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Research Article

The enzyme kinetic studies, DNA protection, and antioxidant activities of furan/ thiophene-2-carboxamide derivatives

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ABSTRACT

Three furan and/or thiophene-2-carboxamide compounds, namely *N*-(furan-2-ylmethyl)thiophene-2-carboxamide (**1**), *N*-(furan-2-carbonyl)furan-2-carboxamide (**2**), and *N*-(Thiophene-2-ylmethyl)furan-2-carboxamide (**3**) were investigated the enzyme kinetic studies by urease, acetylcholinesterase (AChE), and butyrylcholinesterase (BChE). The inhibition constant (K_i) of Compound (CPD)**3** by AChE was determined as 0.10 mM, and the K_i value by BChE was determined as 0.07 mM. In comparison, the K_i value of CPD**1** by urease was determined as 0.10 mM. These CPDs were examined for antioxidant activity by the DPPH˙ scavenging method. CPD**3** exhibited 98.93% DPPH scavenging activity compared to ascorbic acid, the positive control group. Furthermore, the DNA-protective activities of the compounds were investigated, and the DNA protection activity of CPD**1** was observed to be 78%. The findings suggest that thiophene/furan carboxy amide-containing CPD**1** and CPD**3** might be exploited as potential structures for evaluating pharmaceuticals with greater potency.

Keywords: Furan/thiophene-2-carboxamide, acetylcholinesterase, butyrylcholinesterase, urease inhibition, DNA-protective activity.

1. INTRODUCTION

The carboxamide scaffold is one of the well-known privileged building substructures in many clinical modifications and is present in many different bioactive substances.[1] These carboxamide scaffold-based structures have a wide range of biological activities, comprising but not limited to antimicrobial[2], anticancer[3], antibacterial[4], antioxidant[5], and anti-influenza activities.[6] Therefore, there is a special interest in this substance, which exhibits biological functions but also in pharmacological chemistry.

Understanding how free radicals are created is crucial for building and enhancing an effective antioxidant defense system against the harmful effects of oxidant chemicals. When H_2O_2 and transition metals (such as Fe^{3+}) are present, the Fenton reaction can produce hydroxyl radicals. The most reactive reduced form of dioxygen, hydrogen peroxide, may harm practically every molecule in living cells.[7, 8] Additionally, hydroxyl radicals tend to react with nucleotides and then with whole DNA molecules. This process results in breaks in the DNA strands, which can cause cytotoxicity, mutagenesis, carcinogenesis, and many genetic diseases.[9] The phosphodiester chains of supercoiled DNA are broken when plasmid DNA is exposed to H_2O_2, forming a relaxed, open-circular DNA form. DNA molecules with linear double strands are produced by further cleavages that take place close to the first break. Circular DNA forms are recognized as a reliable sign of single-strand breaks in DNA. According to Singh et al. (2014)[8], the emergence of linear shapes in DNA is a sign of double-strand breaking[10].

In a previous study,[11] we synthesized three different enzyme inhibition effect tests and molecular calculations of these three compounds. Our prior research in the

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literature has demonstrated that these compounds with furan carboxyamide and/or thiophene moieties can be used as prospective scaffolds for synthesizing more potent medicinal drugs. Accordingly, in the current study, we investigated the molecules' antioxidant (DPPH˙ scavenging activity was chosen because it is a simple and easy test, and it is thought that the compounds may have a radical scavenging effect) and evaluated the DNA protective activities of these CPDs. We performed enzyme (kinetic studies to predict the probable inhibition mechanism such as urease, acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) and confirmed the experimental findings of the synthesized components presented in the previous article. Since the tests on the CPDs in this study have been performed for the first time, they are thought to contribute to the literature.

2. MATERIAL and METHODS

2.1. Chemistry

The three synthesized furan and/or thiophene-2-carboxamide CPDs (**1-3**) have been described in a previous paper.[11] Table 1 displays the chemical structures of the substances that were investigated.

