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Monooxime reactivators of acetylcholinesterase with (*E*)-but-2-ene linker—Preparation and reactivation of tabun- and paraoxon-inhibited acetylcholinesterase

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Abstract—Acetylcholinesterase reactivators are crucial antidotes for the treatment of organophosphate intoxication. Fifteen new monooxime reactivators of acetylcholinesterase with a (*E*)-but-2-ene linker were developed in an effort to extend the properties of K-oxime (*E*)-1-(4-carbamoylpyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide (K203). The known reactivators (pralidoxime, HI-6, obidoxime, K075, K203) and the new compounds were tested in vitro on a model of tabun- and paraoxon-inhibited AChE. Monooxime reactivators were not able to exceed the best known compounds for tabun poisoning, but some of them did show reactivation comparable with known compounds for paraoxon poisoning. However, extensive differences were found by a SAR study for various substitutions on the non-oxime part of the reactivator molecule.

1. Introduction

The enzyme acetylcholinesterase (AChE; EC 3.1.1.7) plays a very important role in human neurotransmission degrading the neurotransmitter acetylcholine within the synaptic cleft. A considerable number of both natural and artificial AChE inhibitors have been produced, tested and used for various purposes.[1,2] The organophosphorus inhibitors of acetylcholinesterase (OPI; Fig. 1) belong to the artificial class of inhibitors of AChE and some OPI are available worldwide (e.g., pesticides—chlorpyrifos, parathion, diazinon).[3] Some of the highly toxic OPI have been used as chemical warfare agents (such as sarin, soman, tabun, VX) and as such are some of the most toxic artificial compounds known.[3,4] The industrial OPI (e.g., tri-*O*-cresylphosphate) are used as plasticizers or flame retardants, however their effect does not originate from the inhibition of AChE.[5]

The OPI inhibit AChE via a covalent binding to the serine hydroxy group within the enzyme's active site.[3,4] Afterwards, AChE is not able to fulfil its essential role in neurotransmission. The non-degraded acetylcholine accumulates within the synaptic cleft resulting in overstimulation and a subsequent cholinergic crisis, which normally causes serious malfunction of the breathing centre followed by death.[3] Various therapies are used to counteract the toxic effects of OPI. A pre-treatment therapy is used for persons who have been primarily exposed to OPI (e.g., soldiers). It contains weak AChE inhibitors (e.g., pyridostigmine) to sequester the enzyme and may additionally contain oxime reactivators (e.g., oxime HI-6), or other esterases (e.g., human butyrylcholinesterase) to scavenge the OPI.[6–8] The oxime reactivators are the drugs of choice for the post-treatment of OPI intoxication and they are used worldwide.[5] They contain an oxime (hydroxyiminomethyl) group, which

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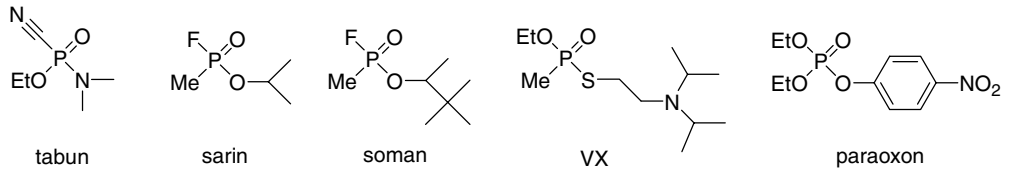


Figure 1. Organophosphorus inhibitors of acetylcholinesterase.

is able to cleave the covalent bond between AChE and OPI and restore the enzyme's vital function. The pralidoxime (**1**, 2-hydroxyiminomethyl-1-methylpyridinium chloride), oxime HI-6 (**2**, 1-(2-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-2-oxapropane dichloride) obidoxime (**3**, Toxogonine®, 1,3-bis(4-hydroxyiminomethylpyridinium)-2-oxapropane dichloride) are commercially available oxime reactivators (Fig. 2).[9–11] However additionally, atropine therapy has to be used to protect neurotransmission against a cholinergic crisis and diazepam is used as an anticonvulsant.[3]

However, the present antidote therapy is not sufficient for the wide spectrum treatment of OPI.[12] Although the oxime HI-6 is the most broad spectrum commercially available reactivator (sarin, soman, VX), it has only limited reactivation capability against tabun (GA) intoxication.[13] Beside GA inhibiting AChE, it also influences the enzyme's active site by intramolecular modifications called 'aging'.[3,4] Namely, GA in a complex with AChE changes the hydrogen bonding of various amino acids (e.g., His447) and consequently the conformational modifications (Pro338) partially close the narrow AChE cleft.[14,15] At this stage (>1 h), AChE is 'conformationally aged' and it is possible to reactivate it. Aftewards, the phosphoramidoyl group of GA is replaced by a molecule of water and the rest of the GA molecule is coordinated in the enzyme's cavity.[14,15] Thereby the 'aged' AChE is insensitive to reactivation by oxime reactivators.[12]

In this paper, we present detailed results on the design, synthesis and in vitro evaluation of a new group of (*E*)-but-2-ene monooxime reactivators. This group of reactivators extends our previous successful compounds against GA intoxication which are currently being tested worldwide.[7,16–18]

2. Design and synthesis of (*E*)-but-2-ene monooxime reactivators

A large amount of the previously identified compounds that counteract GA intoxication were prepared.[9,10,19–27] Due to the steric hindrance caused by GA within the active site of AChE, only spatially narrow compounds were able to reactivate GA-inhibited AChE.[28] Therefore, generally the bispyridinium derivatives bearing at least one oxime functional group at position four were prepared.[20–25] A length of 3 or 4 carbon–carbon bonds was predicted to be optimal for the connecting linker between the two pyridinium rings.[23] The second pyridinium ring usually contained oxime or carbamoyl functional group in position four.[20,21,23–25] The presence of a non-oxime (carbamoyl or other) group means that this group is not necessarily participating in the reactivation per se, but it influences the affinity of the reactivator to AChE via possibly hydrogen bond interactions.[24,25] All of the above-mentioned properties have been shown to be important for the design of potent reactivators of GA-inhibited AChE (Fig. 3). [28]

Previously we prepared the K-oximes (K027, K048, K074, K075, K203; **4**–**8**, Fig. 4). [20,21,23–25] Their design was based on the commercially available reactivator obidoxime (**3**). The obidoxime connecting linker was changed to trimethylene (**4**), tetramethylene (**5**–**6**) and (*E*)-but-2-ene (**7**–**8**) while one oxime group was replaced by a carbamoyl group in several compounds (**4**–**5** and **8**). Some of the K-oximes extend the obidoxime reactivation properties (**7**–**8**) whereas the oxime K203 (**8**) seems to be a relevant candidate for in vivo studies due to its higher reactivation ability and lower toxicity compared to obidoxime (**3**).[25]

The monooxime reactivators with a (*E*)-but-2-ene linker (Fig. 5) were developed in an effort to extend the reactiva-

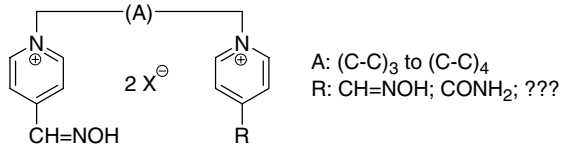


Figure 3. General formula of reactivator able to counteract tabun intoxication.

