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ORIGINAL ARTICLE

ACID DISSOCIATION CONSTANTS AND MOLECULAR DESCRIPTORS OF SOME XYLENE LINKED BISPYRIDINIUM OXIMES

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Summary

The present article is aimed at determination of acid dissociation constants (pK_a), lipophilicity (log$P$) and hydrogen bond acceptor (HBA) and donor (HBD) counts of some novel xylene-linked bispyridiniumoxime based AChE reactivators. The choice was supported by their use in the therapy of acute intoxication with organophosphorus AChE inhibitors. UV-Vis spectrophotometry has been used to measure experimental pK_a values at 27°C, while software Marvin Sketch (chemaxon) has been used to estimate structure based computational pK_a, log$P$ values and hydrogen bonding parameters. The results were compared with standard oximes (HI-6 and obidoxime) under similar conditions. All the calculated pK_a values lie in the range of 7.45-9.85 which is well in agreement with most of the oxime reactivators studied so far.

Key words: Oxime reactivators; acid dissociation constants; lipophilicity; hydrogen bonding; blood brain barrier

INTRODUCTION

Organophosphates including tabun, soman, DFP (diisopropylfluorophosphate), sarin, cyclosarin, and pesticides (paraoxon, chlorpyrifos, TEPP-tetraethyl pyrophosphate) represent an extremely toxic group of compounds [1]. The toxicity of organophosphorus (OP) compounds in mammals results from the inhibition of enzyme acetylcholinesterase (AChE, EC 3.1.1.7) which leads to the accumulation of neurotransmitter acetylcholine (ACh) in the synaptic junction of neurons in both the central and peripheral nervous systems (CNS and PNS). This phenomenon triggers the over-rstimulation of nervous system which can be lethal if not timely controlled [2-4].

