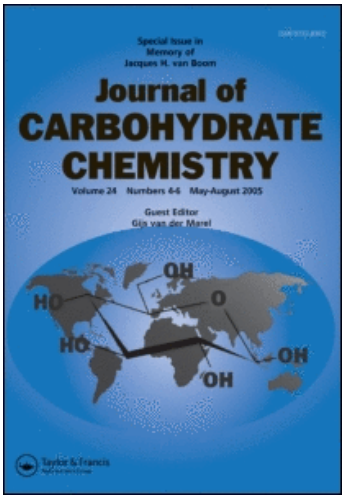

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Dedicated to the memory of Prof. Jacques van Boom.
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The synthesis of new pseudo-*C*-nucleosides was accomplished starting from 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentodialdo-1,4-furanose, aiming to build thiazole, triazole, tetrazole, and thiazolidinone derivatives. The stereochemistry of the thiazolidinone epimers was confirmed by DFT/B3LYP calculations. The well-known diversity of biological activities exhibited by compounds with these heterocyclic moieties in their structure encouraged the investigation of the insecticidal activity against *Musca domestica*. The thiazole derivative was efficient as insecticide with LD50 = 6.7 ng/g, which is in the order of the LD_{50} values for pyrethroids on the same insect. All the compounds tested showed knockdown, mediated through action on the insect nervous system. The neuroactivity found in insects has motivated the evaluation of the inhibition of acetylcholinesterase and butyrylcholinesterase. The two thiazolidinone derivatives tested inhibited butyrylcholinesterase from human serum (36% and 22%), while inhibition of amphiphilic acetylcholinesterase from human erythrocytes at the same concentrations was found to be very low (5% and 8%). This selective activity may indicate potential application of these structures for amelioration of Alzheimer's disease.

Keywords Pseudo-*C*-nucleosides, Synthesis, DFT calculations, Insecticidal activity, Neuroactivity, Alzheimer disease

INTRODUCTION

The use of *C*-nucleosides is well known in medicine, and the research into this group of compounds is likely to yield new applications for essential needs in medicine and other fields, such as pest management. This potential may be further increased by investigating pseudo-*C*-nucleosides, the chemical structure of which differ from that of *C*-nucleosides in the position of the sugar to which the heterocyclic moiety is bound. A variety of biological activities is reported in the literature for these compounds. Tiazofurin, a 2-substituted thiazole-4-carboxamide derivative, and a synthetic *C*-nucleoside, which has demonstrated antiviral activity and potent antitumor activity against several murine tumors, was submitted to clinical trials against lung cancer.[1–3] It was also found to be a potent inducer of leukemia cell differentiation along with other nucleotides containing the thiazole-4-carboxamide unit in their structure.[4]

The thiazole unit is also a component of the structure of the macrocyclic antibiotics thiocilline I[5] and leinamycin, an antitumor natural antibiotic, which is known to promote DNA damage.[6] Other antitumor indolinones contain an imidazothiazole system in their structure.[7]

2,4-Disubstituted thiazole-containing secondary metabolites were isolated from marine sources,[8] including the cytotoxins patellazoles A–C[9] and pateamine,[10] the antimitotic agent curacin A,[11] and the potent anthelmintic agent micothiazole.[12] An anthelmintic thiazole derivative is used to control gastrointestinal nematodes in ruminants.[13]

4-Thiazolidinone derivatives are fungicidal,[14] antibacterial,[15] anti-inflammatory,[15] analgesic,[15] antioxidant,[16] cytotoxic,[17] antiviral[18] and

have potent antituberculosis activity against *Mycobacterium tuberculosis* H (37) Rv (ATCC 27294).[14] These types of compounds are also known as herbicides and have insecticidal activity against *Anopheles* sp.,[19] *Culex* sp.,[19] and cockroaches.[20]

Ribavirin is a broad-spectrum antiviral agent, displaying antitumor activity in mice.[21] Synthesis of this triazole nucleoside and its *C*-nucleoside analogues[22] have attracted considerable attention due to their bioactivities. Preparation of 1,2,4-1*H*-triazolated nucleosides was reported by site-specific triazolation at C-4 of 5-substituted uridines with triazolide, formed from 1,2,4-triazole, $POCl_3$, and triethylamine.[23] Triazole nucleosides were also synthesised by dehydrative cyclization of pentitol-1-yl triazoles.[24] Triazolyl moieties were found to be good leaving groups for the solid-phase synthesis of oligonucleotides containing 4-guanidino-2-pyrimidinone nucleobases, devised to be used in antigene strategies for the control of gene expression.[25] Triazoles with leaf bleaching activity and germination promoting activity were also described in the literature.[26]

The antifungal[27,28] and pesticidal[29] activities of tetrazoles have encouraged the search for new compounds containing these units in their structure. Tetrazole derivatives have also been used as promising activating catalysts of phosphoramidites, resulting in rapid condensation with carboxamides to give *N*-acylphosphoramidate linkages.[30] This linkage is common in nucleotide antibiotics, namely, in phosmidosine, which is a specific inhibitor of *Botrytis cinerea*.

As part of our ongoing search for new compounds derived from sugars focusing on their potential pesticidal applications, we report now the synthesis of new pseudo-*C*-nucleosides possessing thiazole, triazole, tetrazole, and thiazolidinone moieties. In a preliminary evaluation of biological effects, compounds **3**, **4**, and **6** were tested in two invertebrate species, the crustacean *Artemia salina* and the dipteran (insect) *Musca domestica*. This enabled limited comparison of juvenile aquatic and adult terrestrial arthropods based on whole organism toxicity assays. The neuroactivity shown led us to accomplish preliminary experiments on their inhibition of amphiphilic acetylcholinesterase from human erythrocytes and of butyrylcholinesterase from human serum, enzymes that are involved in neurotransmission in the brain, and thus, this type of compound may find value ameliorating Alzheimer's disease.[31]

