Synthesis and Anticholinesterasic Activity of Some α-Thio-Substituted O-(Methylcarbamoyl)oximes

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A series of six oxime N-methylcarbamates, $C_6H_5C(R)$=NOCONHCH$_3$ [R = SCH_3, CH_2SCH_3, (Z)- and (E)-$CH(CH_3)SCH_3$, $C(CH_3)_2SCH_3$, $C(CH_3)_3$], analogues of commercial compounds such as aldicarb, butocarboxim, methomyl, and thiofanox, was synthesized for potential anticholinesterasic activity. NMR, IR, and UV were used to assign the configurations. The kinetic study of the inhibition of acetylcholinesterase from bovine erythrocyte and the determination of the dissociation constant K_d and of the carbamylation rate constant k_2 allowed discussion of the importance of the number of methyl groups on the carbon α to the iminic group. The insertion of the phenyl group generally did not improve the activity but yielded compounds, one isomer of which had a potential activity close to that of the commercial compounds.

In 1965, Weiden et al. first described the O-(methylcarbamoyl)oximes as cholinesterase inhibitors. The replacement of the weakly acidic phenol by another weakly acidic group, such as an aliphatic or alicyclic oxime, in the synthesis broadened the family of the insecticidal carbamates. Further, in order to obtain an optimized acetylcholinesterase inhibition, structure–activity studies showed that conformational similarity to acetylcholine, monomethylation of the carbamate nitrogen, and α-alkylthio substitution were essentials (Fridinger et al., 1971).

The compounds described in this work belong to the group of the α-substituted O-(methylcarbamoyl)acetophenoximes studied by Fukuto et al. (1969) and Jones et al. (1972).

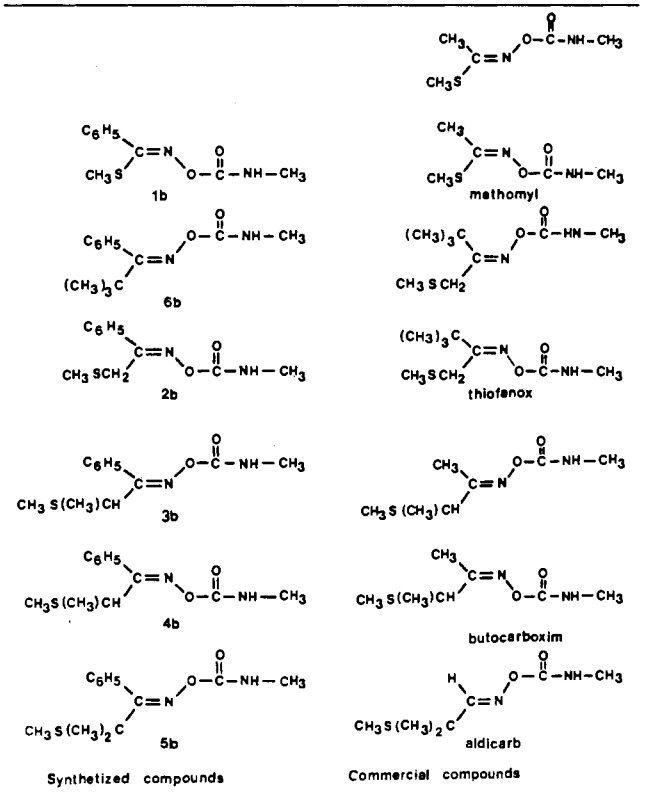
Mrlina and Calmon (1980a,b) characterized the mechanism of hydrolysis for this group and discussed the anticholinesterasic activity versus physicochemical parameters. Following those previous studies, a new series of O-(methylcarbamoyl)oximes was synthesized with a structure resembling those of commercial compounds as aldicarb, butocarboxim, methomyl, and thiofanox (Table I). These new molecules retained the main structure of the commercial analogue to which a phenyl group was added on the carbon bearing the imine bond. This transformation might increase their lipophilic character, thereby promoting the penetration of these molecules in the insect integument (Gerolt, 1970, 1972).

