Bioorganic & Medicinal Chemistry Letters 23 (2013) 4145–4149

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and evaluation of a conditionally-silent agonist for the α7 nicotinic acetylcholine receptor

Kinga Chojnacka [a], Roger L. Papke [b], Nicole A. Horenstein [a,*]

[a] Department of Chemistry, University of Florida, PO Box 117200, Gainesville, FL 32611-7200, United States
[b] Department of Pharmacology and Therapeutics, University of Florida, PO Box 100267, Gainesville, FL 32610-0267, United States

ARTICLE INFO

Article history:
Received 14 February 2013
Revised 8 May 2013
Accepted 13 May 2013
Available online 23 May 2013

Keywords:
Silent-agonist
Allosteric modulator
Ion-channel
Nicotinic receptor

ABSTRACT

We introduce the term 'silent agonists' to describe ligands that can place the α7 nicotinic acetylcholine receptor (nAChR) into a desensitized state with little or no apparent activation of the ion channel, forming a complex that can subsequently generate currents when treated with an allosteric modulator. KC-1 (5′-phenylanabaseine) was synthesized and identified as a new silent agonist for the α7 nAChR; it binds to the receptor but does not activate α7 nAChR channel opening when applied alone, and its agonism is revealed by co-application with the type II positive allosteric modulator PNU-120596 in the *Xenopus* oocyte system. The concise synthesis was accomplished in three steps with the C–C bonds formed via Pd-catalyzed mono-arylation and organolithium coupling with *N*-Boc piperidinone. Comparative structural analyses indicate that a positive charge, an H-bond acceptor, and an aryl ring in a proper arrangement are needed to constitute one class of silent agonist for the α7 nAChR. Because silent agonists may act on signaling pathways not involving ion channel opening, this class of α7 nAChR ligands may constitute a new alternative for the development of α7 nAChR therapeutics.

The α7 nicotinic acetylcholine receptor (α7 nAChR) is a homopentameric ligand gated ion channel that is distinguished from other nAChRs by its rapid desensitization and very low probability of channel opening.[1] The α7 nAChR is currently a drug target for Alzheimer's disease, schizophrenia and inflammatory disorders,[2,3] and there is a great interest in development of α7-selective agonists[4,5] and positive allosteric modulators (PAMs).[6] α7 PAMs have been divided into type I and type II PAMs,[7] and one well-characterized type II PAMs is PNU-120596[7,8] (Fig. 1A). We have reported two forms of α7 desensitization. One is sensitive to type II PAMs such as PNU-120596, (termed D_s), and another is induced by strong episodes of activation and high occupancy and is insensitive to PNU-120596 (termed D_i).[9] The pharmacological relevance of desensitized states is likely to extend beyond their lack of ability to conduct an ion-current. There is growing evidence, especially in non-neuronal cells, that there is α7-mediated signal transduction under conditions when no ion channel activation can be detected.[3,10–13] These findings make compounds that can put the nAChR into the D_s state, with little or no apparent agonism, of considerable potential interest from both mechanistic and therapeutic perspectives. We define a silent agonist[14] as a molecule that does not activate or activates only very weakly α7 nAChR channel opening when used alone, inhibits the response of α7 nAChR to acetylcholine (ACh), and appears as an agonist when a type II PAM is used. NS-6740 is such an agent which, although inactive in an α7-sensitive model for cognitive improvement, has been shown to be effective at modulating the release of pro-inflammatory cytokines.[13,15] Functionally, a silent agonist acts as a desensitizer when applied alone, inducing a significant amount of D_s and a channel activator in the presence of type II PAM. In this Letter we describe the design, synthesis and evaluation of a new compound that acts as a silent agonist.

