Synthesis of 4-(Isothiazol-3-yl)morpholines and 1-(Isothiazol-3-yl)piperazines, and Their Inhibitory Activity towards Acetylcholinesterase

by **Janine Wolf**[a]), **Camino M. González Tanarro**[b]), **Michael Gütschow**[b]), **Joachim Sieler**[c]), and **Bärbel Schulze***[a])

[a]) Institute of Organic Chemistry, University of Leipzig, Johannisallee 29, D-04103, Leipzig (fax: +49-341-9736599; e-mail: bschulze@chemie.uni-leipzig.de)

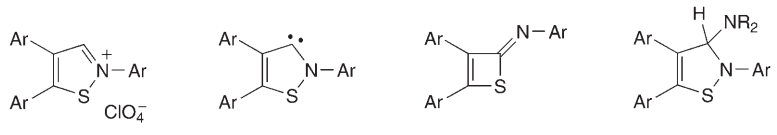
[b]) Pharmaceutical Institute, University of Bonn, An der Immenburg 4, D-53121 Bonn

[c]) Institute of Inorganic Chemistry, University of Leipzig, Johannisallee 29, D-04103 Leipzig

Dedicated to Prof. Dr. *H. Hartmann* on the occasion of his 70th birthday

The synthesis of novel triaryl-substituted 4-(isothiazol-3-yl)morpholines **7** and **8**, and 1-(isothiazol-3-yl)piperazines **9**–**13** by reaction of the corresponding isothiazolium salts **5** and **6** with secondary amines in the presence of *t*-BuOK in absolute THF is described. Some representatives of the isothiazoles were evaluated as inhibitors of acetylcholinesterase from *Electrophorus electricus.*

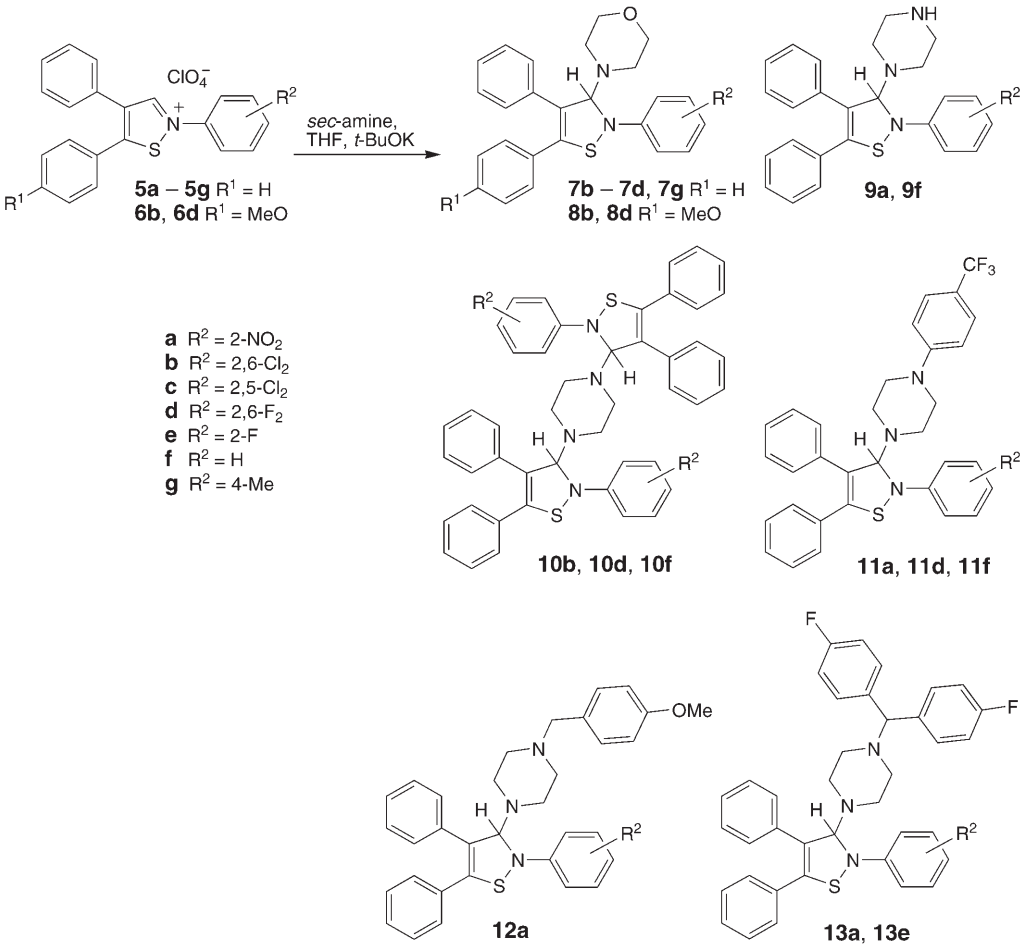
Introduction. – Recent contributions to the chemistry of isothiazoles showed that 2,4,5-triarylisothiazolium perchlorates **1** underwent a transformation to amino-substituted isothiazole derivatives when reacted with secondary amines. It has already been suggested [1] that isothiazolium perchlorates **1** undergo deprotonation in the presence of *t*-BuOK in THF to generate *in situ* the isothiazol-3-ylidenes **2**, which immediately react with morpholine or piperidine to form the corresponding products **4**. Two representatives of **4** have been prepared that way. *Bertrand* and co-workers have demonstrated that carbenes **2** are not stable and cannot be isolated, since they isomerize into the corresponding 2-imino-2*H*-thiete isomers **3** [2]. The reaction of the triphenyl-substituted compound **3** (Ar = Ph) with morpholine at room temperature resulted in ring opening to afford a thioketone derivative [2]. Thus, the conversion of **1** to **4** did not occur *via* the four-membered intermediates **3**. Moreover, the triphenyl-substituted isothiazolium perchlorate **1** (Ar = Ph) was treated with the lithium salt of morpholine to cleanly obtain the corresponding adduct **4** [2]. Whether the formation of **4** involves a base-assisted addition of the secondary amine to the isothiazolium perchlorate **1**, or transient carbenes **2** were trapped by an insertion reaction into the N–H bond of the secondary amine, are alternative explanations for the formation of **4**



[1–3]. Herein, we report the facile transformation of isothiazolium perchlorates **1** to a series of new isothiazolyl-morpholines and -piperazines **4** to demonstrate the scope of this reaction.

Results and Discussion. – *Synthesis.* The new isothiazolium salts **5** and **6** were conveniently synthesized by intramolecular cyclocondensation of 2,3-diaryl-3-thiocyanatoprop-2-enals and variously substituted anilines in the presence of $HClO_4$ and glacial acetic acid [4]. In the *Scheme*, the substituents of the 2-aryl ring (R^2) were graded according to the pK_a value of the corresponding anilinium ions. The structure of the isothiazolium salts **5** was confirmed by X-ray crystal structure determination of **5c** ($R^1 = H$, $R^2 = 2,5\text{-}Cl_2$) and is presented in *Fig. 1*. The crystallographic data are given in the *Exper. Part*.

