Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(5):143-146

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

'*In vivo* assembly' as a method for designing drugs which target central nervous system: An *in silico* analysis

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ABSTRACT

Central nervous system (CNS) is largely inaccessible to drug molecules due to the presence of blood brain barrier (BBB). Existence of BBB is regarded as the single largest bottle neck in developing neuro therapeutic agents. Despite the rising incidence of CNS ailments, the rate of discovery of drugs acting on the nervous system is low. The conventional approach to drug design deems them to be singleton molecules. The present study tests the concept of drugs being designed as two molecules which cross blood brain barrier independently and assemble inside CNS into an active entity through directed hydrogen bonding. Two ligands L_1 and L_2 were designed such that they could form mutual hydrogen bonds. The ability of ligands to cross BBB, as well as the formation of hydrogen bonds between them were tested in silico using appropriate tools/softwares. Results of in silico tests points to the possibility that drugs targeting brain may indeed be designed as two molecules.

Key words: *in vivo* assembly, ensemble, phased delivery

INTRODUCTION

Nervous system gathers and analyse stimuli from external as well as internal environment of an organism, which also initiate responses to maintain homeostasis. Diseases of CNS are often debilitating besides causing high personal, familial, societal and financial burden [1]. As the world's population ages, the number of people suffering from dementia and other CNS diseases are likely to increase [2]. Forms of psychiatric illnesses may also require therapeutic agents targeting CNS for mitigation of symptoms. These facts essentially mean that a large number of drugs which target CNS might be required in the near future. However very few such drugs are tested and still fewer are passed for chemotherapy [1,3].

In higher animals, CNS is effectively sealed off from the rest of the body by an anatomical entity called Blood brain barrier (BBB). The BBB prevents entry of unnecessary molecules and pathogens into nervous tissue, which could jeopardize the homeostasis of CNS [4,5]. This blockade is created by tight junctions in endothelium of capillaries which perfuse brain. The BBB screens out most drugs from entering CNS. It is often regarded as a bottleneck in brain drug development and is the most important factor limiting the future growth of neuro therapeutics [6].

The conventional methods for delivering drugs to CNS were reviewed and all the available techniques utilize drugs which are single molecules [7]. In an attempt to open another avenue before drug designers, the present article explores the possibility of designing drugs as two molecules, which would assemble inside CNS into the active agent. This approach may help developers to escape limitations of BBB block and achieve meaningful drug structures which are otherwise impossible.

EXPERIMENTAL SECTION

Potential component molecules (ligands) were designed using the drawing interface of online tool 'Osiris property explorer', version 2.0 [8,9]. The drug likeness and other properties of the ligands like cLogP, LogS, molecular weights and overall drug scores were also assessed using the same tool. Two ligands, L_1 *viz.* 2-[N-Ethyl(hydroxyamino)]ethanol (Fig 1) and L_2- *viz.* 2-(2-Aminoethylamino)pentanamine (Fig 2) were selected based on their ability to cross BBB. The ability of ligands to permeate BBB was tested using the 'blood brain barrier penetration prediction tool', B_3PP [10,11]. The chemical names in smiles format for use in B_3PP was generated using the tool 'Online SMILES Translator and Structure File Generator' [12]. The structures of the ligands were manually assembled and tested for potential hydrogen bonding between them, using the tool UCSF CHIMERA [13, 14]. The .pdb files of ligands used in 'Chimera' were generated using the online tool 'Marvin' [8].

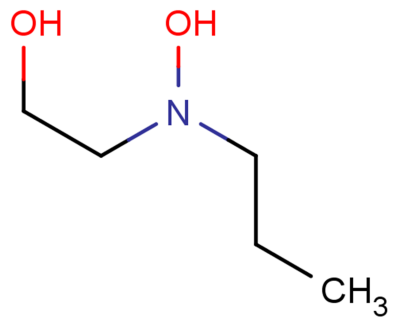

Fig 1: 2-[N-Ethyl(hydroxyamino)]ethanol

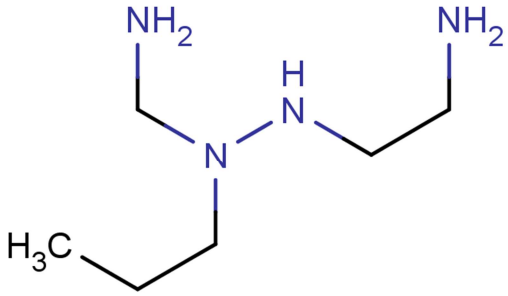

Fig 2: 2-(2-Aminoethylamino)pentanamine

RESULTS AND DISCUSSION

The drug scores and other parameters predicted for the ligands by Osiris property explorer is presented in Table (1). The tool also ruled out mutagenic, tumorigenic, irritant or reproductive effects for both the ligands. The ligands L_1 ($p<0.0468$) and L_2 ($p<0.0271$) were predicted to have the ability to cross blood brain barrier by the tool B_3PP.

