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Original article

Perspectives in designing anti aggregation agents as Alzheimer disease drugs

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ABSTRACT

Ab initio molecular orbital calculations at the Hartree–Fock level have been performed for middle portion of amyloid beta that can be best utilized to design intercalative type of preventive drug molecules. Metal induced self assemblage tendency of 16–23 residues' piece of Aβ and its affinity for different metal ions have been investigated in detail. Based on energetics of self aggregation and charge complementarity aspects, two intercalative type of lead compounds have been designed; their preventive mode of actions have been predicted and compared to other drugs of this category. Designed compounds may also exercise their preventive action by removing metal toxicity. Exact mode of action would perhaps depend on relative concentrations of metal ion and amyloid beta.

1. Introduction

Alzheimer disease (AD) is a commonly occurring dementia amongst the elderly. The growing number of AD patients due to increased life expectancy imposes heavy social and financial burden on families. The majority of approved drugs for AD is acetyl cholinesterase (AchE) inhibitors [1–3]. These drugs can only be used for symptomatic relief as they cannot cure the disease. The AchE inhibitors show many side effects like nausea, vomiting, hepatotoxicity, etc. Research has been on going for several years to find a curative or preventive type of drug. An NMDA antagonist, memantine, has also been recently approved as drug [4–7]. Memantine protects cell against excess glutamate by partially blocking NMDA receptor.

To understand other newer strategies in the design of AD drugs we have to first consider amyloid beta (Aβ) production and plaque formation from it. Neuritic plaques, that is, accumulation of amyloid beta protein in between neurons is the central pathological feature in AD [8]. Aβ is a 39–43 amino acid peptide generated by sequential action of β and γ secretase on β amyloid precursor protein (APP). The commonly occurring isoforms of Aβ are Aβ 40 and Aβ 42. Aβ 42 is fibrillogenic and therefore associated with the diseased state [9]. It is believed that Aβ 42 undergoes metal induced aggregation to form plaques [10]. Recently an obvious drug designing strategy was attempted, that is, designing β and γ secretase inhibitors but was not clinically successful [11]. Other newer strategies include monoamine oxidase inhibitors [12–17], huperzine derivatives [18–20], dual inhibitors [21–27] and anti aggregation agents [27–37].

Recent attempts have also been towards reducing oxidative stress imposed on body by Aβ protein. Aβ can bind divalent ions and reduce them to generate reactive oxygen species [38,39] inducing cross linking. Reparative drugs aim at removal of ion from Aβ leading to its degradation [40,41]. Some low molecular weight compounds that are brain permeable have shown anti aggregation properties [42].

This study is an initial step in the direction of designing anti aggregation agents by understanding the energetics involved in aggregation process. Ab initio molecular orbital computational techniques at quantum mechanical Hartree–Fock level have been utilized to understand and evaluate the energetics involved in the process. The experimental designing procedures lack detailed understanding of energetic aspects involved which may partially be the cause of unsuccessful attempts apart from biodistribution, bioavailability and other pharmacokinetic problems.

2. Methodology

Quantum mechanical ab initio molecular orbital (MO) calculations [43] have been performed at Hartree–Fock 6-31G* [44] level throughout. Complete geometry optimizations [45] have been performed for 16–23 residues' piece of Aβ and some anti aggregation compounds including designed compounds. Intermolecular interaction calculations have been performed to study sites for

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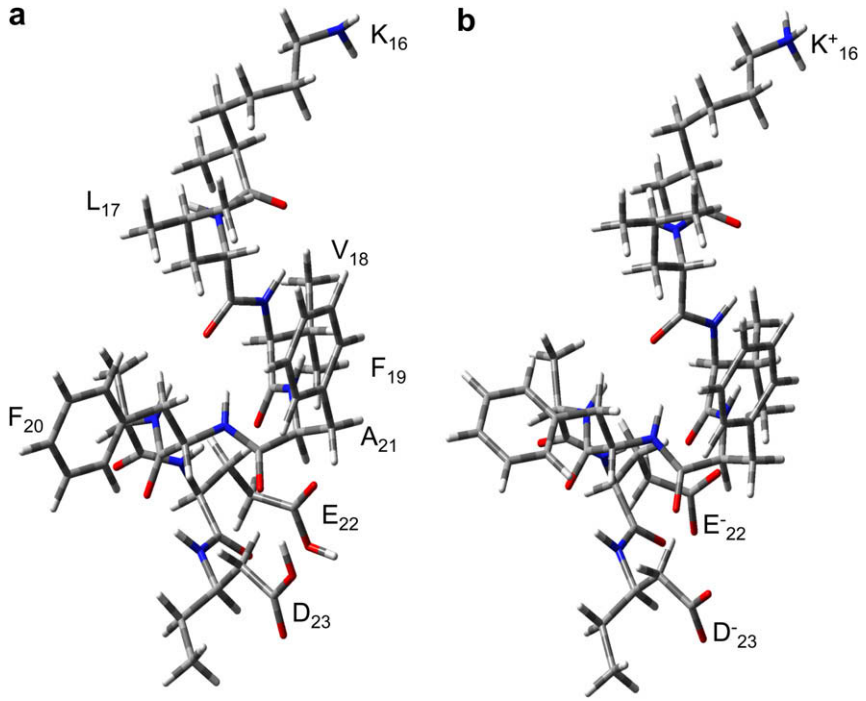