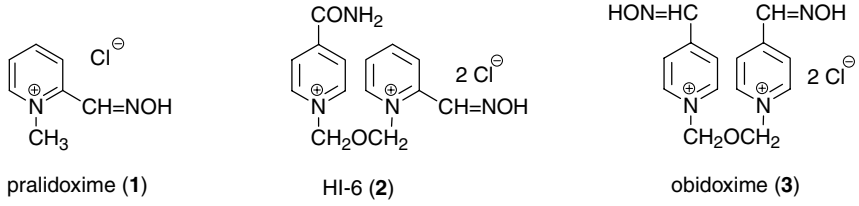


Figure 2. Commercial oxime reactivators used against OPI intoxication.

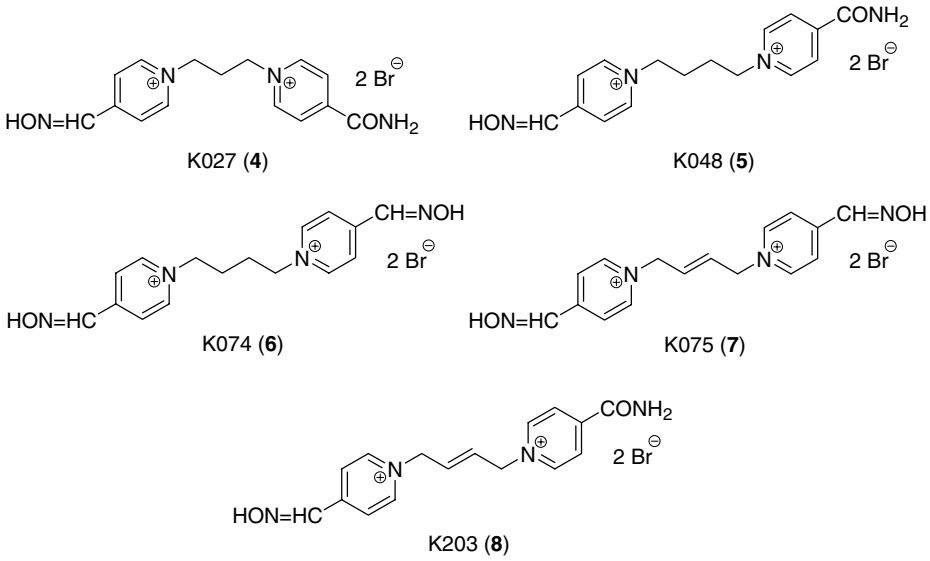


Figure 4. Reactivators tested on tabun-inhibited acetylcholinesterase.

tion of K-oxime **8**. In the first group of compounds, the pyridinium ring bearing the non-oxime moiety was replaced by different heterocycles. In the second group, the carbamoyl functional group was replaced by other functional groups with more lipophilic (methyl, *tert*-butyl, phenyl, benzyl) or hydrophilic properties (hydroxymethyl, carboxyl, methylcarbonyl, ethylcarboxyl, carbonitrile, 1-thia-2-carboxyethyl, 1-aminohydroxyiminomethyl).

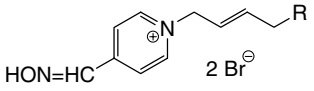
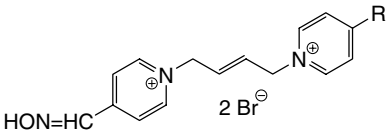
Compound	R
	
9	1-pyridinium
10	1-pyridazinium
11	1-quinolinium
12	1-isoquinolinium
	
13	Me
14	*tert*. butyl
15	phenyl
16	benzyl
17	CH_2OH
18	COOH
19	COMe
20	COOEt
21	CN
22	SCH_2COOH
23	$C(NH_2)$=NOH

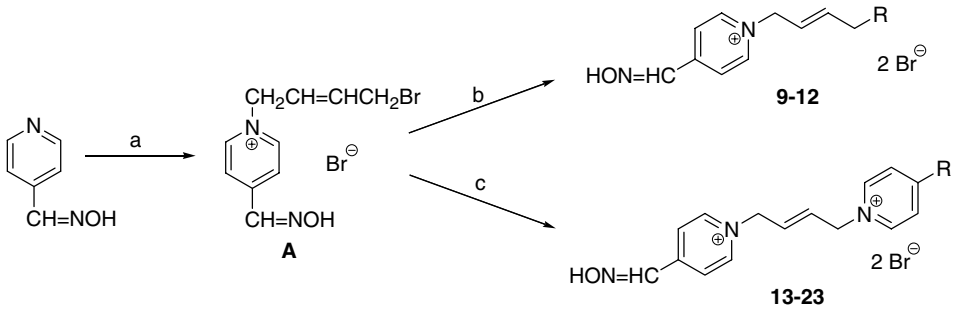
Figure 5. New oxime reactivators tested against tabun- and paraoxon-inhibited AChE.

The monoxime reactivators with a (*E*)-but-2-ene linker were prepared via a two-step synthesis (Scheme 1).[24,25] First, the monoquaternary compound (**A**) was synthesized in 5 molar excess of alkylating agent. The compound **A** was separated from side bisquaternary products by recrystallization from acetonitrile (MeCN), within which the bisquaternary product is almost insoluble.[29] Second, the reactivators were completed by the addition of a corresponding heterocyclic derivative (1.5 molar excess) in various conditions. The recrystallization from MeCN was used again to acquire the non-soluble bisquaternary product (**9**–**23**) in satisfactory yield and purity.[30]

3. AChE reactivation results and SAR discussion

The new monooxime reactivators with a (*E*)-but-2-ene linker (**9**–**23**) and previously known substances (**1**–**3** and **7**–**8**) were assayed for their reactivation potency using a rat brain homogenate inhibited by GA and the pesticide paraoxon (POX).[29] All experiments with GA were performed on conformationally aged AChE. The reactivation results are shown in Table 1.

