

การออกแบบและประเมินศักยภาพของสารสังเคราะห์ชนิดใหม่ซึ่งยับยั้ง เอนไซม์ย่อยโปรตีน

เพื่อรักษาโรคเอมไฟไซมาและอาร์ไธริส

(THE DESIGN, SYNTHESIS AND EVALUATION OF NEW PROTEASE INHIBITORS

AS POTENTIAL DRUGS AGAINST EMPHYSEMA AND ARTHRITIS)



นางสาวนฤมล เครื่ององอาจนกุล

ศูนย์วิทยทรัพยากร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาดำเนินหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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THE DESIGN, SYNTHESIS AND EVALUATION OF NEW PROTEASE INHIBITORS
AS POTENTIAL DRUGS AGAINST EMPHYSEMA AND ARTHRITIS



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หัวข้อวิทยานิพนธ์	การออกแบบและประเมินศักยภาพของสารสังเคราะห์ชนิดใหม่ซึ่งยับยั้ง เอนไซม์ ย่อยโปรตีน เพื่อรักษาโรคเอดส์และอาร์โธรทิส
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บทคัดย่อ

งานวิจัยนี้ได้ศึกษาวิธีการสังเคราะห์กลุ่มของสารที่มีฤทธิ์เฉพาะเจาะจงในการยับยั้ง เอนไซม์ กลุ่มเซรีนโปรติเอส โดยใช้ทริพซินและโคโมทริพซินเป็นแม่แบบ สารยับยั้งที่สังเคราะห์ขึ้นทั้งหมดนี้ เป็นสารใหม่ซึ่งยังไม่เคยมีผู้ใดได้กระทำมาก่อน วิธีการสังเคราะห์สารยับยั้งเหล่านี้เป็นวิธีการที่รู้จักกันมาแล้ว การทำให้ผลิตภัณฑ์ที่ได้บริสุทธิ์โดยการตกผลึกหลายครั้ง ทดสอบความบริสุทธิ์โดยวิธี ทินเลเยอร์ โครมาโตกราฟี ประกอบกับการวิเคราะห์ทางองค์ประกอบของธาตุในสารประกอบ นอกจากนี้ยังได้ทำการพิสูจน์สูตรโครงสร้างของผลิตภัณฑ์ทุกตัวที่เกิดขึ้นได้กระทำอย่างละเอียดรอบคอบ โดยวิธีทางสเปกโตรสโคปี ได้แก่ อินฟราเรด โปรตอนและคาร์บอน 13 นิวเคลียร์แมกเนติก-เรโซแนนซ์

ในการศึกษาคุณสมบัติของสารยับยั้งต่อเอนไซม์ เริ่มต้นโดยการทดสอบสภาวะที่เหมาะสม ในการทำงานของเอนไซม์ และทดสอบผลการยับยั้งต่อการทำงานของ เอนไซม์ทริพซินและโคโมทริพซิน โดยใช้ไลน์ ริเวอร์-เบอร์คพลอต ทำให้ได้ความสัมพันธ์ระหว่างอัตราเร็วของปฏิกิริยากับความเข้มข้นของสารซึ่งทำให้เราทราบได้ว่าสารยับยั้งที่ทำการศึกษานี้มีคุณสมบัติในการยับยั้ง เอนไซม์อย่างไร จากผลของการศึกษานี้พบว่าเราสามารถพัฒนาสารยับยั้งชนิดใหม่ ที่ออกฤทธิ์อย่างเฉพาะเจาะจงที่มี ประสิทธิภาพและเป็นสารยับยั้งที่ไม่มีพิษ ซึ่งแสดงให้เห็นว่าสารยับยั้งเหล่านี้ น่าจะเป็นตัวยาในการ รักษาโรคเอดส์และอาร์โธรทิสในคนต่อไปได้

Thesis Title The Design, Synthesis and Evaluation of New Protease
 Inhibitor as Potential Drugs against Emphysema and
 Arthritis

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ABSTRACT

In the course of this research work, a series of specific inhibitors of serine proteases were synthesised. All of these synthetic inhibitors are novel. The route to these new compounds followed well proven, known methods. Purification was effected by multiple recrystallizations. The purity of the end products was confirmed by thin-layer chromatography and elemental analysis. All the structures were thoroughly elucidated by infrared, proton and carbon 13 nuclear magnetic resonance spectroscopies.

