

**Study on the physicochemical properties and pharmacokinetic
profiles of *bis(7)-tacrine*, a promising anti-Alzheimer's dimer**

by

Hua Yu

A Thesis Submitted in Partial Fulfillment of the
Requirements for the Degree of
Doctor of Philosophy
in
Biomedical Science

A large, faint watermark of the University of Macau seal is centered in the background. The seal is circular, with the text 'UNIVERSIDADE DE MACAU' at the top and '澳門大學' at the bottom. In the center is a shield with a crown on top, and a banner below it with the Chinese characters '經邦濟世'.

© University of Macau

July 2008



UNIVERSITY OF MACAU

INSTITUTE OF CHINESE MEDICAL SCIENCES

The undersigned hereby certify that they have read and recommend to the Institute of Chinese Medical Sciences for acceptance a thesis entitled "Study on the physicochemical properties and pharmacokinetic profiles of *bis(7)-tacrine*, a promising anti-Alzheimer's dimer" by Hua Yu in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Dated: July 2008

Research Supervisor: _____

Professor Yi-fan Han

Professor Yi-tao Wang

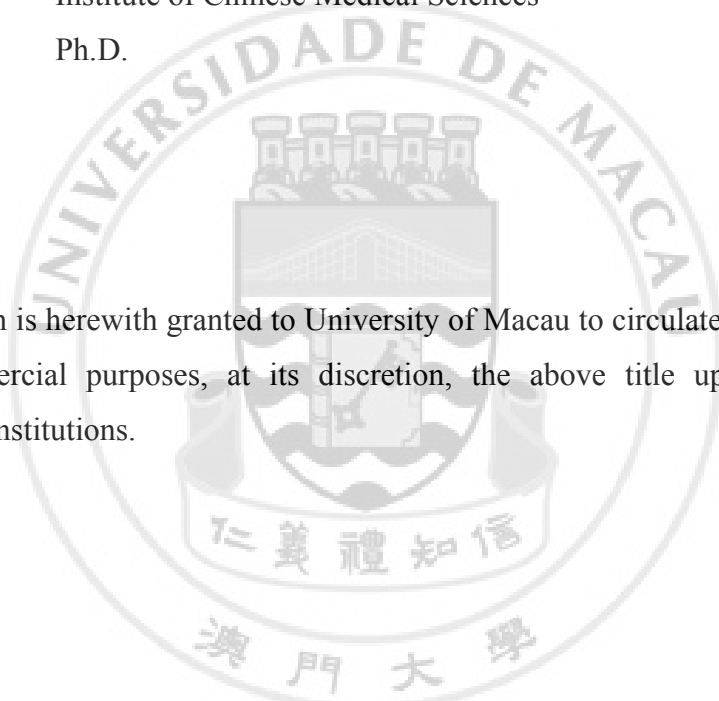
Examining Committee: _____

UNIVERSITY OF MACAU

Date: July 2008

Author: Hua Yu
Title: Study on the physicochemical properties and pharmacokinetic profiles of *bis(7)-tacrine*, a promising anti-Alzheimer's dimer
Faculty: Institute of Chinese Medical Sciences
Degree: Ph.D.

Permission is herewith granted to University of Macau to circulate and to have copied for non-commercial purposes, at its discretion, the above title upon the request of individuals or institutions.



Signature of Author

THE AUTHOR RESERVES OTHER PUBLICATION RIGHTS, AND NEITHER THE THESIS NOR EXTENSIVE EXTRACTS FROM IT MAY BE PRINTED OR OTHERWISE REPRODUCED WITHOUT THE AUTHOR'S WRITTEN PERMISSION.

THE AUTHOR ATTESTS THAT PERMISSION HAS BEEN OBTAINED FOR THE USE OF ANY COPYRIGHTED MATERIAL APPEARING IN THIS THESIS (OTHER THAN BRIEF EXCERPTS REQUIRING ONLY PROPER ACKNOWLEDGEMENT IN SCHOLARLY WRITING) AND THAT ALL SUCH USE IS CLEARLY ACKNOWLEDGED.

