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SYNTHESIS, STRUCTURE, AND CHOLINERGIC EFFECT OF NOVEL NEUROPROTECTIVE COMPOUNDS BEARING THE TACRINE PHARMACOPHORE†

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Abstract – Novel tacrine congeners with side ligands suitable for optimal interaction with the peripheral and catalytic sites of acetyl- and butyrylcholinesterase (AChE and BuChE) have been synthesized using either 9-isothiocyanato-1,2,3,4-tetrahyhroacridine or 9-chloro-1,2,3,4-tetrahydroacridine which represent convenient synthons by reaction with various substituted amines. The synthesized compounds were tested for their inhibition of AChE and BuChE whereby a morpholine and a furfuryl derivative were found to be the most potent AChE and BuChE inhibitors, respectively, with the latter compound comparable in activity to tacrine.

INTRODUCTION

For the therapeutic treatment of Alzheimer's disease, two main strategies are currently being pursued. The first strategy tackles the formation of extracellular β-amyloid protein senile plaques and neurofibrillary tangles of hyperphosphorylated τ-protein[1] whilst the second intensively studied clinical

† *Dedicated to Professor Dr. Ryoji Noyori on the occasion of his 70th birthday.*

method focuses on acetylcholinesterase (AChE) inhibition.[2] The first AChE inhibitor drug approved for the treatment of Alzheimer's disease was 9-amino-1,2,3,4-tetrahydroacridine (tacrine, Cognex®), though it exhibits severe side effects, e.g. hepatotoxicity and gastrointestinal antagonism, which represent important disadvantages.[3] Various tacrine derivatives such as hydroxy[4] and halogenated[5] derivatives, bis[6] and hetero/homodimers,[7] and tetracyclic tacrines,[8] amongst others,[9] have been synthesized in an effort to mitigate the side effects concomitant with increasing efficiency. In previous work we reported the synthesis of a novel AChE inhibitor, viz. 2-[(1,2,3,4-tetrahodroacridin-9-yl)imino]-3-substituted 1,3-thiazolidin-4-ones, using 9-isothiocyanato-1,2,3,4,-tetrahydroacridine (**1**) as synthon.[10] Some of those synthesized compounds, e.g. the 3-cyclohexyl derivative, exhibited 100-fold higher affinity towards AChE in comparison to butyrylcholinesterase (BuChE).[11] Since BuChE too inactivates the neurotransmitter acetylcholine, this enzyme is also a suitable therapeutic target for Alzheimer's disease, which is characterized by a cholinergic deficit. Selective and reversible inhibition of brain BuChE may thus represent a treatment by improving cognition and modulating neuropathological markers.[12,13]

In this work, we focus on constructing novel tacrine derivatives possessing structural requirements for optimal binding to the AChE peripheral site wherein the aim was to synthesize new multipotent compounds that can exhibit greater pharmacological properties leading to a synergic and effective treatment of Alzheimer's disease. For this purpose, we derivatized the pharmacophore tacrine structure with suitable substituents which are able interact with the target sites of AChE, such as the anionic, esterific, and hydrophobic centers. For the synthesis, two synthons, 9-isothiocyanato-1,2,3,4-tetrahydroacridine (**1**) and 9-chloro-1,2,3,4-tetrahydroacridine (**2**) were utilized, with various substituted amines as the side ligands {1-(2-aminoethyl)piperazine (**3a**), 4-(2-aminoethyl)morpholine, 4-methoxyaniline, *N*,*N*-dimethylethylamine, and furfurylamine}.

