T.C.
YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME SULFONAMIDE SUBSTITUTED SCHIFF BASE DERIVATIVES

MASTER OF SCIENCE THESIS

BY
ABDULMAJED BELHULA

ISTANBUL-2022

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

20.7. 2022

MAJED BELHULA

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TABLE OF CONTENT

THESIS APPROVAL FORM iii
DECLARATION iv
ACKNOWLEDGEMENTS v
LIST OF FIGURES viii
LIST OF SCHEMES x
LIST OF TABLES xi
ABBREVIATIONS xii
ABSTRACT xiv
ÖZET xv
1 INTRODUCTION AND PURPOSE 1
2 GENERAL INFORMATION 3
2.1 Schiff Bases 3
2.1.1 Schiff Base Synthesis 3
2.1.2 Biological activity of Schiff bases 5
2.1.2.1 Antibacterial Activity 5
2.1.2.2 Antifungal Activity 8
2.1.2.3 Anticancer activity 8
2.2 Sulfonamides 11
2.2.1 Sulfonamides as anticancer agents 13
2.2.2 Sulfonamides as antimicrobial agents 16
2.3 Sulfonamide substituted Schiff bases 21
2.3.1 Synthetic method 21
2.3.2 Biological activity 22
2.3.2.1 Bioactive sulfonamide Schiff base complex 22
2.3.2.2 Antibacterial activity 24
2.3.2.3 Antifungal activity 25
2.3.2.4 Anticancer activity 25
3 MATERIALS AND METHODS 31
3.1 Chemicals 31
3.2 Synthetic Procedures 31
3.2.1 General procedure for mono schiff base 31
3.2.2 General procedure for bis schiff base 31
3.2.3 General procedure for compound 3-8 31
3.3 Analytical Methods 32
3.3.1 Controls by Thin Layer Chromatography (TLC) solvent systems 32
3.3.2 Spectrometric Analyses 32
3.3.2.1 Infrared Spectra 32
3.3.2.2 ^{1}H-NMR Spectra 32
3.3.2.3 ^{13}C-NMR Spectra 33

3.3.2.4 LC-MS Spectra 33
3.4 Cytotoxicity screening with NCI-Sulforhodamine B assay procedure 33
4 EXPERIMENTAL DATA 34
5 RESULTS AND DISCUSSION 51
5.1 Chemistry 51
5.2 Biology 57
6 CONCLUSION 59
7 REFERENCES 60

LIST OF FIGURES

Figure 1.1 Overview of Schiff base reaction 1
Figure 2.1. Spectroscopy of Schiff base structure. 5
Figure 2.2. Chemical structure of N-(salicylaldehyde)-2-hydroxyaniline 6
Figure 2.3. Chemical structure of bioactive 5-chloro-salicylaldehyde Schiff base derivative 7
Figure 2.4. Compound S12. 8
Figure 2.5. Chitosan-Derived Schiff Base. R = H, OH 8
Figure 2.6. Schiff base derivatives compound 18-2. 9
Figure 2.7. Schiff base Compound SB-12. 9
Figure 2.8. Chemical structure of (VS5-e) 10
Figure 2.9. Chemical structure of compounds 21-17 and 21- 23 10
Figure 2.10. Chemical structure of antitumor Schiff bases 11
Figure 2.11. Prontosil structure 12
Figure 2.12. Example of bioactive sulfonamide derivatives 13
Figure 2.13. Chemical structure of e7070 sulfonamide 14
Figure 2.14. Chemical Structure of HMN-214 sulfonamide 14
Figure 2.15. Catalytic cycle of human carbonic anhydrase. 15
Figure 2.16. The binding mode of sulfonamide-based compound 16
Figure 2.17. Structures of several sulfonamide antimicrobial drugs 18
Figure 2.18. The similarity of sulfanilamide and p-aminobenzoic acid structures. 19
Figure 2.19 Schiff base bioactive complex 23
Figure 2.20. Example of bioactive sulfonamide subistituted shiff base complex. 24
Figure 2.21. 4-[(Hydroxybenzylidene)amino]-N-(pyrimidin-2-yl) benzenesulfonamides structure 25
Figure 2.22 Sulfamethoxazole sulfa drug structure 25
Figure 2.23. Sulfonamide subistituted Schiff base derivatives as anti-tubulin substances. 26
Figure 2.24. Structure of combretastatine- A4 and isoquinoline sulfamate. 26
Figure 2.25 2 Imine-based sulfonamide Coumarin derivatives 29

Figure 2.26 Sulfonamide Schiff base compound A-1 29
Figure 2.27 Schiff base against human breast cancer (MCF7) 30
Figure 5.1 Reaction mechanism for the synthesis of 2-((Z)-(2-aminophenylimino) methyl) phenol 52
Figure 5.2 Chemical structure of bis Schiff base 52
Figure 5.3 ^{1}H-NMR spectrum of compound 1 53
Figure 5.4 ^{13}C-NMR spectrum of compound 1 54
Figure 5.5 FT-IR spectrum of compound 1 54
Figure 5.6 FT-IR spectrum of sulfonamide derivatives 55
Figure 5.7 ^{1}H-NMR spectrum of sulfonamide derivatives 56
Figure 5.8 The ^{13}C-NMR spectrum of sulfonamide substituted schiff base derivative (4) 56
Figure 5.9 Decompositon of the sulfonated bis Schiff base derivative (8) 57

LIST OF SCHEMES

Scheme 2.1. Refluxing of (4-Methoxyphenyl)ethylamine with 3,5-dichlorosalicyla.ldehyde in ethanolic solution..... 3
Scheme 2.2. The solid-state synthesis of various benzylidene aniline derivatives by grinding together solid aniline and solid aromatic aldehydes..... 4
Scheme 2.3. Inhibition of DHF formation by sulfonamide 17
Scheme 2.4. Folic acid synthesis and sulfonamides site of action..... 20
Scheme 2.5. Synthesis of sulfonamide subistituted schiff base at room temperature 22
Scheme 2.6. Synthesis of sulfonamide subistituted schiff base derivatives using refluxing method..... 22
Scheme 2.7. Microwave synthesis of sulfanilamide-imine derivative 26
Scheme 5.1 Aqueous synthesis of the Schiff base 1 51
Scheme 5.2 The reaction of a sulfonyl chloride with Schiff base (1)..... 54

LIST OF TABLES

Table 2.1 Structure of sulfanilamide-immune derivatives with antitumor activity and binding free energies of docking study. 27

Table 5.1 Synthesized sulfonylamide substituted Schiff bases 54

Table 5.2 IC_{50} value obtained for the synthesized compounds (tested on different ovarian cancer cells). 57

ABBREVIATIONS

AAZ	Acetazolamide
CA	Carbonic anhydrase enzyme
CAI	Carbonic anhydrase inhibitors
d	Doublet
DCM	Dichloromethane
DMAP	Dimethylaminopiridine
DMSO	Dimethylsulfoxide
DHF	Dihydrofolate
EtOH	Ethanol
H37RV	Mycobacterium Tuberculosis Cells
hCA	Human carbonic anhydrase
HCC	Hepatocellular carcinoma
HeLa	Cervical Cancer Cell Line
HEPG2	Liver Carcinoma
IC_{50}	Inhibition Concentration which inhibits cell viability by 50%
LAMA-84	Peripheral Chronic Myeloid Leukemia Cells
LC-MS	Liquid Chromatography-Mass Spectrometry
m	Multiplet
MCF-7	Breast Carcinoma
MDR	Mediated drug resistence
MeOH	Methanol
MIC	Minimum inhibition concentration
µM	Micromolar
MRSA	Methicillin-resistant staphylococcus aerous
Mw	Molecular weight
NMR	Nuclear magnetic resonance
OPDA	o-phenylendiamine
ppm	Parts per million

PABA	P-aminobenzoic acid
R_f	Retention factor
rt	Room Temperature
SBDTC	S-benzyldithiocarbazate
SRB	Sulforhodamine B assay
t	Triplet
td	Triplet of Doublet
TEA	Triethylamine
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TNF	Tumor necrosis factor
w/v	Weight per volume
ZBG	Zinc binding group

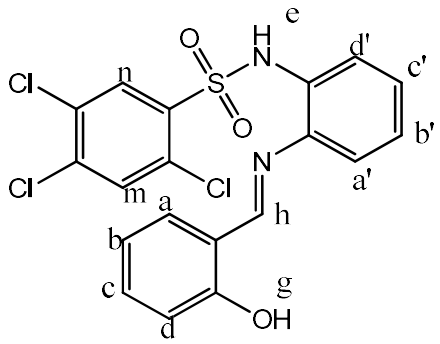
ABSTRACT

BELHULA, M. Synthesis and Biological Evaluation of Some Sulfonamide Substituted Schiff Base Derivatives. Yeditepe University, Institute of Health Sciences, Department of Pharmaceutical Chemistry, MSc Thesis, Istanbul, 2022.

Cancer is a dangerous life-threatening disease that involves uncontrolled cell development and their rapid spread, with the potential to harm normal tissues. Today, search and development of new anticancer agents are required. Recent studies demonstrated the promising antiproliferative activity of sulfonamide substituted Schiff base structures. In this work, we aimed to develop some novel sulfonamide substituted schiff base derivatives to evaluate their cytotoxic activity on a series of ovarian cancer cells.

The schiff bases were synthesized using o-phenylendiamine and salicylaldehyde The obtained Schiff bases were further treated with different sulfonyl chloride derivatives to form the targeted sulfonamide substituted compounds. Structure elucidation of the synthesized compounds was performed by FT-IR, ^{1}H NMR, ^{13}C NMR and LC-MS spectral analyses.

Compounds were then evaluated for their biological activity using the NCI-Sulforhodamine B assay on OVCAR-3, OVSAHO and KUROMOCHI and CAOV-3 ovarian cancer lines. Most of the compound showed moderate to no cytotoxicity against these ovarian cancer cell lines. Yet IC_{50} values of 16.99, 3.31, 17.55 and 6.53µM for compound **5** and 71.52, 9.87, 42.17,and 16.95µM for compound **7** respectively.



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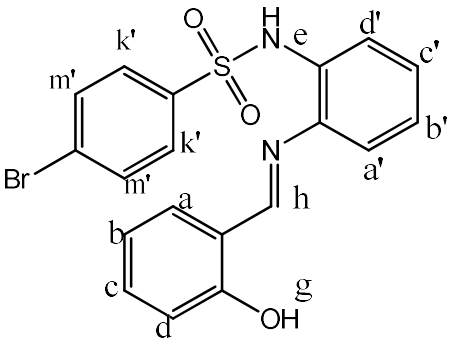

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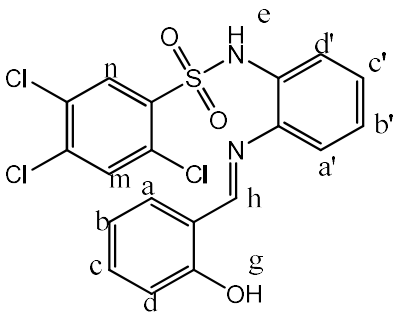
ÖZET

BELHULA, M. Yeni Sülfonamit Sübstitüe Şif Baz Türevlerinin Sentezi ve Biyolojik Değerlendirilmesi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Farmasötik Kimya Anabilim Dalı, Yüksek Lisans Tezi, İstanbul, 2022.

Kanser kontrolsüz hücre bölünmesi ve bu hücrelerin hızlı yayılmasıyla normal dokuyu tehdit eden ölümcül bir hastalıktır. Günümüzde kanserin tedavisinde kullanılacak yeni antikanser yapılarının geliştirilmesine ihtiyaç duyulmaktadır. Literatürde yer alan güncel çalışmalar bazı sülfonamit sübstitüe şif yapılarının kayda değer antiproliferatif etki gösterdiğini kanıtlamıştır. Bu bulgular ışığında, bu çalışmada yumurtalık kanseri hücre hatlarında etkileri değerlendirilmek üzere, bir dizi yeni sülfonamit sübstitüe şif baz yapıları geliştirilmesi hedeflernmiştir.

Şif baz yapıları, o-fenilendiamin ve salisilaldehitten yola çıkılarak elde edilmiştir. Oluşturulan imin yapısı bir dizi sulfonil klorür ile muamele edilmiş ve böylece hedeflenen sülfonamit yapılarının sentezi tamamlanmıştır. Elde edilen bileşiklerin yapıları FT-IR, ^{1}H NMR, ^{13}C NMR ve LC-MS spektral yöntemleriyle aydınlatılmıştır.

Yapıların biyolojik aktiviteleri OVCAR-3, OVSAHO, KUROMOCHI ve CAOV-3 hücre hatlarında Sulforhodamine B sitotoksisite testi aracılığıyla değerlendirilmiştir. Bileşik **5** ve **7** dışında kalan moleküllerin kayda değer bir etki göstermediği belirlenmiştir. Bileşik **5** için IC_{50} değerleri sırasıyla 16.99, 3.31, 17.55 ve 6.53µM, bileşik **7** için ise 71.52, 9.87, 42.17 ve 16.95µM olarak saptanmıştır.



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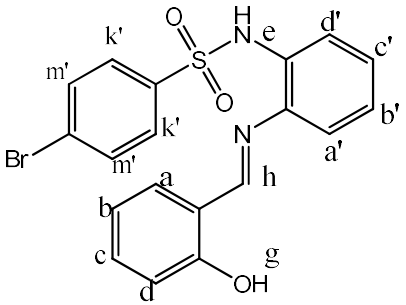


1 INTRODUCTION AND PURPOSE

Schiff bases are a class of synthetically available and structurally diverse chemicals made *via* condensation reactions of aldehyde, or ketone, with primary amines. Chemically, they have an azomethine (-C=N-) functional group that connects two or more physiologically active aromatic, heterocyclic scaffolds to form molecular hybrids. $R_2N = CR$, R_1 is the general formula for a Schiff base, where R, R_1, and R_2 are alkyl, aryl, heteroaryl, or cycloalkyl groups (Figure 1.1) [1].

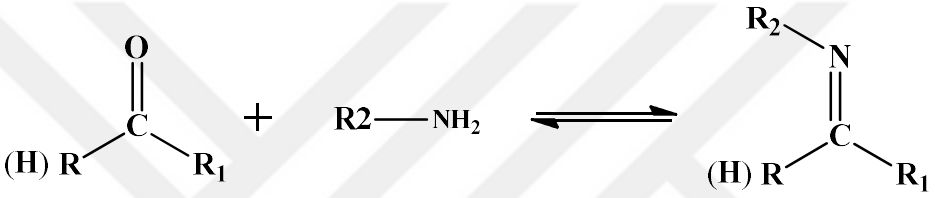


Figure 1.1 Overview of Schiff base reaction

The Schiff base reaction is also important in organic synthesis, bioprocess chemistry, and pharmaceutical chemistry. C=N addition processes, hetero Diels-Alder reactions, utilization of chiral salen metal complexes in asymmetric synthesis, and Staudinger reactions for the formation of lactams compounds are only a few of the reactions in which Schiff bases have been discovered to be particularly useful [2].

Sulfonamide substituted Schiff base derivatives have been gained interest due to their wide variety of biological activity including antibacterial activity [3], antifungal activity [4], and antitumor activity [5].

Imine-based sulfonamides can be made by using the traditional condensation process to treat a sulfonamide derivative containing at least one amine function with a variety of substituted benzaldehydes. The reaction can be carried out using catalytic amounts of acetic acid, and the reaction conditions can be adjusted by carrying out the process in varying polarity solvents. Under reflux conditions, ethanol was determined to be the best solvent for obtaining reasonable yields and a shorter reaction time [4].

The goal of this research was to make a series of sulfonamide substituted Schiff base derivatives and test their anticancer efficacy. Condensing *o*-phenylendiamine with

salicylaldehyde yielded two Schiff bases: 2((Z)-(2-aminophenylimino)methyl)phenol and 2,2'-(1E,1'E)-(1,2-phenylenebis(azan-1-yl-1-ylidene))bis(methan-1-yl-1-ylidene)diphenol The resultant bases were then treated with various arylsulfonylchloride derivatives under nitrogen atmosphere. Spectroscopic investigation (^{1}H-NMR, ^{13}CNMR, FT-IR, and LC-MS) was used to describe the generated compounds. On high-grade serious ovarian cancer cell lines OVCAR3, OVSAHO, and KUROMOCHI ve CAOV3, anticancer activity was examined using the NCI-Sulforhodamine B assay.

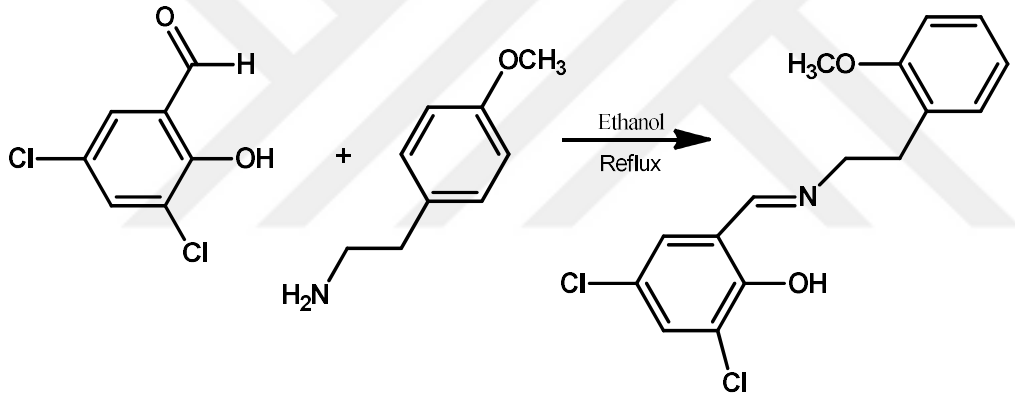
2 GENERAL INFORMATION

2.1 Schiff Bases

2.1.1 Schiff Base Synthesis

Schiff bases are traditionally made by refluxing aldehyde or ketones with primary amines in an organic solvent, such as ethanol or methanol, under controlled conditions. For example, treating the same combination at room temperature, refluxing the mixture in heptane in the presence of acetic acid, or azeotropping the mixture with benzene in an acidic Dean-Stark apparatus. To eliminate water generated during the process, molecular sieves and the addition of dehydrating solvents are described to be utilized. [2,6,7]

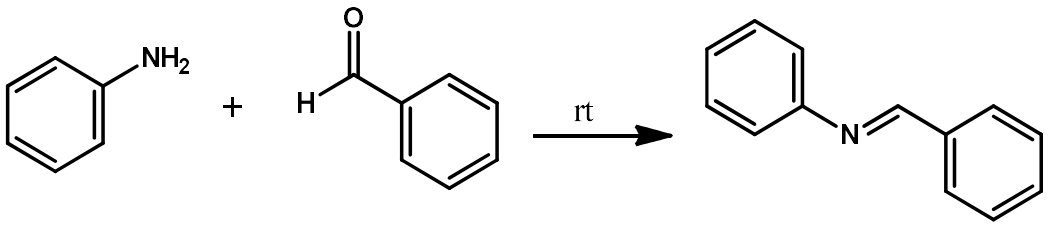
Aggoun et al., for example, have produced Schiff bases by refluxing 2-(4-Methoxyphenyl)ethylamine with 3,5-dichlorosalicylaldehyde in ethanolic solution, yielding the desired product with quantifiable yield (Scheme 2.1) [8].