Scheme



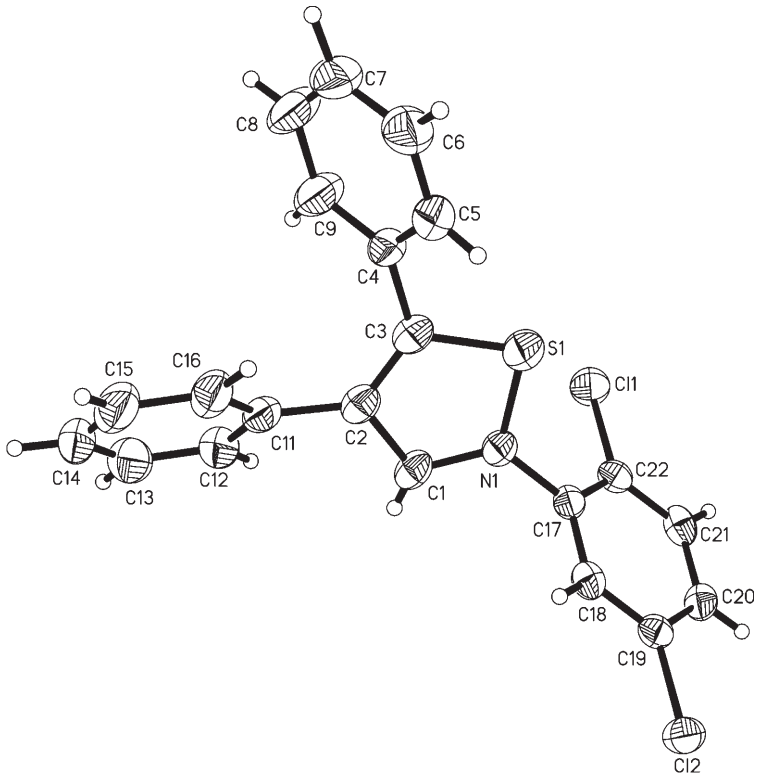


Fig. 1. *Molecular structure of 2-(2,5-dichlorophenyl)-4,5-diphenylisothiazolium perchlorate* (**5c**) *without the perchlorate anion* (thermal ellipsoids at 50% probability)

We have performed the reaction of isothiazolium perchlorates **5** and **6**, respectively, with morpholine, piperazine, or monosubstituted piperazines in the presence of *t*-BuOK in absolute THF to obtain the corresponding isothiazoles **7**–**13** by elimination of $KClO_4$ and *t*-BuOH in good-to-excellent yields. The utility of the reaction is demonstrated in the *Scheme* showing the synthesis of 4-(isothiazol-3-yl)morpholines **7** and **8** and isothiazolyl-piperazines **9**–**13**. The reaction of **5** with piperazine could be controlled to obtain the products **9** with equimolar amounts of the isothiazolium salt and piperazine, whereas the 1,4-bis(diphenyl-isothiazolyl) derivatives **10** were obtained when 2 equiv. of the isothiazolium salt were used.

The structures of the 4-(isothiazol-3-yl)morpholines **7** and 1,4-bis(diphenyl-isothiazolyl)piperazines **10** were confirmed by X-ray crystal-structure analysis of their representatives **7b** and **10b** ($R^1 = H$, $R^2 = 2,6\text{-}Cl_2$), and are presented in *Figs. 2* and *3*, respectively. The crystallographic data are given in the *Exper. Part*. Two molecules of THF are included in the crystal system of **10b**.

The structures of the novel 4-(isothiazol-3-yl)morpholines **7b**–**7d**, **7g**, **8b**, **8d** and isothiazolyl-piperazines **9a, 9f**, **10b**, **10d**, **10f**, **11a**, **11d**, **11f**, **12a**, **13a**, and **13e** were established by IR and NMR spectroscopy, mass spectrometry, as well as elemental analysis. The morpholine derivatives **7** and **8**, and piperazine derivatives **9**–**13** have two

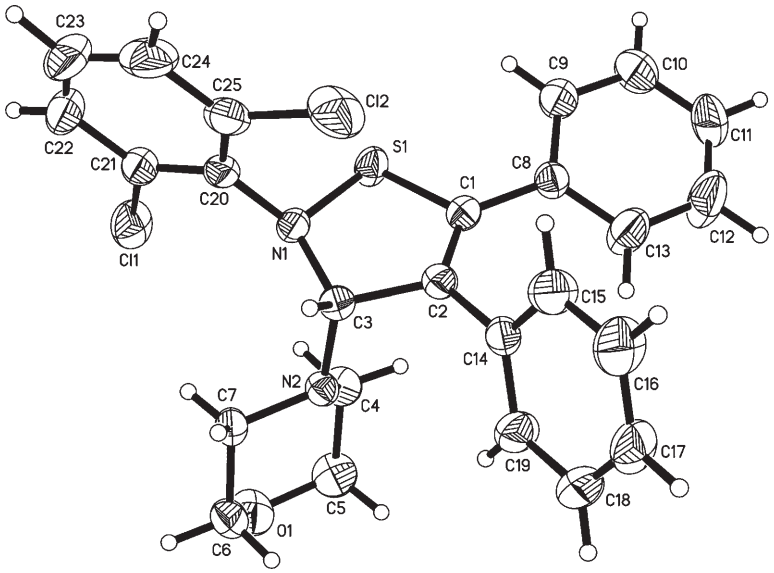


Fig. 2. *Molecular structure of 4-[2-(2,6-dichlorophenyl)-2,3-dihydro-4,5-diphenylisothiazol-3-yl]morpholine* (**7b**; thermal ellipsoids at 50% probability)

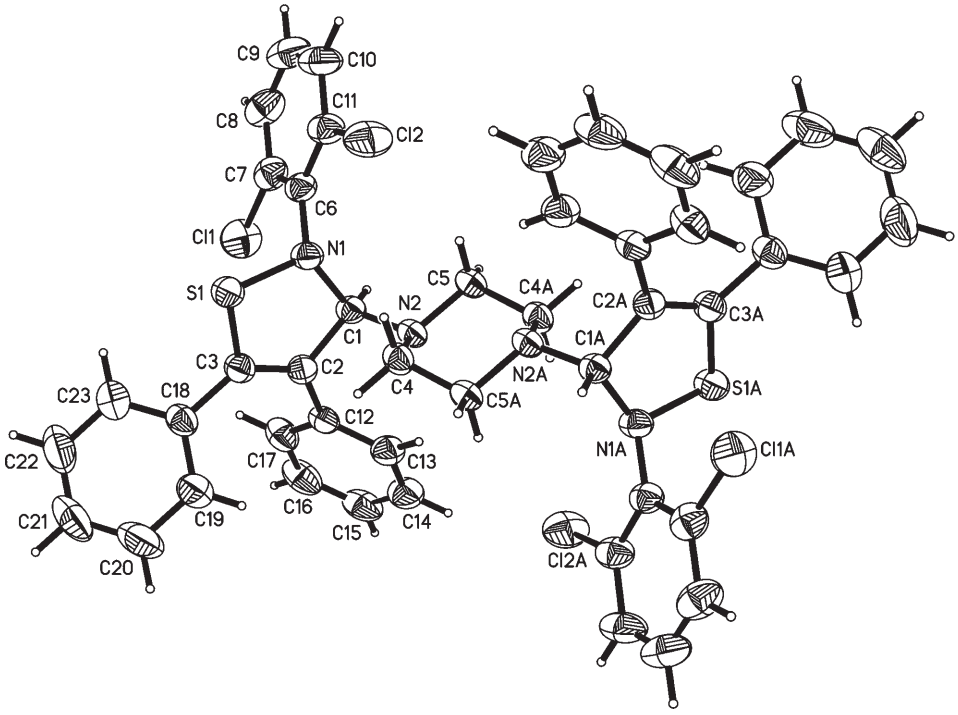


Fig. 3. *Molecular structure of 1,4-bis[2-(2,6-dichlorophenyl)-2,3-dihydro-4,5-diphenylisothiazol-3-yl]piperazine* (**10b**) *without the two solvent molecules* (thermal ellipsoids at 50% probability; symmetry code A: $1-x, 1-y, 1-z$)

