T.C.

YEDİTEPE UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

SYNTHESIS AND BIOLOGICAL STUDIES OF NEW PHTHALAMIDE DERIVATIVES

MASTER OF SCIENCE THESIS

YAPRAK YILDIZ

İSTANBUL-2017

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SUPERVISOR

PROF. DR. HÜLYA AKGÜN

İSTANBUL 2017

APPROVAL

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

Signature

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Institution : Yeditepe University Institute of Health Sciences

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 ..10../..11../2017. and .2017./..23-.06.. 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.

07.11.2017

Yaprak Yıldız

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LIST OF ABBREVIATIONS

RyR	Ryanodine Receptor
MCF7	Human breast cancer cell line
Hep3B	Human liver cancer cell line
HeLa	Cervical Cancer Cell Line
SGC-7901	Cellosaurus Cancer Cell Line
HO-8910	Human Ovarian Carcinoma Cell Line
A-549	Adenocarcinoma Human Alveolar Basal Epithelial Cancer Cell Line
S_1	Solvent system 1
S_2	Solvent system 2
DCM	Dichloromethane
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
TLC	Thin Layer Chromatography
MeOH	Methanol
IR	Infrared
NMR	Nuclear Magnetic Resonance
UV	Ultraviolet
MS	Mass spectrometry
s	Singlet
d	Doublet
t	Triplet
q	Quartet
m	Multiplet
MIC	Minimum Inhibitory Concentration
ppm	Parts per million
R_f	Retention factor
CNS	Central Nervous System
HOA	Human Oral Absorption
PHOA	Percent Human Oral Absorption
ROF	Rule of five
ROT	Rule of three

SUMMARY

Yıldız, Y. 2017. Synthesis and Biological Studies of New Phthalamide Derivatives. Yeditepe University, Institute of Health Science, Pharmaceutical Chemistry Programme, Master Thesis. İstanbul.

In this thesis, eighteen novel derivatives of N^1,N^2-bis[(2-substitutedphenyl)ethyl]phthalamide (compounds **1-11**) and 3-nitro-N^1,N^2-bis[(2-substitutedphenyl)ethyl]phthalamide (compounds **12-22**) were synthesized to screen their biological activities as shown in Table 1.1.

The target compounds (compounds **1-11**); N^1,N^2-bis[(2-substitutedphenyl)ethyl]phthalamide were obtained by the reaction of phthalic anhydride and phenylethylamine derivatives in ethanol. 3-nitro-N^1,N^2-bis[(2-substitutedphenyl)ethyl]phthalamide (compounds **12-22**) were also synthesized by the reaction of 3-nitrophthalic anhydride and phenylethylamine derivatives in methanol.

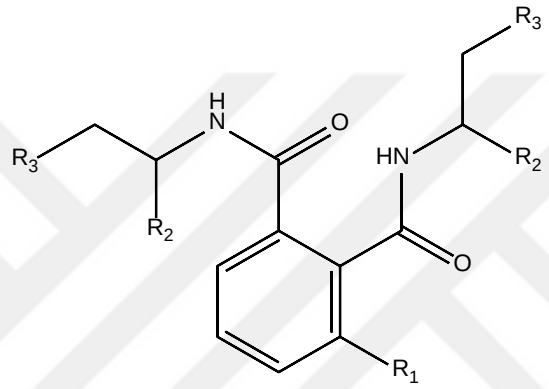
Structure identification of synthesized compounds was elucidated by IR, UV, ^{1}H-NMR, ^{13}C-NMR, and elemental analysis. Antimicrobial activities of the compounds were examined by the conventional agar dilution method. Anticancer activities of the compounds were studied by MTT assay.

