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Ethynylphenyl carbonates and carbamates as dual-action acetylcholinesterase inhibitors and anti-inflammatory agents

Jaya Saxena [a], David Meloni [a,b], Mou-Tuan Huang [c], Diane E. Heck [d], Jeffrey D. Laskin [e], Ned D. Heindel [a], Sherri C. Young [a,f,*]

[a] Department of Chemistry, Lehigh University, Bethlehem, PA 18015, United States
[b] Incyte Corporation, Wilmington, DE 19803, United States
[c] Department of Chemical Biology, Rutgers University, Piscataway, NJ 08854, United States
[d] Department of Environmental Health Science, New York Medical College, Valhalla, NY 10595, United States
[e] Department of Environmental and Occupational Medicine, Rutgers University, Piscataway, NJ 08854, United States
[f] Department of Chemistry, Muhlenberg College, Allentown, PA 18104, United States

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ABSTRACT

Novel ethynylphenyl carbonates and carbamates containing carbon- and silicon-based choline mimics were synthesized from their respective phenol and aniline precursors and screened for anticholinesterase and anti-inflammatory activities. All molecules were micromolar inhibitors of acetylcholinesterase (AChE), with IC_{50}s of 28–86 μM; the carbamates were two-fold more potent than the carbonates. Two of the most potent AChE inhibitors suppressed 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation by 40%. Furthermore, these molecules have physicochemical properties in the range of other CNS drugs. These molecules have the potential to treat inflammation; they could also dually target Alzheimer's disease through restoration of cholinergic balance and inflammation suppression.

The excessive release of proinflammatory cytokines contributes to a multitude of disorders such as multiple sclerosis,[1–3] diabetes,[4] rheumatoid arthritis,[5] Alzheimer's disease (AD),[6,7] and Parkinson's disease.[8,9] While acetylcholinesterase inhibitors (AChEIs) have been extensively explored for the treatment of glaucoma,[10–12] myasthenia gravis,[13] Lewy body dementia,[14,15] and AD,[16–18] there has been an emerging interest in AChEIs for the treatment of inflammatory disorders. Tracey's work supports a physiological link between inflammation and cholinergic imbalance termed the cholinergic anti-inflammatory pathway.[19] This pathway is mediated by the α7 subunit of the nicotinic acetylcholine (ACh) receptor (α7-nAChR) and involves regulation of systemic cytokine release by the vagus nerve. ACh has been shown to decrease both central and peripheral release of proinflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-6 (IL-6).[20] Potentiation of ACh via inhibition of AChE results in inflammation suppression in models of sepsis, obesity, and endotoxemia. For example, intraperitoneal administration of the AChEIs physostigmine and neostigmine improved survival time in a murine model of sepsis.[21] Galantamine, an AChEI on the market for AD treatment, also suppressed the release of TNF and IL-6 in a model of endotoxemia[22] and alleviated obesity-associated inflammation.[23] Snorrason has shown that ointments of AChEIs such as galantamine or donepezil are useful in alleviating skin inflammations.[24] Finally, locally applied neostigmine suppressed inflammation in an inflamed mouse knee model, and topical AChEIs have shown promise in the treatment of hyperhidrosis.[25–27] Clearly, inhibition of AChE is a promising approach to treating inflammatory disorders. Furthermore, considering the well-documented role of both inflammation[6,7] and cholinergic dysfunction[16–18] in AD, anti-inflammatory AChEIs represent a promising class of dual-action AD therapeutics.

In earlier work directed at the development of potential pesticides, we reported the synthesis and development of a mechanism-based, active-site directed, highly specific AChEI, choline *p*-chloromethylphenyl carbonate iodide (**1**, Fig. 1).[28,29] This candidate was modeled after Bechet's classic mechanism-based albeit rather nonspecific chloromethyldihydrocoumarin (**2**, Fig. 1) which inhibited a rather broad set of proteases and esterases. Bechet proposed an electrophilic quinone methide metabolite as the lethal transient which captured the active site nucleophile, presumably serine.[30,31] The anticholinesterase activity of **1** was assessed, and it performed as a classic mechanism-based inhibitor with irreversible, time-dependent, pseudo first-order enzyme inactivation. Additional reversibility and kinetic studies indicated that the quinone methide electrophile was formed on, and remained in,

* Corresponding author. Tel.: +1 484 664 3271; fax: +1 484 664 4113.
E-mail address: young@muhlenberg.edu (S.C. Young).

