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Analysis of electronic structures of physostigmine analogs

HU Zeng-Jian, JIANG Hua-Liang, CHEN Jian-Zhong, CHEN Kai-Xian[1], JI Ru-Yun
(*Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China*)

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AIM: To elucidate the action mechanism and structural prerequisites of 21 physostigmine analogs as acetylcholinesterase inhibitors at the molecular level, and help the rational design of these dihydroindoline inhibitors. **METHODS**: Initial structures of these compounds were built and minimized by SYBYL 6.2 molecular modeling software. Conformations of those molecules with the highest predictive abilities in the Comparative Molecular Field Analysis model were chosen to the semiempirical quantum chemical calculations. **RESULTS**: (1) The highest occupied molecular orbital (HOMO) consisted mainly of the orbitals in phenyl group and N_1 atom; the lowest unoccupied molecular orbital (LUMO) of the molecules was contributed from phenyl group and C_{11} atom. While the HOMO energies did not show any recognizable relationship with activity, the LUMO energies showed a decreased tendency with increasing activity. The active compounds showed lower LUMO energies. (2) The carbon atom (C_{11}) had the most positive net atom charge. The most active compound had the most positive charge on this carbon, but had the lower charges on the carbonyl oxygen (O_{12}) which was the most negative charge atom. (3) The bond order of carbon-oxygen bond ($C_{11} - O_{10}$) was invariant across the series of the compounds. (4) Compounds with too high or too low total dipole moment had lower activities, while the most active one had a lower molecular polarizability. **CONCLUSION**: A molecular model was suggested to explain the possible mode of action by which these compounds inhibit acetylcholinesterase.

[1] Correspondence to Prof CHEN Kai-Xian. Phn 86-21-6431-1833, ext 615. Fax 86-21-6437-0269. E-mail kxchen@iris3.shmm.ac.cn
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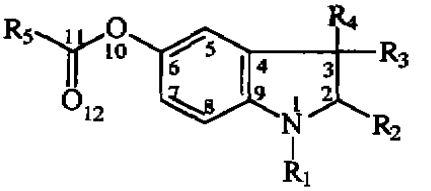
According to the cholinergic hypothesis, memory impairments in Alzheimer's disease patients resulted from a deficit of cholinergic functions in the brain. Inhibition of acetylcholinesterase (AChE) was considered as one of the most promising strategies for activating central cholinergic functions. While physostigmine was shown to improve memory and reverse scopolamine-induced dementia, its use was limited by its serious or potential lethal side effects and a narrow therapeutic index.

The syntheses of physostigmine analogs had been reported[1,2]. The inhibitory activities of these dihydroindoline carbamates (DHIC) also had been measured experimentally[3]. However, their precise modes of action and structural requirements were still unknown. The present work was to elucidate the action mechanism and the structural prerequisites of 21 physostigmine analogs at the molecular level.

COMPUTATIONAL METHODS

The semiempirical quantum chemical calculations on 21 dihydroindoline compounds (Tab 1) were performed with SYBYL 6.2 molecular modeling software[4] running on Silicon Graphics IRIS Indigo XZ4000 workstation. First, the initial structures were built and minimized by molecular mechanical program MAXIMIN2 encoded in SYBYL systems using Tripos force field parameters. A preliminary conformation search was then performed by the system search method implemented in SYBYL system. After a systematic conformational search, 8 series of minimum-energy conformations including 568 different conformers were obtained for all the compounds. Based on each series, 3D-QSAR studies were carried out with Comparative Molecular Field Analysis (CoMFA) method[5]. Consequently, the conformations of these molecules with the highest predictive ability in the CoMFA-QSAR model were chosen as the starting structure to the quantum chemical calculations. The geometries of the 21 compounds were calculated with optimization of all

Tab 1. Structure, numbering, and IC_{50} values of dihydroindoline carbamates (DHIC).



