ASYMMETRIC SYNTHESIS OF 5-SUBSTITUTED-1-(*o*-ARYL)-2-THIOBARBITURIC ACID DERIVATIVES AND THEIR ANTIBACTERIAL ACTIVITIES

by

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ABSTRACT

ASYMMETRIC SYNTHESIS OF 5-SUBSTITUTED-1-(*o*-ARYL)-2-THIOBARBITURIC ACID DERIVATIVES AND THEIR ANTIBACTERIAL ACTIVITIES

The main purpose of the study was to perform asymmetric synthesis of 5-substituted-1-(*o*-aryl)-2-thiobarbituric acid derivatives in order to obtain 5,5-dialkylsubstituted-1-(*o*-aryl)-2-thiobarbituric acid derivatives which are supposed to be biologically active. Many derivatives of barbituric acids act as central nervous system depressants, and can therefore produce a wide spectrum of effects, from mild sedation to total anesthesia. They are also effective as anxiolytics, hypnotics, analgesic and anticonvulsants. Among them, 2-thiobarbituric acid derivatives show a wide range of biological activity, so some of them are useful drugs or agrochemicals. Asymmetric synthesis is an essential process in the field of pharmaceuticals, as different enantiomers or diastereomers of the product often have different biological activity. In this study two types of alkylation reactions were performed as benzylation and allylation reactions. Asymmetric alkylation reactions were performed using (+)-cinchonine as chiral catalyst and enantio-and diastereoselectivity of the reactions in which stereoselective substitution took place at the fifth position of the heterocycle were determined using ^{1}H and ^{13}C NMR spectroscopies, normal phase HPLC and polarimeter. In addition, 5-methyl-1-phenyl-2-thiobarbituric acid was also alkylated to observe the effect of *ortho*-substituent of the aryl group on the enantioselectivity and diasteroselectivity of the reaction. Morever, antibacterial activities of all the synthesized 5,5-disubstituted-1-(*o*-aryl)-2-thiobarbituric acid derivatives and 5-substitued-1-(*o*-aryl)-2-thiobarbituric acid derivatives were measured against E. coli, B. subtiles, P. auregenause and S. aureus by disk diffusion method. Finally, the antibacterial effects of the product 5-benzyl-1(*o*-fluorophenyl)-2-thiobarbituric acid were identified against E. coli and P. aeruginosa by the same method.

ÖZET

5-SUBSTİTÜTİF-1-(*o*-ARİL)-2-TİYOBARBİTÜRİK ASİTLERİN FARKLI TÜREVLERİNİN ASİMETRİK SENTEZLERİ VE ONLARIN ANTİBAKTERİYEL ETKİNLİKLERİ

Projenin temel amacı biyolojik etkinliği beklenen 5,5-dialkilsubstitütif-1-(*o*-aril)-2-tiyobarbitürik asit türevlerini elde etmek için 5-substitütif-1-(*o*-aril)-2-tiyobarbitürik asit türevlerinin asimetrik sentezlerini gerçekleştirmekti. Barbitürik asitlerin birçok türevi, merkezi sinir sistemi depresanları olarak etki eden ilaçlardır ve bu yüzden, onların hafif yatıştırma etkisinden tüm anestezi etkisine kadar geniş bir etki yelpazesi vardır. Barbitürik asitler, ayrıca kaygı giderici, uyuşturucu, ağrı kesici ve antikonvülsanlar olarak etkilidir. Bunlar arasında, 2-tiyobarbitürik asit türevleri geniş bir biyolojik etkinlik aralığı göstermektedirler, öyle ki onlardan bazıları yararlı ilaçlar ve tarım kimyasallarıdırlar. Bir ürünün farklı enantiyomerleri ya da diyastereomerleri çoğu kez farklı biyolojik etkinlik gösterdiklerinden dolayı asimetrik sentez eczacılık alanında temel bir prosestir. Bu projede, allilleme ve benzilleme reaksiyonları olarak iki tür alkilleme reaksiyonları gerçekleştirildi. Asimetrik alkilleme reaksiyonları kiral kataliz olan (+)-cinchonine'i kullanarak gerçekleştirildi ve heterosiklik yapının 5. pozisyonunda stereoseçici sübstitüsyonun gerçekleştiği reaksiyonların enantiyo-ve diyastereo seçicilikleri ^{1}H ve ^{13}C NMR specktroskopileri, normal faz HPLC ve polarimetre kullanılarak belirlendi. Yanısıra, aril grubunun *orto* pozisyonunun alkilleme reaksiyonu üzerindeki enantiyo-ve diyastereoseçicilik etkisini incelemek için, 5-metil-1-fenil-2-tiyobarbitürik asit de alkilleştirildi. Bundan başka, bütün sentezlenen 5,5-disubstitütif-1-(*o*-aril)-2-tiyobarbitürik asit türevlerinin ve 5-substitütif-1-(*o*-aril)-2-tiyobarbituric asit türevlerinin E. coli, B. subtiles, P. auregenause ve S. aureus karşı antibakteriyel aktiviteleridisk difizyon metod ile incelendi. Son olarak, aynı metot ile 5-benzil-1-(*o*-florfenil)-2-tiyobarbitürk asidin E. coli ve P. aeruginosa üzerindeki antibakteriyel etkileri belirlendi.

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LIST OF SYMBOLS/ABBREVIATIONS

CNS	Central Nervous System
HPLC	High Performance Liquid Chromatography
IR	Infrared
LDA	Lithium Diisopropylamide
m	Medium Infrared Spectroscopy Band
MIC	Minimum Inhibition Concentration
mmole	Millimole
nm	Nanometer
NMR	Nuclear Magnetic Resonance
ppm	Parts Per Million
s	Strong Infrared Spectroscopy
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
w	Weak Infrared Spectroscopy Band

1. INTRODUCTION

Barbituric acids refer to the group of 2,4-pyrimidine derivatives [1]. Barbituric acids attract great attention over years because they are essential in a wide spectrum of drugs as anticonvulsant, sedative-hypnotics, anxiolytic, anesthetics and tranquilizer. Barbituric acids have an effect on central nervous system and psychological effects of them are very structure dependent [2,3]. Furthermore, barbituric acids have higher biological activity when aromatic or alkyl groups are bonded to C-5 of the ring [4]. Barbituric acid derivatives also have antibacterial effects against several bacteria and their antibacterial effects can be identified by many methods such as disk diffusion tests in agars and broth dilution methods in agars [5-12, 13].

Asymmetric synthesis is an essential process in the field of pharmaceuticals, as different enantiomers or diastereomers of the product often have different biological activity [14]. Several asymmetric syntheses of barbituric and thiobarbituric acid derivatives were done previously and some of them were found to have antibacterial effects, examples of them are benzenesulfoamide derivatives, 5-benzylidine derivatives and spiroheterobicyclic derivatives of barbituric or thiobarbituric acids. Their antibacterial studies were generally performed with disk diffusion method or dilution methods [5, 7, 8].

The main aim of the study was to perform asymmetric synthesis of 5-alkyl-1-(*o*-aryl)-2-thiobarbituric acid derivatives to obtain optically active 5,5-dialkyl-1-(*o*-aryl)-2-thiobarbituric acid derivatives which were expected to be biologically active. Other objective of the project was to identify the antibacterial activities of all synthesized 5,5-dimethyl-, 5-methyl-1-(*o*-aryl)-2-thiobarbituric, 5,5-dimethyl- and 5-methyl-1-phenyl-2-thiobarbituric acids against E. coli, B. subtiles, P. auregenause, and S. aureus. In addition to that, antibacterial effects of the products, 5-benzyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid were tested against E. coli and P. aeruginosa by disk diffusion method.

In this project, firstly, 5,5-dimethyl-,5-methyl-1-(*o*-aryl)-2-thiobarbituric, 5,5-dimethyl- and 5-methyl-1-phenyl-2-thiobarbituric acid were synthesized from their corresponding thioureas.

Then, asymmetric alkylation reactions of 5-methyl-1-(*o*-aryl)-2-thiobarbituric acids and 5-methyl-1-phenyl-2-thiobarbituric acid were performed at C-5 position of the heterocyclic ring to investigate the stereoselectivity of the reactions and to obtain new biologically active substances. Benzylbromide or allylbromide were used as a base in the asymmetric alkylation reactions. (+)-Cinchonine was used as chiral catalyst and also as alkylating agent. Lithium diisopropylamide (LDA) was used as a base, and some reactions showed diastereoselectivity. Tetrahydrofuran (THF) was used in both alkylation reactions as the reaction solvent. All alkylation reactions were performed at -35 ^{0}C, except benzylation reactions with LDA, they were performed at -78 ^{0}C.

Furthermore, Proton nuclear magnetic resonance (^{1}H NMR Spectroscopy) and melting point analyses were done to characterize all synthesized thiourea derivatives, thiobarbituric acid derivatives and alkylation products. Infrared (IR) spectroscopy analyses were also performed in order to characterize all synthesized thiourea derivatives and thiobarbituric acid derivatives. Besides, ^{13}C NMR spectroscopy analyses were only performed for the new synthesized 5-methyl-1-(*o*-aryl)-2-thiobarbituric acids and their benzylation products. In addition, elemental analyses of all benzylation products synthesized with (+)-cinchonine were done.

Moreover, X-bridge column was used in the reversed phase high performance liquid chromatography (HPLC) analysis in order to check the occurrence of the reactions of thiobarbituric acid derivatives and the purity of the products. Stereoselectivity of the reactions was determined by chiral HPLC analysis or polarimeter. Chiralcel OD-H column was found the most appropriate column for the normal phase HPLC analysis to identify enantioselectivity or diastereoselectivity of the benzylation products. Also, the optical activity measurements of the products, 5-benzyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid and 5-benzyl-1-(*o*-tolyl)-2-thiobarbituric acid were performed by polarimeter to verify their chiral HPLC results.

Finally, antibacterial activities of all the synthesized 5-methyl-1-(*o*-aryl)-2-thiobarbituric acid derivatives were measured against E. coli, B. subtiles, P. auregenause and S. aureus by disk diffusion method. Antibacterial effect of only one alkylation product, 5-benzyl-5-

methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid was tested against E.coli and P. aeruginosa by disk diffusion method.

In the theoretical background part of the M.Sc. thesis, history, properties, structure and applications of thiobarbituric acids, isomerism and types of isomers, asymmetric synthesis, tautomerization, chiral catalyst (+)-cinchonine, antibacterial agents and tests, all bacteria used in disk diffusion method were explained briefly. In the materials and methods part, all chemicals used in the study were presented and also the information about the methods and instruments used, which are thin layer chromatography (TLC), NMR spectroscopy, HPLC, IR spectroscopy, elemental analysis and polarimeter, was given. In the experimental study part, experimental procedures, analysis methods and their conditions were presented. In the result and discussion part, obtained results were discussed in detail. At the end of the thesis, some suggestions for the future work were given in the future study part.

2. THEORETICAL BACKGROUND

2.1. BARBITURIC AND THIOBARBITURIC ACIDS

Barbituric acids refer to group of 2,4-pyrimidine derivatives. The basic structure of barbituric acids consists of four carbon atoms and two nitrogen atoms (Figure 2.1) [1].

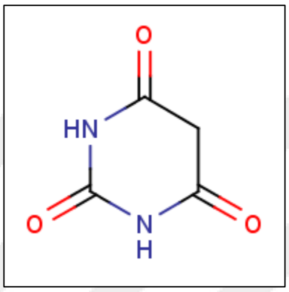


Figure 2.1. Structure of barbituric acid [15]

Barbituric acids attract great attention over the years since they are essential in many drugs [2]. They have an effect on the central nervous system. In addition, they have activity as anticonvulsant, sedative-hypnotics, anxiolytic, anaesthetics and tranquilizer [3]. Physiological effects of barbituric acid derivatives are very structure dependent [1].

N- and C-5 unsubstituted barbituric acid derivatives are very acidic. The acidity of barbituric acids can be decreased by substitution of an alkyl group, which has electron donating effect at position 5 and/or N-atom [3].

Thiobarbituric acid that includes a sulfur atom in place of one of the oxygen atoms is a type of barbiturate derivatives (Figure 2.2). Compounds which include nitrogen and sulfur atoms such as uracil are essential to be used for anticancer and antiviral activities, so thiobarbituric acid derivatives also have pharmacological activities [16]. 2-thiobarbituric acids are also used in sedative and hypnotic drugs as depressants [17]. Moreover thiobarbiturates are used in the medicinal applications such as anesthetics [18].

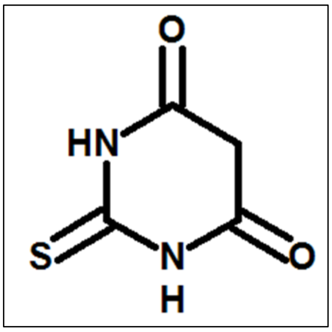


Figure 2.2. The molecular structure of 2-thiobarbituric acid [16]

Substitution of oxygen atom by sulfur atom at Carbon-2 causes higher fat solubility, little period of effectiveness, enhanced hypnotic impact and expected metabolic decline. Thiobarbiturates have a potential to be more toxic than oxybarbiturates with certain alteration in the pharmacodynamics [17].

Barbituric acids have higher biological activity when aromatic or alkyl groups are bonded to C-5 of the ring [5]. C-5-substituted and C-5-disubstituted barbituric acids and -2-thiobarbituric acids have biological impact, so they can be used as helpful drugs or agrochemicals. C-5 position is an active side because it could be acting as both nucleophilic center and electrophilic center [4]. Barbituric acids, of which hydrogen atom at C-5 is replaced by alkyl, cycloalkyl and aromatic radicals, are used widely in medicine as anticonvulsants, hypnotics, narcotics and soporifics. But the barbituric and thiobarbituric acids themselves have no distinct pharmacological activity. In addition, biological effects of barbituric acid derivatives can be varied depending on their relative hydrophobicity, electronic effects and steric effects [3, 19].

Furthermore, 5,5-dimethylbarbituric acids that have a total substitution of two hydrogens by two carbon atoms at C-5 do not have hypnotic effect. In this case, there is no hydrogen atom at the fifth position of the carbon atom on the heterocyclic ring [20]. Besides, two 5-substituents are necessary for depressant effect on Central Nervous System (CNS). The general structural properties which are essential for CNS depressant activity of barbituric acids are indicated in Figure 2.3 [21].

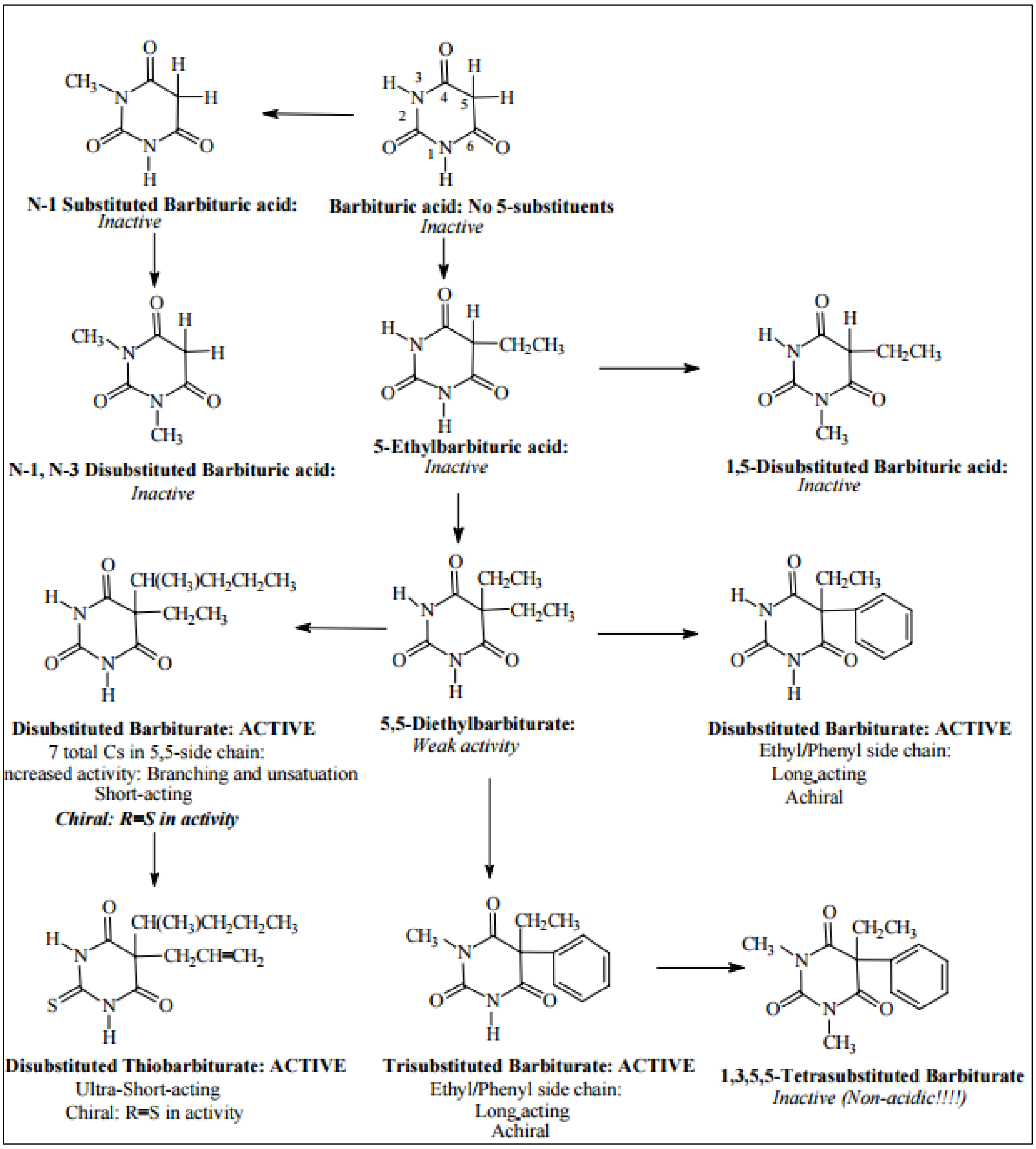


Figure 2.3. CNS depressant activities of essential thiobarbituric acid derivatives [21]

2.1.1. History of Barbituric Acid

The compound barbituric acid was discovered by Adolf von Baeyer on December 4, 1864. Barbituric acid was firstly synthesized by reacting using urea and malonic acid. However, the first synthesized barbituric acid compound did not have sedative effects and was considered as clinically useless [1]. Many thiobarbituric acid derivatives were revealed in 20th century. Emil Fisher and Joseph von Mering synthesized the first therapeuticially active barbituric acid derivative by replacing the C-5 hydrogens of the barbituric acid ring with ethyl substituents in 1903. The synthesized diethyl barbituric acid was the first hypnotic effective barbituric acid and was called as Veronal shown in Figure 2.4 [6].

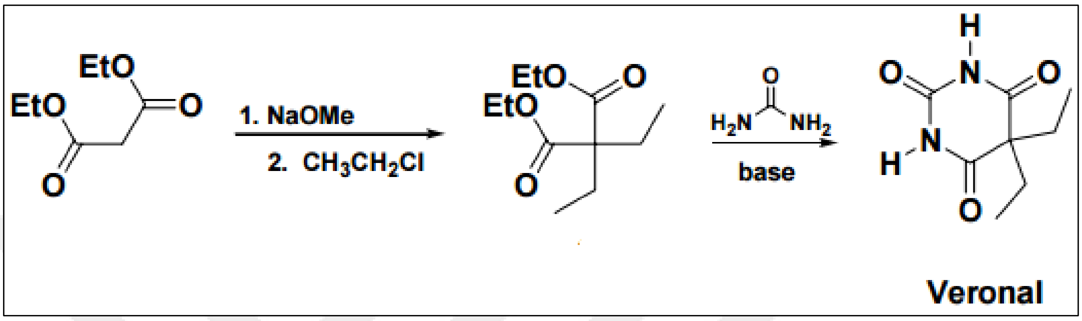


Figure 2.4. Synthesis of Veronal (5,5’-diethylbarbituric acid) [6]

Phenobarbital (Figure 2.5) drug which has a hypnotic and anticonvulsant effect was synthesized in 1912. It is also used for epileptic seizures.

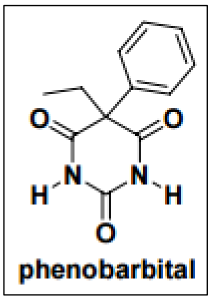


Figure 2.5. Structure of Phenobarbital [6]

Then different derivatives of barbituric acids were synthesized. Amobarbital, pentobarbital, secobarbital and hexobarbital (Figure 2.6) are examples of C-5 substituted derivatives of barbituric acids. Thiopental and thiamylal are examples of C-2 substituted derivatives of barbituric acids (Figure 2.6).

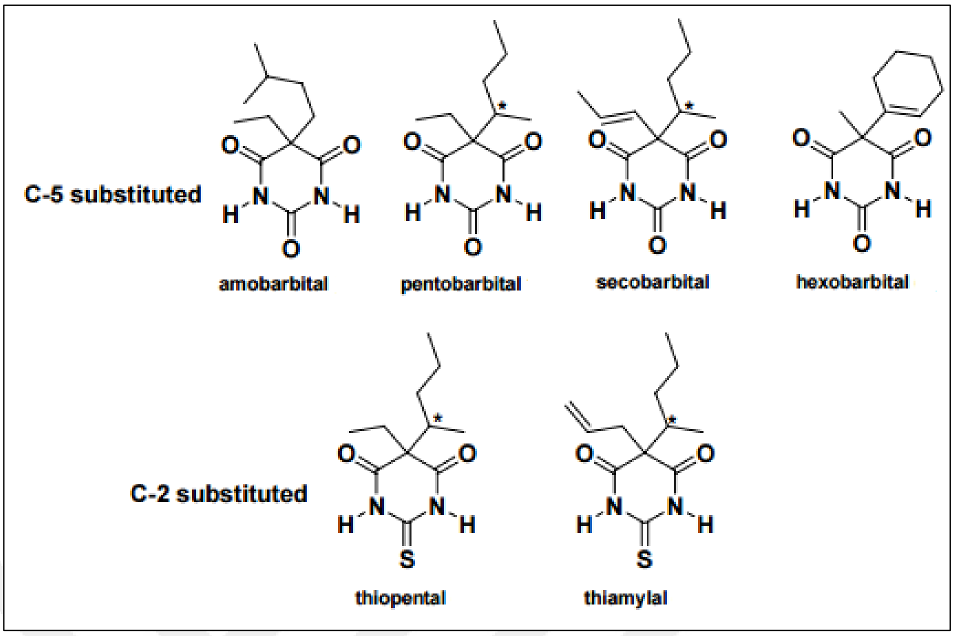


Figure 2.6. Some C-5 and C-2 substituted derivatives of barbituric acids [6]

2.1.2. Antibacterial Activity of Barbiturates and Thiobarbiturates

Some derivatives of barbituric acids and thiobarbituric acids show antibacterial activities. Benzenesulfoamide derivatives of barbituric and thiobarbituric acids were found to indicate antibacterial effect and thiobarbituric acid derivatives showed more antibacterial activity than barbituric acid derivatives [7]. Benzenesulfoamide derivatives of barbituric and thiobarbituric acids (**4-12** in Figure 2.7) were synthesized either from the corresponding ureas or thioureas (**2** and **3**) or barbituric and thiobarbituric acids (**4** and **5**) by cyclization reactions or fluorination reactions or condensation reactions or by the reaction of the derivatives (**4** and **5**) with benzaldeyde and thiourea. Pyrimidine derivatives compounds (**11** and **12**) were also synthesized by cyclization of fluoropyrimidines compounds **6** and **7** [8].

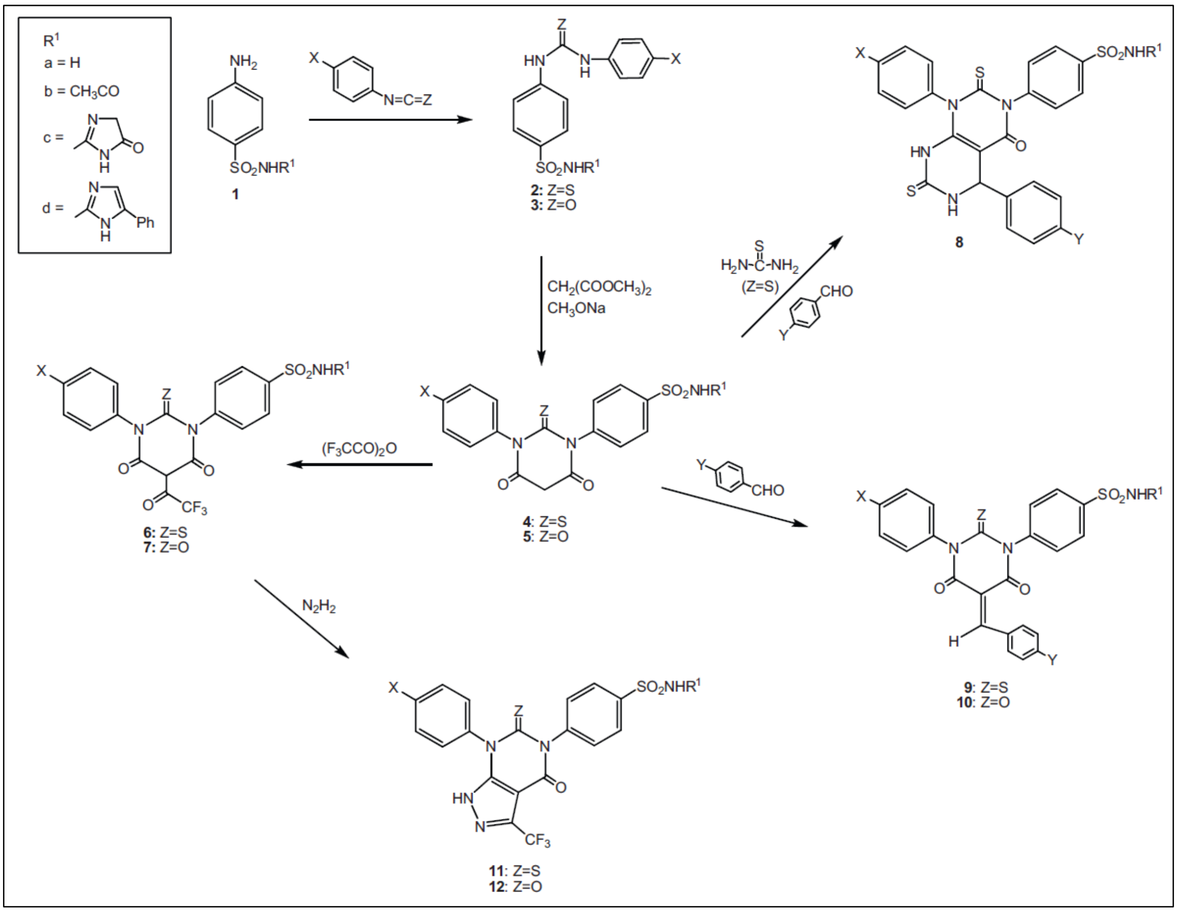


Figure 2.7. Benzenesulfonamide derivatives of barbituric acid derivatives [8]

Other 5-arylidene derivatives of thiobarbituric acids (Figure 2.8), were also found to have antibacterial effect [5].

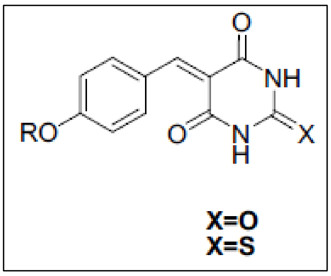


Figure 2.8. Other 5-arylidene derivatives of barbituric acids showing antibacterial activity [5]

In another study, it was proven that 5-benzylidine thiobarbituric acids (Figure 2.9) show more inhibitory impacts than 5-benzylidene barbituric acids in an antibacterial study of barbituric acid derivatives [22].

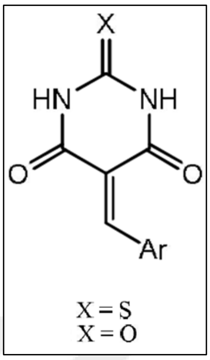


Figure 2.9. General structure of 5-benzylidene thiobarbituric acid derivatives [22]

Besides, thiobarbituric acid derived spiroheterobicyclic compounds (Figure 2.10) possess antibacterial effect against Escherichia coli, Pseudomonas aeruginosa, Staphyllococcus aureus and Staphylococcus epidermidis [23].

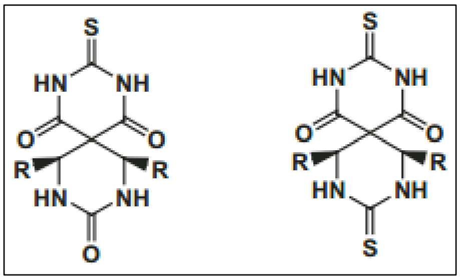


Figure 2.10. Spiroheterobicyclic derivatives of thiobarbituric acids [23]

2.1.3. Synthesis of Barbituric Acid and Thiobarbituric Acid Derivatives

Barbituric acids are obtained from malonic acid and urea as in Figure 2.11. Different derivatives of barbituric acids can be obtained by condensing urea and diethylmalonate [8].

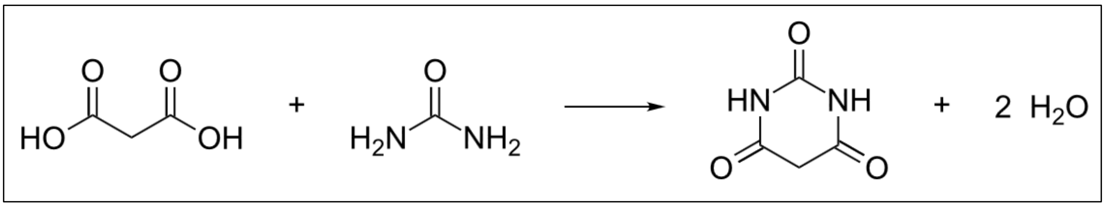


Figure 2.11. The synthesis of barbituric acid from malonic acid and urea [24]

2.2. ISOMERISM AND TYPES OF ISOMERS

The existence of two or more compounds that have the same molecular formula is called as isomerism. Isomers have the same molecular formula, which means, same number of same sorts of atoms belongs to them, but they are different compounds. Their classification comes from that how the atoms are attached to one another, or how they are oriented in the space (Figure 2.12) [25].

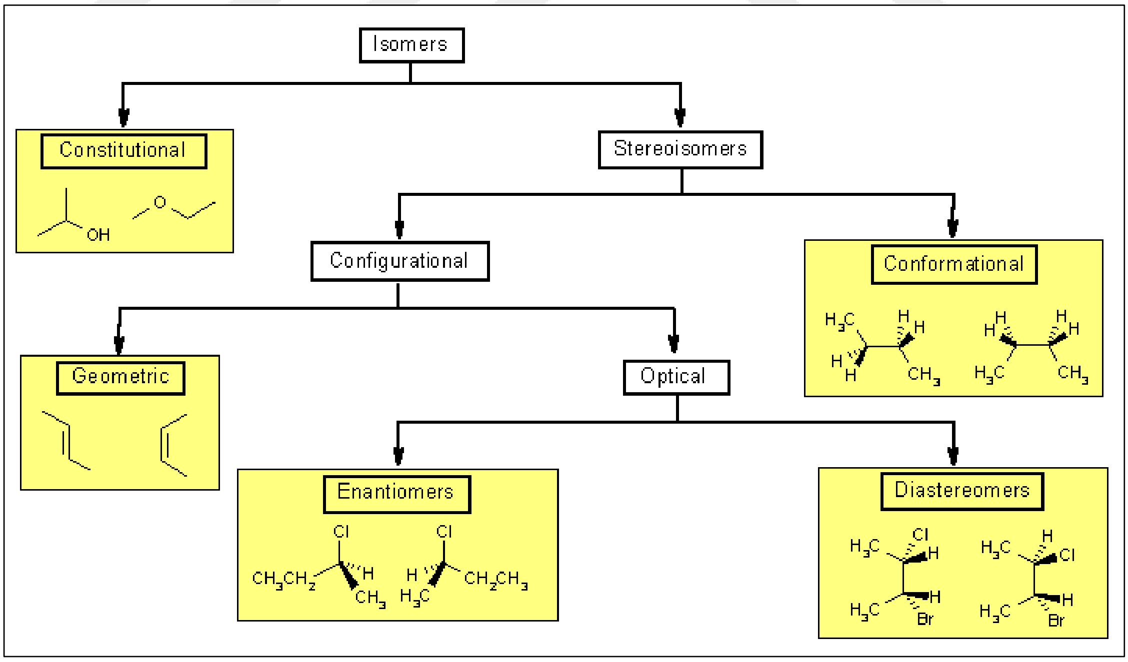


Figure 2.12. Subdivision of isomers [26]

2.2.1. Constitutional Isomers

Constitutional isomers are a type of isomers, where atoms possess a different connectivity. Constitutional isomerism may occur when atoms in a molecule are rearranged as attaching of different atoms to another in different ways (in shorthand different connectivity of atoms causes constitutional isomerism) [27]. In Figure 2.13, an example for constitutional isomers of C_6H_{12} can be seen [27].

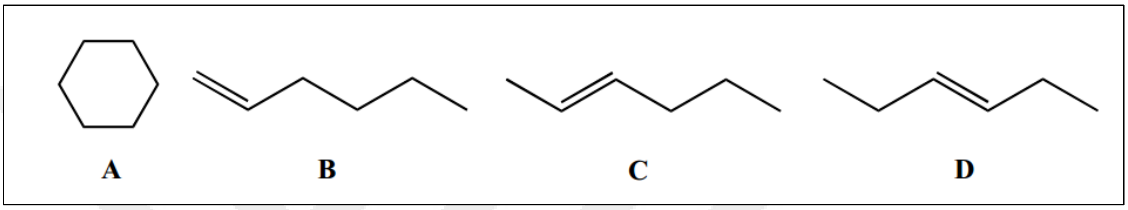


Figure 2.13. Constitutional isomers of C_6H_{12} [27]

2.2.2. Stereoisomers

Stereoisomers have same constitutions which mean same sequence of bonded atoms, so they have same molecular formula. However, stereoisomers are different in the three dimensional orientations of their atoms in space. Stereoisomers can be classified as optical isomers (e.g. enantiomers), geometrical isomers (cis and trans isomers) and conformational isomers (conformers) [25].

An example of cis and trans double bond isomers can be seen in Figure 2.14 [28].

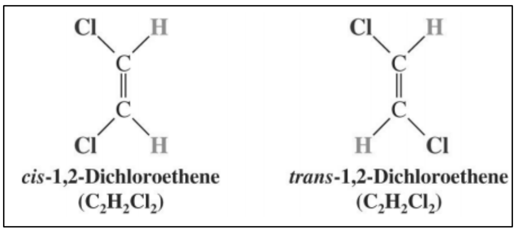


Figure 2.14. Cis and trans double bond isomers of 1,2-dichloroethene [28]

An example of cis and trans cycloalkane isomers is shown in Figure 2.15 [28].

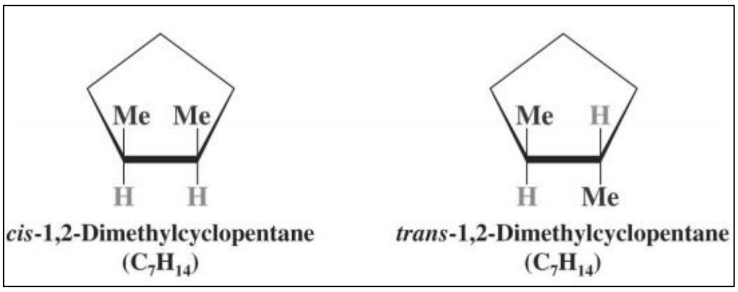


Figure 2.15. Cis and trans cycloalkane isomers [28]

2.2.2.1. Chirality

Optical isomers are chiral molecules. Chiral means hand in Greek. A molecule is called chiral when it is nonsuperimposable on its mirror image (Figure 2.16). It is also called as handedness. For instance, hands and gloves are examples of chiral objects because their right and left sides are nonsuperimposable mirror images [29].

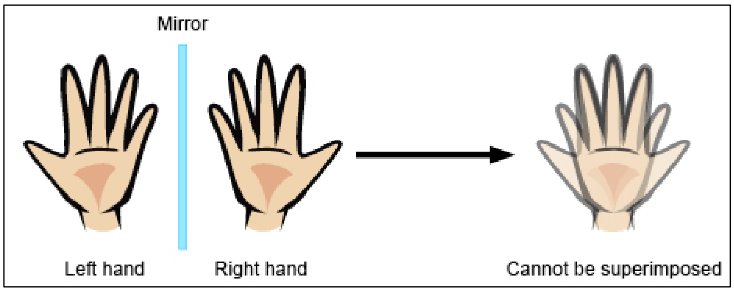


Figure 2.16. Example of a chiral object [29]

Molecules may have chirality due to their three dimensional properties and tetrahedral geometries of saturated carbons [25]. Conformational changes like rotations about single bonds are not considered requirements as acceptable situations for nonsuperimposability [25].

Achiral molecules have a plane of symmetry. A plane of symmetry is an imaginary plane and it divides a molecule into two halves which are mirror images of each other. Achiral molecules do not show stereoisomerism [30]. An achiral compound, which is superimposable on its mirror image, is seen in Figure 2.17.a on the left. In addition to that a chiral compound, which is not superimposable on its mirror image, is seen in Figure 2.17.b on the right [31].

Chiral molecules do not include a plane of symmetry, while achiral molecules include a plane of symmetry. A half of the molecule is a reflection of the other due to plane of symmetry (Figure 2.17.a) [18]. CH_2BrCl has plane of symmetry and two halves of this molecule are identical. Thus, CH_2BrCl is an achiral molecule in Figure 2.17.a. CHBrClF has no plane of symmetry and it is nonsuperimposable on its mirror image. Thus, CHBrClF in Figure 2.17.b is a chiral molecule [25].

The sources of chirality in molecules are chiral center, chiral axis, chiral plane and a helix. The most common is chiral center.

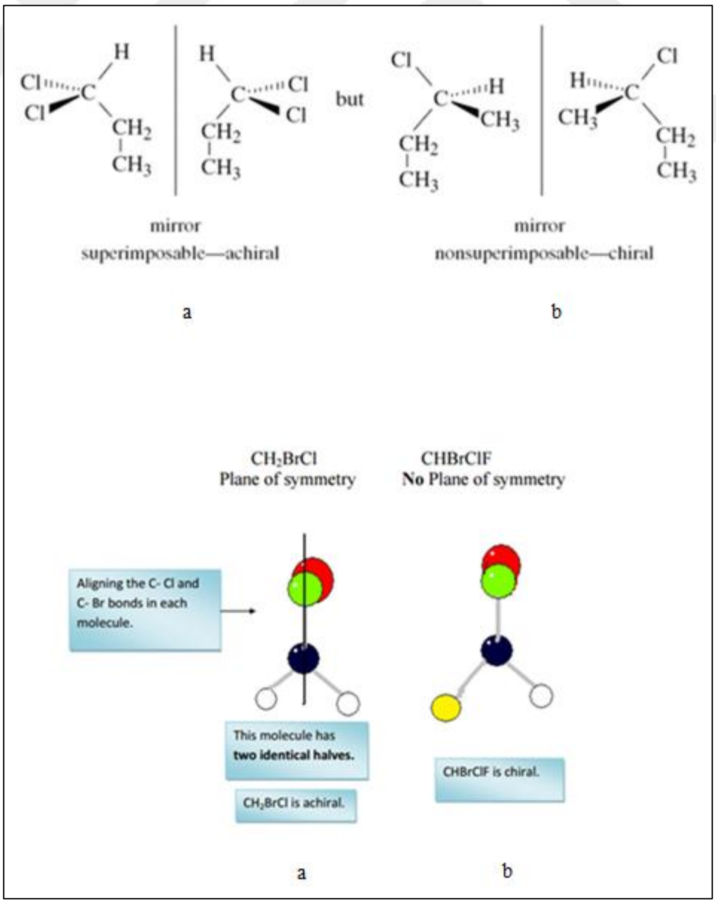


Figure 2.17. Achiral and chiral structures [25]

- ***Chiral Center (Asymmetric Center)***

A tetrahedral carbon atom which bears four distinct groups or atoms is called as a chiral center (Figure 2.18). Carbon atoms which are part of a multiple bond cannot be a chiral center [31].

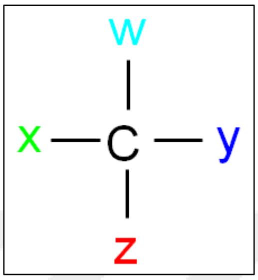


Figure 2.18. Chiral center [30]

A chiral molecule generally gets at least one chiral center. 5-bromodecane and 2,3-dihyroxybutanoic acid are examples of chiral molecules with chiral centers. While 5-bromodecane includes one chiral center (Figure 2.19.a), 2,3-dihyroxybutanoic acid includes two chiral centers (Figure 2.19.b) [32].

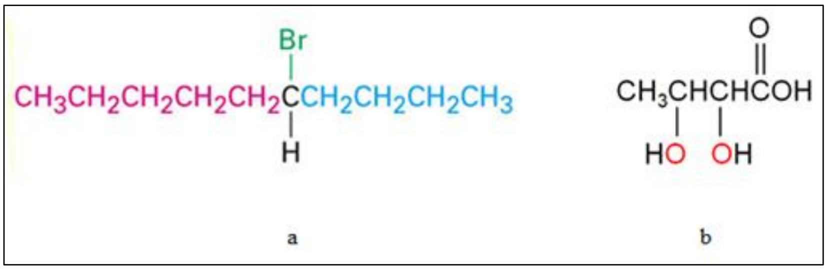


Figure 2.19. a: 5-bromodecane; b: 2,3-dihydroxybutanoic acid [32]

Meso compounds have at least two chiral centers, but they also have plane of symmetry, so they bear superimposable mirror images [30]. An example for this is 2,3-butanediol (Figure 2.20). In this molecule, two chiral centers exist and the top half of the molecule is the mirror image of the bottom half.

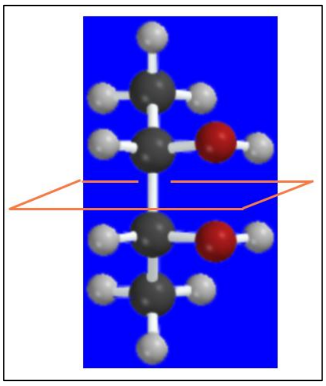


Figure 2.20. 2,3-Butanediol [32]

- ***Chirality Axis***

Although some molecules are chiral, they do not include a chirality center. Some of these molecules include chirality axis, different groups are arranged around an axis. Therefore, spatial arrangement is nonsuperimposable on its mirror image. For example, biphenyls and allenes have chirality axis as shown in Figure 2.21 [32].

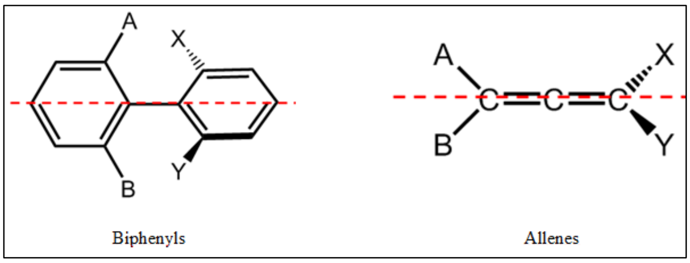


Figure 2.21. Examples of chiral molecules bearing chirality axis [32]

- ***Chiral Plane***

A chiral plane is a plane passing through a molecule so located that placement of a substituent group in that plane destroys a perpendicular plane of symmetry. Bridged aromatics are the largest group of molecules possessing chiral planes (Figure 2.22). The plane of the benzene ring is the chiral plane. Attachment of the Br in that plane in this instance destroys two perpendicular symmetry planes. That is, if the Br were not there, the plane of the page would be a symmetry plane, as would a vertical plane perpendicular to the page [33].

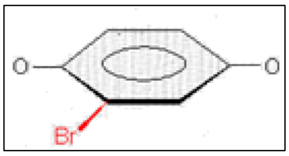


Figure 2.22. An example of the chiral plane [33]

2.2.2.2. Assignment of Configurations of Optical Isomers

- ***Absolute Configuration (R and S configurations)***

Absolute configuration states the exact three-dimensional arrangement of atoms at a stereocenter in space. Stereocenters are labeled as R or S configuration to specify the configuration in each mirror image [25].

In order to determine chirality center as R and S configuration:
Firstly, priorities to the groups that are attached to the chirality center are determined. Then, the group that has the lowest priority is pointed away from the looking side. Finally, the rotation direction of the remaining groups from highest priority to lowest priority is followed. When the direction of the rotation is clockwise, the configuration is called as R configuration (Figure 2.23.a). When the direction of the rotation is counterclockwise, the configuration is called as S configuration (Figure 2.23.b) [25].

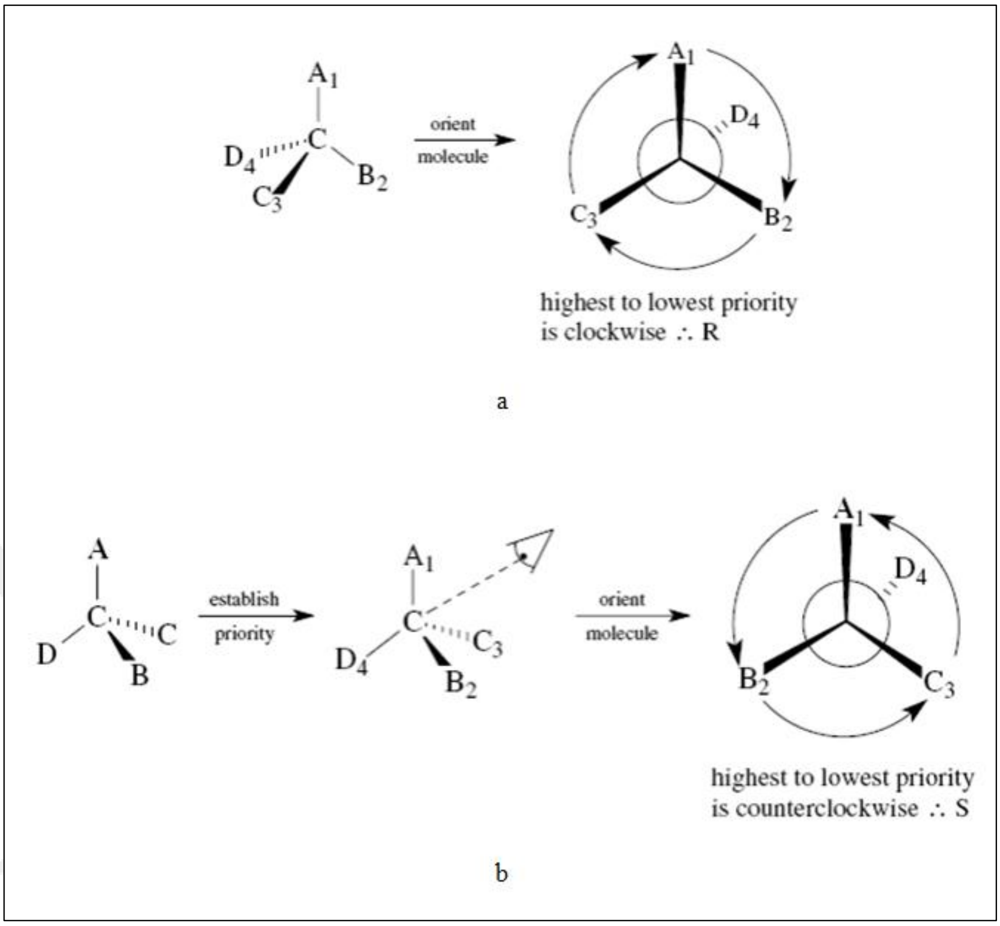


Figure 2.23. R and S configurations [25]

- ***Helical Chirality (M and P Configuration)***

Helical configuration is used for some natural and unnatural linear polymers. Mainly, helical chirality configuration is denominated for right handed B- DNA, left handed Z-DNA, protein alpha-helices and hydrated lipids. The chirality of a compound is assigned by the screw sense of a helix. When the screw is right handed, the chirality is called as P (plus) and when the screw is left handed, the chirality is called as M (minus) (Figure 2.24) [34].

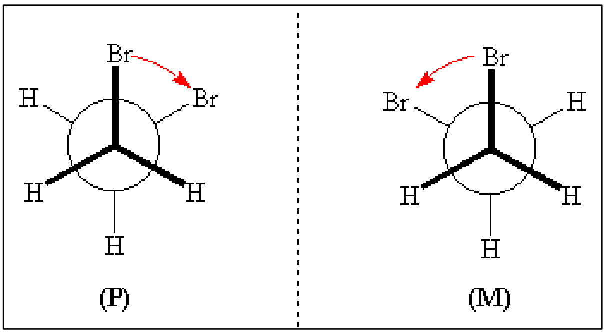


Figure 2.24. M and P configurations [34]

In order to determine M or P configurations to the sense of twist of structures, an axis sight down which will be associated with the helix is chosen, then its far and near substituents are considered separately. Near groups to the axis have higher priority, and highest priority, accordingly highest priority of the near and far groups is identified. While sighting down the axis, if the movement is from the near group of the highest priority, to the highest priority of the far group in a clockwise direction, the helix is a right handed and is called as P (plus) as in Figure 2.24. Besides, counterclockwise rotation means a left handed helix and it is called as M (minus) as in Figure 2.25 [35].

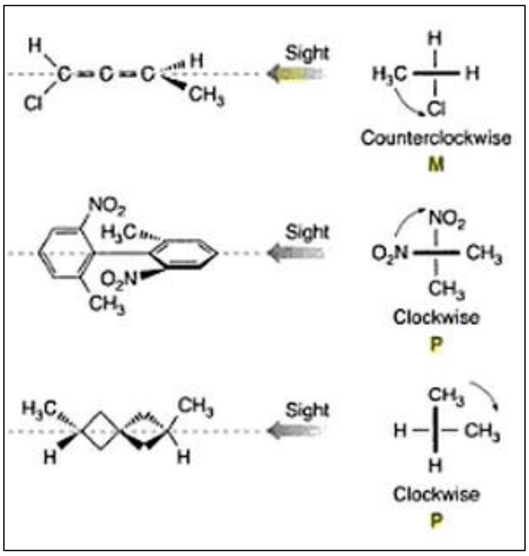


Figure 2.25. Determination of M and P configurations [35]

2.2.2.3. Types of Optical Isomers (Enantiomers and Diastereomers)

Enantiomers are mirror images of each other which are non-superimposable. Therefore, stereogenic centers in enantiomers have the opposite absolute configurations. Besides, enantiomers have the same physical properties; the only difference between them is the rotation direction of the polarized light. When a polarized light interacts with optical isomers of a compound, it rotate counterclockwise or counterclockwise depending on the three dimensional structure of enantiomer. If one enantiomer rotates the polarized light clockwise, the other enantiomer rotates the light counterclockwise [25].

If a molecule contains only one chiral center (Figure 2.11.a), it bears only enantiomers. If the molecule has more than one stereocenter (Figure 2.12), it may have enantiomeric, diastereomeric and meso isomers [36].

Pure enantiomers which are optically active may be separated with a chiral agent. Furthermore, different enantiomers of a compound may have different biological activities [25]. For example, Thalidomide has R and S enantiomers (Figure 2.26) which have different biological effects. While R enantiomer of Thalidomide is teratogen and it causes to birth defects, S enantiomer of Thalidomide is sedative and it is used to calm nervousness [37].

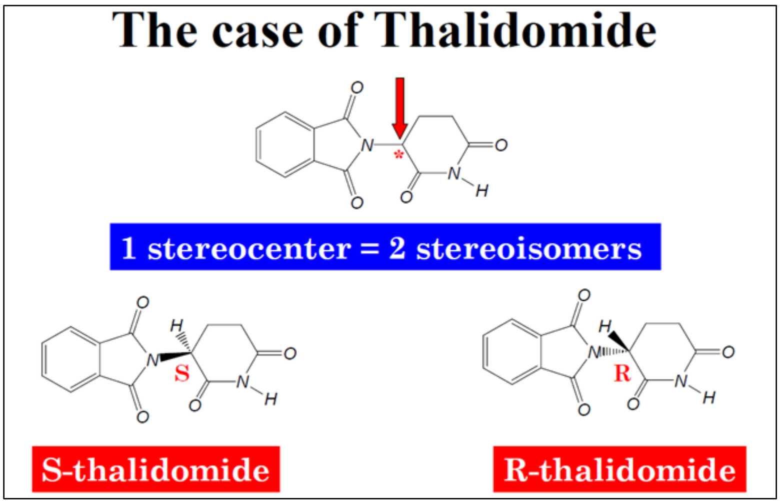


Figure 2.26. R and S enantiomers of Thalidomide [37]

Diastereomers are a type of stereoisomers and are not mirror images of each other (Figure 2.27). Also, diastereomers generally do not have the same physical properties. Meso compounds, cis-trans isomers ad non-enantiomeric optical isomers are examples of diastereomers [38].

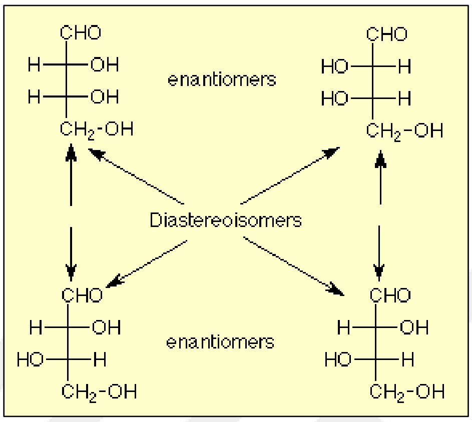


Figure 2.27. Relationship between diastereomers and enantiomers [39]

The studied N-*o*-aryl substituted barbituric and thiobarbituric acid derivatives are axially chiral due to nonplanar ground states of the molecules, the C_{aryl}-N_{sp^2} bond being the chiral axis and they have a pair of thermally interconvertable M and P enantiomers (Figure 2.28) [40].

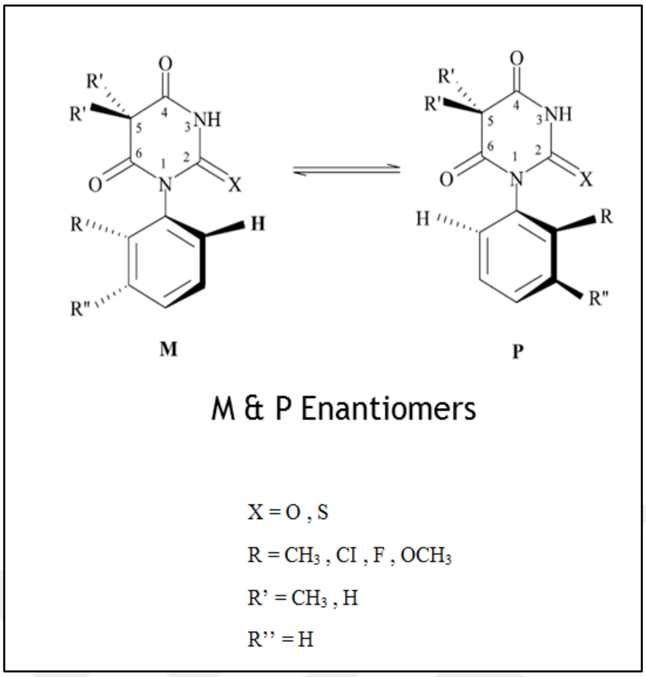


Figure 2.28. The structure of the N-*o*-aryl substituted barbituric acid, and-2-thiobarbituric acid derivatives [40].