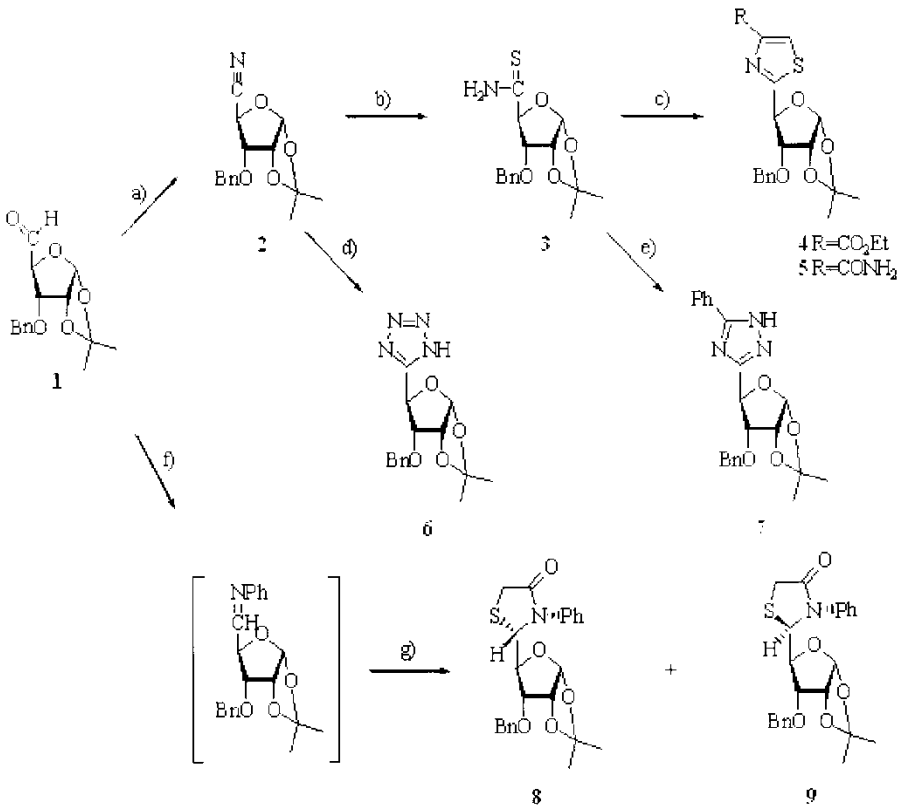
RESULTS AND DISCUSSION

Synthesis of Compounds 2–9

3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentodialdo-1,4-furanose (**1**) was used as synthon for the preparation of the new pseudo-*C*-nucleosides described in this work. The transformation of the aldehyde functionality into nitrile has

been reported in the literature, and the various methods available for that conversion include oxidative transformation of the *N,N*-dimethylhydrazones,[32] oxidation of aldimines,[33] dehydration of aldoximes by using agents such as *N*-methylpyrrolidone,[34] solid-state conversion by alumina-supported potassium peroxymonosulfate,[35] and treatment with hydroxylamine hydrochloride under microwave irradiation,[36] among others. We report now the one-pot synthesis of the sugar nitrile **2** in 78% yield, by reaction of **1** with hydroxylamine hydrochloride and addition of triethylamine, copper(II) sulfate pentahydrate, and the dehydration agent DCC[37] (Sch. 1). The IR band at 2117 cm^{-1} together with the ^{13}C NMR signal at δ 116.82 confirmed the formation of the sugar nitrile **2**.

Reaction of **2** with H_2S in chloroform in the presence of DMAP at 30°C, led to the thioamide derivative **3** in 85% yield. The IR spectrum presented the NH_2 bands at 3447 and 3325 cm^{-1}. The singlets at δ 7.77 and δ 7.59 corresponding to



Scheme 1: Synthesis of thiazole, thiazolidinone, tetrazole, and triazole derivatives. Reagents and conditions: (a) $NH_2OH.HCl$, Pyr., H_2O; $CuSO_4.5H_2O$, Et_3N, DCC, CH_2Cl_2 (78%); (b) H_2S, DMAP, $CHCl_3$ (85%); (c) $BrCH_2COCOOEt$, CH_3CN (50% for **4**); NH_3, MeOH (88% for **5**); (d) NaN_3, DMF, $NH_3.HCl$ (97%); (e) $PhCONH.NH_2$ (87.5%); (f) $PhNH_2$, toluene, *p*-TsOH, powdered molecular sieve 4 Å; (g) $HSCH_2COOH$, toluene (57% for **8**, 13% for **9**, overall yield from **1**).

NH_2 and the C═S signal at δ 203.71 detected in the ^{1}H NMR and ^{13}C NMR spectra, respectively, are in agreement with the introduction of the thioamide moiety in the molecule.

The synthesis of a variety of thiazole-4-carboxylate derivatives was described in the literature using ethyl isocyanoacetate and thiono esters[38] or cyclodehydration of amides.[39] We have constructed the thiazole-4-carboxylate unit using a classical method, by treatment of **3** with ethyl bromopyruvate in acetonitrile at 0°C[40,41] to give the corresponding thiazole-4-carboxylate **4** in 50% yield as major product, separated by flash chromatography from a complex mixture of secondary products. Formation of the heterocyclic ring was detected by the singlet at δ 8.17 corresponding to H-5 and by the ^{13}C NMR signals due to the olefinic carbon atoms C-2, C-4, and C-5 at δ 161.43, δ 147.50, and δ 128.29, respectively. The presence of the ethoxycarbonyl group was confirmed by the resonances at δ 4.20 for the methylene group and the methyl group in a multiplet at δ 1.36–1.43. The signals of the corresponding carbon atoms appeared at δ 61.47 and δ 14.34, respectively, while that of the carbonyl group was present at δ 169.08.

Treatment of **4** with methanolic ammonia led to **5** in 88% yield. The presence of the NH_2 group was confirmed by its IR bands at 3480 and 3405 cm^{-1} and by the singlet at δ 5.82. The carbonyl group appeared at δ 168.39 in the ^{13}C NMR spectrum.

The synthesis of the tiazofurin analogue **5** was thus accomplished in three steps with 37% overall yield, starting from the pentofuranose–4–nitrile **2**, a higher yield than that obtained previously for similar transformations, leading to the synthesis of tiazofurin.[1]

The tetrazole derivative **6** was easily obtained by reaction of the nitrile **2** with sodium azide at 100°C in 97% yield. The assignment of H-4′ at δ 5.42 shifted downfield to the corresponding proton of the nitrile precursor, and the presence of the C = N signal at δ 155.50 confirmed the structure proposed for **6**.