2.2. Urease inhibition activity, AChE and BChE kinetics

The indophenol methods revealed the urease inhibitor effects of furan/thiophene-2-carboxamide derivatives.[12] By employing a BIOTEK (Epoch2) microplate reader, the absorbance values of the blue colors created by the decreasing concentration values of the furan/thiophene-2-carboxamide derivatives were determined at 630 nm. The enzyme kinetic study used nine different substrate concentrations ranging from 20 mM to 0.78 mM.

Through the use of the Ellman method, the inhibitory effects of furan/thiophene-2-carboxamide on AChE and BChE were determined.[13] With a BIOTEK (Epoch2) microplate reader, the absorbance values of the yellow colors created by the decreasing concentration values of the furan/thiophene-2-carboxamide derivatives were determined at 412 nm. The enzyme kinetics study used nine substrate concentrations ranging from 1 mM to 0.0156 mM.

Furthermore, the kinetics of each enzyme were ascertained by drawing Michaelis-Menten and Linewevear-Burk graphs using varying amounts of particular substrates. Using Equation 1, the binding constant of CPD, or K_i, was computed after finding the maximum velocity (V_{max}) and substrate concentration (K_m) values at which half the maximum velocity was attained using the Linewevear-Burk graph.

$$\frac{1}{v}=\frac{K_m}{V_{max}}\left(1+\frac{[I]}{K_i}\right)\frac{1}{[S]}+\frac{1}{V_{max}}\left(1+\frac{[I]}{\alpha K_i}\right) \quad (1)$$

The enzyme reaction rate, v, is the same with and without inhibitors. The most significant velocity is V_{max}. The expressions for the inhibition constant and the Michaelis-Menten constant are K_i and K_m, respectively. The value of α is one for non-competitive inhibition, and A is the ratio of the competitive inhibition constant to the non-competitive inhibition constant. Concentrations of [I] inhibitor and [S] substrate.[14]

2.3. DPPH˙ Scavenging Activity

DPPH˙ was used to measure the scavenging capacity of the sample DMSO solutions.[15] 50 µL of 0.1 mM DPPH˙ and 150 µL of sample solution at various concentrations (512-0.5 µg/mL) were mixed thoroughly in a 96-well plate before being incubated at 25°C in the dark for half an hour. The BIOTEK (Epoch2) microplate reader was used to measure the absorbance values of each combination at 517 nm, and the results were obtained by computing the IC_{50} (µg/mL) values. The activity assay was conducted using ascorbic acid as a standard, and the outcomes were compared.

2.4. DNA Protective Activity

Agarose gel electrophoresis was used to measure DNA protection activities.[16] DNA nicking forms were used to assess samples' capacity to shield plasmid DNA (pBR322, Thermo-Fisher) from the oxidizing effects of H_2O_2 + UV treatment, and the results were then examined in slightly altered agarose gels. The protective capacity was examined from electrophoresis results that were captured using a UV transilluminator (320 nm, 8000 W/cm). The percent protection level of the samples' nicked DNA (Form II) and supercoiled DNA (Form I) was computed using the ImageJ tool.

2.5. Statistical Analysis

The bioactivity studies were expressed as results ± standard deviation. Statistical analysis of whole data was determined using the IBM Statistical Package for Social Studies (SPSS) 20.0 program. For the obtained data, the One Way Anova-Tukey HSD^{a,b} multiple comparisons. The significance level of the analysis result group was expressed with $p<0.05$ values.

3. RESULTS and DISCUSSION

3.1. Synthesis

The three synthesized furans and/or thiophene-2-carboxamide compounds were prepared from acyl chlorides and heterocyclic amine derivatives by the reported procedures.[11] As reported earlier, their structures were confirmed by spectroscopic methods comprising IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis. Furthermore, bioactivity assessment on enzyme and molecular docking calculation were extensively described in the published paper.[11]

3.2. Enzyme Kinetics

Today, many different studies are carried out to use the compounds synthesized or purified from natural products as medicine.[17-19]