Scheme 2.1. Refluxing of (4-Methoxyphenyl)ethylamine with 3,5-dichlorosalicylaldehyde in ethanolic solution

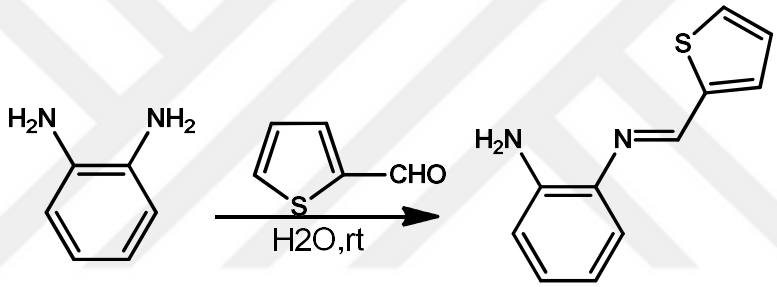
Furthermore, in recent years, ecologically friendly synthetic methods were developed lot, and some solvent-free protocols for synthesizing azomethine molecules have been established.

Schmeyers *et al*. for example, described the solid-state synthesis of different benzylidene aniline derivatives by grinding solid aniline and solid aromatic aldehydes and then slowly heating them at room temperature to remove the water produced. Crystal packing (Scheme 2.2) has been used to simplify this solid-solid reaction [9].



Scheme 2.2. The solid-state synthesis of various benzylidene aniline derivatives by grinding together solid aniline and solid aromatic aldehydes.

Rao and coworkers have described a novel and environmentally friendly condensation reaction technique (scheme 3) that allows the green synthesis of various Schiff's bases by stirring 1,2-diaminobenzene with various aromatic aldehydes in water as a solvent [10].



Scheme 2.3. A novel and eco-friendly condensation reaction method permitting the green synthesis of Schiff's bases.

The spectroscopic methods can detect the presence of a Schiff base in a structure. Indeed, between 8 and 9 ppm, the Schiff base proton (H-C=N) frequently shows as a singlet sharp peak. The azomethine carbon can also be identified via ^{13}C-NMR, which shows a signal of roughly 145-150 ppm. The C=N group can also be identified using an FT-IR spectrum, as the bond's vibration band produces a signal of approximately 1620 cm^{-1}. For example, the predicted singlet signal showed at 8.56 ppm in the ^{1}H NMR spectrum of a Schiff base produced by Fadhil *et al*. and the ^{13}C NMR data indicated a peak at 146.12 ppm attributed to the azomethine carbon (Figure 2.1) [11].

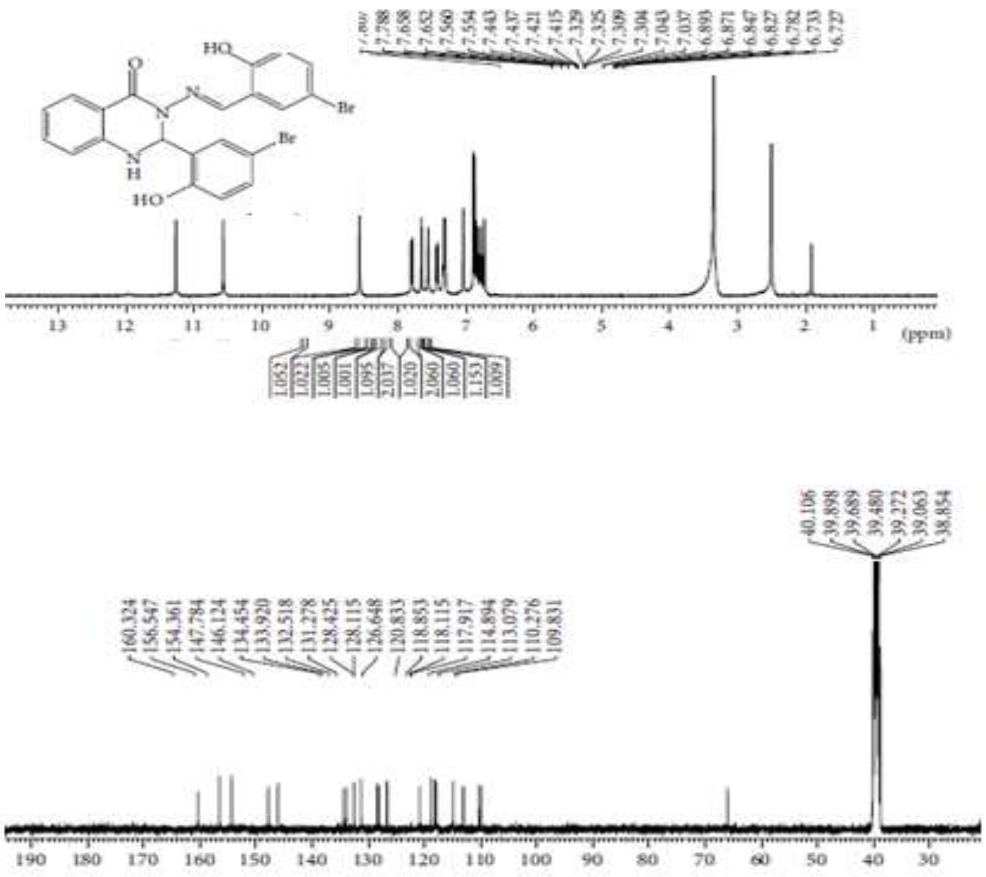


Figure 2.1. Spectroscopy of Schiff base structure**.**

2.1.2 Biological activity of Schiff base

Numerous investigations have been conducted to evaluate the biological activities of Schiff base derivatives. Some of them have been shown to have antibacterial, antifungal, and anticancer properties.

2.1.2.1 Antibacterial Activity

De Souza *et al.* found that the Schiff base N-(salicylidene)-2-hydroxyaniline (Figure 2.2) had a very powerful antibacterial action against *Mycobacterium tuberculosis* **H37RV**, with a MIC value of 8 g/mL. **J774** macrophages were used to assess the selectivity of the discovered chemical. Even at high concentrations in the range of (1000 in micromolar concentration), the chemical had no harmful effect on **J774** macrophages [12].

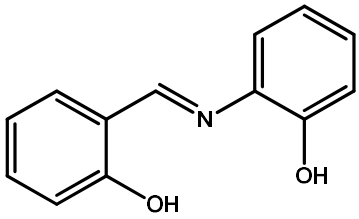


Figure 2.2. Chemical structure of N-(salicylaldehyde)-2-hydroxyaniline

In 2007, Lei and coworkers reported the synthesis and antibacterial activity of a series of Schiff bases generated from salicylaldehyde derivatives. The proposed Schiff base derivatives (Figure 2.3) showed significant activity against a variety of strains, with compounds **13-6**, **13-13**, and **13-15** showing the most activity against *Pseudomnas fluorescence* with MIC values ranging from 2.5 to 5.2 g/mL, whereas the reference medication Kanamycin had a MIC of 3.9 g/mL. MIC values for the Schiff bases **13-6, 13-7, 13-9, 13-14,** and **13-15** against *Escherichia coli* ranged from 1.6 to 5.7 g/mL, while *Bacillus subtilis* was shown to be susceptible to the Schiff base **13-14** (MIC = 1.8 g/mL). Finally, against *Staphylococcus aureus*, the MIC values for compounds **13-6** and **13-7** were 3.1 and 1.6 g/mL, respectively [13].

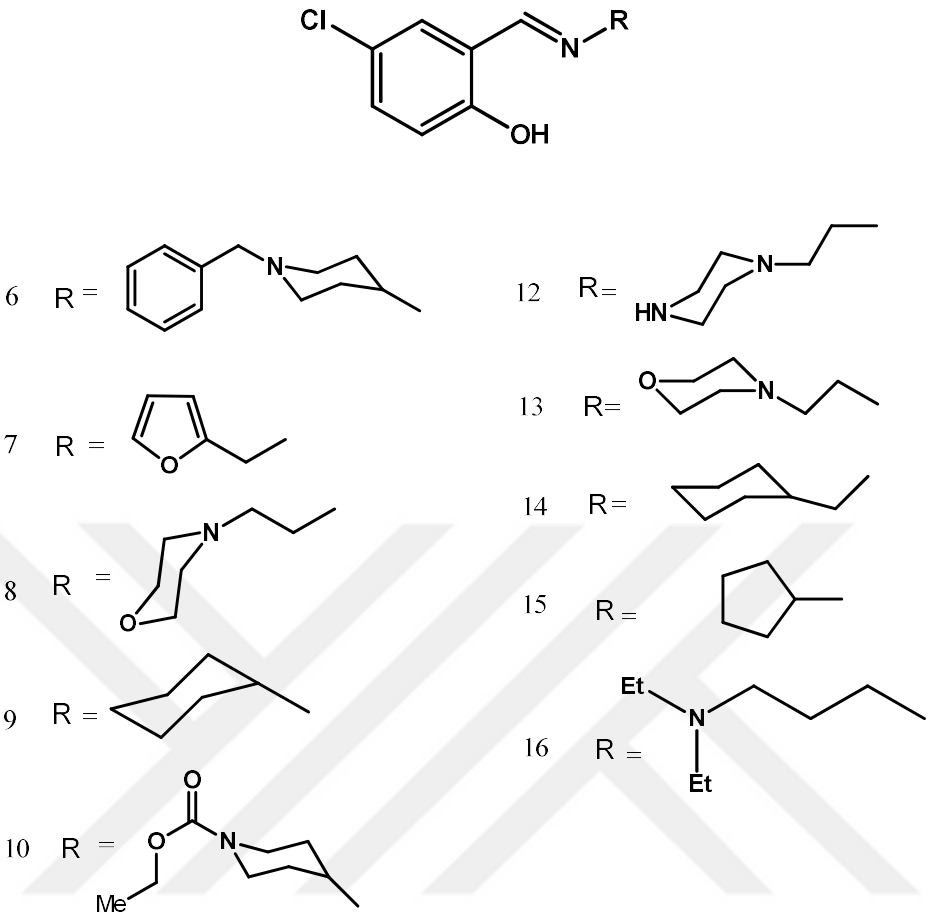


Figure 2.3. Chemical structure of bioactive 5-chloro-salicylaldehyde Schiff base derivative.

The antibacterial activity of an insatin-derived Schiff base (Figure 2.4), has been reported by Pandya and colleagues. When compared to the reference medicine sulfamethoxazole, the chemical (S12) showed promising effectiveness against against *E. coli*, *Vibrio cholera*, *Enterococcus faecalis*, and *Prote shigelloides*, with MIC values of 2.4, 0.3, 1.2, and 4.9 g/mL respectively [14,15].

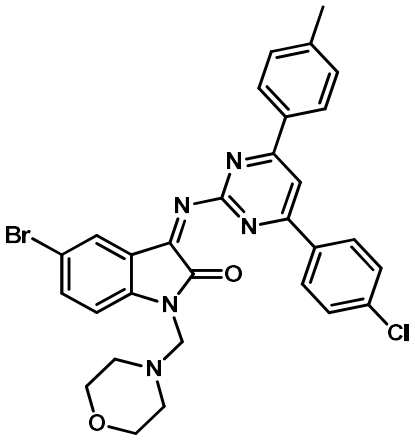


Figure 2.4. Compound S12.

2.1.2.2 Antifungal Activity

It is imperative to find and develop more effective antifungal medications, were several Schiff bases are recognized to be excellent antifungal agents [16].

Guo *et al*. for example, created Chitosan-derived Schiff bases (Figure 2.5), which are naturally occurring amino-polysaccharides, and tested their fungicidal effectiveness against *Botrytis cinerea* and *Colletotrichum lagenarium* [17]. When employed at 1000 ppm, the growth of the investigated fungal strains was decreased by 26-33 percent and 35-38 percent, respectively.

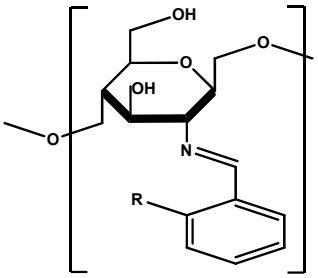


Figure 2.5. Chitosan-Derived Schiff Base. R = H, OH

2.1.2.3 Anticancer activity

Kraicheva et al. investigated several Schiff bases and tested their antiproliferative activity against various leukemic cell lines, including chronic myeloid leukaemia cells (LAMA84), myelogenous leukaemia cells of the erythroleukemia (K562), acute promyelocytic leukaemia cells (HL60), and myeloblastic leukaemia cells (HL60/Dox), using the (MTT) assay. However, compound **18-2** (Figure 2.6) showed promising anti-

proliferative action against LAMA84, K562, and HL60Dox, respectively (IC_{50} = 39.9, 29.9, and 68.6 μM). Furthermore, minimal cytotoxic effects against the leukemic cell line (with an IC_{50} value of 400 μM) were observed [18].

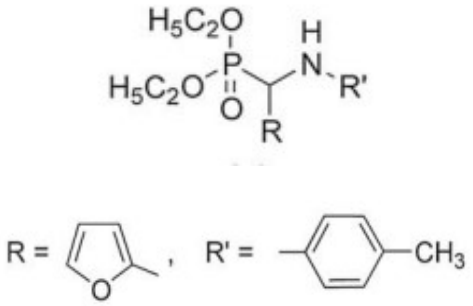


Figure 2.6. Schiff base derivatives compound 18-2.

Shaker *et al*. described the synthesis of azomethine scaffold containing hydrocarbon chains, and testd their anticancer potential against liver carcinoma (HEPG2), breast carcinoma (MCF 7), and colon carcinoma (MCF 7) in mice (HCT116). The antiproliferative effect of compound SBC-12 (Figure 2.7) was studied at various concentrations ranging from 1 to 10 mg/mL. *In vitro*, the chemical showed strong activity on the tumor cell lines studied, with the maximum cytotoxic effect on HEPG2, HCT116, and MCF7, respectively [19].

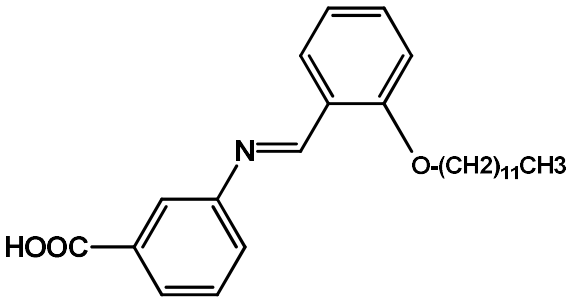


Figure 2.7. Schiff base Compound SB-12.

Mishra *et al*. investigated a number of schiff base derivatives based on benzothiazoles. In order to examine the cytotoxic activity of the synthesized derivatives, the MTT assay was used in their anticancer study, the compound VS5-e inhibited breast cancer cells by 85.8% within the range of (IC_{50} = 973 g/mL) with less cytotoxic effect to normal cells (Figure 2.8) [20].

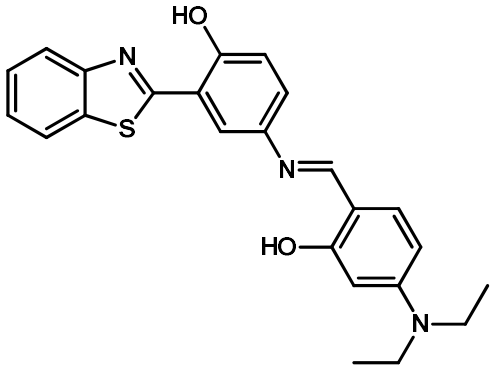
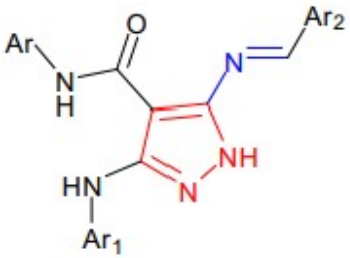


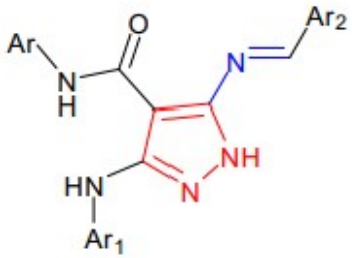
Figure 2.8. Chemical structure of (VS5-e).

Hassan *et al*. described a series of Schiff bases in 2018 and tested their antiproliferative properties in HepG2 liver cells and MCF-7 breast cells. The bulk of the imine compounds was shown to have significant anticancer activity. The most intriguing compounds in the series were **21-17** and **21-23**. (Figure 2.9). When compared to doxorubicin, **22-17** was the most active against HepG2 cells (IC_{50} = 66.3 µM versus 80.9 µM). Compound **22-23**, on the other hand, has a high activity against MCF-7 (IC_{50} = 60.8 µM versus 65.6 µM for doxorubicin). They also exhibited apoptosis in HepG2 and MCF7 cells by raising caspase-3 levels [21].



Ar = 4-CH_3-C_6H_4
Ar_1 = 4-CH_3O-C_6H_4
Ar_2 = C_6H_5

Compound **21-17**

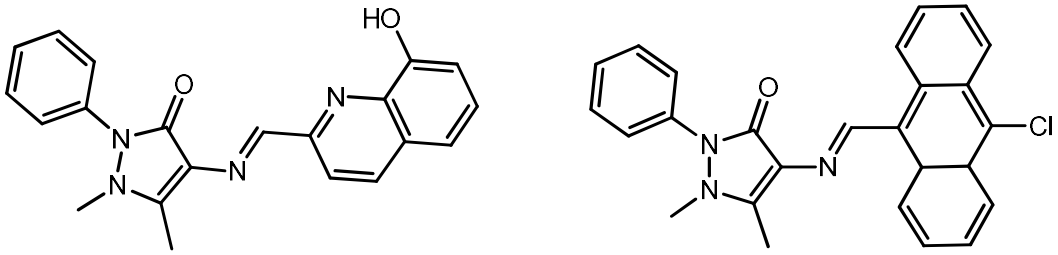


Ar = 4-Cl-C_6H_4
Ar_1 =4-CH_3O-C_6H_4
Ar_2 = C_6H_5

compound **21-23**

Figure 2.9. Chemical structure of compounds **21-17** and **21- 23**

Erturk *et al.* developed and tested two Schiff bases (Figure 2.10), for distinct biological activities, including anticancer activity against the MCF-7 breast cancer cell line, with IC_{50} values of (0.1 µM for **3b**) and (0.14 µM for **3a**). In the presence of chloroanthracene, the molecule was found to have greater activity than hydroxyquinoline for [22].



3a **3b**

Figure 2.10. Chemical structure of antitumor Schiff bases

2.2 Sulfonamides

Sulfonamides have attracted interest in recent decades due to their broad variety of biological effects, including antibacterial, antifungal, hypoglycemic, anti-thyroid, diuretic, anti-HIV capabilities, and anti-HIV features. Various *in vitro* and in *vivo* studies, revealed that a large number of structurally distinct sulfonamides have recently been discovered to exhibit considerable anticancer activity [23]. The anticancer activity is exerted by the sulfonamides through a wide range of mechanisms, such as, matrix metalloproteinase (MMPs) [24], NADH oxidase [25], methionine aminopeptidases (MetAPs) [26], histone deacetylases (HDACs) [27], binding to β-Tubulin, and disruption of microtubule assembly [28].

