SYNTHESIS AND ANTI-HIV ACTIVITY OF OLIGOPEPTIDE-LIKE STRUCTURES CONTAINING CATIONIC FRAGMENTS

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MASTER'S THESIS

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This thesis titled "**Synthesis and Anti-HIV Activity of Oligopeptide-Like Structures Containing Cationic Fragments**" has been prepared and submitted by **Demokrat NUHA** in partial fulfillment of the requirements in "Anadolu University Directive on Graduate Education and Examination" for the Degree of Master of Science in Chemistry Department has been examined and approved on **18/07/2019**.

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ABSTRACT

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Peptides are considered as uprising class of biologically active regulators, which, by preventing various diseases, increase the quality of life. Peptides have a very important function, such as the hormonal role in the organism, in different metabolic functions as well as in human health. Nowadays, about 70 peptide drugs have been approved for use and currently, about 200 peptide therapies are being evaluated in clinical trials.

The aim of this study is to synthesize oligopeptides with cationic fragments that may exhibit potential activity against the HIV virus. Syntheses are initiated by alkoxy benzoates, proceeding to the active intermediate *N*-Acyl benzotriazole, amide derivatives and amino acid amides derives using two amino acid cations N^{ω}-(NO_2–)-arginine and L-histidine as well as various substituents. All of the target compounds that were prepared here are novel and obtained in moderate to high yield.

Key Words: Arginine, Histidine, Peptides, *N*-acyl benzotriazole, Anti-HIV.

ÖZET

KATYONİK UNSURLAR İÇEREN OLİGOPEPTİD BENZERİ YAPILARIN SENTEZ VE ANTİVİRÜS AKTİVİTESİ

Demokrat NUHA

Kimya Anabilim Dalı

Anadolu Üniversitesi, Fen bilimleri Enstitüsü, Temmuz, 2019

Danışman: Doç. Dr. İlker AVAN

Peptitler, çeşitli hastalıkları önleyerek yaşam kalitesini arttıran biyolojik olarak aktif düzenleyicilerin en yeni sınıfı olarak kabul edilir. Peptitler, organizmada hormonal rol gibi, farklı metabolik fonksiyonlarda ve insan sağlığında çok önemli bir fonksiyona sahiptir. Günümüzde, yaklaşık 70 peptid bileşiği ilaç olarak kullanılmaktadır ve şu anda klinik deneylerde yaklaşık 200 peptid bileşiği yeni ilaç adayları olarak değerlendirilmektedir.

Bu çalışmanın amacı, HIV virüsüne karşı potansiyel aktivite gösterebilecek katyonik parçalara sahip oligopeptide yapılarını sentezlemektir. Aktif ara ürün olan *N*-Asil benzotriazole bileşiklerinin bazik sulu ortamda, katyonik amino asitler (His, Arg) ile reaksiyonları sonucu *N*-sübstitüye amino asitler elde edilmiş ve bunların karboksilik uçlarının diğer katyonik ve nört fragmentler (hidrazin, guanidine, birinci aminler) ile eşleştirerek katyonik unsurlar içeren kısa oligopeptid yapılarının sentezi gerçekleştirilmiştir. Bu çalışmada hazırlanan hedef bileşiklerin tümü yenidir ve yüksek verimde elde edilmiştir.

Anahtar Sözcükler: Arginin, Histidin, Peptitler, *N*-asil benzotriazol, Anti-HIV.

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18/07/2019

STATEMENT OF COMPLIANCE WITH ETHICAL PRINCIPLES AND RULES

I hereby truthfully declare that this thesis is an original work prepared by me; that I have behaved in accordance with the scientific ethical principles and rules throughout the stages of preparation, data collection, analysis and presentation of my work; that I have cited the sources of all the data and information that could be obtained within the scope of this study, and included these sources in the references section; and that this study has been scanned for plagiarism with "scientific plagiarism detection program" used by Anadolu University, and that "it does not have any plagiarism" whatsoever. I also declare that, if a case contrary to my declaration is detected in my work at any time, I hereby express my consent to all the ethical and legal consequences that are involved.

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LIST OF ABBREVIATION

Alloc	: Allyloxycarbonyl
AMP	: Antimicrobial peptides
Appx	: Appendix
Arg	: Arginine
Boc	: tert-Butyloxycarbonyl
Bt-*H*	: 1*H*-Benzotriazole
Bzl	: Benzyl
C	: Carbon
CaH_2	: Calcium hydride
Calcd.	: Calculated
Cbz	: Benzyloxycarbonyl
$CDCl_3$	: Deuterated chloroform
CH_2Cl_2	: Dichloromethane
CH_3CN	: Acetonitrile
cHx	: Cyclohexyl
d	: Doublet (spectral)
D_2O	: Deuterium oxide
DCM	: Dichloromethane
dd	: Doublet of doublets (spectral)
ddd	: Doublet of doublet of doublets (spectral)
Dde	: (1-(4,4-dimethyl-2,6-dioxocylohex-1-ylidene)-3-ethyl)
ddq	: Doublets of quartets (spectral)
Diox	: 1,4-Dioxane
DMF	: Dimethylformamide
DMSO	: Dimethyl sulfoxide
DMSO-*d6*	: Deuterated dimethyl sulfoxide
Dnp	: 2,4–Dinitrophenyl
dq	: Quartet of doublets (spectral)

dt	: Triplet of doublets (spectral)
EDC	: *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide
equiv.	: Equivalent(s)
Et	: Ethyl
EtOAc	: Ethyl acetate
EtOH	: Ethanol
Fmoc	: 9-fluorenylmethyloxcarbonyl
FUR protein	: Ferric-uptake regulator protein
g	: Gram(s)
h	: Hour
H	: Hydrogen
H_2	: Hydrogen gas
HCl	: Hydrochloric acid
His	: Histidine
HO-Bt	: 1-Hydroxybenzotriazole
HPLC	: High-performance liquid chromatography
HRMS	: High-resolution mass spectrometry
Hz	: Hertz
I	: Iodine
J	: Coupling constant (NMR spectroscopy)
K_2CO_3	: Potassium carbonate
K_2CO_3	: Potassium carbonate
KOH	: Potassium hydroxide
LiOH	: Lithium hydroxide
m	: meta position
m	: Multiplet (spectral)
m/z	: Mass-to-charge ratio
Me	: Methyl
Me-OH	: Methyl alcohol
MHz	: MegaHertz

min	: Minute(s)
mL	: Milliliter(s)
mol	: Mole(s)
mp	: Melting point
MP	: Melting Point
MS spec	: Mass Spectrometry
N	: Nitrogen
Na	: Sodium
Na_2CO_3	: Sodium carbonate
Na_2SO_4	: Sodium sulfate
NaH	: Sodium hydride
$NaHCO_3$	: Sodium bicarbonate
NaOH	: Sodium hydroxide
NH(CNH)NHPiv	: *N*-carbamimidoylpivalamide
NHBu-*i*	: Isobutyl amine
NHNHCbz	: Benzyl *N*-amino carbamate
nitro-L-Arg	: N^{ω}-(NO_2-)-L-Arginine
NMR	: Nuclear Magnetic Resonance
Nvoc	: 6-nitroveratryloxycarbonyl
O	: Oxygen
^oC	: Degree Celsius
OEt	: Ethoxy
OMe	: Methoxy
p	: para position
Pbf	: Pentamethyl-2,3-dihydrobenzofuran-5-sulfonyl
Pd	: Palladium
Pg	: Protecting group
pH	: The negative logarithm of the hydronium ion concetration (–log[H_3O^+])
Ph	: Phenyl

pNZ	: *p*-Nitrobenzyloxycarbonyl
PPh_3	: Triphenylphosphine
ppm	: Parts per million
psi	: Pounds per square inch
q	: Quartet (spectral)
$SOCl_2$	: Thionyl chloride
t	: Triplet (spectral)
tBu	: *t*-Butyl
td	: Doublet of triplets (spectral)
TEA	: Triethylamine
TFA	: Trifluoroacetic acid
THF	: Tetrahydrofuran
TIPS	: Triisopropylsilane
TLC	: Thin Layer Chromatography
TMS	: Tetramethylsilane
Tos	: *p*-Toluenesulfonyl
Trt	: Trityl group
α	: Alpha
β	: Beta
δ	: Chemical shift in parts per million downfield from tetramethylsilane
ω	: Omega

1. INTRODUCTION

1.1. Amino Acids

Organic compounds that contain the amine group (-NH_2), as well as the carboxylic group (-COOH), are defined as amino acids. Until now, more than 700 amino acids have been discovered in nature(in algae, various plants, bacteria, and fungi), most of them are L-α-amino acids. But only 20 amino acids that contain standard genetic code participate in the construction of peptides and proteins that are also known as standard amino acids [1]. In addition to the amine and carboxylic groups, amino acids also contain another side chain (*R*) which determines the physical and chemical properties and the biochemical functions of amino acids [2-3]. All standard amino acids except glycine exhibit optical activity because they contain asymmetric carbon in their molecules. Due to this, the amino acids are classified into L- or D-isomers by taking glyceraldehyde as a reference [4]. Aditionally, glycine does not contain chiral carbon in its structure because the two hydrogen atoms in its α-carbon are bonded. Just a few exceptions, most amino acids involved in the synthesis of protein are L-stereoisomers.

All standard amino acids contain a central atom (α-carbon) in which a carboxyl group, a hydrogen atom, an amine group and *R* group (side chain) are connected. The proline structure is a little different, where the *R* group as well as the amino acid linkage bond and form a ring that gives the peptide chain **stiffness** because the rotation of the α-carbon in proline is impossible (Figure 1.1.) [5-6].

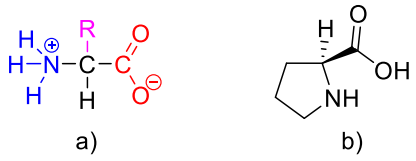


Figure 1.1. a) *General structure of α-amino acid and* **b)** *Structure of proline.*

All amino acids that do not participate in protein synthesis are part of the non-standard amino acid group. The presence of the amine and carboxyl group causes the amino acids to be amphoteric. At low pH values in the amine group, we have a positive charge which is related to cationic amino acids, whereas at high pH values in the carboxylic group we have a

negative charge, which is related to anionic amino acids. At pH 7 in the amino acid structure, we have both loads (positive and negative) referred to as zwitterions (Figure 1.2.) [7].

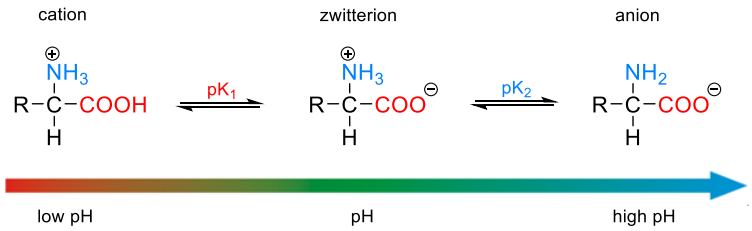


Figure 1.2. *The effect of pH on amino acids*.

Standard amino acids are categorized into four groups considering their interaction with water:

1. *Nonpolar amino acids*: This group includes alanine, cysteine, glycine, isoleucine, leucine, methionine, proline, phenylalanine, tryptophan and valine. The *R* groups in these amino acids have no charge. Due to their poor interaction with water, non-polar amino acids have an important role in biochemical processes as well as storage of three-dimensional protein structures.

2. *Polar amino acids or hydrophilic amino acids*: Glutamine, serine, threonine, and tyrosine are part of this group. In their structures, serine, threonine, and tyrosine contain the hydroxyl group (-OH) which is an important factor in peptide and protein structures. Also, the sulfhydryl (thiol) group (-SH) in the cysteine is important in various enzymatic processes.

3. *Acidic amino acids*: This group includes only two amino acids (aspartic and glutamic acid) that contain an extra carboxyl group in their chains.

4. *Basic amino acids:* They have a positive charge in their structures. They can easily interact with acids via ionic bonds. This group includes arginine, histidine, and lysine [8].

Synthesis of peptides is achieved by the interaction of amino acids via peptide bonds. The peptide bonds are chemical bonds that are formed by the interaction of the amine group of one amino acid with the carboxyl group of another amino acid (Figure 1.3.) [9].

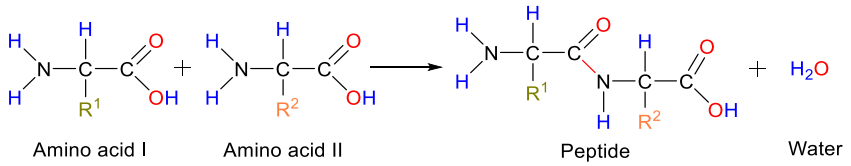


Figure 1.3. *Formation of peptide bonds.*

1.2. Peptides

Peptides are biologically active molecules which consist of 2–50 amino acids linked by peptide or amide bonds. When more than 50 amino acids (> 50) participate in the composition of a chain, is referred to as proteins [10]. Similar to proteins, peptides are synthesized in nature by transcription which involves copying of DNA genetic sequence in an enveloped mRNA molecule [11]. Nowadays, peptides are considered as an ideal class of biologically active regulators, which increase the quality of life, by preventing various diseases [12-13]. Peptides have a very important function, such as the hormonal role in the organism, in different metabolic functions as well as in human health. [14]. The peptide function depends on the structure of peptides, the types of amino acids, the peptide chain transduction sequences and 3D structure in space [15-16].

There are two common ways to synthesize peptides:

1. Solution Phase Synthesis
2. Solid Phase Peptide Synthesis (SPPS)

Solution Phase

This technique is used for the synthesis of small peptides (with a small number of amino acids). After each step of the synthesis during this technique, the intermediate products can be isolated and analyzed. This is the main advantage of using this technique in the synthesis of peptides [17]. Figure 1.4. show the benzotriazole mediated-solution phase peptide synthesis in which benzotriazole is a carboxylic group of activators in peptide synthesis.

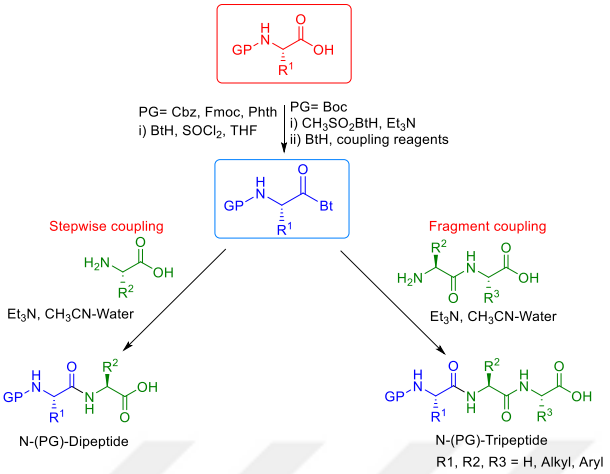


Figure 1.4. *Benzotriazole mediated-solution phase peptide synthesis*.

Solid Phase Peptide Synthesis (SPPS)

Solid phase is used to synthesize large peptides and proteins. In 1984, Merrifield managed to synthesize a nona-peptide (Bradykinin) using the SPPS technique [18]. The sequence and the type of amino acid of this nona-peptide is:

Arg - Pro - Pro - Gly - Phe - Ser - Pro - Phe - Arg (Bradykinin)

The synthesis of this product was completed with 68 % yield in 8 days [19].

Synthesis of dipeptides is achieved by forming an amide bond between two amino acids. Two different amino acids, including two amine groups and two carboxylic groups can be coupled in four different ways to form four different dipeptides. In this process however, they end-up providing peptides which are undesirable for us. Therefore, blocker for N-terminal of one amino acid and a blocker for C-terminal of another amino acid are necessity to prevent undesirable coupling. This enables high selectivity to be achieved, whereby at the end of the synthesis of the peptides, the protective groups can be removed without difficulty [20-21]. In figure 1.5. have shown the schematic synthesis of the solid phase peptide.

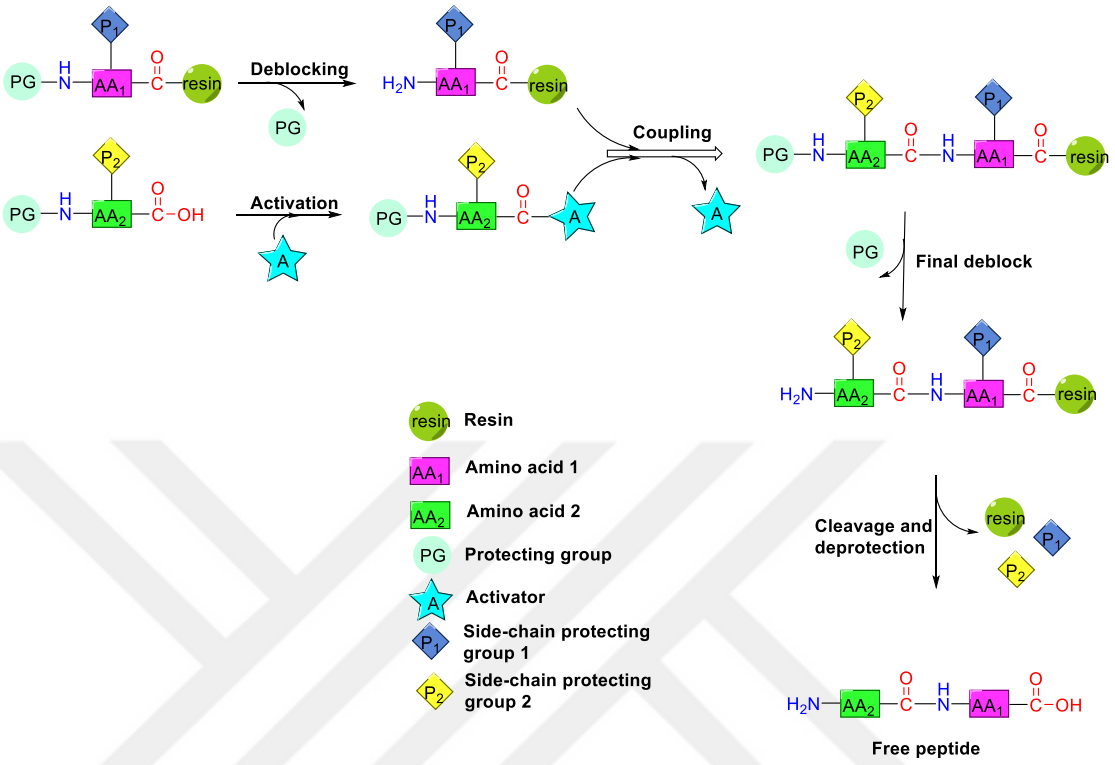


Figure 1.5. *The solid phase-peptide synthesis (SPPS).*

The protecting group (PG) must follow some criteria in order to be used for the synthesis of peptides. PG should have no difficulty in linking with and removing amino acids. The intermediary produced by the PG linkage with the amino acids should be rigid and easily soluble in solution to prevent their dimerization [22]. Most of the PG used in peptide synthesis are shown in Figure 1.6. and 1.7.

In recent years, a new pathway for use in the treatment of various human diseases using peptide drugs has been found. For example, treatment of prostate cancer and breast cancer is made possible by the use of Goserelin (Zoladex, AN: DB00014) [23]. Treatment of neuroendocrine tumors is possible with the use of Octreotide (AN: DB00104) [24] and Lanreotide drugs (AN: DB06791) [25]. For the sclerosis disease a synthetic peptide (Glatiramer acetate, AN: DB05259) [26] is being used. For this reason, there has been a drastic increase in the peptide drug market in recent years and the annual sales of all peptide drugs have reached a value of 20 billion US dollars [27]. Food and Drug Administration (FDA) and Center for Drug Evaluation and Research (CDER), approved approximately 70

peptide drugs for use in the global drug market, about 200 peptide drugs are in clinical trials, 600 peptide drugs in pre-clinical studies, and thousands of peptide drugs in laboratory placement [28].

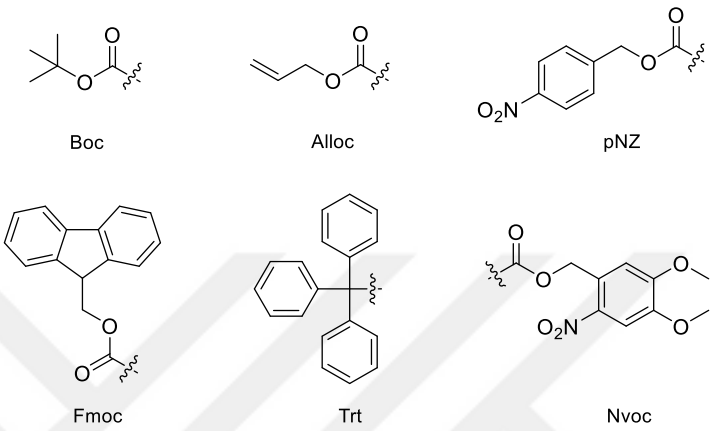


Figure 1.6. *N^{α}–amino protecting groups in peptide synthesis*.

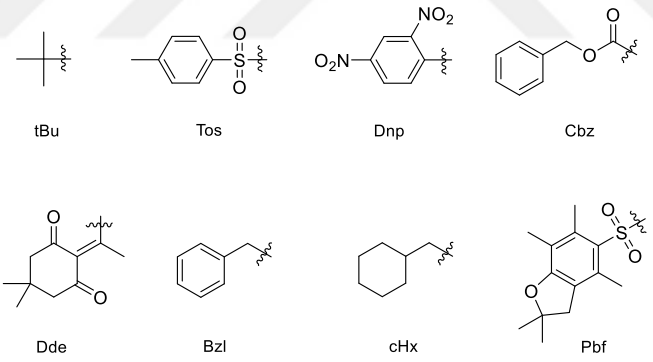


Figure 1.7. *Amino acid side–chain protecting groups.*

1.3. Antimicrobial Peptides

Antimicrobial peptides (AMPs) are organic compounds that are biologically toxic against pathogens. AMPs can also be classified as protective peptides because of their essential role in the composition of the immune system [29]. One of the major factors for the use of AMPs as antibiotics is their broad-spectrum activities against various microorganisms, including Gram-positive and, Gram-negative bacteria, fungi and viruses. Also, they exhibit different mechanisms of action that greatly differ from other antibiotics [30]. Cationic peptides have shown higher anti-microbial activity as compared to the other types of peptides

[31-32]. This activity is related to the physical interaction between opposite charges, the negatively charged phospholipids of pathogenic shingles and the cationic load of peptides [33].

Depending on the structure, transition, function, mechanism, etc. AMPs can be divided into several groups [34]. The construction structure of AMPs can be considered in four divisions:

1. AMPs α-helixes comprise a considerable number of antimicrobial peptides which do not have regular structure into an α-helix.
2. AMPs β-sheet are peptides which contain disulfide in their structures, in that case, high structural stability is achieved.
3. Extended AMPs are antimicrobial peptides, whose structure is constructed from very specific amino acids such as Arginine or histidine.
4. In the end loop or cyclic peptides, whose structure is curved as a result of the disulfide bridges that occurred in them (Figure 1.8.) [35-36].

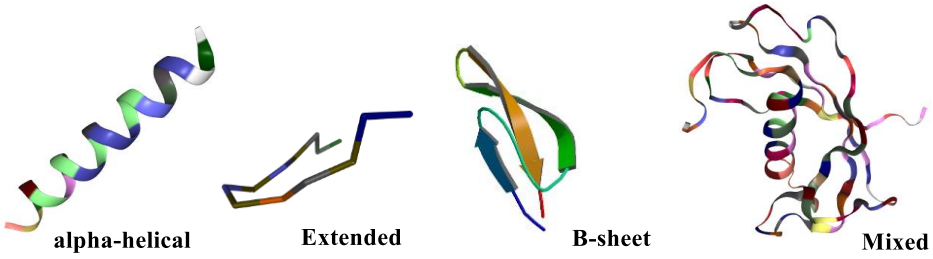


Figure 1.8. *Various structures of antimicrobial peptides*.

Some general features of AMPs: A relatively small number of amino acids (from 12 to 50 amino acids) participate in their structures. Most membrane-active AMPs have a cationic face from the present of Arg or other cationic amino acid. Also AMPs contain different hydrophobic and hydrophilic regions in their structures [37-38].

The use of AMPs as antibiotics also has disadvantages such as potential toxicity, high production cost, etc. In recent years, efforts have been made by scientists to eliminate all these disadvantages and a result has been achieved. In order to decrease the high production costs, short peptides which may contain up to 12 amino acids were synthesized and also the relationship between their three-dimensional structures and antimicrobial activity was

studied in detail [39]. Synthesis short sequences of AMP that exhibit high activity with lower cost was investigated [30].

1.4. Cationic Peptides

Cationic peptides are organic compounds that may contain positive loads from +2 to +9 [40]. Somewhere around half of the amino acids that participate in the construction of cationic peptide chains are hydrophobic amino acids, while the other half are hydrophilic amino acids [33, 41-42]. This is a great advantage, for example, cationic peptides can interact with other compounds in aqueous media as a result of the presence of hydrophilic amino acids, or even in fatty environments due to the presence of hydrophobic amino acids in their chain structures [43-44]. They can also interact with membranes of pathogens which is negatively loaded. If there is a recurrence of cells that are not loaded, as can be the case in animal cells, then the aforementioned peptide does not exhibit a tendency for the interaction of these cells [45].

Cationic peptides have demonstrated antimicrobial activity against both gram-positive and gram-negative bacteria and have shown activity against viruses and fungi [46-47]. Therefore, many chemists and biochemists have been trying to synthesize new structures of cationic peptides to reach elevated anti-microbial activity [48-49].

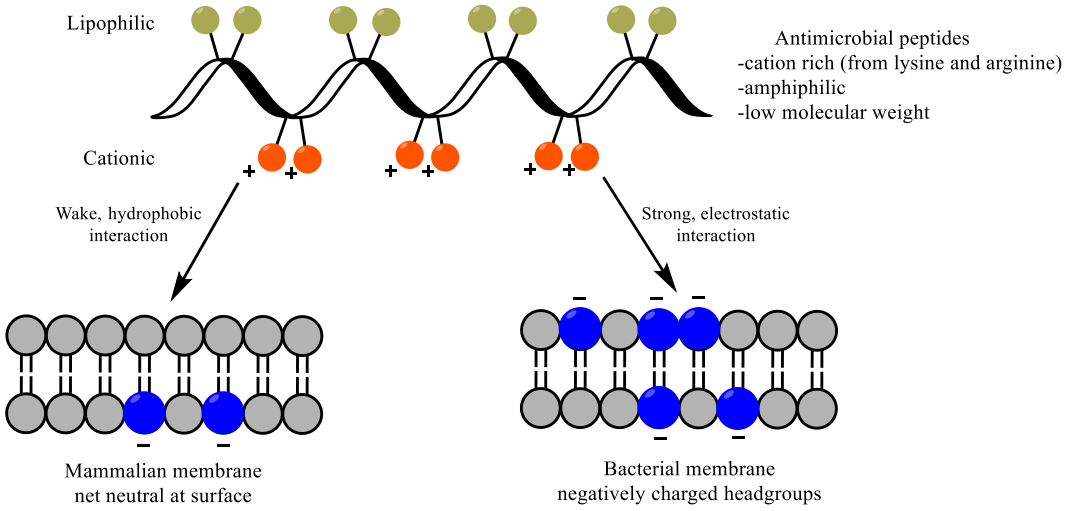


Figure 1.9. *Schematic showing the common structural properties and membrane targets of AMPs.*

1.4.1. The mechanism of cationic peptide in pathogenic cell

To use an organic compound as a microbial antibiotic, it is necessary for the compound to fulfil certain conditions. One of the main conditions is to have high selective toxicity, enabling the distinction between pathogenic cells and mammalian cells [33].

Pathogenic cell membranes mostly negatively charged, which is opposite load of cationic peptides. This enables their electrostatic interaction making the cationic peptides more selective to pathogenic cells. However, mammalian membranes do not possess charge, mostly neutral, they remain indifferent to the possibility of reacting with cationic peptides [50].

Cationic peptides can interact with pathogenic cells in two ways:

1.Via the outer membranes of pathogenic cells and

2.Via their cytoplasmic membranes.

1. *The cationic peptide action mechanism in the outer membranes of pathogenic cells is:* The load on the outer membrane of the pathogenic cells, such as the high negative loads in the gram-negative bacteria, causes electrostatic interaction between the cationic peptides and the outer membranes of these cells [51]. There is a transposition of the magnesium ions from the cationic peptides to the outer membranes of the pathogenic cells, though there is a partial neutralization of negative loads as well as a strong linkage between the cationic peptides and the external membranes of the pathogenic cells. Then the peptides can easily be inserted and translocated into bacterial cells [50, 52].

2. *Cationic peptides can interact electrostatically with external membranes and also with cytoplasmic membranes of pathogenic cells* [46]. After insertion into the cytoplasmic membranes, the hydrophilic and hydrophobic sections of the cationic peptides connect to the internal cytoplasmic part of the bacterial cells. This then marks the beginning of the action of high antibacterial activity inside the pathogenic cells (Figure 1.10.) [45].

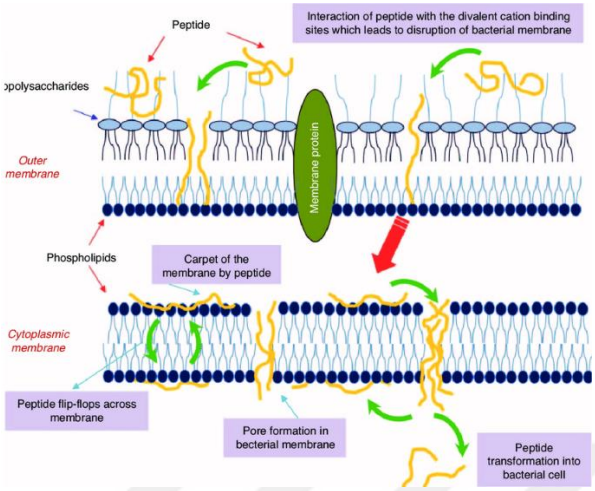


Figure 1.10. *Mechanism of killing by cationic peptides of gram-negative bacteria. Reprinted with permission from* [53]. *Copyright 2015 Reviews in Medical Microbiology*.

1.5. L-Histidine

One of the essential amino acids is L-Histidine (His) [54]. L-histidine has fundamental importance: it contributes to the formation of many important enzymes, it also affects the process of obtaining and using essential metals such as manganese, iron, copper and zinc in mammalian organisms [55]. Histidine also has direct and indirect effects on many biological processes such as fermentation, hemoglobin formation and iron-taking by FUR (Ferric uptake regulator) protein [56].

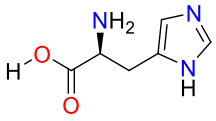


Figure 1.11. *The structure of histidine*.

As seen in Figure 1.11. histidine structure has an imidazole group. This functional group accounts for some special characteristics of the histidine amino acid since it's active during various enzymatic catalyzed processes [57-58]. Because of the presence of free electrons in their structure, the imidazole group can serve as a nucleophilic or as a potential base and, while in the protonic state this group exhibits acidic properties [54]. The most dominant form of histidine in gas phase is in neutral state of imidazole since histidine is in a stable condition due to the two hydrogen bonds present in its structure [59].

Histidine is involved in the construction of a large number of proteins of fundamental importance for human life as well as in enzymatic processes, and this has prompted many scientists in recent years to research more about its structure, properties and potential impact during various biological processes [60].

In a neutral state, histidine may be present in four conformers-tautomer forms. These are schematically shown in figure 1.12. Conformer **a** in figure 1.12. is the lowest in free energy, likely due to two internal hydrogen bonds [61].

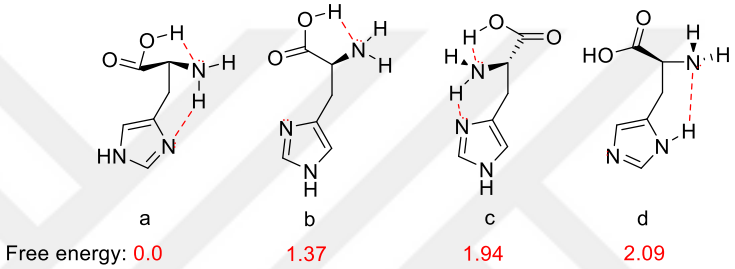


Figure 1.12. *The four conformer-tautomer's of histidine. The relative free energy is in kcal mol^{-1}.*

1.6. L-Arginine

In 1886, Ernst Schulze and Ernst Steiger isolated and determined the structure of L-arginine for the first time [62]. L-arginine is an essential amino acid that contains an α-amino group, an α-carboxylic acid group, and a side chain consisting of a 3-carbon aliphatic straight chain ending with a guanidine group (Figure 1.13). L-arginine is a component of all protein-containing foods and also can be synthesized in the body from glutamine *via* citrulline [62-64].

Arginine's side chain is amphipathic, it contains a positively charged polar guanidinium group in physiological condition, at the end of a hydrophobic aliphatic hydrocarbon chain. When the double bond and the nitrogen lone pairs conjugate, the positive charge is delocalized, enabling the formation of multiple hydrogen bonds (Figure 1.14) [65]. L-arginine is typically found on the outside of the protein because globular proteins have hydrophobic interiors and hydrophilic surfaces [66].

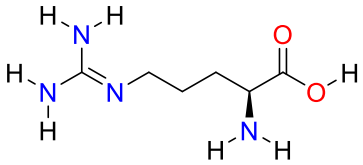


Figure 1.13. *The structure of L-Arginine.*

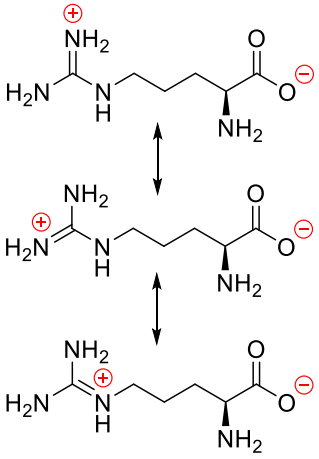


Figure 1.14. *Delocalization of positive charge in guanidinium group of L-Arginine.*

As in the case of some carnivores have a high amount of ammonia in the body because of which, as a result of high protein metabolism. The excess of ammonia content from the organism is removed through the urea cycle, where arginine has an important function. The absence of arginine has a negative effect by increasing ammonia toxicity in the organism that may be deadly [67], [68]. The organism synthesizes arginine in the kidney as well as in the small intestine for its own needs [69]. When its amount is insufficient, arginine should be taken with food to fulfill these needs. The highest amount of this amino acid is in meat, eggs, beans, dairy products and nuts [70].

L-arginine is an essential amino acid because it plays important role in cell division, and the release of hormones [45, 66] making it important in the regulation of blood pressure [71]. It also participates in healing the skin from various injuries and burns [72].

1.7. HIV

Human immunodeficiency virus (HIV) is one of the biggest threats posed to global health. About 70 million people have been infected with the HIV since the beginning of the epidemic and about 35 million people died of HIV, making it one of the deadliest epidemics

in human history. According to WHO statistics, about 44 million people are now living with HIV. Africa is the most affected region with almost 1 in every 25 adults living with HIV and accounting for nearly two-thirds of people living with HIV around the world [73-74].

In the human body, we have T helper cells (lemphosides, called CD4 cell by having CD4 receptor) that help the immune system fight against various infections. The HIV virus is the virus that attacks the immune system by reducing the number of CD4 cells, which makes it impossible to fight various infections and diseases [75-76]. The HIV virus is different from other viruses. There is no realistic cure for treatment of HIV infection but with adequate treatments, HIV medicines (called antiretroviral or ARV) can prevent or decrease the HIV replication. Also, the use of these drugs reduces the HIV transmission [77-78].

There are three stages of HIV infection: Acute HIV infection, chronic HIV infection, and AIDS [79].

Acute HIV infection is the initial phase of HIV infection that lasts for 2 to 4 weeks. People who are affected by this virus at the acute stage exhibit flu-like symptoms such as headaches, rashes, and fevers. At this stage, HIV spreading faster by fighting the CD4 cells, as HIV levels increase in the blood, the risk of HIV transmission significantly increases.

Chronic HIV infection represents the second stage of HIV. At this stage, people affected by HIV do not exhibit HIV symptoms but pose a risk for HIV transmission. At this stage of infection, HIV multiplies but at much lower levels. Without using HIV drugs, chronic HIV infection progresses to the final stage and this takes about 10 years.

AIDS represents the last stage of HIV infection. At this stage, the immune system weakens a lot due to the decrease in the number of CD4 cells, usually less than 200 cells / mm^3 (for someone with a healthy immune system, the number of CD4 is between 500 and 1,600 cells / mm^3). People infected with HIV at this stage are at a high risk by opportunistic infections and illness (like influenza and cancer). Without taking the remedies against HIV, people with AIDS can survive for about 3 years [80-81].

1.7.1. HIV therapy

In fact, there is no complete medication for HIV, but there is a so-called “Highly Active Antiretroviral Therapy” (HAART) method which is the combination of different antiviral

drugs targeting HIV replication steps [82]. Until now, we have a significant number of antiretroviral drugs used in HAART therapy. For example, Biktarvy® consist of bictegravir, emtricitabine and tenofovir alafenamide and Triumeq® consist of abacavir, dolutegravir, lamivudine [83-84].

