Investigation of Collagenase and Elastase Inhibitory Potential of the Novel Coordination Compound

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1. Introduction

The body tissues and organs contain a mixture of cells and non-cellular components, which regulates the cellular functions. The well-organized networks that provides the essential biochemical, biomechanical and physical environment required for tissue morphogenesis, differentiation, homeostasis and cellular components is called extracellular matrix (ECM) (Frantz et al., 2010; Kusindarta & Wihadmadyatami, 2018). ECM has a complex and dynamic structure, comprise various proteins and polysaccharides secreted by some cells in a multicellular organism, filling between cells and functioning as binding agents that hold cells in a defined area (Şen, 2012; Üçgül et al., 2018; Uslu & Dengizek Eltas, 2015; Wong, 2009). ECM consist of a large variety of matrix macromolecules including collagens, elastin, fibronectin (FN), laminins, glycoproteins, proteoglycans (PGs), and glycosaminoglycans (GAGs) whoe composition and specific structure vary from tissue to tissue (Theocharis et al., 2016). The main role of ECM is to maintain the integrity and strength of organs, and tissues with specific mechanical and biochemical properties. Additionally, it acts as a primary barrier to prevent the spread of the tumor cells because is a major component of the cellular microenvironment (Jabłońska-Trypuć et al., 2016). The ECM was initially considered an inert scaffold whose main role is provide mechanical strength to the tissue and organs but nowadays it is accepted as a three-dimensional structure that facilitates the survival of cells by playing an active role in regulating biologic processes, this macromolecules, providing physical protection and signals, directing and facilitating cell behaviours such as proliferation, orientation, gene expression, migration and differentiation (Järveläinen et al., 2009; Onofrı, 2016). The most abundant substance in ECM is collagen, which is the main component of skin and bone, and it makes up approximately 30% of the total mammalian protein mass (Onofrı, 2016; Pereira et al., 2011). Collagen; It is a protein composed of fibrous and non-fibrous proteins synthesized by connective tissue cells such as fibroblasts, chondroblasts, osteoblasts and odontoblasts, and secreted into the ECM through exocytosis (Pereira et al., 2011). Collagen is responsible for the tensile strength in cells, the regulation of cell adhesion and the management of tissue growth (Onofrı, 2016; Rozario & Desimone, 2009). Another important

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ECM protein, elastin, is an extracellular matrix (ECM) protein that provides flexibility and elasticity to tissues and organs. Elastin is roughly 1000 times more flexible than collagen; Therefore, the main function of elastin is the elasticity of tissues. It is the predominant protein in expandable tissues and is found mainly in the lungs, aorta, and skin (Kristensen & Karsdal, 2016).

ECM is distinctly modified in tissue pathologies including atherosclerosis, autoimmune and inflammatory diseases, and cancer. Healthy microenvironment of cells prevents the cancerous outgrowth of epithelial cells, whereas perturbation of homeostasis enables the initiation and progression of malignancy as well as the emergence of resistance (Theocharis et al., 2016). The degradation of ECM involves different types of matrix metalloproteases (MMPs) called matrixins such as collagenase, elastase, gelatinase and hyaluronidase. These enzymes have very important roles regulation and dysregulation, and MMPs play a role in the development, morphogenesis, tissue remodeling, and repair. However, irregularities in physiological conditions in the cell microenvironment raises its dysregulation, and degradation of ECM involved with different types proteases is among the causations of various diseases including cancer, rheumatoid arthritis, chronic ulcers, and fibrosis (Jabłońska-Trypuć et al., 2016).

Collagen and elastin as an extracellular component are necessary to the skin which play major role for the plumpness, flexibility, integrity, and elasticity keeping skin youthful and healthy. However, MMPs involved in degradation of ECM such as collagenase and elastase break down and damage collagen and elastin, respectively (Jiratchayamaethasakul et al., 2020). Also, it is necessary to break down the ECM components that are physical barriers to cell migration because it acts as a primary barrier to prevent the growth of the tumor tissue and the spread unhealthy cells. Malignant tumors use MMPs to overcome this barrier, which cause degradation of ECM and basement membrane components to invade the surrounding tissue (Itoh & Nagase, 2002; Öncel, 2009; Roy et al., 2009).

The chemical and natural molecules can decrease the degradation of ECM by inhibiting MMPs such as collagenase, elastase and gelatinase. Inhibitors of MMPs can prevent breakdown of the ECM and they can be useful in preventing degradation of ECM. Although extensive research has been conducted on enzyme inhibition related to various diseases, new candidate molecules are still needed due to side effects, low efficacy, and limited number of present inhibitors (Genc et al., 2020). Coordination complexes have been used in medicine for their therapeutic properties (Korkmaz et al., 2014, 2017). In the present study, it was aimed to evaluate the inhibitory effect of dicyanidogylene (DSG) complex against collagenase and elastase activities. The complex was previously synthesized for biological applications, but inhibition potency for collagenase and elastase was studied first time with current study.