The effects induced by this modification were determined by measuring acetylcholinesterase inhibition in vitro, which is a good estimation of insecticidal activity for those molecules.

MATERIALS AND METHODS

The commercial oxime N-methylcarbamates submitted to the in vitro anticholinesterase activity test are the following: aldicarb or 2-methyl-2-(methylthio)propionaldehyde O-(methylcarbamoyl)oxime; aldoxycarb or 2-mesyl-2-methylpropionaldehyde O-(methylcarbamoyl)oxime; butocarboxim or 3-(methylthio)butanone O-(methylcarbamoyl)oxime; butoxycarboxim or 3-(methylsulfonyl)-2-butanone O-(methylcarbamoyl)oxime; oxamyl or S-methyl N′,N′-dimethyl-N-[(methylcarbamoyl)oxy]-1-thioxamimidate; methomyl or methyl N-[(methylcarbamoyl)oxy]thioacetimidate; thiofanox or 3,3-dimethyl-1-(methylthio)-2-butanone O-(methylcarbamoyl)oxime.

Table I. Structures and Names of Studied Compounds



The high-grade pesticides were provided by the Cluzeau Info Labo Co. (33220 Ste. Foy La Grande, France), and their purity was checked by NMR and thin-layer chromatography.

SYNTHESIS

The α-thio-substituted ketones (starting molecules for compounds **2b**, **3b**, **4b**, and **5b**) were prepared by reacting thiomethane with the corresponding α-bromo ketone according to Prelog et al. (1944). The intermediate oximes, **2a**, **3a**, **4a**, and **5a** were obtained by the addition of hydroxylamine to the ketones as described by Grob and Ide (1974). The procedure described by Benn (1964) permitted us to synthesize molecule **1a** by chlorination of the benzaldoxime on the α-position and substitution of the chlorine atom by the thiomethyl group. Isobutyrophenoxime was synthesized following the conditions used by Nef (1900). The melting points and the configuration of the oximes are reported in Table II. The resulting oximes were

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Table II. Melting Points and Configuration of Oximes: $C_6H_5(R)C{=}NOH$

no.	R	isomer	mp, °C	lit. mp, °C
1a	SCH_3	Z	83–84	85[a]
2a	CH_2SCH_3	Z	58	59–60.5[b]
3a	$CH(CH_3)SCH_3$	E[d]	100	102–103[b]
4a	$CH(CH_3)SCH_3$	Z[e]	76	94–95[b]
5a	$C(CH_3)_2SCH_3$	E	130	130–130.5[b]
6a	$C(CH_3)_3$	E	166–167	165[c]

[a] Benn (1964). [b] Grob and Ide (1974). [c] Nef (1900). [d] 90% E isomer. [e] 90% Z isomer.

converted to the carbamate by addition of a stoichiometric quantity of methyl isocyanate in anhydrous ethyl ether with triethylamine as catalyst.

α-(Methylthio)acetophenone (Grob and Ide, 1974). To 0.12 mol of thiomethane dissolved in 50 mL of sodium methanolate at 0 °C was added 0.095 mol of α-bromoacetophenone. Stirring was continued for 2 h at that temperature and then stopped; the solution was left at room temperature for 12 h. After extraction with ethyl ether, the oily fraction was distilled under reduced pressure.

α-(Methylthio)acetophenoxime (2a) (Grob and Ide, 1974). A solution containing 0.056 mol of α-(methylthio)acetophenone and 0.17 mol of hydroxylamine hydrochloride in 53 mL of pyridine was stirred for 24 h at room temperature. The reacting mediums was washed with a cold solution of hydrochloric acid (0 °C) and extracted with ethyl ether. The final product was recrystallized from hexane.

α-(Methylthio)acetophenone *O*-(Methylcarbamoyl)oxime (2b). Methyl isocyanate (0.023 mol) was allowed to react with 0.022 mol of α-(methylthio)-acetophenone oxime in 50 mL of anhydrous ethyl ether containing catalytic quantities of triethylamine. After 12 h, the *N*-methylcarbamate was filtered and then recrystallized from a mixture of hexane and toluene (30/50).