№	R_1	R_2	R_3	R_4	R_5	$IC_{50}/\mu mol \cdot L^{-1}$
1	CH_3	H	CH_3	CH_3	$N(CH_3)_2$	0.126
2	CH_3	H	CH_3	CH_3	$NHCH_3$	0.079
3	CH_3	H	CH_3	CH_3	NHC_2H_5	0.200
4	CH_3	H	CH_3	CH_3	$N(C_2H_5)_2$	1.00
5	CH_3	H	CH_3	CH_3	NHC_7H_{15}	0.794
6	H	H	CH_3	CH_3	$N(CH_3)_2$	3.16
7	C_2H_5	H	CH_3	CH_3	$N(CH_3)_2$	0.398
8	C_3H_7	H	CH_3	CH_3	$N(CH_3)_2$	0.631
9	C_4H_9	H	CH_3	CH_3	$N(CH_3)_2$	0.79
10	C_5H_{11}	H	CH_3	CH_3	$N(CH_3)_2$	1.58
11	i-C_5H_{11}	H	CH_3	CH_3	$N(CH_3)_2$	1.26
12	$CH_2C_6H_5$	H	CH_3	CH_3	$N(CH_3)_2$	39.8
13	H	O	CH_3	CH_3	$N(CH_3)_2$	500
14	CH_3	O	CH_3	CH_3	$N(CH_3)_2$	500
15	CH_3	H	CH_3	H	$N(CH_3)_2$	6.31
16	CH_3	H	CH_3	C_2H_5	$N(CH_3)_2$	0.158
17	CH_3	H	CH_3	C_4H_9	$N(CH_3)_2$	1.258
18	CH_3	H	CH_3	C_6H_{13}	$N(CH_3)_2$	39.8
19	CH_3	H	CH_3	$CH_2C_6H_5$	$N(CH_3)_2$	15.8
20	CH_3	H	CH_3	CH_3	H	> 1000
21	CH_3	H	CH_3	CH_3	CH_3	> 1000

bond length, bond angles, and torsion angles using the semiempirical quantum chemical method AM1[6] encoded in MOPAC 6.0. Finally, all the quantum chemical parameters were calculated with AM1 using fully optimized geometries.

RESULTS AND DISCUSSION

The frontier orbital analysis According to frontier molecular orbital (FMO) theory of chemical reactivity[7], the highest occupied molecular orbital (HOMO), and the lowest unoccupied molecular orbital (LUMO) of the molecules played a major role in governing many chemical reactions and were also responsible for the formation of a transition state which was due to an interaction between HOMO and LUMO of reacting species. Thus, the separation of FMO from other orbitals was based on the general principles governing the nature of chemical reactions. The calculation results showed that the HOMO of all the compounds consisted mainly of the orbitals in phenyl group and N_1 atom while the LUMO of the molecules was contributed from phenyl group and C_{11} atom (Tab 2).

These carbamates might inhibit the AChE by 3 key binding interactions involving the carbon atom of the carbamoyl group, the phenyl ring, and the nitrogen atom of indoline.

The HOMO energies did not show any recognizable relationship with activity (Tab 3), while the LUMO energies showed a decreased tendency with increasing activity. The active compounds had lower LUMO energies. Since the energy of LUMO was directly related to the electron affinity and characterized the susceptibility of the molecule toward attack by nucleophiles, these results suggested that the enzyme act as the nucleophile to provide electrons to these inhibitors.

Tab 2. Properties of frontier molecular orbitals of dihydroindoline carbamates (DHIC).

No	NHOMO	HOMO	LUMO	NLUMO
1	C_{phenyl}	C_{phenyl}, N_1, H_{28}	C_{phenyl}, C_{11}	C_{phenyl}
2	C_{phenyl}	C_{phenyl}, N_1, H_{28}	C_{phenyl}, C_{11}	C_{phenyl}
3	C_{phenyl}	C_{phenyl}, N_1, H_{28}	C_{phenyl}, C_{11}	C_{phenyl}
4	C_{phenyl}	C_{phenyl}, N_1, H_{28}, O_{10}	C_{phenyl}, C_{11}	C_{phenyl}
5	C_{phenyl}	C_{phenyl}, N_1, H_{28}	C_{phenyl}, C_{11}	C_{phenyl}
6	C_{phenyl}	C_{phenyl}, N_1, H_{28}, O_{10}	C_{phenyl}, C_{11}	C_{phenyl}
7	C_{phenyl}	C_{phenyl}, N_1, H_{28}	C_{phenyl}, C_{11}	C_{phenyl}
8	C_{phenyl}	C_{phenyl}, N_1, H_{28}	C_{phenyl}, C_{11}	C_{phenyl}
9	C_{phenyl}	C_{phenyl}, N_1, H_{28}	C_{phenyl}, C_{11}	C_{phenyl}
10	C_{phenyl}	C_{phenyl}, N_1, H_{28}	C_{phenyl}, C_{11}	C_{phenyl}
11	C_{phenyl}	C_{phenyl}, N_1, H_{28}	C_{phenyl}, C_{11}	C_{phenyl}
12	C_{phenyl}, C_{phenyl}	C_{phenyl}, N_1, H_{28}, O_{10}	C_{phenyl}, C_{11}, C_{phenyl}	C_{phenyl}, C_{phenyl}
13	C_{phenyl}	C_{phenyl}, N_1, H_{28}, O_{10}, O_{11}	C_{phenyl}, C_{11}	C_{phenyl}
14	C_{phenyl}	C_{phenyl}, N_1, H_{28}, O_{10}, O_{11}	C_{phenyl}, C_{11}	C_{phenyl}
15	C_{phenyl}	C_{phenyl}, N_1, H_{28}, O_{10}	C_{phenyl}, C_{11}	C_{phenyl}
16	C_{phenyl}	C_{phenyl}, N_1, H_{28}	C_{phenyl}, C_{11}	C_{phenyl}
17	C_{phenyl}	C_{phenyl}, N_1, H_{28}	C_{phenyl}, C_{11}	C_{phenyl}
18	C_{phenyl}	C_{phenyl}, N_1, H_{28}	C_{phenyl}, C_{11}	C_{phenyl}
19	C_{phenyl}, C_{phenyl}	C_{phenyl}, N_1, H_{28}	C_{phenyl}, C_{11}	C_{phenyl}
20	C_{phenyl}	C_{phenyl}, N_1, H_{28}	C_{phenyl}, C_{11}	C_{phenyl}
21	C_{phenyl}	C_{phenyl}, N_1, H_{28}	C_{phenyl}, C_{11}	C_{phenyl}

