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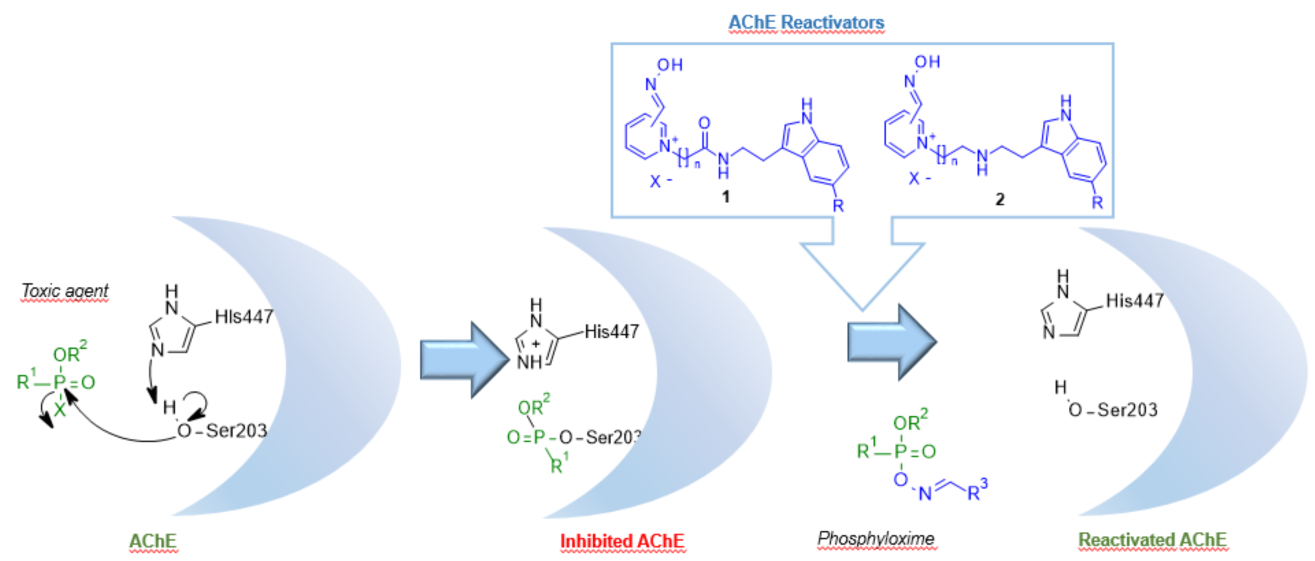
HÍBRIDOS PRALIDOXIMA - MELATONINA COMO ANTÍDOTOS PARA LA INTOXICACIÓN QUÍMICA

PRALIDOXIME – MELATONIN HYBRIDS AS ANTIDOTES FOR CHEMICAL POISONING



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LIST OF ABREVIATIONS

ACh: Acetylcholine

ACh: Acetylcholinesterase

BBB: Blood Brain Barrier

BChE: Butyrylcholinesterase

CNS Central Nervous System

CW: Chemical Wars

CWAs: Chemical War Agents

CWC: Chemical Weapons Convention

d: Doublet

DCM: Dicloromethane

$CDCl_3$: Deuterated chloroform

dd double of doublet

CH_2Cl_2: Diclorometane

DMAP: 4-(Dimethylamino)pyridine

DMSO: Dimethylsulfoxide

EtOAc: Ethyl Acetate

EtOH: Ethanol

F.P. Final Product

^{1}H NMR: Proton Nuclear Magnetic Resonance

H_3CCN: Acetonitrile

HMBC: Heteronuclear Multiple Bond Correlation

HSQC: Heteronuclear Single Quantum Coherence

HPLC-MS Mass-High Performance Liquid Chromatography

ICH_3: Iodomethane

***J*:** Coupling constant

M: Multiplet

MeOH: Methanol

MOH: Melatonine-oxime hybrids

NA: Nerve Agents

NATO: North Atlantic Treaty Organization

NaCl Sodium chloride

Na_2CO_3: Sodium carbonate

NaOH: Sodium hydroxide

$NaHCO_3$ Sodium hydrogen carbonate

Na_2SO_4 Sodium sulfate

$NH_2OH.HCl$ hydroxilamine

OP: Organophosphorus

OPNA: Organophosphorus nerve agents

PCA: Paridincarboxaldehyde

PAS: Peripherical Anionic Site

Rf: Retention Factor

NMR: Nuclear Magnetic Resonance

s: singlet

S.M.: Starting Material

t: triplet

***t*-BuOK:** Potassium tert-butoxide

td: Triplet of doublet

THF: Tetrahydrofuran

TLC: Thin Layer chromatography

$TsOH.H_2O$: monohydrated para-toluensulphonic acid

WHO: World Health Organization

WWI: World War I

WWII: World War II

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ABSTRACT

Despite the prohibition on the use, synthesis and storing of Organophosphorus Nerve Agents (OPNA) as Chemical weapons (CW) by the Chemical Weapons Convention (CWC), there are still a lot of organophosphorus compounds being intentionally used for terrorist purposes. OPNA imply a simple synthesis, are highly lethal and quickly inhibit the AChE causing a major cholinergic syndrome. For these reasons, a treatment against these agents is needed. The current way of mitigating their effects is the administration of an anticonvulsant (Benzodiazepine), a muscarinic acethylcholine receptor antagonist (Atropine), and an oxime to reactivate the inhibited AChE. In this research work, the synthesis, isolation and characterization of different-lenght Pyridinaldoxime-Melatonin/Tryptamine hybrids (**1-2**) have been carried out as a new approach to reactivate the Acetylcholinesterase enzyme.

Key words: AChE reactivators, OPNA, Oximes, Pralidoxime, Melatonin, Tryptamine.

RESUMEN

A pesar de que la Convención sobre Armas Químicas (CAQ) prohibió el uso, la síntesis y el almacenamiento de agentes nerviosos organofosforados (OPNA) como armas químicas (AQ), se siguen usando, intencionadamente, muchos compuestos organofosforados con fines terroristas. Los OPNA implican una síntesis sencilla, son altamente letales e inhiben rápidamente la AChE causando el síndrome colinérgico. Por estas razones, es necesario un tratamiento contra estos agentes. La forma actual de mitigar sus efectos es la administración conjunta de un anticonvulsivante (Benzodiacepina), un antagonista de los receptores muscarínicos de la acetilcolina (Atropina) y una oxima para reactivar la AChE inhibida. En este trabajo de investigación, se ha llevado a cabo la síntesis, aislamiento y caracterización de híbridos de Piridinaldoxima-Melatonina/Triptamina de diferente longitud como un nuevo enfoque para reactivar la enzima Acetilcolinesterasa.

Palabras clave: AChE reactivadores, OPNA, Oximas, Pralidoxima, Melatonina/Triptamina.

1. INTRODUCTION

1.1. CHEMICAL WARS

Some chemical compounds have been used in the last 100 years as chemical weapons (CW) in wars and terrorist attacks. The North Atlantic Treaty Organization (NATO) defines the CW as substances developed to be used in military operations to incapacitate, seriously injure or kill people due to their physiological effects (NATO, 2018). Although the use of these chemical weapon agents (CWAs) has been more popular in recent years, the first use of CW in wars probably goes back to the Peloponnesian war in 431 b.C where a fortification was taken over thanks to the liberation of sulfur smoke.[1] During World War I (WWI), some CW as mustard gas were used and in 1929 Spain used this gas in the Morrocan area. Organophosphorus agents (OP) have been developed as CWAs or pesticides. Intoxication due to these OP is a major global public health problem for both self-harm attempts in rural Asia and for armed conflicts and terrorist attacks. It was in WWII when the first OP agents were used by Germany and Sarin, Tabun, VX and mustard gas were employed in Halabia, Iraq, 1988. Despite the Chemical Weapons Convention (CWC) guaranteeing the prohibition of the developement, production, storage and use of CW in 1993, the Sarin misuse by the sect Aum Shinrikyo in the subway of Tokyo and in Matsumoto, Japan, 1994 and 1995, caused over 6 000 injuries and terrorist attacks continued with the use of Sarin in Damascus, Syria, 2013 and 2017 and with the assasination of Kim Jong-nam by VX agent in Malaysia, 2017. The last incident ocurred in 2018 in the U.K. with the intoxication of a retired Russian army officer and his daughter with the Novichok agent A-234. [2–4] According to the World Health Organization (WHO), there are 3 000 000 OP intoxications and more than 200 000 deaths per year.[5] These historical events have led to greater medical attention and the development of countermeasures against these chemical compounds.

1.2. INTOXICATION BY ORGANOPHOSPHORUS NERVE AGENTS

One type of CWs are the Nerve Agents, which are the most feared mass destruction weapons in the world. They are organophosphorus esters that present high

toxicity. Some of them are fosfonic acid derivatives and others are fosforic acid derivatives. The first generation of Organophosphorus Nerve Agents (OPNAs) (G series) compounds was developed by Germany during WWII, being O-ethyl *N,N*-dimethylphosphoramidocyanidate (GA; Tabun) in 1936 the first NA synthesized, followed by O-isopropyl methylphosphonofluoridate (GB; Sarin) in 1938 and O-pinacolyl methylphosphonofluoridate (GD; Soman) in 1944 (Figure 1).