Table 1: Properties of ligands predicted by Osiris property explorer

Sl No	Property	L_1		L_2	
		Predicted value	Predicted drug score	Predicted value	Predicted drug score
1	c Log P	-0.492	0.995	-2.302	0.999
2	Log S	-0.310	0.990	-0.738	0.986
3	MW	119	0.989	146	0.985
4	Drug likeness	1.057	0.742	0.799	0.689
	Overall score	--	0.860	--	0.832

The formation of hydrogen bonds between L_1 and L_2 was tested *in silico* using the software 'Chimera'. The program identified four intermolecular hydrogen bonds with relaxed settings whereas with stringent settings, a single bond alone was found. The assembled state of the ligands and detected hydrogen bonds (blue lines) with distances between them in Å (as depicted by Chimera) is presented in Fig (3).

Being a preliminary study, 2D structures alone were used for the tests. All tools and softwares used for the present study were available online as freewares. Selection of L_1 and L_2 was based on their ability to cross BBB as predicted by the tool, B_3PP. *In silico* predictions regarding BBB penetration may have pitfalls [15] and is not reliable in all cases.

Hydroxyl groups behave as hydrogen bond donors whereas amino groups act as hydrogen bond acceptors [16]. Therefore the donor and acceptor groups in L_1 (two hydroxyl groups) and L_2 (two amino and one imino groups) gives them the ability to form mutual hydrogen bonds in solution. Molecules which have one or two quadruple hydrogen bonding units were identified to form highly stable hydrogen bonded assemblies in water [17]. The ligands could form four mutual hydrogen bonds as inferred by Chimera (Fig 3) with less stringent software setting. Therefore it is assumed that the ligands would form a hydrogen bonded structure, denoted as 'ensemble'. However a single hydrogen bond (HB_3) alone was observed with stringent software settings. It is difficult to predict whether or not hydrogen bonds between a potential donor and an acceptor in a given system will actually be formed [18]. At this stage, ensemble is merely a concept and aspects like true conformation in solution, its stability, biological activity or even its existence remains cryptic. These properties can be assessed only through experiments after synthesis. The ensemble and component molecules described in this article therefore serves only for the purpose of

illustration of the concept of *in vivo* assembly. In short, the conformation of assembled state (Fig 3) is hypothetical and may not represent the one which they assume under *in vitro* or *in vivo* conditions.