All antiretroviral drugs are divided into seven different groups:

1. Integrase inhibitors (**INIs**) (raltegravir)
2. Nucleotide reverse transcriptase inhibitors (**NtRTIs**) (tenofovir disoproxil fumarate),
3. Nucleoside reverse transcriptase inhibitors (**NRTIs**) (abacavir, didanosine, emtricitabine, lamivudine, stavudine, zalcitabine and zidovudine)
4. Non-nucleoside reverse transcriptase inhibitors (**NNRTIs**) (delavirdine, efavirenz etravirine and nevirapine)
5. Co-receptor inhibitors (**CRIs**) (maraviroc)
6. Fusion inhibitors (**FIs**) (enfuvirtide) and
7. Protease inhibitors (**PIs**) (amprenavir, atazanavir, darunavir, fosamprenavir, indinavir lopinavir, nelfinavir, ritonavir, saquinavir and tipranavir).

The passage of AIDS from a fatal disease to a chronically manageable condition is the attribute of HAART therapy [85].

Also, there are some cationic antiviral peptides: Melittin (26-membered polypeptide) and Indolicidin (13-membered polypeptide). Melittin has been reported to have antitumor activity, inhibitory activity against viruses such as HIV-1, HSV-2 (Herpes simplex virus) and junin virus (JV). The same applies to indolicidin, a peptide isolated from cytoplasmic granules of bovine neutrophils. However, it has shown that it exhibits activity against HIV and HSV through a membrane-mediated antiviral mechanism as well as its DNA-binding ability.

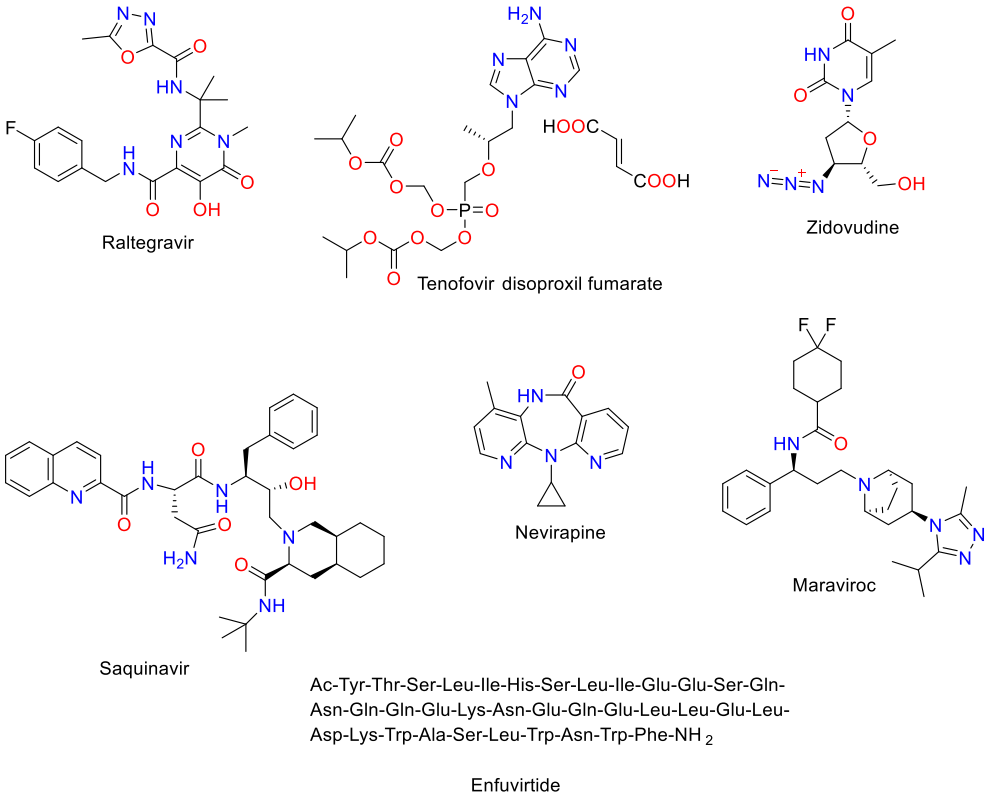


Figure 1.15. *The structure of some antiretroviral drugs used in AIDS therapy*.

Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-
Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-
Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln-NH $_2$

Melittin

H-Ile-Leu-Pro-Trp-Lys-Trp-Pro-
Trp-Trp-Pro-Trp-Arg-Arg-NH $_2$

Indolicidin

Figure 1.16. *The structure of Melittin and Indolicidin*.

2. PREPARATION OF OLIGOPEPTIDE-LIKE STRUCTURES CONTAINING CATIONIC FRAGMENTS

Alkoxy benzoates (**2a–d**) compounds, that were prepared from the hydroxy benzoates, were hydrolyzed to give alkoxy benzoic acids (**3a–d**). Active intermediate *N*-acyl benzotriazole (**4a–d**) was prepared from alkoxy benzoic acids and then coupling with amino acids (**5a–i**) and treated with different substituents to synthesis amino acids amides **6a–l** (**Figure 2.1.).**

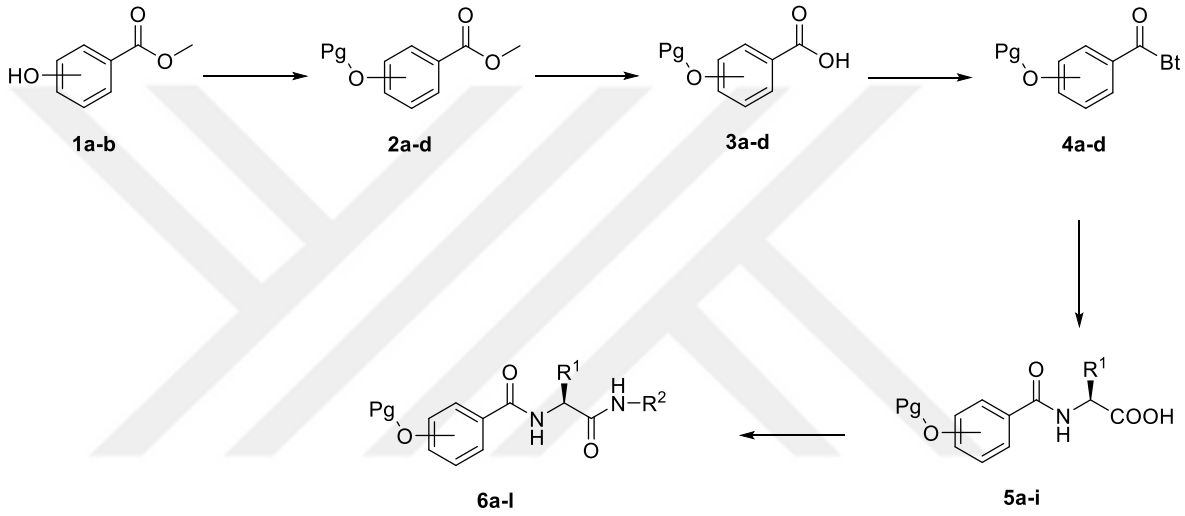


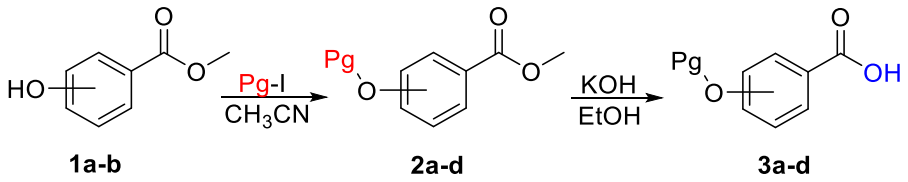
Figure 2.1. *Synthetic route for the preparation of oligopeptides containing cationic fragments*.

2.1. Synthesis of Alkoxy Benzoates 2a–d

Alkoxy benzoates **2a–d** (82–98%) were prepared from the reaction of methyl 3–hydroxybenzoate (**1a**) or methyl 4–hydroxybenzoate (**1b**) with iodoethane or benzyl bromide and K_2CO_3 in dry acetonitrile under reflux conditions for 12h (Scheme 2.1. and Table 2.1.).

2.2. Hydrolysis of Benzoate Ester 3a–d

The hydrolysis of alkoxy benzoates **2a–d** in the KOH solution in ethanol–water gave alkoxy benzoic acids **3a–d** (83–99%) (Scheme 2.1. and Table 2.2.).



Scheme 2.1. *Synthesis of alkoxy benzoates **2a–d** and hydrolysis of benzoate ester **3a–d**.*

Table 2.1. *Alkoxy benzoates **2a–d**.*

Entry	Pg/ Position of Pg	Yield (%)	Mp(ºC)
a	-CH_2CH_3, *m*	**2a**, 96	35–37
b	-CH_2Ph, *m*	**2b**, 82	75–77
c	-CH_2CH_3, *p*	**2c**, 98	35–37
d	-CH_2Ph, *p*	**2d**, 94	95–96

Table 2.2. *Benzoate carboxylic acid **3a–d**.*

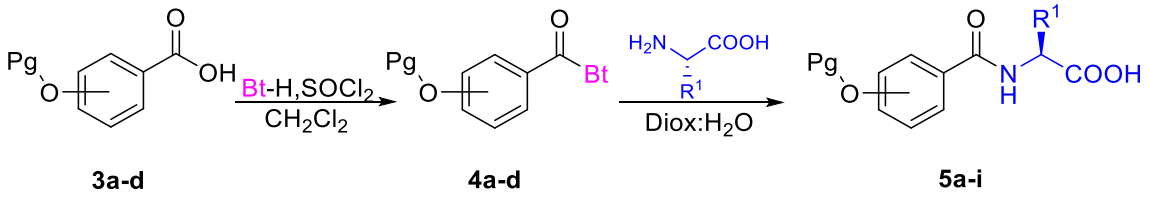
Entry	Pg/ Position of Pg	Yield (%)	Mp(ºC)
a	-CH_2CH_3, *m*	**3a**, 94	136–140
b	-CH_2Ph, *m*	**3b**, 99	133–135
c	-CH_2CH_3, *p*	**3c**, 83	197–198
d	-CH_2Ph, *p*	**3d**, 97	191–192

2.3. Synthesis of Active Intermediate *N*-Acyl Benzotriazole 4a–d

Carboxylic acid derivates **3a–d** was treated with 4 equiv. of 1*H*-benzotriazole and 1.2 equiv. of $SOCl_2$ in CH_2Cl_2 at room temperature for 4h to provide active intermediates, *N*-acyl benzotriazoles **4a–d** (87–97%) (Scheme 2.2. and Table 2.3.).

2.4. Coupling of Amino Acids with Active Benzoic Acid Derivatives 5a–i

Reactions of *N*-acyl benzotriazoles **4a–d** with L-histidine in 1,4–dioxane-water mixture at room temperature for 12–15h or N^{ω}-(NO_2)-L-Arginine in 30 watt microwave power, at 60 °C for 3–5h afford the corresponding amide derivatives **5a–i** (65–89%) (Scheme 2.2. and Table 2.4.).



Scheme 2.2. *Synthesis of active intermediate N-acyl benzotriazoles **4a–d** and amino acids with benzoic acid derivatives **5a–i**.*

Table 2.3. *Active intermediate N-acyl benzotriazoles* ***4a–d****.*

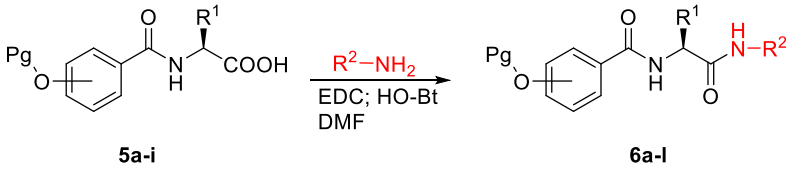
Entry	Pg/ Position of Pg	Yield (%)	Mp(ºC)
a	-CH_2CH_3, *m*	**4a**, 87	78–80
b	-CH_2Ph, *m*	**4b**, 93	73–75
c	-CH_2CH_3, *p*	**4c**, 97	114–117
d	-CH_2Ph, *p*	**4d**, 95	115–117

Table 2.4. *Preparation of N-benzoyl amino acids* ***5a–i****.*

Entry	Pg/ Position of Pg	Amino acid (R^1)	Yield (%)	Mp(ºC)
a	-CH_2CH_3, *m*	L-His	**5a**, 75	115–119
b	-CH_2CH_3, *m*	nitro-L-Arg	**5b**, 70	136–141
c	-CH_2Ph, *m*	L-His	**5c**, 75	146–150
d	-CH_2Ph, *m*	nitro-L-Arg	**5d**, 65	139–141
e	-CH_2CH_3, *p*	L-His	**5e**, 75	241 decomposed
f	-CH_2CH_3, *p*	nitro-L-Arg	**5f**, 75	154–157
g	-CH_2Ph, *p*	L-His methyl ester	**5g**, 89	197–200
h	-CH_2Ph, *p*	L-His	**5h**, 80	194–197
i	-CH_2Ph, *p*	nitro-L-Arg	**5i**, 75	184–186

2.5. Synthesis of Amino Acid Amides 6a–l

Amide derivatives **5a–i** were treated with amine substituents in dry DMF using EDC and HO-Bt at room temperature for 24h to synthesize amino acid amides derivatives **6a–l** (55–90%) (Scheme 2.3. and Table 2.5.). Amide derivative (**5g**) was treated with 1.3 equiv. of trityl chloride and triethylamine in anhydrous toluene at 115 ºC for 4h to provide N^{im}-trt-His derivative **8** (75%) and then hydrolyzed with LiOH 4 equiv. in 10 mL THF:CH_3OH: H_2O (3:2:2) at room temperature for 1h (98%). After synthesized N^{im}-trt-His amides derivatives, Trt group was removed using trifluoroacetic acid in triisopropylsilane and DCM (1:8) at room temperature for 4h (55%) (Figure 2.2.).



5a-i **6a-l**

Scheme 2.3. *Synthesis of amino acid amides* ***6a–l****.*

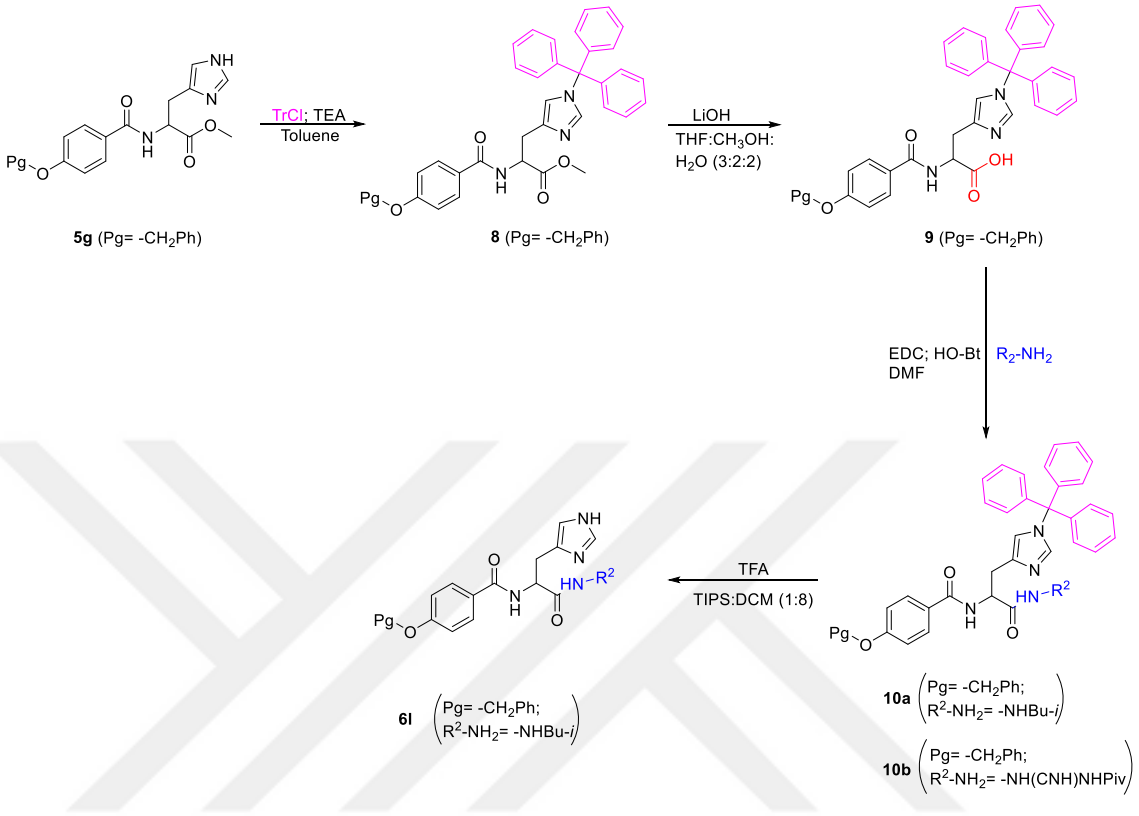


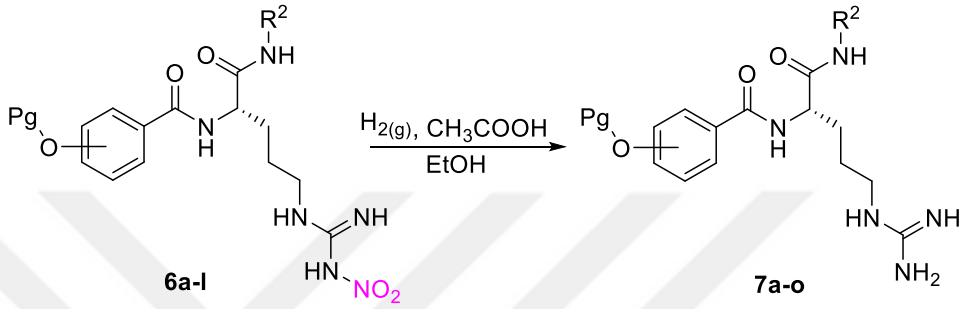
Figure 2.2. *Synthetic route preparation of histidine amides using Trt protecting group.*

Table 2.5. *Synthesis of amino acid amides* ***6a–l.***

Entry	Pg/ Position of Pg	Amino acid (R^1)	R^2–NH_2	Yield (%)	Mp(ºC)
a	-CH_2CH_3, *m*	nitro-L-Arg	-NHBu-*i*	**6a**, 83	129–132
b	-CH_2CH_3, *m*	nitro-L-Arg	-NH(CNH)NHPiv	**6b**, 81	182–186
c	-CH_2CH_3, *m*	nitro-L-Arg	-NHNHCbz	**6c**, 81	hygroscopic
d	-CH_2Ph, *m*	nitro-L-Arg	-NHBu-*i*	**6d**, 82	111–115
e	-CH_2Ph, *m*	nitro-L-Arg	-NH(CNH)NHPiv	**6e**, 76	170–174
f	-CH_2Ph, *m*	nitro-L-Arg	-NHNHCbz	**6f**, 81	hygroscopic
g	-CH_2CH_3, *p*	nitro-L-Arg	-NHBu-*i*	**6g**, 77	85–93
h	-CH_2CH_3, *p*	nitro-L-Arg	-NH(CNH)NHPiv	**6h**, 81	175–179
i	-CH_2CH_3, *p*	nitro-L-Arg	-NHNHCbz	**6i**, 81	hygroscopic
j	-CH_2Ph, *p*	nitro-L-Arg	-NHBu-*i*	**6j**, 90	137–141
k	-CH_2Ph, *p*	nitro-L-Arg	-NH(CNH)NHPiv	**6k**, 80	186–190
l	-CH_2Ph, *p*	L-His	-NHBu-*i*	**6l**, 55	165–170

2.6. Elimination of Nitro Group 7a–o by Hydrogenation

N^{ω}-(NO_2-)-L-Arginine amides were treated with hydrogen gas in the presences of palladium catalyst in acetic acid-ethanol mixture (1:15) at 50 ºC, in 50 psi for 12h to hydrogenate the nitro group **7a–o** (71–90%) (Scheme 2.4. and Table 2.6.).



Scheme 2.4. *Elimination of nitro group **7a–o** by hydrogenation.*

Table 2.6. *The elimination of nitro group **7a–o.***

Entry	Pg/ Position of Pg	R^2–NH_2	Yield (%)	Mp(ºC)
a	-CH_2CH_3, *m*	-OH	**7a**, 78	hygroscopic
b	-CH_2CH_3, *m*	-NHBu-*i*	**7b**, 71	106–110
c	-CH_2CH_3, *m*	-NH(CNH)NHPiv	**7c**, 82	hygroscopic
d	-CH_2CH_3, *m*	-$NHNH_2$	**7d**, 87	hygroscopic
e	-H, *m*	-OH	**7e**, 84	hygroscopic
f	-H, *m*	-NHBu-*i*	**7f**, 84	hygroscopic
g	-H, *m*	-NH(CNH)NHPiv	**7g**, 84	hygroscopic
i	-CH_2CH_3, *p*	-OH	**7i**, 80	66–70
j	-CH_2CH_3, *p*	-NHBu-*i*	**7j**, 75	94–97
k	-CH_2CH_3, *p*	-NH(CNH)NHPiv	**7k**, 80	70–75
l	-CH_2CH_3, *p*	-$NHNH_2$	**7l**, 80	77–81
m	-H, *p*	-OH	**7m**, 90	hygroscopic
n	-H, *p*	-NHBu-*i*	**7n**, 85	74–79
o	-H, *p*	-NH(CNH)NHPiv	**7o**, 85	hygroscopic

2.7. The Preparation of Novel Thiophen Derivates

Suzuki-Miyaura coupling reaction of 5-bromothiophene-2-carboxylic acid (**11**) with phenyl boronic acid gave 5-phenylthiophene-2-carboxylic acid (**12**). Active intermediate thiophen benzotriazole **13** were prepared from 5-phenylthiophene-2-carboxylic acid (**12**) and

then coupling with N^{ω}-(NO_2-)-L-arginine (**14a**) or L-histidine methyl ester (**14b**) and treated with *N*-carbamimidoylpivalamide to synthesis amino acids amides (**15**) (Figure 2.3.).

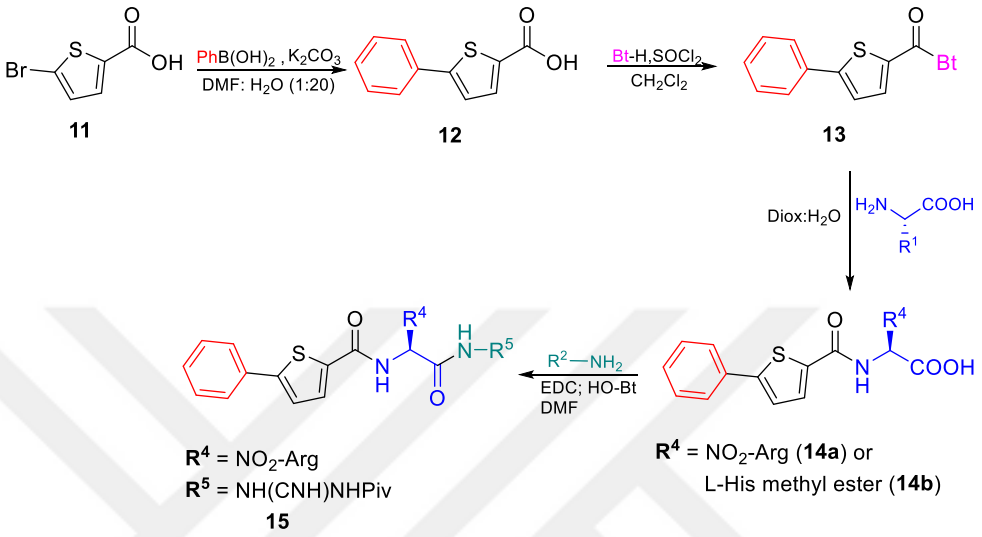


Figure 2.3. *Synthetic route for the preparation of novel thiophen derivatives.*

3. EXPERIMENTAL SECTION

3.1. Materials

3.1.1. Devices used in the study

The melting points of the solid compounds synthesized in this study were measured with Mettler Toledo MP90 Melting Point System.

CEM DISCOVER microwave system was used to synthesize some of the compounds under microwave conditions.

^{1}H (400MHz) and ^{13}C (101MHz) NMR spectra of the synthesized compounds were recorded with Agilent DDR2 400 MHz NMR system in deuterated dimethyl sulfoxide (DMSO-*d*6), deuterated chloroform-d ($CDCl_3$) or their mixture and using TMS as an internal standard.

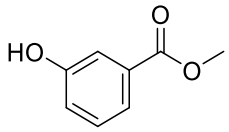
To identify and to elucidate the structure of the synthesized compounds Liquid Chromatograph Mass Spectrometer (LCMS-IT-TOF, Shimadzu, Tokyo,Japan) was used.

3.1.2. Materials used in the study

Materials that are used in this study: N^{ω}-(NO_2-)-Arginine, L-histidine, L-histidine methyl ester, Bt-H, benzyl chloroformate, EDC, HO-Bt, $SOCl_2$, TEA, Pd, hydrazine monohydrate, isobutylamine, ethyl iodide, trityl chloride, triisopropyl silane, pivaloyl chloride, guanidine hydrochloride, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, LiOH, KOH, NaOH, Na_2CO_3, $NaHCO_3$, K_2CO_3, TFA, isopropylamine, benzyl bromide, 1,4-dioxane, ethanol, acetic acid, diethyl ether, acetone, methanol, hexane, ethyl acetate, THF (used after distillation over Na/benzophenone), DMF, DCM, toluene, and acetonitrile (dried by distilling over CaH_2). N-carbamimidoylpivalamide [86] and benzyl N-amino carbamate [87] were prepared by following previously reported procedures.

3.2. Synthesis of Compounds

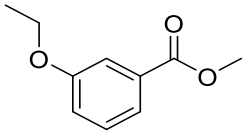
3.2.1. Synthesis of methyl 3-hydroxybenzoate (1a)



Thionyl chloride (14 mmol, 1.67 g) was added to a solution of 3-hydroxybenzoic acid (7 mmol, 1 g) in methanol (80 mL) at room temperature. The mixture was stirred for 7h at 75 ^{o}C. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:3). After the reaction completed, the solvent was removed, and the residue was taken into EtOAc (25 mL). Organic phase was washed with 5% NaHCO3 (2 x 10 mL), 1N HCl (2 x 10 mL), water (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give product (**1a**).

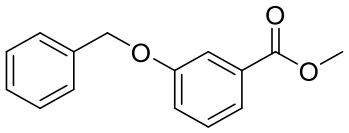
White solid (88%), mp 70–71 ^{o}C (Lit [88], 70–73 ^{o}C); ^{1}H NMR (D_2O) δ 7.34–7.27 (m, 2H), 7.27–7.15 (m, 1H), 7.01–6.89 (m, 1H), 4.63 (s, 3H); 13C NMR (D_2O) δ 167.53, 157.55, 137.91, 136.31, 121.88, 117.94, 115.10, 70.28.

3.2.2. Synthesis of methyl 3-ethoxybenzoate (2a)



Anhydrous potassium carbonate (K_2CO_3, 16 mmol, 2.2 g) was added to a solution of methyl 3-hydroxybenzoate (13 mmol, 2 g) in dry acetonitrile (50 mL). After the mixture stirred for 30 min at room temperature, iodoethane (26 mmol, 4.1 g) was added to this mixture. The mixture was stirred for 10h at 78 ^{o}C under nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, the solvent was removed, and the residue was taken into EtOAc (30 mL). The organic phase was washed with 2N NaOH (2 x 5 mL), 1N HCl (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give the product (**2a**). Colorless oil, yield 96%, melting point 33–35 ^{o}C.

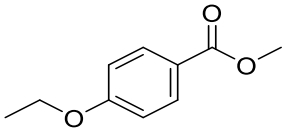
3.2.3. Synthesis of methyl 3-(benzyloxy)benzoate (2b)



Anhydrous potassium carbonate (K_2CO_3, 13 mmol, 1,79 g) was added to a solution of methyl 3-hydroxybenzoate **1a** (13 mmol, 1 g) in dry acetonitrile (20 mL). After the mixture stirred for 30 min at room temperature, benzyl bromide (16 mmol, 2.74 g) was added to this mixture. The mixture was stirred for 12h at 70 ^oC under nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, the solvent was removed, and the residue was taken into EtOAc (25 mL). The organic phase was washed with 1N NaOH (2 x 5 mL), 1N HCl (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give the product (**2b**).

White solid (82%), mp 75–77 ^oC (Lit [89], 74–76 ^oC); ^1H NMR ($CDCl_3$) δ 7.66 (dt, J = 7.0, 1.5 Hz, 1H), 7.48–7.43 (m, 1H), 7.43–7.38 (m, 1H), 7.38–7.32 (m, 1H), 7.20–7.15 (m, 1H), 5.11 (s, 1H), 3.91 (d, J = 0.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 166.93, 158.72, 136.54, 131.47, 129.46, 128.63, 128.11, 127.56, 122.25, 120.22, 115.06, 70.15, 52.20.

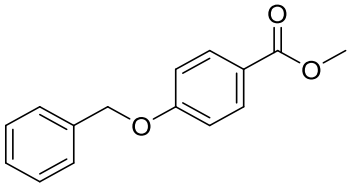
3.2.4. Synthesis of methyl 4-ethoxybenzoate (2c)



Anhydrous potassium carbonate (K_2CO_3, 40 mmol, 5.52 g) was added to a solution of methyl 4-hydroxybenzoate (33 mmol, 5 g) in dry acetonitrile (40 mL). After the mixture stirred for 30 min at room temperature, iodoethane (40 mmol, 6.24 g) was added to this mixture. The mixture was stirred for 12h at 78 ^oC under nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, the solvent was removed, and the residue was taken into EtOAc (30 mL). The organic phase was washed with 2N NaOH (2 x 5 mL), 1N HCl (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The crude

was washed with hexane and dried under high vacuum to give product (**2c**). Yellow oil, yield 98%, melting point 35–37 ºC (Lit [90], 37–38 ºC).

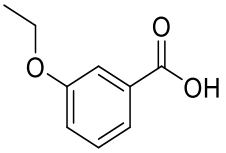
3.2.5. Synthesis of methyl 4-(benzyloxy)benzoate (2d)



Anhydrous potassium carbonate (K_2CO_3, 6.6 mmol, 0.91 g) was added to a solution of methyl 4-hydroxybenzoate (6.6 mmol, 1 g) in dry acetonitrile (10 mL). After the mixture stirred for 15 min at room temperature, benzyl bromide (9.9 mmol, 1.69 g) was added to this mixture. The mixture was stirred for 8h at 70 ºC under nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, the solvent was removed, and the residue was taken into EtOAc (30 mL). The organic phase was washed with saturated solution of Na_2CO_3 (2 x 10 mL), 1N NaOH (2 x 5 mL), 1N HCl (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give the product (**2d**).

White solid (94%), mp 94–96 ºC (Lit [91], 94 ºC); ^{1}H NMR ($CDCl_3$) δ 7.98 (dd, J = 8.9, 2.3 Hz, 2H), 7.47–7.23 (m, 5H), 7.02–6.93 (m, 2H), 5.11 (s, 2H), 3.87 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.75, 162.42, 136.19, 131.54, 128.61, 128.17, 128.14, 127.41, 122.78, 114.40, 70.03, 51.80.

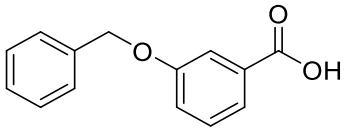
3.2.6. Synthesis of 3-ethoxy benzoic acid (3a)



KOH (28 mmol, 1.57 g) in water (20 mL) was added to a solution of methyl 3-ethoxybenzoate **2a** (11 mmol, 2 g) in ethanol: water (50 mL: 30 mL). The mixture was stirred for 3h at 90 ºC. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:3). After the reaction completed, ethanol was removed, and the solution was diluted with more water (5 mL). The solution was acidified using HCl to pH 2–3. The

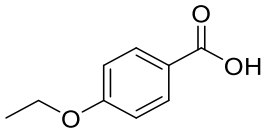
formed precipitate was filtered, washed with water (10 mL) and dried under high vacuum to give the product (**3a)**. White solid, yield 94%, melting point 136–140 ^{o}C(Lit [92], 137–138 ^{o}C).

3.2.7. Synthesis of 3-(benzyloxy)benzoic acid (3b)



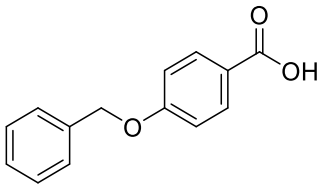
LiOH (16 mmol, 0.38g) was added to a solution of methyl 3-(benzyloxy)benzoate **2b** (4 mmol, 1 g) in 60 mL THF:CH_3OH: H_2O (3:2:2). The mixture was stirred for 12h at room temperature. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, THF and CH_3OH were removed and the solution was diluted with more water (10 mL). The solution was acidified using HCl to pH 2–3. The formed precipitate was filtered, washed with water (5 mL), diethyl ether (3 mL) and dried under high vacuum to give the product (**3b)**. Yield 99%, melting point 133–135 ^{o}C (Lit [93], 132–135 ^{o}C).

3.2.8. Synthesis of 4-ethoxy benzoic acid (3c)



KOH (27.5 mmol, 1.54 g) in water (20 mL) was added to a solution of methyl 4-ethoxybenzoate **2c** (11 mmol, 2 g) in ethanol: water (50 mL: 30 mL). The mixture was stirred for 2h at 90 ^{o}C. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, ethanol was removed, and the solution was diluted with more water (5 mL). The solution was acidified with HCl to pH 2–3. The formed precipitate was filtered, washed with water (10 mL) and dried under high vacuum to give the product (**3c)**. White solid, yield 83%, melting point 197–198 ^{o}C (Lit [94], 195–197 ^{o}C).

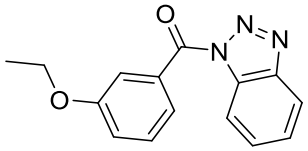
3.2.9. Synthesis of 4-(benzyloxy)benzoic acid (3d)



LiOH (80 mmol, 1.92 g) was added to a solution of methyl 4-(benzyloxy)benzoate **2d** (16 mmol, 4 g) in 30 mL THF:CH_3OH: H_2O (3:2:2). The mixture was stirred for 6h at room temperature. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:1). After the reaction completed, THF and CH_3OH were removed and the solution was diluted with more water (5 mL). The solution was acidified with HCl to pH 2–3. The formed precipitate was filtered and washed with water (10 mL) and dried under high vacuum to give the product (**3d)**.

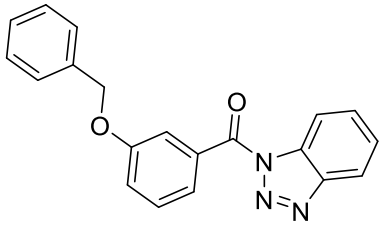
White solid (97%), mp 191–192 ^oC (Lit [95], 191–192 ^oC); ^1H NMR ($CDCl_3$) δ 8.05 (d, J = 8.9 Hz, 2H), 7.56–7.12 (m, 5H), 7.00 (d, J = 8.9 Hz, 2H), 5.12 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 170.87, 163.15, 136.10, 132.34, 128.67, 128.23, 127.46, 121.75, 114.58, 70.14.

3.2.10. Synthesis of (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(3-ethoxyphenyl)methanone (4a)



Thionyl chloride (22 mmol, 2.6 g) was added to a solution of benzotriazole (75 mmol, 8.9 g) in freshly distilled CH_2Cl_2 (60 mL) at 5 ^oC. The reaction mixture was stirred for 30 min at the same temperature. 3-ethoxy benzoic acid **3a** (18 mmol, 3 g) was added to the mixture and stirred for 8h at room temperature. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, white precipitate was filtered off and discarded. The organic phase was washed with saturated solution of Na_2CO_3 (2 x 10 mL), saturated brine (10 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to give the product (**4a**). White solid, yield 87%, melting point 78–80 ^oC.

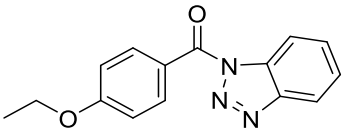
3.2.11. Synthesis of (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(3-(benzyloxy)phenyl)methanone (4b)



Thionyl chloride (11 mmol, 1.3 g) was added to a solution of benzotriazole (37 mmol, 4.4 g) in freshly distilled CH_2Cl_2 (50 mL) at 5 ^oC. The reaction mixture was stirred for 30 min at the same temperature. 3-(benzyloxy)benzoic acid **3b** (8.8 mmol, 2 g) was added to the mixture and stirred for 4h at room temperature. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). The white precipitate was filtered off and discarded. The organic phase was washed with saturated solution of Na_2CO_3 (2 x 10 mL), saturated brine (10 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure. The crude was washed with diethyl ether and then dried under high vacuum to give product (**4b**).

White solid (93%), mp 73–75 ^oC; ^1H NMR ($CDCl_3$) δ 8.39 (dt, J = 8.3, 0.9 Hz, 1H), 8.17 (dt, J = 8.3, 0.9 Hz, 1H), 7.84–7.79 (m, 1H), 7.71 (ddd, J = 8.1, 7.2, 1.1 Hz, 1H), 7.55 (ddd, J = 8.1, 7.1, 1.0 Hz, 1H), 7.51–7.23 (m, 8H), 5.16 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 166.50, 158.60, 145.75, 136.29, 132.60, 132.36, 130.45, 129.56, 128.68, 128.20, 127.60, 126.39, 124.56, 120.94, 120.22, 117.23, 114.81, 70.33.

