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A new strategy to combat Alzheimer's disease. Combining radical-scavenging potential with metal-protein-attenuating ability in one molecule

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Abstract—Oxidative stress and excessive redox metals have been implicated in the pathogenesis of Alzheimer's disease (AD), which leads to the tentative employment of radical scavengers and metal chelators in clinical therapy of AD. The preliminary successes of both therapy strategies inspire us to propose that better clinical effects can be expected for a compound combining radical-scavenging potential with metal-protein-attenuating ability. Based on theoretical investigation, we indicate that two novel metal chelators (1-(benzimidazole-2-ylmethyl)-1,4,7-triazacyclononane and 1,4-bis(benzimidazole-2-ylmethyl)-1,4,7-triazacyclonone), especially the latter, are promising to fulfill this new strategy.

Alzheimer's disease (AD), characterized by progressive memory loss, decline in language skills and other cognitive impairments, has been a major threat to ageing population.[1,2] Although the etiology of AD is not very clear, oxidative stress is believed to play an important role in the pathogenesis of AD.[3–5] The excessive reactive oxygen species (ROS) likely result from the ageing-related decline of ROS-defensing system and the abnormal interaction between metal ions, such as Cu^{2+} and Fe^{3+} and amyloid-β peptide (Aβ), a major pathogenetic factor in AD.[3–6] Therefore, radical scavengers and metal chelators have been scrutinized in clinical studies of AD and positive results have been gained in the past few years.[3,5,7] For instance, popular chain-breaking antioxidants, for example, α-tocopherol, selegiline and *Ginkgo biloba* extract EGb 761, have shown beneficial effects on AD patients.[3,5] In addition, a hydrophobic moderate metal chelator (5-chloro-7-iodo-8-hydroxyquinoline, Clioquinol, Fig. 1), termed as a metal-protein-attenuating compound (MPAC), has exhibited promising treatment effect in a Phase II clinical trial of moderately severe AD patients.[5,7,8] Inspired by the preliminary successes of radical scavengers and MPAC, we propose that if one compound holds radical-scavenging and metal-protein-attenuating properties simultaneously, it may be more potent than that with single property. Considering the fact that some superoxide dismutase (SOD) mimics are metal chelates,[9] we consider that a SOD-mimic ligand with similar metal-binding ability to clioquinol may be an expected lead compound.

However, it is not easy to find a molecule with good metal-binding ability and high SOD-like activity at the same time, because, taking chelating copper ions as an example, the former property means the Cu^{2+}-chelator complex is rather stable, while the latter property implies that the complex is prone to be reduced to Cu^{+}-chelator state. Apparently, the two properties are contradictory.

To theoretically design the expected molecule, we calculated binding energies (BEs) and electron affinities (EAs, which is a proper parameter to characterize the superoxide-anion-scavenging activity of SOD mimics)[10] for various metal chelates by density functional theory (DFT) at B3LYP/LANL2DZ level. The detailed calculation

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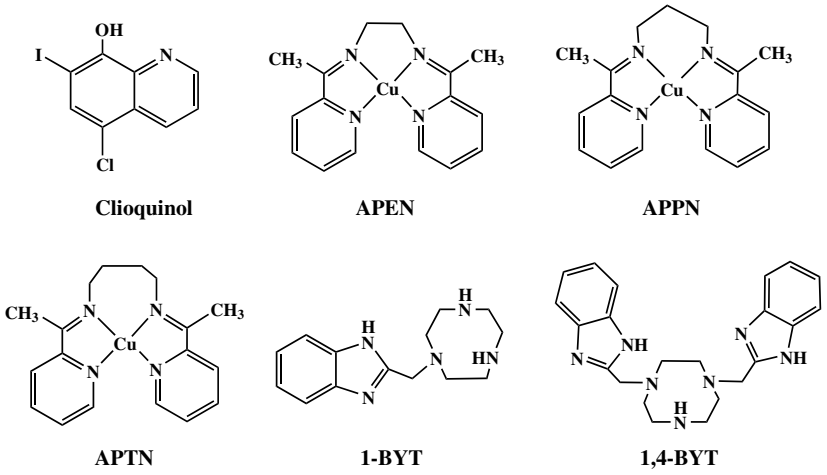