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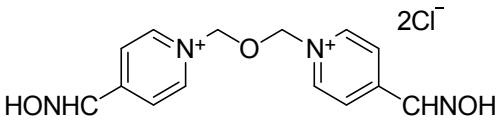
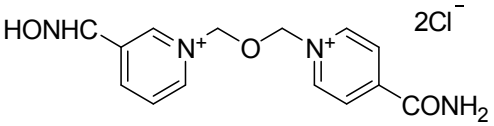
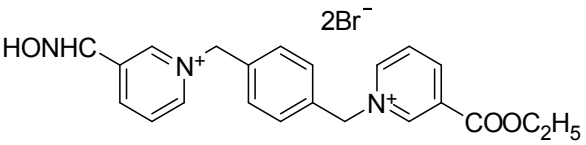
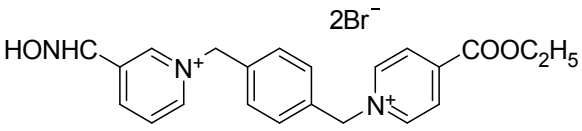
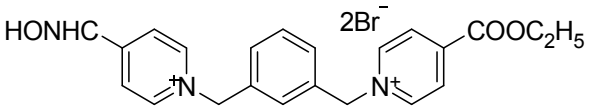
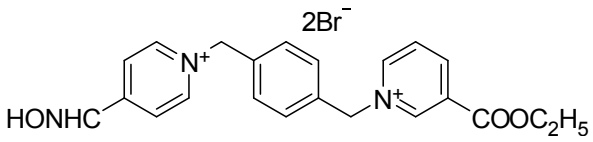
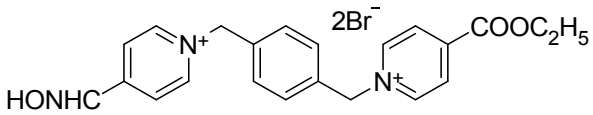
Assessment of physicochemical properties or molecular descriptors such as acid dissociation constant (pK_a), lipophilicity (log*P*), and hydrogen bond donor and acceptor counts (HBD and HBA) are essentially required for development of potent antidotes with substantial blood brain barrier (BBB) penetration [5]. Determination of acid dissociation constants and lipophilicity is important towards the understanding of the dissolution abilities of the drug like compound (for solid dosage forms) and their subsequent transport through cell membrane [6]. Compounds that are too lipophilic are not only more likely to be rapidly metabolized and bioaccumulate, they also have low aqueous solubility and often poor absorption properties. Early determination of lipophilicity (either through experimental measurement or prediction) highlights potential liabilities, and aids better decision-making both in hit-to-lead identification and lead optimization. An investigation of the physicochemical properties is also an essential step in understanding the pharmacokinetic profiles *in vivo*. It is mandatory to measure pK_a value of an oxime to identify the effective pH at which the molecule can ionize and attacks the OP-AChE adduct to reactivate the enzyme. Lipophilicity, accepted as an extension of the hydrophobic character, includes all favorable interactions that contribute to the distribution of a chemical entity between water and other solubilizing media, and represents a manifestation of the characteristics of the system in which the solute is placed [7]. Since last few decades, medicinal chemistry efforts have led to the development of derivatives of currently available standard oxime reactivators (HI-6, obidoxime, 2-PAM etc.) but there is a relative lack of data for physicochemical parameters and computational molecular descriptors of these oximes. We have reported previously [8,9] pK_a of mono- and bis-pyridinium oximes and their alpha nucleophilicity towards decontamination reactions. Our group [5] has also recently studied physicochemical parameters of two tertiary oximes (monoisonitrosoacetone; MINA and Butane-2,3-dione monooxime; BDMO) and thirteen quaternary pyridinium aldoxime derivatives and analyzed their structure-activity relations. Esposito et al. [10] have studied the physicochemical properties and molecular descriptors of oximes towards cyclosarin, sarin, tabun, and VX inactivated-AChE and concluded that oxime therapeutics for the reactivation of sarin-inactivated AChE are conformationally dependent while oxime reverse therapeutics for VX require a compact region with a highly hydrophilic region and two positively charged pyridine rings. Medvedovici et al. [7] have reported chromatographic retention data obtained from interactions between oxime-type compounds and different stationary phases (involving hydrophobic interaction, ion pairing formation availability, H-bonding, dipole-dipole, ion-dipole, electrostatic interaction and glycoprotein binding ability). They have discussed some correlations between the determined descriptors and computational values. Hydrogen bond donor (expressed as the sum of OHs and NHs) and acceptor (denotes the sum of Ns and Os) counts also play vital role in predicting minimal distribution of a molecule in BBB. According to a review by Voicu et al. [6] the penetration of oximes at the CNS level somehow conditioned by the quaternary ammonium-based chemical structure, is undoubtedly necessary for improvement of the survival rate of organisms exposed to OP intoxication. Through some collective data they proved that oximes are compounds with a very low lipophilic character (negative log *P*). Consequently, their digestive absorption and bioavailability are very low, distribution is mainly plasmatic and BBB penetration is very weak. They are weak acids, partially ionized at the physiological pH. The ionized state (characteristic for quaternary ammonium structure) explains their nucleophilic properties (OP displacement at AChE level). They concluded that the oximes have similar PK/PD characteristics, involving reactivation ability of OP phosphorylated/phosphonylated AChE at the PNS level, however, the reactivating efficacy strongly depends on the intrinsic toxic character of the congener, the chemical structure and the targeted tissue. A recent attempt by Voicu et al. [11] to evaluate the transport of oximes through biological barriers from chromatographic retention data revealed complex interaction models mediated through hydrophobic/hydrophilic interactions, charge transfer (including π-π interactions) and protein binding ability. Another chromatographic retention study designed for evaluation of the ion pairing ability of oximes [12] highlighted a complex and unexpected behavior. The chemistry of oximes in physiological conditions is undoubtedly extremely complicated and needs further clarification. Their complicated chemistry strongly influences their ability to penetrate through biological barriers. Oximes, in first instance, due to their polar quaternary ammonium-based chemical structure, are far from fulfilling the minimum conditions requested for BBB penetration at an efficient concentration level. Cerebral AChE reactivation is not necessarily illustrative of oxime protective effect [13]. The explanation for the synergistic therapeutic effects of oximes

together with other antidotes, resides in the peripheral effects on respiratory muscles and the increase in oxygen supply, also having indirect effects on seizures in OP intoxication, but also on other effects not correlated with the tissue.

