Ahmed Omar Attar

PHARMACEUTICAL CHEMISTRY

MASTER OF SCIENCE THESIS

2023

T.C.

YEDITEPE UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

SYNTHESIS AND ACTIVITY STUDIES OF 4- HYDROXYACETOPHENONE-PIPERAZINE MANNICH BASES

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SUPERVISOR
Assist. Prof. Dr. Enise Ece GÜRDAL

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TEZ ONAYI FORMU

Kurum : Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü

Program : Farmasötik Kimya Yüksek Lisans

Tez Başlığı : 4- Hidroksiasetofenon-Piperazin Mannich Bazlarının Sentez ve Aktivite Çalışmaları

Tez Sahibi : Ahmed Omar ATTAR

Sınav Tarihi : 27.1.2023

Bu çalışma jurimiz tarafından kapsam ve kalite yönünden Yüksek Lisans/Doktora Tezi olarak kabul edilmiştir

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Prof. Dr. Bayram YILMAZ

Sağlık Bilimleri Enstitüsü Müdürü

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Owner of the Thesis : Ahmed Omar ATTAR

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This study has approved as a Master/Doctorate Thesis in regard to content and quality by the Jury.

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This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated ..... and numbered .....

Prof. Dr. Bayram YILMAZ

Director of Institute of Health Science

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.

27.1.2023

Ahmed Omar Attar

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LIST OF ABBREVIATIONS

$AlCl_3$	Aluminum Chloride
$CDCl_3$	Deuterated Chloroform
COX	Cyclooxygenase Enzyme
Cu/Wo_3	Copper Doped Tungsten Oxide
DMF	Dimethylformamide
DNA	Deoxyribonucleic Acid
ED_{50}	Effective Dose
EGFR	Epidermal Growth Factor Receptor
Et_3N	Triethylamine
FT-IR	Fourier Transform Infrared
5-FU	5-Fluorouracil
GI_{50}	Growth Inhibitory Concentration
HCl	Hydrochloric Acid
HCT-115, HCT116	Human Colon Cancer Cells
HeLa	Human Cervical Cancer Cells
HEP3B	Liver Cancer Cell Lines
HepG2	Hepatocellular Carcinoma Cell Lines
HIV	Human Immunodeficiency Virus
5-HT	5-Hydroxytryptamine
HUH-7	Hepatocyte Derived Cellular Carcinoma Cell Lines
IC_{50}	The Half Maximal Inhibitory Concentration

K-562	Myelogenous Leukemia Cell Line
RNA	Ribonucleic Acid
LC_{50}	Lethal Concentration 50
MCF-7	Human Breast Cancer Cell Lines
MDA-MB-231	Human Breast Cancer Cell Lines
MIC	Minimum Inhibitory Concentration
M.P	Melting Point
MS	Mass Spectroscopy
M.W.	Microwave
NaOH	Sodium Hydroxide
NMR	Nuclear Magnetic Resonance
Pd	Palladium
P. aeruginosa	Pseudomonas Aeruginosa
ppm	Parts Per Million
SK-LU-1	Lung Adenocarcinoma Cell Lines
$SnMe_4$	Tetramethylstannane
S. aureus	Staphylococcus Aureus
TLC	Thin Layer Chromatography
UV	Ultraviolet

ABSTRACT

Attar, A.O. (2023). Synthesis and activity studies of 4- hydroxyacetophenone-piperazine Mannich bases. Yeditepe University Institute of Health Sciences, Department of Pharmaceutical Chemistry, M.Sc. Thesis, Istanbul.

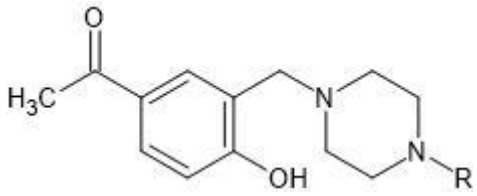
In this study, ten novel compounds having 1-[4-hydroxy-3-[(substituted-1-piperazinyl)methyl]phenyl]ethanone backbone were prepared by Mannich reaction using conventional and microwave assisted synthesis methods. To obtain the target compounds in conventional method a mixture of 4-hydroxyacetophenone, formaldehyde and suitable piperazine derivative were reacted in methanol for 48 hours under reflux at 65 °C. For the microwave assisted synthesis a mixture of 4-hydroxyacetophenone, formaldehyde and suitable piperazine derivative were reacted in 1,4-dioxane for 30 minutes at 120 °C. A comparison between the two methods were carried out yield wise.

Structure elucidation of the synthesized compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, and elemental analysis. The cytotoxicity of the compounds was examined against human breast cancer cell line (MCF-7) by MTT assay.

According to the activity data, compounds **4** and **7** had the highest cytotoxic effect.

Keywords: Cytotoxicity, piperazine, 4-hydroxyacetophenone, Mannich base, microwave.

Table 1.1. Structures of the synthesized compounds.



Compound		Yield (%)		M.P. (°C)
No.	**R**	**Conventional**	**Microwave**	
1	Allyl	19	72	N/A
2	Phenylethyl	26	36	116.1
3	2-Fluorophenyl	21	24	115.6
4	4-Methoxyphenyl	23	33	130
5	Furoyl	21	43	120
6	Benzoyl	15	24	128
7	Cyclohexyl	27	33	110.4
8	2-Methylphenyl	22	25	126.2
9	4-Methylphenyl	29	32	143
10	3-Chlorophenyl	13	30	138.1

ÖZET

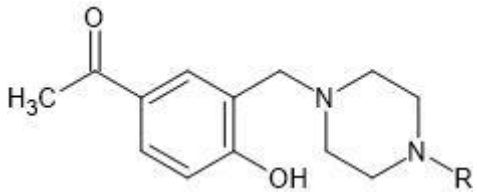
Attar, A.O. (2023). 4- Hidroksiasetofenon-piperazin Mannich bazlarının sentez ve aktivite çalışmaları. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Farmasötik Kimya Anabilim Dalı, Yüksek Lisans Tezi, İstanbul.

Bu çalışmada, 1-[4-hidroksi-3-[(sübstitüe-1-piperazinil)metil]fenil]etanon iskeletine sahip on yeni bileşik, geleneksel ve mikrodalga destekli sentez yöntemleri kullanılarak Mannich reaksiyonu ile hazırlanmıştır. Geleneksel yöntemde hedef bileşikleri elde etmek için 4-hidroksiasetofenon, formaldehit ve uygun piperazin türevi karışımı 65 °C'de geri akış altında 48 saat boyunca metanol içinde reaksiyona sokulmuştur. Mikrodalga destekli sentez için 4-hidroksiasetofenon, formaldehit ve uygun piperazin türevi karışımı 1,4-dioksan içinde 120 °C'de 30 dakika reaksiyona sokulmuştur. İki yöntem arasında verim açısından bir karşılaştırma yapılmıştır.

Sentezlenen bileşiklerin yapıları IR, ^{1}H-NMR, ^{13}C-NMR ve element analizi ile doğrulanmıştır. Bileşiklerin sitotoksisiteleri insan meme kanseri hücre hattı MCF-7'ye karşı MTT deneyi ile incelenmiştir. Aktivite verilerine göre, **4** ve **7** numaralı bileşikler en yüksek sitotoksik etkiye sahiptirler.

Anahtar Kelimeler: Sitotoksisite, piperazin, 4-hidroksiasetofenon, Mannich bazı, mikrodalga.

Tablo 1.1. Sentezlenen bileşiklerin yapıları.



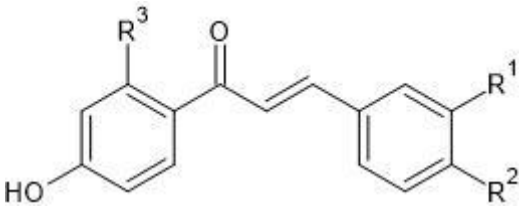
Bileşik		Verim (%)		E.D. (°C)
No.	**R**	**Konvansiyonel**	**Mikrodalga**	
1	Allyl	19	72	N/A
2	Phenylethyl	26	36	116.1
3	2-Fluorophenyl	21	24	115.6
4	4-Methoxyphenyl	23	33	130
5	Furoyl	21	43	120
6	Benzoyl	15	24	128
7	Cyclohexyl	27	33	110.4
8	2-Methylphenyl	22	25	126.2
9	4-Methylphenyl	29	32	143
10	3-Chlorophenyl	13	30	138.1

1. INTRODUCTION

Cancer is a major concern and a significant barrier to raising life expectancy globally [1]. Moreover, as elder people are more vulnerable to cancer and the aging of population carries on, cancer is predicted to become the main death cause in most countries. Despite recent advancements in curing cancer, conventional cytotoxic treatments including radiotherapy and chemotherapy have a number of drawbacks which may result in cancer relapse, therapy failure due to drug resistance, and unpleasant side effects [2]. Therefore, efforts to find new anticancer agents are needed.

Acetophenone is a major synthon in many organic reactions. Numerous heterocyclic compounds can be prepared by utilizing this synthon. Biological activities of many compounds bearing acetophenone were investigated [3], and the findings indicated that these compounds have a broad spectrum of biological activities such as: anticancer [4], antibacterial [5], antifungal [6], antioxidant [7], anticonvulsant [8], and anti-inflammatory [9].

Several studies reported that compounds bearing 4-hydroxyacetophenone in their chemical structures have anticancer activity. Compounds shown below were found to have cytotoxic activity against breast (T47D) and colon (WiDr) cancer cell lines [10].



R_1= H, OCH_3; R_2= OCH_3, Cl; R_3= H, OH

Figure 1.1. Cytotoxic 4-hydroxyacetophenones.

Piperazine is ranked third in popularity among N-heterocycles used in bioactive molecules. The piperazine scaffold is considered a remarkable structural component in drug synthesis and therapeutic development, that keeps on having an increased attention in many drug structures, since it can provide a relative structural rigidity, large polar surface area, and more hydrogen bond acceptors and donors that may result in improved

target affinities and specificities [11]. Drugs containing piperazine moiety were found to have many therapeutic uses such as: anticancer [12], antimicrobial [13], anti-inflammatory [14], antidepressant [15], and antihistaminic [16].

El-Abadelah *et al.* reported the cytotoxic activity of amidrazones carrying piperazine motif. The compound below showed potent activity against MCF-7 with IC_{50} value of 7.26 µM, and 9.91 µM against leukemia cell lines (K-562) [17].

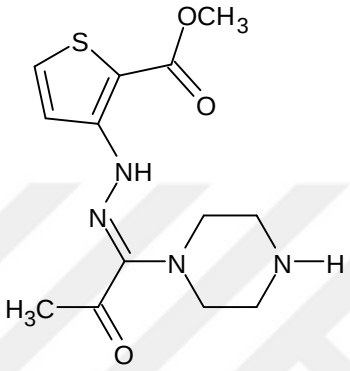


Figure 1.2. Cytotoxic amidrazones with piperazine.

Thuy *et al.* prepared novel Mannich bases bearing 4-hydroxyacetophenone and piperazine in their structures. The cytotoxicity of those compounds was examined against several cell lines. Compound **a** exhibited significant activity against human lung cancer cell lines (SK-LU-1) and, human hepatoma cell lines (HepG2) having IC_{50} values of 3.72 µg/mL, and 3.16 µg/mL, respectively [18].

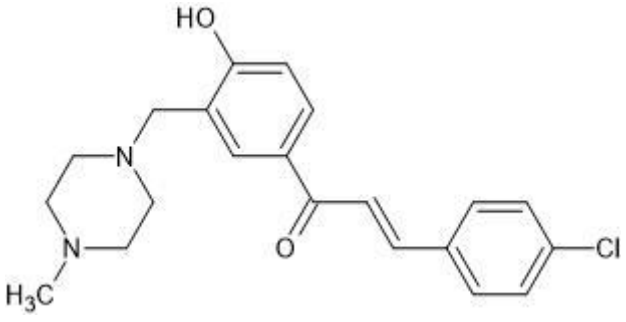


Figure 1.3. Compound a.

In view of these studies, we aimed to synthesize and purify novel Mannich bases bearing 4-hydroxyacetophenone and piperazine as a backbone of their chemical structures and study their cytotoxic activities against breast cancer cell line (MCF-7). In addition, a

comparison was carried out between conventional and microwave methods regarding the reaction conditions and yields obtained.

2. GENERAL DESCRIPTION

2.1. Acetophenone

Acetophenone is a ketone which has both an aromatic and aliphatic moiety. It's considered the simplest aromatic ketone [19]. The molecular formula of acetophenone is $C_6H_5COCH_3$.

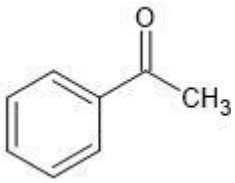
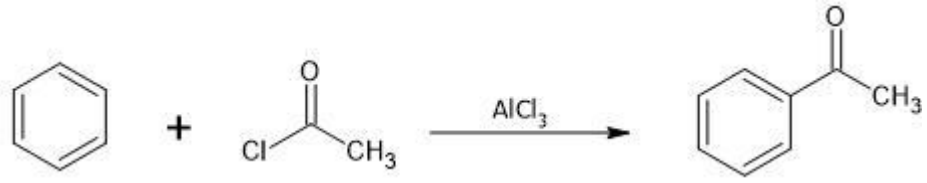


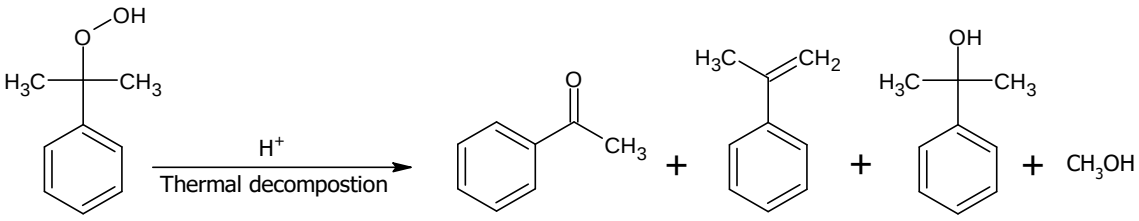
Figure 2.1. Structure of acetophenone.

2.1.1 Method of Synthesis

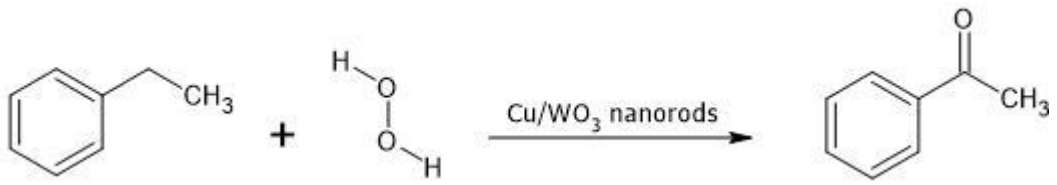
Acetophenone can be synthesized through Friedel-Crafts reaction of benzene with acetyl chloride by utilizing $AlCl_3$ as a Lewis acid catalyst [20].



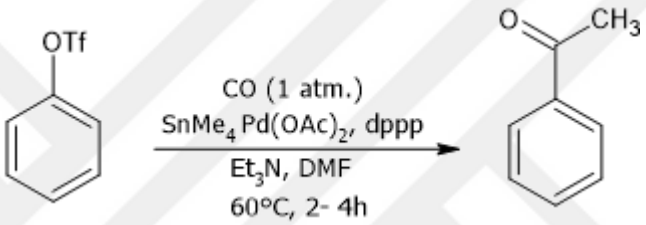
Acetophenone can also be prepared from the thermal decomposition of cumene hydroperoxide (above 90°C), in the presence of acid [21].



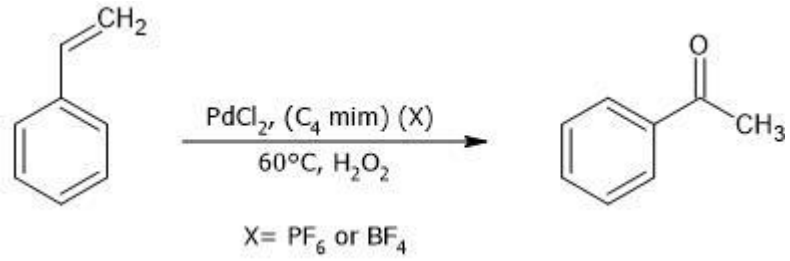
Another approach to obtain acetophenone in acceptable yield is by the oxidation of ethylbenzene using hydrogen peroxide in the presence of Cu/Wo_3 as metallic catalyst [22].



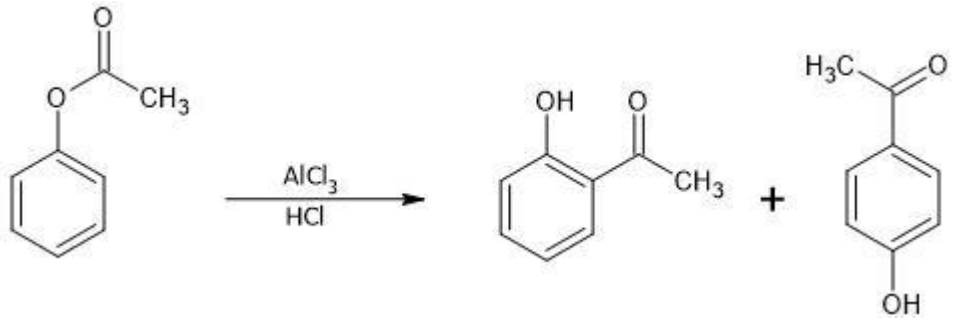
The reaction of aryl triflates with a mixture of $SnMe_4$, Pd (0), CO (balloon), and Et_3N in DMF at 60°C [23].



Acetophenone is also prepared from Palladium-catalyzed oxidation of styrene (Wacker reaction) in the presence of ionic liquids using hydrogen peroxide [24].



Phenyl acetate is subjected to Fries rearrangement to obtain a mixture of *p*-hydroxyacetophenone and *o*-hydroxyacetophenone in the presence of $AlCl_3$ and HCl. The *o*-hydroxyacetophenone can be separated from the mixture by steam distillation after adding a little amount of distilled water, then the *p*-hydroxyacetophenone can be washed and crystallized [25].



2.1.2. Spectral Properties of Acetophenone

2.1.2.1. UV Spectroscopy

Three absorption bands can be observed on the UV spectrum of acetophenone at wave lengths 193 nm, 207 nm, and 243 nm [26].

2.1.2.2. IR Spectroscopy

The C-H band of IR spectra of acetophenone shows approximately at 3040 cm^{-1}. The carbonyl C=O stretching band is seen at 1686 cm^{-1} [27].

2.1.2.3. ^{1}H-NMR Spectroscopy

Signals at 2.567 ($-CH_3$), 7.552, 7.69, and 7.994 (phenyl) ppm are visible in the ^{1}H-NMR spectra of acetophenone in deuterated water (D_2O) [28].

2.1.2.4. ^{13}C-NMR Spectroscopy

Five different signals can be detected in the ^{13}C-NMR spectra of acetophenone at 26.47 ($-CH_3$), 128.56, 133.04, 137.23, and 200.7 ppm in deuterated water (D_2O). The signal at 200.7 ppm is resulted from the carbonyl bond [28].

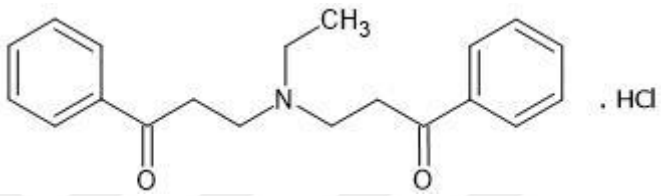
2.1.3. Biological Properties of Acetophenone

The structure of acetophenone attracted many studies in the last decades, since it can be employed in the synthesis of various compounds, which were found to have

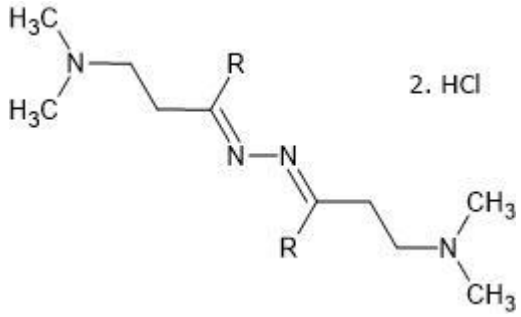
numerous biological activities such as: anticancer [4], antibacterial [5], antifungal [6], antioxidant [7], anticonvulsant [8], and anti-inflammatory [9].

2.1.3.1. Anticancer Activity

3,3'-Ethylimino bis(1-phenyl-1-propanone) hydrochloride was found to have cytotoxic activity against Jurkat cells with LC_{50}= 3.09 µg/mL compared to 4.32 µg/mL for the 5-fluorouracil (5-FU), which makes it more potent than the reference compound by 1.40-fold [29].



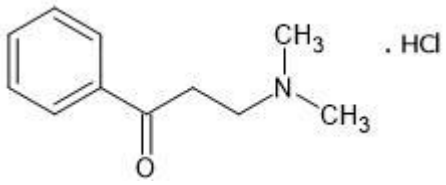
Hydrazones of mono Mannich bases carrying acetophenone synthon in their structures were synthesized and their cytotoxicity were examined against Jurkat cells by Kazaz *et al.* The results showed that these compounds had superior cytotoxic effect compared to the reference drug 5-FU [30].



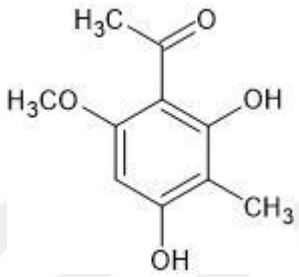
R= C_6H_5, 4-$CH_3C_6H_4$, 4-$CH_3OC_6H_4$, 4-HOC_6H_4, 4-ClC_6H_4, 3-$CH_3OC_6H_4$

4-FC_6H_4, 4-BrC_6H_4, 3-HOC_6H_4, C_4H_3S (2-yl)

Gul *et al.* tested the cytotoxicity of several mono Mannich bases bearing acetophenone nucleus by brine shrimp bioassay. The cytotoxic activity of the compound below was found to be more potent than 5-fluorouracil [31].

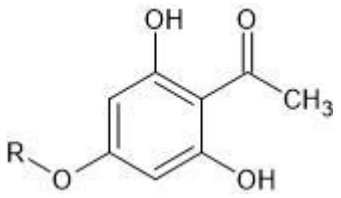


An acetophenone derivative obtained from *Euphorbia ebracteolata Hayata* exhibited selective cytotoxicity against human HeLa cells [32].



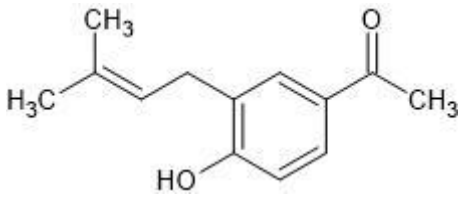
2.1.3.2. Antimicrobial Activity

Bonifait *et al.* reported that compound 2′,6′-dihydroxy-4′-geranyloxyacetophenone has good antimicrobial activity against some Gram-negative bacteria (*Prevotella intermedia, Porphyromonas gingivalis*), Gram-positive bacteria (*Streptococcus mutans, Streptococcus sobrinus*), and *Candida albicans* [33].

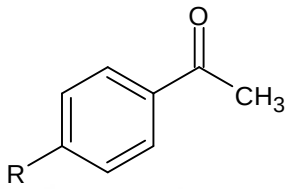


R= Geranyl

Compound below is an acetophenone derivative obtained from *Helichrysum* species. Its antimicrobial activity was good against Gram-negative bacteria (*Escherichia coli*), in addition it exhibited antifungal activity against *Penicillium sp., Cladosporium herbarum* and *Phythophtora capsicii* [34].



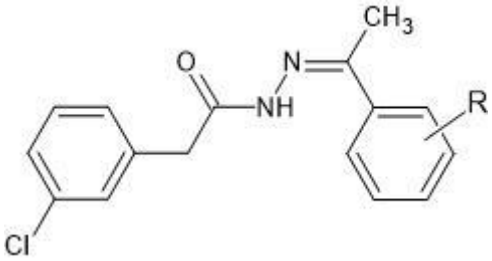
p-Substituted acetophenone derivatives have been reported by Rajabi *et al.* for their moderate growth inhibitory against mycobacteria at 246 µM [35].



R= C_6H_{11}, $C_5H_{10}N$

2.1.3.3. Anticonvulsant Activity

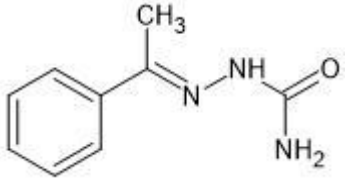
Sameem *et al.* prepared a set of 4-(3-chlorophenyl)-1-(substituted acetophenone) semicarbazones and reported their anticonvulsant effect [36].



R= 4-F, 2-Cl

2.1.3.4. Other Activities

Acetophenone semicarbazone was found to have potent anti-inflammatory and analgesic activities [37].



2.2. Piperazine

Piperazine is a six membered ring heterocyclic compound with its nitrogen atoms at 1,4 positions of the ring [11].

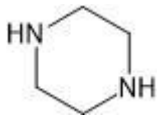
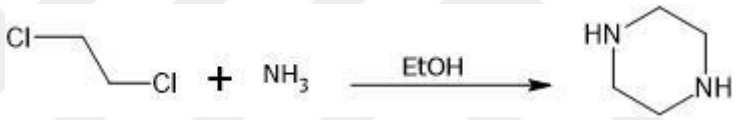


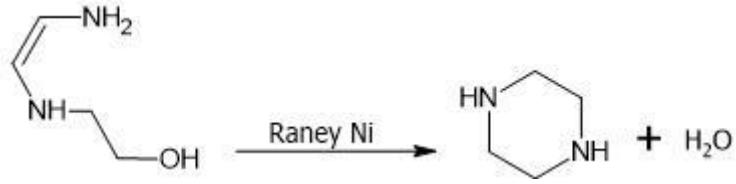
Figure 2.2. Structure of piperazine.

2.2.1. Methods of Synthesis

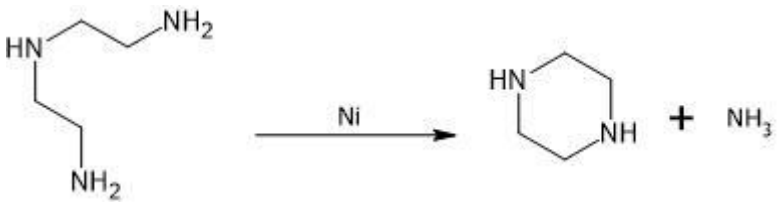
Piperazine was first obtained by the reaction of alcoholic ammonia and ethylene chloride [38].



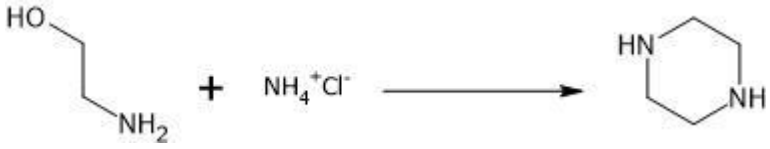
Piperazine can be obtained after the cyclodehydration of N-(2-hydroxyethyl)ethenediamine under high temperatures (200- 300 °C) by utilizing Raney nickel as a catalyst. [39].



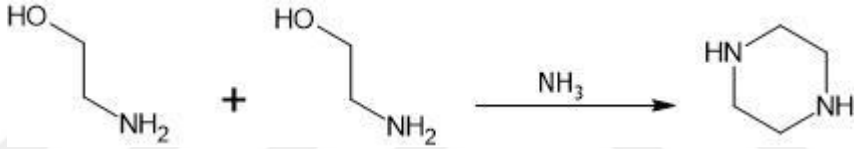
Heating diethylenetriamine by autoclave in the presence of Raney nickel can lead to high yields of piperazine [39,40].



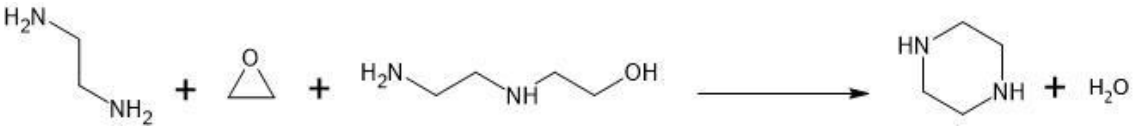
Piperazine is prepared from the reaction of ethanolamine and ammonium chloride at high temperature (250°C) [41].