3.2.12. Synthesis of (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(4-ethoxyphenyl)methanone (4c)

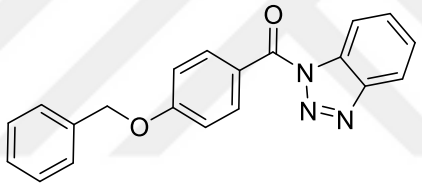


Thionyl chloride (22 mmol, 2.6 g) was added to a solution of benzotriazole (75 mmol, 8.9 g) in freshly distilled CH_2Cl_2 (80 mL) at 5 ^oC. The reaction mixture was stirred for 30 min at the same temperature. 4-ethoxy benzoic acid **3c** (18 mmol, 3 g) was added to the mixture and stirred for 4h at room temperature. The reaction was monitored with TLC using

silica on Al-plates and using EtOAc/hexane (1:2). The white precipitate was filtered off and discarded. The organic phase was washed with saturated solution of Na_2CO_3 (2 x 10 mL), saturated brine (10 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to give the product (**4c**).

White solid (97%), mp 114–117 ^oC; ^1H NMR ($CDCl_3$) δ 8.36 (dt, J = 8.3, 1.0 Hz, 1H), 8.33–8.22 (m, 1H), 8.15 (dt, J = 8.2, 1.0 Hz, 1H), 7.67 (ddd, J = 8.3, 7.1, 1.1 Hz, 1H), 7.52 (ddd, J = 8.2, 7.1, 1.1 Hz, 1H), 7.47–7.33 (m, 1H), 7.10–6.94 (m, 2H), 4.14 (q, J = 7.0 Hz, 2H), 1.46 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 165.68, 163.65, 145.62, 134.44, 132.59, 130.15, 126.11, 123.13, 120.03, 114.83, 114.30, 63.92, 14.62.

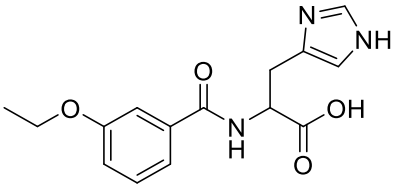
3.2.13. Synthesis of (1*H*-benzo[*d*][1,2,3]-triazol-1-yl)-(4-(benzyloxy)phenyl) methanone (4d)



Thionyl chloride (13 mmol, 1.53 g) was added to a solution of benzotriazole (46 mmol, 5.47 g) in freshly distilled CH_2Cl_2 (40 mL) at 5 ^oC. The reaction mixture was stirred for 30 min at the same temperature. 4-(benzyloxy) benzoic acid **3d** (11 mmol, 2.5 g) was added to the mixture and stirred for 4h at room temperature. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). The white precipitate was filtered off and discarded. The organic phase was washed with saturated solution of Na_2CO_3 (2 x 10 mL), saturated brine (10 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to give the product (**4d**).

White solid (95%), mp 115–117 ^oC; ^1H NMR ($CDCl_3$) δ 8.35 (d, J = 8.2 Hz, 1H), 8.27 (d, J = 9.0 Hz, 2H), 8.14 (d, J = 8.3 Hz, 1H), 7.67 (ddd, J = 8.2, 7.1, 1.1 Hz, 1H), 7.52 (ddd, J = 8.2, 7.1, 1.0 Hz, 1H), 7.46–7.29 (m, 5H), 7.15–7.08 (m, 2H), 5.18 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 165.65, 163.31, 145.66, 135.93, 134.42, 132.55, 130.19, 128.74, 128.33, 127.51, 126.13, 123.64, 120.09, 114.82, 114.71, 70.28.

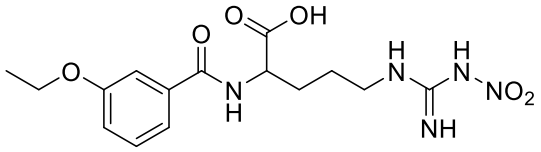
3.2.14. Synthesis of (3-ethoxybenzoyl)histidine (5a)



Triethylamine (4.5 mmol, 0.45 g) was added to a solution of L-histidine (2.2 mmol, 0.34 g) in water (10 mL) at room temperature. The reaction mixture was stirred for 15 min at the same temperature. (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(3-ethoxyphenyl)methanone **4a** (1.5 mmol, 0.4 g) in 1,4-dioxane (30 mL) was added dropwise to this mixture and stirred for 12h at room temperature. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, 1,4-dioxane was removed and the solution was diluted with more water (5 mL). The solution was alkalified using saturated solution of Na_2CO_3 to pH 9–10 and washed with ethyl acetate (2 x 5 mL) to take off the surplus of benzotriazole. After washing, the solution was acidified using HCl to pH 2–3. The water was removed, the residue was taken into DMF and evaporated under reduced pressure to give the product (**5a**).

Grey solid (75%), mp 115–119 ^{o}C; ^{1}H NMR (DMSO-*d*6) δ 8.86 (d, J = 7.7 Hz, 2H), 7.91 (s, 1H), 7.39 (dd, J = 24.6, 10.2 Hz, 4H), 7.09 (d, J = 7.9 Hz, 1H), 4.71 (q, J = 11.3 Hz, 1H), 4.07 (q, 2H), 3.22 (d, J = 13.3 Hz, 2H), 1.33 (t, 3H); ^{13}C NMR (DMSO-*d*6) δ 172.23, 166.09, 158.41, 134.98, 133.79, 130.32, 129.49, 119.57, 117.74, 113.09, 63.28, 51.99, 26.08, 14.64. HRMS Calcd. for $C_{15}H_{17}N_3O_4$ m/z 304.1297 $[M+H]^+$. Found: m/z 304.1291.

3.2.15. Synthesis of N^2-(3-ethoxybenzoyl)-N^{ω}-nitroarginine (5b)

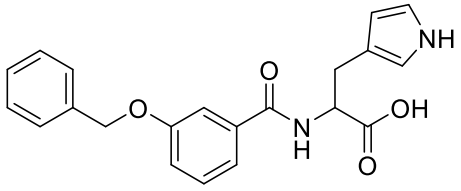


Triethylamine (4.4 mmol, 0.44 g) was added to a solution of N^{ω}-nitro-L-arginine (4.4 mmol, 0.96 g) in water (10 mL) at room temperature. The reaction mixture was stirred for 30 min at the same temperature. (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(3-ethoxyphenyl)methanone **4a** (3.7 mmol, 1 g) in 1,4-dioxane (35 mL) was added dropwise to this mixture and then

mixture was heated at 60 ºC in microwave oven with 30 watt power and stirred for 3h. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, 1,4-dioxane was removed and the solution was diluted with more water (5 mL). The solution was alkalified using saturated solution of Na_2CO_3 to pH 9–10 and washed with ethyl acetate (2 x 5 mL) to take off the surplus of benzotriazole. After washing, the solution was acidified using HCl to pH 3–4. The formed precipitate was filtered, washed with water (10 mL) and dried under high vacuum to give the product (**5b**). For purification, the product was washed with diethyl ether and then dried under high vacuum.

White solid (70%), mp 136–141 ºC; ^{1}H NMR (DMSO-*d*6) δ 12.64 (s, 1H), 8.60 (d, J = 7.7 Hz, 1H), 8.53 (s, 1H), 7.84 (s, 1H), 7.49–7.31 (m, 3H), 7.09 (d, J = 8.1 Hz, 1H), 4.36 (q, J = 7.4, 5.2 Hz, 1H), 4.07 (q, J = 6.9 Hz, 2H), 3.17 (t, J = 6.9 Hz, 2H), 1.79 (q, 2H), 1.66–1.48 (m, 2H), 1.34 (t, J = 6.9 Hz, 3H); ^{13}C NMR (DMSO-*d*6) δ 174.03, 166.68, 159.75, 158.82, 135.73, 129.84, 120.05, 117.95, 113.60, 63.67, 52.79, 46.04, 28.28, 25.66, 15.05. HRMS Calcd. for $C_{15}H_{21}N_5O_6$ m/z 368.1570 $[M+H]^+$. Found: m/z 368.1576.

3.2.16. Synthesis of 2-(3-(benzyloxy)benzamido)-3-(1*H*-pyrrol-3-yl)propanoic acid (5c)

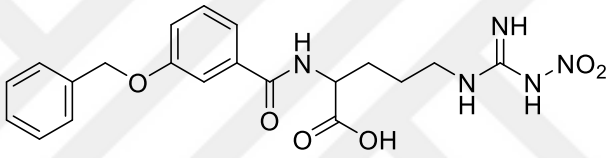


Triethylamine (4.5 mmol, 0.45 g) was added to a solution of L-histidine (3 mmol, 0.46 g) in water (5 mL) at room temperature. The reaction mixture was stirred for 20 min at the same temperature. (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(3-(benzyloxy)phenyl)methanone **4b** (1.5 mmol, 0.5 g) in 1,4-dioxane (20 mL) was added dropwise to this mixture and stirred for 15h at room temperature. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:1). After the reaction completed, 1,4-dioxane was removed and the solution was diluted with more water (5 mL). The solution was alkalified using saturated solution of Na_2CO_3 to pH 9–10 and washed with ethyl acetate (2 x 5 mL) to take off the surplus of benzotriazole. After washing, the solution was acidified using HCl to pH 2–3. The

water was removed, the residue was taken into DMF and evaporated under reduced pressure to give the product (**5c**).

White solid (75%), mp 146–150 °C; ^{1}H NMR (DMSO-*d*6) δ 8.75 (d, *J* = 7.2 Hz, 1H), 7.91 (s, 1H), 7.64 (d, *J* = 4.3 Hz, 1H), 7.44 (s, 7H), 7.17 (d, *J* = 7.7 Hz, 1H), 6.88 (d, *J* = 4.2 Hz, 1H), 5.15 (s, 2H), 4.60 (s, 1H), 3.05 (d, 2H); ^{13}C NMR (DMSO-*d*6) δ 173.05, 165.76, 158.27, 136.85, 135.45, 134.83, 129.56, 128.48, 127.94, 127.76, 123.46, 119.67, 117.90, 113.52, 69.36, 52.88, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 28.38. HRMS Calcd. for $C_{20}H_{19}N_3O_4$ m/z 366.1454 $[M+H]^+$. Found: m/z 366.1447.

3.2.17. Synthesis of N^2-(3-(benzyloxy)benzoyl)-N^{ω}-nitroarginine (5d)

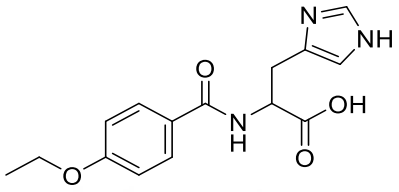


Triethylamine (3.6 mmol, 0.36 g) was added to a solution of N^{ω}-nitro-L-arginine (3.6 mmol, 0.79 g) in water (6 mL) at room temperature. The reaction mixture was stirred for 30 min at the same temperature. (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(3-(benzyloxy)phenyl) methanone **4b** (3 mmol, 1 g) in 1,4-dioxane (20 mL) was added dropwise to this mixture and then mixture was heated at 60 °C in microwave oven with 30 watt power and stirred for 5h. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:1). After the reaction completed, 1,4-dioxane was removed and the solution was diluted with more water (5 mL). The solution was alkalified using saturated solution of Na_2CO_3 to pH 9–10 and washed with EtOAc (2 x 5 mL) to take off the surplus of benzotriazole. After washing, the solution was acidified using HCl to pH 3–4. The formed precipitate was filtered, washed with water (10 mL) and dried under high vacuum to give the product (**5d**). The product was washed with diethyl ether for purification and then dried under high vacuum.

White solid (65%), mp 139–141 °C; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 8.24 (s, 1H), 8.10 (s, 1H), 7.66 (s, 1H), 7.47 (t, *J* = 2.0 Hz, 1H), 7.41 (d, *J* = 7.7 Hz, 1H), 7.37 (d, *J* = 7.5 Hz, 2H), 7.33–7.20 (m, 4H), 7.03 (dd, *J* = 8.2, 2.6 Hz, 1H), 6.75 (s, 1H), 5.04 (s, 2H), 4.50 (td, *J* = 8.5, 4.4 Hz, 1H), 3.30–3.08 (m, 2H), 3.04 (td, *J* = 7.3, 4.9 Hz, 1H), 2.01–1.85 (m, 1H), 1.86–1.70 (m, 1H), 1.70–1.51 (m, 2H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 173.91, 167.02,

162.80, 158.58, 136.66, 135.57, 129.40, 128.56, 128.05, 127.64, 120.07, 117.96, 113.96, 69.95, 52.37, 46.01, 28.71, 24.99. HRMS Calcd. for $C_{20}H_{23}N_5O_6$ m/z 430.1727 $[M+H]^+$. Found: m/z 430.1722.

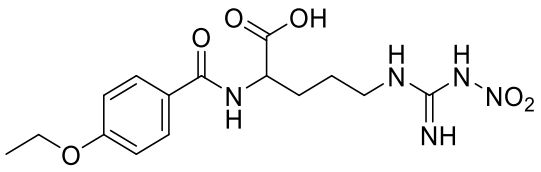
3.2.18. Synthesis of (4-ethoxybenzoyl)histidine (5e)



Triethylamine (6 mmol, 0.6 g) was added to a solution of L-histidine (3 mmol, 0.46 g) in water (10 mL) at room temperature. The reaction mixture was stirred for 15 min at the same temperature. (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(4-ethoxyphenyl)methanone **4c** (2 mmol, 0.53 g) in 1,4-dioxane (30 mL) was added dropwise to this mixture and stirred for 12h at room temperature. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:1). After the reaction completed, 1,4-dioxane was removed and the solution was diluted with more water (5 mL). The solution was alkalified using saturated solution of Na_2CO_3 to pH 9–10 and washed with ethyl acetate (2 x 5mL) to take off the surplus of benzotriazole. After washing, the solution was acidified with HCl to pH 2–3. The water was removed and the residue was transferred into DMF and evaporated under reduced pressure to give the product (**5e**).

White solid (75%), mp 241 ºC decomposed; ^{1}H NMR (DMSO-*d*6) δ 8.48 (t, *J* = 6.2 Hz, 1H), 7.80 (d, *J* = 7.7 Hz, 2H), 7.58 (d, *J* = 5.8 Hz, 1H), 6.98 (d, *J* = 7.6 Hz, 2H), 6.84 (d, *J* = 5.8 Hz, 1H), 4.59 (q, *J* = 7.1 Hz, 1H), 4.08 (q, *J* = 6.7 Hz, 2H), 3.06 (d, *J* = 7.0 Hz, 2H), 1.34 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (DMSO-*d*6) δ 173.73, 165.94, 161.35, 135.17, 134.24, 129.47, 126.56, 117.18, 114.34, 63.72, 53.41, 28.90, 14.96. HRMS Calcd. for $C_{15}H_{17}N_3O_4$ m/z 304.1297 $[M+H]^+$. Found: m/z 304.1273.

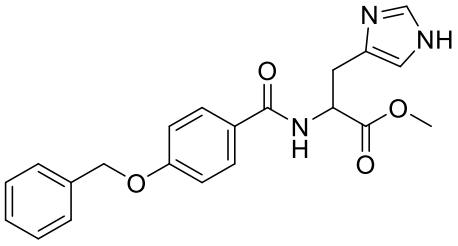
3.2.19. Synthesis of N^2-(4-ethoxybenzoyl)-N^{ω}-nitroarginine (5f)



Triethylamine (4.8 mmol, 0.48 g) was added to a solution of N^{ω}-nitro-L-arginine (4.5 mmol, 0.98 g) in water (10 mL) at room temperature. The reaction mixture was stirred for 30 min at the same temperature. (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(4-ethoxyphenyl)methanone **4c** (3.7 mmol, 1 g) in 1,4-dioxane (35 mL) was added dropwise to this mixture and then mixture was heated at 60 ^{o}C in microwave oven with 30 watt power and stirred for 3h. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, 1,4-dioxane was removed and the solution was diluted with more water (5 mL). The solution was alkalified using saturated solution of Na_2CO_3 to pH 9–10 and washed with ethyl acetate (2 x 5 mL) to take off the surplus of benzotriazole. After washing, the solution was acidified using HCl to pH 3–4. The formed precipitate was filtered, washed with water (10 mL) and dried under high vacuum to give the product (**5f**). For purification, the product was washed with EtOAc: hexane and dried under high vacuum.

White solid (75%), mp 154–157 ^{o}C; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 8.28 (s, 1H), 7.96 (s, 1H), 7.85 (d, *J* = 8.6 Hz, 1H), 7.79 (d, *J* = 8.6 Hz, 2H), 6.82 (d, *J* = 6.0 Hz, 2H), 4.54–4.43 (m, 1H), 4.01 (q, *J* = 10.7, 5.4 Hz, 2H), 3.21 (d, *J* = 6.6 Hz, 2H), 1.98–1.85 (m, 1H), 1.80 (s, 1H), 1.64 (m, 2H), 1.34 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 173.55, 167.26, 161.91, 160.94, 131.05, 128.89, 113.41, 113.32, 78.08, 77.76, 77.43, 62.99, 45.60, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 28.21, 24.45, 14.22. HRMS Calcd. for $C_{15}H_{21}N_5O_6$ m/z 368.1570 $[M+H]^+$. Found: m/z 368.1575.

3.2.20. Synthesis of methyl (4-(benzyloxy)benzoyl)histidinate (5g)

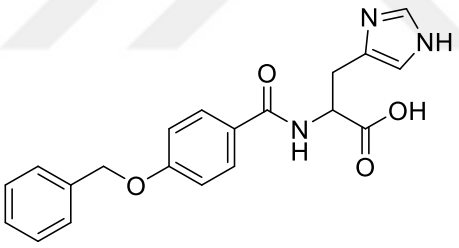


Triethylamine (12 mmol, 1.21 g) was added to a solution of L-histidine methyl ester (4.5 mmol, 1.1 g) in water (10 mL) at room temperature. The reaction mixture was stirred for 15 min at the same temperature. (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(4-(benzyloxy)phenyl) methanone **4d** (3mmol, 1 g) in 1,4-dioxane (30 mL) was added dropwise to this mixture. The mixture was heated at 70 ^{o}C in microwave oven with 50 watt power and stirred for 2h. The

reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, 1,4-dioxane was removed and the solution was diluted with more water (5 mL). The solution was alkalified using saturated solution of Na_2CO_3 to pH 9–10 and washed with ethyl acetate (2 x 5 mL) to take off the surplus of benzotriazole. After washing, the solution was acidified with HCl to pH 2–3. The formed precipitate was filtered and washed with water (10 mL) and dried under high vacuum to give the product (**5g**).

White solid (89%), mp 197–200 ºC; ^{1}H NMR (DMSO-*d*6) δ 11.83 (d, 1H), 8.70 (d, *J* = 7.3 Hz, 1H), 7.79 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 1.2 Hz, 1H), 7.47–7.41 (m, 2H), 7.41–7.35 (m, 2H), 7.34–7.28 (m, 1H), 7.10–7.05 (m, 2H), 6.83 (s, 1H), 5.15 (s, 2H), 4.62 (q, *J* = 7.0 Hz, 1H), 3.59 (s, 3H), 3.01 (d, *J* = 6.9 Hz, 2H); ^{13}C NMR (DMSO-*d*6) δ 172.76, 166.12, 161.29, 137.08, 135.48, 129.59, 128.92, 128.41, 128.25, 126.51, 114.85, 69.79, 53.52, 52.28, 31.15.

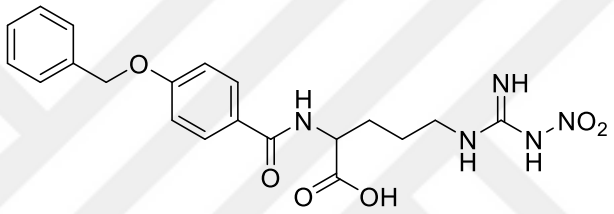
3.2.21. Synthesis of (4-(benzyloxy)benzoyl)histidine (5h)



Triethylamine (7 mmol, 0.71 g) was added to a solution of L-histidine (4.5 mmol, 0.7 g) in water (10 mL) at room temperature. The reaction mixture was stirred for 15 min at the same temperature. (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(4-(benzyloxy)phenyl) methanone **4d** (3 mmol, 1 g) in 1,4-dioxane (30 mL) was added dropwise to this mixture and stirred for 12h at room temperature. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, 1,4-dioxane was removed and the solution was diluted with more water (5 mL). The solution was alkalified with saturated solution of Na_2CO_3 to pH 9–10 and washed with ethyl acetate (2 x 5mL) to take off the surplus of benzotriazole. After washing, the solution was acidified with HCl to pH 2–3. The formed precipitate was filtered and washed with water (10 mL) and dried under high vacuum to give the product (**5h**).

White solid (80%), mp 194–197 ºC; ^{1}H NMR (DMSO-*d*6) δ 8.76 (d, *J* = 1.4 Hz, 1H), 8.46 (d, *J* = 8.2 Hz, 1H), 7.90–7.83 (m, 1H), 7.82–7.76 (m, 2H), 7.41–7.23 (m, 5H), 7.20 (d, *J* = 1.3 Hz, 1H), 6.93 (dd, *J* = 8.9, 7.5 Hz, 2H), 5.07 (s, 2H), 4.79 (q, *J* = 9.7, 8.1, 5.1 Hz, 1H), 3.24 (d, *J* = 15.5, 9.8 Hz, 2H); ^{13}C NMR (DMSO-*d*6) δ 172.03, 166.04, 160.66, 136.01, 132.86, 131.07, 129.76, 129.03, 128.05, 127.55, 127.11, 127.05, 125.75, 116.38, 113.89, 113.79, 69.25, 51.47, 25.93. HRMS Calcd. for $C_{20}H_{19}N_3O_4$ m/z 366.1454 $[M+H]^+$. Found: m/z 366.1439.

3.2.22. Synthesis of N^2-(4-(benzyloxy)benzoyl)-N^{ω}-nitroarginine (5i)

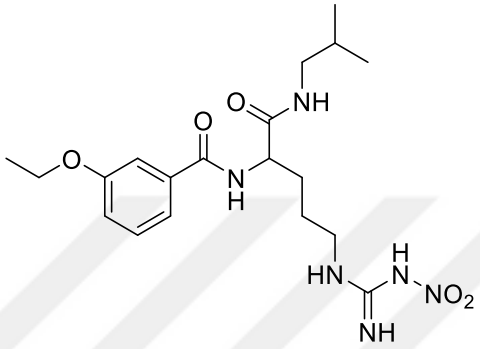


Triethylamine (3.6 mmol, 0.36 g) was added to a solution of N^{ω}-nitro-L-arginine (3.6 mmol, 0.8 g) in water (10 mL) at room temperature. The reaction mixture was stirred for 30 min at the same temperature. (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(4-(benzyloxy)phenyl) methanone **4d** (3 mmol, 1 g) in 1,4-dioxane (30 mL) was added dropwise to this mixture and then mixture was heated at 60 ºC in microwave oven with 30 watt power and stirred for 5h. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, 1,4-dioxane was removed and the solution was diluted with more water (5 mL). The solution was alkalified using saturated solution of Na_2CO_3 to pH 9–10 and washed with ethyl acetate (2 x 5 mL) to take off the surplus of benzotriazole. After washing, the solution was acidified with HCl to pH 3–4. The formed precipitate was filtered and washed with water (10 mL) and dried under high vacuum to give the product (**5i**).

White solid (75%), mp 184–186 ºC; ^{1}H NMR (DMSO-*d*6) δ 8.49 (s, 1H), 8.45 (d, *J* = 7.7 Hz, 1H), 7.86 (d, *J* = 8.6 Hz, 2H), 7.46 (d, *J* = 7.5 Hz, 2H), 7.37 (dt, *J* = 24.8, 7.3 Hz, 3H), 7.09 (d, *J* = 8.4 Hz, 2H), 5.18 (s, 2H), 4.34 (q, *J* = 7.6, 6.2 Hz, 1H), 3.17 (q, *J* = 6.6 Hz, 2H), 1.78 (q, 2H), 1.66–1.47 (m, 2H); ^{13}C NMR (DMSO-*d*6) δ 173.77, 170.97, 166.00,

160.75, 136.69, 129.33, 128.48, 127.96, 127.80, 126.35, 114.31, 69.35, 52.28, 40.25, 30.57, 27.91. HRMS Calcd. for $C_{20}H_{23}N_5O_6$ m/z 430.1727 $[M+H]^+$. Found: m/z 430.1721.

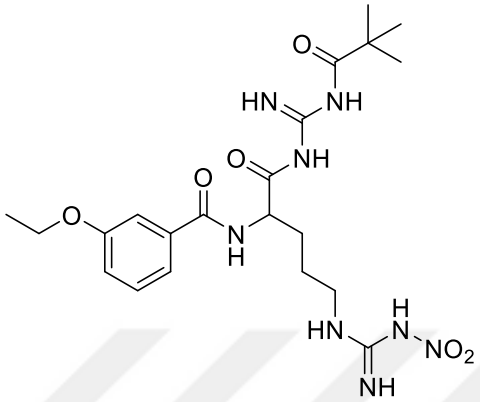
3.2.23. Synthesis of 3-ethoxy-*N*-(1-(isobutylamino)-5-(3-nitroguanidino)-1-oxopentan-2-yl) benzamide (6a)



N-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide (**EDC**) (1.3 mmol, 0.2 g) and hydroxy-benzotriazole (**HO-Bt**) (1.3 mmol, 0.18 g) were added to the solution of N^2-(3-ethoxybenzoyl)-N^{ω}-nitroarginine **5b** (1.1 mmol, 0.4 g) in dry DMF (2 mL). After the mixture stirred for 20 min at 5 ºC, isobutyl amine (1.4 mmol, 0.1 g) was added to this mixture. The mixture was stirred for 24h at room temperature under a nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, the solvent was removed, and the residue was taken into EtOAc (30 mL). The organic phase was washed with 1N HCl (2 x 5 mL), saturated solution of Na_2CO_3 (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The crude was washed with diethyl ether and then dried under high vacuum to give the product (**6a**).

White solid (83%), mp 129–132 ºC; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 7.88 (s, 1H), 7.55 (d, J = 23.9 Hz, 2H), 7.32 (d, J = 9.6 Hz, 2H), 7.21 (t, J = 7.7 Hz, 1H), 6.91 (d, J = 8.1 Hz, 1H), 4.53 (td, 1H), 3.97 (q, J = 7.3 Hz, 2H), 2.90 (dd, J = 6.5 Hz, 2H), 2.77–2.70 (m, 1H), 1.87–1.67 (m, 2H), 1.62–1.45 (m, 2H), 1.30 (t, J = 7.0 Hz, 3H), 0.78 (d, J = 6.7 Hz, 6H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 171.88, 171.44, 167.64, 158.85, 135.55, 129.37, 119.47, 117.70, 113.44, 63.53, 60.28, 46.65, 40.73, 29.54, 28.36, 24.89, 20.20, 14.82. HRMS Calcd. for $C_{19}H_{30}N_6O_5$ m/z 423.2356 $[M+H]^+$. Found: m/z 423.2338.

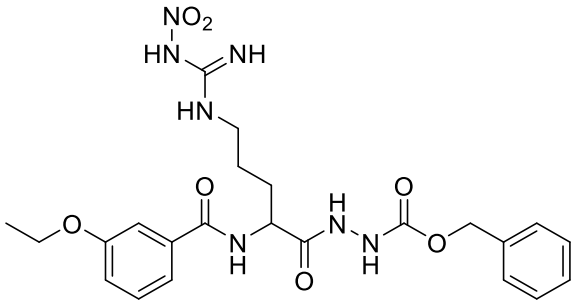
3.2.24. Synthesis of 3-ethoxy-*N*-(5-(3-nitroguanidino)-1-oxo-1-(3-pivaloylguanidino) pentan-2-yl)benzamide (6b)



N-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide (**EDC**) (1.6 mmol, 0.25 g) and hydroxy-benzotriazole (**HO-Bt**) (1.6 mmol, 0.22 g) were added to a solution of N^2-(3-ethoxybenzoyl)-N^{ω}-nitroarginine **5b** (1.1 mmol, 0.4 g) in dry DMF (1 mL). After the mixture stirred for 20 min at 5 ^{o}C, *N*-carbamimidoyl pivalamide (1.9 mmol, 0.27 g) was added to this mixture. The mixture was stirred for 24h at room temperature under a nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using EtOAc. After the reaction completed, the solvent was removed, and the residue was taken into EtOAc (30 mL). The organic phase was washed with saturated solution of Na_2CO_3 (2 x 5 mL), 1N HCl (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The crude was washed with diethyl ether and then dried under high vacuum to give product (**6b**).

White solid (81%), mp 182–186 ^{o}C; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 11.93 (s, 1H), 10.18 (d, *J* = 54.8 Hz, 2H), 8.86 (s, 1H), 8.37 (s, 1H), 8.02 (s, 1H), 7.52–7.38 (m, 2H), 7.30 (t, *J* = 8.1 Hz, 1H), 7.01 (ddd, *J* = 8.3, 2.5, 1.1 Hz, 1H), 4.43 (td, 1H), 4.05 (q, *J* = 6.9 Hz, 2H), 3.22 (td, *J* = 6.5 Hz, 2H), 1.97 (td, 2H), 1.78–1.53 (m, 2H), 1.35 (t, *J* = 6.9 Hz, 3H), 1.26 (s, 9H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 181.21, 175.46, 168.50, 167.81, 158.77, 134.79, 129.45, 120.18, 117.96, 114.02, 63.58, 55.79, 41.18, 40.74, 27.31, 26.42, 14.95. HRMS Calcd. for $C_{21}H_{32}N_8O_6$ m/z 493.2523 $[M+H]^+$. Found: m/z 493.2511.

3.2.25. Synthesis of benzyl 2-(N^2-(3-ethoxybenzoyl)-N^{ω}-nitroarginyl)hydrazine-1-carboxylate (6c)



N-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide (**EDC**) (1.3 mmol, 0.2 g) and hydroxy-benzotriazole (**HO-Bt**) (1.3 mmol, 0.18 g) were added to the solution of N^2-(3-ethoxybenzoyl)-N^{ω}-nitroarginine **5b** (1.1 mmol, 0.4 g) in dry DMF (1 mL). After the mixture stirred for 20 min at 5 ^{o}C, benzyl hydrazinecarboxylate (1.4 mmol, 0.23 g) was added to this mixture. The mixture was stirred for 24h at room temperature under a nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using MeOH/EtOAc (1:4). After the reaction completed, the solvent was removed, and the residue was taken into EtOAc (30 mL). The organic phase was washed with 1N HCl (2 x 5 mL), saturated solution of Na_2CO_3 (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give the product (**6c**).

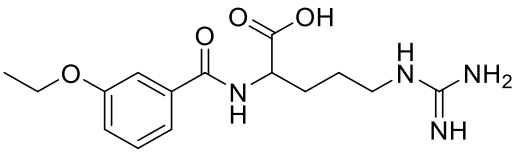
White solid (81%), mp very hygroscopic; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 9.77 (s, 1H), 8.15 (d, *J* = 8.0 Hz, 1H), 7.86 (d, *J* = 21.5 Hz, 1H), 7.37 (d, *J* = 7.7 Hz, 2H), 7.32–7.15 (m, 7H), 6.99–6.89 (m, 1H), 5.01 (s, 2H), 4.54 (td, 1H), 3.99 (q, *J* = 7.0 Hz, 2H), 2.87 (s, 1H), 2.74 (s, 1H), 2.54–2.43 (m, 2H), 1.93–1.74 (m, 2H), 1.67–1.53 (m, 2H), 1.32 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 171.92, 166.89, 162.38, 158.78, 156.39, 136.58, 135.55, 129.32, 128.48, 128.12, 128.02, 119.81, 117.59, 113.71, 78.80, 66.56, 63.51, 51.77, 36.39, 31.27, 14.91. HRMS Calcd. for $C_{23}H_{29}N_7O_7$ m/z 516.2207 $[M+H]^+$. Found: m/z 516.2196.

3.2.26. General procedure reduction for 5b, 6a, 6b and 6c compounds

Palladium (25 mg) and acetic acid (2 mL) were added to the solution of **5b**, **6a, 6b** or **6c** (0.25 mmol) in ethanol (25 mL) at room temperature. The mixture was stirred for 24h, at 50 ^{o}C in 50 psi. The reaction was monitored with TLC using silica on Al-plates and using

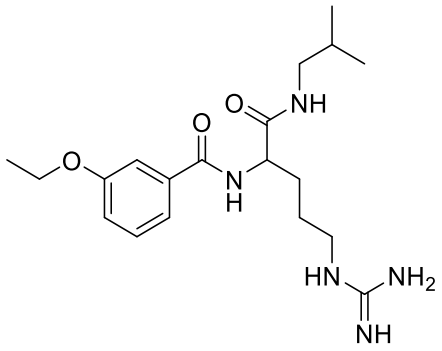
MeOH/EtOAc (1:3). After the reaction completed, the mixture was filtered using silica to take off Pd and the solvent was evaporated under reduced pressure to give product **7a**, **7b,** **7c**, and **7d.**

3.2.26.1. Synthesis of (3-ethoxybenzoyl)arginine (7a)



The crude was washed with DCM : Hexane and then dried under high vacuum. White solid (78%), mp very hygroscopic; ^{1}H NMR (DMSO-*d*6) δ 9.29 (s, 1H), 8.83 (d, *J* = 8.1 Hz, 1H), 7.95–7.83 (m, 1H), 7.39 (dd, *J* = 6.4, 3.1 Hz, 1H), 7.30 (d, *J* = 7.6 Hz, 1H), 7.24 (d, *J* = 2.4 Hz, 1H), 7.10 (t, *J* = 7.9 Hz, 1H), 6.86 (dd, *J* = 8.0, 2.5 Hz, 1H), 4.20 (td, *J* = 8.6, 5.0 Hz, 1H), 3.94 (q, *J* = 7.0 Hz, 2H), 3.10 (d, *J* = 12.1 Hz, 1H), 2.99 (s, 1H), 1.85 (s, 5H), 1.53 (s, 2H), 1.29 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (DMSO-*d*6) δ 176.91, 172.73, 164.87, 158.13, 157.47, 135.80, 128.79, 119.01, 117.28, 112.09, 62.98, 54.36, 40.42, 29.15, 24.61, 22.02, 14.69. HRMS Calcd. for $C_{15}H_{22}N_4O_4$ m/z 323.1719 $[M+H]^+$. Found: m/z 323.1708.

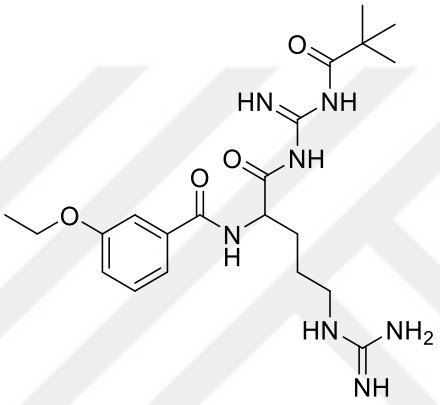
3.2.26.2. Synthesis of 3-ethoxy-N-(5-guanidino-1-(isobutylamino)-1-oxopentan-2-yl)benzamide (7b)



The crude was washed with DCM : Hexane and then dried under high vacuum to give product (**7b**). Grey solid (71%), mp 106–110 ^{o}C; ^{1}H NMR ($CDCl_3$) δ 8.63 (s, 1H), 8.14–7.89 (m, 2H), 7.38 (d, *J* = 13.4 Hz, 3H), 7.29 (dd, *J* = 13.7, 4.6 Hz, 3H), 7.09–6.97 (m, 1H), 4.67 (s, 1H), 4.03 (q, 2H), 3.42–3.14 (m, 2H), 3.03 (d, 2H), 2.69–2.56 (m, 1H), 1.97 (s, 6H), 1.78–

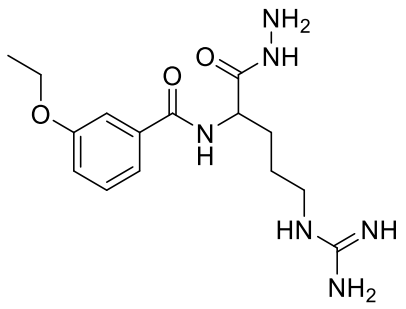
1.60 (m, 2H), 1.40 (t, *J* = 7.0 Hz, 3H), 1.26 (tt, 2H), 0.87 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 177.36, 172.17, 168.31, 159.11, 157.68, 134.85, 129.61, 119.29, 118.36, 113.25, 63.67, 52.61, 47.01, 40.59, 29.82, 28.48, 24.64, 22.69, 20.11, 14.74. HRMS Calcd. for $C_{19}H_{31}N_5O_3$ m/z 378.2505 $[M+H]^+$. Found: m/z 378.2497.

3.2.26.3. Synthesis of 3-ethoxy-N-(5-guanidino-1-oxo-1-(3-pivaloyl guanidino) pentan-2-yl) benzamide (7c)



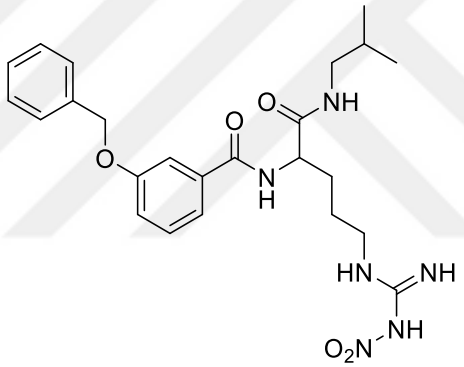
The crude was washed with DCM : Hexane and then dried under high vacuum to give product (**7c**). White solid (82%), mp very hygroscopic; ^{1}H NMR (DMSO-*d*6) δ 8.80 (d, *J* = 7.3 Hz, 1H), 8.71 (d, *J* = 7.5 Hz, 1H), 8.04 (s, 1H), 7.49–7.30 (m, 5H), 7.13 (s, 2H), 7.07 (d, *J* = 8.4 Hz, 2H), 4.36 (td, *J* = 7.6 Hz, 1H), 4.05 (q, 2H), 3.16–3.01 (m, 2H), 2.47 (s, 3H), 1.63–1.46 (m, 2H), 1.31 (t, 3H), 1.16 (tt, *J* = 7.1 Hz, 2H), 1.07 (s, 9H); ^{13}C NMR (DMSO-*d*6) δ 173.23, 172.49, 172.45, 166.87, 161.55, 158.83, 157.41, 135.47, 129.88, 120.11, 118.08, 113.64, 63.68, 60.95, 52.97, 40.82, 28.12, 25.78, 22.17, 15.05, 14.55.

