

Synthesis and biological evaluation of a novel series of phosphonate-functionalized 1,2,3-triazoles as allosteric modulators of $\alpha 7$ nicotinic acetylcholine receptors.

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Abstract:

Nicotinic acetylcholine receptors (nAChR) are ligand gated ion channels formed by the assembly of five subunits. Receptor activity could be subjected to both positive and negative modulation at allosteric sites by endogenous neurotransmitters as well as synthetic ligands such as steroids, bivalent cations, alcohols, and a range of drugs. The subtype of $\alpha 7$ nAChR has been considered a potential therapeutic target for Alzheimer's disease, schizophrenia and other neurological and psychiatric disorders. In this work we present the synthesis of a novel series of phosphonate-functionalized 1,4-disubstituted 1,2,3-triazoles with potential activity over $\alpha 7$ AChR.

These compounds were synthesized through the copper-catalyzed Huisgen 1,3-dipolar cycloaddition of organic azides and alkynes. Copper nanoparticles (CuNPs) immobilized on different supports were prepared using the CuCl₂-Li-DTBB reducing system previously reported by our group.

Keywords: 1,2,3-triazoles ; Phosphonates; Nicotinic receptors.

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are transmembrane receptors that are activated by the neurotransmitter acetylcholine. These receptors are distributed throughout the central and peripheral nervous systems and play important roles in several (patho)physiological processes.[1] Seventeen subunits have been identified in vertebrates ($\alpha 1-10$, $\beta 1-4$, γ , δ and ϵ), which co-assemble to form a diverse family of receptor subtypes. In particular, the human $\alpha 7$ nAChR has been identified as a potential target for therapeutic drug discovery and has been implicated in a number of neurological and psychiatric disorders. [2] Considering the fact that $\alpha 7$ nAChR undergoes very rapid desensitization in response to agonist activation, several positive allosteric modulators (PAMs) were synthesized and evaluated. $\alpha 7$ nAChR PAMs are classified in two types: those that do not change the AChR desensitization properties are called type I whereas those that decrease the level of desensitization are labeled as type II.

The identified $\alpha 7$ PAMs have considerable diversity, ranging from proteins to small molecules. Even the small molecule PAMs vary significantly in structure, as well as in properties and probably mechanisms of the modulation. Nitrogen-containing heterocycles such as indoles, isoxazoles, thiazoles, pyridines and pyrroles are largely present in both type I and type II PAMs.[3]

Frequently, isosteric replacement of these heterocycles, or amide linkers, with triazole rings allows the efficient generation of small libraries of ligands for nAChR via one-pot copper(I)-catalyzed azide-alkyne cycloaddition. In this way, several triazole-containing agonists of nAChR were synthesized.[4] 1,2,4-triazoles are also present in structures acting over nicotinic receptors. Derivatives of 4BP-TQS, a potent allosteric agonist of $\alpha 7$ nAChRs, showed properties of type I or II PAMs.[5]

Our group developed methodologies to synthesize triazoles. Copper nanoparticles (CuNPs) can be obtained through the reducing system $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, lithium metal, and a catalytic amount of 4,4'-di-tert-butylbiphenyl (DTBB) in THF at room temperature. We showed that these unsupported copper nanoparticles (10 mol%) effectively catalyzed the 1,3-dipolar cycloaddition of organic azides and terminal alkynes in the presence of triethylamine at 65 °C in THF. Moreover, these CuNPs can be supported over activated carbon and perform the multicomponent synthesis of 1,2,3-triazoles from organic halides in water, allowing the recycle and reuse of the catalyst. [6]

In the other hand phosphonates and related compounds exhibit a wide range of applications in medicinal chemistry. For example, some of these compounds are potent inhibitors of human acetylcholinesterase. [7] Thus, in this work we present the synthesis of a novel series of phosphonate-functionalized 1,4-disubstituted 1,2,3-triazoles.