Piperazine can be obtained after heating two moles of 2-aminoethanol at 150-220°C under high pressure in the presence of ammonia [42].



The reaction of ethylenediamine and oxirane can be utilized to synthesize piperazine [42].



2.2.2. Spectral Properties of Piperazine

2.2.2.1. UV Spectroscopy

Max absorption band can be observed on the UV spectra of piperazine at 196 nm (Log E= 3.7) [43].

2.2.2.2. IR Spectroscopy

At 3250 cm^{-1}, piperazine IR spectrum exhibits sharp singlet N-H stretching bands, and the stretching bands of alicyclic C-H show between 2950-2700 cm^{-1} [44].

2.2.2.3 ^{1}H-NMR Spectroscopy

Piperazine ^{1}H-NMR spectrum exhibits signal at 2.84 ppm in deuterium chloroform [42].

2.2.2.4. ^{13}C-NMR Spectroscopy

Piperazine signals are shown at 47.9 ppm in deuterium chloroform on the ^{13}C-NMR spectra [42].

2.2.2.5. Mass Spectroscopy

Piperazine.$6H_2O$ shows four m/z values in the mass spectrum. These values are: 86, 56, 44, and 30. Base peak is the m/z= 40 that corresponds to the fraction of $NHCH_2CH_2^+$ [45].

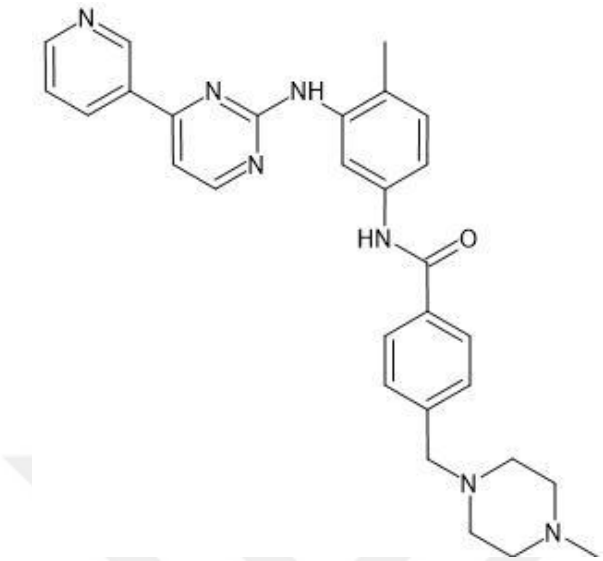
2.2.3. Biological Properties of Piperazine

Piperazine was initially utilized as antihelmintics drug. However, drugs containing piperazine derivatives were not limited to the anthelmintics uses, as numerous studies reported the wide range of biological applications of piperazine in other therapeutic areas [46] such as: antihistamines (hydoxyzine) [47], antibacterial (ciprofloxacin) [48-52], anticancer (imatinib) [53], and antifungal (itraconazole) [54].

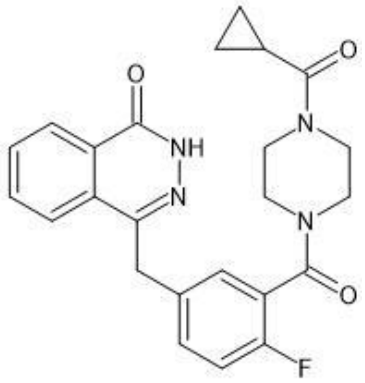
2.2.3.1. Anticancer Activity

Several anticancer medications that have received FDA approval containing the piperazine ring include: abemaciclib which is used to treat different cancer types through the inhibition of D-cyclin dependent kinase (CDK4/6), imatinib inhibits protein kinase,

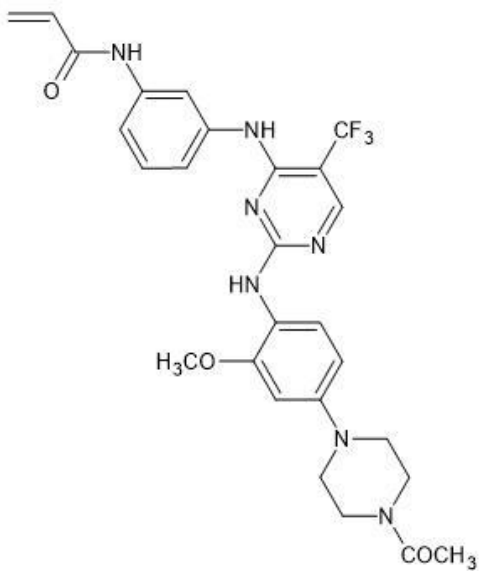
olaparib is used in breast and ovarian cancer therapy, and rociletinib is considered a potent inhibitor of EGFR and is effective against non-small-cell lung cancer [17].



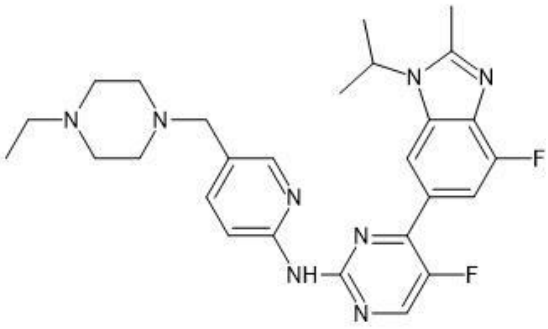
Imatinib



Olaparib



Rociletinib

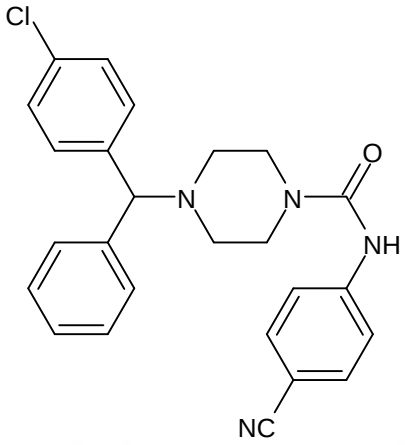


Abemaciclib

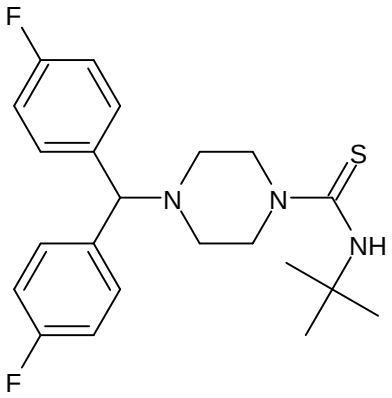
Figure 2.3. Anticancer drugs with piperazine ring.

A set of new benzhydrylpiperazine carboxamide and thioamide derivatives were prepared and screened for their cytotoxicity by Gurdal *et al.* Compounds below exhibited the highest potency against human hepatoma-derived cell line (HUH-7) with GI_{50} values

of 1.29 µM and 5.97 µM. In comparison to the reference drug 5-fluorouracil (30.66 µM), most of the derivatives showed greater cytotoxicity against HUH-7 [55].

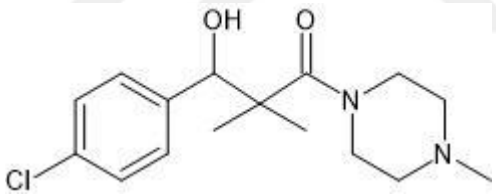


GI_{50}= 1.29 µM

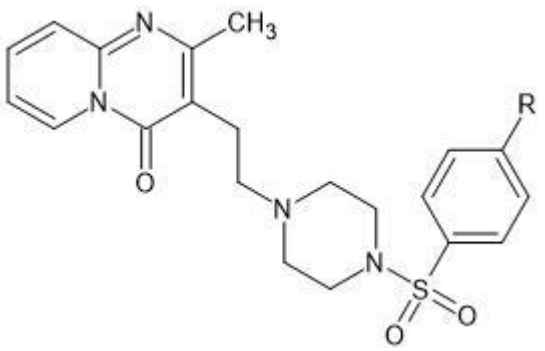


GI_{50}= 5.97 µM

Compound below was synthesized and its antiproliferative activity was studied by molecular docking. The results showed good inhibitory effect against human colon cancer cell line (HCT-116) cells having IC_{50} value of 0.13 mg mL^{-1} [56].

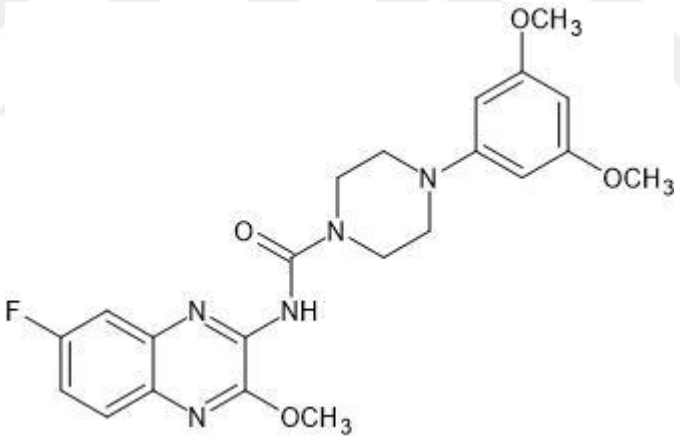


Mallesha *et al.* prepared different pyridopyrimidine-4-one bearing the piperazine ring. The trifluoromethyl phenyl sulfonyl piperazine derivative showed potent cytotoxicity against human neuroblastoma cell line (IMR-32) and cancer cell lines of parental human breast (MDA-MB-231). Nitro-substituted phenyl sulfonyl piperazine derivative also exhibited good inhibition of the cell proliferation [57].



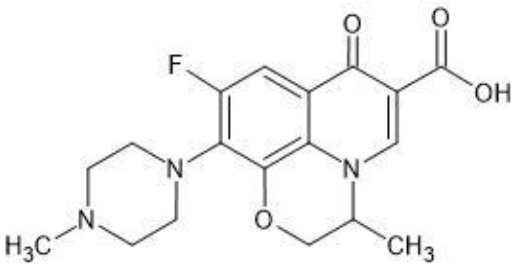
R= CF_3, NO_2

New quinoxalinyl piperazine derivatives were prepared and examined for cytotoxicity effect. Among these derivatives compound below exhibited superior cytotoxicity against eleven different cell lines, having IC_{50} values between 0.011- 0.021 µM. This compound showed high activity against drug-resistant human colorectal carcinoma cell lines (HCT-15) and (HCT-116) having IC_{50} values of 21 ± 0.98 nM and 29 ± 1.4 nM [58].

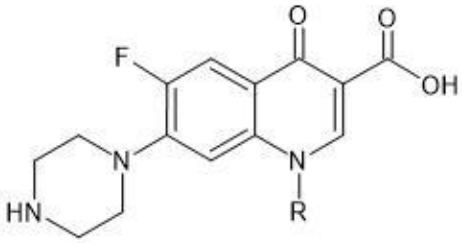


2.2.3.2. Antibacterial Activity

Piperazine scaffold can be found in many antibacterial marketed drugs such as: norfloxacin, ofloxacin and ciprofloxacin [59].



Ofloxacin

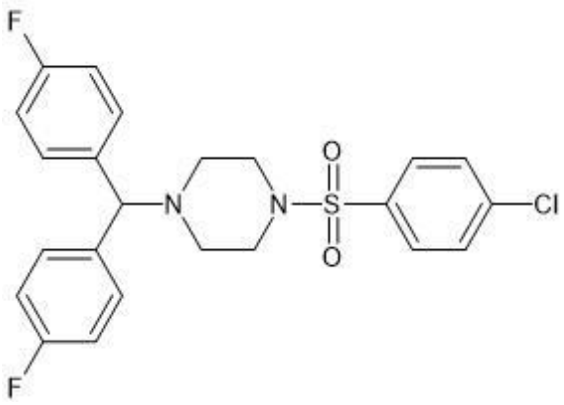


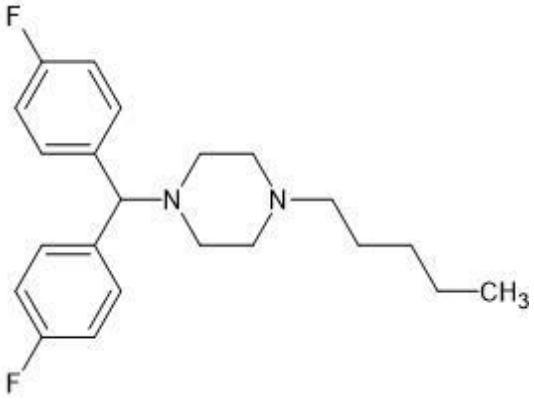
Norfloxacin, R= Ethyl

Ciprofloxacin, R= Cyclopropyl

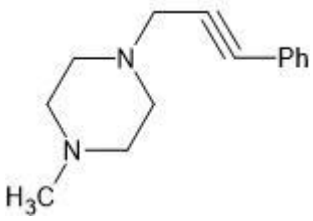
Figure 2.4. Antibacterial drugs with piperazine ring.

Compounds below were prepared and examined for their antibacterial activity by Marella *et al.* These compounds showed high activity against Gram-negative and Gram-positive bacteria. MIC values ranged between 30-169 µg [53].

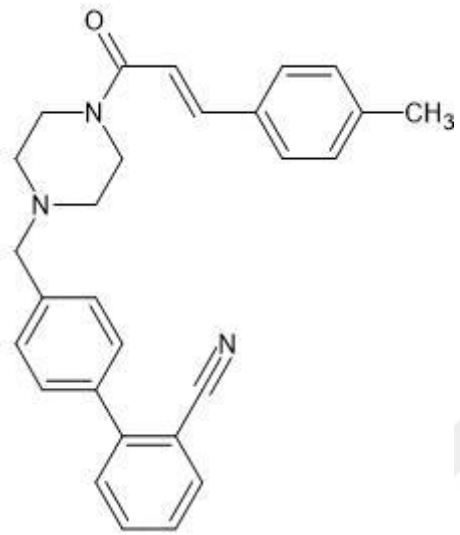




Verma *et al.* prepared numerous piperazine derivatives. Compound below exhibited maximum antibacterial activity against *E. coli, P. aeruginosa, and S. aureus* [60].

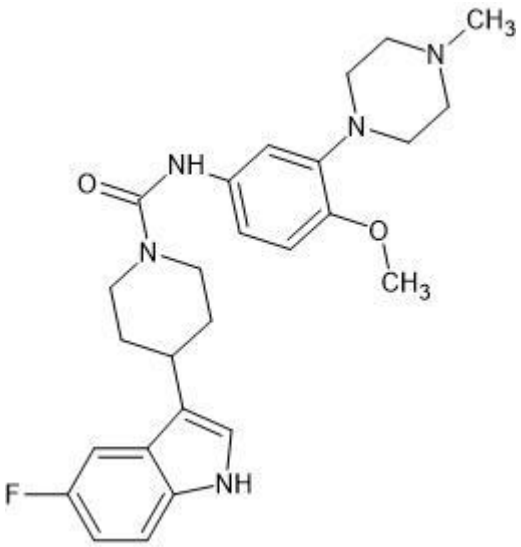


Malani and Dholakiya reported antibacterial activity of compound below against *Escherichia coli*, showing higher activity than standard drug ampicillin with MIC of 62.5 µg/mL [61].

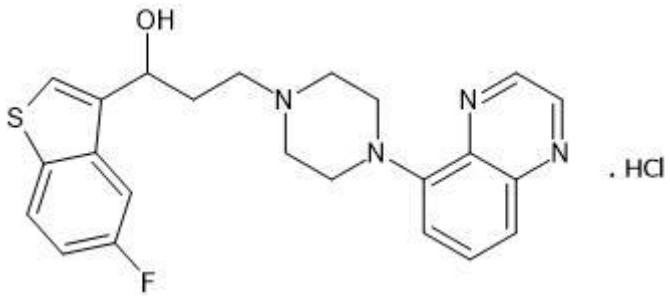


2.2.3.3. Antidepressant Activity

Various piperazine derivatives were prepared and tested for antidepressant activity by Rautenberg *et al*. The results showed significant inhibition of 5-HT reuptake [62].

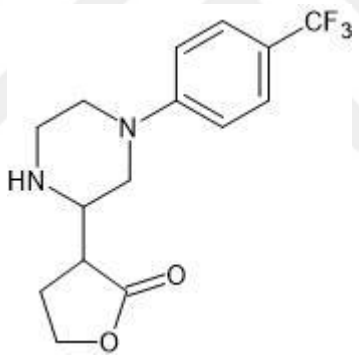


Oficialdegui *et al.* prepared numerous piperazine derivatives to examine their selective serotonin reuptake inhibition. Compound below was found to be potent serotonin reuptake inhibitor [63].



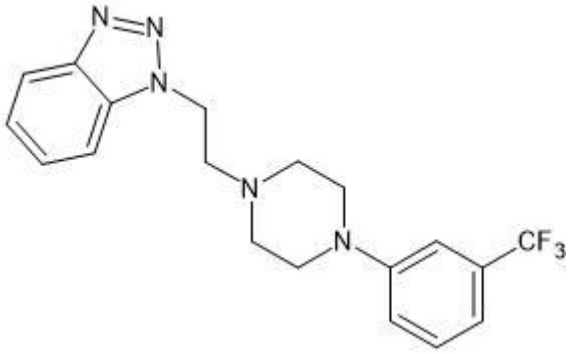
2.2.3.4. Anticonvulsant Activity

Compound below was reported by Salat *et al.* to be a good anticonvulsant drug having an ED_{50} values of 112 mg/Kg [64].

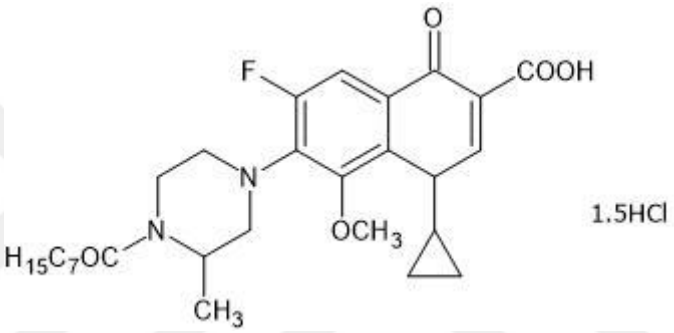


2.2.3.5. Analgesic and Anti-inflammatory Activity

Boido *et al.* synthesized several piperazine analogues. Compound below showed good analgesic activity having IC_{50} value of 580 nM [65].

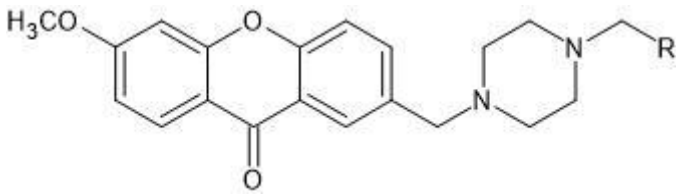


Piperazine derivatives were prepared by Sultana *et al.,* exhibited high anti-inflammatory effect having IC_{50} value less than 12.5 µg/mL [66].



2.2.3.6. Antihistaminic Activity

Perli and Govindarajan prepared new xanthone derivatives bearing piperazine ring and tested them for their biological activity. Compound below showed better affinity for serotoninergic 5- HT_{1A} receptors [67].



R= Ph, CH_3

2.3. Mannich Bases

Mannich bases are produced by a chemical reaction called the *Mannich* reaction, which involves three different reactants: a substrate with one or more active hydrogen atoms (X-H), an aldehyde, and an amine. The reactants are combined in a condensation reaction to form the Mannich base.

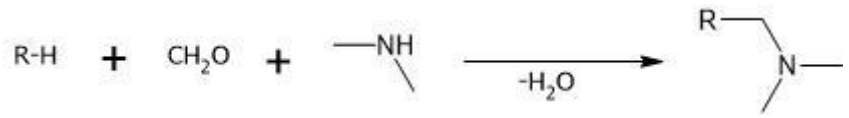


Figure 2.5. General Mannich base reaction.

Mannich bases are considered as substrate derivatives because they are produced by substituting an aminomethyl component when formaldehyde is used or an aminoalkyl component in case of using another type of aldehyde.

Secondary aliphatic amines (R_2NH) are the most frequently used amine reagents in Mannich reaction, primary amines and ammonia can be also used.

The substrates must have a functional group with one or more active hydrogens to undergo Mannich reaction. Commonly used functional groups as substrates in Mannich reactions may include: the hydroxyl functional group in phenols, the heteroatom in heterocycles, the terminal C≡C in alkynes, and the carbonyl group in ketones [68-70].

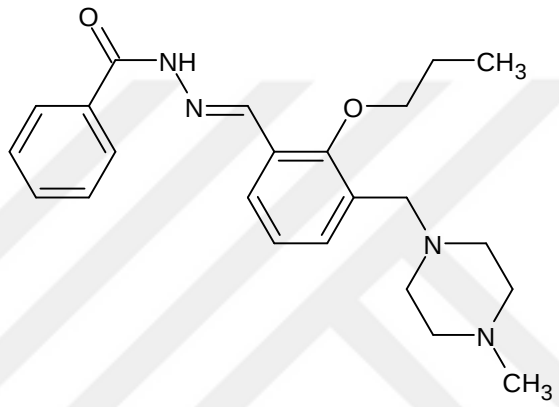
2.3.1. Biological Activities of Mannich Bases

Mannich bases have numerous interesting applications, for example, they can be used as polymers, surface active agents, resins [71], and detergent additives [72], Mannich bases prodrugs of different compounds were developed to get over some drawbacks of these compounds [73]. However, the increased attention Mannich bases have received derives from their importance in the pharmaceutical chemistry field. [74, 75]. Many studies revealed the wide array of biological activates Mannich bases can exhibit such as: anticancer [76, 77], antifungal [78, 79], anti-inflammatory [80, 81],

anticonvulsant [82], antibacterial [83], anthelmintic [84], antiviral [85] antipsychotic [86], antimalarial [87], anti-HIV [88], analgesic [89]**.**

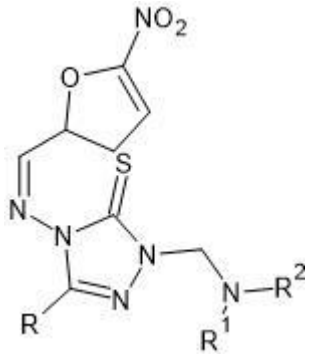
2.3.1.1 Anticancer Activity

Malhotra *et al.* prepared Mannich bases of 2-propoxybenzylideneisonicotinohydrazide and tested them for their cytotoxicity effect against the A-549 human lung adenocarcinoma. The compound below showed potent cytotoxic activity higher than the reference drug, gemcitabine [90].



2.3.1.2. Antimicrobial Activity

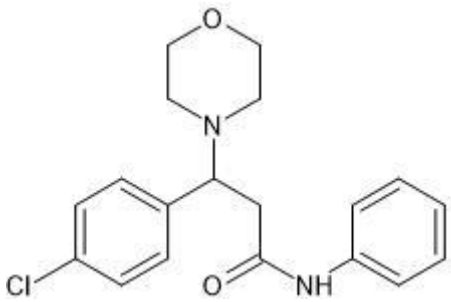
Compounds below were prepared and tested against *C. albicans* for their antifungal activity, these compounds showed better antifungal activity compared to standard drug fluconazole [91].



R= H, Me, Et, Ph

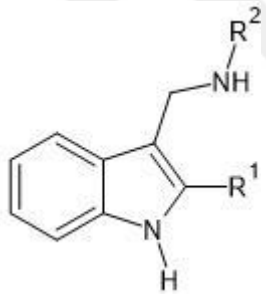
NR_1R_2= 3-Cl, 4-F-aniline, Morpholine, 3-Cl, 4F-aniline, Morpholine, 3-Cl, 4-F-aniline, 3-Cl, 4-F-aniline

Compound below exhibited superior antibacterial activity against *S. epidermidis* compared to reference drug ciprofloxacin [92].



2.3.1.3. Anti-Inflammatory Activity

Mannich bases derived from indole were prepared and evaluated for their anti-inflammatory effect on albino rats. The results showed that the newly obtained Mannich bases had better anti-inflammatory activity than diclofenac sodium [93].

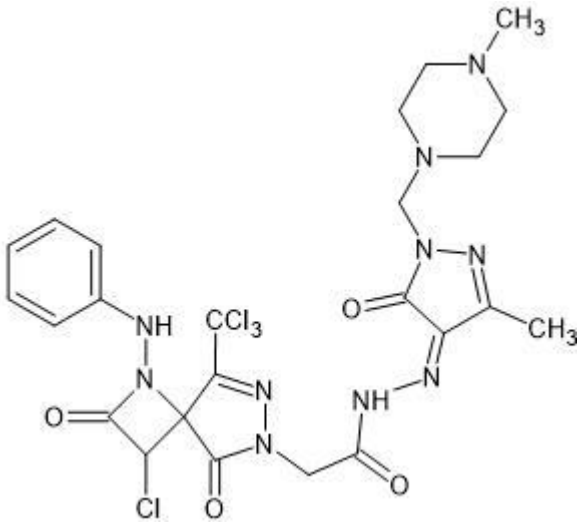


R_1= H, CH_3

R_2= p- $NO_2C_6H_5$, ClC_6H_5NS, ClC_6H_5NS

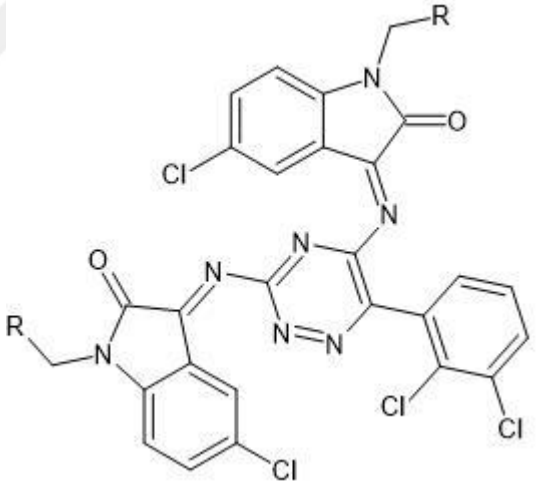
2.3.1.4. Anthelmintic Activity

Mannich base below was synthesized and screened for anthelmintic activity against *P. posthuma*. The compound exhibited potent anthelmintic activity [94].



2.3.1.5. Anticonvulsant Activity

Two Mannich bases derived from lamotrigine were prepared and examined for anticonvulsant activity. Compounds below had higher activity than the reference drugs phenobarbitone sodium and lamotrigine [95].



R= N $(Et)_2$, NC_4H_8O

2.4. Mono Mannich Bases of Acetophenone-Piperazine Derivatives

2.4.1. Method of Synthesis

The preparation of compound (E)-3-[3-(4-ethyl-piperazin-1-ylmethyl)-4-hydroxyphenyl]-1-(methoxyphenyl)propenon was carried out in two stages. First stage

included the preparation of chalcone by *Claisen-Schmidt* reaction between 4-hydroxyacetophenone and 4- methoxybenzaldehyde at room temperature.

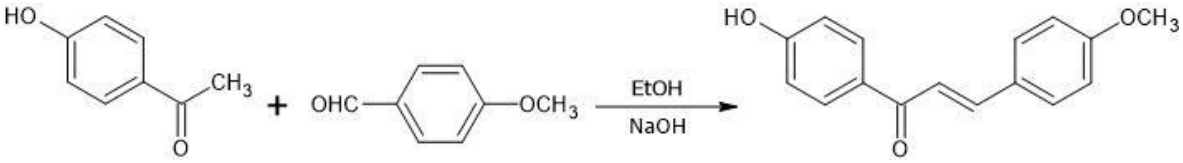


Figure 2.8. First step synthesis of chalcone.

In the second step, a mixture of 1-ethylpiperazine and paraformaldehyde was refluxed in ethanol. Chalcone was added to the reaction mixture, then the mixture was heated under reflux to yield the target compound. [18].

