The pharmacological profile of ELIC, a prokaryotic GABA-gated receptor

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ABSTRACT

The Erwinia ligand-gated ion channel (ELIC) is a bacterial homologue of vertebrate Cys-loop ligand-gated ion channels. It is activated by GABA, and this property, combined with its structural similarity to GABA A and other Cys-loop receptors, makes it potentially an excellent model to probe their structure and function. Here we characterise the pharmacological profile of ELIC, examining the effects of compounds that could activate or inhibit the receptor. We confirm that a range of amino acids and classic GABA A receptor agonists do not elicit responses in ELIC, and we show the receptor can be at least partially activated by 5-aminovaleric acid and γ-hydroxybutyric acid, which are weak agonists. A range of GABA A receptor non-competitive antagonists inhibit GABA-elicited ELIC responses including α-endosulfan (IC 50 = 17 μM), dieldrin (IC 50 = 66 μM), and picrotoxinin (IC 50 = 96 μM) which were the most potent. Docking suggested possible interactions at the 2′ and 6′ pore-lining residues, and mutagenesis of these residues supports this hypothesis for α-endosulfan. A selection of compounds that act at Cys-loop and other receptors also showed some efficacy at blocking ELIC responses, but most were of low potency (IC 50 > 100 μM). Overall our data show that a number of compounds can inhibit ELIC, but it has limited pharmacological similarity to GLIC and to Cys-loop receptors.

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1. Introduction

The Cys-loop family of ligand-gated ion channels are membrane proteins responsible for fast excitatory and inhibitory synaptic neurotransmission in the central and peripheral nervous systems. Members of this family share a common quaternary structure of five subunits that can be homomeric or heteromeric. Each of the subunits has three distinct regions that are known as the extracellular, transmembrane and intracellular domains. The N-terminal extracellular domain contains the neurotransmitter binding sites, which are located at subunit interfaces. They are created by the convergence of three amino acid loops (loops A–C) from the principal subunit and three β-sheets (loops D–F) from the adjacent complementary subunit (Brejc et al., 2001; Unwin, 2005). The transmembrane domain consists of 4 transmembrane α-helices from each subunit (M1–M4) that span the membrane, with the M2 helices surrounding the central ion pore. The intracellular domain is largely unstructured, and is responsible for receptor trafficking, regulation by intracellular modulators, and has a role in channel conductance (Hales et al., 2006; Deeb et al., 2007; Carland et al., 2009).

One of the major problems in understanding the mechanisms of action of this family of channels is the paucity of high resolution structures. Nevertheless the identification of prokaryotic Cys-loop receptor homologues has significantly improved our understanding of many structural details (Tasneem et al., 2005). An X-ray crystal structure of a Cys-loop receptor homologue from Erwinia chrysanthemi (Erwinia ligand-gated ion channel or ELIC) was solved in 2008, and one from Gloeobacter violaceus (Gloeobacter ligand-gated ion channel, or GLIC) in 2009 (Hilf and Dutzler, 2008, 2009; Bocquet et al., 2009). These prokaryotic receptors share many of their structural features with Cys-loop receptors, although they do not possess an N-terminal α-helix, an intracellular domain, or the disulphide bonded loop that gives the eukaryotic family its name. The crystallisation conditions of these proteins (ELIC unliganded; GLIC at high pH) led to the proposal that ELIC is in a closed conformation, while GLIC is in an open conformation, although recent work suggests that the structure of GLIC may represent a desensitized state (Parikh et al., 2011). GLIC is activated by protons and ELIC is activated by a range of small amine molecules, including GABA (Ulens et al., 2011; Zimmermann and Dutzler, 2011). The

Abbreviations: nACh, nicotinic acetylcholine; AChBP, acetylcholine binding protein; GABA, γ-aminobutyric acid; ELIC, Erwinia ligand-gated ion channel; GLIC, Gloeobacter ligand-gated ion channel; 5-AY, 5-aminovaleric acid; GHB, gamma-hydroxybutyric acid; PXN, picrotoxinin; ACh, acetylcholine; 5-HT, 5-hydroxytryptamine.

