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Structure-based design, synthesis and structure–activity relationships of dibenzosuberyl- and benzoate-substituted tropines as ligands for acetylcholine-binding protein

Ewald Edink [a,†], Atilla Akdemir [b,†], Chimed Jansen [a], René van Elk [c], Obbe Zuiderveld [a], Frans J.J. de Kanter [d], Jacqueline E. van Muijlwijk-Koezen [a], August B. Smit [c], Rob Leurs [a], Iwan J.P. de Esch [a,*]

[a] Leiden/Amsterdam Center of Drug Research (LACDR), Division of Medicinal Chemistry, Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), VU University Amsterdam, The Netherlands
[b] Division of Pharmacology, Faculty of Pharmacy, Bezmialem Vakif University, Istanbul, Turkey
[c] Department of Molecular and Cellular Neurobiology, Center for Neurogenomics & Cognitive Research, VU University Amsterdam, The Netherlands
[d] Division of Organic and Inorganic Chemistry, Faculty of Sciences, VU University Amsterdam, The Netherlands

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ABSTRACT

Using structure-based optimization procedures on in silico hits, dibenzosuberyl- and benzoate substituted tropines were designed as ligands for acetylcholine-binding protein (AChBP). This protein is a homolog to the ligand binding domain of the nicotinic acetylcholine receptor (nAChR). Distinct SAR is observed between two AChBP species variants and between the α7 and α4β2 nAChR subtype. The AChBP species differences are indicative of a difference in accessibility of a ligand-inducible subpocket. Hereby, we have identified a region that can be scrutinized to achieve selectivity for nicotinic receptor subtypes.

The neuronal nicotinic acetylcholine receptors (nAChRs) belong to the Cys-loop receptor family of the ligand-gated ion channels (LGICs). The Cys-loop receptors are characterized by a pentameric assembly of subunits. Until now, 12 human neuronal nAChR subunits (α2–α10 and β2–β4) have been identified. These subunits combine to form either homopentamers (e.g., α7) or heteropentamers (e.g., α4β2), resulting in different pharmacological characteristics.[1–3]

Several important physiological and mental processes are regulated by nicotinic receptors and they are therefore potential therapeutic targets for a wide variety of neurodegenerative and psychiatric disorders. The human α4β2 and α7 receptors play a role in the pathophysiology of Alzheimer's disease, Parkinson's disease, schizophrenia, epilepsy and anxiety.[1–3] In addition, the human α4β2 nAChR is involved in nicotine addiction and pain.[2] Furthermore, the human α7 may also be of value as a pharmacological target in inflammation.[4,5] Several nicotinic receptor ligands are being investigated for clinical use. The first clinical breakthrough was reported in 2006, when Varenicline, a partial agonist on the α4β2 nAChR, was approved as a drug for smoking cessation.[6,7]

Due to high sequence identity of the binding pockets of the different subtypes and lack of detailed structural information on nAChRs, the development of selective ligands remains a challenge. Fortunately, the water-soluble acetylcholine binding protein (AChBP) from the fresh water snail *Lymnaea stagnalis* (Ls-AChBP) has been characterized and crystallized. This protein is a widely accepted structural homolog of the extracellular domain (ECD) of nicotinic receptors[8,9] The binding pocket of this homopentameric protein is situated at the interface of two adjacent subunits and is formed by the principal and complementary side (Supplementary data Fig. 1A–D).[8] The principal side is formed by mostly aromatic residues of loops A (Tyr89, Ls-AChBP amino acid numbering), B (Trp143) and C (Tyr185 and Tyr192). In addition, the flexible loop C has two vicinal cysteines (Cys187-188) that form a disulfide bond. The complementary side is formed by loops D, E and F and only loop D donates an aromatic residue (Trp53) (Supplementary data Fig. 1C–E).

After the identification of Ls-AChBP, similar binding proteins have been identified in other molluscan species, for example, *Aplysia californica* (Ac-AChBP) and *Bulinus truncatus* AChBP (Bt-AChBP) and these proteins have been co-crystallized in the

* Corresponding author. Tel.: +31 20 59 87841; fax: +31 20 59 87610.
E-mail address: i.de.esch@few.vu.nl (I.J.P. de Esch).
† These authors contributed equally to this work.

