Experimental Procedures to Organic Synthesis of 2- Hydroxyalkyl Dithioanilines

Amit Kumar(M.Sc Chemistry), Dr.Amit Kumar (Associate Professor & Head)
Deptt of Chemistry
JS university, Shikohabad

Abstract:
As a result of the significance of the antifungal activity of thio compounds, the researcher in this study designed and synthesised a novel aniline compound, "2-Hydroxyalkyl dithio aniline" that contained Sulphur-sulphur groups with alkyl moieties. Their goal was to discover potent antifungal and antiviral agents from these compounds. Infrared spectroscopy and elemental analyses both backed up their constructions. Computational chemistry was utilized in the process of developing the product. In this paper, we also discussed computational chemistry and highlighted how it might be useful in the theoretical creation of new compounds. Specifically, author focused on the latter topic. Since the beginning of the 1980s, too develop tiny molecules with great therapeutic promise, computer-aided drug discovery as well as design technologies have proved crucial. Different approaches may be classified as either structure-based or ligand-based. Having knowledge of the target as well as ligand structures is crucial for structure-based techniques, just as it is for high-throughput screening. Methods like as ligand docking, pharmacophore, and ligand design fall under the category of structure-based techniques. In this study, we also explored the mechanism of antimicrobial and antiviral action, which is how it demonstrates its therapeutic potential.

Keywords: 2-Hydroxyalkyl dithio aniline; Computational chemistry; Infrared spectroscopy; antifungal activity; ligand docking.

1. Introduction

In living organisms, sulfur is one of the most fundamental elements and the seventh most abundant mineral in the human body. Sulfur belongs to chalcogens, elements of the 16 group of the periodic table, which display the awesome characteristic of having a variety of redox states and redox potentials allowing them to form inter-chalcogen bonds and atom exchange reactions, giving rise to a vast number of sulfur species that take part in biological processes. Noteworthy, the bulk of biomolecules consists only of carbon, hydrogen, nitrogen, and oxygen atoms, and the presence of sulfur accounts for the distinctive properties of sulfur compounds. Actually, sulfur and oxygen belong to the same group in the periodic table; however, Met and Cys analogues with the sulfur atom replaced by oxygen do not serve the same function. Sulfur has unique characteristics that differentiate it from oxygen. The increased atomic size confers to sulfur a lower electronegativity than oxygen. The thioether (R_2S) moiety of Met is more reactive than the analogue ether (R_2O). Thioethers can form sulfonium ions (R_3S^+) by donating electrons to other organic species thanks to their ability to sink electrons and stabilize a negative charge on a neighboring carbon (Ward & DeNicola, 2019). These compounds undergo sequential oxidation. to sulfoxides (R_2SO) and sulfones (R_2SO_2), conferring to these derivatives novel unexpected roles. In cell metabolism, a sulfonium compound such as S-adenosylmethionine (SAM) mediates most biochemical methylation reactions. It is doubtful whether other amino acid derivatives or other "-onium" compounds could play this role: quaternary ammonium compounds are unable to effectively methylate acceptor compounds, and oxonium compounds, such as a hypothetical oxygen analogue of SAM, would produce such a powerful methylating agent that it would methylate cellular nucleophiles without the need for an enzyme [1] and [2]. The sulfur compounds contained in food are amino acids or vitamins including

methionine (Met), cysteine (Cys), homocysteine (HCy), cystine (Cys-Cys), taurine (Tau), lipoic acid, thiamine, and biotin as well as the glucosinolates and allylic sulfur compounds that are contained in cabbage and cauliflower (cruciferous vegetables). The amount of sulfur compounds in food greatly varies depending on the type of food: 8% for egg white, 5% for beef as well as for chicken and fish, and 4% for dairy products and plant protein [3]. The recommended dietary allowance (RDA) for sulfur has been estimated to be 13-14mg/kg of body weight per day. Considering 70kg weight for a person, not affected by sex or age, this means 1.1g of sulfur per day. Among the sulfur compounds ingested with food, Met and Cys represent the largest part and are extensively metabolized by the organisms [4].The Met/Cys ratio in food is 3/1 for dairy products, fish, and meat and 4/3 for eggs and plant products such as soybeans [5][6]. Met is an essential amino acid assumed by diet and cannot be synthesized contrary to nonessential Cys. Numerous key metabolic intermediates such as HCy, Cys-Cys, and Tau are generated by these sulfur amino acids[3][7]. Throughout the transsulfuration pathway, Met can be converted to Cys with a yield depending on cell needs. Interestingly, both these two sulfur amino acids cannot be stored as such in the body, but cysteine can be stocked as glutathione (GSH) and sulfur excess is promptly excreted in the urine after its oxidation to sulfate or reabsorbed if required [8].