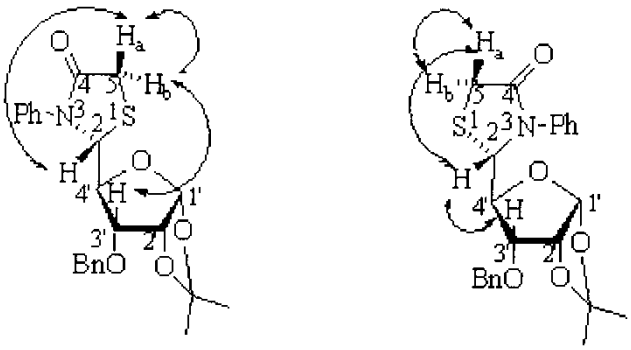
Reaction of benzoyl hydrazide with the thioamide **3** gave the phenyl 1,2,4-triazole derivative **7** in 87.5% yield, following the procedure described by Pesson et al.[42] The triazole moiety was characterized by the presence of the signals at δ 165.54 corresponding to C-5 and at δ 163.16 corresponding to C-3. The assignment was established by observation of the COLOC spectrum, which presented a correlation between C-5 and the double doublet at δ 7.99, the resonance due to the protons at positions 2 and 6 of the phenyl group bound to C-5, and the correlations of C-3 with H-4′ at δ 5.29 and with H-3′ at δ 4.31. The presence of NH was confirmed by its IR band at 3490 cm^{-1}.

Compound **1** reacted with aniline in the presence of *p*-toluenesulphonic acid and powdered molecular sieves 4 Å to afford an intermediate phenylimine, which was treated with sulfanylacetic acid in toluene to give a mixture of the thiazolidin-4-one diastereoisomers **8** and **9**, which could be separated by CC and isolated in 57% and 13% yield, respectively. The ^{1}H NMR experimental

Table 1: Comparison of experimental and calculated NMR chemical shifts using the GIAO algorithm[50] and referenced to TMS, for diasteroisomers **8** and **9.**

	8		**9**	
Proton	**Exp.**	**Calc.**	**Exp.**	**Calc.**
H-2	5.37	5.14	4.85	4.40
H-5a	3.42, 3.48	3.19	3.38, 3.43	3.32
H-5b	3.31, 3.36	3.76	3.72–3.81	3.76
H-1′	5.71	5.82	5.73	4.99
H-2′	4.56	4.52	4.56	4.33
H-3′	3.91	3.68	3.72–3.81	3.65
H-4′	4.28–4.36	4.00	4.18	4.34
CH_2 (Bn)	4.28–4.36	4.51	4.43, 4.39	4.54
CH_2 (Bn)	4.61–4.65	5.12	4.72, 4.68	4.92
Ph (Bn)	7.31–7.38	7.18–7.52	7.24–7.42	7.38–7.83
Ph (N)		7.43–7.82		7.13–7.74

data of both isomers is given in Table 1. The resonances of H-2 and H-5b, which are on opposite sides of the heterocyclic ring (Fig. 1) are significantly different in both isomers. The signal of H-5b is part B of an AB system, showing a coupling constant with H-2 of 1.5 Hz in both compounds and appearing at δ 3.31, 3.36 (for **8**), and in the multiplet at δ 3.72–3.81, together with the signal of H-3′ (for **9**), while the signals of H-5a (part A of the AB system) do not change substantially in both compounds. The configuration of the stereogenic center at C-2 in **8** was first inferred by means of NOE experiments. Detection of the interaction between H-5b and H-3′ indicated that H-5b has the orientation depicted in Figure 1. H-5a presented an interaction with H-2 at δ 5.37 and did not show interaction with sugar protons, inferring that H-2 and



8 **9**

Figure 1: Some NOE interactions relevant for the structure elucidation of compounds **8** and **9**.

H-5a are on the same side of the thiazolidinone ring. These findings are in agreement with the *S* configuration for C-2. NOE experiments for **9** showed an interaction of H-2 at δ 4.85 with the H-3′ signal included in the multiplet at δ 3.73–3.78, which contains also H-5b. In addition a small interaction of H_2 with H-5a was observed, as expected.

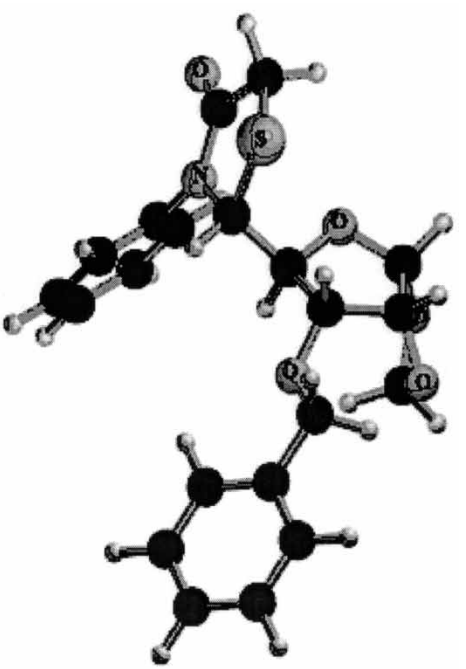
To confirm the structure and NMR signals' assignment for the thiazolidin-4-one diastereoisomers **8** and **9**, DFT (B3LYP/6-311G**) calculations were performed (see Computational Details). The methyl groups of the isopropylidene group were substituted by hydrogen atoms, to simplify the models, since they are not involved in the relevant interactions. The geometries of **8** and **9** were fully optimized and the minimum energy structures are presented in Figure 2. Diastereoisomer **8** was found to be more stable than **9** by 16.2 kJ mol^{-1}, a difference low enough to predict both isomers to occur. NMR chemical shifts were calculated using the GIAO option and the results show a general good agreement with the experimental ones. A comprehensive list of calculated and experimental values is given in Table 1. However, for compound **8** the experimental signal at δ 3.31, 3.36, assigned to proton H-5b, is not consistent with the calculated value at δ 3.76. The experimental chemical shift of H-5b is lower than that of H-5a, while for the calculated chemical shifts the opposite was observed. This can be explained by the existence of an intramolecular C–H$\cdots$O hydrogen bond (H$\cdots$O distance = 2.9 Å) between the oxygen atom of the furanose ring and this endocyclic proton attached to C-5 (see Fig. 2). Such weak interactions are known to be underestimated by hybrid DFT methods.[43] Therefore, the calculations confirm the assignment of the experimental NMR data, showing also that the predicted configuration of the C-2 stereogenic center of isomers **8** and **9** is correct.

Biological Results

Toxicological assays against Musca domestica and Artemia salina

The aquatic bioassays using the brine shrimp larvae showed no effects at all, even when the concentration of compounds **3**, **4**, and **6** was increased to 100 mg/L. This indicated a very low level of activity even if the uptake by the shrimp was limited. This organism is often used for assessing environmental toxicity and cytotoxicity and can be viewed as a reference species.

