.0 (CH), 128.4 (2 × CH), 128.2 (CH), 125.8 (C_q), 125.5 (CH), 115.8 (CH), 93.1 (C_q), 57.1 (CH), 45.72 (CH), 45.71 (CH), 31.5 (CH_2); HRMS (EI) *m/e* calcd. for $C_{18}H_{17}O_2N_2SI$ 452.00499, found 452.00478.

4MP-TQS

4-p-tolyl-3a,4,5,9b-tetrahydro-1H-cyclopenta[c]quinoline-8-sulphonamide

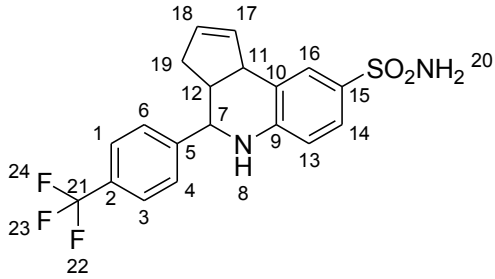
Purified by flash chromatography from 2:3 EtOAc:petrol to give the title compound as a dark green viscous oil (67 mg, 13%).



ν_{max} 3320 (N-H), 2924 (N-H), 2862 (N-H), 1599.5 (aromatic), 1500 (aromatic), 1324.8 (SO_2-N), 1163.7 (S=O), 1086.3 (S=O), 787 (CH=CH bend) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.59 (d, 1H, *J* = 1.9 Hz, H-17), 7.51 (dd, 1H, *J* = 8.7, 1.9 Hz, H-15), 7.29 (d, 2H, *J* = 8.2 Hz, H-1,3), 7.20 (d, 2H, *J* = 8.2 Hz, H-4,6), 6.64 (d, 1H, *J* = 8.7 Hz, H-14), 5.89-5.87 (m, 1H, H-18), 5.69-5.67 (m, 1H, H-19), 4.68 (br d, 1H, *J* = 2.5 Hz, H-8), 4.63 (br s, 2H, NH_2), 4.21 (br s, 1H, N*H*), 4.10 (br d, 1H, *J* = 8.9 Hz, H-12), 3.00 (dtd, 1H, *J* = 10.9, 8.9, 2.5 Hz, H-13), 2.56 (ddd, 1H, *J* = 16.5, 10.9, 2.5 Hz, H-20), 2.36 (br s, 3H, H-7), 1.84 (br dd, 1H, *J* = 16.5, 8.9 Hz, H-20); δ_C (150 MHz, $CDCl_3$) 149.9 (C_q), 138.7 (C_q), 133.5 (CH), 131.2 (CH), 130.6 (C_q), 129.5 (2 × CH), 128.3 (CH), 126.4 (2 ×CH), 125.9 (C_q), 125.4 (CH), 115.6 (CH), 60.5 (CH), 57.3 (CH), 46.0 (CH), 45.9 (CH), 31.6 (CH_2), 21.2 (CH_3). HRMS (EI) *m/e* calcd. for $C_{19}H_{20}O_2N_2S$ 340.12400, found 340.12441.

4TP-TQS

4-(4-(trifluoromethyl)phenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide

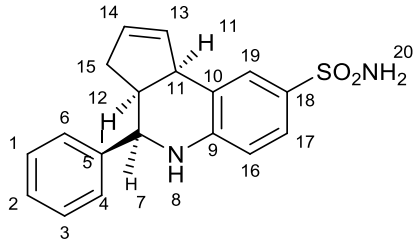


m.p. 112-115˚C; R_f = 0.42 (1:1 EtOAc : Petroleum Ether 50-60 ˚C); ν_{max} 3359-3262 (2˚ amine), 1598 (aromatic C=C), 1499 (CH_2), 1300 (CF_3), 1313 (SO_2-N), 1153-1124 (O=S=O), 732 (CH=CH bend) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.70 (d, 2H, *J* = 8.0 Hz, H-1,3), 7.62 (d, 1H, *J* = 1.5 Hz, H- 16), 7.57-7.54 (m, 3H, H-4, 6, 14), 6.69 (d, 1H, *J* = 8.6 Hz, H-13), 5.91-5.89 (m, 1H, H-17), 5.68 (br d, 1H, *J* = 4.6 Hz, H-18), 4.78 (d, 1H, *J* = 3.2 Hz, H-7), 4.76 (s, 2H, NH_2), 4.13 (d, 1H, *J* = 8.5 Hz, H-11), 3.03 (dtd, 1H, *J* = 9.4, 8.5, 3.2 Hz, H-12), 2.53 (dtd, 1H, 16.1, 9.4, 1.8 Hz, H-19), 1.79 (br dd, 1H, *J* = 16.1, 8.5 Hz, H-19); δ_C (150 MHz, $CDCl_3$) 149.3 (C_q), 145.8 (C_q), 133.4 (CH), 131.3 (C_q), 130.9 (CH), 130.1 (q, C_q, *J* = 32.8 Hz), 128.2 (CH), 126.8 (3 × CH), 125.8 (q, 2 × CH , *J* = 3.7 Hz), 125.7 (C_q), 124.1(q, C_q, *J* = 271.7 Hz), 115.9 (CH), 57.3 (CH), 45.7 (CH), 45.4 (CH), 31.5 (CH_2); HRMS (EI) *m/e* calcd. for $C_{19}H_{17}F_3N_2O_2S$ 394.09573, found 394.09630.