typical CH_2 absorptions in the IR spectra in the range of 2810–2858 and 2929–2962 cm^{-1}, whereas **7** and **8** exhibit an additional absorption between 1112 and 1117 cm^{-1} for the C–O–C group. The ^{1}H-NMR spectra of **7**–**13** show a chemical shift of H–C(3) in the range of 5.28–6.06 ppm, and, in their ^{13}C-NMR spectra, the chemical shift of C(3) is between 98.5 and 101.9 ppm. The parent peaks of the mass spectra correspond to the expected masses, and the results of elemental analysis agree with the calculated values.

All synthesized compounds **7**–**13** were evaluated as inhibitors of acetylcholinesterase from *Electrophorus electricus*. Acetylcholine is one of the major neurotransmitters in the brain and is hydrolyzed by the enzyme acetylcholinesterase. *Morbus Alzheimer*, a progressive, neurodegenerative disease, is characterized by a selective loss of cholinergic neurons in the basal forebrain. Further pathological hallmarks are the formation of intracellular neurofibrillary tangles and extracellular deposition of β-amyloid protein. At present, the administration of cholinesterase inhibitors is the accepted therapeutic approach to compensate the cholinergic deficit [5].

Inhibition of Acetylcholinesterase. The activity of acetylcholinesterase from *Electrophorus electricus* was determined in a coupled assay with the substrate acetylthiocholine (ATCh) and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB). The IC_{50} values are given in the *Table*. Dose-dependent inhibition of the most potent compound, *i.e.*, **12a**, is shown in *Fig. 4*. The morpholine derivatives **7** and **8** failed to inhibit acetylcholinesterase. For compounds **9**, with an unsubstituted piperazine NH group, the introduction of a 2-nitro group into the *N*-phenyl ring improved the activity (*i.e.*, **9a** *vs.* **9f**). The two most potent compounds exhibit structural similarities in that they have a lipophilic substituent at the second piperazine N-atom. Again, a 2-nitrophenyl group at N(2) of the isothiazole was advantageous (**12a**; **11a** *vs.* **11d** and **11f**). A benzyl moiety attached to a basic N-atom is present in potent acetylcholinesterase inhibitors such as donepezil [6] and AP2238 [7]. The 2-NO_2-substituted **13a** is less potent than **11a** and **12a**, probable due to the sterically demanding diarylmethyl moiety.

Table. *Inhibition of Acetylcholinesterase*

Compound	IC_{50}[a]) ± SEM [μM]	Compound	IC_{50}[a]) ± SEM [μM]
7a	n.d.[b])	**10d**	30
7b	31	**10f**	61
7c	30	**11a**	10.1 ± 0.8
7d	> 100	**11d**	56
7f	> 100	**11f**	77
7g	> 100	**12a**	7.58 ± 0.53
8b	> 100	**13a**	44
8d	> 100	**13e**	> 100
9a	16	Tacrine	0.027 ± 0.001[c])
9f	> 100	Galanthamine	2.20 ± 015[c])
10b	16		

[a]) IC_{50} Values with standard error were calculated from duplicate experiments at five inhibitor concentrations. Data were fitted to *Eqn. 1* (see *Exper. Part*). Values without standard error were calculated from duplicate inhibition experiments at a single inhibitor concentration. [b]) Not determined due to low solubility. [c]) Taken from [8].

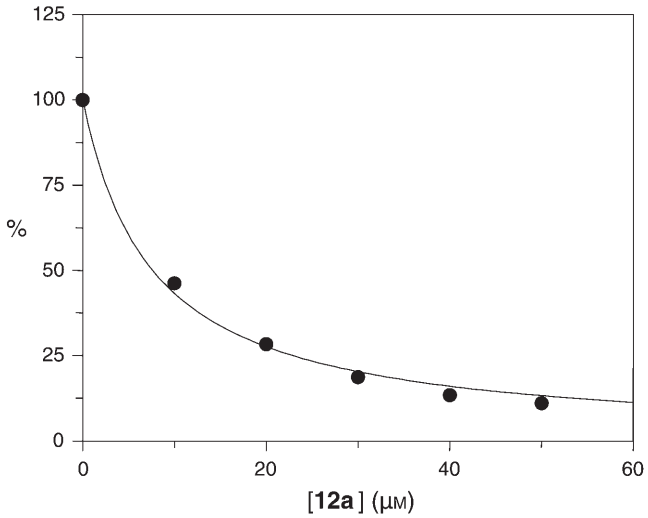


Fig. 4. *Plot of the rates* vs. *[I] for the inhibition of acetylcholinesterase by compound* **12a**. The rate in the absence of the inhibitor was set 100%. The solid line was drawn using the best-fit parameters from a fit according to *Eqn. 1*, which gave $IC_{50} = 7.58 \pm 0.53$ μM.

Conclusions. – In summary, the reaction of isothiazolium salts **5** and **6**, respectively, with secondary amines represents a convenient method to synthesize 4-(isothiazol-3-yl)morpholines **7** and **8**, and isothiazolyl-piperazines **9**–**13** by a one-step procedure without difficult purification. Among the newly synthesized isothiazoles **7**–**13**, some derivatives showed inhibitory properties towards acetylcholinesterase. Ongoing investigations in our laboratories are intended to elucidate the mechanism of this reaction and to further evaluate its potential to produce biologically active compounds. Beyond the morpholine and piperazine derivatives reported herein, the reaction is expected to allow an access to a variety of heterocyclic compounds.

Financial support of this work by the *Deutsche Forschungsgemeinschaft* and *Graduiertenkolleg 677* is gratefully acknowledged.

Experimental Part

General. Acetylcholinesterase from *Electrophorus electricus* was purchased from *Fluka* (D-Deisenhofen). Acetylthiocholine (ATCh) and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from *Sigma* (D-Steinheim). The spectrophotometric assay was performed on a *Varian Cary 50 Bio* UV/VIS spectrometer with a cell holder equipped with a constant-temp. water bath. M.p.: *Boetius* micro-melting-point apparatus; corrected. IR Spectra: *Genisis FTIR Unicam Analytical System* (*ATI Mattson*); KBr pellets; in cm^{-1}. ^1H-, ^{19}F-, and ^{13}C-NMR Spectra: *Varian Gemini-200*, *-300*, and *Bruker Avance DRX-400*; recorded in (D_8)THF; δ in ppm relative to Me_4Si as internal standard, *J* in Hz. MS: *Quadrupole-MS VG 12-250*; 70 eV. Elemental analysis: *Heraeus CHNO Rapid Analyzer*.

