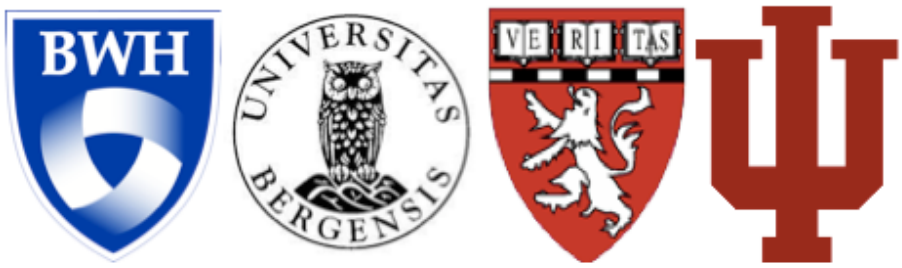
Synthesis of substituted imidazoles that stabilizes Survival Motor Neuron Protein for treatment of Spinal Muscular Atrophy

Maren Heskestad Clementsen



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Maren Heskestad Clementsen

Abstract

Spinal muscular atrophy (SMA) is a common autosomal recessive disorder and the leading cause of infant mortality. Patients with this disorder are missing the SMN1 gene that is producing 80-90 % of the Spinal motor neuron (SMN) protein. The SMN2 gene is not able to produce the full-length protein, due to a small difference in the splicing pattern. This leads to loss of muscle function and physical disability.

Different treatments ave been performed on patients with SMA, and one of them is to increase the SNM2 gene. In 2012 Androphy *et al.*, in collaboration with Dr. Hodgetts at the LDDN, discovered two hit compounds that increase the SMN protein. Based on one of these compounds, the research group at LDDN optimized the molecule and produced a better probe for the treatment of SMA. In this project, the synthesized probe will be optimized by changing the heterocycle, with the same substituents and amide linker.

Different synthesis has been performed, before reaching the target molecule. Amidation with acid chloride and an amine is the main reaction leading to the target molecule. In addition to the target molecule, different analogs have also been made. The substituents on the heterocycle and the amide linker have been modified, leading to several new compounds.

All of the compounds that were synthesized, were also sent for testing at the Department of Dermatology, Indiana University School of Medicine. The main reason for the testing is to see if the compounds are active or not in the treatment for SMA. The % activation and the EC_{50} value for each compound in mouse models were determined. Based on the previously synthesized molecule, the target molecule was expected to be active. This turned out to be wrong, and the target molecule was inactive. However, one of the other compounds showed good activity.

Abbreviations

μw	Microwave
DCM	Dichloromethane
DIH	N,N'-diiodo-5,5-dimethylhydantoin
EC_{50}	Halv-maximum respons consentration
ESI	Electron spray ionisation
GC	Gas chromatography
h	Hour(s)
HPLC	High performance liquid chromatography
LC	Liquid chromatography
LDDN	Laboratory for Drug discovery in Neurodegeneration
m/z	Mass to charge ratio
MS	Mass spectrometry
NA	Not active
NC	No conversion
NMR	Nuclear magnetic resonance
ppm	Parts per million
RT	Room temperature
SAR	Structure-activity relationship
SMA	Spinal muscular atrofi
SMN	Suvival motor neuron
TEA	Triethylamine
THF	Tetrahydrofuran
TLC	Thin layer chromatography
Tos	Tosyl group

Contents

1 Introduction

1.1 Spinal muscular atrophy (SMA)

Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disorder characterized by muscle weakness and eventual loss of motor function [1]. This disorder can occur in different forms and is caused by low levels of the Survival motor neuron (SMN) protein. The presence of this disease is most common in infancy or childhood, but can also be onset in adulthood and leading to different levels of physical disability [2].

Telomeric SMN1 and centromeric SMN2 are two nearly identical SMN genes that are found on chromosome 5q11-13 and produce the SMN protein that is important for the function of the nerves that control the muscles [3]. The normal population typically have two copies of the SMN1 gene and up to four copies of the SMN2 gene. SMA carriers are missing the two SMN1 copies or have a mutation in the gene. The mutation causes a sequence difference between the two genes and leads to a skipping of exon 7 during transcription. This results in the SMN2 gene producing a truncated, non-functional, and rapidly degrading unstable protein. A change in this gene causes reduced levels of the SMN protein and loss of muscle function.

The SMN1 gene is the main source of the SMN protein, and is responsible for producing 80-90 % of the protein, while SMN2 is responsible for 10-20 %, due to a small difference in the splicing pattern that makes it less functional [4]. Copies of SMN2 alone will therefore be unable to express the full-length protein and produce an unstable protein that will not generate full function of motor neurons [2]. Figure 1.1 shows the encode of the SMN protein and the difference between an SMN1 and SMN2 gene.

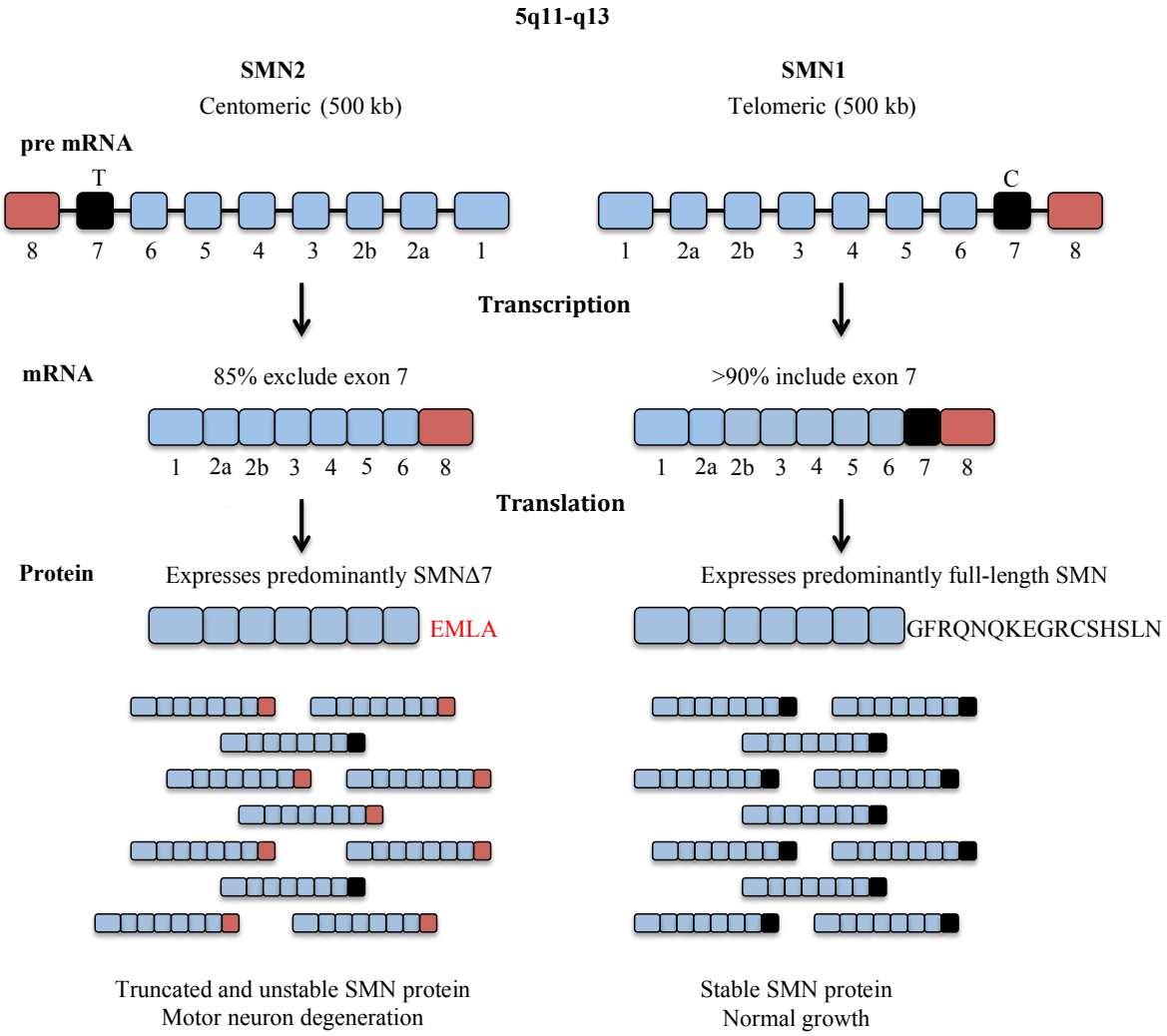


Figure 1.1: Encode of SMN protein, where you see the difference between the two SMN genes [6]

Based on the level of muscle function and the age of onset, SMA has been classified into four types [5]. The most severe form of SMA is called Type I and is one of the leading causes of infant mortality. Infants with this type will show muscle weakness within 6 months, and death often occurs within 2 years due to respiratory failure [3]. Type I patients only possess 2-3 copies of the SMN2 gene, while patients with Type II, possess 3-4 copies, and will show muscle weakness in early childhood. Type III and IV are in general a milder form of SMA and will not affect the patients before later adulthood, due to more copies of SMN2 [6].

There is no genetic way to cure SMA, but there are different treatments that have been performed on individuals. Physical treatments such as respiratory support, has been used to extend the life of a patient. It does not slow down the progression of the disease, but it can improve the quality of the life [4]. Since these types of methods do not stop or slow down the progression of SMA, different research groups are exploring methods to produce small molecules that can treat affected individuals. Spinraza is one example of a drug that increases the length of the SMN protein [7], but there is clearly need for more drug candidates.

1.2 Previous work

From the different treatments that have been performed on patients with SMA, therapeutic treatment focuses on the increase of the full-length protein. An increase in the SNM2 gene will increase the full-length protein [5]. This has been confirmed in mouse models, by introducing two copies of human SMN2 genes to the mouse [8]. Even if the amount of SMN2 copies is a high-priority target for the increase of the full-length protein, there are other options. Some of these methods involve SMN1 gene replacement and motor neuron replacement with stem cell therapy [5]. Another option is to produce small molecules and compounds that post-transitionally stabilizes the SMN protein.

In 2012 Androphy *et al.* [9, 10] in collaboration with the LDDN, published an article where they discovered two hit compounds from a large screening of over 150,000 compounds. The two hit compounds had the best activity and increased the SMN protein. Figure 1.2 shows some of the different analogs that have been tested. Compound **LDN-75654** was one of the two hit compounds and was chosen for further characterization and optimization.

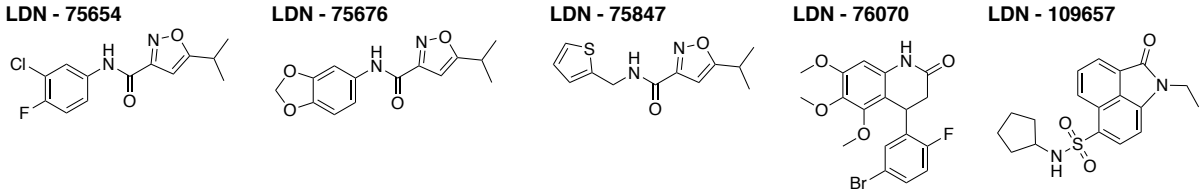


Figure 1.2: Different analoges synthesized and tested by Jonathan J. Cherry *et al.* [9]

Dr. Hodgetts and the research group at LDDN continued the optimization of **LDN-75654**. Figure 1.3 shows that **LDN-75654** has been optimized to a small molecule probe that has been synthesized (**LDN-27**). The amide bond was reversed and the heterocycle was changed to achieve this compound. This compound post-transnationally stabilizes the SNM protein and will increase the levels of the full-length SMN protein.

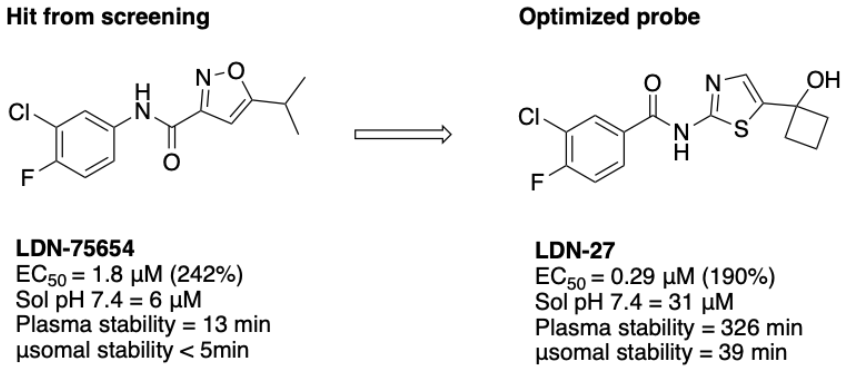


Figure 1.3: Hit compound and the optimized probe synthesized by Dr. Hodgetts *et al.* [3]

Figure 1.4 shows the % activation graphs for **LDN-75654** and **LDN-27**. This shows that both of the compounds are active and good options to increase the SMN protein for treatment of SMA.

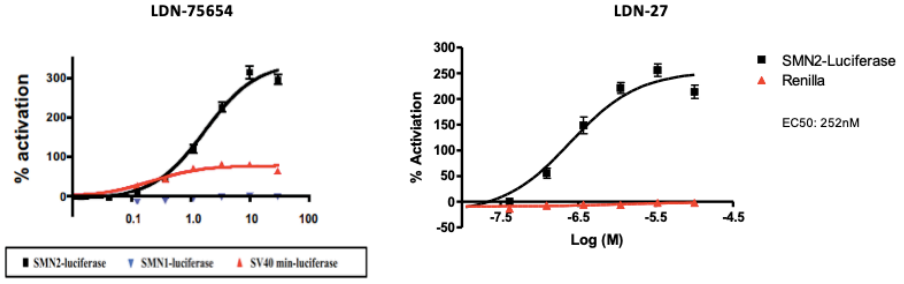


Figure 1.4: % activation graphs for **LDN-75654** and **LDN-27**

The optimization was based on a structure-activity relationship (SAR) study, where different analogs were synthesized and tested. Various substituents at the heterocycle have been tried, in addition to different groups attached to the carbonyl in the amide. An important functional group that was required for the activity was the amide linker. Several different heterocycles have been tried, with different substituents (Figure 1.5). Based on the result and the activity of the compounds, **LDN-27** (Figure 1.3) was the probe that Hodgetts *et al.* ended up with [3].

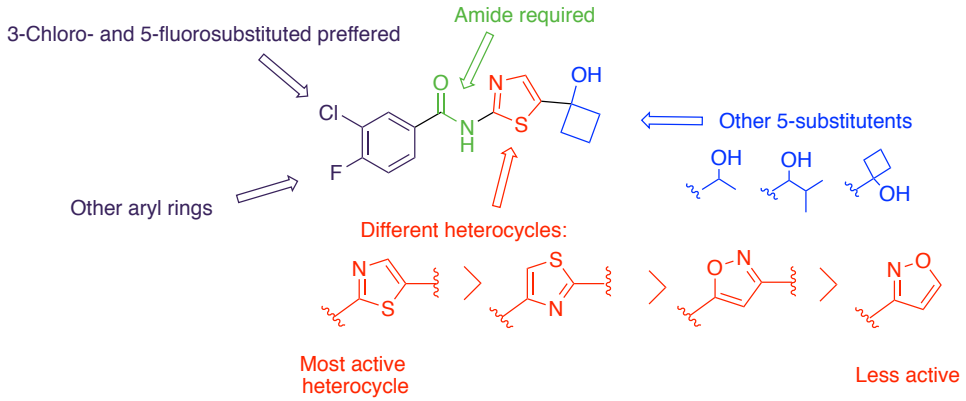


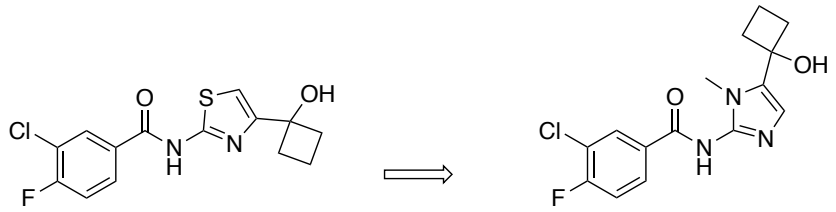
Figure 1.5: Different analogs from SAR study

Even though this compound showed good results in a mouse model of SMA, it will still need additional optimization to be used as a drug for SMA. Further optimization and new analogs were synthesized and tested in this project.

1.3 Aim of study

The aim of this project was to develop and synthesize analogs of the previously synthesized molecule (**LDN-27**) at LDDN (Figure 1.3, page 4). The goal was to develop new derivatives that will be tested for activity in the stabilization of SMN protein, for the treatment of SMA.

The new approach was to change the thiazol to a new heterocycle. Isoxazole and thiazole have been tried, but never imidazoles. The new analogs have an imidazole as the heterocycle and different substituents, both at the backbone and at the nitrogen in the imidazole, have been tried and synthesized. The goal was to optimize the molecule and create different analogs for testing. The molecule synthesized at LDDN (**LDN-27**), was optimized to the target molecule (**A**) of this project (Figure 1.6).



LDN-27 **A**

Figure 1.6: Previously syntesized molecule (**LDN-27**) and target molecule (**A**)

Imidazole and derivatives are important substances that are included in both biological and medicinal chemistry and can be a good approach for the new analogs. The imidazole ring and derivatives are found in several compounds that show biological activity and are of pharmaceutical importance [11]. Based on this, analogs with imidazole as the center of the compound may show activity in the stabilization of the SMN protein.

2 Theory of instrumentation

2.1 Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) has been used as an analytical instrument for many years and is today an important technique for both chemists, biochemists and physicists [12]. NMR is based on the nuclear magnetic field in the instrument. Radiofrequency waves are used to get information about the different magnetic and chemical environment around the NMR active nucleus [13]. This is used to find the composition and molecular structure of a component. ^{1}H and ^{13}C are the most common NMR active nuclei that are analyzed and relevant for this project [14].

Figure 2.1 shows a schematic diagram of a NMR spectrophotometer and how it operates. The sample is placed in a tube between the poles of a magnet and irradiated with radiofrequency waves from the generator. For all of the nuclei to come into resonance in different field strength, the frequency of the radiation is constant and the applied magnetic field is varied. A detector monitors the absorption of radiofrequency energy, and the signals are amplified and displayed as a peak in the NMR spectrum [15].

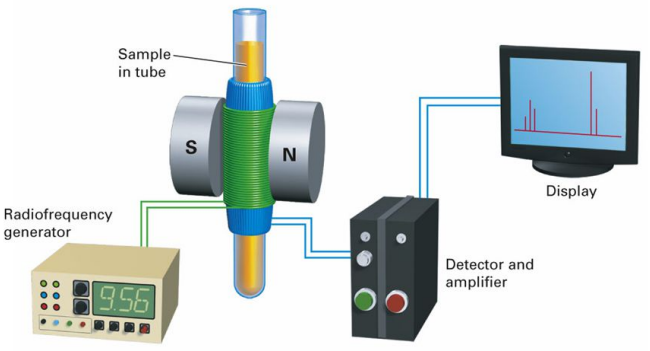


Figure 2.1: Shematic operation of a basic NMR spectrometer [15]

To record an NMR spectrum the nuclei in the molecule that is analyzed needs to have a spin. This is created when a positively charged nucleus, moves from one spin state to another due to the radiofrequency waves. This can only happen when a magnetic field

is applied, and the nucleus interacts with this field [13]. This means that the spherical nucleus rotates around their own axis and produce a nuclear spin.

If a nucleus is placed in a static magnetic field, B_o, a nuclear spin will be produced. This creates different energy levels, depending on the different number of spins each nucleus have. Transitions between these levels can be induced by irradiating the nucleus with electromagnetic waves of the appropriate frequency, which corresponds with the difference in energy levels [16]. The transition between two levels corresponds to the absorption of energy. Absorption occurs between the transition from the lower level to the higher level, and the nucleus will be in resonance with the applied radiation [15].

All ^{1}H and ^{13}C nuclei in a molecule do not absorb energy at the same frequency. This leads to a different NMR spectrum for each molecule, which is why NMR analyzes is so useful. Electrons surround the nucleus in all molecules, and these electrons will move around when the magnetic field is applied. The electrons make their own local magnetic field and make the nucleus shielded by the external field. Each nucleus in a molecule is surrounded by different electronic environments, and will therefore be shielded to a different extent. The difference between the applied magnetic field and the local magnetic field can be detected. The detected absorption can be amplified and displayed as an NMR spectrum [15].

For each ^{1}H and ^{13}C nucleus in a molecule, there is a distinct NMR signal, based on the unique absorption. The exact position of each signal is called a chemical shift. Based on the electronic environment, different ^{1}H and ^{13}C nuclei have different chemical shifts. Nuclei that are more shielded from surrounding electrons require higher field strength for resonance and will absorb in the upfield part of the NMR spectrum. For nuclei that are less shielded, the required field strength for resonance is lower and will absorb in the downfield part of the NMR spectrum [15].

2.2 Masspectroscopy

Mass spectroscopy (MS) is a powerful technique to determine the molecular weight of a compound. In some instruments, it is possible to measure the mass of the fragments produced when molecules are broken apart through the system. The mass of the fragments can then be used to determine the exact structure of a molecule.

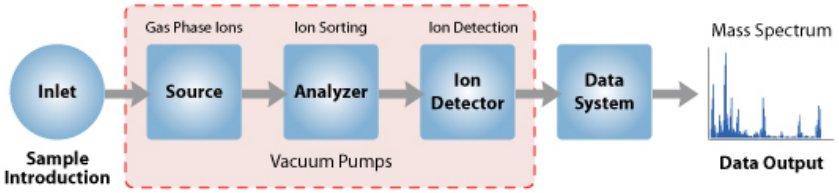


Figure 2.2: Shematic operation of a basic mass spectrometer [17]

The most common mass spectrometers consist of an ionization source, a mass analyzer and a detector. In addition to this, there is a sample inlet and a data system that display the mass spectrum. Figure 2.2 shows the schematic operation of a mass spectrometer. The most used ionization sources are Electron Impact (EI), Electrospray Ionization (ESI) and Matrix-Assisted Laser Desorption Ionization (MALDI). The main function of the ionization source is to bombard the sample with high-energy electrons, which ionizes the molecule and creates different fragments. The fragments will then be extracted into the mass analyzer and separated according to their mass to charge ratio (m/z). The most common mass analyzer are magnetic sector, Time-of-flight (TOF) and Quadrupole (Q). A detector will then detect the separated ions, and the signals are sent to a data system, where the m/z ratios are stored together with their relative abundance. Based on the m/z ratios the ions produce different peaks with different relative abundances. The number of charge on each ion is normally 1, which means that the value of m/z for each ion represents the mass of the molecule or fragment [15].

In some instances where you only need the mass of a compound, ESI as the ionization source is most common. Mostly one ion of each molecule in a sample is produced, which is very useful in synthesis, where you often have other compounds than the desired product. This can then be used to determine the composition of your sample.

2.3 Microwave

The use of microwave heating in chemistry is an increasingly more common approach to drive a chemical reaction. The main reason for this is that the rapid and appropriate heat increases the rate of the reaction significantly [18]. Another advantage of microwave heating is that the reaction time is reduced. Reactions that take days can be carried out in just a few hours (h) in a microwave [19].

The irradiation from a microwave is electromagnetic irradiation, and for chemical synthesis the most common frequency is at 2.45 GHz, corresponding to a wavelength of 12.25 cm. The energy produced by the microwave photon at this frequency is too low to cleave molecular bonds. This means that, based on direct absorption of electromagnetic energy, the microwave can not induce chemical reactions [19]. A solution to this is to use a solvent or a reagent that absorbs microwave energy and converts it into heat [20]. Each solvent converts a different amount of electromagnetic energy, due to different dielectric characteristics. How much of the microwave energy that is absorbed depends on the degree of penetration of microwaves in the solvent [20]. The more polar the reaction mixture is, the greater it's ability to absorb microwave energy. The loss tangent of a solvent is a factor that tells you how efficient the electrical energy is converted into heat. The higher the factor gets, the more energy is converted into heat. The loss tangent of 12 common solvents is listed in Table 2.1 [21].

Table 2.1: Tan δ for 12 common solvents (measured at RT and 2.45 GHz) [21]

Solvent	**Tan δ**	**Solvent**	**Tan δ**
Ethylene Glycol	1.350	Water	0.123
Ethanol	0.941	Acetonitrile	0.062
2-propanol	0.799	Acetone	0.054
1-propanol	0.757	THF	0.047
Methanol	0.659	Toluen	0.040
1-butanol	0.571	Hexane	0.020

2.4 Flash column chromatography

Column chromatography is an intelligent and important purification and separation method used in organic synthesis. The traditional and manual column chromatography can be very time consuming, but the automated flash column chromatography speeds up the process (Figure 2.3). The principle is based on pressurized gas that forces the solvent and sample through the column. The flash column system often uses an ultraviolet (UV) or infrared (IR) detector to detect the different peaks and analyze a sample [22].

Figure 2.3: Instrumentation of flash column chromatography

The most common stationary phase in the column is silica particles, but columns packed with other particles can also be used. Based on the polarity of the sample, different solvent systems can be used. Dichloromethane (DCM)/methanol and hexane/ethyl acetate are some of the common mixtures of solvent, where one of the solvents has higher polarity than the other. Higher polarity of the solvent increases the rate of elution of the compounds in the sample that are being separated. The sample that is analyzed should have a thin layer chromatography (TLC) R_f value of 0.15 to 0.20, in the solvent system that is used in the flash column [22].

When the solvent is pushed through the column, all the compounds that absorb UV-light will show as a peak in the display. Different compounds come out at different times based on polarity, and for each compound, a peak will be made and lead to separation. The different peaks can then be checked by TLC or LC-MS to confirm the identity of the product. In this way, the different compounds in a sample are separated and purified.

2.5 High performance liquid chromatography

High-Performance Liquid Chromatography (HPLC) is an analytical instrument that separates compounds and can identify each product in a mixture by using chromatographic principles. Reverse-phase chromatography is the most common method, where the column is non-polar and the solvent is more polar. The most common column is a C18 column, that is packed with silica particles [23].

If the mobile phase is a mixture of water and an organic solvent, the elution strength of the mobile phase will increase with an increasing percentage of organic solvent. When the percent of organic solvent is increasing during a run, it is called a gradient. Different compounds use different time through the column, based on the gradient and the polarity of each compound [23]. Figure 2.4 shows the instrumentation of a HPLC instrument [24].

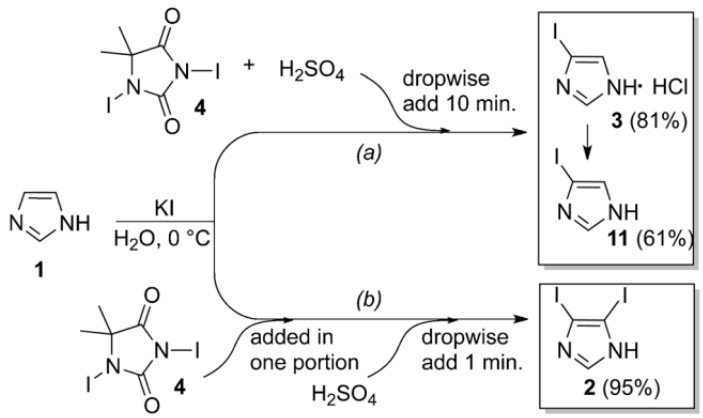
Figure 2.4: Instrumentation of HPLC [24]

In this project, the HPLC-instrument was used in the synthesis of a positron emmission tomography (PET) tracer precursor. The aim was to seperate the products after the methylation with radiolabelling. The HPLC was connected to a radioactivity detector, so a possible ^{11}C activity could be detected, in addition to separating the products.

3 Method

3.1 Halogenation of imidazole

Halogenated imidazoles are of importance in a lot of chemical synthesis and medicinal chemistry. Functionalization of imidazoles is important in transmetalation coupling reactions to substitute the C-backbone of the imidazole skeleton [25]. Several procedures for halogenation exist, and Bjorsvik *et al.* has described methods using both N,N'-diiodo-5,5-dimethyl-hydantoin (DIH, **4**) and iodine with potassium iodide as halogenation reagent. DIH is activated by a strong Brønsteds acid, and iodine is reacting in the presence of a strong base [26, 27]. The method using DIH selectively produces mono- or di-substituted imidazole. This reaction is controlled by the addition and quantity of DIH and acid. Scheme 3.1 shows the two different paths producing halogenated imidazole.



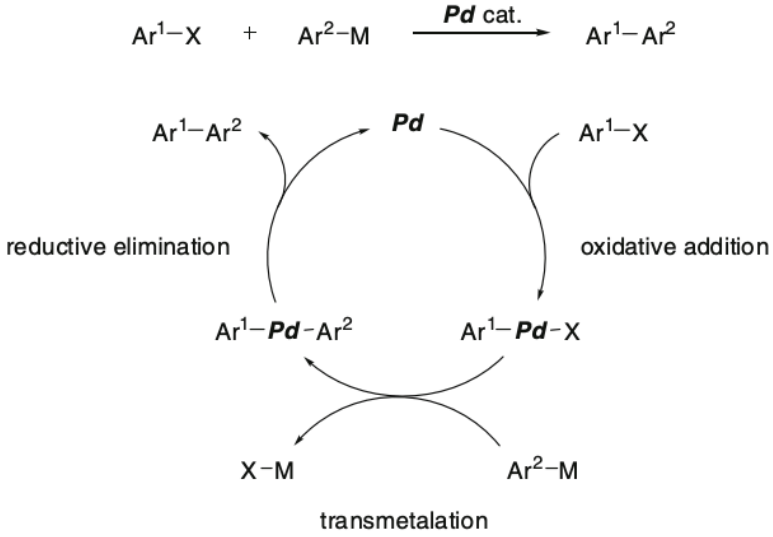
Scheme 3.1: Halogenation of imidazole with DIH, *a)* mono- *b)* di-substituted imidazole [26]

To selectively produce mono- or diiodoimidazole with DIH, is a very useful and powerful method. In the preparation of monoiodoimidazole DIH and acid is mixed together and added to the imidazole. In this was mostly one of the protons in the backbone will be activated. For the diiodoimidazole preparation, DIH is first added to the imidazole, before the acid is added dropwise, to activate both of the protons. The halogenated imidazole can then be used in several different substitution reactions to produce larger molecules.

3.2 Suzuki cross-coupling reaction

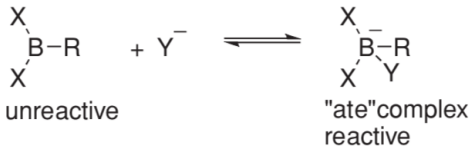
Cross-coupling reactions have been developed for many years, and through this development, it is now possible to produce complex molecules. This is a highly selective reaction and the result is carbon-carbon bonds between unsaturated organic compounds [28].

A Suzuki cross-coupling uses organoboron compounds to couple two hydrocarbon and creates a carbon-carbon bond. The first step is an oxidative addition in which an organic electrophile (Ar^1-X), reacts with the palladium catalyst. The second step is the transmetalation between the product from the oxidative addition and an organometallic reagent (Ar^2-M). The last step to form the cross-coupled product (Ar^1-Ar^2) is a reductive elimination [29]. Scheme 3.2 shows the catalytically cycle of the Suzuki-cross coupling.



Scheme 3.2: A catalytic cycle of the palladium-catalyzed Suzuki-cross-coupling reaction [28]

Boron compounds by themselves are neutral, have low reactivity and make the transmetalation limited. The poor nucleophilicity of boron compounds makes it difficult to transfer the organic groups from boron to the palladium center. However, in 1979, Suzuki and Miyaura disclosed a method that enhances the transmetalation. A Lewis base was added to create an "ate" complex (Scheme 3.3), of the boron complex [28].



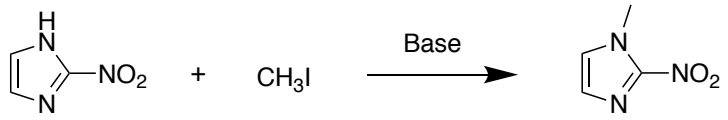
Scheme 3.3: Activation of the boron compound with a Lewis base [29]

This "ate" complex is now more reactive than the neutral boron compound, and is able to transfer the organic part to the palladium through transmetalation. The palladium is now attached to two hydrocarbons and followed by reductive elimination a carbon-carbon bond is formed. This completes the Suzuki cross-coupling reaction, and the palladium catalyst can be reused in a new catalytic cycle [29].

3.3 N-methylation of nitroimidazole

Imidazoles by themselves are of great interest in many reactions and various biological active compounds contain an imidazole in their structures [30]. The reactivity of the imidazole can change by adding substituents to the ring. Either to one of the nitrogen, in the backbone, in between the two nitrogens or a combination of them. One common method is to methylate the nitrogen in the imidazole. From a medicinal chemistry perspective, this will decrease the amount of hydrogen bond donors and make it less polar. This may also increase the lipophilicity of the final drug, which can be an advantage for example to increase brain penetrations [31].

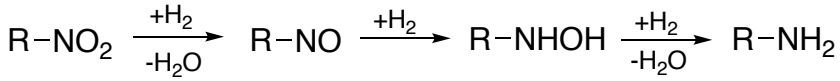
In most cases, a halogenated methyl compound is used in the preparation of N-methylimidazole. In the presence of a base, the imidazole nitrogen will be deprotonated and the methyl will add to this nitrogen. Scheme 3.4 shows the general procedure of the N-methylation of 2-nitroimidazole [32].



Scheme 3.4: General procedure for N-methylation of 2-nitroimidazole

3.4 Reduction of a NO_2 compound

The reduction of nitro compounds is a well-known reaction and a very useful synthetic transformation, leading to the corresponding amine. Different methods exist and one of the most common uses hydrogen gas in the presence of palladium or platinum, as a reducing agent [33]. Scheme 3.5 shows a general procedure for the reduction of a nitro compound.

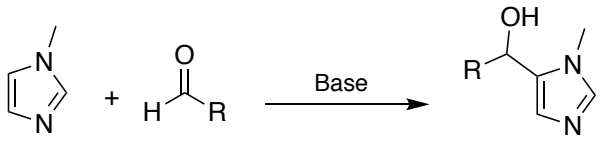


Scheme 3.5: General mechanism of hydrogenation of a nitro-group [33]

Reducing the nitro compound to the corresponding amine makes it available for amidation. The amidation is an important step in this project, due to the required amide linker in the target molecule.

3.5 Synthesis of imidazole-5-yl-alkyl-1-ol derivatives

Functionalization of the backbone of the imidazole is of great interest in medicinal chemistry. Many coupling reactions exist and carbon-carbon bond formation is a well-known reaction. In the synthesis of imidazole-5-yl-alkyl-1-ol derivatives, an aldehyde or ketone is involved in the reaction. A base activates the backbone of the imidazole, and a new carbon-carbon bond occurs and an alcohol is formed.

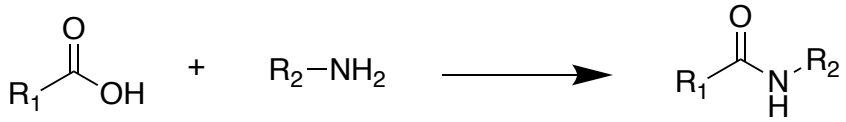


Scheme 3.6: General scheme of the functionalization of the backbone of the imidazole with aldehyde

The formation of hydroxyalkylimidazole is an important reaction, leading to new compounds. By using different derivatives of imidazole, a lot of different compounds can be made. One example is to use 2-aminoimidazole and derivatives of that, in the reaction with aldehydes or ketone [34]. From these derivatives, different coupling reactions, such as amidation, can be performed.

