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Cholinesterase inhibitors: SAR and enzyme inhibitory activity of 3-[ω-(benzylmethylamino)alkoxy]xanthen-9-ones

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Dedicated to prof. Piero Valenti on the occasion of his retirement

Abstract—In this work, we further investigated a previously introduced class of cholinesterase inhibitors. The removal of the carbamic function from the lead compound xanthostigmine led to a reversible cholinesterase inhibitors **3**. Some new 3-[ω-(benzylmethylamino)alkoxy]xanthen-9-one analogs were designed, synthesized, and evaluated for their inhibitory activity against both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The length of the alkoxy chain of compound **3** was increased and different substituents were introduced. From the IC_{50} values, it clearly appears that the carbamic residue is crucial to obtain highly potent AChE inhibitors. On the other hand, peculiarity of these compounds is the high selectivity toward BuChE with respect to AChE, being compound **12** the most selective one (6000-fold). The development of selective BuChE inhibitors may be of great interest to clarify the physiological role of this enzyme and to provide novel therapeutics for various diseases.

1. Introduction

Alzheimer's disease (AD), the most common form of dementia, is a chronic, neurodegenerative disorder, which is characterized by loss of cognitive ability, severe behavioral abnormalities and ultimately total degradation of intellectual and mental activities.

Although the origin of AD is still unknown, three pathological hallmarks have been identified, for example, amyloid-β plaques, neurofibrillary tangles (NFTs) and synaptic loss.[1,2] The neuritic senile plaques consist of a fibrillar amyloid core surrounded by dystrophic neurites and reactive microglia.

The ante mortem diagnosis of AD is clinically problematic, and ambiguity arises from numerous other causes of dementia that are pathologically unrelated to AD. At autopsy, the AD brain is characterized by a number of important pathological changes. There is a marked loss of neurons and synapses in many areas of the CNS, especially in the regions involving higher order cognitive functions, such as hippocampus and the association cortex.[3] In addition, there is a global and dramatic reduction of the neurotransmitters level, for example, noradrenaline, dopamine, serotonin, glutamate, substance P, and acetylcholine (ACh). This depletion of neurotransmitters, among which ACh is the most important one, is almost certainly the cause for the broad clinical manifestations of AD: memory impairment, hallucinations, paranoia, restlessness and depression.[4]

These findings led to the development of the cholinergic hypothesis that, at least in its earlier formulation, predicted a favourable effect for compounds able to sustain the central cholinergic tone.[5–7] The cholinergic hypothesis provided the first rational approach to the treatment of AD. To date only acetylcholinesterase (AChE) inhibitors, such as tacrine, donepezil, rivastigmine, and galantamine and uncompetitive moderate affinity NMDA receptor antagonist, memantine, are available for AD treatment. On the other side in recent times, the role of butyrylcholinesterase (BuChE) inhibition in AD has received increasing attention, both from the medicinal chemistry and the clinical points of view.[8,9] Several lines of evidence indicate that BuChE might be a co-regulator

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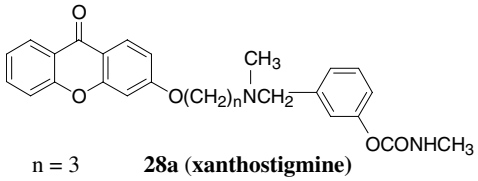
of the activity of the neurotransmitter acetylcholine.[10] Remarkably, cortical levels of BuChE show a significant increase in AD. In this respect, the research efforts are focused on the development of selective inhibitors that are necessary to clearly evaluate the role of the enzyme and the therapeutic feasibility of its inhibition.

Our research group has been involved for many years in the development of AChE inhibitors as potential drugs for AD. We designed and studied a class of *N*-methyl-*N*-(3-carbamoyloxyphenyl)methylamino derivatives characterized by the presence of a heteroaryl moiety linked to the tertiary amino nitrogen through an alkoxy chain. Xanthostigmine (**28a**, Chart 1), the lead compound, showed the highest activity (IC_{50} = 0.3 nM).[11,12]



n = 3 **28a** (**xanthostigmine**)

n = 7 **28b**

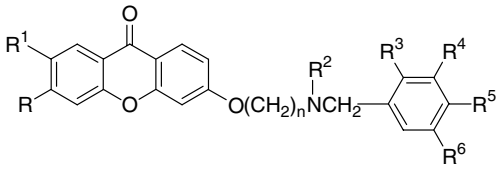
Chart 1.

In previous papers we introduced modifications in the linker chain, the carbamoyl substituent and the aryl moiety of xanthostigmine in order to investigate the structure–activity relationships (SAR) of this series of compounds.[13,14]

A three-dimensional model of the quaternary complex between AChE and xanthostigmine showed that, by properly extending the alkoxy chain of aminoalkoxyaryl derivatives, it might be possible to reach the peripheral anionic binding site in such a way that the heteroaryl moiety would have the possibility to establish π-stacking interactions with the indole ring of Trp286 located at the entrance of the human AChE gorge. This aromatic residue, together with other ones, is considered to constitute an essential part of the AChE peripheral binding site,[15] and recent studies have shown that inhibitors binding to this site are able to block the Aβ aggregation induced by AChE.[16,17]

In this work, we intended to further investigate the interactions of xanthostigmine analogues with the most important residues of the AChE gorge by removing the carbamic function from xanthostigmine. With this modification, a reversible inhibitor was obtained (**3**, Table 1). The length of the alkoxy chain of compound **3** was increased up to 12 methylene units (compounds

Table 1. Physicochemical and analytical data of the compounds studied



Compound	*n*	R	R^1	R^2	R^3	R^4	R^5	R^6	Mp (°C)	Yield (%)
3	3	H	H	Me	H	H	H	H	51–52[a]	60
4	4	H	H	Me	H	H	H	H	67–69[a]	50
5	5	H	H	Me	H	H	H	H	55–58[a]	50
6	6	H	H	Me	H	H	H	H	158–160[b]	50
7	7	H	H	Me	H	H	H	H	50–55[a]	50
8	8	H	H	Me	H	H	H	H	86–89[b]	50
9	9	H	H	Me	H	H	H	H	132–133[a]	40
10	10	H	H	Me	H	H	H	H	108–109[b]	60
11	11	H	H	Me	H	H	H	H	140–141[b]	80
12	12	H	H	Me	H	H	H	H	100–102[b]	50
13	7	OCH_3	H	Me	H	H	H	H	110–113[b]	50
14	7	OCH_3	OCH_3	Me	H	H	H	H	97–98[b]	50
15	3	H	H	Me	OCH_3	H	H	H	189–191[a]	90
16	7	H	H	Me	OCH_3	H	H	H	120–122[b]	90
17	7	H	H	Me	H	OCH_3	H	H	101–103[b]	70
18	7	H	H	Me	H	H	OCH_3	H	153–154[b]	70
19	7	H	H	Me	Cl	H	H	H	131–133[b]	40
20	7	H	H	Me	H	Cl	H	H	144–147[b]	30
21	7	H	H	Me	H	H	Cl	H	88–90[b]	30
22	7	H	H	Me	CH_3	H	H	H	131–133[b]	25
23	7	H	H	Me	NO_2	H	H	H	161–164[b]	30
24	7	H	H	Me	OCH_3	OCH_3	H	H	138–139[b]	90
25	7	H	H	Me	OCH_3	H	H	OCH_3	oil[a]	40
26	7	H	H	Me	OCH_3	OCH_3	OCH_3	H	54–55[b]	40
27	7	H	H	Et	OCH_3	H	H	H	124–126[b]	70

[a] Free base (crystallizing from ligroin).

