T.C.

YEDITEPE UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

SYNTHESIS AND ACTIVITY STUDY OF 4-HYDROXYACETOPHENONE- PIPERIDINE-MANNICH BASES

MASTER OF SCIENCE THESIS

SARA JIHAD AL MAHAMID, MSc.

İSTANBUL-2023

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SUPERVISOR

PROF. DR. HÜLYA AKGÜN

İSTANBUL 2023

THESIS APPROVAL FORM

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Owner of the Thesis: Sara Jihad AL Mahamid

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

Chair of the Jury: Prof. Dr. Hülya Akgün

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Member : Prof. Dr. Meriç Köksal Akkoç

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APPROVAL

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 Yılmaz

Director of Institute of Health Sciences

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.

27.01.2023

Sara Jihad AL Mahamid

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All praises and thanks be to Allah, the most ever merciful for always giving me the wisdom, strength and health to complete this study.

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This thesis is gratefully dedicated to my dear parents, they have been my main support in difficult times, they are the ones who lights my way, they inspired me and gave me hope when I wanted to give up, what they have given me is greater than any words of thanks. Without their moral, spiritual and emotional support, I would not have succeeded.

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LIST OF ABBREVIATIONS

MCF7	Human breast cancer cell line
SGC-7901	Type of human gastric cancer cell lines
MGC-803	Type of human gastric cancer cell lines
CHK1	Checkpoint kinases
MTT	3-(4,5-dimethylthial-2-yl)-2,5-diphenyltetraolium bromide
WHO	World Health Organization
PC-3	Human prostate cancer cell line
IC_{50}	Half maximal inhibitory concentration
E. coli	*Escherichia coli*
K. pneumonia	*Klebsiella pneumonia*
CHK1	Checkpoint kinases
SK-LU-1	Human lung carcinoma
$AlCl_3$	Aluminum chloride
Hf	Hydrogen Fluoride
TFOH	Tri-fluoromethanesulfonic
Ar	Aromatic
KB	Nasopharynx
P388	Leukemia cell line
FDA	Food and Drug Administration
PLP	Pyridoxal Phosphate
SN	Nucleophilic substitution
A549	Lung cancer
DU145	Prostate cancer
HeLa	Cervical cancer
SGC-7901	Human gastric cancer cell line
MGC-803	Gastric carcinoma cell line
Bcap-37	Human Breast Cancer
DTPEP	1-(2-(4-(Dibenzo[b,f]thiepin-10-yl)phenoxy)ethyl) piperidine
AChE	Acetylcholinesterase
P. falciparum	Plasmodium falciparum
C-mannich bases	Carbone mannich bases

N-mannich bases	Nitrogen mannich bases
O-hydrogen	Ortho hydrogen
P-hydrogen	Para hydrogen
GSH	Glutathione
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
OSCC	Human oral squamous cell carcinoma
TS	Tumor selectivity
MW	Microwave radiation
EtOH	Ethanol
$CDCl_3$	Deuterated chloroform
AChE	Acetylcholinesterase inhibitor
S. aureus	*Staphylococcus aureus*
PA	*Pseudomonas aeruginosa*
B. subtilis	*Bacillus subtilis*
KBr	Potassium bromide
TMS	Tetramethyl silane
DMSO-d6	Deuterated dimethyl sulfoxide
M.p	Melting point
S_1	Solvent system 1
S_2	Solvent system 2
S_3	Solvent system 3
DCM	Dichloromethane
DMF	Dimethyl formamide
IR	Infrared
NMR	Nuclear Magnetic Resonance
UV	Ultraviolet
s	Singlet
d	Doublet
t	Triplet
m	Multiplet
ppm	Parts per million
Rf	Retention factor

ABSTRACT

Al Mahamid, S.J. (2023). Synthesis and activity study of 4-hydroxyacetophenone-piperidine-Mannich bases. Yeditepe University Institute of Health sciences, Department of Pharmaceutical Chemistry, M.Sc. Thesis, Istanbul.

In this thesis study, 13 new compounds bearing 1-[4-hydroxy-3-[(substituted-1-piperidinyl)methyl]phenyl]ethanone backbone were synthesized by *Mannich* reaction using conventional and microwave irradiation methods. In the first method, the target compounds were synthesized by reaction of the 4-hydroxyacetophenone, formaldehyde and some piperidine derivatives under reflux for seven hours in ethanol. In the second method, microwave radiation was applied on the 4-hydroxyacetophenone, formaldehyde and piperidine derivatives in 1, 4-dioxane with 15 min at 120°C and power 300 W. The results of two methods were compared.

Structure elucidation of the 13 new compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, and elementary analysis techniques. In addition, the cytotoxicity of synthesized compounds was tested on human breast cancer cell line (MCF7) by MTT assay.

According to the activity result, 1-[4-hydroxy-3-[(4-phenylpiperidin-1-yl) methyl] phenyl]ethanone (compound **2**) and 1-[4-hydroxy-3-[(4-cyano-4-phenylpiperidin-1-yl) methyl] phenyl]ethanone (compound **11**) have high cytotoxic activity on MCF7 with IC_{50} values 22 and 29 µM, respectively. While compounds **1, 4, 5, 7, 9, 12** and **13** have moderate activity on the MCF7 cell line.

Keywords: Cytotoxicity, piperidine, 4-hydroxyacetophenone, Mannich bases, microwave

ÖZET

Al Mahamid, S.J. (2023). 4- hidroksiasetofenon-piperidin-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-[(substitüe-1-piperidinil)metil] fenil] etanon yapısı taşıyan 13 yeni bileşik Mannich reaksiyonu kullanılarak konvensional ve mikrodalga tekniği ile hazırlandı. İlk teknikte hedef bileşikler 4-hidroksiasetofenon, formaldehit ve bazı sübstitüe piperidin türevlerinin etanol içerisinde 7 saat ısıtılmasıyla elde edildi. İkinci metot da 4- hidroksiasetofenon, formaldehit ve bazı sübstitüe piperidin türevlerinin dioksan içindeki çözeltisi 15 dakika süreyle, 120°C da power 300 W uygulanarak sentezlendi. İki metotla da hazırlanan bileşiklerin sonuçları karşılaştırıldı.

13 yeni bileşiğin yapı aydınlatması, IR, ^{1}H-NMR, ^{13}C-NMR ve temel analiz teknikleriyle doğrulandı. Ek olarak, sentezlenen bileşiklerin sitotoksik, MTT metot kullanılarak MCF7 üzerinde test edildi.

Aktivite sonucuna göre sentezlenen bileşikler MCF7 hücre hattına karşı yüksek ve orta aktivite göstermiştir. Bunların arasında 1-[4-hidroksi-3-[(4-fenil piperidin-1-il)metil]fenil] etanon (bileşik **2**) ve 1-[4-hidroksi-3-[(4-siyano-4-fenilpiperidin) -1-il)metil]fenil]etanon (bileşik **11**), sırasıyla 22 ve 29 µM IC_{50} değerleri ile MCF7 hücre hattına karşı yüksek sitotoksik aktivite sergiledi. Bileşik **1, 4, 5, 7, 9, 12** ve **13** ise MCF7 hücre hattında orta düzeyde aktiviteye gösterdi.

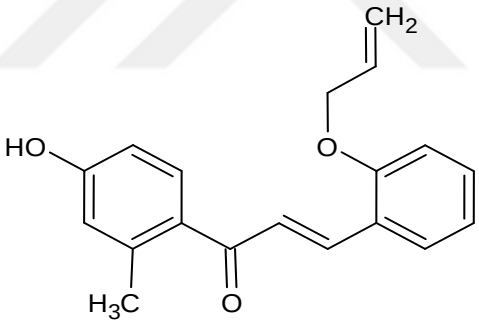
Anahtar Kelimeler: Sitotoksisite, piperidin, 4-hidroksiasetofenon, Mannich bazı, mikrodalga.

1. INTRODUCTION AND AIM

Cancer is the most common disease that leads to mortality in the world. In 2020, 19.29 million cancer patients were reported, including 10 million deaths [1-4].

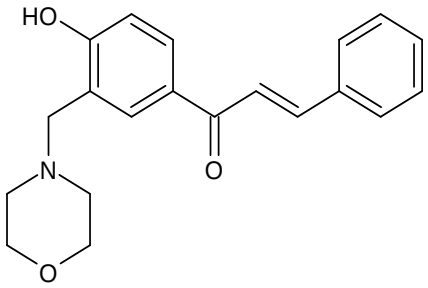
Cancer is uncontrolled cell proliferation due to mutations and genetic changes. The treatment of cancer is still a big issue these days because of current chemotherapeutic drugs have severe side effects, multidrug resistance, and no selectivity [1, 4-6].

p-Hydroxyacetophenone is an alkyl-phenyl ketone with multiple uses. The p-hydroxyacetophenone nucleus is useful in the synthesis of several therapeutic drugs [7]. They have a variety of biological characteristics that have been proven, including antibacterial [8], antifungal [8], anti-tuberculosis [9], anti-inflammatory [10, 11], analgesic [11], and anticancer activity [7, 12-14]. For examples, Mamedov *et al*. studied the biological activities of the 4-hydroxyacetophenone derivatives. Compound **1** showed good antibacterial activity against *E. coli* and *K. pneumonia* with inhibition zones 15 mm and 13 mm, respectively [8].

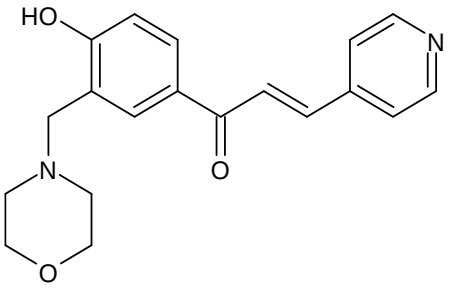


Compound **1**

1-[4-Hydroxy-3-(morpholinomethyl)phenyl]-3-(pyridine-4-yl)prop-2-en-1-one (compound **2**) and 1-[4-hydroxy-3-(morpholinomethyl)phenyl]-3-phenyl-prop-2-en-1-one (compound **3**) synthesized by Reddy *et al.* and these compounds showed activity against PC-3 with IC_{50} 0.10 µg/ml, 0.18 µg/ml and against MCF7 with IC_{50} 0.15 µg/ml, 0.15 µg/ml respectively [14].



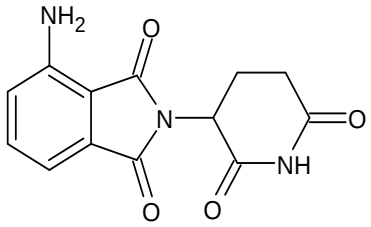
Compound **2**



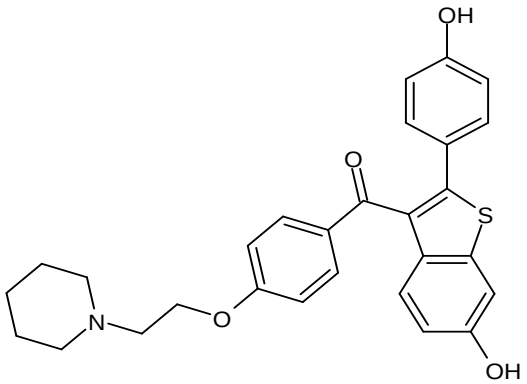
Compound **3**

Numerous researchers have described the characteristics of piperidine in both chemical and biological perspectives. Piperidine-derived compounds have a variety of biological activities such as anticancer [15-17], antiviral [6, 18], analgesic [6, 19], anti-inflammatory [6, 19, 20], antipsychotic [6, 21], antimalarial [6, 22], antifungal [23], antihypertension [24], anti-Alzheimer's [6, 25], antimicrobial [26], and anticoagulant activity [6, 27].

Several drugs with a piperidine nucleus are currently used as drugs in clinics to treat diseases. Pomalidomide is used to treat multiple myeloma [17]. Raloxifene acts as an estrogen blocker to reduce malignant breast cancer [6].

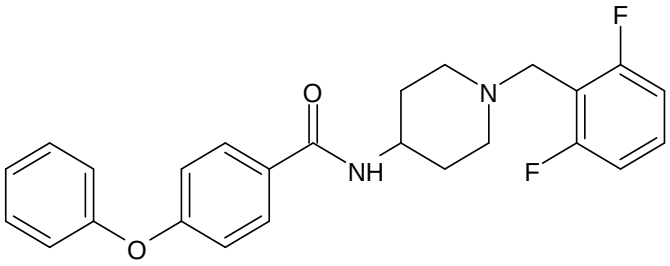


Pomalidomide



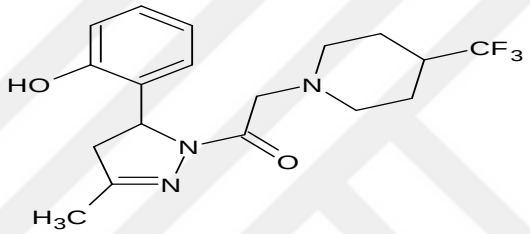
Raloxifene

Hou *et al*. produced a number of N-(4-piperidinyl) benzamide derivatives were synthesized, and their anticancer activity was studied. Among them, compound **4** had an IC_{50} value that was 15 times greater activity than that of sorafenib [28].



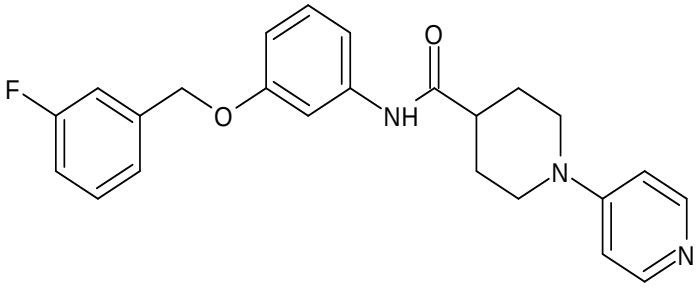
Compound **4**

5-Phenyl-N-piperidine ethanone-4, 5-dihydropyrazole derivatives were synthesized and tested by Liu *et al.* Compound **5** was the one with the greatest potency against the SGC-7901 and MGC-803 cell lines [29].



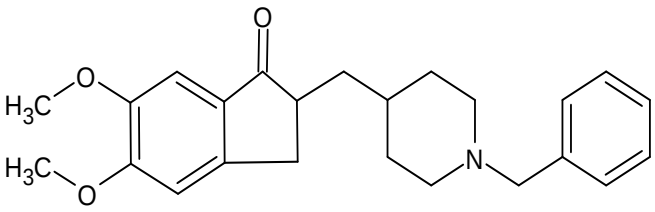
Compound **5**

A series of 4-(piperidin-1-yl) pyridine compounds were synthesized by Candia *et al.* Compound **6** exhibited potent factor IIa inhibition as well as potent anticoagulant effects, according to structure-activity studies [27].



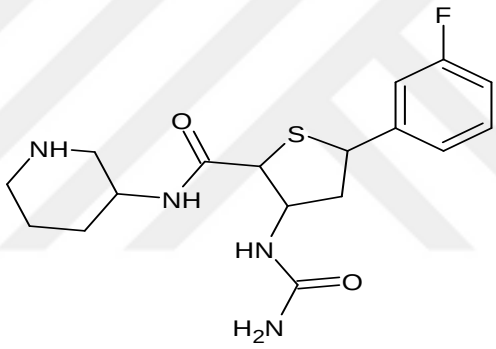
Compound **6**

Donepezil is one of the piperidine derivatives that have anti- Alzheimer's activity [6].



Donepezil

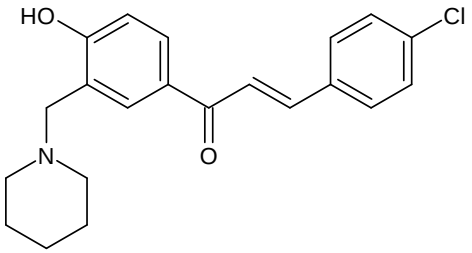
(S)-5-(3-Fluorophenyl)-N-(piperidin-3-yl)-3-ureidothiophene-2-carboxamide was prepared and they reported that the compound was checkpoint kinase CHK1 inhibitors [30].



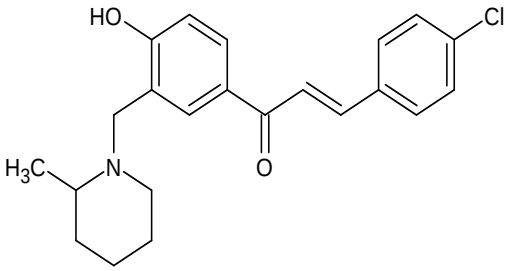
Compound **7**

The *Mannich* reaction is a one cup organic reaction of a compound having an active hydrogen atom next to a functional group, a primary or secondary amine and formaldehyde. The final product has a β-amino structure [31, 32]. In the literature, β-amino structure carrying compounds have a variety of biological activities such as antimicrobial [33], anticancer [34, 35], antifilarial [36], antimalarial [36], antibacterial [36, 37], antifungal [38], anticonvulsant [39], anthelmintic [40], antitubercular [41], anti-HIV [41], analgesic [42], antipsychotic [43], antiviral activities [44], carbonic anhydrase inhibitory [45], acetylcholine esterase inhibitory [45], anti-inflammatory [46], and diuretic activity [47].

In addition, *Mannich* bases of 4-hydroxyacetophenone derivatives and piperidine derivatives were also prepared by Hieu *et al.* and screened for biologic activity. Among them, compound **8** has high cytotoxic activity on MCF7 cell line with IC_{50} value of 2.0 μg/mL and compound **9** has high cytotoxic activity on SK-LU-1 with IC_{50} value of 2.8 μg/mL [48].

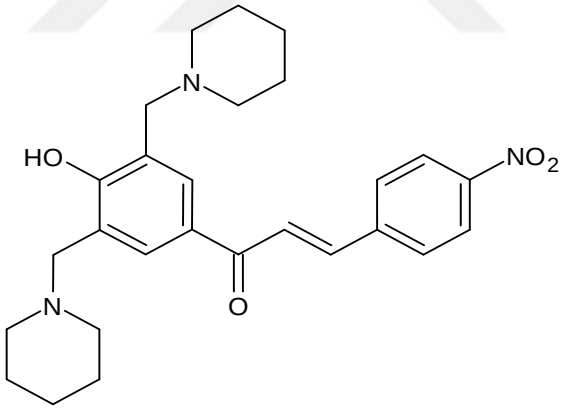


Compound **8**



Compound **9**

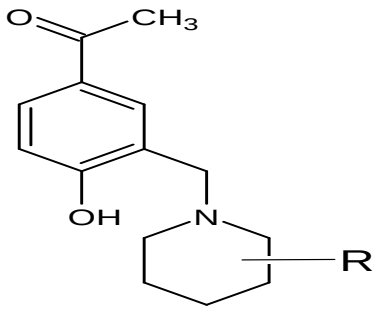
Compound **10,** synthesized by Yamali and studied for cytotoxicity, showed the highest tumor selectivity values and most promising selective cytotoxicity towards the cancer cell line [1].



Compound **10**

After these literature studies, we aimed to synthesize 13 new compounds bearing 1--[4-hydroxy-3-[(substituted-1-piperidinyl)methyl]phenyl]ethanone backbone by conventional and microwave irradiation methods. Using IR, ^{1}H-NMR, ^{13}C-NMR, and an elementary analysis technique, the structures of 13 new compounds were elucidated. The anticancer activity of synthesized compounds on MCF7 were tested by the MTT assay.

Table 1.1. Structure of the synthesized compounds



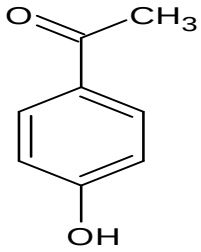
Compound	R
1	4-(3-phenylpropyl)
2	4-phenyl
3	4-benzyl
4	4-hydroxy-4-phenyl
5	4-methyl
6	4-hydroxymethyl
7	3,5dimethyl
8	3-hydroxymethyl
9	4-hydroxy
10	4-morpholine
11	4-cyano-4-phenyl
12	4-piperidino
13	4-(2-hydroxyethyl)

2. LITERATURE REVIEW

2.1. Hydroxyacetophenone

1-(4-Hydroxyphenyl) ethanone (Piceol) is a phenolic compound isolated from the mycorrhizal roots of Norway spruces (*Picea abies*).

4-Hydroxyacetophenone has a 4-hydroxy phenyl methyl ketone structure [7].

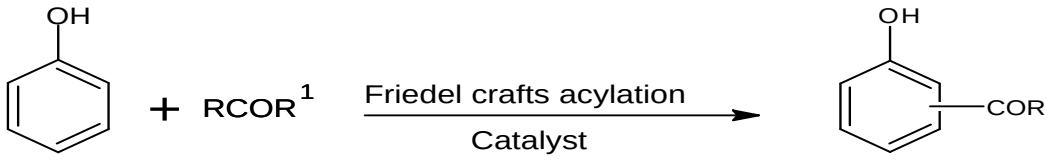


4-Hydroxyacetophenone

It is a white to pale yellow crystalline powder. Melting point: 108-110 °C, density: 1.109 and boiling point: 147-148 °C [49].

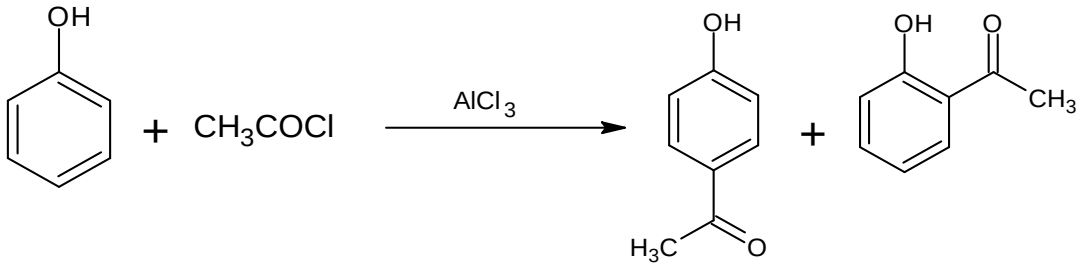
2.1.1. Methods of Synthesis

4-Hydroxyacetophenone is formed by Friedel–Crafts acylation (C-acylation reaction). Generally, Friedel-Crafts acylation occurs when benzene and its derivatives react with acylating reagents such as acyl chlorides, carboxylic anhydrides or carboxylic acids in the presence of a suitable catalyst to produce aryl ketones through the C-acylation of the aromatic ring.



$RCOR^1 = CH_3COCl, CH_3COOH$

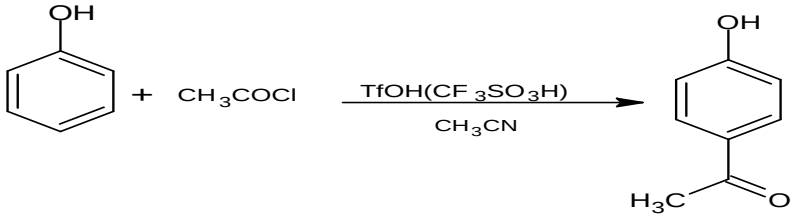
Ortho- and para-hydroxyacetophenone are prepared through the reaction of phenol with acetyl chloride or acetic anhydride in the presence of $AlCl_3$ as a catalyst [50].



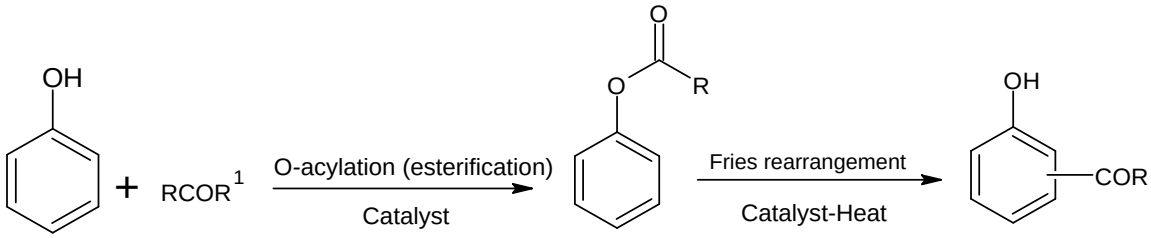
4-Hydroxyacetophenone can be obtained by the acetylation of phenol with acetic acid and using hydrogen fluoride as a catalyst at temperatures ranging from 50 to 80 °C [51].

Another method reported for the synthesis of 4-hydroxyacetophenone was the reaction of phenol and acetic anhydride in the presence of hydrogen fluoride as a catalyst at temperatures ranging from 40 to 90 °C [51].

Tri-fluoromethanesulfonic acid (TfOH) was utilized as a catalyst in Friedel–Crafts acylation by Murashige *et al.* to produce 4-hydroxyacetophenone in the presence of acetyl chloride as an acylation agent in CH_3CN at room temperature [52].

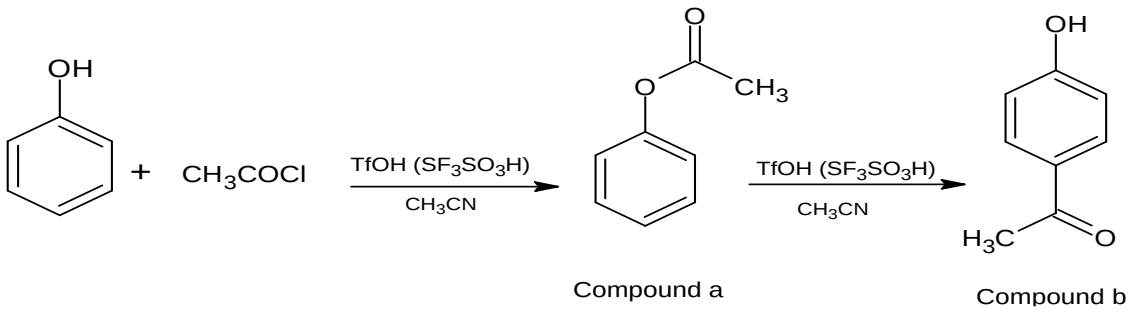


4-Hydroxyacetophenone may be obtained from phenol in two steps, the first being O-acylation, followed by Fries rearrangement. Phenyl esters can be created by O-acylation when phenol and its derivatives react with acylating agents in the presence a suitable catalyst. Then, with the present catalyst, phenyl esters quickly convert into aryl ketones in a process known as the Fries rearrangement [50, 52].

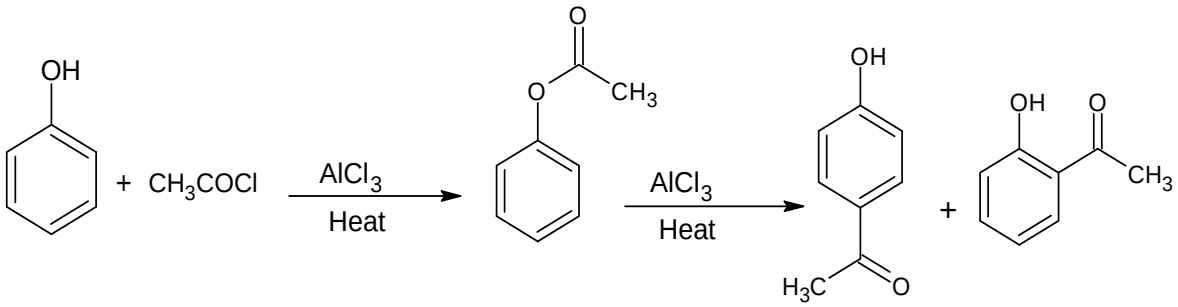


$RCOR^1$ = RCOCl, RCOOH

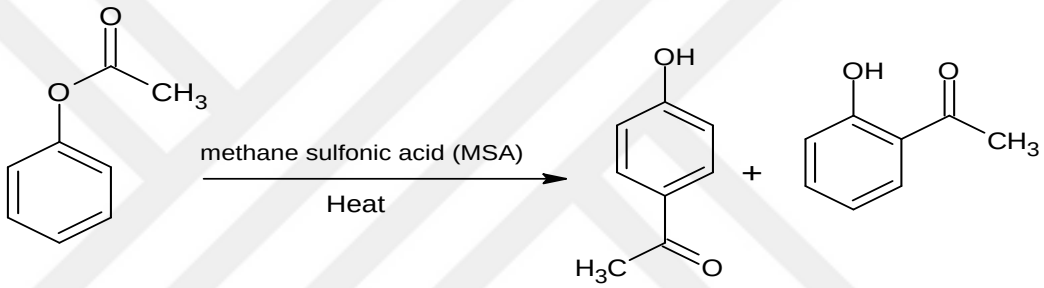
Murashige *et al.* reported that the synthesis of 4-hydroxyacetophenone from phenol occurs via an O-acylation reaction in which phenol and acetyl chloride react in the presence of trifluoromethanesulfonic (TfOH) as a catalyst in CH_3CN at room temperature. According to the O-acylation step, phenyl acetate (compound **a**) is produced. Then, the Fries rearrangement step occurred when phenyl acetate (compound **a**) was completely rearranged to para-acetyl phenol (compound **b**) in the presence of TfOH as a catalyst in CH_3CN at room temperature [52].