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potency of GABA on ELIC is low compared to its eukaryotic counterparts, but works on bacterial receptors in other systems (e.g. Singh et al., 2007; Zhou et al., 2007), suggest that even if the potencies are not in the same range, their mechanism of action at homologous proteins are similar, making ELIC an attractive model system to understand the molecular mechanisms of Cys-loop receptors. Although ELIC shows low sequence similarity with Cys-loop homologous proteins are similar, making ELIC an attractive model system to understand the molecular mechanisms of Cys-loop receptors.

2. Materials and Methods

2.1. Cell culture and oocyte Maintenance

Xenopus laevis oocyte-positive females were purchased from NASCO (Fort Atkinson, Wisconsin, USA) and maintained according to standard methods. Harvested stage V–VI Xenopus oocytes were washed in four changes of ND96 (96 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 5 mM HEPES, pH 7.5), de-folliculated in 1.5 mg ml⁻¹ collagenase Type 1A for approximately 2 h, washed again in four changes of ND96 and stored in ND96 containing 2.5 mM sodium pyruvate, 50 mM gentamycin, 0.7 mM theophylline.

2.2. Receptor expression

The ELIC sequence (Genbank accession number PO7CB7) was purchased from Genscript as a synthetic gene with optimized codon usage for expression in Escherichia coli. For electrophysiological recordings from Xenopus oocytes, the mature sequence of ELIC (residue numbers 8-322) was cloned into pGEMHE with the signal sequence (MRCSPGCVVLAASLHVSLQ) of the human α7 nACh receptor (Liman et al., 1992). cRNA was in vitro transcribed from linearised pGEMHE cDNA template using the mMessage mMACHINE T7 Transcription kit (Ambion, Austin, Texas, USA). Stage V and VI oocytes were injected with 20 ng cRNA, and currents were recorded 1–3 days post-injection.

2.3. Electrophysiology

Using two-electrode voltage-clamp, Xenopus oocytes were clamped at –60 mV using an OC-725 amplifier (Warner Instruments, Connecticut, USA). Data were acquired with the Strathclyde Electrophysiology Software Package (Department of Physiology and Pharmacology, University of Strathclyde, UK). Currents were recorded at 5 kHz and filtered at a frequency of 1 kHz. Micro-electrodes were fabricated from borosilicate glass (GC120TF-10, Harvard Apparatus, Edenbridge, Kent, UK) using a one stage horizontal pull (P-87, Sutter Instrument Company, California, USA) and filled with 3 M KCl. Pipette resistances ranged from 1.0 to 2.0 MΩ. Oocytes were perfused with ND96 at a constant rate of 12 ml min⁻¹. Drug application was via a simple gravity fed system calibrated to run at the same rate. Inhibition by test compounds was measured at the GABA EC₅₀ (1.6 mM).

Analysis and curve fitting was performed using Prism v.4 (GraphPad Software, San Diego, California, USA). Concentration–response data for each oocyte were normalised to the maximum current for that oocyte. The mean and S.E.M. for a series of oocytes were plotted against agonist or antagonist concentration and iteratively fitted to the following equation:

\[ I_A = \frac{I_{max} - I_{min}}{1 + 10^{\frac{EC_{50} - \log ACE}{nH}}} \]

where A is the concentration of ligand present; \( I_A \) is the current in the presence of ligand concentration A; \( I_{min} \) is the current when A = 0; \( I_{max} \) is the current when A = ∞, \( EC_{50} \) is the concentration of A which evokes a current equal to \( (I_{max} + I_{min})/2 \); and \( nH \) is the Hill coefficient. The relative current amplitudes (\%max) were expressed as the maximal current amplitude evoked by the test compound divided by the maximal current amplitude evoked by GABA.