Despite sustained efforts to discover improved reactivators, there has been little success to discover effective broad-spectrum AChE reactivators. For the evaluation of a potential reactivator as an antidote, knowledge of physicochemical parameters is not sufficient, *in-vitro* and *in-vivo* reactivation efficacies of reactivators toward inhibited AChE are equally significant. This work can be considered as an extension of our previous findings [5] and the aim of the present approach was to determine the various physicochemical properties, ionization constants; pK_a, lipophilicity; log*P* and hydrogen bond donor (HBD) and acceptor (HBA) counts of oxime based xylene linkedAChE reactivators using experimental and computational methodology. Xylene linked bis-pyridinium oximes have gained considerable interest in the recent past owing to their improved reactivation potential for OP inhibited AChE [14]. Further, structure-activity relationship (SAR) and docking studies with xylene linked pyridinium oximes revealed π-π and cation-π interactions within the enzyme active-sites [15]. Acharya et al. have also reported symmetrical bis-pyridinim oximes linked by a xylene, methoxyalkane and methoxymethyl benzene as AChE reactivators [16-18]. Some of these oximes have shown promising reactivation potential against OP inhibited AChE. Reactivation potential of such oximes attracted our attention to further explore xylene linked pyridinium oximes. In this context they [19] reported carbamoyl bis-pyridinium mono oximes having a xylene linker between two pyridinium rings with an aim to enhance the lipid solubility and stability, which is found to be low with the currently available antidotes [20] as well as to allow the hydrophobic interactions with some of the aromatic residues that remain in the active site gorge of the enzyme acetylcholinesterase [21].

Table 1. Chemical structure of investigated oximes

Compound	M.W.	Structure
Obidoxime	359.21	
HI-6	478.50	
K740	537.24	
K741	537.24	

Compound	M.W.	Structure
K745	537.24	
K746	537.24	
K747	537.24	

MATERIALS AND METHODS

Chemicals

All the investigated oximes (Table 1) were prepared and characterized at the Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defence, Czech Republic. All the reagents used were of analytical grade. Triply distilled water was used throughout.

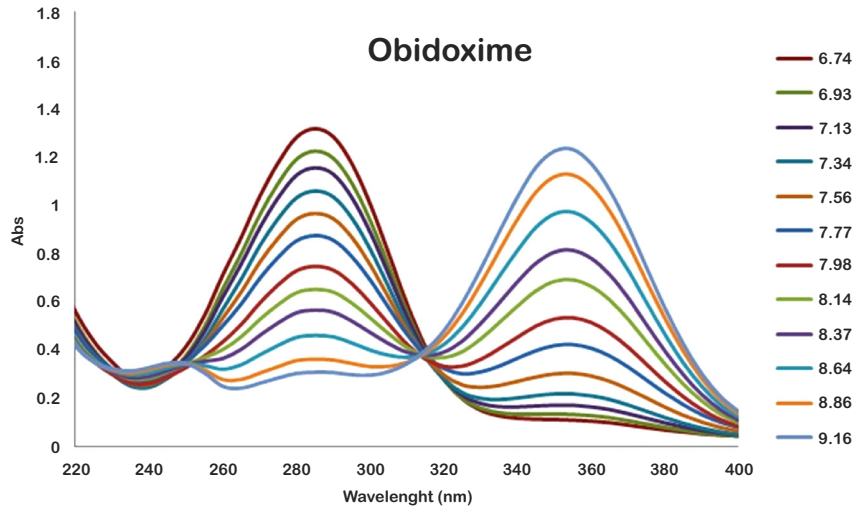
Determination of acid dissociation constant (pK_a) by Albert and Sergeant Method

