Chinese Chemical Letters 24 (2013) 609–612

Contents lists available at SciVerse ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Original article

Synthesis and molecular modeling study of Cu(II) complexes derived from 2-(diphenylmethylene)hydrazinecarbothioamide derivatives with cholinesterase inhibitory activities

Yi Chen Chan [a], Abdussalam Salhin Mohamed Ali [a,*], Melati Khairuddean [a], Kooi Yeong Khaw [b], Vikneswaran Murugaiyah [b], Alireza Basiri [b]

[a] School of Chemical Sciences, Universiti Sains Malaysia, Penang 11800, Malaysia
[b] Discipline of Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang 11800, Malaysia

ARTICLE INFO

Article history:
Received 29 January 2013
Received in revised form 22 March 2013
Accepted 1 April 2013
Available online 17 May 2013

Keywords:
Thiosemicarbazone
Cu(II) complex
Cholinesterase
Molecular modeling

ABSTRACT

Thiosemicarbazones of 2-amino-5-chlorobenzophenone and 3-aminobenzophenone (L^1–L^4) have been synthesized and their Cu(II) complexes (**1**–**4**) were afforded *via* coordination with cupric chloride. All these compounds were characterized by UV–vis and IR spectroscopy together with CHN elemental analysis. NMR spectroscopy was also applied to characterize the ligands. *In vitro* cholinesterase inhibitory assays for the complexes (**1**–**4**) showed IC_{50} values less than 10 μmol/L, with complex **1** exhibiting the most activity, IC_{50} = 2.15 μmol/L and 2.16 μmol/L for AChE and BuChE, respectively. Molecular modeling simulation revealed the binding interaction template for complex **1** with the AChE and BuChE receptors. In DPPH assay, the complexes also showed more *in vitro* antioxidant activities in comparison to their parent ligands.

1. Introduction

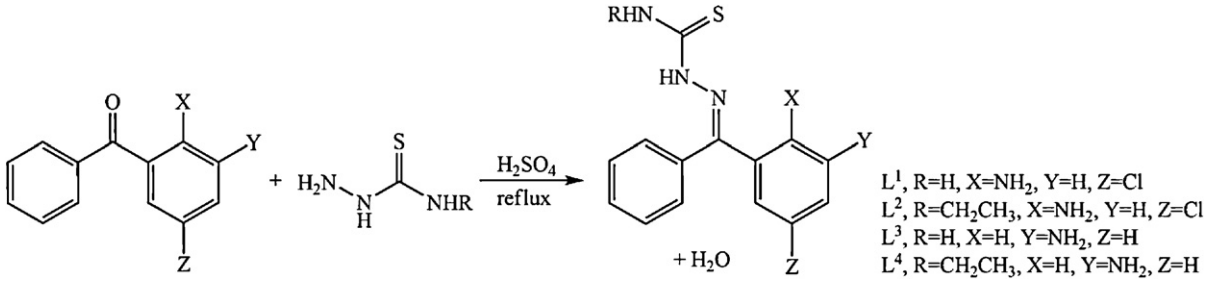
Alzheimer's disease (AD) is the most common cause of dementia among people over the age of 65 years. 25 million individuals are now estimated to suffer from this disease throughout the world [1]. Due to a lack of significant advances in the treatment of AD, the number of symptomatic AD cases is predicted to increase in the following years [2].

One of the leading therapeutic strategies in AD treatment is the use of cholinesterase inhibitors which were shown to ameliorate the cognitive function and activities of daily living of patients with AD [3]. According to the cholinergic hypothesis, the pathogenesis of AD has been linked to a deficit of acetylcholine (ACh), a key neurotransmitter in learning and memory [4]. In the brain, acetylcholinesterase (AChE) terminates the activity of ACh by hydrolyzing it into acetal and choline while butyrylcholinesterase (BuChE) plays a secondary role by regulating the ACh level. It was demonstrated that as AD progresses, BuChE activity increases while AChE activity remain unchanged or decreases [5]. Hence, either selective BuChE inhibition or dual inhibition of these enzymes constitutes a promising approach to increase the ACh level [6]. Four cholinesterase inhibitors (tacrine, donepezil, rivastigmine, and galantamine) are approved by the US Food and Drug Administration [7]. Nevertheless, the applicability of these drugs is limited due to their adverse side-effects [8]. Consequently, there is still a need to develop new drugs to antagonize AD.