The results obtained with the housefly are summarized in Table 2. All three compounds exhibited knockdown at the concentrations used. However, compound **4** gave this effect at much lower dosages ($\times 10^{-3}$) and was the only compound to be insecticidal. By performing regression analysis on the 24-hr mortality data for this compound based on percent mortality and log dosage (presented in Fig. 3) an estimate for the median lethal dosage is as follows: $LD_{50} = 6.7$ ng/g (0.67 μg/g body weight), 95% confidence limits



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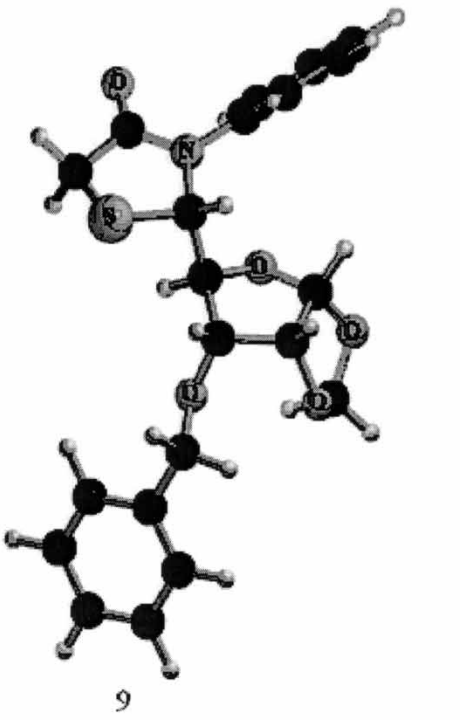

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Figure 2: DFT energy minimized geometries of diastereoisomers **8** and **9** at the B3LYP/6-311G** level.

Table 2: Responses of adult houseflies to topical application of compounds **3, 4**, and **6**.

Compound nr.	Dosage (ng/insect)	Time to 100% knockdown (min)	Duration of knockdown (min)	Time to 100% recovery (min)	% Mortality at 24 hr
3 (Thioamide)	1	15–17	20–21	15–20	0
	10	15–20	20–22	20–28	0
	100	15–20	20–25	30–33	0
4 (Thiazole)	1	10–12	13–17	10–11	0
	2	10–13	14–15	10–15	0
	4	10–12	15–20	12–15	20
	6	10–15	18–21	20–22	40
	8	10–15	17–30	20–23	60
	10	10–15	—	20% recovery	80
	12	10–20	—	no recovery	100
	14	10–20	—	no recovery	100
6 (Tetrazole)	1	15–20	8–10	5–10	0
	10	15–20	10–12	10–12	0
	100	15–19	15–20	15–20	0

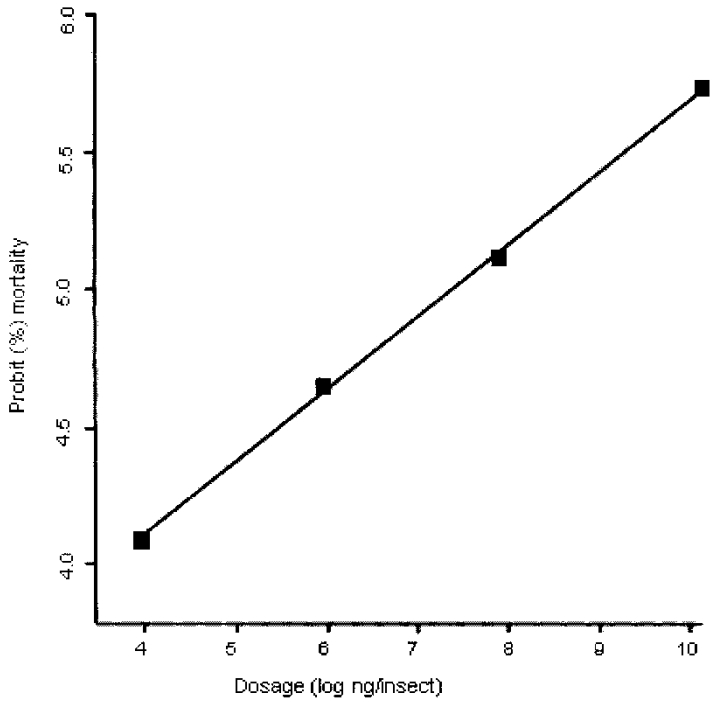

Figure 3: Mortality response (probit percent) of adult housefly with thiazole solutions in acetone applied topically.

4.8–9.3 ng/insect, which is in the order of the typical value for the LD_{50} value for pyrethroids on the same insect (e.g., 0.3 ng/g for the highly potent deltamethrin).[44] For this compound complete knockdown was achieved at the lowest dosage used (1.0 ng/insect), and therefore the estimate for the median effective dosage (ED_{50}) for knockdown must be less than this, and there is likely to be a factor of about 10 between the dosages for these two responses.

The other two compounds (**3** and **6**) were not toxic to the adult housefly, causing no mortality at all up to 10 ng/insect (1 mg/g body weight). This might be due to lack of cuticle penetration, but as complete knockdown was caused at all dosages used, this explanation is unlikely.

Knockdown, or temporary incapacity, is exhibited by a number of compounds, especially insecticides such as some organochlorines, carbamates, and pyrethroids.[45] Members of this latter group that act rapidly are considered to have good knockdown. Knockdown here is a prepoisoning symptom and is mediated through action on the central or on the peripheral nervous system. Structural variation within the pyrethroids influenced knockdown, but the thiolactone kadethrin acted more rapidly than any other pyrethroid, which usually lacks this moiety.[44] Whether these compounds act through a similar mechanism to the pyrethroids, or even if the symptoms are

related, is unclear, but their lack of lethal activity would indicate that another process is operating. Additional support for this view comes from the distinct structural differences between these nucleosides and the pyrethroids. This effect is also similar to that of anaesthesia, such as is provided by carbon dioxide in insects.

Enzyme inhibition

The activity of the two diastereoisomers **8** and **9** toward amphiphilic acetylcholinesterase from human erythrocytes and butyrylcholinesterase from human serum was investigated. The relative inhibition of these enzymes has been found to exert a beneficial therapeutic effect in some patients suffering from Alzheimer's disease. Although in the assay acetylthiocholine and butyrylthiocholine were used as substrates, it is considered that they adequately represent the natural substrates for these hydrolytic enzymes.[46] Enzyme activity was measured by the increase of yellow color produced from thiocholine when it reacted with the dithiobisnitrobenzoate ion. Inhibition of acetylcholinesterase activity at 1.7 mg/mL was 5% for **8** and 8% for **9**. Inhibition of butyrylcholinesterase activity at 1.0 mg/mL was 36% for **8** and at 1.7 mg/mL was 22% for **9**.