Fig. 1. a. Optimized conformation of middle piece of Aβ i.e. $A\beta_{16-23}$(KLVFFAED). b. $A\beta_{16-23}$ at physiological pH (ionized form).

aggregation in Aβ and anti aggregation power of drugs. Mode of action of these drugs has been investigated in detail. Intermolecular interaction calculations have been performed utilizing super-molecule type approach where $E_{int} = E_{compl} - (E_A + E_B)$. More attractive interaction with metal ion indicates better ion carriage property. Basis set superposition error has not been estimated but it is assumed to be similar in all cases and hence should not affect relative inferences. All calculations have been performed using GAUSSIAN '03 software [46].

3. Results and discussion

16–23 residues' piece of Aβ has been chosen as target to design lead anti aggregation compounds as this piece contains maximum

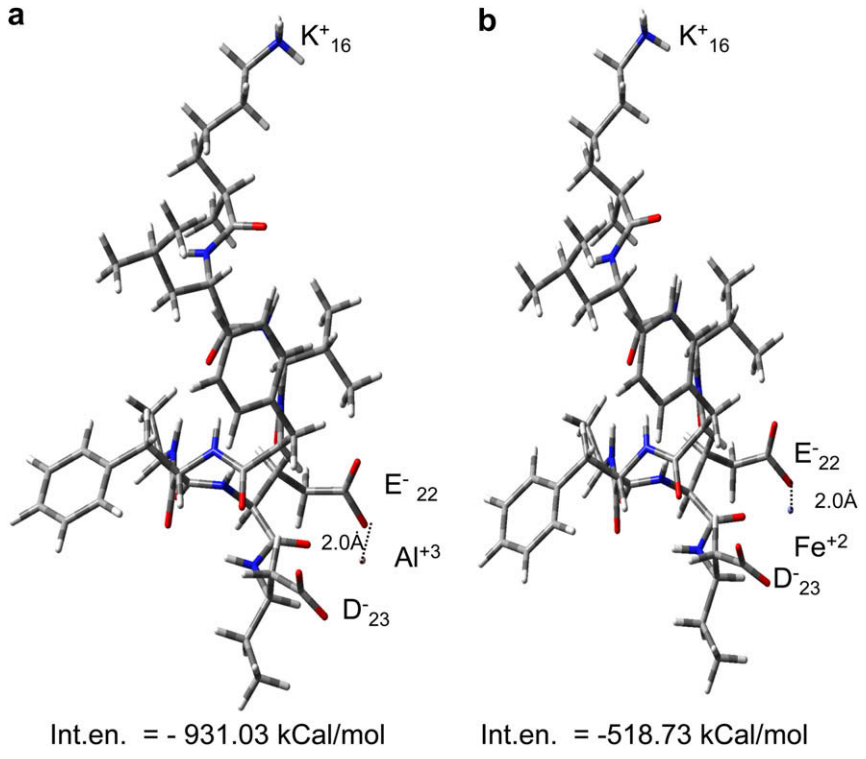

Fig. 2. a. Al^{+3} interacting with Aβ E^{-}_{22} D^{-}_{23}. b. Fe^{+2} interacting with Aβ E^{-}_{22} D^{-}_{23}.