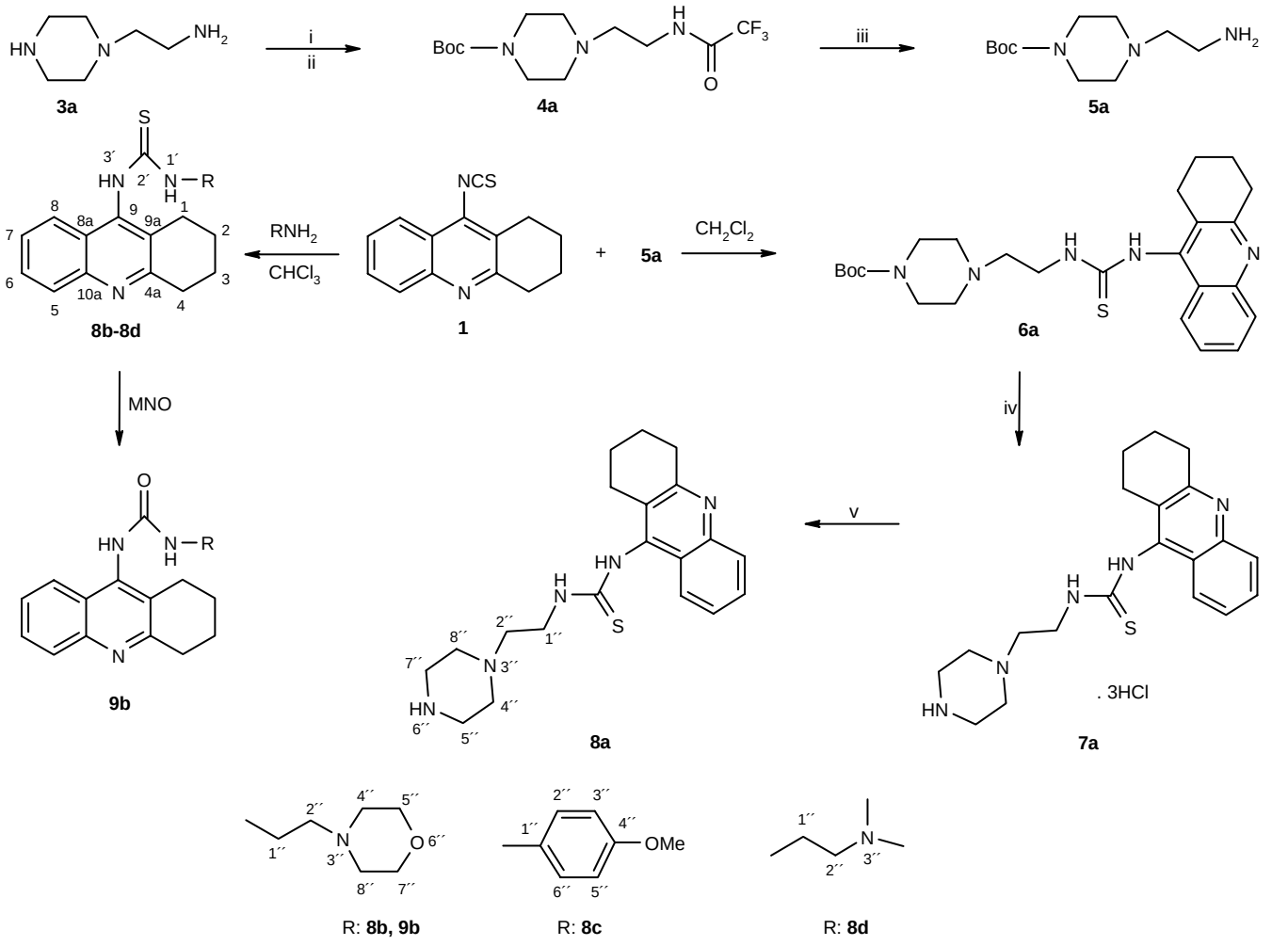
RESULTS AND DISCUSSION

Synthesis

Synthon 9-isothiocyanato-1,2,3,4-tetrahydroacridine (**1**, Scheme 1) was used to prepare four derivatives, **8a**–**d**, using the amines 1-(2-aminoethyl)piperazine (for **8a**), 4-(2-aminoethyl)morpholine (for **8b**), 4-methoxyaniline (for **8c**), and *N*,*N*-dimethylethylamine (for **8d**). Because of the presence of both primary and secondary amino groups in 1-(2-aminoethyl)piperazine (**3a**), the preparation of derivative **8a** required selective protection of the primary and secondary amino groups in 1-(2-aminoethyl)piperazine (**3a**) according to literature methods[14,15] prior to reaction of the amine with **1**. Thus, **3a** was first treated with ethyl trifluoroacetate to selectively protect the NH_2 group followed by treatment with di-*tert*-butyl dicarbonate [$(Boc)_2O$] to protect the NH group to yield piperazine **4a**. Removal of the trifluoroacetyl grouping under basic conditions then yielded **5a** which was subsequently reacted with synthon **1** to yield

thiourea **6a**. Removal of the Boc group under acidic conditions yielded the hydrochloride **7a**. To alleviate the inherent lipophilicity and improve the aqueous solubility of the synthesized derivatives, as an example, the hydrochloride **7a** was also examined after isolation from the reaction mixture. Treatment of **7a** with NH_4OH then yielded the desired product **8a**.

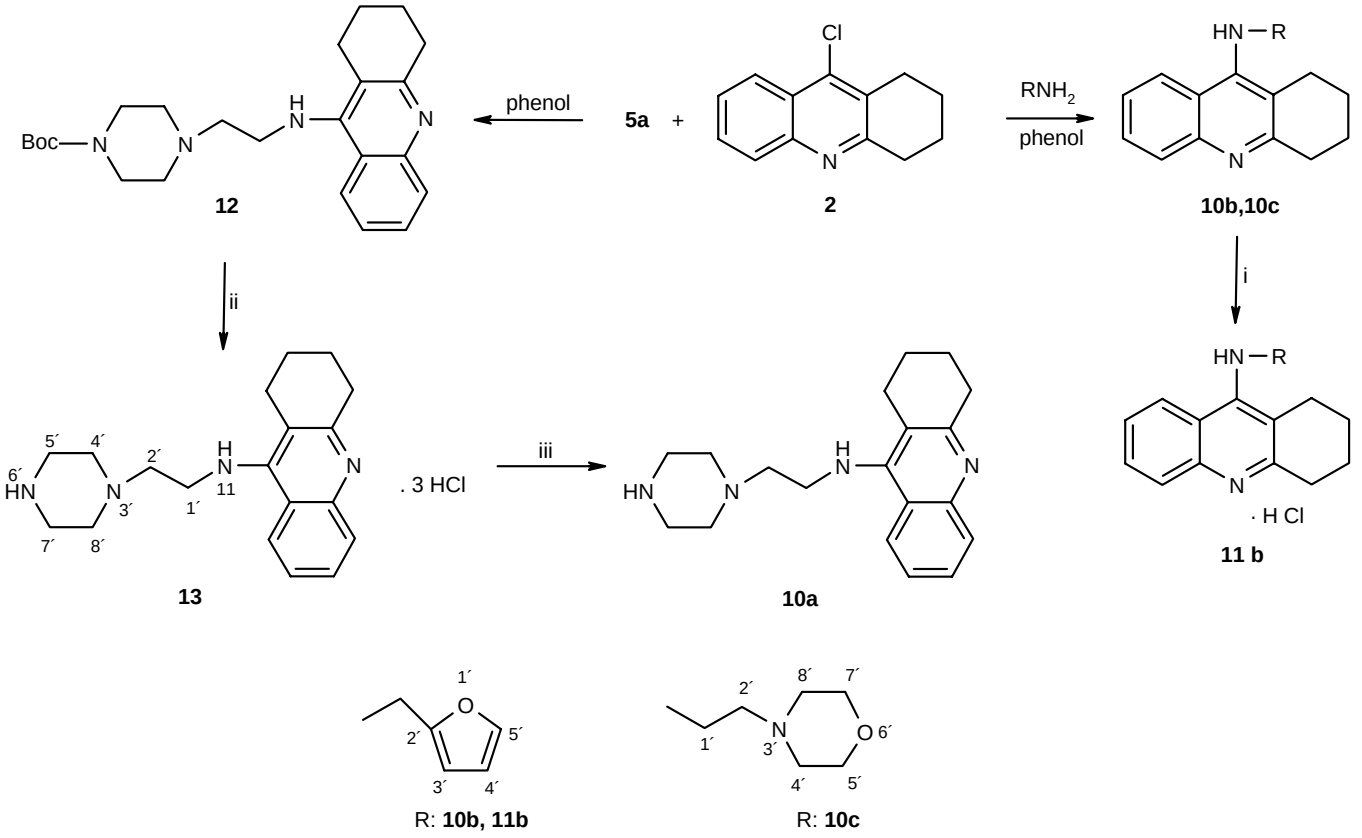
By contrast, derivatives **8b**–**d** were formed directly from the reaction of **1** with the appropriate amines. The oxo analog of **8b** was also afforded by the exchange reaction with mesitylnitrile oxide (MNO) to yield **9b**. Characterization of all of the synthesized compounds was afforded by ^1H and ^{13}C NMR spectroscopy (vide infra) and elemental analysis for which acceptable results were obtained for all compounds.



Reagents/conditions: (i) CF_3CO_2Et, THF < 10 °C; (ii) $(Boc)_2O$, THF < 10 °C; (iii) NaOH, 36 h, 35 °C; (iv) AcOH/HCl; (v) NH_4OH.

Scheme 1

Synthon 9-chloro-1,2,3,4-tetrahydroacridine (**2**, Scheme 2) was used to prepare three derivatives, **10a**–**c**, using the amines, 1-(2-aminoethyl)piperazine (for **10a**), 4-furfurylamine (for **10b**), and 4-(2-aminoethyl)morpholine (for **10c**). The same protective strategy was also required for **3a** prior to its reaction with **2**. Thus, **5a** was also used for the reaction with **2** to yield **12**, which after removal of the Boc group under acidic conditions yielded the hydrochloride **13**, which was also isolated for comparative reasons relating to solubility limitations. Treatment of **13** with NH_4OH then yielded the desired product **10a**. Derivatives **10b**,**c** were formed directly from the reaction of **2** with 4-furfurylamine and 4-(2-aminoethyl)morpholine, respectively, in phenol.[16] For the same considerations regarding solubility restrictions, the hydrochloride of **10b** was deliberately prepared by treatment of **10b** with HCl in methanol to yield **11b**.



Reagents/conditions: (i) MeOH/HCl; (ii) AcOH/HCl; (iii) NH_4OH.

