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## Original article

# Synthesis of tacrine-lophine hybrids *via* one-pot four component reaction and biological evaluation as acetyl- and butyrylcholinesterase inhibitors



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## ABSTRACT

A novel series of tacrine-lophine hybrids was synthesized and tested for their ability to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) with IC<sub>50</sub> in the nanomolar concentration scale. The key step is the one-pot four component condensation reaction of 9-aminoalkylamino-1,2,3,4-tetrahydroacridines, benzil, different substituted aromatic aldehydes and NH<sub>4</sub>OAc, using InCl<sub>3</sub> as the best catalyst. Tacrine-lophine hybrids were found to be potent and selective inhibitors of cholinesterases. As an extension of the four component approach to tetrasubstituted imidazoles, a new series of bis-(2,4,5-triphenyl-1H-imidazoles) or bis(n)-lophines was tested against AChE and BuChE.

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## 1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder of the central nervous system (CNS), characterized by deposits of aberrant proteins namely  $\beta$ -amyloid (A $\beta$ ) and  $\tau$ -protein, oxidative stress, loss of synapses and death of cholinergic neurons [1]. The etiopathogenesis of AD still remains unknown although, in the last decades, several involved factors have been identified and found consistent with its onset. Among them is the impairment of the cholinergic system, as indicated by the presence of altered cholinergic markers in AD patients, resulting in a pronounced acetylcholine (ACh) deficiency which translates into a generalized withdrawal of cholinergic tone [2–4]. Nonetheless, interest in developing improved ChE inhibitors for treatment of AD has increased recently, likely due to the body of empirical evidence showing the benefits of ChE inhibitors in AD patients as well as the lack of successful alternative approaches [5–7].

Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are enzymes which constitute the group of the cholinesterases. Acetylcholinesterase hydrolyses acetylcholine and is mainly associated with nerves and muscles, being typically found on the synapses, while butyrylcholinesterase hydrolyses butyrylcholine and is synthesized by the liver, being found in large concentration in serum [8].

The current therapeutic options for the treatment of AD are limited to four AChE inhibitors [9,10], namely donepezil, rivastigmine, galantamine and tacrine, and one N-methyl-D-aspartate receptor antagonist, memantine [11]. These compounds have been approved by the U.S. Food and Drug Administration for the treatment of AD.

In healthy brains, AChE hydrolyzes the majority of acetylcholine while BuChE plays a secondary role. However, as AD progresses, the activity of AChE decreases, while that of BuChE significantly increases in the hippocampus and temporal cortex [12].

All current kinetic models for AChE propose the existence of at least two substrate-binding sites; the active site and the peripheral anionic site (PAS). Inhibitors directed to the active site prevent the binding of the acetylcholine, or its hydrolysis, by occupying the site with a high affinity molecule, such as tacrine. The active site is composed of two subsites. In the catalytic anionic subsite (CAS), it

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has been proposed that the choline moiety in AChE is stabilized principally *via* a cation- $\pi$  interaction with Trp84, and also interacts with Glu199 and Phe330 [5,13]. A similar cation- $\pi$  interaction occurs in human BuChE, where Trp82 interacts with the product choline and the substrate butyrylthiocholine [14].

Tacrine, the first approved drug for AD, is a potent nonselective inhibitor of both AChE and BuChE. Although this lack of selectivity, and hepatotoxicity, has reduced its therapeutic use, it has been the most studied compound and remains a reference structure in the development of new ChEs inhibitors as potential drugs for AD.

About fifteen years ago, bis(*n*)-tacrine analogues linked by an alkylene chain were prepared, and it was proved that these dimeric molecules of tacrine offered a much stronger potency and selectivity toward AChE [15]. Bis(7)-tacrine simultaneously binds at both the CAS and the PAS sites and provides a higher selectivity towards AChE over BuChE. Since then, most of the dual binding site AChE inhibitors developed so far contain at least one unit related to tacrine, probably because of its ease of synthesis and its affinity for both the CAS and PAS sites of AChE, and an enlarged biological profile [16].

Among achievements in synthesis and pharmacomodulation, various tacrine-based heterodimers have been designed and studied. These tacrine heterodimers are usually obtained by connecting natural or synthetic compounds by a linker of suitable length. Examples of tacrine-based heterodimer families include the combination of tacrine with structural units such as tacrine-huperzine A [17–19], tacrine-donepezil [16,20,21], tacrine-indole [22], tacrine–4-Oxo-4*H*-chromene [10] and tacrine-8-hydroxyquinoline [23].

In continuation with our search for new ChE inhibitors based on tacrine units [24,25] as well as the development of new synthetic methods using Lewis acids as catalysts [25–27], we describe in the present study, the synthesis of a novel class of hybrids tacrine-2,4,5-triphenyl-1*H*-imidazole (or tacrine-lophine hybrids) as inhibitors of AChE and BuChE (Scheme 1). Compounds containing imidazole moiety have many pharmacological properties and play important roles in biochemical processes [28,29]. As an extension of the four component approach to tetrasubstituted imidazoles, we also synthesized a new series of bis-(2,4,5-triphenyl-1*H*-imidazoles) or bis(*n*)-lophines (Scheme 2). These non-based tacrine dimers were also subjected to inhibitory essays to AChE and BuChE.

The key step is a one-pot four component synthesis of 1,2,4,5-tetrasubstituted imidazoles catalyzed by Lewis acid.

## 2. Results and discussion

### 2.1. Chemistry

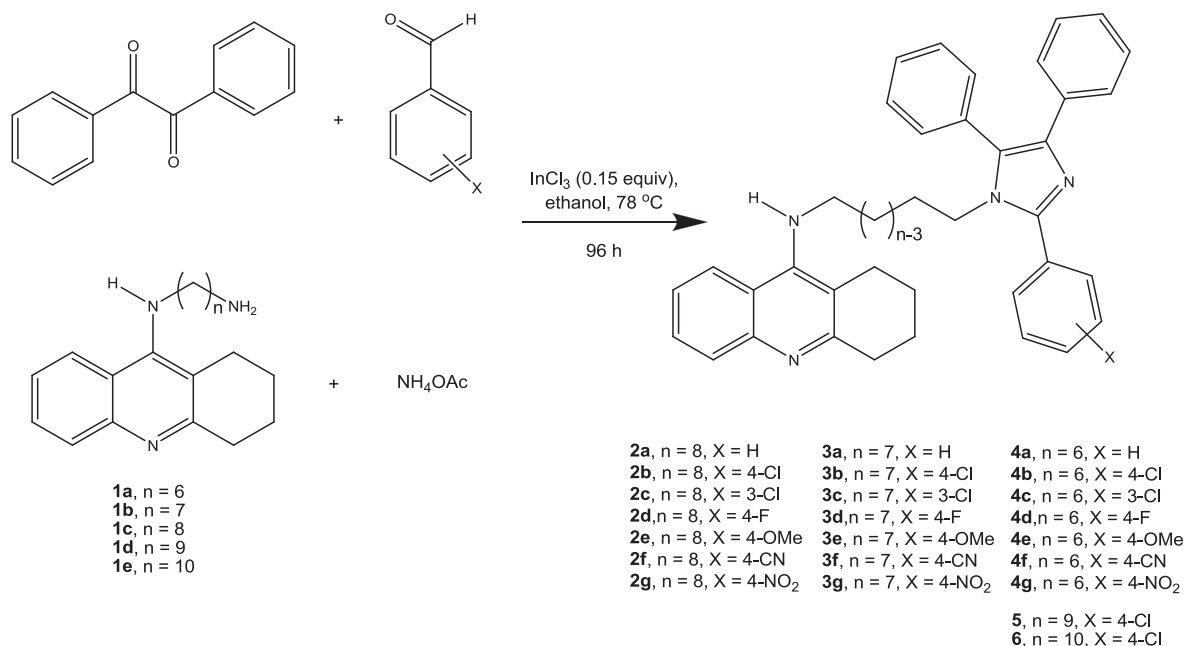
The general synthesis for the novel hybrids tacrine-2,4,5-triphenylimidazole **2a–g**, **3a–g**, **4a–g**, **5** and **6** is depicted in Scheme 1. The key step is the one-pot four component condensation reaction of 9-aminoalkylamino-1,2,3,4-tetrahydroacridines, benzil, different substituted aromatic aldehydes and NH<sub>4</sub>OAc using Lewis acid as catalyst. According to a previously reported protocol, 9-chloro-1,2,3,4-tetrahydroacridine was first prepared by the cyclization of anthranilic acid with cyclohexanone [30,31]. In order to introduce the side chain, different alkylenediamines reacted with 9-chloro-1,2,3,4-tetrahydroacridine to generate the 9-aminoalkylamino-1,2,3,4-tetrahydroacridines in good yields (87–95%) [32].