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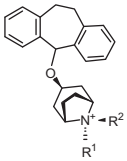
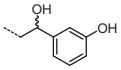
Table 1
The binding affinity (pK_i) of dibenzosuberyl substituted tropines for Ls- and Ac-AChBP, α7 and α4β4 nAChRs



Compd	R^1	R^2	Ls-AChBP pK_i ± SEM[a]	Ac-AChBP pK_i ± SEM[a]	α7 pK_i ± SEM[b]	α4β2 pK_i ± SEM[a]
Nicotine			6.5 ± 0.1	5.6 ± 0.1	6.0 ± 0.1	7.9 ± 0.1
α-Lobeline			6.2 ± 0.1	8.6 ± 0.1	5.1 ± 0.1	8.3 ± 0.1
9	H	H	6.0 ± 0.1	5.0 ± 0.1	n.d.	n.d.
8	Me	H	5.7 ± 0.1	5.2 ± 0.1	n.d.	n.d.
1	Me	Me	6.5 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	<4.5
2		H	6.6 ± 0.1	5.0 ± 0.1	n.d.	n.d.
4		H	7.0 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	<4.5
10		Me	7.4 ± 0.1	6.2 ± 0.1	5.8 ± 0.1	<4.5
5		H	5.7 ± 0.1	4.9 ± 0.1	4.7 ± 0.1	<4.5
6		H	5.7 ± 0.1	4.5 ± 0.1	4.4 ± 0.1	<4.5

n.d. = not determined; Compd = compound.
[a] [^{3}H]epibatidine displacement studies, pH 7.4.
[b] [^{3}H]MLA displacement studies, pH 7.4.

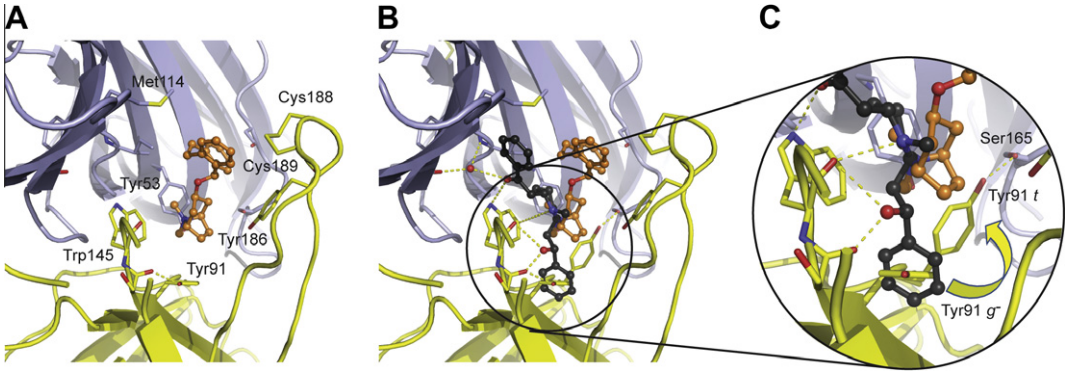


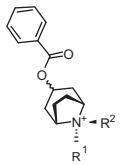
Figure 1. (A) Binding mode of compound **1** (orange balls and sticks) in Ac-AChBP. (B) Superposition of Ac-AChBP in complex with **1** (orange balls and sticks) and lobeline (**3**, black balls and sticks) shows that fragment merging may afford a novel chemotype capable of addressing the lobeline pocket. (C) Lobeline addresses the lobeline pocket after a change in rotameric state of Tyr91 (Y-flip, yellow arrow).

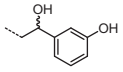
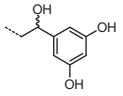
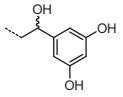
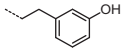
presence of nicotinic receptor ligands or buffer molecules.[10,11] The crystal structures of the three different AChBPs show a conserved architectural fold that has been recognized as a template to understand the ligand binding domains of nicotinic receptors and other mammalian Cys-loop receptors. Crucial information on ligand-receptor interactions has been obtained from agonist-bound structures of AChBP, i.e., carbamylcholine, nicotine and epibatidine.[11,12] Similar cation-π interactions between a conserved tryptophan (loop B) and cationic centers of several nonpeptidic ligands are observed in these co-crystal structures. In addition, nicotine forms a hydrogen bond between its pyrolidine nitrogen atom and the carbonyl backbone of Trp143 (Fig. 1F). Experimental evidence has been obtained that identical interactions are present in neuronal nicotinic receptors, illustrating the use of AChBP in nAChR research.[13]

Recently, we have described a study in which a benzoate substituted tropine-containing fragment (**18**, Scheme 3) and several derivatives that interact with a ligand-inducible subpocket of the binding site of AChBP were thoroughly characterized using thermodynamic and structural analysis.[14] In another recent study, we have reported an in silico screening protocol that resulted in the identification of ligands with affinity for AChBP and the α7 but no affinity for the α4β2 nAChR.[15] Two of the identified hits contained a dibenzosuberyl-substituted tropine scaffold (**1** and **2**, Table 1) and were co-crystalized with Ac-AChBP. In the current study, we describe the structure-based design and synthesis of dibenzosuberyl- and benzoate substituted tropine derivatives and their SAR on Ac- and Ls-AChBP and on α7 and α4β2 nicotinic receptors. The hit optimization was monitored and guided by ligand efficiency and group efficiency considerations (LE and GE) as well as structural information.