2.4. Docking

Docking was performed using an ELIC crystal structure (pdbid: 2VL0) downloaded from the RCSB Protein Data Bank. A three-dimensional structure of β-endosulfan was extracted from the Cambridge Structural Database (Ref. code: β-Endosulfan – ENSULF). β-Endosulfan was converted into the α conformer and the protonated form constructed in Chem3D Ultra 7.0 and energy-minimized using the MM2 force field.

Docking of the protonated ligand into ELIC was carried out using GOLD 3.0 (The Cambridge Crystallographic Data Centre, Cambridge, UK). The binding site was constrained as a docking sphere with a 20 Å radius surrounding the C₆ residues of oocytes were plotted against agonist or antagonist concentration and iteratively fitted to the following equation:

\[ I_A = \frac{I_{max} - I_{min}}{1 + 10^{\frac{EC_{50} - \log ACE}{nH}}} \]

where A is the concentration of ligand present; \( I_A \) is the current in the presence of ligand concentration A; \( I_{min} \) is the current when A = 0; \( I_{max} \) is the current when A = ∞, \( EC_{50} \) is the concentration of A which evokes a current equal to \( (I_{max} + I_{min})/2 \); and \( nH \) is the Hill coefficient. The relative current amplitudes (\%max) were expressed as the maximal current amplitude evoked by the test compound divided by the maximal current amplitude evoked by GABA.

3. Results

3.1. ELIC agonists

Application of GABA produced large, reversible inward currents (Fig. 2). These were predominantly Na⁺ currents (Zimmermann and Dutzler, 2011). Plotting current amplitude against a range of GABA concentrations yielded an EC₅₀ of 1.6 mM (pEC₅₀ = 2.78 ± 0.04, n = 6) and Hill slope of 2.1 ± 0.6. At 1 mM, the amino acid Ala, Arg, Asn, Asp, Cys, Glu, Gln, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val) had no effect on ELIC. At 10 mM several native Cys-loop receptor ligands (ACh, Gly and 5-HT) also yielded no ELIC responses (Table 1).

![Image](image-url)

**Fig. 1.** An alignment of channel-lining residues for a range of eukaryotic Cys-loop receptors and prokaryotic homologues. As is common for these receptors, a prime notation is used to facilitate comparison between different subunits, with 0’ being the conserved charged residue at the start of M2. Grey indicates residue conservation. Accession numbers are: ELIC P07CB7, GLIC Q7N98, 5-HT₁ P46998, nACh α1 P27088, Gly P23415, GABA α1 P14860, GABA β2 P47870, GABA γ2 P18507, GluCl Q94900.

![Image](image-url)

**Fig. 2.** GABA and 5-AV agonist concentration–response curves (A) and example responses (B). The black bar is the application of agonist. Data = mean ± SEM, n ≥ 4.
A range of amino acids (Pro, His, Gln and Tyr at 1 mM) were the most potent, followed by dieldrin, picrotoxinin and rimantadine. A range of other compounds (acetic acid, arginase, 3-aminopropylphosphonic acid, pyroglutamate, L-glutamate, and pyroglutamate) also activated ELIC, but required high concentrations (all at 10 mM), and 3-aminopropylphosphonic acid and pyroglutamate, both at 100 mM, activated ELIC (Table 1). Of the 25 compounds shown in Table 1, 12 inhibited ELIC responses: 2 had IC50s < 20 μM, 3 had IC50s 20–100 μM, 5 had IC50s of 100–1000 μM, and 2 had IC50s > 1 mM. Proadifen and α-endosulfan were the most potent, followed by dieldrin, picrotoxinin and rimantadine. A range of amino acids (Pro, His, Gln and Tyr at 1 mM) and the GABAa receptor competitive antagonists bicuculline and gabazine at 100 μM had no effect when co-applied in the presence of GABA. We also tested the quaternary ammonium compounds tetramethylammonium and tetraethylammonium at much higher concentrations, and these compounds inhibited GABA-evoked ELIC responses with IC50s close to 20 mM (pEC50 = 1.76 ± 0.28 and 1.65 ± 0.06 respectively, n = 3). None of the compounds had an effect when applied alone.

