# The Development of New Oximes and the Evaluation of their Reactivating, Therapeutic and Neuroprotective Efficacy Against Tabun

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**Abstract:** Tabun (O-ethyl-N,N-dimethyl phosphoramidocyanidate) belongs to highly toxic organophosphorus compounds misused as chemical warfare agents for military as well as terroristic purposes. The antidotal treatment of tabun acute poisonings still represents a serious problem and the development of new, more effective AChE reactivators to achieve the satisfactorily effective antidotal treatment of acute poisonings with tabun still represents very important goal. Since 2003, we have prepared around 200 new AChE reactivators. Their potency to reactivate tabun-inhibited acetylcholinesterase has been subsequently evaluated using our *in vitro* screening test. Afterwards, promising compounds were selected and kinetic parameters and reactivation constants were determined. Then, the best reactivators were subjected to the *in vivo* studies (toxicity test, the evaluation of therapeutic, reactivating and neuroprotective efficacy) and their potency to counteract the acute toxicity of tabun is compared to the therapeutic, reactivating and neuroprotective efficacy of commonly used oximes – obidoxime and the oxime HI-6.

According to the results obtained, the newly synthesized oxime K075 showed the highest potency to reduce tabun-induced acute lethal toxicity while the therapeutic potency of obidoxime and the oxime HI-6 was significantly lower. The therapeutic efficacy of oximes studied corresponds to their reactivating efficacy *in vivo* as well as *in vitro*. The potency of all newly synthesized oximes to reactivate tabun-inhibited AChE is comparable with obidoxime with the exception of K074 that is significantly more efficacious in the brain. In addition, all newly synthesized oximes combined with atropine seem to be effective antidotes for a decrease in tabun-induced acute neurotoxicity. While the neuroprotective efficacy of obidoxime in combination with atropine is similar to the potency of newly synthesized oximes, the ability of the oxime HI-6 combined with atropine to counteract tabun-induced acute neurotoxicity is significantly lower. Due to their therapeutic, reactivating and neuroprotective efficacy, all newly synthesized oximes appear to be suitable oximes for the antidotal treatment of acute tabun poisonings.

**Key Words**: Tabun, acetylcholinesterase, K oximes, HI-6, obidoxime, atropine, rats, mice.

## INTRODUCTION

The current standard treatment for poisoning with organosphosphorus compounds called nerve agents usually consists of the combined administration of anticholinergic drugs (preferably atropine) and oximes (preferably pralidoxime or obidoxime). Anticholinergic drugs block the effects of overstimulation by acetylcholine accumulated at muscarinic receptor sites while oximes, compounds with nucleophilic bases, repair biochemical lesions by dephosphorylating tabun-inhibited acetylcholinesterase (AChE, EC 3.1.1.7) and restoring its activity [1,2].

Tabun (O-ethyl-N,N-dimethyl phosphoramidocyanidate; GA) Fig. (**1**) is an organophosphorus compound used as chemical warfare agent for military as well as terroristic purposes. It differs from other organophosphates in its chemical structure and that tabun-inhibited AChE is difficult to reactivate. Its deleterious effects are extraordinarily difficult to counteract because of the existence of a lone electron pair located on a dimethylamide group that makes nucleophilic attack almost impossible [3-5]. Another view on the resistence of tabun-inhibited AChE treatment was provided by Ekström and co-workers [6]. According to this study, the crystal structures of murine AChE showed that non-aged tabun conjugate induces structural changes in H447 and its hydrogen bonds. Moreover, the conformational change of P338 position partially closes the narrow AChE gorge. After aging reaction, the tabun molecule is coordinated in the AChE gorge and phosphoroamidoyl group is replaced by a water molecule. Due to these structural changes, the potency of AChE reactivators to split the bond between inhibitor and enzyme is lower in comparison with other nerve agents.

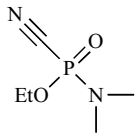


**Fig. (1).** Chemical structure of tabun.

While anticholinergic drugs such as atropine are able to counteract the effects of tabun at peripheral cholinergic receptors [7], commonly used reactivators of phosphorylated AChE based on monopyridinium (e.g. pralidoxime) and bispyridinium oximes (e.g. obidoxime, methoxime) are not able

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to counteract the adverse effects of tabun because of minimal reactivating efficacy [8]. In addition, the reactivating efficacy of the oxime HI-6 which is relatively efficacious against acute toxicity of soman [9], is not as efficient for tabun-inhibited AChE [10,11]. Therefore, the replacement of commonly used oximes (pralidoxime, obidoxime) as well as H oximes (the oxime HI-6) with a more effective oxime has been a long-standing goal for the treatment of tabun poisoning. Our attention was focused on the development of new accessible, non-toxic and *in vitro* and *in vivo* potent AChE reactivator of tabun-inhibited AChE. Our development of new reactivators of tabun inhibited-AChE was divided into three steps:

- Synthesis of new AChE reactivators based on structure-activity relationship studies
- *In vitro* evaluation of new AChE reactivators potency
- *In vivo* evaluation of their therapeutic, reactivating and neuroprotective efficacy

Since 2003, we have prepared around 200 new AChE reactivators. For this purpose, general organic chemistry approaches were used. Then, many results in synthesis and *in vitro* screening evaluation of tabun-inhibited AChE reactivators were obtained during last years [12-19]. Based on these results, structural requirements needed for sufficient reactivation of tabun-inhibited AChE were determinated. There are five most important structural factors influencing the affinity of the AChE reactivators toward inhibited AChE and subsequent oxime reactivity [20]: presence of the quaternary nitrogen in the reactivator molecule, length of the connection chain between two pyridinium rings, presence of the oxime group, position of the oxime group at the pyridinium ring and number of oxime groups in the reactivator structure.