The studied 5-methyl-substituted N-*o*-aryl substituted-2-thiobarbituric acid derivatives have besides chiral axis, a chiralcenter at C-5, thus they have four stereoisomers as MR, PS, PR and MS, of which PR&MS and PS&MR are two enantiomeric pairs. PR&MR and PS&MR are two diastereomeric pairs. The relationship between diastereomers and enantiomers of 5-methyl-1-(*o*-aryl)-2-thiobarbituric acid derivatives is shown in Figure 2.29.

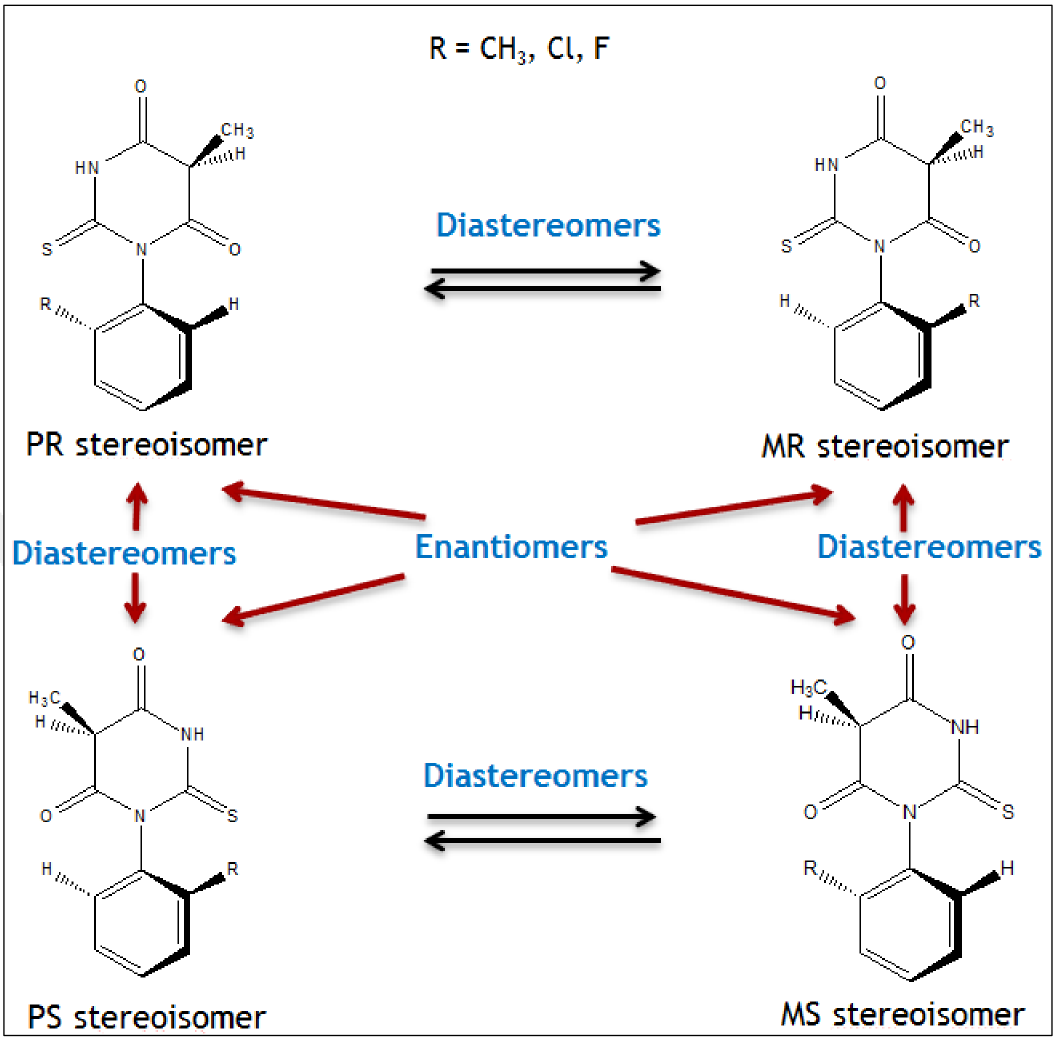


Figure 2.29. The relationship between diastereomers and enantiomers of 5-methyl-1-(*o*-aryl)-2-thiobarbituric acid derivatives

2.2.2.4. Optical Activity

When a compound rotates the plane polarized light, it has optical activity. In order to observe the optical activity, the compound must be chiral and one enantiomer of the compound must exist in excess. When an enantiomer rotates the plane polarized light in the clockwise direction, that enantiomer is called as positive (+)-enantiomer. Besides, when an enantiomer rotates the plane polarized light in the counterclockwise direction, that enantiomer is called as negative (-)-enantiomer (Figure 2.30). Furthermore, if two enantiomers are present in the mixture as 50 %, this phenomenon is called as racemic mixture or racemate. Racemic mixtures cannot rotate plane polarized light [25].

An optically active substance is chiral and the amount of one enantiomer is greater than that of the other [32].

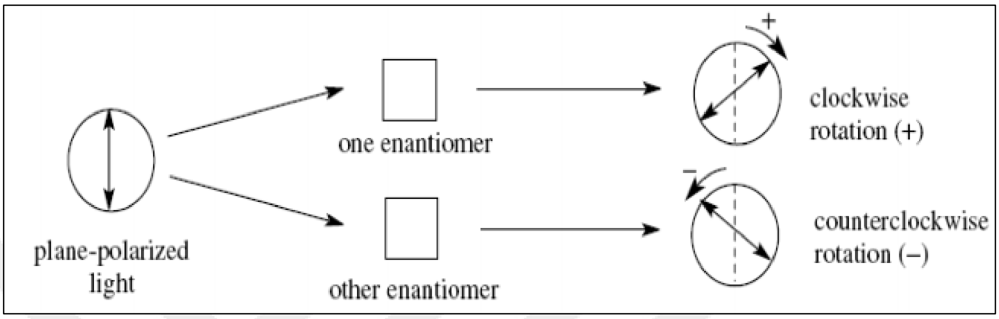


Figure 2.30. Positive and negative enantiomers [25]

2.3. ASYMMETRIC SYNTHESIS

Asymmetric synthesis is important in the chemical production of pharmaceuticals. It is also called as stereospecific reaction since only one stereoisomer is obtained. Generally pharmacological and natural products are obtained in the form of one enantiomer [14].

The reactions of asymmetric synthesis generally include organic compounds that have a symmetrical structural property. In that property, a carbon atom is bonded to four other atoms or groups of atoms of which two are alike. One of the two identical groups is replaced preferably. Therefore, the obtained product is a mixture of two dissymmetric compounds and one of them dominates in the asymmetric synthesis [41].

Asymmetric reactions are occurred with the effect of the some dissymmetry in the reacting system such as the existence of the dissymmetric center in the molecule, a dissymmetric catalyst, dissymmetric solvent or polarized light [41].

If starting material are achiral as reactants or catalyst or solvents, a chiral product can be obtained as a racemic mixture [27].

There are two types of asymmetric synthesis. In the first type of asymmetric synthesis, a new stereogenic center is obtained in an achiral molecule and and two enantiomeric transition states of equal energy form. Hence two enantiomeric products are obtained in equal amounts (Figure 2.31) [42].

Enantioselective reactions are performed directly on achiral starting materials, hence chiral reagent causes to obtain energetically distinct transition states if it is approached to prochiral groups on a molecule equally from both sides [42].

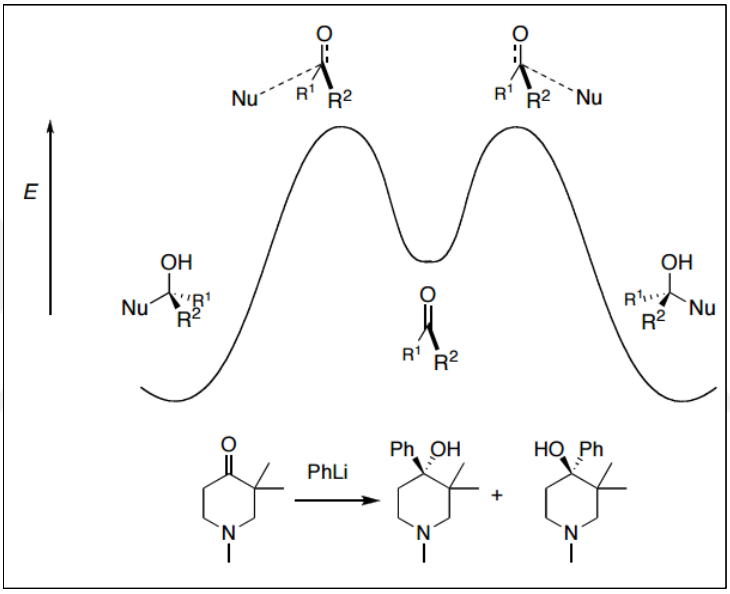


Figure 2.31. Two enantiomeric transition states of equal energy [42]

In the second type of asymmetric synthesis, when there is an existential chiral center, two possible enantiomeric pairs can be obtained with different energies. Thus, one isomer of the new chirality center can be produced in a larger amount (Figure 2.32) [42].

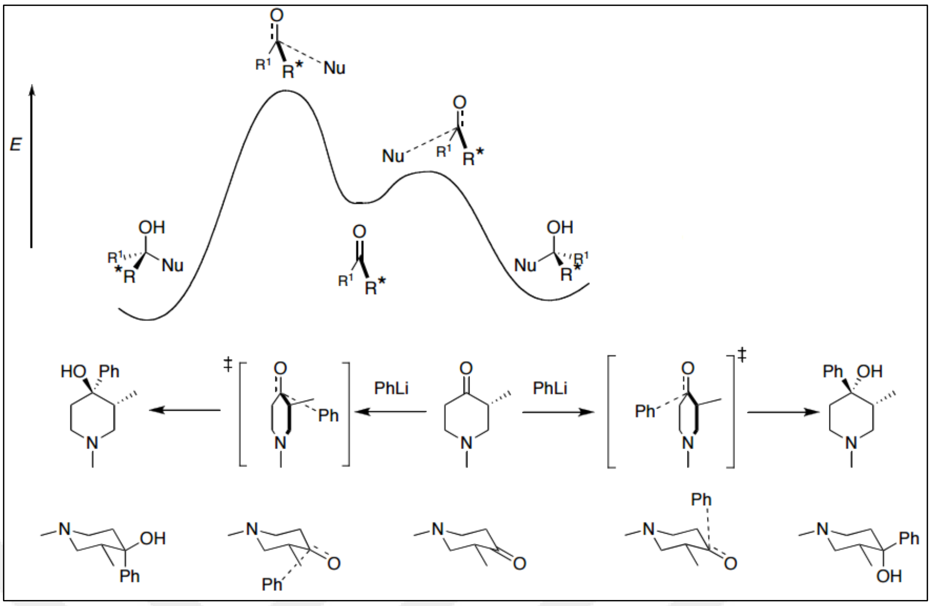


Figure 2.32. Isomers of enantiomeric pair in different energies [42]

Asymmetric enolate alkylation was explained as below [42].

2.3.1. Enantioselective Enolate Alkylation

There are three steps of enantioselective enolate alkylation as installation of auxiliary, reaction with chiral induction and removal of auxiliary, examples of which are shown in Figures 2.33, 2.334 and 2.35, respectively [42]. In this example, acid chloride reacts with oxazolidone used as a chiral catalyst to produce imide as in Figure 2.33. Amide reacts with LDA (Lithium Di-isopropyl Amide) to form enolate. Then, enolate reacts with ethyl iodide as in Figure 2.34 and forms two enantiomeric products, one in a higher amount. Oxazolidones are obtained by the addition of lithium hydroxide or lithium methoxide to enolates as in Figure 2.35 [42].

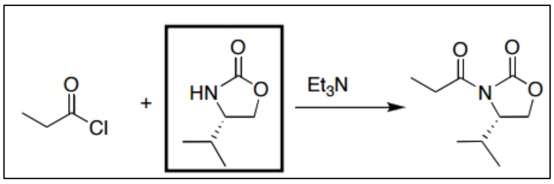


Figure 2.33. Example of installation of auxiliary [42]

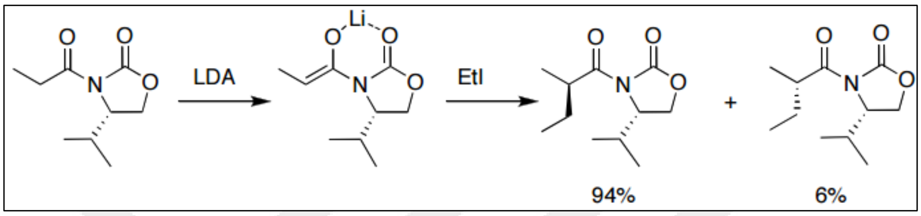


Figure 2.34. Example of reaction with chiral induction [42]

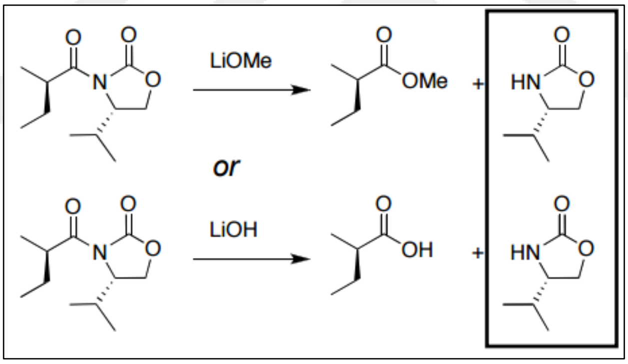


Figure 2.35. Example of removal of auxiliary [42]

2.3.2. Enantiomeric Excess

Enantiomeric excess is used to determine the degree of enantioselectivity for a chemical reaction. It is the amount (%) of subtraction of percentage of one enantiomer from the percentage of the other existing enantiomer. For example, when enantiomers are in the percentage of 80:20, then the enantiomeric excess is 60 % [42].

2.3.3. Nucleophilic Substitution Reaction

In an alkylation reaction, a hydrocarbon is introduced into a compound [43].
''Nucleophile means electron rich species which will react with electron poor species''. Nucleophiles are rich in electrons. Substitution reactions take place when one group in the molecule is replaced with another group. ''Substitution means that one group replaces with another''. Nucleophilic substitution reactions happen when a nucleophile reacts at an electrophilic saturated carbon atom added to an electronegative group. When there is an electrophilic saturated carbon atom added to an electronegative group, a nucleophile may attack this carbon and replace the electronegative element or group. This type of reactions is called nucleophilic substition reaction. The general scheme of the reaction is seen in Figure 2.36 [44].

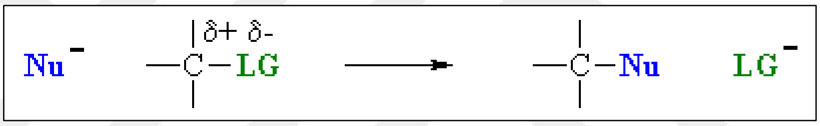


Figure 2.36. General scheme of nucleophilic substitution reaction [44].

Electrophilic carbon atom has polar σ bond because of the existence of electronegative substituent. (example : C-Cl, C-Br, C-I, C-O are polar bonds) [44].

Nucleophilic substitution reactions are essential because they permit the interconversion of functional group transformation. Example of nucleophilic substitution reactions are reactions of alkyl halides (R-X) with Lewis bases and reactions of alcohols (R-OH) with hydrogen halides [44].

2.4. TAUTOMERS and TAUTOMERIZATION

Tautomers are structural isomers of organic compounds which are in dynamic equilibrium with themselves because of migration of a proton [45]. ''The isomerization reaction by which tautomers are interconverted to each other is called as tautomerization'' [38].

X, Y and Z atoms can be C, H, O or S atoms and H is an electrophile center during isomerization (Figure 2.37) [45].

$$H-X-Y=Z \rightleftharpoons X=Y-Z-H$$

Figure 2.37. An Example of Tautomerization (isomerization) [45]

Tautomerization can be observed in solutions. A chemical equilibrium between the isomers can be reached when hydrogen atom migrates and meanwhile an exchange of single and the neighbor double bond takes place. Generally the catalysts of the tautomerization reactions are acids or bases [45].

The chemical equilibrium between tautomers and the schematic representation of a tautomerization mechanism can be seen in Figure 2.38. Also, generation of different tautomer forms for a molecule was represented in Figure 2.39 [45].

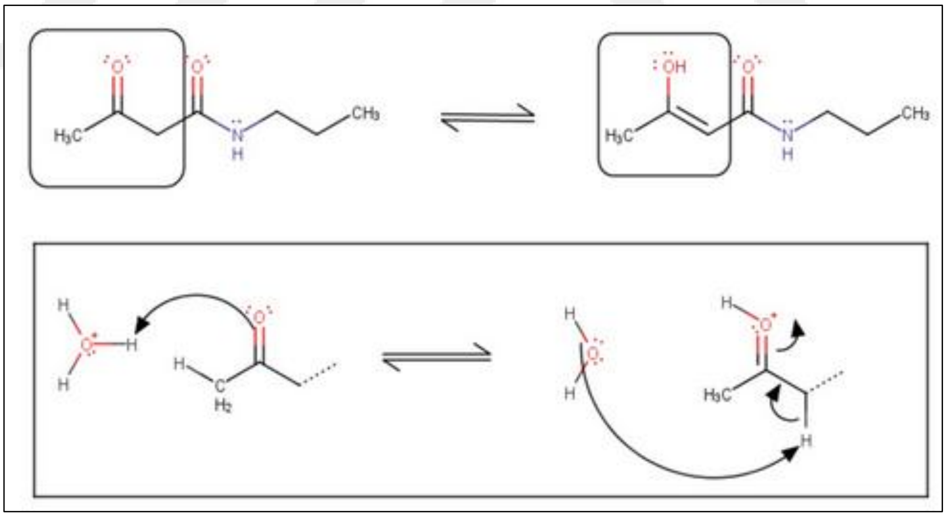


Figure 2.38. Schematic representation of tautomerization mechanism [45]

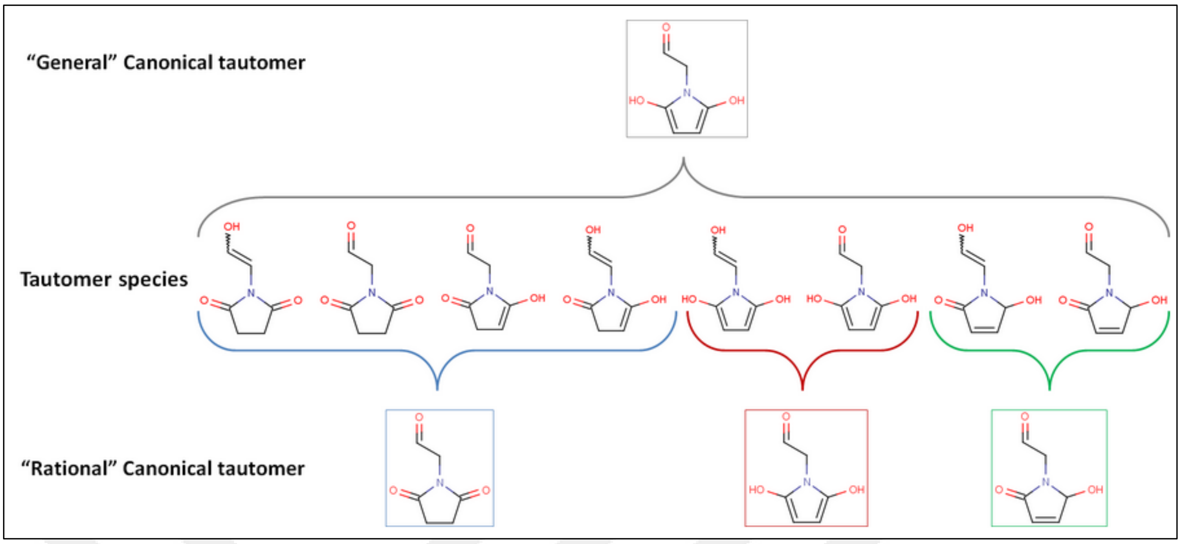


Figure 2.39. Generation of different tautomer forms for a molecule [45].

2.5. CINCHONINE

The chemical formula of Cinchonine is $C_{19}H_{22}N_2O$ (Figure 2.40). Also, cinchonine is used with quinine, quinidine and cinchonidine in the antimalarial drugs. These alkaloids show multidrug resistance in the distinct kinds of tumors. Cinchonine has lower toxicity and higher activity with respect to quinine. It also decreases high-fat-diet (HFD) [46]. Cinchonine catalyst is used highly in the asymmetric reactions such as addition reactions [47].

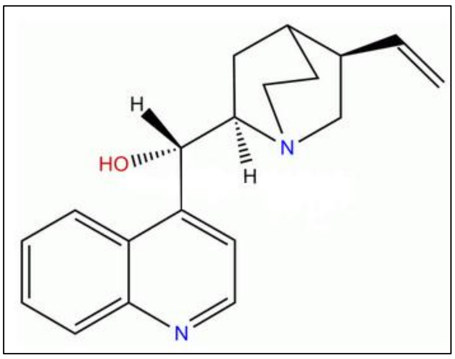


Figure 2.40. Chemical structure of cinchonine, $C_{19}H_{22}N_2O$ [48]

2.6. ANTIBACTERIAL AGENTS AND TESTS

2.6.1. Antibacterial Agents

‘‘Antibacterial agent is any of various chemical compounds and physical agents that are used to kill microorganisms or to obstruct their development’’. Modern antimicrobial therapy started with the production and use of antibiotic penicillin in 1940. Different types of antibiotics and antimicrobials have been found with the discovery of Streptomycin in 1944. Basic structures of the antibacterial agents can be modified chemically in order to enhance their characteristics. This effect was improved with the introduction of antibacterial agents into medication [49].

2.6.2. Bacterial Resistance

‘‘Bacterial resistance to antibacterial agents is a quantitative measurement of the efficiency (concentrated expressed in micrograms per milliliter or as inhibition zones in millimeters for the diffusion techniques) of an antibacterial agent against a specific bacterium’’ [50].

‘‘Minimum inhibitory concentration (MIC) is a relative measurement of the smallest amount of antibacterial agent that is required to inhibit the growth (cell division) of a bacterium’’ [51].

Bacterial resistance to antibacterial agents is a situation that there is not susceptibility or declined susceptibility to antibacterial cell growth or cell death [51].

There are two types of bacterial resistance as natural and acquired. ‘‘The bacterial population is initially susceptible to antibacterial agents, but the bacteria undergo changes by acquisition of plasmid and transposon or chromosome mutation and strains emerge that are less susceptible or not at all susceptible to these antibacterial drugs in the acquired resistance’’ [51].

2.7. METHODS of ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility tests are based on diffusion, dilution and diffusion and dilution [9].

There are three types of diffusion method as Stokes disk diffusion method, Kirby-Bauer disk method and primary disk diffusion test [9]. They were explained briefly in section 2.5.1.

The dilution methods are used to calculate minimum inhibition concentration. There are two types of dilution methods as Broth Dilution and Agar Dilution [9]. They were explained in section 2.5.2.

E-Test method is based on both dilution and diffusion [9]. In this method, a plastic test strip which impregnated with an antibiotic, of step by step declining concentration is used. Numerical scale of the strip is used to determine the antibiotic concentration in the strip. The cost of E-test is high because different strips are required for each antibacterial test [52].

2.7.1. Disk Diffusion Method

In the disk diffusion method, an antimicrobial agent of a specified concentration diffuses from disks, tablets or strips into the solid culture medium which has been seeded with the selected inoculum isolated in a pure culture [10].

The principle of disk diffusion method depends on the identification of an inhibition zone proportional to the bacteria susceptibility to the antimicrobial existing in the disk [10].

Besides, the diffusion of the antimicrobial agent into the seeded culture media causes gradient change of the microbial. If the concentration of the antimicrobial agent is very dilute, inhibition of the growth of the test bacterium takes longer. The diameter of the zone of inhibition around the antimicrobial disk correlates inversely with the minimum inhibitory concentration of the bacterium. In order to obtain larger zone of inhibition, lower concentration of the bacterium and/or higher concentration of the antimicrobial agent can be used.

Disk diffusion method is easy, reproducible and inexpensive. Main advantages of disk diffusion methods are:

- It is cheap.
- It is easy to change antimicrobial test disks when needed.
- It can be used as a screening test toward large number of isolates.
- It can assign a subset of isolates for further testing by other methods such as minimum inhibitory concentrations (MICs) [10].

Some disadvantages of disk diffusion method are:

- Measurements of inhibition zone manually may be time consuming.
- If disks are distributed, the inhibition zones around the disk cannot be determined accurately [49].

In the disk diffusion method, firstly the standardized bacterial isolate is spread on the agar plate. Then, blank paper disks that include certain concentration of agents or antibiotics are located on the agar plate. Finally, this agar plate is incubated at 37 ^{0}C for 24 hours in the incubator. If the isolate is susceptible to agent or antibiotics, it does not grow around the disk. Thus, the zone of inhibition is observed. Strains resistant to the antibiotic or agents grow up to the margin of the disk plate. The diameter of forming zone of inhibition is measured. The result can be read from the chart as sensitive, intermediate or resistant [53]. The measurement of the diameter of the zone is shown in Figure 2.41 [11].

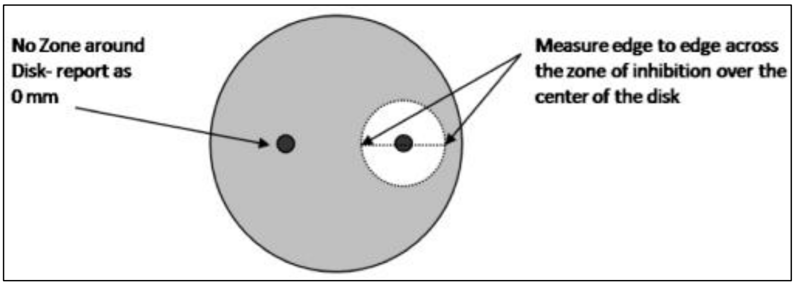


Figure 2.41. The evaluation of the zone around the disk [11]

There are three types of disk diffusion method as Kirby-Bauer disc diffusion method, Stokes disk diffusion method and primary disk diffusion test [12].

2.7.1.1. Kirby-Bauer Disk Diffusion Method

Kirby-Bauer method (Figure 2.42) is the most common used method to determine the antimicrobial agents or antibiotics sensitivity of bacteria [12]. A bacterium is swabbed on an agar and the antibiotic disk is put on the top. The antibiotic or agents diffuse from the disc into the agar in declining amounts the further it is away from the disc. If the organism is killed or inhibited by the concentration of antibiotics or antimicrobial agents, zone of inhibition will be observed. This means that there is no growth of bacteria around the disc [12].

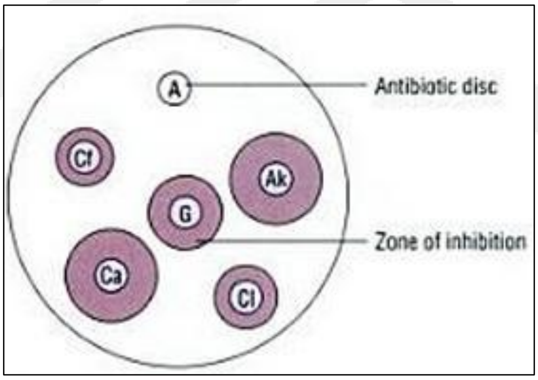


Figure 2.42. Kirby-Bauer disk diffusion method [12]

2.7.1.2. Stokes Disk Diffusion Method

Stokes disk diffusion method is used for inbuilt controls towards many variables. Petri dishes that include Mueller-Hilton agar are divided into three parts horizontally. The test strain is inoculated in the central area and the control strains are inoculated on the upper and the lower third part of the plate (Figure 2.43) [12].

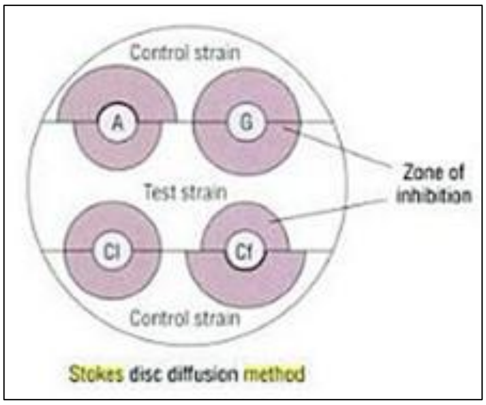


Figure 2.43. Stokes disk diffusion method [12]

In the Stokes disk diffusion method, standard strains are used for the quality control of the antimicrobial work. Also, standard strains are compared to control strains such as Escherichia coli NCTC 10418, Pseudomonas aeruginosa NCTC 10662 and Staphylococcus aureus NCTC [51].

2.7.1.3. Primary Disk Diffusion Test

In contrast to Kirby-Bauer Stokes disk diffusion tests, which are performed on pure cultures of bacterial isolates from clinical specimens, primary disk diffusion test is directly performed on clinical specimens [11].

2.7.2. Dilution Methods

A series of concentrations of antibacterial agents must be isolated in a broth medium for the broth dilution method. Total broth volume is usually in the range of 0.005 and 0.1 mL in microdilution test, but microdilution tests are generally performed as microliter. In the macrodilution method, the broth volume was about 1.0 mL in the standart test tubes. The inhitions of lowest concentrated antibacterial agents are obtained as MIC in the both dilution methods. Microdilution method is valid where positive control demonstrates growth and negative control demonstrates no growth. Same procedure was also used for agar dilution method. Lowest concentrations of serially diluted antibacterial agent are found for the inhibition of the bacterial growth [13].

2.8. SOME BACTERIES USED IN DISK DIFFUSION METHOD

2.8.1. Escherichia Coli (E. Coli)

Escherichia Coli is very widespread bacterium in the digestive system. It is also a part of normal bacterial flora. E. coli can produce toxin which leads to a serious infection. Humans get the infection from contaminated foods or water. The incubation period of E. coli is about 3-4 days. Besides, various gastrointestinal sicknesses from E. coli appear like bloody diarrhoea. Shiga toxin is produced from E. coli that leads to various systemic illnesses on humans such as Haemolytic uremic syndrome [54].

2.8.2. Bacillus Subtilis (B. Subtilis)

Bacillus Subtilis is also very common bacterium that is found in soil, water and decomposing plant matter. It is resistant to high temperatures, chemicals and environmental factors because Genus of B. subtilis bacteria produce spore that generate a thick wall surrounding the DNA and other inner cell structures. Thus, they are used in different industrial processes [55].

Bacillus Subtilis is used for many industrial applications such as various enzyme productions, textile and starch modification for the sizing of paper, leather industry and detergents. Besides, it used for the production of antibiotics like difficidin, oxydifficidin, bacilli, bacillomyin B, and Bacitracin. Additionally, it is used as fungicide and as agricultural seeds of vegetables, soybeans, cotton, peanuts, and flowers. It is also used in the production of toxins to kill malarial mosquito larvae [55].

Bacillus Subtilis is not pathogenic or toxigenic to humans, animals and plants. [55].

2.8.3. Pseudomonas Aeruginase (P. Aeruginase)

Pseudomonas Aeruginosa is a blue-green pus bacterium that rarely leads to infection in healthy individuals. Infection with P. Aeruginase can cause urinary tract infections, blood stream infection, pharyngitis and many diseases as chronic pulmonary illnesses. Besides, soil, marshes and coastal marine habitats contain P. Aeruginase. P. Aeruginase is resistant to most antibiotics. This bacterium can grow in eye drops, soaps, sinks, anesthesia and resuscitation equipment, fuels, humidifiers, dialysis machines and may be stored in distilled water [56].

2.8.4. Staphylococcus Aureus (S. Aureus)

Staphylococcus aureus is a common type of a bacterium. It exists on the skin, hair, noses and throats of people and animals. Besides, S. aureus can lead to food poisoning if the food is not correctly refrigerated and is contaminated. Also, S. Aureus bacteria may multiply at the room temperature to form a toxin which leads to sicknesses. The bacteria of Staphylococcus aureus can be killed by pasteurization and cooking [57].

2.9. FACTORS INFLUENCING ANTIMICRONIAL SUSCEPTIBILITY TESTING

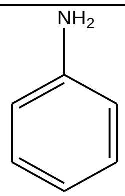
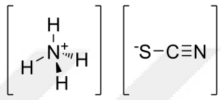
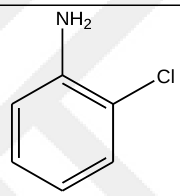
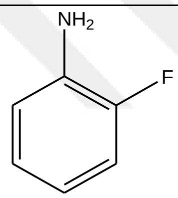
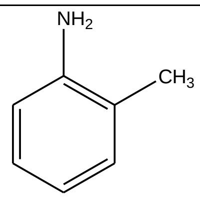
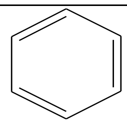
There are some factors affecting antimicrobial susceptibility testing. These are pH, moisture, cation content, amount of organism, temperature, atmosphere, duration of incubation, antimicrobial content of the disk, storage conditions, the source of agars, supplements, the age and turbidity of the bacterial column, the way in which the bacterium is spread on the plate, effects of medium components and the method of reading results [58].

3. MATERIALS AND METHODS

3.1. CHEMICALS

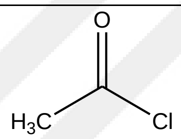
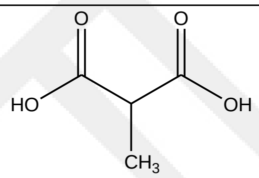
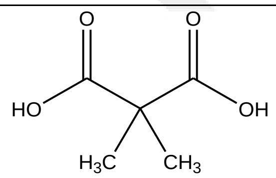
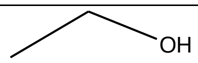
While aniline was used for the phenylthiourea synthesis, *o*-chloroaniline, *o*-fluoroaniline, *o*-toluidine were used for the synthesis of *ortho*-substituted phenylthioureas. Also, ammonium thiocyanate and hydrochloric acid were used for the synthesis of phenylthiourea and *ortho*-substituted phenylthioureas. The chemicals used in the thiourea syntheses were listed in Table 3.1.

Table 3.1. Chemical structures and the origins of the chemicals used in the thiourea syntheses

Chemical Name	**Chemical Formula**	**Chemical Structure**	**Origin**
Aniline, 99 %	C_6H_7N		Sigma Aldrich
Ammonium thiocyanate, 97.5 %	NH_4SCN		Sigma Aldrich
o-chloroaniline, 98 %	C_6H_6ClN		Sigma Aldrich
o-fluoroanilin, 99 %	C_6H_6FN		Sigma Aldrich
o-toluidine, 99 %	C_7H_9N		Acros
Hydrochloric Acid, 37 %	HCI	H—Cl	Sigma Aldrich
Benzene	C_6H_6		Merck
Petroleum Ether	mixture of C_5 and C_6 hydrocarbons		Merck

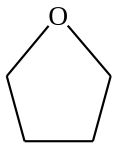
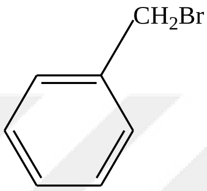
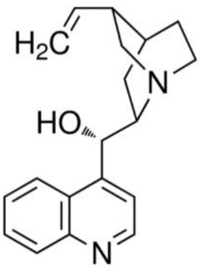
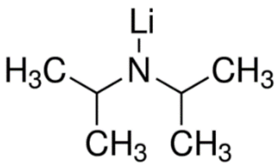
Methylmalonic acid, dimethylmalonic acid, *o*-chlorophenylthiourea, *o*-fluorophenylthiourea, *o*-tolylthiourea, phenylthiourea and acetyl chloride were used in the synthesis of 5,5-dimethyl,-5-methyl-1-(*o*-aryl)-2-thiobarbituric acid derivatives. The chemicals used in this synthesis were listed in Table 3.2.

Table 3.2. Chemical structures and the origins of the chemicals used in the synthesis of 5,5-dimethyl, and- 5-methyl-1-(*o*-aryl)-2-thiobarbituric acid

Chemical Name	**Chemical Formula**	**Chemical Structure**	**Origin**
Acetyl Chloride, 99 %	C_2H_3ClO		Sigma Aldrich
Methylmalonic Acid, 99 %	$C_4H_6O_4$		Sigma Aldrich
Dimethylmalonic Acid, 98 %	$C_5H_8O_4$		Sigma Aldrich
Absolute Ethanol, 99.8 %	C_2H_6O		Sigma Aldrich

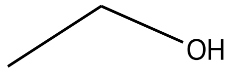
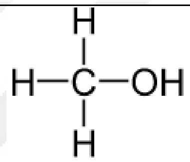
Tetrahydrofuran (THF), benzyl bromide, allyl bromide, (+)-cinchonine and lithium diisopropylamide (LDA) were used in the alkylation reactions of 5-methyl-1-(*o*-aryl)-2-thiobarbituric acids at C-5 position. The chemicals used in the alkylation reactions of 5-methyl-1-(*o*-aryl)-2-thiobarbituric acids were listed in Table 3.3.

Table 3.3. Chemical structures and the origins of the chemicals used in the alkylation reactions of 5-methyl-1-(*o*-aryl)-2-thiobarbituric acids

Chemical Name	**Chemical Formula**	**Chemical Structure**	**Origin**
Tetrahydrofuran (THF), 99.9 %	C_4H_8O		Merck
Benzyl bromide, 98 %	C_7H_7Br		Merck
(+)-cinchonine, 85 %	$C_{19}H_{22}N_2O$		Sigma Aldrich
Lithium diisopropylamide (LDA)	$C_6H_{14}LiN$		Sigma Aldrich
Allyl bromide, 97 %	C_3H_5Br		Sigma Aldrich

Also, chemicals used in TLC and HPLC analyses were listed in Table 3.4.

Table 3.4. Used Solvents in TLC and HPLC Analyses

Chemical Name	Chemical Formula	Chemical Structure	Origin
Ethanol (HPLC Analyzed)	C_2H_6O		J.T.Baker
Hexane (Gradient Grade for HPLC)	C_6H_{14}		Sigma Aldrich
Methanol (Gradient Grade for HPLC)	CH_3OH		Sigma Aldrich

3.2. METHODS

3.2.1. Thin Layer Chromatography (TLC)

Thin layer chromatography is a beneficial method for the separation and identification of compounds in the mixtures. It is generally used to pursue the progress of reactions by observing the depletion of starting materials and also the existence of the products. Trading applications of TLC methods cover the analysis of urine for the evidence of doping, the analysis of drugs to check impurity and identity of the components and analysis of foods to detect the existence of contaminants like pesticides [59].

The same principles as extraction are used to separate and purify the compounds in the TLC analysis. The reason for the different distribution of compounds between two phases is differences in solubility of these compounds in the two phases. In the TLC analysis, one phase is the mobile liquid phase, (the eluent) which is allowed to flow up the plate by capillary action. The other phase is the stationary solid phase, (the adsorbent) with a high surface area. The stationary phase generally comprises of silica or alumina powder, and the mobile phase generally comprises of a volatile organic solvent or mixtures of solvents [59].

The eluting strength increases while solvent polarity increases since eluting strength of the solvent directly related to its adsorption on the adsorbent and adsorbents are generally highly polar. The solvents used as mobile phase are listed in Table 3.5 in order of increasing eluting strength [59].

Table 3.5. Eluting power of solvents used in the chromatography [59].

Less and More Polar Solvents	
Solvents with Less Eluting Strength (less polar solvents)	Pentane, hexane, heptane
	Toluene, *p*-xylene
↓ Increasing Eluting Strenght	Dichloromethane
	Diethyl ether (anhydrous)
	Ethyl acetate (anhydrous)
	Acetone (anhydrous)
	Acetic acid
Solvents with Greater Eluting Strength (more polar solvents)	Ethanol (anhydrous)

With the help of the capillary tube, sample from the solution is put on the marked points on the TLC plate. Then the plate is put into the developing chamber containing the mobile phase as shown in the Figure 3.1. After that the solvent moves by the capillary action up the plate. When the solvent passes through the spot, equilibrium is formed between the molecules of each component of the mixture. The molecules of the components that are highly adsorbed on the solid phase are moving slower than the components that are adsorbed weakly on the solid phase. After the solvent is reached the top of the plate, the plate is taken from the developing chamber. After the TLC plate dried, the separated compounds are visualized with an UV lamp (Figure 3.2). However, if the compounds are colored, it is not necessary using UV lamp [60].

Figure 3.1. Thin layer chromatography analysis [60]

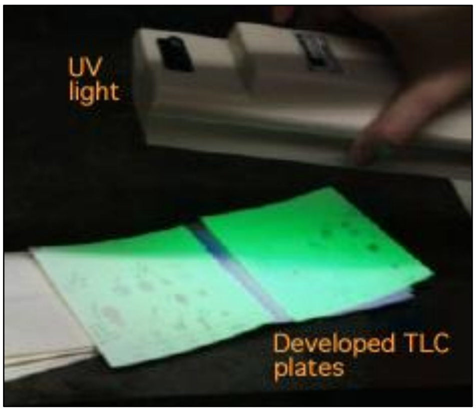


Figure 3.2. UV analysis for TLC [59]

If the prepared solutions which are applied onto TLC plate are too concentrated, the spots will be streaked or components will be carried out jointly. If this situation occurs, less concentrated samples should be prepared in order to observe separated spots on the thin layer plate [61].

3.2.2. High Performance Liquid Chromatography (HPLC)

High pressure liquid chromatography (HPLC) is a separation method that requires the injection of a smaller volume of liquid sample into the stationary phase where each distinctive component of a sample is transported along a column with the liquid mobile phase forced through the column by high pressure delivered by a pump. A tube, which is packed with porous particles is called as stationary phase [62].

HPLC is used for analysis and separation of nonvolatile chemical and biological compounds. Besides, HPLC is used for qualitative and quantitative analysis, trace analysis and purification [63].

Individual compounds in the sample can be identified by qualitative analysis (Figure 3.3). Retention time is the most common parameter in the qualitative analysis [63].

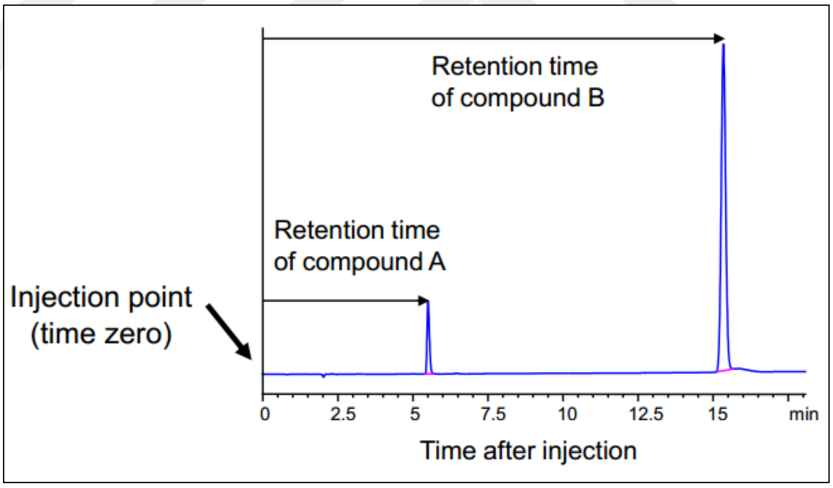


Figure 3.3. Qualitative analysis [63]

Quantitative analyses are performed by the integration of the peak areas of the compounds (Figure 3.4), because there is linearity between the peak height, the peak area and the amount of the sample. The type of column packing material and the mobile phase determine the retention time of the sample [63].

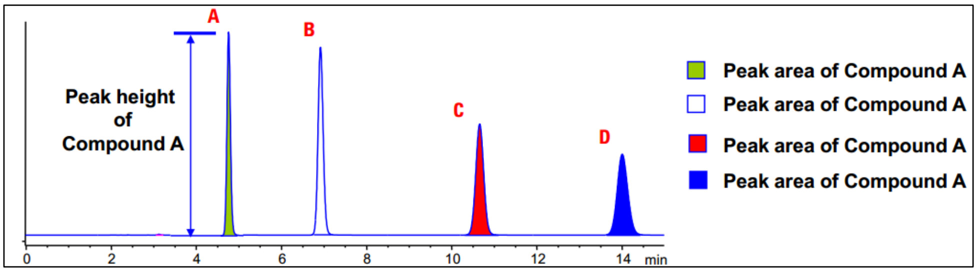


Figure 3.4. Quantitative analysis [63]

In the columns various chemicals and physical interactions take place between the molecules and the packing materials, which result in the divergent distribution of components of a mixture through the column. The separated components are detected at the exit of the column by using a detector which may be used for quantification of the sample [63]. The HPLC system is shown in Figure 3.5 [62].

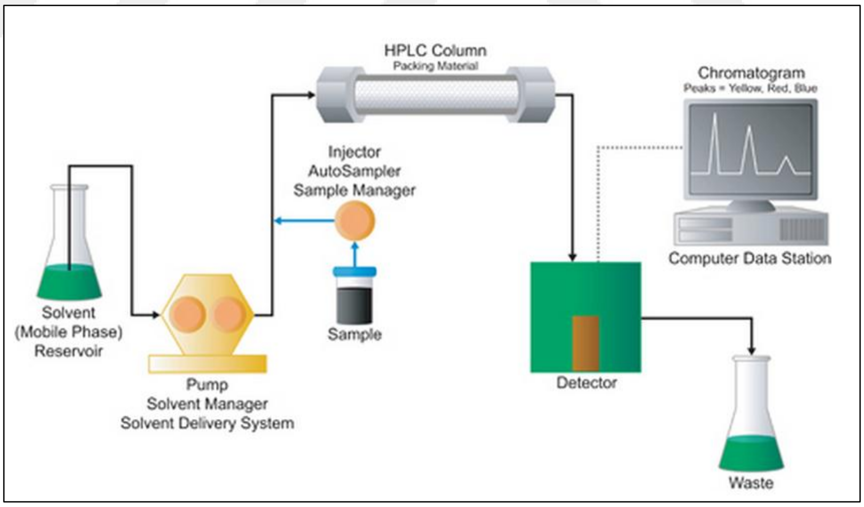


Figure 3.5. High-performance liquid chromatography (HPLC) system [62]

HPLC has five major components as pump, injector, column, detector and computer as shown in Figure 3.6 in a new model of HPLC [63].

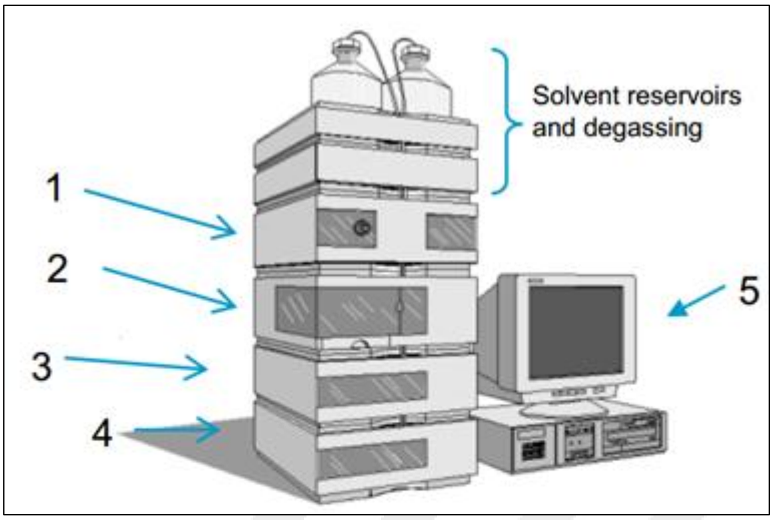


Figure 3.6. Major components of HPLC, 1: pump ; 2: injector, 3: column ; 4: detector, 5: computer [63]

3.2.2.1. Pump

The pump is used to force the mobile phase along the chromatography at a specific flow rate in mL/min. The flow rate range in the HPLC analysis is generally between 1 and 2 mL/min. Besides, the pressure range of a general pump is between 6000 and 9000 psi (400 and 600 bar). During the HPLC analysis, the pump delivers isocratic or gradient mobile phase. Furthermore, binary gradient pump and quaternary gradient pump are examples of gradient pump types [63].

3.2.2.1.1. Isocratic and Gradient Conditions

In the gradient conditions, mobile phase solvent composition changes with time. They are mainly used for the analyses of complex samples especially in the method development for unknown mixtures [63].

An example for the change in the percentage solvent compositions in the gradient elution is shown in Figure 3.7.

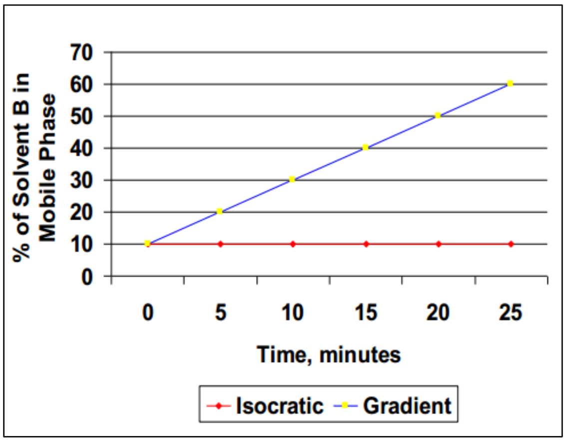


Figure 3.7. Gradient vs. isocratic conditions [63]

3.2.2.2. Injector

The liquid sample is injected to flow stream of mobile phase by using injector. The injection volume range is between 5 and 20 µL. Besides, the injector must be suitable for high pressure [63].

Injection occurs in two different ways by manual injection or by autosampler in the HPLC devices (Figure 3.8). An autosampler is an automatic type injector and provides many sample analyses consecutively, whereas personal intervention is needed for each injection by the manual type. Thus manual injection is unpractical [63].

In the manual injection sample (on left, Figure 3.8) is loaded into the injector by using a syringe. Then, the handle is turned appropriately to inject sample into the flowing mobile phase that transports the sample into the head of the column. When autosampler (on right, Figure 3.8) is used, vials filled with sample solutions are loaded into the autosampler tray. Then, the solutions are injected to the system automatically as defined by the user to the program of the instrument [63].

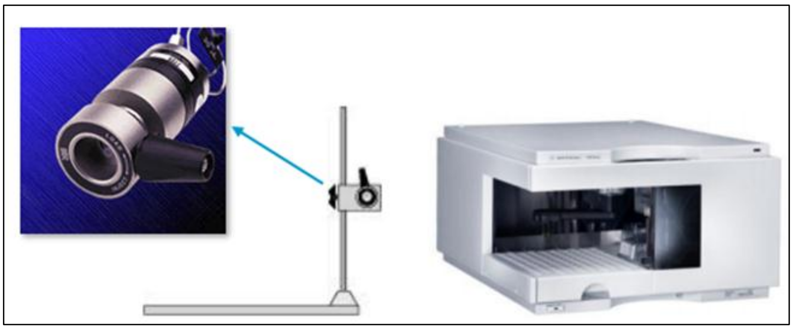


Figure 3.8. Manual injector and autosampler [63]

3.2.2.3. Column

The column is a heart of the chromatography. The column as a stationary phase separates components of the sample by using chemical and physical factors. The tiny particles in the column lead to high backpressure at the normal flow rates. The mobile phase is pushed along the column by the pump. High pressure occurs inside the column due to pump. The selection of column is very essential for the achievement of a good HPLC analysis. Analytical, preparative, capillary and nano are types of columns. Stainless steel, glass and PEEK polymer are types of construction material of the HPLC columns. HPLC columns are packed with porous particles. Porous particles within the columns have a chemically bonded phase on their surface that interacts with the sample components in order to separate them from one another (eg. C18). Besides, the retention time of the sample components depends on the choice of the column packing material [63].

3.2.2.4. Detector

Detectors monitor the concentration or quantitity of the components of the sample emerging from the column. There are two types of detectors as selective property detectors and bulk property detectors. Selective property detectors monitor the concentration of the compounds by measuring a property that is typical only to the compounds in question. UV-VIS detector is an example of selective property detectors. Bulk property detectors measure the changes in a property that is typical of the solvent and the solute as a whole. Refractive index is an example of the bulk property detectors [63].

3.2.2.5. Computer

The computer is used as a data system. Computers get signals which come from the detector. They use the signals to determine the retention time of the sample components for qualitative analysis, and also the amounts of the samples for quantitative analysis [63].

3.2.2.6. Separation Modes of HPLC

There are four different types of separation modes used in HPLC analysis. They are reversed phase chromatography, normal phase and adsorption chromatography, ion exchange chromatography and size exclusion chromatography [63].

3.2.2.6.1. Reversed Phase HPLC

The column packing material is nonpolar in the reversed phase chromatograhy. C18, C8, C3 and phenyl are examples of nonpolar column packing materials. The mobile phase used is water (or buffer) and water-miscible organic solvent mixture (e.g. methanol, acetonitrile). Reversed phase HPLC is the most popular mode used in HPLC. This type of HPLC is generally used for nonpolar, polar, ionizable and ionic molecules. Moreover, gradient elution is generally used for samples containing a wide range of compound [63].

3.2.2.6.2. Normal Phase or Adsorption Chromatography

The column packing is polar material in the normal phase. Silica gel, cyanopropyl-bonded, amino-bonded polymers are examples of polar column packing materials. Examples of the mobile phase used as nonpolar solvents are hexane, isooctane, methylene chloride, and ethyl acetate. In addition, normal phase HPLC studies are beneficial for the analysis of chiral compounds, geometric isomers, cis-trans isomers, water sensitive compounds and classification separations [63].

3.2.3. NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (NMR)

Nuclear magnetic resonance spectroscopy (NMR) is an analytical method which is used in the quality control and research laboratories to determine contents of the products or samples by defining molecular structure of the products. Mixtures which include known compounds can be analyzed as quantitatively with NMR spectroscopy. Additionally, NMR spectroscopy is used to determine molecular conformation in the solution. Besides, NMR spectroscopy can be used to examine physical properties at the molecular level [64].

3.2.3.1. The Basis of NMR

Many nuclei have spins and they are electrically charged. Therefore, the spinning of charged nuclei generates magnetic fields. When an external magnetic field is applied, energy transfer occurs from the ground state energy to a higher energy level. The energy source in the NMR spectroscopy is radio waves. When spin returns to its ground state energy level, energy is emitted at the same frequency. The signal corresponds to the energy transfer can be measured and processed to obtain an NMR spectrum of the nucleus. When low energy radio waves interact with a molecule, nuclear spins of elements which are ^{1}H and ^{13}C can be changed. The basis of the NMR spectroscopy is shown in Figure 3.9 [64].