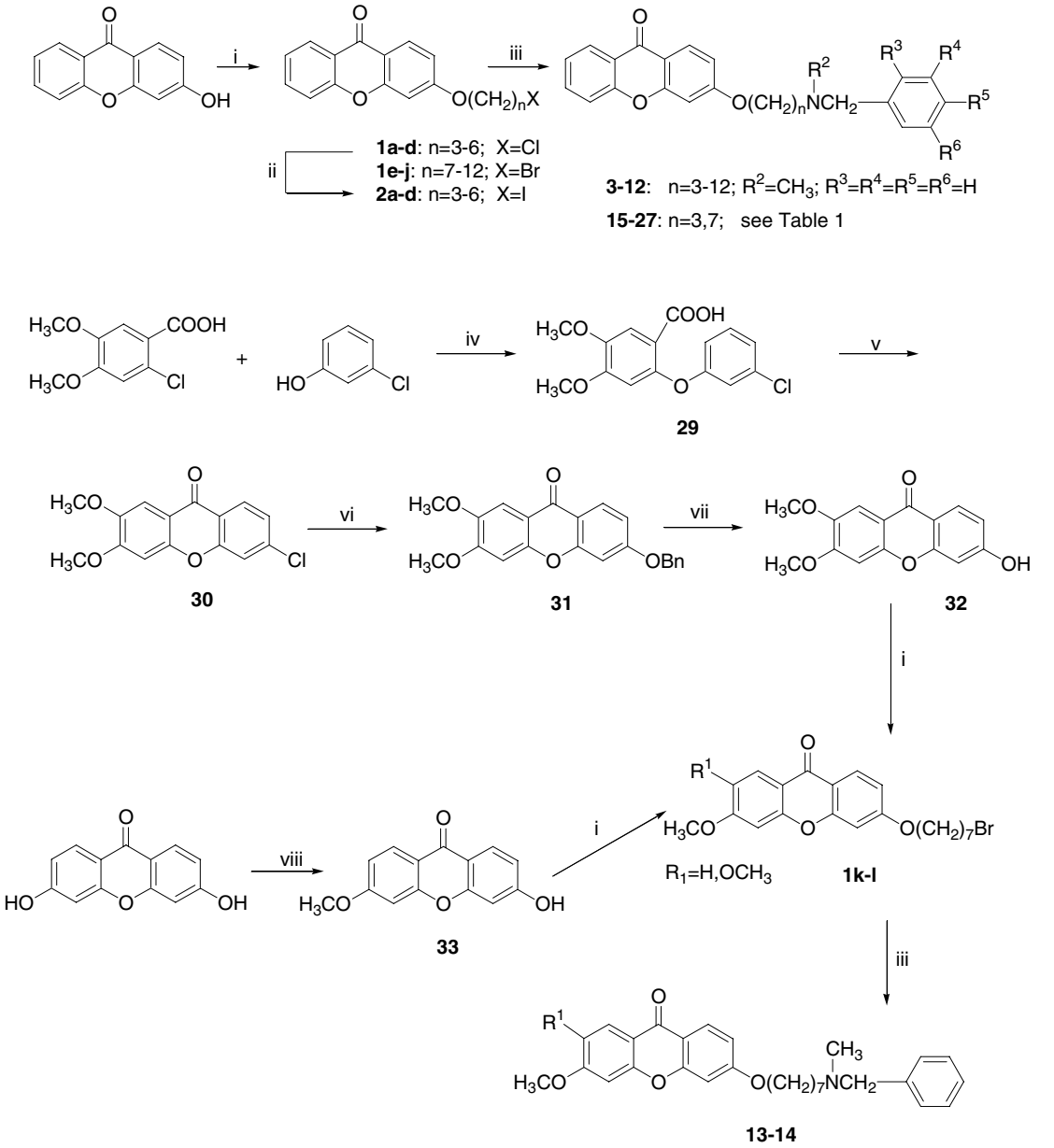
[b] Hydrochlorides (crystallizing from methanol–ether).

4–12) to identify the optimal distance between the xanthone moiety and the amino function. One or two methoxy groups were introduced in positions 6 and 7 of the xanthone nucleus (compounds **13** and **14**), as these substituents could favour the interaction with the PAS, as seen for both Donepezil[18] and the inhibitor AP2238.[19] The basicity of the amino function is crucial for the inhibitory activity: the nitrogen has not to be protonated to cross the BBB, but has to be protonated for the interaction with the central anionic site (AS). Therefore we modified the basicity of the amino group introducing some substituents on the phenyl ring (compounds **15–26**). Finally, the importance of the substituent on the protonable amine was studied by replacing the methyl group with an ethyl group (compound **27**).

2. Chemistry

The synthesis of the studied compounds was accomplished as illustrated in Scheme 1. The 3-hydroxyxanthen-9-one was treated with 1-bromo-ω-chloroalkanes or ω-dibromoalkanes (according to commercial availability) in the presence of K_2CO_3 to afford ω-haloalkoxy derivatives **1a–j**. The ω-chloroalkyl derivatives **1a–d** were treated with NaI in refluxing methyl ethyl ketone to give the corresponding ω-iodoalkoxy intermediates **2a–d**.

For the synthesis of **1k** and **1l** different routes were followed. The 6-chloro-2,3-dimethoxyxanthen-9-one **30** was synthesized from 2-chloro-4,5-dimethoxybenzoic



Scheme 1. Reagents and conditions: (i) $Br(CH_2)_nCl$ or $Br(CH_2)_nBr$, K_2CO_3 reflux, 24 h; (ii) NaI, methylethylketone, reflux 4 h; (iii) selected *N*-benzylalkylamine, toluene, reflux 15 h; (iv) K_2CO_3, Cu, CuI, nitrobenzene, 170 °C, 8 h; (v) P_2O_5, H_3PO_4, 110 °C, 4 h; (vi) $C_6H_5CH_2OH$, KOH, $N(C_4H_8)_4Br$; (vii) H_2, Pd/C, rt; (viii) CH_3I, $C_6H_5CH_2N(C_2H_5)_3Cl$.

acid through reaction with 3-chlorophenol in nitrobenzene in the presence of K_2CO_3, Cu and CuI affording the corresponding diphenyl ether **29** and subsequent cyclization by means of polyphosphoric acid. The chloroxanthone **30** was then treated with benzyl alcohol and KOH in the presence of the transfer-phase catalyst tetrabutylammonium bromide to afford the benzyloxy derivative **31**, which was then debenzylated by H_2 over Pd/C at room temperature and pressure to give the hydroxy derivative **32**.

3,6-Dihydroxyxanthen-9-one[20] was methylated by means of methyl iodide and NaOH in the presence of the transfer-phase catalyst benzyltriethylammonium chloride to afford 3-hydroxy-6-methoxyxanthen-9-one **33**. Compounds **32** and **33** were then alkylated as previously described to give **1k** and **1l**. The ω-haloalkoxy derivatives **1e–l** and **2a–d** were then condensed with the selected *N*-benzylalkylamine to give the final compounds **3–27**.

The *N*-benzylalkylamines were prepared by condensation of the selected benzaldehydes and amines followed by $NaBH_4$ reduction.

The structure and the physicochemical data of compounds **3–27** are reported in Table 1.

3. Enzyme inhibition

The inhibitory activity of the newly synthesized compounds against both cholinesterases was studied using the method of Ellmann[21] to determine the rate of hydrolysis of acetylthiocholine or butyrylthiocholine in the presence of the inhibitor.

4. Results and discussion

The inhibitory activities against both erythrocyte AChE and serum BuChE of 3-[ω-(benzylmethylamino)alkoxy]xanthen-9-one derivatives together with that of the reference compounds xanthostigmine (**28a**) and **28b** are reported in Table 2, expressed as IC_{50} values.

From the IC_{50} values of compounds **3–12**, it appears that variations of the chain length (*n* in the general formula) influenced both AChE and BuChE activities. Remarkably, the behavior of the series is rather uniform for AChE and BuChE inhibition up to a chain length of seven methylene units, while it dramatically diverges from eight carbon units forward. This clearly appears from the plot of Figure 1, where the variations of inhibitory potency (expressed as pIC_{50}) against both AChE and BuChE are reported as a function of the number of methylene units of compounds **3–12**. As already discussed in previous works,[12,13] in which **28a** and its seven methylene analogue **28b** showed the highest inhibitory potency against AChE, also in this series of non-carbamic analogues the best results were obtained for the same cholinesterase with compounds **3** and **7**, having three and seven methylene units, respectively (**3** IC_{50} = 2.68 μM and **7** IC_{50} = 2.82 μM). However, a peculiar feature of compounds **8–12** is the high inhibitory activity for BuChE, and in particular compound **8** (eight

Table 2. Inhibitory activity on isolated AChE and BuChE and IC_{50} ratio of the compounds studied

Compound	IC_{50}(μM) AchE	IC_{50} (μM) BuChE	Ratio IC_{50} BuChE/AchE	Ratio IC_{50} AChE/BuChE
3	2.68 ± 0.09	8.33 ± 0.89	3.11	0.32
4	6.25 ± 0.85	2.65 ± 0.14	0.42	2.36
5	7.78 ± 0.54	2.49 ± 0.22	0.32	3.12
6	2.89 ± 0.23	1.43 ± 0.06	0.49	2.02
7	2.82 ± 0.61	0.80 ± 0.04	0.28	3.52
8	10.3 ± 6.50	0.08 ± 0.01	0.008	129
9	152	0.10 ± 0.02	6.6	1520
10	388	0.10 ± 0.01	2.6	3880
11	626	0.12 ± 0.01	1.92	5217
12	908	0.15 ± 0.02	1.65	6053
13	7.84 ± 0.51	46.1 ± 3.90	5.9	0.17
14	2.32 ± 0.13	3.08 ± 0.35	1.33	0.75
15	3.40 ± 0.51	1.91 ± 0.17	0.56	1.78
16	2.18 ± 0.16	0.93 ± 0.03	0.43	2.34
17	40.0 ± 3.80	1.85 ± 0.08	0.05	21.6
18	360	2.11 ± 0.13	0.006	171
19	433	269 ± 75	0.62	1.6
20	594	63.8 ± 3.1	0.10	9.3
21	1050	127 ± 48	0.12	8.26
22	565	29.5 ± 1.1	0.05	19.15
23	413	14.0 ± 1.1	0.03	29.5
24	138	2.01 ± 0.10	0.014	68.65
25	127	1.27 ± 0.08	0.01	100
26	155	1.00 ± 0.04	0.006	155
27	3.15 ± 0.20	0.32 ± 0.01	0.10	9.8
28a	0.30 ± 0.01 nM	48 ± 4 nM	160	0.0062
28b	0.32 ± 0.09 nM	16.5 ± 1.4 nM	51.6	0.019