The previously obtained co-crystal structures of Ac-AChBP in complex with hit compounds **1** and **2** (PDB: 2W8F and 2W8G, respectively) show that the ligands have a comparable binding pose with their cationic nitrogen atoms forming cation-π interactions

Table 2
The binding affinity (pK_i) of benzoate substituted tropines for Ls- and Ac-AChBP, α7 and α4β2 nAChRs

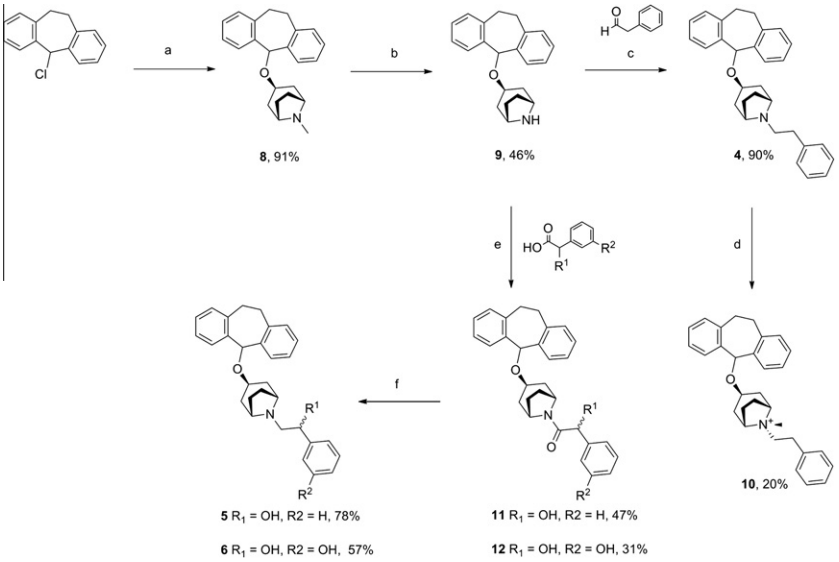


Compd		R^1	R^2	Ls-AChBP pK_i ± SEM[a]	Ac-AChBP pK_i ± SEM[a]	α7 pK_i ± SEM[b]	α4β2 pK_i ± SEM[a]
18[14]	*endo*	H	H	6.1 ± 0.1	5.3 ± 0.1	5.5 ± 0.1	<4.5
19	*exo*	H	H	5.1 ± 0.1	4.8 ± 0.1	n.d.	n.d.
16	*endo*	Me	H	5.7 ± 0.1	5.4 ± 0.1	4.8 ± 0.1	<4.5
17	*exo*	Me	H	5.2 ± 0.1	4.8 ± 0.1	n.d.	n.d.
20[14]	*endo*		H	7.1 ± 0.1	7.5 ± 0.1	5.1 ± 0.1	<4.5
21	*exo*		H	6.4 ± 0.1	6.0 ± 0.1	5.3 ± 0.1	<4.5
22	*endo*		Me	7.5 ± 0.1	6.8 ± 0.1	5.2 ± 0.1	<4.5
24	*endo*		H	6.2 ± 0.1	6.8 ± 0.1	4.9 ± 0.1	<4.5
31	*endo*		H	5.9 ± 0.1	6.1 ± 0.1	n.d.	n.d.
32[14]	*endo*		H	6.1 ± 0.1	7.0 ± 0.1	n.d.	n.d.
23	*endo*		Me	6.9 ± 0.1	5.7 ± 0.1	5.0 ± 0.1	<4.5
33[14]	*endo*		Me	6.9 ± 0.1	6.1 ± 0.1	n.d.	n.d.
26	*endo*		H	6.1 ± 0.1	6.2 ± 0.1	4.6 ± 0.1	<4.5
29	*endo*		H	6.4 ± 0.1	5.1 ± 0.1	4.8 ± 0.1	<4.5
30	*exo*		H	5.7 ± 0.1	4.7 ± 0.1	4.9 ± 0.1	<4.5
25	*endo*		H	7.0 ± 0.1	6.7 ± 0.1	4.9 ± 0.1	<4.5

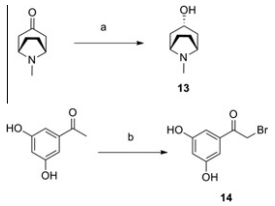
n.d. = not determined; Compd = compound.
[a] [^{3}H]epibatidine displacement studies, pH 7.4.
[b] [^{3}H]MLA displacement studies, pH 7.4.