Transition metal ions such as Cu(II), Zn(II) and Fe(III) accomplish a wide range of biological tasks in the brain due to their involvement in redox reactions. However, in AD patients, these metal ions were found to be involved in amyloid-β aggregation and oxidative stress which are the two major pathogenesis of Alzheimer's disease [9]. Accordingly, metal chelators which bind and deactivate transition metal ions in the brain are a feasible alternative to treat AD patients [10]. Our interest to apply metal chelator in AD therapy comes from the study by Ikram *et al.* [11] where metal based drugs are suggested as potential inhibitors of AChE and BuChE. Thiosemicarbazones are an important class of multidentate ligands which provide potential binding sites for a variety of transition metal ions [12]. Due to their remarkable biological properties, thiosemicarbazones and their metal complexes have been extensively studied. Cu(II) complexes of thiosemicrabazones exhibited more significant biological properties rather than the free ligands [13]. Thiosemicarbazones were also reported as potent cholinesterase inhibitor [14]. Herein, we report the synthesis and characterization of thiosemicarbazide

* Corresponding author.
E-mail address: abdussalam@usm.my (A.S. Mohamed Ali).

1001-8417/$ – see front matter © 2013 Abdussalam Salhin Mohamed Ali. Published by Elsevier B.V. on behalf of Chinese Chemical Society. All rights reserved.
http://dx.doi.org/10.1016/j.cclet.2013.04.013



Scheme 1. The reaction scheme for the synthesis of L^1–L^4.

substituted benzophenone derivatives and their respective Cu(II) complexes. These compounds were subsequently evaluated for the cholinesterase inhibitory activity and antioxidant activity. A molecular docking simulation was applied to reveal possible ligand–receptor interactions which give rise to high inhibitory activity in the most potent compound.

2. Experimental

2.1. Synthesis of thiosemicarbazones

To an ethanolic solution (20 mL) of thiosemicarbazide or 4-ethyl-3-thiosemicarbazide (3 mmol) was added the respective benzophenone (3 mmol) dissolved in the same solvent (5 mL). The mixture was refluxed for 7–8 h in the presence of 2–3 drops of concentrated H_2SO_4. This was then cooled to room temperature and slowly evaporated until sufficient solid was formed.

2.2. Synthesis of Cu(II) complexes

Into a hot ethanolic solution (10 mL) of the respective ligand (1 mmol) was added cupric chloride (1 mmol) dissolved in a minimum quantity of ethanol. The mixture was refluxed for 2–3 h. After keeping the mixture at room temperature for overnight, the colored solid was filtered from the solution, washed with small quantity of ethanol and then air dried.

Physical properties and analytical data, IR and electronic spectral data of the studied ligands and Cu(II) complexes are showed in supporting information (Table S1–3).

3. Results and discussion

Thiosemicarbazones (L^1–L^4) were afforded using acid-catalyzed condensation between 2-amino-5-chlorobenzophenone/3-amino-benzophenone and thiosemicarbazide analogs in 1:1 molar ratio (Scheme 1).

Cu(II) complexes (**1**–**4**) were obtained through the interaction of the ligands with $CuCl_2$ in 1:1 or 2:1 molar ratio in ethanolic solution. As a representative case, Fig. 1 displays the proposed structure of complex **1**. All of the synthesized compounds were obtained in satisfactory yields and had been properly characterized by ^{1}H NMR and ^{13}C NMR, FTIR, UV–vis, and elemental analysis (CHN) [15]. Since the Cu(II) complexes are paramagnetic, their ^{1}H NMR spectra could not be obtained.

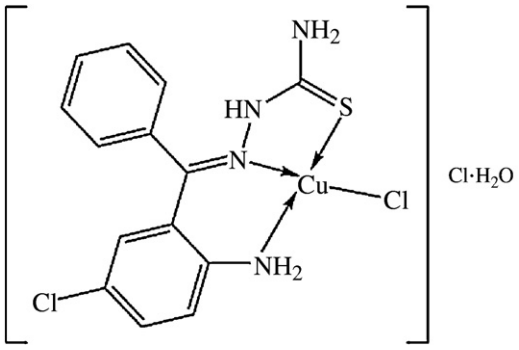


Fig. 1. The proposed structure of complex **1**.