The pK_a's of oximes have been determined by spectrophotometric method using a Synergy HT spectrophotometer (Biotek, USA). All the spectrophotometric measurements were made at 27±0.5°C and the spectra were recorded within the range of 200-400 nm. The electronic absorption spectra of the examined compounds were recorded in aqueous solutions of different pH, as represented in Figure 1. The shorter wavelength absorption maximum, appearing at lower pH values, reflects the absorption of the non-ionized oxime group(s) in all investigated compounds, whereas the longer wavelength maximum, observed at higher pH values, is due to the absorption of the reactivator with an ionized oxime group(s). The pH-dependent absorption spectra show the presence of isosbestic point. The pK_a values have been evaluated from the absorbance *vs*. pH data by the general method of Albert and Sergeant [22] using eq 3 where, Abs_{Ψ}, Abs_{HOx} and Abs_{Ox} represent the absorbance of partially ionized, unionized and completely ionized form of oxime respectively at particular pH.

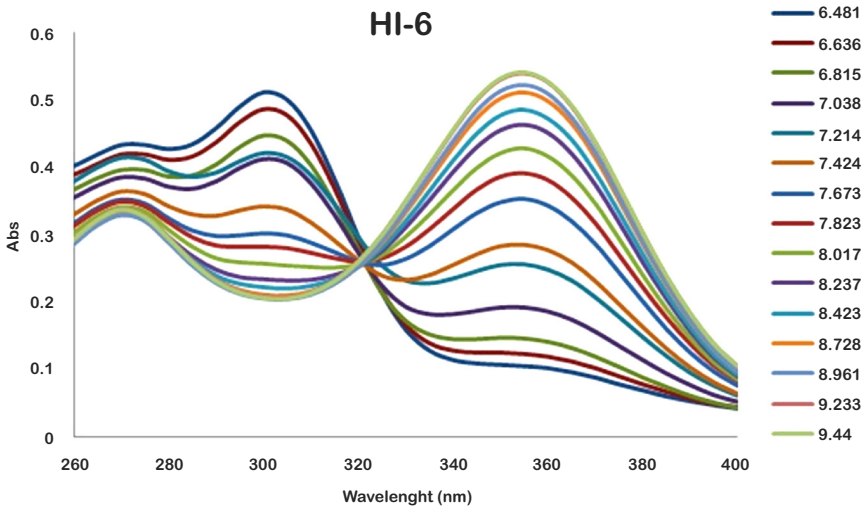
$$HA \leftrightarrow H^+ + A^- \quad (1)$$

$$K_a = \frac{[H^+][A^-]}{[HA]} \quad (2)$$

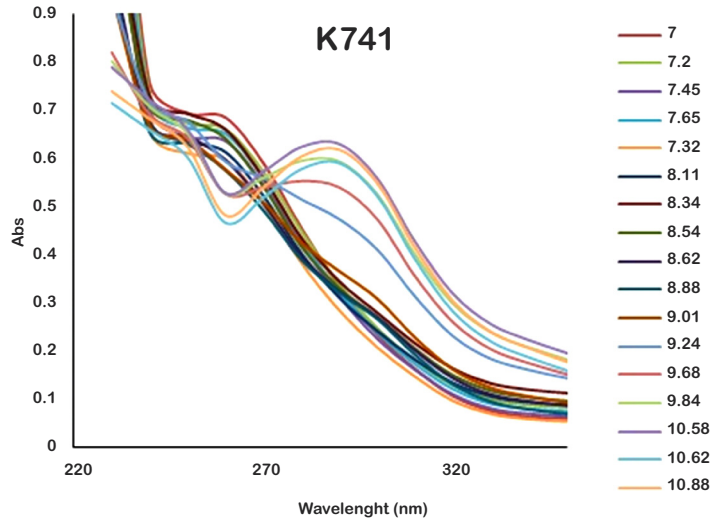
$$pK_a = pH_{exp} - \log \frac{Abs_{\Psi} - Abs_{HOx}}{Abs_{Ox} - Abs_{\Psi}} \quad (3)$$