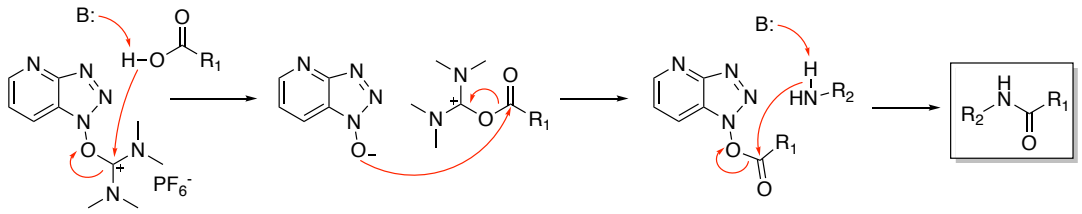
3.6 Amidation

The synthesis of amides is of great interest in organic synthesis and medicinal chemistry. Amides are present in many pharmaceuticals and bioactive natural compounds. For the preparation of amides, several different methods exist. The most common way is to use an amine and a carboxylic acid. This reaction requires high temperature because the -OH is a poor leaving group. Scheme 3.7 shows the general reaction of an amidation.



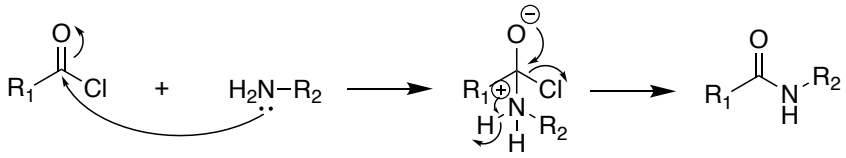
Scheme 3.7: General scheme of an amidation

An option to avoid the high temperature is to use conditions that create a better leaving group. One method involves the coupling reagent HATU that reacts with the carboxylic acid and forms the ester. The amine can then react with the ester, forming the amide bond and cleaving of a better leaving group. Scheme 3.8 shows the general mechanism for the amidation in the presence of HATU [35].



Scheme 3.8: General mechanism for amidation with HATU [35]

Instead of using carboxylic acid as the reagent, a much more reactive compound is the acid chloride. Chloride is a much better leaving group than the hydroxyl group and does not require heat. The only reagent that is necessary in addition to the acid chloride, is the amine and a base, most cases an amine. Scheme 3.9 shows the general mechanism of an amidation.

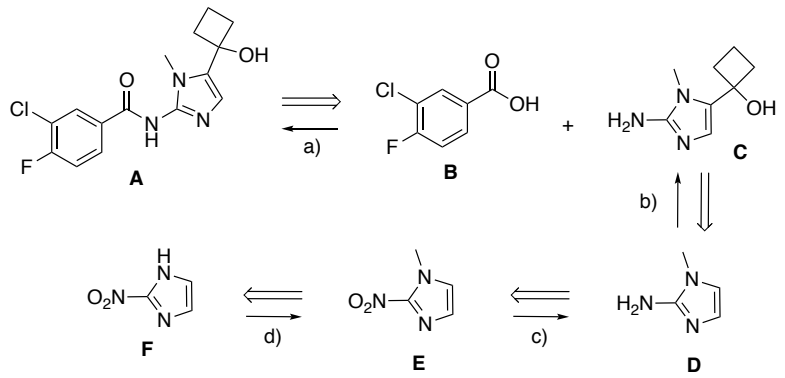


Scheme 3.9: General mechanism of amidation [36]

An amine is a good nucleophile, so the first step will be a nucleophilic attack on the carbonyl. This forms the intermediate and the base removes a proton that neutralizes the hydrochloric acid that is the by-product from the reaction. In the next step the chloride leaves, due to better stability of an amide than the acid chloride [36].

4 Results and discussion

Based on the previously synthesized molecule by Hodgetts *et al.*, the thiazole in the molecule was replaced by an imidazole to make the target molecule (**A**/compound **14**). From an imidazole or an imidazole derivative as the starting material, several different syntheses have been tried. The result of the different methods has led to the retrosynthesis of the target molecule (Scheme 4.1). Disconnection of the amide bond of the target molecule (**A**) gives the aryl acid chloride (**B**) and the 2-aminoimidazole derivative (**C**). Further, the substituent on the backbone of 2-aminoimidazole was disconnected to give a new derivative of 2-aminoimdazole (**D**). Oxidation of the 2-aminoimidazole gives the corresponding nitroimidazole (**E**). A protonation of **E** gives the starting material, 2-nitroimidazole (**F**), in the synthesis of the target molecule (**A**).



Scheme 4.1: Proposed retrosynthesis for the target molecule. a) Amidation b) 2-aminoimidazole with ketone, c) Reduction of nitro group, d) Methylation of 2-nitroimidazole

Before reaching the retrosynthesis (Scheme 4.1), other reaction routes were tried and are also explained in the sections below. The synthesis path that is shown above has led to many different analogs of the target molecule. Figure 4.1 shows the different analogs that were made of the target molecule. Different substituents were used in the backbone of the imidazole, and two different aromatic substituents were attached to the carbonyl in the amide. All of these are explained and shown in the sections below.

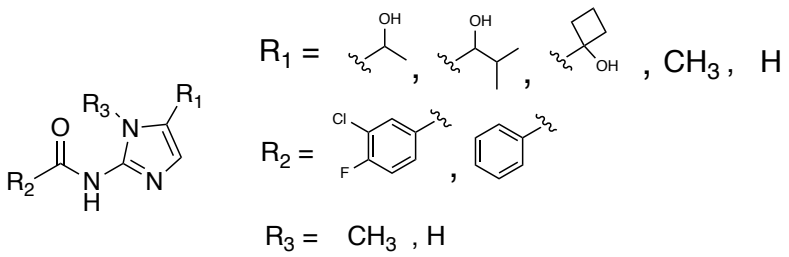
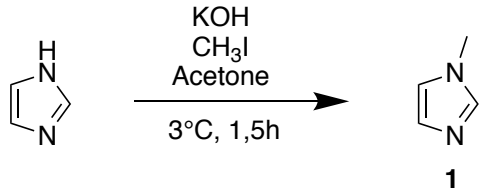


Figure 4.1: All of the analogs that were made of the target molecule

4.1 Synthesis of 1-methyl-1*H*-imidazole (1)

Substitution on the imidazole can change the activity of the compound. Methyl is a common substituent and a good electrophile. Methylation of the imidazole was therefore a good option to change the activity of the imidazole. Several procedures for the methylation of imidazole exist. The most common way is to use a halogenated methyl compound and a good base. The reaction time is not too long and is carried out under low temperature.

Imidazole, potassium hydroxide and acetone were mixed together and when the temperature reached 3 °C, methyl iodide was added dropwise to the mixture. The result will be a deprotonated imidazole, with the methyl group attatched to the nitrogen. Scheme 4.2 shows a general representation of the method.



Scheme 4.2: Reaction scheme for the synthesis of 1-methyl-1*H*-imidazole (**1**)

^{1}H NMR and LC-MS analysis was performed and confirmed conversion and a good yield after workup (84 %). LC-MS indicated that the isolated product contained iodine. This was seen at the LC-MS spectrum in negative ESI, where the peak with a m/z of 126.9 is equivalent to the adduct ion [M-H$^+$] of iodine and corresponds to iodine. Figure 4.2 shows the LC-MS spectrum of the crude product.

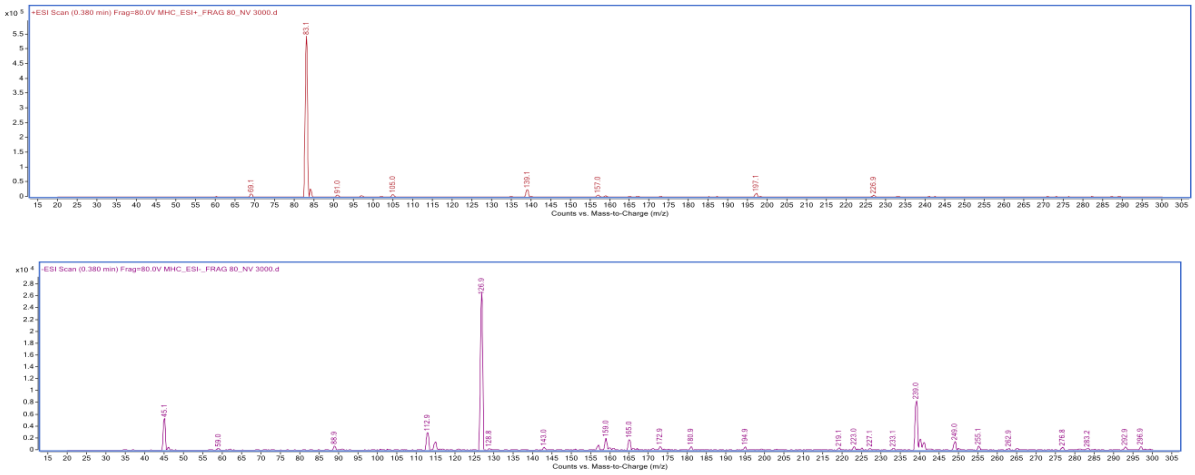


Figure 4.2: MS spectrum in positive and negativ ESI of 1-methyl-1*H*-imidazole (**1**). m/z of 83.1 corresponds to the product, whereas m/z of 126.9 corresponds to iodine

The ^{1}H NMR spectrum of compound **1** (Figure 4.3) shows a clean product, with some traces of contaminated $CDCl_3$ (2.63, 2.18 and 1.26 ppm). The three protons in the aromatic region, (7.44, 7.05, 6.89 ppm) represent the hydrogen in between the two nitrogens and the two in the backbone of the imidazole. The last peak that is more upfield in the spectrum (3.59 ppm), represent the three protons in the methyl group attached to the nitrogen.

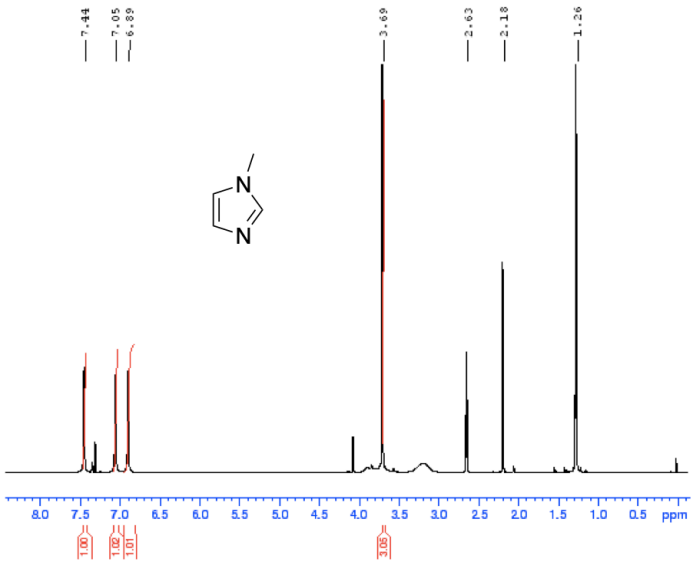
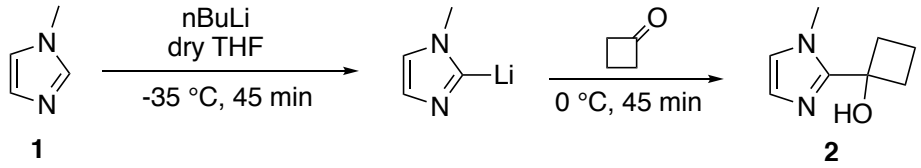


Figure 4.3: ^{1}H NMR spectrum of 1-methyl-1*H*-imidazole (**1**)

4.2 Synthesis of 1-(1-methyl-1*H*-imidazol-2-yl)cyclobutan-1-ol (2)

Based on the previously synthesized probe, the best result was obtained when the thiazol was substituted with a hydroxyl group and a cyclobutyl group. For the imidazole, the 2-position is one of the easiest places to substitute the molecule, and the chosen position for this substituent. This is because the electrons there are most loosely bounded, due to the two nitrogens that are electron-withdrawing and are pulling the electrons. Several procedures for this substitution exist and a reaction with *n*-BuLi is a powerful method that will attack the most loosely bounded electrons (Scheme 4.3) [37].



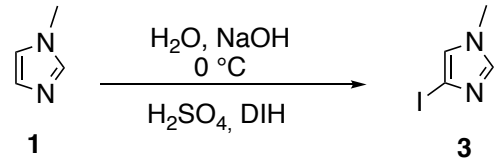
Scheme 4.3: Reaction scheme for synthesis of 1-(1-methyl-1*H*-imidazol-2-yl)cyclobutan-1-ol (**2**)

In the preparation of 1-(1-methyl-1*H*-imidazol-2-yl)cyclobutan-1-ol (**2**), methylimidazole (**1**) and *n*-BuLi was stirred for 45 min under very low temperature. This created a lithium intermediate that reacted with cyclobutanone for 45 min to complete the reaction.

The reaction gave conversion and a good yield with some impurities (68 %). LC-MS analysis indicated that the isolated product contained a small trace of the starting material. This was seen as the LC-MS spectrum in positive ESI consisted of a small peak that corresponds to the m/z of the starting material. This peak has an m/z of 83.1, equivalent to the adduct ion [M+H$^+$] of the starting material. However, the main peak in the spectrum has an m/z of 153.1 that corresponds to the product. The product was not purified, so the yield is not completely accurate.

4.3 Synthesis of 4(5)-iodo-1-methyl-1*H*-imidazole (3)

In the target molecule of this project, the cyclobutyl substituent is attached in the backbone of the imidazole. Therefore the new approach was to halogenate the backbone and try different substitution reactions. Bjørsvik *et al.* [26] disclosed a palladium-catalyzed method for halogenation of imidazole. This is a selective halogenation method that can substitute only one or both of the carbons in the backbone. Substitution at the N-position in the imidazole can sometimes be an issue when it comes to reactivity. This was discovered when carrying out the halogenation procedure with methylimidazole instead of imidazole (Scheme 4.4).

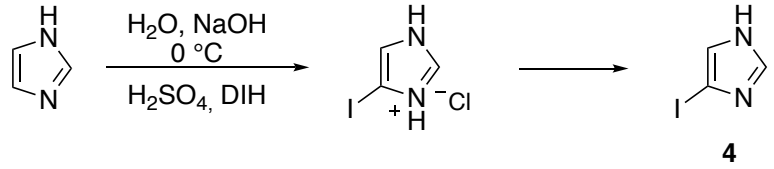


Scheme 4.4: Reaction scheme for synthesis of 4(5)-iodo-1-methyl-1*H*-imidazole (**3**)

In the preparation of 4(5)-iodo-1-methyl-1*H*-imidazole (**3**), methylimidazole (**1**) was dissolved in H_2O and NaOH, and reacted with a mixture of DIH and sulphuric acid. This reaction gave no conversion and both ^{1}H NMR and LC-MS analysis confirmed starting material in the crude product. The target molecule was not observed. Different types of halogenation reagents, iodine monochloride and KI, and I_2, have also been tried but gave no conversion.

4.4 Synthesis of 4(5)-iodo-1*H*-imidazole (4)

Based on the results from the synthesis of compound **3**, the procedure from Bjørsvik *et al.* [26] was carried out in the same manner as the article described, using imidazole as starting material. Scheme 4.5 shows the general procedure for the halogenation of imidazole.



Scheme 4.5: Reaction scheme for the two step synthesis of 4(5)-iodo-1*H*-imidazole (**4**) with DIH as an iodinating agent

NaOH, water and imidazole were added together and cooled down. Sulphuric acid was added to DIH and mixed together before added to the mixture with imidazole. This is a very fast reaction and a good method for halogenation of imidazole at the backbone.

^{1}H NMR and LC-MS analysis confirmed conversion and a good yield after workup (49 %). The ^{1}H NMR spectrum of compound **4** reveals two singlets in the aromatic region (9.12 and 7.81 ppm) and two peaks more upfield (2.51 and 1.25 ppm) that is not explained yet. LC-MS spectrum (Figure 4.4) confirmed the product with a molecular mass of 194 g/mol. This corresponds to the positive ESI spectrum with a peak that has an m/z of 195.0 and negative ESI spectrum with a peak that has an m/z at 193.0.

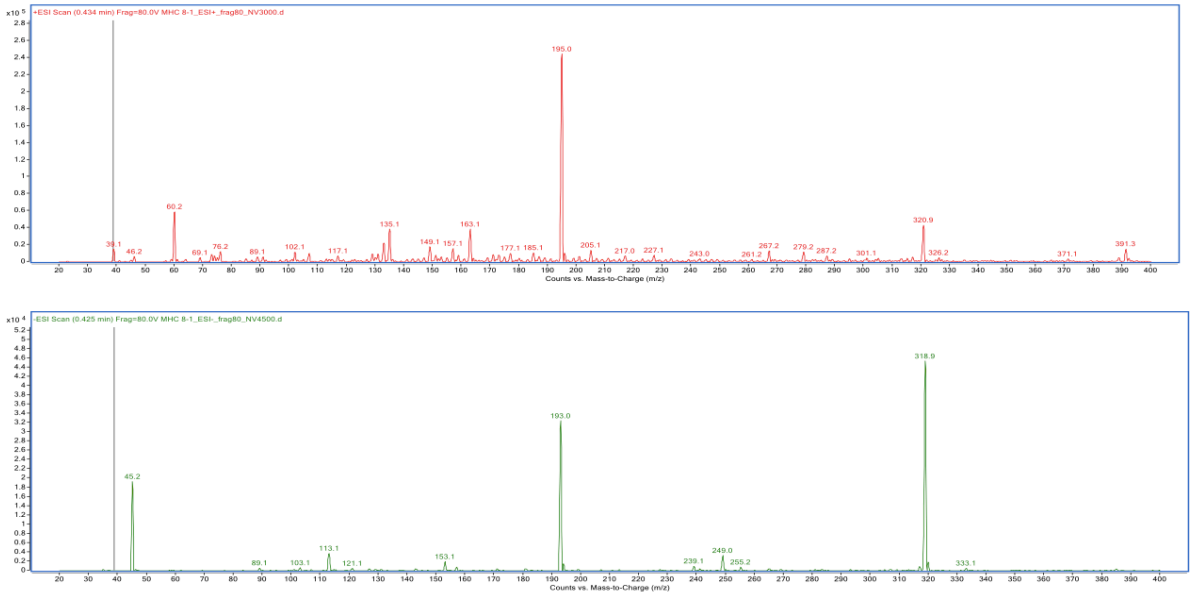


Figure 4.4: MS spectrum in positive and negativ ESI of 4(5)-iodo-1*H*-imidazole (**4**). m/z of 195.0 (+ESI) and 193.0 (-ESI) corresponds to the product, whereas m/z of 320.9 (+ESI) and 318.9 (-ESI) corresponds to 4,5-diiodoimidazole

The LC-MS spectrum also indicated that the product contained the 4,5-diiodoimidazole compound, due to a small peak with an m/z at 320.9 in positive ESI and a big peak with m/z at 318.9 in negative ESI. This corresponds to the mass at 319 g/mol for 4,5-diiodoimidazole. In this type of halogenation of imidazole, it is normal to see both mono- and diiodoimidazole in the product. This is due to two identical hydrogens at the backbone, where both of them can be exchanged.

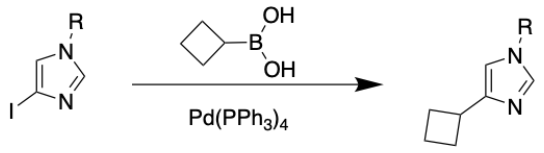
4.5 Synthesis of 4-cyclobutyl-1*H*-imidazole (5)

Several coupling reactions for carbon-carbon bond formation exist. The Suzuki coupling is one example that is used for bond formation in imidazole. Bjørsvik *et al.* [38] disclosed a Suzuki-cross coupling reaction with imidazole, producing aryl-substituted imidazole. This type of Suzuki-cross coupling has not been achieved using cycloalkyl boronic acids and was therefore interesting to try.

The reaction involves a boron compound, in this case, a boronic acid, and an organic electrophile. To activate the boron compound, a base needs to be present in the reaction. In this case two different bases have been tried, K_2HPO_4 and K_2CO_3. A catalyst, in this case, $Pd(PPh_3)_4$, was used to increase the rate of the reaction together with the use of a microwave.

Different attempts on the suzuki coupling were performed using different reagents, temperatures, reaction times and approaches. The first two attempts (Table 4.1) were carried out using the procedure from Bjørsvik *et al.* [38] and the rest followed the procedure from Zeng *et al.* [39], with some changes in solvent, base and reaction time. Two of the reactions were carried out in an oil bath over a longer time, to see if that was the best approach. The temperature was also adjusted, but that did not change the outcome of the reaction. None of the reactions in Table 4.1 gave conversion and the target molecule was not observed.

Table 4.1: Experimental table for the Suzuki-cross coupling in the synthesis of 4-cyclobutyl-1*H*-imidazole (**5**)

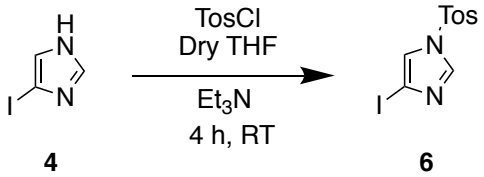


4 / 6 **5**

Number	Substituent, R	Temperature (°C)	Time (h)	Approach	Base	Solvent	Result
1	H	100	1	μw	K_2HPO_4	MeOH	NC
2	Tos	100	1	μw	K_2HPO_4	MeOH	NC
3	H	100	1	μw	K_2CO_3	Toluen	NC
4	H	120	1	μw	K_2CO_3	Toluen	NC
5	H	120	23	Oilbath	K_2CO_3	Toluen	NC
6	H	120	1	μw	K_2CO_3	MeOH	NC
7	H	130	2	μw	K_2CO_3	EtOH	NC
8	H	130	48	Oilbath	K_2CO_3	EtOH	NC

4.6 Synthesis of 4-iodo-1-tosyl-1*H*-imidazole (6)

Protective groups on imidazole are necessary in many reactions, due to high reactivity on the proton in the nitrogen. This can then react with other reagents, and produce unwanted products. Tosyl is a common protective group and has been used several times in Bjørsviks groups in the synthesis with imidazole. Scheme 4.6 shows the general procedure for N-tosylation of 4-(5)-iodoimidazole [27].



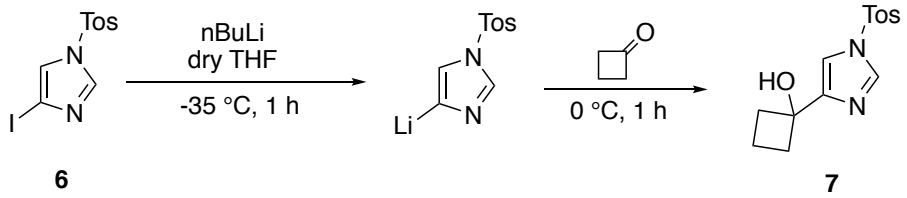
4 **6**

Scheme 4.6: Reaction scheme for the synthesis of 4-iodo-1-tosyl-1*H*-imidazole (**6**)

4-(5)-iodoimidazole (**4**), TosCl, dry THF and Et_3N were mixed together and stirred for 4 h in RT. The crude product was re-crystalized with DCM in a yield of 42 %. ^{1}H NMR spectrum was in accordance with the literature [27].

4.7 Synthesis of 1-(1-tosyl-1*H*-imidazole-4-yl)cyclobutan-1-ol with n-Buli (7)

From the synthesis of compound **2**, we know that the reactivity of nBuLi with imidazole works well and gave the desired product. An exchange of iodine with lithium is a very fast transmetalation, that is used to prepare lithium reagents [40]. To try and get the substituent at the backbone instead of the in second position, compound **6** was used as starting material. Scheme 4.7 shows the general procedure.



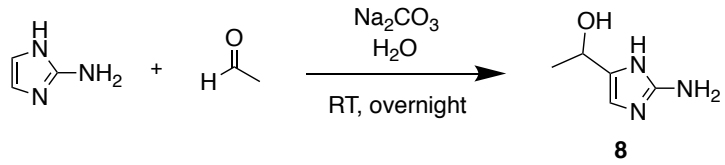
6 **7**

Scheme 4.7: Reaction scheme for the two-step synthesis of 1-(1-tosyl-1*H*-imidazole-4-yl)cyclobutan-1-ol (**7**)

Compound **6** and *n*-BuLi was used to prepare the lithium reagent under very low temperature, before reacting with the cyclobutanone for 1 h. In this reaction, the best outcome would be if the iodine and lithium exchange took place at the backbone. This was not the case and LC-MS analysis did not show any peak of the target product. An LC-MS sample of the intermediate was also checked but did not show the right peak. The desired product was not observed.

4.8 Synthesis of 1-(2-amino-1*H*-imidazol-4-yl)ethan-1-ol (8)

Since the reaction above did not give the desired product, a new approach was tried. This time the starting material was 2-aminoimidazole, trying to substitute the backbone. Reactions of 2-aminoimidazole with the intention of substitute the backbone has not been described much in the literature. Horne *et al.* [34] published an article in 1993 about 2-aminoimidazole and aldehydes to prepare hydroxyalkylaminoimidazole and other derivatives. Scheme 4.8 shows the procedure Horne *et al.* used.



Scheme 4.8: Reaction scheme for the synthesis of 1-(2-amino-1*H*-imidazol-4-yl)ethan-1-ol (**8**)

In the preparation of compound **8**, 2-aminoimidazole was dissolved in water and sodium carbonate was added. After 10 min acetaldehyde was added and the reaction stirred for 4 h. After the reaction, LC-MS analysis (Figure 4.5) was performed and confirmed product formation (m/z = 128.1), but showed mainly starting material (m/z = 84.1). Automated flash chromatography was used to separate the products, but the desired product was not able to be isolated.

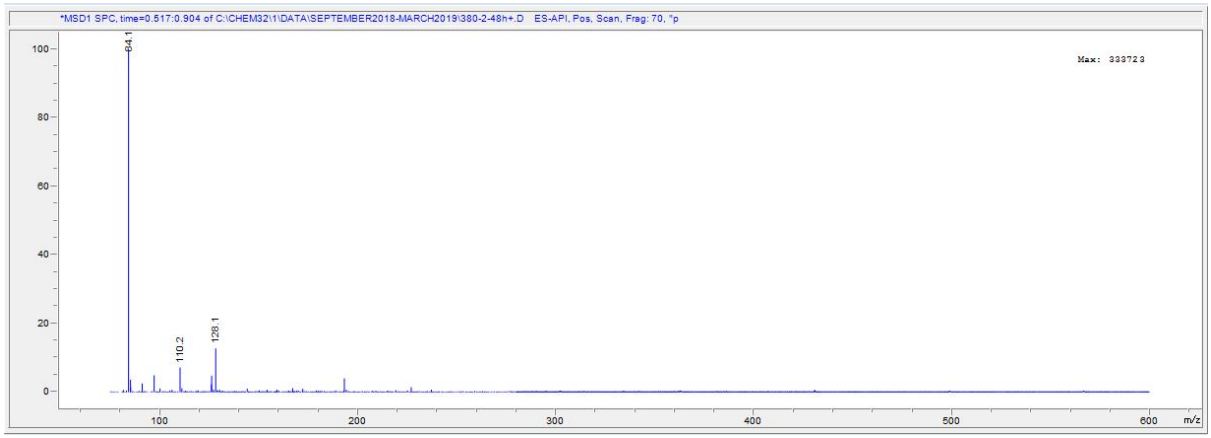
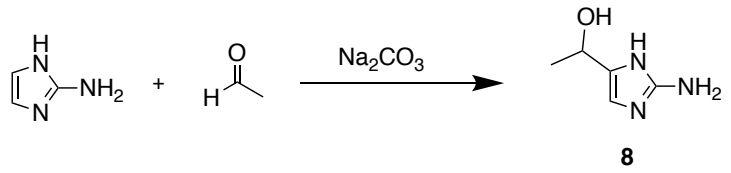


Figure 4.5: MS spectrum of 1-(2-amino-1*H*-imidazol-4-yl)ethan-1-ol (**8**). m/z of 128.1 corresponds to the product, whereas as m/z of 84.1 corresponds to the starting material

A new reaction was set up and the conditions used in the procedure were modified, and described in Table 4.2. Different reaction times and temperatures were changed to try to increase the yield of the desired product.

Table 4.2: Experimental table for synthesis of 1-(2-amino-1*H*-imidazol-4-yl)ethan-1-ol (**8**)



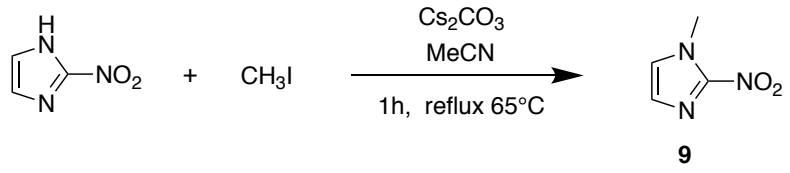
8

Experiment	Time (h)	Temperature (°C)	Result, LC-MS
1	4	RT	peak observed, mostly SM
2	19	RT	peak observed, mostly SM
3	4	60	peak observed, mostly SM
4	17	60	NC

As Table 4.2 shows, the desired product was observed in most cases, but the main peak in the LC-MS spectra was the starting material. The changes showed in Table 4.2 did not increase the yield of the desired product, so the method had to be modified. From previously synthesis, we know that substitution on the nitrogen in the imidazole can change the reactivity of the compound. The next approach was then to substitute the nitrogen in the imidazole, and then try the same reaction.

4.9 Synthesis of 1-methyl-2-nitro-1*H*-imidazole (9)

Direct methylation of the starting material used in the synthesis of compound **8**, will lead to methylation of both the amine group and nitrogen in the imidazole. Due to this, the next step will be to methylate 2-nitroimidazole. Several procedure for the methylation exist and in this case Scheme 4.9 shows the procedure [32].



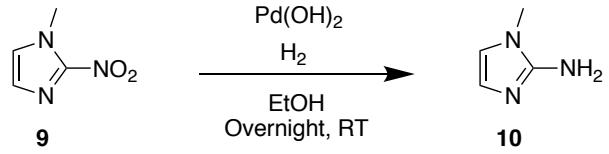
9

Scheme 4.9: Schematic prosedure for the preparation of 1-methyl-2-nitro-1*H*-imidazole (**9**)

In the preparation of compound **9**, 2-nitroimidazole and Cs_2CO_3, were dissolved in MeCN and iodomethane was added dropwise to the mixture at O °C. After 1 h LC-MS analysis was performed and confirmed product formation. The product was purified with silica gel column chromatography, to give the title compound as white solid, in a good yield (68 %). ^{1}H NMR analysis confirmed the product and the spectrum was in accordance with the literature [32].

4.10 Synthesis of 1-methyl-1*H*-imidazole-2-amin (10)

The reduction of a nitro-group is a well-known method and several procedures exist. One of the most common uses palladium in the presence of hydrogen gas to reduce the nitro group to the amine. Scheme 4.10 shows the general reaction procedure [41].

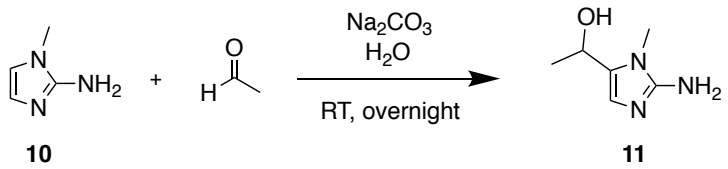


Scheme 4.10: Schematic prosedure for the preparation of 1-methyl-1*H*-imidazole-2-amin (**10**)

In the preparation of compound **10**, compound **9** and $Pd(OH)_2$ were dissolved in ethanol and equipped with a H_2 gas ballon. The reaction stirred overnight and LC-MS analysis confirmed the conversion. After workup the title compound did not need any further purifications, to give the title compound in a good yield (63 %). ^{1}H NMR analysis confirmed the product and the spectrum was in accordance with the literature [42].

4.11 Synthesis of backbone substituted amino imidazole (11-13)

Due to almost no conversion and low yield of compound **8**, it was decided to do the reaction with a substituent at the nitrogen. The reaction was carried out in the same manner as for compound **8**, having compound **10** as the starting material. A methyl group at the nitrogen was added, to see if that made the compound more reactive. Scheme 4.11 shows the general procedure [34].



Scheme 4.11: Reaction scheme for the synthesis of 1-(2-amino-1-methyl-1*H*-imidazol-4-yl)ethan-1-ol (**11**)

In the preparation of compound **11**, the same amount and same equivalents as for compound **8** were used, and stirred overnight. After 19 hours LC-MS analysis was performed and showed a good conversion of the product, but there was still some unreacted starting material. A new amount of Na_2CO_3 and acetaldehyde, the same equivalents as in the beginning, was therefore added and LC-MS was checked again after 24 h. LC-MS showed more conversion and the same amount of the same reactants were added one more time, and the reaction stirred overnight. After 48 h the reaction showed full conversion of the product (m/z=142.1 in Figure 4.6), with some impurities, as was seen in the LC-MS spectrum (Figure 4.6).

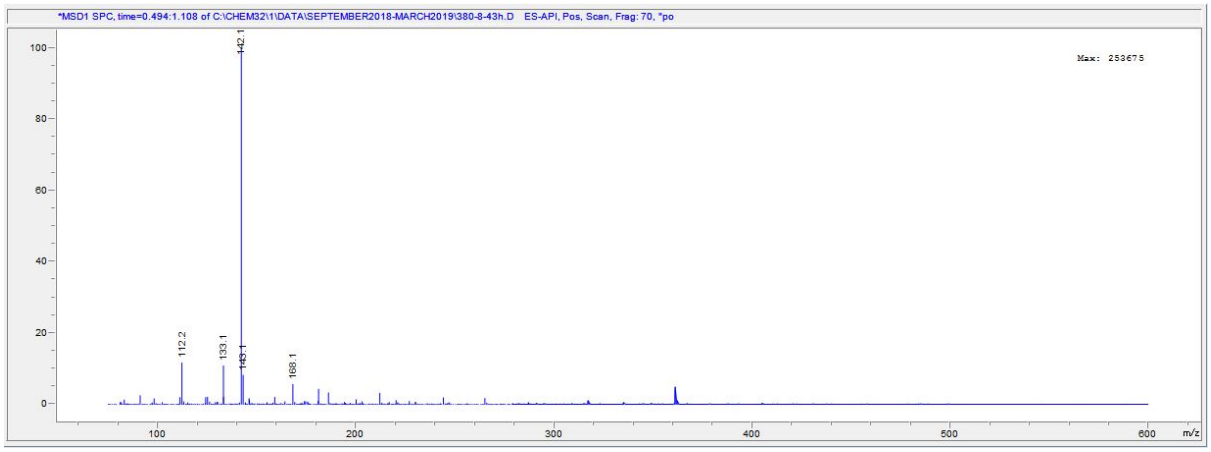


Figure 4.6: LC-MS spectrum of 1-(2-amino-1-methyl-1*H*-imidazol-5-yl)ethan-1-ol (**11**)

Table 4.3 show different attempts of the preparation of compound **11**, where the equivalents of sodium carbonate and the aldehyde have been modified. Based on the first attempt described above, different amounts of the reagents have been tried to reduce the reaction time of 43 h. The only experiment that gave full conversion after 24 h was number 7 where the total amount of Na_2CO_3 and acetaldehyde from experiment number 1 was added at the beginning of the reaction.

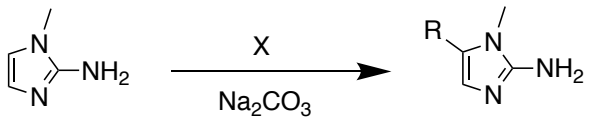
Table 4.3: Experimental table for synthesis of compound **11**

Experiment	Time (h)	Na_2CO_3(mmol)	CH_3CHO(mmol)	Result, LC-MS
1	48	0.6+0.6+0.6	1.2+1.2+1.2	Mostly product
2	30	1.2	2.6	Mostly product, SM observed
3	24	1.5	2.8	Mostly product, SM observed
4	24	1.2	2.8	Mostly product, SM observed
5	24	0.6	3.0	Mostly product, SM observed
6	24	2.0	3.0	Mostly product, SM observed
7	24	1.8	3.6	Mostly product

Even if the reaction gave full conversion, some side-products was formed in the reaction. The crude product was tried purified with silica gel column chromatography, but isolation of the product was not accomplished. Due to no purifications and some contaminations present in the product, an accurate yield can not be given.