Synthesized compounds were screened against gram-positive bacteria strain of *Staphylococcus aureus* and gram-negative bacteria strains of *Escherichia coli* and *Pseudomonas aeruginosa* with the conventional agar dilution method. Synthesized compounds generally showed moderate or low activity compared with Ofloxacin as a reference drug. N^1,N^2-bis[2-(4-chlorophenyl)ethyl]phthalamide (compound **4**), 3-nitro-N^1,N^2-bis[2-(2-chlorophenyl)ethyl]phthalamide (compound **13**), 3-nitro-N^1,N^2-bis[2-(4-chlorophenyl)ethyl]phthalamide (compound **15**) and 3-nitro-N^1,N^2-bis[2-(2,4-dichlorophenyl)ethyl]phthalamide (compound **16**) exhibited the highest zone of inhibition value against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* with moderate antibacterial activities with minimum inhibitory concentration of 25 µg/ml.

Synthesized compounds generally showed moderate or no cytotoxic activity. Among the synthesized compounds (compounds **1-11**), N^1,N^2-bis[2-(2,4-dichlorophenyl)ethyl]phthalamide (compound **5**) was the most active compound against MCF7 (human breast cancer cell line) and Hep3B (human liver cancer cell line)

cancer cell lines with IC_{50} values of 87.6 and 49.6 μM. From the synthesized compounds (compounds **12-22**), 3-nitro-*N*[1],*N*[2]-bis[2-(2,4-dichlorophenyl)ethyl]phthalamide (compound **16**) and 3-nitro-*N*[1],*N*[2]-bis[2-(2-fluorophenyl)ethyl]phthalamide (compound **17**) presented the highest activity against MCF7 and Hep3B cancer cell lines with IC_{50} values of 46.0, 45.1, 50.5 and 37.6 μM, respectively.

Table 1.1. Structure of synthesized compounds



Compound	R_1	R_2	R_3	Compound	R_1	R_2	R_3
1	H	H	phenyl	**12**	NO_2	H	phenyl
2	H	H	2-chlorophenyl	**13**	NO_2	H	2-chlorophenyl
3	H	H	3-chlorophenyl	**14**	NO_2	H	3-chlorophenyl
4	H	H	4-chlorophenyl	**15**	NO_2	H	4-chlorophenyl
5	H	H	2,4-dichlorophenyl	**16**	NO_2	H	2,4-dichlorophenyl
6	H	H	2-fluorophenyl	**17**	NO_2	H	2-fluorophenyl

7	H	H	3-fluorophenyl	**18**	NO_2	H	3-fluorophenyl
8	H	H	4-fluorophenyl	**19**	NO_2	H	4-fluorophenyl
9	H	H	4-methoxyphenyl	**20**	NO_2	H	4-methoxyphenyl
10	H	H	morpholine	**21**	NO_2	H	morpholine
11	H	phenyl	H	**22**	NO_2	phenyl	H

Keywords: Phthalic Anhydride, Phthalamide, Phenylethylamine, Antimicrobial, Anticancer, Cytotoxicity.

This work was supported by Yeditepe University.

ÖZET

Yıldız, Y. 2017. Yeni Ftalamit Türevlerinin Sentezi ve Biyolojik Aktivitelerinin İncelenmesi. Yeditepe Üniversitesi, Sağhk Bilimleri Enstitüsü, Farmasötik Kimya Programı, Master Tezi. İstanbul.

Bu tez çalışmasında, *N*[1],*N*[2]-bis[(2-sübstitüefenil)etil]ftalamit (bileşik **1-11**) ile 3-nitro-*N*[1],*N*[2]-bis[(2-sübstitüefenil)etil]ftalamit (bileşik **12-22**) türevi on yedi adet yeni bileşik biyolojik aktiviteleri test edilmek üzere sentezlenmiştir. Bileşiklerin genel yapıları Tablo 1.1'de gösterilmiştir.

N[1],*N*[2]-bis[(2-sübstitüefenil)etil]ftalamit yapısında bileşikler (bileşik **1-11**) ftalik anhidrit ile feniletilamin türevlerinin etanol içerisinde reaksiyonu ile sentezlenmiştir. 3-nitro-*N*[1],*N*[2]-bis[(2-sübstitüefenil)etil]ftalamit yapısında bileşikler ise (bileşik **12-22**), 3-nitroftalik anhidrit ile feniletilamin türevlerinin metanol içerisinde reaksiyonu ile elde edilmiştir.