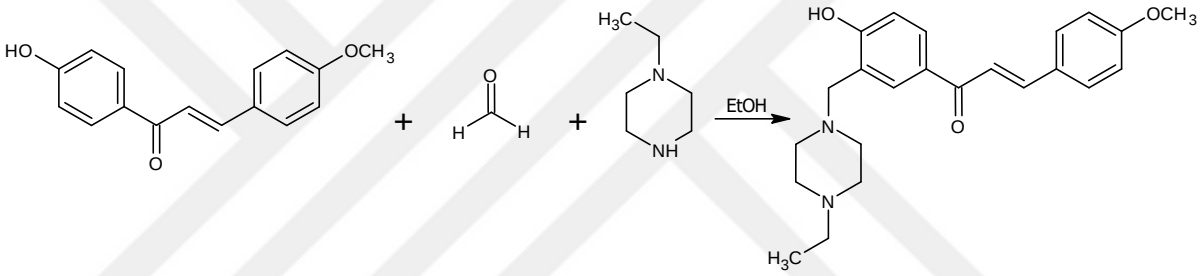
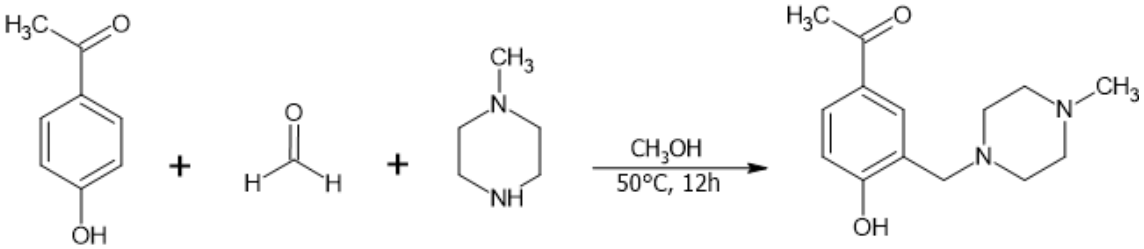
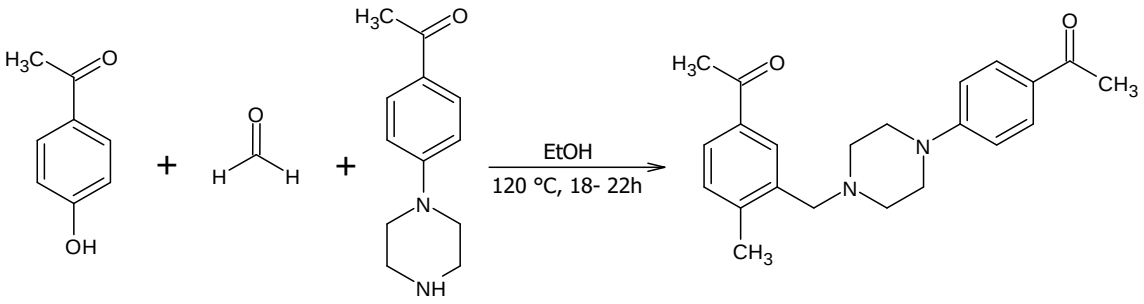


Figure 2.9. Second step synthesis of mono Mannich base.

1-Methylpiperazine, 4-hydroxyacetophenone, and formaldehyde solution were mixed in methanol. The mixture was refluxed, then formaldehyde was added after 6 h to the reaction mixture to yield the target compound [96].



Piperazinoacetophenone was added to a mixture of paraformaldehyde, and 4-hydroxyacetophenone and mixed in ethanol. The resulted mixture was then refluxed to yield the target compound [97].



2.4.2. Spectral Properties of Mono Mannich Bases of Acetophenone-Piperazine Derivatives

2.4.2.1. IR Spectroscopy

IR spectra of 1-{4-[4-(5-acetyl-2-hydroxybenzyl)piperazin-1-yl]phenyl}ethenone shows C-H stretching bands show at 3005 cm^{-1}. Aromatic stretching vibrations can be observed at 1666 cm^{-1} [97].

2.4.2.2. ^{1}H-NMR Spectroscopy

^{1}H-NMR spectra of 2-[(4-methylpiperazin-1-yl)methyl]-4-acetylphenol shows signals at: 2.23 ppm, 2.44 ppm, 2.56 ppm, 3.70 ppm, 6.75–7.73 ppm in $CDCl_3$ [96].

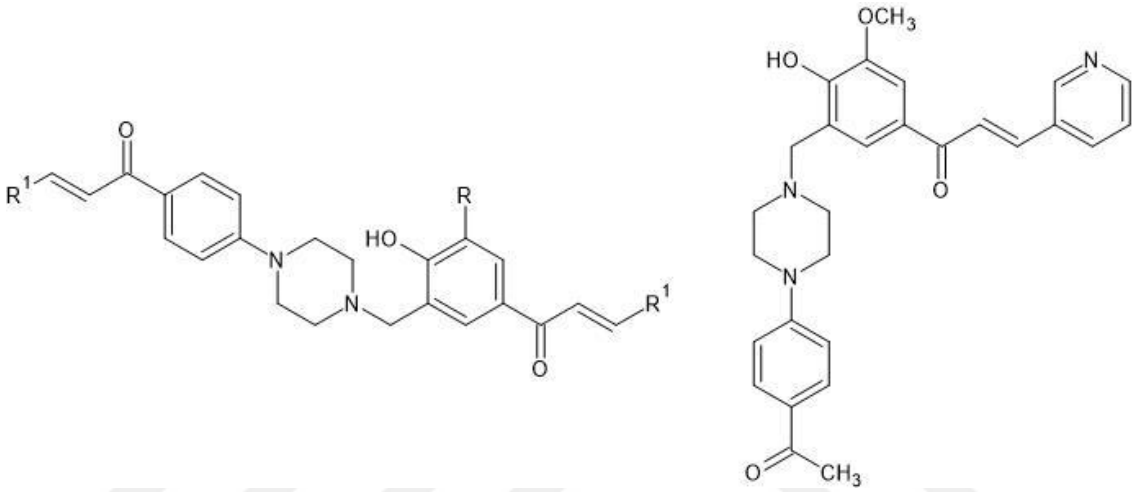
2.4.2.3. ^{13}C-NMR Spectroscopy

Signals of the ^{13}C-NMR spectra for 1-(4-(4-(5-acetyl-2-hydroxybenzyl)piperazin-1-yl)phenyl)ethenone appear at:196.6 ppm (C=O), 196.4 ppm (C=O), 162.3, 153.6, 130.4, 130.3 (x2), 129.4 , 129.1, 128.2 , 120.5 , 116.0, 113.8 (x2) (Ar-C), 61.0 (CH_2-piperazine), 52.0 (x2), 47.2 (x2) (4C, piperazine), 26.2 (CH_3), 26.1 (CH_3) in $CDCl_3$ [97].

2.4.3. Biological Activities of Mono Mannich Bases of Acetophenone-Piperazine Derivatives

2.4.3.1. Anticancer Activity

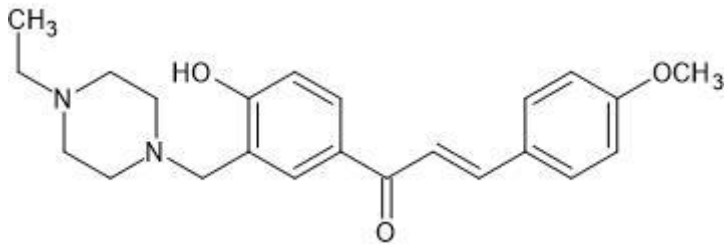
Yang *et al*. synthesized and evaluated the cytotoxicity of novel bichalcone derivatives. Four compounds showed potent *in vitro* cytotoxic activity with GI_{50} values ranging between 0.70-13.10 μM [97].



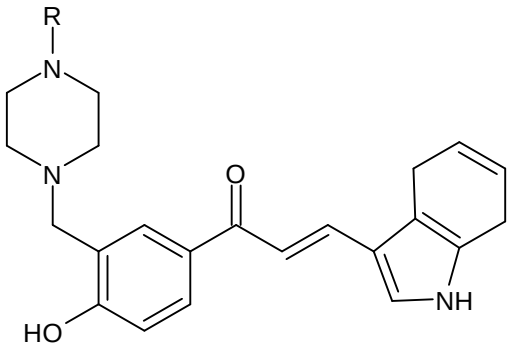
R= H, OMe

R_1= 2-pyridyl, 3-pyridyl

The anticancer activity of the compound below was tested. The results showed that this compound has potent effect against breast cancer cell line (MCF-7) having IC_{50} value of 2.0 μg/mL, while exerted modest anticancer effect against HepG2 cell line [18].



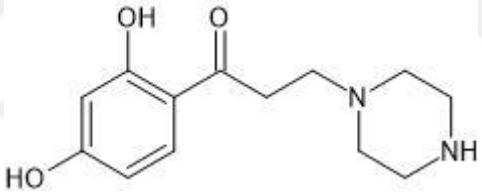
Novel Mannich bases from chalcones were prepared by Kandeel *et al.* and examined for their *in vitro* anticancer activity. Compounds below exhibited significant cytotoxic activity against renal cancer cell line (UO-31) [98].



R= methyl, 4-fluorophenyl, 4-methoxyphenyl

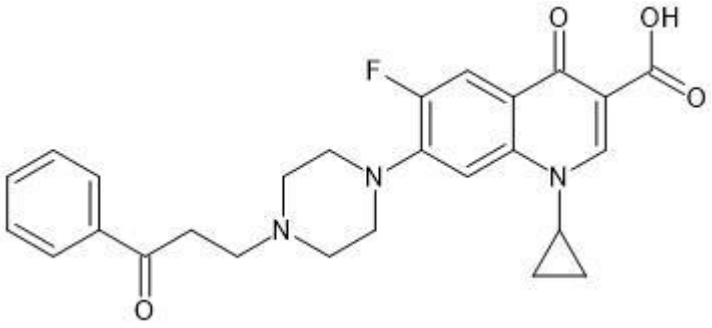
2.4.3.2. Antibacterial Activity

Meera *et al*. prepared various Mannich bases bearing acetophenone derivatives and examined their antibacterial activity. The following compound showed activity against some Gram- positive organisms and Gram- negative organism such as: *Staphylococcus aureus and Escherichia coli* [99].



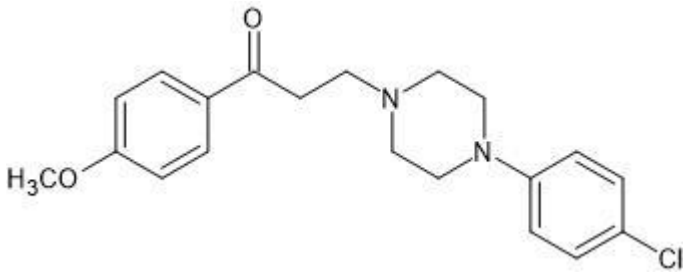
2.4.3.3. Antifungal Activity

Compound below was screened for antifungal effects. The results exhibited the compound activity against *Candida albicans* [100].



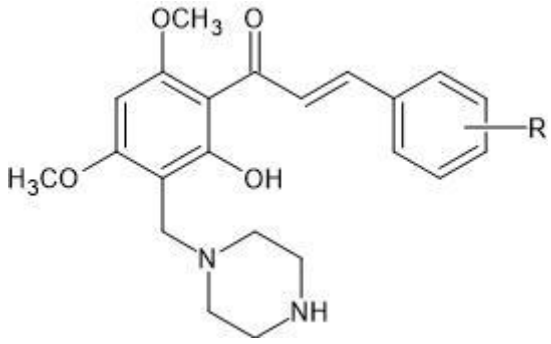
2.4.3.4. Antiparasitic Activity

In vivo antiparasitic activity of different Mannich bases derivatives was screened against *Trypanosoma cruzi*. Compound presented below showed lower levels of toxicity and parasitemia than benznidazole, while having higher therapeutic rates [101].



2.4.3.5. Anti-inflammatory Activity

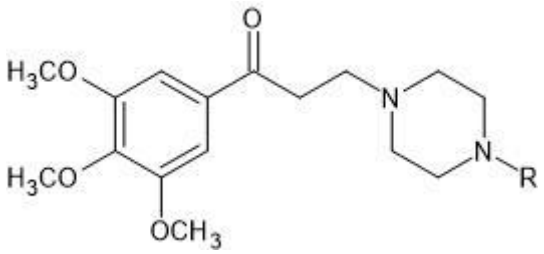
Nitrogen-containing chalcone derivatives were reported by Gacche *et al.* Compound (R= 3-Br) exhibited superior activity against trypsin and ß-glucuronidase enzymes. In addition, compounds (R= 3-Cl, 2-F, 2-Cl) showed potent selective inhibitory effect against COX-2 [102].



R= 3-Cl, 3-Br, 2-F, 2-Cl

2.4.3.6. Sedative Activity

Two Mannich bases which were synthesized to be investigated for their sedative effects. The studies demonstrated these compounds to have potent sedative activity [103].



R= *o*-Methoxyphenyl, *o*-Tolyl

2.5. Microwave- Assisted Synthesis

Microwave-assisted synthesis is a type of chemical synthesis that uses microwaves to produce chemical reactions. There are several benefits to this approach:

- Increased reaction speed: Microwaves can heat materials more quickly than traditional heating methods, which can greatly increase the speed of chemical reactions.
- Improved yield: Because microwaves can heat materials more evenly, they can often improve the yield of a chemical reaction.
- More selective reactions: Microwaves can create local heating and cooling effects within a reaction mixture, which can make certain chemical reactions more selective.
- Reduced solvent usage: Microwave-assisted synthesis can often be carried out in smaller amounts of solvent, which can save resources and reduce waste.

These reasons helped the microwave irradiation to become more popular in the organic synthesis field [104, 105].

2.6. Biological Activity

2.6.1. Cancer

Nearly 10 million people died from cancer worldwide in 2020, making it one of the top causes of death globally [106]. Cancer develops when a clonal cells group from tissue proliferates. There are various ways to represent and describe carcinogenesis, the process by which cancer develops. The “hallmarks” of cancer, which are the key

characteristics of both cancer cells and tumors, can be used to highlight this process. Cancer cells typically produce their own growth signals, ignore signals that prevent growth, evade cell death, reproduce without regulations or limits, support angiogenesis, and penetrate tissues through capillary and basement membranes [107, 108].

A major challenage in cancer treatment is the adverse effects related to the chempotherapy such as: bone marrow inhibition, lesions of gastrointestinal tract, lossing hair, and nausea which are mainly caused by the ablility of these agents to act on both cancer and normal cells [109].

Another obstacle in cancer managment is the Chemotherapy resistance. Recent reports stated that 90% of chemotherapy failures which occur during the invasion and spread of malignancies are linked to drug resistance. Hence, the urge to develop new anticancer agents [110].

2.6.2. History of Chemotherapy

The term “chemotherapy” was first introduced by the chemist Paul Ehrlich who described it as the utilization of chemicals to treat diseases. Although Ehrlich was mainly focusing on developing drugs to cure infectious diseases, he tried to utilize some aniline dyes and alkylating agents to treat cancer. Additionally, he was the first to demonstrate the possibility of using animal models to test the potential activity of different chemicals in the treatment of disease, which offered significant benefits for the development of new cancer agents later [111].

The middle forties and fifties of the last century witnessed the first phase of chemotherapy development with the clinical applications of nitrogen mustard and folic acids antagonists in the treatment of cancer. The utilization of these agents was based on clinical observations [112]. The most remarkable clinical drugs of this phase include: the alkylation agents nitrogen mustards, cyclophosphamide and the folic acid antagonists amethopterin, aminopterin.

During the seventies and following the discovery of the helical structure of DNA which helped in forming rational hypotheses about the mechanisms of action of the drugs

that were employed in cancer therapy [113,114], many new types of anticancer drugs were discovered such as: cisplatin, antibiotics (e.g. anthracyclines, bleomycin), nucleobases and nucleoside analogues (6-thioguanine and fluoropyrimidines, 6-mercaptopurine), antimitotic (vinca alkaloids), procarbazine or etoposide [115-128]. This period also witnessed the first applications of adjuvant chemotherapy.

In 1980s, the third phase of chemotherapy development began with focusing on the concept of improving the clinical use of the available anticancer agents rather than introducing new ones.

The targeted therapy was the title of the fourth phase of chemotherapy development. In the late 1990s, the idea of discovering new pharmacological targets to direct the design of chemotherapeutic drugs emerged after the discovery of various genes which act as tumor suppressor, several oncogenes, different signaling pathways that are related to the angiogenic process and carcinogenesis. One of the examples of a targeted drug is imatinib which is a BCR-ABL tyrosine kinase inhibitor that can be used to treat chronic myelocytic leukemia (CML), and gastrointestinal stromal tumors (GIST) [129].

2.6.3. Chemotherapy Resistance

Cancer patients frequently experience cytotoxic chemotherapy resistance, which is a significant obstacle in treating disseminated neoplasms successfully. Chemotherapy resistance can be expressed in two ways either acquired or intrinsic. Intrinsically resistant tumors do not react to the initial chemotherapy treatment. On the other hand, acquired resistance responds initially to chemotherapy but gets worse ultimately despite the medication. In both cases, tumors are frequently shown to be resistant to a wide range of anticancer agents with various structural and functional differences [129, 130].

Many different molecular processes have been linked to the chemotherapy resistance including:

- Enhanced drug efflux by transporter proteins like the human multidrug resistance-associated protein (MRP) or P-glycoprotein (P-gp).
- Modifications in the drug targets like DNA topoisomerase II (topo II).

- Enhanced substance detoxification such as detoxification by (GSH) the glutathione system.
- Increased the repairing capacity of DNA [131].

3. MATERIALS AND METHODS

3.1. Chemistry

3.1.1. Materials

For this study, 4-hydroxyacetophenone, formaldehyde (37%), 1-allypiperazine, 1-(2-phenylethyl)piperazine, 1-(2-fluorophenyl)piperazine, 1-(4-methoxyphenyl)piperazine, 1-(2-furoyl)piperazine, 1-benzoylpiperazine hydrochloride, 1-cyclohexylpiperazine, 1-(*o*-tolyl)piperazine hydrochloride, 1-(4-methylphenyl)piperazine, 1-(3-chlorophenyl)piperazine hydrochloride, 1-[2-(2-hydroxyethoxy)ethyl]piperazine, ethyl acetate, dichloromethane, chloroform, methanol, 1,4-dioxane were obtained from Sigma-Aldrich.

3.1.2 Methods of Synthesis

3.1.2.1. Synthesis Method of Target Compounds: 1-{4-hydroxy-3-[(4-substitutedpiperazin-1-yl)methyl]phenyl}ethan-1-one by Conventional Heating

4-Hydroxyacetophenone 1.1 mmol (0.153 g) is added to 1.1 mmol of appropriate piperazine and 1.1 mmol (0.082 ml) of formaldehyde (37%) then mixed in methanol (10 ml). The mixture is refluxed, and the temperature of the reaction is kept around 60-65 °C, after 6 hours 1.1 mmol (0.082 ml) of formaldehyde (37%) is added to the reaction mixture. The reaction is followed by thin layer chromatography in silica gel stationary phase and different mobile phase mixtures which are mentioned in (3.1.3.2). The reaction is completed in 48 hours on reflux, then the reaction solvent is evaporated using rotary evaporator. After that, column chromatography is carried out using different solvent systems which are mentioned in (3.1.3.2) to purify the obtained compound.

3.1.2.2. Synthesis Method of Target Compounds: 1-{4-hydroxy-3-[(4-substitutedpiperazin-1-yl)methyl]phenyl}ethan-1-one by Microwave Assisted Synthesis

4-Hydroxyacetophenone 1.1 mmol (0.153 g) is added to 1.1 mmol of appropriate piperazine in the presence of 2.2 mmol (0.164 ml) of formaldehyde (37%) and mixed in

1,4-dioxane (10 ml). The mixture is placed in the microwave sealed vessel with stirring. The reaction mixture will be irradiated at 120° C (power 300 W). TLC is used to track the reaction progression in silica gel stationary phase and different mobile phase mixtures which are mentioned in (3.1.3.2). The reaction is completed after 30 min, then the vessel is removed, and the mixture is dried under reduced pressure. After that, column chromatography is carried out using different solvent systems which are mentioned in (3.1.3.2) to purify the obtained compound.

3.1.3 Analytical Methods

3.1.3.1. Melting Point

Melting Point Meter (A.KRÜSS Optronic Melting Point Meters) apparatus was used to measure the melting points (°C) of the compounds.

3.1.3.2. Controls by Thin Layer Chromatography

Material:

TLC aluminum sheets 20x20 cm Silica gel 60 F254 (Merck) were used as stationary phase, and several mobile phases were prepared to be utilized in TLC and column chromatography.

S1: Methanol: Ethyl acetate (50:50).
S2: Chloroform: Methanol (95:5).
S3: Dichloromethane: Methanol (95:5).
S4: Ethyl acetate: Dichloromethane (80:20)
S5: Methanol: Ethyl acetate (80:20).

TLC Method:

A chamber was loaded with the appropriate mobile phase and left for sufficient time to get homogeneous saturation. After dissolving the reactants and synthesized compound in suitable solvents, Pasteur pipettes were used to apply the solutions on the TLC plate. The TLC plate was then left inside the chamber for adequate time, next the

TLC plate was taken out and left to dry, then the spots were detected using UV light (254 nm), and the R_f values were recorded.

3.1.3.3. Column Chromatography

Column chromatography was utilized to purify the synthesized compounds. Stationary phase was silica gel (60 mesh) with an appropriate solvent system. The purification was monitored by TLC.

3.1.4. Spectral Analyses

NMR and elemental analyses were performed in Ankara University, Faculty of Pharmacy.

3.1.4.1. Infrared Spectroscopy

Perkin-Elmer Spectrum One series FT-IR apparatus (Version 5.0.1) was used to obtain the infrared spectra of the synthesized compounds, potassium bromide (KBr) was applied as the background, and the frequencies were measured in cm^{-1}.

3.1.4.2. ^{1}H-NMR Spectroscopy

Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA USA) was used to conduct the ^{1}H-NMR Spectra analysis. Tetramethyl silane (TMS) was the internal reference, the applied solvent was either deuterated dimethyl sulfoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$), the chemical shifts were recorded in parts per million (ppm).

3.1.4.3. ^{13}C-NMR Spectroscopy

Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA, USA) was used for ^{13}C-NMR spectra analysis. Tetramethyl silane (TMS) was the internal reference, the applied solvent was either deuterated dimethyl sulfoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$), and the chemical shifts were recorded in parts per million (ppm).

3.1.4.4. Elemental Analysis

Elemental analysis was conducted on a LECO 932 CHNS (LECO-932, St. Joseph, MI, USA).

3.2. Cytotoxic Activity

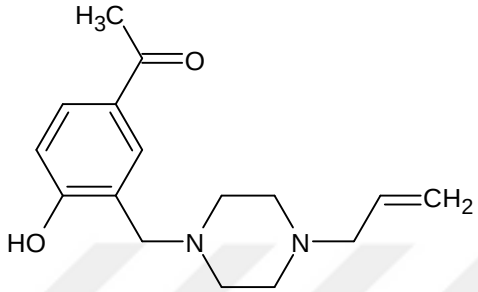
3.2.1 MTT Assay

MCF-7 breast cancer cell line was used for testing compounds cytotoxicity. For this aim, 1mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide (MTT) , a yellow, water-soluble compound, was applied to the cells. When MTT is applied to living cells, it transforms into a blue-violet, water-insoluble reduced form, formazan. Determination of viable cell number was calculated by determining the color intensity obtained after dissolving formazan in alcohol by photometric measurements. To investigate the effect of compounds on MCF-7 cell proliferation, each compound was applied at 5 different concentrations (2.5-50 µM). Serial dilutions of each compound were prepared. The cells were seeded at a density of 3500 cells/well a day before treatment with the samples. Subsequently, different concentrations of compounds were administered to cells. After 72 hours of incubation, formazan formation was determined for each concentration and formazan crystals were dissolved by addition of 150 µL isopropanol. The percentage of viable cells was calculated based on the values acquired by colorimetric methods using Ascent spectrophotometer at 570 nm.

4. RESULTS

4.1. Chemical Data

1-(4-Hydroxy-3-((4-(prop-2-en-1-yl)piperazin-1-yl)methyl)phenyl)ethan-1-one (Compound 1)



Conventional:

4-Hydroxyacetphenone 1.1 mmol (0.153 g), 1-allylpiperazine 1.1 mmol (0.157 ml), and formaldehyde 2.2 mmol (0.1642 ml) were mixed in methanol in accordance with the general synthesis procedure mentioned in (3.1.2.1). Column chromatography was carried out to purify the obtained compound using S1 as solvent system. The yield is 0.058 g (19%).

Microwave:

4-Hydroxyacetphenone 1.1 mmol (0.153 g), 1-allylpiperazine 1.1 mmol (0.157 ml), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in 1.4-dioxane according to the general synthesis procedure mentioned in (3.1.2.2). Column chromatography was carried out to purify the obtained compound using S1 as a solvent system. The yield is 0.1456 g (72%).

The compound appearance is yellow colored oil, soluble in chloroform, ethyl acetate, and methanol. Rf values using S1 and S2 mobile phases in TLC are 0.551, 0.437 respectively.

FT-IR (KBr, cm^{-1}): 3400-3600 (O-H), 2945 (C-H, aromatic), 2817 (C-H, aliphatic), 1673 (C=O), 1640 (C=C), 1594 (C=C, aromatic), 1126 (C-N).

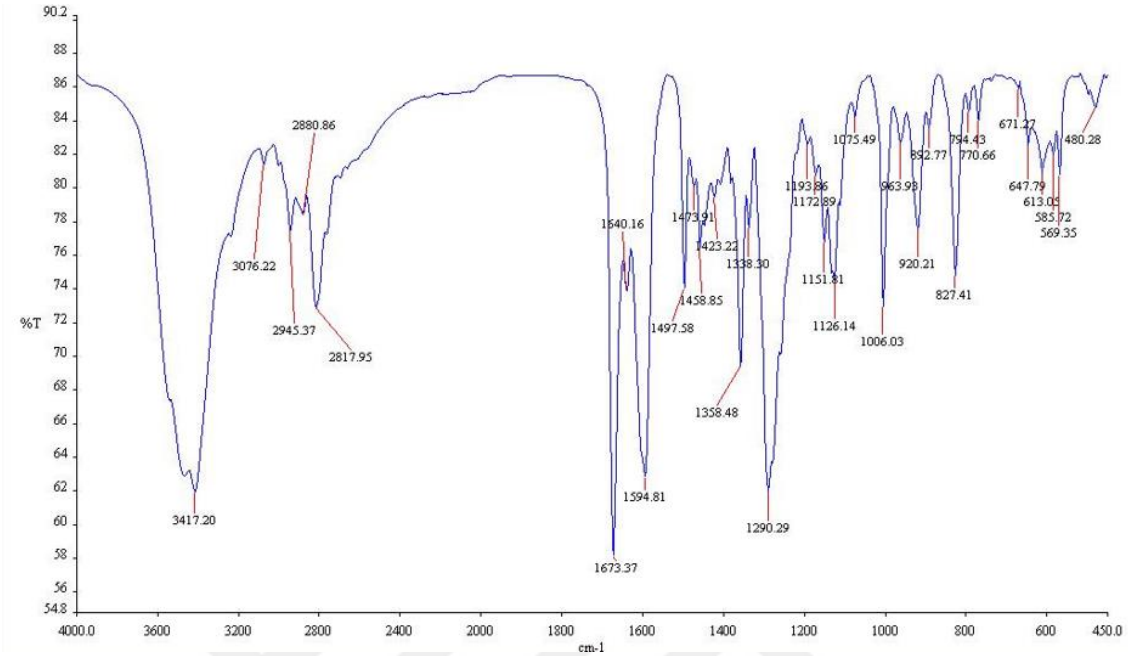


Figure 4.1. IR spectrum of compound 1.

^{1}H-NMR (ppm/δ, 500 MHz, DMSO-d_6): 2.27-2.5 (m, 8H, piperazine), 2.46 (s, 3H, -COCH3), 2.9 (d, 2H, -N-$\underline{CH_2}$CH=CH_2, *J*= 6 Hz), 3.67 (s, Ar-CH_2-N-), 5.1-5.18 (dd, 2H, -CH_2CH=$\underline{CH_2}$, J_1= 31.9 Hz, J_2= 17.25 Hz), 5.76-5.82 (m, 1H, -$CH_2\underline{CH}$=CH_2), 6.81-6.82 (d, 1H, 4-hydroxyacetohpenone H_5, *J*= 8 Hz), 7.74 (s, 1H, 4-hydroxyacetophenone H_2), 7.76 (d, 1H, 4-hydroxyacetophenone H_6, *J*= 7.55 Hz).