3.2.26.4. Synthesis of 3-ethoxy-N-(5-guanidino-1-hydrazinyl-1-oxopentan-2-yl)benzamide (7d)



The crude was washed with DCM : Hexane and then dried under high vacuum to give product (**7d**). White solid (87%), mp very hygroscopic; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 9.21 (s, 1H), 8.43 (d, *J* = 7.9 Hz, 1H), 7.82 (s, 1H), 7.50–7.32 (m, 3H), 7.22 (t, *J* = 7.9 Hz, 3H), 6.91 (dd, *J* = 8.0, 2.7 Hz, 2H), 4.58 (td, *J* = 11.2 Hz, 1H), 3.98 (q, *J* = 7.0 Hz, 2H), 3.08 (d, *J* = 14.7 Hz, 2H), 1.84 (d, *J* = 3.9 Hz, 2H), 1.80 (s, 6H), 1.70–1.52 (m, 2H), 1.31 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 174.82, 171.07, 168.42, 167.09, 158.78, 135.62, 129.30, 119.81, 117.62, 113.66, 63.49, 51.52, 29.01, 24.94, 22.79, 20.81, 14.91. HRMS Calcd. for $C_{15}H_{24}N_6O_3$ m/z 337.1988 $[M+H]^+$. Found: m/z 337.1970.

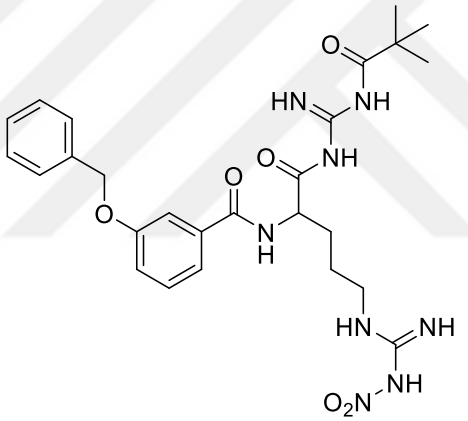
3.2.27. Synthesis of 3-(benzyloxy)-*N*-(1-(isobutylamino)-5-(3-nitroguanidino)-1-oxopentan-2-yl)benzamide (6d)



N-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide (**EDC**) (1.4 mmol, 0.22 g) and hydroxy-benzotriazole (**HO-Bt**) (1.4 mmol, 0.19 g) were added to a solution of N^2-(3-(benzyloxy)benzoyl)-N^{ω}-nitroarginine **5d** (1.2 mmol, 0.5 g) in dry DMF (2 mL). After the mixture stirred for 15 min at 5 ^{o}C, isobutylamine (1.6 mmol, 0.12 g) was added to this mixture. The mixture was stirred for 24h at room temperature under a nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, the solvent was removed, and the residue was taken into EtOAc (20 mL). The organic phase was washed with 1N HCl (2 x 5 mL), saturated solution of Na_2CO_3 (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The crude was washed with DCM: hexane and then dried under high vacuum to give product (**6d**).

White solid (82%), mp 111–115 ^{o}C; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 8.24 (s, 1H), 7.99 (s, 1H), 7.88 (s, 1H), 7.67 (d, *J* = 1.0 Hz, 1H), 7.57 (s, 1H), 7.46–7.14 (m, 7H), 7.01 (dd, *J* = 8.2, 2.5 Hz, 1H), 5.02 (s, 2H), 4.52 (td, *J* = 6.9 Hz, 1H), 3.18 (d, 1H), 2.99–2.81 (m, 1H), 2.73 (td, *J* = 15.1 Hz, 2H), 2.63 (d, *J* = 15.5 Hz, 1H), 1.80 (q, 2H), 1.64–1.45 (m, 2H), 0.78 (d, 6H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 175.26, 171.89, 162.38, 158.62, 136.68, 135.68, 129.45, 128.58, 128.07, 127.65, 120.01, 117.98, 113.93, 72.76, 69.96, 46.64, 43.00, 31.45, 28.38, 22.53, 20.25. HRMS Calcd. for $C_{24}H_{32}N_6O_5$ m/z 485.2512 $[M+H]^+$. Found: m/z 485.2494.

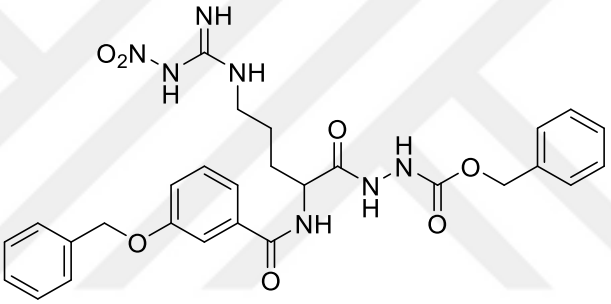
3.2.28. Synthesis of 3-(benzyloxy)-*N*-(5-(3-nitroguanidino)-1-oxo-1-(3-pivaloylguanidino) pentan-2-yl)benzamide (6e)



N-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide (**EDC**) (1.4 mmol, 0.22 g) and hydroxy-benzotriazole (**HO-Bt**) (1.4 mmol, 0.19 g) were added to the solution of N^2-(3-(benzyloxy)benzoyl)-N^{ω}-nitroarginine **5d** (1.2 mmol, 0.5 g) in dry DMF (1 mL). After the mixture stirred for 15 min at 5 ^{o}C, *N*-carbamimidoyl pivalamide (1.6 mmol, 0.23 g) was added to this mixture. The mixture was stirred for 24h at room temperature under a nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using EtOAc. After the reaction completed, the solvent was removed, and the residue was taken into EtOAc (30 mL). The organic phase was washed with saturated solution of Na_2CO_3 (2 x 5 mL), 1N HCl (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The crude was washed with DCM and then dried under high vacuum to give product (**6e**).

White solid (76%), mp 170–174 ºC; ^{1}H NMR (DMSO-*d*6) δ 10.05 (s, 1H), 8.92 (s, 1H), 8.49 (s, 1H), 7.56 (t, *J* = 2.0 Hz, 1H), 7.52–7.41 (m, 3H), 7.40–7.23 (m, 5H), 7.19 (dd, *J* = 8.2, 2.6 Hz, 1H), 5.14 (s, 2H), 4.40 (dt, *J* = 10.7, 5.3 Hz, 1H), 3.24–3.09 (m, 2H), 2.86 (s, 1H), 2.70 (s, 1H), 1.96 (s, 1H), 1.92–1.75 (m, 2H), 1.65–1.49 (m, 2H), 1.21 (s, 9H); ^{13}C NMR (DMSO-*d*6) δ 167.40, 159.78, 159.76, 158.63, 137.24, 135.09, 129.92, 128.91, 128.37, 128.20, 120.65, 118.57, 115.85, 114.48, 69.88, 55.82, 41.15, 40.60, 27.50, 26.49, 26.36. HRMS Calcd. for $C_{26}H_{34}N_8O_6$ m/z 555.2680 $[M+H]^+$. Found: m/z 555.2663.

3.2.29. Synthesis of benzyl 2-(N^2-(3-(benzyloxy)benzoyl)-N^{ω}-nitroarginyl)hydrazine-1-carboxylate (6f)



N-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide (**EDC**) (2.4 mmol, 0.37 g) and hydroxy-benzotriazole (**HO-Bt**) (1.4 mmol, 0.19 g) were added to a solution of N^2-(3-(benzyloxy)benzoyl)-N^{ω}-nitroarginine **5d** (1.2 mmol, 0.5 g) in dry DMF (1 mL). After the mixture stirred for 20 min at 5 ºC, benzyl hydrazinecarboxylate (1.7 mmol, 0.28 g) was added to this mixture. The mixture was stirred for 24h at room temperature under a nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using MeOH/EtOAc (1:4). After the reaction completed, the solvent was removed, and the residue was taken into EtOAc (30 mL). The organic phase was washed with 1N HCl (2 x 5 mL), saturated solution of Na_2CO_3 (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give the product (**6f**).

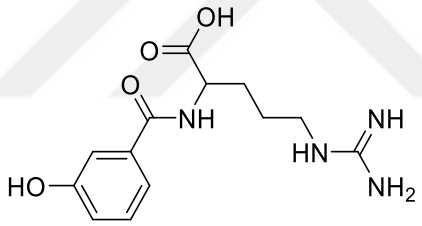
White solid (81%), mp very hygroscopic; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 9.79 (s, 1H), 9.02 (s, 1H), 8.19 (d, *J* = 8.0 Hz, 1H), 7.89 (s, 1H), 7.83 (s, 1H), 7.48 (t, *J* = 2.1 Hz, 1H), 7.45–7.34 (m, 3H), 7.34–7.14 (m, 10H), 7.03 (dd, *J* = 8.2, 2.6 Hz, 1H), 5.04 (s, 2H), 5.02 (s, 2H), 4.65–4.45 (m, 1H), 3.17 (s, 2H), 2.87 (s, 1H), 2.74 (s, 1H), 1.93–1.74 (m, 2H), 1.61 (s,

2H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 171.92, 166.77, 162.39, 158.57, 156.40, 136.83, 135.61, 129.41, 128.61, 128.49, 128.09, 128.03, 127.73, 120.27, 117.99, 114.09, 69.92, 66.56, 51.79, 36.39, 31.46, 22.55. HRMS Calcd. for $C_{28}H_{31}N_7O_7$ m/z 578.2363 $[M+H]^+$. Found: m/z 578.2351.

3.2.30. General procedure reduction for 5d, 6d, 6e and 6f compounds

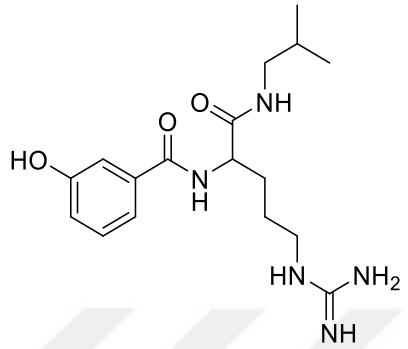
Palladium (50 mg) and acetic acid (2 mL) were added to the solution of **5d**, **6d**, **6e** or **6f** (0.28 mmol) in ethanol (25 mL) at room temperature. The mixture was stirred for 48h, at 70 ^{o}C in 50 psi. The reaction was monitored with TLC using silica on Al-plates and using MeOH/EtOAc (1:3). After the reaction completed, the mixture was filtered using silica to take off Pd and the solvent was evaporated under reduced pressure to give product **7e**, **7g** and **7f**.

3.2.30.1. Synthesis of (3-hydroxy benzoyl)arginine (7e)



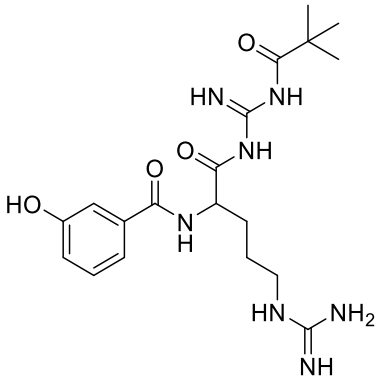
The crude was washed with DCM and then dried under high vacuum to give product (**7e**). Grey solid (84%), mp very hygroscopic; ^{1}H NMR (DMSO-*d*6) δ 9.13 (s, 1H), 7.99–7.77 (m, 2H), 7.60 (s, 1H), 7.28–7.08 (m, 3H), 6.82 (dd, *J* = 7.5, 2.0 Hz, 1H), 4.30 (td, 1H), 4.07 (t, *J* = 6.5 Hz, 1H), 3.06–2.97 (m, 2H), 1.81 (s, 6H), 1.76–1.58 (m, 2H), 1.55–1.35 (m, 2H); ^{13}C NMR (DMSO-*d*6) δ 175.77, 173.46, 165.60, 157.92, 157.77, 136.56, 129.71, 118.36, 117.69, 114.27, 54.35, 45.97, 29.79, 25.54, 22.57. HRMS Calcd. for $C_{13}H_{18}N_4O_4$ m/z 295.1406 $[M+H]^+$. Found: m/z 295.1401.

3.2.30.2. Synthesis of N-(5-guanidino-1-(isobutylamino)-1-oxopentan-2-yl)-3-hydroxybenzamide (7f)



For purification, the product was washed with DCM and then dried under high vacuum. White solid (84%), mp very hygroscopic; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 9.03 (s, 1H), 8.23 (d, *J* = 7.9 Hz, 1H), 7.96–7.84 (m, 1H), 7.41 (s, 1H), 7.33–7.20 (m, 3H), 7.12 (t, *J* = 7.8 Hz, 1H), 6.89–6.74 (m, 1H), 4.44 (td, 1H), 3.21–3.01 (m, 2H), 2.89 (ddq, *J* = 19.1, 12.7, 6.3 Hz, 2H), 1.79 (s, 6H), 1.68 (td, *J* = 13.6, 6.7 Hz, 2H), 1.55 (dq, *J* = 29.7, 7.3 Hz, 2H), 1.23–1.06 (m, 1H), 0.79 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 175.34, 172.15, 167.56, 157.87, 157.78, 135.80, 129.28, 118.65, 118.13, 114.80, 52.95, 46.58, 41.58, 29.26, 28.48, 25.18, 23.31, 20.33. HRMS Calcd. for $C_{17}H_{27}N_5O_3$ m/z 350.2192 $[M+H]^+$. Found: m/z 350.2171

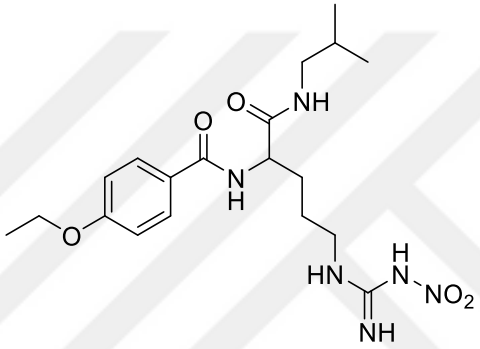
3.2.30.3. Synthesis of N-(5-guanidino-1-oxo-1-(3-pivaloyl guanidino)pentan-2-yl)-3-hydroxybenzamide (7g)



For purification, the product was washed with DCM and then dried under high vacuum. Brown solid (84%), mp very hygroscopic; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 8.63 (d, *J* = 7.5

Hz, 1H), 7.87 (d, *J* = 0.9 Hz, 6H), 7.26 (d, *J* = 7.9 Hz, 2H), 7.11 (t, *J* = 7.8 Hz, 2H), 6.84 (d, *J* = 8.1 Hz, 1H), 4.36 (d, *J* = 7.1 Hz, 1H), 4.12–4.01 (m, 2H), 3.17–2.97 (m, 2H), 2.47 (s, 3H), 2.10–2.00 (m, 1H), 1.78 (s, 9H), 1.62 (tt, *J* = 15.6, 8.0, 7.3 Hz, 2H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 175.18, 172.30, 167.85, 159.04, 157.65, 135.49, 129.25, 118.70, 118.34, 114.85, 78.89, 60.82, 52.76, 28.02, 25.33, 23.19, 14.36.

3.2.31. Synthesis of 4-ethoxy-*N*-(1-(isobutylamino)-5-(3-nitroguanidino)-1-oxopentan-2-yl) benzamide (6g)

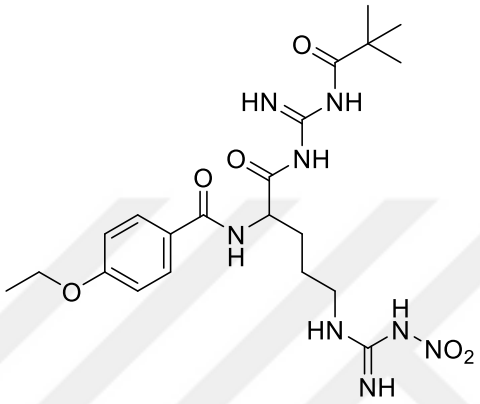


N-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide (**EDC**) (1.2 mmol, 0.19 g) and hydroxy-benzotriazole (**HO-Bt**) (1.2 mmol, 0.16 g) were added to a solution of N^2-(4-ethoxybenzoyl)-N^{ω}-nitroarginine **5f** (1 mmol, 0.4 g) in dry DMF (2 mL). After the mixture stirred for 20 min at 5 ^{o}C, isobutylamine (1.3 mmol, 95 mg) was added to this mixture. The mixture was stirred for 24h at room temperature under a nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, the solvent was removed, and the residue was taken into EtOAc (30 mL). The organic phase was washed with 1N HCl (2 x 5 mL), 2 N NaOH (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The crude was washed with DCM and dried under high vacuum to give product (**6g**).

White solid (77%), mp 85–93 ^{o}C; ^{1}H NMR ($CDCl_3$) δ 9.50 (s, 1H), 7.72 (dd, *J* = 8.8, 2.4 Hz, 2H), 7.62–7.37 (m, 3H), 6.43 (d, *J* = 29.6 Hz, 2H), 5.74 (s, 1H), 4.30–4.18 (m, 1H), 4.09 (s, 1H), 3.91–3.72 (m, 2H), 3.41–3.25 (m, 1H), 3.17 (ddd, *J* = 10.7, 8.3, 5.4 Hz, 1H), 2.97 (dq, *J* = 12.7, 6.3 Hz, 1H), 1.99–1.66 (m, 5H), 1.37 (td, *J* = 7.0, 4.0 Hz, 3H), 0.90 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 171.73, 168.21, 162.18, 159.65, 129.21, 124.50, 114.42,

68.13, 63.57, 47.10, 41.11, 29.69, 28.33, 20.13, 20.03, 14.59. HRMS Calcd. for $C_{19}H_{30}N_6O_5$ m/z 423.2356 $[M+H]^+$. Found: m/z 423.2329.

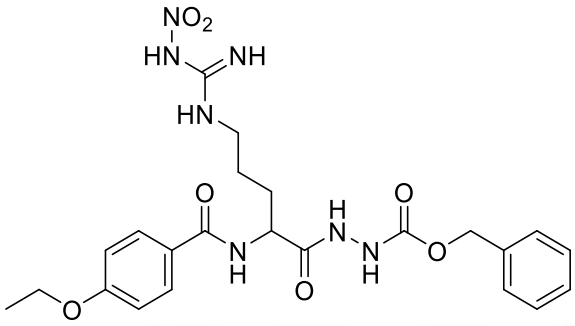
3.2.32. Synthesis of 4-ethoxy-*N*-(5-(3-nitroguanidino)-1-oxo-1-(3-pivaloyl guanidino) pentan-2-yl)benzamide (6h)



N-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide (**EDC**) (1.2 mmol, 0.19 g) and hydroxy-benzotriazole (**HO-Bt**) (1.2 mmol, 0.16 g) were added to a solution of N^2-(4-ethoxybenzoyl)-N^{ω}-nitroarginine **5f** (1 mmol, 0.4 g) in dry DMF (1 mL). After the mixture stirred for 20 min at 5 ^oC, *N*-carbamimidoyl pivalamide (1.8 mmol, 0.26 g) was added to this mixture. The mixture was stirred for 24h at room temperature under a nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using EtOAc. After the reaction completed, the solvent was removed, and the residue was taken into EtOAc (30 mL). The organic phase was washed with saturated solution of Na_2CO_3 (2 x 5 mL), 1N HCl (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The crude was washed with diethyl ether and dried under high vacuum to give the product (**6h**).

White solid (81%), mp 175–179 ^oC; ^1H NMR ($CDCl_3$+DMSO-*d*6) δ 12.82 (s, 1H), 11.91 (s, 1H), 10.16 (d, J = 56.6 Hz, 3H), 8.65 (s, 1H), 8.33 (s, 1H), 7.90–7.81 (m, 2H), 6.91–6.81 (m, 2H), 4.42 (q, 1H), 4.03 (q, J = 6.9 Hz, 2H), 3.23–3.14 (m, 2H), 1.95 (q, J = 7.1, 6.2 Hz, 2H), 1.80–1.57 (m, 2H), 1.35 (t, J = 7.0 Hz, 3H), 1.25 (s, 9H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 181.68, 176.09, 166.96, 163.93, 161.20, 153.55, 129.39, 124.83, 113.36, 63.03, 55.19, 40.61, 36.72, 26.85, 25.89, 24.55, 14.28. HRMS Calcd. for $C_{21}H_{32}N_8O_6$ m/z 493.2523 $[M+H]^+$. Found: m/z 493.2509.

3.2.33. Synthesis of benzyl 2-(N^2-(4-ethoxybenzoyl)-N^{ω}-nitroarginyl)hydrazine-1-carboxylate (6i)



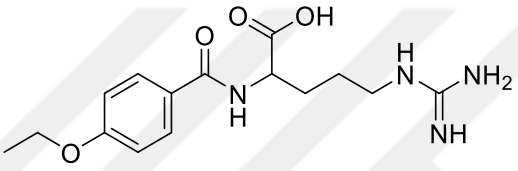
N-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide (**EDC**) (1.3 mmol, 0.2 g) and hydroxy-benzotriazole (**HO-Bt**) (1.3 mmol, 0.17 g) were added to a solution of N^2-(4-ethoxybenzoyl)-N^{ω}-nitroarginine **5f** (1 mmol, 0.4 g) in dry DMF (1 mL). After the mixture stirred for 20 min at 5 ^{o}C, benzyl hydrazinecarboxylate (1.4 mmol, 0.23 g) was added to this mixture. The mixture was stirred for 24h at room temperature under a nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using MeOH/EtOAc (1:4). After the reaction completed, the solvent was removed, and the residue was taken into EtOAc (30 mL). The organic phase was washed with 1N HCl (2 x 5 mL), saturated solution of Na_2CO_3 (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The crude was washed with DCM and dried under high vacuum to give product (**6i**).

White solid (81%), mp very hygroscopic; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 9.73 (s, 1H), 8.94 (s, 1H), 8.23 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.76 (d, *J* = 8.5 Hz, 3H), 7.40–7.11 (m, 5H), 6.79 (d, *J* = 8.7 Hz, 2H), 5.00 (s, 2H), 4.55 (s, 1H), 3.98 (q, *J* = 7.0 Hz, 2H), 2.47 (p, *J* = 1.8 Hz, 2H), 1.89–1.69 (m, 2H), 1.59 (s, 2H), 1.31 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 172.07, 166.67, 161.50, 159.61, 156.40, 136.48, 129.49, 128.46, 128.10, 128.00, 126.12, 113.86, 66.61, 63.54, 51.55, 40.78, 29.47, 24.62, 14.78. HRMS Calcd. for $C_{23}H_{29}N_7O_7$ m/z 516.2207 $[M+H]^+$. Found: m/z 516.2190.

3.2.34. General procedure reduction for 5f, 6g, 6h and 6i compounds

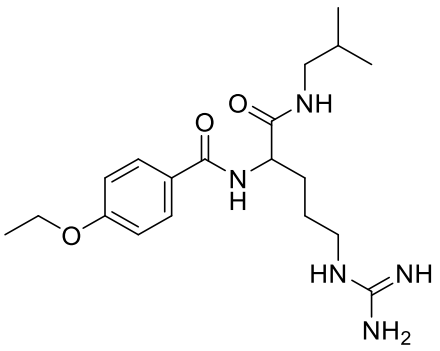
Palladium (25 mg) and acetic acid (2 mL) were added to the solution of **5f**, **6g**, **6h** or **6i** (0.2 mmol) in ethanol (25 mL) at room temperature. The mixture was stirred for 24h, at 70 ºC in 50 psi. The reaction was monitored with TLC using silica on Al-plates and using EtOAc. After the reaction completed, the mixture was filtered using silica to take off Pd and the solvent was evaporated under reduced pressure to give product **7j**, **7k**, **7i** and **7l**.

3.2.34.1. Synthesis of (4-ethoxybenzoyl)arginine (7i)



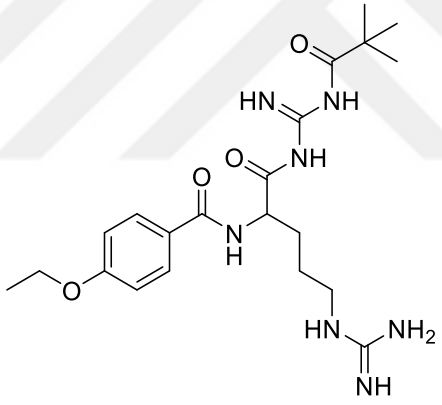
The crude was washed with DCM : Hexane and then dried under high vacuum to give product (**7i**). White solid (80%), mp 66–70 ºC; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 8.70 (s, 1H), 8.04 (d, *J* = 7.1 Hz, 1H), 7.66 (d, *J* = 8.5 Hz, 2H), 7.20 (s, 2H), 6.67 (d, *J* = 8.5 Hz, 2H), 4.33 (d, *J* = 6.5 Hz, 1H), 3.95 (q, *J* = 6.8 Hz, 2H), 3.16–3.04 (m, 2H), 2.74 (d, *J* = 7.7 Hz, 1H), 1.86–1.77 (m, 2H), 1.69–1.54 (m, 2H), 1.32 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 172.50, 165.09, 160.36, 157.20, 128.26, 126.18, 113.16, 78.00, 77.67, 77.35, 62.84, 53.65, 40.19, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 29.07, 20.77, 14.23. HRMS Calcd. for $C_{15}H_{22}N_4O_4$ m/z 323.1719 $[M+H]^+$. Found: m/z 323.1720.

3.2.34.2. Synthesis of 4-ethoxy-N-(5-guanidino-1-(isobutylamino)-1-oxopentan-2-yl) benzamide (7j)



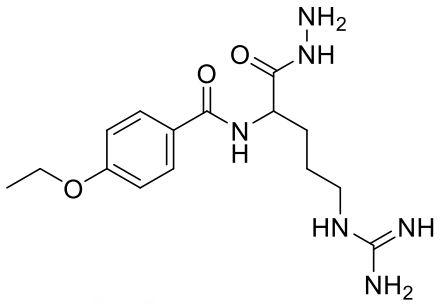
The crude was washed with diethyl ether and then dried under high vacuum to give product (**7j**). Grey solid (75%), mp 94–97 °C; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 7.98 (d, *J* = 8.0 Hz, 1H), 7.93 (t, *J* = 5.7 Hz, 1H), 7.79 (d, *J* = 8.5 Hz, 2H), 7.67 (t, *J* = 6.0 Hz, 1H), 7.15 (d, *J* = 20.9 Hz, 3H), 6.82 (d, *J* = 8.5 Hz, 2H), 4.51 (td, *J* = 9.0, 5.1 Hz, 1H), 4.00 (q, *J* = 6.9 Hz, 2H), 3.83 (s, 3H), 3.14 (dp, *J* = 20.2, 6.7 Hz, 2H), 2.91 (dq, *J* = 15.6, 6.9 Hz, 2H), 1.90–1.79 (m, 1H), 1.76–1.64 (m, 2H), 1.55 (tt, *J* = 14.1, 7.0 Hz, 2H), 1.34 (t, *J* = 6.9 Hz, 3H), 0.79 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 171.60, 166.35, 160.99, 160.38, 156.98, 128.90, 125.63, 117.68, 113.39, 63.03, 52.47, 46.08, 40.08, 28.97, 27.86, 24.88, 19.72, 14.26. HRMS Calcd. for $C_{19}H_{31}N_5O_3$ m/z 378.2505 $[M+H]^+$. Found: m/z 378.2496.

3.2.34.3. Synthesis of 4-ethoxy-N-(5-guanidino-1-oxo-1-(3-pivaloyl guanidino) pentan-2-yl) benzamide (7k)



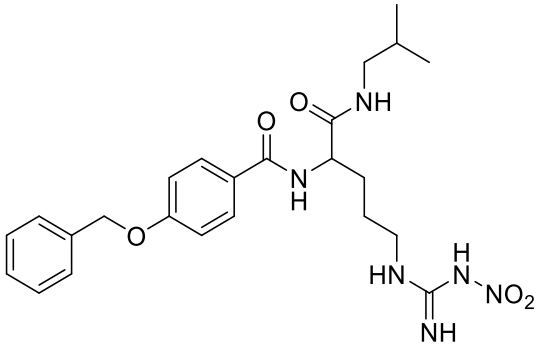
The crude was washed with DCM : Hexane and then dried under high vacuum to give product (**7k**). White solid (80%), mp 70–75 °C; ^{1}H NMR (DMSO-*d*6) δ 8.57 (d, *J* = 7.3 Hz, 2H), 7.84 (d, *J* = 8.5 Hz, 2H), 7.76 (s, 1H), 7.38–7.22 (m, 2H), 6.80 (d, *J* = 8.7 Hz, 2H), 4.40 (q, *J* = 7.2 Hz, 1H), 4.06 (qd, *J* = 7.1, 1.5 Hz, 2H), 3.08 (dd, *J* = 33.4, 6.4 Hz, 2H), 2.56 (q, *J* = 7.1 Hz, 1H), 2.48 (p, *J* = 1.8 Hz, 2H), 1.88–1.79 (m, 2H), 1.78 (s, 3H), 1.69–1.52 (m, 2H), 1.32 (t, J = 6.9 Hz, 3H), 1.16 (s, 9H); ^{13}C NMR (DMSO-*d*6) δ 180.58, 172.63, 172.47, 166.58, 161.50, 157.30, 156.09, 129.85, 126.11, 114.28, 63.75, 60.89, 40.78, 40.71, 28.00, 26.54, 25.87, 21.52, 14.57.

3.2.34.4. Synthesis of 4-ethoxy-N-(5-guanidino-1-hydrazinyl-1-oxopentan-2-yl) benzamide (7l)



For purification, the product was washed with DCM:Hexane and then dried under high vacuum. White solid (80%), mp 77–81 ºC; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 9.23 (m, 1H), 8.40–8.29 (m, 1H), 7.97 (s, 1H), 7.89–7.74 (m, 3H), 6.88–6.76 (m, 3H), 4.00 (q, *J* = 7.0 Hz, 2H), 3.08 (d, *J* = 16.2 Hz, 2H), 1.83 (d, *J* = 3.7 Hz, 2H), 1.80 (s, 6H), 1.67–1.47 (m, 2H), 1.32 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 174.65, 171.34, 168.32, 166.67, 161.39, 129.66, 126.37, 113.86, 63.53, 51.42, 31.45, 22.96, 22.54, 20.84, 14.87.

3.2.35. Synthesis of 4-(benzyloxy)-*N*-(1-(isobutylamino)-5-(3-nitroguanidino)-1-oxopentan-2-yl)benzamide (6j)

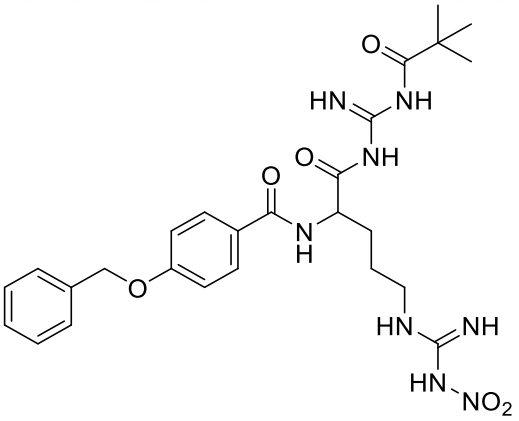


N-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide (**EDC**) (1.2 mmol, 0.19 g) and hydroxy-benzotriazole (**HO-Bt**) (1.2 mmol, 0.16 g) were added to a solution of N^2-(4-(benzyloxy)benzoyl)-N^{ω}-nitroarginine **5i** (1 mmol, 0.43 g) in dry DMF (2 mL). After the mixture stirred for 15 min at 5 ºC, isobutylamine (1.3 mmol, 95 mg) was added to this mixture. The mixture was stirred for 24h at room temperature under a nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using EtOAc. After the

reaction completed, the solvent was removed, and the residue was taken into EtOAc (30 mL). The organic phase was washed with 1N HCl (2 x 5 mL), saturated solution of Na_2CO_3 (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The crude was washed with DCM and then dried under high vacuum. to give product (**6j**)

White solid (90%), mp 137–141 ºC; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 9.51 (s, 1H), 7.76 (d, J = 8.6 Hz, 2H), 7.68–7.36 (m, 3H), 7.34–6.97 (m, 5H), 6.49 (d, J = 7.9 Hz, 2H), 5.75 (s, 1H), 4.72 (s, 2H), 4.08 (td, 1H), 3.39–3.24 (m, 1H), 3.17 (td, J = 13.4, 6.8 Hz, 1H), 2.97 (td, J = 12.5, 11.6, 5.4 Hz, 1H), 1.98–1.74 (m, 2H), 1.62 (dd, 2H), 1.32–1.17 (m, 2H), 0.89 (d, 6H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 171.98, 165.02, 161.15, 154.34, 136.41, 129.33, 128.56, 128.06, 127.51, 126.64, 114.34, 69.83, 52.75, 46.60, 39.05, 29.67, 28.35, 20.19. HRMS Calcd. for $C_{24}H_{32}N_6O_5$ m/z 485.2512 $[M+H]^+$. Found: m/z 485.2506.

3.2.36. Synthesis of 4-(benzyloxy)-*N*-(5-(3-nitroguanidino)-1-oxo-1-(3-pivaloylguanidino) pentan-2-yl)benzamide (6k)

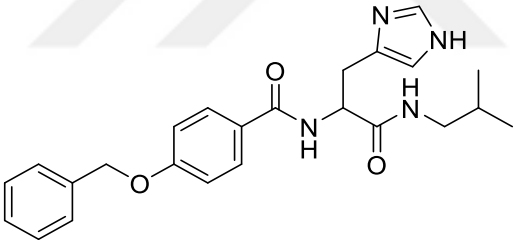


N-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide (**EDC**) (1.2 mmol, 0.19 g) and hydroxy-benzotriazole (**HO-Bt**) (1.2 mmol, 0.16 g) were added to a solution of N^2-(4-(benzyloxy)benzoyl)-N^{ω}-nitroarginine **5i** (1 mmol, 0.43 g) in dry DMF (1 mL). After the mixture stirred for 15 min at 5 ºC, *N*-carbamimidoyl pivalamide (1.3 mmol, 0.19g) was added to this mixture. The mixture was stirred for 24h at room temperature under a nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using EtOAc. After the reaction completed, the solvent was removed, and the residue was taken

into EtOAc (40 mL). The organic phase was washed with saturated solution of Na_2CO_3 (2 x 5 mL), 1N HCl (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The crude was washed with DCM and then dried under high vacuum to give product (**6k**).

White solid (80%), mp 186–190 °C; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 10.04 (s, 2H), 7.84 (d, *J* = 8.3 Hz, 3H), 7.46–7.15 (m, 5H), 6.93 (d, *J* = 8.3 Hz, 2H), 5.03 (s, 2H), 4.74 (s, 1H), 3.68–3.35 (m, 1H), 3.22 (s, 1H), 2.90 (d, *J* = 30.0 Hz, 1H), 2.22–1.97 (m, 2H), 1.86 (s, 2H), 1.73–1.47 (m, 2H), 1.24 (d, *J* = 8.1 Hz, 9H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 184.51, 175.13, 171.75, 156.03, 141.76, 136.64, 129.87, 128.65, 128.14, 127.72, 121.69, 114.42, 72.81, 69.82, 43.13, 40.75, 35.66, 27.94, 26.63. HRMS Calcd. for $C_{26}H_{34}N_8O_6$ m/z 555.2680 $[M+H]^+$. Found: m/z 555.2660.

3.2.37. Synthesis of *N*-(3-(1*H*-imidazol-4-yl)-1-(isobutylamino)-1-oxopropan-2-yl)-4- (benzyloxy)benzamide (6l)



Trifluoroacetic acid (0.5 mL) was added to a solution of 4-(benzyloxy)-*N*-(1-(isobutylamino)-1-oxo-3- (1-trityl-1*H*-imidazol-4-yl)-propan-2-yl)benzamide **10a** (0.15 mmol, 0.1 g) in triisopropylsilane and DCM (0.5 mL/4 mL). The mixture was stirred for 4h at room temperature. The reaction was monitored with TLC using silica on Al-plates and using EtOAc. After the reaction completed, the solvent was removed, and the residue was transferred into water (5 mL). The solution was alkalified using saturated solution of Na_2CO_3 to pH 8–9. The formed precipitate was filtered and washed with water (5 mL). The crude was washed with diethyl ether and dried under high vacuum to give product (**6l**).

