The Effect of Some Organic Compounds on Monoamino oxidase Activity

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Abstract

Imidazole derivatives have occupied a unique place in the field of medicinal chemistry. The incorporation of the imidazole nucleus is an important synthetic strategy in drug discovery. The high therapeutic properties of the imidazole related drugs have encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents. This study was designed to show the effects of new compounds derivatives from 2-Imidazolidine thione on the activity of Monoamino oxidase in sera. All compounds were studied demonstrated inhibitory effects on the enzyme activity and these effects are increased with increasing the concentrations of the compounds. The compound 2-(4,5-dihydro-1H-imidazol-2-ylthio)-N'-(2-hydroxy benzylidene) acetohydrazide has the highest ability to inhibit MAO than the other compounds.Kinatic properties of these inhibitors reveal that compound 4 causes mixed type of inhibtion,componds 1,3 act as non - competitive inhibitor and compound 2 behaves as un- competitive inhibitor.

Keywords: Monoamino oxidase, Imidazole compounds, inhibitors, non–competitive inhibition.

Introduction

(Monoamino oxidases MAO) (EC 1.4.3.4) are an outer mitochondrial membrane-bound flavoenzymes that catalyzes the oxidation of the neurotransmitters serotonin, dopamine, and norepinephrine [1,2]. Changes in the activity of this enzyme have been observed in numerous neuropsychiatric disorders, and the employment of MAO inhibitors often produces a therapeutic effect[3). In mammals MAO exists in two forms, MAO A and MAO B, which are encoded by two different genes, that differ in their substrate specificity and sensitivity to inhibitors[2,4]. Recent studies have demonstrated that a deficiency or low level of expression of this enzyme results in a phenotype of aggressive behavior[5, 6]. Because of the vital role that MAOs play in the inactivation of neurotransmitters, MAO dysfunction (too much or too little MAO activity) is thought to be responsible for a number of neurological disorders. For example, unusually high or low levels of MAOs in the body have been associated with depression,[7] schizophrenia[8,9] substance abuse, attention deficit disorder, migraines, and irregular sexual maturation. Monoamino oxidase inhibitors are one of the major classes of drug prescribed for the treatment of depression, although they are last-line treatment due to risk of the drug's interaction with diet or other drugs[10]. Excessive levels of catecholamines (epinephrine, norepinephrine, and dopamine) may lead to a hypertensive crisis, and excessive levels of serotonin may lead to serotonin syndrome. Positron emission tomography (PET) research which is a nuclear medicine imaging technique that produces a three-dimensional image or picture of functional processes in the body has shown that MAO is also heavily depleted by use of tobacco cigarettes[10] .

This study included study the effect Imidazole derivatives on (MAO) and these compounds have occupied a unique place in the field of medicinal chemistry. The incorporation of the imidazole nucleus is an important synthetic strategy in drug discovery. The high therapeutic properties of the imidazole related drugs have encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents [11].

Material and Methods

Monoamino oxidase activity was assayed by (Mcwen and Cohen method 1963)[12].The principle of the method is the measurement of the benzaldehyde from reaction which is absorbed at wave length 242 nm after extraction by cyclohexan.

The Used Solution

1-Phosphate buffer solution (0.2 M), this solution can prepare by

(a) Dissolve(21.999gm)from NaH_2PO_4 in distill water and then complete the volume to 1000 ml**.**

(b) Dissolve (28.16gm) from Na_2HPO_4 in distill water and then complete the volume to 1000 ml**.**

And add 28 ml from solution **(a)** to 72 ml from solution **(b)** and complete the volume to(200ml)by distill water to give (pH=7.2).

2-Substrate solution (0.008), this solution can be prepared by dissolve (0.21422gm) from benzylamine in 250 ml from phosphate buffer solution (pH 7.2).

A-The assay procedure is described in this table :-

Solution	Test	Control
Serum	Lμ600	Lμ600
MAO buffer	Lμ750	Lμ750
Benzalamine	Lμ150	…….

Water bath shaking for 3 hrs. at 37c

benzalamine	…….	L μ150
Perchloric acid	L μ150	L μ150
cyclohexane	1.5 ml	1.5 ml

All solution were mixed and centrifuged for 10 min. Then the absorbance of the supernatant was measured at 242 nm. Enzyme activity were measured through aldehyde formed in 3 hrs. by reading absorbance of test against control at wave length 242 nm by U.V spectrophotometer in the laboratory of biochemistry on February 2010.