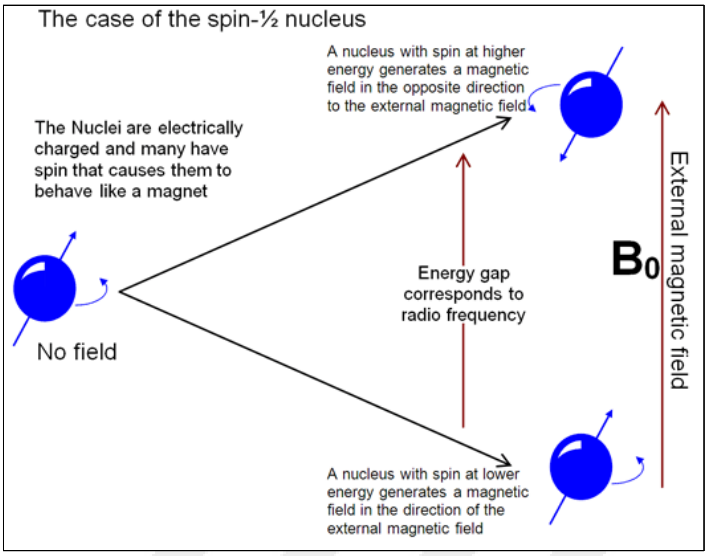


Figure 3.9. The basis of NMR [64]

^{1}H NMR and ^{13}C NMR are most common used types of NMR spectroscopy. They are used to characterize organic structures [65].

In order to obtain a NMR spectrum, firstly a sample is dissolved in a NMR solvent in a thin glass NMR tube. Then, the sample tube is placed in the superconducting magnet. The sample tube is rotated in a magnetic field and is irritated with a short pulse of radiofrequency radiation. Finally, a NMR spectrum can be obtained by changing or sweeping the magnetic field over a tiny range while collecting the radiofrequency signals from the sample. Schematic representation of NMR spectrometer is shown in Figure 3.10 [66].

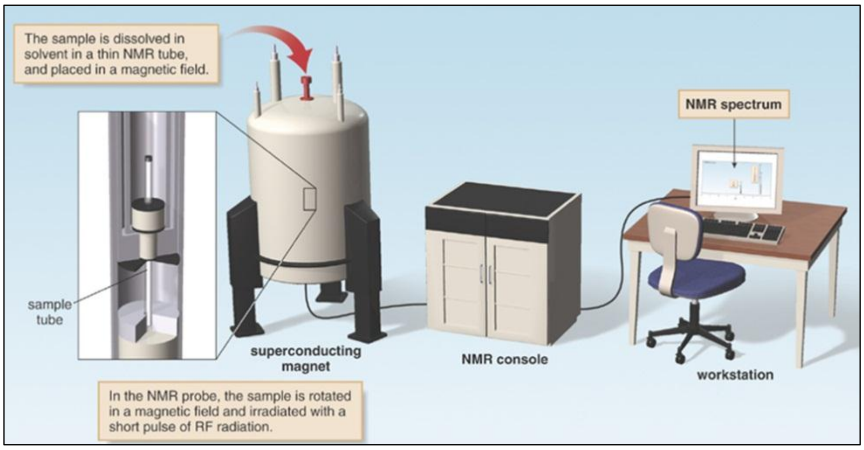


Figure 3.10. Schematic representation of a NMR spectrometer [66]

3.2.3.2. ^{1}H NMR Spectroscopy (Proton Nuclear Magnetic Resonance Spectroscopy)

Proton nuclear magnetic resonance spectroscopy is used to determine the structure of unknown organic compounds.

The following items help to determine the chemical structure:

- Number of different types of hydrogens exist in the molecule
- Relative ratio of hydrogens of the different types of hydrogens
- The electronic environment of the different types of hydrogens
- The number of hydrogens at the neighbour carbon(s) [65].

The tasks of number of signals, position of signals, intensity of signals and spin-spin splitting of signals in the determination of the structures of the compounds are explained in detail [65].

Same or different chemical shifts for hydrogen atoms bonded to the same carbon atom may be observed due to the chemical environment. Besides, there are three types of protons for the hydrogen atoms on the same carbon atom classified as homotopic, enantiotopic and diastereotopic protons (hydrogens) [66].

3.2.3.2.1. Enantiotopic and Diastereotopic Protons in ¹H NMR

The same or different chemical environment of the hydrogen atoms may be determined in the proton NMR analysis for the same or different signals.

- Homotopic Protons: Exact and same chemical shifts are observed.
- Enantiotopic Protons: Same chemical shifts are generally observed, but different chemical shifts may be observed in a chiral environment.
- Diastereotopic Protons: Different chemical shifts are usually observed [66].

When a carbon atom has only two hydrogen atoms, and substitution of one of the two hydrogen atoms (H) by Z form enantiomers, two hydrogen atoms are equivalent (Figure 3.11). Therefore, two hydrogen atoms give a single NMR signal. These two hydrogen (H) atoms are called as enantiotopic protons [67].

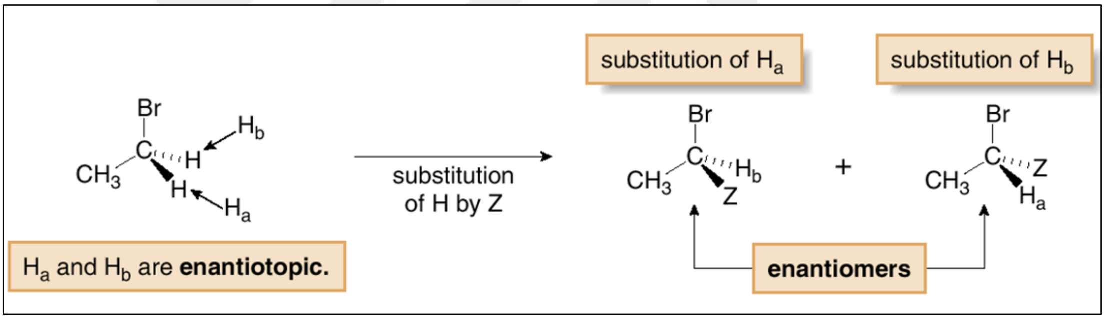


Figure 3.11. Determination of enantiotopic protons in ¹H NMR [67]

When substitution of one of the two hydrogen (H) atoms by Z forms diastereomers, two H atoms are not equivalent (Figure 3.12). Therefore, they give two NMR signals. These two hydrogen atoms are called as diastereotopic protons [67].

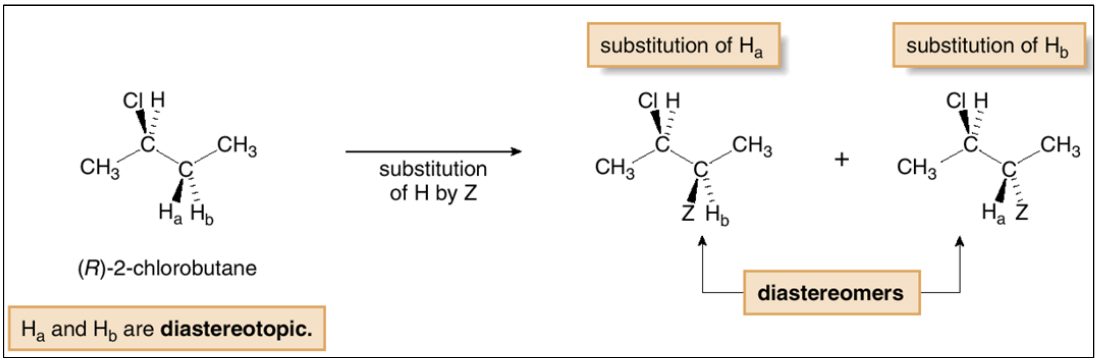


Figure 3.12. Diastereotopic protons in ^{1}H NMR [67]

3.2.3.2.2. Number of Signals

Protons which are different are not chemically equivalent in a compound. Therefore, they will absorb at the different frequencies and thus they will give different signals on the NMR spectrum [65].

3.2.3.2.3. Position of Signals (Chemical Shift)

Equivalent proton signal occurs at the horizontal frequency scale. That frequency scale is called as chemical shift and is measured in ppm. Also, chemical shift depends on the variation of magnetic field from the neighboring protons. As an example, electronegativity of the neighboring carbon affects the chemical shift [65]. Chemical shift ranges of some typical protons are given in Table 3.6.

Table 3.6. NMR chemical shifts of characteristic protons [68]

Type of Proton	Type of Compound	Chemical Shift Ranfe
RCH_3	1^o aliphatic	0.9
R_2CH_2	2^o aliphatic	1.3
R_3CH	3^o aliphatic	1.5
C=C-H	Vinylic	4.6–5.9
C=C-H	vinylic, conjugated	5.5–7.5
C≡C-H	acetylenic	2–3
Ar-H	Aromatic	6–8.5
Ar-C-H	Benzylic	2.2–3
$C=C-CH_3$	Allylic	1.7
HC-F	fluorides	4–4.5
HC-Cl	chlorides	3–4
HC-Br	bromides	2.5–4
HC-I	Iodides	2–4
HC-OH	Alcohols	3.4–4
HC-OR	Ethers	3.3–4
RCOO-CH	Esters	3.7–4.1
HC-COOR	Esters	2–2.2
HC-COOH	Acids	2–2.6
HC-C=O	carbonyl compounds	2–2.7
RCHO	aldehydic	9–10
ROH	hydroxylic	2–4
ArOH	Phenolic	4–12
C=C-OH	Enolic	15–17
RCOOH	carboxylic	10–13.2
HC-NHR	Amine	1.5–2.0
RNH_2	Amino	1–5
RNHC(=O)R’	Amides	5–8.5

3.2.3.2.4. Relative Intensity of Signals (Integration)

The integration in the NMR spectrum gives information about the relative number of different hydrogens. Also, integration provides ratios of protons and it does not provide absolute number. Relative intensities of the signals are directly proportional to the relative number of proton equivalents [66].

3.2.3.2.5. Splitting of Signals (Spin-Spin Coupling)

Complex pattern of splitting can be seen as doublets (2 peaks), triplets (3 peaks), and quartets (4 peaks) [69]. Spin-spin coupling effect is quantified by coupling constant, J which is the distance between two neighbor sub-peaks in a split signal [70]. The interaction between nearby protons produces different spin flip energies. If the spin of the nucleus of one proton is close enough in order to affect the spin of another, spin-spin coupling occurs. By spin-spin coupling the information on the number of protons of the neighbour carbon within the molecule can be obtained [69].

3.2.3.2.6. N+1 Rule

The signal will be split into n+1 lines for a proton with n neighbours as in Figure 3.13.

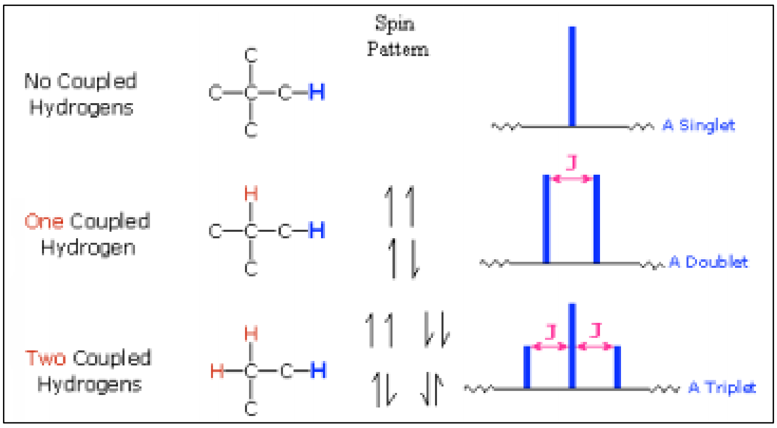


Figure 3.13. N+1 Rule [72]

One neighbour hydrogen atom gives a doublet. Two neighours hydrogen atoms give a triplet. Three neighbour hydrogen atoms give a quartet. Besides, if splitting patterns are very diffucult for analysis, they are called as multiplets [71].

Intensity ratios between the split signals can be determined by using Pascal's triangle (Figure 3.14).

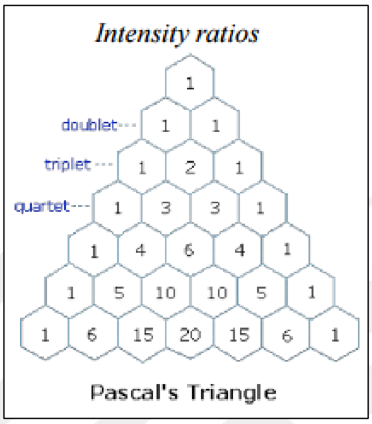


Figure 3.14. Pascal's triangle and intensity ratios between the split signals [71]

Intensity ratios are bionomial coefficients and can be easily found from Pascal's triangle (Figure 3.15). For example, if n is equal to three (it means, there are three protons on neighbour carbons), a quartet is obtained, the ratio of the intensity of the outer lines to that of the inner lines is 3:1 [71].

3.2.3.2.7. First Order Coupling Rules

- Equivalent protons are coupled to each other. However, proton NMR spectra cannot show it.
- J coupling is mutual. ($J_{AB} = J_{BA}$)
- Two closely spaced lines can be coupled or shifted. There are many cases as for decoupling the spectrum,
    - obtaining decoupling at a different field strength
    - measuring the spectrum in different solvents

- Chemical shifts are generally represented in δ (units: ppm). Coupling constants, J are represented in Hz (cycles per second). While chemical shifts depend on magnetic field, coupling constants is field independent.
- Doubling rule is seen for first order patterns. If all couplings to a particular proton are the same, there will be 2nI+1 lines. I is the spin and n is the number of neighbour nuclei (n+1 for ^{1}H I = 1/2). Intensities obey the rule of Pascal's triangle (Figure 3.16).

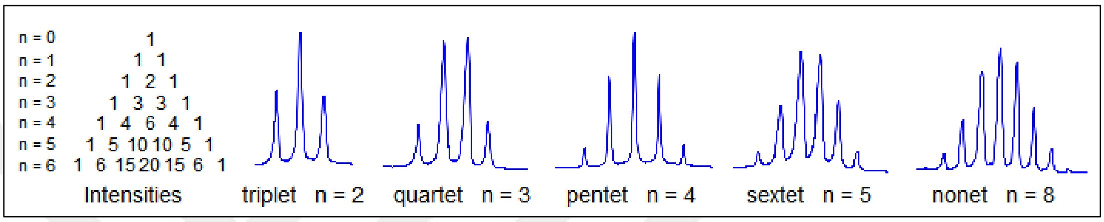


Figure 3.15. Intensities and multiplets [72]

- If all coupling constants are different, then the number of peaks is calculated as 2^n. All the intensities are the same in that case. Thus, a proton which is coupled to two others by different coupling constants gives doublets of doublets. This pattern is never called as a quartet. This situation is called as AB or AX spectra. As the number of couplings gets larger, lines of accidental superposition will sometimes occur (Figure 3.16).

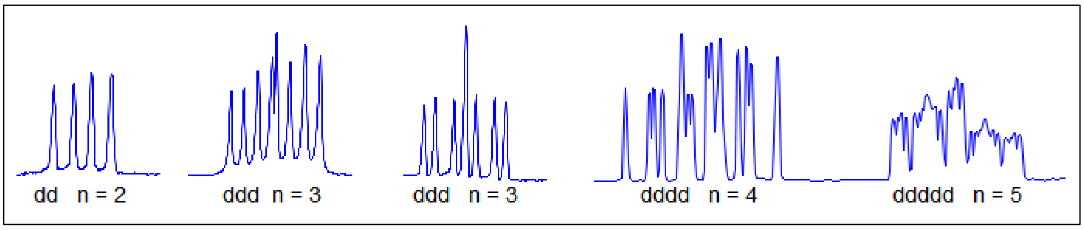


Figure 3.16. Patterns of peaks of the hydrogens coupled to two or more sets of nonequivalent neighbor protons [72]

- If some of the coupling constants are the same and other are different, a variety of patterns can be obtained (Figure 3.17) [72].

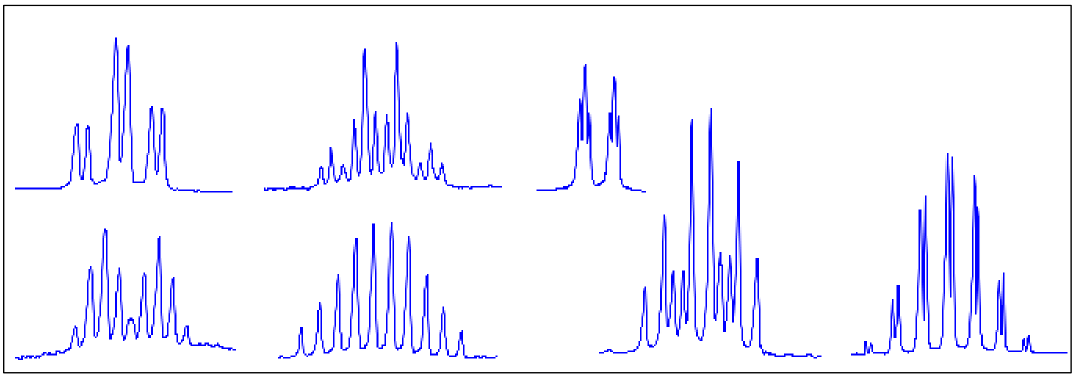


Figure 3.17. Patterns of peaks of the protons coupled with other protons forming complex coupling [72]

3.2.3.3. ^{13}C NMR Spectroscopy

Carbon-13 NMR spectroscopy is important in the analysis of large, biochemically essential molecules because ^{13}C NMR spectroscopy analysis is much simpler than proton NMR spectroscopy analysis [73].

There are some similarities and differences between ^{13}C NMR and ^{1}H NMR spectroscopies. While ^{13}C atom has 1.1% natural abundance and a spin ½ nucleus, NMR behavior is not observed for ^{12}C atom. In addition, ^{13}C nucleus is much sensitive than ^{1}H nucleus for NMR spectrum. ^{13}C - ^{13}C coupling are not generally observed because of the low abundance. Also, the factors, which affect chemical shifts of ^{13}C NMR and ^{1}H NMR, are similar. Furthermore, there is no integration possibility on the ^{13}C NMR due to long relaxation times. Besides, ^{13}C NMR spectrum is generally "broadband, proton decoupled". Therefore, single lines are seen on the ^{13}C NMR spectrum. The number of peaks, which are single lines, gives the number of types of C [74].

While chemical shifts range is seen between 1 ppm and 10 ppm in the ^{1}H NMR spectroscopy, chemical shift range is seen between 0 ppm and 220 ppm in the ^{13}C NMR spectroscopy [74].

^{13}C nuclei may be split by neighbor hydrogen atoms, but interpretation of splitting is very complicated, therefore a technique is used to observe each carbon atom as a single line. In this broad band decoupling technique, couplings are removed with a continuous second radiofrequency signal of a wide frequency range. That frequency range excites all hydrogen nuclei and also coupling patterns are canceled out due to the interactions between proton and ^{13}C. Besides, the off-resonance decouplings may be observed in ^{13}C NMR spectrum. In that case, one bond C-H couplings are retained and n+1 rule is valid for certain carbon atoms attached to hydrogen atoms [74]. Typical chemical shifts in ^{13}C NMR Spectroscopy are given in Table 3.7 [75].

Table 3.7. Typical chemical shifts in ^{13}C NMR spectroscopy [76] (R=alkyl or H, Ar=aryl)

Carbon environment	**Chemical shift (ppm)**
C=O (in ketones)	205-220
C=O (in aldehydes)	190-200
C=O (in acids and esters)	160-185
C in aromatic rings	125-150
C=C (in alkenes)	115-140
RCH_2O-	50-90
RCH_2CI	30-60
RCH_2NH_2	30-65
R_3CH	25-35
CH_3CO-	20-50
R_2CH_2	16-25
RCH_3	10-15

3.2.4. Infrared (IR) Spectroscopy Analysis

Infrared spectroscopy is used mainly to determine the structure of organic compounds. Besides, IR spectroscopy is used for measuring the concentration of compounds in samples, quantitative and qualitative analysis, detection of impurities, kinetic study, isomerism and carbonyl bending depletion [77].

Infrared radiation, the range of which is between 4000 cm^{-1} and 400 cm^{-1}, is a region of the electromagnetic radiation. Infrared spectroscopy is the measurement of the intensity and wavelength of the absorption of mid-infrared light by a sample. Mid-infrared radiation is enough to excite molecular vibrations to higher energy levels. Also, wavelength of the infrared absorption bands is characteristic of specific types of chemical bonds. Besides, chemical bonds in the different environment absorb different intensities at the different frequencies. Bonds in the organic and organometallic molecules may be investigated by IR radiation [78].

In the infrared spectroscopic process, molecular vibration is taken as base. There are two types of bond vibration as stretching and bending [78].

Stretching: The change in inter-atomic distance along bond axis is seen. Vibrations and oscillation along the line of the bond are observed. There are two types of stretching: symmetric and asymmetric (Figure 3.18) [78].

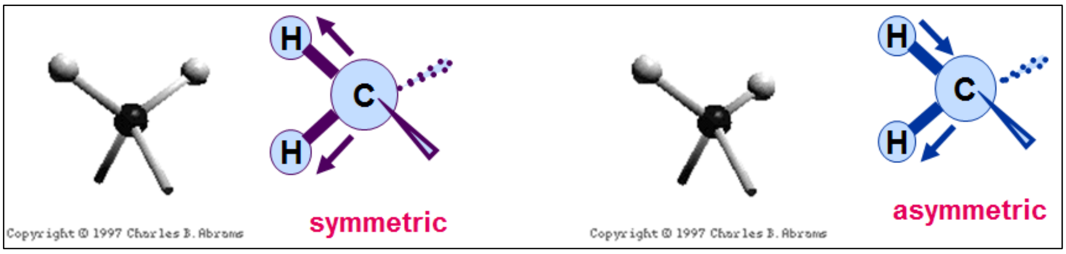


Figure 3.18. Types of stretching vibrations [78]

Bending: The change is observed between two bonds. Vibrations or oscillation are not along the bond. There are four types of bending: scissor, rock, twist and wag (Figure 3.19) [78].

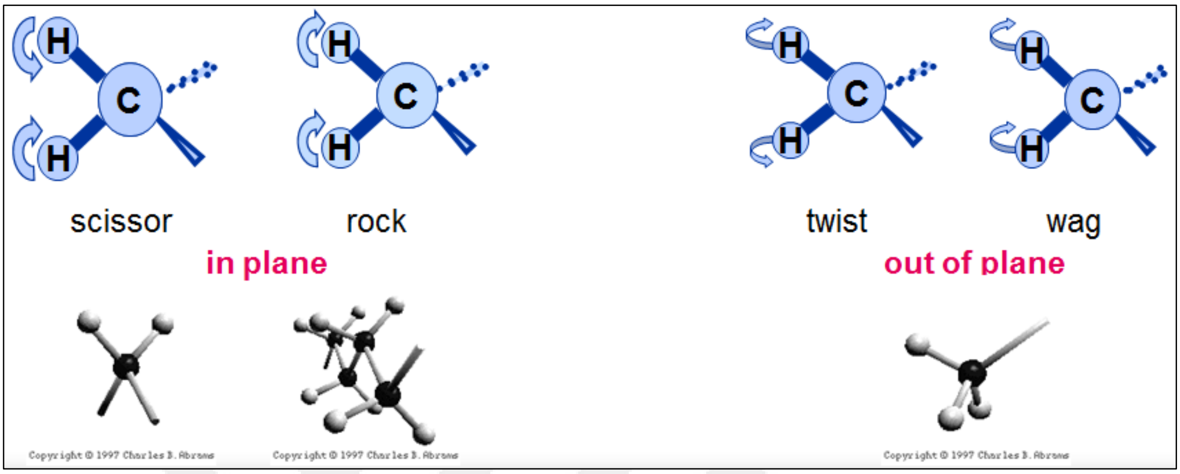


Figure 3.19. Bending vibrations [78]

A varying electromagnetic field is obtained when a covalent bond oscillates. When dipole moment changes through the vibration, electromagnetic field occurs. When an infrared light encounters the oscillating electromagnetic field, which is obtained by dipole of the equivalent frequency, two waves are coupled and infrared light is absorbed. The coupled wave vibrates twice the amplitude. The representation of IR beam from spectrometer and coupled wave are given in Figure 3.20 [78].

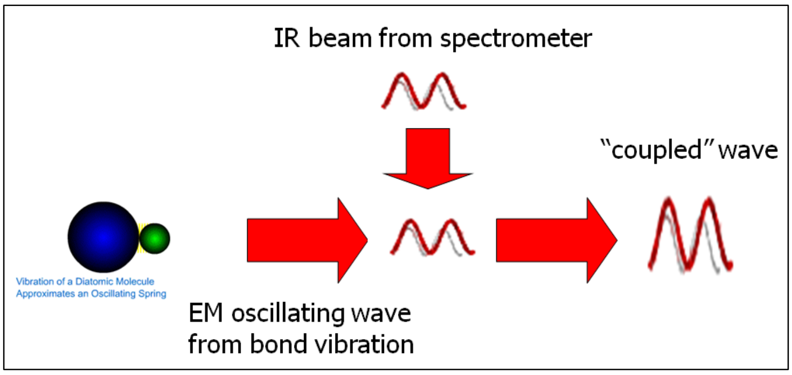


Figure 3.20. IR beam from spectrometer and coupled wave [78]

When atoms and charges are different for different bonds, peaks for each bending and stretching vibrations are observed by a characteristic frequency. There are three types of peak intensities as strong (s), medium (m), weak (w) and broad (Figure 3.21) [78].

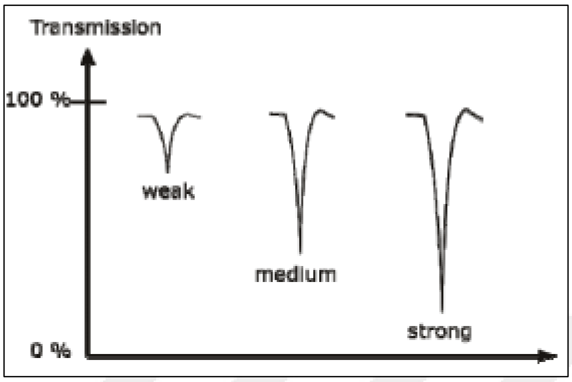


Figure 3.21. Classification of IR bands [78]

Polar bonds are infrared active. Strong intensities are seen for strong polar bonds like carbonyl groups (C=O). Medium intensities are seen for medium polarity bonds and asymmetric bonds. Weakly polar bonds and symmetric bonds give peaks with weak intensities or peaks of them are not observed (Figure 3.22) [79]. In Figure 3.23, the four primary regions of the IR spectrum are given. In Figure 3.24 and Table 3.8, infrared absorption frequencies belonging to some special groups can be seen. The peak assignments can be done according to its position and intensity, so functional groups in the molecule can be determined.

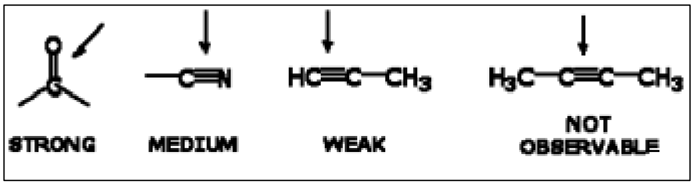


Figure 3.22. Intensity of infrared active bonds [78]

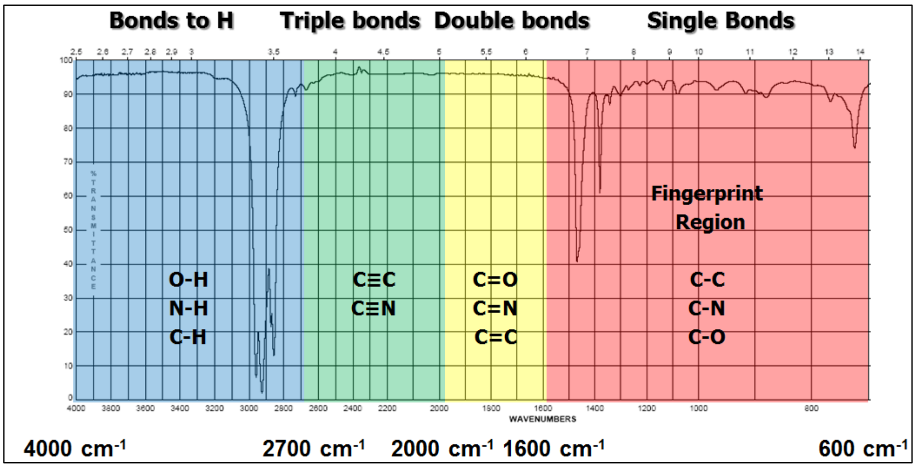


Figure 3.23. The four primary regions of the IR spectrum [78]

Typical Infrared Absorption Frequencies

	Stretching Vibrations			Bending Vibrations		
Functional Class	**Range (cm^{-1})**	**Intensity**	**Assignment**	**Range (cm^{-1})**	**Intensity**	**Assignment**
Alkanes	2850-3000	str	CH_3, CH_2 & CH 2 or 3 bands	1350-1470 1370-1390 720-725	med med wk	CH_2 & CH_3 deformation CH_3 deformation CH_2 rocking
Alkenes	3020-3100 1630-1680 1900-2000	med var str	=C-H & =CH_2 (usually sharp) C=C (symmetry reduces intensity) C=C asymmetric stretch	880-995 780-850 675-730	str med med	=C-H & =CH_2 (out-of-plane bending) cis-RCH=CHR
Alkynes	3300 2100-2250	str var	C-H (usually sharp) C≡C (symmetry reduces intensity)	600-700	str	C-H deformation
Arenes	3030 1600 & 1500	var med-wk	C-H (may be several bands) C=C (in ring) (2 bands) (3 if conjugated)	690-900	str-med	C-H bending & ring puckering
Alcohols & Phenols	3580-3650 3200-3550 970-1250	var str str	O-H (free), usually sharp O-H (H-bonded), usually broad C-O	1330-1430 650-770	med var-wk	O-H bending (in-plane) O-H bend (out-of-plane)
Amines	3400-3500 (dil. soln.) 3300-3400 (dil. soln.) 1000-1250	wk wk med	N-H (1°-amines), 2 bands N-H (2°-amines) C-N	1550-1650 660-900	med-str var	NH_2 scissoring (1°-amines) NH_2 & N-H wagging (shifts on H-bonding)
Aldehydes & Ketones	2690-2840(2 bands) 1720-1740 1710-1720 1690 1675 1745 1780	med str str str str str str	C-H (aldehyde C-H) C=O (saturated aldehyde) C=O (saturated ketone) aryl ketone α, β-unsaturation cyclopentanone cyclobutanone	1350-1360 1400-1450 1100	str str med	α-CH_3 bending α-CH_2 bending C-C-C bending
Carboxylic Acids & Derivatives	2500-3300 (acids) overlap C-H 1705-1720 (acids) 1210-1320 (acids) 1785-1815 (acyl halides) 1750 & 1820 (anhydrides) 1040-1100 1735-1750 (esters) 1000-1300 1630-1695(amides)	str str med-str str str str str str str	O-H (very broad) C=O (H-bonded) O-C (sometimes 2-peaks) C=O C=O (2-bands) O-C C=O O-C (2-bands) C=O (amide I band)	1395-1440 1590-1650 1500-1560	med med med	C-O-H bending N-H (1ⱼ-amide) II band N-H (2ⱼ-amide) II band
Nitriles	2240-2260	med	C≡N (sharp)			
Isocyanates,Isothiocyanates, Diimides, Azides & Ketenes	2100-2270	med	-N=C=O, -N=C=S -N=C=N-, -N_3, C=C=O			

Figure 3.24. Typical infrared absorption frequencies [79]

Table 3.8. IR data of sulfur, phosphorous and oxidized nitrogen functional groups [80]

Functional Class	**Characteristic Absorptions**
Sulfur Functions	
S-H thiols	2550-2600 cm^{-1} (wk & shp)
S-OR esters	700-900 (str)
S-S disulfide	500-540 (wk)
C=S thiocarbonyl	1050-1200 (str)
S=O sulfoxide sulfone sulfonic acid sulfonyl chloride sulfate	1030-1060 (str) 1325± 25 (as) & 1140± 20 (s) (both str) 1345 (str) 1365± 5 (as) & 1180± 10 (s) (both str) 1350-1450 (str)
Phosphorous Functions	
P-H phosphine	2280-2240 cm^{-1} (med & shp) 950-1250 (wk) P-H bending
(O=)PO-H phosphonic acid	2550-2700 (med)
P=O phosphine oxide phosphonate phosphate phosphoramide	1100-1200 (str) 1230-1260 (str) 1100-1200 (str) 1200-1275 (str)
Oxidized Nitrogen Functions	
=NOH oxime O-H (stretch) C=N N-O	 3550-3600 (str) 1665± 15 945± 15
N-O amine oxide aliphatic aromatic	 960± 20 1250± 50
N=O nitrose nitro	1550± 50 (str) 1530± 20 (as) & 1350± 30 (s)

3.2.5. Elemental Analysis

Percentage elemental composition of an element in the pure state in a compound may be found from elemental analysis. Elemental analysis is essential for the qualitative analysis of molecular compounds including carbon (C), hydrogen (H), nitrogen (N) and oxygen (O) in their structure, which are used in research, academic and industrial applications. Petrochemicals pharmaceuticals and agrochemicals may be given as examples for this type of industrial compounds. The principle of elemental analysis is based on burning of a small amount of sample by rising heat in the existence of oxygen. The elements H, C, N, O and S are obtained as gassy oxidation products. The instrument gives directly the percentage of the elements in the sample [81].

3.2.6. Polarimeter

Polarimeter is an instrument that is utilized to measure the rotation of plane-polarized light due to optically active samples such as some inorganic, organic and biological compounds. During polarimer measurements optically active chemicals are not destroyed or changed chemically [82].

The plane polarized light is firstly introduced through the solvent used to dissolve the sample. Then the sample dissolved in an appropriate solvent is placed in the polarimeter tube. The plane polarized light goes through the polarimeter tube containing the sample and the rotation angle is measured and displayed by the polarimeter (Figure 3.25) as negative or positive value depending on the stereochemistry of the excess isomer [83].

Figure 3.25. Polarimeter [84]

4. EXPERIMENTAL STUDY

4.1. ORGANIC SYNTHESIS

4.1.1. Procedure for The Preparation of Phenylthiourea and *Ortho*-Substituted Phenylthioureas

The preparation procedure of phenylthiourea and *ortho*-substituted phenylthioureas was given below.

- 0.30 moles of aniline or *ortho*-substituted aniline was put in a 300 mL of warm water and 27.5 mL (0.33 moles) of concentrated hydrochloric acid (HCI) was added into this solution. If the stated amount of hydrochloric acid was not enough to obtain a clear homogenous solution, more concentrated HCI solution could be added until a clear homogenous solution was obtained.
- When the homogeneity was obtained, 25 g of ammoniumthiocyanate was added into the prepared solution.
- The prepared solution was divided into four different porcelain evaporating dishes equally.
- After heating the resulting solution on the steam bath for one hour, the dishes were put one hour aside to let the product crystallization begin in the solution. Then the solution was heated on the steam bath slowly, until all solvent was evaporated and dry product was obtained.
- In the end of the evaporation, the crystalline residue, which consisted of *ortho*-substituted phenylthioureas or phenylthiourea and ammonium chloride, was powdered finely. After that, 300 mL of water at room temperature were added onto the crystals and again the solvent was evaporated on the steam bath.
- Then, 300 mL of water was put onto the resulting mixture in a beaker and was heated until the temperature of the mixture reached 70^{o}C.
- After that, the beaker was put on the bench to cool the mixture to 35^{o}C

- Then, the vacuum filtration was done to separate the crystals from the solution.
- The crystals were dissolved in 60 mL boiling ethanol.
- Then, the solution was diluted with 100 mL hot benzene and 20 mL petroleum ether and crystals began to precipitate.

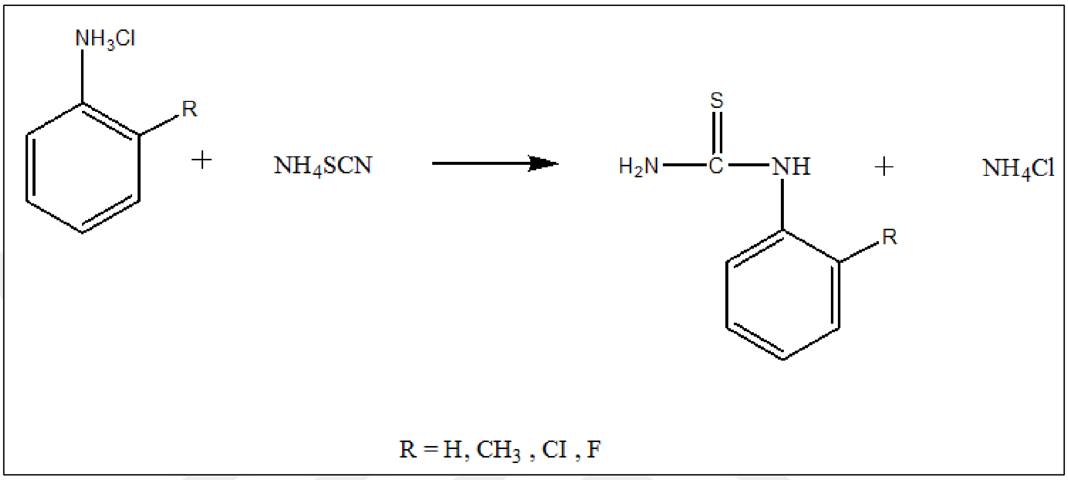


Figure 4.1. Synthesis reaction of phenylthiourea and *ortho*-substituted phenylthioureas

4.1.1.1. o-Chlorophenylthiourea

o-chlorophenylthiourea was synthesized according to the general procedure. The starting chemicals were listed for *o*-chlorophenylthiourea as below.

- *o*-chloroaniline: 38.3 gram (0.30 moles)
- Hydrochloric acid: 27.5 mL
- Ammoniumthiocyanate: 25 gram (0.328 moles)
- Water: 300 mL

Yield of the purified product: 17.23 gram, (31 %)
Melting point of the purified product: 140-144^{o}C
Color of the purified product: white (Figure 4.2)

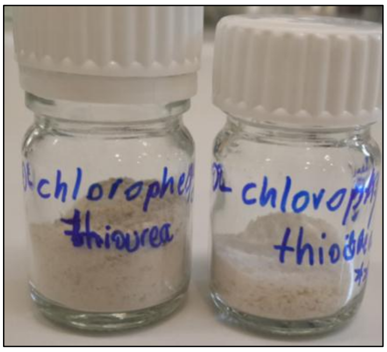


Figure 4.2. *o*-chlorophenylthiourea ($C_7H_7ClN_2S$)

4.1.1.2. o-Fluorophenylthiourea

o-fluorophenylthiourea was synthesized according to the general procedure. The starting chemicals were listed for *o*-fluorophenylthiourea as below.

- *o*-fluoroaniline: 33.3 gram (0.30 moles)
- Hydrochloric acid: 27.5 mL
- Ammoniumthiocyanate: 25 gram (0.328 moles)
- Water: 300 mL

Yield of the purified product: 27.50 gram, (54 %)
Melting point of the purified product: 146^{o}C
Color of the purified product: white (Figure 4.3)

Figure 4.3. *o*-fluorophenylthiourea ($C_7H_7FN_2S$)

4.1.1.3. o-Tolylthiourea

o-toylthiourea was synthesized according to the general procedure. The starting chemicals were listed for *o*-tolylthiourea as below.

- *o*-toluidine: 32.1 gram (0.30 moles)
- Hydrochloric acid: 27.5 mL
- Ammoniumthiocyanate: 25 gram (0.328 moles)
- Water: 300 mL

Yield of the purified product: 37.12 gram, (74 %)
Melting point of the purified product: 152-158^{o}C
Color of the purified product: white (Figure 4.4)

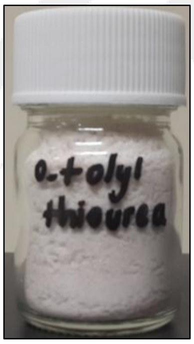


Figure 4.4. *o*-tolylthiourea ($C_8H_{10}N_2S$)

4.1.1.4. Phenylthiourea

Phenylthiourea was synthesized according to the general procedure. The starting chemicals were listed for phenylthiourea as below.

- Aniline: 27.9 gram (0.30 mol)
- Hydrochloric acid: 20 mL
- Ammoniumthiocyanate: 25 gram (0.328 moles)
- Water: 300 mL

Yield of the purified product: 30.03 gram, (66 %)

Melting point of the purified product: 136^{o}C

Color of the purified product: white (Figure 4.5)

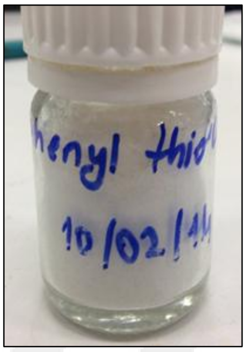


Figure 4.5. Phenylthiourea ($C_7H_8N_2S$)

4.1.2. Synthesis of 5-substituted-1-(*o*-aryl)-2-Thiobarbituric Acid Derivatives

For the synthesis of 5-methyl-1-(*o*-aryl)-2-thiobarbituric acid, methylmalonic acid and *ortho*-substituted thioureas were used usually in 1:1 ratio. In addition to that, methylmalonic acid and *ortho*-substituted thiourea were also used in 1:1.2 ratio in order to increase the yield of thiobarbituric acid derivatives. Dimethylmalonic acid and *o*-substituted phenylthioureas were used in 1:1 ratio to obtain 5,5-dimethyl-1-(*o*-aryl)-2-thiobarbituric acids.

The reaction solvent was acetyl chloride. The reaction completed in approximately twenty four hours. In the end of the reaction, the reaction solution was poured into a beaker containing ice cubes of water. The solvent of the solution was evaporated by using rotary evaporator. In order to take the residual crystalline solids more easily, approximately 10 mL absolute ethanol was added to the flask. After removing the crystals from the flask with ethanol, the ethanol solution was filtered by using vacuum filtration apparatus to obtain the crystals of 5-methyl-1-(*o*-aryl)-2-thiobarbituric acids or 5,5-dimethyl-1-(*o*-aryl)-2-thiobarbituric acids. Then, the crystals were recrystallized from absolute ethanol to obtain purer products. The ethanol solution was left for one day in the fridge to recover maximum amount of crystals from the solution. The following day, the pure crystals of thiobarbituric acid derivatives were collected by the help of the vacuum filtration apparatus.

To prove the formation of the 5-substituted-1-(*o*-aryl)-2-thiobarbituric acid derivatives, NMR and HPLC analyses were performed.

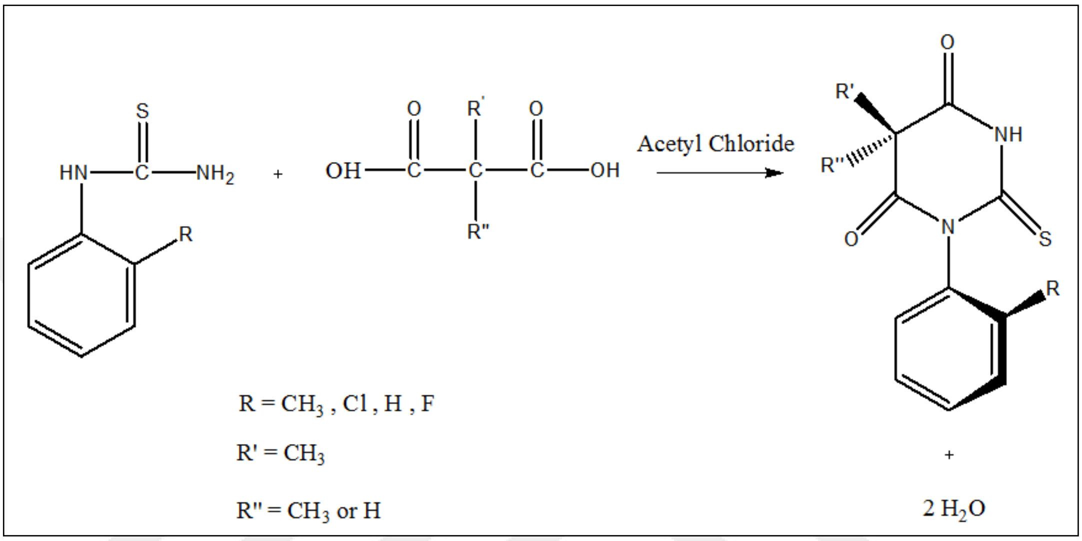


Figure 4.6. Synthesis reactions of 5-methyl-, 5,5-dimethyl-1-phenyl- and -1-(*o*-aryl)-2-thiobarbituric acids

4.1.2.1. 5-methyl-1-(o-chlorophenyl)-2-thiobarbituric Acid

To synthesize 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, the following chemicals were used.

- *o*-chlorophenylthiourea: 4.11 gram (0.022 moles)
- Methylmalonic Acid: 2.60 gram (0.022 moles)
- Acetyl Chloride: 60 mL

Yield of the purified product: 1.13 gram, (19 %)

Melting point of the purified product: 168 – 174^{o}C

Color of the purified product: white (Figure 4.7)

4.1.2.1.1. Synthesis of 5-methyl-1-(o-chlorophenyl)-2-thiobarbituric Acid with Excess Reagent

In this synthesis of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, excess amount of one reactant was used. The chemicals used are as follows:

- *o*-chlorophenylthiourea : 4.11 grams (0.022 moles)
- Methylmalonic Acid : 3.12 gram (0.0264 moles)
- Acetyl Chloride : 75 mL

Yield of the purified product: 0.11 gram, (2%)

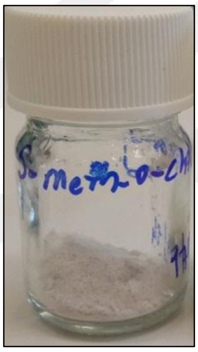


Figure 4.7. 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid ($C_{11}H_9CI\ N_2O_2S$)

4.1.2.2. 5-methyl-1-(o-fluorophenyl)-2-thiobarbituric Acid

To synthesize 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid, the following chemicals were used.

- *o*-fluorophenylthiourea: 3.74 gram (0.022 moles)
- Methylmalonic Acid: 2.60 gram (0.022 moles)
- Acetyl Chloride: 60 mL

Yield of the purified product : 2.25 gram, (40 %)

Melting point of the purified product: 176^{o}C

Color of the purified product: white (Figure 4.8)

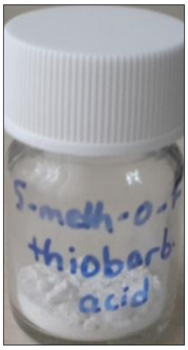


Figure 4.8. 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid ($C_{11}H_9FN_2O_2S$)

4.1.2.3. 5-methyl-1-(o-tolyl)-2-thiobarbituric Acid

To synthesize 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid, the following chemicals were used.

- *o*-tolylthiourea: 3.66 gram (0.022 moles)
- Methylmalonic acid: 2.60 gram (0.022 moles)
- Acetyl Chloride: 60 mL

Yield of the purified product: 1.85 gram, (34 %)
Melting point of the purified product: 169^{o}C
Color of the purified product: white (Figure 4.9)

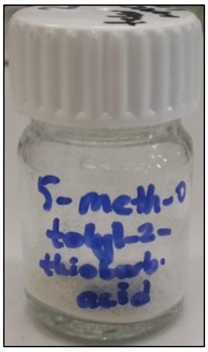


Figure 4.9. 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid ($C_{12}H_{12}N_2O_2S$)

4.1.2.4. 5-methyl-1-phenyl-2-thiobarbituric Acid

To synthesize 5-methyl-1-phenyl-2-thiobarbituric acid, the following chemicals were used.

- Phenylthiourea: 3.35 gram (0.022 moles)
- Methylmalonic acid: 2.60 gram (0.022 moles)
- Acetyl chloride: 60 mL

Yield of the purified product: 1.60 gram, (31 %)
Melting point of the purified product: 201-207^{o}C
Color of the purified product: white (Figure 4.10)

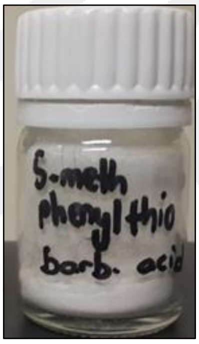


Figure 4.10. 5-methyl-1-phenyl-2-thiobarbituric acid ($C_{11}H_{10}N_2O_2S$)

4.1.2.5. 5,5-dimethyl-1-(o-chlorophenyl)-2-thiobarbituric Acid

To synthesize 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, the following chemicals were used.

- *o*-chlorophenylthiourea : 4.14 gram (0.022 moles)
- Dimethylmalonic Acid : 2.90 gram (0.022 moles)
- Acetyl Chloride : 60 mL

Yield of the purified product: 2.75 gram, (44 %)
Melting point of the purified product: 178^{o}C
Color of the purified product: yellow (Figure 4.11)

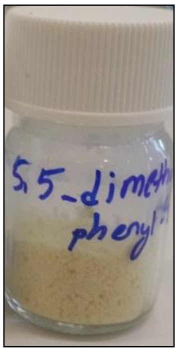


Figure 4.11. 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid ($C_{12}H_{11}ClN_2O_2S$)

4.1.2.6. 5,5-dimethyl-1-(o-fluorophenyl)-2-thiobarbituric Acid

To synthesize 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid, the following chemicals were used.

- *o*-fluorophenylthiourea: 3.64 gram (0.022 moles)
- Dimethylmalonic acid: 2.90 gram (0.022)
- Acetyl chloride: 60 mL

Yield of the purified product: 1.99 gram (34 %)
Melting point of the purified product: 159^{o}C
Color of the purified product: white (Figure 4.12)

Figure 4.12. 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid ($C_{12}H_{11}FN_2O_2S$)

4.1.2.7.5,5-dimethyl-1-(o-tolyl)-2-thiobarbituric Acid

To synthesize 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid, the following chemicals were used.

- *o*-tolylthiourea : 3.64 gram (0.022 moles)
- Dimethylmalonic acid : 2.90 gram (0.022 moles)
- Acetyl chloride : 60 mL

Yield of the purified product: 1.46 gram, (25 %)
Melting Point: 147-150^{o}C (dec.)
Color: white (Figure 4.13)

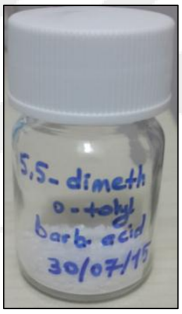


Figure 4.13. 5,5-dimethyl-1-(*o*-tolyl)-2-thiobarbituric acid ($C_{13}H_{14}N_2O_2S$)

4.1.2.8. 5,5-dimethyl-1-phenyl-2-thiobarbituric Acid

To synthesize 5-methyl-1-phenyl-2-thiobarbituric acid, the following chemicals were used.

- Phenylthiourea: 3.35 (0.022 moles)
- Dimethylmalonic acid: 2.91 gram (0.022 moles)
- Acetyl chloride: 62 mL

Yield of the purified product: 0.72 gram, (13 %)
Melting point of the purified product: 139^{o}C
Color of the purified product: white (Figure 4.14)

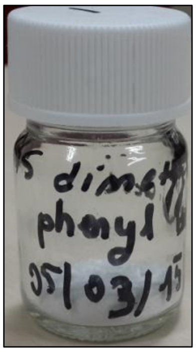


Figure 4.14. 5,5-dimethyl-1-phenyl-2-thiobarbituric acid ($C_{12}H_{12}N_2O_2S$)

4.1.3. General Procedure for The Alkylation Reaction of 5-substituted-1-(*o*-aryl)-2-Thiobarbituric Acid Derivatives at C-5 Position

Alkylation reactions were performed at the Chemistry Department, in Boğaziçi University. First benzylation reactions were conducted by reacting 5-methyl-1-(*o*-aryl)-2-thiobarbituric acids with benzylbromide in the presence of LDA or cinchonine in THF at low temperatures as shown in Figure 4.15. Secondly allylation reactions were performed in the presence of cinchonine at -35°C in THF as shown in Figure 4.16.

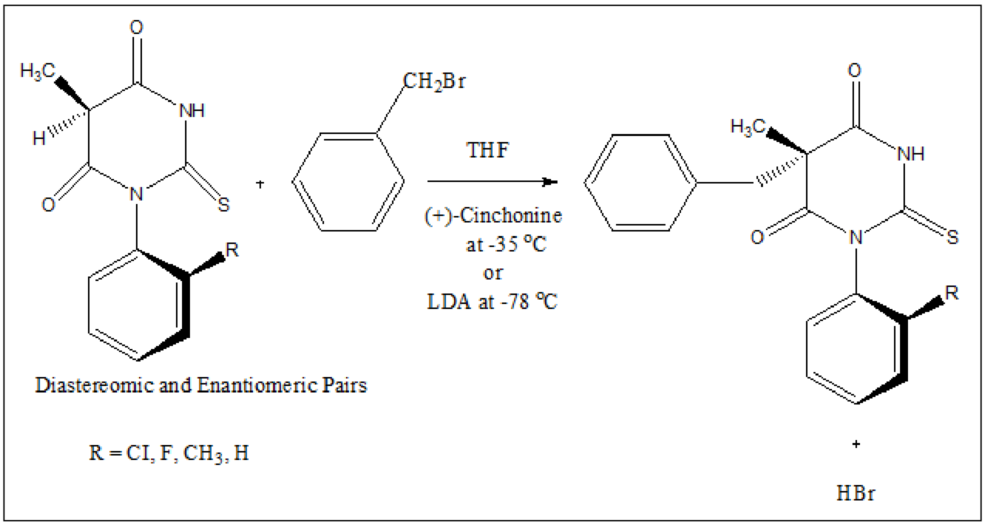


Figure 4.15. Benzylation Reactions at C-5 position of 5-methyl-1-(*o*-aryl)-2- thiobarbituric acid and 5-methyl-1-phenyl-2-thiobarbituric acid

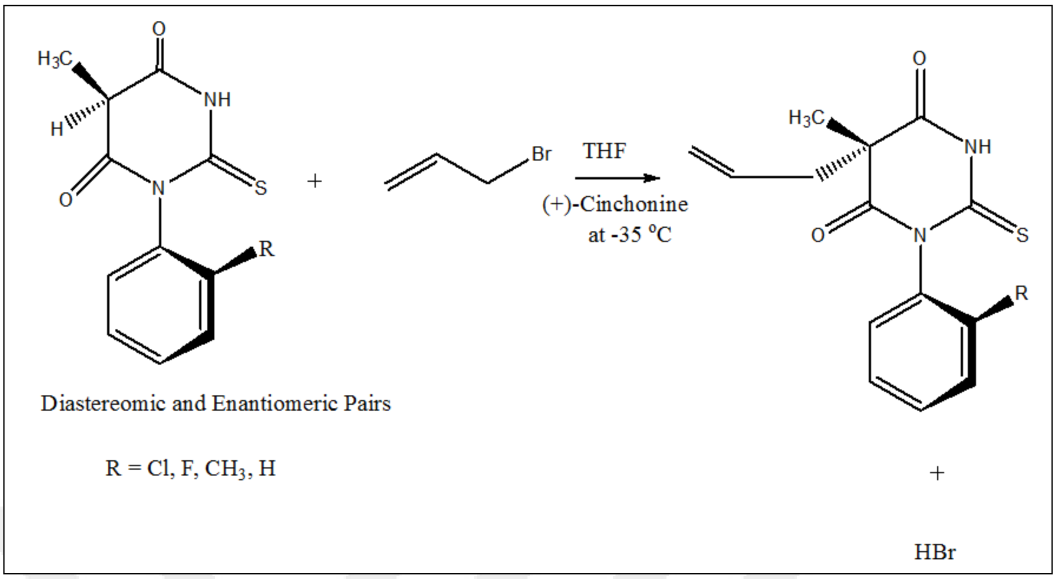


Figure 4.16. Allylation Reactions at C-5 position of 5-methyl-1-(*o*-aryl)-2-thiobarbituric acid and 5-methyl-1-phenyl-2-thiobarbituric acid

4.1.3.1. General Procedure for Alkylation Reactions with (+)-Cinchonine Catalyst

5-methyl-1-(*o*-aryl)-2-thiobarbituric acids and 5-methyl-1-phenyl-2-thiobarbituric acid were alkylated according to the following procedure:

In the reaction, 0.20 equivalents of (+)-cinchonine and 4 equivalents of benzyl bromide or allyl bromide based on the amount of 5-methyl-1-(*o*-aryl)-2-thiobarbituric acid or 5-methyl-1-phenyl-2-thiobarbituric acid were used.