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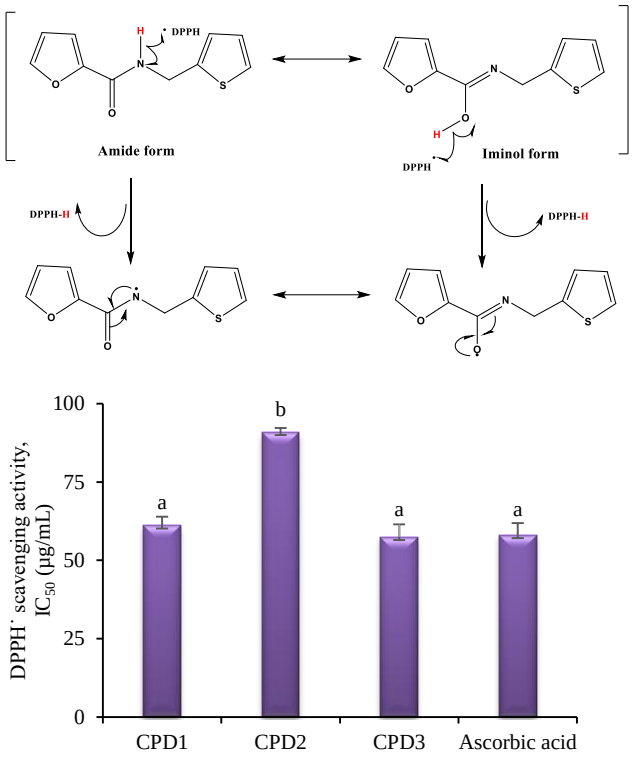


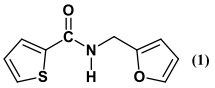
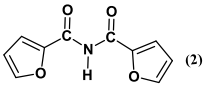
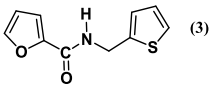
Figure 1. Proposed mechanism for DPPH˙ scavenging activity of CPD**3** and the IC_{50} values of CPDs (**1-3**).

These include enzyme inhibitions, inhibition kinetics, and enzyme-molecule interactions.[20-25] For this purpose, enzyme kinetics of compounds with high enzyme inhibition activity were investigated. The inhibition type of the urease enzyme of CPD**1** was calculated as a non-competitive, and the binding constant (K_i) was 0.10 mM. The inhibition constant K_i value of CPD**3** against the AChE enzyme was determined as 0.10 mM, and the inhibition K_i value of the BChE enzyme was determined as 0.07 mM. Also, this compound was found to be a noncompetitive inhibition towards both enzymes (Table 2 and Figure 3).

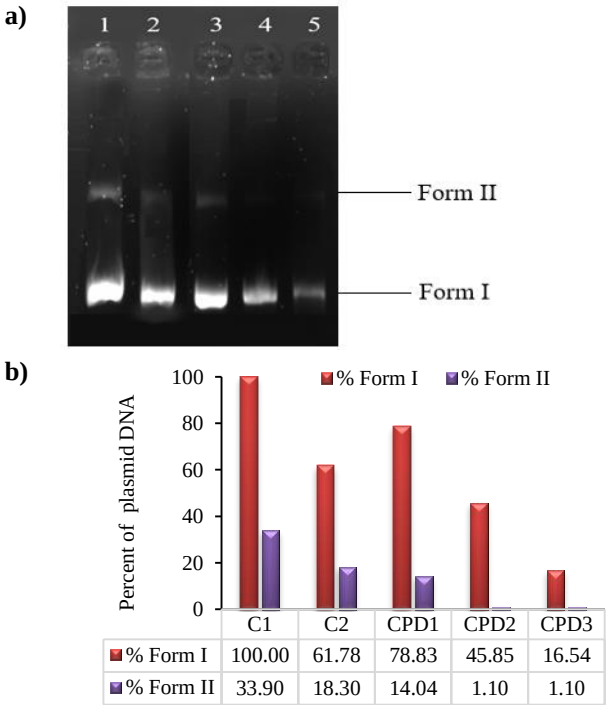
3.3. Determination of DPPH˙ scavenging activity