Scheme 2

Biological activity

The synthesized compounds **7a**, **8a**–**d**, **9b**, **10b**,**c**, and **11b** were all tested for their ability to inhibit the activity of both AChE and BuChE and the results are presented in Table 1. For comparison, tacrine and

9-amino-7-methoxy-1,2,3,4-tetrahydroacridine hydrochloride (7-MEOTA) were used as standards. The most promising inhibition, i.e. the lowest IC_{50} value, of AChE by the compounds synthesized in this work was exhibited by **10c**, closely followed by **10b** and **11b**. Though compounds **10b**,**c**, and **11b** failed to match the inhibition displayed by tacrine, which was ca. an order of magnitude more effective, they were, in fact, more effective in inhibiting AChE than 7-MEOTA. For the inhibition of BuChE, several compounds were comparable to tacrine, viz. **8c**, **9b**, **10b**,**c**, and **11b**. Though none exceeded the capability of tacrine, the IC_{50} value for **10b** was only ca. 50% larger than tacrine. Notably, all of the tested compounds exhibited much higher inhibitory activity towards BuChE, the only exception was 7-MEOTA. Indeed, all compounds were more effective inhibitors of BuChE in comparison to 7-MEOTA.

Table 1. Cholinesterase-inhibitory activities[a] of **7a**, **8a**–**d**, **9b**, **10b**,**c**, and **11b**

Compound	AChE		BuChE	
	IC_{50} [μM]	95% CI [μM]	IC_{50} [μM]	95% CI [μM]
7a	106	69.4–162	17.4	8.74–34.6
8a	11.8	8.96–15.4	1.82	1.25–2.66
8b	398	252–628	66.7	17.9–241
8c	> 5, limited by solubility		0.503	0.457–0.553
8d	> 50, limited by solubility		4.62	1.06–20.1
9b	133	118–149	0.812	0.421–1.57
10b	1.21	1.02–1.43	0.0204	0.0149–0.0279
10c	0.972	0.860–1.01	0.164	0.0799–0.335
11b	1.26	0.951–1.66	0.0823	0.0758–0.0894
tacrine	0.143	0.131–0.156	0.0139	0.00434–0.0448
7-MEOTA	3.68	3.08–4.40	131	55.4–311

[a] IC_{50}: concentration required to elicit a 50% inhibition of the enzyme; 95% CI: the 95% confidence interval range.

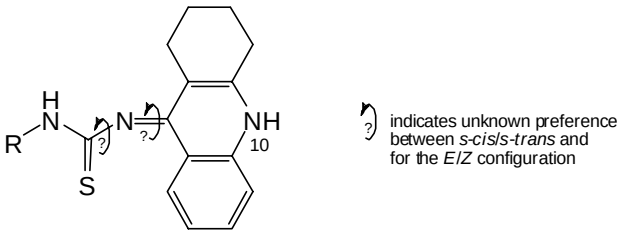
As mentioned above, the most potent inhibitor of AChE was the morpholine aminoderivative **10c**, but contrastingly, the morpholine thiourea **8b** was the least active inhibitor. Moreover, the thiourea derivatives studied here were significantly less active. For example, the replacement of sulfur by oxygen (**8b** → **9b**) produced a compound with a three-fold increase in AChE activity and a nearly two orders of magnitude increase in BuChE activity. Similar increases in activity have been observed for benzisoxazole derivatives in comparison to analogous benzthiazoles.[17] The limited activity of thioureas derivatives **7a** and **8a**–**d** may be explained by two factors: the length of the bridge between the tetrahydroacridine and the azasubstituents, which seems to be optimal for **10c**, and the charge distribution within the molecule

whereby significant negative charge is present on the thiourea sulfur (vide infra).

Structure

To prove the structure of the compounds and facilitate signal assignments, sets of 1D proton and carbon spectra and 2D correlation spectra (H,H-COSY, NOESY, gH,C-HSQC, and gH,C-HMBC) were acquired wherein the tetrahydroacridine skeleton of the compounds was observed to exhibit typical aliphatic and aromatic spin systems. The structure of the quinoline moiety in the tetrahydroacridine skeleton, in particular, as well as the NMR chemical shifts is in agreement with the NMR and X-ray findings for 4-aminosubstituted quinolines.[18] Of note, the three-bond H,C correlations allowed assignment of the quaternary acridine carbons C-4a, 8a, 9, 9a, and 10a signals which are very sensitive to structural changes or to hydrochlorination. Thioureas **6a** and **8a**–**d** displayed a typical C=S carbon resonance at 180 ppm which was significantly shielded upon transformation to urea **9b** (160 ppm) due to the S → O transformation. A smaller electron-withdrawing effect by the NHCONH group (**9b**) in comparison to NHCSNH (**8b**) reduces the proton and carbon chemical shifts of the C-1" segment by 0.28 and 4.6 ppm, respectively. The chemical shift of the acridine C-9 carbon of the thioureas lies in the range 137–141 ppm, but this is deshielded to 149–151 ppm when an amino substituent (**10a**–**c**) is introduced.

The N-10 atom of the acridinyl moiety is well known[19] for a propensity to retain a proton, i.e. the HN-10 tautomer dominates (see Figure) and the presence of this tautomer is evident by the diagnostically shielded[19a,b] C-5 signal in this instance (typically ca. 119 ppm). Of the compounds examined here, only **6a** and the three hydrochlorides **7a**, **11b**, and **13** displayed shielded signals for C-5 (within the range 119–122 ppm), for the rest C-5 resonated within the range 128–129 ppm. Clearly for the protonated



compounds, N-10 is one of the sites of protonation. For the enigmatic **6a**, the stereochemistry regarding *E*/*Z* configuration about the C_9=N double bond and with respect to *s-cis*/*s-trans* conformation could not be ascertained. Thus, with the exception of **6a**, 1,2,3,4-tetrahodroacridinyl systems do not possess the same propensity to retain a proton under neutral conditions but do so upon protonation. To elucidate structure–reactivity relationships with regard to AChE or BuChE inhibition, modeling studies of selected compounds were conducted using DFT at the B3LYP/6-311+G(d,p) level of theory. In particular, the

length of the connecting bridge, the introduction of additional heteroatoms, and electronic effects (e.g. partial atomic charges) all play a significant role in altering cholinesterase inhibition, e.g. for benzisoxazole derivatives.[17] Figure 1 depicts example structures.

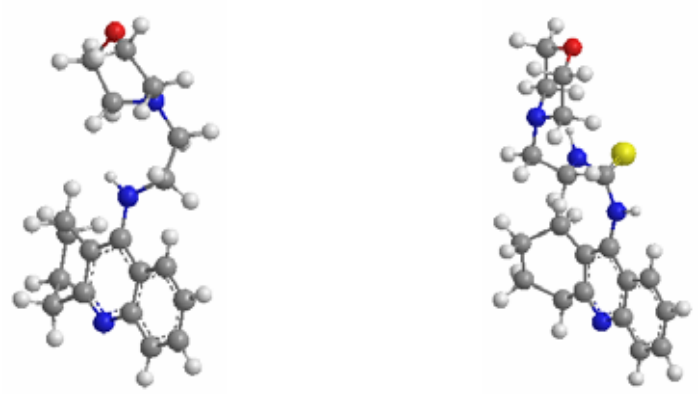


Figure 1. Optimized structures for the most and least active morpholine derivatives **10c** and **8b**, respectively, using DFT at the B3LYP/6-311+G(d,p) level of theory.