Despite the use of Lewis acid catalyst on the multicomponent synthesis of 1,2,4,5-tetrasubstituted imidazoles, Sharma and co-workers found InCl<sub>3</sub>·3H<sub>2</sub>O as a mild and effective catalyst [29]. Thus, in order to evaluate the scope of the multicomponent synthesis of hybrids tacrine-2,4,5-triphenyl-1*H*-imidazole, we chose compound **1c** as the substrate, the aim being to examine some of the general features of these reactions, such as Lewis acid catalysts, stoichiometry, solvent, temperature and product conversion.

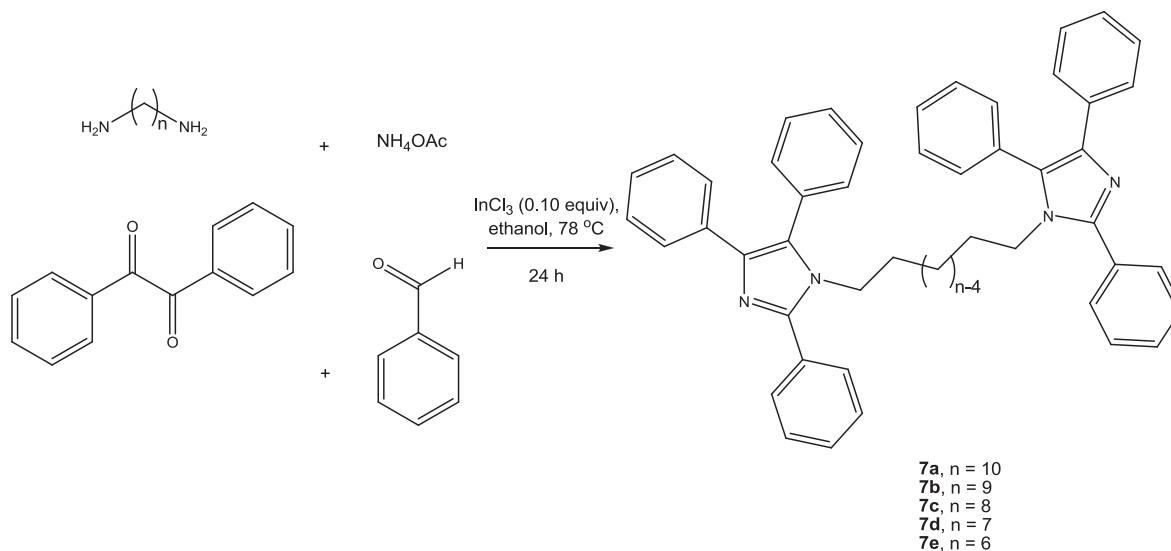
As shown in Table 1 (entry 1), we found that the use of InCl<sub>3</sub> (15 mol%) under ethanol reflux for 96 h, afforded **2a** in quite good yield (55%). This developed protocol was applied to the synthesis of tacrine-2,4,5-triphenyl-1*H*-imidazole **2a–g**, **3a–g**, **4a–g**, **5** and **6** which have a lophine framework substituted at the *meta*- or *para*-position of the 2-phenyl imidazole ring, as shown in Table 2. Next, bis(*n*)-lophines **7a–e** were synthesized by using a similar protocol as depicted in Scheme 2.

### 2.2. AChE and BuChE inhibition evaluation

The inhibitory activities against AChE and BuChE of new tacrine-lophine hybrids and bis(*n*)lophines, together with that of bis(7)-



Scheme 1. Synthesis of tacrine-lophine hybrids.



**Scheme 2.** Synthesis of bis(n)-lophines.

tacrine taken as reference, are reported in Table 2, and are expressed as  $IC_{50}$  values. Tacrine-lophine hybrids were found to be potent inhibitors of cholinesterases with  $IC_{50}$  in the nanomolar concentration scale. The most active AChE inhibitor in the assayed series of tacrine-lophines was hybrid **2b** ( $X = 4\text{-Cl}$ ) with its  $IC_{50}$  of 5.87 nM (entry 2), bearing an octane chain between the tacrine and lophine moieties, pointing out that this linker allows optimal interaction between the aromatic fragments of such an inhibitor and the CAS and PAS of AChE. Moreover, compound **2b** showed very high selectivity for AChE over BuChE (18.5-fold), comparing to only 2.2-fold BuChE selectivity seen with bis(7)-tacrine (entry 29). From this dataset with octamethylene linker, compounds **2d** ( $X = 4\text{-F}$ ) and **2e** ( $X = 4\text{-OMe}$ ) were active for both AChE and BuChE, compounds **2f** ( $X = 4\text{-CN}$ ) and **2g** ( $X = 4\text{-NO}_2$ ) were active only for BuChE while **2a** ( $X = \text{H}$ ) and **2c** ( $X = 3\text{-Cl}$ ) were inactive toward both enzymes.

In the dataset of tacrine-lophines with the heptane chain, compound **3f** ( $R = 4\text{-CN}$ ) entry 13, showed the best inhibition for BuChE in the assayed series with  $IC_{50}$  of 7.10 nM and was inactive toward AChE. It is noteworthy that **3f** was an effective inhibitor of BuChE and exhibited more potent inhibition value compared with prototype bis(7)-tacrine. With the same chain length, compounds **3a** ( $R = \text{H}$ ), **3b** ( $R = 4\text{-Cl}$ ), **3d** ( $R = 4\text{-F}$ ), **3e** ( $R = 4\text{-OMe}$ ), **3g** ( $R = 4\text{-NO}_2$ ) were active for both AChE and BuChE, while **3c** ( $X = 3\text{-Cl}$ ) was the only compound inactive for both enzymes in this dataset. In the case of hexane chain, none of the compounds **4a–4g** exhibited AChE activity while compounds **4a–4g** exhibited BuChE inhibitory  $IC_{50}$  values ranging from 12.44 to 58.00 nM. Comparing products possessing the same substituents in the 2-phenyl group fragment, it was possible to detect the effective influence of changes in the substructure of lophine on the inhibition of ChEs. In the case of chloride substituent in the *para*-position of 2-phenyl group (entries 2, 9 and 16) when the length of the linker is made smaller from the optimum octane chain (entry 2), the ability to inhibit AChE progressively diminished. On the other hand, for compounds containing the chloride substituent in the *para*-position (entries 2, 9 and 16), an increased inhibitory activity against BuChE was observed. Also, hybrids with chloride substituent in the *meta*-position (entries 3 and 10) were inactive toward AChE. This result could be explained as a consequence of the fact that all phenyl rings bonded to the imidazole core are not coplanar and the dihedral angles between the phenyl ring planes can be increased with

substituent groups in the *meta*-position [33]. So, it can be inferred that the loss of AChE activities with substituent at phenyl *meta*-substituted hybrids results from a smaller  $\pi$ – $\pi$  interaction of lophine framework with the active sites of the enzyme. On the other hand, this can explain the improved AChE inhibitor **2b** bearing a phenyl *para*-substituted which has smaller dihedral angles on the phenyl ring planes of the lophine framework, favoring binding interactions.

On the bis(n)-lophines dataset, homodimer **7c** (entry 26) proved to be an interesting compound as a non-tacrine dimer, showing selective AChE inhibitory activity with its  $IC_{50}$  of 42.55 nM.

### 3. Conclusion

In summary, we have developed a simple and convenient approach for the synthesis of tacrine-lophine and lophine-lophine dimers via one-pot four component condensation reaction using  $\text{InCl}_3$  as catalyst. Several tacrine-lophine hybrids were found to be potent and selective inhibitors of AChE and BuChE with  $IC_{50}$  in the nanomolar concentration scale. The non-tacrine bis(8)-lophine (**7c**) showed a selective AChE inhibitory activity with its  $IC_{50}$  value of

**Table 1**

One-pot four component condensation reaction for the synthesis of hybrid **2a** under different conditions.

Entry	Catalyst (15 mol%)	Solvent	Temp. (°C)	Reaction time (h)	Yield (%) <sup>a,b</sup>
1	$\text{InCl}_3$	Ethanol	78	96	55 <sup>c</sup>
2	$\text{FeCl}_3$	Ethanol	78	96	45 <sup>c</sup>
3	$\text{SnCl}_4 \cdot 2\text{H}_2\text{O}$	Ethanol	78	96	41 <sup>c</sup>
4	$\text{AlCl}_3$	Ethanol	78	96	44 <sup>c</sup>
5	$\text{BF}_3 \cdot \text{Et}_2\text{O}$	Ethanol	78	96	33 <sup>c</sup>
6	$\text{InCl}_3$	Free solvent	120	19	3
7	$\text{InCl}_3$	<i>n</i> -Pentanol	120	48	11 <sup>d</sup>
8	$\text{InCl}_3$	Ethanol	25	180	12
9	$\text{InCl}_3$	Ethanol	78	48	31 <sup>d</sup>
10	$\text{InCl}_3$	Ethanol	78	96	35

<sup>a</sup> All yields refer to purified product **2a**.