Organosulfur compounds can be derived from both plant and animal sources; sulfur is essential for life, and these compounds are abundant in nature. According to the iron–sulfur world hypothesis, it even played a key role in the evolution of life [9]. The most common source of sulfur for humans is through a diet composed of broccoli, cauliflower, cabbage, garlic, onion, meat, eggs, and fish [10]. Organosulfur compounds can be classified based on the functional groups to which sulfur is attached. The allium genus of flowering plants, which includes garlic and onions, contains important compounds such as cysteine sulfoxides and g-glutamylcysteines. The hydrolysis of cysteine sulfoxides accounts for the flavor and pungency of garlic and onions [10]. The cysteine sulfoxides are of four types: alliin, methiin, propiin, and isoalliin. Onions are especially rich in isoallin, whereas garlic is rich in alliin. Alliin is converted into allicin by a hydrolyzing enzyme when garlic cells are crushed during chewing or cooking. Allicin is a very unstable compound that can be readily converted to more stable compounds depending on the conditions; for example, it is converted to dithiin when extracted with oil, ajoene when extracted with ethanol, and diallyl disulfide/diallyl trisulfide or S-allylcysetine/S-allylmercaptocysteine when extracted with aqueous solutions. Numerous essential biochemical processes in prokaryotic and eukaryotic cells are tightly linked to this particular element. Enzymes not only harbor iron sulfur clusters but also depend on cofactors that contain sulfur, such as thiamine, molybdopterin, biotin, and lipoic acid[11]. Furthermore, many biosynthetic building blocks are activated as coenzyme A thioesters, and important detoxification processes, such as the conversion of cyanide to isothiocyanate [12] and the conjugation of electrophilic toxins to glutathione and related compounds, depend on sulfur. Thio modifications are also important for stabilizing the tRNA structure and for accurate and efficient translation [13] Sulfur's prime position in primary metabolism is indisputable, which is reflected by a large body of knowledge that has been presented in various review articles [13]. In contrast, the role of sulfur in natural product biosynthesis has been somewhat neglected. One may recall that intermediates of fatty acids, polyketides, and nonribosomal peptides are tethered to phosphopantetheinylated carrier proteins through thioester bonds [14], as well as that *S*-adenosylmethionine is an important cofactor of methylations. Even so, apart from cysteine and methionine residues in peptides, secondary metabolites are mainly composed of carbon, hydrogen, oxygen, and nitrogen—whereas sulfur atoms are scarce. However, there are innumerable examples of natural products containing

diverse sulfur moieties that are pivotal for biological function, and, as might be expected, varied enzymatic mechanisms have evolved for the formation of the carbon–sulfur bonds in these molecules.Over the past decade, our understanding of enzymatic C–S bond formations in natural products has dramatically improved. Studies at the genetic, biochemical, and chemical levels have elucidated the sources of sulfur, the reaction mechanisms, and the types of biocatalysts involved. As in primary metabolism, persulfidic sulfur (R–S–SH) and thiocarboxylate groups on sulfur-donor proteins represent major ionic sulfur sources. In addition, thiols of cysteine and glutathione—and even sulfur dioxide—may serve as S-donors in secondary metabolism. Besides substitution reactions with sulfur nucleophiles, (conjugate) additions, and radical reactions are frequently observed.