B-Four compounds that found in Table (1) were prepared by Zainab Muhammad Ali [13]. The following concentrations (10^{-2},10^{-3}, 10^{-4},10^{-5},10^{-6})M of these compounds are prepared by diluting with ethanol. MAO activity is measured in human serum by using the same method in previous paragraph with replace 750 µl of MAO buffer solution with 500 µl MAO buffer +250 µl of inhibitor(four compounds found in Table (1). The inhibition percentage was calculated by comparing the activity with and without the inhibitor and under the same conditions, according to the equation:-

$$\text{Inhibition}\% = \frac{\text{The activity in the presence of inhibitor}}{\text{The activity in the absence of inhibitor}} \times 100$$

C-Constant concentration of inhibitor 10^{-4} to study the type of inhibition. These different concentrations were prepared from the stock solution of (0.008 M) MAO substrate. The enzyme activity was determined with and without the inhibitor, by using the Lineweaver–Burk equation and plotting 1/v against 1/s were evaluated values:

a) ki, b) Apparent Vmax (vmap),c) Apperent km (k map),d) Type of inhibition.

D- Study MAO activity in the presence of only solvent (ethanol).

Result and Discussion

The organic compounds derivatives that we are used have Imidazole ring .Imidazole ring is a planer five-member heterocyclic ring with 3C and 2N atoms and in ring N is present in 1st and 3rd positions. The imidazole ring is a constituent of several important natural products, including purine, histamine, histidine and nucleic acid. [13]. Many researchers study imidazole derivative compounds properties such as, Gaba M. *etal*[14] study Novel 5-Substituted -1-(phenylsulphonyl) methyl benzimidazole derivatives, they have synthesized and screened for their anti-inflammatory as well as analgesic activity, in other study Hranjec M *etal*[15]., showed newly synthesized benzimidazole derivatives exerted pronounced antiproliferative activity. Among a synthesized series of aminothiazolo [3,2-a] benzimidazole derivatives N-acetylaminothiazolo [3,2-a] benzimidazole -2-carbonitrile revealed significant in vitro antiproliferative activity which attributed to its ability to arrest G2/M phase and to induce apoptosis in time dependant manner[16]. One of these compounds derivatives from 2-Imidazolidine thione, in this research we investigate of the effects of four compounds, stated in Table (1). The effect of the solvent (ethanol) showed a slight inhibitory effect on serum of MAO activity, which was calculated to be 0.5% of original activity. The biochemical tests revealed that all compounds

caused (moderate to good) inhibitory effects on the enzyme activity, Table (2) the normal value of the enzyme activity ranges between (43-110) µmole/3 min/ml. The relationships between compounds concentrations and the activity of enzyme are shown in Fig.(1). From these results it is observed that any increase in compounds concentrations causes increases in percentage of inhibition of enzyme. The greater inhibition of each compound is demonstrated at concentration (0.001 M) as Fig.(2). From this figure it is observed that compound (1) revealed higher percentage of inhibition (85.714%).In contrast compound (2) exhibits lower percentage of inhibition (37.209%) than other compounds.