We also tested PXN and rimantadine against cysteamine-induced responses as cysteamine is a slightly more efficacious agonist (\(R_{\text{max}} = 1.3 \pm 0.1, n = 3\), cf to GABA; similar to data reported in Zimmermann and Dutzler, 2011). There were no significant differences when compared to inhibition of GABA-induced responses (data not shown).

3.3. Ligand docking

To probe possible locations for ligand binding α-endosulfan was docked into the ELIC structure (Fig. 4). It docked close to the 6' location where it was stabilised by hydrogen bond interactions with Q2' (2/10 poses) and/or T6' (6/10) pore-lining residues; in Fig. 4A ten poses are superimposed to show the volume that the docked ligand occupies.

3.4. Effects of pore mutations on antagonist potency

To test the predictions of ligand docking, conservative substitutions were made within the ELIC pore at the 2' and 6' positions, and the effect on inhibition of the two most potent compounds were examined (Table 3). At both Q2' and T6'S mutant receptors, the IC50 of α-endosulfan was increased >10 fold, supporting a binding location in the pore close to these two residues (Fig. 4D). In contrast, IC50s for proadifen were close to wild type, consistent with this compound not being a channel blocking antagonist, as reported for other Cys-loop receptors. At both Q2' and T6'S mutant receptors GABA EC50s and Hill slopes were similar to wild type receptor values.

4. Discussion

ELIC is a cationic GABA-gated prokaryotic ligand-gated ion channel that is structurally similar to vertebrate GABA-gated receptors, and, like GABAa receptors, can be modulated by benzodiazepines (Ulens et al., 2011). ELIC can readily be expressed and functionally characterised in Xenopus oocytes, but unlike homologous vertebrate receptors, the structure of ELIC at high resolution has been solved (Hilf and Dutzler, 2008). This potentially makes ELIC a good model system for studying structure–function relationships. Here we examine the pharmacology of ELIC. We show that compounds that efficiently activate the receptor are difficult to find, and the novel agonists we identified are of low potency. We also show that classic GABAa competitive antagonists do not inhibit the functional response. However, a range of compounds that act as non-competitive antagonists at GABAa and a range of other Cys-loop receptors also inhibit ELIC responses, suggesting that the pore of ELIC shares some pharmacological similarities to homologous eukaryotic receptors.

It has been previously shown that GABA evokes concentration-dependent responses when ELIC mRNA is injected into Xenopus oocytes (Zimmermann and Dutzler, 2011). Our data show similar effects of GABA, and the values obtained from concentration–response curves are comparable. Other compounds that have been previously identified as agonists at ELIC are a range of primary amines, including amino-alcohols and alkylamines (Zimmermann and Dutzler, 2011). New agonists that
we identified are 5-AV and GHB, although these are less potent than GABA, and may be partial agonists, as we did not achieve responses > 20% \( R_{\text{max}} \). 5-AV, which is one \( \text{CH}_2 \) group longer than GABA, is a low potency partial agonist (EC\(_{50} = 1.6 \text{ mM}, R_{\text{max}} = 0.85 \) of RDL, a GABA-activated insect receptor (McGonigle and Lummis, 2010). GHB is equivalent to GABA with a hydroxyl group replacing the amino group, and its very low efficacy at ELIC (\( R_{\text{max}} < 0.05 \) at 100 mM) demonstrates the importance of the amino group; this compound has no effect on RDL, supporting a role for the amino group in receptor activation in both classes of GABA-activated receptor (McGonigle and Lummis, 2010). None of the other compounds tested in this study activated ELIC, and the GABA\(_A\) receptor competitive antagonists were ineffective, suggesting that the ELIC pharmacophore differs significantly from that in the GABA\(_A\) receptor. Some of the tested compounds are intermediates in quorum sensing, a method of bacterial communication in which ELIC could participate. The absence of effects from these compounds suggests that if ELIC is associated with this mechanism, it is not activated by any of these signalling molecules.