2. METHOD

2.1 Collagenase inhibition assay

The inhibitory effect of $[Ni(edbea)Ag_3(CN)_5]$ (DSG) on collagenase (MMP-8) was determined by reading spectrophotometrically at 340 nm. For the measurement of collagenase (ChC) enzyme activity, 20 µL ChC from *C. histolycum* prepared in tricine buffer (50 mM, pH = 7.5) and 25 µL of test compound was added to each well. The plate left for incubation at 25°C for 15 min. Then 0.8 mM

substrate solution (FALGPA) was added to reaction medium and absorbance was measured in 5 minutes by using a microplate reader (Multiskan GO, Thermo Scientific, Ezgi Ersoy, 2019; Özbilgin, 2015; Thring et al., 2009).

2.2 Elastase inhibition assay

The inhibitory potency of the compound on elastase activity was evaluated according to the method previously described with some modifications (Ezgi Ersoy, 2019; Özbilgin, 2015; Süntar, 2011; Thring et al., 2009). The reaction mixture was created by adding 0.2 mM (pH = 8.0) 70 µL Tris-HCl buffer, 10 µL elastase and 10 µL of test compound. The plate was incubated at 25 ° C for 20 min. then 4.4 mM STANA (N-succinyl-Ala-Ala-Ala-Val-pnitroanilide substrate) was added to each well, and the increasing change in absorbance was read for 5 min.

The IC_{50} value of enzymes was calculated using the equation obtained from the linear cut of the curve drawn by entering the substance concentration and % activity data. Collagenase and Elastase enzyme activities were calculated using the following equation ($\mathcal{E}$= 0.53 and 8.8 for collagenase and elastase, respectively, df = dilution factor).

Unite/ml enzyme = $([\Delta A_{340/410}/\text{min test} - \Delta A_{340/410}/\text{min blank}] * \text{Total volume} * df) / (\mathcal{E} * \text{enzyme volume})$

3. RESULTS AND DISCUSSION

In this study, the inhibition effects of novel compound ($[Ni(edbea)Ag_3(CN)_5]$) on the activity of elastase and collagenase were investigated. IC_{50} and Ki values of the complex for collagenase and elastase enzyme are shown in the Table [1] below.

Table 1. Enzyme inhibition results of $[Ni(edbea)Ag_3(CN)_5]$ against to collagenase and elastase

Compounds	IC_{50} (µM)				Ki (µM)	
	Collagenase	r^2	Elastase	r^2	Collagenase	Elastase
DSA	10.66 µM	0.991	49.5 µM	0.9872	16.73+1.07 µM	42.81 ± 9.62 µM

As a result of spectrophotometric measurements of $[Ni(edbea)Ag_3(CN)_5]$, it was exhibited that the compound has inhibitory potency on collagenase and elastase activity on collagenase compared to the control group. IC_{50} values of the DSG were calculated as 10.66 µM and 49.5 µM collagenase and elastase, respectively. Inhibition constant (Ki) of DSG for both enzymes was obtained from drawn Lineweaver Burk (LB) plots. The Ki values of the compound for collagenase and elastase enzymes were calculated as 16.73 ± 1.07 µM and 42.81 ± 9.62 µM, respectively. According to present results, it is understood that these chemical compounds are effective on the mentioned enzymes at micromolar level and LB graphs for collagenase and elastase were shown below in Fig. 1 and Fig. 2. In addition, inhibition type of the compound was competitive against to collagenase, but it exhibited

noncompetitive inhibition against to elastase. According to the results of the work, it has been seen that the inhibitory property of the $[Ni(edbea)Ag_3(CN)_5]$ is higher on the collagenase enzyme activity compared to the elastase enzyme activity.