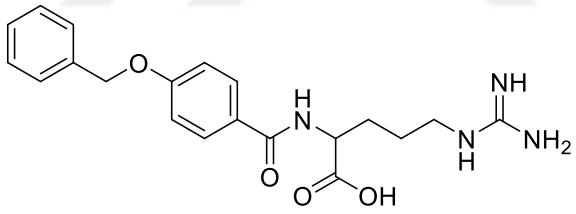
Brown solid (55%), mp 165–170 °C; ^{1}H NMR (DMSO-*d*6) δ 8.49 (d, *J* = 7.8 Hz, 1H), 7.97 (s, 1H), 7.90 (s, 1H), 7.82 (d, *J* = 8.3 Hz, 2H), 7.50–7.28 (m, 5H), 7.08 (d, *J* = 8.5 Hz, 3H), 6.96 (s, 1H), 5.17 (s, 2H), 4.63 (q, *J* = 9.1 Hz, 1H), 3.00 (t, *J* = 14.8 Hz, 2H), 2.87 (d, *J*

= 6.4 Hz, 2H), 1.71–1.59 (m, 1H), 0.78 (d, 6H); ^{13}C NMR (DMSO-*d*6) δ 170.87, 165.63, 160.73, 136.70, 134.47, 129.27, 129.05, 128.49, 127.97, 127.78, 126.48, 121.85, 114.30, 69.33, 53.36, 46.07, 28.68, 28.02, 20.02. HRMS Calcd. for $C_{24}H_{28}N_4O_3$ m/z 421.2240 $[M+H]^+$. Found: m/z 421.2240.

3.2.38. General procedure reduction for 5i, 6j and 6k compounds

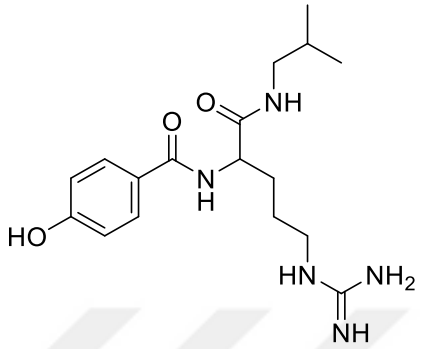
Palladium (50 mg) and acetic acid (2 mL) were added to a solution of **5i**, **6j** or **6k** (0.23 mmol) in ethanol (25 mL) at room temperature. The mixture was stirred for 36h, at 70 ^{o}C in 50 psi. The reaction was monitored with TLC using silica on Al-plates and using MeOH/EtOAc (1:4). After the reaction completed, the mixture was filtered using silica to take off Pd and the solvent was evaporated under reduced pressure to give product **7n**, **7o** and **7m.**

3.2.38.1. Synthesis of (4-(benzyloxy)benzoyl)arginine (7m)



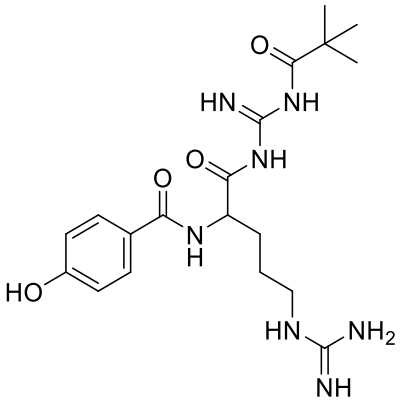
The crude was washed with DCM: Hexane and dried under high vacuum to give product (**7m**). White solid (90%), mp very hygroscopic; ^{1}H NMR (DMSO-*d*6) δ 9.24–8.99 (m, 1H), 8.10 (d, *J* = 7.5 Hz, 1H), 7.61 (d, *J* = 8.2 Hz, 3H), 7.44 (s, 1H), 6.65 (d, *J* = 8.2 Hz, 2H), 4.13 (td, *J* = 7.2 Hz, 1H), 3.02 (td, *J* = 20.5 Hz, 2H), 2.54 (q, *J* = 7.2 Hz, 1H), 1.83 (s, 6H), 1.79–1.61 (m, 2H), 1.54–1.38 (m, 2H); ^{13}C NMR (DMSO-*d*6) δ 176.77, 173.20, 165.44, 160.28, 157.80, 129.23, 125.62, 115.03, 54.34, 40.82, 29.79, 25.42, 22.31.

3.2.38.2. Synthesis of N-(5-guanidino-1-(isobutylamino)-1-oxopentan-2-yl)-4-hydroxy benzamide (7n)



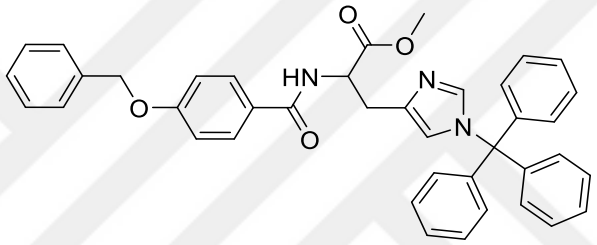
The crude was washed with diethyl ether, crystallized in ethyl acetate:hexane and dried under high vacuum to give product (**7n**). White solid (85%), mp 74–79 ^{o}C; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 8.04 (d, *J* = 8.0 Hz, 1H), 7.84–7.60 (m, 4H), 7.40 – 7.19 (m, 5H), 6.92 (d, *J* = 8.3 Hz, 3H), 6.73 (d, *J* = 8.1 Hz, 1H), 5.04 (s, 2H), 4.48 (d, *J* = 8.7 Hz, 1H), 3.10 (s, 2H), 2.89 (h, *J* = 6.8 Hz, 2H), 1.86 (s, 1H), 1.80 (s, 1H), 1.68 (ddt, *J* = 20.1, 13.4, 5.8 Hz, 2H), 1.53 (s, 2H), 0.78 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 177.60, 172.25, 172.19, 172.12, 172.07, 167.03, 166.75, 161.18, 160.79, 157.33, 136.57, 129.53, 129.49, 128.61, 128.11, 127.65, 126.65, 115.13, 114.39, 69.82, 60.18, 46.60, 40.73, 29.46, 28.38, 25.45, 20.30. HRMS Calcd. for $C_{24}H_{33}N_5O_3$ m/z 440.2662 $[M+H]^+$. Found: m/z 440.2659.

3.2.38.3. Synthesis of N-(5-guanidino-1-oxo-1-(3-pivaloylguanidino)pentan-2-yl)-4-hydroxy benzamide (7o)



The product was washed with diethyl ether for purification and dried under high vacuum. White solid (85%), mp very hygroscopic; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 8.56 (d, *J* = 7.2 Hz, 1H), 8.17–8.08 (m, 5H), 7.76 (d, *J* = 8.5 Hz, 2H), 7.37 (s, 2H), 6.76 (d, *J* = 8.5 Hz, 2H), 4.37 (q, *J* = 6.7, 6.2 Hz, 1H), 4.08 (qq, *J* = 5.9, 3.8 Hz, 2H), 3.11 (dd, *J* = 12.7, 6.5 Hz, 2H), 1.81 (s, 8H), 1.71–1.50 (m, 2H), 1.18 (d, *J* = 2.9 Hz, 9H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 182.97, 174.78, 172.59, 167.11, 160.91, 157.93, 157.52, 129.79, 124.63, 115.06, 60.74, 52.79, 40.75, 28.09, 26.90, 25.58, 23.03, 14.46.

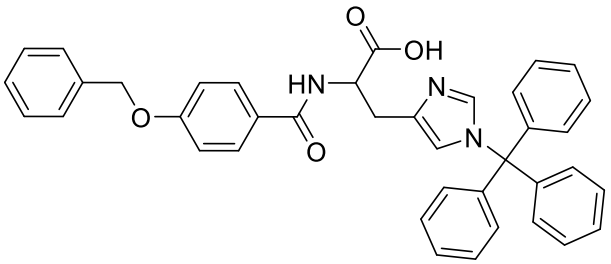
3.2.39. Synthesis of methyl N^{α}-(4-(benzyloxy)benzoyl) -N^{t}-tritylhistidinate (8)



Triethylamine (1.8 mmol, 0.18 g) was added to a solution of trityl chloride (1.8 mmol, 0.5 g) in anhydrous toluene (20 mL). After the mixture stirred for 10 min at room temperature, methyl (4–(benzyloxy)benzoyl)histidinate **5g** (1.6 mmol, 0.6 g) was added to this mixture. The mixture was stirred for 4h at 115 ^{o}C. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:1). After the reaction completed, triethylammonium chloride was filtered off and discarded. The organic phase was washed with saturated solution of Na_2CO_3 (2 x 5 mL), 0.2N HCl (2 x 5 mL) and saturated brine (5 mL) and then dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure. The crude was washed with hexane to give the product (**8**).

White solid (75%), mp 138–142 ^{o}C; ^{1}H NMR ($CDCl_3$) δ 8.23 (d, *J* = 7.7 Hz, 1H), 7.99 (d, *J* = 8.4 Hz, 2H), 7.80 (s, 1H), 7.38–7.21 (m, 13H), 7.05 (d, *J* = 7.2 Hz, 5H), 6.97 (d, *J* = 8.2 Hz, 3H), 6.79 (s, 2H), 5.08 (s, 2H), 5.01 (td, *J* = 7.9, 4.0 Hz, 1H), 3.66 (s, 3H), 3.46 (d, *J* = 15.1, 9.0 Hz, 1H), 3.35 (d, *J* = 15.3 Hz, 1H).

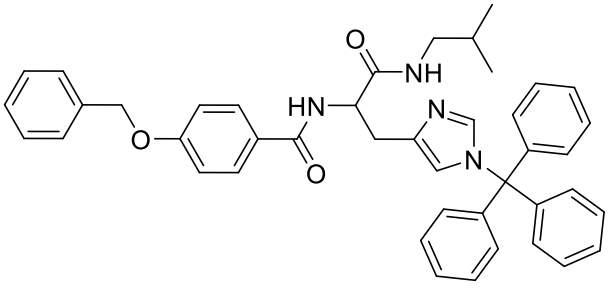
3.2.40. Synthesis of N^{α}-(4-(benzyloxy)benzoyl)-N^{t}-tritylhistidine (9)



LiOH (4 mmol, 0.1 g) was added to a solution of methyl N^{α} -(4-(benzyloxy)benzoyl)-N^{t}-tritylhistidinate **8** (1 mmol, 0.6 g) in 10 mL THF:CH_3OH: H_2O (3:2:2). The mixture was stirred for 1h at room temperature. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, THF and CH_3OH were removed and the solution was diluted with more water (5 mL). The solution was acidified with HCl to pH 2–3. The formed precipitate was filtered and washed with water (10 mL), diethyl ether (5 mL) and dried under high vacuum to give the product (**9)**.

White solid (98%), mp 125–128 ^{o}C; ^{1}H NMR ($CDCl_3$) δ 8.13 (s, 1H), 8.08–7.95 (m, 1H), 7.76 (d, J = 8.2 Hz, 2H), 7.45–7.21 (m, 17H), 6.99 (d, J = 7.4 Hz, 5H), 6.91–6.81 (m, 2H), 5.18 (td, J = 11.0 Hz, 1H), 5.02 (s, 2H), 3.55 (d, J = 14.9 Hz, 1H), 3.36 (d, J = 12.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 171.91, 166.59, 161.34, 141.93, 138.36, 136.46, 136.11, 129.70, 129.21, 128.64, 128.18, 128.13, 128.10, 127.43, 126.50, 119.86, 114.50, 70.01, 52.98, 52.19, 29.38.

3.2.41. Synthesis of 4-(benzyloxy)-*N*-1-(isobutylamine)-1-oxo-3-(1-trityl-1*H*-imidazole-4-yl)propan-2-yl)benzamide (10a)

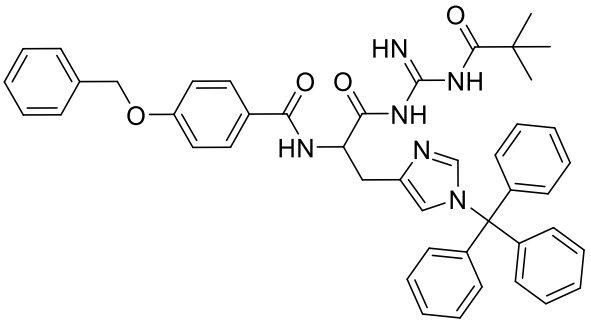


N-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide (**EDC**) (0.26 mmol, 40 mg) and hydroxy-benzotriazole (**HO-Bt**) (0.26 mmol, 35 mg) were added to a solution of N^{α}-(4-

(benzyloxy)benzoyl)-N^t-tritylhistidine **9** (0.16 mmol, 0.1 g) in dry DMF (1 mL). After the mixture stirred for 15 min at 5 ^oC, isobutylamine (0.48 mmol, 35 mg) was added to this mixture. The mixture was stirred for 24h at room temperature under a nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using EtOAc . After the reaction completed, the solvent was removed, and the residue was taken into EtOAc (30 mL). The organic phase was washed with saturated solution of Na_2CO_3 (2 x 5 mL), 1N HCl (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give the product (**10a**).

White solid (95%), mp 157–160 ^oC; ^1H NMR ($CDCl_3$) δ 8.22 (s, 1H), 8.11 (s, 1H), 7.84–7.69 (m, 3H), 7.46–7.37 (m, 4H), 7.37–7.29 (m, 4H), 7.24 (t, J = 7.5 Hz, 6H), 7.03 (d, J = 7.7 Hz, 5H), 6.97 (dd, J = 8.2, 3.2 Hz, 3H), 6.69 (s, 1H), 5.28 (d, J = 6.9 Hz, 1H), 5.12 (s, 2H), 3.60 (d, J = 13.9 Hz, 1H), 3.07 (d, J = 6.1 Hz, 2H), 2.96 (d, J = 12.8 Hz, 1H), 1.84 (dt, J = 13.2, 6.7 Hz, 1H), 0.91 (d, J = 6.4 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 170.39, 166.45, 161.49, 139.55, 136.32, 134.19, 132.54, 129.49, 129.22, 129.17, 128.74, 128.69, 128.19, 127.43, 126.10, 120.52, 114.58, 78.53, 70.05, 53.16, 47.15, 31.58, 28.40, 20.29.

3.2.42. Synthesis of 4-(benzyloxy)-*N*-(1-oxo-1-(3-pivaloyl guanidino)-3-(1-trityl-1*H*-imidazol-4-yl)propan-2-yl)benzamide (10b)

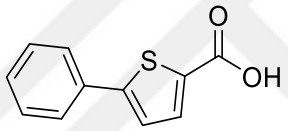


N-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide (**EDC**) (0.19 mmol, 29 mg) and hydroxy-benzotriazole (**HO-Bt**) (0.19 mmol, 26 mg) were added to a solution of N^{α}-(4-(benzyloxy)benzoyl)-N^t-tritylhistidine **9** (0.16 mmol, 1 g) in dry DMF (1 mL). After the mixture stirred for 15 min at 5 ^oC, *N*-carbamimidoyl pivalamide (0.21 mmol, 30 mg) was added to this mixture. The mixture was stirred for 24h at room temperature under a nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using

EtOAc. After the reaction completed, the solvent was removed, and the residue was taken into EtOAc (30 mL). The organic phase was washed with saturated solution of Na_2CO_3 (2 x 5 mL), 1N HCl (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The crude was washed with EtOAc: hexane and then dried under high vacuum to give product (**10b**)..

White solid (52%), mp 145–150 ºC; ^{1}H NMR ($CDCl_3$) δ 7.99–7.88 (m, 3H), 7.45–7.28 (m, 15H), 7.25 (s, 1H), 7.08–6.90 (m, 10H), 5.10 (s, 2H), 4.11 (q, J = 7.2 Hz, 1H), 3.53 (d, J = 14.5 Hz, 2H), 2.04 (s, 1H), 1.31 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 186.68, 182.59, 162.91, 161.80, 140.06, 136.35, 129.69, 129.58, 129.49, 129.03, 128.67, 128.16, 127.45, 114.60, 77.21, 70.04, 55.93, 41.28, 31.58, 26.51.

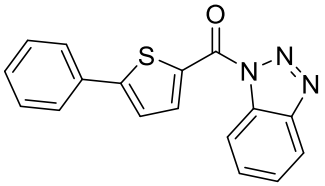
3.2.43. Synthesis of 5-phenylthiophene-2-carboxylic acid (12)



5-bromothiophene-2-carboxylic acid (2 mmol, 0.4 g) and phenylboronic acid (2.4 mmol, 0.29 g) were added to the mixture of K_2CO_3 (2 mmol, 0.28 g) in 10 mL DMF: H_2O (1:20) at room temperature and stirred for 15 min under nitrogen atmosphere. $Pd(Cl)_2(PPh_3)_2$ (0.01 mmol, 7 mg) was added and the mixture was stirred for 12h at 75 ºC. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:3). After the reaction was completed, the solvent was removed, the residue was taken into water (15 mL) and was acidified using HCl to pH 2–3. The formed precipitate was filtered, washed with water (5 mL) and dried under high vacuum to give the product (**12**).

White solid (97%), mp 186–187 ºC (Lit [96], 187–188 ºC); ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 7.58 (dd, J = 8.3, 1.3 Hz, 2H), 7.48 (d, J = 3.8 Hz, 1H), 7.34 (dd, J = 8.3, 6.9 Hz, 2H), 7.29 (d, J = 3.8 Hz, 1H), 7.28–7.20 (m, 1H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 164.21, 133.97, 132.05, 129.29, 128.37, 127.39, 125.86, 123.82.

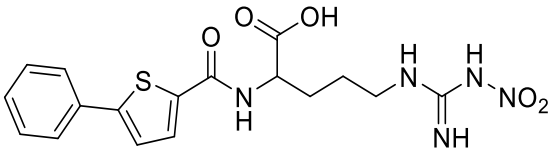
3.2.44. Synthesis of (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(5-phenylthiophen-2-yl)methanone (13)



Thionyl chloride (1.2 mmol, 0.14 g) was added to a solution of benzotriazole (4.2 mmol, 0.5 g) in freshly distilled CH_2Cl_2 (10 mL) at 5 ^oC. The reaction mixture was stirred for 30 min at the same temperature. 5-phenylthiophene-2-carboxylic acid **9.1** (0.88 mmol, 0.18 g) in CH_2Cl_2 (1 mL) was added dropwise to this mixture and stirred for 4h at room temperature. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, a white precipitate was filtered off and discarded. The organic phase was washed with saturated solution of Na_2CO_3 (2 x 5 mL), saturated brine (5 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to give the product (**13**).

White solid (60%), mp 280 ^oC decomposed; ^1H NMR ($CDCl_3$) δ 8.55 (dd, J = 4.2, 1.4 Hz, 1H), 8.42 (dd, J = 8.2, 1.4 Hz, 1H), 8.18 (dd, J = 8.2, 1.3 Hz, 1H), 7.76 (dt, J = 7.8, 1.5 Hz, 2H), 7.70 (dd, J = 8.3, 7.0 Hz, 1H), 7.55 (dd, J = 8.1, 6.8 Hz, 1H), 7.50–7.39 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 159.14, 156.24, 145.83, 139.60, 133.01, 130.47, 129.48, 129.23, 126.45, 126.35, 124.05, 120.27, 114.90.

3.2.45. Synthesis of N^{ω}-nitro-N^2-(5-phenylthiophene-2-carbonyl)arginine (14a)

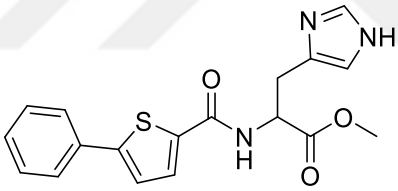


Triethylamine (4 mmol, 0.4 g) was added to a solution of N^{ω}-nitro-L-arginine (3.6 mmol, 0.8 g) in water (10 mL) at room temperature. The reaction mixture was stirred for 30 min at the same temperature. (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(5-phenylthiophen-2-yl)methanone **13** (3 mmol, 0.9 g) in 1,4-dioxane (20 mL) was added dropwise to this mixture

and then mixture was heated at 60 ºC in microwave oven with 30 watt power and stirred for 5h. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:2). After the reaction completed, 1,4-dioxane was removed and the solution was diluted with more water (5 mL). The solution was alkalified using saturated solution of Na_2CO_3 to pH 9–10 and washed with ethyl acetate (2 x 5 mL) to take off the surplus of benzotriazole. After washing, the solution was acidified using HCl to pH 3–4. The formed precipitate was filtered, washed with water (10 mL) and dried under high vacuum to give the product (**14a**).

White solid (62%), mp 163–166 ºC; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 8.48–8.41 (m, 1H), 7.79 (s, 1H), 7.67–7.53 (m, 3H), 7.42–7.23 (m, 5H), 4.44 (s, 1H), 3.20 (s, 1H), 2.12–2.02 (m,3H), 1.84 (d, J = 47.3 Hz, 1H), 1.64 (s,1H), 1.18 (s, 1H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 173.87, 163.41, 161.81, 150.33, 148.43, 138.43, 134.18, 133.54, 133.35, 129.87, 129.30, 129.26, 128.88, 128.57, 126.07, 125.92, 124.01, 123.80, 52.40, 40.60, 30.97, 28.55.

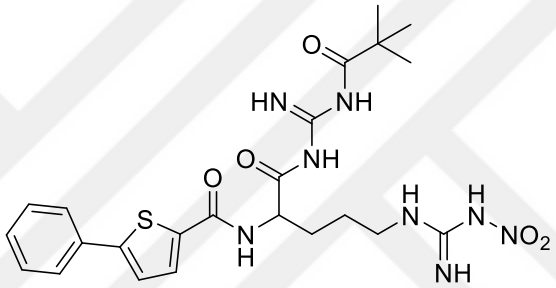
3.2.46. Synthesis of methyl (5-phenylthiophene-2-carbonyl) histidinate (14b)



Triethylamine (12 mmol, 1.21 g) was added to a solution of L-histidine methyl ester (4.5 mmol, 1.1 g) in water (10 mL) at room temperature. The reaction mixture was stirred for 15 min at the same temperature. (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(5-phenylthiophen-2-yl)methanone **13** (3mmol, 0.92 g) in 1,4-dioxane (30 mL) was added dropwise to this mixture. The mixture was heated at 70 ºC in microwave oven with 50 watt power and stirred for 6h. The reaction was monitored with TLC using silica on Al-plates and using EtOAc/hexane (1:1). After the reaction completed, 1,4-dioxane was removed and the solution was diluted with more water (5 mL). The solution was alkalified using saturated solution of Na_2CO_3 to pH 9–10 and washed with ethyl acetate (2 x 5 mL) to take off the surplus of benzotriazole. After washing, the solution was acidified using HCl to pH 2–3. The formed precipitate was filtered, washed with water (10 mL) and dried under high vacuum to give the product (**5g**).

White solid (76%), mp 195 °C decomposed; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 8.78 (d, *J* = 7.7 Hz, 1H), 7.88 (d, *J* = 3.1 Hz, 1H), 7.70 (d, *J* = 3.9 Hz, 1H), 7.62–7.54 (m, 2H), 7.35 (t, *J* = 7.5 Hz, 2H), 7.32–7.26 (m, 2H), 6.92 (s, 1H), 4.74 (q, *J* = 7.1 Hz, 1H), 3.64 (s, 3H), 3.11 (d, *J* = 6.4 Hz, 2H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 171.83, 161.59, 148.66, 137.97, 134.75, 133.45, 129.86, 129.27, 128.65, 126.06, 125.94, 123.82, 116.61, 52.77, 52.28, 28.17. HRMS Calcd. for $C_{18}H_{17}N_3O_3S$ m/z 356.1069 $[M+H]^+$. Found: m/z 356.1051.

3.2.47. Synthesis of *N*-(5-(3-nitroguanidino)-1-oxo-1-(3-pivaloylguanidino)pentan-2-yl)-5-phenylthiophene-2-carboxamide (15)

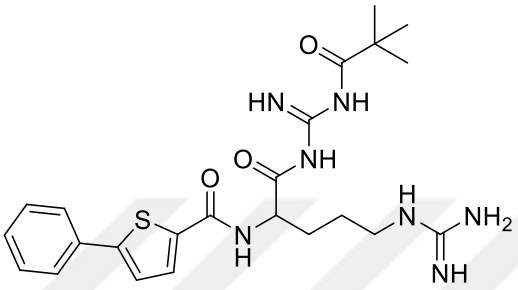


N-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide (**EDC**) (1.6 mmol, 0.25 g) and hydroxy-benzotriazole (**HO-Bt**) (1.6 mmol, 0.22 g) were added to a solution of N^{ω}-nitro-N^{2}-(5-phenylthiophene-2-carbonyl)arginine **14a** (1.1 mmol, 0.44 g) in dry DMF (1 mL). After the mixture stirred for 20 min at 5 °C, *N*-carbamimidoyl pivalamide **D**(1.9 mmol, 0.27 g) was added to this mixture. The mixture was stirred for 24h at room temperature under a nitrogen atmosphere. The reaction was monitored with TLC using silica on Al-plates and using EtOAc. After the reaction completed, the solvent was removed, and the residue was taken into EtOAc (30 mL). The organic phase was washed with saturated solution of Na_2CO_3 (2 x 5 mL), 1N HCl (2 x 5 mL), brine (5 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure The crude was washed with diethyl ether and then dried under high vacuum to give product (**15**).

White solid (83%), mp 188–190 °C; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 10.29 (s, 1H), 10.13 (s, 1H), 8.89 (s, 1H), 7.97–7.87 (m, 1H), 7.59 (dd, *J* = 8.2, 1.3 Hz, 2H), 7.37 (dd, *J* = 8.5, 7.0 Hz, 3H), 7.34–7.24 (m, 1H), 4.45 (s, 1H), 3.24 (p, *J* = 6.6 Hz, 2H), 2.90 (s, 1H), 2.76 (s, 1H), 1.95 (ddd, *J* = 25.7, 16.7, 9.1 Hz, 2H), 1.72 (s, 2H), 1.35–1.31 (m, 1H), 1.27 (s, 9H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 181.20, 175.39, 162.49, 153.89, 149.05, 137.18, 133.41,

131.04, 129.45, 129.30, 128.73, 126.42, 125.98, 125.12, 123.95, 55.63, 46.14, 41.21, 36.37, 27.42, 26.43. HRMS Calcd. for $C_{23}H_{30}N_8O_5S$ m/z 531.2138 $[M+H]^+$. Found: m/z 531.2132.

3.2.48. Synthesis of *N*-(5-guanidino-1-oxo-1-(3-pivaloylguanidino)pentan-2-yl)-5-phenylthiophene-2-carboxamide (16)



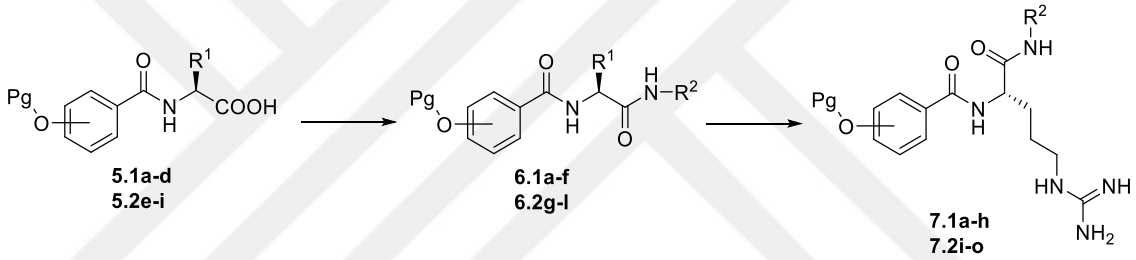
The crude was washed with DCM : Hexane and then dried under high vacuum to give product (**16**). White solid (82%), mp 71–73 ºC; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) δ 8.66 (d, *J* = 7.7 Hz, 1H), 7.91 (d, *J* = 3.9 Hz, 1H), 7.82 (s, 1H), 7.56 (dd, *J* = 8.2, 1.3 Hz, 2H), 7.34 (dd, *J* = 8.4, 6.9 Hz, 2H), 7.31–7.24 (m, 3H), 6.92 (s, 2H), 4.54–4.39 (m, 1H), 4.17–4.04 (m, 1H), 3.37 (q, *J* = 7.0 Hz, 1H), 3.14 (dd, *J* = 13.2, 6.4 Hz, 3H), 1.88 (s, 3H), 1.74–1.54 (m, 2H), 1.21 (d, 8H), 0.87–0.72 (m, 2H); ^{13}C NMR ($CDCl_3$+DMSO-*d*6) δ 172.06, 162.11, 157.48, 148.63, 138.10, 133.51, 130.23, 129.22, 128.58, 125.92, 123.79, 61.03, 52.47, 40.79, 31.46, 28.20, 28.20, 26.75, 25.60, 25.60, 22.55, 21.55, 14.38.

3.3. Biological Activity Studies

The biological activity studies including antiviral activity in a single cycle and toxicity assay were done by Prof. Dr. Asim K. Debnath and Dr. Francesca Curreli at Lindsley F. Kimball Research Institute, New York Blood Center, New York 10065, United States.

The HIV-1-inhibitory activity and cytotoxicity some of the novel compounds that are synthesized in this study were analyzed by following previously reported article titled with "*Synthesis, Antiviral Activity, and Structure–Activity Relationship of 1,3-Benzodioxolyl Pyrrole-Based Entry Inhibitors Targeting the Phe43 Cavity in HIV-1 gp120*" [97].

Table 3.1. *Anti- HIV-$_{1HXB2}$ activity, cytotoxicity and selectivity indexes of some novel compounds*.



Code	Pg, Position of Pg	Amino acid (R^1)	R^2-NH_2	MW	IC50 (µM)	CC50 (µM)
5a	-CH_2CH_3, *p*	L-His	/	303.3	33	131.9
5b	-CH_2CH_3, *m*	nitro-L-Arg	/	367.4	27.2	108.9
5c	-CH_2Ph, *m*	L-His	/	365.4	27.4	109.5
5e	-CH_2CH_3, *p*	L-His	/	303.3	33	131.87
5f	-CH_2CH_3, *p*	nitro-L-Arg	/	367.4	27.2	108.9
5h	-CH_2Ph, *p*	L-His	/	465.4	27.4	109.5
5i	-CH_2Ph, *p*	nitro-L-Arg	/	429.4	23.3	93.2
6e	-CH_2Ph, *m*	nitro-L-Arg	-NH(CNH)NHPiv	554.6	18.03	72.12
6g	-CH_2CH_3, *p*	nitro-L-Arg	-NHBu-*i*	422.5	23.7	94.68
6h	-CH_2CH_3, *p*	nitro-L-Arg	-NH(CNH)NHPiv	492.5	20.3	81.2
6l	-CH_2Ph, *p*	L-His	-NHBu-*i*	420.5	23.8	95.12
7a	-CH_2CH_3, *m*	L-Arg	-OH	382.4	26.2	104.6
7i	-CH_2CH_3, *p*	L-Arg	-OH	382.4	26.15	104.6
7j	-CH_2CH_3, *p*	L-Arg	-NHBu-*i*	491.5	20.4	81.38
NBD-14111	/	/	/	400.1	7.0	145.8

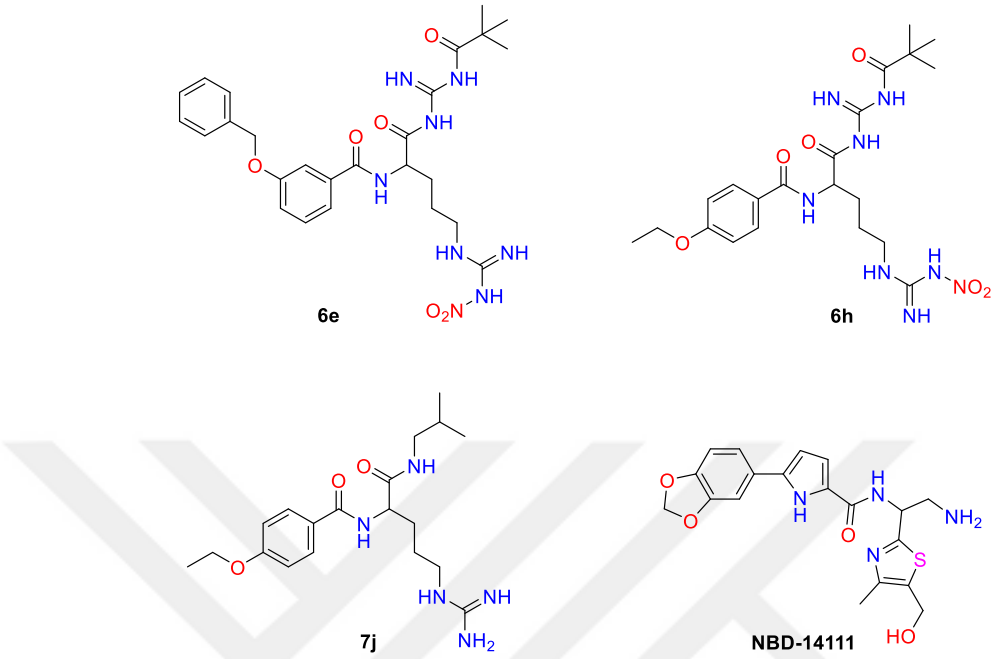


Figure 3.1. *The structure of novel compounds with high biological activity and the structure of reference compound NBD-14111.*

4. SUMMARY OF ACHIEVEMENTS

In this study, alkoxy benzoates, active intermediate *N*-acyl benzotriazole and amino acids amides were synthesized by nucleophilic substitution reaction (yields 55–98%). The protected amino acids (N^{im}-trt-His and N^{ω}-nitro-Arg) were coupled with *N*-benzoyl benzotriazoles to give *N*-benzoyl amino acids **5a-i** (65–89%). Amide derivatives were treated with amine substituents in dry DMF using EDC and HO-Bt to synthesize amino acid amides derivatives **6a–l** (55–90%). After synthesized N^{im}-trt-His amides derivatives, Trt group was removed using trifluoroacetic acid (55%). N^{ω}-(NO_2-)-L-Arginine amides were treated with hydrogen gas in the presences of palladium catalyst to eliminate nitro group **7a–o** (71–90%)

Intermediate compounds 5-phenylthiophene-2-carboxylic acid was synthesized by using Suzuki-Miyaura coupling reaction (yield 97%). Active intermediate thiophen benzotriazole (**13**) were prepared from 5-phenylthiophene-2-carboxylic acid (60%) and then coupling with N^{ω}-(NO_2-)-L-arginine or L-histidine methyl ester and treated with *N*-carbamimidoylpivalamide to synthesis amino acids amides **15** (83%).

Compounds that tested for potential anti-HIV-1 activity showed good result. The compounds, 3-(benzyloxy)-N-(5-(3-nitroguanidino)-1-oxo-1-(3-pivaloylguanidino) pentan-2-yl)benzamide **(6e)**, 4-ethoxy-N-(5-(3-nitroguanidino)-1-oxo-1-(3-pivaloyl guanidino) pentan-2-yl)benzamide **(6h)** and Synthesis of 4-ethoxy-N-(5-guanidino-1-(isobutylamino)-1-oxopentan-2-yl) benzamide **(7j)** showed the best anti-HIV-1 activity, with **IC_{50} (µM)** of 18.03, 20.3 and 20.4 and with **CC_{50} (µM)** of 72.12, 81.2 and 81.38 respectively.