Conclusion

According to the data given in Table 2, the thiazole derivative **4** led to 100% mortality of adult houseflies with dosages of 12 and 14 ng/insect and was the only pseudo-nucleoside tested showing insecticidal activity. Regression analysis on 24-hr mortality data for this compound based on percent mortality and log dosage (Fig. 3) gave an estimate for the median lethal dosage $LD_{50} = 6.7$ ng/g (0.67 μg/g body weight), which is in the order of magnitude of LD_{50} values for pyrethroids.

All three compounds exhibited knockdown at the concentrations used. No estimate for ED_{50} for these compounds was found, but clearly it is less than 100 ng/insect. This property is clearly unusual and novel.

The thiazolidinone derivatives have demonstrated some efficacy for the inhibition of butyrylcholinesterase (36% for **8** and 22% for **9**). The enzyme acetylcholinesterase was not significantly affected by these compounds (5% for **8** and 8% for **9**).

EXPERIMENTAL

General Methods

Melting points were determined by using either a Leitz-Biomed apparatus or a Buchi 530. Optical rotations were determined with an Atago Polax-D

polarimeter, or in a Perkin Elmer 343. ^{1}H NMR, ^{13}C NMR, NOE, HMQC, and COLOC experiments were recorded in $CDCl_3$ either with a Bruker AC-P 250 (250 MHz) or with a Varian Unity-300 MHz, with Me_4Si as internal standard. Chemical shifts are expressed in ppm (δ) and coupling constants J in Hz. The ^{13}C NMR spectra were recorded at 62.9 MHz.

IR spectra were recorded with a Biorad FTS (FT) or a Hitachi 270-50 spectrometer. Electron impact mass spectra were determined using a VG Trio 1000 spectrometer, by direct injection. Elemental analyses were performed with a Fisons EA 1108 analyser.

The progress of all reactions was monitored by thin-layer chromatography (TLC) using aluminum sheets precoated with silica gel $60F_{254}$ to a thickness of 0.2 mm (Merck). Compounds were detected with UV light (254 nm) and/or by spraying the sheets with a 3% vanillin-sulphuric acid solution. Column chromatography (CC) was conducted under low pressure by elution from silica gel (0.040–0.063 mm, Merck) columns.

Acetylcholinesterase from human erythrocytes and butyrylcholinesterase from human serum were purchased from Sigma, Poole, UK.

Computational Details

DFT[47] calculations were performed using the Gaussian 98 program, rev. A.11.[48] Molecular structures were fully optimized without any symmetry constraints at the B3LYP[49] level with the 6-311G** basis set[50] for all atoms. Frequency calculations were performed to confirm the nature (minima) of the stationary points determined. NMR chemical shifts were calculated using the GIAO algorithm[51] and referenced to TMS (Tetramethylsilane).

Synthesis of Compounds 2–9

3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentofuranose-4-nitrile (2). A solution of aldehyde **1** (7.25 mmol, 2 g) in pyridine (4 mL) was added slowly to a solution of hydroxylamine hydrochloride (7.93 mmol, 0.55 g) in water (2 mL). After stirring for 15 min at rt, pyridine (0.4 mL) was added to dissolve the precipitated oxime. The mixture remained under stirring at rt for 1 hr. $CuSO_4.5H_2O$ was added (14.4 mmol, 3.6 g), followed by a solution of triethylamine (14.4 mmol, 2 mL) in dichloromethane (3.6 mL) and DCC (8.72 mmol, 1.8 g) in dichloromethane (15 mL). After 2 hr at rt, formic acid was added (1.3 mL) to eliminate DCC. The mixture was filtered, and the filtrate was concentrated under vacuum. The residue was treated with water (50 mL), then extracted with dichloromethane (3 × 50 mL). The organic phase was washed with a solution of HCl 5% (v/v) (50 mL), dried with sodium sulfate, and concentrated under vacuum to give **2** (1.54 g, 78%); m.p. 123–124°C; $R_f = 0.74$ (ethyl acetate/toluene 1:2); $[\alpha]_D^{25} = +59^\circ$ (c1.9, $CHCl_3$); IR (KBr) (cm^{-1}): 2117 (CN); UV ($\lambda_{máx.}$ nm) ethanol: 209 ($\varepsilon = 6887$); ^{1}H NMR (250 MHz): 7.32 (s, 5

H, Ph), 5.74 (d, 1H, H-1, $J_{1,2} = 3.4$), 4.78, 4.71 (AB system, OCH_2, Bn), 4.62 (d, 1H, H-4, $J_{3,4} = 9$), 4.53 (t, 1H, H-2, $J_{2,3} = 4.1$), 4.10 (dd, 1H, H-3), 1.56 (s, 3H, CH_3 isop), 1.33 (s, CH_3 isop); ^{13}C NMR: 136.08 (Cq, Ph), 128.45, 128.23, 127.89 (CH, Ph), 116.82 (Cq, C-5), 113.98 (Cq, isop), 104.56 (CH, C-1), 80.03 (CH, C-3), 76.74 (CH, C-2), 72.55 (OCH_2, Bn), 66.07 (CH, C-4), 26.59 (CH_3, isop), 26.05 (CH_3, isop).

Anal. Calcd for $C_{15}H_{17}O_4N$: C, 65.44; H, 6.22; N 5.09. Found: C, 64.95; H, 6.38; N, 5.52.