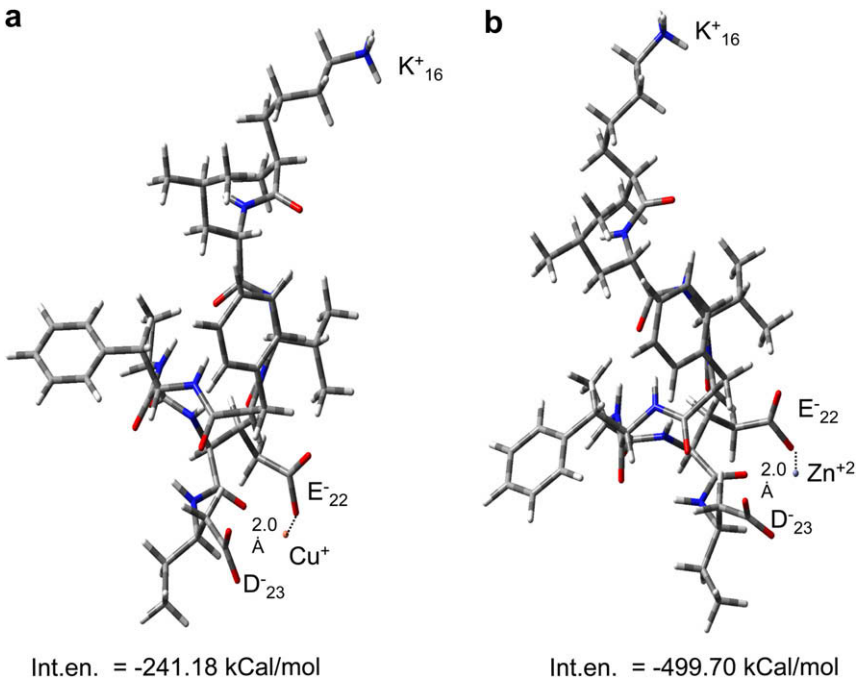

Fig. 3. a. Cu^{+} interacting with Aβ E^{-}_{22} D^{-}_{23}. b. Zn^{+2} interacting with Aβ E^{-}_{22} D^{-}_{23}.

number of ionized residues and hence it is easiest to design intercalative compound for this piece. The added advantage is that when middle piece is intercalated, remaining portion is automatically rendered incapable of participating in aggregation. Completely optimized conformation of middle piece of Aβ 16–23 residues (KLVFFAED) is shown in Fig. 1. Initial geometry was based on solution NMR data [47] for Aβ (pdb file 1Z0Q). Hydrogens were added and complete minimization was performed to remove bad contacts. Optimized structure retains the helical form of peptide in solution. In the presence of metal ion it may become cyclic or in other words prion like (badly structured) [48]. To design preventive drug we must design a compound that can interact with Aβ in solution form. At the same time it should efficiently compete with self aggregation. This piece contains residues occurring in ionized form at physiological pH (cf. Fig. 1b).

To investigate energetics involved in self aggregation of this portion of Aβ we have first studied affinity of Aβ towards different metal ions (as the self aggregation may or not be metal ion induced).

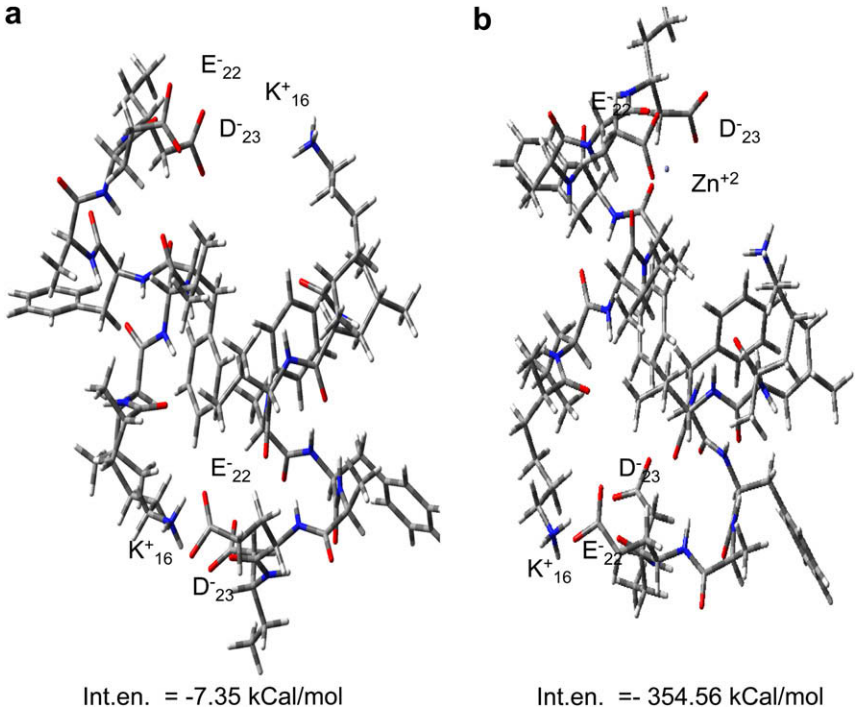

Fig. 4. a. Dimerization tendency of $Aβ_{16–23}$. b. Metal induced dimerization tendency of $Aβ_{16–23}$.