The structure-activity relationship study helps us to design new structures of promising reactivators of tabun-inhibited AChE: 1-(4-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-propane dibromide (K027), 1-(4-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-butane dibromide (K048), 1,4-bis(4-hydroxyiminomethylpyridinium) -butane dibromide (K074) and 1,4-bis(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide (K075) were synthesized at our Department of Toxicology [21-23] Fig. (**2**) to improve the efficacy of antidotal treatment in reactivating tabun-inhibited AChE and eliminating tabun-induced acute lethal toxicity including neurotoxicity. The evaluation of their potency to reactivate tabun-inhibited AChE using *in vitro* methods showed that the reactivating efficacy of all newly developed oximes is similar to the effectiveness of obidoxime. They seem to be better reactivators of tabun-inhibited AChE than HI-6 especially at concentrations corresponding to human-relevant concentrations ($10^{-5}$ - $10^{-4}$ M) [23,24]. *In vitro* assessment of reactivating efficacy of oximes is usually followed by the evaluation of their reactivating efficacy *in vivo* and their therapeutic and neuroprotective efficacy against lethal nerve agent poisoning [1-2,25-27]. The aim of this review was to summarize the reactivating, therapeutic and neuroprotective efficacy of newly synthesized oximes (K027, K048, K074, K075) and currently available oximes (obidoxime, the oxime HI-6) against tabun using *in vitro* and *in vivo* methods.

## THE EVALUATION OF REACTIVATING EFFICACY OF NEW OXIMES *IN VITRO*

The *in vitro* evaluation of the reactivating efficacy of newly synthesized and currently available oximes was conducted by the standard potentiostatic method described by Kuca and co-workers [28,29].

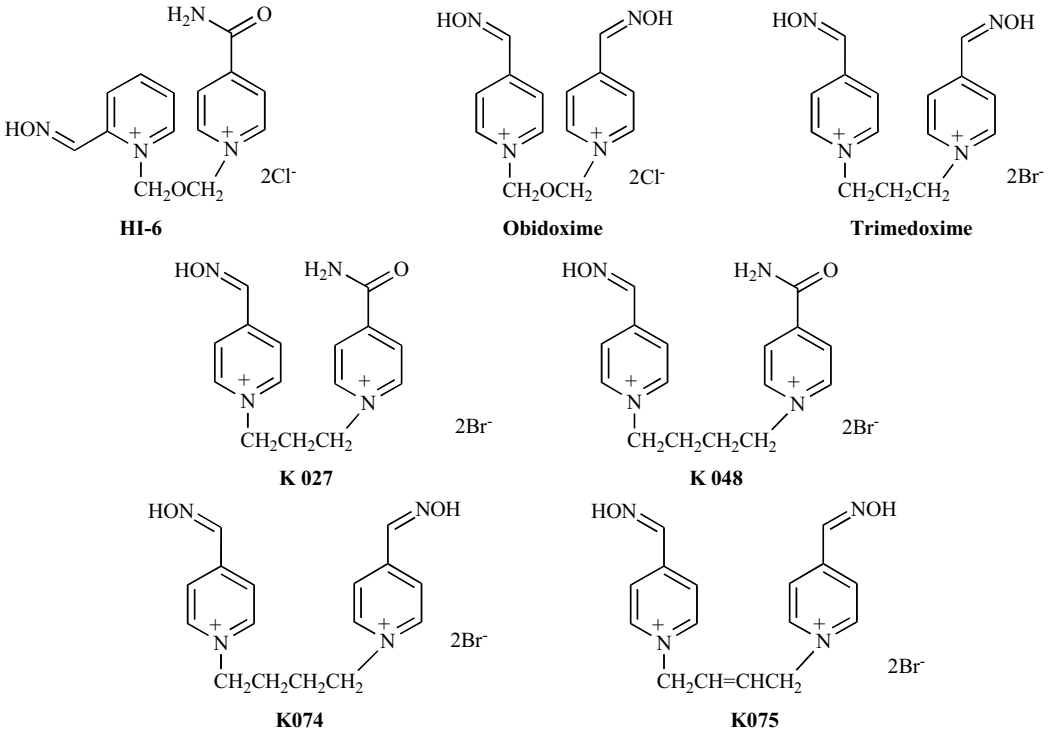


**Fig. (2).** Chemical structure of oximes.

The screening test was used as the first step of evaluation of the large quantity of new oximes to reactivate tabun-inhibited AChE *in vitro*. As the results, we have obtained a percentage of reactivation. Only promising oximes together with standards were tested in the second step of *in vitro* investigation where their kinetic parameters of the reactivation were calculated (Table **1**). According to the dissociation constant of inhibited enzyme-reactivator complex values, the oxime HI-6 had higher affinity towards tabun-inhibited enzyme if compared to K075. On the contrary, the first-order rate constant of reactivation ($k_R$) is higher for K075 than for HI-6. Regarding to the second-order rate constant of reactivation ($k_r$), calculated as ratio $k_R/K_R$, that characterizes the whole reactivation process, the oxime K075 seems to be better reactivator than HI-6. Generally, all above-mentioned, newly synthesized oximes seemed to be very promising and they were recommended for their *in vivo* investigation [23,28,30-33].

