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SAR of α7 nicotinic receptor agonists derived from tilorone: Exploration of a novel nicotinic pharmacophore

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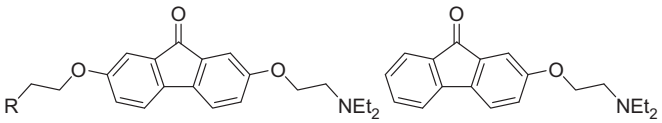
ABSTRACT

The well-known interferon-inducer tilorone was found to possess potent affinity for the agonist site of the α7 neuronal nicotinic receptor (K_i = 56 nM). SAR investigations determined that both basic sidechains are essential for potent activity, however active monosubstituted derivatives can also be prepared if the flexible sidechains are replaced with conformationally rigidified cyclic amines. Analogs in which the fluorenone core is replaced with either dibenzothiophene-5,5-dioxide or xanthenone also retain potent activity.

Neuronal nicotinic acetylcholine receptors (neuronal nAChRs, or NNRs) are ligand-gated ion channels that modulate the release of neurotransmitters in the brain and peripheral nervous system.[1] They are pentameric structures composed of α (i.e., α2–α10) and β (i.e., β2–β4) subunits that can assemble in multiple combinations, with each permutation exhibiting its own unique pharmacological properties. The prevailing hypothesis is that distinct biological roles can be assigned to individual NNR subtypes, and that unraveling these roles will enable the development of subtype-selective compounds for therapeutic use.[2]

In this context, the homopentameric α7 NNR [i.e., $(\alpha7)_5$] has emerged as a popular target with potential utility in the treatment of Alzheimer's disease, schizophrenia, and other neurological disorders.[3] It is prevalent in brain regions (hippocampus, cortex) that are critical for cognitive functioning. Genetic abnormalities in the α7 gene are correlated with sensory gating deficits in schizophrenics.[4] Experiments with selective α7 agonists in animal behavioral models support the role of the α7 NNR in learning, attention and memory processes.[5] Recently presented clinical data with DMXB (GTS-21),[6] MEM-3454 (R3487)[7] and EVP-6124[8] have demonstrated proof-of-concept for this mechanism in human disease.

We have discovered that tilorone (**1**, Fig. 1), a well-known orally-active interferon inducer,[9,10] has potent affinity for the α7 NNR (K_i = 56 nM) and is an agonist at recombinant human α7 receptors expressed in oocytes (EC_{50} = 2.5 μM, 67% efficacy relative to 10 mM acetylcholine).[11] It is selective with respect to the α3β2, α3β4, α4β2 and α4β4 NNR subunit combinations (EC_{50} >10 μM in FLIPR assays) as well as rat brain muscarinic receptors (K_i = 420 nM). The antiviral,[9] antitumor[12] and anti-inflammatory[13] properties of tilorone have been investigated extensively, and are generally ascribed to the compound's ability to induce interferon release. However, there are no previous reports linking this biological activity to the α7 NNR or any other neurotransmitter receptor. It is intriguing that tilorone does not easily overlay with the structures of known α7 ligands, many of which are constructed from quinuclidine or related amine scaffolds[14–16] (Fig. 2). In an effort



1 R= NEt_2 α7 K_i = 56 nM
2 R= OMe α7 K_i = 2000 nM
3 R= H α7 K_i = 1800 nM

Figure 1. Tilorone (**1**) and two monobasic analogs (**2**, **3**).

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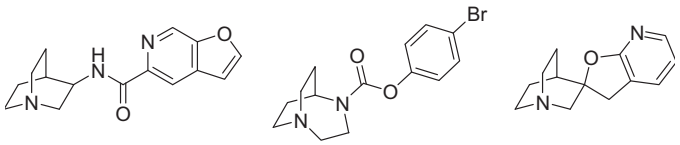


Figure 2. Representative α7 NNR ligands.

to understand this novel pharmacophore and determine the potential of tilorone analogs as α7 ligands, we investigated the SAR surrounding this unique NNR agonist.

Tilorone is an amphiphilic molecule that consists of three structural domains: two diethylaminoethoxy substituents (protonated at physiological pH), and the tricyclic fluorenone nucleus (with a partial negative charge on the carbonyl oxygen). It has been demonstrated that both sidechains are required for antiviral activity,[17] but also that some of tilorone's undesirable effects (such as lipidosis and lysosomal storage of sulfated glycosaminoglycans) are correlated with its dibasic nature.[18] As a first step towards understanding the SAR related to NNR activity, analogs were prepared in which the substituent on one side was either modified to attenuate its basicity (**2**), or completely removed (**3**).[17] Both compounds have significantly diminished α7 NNR binding affinity[19] relative to tilorone (36× and 32×, respectively), establishing that both basic moieties are necessary for potent nicotinic activity.