In the course of testing the inhibitory activities of these new inhibitors, firstly the optimum conditions for the enzyme-assays were established. Then the assays were carried out to determine the effectiveness of the inhibitors against trypsin and chymotrypsin. This was followed by accurate rate measurements as a function of concentration, in order to establish the mode of action of the inhibitors, by the interpretation of Lineweaver-Burk plots. As a result of these investigations new, effective, specific and non-toxic inhibitors were developed, which show promising as drugs against emphysema and arthritis in man.



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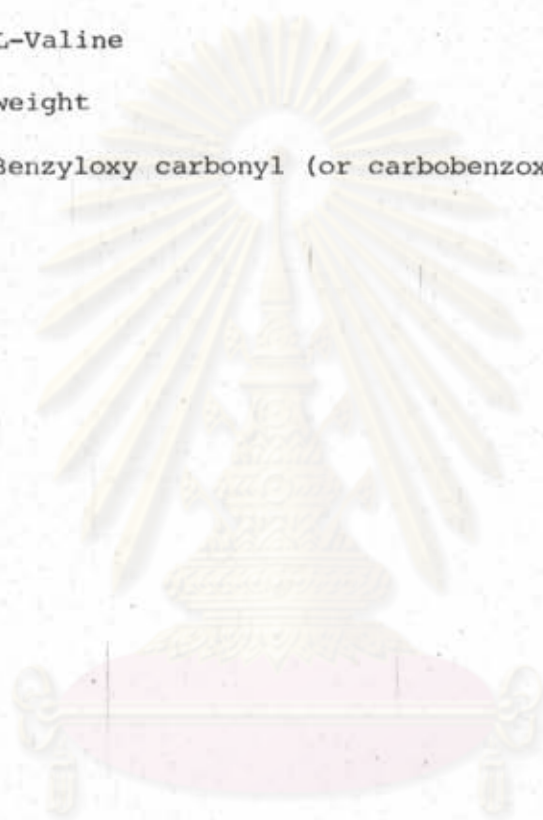


List of Abbreviations

A	L-Alanine
Abs	Absorbance
BAN	t-butyloxy carbonyl-L-alanine 4-nitrophenyl ester
b.p.	boiling point
br	broad
°C	degree celsius
cm	centimeter
cm ³	cubic centimeter
¹³ C NMR	carbon 13 Nuclear Magnetic Resonance
d	doublet
DMSO	dimethyl sulfoxide
E	enzyme
ε	molar extinction coefficient
ES	enzyme-substrate complex
ESI	enzyme-substrate-inhibitor complex
g	gram
HEPES	N-2-hydroxyethyl piperazine-N'-2-ethane sulfonic acid
HLE	hyman leukocyte elastase
¹ H NMR	proton Nuclear Magnetic Resonance
hrs	hours
I	inhibitor
ID ₅₀	50% inhibition dose (the concentration which inhibits 50% of the enzyme activity under assay conditions specified in the experimental section)
IR	Infrared
Kg	kilogram

lit	liter
LD ₅₀	lethal dose
M	mole per liter (or molar)
m	multiplet
mol	gram-molecule
m mole	milli gram-molecule
mm	millimeter
mM	millimolar
ml	milliliter
min	minute
mg	milligram
m.p.	melting point
nm	nanometer
P _n	amino acid position in polypeptide chain of substrate or inhibitor
PPE	porcine pancreatic elastase
ppm	parts per million
q	quartet
R _f	ratio of distance a compound moves to distance solvent front moves
s	singlet
S	substrate
S _n	subsite in active site of enzyme
Suc	succinyl
t	triplet
t-BOC	tertiary butyloxycarbonyl
THF	tetrahydrofuran

TLC	thin layer chromatography
μl	microliter
μM	micromolar
UV	ultraviolet
v	volume
V	L-Valine
w	weight
Z	Benzyloxy carbonyl (or carbobenzoxy)



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