From the structures of the compounds it follows that there are the potential for 3 (**10a**–**c**) to 6 (**8a**–**d**, **9b**) hydrogen bonds to form utilizing the tacrine moiety nitrogens, the oxygens of furfuryl or morpholine, the nitrogen of piperazine, and the sulfur (oxygen) and nitrogens of the thiourea (urea) segments. However, the charges on the nitrogen atoms of the tacrine moiety as determined by the quantum chemical calculations do not correlate with the observed inhibitions. For example, in comparison to tacrine, the most active (**10c**) and the least active (**8b**) derivatives provide the order tacrine > **10c** > **8b**, but the charges on N-10 yield the order tacrine > **10c** = **8b** whilst for N-3', the order is tacrine > **8b** > **10c** (see Tables 1–3). Similarly, the charges on the R substituent cannot be related to the AChE/BuChE inhibitory activity. An interesting result is that a large decrease in the inhibition of the enzymes occurs if thiourea/urea is present in the molecule, e.g. derivative **10c** is about 400× more active than **8b** which sports a thiourea group. As the charge differences on the aforesaid atoms (Tables 2 and 3) are not significant, we can suppose that the differences in inhibition are not dependent on electron distribution, but rather on structural factors. One such factor could be prolongation of the side chain (*r* values are presented in Tables 2 and 3) with another the possible conjugation of the nitrogen and sulfur/oxygen atoms of the thiourea/urea mioety. A consequence of this could be the finding from the quantum chemical optimizations of **8a**–**d** and **9b** that the C9-N3′-C2′-S atoms lie practically in the same plane. Beside this in the same plane lie also the C9-N3′-C2′-N1′-C atoms (Table 2).Results for the structural parameter values in relation to partial atomic charges, internuclei distances and dihedral angles are collected in

Tables 2 and 3.

Table 2. Parameter values derived from DFT calculations of compounds **8a**–**d**, **9b** and tacrine

	Atomic charge, *Q*										Dihedral angles [o]	
Cmpd	N-10	N-1'	C-2'	N-3'	N-3''	N-6''	S	O	O(=C)	*r*[Å][a]	C9N3'C2'S	N3'C2N1'C1''
8a	–0.29	-0.09	+0.59	-0.21	+0.03	-0.18	-0.83	-	-	5.89	6.9	179.0
8b	–0.29	-0.08	+0.53	-0.17	-0.09	-	-0.85	-0.37	-	5.88	0.7	178.9
8c	–0.31	-0.01	+0.60	-0.20	-	-	-0.80	-0.44	-	3.69[b]	2.9	179.3
8d	–0.28	-0.09	+0.56	-0.26	-0.05	-	-0.83	-	-	5.89	11.1	177.8
9b	–0.29	-0.16	+0.41	-	-0.09	-	-	-	-0.38	5.85	5.3[c]	177.1
t[d]	–0.40	-	-	-0.39	-	-	-	-	-	-	-	

[a] Internuclei distances are between the thiourea nitrogens N-3' and X where X represents the N, O, or C atoms of the R group. [b] Value is for the *ipso* carbon C-1''. [c] Dihedral angle C9N3'C2'O. [d] t = tacrine.

Table 3. Parameter values derived from DFT calculations of compounds **10a**–**c**

Cmpd	Atomic charge, *Q*						
	N-10	N-11	N-3'	N-6'	O-1'	O-6'	*r* [Å]
10a	–0.30	–0.24	–0.15	–0.22	–	–	2.908
10b	–0.29	–0.20	–	–	–0.32	–0.10	2.51[a]
10c	–0.29	–0.23	–0.09	–	–0.37	–0.27	2.886

[a] Internuclei distance for the N-11 furfuryl C-2'.

EXPERIMENTAL

Melting points were determined on a Boetius hot-stage apparatus and are uncorrected. NMR spectra were recorded on a Varian Mercury Plus spectrometer. ^{1}H and ^{13}C NMR spectra were acquired at 400 and 100 MHz, respectively, in deuteriochloroform ($CDCl_3$) or hexadeuteriodimethylsulfoxide (DMSO-d_6). Chemical shifts are given in δ relative to tetramethylsilane (= 0 ppm for both nuclei). The biological evaluation of products was conducted in the Department of Toxicology, Hradec Králové. DFT quantum-chemical calculation were performed using the *Gaussian 03* program at the B3LYP/6-311+G(d,p) level of theory.[20,21] Atomic charges were calculated using the 6-311++G(2d,2p) basis set.

Chemicals

Chemicals used for synthetic work were of laboratory purity grade. Human recombinant acetylcholin esterase (AChE; EC 3.1.1.7), human plasma butyrylcholinesterase (BuChE; EC 3.1.1.8), bovine serum

albumin (BSA), 9-amino-1,2,3,4-tetrahydroacridine hydrochloride hydrate (tacrine), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), acetylthiocholine iodide, and butyrylthiocholine iodide were bought from Sigma-Aldrich (Prague, Czech Republic). 9-Amino-7-methoxy-1,2,3,4-tetrahydroacridine hydrochloride (7-MEOTA) had been synthesized previously at the Department of Toxicology, Hradec Králové. Other chemicals were purchased from Lach-Ner (Neratovice, Czech Republic).

Cholinesterase-inhibitory activities

Cholinesterase-inhibitory activities were evaluated spectrophotometrically according to the method of Ellman.[22] Enzymes were dissolved in phosphate buffer (PB; 0.1 M, pH 7.4) containing BSA (1 g/L) and their activity before inhibition was set to 1000 ± 50 U/L. Tested compounds were dissolved in PB or *iso*-propanol with the final concentration in the mixture not exceeding 5%. The inhibition of the cholinesterases was started in 1.5 mL plastic cuvettes by the addition of the compound solution to the mixture of PB, DTNB, and cholinesterase. An incubation time of 5 min was followed by the enzymatic reaction upon addition of AChE substrate acetylthiocholine or BuChE substrate butyrylthiocholine. The final concentration of the compounds lay in the range 10^{-3}–10^{-10} M and at least 12 concentrations were employed, the final concentration of DTNB and acetyl- or butyrylthiocholine was 1 mM. Control samples (100% enzyme activity) contained only PB or *iso*-propanol. The absorbance of the sample in the cuvette was measured in triplicate at 412 nm for 1 min at 25 °C using a spectrophotometer (Helios Alfa, Thermo Fisher Scientific, Inc., USA). IC_{50} values (the concentration producing 50% enzyme-activity inhibition) were calculated by nonlinear regression analysis using *GraphPad Prism* software.[23]

4-[2-(2,2-Trifluoroacetylamino)ethyl]piperazino-1-carboxylic acid tert-butyl ester (4a). To 1-(2-aminoethyl)piperazine (1 g, 7.7 mmol) in dry THF (1 mL), ethyl trifluoroacetate (1.09 g, 7.7 mmol) was added dropwise at 5 °C. The solution was stirred for 30 min followed by the addition of di-*tert*-butyl dicarbonate [$(Boc)_2O$] (1.68 g, 7.7 mmol) in dry THF (2 mL). Stirring was continued at rt for 24 h after which the solvent was removed in vacuo and the residue was redissolved in EtOAc. The organic layer was washed with saturated aqueous NaCl soln., then dried by solid Na_2SO_4 and evaporated to dryness in vacuo. Yield 90%; mp 85–89 °C; ^{1}H NMR ($CDCl_3$) δ: 1.47 (s, 9H, 3 × CH_3), 2.46 (m, 4H, CH_2NCH_2), 2.59 (m, 2H, CH_2N), 3.41–3.47 (m, 6H, $CH_2N(CO_2)CH_2$ + CH_2NHCO), 7.15 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 28.3 $(CH_3)_3$, 36.0 (CH_2NH), 43.1 ($\underline{C}H_2N(CO_2)\underline{C}H_2$), 52.5 ($CH_2NCH_2$), 55.5 ($CH_2N$), 79.9 ($C_{qt}$), 115.8 (q, CF_3, $^1J_{CF}$ = 285.8 Hz, 154.6 (CO_2), 157.1 ($\underline{C}OCF_3$, $^2J_{CF}$ = 36.6 Hz). Anal. Calcd for $C_{13}H_{22}N_3O_3F_3$ (325.32): C, 48.00; H, 6.82; N, 12.92. Found: C, 48.11; H, 6.71; N, 13.03%.