## THE EVALUATION OF REACTIVATING, THERAPEUTIC AND NEUROPROTECTIVE EFFICACY OF NEW OXIMES *IN VIVO*

To realize *in vivo* experiments, male albino Wistar rats weighing 180-220g and NMRI mice weighing 15-17g were used. Tabun was obtained from Military Technical Institute in Brno (Czech Republic) while obidoxime, the oxime HI-6 and K oximes (K027, K048, K074, K075) were synthesized at the Department of Toxicology of the Faculty of Military Health Sciences in Hradec Kralove (Czech Republic). All other drugs and chemicals of analytical grade were obtained commercially and used without further purification. All substances were administered intramuscularly (i.m.) at a volume of 1 mL/kg body weight (b.w.) in rats and 10mL/kg b.w. in mice.

Before starting the evaluation of reactivating, therapeutic and neuroprotective efficacy of all newly synthesized oximes, the acute toxicity of oximes in mice was evaluated by the assessment of the $LD_{50}$ values and their 95% confidence limit using probit-logarithmical analysis of death occurring within 24 hr after i.m. administration of an oxime at 5 different doses with 8 animals per dose [34]. The acute i.m. toxicity of all oximes studied is summarized in Table **2**. The results show that the acute toxicity of newly synthesized oxime K048 for mice corresponds to the acute toxicity of obidoxime, while another newly developed oxime K027 is less toxic and its $LD_{50}$ value is approaching to $LD_{50}$ value of the oxime HI-6 that is considered to be the least toxic for mammals among currently available oximes. On the other hand, the acute toxicity of other newly synthesized K oximes (K074, K075) for mice is markedly higher in comparison

**Table 1.** ***In Vitro*** **Reactivation of Tabun-Inhibited AChE**

| Oxime | *Enzyme* | $K_R$ [μM] | $k_R$ [$min^{-1}$] | $k_r$ [$min^{-1}. M^{-1}$] |
|---|---|---|---|---|
| Pralidoxime | Rat | 575 | 0.006 | 10 |
| Methoxime | Rat | -* | -* | -* |
| Trimedoxime | Rat | 460 | 0.079 | 172 |
| Obidoxime | Rat | 3 | 0.020 | 6250 |
| HI-6 | Rat | 6 | 0.007 | 1111 |
| K027 | Rat | 54 | 0.015 | 273 |
| K048 | Rat | 93 | 0.032 | 348 |
| K074 | Rat | 29 | 0.056 | 1931 |
| K075 | Rat | 19 | 0.056 | 2947 |
| Pralidoxime | Human | -* | -* | -* |
| Methoxime | Human | 2512 | 0.05 | 20 |
| Trimedoxime | Human | 1585 | 0.08 | 50 |
| Obidoxime | Human | 1412 | 0.06 | 42 |
| HI-6 | Human | -* | -* | -* |
| K027 | Human | 2512 | 0.05 | 20 |
| K048 | Human | 251 | 0.06 | 239 |
| K074 | Human | 1995 | 0.08 | 40 |
| K075 | Human | 200 | 0.02 | 100 |

$K_R$ - dissociation constant of inhibited enzyme-reactivator complex;

$k_R$ - the first-order rate constant of reactivation;

$k_r$ - the second-order rate constant of reactivation

* appropriate constants cannot be calculated due to extremely low potency of this oxime to reactivate tabun-inhibited acetylcholinesterase

**Table 2. $LD_{50}$ Values of Oximes Following i.m. Administration in Mice**

| Antidote | $LD_{50}$ (mg/kg) ± 95% Confidence Limit |
|---|---|
| HI-6 | 671.3 (627.4 – 718.3) |
| Obidoxime | 188.4 (156.3 – 208.0) |
| K027 | 577.0 (539.0 – 617.8) |
| K048 | 233.5 (217.8 – 250.3) |
| K074 | 23.3 (20.6-26.4) |
| K075 | 43.0 (38.6-47.8) |

with other oximes studied. Their $LD_{50}$ value is more than fifteen times lower than $LD_{50}$ value of the oxime HI-6. The high toxicity of oximes K074 a K075 is probably caused by the presence of two oxime groups in their chemical structure. Generally, the toxicity of reactivators having in their chemical structure two oximes groups is higher in comparison with oximes having carbamoyl group instead of one oxime group [35].

To evaluate the therapeutic efficacy of newly synthesized oximes against supralethal poisoning with tabun in mice was evaluated by above mentioned method [34]. To describe their therapeutic efficacy, the oximes were i.m injected at equitoxic doses corresponding to 5% of their $LD_{50}$ value in combination with atropine (21 mg/kg) 1 min after tabun administration. The influence of the nature of the antidotal treatment was expressed as protective ratio ($LD_{50}$ value of treated mice/ $LD_{50}$ value of untreated mice). The therapeutic efficacy of all oximes studied in combination with atropine against tabun is presented in Table **3a**,**b**. Tabun–poisoned mice showed wide spectrum of clinical signs of poisoning including muscarinic (lacrymation, salivation, chewing, miosis) and niconitic (tremor, tonic-clonic convulsions) signs within a few minutes. In the case of antidotal treatment, the clinical signs appeared later and their intensity was diminished compared to untreated poisoning regardless of the type of oxime. They died within 20-30 minutes after poisoning with tabun if they were not treated with antidotes. All antidotal mixtures used for the antidotal treatment of acute tabun poisoning were potent to significantly decrease acute toxicity of tabun with the exception of the oxime HI-6 in combination with atropine (Table **3b**). All newly synthesized oximes (K027, K048, K074, K075) were able to significantly decrease the acute toxicity of tabun almost 2-fold and their therapeutic efficacy was higher than the therapeutic efficacy of obidoxime and HI-6 [36,37].