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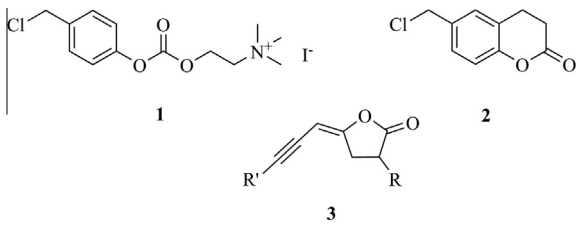


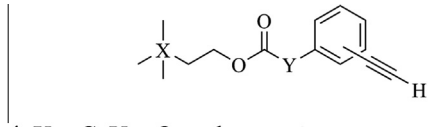
Figure 1. Choline *p*-chloromethylphenyl carbonate iodide (**1**),[28,29] Bechet's chloromethyldihydrocoumarin antiprotease (**2**),[30,31] and a generic ynenol furyl lactone (**3**).[35]

the active site of the enzyme.[28,29] Considering the structural and functional similarities between **1** and **2**, it was proposed that the AChE inactivation mechanism by **1** involves a quinone methide intermediate.[28] Unfortunately, **1** proved ineffective when screened against fully-developed adult insects, and it was only modestly effective against immature larvae. Furthermore, in control experiments, **1** was unable to cross biological membranes and proved unstable both in solution and in the solid state, an occurrence traced to its chloromethyl moiety.[28,29] Despite the limitations of this molecule as a pesticide, it has served as a structural platform for the synthesis and optimization of selective and potent AChEIs by our group.[32–34]

It is well known in other mechanism-based inhibitors that a terminal unsubstituted ethynyl group on an aromatic ring or on an sp^2 carbon can act as an electrophilic surrogate for a halomethyl. For example, ynenol furyl lactones (**3**, Fig. 1) serve as irreversible inhibitors of human leukocyte elastase apparently through transient formation of an electrophilic allenone.[35] Considering the documented similarities in reactivity between conjugated ethynyls and halomethyls, we chose to design a new class of ethynylphenyl candidate inhibitors (Fig. 2).

Uncharged isosteres of choline have moderate affinities for AChE, with inhibition constants (K_i) of 7.5 mM and 3.3 mM reported for 3,3-dimethyl-1-butanol and 2-(trimethylsilyl)ethanol, respectively.[36] As such, many of the molecules designed by our group, including the molecules presented herein (Fig. 2), contain one of these moieties to provide cholinesterase recognition.[32–34] Substitution of the nitrogen-based choline analog with a carbon- or silicon-based analog may also address the inherent instability and poor membrane permeability of carbonate **1**, while maintaining potent anticholinesterase activity. Furthermore, we decided to replace the *p*-chloromethyl moiety of **1** with an ethynyl group (vide infra) because the chloromethyl proved to be a source of instability in other studies.[28,29]

Presented herein is the synthesis of a series of ethynylphenyl carbonates and carbamates linked to lipophilic ACh mimics. Not only do these molecules have the potential to target inflammation, they may also dually target AD through AChE inhibition and inflammation suppression, potentially through the cholinergic anti-inflammatory pathway.



4, X = C, Y = O; ortho- **8**, X = C, Y = NH; ortho-
5, X = C, Y = O; para- **9**, X = C, Y = NH; para-
6, X = Si, Y = O; ortho- **10**, X = Si, Y = NH; ortho-
7, X = Si, Y = O; para- **11**, X = Si, Y = NH; para-

Figure 2. Ethynylphenyl carbonates (**4**–**7**) and carbamates (**8**–**11**).