Aniline is a simplest aromatic amine compound consisting of a phenyl group attached to an amino group. It has an industrially significant commodity chemical, as well as a versatile starting material for fine chemical synthesis. It is mainly used in manufacturing of precursors to polyurethane, dyes, and other industrial chemicals. It smells like odor of rotten fish like other volatile amines. Chemically, it is an electron-rich benzene derivative as it is also prone to oxidation: while freshly purified aniline is an almost colorless oil, exposure to air results in gradual darkening to yellow or red, due to the formation of strongly colored, oxidized impurities. It can be diazotized to give a diazonium salt, which can then undergo various nucleophilic substitution reactions. There are many analogues known where the phenyl group is further substituted. These include toluidines, xylidines, chloroanilines, aminobenzoic acids, nitroanilines, and many others. They often are prepared by nitration of the substituted aromatic compounds followed by reduction. For example, conversion of toluene into toluidines and chlorobenzene into 4-chloroaniline. Addition of aniline with carbon disulfide leads to the formation of Dithio aniline.
The remaining part of this article is divided into two different sections. Section 2 is on materials and methods, including experimental datasets, the proposed experiments, and experimental setup. In section 3 of this article, the obtained results from the experiment based on various parameters are presented. And finally, section 4 describes the conclusion of the experiment.

2. Material and Method

The study's primary aim is the production of a novel chemical which is 2-hydroxyalkyl dithio aniline. For this synthesis author has worked based on different strategies such as

I. Certain chemical compounds have a relationship to ordinary differential reaction and partial differential reaction.
II. Computer approaches, including chemistry lab, to tackle the problem of diverse processes involving chemical reactions and the data was collected from different organizations.

The organizations under consideration for the data collection are

A. Science Library and different Science universities like DCRUST university sonipat (Haryana), MDU rohtak (Haryana) etc.
B. “Digital library and chemistry lab, in dept. of chemistry, JS university shikohabad”.

Further for the synthesis of the compound author has followed different criteria such as

I. Approached the literature review to know about the synthesis of compound

II. Visited the library and research centers

III. Had a research on how to establish a new chemical compound

IV. CHMLAB was used to solve the governing reaction.

The different chemicals that were taken to conduct the study are

- Aniline
- Carbondisulfide
- Propylene oxide (oxirane)
- Aldehyde
- Ether
- Ketone

The author worked on 2-hydroxyalkyl dithio-aniline in this work and considered its applications such as fungicides and viral illnesses. It is also used in polyurethane production. Many research papers, journals, and review papers have been published on this issue and may be found on the Science Direct and Bentham Science websites. After the rigorous process, the author came to this particular problem area , which is mostly concerned with the treatment of viruses and fungicides. During the course of our investigation, we learned that working in a chemical lab requires caution. As per the findings, this chemical compound can be completed in two phases. The first step is to react aniline with carbon disulfide, resulting in the chemical dithio-aniline. After that, in its second phase, dithio-aniline interacted with propylene oxide (oxirane) at low temperatures to generate the 2-hydroxyalkyl dithio-aniline molecule.

The treatment of fungicides and viral illnesses is the major focus of this topic. The author searched for its application in the rubber and chemical industries. They're used in the dyeing process. " An important issue in green chemistry that is currently receiving increased attention is the use of alternative reaction media that circumvent the problems associated with many of the traditional toxic and volatile organic solvents. As part of our research to develop practical, simple, and green methodologies in organic synthesis, the author has described an efficient, catalyst-free synthesis of 2-hydroxyalkayl dithio-aniline from aniline and carbon disulfide in this work.

The followings are the methodologies by which author has supposed to develop this particular compound.

I. General Experimental Procedure for the Preparation of 2-Hydroxyalkyl Dithioanilin

II. Preparation of 2-Hydroxyalkyl dithio aniline

III. Preparation of 2-Hydroxyalkyl dithio aniline from aldehyde

IV. Preparation of 2-Hydroxyalkyl dithio aniline from Ketone

3. Experiment Setup for the formation of 2-Hydroxyalkyl dithio

In this section, the detailed description of various method adopted for the formation of 2-Hydroxyalkyl dithio has been described in detail.

3.1 General Experimental Procedure for the Preparation of 2-Hydroxyalkyl Dithioaniline

In general experimentation for the preparation of 2-Hydroxyalkyl Dithioaniline, after slowly adding the amine (1.2 mmol) to a solution of carbon disulfide (1.3 mmol) in EtOH (1.5 ml), the mixture was stirred at room temperature for 15 minutes before the addition of the epoxides (1.0 mmol) in one portion. The reaction was then allowed to proceed at room temperature for a set period of time. Upon completion of the reaction, ethanol was allowed to evaporate, 10 mL of ethyl toluene was added, the mixture is washed with 2.5 mL of water, and the organic layer was dried (Na2SO4). Preparative TLC (silica gel: eluent, n-hexane/EtOAc = 2:1) was used to remove impurities from the crude mixture after the solvent had been evaporated at reduced pressure.