<sup>b</sup> The reactions were performed using 1.0 equivalent of 9-octylamino-1,2,3,4-tetrahydroacridine **1c**, benzaldehyde, benzil and ammonium acetate.

<sup>c</sup> After 48 h, one more equivalent of benzaldehyde, benzil and ammonium acetate was added.

<sup>d</sup> After 24 h, one more equivalent of benzaldehyde, benzil and ammonium acetate was added.

**Table 2**Inhibitory activity on AChE and BuChE, and IC<sub>50</sub> ratio of the studied compounds.

Entry	Compd	n	X	Yield (%) <sup>a</sup>	IC <sub>50</sub> (nM) <sup>b</sup>		IC <sub>50</sub> ratio BuChE/AChE
					AChE	BuChE	
1	<b>2a</b>	8	H	55	n.a.	n.a.	—
2	<b>2b</b>	8	4-Cl	57	5.87 ± 0.30	108.97 ± 6.41	18.5
3	<b>2c</b>	8	3-Cl	37	n.a.	n.a.	—
4	<b>2d</b>	8	4-F	41	25.78 ± 4.66	57.30 ± 4.15	2.2
5	<b>2e</b>	8	4-OMe	74	125.90 ± 15.84	82.34 ± 4.51	0.6
6	<b>2f</b>	8	4-CN	33	n.a.	30.47 ± 3.65	—
7	<b>2g</b>	8	4-NO <sub>2</sub>	39	n.a.	120.13 ± 29.25	—
8	<b>3a</b>	7	H	54	80.65 ± 4.90	92.42 ± 1.95	1.1
9	<b>3b</b>	7	4-Cl	33	59.76 ± 2.09	37.57 ± 6.24	0.6
10	<b>3c</b>	7	3-Cl	42	n.a.	n.a.	—
11	<b>3d</b>	7	4-F	52	38.51 ± 3.63	67.31 ± 4.79	1.7
12	<b>3e</b>	7	4-OMe	44	68.74 ± 7.10	61.69 ± 6.71	0.9
13	<b>3f</b>	7	4-CN	53	n.a.	7.10 ± 2.49	—
14	<b>3g</b>	7	4-NO <sub>2</sub>	44	72.10 ± 2.18	76.89 ± 28.13	1.0
15	<b>4a</b>	6	H	53	n.a.	58.00 ± 5.37	—
16	<b>4b</b>	6	4-Cl	37	n.a.	29.28 ± 2.67	—
17	<b>4c</b>	6	3-Cl	29	n.a.	34.75 ± 2.51	—
18	<b>4d</b>	6	4-F	50	n.a.	55.25 ± 3.66	—
19	<b>4e</b>	6	4-OMe	33	n.a.	35.98 ± 3.30	—
20	<b>4f</b>	6	4-CN	53	n.a.	29.56 ± 4.37	—
21	<b>4g</b>	6	4-NO <sub>2</sub>	55	n.a.	12.44 ± 2.84	—
22	<b>5</b>	9	4-Cl	43	n.a.	n.a.	—
23	<b>6</b>	10	4-Cl	33	n.a.	n.a.	—
24	<b>7a</b>	Bis(10)-lophine		22	n.a.	n.a.	—
25	<b>7b</b>	Bis(9)-lophine		42	n.a.	n.a.	—
26	<b>7c</b>	Bis(8)-lophine		37	42.55 ± 2.54	n.a.	—
27	<b>7d</b>	Bis(7)-lophine		29	n.a.	n.a.	—
28	<b>7e</b>	Bis(6)-lophine		30	n.a.	n.a.	—
29		Bis(7)-tacrine			4.12 ± 0.70	9.03 ± 1.67	2.2

n.a. = not active.

<sup>a</sup> All yields refer to purified product.<sup>b</sup> ±95% Confidence limits.

42.55 nM. We can infer that several new hybrids synthesized have targeted both the catalytic active site and the peripheral anionic site of AChE.

#### 4. Experimental protocols

All melting points were determined in open glass capillaries using a Büchi M-565 apparatus. IR spectra were recorded on a Varian 640-IR spectrometer in KBr disks. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded in CDCl<sub>3</sub> solution on a Varian VNMRs 300 MHz spectrometer. The assignment of chemical shifts is based on standard NMR experiments (<sup>1</sup>H; <sup>13</sup>C-APT; <sup>1</sup>H, <sup>1</sup>H-COSY; <sup>1</sup>H, <sup>13</sup>C-HMQC). Chemical shifts (δ) are given in part per million from the peak of tetramethylsilane (δ = 0.00 ppm) as internal standard in <sup>1</sup>H NMR or from the solvent peak of CDCl<sub>3</sub> (δ = 77.23 ppm) in <sup>13</sup>C NMR; and multiplicities are given as s (singlet), d (doublet), t (triplet), qn (quintet), m (multiplet) or br (broad); coupling constants (*J*) are given in Hz. High Resolution Mass Spectrometry with Elektrospray Ionization (HRMS-ESI) data on the positive mode was collected on a Micromass Q-ToF instrument from Waters (Manchester, UK). Samples were infused from a 100 μL Hamilton syringe at flow rate range from 5 to 10 μL/min, depending on the sample. The instrument settings were the following: capillary voltage 3000 V, cone voltage 33 V, extraction cone voltage 2.5 V, desolvation gas temperature 100 °C. Nitrogen was used as the desolvation gas. Methanol (Tedia HPLC grade) was used as solvent for the analysed samples and filtered prior to injection. Purification by column chromatography was carried out on silica gel 60 (70–230 mesh). Analytical thin layer chromatography (TLC) was conducted on aluminum plates with 0.2 mm of silica gel 60F-254 (Macherey–Nagel).

#### 4.1. Synthesis

##### 4.1.1. General procedure for the preparation of 9-alkylamino-1,2,3,4-tetrahydroacridines

Intermediates **1a–e** were prepared according to the previous literature procedure [32].

##### 4.1.2. General procedure for the preparation of hybrids **2a–g**, **3a–g**, **4a–g**, **5**, **6**

A mixture of 9-alkylamino-1,2,3,4-tetrahydroacridines **1a–e** (0.5 mmol), benzil (0.5 mmol), aldehyde (0.5 mmol), ammonium acetate (0.5 mmol) and InCl<sub>3</sub> (15 mol%) in absolute ethanol (1 mL), placed in a round bottom flask fitted with a condenser and drying tube, was stirred at 78 °C. After 48 h, one more equivalent of benzil, aldehyde and ammonium acetate was added and the mixture was maintained at 78 °C for an additional 48 h. The solvent was removed under reduced pressure, and the crude product purified by column chromatography (eluting with hexane-ethyl acetate-triethylamine, 60:39:1) to give the desired product.

**4.1.2.1. 1,2,3,4-Tetrahydro-N-(8-(2,4,5-triphenyl-1H-imidazol-1-yl)octyl)acridin-9-amine (2a).** Intermediate **1c** was treated with benzil, benzaldehyde and ammonium acetate according to general procedure to give the desired product **2a** as a yellow solid (55% yield): m.p. 71–72 °C; IR (KBr)  $\nu_{\max}$ /cm<sup>-1</sup>: 3346, 3058, 2927, 2854, 1579, 1500, 762, 698; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.97–7.87 (m, 2H), 7.73–7.66 (m, 2H), 7.58–7.28 (m, 12H), 7.24–7.10 (m, 3H), 4.00–3.80 (br, 1H), 3.87 (t, 2H, *J* = 7.5 Hz), 3.40 (t, 2H, *J* = 7.2 Hz), 3.12–3.00 (m, 2H), 2.74–2.64 (m, 2H), 1.98–1.84 (m, 4H), 1.53 (qn, 2H, *J* = 7.2 Hz), 1.41–1.12 (m, 4H), 1.12–0.86 (m, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 158.6, 150.8, 147.8, 147.7, 137.8, 134.7, 131.7, 131.6, 131.1, 129.7, 129.3, 129.1, 129.0, 128.9, 128.7, 128.3, 128.1, 126.9, 126.3, 123.7, 122.9,

120.4, 116.0, 49.6, 44.7, 34.2, 31.8, 30.3, 29.0, 28.6, 26.8, 26.1, 24.9, 23.2, 22.9; HRMS-ESI: calcd for  $[M - H]^+$  605.8463, found 605.8474.