A reactivation in vitro should exceed 10% to suggest a promising compound warranting further testing.[3,12] Noticeably, not all tested compounds were able to fulfil this criterion for GA-inhibited AChE. The commercially available reactivators pralidoxime and oxime HI-6 (**1** and **2**) are known to be not suitable for treatment of GA intoxication.[12,13,32] Although obidoxime (**3**) is the most potent commercially available compound against GA, it has increased toxicity.[25] The K-oximes (**7** and **8**) showed increased reactivation compared to **3** at



Scheme 1. Preparation of new oxime reactivators via two-step synthesis. Reagents and conditions: (a) (*E*)-1,4-dibromobut-2-ene (5 equiv), acetone, reflux, 2 h, 95%; (b and c) corresponding heterocyclic derivative (1.5 equiv), DMF or MeCN, 50–100 °C, 2–10 h, 43–89%.

Table 1. Reactivation potencies of tested oximes (%, mean value of three independent determinations): time of inhibition, 30 min; time of reactivation by AChE reactivators, 10 min; pH 7.6; temperature 25 °C

Inhibitor:	% Reactivation ± SD			
	Tabun		Paraoxon	
Reactivator/concentration	10^{-3} M	10^{-5} M	10^{-3} M	10^{-5} M
Pralidoxime (**1**)	4 ± 1	0	42 ± 1	0
HI-6 (**2**)	2 ± 1	4 ± 1	35 ± 2	0
Obidoxime (**3**)	37 ± 1	28 ± 2	76 ± 2	37 ± 2
K075 (**7**)	42 ± 1	19 ± 0	60 ± 1	46 ± 2
K203 (**8**)	55 ± 1	14 ± 0	64 ± 2	23 ± 1
9	4 ± 0	0	57 ± 0	22 ± 2
10	0	0	35 ± 3	23 ± 0
11	0	0	0	15 ± 1
12	0	3 ± 0	43 ± 0	48 ± 3
13	0	0	61 ± 1	26 ± 2
14	7 ± 2	0	52 ± 1	14 ± 1
15	0	4 ± 1	13 ± 0	22 ± 0
16	0	0	0	14 ± 0
17	2 ± 0	0	50 ± 0	42 ± 0
18	11 ± 3	3 ± 0	63 ± 1	15 ± 3
19	11 ± 3	2 ± 0	68 ± 1	36 ± 2
20	9 ± 1	2 ± 0	68 ± 1	30 ± 2
21	0	0	54 ± 3	28 ± 0
22	0	0	13 ± 1	6 ± 0
23	14 ± 0	9 ± 1	52 ± 2	32 ± 1

10^{-3} M, while their potency was decreased compared to **3** at 10^{-5} M. Since the maximal attainable plasma concentration in humans is 10^{-4} M, **3** is therefore the best compound among the previously known reactivators.[33] Moreover, compound **7** had three times increased toxicity when compared to **3**.[25] Although compound **8** had a decreased reactivation potency at 10^{-5} M, it showed less toxicity when compared to **3**.[25]

The new monooxime compounds (**9**–**23**) had decreased reactivation capability when compared to the known compounds for GA-inhibited AChE. Some of these compounds (**18**–**20** and **23**) showed a borderline acceptable (10%) reactivation at 10^{-3} M, and compound **23** also at 10^{-5} M (which is at a concentration that is possible to attain in vivo). Compared to their relative K-oxime **8**, the new monooxime reactivators had no greater reactivation potency.

The reactivation did change for POX-inhibited AChE. The commercial compounds **1** and **2** had half the reactivation capability when compared to **3** at 10^{-3} M and no reactivation at 10^{-5} M. The best reactivation from known oximes at 10^{-3} M was compound **3** whereas the K-oximes (**7** and **8**) and some monooxime reactivators (**13** and **18**–**20**) had a slightly decreased potency. For the concentration attainable in vivo (10^{-5} M), K-oxime **7** and monooxime **12** presented as the best reactivators whereas commercial compound **3** and monooximes (**17**, **19**, **20** and **23**) showed also promising reactivation of POX-inhibited ACHE. Surprisingly, some of the new compounds (**1**–**12**, **15** and **16**) had a higher reactivation potency at lower concentrations. This phenomenon has been previously described for reactivators with a xylene connecting linker.[34,35] The reactivation curve for all concentrations is bell-shaped. Highly concentrated reactivators (10^{-3} M) are both able to reactivate and inhibit the AChE themselves, whereas the more diluted ones (10^{-5} M) only reactivate the enzyme.

Furthermore, the structural factors necessary for reactivation of GA and POX-inhibited AChE were estab-

lished. The main structural features which influence the reactivation potency are the oxime functional group (its position and amount), the connecting linker for bisquaternary reactivators and other substituent(s) on the second heteroaromatic ring.[25,26] For GA-inhibited AChE, at least one oxime in position four on the heteroaromatic ring is necessary for substantial reactivation (**3**, **7** and **8**) whilst an oxime in position two has a low or no reactivation capability (**1** and **2**). Additionally, the optimal linker length suitable for GA intoxication varies from 3 to 4 carbon–carbon bonds.[36] Notably, the (*E*)-but-2-ene linker (**7**–**23**) is slightly longer than 3 but slightly shorter than 4 carbon–carbon bonds due the presence of double bond, which also restricts the conformational flexibility of (*E*)-but-2-ene linker.[37] Moreover, the necessity of (*E*)- instead of (*Z*)-but-2-ene linker has also been established.[38] The new monooxime reactivators (**9**–**23**) indicated the strong influence of the second heteroaromatic ring or its substitution for GA intoxication. The replacement of the second pyridinium ring and its functional group (**7** and **8**) by another heteroaromatic ring (**9**–**12**; pyridinium, pyridazinium, quinolinium, isoquinolinium) decreased the reactivation potency to zero levels. While the lipophilic substitution on pyridinium ring (**13**–**16**; methyl, *tert*-butyl, phenyl, benzyl) showed minimal reactivation of GA-inhibited AChE, some hydrophilic substituents (**18**–**20**, **23**; carboxyl, methylcarbonyl, ethylcarboxyl, 1-aminohydroxyiminomethyl) had decreased, but still showed notable reactivation ability. Although the mechanism of the interaction of the hydrophilic substituents with inhibited enzyme is not clear (compounds **17**, **21** and **22** with minimal vs **8** with adequate reactivation), weak hydrogen bond interactions are predicted.

