

การยับยั้งไตไฮโดรฟเทอโรเอคซิมเทสจาก Escherichia coli โดยสารจำพวก  
กรดพารา-อะมิโนเบนซีนซัลโฟนามิโดซัลคาโนอิก



นางสาว นุชรินทร์ เตียวปียกุล

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INHIBITION OF DIHYDROPTEROATE SYNTHASE FROM ESCHERICHIA COLI  
BY p-AMINOBENZENESULFONAMIDOALKANOIC ACIDS



Miss Nutcharat Tiewpiyakul

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By                      Miss Nutcharat Tiewpiyakul  
Department        Biochemistry  
Thesis Advisor    Associate Professor Wichai Suttimool, Ph.D.



Accepted by the Graduate School, Chulalongkorn University in  
Partial Fulfillment of the Requirements for the Master's Degree.

*Thavorn Vajrabhaya* ..... Dean of Graduate School  
(Professor Thavorn Vajrabhaya, Ph.D.)

Thesis Committee

*Jariya Boonjawat* ..... Chairman  
(Associate Professor Jariya Boonjawat, Ph.D.)

*Wichai Suttimool* ..... Thesis Advisor  
(Associate Professor Wichai Suttimool, Ph.D.)

*Siriporn Sitthipraneed* ..... Member  
(Assistant Professor Siriporn Sitthipraneed, Ph.D.)

*Sunanta Pongsamart* ..... Member  
(Associate Professor Sunanta Pongsamart, Ph.D.)

หัวข้อวิทยานิพนธ์ การยับยั้งไตไฮโดรฟเทอโรเอคซิมเทสจาก Escherichia coli โดยสาร  
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ชื่อนิสิต นางสาว นุชรัตน์ เตียววิญกุล

อาจารย์ที่ปรึกษา รองศาสตราจารย์ ดร.วิชัย สุทธิมูล

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บทคัดย่อ

ได้ศึกษาผลการยับยั้งการทำงานของไตไฮโดรฟเทอโรเอคซิมเทส จาก Escherichia coli โดยสารจำพวกกรด พารา-อะมิโนเบนซีนซัลโฟนามิโคอัลคาโนอิก จำนวน 7 ชนิด โดยการหาค่า inhibitor constant เอนไซม์ที่ใช้ได้ทำให้บริสุทธิ์ขึ้นบางส่วนซึ่งเท่ากับ 417 เท่าโดยใช้ affinity chromatography ซึ่งมี 4-(4-aminobenzenesulfonamido) benzenesulfonylglycine เป็น ligand พบว่าอุณหภูมิและ pH ที่เหมาะสมของการทำงานของเอนไซม์เท่ากับ 42 องศาเซลเซียสและ pH 8.55 ตามลำดับ บริเวณ active site ของเอนไซม์อาจจะมีกรดอะมิโนชนิด cysteine, lysine และ arginine ค่า  $K_m$  ของกรดพารา-อะมิโนเบนโซอิกเท่ากับ  $1.30 \times 10^{-6}$  โมล/ลิตร ค่า  $K_i$  ของ sulfanilamide, N-(p-aminobenzenesulfonyl) glycine, N-(p-aminobenzenesulfonyl)tyrosine, N-(p-aminobenzenesulfonyl) alanine, N-(p-aminobenzenesulfonyl) phenylalanine, N-(p-aminobenzenesulfonyl)methionine, N-(p-aminobenzenesulfonyl) leucine และ N-(p-aminobenzenesulfonyl) valine เท่ากับ  $4.8 \times 10^{-5}$ ,  $4.0 \times 10^{-5}$ ,  $13.8 \times 10^{-5}$ ,  $23.5 \times 10^{-5}$ ,  $24.0 \times 10^{-5}$ ,  $49.3 \times 10^{-5}$ ,  $84.0 \times 10^{-5}$ , และ  $100.0 \times 10^{-5}$  โมล/ลิตร ตามลำดับ จาก Dixon plot พบว่าสารที่นำมาศึกษาทุกตัวเป็นตัวยับยั้งเอนไซม์แบบแข่งขัน (competitive inhibitor) โดยแข่งขันกับกรดพารา-อะมิโนเบนโซอิก ในการจับกับ active site ของเอนไซม์ เมื่อลงแผนระหว่าง  $1/K_i$  กับค่าไฮโดรโฟบิซิตีของ side chains ของกรดอะมิโนของกรด พารา-อะมิโนเบนซีนซัลโฟนามิโคอัลคาโนอิก (ค่า  $\Delta f_t$  และ  $\pi$ ) พบว่า  $1/K_i$  จะลดลงเมื่อค่า  $\Delta f_t$  และ  $\pi$  มีค่า 0-1500