Based on the result of the preparation of compound **11**, a few more analogs were prepared. They were carried out in the same manner as with compound **11**, but with a change of reagent. Table 4.4 shows the different reagents that were used and the resulting substituents.

Table 4.4: Experimental table for synthesis of compounds **11-13**



Compd	Reagent, X	Substituent, R
11		
12		
13		

Isobutyraldehyde was used instead of acetaldehyde, to make a more branched alcohol in the backbone. The LC-MS analysis of this reaction did not show full conversion, but the target molecule had the highest peak in the LC-MS spectrum (m/z=170.1) (Figure 4.7). Isobutyaldehyde is a more branched aldehyde, which makes it more steric hindered in the reaction and may be the reason for not a full conversion. Isolation of the product was not accomplished, but product formation was confirmed and the crude mixture was used directly in the next step.

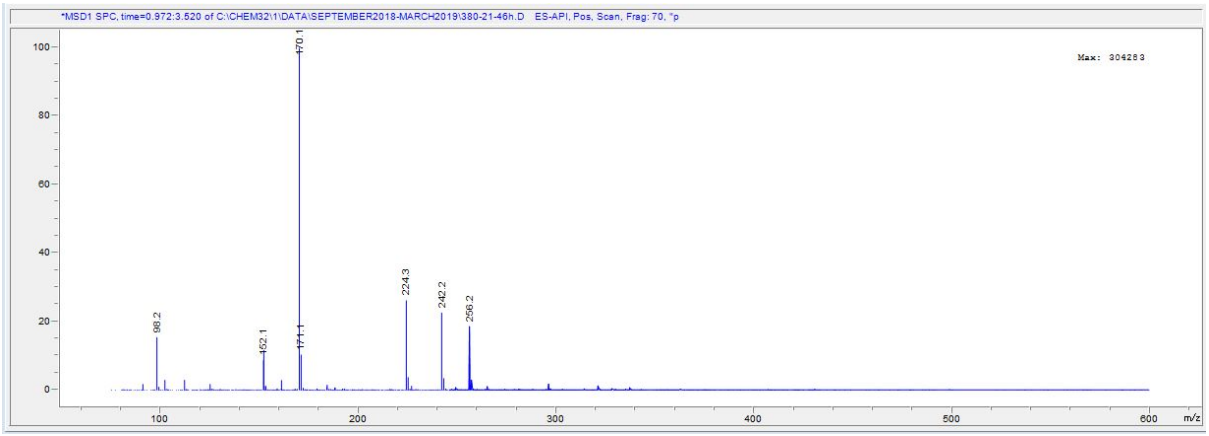


Figure 4.7: LC-MS spectrum of 1-(2-amino-1-methyl-1*H*-imidazol-5-yl)-2-methylpropan-1-ol (**12**)

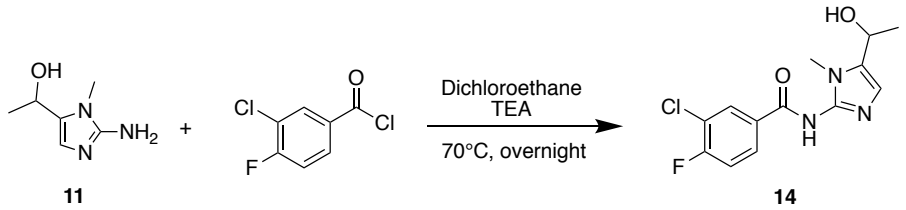
Based on the procedure from Horne *et al.* [34], an aldehyde was used as reagent to prepare compound **11** and **12**. To achieve the analog of the compound that Hodgetts *et al.* synthesized, a ketone was the only option as a reagent. Based on the literature, it is difficult to find a procedure using a ketone instead of an aldehyde. This was therefore an interesting investigation, leading to new and good results. Also here the reaction did not show full conversion, even if the reaction time was longer and was carried out with heat. The target molecule (**13**) was observed and the crude mixture was used in the next step without any purifications.

4.12 Synthesis of amide analogs (14-27)

Several procedures for an amidation exist. The standard method involves a carboxylic acid and an amine. Hodgetts *et al.* [3] used carboxylic acid and aniline in the presence of the coupling agent HATU, to create the amide bond in their synthesized molecule. Another option is to use acid chloride instead of carboxylic acid and HATU. The acid chloride is more reactive then carboxylic acid, and chloride is a better leaving group in the coupling with the amine.

Synthesis of compound 14 and 15

Scheme 4.12 shows the general procedure for the first attempt of amidation used in the synthesis of compound **14**. Compound **11** was dissolved in dichloroethane, TEA and the acid chloride was added. The reaction stirred for overnight at 70 °C. After the reaction, LC-MS analysis was performed and showed a peak with an m/z of 280, which is a loss of m/z = 18 from the desired product. The product was purified with silica gel column chromatography with a mixture of hexane and ethyl acetate, with a 100:0 % - 50:50 % gradient and ^{1}H NMR was collected.



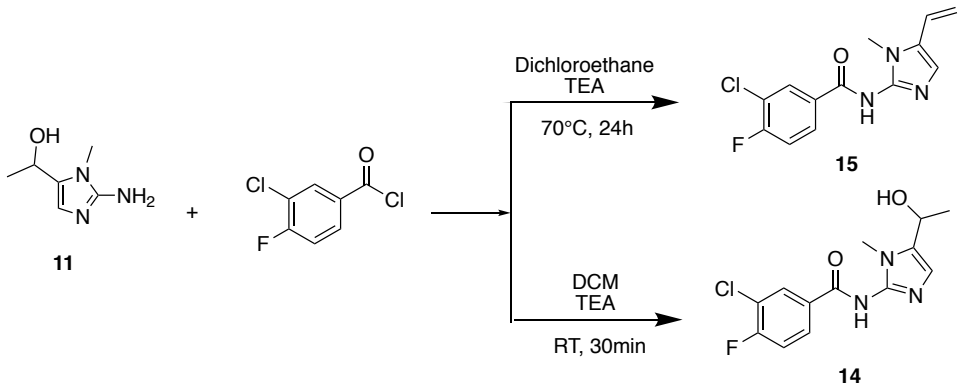
Scheme 4.12: Synthesis of 3-chloro-4-fluoro-*N*-(4-(1-hydroxyethyl)-1*H*-imidazol-2-yl)benzamide (**14**)

Based on the loss of m/z = 18 and the existence of a hydroxyl group in the starting material, there was a possibility that the alcohol has been eliminated and an alkene was formed. An elimination happens in the presence of heat, which was the case in this reaction. This means that the alcohol was most likely eliminated to an alkene. This assumption corresponds well with the LC-MS analysis, where the peak shows a loss of water, which occur during the elimination.

The ^{1}H NMR spectrum (Figure 4.8) also indicates that the alkene was formed, due to more peaks in the aromatic region than the desired product was supposed to have. The most downfield peak (12.45 ppm) represents the hydrogen at the amide, and the next three peaks in the aromatic region represent the three protons in the benzene ring (8.22, 7.42 and 7.11 ppm). In addition to this, there was no sign of the hydrogen in the alcohol, instead, there were three peaks, two doublets (5.32 and 5.76 ppm) and one triplet (6.61 ppm) that corresponds well with the three protons in an alkene.

Figure 4.8: ^{1}H NMR spectrum of 3-chloro-4-fluoro-*N*-(1-methyl-5-vinyl-1*H*-imidazol-2-yl)benzamide (**15**)

Another observation was that there was just one peak (3.58 ppm) that represents a methyl group, instead of two that the desired product was supposed to have. This confirms that compound **15** was synthesized instead of compound **14**. A new method was developed for the preparation of compound **14**. Scheme 4.13 shows the two different amidation methods for the synthesis of compound **14** and **15**.



Scheme 4.13: Two different methods for the amidation in the synthesis of compound **14** and **15**

In the preparation of compound **14** the reaction was carried out in room temperature instead of 70 °C. The solvent was changed to DCM and the equivalent of the acid chloride was also reduced from 2.2 mmol to 1.0 mmol. After stirring for 30 min, LC-MS analysis showed full conversion. Mostly the product was formed, but a small peak in the LC-MS spectrum showed that the alkene was formed too. The two products were separated and purified with silica gel column chromatography with a mixture of DCM and MeOH on a 100:0 % - 90:10 % gradient. The ^{1}H NMR spectrum of the product showed some impurities, so the product was completely purified with crystallization with DCM.

After the crystallization the ^{1}H NMR spectrum (Figure 4.9), shows a clean product. In the downfield part of the spectrum, you will find the hydrogen at the amide (12.12 ppm) and in the aromatic region, you will see the three hydrogens at the phenyl (8.23, 8.12 and 7.42 ppm) and the hydrogen at the backbone of the imidazole (6.68 ppm). More upfield in the spectrum, you will find the hydrogen in the alcohol and the hydrogen in the secondary carbon, which are the differences from the spectrum of the alkene. In addition to this, you can see the hydrogens in both of the methyl groups in the upfield part of the spectrum (3.57 and 1.42 ppm), which was a strong indication that the right molecule was prepared.

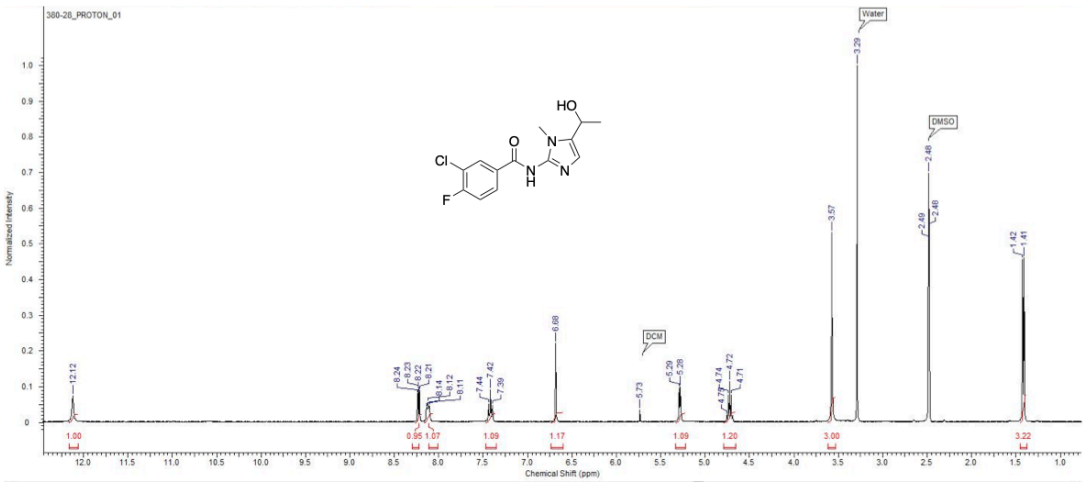


Figure 4.9: ^{1}H NMR spectrum of 3-chloro-4-fluoro-*N*-(5-(1-hydroxyethyl)-1-methyl-1*H*-imidazol-2-yl)benzamide (**14**)

Since the starting material for the amidation, compound **11**, was not pure, a ^{1}H NMR analysis was not performed. Due to this, it was difficult to determine in which position of the backbone the substituent was attached. A more detailed NMR analysis was therefore collected of the target molecule (compound **14**). Based on the chemistry of the methyl-imidazole, the substituent was most likely attached to the 5th position, closest to the nitrogen with the methyl group. To confirm this, a NOESY spectrum of the final product (compound **14**) was collected (Figure 4.10).

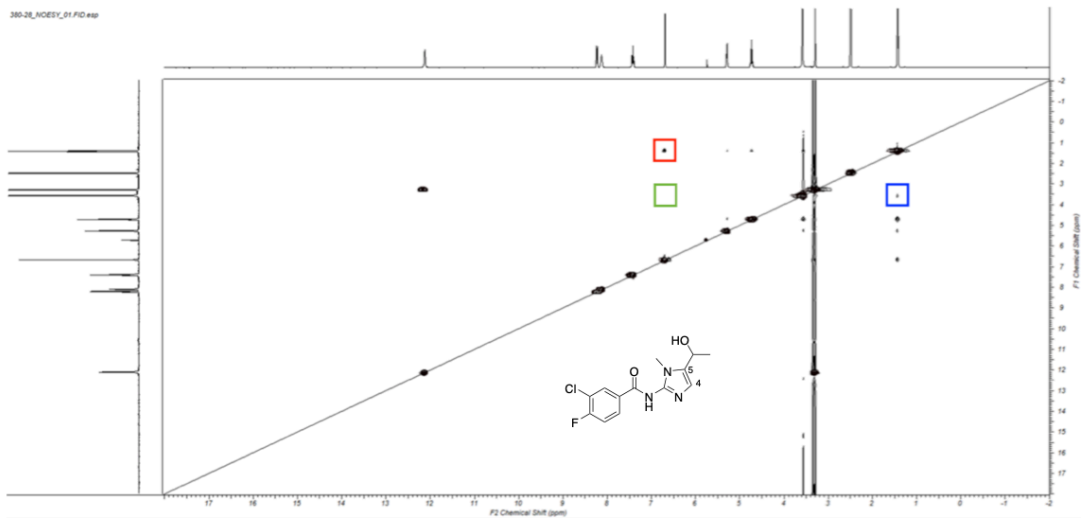
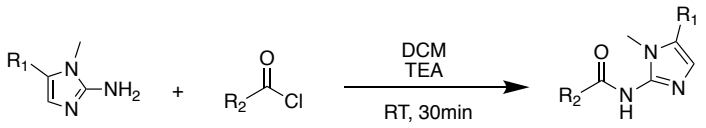


Figure 4.10: NOESY spectrum of 3-chloro-4-fluoro-*N*-(5-(1-hydroxyethyl)-1-methyl-1*H*-imidazol-2-yl) benzamide (**14**)

In the NOESY spectrum you can see interactions between the different hydrogens in the molecule. Hydrogens that are close to each other will interact and produce a spot in the spectrum. A good example is the hydrogen in the backbone of the imidazole and the hydrogen in the methyl group in the backbone. They are close to each other and will therefore produce a spot that is shown in the NOESY spectrum in Figure 4.10 (red square). Based on the two options of attachment for the substituent, the NOESY spectrum will show different interactions. If the substituent is attached in the 4-position in the backbone, the hydrogen in 5-position would be interacting with the methyl group in the nitrogen. The spectrum does not show any interactions between these hydrogens (green square). However, the spectrum shows a weak interaction between the methyl group in the substituent and the methyl group in the nitrogen (blue square). This means that the substituent in the backbone is close to the methyl group in the nitrogen, and is most likely attached to the imidazole in the 5th position.

Based on the result of the amidation of compound **11**, compound **12** and **13** were also used in amidation, to produce analogs of the target molecule. The reactions were carried out in the same manner as for compound **14**. Table 4.5 shows the different analogs that were made.

Table 4.5: Synthesis of the different amide analoges



Compd	R_1	R_2	Compd	R_1	R_2
14			**19**		
15			**20**		
16			**21**		
17			**22**		
18			**23**		

Synthesis of compound 16

In the preparation of compound **16**, compound **12** was used directly without any purification. Since the crude product still contained some starting material (compound **10**), the expected result will be to get two products after the amidation. After 1 h of stirring in RT, the reaction showed full conversion. The crude reaction mixture was analyzed by LC-MS and confirmed the assumption that two products were formed. The spectrum showed mainly two peaks, one with an m/z at 326.1 and one with an m/z at 254.1, which represent the two molecules in Figure 4.11. The two products were successfully separated with silica gel column chromatography and the desired product was crystallized with a mixture of hexane and ethyl acetate.

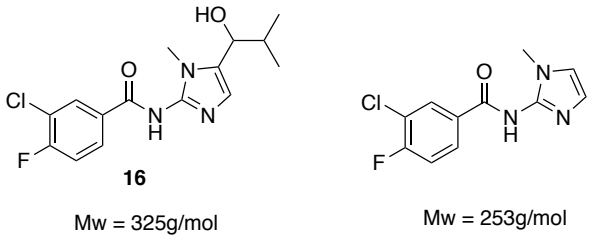


Figure 4.11: Product (**16**) and side-product produced in the preparation of compound **16**

The ^{1}H NMR spectrum of the isolated product (Figure 4.12), indicates a clean product with no impurities. The main difference in this spectrum compared to the one of compound **14**, is that there is more peaks in the upfield part due to more carbon and hydrogens in the backbone of the imidazole. The two doublets (0.97 and 0.81 ppm) represent the two methyl groups and the multiplet (1.98 ppm) represents the hydrogen in the tertiary carbon. The rest of the peaks more downfield in the spectrum are very similar to the spectrum of compound **14**.

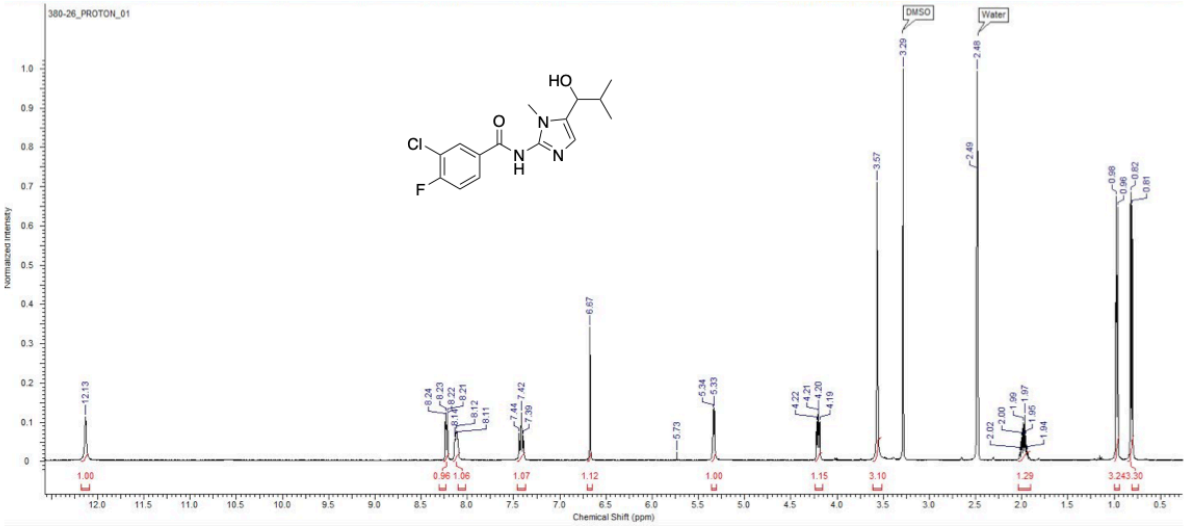


Figure 4.12: ^{1}H NMR spectrum of 3-chloro-4-fluoro-*N*-(5-(1-hydroxy-2-methylpropyl)-1-methyl-1*H*-imidazol-2-yl)benzamide (**16**)

Synthesis of compound 17

In the preparation of compound **17**, the amidation was carried out in the same manner as with compound **14** and **16**. Also here the crude product of compound **13** was used directly without any purifications, which means that it contains both the starting material (compound **10**) and the product (compound **13**). In the amidation both of these compounds will be available for coupling reaction and the crude product will most likely contain two compounds. One of the compounds will be the desired product and the other one will be the same side-product that is shown in Figure 4.11 (p. 40). The LC-MS analysis of the crude mixture (Figure 4.13) confirms formation of both the side-product (m/z = 254.0), and the desired product (m/z = 324.1). Silica gel column chromatography was used to purify and separate the two products, with a mixture of DCM/MeOH (100:0 % - 90:10 % gradient).

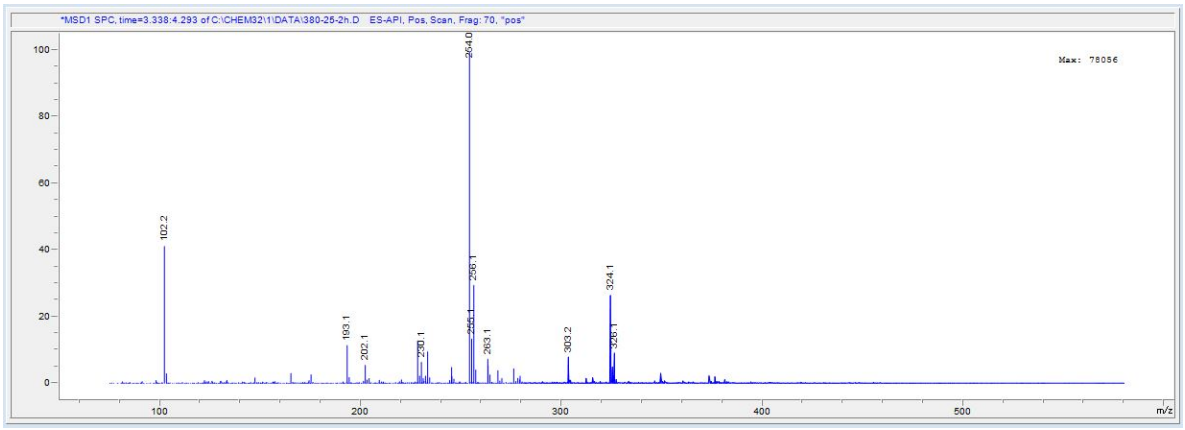


Figure 4.13: LC-MS spectrum of the crude mixture of 3-chloro-4-fluoro-*N*-(5-(1-hydroxycyclobutyl)-1-methyl-1*H*-imidazol-2-yl) benzamide (**17**)

The ^{1}H NMR spectrum of compound **17** (Figure 4.14) is very similar to the spectrum of compound **14** and **16**. The aromatic region has the same peaks, due to the same phenyl ring in the amide and one hydrogen in the backbone of the imidazole. The main difference is in the downfield part of the spectrum. For compound **17**, there will be more peaks in this area, due to more hydrogens in different chemical environment for the cyclobutyl. The four multiplets most downfield represent the six hydrogens in the cyclobutyl that is attached in the backbone of the imidazole.

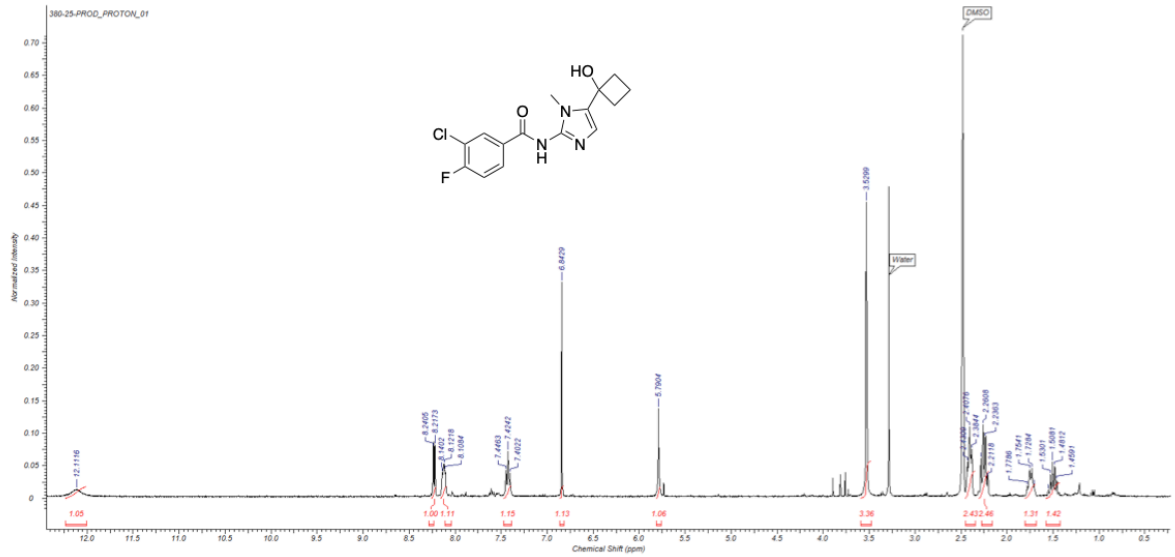


Figure 4.14: ^{1}H NMR spectrum of 3-chloro-4-fluoro-*N*-(5-(1-hydroxycyclobutyl)-1-methyl-1*H*-imidazol-2-yl)benzamide (**17**)

Synthesis of compound 18

In addition to compound **14-17**, an analog without any substituent on the backbone of the imidazole, compound **18** in Table 4.5, were also made. This was a one-step amidation reaction with 1-methylimidazole-2-amin, and the preparation was carried out in the same manner as for compound **14-17**. LC-MS analysis confirmed product formation and the product was purified with crystallization with ethyl ether to give the title compound in an overall yield of 38 %.

The ^{1}H NMR spectrum (Figure 4.15) of compound **18** is also very similar to the spectra of compound **14-17**. The main difference is that compound **18** does not have any substituents in the backbone, and will therefore have two hydrogens instead of one hydrogen in the backbone. This will give an extra peak in the aromatic region in addition to all the other peaks that are similar to the other spectra. Due to no substituents, there will not be any peaks in the most upfield region of the spectrum.

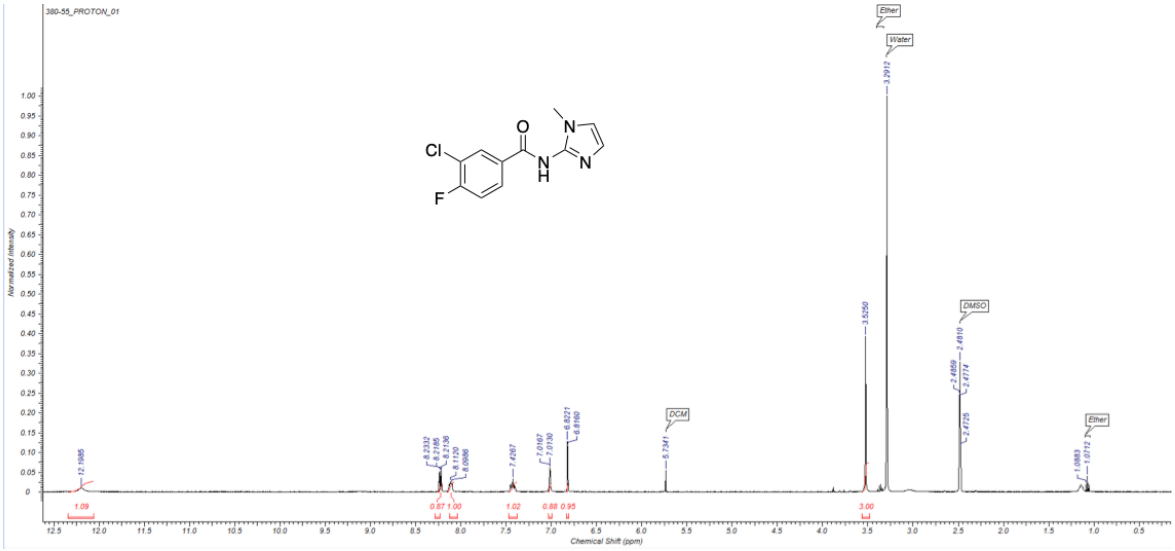


Figure 4.15: ^{1}H NMR spectrum of 3-chloro-4-fluoro-*N*-(1-methyl-1*H*-imidazol-2-yl)benzamide (**18**)

Synthesis of compound 19-23

From the different compounds Hodgetts *et al.* [3] have been tested, they tried different aromatic compounds in the amide bond. The one that gave the best results was with chloride and fluoride in the 5th and 3rd position of the phenyl ring. Now when the thiazole is replaced with an imidazole, that might change the activity and the aromatic ring with two substituents might not be the best option anymore. Based on this compound **14-18** were also synthesized without any substituent on the aromatic ring, only a phenyl ring. This lead to compound **19-23** in Table 4.5 (page 39).

Compound **19-23** were synthesized in the same manner as for compound **14-18**, with a new acid chloride. Benzoyl chloride was now used to prepare compounds with the aromatic substituent as a phenyl ring without any substituent. The ^{1}H NMR spectrum of compound **19** is almost identical to the one of compound **14**. The only difference is that compound **19** has five protons in the aromatic region instead of three which compound **14** has. This will be the case for compound **15-18** as well. The ^{1}H NMR spectrum of compound **19** (Figure 4.16), shows that the aromatic region contains five protons, instead of three. The other peaks are similar to compound **14** and will represent the same hydrogens.

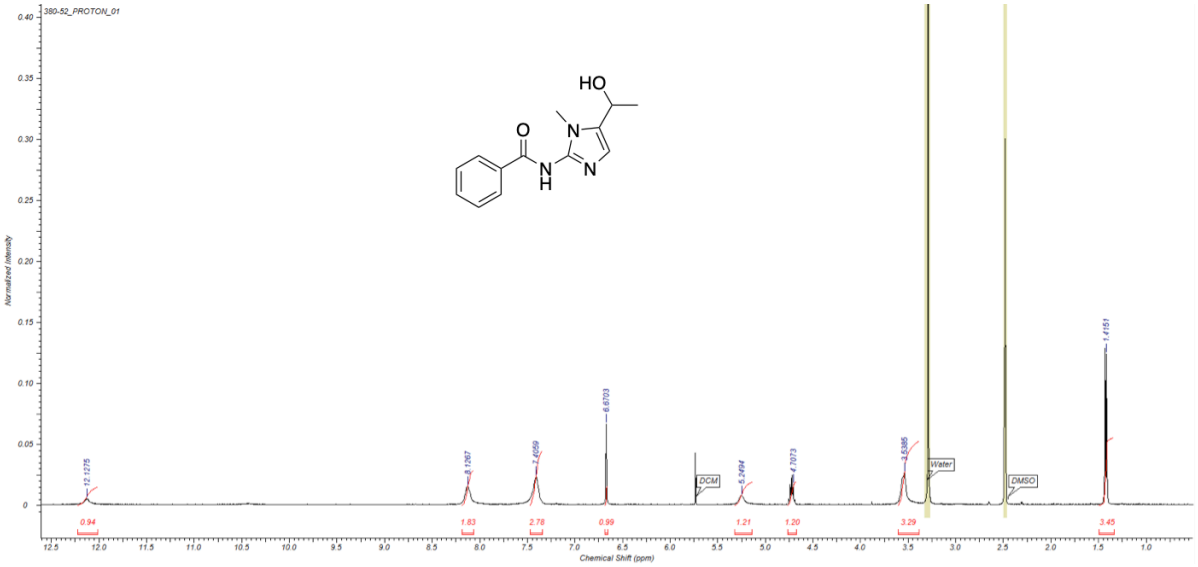
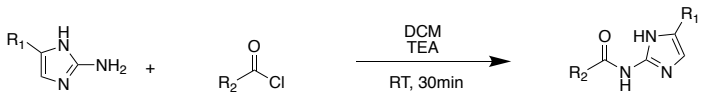


Figure 4.16: ^{1}H NMR spectrum of *N*-(5-(1-hydroxyethyl)-1-methyl-1*H*-imidazol-2-yl)benzamide (**19**)

Synthesis of compound 24-27

All of the analogs described above have a methyl group as a substituent on the nitrogen in the imidazole. From the previously synthesized molecule by Hodgetts, neither the nitrogen nor the sulfur in the thiazol had any substituents. It would therefore be an interesting approach to make a few analogs without any N-substitution in the imidazole. Two different molecules were used as starting material, 2-aminoimidazole and 5-methylimidazole-2-amine. From these two compounds, four new analogs were made. All of them were synthesized in the same manner as for the compounds in Table 4.5 (page 39). Table 4.6 shows the different substituents and products.

Table 4.6: Synthesis of amide analoges without N-substitution



Compd	R_1	R_2	Compd	R_1	R_2
24			**26**		
25			**27**		

In the preparation of the compounds in Table 4.6, the reaction showed almost full conversion, due to a one-step reaction. Compound **24** had low solubility in DCM and was not able to be purified with silica gel column chromatography. However, extraction with ethyl acetate instead of DCM gave a pure product. LC-MS and ^{1}H NMR analysis confirmed product formation and a clean product in an overall yield of 36 % (86 mg). Compound **25** was purified with silica gel column chromatography and crystallized with ethyl ether to give the title compound as a pure product in a overall yield of 8 % (19 mg).

The ^{1}H NMR spectrum of compound **25** (Figure 4.17) has the same peaks in the aromatic region of the spectrum as for compound **14-17**. The most downfield peak will now integrate into two instead of one, due to the proton in the nitrogen in the imidazole in addition to the proton in the amide. The upfield part of the spectrum will only have the three hydrogens in the methyl group at the backbone of the imidazole. The chemical shift of these protons will be more downfield than the methyl group in the nitrogen due to less shielding from a carbon instead of nitrogen.

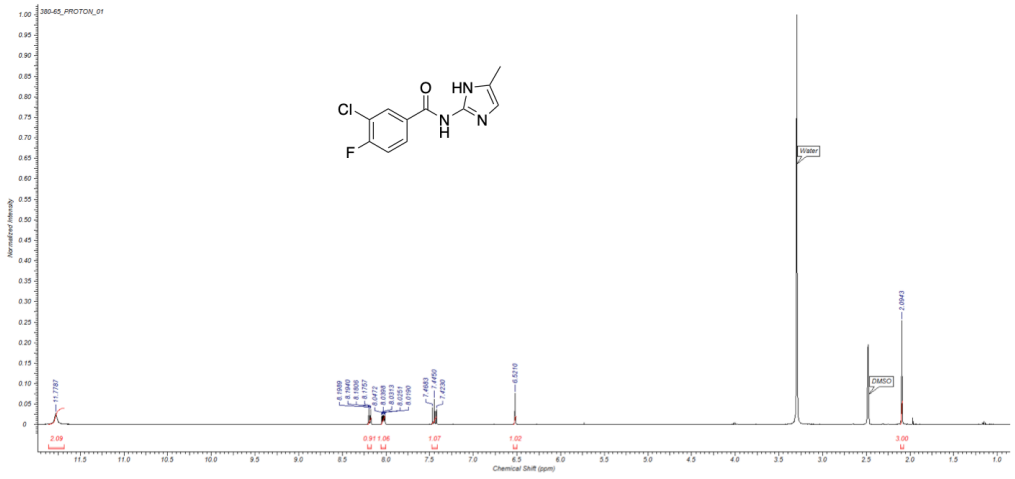


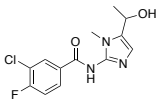
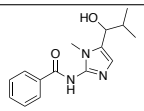
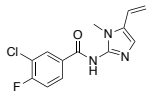
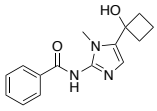
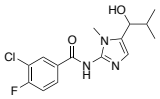
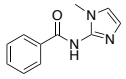
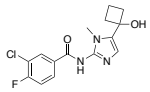
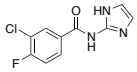
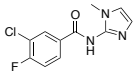
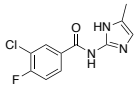
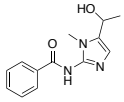
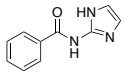
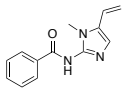
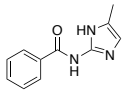
Figure 4.17: ^{1}H NMR spectrum of 3-chloro-4-fluoro-N-(5-methyl-1H-imidazol-2-yl)benzamide (**25**)

For compound **26** and **27** purification was a problem, due to low solubility in DCM. Silica gel column chromatography was used to purify the products. Compound **27** gave the best yield (12 %, 23 mg) but the yield of the purified product for compound **26** was lower (6 %, 11 mg). Different crystallization methods were also tried but did not completely purify the products.

Result from preliminary testing

All of the compounds described above were synthesized and tested at the Department of Dermatology, Indiana University School of Medicine. Based on the testing, the result is presented by an EC_{50} value and % activation. The EC_{50} value describes the concentration of the molecule that gives the half-maximum response or % activation. A lower EC_{50} value and higher % activation makes a better and more potent compound. Table 4.7 shows the different molecules that were tested, with the corresponding EC_{50} value and % activation.

