

Article Type: Communication

A small library of 1,2,3-triazole analogues of CAP-55: synthesis and binding affinity at nicotinic acetylcholine receptors

Luca Rizzi^a, Cecilia Gotti^b, Marco De Amici^a, Clelia Dallanocce^a, and Carlo Matera^{*,a,c}

^a Dipartimento di Scienze Farmaceutiche, Sezione di Chimica Farmaceutica "Pietro Pratesi",
Università degli Studi di Milano, Via L. Mangiagalli 25, 20133 Milano, Italy.

^b Consiglio Nazionale delle Ricerche (CNR), Istituto di Neuroscienze, Via Vanvitelli 32, 20129 Milano, Italy; Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università degli Studi di Milano, Via Vanvitelli 32, 20129 Milano, Italy.

^c Current address: Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST), Parc Científic de Barcelona, Carrer Baldri Reixac 15-21, 08028 Barcelona, Spain; * email: cmatera@ibecbarcelona.eu

Alpha7 nicotinic acetylcholine receptor is emerging as a central regulator in inflammatory processes, as documented by increasing studies reported in the literature. For instance, the activation of this nicotinic receptor subtype in resident macrophages inhibits the production of pro-inflammatory cytokines, thereby attenuating local inflammatory responses, and may open a new window in the treatment of chronic inflammatory disease, such as Crohn's disease, rheumatoid arthritis, psoriasis and asthma. In continuation of our ongoing research for the development of new cholinergic drug candidates, we selected the nicotine derivative CAP55, which was previously shown to exert anti-inflammatory effects via nicotinic stimulation, as a suitable compound for lead optimization. Through the isosteric replacement of its 3,5-disubstituted 4,5-dihydroisoxazole core with a 1,4-disubstituted 1,2,3-triazole ring, we could rapidly generate a small library of CAP55-related analogues via a one-pot copper(I)-catalyzed azide-alkyne cycloaddition. Receptor binding assays at nAChRs led to the identification of two promising derivatives, compounds **4** and **10**, worthy of further pharmacological studies.

Keywords: click chemistry • nicotine • alpha7 • cholinergic • inflammation

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/cbdv.201800210

This article is protected by copyright. All rights reserved.

Introduction

Convergence from many unrelated research areas has identified a primary role in health and disease for one of the most abundant subtype of the nicotinic acetylcholine receptor (nAChR), the $\alpha 7$ nAChR. The $\alpha 7$ nAChR belongs to the family of acetylcholine-gated ion channels and exhibits distinct biophysical and pharmacological profiles relative to other nAChR subtypes. This receptor, expressed in the CNS, in the autonomous nervous system, and in the vascular system, is emerging as a central regulator in processes ranging from cognitive mechanisms through modulation of specific neurotransmitters, neuroprotection following various insults (e.g., chemical toxicity and β -amyloid-induced cell death), normalization of sensory gating in schizophrenic patients, and inflammatory processes. Resident macrophages express the $\alpha 7$ nAChR, and activation of this receptor inhibits the production of pro-inflammatory cytokines (e.g., TNF, IL-1, IL-6, HMGB-1), but does not inhibit anti-inflammatory cytokine production (e.g., IL-10 and TGF- β), thereby attenuating local inflammatory responses.^{1,2,3} Previous studies have demonstrated that targeting pro-inflammatory cytokines, such as TNF, IL-1 and IL-6, represents a promising approach in the treatment of inflammatory disorders, such as Crohn's disease, psoriasis, rheumatoid arthritis and asthma.^{4,5,6} Therefore, $\alpha 7$ nAChRs may represent a relevant target in view of new therapeutics for the treatment of such inflammatory diseases.

Several studies supporting a role for $\alpha 7$ nAChR in inflammatory reflexes can be found in the literature. For instance, GTS-21, an $\alpha 7$ nAChR partial agonist, was shown to inhibit levels of serum TNF- α , which was associated with improved survival in a murine endotoxemia and severe sepsis model,⁷ and strongly decreased the severity of pancreatitis-associated pulmonary inflammation.^{8,9} Moreover, GTS-21 strongly inhibited LPS-induced TNF- α release into the peritoneal cavity and the circulation.¹⁰ In 2011, Li and co-workers described the $\alpha 7$ -mediated anti-inflammatory effects of A-833834, a new-generation $\alpha 7$ nAChR agonist endowed with exceptionally high binding affinity for this receptor subtype.¹¹ A-833834 inhibited LPS-induced TNF- α release in a methyllycaconitine (MLA)-sensitive manner in macrophages. These effects were also confirmed using human whole blood assay where A-833834 was active. The role for $\alpha 7$ nAChR in regulating TNF- α release was further confirmed in two different *in vivo* mouse models, the i.v. LPS-induced TNF- α release from whole blood and the i.p. zymosan-induced peritonitis models. Li and coll. also showed that A-833834 reduced LPS-induced release of IL-1 β and IL-6 in human blood. This is consistent with recent reports that acetylcholine and other $\alpha 7$ nAChR agonists including PNU-282987 and AR-R17779 reduced the release of TNF- α and IL-6 in human fibroblast-like synoviocytes via $\alpha 7$ nAChR,^{12,13} even though the precise mechanisms underlying $\alpha 7$ nAChR-mediated cytokine reduction remain to be elucidated.¹¹

In 2005, Saeed and coll. developed a new synthetic derivative of the major tobacco alkaloid nicotine (from *Nicotiana tabacum*), named CAP55, and investigated its anti-inflammatory effects, which were shown to be mediated by selective activation of the $\alpha 7$ nAChR subtype (**Figure 1**).^{5,14} CAP55 significantly inhibited endothelial cell inflammatory responses *in vitro* and *in vivo*. Using the localized Shwartzman reaction model, characterized by sustained E-selectin and VCAM-1 expression,¹⁵ CAP55 was found to be very effective in inhibiting endothelial cell activation *in vivo*. Moreover, CAP55 significantly blocked TNF-induced adhesion molecule expression and chemokine expression by human microvascular endothelial cells (HuMVECs) in a dose-dependent manner. The inhibitory effects of CAP55 on endothelial cell activation were reverted both *in vitro* and *in vivo* by

mecamylamine, a noncompetitive nAChR blocker, suggesting that the observed anti-inflammatory effects were mediated by nAChRs. In the carrageenan air pouch model, CAP55 was also found to suppress leukocyte migration during inflammation *in vivo*, and again this effect was blocked by administration of mecamylamine.⁵