Benzohydroxamoyl Chloride (Benn, 1964). Benzaldoxime (0.099 mol) was dissolved in chloroform (200 mL) and the resultant mixture cooled to 0 °C in an ice salt bath. A fairly slow stream of dry chlorine was bubbled through the solution until the starting blue solution turned yellow.

***S*-Methyl Benzothiohydroximate (Benn, 1964).** To a mixture of benzohydroxamoyl chloride (0.05 mol) and methanethiol (0.042 mol) dissolved in anhydrous ethyl ether (200 mL) was added triethylamine (0.1 mol). A white precipitate of triethylamine hydrochloride appeared instantaneously. The reaction medium was washed with a cold 0.5 N sulfuric acid first and then extracted with ethyl ether. The final product was recrystallized from an ethyl ether/petroleum ether mixture (1/1).

Isobutyrophenoxime (Nef, 1900). The oxime was obtained by reacting two molecules of hydroxylamine hydrochloride with one molecule of isobutyrophenone in a mixture of ethanol and water (15/25). The oxime crystals were recrystallized in hexane and in ethanol.

The two last oximes were converted into the *N*-methylcarbamate in the same way as described for compound **2b**.

In every case, except for compound **3a**, a single isomer was obtained for the oxime and the configurations were in agreement with those previously described. The chromatographic method used by Grob and Ide (1974) did not lead to a good separation of the two isomers *E* and *Z*. After repeated recrystallizations from hexane, two fractions, each containing a single isomer of 90% purity, could be obtained. A complete separation of the carbamate derivatives could be achieved by flash chromatography (Still et al., 1978). In a 40-cm-high column (4-cm i.d.) containing 15 cm of 230–400-mesh silica gel (Merck 9385), the isomers were eluted with a mixture of ethyl acetate/petroleum ether (45/55) at a rate of 10 cm/min. Thin-layer chromatography (Merck 5526) of the isomers, using as eluant ethyl acetate/petroleum ether (50/50), ensures an isomeric purity of 99% (ΔR_f = 0.16) for the carbamic derivatives.

The structures were confirmed by elemental analysis and by their IR, UV, and NMR spectra. The results are shown in Tables III–V. The infrared spectra were taken on a Perkin-Elmer Model 783 either in solution (chloroform or carbon tetrachloride) or as a KBr pellet. The UV spectra were obtained in ethanol with a Cary 210 spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian A 56/60 spectrometer with tetramethylsilane as internal standard and deuteriated chloroform or dimethyl sulfoxide as solvents.

ACETYLCHOLINESTERASE INHIBITION

The anticholinesterase activity was measured with purified bovine erythrocyte acetylcholinesterase, XII S 0.4–1

Table III. Physicochemical Characteristics of Oxime *N*-Methylcarbamates: $C_6H_5(R)C{=}NOC(=O)NHCH_3$

				elemental analyses							
				calculated, %				found, %			
no.	R	mp, °C	isomer	C	H	N	S	C	H	N	S
1b	SCH_3	119[a]	Z	53.56	5.40	12.49	14.29	53.52	5.43	12.67	14.83
2b	CH_2SCH_3	92–94	Z	55.44	5.92	11.75	13.45	55.30	5.72	12.01	13.19
3b	$CH(CH_3)SCH_3$	102	E	57.12	6.39	11.10	12.70	57.24	6.39	10.84	13.40
4b	$CH(CH_3)SCH_3$	98	Z	57.12	6.39	11.10	12.70	57.03	6.34	11.63	12.62
5b	$C(CH_3)_2SCH_3$	205	E	58.62	6.81	10.51	12.03	58.61	6.69	10.36	12.62
6b	$C(CH_3)_3$	158	E	66.66	7.69	11.96		66.18	7.76	11.86	

[a] Lit. mp 119 °C (Fukuto et al., 1969).

