Bioorganic & Medicinal Chemistry Letters 19 (2009) 5528–5530

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis of organophosphates with fluorine-containing leaving groups as serine esterase inhibitors with potential for Alzheimer disease therapeutics

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ARTICLE INFO

Article history:
Received 4 July 2009
Revised 14 August 2009
Accepted 18 August 2009
Available online 21 August 2009

Keywords:
Acetylcholinesterase
Alzheimer disease
Butyrylcholinesterase
Carboxylesterase
Esterase inhibitors
Fluorine chemistry
Organophosphorus esters

ABSTRACT

Acetylcholinesterase and butyrylcholinesterase inhibitors are potential cognition enhancers in Alzheimer disease. *O,O*-Dialkylphosphate inhibitors with 1-substituted 2,2,2-trifluoroethoxy leaving groups were synthesized by phosphonate–phosphate rearrangement. Substituents in the 1-position of the leaving group along with the *O*-alkyl groups modulated potency and selectivity against acetylcholinesterase, butyrylcholinesterase, and carboxylesterase.

Dialkyl phosphates possessing electron-withdrawing (acidic) leaving groups are anticholinesterases, a class of drugs that can be used in the treatment of Alzheimer disease (AD), a progressive neurodegenerative disorder characterized by global age-dependent deficits in brain cholinergic pathways.[1,2] According to recent data, it may not be an advantage for a cholinesterase inhibitor to be selective for acetylcholinesterase (AChE); on the contrary, striking a certain balance between AChE and butyrylcholinesterase (BChE) inhibition may result in higher efficacy in AD treatment.[3–5] Anticholinesterase compounds can also inhibit other serine esterases, for example, carboxylesterases (CaEs). CaEs play major roles in the activation, detoxification, and biodistribution of numerous drugs and xenobiotics.[6] These enzymes metabolize numerous clinically useful drugs, for example, CPT-11, capecitabine, and meperidine.[7,8] Therefore, the application of CaE inhibitors to increase bioavailability and/or extend the half-life of drugs may represent a viable therapeutic option.[8]

Fluorine has a unique combination of atomic physical properties compared to other halogens; in particular, it has the highest electronegativity and smallest size among these elements.[9] Although the average van der Waals radius of fluorine (1.47 Å) is greater than that of hydrogen (1.20 Å),[10] substitution of fluorine for hydrogen is regarded as a bioisosteric way to introduce strong electron-withdrawing properties into molecules.[11] In so doing, desirable pharmacodynamic attributes, such as inhibition of target enzymes, can be produced.[12,13] In addition, fluorination can enhance pharmacokinetic characteristics such as absorption and biological half-life by increasing lipophilicity and resistance to biotransformation.[14] For example, -CF_3 not only has high electronegativity; it also has high metabolic stability and hydrophobicity (Hansch–Leo substituent parameter $\pi = 0.88$ for –CF_3 compared to $\pi = 0.56$ for –CH_3).[14,15]

Some fluorine-containing esters of phosphonic acid and phosphine oxides, which do not have leaving groups typical of anticholinesterases, have been shown to inhibit AChE and BChE,[16–19] CaE,[18,19] neuropathy target esterase[18,19], and thrombin,[20] in contrast to their non-fluorinated analogues. Furthermore, our previous studies showed that introduction of CF_3-containing substituents into one of the *O*-alkyl groups of trialkylphosphates transforms the formerly unreactive alkoxy group into an electron-withdrawing fluoroalkoxy leaving group. Thus, *O,O*-dialkyl-*O*-(1-methoxycarbonyl-2,2,2-trifluoroethyl) phosphates[21] and *O,O*-dialkyl-*O*-(1-trifluoromethyl-2,2,2-trifluoroethyl) phosphates[22] were shown to inhibit irreversibly three serine hydrolases: AChE, BChE, and CaE. Therefore, it was reasonable to extend the variety of structures of fluorine-containing leaving groups in such compounds.

It is well known that α-hydroxyphosphonates with electron-withdrawing groups at the α-carbon atom can rearrange into phosphates in the presence of bases; moreover, the starting

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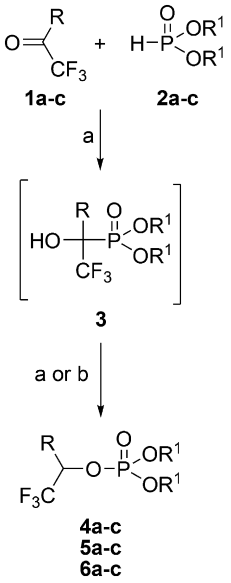
doi:10.1016/j.bmcl.2009.08.065



1a: R = Ph
1b: R = CF_3
1c: R = C(O)OEt
2a: R^1 = Me
2b: R^1 = Et
2c: R^1 = Bu
4a: R = Ph, R^1 = Me
4b: R = Ph, R^1 = Et
4c: R = Ph, R^1 = Bu
5a: R = CF_3, R^1 = Me
5b: R = CF_3, R^1 = Et
5c: R = CF_3, R^1 = Bu
6a: R = C(O)OEt, R^1 = Me
6b: R = C(O)OEt, R^1 = Et
6c: R = C(O)OEt, R^1 = Bu

Scheme 1. Reagents and conditions: (a) cat. Et_3N (for **1b**—sealed ampoule); (b) for **1a**, 120 °C, DMF.