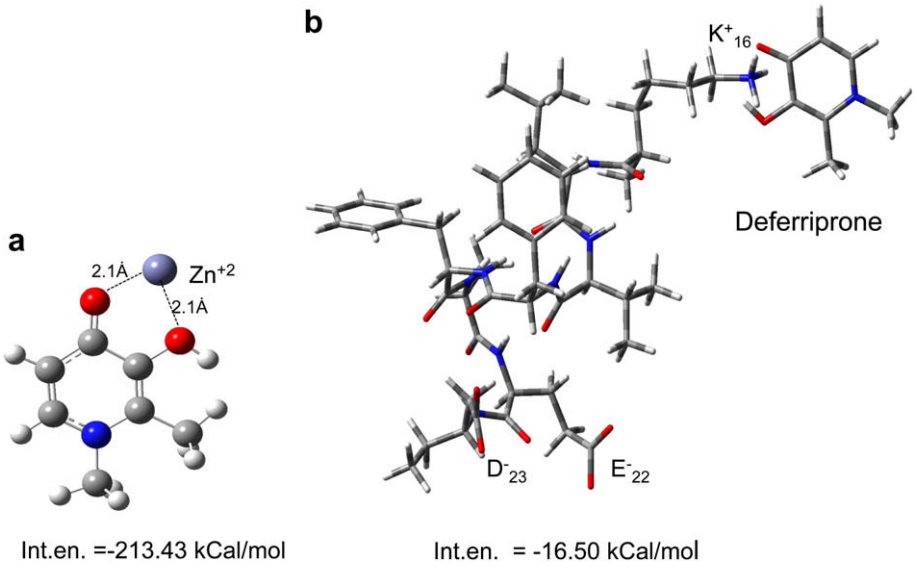

Fig. 5. a. Deferriprone holding Zn^{+2}. b. Deferriprone masking K^{+}_{16}.

Interaction with different metal ions at several positions along Aβ has been evaluated to find out possible sites for metal induced aggregation. Metal ions which have been detected in brain (mostly in traces) during neurotransmitter release and are suspected to be involved in aggregation process have been considered. Figs. 2 and 3 depict best interactions obtained with various metal ions. Metal ion affinity as expected is maximum in vicinity of $E_{22}D_{23}$ due to strong electrostatic interactions. Affinity for metal ion depends on the size of metal ion and charge carried by it. Small metal ions can interact better and higher charge obviously leads to stronger electrostatic interactions. Thus aggregation tendency would be strongest in the presence of Al^{+3} and moderate in presence of ferrous ions provided they are present in similar concentrations. Strong interaction is necessary for inducing aggregation as only traces of ion may be available. Past literature does not indicate decisively which ion is involved in aggregation.

Next we have studied self assemblage tendency of Aβ by considering dimerization with and without metal ion (cf. Fig. 4). Higher order aggregation will be studied later after understanding energetics involved. Aβ is a peptide with usual helical secondary structure, therefore, self assemblage without metal ion (Fig. 4a) is not favored as it intervenes the stable secondary structure of peptide whereas, metal induced self assemblage (cf. Fig. 4b) is highly favored due to strong electrostatic interactions with metal ion. Dimerization tendency is equivalent to evaluating anti aggregation tendency of KLVFFA motif containing anti aggregation peptidic compounds.

Let us now consider role of an anti aggregation compound. An anti aggregation compound may act by removing metal toxicity or by intercalating itself onto Aβ thus masking ionized residues involved in aggregation. We now try to understand why metal chelator drugs commonly used in chelation therapy for metal