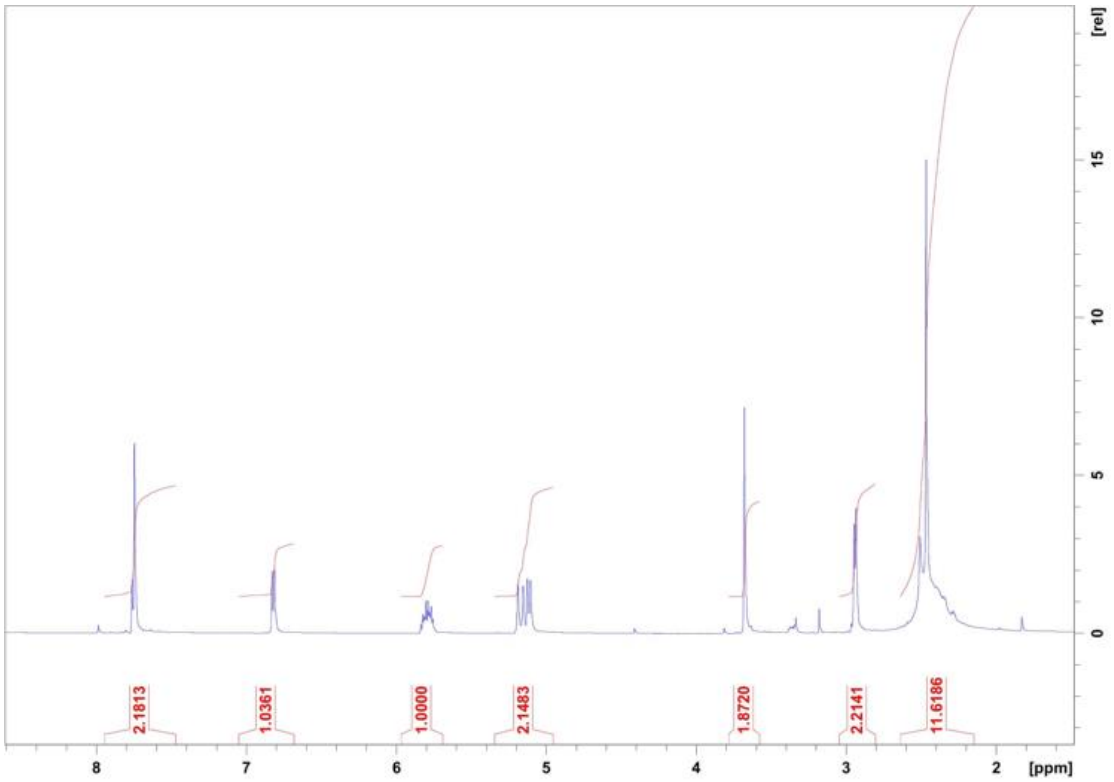
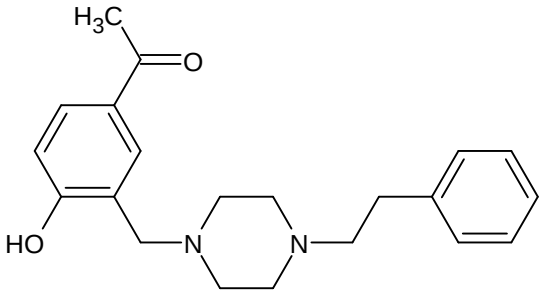


Figure 4.2. ^{1}H-NMR spectrum of compound 1.

1-(4-Hydroxy-3-((4-phenethylpiperazin-1-yl)methyl)phenyl)ethan-1-one (Compound 2)



Conventional:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-(2-phenylethyl)piperazine 1.1 mmol (0.2122 ml), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in methanol according to the general synthesis procedure mentioned in (3.1.2.1). Column chromatography was carried out to purify the obtained compound using S2 as a solvent system. The yield is 0.064 g (26%).

Microwave:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-(2-phenylethyl)piperazine 1.1 mmol (0.2122 ml), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in 1,4-dioxane according to the general synthesis procedure mentioned in (3.1.2.2). Column chromatography was carried out to purify the obtained compound using S2 as a solvent system. The yield is 0.1352 g (36%).

The compound appearance is yellowish-white colored powder crystals, melting point is 116.1 °C, soluble in chloroform and methanol. Rf values using S2 and S3 mobile phases are 0.5625, 0.725 respectively.

FT-IR (KBr, cm^{-1}): 3419-3600 (O-H), 2947 (C-H, aromatic), 2824 (C-H, aliphatic), 1669 (C=O), 1594 (C=C, aromatic), 1129 (C-N).

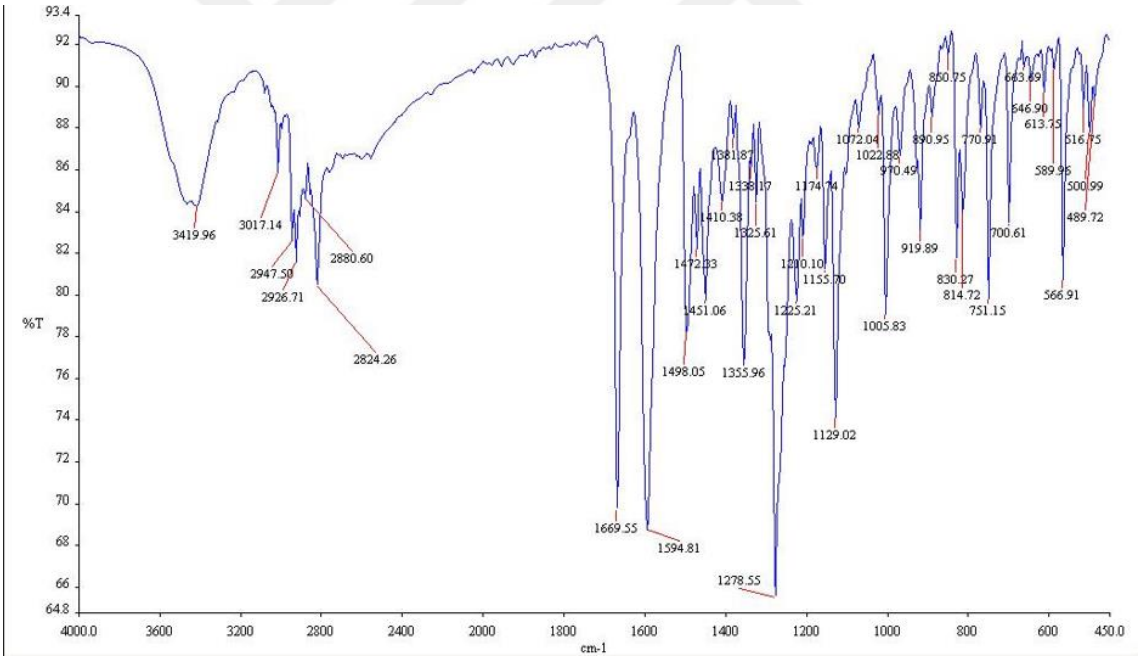


Figure 4.3. IR spectrum of compound 2.

^{1}H-NMR (ppm/δ, 500 MHz, DMSO-d_6): 2.47 (s, 3H, -$COCH_3$), 2.503- 2.507 (m, 8H, piperazine), 2.51- 2.53 (t, 2H, -$CH_2\underline{CH_2}$-Ar, *J*= 8.4 Hz), 2.7- 2.73 (t, 2H,- N-$\underline{CH_2}CH_2$-, *J*= 7.3 Hz), 3.7 (s, 2H, Ar-CH_2-N-), 6.81- 6.83 (d, 1H, 4-hydroxyacetohpenone H_5, *J*= 8.3 Hz), 7.16- 7.28 (m, 5H, phenyl), 7.75- 7.76 (d, 1H, 4-hydroxyacetophenone H_2, *J*= 2.7 Hz), 7.76- 7.78 (d, 1H, 4-hydroxyacetophenone H_6, *J*= 8.45 Hz).

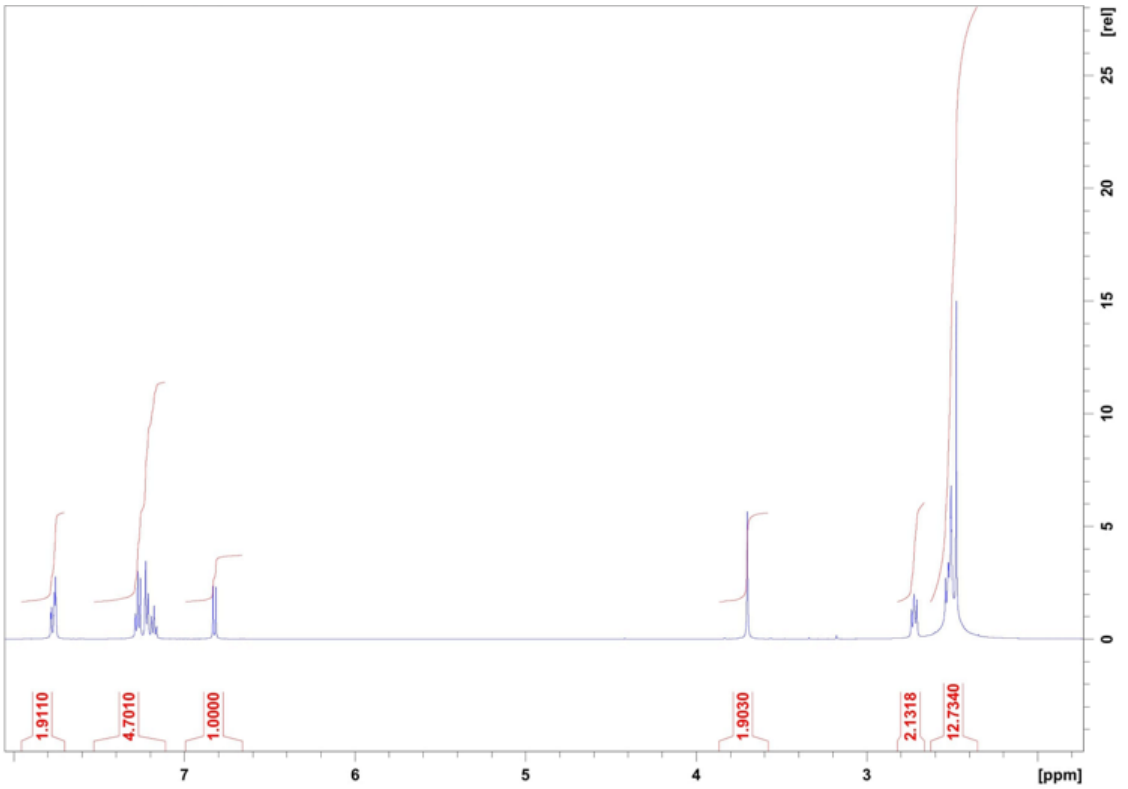


Figure 4.4. ^{1}H-NMR spectrum of compound 2.

^{13}C-NMR (ppm, DMSO-d_6): 26.25 (<u>CH_3</u>CO-), 32.71 (CH_2<u>CH_2</u>- Ar), 52.11 (piperazine $C_{2,6}$), 52.51 (piperazine $C_{3,5}$), 58.27 (Ar-<u>CH_2</u>-N-), 59.53 (N-<u>CH_2</u>CH_2-), 115.23, 122.21, 125.83, 128.21, 128.37, 128.62, 129.49, 129.87, 140.35 (Ar-C), 161.76 (Ar-C-OH), 196.07 (C=O).

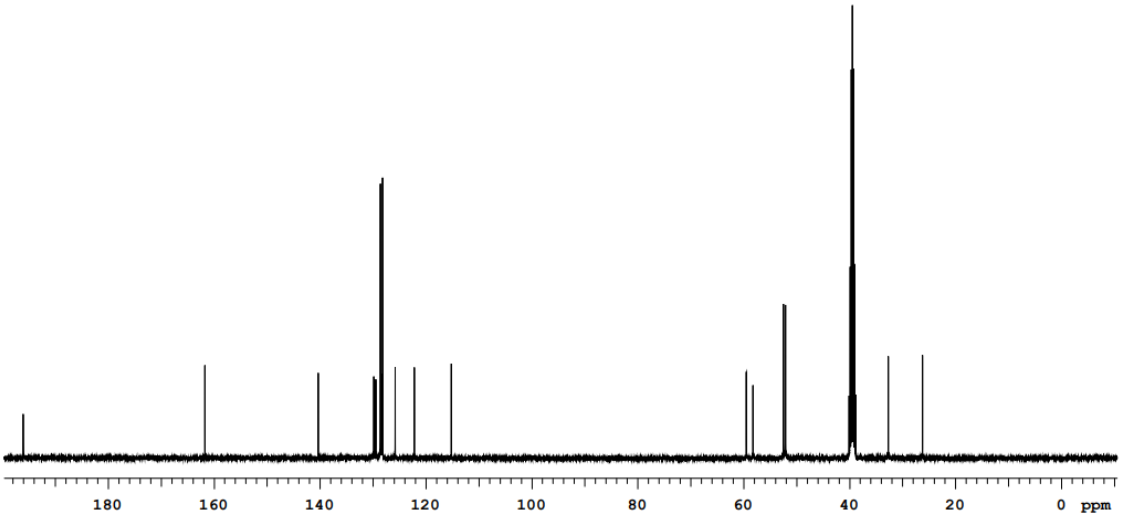
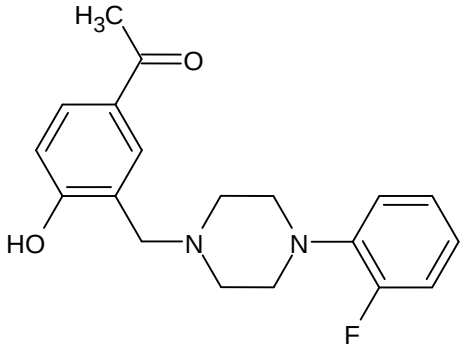


Figure 4.5. ^{13}C-NMR spectrum of compound 2.

Elemental analysis of $C_{21}H_{26}N_2O_2$ (MW: 338.4433 g/mol):

	%C	%H	%N
Calculated	74.52	7.74	8.28
Found	74.58	7.99	8.29

1-(3-((4-(2-Fluorophenyl)piperazin-1-yl)methyl)-4-hydroxyphenyl)ethan-1-one (Compound 3)



Conventional:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-(2-fluorophenyl)piperazine 1.1 mmol (0.179 ml), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in methanol according to the general synthesis procedure mentioned in (3.1.2.1). Column chromatography was carried out to purify the obtained compound using S3 as a solvent system. The yield is 0.075 g (21%).

Microwave:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-(2-fluorophenyl)piperazine 1.1 mmol (0.179 ml), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in 1,4-dioxane according to the general synthesis procedure mentioned in (3.1.2.2). Column chromatography was carried out to purify the obtained compound using S3 as a solvent system. The yield is 0.086 g (24%).

The compound appearance is white colored crystals, melting point is 115.6 °C, soluble in chloroform, dichloromethane and methanol. Rf values using S3 and S4 mobile phases are 0.871, 0.794 respectively.

FT-IR (KBr, cm^{-1}): 3300-3600 (O-H), 2951 (C-H, aromatic), 2837 (C-H, aliphatic), 1670 (C=O), 1591 (C=C, aromatic), 1113 (C-N).

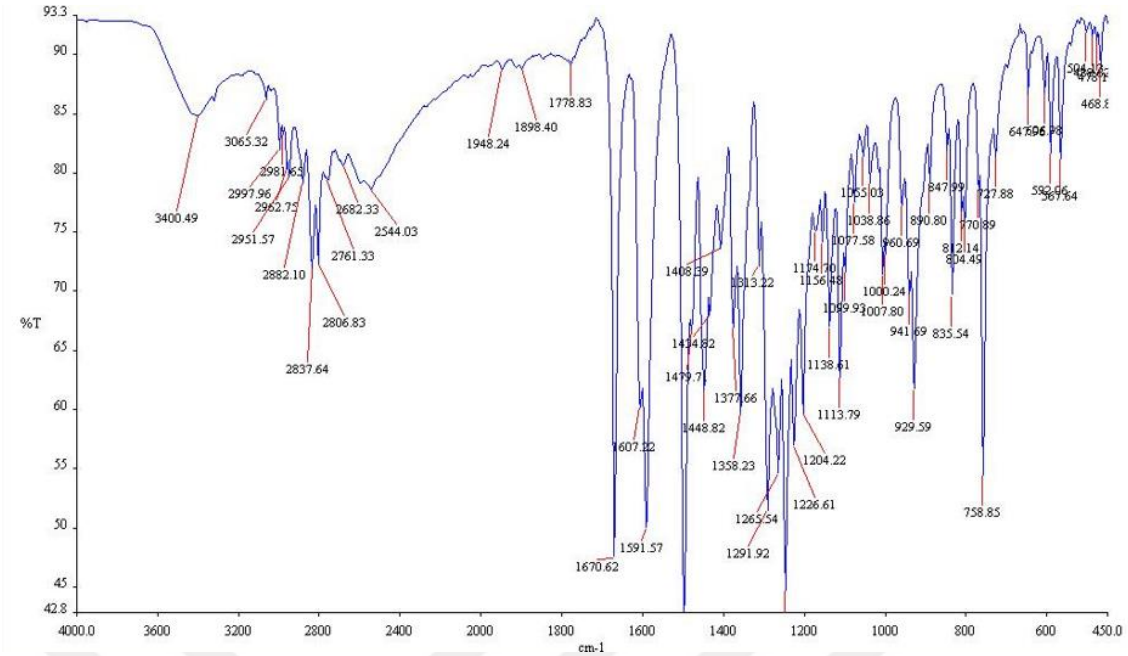


Figure 4.6. IR spectrum of compound 3.

^{1}H-NMR (ppm/δ, 500 MHz, DMSO-d_6): 2.49 (s, 3H, CH_3CO-), 2.46 (s, 4H, piperazine $H_{2,6}$), 3.05 (s, 4H, piperazine $H_{3,5}$), 3.73 (s, 2H, Ar-CH_2-N-), 6.85- 6.87 (d, 1H, 4-hydroxyacetophenone H_5, J= 8.4 Hz), 6.97- 6.98 (m, 1H, phenyl H_5), 7.05- 7.06 (t, 1H, phenyl, H_4, J= 8.35 Hz), 7.09 (d, 1H, phenyl, H_6, J= 1.25 Hz), 7.1- 7.14 (m, 1H, phenyl, H_3), 7.77- 7.79 (dd, 1H, 4-hydroxyacetophenone H_2 J_1= 8.45 Hz, J_2= 2.15 Hz), 7.81 (d, 1H, 4-hydroxyacetophenone H_6 J= 1.85 Hz).

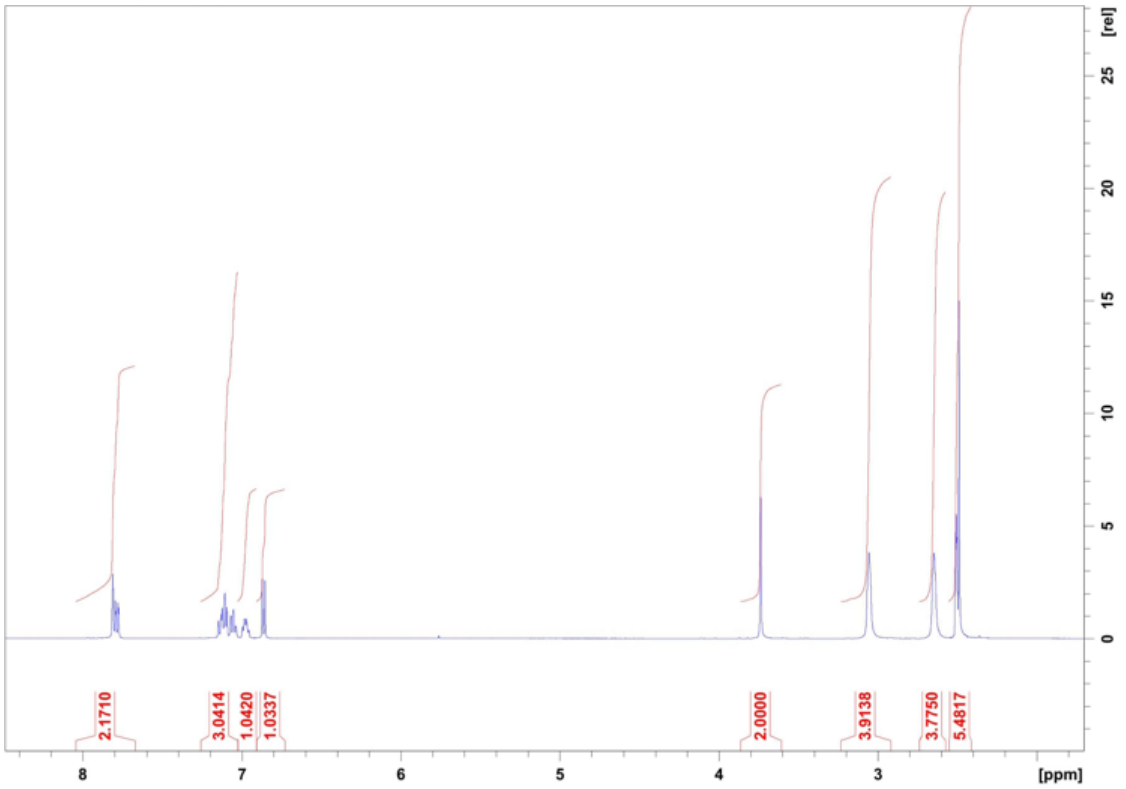
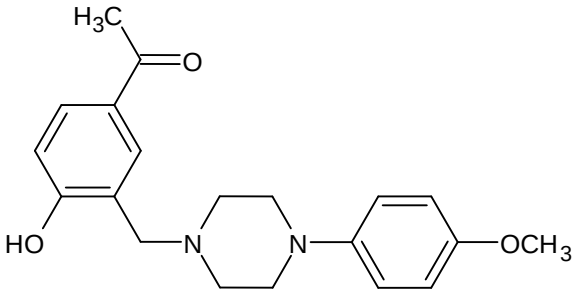


Figure 4.7. ^{1}H-NMR spectrum of compound 3.

1-(4-Hydroxy-3-((4-(4-methoxyphenyl)piperazin-1-yl)methyl)phenyl)ethan-1-one (Compound 4)



Conventional:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-(4-methoxyphenyl)piperazine 1.1 mmol (0.21 g), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in methanol according to the general synthesis procedure mentioned in (3.1.2.1). Column chromatography was carried out to purify the obtained compound using S3 as a solvent system. The yield is 0.085 g (23%).

Microwave:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-(4-methoxyphenyl)piperazine 1.1 mmol (0.21 g), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in 1,4-dioxane according to the general synthesis procedure mentioned in (3.1.2.2). Column chromatography was carried out to purify the obtained compound using S3 as a solvent system. The yield is 0.122 g (33%).

The compound appearance is yellow colored crystals, melting point is 130 °C, soluble in chloroform, dichloromethane and methanol. Rf values in TLC using S3 and S4 solvent systems are 0.807, 0.743 respectively.

FT-IR (KBr, cm^{-1}): 3200-3600 (O-H), 2942 (C-H, aromatic), .2818 (C-H, aliphatic), 1672 (C=O), 1593 (C=C), 1127 (C-N).

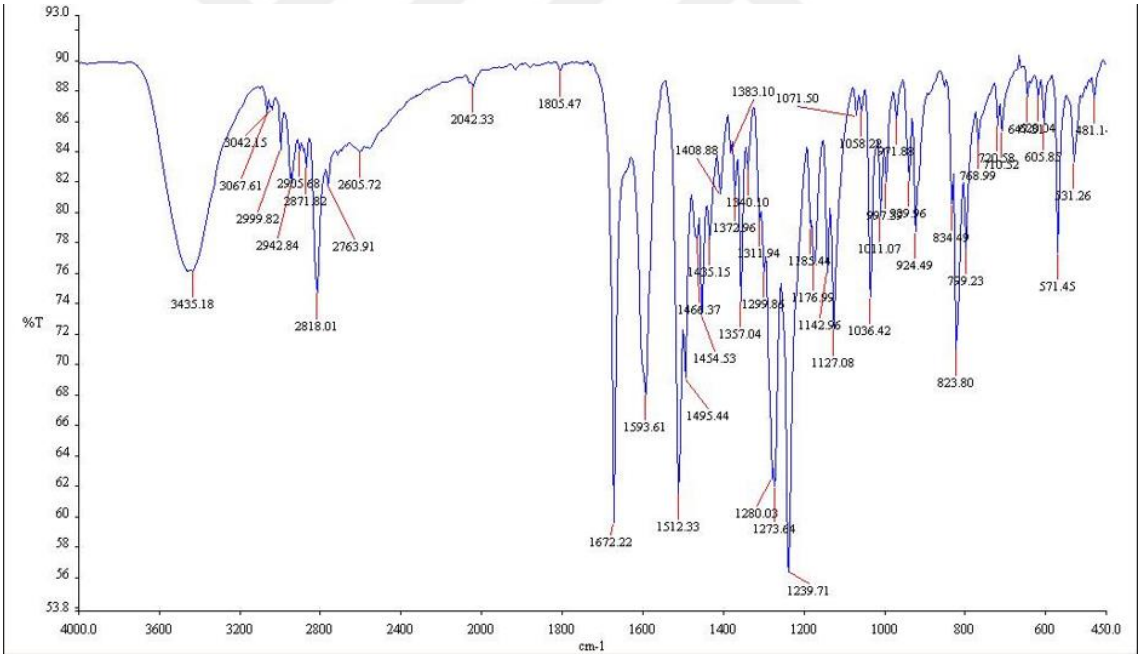


Figure 4.8. IR spectrum of compound 4.

^{1}H-NMR (ppm/δ, 500 MHz, DMSO-d_6): 2.48 (s, 3H, CH_3CO-), 2.61- 2.63 (t, 4H, piperazine $H_{2,6}$, *J*= 9.45 Hz), 3.04- 3.06 (t. 4H, piperazine $H_{3,5}$, *J*= 8.95 Hz), 3.68 (s, 3H, OCH_3), 3.73 (s, 2H, Ar-CH_2-N-), 6.81- 6.82 (m, 2H, phenyl $H_{3,5}$), 6.84- 6.86 (d, 1H, 4-hydroxyacetophenone H_5, *J*= 8.35 Hz), 6.88- 6.9 (m, 2H, phenyl $H_{2.6}$), 7.77- 7.79 (dd, 4-

hydroxyacetophenone H_2, J_1= 8.35 Hz, J_2= 2.25 Hz), 7.8 (d, 4-hydroxyacetophenone H_6, J= 2.05 Hz).

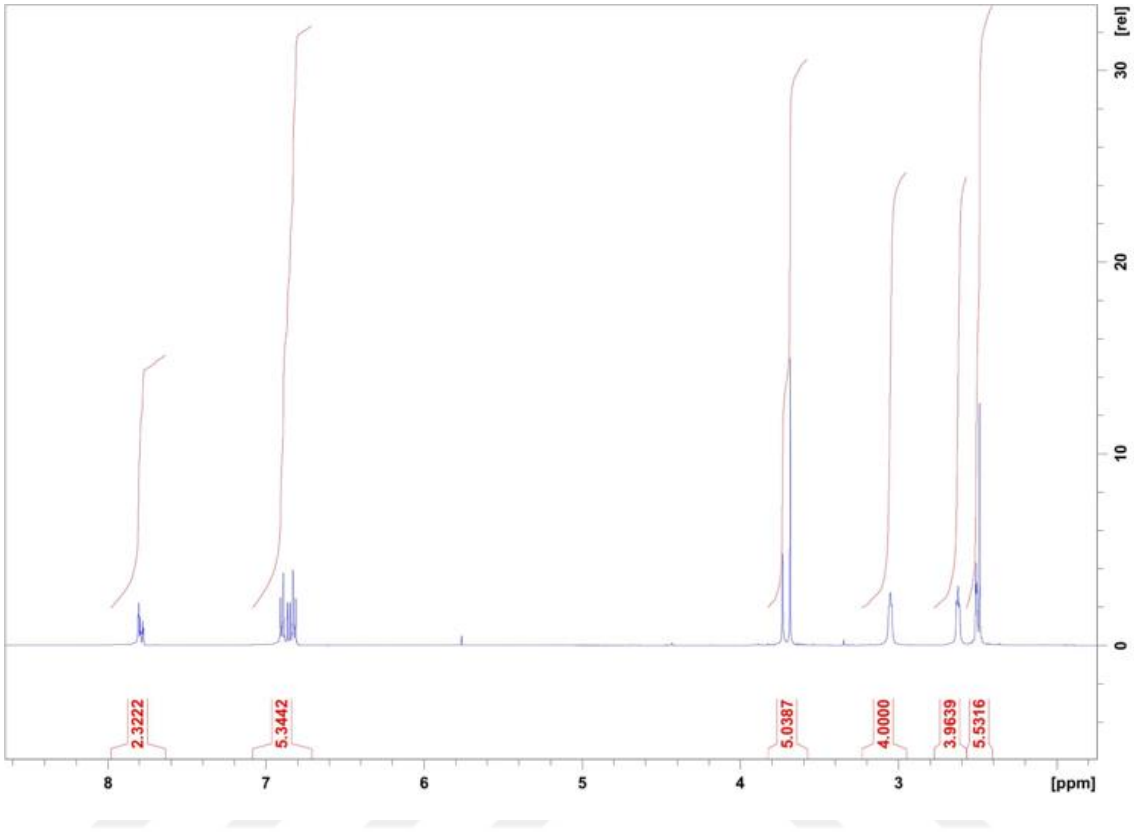


Figure 4.9. ^{1}H-NMR spectrum of compound 4.