In this study, we determined the type of inhibition and kinetic parameters (K map, Vmap and Ki) at different concentrations of substrate under the same conditions by using Lineweaver-burk equation and one plotted as shown in Fig.(3), and Table (3). Our results revealed that compounds (1,3) have the same type of inhibition(non-competitive inhibition) this type of inhibition is recognized by its characteristic effect on Vmax, it occurs when the inhibitor and substrate bind at different sites on enzyme. The non-competitive inhibitor can bind either free enzyme or ES complex, there by preventing the reaction from occurring[17], while compound (2) exhibit observed from all of previous that the differences in potency of inhibition from compound to another belong to the differences in the nature of groups substituted instead of hydrogen atom in 2-Imidazolidine thione compound .Over the past ten years, efforts have been directed to the design, synthesis and study of new monoamine oxidase inhibitors (MAOIs) for the treatment of various mental and neurological disorders. MAO inhibitors of the new generation are usually characterized by their relatives specificities for the MAO subtypes and in some cases by the reversibility of their actions. Despite considerable progress in understanding the interactions of the two enzyme forms with their preferred substrates and inhibitors, no general rules are yet available for the rational design of potent and selective inhibitors of MAO possibly due to the fact that the mechanism of interaction of the new drugs with MAO isoforms have not been fully characterized. Preferential MAO-A inhibitors have been recognized as therapeutically useful antidepressants while MAO-B inhibitors have been found to be beneficial in the treatment of Parkinson's disease and Alzheimer [18,19,20]. Bellik L.etal [21], reported that the novel MAO-B inhibitor PF9601N, its cytochrome P450-dependent metabolite FA72 and l-deprenyl were studied as potential peroxynitrite (ONOO) scavengers and nitric oxide synthase (NOS) inhibitors both the novel MAO-B inhibitor as well as its metabolite were able to strongly inhibit rat brain neuronal NOS (IC50 of 183 µM and 192 µM, respectively), while l-deprenyl at the highest concentration used (3 mM), caused only a slight decrease of the enzyme activity. All these results suggest that PF9601N could be a promising therapeutic agent in neurodegenerative disorders such as Parkinson's disease. In this context, the development of new molecules able to reduce the oxidative stress, thereby slowing the rate of neuronal degeneration, appears to be necessary. A new series of acetylenic and allenic derivatives of tryptamine synthesized as MAO-B inhibitors (MAOIs) have been described [22,23]. Among these, N- (2-propynyl) -2- (5-benzyloxy indolyl) methylamine (PF9601N) was shown to have a neuroprotective effect in several experimental models of Parkinson's disease (PD). PF9601N protects nigrostriatal dopamine neurons against MPTP neurotoxicity in C57bl /6 adult mice [24]. and protects rat nigral neuronsafter 6-hydroxydopamine striatal lesion [25). One of these enzymes that act as MAO is Semicarbazide-sensitive amino oxidase (SSAO) is a common name for a group of enzymes, containing copper and quinoneco factor[26]. and sensitive to semicarbazide, that converts primary amines into the corresponding aldehydes, while releasing ammonia and hydrogen peroxide[27]. is found in a great variety of species from prokaryotes to eukaryotes. In mammals, two forms of the SSAO protein have been identified: a tissue-bound form and a soluble plasma form[28]. The tissue-bound SSAO contains a short intracellular domain, a single transmembrane domain, and a long extracellular domain that

contains the active site[29]. It has been reported that increases in the levels of plasma and/or membrane-associated SSAO occur in many inflammation-associated diseases, including rheumatoid arthritis inflammatory bowel disease, 2 types of diabetes mellitus, atherosclerosis, and chronic heart failure [30]. The possible involvement of SSAO in the inflammatory processes associated with Alzheimer's disease has also been reported [31]. The in- vitro biological results revealed that compounds **4a**,**c** are highly potent SSAO inhibitors with notable selectivity toward SSAO over monoamine oxidases A and B (MAO-A and MAO-B). The most potent and selective compound, **4a** (IC50) 2 nM), is an orally active, competitive, and apparently irreversible inhibitor of SSAO that is effective at reducing disease[32]. On other hand Gastagnoli K.*etal* [33]. found that smokers have lowered brain and blood platelet MAO-A and MAO-B activites compared to non-smokers and that smokers have a lowered incidence of parkinsons disease because the neuroprotective properties of an MAO inhibitor.2,3,6-trimethyl-1,4-naphthoquinone (TMN) which is present in the tobacco plant and smoke in the 1-methyl -4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) C57BL/ 6mouse model of neurodegeneration.

Table (1)
Compounds were used to effec on MAO activity.