α-hydroxyphosphonates can be obtained by reacting dialkylphosphites with the appropriate carbonyl compounds[23–26] (Scheme 1).

The aims of the present work were (1) to exploit the synthetic potential of the phosphonate–phosphate rearrangement for obtaining organophosphates with various fluorine-containing leaving groups as possible inhibitors of serine esterases; and (2) to determine the inhibitory activities of these organophosphates against three serine esterases: AChE, BChE, and CaE.

The synthetic route used to obtain the titled compounds is outlined in Scheme 1. Dialkylphosphites **2** (shown as the phosphonate tautomer, likely to be the predominant form)[27] were added to trifluoromethyl ketones **1** to form phosphonates **3**. This reaction may proceed via formation of phosphinate anions, at least in the presence of a base catalyst.[27–29] The subsequent phosphonate–phosphate rearrangement was effected by including catalytic amounts of base (triethylamine). The first stage, the formation of phosphonate **3**, was exothermic for **1c** even without a catalyst,[30] while for **1a** addition of a base was required. During one week in the absence of the catalyst, no reaction proceeded in the mixture of **1a** and **2a** according to ^{19}F and ^{31}P NMR spectra. After the addition of Et_3N, the formation of **3a** was exothermic. It should be noted that there were differences in the conditions of rearrangement of the intermediates **3**. To obtain phosphates **5** and **6**, it was sufficient to raise the temperature of the reaction mixture during a half-hour to 80 °C, whereas the rearrangement of **3** into **4a–c** required conditions that were more energetic: heating the reaction mixture at 120 °C for 1 h. The chemical structures of synthesized compounds were confirmed by NMR (see Supplementary data).

Our further attempts to extend the structures of leaving groups, namely, to obtain phosphates with R = Me were unsuccessful. Phosphonates **3** in this case were isolated, but the phosphonate–phosphate rearrangement of **3** into **4** did not take place even after refluxing the reaction mixture in DMF in the presence of either Et_3N or CsF during 4 h.

The inhibitory activity of dialkylphosphates **4–6** was evaluated using human erythrocyte AChE, horse serum BChE and pig liver CaE ('Sigma', USA).[31]

Kinetic studies showed that phosphates **5–6** [R = CF_3 and C(O)OEt] were irreversible progressive inhibitors for all the studied esterases. In addition, phosphates **4** (R = Ph) irreversibly inhibited AChE and BChE; however, their inhibition of CaE was reversible and competitive. Accordingly, Table 1 presents the bimolecular kinetic constants of inhibition (k_i, $M^{-1}\,min^{-1}$) and corresponding selectivity for BChE and CaE relative to AChE for compounds that exhibited irreversible progressive inhibition of these esterases. However, in the case of phosphates **4** and CaE, only the constants for the observed reversible inhibition are listed (K_i, M). Inhibitors of CaEs include organophosphates that acylate the active site serine,[32] trifluoromethyl ketones that react with the active site serine to form transition state analogs,[33] aromatic ethane-1,2-diones such as benzil that form a reversible covalent bond with the active site serine,[8] and derivatives of the AD drug tacrine that act as truly reversible inhibitors,[34] presumably through noncovalent interactions with hydrophobic residues in the active site gorge of the enzyme. The fact that CaE undergoes irreversible inhibition by phosphates **5** and **6** and reversible inhibition by phosphates **4** remains unexplained and will require further research to elucidate the mechanism.

Compounds **4–6** were weak (**4a**, **4b**, **5a**, **5b**, **6a**, **6b**) or moderate (**4c**, **5c**, **6c**) irreversible inhibitors of AChE. Anti-AChE, anti-BChE, and anti-CaE activities (the last one for compounds **5–6**) increased in each series with rising hydrophobicity of R^1: Me < Et < Bu. In each series, the more hydrophobic and bulky Bu derivatives (**4c**, **5c**, **6c**) had the highest selectivity for BChE relative to AChE, consistent with the larger active site volume of BChE.[35] Phosphates **4** containing R = Ph in the leaving group were competitive reversible inhibitors of CaE; compound **4c** (R^1 = Bu) exhibited maximal affinity (K_i = 156 nM).

Phosphates **6** with R = $C(O)OC_2H_5$ were more pronounced selective inhibitors of BChE versus AChE. The most selective BChE inhibitor was compound **6c** (B/A = 35). In addition, this compound had similar selectivity for CaE (C/A = 58).