The inhibitory activities of the test compounds against AChE and BuChE enzymes were evaluated *in vitro* by means of modified Ellman's method [16] (See supporting information). Results with physostigmine as the positive control drug show IC_{50} values of (0.18 ± 0.005) μmol/L and (0.29 ± 0.02) μmol/L for AChE and BuChE, respectively. Meanwhile, IC_{50} data of the synthesized compounds against both enzymes are summarized in Table 1. Due to the low percentage of inhibition (<50%) exhibited by L^1 at 50 μg/mL, its IC_{50} value was not further assessed. Cu(II) complexes generally show higher inhibitory potency against the cholinesterase enzymes as compared to the parent ligands, as previously reported by Ikram *et al.* [11].

Complex **1** shows identical selectivity for AChE and BuChE. Other compounds display more selectivity on AChE rather than BuChE, except for L^3 and complex **2**, which exhibit more selectivity toward BuChE. Recent studies had clarified that BuChE activity

Table 1
IC_{50} values of the cholinesterase inhibitory activities and radical-scavenging assays for the test samples.

Compound	AChE		BuChE		Selectivity		DPPH assay	
	IC_{50} (μmol/L)	S.D.[a]	IC_{50} (μmol/L)	S.D.[a]	AChE	BuChE	IC_{50} (μmol/L)	S.D.[a]
L^1	–	–	–	–	–	–	964.59	6.76
L^2	38.09	5.28	122.23	10.82	3.21	0.31	505.46	6.71
L^3	183.93	11.11	171.07	11.71	0.93	1.08	–	–
L^4	115.98	15.51	148.21	10.62	1.28	0.78	–	–
1	2.15	0.18	2.16	0.46	1.00	1.00	33.90	0.04
2	3.22	0.26	2.57	0.19	0.80	1.25	35.69	0.33
3	4.48	0.27	5.52	0.34	1.23	0.81	40.19	0.40
4	5.32	0.11	7.82	0.35	1.47	0.68	237.79	1.05

[a] Standard deviation.

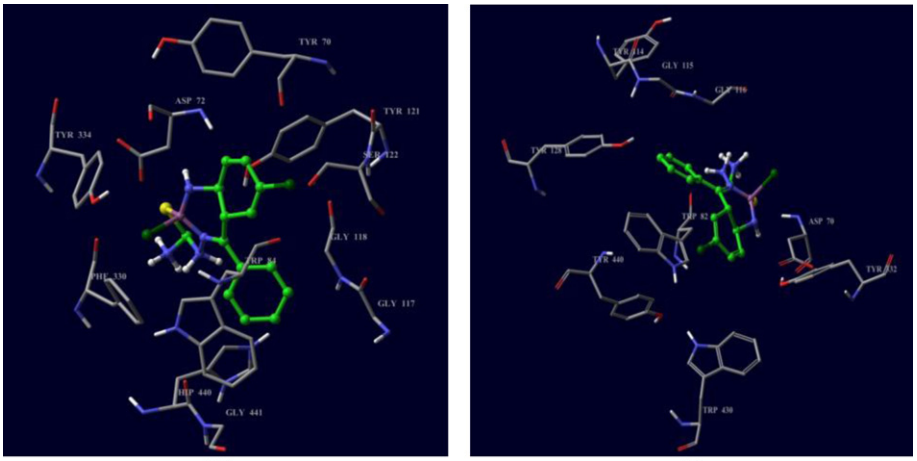


Fig. 2. Complex **1** docked into (a) active site of TcAChE (b) active site of hBuChE.

raises drastically as AD progresses. BuChE are also found at high concentrations in amyloid plaques and neurofibrillary tangles [17]. Thus, dual inhibitors or selective BuChE inhibitors such as compounds L^3, **1** and **2** may enhance ACh availability in AD patients.