3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentofuranose-4-thioamide (3). DMAP (5.92 mmol, 0.072 g) was added to a solution of **2** (3.64 mmol, 1 g) in chloroform (18 mL). Hydrogen sulfide was bubbled into the solution until full saturation, and the reaction mixture was kept under stirring at 30°C for 6 hr 30 min. After evaporation of the hydrogen sulfide that did not react, dichloromethane (50 mL) was added, and the solution was extracted with sodium chloride solution 10% (w/v) (2 × 30 mL). The organic phase was dried with magnesium sulfate, filtered, and concentrated in vacuum to give **3** (0.96 g, 85%); m.p. 194.3–194.6°C; $R_f = 0.51$ (ethyl acetate/toluene 1:1); $[\alpha]_D^{25} = +60^\circ$ (*c*2.2, $CHCl_3$); IR (KBr) (cm^{-1}): 3447, 3325 (NH_2); UV ($\lambda_{máx.}$ nm) ethanol: 271 ($\varepsilon = 10292$), 208 ($\varepsilon = 9372$); ^1H NMR (250 MHz): 7.77 (s, 1H, NH_2), 7.59 (s, 1H, NH_2), 7.46–7.26 (m, 5 H, Ph), 5.80 (d, 1H, H-1, $J_{1,2} = 3.5$), 4.87–4.74 (m, 3H, H-4, OCH_2, Bn), 4.52 (t, 1H, H-2, $J_{2,3} = 4$), 3.77 (dd, 1H, H-3, $J_{3,4} = 8.6$), 1.59 (s, 3H, CH_3, isop), 1.37 (s, 3H, CH_3, isop); ^{13}C NMR: 203.71 (Cq, C-5), 136.22 (Cq, Ph), 128.34, 128.20, 128.13 (CH, Ph), 113.90 (Cq, isop), 103.43 (CH, C-1), 83.15 (CH, C-4), 81.96 (CH, C-3), 78.50 (CH, C-2), 72.94 (OCH_2, Bn), 26.96 (CH_3, isop), 26.62 (CH_3, isop).

Anal. Calcd for $C_{15}H_{19}O_4NS$: C, 58.23; H, 6.19; N, 4.53; S, 10.36. Found: C, 57.85; H, 6.08; N, 4.52; S, 10.71.

Ethyl 2-[(4*R*)-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl]thiazole-4-carboxylate (4). To a solution of ethyl bromopyruvate (9.93 mmol, 1.24 mL) in anhydrous acetonitrile (6 mL) cooled at 0°C, a solution of **3** (3.24 mmol, 1 g) in anhydrous acetonitrile (15 mL) was added at 0°C. The reaction mixture was ice-cooled. After 5 min stirring, the ice bath was removed, and the solution was stirred at 20°C for 1 hr. Evaporation of the solvent under vacuum (20°C) gave a residue, which was treated with a solution of sodium hydrogen carbonate (100 mL) and extracted with dichloromethane (3 × 100 mL). The organic phase was washed with water and dried with magnesium sulfate. The dichloromethane was evaporated under vacuum to give a residue, which was purified by CC eluted with ethyl acetate/toluene 1:5 (v/v) to give **4** (0.65 g, 50%) as a syrup; $R_f = 0.57$ (ethyl acetate/toluene 1:2); $[\alpha]_D^{25} = +66^\circ$ (*c*1.5, $CHCl_3$); IR (neat) (cm^{-1}): 1741 (C═O), 1596 (C = C); UV ($\lambda_{máx.}$ nm) ethanol: 200 ($\varepsilon = 8950$); ^1H NMR

(250 MHz): 8.17 (s, 1H, H-5), 7.24 (s, 5 H, Ph), 5.88 (d, 1H, H-1′, $J_{1',2'} = 3.3$), 5.41 (d, 1H, H-4′, $J_{3',4'} = 8.6$), 4.74-4.59 (m, 3H, H-2′, OCH_2, Bn), 4.20 (q, 2 H, CH_2, Et, $J = 7.1$), 3.99 (dd, 1H, H-3′, $J_{2',3'} = 4.1$), 1.66 (s, 3H, CH_3, isop), 1.43–1.36 (m, 6 H, CH_3, isop, CH_3, Et); ^{13}C NMR: 169.08 (Cq, C═O), 161.43 (Cq, C-2), 147.50 (Cq, C-4), 137.01 (Cq, Ph), 128.29 (CH, C-5), 128.11, 128.03, 127.90 (CH, Ph), 113.69 (Cq, isop), 104.04 (CH, C-1′), 82.55 (CH, C-3′), 78.22 (CH, C-2′), 77.51 (CH, C-4′), 72.36 (OCH_2, Bn), 61.47 (OCH_2, Et), 26.89 (CH_3, isop), 26.47 (CH_3 isop), 14.34 (CH_3, Et).

Anal. Calcd for $C_{20}H_{23}O_6NS$: C, 59.25; H, 5.72; N, 3.45; S, 7.91. Found: C, 59.61; H, 5.79; N, 3.13; S, 7.73.

2-[(4*R*)-3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl]thiazole-4-carboxamide (5). A solution of **4** (2.47 mmol, 1 g) in anhydrous methanol was cooled at 0°C. Ammonia gas was bubbled into the solution until complete saturation. After stirring for 48 hr at rt, the solvent was evaporated to give **5** as a solid (0.79 g, 88%); m.p. 161–161.5°C; $R_f = 0.43$ (ethyl acetate/toluene 2:1); $[\alpha]_D^{25} = +40^\circ$ (*c*1.25, $CHCl_3$); IR (KBr) (cm^{-1}): 3480, 3405 (NH_2), 1683 (C═O); UV ($\lambda_{máx.}$ nm) ethanol: 233 ($\varepsilon = 11520$), 219 ($\varepsilon = 11330$); ^{1}H NMR (250 MHz): 8.15 (s, 1H, H-5), 7.35–7.20 (m, 5 H, Ph), 5.91 (d, 1H, H-1′, $J_{1',2'} = 3.5$), 5.82 (s, 1H, NH), 5.31 (d, 1H, H-4′, $J_{3',4'} = 8.8$), 4.74–4.70 (m, 2H, H-2′, part A of AB system, OCH_2, Bn), 4.59 (1H, part B of the AB system, OCH_2, Bn, $J_{AB} = 12$), 3.92 (dd, 1H, H-3′, $J_{2',3'} = 4$), 1.68 (s, 3H, CH_3, isop), 1.42 (s, 3H, CH_3, isop); ^{13}C NMR: 168.39 (Cq, C═O), 162.75 (Cq, C-2), 149.40 (Cq, C-4), 136.78 (Cq, Ph), 128.51, 128.14, 127.89 (CH, Ph), 125.00 (CH, C-5), 113.80 (Cq, isop), 104.16 (CH, C-1′), 82.56 (CH, C-3′), 77.88 (CH, C-2′), 77.52 (CH, C-4′), 76.48 (OCH_2, Bn), 26.89 (CH_3, isop), 26.47 (CH_3 isop).

Anal. Calcd for $C_{18}H_{20}O_5N_2S$: C, 57.43; H, 5.35; N, 7.44; S, 8.52. Found: C, 57.12; H, 5.49; N, 7.88; S, 8.34.