Table IV. NMR and UV Spectra of Oximes $C_6H_5(R)C{=}NOH$

	^1H NMR[a]								UV[b]			
no.	C_6H_5	H(Cα)	SCH_3	OH	HC(Cα) (d[c])	OH (d[c])	HC(Cα) (d[c])	OH (d)	λ_{max}	lit.[c] λ_{max}	ε	lit.[c] ε
1a	7.43 (s)		2.09 (s)			11.55			219		10320	
2a	7.56 (m)	3.86 (s)	2.15 (s)	8.23 (s)	3.81 (s)	11.36 (s)	3.84 (s)	11.44 (s)	249	250	10560	10964
3a	7.43 (s)	3.66 (q)	2.10 (s)	8.33 (s)	3.80 (q)	10.63 (s)	3.86 (q)	10.76 (s)	221	221	6080 (sh)	6918 (sh)
4a	7.50 (m)	4.81 (q)	2.16 (s)	9.08 (s)	4.73 (q)	11.16 (s)	4.74 (q)	11.36 (s)	236.7	236.5	7760	7762
5a	7.39 (s)		2.06 (s)	7.86 (s)		10.53 (s)		10.66 (s)				
6a	7.19 (m)			8.13 (s)		10.26 (s)						

[a] Chemical shifts in ppm; $(CH_3)_4Si$ internal standard; $CDCl_3$ solvent since DMSO was used (d). Key: s, singlet; d, doublet; q quartet; m, multiplet; sh, shoulder. [b] Wavelengths in nm; EtOH solvent. [c] Grob and Ide (1974).

Table V. NMR and UV Spectra of Oxime N-Methylcarbamates: $C_6H_5(R)C{=}NOC(=O)NHCH_3$

	^1H NMR[a]						UV[b]	
no.	C_6H_5	H(C^α)	H(C^β)	NH	NCH_3	SCH_3	λ_{max}	ϵ
1b	7.45 (s)			6.10 (br)	2.83 (d)	2.06 (s)	215	9920
2b	7.53 (m)	3.89 (s)		6.39 (br)	2.93 (d)	2.13 (s)	248.7	12080
3b	7.43 (s)	3.81 (q)	1.33 (d)	6.06 (br)	2.89 (d)	2.16 (s)	222	6400 (sh)
4b	7.56 (m)	4.86 (q)	1.43 (d)	6.39 (br)	2.93 (d)	2.16 (s)	234.6	7600
5b	7.35 (s)		1.46 (s)	5.93 (br)	2.86 (d)	2.10 (s)		
6b	7.26 (m)		1.25 (s)	6.16 (br)	2.86 (d)			

[a] Chemical shifts in ppm; $(CH_3)_4Si$ internal standard; $CDCl_3$ solvent. Key: s, singlet; d, doublet; q, quartet; m, multiplet; br, broad; sh shoulder. [b] Wavelengths in nm; EtOH solvent.

unit/mg of solid, purchased from Sigma Chemical Co. and acetylcholinesterase, XII S 0.4–1 unit/mg of solid, purchased from Sigma Chemical Co. and acetylcholine hydrochloride provided by Koch Light Laboratories as substrate. The residual activity was determined by the method of pH–stat as described by Nishioka et al. (1976). Both enzyme and substrate were dissolved in a phosphate buffer, 10 mM, pH 7.6, containing 0.1 M NaCl (13.9 mg of AChE, activity 0.54 unit/mg of solid in 1 mL of buffer). The inhibitors, commercial or previously described oxime carbamates, were added to the reaction medium as freshly prepared ethanolic solutions. The final concentration of ethanol in the enzyme reaction medium did not exceed 2.5%.

To 2 mL of phosphate buffer were added first 10–50 μL of the ethanolic solution of the inhibitor and then only 50 μL of the enzymatic solution. After 3 min of incubation time, the pH was adjusted to its initial value of 7.6 with a 2×10^{-2} N NaOH solution and then the substrate added (0.5 mL of 10 mM acetylcholine). The reaction was followed by an automatic titrator (Radiometer pHM 64, combined with a TTT60 titrator and Autoburet ABU13) with a 2×10^{-2} N NAOH carbonate-free solution as the titrant. Incubation and reaction temperatures were held at 25 ± 0.2 °C.