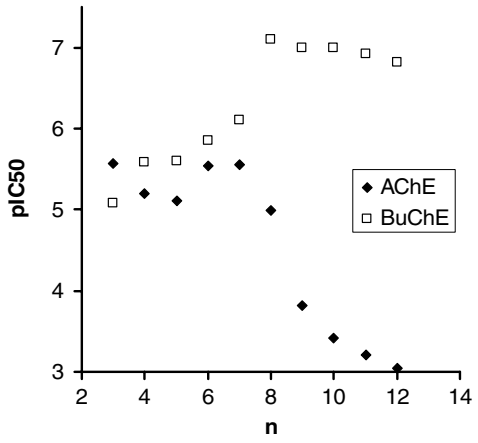


Figure 1. Plot of pIC_{50} values for AChE (♦) and BuChE (□) inhibition against the number of methylene units of compounds **3**–**12**.

methylene units) is a potent BuChE inhibitor having IC_{50} = 80 nM. For these compounds, it appears that the loss of the carbamic function and of the consequent ability to covalently bind the BuChE active site is at least partly compensated by a higher propensity to fill the enzyme cavity and to stabilize its occupancy possibly by means of hydrophobic interactions. In fact, the previously reported carbamic analogs of **8**–**12** showed IC_{50} values against BuChE in the range of 13.9–31.6 nM,[13] which are only 3–6 times lower than those of the present derivatives. On the contrary, the loss of activity against AChE resulting from the elimination of the carbamic function is of the order of thousands of times. Summarizing, the effects of varying the chain length of the present non-carbamic xanthostigmine derivatives, we point out that the elongation of the chain proportionally increased the selectivity of these compounds for BuChE, which resulted more than 100-fold for **8** and 6000-fold for **12**, bearing a 12 methylene units chain.

We further investigated the interaction of non-carbamic xanthostigmine analogues with AChE and BuChE by introducing one or two methoxy groups in positions 6 and 7 of the xanthone moiety. Both the methoxy and the dimethoxy derivatives **13** and **14** inverted the selectivity of the parent compound **7**, allowing us to speculate about a possible interaction of the methoxy substituent with aminoacids that are missing in BuChE.

To complete the SAR of this class of compounds, we introduced different substituents on the benzyl ring, in particular methoxy and chlorine groups in *ortho*, *meta* and *para* position (**15**–**21**). Compounds **15**–**16**, with the methoxy group in the *ortho* position, were the only compounds that retained the inhibitory potency with respect to the unsubstituted parent compounds **3** and **7**, respectively. Substituents in *meta* and in particular in *para* position drammatically reduced the activity. The same trend was also shown with the chlorine substitution, but in this case even the *ortho* substitution was detrimental for activity, though less than *meta* and *para*.

Considering these results, we introduced electron withdrawing and electron donating substituents in the *ortho* position, to evaluate the importance of this modification in stabilizing the formal positive charge on the amine function, that is known to be crucial for the interaction with the AS. As already shown in the literature for AChE activity,[22] for this class of compounds as well, the only accepted substituent on the benzylamino moiety seems to be the *ortho* methoxy group. This hypothesis was confirmed by the introduction of one or two methoxy groups in compound **16**, which led to less active compounds (**24**–**26**). Any substituent which decreased activity toward AChE increased the selectivity to BuChE. In an attempt to rationalize this SAR, we considered that in this latter enzyme, Lys286 and Val288 line the acyl pocket and that these aminoacid residue side chains are not large, compared to the phenylalanine side chains of the corresponding residues of AChE. For this reason, it may be that BuChE tolerated all the substituents we introduced in the phenyl ring positions, with the exception of chlorine atom. Accordingly to this hypothesis, also the introduction of two or three methoxy groups led to compounds showing a good BuChE inhibitory activity: in particular **26** was 155 times more active on BuChE then on AChE.

Finally, the substitution of the methyl group on the amino function (**27**) with an ethyl group maintained the activity for AChE, while it increased the potency toward BuChE with respect to compound **7**.

5. Conclusions

A SAR study of non-carbamic derivatives of xanthostigmine (**28a**) was performed with the aim of exploring the anti-cholinesterase potential of corresponding reversible inhibitors. From the IC_{50} values shown in Table 2, it clearly appears that the carbamic residue is crucial to obtain highly potent AChE inhibitors. However, a peculiar feature of compounds **8**–**12** of this class is the high selectivity for BuChE shown by derivatives in which the distance between the xanthone moiety and the benzylamino group is more than seven methylene units. This could be explained by the presence of a larger cavity in BuChE with respect to the narrow gorge of AChE, that could allow longer compounds to fold and better fill the hydrophobic cavity.

Unlike AChE, the physiological function of BuChE in healthy and diseased humans is still unclear, and recently its involvement in developmental and neurobiological processes has been suggested. In particular, BuChE is thought to be involved in neurogenesis and regulation of cell proliferation and differentiation, as well as in changes in apoptotic events.[23]

Thus, the development of selective BuChE inhibitors, as compounds **8**–**12** and **24**–**26**, may be of great interest to clarify the physiological role of this enzyme and to provide novel therapeutics for various diseases.

6. Experimental

6.1. Chemistry

6.1.1. General methods. All melting points were determined in open glass capillaries using a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were recorded in $CDCl_3$ solution on a Varian Gemini 300 spectrometer with Me_4Si as the internal standard. Mass spectra were recorded on a V.G. 7070 E spectrometer. Silica gel Merck 230–400 mesh was used for purification with flash chromatography. Wherever analyses are only indicated with element symbols, analytical results obtained for those elements were within 0.4% of the theoretical values. Compounds' names were obtained using AUTONOM, PC software for nomenclature in organic chemistry, Beilstein-Institut and Springer.

6.1.2. 3-(3-Chloropropoxy)xanthen-9-one (1a). A stirred suspension of 2.12 g (0.01 mol) of 3-hydroxyxanthen-9-one, 3.15 g (0.02 mol) of 1-bromo-3-chloropropane, and 2.76 g (0.02 mol) of K_2CO_3 in dry acetone was refluxed for 24 h. The reaction was monitored by TLC. The hot reaction mixture was filtered and evaporated to dryness. The residue was crystallized from EtOH to give 2.02 g (70%) of **1a**: mp 122–125 °C; ^{1}H NMR δ 2.30–2.40 (m, 2H), 3.90 (t, 2H), 4.42 (t, 2H), 6.98–8.40 (m, 7H, Ar). Anal. ($C_{16}H_{13}ClO_3$): C, H.

6.1.3. 3-(4-Chlorobutoxy)xanthen-9-one (1b). Using the previous procedure and starting from 2.12 g (0.01 mol) of 3-hydroxyxanthen-9-one and 3.43 g (0.02 mol) of 1-bromo-4-chlorobutane, 2.1 g (70%) of **1b** were obtained: mp 123–125 °C (EtOH); ^{1}H NMR δ 2.01–2.15 (m, 4H), 3.65 (t, 2H), 4.15 (t, 2H), 6.85–8.40 (m, 7H, Ar). Anal. ($C_{17}H_{15}ClO_3$): C, H.

6.1.4. 3-(5-Chloropentyloxy)xanthen-9-one (1c). Using the previous procedure and starting from 2.12 g (0.01 mol) of 3-hydroxyxanthen-9-one and 3.71 g (0.02 mol) of 1-bromo-5-chloropentane, 2.2 g (70%) of **1c** were obtained: mp 128–130 °C (EtOH); ^{1}H NMR δ 1.65–1.75 (m, 2H), 1.85–1.95 (m, 4H), 3.60 (t, 2H), 4.10 (t, 2H), 6.85–8.40 (m, 7H, Ar). Anal. ($C_{18}H_{17}ClO_3$): C, H.

6.1.5. 3-(6-Chlorohexyloxy)xanthen-9-one (1d). Using the previous procedure and starting from 2.12 g (0.01 mol) of 3-hydroxyxanthen-9-one and 3.99 g (0.02 mol) of 1-bromo-6-chloroexane, 2.64 g (80%) of **1d** were obtained: mp 96–97 °C (EtOH); ^{1}H NMR δ 1.45–1.55 (m, 4H), 1.80–1.90 (m, 4H), 3.55 (t, 2H), 4.05 (t, 2H), 6.85–8.35 (m, 7H, Ar). Anal. ($C_{19}H_{19}ClO_3$): C, H.