5-[(4*R*)-3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl]tetrazole (6). Ammonium chloride (4.6 mmol, 0.25 g) and sodium azide (4 mmol, 0.342 g) were added to a solution of **2** (3.64 mmol, 1.0 g) in DMF (8 mL). The mixture was heated at 100°C for 3 hr 30 min. The solution was concentrated, and the residue was extracted with ethyl acetate (3 × 50 mL) and water (50 mL). The organic phase was washed with water and dried with magnesium sulfate. The ethyl acetate was evaporated under vacuum to give **6** (1.23 g, 97%); mp. 149.8–150.1°C; $R_f = 0.67$ (ethyl acetate/methanol: 1/1); $[\alpha]_D^{25} = +69^\circ$ (*c*1.8, $CHCl_3$); IR (KBr) (cm^{-1}): 3480 (NH); UV ($\lambda_{máx.}$ nm) ethanol: 208 ($\varepsilon = 3390$); ^{1}H-NMR (250 MHz): 7.27 (s, 5 H, Ph), 5.93 (d, 1H, H-1′, $J_{1',2'} = 3$), 5.42 (d, 1H, H-4′, $J_{3',4'} = 8.8$), 4.73–4.58 (m, 3H, H-2′, OCH_2, Bn), 4.19 (dd, 1H, H-3′, $J_{2',3'} = 4.2$), 1.62 (s, 3H, CH_3, isop), 1.40 (s, 3H, CH_3, isop); ^{13}C-NMR: 155.50 (Cq, C-5), 136.50 (Cq, Ph), 128.49, 128.28, 128.09

(CH, Ph), 114.03 (Cq, isop), 104.57 (CH, C-1′), 81.36 (CH, C-3′), 77.79 (CH, C-2′), 72.81 (OCH_2, Bn), 71.48 (CH, C-4′), 26.75 (CH_3, isop), 26.36 (CH_3, isop).

Anal. Calcd for $C_{15}H_{18}O_4N_4$: C, 56.60; H, 5.70; N, 17.60. Found: C, 56.86; H, 5.73; N, 17.21.

5-[(4*R*)-3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl]-5-phenyl-1,2,4-triazole (7). Compound **3** (3.24 mmol, 1 g) was added to benzoyl hydrazine (3.7 mmol, 0.5 g), and the mixture was heated at 170°C for 2 hr. After cooling, the crude product was purified by CC eluted with ethyl acetate/toluene 1:5 (v/v) to give **7** as syrup (1.11 g, 87.5%); $R_f = 0.72$ (ethyl acetate/toluene: 1/1). $[\alpha]_D^{25} = +78^\circ$ (*c*1.6, $CHCl_3$); IR (neat) (cm^{-1}): 3490 (NH_2); UV ($\lambda_{máx.}$ nm) ethanol: 277 ($\varepsilon = 14600$), 235 ($\varepsilon = 13350$); ^{1}H-NMR (250 MHz): 7.99 (dd, 2 H, Ph-5, $J_{2'',3''} = 7.7$, $J_{2'',4''} = 1.4$), 7.54–7.49 (m, 3H, Ph-5), 7.21–7.15 (m, 5 H, Ph, Bn), 5.88 (d, 1H, H-1′, $J_{1',2'} = 3.5$), 5.29 (d, 1H, H-4′, $J_{3',4'} = 9$), 4.76, 4.62 (AB system, 2 H, OCH_2, Bn), 4.71 (t, 1H, H-2′, $J_{2',3'} = 4.2$), 4.31 (dd, 1H, H-3′), 1.69 (s, 3H, CH_3, isop), 1.41 (s, 3H, CH_3, isop); ^{13}C-NMR: 165.54 (Cq, C-5), 163.16 (Cq, C-3), 136.69 (Cq Ph, Bn), 131.91, 128.92, 126.99 (3 CH, Ph-5), 128.37, 128.06, 127.89 (3 CH, Ph, Bn), 123.36 (Cq, Ph-5), 113.79 (Cq, isop), 104.38 (CH, C-1′), 79.84 (CH, C-3′), 77.58 (CH, C-2′), 72.65 (OCH_2, Bn), 71.37 (CH, C-4′), 26.85 (CH_3, isop), 26.35 (CH_3, isop).

Anal. Calcd for ($C_{22}H_{23}O_4N_3$): C, 67.16; H, 5.89; N, 10.68. Found: C, 67.52; H, 6.45; N, 10.25.

(2*S*)-2-[(4*R*)-3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl]-3-phenyl-2*H*-thiazolidin-4-one (8) and (2*R*)-2-[(4*R*)-3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl]-3-phenyl-2*H*-thiazolidin-4-one (9). To a solution of **1** (0.18 mmol, 0.05 g) in dry toluene (2 mL), activated powdered molecular sieves (4 Å, 0.18 g), *p*-toluenesulfonic acid (catalytic amount), and aniline (1.4 mmol, 0.13 g) in dry toluene (0.5 mL) were added. The reaction mixture was refluxed under argon atmosphere with stirring, at 55–65°C, in a reaction flask equipped with a Dean-Stark apparatus. After 3 hr, the mixture was cooled to rt, dry dichloromethane (20 mL) was added, the mixture was filtered, and the filtrate was neutralized with a saturated solution of sodium carbonate. The organic phase was dried with sodium sulfate, filtered, and concentrated. Sulfanylacetic acid (0.628 mmol, 0.05 mL) was added to the solution of the compound previously synthesized (0.111 g), without purification, in dry toluene (15 mL). The mixture was heated at 55–65°C under stirring in argon atmosphere, again with a Dean-Stark apparatus coupled to the reaction flask. The mixture was cooled to rt after 50 hr 20 min heating, and dry toluene (15 mL) was added. After extraction with water, the organic phase was dried over sodium sulfate, filtered, and concentrated. The isomers **8** and **9** were purified by CC eluted with ethyl acetate/toluene 1:5 (v/v), to give **8** (0.044 g, 57 %) as a syrup and **9** (0.010 g, 13%).