Sulfonamides are a biologically important class of compounds because they are easily absorbed and eliminated in the urine, resulting in lower toxicity, higher reactivity, and more cost-effective molecules [29, 30].

Prontosil (Figure 2.11), the first approved sulfonamide medicine, was discovered in 1932, and used as an antibacterial agent. Since then Sulfonamides have become the most widely utilized anti-infective class on the planet. Furthermore, sulfonamides are widely used in medicinal chemistry to develop new drugs to treat a variety of pathological disorders and chronic diseases [31, 32].

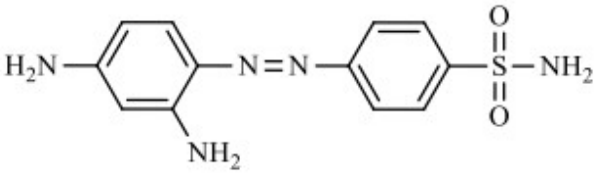


Figure 2.11. Prontosil structure

Sulfonamide group coupled with different functional groups such as acetamides and another aryl, heteroaryl, and alkyl substituents, have enormous pharmacological potential. Sulfonamides containing short amine fragments, in particular, have promising anticancer activity. This explains the increased interest in sulfonamide-acetamide derivatives' production, biological characteristics, and structure-activity connections. Based on previous findings relating to the production of sulfonamide derivatives and bioactive nitrogen-containing heterocyclic compounds, sulfonamides with acetamide pharmacophores may be highly effective antibacterial and anticancer medicines [33,34].
Sulfonamide derivatives are valuable therapeutic chemical scaffolds, chemically identified by the sulfamoyl (–SO2NH–) group, which is related to the amide derivatives. However, because sulfonamides are classified as an important structural component of carbonic anhydrase inhibitor drugs, the incorporation of sulfonamide with other functional groups conceded as important drug targets in a wide range of pathological conditions such as antitumor (Figure 2.12) [35].

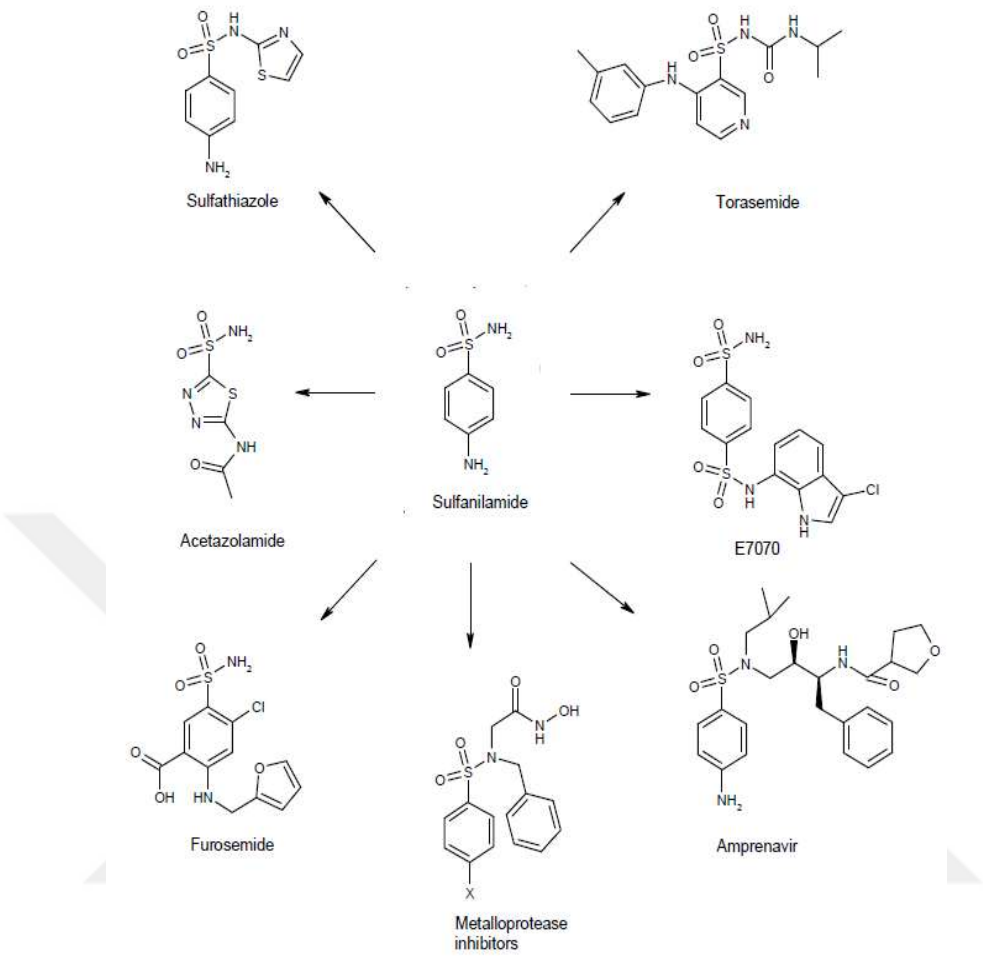


Figure 2.12. Example of bioactive sulfonamide derivatives**.**

2.2.1 Sulfonamides as anticancer agent

Many new sulfonamide compounds have recently been found to exhibit potent anticancer properties. N-(3-chloro-1H-indol-7-yl)-1,4-benzenedisulfonamide (E7070), for example, is considered as breakthrough in the development of novel sulfonamides with potent anticancer properties. E7070 is a new cell cycle inhibitor that inhibits cell cycle progression at numerous sites, while its target is unknown [36,37]. E7070 has been shown to have anticancer activity in rat and human tumor xenografts and is currently being tested in phase I/II clinical trials (Figure 2.13). N-(4-methoxyphenyl)sulfonyl-N-[2-[2-(1-oxido-4-pyridin-1-iumyl)ethenyl]phenyl]acetamide (HMN-214) is another sulfonamide that arrests cells in the G2/M phase and has anticancer activity (Figure 2.14). This anticancer action is mediated via cytotoxicity, which is mediated by inhibiting polo-

like kinase, and MDR downregulation, which is mediated by binding to the B-subunit of the important ranscription factor NF-Y [38].

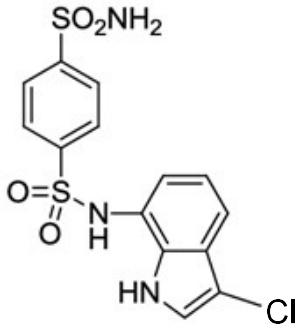


Figure 2.13. Chemical structure of e7070 sulfonamide

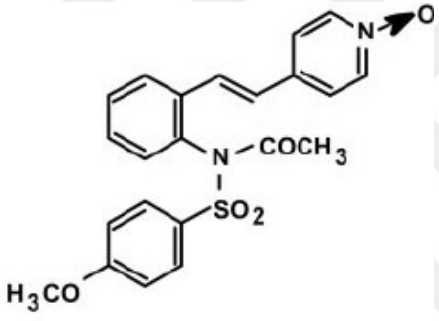


Figure 2.14. Chemical Structure of HMN-214 sulfonamide

In addition, CA are zinc metalloenzymes that catalyze reversible carbon dioxide hydration to produce bicarbonate and proton. CAs involved in pH regulation, electrolyte secretion, and biosynthetic respiration that uses CO_2 and bicarbonate as a substrate, such as gluconeogenesis, lipogenesis, and nucleotide synthesis. The Zn^{+2} metal active site, a strong Lewis acid that binds to and activates a substrate H_2O molecule to catalyze the hydration reaction of CO_2, is found in the catalytic domain of CAs. In the absence of hCA enzymes, this process does not develop at a significant rate within physiological conditions. The hCA enzyme inhibitor such as sulfonamide derivatives has been discovered to have an important role in tumor therapy by limiting the supply of bicarbonate for nucleotide synthesis and other cell processes like membrane lipids (Figure 2.15).

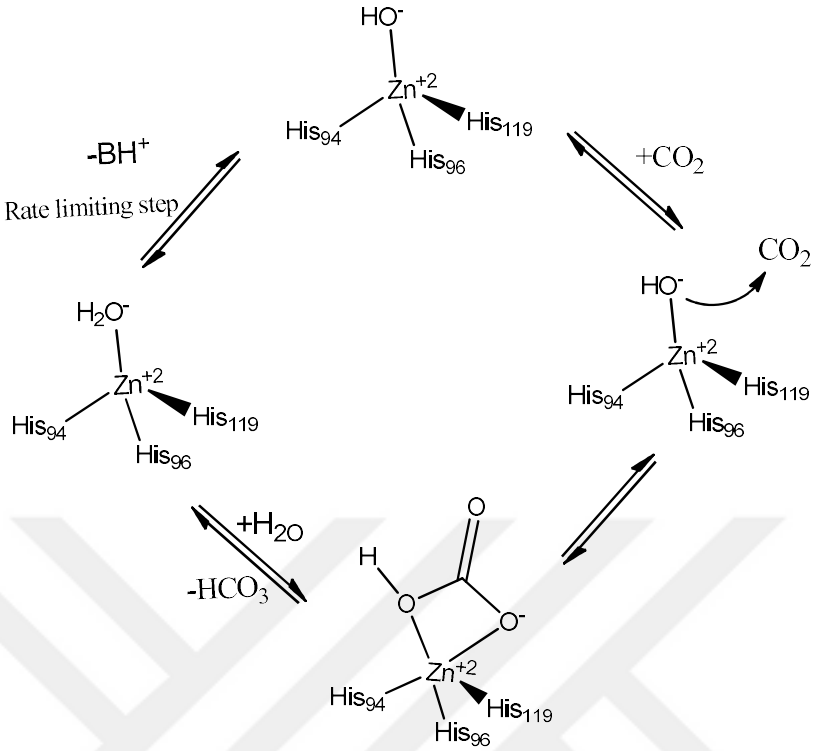


Figure 2.15. Catalytic cycle of human carbonic anhydrase**.**

According to scientific research, over-expression of (hCA IX and XII) isoforms is reported in numerous solid tumors as a result of decreased oxygen tension in the inner tumoral regions, which is one of the most anticancer successful studies. The (hCA IX) isoform is the most studied, and it plays a key role in the genesis of cancer. One of the more prominent structural designs for selective suppression of tumor-related CA isoforms is sulfonamide derivatives, specifically (Figure 2.16) [35]. The sulfonamide-binding mood in the structure is compatible with the enzyme biniding requirement, and has the ability to link to and coordinate zinc metal ions, limiting CA isoform enzyme IX and XII catalytic activity at low concentrations. The ionized NH- sulfonamides coordinate the enzyme zinc ion to create a tetrahedral complex in the classic binding mode. The combination is further stabilized via a hydrogen bond created by the nitrogen atom and the OH function of the Thr199 amino acid residue, which interacts with one of the sulfonamide group's oxygen atoms through its NH group [39,40].

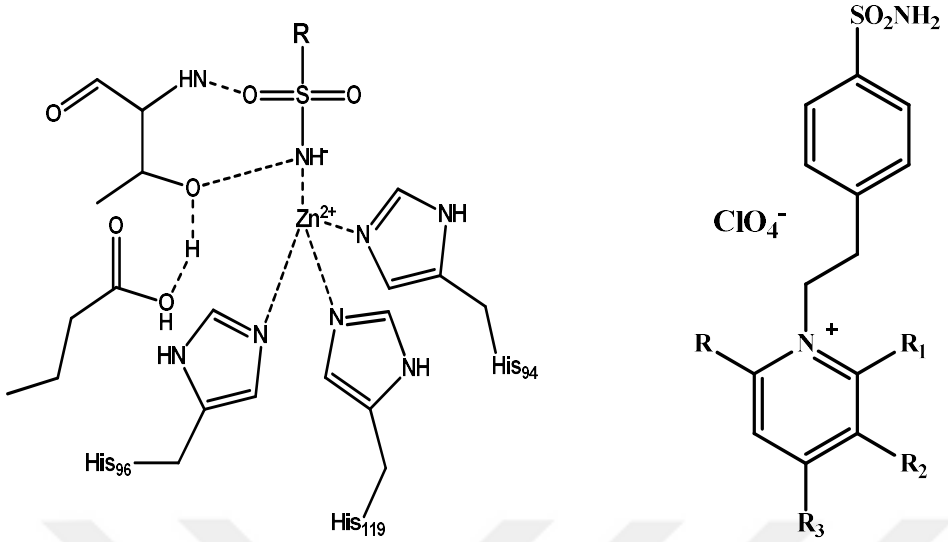


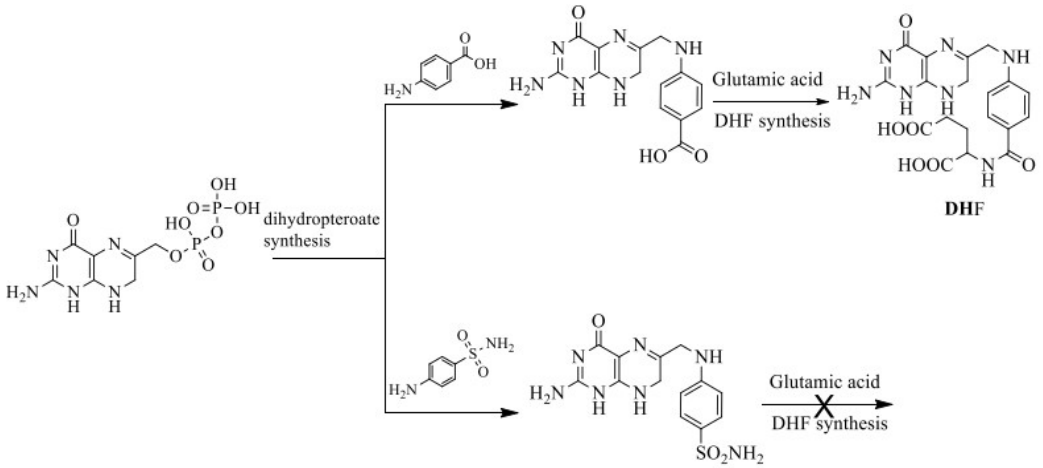
Figure 2.16. The binding mode of sulfonamide-based compound

2.2.2 Sulfonamide Antimicrobial agent

Sulfonamide compounds are a large class of synthetic bacteriostatic antibiotics that are still used to treat bacterial infections and infections caused by other microorganisms today. Prior to the introduction of penicillin in 1941, they were known as sulfa medicines and were the most common form of treatment for bacterial infections [41].

Sulfa medicines, which contain sulfonamide functional groups and have a wide range of biological actions, have transformed medical science. Sulfonamides prevent folic acid from being used in the creation of bacterial DNA and RNA, which is hindered by sulfonamides. Tetrahydrofolate deficiency reduces the creation of new DNA and RNA, which eventually causes the bacteria to die. Because bacteria mistakenly convert sulfonamide instead of p-amino benzoic acid for folic acid synthesis, normal microorganism growth is impeded. Sulfonamides are also effective in antibacterial activities as a result of these actions [42]. To combat current drug-resistant infections, innovative antibacterial medicines with distinct modes of action and mechanisms are becoming increasingly popular. Pathogenic organisms (bacteria, fungus, and mold) become increasingly resistant to the development of new species as a result of permutation, conjugation, transduction, or transformation as they are exposed to or treated medically with conventional antibiotics drug molecules [43].

Sulfonamides are antimicrobials with a broad spectrum of action that works against Gram-positive and Gram-negative bacteria, including those found in the intestine. Sulfonamides have high antimicrobial activity against E. coli, moderate antimicrobial activity against Proteus mirabilis and Enterobacter species, and weak antimicrobial activity against Klebsiella; however, they have no antimicrobial activity against pseudomonas aeruginosa and Serratia species. They are efficient against Chlamydia genus species. Sulfonamides have also been shown to be effective against fungi (Pneumocystis carinii) and protozoa (Toxoplasma gondii). Sulfonamides differ in potency but not in the antibacterial activity spectrum . Inhibition of dihydrofolic acid synthesis is a desirable target for bacteriostatic drugs due to bacteria's inability to collect dihydrofolic acid from their environment as part of DNA construction (Scheme 2.3). Early sulfonamides, such as sulfanilamide, have been used to inhibit these enzymes. Dihydrofolic acid is formed by combining pteridine diphosphate with p-aminobenzoic acid, which can then be further modified by amide coupling with glutamic acid to generate dihydrofolic acid. Sulfanilamide is a competitive inhibitor with a core structure similar to that of p-aminobenzoic acid. The creation of dihydrofolic acid is prevented during the second phase due to a shortage of acidic terminals to link with glutamic acid; thus, dihydrofolic acid formation is interrupted [44,45].



Scheme 2.3. Inhibition of DHF formation by sulfonamide

The sulfonamide group and the amino group in the para position of the benzene ring make form the basic sulfonamide structure. Substituting the hydrogen atom on the

nitrogen of the sulfonamide group (N1) yielded a significant number of sulfonamide derivatives, but substituting the hydrogen atom on the nitrogen of the aromatic amino group yielded a small number of active sulfonamide medicines (N4). The addition of various substituents resulted in compounds with a variety of physicochemical, pharmacokinetic (protein binding, metabolism, and excretion), and pharmacodynamic properties. The structures of a number of key sulfonamide antibacterial medicines (Figure 2.17).

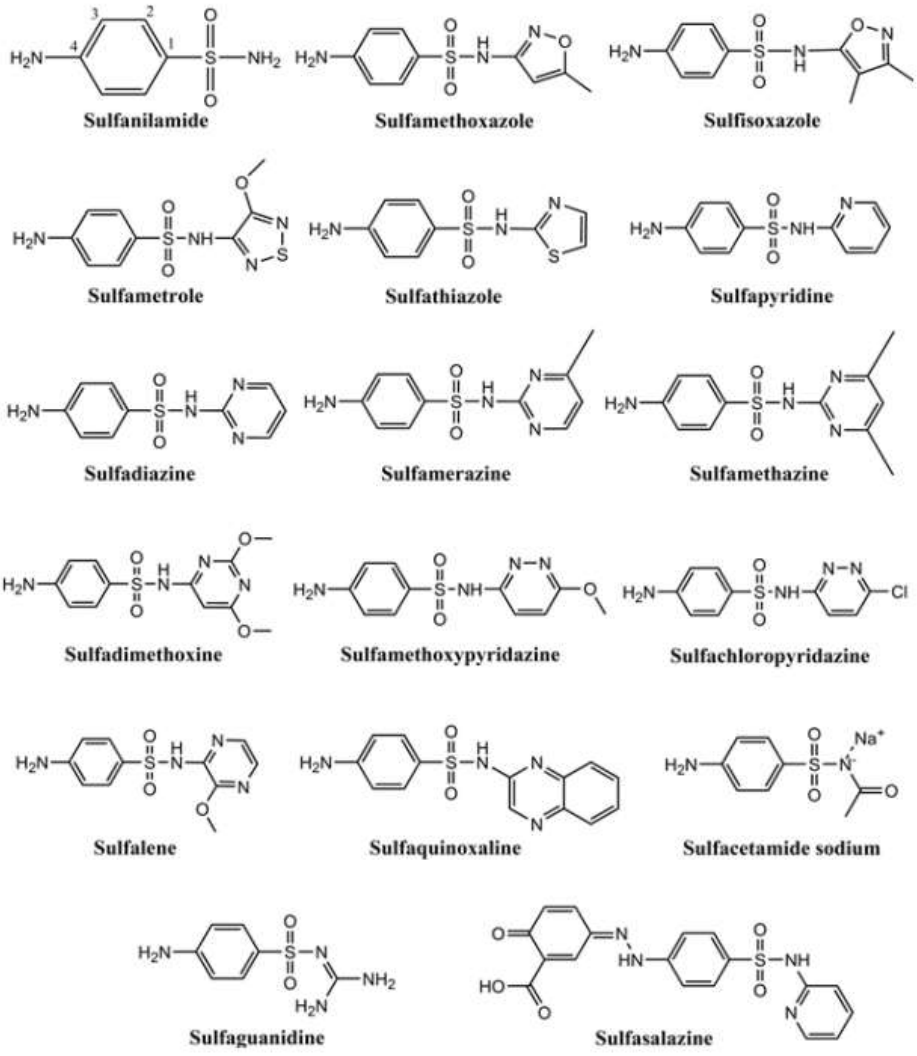


Figure 2.17. Structures of several sulfonamide antimicrobial drugs

Sulfonamides are competitive antagonists of p-aminobenzoic acid (PABA), a chemical required for bacterial growth and reproduction and hence for the synthesis of folic acid.