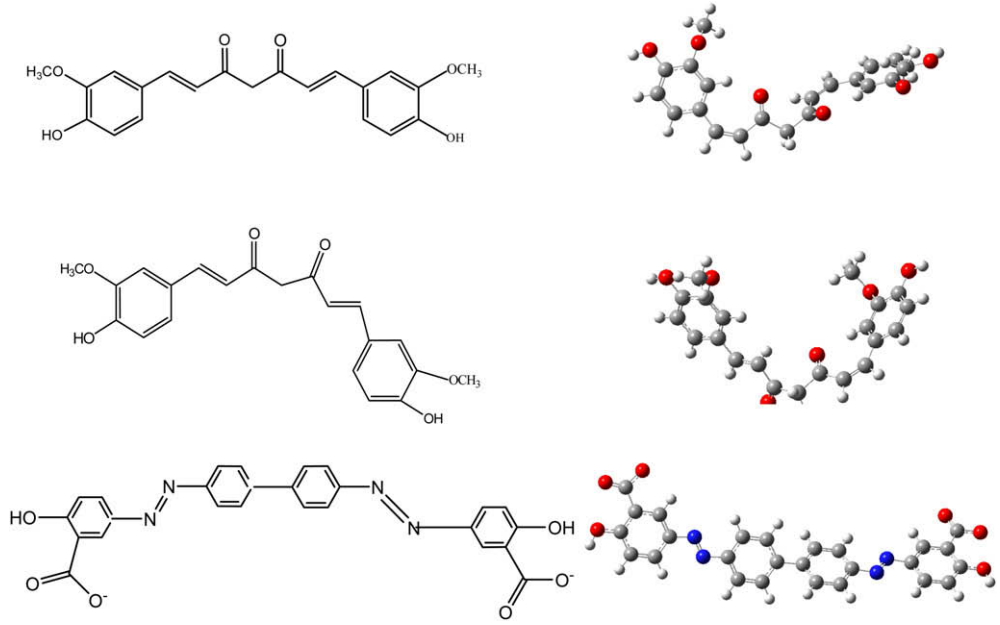

Fig. 6. Line diagrams and corresponding optimized conformations of anti aggregation compounds Curcumin and Chrysamine G.

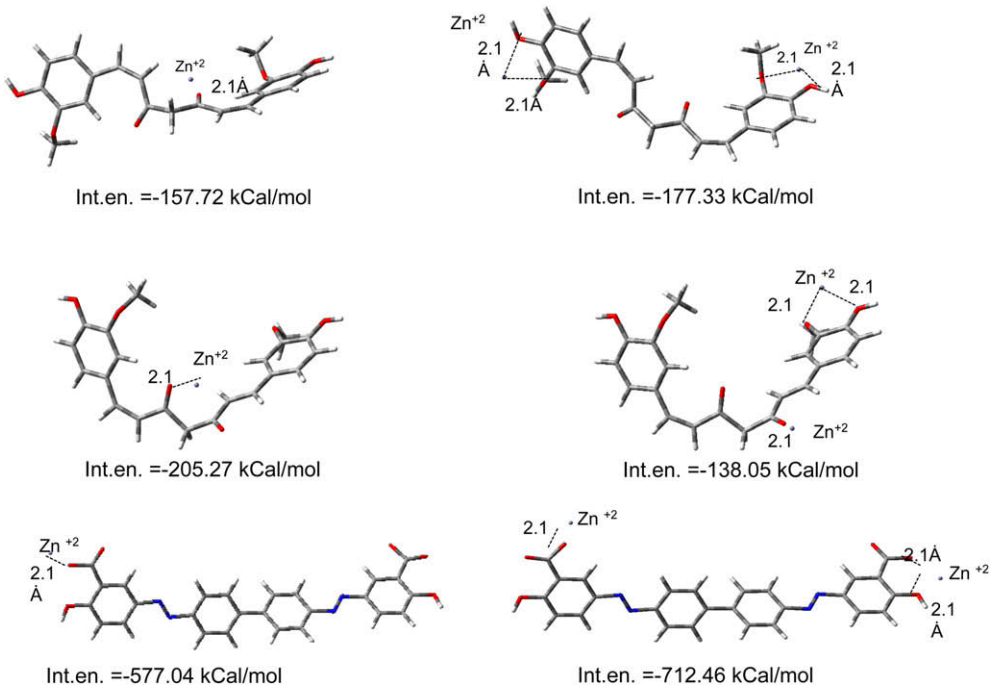

Fig. 7. Single and multiple ion carriage by Curcumin and Chrysamine G.

toxicity cannot be used as effective anti aggregation agents in this case apart from the problems of effectively crossing blood–brain barrier (BBB). Fig. 5a depicts affinity of metal chelator drug Deferriprone towards Zn^{2+} and Fig. 5b depicts Deferriprone trying to mask a portion of Aβ in the absence of metal ion. Results indicate that Deferriprone cannot compete with metal ion for Aβ. Aβ has strong affinity for metal ions due to its ionized residues. Once metal ion is interacting with Aβ it is almost impossible for any drug to snatch it away. In order for any compound to be an efficient anti aggregation agent it must be competitive with metal ion for interaction at Aβ. Fig. 6 depicts optimized conformations of Curcumin and Chrysamine G. These compounds are known to possess anti aggregation property as well as brain permeability [42,49]. We have studied possible mode of action of these compounds for comparison sake. Fig. 7 depicts efficient single and multiple ion carriage by Chrysamine G as compared to Curcumin. These results