Table 4.7: Analogs that are synthezised and tested, with the corresponding EC_{50} value and % activation

Compd	Structure	EC_{50}	% Activation	Compd	Structure	EC_{50}	% Activation
14		1.75	193	**21**		9.95	240
15		0.45	158	**22**		2.8	115
16		NA	48	**23**		NA	0
17		NA	0	**24**		NA	0
18		27.5	98	**25**		32	198
19		NA	157	**26**		NA	0
20		-	-	**27**		NA	72

Based on the results from the previously tested molecule (**LDN-27**), compound **17** was the target molecule and was expected to be active. The result was compared with compound **LDN-27**, and compound **17** turned out to be inactive. However, compound **15** showed good activity (158 %) and a low EC_{50} value (0.45). Figure 4.18 shows the % activation and the EC_{50} value presented in graphs for the three compounds (**LDN-27**, compound **17** and compound **15**).

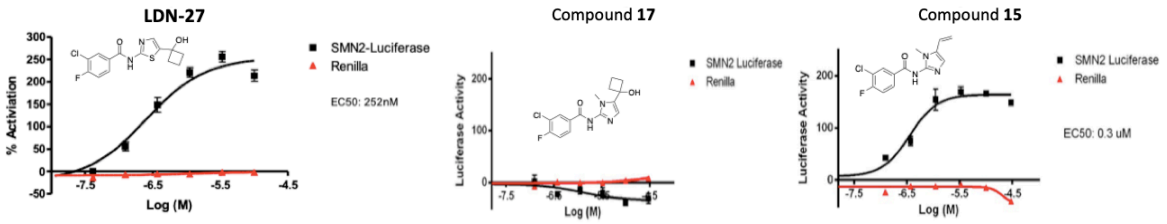


Figure 4.18: Results of the previously tested molecule (**LDN-27**) and compound **15** and **17**

Compounds with low % activation and high EC_{50} value (compound **18**), will not be good and potent molecules, because you do not want a high concentration of the drug at the half-maximum response. Figure 4.19 shows the different substituents that have been used in the synthesized molecules. A summary of the results shows that a methyl group at the nitrogen, an aryl ring with chloride and fluoride as substituents attached to the carbonyl and an alkene at the backbone of the imidazole showed the best activity (Figure 4.19).

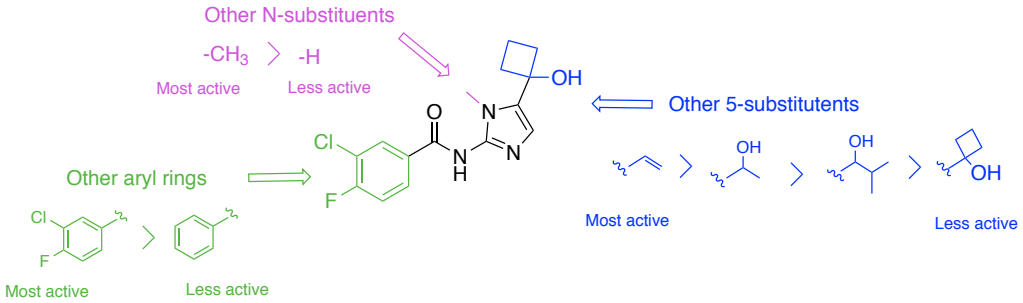
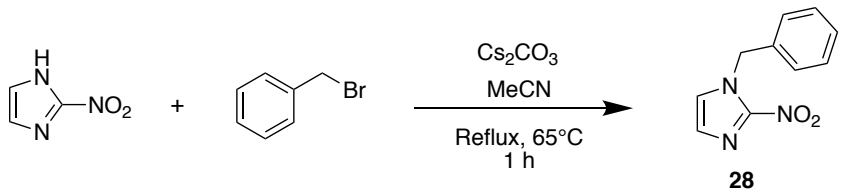


Figure 4.19: Summary of the result from the preliminary testing

The conclusion of this testing will therefore be that compound **15** is the most potent molecule, but due to the alkene, it might not be the best solution as a drug. If you compare it to the previously synthesized molecule (**LDN-27**), the EC_{50} value is almost the same, but the % activation is a bit lower for compound **15**. This means that further optimization can be done, either to make this compound more potent or synthesized analogs that are more active. One option is to change the substituents on the phenyl ring. Another change can be to try other substituents on the nitrogen and the backbone of the imidazole. In the nitrogen, it would be interesting to try a phenyl ring or a longer carbon chain, for example, ethyl instead of a methyl group. At the backbone, it would also be interesting to try a longer carbon chain without the alcohol or a phenyl ring.

4.13 Synthesis of 1-benzyl-2-nitro-1*H*-imidazole (28)

In this project, most of the analogs have a methyl group at the nitrogen in the imidazole. By changing this to a benzyl group, more analogs can be made. The preparation of compound **28** was carried out in the same manner as for compound **9**, using benzyl bromide instead of iodomethane (Scheme 4.14) [32].



Scheme 4.14: Schematic prosedure for the preparation of 1-benzyl-2-nitro-1*H*-imidazole (28)

In the preparation of compound **28**, 2-nitroimidazole and Cs_2CO_3 were dissolved in acetonitrile and cooled down in an ice bath. Benzyl bromide was added dropwise before the reaction stirred with reflux at 65 °C for 1 h, to give the crude mixture containing the product. The product was purified with silica gel column chromatography with a mixture of hexane/ethyl acetate, to give the title compound in a yield of 43 % (88 mg). Both LC-MS and ^{1}H NMR analysis confirmed a clean product. Figure 4.20 shows the ^{1}H NMR spectrum of compound **28**.

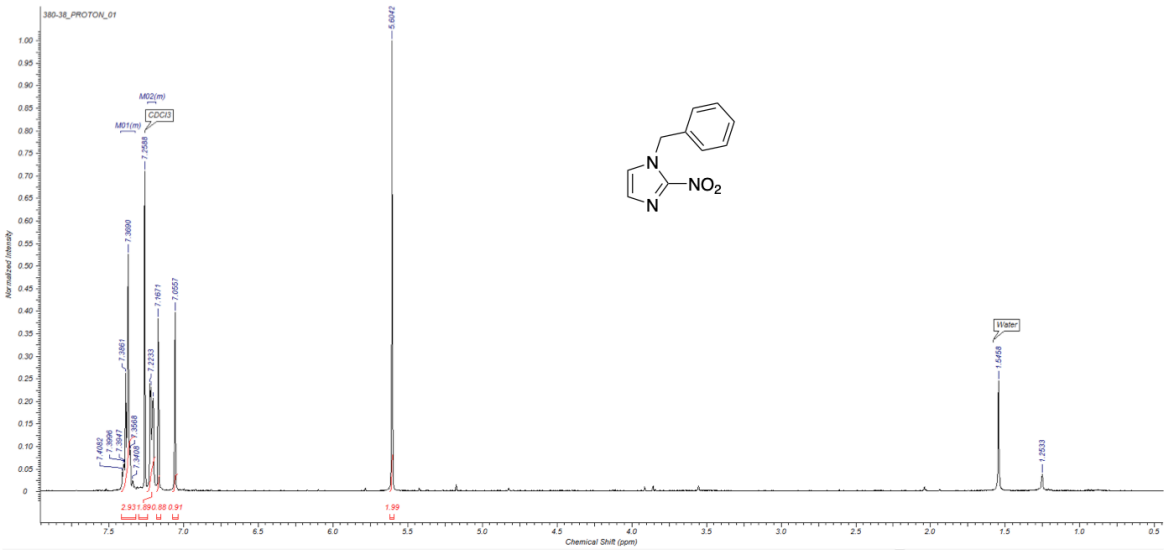
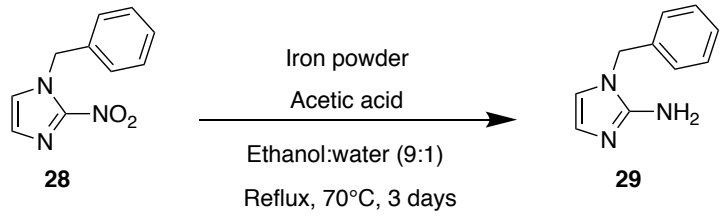


Figure 4.20: ^{1}H NMR spectrum of 1-benzyl-2-nitro-1*H*-imidazole (**28**)

The ^{1}H NMR spectrum (Figure 4.20) shows the five hydrogens in the phenyl (7.38 and 7.21 ppm) and the two hydrogens in the backbone of the imidazole (7.17 and 7.05 ppm) in the aromatic region. The two hydrogens more upfield (5.60 ppm) represent the two hydrogens in the benzylic position.

4.14 Synthesis of 1-benzyl-1*H*-imidazole-2-amin (29)

To reduce compound **28** to the corresponding amine, the presence of Palladium (Pd) will cleave off the benzyl at the nitrogen. Instead of using Pd as a catalyst, iron powder was used in the presence of acetic acid. The reaction time had to be increased, compared to the reduction with H_2 gas and Pd, to prepare the title compound (**29**). Scheme 4.15 shows the schematic procedure for the preparation of compound **29**.

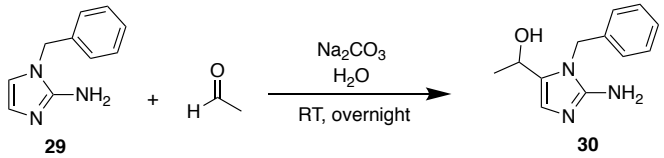


Scheme 4.15: Schematic prosedure for the preparation of 1-benzyl-1*H*-imidazole-2-amin (**29**)

In the preparation of compound **29**, compound **28** was dissolved in ethanol and water, before iron powder and acetic acid was added. After three days at 70 °C with reflux, LC-MS analysis was performed and showed full conversion. The product was tried purified on silica gel column chromatography, but the desired product was not able to be separated. Due to no purifications and some contaminations present in the product, an accurate yield cannot be given.

4.15 Synthesis of 1-(2-amino-1-benzyl-1*H*-imidazol-5-yl)ethan-1-ol (30)

Based on the results on the preparation of compound **11-13**, another analog was possible to make. As we know from before, the substituent on the nitrogen in the imidazole may change the activity of the imidazole. We know that the reaction works well with a methyl group at the nitrogen, but we do not know what will happen with a benzyl group attached to the nitrogen. Compound **29** was used to prepare compound **30**, in the same manner as for compound **11-13**. Scheme 4.16 shows the schematic procedure for the preparation of compound **30**.



Scheme 4.16: Schematic prosedure for the preparation of 1-(2-amino-1-benzyl-1*H*-imidazol-5-yl)ethan-1-ol (**30**)

After 24 h LC-MS analysis was performed and the desired product and the starting material were not observed. Some side-reactions may have happened, producing other compounds. The benzyl group is most likely the reason the backbone substitution did not happen. The benzyl may be more reactive, and reactions may have happened there instead of in the backbone of the imidazole.

4.16 Synthesis of 3-chloro-*N*-(1,5-dimethyl-1*H*-imidazol-2-yl)-4-fluorobenzamide (31)

Another part of this project was to see if some of the compounds worked as PET tracer precursors. This means that one part of the molecule needs to have a radioactive atom, making them active in PET analysis. In this project, the radioactive part was a ^{11}C in form of a CH_3-group. To achieve this, the last step of the synthesis had to be the methylation of the N-H in the imidazole. Some of the synthesized compounds have a free

N-H, leaving them as a good option. With methylation in a "hot cell" with $[^{11}C]CH_3$, it might be possible to make them as PET tracer precursors. Compound **25** was a good option to choose for a precursor. Before doing the methylation in the "hot cell" introducing the $[^{11}C]CH_3$, the synthesis needs to work with regular CH_3I. Figure 4.21 shows the procedure for the methylation of compound **25**.

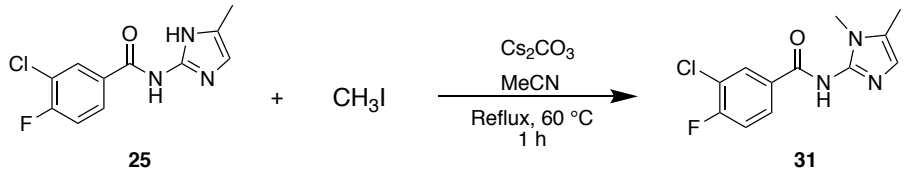


Figure 4.21: Schematic procedure for the synthesis of 3-chloro-*N*-(1,5-dimethyl-1*H*-imidazol-2-yl)-4-fluorobenzamide (**31**)

In the preparation of compound **31**, compound **25** and Cs_2CO_3 were dissolved in acetonitrile and iodomethane was added dropwise at O °C. The reaction stirred with reflux at 60 °C for 1 h, to give the crude mixture containing the product. The product was purified with silica gel column chromatography with a mixture of hexane and ethyl acetate. The product was crystallized with ethyl ether, to give the title compound in a yield of 18 % (23 mg). Both LC-MS and ^{1}H NMR analysis confirmed the formation of a clean product. Figure 4.22 shows the ^{1}H NMR spectrum for compound **31**.

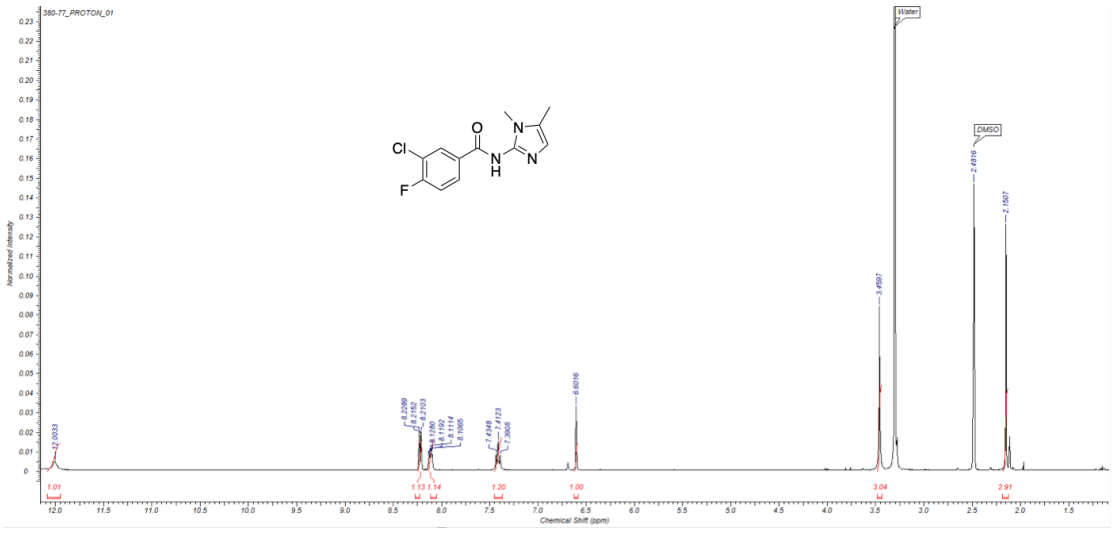


Figure 4.22: ^{1}H NMR spectrum for compound 3-chloro-*N*-(1,5-dimethyl-1*H*-imidazol-2-yl)-4-fluorobenzamide (**31**)

The ^{1}H NMR spectrum of compound **31** (Figure 4.22) was very similar to the spectrum for compound **25** (Figure 4.17, page 45). Both have the hydrogen in the amide (12.00 ppm), the three hydrogens in the phenyl (8.21, 8.11 and 7.41 ppm) and the hydrogen in the backbone of the imidazole (6.60 ppm). The difference is that compound **31** has a methyl group instead of a proton at the nitrogen. This is shown with a singlet with three protons at 3.45 ppm. In addition to this, there are the three hydrogens that represent the methyl group in the backbone (2.15 ppm) in the upfield region of the spectrum.

Preparation of the PET tracer precursor

To achieve a radiolabelled molecule, the reaction needs to take place in a "hot cell". This is a highly shielded fume hood that protects individuals from radioactivity. Before the compound was ready for the "hot-cell", an HPLC method that separates the precursor (compound **25**) and the radiolabelled product (compound **31** with $[^{11}C]CH_3$), needed to be made. This was done by making a method and run the precursor and a standard of compound **31** (without radiolabelling), separately and together. The standard and the radiolabelled product will have the same retention time with the same method. This can therefore be used after the radiolabelling reaction, to separate the two compounds and to see if the reaction worked.

The method that worked well and gave a good separation between the two products, used the gradient that is shown below (Figure 4.23). The gradient starts with 95 % water and 5 % acetonitrile, and after 5 min it increases to 50 % acetonitrile.

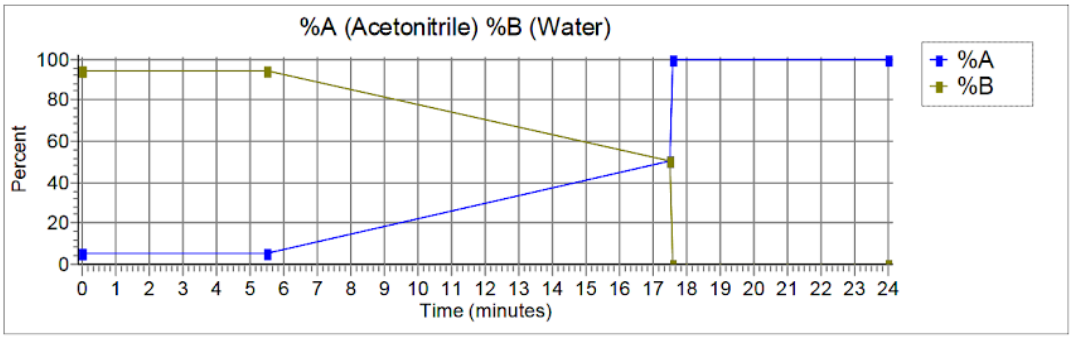


Figure 4.23: Solvent gradient for the separation of the precursor and the standard on HPLC

The method was first run with the standard without radiolabelling (compound **31**) and the compound got a retention time of 11:32 (left in Figure 4.24). Then the precursor (compound **25**) was run with the same method, and got a retention time of 14:06 min (right in Figure 4.24). The peak with a retention time of 10:50 in the right chromatogram (Figure 4.24), is an impurity in the precursor. In the chromatogram of the standard (left in Figure 4.24), there seems to be a small impurity too that might be the same as for the precursor, since the retention time is very similar.

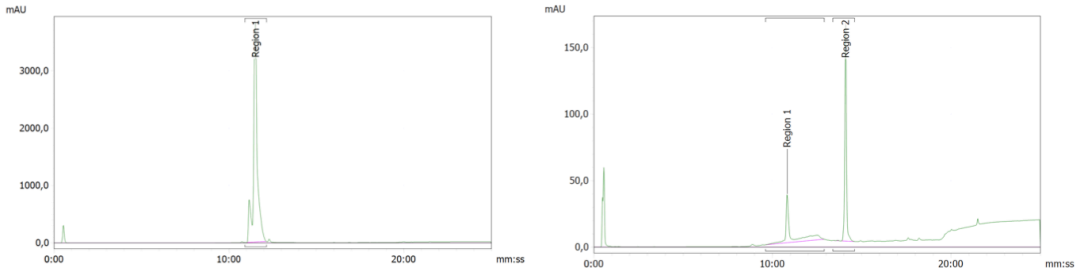


Figure 4.24: Chromatogram of the standard and the precursor

To confirm that this method worked after the radiolabelling reaction, where we will see both of these products, the method was run with a mixture of the two products. The chromatogram (Figure 4.25) shows a good separation between the products. The first peak (Region 1) had a retention time of 10:50, that corresponds with the retention time of the standard (compound **31**). The second peak (Region 2) had a retention time of 14:06, which corresponds to the retention time of the precursor (compound **31**). Also here we can see the peak from the impurity, the first peak that overlaps with the standard.

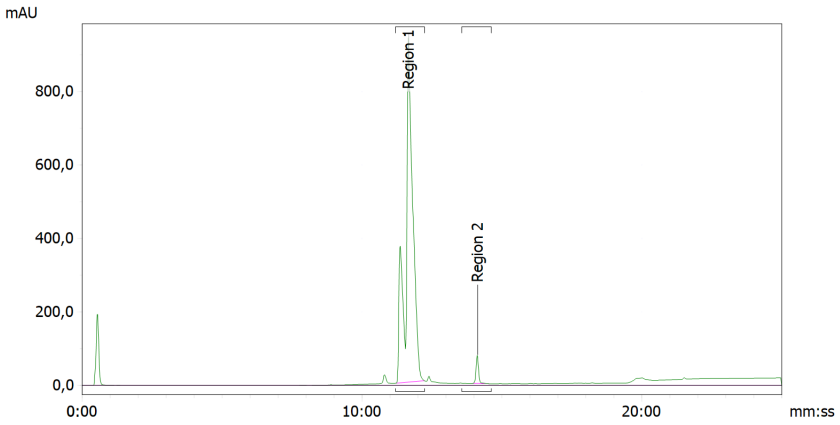


Figure 4.25: Chromatogram of a mixture of the standard and the precursor

Compound **31** was prepared in the "hot lab" using the same procedure as mentioned above. The only change that was made was the temperature. Instead of using reflux at 60 °C, the reaction was run in RT. This is the temperature that is most used in "hot cells", and was therefore tried in this case too. After the radiolabelling, HPLC was run with the prepared method and if the radiolabelling worked it would be visible in the chromatogram that detects the ^{11}C activity. All compounds with ^{11}C activity will give a peak in this chromatogram.

Figure 4.26 shows the ^{11}C chromatogram of the reaction after the methylation. The big peak in the chromatogram does not represent the product. It is a very common peak for the $[^{11}C]CH_3I$, and since this is the only peak in the chromatogram it means that the reaction did not work. The reason for this might be the change in temperature.

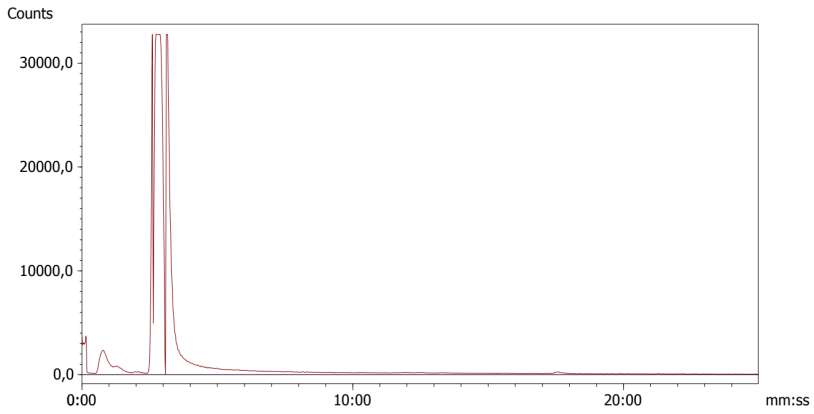


Figure 4.26: Chromatogram for the ^{11}C activity of the product after the methylation of compound **25**

For future work with this reaction, a change in temperature would be a good start. By increasing the temperature, the reaction is more similar to the original procedure. Another solvent may also be a better solution.

5 General methods

5.1 Chemicals

Most chemicals were purchased commercially and used as received. One exception is 1,3-diiodo-5,5-dimethylhydantoin (DIH) [43], that previously has been synthesized in our group using continuous flow.

5.2 Experimental description

TLC analyses were performed on silica plates, Camlab Polygram SIL G/UV254. Various mixtures of hexane/ethyl acetate and dichloromethane/methanol were used as mobile phases. Solvents were used as received, without any purifications.

Microwave synthesis was carried out on a Biotage Initiator Sixty EXP microwave system. The temperature scale vary from 40 - 250 °C and the pressure vary from 0 - 20 bar. The microwave operates at 0 - 400 W at 2.45 GHz. The reactor vials that were used in the synthesis were 10 mL.

5.3 Spectrometric and spectroscopic descriptions

NMR analysis was performed on a Bruker Biospin AV500, 500 MHz for ^{1}H NMR and a Bruker 400 MHz. Chemical shifts are given in ppm, coupling constants are given in Hz and multiplicity are given as singlet (s), doublet (d), doublets of doublets (dd), triplet (t) and multiplet (m).

LC-MS spectrum were obtained on an Agilent 6420A triple quadrupole mass analyzer with electrospray ionization (ESI), which is connected to a 1200 series LC module (binary pump, column compartment/oven, and autosampler). Agilent ZORBAX SB-C18, RRHT; 2.1 × 50 mm × 1.8 μL was used as column. Agilent 1260 Infinity Quaternary LC system

interfaced to an Agilent 6120 single Quadrupole mass spectrometer. Poroshell 120 EC-C18; 4.6 mm $\times$ 50 mm $\times$ 2.7 μL.

GC-MS analysis were performed on a capillary gas chromatograph with a fused silica column and helium as the carrier gas. The gas chromatograph was connected to a mass spectrometer using electron ionization (EI) as an ionization source.

Automatic flash column chromatography was carried out on Teledyne CombiFlash Rf chromatograph, with Agela Flash Column Silica-CS 60 Å.

HPLC analysis for PET tracer percursor were performed on Agilent 1260 infinity with a radio-HPLC detector Posi-RAM model 4 from LabLogic. The column used was a Luna Omega 3 μm polar C18 LC column 50 $\times$ 2.1 mm

6 Experimental procedures

1-metyl-1*H*-imidazole (1)

Imidazole (1.36 g, 20 mmol), KOH (1.50 g, 26.7 mmol) and acetone (20 mL) were placed in a round bottle flask with a magnetic stirrer, at 3 °C. Methyl iodide (1.33 mL, 21.4 mmol) was added dropwise to the mixture. The mixture changed color from blank to white, when methyl iodide was added. After 1.5 h the mixture was extracted with ethyl acetate and the organic phase was dried over Na_2SO_4. The solvent was evaporated under reduced pressure to provide the title compound in a yield of 84 % (1.38 g, 16.7 mmol). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.44 (s, 1H), 7.05 (s, 1H), 6.89 (s, 1H), 3.59 (s, 3H) ppm. Traces of contaminated $CDCl_3$ were observed with a chemical shift at 2.63, 2.18 and 1.26 ppm. MS found 83.1 [M+H$^+$].

1-(1-methyl-1*H*-imidazol-2-yl)cyclobutan-1-ol (2)

Methyl imidazole (5 mmol, 0.4 mL), was added to a round bottle flask that was flushed with nitrogen before and after the addition, to avoid moisture. Dry THF (10 mL) was added to the flask while stirring. The flask was placed in a bath with dry ice and acetone. When the mixture had cooled down, *n*-BuLi (5 mmol, 3.125 mL), was added dropwise to the mixture. After the addition, the reaction stirred for 45 min, before cyclobutanone (5 mmol, 0.376 mL) was added and the mixture stirred for another 45 min. After the reaction, the mixture was quenched with H_2O (15 mL) and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure to provide the title compound in a yield of 68 % [37]. MS found 153.1 [M+H$^+$].

4(5)-iodo-1-methyl-1*H*-imidazole (3)

The desired product was not synthesized

Method 1. Methylimidazole (0.45 mL, 5.7 mmol) and water (50 mL) was added to a 250 mL round bottle flask while stirring and immersed in an ice-water bath. NaOH (50 mL) was added. In a separate beaker DIH (0.352 g, 1.4 mmol) was weighed in and sulphuric acid (5 mL) was added dropwise to the beaker. The mixture became a black slurry and this was added to the flask dropwise in a period of 10 min. Immediately after the addition, acetic acid (50 % acid and 50 % water) was added until pH 6. The mixture was saturated with sodium chloride, so a small portion of NaCl was not dissolved. The water phase was extracted with diethyl ether (3 × 40 mL) and the combined organic phase was extracted with 10 % HCl (3 × 10 mL). This made the product as a salt that was in the aqueous phase, so the water was concentrated down. The crude was dissolved in water (5 mL), and neutralized with a saturated $NaHCO_3$ solution until pH 6. The solution was saturated with NaCl and extracted with ether (3 × 35 mL). The organic phase was combined and dried over Na_2SO_4, filtered and concentrated down under reduced pressure. ^{1}H NMR, LC-MS and GC analysis of the crude product did not show the desired product [26].

Method 2. To a solution of methylimidazole (0.45 mL, 5.7 mmol) in NaOH (3.9 M, 21 mL), a solution of KI (5.12 g, 31 mmol) and I_2 (3.04 g, 12.6 mmol) was added dropwise over a period of 30 min. The reaction mixture was stirred for 23 h in RT, followed by neutralization with acetic acid, which resulted in precipitation. The mixture was cooled on ice, filtered with Buchner funnel and washed with cold water and saturated Na_2SO_4. Most of the product were dissolved in the washing prosses and ^{1}H NMR and LC-MS analysis did not show the desired compound [27].

4(5)-iodoimidazole (4)

Water (50 mL) was added to a round bottle flask and immersed in an ice-bath. Imidazole (0.390 g, 5.7 mmol) was transferred to the flask, stirred until dissolved and NaOH (3.9 M, 50 mL) was added. In a separate beaker, sulphuric acid (5 mL) was added to DIH (0.517 g, 1.4 mmol), and vigorously stirred. The resulting viscous mixture was added dropwise to the imidazole solution over 10min. Immediately following the addition, the reaction mixture was neutralized until pH 6. The mixture was saturated with sodium chloride, so a small portion of NaCl was not dissolved. The water phase was extracted with diethyl ether (3 × 40 mL). The combined organic phase was extracted with 10% HCl (3 × 10 mL), and the product became a salt. The aqueous phase was combined and the water was evaporated. The crude was dissolved in water (5 mL), and neutralized with a saturated $NaHCO_3$ solution until pH 6. The solution was saturated with NaCl and extracted with ether (3 × 35 mL). The organic phase was combined and dried over Na_2SO_4, filtered and evaporated under reduced pressure to provide the title compound in a yield of 49 % (0.133 g, 0.69 mmol) [26]. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.12 (s, 1H), 7.81 (s, 1H) ppm. MS found 195.0 [M+H^+].

4-cyclobutyl-1*H*-imidazole (5)

The desired product was not synthesized

Method 1. 4-(5)-iodo-1*H*-imidazole (68 mg, 0.34 mmol), cyclobutylboronic acid (1.5 equiv), TBAB (0.008 g, 0.025 mmol) K_2HPO_4 (0,65 mmol) and $Pd(PPh_3)_4$ (3 mol %), were transferred to a microwave reactor tube equipped with a magnetic stirring bar. The tube was sealed and carefully flushed with argon. Methanol/water (4:1)(5 mL) was added to the tube and the tube was placed in the microwave cavity and heated at 100 °C for 60 min. After the reaction time, the crude reaction mixture was dissolved in HCl (10 %, 20 mL) and stirred vigorously. Not all the solid dissolved in the HCl. The aqueous phase was washed with diethyl ether (20 mL) to remove the boroxxine and PPh_3. NaOH was added to the aqueous phase until pH 12, and the solution became white and milky. The

alkaline solution was then extracted with diethyl ether (3 × 20 mL), organic extracts were combined and dried over anhydrous Na_2SO_4. The drying agent was removed and the solvent was evaporated under reduced pressure. LC-MS and ^{1}H NMR analysis of the product was collected, but the desired product was not observed [38].

Method 2. 4-(5)-iodo-1*H*-imidazole (68 mg, 0.34 mmol), cyclobutylboronic acid (0,41 mmol, 41 mg), K_2CO_3 (1.02 mmol, 141 mg) was added to a microwave tube, dissolved in toluene/water (2 mL/0.6 mL) and protected in an atmosphere of nitrogen. $Pd(PPh_3)_4$ (3 mg) was dissolved in toluene (0.5 mL) and added to the reaction tube. The reaction stirred at 90 °C in a microwave for 1 h. After the reaction, the solvent was evaporated under reduced pressure. The crude mixture was dissolved in water and extracted with DCM (3 × 20 mL). The organic phases were combined and dried over Na_2SO_4. The drying agent was removed and the solvent was evaporated under reduced pressure. LC-MS and ^{1}H NMR analysis of the product was collected, but the desired product was not observed [39].

***N*-Tosyl-4-iodoimidazole (6)**

4(5)-iodoimidazole (5.00 g, 25.8 mmol) and TosCl (4.91 g, 25.8 mmol) where transferred to a round bottle flask (100 mL) and flushed with argon gas. Dry THF (40 mL) was added and stirred to dissolve the solid. Et_3N was added the mixture stirred for 4 h in RT. After the reaction, the mixture was filtrated, and the solvent evaporated under reduced pressure. Yellow/white precipitate formed and the crude product was re-crystallized with DCM to provide the title compound as white crystals in a yield of 42 % [27]. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.87 (s, 1H), 7.82 (d, 2H, J=8.66Hz), 7.37 (m, 2H), 7.26 (s, 1H), 2.45 (s, 3H).

1-(1-tosyl-1*H*-imidazole-4-yl)cyclobutan-1-ol (7)

The desired product was not synthesized

4-iodo-1-tosyl-1*H*-imidazole (0.57 mmol, 198 mg), was added to a microwave tube and flushed with argon. Dry THF (2 mL) was added to the tube while stirring. The tube was placed in a bath with dry ice and acetone, and nBuLi (1.15 mmol, 0.46 mL) was added dropwise to the mixture. After the addition, the reaction stirred for 45 min before cyclobutanone (0.86 mmol, 65 μL) was added and the mixture stirred for 1 h. After the reaction, the mixture was quenched with H_2O (15 mL) and extracted with ethyl acetate (3 $\times$ 20 mL). The organic phase was combined, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. ^{1}H NMR and LC-MS analysis of the product was collected, but the desired molecule was not observed [37].

1-(2-amino-1*H*-imidazol-5-yl)ethan-1-ol (8)

2-aminoimidazole (1 mmol, 83 mg) was weighted in a vial and dissolved in H_2O (5 mL). Na_2CO_3 (0.6 mmol, 63 mg) was added and stirred for 10 min. After 10 min acetaldehyde (1.2 mmol, 68 μL) was added and stirred over night. After 19 h LC-MS was checked and showed starting material and a small peak of product. Automated flash chromatography was used to separate the product, but the desired product was not able to be isolated [34]. MS found 128.1 [M+H$^+$].

1-methyl-2-nitro-1*H*-imidazole (9)

2-nitroimidazole (1.76 mmol, 200 mg) and Cs_2CO_3 (2.64 mmol, 860 mg, 1.5 equiv) were dissolved in MeCN (15 mL) and CH_3I (2.11 mmol, 131 μL, 1.2 equiv) was added dropwise at 0 °C. After the addition the reaction mixture stirred for 1 h at 65 °C with reflux. After 1 h the reaction was quenched with water and concentrated down under reduced pressure. The resulting residue was diluted in water and extracted with ethyl acetate and the combined organic layers were dried over Na_2SO_4 and concentrated down under

reduced pressure. The product was purified with silica gel column chromatography with a mixture of hexane/ethyl acetate (100:0 % - 40:60 % gradient) to give the title compound as a white solid in a yield of 68% (152 mg) [32]. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.14 (s, 1H), 7.05 (s, 1H), 4.07 (s, 3H). MS found 128.1 [M+H^+].

1-methyl-1*H*-imidazol-2-amine (10)

Compound 9 (0.37 mmol, 47 mg) and $Pd(OH)_2$ (0.063 mmol, 9 mg) was added to a round bottle flask and dissolved in EtOH (15 mL). The flask was capped and flushed with argon gas. A balloon with H_2 gas was added and the reaction stirred overnight in RT. After the reaction the mixture was filtrated with celite and the solvent was concentrated down under reduced pressure, to give the title compound in a yield of 63 % (61mg) [41]. ^{1}H NMR (400 MHz, DMSO): δ = 6.48 (s, 1H), 6.29 (s, 1H), 5.17 (s, 2H), 3.29 (s, 3H). MS found 98.1 [M+H^+].