According to other articles, ortho- and para- hydroxyacetophenone produce by O-acylation and Fries rearrangement when phenol reacts with acetyl chloride in the presence of $AlCl_3$ as a catalyst and heat [53].



Commarieu reported the Fries rearrangement of phenyl acetate to produce the para-hydroxyacetophenone with high conversion and selectivity (up to 92% of the para-isomer and 8% of ortho-isomer at 100% conversion) in in the presence of methane sulfonic acid (MSA), a strong, stable and biodegradable acid as a catalyst [54].



2.1.2. Spectral Properties of Hydroxyacetophenone

2.1.2.1. UV Spectroscopy

UV absorption spectra of the 4-hydroxyacetophenone show three absorption bands at wave lengths of 202, 219, and 276 nm [7].

2.1.2.2. IR Spectroscopy

IR spectra of 4-hydroxyaceophenone shows peaks at 3311 cm^{-1} (O-H stretching), 3217cm^{-1} (Ar-CH stretching), 2997 cm^{-1} (Aliphatic-CH stretching), 1660 cm^{-1} (C=O stretching), 1600 cm^{-1} (C=C stretching) [7].

2.1.2.3. ^{1}H-NMR Spectroscopy

^{1}H-NMR spectra of 4-hydroxyacetophenone shows peaks at 7.99 (s, 1H, Ar-OH), 7.91 (d, 2H, Ar-CH), 6.95 (d, 2H, Ar-CH), 2.60 (s, 3H, CH_3-C=O) ppm in deuterated chloroform ($CDCl_3$) [49].

2.1.2.4. ^{13}C-NMR Spectroscopy

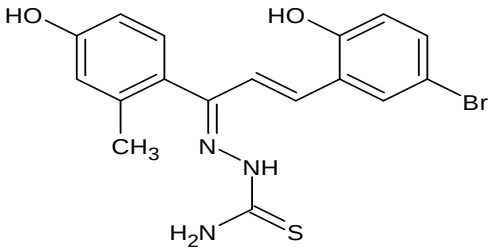
^{13}C-NMR spectra of 4-hydroxyacetophenone shows peak at 198.6, 161.6, 131.3, 129.6, 115.6, 26.4 ppm [49].

2.1.3. Biological Properties of Para-Hydroxyacetophenone

Para-hydroxyacetophenone nucleus have a number of biological activities.

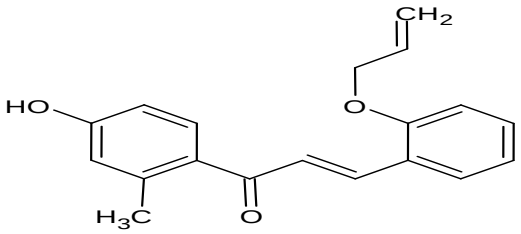
2.1.3.1 Antibacterial activity

(E,E)-3-(5-bromo-2-hydroxyphenoyl)-1-(4-hydroxy-2-methylphenyl)prop-2-en-1-one thiosemicarbazone (compound **11**) was reported by Mamedov was found to have high effective antibacterial activity against both *Escherichia coli (E.coli) and Klebsiella pneumonia (K. pneumonia)* more than antibiotics *Gentamicin, Amoxicillin and Cefazolin* [8].



Compound **11**

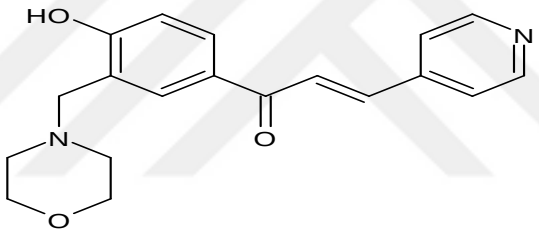
(2E)-1-(4-hydroxy-2-methylphenyl)-3-[2-(prop-2-en-1-yloxy) phenyl] prop-2-en-1-one (compound **12**) has been shown to be more effective against *E. coli* than the antibiotics *Amoxicillin* and *Cefazolin* [8].



Compound **12**

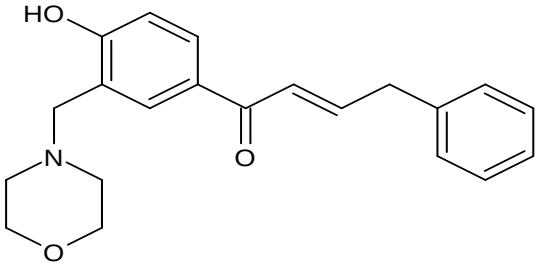
2.1.3.2. Anticancer activity

(E)-1-[4-Hydroxy-3-(morpholinomethyl)phenyl]-3-(pyridin4-yl)prop-2-en-1-one (compound **13**) was synthesized and evaluated the cytotoxicity activity against three human cancer cell lines PC-3, MCF-7, and nasopharynx (KB) by M. Vijaya Bhaskar Reddy, was found to have cytotoxicity against PC-3, MCF-7 and KB with IC_{50} values of 0.10, 0.15 and 0.74 μg/ml respectively [14].



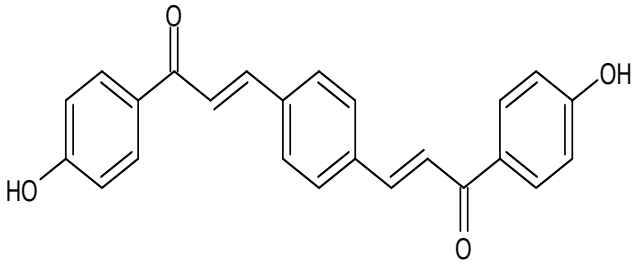
Compound **13**

(E)-1-[4-Hydroxy-3-(morpholinomethyl)phenyl]-3-phenylprop-2-en-1-one (compound **14**) was synthesized and reported have cytotoxicity activity against human cancer cell lines (prostate, PC-3 and breast, MCF7) with IC_{50} values of 0.18 and 0.15 μg/ml respectively [14].



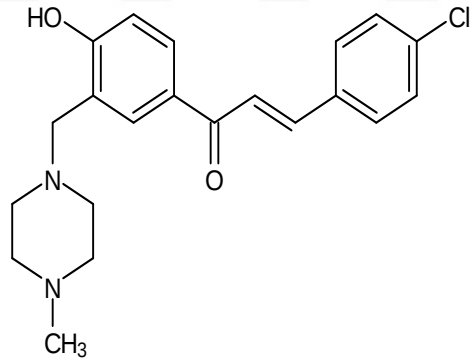
Compound **14**

Compound **15** containing the 4-hydroxyacetophenone structure was synthesized and tested for anticancer activity against P388 (a leukemia cell line) by Dimmock, the compound showed activity against P388 with IC_{50} values of 0.564 µM [13].

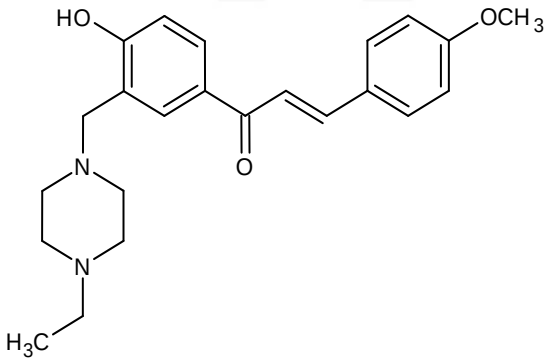


Compound **15**

Compound **16** 1-(4-chloro-phenyl)-3-[3-(4-methyl-piperazin-1-yl-methyl)-4-hydroxy-phenyl]-propenone and compound **17** 3-[3-(4-Ethyl-piperazin-1-ylmethyl)-4-hydroxy-phenyl]-1-(methoxy-phenyl)-proprenone exhibited very significant activity against MCF-7 with IC_{50} value of 2.0 µg/ml [48].



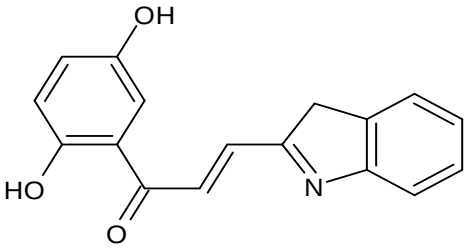
Compound **16**



Compound **17**

2.1.3.3 Anti-inflammatory activity

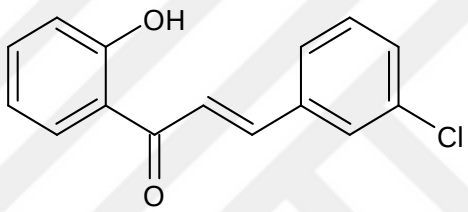
2, 5- Dihydroxychalcone (compound **18**) was synthesized and reported by Won *et al.* to have high anti-inflammatory effects [10].



Compound **18**

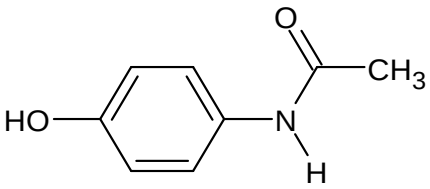
2.1.3.4. Other Activities

Chalcones 1-(2-hydroxyphenyl)-3-(3-chlorophenyl)-2-propen-1-one (compound **19**) exhibited 90% inhibitory activity against *Mycobacterium tuberculosis* H37Rv [9].



Compound **19**

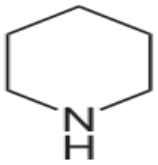
The production of paracetamol (compound **20**) and p-hydroxyacetanilide use para-hydroxyaceophenone as a precursor (analgesic drug) [7, 54].



Compound **20**

2.2. Piperidine

Piperidine (hexahydropyridine) is a major alkaloid extracted from barley (Hordeum vulgare L., Poaceae) and black pepper (Piper nigrum L., Piperaceae). Piperidine ring is one of the most common heterocyclic fragments present in FDA approved medications [6, 15].

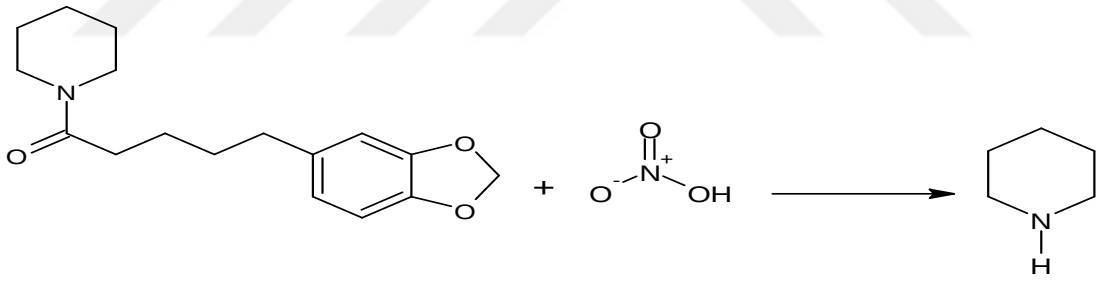


Piperidine

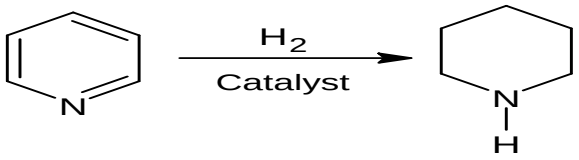
It has a boiling point of 106°C and a melting point of 10.5°C. It is an inert liquid that is miscible with water and has an unpleasant smell. It is a stronger base (pKa = 11.2) and has features of a secondary amine. Commercially, it is made using the catalytic hydrogenation of pyridine [55, 56].

2.2.1. Synthesis methods

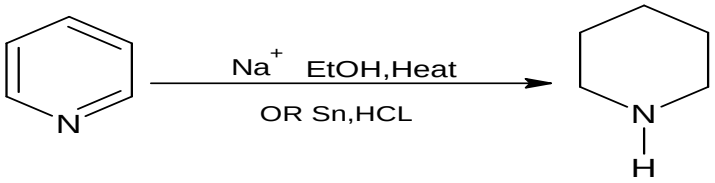
Thomas Anderson, a Scottish chemist, discovered piperidine for the first time in 1850. Auguste Cahours, a French scientist, reported piperidine for the second time independently in 1852 and gave it its name. By mixing piperine with nitric acid, they both produced piperidine [57, 58].



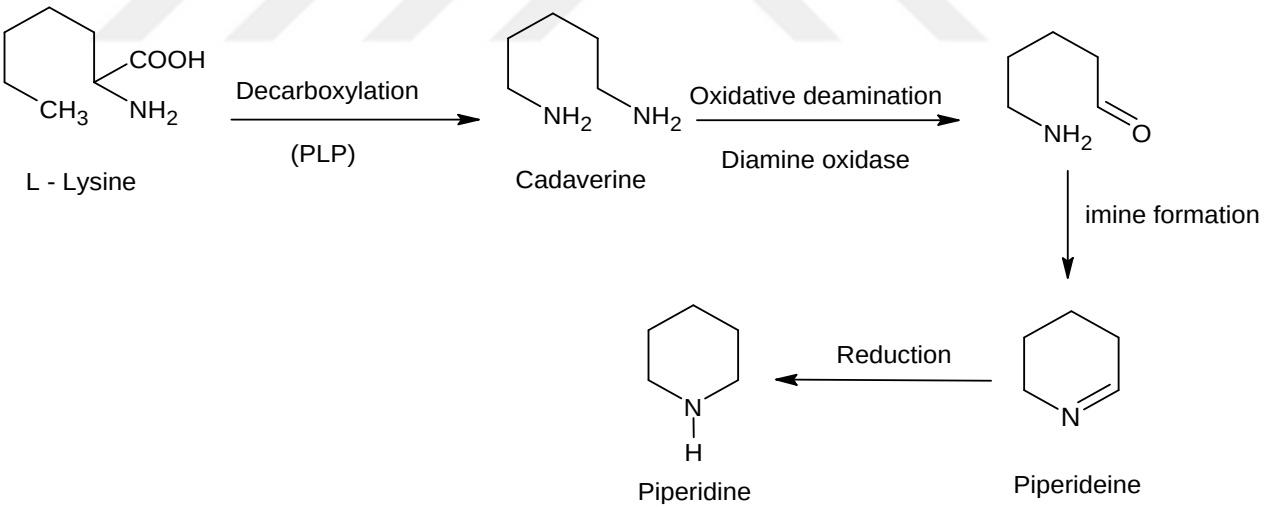
Piperidine is easily produced by catalytic hydrogenation of the corresponding pyridine derivatives over nickel, palladium or ruthenium as catalysts. Pyridine produced from tar distillation is contaminated by molecules containing sulfur. Therefore, hydrogenation can only be economically done in two steps. The crude pyridine is first hydroraffinated over a sulfidic metal catalyst at 280–310°C, and then it is quantitatively hydrogenated to piperidine over a Ni–Al_2O_3 catalyst at 120–160°C. Pyridine without sulfur can be hydrogenated using a cobalt or ruthenium catalyst [55, 56, 59].



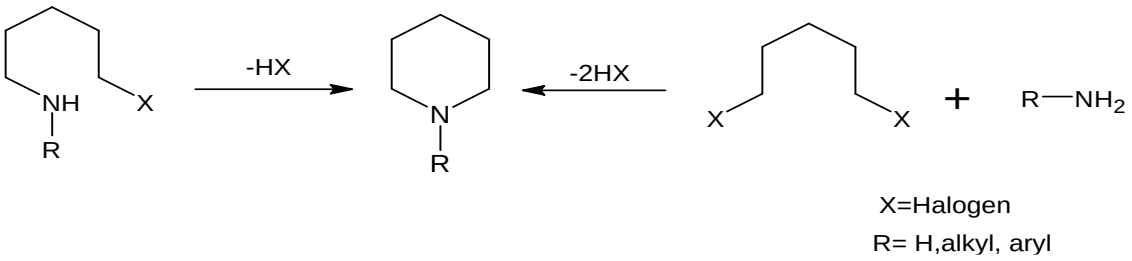
Piperidine can also be produced by reducing pyridine via sodium in ethanol or tin in hydrochloric acid [59].



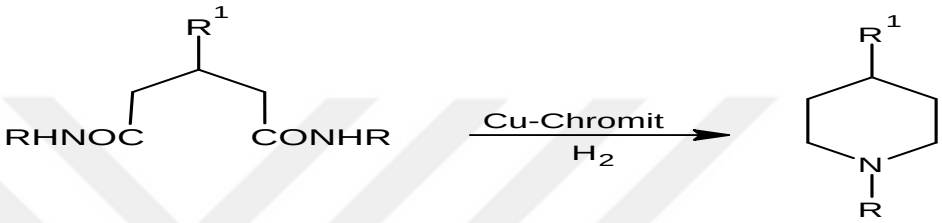
The amino acid L-lysine is decarboxylated to produce the N-heterocycle piperidine in the presence of pyridoxal phosphate (PLP). Cadaverine then undergoes direct oxidative deamination by the enzyme diamine oxidase to produce an amino aldehyde. After that, this amino aldehyde undergoes additional cyclization to produce the imine, 1-piperidine, which was subsequently reduced to piperidine [60].



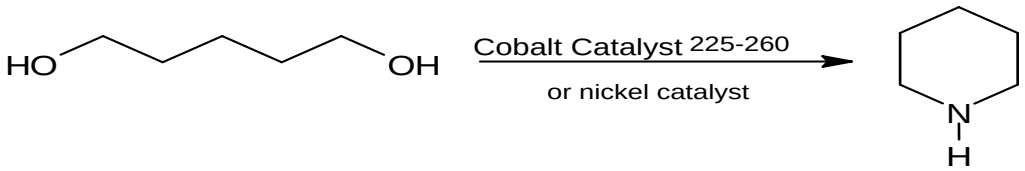
Piperidine is formed by the cyclization of 1-amino-5-haloalkanes, which involves an intramolecular SN reaction. Thus, piperidines are also formed by cyclizing 1, 5-dihaloalkanes with primary amines [56].



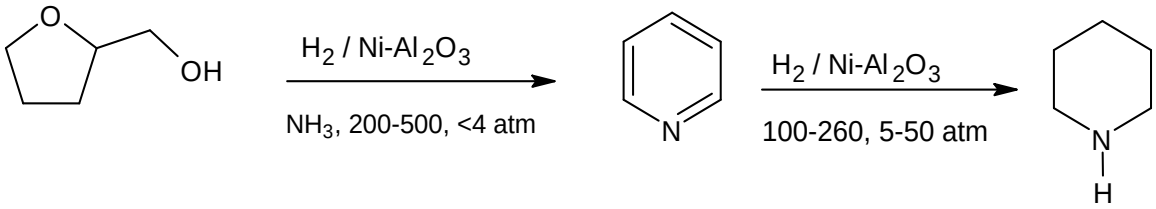
Piperidine reductive cyclization of pentanediamides or -dinitriles with H_2 over Cu chromite [56].



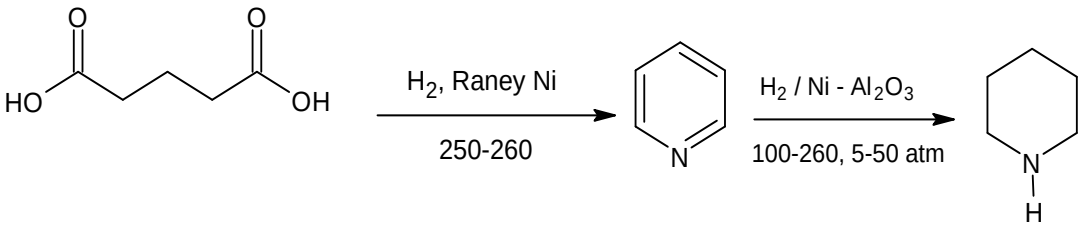
The production of piperidine is accomplished through the aminolysis of 1, 5-pentanediol under hydrogenation conditions at 225-260°C over a cobalt catalyst, while nickel catalysts can also have an effect [55].



Piperidine can be prepared from tetrahydrofurfuryl alcohol under hydrogenation conditions over a cobalt catalyst or nickel catalyst [55, 59].



When piperidine is prepared by hydrogenating glutaric acid, the reaction is carried out in the presence of a large excess of ammonia [55].



2.2.2. Spectral Properties of Piperidine

2.2.2.1. IR Spectroscopy

IR spectra of piperidine shows peaks at 3263 cm^{-1} (N-H stretching), alicyclic C-H stretching bands appear at 2790-2635 cm^{-1} [61].

2.2.2.2. ^{1}H-NMR Spectroscopy

^{1}H-NMR spectra of piperidine shows multiple peaks at 2.77 ppm and 1.52 ppm in deuterated chloroform ($CDCI_3$) [56].

2.2.2.3. ^{13}C-NMR Spectroscopy

^{13}C-NMR spectra of piperidine shows peaks at 47.5 ppm, 27.2 ppm and 25.5 ppm in deuterated chloroform ($CDCl_3$) [56].

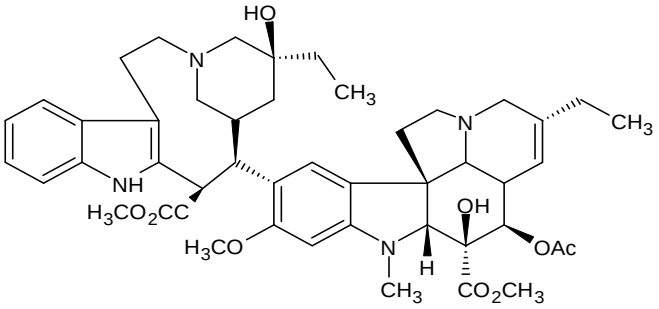
2.2.3. Biological Properties of Piperidine

Several substances with a piperidine nucleus are currently used as drugs in clinical settings to treat disease. The piperidine moiety is included in 23 of the top 200 brand-name drugs according to retail sales, and it is the most common heterocyclic in drugs that have received FDA approval [25].

2.2.3.1. Anticancer Activity

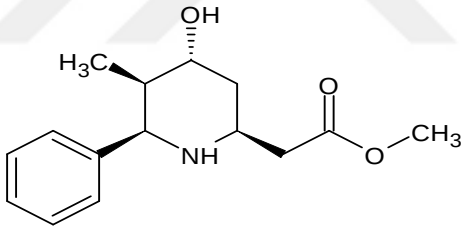
Piperidine derivatives act as a potential therapeutic drug against cancers, including ovarian, colon, prostate, lung, and breast cancer, when used alone or in combination with some novel drugs. These compounds have the ability to activate several molecular pathways, which further leads to the apoptosis of cancer cells [15].

Vinblastine is an alkaloid that naturally occurs and derives from *Catharanthus roseus*. Vinblastine is used as an anticancer drug for a wide range of cancers, including non-small cell lung cancer, breast cancer, bladder cancer, lymphomas, and leukemia [6, 25].



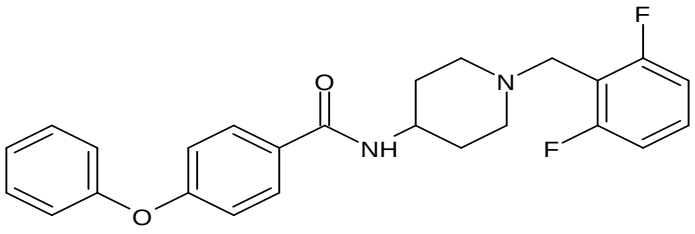
Vinblastine

The compound **21** was synthesized and displayed antiproliferative activity with IC_{50} values from 5.4 µm to 8.5 µm against A549, MCF7, DU145, and HeLa cell lines [17, 61].



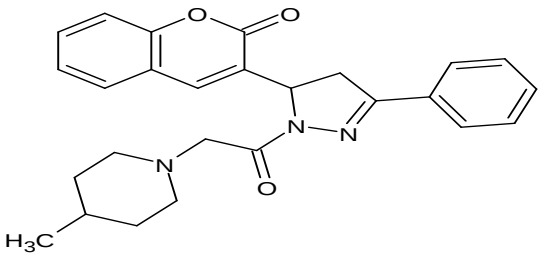
Compound **21**

N-(piperidine-4-yl) benzamide carrying molecules were synthesized by Hou *et al.* and evaluated their effects on cancer cells. Compound **22** showed the most potent effect with an IC_{50} value 15 times greater than sorafenib [28].



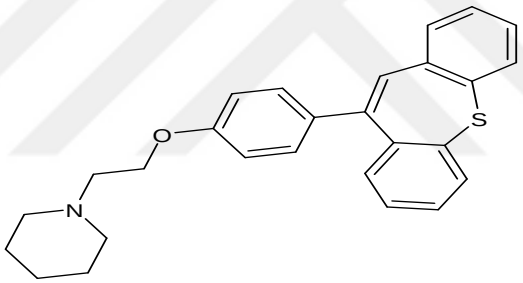
Compound **22**

High antiproliferative activities were occupied by a 4, 5-dihydropyrazole derivative of 5-phenyl-N-piperidine ethanone (compound **23**) against SGC-7901, MGC-803, and Bcap-37 cells [17].



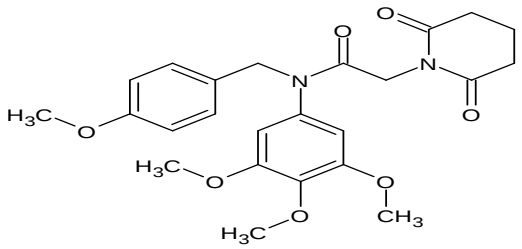
Compound **23**

The piperidine derivative 1-(2-(4-(dibenzo[b,f]thiepin-10-yl)phenoxy)ethyl) piperidine (DTPEP) (compound **24)** was synthesized from Tamoxifen. According to the research by Arun *et al.* the compound showed anti-breast cancer effect [15, 16].



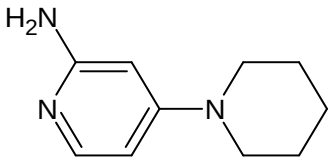
Compound **24**

Several piperidine derivatives were synthesized by Jun Fu *et al.* among them, one such derivative is 2–(2,6-dioxopiperidin-1-yl)-N-(4-methoxybenzyl)-N-(3,4,5-trimethoxy phenyl) acetamid (compound **25),** which showed powerful anticancer activity with an IC_{50} value of 0.81 μm against PC3 cells. [15, 17].



Compound **25**

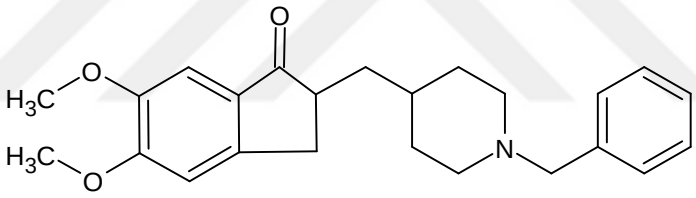
The effect of 2-amino-4-(1-piperidine)pyridine (compound **26**) on colon cancer cells was studied by Wang *et al.* and this compound showed that it is reduced the spread and growth of colon cancer cells [62].



