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Design, synthesis, and evaluation of indanone derivatives as acetylcholinesterase inhibitors and metal-chelating agents

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ABSTRACT

A series of novel indanone derivatives was designed, synthesised and evaluated as potential agents for Alzheimer's disease. Among them, compound **6a**, with a piperidine group linked to indone by a two-carbon spacer, exhibited the most potent inhibitor activity, with an IC_{50} of 0.0018 μM for AChE; the inhibitory activity of this compound was 14-fold more potent than that of donepezil. Furthermore, these compounds also exhibited good metal-chelating ability.

Alzheimer's disease (AD) is a neurodegenerative disorder associated with a selective loss of cholinergic neurons in the brain and decreasing levels of acetylcholine (ACh).[1] AD leads to a progressive decline in cognitive, executive and memory functions and eventually to incapacitating dementia before death.[2] The prevalence of AD increases dramatically with age and doubles for every five-year interval after the age of 65.[3] Although several diverse hallmarks such as β-amyloid (Aβ) deposits, τ-protein aggregation, oxidative stress, and low levels of acetylcholine (ACh) have been thought to play significant roles in the pathophysiology of AD, acetylcholinesterase inhibitors (AChEIs) are the only drugs used clinically for the treatment of this disease (Fig. 1).[4,5] According to the 'cholinergic hypothesis', AChEIs could increase the levels of ACh in AD patients through the inhibition of acetylcholinesterase (AChE) and, therefore, relieve some symptoms experienced by AD patients.[6]

Among the several AChEIs drugs approved by the US Food and Drug Administration (FDA) for the treatment of AD, donepezil (Fig. 1) has been demonstrated to improve the cognitive function of patients with mild to severe moderate AD.[7] This drug shows excellent tolerability without hepatotoxicity and is thought to be the most effective AChE inhibitor in the world at present.[8,9] Recently, a number of donepezil analogues have been synthesised and evaluated as AChE inhibitors.[10,11] However, from academic and practical standpoints, it is still desirable to develop more effective agents for the treatment of Alzheimer's disease. It is well known that the indanone donepezil moiety can interact with the PAS of AChE via aromatic stacking interactions, which contributes greatly to the high affinity and selectivity of donepezil for AChE.[12] Inspired by this fact, we combined the indanone moiety with an aromatic ring via an aldol condensation to enhance the affinity for the PAS. In addition, the introduction of the double bonds may endow this type of compound with other activities, such as metal-chelating activity. Based on this strategy, we designed a series of indanone derivatives with various amine groups linked to the 6-position of indanone by carbon spacers of different lengths. Herein, we report the design and synthesis of these molecules and the biological evaluation of the abilities of these molecules to serve a highly effective inhibitor of acetylcholinesterase and metal-chelating agents.

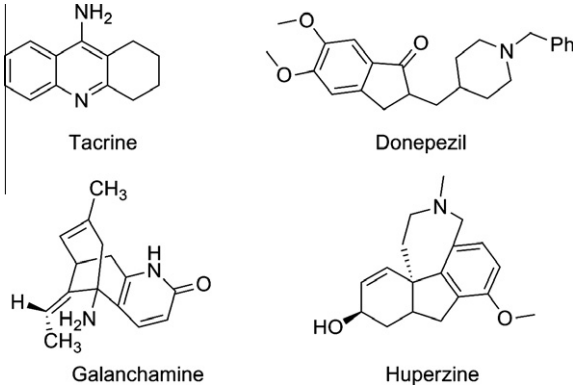

Figure 1. Stucture of reported AChE inhibitors.