1-(2-amino-1-methyl-1*H*-imidazol-5-yl)ethan-1-ol (11)

1-methyl-1*H*-imidazole-2-amine (1 mmol, 97 mg) was weighted in a vial and dissolved in H_2O (5 mL). Na_2CO_3 (1.8 mmol, 189 mg) was added and stirred for 10 min. After 10 min acetaldehyde (3.6 mmol, 0.2 mL) was added and stirred for 24 h, to give a crude mixture containing product, with some impurities. After the reaction, the solvent was evaporated under reduced pressure, and the product was dissolved in methanol to filter off undissolved salt. Methanol was evaporated under reduced pressure and the crude product was dried [34]. MS found 142.1[M+H^+].

1-(2-amino-1-methyl-1*H*-imidazol-5-yl)-2-methylpropan-1-ol (12)

1-methyl-1*H*-imidazole-2-amine (1 mmol, 97 mg) was weighted in a vial and dissolved in H_2O (5 mL). Na_2CO_3 (1.8 mmol, 189 mg) was added and stirred for 10 min. After 10 min isobutyraldehyde (3.6 mmol, 0.328 mL) was added and stirred for 24 h, to give a crude mixture containing product, with some impurities and starting material. After the reaction the solvent was evaporated under reduced pressure, and the product was dissolved in methanol to filter off undissolved salt. Methanol was evaporated under reduced pressure and the crude product was dried [34]. MS found 170.1 [M+H^+].

1-(2-amino-1-methyl-1*H*-imidazol-5-yl)cyclobutan-1-ol (13)

1-methyl-1*H*-imidazole-2-amine (1 mmol, 97 mg) was weighted in a vial and dissolved in H_2O (5 mL). Na_2CO_3 (1.8 mmol, 189 mg) was added and stirred for 10 min. After 10 min cyclobutanone (3.6 mmol, 0.27 mL) was added and stirred for 48 h at 40 °C, to give a crude mixture containing product, with some impurities and starting material. After the reaction the solvent was evaporated under reduced pressure, and the product was dissolved in methanol to filter off undissolved salt. Methanol was evaporated under reduced pressure and the crude product was dried [34]. MS found 168.2 [M+H^+].

3-chloro-4-fluoro-*N*-(5-(1-hydroxyethyl)-1-methyl-1*H*-imidazol-2-yl) benzamide (14)

Crude solid from the synthesis of compound 11 (90 mg) was dissolved in DCM (10 mL). TEA (4 mmol, 0.56 mL) and 3-chloro-4-fluoro benzoyl chloride (1 mmol, 193 mg) was added and the reaction stirred in RT for 30 min to give a crude mixture containing product. After the reaction time the reaction was quenched with sodium bicarbonate (15 mL) and extracted with DCM (3 × 20 mL). The organic phase was combined, washed

with saturated NaCl solution, dried over anhydrous $MgSO_4$ and concentrated down under reduced pressure. The crude mixture was purified with silica gel column chromatography with a mixture of DCM/MeOH (100:0 % - 90:10 % gradient). The product was crystalized and completely purified with DCM to give the title compound as white crystals in a overall yield of 6 % (18 mg) [3]. ^{1}H NMR (400 MHz, DMSO): δ = 12.12 (s, 1H), 8.23 (dd, J=7.8, 2, 1H), 8.12 (m, 1H), 7.42 (t, J=8.8, 1H), 6.68 (s, 1H), 5.29 (d, J=5.9, 1H), 4.73 (m, 1H), 3.57 (s, 3H), 1.42 (d, J=6.4, 3H). MS found 298.1 [M+H$^+$].

3-chloro-4-fluoro-*N*-(1-methyl-5-vinyl-1*H*-imidazol-2-yl) benzamide (15)

The crude solid from the synthesis of compound 11 (0,300 g) was dissolved in 1,2-dichloroethane (10 mL), TEA (4 mmol, 0.56 mL) and 3-chloro-4-fluoro benzoyl chloride (2.2 mmol, 424 mg) was added and stirred over night at 70 ^{o}C, to give a crude mixture containing product. After the reaction time the reaction was quenched with sodium bicarbonate (15 mL) and extracted with DCM (3 $\times$ 20 mL). The organic phase was combined, washed with saturated NaCl solution, dried over anhydrous $MgSO_4$ and concentrated down under reduced pressure. The crude mixture was purified with silica gel column chromatography with a mixture of hexane/ethyl acetate (100:0 % - 50:50 %) to give the title compound as a white solid in a overall yield of 9 % (25 mg) [3]. ^{1}H NMR (400 MHz, DMSO): δ = 12.33 (s, 1H), 8.23 (d, J=7.3, 1H), 8.13 (m, 1H), 7.42 (t, J=8.8x2, 1H), 7.11 (s, 1H), 6.61 (dd, J=17.6,11.7, 1H) 5.77 (d, J=17.6, 1H), 5.32 (d, J=11.2, 1H), 3.58 (s, 3H). MS found 280.1 [M+H$^+$].

3-chloro-4-fluoro-*N*-(5-(1-hydroxy-2-methylpropyl)-1-methyl-1*H*-imidazol-2-yl)benzamide (16)

Crude solid from the synthesis of compound 12 (230 mg) was dissolved in DCM (10 mL). TEA (4 mmol, 0.56 mL) and 3-chloro-4-fluoro benzoyl chloride (1 mmol, 193 mg) was added and the reaction stirred in RT for 30 min to give a crude mixture containing product. After the reaction time the reaction was quenched with sodium bicarbonate (15 mL) and extracted with DCM (3 × 20 mL). The organic phase was combined, washed with saturated NaCl solution, dried over anhydrous $MgSO_4$ and concentrated down under reduced pressure. The crude mixture was purified with silica gel column chromatography with a mixture of DCM/MeOH (100:0 % - 90:10 % gradient). The product was crystalized with a mixture of hexane/ethyl acetate to get it completely pure and to give the title compound as white crystals in a overall yield of 5 % (14 mg) [3]. ^{1}H NMR (400 MHz, DMSO): δ = 12.13 (s, 1H), 8.22 (dd, J=7.6, 1.7, 1H), 8.13 (m, 1H), 7.42 (t, J=8.8, 1H), 6.67 (s, 1H), 5.33 (d, J=5.4, 1H), 4.20 (dd, J=7.3, 5.9, 1H), 3.56 (s, 3H), 1.98 (m, 1H), 0.97 (d, 3H, J=6.4), 0.81 (d, 6.4, 3H). MS found 326.1 [M+H$^+$].

3-chloro-4-fluoro-*N*-(5-(1-hydroxycyclobutyl)-1-methyl-1*H*-imidazol-2-yl)benzamide (17)

Crude solid from the synthesis of compound 13 (0.307 g) was dissolved in DCM (10 mL). TEA (4 mmol, 0.56 mL) and 3-chloro-4-fluoro benzoyl chloride (1 mmol, 193 mg) was added and the reaction stirred for 1 h in RT, to give a crude mixture containing product. After the reaction time the reaction was quenched with sodium bicarbonate (15 mL) and extracted with DCM (3 × 20 mL). The organic phase was combined, washed with saturated NaCl solution, dried over anhydrous $MgSO_4$ and concentrated down under reduced pressure. The crude mixture was purified with silica gel column chromatography with a mixture of DCM/MeOH (100:0 % - 90:10 % gradient). The product was completely purified with crystallization with a mixture of hexane/ethyl acetate to give the title compound as white crystals in a overall yield of 11 % (36 mg) [3]. ^{1}H NMR (400 MHz,

DMSO): δ = 12.11 (s, 1H), 8.23 (dd, J=7.6, 1.17, 1H), 8.12 (m, 1H), 7.42 (t, J=8.8, 1H), 6.84 (s, 1H), 5.79 (s, 1H), 3.53 (s, 3H), 2.41 (m, 2H), 2.25 (m, 2H), 1.74 (m, 1H), 1.49 (m, 1H). MS found 324.1 [M+H^+].

3-chloro-4-fluoro-*N*-(1*H*-imidazol-2-yl)benzamide (18)

1-methylimidazole-2-amin (1 mmol, 97 mg) was dissolved in DCM (10 mL). TEA (4 mmol, 0.56 mL) and 3-chloro-4 fluoro benzoyl chloride (1 mmol, 193 mg) was added and the reaction stirred in RT for 30 min to give a crude mixture containing product. After the reaction time the reaction was quenched with sodium bicarbonate (15 mL) and extracted with DCM (3 $\times$ 20 mL). The organic phase was combined, washed with saturated NaCl solution, dried over anhydrous $MgSO_4$ and concentrated down under reduced pressure. The product was purified with crystallization with ethyl ether to give the title compound as white crystals in a overall yield of 38 % (96 mg) [3]. ^{1}H NMR (400 MHz, DMSO): δ = 12.19 (s,1H), 8.22 (dd, J=7.8,2, 1H), 8.10 (m, 1H), 7.43 (t, J=8.8x2, 1H), 7.01 (d, J=1.5x2, 1H), 6.82 (d, J=2.4, 1H), 3.52 (s, 3H). MS found 254.0 [M+H^+].

N-(5-(1-hydroxyethyl)-1-methyl-1*H*-imidazol-2-yl)benzamide (19)

Crude mixture of compound 11 (222 mg) was dissolved in DCM (10 mL). TEA (4 mmol, 0.56 mL) and benzoyl chloride (1 mmol, 116 μL) was added and the reaction stirred for 30 min in RT, to give a crude mixture containing product. After the reaction time the reaction was quenched with sodium bicarbonate (15 mL) and extracted with DCM (3 $\times$ 20 mL). The organic phase was combined, washed with saturated NaCl solution, dried over anhydrous $MgSO_4$ and concentrated down under reduced pressure. The crude mixture was purified with silica gel column chromatography with a mixture of DCM/MeOH (100:0 % - 90:10 % gradient). The product was completely purified by crystallization with DCM to give the title compound as white crystals in a overall yield of 5 % (11 mg) [3]. ^{1}H NMR (400 MHz, DMSO): δ = 12.12 (s, 1H), 8.12 (s, 2H), 7.40 (s, 3H), 6.67 (s, 1H), 5.24 (s, 1H), 4.71 (m, 1H), 3.53 (s, 3H), 1.41 (d, J=6.4, 3H). MS found 246.1 [M+H^+].

N-(1-methyl-5-vinyl-*H*-imidazol-2-yl)benzamide (20)

Crude product of compound 11 (222 mg) was dissolved in 1,2-dichloroethane (10 mL). TEA (4 mmol, 0.56 mL) and benzoyl chloride (2.2 mmol, 0.25 mL) was added and stirred over night at 70 °C, to give a crude mixture containing product. After the reaction time the reaction was quenched with sodium bicarbonate (15 mL) and extracted with DCM (3 × 20 mL). The organic phase was combined, washed with saturated NaCl solution, dried over anhydrous $MgSO_4$ and concentrated down under reduced pressure. The crude mixture was purified with silica gel column chromatography with a mixture of DCM/MeOH (100:0 % - 90:10 % gradient). The product was not completely pure [3]. MS found 228.1 [M+H^+].

N-(5-(1-hydroxy-2-methylpropyl)-1-methyl-1*H*-imidazol-2-yl) benzamide (21)

The crude mixture of compound 12 (275 mg) was dissolved in DCM (10 mL). TEA (4 mmol, 0.56 mL) and benzoyl chloride (1 mmol, 116 μL) was added and the reaction stirred for 30 min at RT, to give a crude mixture containing product. After the reaction time, the reaction was quenched with sodium bicarbonate (15 mL) and extracted with DCM (3 × 20 mL). The organic phase was combined, washed with a saturated NaCl solution, dried over anhydrous $MgSO_4$ and concentrated down under reduced pressure. The crude mixture was purified with silica gel column chromatography with a mixture of DCM/MeOH (100:0 % - 90:10 % gradient). The product was completely purified by crystallization with ethyl ether to give the title compound as white crystals in an overall yield of 3 % (9 mg) [3]. ^{1}H NMR (400 MHz, DMSO): δ = 12.14 (s, 1H), 8.15 (d, J=6.4, 2H), 7.39 (d, J=7.3, 3H), 6.65 (s, 1H), 5.32 (s, 1H), 4.19 (t, J=6.6x2, 1H), 3.56 (s, 3H), 1.98 (m, 1H) 0.98 (d, J=6.4, 3H), 0.82 (d, J=6.8 3H). MS found 274.2 [M+H^+].

N-(5-(1-hydroxycyclobutyl)-1-methyl-1*H*-imidazol-2-yl) benzamide (22)

Crude solid from the synthesis of compound 13 (0.243 g) was dissolved in DCM (10 mL). TEA (4 mmol, 0.56 mL) and benzoyl chloride (1 mmol, 116 μL) was added and the reaction stirred for 30 min at RT, to give a crude mixture containing product. After the reaction time the reaction was quenched with sodium bicarbonate (15 mL) and extracted with DCM (3 $\times$ 20 mL). The organic phase was combined, washed with a saturated NaCl solution, dried over anhydrous $MgSO_4$ and concentrated down under reduced pressure. The crude mixture was purified with silica gel column chromatography with a mixture of DCM/MeOH (100:0 % - 90:10 % gradient). The product was completely purified by crystallization with DCM to give the title compound as white crystals in a overall yield of 3 % (8 mg) [3]. ^{1}H NMR (400 MHz, DMSO): δ = 12.14 (s, 1H) 8.14 (s, 2H), 7.40 (s, 3H), 6.82 (s, 1H), 5.77 (s, 1H), 3.53 (s, 3H), 2.41 (m, 2H), 2.25 (m, 2H), 1.74 (m, 1H) 1.51 (m, 1H). MS found 272.1 [M+H$^+$].

N-(1-methyl-1*H*-imidazol-2-yl)benzamide (23)

1-methylimidazole-2-amin (1 mmol, 97 mg) was dissolved in DCM (10 mL). TEA (4 mmol, 0.56 mL) and benzoyl chloride (1mmol, 116μL) was added and the reaction stirred for 30 min at RT, to give a crude mixture containing product. After the reaction time the reaction was quenched with sodium bicarbonate (15 mL) and extracted with DCM (3 $\times$ 20 mL). The organic phase was combined, washed with saturated NaCl solution, dried over anhydrous $MgSO_4$ and concentrated down under reduced pressure. The crude mixture was purified with silica gel column chromatography with a mixture of DCM/MeOH (100:0 % - 90:10 % gradient). The product was completely purified by crystallization with ethyl ether to give the title compound as white crystals in a overall yield of 17 % (33 mg) [3]. ^{1}H NMR (400 MHz, DMSO): δ = 12.21 (s, 1H) 8.10 (s, 2H), 7.42 (s, 3H), 7.01 (s, 1H) 6.80 (s, 1H), 3.49 (s, 3H). MS found 202.1 [M+H$^+$].

3-chloro-4-fluoro-*N*-(1*H*-imidazol-2-yl)benzamide (24)

2-aminoimidazole (1.08 mmol, 90 mg) was dissolved in DCM (10 mL). TEA (4 mmol, 0.56 mL) and 3-chloro-4-fluoro benzoyl chloride (1 mmol, 193 mg) was added and the reaction stirred for 30 min at RT, to give a crude mixture containing product. After extracting with DCM, the water phase still contained the product, due to low solubility of the product in DCM. The water phase was therefor extracted with ethyl ether and concentrated down. The crude solid of the DCM layer were dissolved in ethyl acetate and the solid was filtrated off. The ethyl acetate layer was dried over Na_2SO_4 and concentrated down to give the title compound in a overall yield of 36 % (86 mg) [3]. ^{1}H NMR (400 MHz, DMSO): δ = 7.98 (dd, J=6.8,2, 1H), 7.76 (m, 1H), 7.59 (t, J=8.8x2, 1H), 6.75 (s, 2H), 6.69 (d, J=2, 1H), 6.47 (d, J=2, 1H). MS found 240.1 [M+H$^+$].

3-chloro-4-fluoro-*N*-(5-methyl-1*H*-imidazol-2-yl)benzamide (25)

5-methylimidazole-2-amine (1,02 mmol, 99 mg) was dissolved in DCM (10 mL). TEA (4 mmol, 0.56mL) and 3-chloro-4-fluoro benzoyl chloride (1 mmol, 193 mg) was added and the reaction stirred for 30 min at RT, to give a crude mixture containing product. After the reaction time the reaction was quenched with sodium bicarbonate (15 mL) and extracted with DCM (3 $\times$ 20 mL). The organic phase was combined, washed with saturated NaCl solution, dried over anhydrous $MgSO_4$ and concentrated down under reduced pressure. The crude mixture was purified with silica gel column chromatography with a mixture of hexane/ethyl acetate (100:0 % - 50:50 % gradient). The product was completely purified by crystallization with ethyl ether to give the title compound as white crystals in a overall yield of 8 % (19 mg) [3]. ^{1}H NMR (400 MHz, DMSO): δ = 11.77 (s, 2H) 8.19 (dd, J=7.3,2, 1H), 8.03 (m, 1H), 7.45 (t, J=9x2, 1H), 6.52 (s, 1H), 2.09 (s, 3H). MS found 254.0 [M+H$^+$].

N-(1*H*-imidazol-2-yl)benzamide (26)

1*H*-imidazole-2-amine (1 mmol, 83 mg) was dissolved in DCM (10 mL). TEA (4 mmol, 0.56 mL) and benzoyl chloride (1mmol, 116 μL) was added and the reaction stirred for 30 min at RT, to give a crude mixture containing product. After the reaction time the reaction was quenched with sodium bicarbonate (15 mL) and extracted with DCM (3 $\times$ 20 mL). The organic phase was combined, washed with saturated NaCl solution, dried over anhydrous $MgSO_4$ and concentrated down under reduced pressure. The product was purified with silica gel column chromatography with a mixture of hexane/ethyl acetate (100:0 % - 50:50 % gradient), to give the title compound as white crystals in a overall yield of 6 % (11 mg) [3]. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.03 (m, 2H), 7.60 (m, 1H), 7.51 (m, 2H), 6.65 (s, 2H). MS found 188.1 [M+H^+].

N-(5-methyl-1*H*-imidazol-2-yl)benzamide (27)

5-methylimidazole-2-amine (1 mmol, 97 mg) was dissolved in DCM (10 mL). TEA (4 mmol, 0.56 mL) and benzoyl chloride (1 mmol, 116μL) was added and the reaction stirred for 30 min at RT, to give a crude mixture containing product. After the reaction time the reaction was quenched with sodium bicarbonate (15 mL) and extracted with DCM (3 $\times$ 20 mL). The organic phase was combined, washed with saturated NaCl solution, dried over anhydrous $MgSO_4$ and concentrated down under reduced pressure. The crude mixture was purified with silica gel column chromatography with a mixture of hexane/ethyl acetate (100:0 % - 40:60 % gradient), to give the title compound as white crystals in a overall yield of 12 % (23 mg) [3]. ^{1}H NMR (400 MHz, MeOH): δ = 7.97 (m, 2H), 7.56 (m, 1H), 7.49 (m, 2H), 6.56 (s, 1H), 2.19 (s, 3H). MS found 202.1 [M+H^+].

1-benzyl-2-nitro-1*H*-imidazole (28)

2-nitroimidazole (0.5 mmol, 57 mg) and Cs_2CO_3 (0.75 mmol, 244 mg, 1.5 equiv) was dissolved in MeCN (3 mL) and benzyl bromide (0.6 mmol, 71 μL, 1.5 equiv) was added dropwise at 0 °C. The reaction mixture stirred for 1 h with reflux at 65 °C. After 1 h the reaction was quenched with water and concentrated down under reduced pressure. The resulting residue was diluted in water and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and concentrated down under reduced pressure. The product was purified with silica gel column chromatography with a mixture of hexane/ethyl acetate (100:0 % - 60:40 % gradient) to give the title compound as a yellow solid in a overall yield of 43 % (88 mg) [32]. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38 (m, 3H), 7.21 (m, 2H), 7.17 (s, 1H), 7.05 (s, 1H), 5.60 (s 2H). MS found 204.1 [M+H$^+$].

1-benzyl-1*H*-imidazol-2-amine (29)

Compound 28 (0.23 mmol, 47 mg) was dissolved in 5 mL of ethanol:water (9:1), iron powder (5 equiv, 1.15 mmol, 65 mg) and acetic acid (10 drops) was added and the reaction stirred at 70 °C under reflux over the weekend. After 3 days LC-MS confirmed conversion and the mixture was filtrated with celite and concentrated down. MS found 174.1 [M+H$^+$].

1-(2-amino-1-benzyl-1*H*-imidazol-5-yl)ethan-1-ol (30)

The desired product was not synthesized

Crude mixture of compound 29 (153 mg, 0.88 mmol) was dissolved in H_2O (5 mL). Na_2CO_3 (1.8 mmol, 189 mg) was added and stirred for 10 min. After 10 min acetaldehyde (3.6 mmol, 0.2 mL) was added and stirred for 24 h in RT. After the reaction, the solvent was evaporated under reduced pressure, and the product was dissolved in methanol to filter off undissolved salt. Methanol was evaporated under reduced pressure and the crude product was dried [34]. LC-MS was collected but the desired product was not observed.

3-chloro-*N*-(1,5-dimethyl-1*H*-imidazol-2-yl)-4-fluorobenzamide (31)

Compound 25 (0.46 mmol, 117 mg) and Cs_2CO_3 (1.5 equiv, 0.69 mmol, 225 mg) was dissolved in MeCN (15 mL) and CH_3I (1.2 equiv, 0.55 mmol, 34 μL) was added dropwise at 0 °C. The reaction mixture stirred for 1 h with reflux at 65 °C. After 1h the reaction was quenched with water and concentrated down under reduced pressure. The resulting residue was diluted in water and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and concentrated down under reduced pressure. The product was purified with silica gel column chromatography with a mixture of hexane/ethyl acetate (100:0 % - 40:60 % gradient) and crystallized with ethyl ether to give the title compound as white crystals in a overall yield of 18 % (23 mg) [32]. ^{1}H NMR (400 MHz, DMSO): δ = 12.00 (s, 1H), 8.22 (dd, J=7.4,2, 1H), 8.11 (m, 1H), 7.41 (t, J=8.8x2, 1H), 6.60 (s, 1H), 3.46 (s, 3H), 2.15 (s, 3H). MS found 268.0 [M+H$^+$].

7 References

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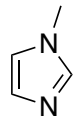
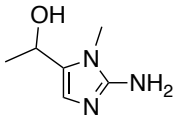
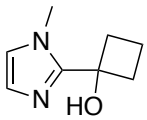
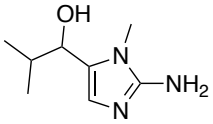
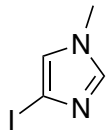
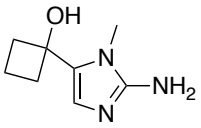
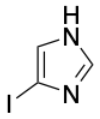
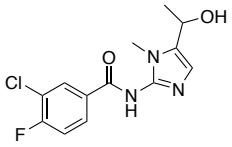
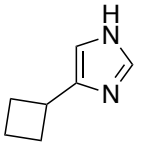
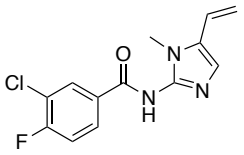
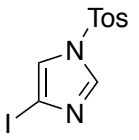
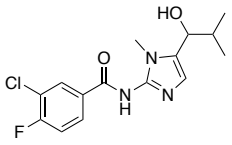
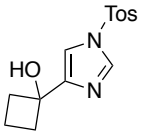
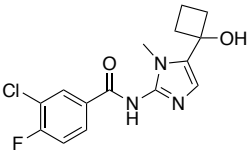
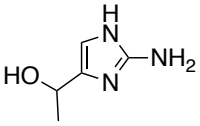
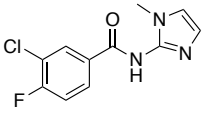
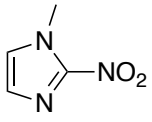
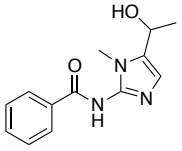
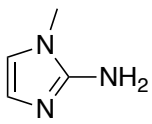
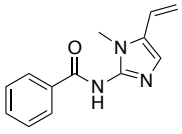
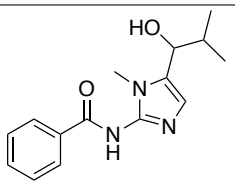
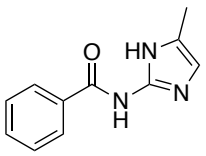
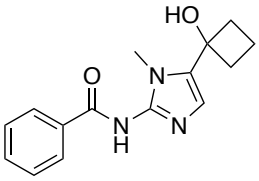
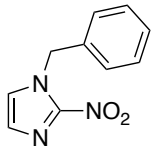
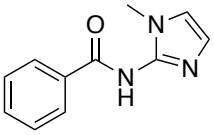
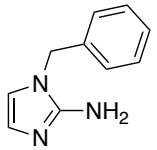
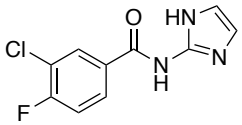
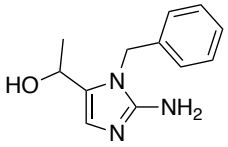
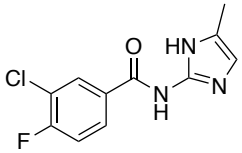
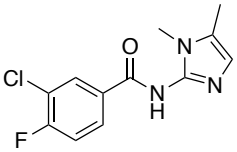
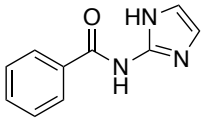
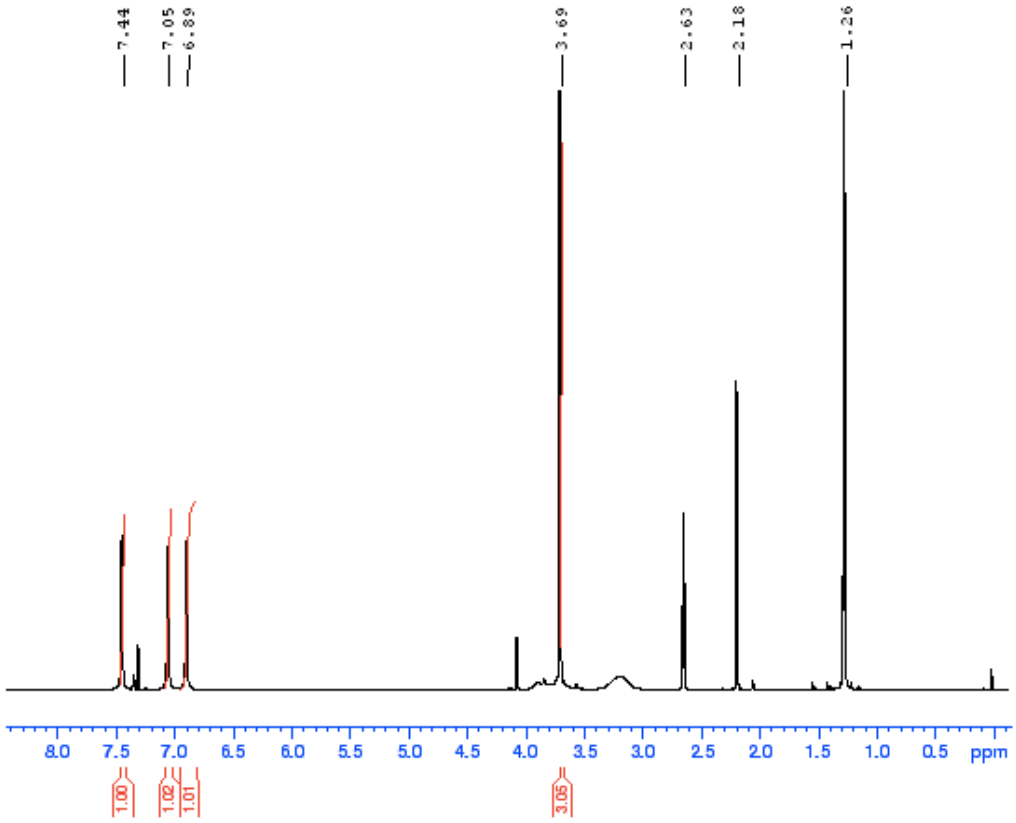
Appendix A List of compounds

Compound #	Structure	Compound #	Structure
1		**11**	
2		**12**	
3		**13**	
4		**14**	
5		**15**	
6		**16**	
7		**17**	
8		**18**	
9		**19**	
10		**20**	

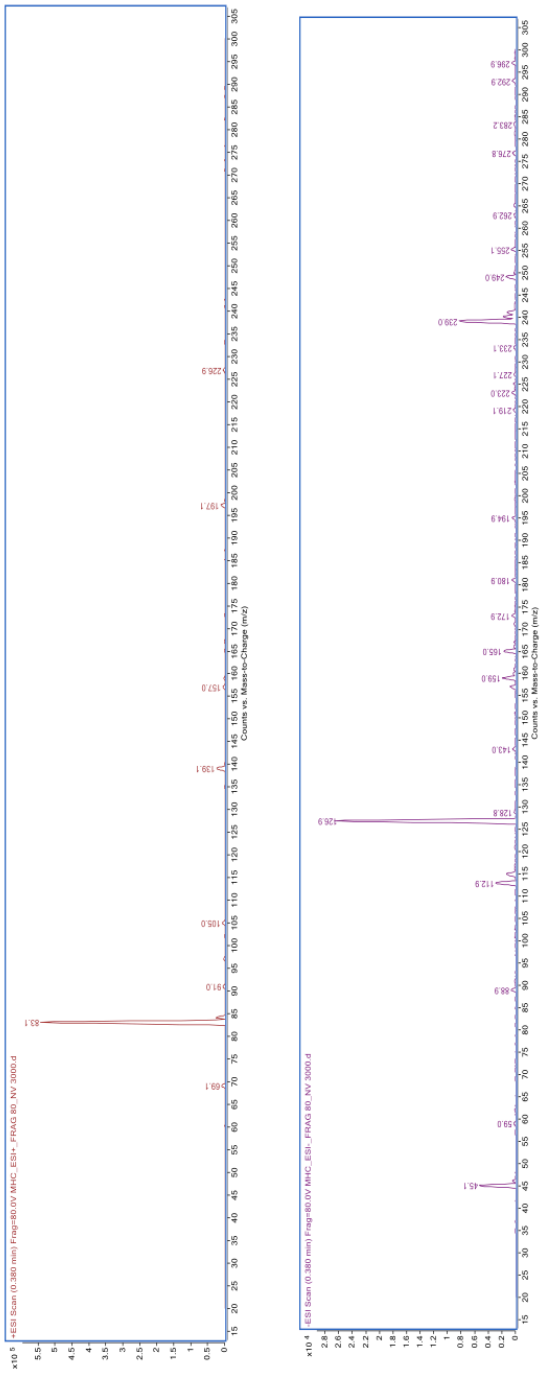
Compound #	Structure	Compound #	Structure
21		**27**	
22		**28**	
23		**29**	
24		**30**	
25		**31**	
26			

Appendix B Spectral data

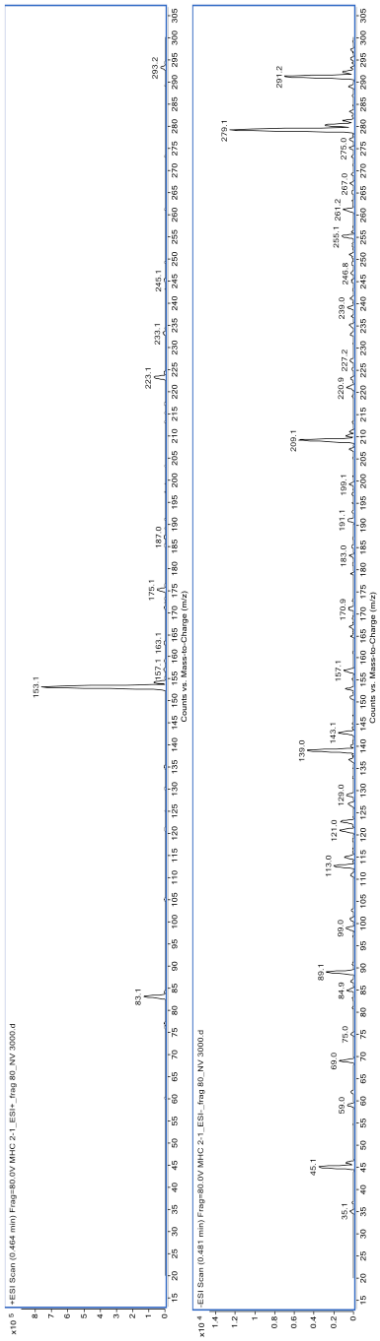
Proton spectrum of 1-methyl-1*H*-imidazole (1)



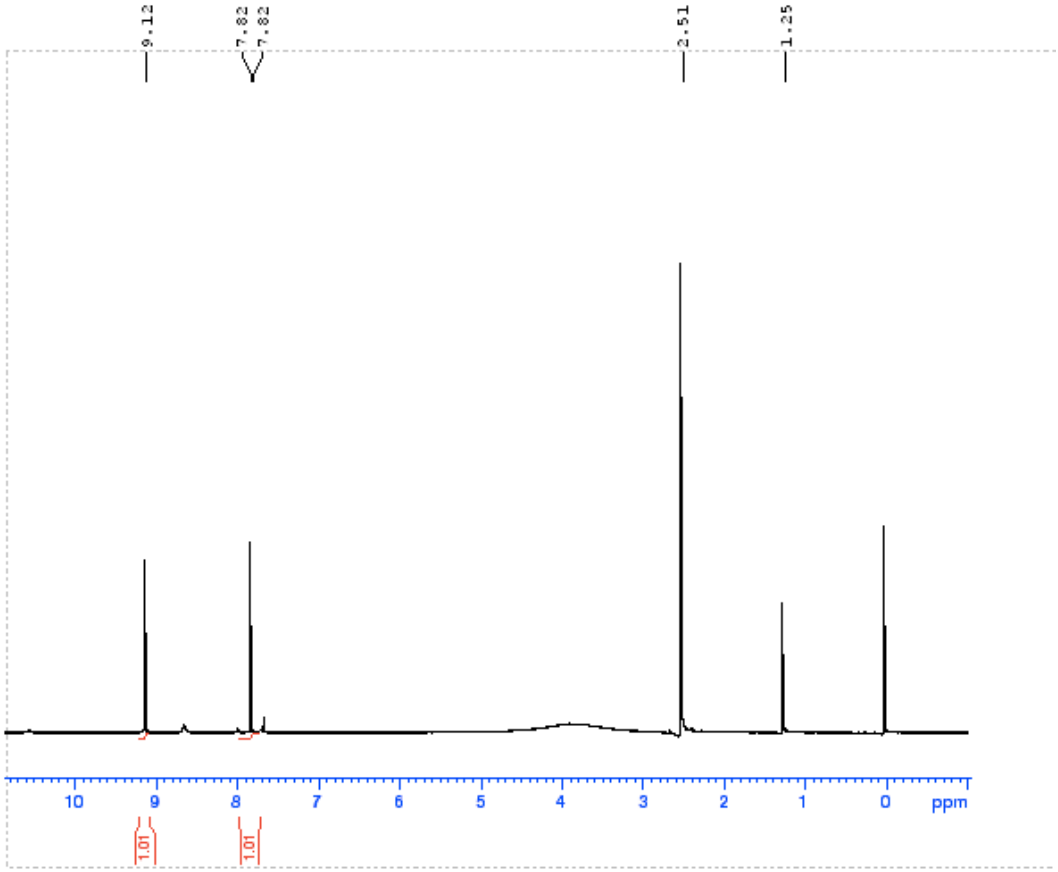
LC-MS spectrum of of 1-methyl-1*H*-imidazole (1)