Firstly, 5-methyl-1-(*o*-aryl)-2-thiobarbituric acid (2 mmol) or 5-methyl-1-phenyl-2-thiobarbituric acid (2 mmol) and (+)-cinchonine (20 % moles of thiobarbituric acid, 0.4 mmol) were put into a septum-capped, oven dried flask. The reaction was performed under nitrogen gas with the help of a balloon filled with nitrogen gas. Then, required amount of THF (12.5 mL) was added to the septum capped flask to obtain 0.16 M thiobarbituric acid solution. After that, the septum capped flask is placed into the cryostat machine at -35^{o}C. That reaction mixture was stirred for one hour at -35^{o}C. After one hour, benzyl bromide or allyl bromide (8 mmol) was added to the flask. Four hours later, the reaction solution was taken from the cryostat machine and it was quenched by adding saturated ammonium chloride solution (NH_4Cl solution) at room temperature (2 mL/mmol of 1-(*o*-aryl)-2-thiobarbituric acid was used).

The solution was extracted three times with diethyl ether. The organic layer was dried over anhydrous $MgSO_4$ and after evaporation of diethyl ether, the crude product was recrystallized from absolute ethanol or hexane.

Finally, the aqueous phase of the reaction solution was extracted three times with diethyl ether so that any remaining organic compound passed into the aqueous phase. The obtained crystals were further analyzed.

4.1.3.1.1. Benzylation Reaction of 5-methyl-1-(o-chlorophenyl)-2-thiobarbituric acid with (+)-Cinchonine

Benzylation reaction of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid with (+)-cinchonine was performed according to the general procedure for the alkylation reactions with (+)-cinchonine-catalyst. For this reaction, the following chemicals were used.

- 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid: 0.54 gram (2 mmole)
- (+)-Cinchonine: 0.118 gram (0.4 mmole)
- Tetrahydrofuran (THF): 12.5 mL
- Benzyl bromide (C_7H_7Br): 0.95 mL (8 mmole)
- Saturated ammonium chloride solution (NH_4Cl – water solution): 4 mL

Yield of the purified product: 0.140 gram, (20 %)
Melting point of the purified product: 198^{o}C
Color of the purified product: orange (Figure 4.17)

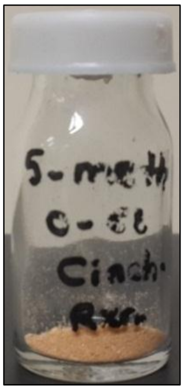


Figure 4.17. Crystals of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid ($C_{1815}ClN_2O_2S$)

4.1.3.1.2. Benzylation Reaction of 5-methyl-1-(o-fluorophenyl)-2-thiobarbituric acid with (+)-Cinchonine

Benzylation reaction of 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid with (+)-cinchonine was performed according to the general procedure for alkylation reactions with (+)-cinchonine catalyst. For this reaction, the following chemicals were used.

- 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid: 0.505 gram (2 mmole)
- (+)-Cinchonine: 0.118 gram (0.4 mmole)
- Tetrahydrofuran (THF): 12.5 mL
- Benzyl bromide: 0.95 mL (8 mmole)
- Saturated ammonium chloride solution (NH_4Cl – water solution): 4 mL

Yield of the purified product: 0.0431 gram, (6 %)
Melting point of the purified product: 233 ^oC
Color of the purified product: white (Figure 4.18)

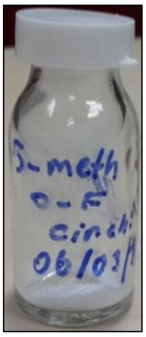


Figure 4.18. Crystals of 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid ($C_{18}H_{15}FN_2O_2S$)

4.1.3.1.3. Benzylation Reaction of 5-methyl-1-(o-tolyl)-2-thiobarbituric acid with (+)-Cinchonine

Benzylation reaction of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid with (+)-cinchonine was applied according to the general procedure for alkylation reactions with (+)-cinchonine catalyst. For this reaction, the following chemicals were used.

- 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid: 0.50 gram (2 mmole)
- (+)-Cinchonine: 0.118 gram (0.4 mmole)
- Tetrahydrofuran (THF): 12.5 mL
- Benzyl bromide: 0.95 mL (8 mmole)
- Saturated ammonium chloride solution (NH_4Cl – water solution): 4 mL

Yield of the purified product: 0.066 gram, (21 %)
Melting point of the purified product: 223 ^oC
Color of the purified product: white (Figure 4.19)

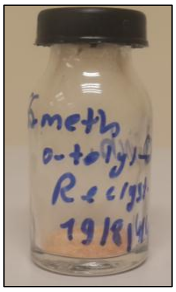


Figure 4.19. Crystals of 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid ($C_{19}H_{18}N_2O_2S$)

4.1.3.1.4. Benzylation Reaction of 5-methyl-1-phenyl-2-thiobarbituric Acid with (+)-Cinchonine

Benzylation reaction of 5-methyl-1-phenyl-2-thiobarbituric acid was performed according to the general procedure for alkylation reactions with (+)-cinchonine catalyst. For this reaction, the following chemicals were used.

- 5-methyl-1-phenyl-2-thiobarbituric acid: 0.469 gram (2 mmole)
- (+)-Cinchonine: 0.118 gram (0.4 mmole)
- Tetrahydrofuran (THF): 12.5 mL
- Benzyl bromide: 0.95 mL (8 mmole)
- Saturated ammonium chloride solution (NH_4Cl – water solution): 4 mL

Yield of the purified product: 0.5035 gram, (78 %)
Melting point of the purified product: 225^{o}C
Color of the purified product: white (Figure 4.20)

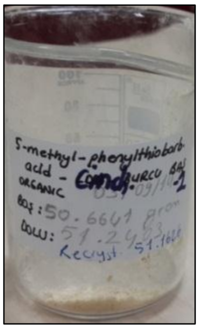


Figure 4.20. Crystals of 5-benzyl-5-methyl-1-phenyl-2-thiobarbituric acid ($C_{18}H_{16}N_2O_2S$)

4.1.3.1.5. Allyllation Reaction of 5-methyl-1-(o-chlorophenyl)-2-thiobarbituric Acid with (+)-Cinchonine

Allylation reaction of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric with (+)-cinchonine acid was applied according to the general procedure for alkylation reactions with (+)-cinchonine catalyst. For this reaction, the following chemicals were used.

- 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid: 0.54 gram (2 mmole)
- (+)-Cinchonine: 0.118 gram (0.4 mmole)
- Tetrahydrofuran (THF): 12.5 mL
- Benzyl bromide (C_7H_7Br): 0.69 mL (8 mmole)
- Saturated ammonium chloride solution (NH_4Cl – water solution): 4 mL

Despite the purification process, NMR results of the all allylation products have shown (Section 5.8.5) that all synthesized allylation products have impurities. Therefore, a new purification process should be involved.

Yield of the product: 0.4336 gram, (70 %)
Melting point of the product: 178^{o}C
Color of the product: white (Figure 4.21)

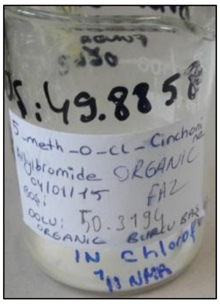


Figure 4.21. Crystals of 5-allyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid ($C_{14}H_{13}ClN_2O_2S$)

4.1.3.1.6. Allylation Reaction of 5-methyl-1-(o-fluorophenyl)-2-thiobarbituric acid with (+)-Cinchonine

Allylation reaction of 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid with (+)-cinchonine was performed according to the general procedure for alkylation reactions with (+)-cinchonine catalyst. For this reaction, the following chemicals were used.

- 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid: 0.505 gram (2 mmole)
- (+)-Cinchonine: 0.118 gram (0.4 mmole)
- Tetrahydrofuran (THF): 12.5 mL
- Allyl bromide: 0.69 mL (8 mmole)
- Saturated ammonium chloride solution (NH_4Cl – water solution): 4 mL

Yield of the product: 0.4972 gram, (85 %)
Melting point of the product: 147^oC
Color of the product: white (Figure 4.22)

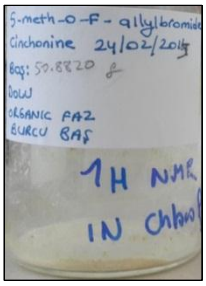


Figure 4.22. Crystals of 5-allyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric Acid ($C_{14}H_{13}FN_2O_2S$)

4.1.3.1.7. Allylation Reaction of 5-methyl-1-(o-tolyl)-2-thiobarbituric acid with (+)-Cinchonine

Allylation reaction of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid with (+)-cinchonine was performed according to the general procedure for alkylation reactions with (+)-cinchonine catalyst. For this reaction, the following chemicals were used.

- 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid: 0.50 gram (2 mmole)
- (+)-Cinchonine: 0.118 grams (0.4 mmole)
- Tetrahydrofuran (THF): 12.5 mL
- Allyl bromide: 0.69 mL (8 mmole)
- Saturated ammonium chloride solution (NH_4Cl – water solution): 4 mL

Yield of the product: 0.1665 gram, (29 %)
Melting point of the product: 155^{o}C
Color of the product: white (Figure 4.23)

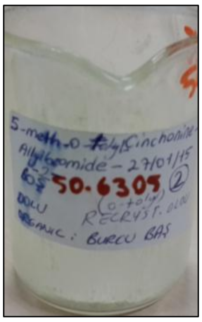


Figure 4.23. Allylation Reaction of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric Acid with (+)-Cinchonine ($C_{15}H_{16}N_2O_2S$)

4.1.3.1.8. Allylation Reaction of 5-methyl-1-phenyl-2-thiobarbituric Acid with (+)-Cinchonine

Alkylation reaction of 5-methyl-phenyl-thiobarbituric acid with (+)-cinchonine was performed according to the general procedure for alkylation reactions with (+) cinchonine catalyst. For this reaction, the following chemicals were used.

- 5-methyl-1-phenyl-2-thiobarbituric acid: 0.469 gram (2 mmole)
- (+)-Cinchonine: 0.118 gram (0.4 mmole)
- Tetrahydrofuran (THF): 12.5 mL
- Allyl bromide: 0.69 mL (8 mmole)
- Saturated ammonium chloride solution (NH_4Cl – water solution): 4 mL

Yield of the product: 0.3723 gram, (68 %)
Melting point of the product: 168^{o}C
Color of the product: white (Figure 4.24)

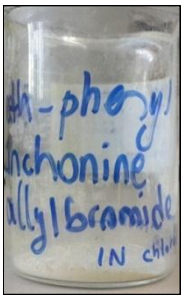


Figure 4.24. Crystals of 5-allyl-5-methyl-1-phenyl-2-thiobarbituric Acid ($C_{14}H_{14}N_2O_2S$)

4.1.3.2. General Procedure For Benzylation Reactions with LDA

When benzylation reactions were performed with LDA base, same procedure was applied as that in alkylation reactions with (+)-cinchonine catalyst. However, the reactions were performed at -78^{o}C instead of at -35^{o}C and 2.4 equivalents of LDA and 4 equivalents of benzyl bromide based on the amount of 5-methyl-1-(*o*-aryl)-2-thiobarbituric acid or 5-methyl-1-phenyl-2-thiobarbituric acid were used.

4.1.3.2.1. Benzylation Reaction of 5-methyl-1-(o-chlorophenyl)-2-thiobarbituric Acid with LDA

Benzylation reaction of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid with LDA was performed according to the general procedure for alkylation reactions with LDA. For this reaction, the following chemicals were used.

- 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid: 0.537 gram (2 mmole)
- Tetrahydrofuran (THF): 12.5 mL
- LDA: 0.6 mL (4.8 mmole)
- Benzyl bromide: 0.95 mL (8 mmole)
- Saturated ammonium chloride solution (NH_4Cl – water solution): 4 mL

Yield of the purified product: 0.1674 gram, (23 %)
Melting point of the purified product: 97^{o}C

Color of the purified product: light brown

The color of the product in the presence of (+)-cinchonine was orange and its melting point was measured as 198^0C. The reason for the difference of the melting points of the products obtained with LDA and (+)-cinchonine is that the product obtained in the presence of (+)-cinchonine was purer than the product obtained in the presence of LDA according to the integral ratios of the peaks in their ^{1}H NMR results. (Section 5.8.1 and 5.8.3)

4.1.3.2.2. Benzylation Reaction of 5-methyl-1-(o-fluorophenyl)-2-thiobarbituric Acid with LDA

Benzylation reaction of 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid with LDA was applied according to the general procedure for alkylation reactions with LDA. For this reaction, the following chemicals were used.

- 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid: 0.505 gram (2 mmole)
- Tetrahydrofuran (THF): 12.5 mL
- LDA: 0.6 mL (4.8 mmole)
- Benzyl bromide: 0.95 mL (8 mmole)
- Saturated ammonium chloride solution (NH_4Cl – water solution): 4 mL

Yield of the purified product: 0.1379 gram, (20 %)
Melting point of the purified product: 195^{o}C (crystals extracted from the aqueous phase were analyzed)
Color of the purified product: white

The color of the product obtained with (+)-cinchonine was also white. However, its melting point was measured as 233^0C. The reason for the difference in the melting points of the same product obtained with different bases can be explained by the fact that the product synthesized with (+)-cinchonine was purer than the synthesized product with LDA according to the integral ratios of the peaks in their ^{1}H NMR results (Section 5.8.1 and 5.8.3). Furthermore, normal phase HPLC results of the products showed that the product with (+)-cinchonine was purer than the product with LDA (Section 5.9).

4.1.3.2.3. Benzylation Reaction of 5-methyl-1-(o-tolyl)-2-thiobarbituric Acid with LDA

Alkylation reaction of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid was performed according to the general procedure for alkylation reactions with LDA. For that alkylation reaction, the following chemicals were used.

- 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid: 0.50 gram (2 mmole)
- Tetrahydrofuran (THF): 12.5 mL
- LDA: 0.6 mL (4.8 mmole)
- Benzyl bromide: 0.95 mL (8 mmole)
- Saturated ammonium chloride solution (NH_4Cl – water solution): 4 mL

Yield of the purified product: 0.1018 gram, (32 %)
Melting point of the purified product: 182^{o}C (crystals extracted from the aqueous phase were analyzed)
Color of the purified product: white

The color of the product synthesized with (+)-cinchonine was orange and its melting point was 223^{0}C. The reason for the difference in melting points of the products obtained with (+)-cinchonine and LDA is that the synthesized product with (+)-cinchonine was purer than the synthesized product with LDA according to the integral ratios of the peaks in their ^{1}H NMR results (Section 5.8.1 and 5.8.3). Besides, overlapping of the second peak with a peak of another compound in HPLC (Figure 5.50) showed that the product obtained with LDA is not as pure as that product obtained with (+)-cinchonine (Section 5.9).

4.1.4. Procedure for Testing Antibacterial Effect

Antibacterial effects of the synthesized thiobarbituric acid derivatives and the products of alkylation reactions were tested by using different types of bacteria. These bacteria were E.Coli, B. Subtiles, P. Auregenause, B. Megaterium and S.Aureus bacteria. Besides TSA agars were used.

First one bacterium was taken and spread on a TSA agar on every side by using cotton swab. The same procedure was carried out for other bacteria. Blank disks were wetted with 10 μL of sterile distilled water. Wet blank disks were used to get compounds with the help of tweezers. After taking the compounds with wet blank disks, they were put into the TSA agar cap which contained a bacterium. Additionally, oflaxin disk was used as a controller for each agar having a bacterium. These TSA agar caps were coated with parafilm to ensure air-tightness and then were left for 24 hours in an incubator at 37 ^{o}C.

4.2. ANALYSIS METHODS

TLC, HPLC, ^{1}H NMR, ^{13}C NMR, IR and polarimeter analyses were performed.

4.2.1. Thin Layer Chromatography (TLC)

Mobile Phase: 3:2, 4:3 Hexane – Ethyl Acetate
Sample is dissolved in absolute ethanol

4.2.2. High Pressure Liquid Chromatography (HPLC)

Reverse and normal phase studies were performed with HPLC.

4.2.2.1. Bridge Column (Reversed Phase)

Bridge column was used to check the purity of the thiobarbituric acid derivatives and their corresponding thioureas.

Mobile Phase: 50:50 Methanol – Deionized Water
Sample: 1.5 mg sample was dissolved in 3 mL methanol.
Flow Rate: 0.5 mL/min, 0.6 mL/min or 0.7 mL/min
Wavelength (λ): 254 nm

4.2.2.2. Chiral OD-H Column, Chiralpak IA, IB and IC Column (Normal Phase)

They are chiral columns and they were used for the determination of the enantioselectivity and diastereoselectivity of the alkylation reactions by finding the ratio of the peaks belonging to enantiomers and diastereomers of the products.

4.2.2.2.1. Chiral OD-H Column (Normal Phase)

Mobile Phase: 90:10 Hexane - Ethanol
Sample: 4.8 mg sample is dissolved in 2 mL ethanol.
Flow rate: 0.6 mL/min
Wavelength (λ): 254 nm

4.2.2.2.2. Chiralpak-IA, IB and IC Column (Normal Phase)

Mobile Phase: 95:5, 90:10, 80:20 or 70:30 Hexane - Ethanol
Sample: 4.8 mg sample is dissolved in 2 mL ethanol.
Flow rate: 0.4, 0.6, 0.8, 1.0 or 1.2 mL/min
Wavelength: 254 nm

4.2.3. NMR Spectroscopies

^{1}H NMR and ^{13}C NMR analyses were performed.

4.2.3.1. ^{1}H NMR Spectroscopy

^{1}H NMR spectra of thiourea derivatives were taken in acetone-d_6 and in 400 MHz instrument.

^{1}H NMR spectra of 5,5-dimethyl-,5-methyl-1-(*o*-aryl)-2-thiobarbituric acids and 5,5-dimethyl-, 5-methyl-1-phenyl-2-thiobarbituric acids were taken in acetone-d_6 in 400 MHz instrument.

^{1}H NMR spectra of the benzylation and allylation products were taken in chloroform-d or dimethyl sulfoxide-d_6 in 400 MHz instrument.

4.2.3.2. ^{13}C NMR Spectroscopy

^{13}C NMR spectra of 5,5-dimethyl-,5-methyl-1-(*o*-aryl)-2-thiobarbituric acids and 5,5-dimethyl-,5-methyl-1-phenyl-2-thiobarbituric acids were taken in acetone-d_6 or chloroform-d in 400 MHz instrument.

^{13}C NMR spectra of the benzylation products were taken in chloroform-d or dimethyl sulfoxide-d_6 in 400 MHz instrument.

4.2.3. Polarimeter

0.004 grams of the synthesized substances which could be analyzed with the polarimeter were dissolved in 8 mL ethanol. The polarimeter measurements were performed at the room temperature.

5. RESULTS AND DISCUSSION

The main objective of this study was to perform asymmetric alkylation via complex formation of 5-substituted-1-(*o*-aryl)-2-thiobarbituric acids with (+)-cinchonine to obtain 5,5-dialkylsubstituted-1-(*o*-aryl)-2-thiobarbituric acids, which may support to produce different biologically active derivatives of thiobarbiturates. Another objective of the project was to measure the antibacterial activity of different 5,5-disubstituted-1-(*o*-aryl)-2-thiobarbituric acids and 5-substitued-1-(*o*-aryl)-2-thiobarbituric acids. Besides, the 1-*o*-aryl-derivatives, 5-methyl-1-phenyl-2-thiobarbituric acid was also synthesized to be used in the asymmetric alkylation reaction at C-5 in the presence of (+)-cinchonine to observe the effect of the *ortho* substitution on the enantioselectivity and diastereoselectivity of the asymmetric reaction of thiobarbiturates. 5-methyl-1-phenyl-2-thiobarbituric acid and 5,5-dimethyl-1-phenyl-2-thiobarbituric acid were also tested for their antibacterial activity to see the effect of *ortho*-substituent and proton at C-5 on the antibacterial activity.

In this project, firstly, 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid, 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid, 5-methyl-1-phenyl-2-thiobarbituric acid, 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid, 5,5-dimetyl-1-(o-tolyl)-2-thiobarbituric acid and 5,5-dimethyl-1-phenyl-2-thiobarbituric acid were synthesized from the corresponding thioureas.

Then, alkylation reactions of 5-methyl-1-(*o*-aryl)-2-thiobarbituric acids, which were 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric, 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric, 5-methyl-1-(*o*-tolyl)-2-thiobarbituric and 5-methyl-1-phenyl-2-thiobarbituric acids were performed in order to investigate the stereoselectivity of the reaction and to obtain new biologically active compounds. Asymmetric benzylation and allylation reactions were performed at carbon-5 of thiobarbituric acid derivatives using chiral catalyst cinchonine. The benzylation reactions with LDA were performed at -78 ^{0}C, while all alkylation reactions with (+)-cinchonine were performed at -35 ^{0}C. LDA and (+)-cinchonine were used as bases for hydrogen abstraction. (+)-Cinchonine was also used as chiral catalyst in the enantioselective reaction. THF was used as the reaction solvent in the alkylation and

benzylation reactions. Allylbromide was used in the asymmetric allylation reactions, while benzyl bromide was used in the asymmetric benzylation reactions as alkylating agents.
All the synthesized compounds were characterized by NMR, IR spectroscopies and melting point analyses. Some of the new synthesized compounds were also analyzed by elemental analysis technique.

In addition, antibacterial activities of the synthesized thiobarbituric acid derivatives and the products of their alkylation reactions were measured by disk diffusion method.

In order to obtain higher yield of 5-methyl-1-(*o*-aryl)-2-thiobarbituric acids, different stoichiometry of the reactants was tested, so 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid and methylmalonic acid were used in 1:1.2 ratio and the percentage yield of this reaction was calculated as 2 %. According to this result, it can be concluded that a lower yield (2%) of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid can be obtained when methymalonic acid and *ortho*-chlorophenylthiourea react in 1:1.2 ratio compared to when they react in 1:1 ratio, where the reaction yield was 19 %.
In the synthesis of thiobarbituric acid derivatives, products were sometimes obtained in light brown, yellow or orange colours because of probable side reactions. However, after one or two recrystallization processes, the products were obtained in white colours.

OD-H column was used for the chiral HPLC analysis of benzylation and allylation products to find enantioselectivities or diastereoselectivities of the alkylation reactions. Additionally, Chiralpak IA, Chiralpak IB and Chiralpak IC columns were used to determine the enantiomeric ratio of the products, but the desired results could not be achieved by these columns due to improper resolution of the peaks.

X-Bridge column was used to control the purity and occurrence of the synthesis reactions of thiobarbituric acid derivatives in the reversed phase HPLC.

5.1. ^{1}H NMR RESULTS OF THIOUREA DERIVATIVES

^{1}H NMR analyses were performed for all synthesized thiourea derivatives. In the NMR analyses, the following solvents (Table 5.1) were used. The integral values of the protons were consistent with the proton ratios of the compounds. In the spectra, except the peaks of the compound some residual peaks belonging to NMR solvent or water in the NMR solvent were seen, their chemical shift values are also given in Table 5.1.

Table 5.1. Residual peaks due to solvent or water

Solvent	**^{1}H NMR Chemical Shift (# of signals)**	**^{1} H NMR Water Signal**
Acetone-d_6	2.05 (5)	2.8
Chloroform-d	7.26 (1)	1.6
Dimethyl Sulfoxide-d_6 (DMSO)	2.50 (5)	3.3

In the ^{1}H NMR analysis of *ortho*-substituted phenylthioureas and phenylthiourea, two singlet peaks due to NH protons and multiplet peaks due to aromatic protons were expected and were seen in the spectra (Figure 5.1-5.4). One singlet peak belonging to one hydrogen atom of the NH group, and the other singlet peak belonging to two hydrogen atoms of the NH_2 group were observed in the spectra (Figure 5.1-Figure 5.4). Methyl protons (3 H) were also observed as a singlet in the ^{1}H NMR spectrum of *o*-tolylthiourea (Figure 5.3). The assignments of the peaks were shown in Table 5.2-5.5.

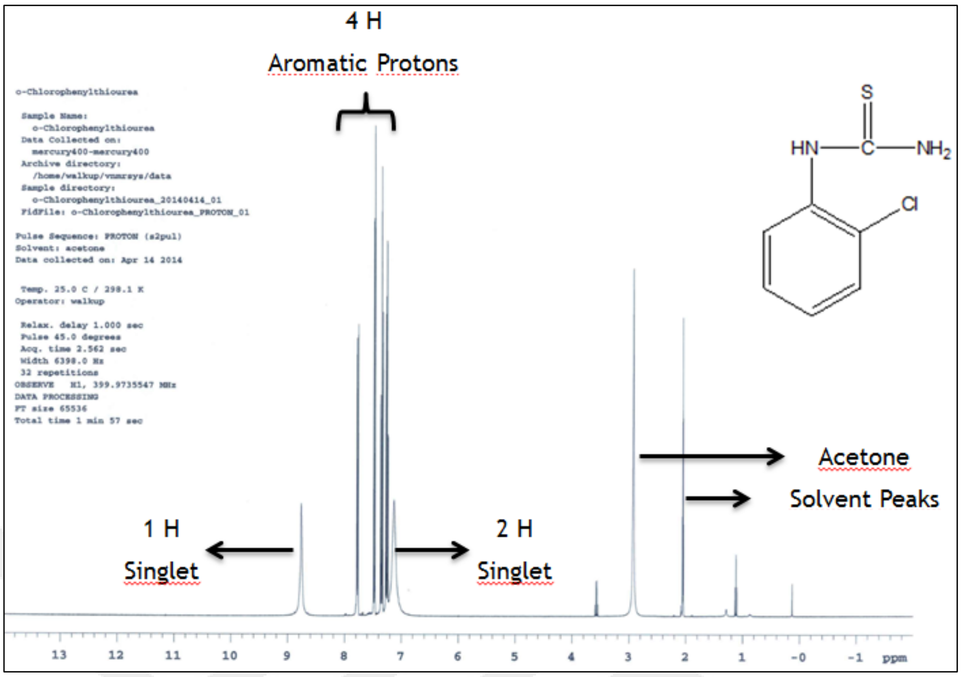


Figure 5.1. [1]H NMR spectrum of *o*-chlorophenylthiourea ($C_7H_7ClN_2S$), NMR solvent: acetone-d_6

Table 5.2. [1]H NMR (400 MHz) data of *o*-chlorophenylthiourea, solvent: acetone-d_6

Multiplicity, Number of Protons	**δ (ppm)**
(s, 1 H)	8.77
(m, 4 H)	7.79-7.24
(s, 2 H)	7.13

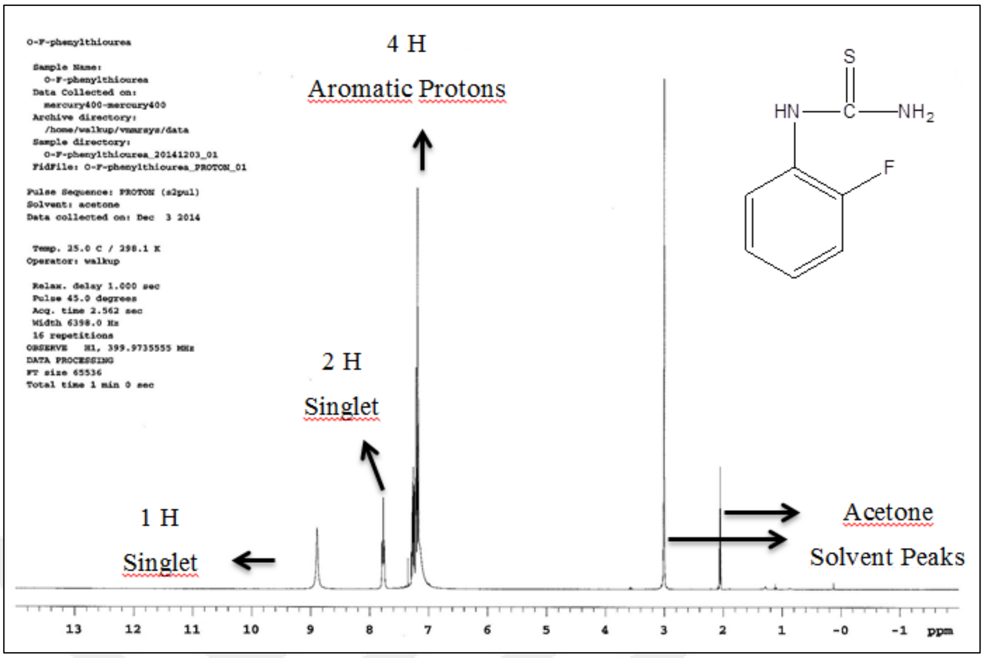


Figure 5.2. [1]H NMR spectrum of *o*-fluorophenylthiourea ($C_7H_7FN_2S$), NMR solvent: acetone –d_6

Table 5.3. [1]H NMR (400 MHz) data of *o*-fluorophenylthiourea, solvent: acetone-d_6

Multiplicity, Number of Protons	**δ (ppm)**
(s, 1 H)	8.89
(m, 4 H)	7.30-7.17
(s, 2 H)	7.77

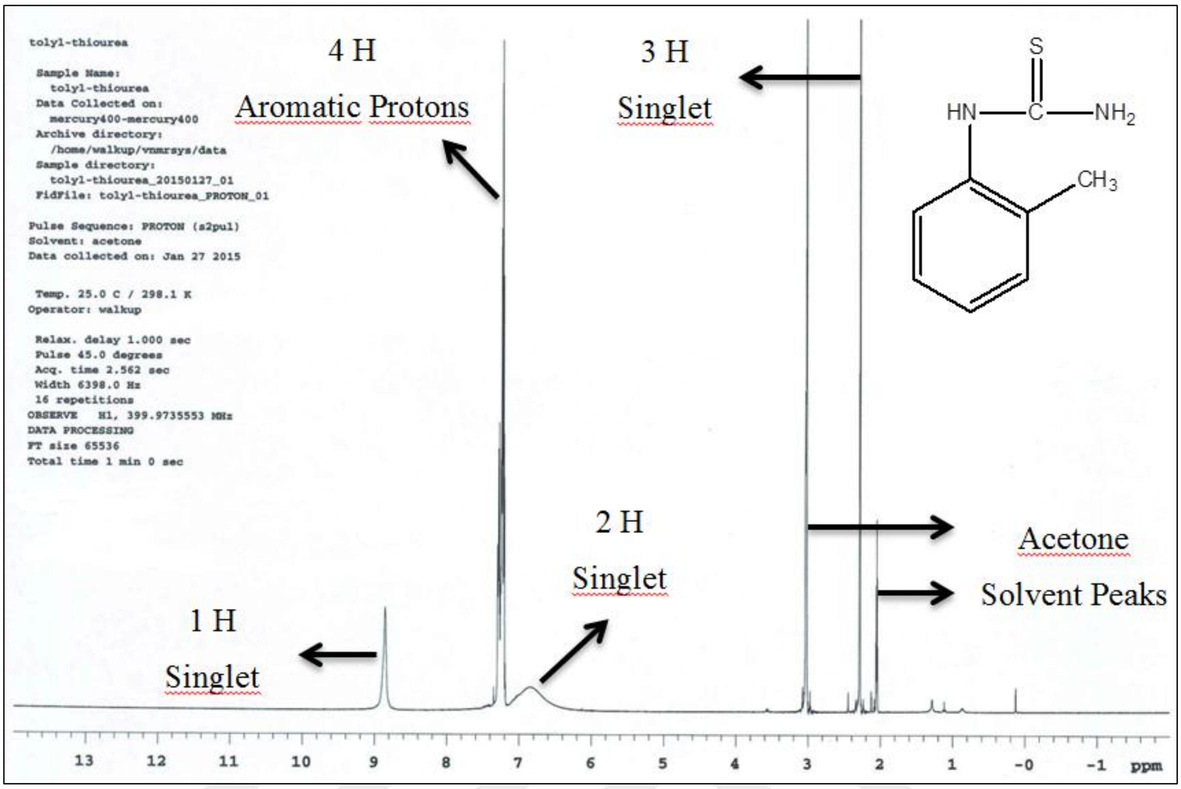


Figure 5.3. ^{1}H NMR spectrum of *o*-tolylthiourea ($C_8H_{10}N_2S$),

NMR solvent : acetone – d_6

Table 5.4. ^{1}H NMR (400 MHz) data of *o*-tolylthiourea, solvent: acetone-d_6

Multiplicity, Number of Protons	**δ (ppm)**
(s, 1 H)	8.85
(m, 4 H)	7.30-7.19
(s, 2 H)	6.84
(s, 3 H)	2.29

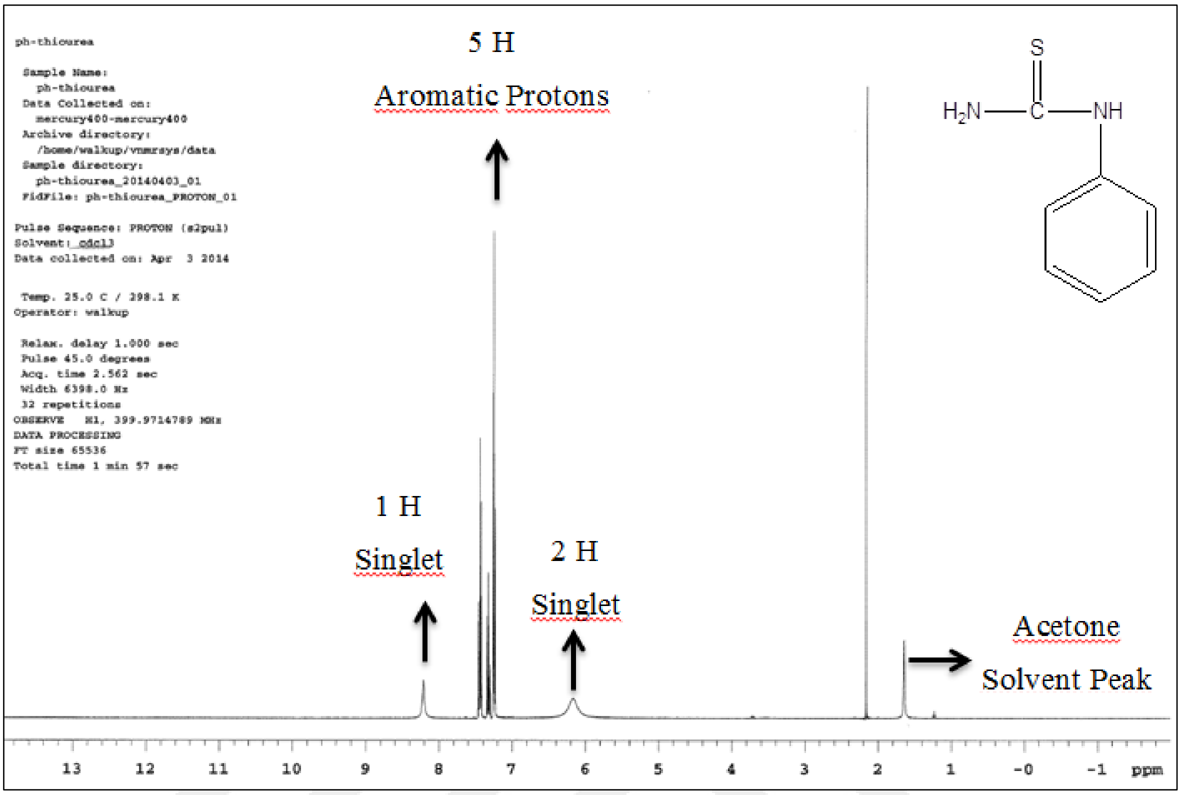


Figure 5.4. ^{1}H NMR spectrum of phenylthiourea ($C_7H_8N_2S$),

NMR solvent: acetone – d_6

Table 5.5. ^{1}H NMR (400 MHz) data of phenylthiourea, solvent: acetone-d_6

Multiplicity, Number of Protons	δ (ppm)
(s, 1 H)	8.21
(m, 5 H)	7.46-7.22
(s, 2H)	6.17

5.2. HPLC ANALYSES RESULTS OF THIOUREA DERIVATIVES

The results of the HPLC analyses of the synthesized *ortho*-substituted phenylthioureas and phenylthiourea were shown in Appendix A. Only one sharp peak was seen in the chromatograms, which is a sign of the purity of the tested compound.

Table 5.6. Reversed phase HPLC results of *ortho*-substituted phenylthioureas and phenylthiourea

Column type	Name of the compound	Mobile phase composition	Flow rate (mL/minute)	Retention time (minutes)
X-Bridge Column	*o*-chlorophenyl thiourea	methanol:water = 50:50	0.5	5.184
X-Bridge Column	*o*-fluorophenyl thiourea	methanol:water = 50:50	0.5	4.196
X-Bridge Column	*o*-tolylthiourea	methanol:water = 50:50	0.5	4.814
X-Bridge Column	phenylthiourea	methanol:water = 50:50	0.5	4.190

5.3. IR RESULTS OF THIOUREA DERIVATIVES

The results of IR spectra of thiourea and thiobarbituric acid derivatives were evaluated as in Table 5.7.

Table 5.7. Table for characteristic IR absorptions expected in thiourea and thiobarbituric acid derivatives

Assignment	**Frequency (cm^{-1})**
$\bar{\nu}$ of N-H stretching	3700-3100
$\bar{\nu}$ of aromatic C-H stretching	3000-3100
$\bar{\nu}$ of C=S stretching	1050-1200 or ~1200
$\bar{\nu}$ of aromatic C=C stretching	1400-1600 or 1650-1550
$\bar{\nu}$ of aromatic C-H bend (mono)	770-730 or 715-685
$\bar{\nu}$ of C-N	1360-1250
$\bar{\nu}$ alkyl C-CI stretching	785-540
$\bar{\nu}$ alkyl C-F stretching	1400-1000
$\bar{\nu}$ *ortho*-disubstituted benzene C-H stretching	~ 750
$\bar{\nu}$ C=O stretching	1650-1690 or 1650-1870

IR spectra of the synthesized *ortho*-substituted phenylthioureas and phenylthiourea were shown in Figures 5.5-5.8 and their results were analyzed in Tables 5.8-5.11.

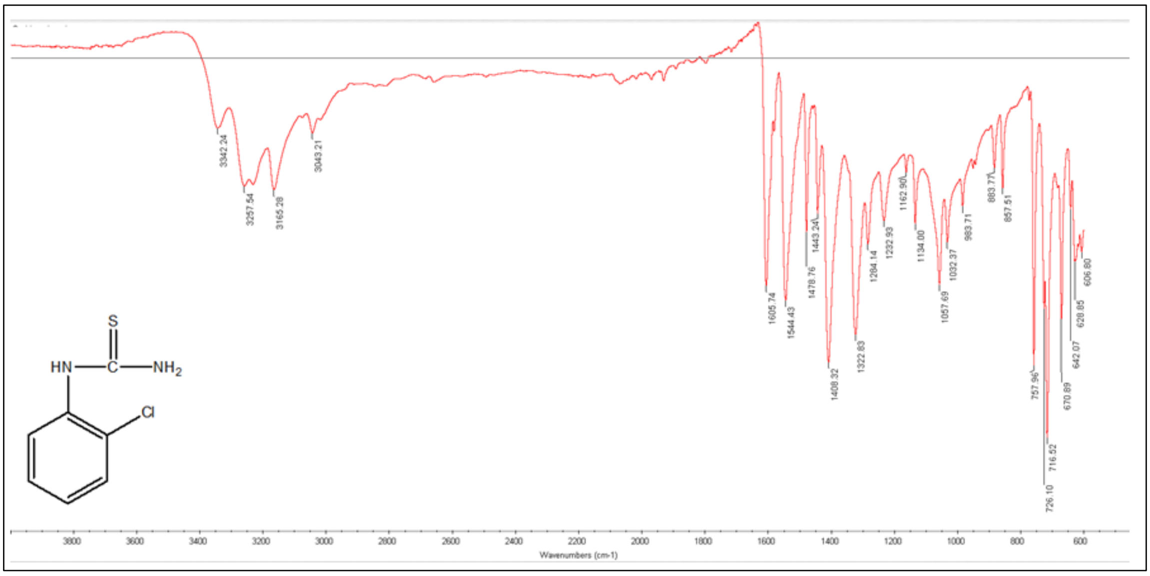


Figure 5.5. IR spectrum of *o*-chlorophenylthiourea ($C_7H_7ClN_2S$)

Table 5.8. Assignments of IR absorption frequencies of *o*-chlorophenylthiourea ($C_7H_7CIN_2S$)

Assignment	**Frequency (cm^{-1})**	**Relative intensity**
$\bar{\nu}$ of N-H Stretching	3257.54, 3342.24	w
$\bar{\nu}$ of aromatic C-H stretching	3043.21	w
$\bar{\nu}$ of C=S stretching	1057.69	m
$\bar{\nu}$ of aromatic C=C stretching	1408.32, 14443.24, 1478.76, 1544.43, 1605.74	m
$\bar{\nu}$ of aromatic C-H bend (mono)	757.96	m
$\bar{\nu}$ of C-N stretching	1284.14, 1322.83	m
$\bar{\nu}$ alkyl C-CI stretching	716.52	s

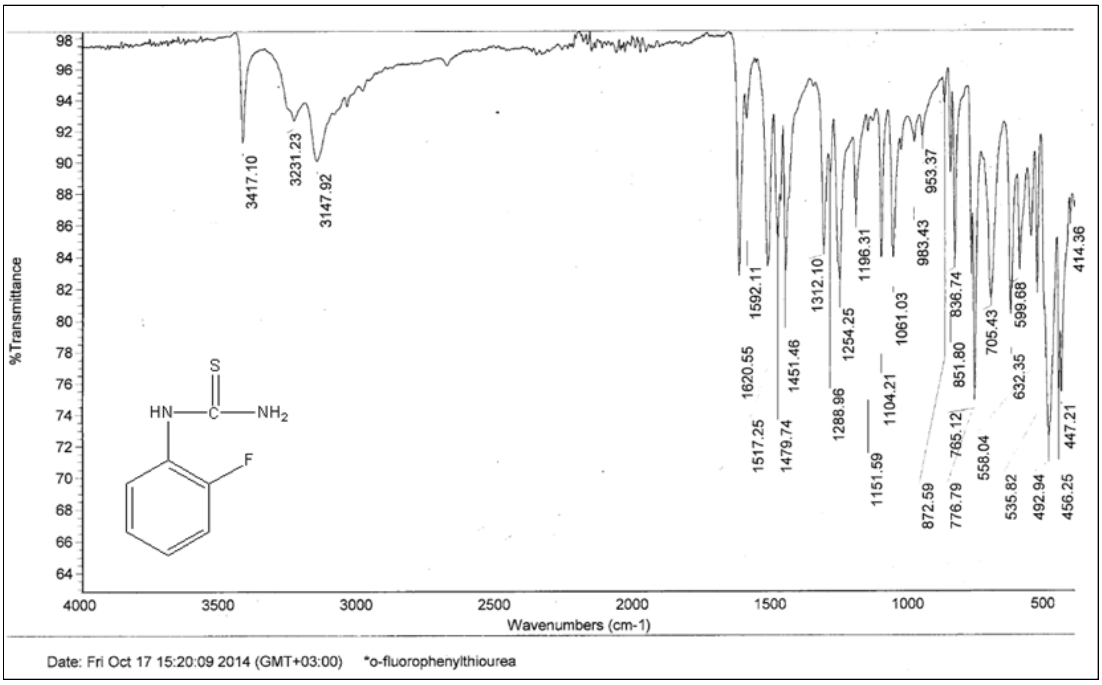


Figure 5.6. IR Spectrum of *o*-fluorophenylthiourea ($C_7H_7FN_2S$)

Table 5.9. Assignments of IR absorption frequencies of *o*-fluorophenylthiourea ($C_7H_7FN_2S$)

Assignment	Frequency (cm^{-1})	Relative intensity
$\bar{\nu}$ of N-H Stretching	3417.10, 3231.23	w
$\bar{\nu}$ of aromatic C-H stretching	3147.92	w
$\bar{\nu}$ of C=S stretching	1061.03	m
$\bar{\nu}$ of aromatic C=C stretching	1620.55, 1592.11, 1517.25, 1479.74, 1451.46	m
$\bar{\nu}$ of aromatic C-H bend (mono)	705.43, 765.12	m
$\bar{\nu}$ of C-N stretching	1254.25, 1288.96, 1312.10	m
$\bar{\nu}$ alkyl C-F stretching	1312.10	m

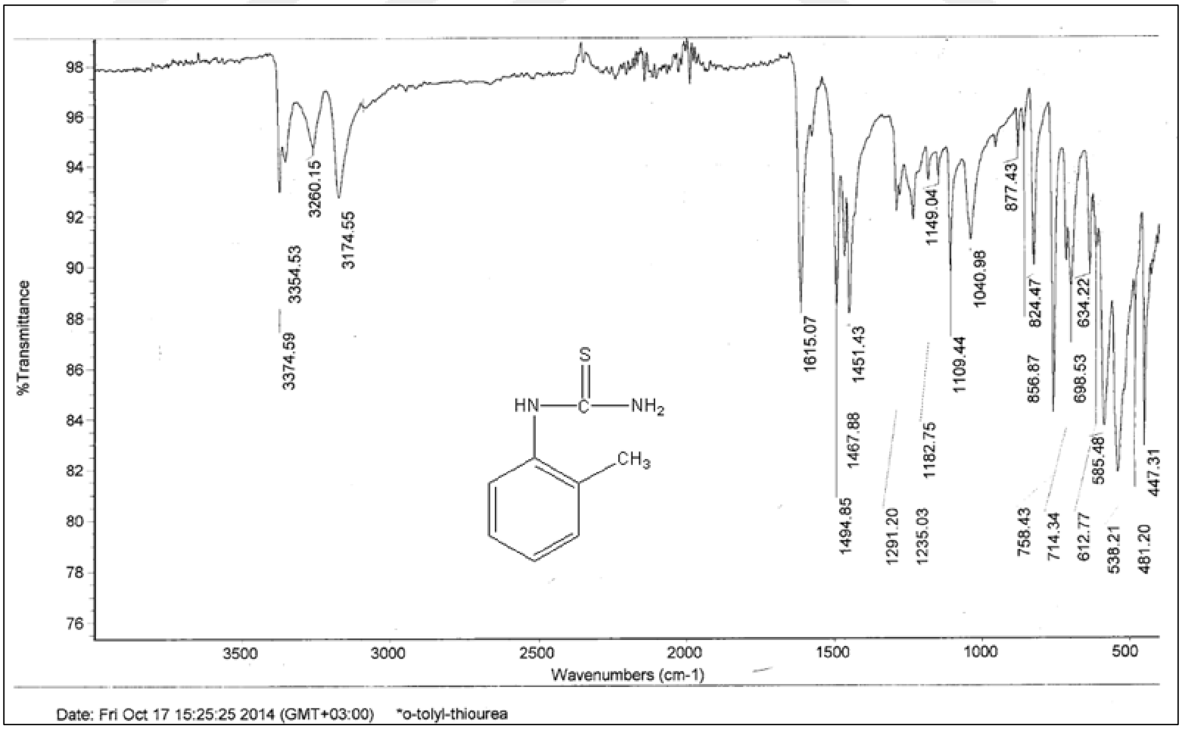


Figure 5.7. IR Spectrum of *o*-tolylthiourea ($C_8H_{10}N_2S$)

Table 5.10. Assignments of IR absorption frequencies of *o*-tolylthiourea ($C_8H_{10}N_2S$)

Assignment	Frequency (cm^{-1})	Relative intensity
$\bar{\nu}$ of N-H Stretching	3354.53 , 3374.59	w
$\bar{\nu}$ of aromatic C-H stretching	3174.55	w
$\bar{\nu}$ of C=S stretching	1109.44	m
$\bar{\nu}$ of aromatic C=C stretching	1451.43, 1467.88, 1494.85, 1615.07	m
$\bar{\nu}$ of aromatic C-H bending (mono)	758.43, 698.53, 714.34	m
$\bar{\nu}$ of C-N stretching	1291.20	w
$\bar{\nu}$ ortho disubstituted benzene C-H	758.43	m

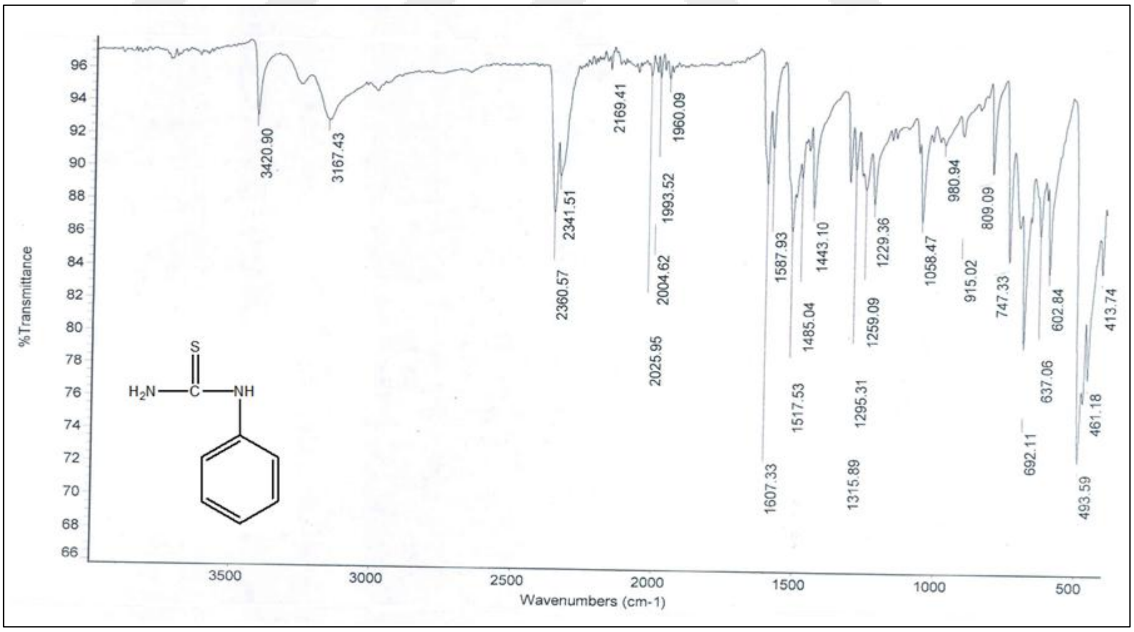


Figure 5.8. IR Spectrum of phenylthiourea ($C_7H_8N_2S$)

Table 5.11. Assignments of IR absorption frequencies of phenylthiourea ($C_7H_8N_2S$)

Assignment	**Frequency (cm^{-1})**	**Relative intensity**
$\bar{\nu}$ of N-H Stretching	3420.90	w
$\bar{\nu}$ of aromatic C-H stretching	3167.43	w
$\bar{\nu}$ of C = S stretching	1058.47	m
$\bar{\nu}$ of aromatic C = C stretching	1443.10, 1485.04, 1517.53, 1587.93, 1607.33	m
$\bar{\nu}$ of aromatic C-H bend (mono)	747.33, 692.11	m
$\bar{\nu}$ of C-N stretching	1259.09, 1315.89	m

5.4. ^{1}H NMR RESULTS of 5,5-DIMETHYL-, 5-METHYL-1-(*o*-ARYL)-2-THIOBARBITURIC ACIDS and 5,5-DIMETHYL-, 5-METHYL-1-PHENYL-2-THIOBARBITURIC ACIDS

As described in the theory part (section 2.2.3), 5-methyl-1-(*o*-aryl)-2-thiobarbituric acids have two stereogenic units, one chiral center and one chiral axis, which cause them to form four stereoisomers with diastereomeric and enantiomeric relationships: M-R & P-S (one enantiomeric pair) and P-R & M-S (one enantiomeric pair).

Enantiomers have the same NMR spectrum, whereas the diastereomers may give rise to different NMR spectra. Since the studied thiobarbituric acids have dieastereomeric forms, in the ^{1}H NMR analysis of thiobarbituric acid derivatives, two peaks were seen for some proton types, one of which belongs to M-R & P-S pairs and the other belongs to P-R & M-S pair. For example two quartets for hydrogen atom at C-5 and two doublets for 5-methyl protons were observed due to the presence of two diastereomeric forms.

Depending on the recrystallization process, only one peak may be observed for the same type of hydrogens of the recrystallized product. It can be explained by the difference in the crystallization process of two diastereomers. One diastereomeric form might crystallize more rapidly than the other form, so the first precipitate will be richer in one of the diastereomeric forms. ^{1}H NMR spectrum of 5-benzyl-5-methyl-1-tolyl-2-thiobarbituric acid might be an example for this case (Figure 5.29).

The expected peaks for the 5-methyl-1-(o-aryl)-2-thiobarbituric acids are as follows: One quartet due to the proton at C-5, one doublet due to the methyl group at C-5, one singlet due to the proton of NH group, multiplet peaks due to aromatic protons, and one singlet due to the *ortho*-methyl group in the aromatic group if present. Double of these peaks may occur depending on the presence of diastereomeric forms of the compound and NMR conditions.

Before starting synthesis of thiobarbituric acid derivatives, the purity of the synthesized phenylthiourea and *ortho*-substituted phenylthioureas were checked by ^{1}H NMR spectroscopy and HPLC analyses.

In the NMR spectrum of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid (Figure 5.9), two singlets for NH group (for 1 H of NH group) were obtained. Besides, two quartets were obtained for the proton at C-5 and two doublets were seen for the three protons of methyl group bonded to C-5. This proves the presence of diastereomeric forms of the compound. The assignments of the peaks were given in Table 5.12.