6.1.6. 3-(7-Bromoheptyloxy)xanthen-9-one (1e). Using the previous procedure and starting from 2.12 g (0.01 mol) of **32** and 5.16 g (0.02 mol) of 1,7-dibromoheptane, 3.1 g (80%) of **1e** were obtained: mp 90–93 °C; ^{1}H NMR δ 1.45–1.55 (m, 6H), 1.80–1.90 (m, 4H), 3.45 (t, 2H), 4.10 (t, 2H), 6.85-8.35 (m, 7H, Ar). Anal. ($C_{20}H_{21}BrO_3$): C, H.

6.1.7. 3-(8-Bromooctyloxy)xanthen-9-one (1f). Using the previous procedure and starting from 2.12 g (0.01 mol) of 3-hydroxyxanthen-9-one and 5.44 g (0.02 mol) of 1,8-dibromooctane, 2.8 g (70%) of **1f** were obtained: mp 138–141 °C; ^{1}H NMR δ 1.45–1.55 (m, 8H), 1.80–1.90 (m, 4H), 3.45 (t, 2H), 4.10 (t, 2H), 6.85–8.35 (m, 7H, Ar). Anal. ($C_{21}H_{23}BrO_3$): C, H.

6.1.8. 3-(9-Bromononyloxy)xanthen-9-one (1g). Using the previous procedure and starting from 2.12 g (0.01 mol) of 3-hydroxyxanthen-9-one and 5.72 g (0.02 mol) of 1,9-dibromononane, 3.3 g (80%) of **1 g** were obtained: mp 100–102 °C; ^{1}H NMR δ 1.35–1.55 (m, 10H), 1.80–1.90 (m, 4H), 3.45 (t, 2H), 4.10 (t, 2H), 6.85–8.35 (m, 7H, Ar). Anal. ($C_{22}H_{25}BrO_3$): C, H.

6.1.9. 3-(10-Bromodecyloxy)xanthen-9-one (1h). Using the previous procedure and starting from 2.12 g (0.01 mol) of 3-hydroxyxanthen-9-one and 6.00 g (0.02 mol) of 1,10-dibromodecane, 3.01 g (70%) of **1h** were obtained: mp 124–126 °C; ^{1}H NMR δ 1.35–1.45 (m, 12H), 1.80–1.90 (m, 4H), 3.45 (t, 2H), 4.10 (t, 2H), 6.85–8.35 (m, 7H, Ar). Anal. ($C_{23}H_{27}BrO_3$): C, H.

6.1.10. 3-(11-Bromoundecyloxy)xanthen-9-one (1i). Using the previous procedure and starting from 2.12 g (0.01 mol) of 3-hydroxyxanthen-9-one and 6.28 g (0.02 mol) of 1,11-dibromoundecane, 3.1 g (70%) of **1i** were obtained: mp 84–86 °C; ^{1}H NMR δ 1.30–1.50 (m, 14H), 1.80–1.90 (m, 4H), 3.45 (t, 2H), 4.10 (t, 2H), 6.85–8.35 (m, 7H, Ar). Anal. ($C_{24}H_{29}BrO_3$): C, H.

6.1.11. 3-(12-Bromododecyloxy)xanthen-9-one (1j). Using the previous procedure and starting from 2.12 g (0.01 mol) of 3-hydroxyxanthen-9-one and 6.56 g (0.02 mol) of 1,12-dibromododecane, 3.2 g (70%) of **1j** were obtained: mp 80–81 °C; ^{1}H NMR δ 1.25–1.50 (m, 16H), 1.80–1.90 (m, 4H), 3.45 (t, 2H), 4.10 (t, 2H), 6.85–8.35 (m, 7H, Ar). Anal. ($C_{25}H_{31}BrO_3$): C, H.

6.1.12. 3-(7-Bromoheptyloxy)-6-methoxyxanthen-9-one (1k). Using the previous procedure and starting from 2.43 g (0.01 mol) of 3-hydroxy-6-methoxyxanthen-9-one **33** and 5.16 g (0.02 mol) of 1,7-dibromoheptane, 3.1 g (80%) of **1k** were obtained: mp 148–150 °C; ^{1}H NMR δ 1.45–1.55 (m, 6H), 1.80–1.90 (m, 4H), 3.45 (t, 2H), 3.95 (s, 3H), 4.10 (t, 2H), 6.85–8.25 (m, 6H, Ar). Anal. ($C_{21}H_{23}BrO_4$): C, H.

6.1.13. 3-(7-Bromoheptyloxy)-6,7-dimethoxyxanthen-9-one (1l). Using the previous procedure and starting from 2.72 g (0.01 mol) of 3-hydroxy-6,7-dimethoxyxanthen-9-one **32** and 5.16 g (0.02 mol) of 1,7-dibromoheptane, 3.5 g (80%) of **1l** were obtained: mp 158–160 °C; ^{1}H NMR δ 1.45–1.55 (m, 6H), 1.80–1.90 (m, 4H), 3.45 (t, 2H), 3.90 (s, 3H), 3.95 (s, 3H), 4.10 (t, 2H), 6.85–8.30 (m, 5H, Ar). Anal. ($C_{22}H_{25}BrO_5$): C, H.

6.1.14. 3-(3-Iodopropoxy)xanthen-9-one (2a). A mixture of 2.88 g (0.01 mol) of **1a** and 1.5 g (0.01 mol) of NaI in 30 mL of methylethylketone was refluxed for 4 h. After cooling, the separated solid was collected by filtration: 2.66 g (70%) of **2a** were obtained: mp 119–121 °C;

^{1}H NMR δ 2.30–2.40 (m, 2H), 3.45 (t, 2H), 4.30 (t, 2H), 6.90-8.40 (m, 7H, Ar). Anal. ($C_{16}H_{13}IO_3$): C, H.

6.1.15. 3-(4-Iodobutoxy)xanthen-9-one (2b). Using the previous procedure and starting from 3.02 g (0.01 mol) of **1b**, 2.8 g (70%) of **2b** were obtained: mp 113–115 °C; ^{1}H NMR δ 2.01–2.09 (m, 4H), 3.30 (t, 2H), 4.15 (t, 2H), 6.85–8.40 (m, 7H, Ar). Anal. ($C_{17}H_{15}IO_3$): C, H.

6.1.16. 3-(5-Iodopentyloxy)xanthen-9-one (2c). Using the previous procedure and starting from 3.16 g (0.01 mol) of **1c**, 2.9 g (70%) of **2c** were obtained: mp 129–131 °C; ^{1}H NMR δ 1.65–1.75 (m, 2H), 1.80–1.95 (m, 4H), 3.30 (t, 2H), 4.15 (t, 2H), 6.85–8.40 (m, 7H, Ar). Anal. ($C_{18}H_{17}IO_3$): C, H.

6.1.17. 3-(6-Iodohexyloxy)xanthen-9-one (2d). Using the previous procedure and starting from 3.3 g (0.01 mol) of **1d**, 3.8 g (90%) of **2d** were obtained: mp 75–78°C; ^{1}H NMR δ 1.40–1.55 (m, 4H), 1.80–1.90 (m, 4H), 3.20 (t, 2H), 4.05 (t, 2H), 6.80–8.35 (m, 7H, Ar). Anal. ($C_{19}H_{19}IO_3$): C, H.

6.1.18. General method for the preparation of compounds (3–27). A solution of the selected ω-haloalkoxyxanthen-9-one (5 mmol) and selected amine (10 mmol) in toluene (100 mL) was refluxed for 30 h. After cooling, the toluene was evaporated and the residue was purified by flash chromatography (toluene/acetone 3:2).

6.2. ^{1}H NMR, mass spectrum data and elemental analysis for compounds 3–27

6.2.1. 3-[3-(Benzylmethylamino)propoxy]xanthen-9-one (3). δ 2.00–2.10 (m, 2H, alkyl), 2.28 (s, 3H, N*CH*$_3$), 2.61 (t, 2H, *CH*$_2$N), 3.56 (s, 2H, *CH*$_2$Ar), 4.16 (t, 2H, O*CH*$_2$), 6.89–8.48 (m, 12H, *Ar*). MS: *m*/*z* (rel.abundance): 373 (M^+, 9.55), 134 (100), 91 (91.92). Anal. ($C_{24}H_{23}NO_3$): Calcd C, 77.19; H, 6.21; N, 3.75. Found: C, 77.08; H, 6.19; N, 3.71.

6.2.2. 3-[4-(Benzylmethylamino)butoxy]xanthen-9-one (4). δ 1.60–1.92 (m, 4H, alkyl), 2.18 (s, 3H, N*CH*$_3$), 2.44 (t, 2H, *CH*$_2$N), 3.45 (s, 2H, *CH*$_2$Ar), 3.98 (t, 2H, O*CH*$_2$), 6.73–8.29 (m, 12H, *Ar*). MS: *m*/*z* (rel.abundance): 378 (M^+, 14.10), 134 (100), 91 (55.52). Anal. ($C_{25}H_{25}NO_3$): Calcd C, 77.49; H, 6.50; N, 3.61. Found: C, 77.41; H, 6.49; N, 3.69.

