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Design and synthesis of new agents for neuronal nicotinic acetylcholine receptor (nAChRs) imaging

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ABSTRACT

Introduction: The most abundant subtype of cerebral nicotinic acetylcholine receptors (nAChR), α4β2, plays a critical role in various brain functions and pathological states. Due to rapid technological progress in chemistry, bioinformatics, structural biology and computer technology, computer aided drug design (CADD) plays a more and more important role in today's drug discovery.

Methods: Two novel 3-pyridyl ether nicotinic ligands-3-((pyridine-2-yl)methoxy)-5-iodopyridine, and 3-(((*S*)-pyrrolidin-2-yl)methoxy)-5-((4-iodobenzyloxy)-methyl)pyridine were designed and synthesized and radiolabeled with I-125 based on our 3D-QSAR models reported previously. Their ability to label high-affinity brain nicotinic acetylcholine receptors (nAChRs) was evaluated.

Results: [^{125}I]3-((pyridin-2-yl)methoxy)-5-iodopyridine shows rapid accumulation and elimination with peak (1.86%ID/g) at 5 min post injection, but has high blood uptake. [^{125}I]3-(((*S*)-pyrrolidin-2-yl)methoxy)-5-((4-iodobenzyloxy)methyl)pyridine entered the brain with maximal uptake value 3.01%ID/g at 15 min after injection, and showed approximately 27% inhibition of radioactivity uptake in thalamus in mice pretreated with nicotine.

Conclusions: The results of this preliminary study show that [^{125}I]3-(((*S*)-pyrrolidin-2-yl)methoxy)-5-((4-iodobenzyloxy)methyl)pyridine shows relatively high uptake to the brain, however, since the in vivo selectivity for α4β2 nAChRs was not enough, [^{125}I]3-(((*S*)-pyrrolidin-2-yl)methoxy)-5-((4-iodobenzyloxy)methyl)pyridine does not have the required properties for imaging nAChRs using SPECT. Structure optimization is needed for specific visualization of brain α4β2 nAChRs in vivo.

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are found throughout the central and peripheral nervous systems, as well as in the neuromuscular junction [1–3]. nAChRs are a class of excitatory, ligand-gated ion channels formed by combinations of α and β subunits, or as homopentamers in the case of α7, α8 and α9. Abnormal opening–closing of these channels contributes to neuro-degenerative disorders, resulting in several kinds of severe diseases, including Alzheimer's diseases, Parkinson's diseases, dyskinesias, Tourette's syndrome, schizophrenia, attention deficit disorder, anxiety, and pain, as well as nicotine addiction [4]. To date, 10α and 4β isoforms have been discovered, resulting in a huge diversity of possible compositions, though only a small subset of combinations has been shown to give rise to functionally and physiologically relevant channels [5]. Subtype selectivity is a critical issue for the effectiveness and safety of drugs. Development of selective agonists or antagonists may therefore result in new and potentially useful therapeutic agents. One of the important targets for selective drugs is the α4β2 nAChR subtype, which is the most abundant nAChR in the brain [27].

Development of α4β2 selective nAChR ligands that offer potential as therapeutic agents has led also to the development of highly specific tomographic imaging agents for these important neuroreceptor sites [6]. Much of the current radiotracer development work has focused on tomographic agents from the 3-pyridyl ether series. Though the *N*-methyl pyrrolidine ether, A-84543 labeled with C-11 [7,8], having high affinity with α4β2 subtype and low toxicity, but for its high nonspecific binding with other ganglionic nAChR population, it is not suitable as imaging probe. In 1996, Abbott Lab. synthesized a series of 3-pyridyl ether compounds, including A-83580 which showed more selective in binding to the α4β2 subtype than to other nAChRs subtypes, such as α3β4 subtype [9], the main ganglionic nAChR population. In general, radiolabeled ligands based on A-85380 show exceptional specificity to nAChRs [11–20]. However, the pharmacokinetic profile of these ligands in baboons is relatively slow and shows poor reversibility over the time-course for the most commonly used positron emitters C-11 (t1/2 = 20 min) and F-18 (t1/2 = 110 min).All these have limited the wide use of these analogues as brain imaging agents.

In 2004, Brown etc. reported a series of 6-chloro-pyrrolidinyl-methoxy-pyridine analogues. In in vitro binding essay, ^{11}C-Me-p-PVC

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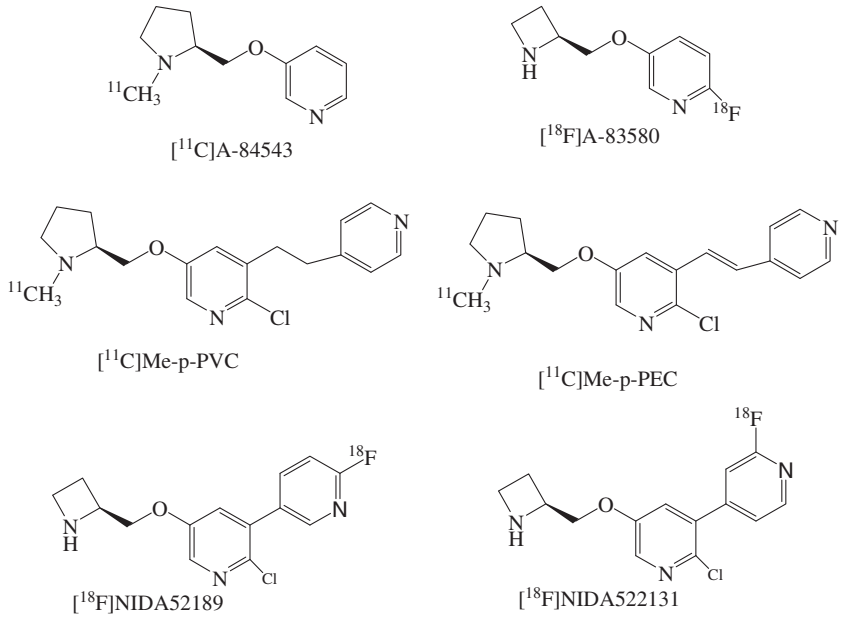


Fig. 1. High-affinity α4β2 nAChR ligands.