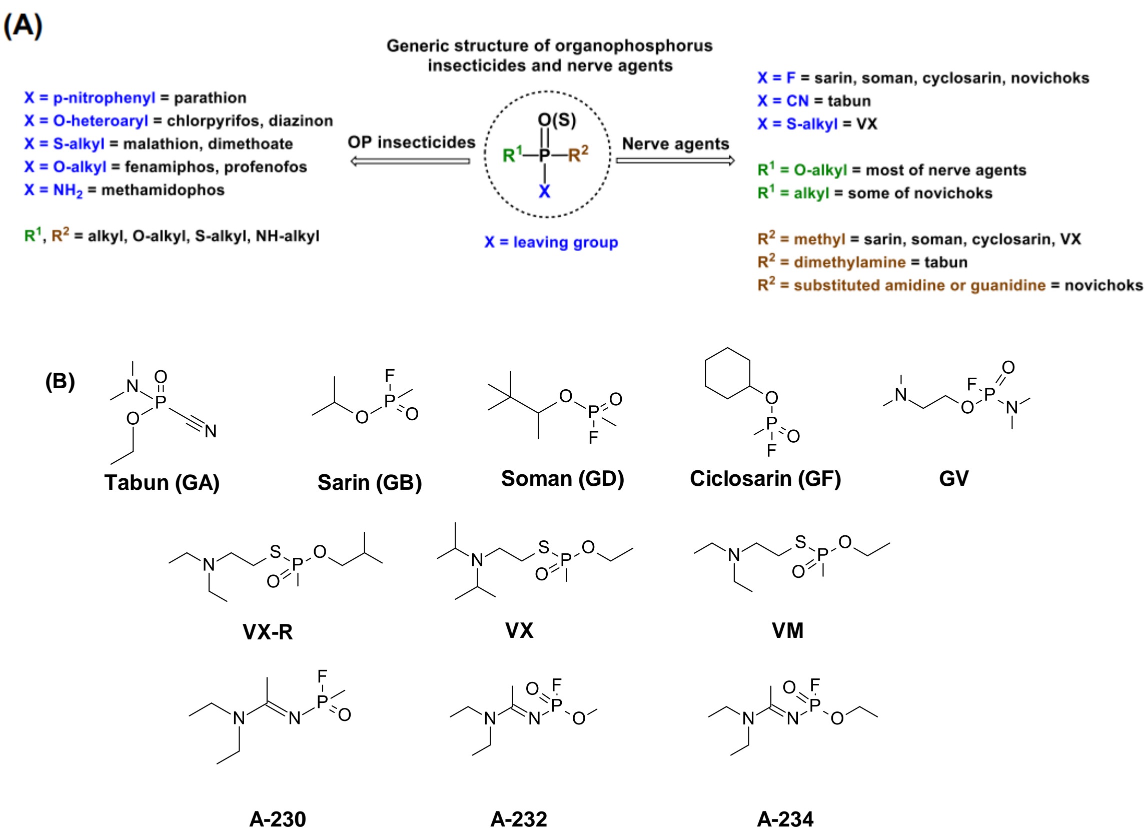


Figure 1. A) General structure of OP compounds representing most of the NA and OP insecticides[6]; B) Organophosphorus nerve agents.

After WWII, The V series, which stands for victory, venomous or viscous, was developed by the allied forces including O-ethyl methylphosphonothiolate and S-2-diisopropylaminoethyl methylphosphonothiolate (VX) and O-ethyl methylphosphonothiolate and S-2-diethylaminoethyl methylphosphonothiolate (VM). These were supposed to be designed in secret, but the Soviets discovered the V agents which led to the synthesis of O-Isobutyl methylphosphonothiolate and S-2-diethylaminoethyl methylphosphonothiolate (Russian VX) by the Ancient Soviet Union

in 1957. Other OPNAs that were developed are GF, GV and A-agents or Novichok's agents (A-230, A-232, A-234) GB, GD and GF have the same core as diisopropylfluorophosphate (DFP) which is a commercial pesticide. They have a phosphonate methyl group and a fluoride leaving group. In contrast, GA has a cyanide as the leaving group. In the V-agents, the leaving group is a thyolate, instead.[4,7] The different substituents attached to the central phosphorus atom deliver divergent physicochemical properties and biological effects.

An in-depth understanding of the physico-chemical properties of OP is essential for comprehending how they act, distribute and are exposed as well as their biological stability to know the efficiency of their elimination process. G-agents are known to be liquid at room temperature and highly volatile compounds. For this reason, they have low persistence in their objective and low biological stability. Inhalation of these agents results in a rapid intoxication. V-agents are also liquid at room temperature but they are low volatile compounds, therefore, they present high persistence. Poisoning is expected after percutaneous absorption, mainly through the skin or the mucous membranes. As for Novichoks agents, there is no available information, but the incidents in the United Kingdom in 2018 suggest they present low steam pressure and high persistance. Persistence not only depends on the agent's intrinsic characteristics, but also on the metereological conditions like an increase in temperature and wind cause a decrease in persistence. In general, Nerve Agents are moderately soluble in water where they are quickly hydrolyzed. Since they present lipophilicity they are also soluble in organic solvents and get inactivated in basic surroundings and in the presence of chlorinated compounds, affording less toxic oxidated and hydrolyzed products, except a VX hydrolyzed product which shows higher toxicity in mammals.[8] As for their organoleptic properties, fruity odors are described and if the agents are pure, they are colorless liquids, but if they contain any impurities, they present yellow and brown colors. All NA in their vapor state are less dense than air, so the vapor accumulates at low levels of the affected areas.[4,9]

Knowledge of how the OPNAs cause the intoxication is crucial for the development of antidotes capable of reverting the action. Organophosphorus nerve

agents inhibit the AChE causing a cholinergic syndrome. The AChE is the enzyme in charge of ending the neurotransmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter, acetylcholine, into acetate and choline to regulate its concentration. The ACh plays a key role when transmitting the electric nerve signals from the presynaptic to the postsynaptic membrane since there is a space between them which signals cannot cross. AChE acts in the Central Nervous System (CNS), in the peripheral lymph nodes and in the neuromuscular plaque. Its function is crucial for the CNS activity and its interruption would be lethal. Acetylcholinesterase role finishes with the AChE activity. The AChE belongs to the cholinesterases enzyme family, which are able to hydrolyze choline esters. AChE is codified by a single gene in chromosome 7 and predominates in muscular tissue and the CNS, where the concentration of BChE is lower. It also participates in development functions and changes in its properties or concentration are present in some neuropathologies such as Alzheimer or Parkinson.[10,11,12] Its active site is located at the bottom of a deep and narrow gorge of 20 Å that connects with the enzyme surface. It is lined by 14 aromatic residues, with W84 and F330 contributing to the catalytic anionic site (CAS), and Y70, Y121 and W279 to the peripheral anionic site (PAS). The active site consists of a catalytic triad of three aminoacids, glutamic acid (E334), histidine (H447) and serine (S203). The reason it is thought that the active site is buried in this way, is for the substrate, acetylcholine (ACh), to be surrounded by the protein almost 360°, allowing multiple substrate/enzyme interactions, which will create a more effective transition state.[4,11]

The hydrolysis of acetylcholine occurs by hydrolyzing the serine residue by the AChE active site. Hence, the inhibition of the AChE causes major problems.[13] OP compounds are the major inhibitors of AChE in the peripheral and central nervous systems which by covalently binding at serine residue (S203) hydroxyl group within the enzyme's active site, block the action of AChE (Figure 2). The inhibition includes a nucleophilic, rapid and time dependent attack by the serine to the electrophilic phosphorus moiety in the OP agents. This nucleophilic activity is enhanced by the formation of a hydrogen bond with the imidazole ring of the histidine which also forms another hydrogen bond with the glutamic acid. A low stable and negatively charged intermediate is formed, which finally allows the phosphorilated AChE formation. Once the AChE-OP conjugate is formed, a

spontaneous dealkylation can occur, with the exit of a carbocation, which affords a stable, irreversible ester adduct from which the AChE activity cannot be restored. This process is known as aging. In contrast, the inhibited AChE can undergo a spontaneous hydrolysis mediated by water, providing the recovered enzyme. Normally, the aged enzyme is yielded instead of the recovered enzyme due to the slow hydrolysis and the rapid formation of the aged AChE. However, the quick administration of an antidote could restore the enzyme activity by forming a phosphyloxime.[7,13,14,15,16]

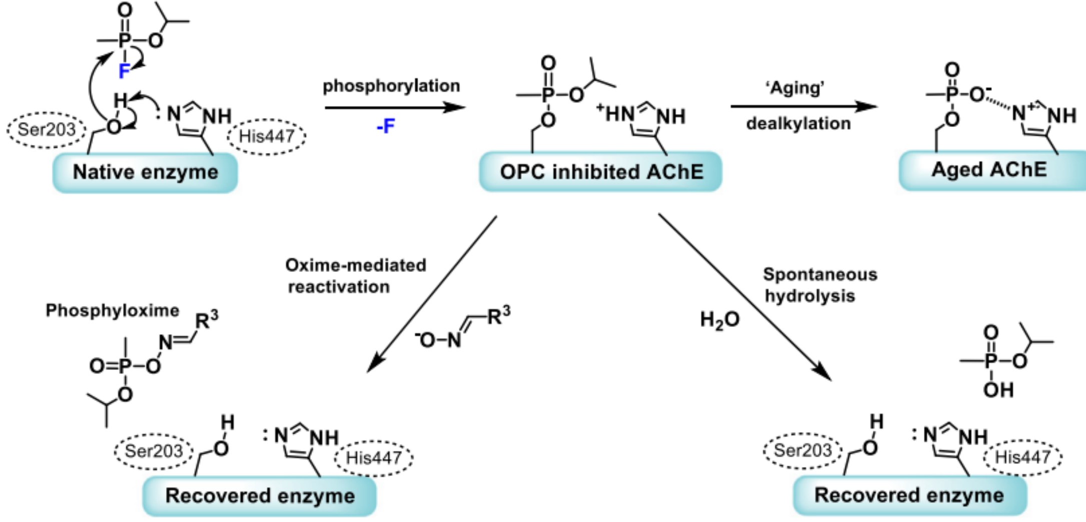


Figure 2. Acetylcholinesterase (AChE) inhibition exemplified by interaction with Sarin.[6]

The inhibition of AChE leads to an incapability of hydrolyzing acetylcholine which causes the accumulation of the neurotrasmitter in the synaptic cleft, producing a cholinergic over-stimulation and launching the so called cholinergic crisis, which manifest itself as breathing failure, paralysis and even death. Intermediate syndrome and OP-induced delayed neuropathy are also associated with OPNA intoxication. The former occurs after the resolution of acute cholinergic crisis (24-96 hours after OP exposure) and includes weakness or the paralysis of motor cranial nerves, respiratory and proximal limb muscles and the latter involves week-delayed paralysis with swelling and degeneration of distal long axons in the legs and spinal cord.[3,6,15]

1.3. TREATMENT WITH ANTIDOTES

In case of intoxication, immediate antidotal measures must be applied to mantain the cardiorespiratory function and to protect from CNS damage. The sooner the antidotes are provided, the better the prognosis will be. The treatment must include a muscarinic antagonist like Atropine[17], an AChE reactivating oxime and a seizure controller, Benzodiazepine.[18] Because some OPNA leads to the aging of the AChE and the reactivating oxime is not useful there, the problem can be mitigated by a pretreatment with a carbamate which irreversibly inhibits part of the peripheric enzyme and protects it from the irreversible fosfonilation. The pyridostigmine bromide is the most commonly used carbamate for pretreatment.[4,7]