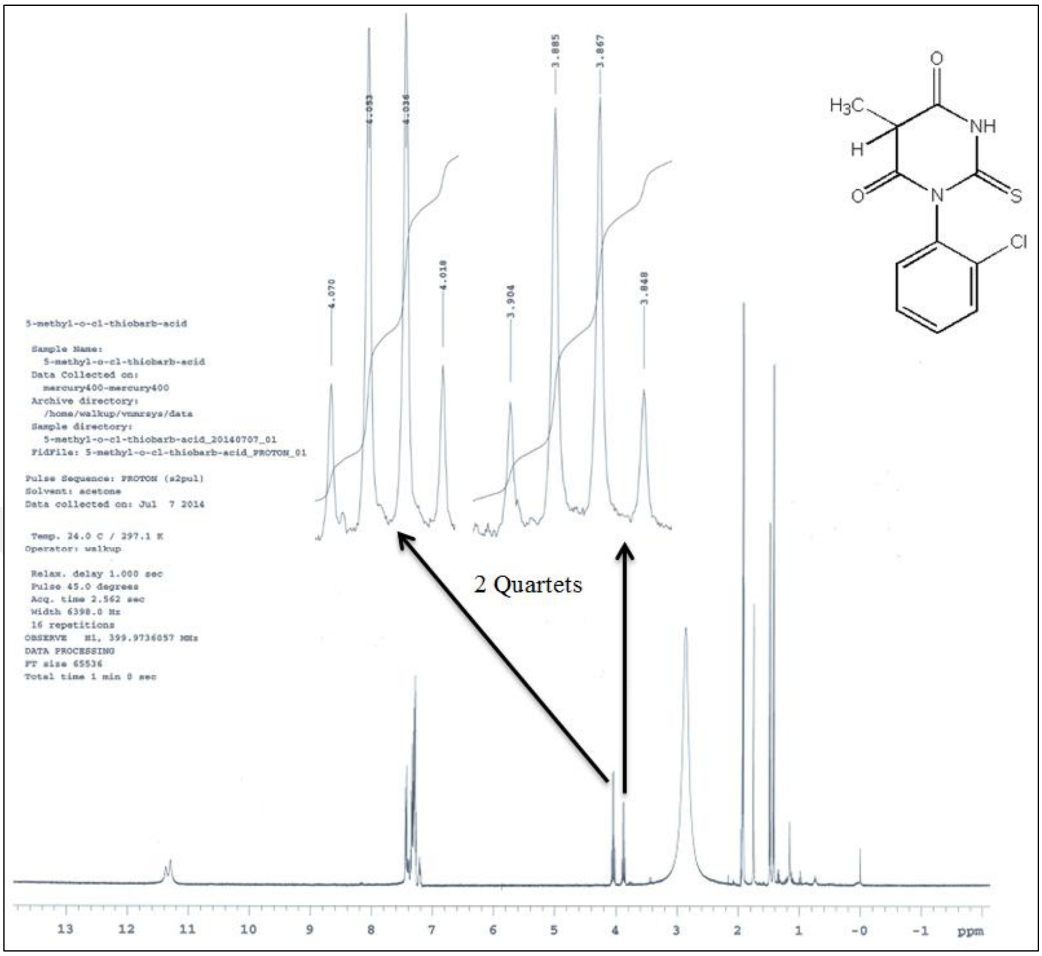


Figure 5.9. ^{1}H NMR Spectrum of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric Acid ($C_{11}H_9CI\ N_2O_2S$), NMR solvent: acetone-d_6

Table 5.12. ^{1}H NMR (400 MHz) data of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, solvent: acetone-d_6

Multiplicity, Number of Protons	**δ (ppm)**
(s, 1 H)	11.36 & 11.28
(m, 4 H)	7.45-7.20
(q, J = 6.8 Hz and J = 7.6 Hz, 1 H)	4.044 & 3.876
(d, J= 7.2 Hz and J = 7.2 Hz, 3 H)	1.482 & 1.423

In the [1]H NMR spectrum of 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid (Figure 5.10), two quartets for the proton at C-5, two doublets peaks for the methyl protons at C-5 and one singlet for the of NH proton were observed. The assignments of the peaks were given in Table 5.13. Plus, the water peak of acetone solvent was observed at 2.97 ppm.

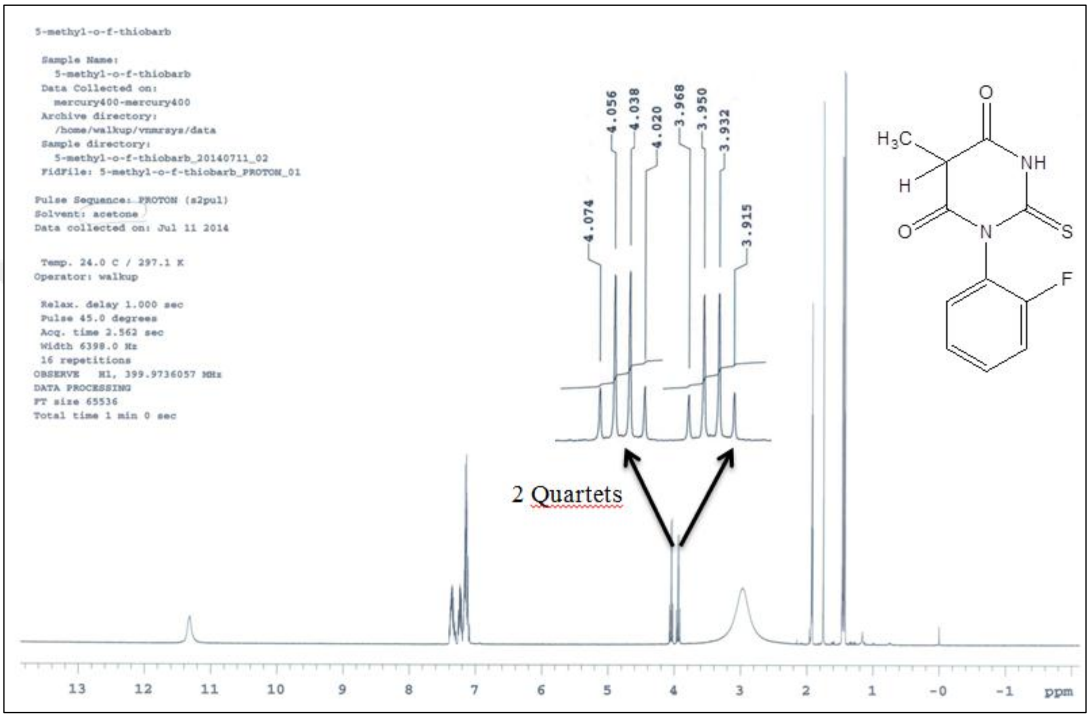


Figure 5.10. [1]H NMR spectrum of 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid ($C_{11}H_9FN_2O_2S$): NMR solvent: acetone-d_6

Table 5.13. [1]H NMR (400 MHz) data of 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid, solvent: acetone-d_6

Multiplicity, Number of Protons	**δ (ppm)**
(s, 1 H)	11.32
(m, 4 H)	7.396-7.087
(q, J=7.2 Hz and J=7.2Hz, 1 H)	4.047 & 3.942
(d, J=7.2 Hz and J=7.2 Hz, 3 H)	1.457 & 1.431

In the [1]H NMR spectrum of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid (Figure 5.11). Two quartets (for 1 H at C-5), one singlet (for 1 H of NH group), one doublet (for 3 H's of the methyl group bonded to C-5) and one singlet (for 3 H's of CH_3 at *ortho* position) peaks were observed. Although two quartets were seen for the proton at C-5, only one doublet peak was observed for 3 H's of the methyl group bonded to C-5, this occurs probably due to the close chemical shift values of these protons of the two diastereomers in acetone-d_6 and the magnetic field strength of the instrument. The assignments of the peaks were given in Table 5.14.

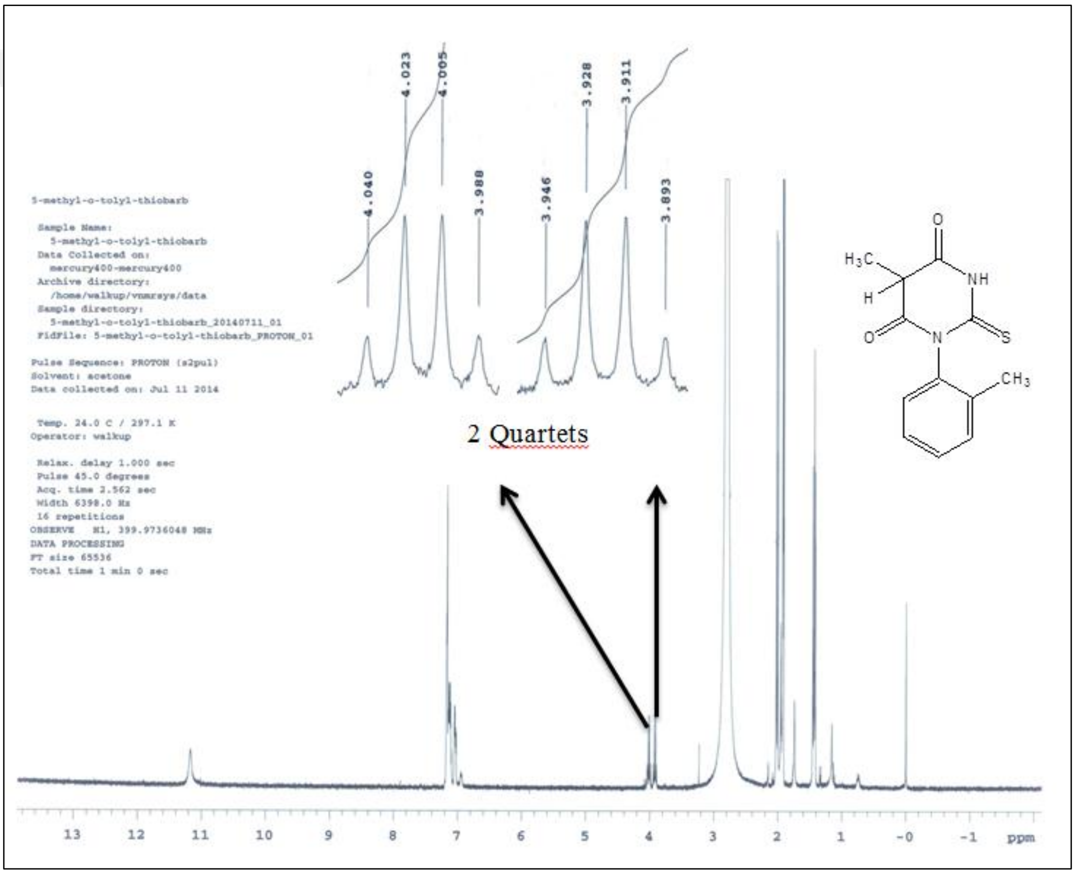


Figure 5.11. [1]H NMR spectrum of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid ($C_{12}H_{12}N_2O_2S$), NMR solvent : acetone-d_6

Table 5.14. ^{1}H NMR (400 MHz) Data of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid, solvent: acetone-d_6

Multiplicity, Number of Protons	**δ (ppm)**
(s, 1 H)	11.20
(m, 4 H)	7.176-6.951
(q, J=6.8 Hz, 1 H)	4.014
(q, J=7.2 Hz, 1 H)	3.920
(d, J=9.2 Hz, 3 H)	2.018
(s, 3H)	1.924

In the NMR spectrum of 5-methyl-1-phenyl-2-thiobarbituric acid, only one quartet was observed, since there is no *ortho*-substituent in the aromatic ring and consequently the chiral axis does not exist.

One quartet (for 1 H of C-5), one singlet (for 1 H of NH group), one doublet (for 3 H's of C-5) peaks were observed in Figure 5.12. The assignments of the peaks were given in Table 5.15.

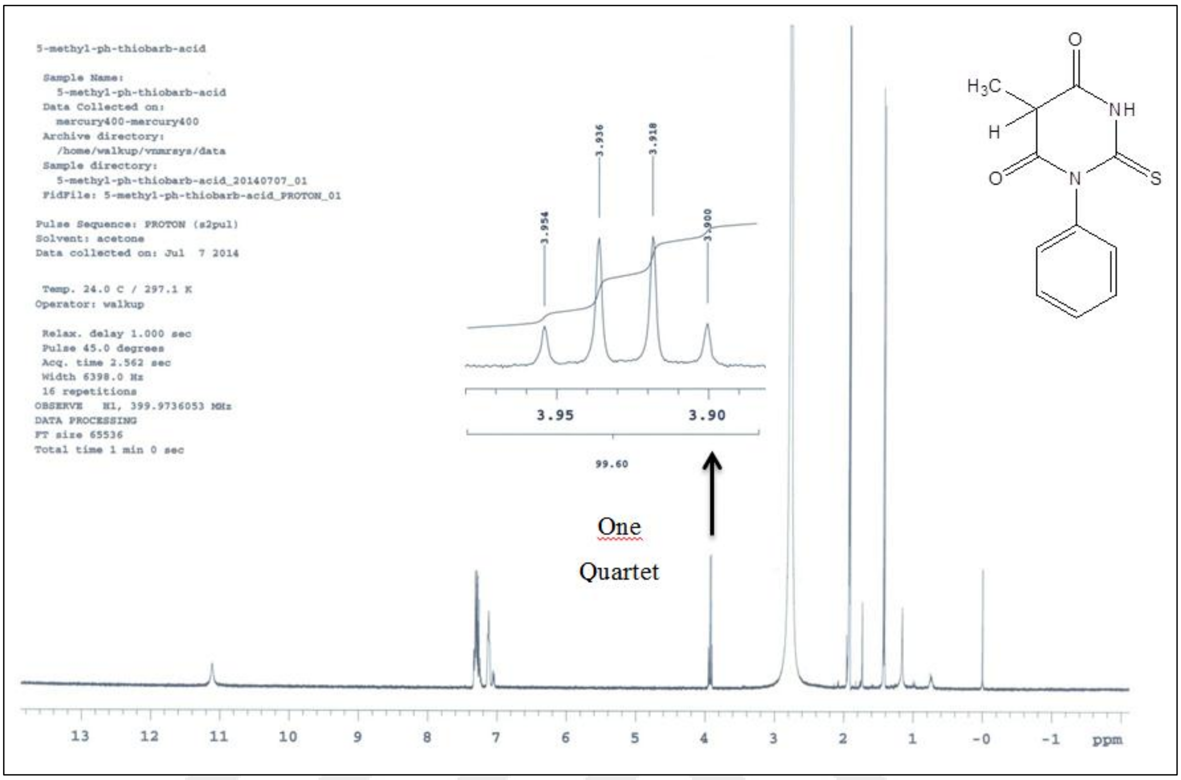


Figure 5.12. [1]H NMR spectrum of 5-methyl-1-phenyl-2-thiobarbituric acid ($C_{11}H_{10}N_2O_2S$), NMR solvent: acetone-d_6

Table 5.15. [1]H NMR (400 MHz) data of 5-methyl-1-phenyl-2-thiobarbituric acid, solvent: acetone-d_6

Multiplicity, Number of Protons	**δ (ppm)**
(s, 1 H)	11.11
(m, 4 H)	7.340-7.043
(q, J= 7.2 Hz, 1 H)	3.927
(d, J= 7.2 Hz, 3 H)	1.424

In the NMR spectra of 5,5-dimethyl-1-(*o*-aryl)-2-thiobarbituric acids, two singlets for the methyl substituents at C-5 were observed, since these methyl groups are diastereotopic due to the presence of the C_{aryl}-$N_{heterocycle}$ chiral axis and diasteretopic protons (groups) have different chemical shifts. Because of restricted rotation around the C_{aryl}-$N_{heterocycle}$ chiral axis two rings in the structure are nonplanar, that gives the molecule dissymmetry.

In the ^{1}H NMR spectrum of 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid (Figure 5.13), one singlet peak for one hydrogen atom of NH group and two singlets for the two methyl groups at C-5 were seen. Two diastereotopic 5-methyl protons gave rise to two singlets at 1.664 ppm and 1.574 ppm. The data for all the peaks in the spectrum were given in Table 5.16.

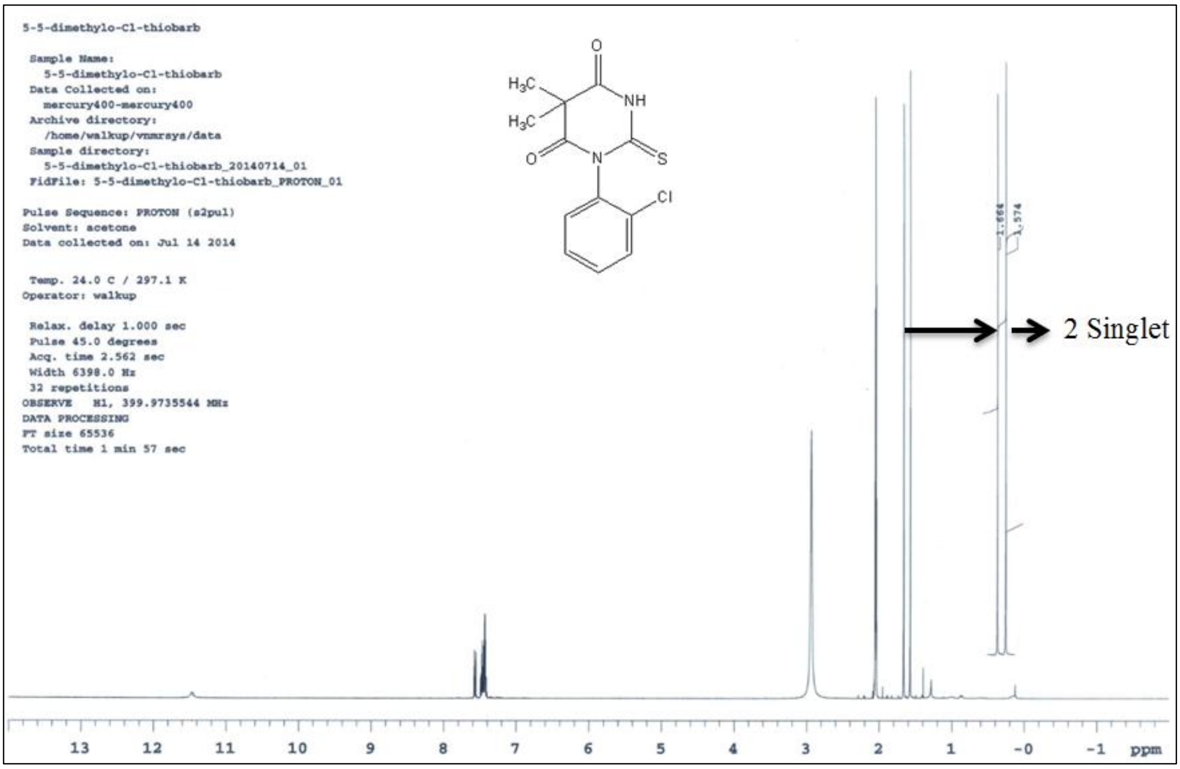


Figure 5.13. ^{1}H NMR spectrum of 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid ($C_{12}H_{11}ClN_2O_2S$), NMR solvent: acetone-d_6

Table 5.16. [1]H NMR (400 MHz) data of 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, solvent: acetone-d_6

Multiplicity, Number of Protons	δ (ppm)
(s, 1 H)	11.47
(m, 4 H)	7.580-7.412
(3H, s)	1.664
(3H, s)	1.574

In the [1]H spectrum of 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid (Figure 5.14), two singlet peaks for diastereotopic methyl groups at C-5 were seen. The chemical shift values of the peaks were given in Table 5.17.

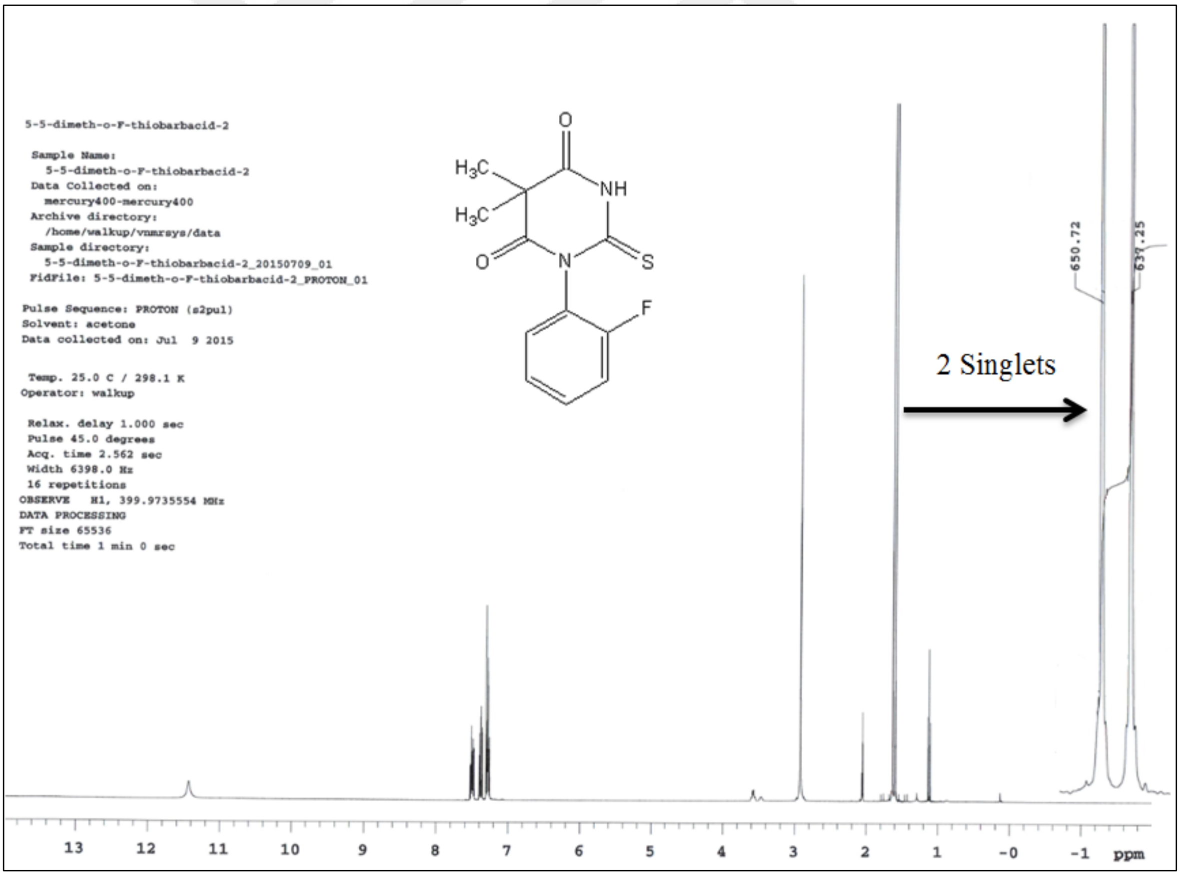


Figure 5.14. [1]H NMR spectrum of 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid ($C_{12}H_{11}FN_2O_2S$), NMR solvent: acetone-d_6

Table 5.17. [1]H NMR (400 MHz) data of 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid, solvent: acetone-d_6

Multiplicity, Number of Protons	**δ (ppm)**
(s, 1 H)	11.44
(m, 4 H)	7.53-7.25
(3H, s)	1.63
(3H, s)	1.60

In [1]H NMR Spectrum of 5,5-dimethyl-1-(*o*-tolyl)-2-thiobarbituric acid (Table 5.18), again two singlet peaks for the diastereotopic methyl groups at C-5 were seen. Besides one singlet peak for *o*-methyl substituent on the aromatic ring was observed [71].

Table 5.18. [1]H NMR result of 5,5-dimethyl-1-(*o*-tolyl)-2-thiobarbituric acid, solvent: chloroform-d [71]

Multiplicity, Number of Protons	**δ (ppm)**
(s, 1 H)	9.08
(m, 4 H)	7.04-7.39
(s, 3H)	1.70
(s, 3H)	1.69
(s, 3H)	2.16

In the [1]H NMR spectrum of 5,5-dimethyl-1-phenyl-2-thiobarbituric acid (Figure 5.15), one singlet peak for one hydrogen atom of NH group and one singlet peak for two methyl groups at C-5 were observed. Two methyl groups give only one singlet peak, since there is no chiral axis in the compound, so these two methyl groups are enantiotopic groups and give the same peak. The assignments of the peaks were given in Table 5.19.

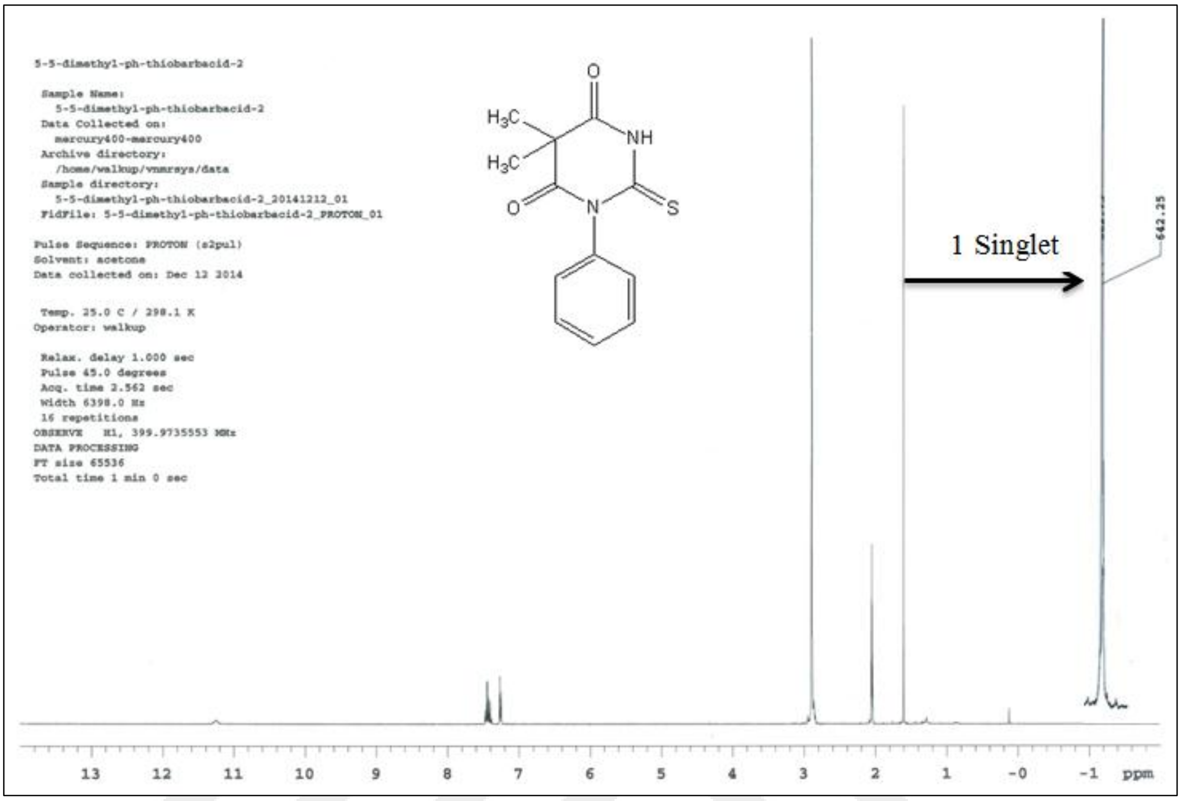


Figure 5.15. [1]H NMR spectrum of 5,5-dimethyl-1-phenyl-2-thiobarbituric acid ($C_{12}H_{12}N_2O_2S$), NMR solvent: acetone-d_6

Table 5.19. ^{1}H NMR (400 MHz) data of 5,5-dimethyl-1-phenyl-2-thiobarbituric acid, solvent: acetone-d_6

Multiplicity, Number of Protons	**δ (ppm)**
(s, 1 H)	11.25
(m, 5 H)	7.48-7.25
(s, 6 H)	1.61

5.5. ^{13}C NMR RESULTS of 5,5-DIMETHYL-, 5-METHYL-1-(*o*-ARYL)-2-THIOBARBITURIC ACIDS and 5,5-DIMETHYL-, 5-METHYL-1-PHENYL-2-THIOBARBITURIC ACIDS

5-methyl-1-(*o*-aryl)-2-thiobarbituric acids were synthesized for the first time. Therefore the C-13 NMR analyses were done for all 5-methyl derivatives. Since 5-methyl derivatives have diastereomeric isomers, some of the carbon atoms gave two peaks at different chemical shift values due to diastereomeric pairs. In the NMR analyses the following solvents (Table 5.20) were used. In the spectra, except the peaks of the compounds some residual peaks belonging to NMR solvents were seen, their chemical shift values were given in Table 5.20.

Table 5.20. Residual peaks due to solvent or water

Solvent	**^{13}C NMR Chemical Shift (# of signals)**
Acetone-d_6	29.92 (7), 206.68 (1)
Chloroform-d	77.23 (3)

^{13}C NMR spectrum of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid was given in Figure 5.16. The assignments of the peaks to specific carbons were listed in Table 5.21. Peaks were observed for each diastereomer with different chemical shift value for carbon atoms in the ^{13}C NMR spectrum of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid (Figure 5.16).

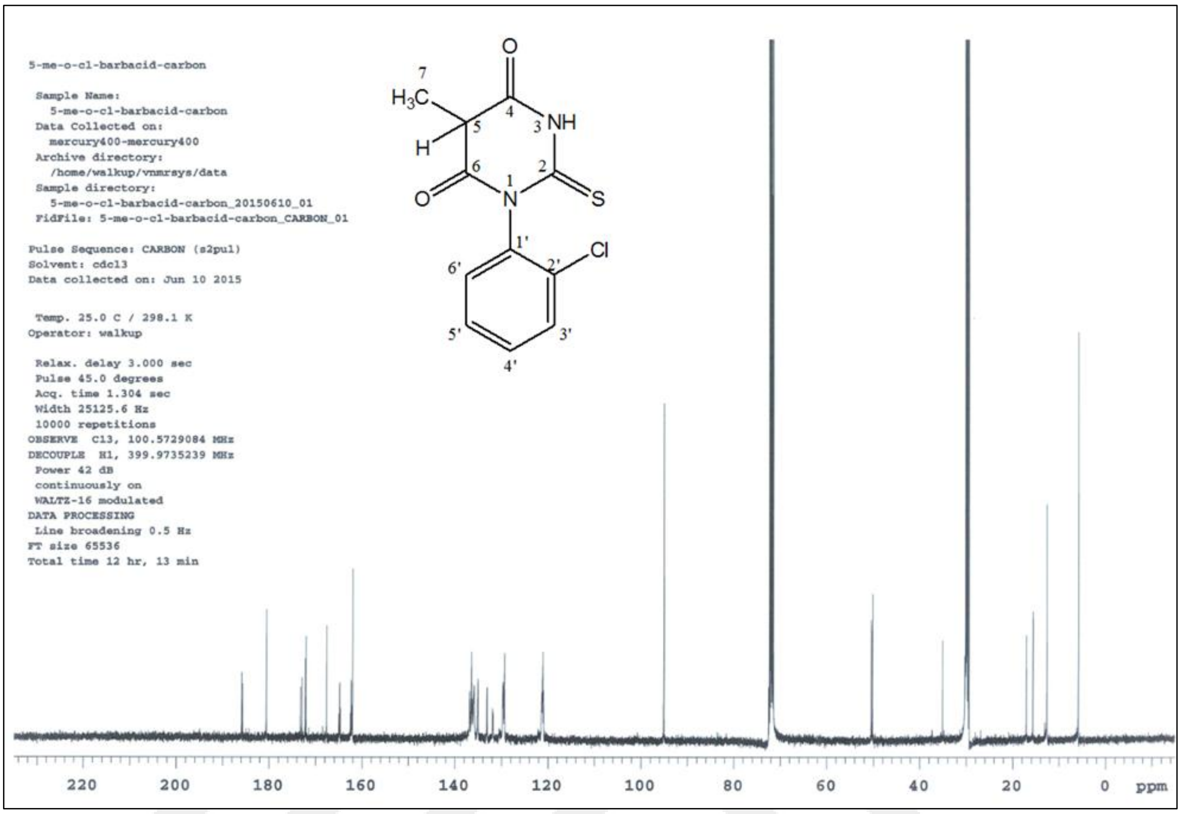


Figure 5.16. [13]C NMR Spectrum of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid ($C_{11}H_9CI\ N_2O_2S$), NMR solvent: chloroform-d

Table 5.21. Assignments of [13]C NMR peaks of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid to specific carbons, solvent: chloroform-d

Number of Carbons	**δ (ppm)**
C-2	185.928 & 185.737
C-4 or C-6	173.242 & 172.959
C-4 or C-6	172.266 & 172.083
C-5	50.464 & 50.212
C-7 (CH_3 group at C-5)	17.078 & 15.683
Aromatic C's	136.829 - 129.434

^{13}C NMR spectrum of 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid was given in Figure 5.17. The assignments of the peaks to specific carbons were listed in Table 5.22. Peaks of two diastereomers were observed in the ^{13}C NMR spectrum of 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid for C-5 and methyl carbon at C-5.

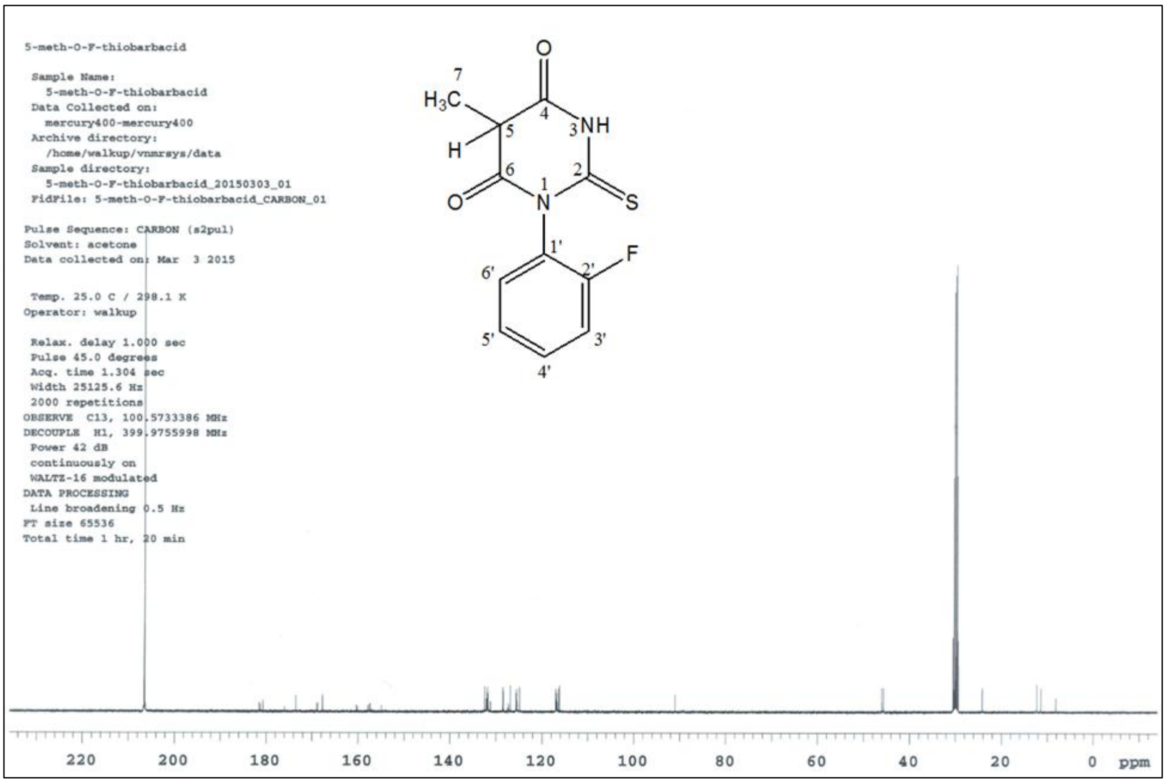


Figure 5.17. ^{13}C NMR spectrum of 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid ($C_{11}H_9FN_2O_2S$), NMR solvent: acetone-d_6

Table 5.22. Assignments of ^{13}C NMR peaks of 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid to specific carbons, solvent: acetone-d_6

Number of Carbons	**δ (ppm)**
C-2	182.49 & 182.26
C-4 or C-6	169.95 & 169.69
C-4 or C-6	168.69 & 168.61
C-5	45.986 & 45.650
C-7 (CH_3 group at C-5)	12.303 & 11.342
Aromatic C's	132.381 - 116.173

Not all peaks of diastereomers of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid could be observed in Figure 5.18. From that result, it can be said that carbon peaks of diastereomers were not separated well in ^{13}C NMR in the specified solvent. The assignments of the peaks were shown in Table 5.23.

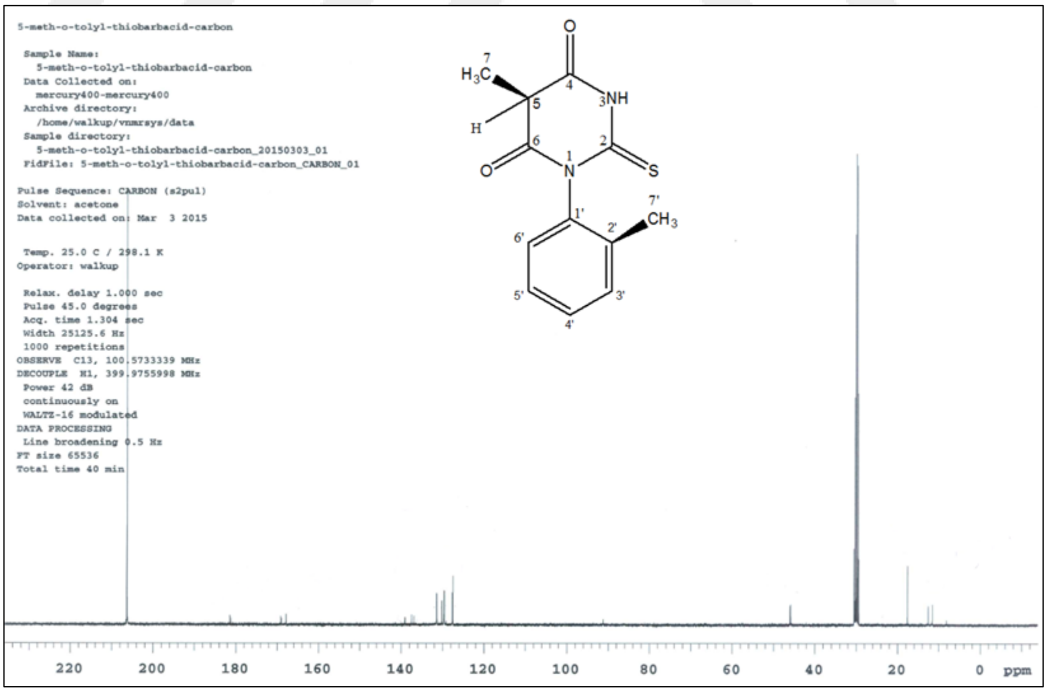


Figure 5.18. ^{13}C NMR spectrum of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid ($C_{12}H_{12}N_2O_2S$), NMR solvent: acetone-d_6

Table 5.23. Assignments of ^{13}C NMR peaks of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid to specific carbons, solvent: acetone-d_6

Number of Carbons	δ (ppm)
C-2	182.45 & 182.21
C-4 or C-6	170.13 or 168.84 & 168.79
C-7'	17.68
C-5	46.29 & 46.06
C-7 (CH_3 group at C-5)	12.303 & 11.342
Aromatic C's	139.94 - 128.242 (12 C atoms)

In the ^{13}C NMR spectrum of 5-methyl-1-(phenyl)-2-thiobarbituric acid, one peak for each carbon atom was observed as expected due to the absence of diastereomeric isomers (Figure 5.19). The assignments of the peaks were shown in Table 5.24.

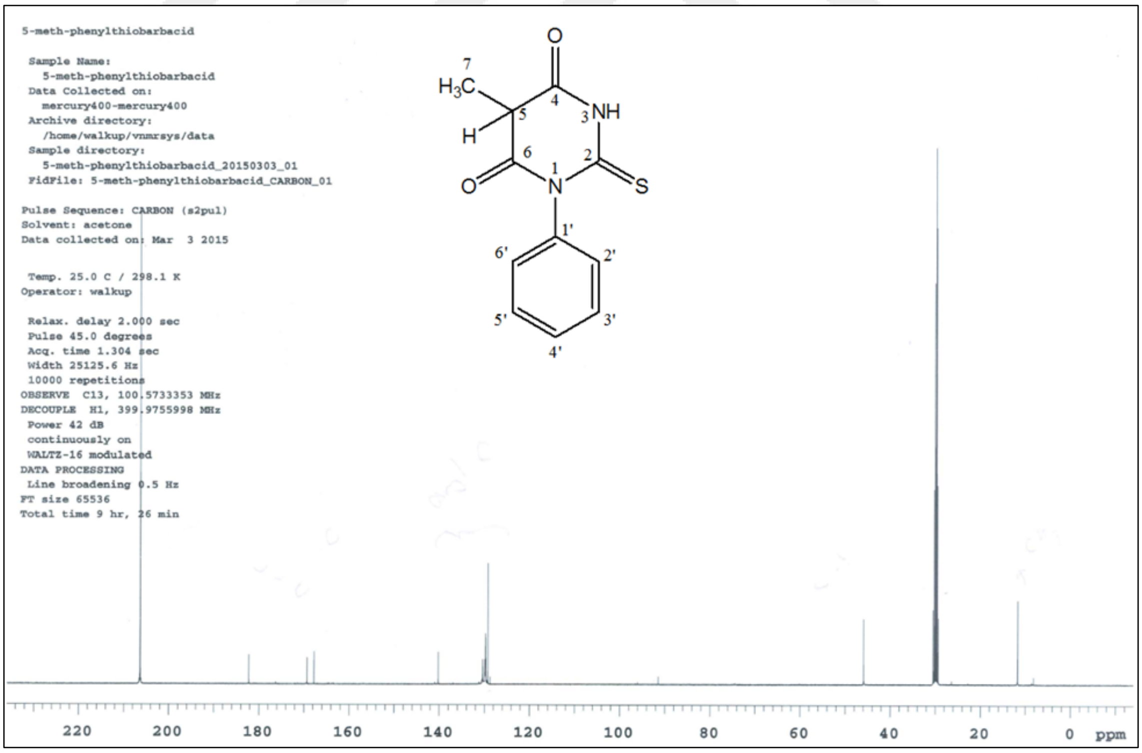


Figure 5.19. ^{13}C NMR Spectrum of 5-methyl-1-(phenyl)-2-thiobarbituric acid ($C_{11}H_{10}N_2O_2S$), NMR solvent: acetone-d_6

Table 5.24. Assignments of ^{13}C NMR peaks of 5-methyl-1-(phenyl)-2-thiobarbituric acid to specific carbons, solvent: acetone-d_6

Number of Carbons	**δ (ppm)**
C-2	182.199
C-4 or C-6	169.314
C-4 or C-6	167.805
C-5	45.935
C-7 (CH_3 group at C-5)	11.749
Aromatic C’s	140.282 - 129.220

5.6. REVERSED PHASE HPLC ANALYSES of THIOBARBITURIC ACID DERIVATIVES

Reversed Phase HPLC analysis data of all the synthesized thiobarbituric acid derivatives were listed in Tables 5.25 and 5.26. The chromatograms of the compounds can be seen in Appendix. All the chromatograms contain only one sharp peak, which is a sign of the purity of the synthesized compounds.

Table 5.25. HPLC data of 5-methyl -1-(*o*-aryl)-2-thiobarbituric acid derivatives and 5-methyl-1-phenyl-2-thiobarbituric acid

Name of the compound	**Column type**	**Mobile phase composition**	**Flow rate (mL/minute)**	**Retention time (minutes)**
5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid	X-Bridge Column	methanol:water = 50:50	0.6	2.414
5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid	X-Bridge Column	methanol:water = 50:50	0.6	2.229
5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid	X-Bridge Column	methanol:water = 50:50	0.6	2.468
5-methyl-1-phenyl-2-thiobarbituric acid	X-Bridge Column	methanol:water = 50:50	0.5	3.222

Table 5.26. Reversed phase HPLC data of 5,5-dimethyl-1-(*o*-aryl)-2-thiobarbituric acid derivatives

Name of the compound	**Column Type**	**Mobile phase composition**	**Flow rate (mL/minute)**	**Retention time (minutes)**
5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid	X-Bridge Column	methanol:water = 50:50	0.6	2.536
5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid	X-Bridge Column	methanol:water = 50:50	0.6	3.521
5,5-dimethyl-1-(*o*-tolyl)-2-thiobarbituric acid	X-Bridge Column	methanol:water = 50:50	0.6	3.472
5,5-dimethyl-1-phenyl-2-thiobarbituric acid	X-Bridge Column	methanol:water = 50:50	0.6	3.550

5.7. IR RESULTS of THIOBARBITURIC ACID DERIVATIVES

Results of the IR analyses of the synthesized *ortho*-substituted thiobarbituric acids were shown in Figures 5.20– 5.24 and the assignments of the peaks were listed in Tables 5.27-5.31.

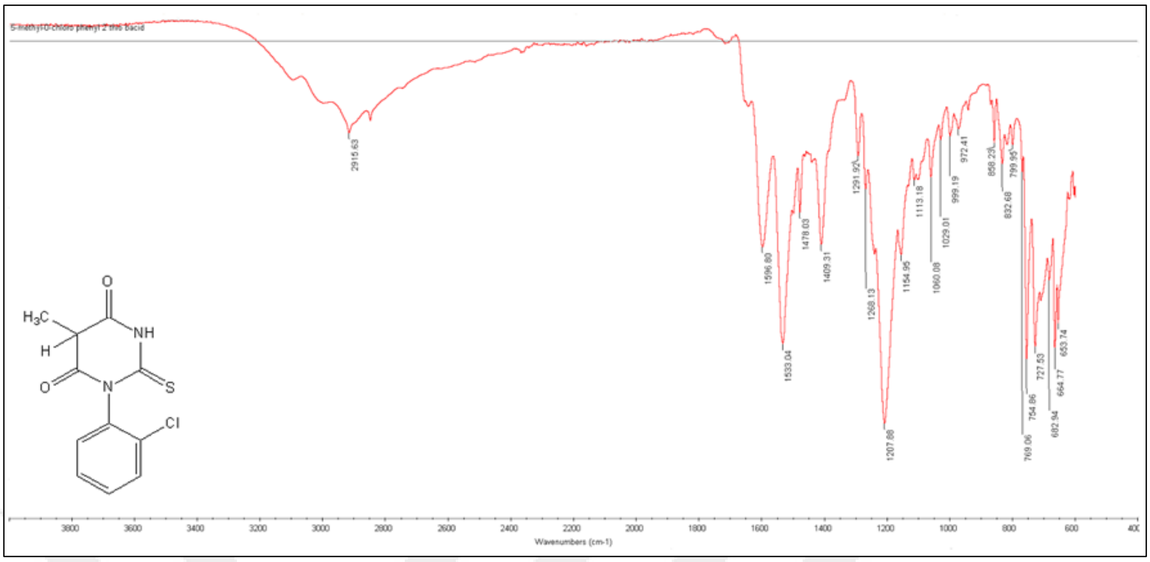


Figure 5.20. IR spectrum of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid ($C_{11}H_9CI\ N_2O_2S$)

Table 5.27. Assignments of IR absorption frequencies of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid ($C_{11}H_9CI\ N_2O_2S$)

Assignment	**Frequency (cm^{-1})**	**Relative intensity**
$\bar{\nu}$ of N-H stretching	3100	w
$\bar{\nu}$ of aromatic C-H stretching	2915.63, 3000	w
$\bar{\nu}$ of C=S stretching	1207.88	m
$\bar{\nu}$ of C-N stretching	1268.13, 1291.92	m
$\bar{\nu}$ C=O stretching	1650, 1675	w
$\bar{\nu}$ Aromatic C=C stretching	1596.80, 1478.03	m
$\bar{\nu}$ alkyl C-CI stretching	754.86	m

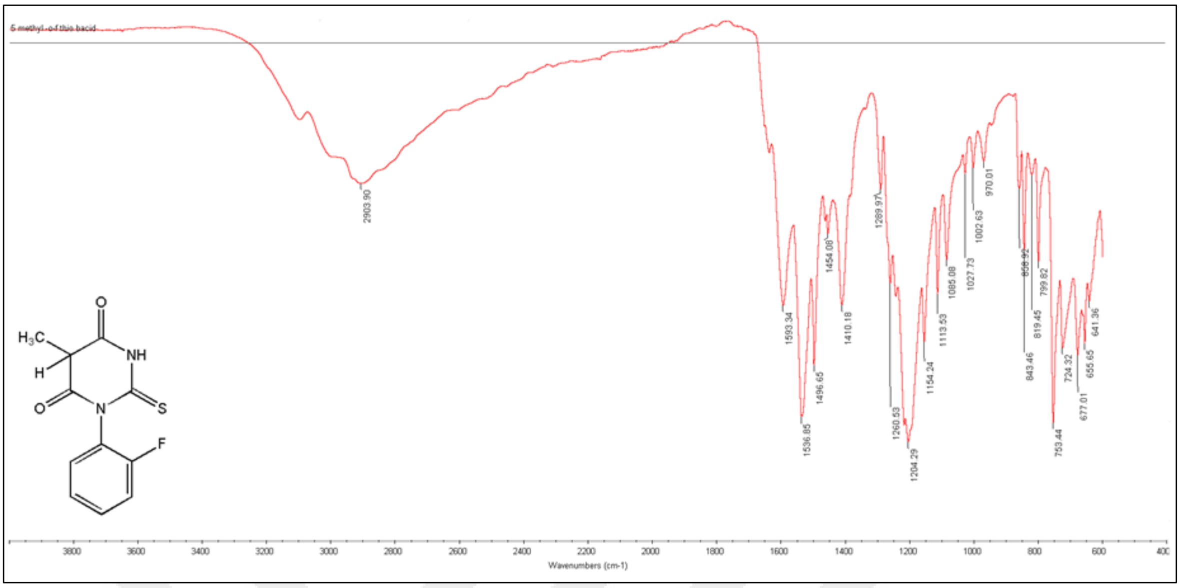


Figure 5.21. IR spectrum of 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid ($C_{11}H_9FN_2O_2S$)

Table 5.28. Assignments of IR absorption frequencies of 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid ($C_{11}H_9CI\ N_2O_2S$)

Assignment	**Frequency (cm^{-1})**	**Relative intensity**
$\bar{\nu}$ of N-H stretching	3100	w
$\bar{\nu}$ of aromatic C-H stretching	2850, 3000	w
$\bar{\nu}$ of C=S stretching	1204.29	m
$\bar{\nu}$ of C-N stretching	1260.53, 1289.97	m
$\bar{\nu}$ C=O stretching	1650, 1700	w
$\bar{\nu}$ Aromatic C=C stretching	1593.34, 1536.85	m
$\bar{\nu}$ alkyl C=F stretching	1002.63	m

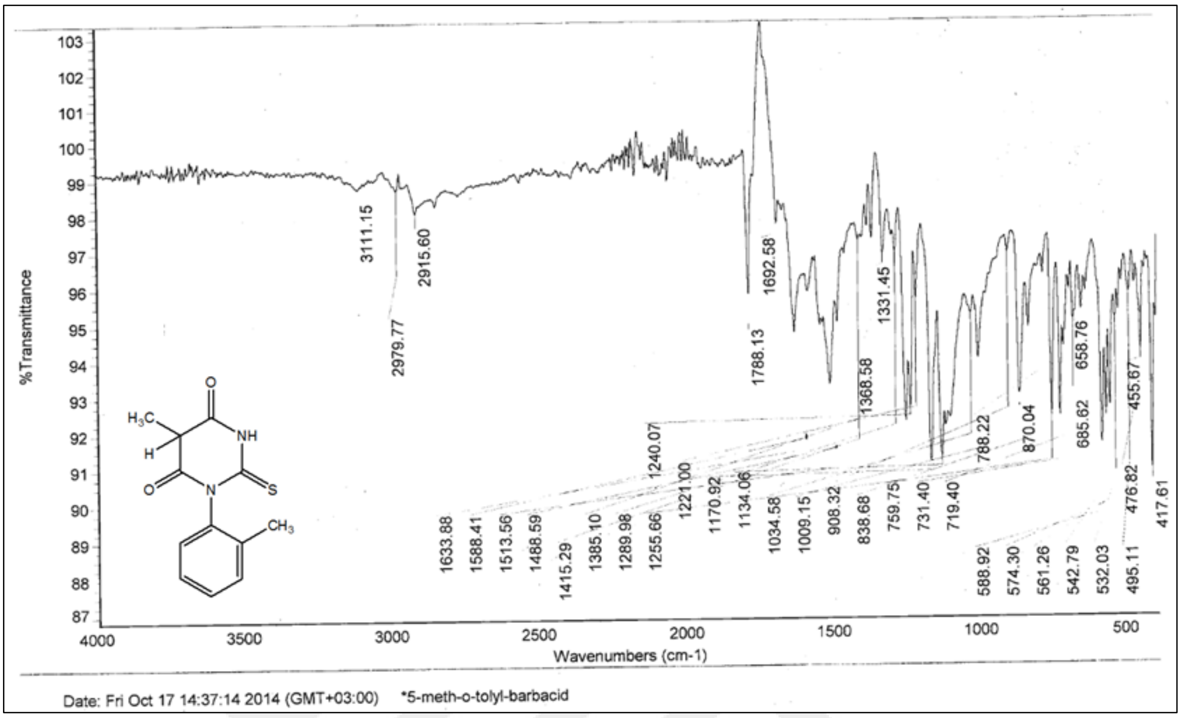


Figure 5.22. IR spectrum of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid ($C_{12}H_{12}N_2O_2S$)

Table 5.29. Assignments of IR absorption frequencies of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid ($C_{12}H_{12}N_2O_2S$)

Assignment	**Frequency (cm^{-1})**	**Relative intensity**
$\bar{\nu}$ of N-H stretching	3111.15	w
$\bar{\nu}$ of aromatic C-H stretching	2915.60	w
$\bar{\nu}$ of C = S stretching	1170.90	m
$\bar{\nu}$ of C-N stretching	1289.98	m
$\bar{\nu}$ C=O stretching	1788.13, 1692.58	m
$\bar{\nu}$ Aromatic C=C stretching	1588.41, 1513.56	m
$\bar{\nu}$ *ortho* disubstituted benzene C-H	759.75	m

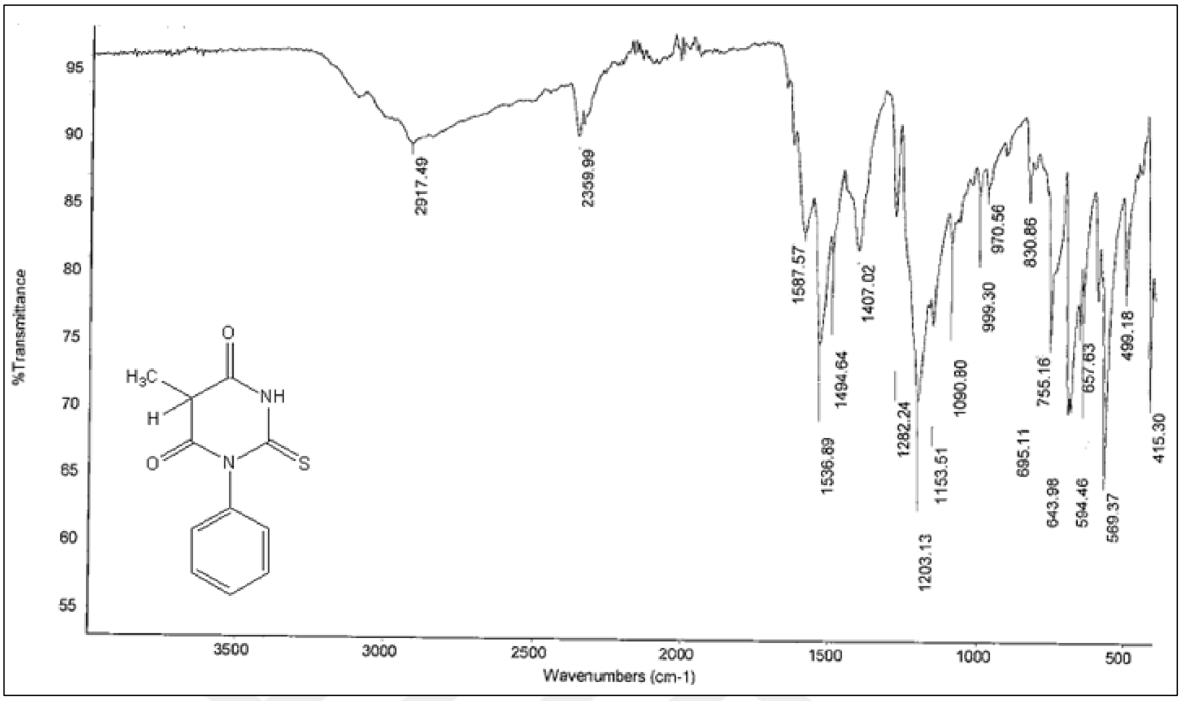


Figure 5.23. IR spectrum of 5-methyl-1-phenyl-2-thiobarbituric acid ($C_{11}H_{10}N_2O_2S$)

Table 5.30. Assignments of IR absorption frequencies of 5-methyl-1-phenyl-2-thiobarbituric acid ($C_{11}H_{10}N_2O_2S$)

Assignment	**Frequency (cm^{-1})**	**Relative intensity**
$\bar{\nu}$ of N-H stretching	3100	w
$\bar{\nu}$ of aromatic C-H stretching	3000	w
$\bar{\nu}$ of C = S stretching	1203.13	m
$\bar{\nu}$ of C-N stretching	1282.90	m
$\bar{\nu}$ C=O stretching	1600.01, 1600.03	w
$\bar{\nu}$ Aromatic C=C stretching	1587.57, 1494.64	m

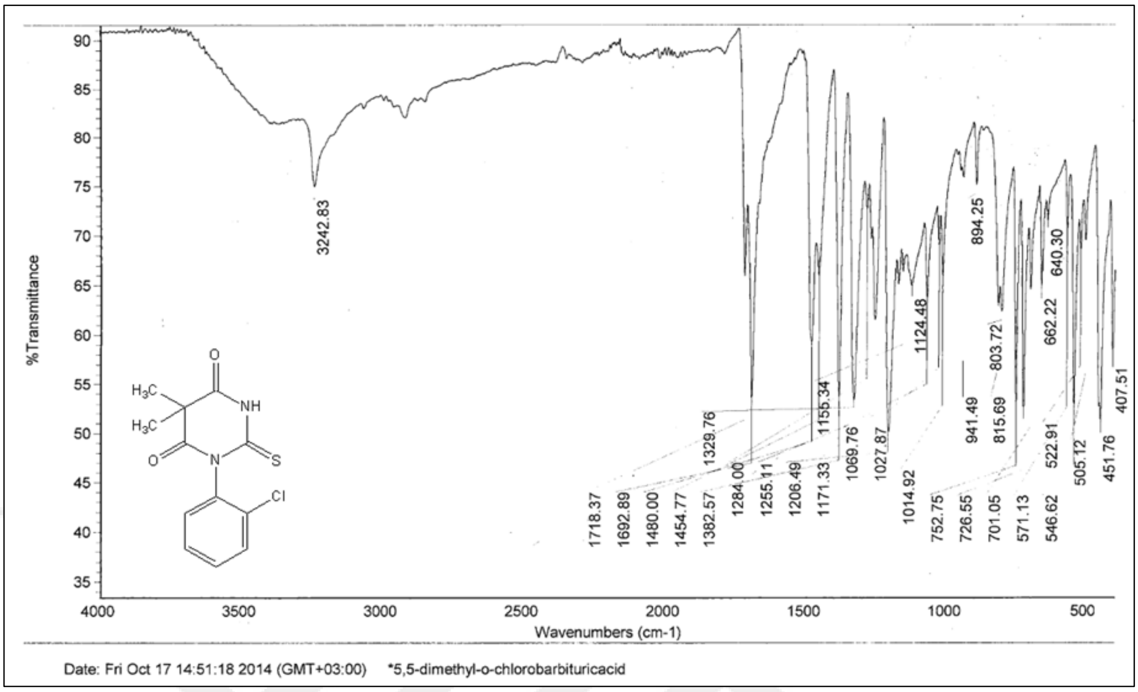


Figure 5.24. IR spectrum of 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid ($C_{12}H_{11}ClN_2O_2S$)

Table 5.31. Assignments of IR absorption frequencies of 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid ($C_{12}H_{11}ClN_2O_2S$)

Assignment	**Frequency (cm^{-1})**	**Relative intensity**
$\bar{\nu}$ of N-H Stretching	3242.83	w
$\bar{\nu}$ of aromatic C-H stretching	3071.4	w
$\bar{\nu}$ of C=S stretching	1206.49	m
$\bar{\nu}$ of C-N stretching	1329.76	m
$\bar{\nu}$ C=O stretching	1692.89, 1718.37	m
$\bar{\nu}$ Aromatic C=C stretching	1480.00	m
$\bar{\nu}$ alkyl C-CI stretching	752.75	m

5.8. ALKYLATION REACTIONS

Before performing the synthesis of the alkylation reactions, ^{1}H NMR analyses of the reactant thiobarbituric acid derivatives were done and the ratio of the diastereomers of the thiobarbituric acid derivatives was determined. In all spectra, the diastereomeric ratio was found as 1:1 regarding the integral ratios of two quartets of the proton at C-5 in all thiobarbiturates.