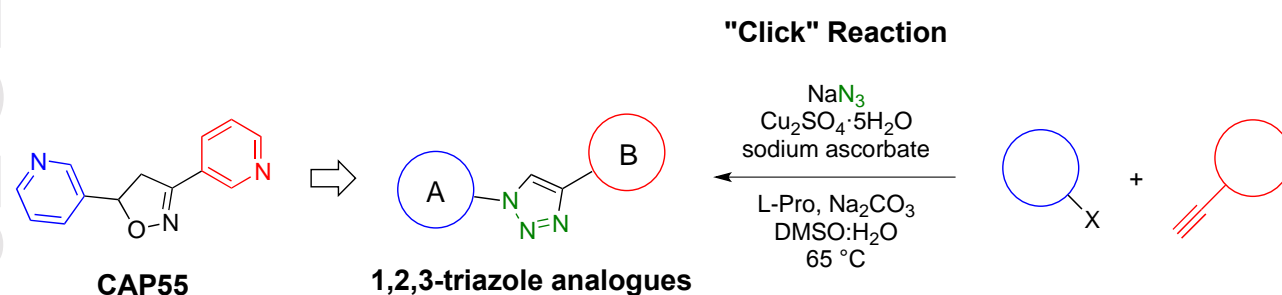


Figure 1. Chemical structure of the cholinergic agonist CAP55, design strategies of the 1,2,3-triazole analogues reported in this study and general scheme of the “click” reaction applied. Blue rings represent aryl, pyridyl, benzyl and pyridylmethyl halides; red rings represent aryl and pyridyl alkynes.

The above discussed results demonstrate the suppressive effects of $\alpha 7$ nAChR agonists on inflammation-related events and highlight the potential therapeutic usefulness of such agents in the treatment of a variety of inflammatory disorders. In continuation of our ongoing research for the development of selective nicotinic agents,^{16,17,18,19,20,21} we identified CAP55 as a suitable candidate for lead optimization. In order to rapidly generate a small library of CAP55 analogues, we aimed at applying a “click chemistry” approach.²² For its molecular structure, CAP55 is well-suited to this purpose: in fact, we observed that the replacement of its 3,5-disubstituted 4,5-dihydroisoxazole core with a 1,4-disubstituted 1,2,3-triazole ring would have allowed us to prepare a set of new derivatives via one-pot copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) (**Figure 1**).²³ Herein, we report the synthesis of a “click chemistry”-based small library of 1,2,3-triazole analogues of CAP55 and the assessment of their binding affinity at $\alpha 7$ and $\alpha 4\beta 2$ nAChR subtypes.

Results and Discussion

The desired 1,4-disubstituted 1,2,3-triazole derivatives **1-10** (**Figure 2**) were all prepared via a copper(I)-promoted 1,3-dipolar cycloaddition between an *in situ* generated azide and a terminal alkyne, following the one-pot two-step procedure described by Fokin and co-workers.²⁴ The starting halide and alkyne building blocks were all selected in such a way that the new derivatives differed for the presence and/or the position of the two pyridine nitrogen atoms, which normally play an important role in the orthosteric binding at nAChRs (**Figure 2**).^{25,26} Our hypothesis that a 1,2,3-triazole could work as an effective isosteric replacing function for the 4,5-dihydroisoxazole ring was supported by *in silico* conformational analyses (see Experimental section for details). In fact, molecular overlay of the lowest energy conformers of a representative ligand (compound **4**) with CAP55 revealed a high degree of structural similarity, with an average root mean square deviation (RMSD) of 1.785 Å, as calculated on the corresponding heavy atoms after best superimposition (**Figure 3**).

All the new derivatives were obtained by simply mixing and stirring the needed reagents in a sealed vial at 65 °C and isolated in a pure form by filtration (see Experimental section for details). Through this experimental protocol, we did not observe the formation of any undesired N–H triazole byproduct, which may result from the competing reaction between inorganic azide and an alkyne. Moreover, yields ranged from fair to excellent and the expected regioselectivity was maintained.²⁴ It is indeed well known that copper(I)-catalyzed azide-alkyne cycloadditions lead exclusively to the 1,4-regioisomer.²⁷ On the other hand, the ruthenium-catalyzed version of the reaction affords mainly the 1,5-regioisomer.^{28,29} Finally, we tested the binding affinity of CAP55 (so far not reported in the literature) and the new analogues **1-10** towards $\alpha 7$ and $\alpha 4\beta 2$ nAChR subtypes. K_i values obtained from the competition assays are reported in **Table 1**.