Data for **8**: $R_f = 0.69$ (ethyl acetate/toluene 1:1); $[\alpha]_D^{20} = -1^\circ$ (*c* 0.05, $CHCl_3$); ^1H NMR (300 MHz): 7.38–7.31 (m, 10H, 2Ph), 5.71 (d, 1H, H-1′, $J_{1',2'} = 3.6$), 5.37 (t, 1H, H-2, $J_{2,4'} = 1.8$), 4.65, 4.61 (1H, part A of AB system, OCH_2, Bn, $J_{A,B} = 11.7$), 4.56 (t, 1H, H-2′, $J_{2',3'} = 4.5$), 4.36–4.28 (m, 2H, H-4′, part B of AB system, OCH_2, Bn,), 3.91 (dd, 1H, H-3′, $J_{3',4'} =$ 8.4), 3.48, 3.42 (each s, 1H, H-5a, part A of AB system, $J_{5a,5b} = 15.6$), 3.36, 3.31 (each d, 1H, H-5b, part B of AB system, $J_{2,5b} = 1.5$), 1.45 (s, 3H, CH_3, isop), 1.31 (s, 3H, CH_3, isop); ^{13}C NMR: 170.8 (C═O); 137.8, 136.7 (Cq, Ph), 129.4, 128.6, 128.3, 127.7, 126.5, 125.5 (CH, Ph), 113.5 (Cq, isop), 104.1 (CH, C-1′), 79.3 (CH, C-4′), 77.6 (CH, C-2′), 77.1 (CH, C-3′), 72.0 (OCH_2, Bn), 63.8 (CH, C-2); 32.4 (CH_2, C-5), 26.8 (CH_3, isop), 26.6 (CH_3, isop).

Anal. Calcd for $C_{23}H_{25}NO_5S$: C, 64.62; H, 5.89; N, 3.28; S, 7.50. Found: C, 64.92; H, 5.79; N, 3.35; S, 7.62.

Data for **9**: $R_f = 0.59$ (ethyl acetate/toluene 1:2); $[\alpha]_D^{20} = +8^\circ$ (*c*0.05, $CHCl_3$); ^1H NMR (300 MHz): 7.42–7.24 (m, 10H, 2Ph), 5.73 (d, 1H, H-1′, $J_{1',2'} =$ 3.3), 4.85 (t, 1H, H-2, $J_{2,4'} = 1.5$, $J_{2,5a} = 1.5$), 4.72, 4.68 (1H, part A of AB system, OCH_2, Bn), 4.56 (t, 1H, H-2′, $J_{2',3'} = 3.9$), 4.43, 4.39 (1H, part B of AB system, OCH_2, Bn, $J_{A,B} = 12.3$), 4.18 (dd, 1H, H-4′, $J_{3',4'} = 9.0$), 3.81–3.72 (m, 2H, H-3′, H-5a, part A of AB system), 3.43–3.38 (each s, 1H, H-5b, part B of AB system, $J_{5a,5b} = 15.3$), 1.37 (s, 3H, CH_3, isop), 1.32 (s, 3H, CH_3, isop); ^{13}C NMR: 171.1 (C═O), 137.0 (Cq, Ph), 129.6, 129.0, 128.8, 128.5, 128.2, 127.3 (CH, Ph), 113.8 (Cq, isop), 104.2 (CH, C-1′), 78.5 (CH, C-3′), 77.9 (CH, C-2′), 76.6 (CH, C-4′), 72.4 (OCH_2, Bn), 0.65.3 (CH, C-2), 33.1 (CH_2, C-5), 26.9 (CH_3, isop), 26.7 (CH_3, isop).

Anal. Calcd for $C_{23}H_{25}NO_5S$: C, 64.62; H, 5.89; N, 3.28; S, 7.50. Found: C, 64.78; H, 5.73; N, 3.44; S, 7.28.

Biological Assays

Aquatic brine shrimp bioassay

Newly hatched brine shrimp larvae, *Artemia salina*, were bioassayed using a method based on microtitre plates.[52] Brine shrimp larvae were reared from eggs in brine (15 g/L sodium chloride) in a small glass tank at 30°C. Using their phototropic response the emerging larvae were separated from the eggs. Larvae up to 24 hr old only were used. Using a dissecting microscope the larvae were carefully transferred (in 75 μL), in batches of five, using a piston micropipette from the tank to the wells of the microtitre plate (each well volume 300 μL). Solutions of the test substances in saline were then added to the wells, and the plate was covered and kept at 30°C. Eight replicates for each concentration of each test compound and untreated control were used. Percent mortality (no movement or response to a probe) was determined at 24 hr.

Housefly bioassay

A pyrethroid susceptible strain (Cooper) of the housefly *Musca domestica* was cultured at 25°C.[53] This method included feeding the adults on milk with 5% sucrose and egg laying and larval rearing on moist bran. Three-day-old adults were selected (both sexes) and anaesthetized with carbon dioxide. Each compound was applied to the ventral thorax in 1.0 μL acetone using a microapplicator. Five insects (mean adult fly weight was 10 mg) were used for each dosage of each compound. The responses were determined continuously up to 1 hr after treatment; recovery from anaesthesia, knockdown (inability to recover normal posture). Final mortality was determined at 24 hr after treatment, keeping the temperature at 25°C and food available.

Inhibition of Acetylcholinesterase and of Butyrylcholinesterase[54]

Incubation time (with inhibitor): 15 min at 30°C ± 1.

Reaction time: 6 min at 30°C ± 1.

In each well of a microtiter plate, enzyme solution (5 μL), pH 8 buffer (200 μL), dithiobisnitrobenzoate (DTNB) reagent (5 μL), and the solution of the tested compounds (5 μL) were placed. The solution of amphiphilic acetylcholinesterase from human erythrocytes was used with the concentration 1.26 mg enzyme/mL pH 8 buffer, the solution of butyrylcholinesterase from human serum had a concentration of 0.1 mg/mL, DTNB had a concentration of 5.2 mg/mL pH 7 buffer, and the tested compounds were used with the concentration of 1.7 mg/mL ethanol 86%, except when the inhibition of butyrylcholinesterase was tested with compound **8**, whose solution had the concentration of 1.0 mg/mL ethanol 86%. After 15 min incubation the substrate (5 μL), which was acetylthiocholine (6.4 mg/mL water) or butyrylthiocholine (7 mg/mL water), was added. The reaction time was 6 min, and during this time the reaction kinetics were measured by a UV spectrophotometer at 405 nm with a temperature control of 30°C ± 1. This procedure took place six times. The three blank assays for such run consisted of buffer, DTNB, and substrate. The enzyme solution was replaced by the same amount of water, and the solution of compounds **8** or **9** was replaced by ethanol 86%.

There were also six control assays with the enzyme, buffer, and DTNB, adding ethanol 86% to replace the solution of the tested compounds.

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