In the alkylation reactions, (+)-cinchonine or LDA were used for abstracting the acidic proton. (+)-Cinchonine was also used as chiral catalyst for enantioselective synthesis. Alkylating agents were benzyl bromide or allyl bromide.

In the ^{1}H NMR analyses of benzylation products (Figure 5.25), an AB spectrum was expected due to diastereotopicity of CH_2 protons of the benzyl group connected to the C-5 of the heterocycle. In most spectra an AB spectrum was observed, but in some of them only one singlet peak was seen instead of an AB spectrum. Observation of a singlet or an AB spectrum may depend on the type of the NMR solvent used.

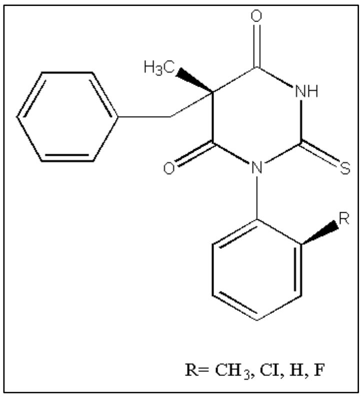


Figure 5.25. Benzylation products

Additionally, the methyl group at C-5 gives a singlet peak. Besides, a singlet peak was expected for one hydrogen atom of NH group (Figures 5.27, 5.28, 5.35 and 5.37).
NH protons of the products of benzylation reactions with (+)-cinchonine and LDA that were seen in the ^{1}H NMR spectra of crude products were not observed in some ^{1}H NMR

spectra of the purified products (Figures 5.26, 5.29, 5.30 and 5.36) NH protons may exchange with deuterium atom in the deuterated solvent.

Although two quartets for the proton at C-5 were observed in the ^{1}H NMR spectra of all synthesized 5-methyl derivatives of thiobarbituric acid derivatives (Figures 5.9 - 5.11), only one AB spectrum for 5-CH_2 protons was observed in their alkylation products (Figures 5.27, 5.28, 5.29, 5.36 and 5.37). The reason could be that the alkylation reactions are diastereoselective because of steric conditions during the process, so one diastereomeric pair dominates. Another reason could be that one of the diastereomeric pair precipitates selectively during the crystallization process or NMR solvent does not differentiate between the diastereomers. The reason was tried to be clarified with HPLC analyses.

Only one quartet spectrum was observed in the ^{1}H NMR spectra of 5-methyl-1-phenyl-2-thiobarbituric acids as mentioned before in Section 5.4. This derivative contains only enantiomeric isomers.

Benzylation reactions of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid, 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid and 5-methyl-1-(phenyl)-2-thiobarbituric acid were performed. ^{1}H NMR analyses of the benyzlation products were discussed in Sections 5.8.1 and 5.8.3. ^{13}C NMR analyses of the benzylation products were discussed in Section 5.8.2. Additionally, normal phase HPLC analyses of benzylation reactions were interpreted in Section 5.9.

5.8.1. ^{1}H NMR Results of The Products of Benzylation Reactions with (+)-Cinchonine

In the ^{1}H NMR spectrum of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid (Figure 5.26.), an AB spectrum was not observed when chloroform-d was used as NMR solvent, instead of that a singlet peak was observed as seen in Figure 5.26. However, one AB spectrum appeared when dimethyl sulfoxide-d_6 was used as NMR solvent as seen in Figure 5.27. Besides, a proton of NH group was seen in Figure 5.27, although it was not seen in Figure 5.26. It was concluded that the appearance of the AB peak and NH peak is solvent dependent.

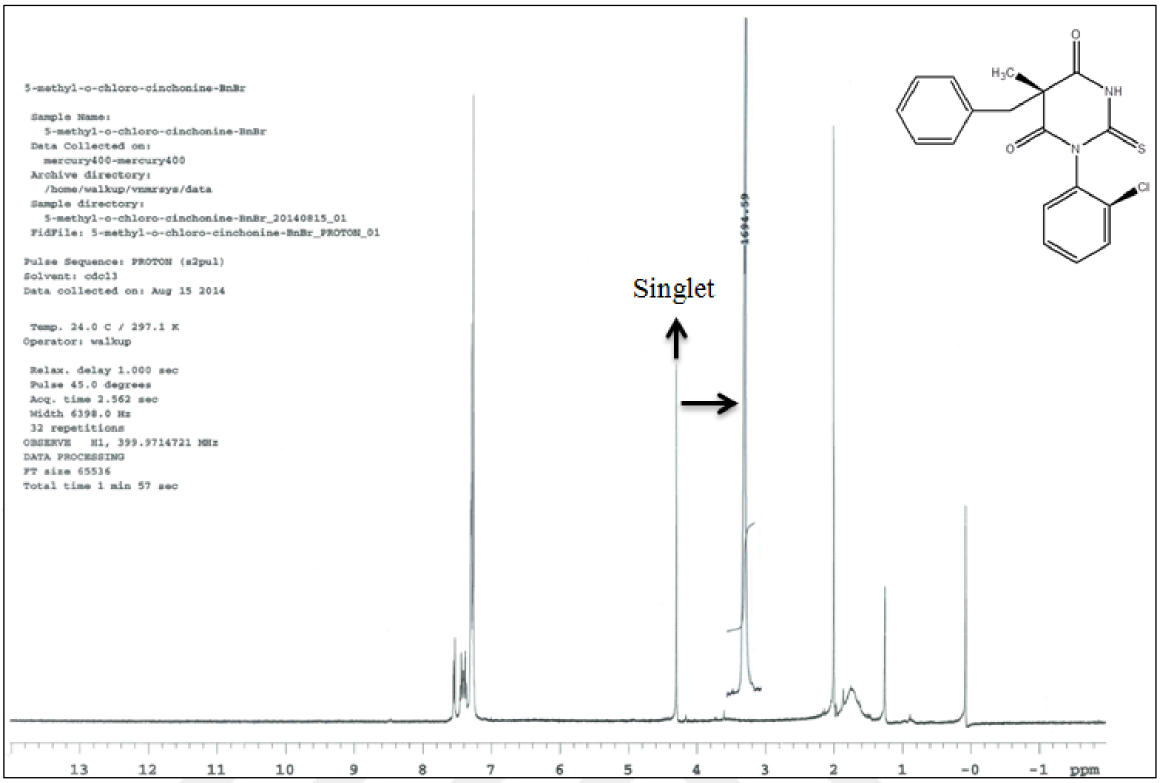


Figure 5.26. ^{1}H NMR spectrum of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid ($C_{18}H_{15}ClN_2O_2S$), NMR solvent: chloroform-d

In Tables 5.32 and 5.33, the peaks in the ^{1}H NMR spectra of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid in different solvents were expressed with the chemical shift values, multiplicities and integral values.

Table 5.32. ^{1}H NMR (400 MHz) data of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid), solvent: chloroform-d

Multiplicity, Number of Protons	δ (ppm)
(m, 9 H)	7.55-7.27
(s, 2 H)	4.30
(s, 3 H)	2.00

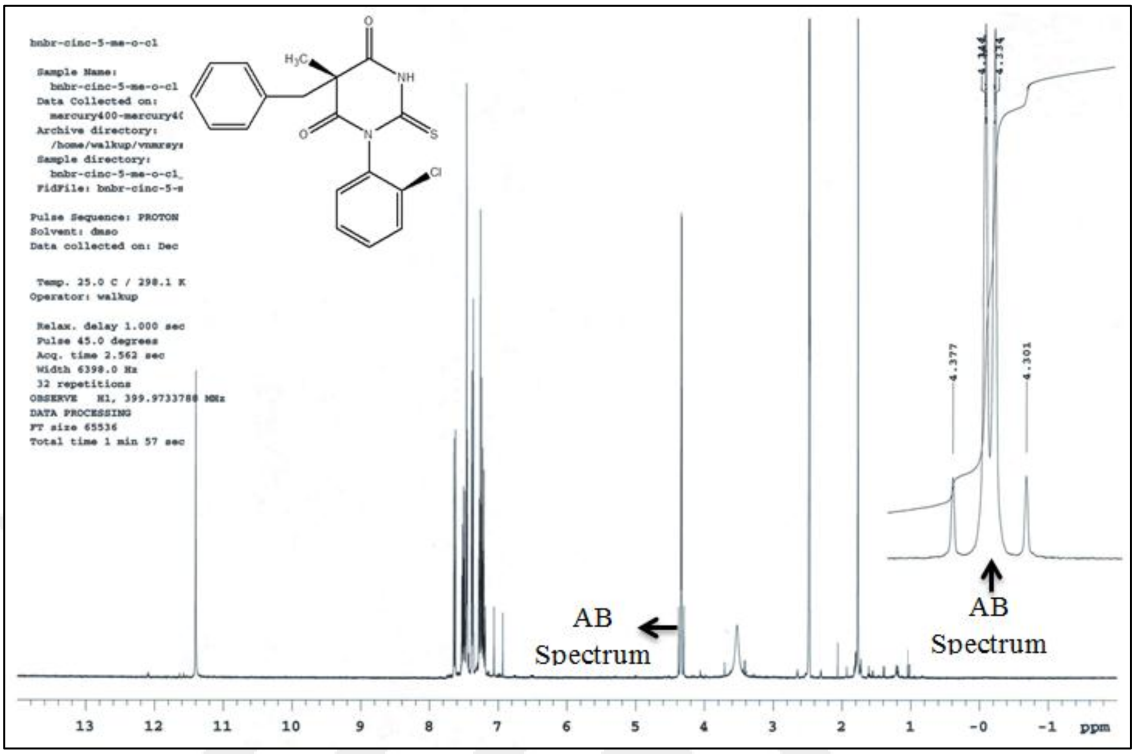


Figure 5.27. ¹H NMR spectrum of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid ($C_{18}H_{15}ClN_2O_2S$), NMR solvent: dimethyl sulfoxide-d_6

Table 5.33. ¹H NMR (400 MHz) data of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, solvent: dimethyl sulfoxide-d_6

Multiplicity, Number of Protons	δ (ppm)
(s, 1 H)	11.40
(m, 9 H)	7.649-6.934
(AB Spectrum, J=17.2 Hz, J=13.2 Hz, 2 H)	4.377-4.301
(s, 3 H)	1.774

In the ¹H NMR spectra of 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid and 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid, one AB peak of benzylic protons could be seen when chloroform-d was used as NMR solvent (Figure 5.28 and 5.29). Lists of the peaks with their chemical shift values, multiplicities and integral values can be seen in Tables 5.34 and 5.35.

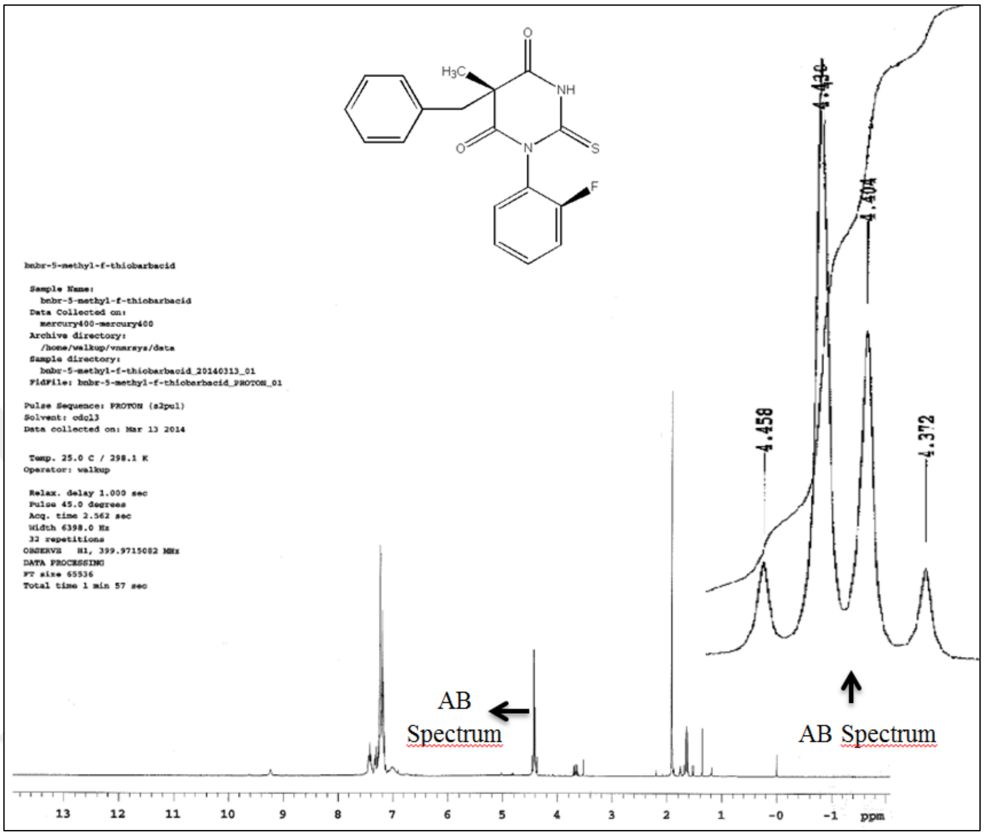


Figure 5.28. ^{1}H NMR spectrum of 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid ($C_{18}H_{15}FN_2O_2S$), NMR solvent: chloroform-d

Table 5.34. ^{1}H NMR (400 MHz) data of 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid, solvent: chloroform-d

Multiplicity, Number of Protons	**δ (ppm)**
(s, 1 H)	9.232
(m, 9 H)	7.488-6.907
(AB Spectrum, J=22.4 Hz, J=11.2 Hz, 2 H)	4.458-4.372
(s, 3 H)	1.916

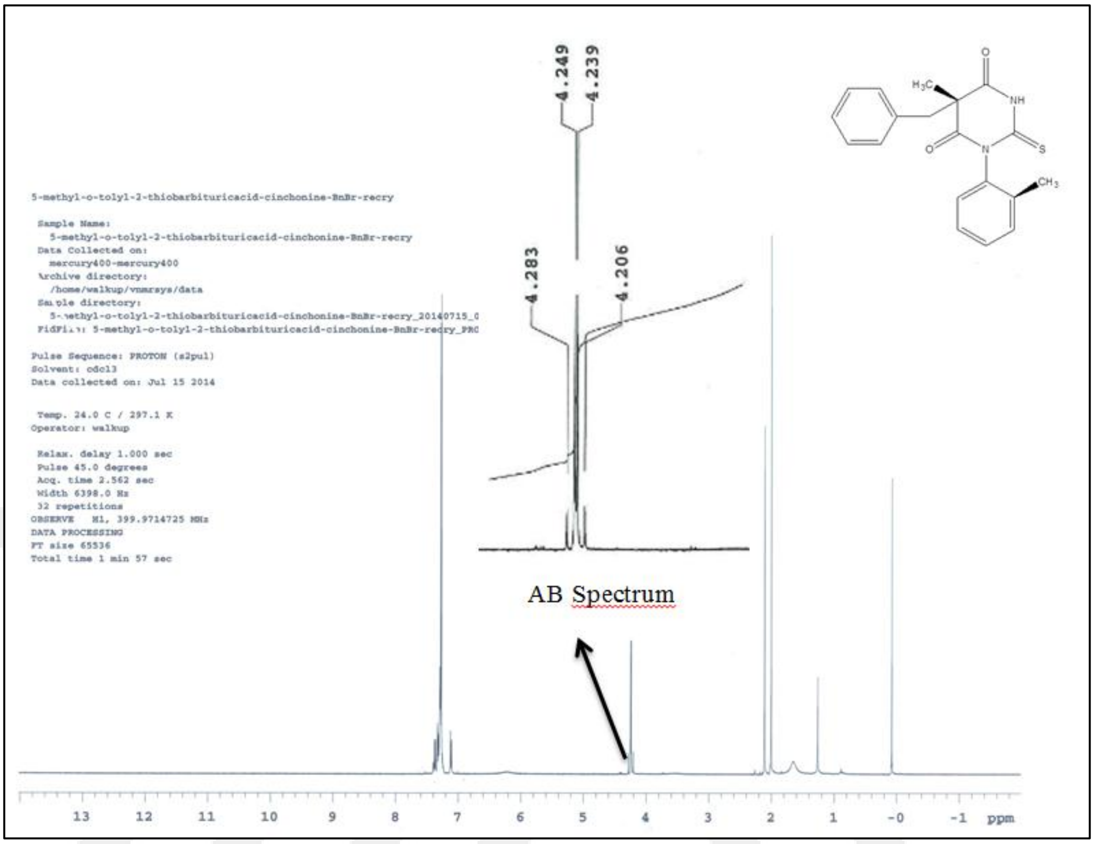


Figure 5.29. [1]H NMR spectrum of 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid ($C_{19}H_{18}N_2O_2S$), NMR solvent: chloroform-d

Table 5.35. [1]H NMR (400 MHz) data of 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid, solvent: chloroform-d

Multiplicity, Number of Protons	**δ (ppm)**
(m, 9 H)	7.395-7.104
(AB Spectrum, J=17.4 Hz, J=13.2, 2 H)	4.283-4.026
(s, 3 H)	2.111
(s, 3 H)	2.004

In the [1]H NMR spectra of 5-benzyl-5-methyl-1-phenyl-2-thiobarbituric acid, AB peak for the benzylic protons could not observed in chloroform-d (Figure 5.30), acetone- d_6 and dimethyl sulfoxide-d_6 (Appendix E) were used as NMR solvent, although the benzylic protons are diastereotopic. The *ortho*-substituent on the aromatic ring in the other derivatives generates AB spectrum in the mentioned solvents.

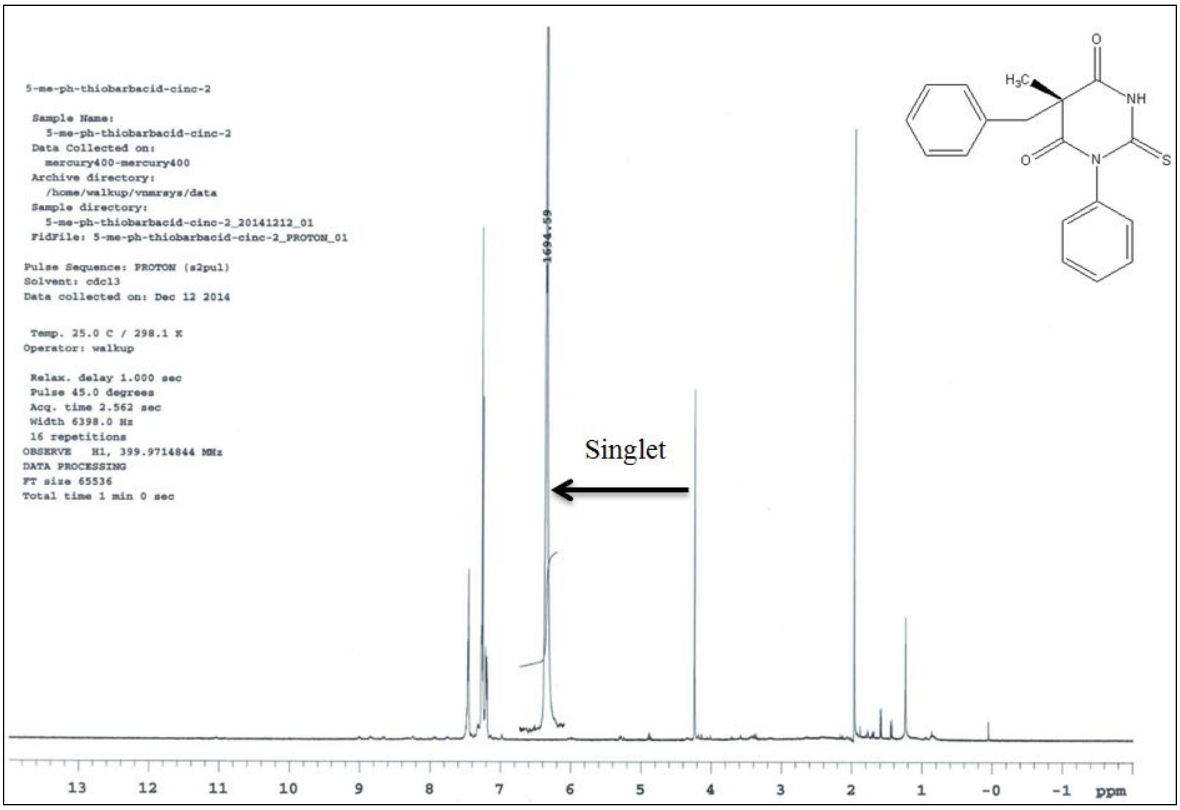


Figure 5.30. [1]H NMR spectrum of 5-benzyl-5-methyl-1-phenyl-2-thiobarbituric acid ($C_{18}H_{16}N_2O_2S$), NMR solvent: chloroform-d

The peaks in [1]H NMR spectrum of 5-benzyl-5-methyl-1-phenyl-2-thiobarbituric acid were listed with their chemical shift values and integral values in Table 5.36.

Table 5.36. ^{1}H NMR (400 MHz) data of 5-benzyl-5-methyl-1-phenyl-2-thiobarbituric acid (solvent: $CDCl_3$)

Multiplicity, Number of Protons	**δ (ppm)**
(m, 10 H)	7.49-7.19
(s, 2 H)	4.24
(s, 3 H)	1.96

5.8.2. ^{13}C NMR Results of The Products of Benzylation Reactions with (+)-Cinchonine

The benzylation products of 5-methyl-1-(*o*-aryl)-2-thiobarbituric acids were synthesized for the first time. All of them were characterized by C-13 NMR spectroscopy besides ^{1}H NMR spectroscopy.

In most of the ^{13}C NMR spectra, only one peak for each carbon was observed, that means that only one enantiomeric pair (one diastereomer) formed supporting the result of ^{1}H NMR.

^{13}C NMR spectrum of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid was seen in Figure 5.31. The assignments of the peaks were listed in Table 5.37.

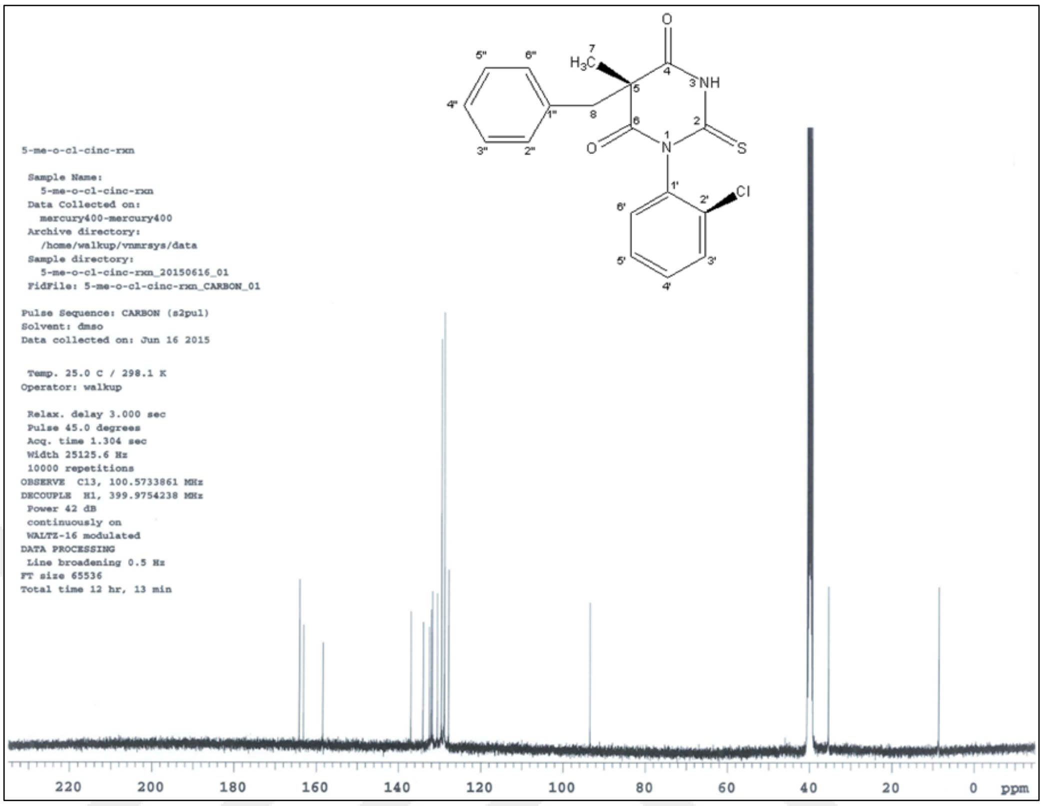


Figure 5.31. ^{13}C NMR Spectrum of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid ($C_{18}H_{15}ClN_2O_2S$), NMR solvent: dimethyl sulfoxide-d_6

Table 5.37. Assignment of ^{13}C NMR peaks of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid to specific carbons, solvent: dimethyl sulfoxide-d_6

Multiplicity, Number of Protons	δ (ppm)
C-2	164.146
C-4 or C-6	163.139
C-4 or C-6	158.412
C-5	93.494
C-7 (CH_3 group at C-5)	8.608
C-8 ($-CH_2-C_6H_5$: Benzyl group)	35.452
Aromatic C's	137.058 – 127.848

^{13}C NMR spectrum of 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid was seen in Figure 5.32. The assignments of the peaks were listed in Table 5.38. Some carbon atoms give two peaks. The reason might be the existence of two diastereomers. Peaks of two diastereomers were only observed in the ^{13}C NMR spectrum of 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid among all benzylation products (Figure 5.32).

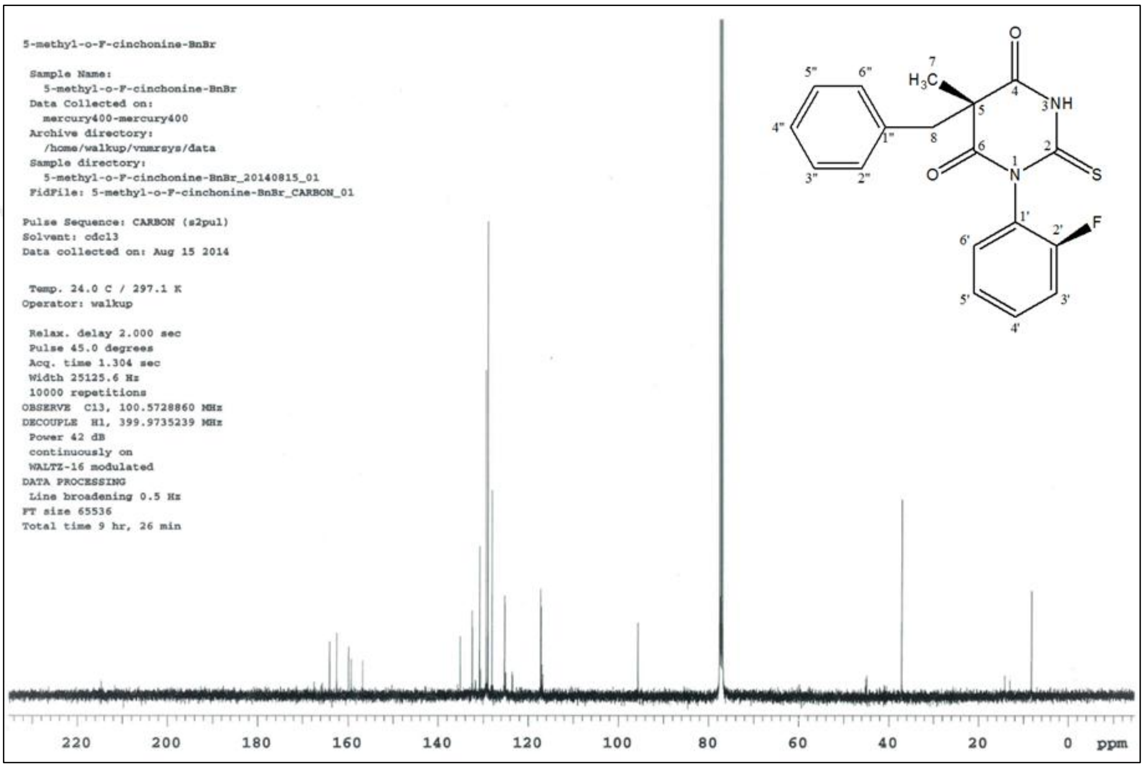


Figure 5.32**.** ^{13}C NMR Spectrum of 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid ($C_{18}H_{15}FN_2O_2S$), NMR solvent: chloroform-d

Table 5.38. Assignment of ^{13}C NMR peaks of 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid to specific carbons, solvent: chloroform-d

Multiplicity, Number of Protons	**δ (ppm)**
C-2	164.056 and 162.516
C-4 or C-6	159.855 or 159.291
C-4 or C-6	156.775
C-5	95.661
C-7 (CH_3 group at C-5)	8.228
C-8 ($-CH_2-C_6H_5$: Benzyl group)	37.27
Aromatic C's	135.107 – 117.122

^{13}C NMR spectrum of 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid is seen in Figure 5.33. The assignments of the peaks are listed in Table 5.39.

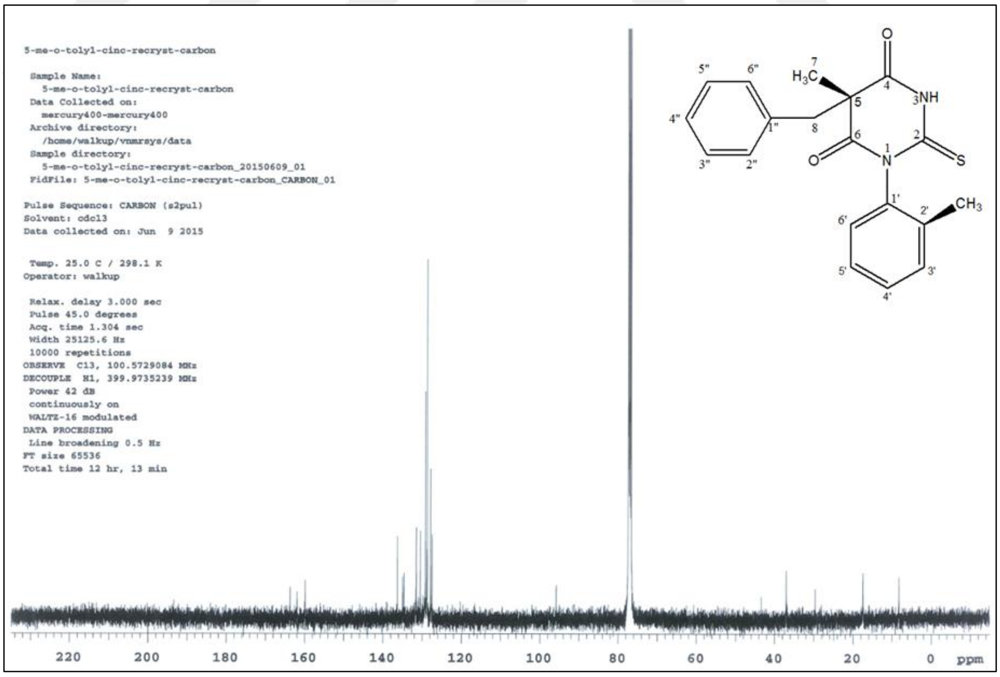


Figure 5.33. ^{13}C NMR Spectrum of 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid ($C_{19}H_{18}N_2O_2S$), NMR solvent : chloroform-d

Table 5.39. Assignment of ^{13}C NMR peaks of 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid to specific carbons, solvent: chloroform-d

Multiplicity, Number of Protons	**δ (ppm)**
C-2	163.711
C-4 or C-6	161.981 or 159.892
C-5	95.781
C-7 (CH_3 group at C-5)	8.265
C-8 (-CH_2-C_6H_5 : Benzyl group)	37.045
2' (*ortho* position, CH_3)	17.505
Aromatic C's	136.318 – 127.535

^{13}C NMR spectrum of 5-benzyl-5-methyl-1-phenyl-2-thiobarbituric acid is seen in Figure 5.34. The assignments of the peaks are listed in Table 5.40.

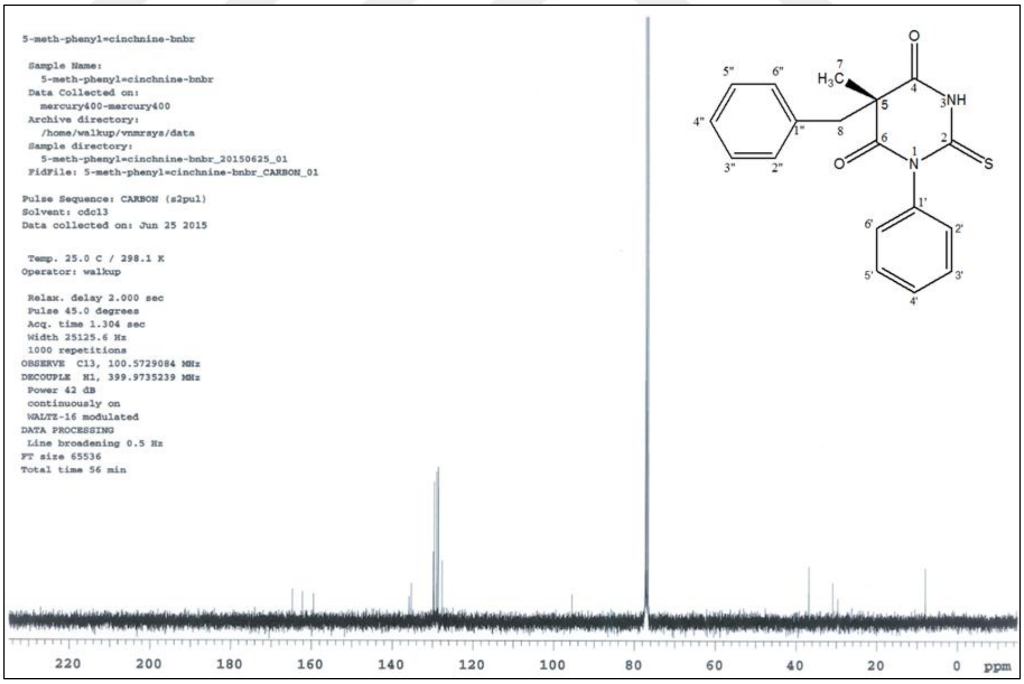


Figure 5.34. ^{13}C NMR Spectrum of 5-benzyl-5-methyl-1-phenyl-2-thiobarbituric acid ($C_{18}H_{16}N_2O_2S$), NMR solvent: chloroform-d

Table 5.40. Assignment of ^{13}C NMR peaks of 5-benzyl-5-methyl-1-phenyl-2-thiobarbituric acid, solvent: chloroform-d

Multiplicity, Number of Protons	**δ (ppm)**
C-2	164.718
C-4 or C-6	162.293 or 159.495
C-5	95.476
C-7 (CH_3 group at C-5)	8.013
C-8 ($-CH_2-C_6H_5$: Benzyl group)	36.878
Aromatic C's	135.838 – 127.718

5.8.3. ^{1}H NMR Results of The Products of Benzylation Reactions with LDA

The products of the benzylation reaction of the 5-methyl-1-(o-aryl)-2-thiobarbituric acids with LDA were analyzed by ^{1}H NMR to characterize the compounds and find the diastereomeric ratio if present.

Only one AB peak for benzylic protons in all products was obtained as in the benzylation reactions with (+)-cinchonine. This might be a proof for the formation of only one enantiomeric pair. The reaction is further analyzed for its diastereoselectivity by HPLC described in Section 5.9.

The ^{1}H NMR spectrum of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid was shown in Figure 5.35. In this spectrum one singlet peak was seen for benzylic protons instead of AB peak due to solvent effect. *Ortho*-chloro derivative gave singlet peak for benzylic protons when chloroform-d was used as NMR solvent, but an AB peak was observed when dimethyl sulfoxide (DMSO)-d_6 was used as NMR solvent as described in Section 5.8.1 (Figure 5.27). The peaks were listed with their multiplicity and chemical shift values in Table 5.41.

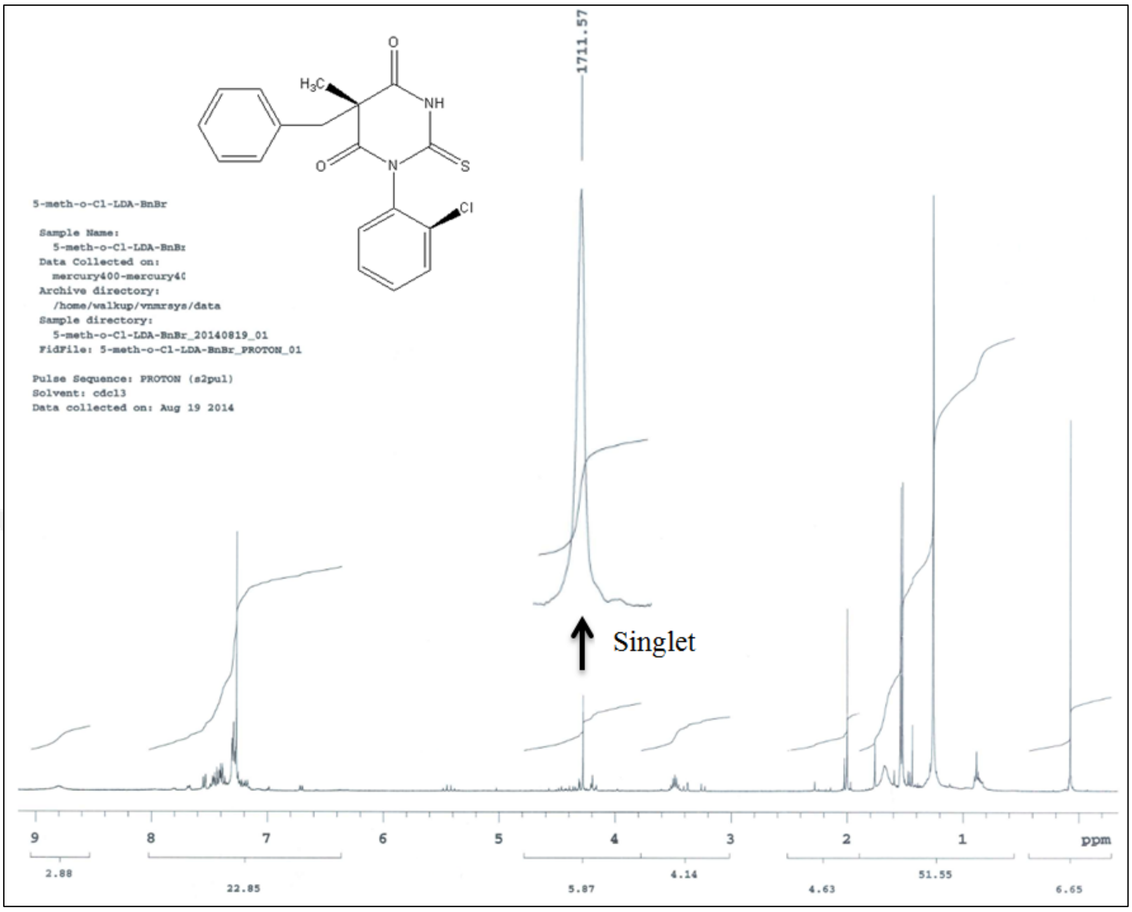


Figure 5.35. [1]H NMR spectrum of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid ($C_{18}H_{15}ClN_2O_2S$), NMR solvent: chloroform-d

Table 5.41. [1]H NMR (400 MHz) data of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, solvent: chloroform-d

Multiplicity, Number of Protons	**δ (ppm)**
(s, 1 H)	8.80
(m, 9 H)	7.81-7.15
(s, 2 H)	4.28
(s, 3 H)	2.00

The [1]H NMR spectrum of 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid was shown in Figure 5.36. In this spectrum, one AB peak was observed for benzylic protons. The peaks were listed with their multiplicity and chemical shift values in Table 5.42.

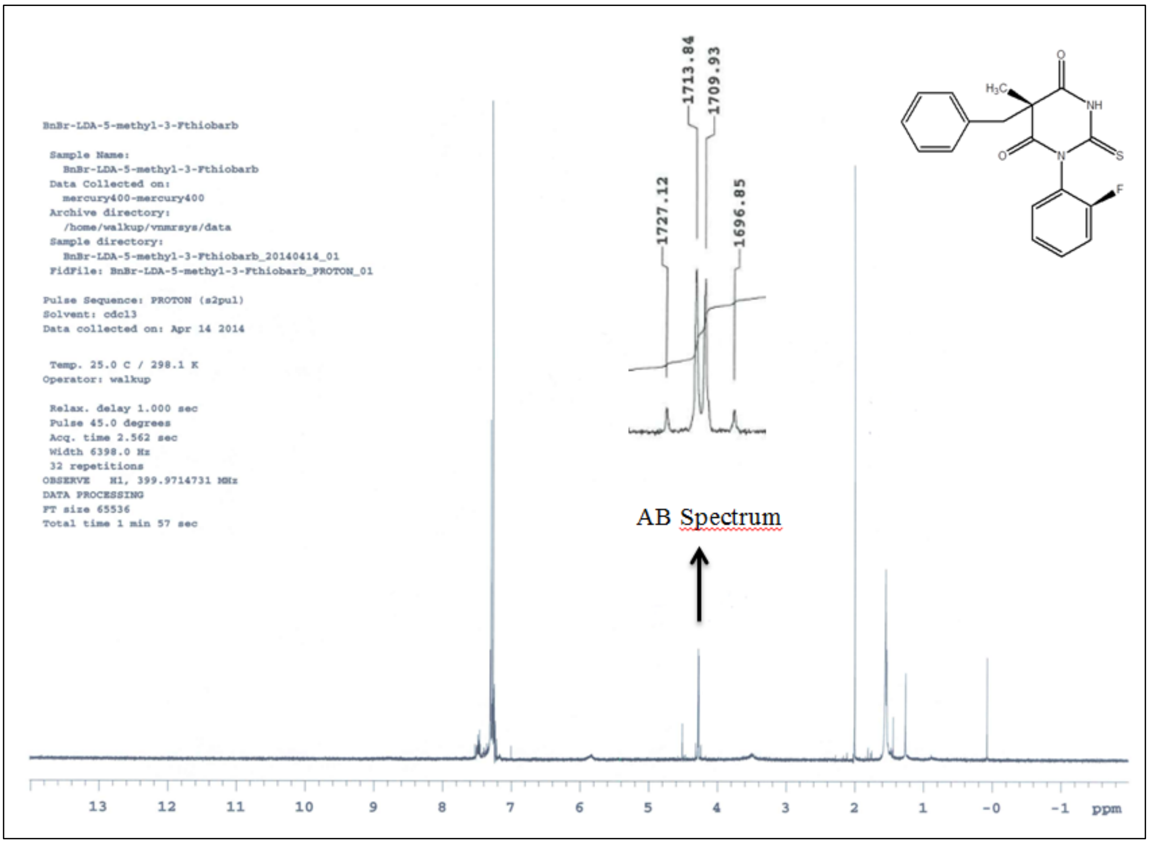


Figure 5.36. [1]H NMR spectrum of 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid ($C_{18}H_{15}FN_2O_2S$), NMR solvent: chloroform-d

Table 5.42. [1]H NMR (400 MHz) data of 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid, solvent: chloroform-d

Multiplicity, Number of Protons	**δ (ppm)**
(m, 9 H)	7.450-7.133
(AB Spectrum, J=17.09 Hz, J=13.08 Hz, 2 H)	4.24-4.17
(s, 3 H)	1.93

The [1]H NMR Spectrum of 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid was shown in Figure 5.37. In this spectrum, one AB peak was observed for benzylic protons. The peaks were listed with their multiplicity and chemical shift values in Table 5.43.

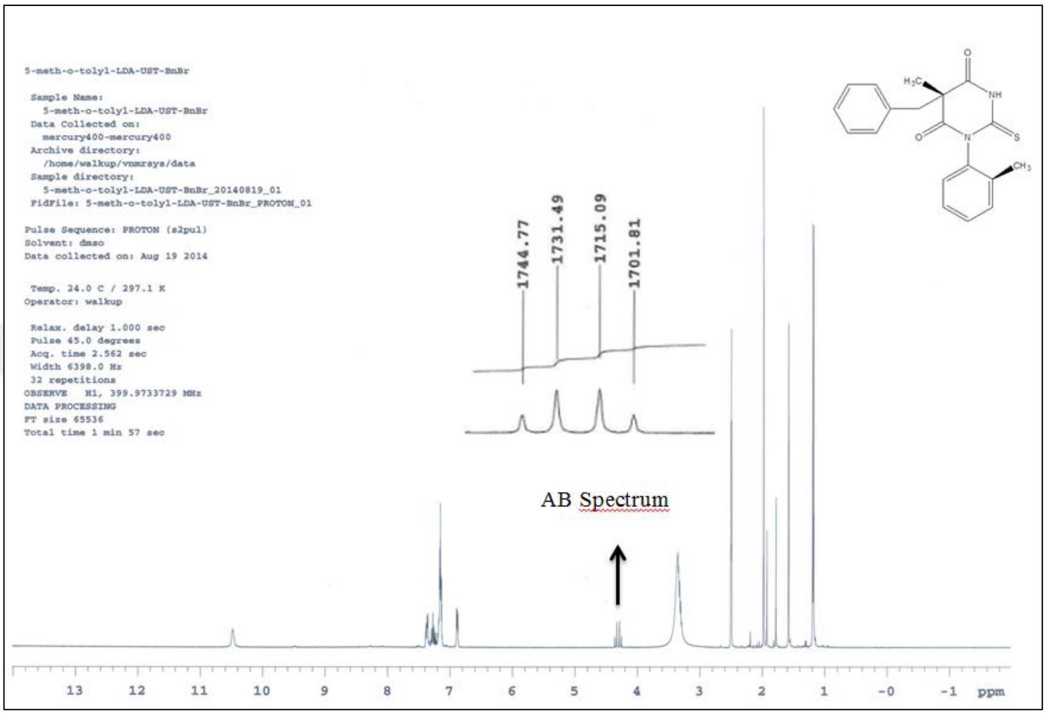


Figure 5.37. [1]H NMR of 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid ($C_{18}H_{15}FN_2O_2S$), NMR solvent: DMSO-d_6

Table 5.43. [1]H NMR (400 MHz) Result of 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid, solvent: DMSO-d_6

Multiplicity, Number of Protons	**δ (ppm)**
(s, 1 H)	10.481
(m, 9 H)	7.385-7.126
(AB Spectrum, J=13.28 Hz and J=29.68 Hz, 2 H)	4.36-4.25
(s, 3 H) at C-5	1.98
(s, 3 H), *ortho* position	1.58

5.8.4. Elemental Analysis of The Products of Benzylation Reactions with (+)-Cinchonine

For further characterization of the products of benzylation reactions, elemental analysis was also done. Theoretical values were compared with experimental results. The results were shown in Tables 5.44-5.47. The difference between theoretical and experimental values of the elemental analysis should be at least 0.4 or less than 0.4 to be in the acceptable range. But as seen from the tables, mainly % C and also some other % elements were not in the accepted range. Therefore, further purification of the products 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid, 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid and 5-benzyl-5-methyl-1-phenyl-2-thiobarbituric acid must be done. After that, elemental analyses of all the compounds must be repeated.

Table 5.44. Elemental analysis results of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid ($C_{18}H_{15}ClN_2O_2S$)

Elements (%)	Experimental	Theoretical	Difference
N	7.9769	7.8066	0.1703
C	56.0774	60.2474	4.17
H	3.8489	4.2131	0.3642
S	8.3794	8.9357	0.5563

Table 5.45. Elemental analysis results of 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid ($C_{19}H_{18}FN_2O_2S$)

Elements (%)	Experimental	Theoretical	Difference
N	8.7429	8.1818	0.5611
C	60.8804	63.1428	2.2624
H	4.3117	4.4156	0.1039
S	8.4891	9.3651	0.876

Table 5.46. Elemental analysis results of 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid ($C_{18}H_{15}FN_2O_2S$)

Elements (%)	Experimental	Theoretical	Difference
N	8.7820	8.2776	0.5044
C	61.9075	67.4313	5.5238
H	4.6805	5.3608	0.6803
S	8.6446	9.4748	0.8302

Table 5.47. Elemental analysis results of 5-benzyl-5-methyl-1-phenyl-2-thiobarbituric acid ($C_{18}H_{16}N_2O_2S$)

Elements (%)	Experimental	Theoretical	Difference
N	9.4579	8.6356	0.8223
C	61.7407	66.6445	4.9038
H	4.6342	4.9712	0.337
S	2.8809	9.8845	7.0036

5.8.5. ^{1} H NMR Results of Products of Allylation Reactions

Allylation reactions of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid, 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid and 5-methyl-1-phenyl-2-thiobarbituric acid were performed. The ^{1}H NMR spectra of the products were shown in Figures 5.39, 5.42-5.44.

In the ^{1}H NMR spectra of the allylation products, one singlet peak was expected around 9 ppm due to NH proton of thiobarbituric acid derivatives. Besides an AB spectrum was expected between 1.6 ppm and 1.7 ppm due to diastereotopic protons of methylene group bonded to C-5. Also the peak of three protons of methyl groups (*ortho* position) was expected to appear at around 2 ppm. Multiplet peaks belonging to aromatic protons were expected to be between 7.0 ppm and 7.6 ppm.

Spectra of allylation products were compared with [1]H NMR spectrum of allylbromide (Figure 5.38) to be able to identify peaks belonging to excess reactant and peaks belonging to the product. Peak of "A" type allyl bromide proton was expected to appear at around 6 ppm. Peaks of B and C types allyl bromide protons were expected to be seen at around 5 ppm. Peak of D type of allylbromide proton was expected to be observed at around 4 ppm.