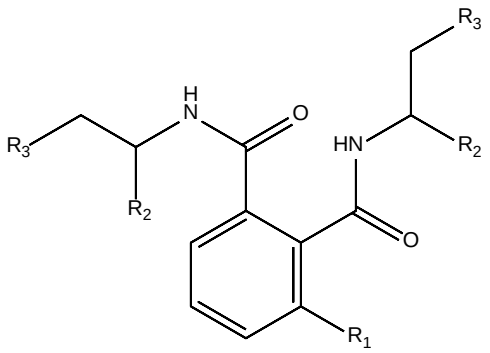
Sentezlenen bileşiklerin yapıları UV, ^{1}H-NMR, ^{13}C-NMR ve elementel analiz yöntemleri ile aydınlatılmıştır. Sentezlenen bileşiklerin antimikrobiyal aktivite çalışmaları agar seyreltme yöntemi ile antikanser aktivite çalışmaları ise MTT testi ile yapılmıştır.

Sentezlenen bileşiklerin antimikrobiyal aktiviteleri gram-pozitif *Staphylococcus aureus* ve gram-negatif *Escherichia coli* ve *Pseudomonas aeruginosa* bakterilerine karşı incelenmiştir. Ofloksasin referans bileşik olarak kullanılmıştır. *N*[1],*N*[2]-bis[2-(4-klorofenil)etil]ftalamit (bileşik **4**), 3-nitro-*N*[1],*N*[2]-bis[2-(2-klorofenil)etil]ftalamit (bileşik **13**), 3-nitro-*N*[1],*N*[2]-bis[2-(4-klorofenil)etil]ftalamit (bileşik **15**) ve 3-nitro-*N*[1],*N*[2]-bis[2-(2,4-diklorofenil)etil]ftalamit (bileşik **16**) bileşikleri *Staphylococcus aureus, Escherichia coli* ve *Pseudomonas aeruginosa* bakterilerine karşı 25 μg/ml ile en aktif bileşikler olarak saptanmıştır.

Sitotoksisite sonuçları incelendiğinde sentezlenen bileşiklerin genellikle orta seviyede aktivite gösterdikleri veya aktivite göstermedikleri belirlenmiştir. Sentezlenen bileşikler (bileşik **1-11**) arasından *N*[1],*N*[2]-bis[2-(2,4-diklorofenil)etil]ftalamit (bileşik **5**) 87.6 ve 49.6 μM IC_{50} değerleri ile MCF7 (meme kanseri hücre hattı) ve Hep3B (karaciğer kanseri hücre hattı) kanser hücre hatlarında en aktif bileşik olarak bulunmuştur. Sentezlenen bileşikler (bileşik **12-22**) içinden, 3-nitro-*N*[1],*N*[2]-bis[2-(2,4-diklorofenil)etil]ftalamit (bileşik **16**) ve 3-nitro-*N*[1],*N*[2]-bis[2-(2-florofenil)etil]ftalamit

(bileşik **17**) MCF7 ve Hep3B kanser hücre hatlarına karşı sırasıyla 46.0, 45.1, 50.5 ve 37.6 µM IC_{50} değerleri ile en yüksek seviyede aktivite göstermiştir.

Tablo 1.1. Sentezlenen bileşiklerin yapıları



Bileşik	R_1	R_2	R_3	Bileşik	R_1	R_2	R_3
1	H	H	fenil	**12**	NO_2	H	fenil
2	H	H	2-klorofenil	**13**	NO_2	H	2-klorofenil
3	H	H	3-klorofenil	**14**	NO_2	H	3-klorofenil
4	H	H	4-klorofenil	**15**	NO_2	H	4-klorofenil
5	H	H	2,4-diklorofenil	**16**	NO_2	H	2,4-diklorofenil
6	H	H	2-florofenil	**17**	NO_2	H	2-florofenil
7	H	H	3-florofenil	**18**	NO_2	H	3-florofenil
8	H	H	4-florofenil	**19**	NO_2	H	4-florofenil
9	H	H	4-metoksifenil	**20**	NO_2	H	4-metoksifenil

10	H	H	morfolin	**21**	NO_2	H	morfolin
11	H	fenil	H	**22**	NO_2	fenil	H

Anahtar Kelimeler: Ftalik Anhidrit, Ftalamit, Feniletilamin, Antimikrobiyal, Antikanser, Sitotoksisite.