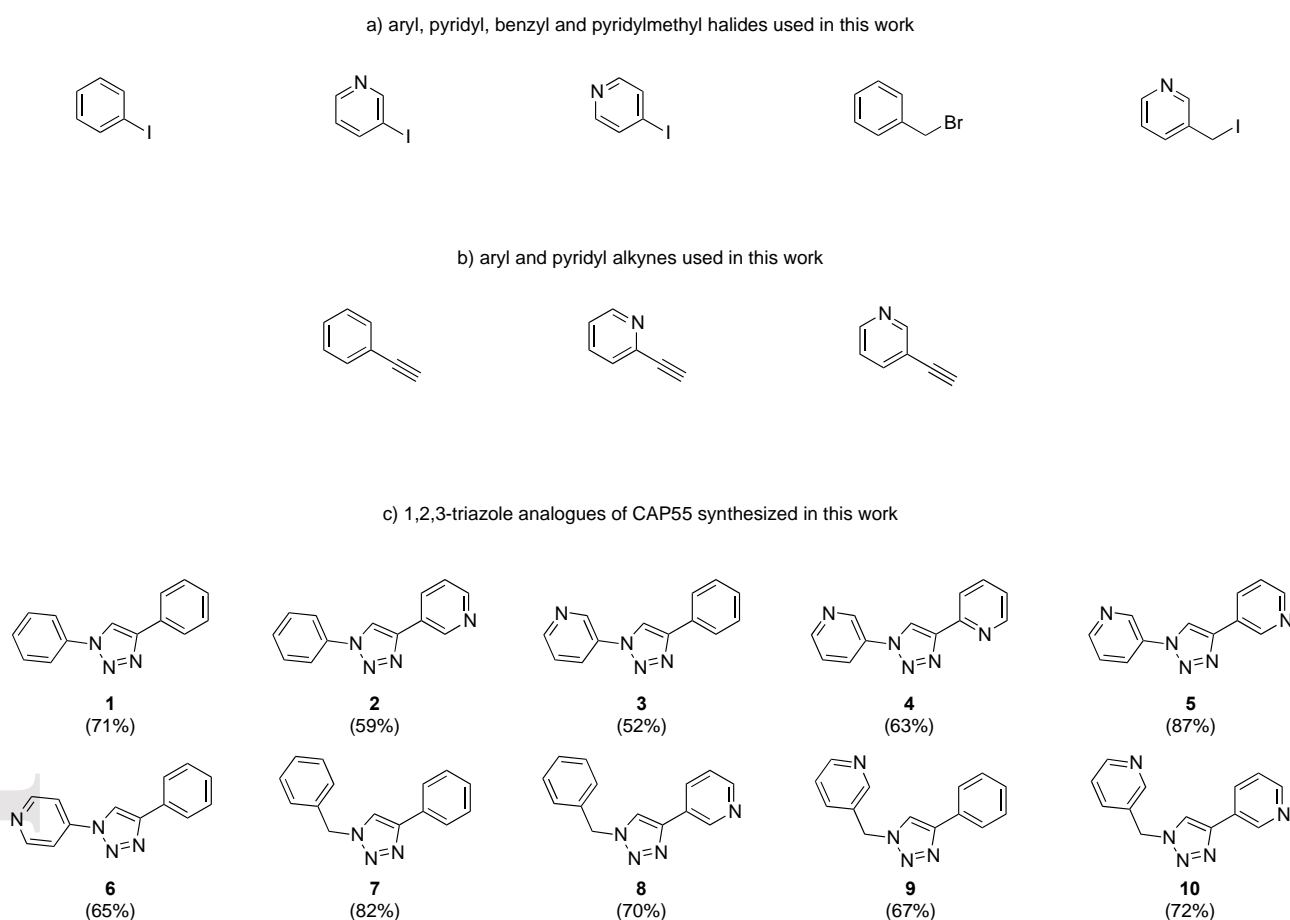


Figure 2. Chemical structure of the CAP55 1,2,3-triazole analogues synthesized and their building blocks for the one-pot copper(I)-catalyzed azide-alkyne cycloaddition. Reaction yields are given in brackets.

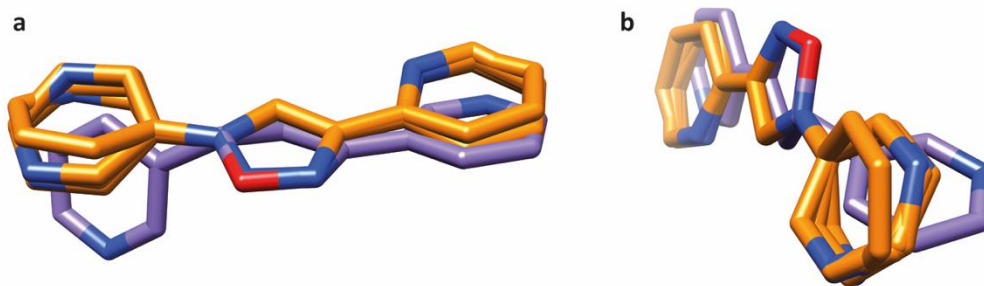


Figure 3. Superimposition of CAP55 (lowest energy conformer, in purple) and a representative member of our set of 1,2,3-triazole analogues (compound 4, five lowest energy conformers, in orange), showing a high degree of similarity (average RMSD = 1.785 Å; single RMSD values were calculated with UCSF Chimera). Panels a and b are different views of the same superimposition. Oxygen and nitrogen atoms are coloured in red and blue, respectively; hydrogen atoms are hidden for the sake of clarity.

Table 1. CAP55 and compounds 1–10: binding affinity for native receptor subtypes present in rat cortex and labelled by [125 I] α Bungarotoxin ($\alpha 7$ nAChRs) or [3 H]epibatidine ($\alpha 4\beta 2$ nAChRs). Numbers in brackets represent the % coefficient of variation (CV).

Compound	Structure	$\alpha 7$	$\alpha 4\beta 2$
		[125 I] α -Bungarotoxin (K_i , μ M)	[3 H]Epibatidine (K_i , μ M)
CAP55		407 (83)	70 (28)
1		686 (88)	76 (12)
2		100 (65)	48 (46)
3		> 100	> 100
4		6.5 (48)	20 (75)
5		> 100	50 (53)
6		346 (125)	178 (14)
7		189 (94)	150 (15)
8		91 (85)	47 (55)
9		73 (64)	34 (58)
10		24 (63)	> 100

The parent compound CAP55 showed a moderate affinity for both receptor subtypes, with a certain preference for $\alpha 4\beta 2$. Based on the effects reported for this compound as an $\alpha 7$ activator in *in vitro* and *in vivo* models of inflammation,⁵ its binding profile may seem quite surprising. However, similar nicotinic ligands, such as GTS-21, have been proven to selectively stimulate $\alpha 7$ nAChRs but also inhibit some other nicotinic receptors, including those containing $\beta 2$ subunits, and, in our opinion, it is reasonable to hypothesize a similar scenario for CAP55.^{30,31} Most of the new derivatives showed a similar binding profile, with moderate or poor affinity for both receptor subtypes. Indeed, compounds **4**, **8**, **9** and **10** displayed an increased affinity for $\alpha 7$, with compounds **4** (60-fold increase) and **10** (17-fold increase) —both characterized by the presence of a pyridine ring on the two substituted positions of the triazole core— emerging as the most interesting analogues. In addition, compound **10** also showed a moderate gain in selectivity versus the $\alpha 4\beta 2$ subtype. Overall, the replacement of the 4,5-dihydroisoxazole core of CAP55 with a triazole system turned out to be a well-tolerated and in some cases a beneficial substitution. Interestingly, increasing the distance between the two pyridine nitrogen atoms produced an inversion of the selectivity profile in compound **10** compared to its lower homologue **5**.