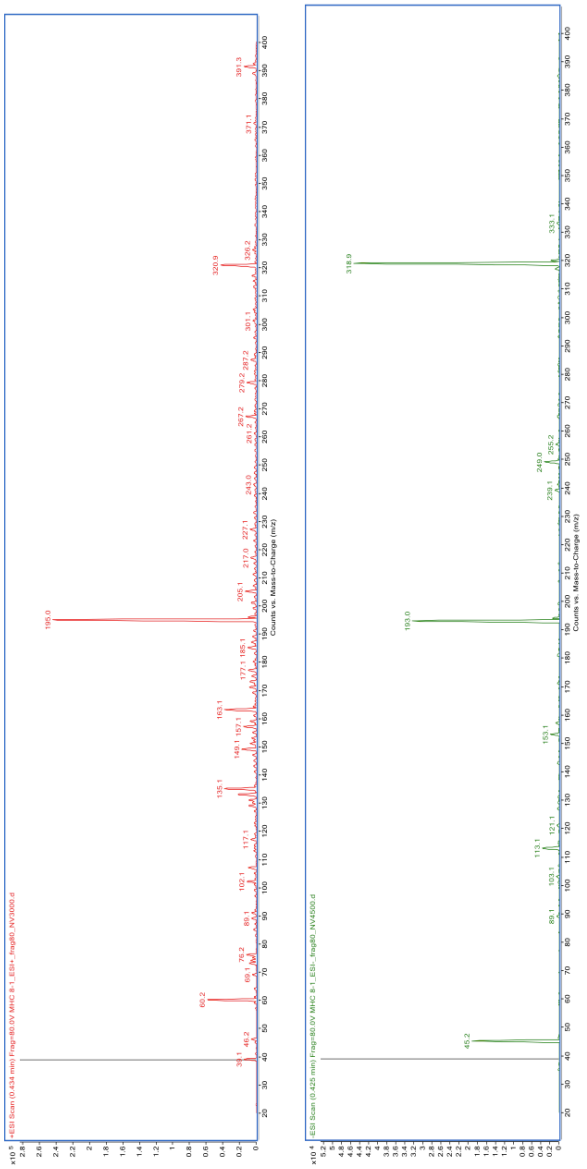
LC-MS spectrum of 1-(1-methyl-1*H*-imidazol-2-yl)cyclobutan-1-ol (2)



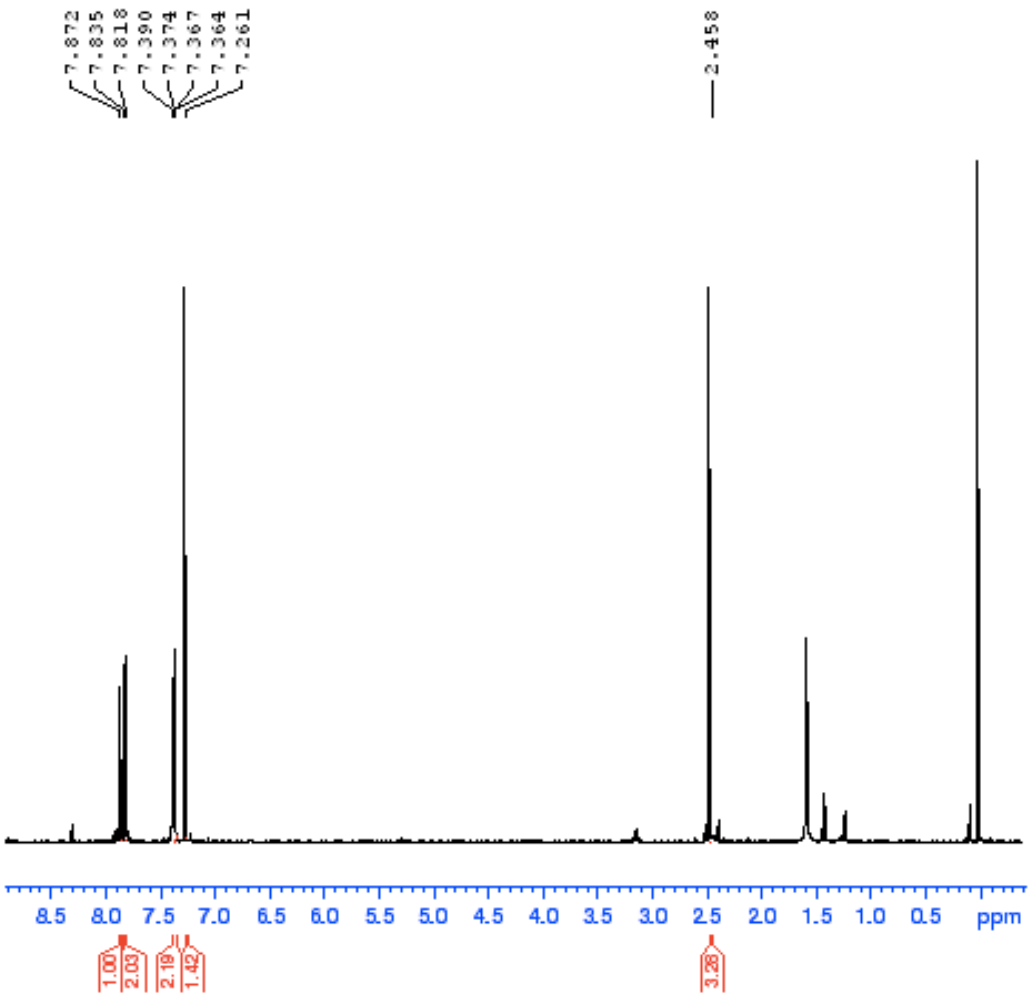
Proton spectrum of 4(5)-iodoimidazole (4)



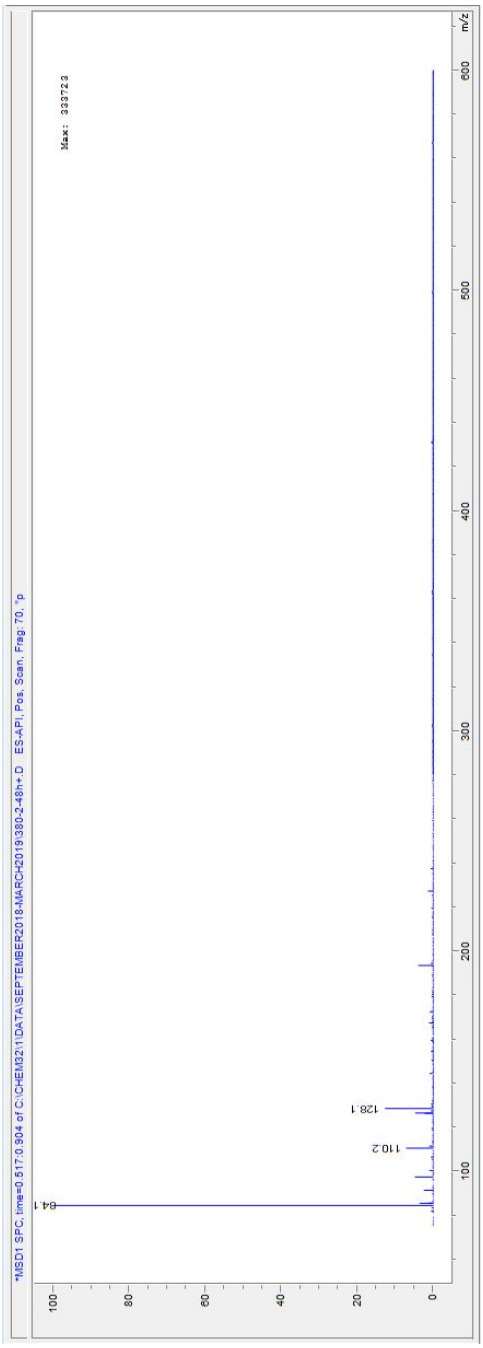
LC-MS spectrum of 4(5)-iodoimidazole (4)



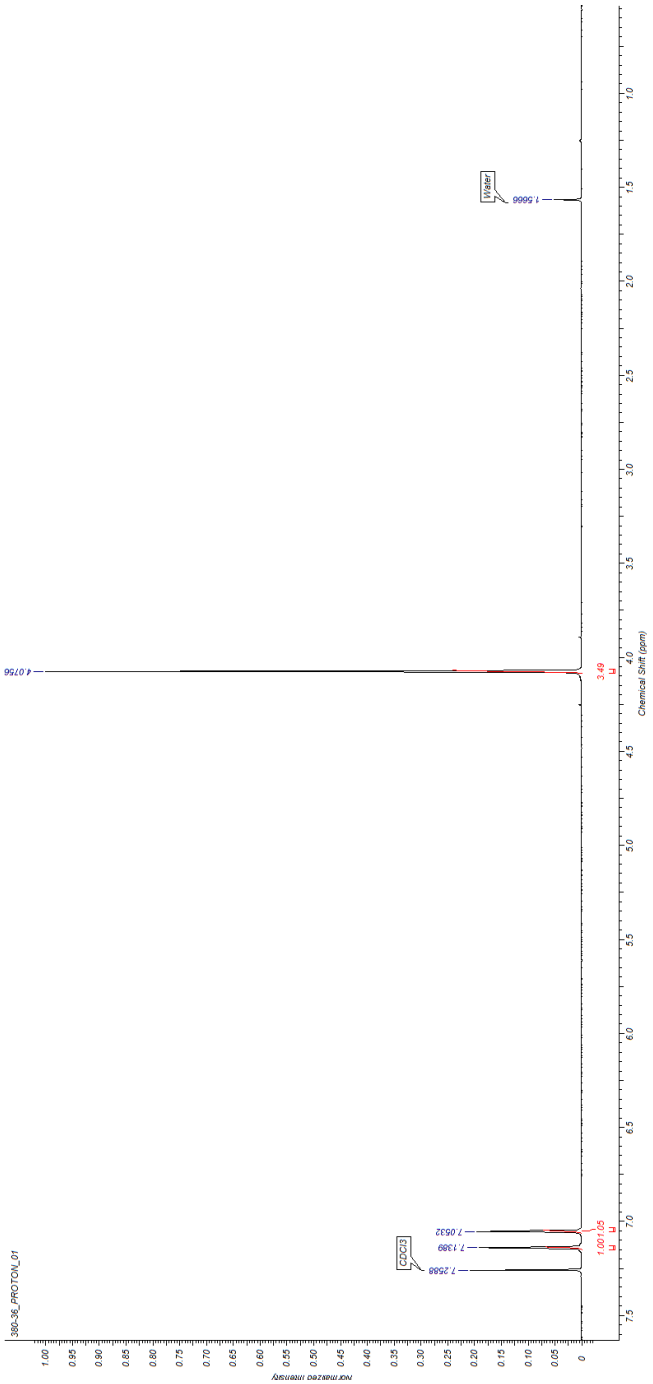
Proton spectrum of *N*-Tosyl-4-iodoimidazole (6)



LC-MS spectrum of 1-(2-amino-1*H*-imidazol-5-yl)ethan-1-ol (8)



Proton spectrum of 1-methyl-2-nitro-1*H*-imidazole (9)



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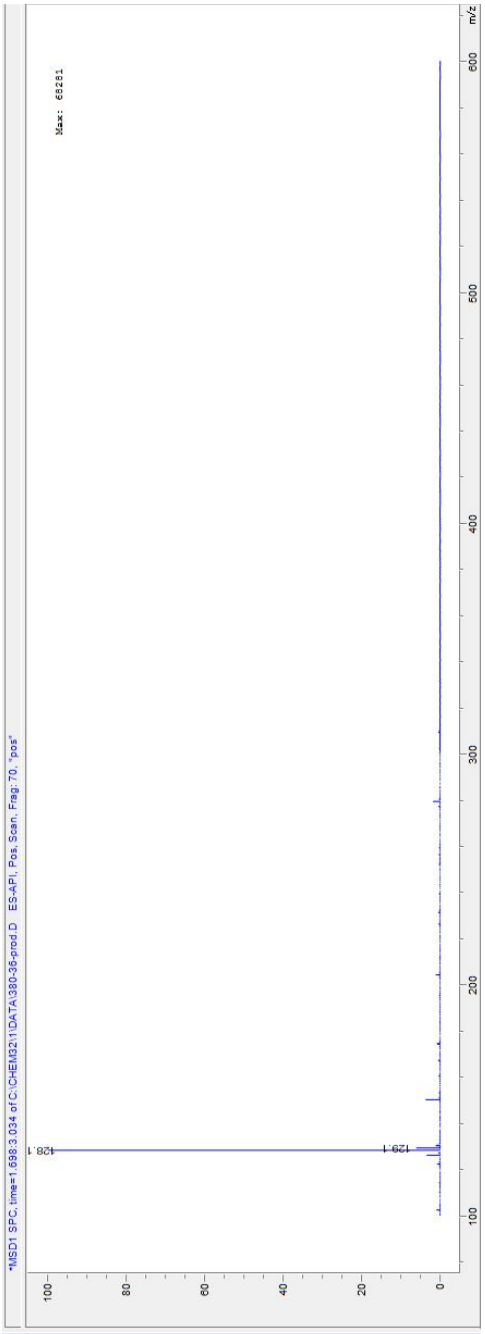
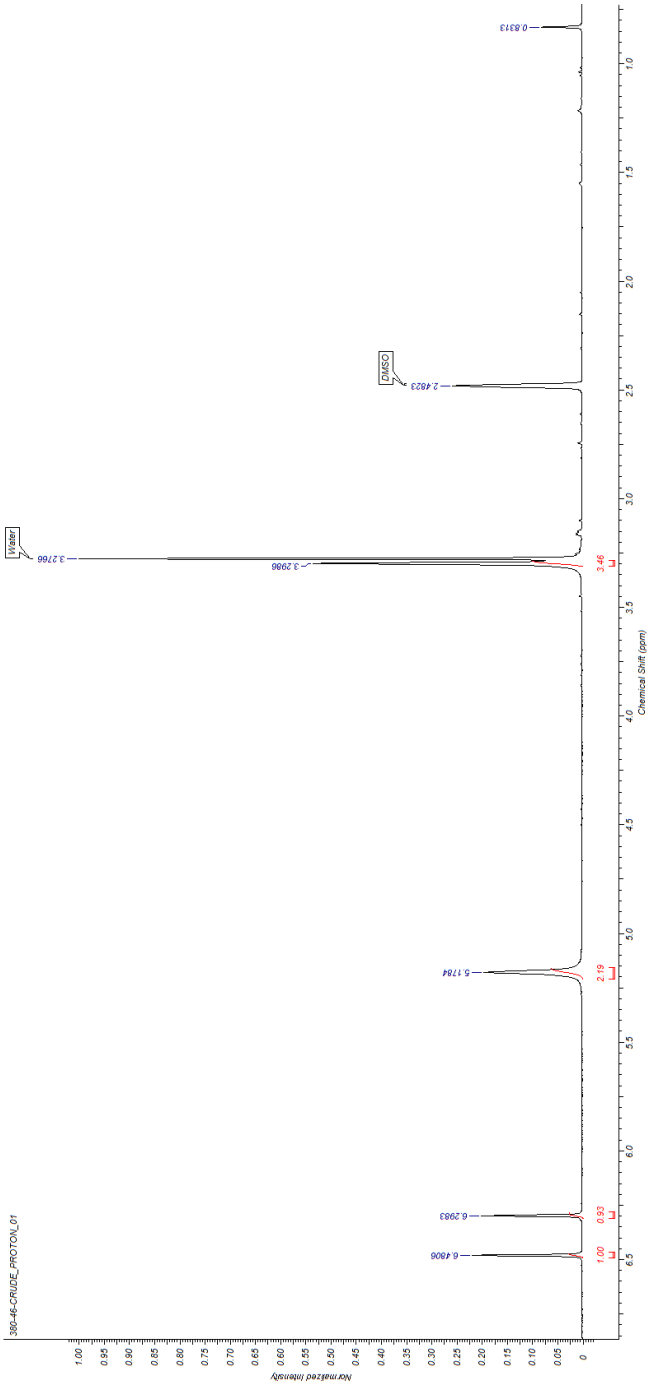
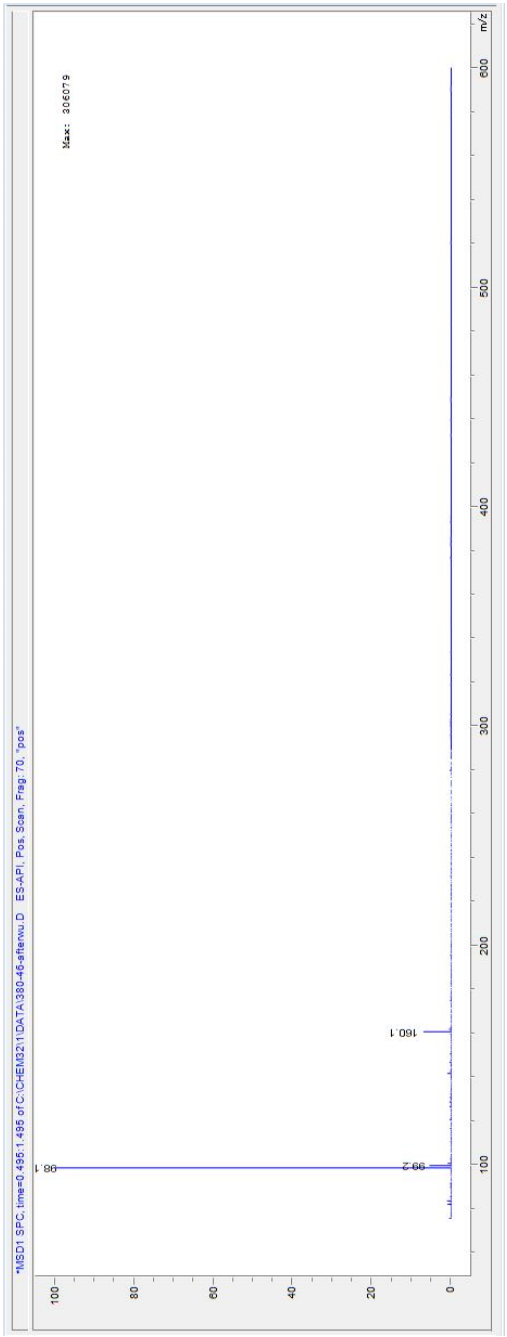


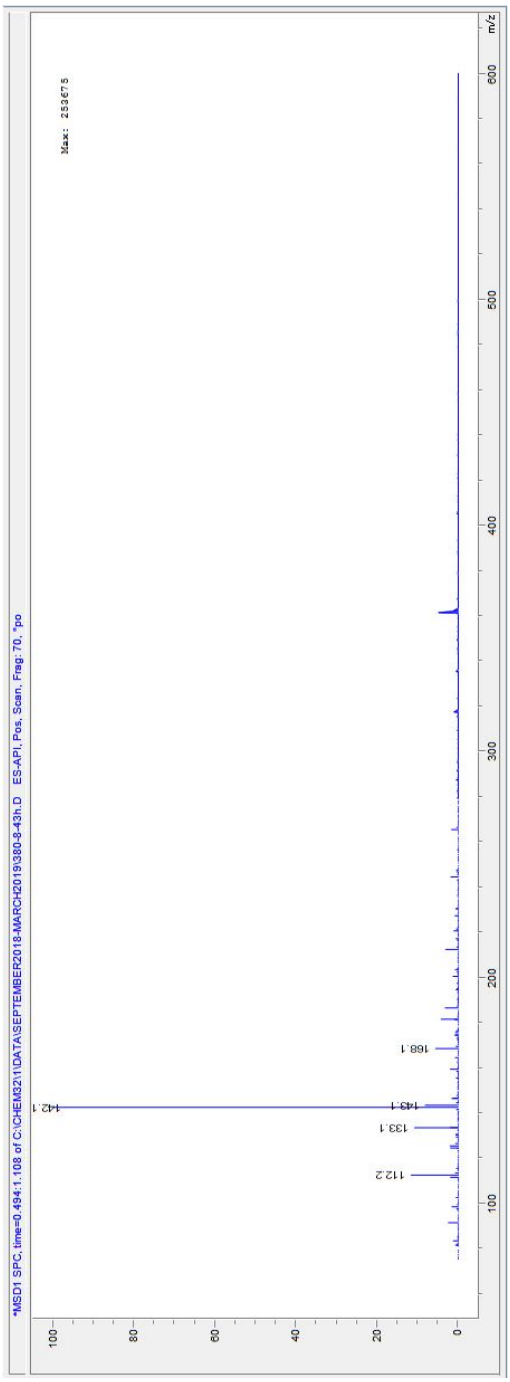
Proton spectrum of 1-methyl-1*H*-imidazol-2-amine (10)



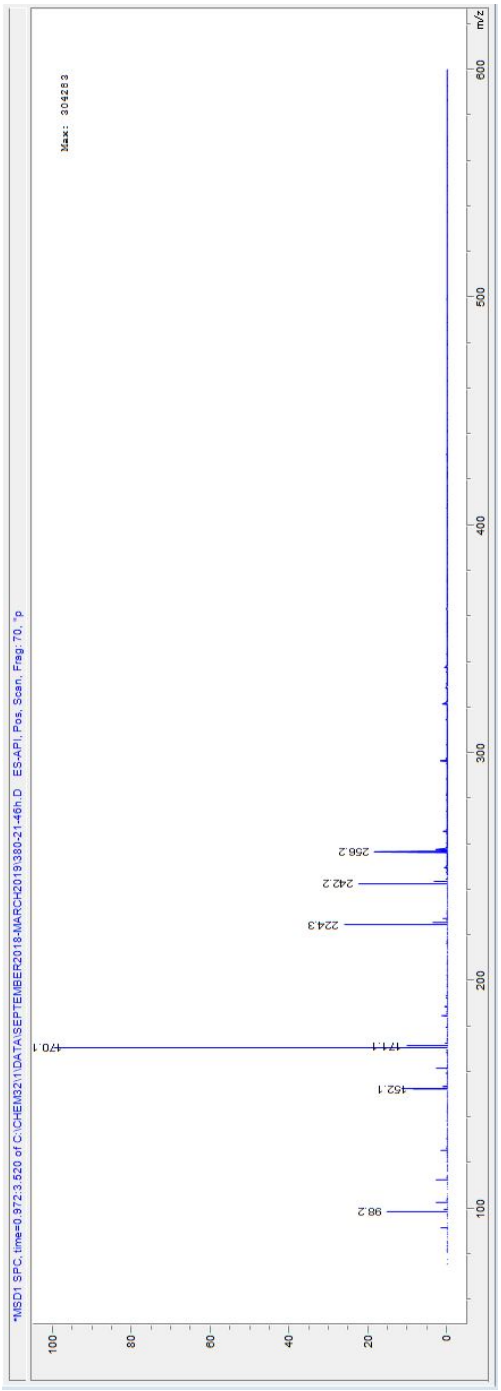
LC-MS spectrum of 1-methyl-1*H*-imidazol-2-amine (10)



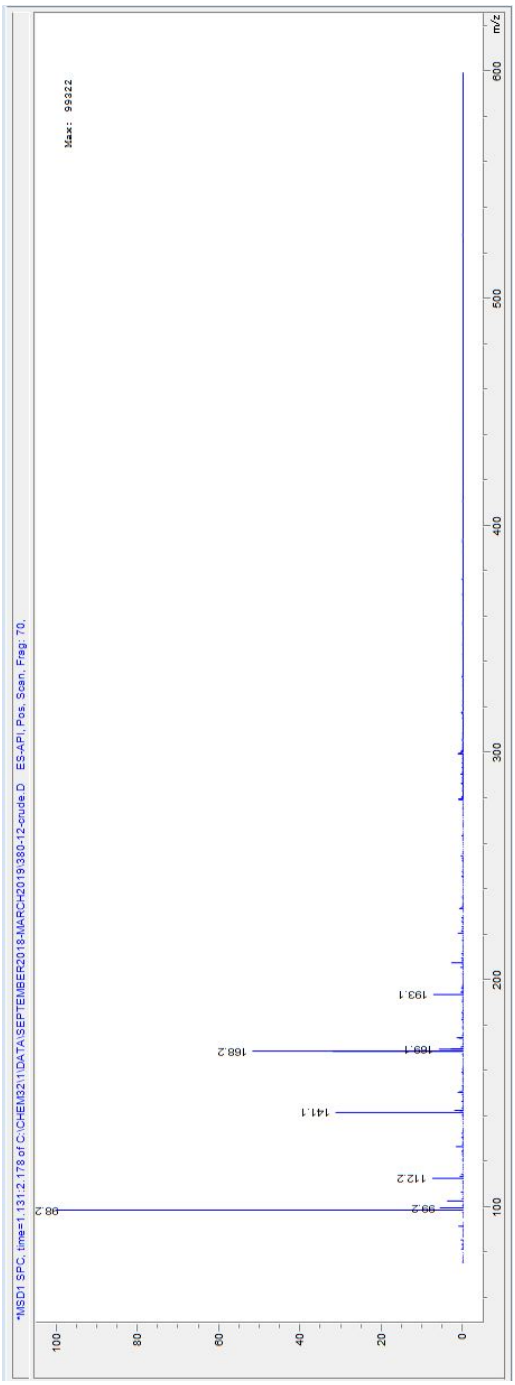
LC-MS spectrum of 1-(2-amino-1-methyl-1*H*-imidazol-5-yl)ethan-1-ol (11)



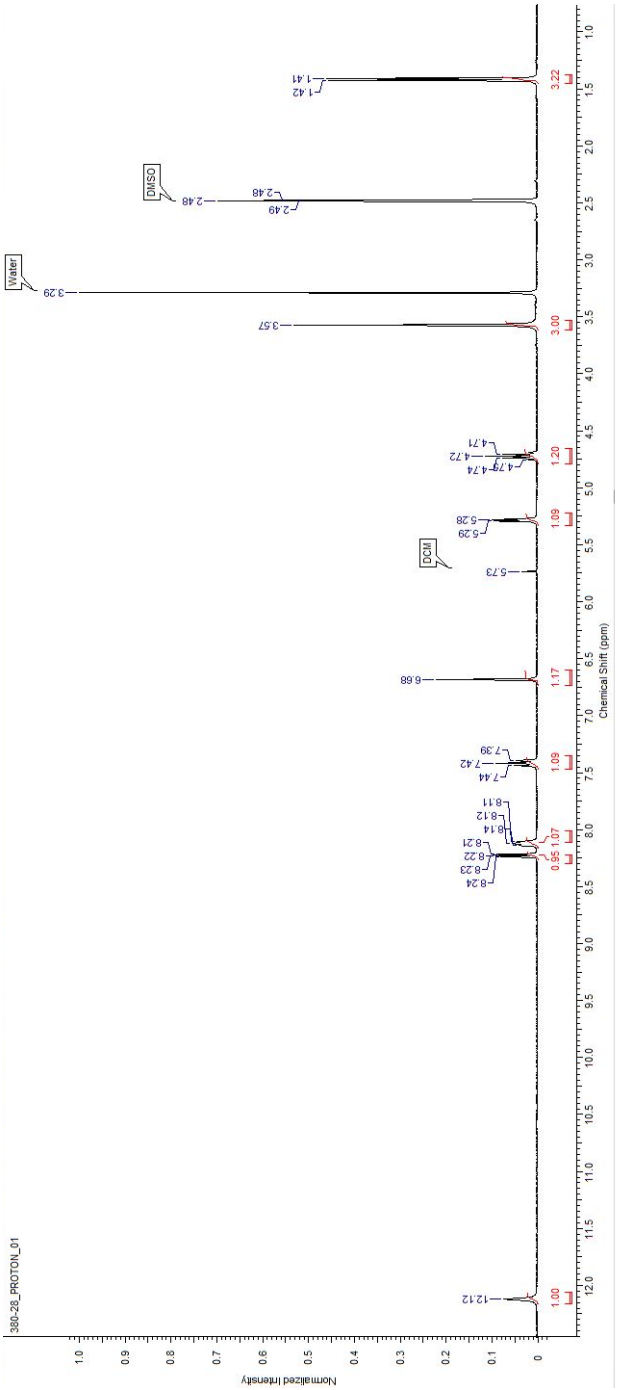
LC-MS spectrum of 1-(2-amino-1-methyl-1*H*-imidazol-5-yl)-2-methylpropan-1-ol (12)



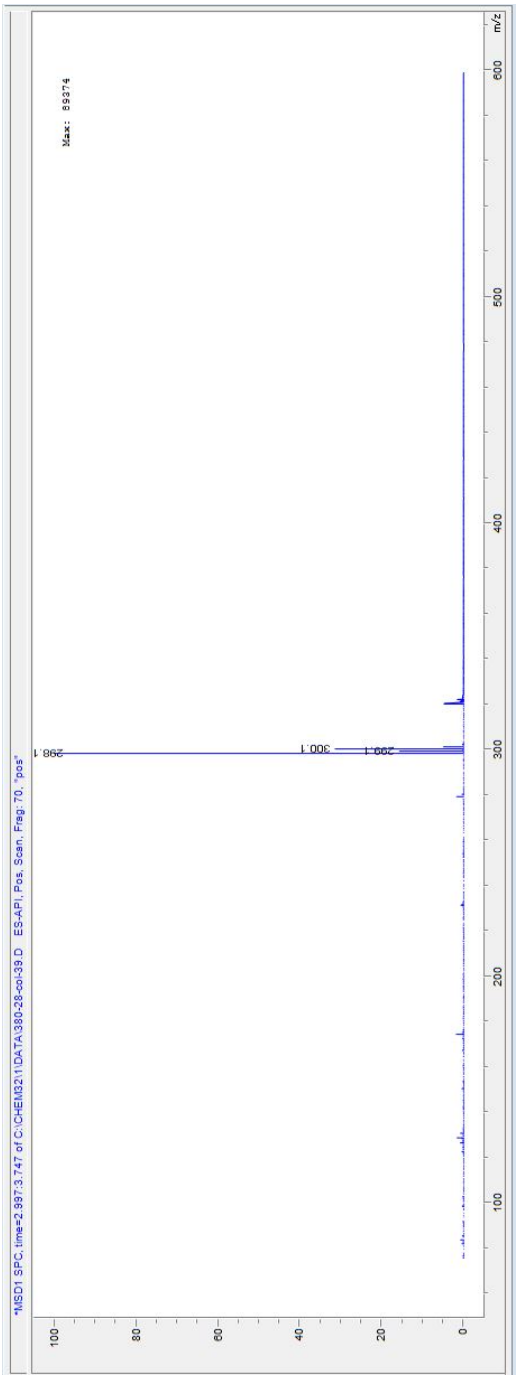
LC-MS spectrum of 1-(2-amino-1-methyl-1*H*-imidazol-5-yl)cyclobutan-1-ol (13)



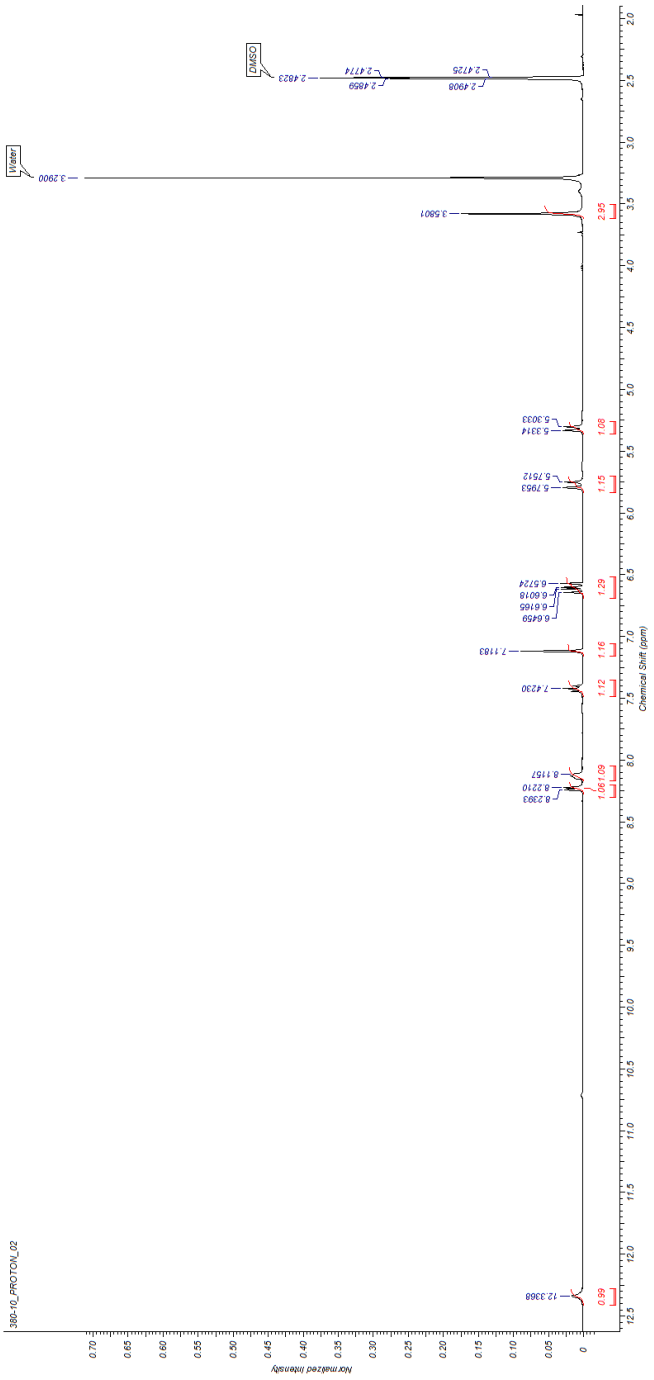
Proton spectrum of 3-chloro-4-fluoro-*N*-(5-(1-hydroxyethyl)-1-methyl-1*H*-imidazol-2-yl) benzamide (14) (380-28)



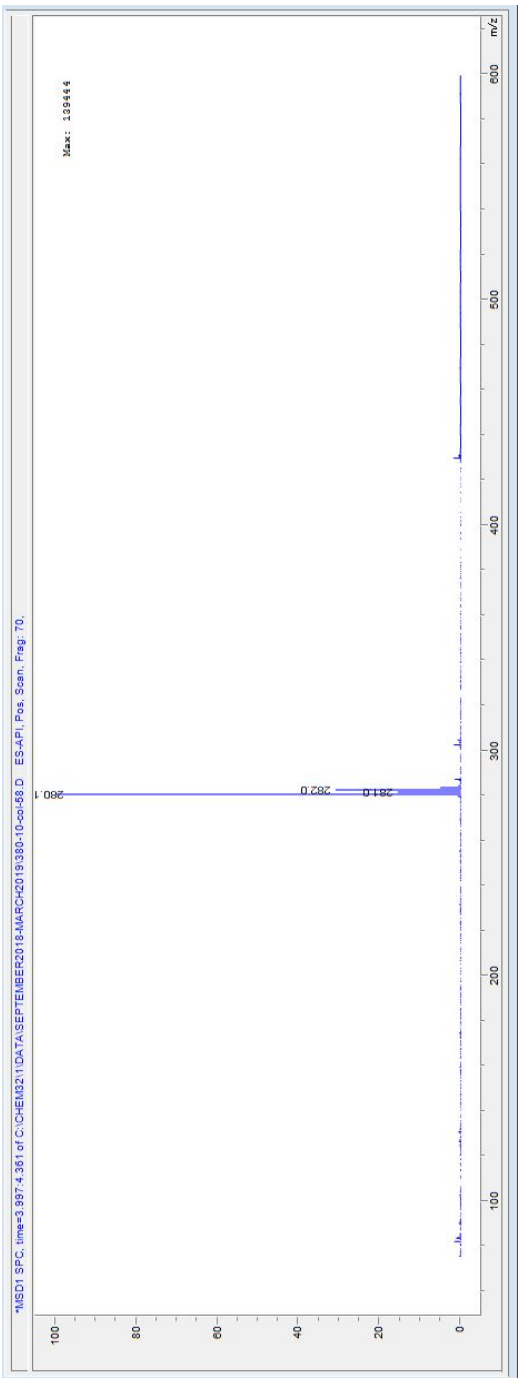
LC-MS spectrum of 3-chloro-4-fluoro-*N*-(5-(1-hydroxyethyl)-1-methyl-1*H*-imidazol-2-yl) benzamide (14)