Obidoxime
Abs
Wavelenght (nm)
6.74
6.93
7.13
7.34
7.56
7.77
7.98
8.14
8.37
8.64
8.86
9.16

a


HI-6
Abs
Wavelenght (nm)
6.481
6.636
6.815
7.038
7.214
7.424
7.673
7.823
8.017
8.237
8.423
8.728
8.961
9.233
9.44

b


K741
Abs
Wavelenght (nm)
7
7.2
7.45
7.65
7.32
8.11
8.34
8.54
8.62
8.88
9.01
9.24
9.68
9.84
10.58
10.62
10.88

c

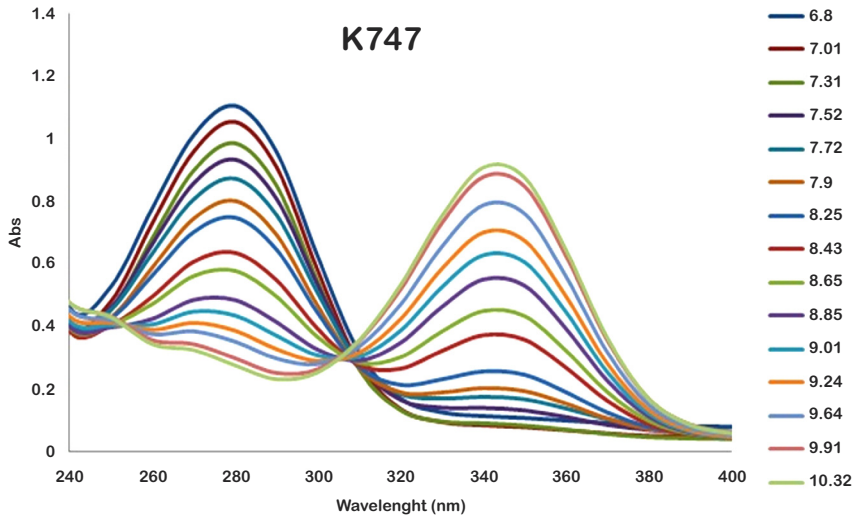


Figure 1. Representative absorption spectra of (a) obidoxime (b) HI-6 (c) K741 (d) K747in aqueous solutions of different pH values (indicated by vertical column), c= 5.3×10^{-5} M; at 27 °C.

RESULTS and DISCUSSIONS

Acid dissociation constants and molecular descriptors were calculated for five bis-pyridinium monooximes with xylene linkers and carbamoyl functionality. HI-6 and obidoxime were chosen for comparative analysis of results. The selected pH range for recording the spectra of examined reactivators was from 6.0-11.0 pH. As represented in Fig. 1 (a, b), absorption electronic spectra showed two absorption maxima for obidoxime (285 and 355 nm) and HI-6 (300 and 355 nm). For K740 and K741 maxima were observed at wavelength of 260 and 290 nm but effect of pH on shorter wavelength (260 nm) was not much pronounced. For K745, 746 and 747 maxima were obtained at wavelength of 280 and 350 nm but both the wavelengths were adequately pH sensitive. Effect of pH on the absorption spectra was reflected by the dissociation of either oxime groups and was represented by two overlapping ionization equilibria (isosbestic point). It was observed that the calculated pK_a values for oximes lie in the range of 7.45-9.85 which is well in agreement with those of known oxime reactivators. pK_a values of structurally different xylene linked bis-pyridinium oximes which were reported by Acharya et al. [19,23]. The group has experimentally determined the pK_a with the help of Albert and Sergeant method. The observed range for the pK_a was 7.66-8.65.

Experimental and software aided pK_a values were in agreement for HI-6, obidoxime and K740 but this was not the case with rest of the oximes. For K-741, -745, -746 and -747 a substantial difference was noted. Oximes K740 and K747 had their pK_a values quite similar to that of obidoxime and all the tested oximes showed higher pK_a as compared to that of HI-6. Obidoxime showed two pK_a values (in agreement) due to the presence of two oxime groups at symmetrical position in the pyridinium rings. Since ethyl carboxyl group is not a proton donor, bis-pyridinium oximes with mono-carbamoyl functions provided only one pK_a value.