Sulfonamides have a bacteriostatic effect by blocking the synthesis of folic acid in a reversible manner. The inhibitory action is based on the structural similarities of sulfonamide and PABA, as shown in the example of sulfanilamide (Figure 2.18).

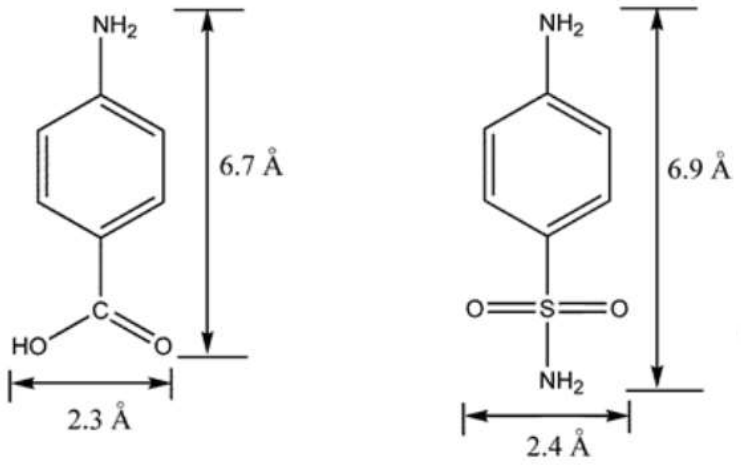
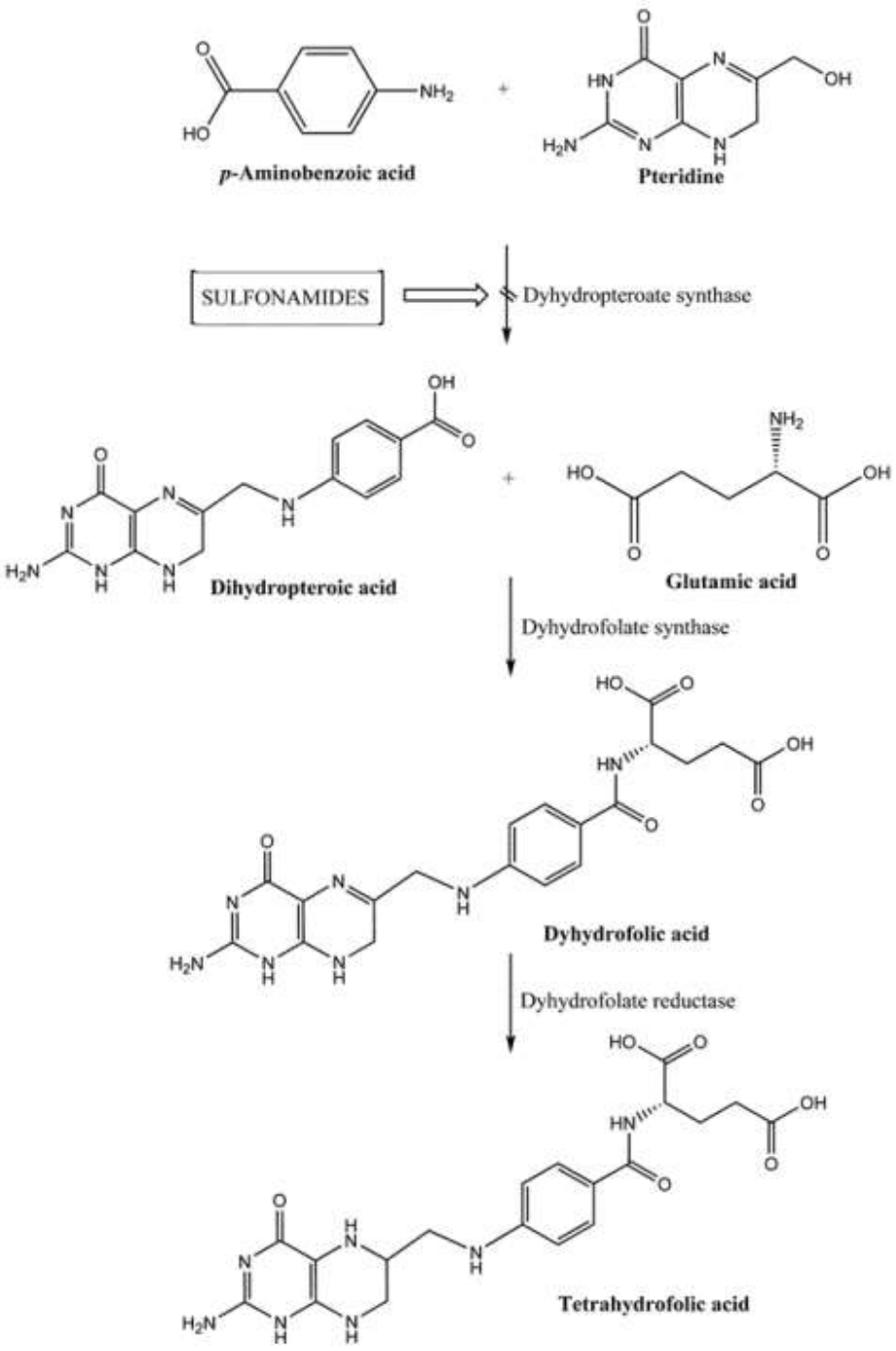


Figure 2.18. The similarity of sulfanilamide and p-aminobenzoic acid structures.

The interaction between PABA with pteridine in the presence of the enzyme dihydropteroate synthase is normally the initial step in the creation of folic acid. Dihydro pteroic acid is created during this process. Under the action of the enzyme dihydrofolate synthase, the produced intermediate combines with glutamic acid-producing dihydrofolic acid to produce glutamic acid. Dihydrofolate is converted to tetrahydrofolate in the presence of the enzyme dihydrofolate reductase, which is employed by bacteria in the manufacture of methionine, purine, and pyrimidine bases. The enzyme dihydropteroate synthase attaches to these structural PABA analogues in the presence of sulfonamides, preventing folic acid and DNA synthesis in the end (Scheme 2.4) [46].



Scheme 2.4. Folic acid synthesis and sulfonamides site of action

2.3 Sulfonamide substituted Schiff bases

Schiff base molecules have been used in biological, industrial, pharmaceutical, and many other fields of research, and their chemistry has attracted people's attention. Sulfonamide Schiff base derivatives can be made by condensing sulfonamide compounds with at least a –NH2 group and an aldehyde, which could lead to physiologically active molecules. Schiff base compounds generated from sulfa pharmaceuticals have gotten a lot of attention because of their pharmacological properties [47].

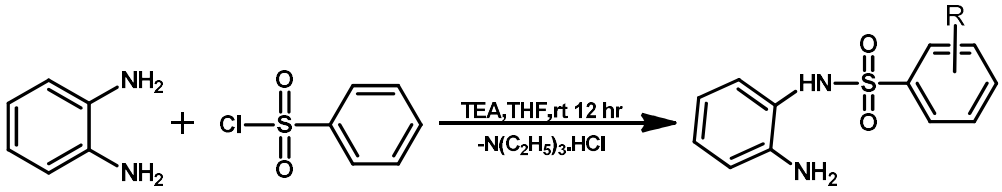
Antibacterial, anticancer, diuretic, anti-carbonic anhydrase (anti-CA), hypoglycemic, anti-thyroid, and protease inhibitor properties are all common uses for Sulfonamide-derived Schiff base compounds. Many drugs have several pharmacological and toxicological effects when delivered as sulfonamide incorporated with schiff base metal complexes[48].

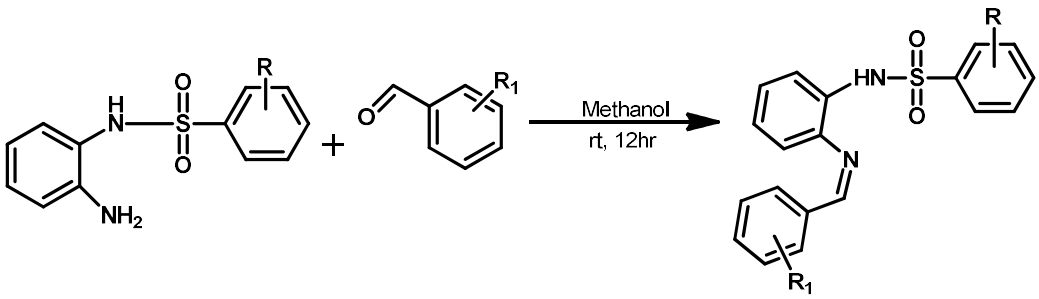
It has been established that the Schiff base imine group is required for biological processes. By creating a hydrogen bond with the active centers of cell constituents, the nitrogen atom of azomethine may interfere with normal cell processes; also, the SO_2NH moiety of sulfa medications acts as a crucial toxophoric function.

2.3.1 Synthetic method:

The reaction of a sulfonyl chloride with primary or secondary amines in an alkaline media yields Schiff base sulfonamide derivative.

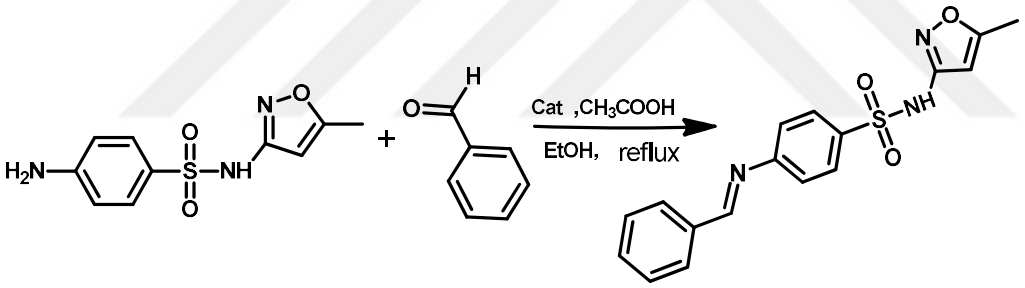
Dayan and colleagues have synthesized a series of sulfonamide substituted Schiff derivatives by treating *o*-phenylendiamine with arylsulfonylchloride in triethylamine solution. The resultant N-(2-aminophenyl) arylsulfonamides were further condensed with aromatic aldehyde in methanol, for 24 hours at room temperature to yield the Schiff base sulfonamide derivative (Scheme 2.5) [49].





Scheme 2.5. Synthesis of sulfonamide subistituted schiff base at room temperature

In addition, employing an acetic acid catalyst, Hamad et al. produced Schiff base sulfonamide derivatives by refluxing 4-amino-N-(5-methylisoxazol-3-yl) benzensulfonamide with an appropriate aromatic aldehyde in methanol (Scheme 2.6) [45].



Scheme 2.6. Synthesis of sulfonamide subistituted schiff base derivatives using refluxing method

2.3.2 Biological activity

2.3.2.1 Bioactive Sulfonamide Schiff Base Complex

Azomethine compound has the ability to interact and form a ligand in a stable complex, with various metal ions in many coordination geometries and oxidation states. These complexes have been widely used in chemistry due to their importance in different therapeutics and biological purposes (Figure 2.19) [1].

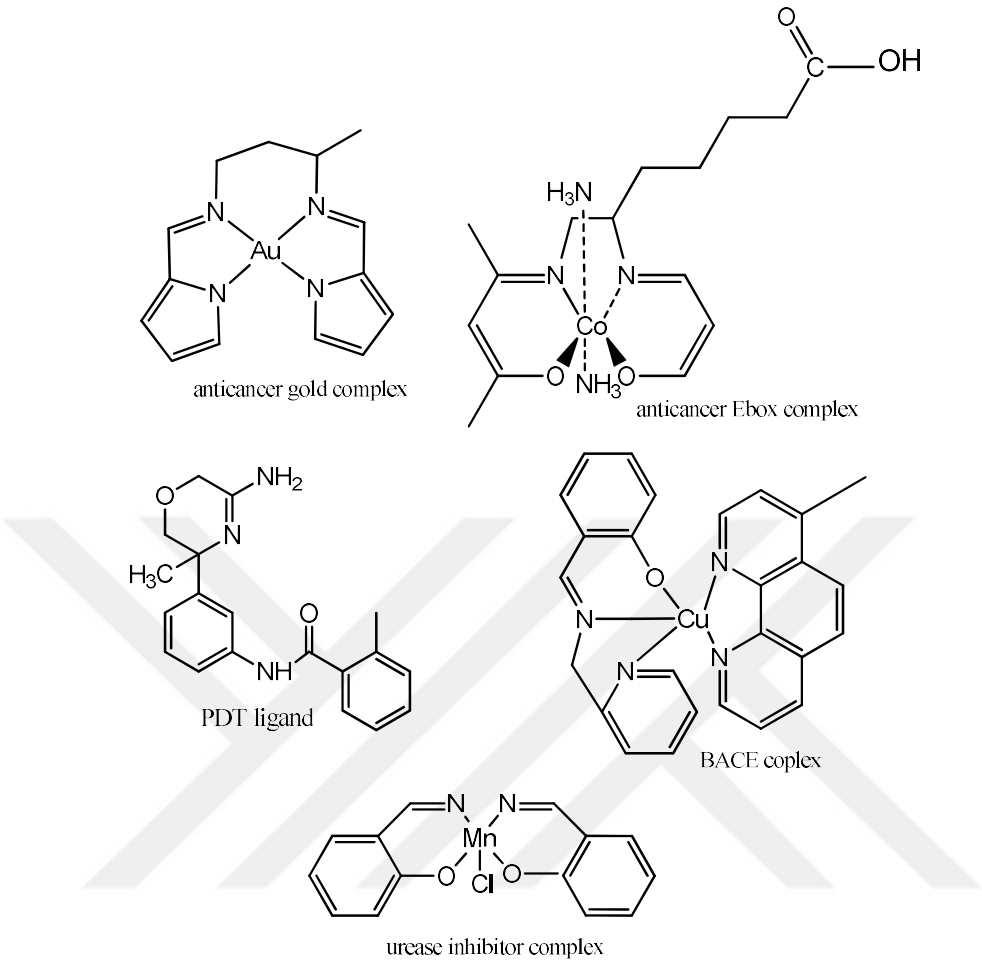


Figure 2.19 Schiff base bioactive complex

Sulfonamides and their Schiff metal complexes have been studied in medicinal chemistry,and found to be effective against a variety of bathological conditions. For example, Sulfonamide substituted Schiff base complexes such as 5-chloro-2-hydroxybenzyliedine sulfonamide derivatives, 4-(2-aminoethyl)benzenesulfonamide, and sulfisoxazole derivatives were found to have promising antibacterial and antifungal properties (Figure 2.20).

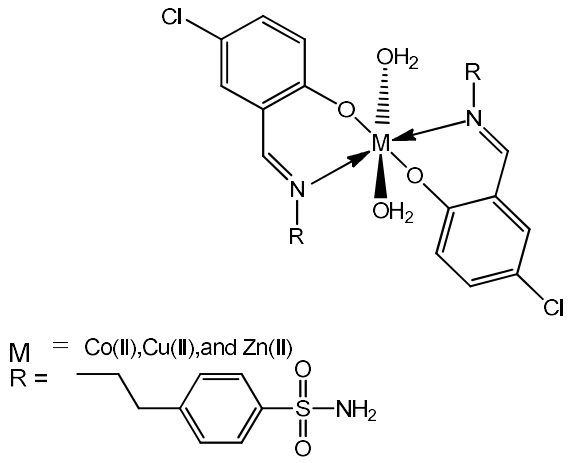


Figure 1.20. Example of bioactive sulfonamide subistituted shiff base complex.

Furthermore, the Schiff base complex formed from sulfonamides was reported to be more active than the Schiff base complex itself. The vanadium complex, for example, is a well-known transitional metal Schiff base complex with promising urease inhibitor action that exists in multiple oxidation states. The presence of ions in these complexes is frequently attached to proteins. However, the activity is result from presence of the sulfamoyl groyp and bimetallic imine complex that improve the geometric shape and stability of the complex (Figure 2.21) [51].

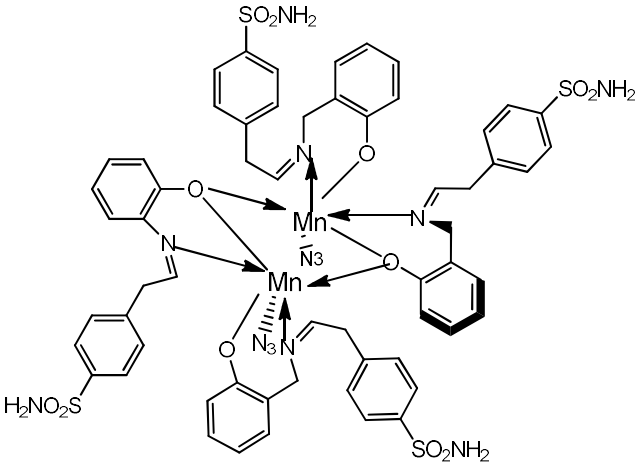


Figure 2.21. Chemical structure of sulfonamide schiff base vandium complex.

2.3.2.2 Antibacterial activity:

Kratky and colleagues synthesized and tested Hybrid Schiff bases with salicylidene and sulfonamide moieties in 2017. The compound 4-[(Hydroxybenzylidene)amino]-N-

(pyrimidin-2-yl)benzenesulfonamides (Figure 2.21) showed promising antimicrobial activity against *S. aureus*, including methicillin-resistant strain (MRSA) (MIC values in the range of 0.125 – 0.250 in micromolar concentration [3].

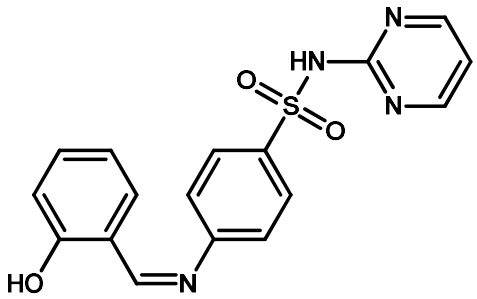


Figure 2.21. 4-[(Hydroxybenzylidene)amino]-N-(pyrimidin-2-yl) benzenesulfonamides structure

2.3.2.3 Antifungal activity:

Hamad et al. compared the potential of many commercially available sulfa drugs to their manufactured Schiff bases. Sulfamethoxazole (Figure 2.22), sulfamethoxypridazine, and sulfamethazine, all parent sulfa drugs, were found to be efficacious against all pathogenic Candida species tested. Their Schiff bases, on the other hand, demonstrated promising antifungal activity against a wide range of pathogenic Candida strains, with MICs ranging from 4-32 g/ml [4].