It was discovered that the expected dithioaniline could be obtained in a very high yield of 92 percent in a very short amount of time through the straightforward initial mixing of benzylamine (1.2 mmol) and carbon disulphide (1.3 mmol) in ethanol (1.5 mL) at room temperature. This was followed by the addition of the epoxide (1.0 mmol) at that temperature. However, despite being carried out in water and subjected to the identical reaction conditions as described above, the reaction did not produce the anticipated result after 3.5 hours had passed. Under the circumstances indicated above, a variety of amines (primary, secondary, benzylic, and aromatic) in addition to epoxides were investigated in order to investigate the generalizability and applicability of this approach.

3.2 Preparation of 2-Hydroxyalkyl dithio aniline

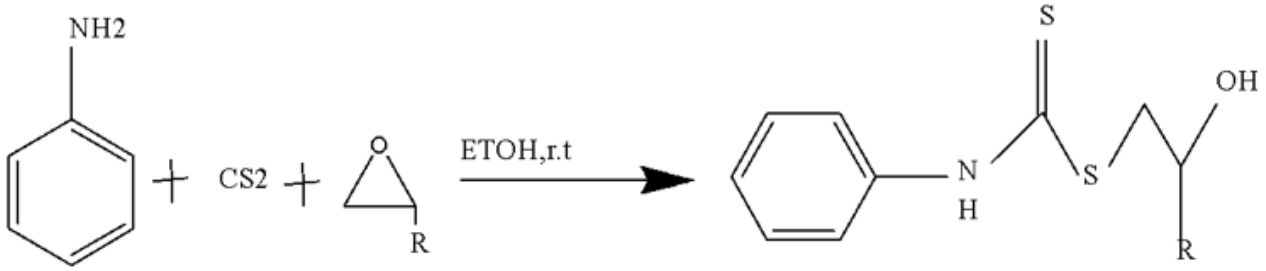

Figure 1: Preparation of 2-Hydroxyalkyl dithio aniline

The above reaction is the general reaction of synthesis of 2-hydroxyalkyl dithio aniline. The Aniline when reacted with carbondisulfide and propylene oxide it gives 2-hydroxyalkyl dithio aniline. This reaction was done in two steps. The following are the two steps:

Step-1: Preparation of dithioaniline

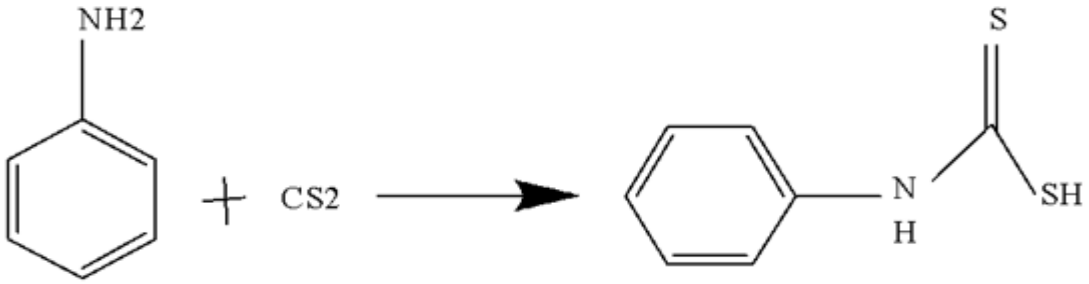

Figure 2: Preparation of dithioaniline

Step 2: Preparation of 2-Hydroxyalkyl dithio aniline

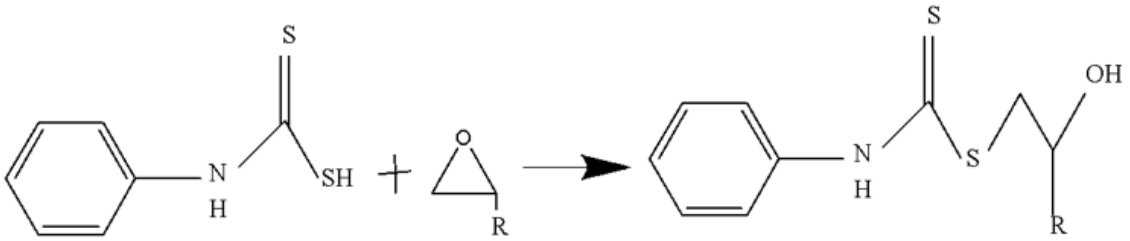

Figure 2: Preparation of full compound (2-Hydroxyalkyl dithioaniline)