Destekleyen Kurum: Yeditepe Üniversitesi

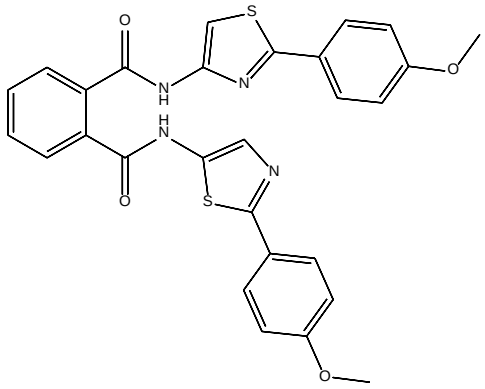
1. INTRODUCTION

Cancer and infectious diseases are the most important health problems today. Cancer is a fatal disease standing next to the cardiovascular diseases in terms of morbidity and mortality [1]. World Health Organization showed that 4% of all cancers are related to several environment conditions like radiation, air pollutions, insecticides, hormones, infectious agents, obesity, immunosuppression, alcohol and age [2]. Although, research has led to a number of new and effective solutions for cancer therapy, chemotherapy is still one of the best solutions. Currently, there is a huge scientific and commercial interest in the discovery of potent, safe and selective anticancer agents [3].

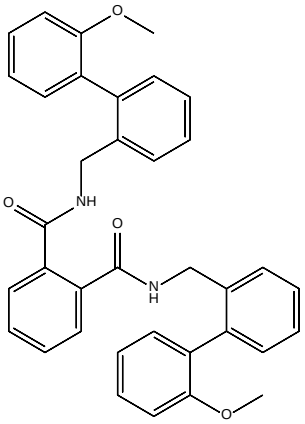
On the other hand, infectious diseases are also on the top of list of worldwide deaths according to the World Health Organization [4]. A major problem has been occurred by extensive use of antibiotics as a result of multi-drug resistant microbial pathogens [5]. A number of different classes of antibacterial agents have been discovered and new agents are still being discovered to treat infectious diseases [6].

Insecticides are agents of chemical or biological origin that control insects. They should be less toxic to fish and mammals in order to protect the environment. Resistance has often been a potential problem for insecticides although many scientists have devoted a lot of effort to find new molecules with a new mode of action. Therefore, new synthetic molecules are targeted to gain specific attention for these years.

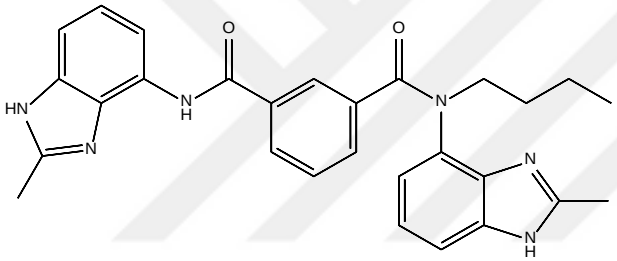
Phthalamides are a class of well-known compounds for a long time and have attracted the scientists' attention, in different fields particularly in organic synthesis [7-23]. Typically, the most important biological studies that have been reported for phthalamide derivatives are anticancer, antimicrobial and insecticidal activities [5-37]. For example; N^1-(2-(4-methoxyphenyl)thiazol-4-yl)-N^2-(2-(4-methoxyphenyl)thiazol-5-yl)phthalamide and N^1,N^2-bis[(2-methoxy-(1,1'-biphenyl)-2-yl)methyl]phthalamide are compounds which showed activity on MCF7 and HeLa cell lines with IC_{50} of 81.46 and 4.37 µM [35]. N^1-butyl-N^1,N^3-bis(2-methyl-1H-benzo[d]imidazol-4-yl)isophthalamide and N^2,N^3-diphenylpyrazine-2,3-dicarboxamide are highly potent antibacterial compounds with MIC of 6, 3, 4 and 5 µg/ml against *Staphylococcus aureus* and *Escherichia coli*, respectively [31].