General Procedure for the Preparation of Salts **5** *and* **6**. The new salts were prepared according to the procedure described in [4]. Compounds **5a** and **5f** were described in [1] and [8], resp. Yields and melting points are **5b**: 61%, 207–210°; **5c**: 61%, 214–217°; **5d**: 71%, 191–193°; **5e**: 55%, 148–151°; **5g**: 53%, 129–132°; **6b**: 52%, 139–143°; **6d**: 74%, 195–197°.

General Procedure for the Preparation of 4-(Isothiazol-3-yl)morpholines **7** *and* **8**. A suspension of the corresponding isothiazolium salts **5** or **6** (0.30 mmol) and morpholine (26 mg, 0.30 mmol) in abs. THF (6 ml) was treated with *t*-BuOK (37 mg, 0.33 mmol). The soln. was stirred in an ice bath under N_2. After 30 min, the org. solvent was evaporated. The crude product was extracted with dry hexane (4×10 ml), and the combined org. soln. was evaporated to a small volume. The precipitate was filtered to obtain the crystalline compounds **7** and **8**. Compounds **7a** and **7f** have been described in [1].

4-[2-(2,6-Dichlorophenyl)-2,3-dihydro-4,5-diphenylisothiazol-3-yl]morpholine (**7b**). Yield: 94%. Yellow crystals. M.p. 130–133°. IR: 1112 (C–O–C), 1430, 2858 (CH_2), 2960 (CH_2). ^{1}H-NMR: 2.72–2.81 (*m*, CH_2); 3.02–3.12 (*m*, CH_2); 3.57–3.67 (*m*, CH_2); 5.55 (*s*, H–C(3)); 7.14–7.24 (*m*, arom. H); 7.41–7.51 (*m*, arom. H). ^{13}C-NMR: 48.0 (CH_2); 67.6 (CH_2); 100.1 (C(3)); 121.3 (C(5)); 127.4; 128.5; 129.0; 129.2; 129.6; 130.1; 130.9 (C(Cl)); 132.3; 136.1; 136.2 (C(4)); 138.4; 142.2; 146.1 (C_q(N)). EI-MS: 382.0 ([*M* – morpholine]$^{+\bullet}$). Anal. calc. for $C_{25}H_{22}Cl_2N_2OS$ (469.44): C 63.97, H 4.72, N 5.97, S 6.83; found: C 64.30, H 4.89, N 5.75, S 7.03.

4-[2-(2,5-Dichlorophenyl)-2,3-dihydro-4,5-diphenylisothiazol-3-yl]morpholine (**7c**). Yield: 92%. Colorless crystals. M.p. 126–129°. IR: 1117 (C–O–C), 1461, 1574, 2850 (CH_2), 2962 (CH_2). ^{1}H-NMR: 2.72–2.84 (*m*, CH_2); 3.03–3.18 (*m*, CH_2); 3.63–3.70 (*m*, CH_2); 5.66 (*s*, H–C(3)); 7.13–7.29 (*m*, arom. H); 7.45 (*d*, $J = 8.5$, arom. H); 7.53–7.58 (*m*, arom. H). ^{13}C-NMR: 49.8 (CH_2); 68.5 (CH_2); 99.9 (C(3)); 122.1 (C(5)); 126.7 (C(Cl)); 128.3; 129.1; 130.1; 130.7; 131.0; 131.1; 131.6; 131.8; 133.2 (C(Cl)); 135.0 (C(4)); 137.0; 144.3; 154.2 (C_q(N)). EI-MS: 469.0 ($M^{+\bullet}$). Anal. calc. for $C_{25}H_{22}Cl_2N_2OS$ (469.44): C 63.97, H 4.72, N 5.97, S 6.83; found: C 64.50, H 4.97, N 5.71, S 6.74.

4-[2-(2,6-Difluorophenyl)-2,3-dihydro-4,5-diphenylisothiazol-3-yl]morpholine (**7d**). Yield: 94%. Colorless crystals. M.p. 170–173°. IR: 1116 (C–O–C), 1136 (C–F), 1280 (C–F), 1468, 2850 (CH_2), 2957 (CH_2). ^{1}H-NMR: 2.85–2.90 (*m*, CH_2); 3.23–3.28 (*m*, CH_2); 3.77–3.82 (*m*, CH_2); 5.46 (*s*, H–C(3)); 7.11 (*t*, $J = 8.6$, arom. H); 7.27–7.38 (*m*, arom. H); 7.60–7.63 (*m*, arom. H). ^{19}F-NMR: –115.59 (*t*, arom. F). ^{13}C-NMR: 48.0 (CH_2); 67.6 (CH_2); 100.7 (C(3)); 112.9; 120.8 (C(5)); 127.4; 128.2; 128.4; 128.5; 129.2; 129.4; 129.9; 130.1; 132.3; 136.1 (C(4)); 142.6 (C_q(N)); 161.5 ($J = 250$, arom. F). EI-MS: 436.0 ($M^{+\bullet}$). Anal. calc. for $C_{25}H_{22}F_2N_2OS$ (436.53): C 68.79, H 5.08, N 6.42, S 7.35; found: C 68.60, H 5.12, N 5.99, S 7.04.

4-[2,3-Dihydro-2-(4-methylphenyl)-4,5-diphenylisothiazol-3-yl]morpholine (**7g**). Yield: 79%. Colorless crystals. M.p. 181–184°. IR: 1116 (C–O–C), 1443, 1503, 1589, 2849 (CH_2), 2953 (CH_2). ^{1}H-NMR: 2.24 (*s*, Me); 2.89–2.93 (*m*, CH_2); 3.16–3.20 (*m*, CH_2); 3.64–3.66 (*m*, CH_2); 5.37 (*s*, H–C(3)); 7.05–7.47 (*m*, arom. H). ^{13}C-NMR: 20.1 (Me); 47.5 (CH_2); 68.6 (CH_2); 101.3 (C(3)); 114.9; 122.2; 125.7 (C(5)); 127.7; 128.5; 129.0; 129.3; 129.9; 130.6; 131.7; 132.3; 136.2 (C(4)); 138.0 (Me*C*); 159.3 (C_q(N)). EI-MS: 413.0 ([*M* – H]$^{+\bullet}$). Anal. calc. for $C_{26}H_{26}N_2OS$ (414.57): C 75.33, H 6.32, N 6.76, S 7.73; found: C 75.08, H 6.17, N 6.56, S 7.47.