Drug properties like lipophilicity are of supreme importance in several other ADME aspects, that is, absorption, distribution, metabolism, and excretion. It is generally held that very lipophilic compounds are "preferred" targets for metabolism, often leading to high clearance values and, quite often, lipophilicity positively correlates with a high plasma protein binding. A large volume of distribution, probably due to a high fraction of the compound bound to tissues, is often observed for lipophilic compounds. Thus, a method that can accurately and rapidly yield log*P* values would be a welcome addition to the experimental tools available for physicochemical properties screening in the discovery setting. It should be considered here that experimental and software calculated parameters may be often disputed. Values may also differ from software to other softwares [24]. It has been very rationally discussed by Lombardo et al. [25] that, for drug molecules, computed values are often inaccurate and, depending on the software used, they may differ by as much as two log*P* units, among

different software packages and from the experimental values, for an entire class of compounds.

For newly synthesized compounds, properties of absorption, distribution, metabolism and excretion ("ADME") are critical for determining the future potency of compounds in clinical practice. Indeed, the "Lipinski rule of 5" (Ro5) quantifies the properties that compounds should possess to be eligible for successful penetration through biological membranes [26,27]. This rule states that poor absorption or permeation is more likely when there are more than 5 H-bond donors, 10 H-bond acceptors, the molecular weight is greater than 500 and the calculated Log *P* is greater than 5. Lipinski et al. [26] stated these properties for drug candidates to be used orally, however it can be more or less applied to all drug formulations with appropriate modifications. This simple set of rules does not provide an exhaustive study of all the descriptors that define overall compound drug-likeness, the Ro5 specifically offers a fair estimation of drug-likeness with regard to good *in vivo* permeability and is the most quick profiling tool for drug likeness [28]. Although the charged structure and hydrophilic nature of oxime moiety makes pyridinium oximes rather less permeable to BBB, study of molecular descriptors is essential for prediction of their acute properties [29-32]. Herein, we have reported lipophilicity and hydrogen bonding parameters of tested oximes calculated by MarvinSketch version 6.2.2, 2014 (http://www.chemaxon.com). Sometimes, ionization of a molecule may result in decreased lipophilicity with respect to the neutral state, it may be more appropriate to take the ionic state of the compound into account when describing the lipophilicity of potential drugs. In this context log *D* may be a more reliable parameter than log *P* and is defined as a descriptor of the octanol/water distribution coefficient for partitioning of ionizable species in biphasic media. Hydrogen bonding donor counts were found in the range of 1-2 and hydrogen bonding acceptor counts were in the range of 3-5 for investigated oximes. We have already proved in our previous findings [5, 33] that in case of bis-pyridinium oximes, connecting linkers as well as presence of oxime and other functionality along with their position have a pivotal role on their physicochemical properties and reactivation potencies. Oximes with aliphatic linkers are superior reactivators than the oximes with unsaturated and aromatic linkers. All the bis-quaternary oximes showed negative log *P* values and this can be attributed to the presence of quaternary nitrogen. Calculated log *P* value of obidoxime (-3.40) was in good agreement with computational predicted values reported in literature [6,34]; however the values were much lower as compared to our experimental calculations reported previously [5]. It should be considered that the high molecular weight and negative log *P* of tested xylene linked oximes could be a discouraging factor while exploring their reactivation potencies because these can lead to low lipophilic character consequently leading to lower BBB permeability. However many other factors (physical and chemical properties) contribute towards the reactivation ability and this should be studied at a broader scale to properly understand the probability of their role in CNS. Voicu et al. [6] well argued that oxime structures especially designed for enhanced BBB penetration have no real viewpoints. The improved penetration of BBB produce slow AChE reactivation and not exactly resulting in a sensible increase of the survival rate of organisms exposed to OP intoxication. They also commented that increasing penetration through the BBB may also produce significant neurotoxic effects. Although, the study of physicochemical parameters is the first preferred characterization step to analyze any oxime as reactivator but the quantification of its maximum reactivation ability as an antidote involves detailed investigation of reactivation kinetics against inhibited AChE and also the evaluation of its intrinsic inhibitory potency. Hence, the detailed *in-vitro* study will be reported in due course of time.

