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Identification by virtual screening and functional characterisation of novel positive and negative allosteric modulators of the α7 nicotinic acetylcholine receptor

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ABSTRACT

Several previous studies have demonstrated that the activity of neurotransmitters acting on ligand-gated ion channels such as the nicotinic acetylcholine receptor (nAChR) can be altered by compounds binding to allosteric modulatory sites. In the case of α7 nAChRs, both positive and negative allosteric modulators (PAMs and NAMs) have been identified and have attracted considerable interest. A recent study, employing revised structural models of the transmembrane domain of the α7 nAChR in closed and open conformations, has provided support for an inter-subunit transmembrane allosteric binding site (Newcombe et al 2017). In the present study, we have performed virtual screening of the DrugBank database using pharmacophore queries that were based on the predicted binding mode of PAMs to α7 nAChR structural models. A total of 81 compounds were identified in the DrugBank database, of which the 25 highest-ranked hits corresponded to one of four previously-identified therapeutic compound groups (carbonic anhydrase inhibitors, cyclin-dependent kinase inhibitors, diuretics targeting the Na^+-K^+-Cl^- cotransporter, and fluoroquinolone antibiotics targeting DNA gyrase). The top-ranked compound from each of these four groups (DB04763, DB08122, furosemide and pefloxacin, respectively) was tested for its effects on human α7 nAChR expressed in *Xenopus* oocytes using two-electrode voltage-clamp electrophysiology. These studies, conducted with wild-type, mutant and chimeric receptors, resulted in all four compounds exerting allosteric modulatory effects. While DB04763, DB08122 and pefloxacin were antagonists, furosemide potentiated ACh responses. Our findings, supported by docking studies, are consistent with these compounds acting as PAMs and NAMs of the α7 nAChR via interaction with a transmembrane site.

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1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are members of the superfamily of pentameric ligand-gated ion channels, that also includes receptors for 5-hydroxytrptamine (5-HT), γ-aminobutyric acid (GABA) and glycine (Changeux, 2012). Seventeen nAChR subunits have been identified in vertebrates (α1-α10, β1- β4, γ, δ and ε) that can co-assemble to generate a diverse family of pharmacologically distinct nAChR subtypes (Millar and Gotti, 2009). The human α7 nAChR has attracted interest as a target for therapeutic drug discovery, which has arisen, in part, from evidence that α7 nAChRs may play a role in a range of neurological and psychiatric disorders (Parri et al., 2011; Wallace and Porter, 2011). In particular, considerable attention has focussed on studies of positive allosteric modulators (PAMs) that are thought to bind within the receptor's transmembrane domain (Williams et al., 2011; Chatzidaki and Millar, 2015).

Abbreviations: 5-HT, 5-hydroxytryptamine; DB04763, 1-N-(4-sulfamoylphenethyl)-2,4,6-trimethylpyridinium tetrafluoroborate; AUC, area under the curve; CAII, carbonic anhydrase II; CDK2, cyclin-dependent kinase 2; DB08122, *N*-methyl-4-{[(2-oxo-1,2-dihydro-3h-indol-3-ylidene)methyl]amino} benzenesulfonamide; GABA, γ-aminobutyric acid; NAM, negative allosteric modulator; nAChR, nicotinic acetylcholine receptor; NKCC, Na^+-K^+-Cl^- cotransporter; PAM, positive allosteric modulator; ROC, receiver operator characteristic.

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The nAChR orthosteric binding site is located in the extracellular domain, at the interface between subunits (Changeux, 2012). Therefore, we consider allosteric binding sites to be any binding site that is topographically distinct from the binding site of the endogenous agonist (the orthosteric site). In addition to PAMs, which are allosteric ligands that potentiate agonist-evoked responses, negative allosteric modulators (NAMs) reduce agonist-evoked responses. Homomeric α7 nAChRs are characterised by their relatively low ACh sensitivity, rapid activation and fast desensitisation (Couturier et al., 1990). By convention, α7 nAChR PAMs have been classified as either 'type I', which have little or no effect on desensitisation kinetics, or 'type II', which reduce the rate of receptor desensitisation (Bertrand and Gopalakrishnan, 2007). However, there is also evidence for α7-selective PAMs with intermediate properties (Chatzidaki et al., 2015).

We have recently generated revised structural models of the human α7 nAChR, based on the cryo-EM structure of the *Torpedo* electric organ nAChR in its closed and open conformations, in which an error in the transmembrane domain of the *Torpedo* nAChR structure has been corrected (Newcombe et al., 2017). Previous computer docking studies performed with our revised human α7 nAChR structural models identified an inter-subunit transmembrane site for allosteric modulators (Newcombe et al., 2017). In the present study, we have extended these findings by generating pharmacophore models to perform virtual screening of the DrugBank database (Wishart et al., 2006). DrugBank is a relatively small database, containing approximately 11,000 compounds that act on identified drug targets, of which a relatively high proportion (approximately 2500) are approved small molecule drugs. Our goal in performing virtual screening with pharmacophore queries based on a series of known α7 nAChR PAMs was to identify compounds that may interact with the predicted allosteric transmembrane site and may therefore act as α7 nAChR allosteric modulators.