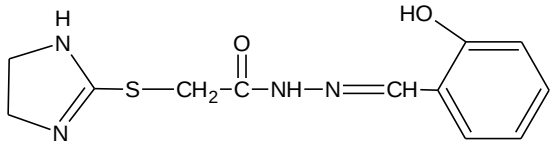
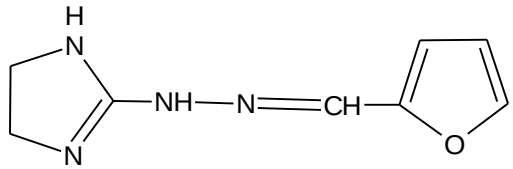
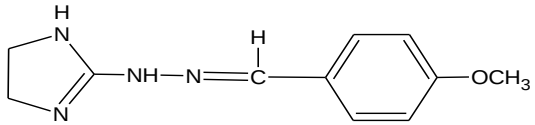
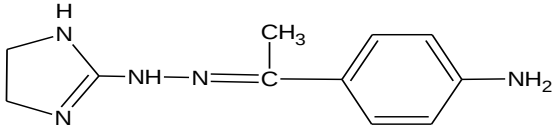
Compound number	***Compound name***	***Structure of Compound***
1	2-(4,5-dihydro-1H-imidazol-2-ylthio)-N'-(2-hydroxy benzylidene)acetohydrazide	
2	2-furaldehyde4,5-dihydro-1H-imidazol-2-ylhydrazone	
3	4-methoxybenzaldehyde4,5-dihydro-1H-imidazol-2-ylhydrazone	
4	1-(4-aminophenyl)ethanone4,5-dihydro-1H-imidazol-2-ylhydrazone	

Table (2)
The effect of different concentrations of compounds (1-4) on the activity of MAO enzyme in human serum.

Compound conc.(M)	*Enzyme activity conc.(M) Mol/ min/3ml*	*Inhibition %*
Compound (1)		
Nil	105	0.00
10^-7	83.7	20.476
10^-6	57.3	45.428
10^-5	27.15	74.142
10^-4	21.75	79.285
10^-3	15	85.714
Compound (2)		
Nil	43	0.00
10^-7	42	2.325
10^-6	33	23.255
10^-5	30	30.232
10^-4	28.5	33.720
10^-3	27	37.209
Compound (3)		
Nil	60	0.00
10^-7	54	10
10^-6	49.5	17.5
10^-5	46.5	22.5
10^-4	31.5	47.5
10^-3	21	65
Compound (4)		
Nil	110	0.00
10^-7	108	1.818
10^-6	103	6.363
10^-5	70	36.363
10^-4	65.25	37.945
10^-3	59.5	45.909