RESULTS AND DISCUSSION

Prior to any activity interpretation, synthesized oxime N-methylcarbamates were checked for their structure and, in some cases, their configurations could be confirmed.

NMR Spectra. The chemical shifts concerning the oximes (series **a**) and the corresponding carbamates (series **b**) are reported in Tables IV and V. The results observed for the oximes are close to those observed by Grob and Ide (1974). For the hydroxyl proton, the difference in the chemical shifts between those obtained in dimethyl sulfoxide and those in chloroform fluctuates from 1.68 to 3.21 ppm. Such a difference could be explained by a solvent effect. Dimethyl sulfoxide used by Grob and Ide seems to be more effective for the disrupting of the intermolecular hydrogen bond than the deuteriated chloroform. Thereby, the protons beared by the hydroxyl group are more protected and resonate at higher fields (7.8–9 ppm) than in dimethyl sulfoxide (10.7–11.4 ppm). A similar phenomenon was earlier described by Karabatsos and Taller (1968). For that series of oximes, the chemical shift observed in deuteriated dimethyl sulfoxide in typical of the configuration; the hydroxyl proton of the *E* isomer appears at lower fields than that of the *Z* isomer (over 10.63 ppm).

For both oximes and carbamates, a difference appeared in the behavior of the phenyl protons that could give either a singlet or a multiplet. The bulkiness of the substituents neighboring the carbon α to the imine bond disturbs the coplanarity of the phenyl ring with the imine bond, modifying the spatial interactions between the protons of the substituent and those of the phenyl ring. The singlet seems to be linked to the noncoplanar position. The influence on the protons belonging to the carbon α to the C=N, owed to the position of the hydroxyl group on one or on the other side of the imine double bond, is well shown by the spectra of compounds **3a** and **4a**. The proton in α-position resonates as a quartet at 4.81 ppm for molecule **4a** and 3.66 ppm for molecule **3a**. According to Karabatsos and Taller (1968) and Berlin and Rengaraju (1971), the protons on the carbon α to the imine bond cis to the hydroxyl group resonate at lower fields than the corresponding protons in trans position. So we can conclude that molecule **4a** is really the *Z* isomer and molecule **3a** the *E* isomer. For those protons, the variation between the chemical shifts due to isomerism is smaller for the carbamate series than for the oxime series. In the same way, protons of the aromatic ring resonate under 7.43 ppm for the *E* isomers and over 7.50 ppm for the *Z* isomers. For the proton on the carbamoyl nitrogen, the effects of the oximino function are expressed by higher chemical shifts for the *Z* isomer (6.39 ppm) than for the *E* isomer (below 6.16 ppm).

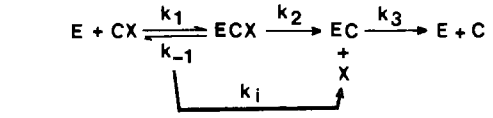
Infrared Spectrophotometry. In the infrared spectrum of molecule **4a**, done in a KBr pellet, the O–H stretching band appears at 3160 cm^{-1} for the isomer trans (phenyl and OH in trans position) and at 3200 cm^{-1} for its cis homologue (molecule **3a**). This change in frequencies of the absorption of the OH bond is in agreement with the observations of Palm and Werbin (1953, 1954) who found this difference in the frequencies for oximes between *E* and *Z* forms.

In chloroform solution as in carbon tetrachloride diluted solution, the oximes are characterized by a strong, sharp band at 3580 cm^{-1} for the absorption of the "free" OH and by a medium–broad band at 3280–3300 cm^{-1} due to the absorption of the bonded OH itself. The aromatic band is slightly shifted to 3000 cm^{-1} because of the conjugation of the phenyl group with the C=N double bond. The absorption of this last bond appears at 1620 and 1500 cm^{-1} as sharp, medium bands. For molecule **1a**, the absorption at 1620 cm^{-1} is in agreement with the observations of Goulden (1953). The *N*-methylcarbamates corresponding to those oximes stand out by the stretching band of free N–H bond at 3425 or 3440 cm^{-1} (strong and very sharp), a very strong vibration of the carbonyl group at 1740 cm^{-1}, a bending band at 1500–1530 cm^{-1} for the N–H bond, and a strong absorption of C–O bond of the carbamoyl function near 1200–1240 cm^{-1} (Nyquist, 1973).