For POX-inhibited AChE, at least one oxime group in position four seemed to be beneficial (**3**, **7** and **8**) when compared to an oxime in position two (**1** and **2**), especially at 10^{-5} M. Not surprisingly, the reactivation ability of monooxime compounds increased for POX compared to GA-inhibited AChE due to the slower 'aging' process resulting in less changes in the enzyme's active site. But surprisingly for monooximes, the differences among other heteroaromatic rings (**9**–**12**), lipophilic (**13**–**16**) and hydrophilic substituents (**17**–**23**) were not as distinctive as in the case of GA. Particularly, the isoquinolinium monooxime reactivator (**12**) was the best compound from those with various heteroaromatic rings or all of the new monooximes. Moreover, compound **12** was comparable with **7** at the more suitable concentration of 10^{-5} M, while other substitutions (including quinolinium) showed a decreased reactivation. Among the lipophilic substituents on pyridinium ring, the methyl and phenyl derivatives (**13** and **15**) exceeded the properties of *tert*-butyl and benzyl (**14** and **16**) reactivators at 10^{-5} M. For hydrophilic derivatives, hydroxymethyl, methylcarbonyl, ethylcarboxyl, carbonitrile and 1-aminohydroxyiminomethyl (**17**, **19**–**21** and **23**) improved the reactivation of carboxyl and 1-thia-2-carboxyethyl (**18** and **22**) at 10^{-5} M. Among all tested reactivators for POX-inhibited AChE, it is not clear if weak hydrophobic or hydrophilic (**12** compared to **11**, both compared to **7** or **17**) interactions play the main role in the different reactivation potency.

4. Conclusions

The new monooxime reactivators with (*E*)-but-2-ene linker were developed in an effort to extend the properties of the K-oxime K203. Their reactivation was tested on the model of tabun- and paraoxon-inhibited AChE in vitro. Although monooxime reactivators were weak reactivators of tabun compared to known oximes (obidoxime, K075, K203), there were several promising new compounds against paraoxon poisoning comparable to known oximes at concentrations attainable for human use. The SAR results showed interesting differences between reactivation of tabun and paraoxon. While hydrophilic substitution on non-oxime pyridinium ring was superior for tabun intoxication, both hydrophilic and lipophilic substituents or other heterocyclic moieties in the molecule of reactivator were able to reactivate paraoxon-inhibited AChE. These preliminary in vitro findings suggest an apparent influence of weak interactions within the AChE active site for the main reactivation process and suggest further necessary studies (lipophilicity, X-ray, docking studies).

5. Experimental

5.1. Chemical preparation

Solvents (acetone, DMF, MeCN) and reagents were purchased from Fluka and Sigma–Aldrich (Czech Republic) and used without further purification. Reactions were monitored by TLC using DC-Alufolien Cellulose F (Merck, Germany) and mobile phase BuOH/CH_3COOH/H_2O 5:1:2, detection by solution of Dragendorff reagent (solution containing 10 mL CH_3COOH, 50 mL H_2O and 5 mL of basic solution prepared by mixing of two fractions—fraction A: 850 mg $Bi(NO_3)_3$, 40 mL H_2O, 10 mL CH_3COOH; fraction B: 8 g KI, 20 mL H_2O). Melting points were measured on micro heating stage PHMK 05 (VEB Kombinat Nagema, Radebeul, Germany) and are uncorrected.

NMR spectra were generally recorded at Varian Gemini 300 (^{1}H 300 MHz, ^{13}C 75 MHz, Palo Alto, CA, USA). In all cases, the chemical shift values for ^{1}H spectra are reported in ppm (δ) relative to residual $CHD_2SO_2CD_3$ (δ 2.50) or D_2O (δ 4.79), shift values for ^{13}C spectra are reported in ppm (δ) relative to solvent peak dimethylsulfoxide-d_6 δ 39.43. Signals are quoted as s (singlet), d (doublet), t (triplet) and m (multiplet).

Mass spectra were recorded using combination of high performance liquid chromatography and mass spectrometry. HP1100 HPLC system was obtained from Agilent Technologies (Waldbronn, Germany). It consisted of vacuum degasser G1322A, quaternary pump G1311A, autosampler G1313A and quadrupole mass

spectrometer MSD1456 VL equipped with electrospray ionization source. Nitrogen for mass spectrometer was supplied by Whatman 75–720 nitrogen generator. Data were collected in positive ion mode with an ESI probe voltage of 4000 V. The pressure of nebulizer gas was set up to 35 psig. Drying gas temperature was operated at 335 °C and flow at 13 L/min.

5.2. Preparation of monoquaternary salt

(*E*)-1-(4-Bromobut-2-enyl)-4-hydroxyiminomethylpyridinium bromide (**A**). Preparation and spectral data are consistent with literature data.[27]

5.3. Preparation of bisquaternary salts

A solution of the (*E*)-1-(4-bromobut-2-enyl)-4-hydroxyiminomethylpyridinium bromide (**A**) (0.50 g, 1.5 mmol) and corresponding heterocyclic derivative (2.2 mmol) in DMF (10 mL) or MeCN (50 mL) was stirred at 50–100 °C. The reaction mixture was cooled to the room temperature and portioned with acetone (50 mL); the crystalline crude product was collected by filtration, washed with acetone (3× 20 mL) and recrystallized from MeCN.[24,27]

5.3.1. (*E*)-1-(4-Hydroxyiminomethylpyridinium)-4-pyridinium-but-2-ene dibromide (9). The reaction mixture was stirred at 80 °C (DMF) and stopped after 4 h. Yield 0.51 g (82%), mp 232–233 °C. ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ (ppm) 9.15 (d, 2H, J = 6.0 Hz, H-2,6), 9.09 (d, 2H, J = 6.0 Hz, H′-2,6), 8.70–8.62 (m, 1H, H′-4), 8.47 (s, 1H, –*CH*═NOH), 8.27 (d, 2H, J = 6.0 Hz, H-3,5), 8.24–8.17 (m, 2H, H′-3,5), 6.30–6.16 (m, 2H, –*CH*═), 5.45–5.33 (m, 4H, –CH_2–). ^{13}C NMR spectrum (75 MHz, DMSO d_6): δ (ppm) 148.63, 145.91, 145.10, 145.02, 144.86, 130.22, 130.00, 128.11, 124.02, 60.68, 60.23. EA: calculated 43.40% C, 4.13% H, 10.12% N; found 43.14% C, 4.19% H, 10.18% N. ESI-MS: *m*/*z* 127.7 $[M/2]^{2+}$ (calculated for $[C_{15}H_{17}N_3O/2]^{2+}$ 127.57).