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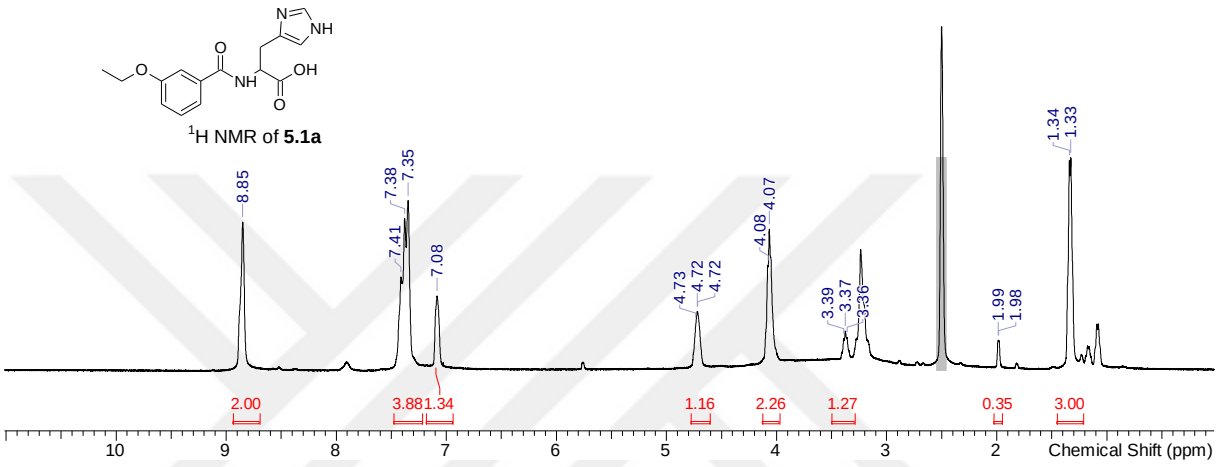
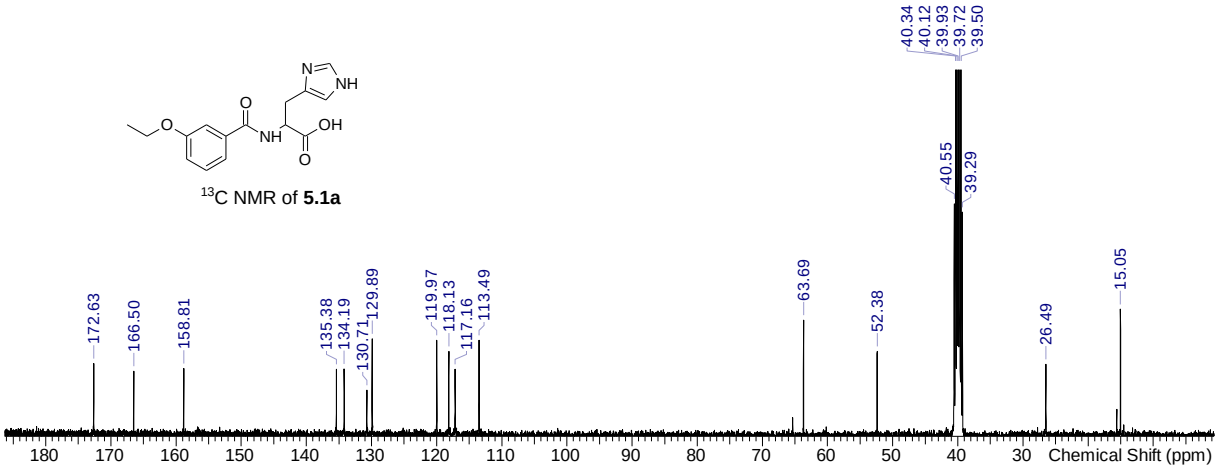
APPENDIXES

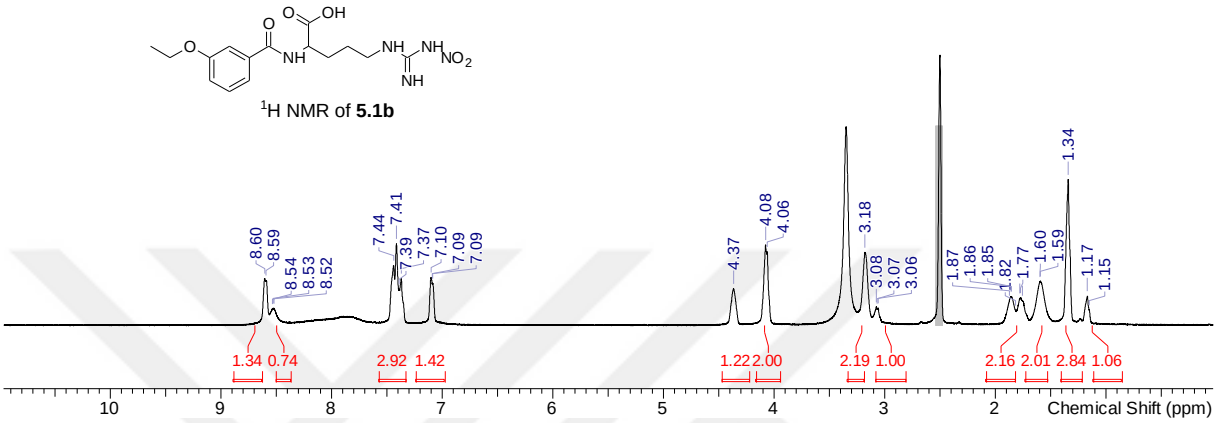
Appex–1. *[1]H NMR spectra of (3–ethoxybenzoyl)histidine (**5a**).*



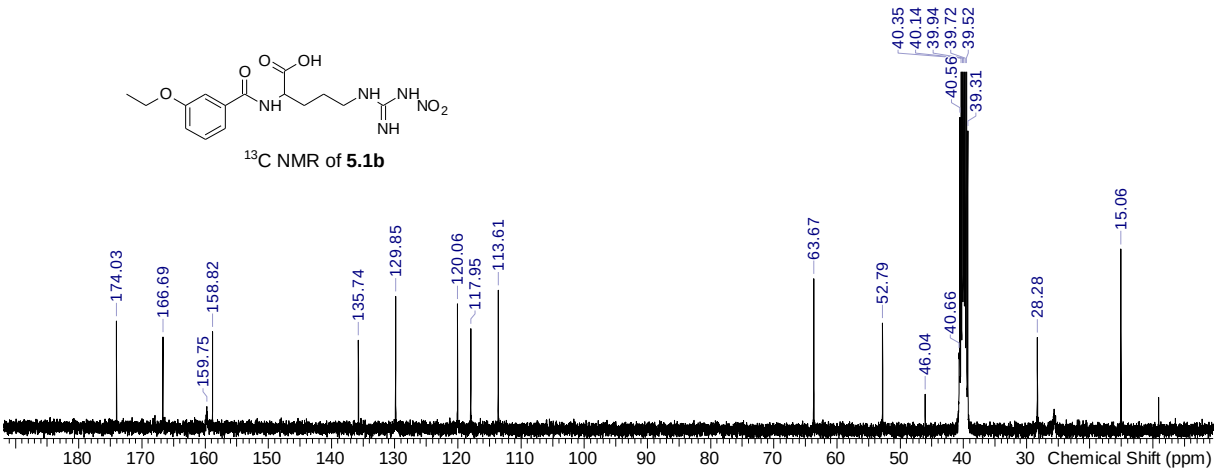
Appex–2. *[13]C NMR spectra of (3–ethoxybenzoyl)histidine (**5a**).*



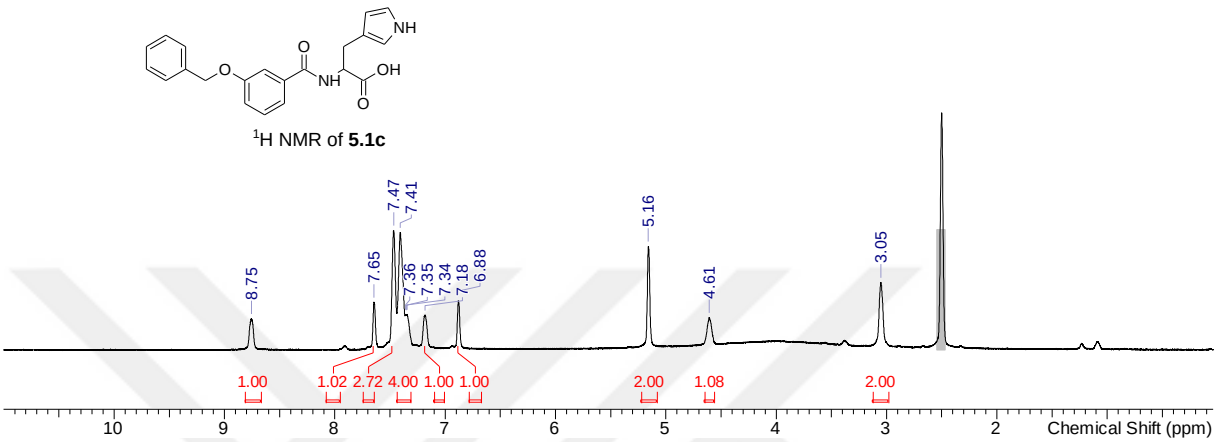
Appex–3. *^{1}H NMR spectra of N^{2}–(3–ethoxybenzoyl)–N$^{\omega}$–nitroarginine (**5b**).*



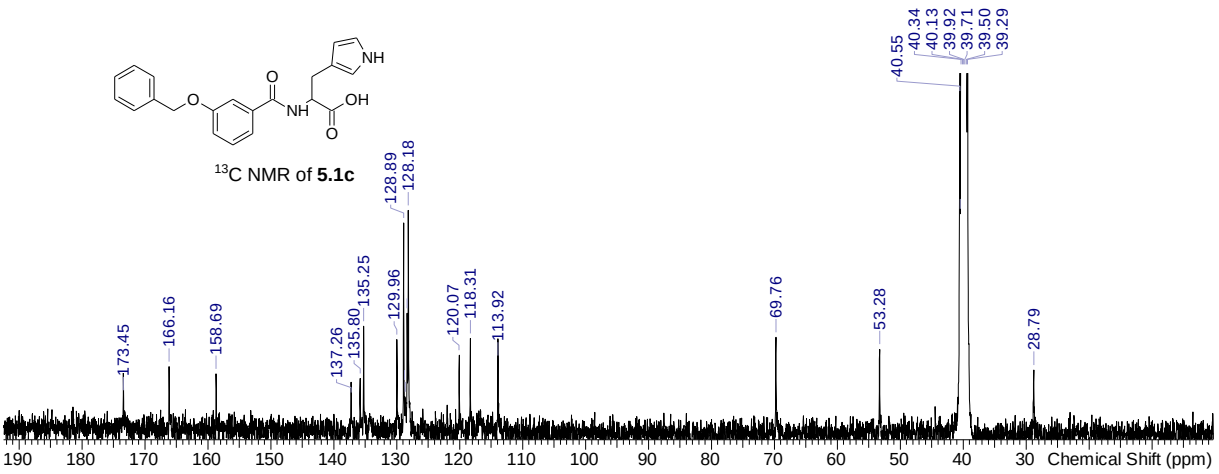
Appex–4. *^{13}C NMR spectra of N^{2}–(3–ethoxybenzoyl)–N$^{\omega}$–nitroarginine (**5b**).*



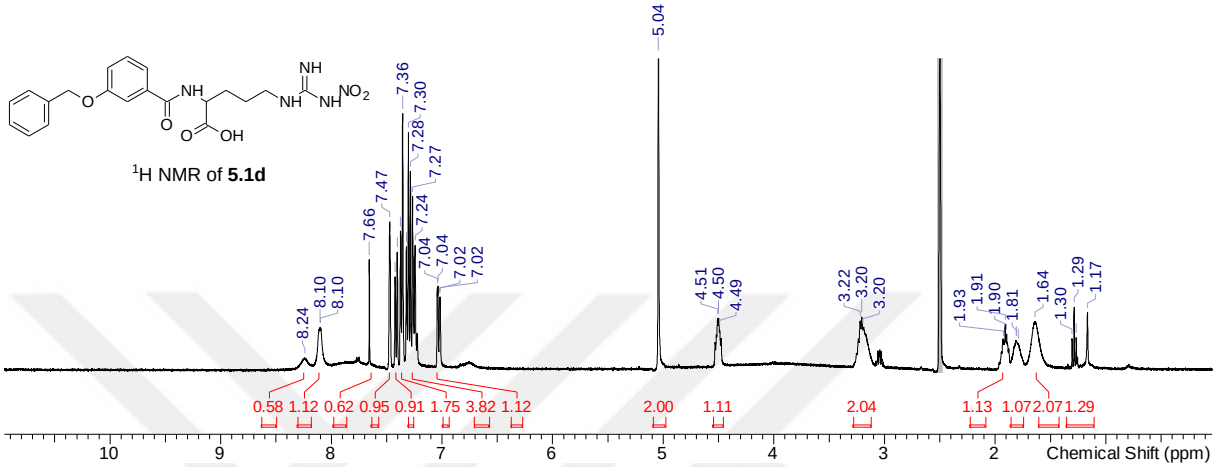
Appex–5. *[1]H NMR spectra of 2–(3–(benzyloxy)benzamido)–3–(1H–pyrrol–3–yl)propanoic acid (**5c**).*



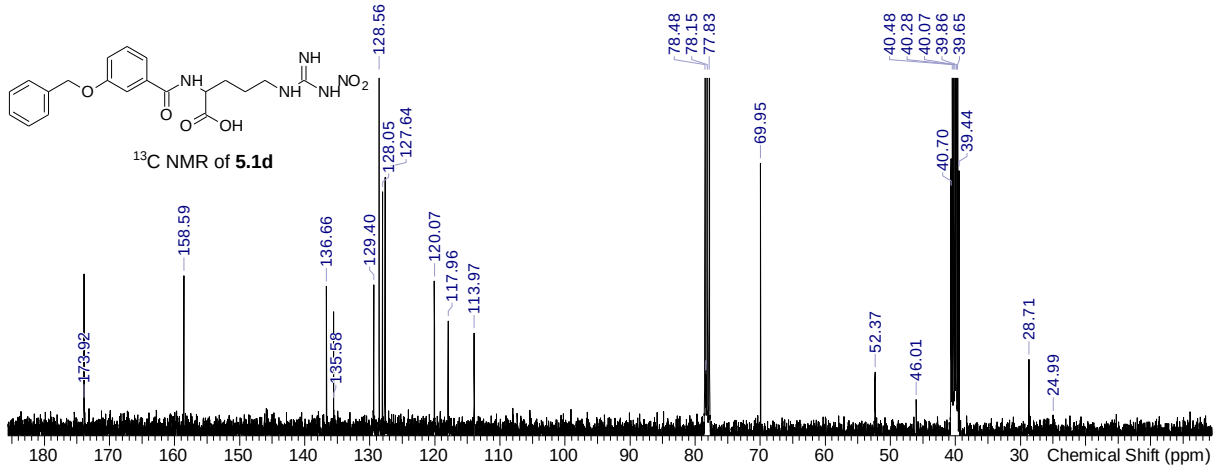
Appex–6. *[13]C NMR spectra of 2–(3–(benzyloxy)benzamido)–3–(1H–pyrrol–3–yl)propanoic acid (**5c**).*



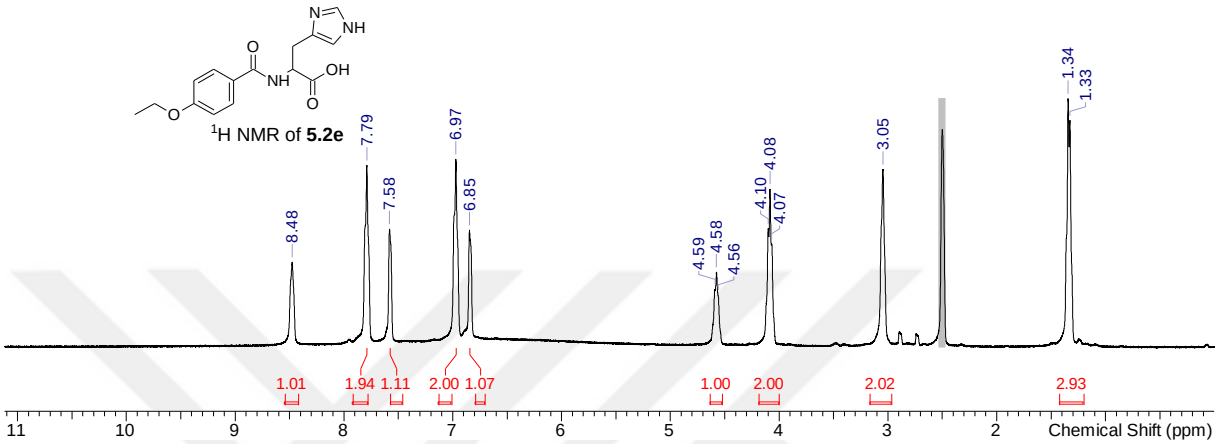
Appex–7. *^{1}H NMR spectra of N^{2}–(3–(benzyloxy)benzoyl)–N^{ω}–nitroarginine (**5d**).*



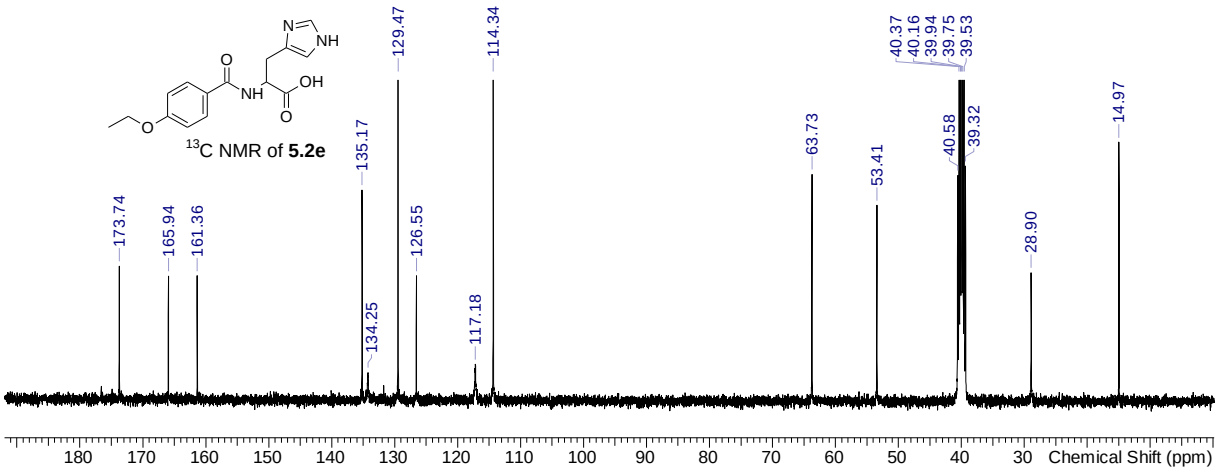
Appex–8. *^{13}C NMR spectra of N^{2}–(3–(benzyloxy)benzoyl)–N^{ω}–nitroarginine (**5d**).*



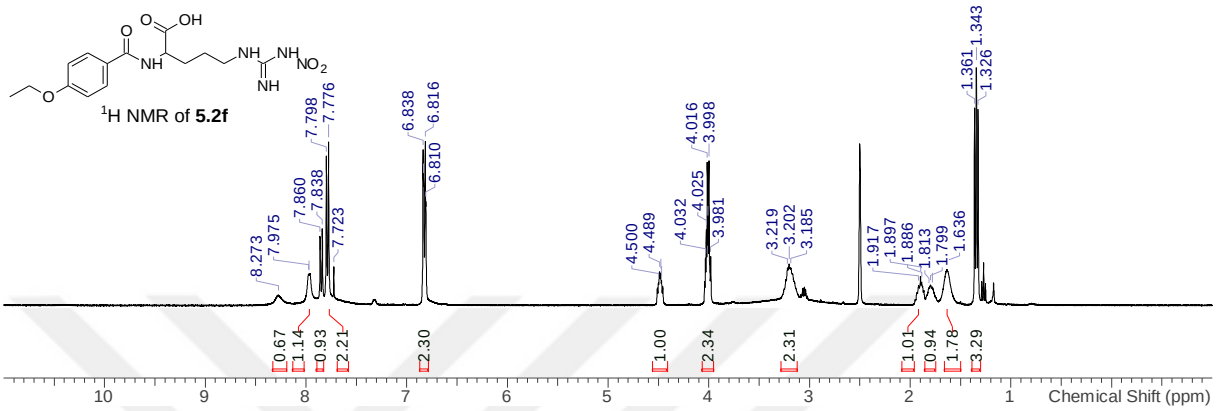
Appex–9. *^{1}H NMR spectra of (4–ethoxybenzoyl)histidine (**5e**).*



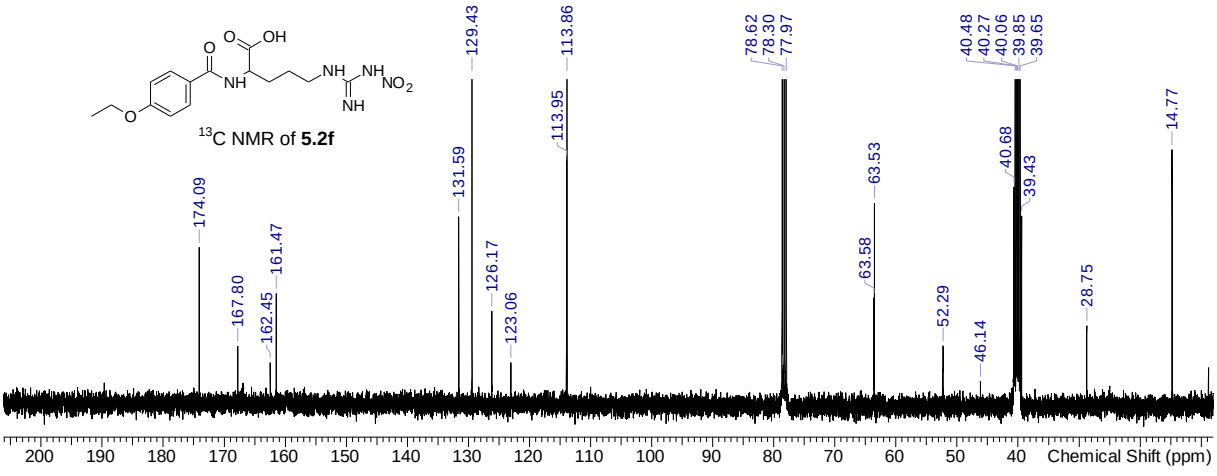
Appex–10. *^{13}C NMR spectra of (4–ethoxybenzoyl)histidine (**5e**).*



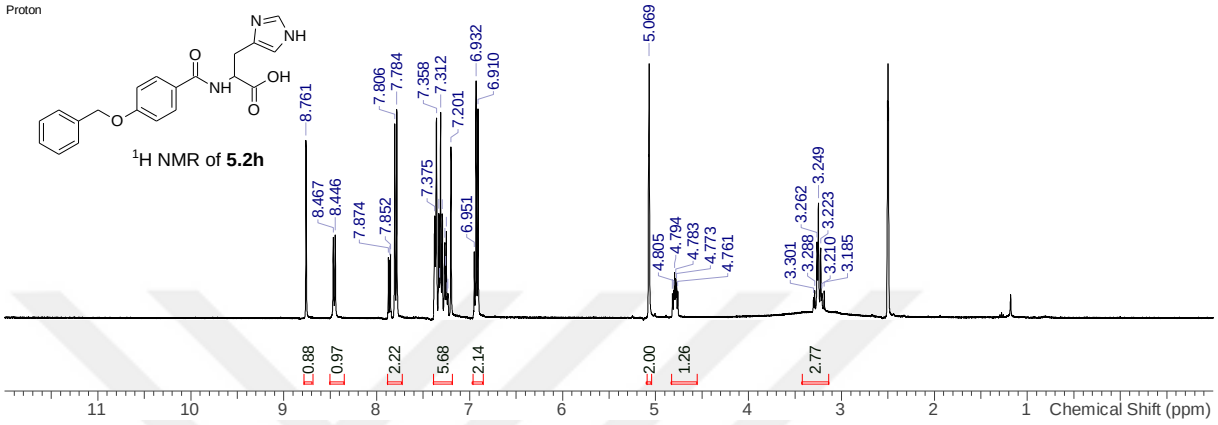
Appex–11. *^{1}H NMR spectra of N^{2}–(4–ethoxybenzoyl)–N^{ω}–nitroarginine (**5f**).*



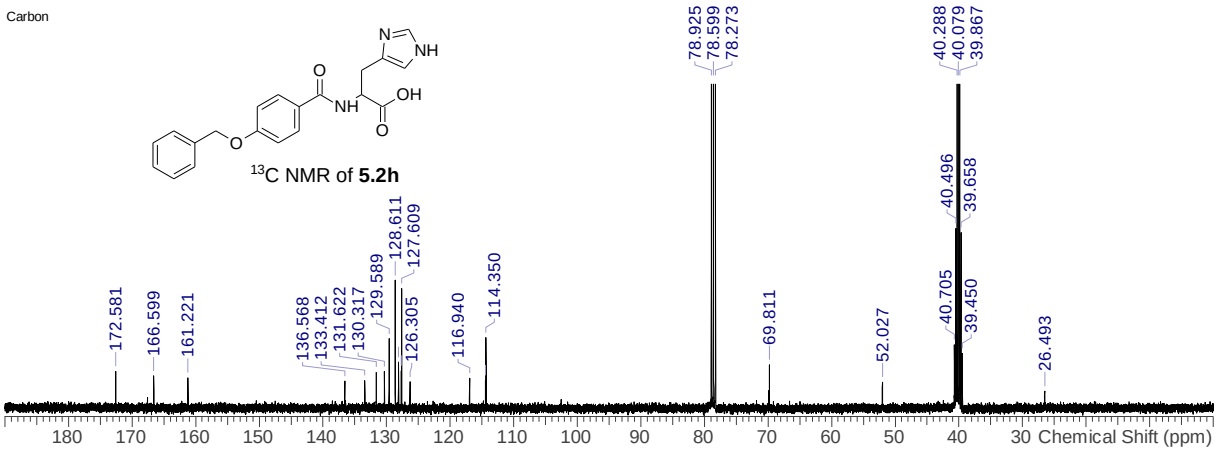
Appex–12. *^{13}C NMR spectra of N^{2}–(4–ethoxybenzoyl)–N^{ω}–nitroarginine (**5f**).*



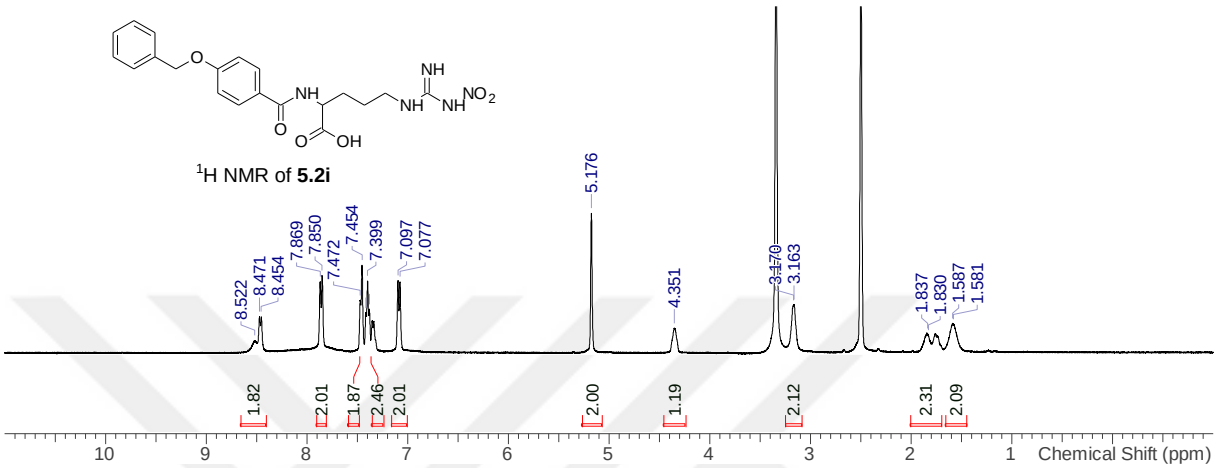
Appex–13. *[1]H NMR spectra of (4–(benzyloxy)benzoyl)histidine (**5h**).*



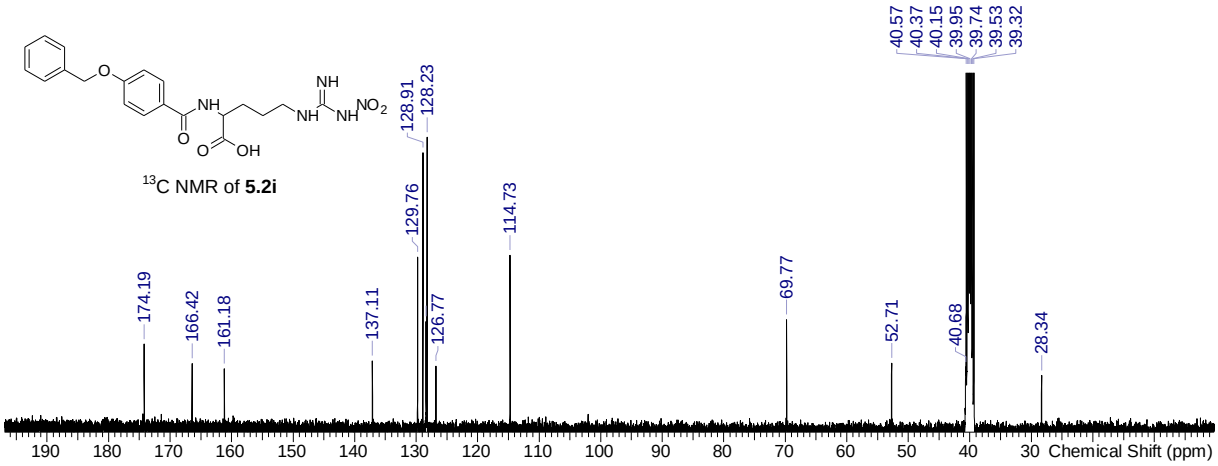
Appex–14. *[13]C NMR spectra of (4–(benzyloxy)benzoyl)histidine (**5h**).*



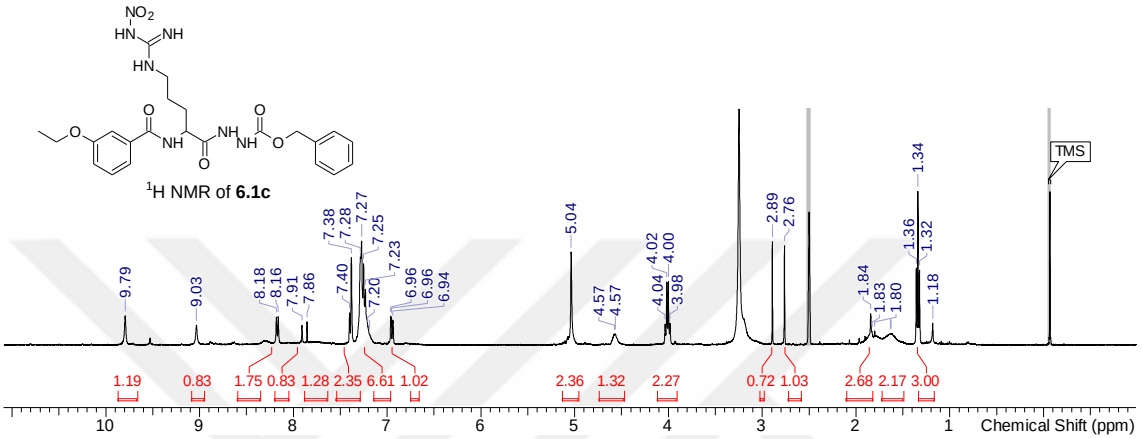
Appex–15. *^{1}H NMR spectra of N^{2}–(4–(benzyloxy)benzoyl)–N^{ω}–nitroarginine (**5i**).*



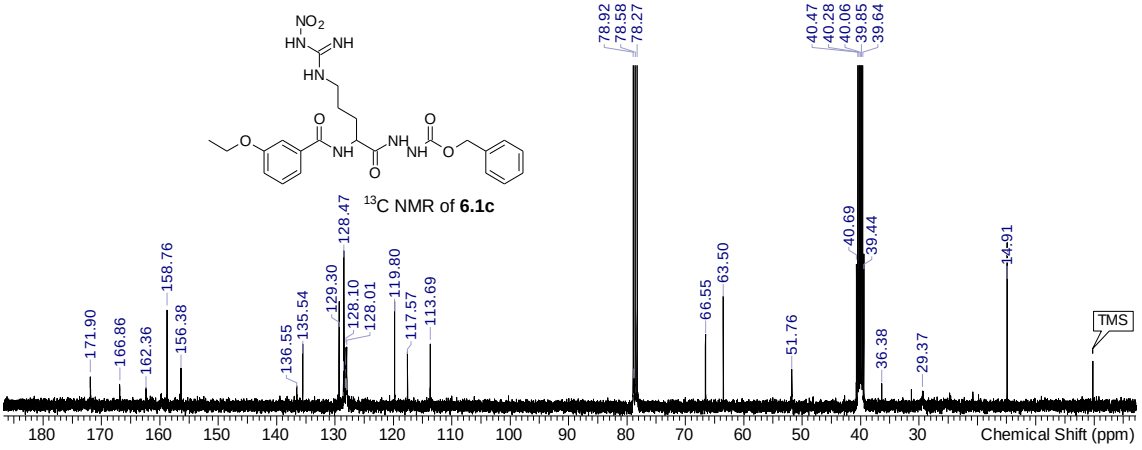
Appex–16. *^{13}C NMR spectra of N^{2}–(4–(benzyloxy)benzoyl)–N^{ω}–nitroarginine (**5i**).*



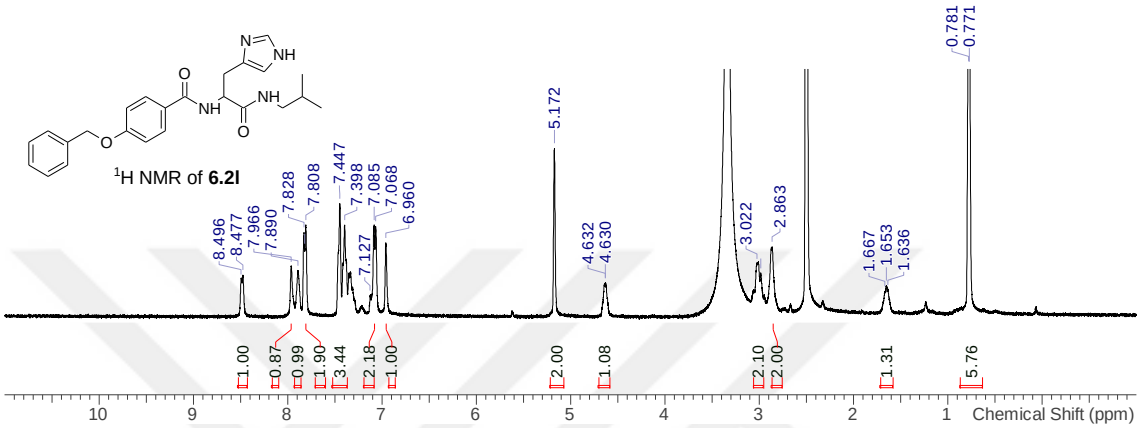
Appex–17. *^{1}H NMR spectra of benzyl 2–(N^{2}–(3–ethoxybenzoyl)–N^{ω}–nitroarginyl)hydrazine–1–carboxylate (**6c**).*



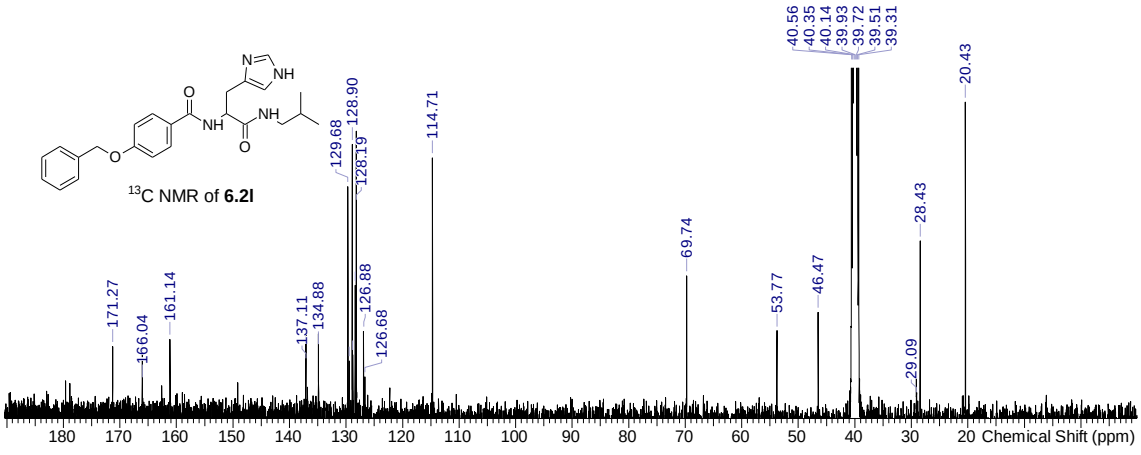
Appex–18. *^{13}C NMR spectra of benzyl 2–(N^{2}–(3–ethoxybenzoyl)–N^{ω}–nitroarginyl)hydrazine–1–carboxylate (**6c**).*



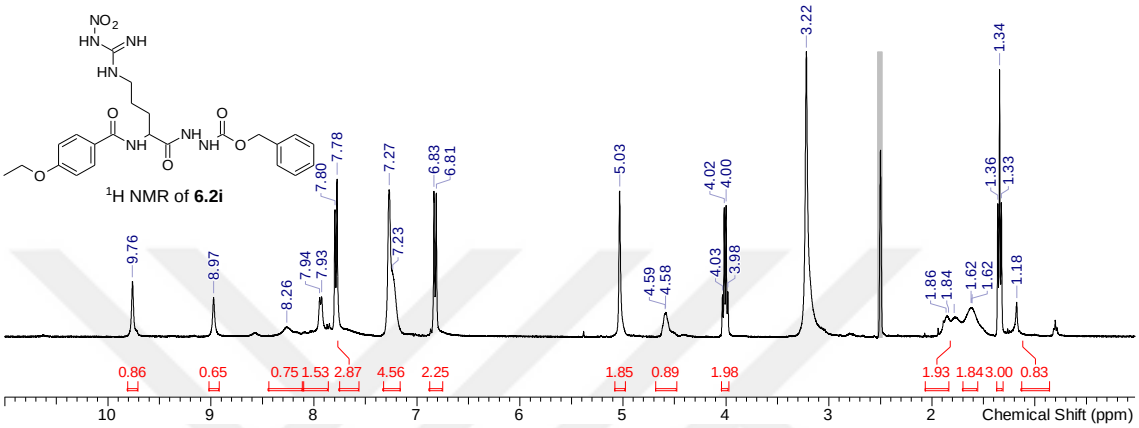
Appex–19. *[1]H NMR spectra of N–(3–(1H–imidazol–4–yl)–1–(isobutylamino)–1–oxopropan–2–yl)–4–(benzyloxy)benzamide (**6l**).*