with Trp145 (Ac-AChBP amino acid numbering, Trp143 in Ls-AChBP).[15] Comparing the structures of Ac-AChBP in complex with **1** and lobeline (**3**, PDB: 2BYS, Fig. 1A–B)[11], reveals the opening of a subpocket by the rotameric change of Tyr91 (Tyr93 in 2BYS corresponds to Tyr91 in 2W8F), see Fig. 1C. In addition, the superposition of both X-ray structures shows that the cationic nitrogen atoms of compound **1** and lobeline are at a similar position and engaged in cation-π interactions with Trp145 (Fig. 1B). The structural overlay inspired us to pursue a fragment merging approach[16] with the aim of increasing the binding affinity of in silico hit **1** (Supplementary data Figs. 2A–C). It was anticipated that the merging of lobeline's α-hydroxyphenetyl moiety with the dibenzosuberyl substituted tropine part of **1** would afford a hybrid ligand (e.g., **5**) with improved binding affinity due to additional interactions with the ligand-inducible subpocket to which we will refer to as the lobeline pocket.

To further optimize the interactions of the α-hydroxyphenetyl moiety of hybrid compound **5** with the lobeline pocket, we designed analogs of **5** (**4**–**7**, Supplementary data Figs. 2A, Table 1). These analogs were subsequently docked into the crystal structure of **1** (PDB: 2W8F) after opening of the lobeline pocket by manually changing the rotameric state of Tyr91 from the *g*- to *t* conformation).[17,18] The obtained binding poses indicate that the lobeline pocket can indeed be addressed by the α-hydroxyphenetyl moiety (Supplementary data Fig. 2B). Further investigation of the docking results suggests that additional interactions with the binding site can be established by incorporation of hydroxyl moieties at the *meta* positions (one or both) of the phenyl ring. By doing so, it was anticipated that hydrogen bonds could be formed with the backbone carbonyl oxygen of Thr89 and the sidechain of Asp195 (Supplementary data Fig. 2C). Our previously performed in silico screening study has provided an indication that compounds **1** and **2** bind to both the orthosteric site and the ion pore of nAChRs.[15] The interaction with the ion pore may arise from the structural resemblance to tricyclic antidepressants that have been shown to block Na^+ channels.[19] In order to abolish the putative channel blockade of the tricyclic ligands, we performed an



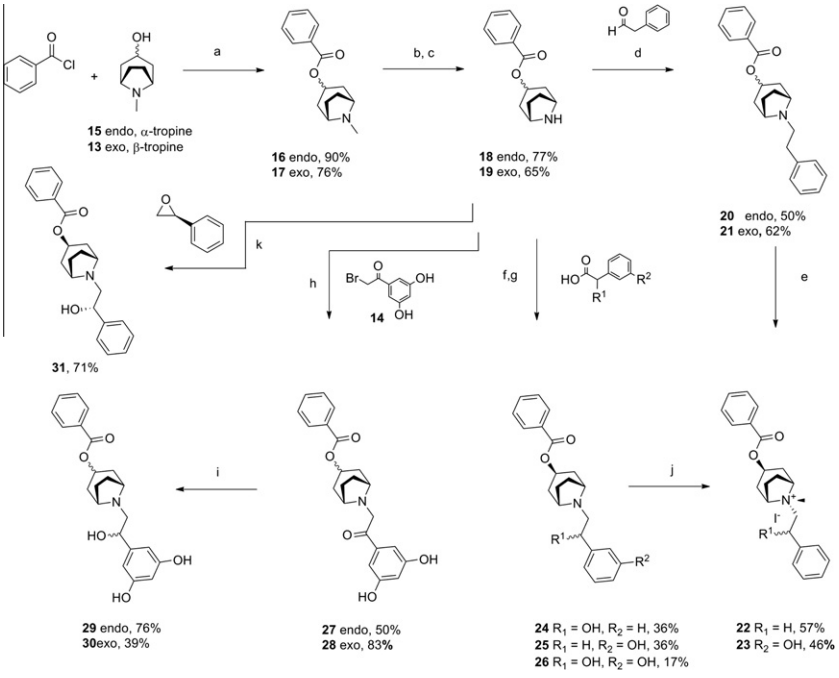
Scheme 1. Reagents and conditions: (a) α-tropine (2 eq.), toluene, rt, 16 h, reflux, 1 h; (b) $KMnO_4$, KOH, pyridine/water, rt, 3 h; (c) $NaBH(OAc)_3$, DCE, rt, 16 h; (d) MeI, toluene, rt, 24 h; (e) TBTU, Et_3N, DMF, rt, 1 h; (f) $LiAlH_4$, THF, rt, 2 h.