2. Materials and Methods

2.1. Synthetic procedures

Reagents employed were analytical grade. THF and CH_2Cl_2 were freshly distilled over sodium/benzophenone or CaH_2 respectively. Moisture sensitive reactions were conducted under inert atmosphere of dry nitrogen using oven dried glassware. Reactions were monitored by analytical TLC performed on Merck silica gel 60 F254 plates and visualized under UV light and/or using phosphomolybdic acid or p-anisaldehyde solutions. Purification of crude materials was performed by flash chromatography on silica gel (200–400 mesh, Merck).

2.2. General procedure for azide-alkyne cycloaddition employing CuNPs/C in water

NaN_3 (1.2 eq.), the organic halide (1 eq.), and the alkyne (1 eq.) were added to a suspension of CuNPs/C (5 mol% Cu) in H_2O . The reaction mixture was warmed to 70 °C overnight. Then, the mixture was diluted with water and extracted with EtOAc. The collected organic phases were dried with anhydrous Na_2SO_4 , dried and purified by flash chromatography.

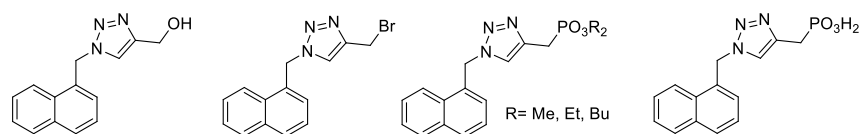
2.3. General procedure for azide-alkyne cycloaddition employing CuNPs/C in THF

Organic azide (1 eq.), alkyne (1 eq.) and triethyl amine (1 eq.) were added to a suspension of CuNPs/C (5 mol% Cu) in THF. The reaction mixture was heated under reflux overnight. Then, THF was removed and the mixture was washed with water, followed by extractions with EtOAc. The collected organic phases were dried with anhydrous Na_2SO_4 , dried and purified by flash chromatography.

3. Results and discussion

The 1,4-disubstituted triazole derivatives synthesized in this work are shown in Figure 1. We have successively synthesized two series of derivatives, taking into account the preliminary results in their evaluation as $\alpha 7$ nAChRs PAMs. In the first series, structure activity relationship strategies (SAR) were employed and we observed that compounds bearing a phosphonate moiety presented the desired activity. Thus, we continued synthesizing phosphonate derivatives in order to enhance the activity.

SERIES I



SERIES II

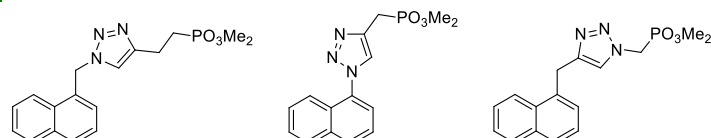
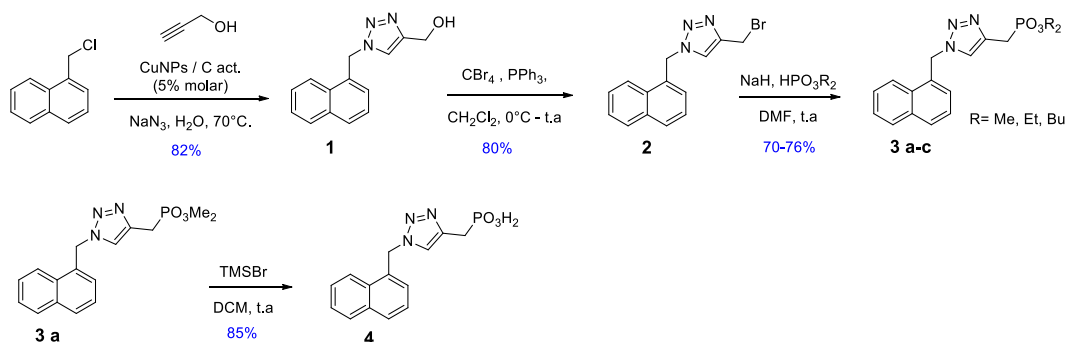


Figure 1. 1,4-disubstituted-1,2,3-triazoles synthesized in this work.