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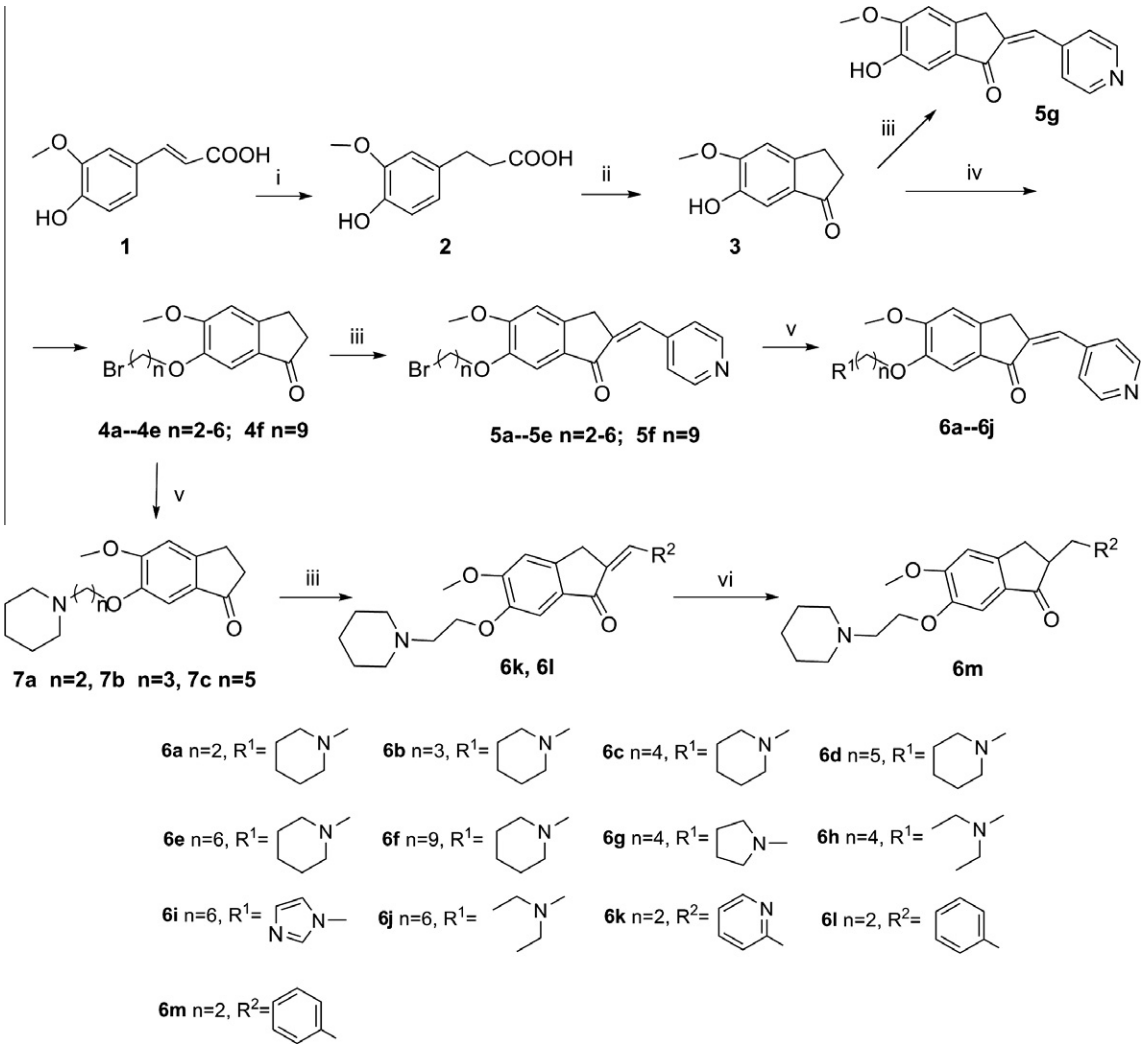

Scheme 1. Reagents and conditions: (i) Pd/C, H_2, MeOH, 40 °C; (ii) methanesulfonic acid, 120 °C; (iii) aldehyde, PTSA, toluene, 120 °C; (iv) $Br(CH_2)_nBr$, K_2CO_3, acetonitrile, 80 °C; (v) amines, acetonitrile, 80 °C; (vi) 10% Pd/C, H_2, CH_3OH.

The synthetic routes for 6-hydroxy-5-methoxy indanone **3**, pyridinylmethylene-indanone **5** and their derivatives **6** and **7** are shown in Scheme 1. First, ferulic acid **1** was hydrogenated in the presence of Pd/C and then cyclised at 120 °C, catalysed by methanesulfonic acid, to yield the indone **3** with 70% yield. The alkylation of **3** with different α,ω-dibromoalkanes compounds in acetonitrile provided **4a**–**4f** with 50–80% yields, and these compounds were then treated with different aldehydes in the presence of *p*-toluenesulphonic acid to afford compounds **5a**–**5f** in approximately 80% yields. Finally, products **6a**–**6g** were obtained by the reaction of **5a**–**5f** with commercially available secondary amines (e.g., diethylamine and piperidine) in 55–80% yields.[13] Compound **5g** was prepared by the condensation of **3** with pyridylaldehyde.[14] Compounds **7a**–**7c**, were prepared via a displacement reaction of **4a**–**4c** with piperidine.[15] Compounds **6k** and **6l**, which have the piperidine group linked to pyridin-2-ylmethylene-indanone and to pyridin-2-ylmethylene-indanone and benzylidene by two- to nine-carbon spacers, respectively, were obtained by the reaction of **7a** with corresponding aldehydes under the same reaction conditions as those used to prepare **5**. The reduction product (**6m**) from **6l** was prepared by reducing the double bond using 10% Pd/C as catalyst. All of the compounds were characterised using analytical and NMR spectroscopic data. The Z configuration at the double bond was justified by NMR experiments of compound **6a**, which the hydrogen at double bond and methylene display strong coupling in NOESY spectra (See Supplementary data).