A series of *ortho*- and *para*-substituted ethynylphenyl carbonates (**4**–**7**) and carbamates (**8**–**11**) was synthesized from commercially available phenol and aniline precursors (see Supporting information). The carbonates were isolated via a three-step process, with yields ranging from 9% to 57%. The carbamates were synthesized in a single step, resulting in 37% to 65% yields. The identities of these novel molecules were confirmed by nuclear magnetic resonance (NMR) spectroscopy and elemental analysis.

When screened in an Ellman assay,[37] all compounds inhibited AChE in the micromolar range, with IC_{50}s ranging from 28 to 86 μM (Table 1). Out of the series, carbamate **8** was the most potent inhibitor and represents a promising lead for further study. The carbamates proved to be approximately two times more potent AChEIs than the carbonates. The mode of AChE binding of many known carbamate AChEIs (e.g., rivastigmine, physostigmine) involves carbamoylation of the active site serine residue followed by restoration of the active enzyme.[38] A critical hydrogen bonding interaction between the nitrogen atom of the carbamate and the histidine residue of the catalytic triad could therefore be responsible for the enhanced potency of the carbamates relative to the carbonates.[38] When all other structural elements are conserved, changing the choline mimic from a 3,3-dimethylbutyl to a 2-(trimethylsilyl)ethyl moiety does not have a significant impact on AChE inhibition. It is interesting to note that the *para*-substituted ethynyls were slightly more potent than the *ortho*-ethynyls in the carbonate series, perhaps due to steric constraints in the active site gorge of the enzyme.[39]

Following incubation of AChE with each inhibitor and subsequent gel filtration, 80–100% of enzyme activity was recovered (Table 1). Interestingly, a higher percentage of enzyme activity was recovered following incubation of AChE with the carbamates (85–100%) compared to the carbonates (60–83%), with the exception of carbamate **11**. This difference may be because the more hydrophobic carbonates lead to formation of an acylated enzyme complex which is slower to reactivate than the corresponding carbamylated enzyme complex.[40] Slow reactivation of other lipophilic acyl complexes of various esterases has been reported previously.[40–42]

Table 1
IC_{50} values, percent recoveries of AChE activities, percent reductions of TPA-induced edema, *c*Log*P*, and PSA values for ethynylphenyl carbonates and carbamates

#	IC_{50}[a] (μM)	% AChE recovery	% red. (TPA)[b]	*c*Log*P*[e]	PSA[h] (Å^2)
Ethynylphenyl carbonates					
4	86 ± 1	83 ± 8	18	4.41	24.1
5	59 ± 2	83 ± 5	22	4.41	25.1
6	80 ± 3	78 ± 4	nd	—[f]	24.2
7	62 ± 2	60 ± 11	nd	—[f]	25.2
Ethynylphenyl carbamates					
8	28.4 ± 9	100 ± 9	39[c]	3.73	27.5
9	34.3 ± 1	95 ± 3	nd	3.73	29.4
10	38.1 ± 1	96 ± 5	Irritant	—[f]	27.6
11	34.4 ± 1	85 ± 5	40[d]	—[f]	29.5
Tacrine	0.058± 0.005	95	nd	2.91[g]	28.4[g]

[a] IC_{50} values represent mean ± S.D. Individual experiment is performed at least in duplicate; source of AChE is electric eel (*Electrophorus electricus*); enzyme was incubated for 30 min with 52 μM inhibitor at 25 °C.
[b] nd: not determined.
[c] Value differs from positive control (TPA only) based on one-way ANOVA, with $P < 0.05$.
[d] Value differs from positive control (TPA only) based on one-way ANOVA, with $P < 0.10$.
[e] *c*Log*P* values calculated using ChemBioDraw Ultra 14.0.
[f] *c*Log*P* values could not be calculated.
[g] Calculations performed on free base.
[h] PSA values calculated using Spartan'14 V1.1.4.