Figure 1. Molecular structures of some metal chelators and Cu,Zn-SOD mimics.

procedure can be found elsewhere.[10] The accuracy of the method was demonstrated in a previous study that the B3LYP/LANL2DZ-calculated BEs can distinguish the most possible Cu^{2+}-chelating modes from various candidates and there exists a very good linear correlation between B3LYP/LANL2DZ-calculated EAs and logarithm of superoxide-anion-scavenging rate constants of SOD mimics ($r = 0.996$).[10] The accuracy of the method may partially result from its reliability in optimizing geometries of metal chelates. For instance, there is a high similarity between crystal structures and theoretical geometries of metal chelates (Tables 1–3, Fig. 2). All of the calculations were performed with GAUSSIAN 98 package of programs.[11]

As shown in Table 4, the BE for Cu^{2+}-clioquinol complex is rather high and comparable with that of Cu^{2+}-Aβ complex (752.56 kcal/mol),[10] which provides a theoretical evidence to support clioquinol's metal-Aβ-attenuating property. However, the electron-accepting ability of Cu^{2+}-clioquinol complex is rather weak, reflected by its high EA (Table 4), which suggests that the complex is very inert in scavenging superoxide radical. On the other hand, although some SOD mimics (*N*,*N*′-ethylene bis-(2-acetylpyridine iminato) copper(II), APEN; *N*,*N*′-propylene bis-(2-acetylpyridine iminato) copper(II), APPN; *N*,*N*′-butylene bis-(2-acetylpyridine iminato) copper(II), APTN; Fig. 1)[12] show low EA and high superoxide-scavenging activity (Table 4), their Cu^{2+}-binding abilities are rather poor (<500 kcal/mol, Table 4), which cannot be anticipated to compete with Aβ to sequester metals.

After frustrated in designing an expected molecule on the basis of the above compounds, we serendipitously noted that Li et al. presented recently two novel Cu^{2+}-chelator complexes with high SOD-like activity and good thermodynamic stability.[13] The chelators (1-(benzimidazole-2-ylmethyl)-1,4,7-triazacyclononane (1-BYT) and 1,4-bis(benzimidazole-2-ylmethyl)-1,4,7-triazacyclononе (1,4-BYT), Fig. 1) were designed by introducing benzimidazole into tridentate macrocycle 1,4,7-triazacyclononane. The B3LYP/LANL2DZ-calculated BEs for 1-BYT and 1,4-BYT are 798.46 and 709.08 kcal/mol, respectively (Table 4), higher than that of clioquinol and in accord with their equilibrium constant difference (19.50 vs 18.86).[13] The calculated EAs for both complexes are also comparable with that of Cu,Zn-SOD

Table 1. Selected bond distances (Å) of metal–cliquinol complexes

	Theoretical value	Experimental value[a]	Error[b]
Cu-N1	1.960	1.964(10)	0.004
Cu-N2	1.979	1.984(10)	0.005
Cu-O1	1.910	1.915(9)	0.005
Cu-O2	1.920	1.922(9)	0.002
Zn-N1	2.043	2.039(14)	−0.004
Zn-N2	2.050	2.040(16)	−0.010
Zn-O1	2.090	2.085(13)	−0.005
Zn-O2	2.060	2.060(12)	0.000
Zn-O3	2.028	2.022(14)	−0.006

[a] Data from Ref. 19.
[b] Difference between experimental and theoretical values.

Table 2. Selected bond distances (Å) of 1-BYT-Cu^{2+} chelate

	Theoretical value	Experimental value[a]	Error[b]
Cu-N1	1.969	1.964(2)	−0.005
Cu-N2	2.150	2.1398(19)	−0.010
Cu-N3	2.161	2.170(2)	0.009
Cu-N4	1.990	2.000(2)	0.010
Cu-Cl	2.290	2.2827(7)	−0.007

[a] Data from Ref. 10.
[b] Difference between experimental and theoretical values.