The AChE-inhibitory effects of these pyridinylmethylene-indanone derivatives were determined by the spectroscopic method of Ellman et al.[16] using tacrine donepezil and galantamine as the reference standards. The BuChE inhibitory activity against equine serum BuChE was also determined using the same method. The IC_{50} values for AChE and BuChE inhibition are summarised in Table 1.

The lead compound 6-hydroxy-5-methoxy indanone **3** exhibited poor inhibitory activity for AChE and BuChE (Table 1, entry 1, IC_{50} value = 91.96 μM for AChE and 258.03 μM for BuChE). Compound **5g**, the condensation product of **3** with pyridylaldehyde, provided better results (Table 1, entry 2, IC_{50} value = 49.55 μM for AChE). It is exciting that all of the indanone **6** derivatives exhibited much better inhibitory activities against AChE than the lead compounds **3** and **5**. The most potent inhibitor was **6a,** in which the piperidine group was linked to indanone by two-carbon spacers; this compound had an IC_{50} value of 0.0018 μM (i.e., it was 47-fold more potent than tacrine and 14-fold more potent than donepezil). The carbon spacers seem to be very important for the inhibitory activities of these compounds. For example, compounds **6a–f**, which have the piperidine group linked with pyridinylmethylene-indanone by two to nine-carbon spacers, exhibited very

Table 1
In vitro inhibition IC_{50} (μM) and selectivity of indanone derivatives, tacrine and donepezil for AChE and BuChE

Entry	Compounds	*n*	IC_{50} (μM)		Selectivity for AChE[c]
			AChE ± SEM[a]	BuChE ± SEM[b]	
1	**3**	0	91.96 ± 0.867	258.03 ± 1.334	2.8
2	**5g**	0	49.55 ± 0.430	246.8 ± 1.506	4.9
3	**7a**	2	3.11 ± 0.362	136.5 ± 1.372	43.9
4	**7b**	3	0.916 ± 0.061	32.95 ± 0.148	35.9
5	**7c**	5	0.361 ± 0.084	114.97 ± 0.969	319.4
6	**6a**	2	0.0018 ± 0.00007	9.5 ± 0.241	5248.6
7	**6b**	3	0.062 ± 0.0001	42.72 ± 0.347	684.6
8	**6c**	4	0.081 ± 0.002	13.84 ± 0.467	170.2
9	**6d**	5	0.288 ± 0.004	9.15 ± 0.169	31.8
10	**6e**	6	0.232 ± 0.005	4.47 ± 0.005	19.3
11	**6f**	9	0.734 ± 0.053	1.24 ± 0.142	1.7
12	**6g**	4	0.156 ± 0.006	17.48 ± 0.236	112.2
13	**6h**	4	0.205 ± 0.009	28.92 ± 0.672	141.1
14	**6i**	6	2.87 ± 0.351	18.50 ± 0.832	6.4
15	**6j**	6	0.271 ± 0.002	13.36 ± 0.569	49.3
16	**6k**	2	0.0044 ± 0.00081	7.48 ± 0.432	1703.9
17	**6l**	2	0.0091 ± 0.00039	2.24 ± 0.332	246.4
18	**6m**	2	1.27 ± 0.12	9.20 ± 0.31	7.24
18	Tacrine		0.0858 ± 0.0049	0.013 ± 0.001	0.2
19	Donepezil		0.026 ± 0.0005	4.66 ± 0.503	179.5
20	Galantamine		0.623 ± 0.099	15.7 ± 0.787	25.3

SEM: standard error of the mean.
[a] 50% inhibitory concentration (means ± SEM of three experiments) of AChE from electric eel.
[b] 50% Inhibitory concentration (means ± SEM of three experiments) of BuChE from equine serum.
[c] Selectivity for AChE = IC_{50} (BuChE)/IC_{50} (AChE).