Scheme 2. Reagents and conditions: (a) Al(*i*-OPr)$_3$, 2-propanol, reflux, 24 h; (b) $CuBr_2$, EtOAc/DCM, reflux, 6.5 h.

additional fragment-merging exercise affording new ligands, in which the dibenzosuberyl moiety was replaced by a smaller benzoate group (Table 2). Thus, a set of benzoate substituted analogs was synthesized in order to determine the influence on the binding affinity of (1) the configuration of the epimeric C(3) position of the tropine spacer, (2) the quaternization of the tropine nitrogen atom, and (3) the introduction of hydroxyl substituted phenetyl moieties.

The dibenzosuberyl substituted tropinyl ethers were synthesized according to Scheme 1. Treatment of tropine with 5-chlorodibenzosuberane followed by oxidative demethylation with $KMnO_4$ afforded dibenzosuberyl ether **9**.[18,20,21] The corresponding phenetylamine **4** was obtained by reductive amination of **9** with phenylacetaldehyde.[22] Methylation using iodomethane afforded the quaternary ammonium salt **10** with the phenetyl moiety in



Scheme 3. Reagents and conditions: (a) TEA, toluene, reflux, 4-6 h; (b) 1-chloroethyl chloroformate, DCE, reflux, 5–19 h; (c) MeOH, reflux/rt, 1-4 h (d) $NaBH(OAc)_3$, DCE, rt, 16–48 h; (e) MeI, acetonitrile, rt, 16 h; (f) TBTU, Et_3N, DMF, rt, 3–16 h; (g) BH_3.THF, THF, rt, 16 h; (h) DIPEA, acetonitrile, rt, 2–16 h; (i) BH_3·THF, THF, 0 °C, 0.75–1.5 h; (j) MeI, toluene, rt, 2 weeks; (k) NMP, microwave 150 °C, 50 min.

an *endo*-configuration as determined by 2D NMR spectroscopy at 393 K (Supplementary data). The mono and di-hydroxyl substituted phenetylamines **5** and **6** were obtained via TBTU-mediated coupling with the corresponding racemic mandelic acids, followed by LAH reduction. β-tropine (**13**) was prepared via Meerwein–Ponndorf–Verley reduction of tropinone using $Al(i\text{-}OPr)_3$ (Scheme 2).[23] The benzoate esters of α- and β-tropine (**16**, **17**) were obtained by acylation with benzoyl chloride (Scheme 3).[24] Subsequent demethylation using α-chloroethyl chloroformate afforded the corresponding nortropinyl esters (**18**, **19**).[25] Similar as in the tricyclic series, phenetylamines **20** and **21** were obtained by reductive amination with phenylacetaldehyde. Treatment of *endo*-epimer **20** with iodomethane resulted in formation of the *endo*-phenetyl substituted quaternary ammonium derivative **22**. The epimeric configuration was confirmed by performing 2D NMR (Supplementary data). The mono and di-hydroxyl substituted phenetylamines **24**–**26** were synthesized via TBTU-mediated coupling with the corresponding phenylacetic acids, followed by BH_3 reduction. Reaction of α-hydroxyl substituted phenetylamine **24** with iodomethane afforded similar as before the *endo*-phenetyl substituted quaternary ammonium derivative **23** as confirmed by 2D NMR (Supplementary data). The tri-hydroxyl substituted *endo*- and *exo*-benzoate substituted N-phenetyltropines (**29**, **30**) were obtained through alkylation of nortropinyl esters **18** and **19** with 2-bromo-1-(3,5-dihydroxyphenyl)ethanone (**14**) followed by BH_3 reduction of the obtained ketone to the corresponding alcohol. 2-Bromo-1-(3,5-dihydroxyphenyl)ethanone (**14**) was prepared from the corresponding acetophenone using CuBr (Scheme 2).[26]

The binding affinity data of the dibenzosuberyl-substituted compounds shows that removing methyl groups from the quaternary tropine nitrogen atoms reduces binding affinity for Ls-AChBP (compare **1**, **8** and **9**).[18] Extension of the methylene spacer of benzyl derivative **2** with one methylene unit affording the phenetyl substituted compound **4** resulted in a 0.4 log unit increase in affinity. Subsequent quaternization of the tropine nitrogen atom of **4** gave rise to an additional 0.4 log unit increase in binding affinity. In total, the affinity of the in silico identified hit **1** was increased 6-fold (**10**, $pK_i = 7.4$). Introduction of hydroxyl groups at the α- and *meta* positions of the phenetyl group of compound **4** diminished the affinity. It should be noted that both α-hydroxyl substituted derivatives (**5** and **6**) were tested as racemic mixtures. Taken together, these results illustrate that for Ls-AChBP affinity, a quaternary tropine nitrogen atom is preferred and a lipophilic substituent on the nitrogen atom such as a phenetyl moiety is beneficial for affinity. Introduction of hydroxyl functionalities on the phenetyl moiety decreases affinity significantly. For the benzoate-substituted series, we observed that the *endo*-epimers exhibit higher affinity than the *exo*-epimers for Ls-AChBP (compounds **16**–**21** and **29**, **30**). Interestingly, for Ls-AChBP, the SAR of the *endo*-benzoate tropine esters coincides with the SAR of the dibenzosuberyl-substituted tropinyl ethers. Similar binding affinities are observed but due to a decrease in molecular weight, the benzoate esters exhibit better ligand efficiencies[27] (LE) than the dibenzosuberyl ethers (Fig. 2). Particularly, fragment **18** with an LE >0.4 $kcal \cdot mol^{-1}$ for both AChBP species variants, serves as an ideal starting point for further optimization. Similar to the tricyclic series, quaternization of the tropine nitrogen atom of benzoate substituted tropines results in increased affinity for Ls-AChBP (**21** vs **22** and **24** vs **23**). Extending fragment **18** with a phenetyl moiety on the tropine nitrogen atom affords compound **20** with a 10-fold increase in affinity. As seen with the dibenzosuberyl substituted derivatives, introduction of an α-hydroxyl group to the phenetyl moiety results in a significant loss in affinity (**20** vs **24** and **25** vs **26**). Additional introduction of hydroxyl groups at the meta positions to α-hydroxyphenetyl-substituted *endo*-epimer **24** resulted in minor or no increases in affinity (**24** vs **26** vs **29**). As such, the