^{13}C-NMR (ppm, DMSO-d_6): 26.26 ($\underline{CH_3}CO$-), 49.60 (piperazine $C_{2,6}$), 52.23 (piperazine $C_{3,5}$), 55.14 (-OCH_3), 57.83 (Ar-$\underline{CH_2}$-N-), 114.23, 115.23, 117.54, 122.42, 128.44, 129.51, 130.16, 145.17 (Ar-C), 153.03 (Ar-C-OCH_3), 161.49 (Ar-C-OH), 196.13 (C=O).

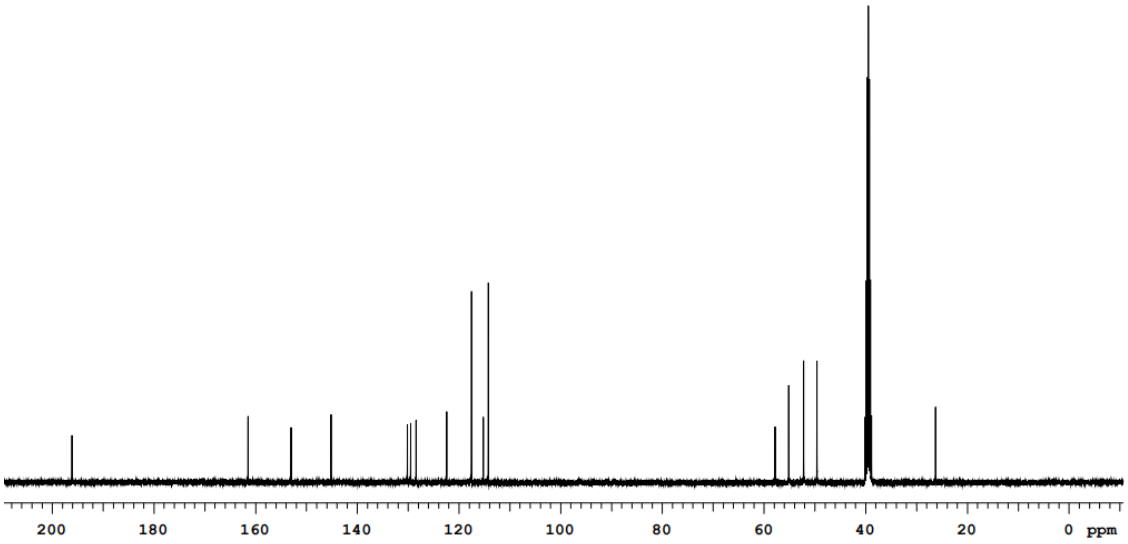
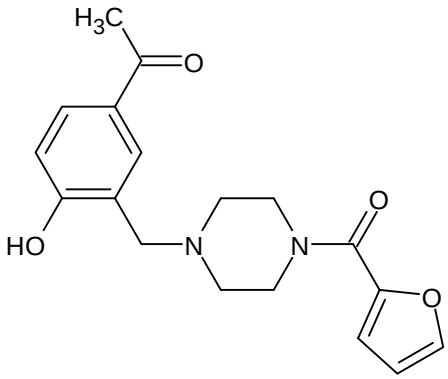


Figure 4.10. ^{13}C-NMR spectrum of compound 4.

1-(3-((4-(Furan-2-carbonyl)piperazin-1-yl)methyl)-4-hydroxyphenyl)ethan-1-one (Compound 5)



Conventional:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-(2-furoyl)piperazine 1.1 mmol (0.2 g), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in methanol according to the general synthesis procedure mentioned in (3.1.2.1). Column chromatography was carried out to purify the obtained compound using S4 as a solvent system. The yield is 0.051 g (21%).

Microwave:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-(2-furoyl)piperazine 1.1 mmol (0.2 g), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in 1,4-dioxane according to the general synthesis procedure mentioned in (3.1.2.2). Column chromatography was carried out to purify the obtained compound using S4 as a solvent system. The yield is 0.157 g (43%).

The compound appearance is yellowish white colored powder, melting point is 120 °C, soluble in ethyl acetate, dichloromethane, and methanol. Rf values using S2 and S4 mobile phases are 0.65, 0.525 respectively.

FT-IR (KBr, cm^{-1}): 3221-3600 (O-H), 2947 (C-H, aromatic), 2859 (C-H, aliphatic), 1667 (C=O), 1572 (C=C, aromatic), 1128 (C-N).

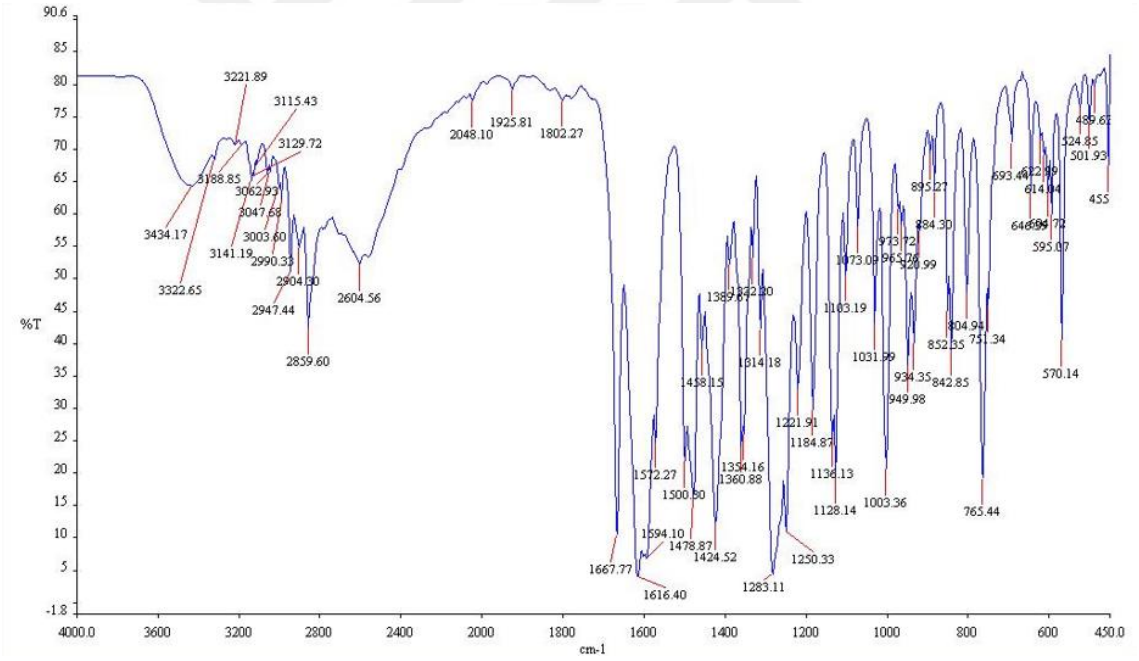


Figure 4.11. IR spectrum of compound 5.

^{1}H-NMR (ppm/δ, 500 MHz, DMSO-d_6): 2.5 (s, 3H, CH_3CO-), 2.507- 2.52 (t, 4H, piperazine $H_{2,6}$, *J*= 4.5 Hz), 3.66 (s, 2H, Ar-CH_2-N-), 3.69 (s, 4H, piperazine $H_{3,5}$), 6.61-6.62 (dd, 1H, furoyl H_4, J_1= 3.45 Hz, J_2= 1.75 Hz), 6.86- 6.88 (d, 1H, 4-hydroxyacetophenone H_5, *J*= 8.4 Hz), 6.98- 6.99 (d, 1H, furoyl H_5, J_1= 3.45), 7.77- 7.78

(dd, 1H, 4-hydroxyacetophenone H_2, *J*= 8.45 Hz), 7.81 (d, 1H, 4-hydroxyacetophenone $H_{6,}$ *J*= 2.15 Hz), 7.83 (d, 1H, furoyl H_3, *J*= 1.35 Hz).

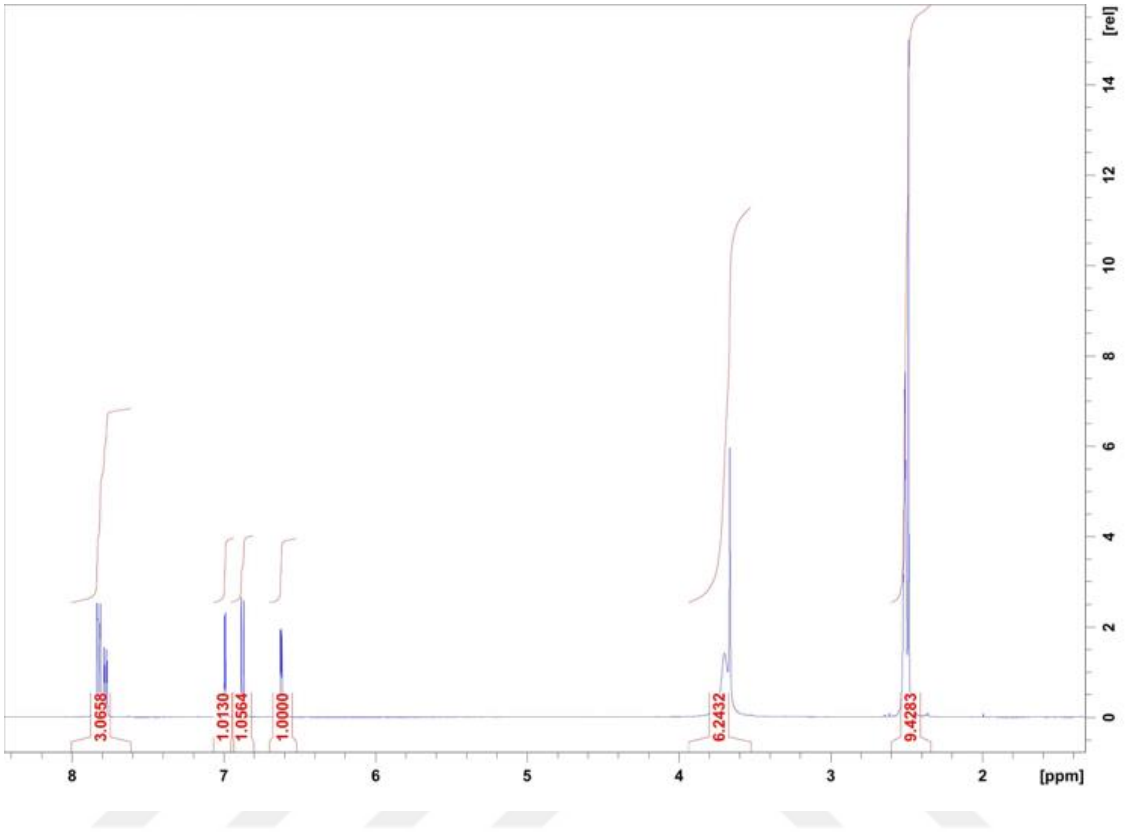
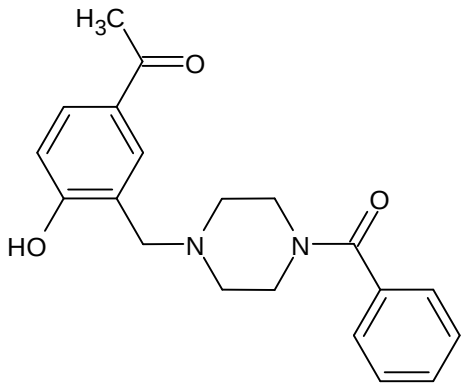


Figure 4.12. ^{1}H-NMR spectrum of compound 5.

1-(3-((4-Benzoylpiperazin-1-yl)methyl)-4-hydroxyphenyl)ethan-1-one (Compound 6)



Conventional:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-benzoylpiperazine 1.1 mmol (0.2 ml), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in methanol according to the general synthesis procedure mentioned in (3.1.2.1). Column chromatography was carried out to purify the obtained compound using S4 as a solvent system. The yield is 0.057 g (15%).

Microwave:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-benzoylpiperazine 1.1 mmol (0.2 ml), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in 1,4-dioxane according to the general synthesis procedure mentioned in (3.1.2.2). Column chromatography was carried out to purify the obtained compound using S4 as a solvent system. The yield is 0.092 g (24%).

The compound appearance is yellow colored powder, melting point is 128 °C, soluble in ethyl acetate, dichloromethane. Rf values using S2 and S4 mobile phases are 0.725, 0.576 respectively.

FT-IR (KBr, cm^{-1}): 3324 (O-H), 2965 (C-H, aromatic), 2838 (C-H, aliphatic), 1671 (C=O), 1584 (C=C, aromatic), 1119 (C-N).

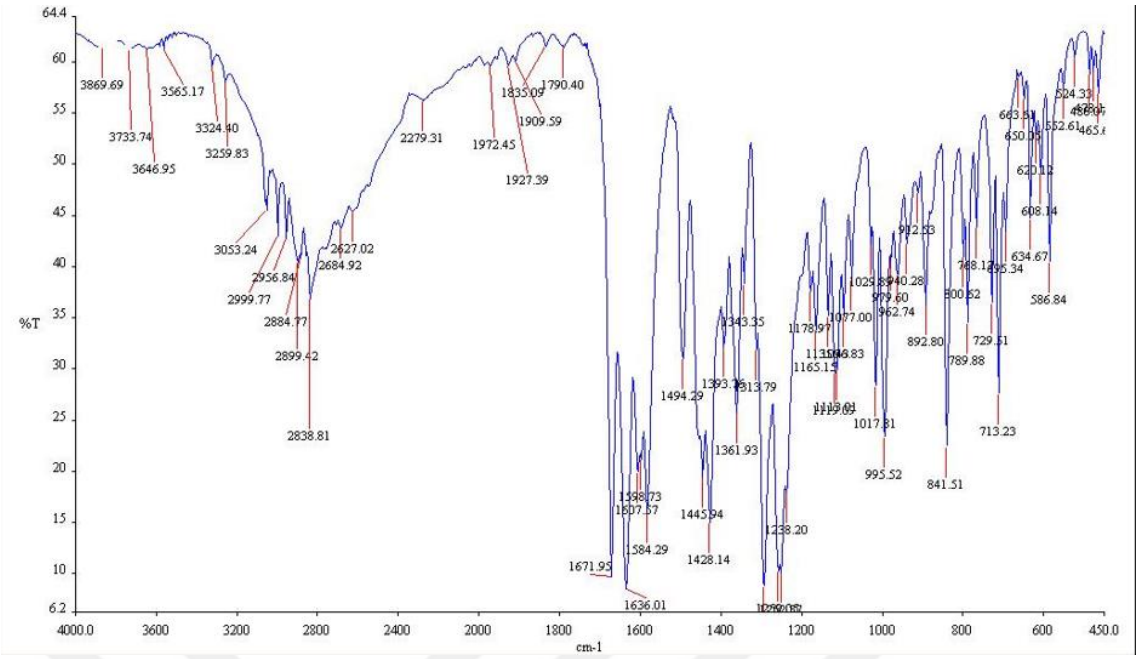


Figure 4.13. IR spectrum of compound 6.

^{1}H-NMR (ppm/δ, 500 MHz, DMSO-d_6): 2.50 (s, 3H, CH_3CO-), 2.51 (t, 4H, piperazine $H_{2.6}$, *J*= 1.75 Hz), 3.36 (s, 2H, Ar-CH_2-N-), 3.65 (s, 4H, piperazine $H_{3,5}$), 6.85-6.87 (d, 1H, 4-hydroxyacetophenone H_5, *J*= 8.4 Hz), 7.38- 7.39 (m, 1H, benzoyl H_4), 7.4-7.43 (m, 2H, benzoyl $H_{3,5}$), 7.44- 7.45 (m, 2H, benzoyl $H_{2,6}$), 7.75- 7.78 (dd, 1H, 4-hydroxyacetophenone H_6, J_1= 8.45 Hz, J_2= 2.25 Hz), 7.8 (d, 1H, 4-hydroxyacetophenone H_2, *J*= 2.1 Hz).

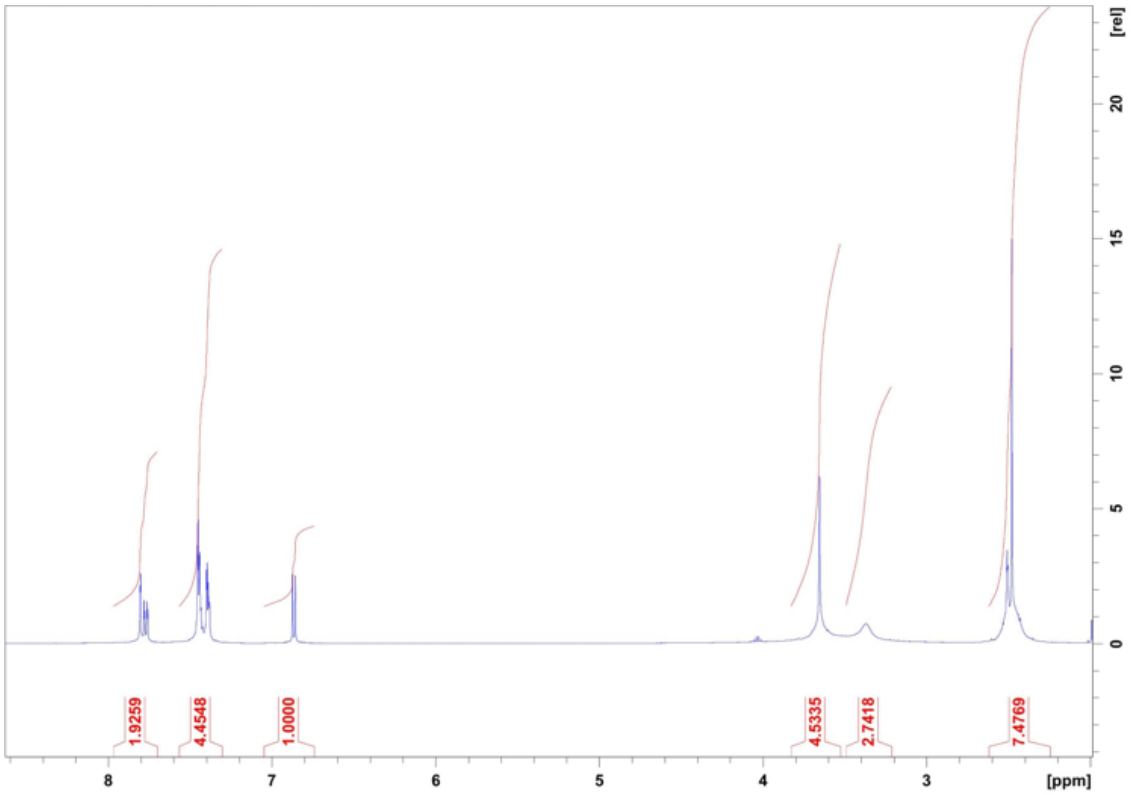
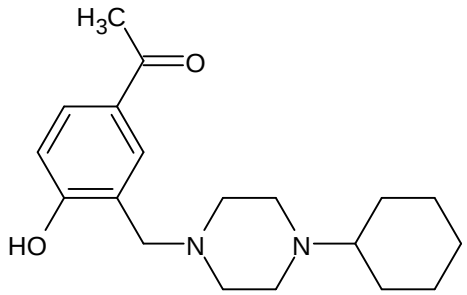


Figure 4.14. ^{1}H-NMR spectrum of compound 6.

1-(3-((4-Cyclohexylpiperazin-1-yl)methyl)-4-hydroxyphenyl)ethan-1-one (Compound 7)



Conventional:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-cyclohexyl piperazine 1.1 mmol (0.19 g), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in methanol according to the general synthesis procedure mentioned in (3.1.2.1). Column chromatography was carried out to purify the obtained compound using S5 as a solvent system. The yield is 0.095 g (27%).

Microwave:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-cyclohexyl piperazine 1.1 mmol (0.19 g), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in 1,4-dioxane according to the general synthesis procedure mentioned in (3.1.2.2). Column chromatography was carried out to purify the obtained compound using S5 as a solvent system. The yield is 0.115 g (33%).

The compound appearance is reddish orange colored powder, melting point is 129.5 °C, soluble in ethyl acetate, and methanol. Rf values using S3 and S5 mobile phases are 0.253, 0.543 respectively.

FT-IR (KBr, cm^{-1}): 3200-3700 (O-H), 2929 (C-H, aromatic), 2852 (C-H, aliphatic), 1674 (C=O), 1598 (C=C, aromatic), 1118 (C-N).

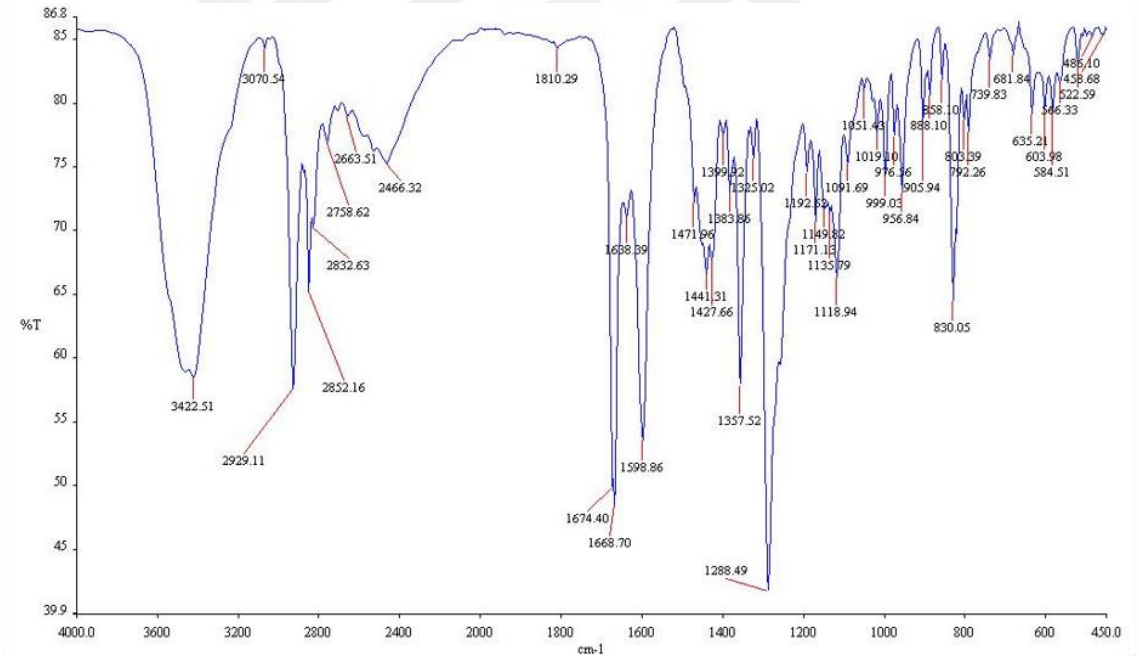


Figure 4.15. IR spectrum of compound 7.

^{1}H-NMR (ppm/δ, 500 MHz, DMSO-d_6): 1.05- 1.2 (m, 6H, cyclohexyl $H_{3,4,5}$), 1.71- 1.75 (m, 4H, cyclohexyl $H_{2,6}$), 2.2 (m, 1H, cyclohexyl, H_1), 2.47 (s, 3H, CH_3CO-), 2.5- 2.51 (t, 8H, piperazine, *J*= 1.7 Hz), 3.68 (s, 2H, Ar-CH_2-N), 6.8- 6.81 (d, 1H, 4-hydroxyacetophenone H_5, *J*= 8.4 Hz), 7.73 (d, 1H, 4-hydroxyacetophenone H_2, *J*= 1.95 Hz), 7.75- 7.77 (dd, 1H, 4-hydroxyacetophenone H_6, J_1= 8.45 Hz, J_2= 2.2 Hz).

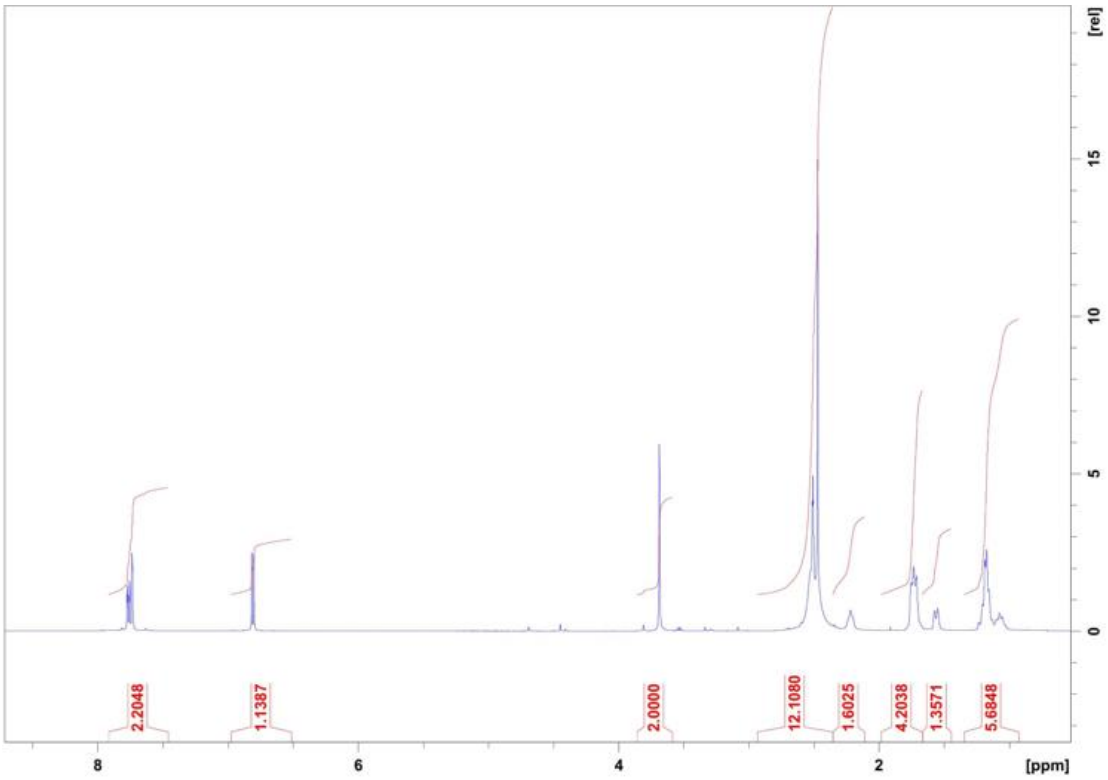


Figure 4.16. ^{1}H-NMR spectrum of compound 7.

^{13}C-NMR (ppm, DMSO-d_6): 25.27 (cyclohexyl $C_{9,11}$), 26.89 (cyclohexyl C_{10}), 26.25 ($\underline{CH_3}CO$-), 28.32 (cyclohexyl $C_{8,12}$), 48.27 (piperazine $C_{2,6}$), 52.61 (piperazine $C_{3,5}$), 58.64 (Ar-$\underline{CH_2}$-N-), 62.46 (cyclohexyl C_7), 115.25, 122.16, 128.33, 129.5, 129.83 (Ar-C), 161.87 (Ar-C-OH), 196.07 (C=O).