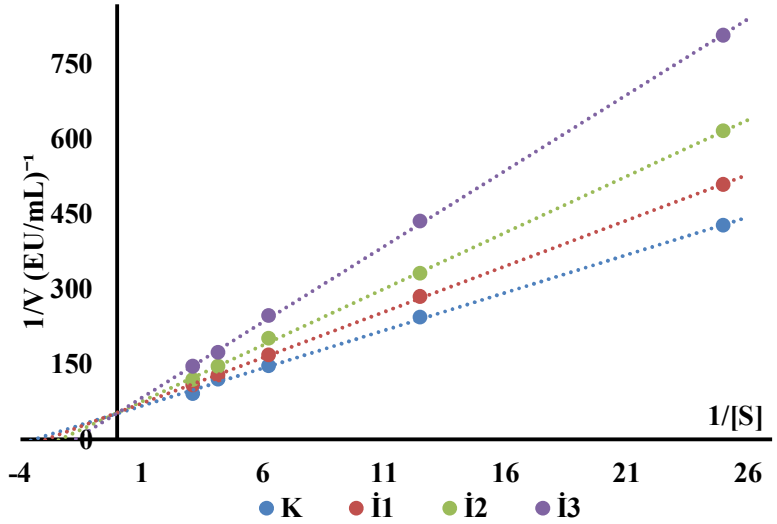

Figure 1. Effect of the $[Ni(edbea)Ag_3(CN)_5]$ on collagenase (MMP-8) activity

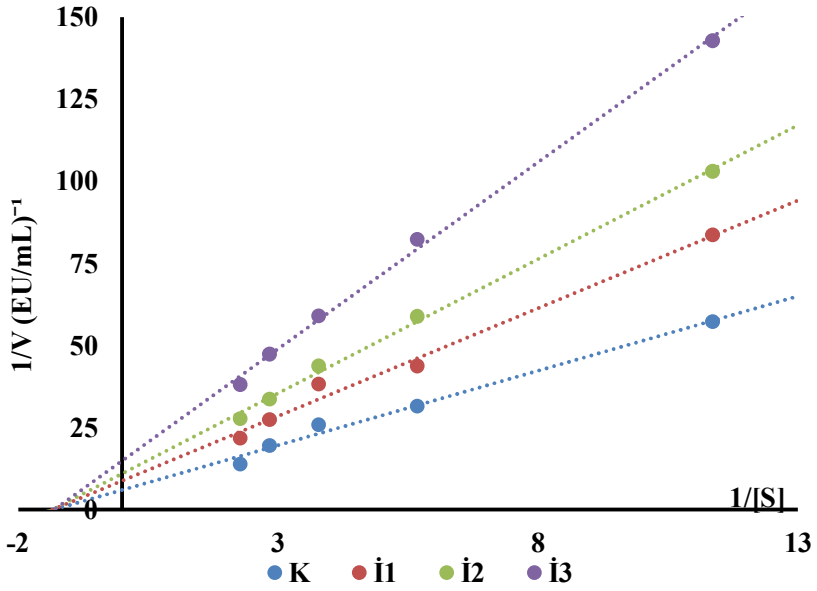

Figure 2. Effect of the $[Ni(edbea)Ag_3(CN)_5]$ on elastase enzyme activity

The results indicate that coordination polymers containing dicyanidogylene have inhibition effects and the work is one of the first report on inhibition of the collagenase and elastase.

Coordination complexes have remarkable therapeutic properties due to their biological activities. Their pharmacological properties involved in biochemical reactions, which are important reactions for human quality of life (Kısa et al., 2020). Previously studies have reported that chemical and plant-based compounds inhibit enzymes (Bahadır Acıkara et al., 2019; Korkmaz et al., 2021; Lee et al., 2020). Genç et al. recorded that constituents of *Plantago major subsp. major* L. exhibited the inhibition against collagenase enzyme (Genc et al., 2020). The another study related with halophyte plants reported that the extracts of *Salicornia europaea* and *Rosa rugosa* showed the highest anti-elastase and anti-collagenase effect, respectively (Jiratchayamaethasakul et al., 2020).

According to the obtained results of present work, DSG have anti-collagenase and anti-elastase activity, suggesting that this compound could be used for the prevent breakdown of the ECM to support the struggle against various diseases such as including cancer, rheumatoid arthritis, and fibrosis. Various therapeutic interventions and medical approaches can affect different processes involved in the progression of disease processes related with MMPs (Boran et al., 2018). The inhibition of these enzymes leading to degradation of ECM can provide to prevent the unhealthy cells from spreading to other medium. The disorders in collagen and elastin metabolism and degradation are important in the course of osteoarthritis, osteoporosis, and oncogenesis. Extensive knowledge of the properties of different enzymes that participate in ECM degradation is very important due to their possible therapeutic use (Jabłońska-Trypuć et al., 2016). The results offered an acceptable inhibitory effect of both ECM-deteriorated enzymes and presumably recommended that DSG may contribute an effective anti-degradation compound by interrupting the activity of collagenase and elastase. According to the obtained results, this study is an important pre-screening for new and effective inhibitor trials. In the future, the inhibitory effect of the molecule on ECM-degrading enzymes can be investigated in cell cultures and animal experiments for beneficial effects and the result may lead to designing potent new inhibitors.

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