Table of contents

Abstract	vi
Acknowledgements	ix
Achievements	xi
Abbreviations	xiii
List of Figures	xvi
List of Tables	xviii

Chapter 1 General Introduction

1.1 New Drug Development	2
1.1.1. From suitable disease to drug target	3
1.1.2. From target to lead compound	3
1.1.3. From lead compound to new candidate compound	3
1.1.4. From new drug candidate to application	4
1.2. Alzheimer's disease (AD)	5
1.3. Strategies for AD treatment	6
1.4. Drug development for AD treatment	8
1.4.1. Current anti-Alzheimer's agents	8
1.4.2. Treatment of AD by Traditional Chinese Medicine (TCM)	10
1.4.2.1. Ginkgo biloba	11
1.4.2.2. Physostigmine	12
1.4.2.3. Huperzine A	13
1.4.3. Structure of AChE and Structure-based Rational Design for Alzheimer's Therapeutics	14
1.4.3.1. Structure of AChE (Dual binding hypothesis)	14
1.4.3.2. Structure-based rational drug design	15
1.4.4. Novel dimers from structure-based rational drug design	16
1.4.4.1. Bis(n)-tacrine — Novel dimers combining two tacrine molecules	16
1.4.4.2. Bis(n)-hupyrindone — Novel dimers combining two huperzine A molecules	18
1.4.4.3. Tacrine-(n)-hupyrindone — Novel dimers combining one molecule of tacrine	

and one molecule of huperzine A	19
1.4.4.4. Selecting dimers for further study	19
1.4.5. Pharmacological mechanisms of bis(7)-tacrine on AD treatment	20
1.4.5.1. AChE inhibition	20
1.4.5.2. Antagonistic actions on N-methyl-D-aspartate (NMDA) receptors	21
1.4.5.3. Inhibition of nitric oxide synthase (NOS)	21
1.4.5.4. Regulation of the L-type calcium channels	22
1.4.5.5. Inhibition of 5-HT-activated current in rat TG neurons	22
1.4.5.6. Multiple effects on AD treatment	23
1.4.6. Toxicity	23
1.5. Significances of this project	24
 Chapter 2 Physicochemical Properties	
<i>Membrane permeability associated physical and chemical properties: Solubility, Stability, Ionization Constants and Lipophilicity</i>	
2.1 Introduction	26
2.1.1. UV-Visible absorption property	27
2.1.2. Stability	27
2.1.3. Dissolution and solubility	27
2.1.4. Ionization (Dissociation constant)	28
2.1.5. Lipophilicity (Partition coefficient)	29
2.1.6. Influences of physicochemical properties on absorption and distribution	31
2.2 Material and Methods	31
2.2.1. Chemicals	31
2.2.2. UV absorption profiles and UV-absorption constants	32
2.2.3. Analytical method	33
2.2.4. Determination of Solubility	33
2.2.5. Determination of Stability	34
2.2.5.1. Stability at different storage conditions	34
2.2.5.2. Stability at different pH conditions	35
2.2.6. Determination of Dissociation Constants	36
2.2.7. Determination of Lipophilicity	38

2.3. Results and discussions	40
2.3.1. UV-absorption profiles and UV-absorption constant	40
2.3.2. Analytical Method	41
2.3.3. Solubility	43
2.3.4. Stability	44
2.3.4.1. Stability to storage conditions	44
2.3.4.2. Stability to pH conditions	46
2.3.5. Dissociation Constants	47
2.3.6. Lipophilicity	49
2.4 Conclusion	51
 Chapter 3 Pharmacodynamic Evaluation	
<i>Selectivity, Potency and Dose Design</i>	
3.1 Introduction	53
3.2. Materials and Methods	54
3.2.1. Chemicals and animals	54
3.2.2. Preparation of AChE/ BChE	55
3.2.3. ChE assay	55
3.2.4. In vitro evaluation on the ChE effect	56
3.2.4.1. Inhibitory effect on AChE	56
3.2.4.2. Inhibitory effect on BChE	56
3.2.4.3. Selectivity on AChE/BChE inhibition	57
3.2.5. In vivo evaluation on the ChE effect	57
3.2.5.1. Dose-response relationship	57
3.2.5.2. Response-time Curve	58
3.3. Results	58
3.3.1. Potency and selectivity	58
3.3.2. Dose-response relationship	60
3.3.3. Response-time curve	61
3.4. Discussion	61
3.5. Conclusion	64
 Chapter 4 Pharmacokinetic study	

Absorption, Distribution, Metabolism, Excretion and pharmacokinetic profiles of bis(7)-tacrine

4.1. Introduction	67
4.1.1. Absorption	68
4.1.2. Distribution	71
4.1.3. Elimination (Metabolism and Excretion)	71
4.1.4. Pharmacokinetics	73
4.2. Chemicals and animals	74
4.2.1. Chemicals	74
4.2.2. Animals	75
4.3. Analytical methods	75
4.3.1. Determination of B7T in blood sample	75
4.3.2. Determination of B7T in organs	77
4.3.3. Results and discussion	79
4.3.4. Conclusion	87
4.4. Absorption	88
4.4.1. Experimental	88
4.4.2. Results	94
4.4.3. Discussion	97
4.5. Distribution	99
4.5.1. Experimental	99
4.5.2. Results and discussion	100
4.6. Metabolism	105
4.6.1. Experimental	105
4.6.2. Results and discussion	107
4.6.3. Conclusion	112
4.7. Pharmacokinetics	112
4.7.1. Experimental	112
4.7.1.1. Comparative pharmacokinetic profiles of B7T via different administration routes	112
4.7.1.2. Effect of surfactant on oral bioavailability	113
4.7.1.3. Sample determination	113
4.7.1.4. Data analysis	114
4.7.2. Results	115