The obtained dithio aniline in the step one reacts with propylene oxide in the presence of low temperature yields 2-Hydroxy alkyl dithio aniline.

By the above reaction it can be stated that the hydrogen group which binds to the sulfur moiety gets substituted to the oxygen to form hydroxy group.

3.3 Preparation of 2-Hydroxyalkyl dithio aniline from aldehyde

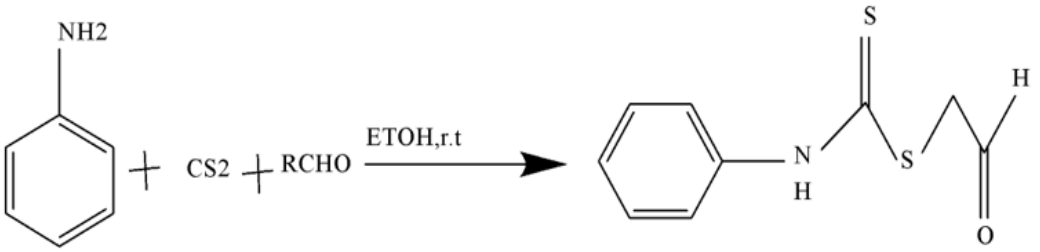

Figure 3: Preparation of 2-Hydroxyalkyl dithioaniline from aldehyde

By approaching to some of the chemical laboratories, the above reaction was obtained by researching, in which in the first step dithio aniline was found by the reaction of anline with carbon disulfide. Then they obtained intermediate was made to react with aldehyde to yield 2-Hydroxyalkyl dithio aniline.

3.4 Preparation of 2-Hydroxyalkyl dithio aniline from ether

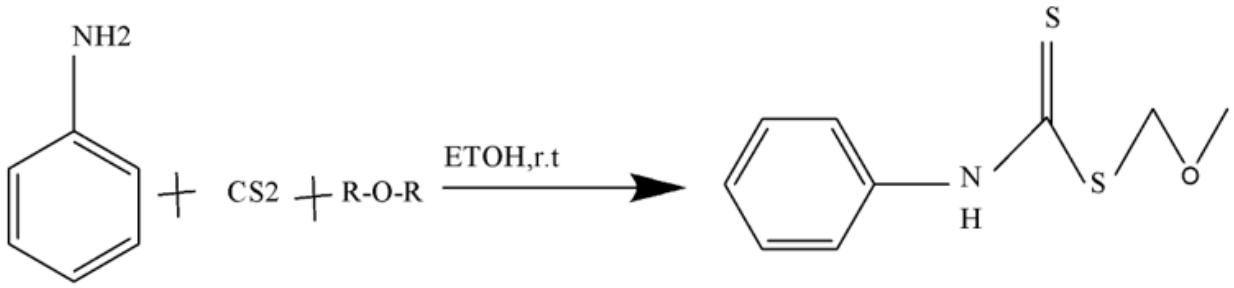

Figure 4: Preparation of 2-Hydroxyalkyl dithioaniline from ether

The aforesaid reaction was acquired by investigating it in a number of chemical laboratories, which is how the first stage of the process, in which dithio aniline was discovered by the reaction of anline with carbon disulphide, was accomplished. The resulting 2-Hydroxyalkyl dithio aniline was prepared by subjecting the intermediate that had been acquired to a reaction with ether.