4-(2-Aminoethyl)piperazine-1-carboxylic acid tert-butyl ester (5a). To the protected amine **4a** (2.9 g, 8.9

mmol) in MeOH (40 mL), 0.22 M aqueous NaOH (45 mL) was added dropwise over the course of 1 h whilst ensuring the temperature of the reaction mixture did not exceed 15 °C. After raising the temperature of the solution to rt, the stirring was continued for 36 h at 35 °C. The MeOH was then removed in vacuo and the water–oil mixture obtained was extracted with $CHCl_3$ (2 × 10 mL). The $CHCl_3$ phases were combined, dried over Na_2SO_4 to which activated carbon had been added, and the mixture stirred for 30 min. After the solvent was removed under reduced pressure, the product **5a** was obtained as a yellow oil. Yield 85%; ^{1}H NMR ($CDCl_3$) δ: 1.46 (s, 9H, 3 × CH_3), 2.39 (t, 4H, CH_2NCH_2, J = 4.8 Hz), 2.43 (t, 2H, CH_2N, J = 6.4 Hz), 2.80 (t, 2H, $C\underline{H}_2NH_2$, J = 6.4 Hz), 3.43 (t, 4H, $CH_2N(CO_2)CH_2$, J = 4.8 Hz); ^{13}C NMR ($CDCl_3$) δ: 28.4 $(CH_3)_3$, 38.6 (CH_2NH_2), 43.1 ($\underline{C}H_2N(CO_2)\underline{C}H_2$), 53.0 ($CH_2NCH_2$), 61.1 ($CH_2N$), 79.5 ($C_{quart}$), 154.7 ($CO_2$).

4-{2-[3-(1,2,3,4-Tetrahydroacridin-9-yl)thioureido]ethyl}-piperazine-1-carboxylic acid tert-butyl ester (6a). To a solution of 9-isothiocyanato-1,2,3,4-tetrahydroacridine **1** (0.41 mmol) in dry CH_2Cl_2 (10 mL), protected amine **5a** (0.41 mmol) was added and the reaction mixture stirred at rt for 20 h. The precipitate that formed was collected by filtration, washed with Et_2O and dried to provide a yellow solid. Yield 85%; mp 178–180 °C; ^{1}H NMR ($CDCl_3$) δ: 1.44 (s, 9H, 3 × CH_3), 1.82-1.97 (m, 4H, 2 × CH_2, H-2, H-3), 2.43 (m, 4H, 2 × CH_2, H-4", H-8"), 2.56–2.68 (m, 4H, 2 × CH_2, H-1, H-2"), 3.11 (t, 2H, CH_2, H-4, J = 6.0 Hz), 3.22–3.41 (m, 6H, 3 × CH_2, H-5", H-7", H-1"), 7.44 (m, 1H, CH, H-7), 7.63 (ddd, 1H, CH, H-6, J = 8.4, 6.8, 1.2 Hz), 7.93 (dd, 1H, CH, H-8, J = 8.4, 1.2 Hz), 7.99 (d, 1H, CH, H-5, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ: 21.7, 22.2 (C-2, C-3), 24.5 (C-1), 28.4 (CH_3), 29.7 (C-4), 39.2 (C-1"), 43.6 (C-5", C-7"), 52.7 (C-4", C-8"), 57.5 (C-2"), 80.0 (C), 120.3 (C-8a), 122.0 (C-5), 124.2 (C-8), 125.8 (C-7), 131.4 (C-6), 132.9 (C-9a), 140.5 (C-9), 154.5, 154.5, 155.6 (C-4a, C-10a, CO_2), 175.7 (C=S). Anal. Calcd for $C_{25}H_{35}N_5O_2S$ (469.65): C, 63.94; H, 7.51; N, 14.91. Found: C, 64.02; H, 7.62; N, 15.03%.

3'-(1,2,3,4-Tetrahydroacridin-9-yl)-1'-piperazinoethylthiourea trihydrochloride (7a). The Boc protecting group of **6a** (0.28 g, 0.25 mmol) was removed by stirring the material in acetic acid (5 mL) and conc. HCl (0.5 mL) at rt for 1.5 h. After removing the acid in vacuo, the resulting oil was treated with a 1:1 mixture of EtOH and Et_2O (1 mL) and the corresponding trihydrochloride was obtained as yellow-brown powder. Yield 90%; mp 171–174 °C; ^{1}H NMR (DMSO-d_6) δ: 1.86, 1.92 (m, 4H, 2 × CH_2, H-2, H-3), 2.92 (m, 2H, CH_2, H-1), 3.34 (m, 2 × 2H, CH_2, H-2", H-4), 3.43 (m, 4H, 2 × CH_2, H-5", H-7"), 3.50 (m, 4H, 2 × CH_2, H-4", H-8"), 3.96 (m, 2H, CH_2, H-1"), 7.84 (dd, 1H, CH, H-7, J = 8.2, 7.4 Hz), 8.03 (dd, 1H, CH, H-6, J = 8.2, 7.4 Hz), 8.17 (d, 1H, CH, H-8, J = 8.2 Hz), 8.36 (d, 1H, CH, H-5, J = 8.2 Hz), 8.85 (t, 1H, NH-1", J = 5.4 Hz), 9.93 (s, 2H, 2 × NH), 10.96 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ:

20.2 (C-3), 20.6 (C-2), 24.4 (C-1), 28.6 (C-4), 38.6 (C-1"), 39.7 (C-5", C-7"), 48.0 (C-4", C-8"), 54.1 (C-2"), 120.0 (C-5), 124.6 (C-8a), 124.9 (C-8), 128.3 (C-7), 129.7 (C-9a), 133.1 (C-6), 137.3 (C-10a), 149.1 (C-9), 157.8 (C-4a), 181.6 (C=S). Anal. Calcd for $C_{20}H_{30}N_5SCl_3$ (478.92): C, 50.16; H, 6.31; N, 14.62. Found: C, 50.45; H, 6.30; N, 14.59%.