4.7.2.1. Comparative pharmacokinetic profiles of B7T via different administration routes	115
4.7.2.2. Effect of surfactant on oral bioavailability	119
4.7.3. Discussion	122
4.7.4. Conclusion	124
 Chapter 5 General Discussion	
5.1 Overview	127
5.2. Contributions of the present study	127
5.2.1. Predicting the in vivo characteristics from physicochemical properties	127
5.2.2. Absorption mechanisms of bis(7)-tacrine	128
5.2.3. Pharmacodynamic evaluation associated with the in vivo distribution	128
5.2.4. Hepatic and intestinal metabolism of bis(7)-tacrine	129
5.2.5. Comparative pharmacokinetic profiles of bis(7)-tacrine after various administration routes	129
5.2.6. Directions for the investigation for the other dimers to structure-activity relationship investigation	130
References	131

Abstract

Alzheimer's Disease (AD) is a chronic neurodegenerative disease that attacks the brain and results in impaired memory, thinking and behavior in the elderly. There are many different theories on the causes of AD and one of the well-established theories suggests that the neurotransmitter acetylcholine levels are too low in the brains of AD patients. Although several acetylcholinesterase (AChE) inhibitors have been developed, they are only useful for treating patients with mild to moderate AD. Tacrine, the first approved AChE inhibitor for AD, has been found to have potential hepatotoxicity and is therefore seldom used nowadays. As a result, the development of more effective anti-AD agents is still an important area of AD-associated research.

With the identification of the three-dimensional structure of AChE and the assistance of computer docking programs, three series of novel dimers have been designed based on the structures of tacrine and huperzine A. Of the newer dimers, bis(7)-tacrine (B7T), bis(12)-hupyrindone (B12H) and tacrine-(10)-hupyrindone (T10H) are three typical compounds from those candidates. Among them, B7T has been demonstrated to have selective and potent AChE inhibitory effects both in vitro and in vivo, but also shown to have multiple actions such as antagonizing N-methyl- d-aspartate (NMDA) receptors and gamma-aminobutyric acid (GABA_A) receptors and inhibiting nitric oxide synthase, which may play synergistical roles in the treatment of AD.

Although these dimers are expected to be the promising drugs for the treatment of AD in clinic, lack of information on the physicochemical properties as well as pharmacokinetic profile and related pharmacokinetic parameters delays its further development. Therefore, the objective of this project is focused on the measurement of the pharmacokinetic profiles and parameters as well as the pharmacokinetics related physicochemical properties for B7T because of its advantages identified in our previous pharmacological studies.

In **Chapter 1**, the background of our project is introduced. The basic principle for the molecular design and development of the novel dimeric agents are also displayed. Although we have gotten three series of different homo- and hetero-dimer from tacrine and huperzine A, but only B7T is selected in this study because of its advantages and clear pharmacological mechanisms. Moreover, the significances of this project are also

presented in this chapter.

In **Chapter 2**, we measured some main physicochemical properties of B7T including solubility, stability, ionization constants and lipophilicity. The results indicate that B7T is poorly soluble in water ($12.71 \text{ mg}\cdot\text{mL}^{-1}$), and sparingly soluble in saline (0.36 mg/ml) and phosphate buffers ($0.33\text{--}0.47 \text{ mg}\cdot\text{mL}^{-1}$). The solubilities are considerably affected by pH value and the ionic strength (main the $[\text{Cl}^-]$) of the solution. Two pK_a values are determined as 8.7 ± 0.1 (pK_{a1}) and 10.7 ± 0.4 (pK_{a2}) for B7T in water. The high $\log P$ value for B7T is found to be 8.2, indicating that the B7T are highly lipophilic. All these physiochemical characteristics suggest that B7T maybe have the favorite properties for membrane permeability in vivo.