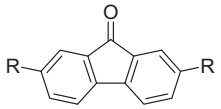
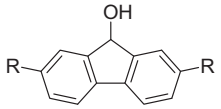
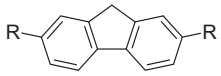
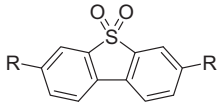
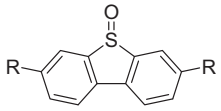
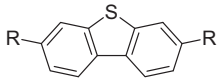
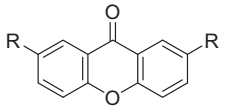
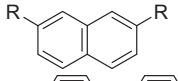
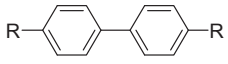
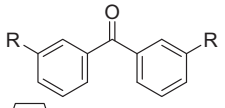
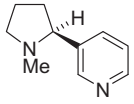
With this in mind, the influence of the central aromatic core on NNR affinity was probed with a set of symmetrical aryl bis-ethers that retain tilorone's diethylaminoethoxy sidechains (Table 1). Compounds **4**–**6**, **9**–**12** have previously appeared in the literature, and were readily prepared by Mitsunobu etherification of the corresponding bis-phenols with diethylaminoethanol using BocN=N-Boc and polymer-bound triphenylphosphine[20] to simplify purification (Scheme 1). The novel dibenzothiophene **8** was synthesized from the known sulfone **6**[21] by reduction with $LiAlH_4$. Subsequent oxidation of **8** with peracid yielded the sulfoxide **7**.

As shown in Table 1, several of the analogs containing non-fluorenone tricyclic aromatics are active at the α7 NNR, however all of these variants are less potent than tilorone itself. Clearly, the carbonyl is essential for good potency since removal of this group by reduction to the fluorenol **4** or fluorene **5** results in five-fold and three-fold diminutions of activity, respectively. The same effect is evident in the dibenzothiophene-dioxide (**6**) series, where successive deoxygenations to the sulfoxide **7** and cyclic sulfide **8** attenuate binding affinity. Expansion of the central aromatic ring of tilorone to a xanthenone (**9**) is tolerated, however the naphthalene derivative **10** containing a shorter bicyclic rather than a tricyclic core is inactive. Further dissecting the center of the molecule into less rigid derivatives (biphenyl **11** or benzophenone **12**) completely abolishes activity.

Having determined that the fluorenone core is preferred in the symmetrically-substituted compounds, the detailed SAR of the sidechains was investigated. An array of aryl ethers (**13**–**17**, **20**–**29**, **32**) was prepared from 2,7-dihydroxyfluorenone[25] and substituted amino alcohols by Mitsunobu reaction, or alternatively from the corresponding primary or secondary mesylates by Williamson etherification (Scheme 1). Compounds **30**–**31** containing the hindered alcohols tropanol and (*R*)-quinuclidinol were synthesized from 2,7-diiodofluorenone using modified Ullman coupling methods (with retention of configuration).[26] A variety of nitrogen-linked analogs (**33**–**38**) were available from 2,7-dibromofluorenone using standard Buchwald–Hartwig palladium-catalyzed amination chemistry.[27]

The diacetylene **19** was synthesized by Sonagashira coupling of 2,7-dibromofluorenone with diethylaminopropyne. Subsequent hydrogenation with Wilkinson's catalyst afforded the saturated analog **18**.

Table 1
Rat α7 NNR affinity for aromatic core replacements

Compound	Structure (R = $OCH_2CH_2NEt_2$)	α7 K_i[a] (nM)
1		56
4[b]		270
5[b]		770
6[c]		230
7		690
8		1600
9[d]		440
10[e]		>10^6
11[f]		>10^6
12[c]		68,000
Nicotine		1400

[a] Displacement of [^{3}H]-methyllycaconitine ([^{3}H]-MLA) from rat whole brain, average of at least three determinations, SEM ⩽ 10% (Ref. 19).
[b] Ref. 17.
[c] Ref. 21.
[d] Ref. 22.
[e] Ref. 23.
[f] Ref. 24.

Several SAR trends are apparent from the binding data in Table 2. For instance, the length of the flexible linker connecting the core to the basic amine is optimal in tilorone, with a loss of potency occurring with each additional methylene group (**1** < **13** < **14**). There is a clear preference for increasing alkyl substitution on nitrogen in the series NH_2 (**15**) < NHEt (**16**) < NEt_2 (**1**) < $NMeEt_2^+$ (**17**) which parallels the trend for increasing stabilization of the positive charge on N. However, the steric demands of the amine substituent cannot be too large, as demonstrated by the observation that NMe_2 (**20**) and NEt_2 (**1**) are essentially equipotent, whereas N^iPr_2 (**21**) is much weaker. An important point in this regard is that both **16** and **21** are as efficacious as **1** in a mouse survival model which was used in early publications describing tilorone, suggesting that the antiviral effectiveness of these compounds is not related to activity at the α7 NNR.[17] Surprisingly, merely