Oxidative stress was extensively reported as one of the main symptoms of AD [18], thus the antioxidant capabilities of the synthesized compounds were also evaluated using DPPH assay (See supporting information). Complex **1** again shows the most promising antioxidant effect with IC_{50} value of 33.90 μmol/L (Table 1) that is approximately two-fold less effective than quercetin [$IC_{50} = (14.73 \pm 0.07)$ μmol/L] and almost as active as morin [$IC_{50} = (24.81 \pm 0.08)$ μmol/L].

The molecular modeling simulation were undertaken for the most active compound, complex **1**, to investigate and disclose feasible binding interactions with AChE and BuChE receptors ensuing its high dual cholinesterase inhibitory properties (See supporting information).

For AChE receptor, mild polar, hydrophobic and π–π stacking are the dominant interactions observed with the active site residues. The π–π stacking interaction with Trp84 at choline binding site is assumed to be a major factor in appropriate accommodation of this compound inside the gorge. Tyr70, Tyr121 and Tyr334 at peripheral anionic and Phe330 at choline binding site are other residues which are possibly assisted docking of complex **1** to the bottom of the gorge (Fig. 2(a)).

For BuChE, docking simulations revealed that aromatic rings existing in complex **1** are totally involved in the π–π stacking interaction with indole moiety of Trp82 at choline binding site. This interaction along with hydrophobic interaction with the backbone residues such as Tyr128 and Tyr332 completely anchored this compound to the bottom of active site gorge (Fig. 2(b)). It can be proposed that a similar interaction template of complex **1** with choline binding site residues (Trp84/Phe330 in AChE and Trp82 in BuChE) in both receptors is the major factor which leads to its high/dual cholinesterase inhibitory activity.

4. Conclusion

In conclusion, complex **1** which is the most active compound in AChE and BuChE inhibitory activities, shows equal selectivity for both enzymes with $IC_{50} = 2.15$ μmol/L and 2.16 μmol/L against AChE and BuChE, respectively. In addition, complex **1** also acts as a potent antioxidant with an IC_{50} value of 33.90 μmol/L. Therefore, thiosemicarbazones in combination with copper are useful in the inhibition of the cholinesterase enzymes, which is in accordance with the relative abundance of copper in the brain. Furthermore, thiosemicarbazones with characteristic chelating properties are expected to cause a reduction of Aβ deposits by removing excess deregulated metal ions accumulate in the brain.

Acknowledgments

The authors would like to thank Universiti Sains Malaysia – Science Fund Grant No. 1001/PKIMIA/823003 and RU Grant No. 1001/PKIMIA/811196 for the financial support of this work.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2013.04.013.

References

[1] G. Small, R. Bullock, Defining optimal treatment with cholinesterase inhibitors in Alzheimer's disease, Alzheimers Dement. 7 (2011) 177–184.
[2] M.A. Kamal, P. Klein, Q.S. Yu, et al., Kinetics of human serum butyrylcholinesterase and its inhibition by a novel experimental Alzheimer therapeutic, bisnorcymserine, J. Alzheimers Dis. 10 (2006) 43–51.
[3] H.J. Han, J.J. Lee, S.A. Park, et al., Efficacy and safety of switching from oral cholinesterase inhibitors to the rivastigmine transdermal patch in patients with probable Alzheimer's disease, J. Clin. Neurol. 7 (2011) 137–142.
[4] A. Adsersen, A. Kjølbye, O. Dall, et al., Acetylcholinesterase and butyrylcholinesterase inhibitory compounds from Corydalis cava schweigg. & kort, J. Ethnopharmacol. 113 (2007) 179–182.
[5] N.H. Greig, D.K. Lahiri, K. Sambamurti, Butyrylcholinesterase: an important new target in Alzheimer's disease therapy, Int. Psychogeriatr. 14 (2002) 77–91.
[6] A. Fallarero, P. Oinonen, S. Gupta, et al., Inhibition of acetylcholinesterase by coumarins: the case of coumarin 106, Pharmacol. Res. 58 (2008) 215–221.
[7] Z. Yang, X. Zhang, D. Duan, et al., Modified TLC bioautographic method for screening acetylcholinesterase inhibitors from plant extracts, J. Sep. Sci. 32 (2009) 3257–3259.
[8] P.K. Mukherjee, V. Kumar, M. Mal, et al., Acetylcholinesterase inhibitors from plants, Phytomedicine 14 (2007) 289–300.
[9] A. Budimir, N. Humbert, M. Elhabiri, et al., Hydroxyquinoline based binders: promising ligands for chelatotherapy? J. Inorg. Biochem. 105 (2011) 490–496.
[10] D.J. Bonda, G. Liu, P. Men, et al., Nanoparticle delivery of transition-metal chelators to the brain: oxidative stress will never see it coming, CNS Neurol. Disord. Drug Targets 11 (2012) 81–85.
[11] M. Ikram, R. Saeed Ur, S. Rehman, et al., Synthesis, characterization and distinct butyrylcholinesterase activities of transition metal complexes of 2-[(E)-(quinolin-3-ylimino)methyl]phenol, Inorg. Chim. Acta 390 (2012) 210–216.
[12] M.C. Rodriguez-Argüelles, M.B. Ferrari, G.G. Fava, et al., Acenaphthenequinone thiosemicarbazone and its transition metal complexes: synthesis, structure, and biological activity, J. Inorg. Biochem. 66 (1997) 7–17.