Ultraviolet Spectrophotometry. The UV spectrophotometry can contribute to the configurational assignment of the isomers: For example, isomer **3a**, possessing a phenyl group trans to the oxygen atom, exhibits an absorption maximum at a lower wavelength with a smaller extinction coefficient than its isomer **4a** (cis isomer), results in agreement with those of Karabatsos and Hsi (1967).

Acetylcholinesterase Inhibition. As acetylcholine is the synaptic mediator of neurotransmission in the central nervous system in insects and vertebrates, the *N*-methylcarbamates act as substrates whose biological cycle

Scheme I



E: ACETYLCHOLINESTERASE. Kd: k_1/k_{-1} EQUILIBRIUM DISSOCIATION CONSTANT.

CX: CARBAMATE.

ECX: REVERSIBLE COMPLEXE. k_2: CARBAMYLATION RATE CONSTANT.

EC: CARBAMYLATED ENZYME. ki: BIMOLECULAR CARBAMYLATION RATE CONSTANT.

lasts longer than that of acetylcholine. The latter, never hydrolyzed, is accumulated in the synaptic junction and perturbs the transmission of the nervous influx.

Scheme I was proposed for acetylcholinesterase inhibition (Hastings et al., 1970; Jones et al., 1972; Hart et al., 1973).

The inhibition efficiency is measured by the residual enzymatic activity with an excess of substrate after an appropriate time of incubation (2–3 min). Under these conditions, the residual activity depends only on the concentration of the free enzyme.

The kinetic parameters determined according to Nishioka et al. (1976, 1977) revealed an anticholinesterase activity for compound **3b** 80 times greater than that of butocarboxim. The inhibitor efficiency depends not only on the ability to cross the biological barriers to the insect neuron, but also on the structural compatibility with the active site of the enzyme. Each isomer can have its proper activity. For compounds **3b** and **4b**, the *E* configuration evinces the greater k_i. In the case of methomyl, the anticholinesterase activity of the *Z* isomer is 100 times that of the *E* isomer and 10 times as toxic to the house fly (Felton, 1968).

A good affinity between enzyme and *N*-methylcarbamate is expressed by a decrease of the dissociation constant K_d. Table VI shows the importance of the sulfur atom and its environment for this parameter. The behavior of the molecule studied here is close to the good association enzyme inhibitor observed by Cohen et al. (1985) for the sulfoxide group, where the volume, the spatial configuration, and the dipolar character are favorable. Thus, aldoxycarb and butoxycarboxim have a smaller dissociation constant and better anticholinesterase action (Durden and Weiden, 1969). However, the increase in hydrophilicity, which increases the departure of water molecules from the hydrolytic site, leads to its inactivation (Cohen et al., 1984) and explains the decrease of the carbamoylation rate constant k_2 observed between aldicarb and butocarboxim versus aldoxycarb and butoxycarboxim.

The insertion of the phenyl group in the commercial compounds allowed estimation of the influence of lipophilicity and steric effects on the residual enzymatic activity. The replacement of the hydrogen atom in aldicarb decreases the general enzymatic activity: even though the binding capacity is increased, the carbamoylation rate is depressed. The phenyl group restricts the penetration of the molecule in the enzymatic site, allowing a good fit of $C(CH_3)_2SCH_3$ part with the "trimethyl" receptor site. This allows an enhanced binding but at the same time orients the carbamoyl group so that the hydrolysis is less favorable. The substitution of the methyl group in methomyl or the substitution of the *tert*-butyl group in thiofanox by the phenyl group does not lead to an improvement in the activity even though a slight increase in the carbamoylation is observed. The introduction of the phenyl group has a positive effect on the anticholinesterase activity only for compound **3b**, an analogue of butocarboxim, for which the affinity is increased by 13 times and the carbamoylation by 7.