Table 1
Inhibitor activity of phosphates (**4–6**) against AChE, BChE and CaE and their selectivity to BChE versus AChE (B/A)[b] and to CaE versus AChE (C/A)[c]

Compounds	k_i(AChE)[a] ($M^{-1}\,min^{-1}$)	k_i(BChE)[a] ($M^{-1}\,min^{-1}$)	k_i(CaE)[a] ($M^{-1}\,min^{-1}$)	K_i(CaE)[a] (M)	B/A	C/A
4a	$(2.13 \pm 0.19) \times 10^2$	$(3.48 \pm 0.26) \times 10^2$		$(6.64 \pm 0.52) \times 10^{-6}$	1.6	
4b	$(6.04 \pm 0.21) \times 10^2$	$(4.20 \pm 0.41) \times 10^3$		$(3.33 \pm 0.29) \times 10^{-6}$	7	
4c	$(1.91 \pm 0.11) \times 10^4$	$(2.29 \pm 0.10) \times 10^5$		$(1.56 \pm 0.16) \times 10^{-7}$	11	
5a[d]	$(2.17 \pm 0.18) \times 10^2$	$(3.30 \pm 0.28) \times 10^2$	$(9.60 \pm 0.92) \times 10^3$		1.5	46
5b[d]	$(8.65 \pm 0.06) \times 10^2$	$(3.16 \pm 0.31) \times 10^3$	$(1.20 \pm 0.12) \times 10^5$		3.7	139
5c[d]	$(7.41 \pm 0.92) \times 10^4$	$(2.38 \pm 0.01) \times 10^6$	$(1.18 \pm 0.11) \times 10^7$		27	159
6a	$(4.19 \pm 0.31) \times 10^2$	$(3.56 \pm 0.17) \times 10^3$	$(2.58 \pm 0.21) \times 10^2$		8.5	0.6
6b	$(1.19 \pm 0.06) \times 10^3$	$(1.59 \pm 0.06) \times 10^4$	$(1.49 \pm 0.09) \times 10^3$		13	1.3
6c	$(6.56 \pm 0.12) \times 10^3$	$(2.32 \pm 0.08) \times 10^5$	$(3.79 \pm 0.09) \times 10^5$		35	58

[a] Values are means of three experiments, (mean ± SEM).
[b] B/A = k_i(BChE)/k_i(AChE).
[c] C/A = k_i(CaE)/k_i(AChE).
[d] From 22.

Phosphates **5** with R = CF_3 were more selective inhibitors of CaE in comparison to AChE and BChE. Inhibitor activities against CaE versus AChE (C/A values) for **5b** and **5c** differed more than 100-fold; compound **5b** also had low selectivity for BChE (B/A = 3.7, C/B = 38). Taking into account the low anti-AChE activity of **5b** (and, consequently, its expected low acute toxicity), this compound could be considered as a selective CaE inhibitor that might be used for modulating the activity of drugs hydrolyzed by CaE.

In summary, *O*,*O*-dialkyl-phosphates with electron-withdrawing 1-substituted 2,2,2-trifluoroethoxy groups were synthesized by phosphonate–phosphate rearrangement and some limitations for this reaction were determined. The *O*-alkyl groups, along with the substituent in 1-position in the leaving group, determined potency and selectivity of the phosphates as inhibitors of AChE, BChE, and CaE. Some selective inhibitors of BChE and especially CaE were found; compounds with 1-phenyl substituents were reversible rather than progressive inhibitors of CaE.

Acknowledgments

The research was supported by Project #3130 of the International Science and Technology Center (ISTC) and the Russian Academy of Science Program 'Biomolecular and Medicinal Chemistry'.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.08.065.

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30. According to the ^{31}P NMR spectra, the ratios of phosphonates **3** to phosphates **5** and **6** in the reaction media after mixing the carbonyls **1b**,**c** and phosphite **2a** without the presence of base were 1:4 (**3b**:**5a**) and 1:3 (**3c**:**6a**), while in the presence of base the formation of phosphates **5a** and **6a** was nearly quantitative.
31. AChE and BChE activities were measured spectrophotometrically using acetylthiocholine and butyrylthiocholine as substrates (25 °C, 0.1 M phosphate buffer, pH 7.5, λ 412 nm). CaE activity was determined spectrophotometrically (λ 405 nm), using the substrate, *p*-nitrophenyl acetate (25 °C, 0.1 M phosphate buffer, pH 8.0). For kinetic studies of irreversible enzyme inhibition, a sample of enzyme was incubated with a corresponding phosphate (4–5 inhibitor concentrations, acetone concentration 1% v/v) for different times. The residual enzyme activity was then assayed in duplicate for each experiment. The slopes (k') of each plot of log (% activity remaining) versus time were calculated by linear regression. These values of k' were then plotted against inhibitor concentration [I], and the slope (k'') of the resultant line was derived by linear regression. The bimolecular rate constant of inhibition (k_i) was calculated as a measure of inhibitory potency using the relationship, $k_i = 2.303\ k'/[I] = 2.303\ k''$.[36] Each value of k' was obtained from a line through 4 to 6 points. Plotting and regression analysis was done using Origin 6.1 software. Mechanism of CaE reversible inhibition was determined in double reciprocal coordinates using the Lineweaver–Burk approach. Inhibition of enzyme activity was measured over a substrate concentration range of 0.2–1 mM and at 4–5 inhibitor concentrations. Reversible inhibitor constants (K_i, M) were determined from dependence of the slopes in double reciprocal coordinates obtained at different inhibitor concentrations on the concentration of inhibitor. Plotting and regression analysis was done using Origin 6.1 software.
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