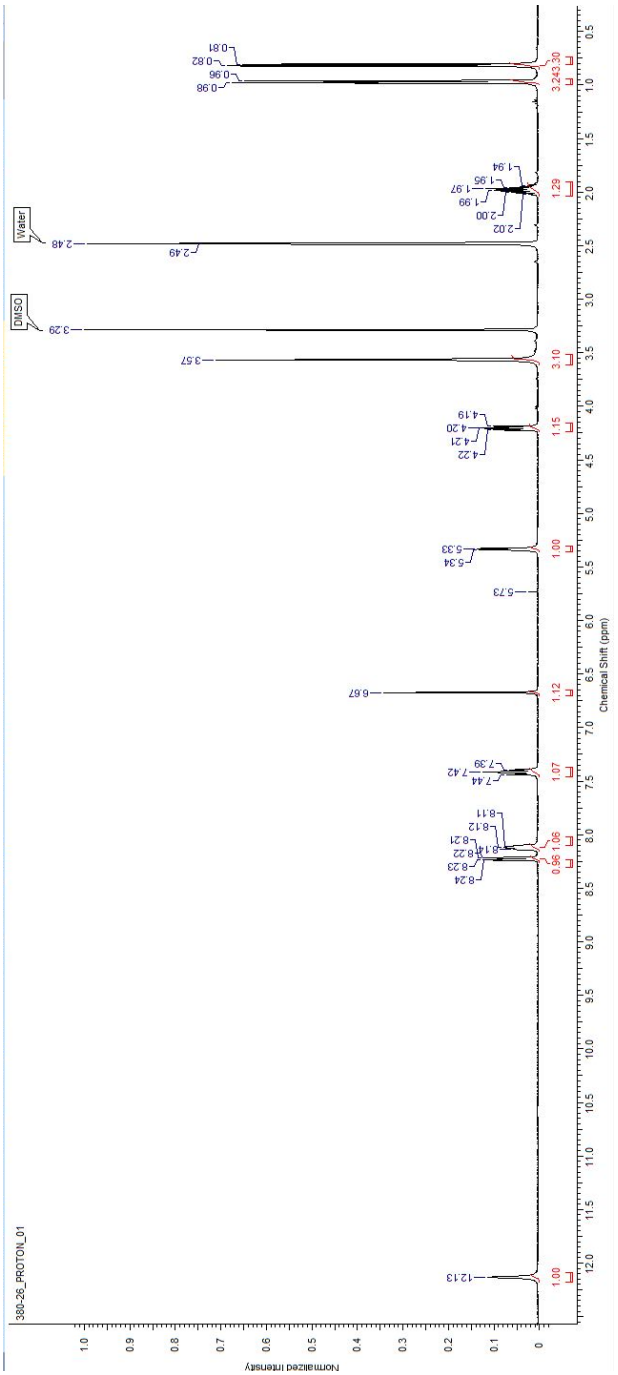
Proton spectrum of 3-chloro-4-fluoro-*N*-(1-methyl-5-vinyl-1*H*-imidazol-2-yl) benzamide (15)



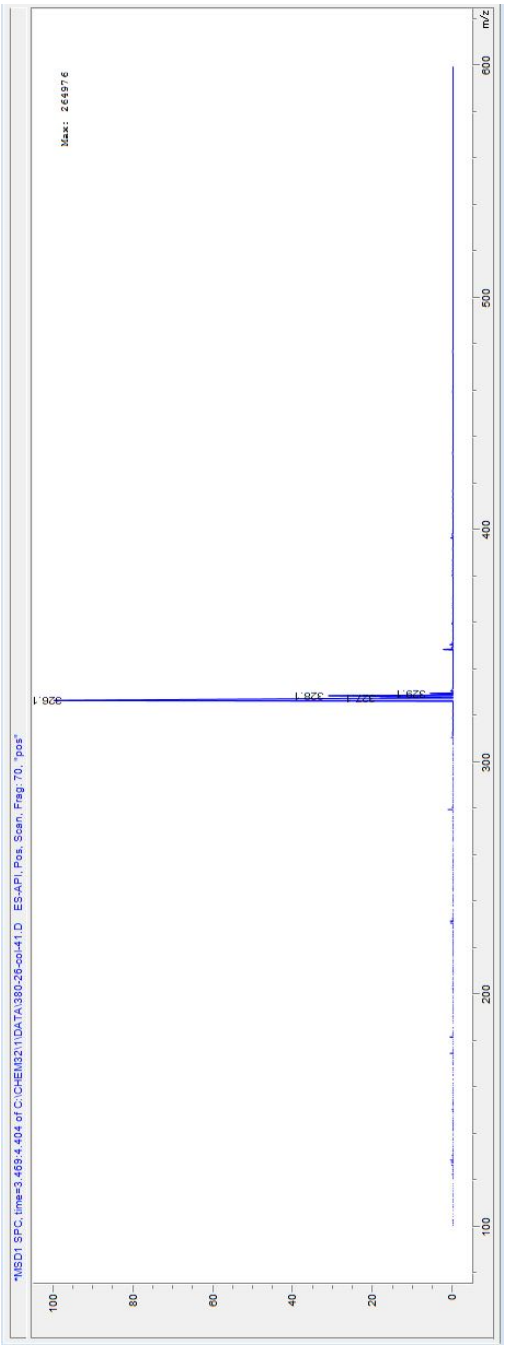
LC-MS spectrum of 3-chloro-4-fluoro-*N*-(1-methyl-5-vinyl-1*H*-imidazol-2-yl) benzamide (15)



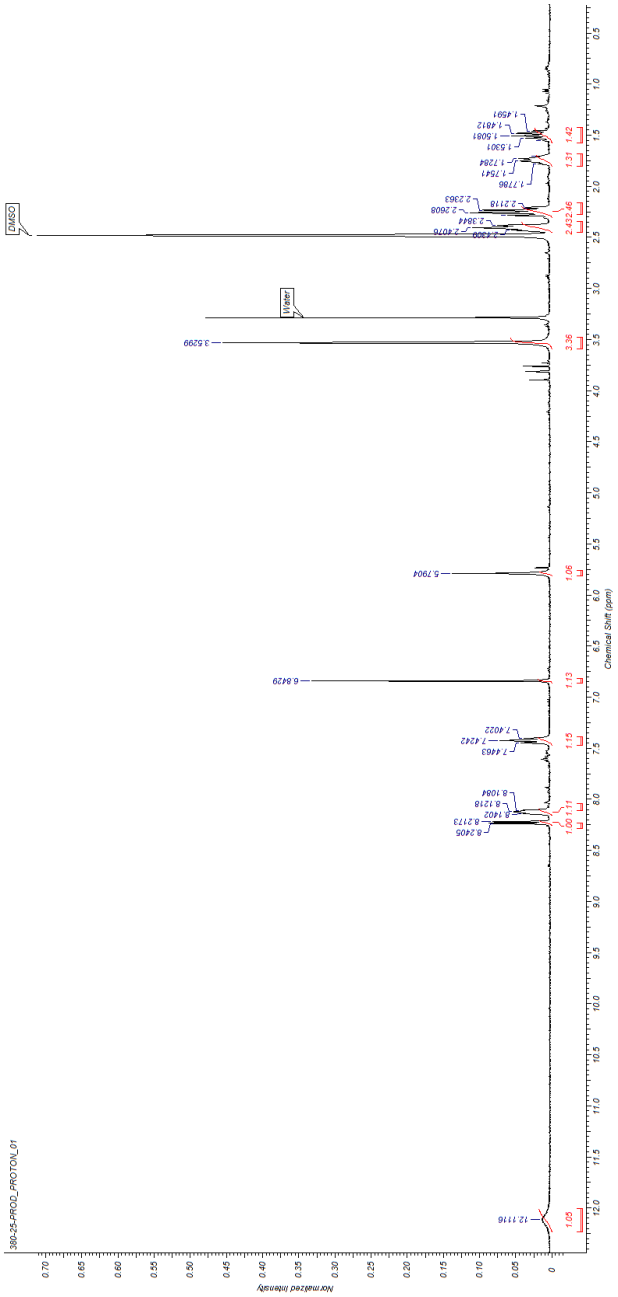
Proton spectrum of 3-chloro-4-fluoro-*N*-(5-(1-hydroxy-2-methylpropyl)-1-methyl-1*H*-imidazol-2-yl) benzamide (16)



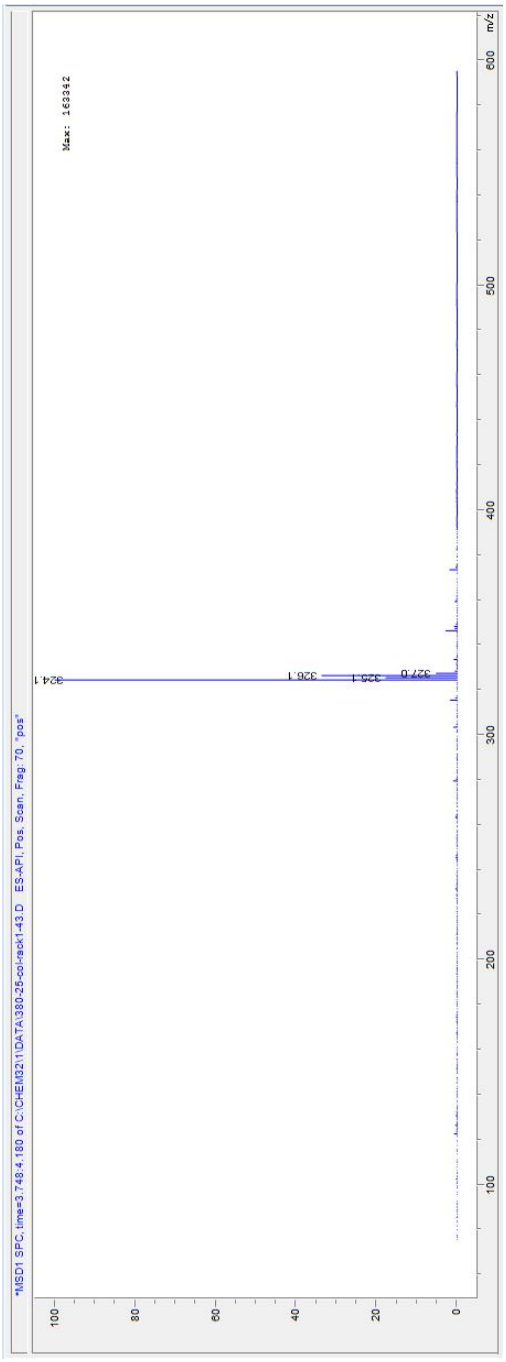
LC-MS spectrum of 3-chloro-4-fluoro-*N*-(5-(1-hydroxy-2-methylpropyl)-1-methyl-1*H*-imidazol-2-yl) benzamide (16)



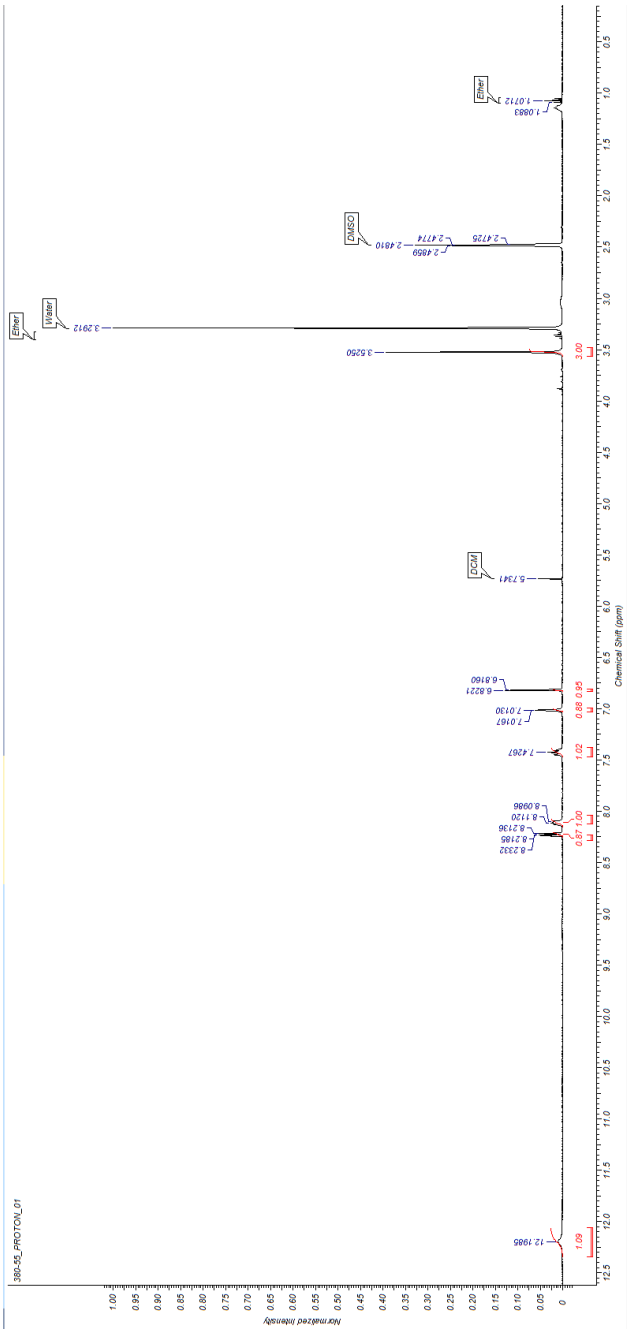
Proton spectrum of 3-chloro-4-fluoro-*N*-(5-(1-hydroxycyclobutyl)-1-methyl-1*H*-imidazol-2-yl) benzamide (17)



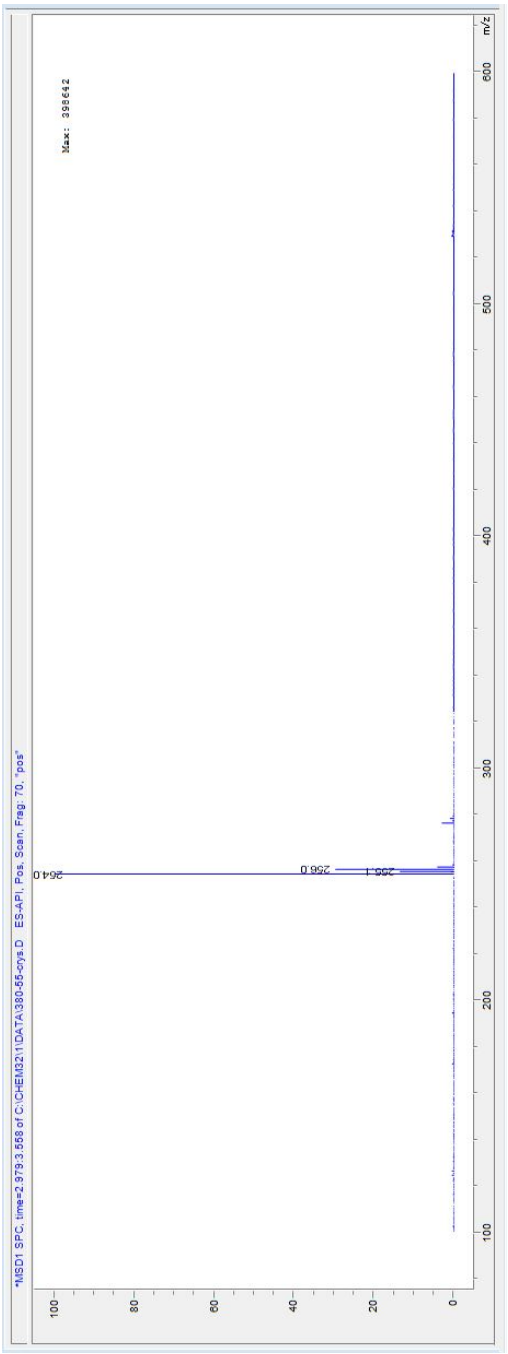
LC-MS spectrum of 3-chloro-4-fluoro-*N*-(5-(1-hydroxycyclobutyl)-1-methyl-1*H*-imidazol-2-yl) benzamide (17)



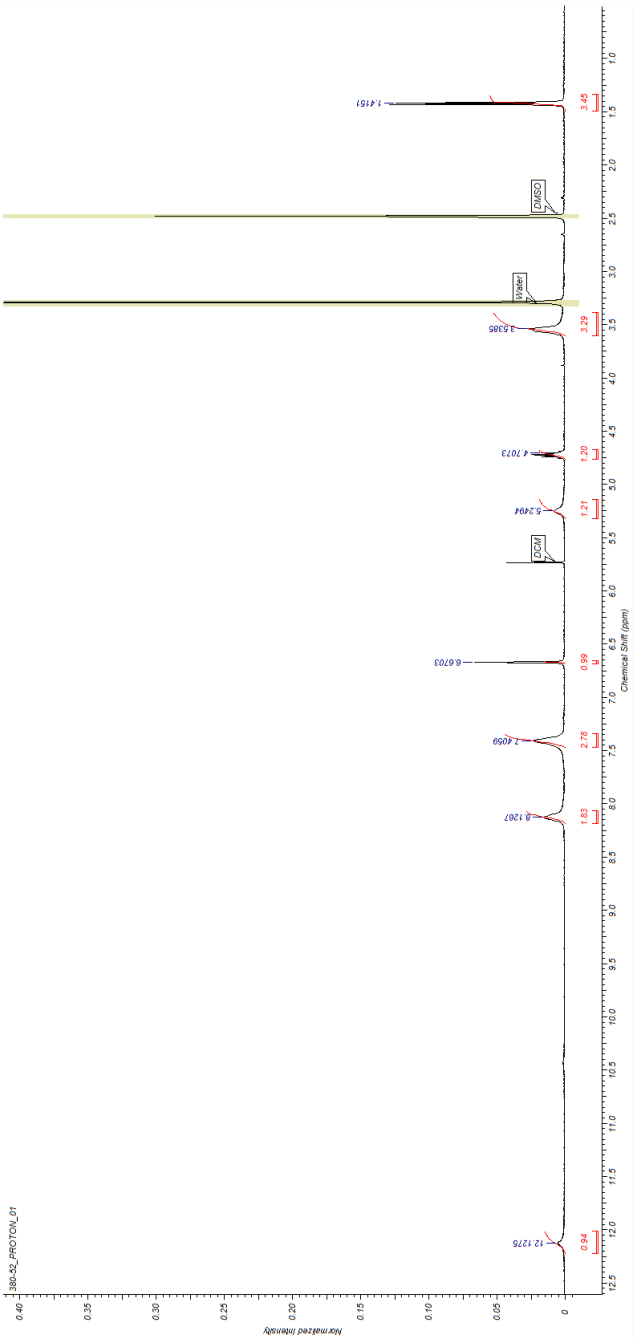
Proton spectrum of 3-chloro-4-fluoro-*N*-(1*H*-imidazol-2-yl)benzamide (18)



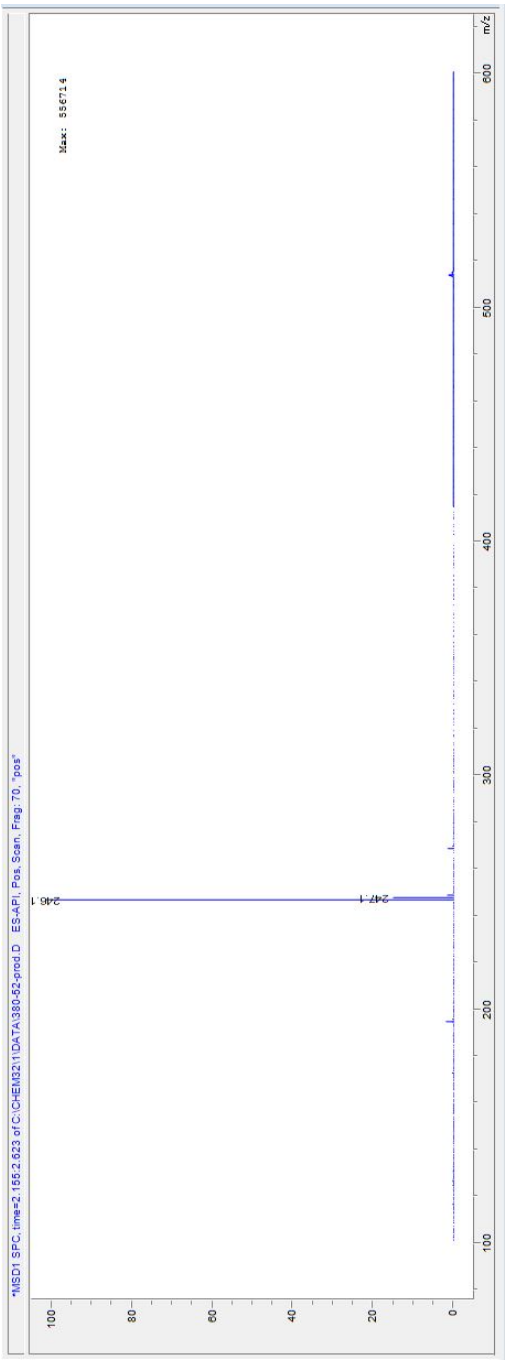
LC-MS spectrum of 3-chloro-4-fluoro-*N*-(1*H*-imidazol-2-yl)benzamide (18)



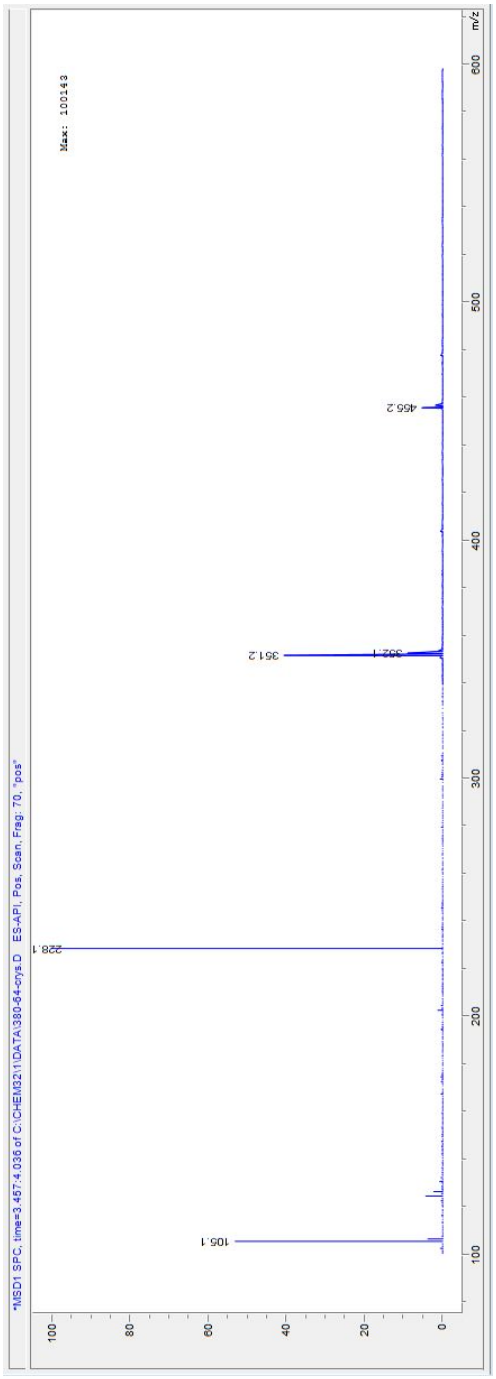
Proton spectrum of *N*-(5-(1-hydroxyethyl)-1-methyl-1*H*-imidazol-2-yl) benzamide (19)



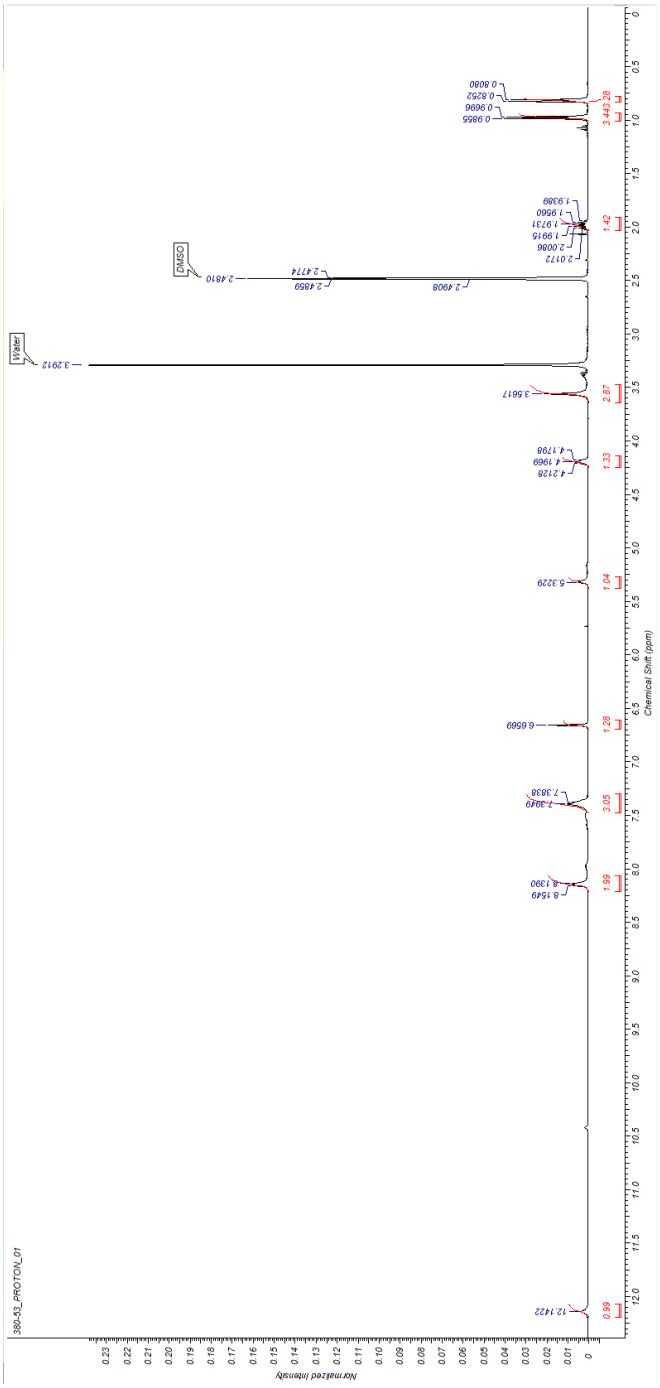
LC-MS spectrum of *N*-(5-(1-hydroxyethyl)-1-methyl-1*H*-imidazol-2-yl) benzamide (19)



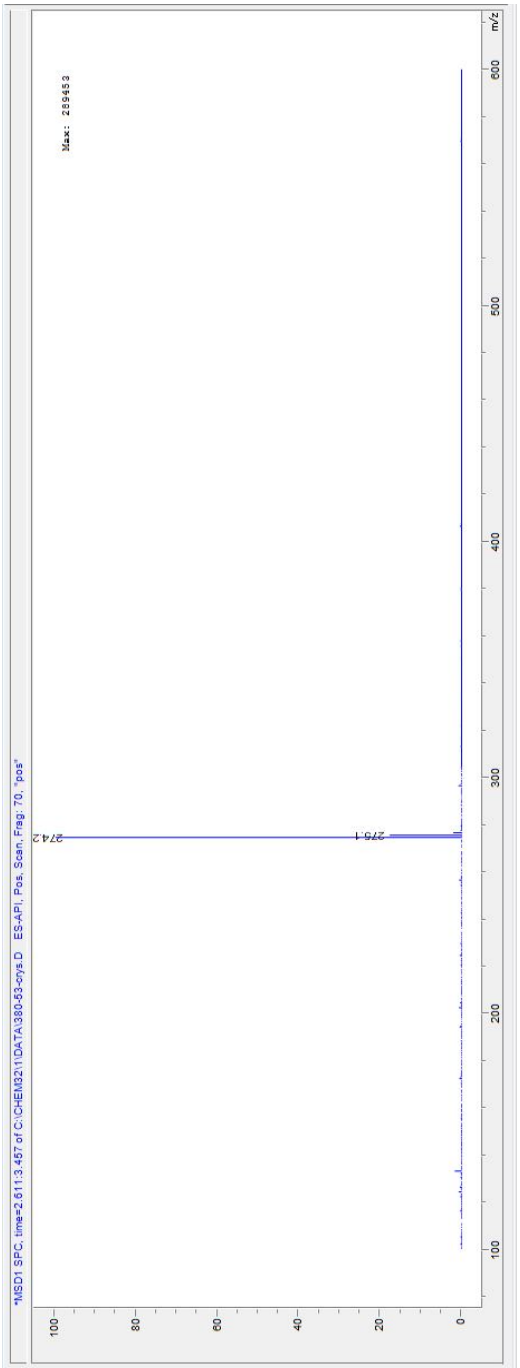
LC-MS spectrum of *N*-(1-methyl-5-vinyl-*H*-imidazol-2-yl)benzamide (20)



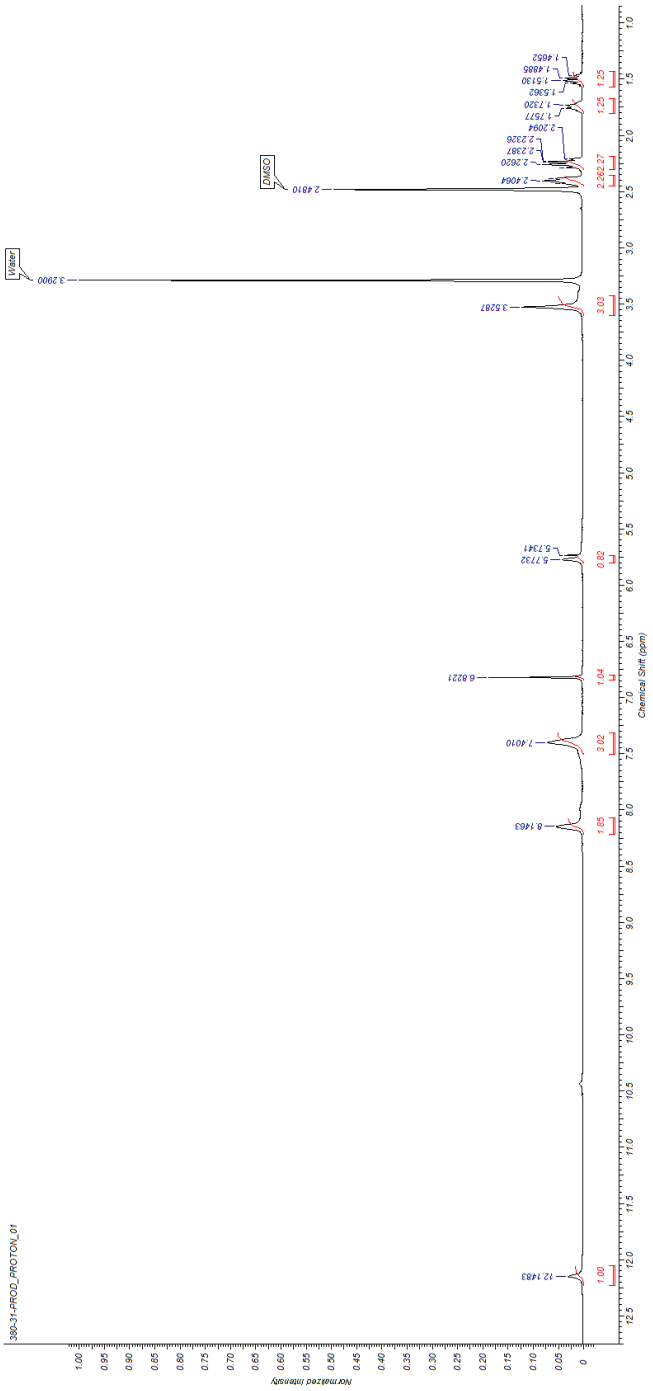
Proton spectrum of *N*-(5-(1-hydroxy-2-methylpropyl)-1-methyl-1*H*-imidazol-2-yl)benzamide (21)



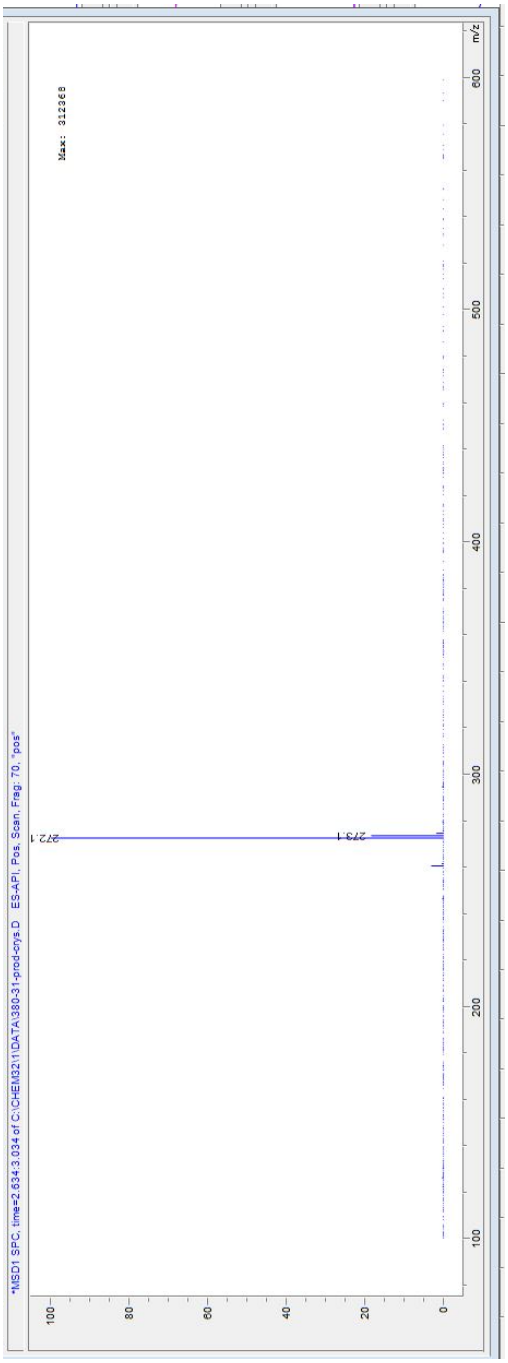
LC-MS spectrum of *N*-(5-(1-hydroxy-2-methylpropyl)-1-methyl-1*H*-imidazol-2-yl)benzamide (21)



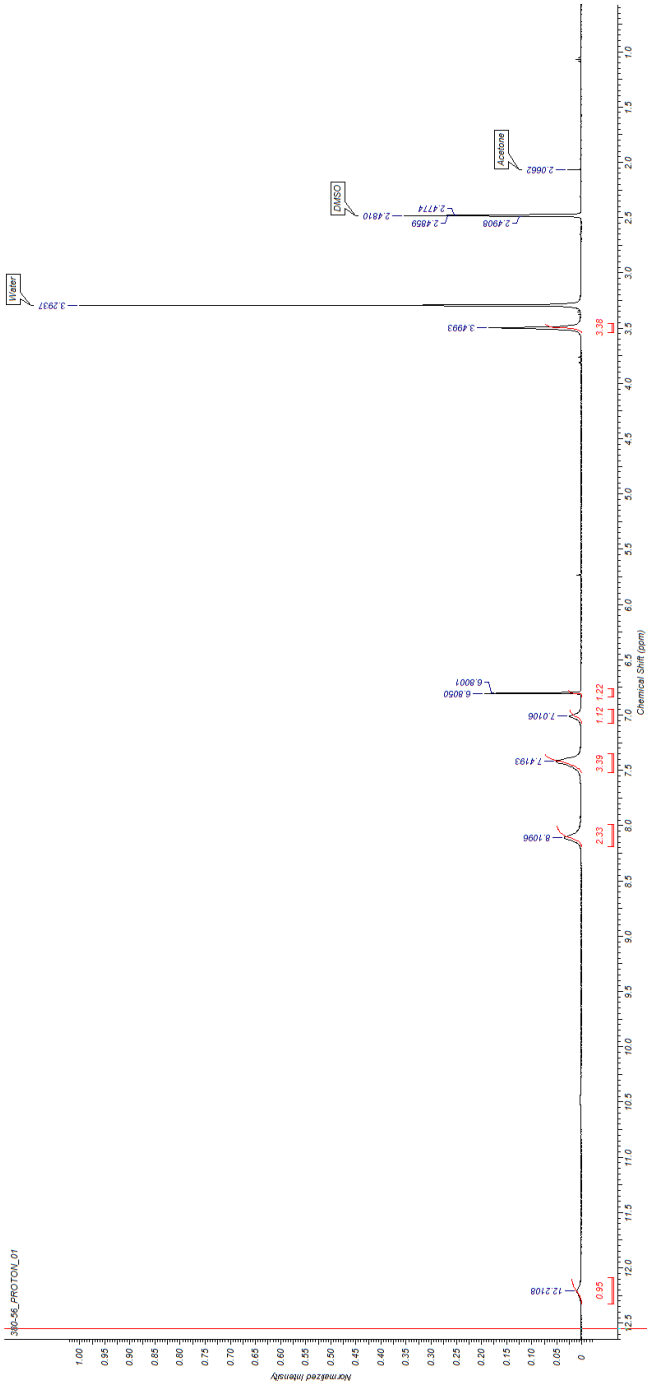
Proton spectrum of *N*-(5-(1-hydroxycyclobutyl)-1-methyl-1*H*-imidazol-2-yl) benzamide (22)