Atropine is a muscarinic competitive antagonist. Although it slowly crosses the BBB, it has a high absorption and distribution to the CNS since it easily permeates membranes. As soon as the intoxication by OPNA occurs, Atropine should be injected either intravenously or intramuscularly, starting with a 2 mg dose and duplicating the dose every two to five seconds until the cholinergic syndrome is over.[6] Respiratory failure, circulatory collapse or incurable CNS damage could be a consequence of a delayed atropinization. The seizure controller must be administered within the first 10 minutes after the exposure to the OPNA, intravenously or intramuscularly. It reduces the probability of seizure and diminishes the morbidity and mortality. The initial indicated dose is 10 mg of Diazepam followed by higher dosis depending on the severety of the patient.[4] The oximes act as AChE reactivators which, by a nucleophilic attack to the phosphorus atom, dephosphorylate the active site, forming a a fully reversible Michaelis-type oxime-phosphyl-AChE-conjugate. Then the displacement of the phosphyl residue from the pentacoordinate transition state as a phosphyloxime takes place, returning the AChE to its native form (Figure 2). Different structured oximes have been synthesized and at least 2000 oximes have been published in open literature in the last years.[9] They can be classified in four big groups: charged or uncharged one or two rings compounds having different oxime positions and substituents with or without different-length linkers. Nevertheless, only some of them are in use. Examples of these are pralidoxime (2-PAM), obidoxime, HI-6, TMB-4, MMB-4 and HLö-7 which are positively

charged pyridinaldoximes (Figure 3). 2-PAM was the first one used in humans. It is a mono-pyridinium oxime with the oxime at position 2. Obidoxime, MMB-4 and TMB-4 are bispyridinium oximes with the oxime at position 4. Each oxime has a different efficacy depending on the OP used.[7] Although the positive charge makes them more hydrophilic, making it harder to cross the BBB, it has been found that this charge is important for the reactivation of the catalytic site.[13] To increase their permeability, different carbon chain linkers have been introduced to enhance their lipophilicity. With this, an increase in BBB permeability and % of reactivation was proven. Other AChE reactivators are the charged non-pyridiniumoxime compounds as some imidazole[19] and benzaldehyde derivatives.[16] As for uncharged reactivators, some examples are the pyridinium[2] and thienostilbene oximes[20] as well as some non-oxime molecules.[6,14,21]

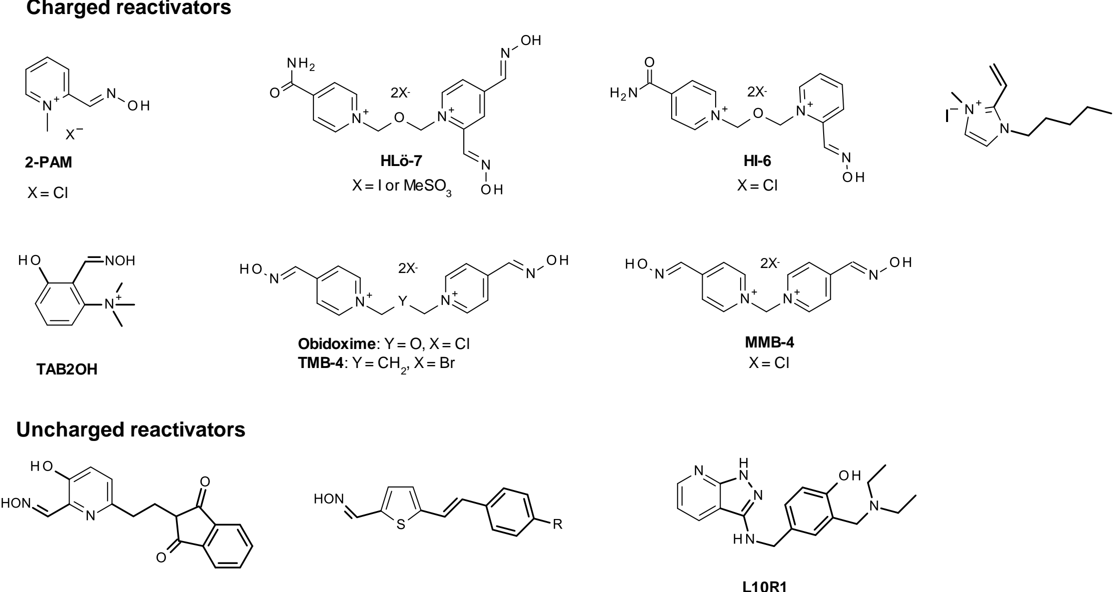


Figure 3. Structures of some oxime, non-oxime, charged or uncharged reactivators.

Another approach is the use of scavengers, enzymes to which the OPNA irreversibly bind preventing the AChE inhibition (stoichiometric scavengers) or which hydrolyze the agents detoxing them before returning to the blood stream (catalytic scavengers). One example of a stoichiometric bioscavenger is the BChE; one BChE molecule interacts with one toxic agent molecule. Endogenous bioscavengers in skin, blood and tissues are present in sufficient quantity to remove a low dose of the OPNA. Phosphotriesterases are an example of catalytic scavengers. These are more efficient

than the stoichiometric ones since each molecule can hydrolyze more than one toxic molecule. Although this works, an administration of a high amount of enzymes could result harmful for some metabolic routs.[4,14]

1.4. MELATONIN HYBRIDS AS A NEW THERAPEUTIC STRATEGY

Melatonin (*N*-acetyl-5-methoxytryptamine) is a hormone secreted by the pineal gland and regulated by the photoperiodic environment. It is an endogenous, immunomodulator molecule involved in the regulation of circadian rythms, sleep - wake cycle and in cellular signalling pathways. Melatonin also presents high efficiency in reducing the oxidative stress, inflammation and excitotoxicity processes and has low toxicity.[22] This molecule pontentiates free radical scavenging by a non-enzymatic process of electron donation. Its indoyl moiety is the one responsible for its antioxidant properties, acting as a free radical (the indoyl or melatonyl cation radical) which neutralizes the free toxic radicals. Its two functional groups are responsible for specificity of receptor binding and for its amphipacity. The last property makes metalonine act independently from its membrane receptors, crossing any cell compartment, including the Blood Brain Barrier (BBB), which allows Melatonin to interact with toxic molecules reducing the oxidative stress. Numerous attempts have been made to mitigate neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's diseases and amyotrophic lateral sclerosis and a new approach with Melatonin involves forming MOH to mitigate the OPNA toxicity at the same time that Melatonin reduces the oxidative damage.[4,23]

1.5. FRAGMENT LINKING

The fragment linking approach is based on the unión, by a linker, of two fragments, which bind at different active sites of a protein, to form a unique compound. These fragments are low molecular weight and highly soluble molecules.[25] Here, their favorable interactions with the active site are maximized, gaining in binding energy and affinity due to the superadditivity concept.[24] It establishes that the biding free energy of the linked compound exceeds the sum of the binding energies of the two fragments.

2. OBJECTIVES

Based on the chemical structure of Pralidoxime or (2-pyridine aldoxime methyl choride), compound which binds to organophosphate inactivated acetylcholinesterase and Melatonin, natural hormone that plays an important role in managing the sleep - wake cycle and circadian rhythm and with multiple biological activities, herein the synthesis, isolation and full characterization of some pyridine-aldoxime and melatonin (**1** and **2**) hybrids are described. As it is shown in Figure 4, in these proposed structures, the pyridine - aldoxime moiety is positively charged due to the connection with the melatonin fragment is through the *N*-1 of the pyridine. It is known that one of the main problems of Pralidoxime is its poor BBB penetration. However, the introduction of an alkyl chain together with a combination with Melatonin or its Tryptamine analogous fragment, these drawbacks can be mitigated or reduced. For growing the final structures, the concept of "fragment linking" used in Fragment Based Drug discovery will be applied.[24,25] For growing both fragments, we must consider that the melatonin moiety must interact with the peripherical anionic site of AChE whereas, the pyridine - aldoxime partner must interact directly with the catalytic site. In structure **1**, the melatonin residue, containing its carboxamide moiety, and Pralidoxime structures will be maintained and the length of the alkyl side chain will be modified. However, in structure **2**, the carboxamide moiety will be removed. Due to the high price of Melatonin, its analogous, Tryptamine, is considered to develop this concep trial.

Finally, the capacity of these compounds in the reactivation of AChE will be evaluated in collaboration with the Pharmacy Department at Complutense University.

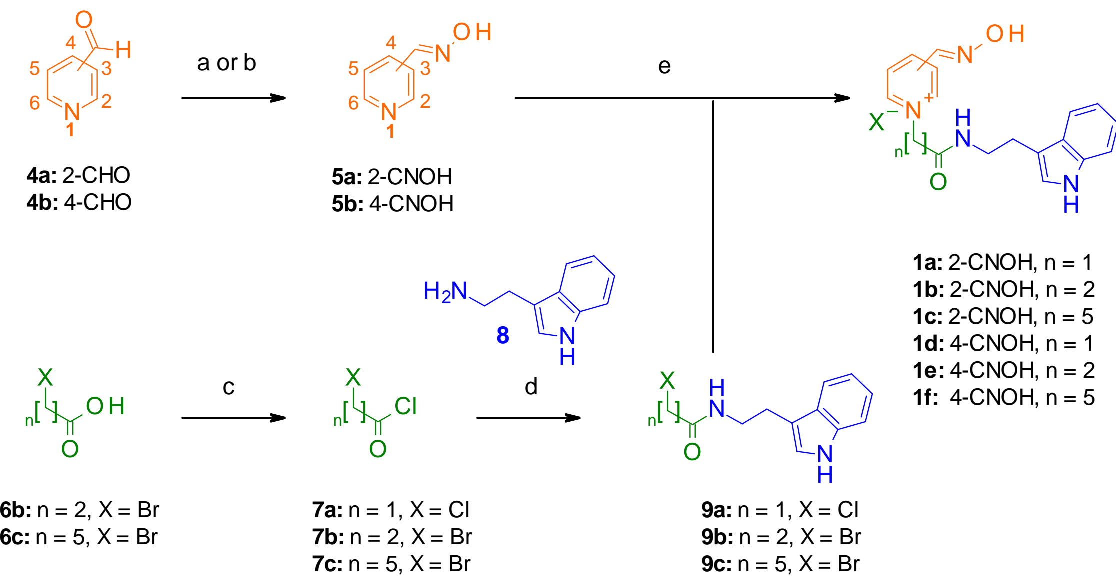
Figure 4. Pralidoxime – Melatonin/Tryptamine hybrids (**1-2**) proposed in this research work.