different inhibitory activities against AChE (**6a**, two-carbon spacer, IC_{50} value = 0.0018 μM; **6b**, *n* = 3, 0.062 μM; **6c**, *n* = 4, 0.081 μM; **6d**, *n* = 5, 0.288 μM; **6f**, *n* = 9, 0.734 μM). These results indicated that long carbon spacers generally had an unfavourable effect on the inhibitory activity of AchE, and it seemed that the shorter carbon spacer is, the better the inhibitory activity that it exhibits. In addition to the length of the carbon spacer, the substituted amino groups had important effects on the inhibitory activity. Compounds **6c**, **6g**, and **6h**, in which pyridinylmethylene-indanone is linked piperidinyl, pyrrolidinyl and *N,N*-diethyl amino groups by a four-carbon spacer, exhibited IC_{50} values of 0.081, 0.156 and 0.205 μM, respectively. Similar phenomenon were also found for compounds **6e**, **6i**, and **6j**, in which pyridinylmethylene-indanone is linked by a six-carbon spacer to pyrrolidinyl, imidazolyl and *N,N*-diethyl amino groups (**6e**: 0.232 μM, **6i**: 2.87 μM, **6j**: 0.271 μM). Compounds **7a** (3.11 μM), **7b** (0.916 μM) and **7c** (0.3599 μM) did not exhibit high levels of activity, a result that indicated that the pyridinylmethylene group in these compounds was very important for the inhibitory activity. In addition, compounds **6k** (0.0044 μM) and **6l** (0.0091 μM) both exhibited better inhibitory activities than donepezil (0.026 μM). However, the reduction product **6m** showed poor inhibitory activity (1.27 μM), which indicated that the double bond is crucial for the activity. The level of in vitro BuChE inhibition was also determined using the same method. Most of these compounds exhibited moderate inhibitory potency against BuChE, but there were no apparent patterns in the levels of inhibition.

To study the inhibitory mechanism for this class of AChEIs, compound **6a** was chosen for kinetic studies based on the in vitro inhibition experiments, and the results are shown in Figure 2. The intersection point of the Lineweaver–Burk reciprocal plot was in the third quadrant, which is indicative of mixed-type inhibition, which consists of both uncompetitive inhibition and non-competitive inhibition,[17] the latter of which involves binding to the allosteric site(s) of the enzyme.

The chelation abilities of compounds **6a, 6d, 6k 6l** and Clioquinol (CQ) towards biometals such as Cu^{2+}, Fe^{3+} and Zn^{2+} in ethanol were studied by UV–vis spectrometry.[18,19] The results in

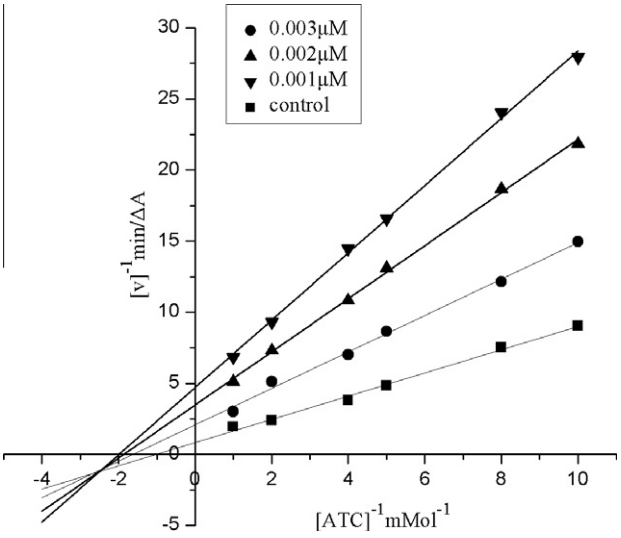

Figure 2. Steady state inhibition by compound **6a** of AChE hydrolysis of ACh; the plots show mixed type inhibition for compound **6a** on AChE.