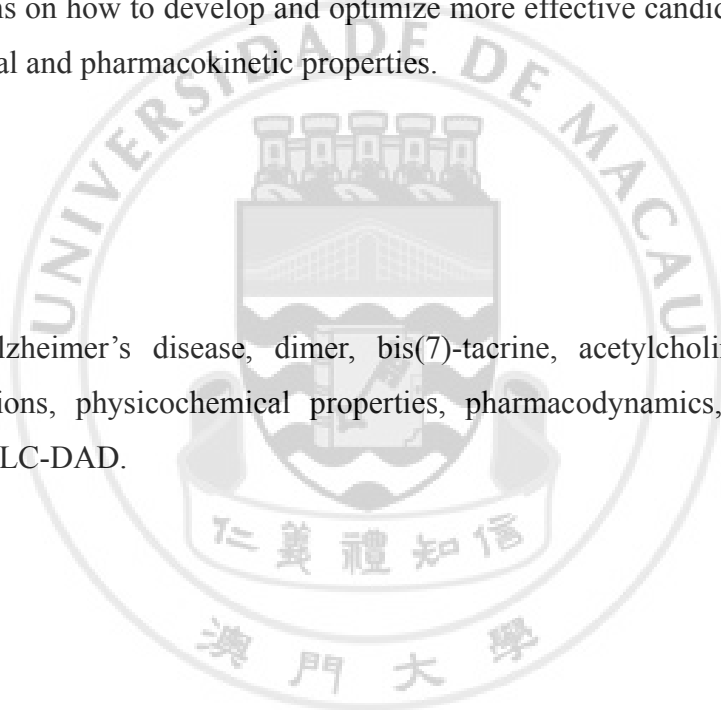
In **Chapter 3**, based on the results obtained from our previous studies, the in vivo dose-response relationship and response-time curve for B7T are evaluated to rats. As the results, the ED_{50} is calculated as 1.44 mg/kg (i.v.) according to the inhibition to the AChE in brain. However, higher dose ($>5 \text{ mg}\cdot\text{kg}^{-1}$, i.v.) may cause to the side-effects even to death ($>7 \text{ mg}\cdot\text{kg}^{-1}$, i.v.). Finally, the dose of 1 mg/kg is selected for the i.v. administration in our later studies. Moreover, the response-time curve suggests that B7T can quickly exhibit its AChE inhibition in rat brain in 15~30 min, and duration of this effect can be kept more than 4 h after administration. However, the fast blood elimination can be observed by measuring the BChE inhibitory effect in rat serum. To directly measure the B7T concentrations in blood and brain, the further pharmacokinetic studies are designed and carried out to establish the relationship between the B7T concentrations to time.

In **Chapter 4**, the analytical methods (HPLC-DAD and HPLC-MS) are established and validated for the bio-sample quantification. Furthermore, the pharmacokinetic profiles of B7T including **A**bsorption, **D**istribution, **M**etabolism and **E**xcretion are systemic studied using different administration routes and the relative parameters are also compared. As results, B7T was found poorly absorbed on the Caco-2 cell monolayer and rat intestinal perfusion models with the passive transport although it has the high lipophilicity. However, much extensive tissue distribution was measured by determining the B7T contents in different organs (heart, liver, spleen, lung, kidney, brain and blood) at different time after dosing. Renal excretion is considered as the main elimination route as presented as the highest $\text{AUC}_{\text{organ}}/\text{AUC}_{\text{blood}}$ value (5789%). The in vitro incubation of B7T with intestine or liver microsomes suggests that B7T is very stable in intestine but first-pass maybe occur in liver, and the hydroxylation is the main metabolic route of B7T in vivo. Further study the

pharmacokinetic profiles of B7T after various administration routes (i.v., i.p., i.m. and p.o.), B7T shows a very low absolute bioavailability after oral administration (12.6% with dose of 20 mg•kg⁻¹ and 9.4% of 50 mg•kg⁻¹). Although we try to increase the oral bioavailability by using of the nonionic surfactant, Pluronic F-68, it only extends the absorption rate of B7T from the gastrointestinal tract but change the absorption extent. Further study on the enhancing of the bioavailability with the assistance of absorption enhancer or pharmaceutical techniques need to be carefully considered.

In **Chapter 5**, the overviews of the present study are discussed. The contributors of this study are not only help us to well understand the physicochemical properties and in vivo characteristics of the promising anti-Alzheimer's dimer, B7T, but also give us many useful directions on how to develop and optimize more effective candidates with favorable physicochemical and pharmacokinetic properties.

Keywords: Alzheimer's disease, dimer, bis(7)-tacrine, acetylcholinesterase inhibitor, multiple functions, physicochemical properties, pharmacodynamics, pharmacokinetics, HPLC-MS, HPLC-DAD.