3.5 Preparation of 2-Hydroxyalkyl dithio aniline from Ketone

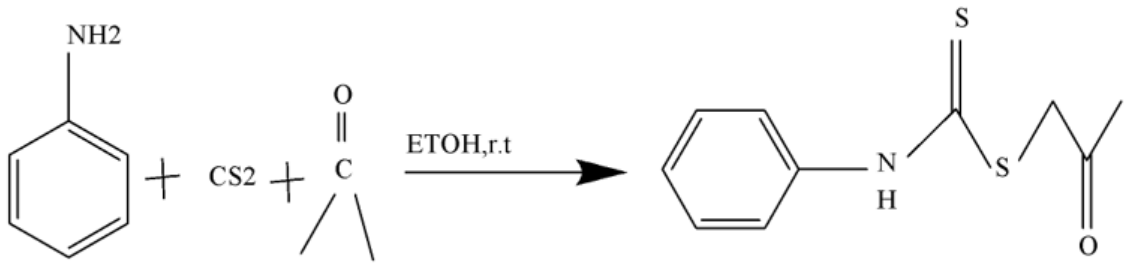

Figure 5: Preparation of 2-Hydroxyalkyl dithioaniline from Ketone

The first stage of the procedure, in which dithio aniline was found via the reaction of aniline with carbon disulfide, was completed by analyzing the aforementioned reaction in a variety of chemical laboratories. The resultant 2-Hydroxyalkyl dithio aniline was obtained by reacting the intermediate with ketone.

4. Experimental Results

The Buchi B-540 device was used to record the melting points, and these readings were not adjusted. An ABB FTLA 2000 was used as the recording device for the IR spectra. NMR spectra were obtained in CDCl3 by employing TMS as an internal standard and recording them with either a Bruker AQS-300 or Bruker DRX-500 spectrometer from Bruker, respectively, at nominal frequencies of 300 MHz and 500 MHz for proton or 75 and 125 MHz for carbon, respectively.

4.1 Selected Physical and Spectral Data

4.1.1 2-Hydroxypropyl n-Butyl dithioaniline

Colorless oil; IR (neat): νmax = 750, 922, 1039, 1125, 1406, 3224, 3373 cm–1; 1 H NMR (300 MHz,

CDCl3): δ = 0.88 (t, J = 7.2 Hz, 3H), 1.23 (d, J = 6.2 Hz, 3H), 1.34 (sext, J = 7.1 Hz, 2H), 1.58 (quin, J = 7.6 Hz, 2H), 3.13 (dd, J = 14.7, 7.1 Hz, 1H), 3.34 (dd, J = 14.7, 3.2 Hz, 1H), 3.5 (br s, 1H), 3.63 (q, J = 6.9 Hz, 2H), 4.06 - 4.09 (br m, 1H), 8.44 (br s, 1H). 13C NMR (75 MHz, CDCl3): δ = 13.6, 20.1, 22.4, 30.2, 43.4, 47.3, 67.7, 197.2.

4.1.2 2-Hydroxybutyl Pyrrolidine dithio aniline

Colorless oil; IR (neat): νmax = 750, 1219, 1432, 1463, 3404 cm–1; 1 H NMR (300 MHz, CDCl3): δ = 1.00 (t, J = 7.4 Hz, 3H), 1.60 (quin, J = 7.3 Hz, 2H), 1.97 (quin, J = 6.9 Hz, 2H), 2.09 (quin, J = 6.9 Hz, 2H), 2.56 (br s, 1H), 3.38 (dd, J = 14.3, 7.4 Hz, 1H), 3.66 - 3.75 (m, 3H), 3.82 - 3.86 (m, 1H), 3.93 (t, J = 6.9 Hz, 2H).

4.1.3 2-Hydroxybutyl Benzyl dithioaniline

Yellow oil; IR (neat): νmax = 703, 750, 938, 1094, 1234,1391, 1499, 3209, 3337 cm–1; 1 H NMR (300 MHz, CDCl3): δ = 0.91 (t, J = 7.3 Hz, 3H), 1.51 (quin, J = 7.4 Hz, 2H), 3.13 (dd, J = 14.7, 7.6 Hz, 1H), 3.36 (dd, J = 14.7, 3.1 Hz, 1H), 3.40 (br s, 1H), 3.72-3.77 (m, 1H), 4.84 (d, J = 5.3 Hz, 2H), 7.25-7.34 (m, 5H), 8.75 (br t, 1H); 13C NMR (75 MHz, CDCl3): δ = 10.0, 29.3, 41.8, 51.1, 72.9, 127.9, 128.2, 128.8, 136.2, 198.1.

