Bioorganic Chemistry 72 (2017) 301–307

Contents lists available at ScienceDirect

Bioorganic Chemistry

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

Efficient synthesis of novel dialkyl-3-cyanopropylphosphate derivatives and evaluation of their anticholinesterase activity

Iyadh Aouani [a], Badreddine Sellami [b], Karima Lahbib [a], Jean-François Cavalier [c], Soufiane Touil [a,*]

[a] Laboratory of Heteroatom Organic Chemistry, University of Carthage, Faculty of Sciences of Bizerte, 7021 Jarzouna, Tunisia
[b] Laboratory of Environment Biomonitoring, Coastal Ecology Unit, University of Carthage, Faculty of Sciences of Bizerte, 7021 Jarzouna, Tunisia
[c] Aix-Marseille University, CNRS, EIPL, Marseille, France

ARTICLE INFO

Article history:
Received 5 February 2017
Revised 30 April 2017
Accepted 4 May 2017
Available online 6 May 2017

Keywords:
Organophosphates
γ-Ketonitriles
Dialkyl-3-cyanopropylphosphates
Phospha-Brook rearrangement
Cholinesterase inhibitors

ABSTRACT

Based on the broad spectrum of biological activities associated with organophosphates, a novel type of this class of compounds was synthesized, bearing a nitrile group, from the sodium alkoxide-catalyzed reaction of dialkylphosphites with γ-ketonitriles at 80 °C under solvent-free conditions. A reaction mechanism involving a phospha-Brook type rearrangement is proposed. Eight title compounds were investigated for their *in vitro* inhibitory potency and selectivity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) using Ellman's spectrophotometric method. The synthesized derivatives exhibited mostly a moderate activity against both cholinesterases. The IC_{50} values for BChE were in a smaller concentration range (5.96–23.35 μM) compared to those for AChE inhibition (9.61–53.74 μM). The diethyl-3-cyano-1-*p*-tolylpropylphosphate which displayed the higher dual inhibitory potency towards both cholinesterases could be considered as a potential candidate for developing new drugs to treat Alzheimer's disease.

1. Introduction

Alzheimer's disease, the most common form of neurodegenerative senile dementia, is associated with the selective loss of cholinergic neurons and a reduced level of the acetylcholine (ACh) neurotransmitter. The cholinergic hypothesis postulates that Alzheimer's disease (AD) is caused by a decrease in ACh levels in the brain, leading to gradual neurodegeneration [1]. In normal brain signaling, ACh is related to the preservation and access to memory as well as function [1]. Acetylcholinesterase (AChE), a drug target for Alzheimer's disease, is an enzyme that belongs to the very large serine hydrolase class of enzymes [2]. AChE catalyzes the hydrolysis of ACh to choline and acetic acid which allows the control of the ACh level and to regulate its action as a cholinergic neurotransmitter interacting with postsynaptic cell receptors. This enzyme is widely distributed throughout the body and is the main cholinesterase in the human brain [3,4]. Additionally, Butyrylcholinesterase (BChE) plays a secondary role in the regulation of the ACh level and compensates for the lack of AChE, allowing the cholinergic neurotransmission to continue [5–7].

Inhibitors of ChE have been used in the treatment of various diseases such as Alzheimer's, myasthenia gravis and some other dementias [8], parasitic infections [9], glaucoma and obstipation [10].

Organophosphates are well-known anticholinesterase agents [11,12]. They cause irreversible inhibition of both AChE and BChE by phosphorylation of the active site catalytic serine hydroxyl group [13], thus inhibiting its physiologic action of hydrolyzing the ACh neurotransmitter at the central and peripheral synapses [8,14]. This results in ACh accumulation and in thus an overstimulation of cholinergic receptors [15]. For instance, salicylanilide diethyl phosphates were found to be excellent inhibitors of both AChE and BChE with IC_{50} values in the micromolar range, and in some cases they exhibited even better inhibitory activity than the current AD medications Rivastigmine and Galantamine [11,16].

In the present study, we report a simple synthesis of the novel dialkyl-3-cyanopropylphosphates starting from dialkyl phosphites and γ-ketonitriles. A preliminary evaluation of the inhibitory activities of AChE and BChE is also reported for eight dialkyl-3-cyanopropylphosphate derivatives. It should be noted that the presence of the nitrile unit in these molecules might regulate important biological functions and could improve the biological activity of such compounds by accentuating the binding to receptor targets, in a similar way to that reported for other pharmaceuticals [17].

* Corresponding author.
E-mail address: soufiane.touil@fsb.rnu.tn (S. Touil).

http://dx.doi.org/10.1016/j.bioorg.2017.05.008
0045-2068/

2. Results and discussion

2.1. Chemistry

We first aimed at optimizing the reaction conditions for the formation of the dialkyl-3-cyanopropylphosphate target compounds, by using γ-ketonitrile **1a** and diethyl phosphite as model substrates. The reaction was studied with various basic catalysts under different conditions. The results of these comparative experiments are summarized in Table 1. Inspired by our previous work regarding the synthesis of γ-cyano-α-hydroxyphosphonates *via* the reaction of γ-ketonitriles with dialkyl phosphites on magnesia support at room temperature and in solvent-free conditions [18], we thought that conducting the same reaction at a higher temperature may lead, after a phospha-Brook type rearrangement [19] promoted by heat, to the corresponding dialkyl-3-cyanopropylphosphate **3a**. Contrary to our expectations, the reaction did not yield the desired product but gave the corresponding α-hydroxyphosphonate **3′a** with less than 30% yields (Table 1, entries 1 and 2). The use of K_2CO_3 and amines as basic catalysts was also tested, but this left the starting materials intact even after prolonged heating (Table 1, entries 3–7). An improvement in the yield of product **3a** was however observed when employing sodium ethoxide as catalyst under solvent-free conditions (Table 1, entry 9). Finally, under the same reaction conditions and by increasing the amount of diethyl phosphite up to 5 molar equivalents, it was gratifying to obtain the desired product **3a** with 85% yield (Table 1, entry 10).