**Table 3a. The Potency of Oximes in Combination with Atropine to Eliminate Acute Lethal Effects of Tabun in Mice**

| Treatment | $LD_{50}$ (µg/kg) ± 95% IS | Protective Ratio |
|---|---|---|
| ---- | 295.2 (275.5 – 317.9) | ---- |
| Obidoxime + atropine | 435.7 (410.8 – 460.5)* | 1.42 |
| HI-6 + atropine | 485.3 (461.9 – 511.5)* | 1.64 |
| K027 + atropine | 497.1 (468.1 – 528.1)*x | 1.68 |
| K048 + atropine | 517.8 (481.4 – 556.6)*x | 1.75 |

* significantly different from non-treated group at the level of $P < 0.05$,

x significantly different from the group treated with obidoxime at the level of $P < 0.05$.

**Table 3b. The Potency of Oximes in Combination with Atropine to Eliminate Acute Lethal Effects of Tabun in Mice**

| Treatment | $LD_{50}$ (µg/kg) ± 95% IS | Protective Ratio |
|---|---|---|
| ---- | 318.0 (305.5 – 331.0) | ---- |
| Obidoxime + atropine | 451.6 (418.8 – 487.5)* | 1.42 |
| HI-6 + atropine | 343.4 (336.9 – 349.5)* | 1.08 |
| K074 + atropine | 551.7 (490.3 – 620.7)*x | 1.74 |
| K075 + atropine | 635.3 (587.9 – 686.6)*x | 2.00 |

* significantly different from non-treated group at the level of $P < 0.05$,

x significantly different from the group treated with obidoxime and HI-6 at the level of $P < 0.05$.

To evaluate the reactivating efficacy of the oximes, the rats were injected i.m. with either atropine (21 mg/kg) alone or atropine (21 mg/kg) in combination with one of the oximes studied in equimolar dose (50 µmol/kg) 5 min before intramuscular tabun poisoning in rats. The control rats were administered i.m. with saline instead of tabun and antidotes at the same volume. The prophylactic administration of antidotes was used because this procedure is suitable for a mechanistic study that compares the reactivating efficacy of various oximes. The technique should give better results than the treatment of animals after poisoning and reduce the influence of aging of nerve agent-AChE complex [38]. The reactivating efficacy of oximes was evalutated in the whole blood hemolysate and brain homogenate 30 min following tabun poisoning at a dose corresponding to $LD_{50}$ (212.5 µg/kg) by the calculation of the reactivation rate (%) using the AChE activity values [38]. Statistical significance was determined by the use of Student`s t-test and differences were considered significant when $P < 0.05$ with the help of relevant computer program PRISM4 [34]. The ability of oximes to reactivate tabun-inhibited AChE in rat blood and brain *in vivo* is shown in Table **4a**,**b**. All newly synthesized oximes seemed to be effective reactivators of tabun-inhibited AChE. While the reactivating effectiveness of all newly synthesized oximes in the peripheral compartment (blood) is similar and corresponds to the efficacy of obidoxime, K074 appeared to be the best reactivator of tabun-inhibited AChE in the central compartment (brain) among all oximes studied. The oxime HI-6 has significantly lower potency in reactivating tabun-inhibited AChE in peripheral as well as central compartments [39,40].

To evaluate neuroprotective efficacy of oximes, the rats were administered with tabun at a sublethal dose (170 µg/kg b.w. - 80% $LD_{50}$). One minute following tabun challenge, the

**Table 4a. Rate of Reactivation of Tabun-Inhibited AChE by Oximes in Rat Blood and Brain *In Vivo*. AChE Activity (μkat/L or μkat/kg)**

| Treatment | Blood | Brain |
|---|---|---|
| Atropine | 2.23 ± 0.59[a] | 82.8 ± 20.5[a] |
| Atropine + obidoxime (% reactivation)[b] | 5.36 ± 0.46 (18.1[*x]) | 126.5 ± 17.1 (20.7[*]) |
| Atropine+ HI-6 (% reactivation) | 3.28 ± 0.61 (6.1) | 104.8 ± 21.3 (10.5) |
| Atropine+ K027 (% reactivation) | 5.14 ± 0.77 (16.9[*x]) | 129.3 ± 23.8 (22.1*) |
| Atropine+ K048 (% reactivation) | 5.94 ± 0.91 (21.5*[x]) | 115.4 ± 20.6 (15.5) |

[a] Means ± S.D., N = 8. The untreated control value for rat blood AChE activity was 19.5 μkat/L and for brain AChE activity 293.3 μkat/kg.

[b] Percent reactivation was determined using the AChE activity values: {1- [((saline control) – (oxime + atropine))/((saline control) – (atropine control))]} x 100.

