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SYNTHESIS AND EVALUATION OF AMINO ACID DERIVATIVES AS
PROTEASE INHIBITORS ON CATHEPSIN G
TRYPSIN AND CHYMOTRYPSIN

MISS WIMON PORNSAWATCHAI

A Thesis Submitted in Partial Fulfillment of the Requirements
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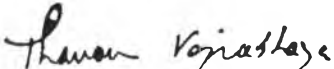
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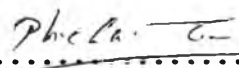
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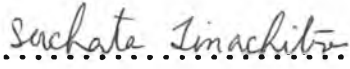
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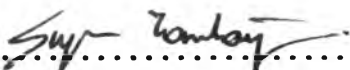
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รวม พลสวัสดิชัย : การสังเคราะห์และประเมินผลการเป็นตัวยับยั้ง เอนไซม์ย่อยโปรตีนของ
อนุพันธ์กรดอะมิโนที่มีต่อ คาเทปซิน-จี ทริปซิน และโคโมทรופן (SYNTHESIS AND EVA-
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งานวิจัยนี้ได้ทำการสังเคราะห์เปปไทด์หรืออนุพันธ์ของกรดอะมิโนโดยวิธี mixed anhydride
สารที่สังเคราะห์ขึ้นทั้งหมดจำนวน 10 ตัวนี้เป็นสารใหม่ซึ่งยังไม่มีใครได้สังเคราะห์มาก่อน การทำเปป-
ไทด์ที่สังเคราะห์ให้บริสุทธิ์ทำได้โดยการตกผลึกหลายครั้ง แล้วทำการทดสอบความบริสุทธิ์ด้วยวิธีทินแลร์โคร-
มาโทกราฟี และวิเคราะห์หาองค์ประกอบของธาตุในสารประกอบ นอกจากนี้ยังได้ทำการพิสูจน์สูตรโครง
สร้างของผลิตภัณฑ์ทุกตัวโดยวิธีทางสเปกโทรสโกปี ได้แก่ อินฟราเรด โพรตอน และ คาร์บอน-13 นิว-
เคลียร์แมกเนติกเรโซแนนซ์

จากการทดสอบคุณสมบัติในการยับยั้ง เอนไซม์กลุ่มเซรินโปรติเอส 3 ชนิด คือ ทริปซิน โคโม-
ทรופן และคาเทปซิน-จีที่สภาวะทางสรีระ พบว่าตัวยับยั้งทั้งหมดให้ค่าการยับยั้งที่ต่ำสำหรับทริปซิน และ
เป็นตัวยับยั้งที่ดีสำหรับโคโมทรופן อย่างไรก็ตามสำหรับคาเทปซิน-จีในสภาพที่ทำการทดสอบให้ค่าการออก
ฤทธิ์ที่ต่ำมากจนไม่สามารถทำการทดสอบกับตัวยับยั้งที่สังเคราะห์ได้ นอกจากนี้ยังได้ทำการทดสอบสารสัง-
เคราะห์ซึ่งกล่าวว่ามีความสัมพันธ์ในการยับยั้ง เอนไซม์โคโมทรופןแบบใดโดยใช้ Lineweaver-Burk Plot
และพบว่าสารสังเคราะห์ทุกตัวเป็นตัวยับยั้งชนิดแข่งขัน (Competitive Inhibitor) ของเอนไซม์โค-
โมทรופן

ภาควิชาเคมี
สาขาวิชาเคมีอินทรีย์
ปีการศึกษา2531

ลายมือชื่อนิสิต พลสวัสดิชัย
ลายมือชื่ออาจารย์ที่ปรึกษา สกนต A

WIMON PORNSAWATCHAI : SYNTHESIS AND EVALUATION OF AMINO ACID DERIVATIVES AS PROTEASE INHIBITORS ON CATHEPSIN G, TRYPSIN AND CHYMOTRYPSIN. THESIS ADVISOR : ASSO. PROF. PHICHAI TOVIVICH, Ph.D., ASSO. PROF. SUCHATA JINACHITRA, PROF. BELA TERNAI, Ph.D., 121 PP.

In the course of this research work, a series of peptides or amino acid derivatives were synthesized by mixed anhydride method. All of these synthetic peptides are novel. These synthetic compounds were purified by fractional recrystallisation. 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.

The effectiveness of the synthetic compounds as inhibitors was determined against three serine proteases namely trypsin, chymotrypsin and cathepsin G at the physiological conditions. The results showed that all the synthetic compounds gave a poor inhibitory activity against trypsin, but they are quite good inhibitors against chymotrypsin. However, at the testing conditions, cathepsin G gave such a low activity that it was impossible to test with the synthetic compounds. All the synthetic inhibitors were also tested to determine the type of inhibition against chymotrypsin by the interpretation of Lineweaver-Burk plots. The results showed that all of them are competitive inhibitors.

ภาควิชาเคมี.....
สาขาวิชาเคมีอินทรีย์.....
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LIST OF ABBREVIATIONS

Abs	absorbance
Ala	L-Alanine
Ar	aromatic
Arg	L-Arginine
BAPNA	N -Benzoyl-DL-arginine p-nitroanilide
b.p.	boiling point
br.	broad
Bz	Benzoyl
calc'd	calculated
°C	degree celcius
cm	centimeter
d	doublet
dd	doublet of doublet
D	Dalton
DMSO	dimethyl sulfoxide
E	enzyme
ES	enzyme-substrate complex
ESI	enzyme-substrate-inhibitor complex
Eq.	equation
Fig.	Figure
g	gram
Gly	glycine
HEPES	N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid
HLC-G	Human leukocyte cathepsin G
HLE	Human leukocyte elastase

I	inhibitor	v	volume
Lit	literature	w	weight
m	multiplet	Z	carbobenzoxy
M	mole per liter or molar		
mg	milligram		
min	minute		
mL	milliliter		
mm	millimeter		
mM	millimolar		
mmole	millimole		
m.p.	melting point		
μ L	microliter		
μ M	micromolar		
M_r	relative molecular weight		
nm	nanometer		
ppm	parts per million		
Phe	L-Phenylalanine		
Pro	L-Proline		
q	quartet		
R_f	rate of flow in chromatography		
s	singlet		
S	substrate		
SDS	sodium dodecyl sulfate		
t	triplet		
THF	tetrahydrofuran		
TLC	thin layer chromatography		
Tyr	tyrosine		