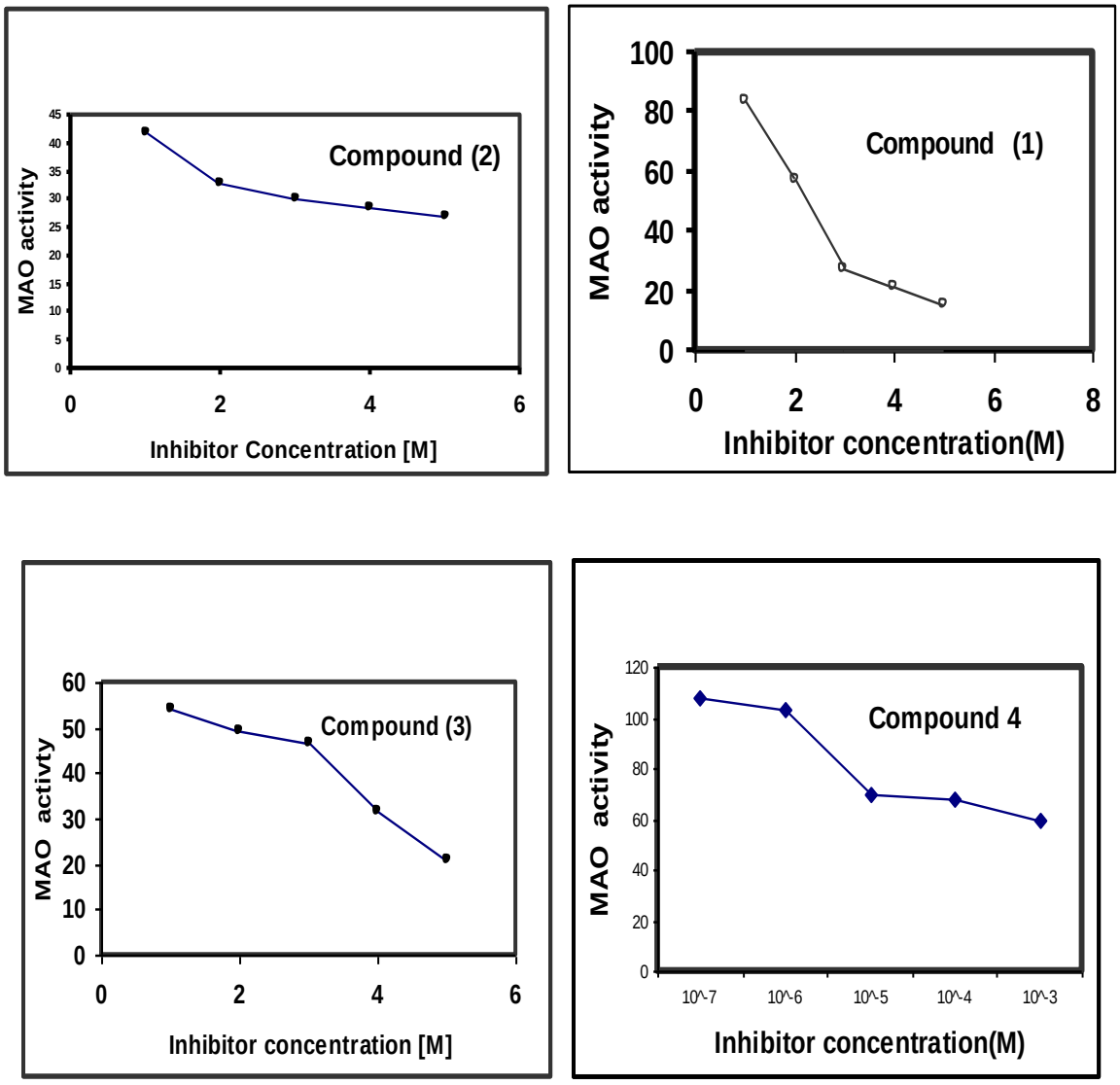


Fig.(1) The relationship between concentration of compounds (1-4) and MAO activity.

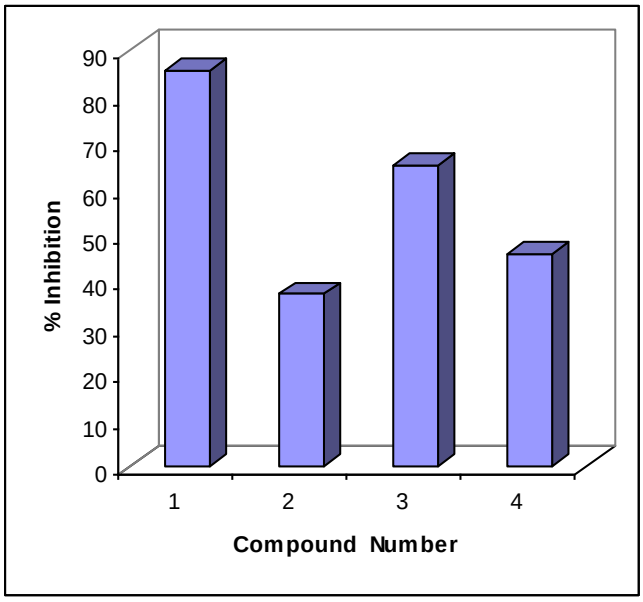


Fig.(2) The percentages of inhibition enzyme and compounds (1-4).