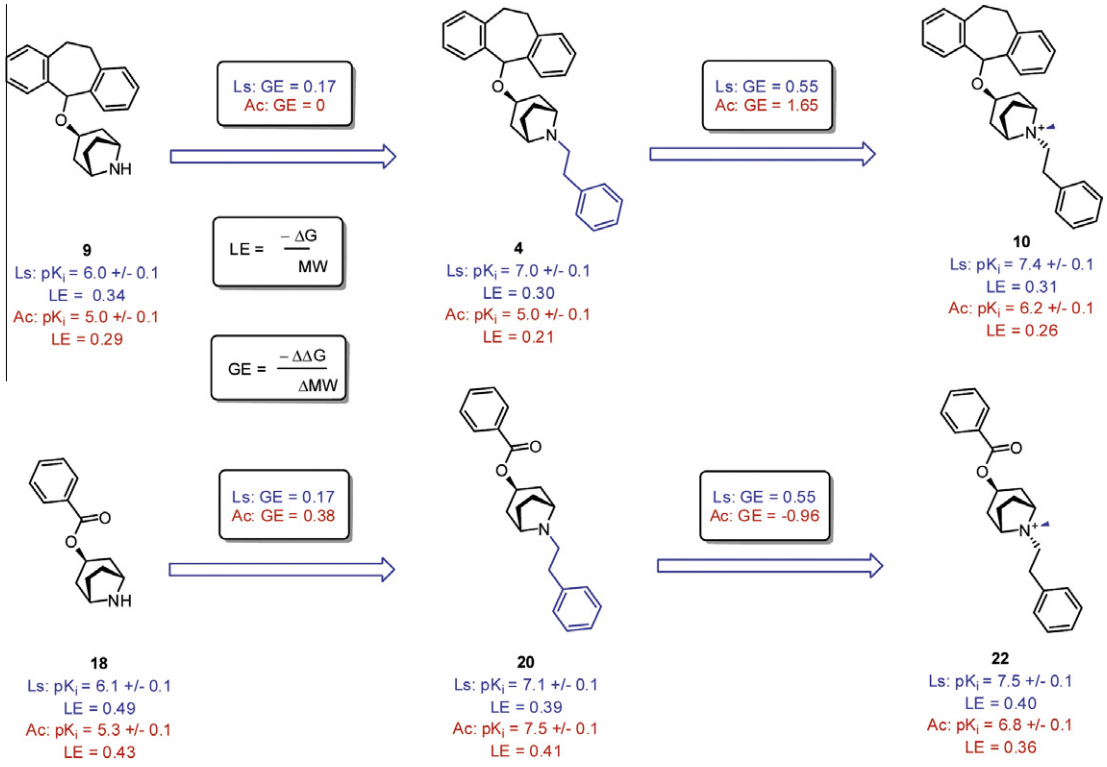


Figure 2. Calculation of LE and GE allows easy comparison of and the average affinity contributions per heavy atom of compounds and functional groups of different sizes. In this example, comparison of LE's of **18** and **9** points out that **18** is a much better starting point for further optimization than **9**. In addition, comparison of GE's between Ls- and Ac-AChBP underlines that there are clear SAR differences between both proteins.

affinity of the tri-hydroxyl-substituted phenetylamine with an *endo*-configuration (**29** $pK_i = 6.4$) is lower than the affinity of the corresponding unsubstituted phenetylamine (**20**, $pK_i = 7.1$). The highest affinity ($pK_i = 7.5$) was observed for the quaternary ammonium derivative **22**.