3'-(1,2,3,4-Tetrahydroacridin-9-yl)-1'-piperazinoethyl thiourea (8a). Compound **7a** (0.2 g, 0.4 mmol) was treated with 2 M NH_4OH solution (13 mL) with stirring for 15 min and then extracted with $CHCl_3$ (8 mL). The organic layer was washed with water (3 mL) and then dried over Na_2SO_4. Removal of the solvent under reduced pressure afforded a yellow powder. Yield 95%; mp 163–166 °C; ^{1}H NMR (DMSO-d_6) δ: 1.80 (m, 2H, CH_2, H-2) 1.88 (m, 2H, CH_2, H-3), 2.25 (m, 4H, 2 × CH_2, H-4", H-8"), 2.37 (m, 2H, CH_2, H-2"), 2.58 (m, 4H, 2 × CH_2, H-5", H-7"), 2.82 (m, 2H, CH_2, H-1), 3.04 (t, 2H, CH_2, H-4, J = 6.4 Hz), 3.51 (m, 2H, CH_2, H-1"), 7.40 (s, 1H, NH), 7.50 (dd, 1H, CH, H-7, J = 8.0, 7.3 Hz), 7.65 (dd, 1H, CH, H-6, J = 8.0, 7.3 Hz), 7.76 (d, 1H, CH, H-8, J = 8.0 Hz), 7.89 (d, 1H, CH, H-5, J = 8.0 Hz); ^{13}C NMR (DMSO-d_6) δ: 21.9 (C-2), 22.3 (C-3), 24.5 (C-1), 33.3 (C-4), 41.1 (C-1"), 45.3 (C-5", C-7"), 53.7 (C-4", C-8"), 56.7 (C-2"), 123.0 (C-8), 124.8 (C-8a), 125.6 (C-7), 128.0 (C-5), 128.5 (C-6), 128.8 (C-9a), 139.8 (C-9), 146.6 (C-10a), 159.6 (C-4a), 180.6 (C=S). Anal. Calcd for $C_{20}H_{27}N_5S$ (369.53): C, 65.01; H, 7.36; N, 18.95. Found: C, 65.11; H, 7.39; N, 18.85%.

General procedure for the synthesis of 3'-(1,2,3,4-tetrahydroacridin-9-yl)-1'-substituted thioureas ***8b–d***

To a solution of 9-isothiocyanato-1,2,3,4-tetrahydroacridine **1** (1 mmol) in $CHCl_3$ (20 mL), the corresponding amine (1 mmol) was added dropwise with stirring. Stirring was continued at rt until a precipitate had been deposited. This was collected by filtration, washed with Et_2O, and then dried.

3'-(1,2,3,4-Tetrahydroacridin-9-yl)-1'-morpholinoethylthiourea (8b). White powder; yield 90%; mp 167–171 °C; ^{1}H NMR ($CDCl_3$) δ: 1.91 (m, 2H, CH_2, H-2), 2.00 (m, 2H, CH_2, H-3), 2.08 (m, 4H, 2 × CH_2, H-4", H-8"), 2.33 (m, 2H, CH_2, H-2"), 2.95 (m, 2H, CH_2, H-1), 3.10 (m, 4H, 2 × CH_2, H-5", H-7"), 3.17 (t, 2H, CH_2, H-4, J = 6.4 Hz), 3.58 (dt, 2H, CH_2, H-1", J = 5.4, 5.2 Hz), 6.30 (s, 1H, NH), 7.50 (dd, 1H, CH, H-7, J = 8.4, 7.2 Hz), 7.68 (dd, 1H, CH, H-6, J = 8.8, 7.2 Hz), 7.88 (d, 1H, CH, H-8, J = 8.4 Hz), 8.04 (d, 1H, CH, H-5, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$) δ: 22.4 (C-2), 22.7 (C-3), 25.3 (C-1), 33.9 (C-4), 41.2 (C-1"), 52.6 (C-4", C-8"), 55.7 (C-2"), 66.5 (C-5", C-7"), 122.5 (C-8), 124.0 (C-8a), 126.8 (C-7), 128.9 (C-9a), 129.1 (C-5), 129.5 (C-6), 137.6 (C-9), 147.5 (C-10a), 160.6 (C-4a), 180.2 (C=S). Anal. Calcd for $C_{20}H_{26}ON_4S$ (370.51): C, 64.83; H, 7.07; N, 15.12. Found: C, 64.91; H, 7.14; N, 15.02%.

3'-(1,2,3,4-Tetrahydroacridin-9-yl)-1'-(4-methoxyphenyl)thiourea (8c). White powder; yield 80%; mp

White powder; yield 80%; mp 189–190 °C; ^{1}H NMR (DMSO-d_6) δ: 1.83 (m, 2H, CH_2, H-2), 1.87 (m, 2H, CH_2, H-3), 2.85, 2.91 (2 × m, 2H, CH_2, H-1), 3.03 (m, 2H, CH_2, H-4), 3.74 (s, 3H, OCH_3), 6.91 (m, 2H, 2 × CH, H-3", H-5"), 7.31 (m, 2H, 2 × CH, H-2", H-6"), 7.52 (dd, 1H, CH, H-7, J = 8.4, 6.0 Hz), 7.65 (dd, 1H, CH, H-6, J = 8.8, 6.0 Hz), 7.83 (d, 1H, CH, H-8, J = 8.4 Hz), 7.89 (d, 1H, CH, H-5, J = 8.8 Hz), 9.50 (bs, 1H, NH) 9.63 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ: 21.8 (C-2), 22.3 (C-3), 24.6 (C-1), 33.3 (C-4), 55.1 ($O\underline{C}H_3$), 113.7 (C-3", C-5"), 123.2 (C-8), 124.9 (C-8a), 125.5 (C-7), 126.6 (C-2", C-6"), 128.0 (C-5), 128.4 (C-6), 128.7 (C-9a), 131.8 (C-1"), 140.8 (C-9), 146.5 (C-10a), 156.8 (C-4"), 159.4 (C-4a), 180.6 (C=S). Anal. Calcd for $C_{21}H_{21}ON_3S$ (363.48): C, 69.39; H, 5.82; N, 11.56. Found: C, 69.42; H, 5.90; N, 11.47%.

3'-(1,2,3,4-Tetrahydroacridin-9-yl)-1'-[2-(dimethylamino)ethyl]thiourea (8d). Yellow powder; yield 80%; mp 178–180 °C; ^{1}H NMR ($CDCl_3$) δ: 1.80–2.04 (m, 10H, 2 × NCH_3, 2 × CH_2, H-2, H-3), 2.29 (m, 2H, CH_2, H-2"), 2.93 (m, 2H, CH_2, H-1), 3.15 (m, 2H, CH_2, H-4), 3.59 (m, 2H, CH_2, H-1"), 6.46 (bs, 1H, NH), 7.50 (m, 2H, CH_2, H-7), 7.66 (m, CH_2, H-6), 7.89 (d, 1H, CH, H-8, J = 7.2 Hz), 8.01 (d, 1H, CH, H-5, J = 8.0 Hz), 8.20 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 22.4 (C-2), 22.7 (C-3), 25.4 (C-1), 34.0 (C-4), 42.7 (C-1"), 44.7 (2 × $N\underline{C}H_3$), 56.9 (C-2"), 122.5 (C-8), 124.0 (C-8a), 126.6 (C-7), 128.7 (C-9a), 128.9 (C-5), 129.4 (C-6), 137.8 (C-9), 147.4 (C-10a), 160.5 (C-4a), 180.2 (C=S). Anal. Calcd for $C_{18}H_{24}N_4S$ (328.48): C, 65.82; H, 7.36; N, 17.06. Found: C, 65.93; H, 7.36; N, 17.13%.