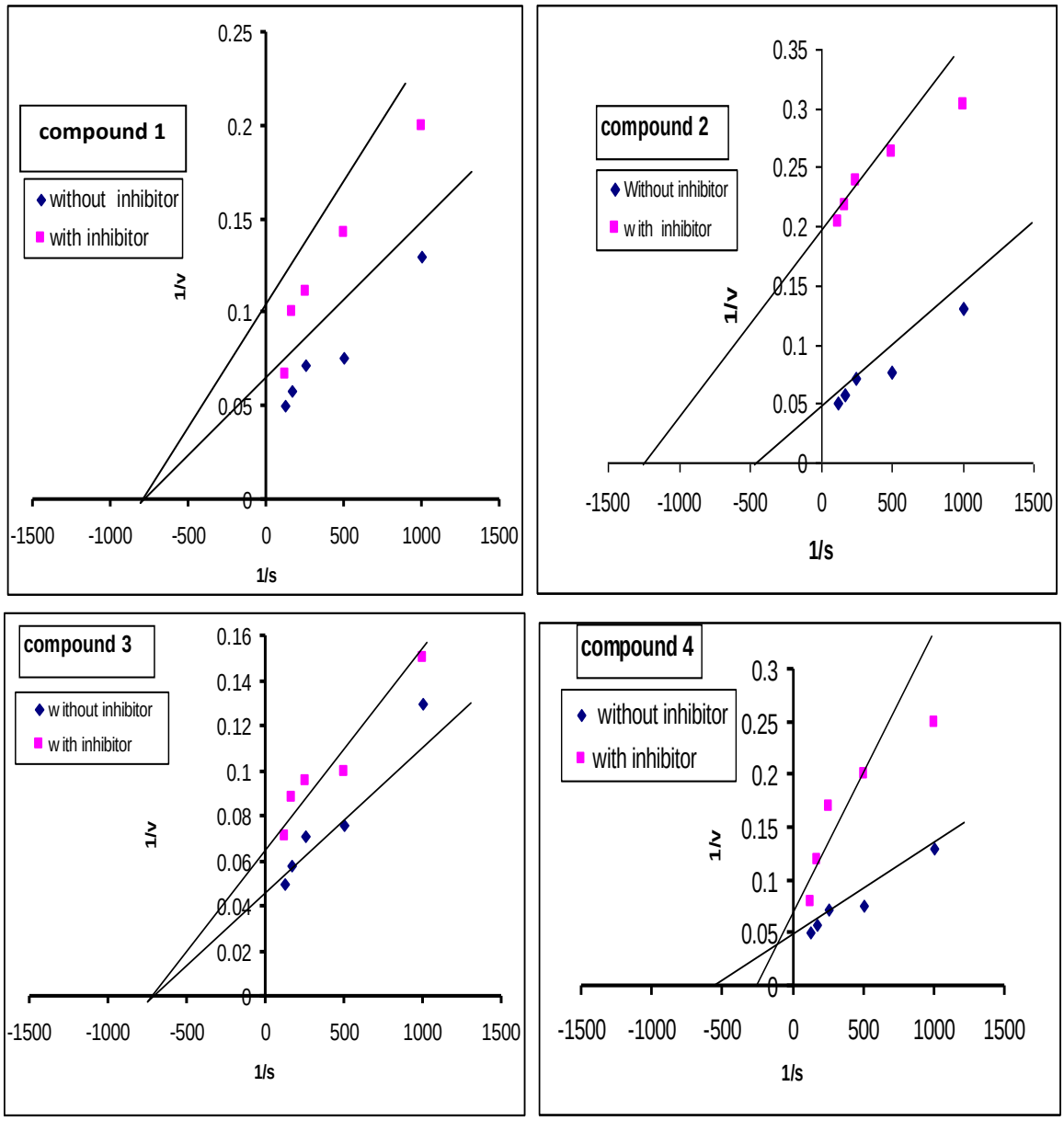


Fig.(3) Lineweaver- Burk plots for compounds (1-4) effects on MAO.

Table (3)
The kinetic properties of MAO with compounds (1-4).

Compound number (10^{-4})	*K_{map} (M)*	*V_{map} (mol/ml/min)*	*Ki(M)*	*Type of inhibition*
1		14.28	0.00013	Non competitive
2	0.0008	5	0.33×10^-4	uncompetitive
3		16.66	0.0002	Non competitive
4	0.004	16.66	0.0005	Mixed

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الخلاصة

يوضح هذا البحث تأثير مشتقات جديدة للأيميدأزوليدين ثايون في فعالية انزيم مونوامينو اوكسيديز في مصل الدم. أضهرت جميع المركبات المدروسة تأثير تثبيطي في فعالية الانزيم وان قدرة هذه المركبات على تثبيط الانزيم تزداد طرديا مع زيادة تركيزها. لقد وجد ان المركب 2-(4,5-dihydro-1H-imidazol-2-ylthio)-N'-(2-hydroxy benzylidene)acetohydrazide يمتلك أعلى قابلية لتثبيط الانزيم مقارنة ببقية المركبات .كما اظهرت دراسة الخواص الحركية لهذه المثبطات ان المركب (٤) يتسبب بتثبيط من النوع المختلط والمركبان ١و٣ يسببان تثبيط من النوع اللاتنافسي اما المركب (٢) فانه يسبب تثبيط من النوع الغير تنافسي.