3. RESULTS AND DISCUSSION

3.1. SYNTHESIS OF PRALIDOXIME– MELATONIN HYBRIDS WITH GENERAL STRUCTURE 1

For the synthesis of Pralidoxime-Melatonin/Tryptamine hybrids with structure **1**, the 2- and 4-pyridincarbaldehydes **4(a,b)**, different-length n-haloalkyl acids **6(a-c)** and Tryptamine (**8**) are used as starting materials. The formation of the hybrids **1(a-f)** is achieved throughout a convergent synthetic pathway (Scheme 1) Firstly, the 2- and 4 pyridincarbaldehydes **4(a,b)** condensate with hydroxylamine hydrochloride to afford the oximes **5(a,b)**.[26] In the next step, the corresponding n-bromoalkyl acids **6(b,c)** are transformed into the n-bromoalkanoyl chlorides **7(b,c)** by treatment with neat $SOCl_2$.[27] The subsequent reaction between these derivatives and the Tryptamine (**8**) provides the *N*-(2-(1*H*-indol-3-yl)ethyl)-n-bromoalkanamides intermediates **9(a-c)**.[7] Finally, the quaternization reaction of the oximes **4(a,b)** with the Tryptamine derivatives **9(a-c)** in presence of a polar solvent (THF, CH_3CN) affords the Pralidoxime – Melatonin/Tryptamine hybrids **1(a-f)** with moderate to good yields.

Scheme 1. Synthesis of Pralidoxime-Melatonin hybrids with general structure **1**.



Reagents and conditions: *(a) H_2O, 10% NaOH, $NH_2OH.HCl$, r.t. or 60 ºC; (b) MeOH, $NH_2OH.HCl$, r.t. or 60 ºC; (c) $SOCl_2$, 0 ºC, 30 min, r.t., and then, 50 ºC, 30 min, 2h; (d) N_2, dry THF, Tryptamine (**8**), 0 ºC, 30 min, purification by chromatographic column; (e) N_2, 2-Pyridinaldoxime, THF, 47 ºC, 3-4 days or 4-Pyridinaldoxime, THF:CH_3CN (1:1), 65 ºC, 3-4 days.*

3.1.1. Synthesis of the pyridinaldoximes 5(a,b)

The first synthetic step involves the generation of the desired pyridinaldoximes **5(a,b)** (Scheme 1). For this purpose, the 2- and 4-pyridine carboxaldehyde **4**(**a,b**) are used as starting materials. The 2-pyridine carboxaldehyde (**4a**) is used without extra purification. However, the 4-pyridine carboxaldehyde (**4b**) is purified by high vacuum distillation (2.3 mbar, 50°C). The formation of the pyridinaldoximes can be carried out by the treatment of the corresponding pyridine carboxaldehyde in the presence of hydroxylamine hydrochloride in both aqueous[28] and organic solvents. [26] When the reaction takes place in water and in the presence of an aqueous 10% NaOH solution at room temperature (*Entry 1*, Table 1), a mixture of **4b** and **5b** is isolated as a white solid. Analysis by ^{1}H-NMR indicates that the ratio **5b**/**4b** is 100:28. In the HPLC recorded for this mixture, a unique peak at t_R = 1,18 min (@214 nm and @254 nm) is observed. It has a *m/z* relationship of 123.1 [M+H]$^+$ and 140.1 [M+H]$^+$. The first *m/z* value belongs to the final product **5b**, whereas the formation of the second one could only be explained by the presence of starting material in the reaction crude. In order to explain these HPLC results, the applied analysis conditions must be considered. In a mixture of water, methanol and under acidic conditions, the hemiacetal **10** is formed and identified (***Table 1***).

Table 1. Experimental conditions for the synthesis of pyridinaldoximes **5**(**a,b**).

Conditions

4a: 2-CHO
4b: 4-CHO

5a: 2-CNOH
5b: 4-CNOH

10

Entry	Aldehyde (mmol)	NH_2OH (mmol)	Solvent	T (ºC)	t (h)	pH	Yield (%)	Ratio **5a/4a or 5b/4b**
1	**4b** (9.33)	7.19	H_2O	25	0.17	*pH < 7*	***4b +5b** (58)*	100:28
2	**4b** (9.33)	7.19	H_2O	60	24	*pH < 7*	***4b+ 5b** (28)*	100:16
3	**4b** (46.68)	35.97	H_2O	60	72	*pH < 7*	***4b + 5b** (35)*	100:20
4	**4b** (18.67)	28.78	H_2O	60	24	*pH < 7*	***4b + 5b** (39)*	100:87
5	**4b** (9.33)	10.07	MeOH	30-60	0.5	*8-9*	***5b** (53)*	100:0
6	**4b** (93.36)	102.17	MeOH	30-60	0.33	*9-10*	***5b** (63)*	100:0
7	**4a** (93.36)	71.95	MeOH	60	23	*9*	***5a*** (22)*	100:0

**We had some problems during the isolation process.*

In order to optimize this experimental procedure, in terms of yield and purity of the final product, some parameters like temperature, reaction time and solvent are modified. An increase in temperature to 60°C as well as reaction time up to 24 hours, the ratio **5b**/**4b** is decreased to 100:16 (*Entry 2*, Table 1). However, an increase in the reaction time to 72 hours does not provide a full conversion to **5b** leading to an increase of ratio **5b**/**4b** to 100:20 (*Entry 3*, Table 1). This result indicates that an increase in temperature does not improve the desired oxime ratio. The same applied conditions but with a decrease in time to 24 hours give a higher ratio of **4b** (**5b**/**4b**, 100:87) (*Entry 4*, Table 1). When the solvent is changed to an organic one (methanol) and the reaction is heated up from 30°C to 60°C, the reaction takes place faster providing a total conversion of **4b** to **5b** at different scales (1 and 10 grams) (*Entries 5 and 6*, Table 1). As shown for **4b**, the shorter the time, the larger the yield. The 30-minute reaction affords the pure oxime **5b** in a 53% yield meanwhile, the 20-minute reaction provides it in a 63% yield.

The reaction between 4-pyridine carboxaldehyde (**4b**) and hydroxylamine hydrochloride in methanol at 60°Cand after 30 minutes of reaction time, the pyridine aldoxime **5b** is isolated as a white solid and 53% yield, in a 1-gram scale.[26] An analysis by ^{1}H-NMR of the isolated solid confirmed the presence of the aimed product **5b**. This product is used in the next step without extra treatment. However, we realized that when **5b** is used in the quaternization reaction of pyridine, it does not work. It is due to the latest experimental conditions affording **5b** as a hydrochloride derivative. So, **5b** is solubilized in H_2O (0.60 g/4 mL) and treated with an aqueous 1M NaOH solution until pH 7-9, providing the pyridine aldoxime **5b** in a free base form, in which the quaternization reactions occurs. The reaction is scaled up to 10 grams, isolating first the pyridine aldoxime **5b** as the hydrochloride and after the described treatment, the pyridine aldoxime **5b** is isolated as a white free base form with a63% yield. Under this optimized conditions the2-pyridinaldoxime (**5a)** is isolated as a white solid in a 22% yield, dissolved in H_2O (60 mL) and neutralized with an aqueous 1M NaOH solution to provide the pyridinaldoxime **5a** in its free base form.

Finally, the characterization of both pyridinaldoximes **5**(**a,b**) is carried out by mono-(^{1}H-, ^{13}C-) and bi-(HSQC and HMBC) NMR experiments, HPLC and FTIR (Annex I).

3.1.2. Synthesis of n-chloro or n-bromoalkanoyl chlorides (7a-c).

The next step in the preparation of the Pralidoxime - Melatonin/Tryptamine hybrids (**1**) is the synthesis of n-chloro or n-bromoalkanoyl chlorides **7**(**a-c**) (Scheme 1). The bromo acids **6**(**b,c**) and the 2-chloroacetyl chloride (**7a**) are commercially available, and they will be used in the next synthetic step without further purification. The 3-bromopropionic acid (**6b**) and the 6-bromohexanoic acid (**6c**) when treated with neat $SOCl_2$ at low temperature (0°C) and then, reflux provide the correspondent n-alkanoyl chlorides **7**(**b,c**) as yellowish oils and with good yields (100%) (Scheme 2).[27] Finally, the characterization of both bromoalkanoyl chlorides **7**(**b,c**) is carried out by mono-(^{1}H, ^{13}C) and bi-(HSQC and HMBC) NMR experiments, HPLC and FTIR. The complete assignment of both **7b** and **7c** is described in Annex I. With a comparison between **6**(**b,c**) and **7**(**b,c**) FTIR analysis, it is concluded the formation of the n-bromoalkyl chlorides has taken place. For **6b,c**, the carbonyl band appears centered at 1688.48 cm^{-1}. However, when **7b,c** are formed the carbonyl band is shifting to 1785.39 cm^{-1}. Due to its instability, the HPLC-Ms recorded is made in MeOH so that the bromoacid chlorides are transformed into the correspondent methyl ethers so they can be observed. Otherwise, they would be hydrolyzed to their correspondent bromoacid. This way, the methyl ester of **7b** is found in HPLC at a t_R = 7.684 min and with a *m/z* relationship of 210.4 $[M+H]^+$, whereas the methyl ester of **7c** appears at a t_R = 13.946min and with a *m/z* relationship of 209.1 $[M+H]^+$. The yields of **7b** and **7c** are considered as 100% since they are not isolated and are directly used in the next step without further purification.

Scheme 2. Synthesis of 3-bromopropanoyl and 6-bromohexanoyl chlorides **7(b,c)**.

Br, n, OH, O — a) → Br, n, Cl, O

6(b,c) **7(b,c)**

Reagents and conditions: *a) N_2, 1 eq. of n-bromoalkano acid, $SOCl_2$ (5.35 eq.), 0°C for 30 min, then r.t. for 50 min and, finally, 50°C for 2h.*

3.1.3. Synthesis of n-bromo-N-[2-(1H-indol-3-yl)ethylalkylamide 9(a-c).

The third step of this synthetic pathway involves the formation of the n-halo-*N*-[2-(1*H*-indol-3yl)ethylalkylamides **9(a-c)**. These products are afforded after the condensation between the Tryptamine (**8**) and the n-haloalkanoyl chlorides **7(a-c)** in THF at low temperature[7] (Scheme 3).

Scheme 3. Synthesis of n-bromo-*N*-[2-(1*H*-indol-3-yl)ethylalkylamides **9(a-c)** and the impurity **(11)**.

7(a-c) + Tryptamine (8) → a) or b) → 9(a-c) + 11

Reagents and conditions: *a) N_2, DMAP (0.1 eq.), Et_3N, THF, 0°C, 30 min, stirring, when n=2, purification by silica gel column chromatography is needed; b) N_2, THF, 0°C, 30 min.*

The chloroacyl chloride (**7a**) reacts with the Tryptamine (**8**) in presence of DMAP and Et_3N in dry THF to afford **9a** as a gold solid in a 55% yield (Scheme 4). In this reaction, the DMAP acts as an activator of the carbonyl group. By being positively charged, it becomes a better leaving group than the chlorine is. When the Tryptamine is added, the proton liberated is caught by the DMAP, leading to an acid compound which is neutralized with the Et_3N added.

Scheme 4. Mechanism of the formation of n-bromo-*N*-[2-(1*H*-indol-3-yl)ethylalkylamides **9(a-c)** by using DMAP and Et_3N.