Tab 3. Energy of HOMO (E_{HOMO}), LUMO (E_{LUMO}), total dipole moment (μ), the most positive net atomic charge (Q_{11}), the most negative net atomic charge (Q_{12}), and molecular polarizability (α) of dihydroxyindoline carbamates.

No	$-\lg IC_{50}$	E_{LUMO}	E_{HOMO}	μ	Q_{11}	Q_{12}	α
1	6.9000	0.3584	−8.3047	2.2920	0.4728	−0.4042	147.5072
2	7.1000	0.3521	−8.3172	2.5590	0.4827	−0.4141	136.8513
3	6.7000	0.3608	−8.3092	2.5920	0.4763	−0.4128	145.9493
4	6.0000	0.3854	−8.2813	2.3970	0.4814	−0.4111	164.6829
5	6.1000	0.3621	−8.3081	2.6520	0.4758	−0.4127	187.3042
6	5.5000	0.3295	−8.4254	2.4780	0.4729	−0.4040	136.8928
7	6.4000	0.3754	−8.2701	2.3420	0.4726	−0.4041	155.9231
8	6.2000	0.3724	−8.2615	2.3580	0.4727	−0.4041	164.3679
9	6.1000	0.3731	−8.2617	2.3800	0.4726	−0.4042	172.4213
10	5.8000	0.3725	−8.2623	2.3870	0.4726	−0.4042	180.6138
11	5.9000	0.3759	−8.2565	2.3850	0.4726	−0.4041	180.2780
12	4.4000	0.3287	−8.2797	2.0830	0.4728	−0.4044	199.9301
13	3.3000	−0.041	−8.7321	4.7410	0.4734	−0.4051	142.2147
14	3.3000	−0.001	−8.6021	4.4790	0.4733	−0.4051	152.6581
15	5.2000	0.3468	−8.3166	2.3770	0.4727	−0.4037	140.0314
16	6.8000	0.3591	−8.2964	2.3210	0.4725	−0.4042	155.1302
17	5.9000	0.3537	−8.3006	2.3720	0.4723	−0.4039	170.7552
18	4.4000	0.3510	−8.3032	2.4090	0.4723	−0.4039	186.6828
19	4.8000	0.2861	−8.3613	2.5860	0.4752	−0.4115	197.3638
20	3.0000	0.4355	−8.1908	2.3080	0.1962	0.0000	105.6422
21	3.0000	0.4997	−8.1290	2.1590	0.2005	0.0000	116.5725

There were 2 compounds in Tab 3 with the LUMO values <0. The physical interpretation of LUMO values <0 still remained controversial. In some cases, when LUMO <0, the molecule

was electron-deficient and stabilized through acquisition of an electron. Since different semiempirical quantum chemical calculation methods and different basis sets in *ab initio* quantum chemical calculations provided not only different magnitudes but different signs for LUMO energies, it was better to rely on trends of orbital properties across a set of molecules rather than on the properties of a single orbital in a single molecule.

The net charge distribution All chemical interactions were by nature either electrostatic (polar) or orbital (covalent). Electric charges in the molecule were obviously the driving force of electrostatic interactions. Thus, net charges on atoms were considered as measures of weak intermolecular interactions.