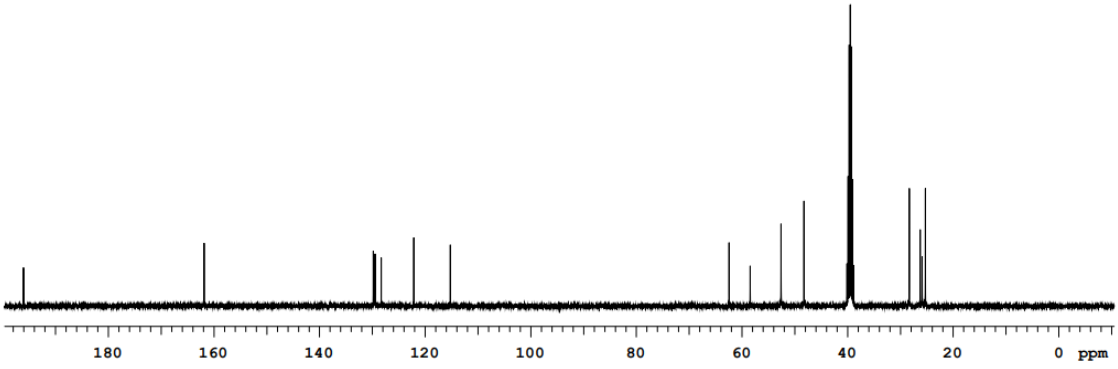
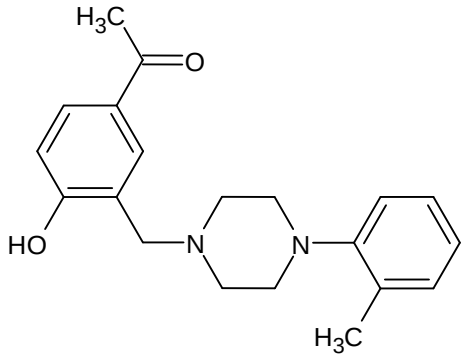


Figure 4.17. ^{13}C-NMR spectrum of compound 7.

1-(4-Hydroxy-3-((4-(*o*-tolyl)piperazin-1-yl)methyl)phenyl)ethan-1-one (Compound 8)



Conventional:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-(*o*-tolyl)piperazine 1.1 mmol (0.2 g), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in methanol according to the general synthesis procedure mentioned in (3.1.2.1). Column chromatography was carried out to purify the obtained compound using S3 as a solvent system. The yield is 0.078 g (22%).

Microwave:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-(*o*-tolyl)piperazine 1.1 mmol (0.2 g), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in 1,4-dioxane according to the general synthesis procedure mentioned in (3.1.2.2). Column chromatography was carried out to purify the obtained compound using S3 as a solvent system. The yield is 0.089 g (25%).

The compound appearance is white colored crystals, melting point is 126.2 °C, soluble in dichloromethane, and hot methanol. Rf values using S2 and S3 mobile phases are 0.708, 0.858 respectively.

FT-IR (KBr, cm^{-1}): 3200-3700 (O-H), 2929 (C-H, aromatic), 2852 (C-H, aliphatic), 1674 (C=O), 1598 (C=C), 1118 (C-N).

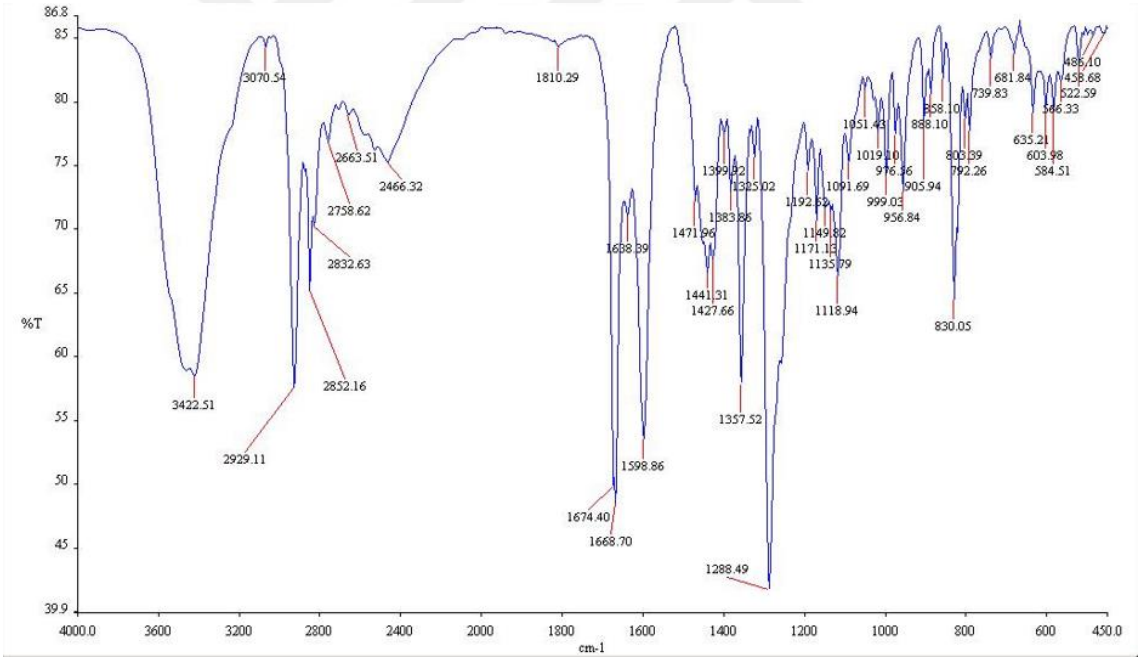


Figure 4.18. IR spectrum of compound 8.

^{1}H-NMR (ppm/δ, 500 MHz, $CDCl_3$): 2.32 (s, 3H, CH_3CO-), 2.56 (s, 3H, CH_3-Ar), 2.78 (bs, 4H, piperazine $H_{2,6}$), 3.01 (s, 4H, piperazine $H_{3,5}$), 3.87 (s, 2H, Ar-CH_2-N-), 6.87- 6.89 (d, 1H, 4-hydroxyacetophenone H_5, *J*= 8.45 Hz), 7.01- 7.05 (m, 2H, Phenyl, $H_{3,6}$), 7.18- 7.21 (t, 2H, phenyl $H_{4,5}$, *J*= 7.5 Hz), 7.73 (d, 1H, 4-hydroxyacetophenone H_2, *J*= 2.15 Hz), 7.83- 7.85 (dd, 1H, 4-hydroxyacetophenone H_6, J_1= 8.45 Hz, J_2= 2.2 Hz).

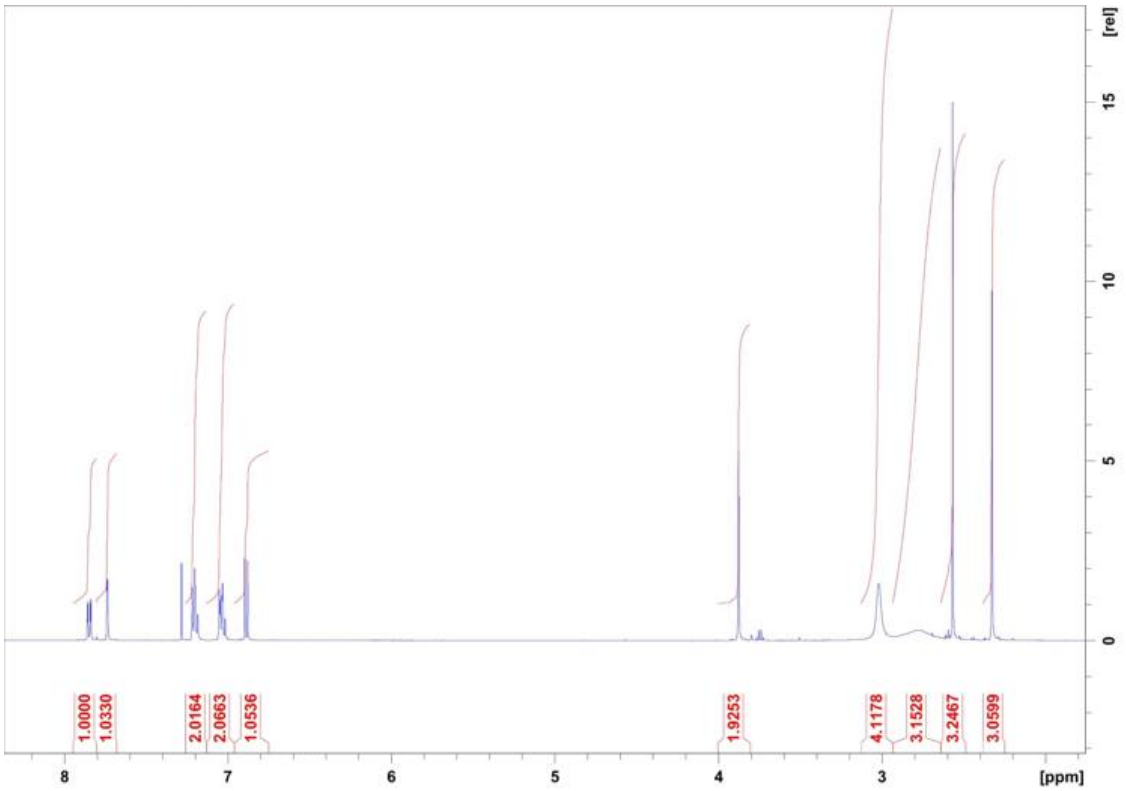


Figure 4.19. ^{1}H-NMR spectrum of compound 8.

^{13}C-NMR (ppm, DMSO-d_6): 17.55 (Ar-CH_3), 26.37 ($\underline{CH_3}$CO-), 51.25 (piperazine $C_{2,6}$), 52.65 (piperazine $C_{3,5}$), 58.01 (Ar-$\underline{CH_2}$-N-), 115.24, 118.83, 122.43, 122.97, 126.56, 128.44, 129.54, 130.16, 130.81, 131.76 (Ar-C), 150.99 (Ar-C-N-), 161.57 (Ar-C-OH), 196.12 (C=O).

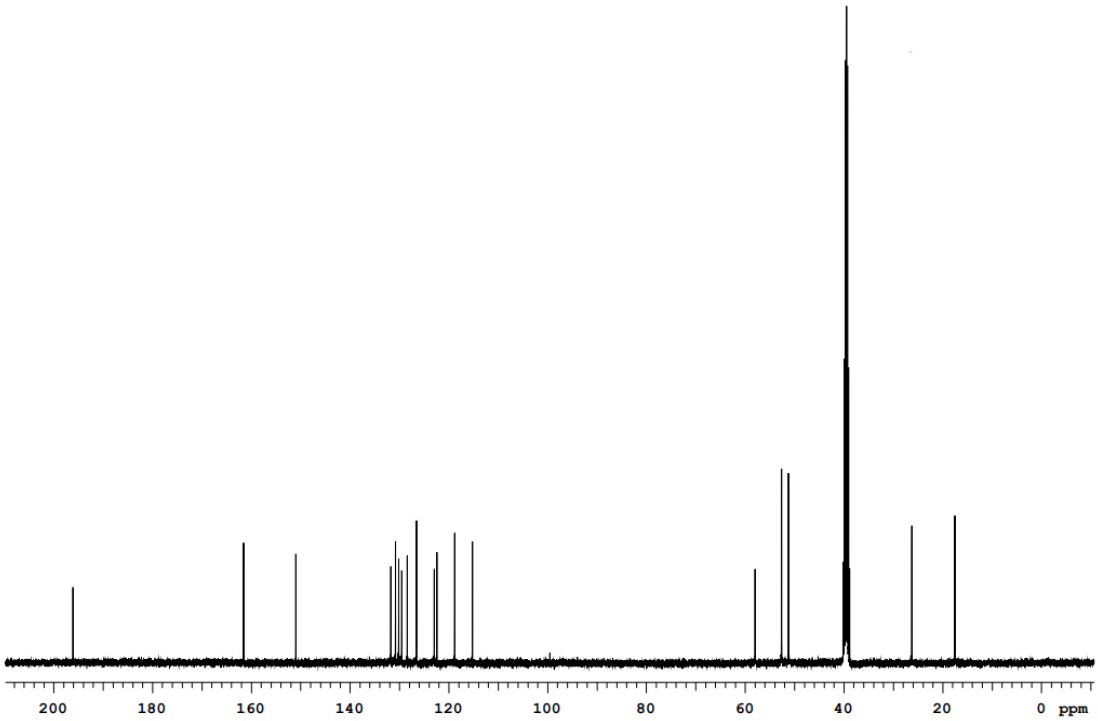
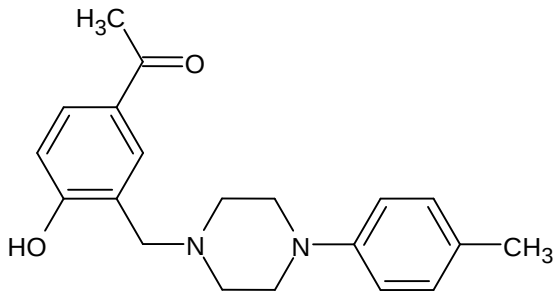


Figure 4.20. ^{13}C-NMR spectrum of compound 8.

Elemental analysis of $C_{20}H_{24}N_2O_2$ (MW: 324.4167 g/mol):

	%C	%H	%N
Calculated	74.04	7.46	8.64
Found	74.23	7.64	8.68

1-(4-Hydroxy-3-((4-(p-tolyl)piperazin-1-yl)methyl)phenyl)ethan-1-one (Compound 9)



Conventional:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-(4-methylphenyl)piperazine 1.1 mmol (0.2 g), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in methanol according to the general synthesis procedure mentioned in (3.1.2.1). Column

chromatography was carried out to purify the obtained compound using S3 as a solvent system. The yield is 0.105 g (29%).

Microwave:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-(4-methylphenyl)piperazine 1.1 mmol (0.2 g), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in 1,4-dioxane according to the general synthesis procedure mentioned in (3.1.2.2). Column chromatography was carried out to purify the obtained compound using S3 as a solvent system. The yield is 0.1125 g (32%).

The compound appearance is yellowish white colored crystals, melting point is 143 °C, soluble in dichloromethane, and hot methanol. Rf values using S2 and S3 mobile phases are 0.641, 0.82 respectively.

FT-IR (KBr, cm^{-1}): 3200-3600 (O-H), 2941 (C-H, aromatic), 2833 (C-H, aliphatic), 1670 (C=O), 1589 (C=C, aromatic), 1124 (C-N).

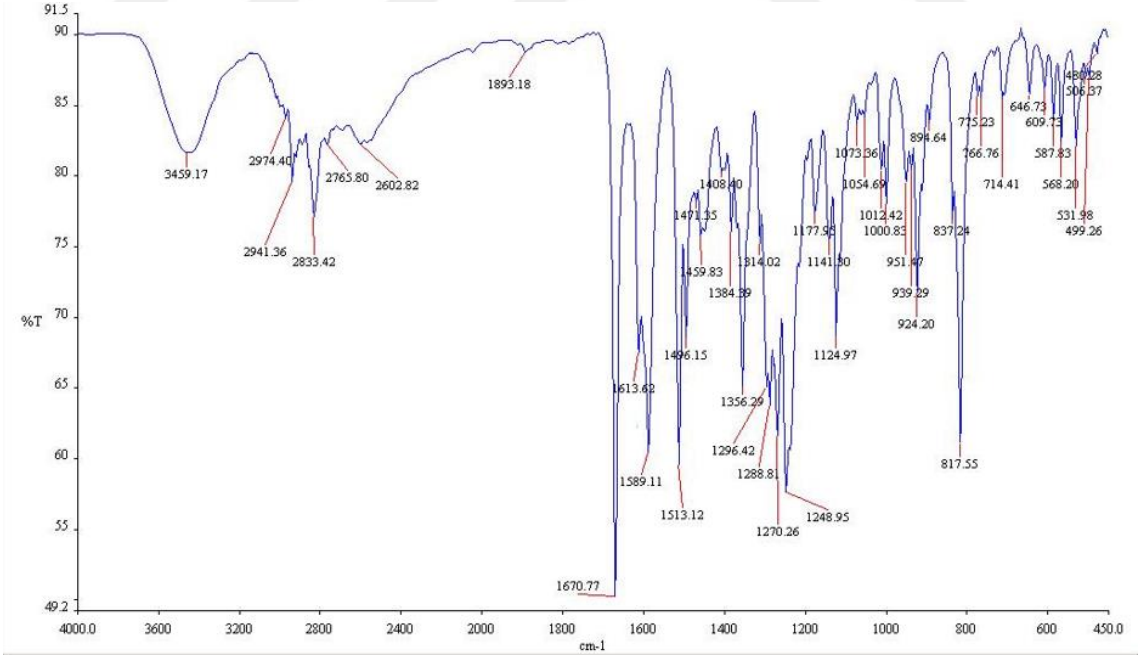


Figure 4.21. IR spectrum of compound 9.

^{1}H-NMR (ppm/δ, 500 MHz, DMSO-d_6): 2.2 (s, 3H, Ar-CH_3), 2.48 (s, 3H, CH_3CO-), 2.6- 2.62 (t, 4H, piperazine $H_{2.6}$, *J*= 4.65 Hz), 3.09- 3.11 (t, 4H, piperazine $H_{3,5}$,

J= 4.2 Hz), 3.72 (s, 2H, Ar-CH_2-N-), 6.83- 6.84 (d, 1H, 4-hydroxyacetophenone, *J*= 8.8 Hz), 6.86 (d, 2H, phenyl, $H_{3.5}$, *J*= 9.4 Hz), 7.01- 7.03 (d, 2H, phenyl $H_{2.6}$, *J*= 8.4 Hz), 7.77 (dd, 1H, 4-hydroxyacetophenone H_6, J_1= 8.4 Hz, J_2= 2.2 Hz), 7.79- 7.8 (d, 1H, 4-hydroxyacetophenone H_2, *J*= 2 Hz).

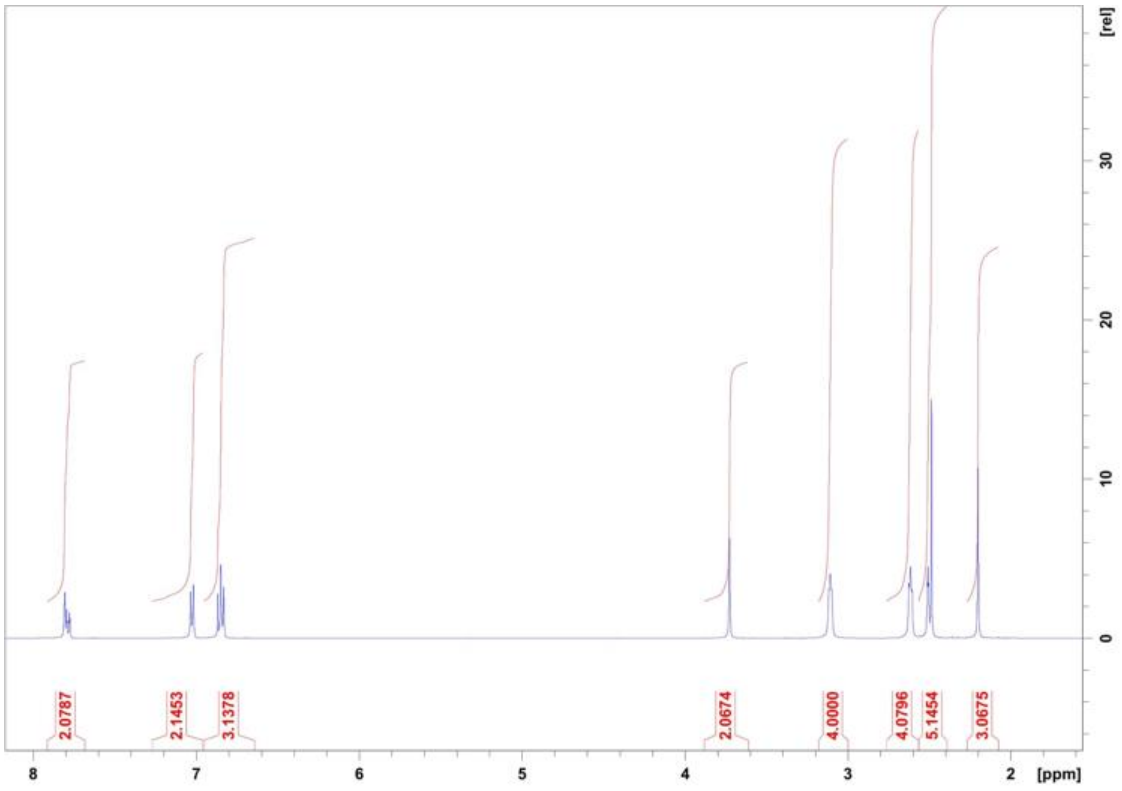


Figure 4.22. ^{1}H-NMR spectrum of compound 9.

^{13}C-NMR (ppm, DMSO-d_6): 20.06 (Ar-CH_3), 26.29 (<u>CH_3</u>CO-), 48.68 (piperazine $C_{2,4}$), 52.16 (piperazine $C_{3,5}$), 57.8 (Ar-<u>CH_2</u>-N-), 115.23, 115.80, 122.46, 127.85, 128.45, 129.38, 129.52, 130.19 (Ar-C), 148.76 (Ar-C-N-), 161.47 (Ar-C-OH), 196.14 (C=O).

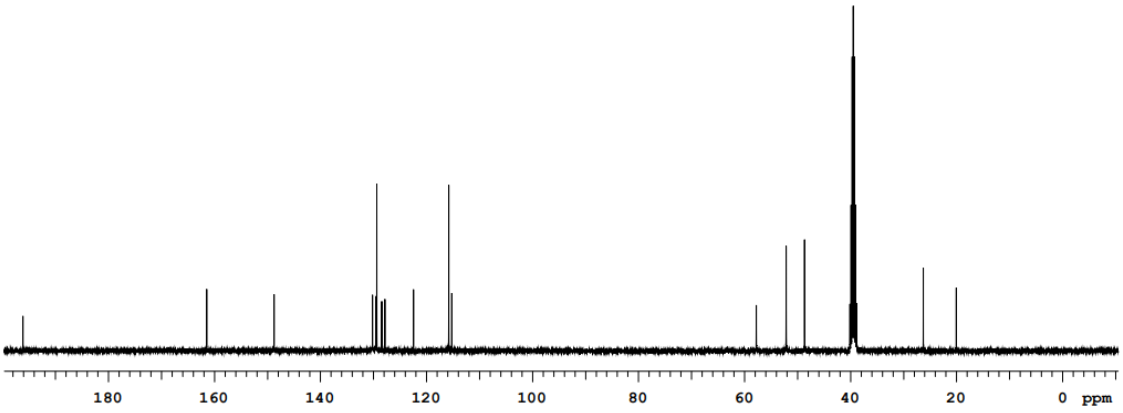
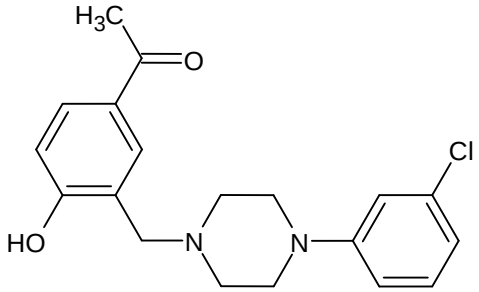


Figure 4.23. ^{13}C-NMR spectrum of compound 9.

1-(3-((4-(3-Chlorophenyl)piperazin-1-yl)methyl)-4-hydroxyphenyl)ethan-1-one (Compound 10)



Conventional:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-(3-chlorophenyl)piperazine 1.1 mmol (0.19 ml), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in methanol according to the general synthesis procedure mentioned in (3.1.2.1). Column chromatography was carried out to purify the obtained compound using S3 as a solvent system. The yield is 0.051 g (13%).

Microwave:

4-Hydroxyacetophenone 1.1 mmol (0.153 g), 1-(3-chlorophenyl)piperazine 1.1 mmol (0.19 ml), and formaldehyde 2.2 mmol (0.1642 ml) were reacted in 1,4-dioxane according to the general synthesis procedure mentioned in (3.1.2.2). Column chromatography was carried out to purify the obtained compound using S3 as a solvent system. The yield is 0.114 g (30%).

The compound appearance is yellowish white colored crystals, melting point is 138.1 °C, soluble in dichloromethane, and hot methanol. Rf values using S2 and S3 mobile phases are 0.769, 0.846 respectively.

FT-IR (KBr, cm^{-1}):3185-3600 (O-H), 2949 (C-H, aromatic), 2836 (C-H, aliphatic), 1.666 (C=O), 1594 (C=C, aromatic), 1127 (C-N).

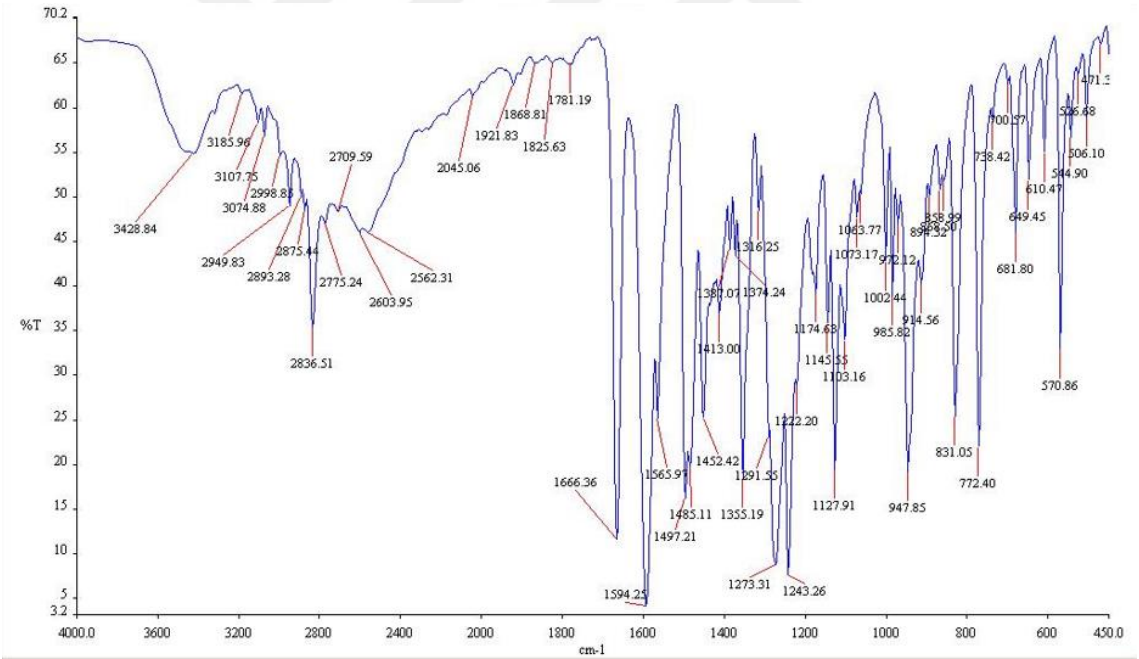


Figure 4.24. IR spectrum of compound 10.

^{1}H-NMR (ppm/δ, 500 MHz, $CDCl_3$): 2.56 (s, 3H, CH_3CO-), 2.73- 2.74 (d, 4H, piperazine $H_{2,6,}$ *J*= 4.6 Hz), 3.25- 3.27 (t, 4H, piperazine $H_{3,5}$, *J*= 4.65 Hz), 3.85 (s, 2H, Ar-CH_2-N-), 6.79- 6.81 (dd, 1H, phenyl H_6, J_1= 6.6 Hz, J_2= 1.75 Hz), 6.85- 6.86 (d, 1H, phenyl $H_{2,}$ *J*= 1.85 Hz), 6.87- 6.89 (d, 1H, 4-hydroxyacetophenone H_5, *J*= 8.4 Hz), 6.9 (d, 1H, phenyl H_4, *J*= 2.1 Hz) 7.18- 7.21 (t, 1H, phenyl $H_{5,}$ *J*= 8.1 Hz), 7.72- 7.73 (d, 1H, 4-

hydroxyacetophenone H_2, J= 2.1 Hz), 7.84- 7.86 (dd, 1H, 4-hydroxyacetophenone H_6, J_1= 8.5 Hz, J_2= 2.2 Hz).