Several observations led to a working model for the design of a silent agonist. Compound NS-6740 (Fig. 1B) has been characterized by Abbott and Neurosearch as a very weak agonist (<2% of the response of to ACh), whose agonist-like properties were revealed by adding the positive allosteric modulator PNU-120596.[15] We found in our laboratories that 3PAB (Fig. 1C),[16] also behaves as a silent agonist, leading to the idea that silent agonists may be groupable into structurally distinct families. To this end, we were intrigued with the divergent behavior of NS-6740 as a silent agonist and the structurally related NS-6784 acting as a typical α7 agonist. (Fig. 1D). Both compounds feature a cationic diaza [3.2.2] bicyclononane group, a central 2,5-disubstituted heterocyclic ring and a phenyl substituent at the 5-position of the central ring. Further, both compounds offer a hydrogen bond acceptor adjacent to the bicyclic ring system; an amide carbonyl in the case of NS-6740, or a nitrogen atom of the 1,2,4-oxadiazole central ring in NS-6784. We therefore initiated a synthesis of a molecule whose structural features and biological activity might shed further light on what constitutes a set of features that would confer silent agonism.

* Corresponding author. +1 352 392 9859.
E-mail address: horen@chem.ufl.edu (N.A. Horenstein).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.05.039

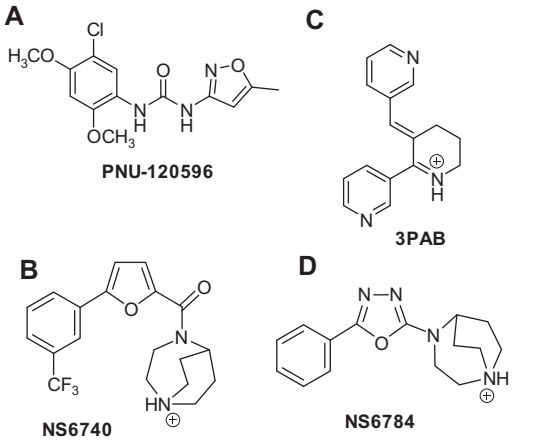


Figure 1. (A) Structure of type II PAM PNU-120596; (B and C) structures of α7 silent agonists NS-6740 and 3PAB; (D) structure of α7 agonist NS-6784.

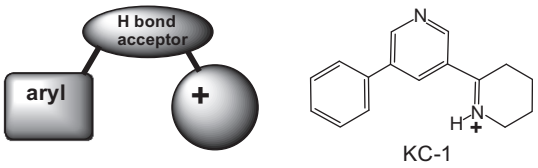
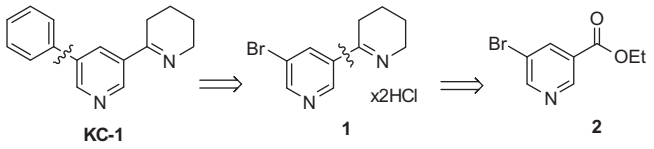


Figure 2. Putative pharmacophoric features for the KC-1/NS-6740 class of silent agonists.



Scheme 1. Initial KC-1 retrosynthesis analysis.

We considered a minimal pharmacophore to feature a positively charged center, a central ring with hydrogen bonding capability, and a flanking aryl substituent with an angular relationship between these elements as embodied in the molecule KC-1 and the cartoon shown in Figure 2. One will note the core anabaseine portion of KC-1 is a non-selective nAChR agonist, and as will be presented we show phenyl substitution on the pyridine ring dramatically changes this profile.