[*] Significantly different from the atropine group at a level of $P < 0.05$, [x] significantly different from the atropine + HI-6 group at a level of $P < 0.05$ as determined by the Student`s test.

rats were treated with atropine (21 mg/kg b.w.) in combination with obidoxime, the oxime HI-6, K027, K048, K074 or K075 at equimolar doses corresponding to 50 μmol/kg b.w. According to our *in vitro* results, this dose level causes almost maximal % reactivation of tabun-inhibited AChE [23]. The neurotoxicity of tabun was monitored using the Functional observational battery at 24 hours following tabun poisoning. The evaluated markers of tabun-induced neurotoxicity in experimental animals were compared with the parameters obtained from control rats, that saline was administered instead of tabun and antidotes at the same volume. The Functional observational battery consists of 47 measurements of sensory, motor and autonomic nervous functions. Some of them are scored, the others are measured in absolute units [41-43]. Data collected with the Functional observational battery include categorial, ordinal and continuous values. Their statistical analyses were performed on a PC with a special interactive programme NTX [41]. The categorial and ordinal values were formulated as contingency tables and judged consecutively by Chi-squared test of homogeneity, Concordance-Discordance test and Kruskal-Wallis test, respectively. The continual data were assessed by successive statistical tests: CI for Delta, Barlett test for Equality of Variance, Williams test and Test for Distribution Functions [44]. The differences were considered significant when $P < 0.05$. The ability of newly synthesized oximes K027 and K048 to eliminate tabun-induced neurotoxicity at 24h following tabun poisoning is shown in Table **5**. The observation of neurotoxic signs indicated that many functional disorders of poisoned organisms outlasted at least 24 hours not only in non-treated tabun-poisoned rats but also in tabun-poisoned rats treated with atropine combined with the oxime HI-6. Tabun caused passive behavior of rats during handling and catching and a decrease in muscular tonus. The exploratory activity (rearing) was significantly decreased, gait and mobility were somewhat impaired and the level of unprovoked activity was reduced. Involuntary clonic movements were also observed. In addition, no reaction during a reflex testing consisting of recording each rat`s response to the frontal approach of the blunt end of a pen or a touch of the pen to the posterior flank was observed. No ability of pupils to constrict in response to light was demonstrated either. A significant decrease in the distance between hindpaws after a jump, forelimb and hindlimb grip strength, food receiving and spontaneous horizontal as well as vertical motor activity were also observed at 24 h following tabun challenge. Both newly synthesized oximes (K027, K048) in combination with atropine was able to eliminate many tabun-induced signs of neurotoxicity observed at 24 hours following tabun challenge with the exception of passive behavior of rats during handling and catching, a decrease in the exploratory activity, body temperature, food receiving and spontaneous motor activity. In addition, the oxime K027 was not able to eliminate a decrease in the distance between hindpaws after a jump and forelimb grip strength. On the other hand, the oxime HI-6 in combination with atropine was not able to eliminate or at least to decrease the intensity of most of above mentioned tabun-induced signs of neurotoxicity. Obidoxime in combination with atropine was almost as effective as newly developed oximes with the exception of approach response and hindlimb grip strength [45]. The potency of other newly synthesized oximes (K074 and K075) to eliminate tabun-induced neurotoxicity at 24h following tabun poisoning is shown in Table **6**. The newly synthesized oxime K075 in combination with atropine was able to prevent many tabun-induced signs of neurotoxicity observed at 24 hours following tabun challenge with the exception of a decrease in the distance between hindpaws after a jump, forelimb grip

**Table 4b. Rate of Reactivation of Tabun-Inhibited AChE by Oximes in Rat Blood and Brain *In Vivo*. AChE Activity (μkat/L or μkat/kg)**

| Treatment | Blood | Brain |
|---|---|---|
| Atropine | 4.55 ± 1.19[a] | 11.63 ± 1.27[a] |
| Atropine + obidoxime (% reactivation)[b] | 7.14 ± 0.66 (24.3*[x]) | 9.25 ± 1.54 (0) |
| Atropine+ HI-6 (% reactivation) | 3.95 ± 0.39 (0) | 12.40 ± 0.71 (6.7) |
| Atropine+ K074 (% reactivation) | 6.56 ± 1.04 (18.8*[x]) | 21.60 ± 3.03 (86.8*[x]) |
| Atropine+ K075 (% reactivation) | 6.64 ± 0.75 (19.6*[x]) | 13.22 ± 0.95 (13.9) |

[a] Means ± S.D., N = 8. The untreated control value for rat blood AChE activity was 15.2 (μkat/L) and for brain AChE activity 23.1 μkat/kg.

[b] Percent reactivation was determined using the AChE activity values: {1- [((saline control) – (oxime + atropine))/((saline control) – (atropine control))]} x 100.

[*] Significantly different from the atropine group at a level of $P < 0.05$, [x] significantly different from the atropine + HI-6 group at a level of $P < 0.05$ as determined by the Student`s test.

**Table 5. The Values of Tabun-Induced Neurotoxic Markers Measured at 24 Hours Following Tabun Challenge by the Functional Observational Battery (No 1-11, 14-36, 44 - Scored Values, No 12-13, 37-43, 45-47 - Values in Absolute Units). Abbreviations: GA – tabun; A – atropine; x/M – mean; -/+s – standard deviation. Statistical significance: * P < 0.05; ** P < 0.01; *** P < 0.001 (comparison with the control values)**