Conclusions

In conclusion, we generated a small library of CAP55 analogues via a click chemistry approach and demonstrated that the 1,4-disubstituted 1,2,3-triazole system may serve as an effective bioisosteric replacement of the 3,5-disubstituted 4,5-dihydroisoxazole nucleus. We have also reported for the first time the binding affinity profile of the cholinergic agonist CAP55 at the two main nAChR subtypes. Compounds **4** and **10**, which both showed a relevant increase in affinity for the $\alpha 7$ subtype, emerged as the most promising derivatives. Since it has been demonstrated that the anti-inflammatory effects of CAP55 are mediated by nAChRs, it is reasonable to expect that structural analogues endowed with a better binding affinity could prove better drug candidates. Further studies are ongoing to elucidate, on one hand, the functional and anti-inflammatory properties of these two nicotinic ligands and, on the other, to deepen the structure-activity relationships in this set of compounds through the rational design of novel analogues.

Experimental Section

Conformational analyses

Methods. The two-dimensional (2D) structures of CAP55 and compound **4** were drawn (.mol files), converted to three-dimensional (3D) representations (.mol2 files), and energy-minimized using the ChemAxon MarvinSketch software.³² Five low energy conformers were generated for compound **4** (DREIDING force field). Energy minimization and conformer generation were carried out using the proper tools implemented in ChemAxon MarvinSketch. Each conformer of **4** was then superimposed to CAP55 using the “Match” function in UCSF Chimera.³³ The carbon-5, oxygen-1 and nitrogen-2 atoms of the 4,5-dihydroisoxazole ring in CAP55 and the corresponding nitrogen-1, nitrogen-2 and nitrogen-3 of the 1,2,3-triazole ring in compound **4** were used for the superimposition. Root mean square deviation (RMSD) values were calculated for each conformer of **4** over CAP55 to evaluate the

goodness of the overall superimposition using the “RMSD” function in UCSF Chimera. Only heavy atoms were considered for these calculations, namely all pyridine nitrogen atoms, oxygen-1 and nitrogen-2 in CAP55, and nitrogen-2 and nitrogen-3 in compound **4**.

Chemistry

Materials and methods. All chemicals were purchased from Sigma-Aldrich and used without any further purification. ^1H NMR and ^{13}C NMR spectra were recorded with a Varian Mercury 300 (^1H , 300.063; ^{13}C , 75.451 MHz) spectrometer at 20 °C. ESI Mass spectra were obtained on a Varian 320 LC-MS-MS instrument; data are reported as mass-to-charge ratio (m/z). TLC analyses were performed on commercial Silica Gel 60 F254 aluminium sheets; the spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution or a phosphomolybdic acid solution (10% in ethanol). Melting points were determined on a model B 540 Büchi apparatus and are uncorrected.

Typical experimental procedure. The aryl- or arylmethyl halide (1 equiv) was mixed with ethynylbenzene or ethynylpyridine (1 equiv) in a sealable round bottom vial. To the mixture were added L-proline (0.2 equiv), Na_2CO_3 (0.2 equiv), NaN_3 (1.2 equiv), sodium ascorbate (0.1 equiv), 9:1 DMSO/ H_2O and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.05 equiv). The vial was sealed and the mixture was stirred overnight at 65 °C. Upon completion (monitored by TLC), the crude mixture was poured into ice-cold water. The off-white precipitate was isolated by filtration and washed with dilute NH_4OH to remove any traces of explosive copper azides before the final drying step.

1,4-Diphenyl-1H-1,2,3-triazole [1]. 71% yield, white powder, mp: 183-185 °C; R_f = 0.79 (DCM/MeOH = 99:1); ^1H NMR ($\text{DMSO}-d_6$): δ (ppm) 9.29 (s, 1H), 7.95 (d, J = 8.1 Hz, 4H), 7.62 (t, J = 7.5 Hz, 2H), 7.52-7.46 (m, 3H), 7.37 (t, J = 7.4 Hz, 1H); ^{13}C NMR ($\text{DMSO}-d_6$): δ (ppm) 148.0, 137.3, 131.0, 130.6, 129.7, 129.4, 128.9, 126.0, 120.7, 120.3; MS (m/z) calcd. for $\text{C}_{14}\text{H}_{12}\text{N}_3^+$ [$\text{M}+\text{H}^+$] 222.10, found 222.1.

3-(1-Phenyl-1H-1,2,3-triazol-4-yl)pyridine [2]. 59% yield, light yellow powder, mp: 170-173 °C; R_f = 0.20 (cyclohexane/AcOEt = 1:1); ^1H NMR (CDCl_3): δ (ppm) 9.09 (s, 1H), 8.62 (d, J = 4.7 Hz, 1H), 8.31 (t, J = 1.9 Hz, 1H), 8.29 (s, 1H), 7.83-7.79 (m, 2H), 7.58 (t, J = 7.2 Hz, 2H), 7.50 (d, J = 7.4 Hz, 1H), 7.42 (dd, J = 8.0 and 5.0 Hz, 1H); ^{13}C NMR (CDCl_3): δ (ppm) 149.7, 147.3, 145.5, 133.4, 130.1, 129.8, 129.3, 126.7, 124.1, 120.9, 118.2; MS (m/z) calcd. for $\text{C}_{13}\text{H}_{11}\text{N}_4^+$ [$\text{M}+\text{H}^+$] 223.10, found 223.1.