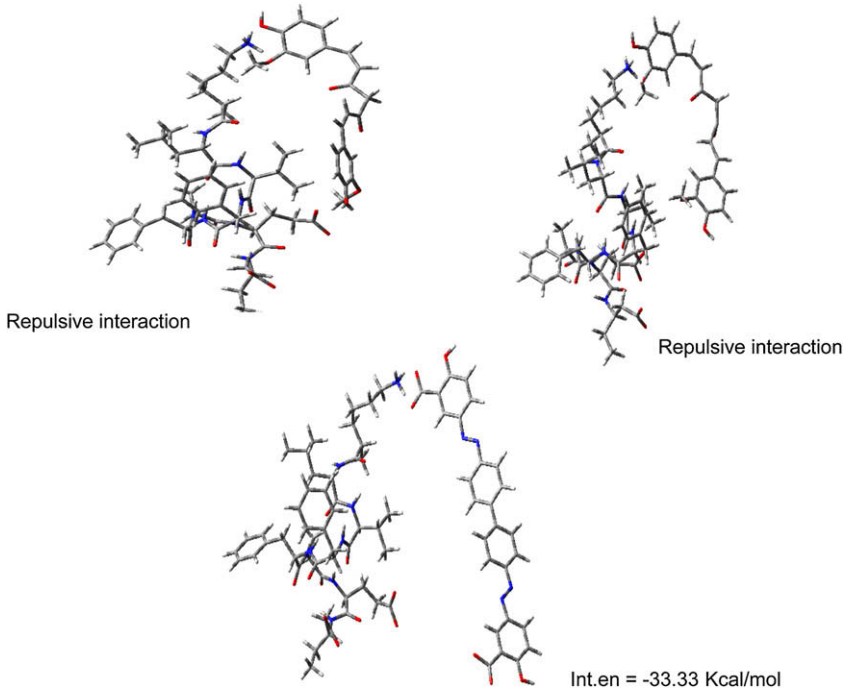

Fig. 8. Exploring intercalative property of Curcumin and Chrysamine G.