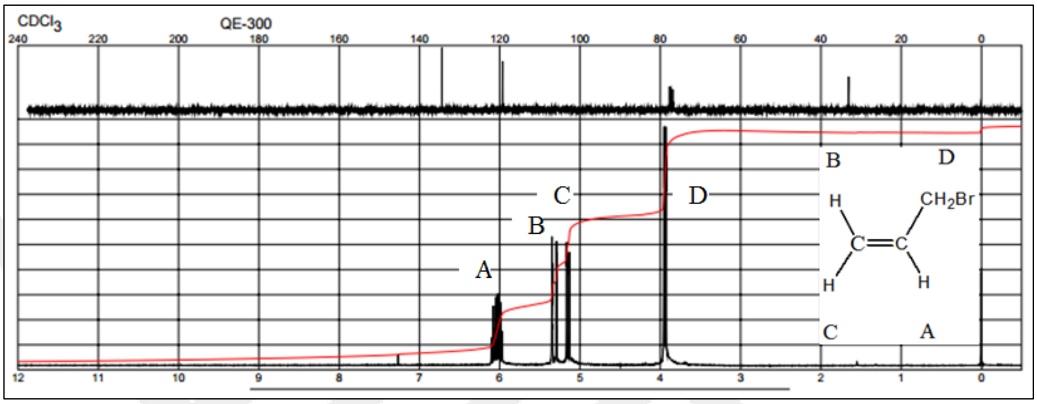


Figure 5.38. [1]H NMR spectrum of allylbromide [85]

In the [1]H NMR spectrum of 5-allyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid (Figure 5.39), two peaks, one small and one big, were observed at around 6 ppm. One belongs probably to the hydrogen bonded to the double bond in allylbromide (reactant, A type proton). Other peak belongs to the H (A' in Figure 5.39) on the double bond of the product. Multiplet peaks were seen between 6.97 and 7.47 ppm due to aromatic protons of the product and the reactants. The peaks of two hydrogens bonded to the same carbon atom of the double bond (B, C and B', C' protons) were observed between 5 and 5.4 ppm. AB spectrum of CH_2 protons (D' protons) was not identified because the peak patterns are very complicated at around 2 ppm. Also a quartet belongs to reactant was observed at around 4 ppm, but it was not identified clearly because of overlap of peaks.

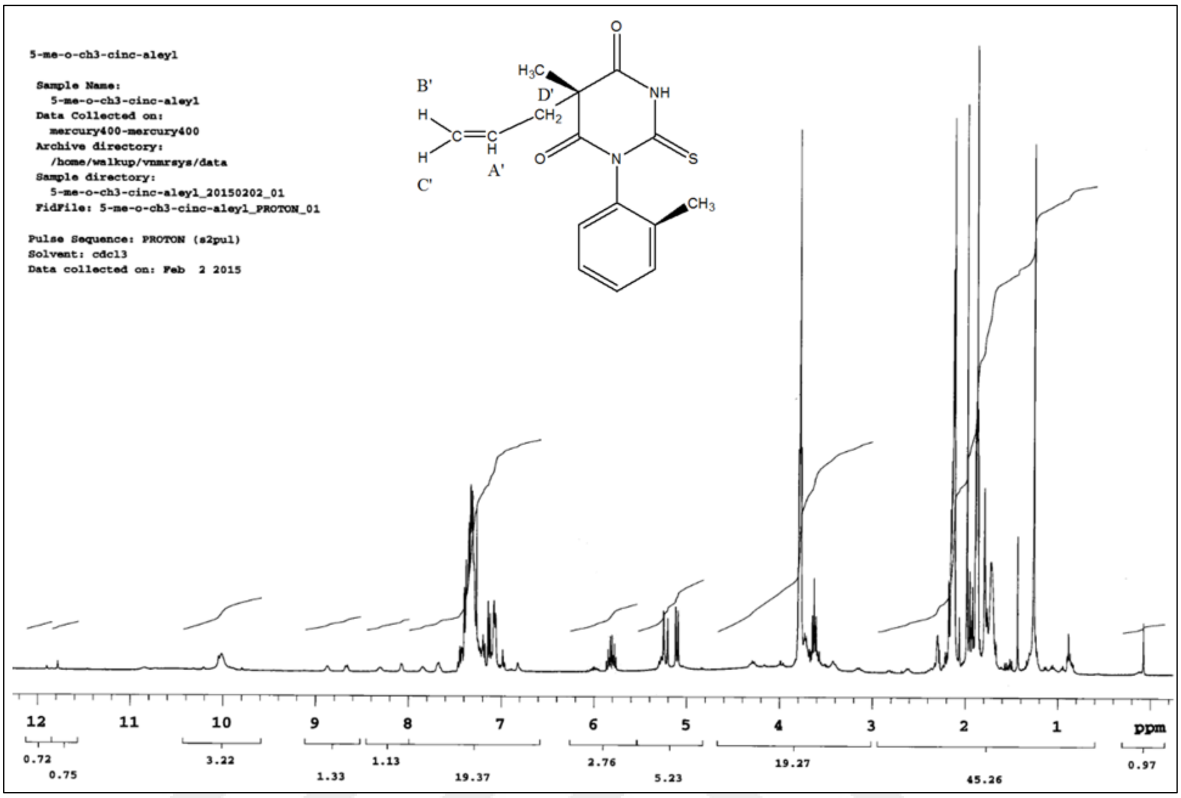


Figure 5.39. ^{1}H NMR spectrum of 5-allyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid, NMR solvent: chloroform-d

The solid product 5-allyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric was tried to be purified by fractional recrystallization method. Therefore, two recystallization processes were performed in absolute ethanol and results of the products were given in Figures 5.40 and 5.41.

In the first recrystallization of the product, A, B, C and D type protons of allylbromide were observed again in the ^{1}H NMR spectrum (Figure 5.40). After the first recrystalization, the smallest peak seen at 6 ppm in the first spectrum has grown in the second spectrum. So the peaks belonging A and A' protons were observed at 6 and 5.8 ppm in almost the same ratio. Two peaks of doublets belonging to B, B' and C, C' types of protons of allylbromide and the product were observed between 5.09 and 5.19 ppm. D type protons of allylbromide was observed at around 4 ppm. The recrystallized solid seems to contain also the reactant thiobarbituric acid since a quartet was observed between 3.75 and 3.70 ppm belonging probably to H bonded to C-5 of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid. Besides, AB spectrum of D type CH_2 protons could not be identified at around 2 ppm due to complicated pattern at this range.

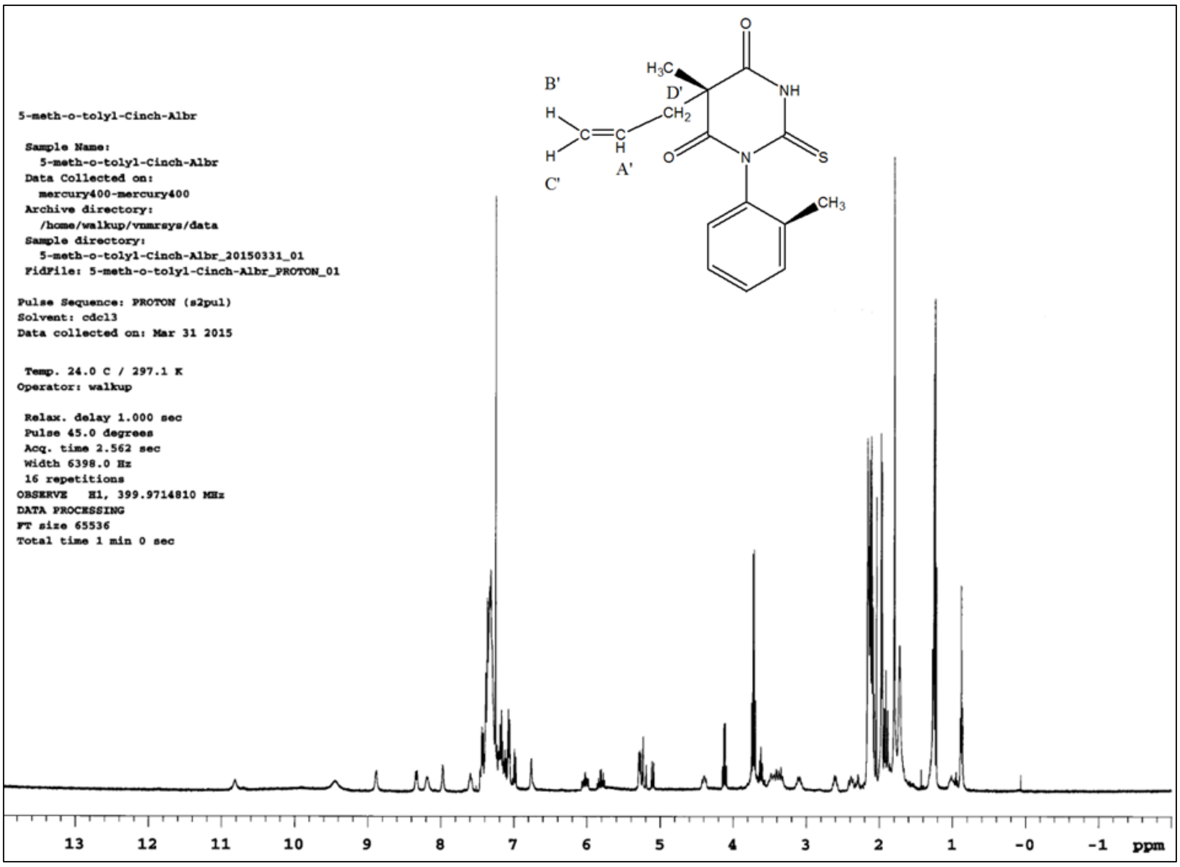


Figure 5.40. ^{1}H NMR spectrum of 5-allyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid after 1st recrystalization, NMR solvent: chloroform-d

In the ^{1}H NMR spectrum of second recrystallization crystals of the product (Figure 5.41), peaks between 5 and 6.1 ppm (product's peaks) became smaller compared to previous spectra. The quartet peak belonging to the thiobarbituric acid enlarged compared to the peak in the spectrum of 1st recrystallization crystals. This result showed that the crystallization rate of the product 5-allyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid is smaller than that of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid. So the fractional crystallization in ethanol is not an adequate purification technique.

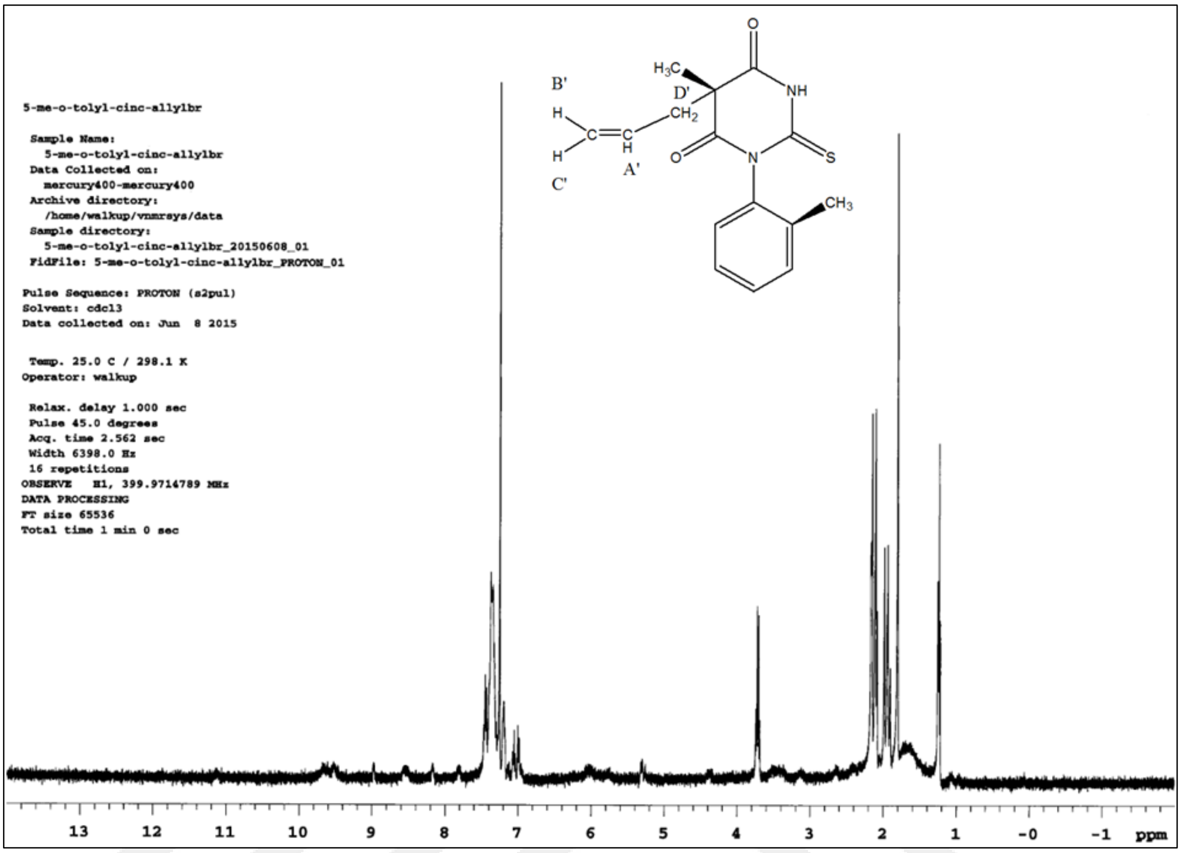


Figure 5.41. ^{1}H NMR spectrum of 5-allyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid after 2nd recrystalization, NMR solvent: chloroform-d

According to NMR results of the recrystallized product, it was proposed that some of the products crystals remain in the solvent during recrystallization process. Therefore the solvent was evaporated and the remaining crystals were analyzed to support this suggestion. After second recrystallization of the product, ethanol of the filtrate was evaporated. Then, the obtained crystals from this filtrate were analyzed by the ^{1}H NMR spectroscopy. The ^{1}H NMR spectrum was shown in Figure 5.42.

One singlet for NH group was observed at 9.17 ppm. Aromatic protons were observed between 7.407 and 6.913 ppm. The peaks belonging to the product was observed between 5.816 and 5.715 ppm. One doublet peak was observed between 5.185 and 5.143 ppm for B type proton of allylbromide. Other doublet peak was observed between 5.070 and 5.045 ppm for C type proton of allylbromide. A quartet that belongs reactant (5-methyl-1-*o*-

tolyl)-2-thiobarbituric acid) was observed between 3.682 and 3.630 ppm. AB spectrum of the allylic protons of the product was observed between 1.666 and 1.640 ppm. Two different methyl group protons were observed at 2.051 and 1.919 ppm. According to that result, the product was observed but it was obtained in a mixture and low yield.

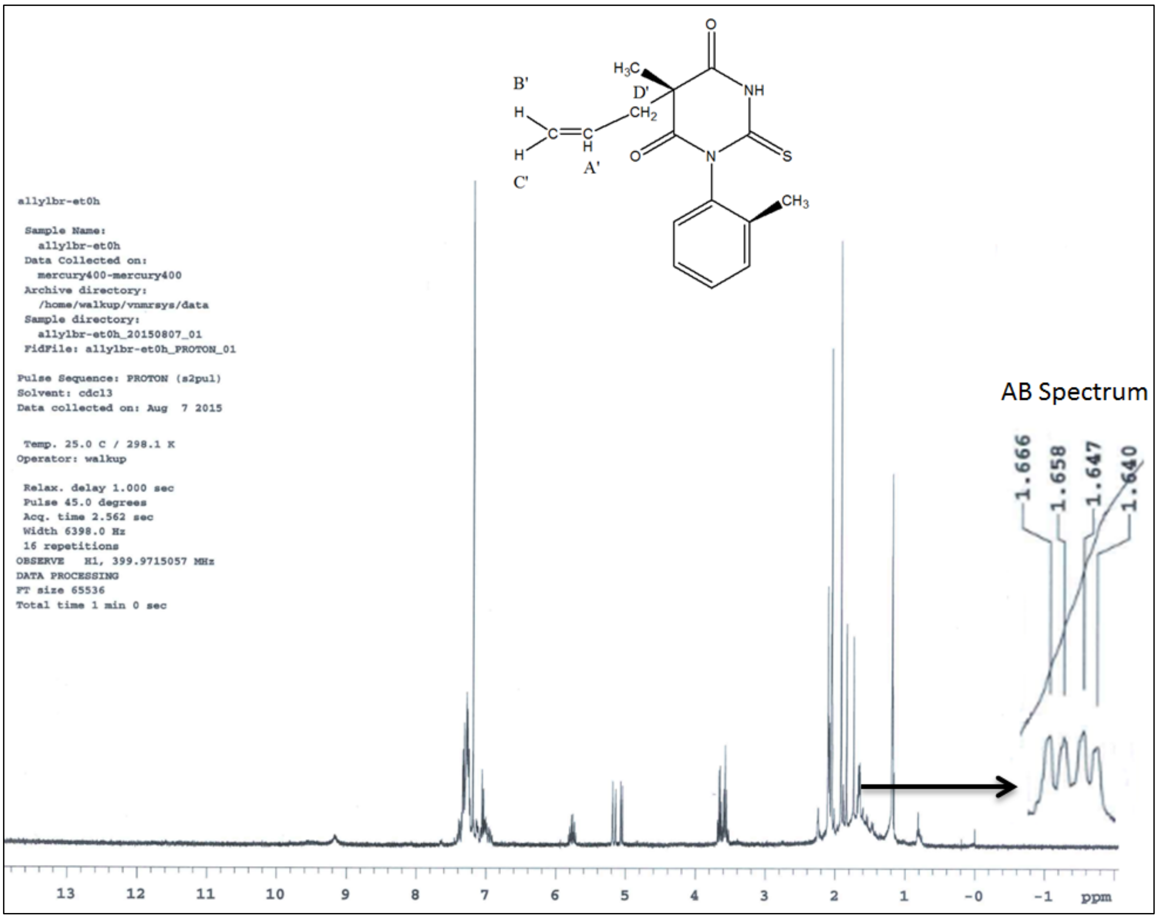


Figure 5.42. ^{1}H NMR spectrum of 5-allyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid, after evaporation of ethanol of filtrate, NMR solvent: chloroform-d

Similar commments for the ^{1}H NMR spectra of the products 5-allyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, 5-allyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid and 5-allyl-5-methyl-1-phenyl-2-thiobarbituric acid were done regarding the ^{1}H NMR comments of 5-allyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid in Figures 5.38, 5.43, 5.44 and 5.45, respectively. However, an AB spectrum peak for the allylic protons of the product and a quartet peak for C-5 proton of the reactant were not identified clearly due to poor resolution (Figures 5.43, 5.44 and 5.45).

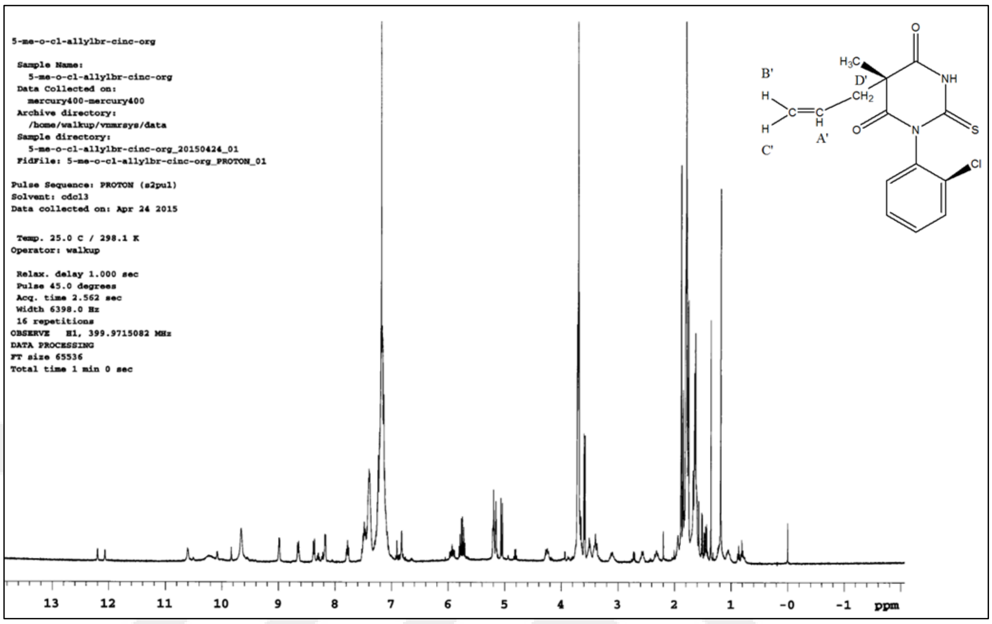


Figure 5.43. [1]H NMR spectrum of 5-allyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, NMR solvent: chloroform-d

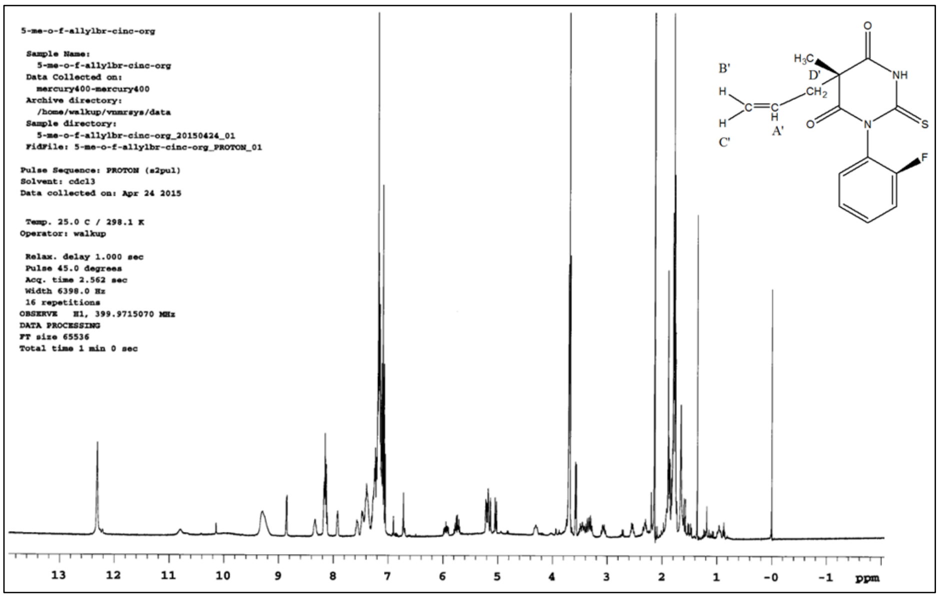


Figure 5.44. [1]H NMR spectrum of 5-allyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid, NMR solvent: chloroform-d

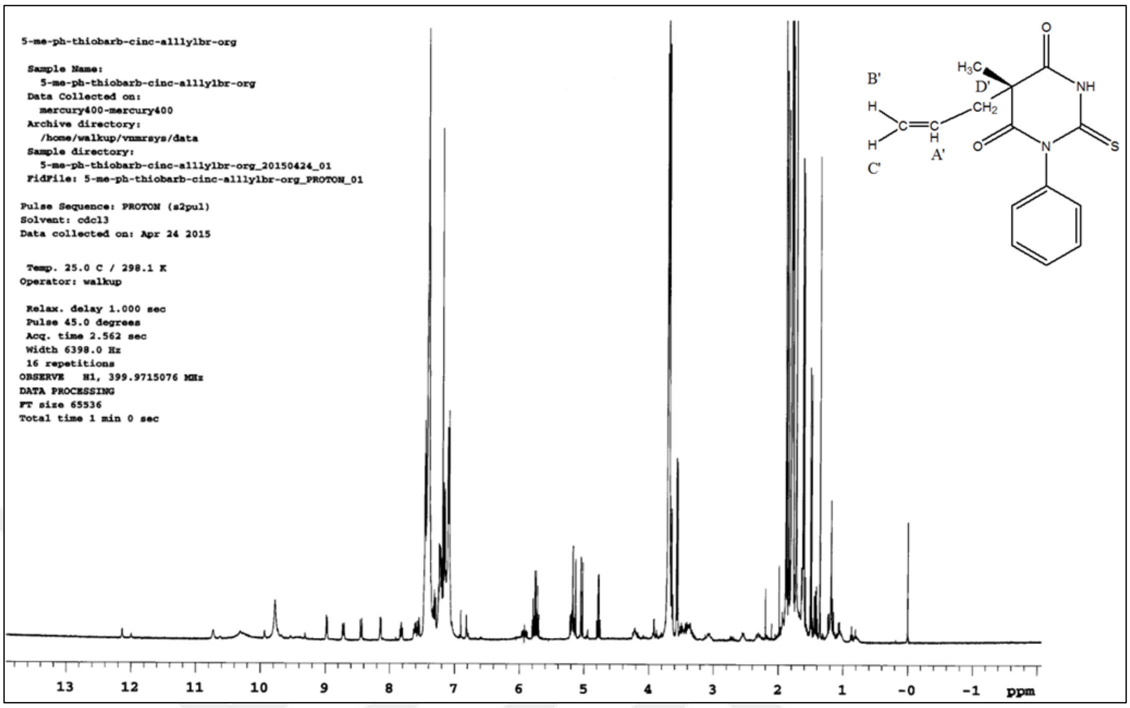


Figure 5.45. ^{1}H NMR spectrum of 5-allyl-5-methyl-1-(phenyl)-2-thiobarbituric acid, NMR solvent: chloroform-d

Besides the products 5-allyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, 5-allyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid and 5-allyl-5-methyl-1-phenyl-2-thiobarbituric acid were lost during recrystalization process as observed in the recrystallization of 5-allyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid. It was concluded that the products remained in the recrystallization solvent absolute ethanol during recrystallization process. Their ^{1}H NMR spectra of the purified peaks were given in Appendix C.

5.9. NORMAL PHASE HPLC RESULTS of THE PRODUCTS of BENZYLATION REACTIONS

In the alkylation reactions of 5-methyl-1-(*o*-aryl)-2-thiobarbituric acid derivatives, the starting materials had diastereomeric isomers in 1:1 ratio regarding the proton and carbon NMR results (Sections 5.4 and 5.5).

When LDA was used as a base in the the benzylation reactions, the products were expected to keep the diastereomeric ratio of the reactants. But the NMR results (Section 5.8.3) revealed that the products have only one type of diastereomer (one type of enantiomeric pair, P-R & M-S or P-S & M-R). This might occur due to diastereoselectivity of the reaction. The product was further analyzed by chiral HPLC spectroscopy to clarify the presence of stereoisomers of the products.

Additionally, the benzylation reactions were performed with (+)-cinchonine, too. (+)-Cinchonine acts as both a base and a chiral catalyst for enantioselective reaction. According to proton and carbon NMR results it has been found that the reactions were also diastereoselective. HPLC analyses with chiral column were done to determine the enantioselectivity of the reactions and also to reconfirm the diastereoselectivity of the reactions.

HPLC analyses conditions were given in Section 4.2.2. Different chiral columns, which are Chiralcel OD-H, Chiralpak IA, Chiralpak IB and Chiralpak IC, were used to be able to separate enantiomers of the alkylation products, and the most efficient column was found as Chiralcel OD-H column.

Also, the optical activity of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid and 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid was measured to confirm normal phase HPLC results. The other products could not be analyzed by polarimeter due to their insufficient amounts.

The HPLC chromatogram of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid obtained with LDA as a base, was presented in Figure 5.46. Besides the HPLC chromatogram of the 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid obtained with (+)-cinchonine as a catalyst was in Figure 5.47.

In order to determine the peaks of the product 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, HPLC chromatograms of alkylation reactions of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid with LDA and (+)-cinchonine were compared. Common peaks were seen around 23 minutes in both chromatograms, so it was concluded that the peak belonged one of the diastereomers. Also some peaks were observed above 25 minutes in the chromatogram of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid obtained in the presence of LDA and that peak was not observed in the chromatogram of benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid obtained in the presence of (+)-cinchonine. The extra peaks in the chromatogram of the product obtained in the presence of LDA might belong to one diastereomer, which was not formed from the reaction with (+)-cinchonine. When LDA is used in the alkylation reaction of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, enantioselectivity is not possible since there is no chiral agent in the medium. Therefore enantiomeric isomers were expected to be formed in 1:1 ratio and peaks were expected to be seen in HPLC chromatogram in 1:1 ratio. However that ratio of the peaks was not observed in the HPLC chromatogram. One possibility might be that the peaks of the enantiomers could not be separated by this column, and another possibility could be the peak areas were not measured correctly by the instrument due to improper baseline.

Enantiomeric and diastereomeric excesses of the 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid reaction were not calculated due to indefinite identification of the peaks in the HPLC chromatogram. However the reaction with (+)-cinchonine was thought to be enantioselective or diastereoselective because some peaks in the chromatogram of the reaction product obtained with LDA were not observed in the chromatogram of the reaction product obtained with (+)-cinchonine, although their NMR spectra were similar.

The optical activity (α) of the product obtained in the presence of (+)-cinchonine was measured as 0.00^{o} by the polarimeter. This shows that there is no enantiomeric excess.

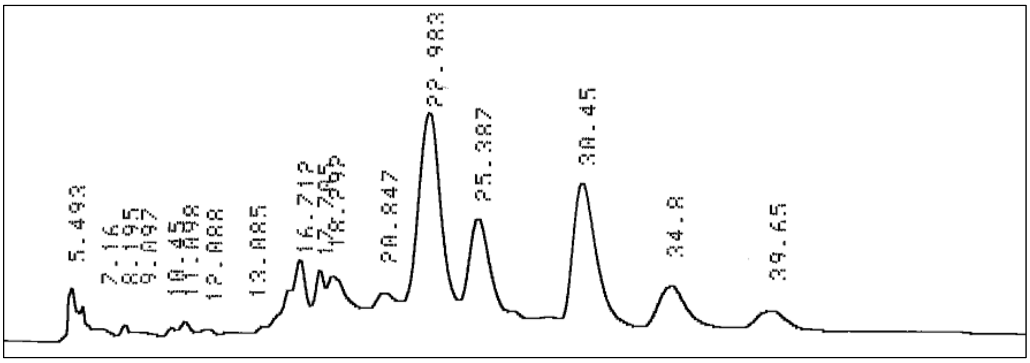


Figure 5.46. HPLC chromatogram of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid; base: LDA; column: Chiralcel OD-H column; mobile phase: 90:10 (Hexane-Ethanol); flow rate: 0.6 mL/min

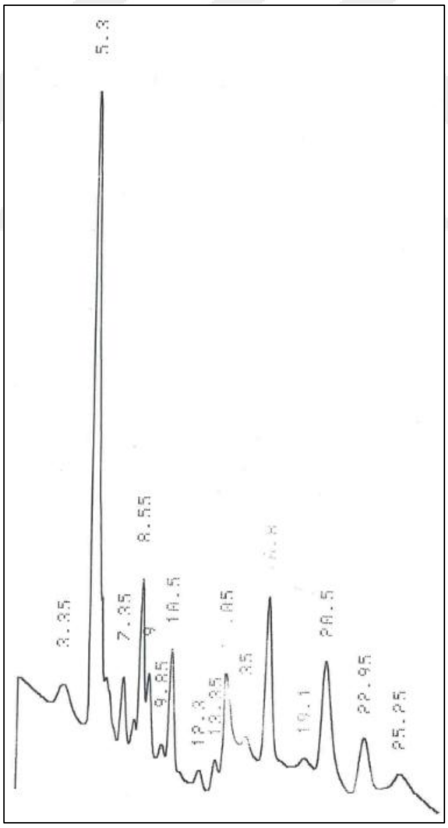


Figure 5.47. HPLC chromatogram of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid; catalyst: (+)-Cinchonine; column: Chiralcel OD-H column; mobile phase: 90:10 (Hexane-Ethanol); flow rate: 0.6 mL/min

The HPLC chromatogram of 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid obtained with LDA was shown in Figure 5.48. The reaction was expected as diastereoselective. However one diastereomer was observed in 29.05 minutes and its percentage concentration was calculated as 34.39% but this peak was not resolved well in HPLC chromatogram, therefore enantiomeric ratio could not be measured. The peaks of the enantiomers of the other diastereomer were observed in 18.3 and 19.45 minutes. Their percentage concentrations are close to 50:50. The percentage concentration of the peak of this diastereomer was calculated as 65.61%. The diastereomeric excess of the reaction was calculated by subtracting 65.61% from 34.39% as 31.22% (Figure 5.48).

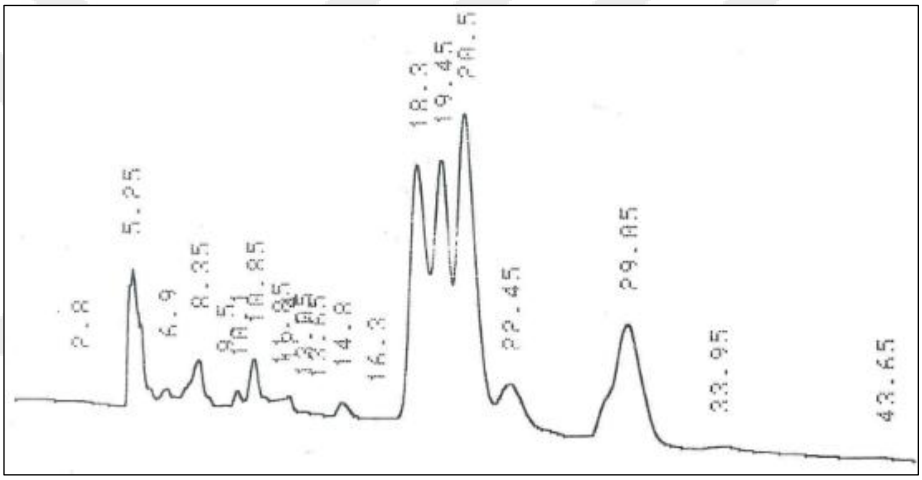


Figure 5.48. HPLC chromatogram of 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid; base: LDA; column: Chiralcel OD-H column; mobile phase: 90:10 (Hexane-Ethanol); flow rate: 0.6 mL/min

The HPLC chromatogram of 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid obtained with (+)-cinchonine was shown in Figure 5.49. Three peaks in 15.2, 19.5 and 28.35 minutes were assigned for diastereoselectivity and enantioselectivity calculation. One diastereomer in 28.5 minutes was not separated into its enantiomeric isomers in HPLC chromatogram and its percentage concentration was calculated as 10.56%. The percentage concentrations of the peaks in 15.2 and 19.5 minutes belonging to enantiomeric isomers of the other diastereomer were calculated as 69.08% and 20.36%, respectively. Thus the total percentage of the other diastereomer in the mixture was calculated as 89.44%.

Diastereomeric excess was found by subtracting 89.44% from 10.56% as 78.88% (Figure 5.49). Two peaks in 15.2 and 19.5 minutes were assigned as peaks of the enantiomers and used for enantiomeric excess calculation. Percentage concentration of the peak of one enantiomer in 15.2 minutes was calculated as 77.23%, the percentage concentration of the peak of the other enantiomer in 19.5 minutes was calculated as 22.77%. The enantiomeric excess of the reaction was calculated by subtracting 77.23% from 22.77% as 54.46% (Figure 5.49).

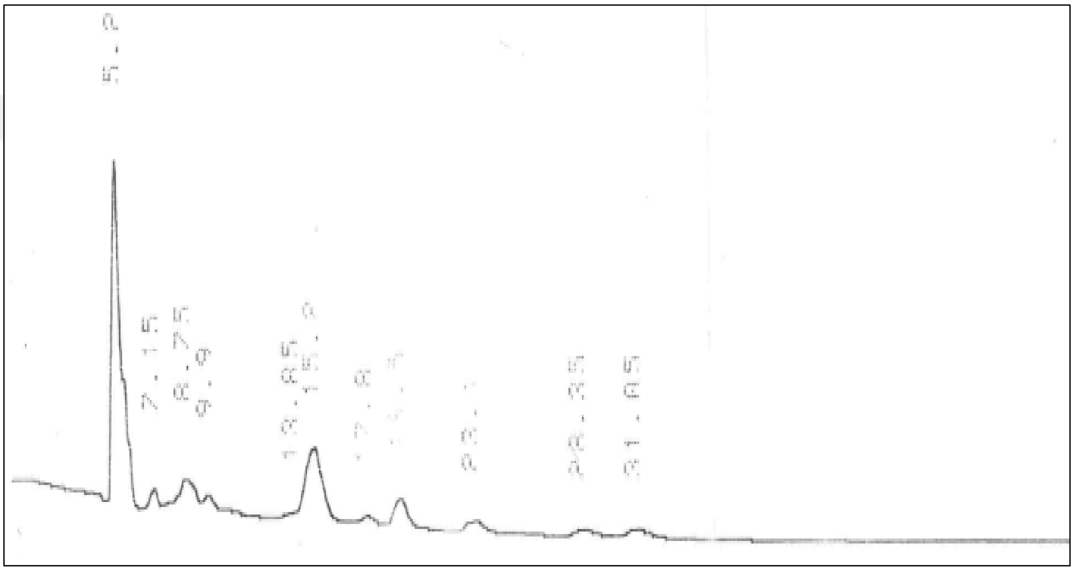


Figure 5.49. HPLC chromatogram of 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid; catalyst: (+)-Cinchonine; column: Chiralcel OD-H column; mobile phase: 90:10 (Hexane-Ethanol); flow rate: 0.6 mL/min

The HPLC chromatogram of 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid obtained with LDA, is presented in Figure 5.50. Two peaks at 19.9 minutes and 28.55 minutes were observed in the chromatogram. They belong to one enantiomeric pair of one diastereomer, so it was concluded that the reaction is diastereoselective. The percentage concentration of the enantiomer appearing at 19.9 minutes was found as 43.34%, the percentage concentration of the other enantiomer appearing at 28.55 minutes was 56.66%. Hence the enantiomeric excess was calculated by subtracting 43.34% from 56.66% as 13.32% (Figure 5.50).

However, in this reaction no enantioselectivity was expected, because there is no optically active reagent in the medium, this difference might be due to inaccurate calculation of the areas by the instrument, or overlapping of the second peak with a peak of another compound.

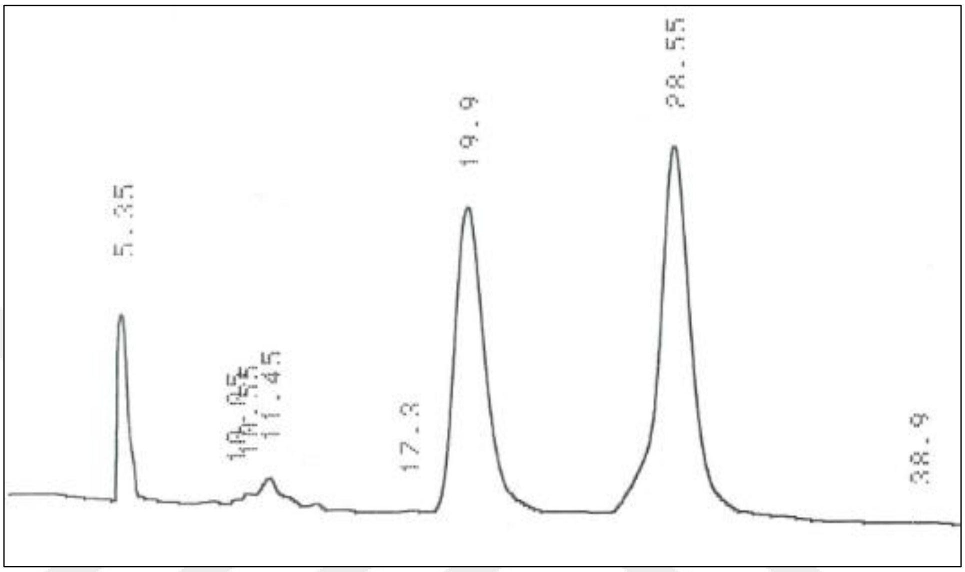


Figure 5.50. HPLC chromatogram of 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid; base : LDA; column: Chiralcel OD-H column; mobile phase: 90:10 (Hexane-Ethanol); flow rate: 0.6 mL/min

The HPLC chromatogram of 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid obtained with (+)-cinchonine, is presented in Figure 5.51. In the chromatogram two peaks at 19.1 minutes and 30.85 minutes (small peak) were observed. They were assigned as the peaks of one enantiomeric pair of one diastereomer type. The percentage concentration of the peak of the enantiomer appearing at 19.1 minutes was found as 94.75%, the percentage concentration of the peak of the enantiomer appearing at 30.85 minutes was 5.25%. Hence the enantiomeric excess was found by subtracting 5.25% from 94.75% as 89.5% (Figure 5.51). The observed optical activity or rotation (α) of the product of the product was measured as 20^{o} by using polarimeter. This is a proof that the reaction is enantioselective that means one enantiomer dominates distinctly in the end of the reaction.

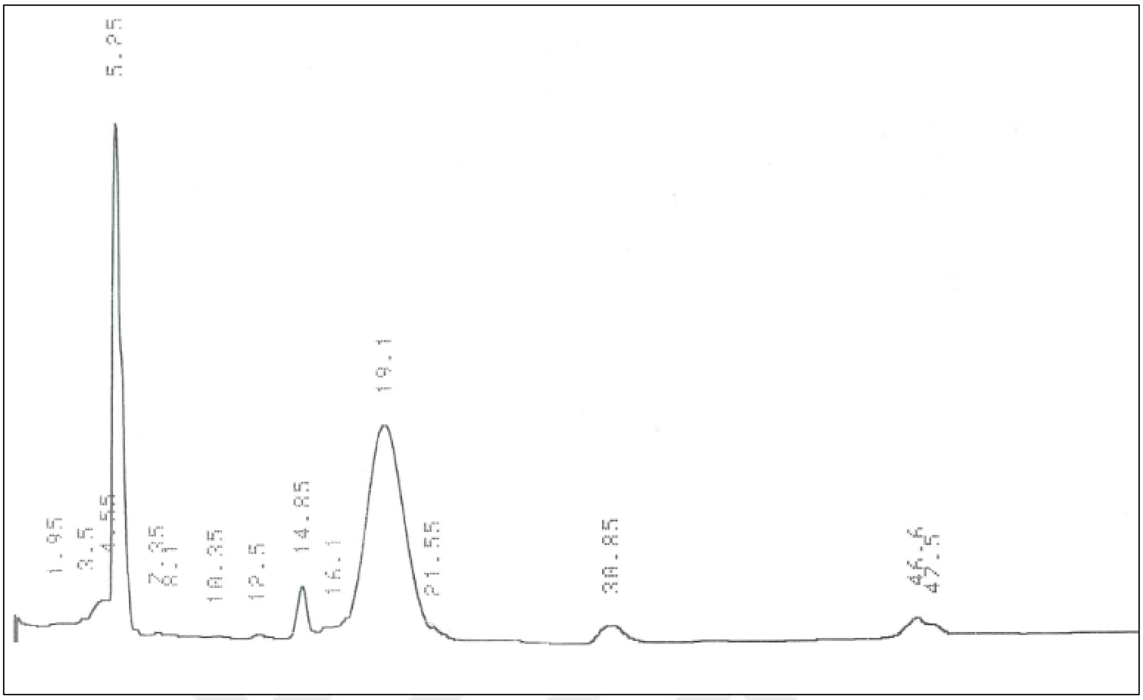


Figure 5.51. HPLC of 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid; catalyst: (+)-Cinchonine; column: Chiralcel OD-H column; mobile phase: 90:10 (Hexane-Ethanol); flow rate: 0.6 mL/min

In the alkylation reaction of 5-methyl-1-phenyl-2-thiobarbituric acid, where (+)-cinchonine was used as a catalyst, and only one enantiomeric pair formation with different percentage values is possible. The percentage concentration of the peak appearing at 18.497 minutes was 59.27%, the percentage concentration of the peak appearing at 38.078 minutes was 40.73% (Figure 5.52). The retention times of the peaks coincide with the retention times of the peaks of 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid. This can be accepted as a proof that the peaks in the chromatogram of 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid belong only to one enantiomeric pair.

The enantiomeric excess was calculated by subtracting 59.27% from 40.73% as 18.9% (Figure 5.52). From that result, it was concluded that the reaction has an enantioselectivity feature.

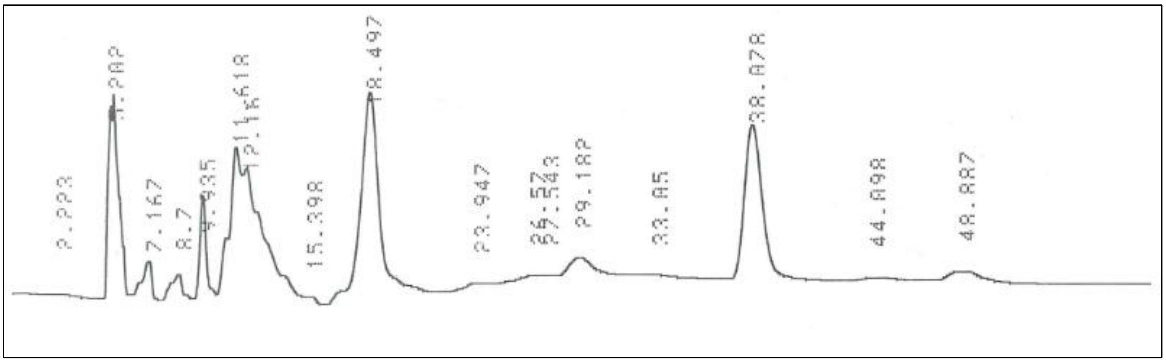


Figure 5.52. HPLC chromatogram of 5-benzyl-5-methyl-1-phenyl-2-thiobarbituric acid; catalyst: (+)-Cinchonine; column: Chiralcel OD-H column; mobile phase: 90:10 (Hexane-Ethanol); flow rate: 0.6 mL/min

The overall evaluation of the benzylation reactions for the stereoisomer formation according to the ^{1}H NMR and ^{13}C NMR analyses of the products were shown in Table 5.48.

Table 5.48. Overall evaluation of benzylation reactions for the stereoisomer formation according to the ^{1}H NMR and ^{13}C NMR analyses of the products

Benzylation Product	**^{1}H NMR Result for the products obtained with (+)-cinchonine&LDA**	**^{13}C NMR Result for the products obtained with (+)-cinchonine**
5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid	One Diastereomer (One AB Spectrum)	One Diastereomer (One peak for each C)
5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid	One Diastereomer (One AB Spectrum)	Two Diastereomers (Two peaks for some C’s)
5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid	One Diastereomer (One AB Spectrum)	One Diastereomer (One peak for each C)

5.10. ANTIBACTERIAL ACTIVITIES of THIOBARBITURIC ACID DERIVATIVES and ALKYLATION REACTIONS of 5-SUBSTITUTED-1-(*o*-ARYL)-2-THIOBARBITURIC ACID DERIVATIVES

5-methyl-1-(*o*-aryl)-2-thiobarbituric acids were expected to bear antibacterial effects regarding the previous studies of thiobarbituric acids (16,17,18). Therefore their antibacterial activity was measured by disc diffusion method.

The antibacterial effects of the synthesized 5-methyl-1-(*o*-aryl)-2-thiobarbituric acids, 5,5 dimethyl-1-(*o*-aryl)-2-thiobarbituric acids, 5-methyl-1-phenyl-2-thiobarbituric acids and 5,5-dimethyl-1-phenyl-2-thiobarbituric acids were tested on the bacteria, Escherichia coli (E. coli), Bacillus subtilis (B. subtilis), Pseudomonas aeruginosa (P. aeruginosa), Bacillus megaterium (B. Megaterium) and Staphylococcus aureus (S. aureus). In these tests, ofloxacin antibiotic disk was used as a controller. Tryptic Soy Agar (TSA) was also used as a medium of various microorganisms to grow.

The antibacterial effects occurred around the disk could be observed by a transparent and circular zone. A zoom around the disk was a sign for a positive result. The positive result means that the bacteria could not grow in the medium containing the bacteria because of the active substance.

In Figure 5.53, the antibacterial test results of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid, 5,5-dimethyl-1-(*o*-tolyl)-2-thiobarbituric acid and 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid and 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid can be observed. According to Figure 5.53, while 5-methyl-1-*o*-tolyl-2-thiobarbiturics acid and 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acids have antibacterial effect against E. coli, P. aeruginosa and S. aureus, 5,5-dimethyl-1-(*o*-tolyl)-2-thiobarbituric acids and 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid have negative antibacterial effect against them according to the result shown in Figure 5.53. The positive and negative results were listed in Table 5.49.

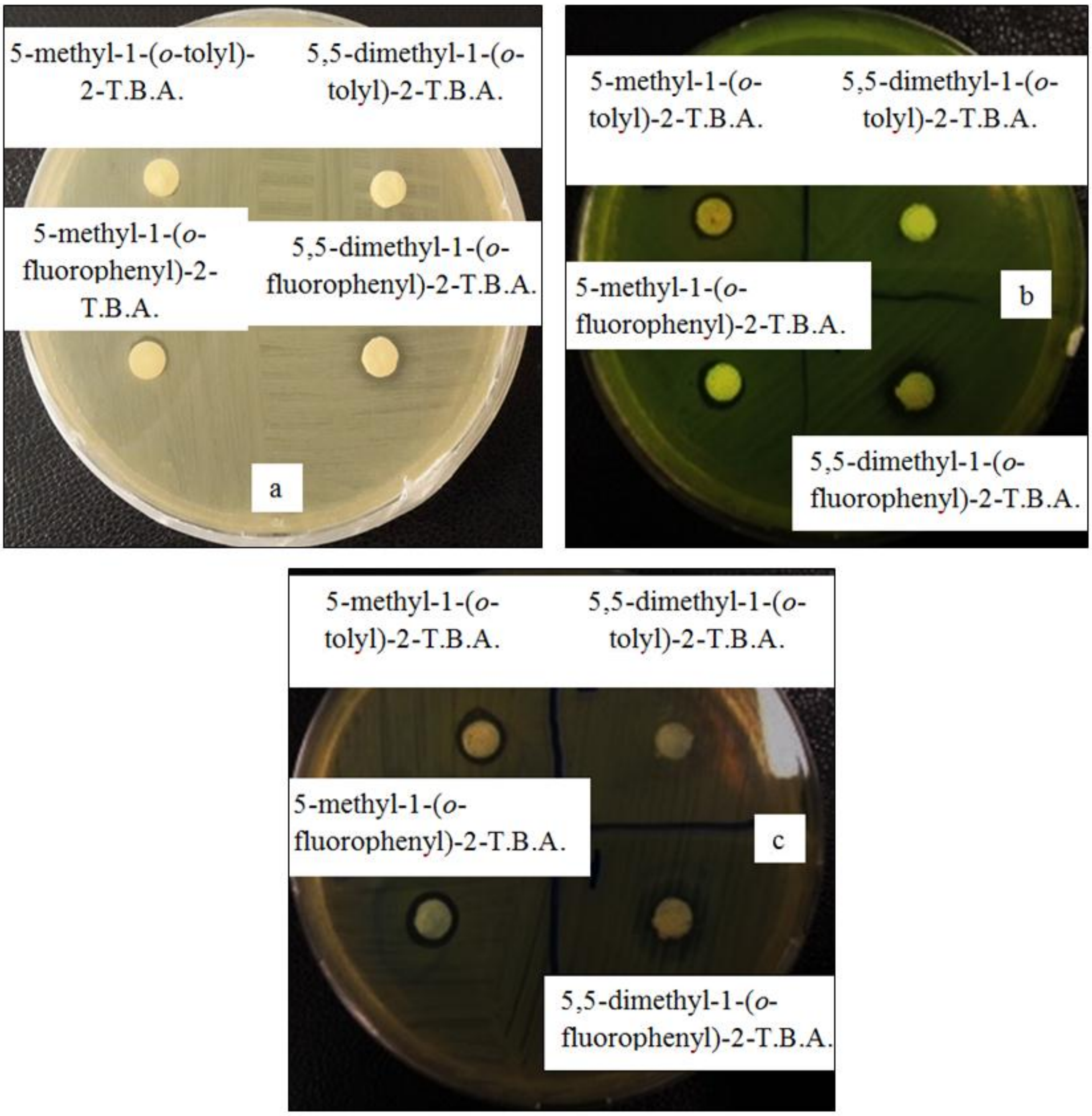


Figure 5.53. Antibacterial test results of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid, 5,5-dimethyl-1-(*o*-tolyl)-2-thiobarbituric acid, 5-methyl-1-(o-fluorophenyl)-2-thiobarbituric acid and 5,5-dimethyl-1-(*o*-tolyl)-2-thiobarbituric acid against bacteria, **a**: E. coli, **b**: P. aeruginosa and **c**: S. aureus

Table 5.49. Interpreatation of antibacterial test results of Thiobarbituric acid derivatives

BACTERIUM	**5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid**	**5,5-dimethyl-1-(*o*-tolyl)-2-thiobarbituric acid**	**5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid**	**5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid**
Escherichia Coli (E. Coli)	+	–	+	+
Pseudomonas Aeruginase (P. Aeruginase)	+	–	+	+
Staphylococus Aureus (S. Aureus)	+	–	+	+

The same test was applied also to 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid and 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid. As seen in Figure 5.54, a clear zone around the applied bacterium can be seen when 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid was used as antibacterial agent, but any zone can be observed aroud the bacterium can be seen in the case of 5,5-dimethyl-*o*-chlorophenyl-2-thiobarbituric acid. Hence it was concluded that 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid shows antibacterial effect against E. coli, P. aeruginosa, B. Subtilis and S. aureus, whereas 5,5-dimethyl-*o*-chlorophenyl-2-thiobarbituric acid shows negative antibacterial effect against them. The positive and negative results were listed in Table 5.50.