6.2.3. 3-[5-(Benzylmethylamino)pentyloxy]xanthen-9-one (5). δ 1.73–1.90 (m, 4H, alkyl), 1.91–1.97 (m, 2H, alkyl), 2.12 (s, 3H, N*CH*$_3$), 2.22 (t, 2H, *CH*$_2$N), 3.50 (s, 2H, *CH*$_2$Ar), 4.10 (t, 2H, O*CH*$_2$), 6.82–8.27 (m, 12H, *Ar*). MS: *m*/*z* (rel.abundance): 401 (M^+, 10.00), 134 (100), 91 (82.23). Anal. ($C_{26}H_{27}NO_3$): Calcd C, 77.78; H, 6.78; N, 3.49. Found: C, 77.72; H, 6.74; N, 3.45.

6.2.4. 3-[6-(Benzylmethylamino)hexyloxy]xanthen-9-one (6). δ 1.42–1.88 (m, 6H, alkyl), 1.78–1.92 (m, 2H, alkyl), 2.22 (s, 3H, N*CH*$_3$), 2.43 (t, 2H, *CH*$_2$N), 3.52 (s, 2H, *CH*$_2$Ar), 4.12 (t, 2H, O*CH*$_2$), 6.86–8.38 (m, 12H, *Ar*). MS: *m*/*z* (rel.abundance): 415 (M^+, 13.00), 134 (100), 91 (68.18). Anal. ($C_{27}H_{29}NO_3$): Calcd C, 78.04; H, 7.03; N, 3.37. Found: C, 77.99; H, 7.01; N, 3.34.

6.2.5. 3-[7-(Benzylmethylamino)-heptyloxy]xanthen-9-one (7). δ 1.32–1.61 (m, 8H, alkyl), 1.77–1.91 (m, 2H, alkyl), 2.24 (s, 3H, N*CH*$_3$), 2.45 (t, 2H, *CH*$_2$N), 3.50 (s, 2H, *CH*$_2$Ar), 4.11 (t, 2H, O*CH*$_2$), 6.80–8.33 (m, 12H, *Ar*). MS: *m*/*z* (rel.abundance): 429 (M^+, 39.00), 134 (100), 91 (55.50). Anal. ($C_{28}H_{31}NO_3$): Calcd C, 78.29; H, 7.27; N, 3.26. Found: C, 78.31; H, 7.04; N, 3.25.

6.2.6. 3-[8-(Benzylmethylamino)octyloxy]xanthen-9-one (8). δ 1.18–1.50 (m, 8H, alkyl), 1.70–1.81 (m, 4H, alkyl), 2.50 (s, 3H, N*CH*$_3$), 2.62 (t, 2H, *CH*$_2$N), 4.10–4.30 (m, 4H, *CH*$_2$Ar and O*CH*$_2$), 6.98–8.22 (m, 12H, *Ar*). MS: *m*/*z* (rel.abundance): 443 (M^+, 6.13), 134 (64.70), 91 (100). Anal. ($C_{29}H_{33}NO_3$): Calcd C, 78.52; H, 7.50; N, 3.16. Found: C, 78.49; H, 7.51; N, 3.14.

6.2.7. 3-[9-(Benzylmethylamino)nonyloxy]xanthen-9-one (9). δ 1.30–1.70 (m, 10H, alkyl), 1.79–1.94 (m, 4H, alkyl), 2.70 (s, 3H, N*CH*$_3$), 2.80–3.15 (m, 2H, *CH*$_2$N), 4.07–4.25 (m, 4H, *CH*$_2$Ar and O*CH*$_2$), 6.86–8.33 (m, 12H, *Ar*). MS: *m*/*z* (rel.abundance): 457 (M^+, 30.30), 442 (14.2), 366 (100). Anal. ($C_{30}H_{35}NO_3$): Calcd C, 78.74; H, 7.71; N, 3.06. Found: C, 78.69; H, 7.70; N, 3.04.

6.2.8. 3-[10-(Benzylmethylamino)decyloxy]xanthen-9-one (10). δ 1.22–1.58 (m, 14H, alkyl), 1.77–1.95 (m, 2H, alkyl), 2.20 (s, 3H, N*CH*$_3$), 2.27 (t, 2H, *CH*$_2$N), 3.50 (s, 2H, *CH*$_2$Ar), 4.06 (t, 2H, O*CH*$_2$), 6.94–8.27 (m, 12H, *Ar*). MS: *m*/*z* (rel.abundance): 471 (M^+, 31.50), 380 (100). Anal. ($C_{31}H_{37}NO_3$): Calcd C, 78.95; H, 7.91; N, 2.97. Found: C, 78.99; H, 7.90; N, 2.94.

6.2.9. 3-[11-(Benzylmethylamino)undecyloxy]xanthen-9-one (11). δ 1.22–1.72 (m, 16H, alkyl), 1.80–1.97 (m, 2H, alkyl), 2.21 (s, 3H, N*CH*$_3$), 2.35 (t, 2H, *CH*$_2$N), 3.46 (s, 2H, *CH*$_2$Ar), 4.06 (t, 2H, O*CH*$_2$), 6.79–8.29 (m, 12H, *Ar*). MS: *m*/*z* (rel.abundance): 485 (M^+, 28.40), 395 (100). Anal. ($C_{32}H_{39}NO_3$): Calcd C, 79.14; H, 8.09; N, 2.88. Found: C, 79.19; H, 8.10; N, 2.84.

6.2.10. 3-[12-(Benzylmethylamino)dodecyloxy]xanthen-9-one (12). δ 1.30–1.61(m, 16H, alkyl), 1.81–1.99 (m, 2H, alkyl), 2.20 (s, 3H, N*CH*$_3$), 2.37 (t, 2H, *CH*$_2$N), 3.48 (s, 2H, *CH*$_2$Ar), 4.10 (t, 2H, O*CH*$_2$), 6.74–8.37 (m, 12H, *Ar*). MS: *m*/*z* (rel.abundance): 499 (M^+, 6.50), 409 (100), 407 (7.7). Anal. ($C_{33}H_{41}NO_3$): Calcd C, 79.32; H, 8.27; N, 2.80. Found: C, 79.39; H, 8.30; N, 2.84.

6.2.11. 3-[7-(Benzylmethylamino)heptyloxy]-6-methoxyxanthen-9-one (13). δ 1.3–1.6 (m, 8H, alkyl), 1.77–1.91 (m, 2H, alkyl), 2.20 (s, 3H, N*CH*$_3$), 2.41 (t, 2H, *CH*$_2$N), 3.44 (s, 2H, *CH*$_2$Ar), 3.95 (s, 3H, O*CH*$_3$), 4.09 (t, 2H, O*CH*$_2$), 6.82–8.25 (m, 11H, *Ar*). MS: *m*/*z* (rel.abundance): 459 (M^+, 37.00), 409 (17.0), 368 (100). Anal. ($C_{29}H_{33}NO_4$): Calcd C, 75.79; H, 7.24; N, 3.05. Found: C, 75.72; H, 7.21; N, 3.04.

6.2.12. 6-[7-(Benzylmethylamino)heptyloxy]-2,3-dimethoxyxanthen-9-one (14). δ 1.3–1.6 (m, 8H, alkyl), 1.77–1.91 (m, 2H, alkyl), 2.20 (s, 3H, NCH_3), 2.39 (t, 2H, CH_2N), 3.44 (s, 2H, CH_2Ar), 3.95 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 4.09 (t, 2H, OCH_2), 6.82–8.25 (m, 10H, *Ar*). MS: *m*/*z* (rel.abundance): 489 (M^+, 100). Anal. ($C_{30}H_{35}NO_5$): Calcd C, 73.59; H, 7.21; N, 2.86. Found: C, 73.58; H, 7.20; N, 2.84.

6.2.13. 3-{3-[(2-Methoxybenzyl)methylamino]propoxy}-xanthen-9-one (15). δ 2.00–2.10 (m, 2H, alkyl), 2.30 (s, 3H, NCH_3), 2.59 (t, 2H, CH_2N), 3.54 (s, 2H, CH_2Ar), 3.89 (s, 3H, OCH_3), 4.12 (t, 2H, OCH_2), 6.75–8.33 (m, 11H, *Ar*). MS: *m*/*z* (rel.abundance): 403 (M^+, 70.00), 164 (60.50), 121 (100). Anal. ($C_{25}H_{25}$-NO_4): Calcd C, 74.42; H, 6.25; N, 3.47. Found: C, 74.49; H, 6.26; N, 3.44.