Compound **26**

2.2.3.2. Anti-Alzheimer's Activity

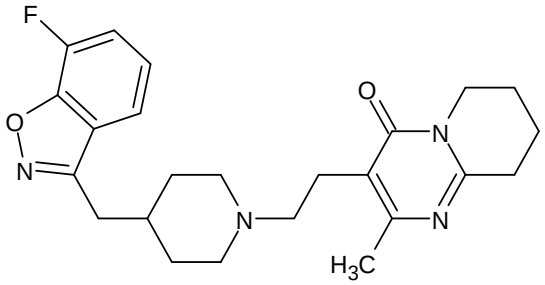
The first medication designated for the symptomatic treatment of Alzheimer's disease was donepezil, which was introduced in March 1997. Donepezil is a piperidine based, non-competitive, potent, specific, and reversible inhibitor of acetylcholinesterase (AChE) [6, 25, 63].



Donepezil

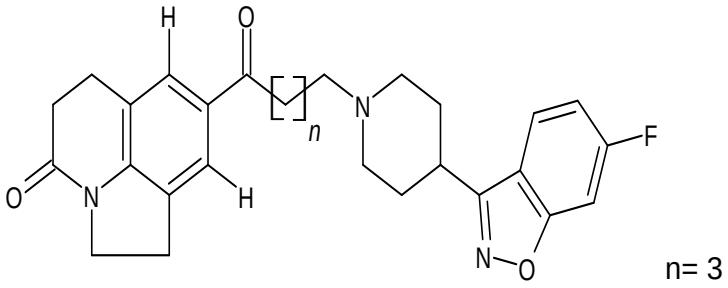
2.2.3.3. Antipsychotic Activity

Risperidone is a common antipsychotic medication used to treat schizophrenia [25].



Risperidone

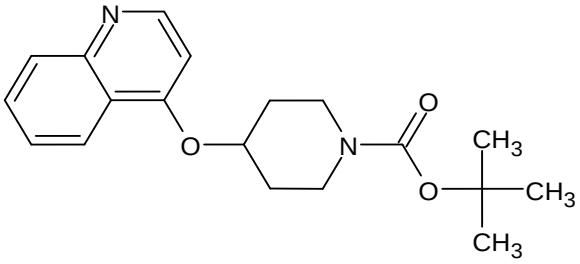
Cao *et al.* developed and produced a series of piperidine derivatives. Among them, piperidinyl fluorobenzoxazole derivatives (compound **27**) had pro-cognitive qualities, significant pharmacokinetic characteristics, and unique pharmacological features. Compound **27** may belong to a new class of atypical antipsychotic medications for schizophrenia [64].



Compound **27**

2.2.3.4. Antiviral Activity

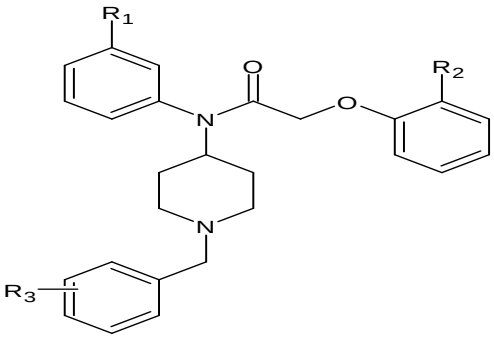
The compound **28** synthesized by Wang *et al.* and showed excellent inhibitory effects on a variety of influenza virus strains and showed a strong ability to inhibit the early to middle stages of influenza virus reproduction [18].



Compound **28**

2.2.3.5. Antimalarial Activity

Novel 1, 4-disubstituted piperidine derivatives designed and tested for their effectiveness against chloroquine-sensitive and chloroquine-resistant strains of P. *falciparum*. Compounds **29** (a, b, c) presented below were the most effective ones [6, 22].



Compound a (R_1=H ,R_2=H,R_3=H)

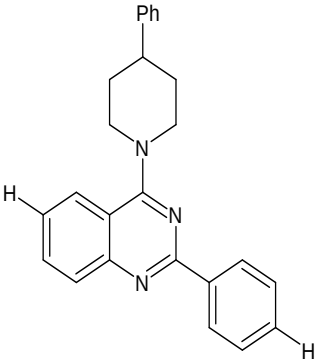
Compound b (R_1= F,R_2=CL, R_3=H)

Compound c (R_1= F,R_2=CL, R_3=Br)

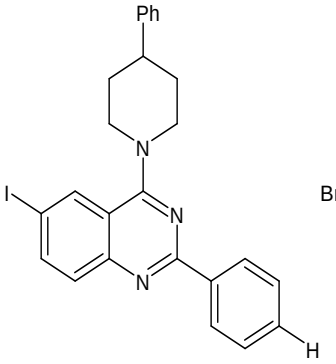
Compounds **29** (a, b, c)

2.2.3.6. Analgesic and Anti-inflammatory activity

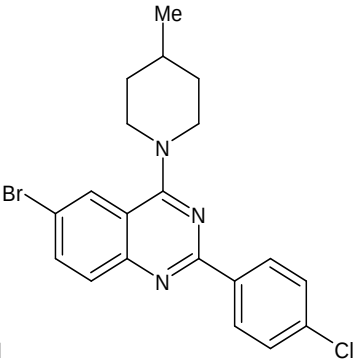
A line of 2, 4, 6-trisubstituted quinazoline derivatives with a piperidine moiety was synthesized by Alafeefy *et al.* and the ability of these substances to reduce pain and inflammation was tested. Compound **30**, compound **31** and compound **32** presented below were found to have a higher analgesic effect than the common medication indomethacin [19].



Compound **30**

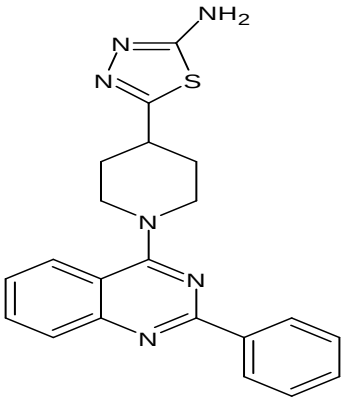


Compound **31**

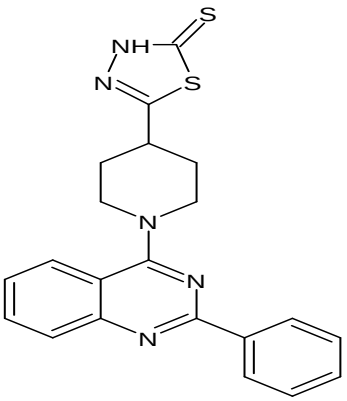


Compound **32**

Compounds **33** and compound **34** presented below showed analgesic and anti-inflammatory effects [19].



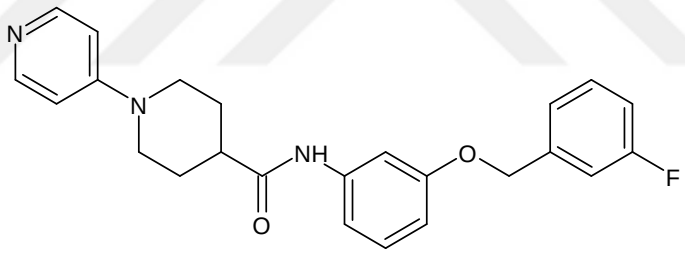
Compound **33**



Compound **34**

2.2.3.7. Anticoagulant Activity

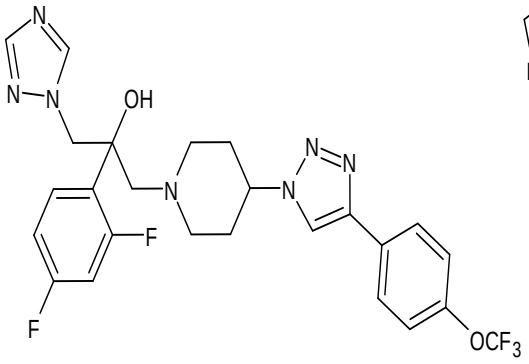
A series of 4-(piperidin-1-yl)pyridine compounds were synthesized by Candia *et al* and studies of the relationships between structure and activity demonstrated that (compound **35)** exhibited strong factor IIa inhibition and showed a good an anticoagulant effect [27].



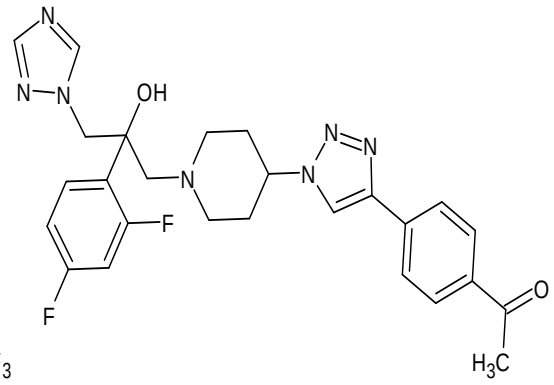
Compound **35**

2.2.3.8. Antifungal Activity

New antifungal piperidinyl triazole compounds were designed and created by Zhigan *et al.,* and several showed outstanding antifungal effectiveness against a variety of significant fungi. Below compounds **36, 37** from this group were found to be extremely effective antifungal medications [23].



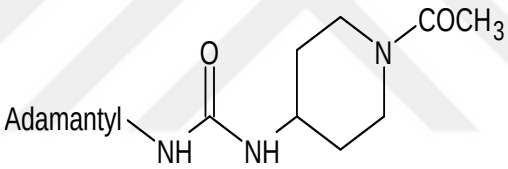
Compounds **36**



Compounds **37**

2.2.3.9. Anti-hypertension Activity

Novel piperidinyl urea compounds synthesized and examined for their effectiveness as epoxide hydrolase inhibitors. Compound **38** was discovered to significantly reduce blood pressure [6].

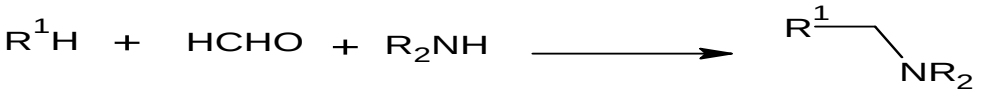


Compound **38**

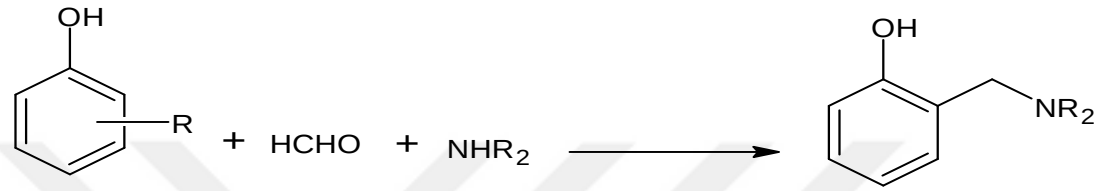
2.3. Mannich bases

The *Mannich* reaction is a nucleophilic addition reaction and it is essential for the direct formation of new C–C and C–N bonds [36, 65, 66]. The *Mannich* reaction was named after the German chemist Carl *Mannich*, who discovered and published it in 1912 [67, 68]. Generally, the *Mannich* reaction process is run as a three-component reaction of active hydrogen compounds, reactive aldehyde, and a primary or secondary amine. The active hydrogen compound is most frequently a ketone, an alkyne and a phenol [32, 69-71].

Mannich bases are an important group of compounds in medicinal chemistry and are synthesized by the *Mannich* reaction. In the below equation, the product represent the general form of the *Mannich* bases [32, 69].



There are different types of *Mannich* bases, including C-*Mannich* bases and N-*Mannich* bases. Phenolic *Mannich* bases are a type of carbon *Mannich* bases. The O- and P-hydrogens in phenols are sufficiently active to enter into the *Mannich* reaction [32, 69, 72, 73].



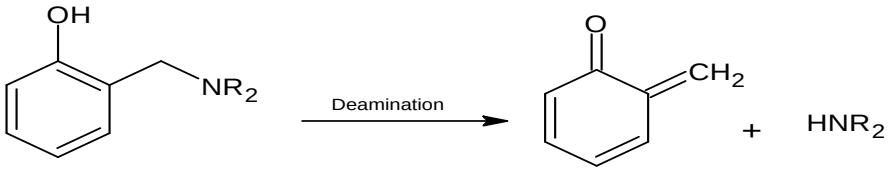
Mannich reactions are also known as amino alkylation reactions because of *Mannich* bases may be regarded as derivatives of the substrate obtained through substitution by an aminoalkyl moiety. In the particular instance when formaldehyde is employed as an aldehyde component, the substrate is converted into the corresponding *Mannich* base through an aminomethylation process [32]. The critical part of the *Mannich* reaction is the replacement of the active hydrogen atom by an aminomethyl or a substituted aminomethyl group [71].

According literature studies, aminomethylation of drugs could be used to improve the delivery of a drug into the human body. Aminomethylation may increase the hydrophilic properties of drug through the introduction of a polar function into chemical structure [1, 32, 73].

Addiatonly, *Mannich* bases are very reactive and easily transform into other compounds. For example, they can be reduced to produce physiologically active amino alcohols [36, 74].

Prodrug of *Mannich* bases have been prepared to overcome the limitations. The majority of the biological activity of *Mannich* bases is attributed to, α, β unsaturated ketone, which can be produced by deamination, the α, β-unsaturated ketone is an active site for nucleophilic activation [32, 75, 76].

The biological effects of *Mannich* bases, including their cytotoxic, antimicrobial, and antitumor properties have been related to these liberated, α, β- unsaturated ketones that have the ability to alkylate nucleophiles, particularly thiol groups like glutathione [1, 32, 76-78]. Below equation show deamination of phenolic *Mannich* bases, which generates an additional alkylating site for cellular nucleophile.



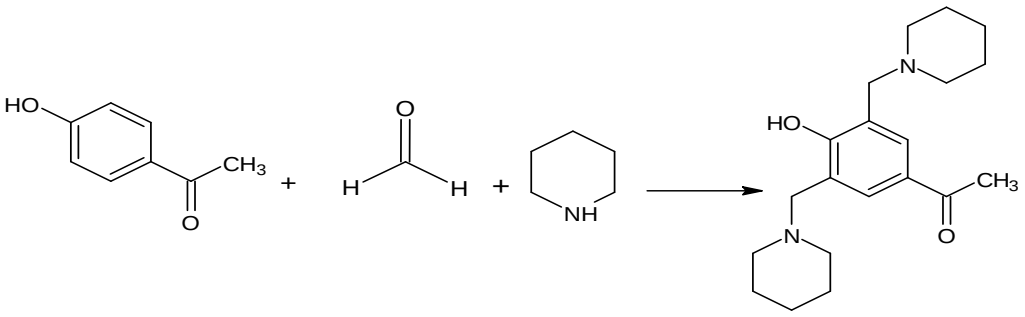
2.3.1 Mannich bases as anticancer agent

Mannich bases were generated as a prodrug to α, β-unsaturated ketones since deamination of *Mannich* bases in vivo and under simulated in vitro conditions have been observed. The α, β- unsaturated ketones are important in cancer chemotherapy because these compounds are thiol alkylators, they often have little to no affinity for hydroxy and amino groups. Enones may therefore avoid interactions with nucleic acids and offer benefits over several alkylating agents used to treat cancer, which have been shown to have carcinogenic and mutagenic effects [1, 77]. Compared to traditional alkylating agents and antimetabolites, which typically suppress the incorporation of substrates into these macromolecules more in normal tissues than in cancers, certain thiol alkylators have demonstrated greater effects against tumors than normal tissues. As a result, modern anticancer drugs from *Mannich* bases and the related α, β-unsaturated ketone might be helpful in showing activity against some tumors for whom the current treatment options are insufficient. Additionally, they may be helpful in treating neoplasms that have developed a resistance to conventional medications [79, 80].

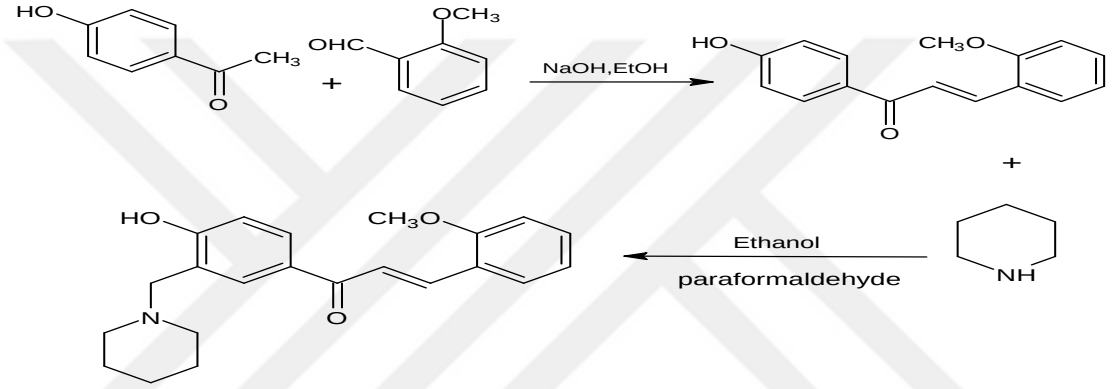
2.3.2. Mannich bases of Para- Hydroxyacetophenone Piperidine derivative

2.3.2.1. Methods of Synthesis

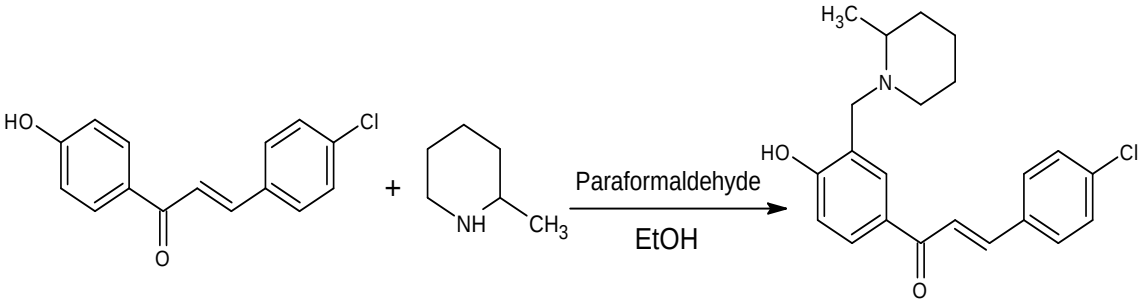
Hasan *et al* synthesized 4-hydroxy-3, 5-(di(piperidin-1-yl)methyl) acetophenone [81].



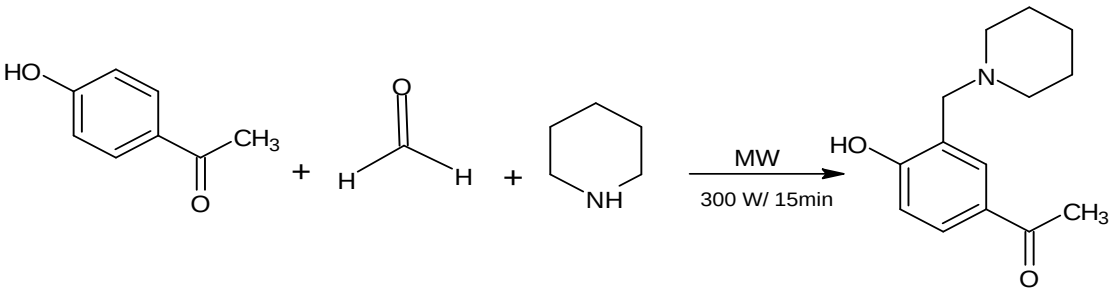
In 2012 Hieu *et al* synthesized (E)-3-(4-hydroxy-3-piperidin-1-ylmethyl-phenyl)-1-(2- methoxy-phenyl)- propenone and screened cytotoxic activity [48].



(E)-1-(4-Chloro-phenyl)-3-[4-hydroxy-3-(2-methyl-piperidin-1-ylmethyl)-phenyl]-propenone is synthesized by using 2-methylpiperidine as secondary amine [48].



In 2017 Aljohani *et al.* used a microwave irradiation for the synthesis 1-{4-hydroxy-3-[(piperidin-1-yl) methyl] phenyl}ethan-1-one [65].



2.3.2.2 Spectral Properties of 4-Hydroxyacetophenone Piperidine Derivatives

2.3.2.2.1. IR Spectroscopy

IR spectrum of *Mannich* bases of 4-hydroxyacetophenone piperidine shows sharp band at 1256 (C–N), 1287 (C–O), 1594 (C=C), 1661 (C=O), 2946 (CH_3, sp_3) cm^{-1} [65].

2.3.2.2.2. ^{1}H-NMR Spectroscopy

In ^{1}H-NMR spectra of of Mannich bases of 4-hydroxyacetophenone piperidine peaks appear at 11.41 (br s, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.68 (s, 1H), 6.84 (d, J = 8.4 Hz, 1H), 3.75 (s, 2H), 2.53 (br s, 7H), 1.67–1.52 (m, 6H) in deuterated chloroform ($CDCl_3$) [65].

2.3.2.2.3. ^{13}C-NMR Spectroscopy

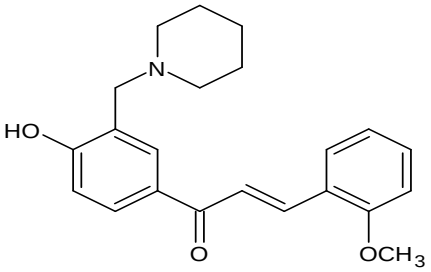
^{13}C-NMR spectra of 4-hydroxyacetophenone piperidine shows peaks at 196.86, 163.32, 130.24, 129.35, 128.73, 121.06, 115.93, 61.51, 53.73, 26.22, 25.6, 23.71 ppm in deuterated chloroform ($CDCl_3$) [65].

2.3.2.3. Biological Properties of Mannich bases of para-hydroxyacetophenone derivative piperidine.

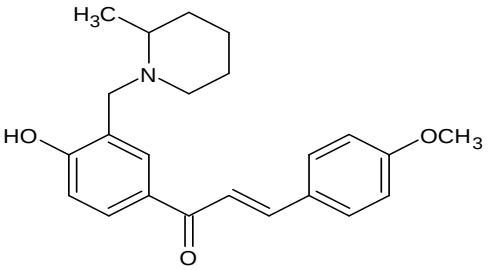
2.3.2.3.1. Anticancer Activity

Below are compounds synthesized by Hieu *et al.* and tested for their effectiveness as an anticancer against MCF7 and SK-LU-1. (E)-3-(4-Hydroxy-3-piperidin-1-ylmethyl phenyl)-1-(2-methoxy phenyl) propenone (compound **39**) showed improved activity against the MCF7 cell line with IC_{50} values of 4.92 μg/mL. (E)-3-[4-Hydroxy-3-(2-methyl piperidin-1-ylmethyl)phenyl]-1-(4-methoxyphenyl)propenone (compound **40**)

showed activity as an anticancer agent against the SK-LU-1 cell line with IC_{50} values of 4.93 µg/mL [48].

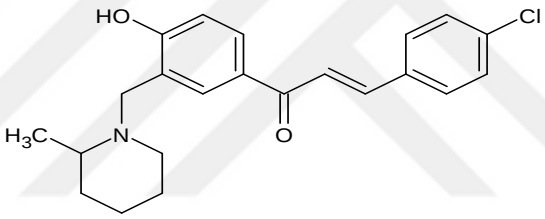


Compound **39**



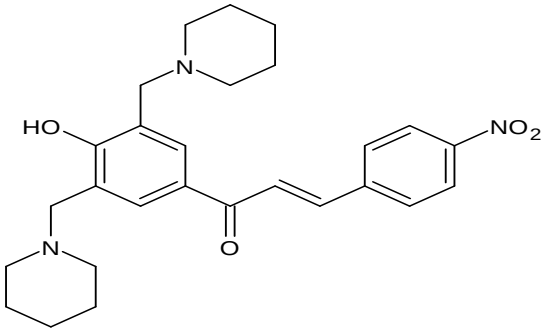
Compound **40**

(E)-1-(4-Chlorophenyl)-3-[4-hydroxy-3-(2-methylpiperidin-1-ylmethyl)phenyl]-propenone (compound **41**) has cytotoxic activity against MCF-7 with IC_{50} values of 4.52 µg/mL and has potent activity against SK-LU-1 with IC_{50} values of 2.8 µg/mL [48].



Compound **41**

Yamali C *et al.* tested the cytotoxicities of the *Mannich* bases of chalcones and piperidine towards human oral squamous cell carcinoma (OSCC). Compound **42** represented below has the highest tumor selectivity (TS) values (TS1:11.2, TS2:15.8) and shows promising selective cytotoxicity towards cancer cell lines [1].

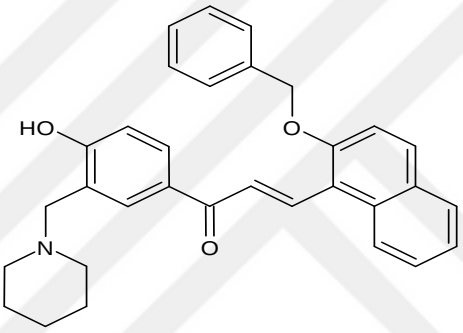


Compound **42**

2.3.2.3.2. Anti-Alzheimer Activity

The design of a potent medication to treat Alzheimer's disease was based on the synthesis of an acetylcholinesterase (AChE) inhibitor with multifunctional features. In order to reach this goal, 1-4-hydroxy-3-[(piperidin-1-yl)methyl]phenylethan-1-one (chalcone) the acetylcholinesterase inhibitor was synthesized and studied. Utilizing the *Claisen-Schmidt* condensation reaction to produce this chalcone [79].

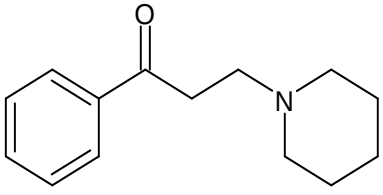
In vitro bioactivity tests of compound **43** showed excellent inhibitory activity against AChE (IC_{50} 1.0 nM), showing 33-fold stronger inhibition than donepezil, biometal chelating activity, and moderate antioxidant activity; therefore, compound **43** can be a good candidate for the development of Alzheimer's disease treatments [82].



Compound **43**

2.3.2.3.3. Antimicrobial Activity

1-Piperidinomethyl-3-phenylethanone (compound **44**) has been synthesized and tested as an anti-bacterial and anti-fungal. The compound showed significant antibacterial activity against gram positive bacteria *Staphylococcus aureus (S. aureus), Bacillus subtilis (B. subtilis),* gram-negative bacteria *Escherichia coli (E.c), Pseudomonas aeruginosa (PA)* and antifungal activity against *Aspergillus niger* [69].



Compound **44**

2.4. Microwave

Green chemistry highlights the synthesis of molecules using waste-free chemical reagents that are friendly to the environment in order to improve environmental performance and decrease the production of hazardous compounds. In the pharmaceutical field, green chemistry is particularly effective in producing many drugs [2, 65, 83, 84].

In the field of green chemistry, microwave irradiation is now a very powerful tool used in the field of drug discovery and it is found between infrared and radio waves in the electromagnetic spectrum. Microwave irradiation offers an alternative to conventional methods for heating or bringing energy into the system. It utilizes the ability of mobile electric charges present in liquid or conducting ions in solid to transform electromagnetic energy into heat. Microwave-assisted reactions are fast, clean, economical, eco-friendly, and microwave-assisted reactions have been called the "technology of future" [83, 85, 86].

In fact, microwaves can be used to perform new reactions and create conditions that are impossible with conventional heating. In comparison to conventional heating, microwave energy's absorption and transmission are completely different. In conventional heating, the reactants are slowly activated by a conventional external heat source. Heat is driven into the substance, passing first through the walls of the vessel in order to reach the solvent and the reactants. Therefore, conventional heating is a superficial heating procedure that transfers energy by conduction and convection from the surface to the bulk. Since the vessel must be overheated to reach the appropriate temperature, this is a slow and inefficient method for transferring energy into the reaction.