7(a-c) + DMAP → → (8) → [ ] → H^+ → 9(a-c)

Reagents and conditions: *a) N_2, THF, DMAP, Et_3N, 0°C, 30 min.*

The HPLC analysis shows a peak at t_R = 13.94 min with a *m/z* relationship of 237.1 $[M+H]^+$ and 100% purity (@254 nm) (*Entry 1*, Table 2). In presence of DMAP (0.1 eq.), Et_3N (1.0 eq.) and Tryptamine (1.0 eq.), the 3-bromopropanoyl chloride (**7b**) provides a mixture of two compounds (*Entry 2*, Table 2) (Scheme 3). As it is shown in the HPLC, the peak at t_R = 11.66 min belongs to 3-bromo-*N*[2(1*H*-indol-3-yl)ethyl)propanamide (**9b**), whereas the peak at t_R = 10.203 min corresponds to 3-bromo-*N*[2(1*H*-indol-3-yl)ethyl)acrylamide (**11**). The formation of this impurity can only be explained because the base was taking a proton leading to HBr elimination with the subsequent formation of the double bond. In order to identify both substances, the obtained mixture was purified by silica gel column chromatography using DCM: MeOH (30:1) as eluyent. From the fractions with R_f = 0.8 (DCM: MeOH, 10:1) **9b** is isolated in a 11% yield. However, from the fractions with a R_f = 0.5 (DCM: MeOH, 10:1) the impurity (**11**) is isolated in a 13% yield. The use of mono-(^{1}H-, ^{13}C-) and bi- (HSQC and HMBC) NMR experiments and HPLC (Annex I) confirm both structures. The complete assignment of both **9b** and the impurity **11** is recorded in Annex I. In order to increase the final yield, some experimental conditions like time and base amount are modified. Maintaining DMAP (0.1) and Et_3N (1.0 eq,), a reduction in reaction time to 15 min also affords a mixture of the product and the impurity in a ratio **9b/11** (43:57) (*Entry 3*, Table 2). For this reason, two more experiments are developed. In the first one, the amount of Et_3N is decreased to 0.5 eq. but it is observed that the impurity still gets formed, affording it in a ratio **9b/11** (100:51). (*Entry 4*, Table 2). In the next experiment, neither Et_3N nor DMAP are added. By NMR and HPLC analysis, it is concluded that no impurity is afforded this time (*Entry 5*, Table 2). Under thede experimental conditions, **9b** is provided in a 25%* yield. In presence of DMPA and Et_3N, the 6-bromoheaxnoyl chloride (**8c**) affords **9c** as a white solid in a 46% yield and at t_R = 14.34 min with a *m/z* relationship of 338.1 and 339.1 $[M+H]^+$ and with 100% purity (@214 and 254 nm) (*Entry 6*, Table 2).

Table 2. Conditions for the synthesis of n-bromo-*N*-[2-(1*H*-indol-3-yl)ethylalkylamides **9(a-c)** at 0°C.

									HPLC (@254 nm)						
									Product (9)			**Impurity (11)**			
										***m/z* [M+H]$^+$**				***m/z* [M+H]$^+$**	
Entry	**S.M**	**S.M (eq.)**	**Tryptamine (8) (eq.)**	**DMAP (eq.)**	**Et_3N (eq.)**	**F.P**	**Yield (%)**	**t_R (min)**	**Purity (%)**	**Calculated**	**Found**	**t_R (min)**	**Purity (%)**	**Calculated**	**Found**
1	**7a**	1.1	1.0	0.1	1	**9a**	55	13.936	100	237.1	237.1	-	-	-	-
2	**7b**	1.1	1.0	0.1	1	**9b**	38	11.667	71	296.0	296.0	10.203	29	215.1	215.1
3	**7b**	1.1	1.0	0.1	1	**9b**	25	11.667	43	296.0	296.0	10.203	57	215.1	215.1
4	**7b**	1.1	1.0	0.1	0.5	**9b**	-	11.667	66	296.0	296.0	10.203	34	215.1	215.1
5	**7b**	1.1	1.0	0	0	**9b**	25*	11.667	96	296.0	296.0	-	-	-	-
6	**7c**	1.1	1.0	0.1	1	**9c**	46	14.336	100	338.1	338.1	-	-	-	-

*We had some problems during the isolation process.

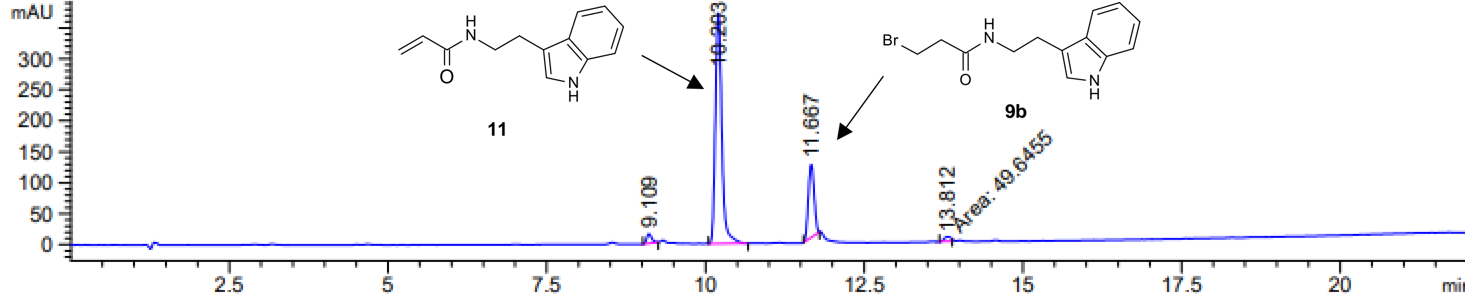


Figure 5. HPLC (@ 254 nm) of the mixture of 3-bromo-*N*-[2-(1*H*-indol-3-yl)ethyl]propanamide **(9b)** and *N*-[2-(1*H*-indol-3-yl)ethyl]prop-2-enamide **(11).**

3.1.4. Synthesis of n-[n'-[(E)-hydroxyiminomethyl]pyridin-1-uid-1-yl]-*N*-[2-(1*H*-indol-3-yl)ethyl]alkylamide;halide 1(a-f).

In the last step, final products **1**(**a-f**) are afforded after the alkylation of the oximes **5**(**a,b**) with each *n*-bromo-*N*-[2-(1*H*-indol-3-yl)ethylalkylamide **9**(**a-c**) in either THF, CH_3CN or a mixture of the two solvents in a 1:1 ratio, under inert atmosphere and different experimental conditions as indicated in Scheme 5 and Table 3.[7]

Scheme 5. Synthesis of the formation of Pralidoxime-Tryptamine hybrids **(1)**.

5(a,b) **9(a-c)** **1(a-f)**

Reagents and conditions: *a) N_2, 2-Pyridinaldoxime, THF, 47 °C, 3-4 days or 4-Pyridinaldoxime, THF:CH_3CN (1:1), 65 °C, 3-4 days.*

2-Pyridinaldoxime (**5a**) does not react with any *n*-bromo-*N*-[2-(1*H*-indol-3-yl)ethylalkylamides **9**(**a-c)** under different experimental conditions (*Entries 1-4*, Table 3). In THF and at 47ºC, 4-Pyridinaldoxime (**5b**) does not react with **9a** after 17 hours (*Entry 5*, Table 3). However, in a mixture of CH_3CN:THF (1:1), it provides, in 65 hours, **1d** as a brown viscous solid in a 93% yield and 95 % purity (@254 nm) (*Entry 6*, Table 3). The treatment of 4-Pyridinaldoxime (**5b**) with 3-bromo-*N*-[2-(1*H*-indol-3-yl)ethyl]propanamide (**9b**) in a mixture of CH_3CN:THF (1:1) affords **1e** in 50 % yield and with a purity of 70% (@254 nm) (*Entry 7*, Table 3). The reaction of 4-pyridinaldoxime (**5b**) with 6-Bromo-*N*-[2-(1*H*-indol-3-yl)ethyl]hexanamide (**9c**) in CH_3CN for 24 hours affords a mixture of products where the ratio **9c**/**5b** is 51%. However, when the mixture of solvents CH_3CN:THF (1:1) is used, the proportion of the final product in the reaction mixture increases up to 81%. After its purification, **1f** is provided with a 65% yield (*Entry 9*, Table 3).

Table 3. Experimental conditions, yield (%) and purity for the Pralidoxime-Melatonin/Tryptamine hybrids (**1**).

	Starting materials								**HPLC (@ 254 nm)**		***m/z* [M+H]⁺**	
						1(a-f)						
Entry	**5**	**9**	**Solvent**	**T (°C)**	**t(h)**	**Final Product**		**Yield (%)**	**t_R (min)**	**Percentage (%)**	**Calculated**	**Found**
1		**9a**	THF	47	70	**1a**	*n* = 1	-	-	-	-	-
2		**9b**	THF	47	144	**1b**	*n* = 2	-	-	-	-	-
3		**9c**	CH_3CN	65	24	**1c**	*n* = 5	-	-	-	-	-
4	**a**	**9c**	THF	47	48	**1c**	*n* = 5	-	-	-	-	-
5		**9a**	THF	47	17	**1d**	*n* = 1	-	-	-	-	-
6		**9a**	CH_3CN:THF, 1:1	65	65	**1d**	*n* = 1	93	6.758	95	323.1	323.1
7		**9b**	CH_3CN:THF, 1:1	65	65	**1e**	*n* = 2	50	5.389	70*	337.1	337.4
8	**b**	**9c**	CH_3CN	65	24	**1f**	*n* = 5	-	6.154	51	380.2	380.2
9		**9c**	CH_3CN:THF, 1:1	65	144	**1f**	*n* = 5	65	6.215	81*	380.2	380.2

**These percentages obtained in HPLC analysis is not in accordance with the one shown by ^{1}HNMR. Right now, due to the purity of the final products, different purification processes are being developed.*

3.2. SYNTHESIS OF PRALIDOXIME– TRYPTAMINE HYBRIDS WITH GENERAL STRUCTURE 2

To obtain the Pradiloxime - Melatonin/Tryptamine hybrids (**2**), the corresponding pyridinaldoximes **5**(**a,b**) react with the dihaloalkanes **12(a-d)** and with the n-bromoalkano acids **13(a-d)** , which contain different chain lengths, to afford the correspondent pyridinium derivatives **14(a-d)** and **15(a-d).**[7,29] Finally, the intermediates 14(a-d) will react with Tryptamine to provide the Pradiloxime-Melatonin/Tryptamine derivatives **2(a-d)** (Scheme 6).