Recently, different methods have been developed by the scientific community for the calculation of the drug properties (especially lipophilicity and solubility) of chemical compounds. Tetko et al. [35] have very finely discussed the aspect that it is possible for some methods to be more or less appropriate for specific types of compounds being analyzed. Thus it may be interesting to compare the calculated properties using several different methods and then one can decide which software should be used for his/her data series. They developed a program for the single-compound mode that displays the lipophilicity results calculated by their own model, ALOGPS, and three other programs, namely, CLOGP, KOWWIN and XLOGP. We can consider this point for our future investigation so as to analyze the difference in predicated data by using different computational programs and discuss the similarity or variances observed. However there are some efforts available in the literature to compare the molecular descriptors calculations based on different softwares [36,37]. The most precise results available

Table 2. Acid dissociation constants, lipophilicity and hydrogen bond counts of investigated oximes

S.N.	Compound	Experimental[#]	Computational*			
		pK_a	pK_a	Log *P*	HBD	HBA
1	HI-6	7.45±0.06	7.44	-6.58	2	4
2	Obidoxime	8.48±0.05	8.55 (pK_{a1}) 8.65 (pK_{a1})	-3.40	2	5
3	K740	8.56±0.04	8.55	-4.13	2	4
4	K741	8.14±0.05	9.81	-4.13	1	3
5	K745	8.01±0.02	9.81	-3.99	1	3
6	K746	8.38±0.04	9.85	-3.99	1	3
7	K747	7.92±0.02	8.56	-3.99	1	3

[#] mean values obtained by Albert & Sergeant Method

[*] values evaluated by Marvin Sketch version 6.2.2, 2014 (http://www.chemaxon.com).

are generally obtained from *ab-initio* calculations, meaning that these calculations tend to converge to an exact mathematical solution. However, since the equations used to describe the system are entirely derived from theoretical principles, any flaw in the model will still produce a result that may deviate from its physical value. For instance, considerable difficulties exist when attempting to calculate the pK_a value in a solvated or aqueous phase, since the problem of how to accurately take solvent-analyte interactions into account remains largely unsolved [38]. The most precise results available are generally obtained from *ab-initio* calculations, however, since the equations used to describe the system are entirely derived from theoretical principles, any flaw in the model will still produce a result that may deviate from its physical value. For instance, considerable difficulties exist when attempting to calculate the pK_a value in a solvated or aqueous phase, since the problem of how to accurately take solvent-analyte interactions into account remains largely unsolved [38]. Also, Semi-empirical quantum-mechanical (QM) methods are there, based on the same formalisms as the *ab-initio* calculations, but make use of further approximations and obtain many parameters from experimental data [39]. More recently, quantum structure-property relationship (QSAR) based methods have been developed and can be considered as a further development of the semi-empirical methods. In terms of the accuracy of their predictions, computational methods suffer where multiple competing models exist, since the choice of parameters and starting assumptions can dramatically affect the outcome of the calculations. In addition, there are often significant deviations from literature values [38]. Despite these problems, there is still considerable interest in computational methods in drug discovery, where the method might be used to reduce uncertainty of the physical/chemical properties of molecules without even having to synthesize them at all. However, for the time being, the method is purely of interest as an estimator, since the reliability of the results leaves much to be desired. This method may find more application in the future, but for now, computational methods are competing with actual measurement in terms of accuracy. In experimental methods, matrix effects are significant, especially ionic interaction between analyte and buffer ions whereas in theoretical calculations these effects are mostly overlooked. These reasons can account for the changes observed in our experimental and computational calculated pK_a values shown in Table 2.

CONCLUSIONS

Acid dissociation constants and molecular descriptors were calculated for five bis-pyridinium mono oximes with xylene linkers and carbamoyl functionality by experimental and software techniques. The results of the present investigation may be useful for the study of inhibited-AChE reactivation with xylene linked bispyridium oximes. Such molecular properties are key parameters in quantitative structure activity relationship studies and can be informative towards the overall understanding of the uptake distribution, biotransformation, and elimination of drug like molecules. Such coefficients may play a pivotal role in the process of drug discovery and the development from molecular

design to pharmaceutical formulation. The report also gives an idea of physicochemical and molecular properties of carbamoyl functionalized bispyridiniumoximes in relation to standard oximes (obidoxime and HI-6).

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