On the other hand, in microwave heating, microwaves interact directly with the molecules of the entire reaction mixture, causing the temperature to rise quickly. Any substance that will respond to either dipole rotation or ionic conductivity will instantly experience localized superheating because the process is not constrained by the thermal conductivity of the vessel. Only the reaction vessel contents are heated, not the vessel itself. In this way, better homogeneity and selective heating of molecules might be achieved [84, 86, 87].

In chemical reactions, microwave radiation has proved to be a very efficient source of heating. Microwaves can improve yields, increase the rate of reaction due to microwave heating, speed up some chemical reactions by ten to one thousand times

compared to conventional heating, achieve a uniform and selective heating, increase reaction repeatability, and aid in the creation of cleaner synthetic pathways [85].

In the literature, the microwave radiation technique is used for the synthesis of many *Mannich* bases. This technique is much greener than the conventional synthesis using less amount and readily recyclable solvents or without solvents and the products become much cleaner in reduced time, and high yield when compared to the conventional method, which has a long reaction time, harsh reaction conditions, toxic vapor, by-products and low yield [83, 88].

3. MATERIALS AND METHODS

3.1. Chemistry

3.1.1. Material

4-hydroxyacetophenone, 4-methylpiperidine, 4-(3-phenylpropyl) piperidine, 4-phenylpiperidine, 4-benzylpiperidine, 4-piperidinemethanol, 3,5-dimethylpiperidine, 3-piperidinemethanol, 4-hydroxy-4-phenylpiperidine, 4-hydroxypiperdine, 4-morpholinepiperidine, 4-cyano-4-phenylpiperidine, 4-piperidinopiperidine, 4-piperidineethanol, dichloromethane, methanol, ethanol, chloroform, 1,4-dioxane and formaldehyde were obtained from Sigma-Aldrich

3.1.2. Methods of Synthesis

3.1.2.1. General Procedure A: Conventional synthesis of Compound 1-13

3.1.2.1.1. General Procedure A1 of Compound 1-9

0.0022 mol (0.306 g) of 4-hydroxyacetophenone and 0.0033 mol piperidine derivative in presence of 0.0033 mol (0.247 ml) formaldehyde were put in a round bottom flask and 10 ml ethanol was added under refluxed for seven hours.

3.1.2.1.2. General Procedure A2 of Compound 10-12

0.0011 mol (0.153 g) of 4-hydroxyacetophenone and 0.0022 mol piperidine derivative in presence of 0.0022 mol (0.164 ml) formaldehyde were put in a round bottom flask and 10 ml ethanol was added under refluxed for seven hours.

3.1.2.1.3. General Procedure A3 of Compound 13

0.0007 mol (0.102 g) of 4-hydroxyacetophenone and 0.0011 mol piperidine derivative in presence of 0.0011 mol (0.082 ml) formaldehyde were put in a round bottom flask and 10 ml ethanol was added under refluxed for seven hours.

The reaction is monitored by TLC with a silica gel plate and various mobile phase mixtures. Under reduced pressure, the reaction mixture was concentrated. The column chromatography was applied to obtain pure target compounds using various solvent

mixtures, including dichloromethane: methanol (10:90), chloroform: methanol (95:5), dichloromethane: methanol (98:2) solution or crystallization was done in ethanol [10, 70].

3.1.2.2. General Procedure B: Microwave-assisted synthesis of Compound 1-13

3.1.2.2.1. General Procedure B1 of Compound 1-9

To a solution of 1, 4-dioxane (10 ml) added 0.0022 mol (0.306 g) of 4-hydroxyacetophenone, 0.0033 mol piperidine derivative and 0.0033 mol (0.247 ml) formaldehyde were added to the MW sealed vessel while being stirred. The reaction contents was exposed to radiation for 15 minutes at 120 °C (power 300 W).

3.1.2.2.2. General Procedure B2 of Compound 10-12

To a solution of 1, 4-dioxane (10 ml) added 0.0011 mol (0.153 g) of 4-hydroxyacetophenone, 0.0022 mol piperidine derivative and 0.0022 mol (0.164 ml) formaldehyde were added to the MW sealed vessel while being stirred. The reaction contents was exposed to radiation for 15 minutes at 120 °C (power 300 W).

3.1.2.2.3. General Procedure B3 of Compound 13

To a solution of 1, 4-dioxane (10 ml) added 0.0007 mol (0.102 g) of 4-hydroxyacetophenone and 0.0011 mol piperidine derivative and 0.0011 mol (0.082 ml) formaldehyde were added to the MW sealed vessel while being stirred. The reaction contents was exposed to radiation for 15 minutes at 120 °C (power 300 W).

Under reduced pressure, the reaction mixture was concentrated. The column chromatography was applied to obtain pure target compounds using various solvent mixtures, include dichloromethane: methanol (10:90), chloroform: methanol (95:5), dichloromethane: methanol (98:2) solution or crystallization was done in ethanol [52].

3.1.3. Analytical Methods

3.1.3.1. Melting Point Determination

Melting points (^{o}C) determination of compounds were done by using a Mettler Toledo FP62 capillary melting point apparatus and were uncorrected.

3.1.3.2. Microwave-assisted synthesis

Microsynth microwave labstatition was used for the synthesis of the compounds.

3.1.3.3. Controls by Thin Layer Chromatography

TLC aluminum sheets 20x20 cm Silica gel 60 F254 (Merck) were used as stationary phase, and preparation of three different solvent systems was done to be applied in chromatographic controls of compounds.

S1: Dichloromethane: Methanol (10:90)

S2: Chloroform: Methanol (95:5)

S3: Dichloromethane: Methanol (98:2)

For TLC method, solvent systems were poured to chamber and kept 1 hour for adequate and homogeneous saturation. Synthesized compounds and their starting materials were dissolved in suitable solvents and solutions were applied by Pasteur pipettes on the silica gel plates. The compounds on plates were dragged for 10 cm at room temperature, before their Rf values were calculated. After drying the plates, determination of spots was performed under UV light (254/365 nm).

3.1.3.4. Column Chromatography.

All Compounds except compounds **3** and **8** were purified by column chromatography using silica gel (60 mesh) as stationary phase and dichloromethane: methanol (10:90), chloroform: methanol (95:5) or dichloromethane: methanol (98:2) solutions as mobile phases. Column was filled according to wet method. Elution was controlled with TLC using silica gel plates.

3.1.4. Spectral Analysis

3.1.4.1. Infrared Spectra

Infrared spectra were collected on a Perkin-Elmer Spectrum One series FT-IR apparatus (Version 5.0.1), by applying potassium bromide (KBr) as a background, and the frequencies were shown in cm^{-1}.

3.1.4.2. ^{1}H-NMR Spectra

^{1}H-NMR Spectra were collected with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA USA) by applying of tetramethyl silane (TMS) as the internal reference, with deuterated dimethyl sulfoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$) as a solvent, and the chemical shifts were reported in parts per million (ppm).

3.1.4.3. ^{13}C-NMR Spectra

^{13}C-NMR spectra were collected with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA, USA) by applying tetramethyl silane (TMS) as the internal reference, with deuterated dimethyl sulfoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$) as a solvent, and the chemical shifts were reported in parts per million (ppm).

3.1.4.4. Elemental analysis

Elemental Analysis were performed on LECO 392 CHNS (Leco-932, St. Joseph, MI, USA) instrument.

3.2. Biological Studies

3.2.1. Cytotoxic Activity (MTT Assay)

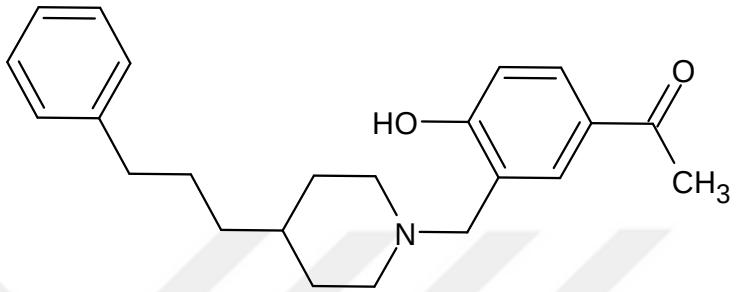
MCF-7 breast cancer cell line was used for testing compounds cytotoxicity. For this aim, 1 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), a yellow, water-soluble compound, was applied to the cells. When MTT is applied to living cells, it transforms into a blue-violet, water-insoluble 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 being treated 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 the addition of 150 µL

isopropanol. The percentage of viable cells was calculated based on the values acquired by colorimetric methods using an ascent spectrophotometer at 570 nm.

4. RESULTS

4.1. Chemical Data

1-[4-Hydroxy-3-[(4-(3-phenylpropyl)piperidin-1-yl)methyl]phenyl]ethanone (Compound 1)



4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 4-(3-phenylpropyl) piperidine (0.0033 moles, 0.714 ml), and formaldehyde (0.0033 moles, 0.247 ml) in 10 ml of ethanol were reacted as explained in procedure **A1**. Column chromatography was applied using **S2** solvent mixtures. The compound is soluble in chloroform, methanol, dichloromethane, and ethanol. The form of the compound is white-colored solid.

4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 4-(3-phenylpropyl) piperidine (0.0033 moles, 0.714 ml), and formaldehyde (0.0033 moles, 0.247 ml) in 10 ml of 1, 4-dioxane were reacted as explained in procedure **B1**. Column chromatography was applied using **S2** solvent mixtures. The form of the compound is white-colored solid.

Rf values: 0.81 (S2)

M.p.: 64.4 °C.

Yields: 37.4 % (procedure A1), 79 % (procedure B1)

FT-IR (KBr, vmax, cm^{-1}): 3600-3300 (O-H), 3025 (Ar C-H), 2932 (Aliphatic C-H), 1660 (C=O), 1600 (C=C).

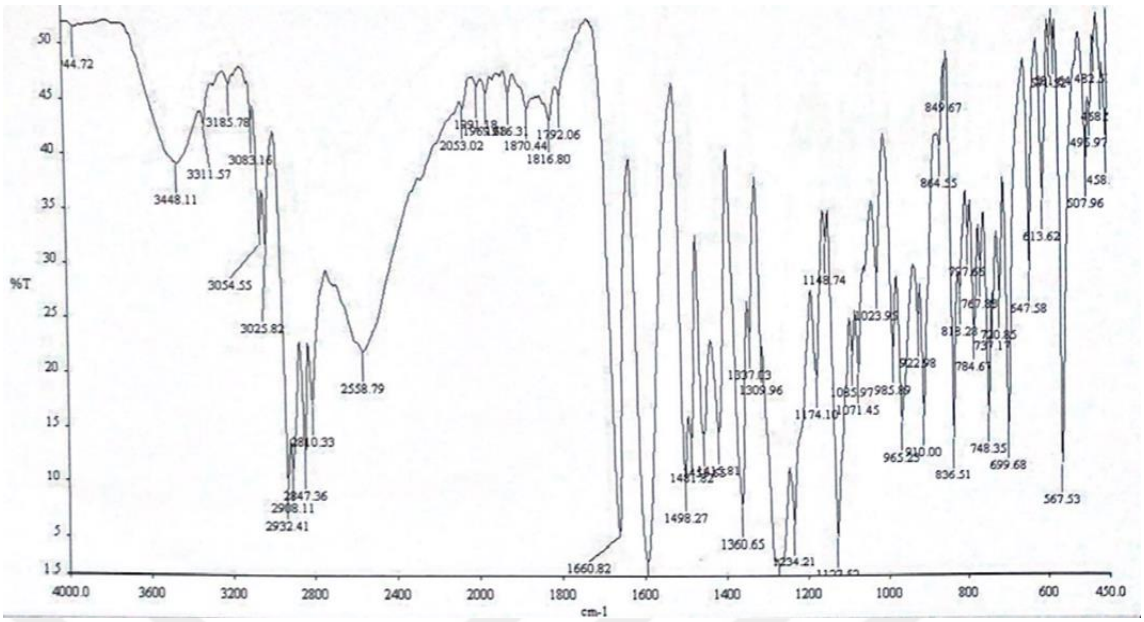


Figure 4.1. IR spectrum of compound **1**

^{1}H-NMR: 7.80-7.77 (dd, 1H, Ar, J_1=2 Hz, J_2=2 Hz), 7.65 (s, 1H, Ar), 7.27 (t, 2H, Ar, J=7.6 Hz), 7.17 (t, 3H, Ar, J=8.8 Hz), 6.81 (d, 1H, Ar, J=8 Hz), 3.74 (s, 2H, CH_2), 2.97 (d, 2H-piperidine, J=11.6 Hz), 2.58 (t, 2H, CH_2- Ar, J=7.6 Hz), 2.521 (s, 3H, CH_3), 2.11 (s, 2H-piperidine), 1.73 (d, 2H-piperidine, J=11.2 Hz), 1.66-1.58 (m, 2H, piperidine), 1.30-1.22 (m, 5H, CH_2 -CH_2, 1H-piperidine).

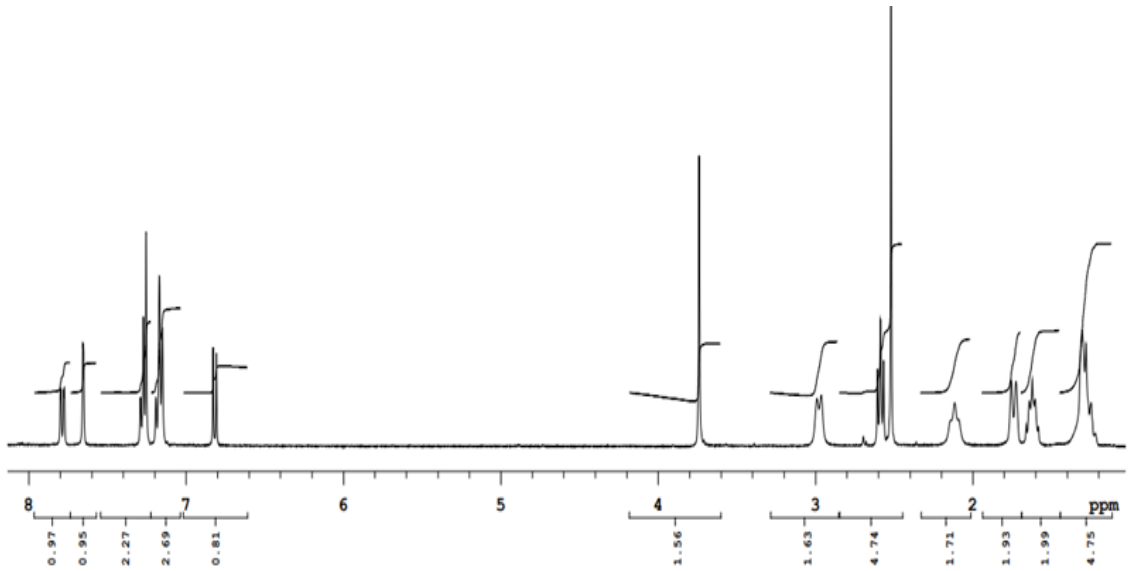


Figure 4.2. ^{1}H-NMR spectrum of compound **1**

^{13}C-NMR: 196.84 (**C**=O), aromatic carbons were seen at 163.34, 142.47, 130.16, 129.07, 128.72, 128.34, 128.28, 125.71, 121.36, 115.86, 61.42 (N-**C**H_2), 53.27(Ar-**C**H_2), 36.06 (Ar-CH_2-**C**H_2). Piperidine carbons found at 35.84, 35.30, 32.09, 28.62 (O=C-**C**H_3), 26.2 (C-**C**H_2).

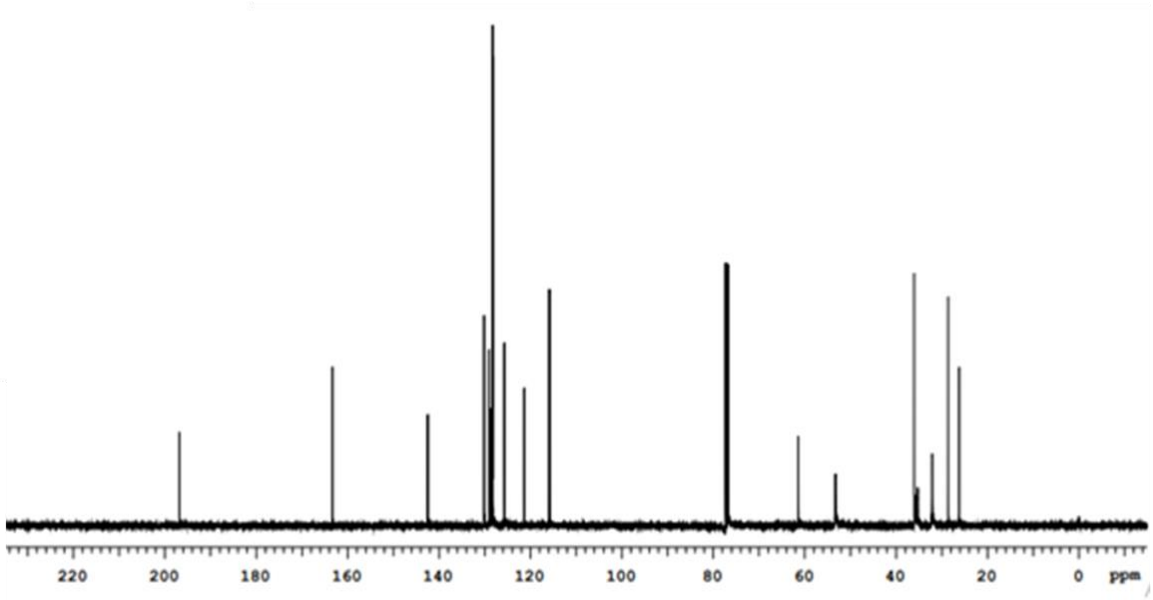


Figure 4.3. ^{13}C-NMR spectrum of compound **1**

Elemental analysis:

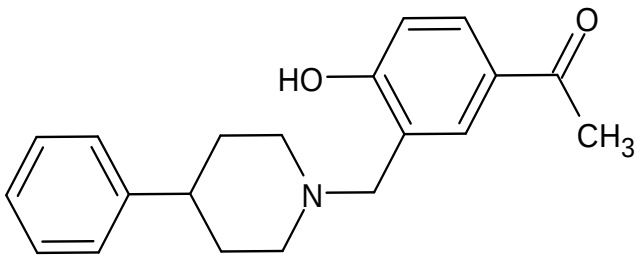
Molecular Formula: $C_{23}H_{29}NO_2$

Molecular weight: 351.48 mole

Calculated %: C 78.59, H 8.32, N 3.99.

Found %: C 78.51, H 8.49, N 4.09.

1-[4-Hydroxy-3-[(4-phenylpiperidin-1-yl)methyl]phenyl]ethanone (Compound 2)



4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 4-phenyl piperidine (0.0033 moles 0.54917 g) and formaldehyde (0.0033 moles, 0.247ml) in 10 ml of ethanol were reacted as explained in procedure **A1**. The pure target compound was crystallized by ethanol. The compound is soluble in chloroform, hot ethanol and methanol. The form of the compound is white-colored crystals.

4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 4-phenyl piperidine (0.0033 moles 0.54917 g) and formaldehyde (0.0033 moles, 0.247ml) in 10 ml of 1, 4-dioxane were reacted as explained in procedure **B1**. The pure target compound was crystallized by ethanol. The form of the compound is white-colored crystals.

Rf values: 0.7 (S2).

M.p.: 136.5 °C

Yields: 60.3 % (procedure A1), 95% (procedure B1).

FT-IR (KBr, vmax, cm-1): 3572-3300 (O-H), 3029 (Ar C-H), 2943 (Aliphatic C-H), 1663 (C=O), 1587 (C=C).

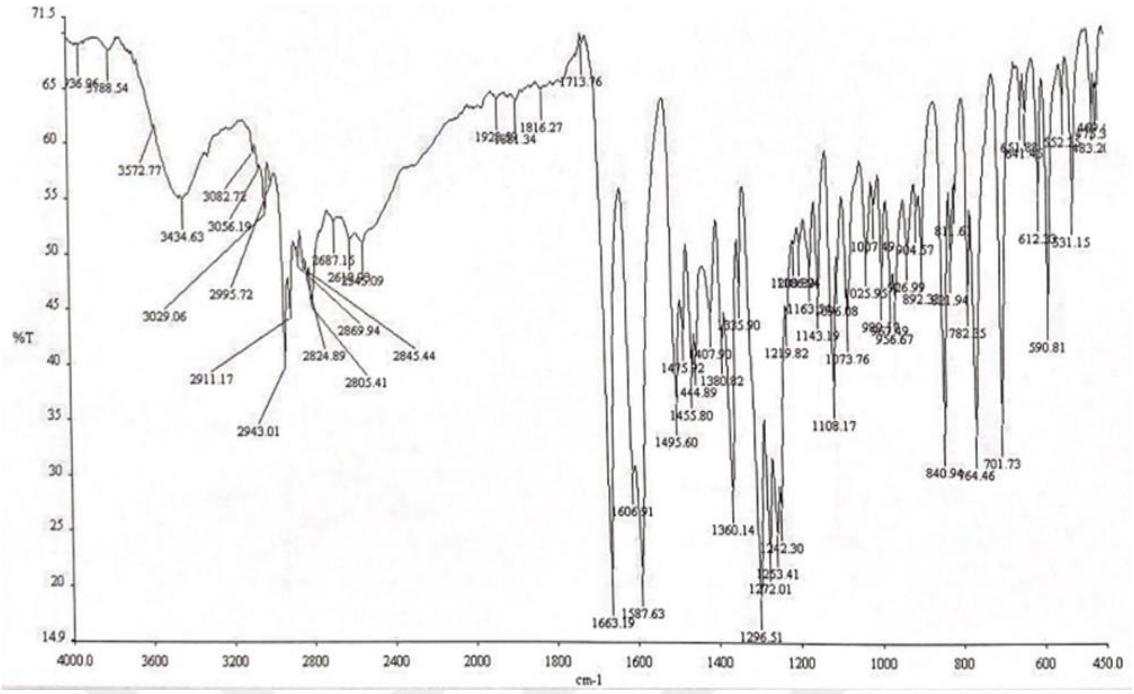


Figure 4.4. IR spectrum of compound **2**

^{1}H-NMR: 7.82-7.79 (dd, 1H, Ar, J_1=2.4 Hz, J_2=2.4 Hz), 7.69 (d, 1H, Ar, J=2.4 Hz), 7.33-7.19 (m, 5H, Ar), 6.84 (d, 1H, Ar, J=8.4 Hz), 3.80 (s, 2H, $CH_{2)}$, 3.12 (d, 2H-piperidine, J=11.6 Hz), 2.60-2.56 (m, 1H-piperidine), 2.53 (s, 3H, CH_3), 2.30-2.24 (m, 2H-piperidine), 1.94-1.80 (m, 4H-piperidine).

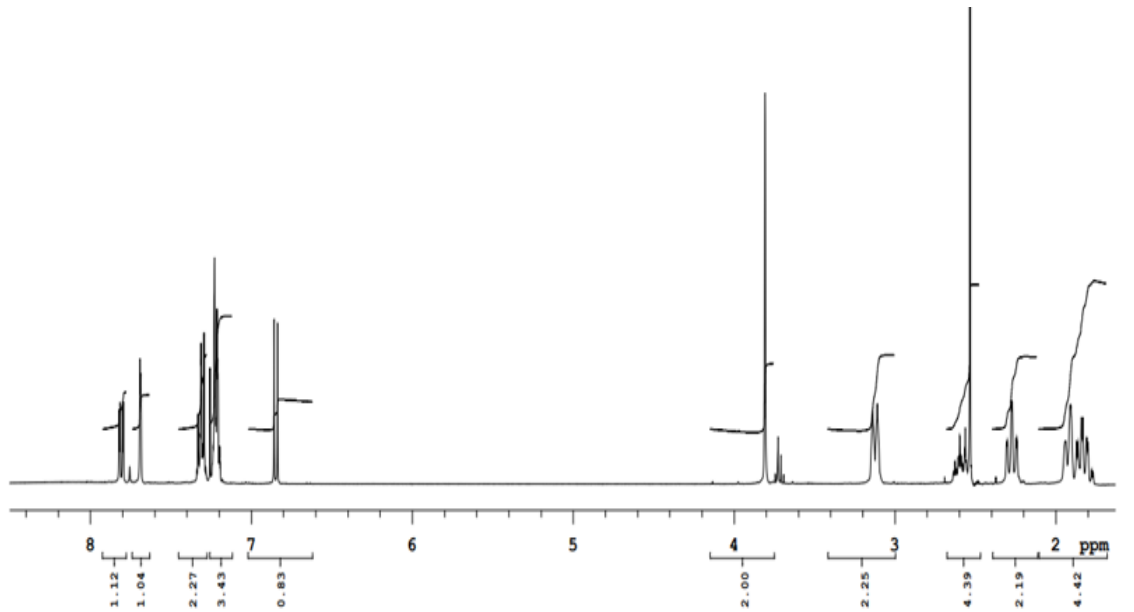


Figure 4.5. ^{1}H-NMR spectrum of compound **2**

Elemental analysis:

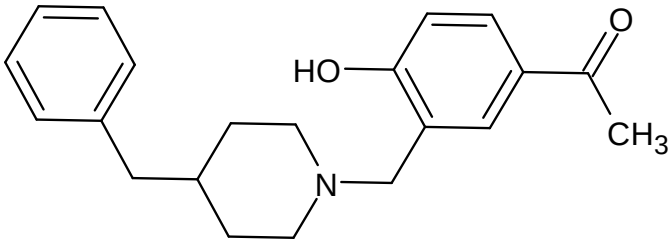
Molecular Formula: $C_{20}H_{23}NO_2$

Molecular weight: 309.40 mole

Calculated %: C 77.64, H 7.49, N 4.53.

Found %: C 77.51, H 7.58, N 4.68.

1-[4-Hydroxy-3-[(4-benzylpiperidin-1-yl)methyl]phenyl]ethanone (Compound 3)



4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 4-benzyl piperidine (0.0033 moles, 0.5968 ml) and formaldehyde (0.0033 moles, 0.247 ml) in 10 ml of ethanol were reacted as explained in procedure **A1**. Column chromatography was applied using **S2** solvent mixtures. The compound is soluble in methanol, chloroform, hot ethanol and dichloromethane. The form of the compound is white-colored solid.

4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 4-benzyl piperidine (0.0033 moles, 0.5968 ml) and formaldehyde (0.0033 moles, 0.247 ml) in 10 ml of 1,4-dioxane were reacted as explained in procedure **B1**. Column chromatography was applied using **S2** solvent mixtures. The form of the compound is white-colored solid.

Rf values: 0.7 (S2).

M.p.: 92 °C

Yields: 38.27 % (procedure A1), 88 %. (procedure B1).

FT-IR (KBr, vmax, cm-1): 3600-3330 (O-H), 3028 (Ar C-H), 2913 (Aliphatic C-H), 1672 (C=O), 1587 (C=C).