A range of non-competitive antagonists were able to block GABA-evoked responses in ELIC. The majority of these also block GABA-activated Cys-loop receptors, with the most potent (IC\(_{50} < 20 \mu\text{M}\)) being \( \alpha \)-endosulfan and proadifen, with dieldrin, picrotoxinin (PXN) and rimantidine having IC\(_{50}\)s < 100 \mu\text{M}. PXN, the more potent component of picrotoxin, blocks a range of Cys-loop receptors including GABA\(_A\) receptors, while \( \alpha \)-endosulfan and dieldrin are cyclodiene insecticides (now rarely used), which block the pore of GABA-activated receptors in both vertebrates and invertebrates (Abalis et al., 1985; Ratra et al., 2001; Chen et al. 2006). Rimantidine and proadifen are not classic channel blockers, although some inhibitory effects have been reported (Spitzmaul et al., 2009; Stouffer et al., 2008). Rimantidine also inhibits GLIC and may act in the pore, although this is unlikely for proadifen, which stabilises the desensitised state. Studies of Cys-loop receptors show interactions with the 2' and 6' pore-lining residues contribute to stabilising many channel blocking compounds (e.g. Chiara et al., 2009 Thompson et al., 2011), and more recently, high resolution co-crystal structures have revealed the binding sites of some of these compounds (Hibbs and Gouaux, 2011; Hilf and Dutzler, 2009). Our docking studies indicate that the 2' and 6' residues are also important for channel blocking compounds that inhibit ELIC, and our data with \( \alpha \)-endosulfan support this hypothesis as mutation of either the 2' or 6' residues significantly reduced the potency of this compound.

Compounds that inhibited ELIC responses less potently (IC\(_{50} > 100 \mu\text{M}\)) were amantadine, bilobalide, chlorpromazine, fipronil and progesterone. Bilobalide and fipronil block several

**Table 2**

| Ligand              | Known LGIC Targets | pIC\(_{50}\) (Mean ± SEM) | IC\(_{50}\) (\mu\text{M}) | \( n_H \) | \( n \) |
|---------------------|--------------------|---------------------------|--------------------------|----------|--------|
| 5-hydroxyindole     | 5-HT\(_5\), \( \alpha \)-7 nACH, GABA\(_A\) | NI                       | 4.77 ± 0.15 | 17       | 0.7 ± 0.2 | 4     |
| \( \alpha \)-endosulfan | GABA\(_A\), Gly | 3.43 ± 0.03              | 370                      | 2.5 ± 0.4 | 4      |
| Amanantidine        | nACH               | NI                       | 4.06 ± 0.04             | 96       | 1.2 ± 0.6 | 3     |
| Bicuculline         | GABA\(_A\)         | NI                       | 5.09 ± 0.04             | 81       | 2.9 ± 0.6 | 4     |
| Bilobalide          | 5-HT\(_3\), GABA\(_A\), Gly | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| Chlorpromazine      | 5-HT\(_3\), nACH  | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| Dexamethasone       | 5-HT\(_3\)         | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| Dieldrin            | GABA\(_A\), Gly   | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| Diltiazem           | 5-HT\(_3\), \( \alpha \)-7 nACH, GABA\(_A\) | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| Estrone             | 5-HT\(_3\)         | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| Fipronil            | GABA\(_A\), Gly   | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| GABazine            | GABA\(_A\)         | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| Ivermectin          | GluCL, GABA\(_A\), Gly | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| Lindane             | GABA\(_A\), Gly   | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| Melphamine          | 5-HT\(_5\), nACH  | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| Pancuronium         | nACH               | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| Picrotoxin          | 5-HT\(_3\), GABA\(_A\), Gly | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| Proadifen           | nACH               | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| Progestosterone     | 5-HT\(_3\)         | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| Quinacrine          | nACH               | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| QX-222              | nACH               | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| Rimantadine         | nACH               | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |
| Tetracaine          | 5-HT\(_3\), nACH  | NI                       | 4.06 ± 0.10             | 96       | 1.2 ± 0.6 | 3     |

NI = no inhibition at 10 \mu\text{M}, NI* = no inhibition at 100 \mu\text{M}.