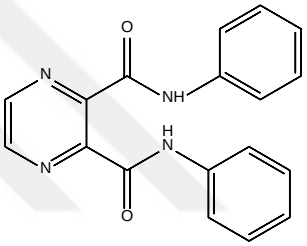
***N*^{1}-(2-(4-methoxyphenyl)thiazol-4-yl)-*N*^{2}-(2-(4-methoxyphenyl)thiazol-5-yl)phthalamide**



***N*^{1},*N*^{2}-bis[(2'-methoxy-(1,1'-biphenyl)-2-yl)methyl]phthalamide**



***N*^{1}-butyl-*N*^{1},*N*^{3}-bis(2-methyl-1H-benzo[d]imidazol-4-yl)isophthalamide**



***N*^{2},*N*^{3}-diphenylpyrazine-2,3-dicarboxamide**

South American plant *Ryania Speciosa* has been recognized for its insecticidal properties long times ago. Its alkaloid known as Ryanodine (Figure 1.1), targets a membrane protein known as the Ryanodine Receptor (RyRs). Ryanodine receptors (Figure 1.2) are ion channels that are responsible for the release of Ca^{2+} from the endoplasmic reticulum. Ryanodine affects calcium release by locking channels in a partially opened state and provide an excellent target for insect control [7].

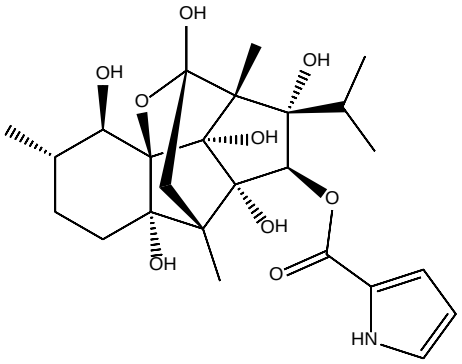


Figure 1.1. Ryanodine

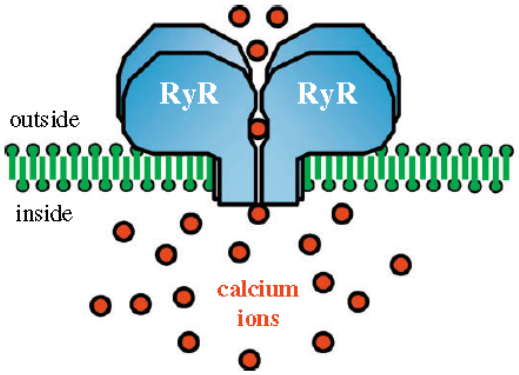


Figure 1.2. Ryanodine Receptor

Some phthalamide derivatives are newly-developed agrochemicals and the first synthetic classes of potent activators of insect RyRs [6, 15-17]. When compared to alkaloid Ryanodine, artificial insecticides are less toxic to fish and mammals, because they selectively induce the release of Ca^{2+} through the insect RyRs [10-12], but have no effect on mammalian RyRs [11,13]. The selectivity of phthalamide insecticides may be the result of large species differences on the RyRs binding sites [9]. These compounds are important for their safety to mammals, high efficiency, and lack of resistance.

Flubendiamide (3-iodo-N^2-[(2-methyl-1-(methylsulfonyl)propan-2-yl)-N^1-(2-methyl-4-(perfluoro)propan-2-yl)phenyl]phthalamide) is one of the phthalamide as insecticide which is discovered by Nihon Nohyaku. This compound is the first synthetic insecticide that controls Lepidoptera effecting on RyRs in muscle cells causes muscle contraction, paralysis and death [7, 14, 15].