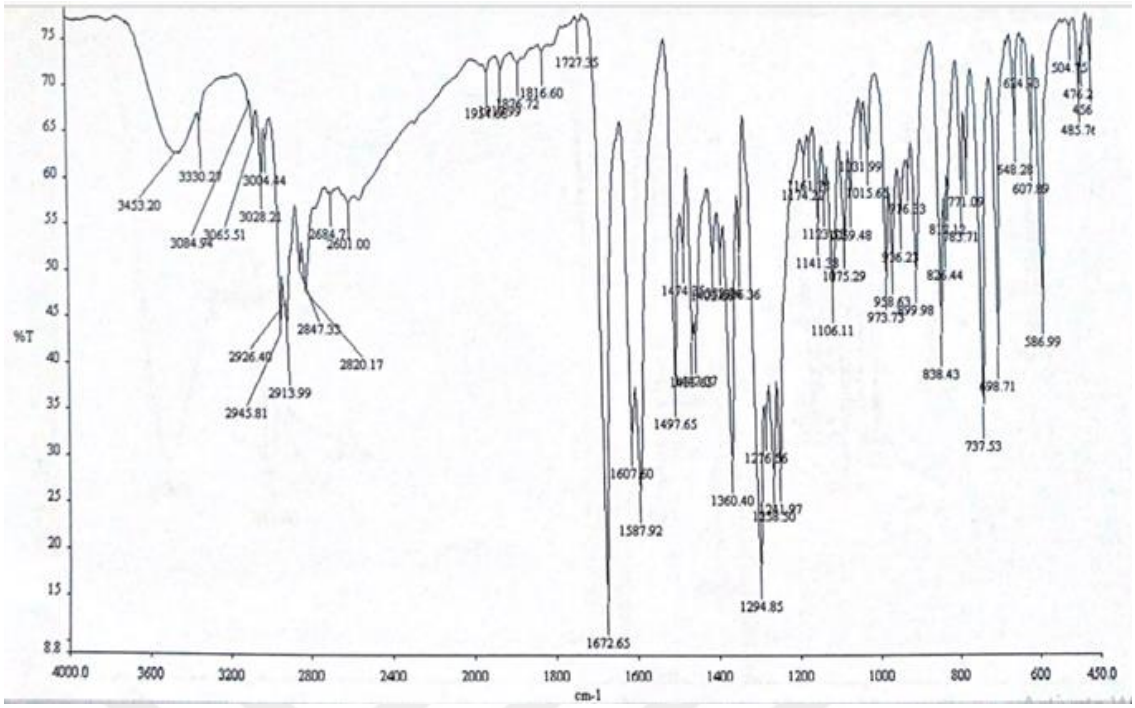


Figure 4.6. IR spectrum of compound **3**

^{1}H-NMR: 7.80-7.77 (dd, 1H, Ar, J_1=2.4 Hz, J_2=2.4 Hz), 7.63 (d, 1H, Ar, J=2 Hz), 7.29-7.12 (m, 5H, Ar), 6.81 (d, 1H, Ar, J=8.4 Hz), 3.72 (s, 2H, N-CH_2), 2.96 (d, 2H-Piperidine, J=11.6 Hz), 2.54 (d, 2H, C-CH_2, J=7.2 Hz), 2.51 (s, 3H, CH_3), 2.07 (t, 2H-Piperidine, J=11.6 Hz), 1.72- 1.59 (m, 3H- Piperidine), 1.38-1.32 (m, 2H-Piperidine).

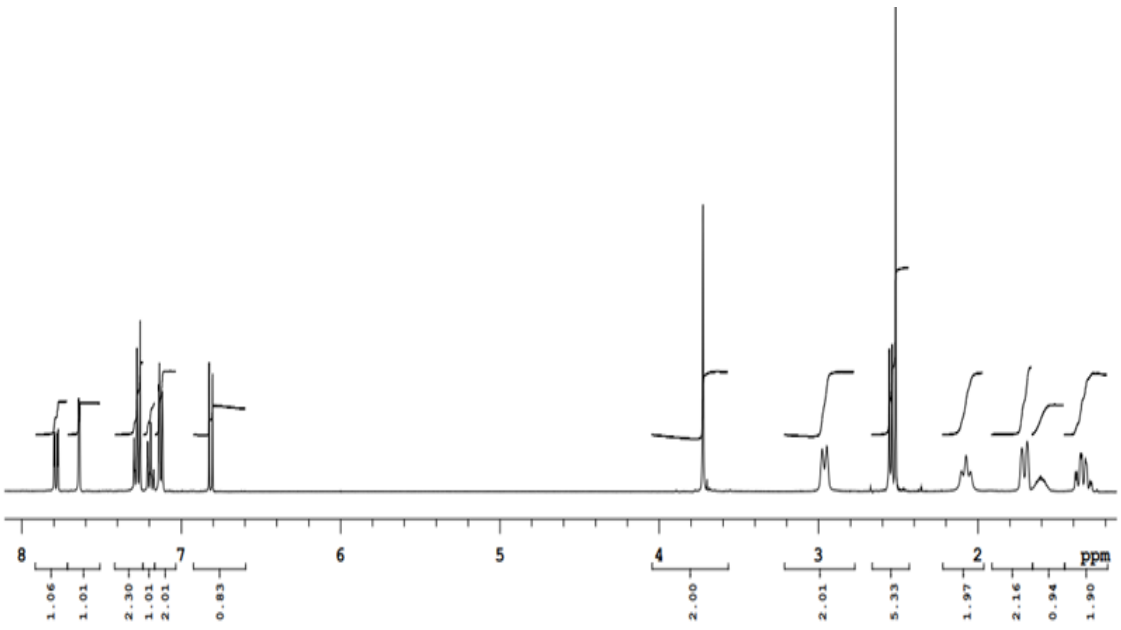


Figure 4.7. ^{1}H-NMR spectrum of compound **3**

^{13}C-NMR: 196.84 (**C**=O), aromatic carbons were seen at 163.28, 140.13, 130.17, 129.08, 129.08, 129.05, 128.76, 128.33, 128.28, 126.00, 121.30, 115.86, 61.36 (N-**C**H_2), 53.16 (Ar-**C**H_2). Piperidine carbons found at 42.88, 37.51, 31.95, 26.20 (O=C-**C**H_3).

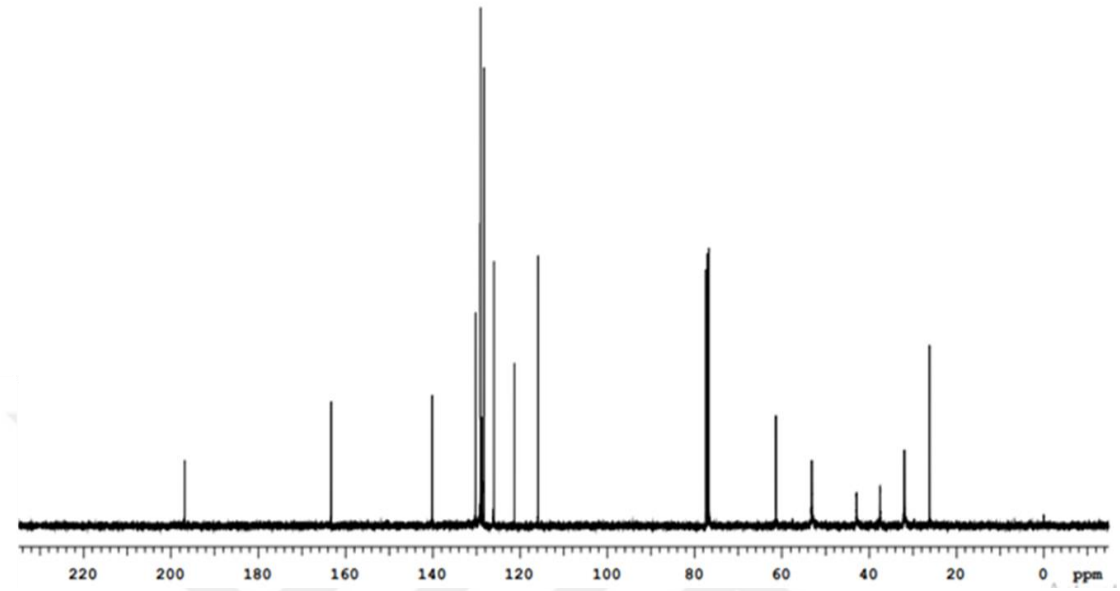


Figure 4.8. ^{13}C-NMR spectrum of compound **3**

Elemental analysis:

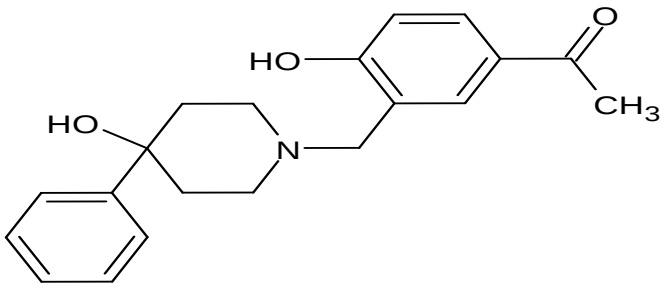
Molecular Formula: $C_{21}H_{25}NO_2$

Molecular weight: 323.42 mole

Calculated %: C 77.98, H 7.79, N 4.33.

Found %: C 77.98, H 7.80, N 4.44.

1-[4-Hydroxy-3-[(4-hydroxy-4-phenylpiperidin-1-yl)methyl]phenyl]ethanone (Compound 4)



4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 4-hydroxy-4-phenylpiperidine (0.0033 moles, 0.5915 g) and formaldehyde (0.0033 moles, 0.247 ml) in 10 ml of ethanol were reacted as explained in procedure **A1**. The pure target compound was crystallized by ethanol. The compound is soluble in methanol, chloroform, dichloromethane and hot ethanol. The form of the compound is white-colored crystals.

4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 4-hydroxy-4-phenylpiperidine (0.0033 moles, 0.5915 g) and formaldehyde (0.0033 moles, 0.247 ml) in 10 ml of 1,4-dioxane were reacted as explained in procedure **B1**. The pure target compound was crystallized by ethanol. The form of the compound is white-colored crystals.

Rf values: 0.7 (**S1**).

M.p.: 166.7 °C

Yields: 18 % (procedure A1), 70 % (procedure B1).

FT-IR (KBr, vmax, cm^{-1}): 3600-3300 (O-H), 3002 (Ar C-H), 2964 (Aliphatic C-H), 1666 (C=O), 1598 (C=C).

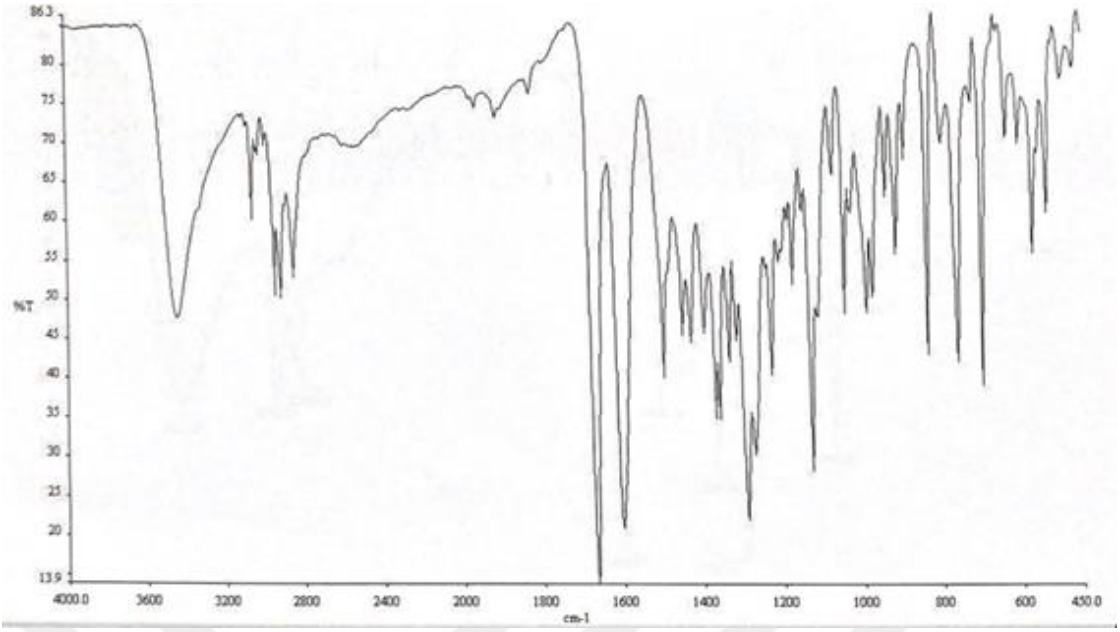


Figure 4.9. IR spectrum of compound **4**

^{1}H-NMR: 7.78-7.76 (dd, 2H, Ar, J_1=2 Hz, J_2=2.4 Hz), 7.51-7.48 (dd, 2H, Ar, J_1=1.2 Hz, J_2=0.8 Hz), 7.32 (m, 2H, Ar), 7.23-7.19 (m, 1H, Ar), 6.82-6.79 (m, 1H, Ar), 3.79 (s, 2H, CH_2), 2.73 (d, 2H-Piperidine, J=11.2 Hz), 2.6 (t, 2H-Piperidine, J=10 Hz), 2.47 (s, 3H, CH_3), 1.99-1.92(m, 2H-Piperidine), 1.65 (d, 2H-Piperidine, J=12.4 Hz).

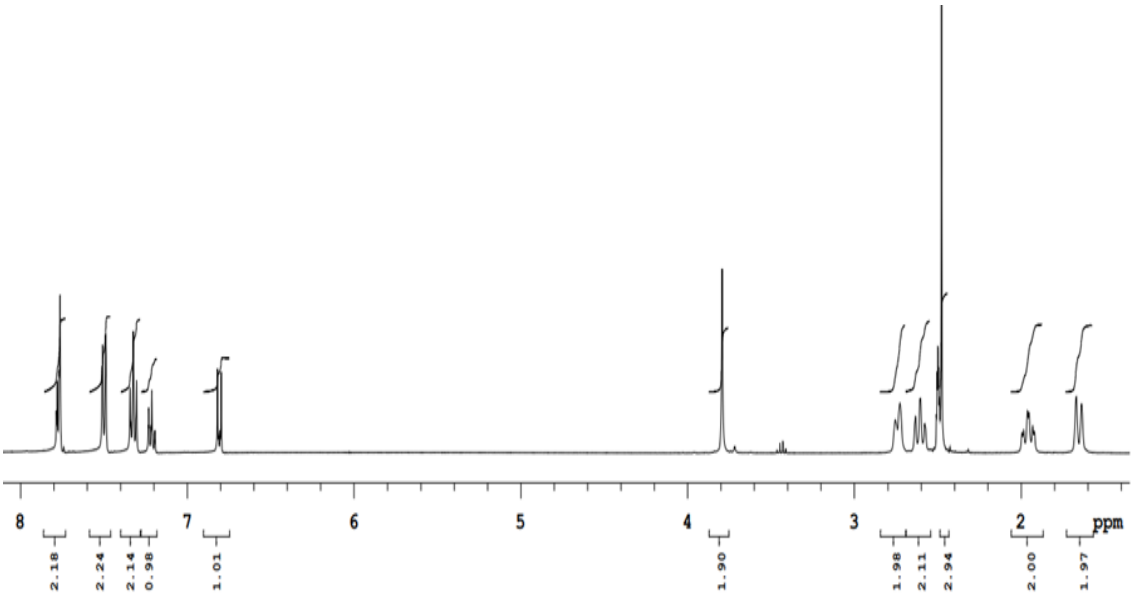


Figure 4.10. ^{1}H-NMR spectrum of compound **4**

^{13}C-NMR: 196.82 (**C**=O), aromatic carbons were seen at 163.19, 147, 130.27, 129.25, 128, 89, 128.51, 127.38, 124.42, 121.21, 115.91, 70.68 (Ar-**C**-OH), 61.25 (N-**C**H_2). Piperidine carbons found at 48.90, 38.14, 26.20 (O=C-**C**H_3).

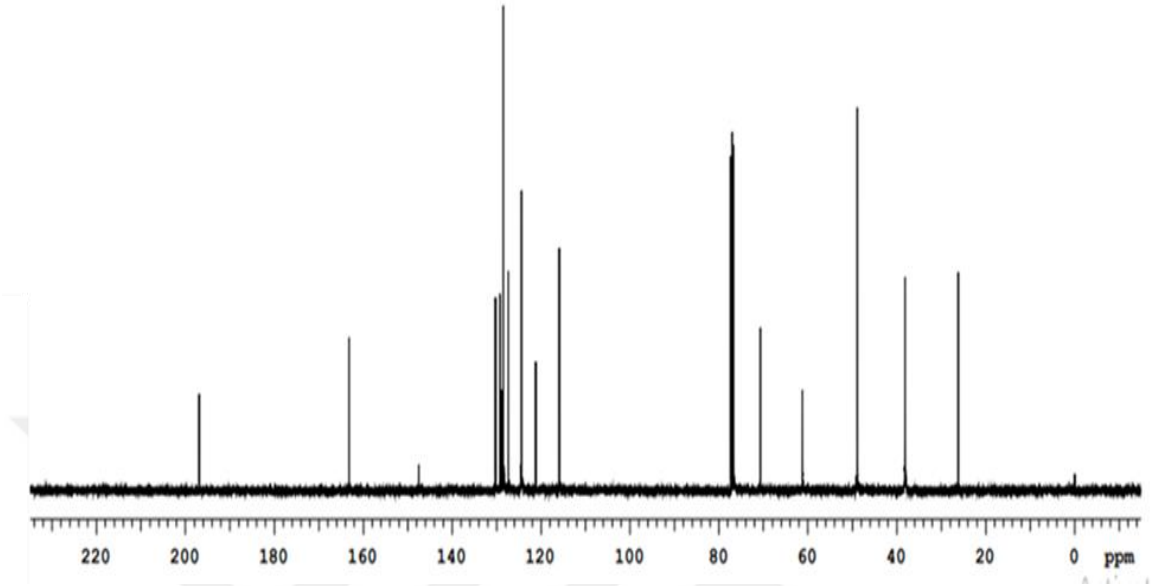
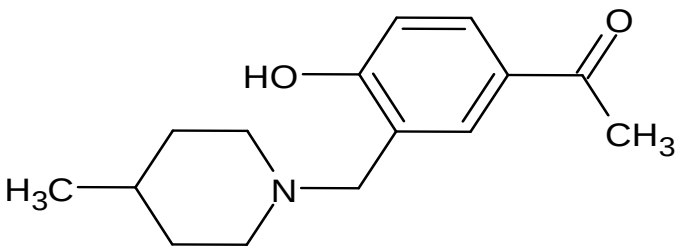


Figure 4.11. ^{13}C-NMR spectrum of compound **4**

1-[4-Hydroxy-3-[(4-methylpiperidin-1-yl)methyl]phenyl]ethanone (Compound 5 - CAS No: 1456243-65-4).



4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 4-methylpiperidine (0.0033 moles, 0.3988 ml) and formaldehyde (0.0033 moles, 0.247ml) in 10 ml of ethanol were reacted as explained in procedure **A1**. Column chromatography was applied using **S1** solvent mixtures. The compound is soluble in chloroform, dichloromethane, methanol and ethanol. The form of the compound is yellow-colored oily.

4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 4-methylpiperidine (0.0033 moles, 0.3988 ml) and formaldehyde (0.0033 moles, 0.247ml) in 10 ml of 1,4-dioxane were reacted as explained in procedure **B1**. Column chromatography was applied using **S1** solvent mixtures. The form of the compound is yellow-colored oily.

Rf value: 0.58 (S1).

Yields: 49.57 % (procedure A1), 90 % (procedure B1).

FT-IR (KBr, vmax, cm^{-1}): 3600-3430 (O-H), 2949 (Ar C-H), 2925 (Aliphatic C-H), 1673 (C=O), 1593 (C=C).

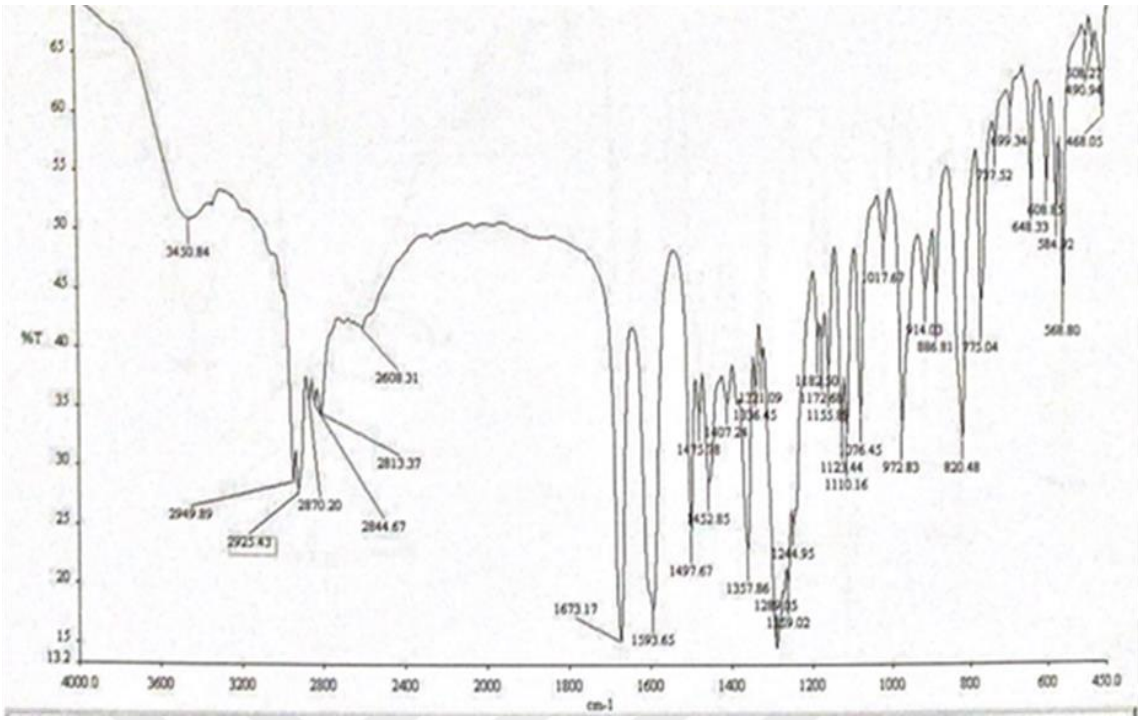


Figure 4.12. IR spectrum of compound **5**

^{1}H-NMR: 7.79 - 7.76 (dd, 1H, Ar, J_1=2.4 Hz, J_2=2 Hz), 7.65 – 7.64 (m, 1H, Ar), 6.80 (d, 1H, Ar, J=8 Hz), 3.73 (s, 2H, CH_2), 2.95 (d, 2H- piperidine, J=11.2 Hz), 2.51 (s, 3H, O=C-CH_3), 2.12 (t, 2H-piperidine, J=10.8 Hz), 1.69 (d, 2H-piperidine, J= 13.6 Hz), 1.32-1.24 (m, 3H- piperidine), 0.93 (d, 3H, CH_3, J=6.8 Hz).

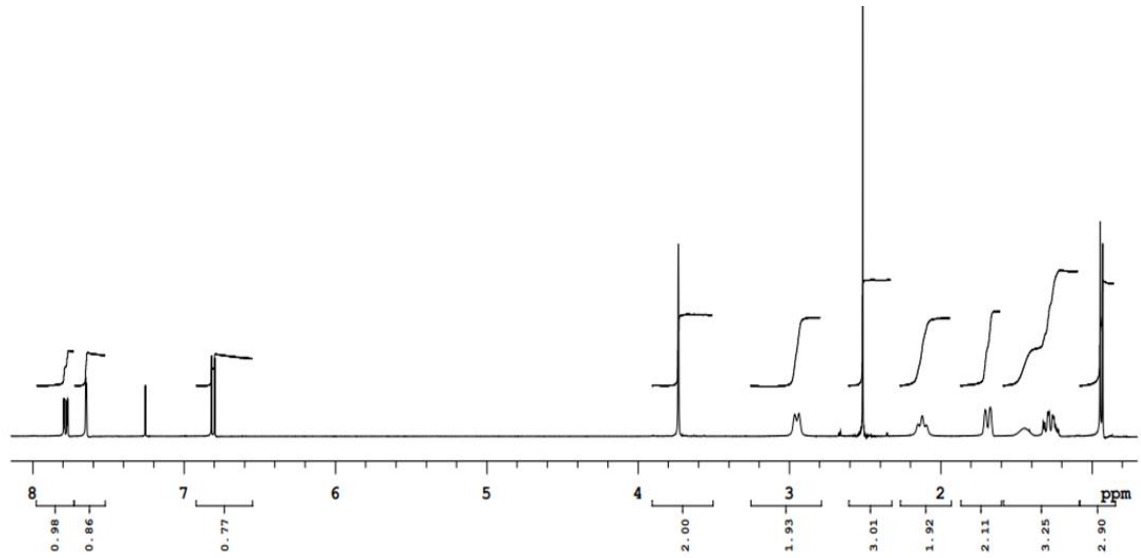


Figure 4.13. ^{1}H-NMR spectrum of compound **5**

^{13}C-NMR: 196.82 (**C**=O), aromatic carbons were seen at 163.37, 130.13, 129.05, 128.69, 121.37, 115.84, 61.40 (N-**C**H_2). Piperidine found at 53.23, 33.99, 30.38, 26.17 (O=C-**C**H_3), 21.57 (C-**C**H_3).

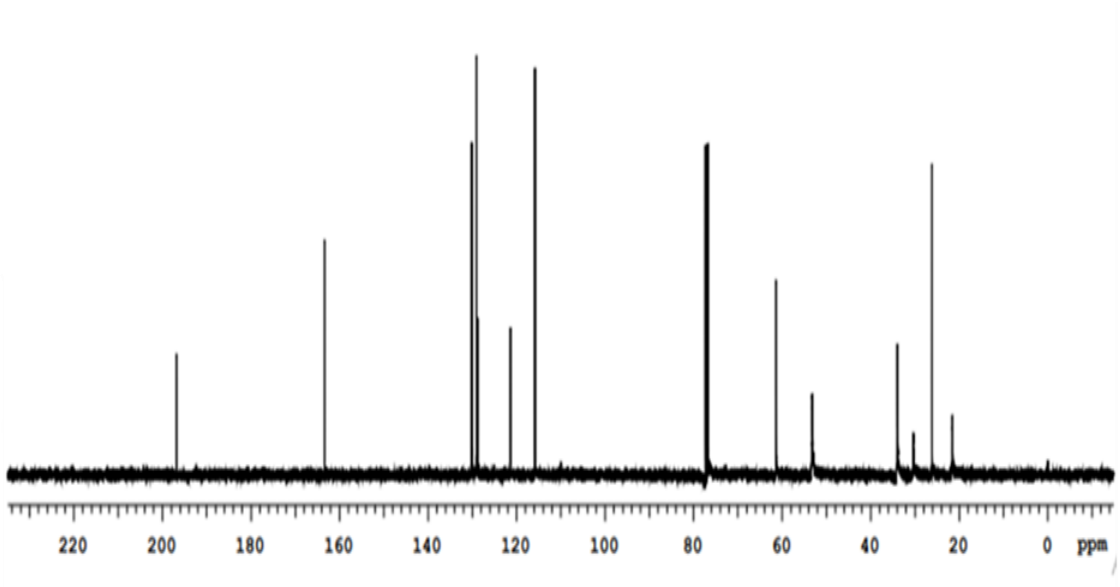


Figure 4.14. ^{13}C-NMR spectrum of compound **5**

Elemental analysis:

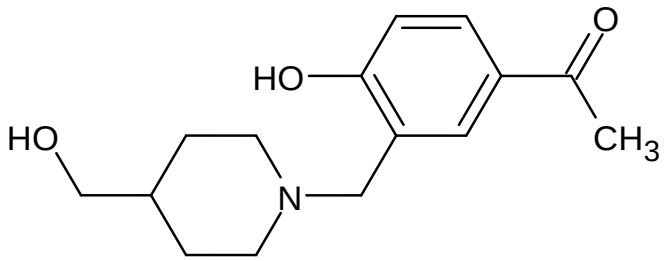
Molecular Formula: $C_{15}H_{21}NO_2$

Molecular weight: 247.33 mole

Calculated %: C 72.81, H 8.56, N 5.66.

Found %: C 72.43, H 8.72, N 5.68.

1-[4-Hydroxy-3-[[4-(hydroxymethyl)piperidin-1-yl]methyl]phenyl]ethanone (Compound 6 - CAS No: 1456243-65-4).



4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 4-piperidinemethanol (0.0033 moles, 0.38828 g) and formaldehyde (0.0033 moles, 0.247 ml) in 10 ml of ethanol were reacted as explained in procedure **A1**. Column chromatography was applied using **S1** solvent mixtures. The compound is soluble in chloroform, methanol, dichloromethane and ethanol. The form of the compound is yellow-colored solid.