Figure 3 showed that the electronic spectra of **6a** exhibited a blue shift (the peak at 360 nm shift to 352 nm) after the addition of Cu^{2+}, whereas **6k** and **6l** exhibited a red shift (from 315 nm to 318 nm and from 320 to 325 nm, respectively). These results indicated that all of the compounds could interact with the Cu^{2+} ion. Similar results were also obtained when Fe^{3+} was added; the peak in the electronic spectra of **6a** at 360 nm shifted to 346 nm, the peaks of **6k** and **6l** changed from 315 nm to 318 nm and from 320 to 326 nm, respectively. The electronic spectra of **6k** and **6d** exhibited no obvious change when Zn^{2+} was added, whereas that of **6a** exhibited a shift from 360 to 357 nm and that of **6l** exhibited a shift from 320 to 324 nm. To determine the key functional motifs responsible for the observed chelator properties, compounds **6d**, which has five carbon linkers between the nitrogen of the

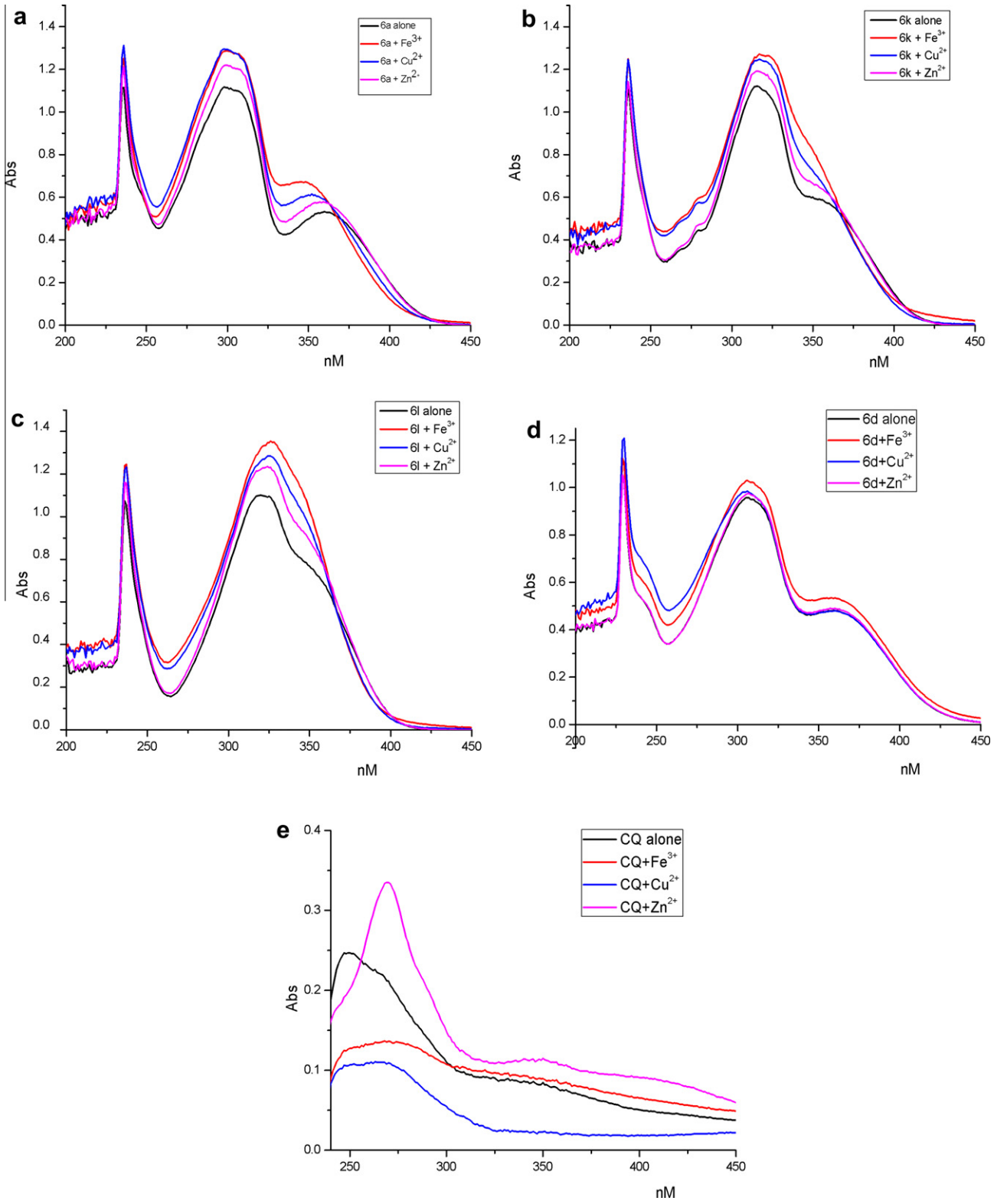

Figure 3. UV spectrum of compound **6a**, **6d**, **6k**, **6l** and CQ (50 μM) alone or at the presence of 50 μM Fe^{3+}, Cu^{2+} and Zn^{2+}.

piperidine ring and the oxygen atom at the phenyl ring, was also evaluated as a chelator with iron (Fig. 3). There is no significant shift was observed in the UV spectrum of **6d** after adding Fe^{3+} and Cu^{2+}. The relatively poor chelating ability of **6d** indicated that the nitrogen of the piperidine ring and oxygen of phenolic group in an appropriate distance was the main role of chelating metal.