6.2.14. 3-{7-[(2-Methoxybenzyl)methylamino]heptyloxy}-xanthen-9-one (16). δ 1.32–1.61 (m, 8H, alkyl), 1.77–1.91 (m, 2H, alkyl), 2.20 (s, 3H, NCH_3), 2.41 (t, 2H, CH_2N), 3.44 (s, 2H, CH_2Ar), 3.79 (s, 3H, OCH_3), 4.09 (t, 2H, OCH_2), 6.75–8.38 (m, 11H, *Ar*). MS: *m*/*z* (rel.abundance): 459 (M^+, 9.00), 121 (100), 164 (45.50). Anal. ($C_{29}H_{33}NO_4$): Calcd C, 75.79; H, 7.24; N, 3.05. Found: C, 75.79; H, 7.23; N, 3.04.

6.2.15. 3-{7-[(3-Methoxybenzyl)methylamino]heptyloxy}-xanthen-9- one (17). δ 1.30–1.60 (m, 8H, alkyl), 1.75–1.90 (m, 2H, alkyl), 2.18 (s, 3H, NCH_3), 2.37 (t, 2H, CH_2N), 3.45 (s, 2H, CH_2Ar), 3.80 (s, 3H, OCH_3), 4.08 (t, 2H, OCH_2), 6.73–8.36 (m, 11H, *Ar*). MS: *m*/*z* (rel.abundance): 459 (M^+, 11.22), 121 (100), 164 (60.79). Anal. ($C_{29}H_{33}NO_4$): Calcd C, 75.79; H, 7.24; N, 3.05. Found: C, 75.75; H, 7.22; N, 3.04.

6.2.16. 3-{7-[(4-Methoxybenzyl)methylamino]heptyloxy}-xanthen-9-one (18). δ 1.18–1.50 (m, 6H, alkyl), 1.62–1.82 (m, 4H, alkyl), 2.60 (s, 3H, NCH_3), 2.80–3.13 (m, 2H, CH_2N), 3.80 (s, 3H, OCH_3), 4.10–4.23 (m, 4H, OCH_2 and CH_2Ar), 6.96–8.22 (m, 11H, *Ar*). MS: *m*/*z* (rel.abundance): 459 (M^+, 8.00), 121 (100), 164 (19.78). Anal. ($C_{29}H_{33}NO_4$): Calcd C, 75.79; H, 7.24; N, 3.05. Found: C, 75.77; H, 7.23; N, 3.03.

6.2.17. 3-{7-[(2-Chlorobenzyl)methylamino]-heptyloxy}-xanthen-9-one (19). δ 1.26-1.63 (m, 8H, alkyl), 1.73-1.90 (m, 2H, alkyl), 2.21 (s, 3H, NCH_3), 2.45 (t, 2H, CH_2N), 3.57 (s, 2H, CH_2Ar), 4.04 (t, 2H, OCH_2), 6.88-8.33 (m, 11H, *Ar*). MS: *m*/*z* (rel.abundance): 463 (M^+, 100), 124 (100), 168 (75.90). Anal. ($C_{28}H_{30}ClNO_3$): Calcd C, 72.48; H, 6.52; N, 3.02. Found: C, 72.49; H, 6.53; N, 3.04.

6.2.18. 3-{7-[(3-Chlorobenzyl)methylamino]-heptyloxy}-xanthen-9-one (20). δ 1.30–1.60 (m, 8H, alkyl), 1.78–1.91 (m, 2H, alkyl), 2.18 (s, 3H, NCH_3), 2.37 (t, 2H, CH_2N), 3.44 (s, 2H, CH_2Ar), 4.07 (t, 2H, OCH_2), 6.85–8.38 (m, 11H, *Ar*). MS: *m*/*z* (rel.abundance): 463 (M^+, 7.85), 167 (100), 124 (75.96). Anal. ($C_{28}H_{30}$-$ClNO_3$): Calcd C, 72.48; H, 6.52; N, 3.02. Found: C, 72.46; H, 6.51; N, 3.01.

6.2.19. 3-{7-[(4-Chlorobenzyl)methylamino]-heptyloxy}-xanthen-9-one (21). δ 1.31–1.56 (m, 8H, alkyl), 1.78-1.91 (m, 2H, alkyl), 2.19 (s, 3H, NCH_3), 2.35 (t, 2H, CH_2N), 3.45 (s, 2H, CH_2Ar), 4.09 (t, 2H, OCH_2), 6.88–8.38 (m, 11H, *Ar*). MS: *m*/*z* (rel.abundance): 463 (M^+, 25.00), 428 (100), 281 (29.20). Anal. ($C_{28}H_{30}$-$ClNO_3$): Calcd C, 72.48; H, 6.52; N, 3.02. Found: C, 72.47; H, 6.53; N, 3.04.

6.2.20. 3-{7-[Methyl-(2-Methylbenzyl)amino]-heptyloxy}-xanthen-9-one (22). δ 1.28–1.64 (m, 8H, alkyl), 1.78-1.92 (m, 2H, alkyl), 2.18 (s, 3H, NCH_3), 2.30–2.43 (m, 5H, OCH_2 and ArCH_3), 3.45 (s, 2H, CH_2Ar), 4.07 (t, 2H, OCH_2), 6.82–8.36 (m, 11H, *Ar*). MS: *m*/*z* (rel.abundance): 443 (M^+, 7.55), 105 (100), 148 (54.64). Anal. ($C_{29}H_{33}NO_3$): Calcd C, 78.52; H, 7.50; N, 3.16. Found: C, 78.50; H, 7.52; N, 3.14.

6.2.21. 3-{7-[Methyl-(2-nitrobenzyl)amino]heptyloxy}-xanthen-9-one (23). δ 1.25–1.58 (m, 8H, alkyl), 1.72–1.90 (m, 2H, alkyl), 2.15 (s, 3H, NCH_3), 2.39 (t, 2H, CH_2N), 3.38 (s, 2H, CH_2Ar), 4.10 (t, 2H, OCH_2), 6.87–8.37 (m, 11H, *Ar*). MS: *m*/*z* (rel.abundance): 474 (M^+, 23.00), 427 (100), 457 (47.50). Anal. ($C_{28}H_{30}$-N_2O_5): Calcd C, 70.87; H, 6.37; N, 5.90. Found: C, 70.89; H, 6.35; N, 5.94.

6.2.22. 3-{7-[(2,3-Dimethoxybenzyl)methylamino]heptyloxy}xanthen-9-one (24). δ 1.80–2.02 (m, 6H, alkyl), 2.24–2.38 (m, 6H, alkyl), 3.12 (s, 3H, NCH_3), 3.22–3.30 (m, 2H, CH_2N), 4.32–4.38 (m, 6H, OCH_3), 4.63–4.77 (m, 4H, OCH_2 and CH_2Ar), 7.43–8.75 (m, 10H, *Ar*). MS: *m*/*z* (rel.abundance): 489 (M^+, 8.31), 150 (100), 194 (80.21). Anal. ($C_{30}H_{35}NO_5$): Calcd C, 73.59; H, 7.21; N, 2.86. Found: C, 73.54; H, 7.23; N, 2.84.

6.2.23. 3-{7-[(2,5-Dimethoxybenzyl)methylamino]heptyloxy}xanthen-9-one (25). δ 1.31–1.57 (m, 8H, alkyl), 1.75-1.88 (m, 2H, alkyl), 2.21 (s, 3H, NCH_3), 2.40 (t, 2H, CH_2N), 3.46 (s, 2H, CH_2Ar), 3.70 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.16 (t, 2H, OCH_2), 6.68–8.35 (m, 10H, *Ar*). MS: *m*/*z* (rel.abundance): 489 (M^+, 15.31), 150 (100), 194 (60.21). Anal. ($C_{30}H_{35}NO_5$): Calcd C, 73.59; H, 7.21; N, 2.86. Found: C, 73.56; H, 7.22; N, 2.83.

6.2.24. 3-{7-[Methyl-(2,3,4-trimethoxybenzyl)amino]-heptyloxy}xanthen-9-one (26). δ 1.27–1.57 (m, 8H, alkyl), 1.68-1.84 (m, 2H, alkyl), 2.18 (s, 3H, NCH_3), 2.37 (t, 2H, CH_2N), 3.41 (s, 2H, CH_2Ar), 3.83 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.06 (t, 2H, OCH_2), 6.59–8.36 (m, 9H, *Ar*). MS: *m*/*z* (rel.abundance): 519 (M^+, 5.85), 180 (100), 182 (13.74). Anal. ($C_{31}H_{37}NO_6$): Calcd C, 71.65; H, 7.18; N, 2.70. Found: C, 71.64; H, 7.20; N, 2.71.