**4.1.2.2. N-(8-(2-(4-Chlorophenyl)-4,5-diphenyl-1H-imidazol-1-yl)octyl)-1,2,3,4-tetrahydroacridin-9-amine (2b).** Intermediate **1c** was treated with benzil, 4-chlorobenzaldehyde and ammonium acetate according to general procedure to give the desired product **2b** as a yellow solid (57% yield): m.p. 62–63 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$ : 3367, 3058, 2927, 2854, 1500, 835, 762, 698;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.97–7.87 (m, 2H), 7.63 (d, 2H,  $J = 8.4$  Hz), 7.58–7.28 (m, 11H), 7.24–7.09 (m, 3H), 4.00–3.75 (br, 1H), 3.85 (t, 2H,  $J = 7.5$  Hz), 3.41 (t, 2H,  $J = 7.3$  Hz), 3.12–2.98 (m, 2H), 2.76–2.60 (m, 2H), 2.00–1.83 (m, 4H), 1.54 (qn, 2H,  $J = 7.3$  Hz), 1.40–1.12 (m, 4H), 1.12–0.86 (m, 6H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  158.6, 150.8, 147.7, 146.6, 138.1, 135.0, 134.5, 131.5, 131.1, 130.6, 130.2, 130.1, 129.2, 129.0, 128.9, 128.8, 128.4, 128.2, 126.9, 126.5, 123.7, 123.0, 120.4, 116.0, 49.6, 44.8, 34.2, 31.8, 30.4, 29.0, 28.6, 26.9, 26.2, 24.9, 23.2, 23.0; HRMS-ESI: calcd for  $[M - H]^+$  640.2910, found 640.2910.

**4.1.2.3. N-(8-(2-(3-Chlorophenyl)-4,5-diphenyl-1H-imidazol-1-yl)octyl)-1,2,3,4-tetrahydroacridin-9-amine (2c).** Intermediate **1c** was treated with benzil, 3-chlorobenzaldehyde and ammonium acetate according to general procedure to give the desired product **2c** as a yellow solid (37% yield): m.p. 43–44 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$ : 3367, 3056, 2927, 2854, 1562, 1498, 767, 696;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.97–7.86 (m, 2H), 7.72 (s, 1H), 7.60–7.27 (m, 12H), 7.23–7.10 (m, 3H), 4.00–3.80 (br, 1H), 3.87 (t, 2H,  $J = 7.5$  Hz), 3.41 (t, 2H,  $J = 7.1$  Hz), 3.12–3.00 (m, 2H), 2.74–2.62 (m, 2H), 2.00–1.83 (m, 4H), 1.54 (qn, 2H,  $J = 7.1$  Hz), 1.40–1.13 (m, 4H), 1.13–0.88 (m, 6H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  158.6, 150.8, 147.7, 146.2, 138.1, 134.7, 134.4, 133.4, 131.4, 131.1, 130.2, 130.0, 129.4, 129.2, 129.0, 128.9, 128.8, 128.4, 128.2, 127.2, 126.9, 126.5, 123.7, 122.9, 120.4, 116.0, 49.6, 44.8, 34.2, 31.8, 30.4, 29.0, 28.6, 26.8, 26.1, 24.9, 23.2, 22.9; HRMS-ESI: calcd for  $[M - H]^+$  640.2910, found 640.2930.

**4.1.2.4. N-(8-(2-(4-Fluorophenyl)-4,5-diphenyl-1H-imidazol-1-yl)octyl)-1,2,3,4-tetrahydroacridin-9-amine (2d).** Intermediate **1c** was treated with benzil, 4-fluorobenzaldehyde and ammonium acetate according to general procedure to give the desired product **2d** as a yellow solid (41% yield): m.p. 57–58 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$ : 3380, 3056, 2927, 1500, 1224, 843, 763, 698;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.97–7.86 (m, 2H), 7.72–7.63 (m, 2H), 7.58–7.28 (m, 9H), 7.23–7.10 (m, 5H), 3.98–3.78 (br, 1H), 3.84 (t, 2H,  $J = 7.6$  Hz), 3.41 (t, 2H,  $J = 7.2$  Hz), 3.12–2.98 (m, 2H), 2.76–2.64 (m, 2H), 2.00–1.84 (m, 4H), 1.54 (qn, 2H,  $J = 7.2$  Hz), 1.40–1.13 (m, 4H), 1.13–0.88 (m, 6H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  163.2 (d,  $J = 247.3$  Hz), 158.6, 150.8, 147.7, 146.8, 137.8, 134.6, 131.5, 131.3, 131.2, 131.1, 129.8, 129.2, 128.9, 128.8, 128.4, 128.2, 127.8 (d,  $J = 3.3$  Hz), 126.9, 126.4, 123.7, 122.9, 120.4, 116.0, 115.8 (d,  $J = 21.4$  Hz), 49.6, 44.7, 34.2, 31.8, 30.3, 29.0, 28.6, 26.8, 26.2, 24.9, 23.2, 22.9; HRMS-ESI: calcd for  $[M - H]^+$  623.8367, found 623.8380.

**4.1.2.5. 1,2,3,4-Tetrahydro-N-(8-(2-(4-methoxyphenyl)-4,5-diphenyl-1H-imidazol-1-yl)octyl)acridin-9-amine (2e).** Intermediate **1c** was treated with benzil, 4-methoxybenzaldehyde and ammonium acetate according to general procedure to give the desired product **2e** as a yellow solid (74% yield): m.p. 56–57 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$ : 3349, 3056, 2929, 1612, 1250, 1027, 837, 700;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.98–7.86 (m, 2H), 7.60 (d, 2H,  $J = 8.7$  Hz), 7.58–7.28 (m, 9H), 7.24–7.08 (m, 3H), 6.99 (d, 2H,  $J = 8.7$  Hz), 3.96–3.78 (m, 3H), 3.84 (s, 3H), 3.41 (t, 2H,  $J = 7.2$  Hz), 3.12–3.00 (m, 2H), 2.74–2.64 (m, 2H), 1.98–1.84 (m, 4H), 1.54 (qn, 2H,  $J = 7.2$  Hz), 1.48–1.13 (m, 4H), 1.13–0.86 (m, 6H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  160.2, 158.7, 150.9, 147.7, 137.6, 134.8, 131.9, 131.2, 130.7, 129.5, 129.2, 129.0, 128.7, 128.4, 128.2, 126.9, 126.3, 124.1, 123.7, 123.0, 120.4, 116.1, 114.2, 55.5, 49.6, 44.8, 34.3, 31.9, 30.4, 29.1, 28.7, 26.9, 26.2, 25.0, 23.2, 23.0; HRMS-ESI: calcd for  $[M - H]^+$  635.8725, found 635.8731.

**4.1.2.6. 4-(1-(8-(1,2,3,4-Tetrahydroacridin-9-ylamino)octyl)-4,5-diphenyl-1H-imidazol-2-yl)benzonitrile (2f).** Intermediate **1c** was treated with benzil, 4-cyanobenzaldehyde and ammonium acetate according to general procedure to give the desired product **2f** as a yellow solid (33% yield): m.p. 93–94 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$ : 3402, 3056, 2927, 2225, 1604, 1579, 771, 698;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.02 (d, 1H,  $J = 8.7$  Hz), 7.96 (d, 1H,  $J = 8.4$  Hz), 7.86 (d, 2H,  $J = 8.5$  Hz), 7.77 (d, 2H,  $J = 8.5$  Hz), 7.62–7.32 (m, 9H), 7.24–7.12 (m, 3H), 3.91 (t, 2H,  $J = 7.6$  Hz), 3.50 (t, 2H,  $J = 7.3$  Hz), 3.20–3.04 (m, 2H), 2.75–2.60 (m, 2H), 2.00–1.83 (m, 4H), 1.56 (qn, 2H,  $J = 7.3$  Hz), 1.44–1.15 (m, 5H), 1.15–0.84 (m, 6H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  157.3, 151.8, 146.0, 145.6, 138.9, 136.1, 134.2, 132.6, 131.1, 131.0, 129.6, 129.4, 129.3, 129.2, 128.4, 126.9, 126.8, 124.1, 123.2, 119.5, 118.8, 115.0, 112.3, 49.4, 45.1, 33.1, 31.8, 30.5, 29.1, 28.7, 26.9, 26.2, 24.7, 23.0, 22.6; HRMS-ESI: calcd for  $[M - H]^+$  630.8561, found 630.8572.