[13] S. Sharma, F. Athar, M.R. Maurya, et al., Novel bidentate complexes of Cu(II) derived from 5-nitrofuran-2-carboxaldehyde thiosemicarbazones with antiamoebic activity against *E. histolytica*, Eur. J. Med. Chem. 40 (2005) 557–562.
[14] G. Vatsa, O.P. Pandey, S.K. Sengupta, Synthesis, spectroscopic and toxicity studies of titanocene chelates of isatin-3-thiosemicarbazones, Bioinorg. Chem. Appl. 3 (2005) 151–160.
[15] The characteristic data for representative compounds L^1: pale yellow solid, 78% yield, mp: 200–202 °C. Anal. Calcd. for $C_{14}H_{13}ClN_4S$ (%): C, 55.17; H, 4.27; N, 18.39. Found: C, 55.13; H, 4.18; N, 18.45. IR (KBr, cm^{-1}): ν $(NH_2)_{aromatic}$ 1622, ν (C=N) 1591, ν (C=S) 818. UV–vis (DMF, nm): 267, 319. ^1H NMR (500 MHz, DMSO-d_6): δ 5.29 (s, 2H, Ar–NH_2), 6.91 (d, 1H, J = 9.0 Hz, Ar–H), 6.92 (d, 1H, J = 2.5 Hz, Ar–H), 7.27–7.30 (dd, 1H, J = 2.5, 9.0 Hz, Ar–H), 7.36–7.41 (m, 3H, Ar–H), 7.70–7.71 (m, 2H, Ar–H), 8.28 (s, 1H, $CSNH_2$), 8.61 (s, 1H, $CSNH_2$), 8.61 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): δ 119.68 (C–Cl), 144.62 (C–NH_2), 146.43 (C=N), 178.07 (C=S). Complex **1**: army green, 60% yield, mp: 186–187 °C. Anal. Calcd. for $[Cu(L^1)Cl]Cl{\cdot}H_2O$ (%): C, 36.76; H, 3.28; N, 12.25. Found: C, 36.84; H, 3.22; N, 12.18. IR (KBr, cm^{-1}): ν $(NH_2)_{aromatic}$ 1635, (C=N) 1580, ν (C=S) 801. UV–vis (DMF, nm): 272, 293, 364, 433, 622. m/z (ESI−MS): 367.8 (M^+ – 2Cl – H_2O, 62%). μeff: 1.78 B.M.
[16] G.L. Ellman, D. Courtney, V.J. Andres, et al., A new and rapid colorimetric determination of acetylcholinesterase activity, BioChem. Pharmacol. 7 (1961) 88–95.
[17] A. Zawadzka, I. Łozińska, Z. Molęda, et al., Highly selective inhibition of butyrylcholinesterase by a novel melatonin–tacrine heterodimers, J. Pineal Res. (2012).
[18] S.M. Salga, H.M. Ali, M.A. Abdullah, et al., Synthesis, characterization, acetylcholinesterase inhibition, molecular modeling and antioxidant activities of some novel Schiff bases derived from 1-(2-ketoiminoethyl)piperazines, Molecules 16 (2011) 9316–9330.