6.2.25. 3-{7-[Ethyl-(2-methoxybenzyl)amino]heptyloxy}-xanthen-9-one (27). δ 1.01–1.18 (m, 3H, CH_2CH_3), 1.32–1.61 (m, 8H, alkyl), 1.77–1.91 (m, 2H, alkyl), 2.20 (s, 3H, NCH_3), 2.41 (t, 2H, CH_2N), 2.61 (t, 2H, CH_2CH_3), 3.44 (s, 2H, CH_2Ar), 3.79 (s, 3H, OCH_3), 4.09 (t, 2H, OCH_2), 6.75–8.38 (m, 11H, *Ar*). MS: *m*/*z* (rel.abundance): 473 (M^+, 5.1), 445 (100). Anal.

($C_{30}H_{35}NO_4$): Calcd C, 76.08; H, 7.45; N, 2.96. Found: C, 76.04; H, 7.43; N, 2.94.

6.2.26. 2-(3-Chlorophenoxy)-4,5-dimethoxybenzoic acid (29). A mixture of 7.4 g (0.034 mol) of 2-chloro-4,5-dimethoxybenzoic acid, 4.4 g (0.034 mol) of 3-chlorophenol, 8 g of K_2CO_3, 0.5 g of Cu and 0.5 g of CuI in 80 mL of nitrobenzene was heated at 170–180 °C for 8 h. The solvent was steam distilled and the residue was filtered and acidified with HCl. The separated solid was filtered, washed with water and suspended in $NaHCO_3$ saturated solution. The suspension obtained was filtered and acidified with HCl. The solid was collected by filtration, washed with water and dried, obtaining 4.22 g (41%) of **29**: mp 170–173 °C (toluene); ^{1}H NMR δ 3.80 (s, 3H), 3.92 (s, 3H), 6.80–7.60 (m, 6H, Ar). Anal. ($C_{15}H_{13}ClO_5$): C, H.

6.2.27. 3-Chloro-6,7-dimethoxyxanthen-9-one (30). 4.20 g of **29** was added portionwise to 420 ml of 85% H_3PO_4 and 42 g of P_2O_5 and the reaction mixture was then heated at 110 °C under stirring for 4 h, cooled and poured into ice. After filtration, the product was dried and crystallized from toluene, affording 3.7 g (93%) of **30**: mp 238–240 °C; ^{1}H NMR δ 3.90 (s, 3H), 3.95 (s, 3H), 6.80–8.20 (m, 5H, Ar). Anal. ($C_{15}H_{11}ClO_4$): C, H.

6.2.28. 3-Benzyloxy-6,7-dimethoxyxanthen-9-one (31). A solution of 3.62 g (0.01 mol) of **30** in 50 mL of benzyl alcohol was added to a solution of KOH (5.60 g, 0.1 mol) in 20 mL of benzyl alcohol. 36,6 g of tetrabutylammonium bromide were then added and the mixture was heated to 75 °C for 6h under stirring. The benzyl alcohol was evaporated, the residue dissolved in HCl 50% and extracted with CH_2Cl_2. The organic phase was washed with HCl 50%, then with water, dried and evaporated. The residue was then purified by flash chromatography (toluene/acetone 3:2) to give 2.4 g (66%) of **31**: mp 186–187 °C; ^{1}H NMR δ 3.90 (s, 3H), 3.95 (s, 3H), 4.05 (s, 2H), 6.70-8.20 (m, 10H, Ar). Anal. ($C_{22}H_{18}O_5$): C, H.

6.2.29. 3-Hydroxy-6,7-dimethoxyxanthen-9-one (32). A solution of **31** (2.4 g, 6.6 mmol) in THF (100 mL) was hydrogenated at room temperature and pressure over Pd/C. The solution was filtered from catalyst and evaporated to dryness to give 1.5 g (83%) of **32**: mp > 300 °C; ^{1}H NMR δ 3.90 (s, 3H), 3.95 (s, 3H), 6.80–8.20 (m, 5H, Ar). Anal. ($C_{15}H_{12}O_5$): C, H.

6.2.30. 3-Hydroxy-6-methoxyxanthen-9-one (33). 5 g (0.022 mol) of 3,6-dihydroxyxanthen-9-one[13] were suspended in 250 mL of toluene, 5 g of *N*-benzyltriethylammonium chloride and 1.5 mL (0.022 mol) of methyl iodide and then 125 mL of 50% NaOH were added. The mixture was refluxed under stirring for 6 h and the phases were separated: the toluene phase gave after evaporation 1.4 g of 3,6-dimethoxyxanthen-9-one. The aqueous phase was acidified with HCl and the solid was filtered and dried. Crystallization from toluene afforded 5.11 g (95%) of 33: mp 268–270 °C; ^{1}H NMR δ 3.9 (s, 3H), 6.80–8.10 (m, 6H, Ar). Anal. ($C_{14}H_{10}O_4$): C, H.

6.3. The amine that were not commercially available were synthesised using the following procedure

6.3.1. *N*-Methyl-*N*-[(2-methoxyphenyl)methyl]imine. Methylamine solution (30%, 10 mL) was rapidly added to a solution of 2-methoxybenzaldehyde (10 g, 0.07 mol) in ethanol (30 mL) at about 40 °C. The mixture was allowed to stand at 0–5 °C for 2 h. The ethanol was evaporated to dryness. The residue was dissolved in CH_2Cl_2; the organic phase was washed with H_2O and evaporated to dryness to give the Schiff base (10.8 g, 100%) as an yellow oil; ^{1}H NMR δ 3.40–3.50 (m, 3H), 3.82 (s, 3H), 6.80–7.35 (m, 4H, Ar), 8.25 (s, 1H, CH).

6.3.2. *N*-Methyl-*N*-[(3-methoxyphenyl)methyl]imine. Using the previous procedure and starting from methylamine solution and 3-methoxybenzaldehyde the Schiff base (90%) was obtained as an oil; ^{1}H NMR δ 3.48–3.52 (m, 3H), 3.84 (s, 3H), 6.92–7.35 (m, 4H, Ar), 8.23 (s, 1H, CH).

6.3.3. *N*-Methyl-*N*-[(4-methoxyphenyl)methyl]imine. Using the previous procedure and starting from methylamine solution and 4-methoxybenzaldehyde the Schiff base (90%) was obtained as an oil; ^{1}H NMR δ 3.41–3.44 (m, 3H), 3.77 (s, 3H), 6.86–7.62 (m, 4H, Ar), 8.15 (s, 1H, CH).

6.3.4. *N*-Methyl-*N*-[(2-chlorophenyl)methyl]imine. Using the previous procedure and starting from methylamine solution and 2-chlorobenzaldehyde the Schiff base (90%) was obtained as an oil; ^{1}H NMR δ 3.48–3.52 (m, 3H), 6.74–8.37 (m, 4H, Ar), 8.67 (s, 1H, CH).

6.3.5. *N*-Methyl-*N*-[(3-chlorophenyl)methyl]imine. Using the previous procedure and starting from methylamine solution and 3-chlorobenzaldehyde the Schiff base (90%) was obtained as an oil; ^{1}H NMR δ 3.45–3.49 (m, 3H), 7.23–7.83 (m, 4H, Ar), 8.26 (s, 1H, CH).

6.3.6. *N*-Methyl-*N*-[(4-chlorophenyl)methyl]imine. Using the previous procedure and starting from methylamine solution and 4-chlorobenzaldehyde the Schiff base (90%) was obtained as an oil; ^{1}H NMR δ 3.48–3.52 (m, 3H), 7.25–7.85 (m, 4H, Ar), 8.25 (s, 1H, CH).

6.3.7. *N*-Methyl-*N*-[(2-methylphenyl)methyl]imine. Using the previous procedure and starting from methylamine solution and 2-methylbenzaldehyde the Schiff base (90%) was obtained as an oil; ^{1}H NMR δ 2.48–2.51 (m, 3H), 3.51-3.55 (s, 3H), 7.15–7.85 (m, 4H, Ar), 8.59 (s, 1H, CH).

6.3.8. *N*-Methyl-*N*-[(2-nitrophenyl)methyl]imine. Using the previous procedure and starting from methylamine solution and 2-nitrobenzaldehyde the Schiff base (90%) was obtained as an oil; ^{1}H NMR δ 3.58–3.62 (m, 3H), 7.52–8.05 (m, 4H, Ar), 8.69 (s, 1H, CH).

6.3.9. *N*-Methyl-*N*-[(2,3-dimethoxyphenyl)methyl]imine. Using the previous procedure and starting from methylamine solution and 2,3-dimethoxybenzaldehyde the Schiff base (90%) was obtained as an oil; ^{1}H NMR δ

2.41–2.45 (m, 3H), 3.82 (s, 3H), 3.90 (s, 3H), 6.80–7.40 (m, 3H, Ar), 8.23 (s, 1H, CH).

6.3.10. *N*-Methyl-*N*-[(2,5-dimethoxyphenyl)methyl]imine. Using the previous procedure and starting from methylamine solution and 2,5-dimethoxybenzaldehyde the Schiff base (90%) was obtained as an oil; ^{1}H NMR δ 2.41–2.45 (m, 3H), 3.83 (s, 3H), 3.87 (s, 3H), 6.70–6.93 (m, 3H, Ar), 8.20 (s, 1H, CH).