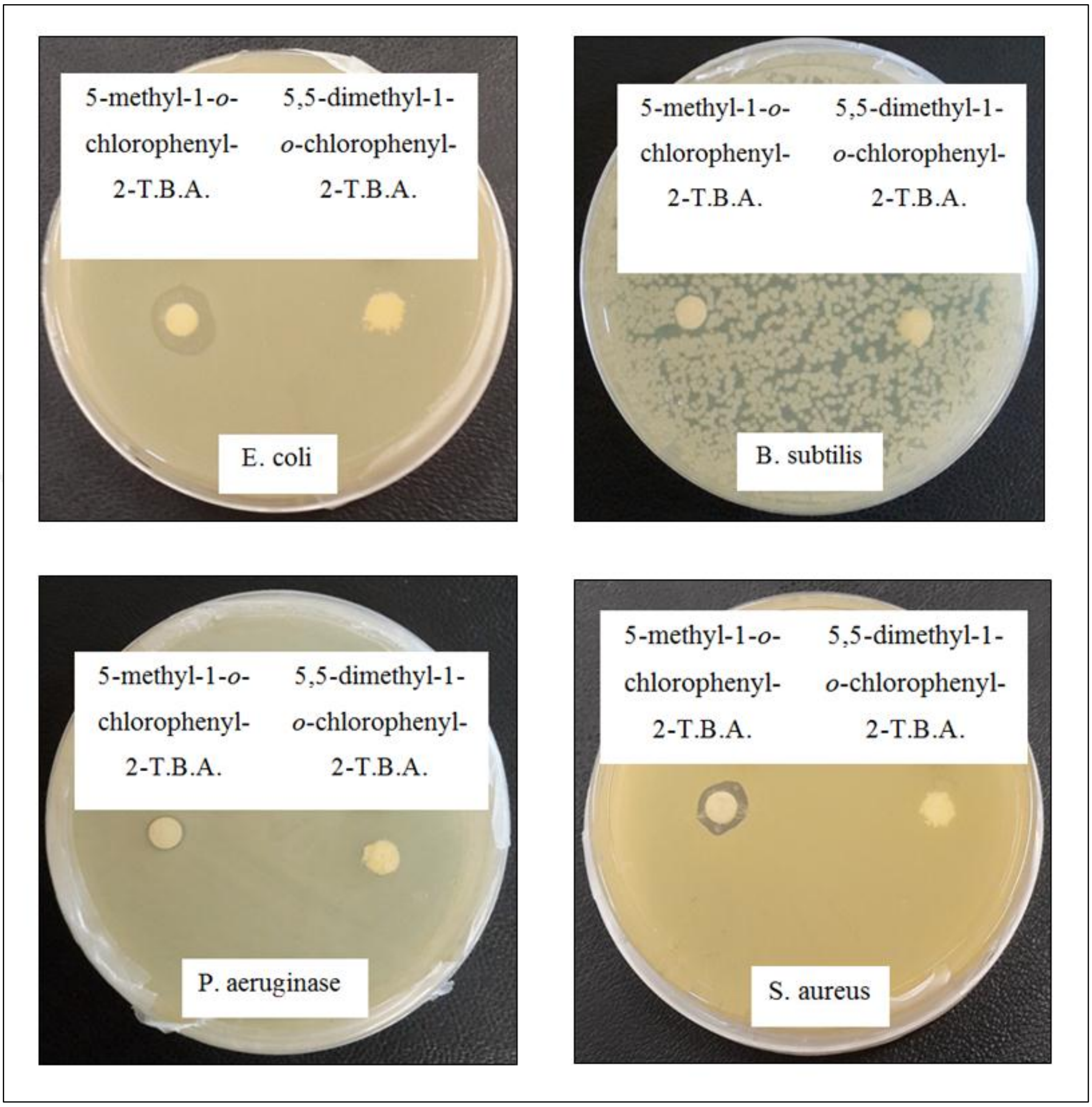


Figure 5.54. Antibacterial test results of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid and 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid against bacteria: E. coli, B. subtilis, P. aeruginosa and S. aureus

Table 5.50. Interpretation of antibacterial test results of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid and 5,5-dimethyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid

BACTERIUM	**5-methyl-1 (*o*-chlorophenyl)-2-thiobarbituric acid**	**5,5-dimethyl-1 (*o*-chlorophenyl)-2-thiobarbituric acid**
Escherichia Coli (E. coli)	+	–
Bacillus Subtilis (B. subtilis)	+	–
Pseudomonas Aeruginosa (P. aeruginosa)	+	–
Staphylococcus Aureus (S. Aureus)	+	–

To be able to compare the *ortho*-substituent effect, 5-methyl-1-phenyl-2-thiobarbituric acid and 5,5-dimethyl-1-phenyl-2-thiobarbituric acid were also tested. According to test results (Figure 5.55) 5-methyl-1-phenyl-2-thiobarbituric acid had an antibacterial effect on E. coli, P. aeruginosa and S. aureus, however, 5,5-dimethyl-1-phenyl-2-thiobarbituric acid gave negative antibacterial effect against them. An accurate comparison of the effects of 5-methyl-1-phenyl-2-thiobarbituric acid and 5-methyl-1-(*o*-aryl)-2-thiobarbituric acids cannot be made, since the amount of the compound to be tested cannot be measured in the applied test. Therefore an accurate comparison may be done if the amount of the compound to be tested was applied the same. The positive or negative results were listed in Table 5.51. The highest circular and transparent zooms were obtained due to ofloxacin antibiotics as seen in Figure 5.55.

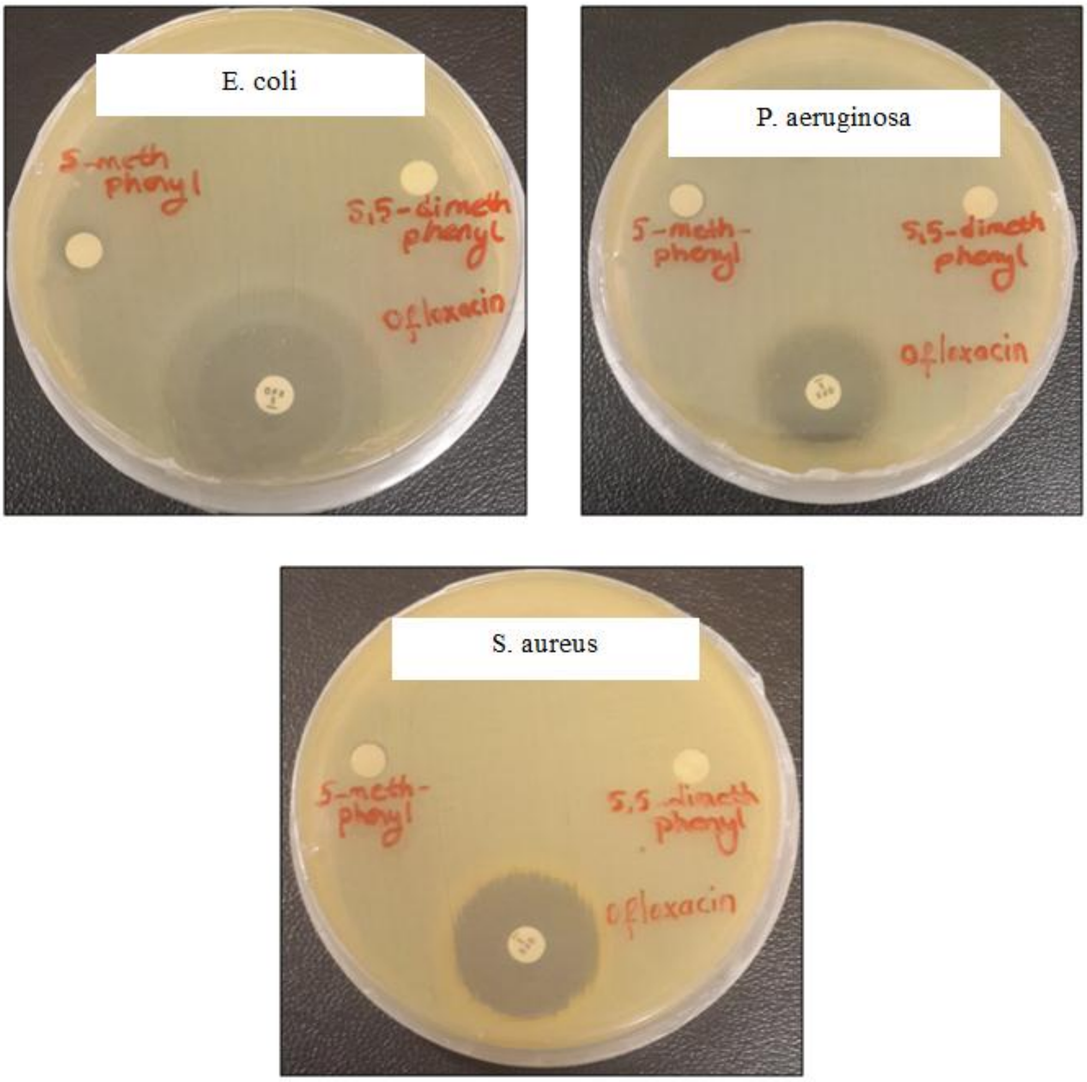


Figure 5.55. Antibacterial test results of 5-methyl-1-phenyl-2-thiobarbituric acid, 5,5-dimethyl-1-phenyl-2-thiobarbituric acid and ofloxacin against bacteria: E. coli, P. aeruginosa and S. aureus

Table 5.51. Interpretation of antibacterial test results of 5-methyl- and 5,5-dimethyl-1-phenyl-2-thiobarbituric acid

BACTERIA	**5-methyl-1-phenyl-2-thiobarbituric acid**	**5,5-dimethyl-1-phenyl-2-thiobarbituric acid**	**Ofloxacin Disk**
Escherichia Coli (E. coli)	+	–	+
Pseudomonas aeruginosa (P. aeruginosa)	+	–	+
Staphylococcus aureus (S. aureus)	+	–	+

Depending on the results, 5-methyl substituted derivatives of 2-thiobarbituric acids were found to possess antibacterial activity, whereas 5,5-dimethyl derivatives of 2-thiobarbituric acids including the alkylation product 5-benzyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid were found to be inactive against the specified bacteria. This behavior can be explained that the proton at C-5 or the enol form has a function for this activity. The test process (the interaction of the bacterium and the compound to be tested) can be examined in detail to clarify the function of the proton at C-5. Among the 5-methyl substituted derivatives, the fluoro-substituted derivatives were discovered to be the most active. In the literature, it has been shown that the fluoro substituent has provided a higher antibacterial activity to specific bacteria compound than other halogen-substituents [13]. A more accurate comparison can be achieved if the same amount of the compound is applied on the disk for each compound. Besides *ortho*-substituted derivatives of thiobarbituric acid derivatives show more antibacterial effect against bacteria than 5-methyl-1-phenyl-2-thiobabrbituric acids and 5,5-dimethyl-1-phenyl-2-thiobarbituric acids regarding the zone sizes appearing around the bacteria when these compounds were applied as antibacterial agents. This is also a proof of the effects of the *ortho*-substituents on the antibacterial activity.

Antibacterial activity of only one product, 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid was tested against two bacteria, E. coli and S. aureus because of the low yield of the alkylation products of thiobarbituric acid derivatives and the absence of other bacteria.

Alkylation product 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid, obtained from the reaction of 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid with benzyl bromide in the presence of (+)-cinchonine, did not show any antibacterial effect against E. coli and S. aureus as observed from Figure 5.56. Negative results can be seen in Table 5.52.

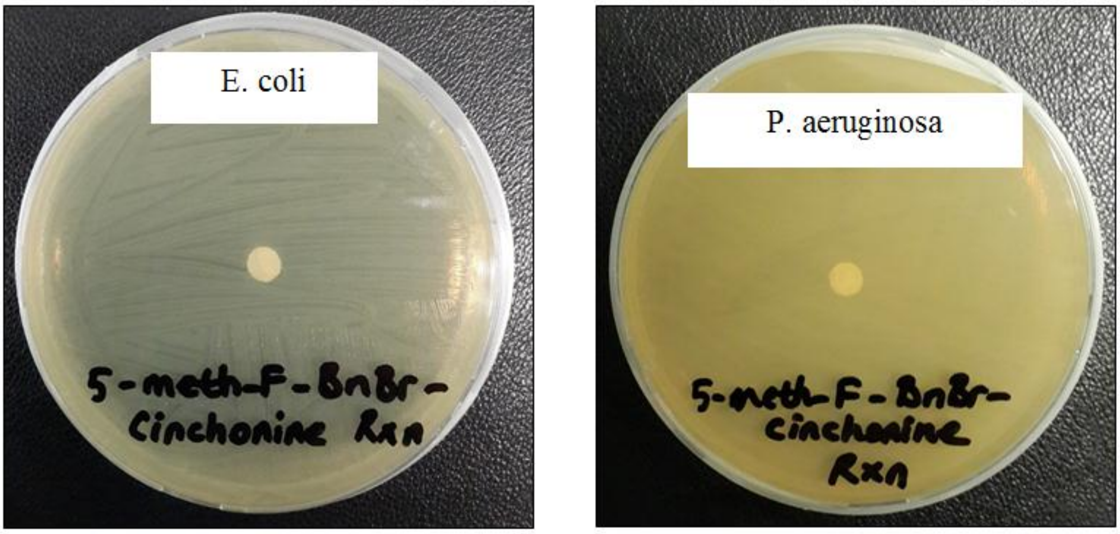


Figure 5.56. Antibacterial test results of 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid with (+) cinchonine against bacteria: E. coli and P. aeruginosa

Table 5.52. Interpretation of antibacterial test results of the 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid

BACTERIUM	**5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid**
Escherichia Coli (E. coli)	–
Pseudomonas aeruginosa (P. aeruginosa)	–

6. CONCLUSION AND FUTURE WORKS

6.1. Conclusion

In this study, firstly phenyltioureas and *ortho*-substituted phenylthioureas were synthesized to perform the syntheses of different 5,5-disubstituted-1-(*o*-aryl)-2-thiobarbituric acids, 5-substitued-1-(*o*-aryl)-2-thiobarbituric acids, 5-methyl-1-phenyl-2-thiobarbituric acids and 5,5-dimethyl-1-phenyl-2-thiobarbituric acids. For the synthesis of the phenylthiourea derivatives, aniline or *o*-substituted aniline, hydrochloric acid, ammoniumthiocyanate and water were used. Then, thiobarbituric acid derivatives were synthesized by refluxing methylmalonic acid or dimethylmalonic acid and phenylthiourea derivatives in acetyl chloride for 24 hours.

Some thiobarbituric acid derivatives were obtained in low yield. Excess reagent was used to improve the yield, but a lower yield of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid was obtained when methymalonic acid and *ortho*-substituted phenylthiourea reacted in 1:1.2 ratio compared to when they reacted in 1:1 ratio. Therefore reactions were performed in 1:1 ratio.

Synthesized thiourea derivatives and their corresponding thiobarbituric acid derivatives were examined by X-bridge column in the reversed phase HPLC chromatography to prove the formation and purity of the products.

Asymmetric alkylation reactions of 5-methyl-1-(*o*-aryl)-2-thiobarbituric acids were performed at carbon-5 position. Two types of asymmetric alkylation reactions were performed as benzylation and allylation. THF was used as a reaction solvent in both alkylation reactions. Benzylation reactions were done with (+)-cinchonine and LDA, but allylation reactions were only performed with (+)-cinchonine. Allylation and benzylation reactions with (+)-cinchonine were performed at -35°C. However, benzylation reactions with LDA were performed at -78°C.

^{1}H NMR spectroscopy, IR spectroscopy and melting point measurements were done to characterize all synthesized thiourea derivatives, thiobarbituric acid derivatives and alkylation products. ^{13}C NMR spectroscopy analyses were only performed for the firstly synthesized 5-methyl-1-(*o*-aryl)-2-thiobarbituric acids and their benzylation products. Also elemental analyses of benzylation products were done.

The existence of the two diastereomeric stereoisomers was determined by examining the multiplicity or the number of the peak of the proton or methylene group bonded to C-5. In ^{1}H NMR spectrum of all synthesized 5-methyl-1-(*o*-aryl)-2-thiobarbituric acids, two quartets due to hydrogen atom at C-5 of two diastereomeric isomers were observed around 4 ppm and all of them had 1:1 integral ratio, and consequently 1:1 diastereomeric ratio. As a result, for the alkylation products of these compounds, two AB spectra were expected for the benzylic protons of their two diastereomers in their proton NMR spectra. However, only one AB peak was observed between 4 and 5 ppm for the benzylation products. The reason of that may be difference in the crystallization process of the two diastereomers. One diastereomeric form might crystallize more rapidly than the other form, so the first precipitate will be richer in one diastereomeric form. Other reason may be that two diastereomers of benzylation products cannot be resolved in ^{1}H NMR spectroscopy. Additionally, one AB spectrum might be formed because of the formation of only one diastereomer in the end of the reaction. Besides one quartet for methyl groups at C-5 was observed in ^{1}H NMR spectrum (in acetone-d_6 solvent) of 5-methyl-1-phenyl-2-thiobarbituric acid as expected due to absence of *ortho*-substitution. In addition to that one singlet for the proton at C-5 was observed in the proton NMR spectrum of benzylation product 5-methyl-1-phenyl-2-thiobarbituric acid in chloroform although a quartet was seen for this proton in acetone-d_6, so it was concluded that the presence of AB peak is solvent dependent.

Furthermore two singlets for methyl protons at carbon-5 were observed around 1.60 ppm in the ^{1}H NMR spectrum of 5,5-disubstituted-1-(*o*-aryl)-2-thiobarbituric acids due to the presence of two diastereomeric forms. In addition, two methyl groups at carbon-5 gave one singlet in the ^{1}H NMR spectrum of 5,5-dimethyl-1-phenyl-2-thiobarbituric acid because there is no chiral axis in the compound. Thus these two methyl groups are enantiotopic groups.

In the analysis of ^{13}C NMR spectra results of 5-methyl-1-(*o*-aryl)-2-thiobarbituric acids, peaks belonging to two diastereomers were observed in the spectra of 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid and 5-methyl-(o-fluorophenyl)-2-thiobarbituric acid except 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid. One peak was observed for each carbon atom in the ^{13}C NMR spectrum of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid, although two quartets were observed for the proton at carbon-5 in its ^{1}H NMR spectrum. From that result, it can be said that the peaks of the diastereomers of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid cannot be resolved well in ^{13}C NMR spectrum. Besides one peak for each carbon atom was observed in the ^{13}C NMR spectrum of 5-methyl-1-phenyl-2-thiobarbituric acid as expected due to absence of diastereomeric isomers.

Normal phase HPLC chromatography was performed to investigate the stereoselectivity of the benzylation reactions. OD-H chiral column was used to separate diastereomeric or enantiomeric isomers and to determine diastereomeric or enantiomeric excess. Moreover Chiralpak IA, Chiralpak IB and Chiralpak IC columns were also used to identify the enantiomeric ratio of the products, but desired separation of the isomers could not be achieved with these chiral columns at different conditions.

Benzylation products were compared in Table 5.48. Peaks of two diastereomers were only observed in the ^{13}C NMR spectrum of the product 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid, some carbon atoms of this product give two peaks. Although only one AB was observed in the ^{1}H NMR spectrum of product 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid, peaks of two diastereomers were observed in the normal phase chromatography. In addition to that, peak of only one diastereomer was observed in both ^{1}H and ^{13}C NMR spectra of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, although peaks of two diastereomers of the product were obtained in the normal phase HPLC chromatogram,. The reason of this may be that peaks of diastereomers of the product 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid were not well resolved in both ^{1}H and ^{13}C NMR spectra in the specified solvent. Besides one diastereomer of the product 5-benzyl-5-methyl-1-phenyl-2-thiobarbituric acid was observed as expected in the HPLC chromatogram, ^{1}H and ^{13}C NMR spectra. Besides peak of only one diastereomer of product 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid was observed in the HPLC chromatogram, ^{1}H and ^{13}C NMR spectra. Hence formation of this product was accepted as enantioselective.

By analyzing the normal phase HPLC results, the alkylation reactions were generally found as diastereoselective in the presence of LDA and as enantioselective and/or diastereoselective in the presence (+)-cinchonine. Diastereomeric and enantiomeric excesses of the benzylation products were calculated by the difference of the peak area percentage values. Diastereomeric excesses of the products 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid, when LDA and (+)-cinchonine were used, and 5-methyl-1-(*o*-tolyl)-2-thiobarbituric aci when LDA was used, were calculated. Diastereomeric excess of the product 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid obtained in the presence of LDA was found as 31.22%. While diastereomeric excess of product 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid obtained in the presence of (+)-cinchonine was found as 78.88%, and enantiomeric excess of the product was found as 54.46%.

Diastereomeric or enantiomeric excess of the product 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid in the presence of LDA and (+)-cinchonine cannot be calculated correctly due to improper normal phase HPLC results. When HPLC chromatograms of alkylation reactions of 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid in the presence of LDA and (+)-cinchonine were compared, common peak was seen at around 23 minutes (Figure 5.46 and 5.47). This result showed that the peak belonged to one of the diastereomers or enantiomers. Besides, some peaks were observed above 25 minutes only in the chromatogram of the product 5-benzyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid obtained in the presence of LDA (Figure 5.46). The extra peaks might belong to one diastereomer or enantiomer, which was not obtained from the benzylation reaction in the presence of (+)-cinchonine. So the benzylation reaction in the presence of (+)-cinchonine was thought to be diastereoselective or enantioselective.

In the HPLC chromatogram of 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid, peaks of only one diastereomer were observed, hence it was concluded that the reaction is 100%

diastereoselective. Enantiomeric excess of product 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid obtained in the presence of LDA was calculated as 13.32%, but it is impossible since there is no chiral agent in the medium. This result is probably due to improper resolution of the second peak. Enantiomeric excess of product 5-benzyl-5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid obtained in the presence of (+)-cinchonine was found as 89.5%. It was concluded that reaction of 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid with benzylbromide in the presence of (+)-cinchonine is enantioselective and this result was proved by the optical activity measurement of the product. The specific rotation of the product was found as $4x10^4$ $cm^3.g^{-1}.dm^{-1}$ at 25^oC.

Enantiomeric excess of product 5-methyl-1-phenyl-2-thiobarbituric acid obtained in the presence of (+)-cinchonine was found as 18.9%.

Besides allylation reactions of 5-substitued-1-(*o*-aryl)-2-thiobarbituric acids were performed. However, ^1H NMR spectra results revealed that the starting materials of the allylation reactions were not separated well from the product and it was further concluded from ^1H NMR spectra results, that most of the products were remained in the recrystallization solvent absolute ethanol during recrystallization process.

In addition, antibacterial activities of the synthesized thiobarbituric acid derivatives and their alkylation products were identified by disk diffusion method. According to antibacterial results, 5-methyl substituted derivatives of 2-thiobarbituric acids were found to possess antibacterial activity, whereas 5,5-dimethyl derivatives of 2-thiobarbituric acids were found to be inactive against the specified bacteria. Among the 5-methyl substituted derivatives, the fluoro-substituted derivatives were found to be the most active.

Also, *ortho*-aryl-substituted thiobarbituric acid derivatives were found to exhibit more antibacterial effect against specified bacteria than 5-methyl-1-phenyl-2-thiobarbituric acid. So *ortho*-substituent effect on the antibacterial activity was proven and will be further studied. Furthermore, product 5-benzyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid does not possess any antibacterial effect against E. Coli and S. Aureus.

6.2. Future Works

Stereoselectivity of some products could not be accurately determined. Different chiral HPLC columns can be tried or the conditions can be changed in order to obtain better enantioseparation or diastereoseparation, hence to determine enantiomeric and diastereomeric excesses more accurately. Allylation products could not be obtained purely. Different purification procedures, different recrystallization solvent or column chromatography can be used to obtain purer allylation products. Also, allylation reactions can be perfomed for a longer time and different reaction solvents can be used instead of tetrahydrofuran. Antibacterial activity of only one product was determined. Antibacterial activities of all alkylation products can be tested. Furthermore, different derivatives of thiobarbituric acids may be synthesized regarding the enantioselectivity and antibacterial test results. An accurate comparison of the antibacterial activity of 5-methyl- and 5,5-dimethyl-1-phenyl-2-thiobarbituric acid and 5-methyl-, 5,5-dimethyl-1-(*o*-aryl)-2-thiobarbituric acids cannot be made, since the amounts of the compounds to be tested cannot be measured in the applied tests. Therefore, minimum inhibitory concentrations of 5-methyl, 5,5-dimethyl-1-phenyl-2-thiobarbituric acid, 5-methyl-, and 5,5-dimethyl-1-(*o*-aryl)-2-thiobarbituric acids against specified bacteria cannot be calculated, but in future studies they can be calculated to make more accurate antibacterial activity comparison of the thiobarbituric acid derivatives.

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APPENDIX A: HPLC ANALYSES OF THIOUREA DERIVATIVES

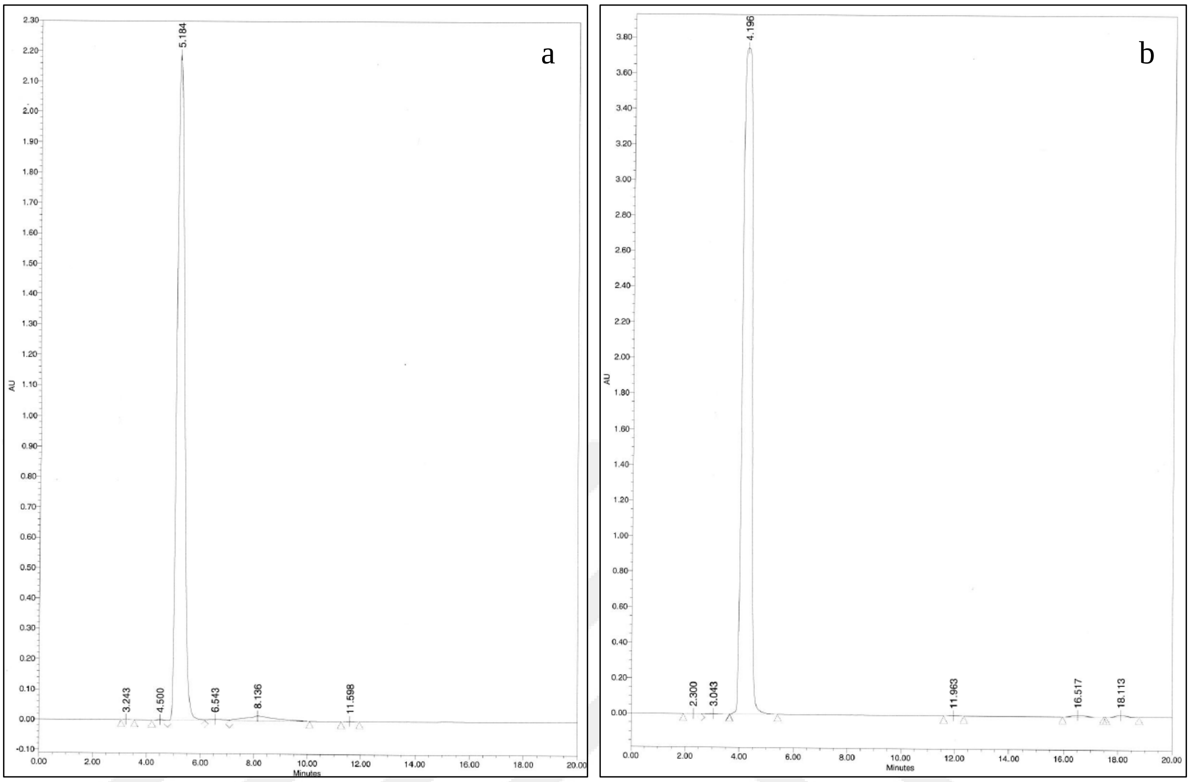

Figure A.1. Reversed phase HPLC result of a: *o*-chlorophenylthiourea, b: *o*-fluorophenylthiourea; column: X-Bridge; mobile phase: 50:50 (methanol-water); flow rate: 0.5 mL/min

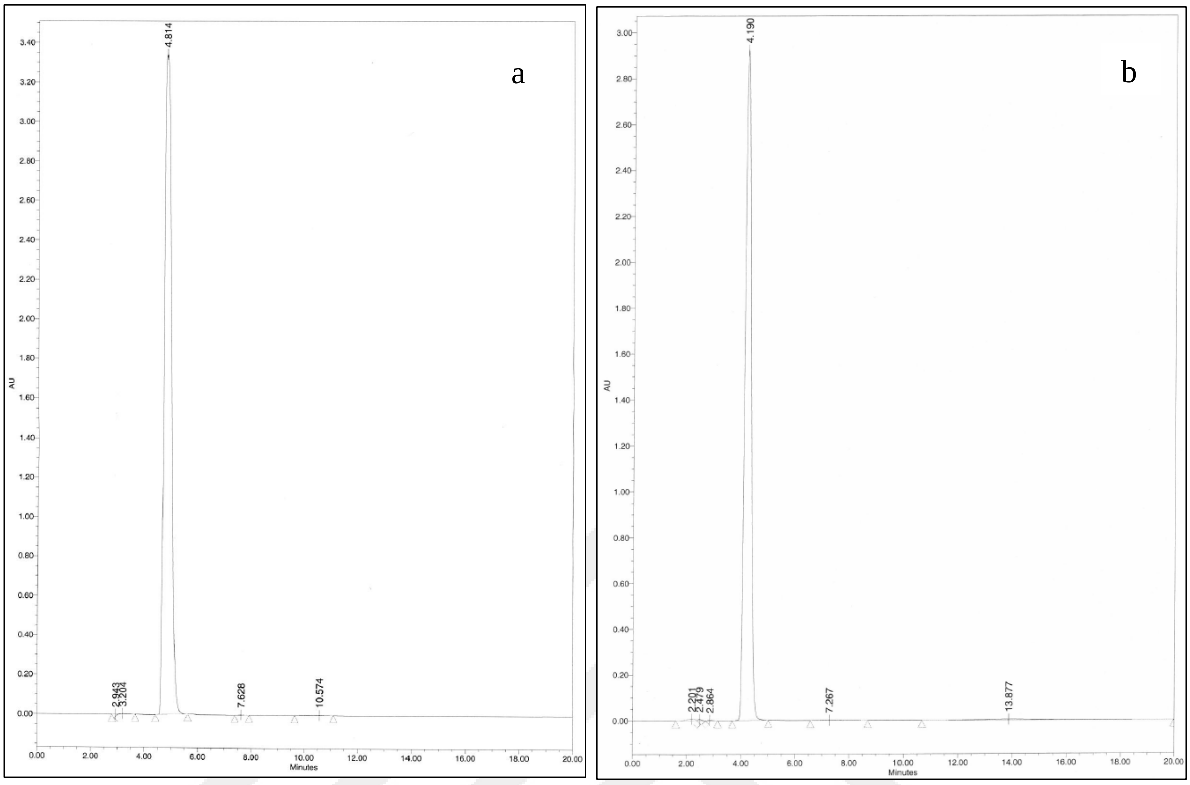

Figure A.2. Reversed phase HPLC result of a: *o*-tolylthiourea, b: phenylthiourea; column: X-Bridge; mobile phase: 50:50 (methanol-water); flow rate: 0.5 mL/min

APPENDIX B: REVERSED HPLC ANALYSES FOR THIOBARBITURIC ACID DERIVATIVES

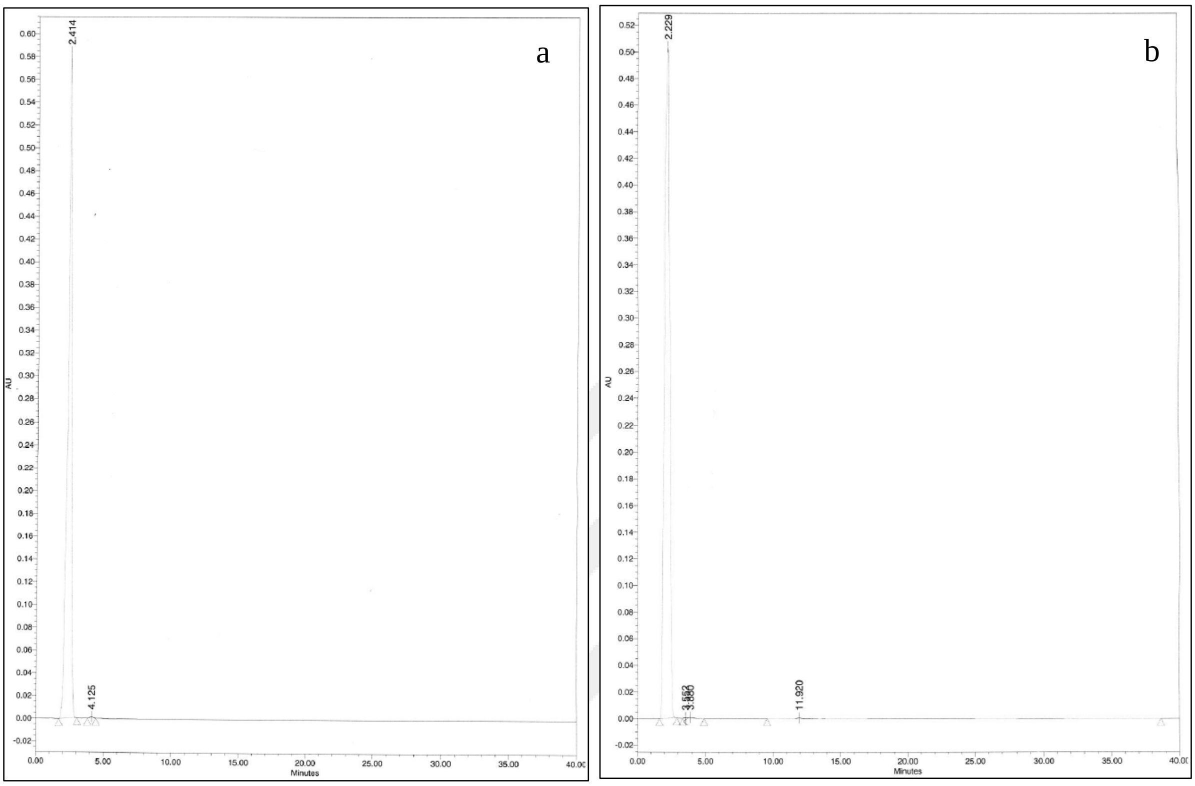

Figure B.1. Reversed HPLC analysis of a: 5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid, b: 5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid ; column: X-Bridge column; mobile phase: 50:50 (methanol-water); flow rate: 0.6 mL/min

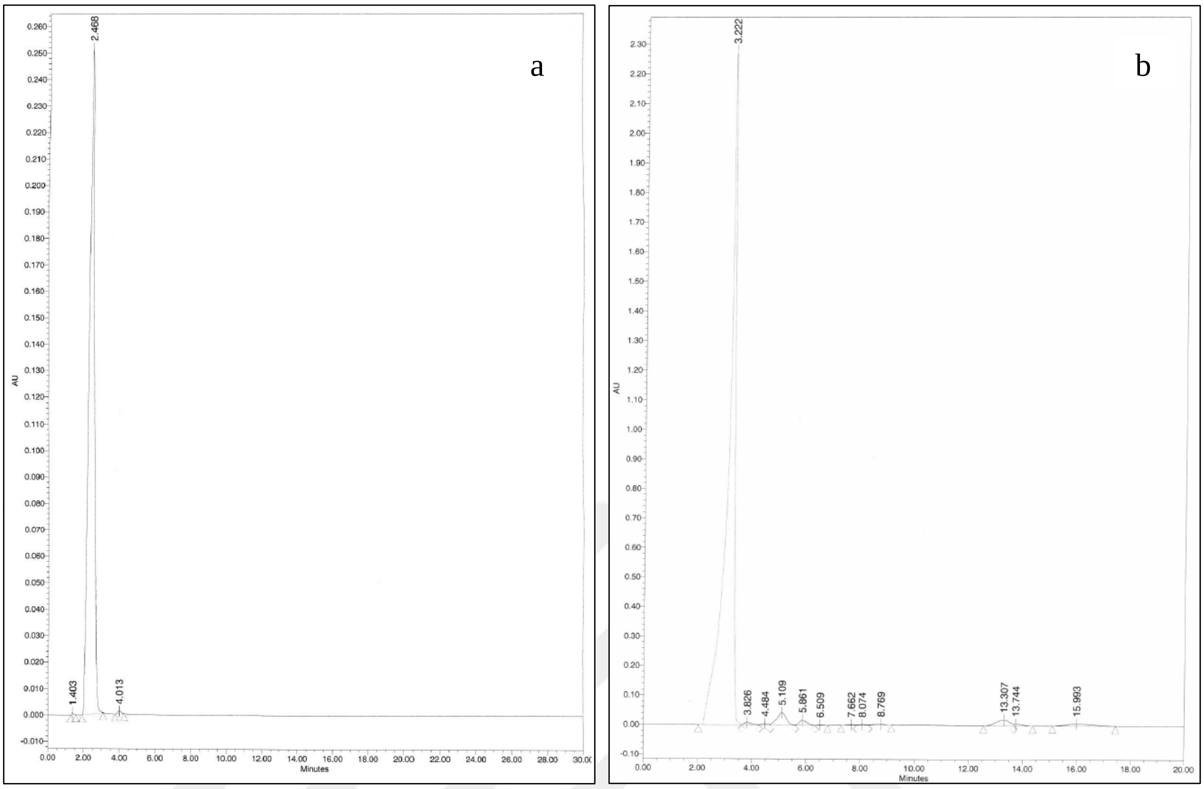

Figure B.2. Reversed HPLC analysis of a: 5-methyl-1-(*o*-tolyl)-2-thiobarbituric acid (flow rate: (0.6 mL/min), b: 5-methyl-1-phenyl-2-thiobarbituric acid (flow rate: 0.5 mL/min); column: X-Bridge column; mobile phase: 50:50 (methanol-water)

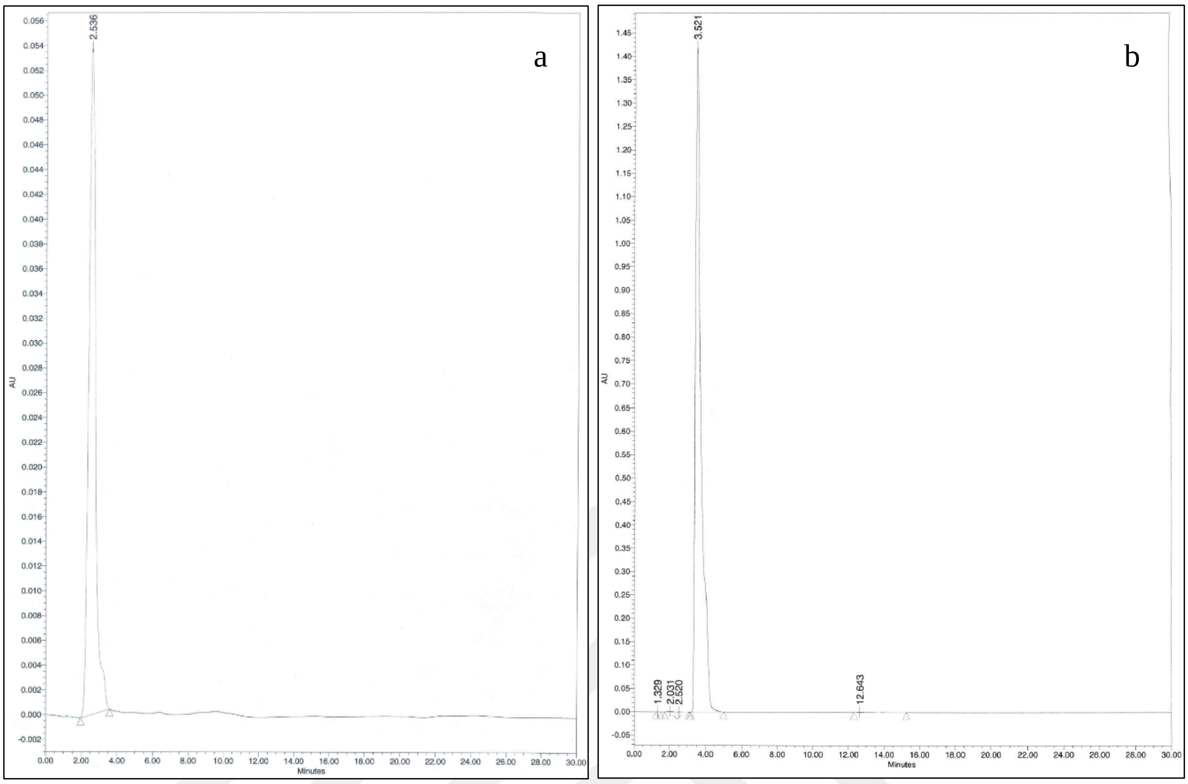

Figure B.3. Reversed HPLC analysis of a: 5,5-dimethyl-(*o*-chlorophenyl)-2-thiobarbituric acid, b: 5,5-dimethyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid; column: X-Bridge column; mobile phase: 50:50 (methanol-water); flow rate: 0.6 mL/min

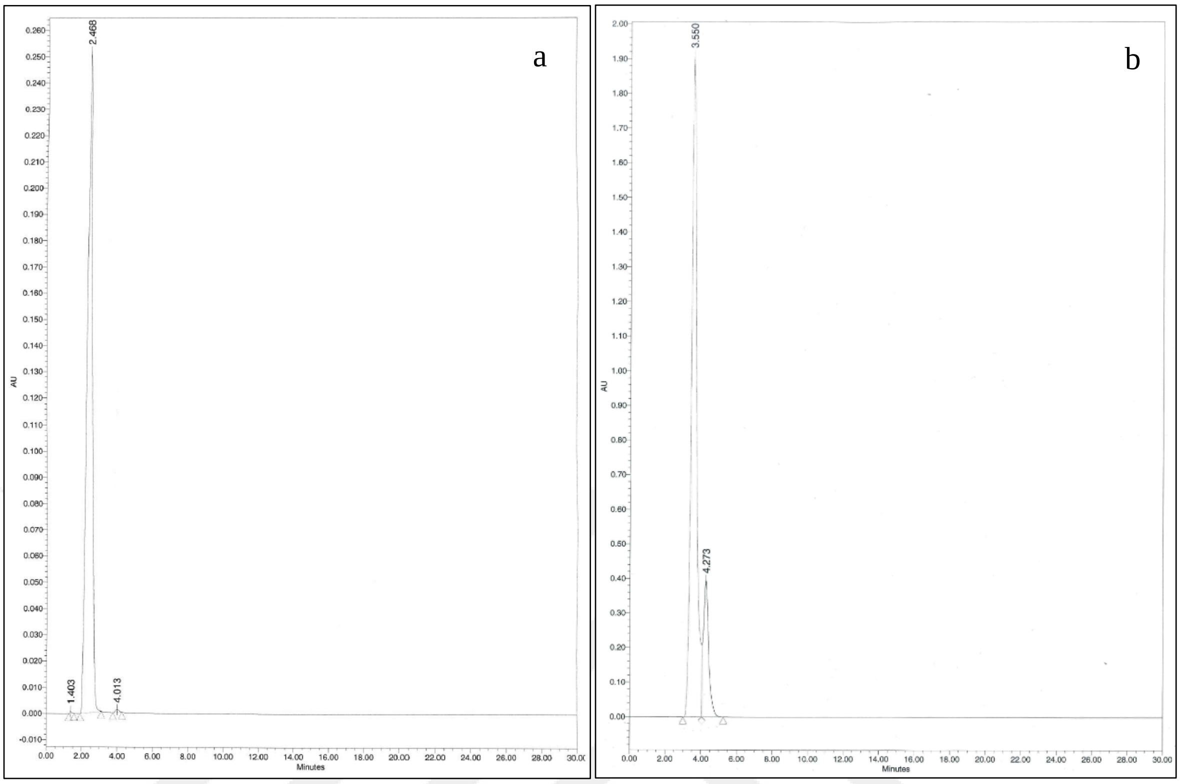

Figure B.4. Reversed HPLC analysis of a: 5,5-dimethyl-1-(*o*-tolyl)-2-thiobarbituric acid, b: 5,5-dimethyl-1-phenyl-2-thiobarbituric acid; column: X-Bridge column; mobile phase: 50:50 (methanol-water); flow rate: 0.6 mL/min

APPENDIX C: ^{1}H NMR SPECTRA OF BENZYLATION REAGENTS

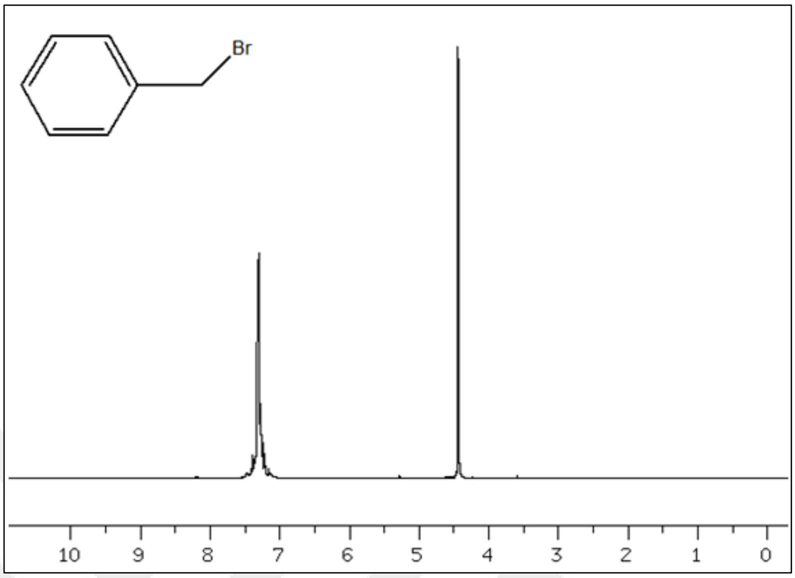

Figure C.1. ^{1}H NMR spectrum of benzyl bromide [86]

APPENDIX D: [1]H NMR SPECTRA OF BENZYLATION REACTIONS

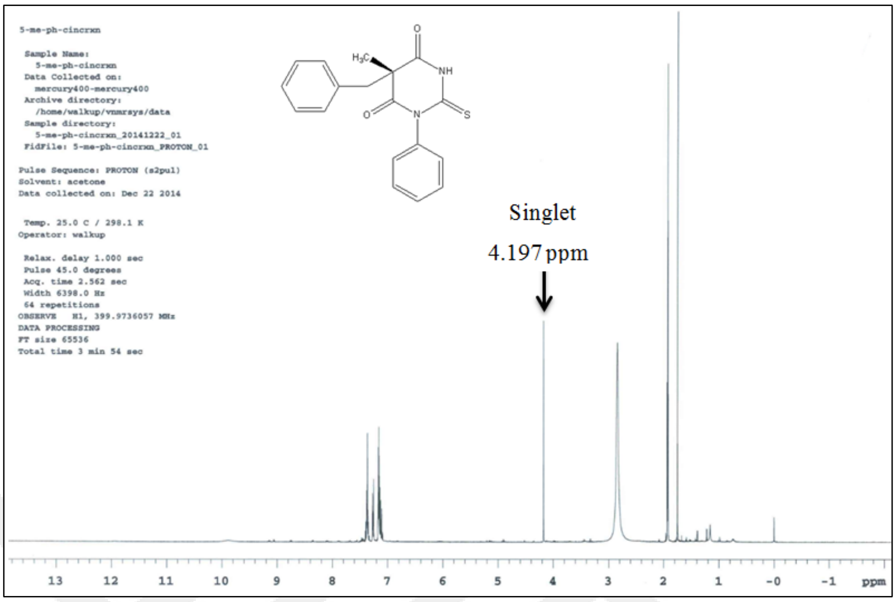

Figure D.1. [1]H NMR spectrum of 5-benzyl-5-methyl-1-phenyl-2-thiobarbituric acid ($C_{18}H_{16}N_2O_2S$), NMR solvent: acetone-d_6

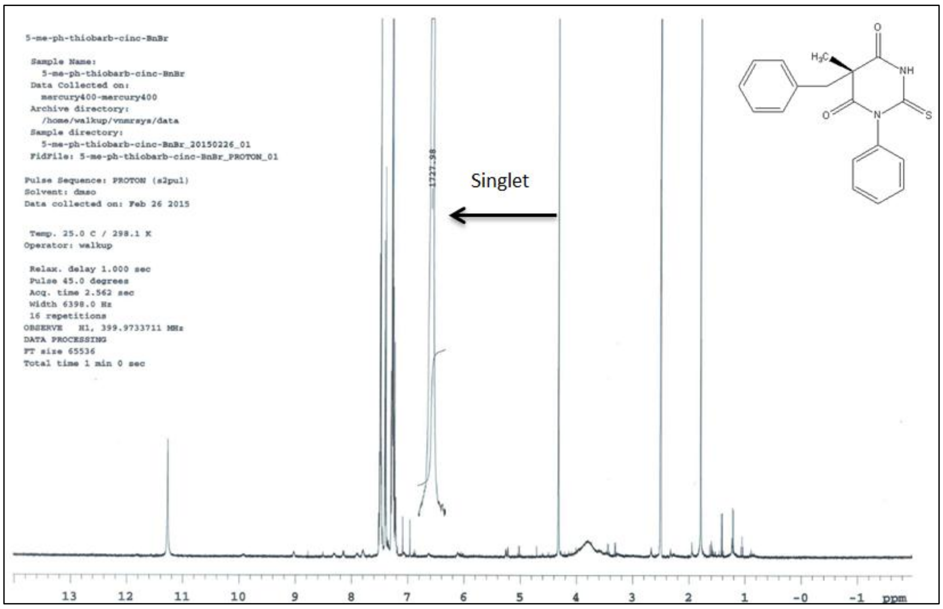

Figure D.2. [1]H NMR spectrum of 5-benzyl-5-methyl-1-phenyl-2-thiobarbituric acid ($C_{18}H_{16}N_2O_2S$), NMR solvent: dimethyl sulfoxide-d_6

APPENDIX E: ^{1}H NMR SPECTRA OF ALLYLATION REACTIONS

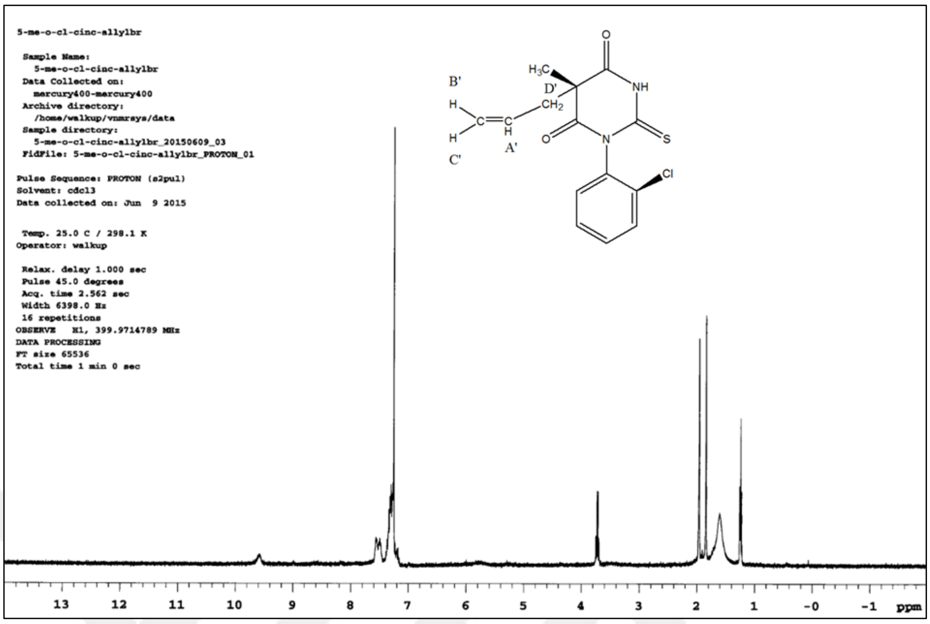

Figure E.1. ^{1}H NMR result of 5-allyl-5-methyl-1-(*o*-chlorophenyl)-2-thiobarbituric acid after recrystalization, NMR solvent: chloroform-d

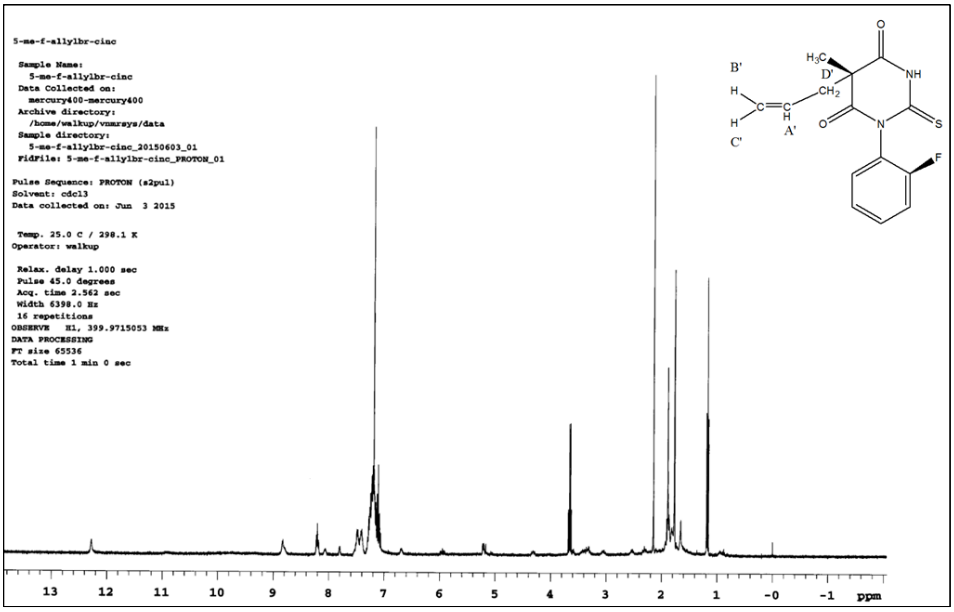

Figure E.2. ^{1}H NMR result of 5-allyl-5-methyl-1-(*o*-fluorophenyl)-2-thiobarbituric acid after recrystalization, NMR solvent: chloroform-d

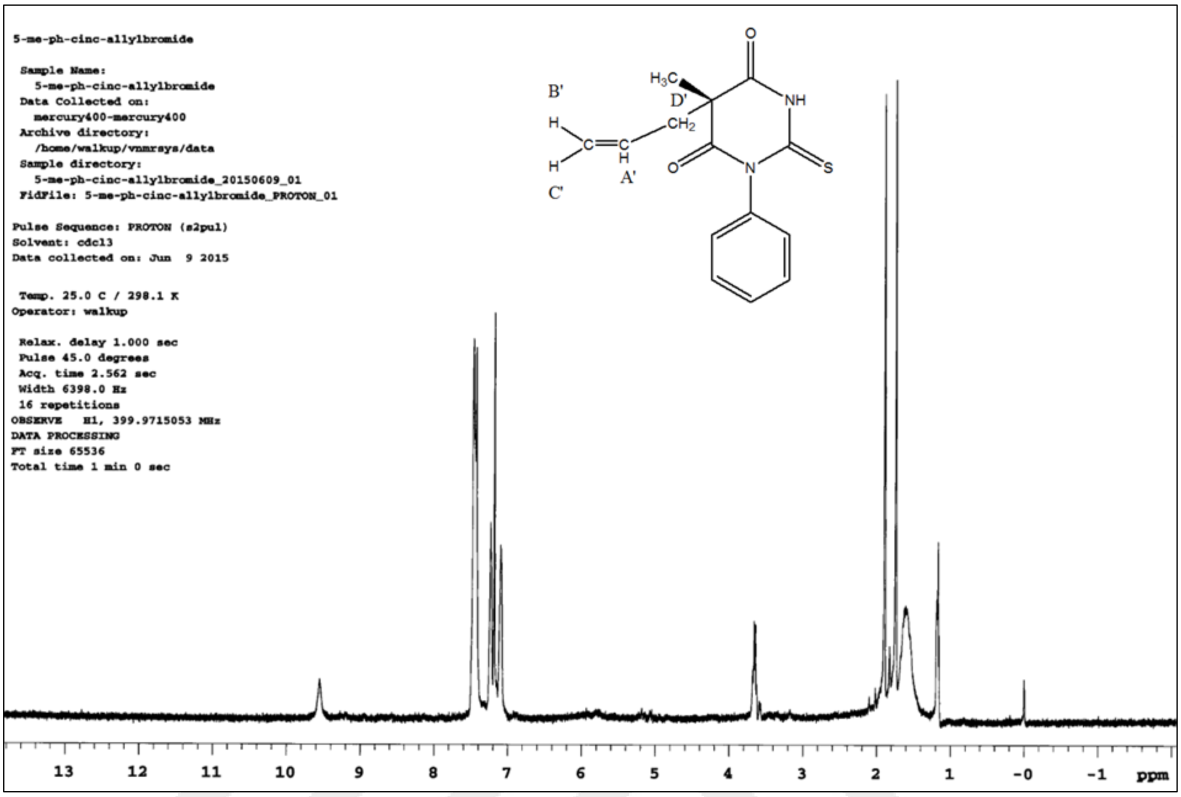

Figure E.3. [1]H NMR result of 5-allyl-5-methyl-1-(phenyl)-2-thiobarbituric acid after recrystalization, NMR solvent: chloroform-d