All of the 25 highest-ranked hits identified by virtual screening were compounds that are known to act as inhibitors of one of four previously identified protein targets: carbonic anhydrase II (CAII), cyclin-dependent kinase 2 (CDK2), Na^+-K^+-Cl^- cotransporter (NKCC) and DNA gyrase (DNAG). Drugs acting on these protein targets have been developed for use in the treatment of glaucoma (CAII inhibitors), as anti-cancer therapies (CDC2 inhibitors), as diuretics (NKCC inhibitors), or as antibiotics (DNA gyrase inhibitors). The highest ranked compounds identified by virtual screening from each of these four drug groups (DB04763, DB08122, DB00695 [furosemide] and DB00487 [pefloxacin], respectively) were tested for their effects on human α7 nAChR expressed in *Xenopus* oocytes. By means of two-electrode voltage-clamp recording, all four of the compounds were observed to have either positive or negative modulatory effects on α7 nAChRs, either potentiating or antagonising responses to acetylcholine. Three of the compounds (DB04763, DB08122 and pefloxacin) were found to act as NAMs of the α7 nAChR, whereas furosemide was an α7 nAChR PAM. The findings provide strong and direct evidence that virtual screening can be an effective approach for the identification of compounds with allosteric modulatory effects on neurotransmitter receptors such as the nAChR, even when employed with relatively small compound libraries.

2. Materials and methods

2.1. Virtual screening

A group of 25 α7 nAChR PAMs sharing close chemical similarity were selected (see the representative 'TQS-family' structure illustrated in Fig. 2 and also the compounds identified as 'TQS-family' in the supplemental Table 1 of Newcombe et al., 2017). These compounds were docked into revised structural models of the α7 nAChR transmembrane domain in both the open and closed conformations (Newcombe et al., 2017). Using a previously described consensus docking protocol (Newcombe et al., 2017), the top five solutions for each of the PAMs were clustered by RMSD with a cut-off of 2.0 Å. The largest cluster found for each of the open and closed docking experiments was taken to represent the active conformation of the ligand in each receptor conformation (Fig. 1). Three 3D pharmacophore queries were created based on each of the two clusters (one from the open form and the other from the closed form of the α7 nAChR structural model). This was done using the ligand model builder tool from the software package Rapid Overlay of Chemical Structures (ROCS) (Rush et al., 2005), allowing a maximum of six ligands to be utilized by the query generation algorithm. ROCS built every variation of possible query models containing between one and six ligands from the supplied binding mode cluster, creating a gaussian volume corresponding to the molecular shape of the overlaid ligands and assigning 'color atoms' at pharmacophoric points associated with hydrogen bond donors, hydrogen bond acceptors, rings and hydrophobes in the ligands that contributed to each of the queries that were built. Every built query was screened against the ligands in the cluster and the three

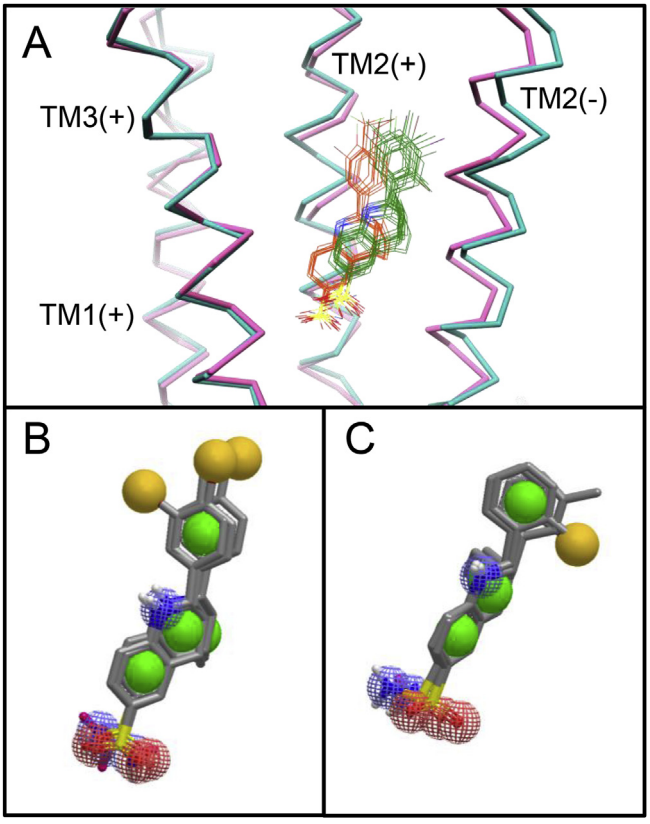

Fig. 1. Generation of pharmacophore queries used for virtual screening. The highest ranked clusters of binding mode solutions with previously characterised PAMs are shown within the α7 nAChR transmembrane domain (A). The Cα trace of TM1-3 helices of the principal subunit and TM2 helix of the complimentary subunit are shown for the open (cyan) and closed (pink) conformations. Also shown are binding mode clusters from which pharmacophore queries were generated for the open (green) and closed (orange) conformations. From the ligands in each cluster, pharmacophore queries were generated for the closed and open conformations (B and C, respectively). Note, only those selected for screening are shown. Features of the pharmacophore are represented as yellow spheres (hydrophobes), green spheres (rings), red hashed spheres (hydrogen bond acceptors) and blue hashed spheres (hydrogen bond donors). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

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