| 24 hours: | | Controls | | GA + A + HI-6 | | GA + A + Obi-doxime | | GA + A + K027 | | GA + A + K048 | | GA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No | Marker | x/M | -/+s | x/M | -/+s | x/M | -/+s | x/M | -/+s | x/M | -/+s | x/M | -/+s |
| 1 | posture | 1.00 | | 3.00 | | 1.00 | | 1.00 | | 1.00 | | 3.00 | |
| 2 | catch difficulty | 2.00 | | 1,00*** | | 1,00* | | 1,00* | | 1,00* | | 1,00*** | |
| 3 | ease of handling | 2.00 | | 1,00*** | | 1,00* | | 1,00* | | 1,00* | | 1,00*** | |
| 4 | muscular tonus | 0.00 | | -2,00*** | | 0.00 | | 0.00 | | 0.00 | | -2,00*** | |
| 5 | lacrimation | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 6 | palpebral closure | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 7 | endo/exophtalmus | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 8 | fur abnormalities | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 9 | skin abnormalities | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 10 | salivation | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 11 | nose secretion | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 12 | rearing | 23.38 | 8.19 | 10,00** | 7.21 | 6,63*** | 5.71 | 6,50*** | 6.28 | 12,50** | 4.50 | 12.83 | 10.30 |
| 13 | urination | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 14 | defecation | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 15 | hyperkinesis | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 16 | tremors | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 17 | clonic movements | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 2,00* | |
| 18 | tonic movements | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 19 | gait | 0.00 | | 1,00** | | 0.00 | | 0.00 | | 0.00 | | 7,00* | |
| 20 | ataxia | 0.00 | | 1,00** | | 0.00 | | 0.00 | | 0.00 | | 2,00* | |
| 21 | gait score | 0.00 | | 1,00*** | | 0.00 | | 0.00 | | 0.00 | | 2,00* | |
| 22 | mobility score | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 23 | arousal (GSC) | 1.00 | | 2,00*** | | 1.00 | | 1.00 | | 1.00 | | 4,00** | |
| 24 | activity | 4.00 | | 3.00 | | 2.00 | | 3.00 | | 4.00 | | 2.00 | |
| 25 | tension | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 26 | vocalisation | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 27 | stereotypy | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 28 | bizzare behavior | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 29 | approach response | 2.00 | | 2.00 | | 2,00* | | 2.00 | | 2.00 | | 1,00*** | |
| 30 | touch response | 2.00 | | 2.00 | | 2.00 | | 2.00 | | 2.00 | | 1,00*** | |
| 31 | click response | 2.00 | | 2.00 | | 2.00 | | 2.00 | | 2.00 | | 1.00 | |
| 32 | tail-pinch response | 2.00 | | 2.00 | | 2.00 | | 2.00 | | 2.00 | | 2.00 | |
| 33 | pupil size | 0.00 | | 2,00*** | | 1,00* | | 0.00 | | 0.00 | | -2,00** | |

**(Table 5. Contd….)**

| 24 hours: | | Controls | | GA + A + HI-6 | | GA + A + Obi-doxime | | GA + A + K027 | | GA + A + K048 | | GA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **No** | **Marker** | **x/M** | **-/+s** | **x/M** | **-/+s** | **x/M** | **-/+s** | **x/M** | **-/+s** | **x/M** | **-/+s** | **x/M** | **-/+s** |
| 34 | pupil response | 1.00 | | 0.50 | | 0,00** | | 1.00 | | 0,50* | | 0,00** | |
| 35 | RRF | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 36 | RRV | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 3,00* | |
| 37 | landing foot splay (mm) | 109.69 | 14.69 | 68,06*** | 31.49 | 81,56*** | 17.82 | 86,00* | 18.59 | 96.00 | 13.27 | 54,75*** | 36.09 |
| 38 | forelimb grip strength (kg) | 5.53 | 0.85 | 3,93*** | 0.77 | 3,79*** | 0.98 | 4,36* | 0.88 | 4.83 | 1.00 | 4,28* | 0.74 |
| 39 | hindlimb grip strength (kg) | 1.24 | 0.24 | 0,87** | 0.19 | 0,96* | 0.18 | 1.01 | 0.15 | 1.21 | 0.23 | 0.98 | 0.54 |
| 40 | grip strength of all limbs (kg) | 14.54 | 1.79 | 10.94 | 4.09 | 11,35* | 3.31 | 13.89 | 1.88 | 13.88 | 2.51 | 11.02 | 2.96 |
| 41 | food receiving (%) | 100.00 | 0.00 | 70,50* | 30.50 | 74,50*** | 6.21 | 77,00*** | 8.90 | 77,50*** | 3.21 | 48,75*** | 30.44 |
| 42 | body weight (g) | 186.00 | 14.74 | 191.71 | 17.57 | 186.63 | 25.86 | 190.38 | 19.42 | 197.13 | 19.97 | 182.67 | 24.33 |
| 43 | body temperature (°C) | 37.41 | 0.23 | 37.01 | 0.55 | 37.21 | 0.39 | 36,89*** | 0.55 | 36,86*** | 0.35 | 37.18 | 0.42 |
| 44 | respiration | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 45 | vertical activity | 362.00 | 90.71 | 139,75*** | 164.13 | 174,13*** | 80.67 | 260.75 | 122.36 | 225,75* | 105.02 | 181,25** | 145.50 |
| 46 | horizontal activity | 92.75 | 15.72 | 23,38*** | 40.66 | 22,50*** | 18.58 | 29,75*** | 14.73 | 36,75*** | 29.84 | 27,38*** | 33.17 |
| 47 | total motor activity | 454.75 | 96.99 | 163,13*** | 199.64 | 196,63*** | 97.59 | 290,50* | 123.64 | 262,50*** | 122.22 | 208,63*** | 175.94 |
| | | n=8 | | n=7 | | n=8 | | n=8 | | n=8 | | n=6 | |

strength, body temperature, food receiving and spontaneous motor activity. Another newly synthesized oxime K074 seems to be less efficacious than K075 because it was not able to eliminate tabun-induced miosis, a decrease in muscular tonus, passive behavior of rats during handling and catching and a decrease in rat`s response to the frontal approach of the blunt end of a pen. Obidoxime in combination with atropine was almost as effective as K075 with the exception of miosis. On the other hand, the oxime HI-6 in combination with atropine was not able to prevent or at least to decrease the intensity of some above mentioned tabun-induced signs of neurotoxicity [46].