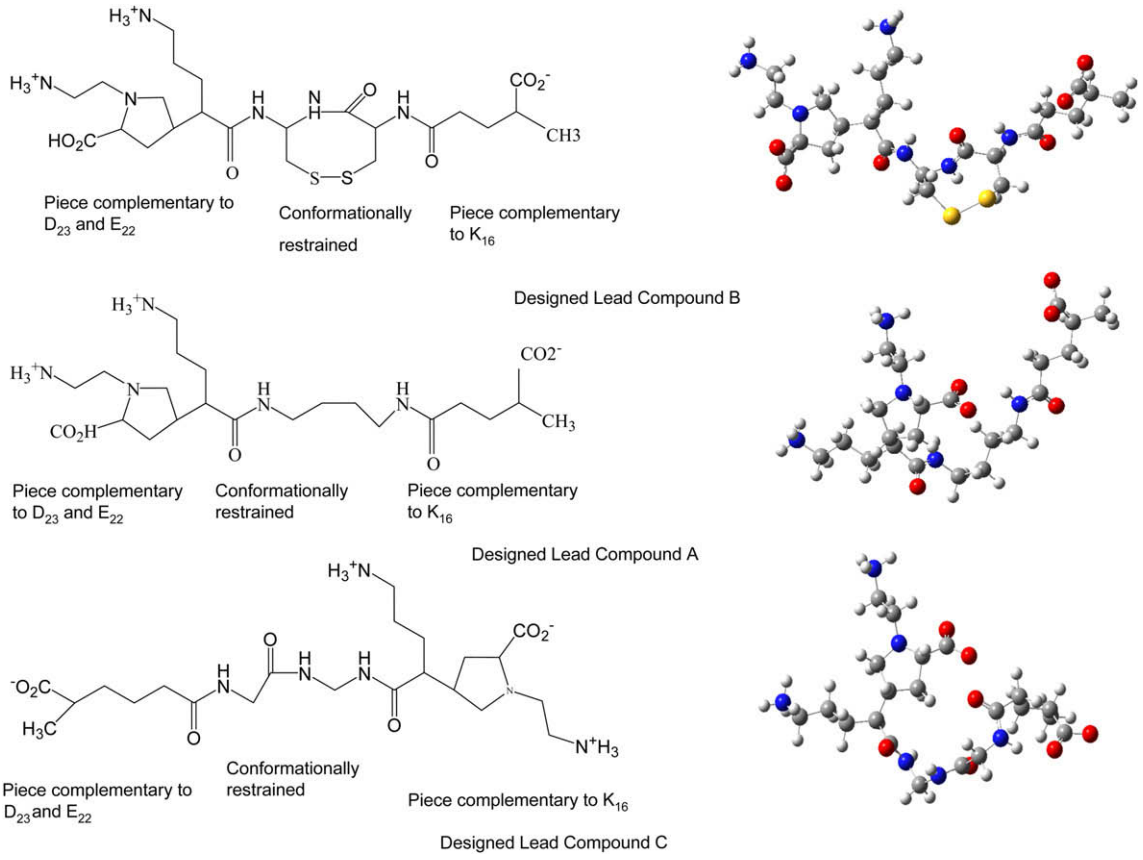

Fig. 9. Line diagrams and corresponding optimized conformations of designed lead compounds.

are in conformity with observed potencies of Curcumin (0.8 μM) [42] and Chrysamine G (0.0253 μM) [49]. Our calculations indicate that Chrysamine G can competitively inhibit metal induced aggregation of Aβ by removing metal ion toxicity. Neither Curcumin nor Chrysamine G can be efficient intercalators (cf. Fig. 8).

We have designed three peptidomimetic lead compounds keeping in mind the charge complementarity and the conformational aspects of middle piece of Aβ. These compounds have been designed as intercalative agents capable of masking charged residues that induce aggregation and are simultaneously capable of acting as metal chelators. Designed compounds and their respective completely optimized conformations are shown in Fig. 9. Lead compound A has two lysine type substituents to interact with E_{22} and D_{23}. At the other end there is a carboxylic substituent to interact with K_{16}. Due to the conformational flexibility inherent in peptidomimetic compound this lead compound shows a bent

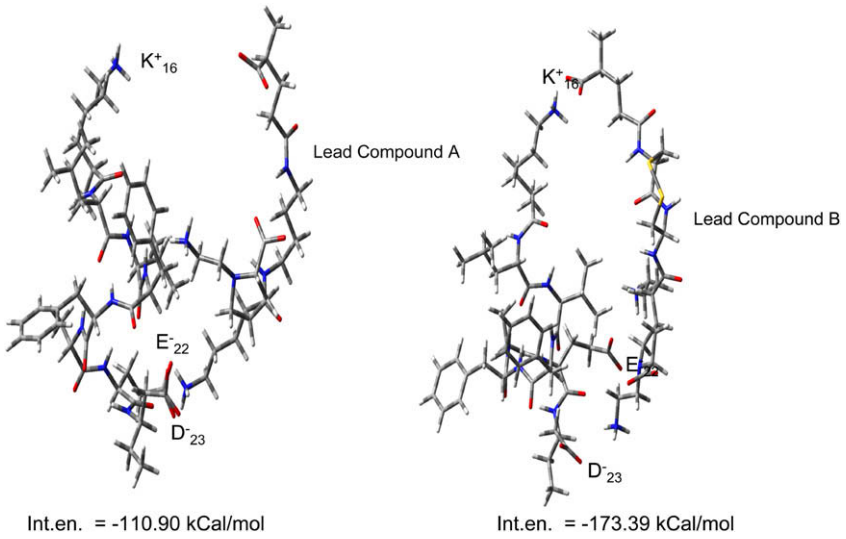

Fig. 10. Intercalative property of designed lead compounds.