The *in vitro* DPPH˙ scavenging activity was examined in the target molecules, and the results are given in Table 1. Based on the experimental results, CPD**3** showed higher scavenging activity towards DPPH˙ among compounds synthesized. As deduced from the IC_{50} data, the lowest radical scavenging capacity was found to be CPD**2** (with 90.93±1.29 µg/mL), followed by CPD**1** (61.11±2.78 µg/mL), and CPD**3** was found to be slightly more active (57.47±4.06 µg/mL) than CPD**1** (Figure 1). The proposed mechanism for the DPPH˙ scavenging activity of the most potent CPD**3**, the mechanism by which there is an amide-iminol tautomerization, is presented in Figure 1. The tautomeric forms in this proposed mechanism are thought to be responsible for the antioxidant activity.[26] According to this mechanism, the activity of CPD**3** is thought to be due to the presence of an N-H group in the carboxamide scaffold, which can easily donate a hydrogen atom to the DPPH˙ compared to an O-H group. [27, 28]

Table 1. Antioxidant activity values of synthesized CPDs (**1-3**) by DPPH˙ scavenging activity

Code	Compounds and standard	DPPH˙ scavenging activity, IC_{50} (µg/mL)
CPD1	 (1)	61.11±2.78[a]
CPD2	 (2)	90.93±1.29[b]
CPD3	 (3)	57.47±4.06[a]
Ascorbic acid		58.09±3.83[a]

*Values are expressed as means (n = 3), The letters a, b, and c are statistically significant indicators



*** 1:**C1, **2:**C2, **3:**CPD1, **4:**CPD2, **5:**CPD3

Figure 2. DNA protection activity of the CPDs a) Agarose gel electrophoresis image b) Percent comparison of the effect intensity of form I (supercoiled circular DNA) and form II (nicked DNA) of plasmid DNA

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Table 2. The inhibition types, V_{max}, K_m, and K_i values of the urease, AChE, and BChE for CPDs **1** and **3**.

Urease	CPD1, mM	V_{max}, µmol/min	K_m, mM	K_i, mM	Inhibition Types
	0.00	0.00254	1.078	0.10	Non-competitive
	0.01	0.00205	1.078		
	0.05	0.00178	1.081		
	0.10	0.00147	1.080		
AChE	**CPD3, mM**	**V_{max}, µmol/min**	**K_m, mM**	**K_i, mM**	**Inhibition Types**
	0.00	0.00211	0.130	010	Non-competitive
	0.01	0.00177	0.130		
	0.05	0.00145	0.131		
	0.10	0.00123	0.131		
BChE	**CPD3, mM**	**V_{max}, µmol/min**	**K_m, mM**	**K_i, mM**	**Inhibition Types**
	0.00	0.00262	0.189	0.07	Non-competitive
	0.01	0.00199	0.185		
	0.05	0.00161	0.189		
	0.10	0.00128	0.187		