Acknowledgements

It is a valuable experience to have participated in the investigation of the novel anti-Alzheimer's dimers, by which I can contribute to the public health. Beside gaining lots of knowledge in the fields of pharmacology, pharmacodynamics and pharmacokinetics, I learnt to undergo research of high quality, to work under pressure and to analyze problems in different points of view. However, performing research is never a smooth and easy task. I would like to express my sincere thanks to the following people, without their help, I could not have finished my PhD studies.

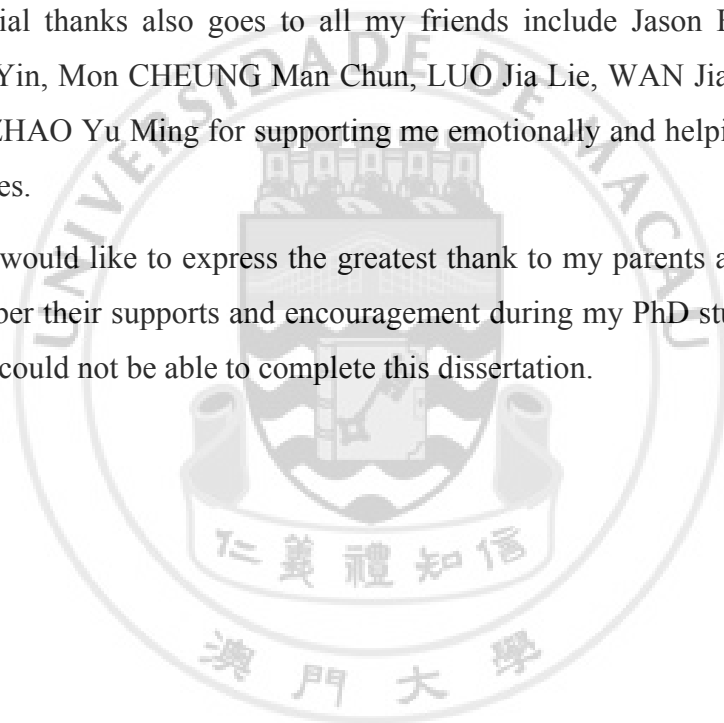
First of all, my graduate must be given to my supervisors Prof HAN Yi Fan, Prof. WANG Yi Tao and Prof. LIANG Chun. Their expertise and guidance not only enlightened me and helped to overcome the obstacles in my project, but also encouraged me when I was confused and frustrated by the failure of experiments. Moreover, I will give my thanks to Prof LIN Ge (Department of Pharmacology, The Hong Kong Chinese University) and Prof ZUO Zhong (School of Pharmacy, The Hong Kong Chinese University). Prof LIN gave me so many directions for me on my first publication, without her help, that paper may not be published as the expected time. Prof. ZUO and her group gave me so much help for the determination of my samples, so that I can finished my project during the period of my PhD studies. In addition, I am thankful to the financial supports of this project by the Research Grants Council of Hong Kong (HKUST 6133/03M, 6441/06M and 6608/07M) and Macao Science & Technology Development Fund (Ref. No. 049/2005/A-R10).

Beside, I would like to thank for Mr. Nelson T.K. Lee (Department of Biochemistry, The Hong Kong University of Science and Technology, Hong Kong SAR, China), Prof. Kelvin CHAN (School of Applied Sciences, University of Wolverhampton, United Kingdom), Prof Nancy IP (Department of Biochemistry, The Hong Kong University of Science and Technology, Hong Kong SAR, China), Dr. LI Wen Ming (Department of Biochemistry, The Hong Kong University of Science and Technology, Hong Kong SAR, China), Dr. FU Hong Jun (Department of Biochemistry, The Hong Kong University of Science and Technology, Hong Kong SAR, China), Dr. Kelvin KAN Ka Wing (Department of Biochemistry, The Hong Kong University of Science and Technology, Hong Kong SAR, China), Prof LI Shao Ping (Institute of Chinese Medical Science,

University of Macau, Macau SAR, China), Prof PANG Yuan Ping (Computer-Aided Molecular Design Laboratory, Mayo Clinic College of Medicine, Rochester, Minnesota, USA), Dr. GU Ze Ming (XenoBiotic Laboratories, Inc., Plainsboro, NJ, USA), Dr. Zhang Li (Department of Biochemistry, The Hong Kong University of Science and Technology, Hong Kong SAR, China) for their patience, advices and criticisms given as the external and internal collaborators of my thesis respectively.

Moreover, I am grateful to all the staffs in Department of Biochemistry (HKUST) and Institute of Chinese Medical Sciences (University of Macau). They provide a comfortable and delightful environment to work and to study. I also thank to the Biotechnology Research Institute of Hong Kong University of Science and Technology for the technical supports. Special thanks also goes to all my friends include Jason HO Man Kit, Peter CHOW Chun Yin, Mon CHEUNG Man Chun, LUO Jia Lie, WAN Jian Bo, LI Peng, HU Yuan Jia and ZHAO Yu Ming for supporting me emotionally and helping me to overcome many difficulties.