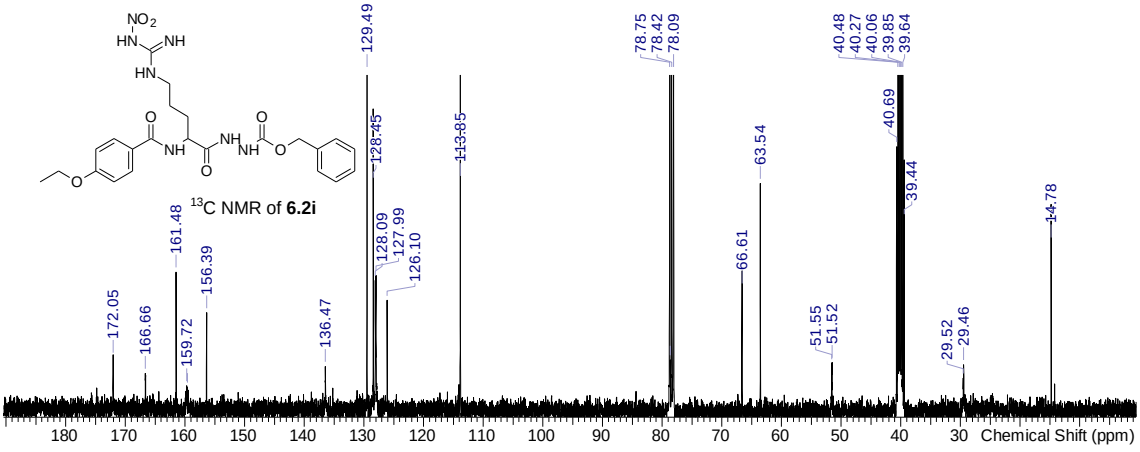
Appex–20. *[13]C NMR spectra of N–(3–(1H–imidazol–4–yl)–1–(isobutylamino)–1–oxopropan–2–yl)–4–(benzyloxy)benzamide (**6l**).*



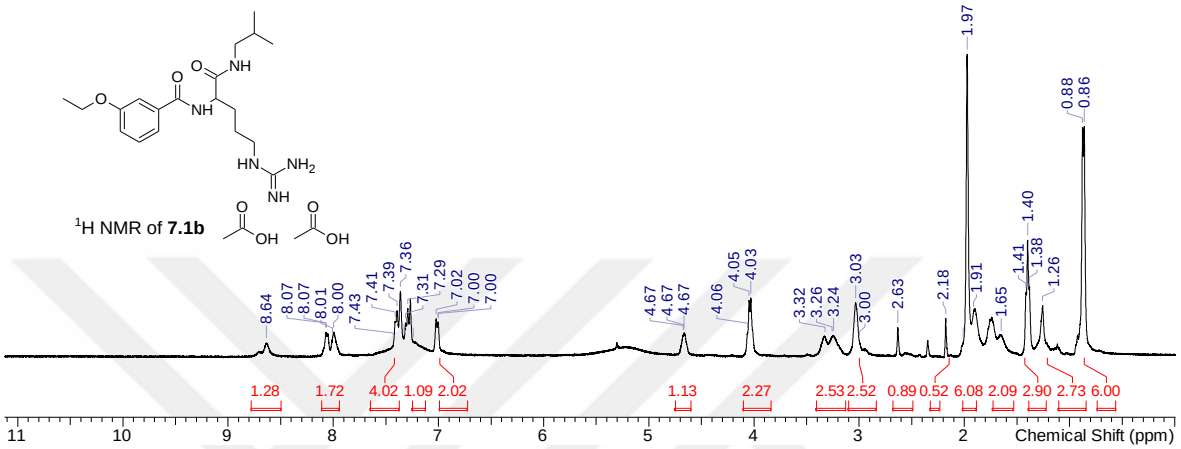
Appex–21. *^{1}H NMR spectra of benzyl 2–(N^{2}–(4–ethoxybenzoyl)–N^{ω}–nitroarginyl)hydrazine–1– carboxylate (**6i**).*



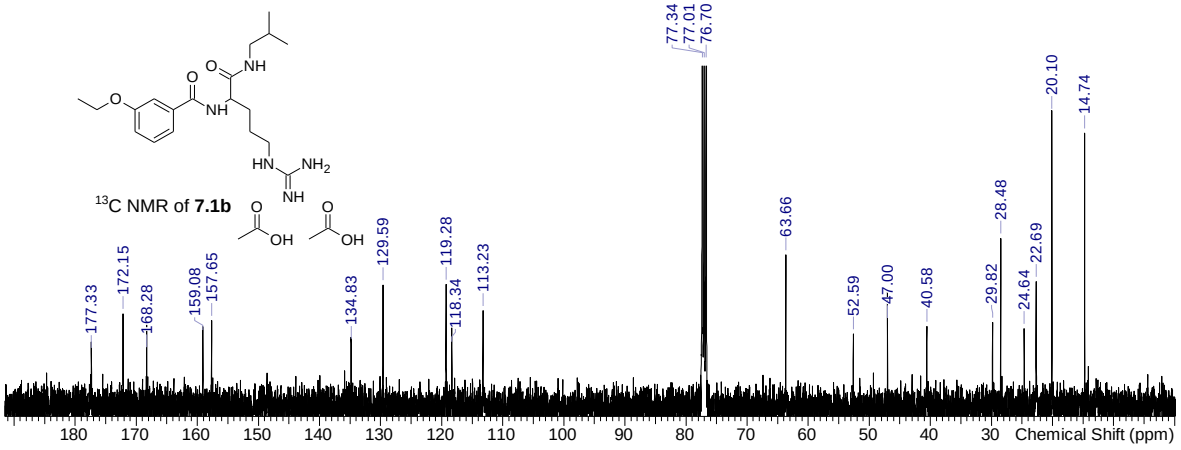
Appex–22. *^{13}C NMR spectra of benzyl 2–(N^{2}–(4–ethoxybenzoyl)–N^{ω}–nitroarginyl)hydrazine–1– carboxylate (**6i**).*



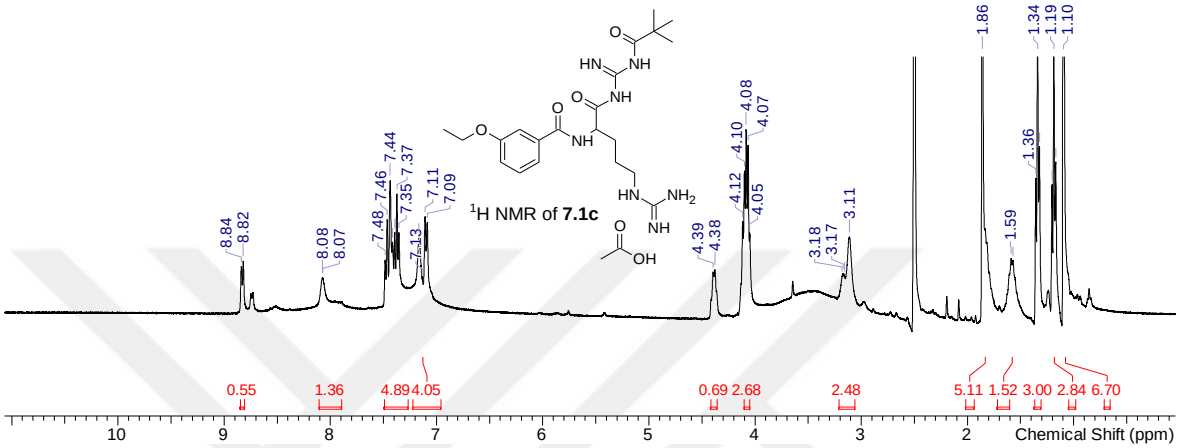
Appex–23. *[1]H NMR spectra of 3–ethoxy–N–(5–guanidino–1–(isobutylamino)–1–oxopentan–2–yl)benzamide (**7b**).*



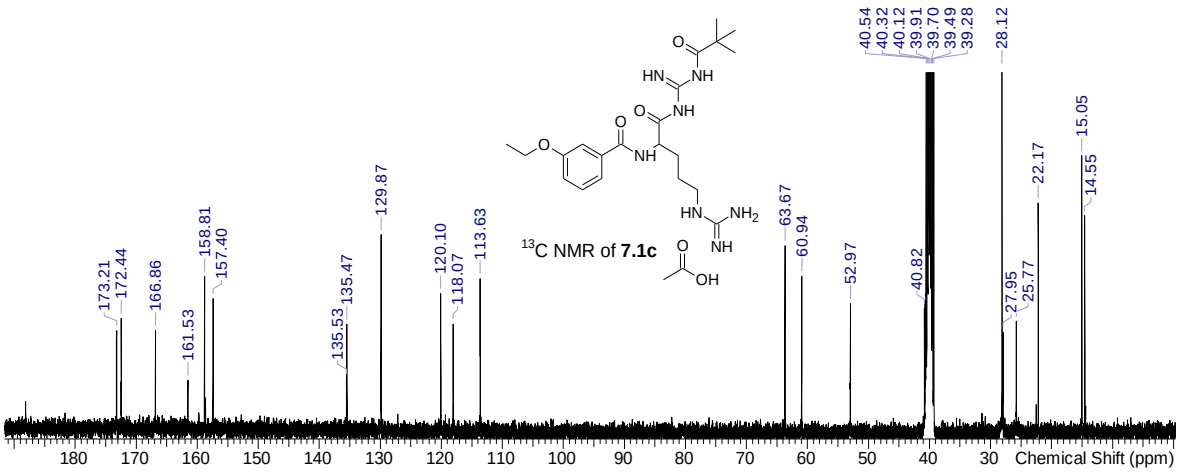
Appex–24. *[13]C NMR spectra of 3–ethoxy–N–(5–guanidino–1–(isobutylamino)–1–oxopentan–2–yl)benzamide (**7b**).*



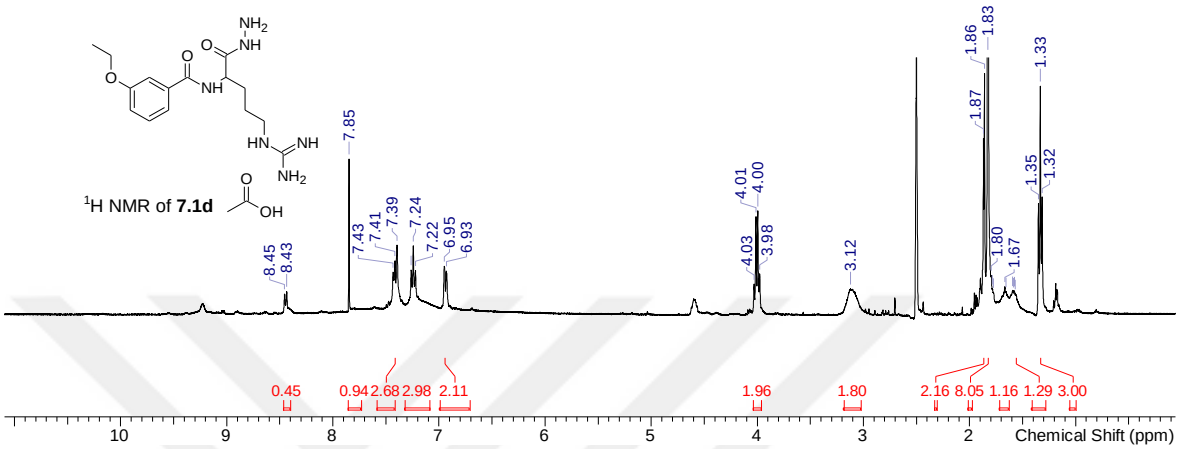
Appex–25. *[1]H NMR spectra of 3–ethoxy–N–(5–guanidino–1–oxo–1–(3–pivaloyl guanidino)pentan–2–yl) benzamide (**7c**).*



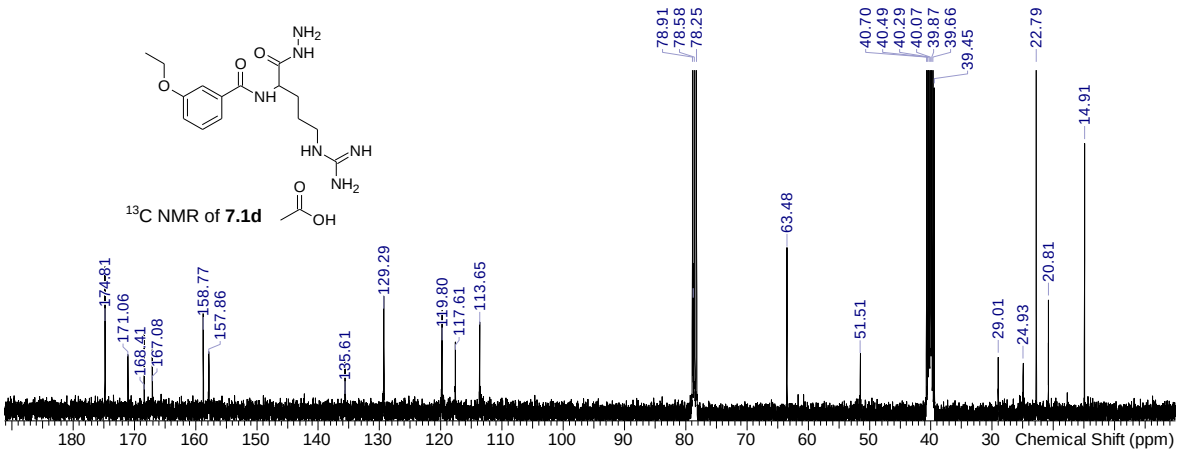
Appex–26. *[13]C NMR spectra of 3–ethoxy–N–(5–guanidino–1–oxo–1–(3–pivaloyl guanidino)pentan–2–yl) benzamide (**7c**).*



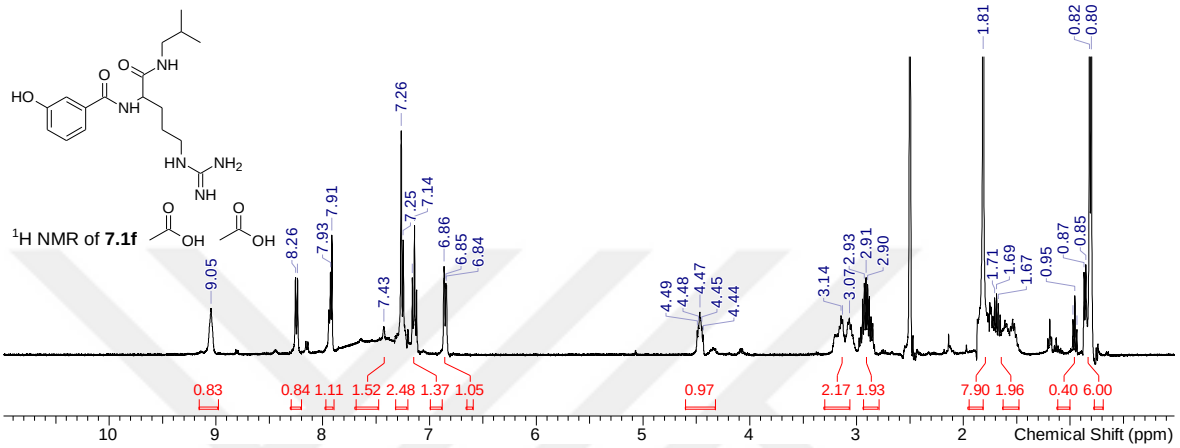
Appex–27. *[1]H NMR spectra of 3-ethoxy-N-(5-guanidino-1-hydrazinyl-1-oxopentan-2-yl)benzamide (**7d**).*



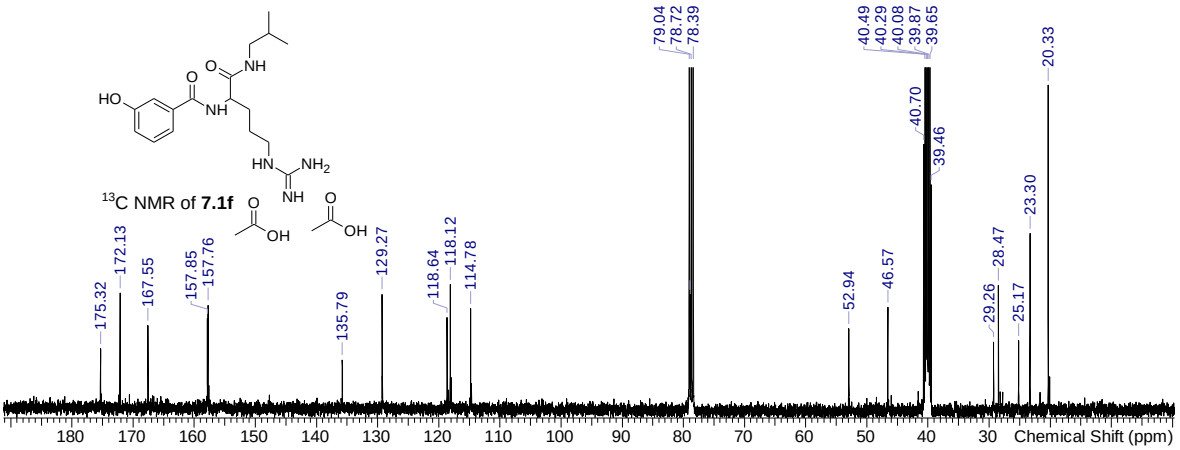
Appex–28. *[13]C NMR spectra of 3-ethoxy-N-(5-guanidino-1-hydrazinyl-1-oxopentan-2-yl)benzamide (**7d**).*



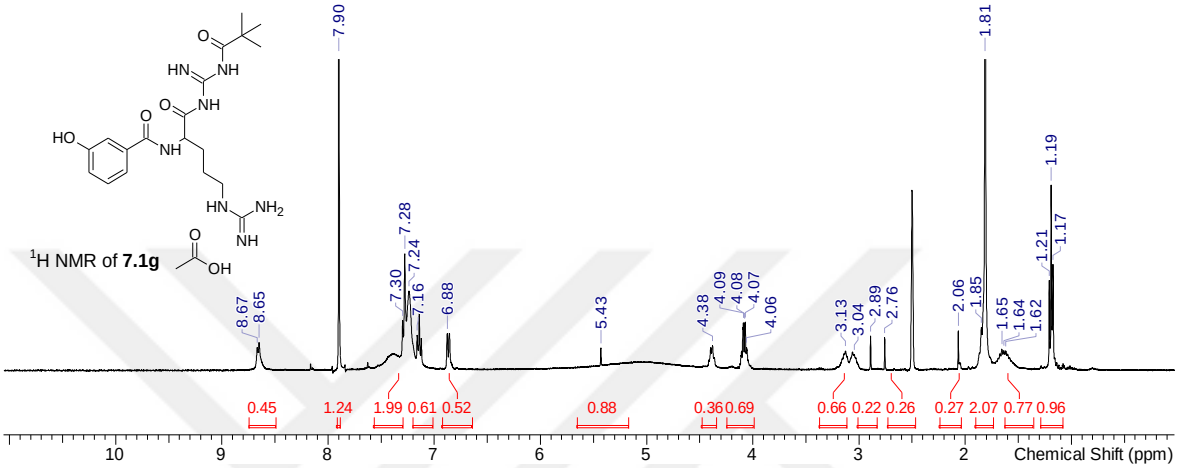
Appex–29. *[1]H NMR spectra of N–(5–guanidino–1–(isobutylamino)–1–oxopentan–2–yl)–3–hydroxybenzamide (**7f**).*



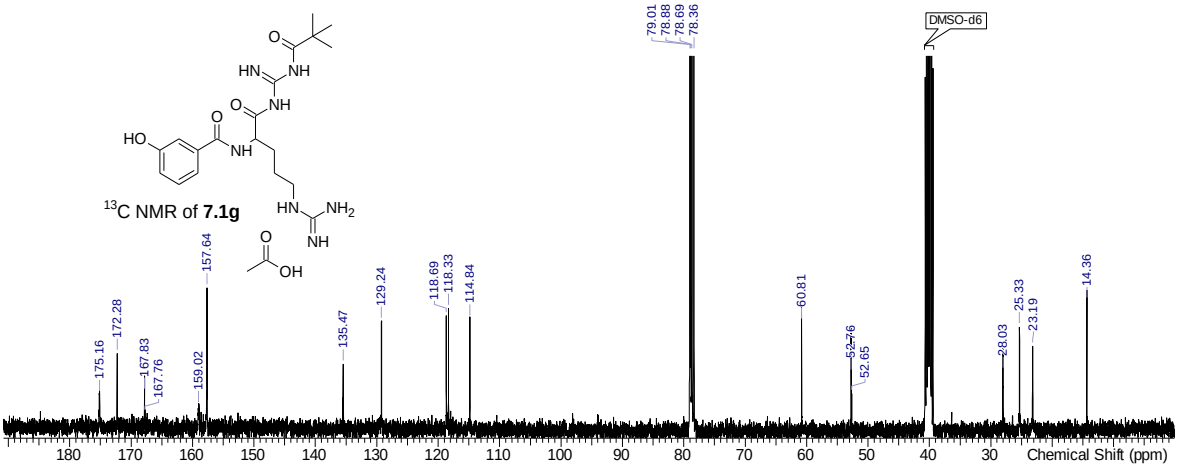
Appex–30. *[13]C NMR spectra of N–(5–guanidino–1–(isobutylamino)–1–oxopentan–2–yl)–3–hydroxybenzamide (**7f**).*



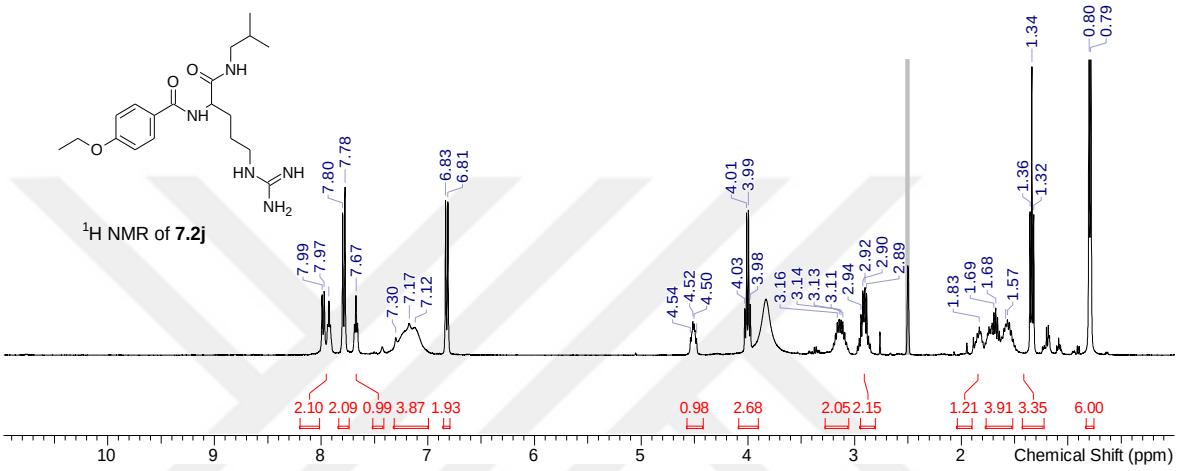
Appex–31. *[1]H NMR spectra of N–(5–guanidino–1–oxo–1–(3–pivaloyl guanidino)pentan–2–yl)–3–hydroxybenzamide* (**7g**).



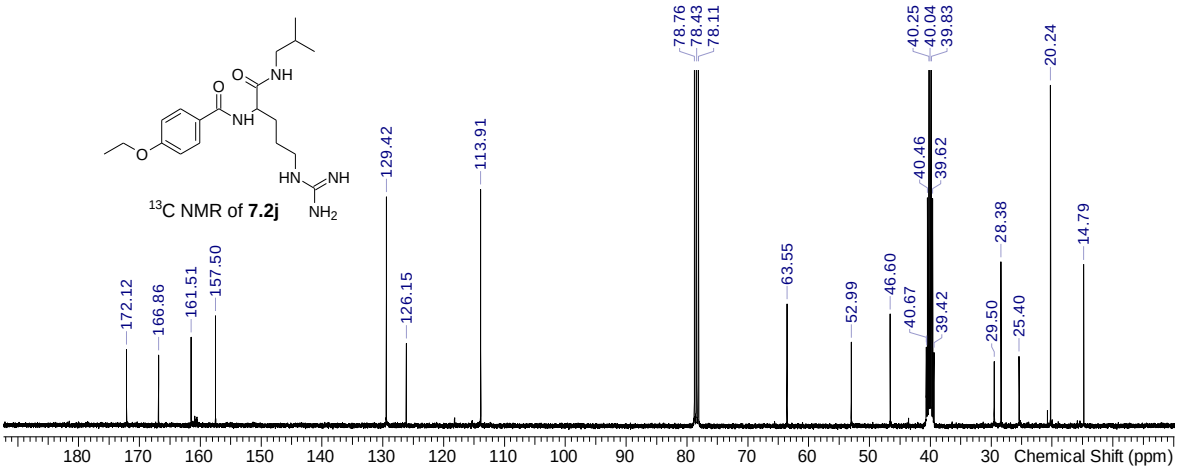
Appex–32. *[13]C NMR spectra of N–(5–guanidino–1–oxo–1–(3–pivaloyl guanidino)pentan–2–yl)–3–hydroxybenzamide* (**7g**).



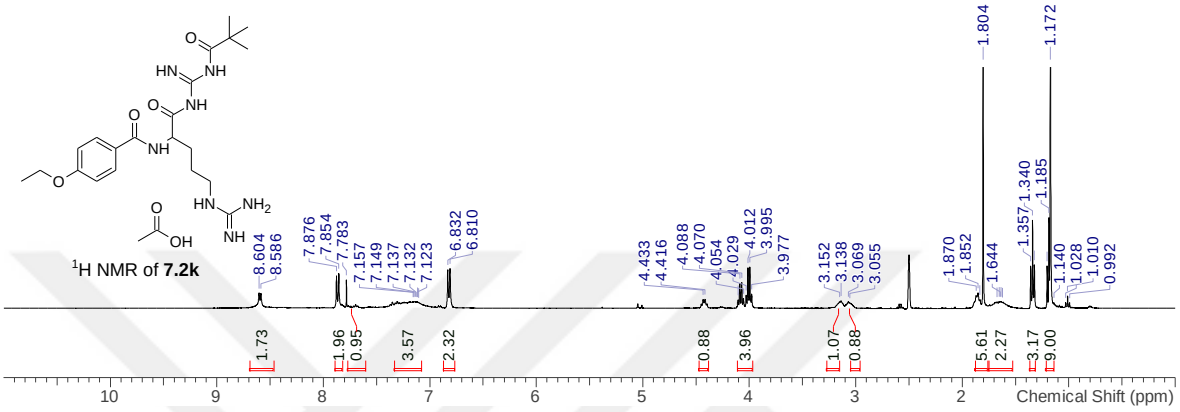
Appex–33. *[1]H NMR spectra of 4–ethoxy–N–(5–guanidino–1–(isobutylamino)–1–oxopentan–2-yl) benzamide (**7j**).*



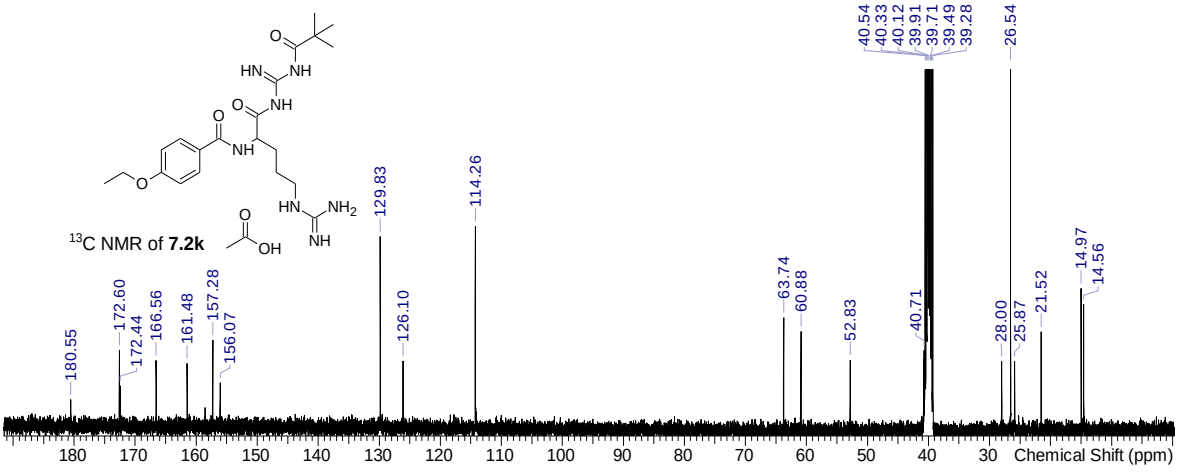
Appex–34. *[13]C NMR spectra of 4–ethoxy–N–(5–guanidino–1–(isobutylamino)–1–oxopentan–2-yl) benzamide (**7j**).*



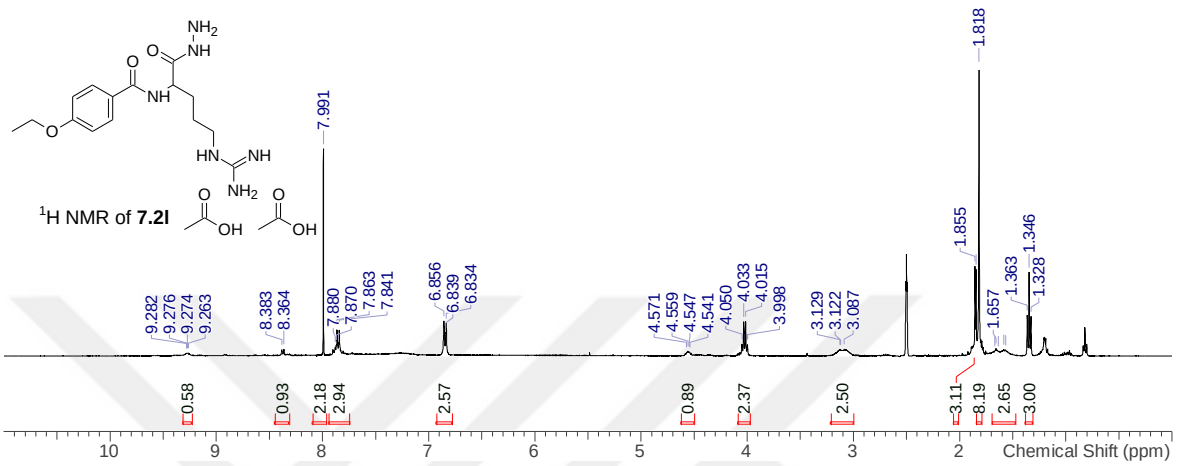
Appex–35. *[1]H NMR spectra of 4–ethoxy–N–(5-guanidino–1–oxo–1–(3–pivaloyl guanidino)pentan–2–yl) benzamide (**7k**).*



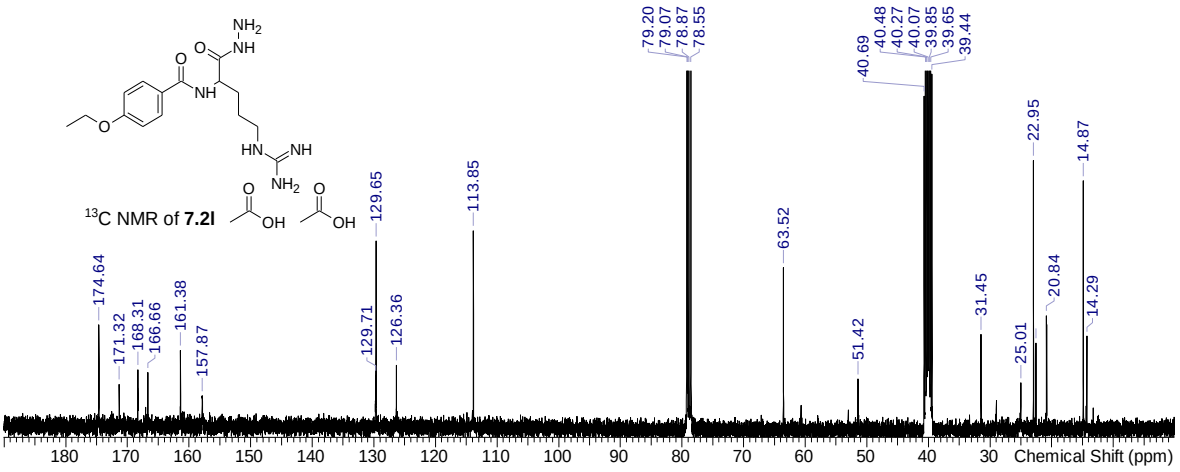
Appex–36. *[13]C NMR spectra of 4–ethoxy–N–(5-guanidino–1–oxo–1–(3–pivaloyl guanidino)pentan–2–yl) benzamide (**7k**).*



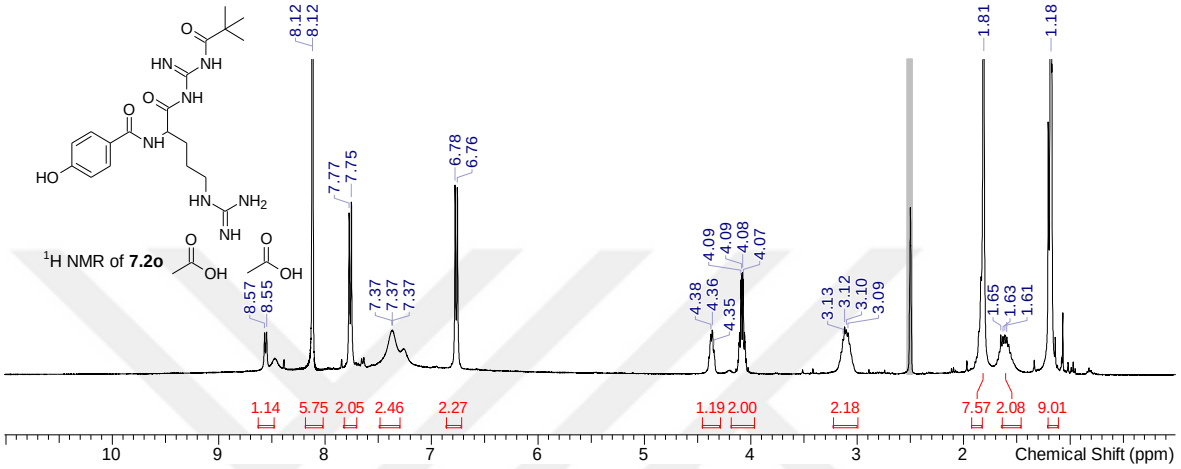
Appex–37. *[1]H NMR spectra of 4-ethoxy-N-(5-guanidino-1-hydrazinyl-1-oxopentan-2-yl)benzamide (**7l**).*



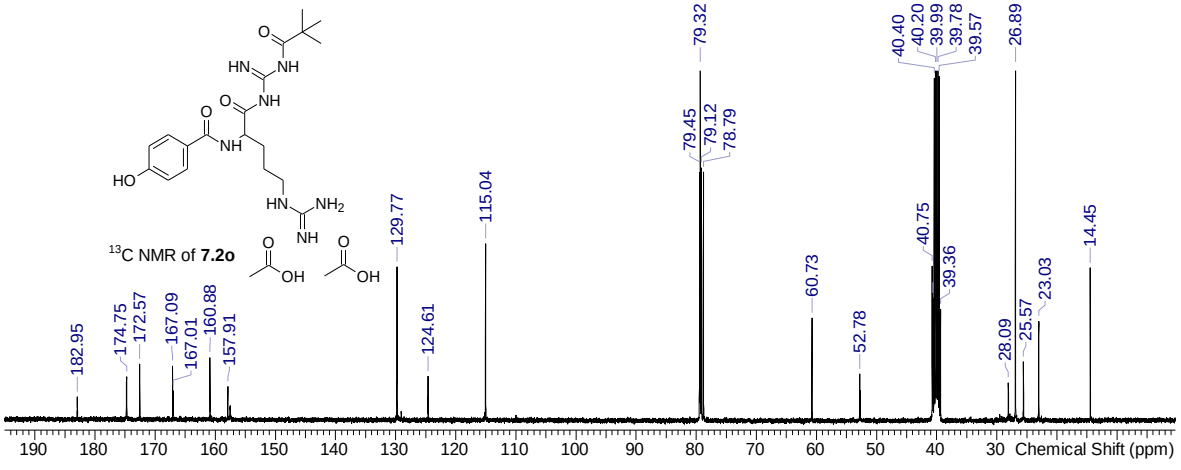
Appex–38. *[13]C NMR spectra of 4-ethoxy-N-(5-guanidino-1-hydrazinyl-1-oxopentan-2-yl)benzamide (**7l**).*



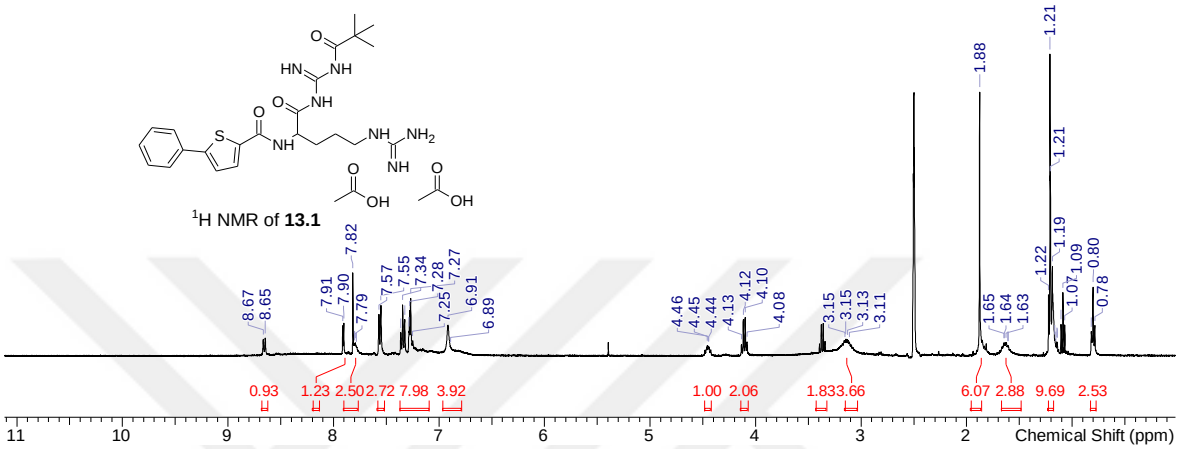
Appex–39. *^{1}H NMR spectra of N–(5–guanidino–1–oxo–1–(3–pivaloylguanidino)pentan–2–yl)–4–hydroxy benzamide (**7o**).*