3-(4-Phenyl-1H-1,2,3-triazol-1-yl)pyridine [3]. 52% yield, yellow powder, mp: 176-178 °C; R_f = 0.22 (cyclohexane/AcOEt = 1:1); ^1H NMR (CDCl_3): δ (ppm) 9.07 (s, 1H), 8.73 (d, J = 4.4 Hz, 1H), 8.25 (s, 1H), 8.23-8.19 (m, 1H), 7.92 (d, J = 8.3 Hz, 2H), 7.53 (dd, J = 8.3 and 5.0 Hz, 1H), 7.48 (t, J = 7.4 Hz, 2H), 7.41 (d, J = 7.7 Hz, 1H); ^{13}C NMR (CDCl_3): δ (ppm) 150.2, 149.2, 141.8, 134.0, 130.0, 129.2, 129.0, 128.3, 126.2, 124.5, 117.6; MS (m/z) calcd. for $\text{C}_{13}\text{H}_{11}\text{N}_4^+$ [$\text{M}+\text{H}^+$] 223.10, found 223.1.

2-(1-(Pyridin-3-yl)-1H-1,2,3-triazol-4-yl)pyridine [4]. 63% yield, light brown powder, mp: 203-206 °C; R_f = 0.50 (DCM/MeOH = 95:5); ^1H NMR (CDCl_3): δ (ppm) 9.20 (s, 1H), 8.81 (m, 1H), 8.69-8.60 (m, 2H), 8.24 (dd, J = 11.8 and 8.0 Hz, 2H), 7.84 (t, J = 7.7 Hz, 1H), 7.64-7.52 (m, 1H), 7.30 (dd, J = 7.2 and 5.0 Hz, 1H); ^{13}C NMR (CDCl_3): δ (ppm) 150.1, 149.5, 149.0, 137.5, 137.3, 134.0, 130.1, 129.2, 128.3, 126.2, 123.4, 117.6; MS (m/z) calcd. for $\text{C}_{12}\text{H}_{10}\text{N}_5^+$ [$\text{M}+\text{H}^+$] 224.09, found 224.0.

3,3'-(1H-1,2,3-Triazole-1,4-diyl)dipyridine [5]. 87% yield, white powder, mp: 218-220 °C; R_f = 0.45 (DCM/MeOH = 96:4); ^1H NMR (CDCl_3): δ (ppm) 9.10 (s, 1H), 9.08 (d, J = 2.7 Hz, 1H), 8.75 (dd, J = 4.7 and 1.1 Hz, 1H), 8.65-8.60 (m, 1H), 8.35 (s, 1H), 8.29 (dt, J = 8.0 and 1.7 Hz, 1H), 8.21 (dq, J = 8.3 and 1.7 Hz, 1H), 7.55 (dd, J = 8.3 and 4.7 Hz, 1H), 7.43 (dd, J = 8.0 and 5.0 Hz, 1H); ^{13}C NMR (CDCl_3): δ (ppm) 150.5, 150.1, 147.4, 146.2, 141.9, 133.7, 133.5, 128.4, 126.3, 124.6, 124.1, 118.1; MS (m/z) calcd. for $\text{C}_{12}\text{H}_{10}\text{N}_5^+$ [$\text{M}+\text{H}^+$] 224.09, found 224.1.

4-(4-Phenyl-1H-1,2,3-triazol-1-yl)pyridine [6]. 65% yield, light brown powder, mp: 170-172 °C; R_f = 0.30 (cyclohexane/AcOEt = 6:4); ^1H NMR (CDCl_3): δ (ppm) 8.83 (m, 2H), 8.30 (s, 1H), 7.92 (d, J = 7.2 Hz, 2H), 7.80 (d, J = 5.0 Hz, 2H), 7.48 (t, J = 7.2 Hz, 2H), 7.41 (d, J = 7.2 Hz, 1H); ^{13}C NMR (CDCl_3): δ (ppm) 152.0, 149.4, 143.2, 129.8, 129.3, 129.1, 126.2, 116.8, 113.9; MS (m/z) calcd. for $\text{C}_{13}\text{H}_{11}\text{N}_4^+$ [$\text{M}+\text{H}^+$] 223.10, found 223.1.

1-Benzyl-4-phenyl-1H-1,2,3-triazole [7]. 82% yield, white powder, mp: 128-130 °C; R_f = 0.38 (cyclohexane/AcOEt = 7:3); ^1H NMR ($\text{DMSO}-d_6$): δ (ppm) 8.63 (s, 1H), 7.83 (d, J = 7.4 Hz, 2H), 7.45-7.30 (m, 8H), 5.64 (s, 2H); ^{13}C NMR ($\text{DMSO}-d_6$): δ (ppm) 136.7, 131.4, 129.6, 129.5, 128.9, 128.6, 128.1, 125.8, 122.3, 120.7, 53.7; MS (m/z) calcd. for $\text{C}_{15}\text{H}_{14}\text{N}_3^+$ [$\text{M}+\text{H}^+$] 236.12, found 236.1.

3-(1-Benzyl-1H-1,2,3-triazol-4-yl)pyridine [8]. 70% yield, light brown powder, mp: 100-102 °C; R_f = 0.25 (cyclohexane/AcOEt = 3:7); ^1H NMR (CDCl_3): δ (ppm) 8.95 (bs, 1H), 8.56 (d, J = 3.4 Hz, 1H), 8.18 (ddd, J = 7.7, 3.9 and 2.2 Hz, 1H), 7.74 (s, 1H), 7.43-7.30 (m, 6H), 5.60 (s, 2H); ^{13}C NMR (CDCl_3): δ (ppm) 149.4, 147.2, 145.4, 134.5, 133.1, 129.4, 129.1, 128.3, 126.9, 123.9, 120.0, 54.6; MS (m/z) calcd. for $\text{C}_{14}\text{H}_{13}\text{N}_4^+$ [$\text{M}+\text{H}^+$] 237.11, found 237.1.