4-[2-(2,6-Dichlorophenyl)-2,3-dihydro-5-(4-methoxyphenyl)-4-phenylisothiazol-3-yl]morpholine (**8b**). Yield: 93%. Colorless crystals. M.p. 140–142°. IR: 1116 (C–O–C), 1510, 1604, 2849 (CH_2), 2943 (CH_2). ^{1}H-NMR: 2.67–2.77 (*m*, CH_2); 2.99–3.12 (*m*, CH_2); 3.61–3.67 (*m*, CH_2); 3.73 (*s*, MeO); 5.50 (*s*, H–C(3)); 6.76, 6.80 (*AA′BB′*, $J = 8.0$, arom. H); 7.13–7.23 (*m*, arom. H); 7.40–7.54 (*m*, arom. H). ^{13}C-NMR: 48.0 (CH_2); 55.2 (MeO); 67.7 (CH_2); 100.1 (C(3)); 114.6; 120.0 (C(5)); 124.2; 128.5; 128.9; 129.5; 130.8; 131.1; 136.0; 136.5 (C(4)); 138.3; 142.2; 146.2; 161.1 (C_q(N)). EI-MS: 499.0 ($M^{+\bullet}$). Anal. calc. for $C_{26}H_{24}Cl_2N_2O_2S$ (499.46): C 62.52, H 4.84, N 5.61, S 6.42; found: C 62.76, H 5.01, N 5.40, S 6.80.

4-[2-(2,6-Difluorophenyl)-2,3-dihydro-5-(4-methoxyphenyl)-4-phenylisothiazol-3-yl]morpholine (**8d**). Yield: 96%. Yellow crystals. M.p. 151–155°. IR: 1115 (C–O–C), 1139 (C–F), 1467, 1608, 2857 (CH_2), 2930 (CH_2). ^{1}H-NMR: 2.74–2.75 (*m*, CH_2); 3.10–3.12 (*m*, CH_2); 3.61–3.66 (*m*, CH_2); 3.73 (*s*, MeO); 5.28 (*s*, H–C(3)); 6.77, 6.80 (*AA′BB′*, $J = 8.4$, arom. H); 6.97 (*t*, $J = 8.4$, arom. H); 7.15–7.23 (*m*, arom. H); 7.52 (*d*, $J = 7.2$, arom. H). ^{19}F-NMR: –118.45 (*d*, arom. F). ^{13}C-NMR: 48.0 (CH_2); 55.2 (MeO); 67.7 (CH_2); 100.7 (C(3)); 112.7; 113.0; 114.6; 119.4; 124.1 (C(5)); 127.3; 128.3; 128.4; 128.5; 130.0; 131.4; 136.4 (C(4)); 161.1 (C_q(N)). EI-MS: 466.0 ($M^{+\bullet}$). Anal. calc. for $C_{26}H_{24}F_2N_2O_2S$ (466.55): C 66.94, H 5.19, N 6.01, S 6.87; found: C 66.50, H 5.49, N 5.71, S 6.57.

General Procedure for the Preparation of 1-(Isothiazol-3-yl)piperazines **9**. A suspension of the isothiazolium salts **5** (0.30 mmol) and piperazine (26 mg, 0.3 mmol) in abs. THF (6 ml) was treated with *t*-BuOK (37 mg, 0.33 mmol). The soln. was stirred in an ice bath under N_2. After 60 min, the org. solvent

was evaporated. The crude product was extracted with dry hexane and Et_2O (3×10 ml), and the combined org. soln. was reduced to a small volume. The precipitate was filtered to obtain the crystalline compounds **9**.

1-[2,3-Dihydro-2-(2-nitrophenyl)-4,5-diphenylisothiazol-3-yl]piperazine (**9a**). Yield: 51%. Orange crystals. M.p. 73–76°. IR: 1345 (NO_2), 1525 (NO_2), 2831 (CH_2), 2947 (CH_2). ^{1}H-NMR: 2.77–2.81 (*m*, CH_2); 2.92–2.96 (*m*, CH_2); 3.08–3.10 (*m*, CH_2); 5.75 (*s*, NH); 6.06 (*s*, H–C(3)); 7.33–7.73 (*m*, arom. H); 7.88 (*d*, $J = 8.0$, arom. H). ^{13}C-NMR: 45.3 (CH_2); 46.5 (CH_2); 47.5 (CH_2); 99.0 (C(3)); 119.2; 122.6 (C(5)); 123.8; 124.4; 124.6; 124.8; 125.8; 126.2; 126.9; 127.5; 127.8; 128.4; 128.5; 132.4; 133.8 (C(4)); 141.6 (C_q(N)). EI-MS: 444.0 ($[M-H]^{+\bullet}$). Anal. calc. for $C_{25}H_{24}N_4O_2S$ (444.56): C 67.54, H 5.44, N 12.60, S 7.21; found: C 67.21, H 5.34, N 12.71, S 7.18.

1-(2,3-Dihydro-2,4,5-triphenylisothiazol-3-yl)piperazine (**9f**). Yield: 94%. Red crystals. M.p. 128–131°. IR: 1345, 1268, 1443, 1488, 2840 (CH_2), 2931 (CH_2). ^{1}H-NMR: 2.68–2.70 (*m*, CH_2); 2.88–2.90 (*m*, CH_2); 3.12–3.18 (*m*, CH_2); 3.38–3.40 (*m*, CH_2); 5.49 (*s*, NH); 5.60 (*s*, H–C(3)); 6.96 (*d*, $J = 7.4$, arom. H); 7.11–7.49 (*m*, arom. H). ^{13}C-NMR: 46.8 (CH_2); 47.4 (CH_2); 48.2 (CH_2); 48.9 (CH_2); 100.6 (C(3)); 114.7; 116.9; 120.3; 121.1 (C(5)); 122.9; 127.4; 129.6; 130.3; 131.6; 131.8; 132.4; 136.1; 138.0 (C(4)); 152.9 (C_q(N)). EI-MS: 397.0 ($[M-H_2]^{+\bullet}$). Anal. calc. for $C_{25}H_{25}N_3S$ (399.56): C 75.15, H 6.31, N 10.52, S 8.03; found: C 74.92, H 6.19, N 10.41, S 7.98.

General Procedure for the Preparation of Bis(isothiazol-3-yl)piperazine Derivatives (**10**). A suspension of the isothiazolium salts **5** (0.40 mmol) and piperazine (17 mg, 0.20 mmol) in abs. THF (6 ml) was treated with *t*-BuOK (51 mg, 0.45 mmol). The soln. was stirred in an ice bath under N_2. After 60 min, the org. solvent was evaporated. The crude product was extracted with dry hexane and Et_2O (3×10 ml), and the combined org. soln. was evaporated to a small volume. The precipitate was filtered to obtain the crystalline compounds **10**.