5.3.2. (*E*)-1-(4-Hydroxyiminomethylpyridinium)-4-pyridazinium-but-2-ene dibromide (10). The reaction mixture was stirred at 80 °C (DMF) and stopped after 2 h. Yield 0.53 g (85%), mp 215–217 °C. ^{1}H NMR spectrum (300 MHz, D_2O): δ (ppm) 9.79 (d, 1H, J = 5.5 Hz, H′-6), 9.55 (d, 1H, J = 4.0 Hz, H′-3), 8.86 (d, 2H, J = 6.0 Hz, H-2,6), 8.74–8.53 (m, 2H, H′-4,5), 8.40 (s, 1H, –*CH*═NOH), 8.25 (d, 2H, J = 6.0 Hz, H-3,5), 6.51–6.30 (m, 2H, –*CH*═), 5.63 (d, 2H, J = 4.9 Hz, –CH_2–N′), 5.36 (d, 2H, J = 4.4 Hz, –CH_2–N). ^{13}C NMR spectrum (75 MHz, D_2O): δ (ppm) 154.30, 149.13, 148.69, 145.73, 144.12, 136.71, 135.63, 130.42, 128.43, 124.50, 65.17, 60.91. EA: calculated 40.41% C, 3.88% H, 13.46% N; found 39.91% C, 4.05% H, 13.08% N. ESI-MS: *m*/*z* 128.0 $[M/2]^{2+}$ (calculated for $[C_{14}H_{16}N_4O/2]^{2+}$ 128.07).

5.3.3. (*E*)-1-(4-Hydroxyiminomethylpyridinium)-4-quinolinium-but-2-ene dibromide (11). The reaction mixture was stirred at 80 °C (DMF) and stopped after 3 h. Yield 0.40 g (57%), mp 200–203 °C. ^{1}H NMR spectrum (300 MHz, D_2O): δ (ppm) 9.33 (d, 1H, J = 5.6 Hz, Q-2), 9.19 (d, 1H, J = 8.4 Hz, Q-8), 8.75 (d, 2H, J = 6.0 Hz, Pyr-2,6), 8.45–8.32 (m, 3H, –*CH*═NOH + Pyr-3,5), 8.29–7.98 (m, 5H, Q-3-7), 6.54–6.40 (m, 1H, Q-*CH*═), 6.13–5.98 (m, 1H, Pyr-*CH*═), 5.82 (d, 2H, J = 5.1 Hz, Q-CH_2–), 5.26 (d, 2H, J = 6.5 Hz, Pyr-CH_2–). ^{13}C NMR spectrum (75 MHz, D_2O): δ (ppm) 148.68, 148.19, 145.67, 143.95, 137.60, 135.73, 130.48, 129.80, 129.74, 127.19, 124.44, 121.41, 117.91, 60.98, 57.80. EA: calculated 49.06% C, 4.12% H, 9.03% N; found 48.60% C, 4.14% H, 9.19% N. ESI-MS: *m*/*z* 152.6 $[M/2]^{2+}$ (calculated for $[C_{19}H_{19}N_3O/2]^{2+}$ 152.58).

5.3.4. (*E*)-1-(4-Hydroxyiminomethylpyridinium)-4-isoquinolinium-but-2-ene dibromide (12). The reaction mixture was stirred at 80 °C (DMF) and stopped after 3 h. Yield 0.55 g (80%), mp 228–230 °C. ^{1}H NMR spectrum (300 MHz, D_2O): δ (ppm) 8.85 (d, 2H, J = 6.0 Hz, Pyr-2,6), 8.57–8.39 (m, 3H, Q-1,3,4), 8.36 (s, 1H, –*CH*═NOH), 8.30–8.15 (m, 5H, Pyr-3,5 + Q-5,7,8), 8.09–7.99 (m, 1H, Q-6), 6.47–6.26 (m, 2H, –*CH*═), 5.49 (d, 2H, J = 5.1 Hz, Q-CH_2–), 5.35 (d, 2H, J = 5.2, Pyr-CH_2–). ^{13}C NMR spectrum (75 MHz, D_2O): δ (ppm) 149.00, 148.72, 145.70, 144.13, 137.29, 136.99, 133.37, 131.08, 130.02, 129.79, 129.27, 127.25, 126.84, 126.23, 124.52, 61.05, 60.97. EA: calculated 49.06% C, 4.12% H, 9.03% N; found 48.64% C, 4.18% H, 8.93% N. ESI-MS: *m*/*z* 152.6 $[M/2]^{2+}$ (calculated for $[C_{19}H_{19}N_3O/2]^{2+}$ 152.58).

5.3.5. (*E*)-1-(4-Hydroxyiminomethylpyridinium)-4-(4-methylpyridinium)-but-2-ene dibromide (13). The reaction mixture was stirred at 80 °C (DMF) and stopped after 3 h. Yield 0.52 g (81%), mp 224–225 °C. ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ (ppm) 9.08 (d, 2H, J = 6.0 Hz, H-2,6), 8.98 (d, 2H, J = 6.0 Hz, H′-2,6), 8.47 (s, 1H, –*CH*═NOH), 8.27 (d, 2H, J = 6.0 Hz, H-3,5), 8.03 (d, 2H, J = 6.0 Hz, H′-3,5), 6.30–6.10 (m, 2H, –*CH*═), 5.48–5.23 (m, 4H, –CH_2–), 2.62 (s, 3H, –CH_3). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ (ppm) 148.63, 145.09, 145.03, 143.83, 130.21, 129.88, 128.35, 124.03, 60.23, 59.87, 21.38. EA: calculated 44.78% C, 4.46% H, 9.79% N; found 44.42% C, 4.45% H, 9.48% N. ESI-MS: *m*/*z* 134.7 $[M/2]^{2+}$ (calculated for $[C_{16}H_{19}N_3O/2]^{2+}$ 134.58).

5.3.6. (*E*)-1-(4-Hydroxyiminomethylpyridinium)-4-(4-*tert*-butylpyridinium)-but-2-ene dibromide (14). The reaction mixture was stirred at 60 °C (MeCN) and stopped after 7 h. Yield 0.35 g (50%), mp 131–133 °C. ^{1}H NMR spectrum (300 MHz, D_2O): δ (ppm) 8.83 (d, 2H, J = 6.0 Hz, H-2,6), 8.71 (d, 2H, J = 6.0 Hz, H′-2,6), 8.39 (s, 1H, –*CH*═NOH), 8.23 (d, 2H, J = 6.0 Hz, H-3,5), 8.11 (d, 2H, J = 6.0 Hz, H′-3,5), 6.37–6.18 (m, 2H, –*CH*═), 5.30 (dd, 4H, J_{1-1} = 4.8 Hz, J_{1-2} = 6.5 Hz, –CH_2–), 1.41 (s, 9H, –CH_3). ^{13}C NMR spectrum (75 MHz, D_2O): δ (ppm) 148.72, 145.74, 144.10, 142.96, 130.01, 129.00, 125.14, 124.52, 60.91, 60.22, 29.80, 28.59. EA: calculated 48.43% C, 5.35% H, 8.92% N; found 48.49% C, 5.61% H, 8.54% N. ESI-MS: *m*/*z* 155.7 $[M/2]^{2+}$ (calculated for $[C_{19}H_{25}N_3O/2]^{2+}$ 155.60).