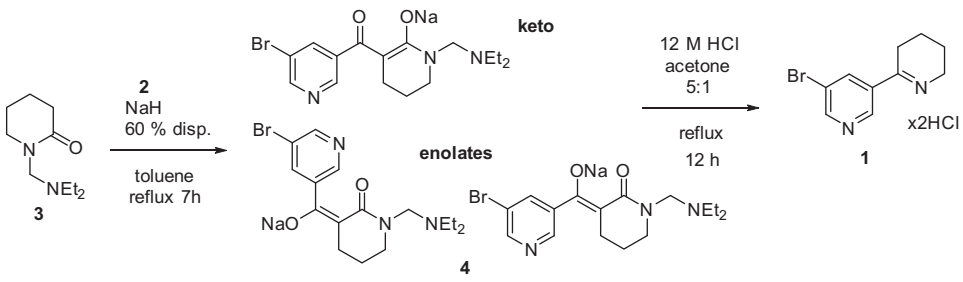
KC-1 (5′-phenylanabaseine) can be considered as an analog of anabaseine, and we decided to make KC-1 by adapting well-known anabaseine synthesis protocols. We first planned to prepare KC-1 from 5′-bromoanabaseine by organometallic coupling (Scheme 1). That approach would have allowed us flexibility in preparation of a series of KC-1 analogs. We envisaged preparing several grams of 5′-bromoanabaseine as its dihydrochloride salt (**1**) from bromonicotinic ethyl ester (**2**), by applying the Zoltewicz anabaseine synthetic protocol[17] that we use in our lab. That approach is based on a mixed Claisen condensation of nicotinic ester and the amide enolate ion of *N*-aminomethyl protected 2-piperidone **3**, followed by hydrolysis of the resulting sodium salt of the 3-nicotinoyl-2-piperidone intermediate in hot acid with concomitant decarboxylation and cyclic imine formation. Anabaseine is isolated by crystallization as its stable dihydrochloride salt.

The reaction of 3-bromonicotinic ethyl ester (**2**) with N-protected 2-piperidone **3**[16,17] and sodium hydride in toluene (Scheme 2) yielded the sodium salt **4** as a complex mixture of Z- and E-enolates and keto forms as supported by ^{1}H NMR, but it could not be isolated from the crude, so the crude product was subjected to the next step, hydrolysis with concentrated hydrochloric acid. The desired 5′-bromoanabaseine dihydrochloride (**1**) was formed in that reaction as shown by ^{1}H NMR, however extensive degradation was observed, many impurities were present, and the product couldn't be obtained in a pure form. We thus tried to synthesize KC-1 from 5-phenylnicotinic ethyl ester (**8**) following the same protocol.

5-Phenylnicotic ethyl ester (**8**) is not commercially available and we prepared it from 3-bromonictotinic acid (**5**) by Suzuki coupling with phenylboronic acid (**6**), using palladium acetate as catalyst, and potassium phosphate as a base, in a 1:1 mixture of water and 2-propanol.[18] The 5-phenylnicotinic acid was then reacted with thionyl chloride, followed by reaction with ethanol to give the product **8** in 63% yield from **5** (Scheme 3).

The 5-phenylnicotinic ethyl ester (**8**) was then subjected to the mixed Claisen condensation with N-protected 2-piperidone **3**, using the same methodology as presented in Scheme 2. Unfortunately, the intermediate sodium salt did not crystallize from the reaction mixture after removal of excess NaH by filtration, and again the crude was subjected to acidic hydrolysis. 5-Phenylcarboxylic acid **7**, resulting from hydrolysis of unreacted ethyl ester **8** from Claisen condensation crystallized first, then the KC-1 salt, which was impure. The KC-1 salt was thus transformed with 1 M sodium hydroxide into its imine free-base form, purified by column chromatography, and then converted back into its dihydrochloride salt to yield the pure compound in 2% yield over two-steps from 5-phenylnicotine ethyl ester (**8**). The results of these two approaches suggest that the synthetic route to anabaseine is not highly tolerant of substitutions.

The third and most successful route to KC-1 employed addition of 5-phenylpyridynyl lithium generated with *n*BuLi from the bromide **10** to *N*-Boc protected 2-piperidone **11** in diethyl ether, followed by deprotection, ring closure, and dehydration (Scheme 4). Organometallic ring-opening reaction of *N*-alkoxycarbonyl lactams has been used to make cyclic imines by Giovanni et al.[19] 3-Bromo-5-phenylpyridine **10** was prepared from 3,5-dibromopyridine **9** by



Scheme 2.