Distinct SAR is observed for Ac-AChBP. One of the best dibenzosuberyl-substituted compounds in the Ls-AChBP assay (**1**) binds with 100-fold lower affinity to Ac-AChBP, suggesting that in contrast to Ls-AChBP, large hydrophobic substituents on a tertiary nitrogen atom are not allowed. Similar to Ls-AChBP, quaternization of the tropine nitrogen atom of *N*-phenetyl derivative **4** (affording **10**) is beneficial, although the increase in affinity upon quaternization is more pronounced for Ac-AChBP, i.e., 1.2 log units compared to 0.4 log units, respectively. Similar to Ls-AChBP, introduction of hydroxyl functionalities on the *N*-phenetyl group's α and meta position (**5** and **6**) does not result in increases in binding affinity. For Ac-AChBP binding affinity, the *endo*-epimer of benzoate-substituted tropines is also preferred. However, in contrast to Ls-AChBP, quaternization of the tropine nitrogen atom results in significant decreases of affinity for Ac-AChBP (**20** vs **22** and **24** vs **23**). Introduction of a hydroxyl group at the α or two meta positions of the phenetyl moiety is not tolerated and the highest Ac-AChBP affinity is obtained for the unsubstituted phenetylamine **20** (pK_i of 7.5). An interesting observation is that in contrast to all the other ligands, benzoate esters **20** and **24** exhibit a preference for Ac-over Ls-AChBP. Since **24** was tested as a racemic mixture, both enantiomers ((*S*)-**31** and (*R*)-**32**) were synthesized revealing that only the (*R*)-enantiomer shows a preference for Ac-AChBP.[14]

Whereas for Ls-AChBP, the SAR of the tropine substituents is almost identical for the dibenzosuberyl and benzoate series, in the case of Ac-AChBP clear SAR differences were observed when focusing on the tropine substituent. To exemplify these SAR differences, we have calculated both the ligand efficiency (LE)[27] and the group efficiency (GE)[28] of several of our newly designed ligands for both AChBP species variants (Fig. 2). For Ls-AChBP, we observed an identical GE value of 0.17 kcal·mol^{-1} per heavy atom for the addition of a phenetyl moiety to dibenzosuberyl nortropinyl ether **9** (affording **4**) and to nortropinyl benzoate **18** (affording **20**). Adding an additional methyl group to the tropine nitrogen atom of *N*-phenetyl derivatives (to obtain compounds **10** and **22**, respectively) results again in identical GE values (0.55 kcal·mol^{-1} per heavy atom, (Fig. 2). Remarkably, for Ac-AChBP, we observed significant differences in the trends for the dibenzosuberyl-substituted tropines and benzoate-substituted tropines (Fig. 2). Addition of a phenetyl group to ether **9** does not result in an increase in Ac-AChBP binding affinity (GE = 0 kcal·mol^{-1} per heavy atom) whereas the same modification for benzoate ester **18** yields a substantial GE of 0.38 kcal · mol^{-1} per heavy atom. Even more pronounced differences are observed for the subsequent methylation of the tropine nitrogen atom of ether **4** and ester **20**. In the case of ether **4**, this minor modification affords a significant increase in Ac-AChBP binding affinity (GE = 1.65 kcal·mol^{-1} per heavy atom), whereas quaternization of ester **20** results in a large drop in binding affinity (GE = −0.96 kcal·mol^{-1} per heavy atom). These SAR differences between dibenzosuberyl tropinyl ethers **9**, **4** and **10** and the benzoate tropine esters **18**, **20** and **22** are indicative of different binding modes between the two compound series. Since we have provided structural evidence that benzoate ester **20** ((*R*)-enantiomer) is interacting with the lobeline pocket in Ac-AChBP (2Y57.pdb),[14] a likely explanation for the observed SAR differences between the compound series is that dibenzosuberyl ether **4** is not interacting with the lobeline pocket. In the same study, we have shown that the loss of Ac-AChBP affinity upon quaternization of the α-hydroxyl-substituted analog of **24** (**23**, (*R*)-enantiomer) is due to loss of interactions with the lobeline pocket. As such, the beneficial effect of quaternization of the dibenzosuberyl ether **4**, is another indication that **4** is not interacting with the lobeline pocket in Ac-AChBP. In addition, we have provided strong evidence that due to the lack of stabilization of the tyrosine-flip of Tyr91, the lobeline pocket in Ls-AChBP is less accessible compared to Ac-AChBP.[14] The current results indicate that in Ls-AChBP, the *N*-phenetyl substituents of ligands **4**–**6** are likely to be accommodated by a (hydrophobic) part of the binding site different than the lobeline pocket.