4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 4-piperidinemethanol (0.0033 moles, 0.38828 g) and formaldehyde (0.0033 moles, 0.247 ml) in 10 ml of 1,4-dioxane were reacted as explained in procedure **B1**. Column chromatography was applied using **S1** solvent mixtures. The form of the compound is yellow-colored solid.

Rf values: 0.6 (S1).

M.p.: 93.9 °C

Yields: 61 % (procedure A1), 94% (procedure B1).

FT-IR (KBr, vmax, cm^{-1}): 3600-3100 (O-H), 3050 (Ar C-H), 2918 (Aliphatic C-H), 1670 (C=O), 1594 (C=C).

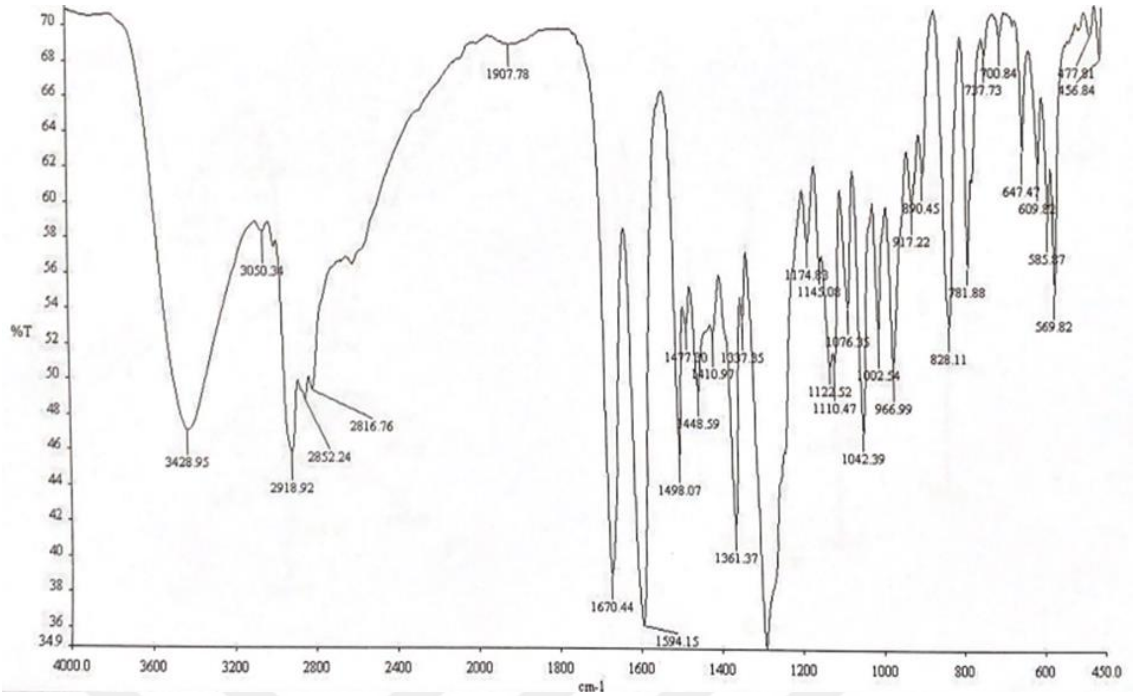


Figure 4.15. IR spectrum of compound **6**

^{1}H-NMR: 7.79-7.76 (dd, 1H, Ar, J_1=2.4 Hz, J_2=2 Hz), 7.64 (d, 1H, Ar, J=2 Hz), 6.8 (d, 1H, Ar, J=8.4 Hz), 3.74 (s, 2H, N-CH_2), 3.51-3.46 (t, 2H, CH_2-OH, J=6.8 Hz), 3.01 (d, 2H-Piperidine, J=12 Hz), 2.51 (s, 3H, CH_3), 2.13 (t, 2H-Piperidine, J=12 Hz), 1.80 (d, 2H-Piperidine, J=13.2 Hz), 1.33-1.29 (m, 3H-Piperidine).

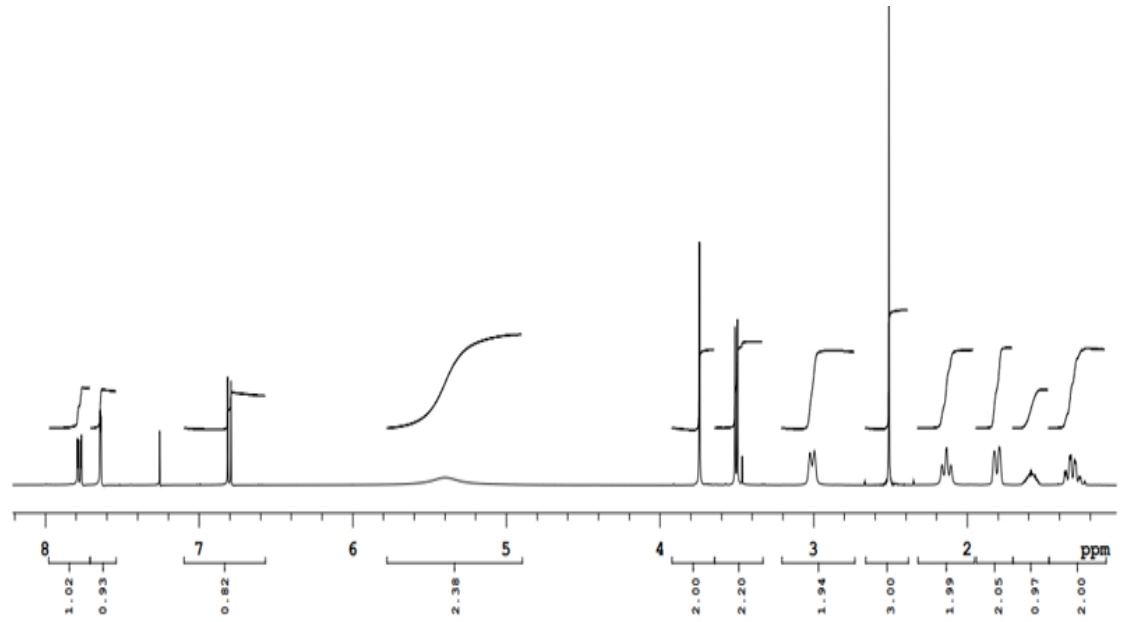
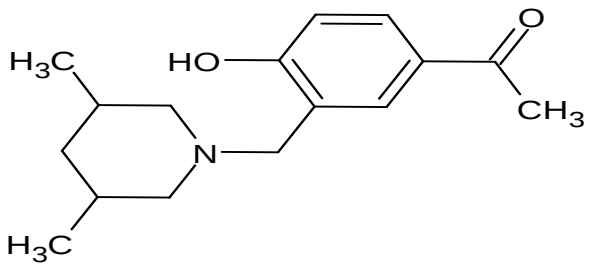


Figure 4.16. ^{1}H-NMR spectrum of compound **6**

1-[4-Hydroxy-3-[(3, 5-Dimethylpiperidin-1-yl)methyl]phenyl]ethanone (Compound 7- CAS No: 1157492-79-9).



4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 3,5-dimethylpiperidine (0.0033 moles, 0.4567 ml) and formaldehyde (0.0033 moles, 0.247 ml) in 10 ml of ethanol were reacted as explained in procedure **A1**. Column chromatography was applied using **S2** solvent mixtures. The compound is soluble in chloroform, methanol, dichloromethane and ethanol. The form of the compound is yellow-colored oily.

4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 3, 5-dimethylpiperidine (0.0033 moles, 0.4567 ml) and formaldehyde (0.0033 moles, 0.247 ml) in 10 ml of 1,4-dioxane were reacted as explained in procedure **B1**. Column chromatography was applied using **S2** solvent mixtures. The form of compound is yellow-colored oily.

Rf values: 0.77 (S2)

Yields: 45.82 % (procedure A1), 80 % (procedure B1).

FT-IR (KBr, vmax, cm^{-1}): 3600-3400 (O-H), 2953 (Ar C-H), 2808 (Aliphatic C-H), 1673 (C=O), 1593 (C=C).

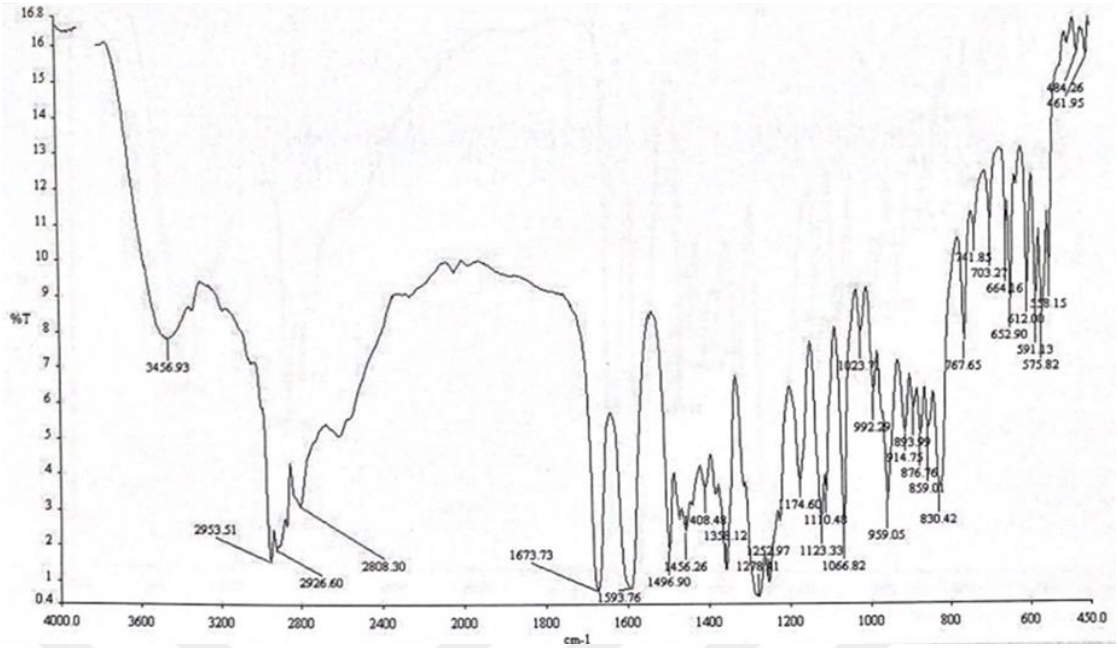


Figure 4.17. IR spectrum of compound **7**

^{1}H-NMR: 7.80-7.77 (dd, 1H, Ar, J_1=2 Hz, J_2=2 Hz), 7.65 (d, 1H, Ar, J=2.4 Hz), 6.83- 6.80 (dd, 1H, Ar, J_1=3.2 Hz, J_2=2.8 Hz), 3.73 (s, 2H, CH_2), 2.91-2.88 (m, 1H-Piperidine), 2.52 (s, 3H, CH_3), 2.00-1.61 (m, 5H-Piperidine), 0.861 (m, 7H, CH_3-CH), 0.62-0.59 (m, 1H-Piperidine).

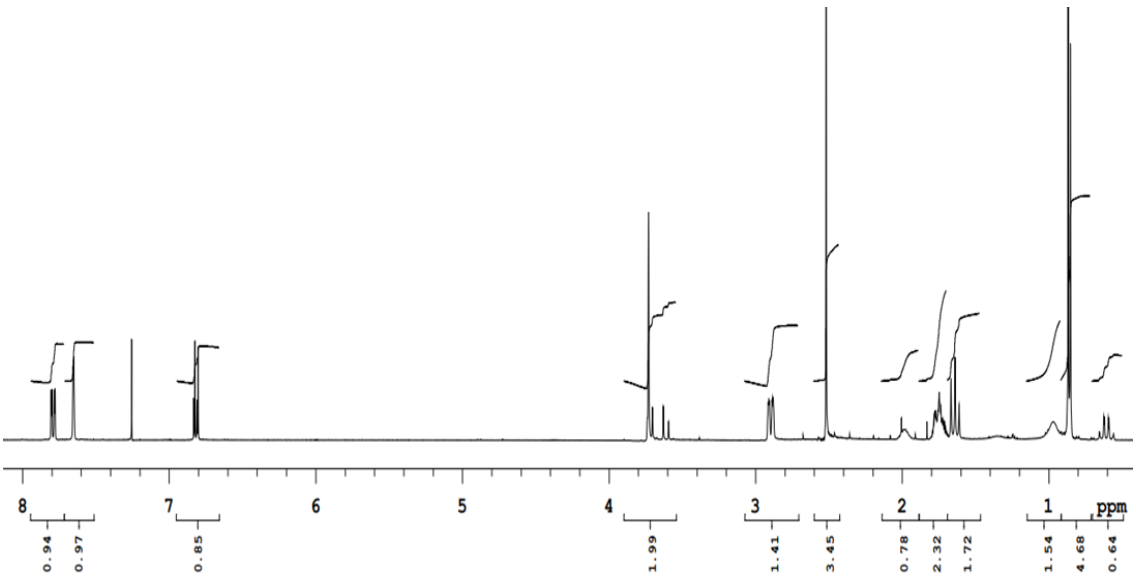
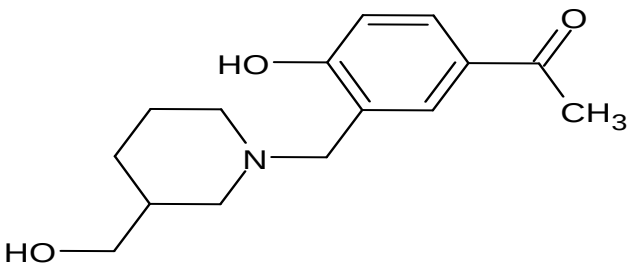


Figure 4.18. ^{1}H-NMR spectrum of compound **7**

1-[4-Hydroxy-3-[[3-(hydroxymethyl)piperidin-1-yl]methyl]phenyl]ethanone Compound 8 - CAS No: 1457824-85-9).



4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 3-piperidinemethanol (0.0033 moles, 0.395 g) and formaldehyde (0.0033 moles, 0.247 ml) in 10 ml of ethanol were reacted as explained in procedure **A1**. Column chromatography was applied using **S1** solvent mixtures. The compound is soluble in chloroform, methanol, dichloromethane and ethanol. The form of the compound is yellow-colored oily.

4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 3-piperidinemethanol (0.0033 moles, 0.395 g) and formaldehyde (0.0033 moles, 0.247 ml) in 10 ml of 1,4-dioxane were reacted as explained in procedure **B1**. Column chromatography was applied using **S1** solvent mixtures. The form of the compound is yellow-colored oily.

Rf values: 0.5 (S2)

Yields: 38 % (procedure A1), 88 % (procedure B1).

FT-IR (KBr, vmax, cm^{-1}): 3600-3200 (O-H), 2929 (Ar C-H), 2855 (Aliphatic C-H), 1668 (C=O), 1593 (C=C).

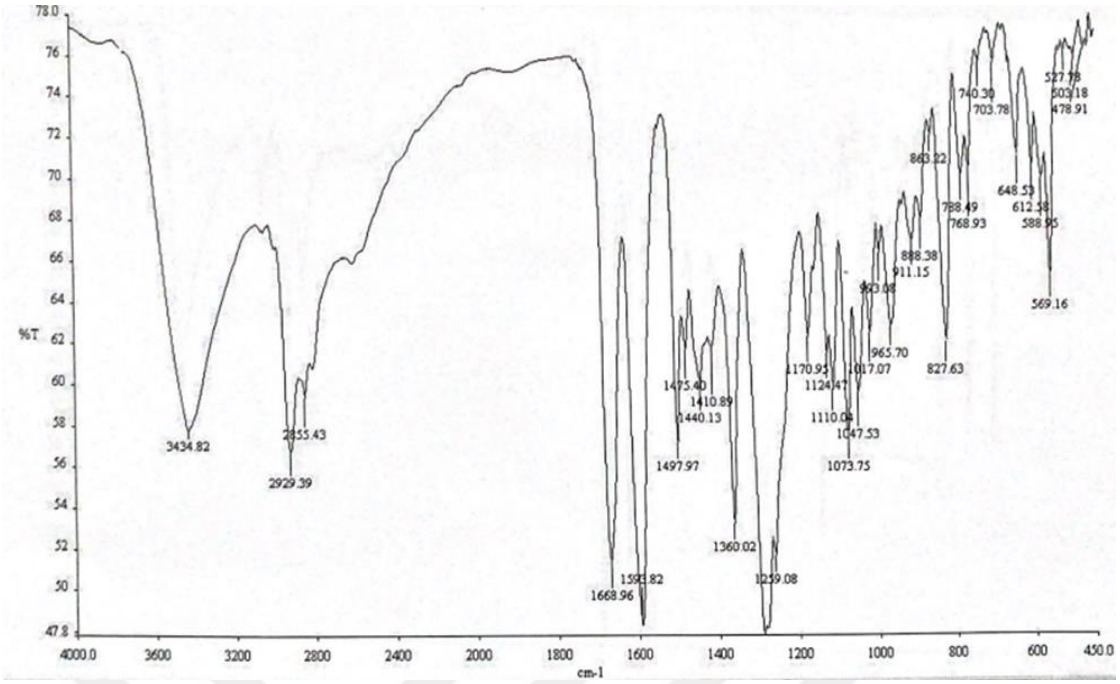


Figure 4.19. IR spectrum of compound **8**

^{1}H-NMR: 7.79-7.76 (dd, 1H, Ar, J_1=2.4 Hz, J_2=2 Hz), 7.64 (d, 1H, Ar, J=2 Hz), 6.8 (d, 1H, Ar, J=8.4 Hz), 3.74 (s, 2H, N-CH_2), 3.51-3.46 (t, 2H, CH_2-OH), 3.01 (d, 2H-Piperidine, J=12 Hz), 2.51 (s, 3H, CH_3), 2.13 (t, 2H-Piperidine, J=12 Hz), 1.80 (d, 2H-Piperidine, J=13.2 Hz), 1.33-1.29 (m, 3H-Piperidine).

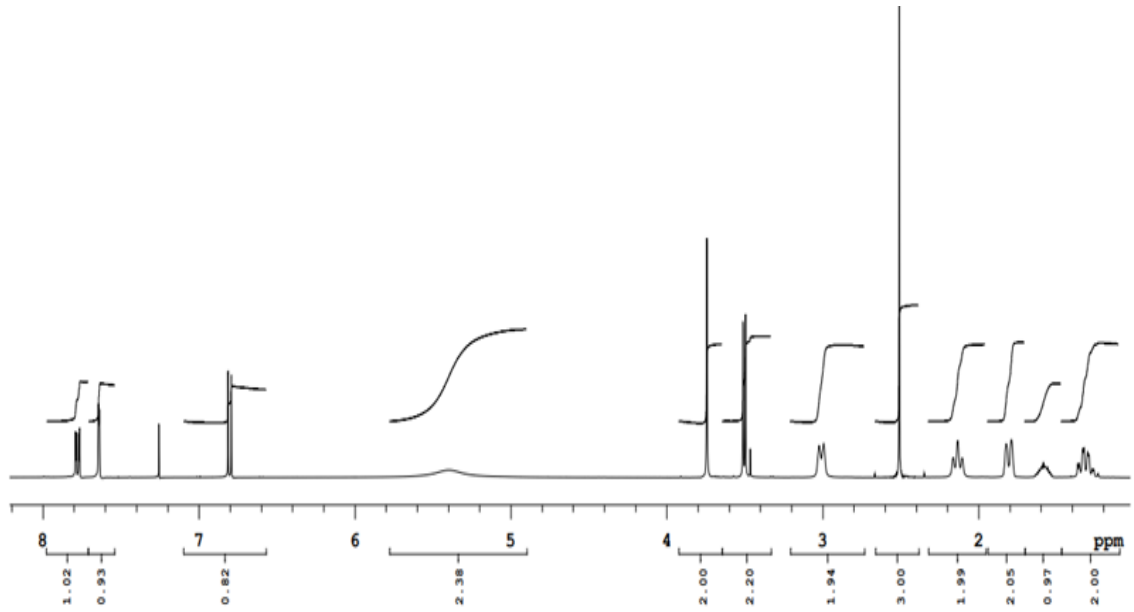


Figure 4.20. ^{1}H-NMR spectrum of compound **8**

^{13}C-NMR: 196.99 (C=O), aromatic carbons were seen at 163.19, 130.22, 129.18, 128.76, 121.19, 115.89, 65.47 (HO-**C**H_2), 61.71 (O=C-**C**H_3). Piperidine carbons found at 56.25, 53.54, 38.84, 26.53, 26.18 (O=C-**C**H_3), 24.61 (Piperidine **C**).

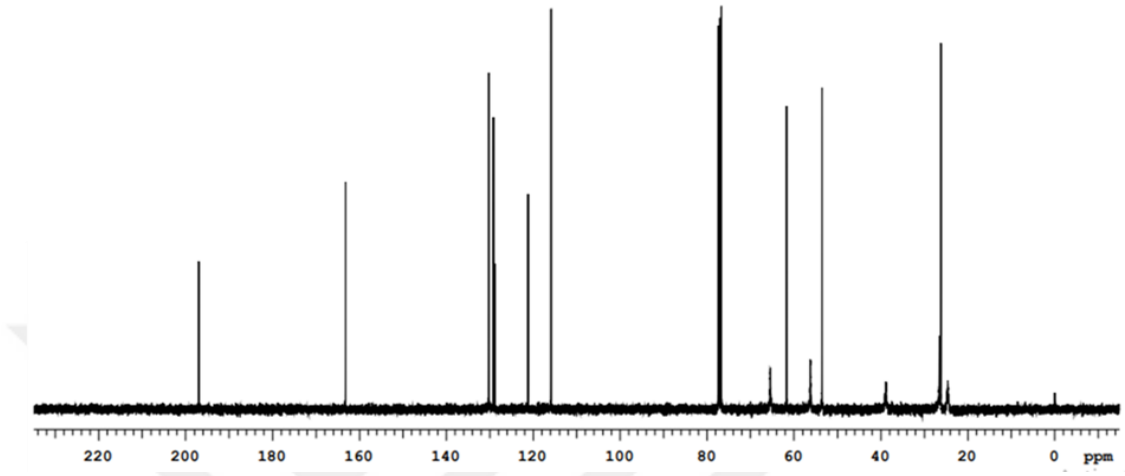
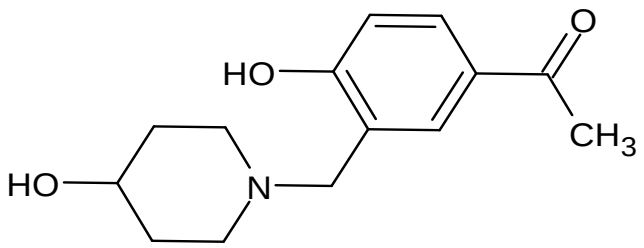


Figure 4.21. ^{13}C-NMR spectrum of compound **8**

1-[4-Hydroxy-3-[(4-hydroxypiperidin-1-yl)methyl]phenyl]ethanone (Compound 9 - CAS No: 1455814-21-7).



4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 4-hydroxypiperidine (0.0033 moles, 0.34101 g) and formaldehyde (0.0033 moles, 0.247 ml) in 10 ml of ethanol were reacted as explained in procedure **A1**. Column chromatography was applied using **S1** solvent mixtures. The compound is soluble in chloroform, methanol, dichloromethane and ethanol. The form of the compound is yellow-colored oily.

4-Hydroxyacetophenone (0.0022 moles, 0.306 g), 4-hydroxypiperidine (0.0033 moles, 0.34101 g) and formaldehyde (0.0033 moles, 0.247 ml) in 10 ml of 1,4-dioxane were reacted as explained in procedure **B1**. Column chromatography was applied using **S1** solvent mixtures. The form of the compound is yellow-colored oily.

Rf values: 0.5 (S1).

Yields: 44.8 % (procedure A1), 92% (procedure B1).

FT-IR (KBr, vmax, cm^{-1}): 3636-3200 (O-H), 2942 (Ar C-H), 2848 (Aliphatic C-H), 1670 (C=O), 1593 (C=C).

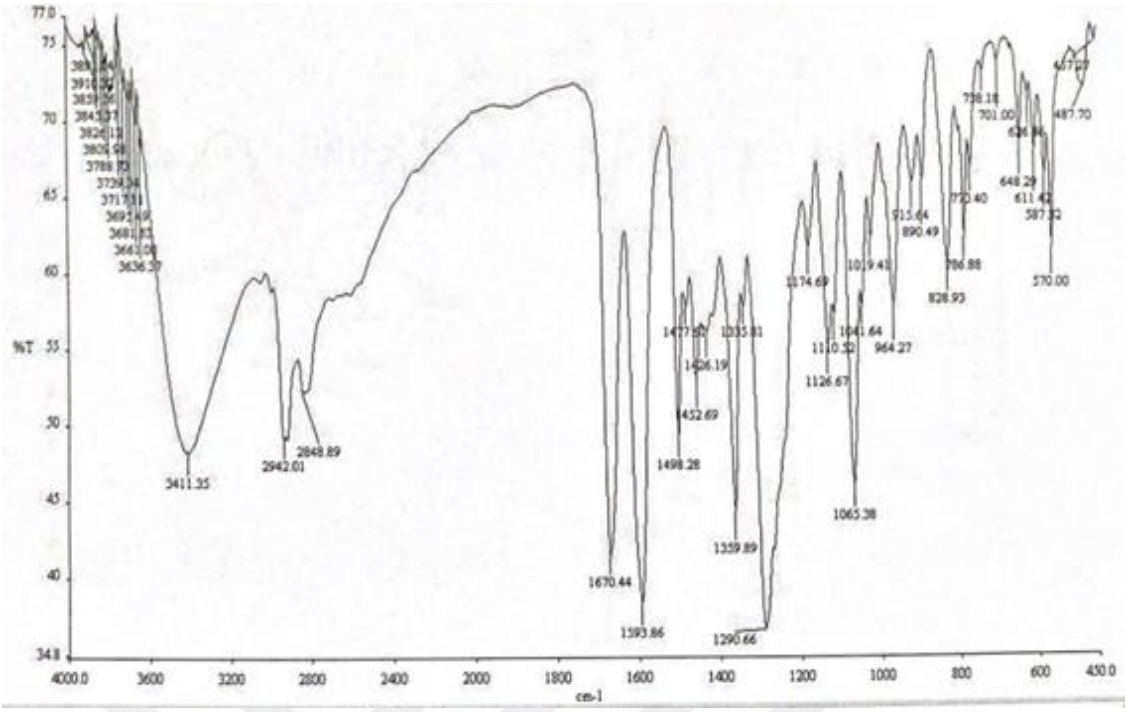


Figure 4.22. IR spectrum of compound **9**

^{1}H-NMR: 7.76-7.70 (m, 2H, Ar), 6.78 (d, 1H, Ar, *J*=8 Hz), 3.68 (s, 2H, CH_2), 3.53 (s,1H, H-C-OH), 2.74-2.72 (m, 2H-Piperidine), 2.46 (s, 3H, CH_3), 2.21 (t, 2H-Piperidine *J*=10 Hz), 1.76-1.73 (m, 2H-Piperidine), 1.45-1.37 (m, 2H-Piperidine).