Once the optimized reaction conditions were obtained, the scope of this methodology was studied. A variety of structurally diverse γ-ketonitriles and dialkyl phosphites were investigated and a series of dialkyl-3-cyanopropylphosphates of type **3** were obtained in good yields (Table 2). Compounds **3g** and **3h**, presenting two stereocenters, were obtained as a mixture of two unseparable diastereoisomers in an approximate 3:2 ratio, as evidenced by their NMR spectral data. The relative proportions of these diastereoisomers were estimated from the ^{31}P NMR spectra where a singlet for each one was found (see Section 4).

A mechanistic rationalization of this reaction is depicted in Scheme 1. In this proposed mechanism, the role of sodium alkoxide is to promote the deprotonation of dialkyl H-phosphonate **2** giving rise to the more nucleophilic dialkylphosphite anion which can react with the carbonyl of the γ-ketonitrile **1** to supply an α-hydroxyphosphonate $\mathbf{I_2}$ as intermediate, in equilibrium with the alkoxide form $\mathbf{I_1}$. When heating, the latter undergoes a phospha-Brook type rearrangement [19] that consists in an intramolecular 1,2-migration of the phosphoryl group from carbon to oxygen. This includes the formation of a three-membered cyclic species from intermediate $\mathbf{I_1}$. Subsequent ring opening and irreversible fast protonation of the carbanion by the dialkyl phosphite, lead to the final dialkyl-3-cyanopropylphosphate **3**.

2.2. Anticholinesterase activity

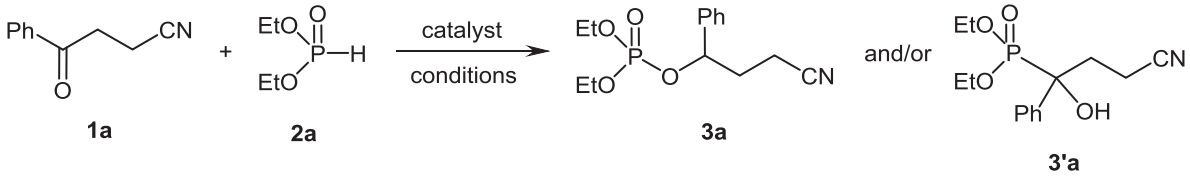
The inhibitory potency of the eight newly synthesized dialkyl-3-cyanopropylphosphates against AChE and BChE were evaluated *in vitro* using modified Ellman's method [20]. The efficiency of these inhibitors is expressed as IC_{50}, representing the concentration of an inhibitor leading to 50% inhibition of the enzyme. The obtained results were compared with Rivastigmine, an acylating pseudo-irreversible carbamate inhibitor that is able to inhibit both AChE and BChE.

With respect to the inhibition of cholinesterases, all tested dialkyl-3-cyanopropylphosphates exhibited a significant inhibitory power towards AChE and BChE, with IC_{50} values ranging from 5.96 to 53.74 μM (Table 3). In addition, and as awaited from previous studies with phosphate inhibitors [11,13b], compounds **3a–h** showed greater inhibition of both enzymes than Rivastigmine. This result is probably related to the chemical structure of the **3a–h** molecules which contains a good leaving group (—O—CH(R^1)—CH(R^2)—CH_2—CN) enabling them to perform the phosphorylation of the nucleophilic serine hydroxyl group in the active site of the ChE enzymes. In addition, the cyano group present in these molecules could contribute to this activity by its electron-withdrawing inductive effect which increases the leaving group ability of the 3-cyanopropyloxy moiety.

Moreover, in agreement with previous studies showing that organophosphorus molecules behave more selectively towards BChE [11,21,22], we also demonstrated that our dialkyl-3-cyanopropylphosphates **3a–h** inhibited BChE more effectively than AChE, by 1.43–2.59-fold for **3h** and **3e**, respectively. Only compound **3d** exhibited comparable IC_{50}s for both enzymes.

Among the eight tested compounds, the most potent inhibitor of AChE was found to be the diethyl-3-cyano-1-*p*-tolylpropylphosphate (**3c**) with an IC_{50} value of 9.61 μM, which is also the best inhibitor of BChE (IC_{50} = 5.96 μM). In contrast, the die

Table 1
Optimization of the reaction conditions.



Entry	Catalyst	Ratio (**1**/**2**/catalyst)	Solvent	Temp. (°C)	Time (h)	Yield[a]
1	MgO	1/2/2	–	80	48	26% **3′a**
2	MgO	1/2/2	–	140	48	15% **3′a**
3	K_2CO_3	1/2/1	–	80	48	ND[b]
4	Et_2NH	1/2/0.2	Toluene	111	48	ND
5	Et_2NH	1/2/0.2	–	80	48	ND
6	Et_3N	1/2/0.2	–	80	48	ND
7	Piperazine	1/2/0.2	–	80	48	ND
8	EtONa	1/2/0.2	Ethanol	78	48	ND
9	EtONa	1/2/0.2	–	80	48	32% **3a**
10	EtONa	1/5/0.2	–	80	24	85% **3a**

[a] Isolated yield.
[b] No products detected.