Finally, I would like to express the greatest thank to my parents and my sister. I will always remember their supports and encouragement during my PhD studies. Without their endless love, I could not be able to complete this dissertation.



Achievements

1. **Yu, H.**; Ho, J.M.; Kan, K.K.; Cheng, B.W.; Li, W.M.; Zhang, L.; Lin, G.; Pang, Y.P.; Gu, Z.M.; Kelvin, C.; Wang, Y.T.; Han, Y.F.

Development of a high performance liquid chromatography-tandem mass method for determination of bis(7)-tacrine, a promising anti-Alzheimer's dimer, in rat blood.

J Pharm Biomed Anal. 2007, **44**(5): 1133-1138.

2. Fu, H.; Li, W.; Liu, Y.; Lao, Y.; Liu, W.; Chen, C.; **Yu, H.**; Lee, N.T.; Chang, D.C.; Li, P.; Pang, Y.; Tsim, K.W.; Li, M.; Han, Y.

Mitochondrial proteomic analysis and characterization of the intracellular mechanisms of bis(7)-tacrine in protecting against glutamate-induced excitotoxicity in primary cultured neurons.

J Proteome Res. 2007, **6**(7): 2435-2446.

3. Luo, J.; Li, W.; Liu, Y.; Zhang, W.; Fu, H.; Lee, N.T.; **Yu, H.**; Pang, Y.; Huang, P.; Xia, J.; Li, Z.W.; Li, C.; Han, Y.

Novel dimeric bis(7)-tacrine proton-dependently inhibits NMDA-activated currents.

Biochem Biophys Res Commun. 2007, **361**(2): 505-509.

4. **Yu, H.**; Li, W.M.; Kan, K.W.; Kelvin C.; Wang, Y.T.; Han, Y.F.

[Research Progress on the Pharmacokinetics of Anti-Alzheimer's Agents].

Chin Pharm J. 2007, **42**(15): 1121-1125.

5. **Yu, H.**; Wang, Y.T.; Han, Y.F.

Progress on the clinical therapeutics for Alzheimer's disease by Traditional Chineses Medicine.

Paper in 2007 Annual Meeting of Specialty Committee of Chinese Medicine Pharmacology World Federation of Chinese Medicine Societies. China, Zhen Zhou. 2007.11 Page 165-169.

6. **Yu, H.**; Li, W.M.; Kan, K.K.; Ho, J.M.; Carlier, P.R.; Pang, Y.P.; Gu, Z.M.; Zuo, Z.; Kelvin, C.; Wang, Y.T.; Han, Y.F.

The physicochemical properties and the in vivo AChE inhibition of two potential anti-Alzheimer agents, bis(12)-hupyrindone and bis(7)-tacrine.

J Pharm Biomed Anal. 2008, **46**(1): 75-81.

7. Zhang, L.; **Yu, H.**; Li, W.M.; Cheung, M.C.; Pang, Y.P.; Lin, G.; Wang, Y.T.; Zuo, Z.; Han, Y.F.

Selective and sensitive determination of bis(7)-tacrine, a high erythrocyte binding acetylcholinesterase inhibitor, in rat plasma by high-performance liquid chromatography-tandem mass spectrometry.

Biomed Chromatogr. 2008, **22**; 414-420

8. Zhang, L.; **Yu, H.**; Li, W.M.; Cheung, M.C.; Pang, Y.P.; Wang, Y.T.; Zuo, Z.; Han, Y.F.

Preclinical characterization of intestinal absorption and metabolism of promising anti-Alzheimer's dimer bis(7)-tacrine.

Int. J. Pharm. 2008, **357**: 85-94.

Submitted Manuscripts

1. **Yu, H.**; Li, W.M.; Cheung, M.C.; Zuo, Z.; Carlier, P.R.; Gu, Z.M.; Huang, M.; Wang, Y.T.; Han, Y.F.

Development of a High-Performance Liquid Chromatographic Method for the Pharmacokinetic Study of a Novel Dimeric Acetylcholinesterase Inhibitor Bis(12)-hupyrindone in Rat after Intravenous Administration.

2. **Yu, H.**; Cheung, M.C.; Li, W.M.; Zhang, L.; Zuo, Z.; Pang, Y.P.; Wang, Y.T.; Han, Y.F.

In vivo ChE inhibition, tissue distribution and comparative pharmacokinetic profiles of bis(7)-tacrine in rat.