5.3.7. (*E*)-1-(4-Hydroxyiminomethylpyridinium)-4-(4-phenylpyridinium)-but-2-ene dibromide (15). The reaction mixture was stirred at 80 °C (DMF) and stopped after 3 h. Yield 0.60 g (82%), mp 247–248 °C. ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ (ppm) 9.15 (d, 2H, J = 6.0 Hz, H-2,6), 9.08 (d, 2H, J = 6.0 Hz, H′-2,6), 8.59 (d, 2H, J = 6.0 Hz, H-3,5), 8.47 (s, 1H, –*CH*═NOH), 8.28 (d, 2H, J = 6.0 Hz, H′-3,5), 8.15–8.06 (m, 2H, Ph-2,6), 7.71–7.60 (m, 3H, Ph-3,4,5), 6.34–6.16 (m, 2H, –*CH*═), 5.46–5.29 (m, 4H, –CH_2–). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ (ppm) 154.93, 148.65, 145.10, 133.36, 132.19, 130.29, 129.83, 129.65, 128.12, 124.49, 124.04, 60.30, 59.88. EA: calculated 51.35% C, 4.31% H, 8.55% N; found 51.53% C, 4.36% H, 8.65% N. ESI-MS: m/z 165.7 $[M/2]^{2+}$ (calculated for $[C_{21}H_{21}N_3O/2]^{2+}$ 165.59).

5.3.8. (*E*)-1-(4-Benzylpyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide (16). The reaction mixture was stirred at 60 °C (MeCN) and stopped after 7 h. Yield 0.32 g (43%), mp 153–155 °C. ^{1}H NMR spectrum (300 MHz, D_2O): δ (ppm) 8.80 (d, 2H, J = 6.0 Hz, H′-2,6), 8.68 (d, 2H, J = 6.0 Hz, H-2,6), 8.36 (s, 1H, –*CH*═NOH), 8.20 (d, 2H, J = 6.0 Hz, H′-3,5), 7.90 (d, 2H, J = 6.0 Hz, H-3,5), 7.46–7.27 (m, 5H, Ph), 6.34–6.14 (m, 2H, –*CH*═), 5.28 (dd, 4H, J_{1-1} = 4.8 Hz, J_{1-2} = 4.4 Hz, –CH_2–), 4.32 (s, 2H, Ph-CH_2–). ^{13}C NMR spectrum (75 MHz, D_2O): δ (ppm) 148.70, 145.71, 144.07, 143.26, 136.49, 129.89, 129.13, 128.95, 128.78, 127.81, 127.02, 124.51, 60.88, 60.47, 40.35. EA: calculated 52.30% C, 4.59% H, 8.32% N; found 52.49% C, 4.62% H, 8.19% N. ESI-MS: m/z 172.7 $[M/2]^{2+}$ (calculated for $[C_{22}H_{23}N_3O/2]^{2+}$ 172.59).

5.3.9. (*E*)-1-(4-Hydroxyiminomethylpyridinium)-4-(4-hydroxymethylpyridinium)-but-2-ene dibromide (17). The reaction mixture was stirred at 60 °C (MeCN) and stopped after 10 h. Yield 0.59 g (89%), mp 182–186 °C. ^{1}H NMR spectrum (300 MHz, D_2O): δ (ppm) 8.95–8.70 (m, 4H, H-2,6 + H′-2,6), 8.39 (s, 1H, –*CH*═NOH), 8.24 (d, 2H, J = 6.0 Hz, H-3,5), 8.05 (d, 2H, J = 6.0 Hz, H′-3,5), 6.42–6.19 (m, 2H, –*CH*═), 5.42–5.21 (m, 4H, –CH_2–), 4.99 (s, 2H, –CH_2–OH). ^{13}C NMR spectrum (75 MHz, D_2O): δ (ppm) 148.73, 145.73, 144.10, 143.40, 129.82, 129.24, 126.11, 124.52, 73.81, 61.11, 60.91. EA: calculated 43.17% C, 4.30% H, 9.44% N; found 42.63% C, 4.33% H, 9.24% N. ESI-MS: m/z 142.6 $[M/2]^{2+}$ (calculated for $[C_{16}H_{19}N_3O_2/2]^{2+}$ 142.58).

5.3.10. (*E*)-1-(4-Carboxypyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide (18). The reaction mixture was stirred at 80 °C (DMF) and stopped after 3 h. Yield 0.51 g (75%), mp 243–244 °C. ^{1}H NMR spectrum (300 MHz, D_2O): δ (ppm) 9.02 (d, 2H, J = 6.0 Hz, H′-2,6), 8.83 (d, 2H, J = 6.5 Hz, H-2,6), 8.46 (d, 2H, J = 6.0 Hz, H′-3,5), 8.38 (s, 1H, –*CH*═NOH), 8.22 (d, 2H, J = 6.5 Hz, H-3,5), 6.42–6.24 (m, 2H, –*CH*═), 5.50–5.28 (m, 4H, –CH_2–). ^{13}C NMR spectrum (75 MHz, D_2O): δ (ppm) 148.77, 147.88, 145.73, 145.04, 144.14, 130.08, 129.21, 127.28, 124.54, 61.53, 60.88. EA: calculated 41.86% C, 3.73% H, 9.15% N; found 41.96% C, 3.86% H, 9.51% N. ESI-M|%1e[OK]S: m/z 149.6 $[M]^{2+}$ (calculated for $[C_{16}H_{17}N_3O_3/2]^{2+}$ 149.57).

5.3.11. (*E*)-1-(4-Hydroxyiminomethylpyridinium)-4-(4-methylcarbonylpyridinium)-but-2-ene dibromide (19). The reaction mixture was stirred at 80 °C (DMF) and stopped after 3 h. Yield 0.51 g (75%), mp 206–208 °C. ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ (ppm) 9.35 (d, 2H, J = 6.0 Hz, H′-2,6), 9.08 (d, 2H, J = 5.9 Hz, H-2,6), 8.54 (d, 2H, J = 5.9 Hz, H′-3,5), 8.47 (s, 1H, –*CH*═NOH), 8.28 (d, 2H, J = 6.0 Hz, H-3,5), 6.34–6.15 (m, 2H, –*CH*═), 5.49 (d, 2H, J = 4.5 Hz, N′–CH_2–), 5.38 (d, 2H, J = 4.4 Hz, N–CH_2–), 2.76 (s, 3H, –CH_3). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ (ppm) 148.65, 148.61, 146.41, 145.12, 145.04, 130.58, 129.71, 125.94, 124.04, 60.93, 60.23, 27.52. EA: calculated 44.66% C, 4.19% H, 9.19% N; found 44.27% C, 4.24% H, 9.30% N. ESI-MS: m/z 148.6 $[M]^{2+}$ (calculated for $[C_{17}H_{19}N_3O_2/2]^{2+}$ 148.58).