4.1.4 2-Hydroxy-3-Phenoxypropyl Piperidine dithioaniline

Yellow oil; IR (KBr): νmax = 1601, 2926, 3404 cm–1; 1 H NMR (300 MHz, CDCl3): δ = 1.64 (br s, 6H), 3.62 (dd, J = 14.3, 6.9 Hz, 2H), 3.80 (dd, J = 14.5, 3.8 Hz, 1H), 3.86 (br s, 2H), 4.05 (t, J = 4.8 Hz, 2H), 4.2-4.31 (m, 3H), 6.90 - 6.96 (m, 3H), 7.23-7.28 (m, 2H); 13C NMR (75 MHz, CDCl3): δ = 24.2, 25.5, 26.0, 39.9, 51.6, 53.5, 69.6,70.5, 114.6, 121.1, 129.5, 158.5, 195.4.

5. Conclusion

In this study we performed the green synthesis of 2-hydroxyalkyl dithio aniline from the compound aniline with carbon disulphide. While studying the process of synthesis which is a novel drug we gone through several reaactions. The computational chemistry helped in many ways as described in the above sections in the synthesis and in this study the applications of the computational chemistry was also mentioned. The compound studied in this research is mainly used for the treatment of fungal infections and viral infections. Hence the mechanisms of the drug act as aantifungal and antivirus also described in the study. When compared to the other studies the 2-hydroxy alkyl di thio aniline produces good therapeutic efficacy as it is anovel drug the NMR studies were also conducted and the observations were recorded.

References

1. Brosnan, J. T., & Brosnan, M. E. (2006). The sulfur-containing amino acids: an overview. *The Journal of nutrition*, *136*(6), 1636S-1640S.
2. Griffith, O. W. (1987). Mammalian sulfur amino acid metabolism: an overview. *Methods in enzymology*, *143*, 366-376.
3. Nimni, M. E., Han, B., & Cordoba, F. (2007). Are we getting enough sulfur in our diet?. *Nutrition & metabolism*, *4*(1), 1-12
4. Griffith, O. W. (1987). Mammalian sulfur amino acid metabolism: an overview. Methods in enzymology, 143, 366-376.

5. Anderson, J. O., Warnick, R. E., & Dalai, R. K. (1975). Replacing dietary methionine and cystine in chick diets with sulfate or other sulfur compounds. *Poultry science*, *54*(4), 1122-1128.
6. Schutte, J. B., & Pack, M. (1995). Effects of dietary sulphur-containing amino acids on performance and breast meat deposition of broiler chicks during the growing and finishing phases. *British Poultry Science*, *36*(5), 747-762
7. Gruhlke, M. C., & Slusarenko, A. J. (2012). The biology of reactive sulfur species (RSS). *Plant Physiology and Biochemistry*, *59*, 98-107.
8. Tateishi, N., HIGASHI, T., NARUSE, A., HIKITA, K., & SAKAMOTO, Y. (1981). Relative contributions of sulfur atoms of dietary cysteine and methionine to rat liver glutathione and proteins. The Journal of Biochemistry, 90(6), 1603-1610.
9. Wächtershäuser, G. (2006). From volcanic origins of chemoautotrophic life to Bacteria, Archaea and Eukarya. Philosophical Transactions of the Royal Society B: Biological Sciences, 361(1474), 1787-1808.
10. Vazquez-Prieto, M. A., & Miatello, R. M. (2010). Organosulfur compounds and cardiovascular disease. Molecular Aspects of Medicine, 31(6), 540-545.
11. Richter, M. (2013). Functional diversity of organic molecule enzyme cofactors. *Natural product reports*, *30*(10), 1324-1345.
12. Cipollone, F., Cicolini, G., & Bucci, M. (2008). Cyclooxygenase and prostaglandin synthases in atherosclerosis: recent insights and future perspectives. *Pharmacology & therapeutics*, *118*(2), 161-180.
13. Shigi, N. (2014). Biosynthesis and functions of sulfur modifications in tRNA. Frontiers in genetics, 5, 67.
14. Beld, J., Sonnenschein, E. C., Vickery, C. R., Noel, J. P., & Burkart, M. D. (2014). The phosphopantetheinyl transferases: catalysis of a post-translational modification crucial for life. *Natural product reports*, *31*(1), 61-108.