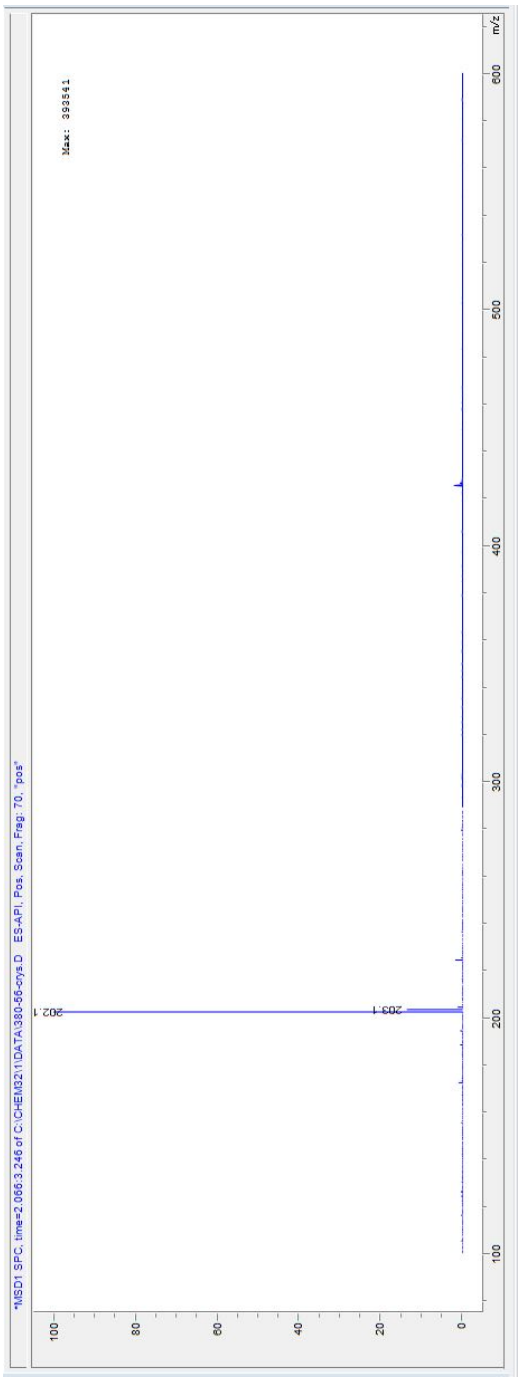
LC-MS spectrum of *N*-(5-(1-hydroxycyclobutyl)-1-methyl-1*H*-imidazol-2-yl) benzamide (22)



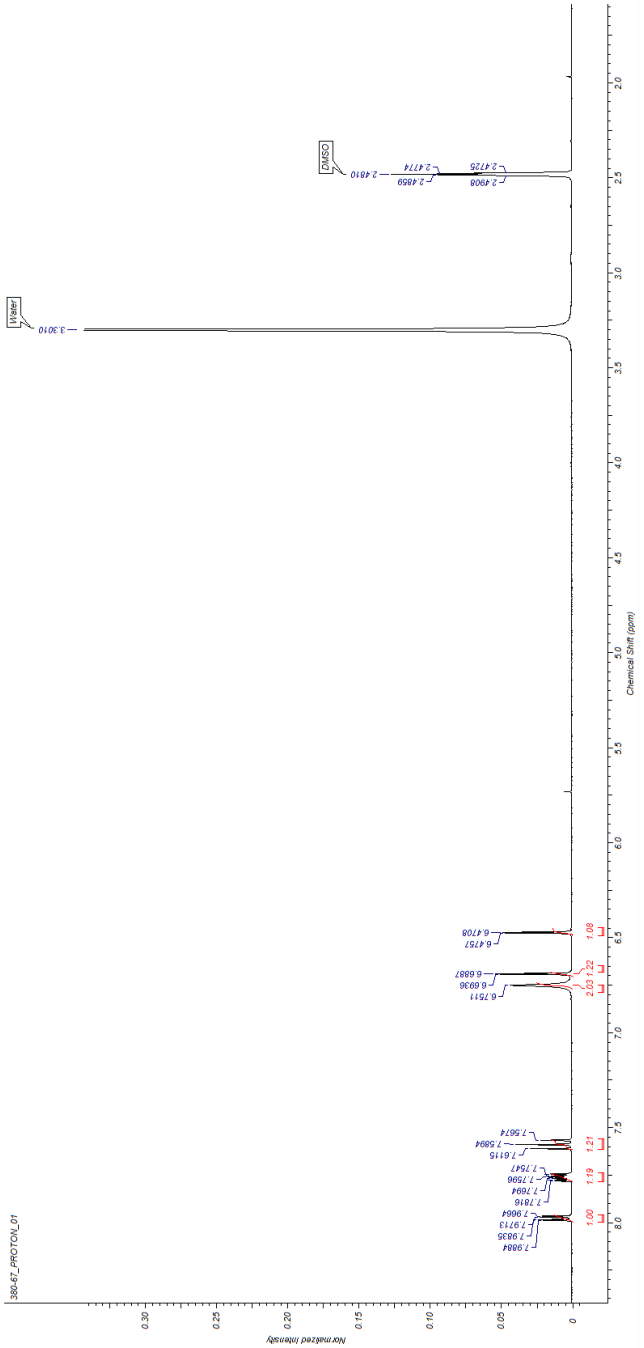
Proton spectrum of *N*-(1-methyl-1*H*-imidazol-2-yl)benzamide (23)



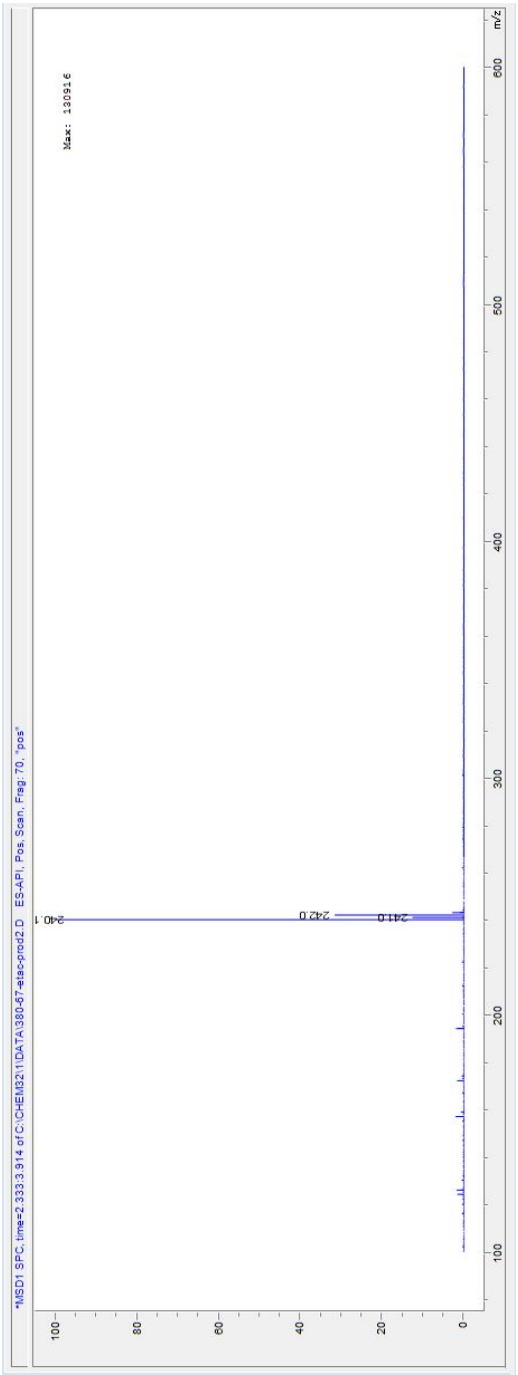
LC-MS spectrum of *N*-(1-methyl-1*H*-imidazol-2-yl)benzamide (23)



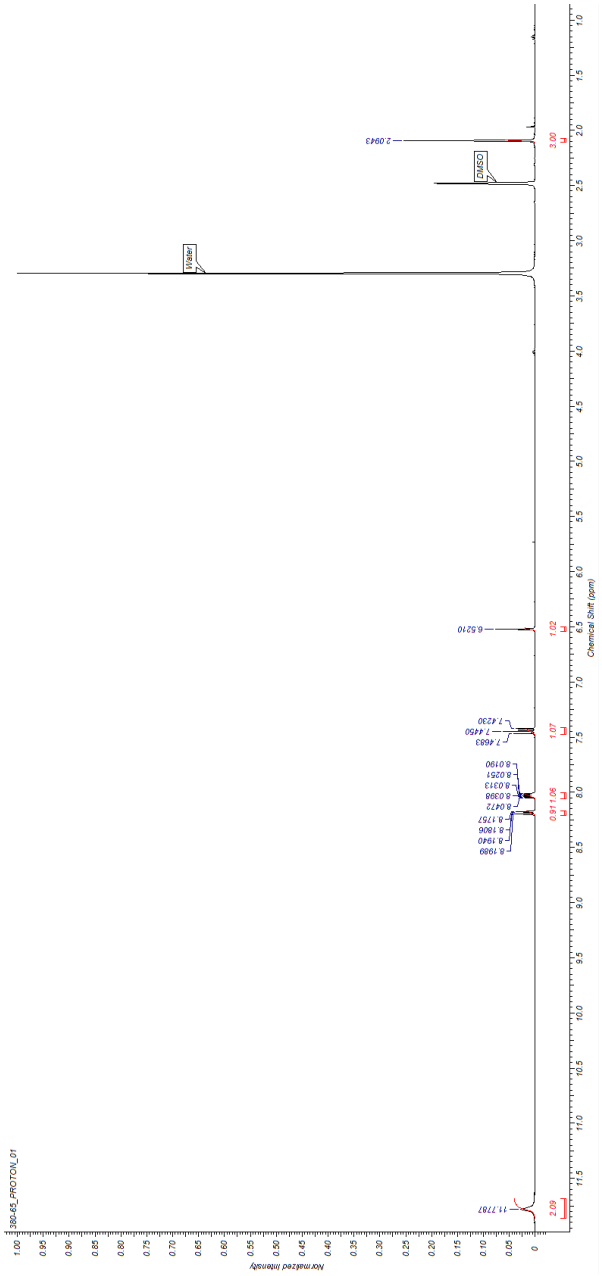
Proton spectrum of 3-chloro-4-fluoro-*N*-(1*H*-imidazol-2-yl)benzamide (24)



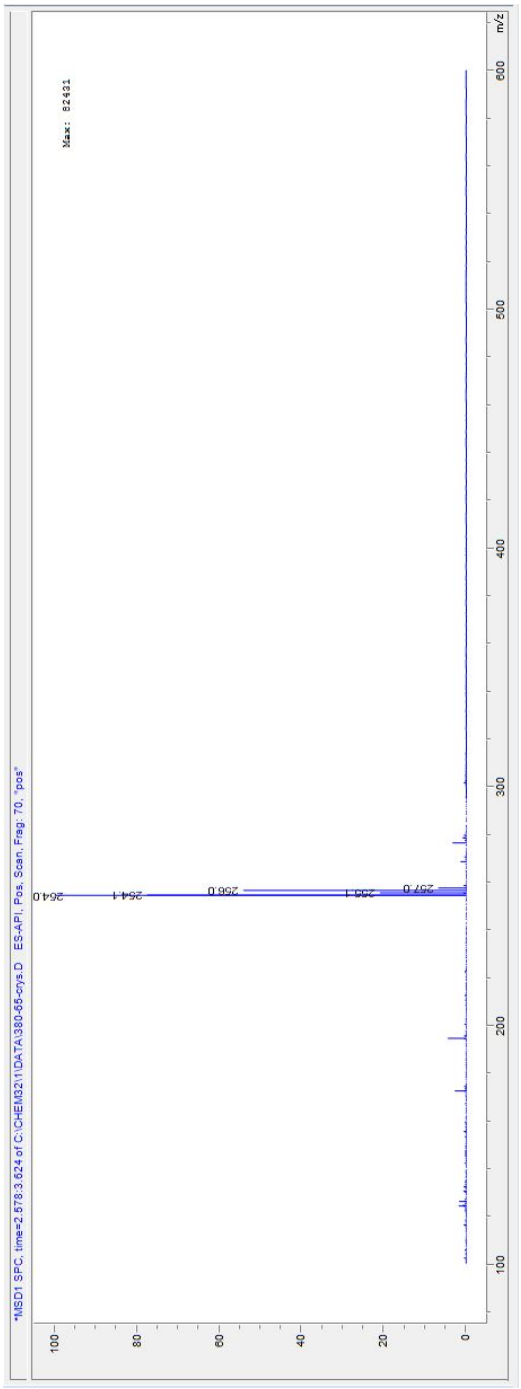
LC-MS spectrum of 3-chloro-4-fluoro-*N*-(1*H*-imidazol-2-yl)benzamide (24)



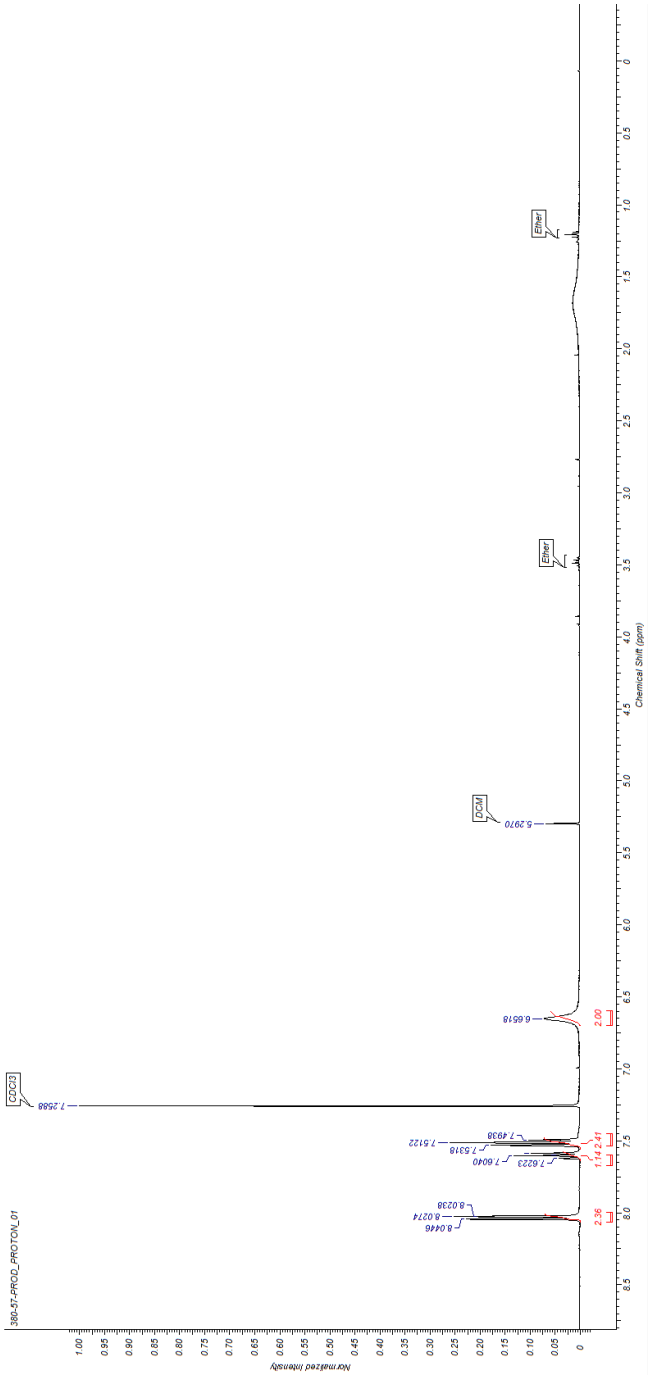
Proton spectrum of 3-chloro-4-fluoro-*N*-(5-methyl-1*H*-imidazol-2-yl) benzamide (25)



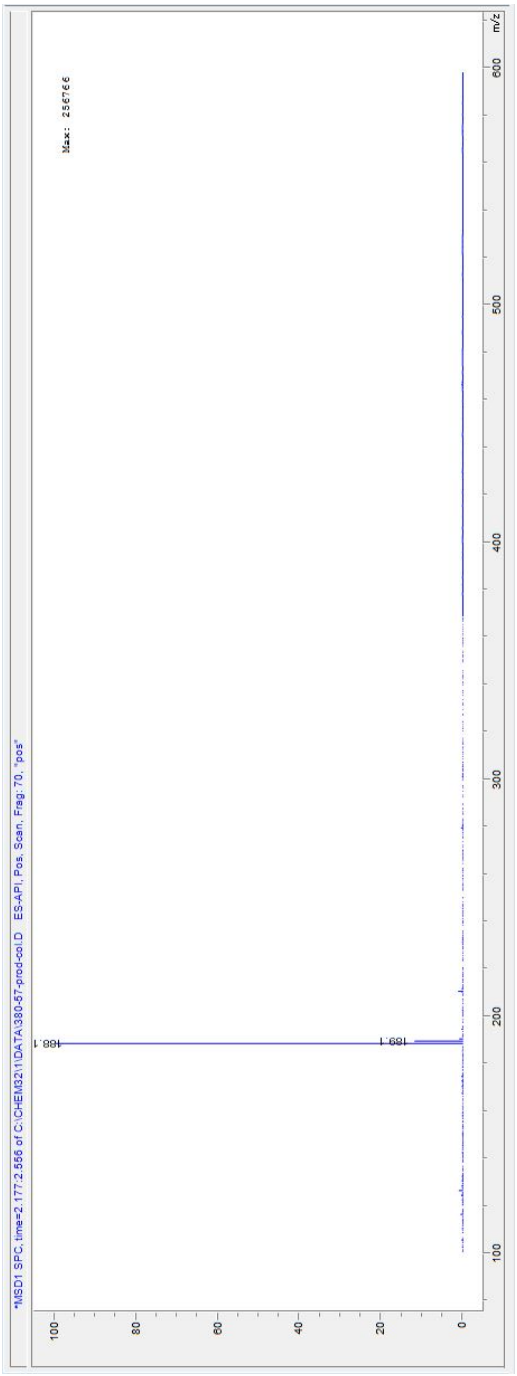
LC-MS spectrum of 3-chloro-4-fluoro-*N*-(5-methyl-1*H*-imidazol-2-yl) benzamide (25)



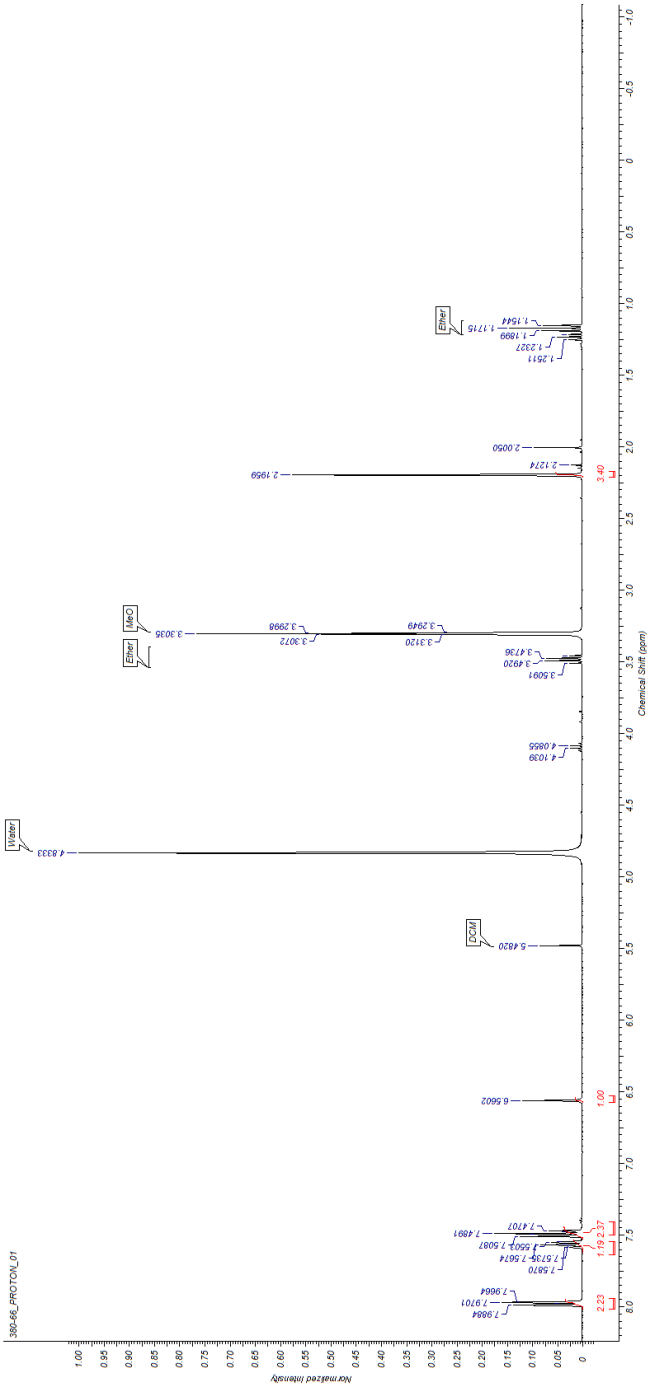
Proton spectrum of *N*-(1*H*-imidazol-2-yl)benzamide (26)



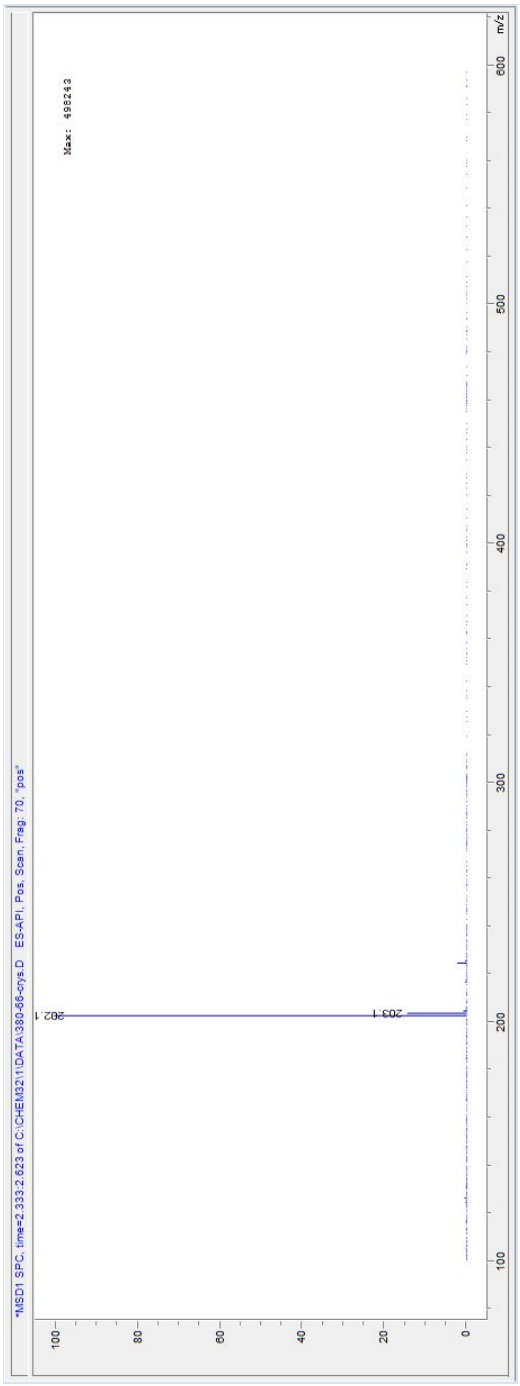
LC-MS spectrum of *N*-(1*H*-imidazol-2-yl)benzamide (26)



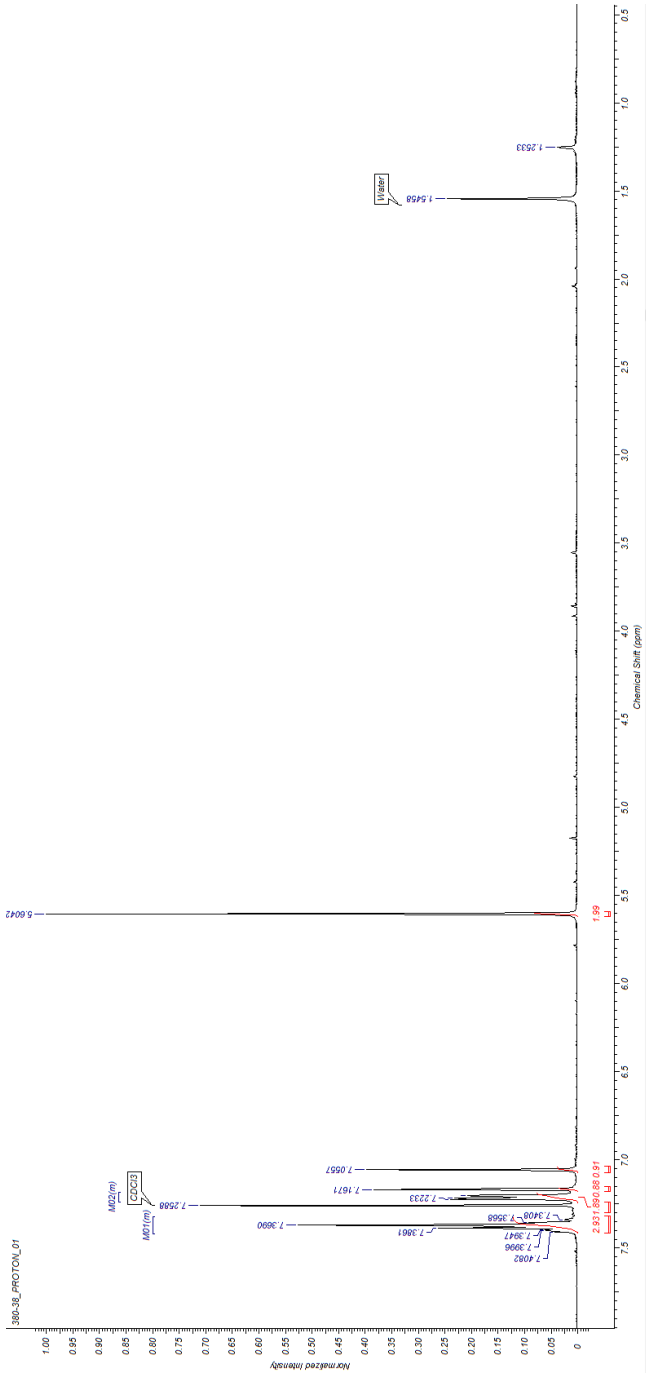
Proton spectrum of *N*-(5-methyl-1*H*-imidazol-2-yl)benzamide (27)



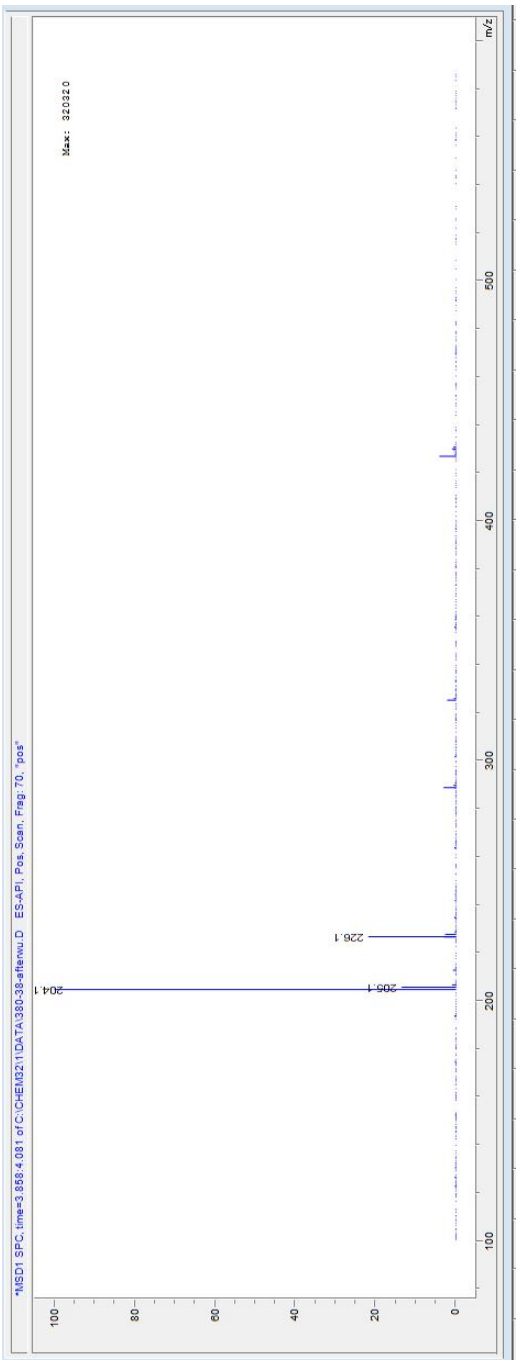
LC-MS spectrum of *N*-(5-methyl-1*H*-imidazol-2-yl)benzamide (27)



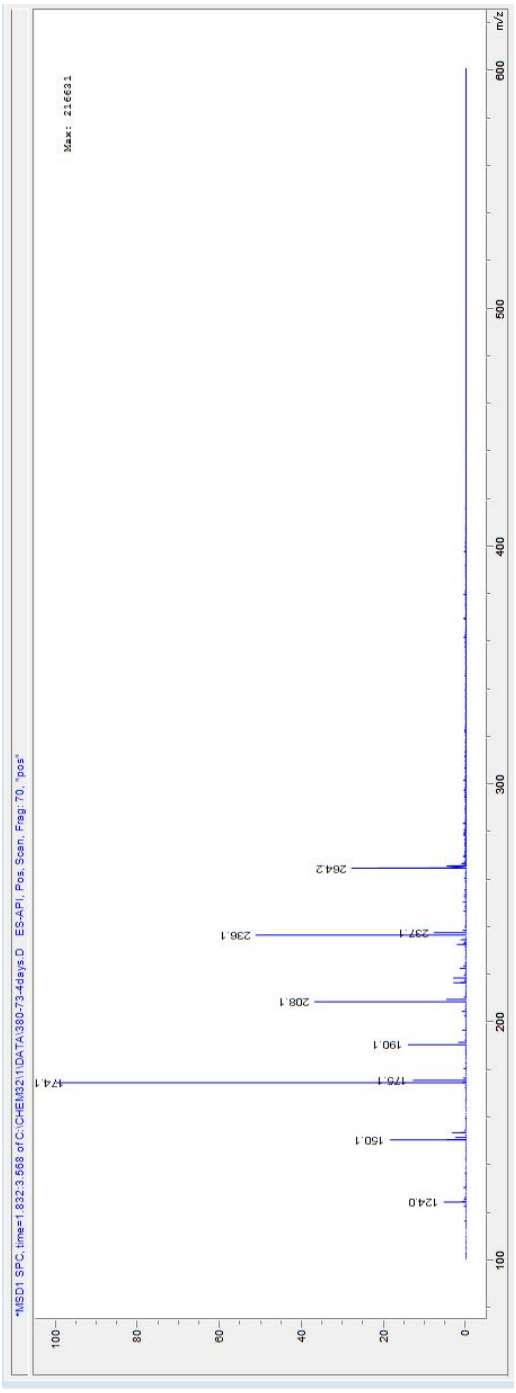
Proton spectrum of 1-benzyl-2-nitro-1*H*-imidazole (28)



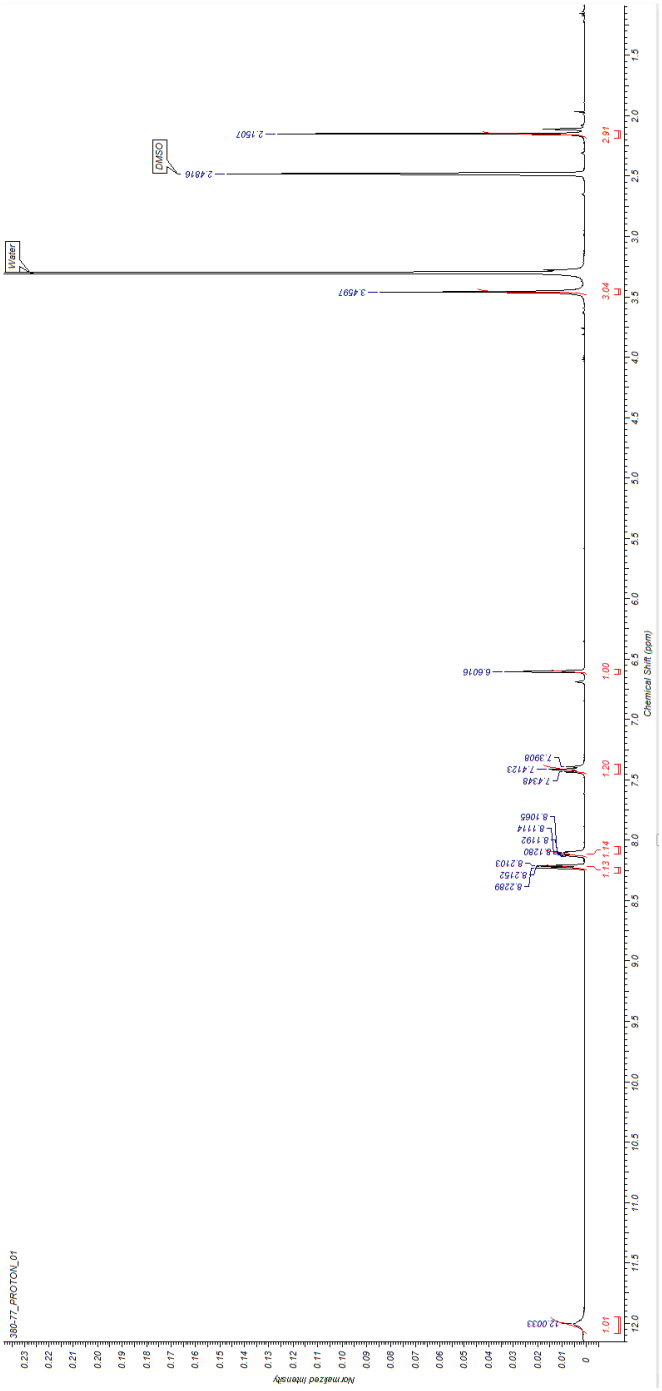
LC-MS spectrum of 1-benzyl-2-nitro-1*H*-imidazole (28)



LC-MS spectrum of 1-benzyl-1*H*-imidazol-2-amine (29)



Proton spectrum of 3-chloro-*N*-(1,5-dimethyl-1*H*-imidazol-2-yl)-4-fluoro benzamide (31)



LC-MS spectrum of 3-chloro-*N*-(1,5-dimethyl-1*H*-imidazol-2-yl)-4-fluoro benzamide (31)