Scheme 6. Synthesis of Pralidoxim – Melatonin hybrids with general Structure **2**.

Reagents and conditions: *a) N_2, THF or CH_3CN, 47°C or 70°C, **12(a-d)** dropwise, 48 h; b) N_2, THF or CH_3CN, 47°C or 70°C, **13(a-d)**, 24 h; c) N_2, CH_3CN, 70°C, Tryptamine (**8**), 24 h.*

3.2.1. Synthesis of (nE)-1-(n-bromoalkyl)pyridin-1-ium-4-carbaldehyde oxime;bromide 14(a-d) and (nE)-1-(n-hydroxalkyl)pyridin-1-ium-n-carbaldehyde oxime;bromide 15(a-d) through the pyridinaldoximes 5(a,b) quaternization reaction.

The formation of **14(a-d)** derivatives is carried out in presence of an excess (3.0 eq.) of the dibromoalkanes **12(a-d)** in order to prevent the dimerization impurity with general structure **16** (Scheme 6). This reaction takes place at reflux of THF or CH_3CN. As indicated in Table 4, when 4-pyridinaldoxime (**5b**) reacts with 1,2-dibromoethane in THF at 47°C does not afford the intermediate **14a**. However, when the reaction takes place using CH_3CN as a solvent 70°C and after 48 h, (4E)-1-(2-bromoethyl)pyridin-1-ium-4-carbaldehyde oxime;bromide (**14a**) is formed in a 23% yield. At 47°C and employing THF as the solvent, the treatment of **5b** with 1,3-dibromopropane (**12b**) affords the corresponding (4E)-1-(3-bromopropyl)pyridin-1-ium-4-

carbaldehyde oxime;bromide (**14b**) with low yield (15%) and a purity of 100% (@254 nm). The reaction of **5b** with 1,2-dibromopentane (**12c**) and 1,2-dibromohexane (**12d**) gives a mixture of the product and the oxime, indicating that this reaction time is not enough for completing the reaction. The the 4-pyridinaldoxime (**5b**) is treated with 2 equivalents of each n-bromoalkano acid **13(a-d)** at reflux using THF or CH_3CN as solvents, resulting in the formation of **15(a-d)** with moderate to good yields and purities in the range (74%-100%) (@254 nm) (Table 5). Finally, the characterization of these products is carried out by mono-(^{1}H-, ^{13}C-) and bi-(HSQC and HMBC) NMR experiments (Annex I).

These 4(E)-1-(n-bromoalkyl)pyridin-1-ium-4-carbaldehyde oxime;bromides **14(a-d)**, after their purification, would be used in the next synthetic step, which is not developed in this research work, but the intermediates are ready to make them react with the Tryptamine to form the aimed hybrids **2(a-d).**

4. BIOLOGICAL TRIALS

To determine the efficacy of the oximes, *in vitro* analysis will be carried out, since these agents suffer a fast hydrolysis *in vivo*. The oxime kinetic properties will be established by the determination of the reactivation constants K_D, kr and kr_2. K_D is the dissociation constant and it is inversely proportional to the affinity of the oxime to the inhibit AChE and calculated by non-linear regression; the kr is a measure of the reactivity of the oxime; and the reactivation constant, kr_2 is the ratio kr/k_D.[9,13] To know the degree of inhibition of the AChE in the CNS, the determination of the erythrocytic AChE activity will be determined as well using a spectrophotometric trial at 25°C in 0.1 M Na_2SO_4 buffer (pH 7.4), containing 0.01% BSA and 10.mM substrate acetylthiocholine when the OP-AChE conjugates are prepared [4,16]

$$[EP]+[OX] \underset{}{\overset{K_D}{\rightleftharpoons}} [EPOX] \underset{(k_{POX})}{\overset{k_r}{\rightleftharpoons}} [E]+[POX]$$

Figure 6. Reaction scheme for the reactivation of inhibited AChE by oximes with [E] active AChE; [EP] phosphylated AChE; [OX] oxime; [EPOX] Michaelis-type phosphyl-AChE-oxime-conjugate; [POX] phosphylated oxime.[9]

Table 4. Reaction conditions for the synthesis of the pyridinaldoxime halides **14(a-d)** through the reaction of the oximes with the dibromoalkanes **12(a-d).**

	Oxime	Dihaloalkane	Solvent	T (°C)	t (h)	Product	Yield (%)	HPLC (@254 nm)		m/z [M+H]$^+$
								t_R (min)	Purity (%)	
1	**5b**	**12a**; 1,2-dibromoethane	THF	47	24	**14a**	-	-	-	-
2	**5b**	**12a**; 1,2- dibromoethane	CH_3CN	70	48	**14a**	23	1.171	62.9	231.0
3	**5b**	**12b**, 1,3-dibromopropane	THF	47	20	**14b**	15	0.974	100.0	245.0
4	**5b**	**12c**; 1,5-dibromopentane	CH_3CN	70	25	**14c**	18	1.192	58.5	273.0
5	**5b**	**12d**; 1,6-dibromohexane	THF	47	23	**14d**	81	7.032	50.0	287.0

Table 5. Reaction conditions for the synthesis of the pyridinaldoxime acids **15(a-d)** through the reaction of the oximes with the n-bromoalkano acids **13(a-d)**.

	Oxime	n-Bromoalkanoacid	Solvent	T (ºC)	t (h)	Product	Yield (%)	HPLC (@254 nm)		m/z [M+H]$^+$
								t_R (min)	Purity (%)	
1	**4a**	**13a**; 2-bromoacetic acid	CH_3CN	70	24	**15a**	75	1.114	100	182.0
2	**4a**	**13b**; 3-bromopropanoic acid	CH_3CN	70	24	**15b**	55	1.203	72.4	196.1
3	**4a**	**13c;** 6-bromo hexanoic acid	THF	47	20	**15c**	66	0.974	100	238.0
4	**4a**	**13d**; 8-bromooctanoic acid	THF	47	23	**15d**	99	4.965	100	266.2

5. CONCLUSIONS

- In order to reactivate the inhibited AChE by OPNAs, the Pyridinaldoxime-Meltonin/Tryptamine hybrids (**1-2**) have been proposed as chemical countermesuares. against the intoxication with OPNAs.
- These hybrids are charged reactivators so that they can interact both with the Peripheral anionic site and the Catalytic anionic site of the active site. To make the Brain Blood Barrier permeation easier, different-length linkers have been introduced to increase the lipophility of the compounds.
- 2- and 4- pyridinaloximes have been used to develop the Tryptamine/Melatonin hybrids. After their quaternization with different linkers we could observe how the reactions between the 4-pyridinaldoxime (**5b**) and the Tryptamine derivatives **9(a-c)** have yielded the different hybrids **1(d-f)**, meanwhile the reactions between the 2-pyridinaldoxime (**5a**) and the correspondent derivatives **9(a-c)** have not worked.
- Currently, experiments with the 2-pyridinaldoxime are being develop to find out the reasons why it has not reacted with the Tryptamine derivatives. It is thought it might be a pH problem. If the aldoxime is in its hydrochloride form, it will not provide the quaternization reaction.
- To sum up, eight intermediates (**14(a-d), 15(a-d)**) and three 4-pyridinaldoxime-Melatonin/Tryptamine hybrids **1(d-f)**, with general structure **1**, have been obtained as final products as OPNA intoxication antidotes. They all will be tested in order to check their efficiency in the AChE activity restauration. Furthermore, the intermediates (**14(a-d), 15(a-d)**) will be evaluated in orden to identify the importance of every fragment/moiety present in the final hybrids.
- Once synthesized and purified, these oximes will be submitted to biological trials to determine their efectiveness in restoring the AChE activity.

6. EXPERIMENTAL SECTION

6.1. REAGENTS AND GENERAL METHODS (Annex I).

6.1.1. General procedure for the synthesis of pyridinaldoximes (5a, b) (Annex I).

6.1.2. Synthesis of bromoacid chlorides (7b,c) (Annex I).

6.1.3. General procedure for the synthesis of n-bromo-N-[2-(1H-indol-3-yl)ethylalkylamide (9a-c). Under N_2 atmosphere, a solution of Tryptamine (1 eq.) in dry THF (15 mL) was treated with DMAP (0.1 eq.), Et_3N (1.0 eq.) and with a solution of n-bromoalkanoyl chloride (1.1 eq.) in THF (2 mL) at 0ºC for 2 hours. After that, the reaction mixture was diluted with DCM (100 mL) and washed sequencially with an aqueous 1M NH_4Cl solution (3 x 100 mL) and saturated aqueous NaCl solution (3 x 100 mL). The organic phase was dried over Na_2SO_4, filtered out and the solvent was removed under reduced pressure. The isolated product was employed in the next step without further purification.

2-bromo-*N*-[2-(1*H*-indol-3-yl)ethyl]acetamide (9a) (RJG-2301-072). The treatment of 2-chloroacetyl chloride (0.353 g, 3.13 mmol, 1.0 eq.) with tryptamine (0.50 g, 3.13 mmol, 1 eq.), DMAP (0.038 g, 0.313 mmol, 0.1 eq.), Et_3N (0.31 g, 3.13 mmol, 1 eq.) in THF (5 mL) and, following the general, procedure gave 0.41 g (55%) of 2-bromo-*N*-[2-(1*H*-indol-3-yl)ethyl]acetamide (**9a**) as a goldish solid. HPLC-MS (@254 nm): t_R = 13.936 min (100%), *m/z* = 237.1 $[M+H]^+$. ^1H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.82 (s, 1H), 8.31 (t, *J* = 5.4 Hz, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.16 (d, *J* = 2.3 Hz, 1H), 7.07 (td, *J* = 7.9 Hz, *J* = 1.1 Hz, 1H), 6.98 (td, *J* = 8.1 Hz, *J* = 1.1 Hz, 1H), 4.06 (s, 2H), 3.38 (m, 2H), 2.85 (t, *J* = 7.5 Hz, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 165.77, 136.24, 127.16, 122.70, 120.94, 118.25, 118.22, 111.50, 111.37, 39.86, 42.71, 24.91.