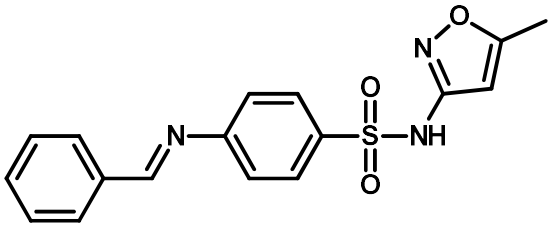


Figure 2.22 Sulfamethoxazole sulfa drug structure

2.3.2.4 Anticancer activity:

Moh *et al*. created a series of sulfanilamide Schiff base derivatives as prospective anti-tubulin compounds (Figure 2.23), based on the chemical structures of combretastatine-A4 and isoquinoline sulfamate (Figure 2.24). The anticancer properties of the proposed compounds, which were synthesized using microwave chemical synthesis and docked in

the colchicine binding site of -tubulin using molecular modeling, were further investigated on tumor cells [52].

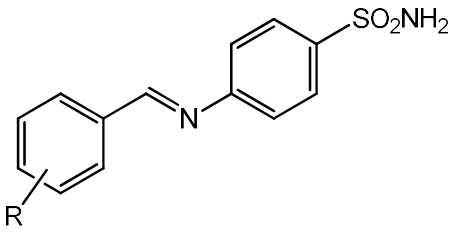


Figure 2.23. Sulfonamide subistituted Schiff base derivatives as anti-tubulin substances.

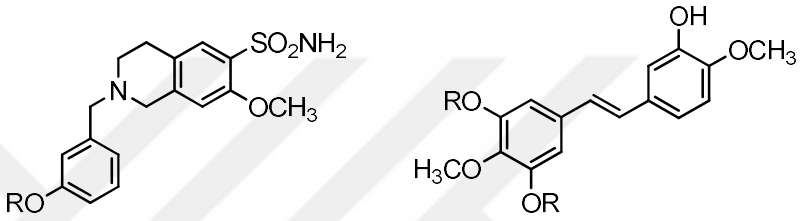
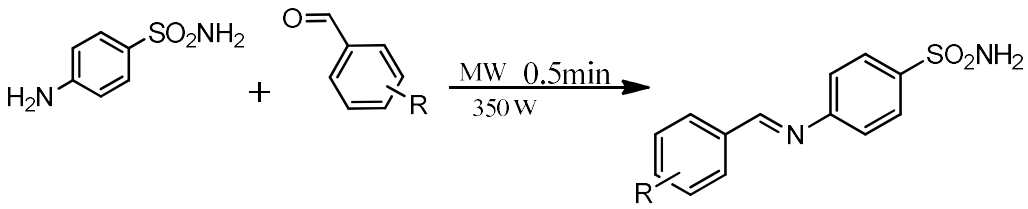


Figure 2.24. Structure of combretastatine- A4 and isoquinoline sulfamate.

The sulfanilamide Schiff base design in this work was described as an inhibitor of microtubule polymerization by attaching to the tubulin subunits colchicine binding site. However, the activity of the synthesized isoquinoline design is due to sulfamoyl group incorporated with immine bond and other electron with drowning groups such as methoxy group. In addition, isoquinoline sulfamate and combretastatin are examples of the most significant compounds under investigation as antimicrotubule.

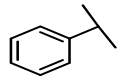
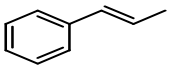
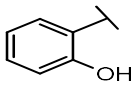
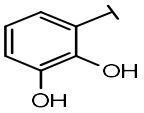
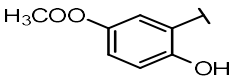
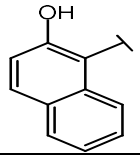
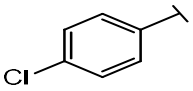
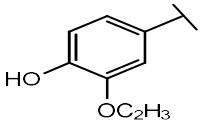
The compounds were made by condensing sulfanilamide with different aromatic aldehyde derivatives using microwave synthetic method (Scheme 2.7).



Scheme 2.7. Microwave synthesis of sulfanilamide-immine derivative

The two phenyl rings linked by Schiff base bonds in their design were chosen as the key structural characteristic in their investigation rather than the alkene moiety of Isoquinoline sulfamate. Sulfonamide activity is also employed to replace the methoxy group of combretastatine A-4. Sulfanilamide Schiff base derivatives with various substituents on the second benzene ring have been designed and produced.

Table 2.1 Structure of sulfanilamide-immune derivatives with antitumor activity and binding free energies of docking study.

cpd.	R(subistetuene)	Breast cancer cell (IC_{50})	lung cancer cell (IC_{50})	docking erergy
1		———	———	-4.6
2		105	———	-5.4
3		91	———	-5.6
4		101	———	-5.8
5		101	119	-5.4
6		130	———	-7.02
7		104	———	-6.05
8		96	130	-6.2

Most of the synthesized sulfanilamide-imines were appropriate to interact with the colchicine active site in the same manner as combretastatine A-4, with extra binding ability via sulfonamide moiety in the presence of azomethine group according to a molecular modeling study of the formulated compounds. The produced sulfonamide derivatives is compatible with the same moiety of the isoquinoline sulfamate backbone. Furthermore, they had a stronger active site reactivity and a greater ability to coordinate various hydrogen bonds with the amino acid histidine 94, histidine 119, threonine 199, and threonine 200 in the colchicine active site.

The discovered structure-activity relationship of the suggested sulfanilamide immune derivatives demonstrated that the inclusion of an additional double bond between the R substituent and the azomethine moiety leads to high anticancer activity for example compound 2. This could be due to the participation of the Schiff base bond within the phenyl resonance. The lone pair of Schiff base bonds improves the interaction of the proposed molecule with cancer tissues. The introduction of an electron with a donating group, such as hydroxyl in the ortho position of the phenyl group of compounds 1 in the presence of a sulfamoyl group and immine designing, results in significant activity against breast cancer cells compound 3. Compound 5 has a small decrease in activity due to the presence of the methoxy group at the same phenyl group, whereas compound 4 has an increase in anticancer activity against cancer cells due to the addition of a hydroxyl group, which is mainly due to the physicochemical properties of the added substituent. Compound 3's lipophilicity is reduced by replacing the phenyl group with a naphthyl group, resulting in a slight drop-in activity, similar to compound 6. The addition of Chloro, hydroxyl, dimethylamino, or nitro substituents increase the activity of compound 8, the flexibility of the schiff base in the synthesized compound is critical for interaction of the compound with the aminoacid residue.

Wang and colleagues also developed and synthesized a series of imine-based sulfonamide Coumarin derivatives in 2013. The most relevant compounds (Figure 2.25) were active in vitro against mouse melanoma and MCF7 cells, yielding IC_{50} values of 0.19μM concentrations [53].

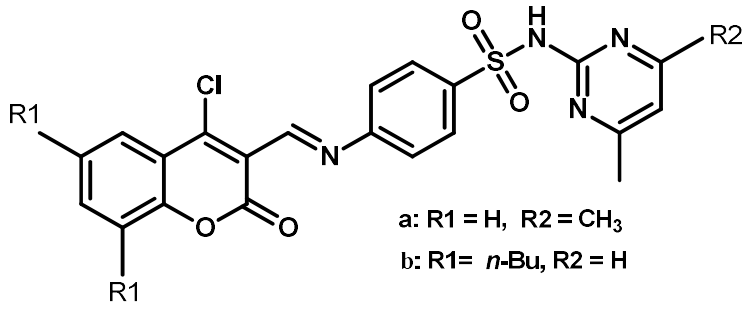


Figure 2.25 2 Imine-based sulfonamide Coumarin derivatives

Koyuncu et al. investigated the effect of sulfonamide Schiff base compound A-1 (Figure 2.26) on Hela cancer cell lines. The compound was found to affect cell division in hell cells at concentrations greater than 25 µM, with a percent slowing down in the G0, G1, S, and G2, M phases of the cell cycle of (65%, 19%, and 13%), respectively [5].

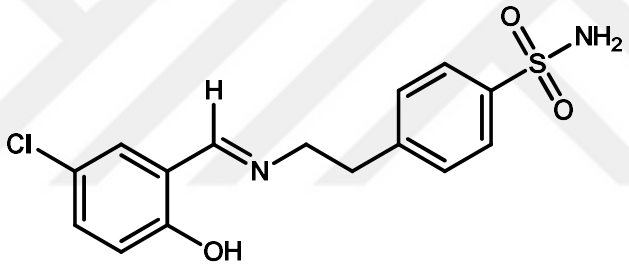
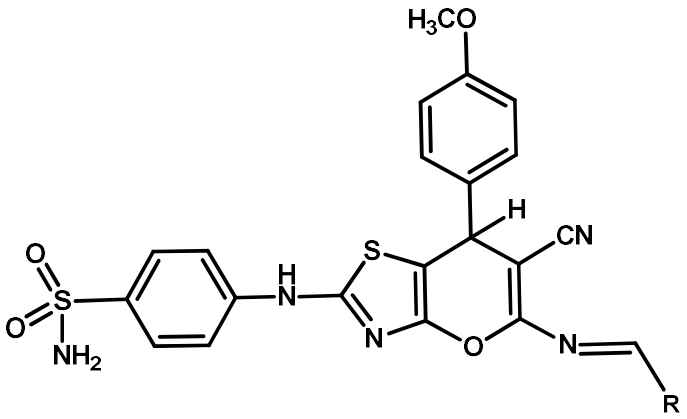


Figure 2.26 Sulfonamide Schiff base compound A-1

Concerning the i*n vitro* anticancer activity of the obtained Schiff bases a, b, and c (Figure 2.27), against oestrogen human breast carcinoma. have been reported by Ghorab *et al*.in their study the range of IC_{50} values was; 10.3 µM for compound (a), 9.6 µM for compound (b), and 9.4 µM for compound (c), the compounds were found to exhibit strong cytotoxic effects against MCF7 cells [54].



a: R= 2-(C_6H_4)Cl

b: R= 4-(C_6H_4)Cl

c: R= 3-(C_6H_4)Br

Figure 2.27 Schiff base against human breast cancer (MCF7)

3 MATERIALS AND METHODS

3.1 Chemicals

All of the starting ingredients in this study were commercially available and were used without further purification. Sigma-Aldrich provided o-Phynylendiamine, salicylaldehyde, anhydrous dimethyl sulfoxide, triethylamine, 4-Nitrobenzenesulfonyl chloride, 2,4,5-Trichlorobenzenesulfonyl chloride, and 4-Bromobenzenesulfonyl chloride. Merck supplied the bensensulfonyl chloride. MAYBRIDGE provided the 3-cynobenzenesulfonyl chloride. ISOLAB Laborgerate GmbH provided diethyl ether, ethyl acetate, hexane, and anhydrous sodium sulphate, while Riedel-deHaen provided sodium bicarbonate.

3.2 Synthetic Procedures

3.2.1 General procedure for mono schiff base:

1. o equivalent salicylaldehyde was added to a solution of 1.0 equivalent OPDA in 10ml water, and the combination was agitated at room temperature for 10 minutes. To obtain the base, the yellow precipitate was filtered using a Buchner funnel, rinsed with water, and dried.

3.2.2 General procedure for bis schiff base:

A solution of (2 eq) salicylaldehyde in 20 ml of ethanol was added to a solution of 1.08 gm of 1,2-diaminobenzene (0.01mole) in 30 ml of ethanol. A few drops of 10% NaOH were added to the reaction mixture, which was then refluxed for two hours with stirring, and the precipitate was collected by filtration through a Buchner funnel, recrystallized from methanol, and dried at room temperature to yield yellow powder.

3.2.3 General procedure for compounds 3-8

2 eq of TEA was added to a 10ml DMSO mixture of (0.1 eq) Schiff base. At room temperature, the mixture was stirred for one hour. The matching arylsulfonylchloride (2.5 eq) was added to the reaction mixture and agitated for 72 hours under a nitrogen atmosphere, with the resulting reaction mixture being partitioned between dethylether and saturated aqueous $NaHCO_3$ (10% w/v). TLC monitoring and column

chromatography purification was performed, using the solvent system (ethylacetate:hexane 1:1).

3.3 Analytical Methods

3.3.1 Controls by Thin Layer Chromatography (TLC) solvent systems

Plates: In this work, Kieselgel 60 F254 (Merck) silica gel plaques were used for thin layer chromatography.

Solvent Systems: Solvent systems used in this work for the chromatographic controls of the compounds are given below.

ethyl acetate: *n*-hexane (30: 70), ethyl acetate: *n*-hexane (50: 50), methanol: dichloromethane (90: 10), ethylacetate: petrolleum ether (30:70).

Elution Conditions: Solvent systems were poured to chambers and waited for saturation. Synthesized compounds and their starting materials dissolved in suitable solvents were applied to thin layer chromatography (TLC) plates, and waited to drag at room temperature.

Identification of TLC spots: UV light (254 nm) was used for the detection of the spots.

Dyes:

Ninhydrin (Riedel-de Haen): A solution of 0.2 g ninhydrin in 100 mL ethanol.

Ferric chloride test for phenolic compound identification.

3.3.2 Spectrometric Analyses

3.3.2.1 Infrared Spectra

Infrared (IR) spectra (10T/cm2 pressure applied potassium bromide discs) were recorded on a Perkin Elmer FT-IR 1720X spectrometer and the frequencies expressed in cm^{-1}

3.3.2.2 ^{1}H-NMR Spectra

The NMR spectra were recorded with a Bruker AC 400 Hz spectrometer, using tetramethylsilane as the internal reference, with chloroform (CDCl3) and dimethylsulfoxide-d6 as solvent and chemical shifts were reported in parts per million (ppm).

3.3.2.3 ^{13}C-NMR Spectra:

The ^{13}C-NMR spectra of some compounds were recorded with a Bruker AC 400 Hz spectrometer.

3.3.2.4 LC-MS Spectra

Spectra were recorded with a Waters 2695 Alliance Micromass ZQ LC-MS.

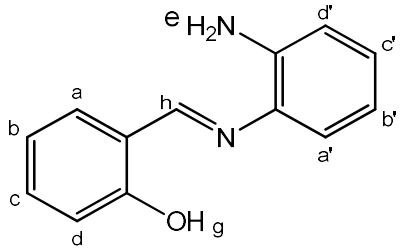
3.4 Cytotoxicity screening with NCI-Sulforhodamine B assay procedure:

OVCAR3, OVSAHO and KUROMOCHI ve CAOV3 high grade serous ovarian cancer cell lines were inoculated into 96-well plates as 3000 cells/well. After 24h incubation, cells were treated with the compounds in increasing concentrations (2.5µM-40µM). Each drug treatment was performed in triplicate. DMSO was used as negative control. After 72h of incubation time, medium was discarded and plates were washed twice with 1xPBS. Then the cells were fixed with 10% (w/v) trichloroacetic acid (TCA) solution for 1hr in dark at +4^{o}C. In order to remove TCA, cells were then washed with ddH_2O about 4-5 times and left air-dry at room temperature. The plates were then stained using 0.4% sulforhodamine B (SRB) solution in 1%acetic acid and incubated in dark at room temperature for 10min. Finally, excess dye was discarded by washing off by 1%acetic acid 4-5 times, until no dye comes out and left air dry at room temperature. Lastly, 10mM cold TBS solution was used to solubilize the protein-bound SRB dye. Absorbance values were measured at 515nm with microplate reader. In order to calculate IC_{50} values, the recorded OD value for each well was normalized to the OD value of its corresponding DMSO control.

4 EXPERİMENTAL DATA

Spectral Data

2-((Z)-(2-aminophenylimino) methyl) phenol (1)



1.o equivalent of salicylaldehyde was added to a solution of 1.0 equivalent OPDA in 10ml of water, the resulting mixture was then stirred for 10 min in room temperature. The yellow precipitate formed was filtered using Buchnner funnel, washed with water, dried to obtain the base.

Molecular weight [g/mol]: 212.2

Molecular formula: $C_{13}H_{12}N_2O$

Yield [%]: 62

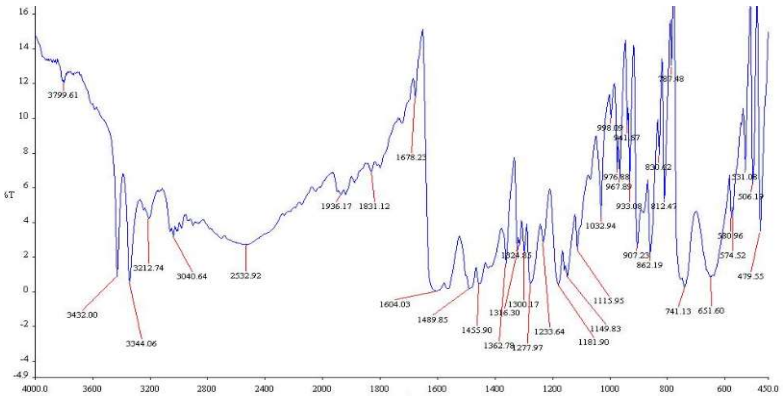
Retention factor (R_f): 0.31(Ethylacetate:n-Hexane=70:30)

Physical appearance: yellow solid powder

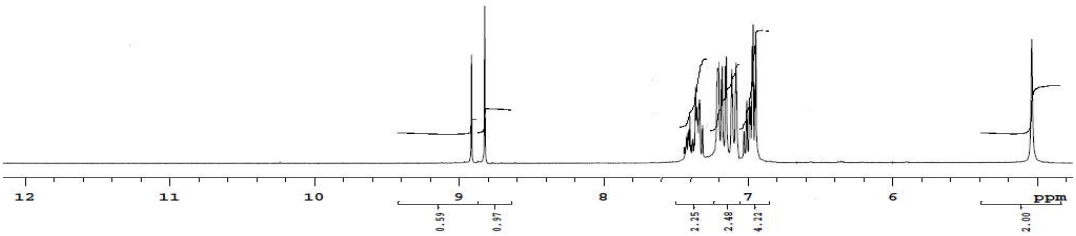
Melting point [^{0}C]: 194

Solubility: DCM, DMSO

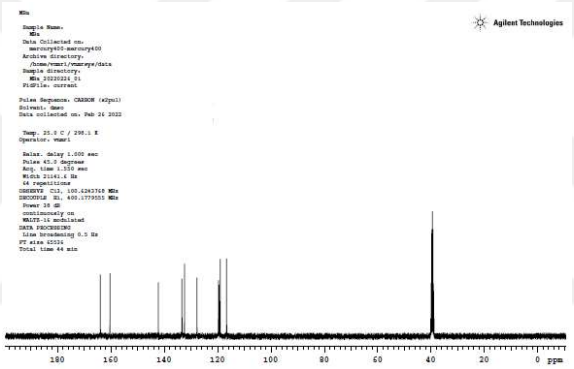
FT-IR (KBr), cm^{-1}: 3392 (OH), 3432-3344 (N-H_2), 1598 (N=C)



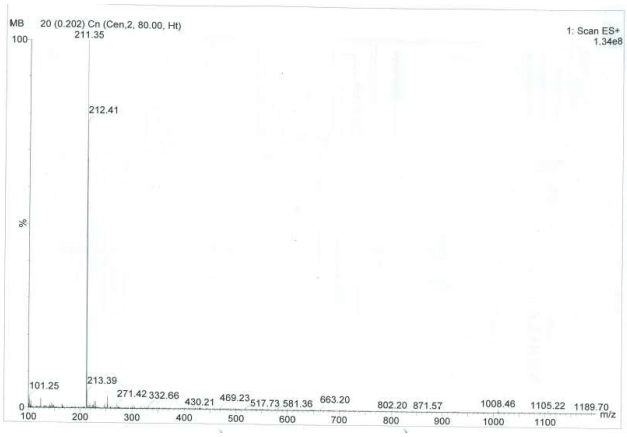
^{1}H NMR (400MHz, DMSO-d6): 8.9 (0.5H,s, H_g), 8.8 (1H, s, Hh), 6.87-7.49 (8H,m, H-arom), 5.03(2H, s, He).