1,4-Bis[2-(2,6-dichlorophenyl)-2,3-dihydro-4,5-diphenylisothiazol-3-yl]piperazine (**10b**). Yield: 43%. Brown crystals. M.p. 84–87°. IR: 1059 (C–Cl), 2844 (CH_2), 2942 (CH_2). ^{1}H-NMR: 2.66–2.68 (*m*, CH_2); 2.80–2.91 (*m*, CH_2); 3.20–3.24 (*m*, CH_2); 5.54 (*s*, H–C(3)); 5.57 (*s*, H–C(3)); 7.23 (*s*, arom. H); 7.41–7.49 (*m*, arom. H); 7.59–7.62 (*m*, arom. H). ^{13}C-NMR: 47.2 (CH_2); 48.2 (CH_2); 100.2 (C(3)); 121.4 (C(5)); 125.7; 127.4; 128.9; 129.2; 129.5; 129.9; 130.2; 130.4; 130.8 (C(Cl)); 132.4; 136.2 (C(4)); 142.2; 146.4 (C_q(N)). EI-MS: 849.1 ($[M-H]^{+\bullet}$). Anal. calc. for $C_{46}H_{36}Cl_4N_4S_2$ (850.76): C 64.94, H 4.27, N 6.59, S 7.54; found: C 64.93, H 4.59, N 6.36, S 7.19.

1,4-Bis[2-(2,6-difluorophenyl)-2,3-dihydro-4,5-diphenylisothiazol-3-yl]piperazine (**10d**). Yield: 46%. Brown crystals. M.p. 74–77°. IR: 1137 (C–F), 2840 (CH_2), 2942 (CH_2). ^{1}H-NMR: 2.26–2.28 (*m*, CH_2); 2.66–2.68 (*m*, CH_2); 2.76–2.88 (*m*, CH_2); 3.05–3.09 (*m*, CH_2); 5.28 (*s*, H–C(3)); 5.38 (*s*, H–C(3)); 6.91–7.00 (*m*, arom. H); 7.14–7.30 (*m*, arom. H); 7.49–7.59 (*m*, arom. H). ^{19}F-NMR: –116.50 (*d*, arom. F). ^{13}C-NMR: 47.8 (CH_2); 48.6 (CH_2); 101.9 (C(3)); 110.5; 114.7; 120.7 (C(5)); 125.1; 126.8; 127.5; 127.9; 128.7; 129.4; 129.7; 131.9; 135.8 (C(4)); 139.6 ($J = 215.6$, arom. F); 158.5 (C_q(N)). EI-MS: 783.2 ($[M-H]^{+\bullet}$). Anal. calc. for $C_{46}H_{36}F_4N_4S_2$ (784.94): C 70.39, H 4.62, N 7.17, S 8.17; found: C 70.02, H 4.45, N 7.19, S 8.39.

1,4-Bis(2,3-dihydro-2,4,5-triphenylisothiazol-3-yl)piperazine (**10f**). Yield: 43%. Red crystals. M.p. 111–114°. IR: 2840 (CH_2), 2930 (CH_2). ^{1}H-NMR: 2.83–2.85 (*m*, CH_2); 3.00–3.02 (*m*, CH_2); 3.24–3.28 (*m*, CH_2); 3.48–3.52 (*m*, CH_2); 5.60 (*s*, H–C(3)); 5.72 (*s*, H–C(3)); 6.66 (*d*, $J = 7.6$, arom. H); 7.06–7.58 (*m*, arom. H). ^{13}C-NMR: 45.7 (CH_2); 48.2 (CH_2); 98.9 (C(3)); 119.5; 120.9 (C(5)); 121.3; 124.1; 125.8; 126.1; 126.9; 127.7; 128.0; 128.6; 129.8; 130.6; 134.3 (C(4)); 151.2 (C_q(N)). EI-MS: 713.3 (M^+). Anal. calc. for $C_{46}H_{40}N_4S_2$ (712.98): C 77.49, H 5.65, N 7.86, S 8.99; found: C 77.01, H 5.71, N 8.04, S 8.49.

General Procedure for the Preparation of (Isothiazol-3-yl)piperazine Derivatives (**11**–**13**). A suspension of the isothiazolium salts **5** (0.30 mmol) and respective piperazine derivatives (0.30 mmol) in abs. THF (6 ml) was treated with *t*-BuOK (37 mg, 0.33 mmol). The soln. was stirred in an ice bath under N_2. After 2 h, the org. solvent was evaporated. The crude product was extracted with dry hexane and Et_2O (3×10 ml), the combined org. soln. was reduced to a small volume, and the precipitate was filtered to obtain the crystalline compounds **11**–**13**.

1-[2,3-Dihydro-2-(2-nitrophenyl)-4,5-diphenylisothiazol-3-yl]-4-[4-(trifluoromethyl)phenyl]piperazine (**11a**). Yield: 96%. Yellow crystals. M.p. 152–155°. IR: 1117 (C–F), 1330 (NO_2), 1529 (NO_2), 2830 (CH_2), 2929 (CH_2). ^{1}H-NMR: 3.16–3.19 (*m*, CH_2); 3.28–3.33 (*m*, CH_2); 5.64 (*s*, H–C(3)); 7.00–7.59

(*m*, arom. H). ^{19}F-NMR: −61.67 (*d*, CF_3). ^{13}C-NMR: 47.0 (CH_2); 47.7 (CH_2); 49.0 (CH_2); 49.8 (CH_2); 101.1 (C(3)); 115.3; 121.3 (C(5)); 124.7; 125.3 (J = 217.9, CF_3); 126.9; 126.9; 127.0; 127.2; 128.0; 129.1; 129.6; 130.1; 130.5; 134.6; 135.7 (C(4)); 144.0 ($C(NO_2)$); 148.3; 148.8; 154.9 (C_q(N)). EI-MS: 587.2 ([M − H]$^{+\cdot}$). Anal. calc. for $C_{32}H_{27}F_3N_4O_2S$ (588.65): C 65.29, H 4.62, N 9.52, S 5.45; found: C 65.02, H 4.38, N 9.23, S 5.25.

1-[2-(2,6-Difluorophenyl)-2,3-dihydro-4,5-diphenylisothiazol-3-yl]-4-[4-(trifluoromethyl)phenyl]piperazine (**11d**). Yield: 88%. Yellow crystals. M.p. 138–141°. IR: 1115 (C−F), 2838 (CH_2), 2953 (CH_2). ^{1}H-NMR: 2.88–2.97 (*m*, CH_2); 3.29–3.35 (*m*, CH_2); 5.46 (*s*, H−C(3)); 6.96–7.04 (*m*, arom. H); 7.16–7.32 (*m*, arom. H); 7.45–7.54 (*m*, arom. H). ^{19}F-NMR: −61.64 (*d*, CF_3); −116.52 (*s*, arom. F). ^{13}C-NMR: 46.8 (CH_2); 47.3 (CH_2); 48.7 (CH_2); 49.6 (CH_2); 101.4 (C(3)); 112.7; 114.8; 115.1; 119.9; 121.1 (C(5)); 122.2; 122.9 (J = 216.9, CF_3); 126.8; 128.6; 129.0; 129.3; 129.5; 130.0; 130.1; 130.2; 132.2; 136.1 (C(4)); 142.6; 154.7 (C_q(N)); 161.5 (J = 250.5, CF). EI-MS: 578.2 ([M − H)$^{+\cdot}$). Anal. calc. for $C_{32}H_{26}F_5N_3S$ (579.64): C 66.31, H 4.52, N 7.25, S 5.53; found: C 66.15, H 4.72, N 7.12, S 5.58.