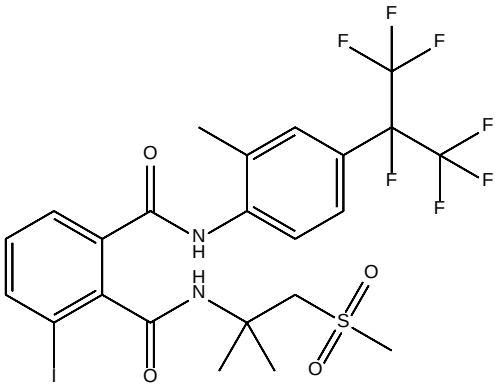
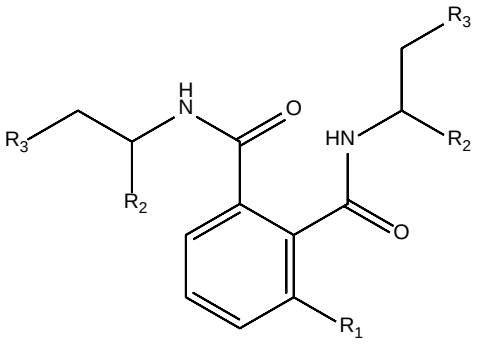


Figure 1.3. Flubendiamide

In the light of these studies, a new series of N^1,N^2-bis[(2-substitutedphenyl)ethyl]phthalamide and 3-nitro-N^1,N^2-bis[(2-substitutedphenyl)ethyl]phthalamide have been synthesized and characterized by spectral studies. All synthesized compounds have been screened the antimicrobial activity against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* by the conventional agar dilution method and screened the anticancer activities using MCF7 and Hep3B cancer cell lines by MTT assay. Yields and melting points of synthesized compounds were given in Table 1.1.

Table 1.2. Yields and melting points of synthesized compounds



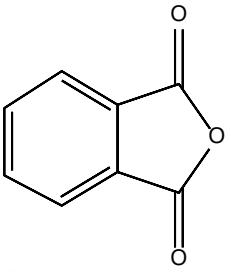
Compound	R_1	R_2	R_3	Yield	Melting Point (°C)
1	H	H	phenyl	79	163.2
2	H	H	2-chlorophenyl	35	198.2

3	H	H	3-chlorophenyl	73	159.5
4	H	H	4-chlorophenyl	47	143.1
5	H	H	2,4-dichlorophenyl	70	164.6
6	H	H	2-fluorophenyl	74	200.7
7	H	H	3-fluorophenyl	36	131.0
8	H	H	4-fluorophenyl	72	123.4
9	H	H	4-methoxyphenyl	55	139.6
10	H	H	morpholine	21	119.7
11	H	phenyl	H	48	liquid
12	NO_2	H	β-phenyl	24	177.2
13	NO_2	H	2-chlorophenyl	38	211.7
14	NO_2	H	3-chlorophenyl	23	248.4
15	NO_2	H	4-chlorophenyl	22	208.3
16	NO_2	H	2,4-dichlorophenyl	9	213.8
17	NO_2	H	2-fluorophenyl	19	143.2
18	NO_2	H	3-fluorophenyl	9	139.8
19	NO_2	H	4-fluorophenyl	16	147.6
20	NO_2	H	4-methoxyphenyl	10	158.8
21	NO_2	H	morpholine	7	liquid
22	NO_2	phenyl	H	14	180.1

2. GENERAL DESCRIPTIONS

2.1. Phthalic Anhydride

Phthalic anhydride is the anhydride form of phthalic acid. It is a white solid crystalline compound in various forms or a clear molten liquid, with an irritating odor. It is slightly soluble in hot water, hydrolyzing to phthalic acid and soluble in alcohol [19].