**4.1.2.7. 1,2,3,4-Tetrahydro-N-(8-(2-(4-nitrophenyl)-4,5-diphenyl-1H-imidazol-1-yl)octyl)acridin-9-amine (2g).** Intermediate **1c** was treated with benzil, 4-nitrobenzaldehyde and ammonium acetate according to general procedure to give the desired product **2g** as a brown solid (39% yield): m.p. 90–91 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$ : 3422, 3058, 2929, 2854, 1599, 1519, 1342, 698;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.35 (d, 2H,  $J = 7.8$  Hz), 8.03 (d, 1H,  $J = 8.4$  Hz), 7.99–7.88 (m, 3H), 7.60–7.30 (m, 9H), 7.25–7.12 (m, 3H), 3.95 (t, 2H,  $J = 7.6$  Hz), 3.51 (t, 2H,  $J = 7.2$  Hz), 3.14–3.02 (m, 2H), 2.70–2.58 (m, 2H), 1.98–1.80 (m, 4H), 1.58 (qn, 2H,  $J = 7.2$  Hz), 1.44–1.15 (m, 5H), 1.15–0.80 (m, 6H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  157.1, 151.9, 147.7, 145.8, 145.2, 139.1, 137.9, 134.2, 131.4, 131.1, 131.0, 129.7, 129.4, 129.2, 128.4, 127.0, 126.9, 124.2, 124.1, 123.3, 119.4, 114.9, 49.4, 45.2, 33.0, 31.7, 30.6, 29.1, 28.7, 26.9, 26.2, 24.7, 23.0, 22.5; HRMS-ESI: calcd for  $[M - H]^+$  650.8439, found 650.8471.

**4.1.2.8. 1,2,3,4-Tetrahydro-N-(7-(2,4,5-triphenyl-1H-imidazol-1-yl)heptyl)acridin-9-amine (3a).** Intermediate **1b** was treated with benzil, benzaldehyde and ammonium acetate according to general procedure to give the desired product **3a** as a yellow solid (54% yield): m.p. 61–62 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$ : 3369, 3058, 2929, 2854, 1579, 1498, 762, 698;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.94–7.86 (m, 2H), 7.67 (d, 2H,  $J = 6.9$  Hz), 7.59–7.26 (m, 12H), 7.22–7.09 (m, 3H), 3.86 (t, 2H,  $J = 7.5$  Hz), 3.96–3.76 (br, 1H), 3.42–3.28 (m, 2H), 3.14–3.00 (m, 2H), 2.70–2.56 (m, 2H), 1.98–1.80 (m, 4H), 1.43 (qn, 2H,  $J = 7.3$  Hz), 1.38–1.24 (m, 2H), 1.17–1.02 (m, 2H), 1.01–0.84 (m, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  158.5, 150.6, 147.7, 147.6, 137.7, 134.6, 131.5, 131.0, 129.6, 129.2, 129.1, 128.9, 128.8, 128.6, 128.2, 128.1, 126.8, 126.3, 123.6, 122.8, 120.3, 116.0, 49.4, 44.6, 34.2, 31.5, 30.1, 28.3, 26.5, 26.0, 24.8, 23.1, 22.8; HRMS-ESI: calcd for  $[M - H]^+$  591.8194, found 591.8218.

**4.1.2.9. N-(7-(2-(4-Chlorophenyl)-4,5-diphenyl-1H-imidazol-1-yl)heptyl)-1,2,3,4-tetrahydroacridin-9-amine (3b).** Intermediate **1b** was treated with benzil, 4-chlorobenzaldehyde and ammonium acetate according to general procedure to give the desired product **3b** as a yellow solid (33% yield): m.p. 64–65 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$ : 3367, 3057, 2927, 2854, 1500, 835, 762, 698;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.94–7.87 (m, 2H), 7.63 (d, 2H,  $J = 8.7$  Hz), 7.58–7.28 (m, 11H), 7.23–7.10 (m, 3H), 3.86 (t, 2H,  $J = 7.5$  Hz), 3.34 (t, 2H,  $J = 7.2$  Hz), 3.12–3.00 (m, 2H), 2.73–2.60 (m, 2H), 1.98–1.84 (m, 4H), 1.48 (qn, 2H,  $J = 7.2$  Hz), 1.40–1.24 (m, 3H), 1.20–1.05 (m, 2H), 1.04–0.88 (m, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  158.7, 150.8, 147.7, 146.6, 138.1, 135.0, 134.5, 131.5, 131.1, 130.6, 130.2, 130.1, 129.2, 129.1, 129.0, 128.9, 128.8, 128.4, 128.3, 126.9, 126.5, 123.8, 122.9, 120.4, 116.1, 49.5, 44.8, 34.2, 31.7, 30.4, 28.5, 26.7, 26.2, 25.0, 23.2, 23.0; HRMS-ESI: calcd for  $[M - H]^+$  626.2641, found 626.2669.

**4.1.2.10. N-(7-(2-(3-Chlorophenyl)-4,5-diphenyl-1H-imidazol-1-yl)heptyl)-1,2,3,4-tetrahydroacridin-9-amine (3c).** Intermediate **1b**



was treated with benzil, 3-chlorobenzaldehyde and ammonium acetate according to general procedure to give the desired product **3c** as a yellow solid (42% yield): m.p. 59–60 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3367, 3056, 2927, 2854, 1562, 1498, 767, 696;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.95–7.86 (m, 2H), 7.72 (s, 1H), 7.60–7.27 (m, 12H), 7.23–7.10 (m, 3H), 4.00–3.78 (br, 1H), 3.87 (t, 2H,  $J = 7.5$  Hz), 3.35 (t, 2H,  $J = 7.1$  Hz), 3.14–3.00 (m, 2H), 2.76–2.58 (m, 2H), 2.00–1.82 (m, 4H), 1.47 (qn, 2H,  $J = 7.1$  Hz), 1.39–1.24 (m, 2H), 1.20–1.15 (m, 2H), 1.15–0.88 (m, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  158.6, 150.7, 147.7, 146.2, 138.1, 134.7, 134.4, 133.3, 131.3, 131.1, 130.1, 130.0, 129.4, 129.2, 129.0, 128.9, 128.8, 128.4, 128.2, 127.2, 126.9, 126.5, 123.7, 122.9, 120.4, 116.1, 49.5, 44.7, 34.2, 31.7, 30.3, 28.4, 26.6, 26.1, 24.9, 23.2, 22.9; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  626.2641, found 626.2654.

**4.1.2.11. N-(7-(2-(4-Fluorophenyl)-4,5-diphenyl-1H-imidazol-1-yl)heptyl)-1,2,3,4-tetrahydroacridin-9-amine (3d).** Intermediate **1b** was treated with benzil, 4-fluorobenzaldehyde and ammonium acetate according to general procedure to give the desired product **3d** as a yellow solid (52% yield): m.p. 61–62 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3361, 3056, 2929, 1500, 1224, 843, 761, 698;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.95–7.86 (m, 2H), 7.70–7.62 (m, 2H), 7.59–7.28 (m, 9H), 7.24–7.09 (m, 5H), 3.96–3.76 (br, 1H), 3.84 (t, 2H,  $J = 7.5$  Hz), 3.42–3.28 (m, 2H), 3.14–2.98 (m, 2H), 2.74–2.58 (m, 2H), 2.00–1.80 (m, 4H), 1.47 (qn, 2H,  $J = 7.2$  Hz), 1.38–1.24 (m, 2H), 1.19–1.05 (m, 2H), 1.05–0.84 (m, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  163.1 (d,  $J = 247.2$  Hz), 158.6, 150.7, 147.6, 146.7, 137.8, 134.5, 131.4, 131.2, 131.1, 131.0, 129.7, 129.2, 128.9, 128.8, 128.3, 128.2, 127.8 (d,  $J = 3.3$  Hz), 126.8, 126.4, 123.7, 122.9, 120.3, 116.1, 115.8 (d,  $J = 22.0$  Hz), 49.4, 44.6, 34.2, 31.6, 30.2, 28.4, 26.5, 26.1, 24.9, 23.1, 22.9; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  609.8098, found 609.8119.

**4.1.2.12. 1,2,3,4-Tetrahydro-N-(7-(2-(4-methoxyphenyl)-4,5-diphenyl-1H-imidazol-1-yl)heptyl)acridin-9-amine (3e).** Intermediate **1b** was treated with benzil, 4-methoxybenzaldehyde and ammonium acetate according to general procedure to give the desired product **3e** as a yellow solid (44% yield): m.p. 67–68 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3378, 3056, 2929, 1500, 1249, 1027, 771, 700;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.94–7.86 (m, 2H), 7.60 (d, 2H,  $J = 9.0$  Hz), 7.56–7.28 (m, 9H), 7.22–7.08 (m, 3H), 6.98 (d, 2H,  $J = 9.0$  Hz), 3.90–3.78 (m, 3H), 3.82 (s, 3H), 3.35 (t, 2H,  $J = 7.2$  Hz), 3.10–3.00 (m, 2H), 2.70–2.62 (m, 2H), 1.96–1.84 (m, 4H), 1.47 (qn, 2H,  $J = 7.2$  Hz), 1.31 (qn, 2H,  $J = 7.2$  Hz), 1.17–1.04 (m, 2H), 1.04–0.88 (m, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  160.2, 158.7, 150.8, 147.7, 147.6, 137.7, 134.8, 131.8, 131.2, 130.7, 129.5, 129.1, 129.0, 128.7, 128.4, 128.2, 126.9, 126.3, 124.1, 123.7, 122.9, 120.4, 116.1, 114.2, 55.5, 49.5, 44.7, 34.2, 31.7, 30.3, 28.5, 26.6, 26.2, 25.0, 23.2, 23.0; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  621.8457, found 621.8468.