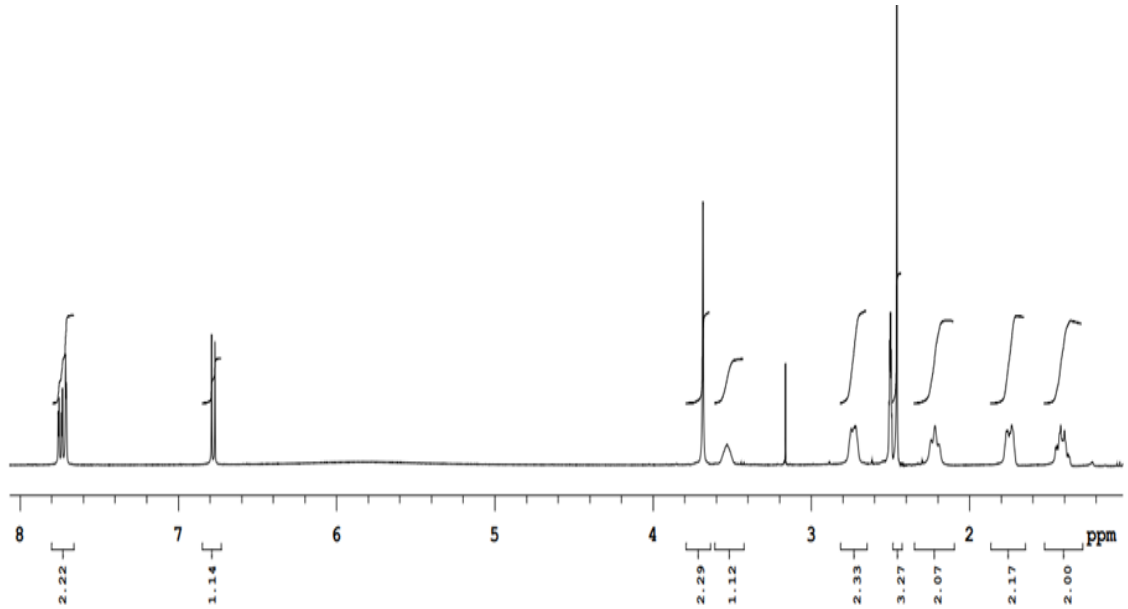
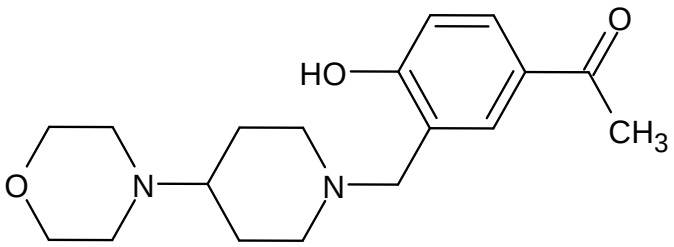


Figure 4.23. ^{1}H-NMR spectrum of compound **9**

1-[4-Hydroxy-3-[(4-morpholinepiperidin-1-yl)methyl]phenyl]ethanone (Compound 10).



4-Hydroxyacetophenone (0.0011 moles, 0.153g), 4-morpholinepiperidine (0.0022 moles, 0.3821g) and formaldehyde (0.0022 moles, 0.1643 ml) in 10 ml of ethanol were reacted as explained in procedure **A2**. Column chromatography was applied using **S3** solvent mixtures. The compound is soluble in chloroform, methanol, dichloromethane and ethanol. The form of the compound is yellow-colored solid.

4-Hydroxyacetophenone (0.0011 moles, 0.153 g), 4-morpholinepiperidine (0.0022 moles, 0.3821g) and formaldehyde (0.0022 moles, 0.1643 ml) in 10 ml of 1,4-dioxane were reacted as explained in procedure **B2**. Column chromatography was applied using **S3** solvent mixtures. The form of the compound is yellow-colored solid.

Rf values: 0.7 (S3).

M.p.: 136.7 °C

Yields: 20 % (procedure A2), 70 % (procedure B2).

FT-IR (KBr, vmax, cm^{-1}): 3600-3200 (O-H), 2965 (Ar C-H), 2858 (Aliphatic C-H), 1670 (C=O), 1594 (C=C).

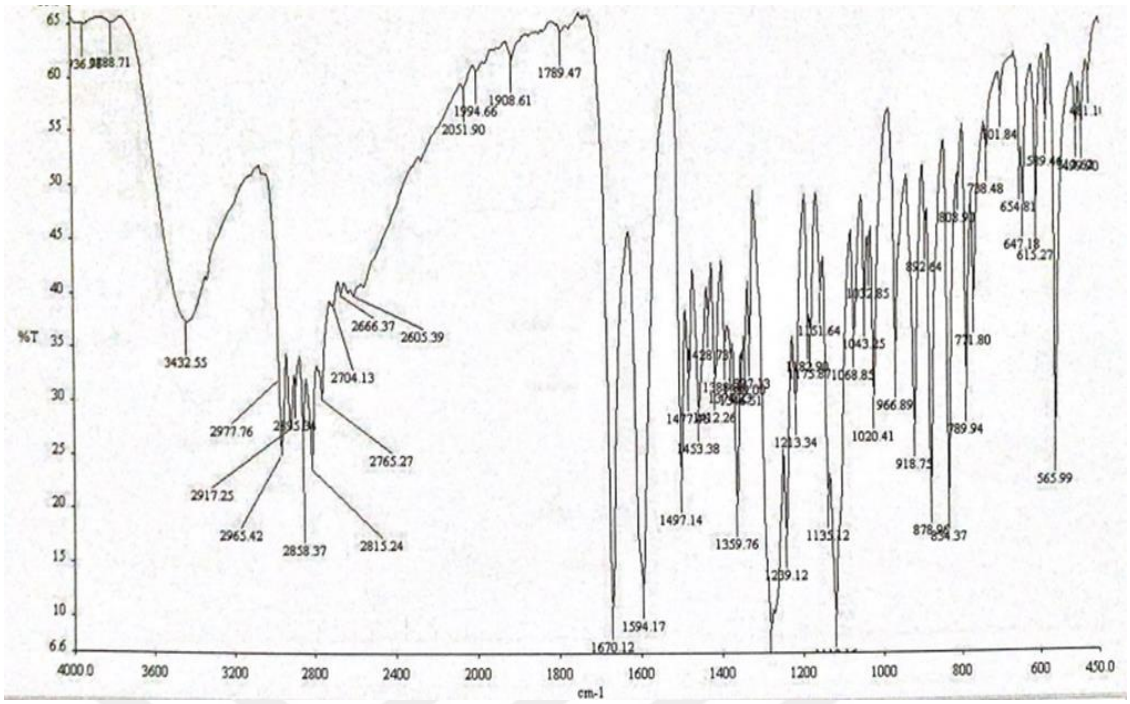


Figure 4.24. IR spectrum of compound **10**

^{1}H-NMR: 7.78-7.75 (dd, 1H, Ar, J_1=2.4 Hz, J_2=1.6 Hz), 7.62 (d, 1H, Ar, J=2 Hz), 6.8 (d, 1H, Ar, J=8 Hz), 3.72 (s, 2H, CH_2), 3.69 (t, 4H, O-CH_2, J=4.4 Hz), 3.01 (d, 2H-Piperidine, J=11.6 Hz), 2.52 (t, 4H-morpholine, J=4.8 Hz), 2.50 (s, 3H, CH_3), 2.24-2.10 (m, 3H-Piperidine), 1.88 (d, 2H-Piperidine, J=12.8 Hz), 1.58-1.55 (m, 2H-Piperidine).

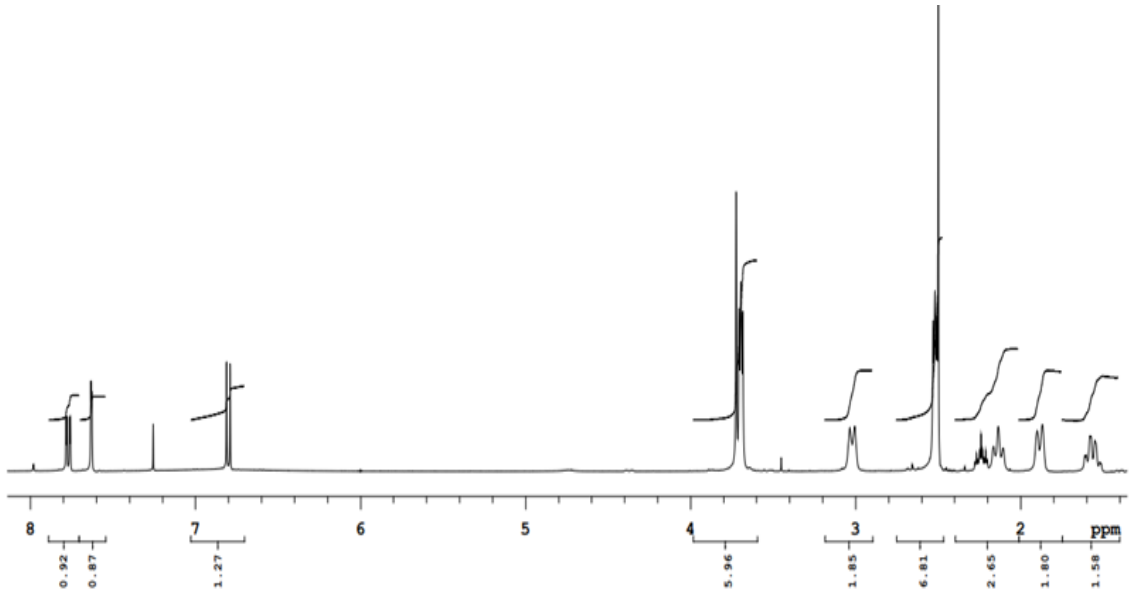
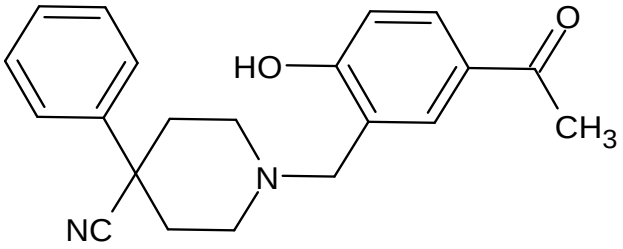


Figure 4.25. ^{1}H-NMR spectrum of compound **10**

1-[4-Hydroxy-3-[(4-cyano-4-phenylpiperidin-1-yl)methyl]phenyl]ethanone (Compound 11).



4-Hydroxyacetophenone (0.0011 moles, 0.153g), 4-cyano-4-phenylpiperidine (0.0022 moles, 0.4739ml) and formaldehyde (0.0022 moles, 0.1643 ml) in 10 ml of ethanol were reacted as explained in procedure **A2**. Column chromatography was applied using **S3** solvent mixtures. The compound is soluble in chloroform, methanol, dichloromethane and hot ethanol. The form of the compound is white-colored solid.

4-Hydroxyacetophenone (0.0011 moles, 0.153g), 4-cyano-4-phenylpiperidine (0.0022 moles, 0.4739ml) and formaldehyde (0.0022 moles, 0.1643 ml) in 10 ml of 1,4-dioxane were reacted as explained in procedure **B2**. Column chromatography was applied using **S3** solvent mixtures. The form of the compound is white-colored solid.

Rf values: 0.7 (S3).

M.p.: 172.8 °C

Yields: 37 % (procedure A), 76 % (procedure B).

FT-IR (KBr, vmax, cm^{-1}): 3572-3318 (O-H), 3002 (Ar C-H), 2929 (Aliphatic C-H), 2237 (C-N) triple bond, 1671 (C=O), 1581 (C=C).

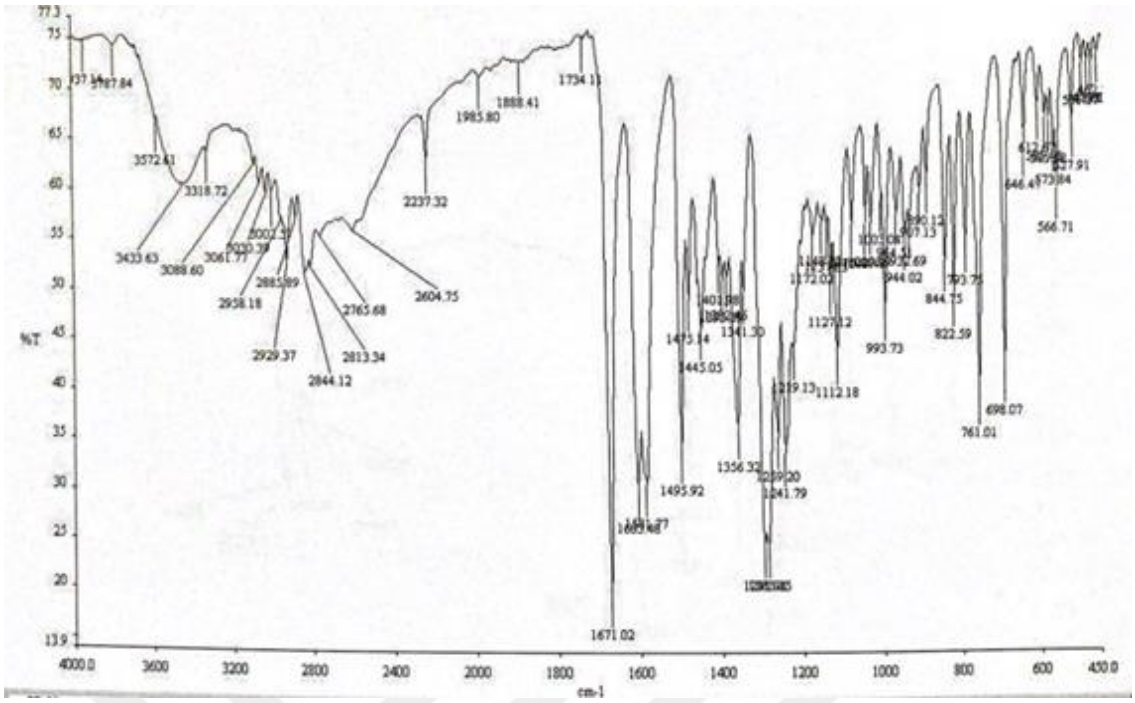


Figure 4.26. IR spectrum of compound **11**

^{1}H-NMR: 7.84-7.81 (dd, 1H, Ar, J_1=2.4 Hz, J_2=2 Hz), 7.70 (d, 1H, Ar, J=2.4 Hz), 7.50-7.47 (m, 2H, Ar), 7.44 -7.33 (m, 3H, Ar), 6.85 (d, 1H, Ar, J=8.4 Hz), 3.89 (s, 2H, CH_2), 3.13 (d, 2H-Piperidine, J=13.2 Hz), 2.74-2.67 (m, 2H-Piperidine), 2.53 (s, 3H, CH_3), 2.23-2.10 (m, 4H-Piperidine).

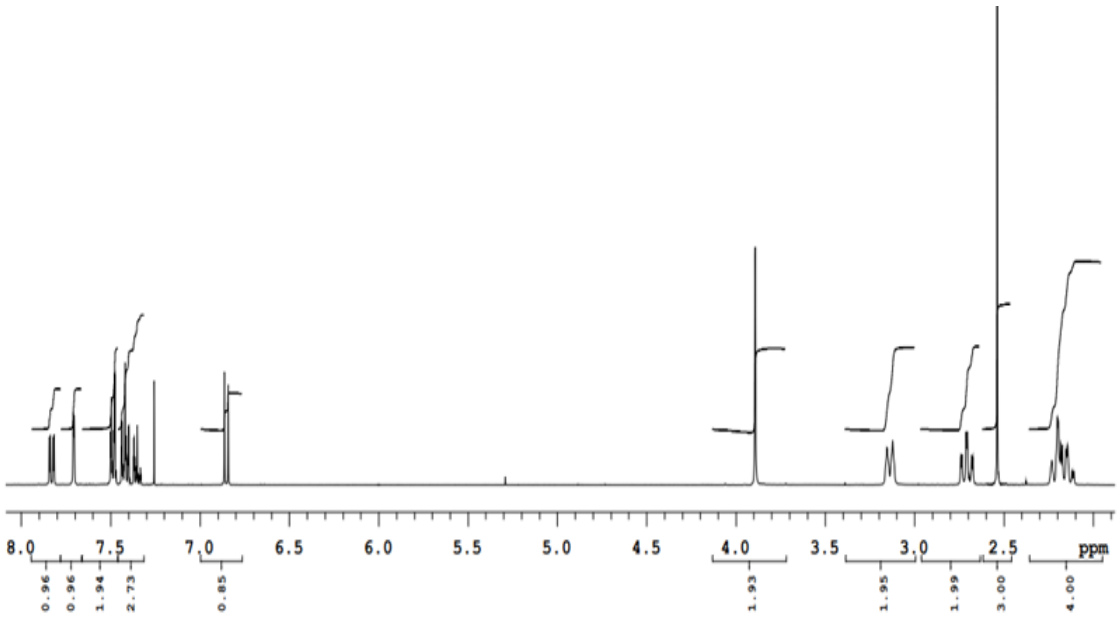
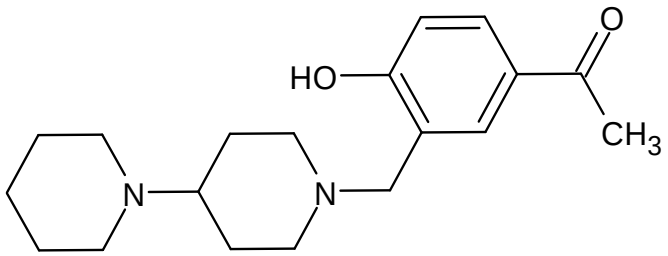


Figure 4.27. ^{1}H-NMR spectrum of compound **11**

1-[4-Hydroxy-3-[(4-piperidinopiperidin-1-yl)methyl]phenyl]ethanone (Compound 12).



4-Hydroxyacetophenone (0.0011 moles, 0.153g), 4-piperidinopiperidien (0.0022 moles, 0.38164g) and formaldehyde (0.0022 moles, 0.1643 ml) in 10 ml of ethanol were reacted as explained in procedure **A2** Column chromatography was applied using **S3** solvent mixtures. The compound is soluble in chloroform, methanol, dichloromethane and ethanol. The form of the compound is white-colored solid.

4-Hydroxyacetophenone (0.0011 moles, 0.153g), 4-piperidinopiperidien (0.0022 moles, 0.38164 g) and formaldehyde (0.0022 moles, 0.1643 ml) in 10 ml of 1,4-dioxane were reacted as explained in procedure **B2**. Column chromatography was applied using **S3** solvent mixtures. The form of compound is white solid.

Rf values: 0.5 (S3).

M.p.: 90.9 °C

Yields: 38 % (procedure A2), 70 % (procedure B2).

FT-IR (KBr, vmax, cm^{-1}): 3600-3330 (O-H), 2926 (Ar C-H), 2889 (Aliphatic C-H), 1677 (C=O), 1590 (C=C).

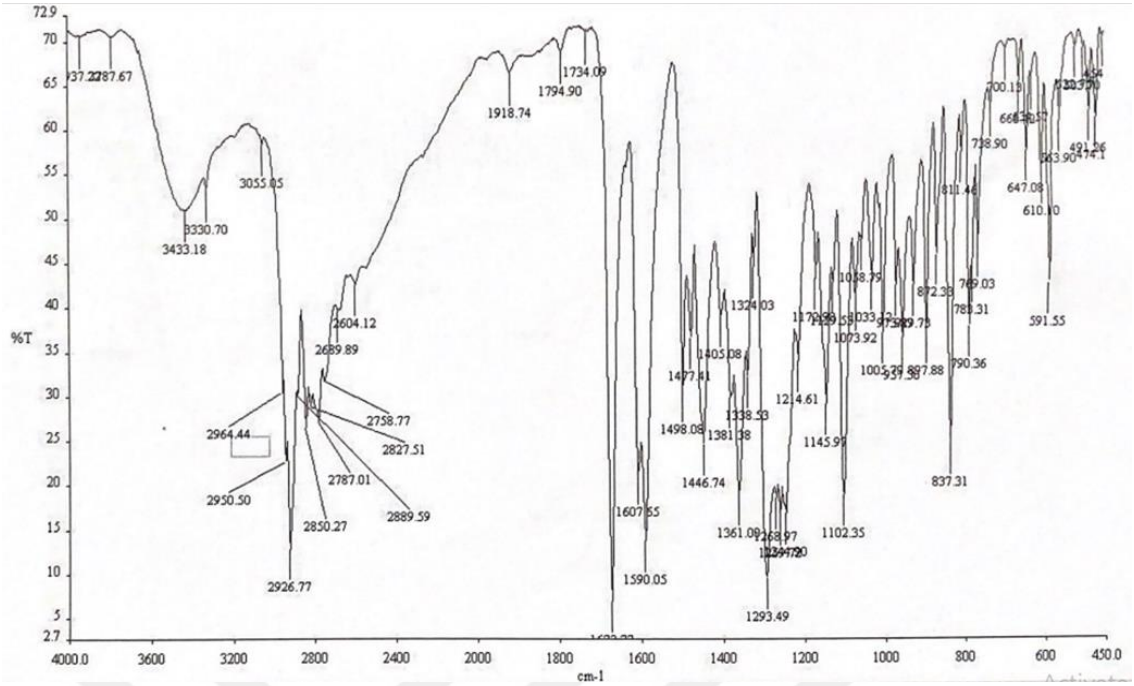


Figure 4.28. IR spectrum of compound **12**

^{1}H-NMR: 7.78- 7.75 (dd, 1H, Ar, J_1=1.6 Hz, J_2=1.6 Hz), 7.62 (d, 1H, Ar, J=2 Hz), 6.79 (d, 1H, Ar, J=8.8 Hz), 3.70 (s, 2H, CH_2), 3.02 (d, 2H-Piperidine, J=11.2 Hz), 2.50 (s, 3H, CH_3), 2.47 (s, 4H-Piperidine), 2.27 (d, 1H-Piperidine, J=11.6 Hz), 2.10 (t, 2H-Piperidine, J=11.2 Hz), 1.84 (d, 2H-Piperidine, J=12.4 Hz), 1.63-1.54 (m, 6H-Piperidine), 1.44-1.41 (m, 2H-Piperidine).

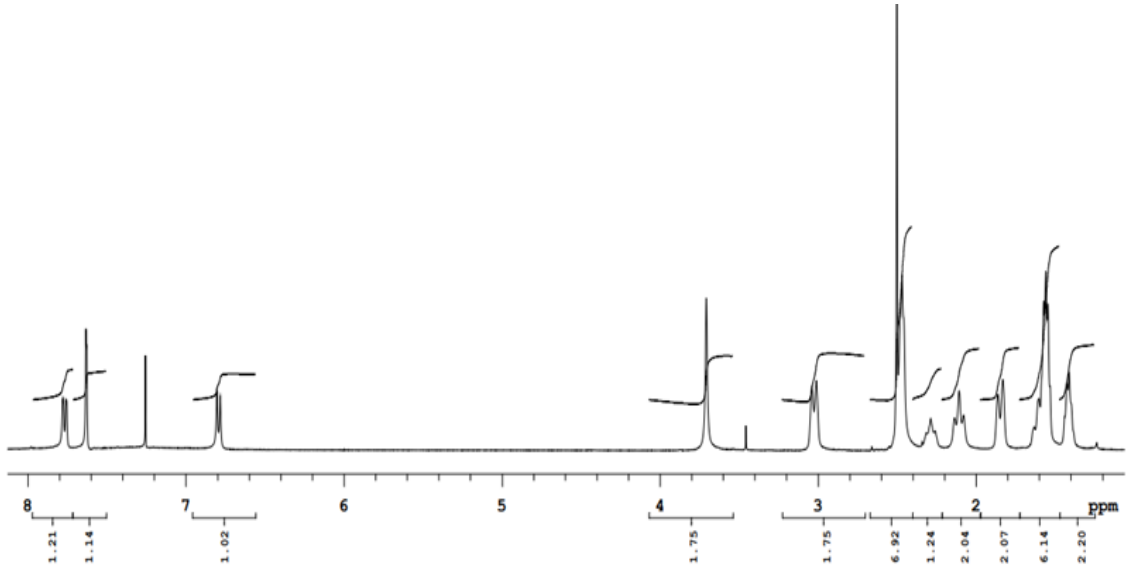


Figure 4.29. ^{1}H-NMR spectrum of compound **12**

Elemental analysis:

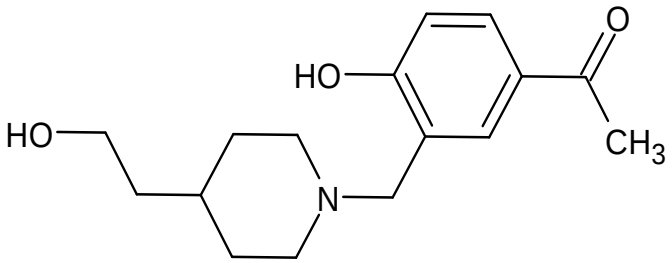
Molecular Formula: $C_{19}H_{28}N_2O_2$

Molecular weight: 316.43 mole

Calculated %: C 72.12, H 8.92, N 8.85.

Found %: C 70.93, H 8.66, N 8.76.

1-[4-Hydroxy-3-[(4-(hydroxyethyl)piperidin-1-yl)methyl]phenyl]ethanone (Compound 13).



4-Hydroxyacetophenone (0.00073 moles, 0.102 g), 4-piperidine ethanol (0.0011 moles, 0.146 g) and formaldehyde (0.0011 moles, 0.0821ml) in 10 ml of ethanol were reacted as explained in procedure **A3**. Column chromatography was applied using **S1** solvent mixtures. The compound is soluble in chloroform, methanol, dichloromethane and ethanol. The form of the compound is yellow-colored oily.

4-Hydroxyacetophenone (0.00073 moles, 0.102 g), 4-piperidine ethanol (0.0011 moles, 0.146 g) and formaldehyde (0.0011 moles, 0.0821ml) in 10 ml of 1,4-dioxane were reacted as explained in procedure **B3**. Column chromatography was applied using **S1** solvent mixtures. The form of the compound is yellow-colored oily.

Rf values: 0.7 (S1).

Yields: procedure A3 (56 %), procedure B3 (89 %).

FT-IR (KBr, vmax, cm^{-1}): 3571-3200 (O-H), 2922 (Ar C-H), 2851 (Aliphatic C-H), 1660 (C=O), 1593 (C=C).

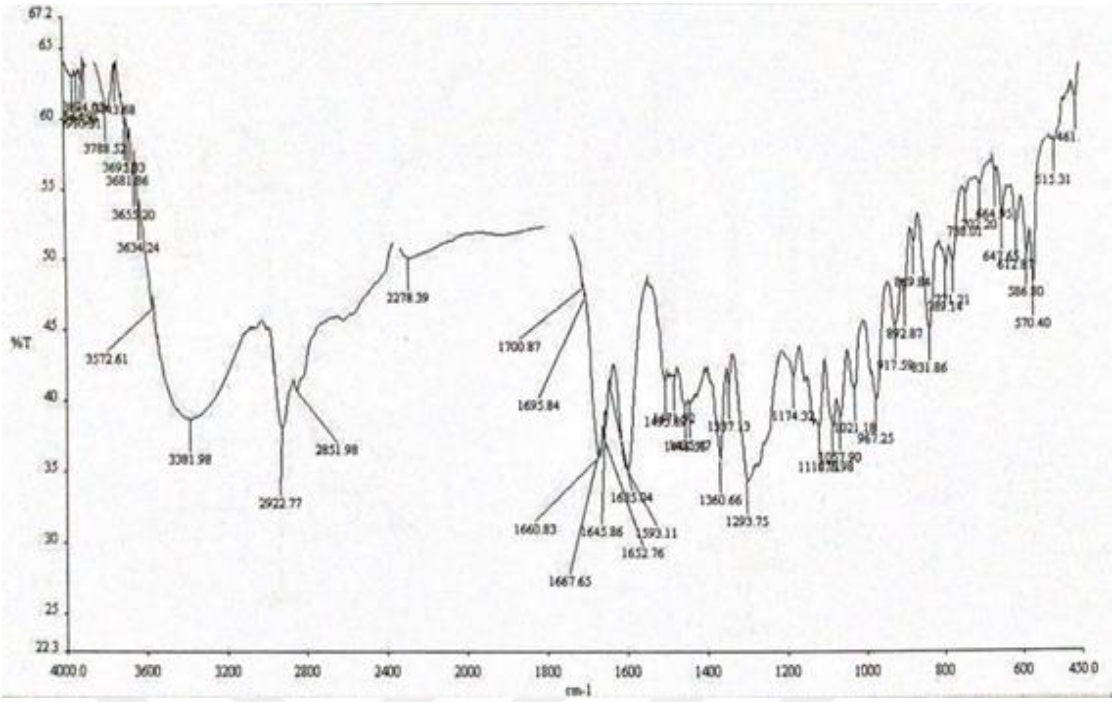


Figure 4.30. IR spectrum of compound **13**

^{1}H-NMR: 7.79- 7.76 (dd, 1H, Ar, J_1=2.4 Hz, J_2=2.4 Hz), 7.63 (d, 1H, Ar, J=2 Hz), 6.8 (d, 1H, Ar, J=8.4 Hz), 3.73 (s, 2H, N-CH_2), 3.70 – 3.67 (m, 2H,O-CH_2), 2.96 (d, 2H-Piperidine, J=12 Hz), 2.51 (s, 3H, CH_3), 2.12 (t, 2H-Piperidine, J=12 Hz), 1.76 (d, 2H-Piperidine, J=13.6 Hz), 1.54 – 1.51 (m, 3H, Piperidine), 1.31–1.28 (m, 2H, C-CH_2).