In conclusion, a series of indanone derivatives was designed, synthesised, and biologically evaluated as inhibitors of AChE and BuChE. Most of these compounds were potent inhibitors of AChE, with IC_{50} values ranging from micromolar to sub-micromolar. In addition, all of these compounds showed moderate activity towards BuChE. Among them, compound **6a**, which contained a

piperidine group linked to indanone by a two-carbon spacer, exhibited the most potent inhibitor activity, with an IC_{50} value of 0.0018 μM against AChE. Compound **6a** was also a good metal chelator, indicating that this compound has strong potential to serve as a multifunctional drug candidate for the treatment of AD.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.04.029.

References and notes

1. Cardozo, M. G.; Kawai, T.; Iimura, Y.; Sugimoto, H.; Yamanishi, Y.; Hopfinger, A. J. *J. Med. Chem.* **1992**, *35*, 590.
2. scarpini, E.; Schelterns, P.; Feldman, H. *Lancet Neurol.* **2003**, *2*, 539.
3. Cuetos, F.; Herrera, E.; Ellis, A. W. *Neuropsychologia* **2010**, *48*, 3329.
4. Auld, D. S.; Kornecook, T. J.; Bastianetto, S.; Quirion, R. *Prog. Neurobiol.* **2002**, *68*, 209.
5. Butterfield, D. A.; Reed, T.; Newman, S. F.; Sultana, R. *Free Radical Biol. Med.* **2007**, *43*, 658.
6. Francis, P. T.; Palmer, A. M.; Snape, M.; Wilcock, G. K. *J. Neurol. Neurosurg. Psychiatry* **1999**, *66*, 137.
7. Colombres, M.; Sagal, J. P.; Inestrosa, N. C. *Curr. Pharm. Design.* **2004**, *10*, 3121.
8. Sugimoto, H.; Yamanishi, Y.; Iimura, Y.; Kawakami, Y. *Curr. Med. Chem.* **2000**, *7*, 303.
9. Van der Zee, E. A.; Platt, B.; Riedel, G. *Behav. Brain Res.* **2011**, *221*, 583.
10. Shen, Y. H.; Sheng, R.; Zhang, J.; He, Q. J.; Yang, B.; Hu, Y. Z. *Bioorg. Med. Chem.* **2008**, *16*, 7646.
11. Rizzo, S.; Bartolini, M.; Ceccarini, L.; Piazzi, L.; Gobbi, S.; Cavalli, A.; Recanatini, M.; Andrisano, V.; Rampa, A. *Bioorg. Med. Chem.* **2010**, *18*, 1749.
12. Kryger, G.; Silman, I.; Sussman, J. L. *Structure* **1999**, *7*, 297.
13. Belluti, F.; Rampa, A.; Piazzi, L.; Bisi, A.; Gobbi, S.; Bartolini, M.; Andrisano, V.; Cavalli, A.; Recanatini, M.; Valenti, P. *J. Med. Chem.* **2005**, *48*, 4444.
14. Sam, J.; Alwani, W.; Aparajithan, K. *J. Heterocycl. Chem.* **1965**, *2*, 366.
15. WO 2004080973.
16. Ellman, G. L.; Courtney, K. D.; Andres, B. J.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.
17. Saieed, P.; Reza, R. M.; Abbas, D.; Seyyedvali, R.; Aliasghar, H. *Chem. Pharm. Bull.* **2006**, *54*, 9.
18. Chen, S.-Y.; Chen, Y.; Li, Y.-P.; Chen, S.-H.; Tan, J.-H.; Ou, T.-M.; Gu, L.-Q.; Huang, Z.-S. *Bioorg. Med. Chem.* **2011**, *19*, 5596.
19. Bolognesi, M. L.; Cavalli, A.; Valgimigli, L.; Bartolini, M.; Rosini, M.; Andrisano, V.; Recanatini, M.; Melchiorre, C. *J. Med. Chem.* **2007**, *50*, 6446.