## GENERAL DISCUSSION

Currently used obidoxime is able to partly reactivate tabun-inhibited AChE and eliminate tabun-induced acute neurotoxicity following i.m. administration of tabun at a lethal dose, nevertheless, its therapeutic, reactivating and neuroprotective efficacy is not satisfactory [25,47]. The oxime HI-6 has been produced and introduced by some countries for the antidotal treatment of severe acute poisonings with soman because of its higher reactivation and therapeutical efficacy compared to currently used oximes such as pralidoxime and obidoxime [2,9,48-50]. Nevertheless, it was demonstrated to be significantly less efficacious to reactivate tabun-inhibited AChE and eliminate tabun–induced acute neurotoxicity than obidoxime [25,47]. The potency of the above mentioned oximes to eliminate tabun-induced acute neurotoxicity is not sufficient because their ability to reactivate tabun-inhibited AChE is very low [4,10,11]. Therefore, new oximes have been synthesized to increase the reactivating potency as well as neuroprotective efficacy of antidotal treatment of acute tabun poisonings. New AChE reactivators able to satisfactorily reactivate AChE inhibited by tabun and counteract tabun-induced acute neurotoxicity have been still developed. There are two very important structural features forming the potency of oximes to reactivate tabun-inhibited AChE – the position of a functional oxime group at the pyridinium ring and the number of methylene groups in linking chain between two quaternary pyridinium rings in the molecule of reactivators [24, 28].

Our results demonstrate that all newly synthesized oximes (K027, K048, K074, K075) appear to be more effective to reactivate tabun-inhibited AChE and reduce tabun-induced acute toxicity than the oxime HI-6 and they are as effective as obidoxime. In addition, there is a small difference between the efficacy of K oximes to reactivate tabun-inhibited AChE and eliminate tabun-induced acute toxicity with the exception of reactivation of tabun-inhibited AChE in the brain by K074. The reason for their relatively high efficacy is probably a special chemical structure of their molecule. All of them are bispyridinium oximes with func-

**Table 6. The Values of Tabun-Induced Neurotoxic Markers Measured at 24 Hours Following Tabun Challenge by the Functional Observational Battery (No 1-11, 14-36, 44 - Scored Values, No 12-13, 37-43, 45-47 - Values in Absolute Units). Abbreviations: GA – tabun; A – atropine; x/M – mean; -/+s – standard deviation. Statistical significance: * P < 0.05; ** P < 0.01; *** P < 0.001 (comparison with the control values)**

| 24 hours: | | Controls | | GA + A + HI-6 | | GA + A + Obi-doxime | | GA + A + K074 | | GA + A + K075 | | GA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No | Marker | x/M | -/+s | x/M | -/+s | x/M | -/+s | x/M | -/+s | x/M | -/+s | x/M | -/+s |
| 1 | posture | 1.00 | | 3,00* | | 1.00 | | 1.00 | | 3.00 | | 3,00* | |
| 2 | catch difficulty | 2.00 | | 1,00*** | | 2.00 | | 1,00*** | | 2.00 | | 1,00*** | |
| 3 | ease of handling | 2.00 | | 1,00*** | | 2.00 | | 1,00*** | | 2.00 | | 1,00*** | |
| 4 | muscular tonus | 0.00 | | ´-1,00* | | 0.00 | | ´-2,00** | | 0.00 | | ´-2,00*** | |
| 5 | lacrimation | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 4,00*** | |
| 6 | palpebral closure | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 7 | endo/exophtalmus | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 8 | fur abnormalities | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 9 | skin abnormalities | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 10 | salivation | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 11 | nose secretion | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 3,00*** | |
| 12 | rearing | 11.75 | 4.68 | 2,00*** | 2.20 | 1,75*** | 0.89 | 2,63*** | 1.85 | 5.63 | 4.34 | 5.67 | 7.74 |
| 13 | urination | 1.00 | 1.69 | 2.63 | 7.03 | 0.00 | 0.00 | 1.75 | 3.24 | 0.75 | 0.71 | 4.00 | 5.90 |
| 14 | defecation | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 15 | hyperkinesis | 0.00 | | 0.00 | | 0.00 | | 3,00* | | 3,00* | | 0.00 | |
| 16 | tremors | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 2.00 | | 0.00 | |
| 17 | clonic movements | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 1.00 | |
| 18 | tonic movements | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 19 | gait | 0.00 | | 1,00** | | 5,00** | | 1,00*** | | 5,00** | | 7,00*** | |
| 20 | ataxia | 0.00 | | 1,00*** | | 1,00** | | 1,00** | | 1,00* | | 1,00** | |
| 21 | gait score | 0.00 | | 1,00*** | | 0.00 | | 0.00 | | 0.00 | | 1,00* | |
| 22 | mobility score | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 2.00 | | 3,00*** | |
| 23 | arousal (GSC) | 1.00 | | 3,00** | | 2,00** | | 2,00*** | | 2,00** | | 4,00*** | |
| 24 | activity | 4.00 | | 1,00*** | | 1,00*** | | 1,00*** | | 2.00 | | 1,00*** | |
| 25 | tension | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 26 | vocalisation | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 27 | stereotypy | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 28 | bizzare behavior | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 29 | approach response | 2.00 | | 1,00*** | | 2.00 | | 1,00* | | 2.00 | | 1,00* | |
| 30 | touch response | 2.00 | | 1,00*** | | 2.00 | | 1.00 | | 2.00 | | 1,00*** | |
| 31 | click response | 2.00 | | 2.00 | | 2.00 | | 3.00 | | 2.00 | | 3,00** | |
| 32 | tail-pinch response | 2.00 | | 2.00 | | 2.00 | | 2.00 | | 2.00 | | 1,00** | |
| 33 | pupil size | 0.00 | | ´-2,00*** | | ´-2,00* | | ´-2,00*** | | 0.00 | | ´-2,00*** | |