3-((4-Phenyl-1H-1,2,3-triazol-1-yl)methyl)pyridine [9]. 67% yield, white powder, mp: 112-115 °C; R_f = 0.40 (AcOEt); ^1H NMR (CDCl_3): δ (ppm) 8.67-8.62 (m, 2H), 7.79 (d, J = 6.9 Hz, 2H), 7.71 (s, 1H), 7.62 (d, J = 7.7 Hz, 1H), 7.41 (t, J = 6.9 Hz, 2H), 7.32 (t, J = 7.2 Hz, 2H), 5.60 (s, 2H); ^{13}C NMR (CDCl_3): δ (ppm) 150.6, 149.4, 148.8, 135.9, 130.8, 130.5, 129.1, 128.6, 126.0, 124.2, 119.6, 51.8; MS (m/z) calcd. for $\text{C}_{14}\text{H}_{13}\text{N}_4^+$ [$\text{M}+\text{H}^+$] 237.11, found 237.1.

3-((4-(Pyridin-3-yl)-1H-1,2,3-triazol-1-yl)methyl)pyridine [10]. 72% yield, white powder, mp: 132-134 °C; R_f = 0.33 (AcOEt); ^1H NMR (CDCl_3): δ (ppm) 8.97 (d, J = 1.9 Hz, 1H), 8.68 (d, J = 2.2 Hz, 1H), 8.65 (dd, J = 5.0 and 1.7 Hz, 1H), 8.58 (dd, J = 4.7 and 1.4 Hz, 1H), 8.19 (dt, J = 8.0 and 1.9 Hz, 1H), 7.80 (s, 1H), 7.66 (dt, J = 8.0 and 2.2 Hz, 1H), 7.39-7.33 (m, 2H), 5.64 (s, 2H); ^{13}C NMR (CDCl_3): δ (ppm) 150.7, 149.5, 148.9, 147.7, 147.4, 136.0, 134.6, 130.8, 130.6, 126.1, 124.2, 120.1, 51.8; MS (m/z) calcd. for $\text{C}_{13}\text{H}_{12}\text{N}_5^+$ [$\text{M}+\text{H}^+$] 238.11, found 238.1.

Receptor binding assays

Animal tissues. Adult male pathogen-free Sprague-Dawley rats (Harlan-Nossan, Milan, Italy) (at least 4-month-old) were used. Rats were reared on a 12 h light/dark cycle (animals were kept in a 12 h light/12 h dark cycle) with free access to food and water. All experiments were conducted according to Ministry of Health (the regulatory authority for controlling the use of laboratory animals and ethics on animal experiments in Italy) guidelines (Legislative Decree n. 116/92) and in accordance with the European Community guidelines European Directive 86/609/EEC). All the experiments were performed according to protocols (n.2/2010) approved by the University of Milan Animal Care and Use Committees and by the Ministry of Health.

Membranes binding of [¹²⁵I]α-Bungarotoxin and [³H]Epibatidine. The cortex tissues were dissected, immediately frozen on dry ice and stored at −80 °C for later use. In each experiment, the cortex tissues from two rats were homogenized in 10 mL of a buffer solution (50 mM Na₃PO₄, 1 M NaCl, 2 mM ethylenediaminetetraacetic acid [EDTA], 2 mM ethylene glycol tetraacetic acid [EGTA] and 2 mM phenylmethylsulfonyl fluoride [PMSF], pH 7.4) using a potter homogenizer; the homogenates were then diluted and centrifuged at 60,000 *g* for 1.5 h. The total membrane homogenization, dilution and centrifugation procedures were performed twice, then the pellets were collected, rapidly rinsed with a buffer solution (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 2.5 mM CaCl₂ and 2 mM PMSF, pH 7), and resuspended in the same buffer containing a mixture of 20 µg/mL of each of the following protease inhibitors: leupeptin, bestatin, pepstatin A, and aprotinin. *K_i* values were calculated from the competition curves with the LIGAND software.³⁴

[¹²⁵I]α-Bungarotoxin binding. The saturation binding experiments were performed using aliquots of cortex membrane homogenates incubated overnight with 0.1-10 nM concentrations of [¹²⁵I]α-bungarotoxin (specific activity 200–213 Ci/mmol, GE Healthcare) at room temperature. Nonspecific binding was determined in parallel by means of incubation in the presence of 1 µM unlabelled α-bungarotoxin. After incubation, the samples were filtered as described above and the bound radioactivity was directly counted in a γ counter.

[³H]Epibatidine binding. (±)-[³H]Epibatidine with a specific activity of 56–60 Ci/mmol was purchased from Perkin–Elmer (Boston MA); the non radioactive α-bungarotoxin and epibatidine were purchased from Sigma. It has been previously reported that [³H]epibatidine also binds to α-bungarotoxin binding receptors with nM affinity.³⁵ To prevent the binding of [³H]epibatidine to the α-bungarotoxin binding receptors, the membrane homogenates were pre-incubated with 2 µM α-bungarotoxin and then with [³H]epibatidine. The saturation experiments were performed by incubating aliquots of cortex membrane homogenates with 0.01-2.5 nM concentrations of (±)-[³H]epibatidine overnight at 4 °C. Nonspecific binding was determined in parallel by means of incubation in the presence of 100 nM unlabelled epibatidine. At the end of the incubation, the samples were filtered on a GFC filter soaked in 0.5% polyethylenimine and washed with 15 mL of a buffer solution (10 mM Na₃PO₄, 50 mM NaCl, pH 7.4), and the filters were counted in a β counter.

Supplementary Material

Supporting information for this article is available on the WWW under <https://XXX.XXX.XXX>

Acknowledgements

ChemAxon MarvinSketch was used for drawing chemical structures and generating low energy conformers (MarvinSketch version 17.28.0, 2017, <http://www.chemaxon.com>). Molecular graphics images and RMSD calculations were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR-01081).