The carbon atom (C_{11}) of the carbamoyl group (Tab 3) had the most positive net atom charge. Since this was the site of nucleophilic attack, the more positive the charge on this carbon, the more susceptible it might be to nucleophilic attack. It was observed that the most reactive compound had the highest (most positive) charge on this carbon, and the least reactive compound had the lowest (less positive) charges on it, and also that the most active compounds had the most negative charges on the carbonyl oxygen (O_{12}) which could form a hydrogen bond with the enzyme.

These features indicated that the carbon atom of the carbamoyl group was more susceptible to nucleophilic attack in compounds with potent anticholinesterase activity; while the carbonyl oxygen had the more negative charges with increasing activity.

At physiologic pH value these carbamates all displayed a positive charge around the N_1 atom due to protonation, which might form a cation-π bond with the ring of aromatic residues in active site of AChE.

The bond order The strength of carbon-oxygen bond ($C_{11} - O_{10}$) might be reflected by its bond order. Since this bond was broken during the reaction, it was reasonable to speculate that the easier it was to sever this bond, the greater the reactivity of the inhibitors was. The results showed that this bond order was practically invariant across the series of the compounds (all are 0.96), so the anticholinesterase activity of these inhibitors was not dependent on the bond order.

The total dipole moment and molecular polarizability The total dipole moment and the molecular polarizability were quantum-chemical parameters widely used to describe the polarity and the possible inductive interactions of a molecule. Although the calculated values of these parameters had no explicit relationship with their activities (Tab 3), it was found that the compounds with too high or too low total dipole moment had lower activities, while the most active compounds had a lower polarizability.

The calculation suggested 3 characteristic features of interaction: the electrostatic and hydrogen binding interactions between the protonated nitrogen (N_1), the carbonyl oxygen (O_{12}) of DHIC and AChE, and the $\pi - \pi$ interaction between phenyl ring of DHIC and the ring of aromatic residues in the active site of AChE, which played important roles in molecular recognition. These interactions led to charge transfer complexes in which electrons transferred from HOMO of AChE active sites to LUMO of carbon atom (C_{11}) of the carbamoyl group of DHIC.

These observations indicated that nucleophilic power and electron density were crucial in determining the activity of these compounds.

According to the 3D structure of AChE isolated from *Torpedo californica* electric organ[8], the active site of AChE contained a catalytic triad formed by Ser-His-Glu at the bottom of a deep and narrow cavity known as the "aromatic gorge" since more than 50 % of its lining was composed of the rings of 14 conserved aromatic amino acids. Associated with the triad, a putative oxyanion hole formed by the 2 amidic nitrogens of 2 Gly residues was identified.

These carbamates inhibited the enzyme by carbamoylating the serine residue of the catalytic triad. The AChE catalytic triad hydrolysed the ester function of the substrate by a nucleophilic attack of the serine hydroxyl group.

Using the results of these quantum chemistry studies, we suggested a molecular model to explain one possible mode of action by which DHIC fitted into the active site of AChE. DHIC were oriented in the active site of AChE with the carbon atom of the carbamoyl group bound to the

hydroxyl oxygen of Ser residues so that the anionic carbonyl oxygen points towards the NH group of 2 Gly residues (oxyanion hole). The ester oxygen of the carbamate was close to the imidazole ring of His residue, while the protonated nitrogen was directed towards the aromatic moieties of Trp and Phe residues. The docking study required to predict the binding sites between DHIC and AChE was now undertaken.

Finally, it should be noted that both electronic features and steric factors controlled the anticholinesterase activity of DHIC, since many electronic properties did not show any recognizable relationship with activity.

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毒扁豆碱类似物电子结构分析

胡增建，蒋华良，陈建忠，陈凯先[1]，秘汝运
（中国科学院上海药物研究所，上海 200031，中国）

关键词 胆碱酯酶抑制剂；毒扁豆碱；分子模型；计算机辅助设计

目的：在分子水平上阐明 21 个毒扁豆碱类似物的作用机制和结构需求. **方法**：用分子模拟软件构建并优化初始分子结构，选比较分子力场分析模型中预测能力最好的分子构象进行半经验量子化学计算. **结果与结论**：(1) 化合物的 HOMO 主要由苯环和 N_1 原子的轨道组成；LUMO 主要由苯环和 C_{11} 原子组成. HOMO 能量与活性无明显线性关系，但 LUMO 能量随活性的增加而降低，LUMO 能量最低的化合物活性最高；(2) 分子中 C_{11} 原子是净电荷最正的原子，其电荷愈正，化合物的活性愈高. O_{12} 原子是净电荷最负的原子，其电荷愈负，化合物的活性愈高；(3) 所有分子的 $C_{11}-O_{10}$ 键的键级均无变化；(4) 分子的总偶极矩过高或过低都对活性不利，分子极化率低对活性有利.