Table VI. Kinetic Parameters for Bovine Erythrocyte AChE Inhibition

compd	K_d, M	k_2, min^{-1}	k_i, M^{-1} min^{-1}	[cx],[g] M
1b	1.68×10^{-4}	0.68	4.10×10^3	$(0.2–1) \times 10^{-4}$
2b	1.25×10^{-2}	10.41	8.26×10^2	$(1–5) \times 10^{-4}$
3b[a]	9.075×10^{-5}	5.098	5.61×10^4	$(0.2–5) \times 10^{-5}$
4b[b]	2.27×10^{-4}	1.004	4.41×10^3	$(0.2–1) \times 10^{-4}$
5b	3.74×10^{-5}	0.13	3.64×10^3	$(0.5–5) \times 10^{-4}$
6b	1.43×10^{-5}	0.66	4.62×10^4	$(0.2–2.5) \times 10^{-4}$
methomyl[c]	5.40×10^{-6}	0.62	1.14×10^5	$(0.2–1) \times 10^{-5}$
thiofanox[d]	1.33×10^{-4}	8.94	6.71×10^4	$(0.7–1) \times 10^{-5}$
butocarboxim[e]	1.12×10^{-3}	0.77	6.85×10^2	$(2–5) \times 10^{-4}$
aldicarb[f]	1.23×10^{-4}	1.72	1.39×10^4	$(0.1–1) \times 10^{-4}$
oxamyl	9.71×10^{-6}	1.28	1.31×10^5	$(2–6) \times 10^{-6}$
butoxycarboxim	2.44×10^{-4}	0.304	1.24×10^3	$(2–5) \times 10^{-4}$
aldoxycarb	6.57×10^{-5}	1.30	1.98×10^4	$(1–5) \times 10^{-5}$

[a] *E* isomer. [b] *Z* isomer. [c] *Z* isomer predominant. [d] *Z* isomer predominant. [e] 85% *E* isomer. [f] *E* isomer. [g] Range of carbamate concentrations.

In the series of compounds **2b**, **3b**, **4b**, and **5b**, the affinity rises with the number of methyl groups carried by the carbon atom (C^α) neighboring the imine bond. The successive methylations of C^α lead this part of the molecule close to the ideal structure able to bind the "trimethyl site" described by Cohen et al. (1985). On the other hand, the increase in number of methyl groups borne by the C^α diminishes the carbamoylation constant k_2. This result is in agreement with the one observed for the alkaline hydrolysis of oxime *N*-methylcarbamate where it was shown that the bimolecular constant k_{OH} is increased by electron-donating groups (Mrlina and Calmon, 1980b). For those molecules, the only electron-donating effect is due to the increase of the number of methyls carried by the C^α.

CONCLUSION

The insertion of the phenyl group in the existing commercial compounds allowed a structure–activity study about a common structure and exhibited the influence of the number of methyl groups fixed on the imine C^α on the affinity and carbamoylation in the inhibition of the acetylcholinesterase. The biological results demonstrate the improvement of the structure of butocarboxim due to the insertion of the phenyl group and the importance of the configuration of the imine double bond when conceptualizing potential acetylcholinesterase inhibitors.

Registry No. **1a**, 57598-95-5; **2a**, 54394-68-2; **2b**, 117652-52-5; **3a**, 54394-71-7; **4a**, 54394-70-6; **5a**, 54394-73-9; **6a**, 100485-49-2; cholinesterase, 9001-08-5; aldicarb, 116-06-3; aldoxycarb, 1646-88-4; butocarboxim, 34681-10-2; butoxycarboxim, 34681-23-7; oxamyl, 23135-22-0; methomyl, 16752-77-5; thiofanox, 39196-18-4; α-(methylthio)acetophenone, 5398-93-6; benzohydroxamoyl chloride, 112129-04-1; *S*-methyl benzothiohydroximate, 61076-36-6; isobutyrophenoxime, 37899-61-9.

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