1-(2,3-Dihydro-2,4,5-triphenylisothiazol-3-yl]-4-[4-(trifluoromethyl)phenyl]piperazine (**11f**). Yield: 85%. Yellow crystals. M.p. 129–132°. IR: 1108 (C−F), 2837 (CH_2), 2940 (CH_2). ^{1}H-NMR: 3.12–3.19 (*m*, CH_2); 3.34–3.43 (*m*, CH_2); 5.66 (*s*, H−C(3)); 6.94–7.51 (*m*, arom. H). ^{19}F-NMR: −61.66 (*d*, CF_3). ^{13}C-NMR: 47.0 (CH_2); 48.3 (CH_2); 49.1 (CH_2); 49.8 (CH_2); 99.9 (C(3)); 115.3; 120.7; 121.2 (C(5)); 124.0 (J = 222.5, CF_3); 125.8; 126.9; 127.0; 127.0; 127.1; 128.9; 129.1; 129.6; 129.9; 130.5; 131.4; 132.5; 136.1 (C(4)); 142.5; 152.9 (C_q(N)); 154.8. EI-MS: 542.2 ([M − H]$^{+\cdot}$). Anal. calc. for $C_{32}H_{28}F_3N_3S$ (543.66): C 70.70, H 5.19, N 7.73, S 5.90; found: C 70.62, H 5.29, N 7.79, S 6.09.

1-[2,3-Dihydro-2-(2-nitrophenyl)-4,5-diphenylisothiazol-3-yl]-4-(4-methoxybenzyl)piperazine (**12a**). Yield: 77%. Colorless crystals. M.p. 135–138°. IR: 1325 (NO_2), 1530 (NO_2), 2834 (CH_2), 2945 (CH_2). ^{1}H-NMR: 2.27–2.34 (*m*, CH_2); 2.72–2.75 (*m*, CH_2); 3.32–3.34 (*m*, CH_2); 3.72 (*s*, MeO); 5.51 (*s*, H−C(3)); 6.78, 6.81 (*AA′BB′*, J = 8.4, arom. H); 7.16–7.52 (*m*, arom. H). ^{13}C-NMR: 47.0 (CH_2); 55.1 (CH_2); 55.4 (MeO); 63.7 (CH_2); 100.0 (C(3)); 114.0; 116.9; 124.5 (C(5)); 126.3; 126.7; 127.7; 128.7; 129.3; 129.6; 129.7; 130.3; 130.5; 131.4; 132.0; 134.3 ($C(NO_2)$); 135.6 (C(4)); 148.3 (MeO*C*); 159.6 (C_q(N)). EI-MS: 563.4 ([M − H]$^{+\cdot}$). Anal. calc. for $C_{33}H_{32}N_4O_3S$ (564.71): C 70.19, H 5.71, N 9.92, S 5.68; found: C 69.86, H 5.86, N 9.80, S 5.58.

1-[Bis(4-fluorophenyl)methyl]-4-[2,3-dihydro-2-(2-nitrophenyl)-4,5-diphenylisothiazol-3-yl]piperazine (**13a**). Yield: 96%. Orange crystals. M.p. 60–63°. IR: 1153 (C−F), 1346 (NO_2), 1527 (NO_2), 2816 (CH_2), 2955 (CH_2). ^{1}H-NMR: 2.75–2.78 (*m*, CH_2); 3.07–3.12 (*m*, CH_2); 4.25–4.27 (*m*, CH); 5.55 (*s*, H−C(3)); 6.95–7.00 (*m*, arom. H); 7.14–7.18 (*m*, arom. H); 7.22–7.32 (*m*, arom. H); 7.37–7.46 (*m*, arom. H); 7.51 (*dd*, ^3J = 8.0, ^4J = 1.4, arom. H); 7.37–7.46 (*m*, arom. H); 7.74 (*dd*, ^3J = 8.2, ^4J = 1.4, arom. H). ^{19}F-NMR: −116.81 (*s*, arom. F). ^{13}C-NMR: 47.2 (CH_2); 53.0 (CH_2); 75.4 (CH); 99.7 (C(3)); 115.6; 115.8; 121.2 (C(5)); 124.5; 126.3; 126.7; 127.7; 128.7; 129.3; 129.6; 129.8; 130.1; 130.2; 132.0; 134.3 (C(4)); 135.6; 139.7; 143.5 ($C(NO_2)$); 148.4 (C_q(N)); 162.6 (J = 243.3, arom. F). EI-MS: 645.3 ([M − H]$^{+\cdot}$). Anal. calc. for $C_{38}H_{32}F_2N_4O_2S$ (646.76): C 70.57, H 4.99, N 8.66, S 4.96; found: C 70.73, H 4.50, N 8.48, S 4.82.

1-[Bis(4-fluorophenyl)methyl]-4-[2-(2-fluorophenyl)-2,3-dihydro-4,5-diphenylisothiazol-3-yl]piperazine (**13e**). Yield: 96%. Yellow oil. IR: 1152 (C−F), 2810 (CH_2), 2955 (CH_2). ^{1}H-NMR: 2.75–2.78 (*m*, CH_2); 2.90–2.93 (*m*, CH_2); 3.24–3.28 (*m*, CH_2); 3.56–3.58 (*m*, CH_2); 4.21–4.23 (*m*, CH); 5.45 (*s*, H−C(3)); 6.94–7.01 (*m*, arom. H); 7.04–7.13 (*m*, arom. H); 7.22–7.25 (*m*, arom. H); 7.36–7.47 (*m*, arom. H). ^{19}F-NMR: −116.88 (*s*, arom. F); −121.39 (*s*, arom. F). ^{13}C-NMR: 47.2 (CH_2); 53.2 (CH_2); 75.4 (CH); 100.4 (C(3)); 115.8; 117.0; 120.8 (C(5)); 125.2; 125.3; 125.8; 126.7; 127.5; 128.6; 129.3; 129.6; 130.1; 130.2; 132.4; 136.0 (C(4)); 139.6; 141.8; 142.9 (C_q(N)); 158.1 (J = 245.5, arom. F); 162.6 (J = 242.0, arom. F). EI-MS: 618.3 ([M − H]$^{+\cdot}$). Anal. calc. for $C_{38}H_{32}F_3N_3S$ (619.76): C 73.65, H 5.21, N 6.78, S 5.17; found: C 73.56, H 5.07, N 6.59, S 5.07.

Acetylcholinesterase Inhibition Assay. Acetylcholinesterase inhibition was assayed spectrophotometrically at 412 nm at 25° [9][10]. Assay buffer was 100 mM sodium phosphate, 100 mM NaCl, pH 7.3. The enzyme stock soln. (*ca.* 100 U/ml) in assay buffer was kept at 0°. Appropriate dilutions were prepared immediately before starting the measurement. ATCh (10 mM) and DTNB (7 mM) were dissolved in assay buffer and kept at 0°. Stock solns. of the inhibitors were prepared in a 1:1 mixture of

MeCN and 0.1M HCl. IC_{50} Values were calculated from the linear steady-state turnover of the substrate using *Eqn. 1*,

$$v = v_0/[1 + ([I]/IC_{50})] \quad (1)$$

where [I] is the inhibitor concentration, and v_0 and v are the rates in the absence and presence of the inhibitor, respectively. Into a cuvette containing 825 µl of assay buffer, 50 µl of the DTNB soln., 55 µl of MeCN, 10 µl of an inhibitor soln., and 10 µl of an enzyme soln. (*ca.* 3 U/ml) were added, and the mixture was thoroughly mixed. After incubation for 15 min at 25°, the reaction was initiated by adding 50 µl of the ATCh soln.