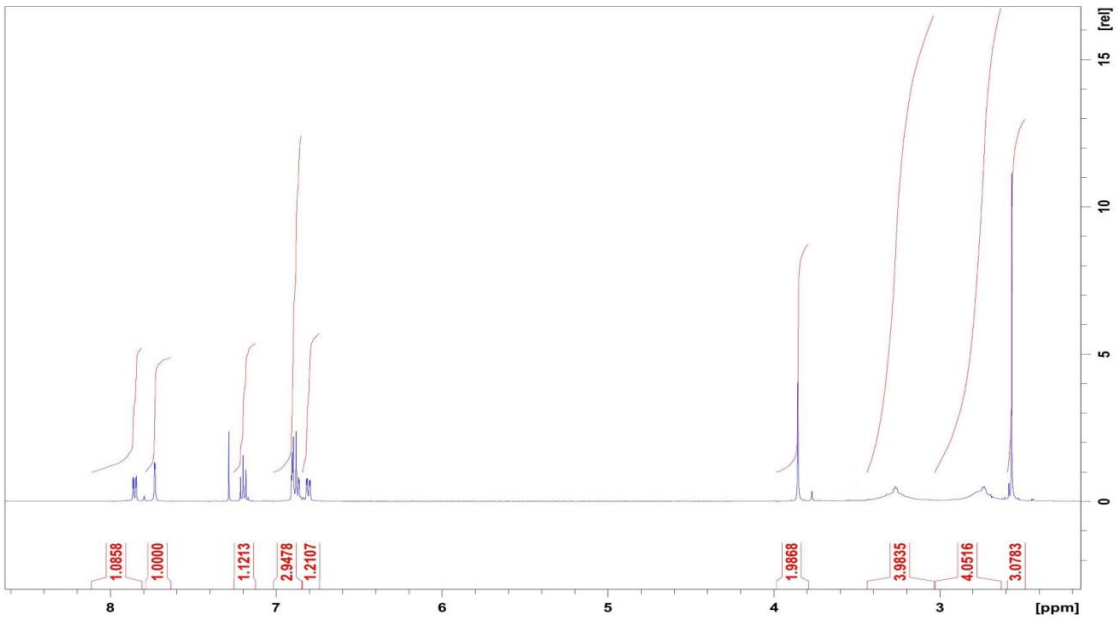


Figure 4.25. ^{1}H-NMR spectrum of compound 10.

^{13}C-NMR (ppm, DMSO-d_6): 26.28 (<u>CH_3</u>CO-), 47.59 (piperazine $C_{2,6}$), 51.96 (piperazine $C_{3,5}$), 57.49 (Ar-<u>CH_2</u>-N-), 113.74, 114.66, 115.20, 118.19, 122.52, 128.46, 129.53, 130.31, 130.42, 133.81 (Ar-C), 152.07 (Ar-C-N-), 161.32 (Ar-C-OH), 196.14 (C=O).

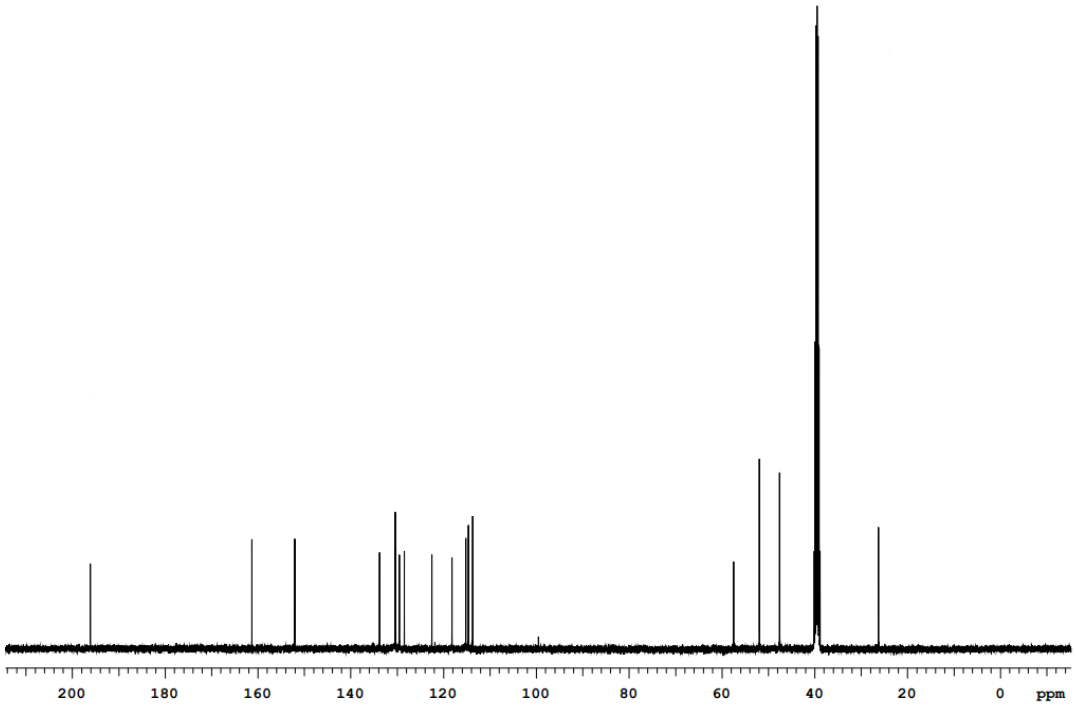
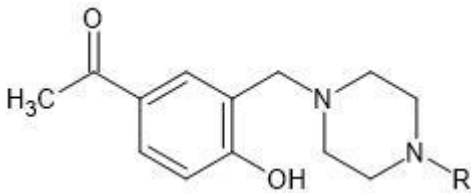


Figure 4.26. ^{13}C-NMR spectrum of compound 10.

4.2. Pharmacological Studies

The cytotoxicity results of the synthesized compound against MCF-7 cell line are given in Table 4.1.

Table 4.1. Cytotoxic activity data for compounds 1-10.

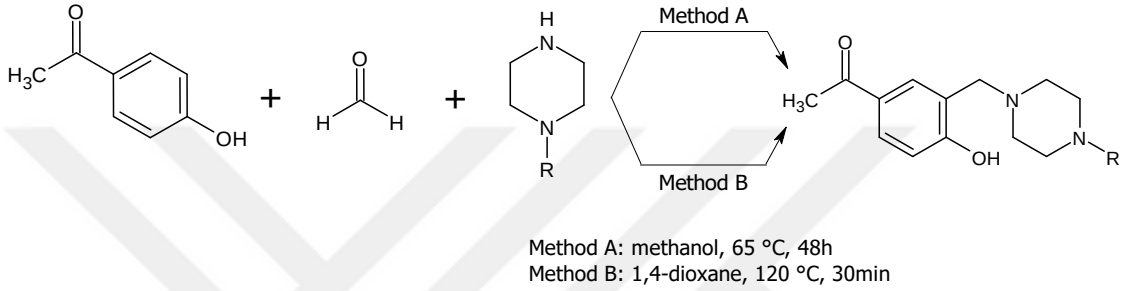


Compound		MCF-7 (IC_{50}, µM)
No.	**R**	
1	Allyl	63
2	Phenylethyl	38
3	2-Fluorophenyl	60
4	4-Methoxyphenyl	29
5	Furoyl	39
6	Benzoyl	35
7	Cyclohexyl	20
8	2-Methylphenyl	N/A
9	4-Methylphenyl	35
10	3-Chlorophenyl	34

5. DISCUSSION AND CONCLUSION

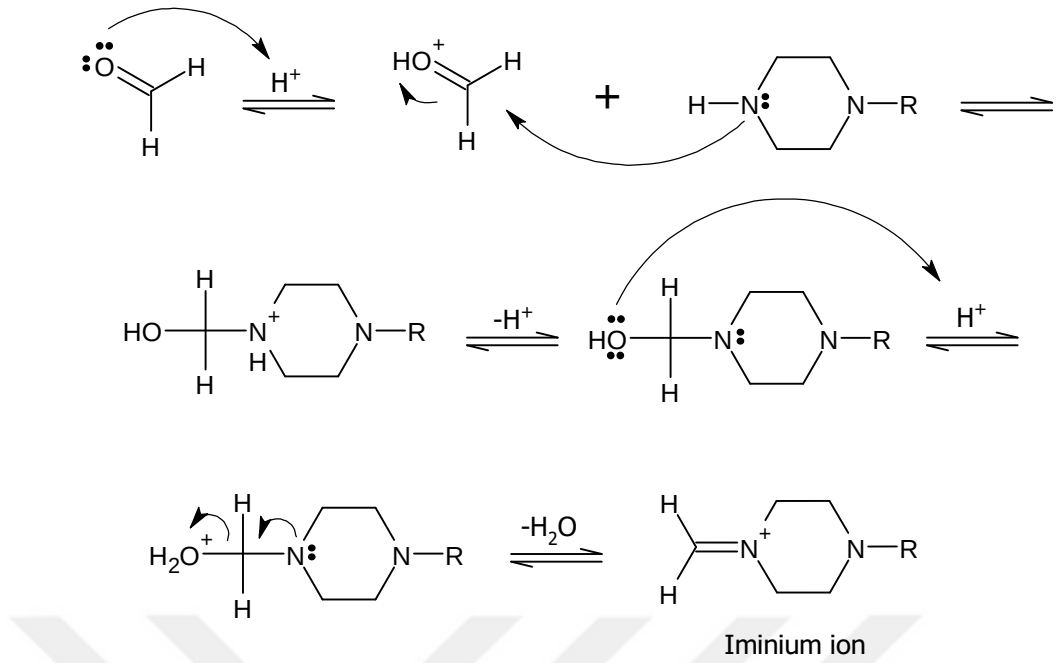
In this thesis, a set of ten new compounds bearing 1-[4-hydroxy-3-[(substituted-1-piperazinyl)methyl]phenyl]ethanone backbone were synthesized and screened for their cytotoxicity against MCF-7 cell lines. The structure illustration of the obtained compounds was carried out by IR, ^{1}H-NMR, ^{13}C-NMR, and elemental analysis.

The synthetic pathway of the target compounds is represented in Scheme 5.1.



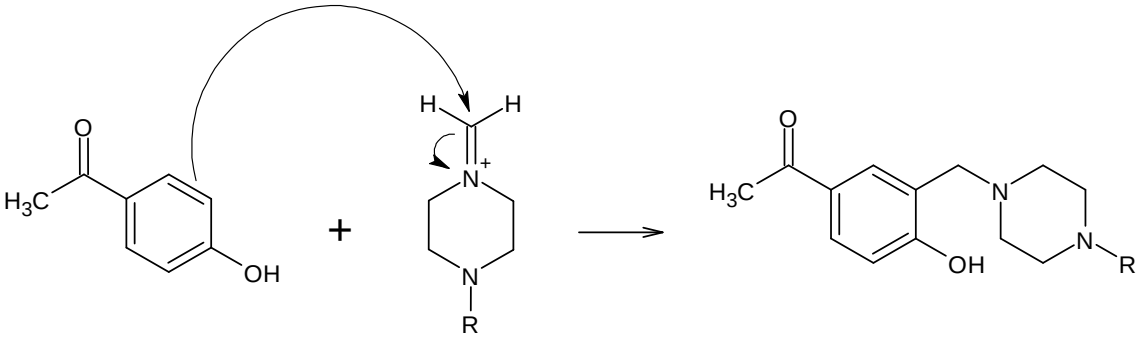
Scheme 5.1. General synthesis pathway of compounds 1-10.

The first step of the reaction involves the formation of the iminium ion due to the reaction between the substituted piperazine and the protonated formaldehyde. The reaction starts with the nucleophilic attack by the pair of electrons of the nitrogen atom of piperazine on the positively charged carbon of the protonated aldehyde, followed by deprotonation and protonation. After that, a loss of water molecule will give the iminium ion.



Scheme 5.2. Formation of the iminium ion.

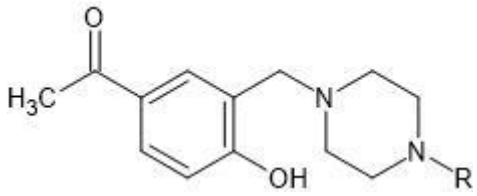
In the second step, an electrophilic addition will occur between the positively charged iminium ion and 4-hydroxyacetophenone, which will result in the formation of a methylene bridge between 4-hydroxyacetophenone and the substituted piperazine on the ortho position to the hydroxyl group of 4-hydroxyacetophenone and give the target compound.



Scheme 5.3. Formation of the Mannich base.

According to the results obtained from the two techniques, which are summarized in Table 5.1. The conventional method produced lower yields in comparison with the yields obtained from the microwave assisted synthesis. In addition, microwave irradiation reactions were completed in shorter time than the conventional reactions.

Table 5.1. Yields of the synthesized compounds.

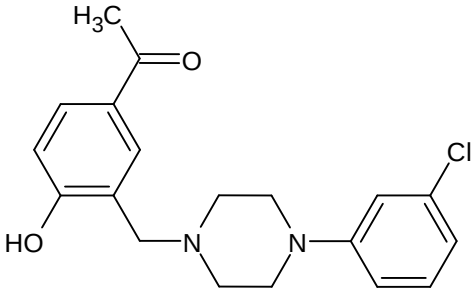


Compound		Yield (%)		M.P. (°C)
No.	**R**	**Conventional**	**Microwave**	
1	Allyl	19	72	N/A
2	Phenylethyl	26	36	116.1
3	2-Fluorophenyl	21	24	115.6
4	4-Methoxyphenyl	23	33	130
5	Furoyl	21	43	120
6	Benzoyl	15	24	128
7	Cyclohexyl	27	33	110.4
8	2-Methylphenyl	22	25	126.2
9	4-Methylphenyl	29	32	143
10	3-Chlorophenyl	13	30	138.1

The compounds structures were verified by IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis. The experimental results were in consistent with predicted data.

FT-IR spectral analysis of the prepared compounds were obtained by using KBr as a carrier. Generally, O-H bands were seen between 3200-3600 cm^{-1}, stretching bands aromatic and aliphatic C-H were observed at 2940 and 2830 cm^{-1} respectively, C=O stretching bands were seen at 1670 cm^{-1}, bands of C=C aromatic were seen at 1594 cm^{-1}, and C-N stretching bands were recorded at 1127 cm^{-1}.

For instance, IR spectra of compound **10** showed mainly stretching bands of O-H between 3185-3600 cm^{-1}, aromatic C-H stretching bands at 2949 cm^{-1}, bands of aliphatic C-H and carbonyl C=O were seen at 2836, 1666 cm^{-1} respectively, 1594 cm^{-1} stretching bands is accounted for (C=C, aromatic), and 1127 cm^{-1} stretching bands for C-N bond.



Compound **10**

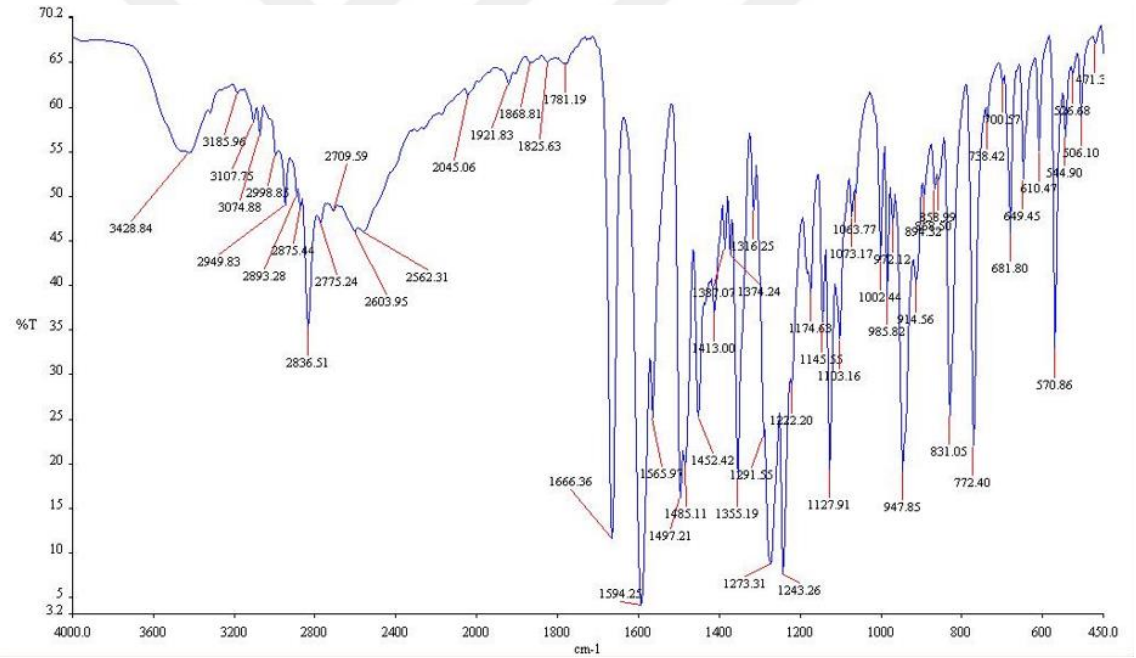
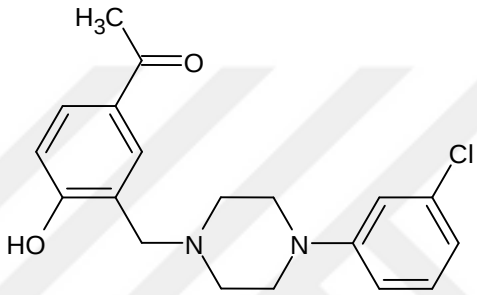


Figure 5.1. IR spectrum of compound **10**.

^{1}H-NMR spectra for the prepared compounds were carried out by using DMSO-d_6 and $CDCl_3$ as solvents and results were recorded in ppm. The compounds mainly showed the following signals: (CH_3CO-) signal showed at 2.32- 2.5 ppm as singlet, piperazine signals were recorded between 2.27- 3.05 ppm, singlet at 3.67- 3.85 ppm related to (Ar-CH_2-N-) which is obtained from Mannich reaction, and CH-aromatic signals at 6.87- 7.86 ppm.

^{1}H-NMR spectrum of compound **10** is shown at Figure 5.5: methyl protons is observed as a singlet at 2.56 ppm (s, 3H, CH_3CO-), piperazine protons is seen at 2.73-2.74 ppm (t, 4H, piperazine $H_{2,6,}$ J= 4.6 Hz), and 3.25- 3.27 ppm (t, 4H, piperazine $H_{3,5}$, J= 4.65 Hz), singlet at 3.85 ppm is related to (s, 2H, Ar-CH_2-N-) which occurred after Mannich reaction, 6.79- 6.81 (dd, 1H, phenyl H_6, J_1= 6.6 Hz, J_2= 1.75 Hz), 6.85- 6.86 (d, 1H, phenyl $H_{2,}$ J= 1.85 Hz), aromatic C-H at 6.87- 6.89 (d, 1H, 4-hydroxyacetophenone H5, J= 8.4 Hz), 6.9 (d, 1H, phenyl H_4, J= 2.1 Hz), 7.18- 7.21 (t, 1H, phenyl $H_{5,}$ J= 8.1 Hz), 7.72- 7.73 (d, 1H, 4-hydroxyacetophenone H_2, J= 2.1 Hz), 7.84- 7.86 (dd, 1H, 4-hydroxyacetophenone $H_{6,}$ J_1= 8.5 Hz, J_2= 2.2 Hz).



Compound **10**

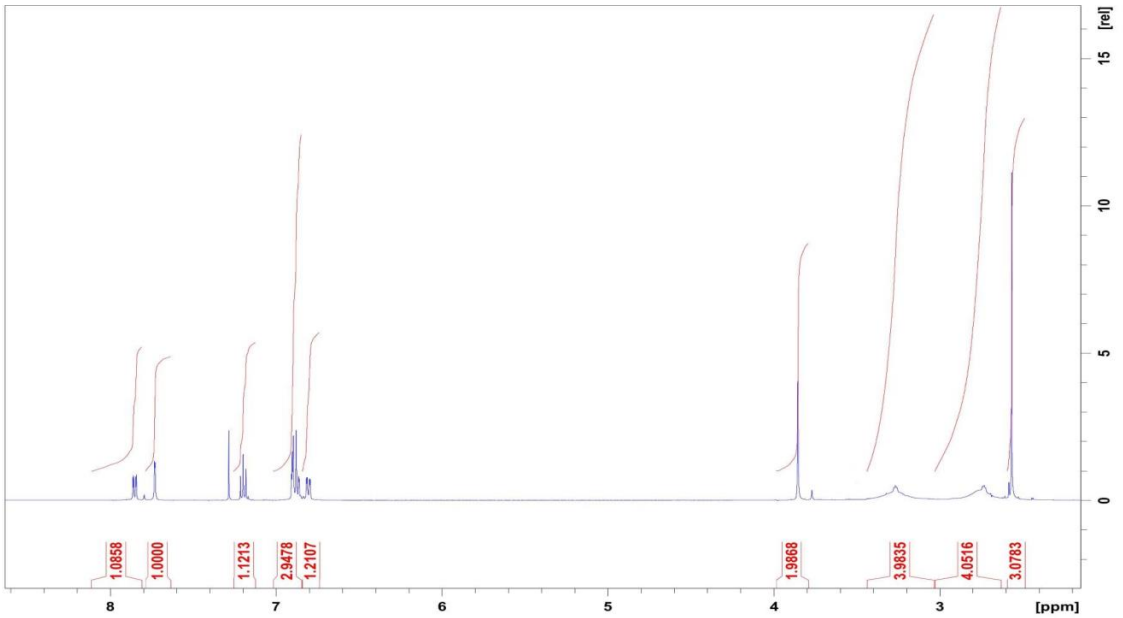
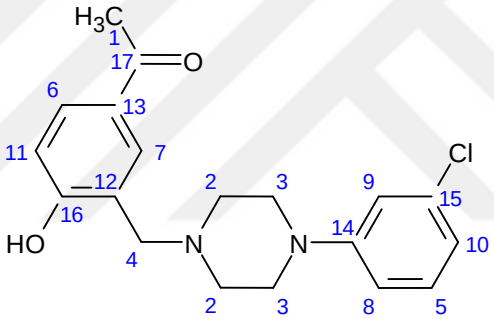


Figure 5.2. ^{1}H-NMR spectrum of compound **10**.

Compounds **2**, **4, 7, 8, 9,** and **10** ^{13}C-NMR spectral analysis was preformed using DMSO-d_6 as a solvent and results were recorded in ppm. The spectrum of the tested compounds showed mainly the following signals: methyl group signal at 26.25 ppm ($\underline{CH_3}CO$-), piperazine ($C_{2,6}$) signals at 47.59- 52.61 ppm, piperazine ($C_{3,5}$) signals at 51.96- 52.65 ppm, signal of (Ar-$\underline{CH_2}$-N-) which is obtained from Mannich reaction at 57.49- 58.64 ppm, carbonyl signal was seen at 196.14 ppm (C=O).

For instance, ^{13}C-NMR spectra analysis of compound **10** shows the following signals: methyl group signal at 26.28 ppm ($\underline{CH_3}CO$-), piperazine signals at 47.59 ppm (piperazine $C_{2,6}$), and 51.96 ppm (piperazine $C_{3,5}$), methylene group signal at 57.49 ppm (Ar-$\underline{CH_2}$-N-) which occurred from Mannich reaction, 113.74, 114.66, 115.20, 118.19, 122.52, 128.46, 129.53, 130.31, 130.42, 133.81 (Ar-C), 152.07 (Ar-C-N-), 161.32 (Ar-C-OH), and carbonyl signal at 196.14 ppm (C=O).



Compound **10**

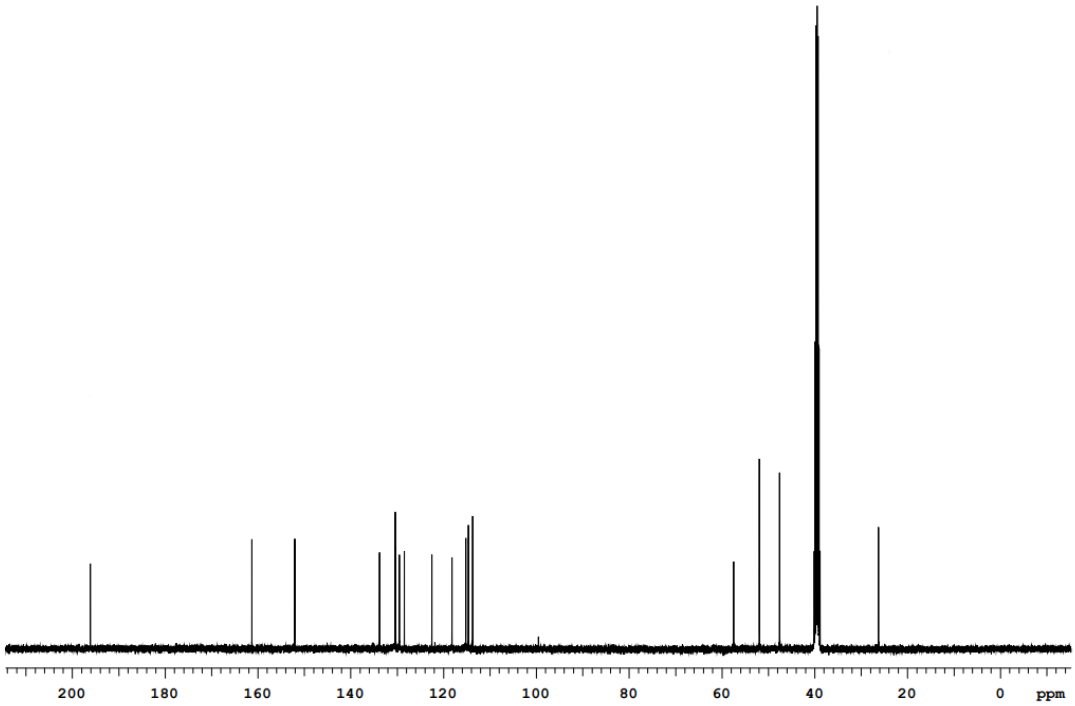


Figure 5.3. ^{13}C-NMR spectrum of compound **10**.

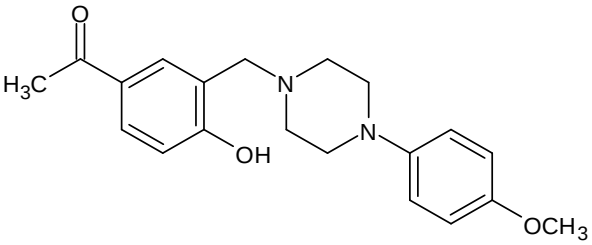
C	Chemical Shift (δ)	C	Chemical Shift (δ)
1	26.28	10	128.46
2	47.59	11	129.53
3	51.96	12	130.31
4	57.49	13	130.42
5	113.74	14	133.81
6	114.66	15	152.07
7	115.2	16	161.32
8	118.19	17	196.14
9	122.52		

Table 5.1. ^{13}C-NMR spectrum of compound **10**.

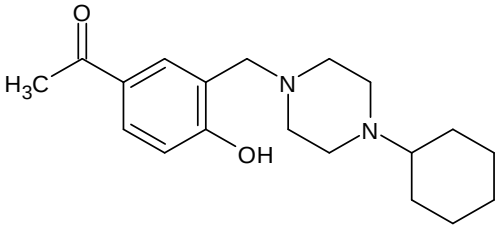
Nine of the synthesized compounds were tested for their cytotoxicities by MTT.

According to the data from Table 4.1., nearly all of the compounds showed cytotoxic effect against MCF-7 breast cancer cell line. The most active compounds were compounds **4** and **7** having IC_{50} values of 29 µM and 20 µM respectively. Compounds **1** (allyl) and compound **3** (2-fluorophenyl) showed the lowest activity having IC_{50} values of 63 µM and 60 µM respectively. In general, other compounds in the 4-

hydroxyacetophenonepiperazine series showed activity with IC_{50} values between 34- 39 µM.



Compound **4**



Compound **7**

In conclusion, ten novel 4-hydroxyacetophenonepiperazine Mannich bases were prepared by both conventional and microwave assisted synthesis. Their structural elucidation was verified by IR, ^{1}H-NMR, ^{13}C-NMR, and elemental analysis. The yields of the prepared compounds were compared, and their cytotoxicity was examined against MCF-7 cell lines, compounds **4** and **7** were found to have the highest cytotoxic effect with IC_{50} values of 29 µM and 20 µM respectively.

6. REFERENCES

1. Lao Y, Shen D, Zhang W, He R, Jiang M. Immune checkpoint inhibitors in cancer therapy—how to overcome drug resistance? *Cancers.* 2022;14(15):3575.

2. D'Eliseo D, Velotti F. Omega-3 fatty acids and cancer cell cytotoxicity: implications for multi-targeted cancer therapy. *J. Clin. Med.* 2016;5(2):15.