3-bromo-*N*-[2-(1*H*-indol-3-yl)ethyl]propanamide (MAG-2307-042) Two fractions: MAG-2307-042F5-8 (9b) and MAG-2307-042F10-14 (11) /MAG-2307-049F5-6 (9b) and MAG-2307-049F8-13 (11). The treatment of 3-bromopropanoyl chloride (1.31 g, 7.65 mmol, 1.1 eq.) with tryptamine (1.11 g, 6.95 mmol, 1 eq.), DMAP (0.085g, 0.69 mmol, 0.1 eq.), Et_3N (0.70 g, 6.95 mmol, 1 eq.) in THF (15 mL), and following the general procedure, gave a mixture of two products. After its purification on silica gel column chromatography and using a mixture of CH_2Cl_2:MeOH (30:1) as eluyent both compounds were isolated. From the fractions with *Rf* =

0.8, (CH_2Cl_2:MeOH, 30:1), 0.27 g (13%) of 3-bromo-*N*-[2-(1*H*-indol-3-yl)ethyl]propanamide (**9b**) was isoltated as a white solid. HPLC-MS (@254 nm): t_R = 11.299 min (96%), *m/z* = 296.1 and 297.0 $[M+H]^+$(positive mode). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.80 (s, 1H), 8.11 (t, *J* = 5.5 Hz, 1H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.15 (d, *J* = 2.3 Hz, 1H), 7.06 (td, *J* = 7.5 Hz, *J* = 1.1 Hz, 1H), 6.98 (td, *J* = 8.0 Hz, *J* = 1.1 Hz, 1H), 3.65 (t, *J* = 6.5 Hz, 2H), 3.36 (m, 2H), 2.83 (t, *J* = 7.4 Hz, 2H), 2.69 (t, *J* = 6.5 Hz, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 168.94, 136.22, 127.20, 122.70, 120.89, 118.21 (2C), 111.72, 111.35, 39.58, 39.52, 29.62, 25.17. From the fractions with *Rf* = 0.5, CH_2Cl_2:MeOH (30:1), 0.23 g (11%) *N*-[2-(1*H*-indol-3-yl)ethyl]prop-2-enamide (**11**) was isolated as a white solid. HPLC-MS (@254 nm): t_R = 9.866 min (94%), *m/z* = 215.1 $[M+H]^+$; 237.1 $[M+Na]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.81 (s, 1H), 8.20 (t, *J* = 5.5 Hz, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.15 (d, *J* = 2.3 Hz, 1H), 7.06 (td, *J* = 7.5 Hz, *J* = 1.1 Hz 1H), 6.98 (td, *J* = 7.5 Hz, *J* = 1.1 Hz 1H), 6.22 (dd, J_{trans} = 17.1 Hz, J_{cis} = 10.0 Hz, 1H), 6.09 (dd, J_{trans} = 17.1 Hz, J_{gem} = 2.4 Hz, 1H), 5.57 (dd, J_{cis} = 10.0 Hz, J_{gem} = 2.4 Hz, 1H), 3.42 (td, *J* = 7.4 Hz, *J* = 5.5 Hz 2H), 2.86 (t, *J* = 7.4 Hz, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 164.53, 136.24, 131.96, 127.21, 124.83, 122.63, 120.92, 118.24 (2C), 111.77, 111.37, 39.98, 25.15.

3-bromo-*N*-[2-(1*H*-indol-3-yl)ethyl]propanamide (9b) (MAG-2307-052) . The treatment of 3-bromopropanoyl chloride (0.50 g, 2.92 mmol, 1.1 eq.) with tryptamine (0.42 g, 2.65 mmol, 1 eq.) in THF (8 mL) afforded the pure 3-bromo-*N*-[2-(1*H*-indol-3-yl)ethyl]propanamide(0.198g, 25%) as a white solid. TLC: *Rf:* 0.6 in DCM:MeOH(30:1).. HPLC-MS (@254 nm): t_R = 11.325 min (96%), *m/z* = 296.1 and 297.0 $[M+H]^+$, *m/z* = 319.0 and 320.1 $[M+Na]^+$, ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.80 (s, 1H), 8.01 (t, *J* = 5.5 Hz, 1H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.33 (d, *J* = 7.6 Hz, 1H), 7.15 (d, *J* = 2.3 Hz, 1H), 7.08 (td, *J* = 7.6 Hz, *J* = 1.1 Hz, 1H), 6.98 (td, *J* = 7.5 Hz, *J* = 1.1 Hz, 1H), 3.65 (t, *J* = 6.5 Hz, 2H), 3.35 (m, 2H), 2.82 (t, *J* = 7.4 Hz, 2H), 2.69 (t, *J* = 6.5 Hz, 2H).

6-bromo-*N*-[2-(1*H*-indol-3-yl)ethyl]hexanamide (9c) (MAG-2307-041). The treatment of 6-bromohexanoyl chloride (1.28 g, 5.99 mmol, 1.1 eq.) with tryptamine (0.87 g, 5.45 mmol, 1 eq.), DMAP (0.066g, 0.54 mmol, 0.1 eq.), Et_3N (0.55g, 5.45 mmol, 1 eq.) in THF (15 mL) and, following the general procedure, gave 0.84 g (46%) of 6-bromo-*N*-[2-(1*H*-indol-3-yl)ethyl]hexanamide (**9c**) as a white solid. TLC: Hexane:EtOAc, (1:2), *Rf:* 0.07 HPLC-MS (@254 nm): t_R = 14.336 min (100%), m/z = 338.1 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.80

(s, 1H), 7.89 (t, *J* = 5.5 Hz, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.33 (d, *J* = 7.5 Hz, 1H), 7.14 (d, *J* = 2.2 Hz, 1H), 7.06 (td, *J* = 7.8 Hz, , *J* = 1.0 Hz, 1H), 6.98 (td, *J* = 7.5 Hz, *J* = 1.0 Hz, 1H), 3.51 (t, *J* = 6.7 Hz, 2H),), 3.37-3.29 (m, 2H), 2.81 (t, *J* = 7.4 Hz, 2H), 2.07 (t, *J* = 7.3 Hz, 2H), 1.87–1.72 (m, 2H), 1.56–1.45 (m, 2H), 1.39–1.28 (m, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.77, 136.22, 127.24, 122.57, 120.86, 118.23, 118.17, 111.87, 111.33, 39.39, 35.26, 35.05, 32.02, 27.21, 25.27, 24.41.

6.1.4. General procedure for the synthesis of n-[n'-[(E)-hydroxyiminomethyl]pyridin-1-uid-1-yl]-N-[2-(1H-indol-3-yl)ethyl]alkanamide;halide (1a-f). Under N_2 atmosphere, a solution of the correspondant pyridinaldoximes (0.95 eq.) in dry THF (10 mL) was treated with *n*-bromo-*N*-[2-(1*H*-indol-3-yl)ethylalkylamide (1.0 eq.) at 47ºC for 2 days. After that, the solvent was dried under reduced pressure. The isolated product was obtained as viscous solid without further purification.

2-[2-[(E)-hydroxyiminomethyl]pyridin-1-ium-1-yl]-*N*-[2-(1H-indol-3-yl)ethyl]acetamide; chloride (1a) (MAG-2307-065). The treatment of 2-pyridinaldoxime (**5a**) (0.09 g, 0.75 mmol, 0.95 eq.) with 2-bromo-*N*-[2-(1*H*-indol-3-yl)ethyl]acetamide (**9b**) (0.18 g, 0.79 mmol, 1 eq.) in THF (5 mL) and following the general procedure, unfortunately did not provide **1a**.

3-[2-[(*E*)-hydroxyiminomethyl]-1-pyridyl]-*N*-[2-(1*H*-indol-3-yl)ethyl]propanamide; hydrobromide (1b) (MAG-2307-062). The treatment of 2-pyridinaldoxime (**5a**) (0.04 g, 0.32 mmol, 0.95 eq.) with 3-bromo-*N*-[2-(1*H*-indol-3-yl)ethyl]propanamide (0.10 g, 0.34 mmol, 1 eq.), in THF:CH_3CN, (1:1) (6 mL) and following the general procedure, unfortunately did not give **1b**.

6-[2-[(E)-hydroxyiminomethyl]-1-pyridyl]-*N*-[2-(1*H*-indol-3-yl)ethyl]hexanamide;bromide (1c) (MAG-2307-066). The treatment of 2-pyridinaldoxime (**5a**) (0.06 g, 0.56 mmol, 0.95 eq.) with 6-bromo-*N*-[2-(1*H*-indol-3-yl)ethyl]hexanamide (**9c**) (0.20 g, 0.59 mmol, 1 eq.), in CH_3CN (5 mL) and following the general procedure, unfortunately did not provide **1c**.

2-[4-[(E)-hydroxyiminomethyl]pyridin-1-ium-1-yl]-N-[2-(1H-indol-3-yl)ethyl]acetamide; chloride (MAG-2307-031) (1d). The treatment of 4-pyridinaldoxime (**5b**) (0.10 g, 0.84 mmol, 1 eq.) with 2-bromo-*N*-[2-(1*H*-indol-3-yl)ethyl]acetamide **(9a)** (0.20 g, 0.845 mmol, 1 eq.) in THF (5 mL) and, following the general procedure gave, 0.28 g (93%) of **1d** as a brown, viscous solid. TLC: Hexane:EtOAc (1:2), *Rf:* 0.0. HPLC-MS (@254 nm): t_R = 6.758 min (95%), *m/z* = 323.1

[M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.96 (s, 1H), 10.93 (s, 1H), 8.97 (t, *J* = 5.3 Hz, 1H), 8.92 (d, *J* = 6.6 Hz, 2H), 8.44 (s, 1H), 8.25 (d, *J* = 6.6 Hz, 2H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 7.9 Hz, 1H), 7.21 (s, 1H), 7.06 (t, *J* = 7.3 Hz, 1H), 6.98 (t, *J* = 7.3 Hz, 1H), 5.44 (s, 2H), 3.41 (t, *J* = 7.3 Hz, 2H), 2.89 (t, *J* = 7.3 Hz, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 166.0, 164.42, 149.06, 146.59, 146.51, 145.34, 136.47, 127.31, 123.13, 121.15, 118.50, 118.35, 111.67, 111.48, 61.32, 42.93, 25.21.

3-[4-[(*E*)-hydroxyiminomethyl]-1-pyridyl]-*N*-[2-(1*H*-indol-3-yl)ethyl]propanamide; hydrobromide (1e) (MAG-2307-057). The treatment of 4-pyridinaldoxime (**5b**) (0.04 g, 0.32 mmol, 0.95 eq.) with 3-bromo-*N*-[2-(1*H*-indol-3-yl)ethyl]propanamide (**9b**) (0.10 g, 0.34 mmol, 1 eq.), in THF:CH_3CN, (1:1) (6 mL) and, following the general procedure, gave 0.14 g (50%) of a mixture of **1e** and **5b** as a solid. The final product was identified in HPLC-MS (@254 nm): t_R = 5.389 min (70%), *m/z* = 337.1 [M+H]$^+$.^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.81 (s, 1H), 10.80 (s, 1H), 9.01 (t, *J* = 6.8 Hz, 2H), 8.41 (s, 1H), 8.19 (d, *J* = 6.8 Hz, 2H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.16-7.13 (m, 1H), 7.10 (d, *J* = 2.1 Hz, 1H), 7.05 (td, *J* = 7.5 Hz, *J* = 1.0 Hz, 1H), 6.98 (td, *J* = 8.0 Hz, *J* = 1.0 Hz, 1H), 4.77 (t, *J* = 6.3 Hz, 2H), 3.65 (t, *J* = 6.5 Hz, 2H), 2.88 (t, *J* = 6.5 Hz, 2H), 2.69 (t, *J* = 6.5 Hz, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 168.37, 150.13, 146.63, 145.45 (2C), 136.20, 127.62, 123.7 (2C), 122.62, 120.61, 118.23, 118.21, 111.39, 111.36, 40.15, 38.51, 30.42, 25.07.

