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INHIBITION OF ELECTRIC EEL ACETYLCHOLINESTERASE BY PORPHIN COMPOUNDS

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Abstractct: Synthetic porphin compounds have been found to be reversible inhibitors of acetylcholinesterase from electric eel with K_i values of μ M range. It seems that the number and position of fluorine on the phenyl ring and metal of an inhibitor play an important role for binding of an inhibitor to the enzyme active site.

Acetylcholinesterase(AChE) is an important enzyme in the central and peripheral nervous systems in the transmission of nerve impulses across nerve-nerve and neuromuscular synapses[1]. The role of the enzyme is to hydrolyze the neurotransmitter acetylcholine(ACh) to acetate and choline.[2,3] Due to the critical role of AChE in the nervous system the design and characterization of AChE inhibitors are quite important for various reasons. Some AChE inhibitors such as sarin and soman are chemical warfare agents, while other AChE inhibitors have been used for patients of Alzheimer's Disease(AD).[4-6] A good inhibitor of AChE having low K_i and cytotoxicity is still required for therapeutic purpose. In this regard, inhibition of AChE by porphin derivatives is of significance since porphin complexes usually have low cytotoxicity and ability to cross the blood brain barrier.[7] Thus, porphin derivatives are expected to cross the blood brain barrier to go to the central nervous system. In the other respect, inhibition of AChE by macromolecules such as inorganic complexes are very interesting. Thus, the inhibition of electric eel AChE by the synthetic inorganic complexes is characterized.

The structures of the porphin inhibitors are shown in the Figure. All the inhibitors have phenyl substituents on the carbon of the porphin skeleton. 5,10,15,20-Tetrakis(2,4-difluorophenyl)-21H,23H-porphin and 5,10,15,20-tetrakis(2,6-difluorophenyl)-21H,23H-porphin are abbreviated as ADD-1 and ADD-2, respectively. The inhibition constant, K_i, of the porphin inhibitors determined by K_m/V_{max} vs. inhibitor concentration replot is shown in the Table. Except ADD-1 and ADD-2 the inhibition constant of the tetraphenylporphins(TPP) is in μ

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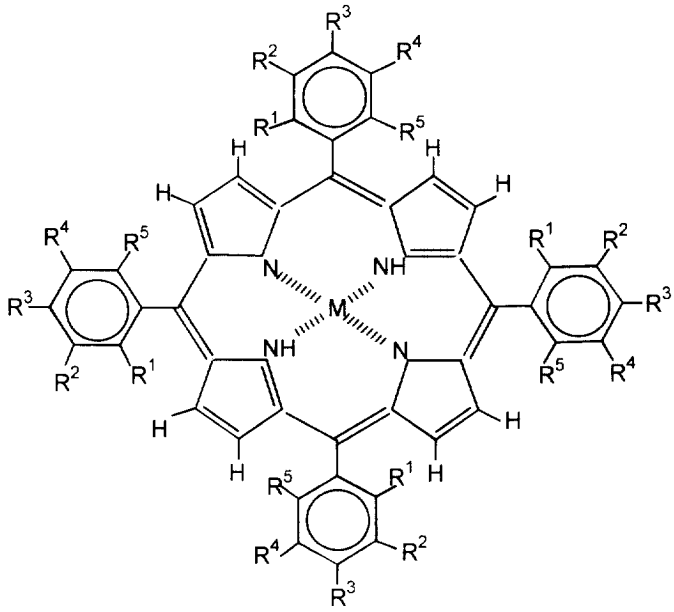
M range. ADD-1 and ADD-2 are much more potent inhibitors than ordinary quaternary ammonium salt inhibitors of AChE. F20TPP is a reversible competitive inhibitor since it increased K_m of the inhibition reactions and it has little effect on V_{max}. All the inhibitors shown in the Table are close to reversible competitive inhibitors. F20TPPFeCl is also a reversible inhibitor with K_i in μM range. The porphin inhibitors bind both free enzyme E and ES complex though they bind E more tightly. Thus, ADD-1 and ADD-2 can be classified as reversible competitive inhibitors of AChE.