Our docking efforts suggested that incorporation of hydroxyl substituents at the α- or *meta* positions of the *N*-phenetyl substituents of the dibenzosuberyl as well as the benzoate series could increase binding affinity by the formation of additional hydrogen bonds with the lobeline pocket. However, for both series of compounds, introduction of hydroxyl moieties at the α- and *meta* positions of the *N*-phenetyl moiety diminishes binding affinity for Ls-AChBP as well as Ac-AChBP. For Ls-AChBP and the dibenzosuberyl-substituted tropines, this can be explained by a different binding mode than predicted, that is no interaction of the *N*-phenetyl moiety with the lobeline pocket. It is noted that the *N*-phenetyl substituted tropine benzoates **20** and **24** have been shown by X-ray co-crystal structures to interact with the lobeline pocket in Ac-AChBP. Nevertheless, the co-crystal complex of AChBP with **24**, shows that the α-hydroxyl group of **24** ((*R*)-enantiomer) is not engaged in a hydrogen bond with the backbone carbonyl oxygen atoms of Ser144 or Trp145 but instead, is involved in van der Waals interactions with Tyr193, providing an explanation for the observed detrimental effect on binding affinity of the α-hydroxyl group (Supplementary data Fig. 3). Due to desolvation penalties, the positioning of hydrogen-bonding groups needs to be near optimal in order to be beneficial in terms of binding affinity.[29,30] Apparently, upon introduction of one or two meta-hydroxyl groups to benzoate ester **20**, the desolvation penalty dominates, indicating that the positioning of the α-hydroxyphenetyl moiety in the lobeline pocket does not allow for strong hydrogen bond formation with the carbonyl backbone of Thr89 and/or the sidechain of Asp195. Interestingly, ionic and hydrogen bond interactions with Asp195 have been observed for α-conotoxins in complex with Ac-AChBP with Tyr91 in a *g*- conformation (closed lobeline pocket, PDB: 2UZ6 and 2BYP), showing that it is possible for ligands to interact with this residue .[11,31]

These ligands were also tested on the α7 and α4β2 nicotinic receptors. None of these compounds showed any affinity for the α4β2 receptor. However, the binding affinities of the dibenzosuberyl-substituted tropines for the α7 receptor were similar to the affinities determined for Ac-AChBP. As seen with the AChBPs, quaternization of the tropine nitrogen atom increases the binding affinity. None of the novel derivatives had higher affinity for the α7 nAChR than the initial in silico hit **1**. No large changes in affinity between the benzoate esters **16**, **18**, **20**–**26**, **29** and **30** for the α7 nAChR were observed. Interestingly, the compound in this series with the highest affinity for the α7 nAChR is fragment **18** ($pK_i = 5.5$). Apparently, all the introduced substituents on the tropine nitrogen atom even as small as methyl decrease affinity for this nAChR subtype, indicating that the lobeline pocket in the α7 nAChR subtype is not being addressed.

It is noted that the optimization of the initial in silico hits that has been achieved for both AChBPs does not translate to increased affinities for the α7 and α4β2 receptors. Even though it has been shown that AChBP can be used to identify new ligands for nicotinic receptors, the current study indicates that AChBP X-ray structures may have their limitations in providing a template for structure-based optimization of ligands for this member of the Cys-loop receptor family. Nevertheless, the current and our previous studies have provided strong evidence that interactions with the lobeline pocket can render ligands selective for Ac-AChBP. These findings may be of interest in the design of subtype-selective ligands for human nicotinic receptors. The gatekeeper tyrosine residue is

conserved among the human nAChR subtypes, whereas the residue (Ser165 in Ac-AChBP) that stabilizes the open lobeline pocket conformation is located in a highly variable region. As a consequence, due to differential stabilization of rotameric states of the gatekeeper tyrosine residue, there may exist pronounced differences in the accessibility of the lobeline pocket between nAChR subtypes. However, additional experiments using site-directed mutagenesis and/or molecular probes are required to determine if the lobeline-pocket can be targeted to obtain nAChR subtype-selectivity.

In summary, novel dibenzosuberyl- and benzoate substituted tropines were designed using a structure-based fragment-merging hit-optimization approach. Distinct SAR of the novel compound series was observed between two AChBP species variants and between the α7 and α4β2 nAChR subtype. The AChBP species differences were indicative of a difference in accessibility of a ligand-inducible subpocket between AChBPs from different organisms. Hereby, we have identified a region within the pocket that can be scrutinized to unravel its importance for obtaining selectivity amongst the fast number of nicotinic receptor subtypes. In addition, our studies are in line with previously published studies that show that structure-based fragment merging can be an efficient method for increasing binding affinity.[32–34] The novel compounds described in this study and the obtained structural understanding can be used to focus on the subtle differences between the AChBPs and nicotinic receptors, possibly the lobeline pocket accessibility, and binding pose differences, that cause the observed selectivity profiles and SAR differences.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.12.008.

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