3. Fu, H.J.; Li, W.M.; Luo, J.L.; Hu, Q.S.; **Yu, H.**; Lee, N.T.K.; Li, M.T.; Tsim, W.K.; Pang, Y.P.; Youdim, M.B.H.; Han, Y.F.

A Promising anti-Alzheimer Drug Dimer, Bis(7)-tacrine, Inhibits β -secretase and Activates α -secretase via the Activation of Protein Kinase C.

Abbreviations and symbols

Symbols

$AUC_{0 \rightarrow \infty}$	Area under the concentration-time curve from time zero to infinity
$AUC_{0 \rightarrow t}$	Area under the concentration-time curve from time zero to time t
CL	Apparent clearance
$C_{\max}$	Maximum blood concentration
EC_{50}	The half maximal effective concentration
ED	Effective dose
F	Asolute bioavailability
IC_{50}	The half inhibitory concentration
LD	The minimum toxic dose
LD_{50}	The median lethal dose
$\log D$	Logarithm of 1-octanol/phosphate buffer coefficient
$\log D_{7.4}$	Logarithm of distribution coefficient (D) at pH 7.4
$\log P$	Logarithm of 1-octanol/water partition coefficient
P_{app}	Permeability coefficient
pK_a	Negative of the ionization constant K_a
$t_{1/2}$	Elimination half-life
$t_{\max}$	Time to peak concentration
V_d	Volume of distribution

Abbreviations

ACh	Acetylcholine
AChE	Acetylcholinesterase
AChEIs	Acetylcholinesterase inhibitors

ACN	Acetonitrile
AD	Alzheimer's disease
ADME	Absorption, distribution, metabolism and excretion
ANOVA	Analysis of variance
AUC	Area under the concentration-time curve
A β	Protein beta amyloid
B6T	Bis(6)-tacrine
B7T	Bis(7)-tacrine
BChE	Butyrylcholinesterase
BSA	Bovine serum albumin
ChE	Cholinesterase
CYP	Cytochrome
DAD	diode array detector
ELSD	Evaporative light scattering detector
EVOM	Epithelial tissue volt-ohmmeter
FBS	Fetal bovine serum
FDA	US Food and Drug Administration
G6P	D-glucose 6-phosphate
G6PDH	Glucose-6-phosphate dehydrogenase
GI	Gastrointestinal
HA	Huperzine A
HBSS	Hanks balanced salt solution
HPLC	High performance liquid chromatography
i.m.	Intramuscular injection
i.p.	Intraperitoneal injection
IS	Internal standard
i.v.	Intravenous injection
LOD	Limit of determination
LOQ	Limited of quantification
MRT	Mean residence time

MS	Mass spectrometry
NCE	Novel chemical entity
NFTs	Neurofibrillary tangles
NMDA	N-methyl-d-aspartate
NOS	Nitric oxide synthase
PAMPA	The parallel artificial membrane permeability assay
PD	Pharmacokinetics
P-gp	P-glycoprotein
PK	Pharmacokinetics
p.o.	Oral administration
RSD	Relative standard deviation
S.D.	Standard deviation
S.E.M.	Standard error of mean
SPIP	Single-pass intestinal perfusion
TCMs	Traditional Chinese Medicines
TEER	Transepithelial electrical resistance
TFA	Trifluoroacetic acid



List of Figures

Fig. 1.1	Phase of new drug discovery and development	2
Fig. 1.2	Number of people with dementia in developed and developing countries	5
Fig. 1.3	Structures of the current Anti-Alzheimer drugs	9
Fig. 1.4	The leaves of <i>Ginkgo biloba</i> and structure of <i>Ginkgo biloba</i> EGb761	11
Fig. 1.5	The seed of <i>physostigma venenosum</i> Balf. and structure of Physostigmine	12
Fig. 1.6	The plant of <i>Huperzia serrata</i> and structure of Huperzine A	13
Fig. 1.7	A diagrammatic illustration of dual-site binding of a dimeric inhibitor L ₁ -L ₂ to the catalytic and peripheral sites of AChE.	15
Fig. 1.8	Structure-based rational drug design for novel dimeric AChE inhibitor	16
Fig. 1.9	Synthesis of analogs derived from tacrine	17
Fig. 1.10	Compound derived from the fragment of huperzine A (huprydone)	18
Fig. 1.11	The structures of bis(7)-tacrine, huprydon(10)-tacrine and bis(12)-huprydone	19
Fig. 2.1	Simplified view of the absorption process	28
Fig. 2.2	Change of pH values in gastrointestinal tract	29
Fig. 2.3	UV-absorption profile of B7T in water from λ_{190} to λ_{400}	40
Fig. 2.4	The graph of solubility versus pH values (2-12) for B7T in isotonic buffer	44
Fig. 2.5	Effects of different pH buffer solutions on the UV absorption of TAC and B7T	48
Fig. 2.6	The graph of partition coefficient (1-octanol/buffer) against pH values of the phosphatic buffers for TAC and B7T	50
Fig. 3.1	Relationships between log[Drug] and AChE or BChE inhibition	59
Fig. 3.2	Inhibitory effect on cortex AChE activity with different dosages of B7T	60
Fig. 3.3	ED ₅₀ of B7T on AChE inhibition	60
Fig. 3.4	The inhibition of AChE/ BChE in different brain tissue/ serum from 5min to 4hr after i.v. administration of B7T at 1 mg•kg ⁻¹	62
Fig. 4.1	The general stages and their relationships in the life cycle of a drug after administration	67