5.3.12. (*E*)-1-(4-Ethylcarboxypyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide (20). The reaction mixture was stirred at 80 °C (DMF) and stopped after 3 h. Yield 0.38 g (53%), mp 175–177 °C. ^{1}H NMR spectrum (300 MHz, D_2O): δ (ppm) 9.10 (d, 2H, J = 6.0 Hz, H-2,6), 8.85 (d, 2H, J = 6.0 Hz, H′-2,6), 8.59 (d, 2H, J = 6.5 Hz, H-3,5), 8.39 (s, 1H, –*CH*═NOH), 8.24 (d, 2H, J = 6.0 Hz, H′-3,5), 6.46–6.26 (m, 2H, –*CH*═), 5.46 (d, 2H, J = 4.9 Hz, N–CH_2–), 5.35 (d, 2H, J = 4.8 Hz, N′–CH_2–), 4.59–4.47 (m, 2H, –CH_2–CH_3), 1.44 (t, 3H, J = 7.0 Hz). ^{13}C NMR spectrum (75 MHz, D_2O): δ (ppm) 148.78, 145.74, 144.15, 130.34, 129.00, 127.37, 124.55, 63.78, 61.75, 60.86, 12.72. EA: calculated 45.88% C, 4.49% H, 8.92% N; found 45.47% C, 4.37% H, 8.66% N. ESI-MS: m/z 163.6 $[M]^{2+}$ (calculated for $[C_{18}H_{21}N_3O_3/2]^{2+}$ 163.58).

5.3.13. (*E*)-1-(4-Carbonitrilepyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide (21). The reaction mixture was stirred at 80 °C (DMF) and stopped after 3 h. Yield 0.49 g (75%), mp 239–240 °C. ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ (ppm) 9.45 (d, 2H, J = 6.0 Hz, H-2,6), 9.08 (d, 2H, J = 6.0 Hz, H′-2,6), 8.77 (d, 2H, J = 6.0 Hz, H-3,5), 8.47 (s, 1H, –*CH*═NOH), 8.27 (d, 2H, J = 6.0 Hz, H′-3,5), 6.35–6.13 (m, 2H, –*CH*═), 5.49 (d, 2H, J = 5.5 Hz, N–CH_2–), 5.37 (d, 2H, J = 5.4 Hz, N′–CH_2–). ^{13}C NMR spectrum (75 MHz, DMSO d_6): δ (ppm) 148.66, 146.33, 145.15, 145.03, 130.96, 129.23, 127.15, 124.01, 114.73, 61.00, 60.23. EA: calculated 43.66% C, 3.66% H, 12.73% N; found 43.11% C, 3.73% H, 12.41% N. ESI-MS: m/z 140.1 $[M]^{2+}$ (calculated for $[C_{16}H_{16}N_4O/2]^{2+}$ 140.07).

5.3.14. (*E*)-1-(4-Hydroxyiminomethylpyridinium)-4-[4-(1-thia-2-carboxyethyl)-pyridinium]-but-2-ene dibromide (22). The reaction mixture was stirred at 50 °C (DMF) and stopped after 6 h. Yield 0.44 g (59%), mp 158–160 °C. ^{1}H NMR spectrum (300 MHz, D_2O): δ (ppm) 8.82 (d, 2H, J = 6.0 Hz, H-2,6), 8.57–8.33 (m, 3H, H′-2,6 + –*CH*═NOH), 8.23 (d, 2H, J = 6.0 Hz, H-3,5), 7.79 (d, 2H, J = 6.0 Hz, H′-3,5), 6.36–6.12 (m, 2H, –*CH*═), 5.40–5.08 (m, 4H, N–CH_2–), 4.08 (d, 2H,

J = 5.4 Hz, S–CH_2–). EA: calculated 40.41% C, 3.79% H, 8.32% N, 6.35% S; found 40.27% C, 3.85% H, 8.43% N, 6.22% S. ESI-MS: *m/z* 172.7 $[M/2]^{2+}$ (calculated for $[C_{17}H_{19}N_3O_3S/2]^{2+}$ 172.56).

5.3.15. (*E*)-1-[4-(1-Aminohydroxyiminomethyl)-pyridinium]-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide (23). The reaction mixture was stirred at 80 °C (DMF) and stopped after 2.5 h. Yield 0.60 g (86%), mp 243–244 °C. ^{1}H NMR spectrum (300 MHz, D_2O): δ (ppm) 8.92 (d, 2H, J = 6.0 Hz, H-2,6), 8.85 (d, 2H, J = 6.0 Hz, H′-2,6), 8.39 (s, 1H, –*CH*═NOH), 8.29 (d, 2H, J = 6.3 Hz, H-3,5), 8.24 (d, 2H, J = 6.3 Hz, H′-3,5), 6.41–6.25 (m, 2H, –*CH*═), 5.45–5.28 (m, 4H, –CH_2–). ^{13}C NMR spectrum (75 MHz, D_2O): δ (ppm) 149.51, 148.73, 147.85, 145.71, 144.25, 144.13, 129.81, 129.38, 124.53, 124.15, 61.06, 60.89. EA: calculated 40.61% C, 4.05% H, 14.80% N; found 40.58% C, 4.20% H, 14.45% N. ESI-MS: *m/z* 156.8 $[M/2]^{2+}$ (calculated for $[C_{16}H_{19}N_5O_2/2]^{2+}$ 156.58).

5.4. In vitro assay

In vitro testing of reactivators has been described in detail.[31] Briefly, the 10% rat brain homogenate in distilled water was used as a source of AChE. The brain homogenate (0.5 mL) was mixed with 20 μL of isopropanol solution of tabun (tabun, *O*-ethyl-*N*,*N*-dimethylphosphoramidocyanidate; obtained from the Military facility Brno, 95% purity) or paraoxon (*O*,*O*-diethyl-*O*-(4-nitrophenyl)phosphate, analytical standard 99.2% from Sigma–Aldrich) and distilled water (0.5 mL). The mixture was incubated at 25 °C for 30 min to achieve 95% inhibition of AChE. The mixture was filled in assay vessel to the volume 23 mL with distilled water and sodium chloride (3 M; 2.5 mL) was added. Finally, 2 mL of acetylcholine iodide (0.02 M; substrate for enzymatic reaction) was added. The enzyme activity (analyzed by potentiometric titration of decomposed acetylcholine iodide) was measured at pH 7.6 and 25 °C using an autotitrator RTS 822 (Radiometer, Denmark).