6.3.11. *N*-Methyl-*N*-[(2,3,4-trimethoxyphenyl)methyl]imine. Using the previous procedure and starting from methylamine solution and 2,3,4-trimethoxybenzaldehyde the Schiff base (90%) was obtained as an oil; ^{1}H NMR δ 2.38–2.42 (m, 3H), 3.85 (s, 3H), 3.88 (s, 3H), 3.91 (s, 3H), 6.58–7.35 (m, 2H, Ar), 8.18 (s, 1H, CH).

6.3.12. *N*-Ethyl-*N*-[(2-methoxyphenyl)methyl]imine. Using the previous procedure and starting from ethylamine solution (70%) and 2-methoxybenzaldehyde the Schiff base (90%) was obtained as an oil; ^{1}H NMR δ 1.01–1.18 (m, 3H), 2.61 (q, 2H), 3.82 (s, 3H), 6.8–7.35 (m, 4H, Ar), 8.25 (s, 1H, CH).

6.3.13. *N*-Methyl-*N*-[(2-methoxyphenyl)methyl]amine. Sodium borohydride (7 g, 0.165 mol) was added portionwise to a solution of the Schiff base (5 g, 3.3 mmol) in ethanol (80 mL) at 0–5 °C. The mixture was stirred for 20 h at room temperature and quenched with water. Ethanol was evaporated and the remaining aqueous solution was extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, and was evaporated to dryness to yield an oil (quantitative); ^{1}H NMR δ 2.45 (s, 3H), 3.65 (s, 2H), 3.80 (s, 3H), 5.10 (broad, 1H, NH), 6.60–7.20 (m, 4H, Ar). Anal. ($C_9H_{13}NO$): C, H, N.

6.3.14. *N*-Methyl-*N*-[(3-methoxyphenyl)methyl]amine. Using the previous procedure and starting from the Schiff base, the product (quantitative) was obtained as an oil; ^{1}H NMR δ 2.45 (s, 3H), 3.73 (s, 2H), 3.80 (s, 3H), 4.63 (broad, 1H, NH), 6.85–7.25 (m, 4H, Ar). Anal. ($C_9H_{13}NO$): C, H, N.

6.3.15. *N*-Methyl-*N*-[(4-methoxyphenyl)methylamine. Using the previous procedure and starting from the Schiff base, the product (quantitative) was obtained: mp 172–174 °C; ^{1}H NMR δ 2.43 (s, 3H), 3.67 (s, 2H), 3.79 (s, 3H), 5.28 (broad, 1H, NH), 6.80–7.22 (m, 4H, Ar). Anal. ($C_9H_{13}NO$): C, H, N.

6.3.16. *N*-Methyl-*N*-[(2-chlorophenyl)methyl]amine. Using the previous procedure and starting from the Schiff base, the product (quantitative) was obtained as an oil; ^{1}H NMR δ 2.44 (s, 3H), 3.81 (s, 2H), 7.18–7.38 (m, 4H, Ar). Anal. ($C_8H_{10}ClN$): C, H, N.

6.3.17. *N*-Methyl-*N*-[(3-chlorophenyl)methyl]amine. Using the previous procedure and starting from the Schiff base, the product (quantitative) was obtained as an oil; ^{1}H NMR δ 2.44 (s, 3H), 3.75 (s, 2H), 7.18–7.25 (m, 4H, Ar). Anal. ($C_8H_{10}ClN$): C, H, N.

6.3.18. *N*-Methyl-*N*-[(4-chlorophenyl)methyl]amine. Using the previous procedure and starting from the Schiff base, the product (quantitative) was obtained as an oil; ^{1}H NMR δ 2.45 (s, 3H), 3.74 (s, 2H), 7.20–7.45 (m, 4H, Ar). Anal. ($C_8H_{10}ClN$): C, H, N.

6.3.19. *N*-Methyl-*N*-[(2-methyphenyl)methyl]amine. Using the previous procedure and starting from the Schiff base, the product (quantitative) was obtained as an oil; ^{1}H NMR δ 2.45 (s, 3H), 2.58 (s, 3H), 3.75 (s, 2H), 4.67 (broad, 1H, NH), 7.13–7.33 (m, 4H, Ar). Anal. ($C_9H_{13}N$): C, H, N.

6.3.20. *N*-Methyl-*N*-[(2-nitrophenyl)methyl]amine. Using the previous procedure and starting from the Schiff base, the product (quantitative) was obtained as an oil; ^{1}H NMR δ 2.77 (s, 3H), 4.37 (s, 2H), 7.60–8.20 (m, 4H, Ar). Anal. ($C_8H_{10}N_2O_2$): C, H, N.

6.3.21. *N*-Methyl-*N*-[(2,3-dimethoxyphenyl)methyl]amine. Using the previous procedure and starting from the Schiff base, the product (quantitative) was obtained as an oil; ^{1}H NMR δ 2.42 (s, 3H), 3.78 (s, 2H), 3.80 (s, 3H), 3.88 (s, 3H), 6.80–7.10 (m, 3H, Ar). Anal. ($C_{10}H_{15}NO_2$): C, H, N.

6.3.22. *N*-Methyl-*N*-[(2,5-dimethoxyphenyl)methyl]amine. Using the previous procedure and starting from the Schiff base, the product (quantitative) was obtained as an oil; ^{1}H NMR δ 2.44 (s, 3H), 3.72 (s, 2H), 3.79 (s, 3H), 3.86 (s, 3H), 4.65 (broad, 1H, NH), 6.75–6.86 (m, 3H, Ar). Anal. ($C_{10}H_{15}NO_2$): C, H, N.

6.3.23. *N*-Methyl-*N*-[(2,3,4-trimethoxyphenyl)methyl]-amine. Using the previous procedure and starting from the Schiff base, the product (quantitative) was obtained as an oil; ^{1}H NMR δ 2.41 (s, 3H), 3.69 (s, 2H), 3.82 (s, 3H), 3.85 (s, 3H), 3.90 (s, 3H), 6.58–6.98 (m, 2H, Ar). Anal. ($C_{11}H_{17}NO_3$): C, H, N.

6.3.24. *N*-Ethyl-*N*-[(2-methoxyphenyl)methyl]amine. Using the previous procedure and starting from the Schiff base, the product (quantitative) was obtained as an oil; ^{1}H NMR δ 1.01–1.18 (m, 3H), 2.61 (q, 2H), 3.53 (m, 2H), 3.82 (s, 3H), 6.8-7.35 (m, 4H, Ar), 8.25 (broad, 1H, NH). Anal. ($C_{10}H_{15}NO$): C, H, N.

6.4. Inhibition of AChE and BuChE

The method of Ellman[20] was followed. Acetylthiocholine ATCh iodide solution (0.037 M) was prepared in water. 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB, Ellman's reagent) (0.01 M) was dissolved in pH 7.0 phosphate buffer, and 0.15% (w/v) $NaHCO_3$ was added. AChE solution was prepared dissolving 20 U in 5 mL of 0.2% aqueous gelatin by sonication at 35 °C. A dilution 1:1 with water was performed before use, in order to obtain the enzyme activity comprised between 0.130 and 0.100 AU/min. Stock solutions of the test compounds (0.5–1 mM) were prepared in ethanol, as well as the physostigmine reference stock solution. The assay solutions were prepared by diluting the stock solutions in water. Five different concentrations of each compound were

used in order to obtain inhibition of AChE activity comprised between 20 and 80%.

The assay solution consisted of a 0.1 M phosphate buffer pH 8.0, with the addition of 340 μM DTNB, 0.035 U/mL AChE derived from human erythrocytes (Sigma Chemical), and 550 μM ATCh iodide. The final assay volume was 1 mL. Test compounds were added to the assay solution and preincubated with the enzyme for 20 min, the addition of substrate following.

Initial rate assays were performed at 37 °C with a Jasco Uvidec-610 double beam Spectrophotometer: the rate of absorbance increase at 412 nm was followed for 5 min. Assays were performed with a blank containing all components except AChE, in order to account for non-enzymatic reaction. The reaction rates were compared and the percent inhibition due to the presence of test compounds was calculated. Each concentration was analyzed in triplicate. The percent inhibition of the enzyme activity due to the presence of increasing test compound concentration was calculated by the following expression: $100 - (v_i/v_0 \times 100)$ where v_i is the rate calculated in the presence of inhibitor and v_0 is the enzyme activity. Inhibition curves were obtained for each compound by plotting the % inhibition versus the logarithm of inhibitor concentration in the assay solution. The linear regression parameters were determined for each curve and the IC_{50} interpolated.

Inhibition of BuChE was measured as described above, substituting 0.035 U/mL of BuChE from human serum and 550 μM butyrylthiocholine (BTCh) for enzyme and substrate, respectively.

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