**4.1.2.13. 4-(1-(7-(1,2,3,4-Tetrahydroacridin-9-ylamino)heptyl)-4,5-diphenyl-1H-imidazol-2-yl)benzonitrile (3f).** Intermediate **1b** was treated with benzil, 4-cyanobenzaldehyde and ammonium acetate according to general procedure to give the desired product **3f** as a yellow solid (53% yield): m.p. 78–79 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3400, 3056, 2929, 2225, 1606, 1500, 771, 700;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.95 (d, 1H,  $J = 8.7$  Hz), 7.91 (d, 1H,  $J = 8.4$  Hz), 7.85 (d, 2H,  $J = 8.5$  Hz), 7.77 (d, 2H,  $J = 8.5$  Hz), 7.59–7.30 (m, 9H), 7.24–7.12 (m, 3H), 4.20–3.80 (br, 1H), 3.91 (t, 2H,  $J = 7.5$  Hz), 3.41 (t, 2H,  $J = 7.2$  Hz), 3.14–3.02 (m, 2H), 2.72–2.60 (m, 2H), 1.98–1.83 (m, 4H), 1.50 (qn, 2H,  $J = 7.2$  Hz), 1.33 (qn, 2H,  $J = 7.5$  Hz), 1.20–1.06 (m, 2H), 1.06–0.88 (m, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  158.1, 151.2, 146.9, 145.6, 138.9, 136.0, 134.2, 132.6, 131.1, 131.0, 129.5, 129.3, 129.1, 128.8, 128.3, 126.9, 126.8, 123.9, 123.0, 120.0, 118.7, 115.7, 112.3, 49.4, 45.1, 33.7, 31.7, 30.5, 28.6, 26.7, 26.2, 24.8, 23.1, 22.8; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  616.8292, found 616.8281.

**4.1.2.14. 1,2,3,4-Tetrahydro-N-(7-(2-(4-nitrophenyl)-4,5-diphenyl-1H-imidazol-1-yl)heptyl)acridin-9-amine (3g).** Intermediate **1b** was

treated with benzil, 4-nitrobenzaldehyde and ammonium acetate according to general procedure to give the desired product **3g** as a brown solid (44% yield): m.p. 84–85 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3411, 3058, 2929, 2856, 1599, 1519, 1342, 696;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.35 (d, 2H,  $J = 8.4$  Hz), 7.86–7.97 (m, 4H), 7.28–7.58 (m, 9H), 7.24–7.12 (m, 3H), 3.94 (t, 2H,  $J = 7.5$  Hz), 3.37 (t, 2H,  $J = 7.2$  Hz), 3.12–2.59 (m, 2H), 2.72–2.60 (m, 2H), 1.98–1.83 (m, 4H), 1.49 (qn, 2H,  $J = 7.2$  Hz), 1.42–1.22 (m, 3H), 1.20–1.08 (m, 2H), 1.08–0.90 (m, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  158.6, 150.9, 147.8, 147.5, 145.2, 139.2, 137.9, 134.1, 131.4, 131.1, 131.0, 129.7, 129.4, 129.2, 128.8, 128.6, 128.4, 127.0, 126.9, 124.2, 123.8, 122.9, 120.3, 116.1, 49.5, 45.2, 34.1, 31.7, 30.6, 28.6, 26.7, 26.3, 25.0, 23.2, 22.9; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  636.8170, found 636.8161.

**4.1.2.15. 1,2,3,4-Tetrahydro-N-(6-(2,4,5-triphenyl-1H-imidazol-1-yl)hexyl)acridin-9-amine (4a).** Intermediate **1a** was treated with benzil, benzaldehyde and ammonium acetate according to general procedure to give the desired product **4a** as a yellow solid (53% yield): m.p. 62–63 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3369, 3058, 2929, 2856, 1579, 1500, 764, 698;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.91 (d, 1H,  $J = 8.4$  Hz), 7.84 (d, 1H,  $J = 8.4$  Hz), 7.66 (d, 2H,  $J = 7.3$  Hz), 7.59–7.25 (m, 12H), 7.23–7.08 (m, 3H), 3.86 (t, 2H,  $J = 7.3$  Hz), 3.79 (br, 1H), 3.32–3.20 (m, 2H), 3.12–3.00 (m, 2H), 2.68–2.52 (m, 2H), 1.98–1.78 (m, 4H), 1.44–1.20 (m, 4H), 1.10–0.88 (m, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  158.3, 150.6, 147.6, 147.4, 137.7, 134.5, 131.4, 130.9, 129.6, 129.1, 129.0, 128.8, 128.7, 128.6, 128.2, 128.0, 126.7, 126.2, 123.5, 122.8, 120.2, 115.9, 49.1, 44.4, 34.0, 31.2, 30.0, 25.8, 25.7, 24.7, 23.0, 22.7; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  577.7925, found 577.7941.

**4.1.2.16. N-(6-(2-(4-Chlorophenyl)-4,5-diphenyl-1H-imidazol-1-yl)hexyl)-1,2,3,4-tetrahydroacridin-9-amine (4b).** Intermediate **1a** was treated with benzil, 4-chlorobenzaldehyde and ammonium acetate according to general procedure to give the desired product **4b** as a yellow solid (37% yield): m.p. 71–72 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3377, 3056, 2931, 2856, 1500, 835, 761, 698;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.90 (d, 1H,  $J = 8.4$  Hz), 7.85 (d, 1H,  $J = 8.4$  Hz), 7.62 (d, 2H,  $J = 8.4$  Hz), 7.56–7.27 (m, 11H), 7.23–7.10 (m, 3H), 3.85 (t, 2H,  $J = 7.5$  Hz), 3.79 (br, 1H), 3.28 (t, 2H,  $J = 7.2$  Hz), 3.10–3.00 (m, 2H), 2.68–2.58 (m, 2H), 1.98–1.80 (m, 4H), 1.44–1.25 (m, 4H), 1.10–0.88 (m, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  158.6, 150.7, 147.6, 146.5, 138.1, 135.0, 134.4, 131.4, 131.0, 130.5, 130.1, 130.0, 129.2, 129.0, 128.9, 128.8, 128.4, 128.2, 126.9, 126.5, 123.7, 122.8, 120.4, 116.2, 49.3, 44.7, 34.2, 31.4, 30.3, 26.1, 26.0, 24.9, 23.1, 22.9; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  612.2373, found 612.2393.

**4.1.2.17. N-(6-(2-(3-Chlorophenyl)-4,5-diphenyl-1H-imidazol-1-yl)hexyl)-1,2,3,4-tetrahydroacridin-9-amine (4c).** Intermediate **1a** was treated with benzil, 3-chlorobenzaldehyde and ammonium acetate according to general procedure to give the desired product **4c** as a yellow solid (29% yield): m.p. 62–63 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3361, 3056, 2929, 2856, 1562, 1498, 767, 696;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.90 (d, 1H,  $J = 8.4$  Hz), 7.86 (d, 1H,  $J = 8.4$  Hz), 7.71 (s, 1H), 7.60–7.27 (m, 12H), 7.23–7.10 (m, 3H), 3.88 (t, 2H,  $J = 7.5$  Hz), 3.79 (br, 1H), 3.28 (t, 2H,  $J = 7.2$  Hz), 3.12–2.98 (m, 2H), 2.72–2.58 (m, 2H), 1.98–1.80 (m, 4H), 1.48–1.25 (m, 4H), 1.12–0.91 (m, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  158.6, 150.7, 147.6, 146.2, 138.2, 134.7, 134.4, 133.3, 131.3, 131.0, 130.1, 130.0, 129.3, 129.2, 129.0, 128.9, 128.4, 128.2, 127.2, 126.9, 126.5, 123.7, 122.8, 120.4, 116.2, 49.3, 44.6, 34.2, 31.5, 30.3, 26.1, 26.0, 24.9, 23.1, 22.9; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  612.2373, found 612.2372.