Table 3. Selected bond distances (Å) of 1,4-BYT-Cu^{2+} chelate

	Theoretical value	Experimental value[a]	Error[b]
Cu-N1	2.017	2.010(4)	−0.007
Cu-N2	2.030	2.028(4)	−0.002
Cu-N3	2.330	2.325(4)	−0.005
Cu-N4	2.094	2.093(4)	−0.001
Cu-N5	1.998	1.995(4)	−0.003
Cu-N6	2.428	2.418(5)	−0.010

[a] Data from Ref. 10.
[b] Difference between experimental and theoretical values.

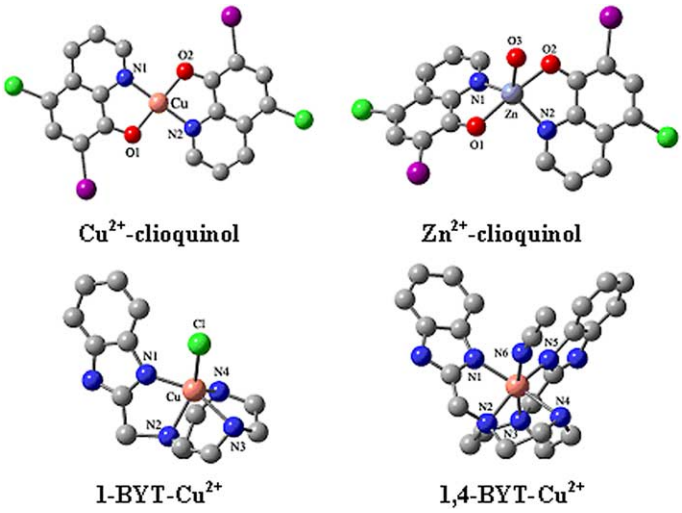

Figure 2. Four metal–chelator complexes. In which, nitrogen is in blue, oxygen is in red, carbon is in gray, chlorine is in green, iodine is in magenta, copper is in orange and zinc is in navy blue.

Table 4. B3LYP/LANL2DZ-calculated electron affinities (EAs, in kcal/mol) and binding energies (BEs, in kcal/mol) for Cu(II) chelates

	BE[a]	BE[b]	EA	EC_{50} (μM)
Cu-clioquinol	685.26	642.57	−64.06	
APEN[c]	461.14		−187.73	11.04[j]
APPN[d]	460.69		−190.58	2.33[j]
APTN[e]	465.90		−192.96	0.56[j]
1-BYT-Cu[f]	798.46	775.49	−164.79	0.90[k]
1,4-BYT-Cu[g]	709.08	665.67	−184.50	0.76[k]
Cu,Zn-SOD	1322.56[h]		−195.79[i]	0.06[k]

[a] Binding energy for copper–chelator complexes.
[b] Binding energy for zinc–chelator complexes.
[c] *N*,*N*′-Ethylene bis-(2-acetylpyridine iminato)-copper(II) chelate.
[d] *N*,*N*′-Propylene bis-(2-acetylpyridine iminato)-copper(II) chelate.
[e] *N*,*N*′-Butylene bis-(2-acetylpyridine iminato)-copper(II) chelate.
[f] 1-(Benzimidazole-2-ylmethyl)-1,4,7-triazacyclononane-copper(II) chelate.
[g] 1,4-Bis(benzimidazole-2-ylmethyl)-1,4,7-triazacyclonone-copper(II) chelate.
[h] Derived from single-point energies calculated on the basis of crystal structure of Cu,Zn-SOD obtained from Protein Data Bank. ID number: 1XSO.
[i] Data from Ref. 10.
[j] Data determined in water.[12]
[k] Data determined in acetonitrile.[13]

(Table 4), in good agreement with their high superoxide-scavenging activity. In addition, the Zn^{2+}-binding ability of 1-BYT and 1,4-BYT can be compared with that of clioquinol (Table 4). Consequently, 1-BYT and 1,4-BYT are the expected compounds that combine radical-scavenging potential with metal-protein-attenuating ability.