3'-(1,2,3,4-Tetrahydroacridin-9-yl)-1'-morpholinoethylurea (9b). To a solution of 3'-(1,2,3,4-tetrahydroacridin-9-yl)-1'-morpholinoethylthiourea (**8b**) (0.45 mmol) in dry MeCN (5 mL), mesitylnitrile oxide (0.45 mmol) was added. The mixture was allowed to stir for 6 h at rt following which the solvent was evaporated under reduced pressure and the solid collected by filtration and dried. Yield 75%; mp 203–205 °C; ^{1}H NMR ($CDCl_3$) δ: 1.87 (m, 2H, CH_2, H-2), 1.96 (m, 2H, CH_2, H-3), 2.28 (m, 4H, 2 × CH_2, H-4", H-8"), 2.37 (m, 2H, CH_2, H-2"), 2.91 (m, 2H, CH_2, H-1), 3.13 (m, 2H, CH_2, H-4), 3.30 (m, 2H, CH_2, H-1"), 3.38 (m, 4H, 2 × CH_2, H-5", H-7"), 5.25 (bs, 1H, NH), 7.44 (dd, 2H, CH_2, H-7, J = 8.4, 6.8 Hz), 7.63 (dd, 2H, CH_2, H-6, J = 8.0, 6.8 Hz), 7.94 (d, 1H, CH, H-8, J = 8.4 Hz), 7.99 (d, 1H, CH, H-5, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ: 22.5 (C-2), 22.7 (C-3), 25.3 (C-1), 33.9 (C-4), 36.6 (C-1"), 53.1 (C-4", C-8"), 57.6 (C-2"), 66.6 (C-5", C-7"), 122.5 (C-8), 124.5 (C-8a), 126.0 (C-7), 127.8 (C-9a), 128.8 (C-5), 129.0 (C-6), 138.9 (C-9), 147.3 (C-10a), 156.3 (C-4a), 160.2 (C=O). Anal. Calcd for $C_{20}H_{26}O_2N_4$ (354.45): C, 67.77; H, 7.39; N, 15.81. Found: C, 67.96; H, 7.49; N, 15.85%.

N-(Piperazinoethyl)-N-(1,2,3,4-tetrahydroacridin-9-yl)amine (10a). Compound **13** (0.2 g, 0.48 mmol) was treated with 2 M NH_4OH solution (13 mL). The mixture was stirred for 15 min and then extracted

with $CHCl_3$ (8 mL). The organic layer was washed with water (3 mL), dried over Na_2SO_4, and the solvent removed under reduced pressure. Yield 80%; oil; ^{1}H NMR ($CDCl_3$) δ: 1.86-1.96 (m, 4H, 2 × CH_2, H-2, H-3), 2.46 (m, 4H, 2 × CH_2, H-4', H-8'), 2.57 (t, 2H, CH_2, H-2', J = 5.2 Hz), 2.77 (t, 2H, CH_2, H-1, J = 5.8 Hz), 2.93 (t, 4H, 2 × CH_2, H-5', H-7', J = 4.6 Hz), 3.06 (t, 2H, CH_2, H-4, J = 5.8 Hz), 3.55 (t, 2H, CH_2, H-1', J = 5.6 Hz), 5.21 (bs, 1H, NH), 7.33 (dd, 1H, CH, H-7, J = 8.4, 7.3 Hz), 7.54 (dd, 1H, CH, H-6, J = 8.0, 7.3 Hz), 7.90 (d, 1H, CH, H-5, J = 8.0 Hz), 8.02 (d, 1H, CH, H-8, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ: 22.9 (C-3), 23.2 (C-2), 25.0 (C-1), 34.0 (C-4), 45.0 (C-1'), 46.3 (C-5', C-7'), 54.0 (C-4', C-8'), 58.2 (C-2'), 116.0 (C-9a), 120.3 (C-8a), 122.8 (C-8), 123.5 (C-7), 128.2 (C-6), 128.7 (C-5), 147.4 (C-10a), 151.1 (C-9), 158.5 (C-4a). Anal. Calcd for $C_{19}H_{26}N_4$ (310.44): C, 73.51; H, 8.44; N, 18.05. Found: C, 73.68; H, 8.53; N, 18.14%.

General procedure for the synthesis of N-substituted-N-(1,2,3,4-tetrahydroacridin-9-yl)amine ***10b,c***

To a homogeneous solution obtained from a mixture of 9-chloro-1,2,3,4-tetrahydroacridine **2** (100 mg, 0.46 mmol) in phenol (405 mg, 4.3 mmol) at 85–90 °C, the corresponding amine **3b** or **c** (1 mmol) was added and heating continued for 4 h at 125–130 °C. After cooling, $CHCl_3$ (5 mL) was added and the solution then treated with 10% aq. NaOH solution (8 mL) and water (8 mL). The organic layer was separated and dried with $CaCl_2$. The solvent was removed under reduced pressure and the residue purified by flash chromatography over silica gel.

N-(Furfuryl)-N-(1,2,3,4-tetrahydroacridin-9-yl)amine (10b). Yellow crystals (cyclohexane–acetone, 1:1); yield 95%; mp 92–94 °C; ^{1}H NMR ($CDCl_3$) δ: 1.82-1.94 (m, 4H, 2 × CH_2, H-2, H-3), 2.65 (t, 2H, CH_2, H-1, J = 6.2 Hz), 3.06 (t, 2H, CH_2, H-4, J = 6.4 Hz), 4.20 (bs, 1H, NH), 4.54 (s, 2H, CH_2), 6.11 (d, 1H, CH, H-3', J = 3.6), 6.29 (dd, 1H, CH, H-4', J = 3.6, 2.0 Hz), 7.37 (d, 1H, CH, H-5', J = 2.0 Hz), 7.39 (m, 1H, H-7), 7.57 (ddd, 1H, CH, H-6, J = 8.4, 6.8, 1.2 Hz), 7.93 (d, 1H, CH, H-5, J = 8.4 Hz), 7.98 (dd, 1H, CH, H-8, J = 8.4, 1.2 Hz); ^{13}C NMR ($CDCl_3$) δ: 22.8 (C-3), 22.9 (C-2), 24.5 (C-1), 34.1 (C-4), 46.1 (CH_2), 107.3 (C-3'), 110.5 (C-4'), 118.4 (C-9a), 120.9 (C-8a), 122.6 (C-8), 124.3 (C-7), 128.4 (C-6), 128.9 (C-5), 142.3 (C-5'), 147.4 (C-10a), 149.8 (C-9), 152.8 (C-2'), 158.8 (C-4a). Anal. Calcd for $C_{18}H_{18}N_2O$ (278.35): C, 77.67; H, 6.52; N, 10.06. Found: C, 77.58; H, 6.67; N, 10.19%.