3.1. Synthesis of 1,2,3-triazoles using supported copper nanoparticles.

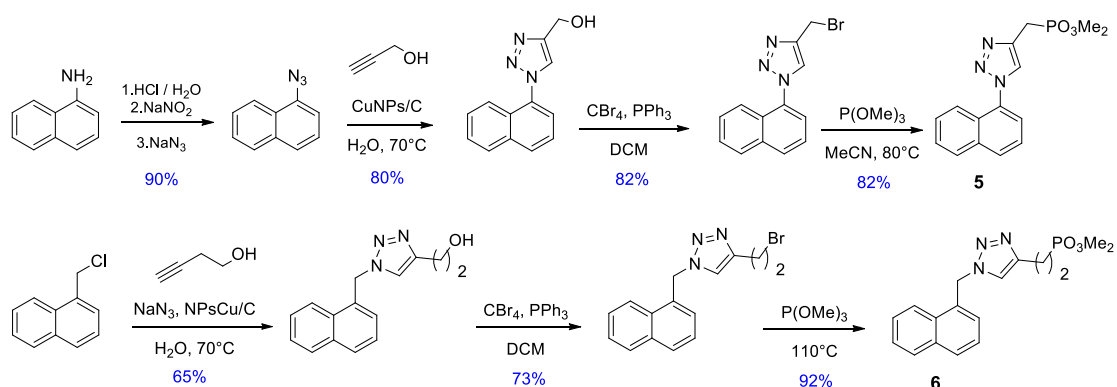
We found that CuNPs supported over activated carbon were able to promote the 1,3-dipolar cycloaddition between 1-azidomethylnaphthalene, formed from the corresponding chloride in the reaction media, and propargyl alcohol in water at 70°C. Afterwards, compound **1** was transformed in the corresponding bromide, which was subjected to Michaelis-Becker reaction with different hydrogen phosphonates, obtaining dialkyl phosphonates esters **3a-c**. Finally, phosphonic acid **4** was obtained through the hydrolysis of compound **3a** in presence of trimethyl silyl bromide (TMSBr).



Scheme 1. Synthesis of 1,4-disubstituted 1,2,3-triazole series I.

3.2. SAR studies. Synthesis of homologs of phosphonate-triazoles and inversion of triazole geometry.

Considering that in preliminary studies of the first series of compounds dimethyl phosphonate ester **3a** exerted the higher activity as $\alpha 7$ nAChRs PAM we decided to synthesize homologs of this compound. We made variations in the distances between the groups' naphthalene-triazole and phosphonate-triazole, leading to derivatives **5** and **6**. In both cases CuNPs/C were used as catalyst for the cycloaddition reaction. 1-azidonaphthalene was obtained via diazonium salt in excellent yields. In this case, dimethyl phosphonates esters were obtained via Arbuzov reaction with higher yields than the former method.



Scheme 2. Synthesis of 1,2,3-triazole series II.

On the other hand, we put our efforts in the synthesis of an analogue of phosphonate **3a** with the inversion of triazole geometry, i.e. with phosphonate ester functionality contained in the organic azide. Retrosynthetic analysis is shown in Figure 2. It is noteworthy that this approach is more convergent than the one used for the synthesis of the previous phosphonates, this is because we were unable to obtain the corresponding propargylic phosphonate through substitution of propargylic bromide with phosphite esters.