X-Ray Crystal-Structure Analysis of **5c**[1]). $C_{21}H_{14}Cl_3NO_4S$, M_r 482.74, $T = 220(2)$ K. Crystal system: monoclinic. Space group: $P2_1/c$, $a = 21.124(6)$ Å, $b = 7.225(4)$ Å, $c = 13.903(4)$ Å, $\beta = 105.140(7)°$, $V = 2048.2(13)$ Å^3, $Z = 4$, $\rho = 1.565$ mg/m^3, Absorption coeff.: 0.579 mm^{-1}. Crystal size: $0.30 \times 0.30 \times 0.08$ mm^3. θ Range for data collection: 2.94–24.08°, index ranges: $-24 \le h \le 24$, $-8 \le k \le 8$, $-15 \le l \le 15$. Reflections collected: 12653, independent reflections: 3218 (R(int) = 0.0424), max./min. transmission: 0.9551/0.8454, data/parameters: 3218/300. Final R indices: [$I > 2\sigma(I)$] $R^1 = 0.0386$, $wR^2 = 0.0728$, R indices (all data): $R^1 = 0.0664$, $wR^2 = 0.0761$, largest diff. peak/hole: 0.424/ – 0.296 e · Å^{-3}

X-Ray Crystal-Structure Analysis of **7b**[1]). $C_{25}H_{22}Cl_2N_2OS$, M_r 469.41, $T = 193(2)$ K. Crystal system: monoclinic. Space group: $P2_1/n$, $a = 11.204(4)$ Å, $b = 10.2838(18)$ Å, $c = 20.010(7)$ Å, $\beta = 100.13(4)°$, $V = 2269.7(11)$ Å^3, $Z = 4$, $\rho = 1.374$ mg/m^3, Absorption coeff.: 0.398 mm^{-1}. Crystal size: $0.20 \times 0.10 \times 0.10$ mm^3. θ Range for data collection: 2.71–28.00°, index ranges: $-14 \le h \le 14$, $-13 \le k \le 13$, $-26 \le l \le 26$. Reflections collected: 21456, independent reflections: 5295 (R(int) = 0.0368), max./min. transmission: 0.9612/0.9246, data/parameters: 5295/280. Final R indices: [$I > 2\sigma(I)$] $R^1 = 0.0355$, $wR^2 = 0.0797$, R indices (all data): $R^1 = 0.0575$, $wR^2 = 0.0840$, largest diff. peak/hole: 0.319/ – 0.271 e · Å^{-3}

X-Ray Crystal-Structure Analysis of **10b**[1]). $C_{46}H_{36}N_4S_2Cl_4 \cdot 2\ C_4H_8O$, M_r 994.92, $T = 213(2)$ K. Crystal system: triclinic. Space group: $P\bar{1}$, $a = 7.527(15)$ Å, $b = 11.339(2)$ Å, $c = 15.007(3)$ Å, $\alpha = 101.10(3)°$, $\beta = 94.17(3)°$, $\gamma = 96.13(3)°$, $V = 1244.0(4)$ Å^3, $Z = 1$, $\rho = 1.328$ mg/m^3, Absorption coeff.: 0.368 mm^{-1}. Crystal size: $0.20 \times 0.10 \times 0.10$ mm^3. θ Range for data collection: 2.07–26.01°, index ranges: $-8 \le h \le 8$, $-13 \le k \le 13$, $-18 \le l \le 18$. Reflections collected: 9849, independent reflections: 4517 (R(int) = 0.0266), data/parameters: 4517/298. Final R indices: [$I > 2\sigma(I)$] $R^1 = 0.0447$, $wR^2 = 0.1310$, R indices (all data): $R^1 = 0.0610$, $wR^2 = 0.1408$, largest diff. peak/hole: 0.235/ – 0.306 e · Å^{-3}

REFERENCES

[1] J. Wolf, W. Böhlmann, M. Findeisen, T. Gelbrich, H.-J. Hofmann, B. Schulze, *Angew. Chem.* **2007**, *119*, 3179.
[2] A. DeHope, V. Lavallo, B. Donnadieu, W. W. Schoeller, G. Bertrand, *Angew. Chem.* **2007**, *119*, 7047.
[3] J. Wolf, W. Böhlmann, M. Findeisen, T. Gelbrich, H.-J. Hofmann, B. Schulze, *Angew. Chem.* **2007**, *119*, 7051.
[4] B. Schulze, U. Obst, G. Zahn, B. Friedrich, R. Cimiraglia, H.-J. Hofmann, *J. Prakt. Chem./Chem. Ztg.* **1995**, *337*, 175.
[5] H. M. Greenblatt, H. Dvir, I. Silman, J. L. Sussman, *J. Mol. Neurosci.* **2003**, *20*, 369.
[6] H. Sugimoto, Y. Yamanishi, Y. Iimura, Y. Kawakami, *Curr. Med. Chem.* **2000**, *7*, 303.

---

[1]) Crystals were obtained from THF and hexane. The intensities were measured on a *Siemens SMART CCD* diffractometer. The structures were solved by direct methods with SHELX-97 [11]. The refinement was performed with SHELXL-97 [12]. Crystallographic data for the structural analyses have been deposited with the *Cambridge Crystallographic Data Centre* (*CCDC*), with No. 617857 for **5c**, No. 617858 for **7b**, and No. 635689 for **10b**. Copies of the data can be obtained, free of charge, from CCDC, 12 Union Road, Cambridge, CB2 1EZ. UK (fax: +441233336033; e-mail: deposit@ccdc.cam.ac.uk; internet: http://www.ccdc.cam.ac.uk/data_request/cif).

[7] L. Piazzi, A. Rampa, A. Bisi, S. Gobbi, F. Belluti, A. Cavalli, M. Bartolini, V. Andrisano, P. Valenti, M. Recanatini, *J. Med. Chem.* **2003**, *46*, 2279.
[8] J. Fahrig, T. H. E. Freysoldt, C. Hartung, J. Sieler, B. Schulze, *J. Sulfur Chem.* **2005**, *26*, 211.
[9] M. Pietsch, M. Gütschow, *J. Med. Chem.* **2005**, *48*, 8270.
[10] G. L. Ellman, K. D. Courtney, V. Andres, R. M. Feather-Stone, *Biochem. Pharmacol.* **1961**, *7*, 88.
[11] G. M. Sheldrick, *Acta Crystallogr.* **1990**, *46*, 467.
[12] G. M. Sheldrick, SHELXL-97, Program for the Solution and the Refinement of X-Ray Crystal Structures, University of Göttingen, Göttingen, 1997.

Received October 1, 2007