N-(Morpholinoethyl)-N-(1,2,3,4-tetrahydroacridin-9-yl)amine (10c). Yellow powder ($CHCl_3$–MeOH, 9:1); yield 90%; mp 103–106 °C; ^{1}H NMR ($CDCl_3$) δ: 1.85–1.98 (m, 4H, 2 × CH_2, H-2, H-3), 2.51 (m, 4H, 2 × CH_2, H-4', H-8'), 2.61 (t, 2H, CH_2, H-2', J = 5.8 Hz), 2.78 (m, 2H, CH_2, H-1), 3.07 (m, 2H, CH_2, H-4), 3.55 (m, 2H, CH_2, H-1'), 3.77 (m, 4H, 2 × CH_2, H-5', H-7'), 5.12 (s, 1H, NH), 7.35 (dd, 1H, CH,

H-7, *J* = 8.4, 6.0 Hz), 7.55 (dd, 1H, CH, H-6, *J* = 8.4. 6.0 Hz), 7.89 (d, 1H, CH, H-5, *J* = 8.4 Hz), 8.01 (d, 1H, CH, H-8, *J* = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ: 22.9 (C-3), 23.2 (C-2), 25.0 (C-1), 34.1 (C-4), 44.9 (C-1'), 53.1 (C-4', C-8'), 58.1 (C-2'), 67.1 (C-5', C-7'), 116.2 (C-9a), 120.4 (C-8a), 122.7 (C-8), 123.5 (C-7), 128.2 (C-6), 128.9 (C-5), 147.6 (C-10a), 150.9 (C-9), 158.6 (C-4a). Anal. Calcd for $C_{19}H_{25}ON_3$ (311.43): C, 73.28; H, 8.09; N, 13.49. Found: C, 73.35; H, 8.02; N, 13.65%.

N(-Furfuryl)-N-(1,2,3,4-tetrahydroacridin-9-yl)amine hydrochloride (11b). To a saturated solution of *N*-furfuryl-*N*-(1,2,3,4-tetrahydroacridin-9-yl)amine **9b** (50 mg, 0.14 mmol) in acetone, a methanolic HCl solution (250 μL) prepared from MeOH (0.9 mL) and concentrated HCl (0.1 mL) was added and stirred at rt 1 h. The residue was treated with Et_2O and the product collected by filtration as a yellow powder. Yield 90%; mp 180–184 °C; ^{1}H NMR (DMSO-d_6) δ: 1.83 (m, 4H, 2 × CH_2, H-2, H-3), 2.75 (m, 2H, CH_2, H-1), 3.06 (m, 2H, CH_2, H-4), 5.08 (d, 2H, CH_2, *J* = 6.4 Hz), 6.41 (m, 1H, CH, H-3'), 6.43 (m, 1H, CH, H-4'), 7.56 (dd, 1H, CH, H-7, *J* = 8.8, 6.8 Hz), 7.64 (m, 1H, CH, H-5'), 7.86 (dd, 1H, CH, H-6, *J* = 8.8, 6.8 Hz), 8.02 (d, 1H, CH, H-5, *J* = 8.8 Hz), 8.35 (t, 1H, NH, *J* = 6.4 Hz), 8.45 (d, 1H, CH, H-8, *J* = 8.8 Hz); ^{13}C NMR (DMSO-d_6) δ: 20.1, 21.3 (C-2, C-3), 24.0 (C-1), 27.9 (C-4), 43.6 (CH_2), 107.9 (C-3'), 110.6 (C-4'), 111.8 (C-9a), 115.7 (C-8a), 119.1 (C-5), 124.7 (C-8), 125.1 (C-7), 132.6 (C-6), 137.5 (C-10a), 142.8 (C-5'), 151.0 (C-2'), 151.3 (C-4a), 155.6 (C-9). Anal. Calcd for $C_{18}H_{19}ON_2Cl$ (314.81): C, 68.67; H, 6.08; N, 8.90. Found: C, 68.80; H, 5.94; N, 8.79%.

4-[2-(1,2,3,4-Tetrahydroacridin-9-ylamino)ethyl]piperazine-1-carboxylic acid tert-butyl ester (12). To a homogeneous solution obtained from a mixture of 9-chloro-1,2,3,4-tetrahydroacridine **2** (100 mg, 0.46 mmol) in phenol (405 mg, 4.3 mmol) at 85–90 °C, the protected amine **5a** (94 mg, 0.41 mmol) was added and heating continued for 4 h at 125–130 °C. After cooling, $CHCl_3$ (5 mL) was added and the solution obtained treated with 10% aq. NaOH solution (8 mL) and water (8 mL). The organic layer was separated and dried over $CaCl_2$. The solvent was removed under reduced pressure and the residue obtained used for the next reaction. Yield 90%; oil; ^{1}H NMR ($CDCl_3$) δ: 1.47 (s, 9H, 3 × CH_3), 1.92-1.98 (m, 4H, 2 × CH_2, H-2, H-3), 2.37-2.46 (m, 6H, 3 × CH_2, H-2', H-4', H-8'), 2.80 (t, 2H, CH_2, H-1', *J* = 6.0 Hz), 3.02 (t, 2H, CH_2, H-1, *J* = 6.2 Hz), 3.13 (t, 2H, CH_2, H-4, *J* = 6.2 Hz), 3.43 (m, 4H, 2 × CH_2, H-5", H-7"), 5.08 (s, 1H, NH), 7.54 (ddd, 1H, CH, H-7, *J* = 8.6, 6.8, 1.4 Hz), 7.66 (ddd, 1H, CH, H-6, *J* = 8.2, 6.8, 1.4 Hz), 7.97 (dd, 1H, CH, H-5, *J* = 8.2, 1.4 Hz), 8.16 (dd, 1H, CH, H-8, *J* = 8.6, 1.4 Hz); ^{13}C NMR ($CDCl_3$) δ: 22.6, 22.7 (C-2, C-3), 27.5 (C-1), 28.4 (3 × $\underline{C}H_3$), 34.2 (C-4), 38.7 (C-1'), 43.7 (C-5', C-7'), 53.1 (C-4', C-8'), 61.2 (C-2'), 79.6 (C), 115.1 (C-9a), 123.7 (C-8), 125.4 (C-8a), 126.5 (C-7), 128.7 (C-5), 129.3 (C-6), 141.4 (C-9), 146.7 (C-10a), 154.8 (C=O), 159.5 (C-4a).

N-(Piperazinoethyl)-N-(1,2,3,4-tetrahydroacridin-9-yl)amine trihydrochloride (13). The same procedure used for the preparation of **7a** was used for **13**. Light brown powder; yeld 90%; mp 187–190 °C; ^{1}H NMR (DMSO-d_6) δ: 1.84 (m, 4H, 2 × CH_2, H-2, H-3), 2.80 (m, 2H, CH_2, H-1), 3.07 (m, 2H, CH_2, H-4), 3.17–3.63 (m, 10H, 5 × CH_2, H-4', H-8', H-5', H-7', H-2'), 4.32 (m, 2H, CH_2, H-1'), 7.60 (dd, 1H, CH, H-7, J = 8.4, 6.4 Hz), 7.89 (dd, 1H, CH, H-6, J = 8.4, 6.4 Hz), 7.94 (bs, 1H, NH), 8.05 (d, 1H, CH, H-5, J = 8.4 Hz), 8.49 (d, 1H, CH, H-8, J = 8.4 Hz), 9.97 (bs, 2H, NH); ^{13}C NMR (DMSO-d_6) δ: 20.2, 21.5 (C-2, C-3), 24.4 (C-1), 28.0 (C-4), 39.1, 48.2 (C-4', C-8', C-5', C-7'), 41,8 (C-1'), 55.2 (C-2'), 112.1 (C-9a), 115.9 (C-8a), 119.2 (C-5), 124.9 (C-8), 125.4 (C-7), 132.7 (C-6), 137.6 (C-9), 151.4 (C-4a), 155.7 (C-10a). Anal. Calcd for $C_{19}H_{29}N_4Cl_3$ (419.83): C, 54.36; H, 6.96; N, 13.35. Found: C, 54.18; H, 6.78; N, 13.40%.

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