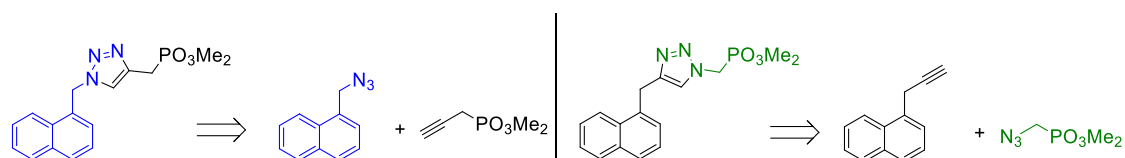
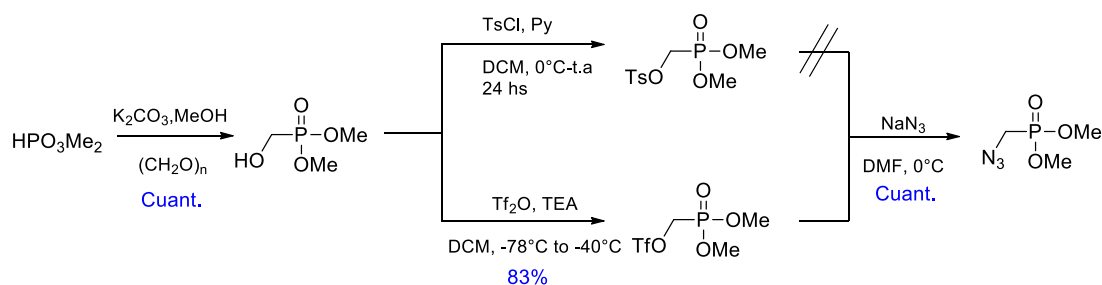
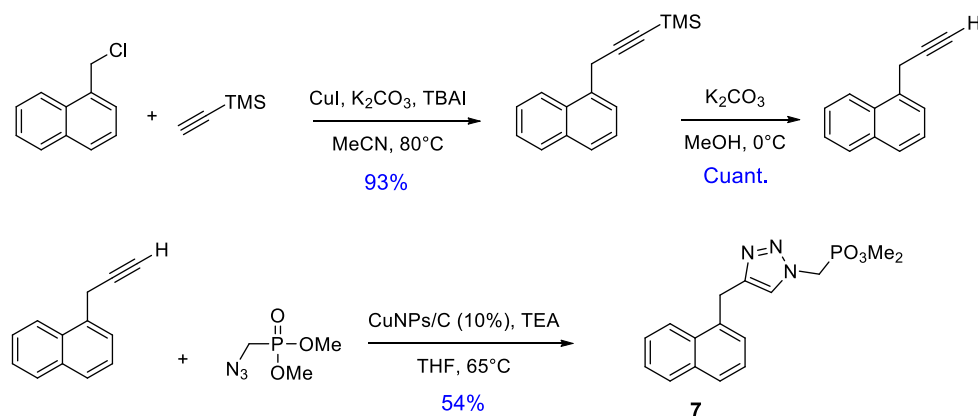


Figure 2. Retrosynthetic analysis of triazole inverted dimethyl phosphonate ester.

Azidomethyl dimethyl phosphonate was synthesized from the corresponding hydroxymethyl phosphonate, which in turn was obtained by the reaction of dimethyl phosphite with paraformaldehyde, in presence of a mild base [8]. It is worthy of mention that it is essential the use of triflates to obtain the azidomethyl phosphonate in good yield, considering the low reactivity of 1-(mesyloxy) or 1-(tosyloxy)alkyl phosphonates and the fact that an increase in temperature could produce mono-dealkylation reactions in both substrate and product [9]. For the synthesis of the desired alkyne we employed a modification of the coupling reaction between benzyl halides and terminal alkynes promoted by CuI described by Wulff and coworkers [10]. Once again, the copper catalyzed azide-alkyne cycloaddition was successfully performed with CuNPs/C.





Scheme 3. Synthesis of the target compound 7.

4. Conclusions

The synthesis of a novel series of phosphonate-functionalized 1,4-disubstituted 1,2,3-triazoles was achieved through the methodologies developed by our group, consisting in the preparation of copper-based catalysts in form of supported nanoparticles. Preliminary studies on their activity as $\alpha 7$ nAChR PAMs show that those with phosphonate functionality, especially dimethyl phosphonate esters, present the desired activity.

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Conflicts of Interest: The authors declare no conflict of interest.

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