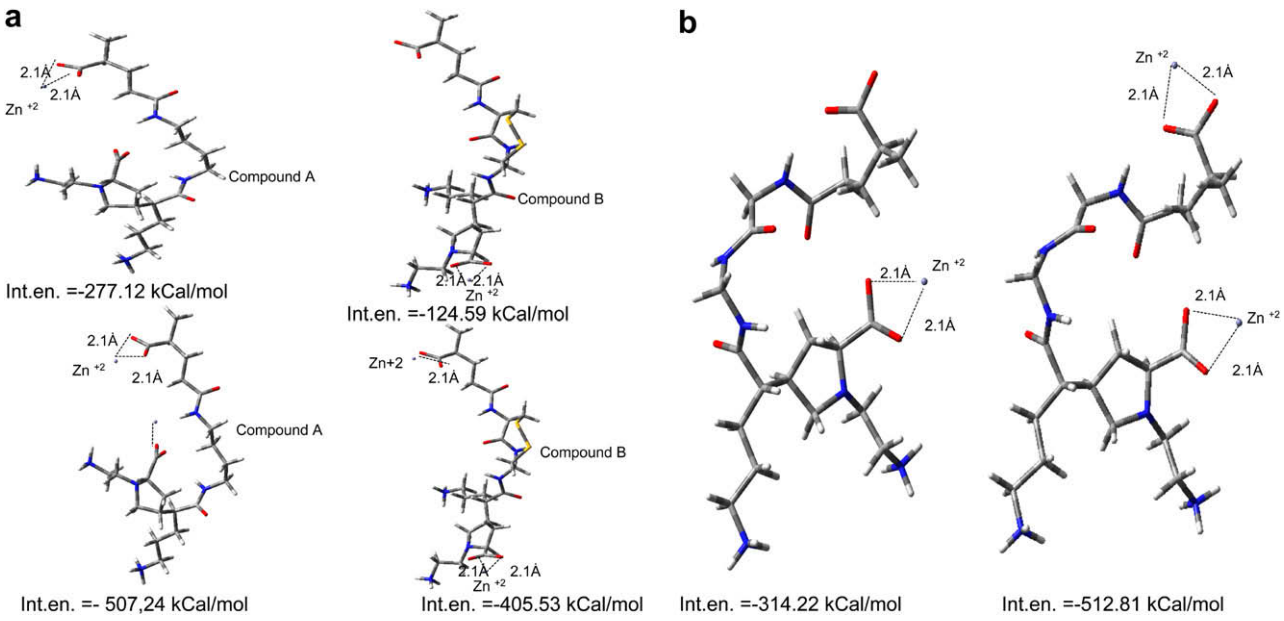

Fig. 11. a. Single and multiple ion carriage by lead compounds A and B. b. Single and multiple ion carriage by lead compound C.

conformation after optimization. Designing was based on simple visual examinations of length required and anionic/cationic sites required for intercalation to Aβ. Another trial compound B was designed by rectifying shortcomings of compound A.

To avoid the bent structure, methylene linkers between cationic and anionic sites were replaced by a more rigid disulfide linkage across a peptidic bond. Again lead compound B was optimized completely. As expected compound B has lesser conformational flexibility and is expected to be a better intercalating agent. Lead compound C being peptidic is conformationally flexible. After optimization compound C possesses intramolecular H-bond rendering it an almost cyclic structure.

An estimate of intercalative property of drugs is obtained by performing intermolecular interaction calculations on Aβ···lead compound complex. Different orientations of lead compounds are tried out until best interaction is obtained. Best intercalation is obtained taking charge complementarity into consideration. Intercalated compound should not be covalently linked so as not to bring about change in physical or chemical state of Aβ. Best results for lead compounds A and B are shown in Fig. 10. Compound C cannot be intercalative due to its almost cyclic conformation.

We have also considered the possibility of these drugs acting as simple ion carriers and helping in removal of toxic metal ions from brain. For efficient usage not only single ion carriage but multiple ion carriage possibilities have also been considered. The availability and concentration of metal ions in brain will decide on the role of compound. Fig. 11a and b shows best possibilities for the three designed lead compounds to act as ion carriers.

Lead compound B seems to be best choice due to its dual characteristics of good ion carrier as well as efficient intercalative property. Lead compounds B and C will be able to compete with Aβ for metal ions. It is desirable to know if these compounds can be synthesized and implications arising due to their pharmacokinetic aspects. Pharmacokinetic aspects can be judged in general by considering compliance with rule of five. Designed lead compounds have molecular weights less than 500 except compound A which is slightly above 500. All compounds have less than 5 hydrogen bond donor groups and less than 10 hydrogen bond acceptor groups. All three designed lead compounds are thus expected to show sufficiently good pharmacokinetics.

Compounds A and B may act as efficient intercalators in the absence of desired concentrations of metal ion. These compounds are predicted to be preventive/reparative type of drugs provided they show moderate pharmacokinetics and reasonably good bioavailability. Synthetic considerations and associated efficacy remain to be verified by experimental chemists.

4. Conclusions

Present study stresses on the need for energetics based designing of preventive type of drugs for Alzheimer's disease. Energetics involved in metal ion induced self assemblage of middle portion of amyloid beta has been investigated. Based on calculations two compounds have been designed as prospective preventive type of anti aggregation compounds. Their efficiencies have been predicted in gas phase and remain to be experimentally verified by synthesis and in vitro analysis. Work is in progress to take into account solvent effects.

Acknowledgements

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