Author Contribution Statement

LR and CM designed and synthesized the new compounds; CG performed receptor binding assays; MDA and CD supervised the project; CM and MDA wrote the manuscript with contributions from all authors.

References

- [1] M.B. Marrero, M. Bencherif, 'Convergence of alpha 7 nicotinic acetylcholine receptor-activated pathways for anti-apoptosis and anti-inflammation: central role for JAK2 activation of STAT3 and NF-kappaB', *Brain Res.* **2009**, 1256, 1-7.
- [2] J.J. Buccafusco, S.R. Letchworth, M. Bencherif, P.M. Lippiello, 'Long-lasting cognitive improvement with nicotinic receptor agonists: mechanisms of pharmacokinetic-pharmacodynamic discordance', *Trends Pharmacol. Sci.* **2005**, 26(7), 352-360.
- [3] M.B. Marrero, M. Bencherif, P.M. Lippiello, R. Lucas, 'Application of alpha7 nicotinic acetylcholine receptor agonists in inflammatory diseases: an overview', *Pharm. Res.* **2011**, 28(2), 413-416.
- [4] M.B. Marrero, R. Lucas, C. Salet, T.A. Hauser, A. Mazurov, P.M. Lippiello, M. Bencherif, 'An alpha7 nicotinic acetylcholine receptor-selective agonist reduces weight gain and metabolic changes in a mouse model of diabetes', *J. Pharmacol. Exp. Ther.* **2010**, 332(1), 173-180.
- [5] R.W. Saeed, S. Varma, T. Peng-Nemerooff, B. Sherry, D. Balakhaneh, J. Huston, K.J. Tracey, Y. Al-Abed, C.N. Metz, 'Cholinergic stimulation blocks endothelial cell activation and leukocyte recruitment during inflammation', *J. Exp. Med.* **2005**, 201(7), 1113-1123.
- [6] H. Wang, M. Yu, M. Ochani, C.A. Amella, M. Tanovic, S. Susarla, J.H. Li, H. Wang, H. Yang, L. Ulloa, Y. Al-Abed, C.J. Czura, K.J. Tracey, 'Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation', *Nature* **2003**, 421(6921), 384-388.
- [7] V.A. Pavlov, M. Ochani, L.H. Yang, M. Gallowitsch-Puerta, K. Ochani, X. Lin, J. Levi, W.R. Parrish, M. Rosas-Ballina, C.J. Czura, G.J. Larosa, E.J. Miller, K.J. Tracey, Y. Al-Abed, 'Selective alpha7-nicotinic acetylcholine receptor agonist GTS-21 improves survival in murine endotoxemia and severe sepsis', *Crit. Care Med.* **2007**, 35(4), 1139-1144.
- [8] D.J. van Westerloo, I.A. Giebelen, S. Florquin, J. Daalhuisen, M.J. Bruno, A.F. de Vos, K.J. Tracey, T. van der Poll, 'The cholinergic anti-inflammatory pathway regulates the host response during septic peritonitis', *J. Infect. Dis.* **2005**, 191(12), 2138-2148.
- [9] D.J. van Westerloo, I.A. Giebelen, S. Florquin, M.J. Bruno, G.J. Larosa, L. Ulloa, K.J. Tracey, T. van der Poll, 'The vagus nerve and nicotinic receptors modulate experimental pancreatitis severity in mice', *Gastroenterology*. **2006**, 130(6), 1822-1830.