3. Ziarani GM, Kheilkordi Z, Mohajer F. Recent advances in the application of acetophenone in heterocyclic compounds synthesis. *J. Iran Chem. Soc.* 2020;17:247-282.

4. Raghavan S, Manogaran P, Kuppuswami BK, Venkatraman G, Narasimha KKG. synthesis and anticancer activity of chalcones derived from vanillin and isovanillin. *Med Chem Res*. 2015;24:4157-4165.

5. Prakash A, Singh BK, Bhojak N, Adhikari D. Synthesis and characterization of bioactive zinc(II) and cadmium(II) complexes with new Schiff bases derived from 4-nitrobenzaldehyde and acetophenone with ethylenediamine. *Spectrochim. Acta A Mol.* 2010;76:356-362.

6. Zaky RR, Ibrahim KM, Gabr IM. Bivalent transition metal complexes of o-hydroxyacetophenone [N-(3-hydroxy-2-naphthoyl)] hydrazone: spectroscopic, antibacterial, antifungal activity and thermogravimetric studies. *Spectrochim. Acta A Mol.* 2011;81:28-34.

7. Victoria FN, Radatz CS, Gerzson MFB *et al.* In vitro antioxidant activity and in vivo antidepressant-like effect of α-(phenylselanyl) acetophenone in mice. *Pharmacol. Biochem. Behav.* 2012;102:21-29.

8. Muthukumar VA , Ramasamy S, Haritha G, Bathala RY, Palani S. Synthesis, characterisation and pharmacological activity of some Mannich bases of acetophenone derivatives. *J. Pharm. Res.* 2011;4(12):4661-4662.

9. Malaviya R, Malaviya R, Uckun F M. Anti-inflammatory activity of 2,4,6-trihydroxy-α-p-methoxyphenyl-acetophenone (CompoundD-58). *J. Dermatol.* 2000;201:337-342.

10. Anwar C, Prasetyo YD, Matsjeh S, Haryadi W, Sholikhah EN , Nendrowati. Synthesis of chalcone derivatives and their in vitro anticancer test against Breast (T47D) and colon (WiDr) cancer cell line. *Indones. J. Chem.* 2018;18(1):102-107.

11. Gettys KE, Ye Z, Dai M. Recent advances in piperazine synthesis. *Synthesis.* 2017; 49(12):2589-2604.

12. Rejmund M, Wilczkiewicz AM, Malarz K, Bulska MP, Gajcy K *et al.* Piperazinyl fragment improves anticancer activity of Triapine. *PloS one.* 2018;13(4):0188767.

13. Kulandaivelu U, Shireesha B, Mahesh C *et al.* Synthesis antimicrobial and anticancer activity of N′- arylmethylidene- piperazine-1- carbothiohydrazide. *Med Chem Res.* 2013;22(6):2802-2808.

14. Manukumar HM, Ananda A. P, Karthik CS *et al.* Synthesis of novel benzodioxane midst piperazine moiety decorated chitosan silver nanoparticle against biohazard pathogens and as potential anti-inflammatory candidate: a molecular docking studies. *Int. J. Biol. Macromol.* 2018;108:489-502.

15. Tantray MA, Hamid H, Khan I *et al.* Synthesis of novel pyrimidin-4-one bearing piperazine ring-based amides as glycogen synthase kinase-3β inhibitors with antidepressant activity. *Chem Biol Drug Des.* 2016;87(5):764-772.

16. Choo HY, Chung BJ, Chung SH. Synthesis of piperazine derivatives and evaluation of their antihistamine and antibradykinin effects. *Bioorg. Med. Chem. Lett.* 1999;9(18):2727-2730.

17. El-Abadelah MM, Sabri SS, Abadleh MM. *et al.* Synthesis and antitumor activity of some N2-(thien-3-yl)amidrazones. *Z Naturforsch B.* 2014;69:811-816.

18. Thuy VT, Thuy LT, Hieu BT *et al.* design, synthesis and *in vitro* cytotoxic activity evaluation of new Mannich bases. *Bull. Korean Chem. Soc.* 2012;33:5.

19. Mir H, Ahmed D, Sharif HMA. Synthesis and spectral studies of acetophenone Schiff bases and evaluation of their antimicrobial activities. *Asian J. Chem.* 2013;25:8105-8110.

20. Singh AP, Pandey AK. Acetylation of benzene to acetophenone over zeolite catalysts. *J Mol Catal A Chem.* 1997;123:141-147.

21. Yang S, Zhou W, Dai X *et al.* Microchannel process for phenol production via the cleavage of cumene hydroperoxide. *Chem. Eng. Sci.* 2019;199:398-404.

22. Nandanwar SU, Rathod S, Bansal V, Bokade VV . A review on selective production of acetophenone from oxidation of ethylbenzene over heterogeneous catalysts in a decade. *Catal Lett*. 2021;151: 2116-2131.

23. Garrido F, Raeppel S, Mann A, Lautens M. Practical synthesis of acetophenones from phenoltriflates. *Tetrahedron Lett.* 2001;42:265.

24. Namboodiri VV, Varma RS, Sahle-Demessie E, Pillai UR. Selective oxidation of styrene to acetophenone in the presence of ionic liquids. *Green Chem.* 2002;4**:** 170-173.

25. Gurnule WB, Rahangdale PK, Paliwal LJ, Kharat RB. Synthesis, characterization and ion-exchange properties of 4-hydroxyacetophenone, biuret and formaldehyde terpolymer resins. *React. Funct. Polym.* 2003;55(3):255-265.

26. Clark LB, Tinoco IJr. Correlations in the ultraviolet spectra of the purine and pyrimidine bases, *J. Am. Chem. Soc.* 1965;87(1):11-15.

27. Coblentz No. 6181/ NIST standard reference database 69: NIST chemistry webbook.

28. Ulrich EL, Akutsu H, Doreleijers JF *et al.* "BioMagResBank". *Nucleic Acids Res. Spec. Publ.* 2008;36:D402-D408 doi: 10.1093/nar/gkm957.

29. Gul HI, Gul M, Erciyas E. Syntheses and stability studies of some Mannich bases of acetophenones and evaluation of their cytotoxicity against Jurkat cells. *ArzneimittelforschungDrugRes.* 2002;52(8):628-635.

30. Kazaz C, Gul HI, Kucukoglu K *et al.* Synthesis of some Mannich bases with dimethylamine and their hydrazones and evaluation of their cytotoxicity against Jurkat cells. *ArzneimittelforschungDrugRes.* 2011;61(6):366-371.

31. Gul HI, Gul M, Hänninen O. Cytotoxic activities of some mono and bis Mannich bases derived from acetophenone in brine shrimp bioassay. *ArzneimittelforschungDrugRes.* 2002;52(11):840-843.

32. Zhang N, Cai H, Cai BC, Yang HJ, Li JS, Yang GM. Two new cytotoxic acetophenone derivatives from Euphorbia ebracteolata Hayata. *Fitoterapia.* 2010;81(5):385-388.

33. Bonifait L, Marquis A, Genovese S, Epifano F, Grenier D. Synthesis and antimicrobial activity of geranyloxy- and farnesyloxy-acetophenone derivatives against oral pathogens. *Fitoterapia.* 2012;83(6):996-999.

34. Barberán FT, Sanmartin EI, Lorente FT, Angel Rumbero A. Antimicrobial phenolic compounds from three Spanish Helichrysum species. *Phytochemistry.* 1990;29(4):1093-1095.

35. Rajabi L, Courreges C, Montoya J, Aguilera RJ,Primm TP. Acetophenones with selective antimycobacterial activity. *Lett. Appl. Microbiol. LETT.* 2005;40:212-217.

36. Sameem S, Kumar N, Pathak D. Synthesis and anticonvulsant activity of some newer semicarbazone derivatives. *IJPSDR.* 2012;4(3):195-198.

37. Mohsin Ali SM, Jesmin M, Abul Kalam Azad M, Islam MK, Zahan R. Anti-inflammatory and analgesic activities of acetophenone semicarbazone and benzophenone semicarbazone. *Asian Pac. J. Trop. Biomed.* 2012;2(2):S1036-S1039.

38. Kour H, Paul S, Singh PP, Gupta M, Gupta RA. Simple one-pot method for the mercuric oxide mediated synthesis of piperazines via oxidative deamination of olefins. *Tetrahedron Lett.* 2013;54:761-764.

39. Martin WB, Martell AE. Preparation of piperazine. *J. Am. Chem. Soc.* 1948;70:1817-1818.

40. Kyrides LP, Groves W. Manufacture of piperazine. US-2267686, 1941.

41. Katrizky AR. Advances in heterocyclic chemistry. *Academic Press.* 1973;15.

42. Theophil-Eicher SH. The chemistry of heterocycles structures, reactions, synthesis and applications. *Wiley-VCH*.2003.

43. Pickett, L.W. *et al. J. Am. Chem. Soc.* 1953;75:161.

44. Lee SP, Mellon N, Shariff AM, Leveque JM. Aromatic polyamines covalent triazine polymer as sorbent for CO2 adsorption. *IRIS.* 2017;226.

45. Kalai T, Khan M, Balog M, Kutala VK, Kuppusamy P, Hideg K. Stracture-activity studies on the protection of trimetazidine derivatives modifies with nitroxides and their precursors from myocardial ischemia-reperfusion injury. *Bioorg. Med. Chem.* 2006;14:5510-5516.

46. Ostrowska K. Coumarin-piperazine derivatives as biologically active compounds. *Saudi Pharm J.* 2020;28(2):220-232.

47. Saxena M, Gaur S, Prathipat P, Saxena A. Synthesis of some substituted pyrazin-opyridoindoles and 3D QSAR studies with related compounds: pipraezines, piperidines, pyrazinoisoquinolines, and diphenylhydramine, and its semi-rigid analogs as antihistamines (H-1). *Bioorg. Med. Chem.* 2006;14:8249-8258.

48. Kaatz GW, Rybak MJ, Kerns RJ *et al.* Piperazinyl-linked fluroquinolone dimers possessing potent antibacterial activity against drug-resistant strains of staphylococcus aureus. *Bioorg Med Chem Lett*. 2003;13:1745-1749.

49. Foroumdi A, Soltani F, Moshafi MH, Asharf-Askari R. Synthesis and *in vitro* antibacterial activity of some N-(5-aryl-1,3,4-thiadiazole-2-yl) piperazinyl quinolone derivatives. *II Farmaco.* 2003;58:1023-1028.

50. Mansouri S, Emami S, Foroumadi A *et al.* Synthesis and antibacterial activity of levofloxacin derivatives with certain bulky 62 residues on piperazine ring. *Eur. J. Med. Chem.* 2007;42:985-992.

51. Thomas KD, Adhikari AV, Chowdhury IH, Sumesh E, Pal NK. New quinolone -4-yl-1, 2, 3-triazoles carrying amides, sulphonamides, and amidopiperazines as potential antitubercular agents. *Eur. J. Med. Chem.* 2001;46:2503-2512.

52. Appelbaum PC, Hunter PA. The fluroquinolone antibacterial: past, present, and future perspectives. *Int. J. Antimicrob. Agents.* 2000;16:5-15.

53. Marella A, Verma G, Shaquiquzzaman M *et al.* Piperazine scaffold: a remarkable tool in generation of diverse pharmacological agents. *Eur. J. Med. Chem.* 2015;102:487-529.

54. Upadhayaya RS, Sinha N, Jain S, Kishore N, Chandra R, Arora SK. Optically active antifungal azoles: synthesis and antifungal activity of (2R,3S)-2-(2,4- diflurophenyl)-3-(5-{2-[4-arryl-piperazin-1-yl]-ethyl}-tetrazol-2-yl\1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol. *Bioorg. Med. Chem.* 2004;12:2225-2238.

55. Gurdal EE, Durmaz I, Atalay RC, Yarim M. Synthesis and cytotoxicity studies of novel benzhydrylpiperazine carboxamide and thioamide derivatives. *J. Enzyme Inhib. Med. Chem.* 2014;29(2):205-214.

56. Aboelmagd A, Gomaa MS, El Rayes SM *et al.* Newly synthesized 3-(4-chloro-phenyl)-3- hydroxy-2,2-dimethyl-propionic acid methyl ester derivatives selectively inhibit the proliferation of colon cancer cells. *RSC Advances.* 2020;10:8825.

57. Mallesha L, Mohana KN, Veeresh B *et al.* Synthesis and *in vitro* antiproliferative activity of 2-methyl-3-(2-piperazin-1-yl-ethyl)-pyrido[1,2-a]pyrimidin-4-one derivatives against human cancer cell lines. *Arch. Pharm. Res.* 2012;35**:**51–57.

58. Lee YB, Gong YD, Yoon H, Ahn CH, Jeon MK, Kong JY. Synthesis and anticancer activity of new 1-[(5 or 6-substituted 2-alkoxyquinoxalin-3-yl)aminocarbonyl]-4-(hetero)arylpiperazine derivatives. *Bioorg. Med. Chem.* 2010;18(22):7966-7974.

59. Kharb R, Bansal K, Sharma AK. A valuable insight into recent advances on antimicrobial activity of piperazine derivatives. *Der Pharma Chemica.* 2012;4(6):2470-2488.

60. Verma AK, Kumar R, Chaudhary P *et al.* Synthesis and antimicrobial activity of N-alkyl and N-aryl piperazine derivatives. *Bioorg. Med. Chem.* 2006;14(6):1819-1826.

61. Malani MH, Dholakiya BZ. Synthesis, characterization and *in vitro* screening on bacterial, fungal and malarial strain of piperazinyl cyano biphenyl based compounds. *Bioorg. Chem.* 2013;51:16-23.

62. Rautenberg W, Amsterdam CV, Matzen L *et al.* 5-HT reuptake inhibitors with 5-HT1B/1D antagonistic activity: a new approach toward efficient antidepressants. *J. Med. Chem.* 2000;43:1149-1157.

63. Oficialdegui AM, Silanes SP, Orus L *et al.* Synthesis and molecular modeling of new 1- aryl-3-[4-arylpiperazin-1-yl]-1-propane derivatives with high affinity at the serotonin transporter and at 5-HT_{1A} receptors. *J. Med. Chem.* 2009;45:4128-4139.

64. Salat R, Moniczewski A, Salat K *et al.* Analgesic, anticonvulsant and antioxidant activities of 3-[4-(3- trifluoromethyl-phenyl)-piperazin-1-yl]-dihydrofuran-2-one dihydrochloride in mice. *Pharmacol. Biochem. Behav.* 2012;101:138-147.

65. Boido A, Boido CC, Sparatore F. Synthesis and pharmacological evaluation of aryl/heteroaryl piperazinyl alkyl benzotriazoles as ligands for some serotonin and dopamine receptor subtypes. *II Farmaco.* 2001;56:263-275.

66. Sultana N, Naz A, Khan B, Arayne MS, Mesaik MA. Synthesis, characterization, antibacterial, antifungal, and immunomodulating activities of gatifloxacin derivatives. *Med. Chem. Res.* 2010;19:1210-1221.

67. Perli M, Govindarajan R. Piperazine derivatives: a review of biological activities. *World J. Pharm. Res.* 2020;9(14):194-204.

68. Tramontini M, Advances in the chemistry of Mannich bases. *Synthesis.* 1973;703-775.

69. Tramontini M, Angiolini L. Further advances in the chemistry of Mannich bases. *Tetrahedron.* 1990;46:1791-1837.

70. Roman G. Mannich bases in medicinal chemistry and drug design. *Eur. J. Med. Chem.* 2015;89:743-816.

71. Sivakami M, Natarajan B, Vijayachandrasekar M. Synthesis, characterization and biological activity of a new Mannich Base and it's metal complexes. *Chem. sci. trans.* 2014;3(3):1110-1114.

72. Kamal RS, Ahmed NS, Nasser AM. Study the efficiency of some compounds as lubricating oil additives. *Appl. Petrochem. Res.* 2013;3:1-8.

73. Dimmock R, Kumar P. Anticancer and cytotoxic properties of Mannich bases. *Curr. Med. Chem.* 1997;4(1):1-22.

74. John Wiley & Sons. Advanced organic chemistry: reactions, mechanisms, and structure. *New York, NY, USA, 3rd edition* 1985.

75. Joshi S, Khosla N, Tiwari P. *In vitro* study of some medicinally important Mannichbases derived from anti-tubercular agent. *Bioorg. Med. Chem.* 2004;12(3):571-576.

76. Ivanova Y, Momekov G, Petrov O, Karaivanova M, Kalcheva V. Cytotoxic Mannich bases of 6-(3-aryl-2-propenoyl)- 2(3H)-benzoxazolones. *Eur. J. Med. Chem.* 2007;42:1382-1387.

77. Gul HI, Vepsalainen J, Gul M, Erciyas E, Hanninen O. Cytotoxic activities of mono and bis Mannich bases derived from acetophenone against renca and Jurkat cells. *Pharm. Acta Helv.* 2000;74(4):393-398.

78. Pandeya SN, Sriram D, Nath G, De Clercq E. Synthesis, antibacterial, antifungal and anti-HIV activities of norfloxacin Mannich bases. *Eur. J. Med. Chem.* 2000;35(2):249-255.

79. Singh BN, Shukla SK, Singh M. Synthesis and biological activity of sulphadiazine Schiff 's bases of isatin and their N-Mannich bases. *Asian J. Chem.* 2007;19(7):5013-5018.

80. Kalluraya B, Chimbalkar RM, Hegde JC. Anticonvulsant activity of nicotinyl/isonicotinyl substituted 1,2,4- triazol-5-thione Mannich bases. *Indian J. Heterocycl. Chem.* 2005;15(1):15-18.

81. Koksal M, Gokhan N, Kupeli E, Yesilada E, Erdogan H. Analgesic and antiinflammatory activities of some new Mannich bases of 5-nitro-2-benzoxazolinones. *Arch. Pharm. Res.* 2007;30(4):419-424.

82. Vashishtha SC, Zello GA, Nienaber KH *et al.* Cytotoxic and anticonvulsant aryloxyaryl Mannich bases and related compounds. *Eur. J. Med. Chem.* 2004;39(1):27-35.

83. Ashok M, Holla BS, Poojary B. Convenient one pot synthesis and antimicrobial evaluation of some new Mannich bases carrying 4-methylthiobenzyl moiety. *Eur. J. Med. Chem.* 2007;42(8):1095-1101.

84. Bennet-Jenkins E, Bryant C. Novel sources of anthelmintics. *Parasitol. Int.* 1996;26:937-947.

85. Edwards ML, Ritter HW, Stemerick DM, Stewart KT. Mannich bases of 4-phenyl-3-buten-2-one: a new class of antiherpes agent. *J. Med. Chem.* 1983; 26 (3):431-436.

86. Scott MK, Martin GE, DiStefano DL *et al.* Pyrrole Mannich bases as potential antipsychotic agents. *J. Med. Chem.* 1992;35(3):552-558.

87. Barlin GB, Jiravinya C. Potential antimalarials. X. DiMannich Bases of 4-(7′-trifluoromethyl-1′,5′-naphthyridin4′-ylamino)phenol and N-(4′ -Diethylamino-1′-methylbutyl)-7-trifluoromethyl-1,5-naphthyridin-4-amine. *Aust. J. Chem.* 1990; 43 (7):1175-1181.

88. Sriram D, Banerjee D, Yogeeswari P. Efavirenz Mannich bases: synthesis, anti-HIV and antitubercular activities. *J. Enzyme Inhib. Med. Chem.* 2009;24(1):1-5.

89. Malinka W, Swia¸tek P, Filipek B, Sapa J, Jezierska A, Koll A. Synthesis, analgesic activity and computational study of new isothiazolopyridines of Mannich base type. *Farmaco.*2005;60:961-968.

90. Malhotra M, Arora M, Samad A, Sahu K, Phogat P, Deep A. Synthesis and evaluation of some novel derivatives of 2- propoxybenzylideneisonicotinohydrazide for their potential antimicrobial activity. *J. Serb. Chem.* Soc. 2012;77(5):589-597.

91. Shivananda MK, Prakash Shet P. Antifungal activity studies of some Mannich bases carrying nitrofuran moiety. *J. Chem. Pharm. Res.* 2011; 3(2):303-307.

92. Idhayadhulla A, Kumar RS, Jamal Abdul Nasser A, Selvin J, Manilal A. Synthesis of some Mannich base derivatives and their antimicrobial activity study. *Arab. J. Chem.* 2014;7(4):994-999.

93. Lohitha P, Giles D, Sreedhar C *et al.* Synthesis and pharmacological evaluation of schiff's and Mannich bases of indole derivatives. *Rguhs. J.Pharm. Sci.* 2011;1(1):70-78.

94. Raju KR, Guru Prasad A, Kumar B, Rao L, Ravindranath KR. Synthesis and medicinal evaluation of Mannich bases carrying azetidinone moiety. *J. Clin. Anal. Med.* 2014;1-5.

95. Kulkarni AA, Wankhede SB, Dhawale ND, Yadav PB, Deore VV, Gonjari ID. Synthesis, characterization and biological behavior of some Schiff 's and Mannich base derivatives of Lamotrigine. *Arab. J. Chem.* 2017;10:S184-S189.

96. Priyay PH, Sreedarany S, Bharathi KS *et al.* Synthesis of new unsymmetrical "end-off" phenoxo bridged copper(II), nickel(II) and zinc(II) complexes: spectral, magnetic, electrochemical, catalytic, and antimicrobial studies. *J Coord Chem.* 2009;62:1356-1372.

97. Yang JS, Shen YC, Vijaya Bhaskar Reddy M *et al.* New bichalcone analogs as NF-κB inhibitors and as cytotoxic agents inducing Fas/CD95-dependent apoptosis. *Bioorg. Med. Chem.* 2011;19(6):1895-1906.

98. Kandeel MM, Abdou NA, Kadry HH, El-Masry RM. Synthesis and *in vitro* antitumor activity of some new Mannich bases. *Int. J. Chemtech Res*. 2013;5(1):401-408.

99. Meera R, Venkataraman S, Muthumani P *et al.* Synthesis and evaluation of anticonvulsant and antimicrobial activities of some Mannich bases of substituted aminophenol and acetophenone. *Int. j. pharm. biomed.* 2010;1(3):78-86.

100. Rabbani MG, Islam MR. Synthesis and characterization of some Mannich base analogues of ciprofloxacin: antibacterial, antifungal, and cytotoxic activities. *J. Sci. Res.* 2021;13(2):545-559.

101. Veliz MS, Viguri EM, Escolano RM *et al.* Second generation of Mannich base-type derivatives with *in vivo* activity against *Trypanosoma cruzi*. *J. Med. Chem.* 2018;61:5643-5663.

102. Gacche RN, Patil SA, Bandgar BP *et al.* Synthesis and biological evaluation of nitrogen-containing chalcones as possible anti-inflammatory and antioxidant agents. Bioorg. *Med. Chem. Lett.* 2010;20(2):730-733.

103. Petigara RB, Deliwala CV, Mandrekar SS, Sheth UK. Synthesis and central nervous system depressant activity of new piperazine derivatives. *I. J. Med. Chem.* 1968; 11(2):332-336.

104. Hayes BL. Recent advances in microwave assisted synthesis. *Aldrichimica ACTA.* 2004;37:66-67.

105. Roberts BA, Strauss CR. Toward rapid, “green”, predictable microwave-assisted synthesis. *Acc. Chem. Res.* 2005;38:653-661.

106. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M *et al.* Global cancer observatory: cancer today. *IARC*; 2020 doi: https://gco.iarc.fr/today.

107. Nahoum SR. Why cancer and inflammation. *Yale J Biol Med.* 2006;79:123-130.

108. Zitvogel L, Tesniere A, Kroemer G. Cancer despite immunosurveillance: immunoselection and immunosubversion. *Nat Rev Immunol.* 2006;6:715-727.

109. Nussbaumerab S, Bonnabryab P, Veuthey JL, Souveraina SF. Analysis of anticancer drugs: a review. Talanta. 2011;85:2265-2289.

110. Mansoori B, Mohammadi A, Davudian S, Shirjang S, Baradaran B. The different mechanisms of cancer drug resistance: a brief review. Adv Pharm Bull. 2017;7(3):339-348.

111. DeVita VT, Chu JrE. A history of cancer chemotherapy. *Cancer Res.* 2008; 68(21): 8643-8653.

112. Farber S, Diamond LK. Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid. *N. Engl. J. Med.* 1948; 238: 787-93.

113. Lajtha LG, Schofield R. Proliferative capacity of hematopoietic stem cells. In: Fry RJ, Griem ML, Kirsten WH, editors. Normal and malignant cell growth. *New York: Springer-Verlag.* 1969.

114. Lesher SW, Bauman J. In: Fry RJ, Griem ML, Kirsten WH, editors. Cell proliferation in the intestinal epithelium. *New York: SpringerVerlag*. 1969.

115. Elion GB, Hitchings GH, Vanderwerff H. Antagonists of nucleic acid derivatives. VI. Purines. *J. Biol. Chem.* 1951;192:505-18.

116. Burchenal JH, Murphy ML, Ellison RR, *et al.* Clinical evaluation of a new antimetabolite, 6-mercaptopurine, in the treatment of leukemia and allied diseases. *Blood*. 1953; 8:965-99.

117. Heidelberger C, Chaudhuri NK, Danneberg P, *et al.* Fluorinated pyrimidines, a new class of tumour-inhibitory compounds. *Nature*. 1957;179:663-6.

118. Pinkel D. Cyclophosphamide in children with cancer. *Cancer Chemother Rep.* 1961; 12:187-90.

119. Bernard J, Boiron M, Jacquillat C, Weil M, Najean Y. A new agent active in the treatment of acute leukemia: cytosine arabinoside. *Presse Med.* 1966;74:799-802.

120. Bonadonna G, Monfardini S, De Lena M, Fossati-Bellani F. Clinical evaluation of adriamycin, a new antitumour antibiotic. *Br. Med. J.* 1969;3:503-6.

121. Dimarco A, Gaetani M, Orezzi P, *et al.* 'Daunomycin', a new antibiotic of the rhodomycin group. *Nature*. 1964;201:706-7.

122. Falkson G, de Villiers PC, Falkson HC, Fichardt T. Natulan (Procarbazine) combined with radiotherapy in management of inoperable malignant melanoma. *Br. Med. J.* 1965;2:1473-4.

123. Johnson IS, Armstrong JG, Gorman M, Burnett Jr JP. The vinca alkaloids: a new class of oncolytic agents. *Cancer Res.* 1963;23:1390-427.

124. Mathe G, Schweisguth O, Brule G *et al.* Trial therapy of Hodgkin's disease and other malignant reticulo-histiocytic diseases by vincaleukoblastin. *Presse Med.* 1962;70:1349-52.

125. Umezawa H, Ishizuka M, Maeda K, Takeuchi T. Studies on bleomycin. *Cancer* 1967;20:891-5.

126. Felix W, Senn HJ. Clinical study of the new podophyllotoxin derivative, 4′ -demethylepipodophyllotoxin 9-(4,6-o-ethylidene-betad-glucopyranoside) (NSC-141540; VP-16-213), in solid tumors. *Cancer Chemother Rep.* 1975;59:737-42.

127. Lippman AJ, Helson C, Helson L, Krakoff IH. Clinical trials of cisdiamminedichloroplatinum (NSC-119875). *Cancer Chemother Rep.* 1973;57:191-200.

128. Holland JF. The chemical control of cancer. *Public Health Rep.* 1954;69:1151-66.

129. Redmond KM, Wilson TR, Johnston PG, Longley DB. Resistance mechanisms to cancer chemotherapy. *Front Biosci (Schol Ed).* 2008;13:5138-5154.

130. Galmarini D, Galmarini CM, Galmarini FC. Cancer chemotherapy: A critical analysis of its 60 years of history. *Crit. Rev. Oncol. Hematol.* 2012;84(2):181-199.

131. Luqmani YA. Mechanisms of drug resistance in cancer chemotherapy. *Med Princ Pract.* 2005;14(1):35-48.

7. CURRICULUM VITAE

Personal Information

Name	Ahmed Omar	**Surname**	Attar

Education

Degree	Department	The name of the Institution Graduated From	Graduation year
Doctorate			
Master			
University	Pharmacy	Philadelphia University	2018
High school	-		

Languages	**Grades (#)**
English	IELTS (7.5)

Work Experience (Sort from present to past)

Position	Institute	Duration (Year - Year)
		-
		-

Computer Skills

Program	Level
Microsoft Office	Good

Scientific works

The articles published in the journals indexed by SCI, SSCI, AHCI

Articles published in other journals

Proceedings presented in international scientific meetings and published in proceedings book.