**4.1.2.18. N-(6-(2-(4-Fluorophenyl)-4,5-diphenyl-1H-imidazol-1-yl)hexyl)-1,2,3,4-tetrahydroacridin-9-amine (4d).** Intermediate **1a** was treated with benzil, 4-fluorobenzaldehyde and ammonium acetate according to general procedure to give the desired product **4d** as a yellow solid (50% yield): m.p. 64–65 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3355, 3056, 2929, 1500, 1224, 843, 764, 698;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.89 (d, 1H,

$J = 8.7$  Hz), 7.85 (d, 1H,  $J = 8.4$  Hz), 7.70–7.60 (m, 2H), 7.58–7.27 (m, 9H), 7.23–7.08 (m, 5H), 3.84 (t, 2H,  $J = 7.5$  Hz), 3.77 (br, 1H), 3.38–3.20 (m, 2H), 3.12–2.98 (m, 2H), 2.68–2.54 (m, 2H), 1.98–1.80 (m, 4H), 1.44–1.20 (m, 4H), 1.10–0.88 (m, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  163.1 (d,  $J = 247.7$  Hz), 158.7, 150.6, 147.7, 146.7, 137.9, 134.5, 131.5, 131.2, 131.1, 131.0, 129.7, 129.2, 129.0, 128.8, 128.3, 128.2, 127.8 (d,  $J = 3.3$  Hz), 126.8, 126.4, 123.7, 122.8, 120.4, 116.2, 115.8 (d,  $J = 22.0$  Hz), 49.3, 44.6, 34.2, 31.4, 30.3, 26.1, 26.0, 24.9, 23.1, 22.9; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  595.7830, found 595.7809.

**4.1.2.19. 1,2,3,4-Tetrahydro-N-(6-(2-(4-methoxyphenyl)-4,5-diphenyl-1H-imidazol-1-yl)hexyl)acridin-9-amine (4e).** Intermediate **1a** was treated with benzil, 4-methoxybenzaldehyde and ammonium acetate according to general procedure to give the desired product **4e** as a yellow solid (33% yield): m.p. 68–69 °C; IR ( $\text{KBr}$ )  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3365, 3056, 2931, 1500, 1249, 1028, 764, 698;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.89 (d, 1H,  $J = 8.7$  Hz), 7.85 (d, 1H,  $J = 8.4$  Hz), 7.60 (d, 2H,  $J = 8.7$  Hz), 7.34–7.55 (m, 8H), 7.34–7.26 (m, 1H), 7.21–7.07 (m, 3H), 6.96 (d, 2H,  $J = 8.7$  Hz), 3.90–3.70 (br, 1H), 3.84 (t, 2H,  $J = 7.5$  Hz), 3.79 (s, 3H), 3.26 (t, 2H,  $J = 7.2$  Hz), 3.10–3.00 (m, 2H), 2.68–2.58 (m, 2H), 1.96–1.82 (m, 4H), 1.44–1.23 (m, 4H), 1.10–0.88 (m, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  160.1, 158.6, 150.6, 147.7, 147.6, 137.6, 134.7, 131.7, 131.0, 130.5, 129.4, 129.1, 128.9, 128.6, 128.3, 128.1, 126.8, 126.2, 124.0, 123.6, 122.8, 120.3, 116.1, 114.1, 55.4, 49.3, 44.5, 34.2, 31.4, 30.1, 26.0, 25.9, 24.8, 23.1, 22.9; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  607.8188, found 607.8179.

**4.1.2.20. 4-(1-(6-(1,2,3,4-Tetrahydroacridin-9-ylamino)hexyl)-4,5-diphenyl-1H-imidazol-2-yl)benzonitrile (4f).** Intermediate **1a** was treated with benzil, 4-cyanobenzaldehyde and ammonium acetate according to general procedure to give the desired product **4f** as a yellow solid (53% yield): m.p. 74–75 °C; IR ( $\text{KBr}$ )  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3384, 3056, 2927, 2225, 1604, 1498, 769, 696;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.90 (d, 1H,  $J = 8.7$  Hz), 7.85 (d, 1H,  $J = 7.8$  Hz), 7.84 (d, 2H,  $J = 8.5$  Hz), 7.75 (d, 2H,  $J = 8.5$  Hz), 7.58–7.28 (m, 9H), 7.24–7.12 (m, 3H), 3.91 (t, 2H,  $J = 7.6$  Hz), 3.79 (br, 1H), 3.30 (t, 2H,  $J = 7.3$  Hz), 3.12–3.00 (m, 2H), 2.68–2.58 (m, 2H), 1.98–1.82 (m, 4H), 1.48–1.24 (m, 4H), 1.12–0.90 (m, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  158.7, 150.7, 147.6, 145.6, 138.9, 136.0, 134.1, 132.6, 131.1, 131.0, 129.5, 129.4, 129.2, 129.0, 128.5, 128.3, 126.9, 126.8, 123.8, 122.8, 120.4, 118.7, 116.3, 112.4, 49.3, 45.0, 34.2, 31.5, 30.5, 26.2, 26.1, 25.0, 23.2, 22.9; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  602.8023, found 602.8043.

**4.1.2.21. 1,2,3,4-Tetrahydro-N-(6-(2-(4-nitrophenyl)-4,5-diphenyl-1H-imidazol-1-yl)hexyl)acridin-9-amine (4g).** Intermediate **1a** was treated with benzil, 4-nitrobenzaldehyde and ammonium acetate according to general procedure to give the desired product **4g** as a brown solid (55% yield): m.p. 73–74 °C; IR ( $\text{KBr}$ )  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3417, 3058, 2929, 2856, 1599, 1519, 1340, 696;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.32 (d, 2H,  $J = 8.7$  Hz), 7.98–7.87 (m, 3H), 7.85 (d, 1H,  $J = 8.1$  Hz), 7.58–7.27 (m, 9H), 7.25–7.12 (m, 3H), 3.94 (t, 2H,  $J = 7.5$  Hz), 3.73 (br, 1H), 3.29 (t, 2H,  $J = 7.0$  Hz), 3.12–2.98 (m, 2H), 2.72–2.56 (m, 2H), 1.98–1.80 (m, 4H), 1.50–1.28 (m, 4H), 1.14–0.90 (m, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  158.6, 150.7, 147.7, 147.5, 145.2, 139.1, 137.7, 134.1, 131.4, 131.0, 130.9, 129.6, 129.4, 129.2, 128.9, 128.5, 128.3, 127.0, 126.9, 124.1, 123.8, 122.8, 120.4, 116.2, 49.3, 45.1, 34.1, 31.5, 30.5, 26.2, 26.1, 24.9, 23.1, 22.9; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  622.7901, found 622.7902.

**4.1.2.22. N-(9-(2-(4-Chlorophenyl)-4,5-diphenyl-1H-imidazol-1-yl)nonyl)-1,2,3,4-tetrahydroacridin-9-amine (5).** Intermediate **1d** was treated with benzil, 4-chlorobenzaldehyde and ammonium acetate according to general procedure to give the desired product **5** as a yellow solid (43% yield): m.p. 153–154 °C; IR ( $\text{KBr}$ )  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3379, 3053, 2931, 2854, 1495, 836, 770, 697;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.93 (d, 1H,  $J = 8.4$  Hz), 7.89 (d, 1H,  $J = 8.1$  Hz), 7.63 (d, 2H,  $J = 8.7$  Hz),

7.58–7.28 (m, 11H), 7.24–7.09 (m, 3H), 4.00–3.78 (br, 1H), 3.85 (t, 2H,  $J = 7.5$  Hz), 3.44 (t, 2H,  $J = 7.2$  Hz), 3.12–2.98 (m, 2H), 2.76–2.63 (m, 2H), 1.98–1.84 (m, 4H), 1.59 (qn, 2H,  $J = 7.2$  Hz), 1.38–1.20 (m, 4H), 1.20–0.86 (m, 8H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  158.7, 150.8, 147.7, 146.5, 138.1, 135.0, 134.5, 131.5, 131.1, 130.5, 130.2, 130.1, 129.2, 129.0, 128.8, 128.3, 128.2, 126.9, 126.5, 123.7, 123.0, 120.4, 116.0, 49.6, 44.9, 34.3, 31.9, 30.4, 29.3, 29.2, 28.6, 27.0, 26.2, 24.9, 23.2, 23.0; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  654.3179, found 654.3195.