**(Table 6. Contd….)**

| 24 hours: | | Controls | | GA + A + HI-6 | | GA + A + Obidoxime | | GA + A + K074 | | GA + A + K075 | | GA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **No** | **Marker** | **x/M** | **-/+s** | **x/M** | **-/+s** | **x/M** | **-/+s** | **x/M** | **-/+s** | **x/M** | **-/+s** | **x/M** | **-/+s** |
| 34 | pupil response | 1.00 | | 0,00*** | | 0,50** | | 0,50*** | | 0,50*** | | 0,00*** | |
| 35 | RRF | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 3,00** | |
| 36 | RRV | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 3,00** | |
| 37 | landing foot splay (mm) | 121.13 | 13.72 | 87,38*** | 21.99 | 101,44* | 21.29 | 85,25*** | 12.48 | 93,13*** | 21.44 | 50,75*** | 33.08 |
| 38 | forelimb grip strength (kg) | 8.55 | 1.57 | 5,34*** | 0.58 | 6,48* | 1.94 | 5,39*** | 1.31 | 5,94*** | 1.49 | 5,58*** | 1.45 |
| 39 | hindlimb grip strength (kg) | 1.23 | 0.15 | 0.99 | 0.32 | 0,99* | 0.20 | 0,71*** | 0.25 | 1.01 | 0.30 | 0,40*** | 0.20 |
| 40 | grip strength of all limbs (kg) | 19.38 | 2.43 | 13,10* | 6.46 | 16.21 | 4.72 | 12,75*** | 4.39 | 14,21** | 4.03 | 8,07*** | 3.05 |
| 41 | food receiving (%) | 100.00 | 0.00 | 72,50* | 29.40 | 65,00*** | 26.73 | 50,00*** | 0.00 | 48,00*** | 2.14 | 7,50*** | 4.63 |
| 42 | body weight (g) | 259.88 | 25.18 | 278.63 | 20.32 | 264.50 | 34.83 | 253.75 | 22.80 | 253.00 | 16.76 | 248.17 | 11.70 |
| 43 | body temperature (°C) | 37.40 | 0.50 | 36.91 | 0.44 | 36,91* | 0.26 | 36.92 | 0.50 | 36,64** | 0.40 | 36,23*** | 0.35 |
| 44 | respiration | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 45 | vertical activity | 391.88 | 127.39 | 126,38*** | 127.79 | 181,00** | 83.61 | 152,00*** | 97.33 | 183,25*** | 95.99 | 110,75*** | 102.96 |
| 46 | horizontal activity | 93.75 | 44.55 | 15,88*** | 22.02 | 23,13*** | 24.79 | 18,13*** | 13.82 | 28,38* | 47.94 | 10,50*** | 11.78 |
| 47 | total motor activity | 485.63 | 160.93 | 142,25*** | 147.31 | 204,13*** | 103.76 | 170,13*** | 107.17 | 211,63*** | 140.60 | 121,25*** | 112.77 |
| | | n=8 | | n=8 | | n=8 | | n=8 | | n=8 | | n=6 | |

tional oxime group in the position four at the pyridinium ring and with three or four methylene groups in linking chain between two quaternary pyridinium rings in the molecule of reactivators. Thus, the position four of functional oxime group at the pyridinium ring and three or four methylene groups in linking chain between two quaternary pyridinium rings seem to be the most important requirements on the structural parameters of new sufficiently effective reactivators of tabun-inhibited AChE [20,51].

## CONCLUSION

In conclusion, there is not any broad spectrum oxime able to satisfactorily counteract acute toxic effects of all nerve agents. The oxime HI-6 is the most efficacious oxime to reactivate soman or cyclosarin-inhibited AChE and to protect soman or cyclosarin-exposed mammals from their acute toxic effects [2,52,53], nevertheless, it is not efficacious to protect tabun-exposed animals from tabun-induced acute toxicity [25]. Obidoxime is suitable oxime for the reactivation of sarin or VX-inhibited AChE [1] but it is not able to sufficiently protect soman or cyclosarin-exposed mammals from their acute toxic effects [52,53]. Newly synthesized K oximes seem to be the very effective reactivators of VX agent-inhibited AChE [30] but they are not suitable oximes for the treatment of cyclosarin poisonings because they are not sufficiently effective to reactivate cyclosarin-inhibited AChE and eliminate cyclosarin-induced toxic effects [25,54]. On the other hand, they seem to be sufficiently effective and suitable oximes for the antidotal treatment of acute tabun poisoning although their reactivating, therapeutic and neuroprotective potency is not significantly higher compared to some currently available oximes (obidoxime).

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