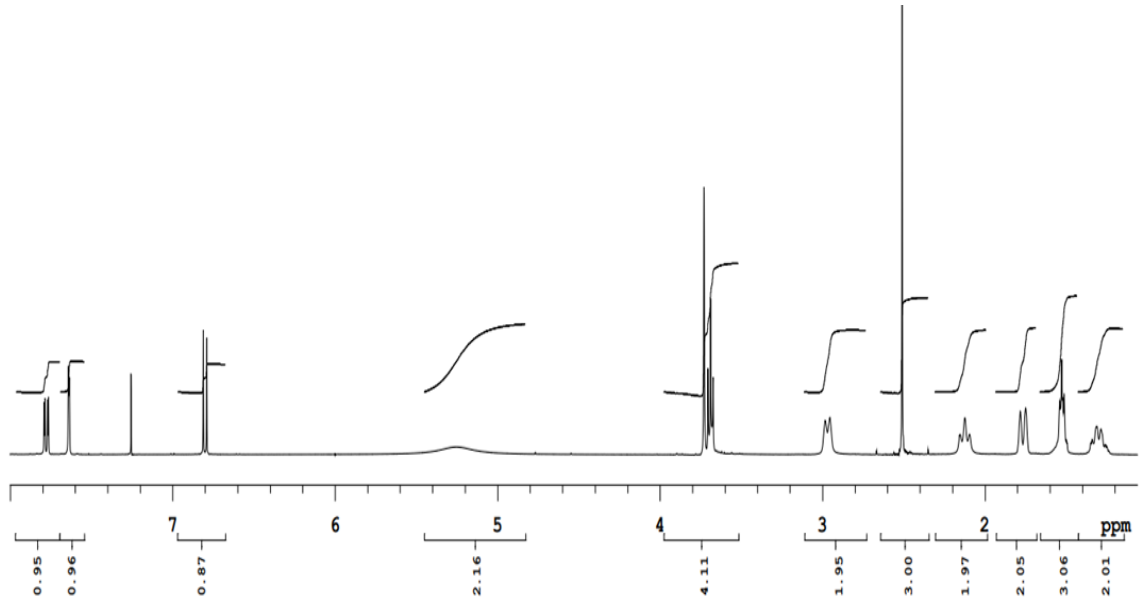


Figure 4.31. ^{1}H-NMR spectrum of compound **13**

4.2. Biological activity

Cytotoxic of synthesized compounds were screened on human breast cancer cell line (MCF7) by MTT assay. The results of cytotoxic activity of our compounds are shown in **Table 4.1.**

Table 4.1. IC_{50} values of target compounds 1-13 against (MCF7) by MTT assay

Compound	R	MCF7 (IC_{50} μM)
1	4-(3-phenylpropyl)	41
2	4-phenyl	**22**
3	4-benzyl	52
4	4-hydroxy-4-phenyl	47
5	4-methyl	42
6	4-hydroxymethyl	105
7	3,5 dimethyl	41
8	3-hydroxymethyl	undetected
9	4-hydroxy	36
10	4-morpholine	undetected
11	4-cyano-4-phenyl	**29**
12	4-piperidino	42
13	4-(2-hydroxyethyl)	45

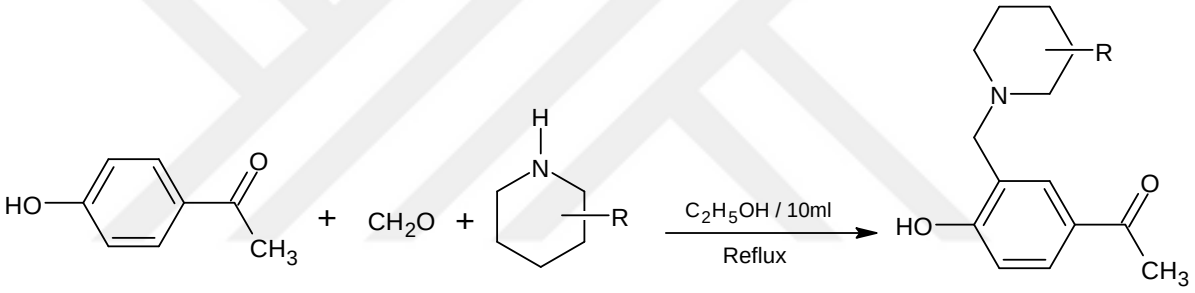
R≥90 for each compound test. Test for each compound was performed with triplicates.

According to activity result, compound **2** and **11** showed high and compounds **1, 4, 5, 7, 9, 12** and **13** showed moderate activity against MCF7 cell line.

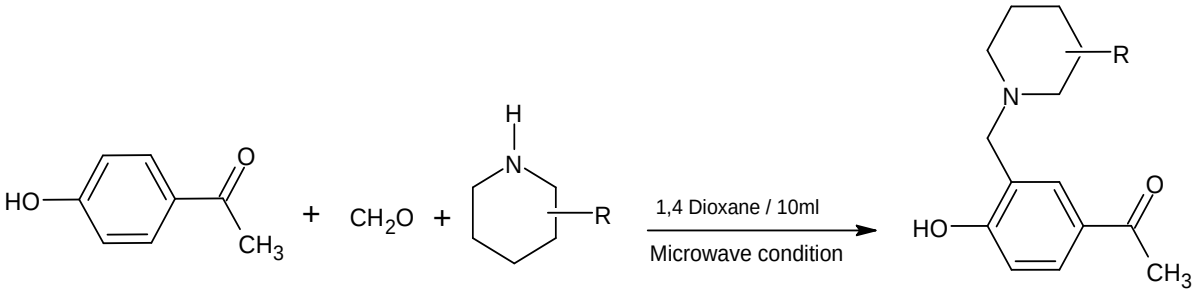
5. DISCUSSION

In this study, 13 compounds have 1-[4-hydroxy-3-[(substituted-1-piperidinyl) methyl] phenyl] ethanone backbone. The compounds 1-4 and 10-13 are originals. The other compounds 5-9 have only CAS number and were not found any articles or information related in the literature. Two different synthetic procedures were used to the synthesis of target compounds. In the first procedure, the target compounds were synthesized by the reaction of 4-hydroxyacetophenone, formaldehyde with piperdine derivatives in ethanol under reflux for 7 hours. In the second procedure, 4-hydroxyacetophenone, formaldehyde with piperdine derivatives were dissolved in 1, 4-dioxane and added to the MW sealed vessel while being stirred. The reaction content was exposed to radiation for fifteen minutes at 120 °C (power 300 W).

1. Conventional method



2. Microwave irradiation method



R: 4-(3-phenylpropyl), 4-phenyl, 4-benzyl, 4-hydroxy-4-phenyl, 4-methyl, 4-hydroxymethyl, 3,5 dimethyl, 3-hydroxymethyl, 4-hydroxy, 4-morpholine, 4-cyano-4-phenyl, 4-piperidino, 4-(2-hydroxyethyl).

Scheme 5.1. General synthesis pathway of compounds.

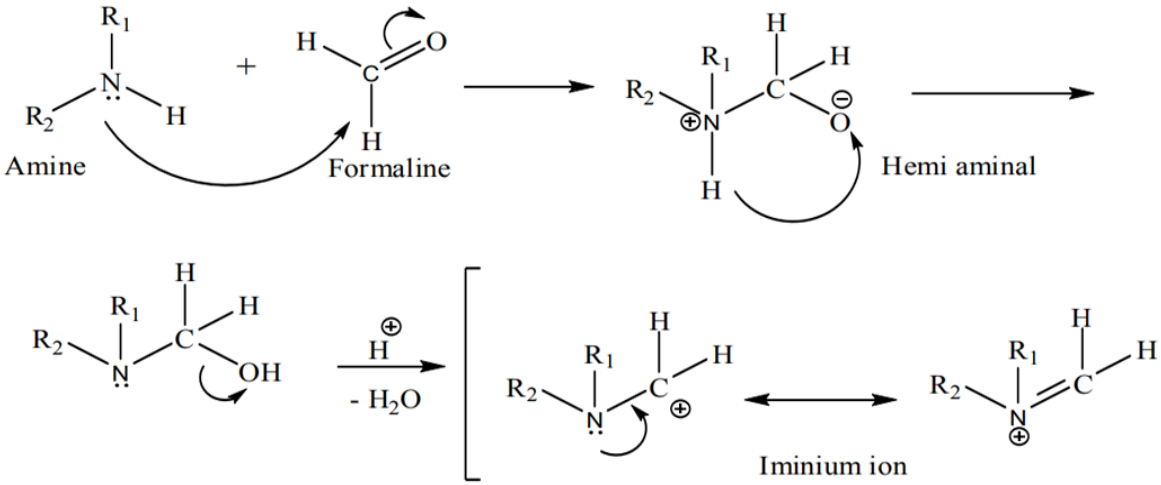
The percent yield of the target compounds in conventional method ranged from 18% to 60.3%, while in microwave method ranged from 70% to 95%. The results, which were obtained from the different techniques, are summarized in Table **5.1.** As seen, the conventional heating method produces low yield, while the microwave irradiation technique results in a high yield of target compounds. Therefore, microwave irradiation offers a higher reaction rate, better product, short time, high yield, and high purity.

Table 5.1. Yield of synthesized compounds.

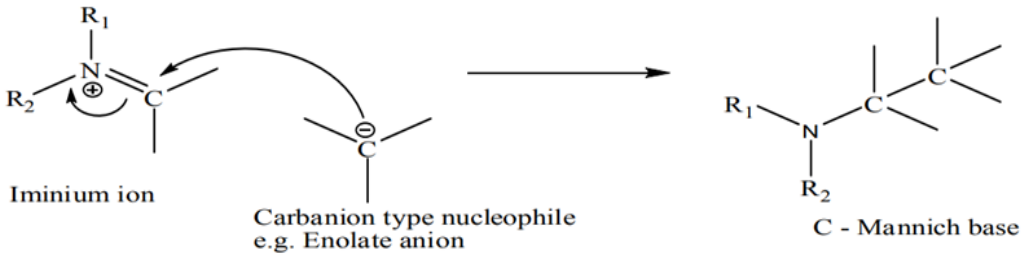
Compound	R	Yield % (Reflux)	Yield% (Mw)*
1	4-(3-phenylpropyl)	37.4%	79%
2	4-phenyl	60%	95%
3	4-benzyl	38%	88%
4	4-hydroxy-4-phenyl	18%	70%
5	4-methyl	49.5%	90%
6	4-hydroxymethyl	61%	94%
7	3,5 dimethyl	45.8%	80%
8	3-hydroxymethyl	38%	88.3%
9	4-hydroxy	44.8%	92.5%
10	4-morpholine	20%	70%
11	4-cyano-4-phenyl	37%	76%
12	4-piperidino	38%	70%
13	4-(2-hydroxyethyl)	56%	89%

* The reaction content was exposed to radiation for fifteen minutes at 120 °C (power 300 W).

In the below scheme shows the general mechanism of action of the *Mannich* reaction. In the first step, the *Mannich* reaction begins with the nucleophilic addition of an amine to the carbonyl carbon followed by dehydration to form an iminium cation. A carbanion is generated from active hydrogen compounds in the second step of the reaction, and then react with the iminium cation to form *Mannich* bases.

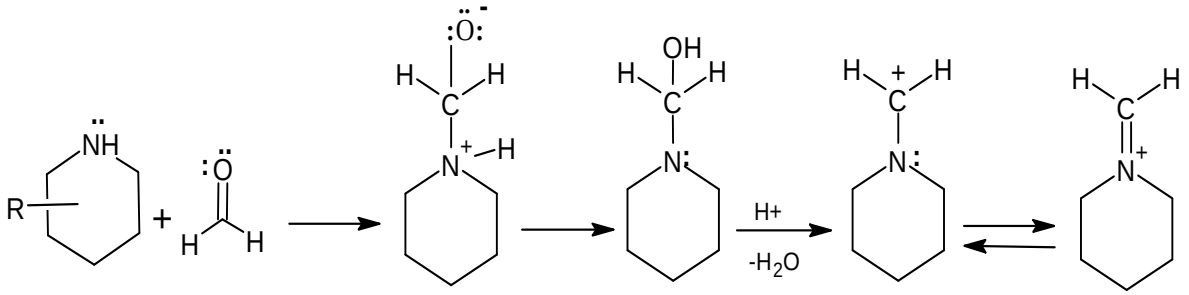


Scheme 5.2. First step in mechanism of *Mannich* reaction: forming iminium ion.

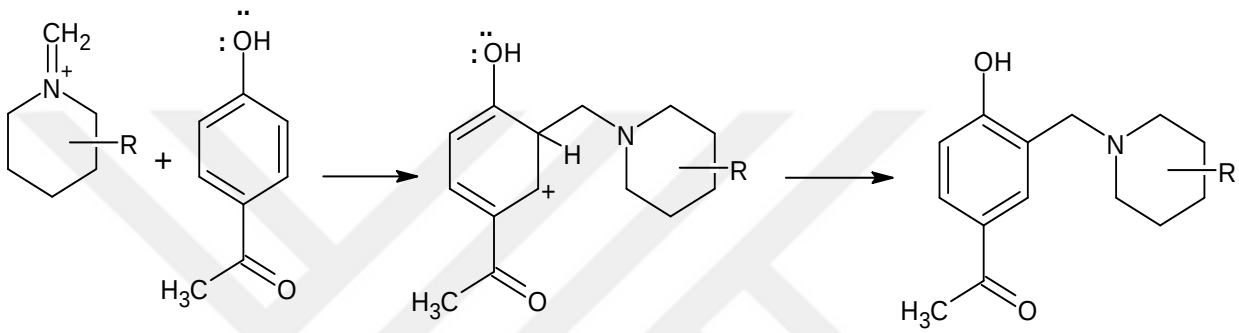


Scheme 5.3. Second step in mechanism of *Mannich* reaction: nucleophilic addition on iminium ion.

Scheme 5.4. shows the mechanism of action of the target compounds, according to the first step of the *Mannich* reaction, nucleophilic addition occurred between piperidine derivatives and formaldehyde then dehydration to form iminium cation (electrophilic center). Then iminium ion react with alpha C of the hydroxyl group of p-hydroxyacetophenone by electrophilic aromatic substitution to give the phenolic *Mannich* base.



1. First step



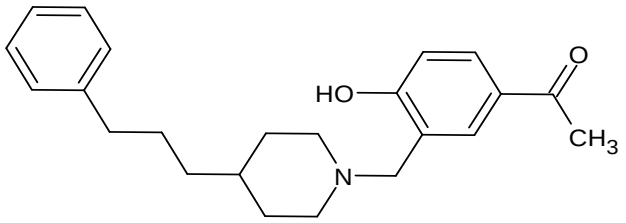
2. Second step

Scheme 5.4. The mechanism of action of the target compounds

By using IR, ^{1}H-NMR, ^{13}C-NMR, and elemental analysis, the structure of the synthesized compounds were confirmed. All spectral data were relevant to the predicted structure and the results are compatible with the literature data [46, 62, 78].

FT-IR spectrum data of synthesized compounds was obtained by using KBr tablets. In general, the stretching band of O-H was seen over 3200, the stretching band of C=O was seen between 1677- 1660 cm^{-1} and the stretching band between 1600- 1580 cm^{-1} indicate to C=C.

For example in the FT-IR spectra of compound **1**, the stretching bands of O-H and aromatic C-H were observed at 3448 cm^{-1} and 3025 cm^{-1}, respectively, while the stretching band of aliphatic C-H was seen at 2932 cm^{-1}. The stretching band of C=O was seen at 1660 cm^{-1} and the stretching band of C=C was observed at 1600 cm^{-1} (**Figure 5.1**.).



Compound **1**

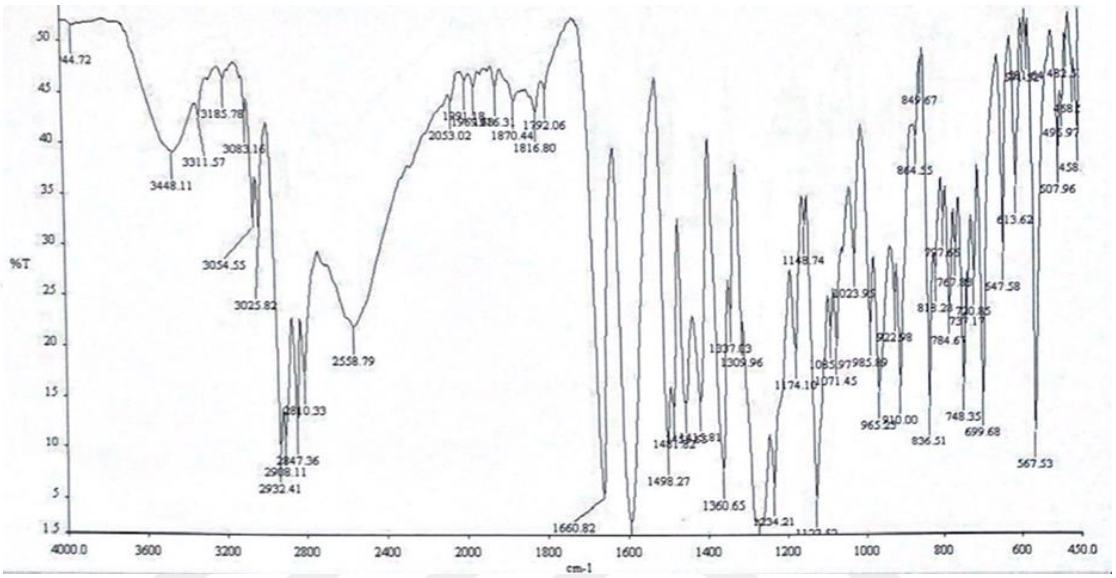
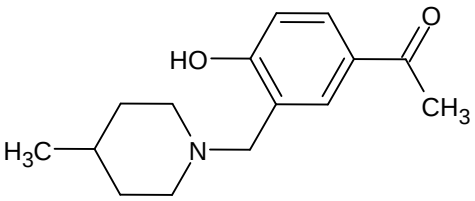


Figure 5.1. IR spectrum of compound **1**

[1]H-NMR spectra of all compounds were taken by using chloroform as solvent and spectrums were recorded in ppm. All synthesized compounds have specific peaks contain: C**H**-aromatics peaks appeared at 7.84- 6.79 ppm, single peak at 3.89- 3.68 ppm was related to C$\mathbf{H_2}$-N which is occurred after mannich reaction, the peak of C$\mathbf{H_3}$ appeared at 2.53- 2.47 ppm as singlet and peaks of protons of piperdine ring between at 3.01- 0.59 ppm .

For example, **Figure 5.2.** shows the [1]H-NMR spectrum of compound **5**, which contains aromatic protons at 7.79–7.76 (dd, 1H, Ar, C**H**-C, J_1=2.4 Hz, J_2=2 Hz), 7.65–7.64 (m, 1H, Ar, C**H**-C), and 6.80. (d, 1H, Ar, C**H**-C-OH, J=8 Hz). Singlet at 3.73 ppm was related to C$\mathbf{H_2}$. Doublet at 2.95 ppm belonged to C$\mathbf{H_2}$ of piperdine ring (J=11.2 Hz). O=C-C$\mathbf{H_3}$ appeared as a singlet at 2.51 ppm. The protons of piperidine ring are seen at 2.12 ppm (t, 2H, C$\mathbf{H_2}$, J=10.8 Hz), 1.69 ppm (d, 2H, C$\mathbf{H_2}$, J= 13.6 Hz). Multiple peaks at 1.32-1.24 ppm was associated to C$\mathbf{H_2}$- C**H** of piperdine ring. C$\mathbf{H_3}$ was seen at 0.93 (d, J=6.8 Hz).



Compound **5**

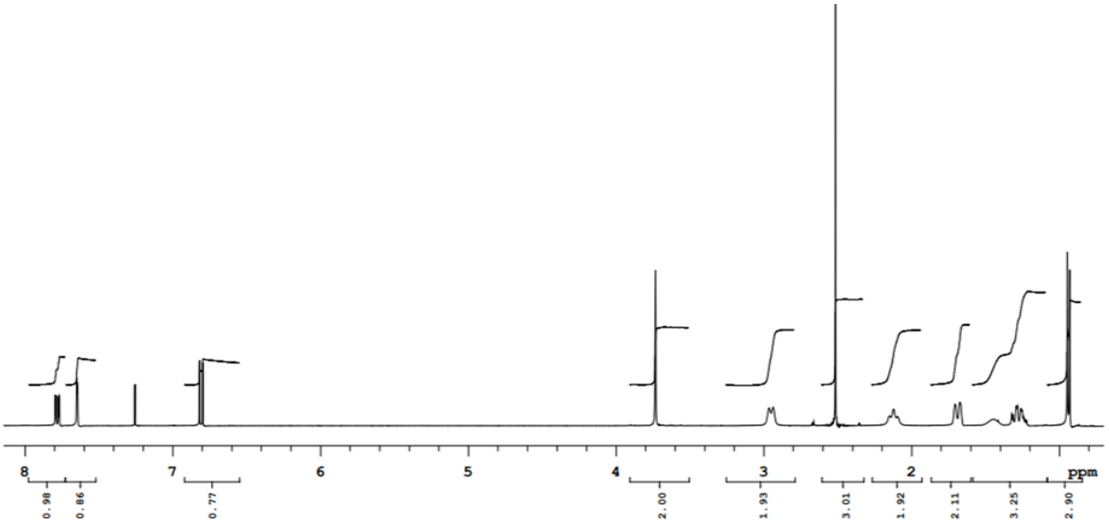
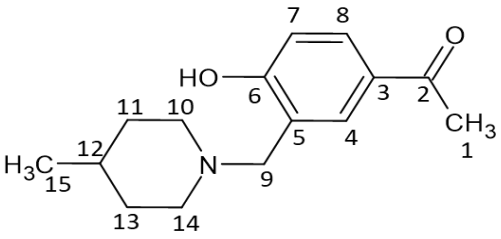


Figure 5.2. ^{1}H-NMR spectrum of the compound **5.**

^{13}C-NMR spectrum of the target compounds 1,2,4,7 and 8 were also taken by using chloroform as solvent and spectrums were recorded in ppm. All synthesized compounds have carbonyl carbon which were showed up at 196.82 ppm, aromatic carbons were seen between 163.37- 115 ppm and the peak of CH_2-N at 61.40 ppm, which is occurred after *Mannich* reaction.

For example ^{13}C-NMR spectrum of the compound **5** showed peak at 196.82 ppm indicated carbonyl carbon (C^2). Aromatic carbons were found at 163.37 ppm C-OH (C^6), 130.13 ppm (C^4), 129.05 ppm (C^8), 128.69 ppm (C^7), 121.37 (C^3), 115 ppm (C^5). The peak of CH_2-N (C^9) showed at 61.40 ppm. Piperdine carbons were seen at 53.23 ppm (C^{10}, C^{14}), 33.99 ppm (C^{11}, C^{13}) and 30.38 ppm (C^{12}) while at 26.17 ppm (C^1) and at 21.57 ppm (C^{15}) (**Figure 5.3.)**.



Compound **5**

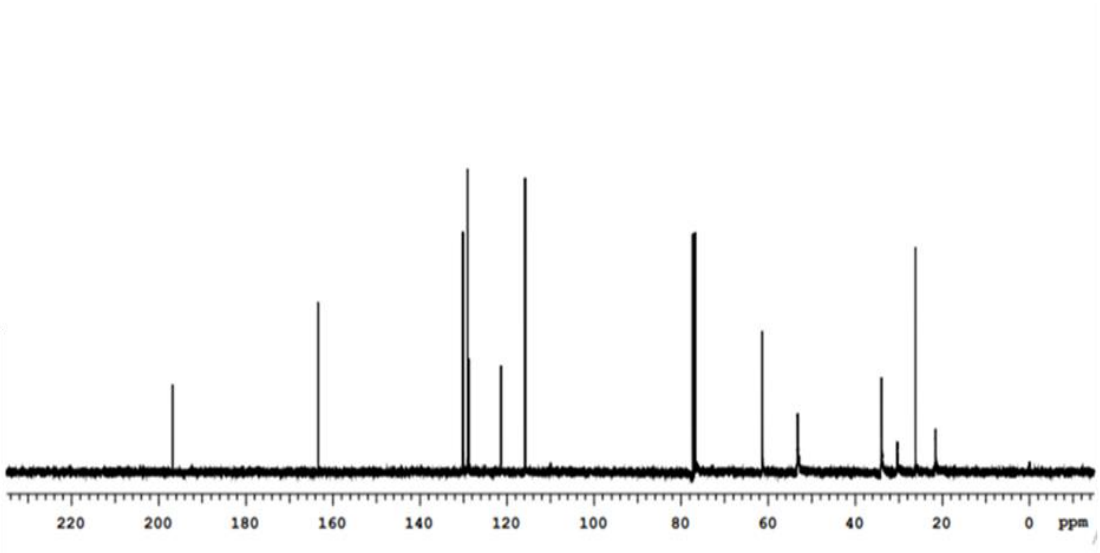


Figure 5.3. ^{13}C-NMR spectrum of the compound **5**.

All target compounds **1-13** were screened on MCF7 cell line by MTT assay. According to the cytotoxic result of the compounds, compound **2** and **11** had shown high activity against (MCF7) cell line with IC_{50} values **22** and **29** µM, respectively. While compounds **1, 4, 5, 7, 9**, **12** and **13** have moderate activity on MCF7 cell line.

6. CONCLUSION

In this thesis study, 13 new compounds bearing 1-[4-hydroxy-3-[(substituted-1-piperidinyl)methyl]phenyl]ethanone backbone were synthesized by *Mannich* reaction using conventional and microwave irradiation methods. The yield of target compounds from microwave method were higher than conventional method.

According to the activity result, 1-[4-hydroxy-3-[(4-phenylpiperidin-1-yl) methyl] phenyl]ethanone (compound **2**) and 1-[4-hydroxy-3-[(4-cyano-4-phenylpiperidin-1-yl) methyl] phenyl]ethanone (compound **11**) have high cytotoxic activity on MCF7 with IC_{50} values 22 and 29 µM, respectively. While compounds **1, 4, 5, 7, 9, 12** and **13** have moderate activity on the MCF7 cell line.

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8. CURRICULUM VITAE

Personal Informations

Name	Sara Jihad	**Surname**	AL Mahamid
Place of Birth		**Date of Birth**	
Nationality	Jordanian	**TR ID Number**	
E-mail		**Phone number**	

Education

Degree	**Department**	**The name of the Institution Graduated From**	**Graduation year**
Doctorate			
Master			
University	Faculty of pharmacy	**University of Jordan**	2020
High school		**Um-Habiba Secondary school**	2015

Languages	**Grades (#))**
English	Intermediate (B2)
Arabic	Native

Work Experience (Sort from present to past)

Position	**Institute**	**Duration (Year - Year)**
Master student	Pharmaceutical chemistry lab at Yeditepe University.	2021-2023
Pharmacist	pharmacy	2019-2020

Computer Skills

Program	**Level**
Microsoft Office	Excellent