Fig. 4.2 Routes by which solutes can traverse cell membranes	69
Fig. 4.3 Illustration for PAMPA, Caco-2 cell monolayer and rat single-pass intestinal perfusion	70
Fig. 4.4 Structures of bis(7)-tacrine, bis(6)-tacrine and pimoziide	74
Fig. 4.5 The full-scan ESI-MS/MS spectra for B7T and pimoziide	81
Fig. 4.6 HPLC-MS/MS chromatograms of B7T and Pimoziide in rat blood	82
Fig. 4.7 Stability of B7T in mobile phase at 25 °C	83
Fig. 4.8 HPLC chromatograms of B7T and B6T in different organs	85
Fig. 4.9 Permeability of B7T across artificial membrane at various pH	94
Fig. 4.10 Permeabilities of B7T and propranolol across Caco-2 cell monolayer	95
Fig. 4.11 Cumulative amount of B7T appearing in the mesenteric blood over time in the rat jejunum single-pass perfusion study	96
Fig. 4.12 Concentrations of B7T in different organs after i.v. administration of B7T	103
Fig. 4.13 Organs and blood concentration-time profiles of B7T after intravenous administration of 1.0 mg•kg ⁻¹ B7T to rats	103
Fig. 4.14 AUC of B7T organs and blood after intravenous administration of 1.0 mg•kg ⁻¹ B7T to rats	104
Fig. 4.15 Percentage of B7T remained during its 2-hour in vitro incubation with rat liver microsome	109
Fig. 4.16 HPLC/UV chromatogram of the samples obtained after 14-hour incubation with rat liver microsome in absence and presence of NADPH	109
Fig. 4.17 The mass spectra and product ion spectra of B7T and its metabolites	110
Fig. 4.18 Postulated fragmentation pathway of B7T and its hydroxylated metabolite under positive ion mode	111
Fig. 4.19 Blood concentration-time profiles of B7T after: i.v., i.p., i.m. and p.o. administrated to rats	117
Fig. 4.20 Effect of Pluronic F-68 on the pharmacokinetic profiles of B7T after orally administrated to rats at the dose of 20 mg•kg ⁻¹	120

List of Tables

Table 1.1	Inhibition of AChE and BChE by tacrine and its dimeric analogs	17
Table 2.1	Storage conditions for the stability test of B7T	35
Table 2.2	UV absorbance of B7T in water at different concentrations	41
Table 2.3	Intra-day, inter-day accuracy and precision for the determination of TAC and B7T	42
Table 2.4	Effect of ionic strength on solubilities for B7T	43
Table 2.5	Chemical stability of B7T under different storage conditions	45
Table 2.6	Pharmacological stability of B7T under different storage conditions	46
Table 2.7	Stability of B7T in water and phosphate buffers	47
Table 2.8	Determination of pKa' values for TAC and B7T	48
Table 2.9	The retention times, logk and logP values of the reference compounds	49
Table 3.1	In-vitro inhibition on AChE and BChE for B7T and tacrine	59
Table 4.1	Intra-day and inter-day precision, and accuracy of the HPLC-MS/MS assay for bis(7)-tacrine in rat blood	83
Table 4.2	Calibration curves for B7T determination in different organs	86
Table 4.3	Intra- and inter-day accuracy and precision for determination of B7T in different organs	87
Table 4.4	Erythrocyte binding of B7T at different blood concentration levels	101
Table 4.5	Contents of B7T in heart, liver, spleen, lung, kidney brain and brain at different time after intravenously administrated to rats	102
Table 4.6	Pharmacokinetic parameters of B7T in rats after i.v., i.p., i.m. and oral administration	118
Table 4.7	Pharmacokinetic parameters of B7T in rats after oral administration of B7T at 20 mg•kg ⁻¹ without or with 0.2%, 1.0% Pluronic F-68	121