K_m/K_i roughly estimates the relative binding affinity of an inhibitor to the enzyme compared to the substrate.[8] As the Table shows metal increased the binding affinity of the inhibitor. The addition of FeCl to F20TPP increased the binding affinity by 7 folds. The addition of Ni to F20TPP lowered the binding affinity to AChE active site(Its not able to determine K_i value within the solubility limit). The addition of fluorine on C2



$R^1 = R^5 = F$ $R^2 = R^3 = R^4 = H$ no metal : ADD - 1
$R^1 = R^3 = F$ $R^2 = R^4 = R^5 = H$ no metal : ADD - 2
M = Fe, $R^1 = R^2 = R^3 = R^4 = R^5 = F$ F20TPPFeCl
M = Zn, $R^1 = R^2 = R^3 = R^4 = R^5 = H$ Zn TPP

Figure. Structures of Porphin Inhibitors

carbon of the phenyl ring has profound effect on the binding affinity of TPP inhibitors. Other substituents on C2 carbon of the phenyl ring have little effect on the potency of the inhibitors(data not shown). It seems that the number and position of fluorine substituent are very critical for the potency of an inhibitor. ADD-1 has two fluorine substituents on C2 and C4 carbon on the phenyl ring and ADD-2 has the substituents on C2 and C6 carbon on the phenyl ring. Thus, the fluorine on C2 carbon of the phenyl ring is very important to increase the potency of tetraphenylporphin. Comparison of ADD-1 and ADD-2 shows that two *ortho* fluorine substituents have greater effect on the inhibition constant than *ortho* and *para* fluorine substituents. Since F20TPP is not as potent as ADD-1, other than electronic effect somehow affects the binding affinity of the inhibitors.

Table. The Inhibition Constant of Porphin Inhibitors

Inhibitor	K_i(μ M)[a]	(K_m/K_i)[b]
F20Tetraphenylporphin	15.2 ± 4.3	9
F20TPPFeCl	2.21 ± 0.49	63
ZnTPP	5.39 ± 1.34	26
2.4-F8TPP(ADD-1)	0.012 ± 0.002	12000
2.6-F8TPP(ADD-2)	0.005 ± 0.001	26000

Timecourses for the AChE-catalyzed hydrolysis of ATCh were followed by monitoring the formation of thio anion of nitrobenzoic acid at 460 nm by Ellman's coupled enzyme assay.[9] Reactions were run in duplicate.
[a] K_i is determined from the K_m/V_{max} vs inhibitor concentration replot.
[b] K_m of acetylthiocholine(ATCh) for AChE is 0.14 mM.[10]

Kenley *et al.* reported the irreversible inhibition of AChE by cobalt(III) complexes.[11] They assumed that the sulfhydryl or other sensitive functional groups on the enzyme are oxidized by the chelates or their solvolysis products. This hypothesis is unlikely for these porphin inhibitors because porphin compounds do not have easily oxidizable metal and no metal containing porphin inhibitors are potent inhibitors of AChE. It is very interesting to note that big and hydrophobic molecules such as porphin compounds are good inhibitors of AChE. It is known that AChE has narrow and long gorge to the enzyme active site.[12] Though the inhibition of AChE by porphins is not very surprising because there are hydrophobic and large molecules which effectively inhibit AChE. A natural alkaloid Huperzine A isolated from *Huperzia serrata* is very potent inhibitor of AChE having

K_i in the range of nM.[13] Huperzine A reversibly inhibit AChE by the interaction of the aromatic amino acids in the active site with the hydrophobic interaction. Very recently, Sugimoto *et al.* proposed a model of AChE active site.[14] According to this model, there are four different site in the enzyme active site; hydrogen bonding site, negative charge site, and two different hydrophobic sites. The porphin compounds can be fit to this model. The phenyl group of the inhibitors can binds to the hydrophobic region 2 and the partial positive charge on the carbon on the main chain generated by the two fluorine atoms on the phenyl ring can interact with the negative charge site. Since F20TPP does not bind as strong as either ADD-1 or ADD-2 to the enzyme active site, the fluorine substitution at the *meta* positions of the phenyl ring somehow lower the binding affinity of the inhibitor compared to ADD-1 and ADD-2. The computer aided modeling study and *in vivo* study with rats are under investigation to see the interaction of these porphin compounds with AChE active site and the efficacy.

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