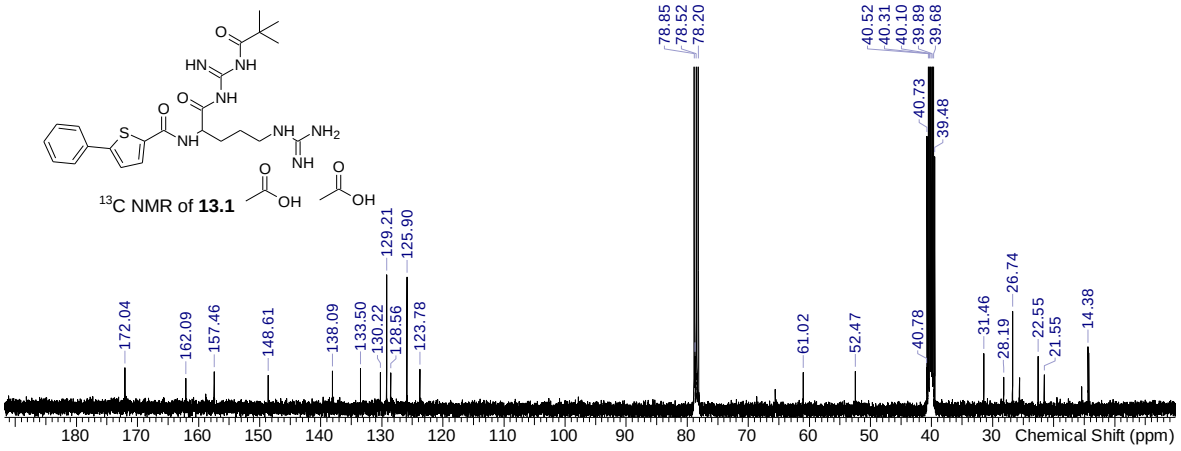
Appex–40. *^{13}C NMR spectra of N–(5–guanidino–1–oxo–1–(3–pivaloylguanidino)pentan–2–yl)–4–hydroxy benzamide (**7o**).*



Appex–41. *[1]H NMR spectra of N-(5-guanidino-1-oxo-1-(3-pivaloylguanidino)pentan-2-yl)-5-phenylthiophene-2-carboxamide (**16**).*



Appex–42. *[13]C NMR spectra of N-(5-guanidino-1-oxo-1-(3-pivaloylguanidino)pentan-2-yl)-5-phenylthiophene-2-carboxamide (**16**).*



Appex–43. *High-Resolution Mass spectrum of (3–ethoxybenzoyl)histidine (**5a**).*

Elmt	Val.	Min	Max
H	1	10	40
C	4	15	30
N	3	0	8

Elmt	Val.	Min	Max
O	2	1	6
F	1	0	0
P	3	0	0

Elmt	Val.	Min	Max
S	2	0	0
Cl	1	0	0
Br	1	0	0

Elmt	Val.	Min	Max
Ru	2	0	0
Pd	2	0	0
I	3	0	0

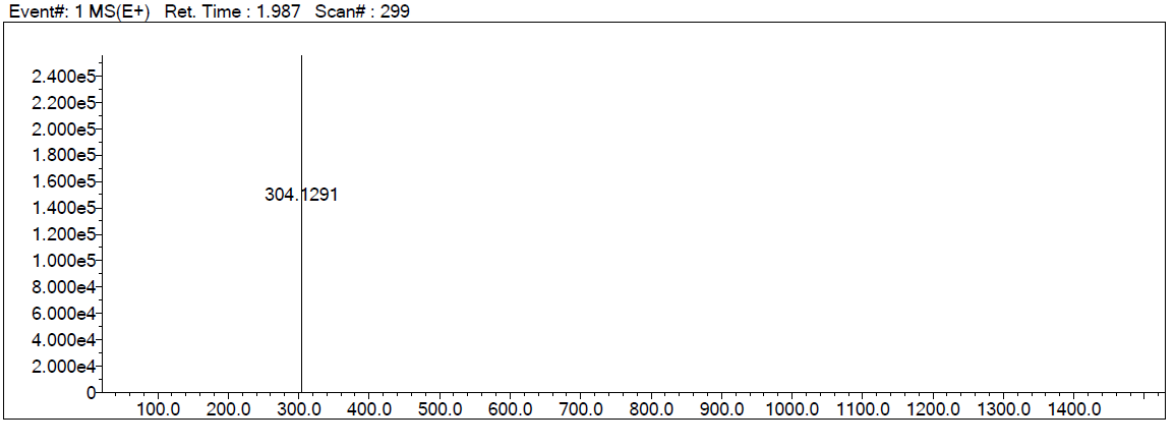
Use Adduct
H

Error Margin (ppm): 5
HC Ratio: unlimited
Max Isotopes: 3
MSn Iso RI (%): 10.00

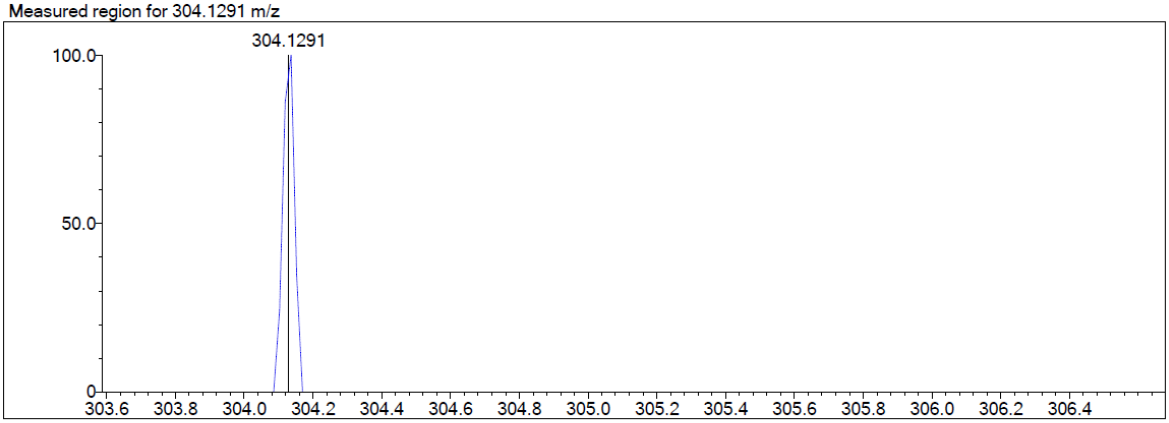
DBE Range: 5.0 - 25.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: yes
Isotope Res: 9000
Max Results: 500

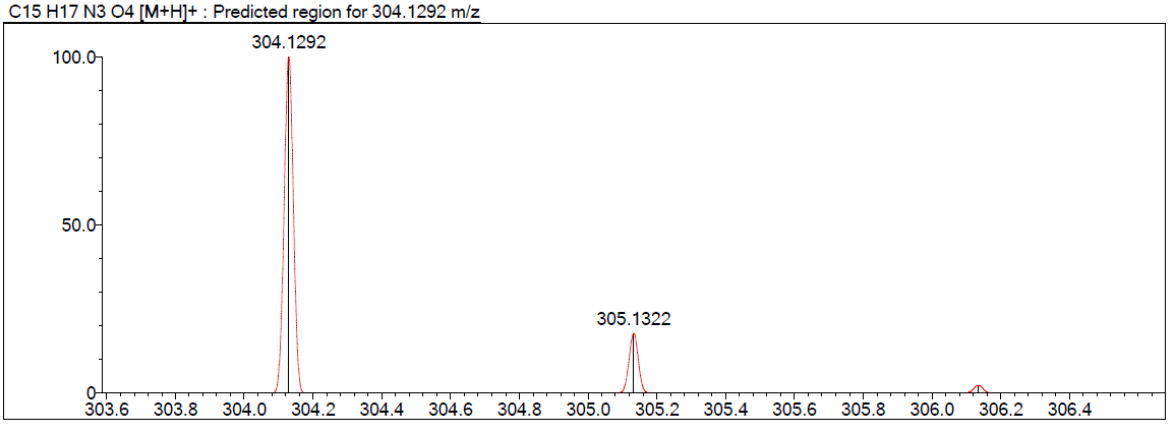
Event#: 1 MS(E+) Ret. Time : 1.987 Scan# : 299



Measured region for 304.1291 m/z



C15 H17 N3 O4 [M+H]+ : Predicted region for 304.1292 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C15 H17 N3 O4	[M+H]+	304.1291	304.1292	-0.1	-0.33	0.00	9.0

Appex–44. *High-Resolution Mass spectrum of N^2–(4–ethoxybenzoyl)–N^{ω}–nitroarginine (**5f**).*

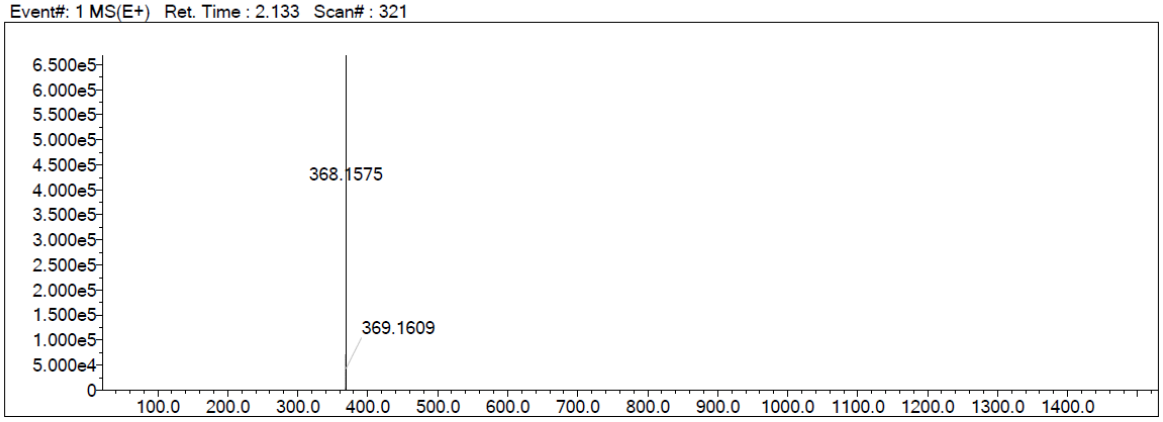
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	40	O	2	1	8	S	2	0	0	Ru	2	0	0	H
C	4	15	30	F	1	0	0	Cl	1	0	0	Pd	2	0	0	
N	3	0	5	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5
HC Ratio: unlimited
Max Isotopes: 3
MSn Iso RI (%): 10.00

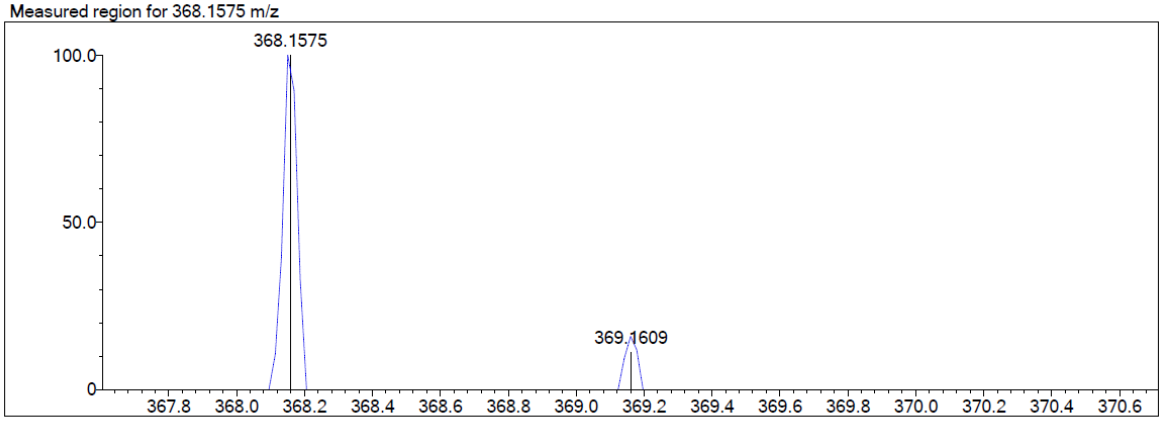
DBE Range: 5.0 - 25.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: yes
Isotope Res: 9000
Max Results: 500

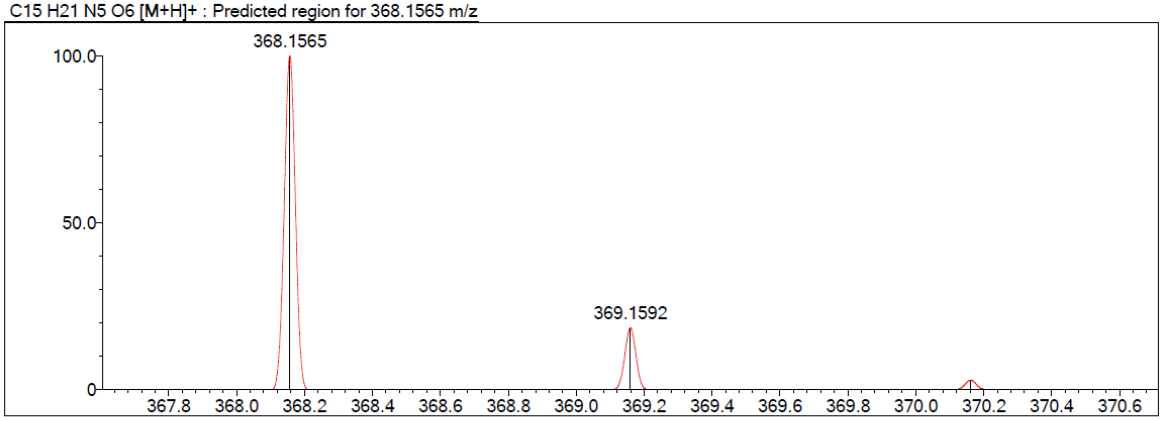
Event#: 1 MS(E+) Ret. Time : 2.133 Scan# : 321



Measured region for 368.1575 m/z



C15 H21 N5 O6 [M+H]+ : Predicted region for 368.1565 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	80.99	C15 H21 N5 O6	[M+H]+	368.1575	368.1565	1.0	2.72	84.62	8.0

Appex–45. *High-Resolution Mass spectrum of N^2–(4–(benzyloxy)benzoyl)–N^{ω}–nitroarginine* ***(5i).***

Elmt	Val.	Min	Max
H	1	10	40
C	4	15	30
N	3	0	8

Elmt	Val.	Min	Max
O	2	1	6
F	1	0	0
P	3	0	0

Elmt	Val.	Min	Max
S	2	0	1
Cl	1	0	0
Br	1	0	0

Elmt	Val.	Min	Max
Ru	2	0	0
Pd	2	0	0
I	3	0	0

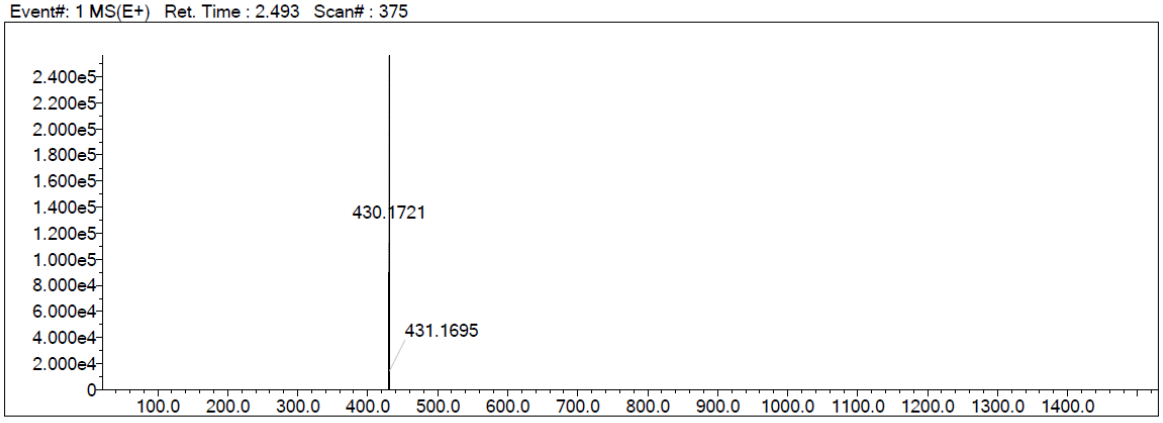
Use Adduct
H

Error Margin (ppm): 5
HC Ratio: unlimited
Max Isotopes: 3
MSn Iso RI (%): 10.00

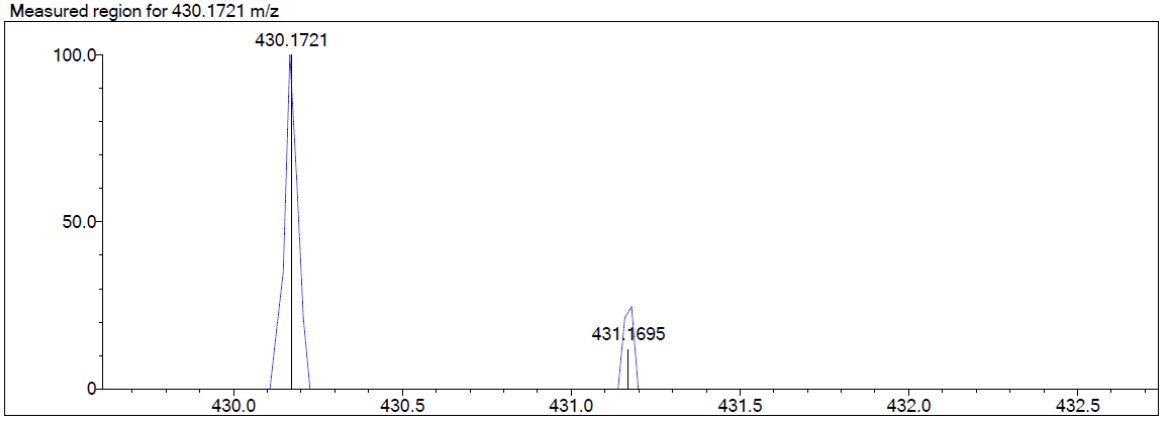
DBE Range: 5.0 - 25.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: yes
Isotope Res: 9000
Max Results: 500

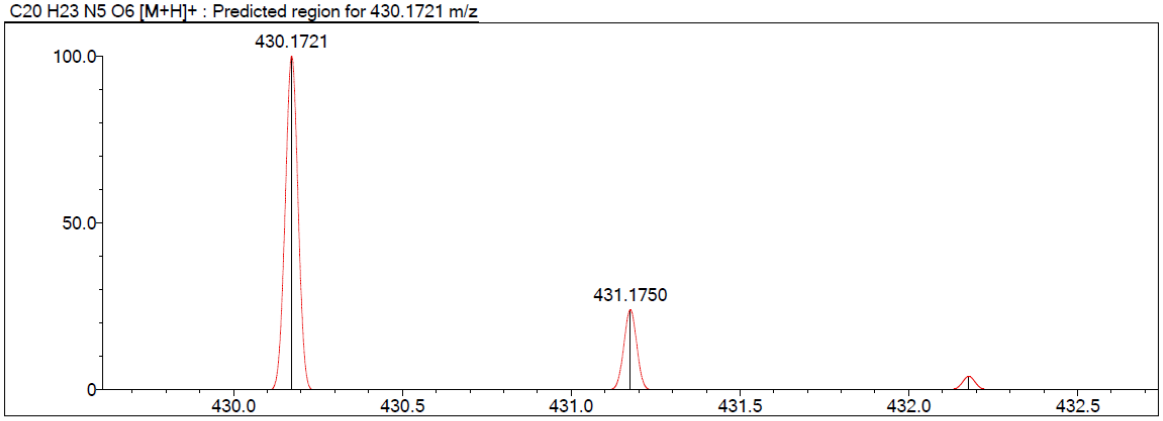
Event#: 1 MS(E+) Ret. Time : 2.493 Scan# : 375



Measured region for 430.1721 m/z



C20 H23 N5 O6 [M+H]+ : Predicted region for 430.1721 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	55.66	C20 H23 N5 O6	[M+H]+	430.1721	430.1721	-0.0	0.00	55.66	12.0

Appex–46. *High-Resolution Mass spectrum of 4–ethoxy–N–(5–(3–nitroguanidino)–1–oxo–1–(3–pivaloyl guanidino) pentan–2–yl)benzamide (**6h**).*

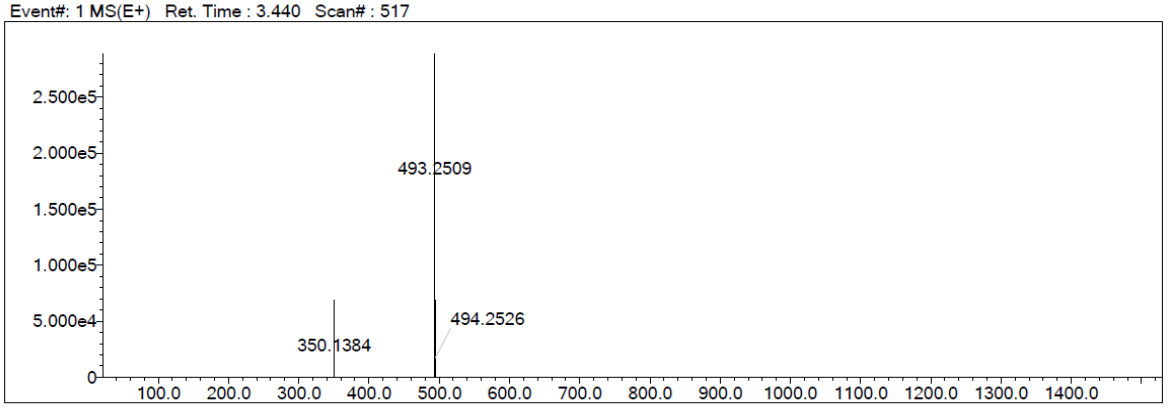
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	40	O	2	1	6	S	2	0	0	Ru	2	0	0	H
C	4	15	30	F	1	0	0	Cl	1	0	0	Pd	2	0	0	
N	3	0	8	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5
HC Ratio: unlimited
Max Isotopes: 3
MSn Iso RI (%): 10.00

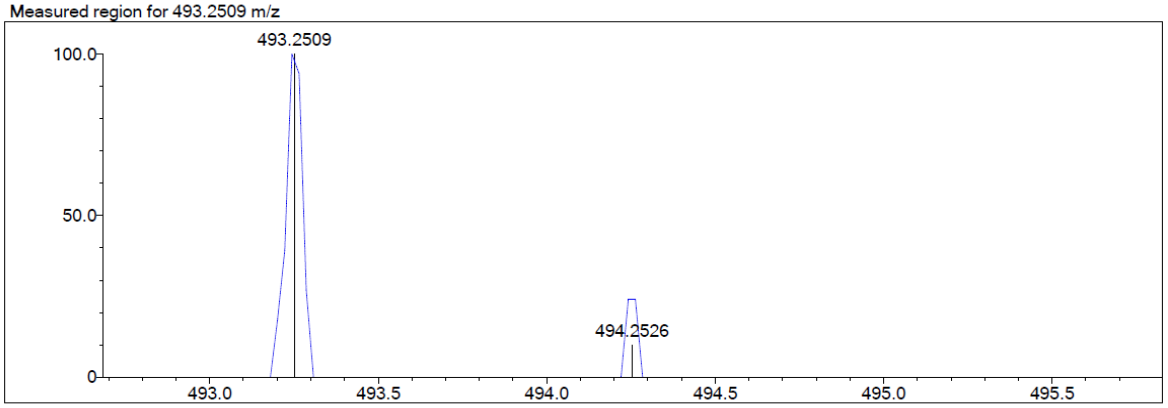
DBE Range: 5.0 - 25.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: yes
Isotope Res: 9000
Max Results: 500

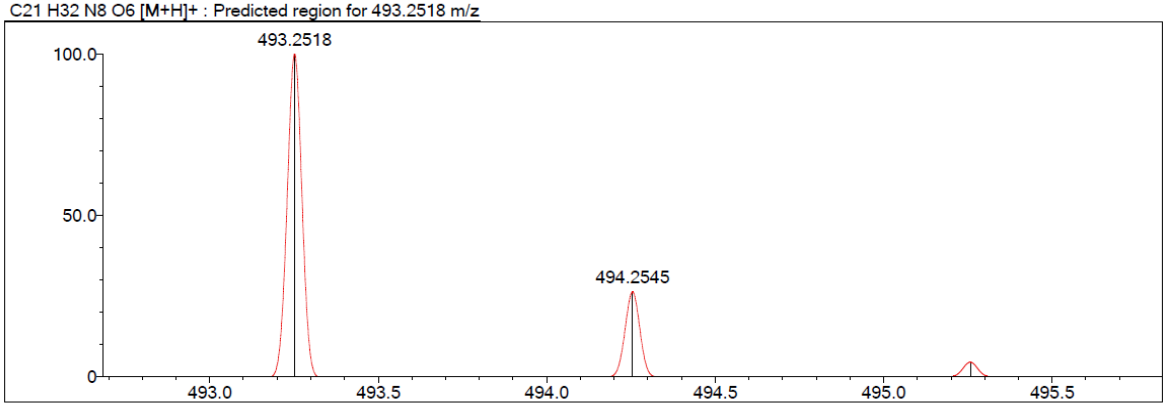
Event#: 1 MS(E+) Ret. Time : 3.440 Scan# : 517



Measured region for 493.2509 m/z



C21 H32 N8 O6 [M+H]+ : Predicted region for 493.2518 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	90.13	C21 H32 N8 O6	[M+H]+	493.2509	493.2518	-0.9	-1.82	92.01	10.0

Appex–47. *High-Resolution Mass spectrum of 4–(benzyloxy)–N–(5–(3–nitroguanidino)–1–oxo–1–(3–pivaloylguanidino) pentan–2–yl)benzamide (**6k**).*

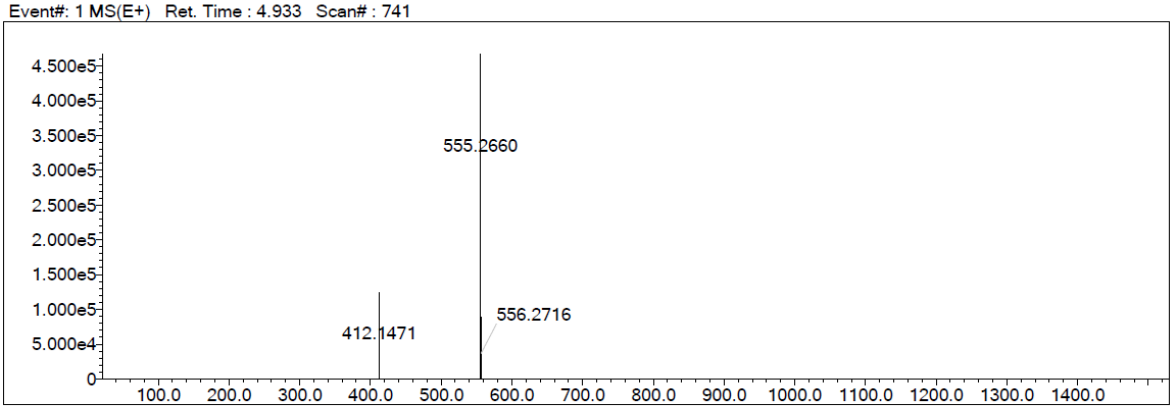
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	40	O	2	1	6	S	2	0	0	Ru	2	0	0	H
C	4	15	30	F	1	0	0	Cl	1	0	0	Pd	2	0	0	
N	3	0	8	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5
HC Ratio: unlimited
Max Isotopes: 3
MSn Iso RI (%): 10.00

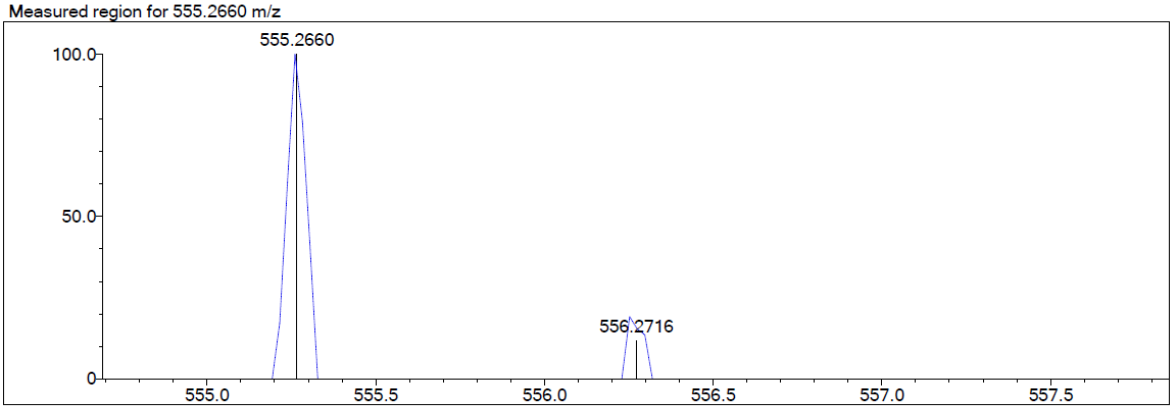
DBE Range: 5.0 - 25.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: yes
Isotope Res: 9000
Max Results: 500

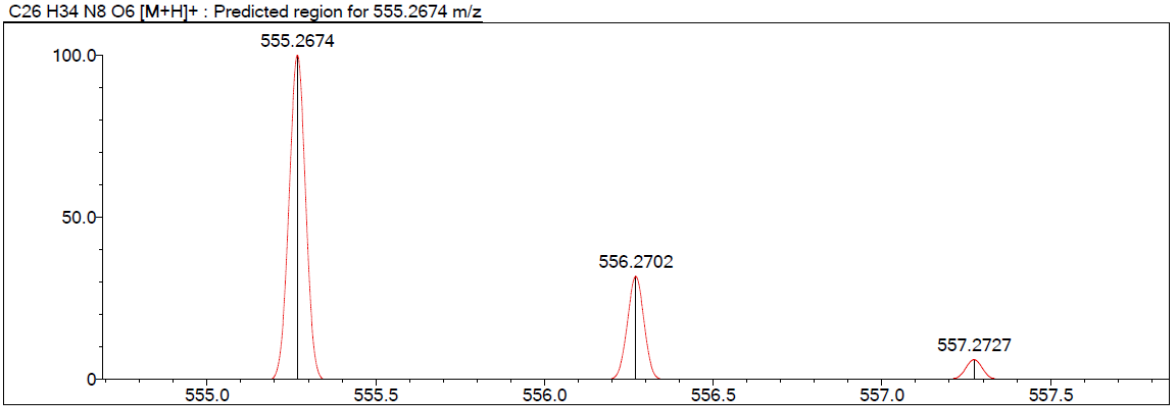
Event#: 1 MS(E+) Ret. Time : 4.933 Scan# : 741



Measured region for 555.2660 m/z



C26 H34 N8 O6 [M+H]+ : Predicted region for 555.2674 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	37.53	C26 H34 N8 O6	[M+H]+	555.2660	555.2674	-1.4	-2.52	39.01	14.0

Appex–48. *High-Resolution Mass spectrum of N–(5–guanidino–1–(isobutylamino)–1–oxopentan–2–yl)–4–hydroxy benzamide (**7n**).*

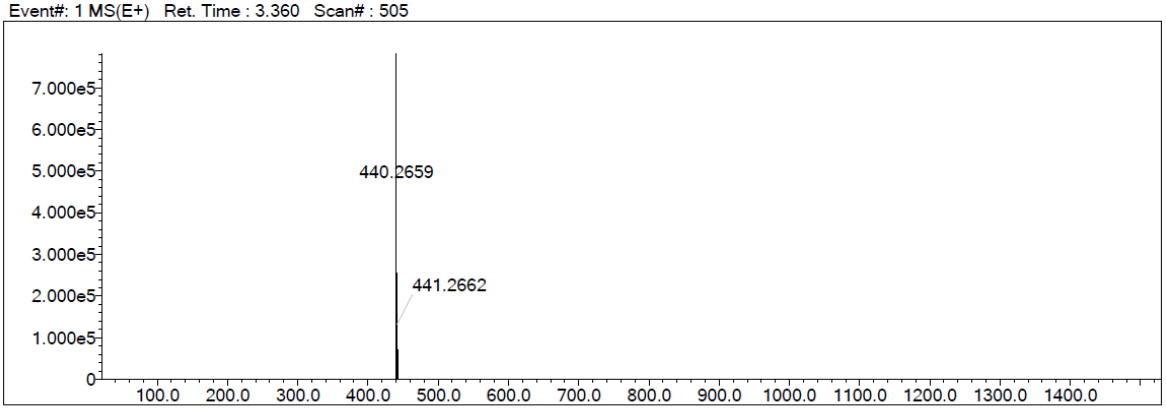
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	40	O	2	1	6	S	2	0	0	Ru	2	0	0	H
C	4	15	30	F	1	0	0	Cl	1	0	0	Pd	2	0	0	
N	3	0	8	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5
HC Ratio: unlimited
Max Isotopes: 3
MSn Iso RI (%): 10.00

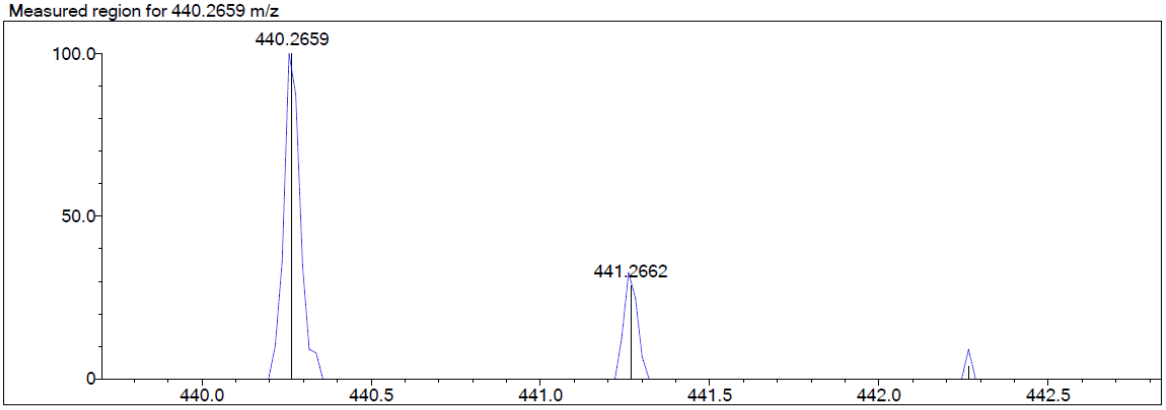
DBE Range: 5.0 - 25.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: yes
Isotope Res: 9000
Max Results: 500

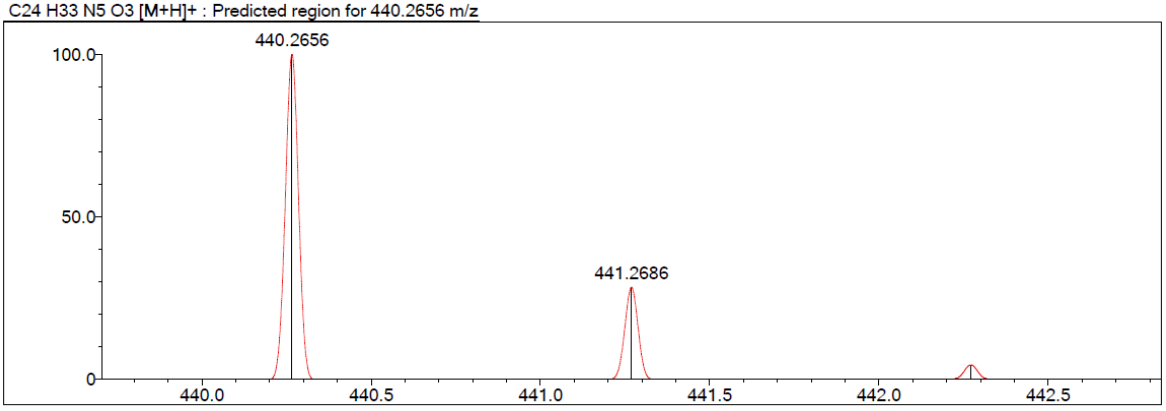
Event#: 1 MS(E+) Ret. Time : 3.360 Scan# : 505



Measured region for 440.2659 m/z



C24 H33 N5 O3 [M+H]+ : Predicted region for 440.2656 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	74.70	C24 H33 N5 O3	[M+H]+	440.2659	440.2656	0.3	0.68	74.70	11.0

CURICULUM VITAE

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EDUCATION BACKGROUND;

Award	Department	Institution	Year
Master's degree	Organic Chemistry	Anadolu University	2016-2019
Bachelors dergree	Chemical Engineering	University of Prishtina "Hasan Prishtina"	2013-2016
High school	Sciences	"Gjergj Kastrioti Skenderbeu"	2010-2013