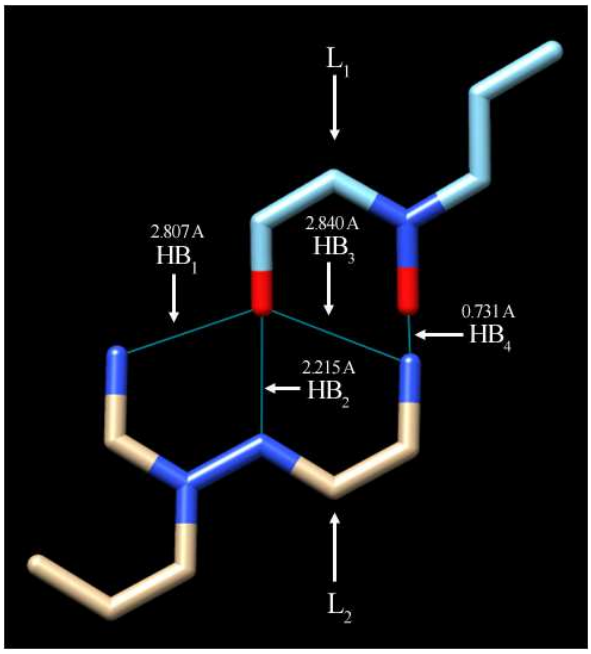

Fig 3: Hydrogen bonds (HB_1 to HB_4) inferred between L_1 and L_2 by Chimera

Drug molecules are mostly designed with rigid structures so as to maximise biological activity [19]. *In vivo* assembled drugs are likely to fall back from this criterion as hydrogen bonds are considerably weaker than covalent bonds. Hydrogen bonds may be switched on and off with energies in the range of thermal fluctuations at physiological temperatures. In such circumstances the vectorial and stereo-chemical properties can prefer specific hydrogen bonding interactions with additive or cooperative strengths [20]. The strength of a hydrogen bond between OH and NH_2 functional groups is 3.8 KCal/mol [18]. In this case, assuming an additive situation with four mutual hydrogen bonds, the strength will increase to 14.8 KCal/mol, which may suffice to stabilize the ensemble. Additional interactions with target protein (with donors/acceptors incorporated into ligands by virtue of design) could further enhance the stability of ensemble.

The real challenge before designers will be to determine the layout of functional groups in hydrogen bond donor and recipient ligands, since self assembly require four mutual hydrogen bonds. If ever any such drugs are materialized, a 'phased delivery' regime will have to be adopted, wherein one of the molecules is introduced first rather than both being delivered as a cocktail. The second molecule then needs to be dispensed after a suitable delay considering the pharmaco-dynamic properties of the first. The molecules cross BBB independently and assemble into the active form (ensemble) inside the CNS and elicit therapeutic effect.

CONCLUSION

In silico tests point to the possibility that drugs could be designed as two molecules which assemble inside the CNS by virtue of directed hydrogen bonding.

Acknowledgements
Inspire fellowship granted by Ministry of Science and Technology, Government of India.

REFERENCES

[1] HK Manji; EB DeSouza. *Neuropsychopharmacology.*, **2008**,1-4.
[2] M Prince; R Brycea; E Albanesea; A Wimoc; W Ribeiroa; CP Ferriae. *Alzheimer's & Dementia.*, **2013**, 9, 63-75.
[3] MS Alavijeh; M Chishty; MZ Qaiser; AM Palmer. *NeuroRx.*, **2005**, 2(4), 554-571.
[4] P Ballabh; A Braun; M Nedergaard. *Neurobiol Dis.*, **2004**, 16(1), 1-13.
[5] BE Grayson; RJ Seeley; DA Sandoval. *Nature reviews Neuroscience.*, **2013**, 14(1), 24-37.
[6] WM Pardridge. *NeuroRx.*, **2005**, 2(1), 3-14.
[7] A Misra; S Ganesh; A Shahiwala. *J Pharm Pharmaceut Sci.*, **2003**, 6(2), 252-273.

[8] OSIRIS property explorer. Accessed from http://www.organic-chemistry.org/prog/peo/. [Last cited on 15/4/**2015**]
[9] P Ertl. *Journal of Cheminformatics.*, **2010**, 2, 1.
[10] IF Martins; AL Teixeira; L Pinheiro; AO Falcao. *Journal of Chemical Information and Modeling.*, **2012**, 52(6), 1686-1697.
[11] Blood brain barrier penetration prediction (B_3PP). Accessed from http://b3pp.lasige.di.fc.ul.pt/. [Last cited on 15/4/**2015**]
[12] Online SMILES Translator and Structure File Generator. Accessed from http://cactus.nci.nih.gov/translate/. [Last cited on 15/4/**2015**]
[13] CC Huang; GS Couch; EF Pettersen; TE Ferrin. *Pacific Symposium on Biocomputing.*, **1996**, 1, 724.
[14] UCSF CHIMERA. Accessed from http://www.cgl.ucsf.edu/chimera. [Last cited on 15/4/**2015**]
[15] JT Goodwin; DE Clark. *The Journal of Pharmacology and Experimental Therapeutics.*, **2005**, 315(2), 477-483.
[16] C Laurence; M Berthelot. *Perspectives in Drug Discovery and Design.*, **2000**, 18(1), 39-60.
[17] JF Xu; LY Niu; YZ Chen; LZ Wu; CH Tung; QZ Yang. *Organic Letters.*, **2014**, 16(15), 4016-4019.
[18] T Steiner. *Angew. Chem. Int. Ed.*, **2002**, 41, 48-76.
[19] H Pajouhesh; GR Lenz. *NeuroRx.*, **2005**, 2,541-553.
[20] B Kojić-Prodić; K Molčanov. *Acta Chim. Slov.*, **2008**, 55, 692-708.