6-[4-[(E)-hydroxyiminomethyl]pyridin-1-uid-1-yl]-*N*-[2-(1*H*-indol-3-yl)ethyl]hexanamide; bromide (1f) (MAG-2307-046). The treatment of 4-pyridinaldoxime (0.06 g, 0.56 mmol, 0.95 eq.) with 6-bromo-*N*-[2-(1*H*-indol-3-yl)ethyl]hexanamide (0.20 g, 0.59 mmol, 1 eq.), in CH_3CN (5 mL) and, following the general procedure, gave 0.18 g (65%) of 6-[4-[(E)-hydroxyiminomethyl]pyridin-1-uid-1-yl]-*N*-[2-(1H-indol-3-yl)ethyl]hexanamide;bromide (**1f**) as a yellowish, viscous solid. TLC: Hexane:EtOAc (1:1), *Rf:* 0.0. HPLC-MS (@254 nm): t_R = 6.215 min (81%), *m/z* = 380.2 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.84 (s, 1H), 10.81 (s, 1H), 9.03 (d, *J* = 6.5 Hz, 2H), 8.42 (s, 1H), 8.22 (d, *J* = 6.5 Hz, 2H), 7.92 (s, 1H), 7.51 (d, *J* = 7.7 Hz, 1H), 7.33 (d, *J* = 7.5 Hz, 1H), 7.13 (s, 1H), 7.05 (t, *J* = 7.7 Hz, 1H), 6.97 (t, *J* = 7.5 Hz, 1H), 4.55 (t, *J* = 7.3 Hz, 2H), 3.60 (t, *J* = 6.2 Hz, 2H), 3.35-3.23 (m, 2H), 2.79 (t, *J* = 7.3 Hz, 2H), 1.95-1.85 (m, 2H), 1.76 (t, *J* = 6.2 Hz, 2H), 1.59-1.46 (m, 2H).^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm):

171.71, 148.34 , 145.10, 144.96, 136.22, 127.23, 124.04, 122.58, 120.89, 118.23, 118.19, 111.84, 111.37, 67.02, 60.11, 39.40, 35.02, 30.37, 25.28, 25.12, 25.00, 24.51.

6.1.5. General procedure for the synthesis of 4(E)-1-(n-bromoalkyl)pyridin-1-ium-4-carbaldehyde oxime; bromide. Under N_2 atmosphere, a solution of 4-pyridinaldoximes (**5b**) (1.0 eq.) in dry THF (6 mL) was treated dropwise with n,n'-dibromoalkane (3.0 eq.), at 47ºC for 20 hours. After that, the solvent was dried under reduced pressure and washed with Et_2O.

(4E)-1-(3-bromopropyl)pyridin-1-ium-4-carbaldehyde oxime;bromide (14b) (MAG-2307-028). The treatment of 4-pyridinaldoxime (**5b**) (0.25 g, 2.05 mmol, 1 eq.) with 1,3-dibromoprapane **(12b)** (1.24 g, 0.62 mL, 6.14 mmol, 3 eq.) in THF (6 mL) gave 0.10 g (15%) of (4E)-1-(3-bromopropyl)pyridin-1-ium-4-carbaldehyde oxime; bromide (**14b**) as a brown, viscous solid. TLC: Hexane:EtOAc (1:1), *Rf:* 0.25. HPLC-MS (@254 nm): t_R = 1.053 min (100%), *m/z* = 245.0 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.84 (s, 1H), 10.93 (s, 1H), 9.05 (d, *J* = 6.5 Hz, 2H), 8.44 (s, 1H), 8.24 (d, *J* = 6.5 Hz, 2H), 4.68 (t, *J* = 7.0 Hz, 2H), 3.58 (t, *J* = 6.6 Hz, 2H), 2.5-2.46 (m, 2H).

(4E)-1-(6-bromohexyl)pyridin-1-ium-4-carbaldehyde oxime;bromide (14d) (MAG-2307-030). The treatment of 4-pyridinaldoxime (**5b**) (0.25 g, 2.05 mmol, 1 eq.) with 1,6-dibromohexane (**12d**) (1.49 g, 0.94 mL, 6.14 mmol, 3 eq.) in THF (6 mL) gave, 0.61 g (81%) of **14d** as a brown, viscous solid. TLC: Hexane:EtOAc (1:1), *Rf:* 0.0. HPLC-MS (@254 nm): t_R = 7.032 min (100%), *m/z* = 287.0 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.82 (s, 1H), 9.10 (d, *J* = 6.4 Hz, 2H), 8.44 (s, 1H), 8.24 (d, *J* = 6.4 Hz, 2 H), 4.59 (t, *J* = 7.2 Hz, 2H), 3.57-3.17 (m, 4 H), 2.01-1.84 (m, 2H), 1.37-1.28 (m, 2H), 1.19 (t, *J* = 7.3 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 148.34 (2C), 145.12, 145.00, 124.03 (2C), 60.03, 45.65, 30.24, 24.70, 8.56.

6.1.6. General procedure for the synthesis of n-[4-[(E)-hydroxyiminomethyl]pyridin-1-ium-1-yl]alkylic acid; bromide. Under N_2 atmosphere, a solution of 4-pyridinaldoximes (**5b**) (1.0 eq.) in dry THF or CH_3CN (6 mL) was treated with n-bromoalkylic acid (2.0 eq.), at 47ºC for 20 hours. After that, the solvent was dried under reduced pressure and washed with acetone or filtered.

2-[4-[(E)-hydroxyiminomethyl]pyridin-1-ium-1-yl]acetic acid;bromide (15a) (MAG-2307-033). The treatment of 4-pyridinaldoxime (**5b**) (0.25 g, 2.05 mmol, 1 eq.) with 2-bromoacetic acid (**13a**) (0.57 g, 4.09 mmol, 2 eq.) in CH_3CN (6 mL) afforded 0.40 g (75%) of **15a**. The product was washed with acetone. TLC: Hexane:EtOAc (1:1), *Rf:* 0.0. HPLC-MS (@254 nm): t_R = 1.206

min (100%), *m/z* = 181.1 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.91 (s, 1H), 9.00 (d, *J* = 6.7 Hz, 2H), 8.45 (s, 1H), 8.30 (d, *J* = 6.7 Hz, 2H), 5.56 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6)) δ(ppm): 167.72, 149.19, 146.36 (2C), 145.19, 123.72 (2C), 60.17.

3-[4-[(E)-hydroxyiminomethyl]pyridin-1-ium-1-yl]propanoic acid;bromide (15b) (MAG-2307-035). The treatment of 4-pyridinaldoxime (**5b**) (0.25 g, 2.05 mmol, 1 eq.) with 3-bromopropanoic acid (**13b**) (0.63 g, 4.09 mmol, 2 eq.) in THF (6 mL) gave 0.31 g (55%) as a mixture of the oxime (**5b**) and 2-[4-[(E)-hydroxyiminomethyl]pyridin-1-ium-1-yl]acetic acid;bromide (**15b**) in a 1:2 ratio FP:SM. The product was washed with acetone. HPLC-MS (@254 nm): t_R =1.145 min (27%), *m/z* = 195.1 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.90 (brs, 1H), 9.10 (d, *J* = 6.6 Hz, 2H), 8.50 (s, 1H), 8.30 (d, *J* = 6.6 Hz, 1H), 4.83 (t, *J* = 6.5 Hz, 2H), 3.15 (t, *J* = 6.5 Hz, 2H), 2.5 (m, 2H).). ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.51, 148.03, 145.64, 145.11, 143.47, 123.72, 122.96, 56.04, 34.35.

6-[4-[(E)-hydroxyiminomethyl]pyridin-1-ium-1-yl]hexanoic acid;bromide (15c) (MAG-2307-027). The treatment of 4-pyridinaldoxime (**5b**) (0.25 g, 2.05 mmol, 1 eq.) with 6-bromohexanoic acid (**13c**) (0.79 g, 4.09 mmol, 2 eq.) in CH_3CN (6 mL) gave 0.43 g (66%) of **15c**. The product was washed with Et_2O. TLC: Hexane:EtOAc (1:1), *Rf:* 0.0. HPLC-MS (@254 nm): t_R = 1.053 min (100%), *m/z* = 237.1 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.82 (s, 1H), 12.29 (s, 1H), 9.06 (d, *J* = 6.6 Hz, 2H), 8.44 (s, 1H), 8.24 (d, *J* = 6.6 Hz, 2H), 4.58 (t, *J* = 7.3 Hz, 2H), 3.52 (t, *J* = 6.7 Hz, 2H), 2.27-2.16 (m, 2H), 1.98-1.85 (m, 2H), 1.59-1.46 (m, 2H), 1.29 (m, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 174.77, 148.80, 145.59, 145.47, 124.51, 60.52 (2C), 33.76 (2C), 30.75, 25.33, 24.23.

8-[4-[(E)-hydroxyiminomethyl]pyridin-1-ium-1-yl]octanoic acid;bromide (15d) (MAG-2307-029). The treatment of 4-pyridinaldoxime (**5b**) (0.25 g, 2.05 mmol, 1 eq.) with 8-bromooctanoic acid **(13d)** (0.91 g, 4.09 mmol, 2 eq.) in THF (6 mL) provided 0.70 g (99%) of **15d** as a White solid. The product was vacuum filtered and dry. HPLC-MS (@254 nm): t_R = 5.065 min (100%), *m/z* = 265.1 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.84 (s, 1H), 11.97 (s, 1H), 9.06 (d, *J* = 6.6 Hz, 2H), 8.43 (s, 1H), 8.23 (d, *J* = 6.6 Hz, 2H), 4.56 (t, *J* = 7.3 Hz, 2H), 3.51-3.21 (m, 4H), 2.18 (t, *J* = 7.3 Hz, 2H),1.96-1.84 (m, 2H), 1.55-1.41 (m, 2H), 1.37-1.16 (m, 2H). ^{13}C NMR (100 MHz, DMSO) δ(ppm): 174.44, 148.34 (2C), 145.11, 144.96, 124.06 (2C), 60.26, 33.58, 30.53, 28.24, 28.06, 25.22, 24.31.

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