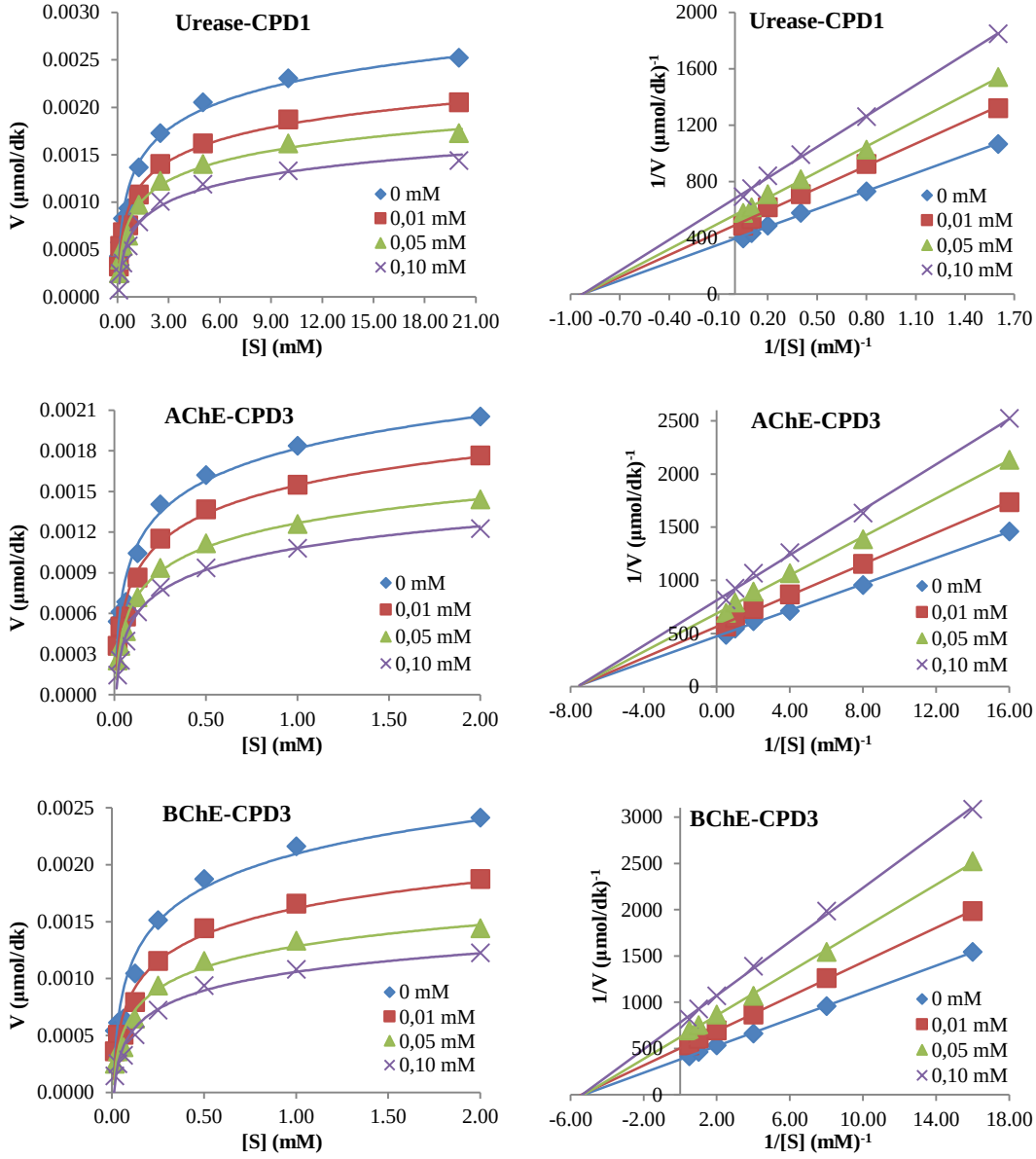

Figure 3. Michaels Menten and Lineweaver Burk plots of CPD **1** and **3**

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3.4. Determination of DNA protective activity

All living organisms contain genetic material called DNA.[29] Normal biological processes, such as transcription and replication, are hampered by DNA damage. Cancer or cell death is caused by DNA damage that compromises cellular function. Chemicals, poisonous substances, and biological materials are just a few of the things that may cause DNA damage. The ability to restore DNA nucleotide sequences to their original condition is provided by DNA repair. [10, 20, 21, 24, 30-36] According to De Almeida et al. [37], oxygen and UV radiation from the sun are two main genotoxic agents for most organisms. Figure 2 illustrates CPD**1** at 78.83%, CPD**2** at 45.85%, and CPD**3** at 16.54% of DNA protection activity Form I.

4.CONCLUSION

This study demonstrates DNA protective properties, antioxidant activity, and enzyme kinetics studies of CPDs (**1**-**3**) with a carboxamide skeleton. Enzyme kinetics of compounds with high activity in enzyme inhibition activity tests examined in our previous study were investigated. Thus, the mechanisms for using these CPDs as drugs were elucidated. In enzyme kinetics, the binding constant (K_i) of CPD **1**, determined by urease, was calculated as 0.10 mM, and the binding constant (K_i) of CPD**3**, determined by AChE and BChE, was calculated as 0.10 mM and 0.07 mM, respectively. In addition, these CPDs showed noncompetitive inhibition with the enzymes studied. The radical scavenging activities of the compounds were compared with the natural antioxidant ascorbic acid standard, and it was observed that CPD**3** had the same level of radical scavenging effect as the standard. According to this result, this compound can be used as an antioxidant and a promising agent that can prevent cells from dying by preventing oxidative damage caused by radicals. In our study, the DNA protection properties of the compounds were investigated, and it was observed that CPDs **1** and **2** protect DNA.

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Conflict of Interest

The authors declare no conflict of interest.

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