As indicated by Barnham et al.,[3] a drug to treat AD must be capable of penetrating the blood–brain barrier (BBB). Therefore, we also evaluated 1-BYT and 1,4-BYT with this respect. The molecular weights of 1-BYT and 1,4-BYT are less than 500 Da and their octanol–water partition coefficients (log *P*) are 0.56 and 2.81, respectively, calculated by Sybyl 6.91, which is a widely used program package for computer-assisted drug design.[14] The latter can be compared with the log *P* of clioquinol (3.73). The polar surface areas (PSAs) of 1-BYT and 1,4-BYT are 48.87 $Å^2$ and 63.61 $Å^2$, respectively, estimated by Sybyl 6.91 program package.[14] The latter also can be compared with the value of clioquinol (74.99). According to the criteria proposed by Barnham et al.,[3] the log *P* and PSA values suggest that 1,4-BYT very likely can penetrate BBB. Furthermore, a theoretical toxicity analysis was also performed for 1-BYT and 1,4-BYT by Topkat (Toxicity Prediction by Komputer Assisted Technology) program package.[15] Both of them showed very weak toxicity in various toxicological tests (unpublished results).

In brief, 1-BYT and 1,4-BYT, especially the latter, will exhibit similar metal-chelating ability to clioquinol and will take the advantage of being a SOD mimic after binding with a transition metal ion. We hope our hypothesis will arouse the interest of pharmacologists to evaluate the effect of 1-BYT and 1,4-BYT by experiments. As the imidazole in the both compounds holds many sites to be modified further, it can be anticipated that other good properties, such as preventing Aβ aggregation, can be introduced into this kind of structure. Taking into account that excessive ROS and disruption of metal homeostasis induce many other diseases than AD, such as Parkinson's disease, prion diseases and amyotrophic lateral sclerosis (ALS),[3,16–18] our strategy is also helpful to designing novel molecules to combat these diseases.

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References and notes

1. Mattson, M. P. *Nature* **2004**, *430*, 631–639.
2. Cummings, J. L. *N. Engl. J. Med.* **2004**, *351*, 56–67.
3. Barnham, K. J.; Masters, C. L.; Bush, A. I. *Nature Rev. Drug Discovery* **2004**, *3*, 205–214.
4. Pratico, D. *Biochem. Pharmcol.* **2002**, *63*, 563–567.
5. Christen, Y. *Am. J. Clin. Nutr.* **2000**, *71*(suppl), 621S–629S.
6. Bush, A. I. *Trends Neurosci.* **2003**, *26*, 207–214.
7. Bush, A. I. *Neurobiol. Aging* **2002**, *23*, 1031–1038.
8. Ritchie, C. W.; Bush, A. I.; Mackinnon, A.; Macfarlane, S.; Mastwyk, M.; MacGregor, L.; Kiers, L.; Cherny, R.; Li, Q. X.; Tammer, A.; Carrington, D.; Mavros, C.; Volitakis, I.; Xilinas, M.; Ames, D.; Davis, S.; Beyreuther, K.; Tanzi, R. E.; Masters, C. L. *Arch. Neurol.* **2003**, *60*, 1685–1691.
9. Salvemini, D.; Riley, D. P.; Cuzzocrea, S. *Nature Rev. Drug Discovery* **2002**, *1*, 367–374.
10. Ji, H. F.; Zhang, H. Y. *Chem. Res. Toxicol.* **2004**, *17*, 471–475.
11. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Cli.ord, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Salvador, P.; Dannenberg, J. J.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaroni, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, Revision A 11.; Gaussian, Inc. Pittsburgh, PA, 2001.
12. Lu, Q.; Shen, C. Y.; Luo, Q. H. *Polyhedron* **1993**, *12*, 2005–2008.
13. Li, Q. X.; Luo, Q. H.; Li, Y. Z.; Shen, M. C. *Dalton Trans.* **2004**, 2329–2335.
14. *SYBYL Molecular Modelling Package*, version 6.91; Tripos, Inc., St. Louis, MO.
15. Enslein, K. *Toxicol. Ind. Health* **1998**, *4*, 479–498.
16. Bush, A. I. *Curr. Opin. Chem. Biol.* **2000**, *4*, 184–191.
17. Lehmann, S. *Curr. Opin. Chem. Biol.* **2002**, *6*, 187–192.
18. Brown, D. R.; Kozlowski, H. *Dalton Trans.* **2004**, 1907–1917.
19. Di Vaira, M.; Bazzicalupi, C.; Orioli, P.; Messori, L.; Bruni, B.; Zatta, P. *Inorg. Chem.* **2004**, *43*, 3795–3797.