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**Fig. 3.** ELIC antagonists. (A) Example traces showing inhibition by picrotoxinin (PXN). Concentration-inhibition curves for PXN (B), \( \alpha \)-endosulfan (C), and rimantidine (D). Inhibition was measured at the GABA EC\(_{50}\) (1.6 \text{ mM}). Data – mean ± SEM, \( n \geq 4 \). Values derived from the curves can be found in Table 2.
Cys-loop receptors, including 5-HT$_3$, GABA$_A$, GluCl, glycine and RDL, by acting at the 6$^0$ residue (Huang et al., 2003; Ratra et al., 2001; Cole et al., 1995; Ikeda et al., 2004; Li and Akk, 2008; Thompson et al., 2011; Islam and Lynch, 2011. Amantadine, chlorpromazine and progesterone also block nACh receptor pores (Buisson and Bertrand, 1998; Chiara et al., 2009; Giraudat et al., 1987, 1989; Matsubayashi et al., 1997; Revah et al., 1990). Quaternary ammonium compounds are open channel blockers of nACh receptors and have been directly observed in co-crystals with GLIC, where they are located close to the 6$^0$ residue (Hilf et al., 2010). Here we show these compounds also block ELIC, albeit at much higher (100-fold) concentrations. The low potency of all these channel blocking compounds at ELIC is puzzling, as the pore-lining M2 residues are broadly conserved; we suggest that future studies examine the roles of residues at or close to the entrance to the pore as these may limit access.

A range of non-competitive antagonists similar to those studied here have also been examined at GLIC (Alqazzaz et al., 2011), but there is limited similarity in the pharmacology of the two receptors (Fig. 5). In general the number of compounds that inhibit ELIC are fewer, and their affinities are lower at ELIC than at GLIC. Only a-endosulfan has a similar IC$_{50}$ at both receptor types (17 µM at both), with 5 other compounds inhibiting both receptors (amantadine, chlorpromazine, fipronil, picrotoxinin, rimantadine) and 5 inhibiting neither (5-hydroxyindole, dexamethasone, imidacloprid, ivermectin, QX-222). This shows that the non-competitive pharmacology of ELIC is less similar to Cys-loop receptors than that of GLIC, and as the majority of ligands studied here are channel blockers in eukaryotes, our data show that the ELIC pore is pharmacologically, as well as structurally, different to those of GLIC, GluCl and the nACh receptor.

In conclusion, we have identified two novel ELIC agonists and a range of compounds that act as antagonists. These are ligands which inhibit a range of Cys-loop receptors (including 5-HT$_3$, GABA$_A$, glycine, GluCl and nACh receptors), consistent with the sequence similarities of the M2 regions in all these proteins. These data will be useful when further characterising the mechanism of action of ELIC, but the limited range of ligands that inhibit ELIC, and their lower potencies, indicate that the ELIC pore structure may not be as good as GLIC or other proteins for inferring molecular interactions in the channels of related receptors.

![Fig. 4.](image)

**Table 3**

Antagonist properties at M2 mutant receptors.

| Ligand        | GABA pEC$_{50}$ (EC$_{50}$) | a-endosulfan pIC$_{50}$ (IC$_{50}$) | Proadifen pIC$_{50}$ (IC$_{50}$) |
|---------------|-----------------------------|-------------------------------------|----------------------------------|
| Wild Type     | 2.78 ± 0.04 (1.6 mM)        | 4.77 ± 0.15 (17 µM)                 | 5.09 ± 0.04 (8.1 µM)             |
| Q2N           | 2.74 ± 0.02 (1.8 mM)        | NI (>100 µM)                        | 5.54 ± 0.09 (2.9 µM)             |
| T6S           | 2.75 ± 0.02 (1.8 mM)        | NI (>100 µM)                        | 5.29 ± 0.04 (5.1 µM)             |

NI – IC$_{50}$ was not reached at the highest concentration tested (10$^{-4}$ M). See Fig. 4D.
Conflicts of interest

None.

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