P-TQS

4-Phenyl-3a, 4, 5, 9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulphonamide

Purified by recrystallisation from isopropanol/petrol to give the title compound as a pale orange solid (169 mg, 51%).

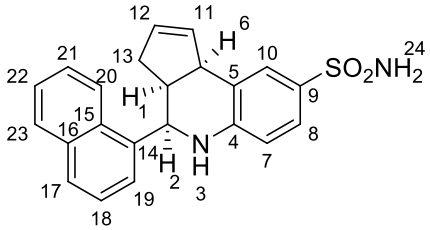


m.p. 142-144°C; ν_{max} 3343 (N-H), 3062 (NH), 2967 (C-H), 1598 (aromatic), 1313 (SO_2-N), 1301 (S=O), 1147-1124 (S=O) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 7.59 (d, 1H, *J* = 1.9 Hz, H-19), 7.52 (dd, 1H, *J* = 8.5, 1.9 Hz, H-17), 7.41 (br s, 2H, H-4, 6), 7.38 (d, 2H, *J* = 7.4 Hz, H-1, 3), 7.32 (m, 1H, H-2), 6.66 (d, 1H, *J* = 8.5 Hz, H-16), 5.90-5.88 (m, 1H, H-13), 5.70-5.67 (m, 1H, H-14), 4.72 (br d, 1H, *J* = 3.1 Hz, H-7), 4.64 (s, 2H, NH_2), 4.24 (br s, 1H, N*H*), 4.12 (br d, 1H, *J* = 8.7 Hz, H-11), 3.03 (dtd, 1H, *J* = 9.2, 8.7, 3.1 Hz, H-12), 2.56 (ddd, 1H, *J* = 16.4, 9.2, 3.1 Hz, H-15), 1.83 (br dd, 1H, *J* = 16.4, 8.7 Hz, H-15); δ_C (150 MHz, $CDCl_3$) 149.8 (C_q), 141.7 (C_q), 133.4 (CH), 131.2 (CH), 130.8 (C_q), 128.8 (2×CH), 128.2 (CH), 127.8 (CH), 126.4 (2×CH), 125.9 (C_q), 125.5 (CH), 115.6 (CH), 57.5 (CH), 45.9 (CH), 45.8 (CH), 31.6 (CH_2). HRMS (EI) *m/e* calcd. for $C_{18}H_{18}O_2N_2S$ 326.10889, found 326.10929.

TQS

4-(naphthalen-1-yl)-3a,4,5,9b-tetrahydro-1H-cyclopenta[c]quinoline-8-sulfonamide

Purified by recrystallisation from isopropanol : petrol to give the title compound as a pale yellow solid (215.8 mg, 22.4 %).



mp 203-205 °C; IR ν_{max} 3344 (N-H), 3268 (N-H), 2930 (N-H), 1598 (aromatic), 1496 (aromatic), 1321-1288 (SO_2-N), 1197 (S=O), 1127 (SO_2–N), 1091 (S=O) cm^{-1}; δ_H (600 MHz, $CDCl_3$) 8.09 (d, 1H, *J* = 8.4 Hz, H-10), 7.92 (d, 1H, *J* = 8.1 Hz, H-), 7.84 (d, 1H, *J* = 8.4 Hz, H-), 7.73 (d, 1H, *J* = 7.1 Hz, H-), 7.66 (d, 1H, *J* = 1.9 Hz, H-), 7.58-7.51 (m, 4H, H-), 6.73 (d, 1H, *J* = 8.2 Hz, H-7), 5.90-5.88 (m, 1H, H-11), 5.67-5.64 (m, 1H, H-12), 5.52 (d, 1H, *J* = 1.56 Hz, H-2), 4.66 (s, 2H, NH_2), 4.25 (d, 1H, *J* = 8.83 Hz, H-6), 4.22 (s, 1H, N*H*), 3.34 (dtd, 1H, *J* = 9.4, 7.3, 3.3 Hz, H-12), 2.58 (ddd, 1H, *J* = 16.7, 9.6, 2.4 Hz, H-15), 1.65 (br dd, 1H, *J* = 16.2, 5.1 Hz, H-15); δ_C (150 MHz, $CDCl_3$) 150.3 (C_q), 137.2 (C_q), 133.9 (C_q), 133.4 (CH), 131.2 (CH), 130.9 (C_q), 130.4 (C_q), 129.3 (CH), 128.2 (d, 5.4 Hz, CH), 126.5 (CH), 126.3 (C_q), 122.7 (CH), 122.3 (CH), 115.9 (CH), 53.3 (CH), 45.9 (CH), 43.7 (CH), 32.0 (CH_2).