^{13} C NMR (400MHz, d6-DMSO): 163.9, 160.2, 142.2, 133.3, 132, 127.7, 119.6, 119.4, 119, 118.9, 116.6.

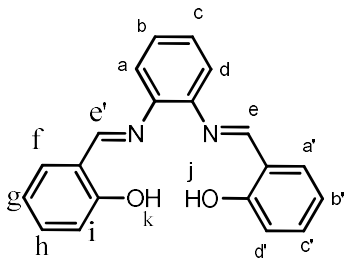


MS spectrum



LC-MS (m/z): 212.41[M^{+}]

2,2-((1E,1'E)-(1,2-phenylenebis(azanylylidene))bis(methnylylidene))diphenol(2)



To a solution of 1.08 gm of 1,2-diaminobenzene (0.01mole) in 30 ml of ethanol, a solution of (2 eq) of salicylaldehyde in 20 ml of ethanol was added. A few drops of 10% NaOH were added, the reaction mixture then refluxed with stirring for two hours and the obtained precipitate was collected by filtration through Buchnner funnel, recrystallized from methanol, and dried at room temperature to afford yellow powder

Molecular weight [g/mol] :316.35

Molecular formula: $C_{20}H_{16}O_2N_2O_2$

Yield [%]:18%

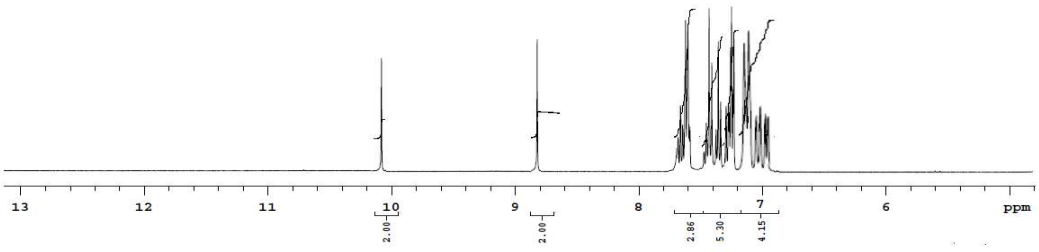
Retention factor (R_f):0.3(Ethylacetate:n-Hexane=70:30)

Physical appearance: yellow solid needle

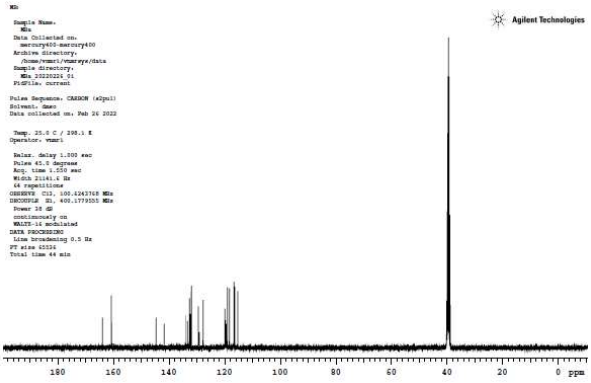
Melting point [^{0}C]: 161

Solubility: DCM

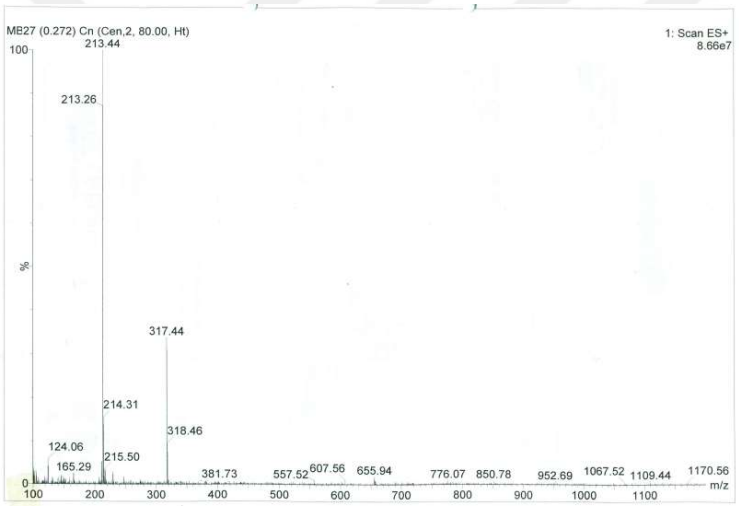
^{1}H NMR (400MHz, DMSO-d6): 10.13 (2H, s,Hj, Hk), 8.81 (2H, s, He,', He), 6.93-7.62 (12H, m,Ar_H).



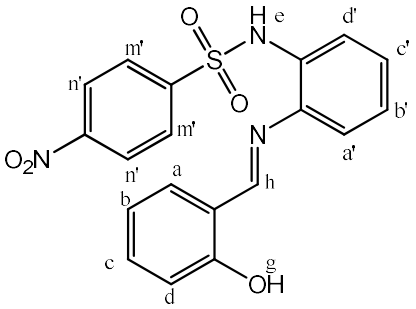
^{13} C NMR (400MHz, d6-DMSO): 162.7, 161.6, 143.2, 141.0, 132.0, 130.5, 128.0, 121.2, 120.2, 117.1, and 116.2.



LC-MS (m/z): 317.4 [M+H].



(E)-N-(2-((2-hydroxybenzylidene)amino)phenyl)-4-nitrobenzenesulfonamide(3)



1 mmol of compound **1** is reacted with 2 mmol of TEA and 2.5 mmol of 4-nitrobenzenesulfonyl chloride in 10mL DMSO, according to the general procedure.

Molecular weight [g/mol]: 397.40

Molecular formula: $C_{19}H_{15}N_3O_5S$

Yield [%]: 20

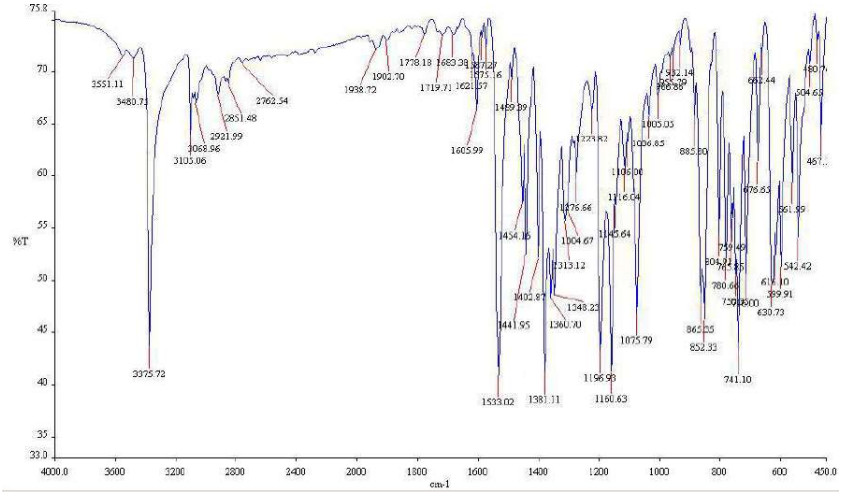
Retention factor (R_f):0.73(Ethylacetate:n-Hexane=70:30)

Melting point [^{0}C]: 203

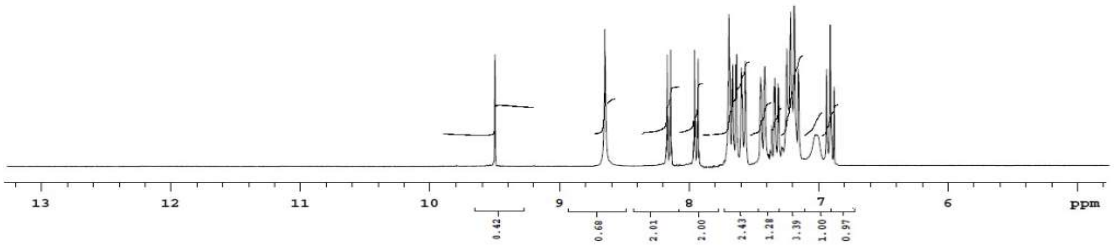
Solubility: DCM, DMSO

Physical appearance: white solid powder

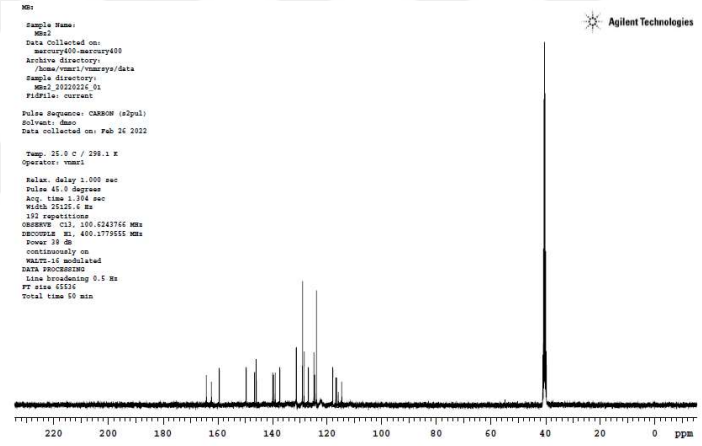
FT-IR (KBr), cm^{-1}: 3375 (NH), 3480 (OH), 1605 (N=C), 1381-1533 (S=O).



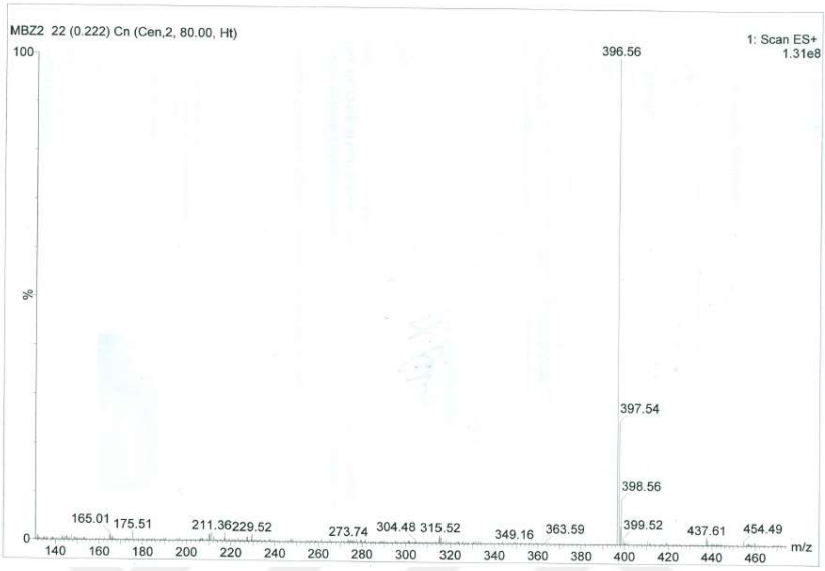
[1]HNMR(400MHz, DMSO-d6): 9.5 (1H, s, Hg), 8.63 (1H,s, Hh), 8.13 (1H, s, Hh), 8.13 (d, 2H *J* =3.3Hz, Hn, Hn'), 7.90 (d, 2H , *J*= 3Hz, Hm,Hm'), 6.86-7.7 (m,H-aromatic).



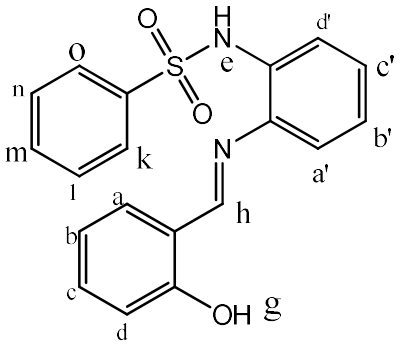
[13]C NMR (400MHz, d6-DMSO): 164.3, 162.2, 161.1, 140.9, 139.5, 137.7, 132.5, 129.4, 128.4, 128.2, 128.2, 128.2, 127.3, 118.9, 117.7, 117, 116.8.



LC-MS (m/z): 396.56 [M^+].



(E)-N-(2-((2-hydroxybenzylidine)amino)phenyl) benzenesulfonaamide (**4**)



1 mmol of compound **1** is reacted with 2 mmol of TEA and 2.5 mmol of benzenesulfonyl chloride in 10mL DMSO, according to the general procedure.

Molecular weight [g/mol]: 352.41

Molecular formula: $C_{19}H_{16}N_2O_3S$

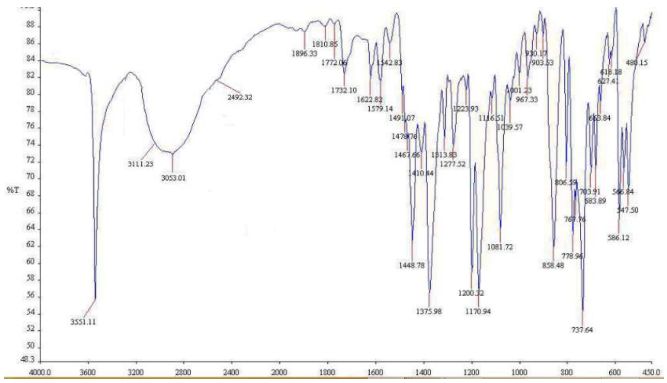
Yield [%]: 14

Retention factor (R_f): 0.65 (Ethylacetate:n-Hexane=70:30)

Solubility: DCM, DMSO,

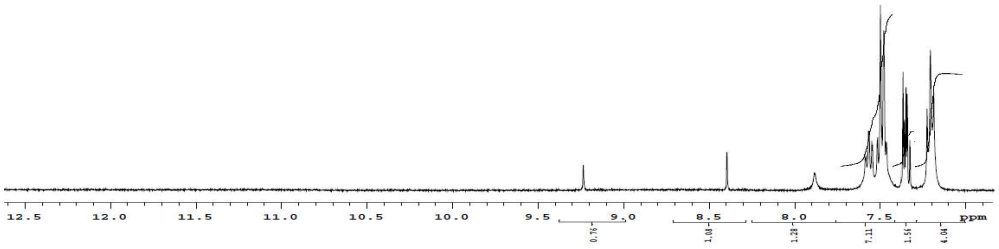
Physical appearance: white solid powder

FT-IR (KBr), cm^{-1}: 3551 (NH), 3111 (OH), 1579-1622 (N=C), 1375-1448 (S=O).

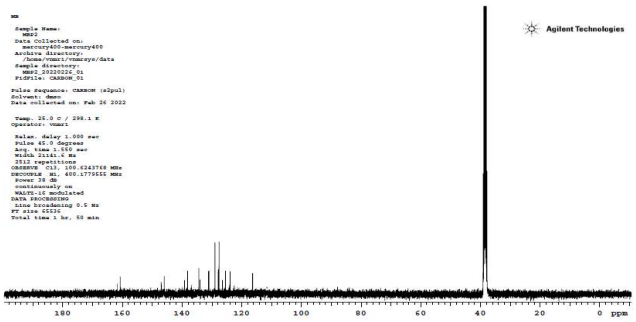


^{1}H-NMR (400MHz, DMSO-d6): 9.43 (1H,s, Hg), 8.43(1H, s, Hh), 7.87 (1H, s, He) 7.4-

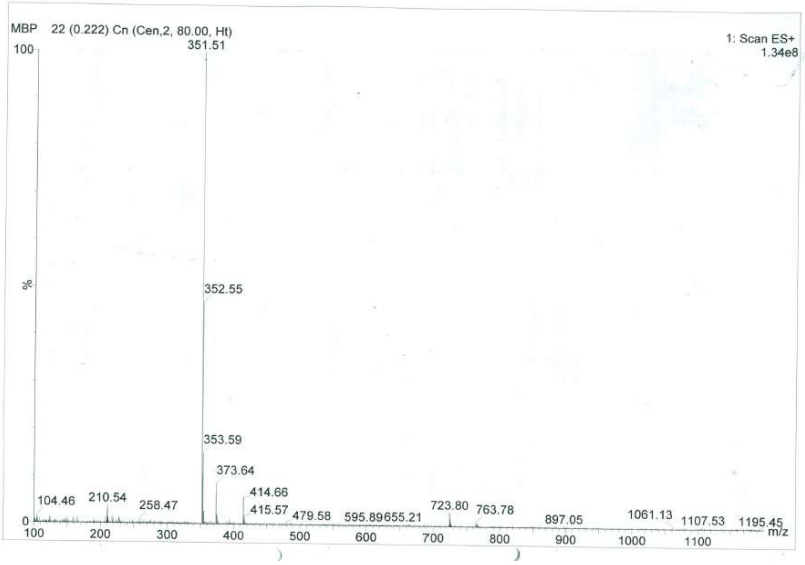
7.62 (m,7H, H-arom), 7.27-7.37 (m, 2H, H-arom), 7.16-7.26 (m,4H, H-arom).



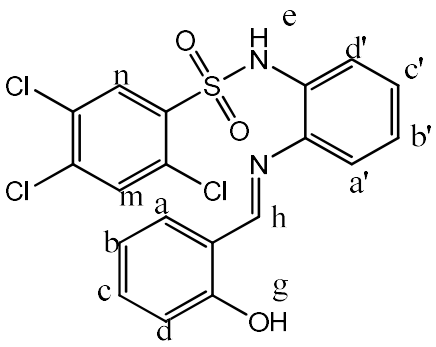
^{13}C NMR (400MHz, d6-DMSO): 148.9, 147, 145, 143.8, 134.4, 134, 132, 131, 130, 128, 127.8, 127.6, 124.9, 123.9, 122,5.



LC-MS (m/z): 351.51[M^+].



(E)-2,4,5-trichloro-N-(2-((2-hydroxybenzylidene)amino)phenyl)benzenesulfonamide (**5**)



1 mmol of compound **1** is reacted with 2 mmol of TEA and 2.5 mmol of 2,4,5-trichlorobenzenesulfonyl chloride in 10mL DMSO, according to the general procedure.