- [10] I.A. Giebelen, D.J. van Westerloo, G.J. LaRosa, A.F. de Vos, T. van der Poll, 'Local stimulation of alpha7 cholinergic receptors inhibits LPS-induced TNF-alpha release in the mouse lung', *Shock* **2007**, 28(6), 700-703.
- [11] J. Li, S.L. Mathieu, R. Harris, J. Ji, D.J. Anderson, J. Malysz, W.H. Bunnelle, J.F. Waring, K.C. Marsh, A. Murtaza, L.M. Olson, M. Gopalakrishnan, 'Role of $\alpha 7$ nicotinic acetylcholine receptors in regulating tumor necrosis factor- α (TNF- α) as revealed by subtype selective agonists', *J. Neuroimmunol.* **2011**, 239(1-2), 37-43.
- [12] J.M. Waldburger, D.L. Boyle, V.A. Pavlov, K.J. Tracey, G.S. Firestein, 'Acetylcholine regulation of synovocyte cytokine expression by the alpha7 nicotinic receptor', *Arthritis Rheum.* **2008**, 58(11), 3439-3449.
- [13] M.A. van Maanen, S.P. Stoof, E.P. van der Zanden, W.J. de Jonge, R.A. Janssen, D.F. Fischer, N. Vandeghinste, R. Brys, M.J. Vervoordeldonk, P.P. Tak, 'The alpha7 nicotinic acetylcholine receptor on fibroblast-like synoviocytes and in synovial tissue from rheumatoid arthritis patients: a possible role for a key neurotransmitter in synovial inflammation', *Arthritis Rheum.* **2009**, 60(5), 1272-1281.
- [14] Y. Al-Abed, K.J. Tracey, 'Isoxazole and isothiazole compounds useful in the treatment of inflammation', WO 2005034952 A2.
- [15] C. Sunderkötter, S. Seeliger, F. Schönlau, J. Roth, R. Hallmann, T.A. Luger, C. Sorg, G. Kolde, 'Different pathways leading to cutaneous leukocytoclastic vasculitis in mice', *Exp. Dermatol.* **2001**, 10(6), 391-404.
- [16] L. Rizzi, C. Dallanoe, C. Matera, P. Magrone, L. Pucci, C. Gotti, F. Clementi, M. De Amici, 'Epiboxidine and novel-related analogues: a convenient synthetic approach and estimation of their affinity at neuronal nicotinic acetylcholine receptor subtypes', *Bioorg. Med. Chem. Lett.* **2008**, 18(16), 4651-4654.
- [17] G. Grazioso, D.Y. Pomè, C. Matera, F. Frigerio, L. Pucci, C. Gotti, C. Dallanoe, M. De Amici, 'Design of novel alpha7-subtype-preferring nicotinic acetylcholine receptor agonists: application of docking and MM-PBSA computational approaches, synthetic and pharmacological studies', *Bioorg. Med. Chem. Lett.* **2009**, 19(22), 6353-6357.
- [18] C. Dallanoe, F. Frigerio, G. Martelli, G. Grazioso, C. Matera, D.Y. Pomè, L. Pucci, F. Clementi, C. Gotti, M. De Amici, 'Novel tricyclic Delta(2)-isoxazoline and 3-oxo-2-methyl-isoxazolidine derivatives: synthesis and binding affinity at neuronal nicotinic acetylcholine receptor subtypes', *Bioorg. Med. Chem.* **2010**, 18(12), 4498-4508.
- [19] C. Dallanoe, P. Magrone, C. Matera, L. Lo Presti, M. De Amici, L. Riganti, F. Clementi, C. Gotti, C. De Micheli, 'Synthesis of novel chiral $\Delta 2$ -isoxazoline derivatives related to ABT-418 and estimation of their affinity at neuronal nicotinic acetylcholine receptor subtypes', *Eur. J. Med. Chem.* **2010**, 45(12), 5594-5601.
- [20] C. Dallanoe, P. Magrone, C. Matera, F. Frigerio, G. Grazioso, M. De Amici, S. Fucile, V. Piccari, K. Frydenvang, L. Pucci, C. Gotti, F. Clementi, C. De Micheli, 'Design, synthesis, and pharmacological characterization of novel spirocyclic quinuclidinyl- $\Delta 2$ -isoxazoline derivatives as potent and selective agonists of $\alpha 7$ nicotinic acetylcholine receptors', *ChemMedChem* **2011**, 6(5), 889-903.
- [21] C. Matera, M. Quadri, M. Sciacaluga, D.Y. Pomè, F. Fasoli, M. De Amici, S. Fucile, C. Gotti, C. Dallanoe, G. Grazioso, 'Modification of the anabaseine pyridine nucleus allows achieving binding and functional selectivity for the $\alpha 3\beta 4$ nicotinic acetylcholine receptor subtype', *Eur. J. Med. Chem.* **2016**, 108, 392-405.
- [22] H.C. Kolb, M.G. Finn, K.B. Sharpless, 'Click Chemistry: Diverse Chemical Function from a Few Good Reactions', *Angew. Chem. Int. Ed. Engl.* **2001**, 40(11), 2004-2021.
- [23] V.V. Rostovtsev, L.G. Green, V.V. Fokin, K.B. Sharpless, 'A stepwise Huisgen cycloaddition process: copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes', *Angew. Chem. Int. Ed. Engl.* **2002**, 41(14), 2596-2599.
- [24] A.K. Feldman, B. Colasson, V.V. Fokin, 'One-pot synthesis of 1,4-disubstituted 1,2,3-triazoles from in situ generated azides', *Org. Lett.* **2004**, 6(22), 3897-3899.
- [25] P.H. Celie, S.E. van Rossum-Fikkert, W.J. van Dijk, K. Brejc, A.B. Smit, T.K. Sixma TK, 'Nicotine and carbamylcholine binding to nicotinic acetylcholine receptors as studied in AChBP crystal structures', *Neuron* **2004**, 41(6), 907-914.
- [26] R.E. Hibbs, G. Sulzenbacher, J. Shi, T.T. Talley, S. Conrod, W.R. Kem, P. Taylor, P. Marchot, Y. Bourne, 'Structural determinants for interaction of partial agonists with acetylcholine binding protein and neuronal alpha7 nicotinic acetylcholine receptor', *EMBO J.* **2009**, 28(19), 3040-3051.
- [27] D. Kumar, V.B. Reddy, and R.S. Varma, 'A facile and regioselective synthesis of 1,4-disubstituted 1,2,3-triazoles using click chemistry', *Tetrahedron Lett.* **2009**, 50(18), 2065-2068.
- [28] K. Takasu, T. Azuma, Y. Takemoto, 'Synthesis of trifunctional thioureas bearing 1,5-disubstituted triazole tether by Ru-catalyzed Huisgen cycloaddition', *Tetrahedron Lett.* **2010**, 51(20), 2737-2740.
- [29] K. Chouaib, S. Delemasure, P. Dutartre, H.B. Jannet, 'Microwave-assisted synthesis, anti-inflammatory and anti-proliferative activities of new maslinic acid derivatives bearing 1,5- and 1,4-disubstituted triazoles', *J. Enzyme Inhib. Med. Chem.* **2016**, 31(sup2), 130-147.
- [30] F. van Haaren, K.G. Anderson, S.C. Haworth, W.R. Kem, 'GTS-21, a mixed nicotinic receptor agonist/antagonist, does not affect the nicotine cue', *Pharmacol. Biochem. Behav.* **1999**, 64(2), 439-444.

- [31] M.D. Meyer, 'AR-R17779', In 'xPharm: The Comprehensive Pharmacology Reference', Elsevier, New York, 2007, pp. 1-4.
- [32] MarvinSketch (version 17.28.0, 2017), calculation module developed by ChemAxon, <http://www.chemaxon.com/products/marvin/marvinsketch/>.
- [33] E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, and T.E. Ferrin, 'UCSF Chimera - A Visualization System for Exploratory Research and Analysis', *J. Comput. Chem.* **2004**, 25(13), 1605-1612.
- [34] P.J. Munson, D. Rodbard, 'Ligand: a versatile computerized approach for characterization of ligand-binding systems', *Anal. Biochem.* **1980**, 107(1), 220-239.
- [35] V. Gerzanich, X. Peng, F. Wang, G. Wells, R. Anand, S. Fletcher, J. Lindstrom, 'Comparative pharmacology of epibatidine: a potent agonist for neuronal nicotinic acetylcholine receptors', *J. Mol. Pharmacol.* **1995**, 48(4), 774-782, and references cited therein.