(Fig. 1) and ^{11}C-Me-p-PEC displayed competitively ideal property [19], including high affinity and high lipotropism, but they are still not suitable for imaging because of their low BP values. On that basis, they synthesized another analogue series with improved property [20], especially [^{18} F]NIDA52189 and [^{18} F]NIDA522131. However, these radioligands exhibited prohibitively slow brain kinetics that requires 8 h of PET scanning in the Rhesus monkey for the tracer radioactivity to reach a spatial–temporal steady state. Such slow and almost irreversible brain kinetics is due to high hydrophilic properties of [^{18} F]NIDA52189 and [^{18} F]NIDA522131(logD7.4≈0.5 for both compounds) and their very high binding affinity (*Ki*≈5 pM for both compounds). An affinity that is too high may be prohibitive for the development of radioligands with sufficiently rapid brain kinetics [21]. Subsequently, they hypothesized that replacement of the azetidine ring of NIDA522131 with an *N*-Me-pyrrolidine moiety should yield a compound with decreased binding affinity and increased lipophilicity. Based on the above rational, 6-chloro-3-((1-methyl)-2-(*S*)-pyrrolidinyl)methoxy)-5-(2-fluoropyridin-4-yl)pyridine(JHU85270), has been synthesized and its binding affinity (Ki= 86, 115 pM) and experimental lipophilicity (logD7.4=1.6) were determined. The ligand was successfully radiolabeled with carbon-11 as a potential PET tracer for imaging of nAChRs [22].

With optimized binding affinity and lipophilicity and, thus, improved brain kinetics (±)-[11C]-NMI-EPI and (±)-[18 F]NPhEP have been synthesized and demonstrated promising properties (including "rapid" brain kinetics) for imaging thalamic nAChR in animals [23,24].

In 2008, Yongjun Gao etc. synthesised two enantiomers (+)-NMI-EPB (Ki=2310, 1680 pM) and (-)-NMI-EPB (Ki=55, 68 pM). The enantiomers were stereoselectively radiolabeled with ^{11}C. In the distribution studies in the rodent brain [^{11}C](-)-NMI-EPB specifically labeled nAChR whereas [^{11}C](+)-NMI-EPB exhibited little specific binding. In the baboon PET study [^{11}C](-)-NMI-EPB did not reach steady-state within 90 min post-injection suggesting that the radioligand may have some limitations for quantitative imaging [37].

Our previous studies of building 3D-QSAR models of 3-pyridyl ethers compound to predict the affinity between ligands and the receptor disclosed that introducing heteroatoms into 5-position of pyridine ring almost had no influence for the activity [31,32], nevertheless, introducing comparative electronegative halogen substituents would significantly improve the activity. Bulk substitution at 5-position of pyridine ring was of benefit to the improvement of the antagonistic activities. The addition of electron-rich and high liposolubility group (such as chlorine atom) was optimal for potency and functional activity. In addition, drug distribution and forecasting models built by our lab provide favorable theoretical basis for the rational design of novel ligands [33,34]. Against this backdrop, the present study explored further the effect of structural modification of the heterocyclic ether series on in vivo brain biodistribution. Specifically, differing cyclic amine substituents on the non-pyridinol portion of the ether were synthesized and their in vivo nAChR binding properties were examined (Fig. 2). As a convenient starting point in this endeavor, a long-lived, economical radionuclide I-125 (t1/2=60 d) was placed. The 5-position of the pyridine nucleus in this series is known to be tolerant to substitution [25]. Indeed, Koren has shown that 5-iodo-A-85380 exhibits roughly two-fold higher affinity than the unsubstituted 3-pyridyl ether [26.35,36]. A key objective was to develop radioligands that exhibited faster brain uptake and clearance

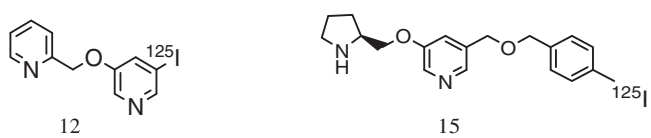
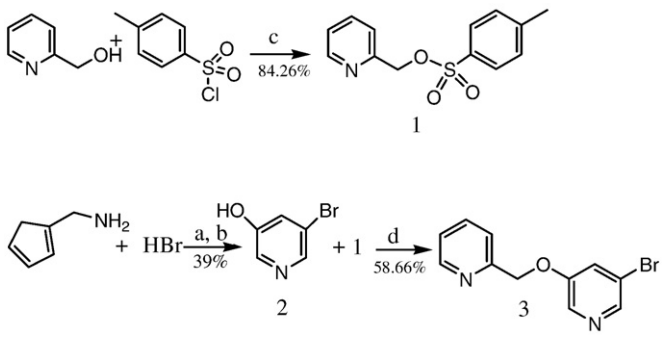


Fig. 2. Target iodo-3-pyridyl ethers as potential nAChR radioprobes.



Scheme 1. Reagents and conditions: (a) Br_2 , −20 °C ~ − 10 °C; (b) 40% NaOH; (c) Et3N, CH_2Cl_2, 0 °C; (d) K_2CO_3, DMF, 80 °C.