Molecular weight [g/mol]: 455.74

Molecular formula: $C_{19}H_{18}Cl_3N_2O_3S$

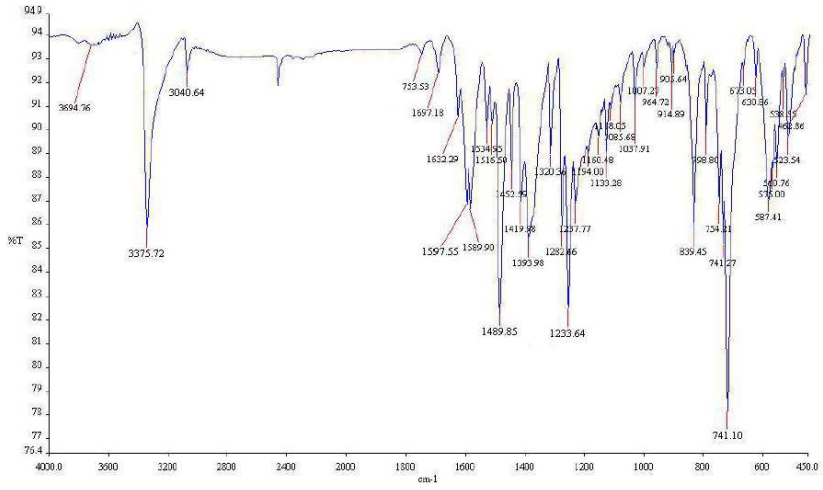
Yield [%]: 18

Retention factor (R_f):o.71(Ethylacetate:n-Hexane=70:30)

Solubility: DCM, DMSO

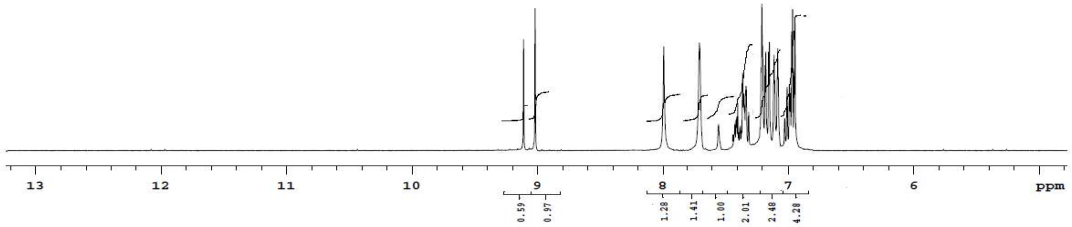
Physical appearance: white powder

FT-IR (KBr), cm^{-1}: 3375.72 (NH), 3694.76 (OH), 1589-1597 (N=C), 1233-1489 (S=O).

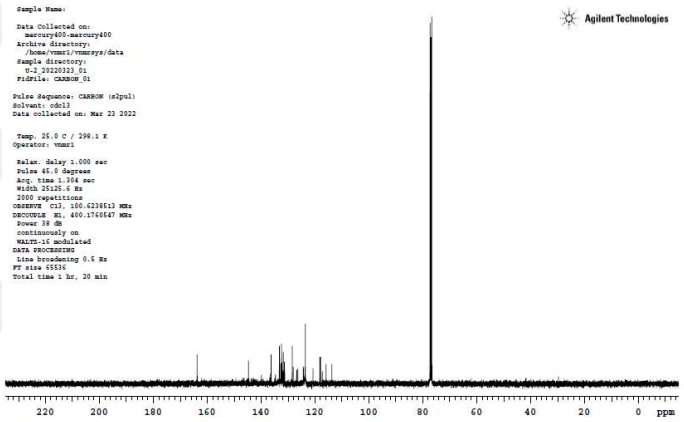


^{1}H-NMR (400MHz, DMSO-d6): 9.15 (1H, s, Hg), 8.98 (1H, s, Hh), 7.95 (s,1H, Hm),

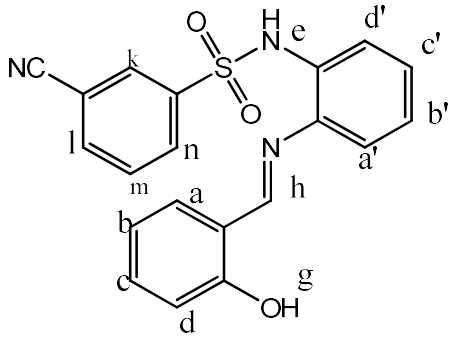
7.68 (s,1H, Hn), 7.54 (1H, s, He), 6.87-7.40 (m, 8H, H-arom).



[13] C NMR (400MHz, d6-DMSO): 162.2, 142.0, 137.7, 136.9, 133.3, 132.5, 132.2, 131.6, 131.3, 129.4, 128.4, 128.3, 128.2, 127, 118.9, 118.9, 116.7, 114.8.



(E)-3-cyano-N-(2-((2-hydroxybenzylidene)amino)phenyl)benzenesulfonamide (**6**)



1 mmol of compound **1** is reacted with 2 mmol of TEA and 2.5 mmol of 3-cyanobenzenesulfonyl chloride in 10mL DMSO, according to the general procedure.

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Molecular weight [g/mol]: 377.42

Molecular formula: $C_{20}H_{15}N_3O_3S$

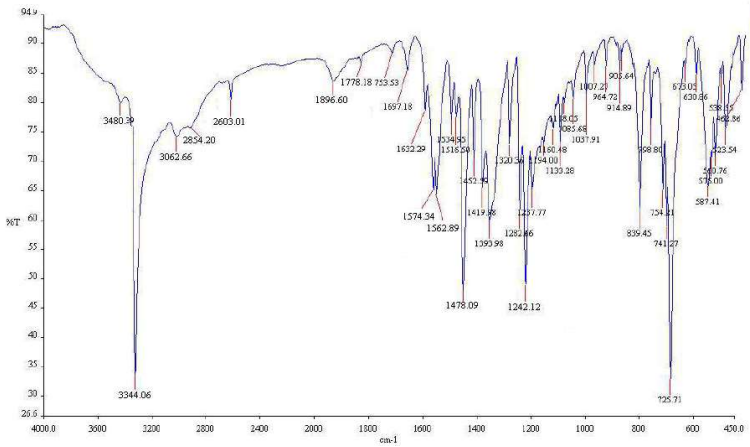
Yield [%]: 27

Retention factor (R_f): 0.59(Ethylacetate:n-Hexane=70:30)

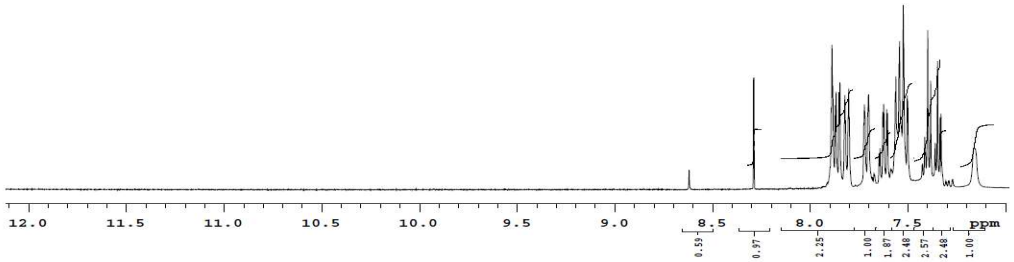
Solubility: DMSO

Physical appearance: white solid powder

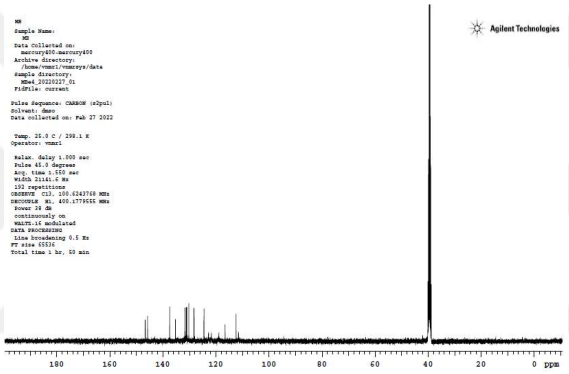
FT-IR (KBr), cm^{-1}: 3344 (NH), 3480 (OH), 1562-1574 (N=C), 1242-1478 (S=O).



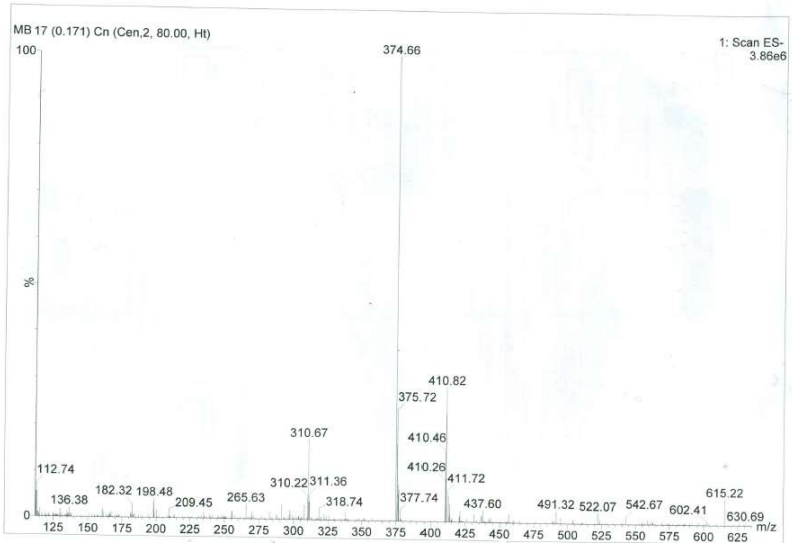
^{1}H NMR (400MHz, DMSO-d6): 8.63 (1H, s, Hg), 8.29 (1H, s, H_h), 7.27-7.89 (m, 12H ,H-aromatic), 7.18(1H, s, H_e).



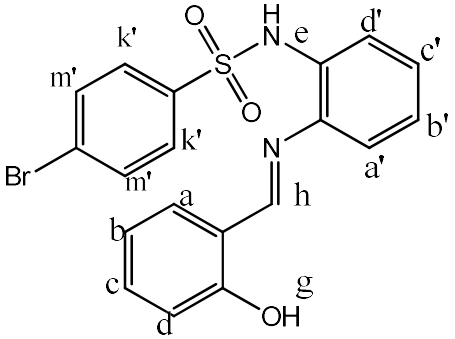
^{13} C NMR (400MHz, d6-DMSO): 146.6, 145.7, 137.4, 135.2, 131.7, 131.2, 131.1, 130.9, 130.2, 128.2, 124.5, 124.4, 122.8, 121.7, 118.9, 116.5, 112.4, 111.5.



LC-MS (m /z): 377.74[M^{+}]



(E)-4-bromo-N-(2-((2-hydroxybenzylidene)amino)phenyl)benzensulfonamide (**7**)



1 mmol of compound **1** is reacted with 2 mmol of TEA and 2.5 mmol of 4-bromobenzenesulfonyl chloride in 10mL DMSO, according to the general procedure.

Molecular weight [g/mol]: 431.30

Molecular formula: $C_{19}H_{15}N_2BrO_3S$

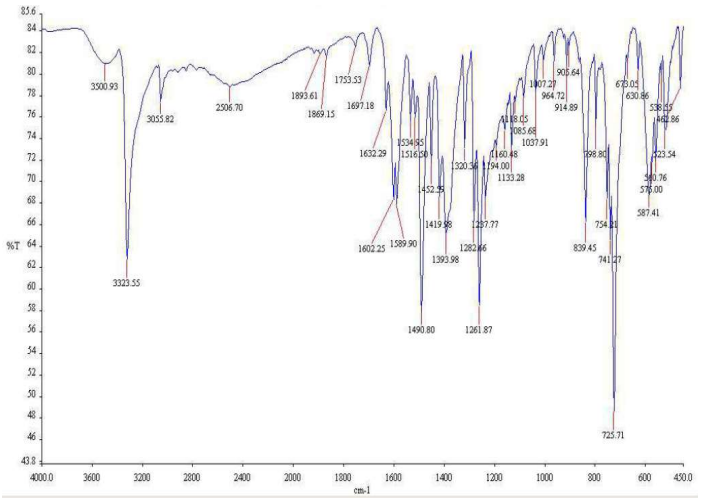
Yield [%]: 30

Retention factor (R_f): 0.67(Ethylacetate: n-Hexane=70:30)

Solubility: DMSO

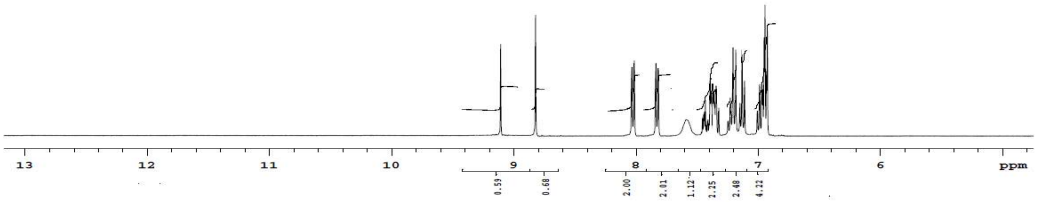
Physical appearance: white powder

FT-IR (KBr), cm^{-1}: 3323 (NH), 3500 (OH), 1589-1602 (N=C), 1261-1490 (S=O).

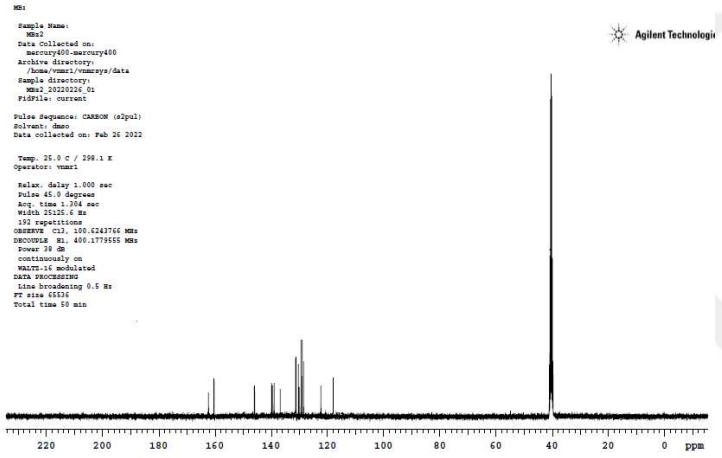


^{1}H NMR (400MHz, DMSO-d6): 9.13 (1H, s, Hg), 8.83 (1H, s, Hh), 8.01 (d, 2H,

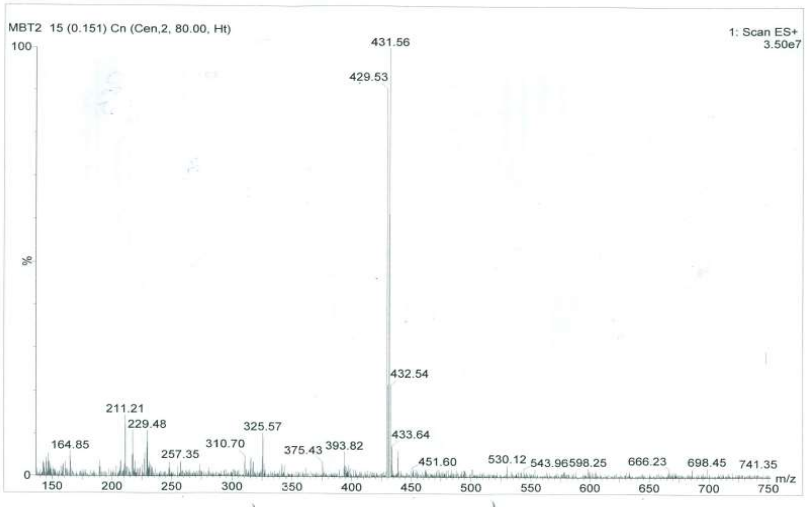
*J=6.*1Hz, Hm'), 7.79 (d, 2H, *J*= 6.4 Hz, Hk'), 7.52 (H, s, He), 6.92-7.43 (m, 8H, H-aromatic).



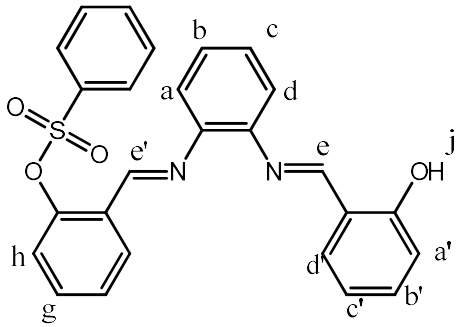
^{13}C NMR (400MHz, d6-DMSO): 162.2, 161.1, 146.2, 139.5, 137.7, 136, 132.7, 132.5, 129.4, 128.4, 128.3, 128.2, 128.1, 127.3, 122.3, 118.9.



LC-MS (m /z): 431.56[M^+].



2-((E)-((2-((E)-(2-hydroxybenzylidene)amino)phenyl)imino)methyl)phenyl benzenesulfonate (**8**)



1 mmol of compound **1** is reacted with 2 mmol of TEA and 2.5 mmol of benzenesulfonyl chloride in 10mL DMSO, according to the general procedure.

Molecular weight [g/mol]: 456.51

Molecular formula: $C_{26}H_{20}N_2O_4S$

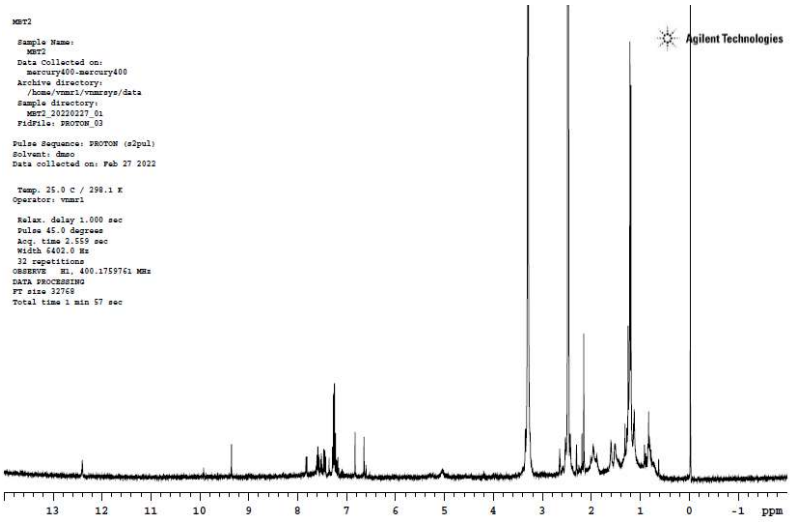
Yield [%]: 21

Retention factor (R_f):0.56

Solubility: DMSO, cloroform

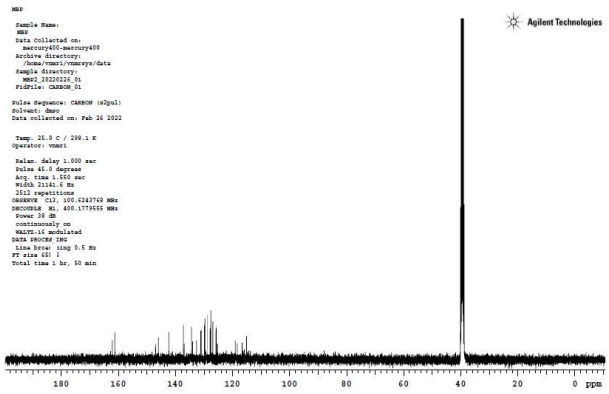
Physical appearance: white powder

^{1}H NMR (400MHz, DMSO-d6: 9.8 (1H, s, Hj), 8.83 (2H, s, He, He'), 7.1-7.67 (m, 15H, H-aromatic), 6.86 (1H, m, Hh), 6.89 (1H, m, Ha').