แคลอรี/โมล และ 0-1.5 ยูนิต ตามลำดับ อย่างไรก็ตาม ค่า  $1/K_i$  จะสูงขึ้นเล็กน้อยเมื่อ  $\Delta ft$  และ  $x$  มีค่า 1500-2500 แคลอรี/โมล และ 1.5-2.63 ยูนิต ตามลำดับ



ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย

Thesis Title      Inhibition of Dihydropteroate Synthase from  
Escherichia coli by p-aminobenzenesulfonamidoalkanoic  
 Acids

Name                Miss Nutcharat Tiewpiyakul

Thesis Advisor    Associate Professor Wichai Suttimool, Ph.D.

Department        Biochemistry

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ABSTRACT

Seven p-aminobenzenesulfonamidoalkanoic acids were selected, and their inhibitory activities against dihydropteroate synthase from Escherichia coli were studied by the determination of the inhibitor constant. The enzyme was partially purified to 417 fold by affinity chromatography in which 4-(4-aminobenzenesulfonamido) benzenesulfonyl-glycine is a ligand. The optimum temperature and pH of the enzyme were 42<sup>o</sup> C and 8.55 respectively. The enzyme active site might contain the amino acid residues of cysteine, lysine and arginine. The  $K_m$  value for p-aminobenzoic acid was  $1.30 \times 10^{-6}$  mol/l. The  $K_i$  values for sulfanilamide, N-(p-aminobenzenesulfonyl) glycine, N-(p-aminobenzenesulfonyl) tyrosine, N-(p-aminobenzenesulfonyl) alanine, N-(p-aminobenzenesulfonyl) phenylalanine, N-(p-aminobenzenesulfonyl) methionine, N-(p-aminobenzenesulfonyl) leucine, and N-(p-aminobenzenesulfonyl) valine were  $4.8 \times 10^{-5}$ ,  $4.0 \times 10^{-5}$ ,  $13.8 \times 10^{-5}$ ,  $23.5 \times 10^{-5}$ ,  $24.0 \times 10^{-5}$ ,  $49.3 \times 10^{-5}$ ,  $84.0 \times 10^{-5}$  and  $100.0 \times 10^{-5}$  mol/l respectively. The Dixon plot indicates that each compounds was an inhibitor competing with p-aminobenzoic acid for the binding to the enzyme active site. The plots of the  $1/K_i$  values versus the hydrophobicity

of the amino acid side chain of p-aminobenzenesulfonamidoalkanoic acids ( $\Delta f t$  and  $\pi$ ) indicate that the  $1/K_i$  values were decreased when  $\Delta f t$  and  $\pi$  were 0-1500 cal/mol and 0-1.5 units respectively. However, the  $1/K_i$  values were slightly increased when  $\Delta f t$  and  $\pi$  were 1500-2500 cal/mol and 1.5-2.63 units respectively.



ศูนย์วิจัยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย

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จุฬาลงกรณ์มหาวิทยาลัย



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## ABBREVIATION

BSA	Bovine serum albumin
DHP	7,8-Dihydropteroate
DHPP	2-Amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine pyrophosphate
Hepes	N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid
$K_i$	Inhibitor constant
$K_m$	Michaelis constant
PABA	p-Aminobenzoic acid
PMB	p-Chloromercuribenzoic acid
PMSF	Phenylmethylsulfonyl fluoride
Tris	Tris (hydroxymethyl) aminomethane



ศูนย์วิทยทรัพยากร  
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