**4.1.2.23. N-(10-(2-(4-Chlorophenyl)-4,5-diphenyl-1H-imidazol-1-yl)decyl)-1,2,3,4-tetrahydroacridin-9-amine (6).** Intermediate **1e** was treated with benzil, 4-chlorobenzaldehyde and ammonium acetate according to general procedure to give the desired product **6** as a yellow solid (33% yield): m.p. 52–53 °C; IR ( $\text{KBr}$ )  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3422, 3058, 2925, 2852, 1499, 834, 760, 696;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.94 (d, 1H,  $J = 8.7$  Hz), 7.90 (d, 1H,  $J = 8.7$  Hz), 7.63 (d, 2H,  $J = 8.4$  Hz), 7.58–7.28 (m, 11H), 7.24–7.09 (m, 3H), 3.92 (br, 1H), 3.85 (t, 2H,  $J = 7.6$  Hz), 3.44 (t, 2H,  $J = 7.2$  Hz), 3.14–2.98 (m, 2H), 2.78–2.60 (m, 2H), 2.00–1.84 (m, 4H), 1.61 (qn, 2H,  $J = 7.2$  Hz), 1.45–0.86 (m, 14H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  158.6, 150.9, 147.7, 146.5, 138.1, 134.9, 134.5, 131.5, 131.1, 130.5, 130.2, 130.1, 129.2, 129.0, 128.9, 128.8, 128.3, 128.2, 126.9, 126.5, 123.7, 123.0, 120.4, 116.0, 49.6, 44.9, 34.2, 31.9, 30.4, 29.4, 29.3, 29.2, 28.7, 27.0, 26.2, 24.9, 23.2, 23.0; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  668.3448, found 668.3459.

#### 4.1.3. General procedure for the preparation of bis(n)-lophines **7a–e**

A mixture of benzil (1.0 mmol), diamine (0.5 mmol), benzaldehyde (1.0 mmol), ammonium acetate (1.0 mmol) and  $\text{InCl}_3$  (10 mol%) in absolute ethanol (1 mL), placed in a round bottom flask fitted with a condenser and drying tube, was stirred under reflux for 24 h. The solvent was removed under reduced pressure, and the crude product purified by column chromatography (eluting with hexane-ethyl acetate-triethylamine, 40:59:1) to give the desired product.

**4.1.3.1. 2,4,5-Triphenyl-1-(10-(2,4,5-triphenyl-1H-imidazol-1-yl)decyl)-1H-imidazole (7a).** Following the general procedure, 1,10-decanodiamine, benzil, benzaldehyde and ammonium acetate afforded product **7a** as a pale yellow solid (22% yield): m.p. 165–166 °C; IR ( $\text{KBr}$ )  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3059, 2925, 2852, 1601, 1501, 1443, 773, 696;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71–7.64 (m, 4H), 7.56–7.37 (m, 20H), 7.23–7.09 (m, 6H), 3.84 (t, 4H,  $J = 7.6$  Hz), 1.38–1.22 (m, 4H), 0.96–0.78 (m, 12H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  147.8, 137.9, 134.8, 131.8, 131.7, 131.2, 129.8, 129.4, 129.2, 129.0, 128.8, 128.2, 127.0, 126.4, 44.9, 30.5, 29.0, 28.6, 26.2; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  732.0039, found 732.0035.

**4.1.3.2. 2,4,5-Triphenyl-1-(9-(2,4,5-triphenyl-1H-imidazol-1-yl)nonyl)-1H-imidazole (7b).** Following the general procedure, 1,9-nonanodiamine, benzil, benzaldehyde and ammonium acetate afforded product **7b** as a pale yellow solid (42% yield): m.p. 83–84 °C; IR ( $\text{KBr}$ )  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3057, 2925, 2852, 1600, 1499, 1442, 773, 695;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.70–7.63 (m, 4H), 7.56–7.34 (m, 20H), 7.23–7.08 (m, 6H), 3.84 (t, 4H,  $J = 7.3$  Hz), 1.38–1.18 (m, 4H), 0.92–0.68 (m, 10H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  147.8, 137.9, 134.7, 131.8, 131.7, 131.2, 129.7, 129.3, 129.2, 128.9, 128.7, 128.2, 127.0, 126.4, 44.8, 30.4, 28.7, 28.5, 26.2; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  717.9770, found 717.9766.

**4.1.3.3. 2,4,5-Triphenyl-1-(8-(2,4,5-triphenyl-1H-imidazol-1-yl)octyl)-1H-imidazole (7c).** Following the general procedure, 1,8-octanodiamine, benzil, benzaldehyde and ammonium acetate afforded product **7c** as a white solid (37% yield): m.p. 150–151 °C; IR ( $\text{KBr}$ )  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3056, 2933, 2856, 1600, 1479, 1442, 773, 696;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.65 (d, 4H,  $J = 7.8$  Hz), 7.52 (d, 4H,  $J = 7.8$  Hz), 7.48–7.34 (m, 16H), 7.23–7.08 (m, 6H), 3.81 (t, 4H,  $J = 7.5$  Hz), 1.21 (qn, 4H,  $J = 7.5$  Hz),



0.81–0.60 (m, 8H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  147.8, 137.8, 134.7, 131.7, 131.6, 131.1, 129.7, 129.3, 129.2, 128.9, 128.7, 128.2, 126.9, 126.4, 44.7, 30.3, 28.2, 26.0; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  703.9501, found 703.9473.

**4.1.3.4. 2,4,5-Triphenyl-1-(7-(2,4,5-triphenyl-1H-imidazol-1-yl)heptyl)-1H-imidazole (7d).** Following the general procedure, 1,7-heptanodiamine, benzil, benzaldehyde and ammonium acetate afforded product **7d** as white solid (29% yield): m.p. 84–85 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3058, 2929, 2856, 1600, 1500, 1442, 773, 696;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.63 (d, 4H,  $J = 7.2$  Hz), 7.54–7.47 (m, 4H), 7.47–7.33 (m, 16H), 7.23–7.08 (m, 6H), 3.77 (t, 4H,  $J = 7.5$  Hz), 1.20–1.08 (m, 4H), 0.74–0.50 (m, 6H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  147.7, 137.8, 134.7, 131.7, 131.6, 131.1, 129.7, 129.3, 129.2, 129.0, 128.7, 128.2, 126.9, 126.4, 44.6, 30.2, 27.7, 25.9; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  689.9233, found 689.9232.

**4.1.3.5. 2,4,5-Triphenyl-1-(6-(2,4,5-triphenyl-1H-imidazol-1-yl)hexyl)-1H-imidazole (7e).** Following the general procedure, 1,6-hexanodiamine, benzil, benzaldehyde and ammonium acetate afforded product **7e** as a white solid (30% yield): m.p. 166–167 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3060, 2937, 2858, 1600, 1500, 1442, 773, 698;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.63–7.57 (m, 4H), 7.54–7.48 (m, 4H), 7.45–7.29 (m, 16H), 7.23–7.08 (m, 6H), 3.71 (t, 4H,  $J = 7.5$  Hz), 1.14–0.98 (m, 4H), 0.64–0.48 (m, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  147.7, 137.8, 134.6, 131.6, 131.5, 131.0, 129.6, 129.2, 129.1, 128.9, 128.7, 128.2, 126.9, 126.4, 44.5, 30.0, 25.3; HRMS-ESI: calcd for  $[\text{M} - \text{H}]^+$  675.8964, found 675.8976.

#### 4.2. In vitro assay of brain AChE and BuChE activities

Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities were measured by the colorimetric method of Ellman et al. [34] with some minor modifications, adapted for determining the effects of analogs on enzyme activity in supernatants of rat's brain homogenates and human plasma, respectively. Adults Wistar male rats (2–3 months) were supplied by Department of Biochemistry-UFRGS. Animals were housed in plastic cages and maintained at 22–23 °C under a 12 h light/dark cycle (lights on at 7:00 a.m.) with free access to food and water. All experiments were following and approved by the Animal Care and Ethics Committee of the Universidade Federal do Rio Grande do Sul. For AChE sources, rats were decapitated, the brain was rapidly dissected on ice into cortex and homogenized in cold 10 mM Tris–HCl buffer, pH 7.2 containing 160 mM sucrose. The homogenates were centrifugated at  $10,000 \times g$  for 10 min at 4 °C; supernatants were used as AChE sources, kept into aliquots and stored at –20 °C. For BuChE sources, human plasma was obtained from 5 healthy volunteers (24–60 years old) of both sexes with written consent and the protocol was approved by the Human Ethic Committee of the Universidade Federal do Rio Grande do Sul. The samples were centrifugated at  $1000 \times g$  for 15 min at room temperature and aliquots were stored at –20 °C.

AChE and BuChE enzyme samples, in 20 mM phosphate buffer pH 7.4, were incubated 150 s with acetylthiocholine iodide or butyrylthiocholine iodide (0.8 mM), respectively, in the presence of 10 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), for color development (all chemicals from Sigma). Production of the yellow anion of 5-thio-2-nitrobenzoic acid was measured with a SPECTRAMax 190, 96-well plate reader, at 415 nm. Protein concentration for both type of samples were measured by determined by Peterson's modification of the procedure of Lowry et al. [35], using bovine serum albumin as standard.

#### 4.3. Statistical analysis

The  $\text{IC}_{50}$  values were determined from a plot of activity value of log (inhibitor) vs. response, which was processed by a software of GraFit 6.0. All results are calculated as mean  $\pm$  SD of five independent measurements, each performed in triplicate.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2013.01.029>.

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