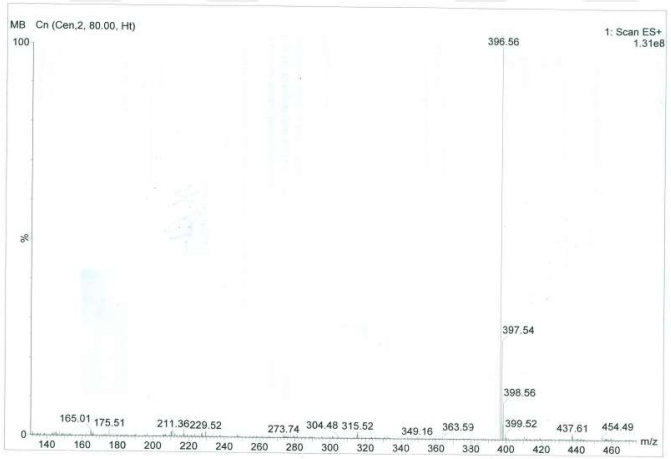


^{13} C NMR (400MHz, d6-DMSO): 162.3, 162.2, 161.1, 142, 137.7, 137.7, 133.3, ;132.4, 132.5, 132.5, 129.4, 129.4, 128.61, 128.6, 128.56, 128.5, 128.3, 128.2, 127.8, 125.8,

118.9, 118.9, 116.1, 14.8.



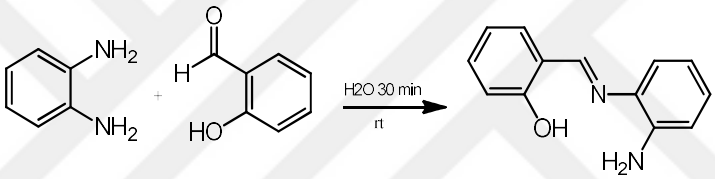
MS spectrum



5 RESULTS AND DISCUSSION

5.1 Chemistry

This study consisted of the synthesis of 2-((Z)-(2-aminophenylimino) methyl) phenol (**1**) and 2,2'-(1E,1'E)-(1,2-phenylenebis(azan-1-yl-1-ylidene))bis(methan-1-yl-1-ylidene) (**2**), two Schiff bases that were further treated with different arylsulfonylchloride derivatives. Two methods were used to synthesize the Schiff bases. For the synthesis of 2-((Z)-(2-aminophenylimino) methyl) phenol (**1**), in the first method the product was obtained in aqueous medium by stirring OPDA with salicylaldehyde to give a yellow solid with a satisfactory yield of 62%. This method is experimentally simple, clean, high yielding, and with reduced reaction times. The product was obtained after simple filtration followed by washing with water and drying processes.



Scheme 5.1 Aqueous synthesis of the Schiff base **1**

In the second method OPDA was condensed with salicylaldehyde in methanol, the reaction mixture was stirred for 3 hours and the obtained precipitate was collected by filtration. The product was obtained yet with a low yield of 14%.

A possible reaction mechanism for the synthesis of 2-((Z)-(2-aminophenylimino) methyl) phenol in these conditions is shown below (Figure 5.1).

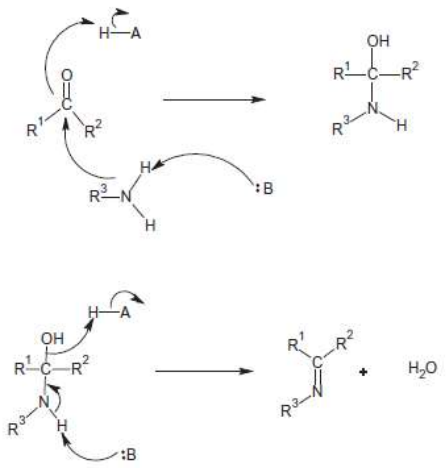


Figure 5.1 Reaction mechanism for the synthesis of 2-((Z)-(2-aminophenylimino) methyl) phenol

According to this mechanism, the formation of an imine molecule undergoes within two different steps. At first, a nucleophilic addition reaction takes place by the attack of the lone pair of electrons of the amine group to the electrophilic carbonyl carbon to form a hemiacetal intermediate. Then in the second step, the deprotonation of the nitrogen atom forms the carbinolamine molecule, wich lead to the expected imine after a final dehaydration step.

When 2 equivalent of salicylaldehyde were added to one equivalent of OPDA to synthesize the bis Schiff base 2,2-((1E,1'E)-(1,2-phenylenebis(azanylylidene))bis(methnylylidene))diphnnol (fig bellow) using both the green synthesis method and the conventional method by mixing the staring material in methanol. The expected bis-Schiff base, compound **2** was obtained with yields of 29 and 18% respectively.

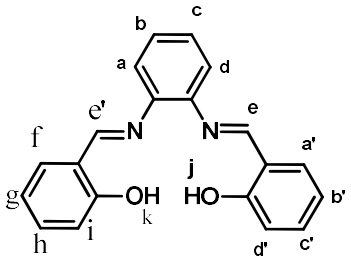


Figure 5.2 Chemical structure of bis Schiff base

Following their synthesis, the structural elucidation of compounds **1** and **2** was carried out using spectral analyses, the detailed analysis for compound 1 is given below.

The ^{1}H NMR spectrum exhibited a singlet peak at 8.82 ppm corresponding to (N=CH) Shiff base proton. An additional singlet peak at 8.92 ppm integrating for one proton was obtained for the signal of the O-H of the compound. The two protons of the amine function gave a signal that was observed as broad singlet at around 5.03 ppm. Also, multiplets were observed in the range of 6.87-7.49 ppm that were related to the aromatic protons.

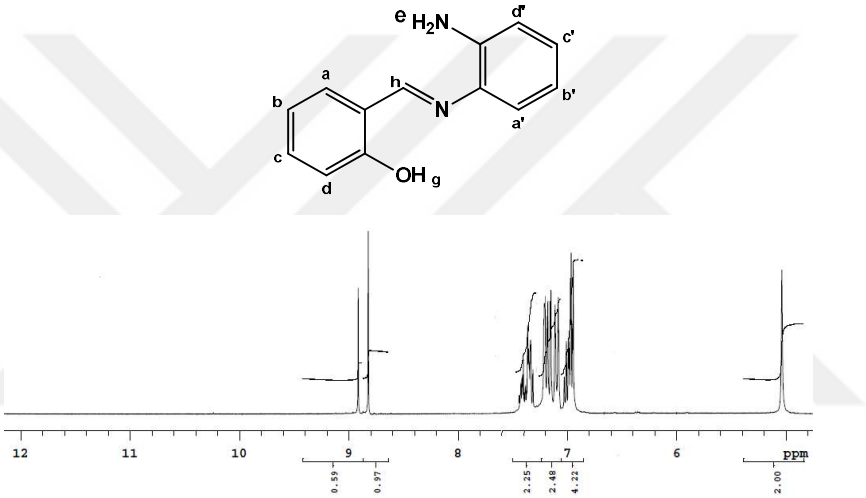


Figure 5.3 ^{1}H-NMR spectrum of Compound 1

Concerning the ^{13}C-NMR spectrum of this Schiff base, the carbon of the imine function gave a signal at 142.2 ppm. For the aromatic carbons, peaks were observed within the aromatic region at 163.9, 160.8, 133.3, 132, 127.7, 119.6, 119.4, 119, 118.9 and 116.6ppm.

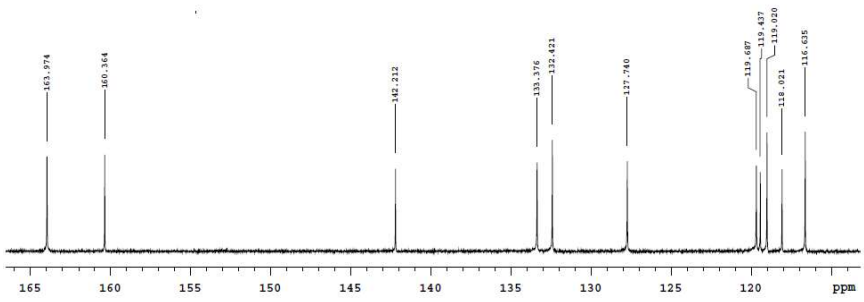


Figure 5.4 ^{13}C-NMR spectrum of compound **1**

The compound was also analyzed by FT-IR and the typical HN_2 asymmetric stretching band was found to appear at 3432-3344 cm^{-1}, also a vibration band at 1604 cm^{-1} corresponding to C=N bond was observed.

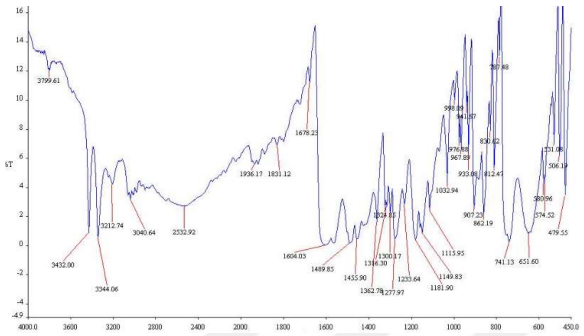
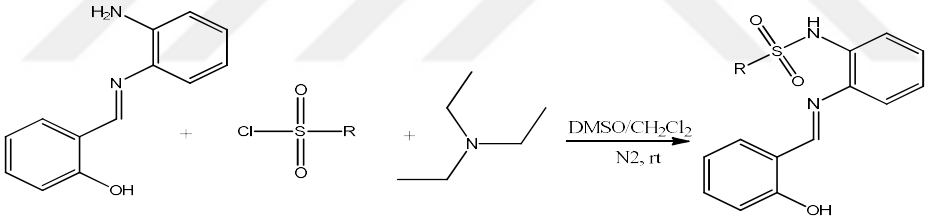


Figure 5.5 FT-IR spectrum of compound **1**

In order to develop sulfonamide substituted Schiff base derivatives, a series of sulfonyl chlorides were used. The results obtained with different sufonyl chlorides are gathered in Table 5.1.



Scheme 5.2 The reaction of a sulfonyl chloride with Schiff base (**1**)

Table 5.1 Synthesized sulfonylamide substituted Schiff bases

	Sulfonyl chloride	**Yield [%]**
3	4-Nitrobenzenesulfonyl chloride	20
4	Benzenesulfonyl chloride	14
5	2,4,5-trichlorolbenzenesulfonyl chloride	18
6	3-cyanobenzenesulfonyl chlorid	27
7	4-Bromobenzenesulfonyl chloride	30

All the synthesized sulfonamide derivatives exhibited a fluorescence property with different intensity under UV lamp. Analysis showed that a complex mixture was obtained for each sulfonylchloride that led to low yields. The procedure and the detailed spectral analyses are given for compound **4** that was obtained from benzenesulfonylchloride.

To obtain compound 4 benzenesulfonyl chloride was reacted with compound **1** in the presence of trimethylamine as base catalyst in anhydrous DMSO, under nitrogen atmosphere. The reaction occurred through an addition-elimination reaction on the highly electrophilic sulfonyl chloride derivative. The reaction of the primary amine on the sulfonyl chloride resulted on the formation of the sulfonamide derivative **4**.

Compound **4** was collected after a column chromatography that was carried out to purify the targeted product using different solvent system.

Compound **4** was first analyzed by FT-IR. A characteristic vibration band corresponding to the N-H appeared at 3500 cm^{1}. The O-H vibration band was found to appear at around 3100 cm^{-1}.

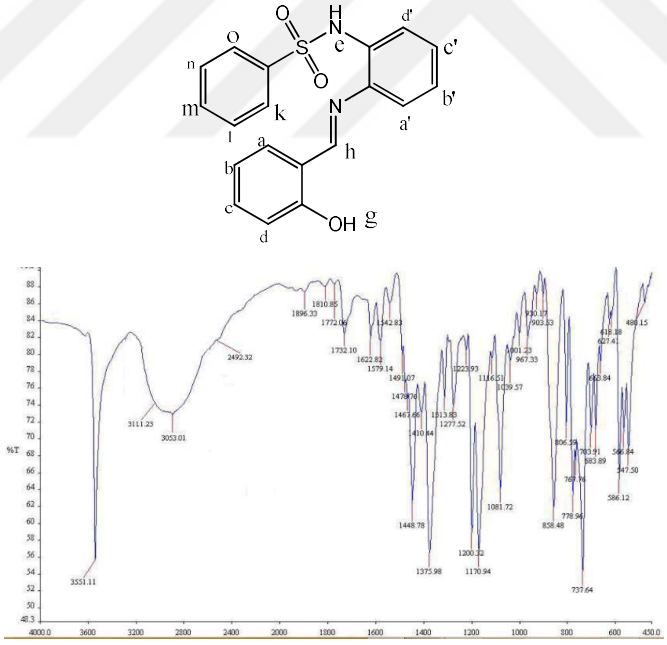


Figure 5.6 FT-IR spectrum of sulfonamide derivatives

The ^{1}H NMR spectrum of the sulfonamide derivative presented a singlet peak in the aromatic region at 7.18 ppm corresponding to sulfamoyl group indicating that the synthesis of sulfonamide derivatives was achieved. Also, a singlet peak appeared at 8.63

ppm corresponding to hydroxyl group. The Shiff base proton gave a sharp singlet signal appearing at 8.29 ppm and the other hydrogen signals were observed in the aromatic region were the signals integrated for a total of 13 hydrogens.

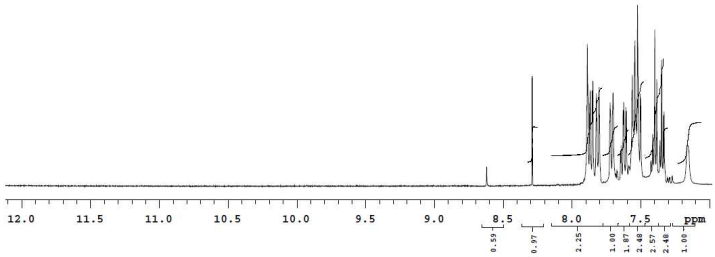


Figure 5.7 ^{1}H-NMR spectrum of sulfonamide derivatives

Concerning the ^{13}C-NMR data of the sulfonamide substituted Schiff base derivative, the presence of Schiff base carbon was characterized by a signal that appeared at 143.8 ppm correspond to Schiff base carbon, the other peaks in the compound observed in the aromatic region were attributed to the sp2 aromatic rings carbons.

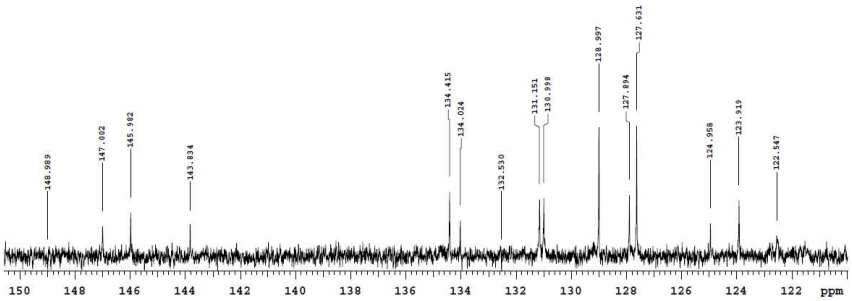


Figure 5.8 The ^{13}C-NMR spectrum of sulfonamide substituted schiff base derivative (**4**)

The compounds **3**, **5**, **6** and **7** were similarly obtained and characterized by the spectral methods. The derivatives presented the typical proton signal corresponding to the imine function at 8.63, 8.98, 8.29 and 8.83 ppm respectively.

In addition, treatment of 2,2-((1E,1'E)-(1,2-phenylenebis(azanylylidene)) bis(methnylylidene))diphenol, compound **2**, with different sulfonylchloride derivative was carried out using dichloromethane and dimethyl sulfoxide as a solvent with use of DMAP and TEA as catalyst. In both conditions, the o-sulfonated products were obtained, when examined by thin layer chromatography, but upon storage for very short time the

compound was found to be decomposed: a solid precipitate was first formed but a further TLC examination revealed a range of spots indicating a stability issue for such structures (Figure 5.9).

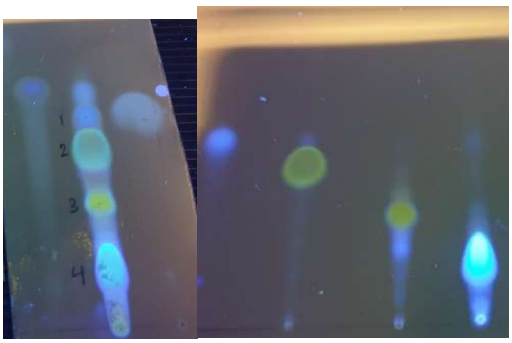


Figure 5.9 Decompositon of the sulfonated bis Schiff base derivative (**8**)

5.2 Biology:

The cytotoxicity of the synthesized compound was tested against high grade serous ovarian cancer cell lines OVCAR-3, OVSAHO and KUROMOCHI ve CAOV-3 using NCI-Sulforhodamine B assay. In order to calculate their IC50 values, at concentration rangig from (2.5µM-40µM) of the tested compound, the IC_{50} values results are gvin in (Table 3).

Table 5.2 IC_{50} value obtained for the synthesized compounds (tested on different ovarian cancer cells)

Compound	KUROMOCHI	OVCAR-3	OVSAHO	CAOV-3
1	NI	64.43	NI	8.02
2	NI	NI	NI	13.15
3	NI	NI	NI	NI
4	NI	NI	NI	NI
5	16.99	3.31	17.55	6.53
6	NI	15.73	NI	NI
7	71.52	9.87	42.17	16.95
8	NI	19.34	NI	NI

Results revealed that compound **5** and **7** exhibit the highest antiproliferative activity against ovarian cancer cell lines OVCAR-3, OVSAHO and KUROMOCHI ve CAOV-3, with IC_{50} values of 16.99, 3.31, 17.55, and 6.53μM respectively for compound **5**. and IC_{50} values of 71.52, 9.87, 42.17, and 16.95μM for compound **7**. These findings suggested that the presence of chlorine and bromine atom may be essential for the antiproliferative activity of such sulfonamide substituted Schiff base structures.

6 CONCLUSION

In this study sulfonamide, substituted Schiff base derivatives were developed under nitrogen atmosphere. The structural information of the synthesized chemical was estimated by FT-IR, 1H NMR, ^{13}C NMR, LC-MS. All compounds were synthesized and tested in order to evaluate their cytotoxic activity. Amongst them compound 5 and, 7 exhibit the highest antiproliferative activity against cancer cells type OVCAR-3, OVSAHO and KUROMOCHI ve CAOV-3, with IC_{50} values of 16.99, 3.31, 17.55, and 6.53µM for compound **5**. And 71.52, 9.87, 42.17, and 16.95µM for compound **7**. The presence of chlorine and bromine substituents in sulfonamide complex combination may be essential for the antiproliferative activity, this is may be attributed to their physicochemical properties, that enhances the chemical interaction with the target proteins.

7 REFERENCES

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Program	Level
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Mendeley EndNote	Very Good