The same procedure was repeated with rat brain homogenate, which was 30 min incubated with tabun or paraoxon and further treated for 10 min with an aqueous solution of reactivator (0.2 mL—replacing same amount of distilled water). Activities of intact AChE (a_0), inhibited AChE (a_i) and reactivated AChE (a_r) were deduced from the amount of NaOH solution (0.01 M) versus time; NaOH reacted with acetic acid released from decomposed acetylcholine iodide. The percentage of reactivation (%) was calculated from the measured data according to the formula:

$$x = \left(1 - \frac{a_0 - a_r}{a_0 - a_i}\right) \cdot 100[\%]$$

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References and notes

1. Giacobini, E. In *Cholinesterases and Cholinesterase Inhibitors*; Giacobini, E., Ed.; Martin Dunitz: London, 2000; pp 1–226.
2. Giacobini, E. *Pharmacol. Res.* **2004**, *50*, 433.
3. Bajgar, J. *Adv. Clin. Chem.* **2004**, *38*, 151.
4. Marrs, T. C. *Pharmacol. Ther.* **1993**, *58*, 51.
5. Gupta, R. In *Toxicology of Organophosphate & Carbamate Compounds*; Elsevier Academic Press: London, 2006; pp 5–69, 703–714.
6. Saxena, A.; Sun, W.; Luo, C.; Myers, T. M.; Koplovitz, I.; Lenz, D. E.; Doctor, B. P. *J. Mol. Neurosci.* **2006**, *30*, 145.
7. Lucic, A.; Radic, B.; Peraica, M.; Mesic, M.; Primozic, I.; Binenfeld, Z. *Arch. Toxicol.* **1997**, *71*, 467.
8. Lucic-Vrdoljak, A.; Calic, M.; Radic, B.; Berend, S.; Jun, D.; Kuca, K.; Kovarik, Z. *Toxicology* **2006**, *228*, 41.
9. Poziomek, E. J.; Hackley, B. E.; Steinberg, G. M. *J. Org. Chem.* **1958**, *23*, 714.
10. Lüttringhaus, A.; Hagedorn, I. *Arzneimittelforschung* **1964**, *14*, 1.
11. Hagedorn, I.; Gündel, W. H.; Schoene, K. *Arzneimittelforschung* **1969**, *19*, 603.
12. Bajgar, J.; Fusek, J.; Kuca, K.; Bartosova, L.; Jun, D. *Mini-Rev. Med. Chem.* **2007**, *7*, 461.
13. Lundy, P. M.; Raveh, L.; Amitai, G. *Toxicol. Rev.* **2006**, *25*, 231.
14. Ekstrom, F.; Akfur, C.; Tunemalm, A. K.; Lundberg, S. *Biochemistry* **2006**, *45*, 74.
15. Ekstrom, F.; Pang, Y. P.; Boman, M.; Artursson, E.; Akfur, C.; Borjegren, S. *Biochem. Pharmacol.* **2006**, *72*, 597.
16. Calic, M.; Lucic, A.; Radic, B.; Jelic, D.; Jun, D.; Kuca, K.; Kovarik, Z. *Toxicology* **2006**, *219*, 85.
17. Park, N.-J.; Jung, Y.-S.; Musilek, K.; Jun, D.; Kuca, K. *Bull. Korean Chem. Soc.* **2006**, *27*, 1401.
18. Kalasz, H.; Hasan, M. Y.; Sheen, R.; Kuca, K.; Petroianu, G.; Ludanyi, K.; Gergely, A.; Tekes, K. *Anal. Bioanal. Chem.* **2006**, *385*, 1062.
19. Odzak, R.; Calic, M.; Hrenar, T.; Primozic, I.; Kovarik, Z. *Toxicology* **2007**, *233*, 85.
20. Kuca, K.; Bielavsky, J.; Cabal, J.; Bielavska, M. *Tetrahedron Lett.* **2003**, *44*, 3123.
21. Kuca, K.; Bielavsky, J.; Cabal, J.; Kassa, J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3545.
22. Pang, Y.-P.; Kollmeyer, T. M.; Hong, F.; Lee, J.-C.; Hammond, P. I.; Haugabouk, S. P.; Brimijoin, S. *Chem. Biol.* **2003**, *10*, 491.
23. Kuca, K.; Cabal, J.; Musilek, K.; Jun, D.; Bajgar, J. *J. Appl. Toxicol.* **2005**, *25*, 491.
24. Musilek, K.; Holas, O.; Kuca, K.; Jun, D.; Dohnal, V.; Opletalova, V.; Dolezal, M. *J. Enzyme Inhib. Med. Chem.* **2007**, MS 238287.
25. Musilek, K.; Jun, D.; Cabal, J.; Kassa, J.; Gunn-Moore, F.; Kuca, K. *J. Med. Chem.* MS JM070653R.
26. Musilek, K.; Kuca, K.; Jun, D.; Dolezal, M. *Curr. Org. Chem.* **2007**, *11*, 229.
27. Musilek, K.; Holas, O.; Hambalek, J.; Kuca, K.; Jun, D.; Dohnal, V.; Dolezal, M. *Lett. Org. Chem.* **2006**, *3*, 831.
28. Kuca, K.; Jun, D.; Musilek, K. *Mini-Rev. Med. Chem.* **2006**, *6*, 269.

29. Musilek, K.; Kuca, K.; Jun, D.; Dohnal, V.; Dolezal, M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 622.
30. Musilek, K.; Holas, O.; Kuca, K.; Jun, D.; Dohnal, V.; Dolezal, M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5673.
31. Kuca, K.; Cabal, J. *Toxicol. Mech. Method* **2005**, *15*, 247.
32. Jokanovic, M.; Stojiljkovic, M. P. *Eur. J. Pharmacol.* **2006**, *553*, 10.
33. Tattersall, J. E. *Br. J. Pharmacol.* **1993**, *108*, 1006.
34. Musilek, K.; Kuca, K.; Jun, D.; Dohnal, V.; Dolezal, M. *J. Enzyme Inhib. Med. Chem.* **2005**, *20*, 409.
35. Musilek, K.; Kuca, K.; Jun, D.; Dohnal, V.; Dolezal, M. *J. Enzyme Inhib. Med. Chem.* **2007**, *22*, 425.
36. Kuca, K.; Patocka, J.; Cabal, J. *J. Appl. Biomed.* **2003**, *1*, 207.
37. Carey, F. A.; Sundberg, R. J. In *The Structure and Mechanisms*, 4th ed.; Plenum Publishers: New York, 2000; p 13.
38. Musilek, K.; Holas, O.; Kuca, K.; Jun, D.; Dohnal, V.; Opletalova, V.; Dolezal, M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3172.