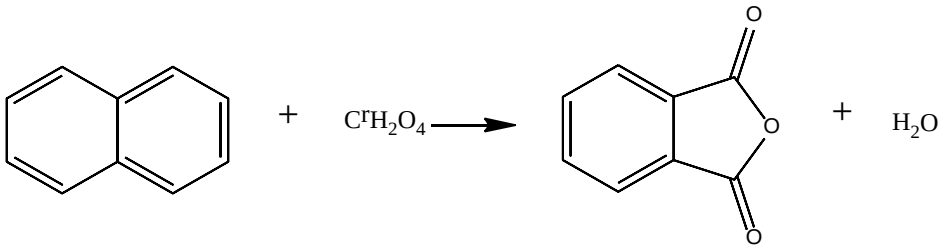


Phthalic anhydride

Phthalic anhydride is a precursor to a variety of reagents useful in organic synthesis. Important derivatives include phthalimide and phthalamide derivatives [19].

2.1.1. Synthesis of Phthalic Anhydride

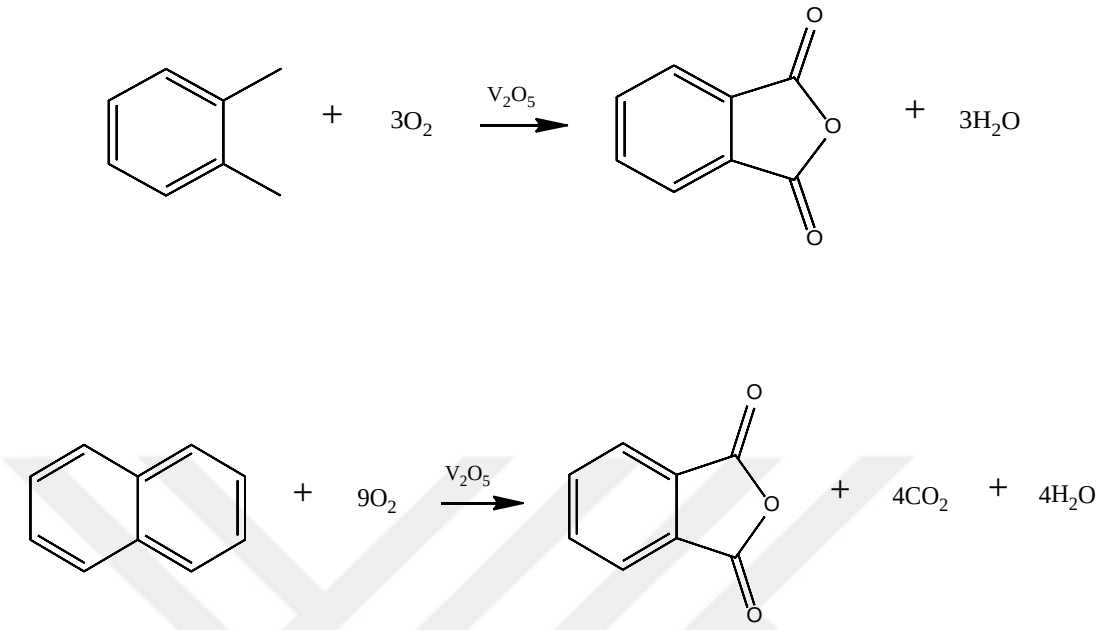
Phthalic anhydride was first synthesized by Auguste Laurent in 1896 and originally prepared by the oxidation of naphthalene with chromic acid which formed anhydride the yield of 83% [20].



Scheme 2.1. Synthetic pathway of phthalic anhydride from naphthalene

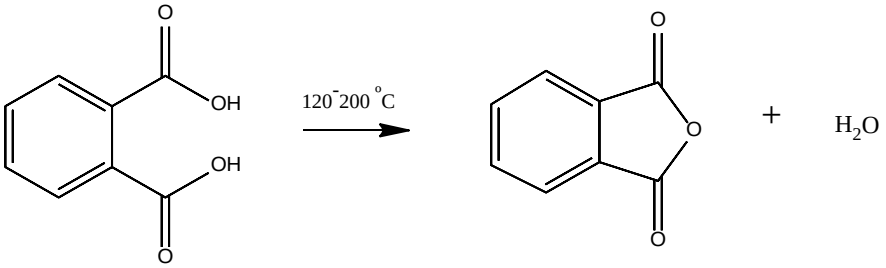
Phthalic anhydride was obtained by two basically different procedures starting from o-xylene or from naphthalene, respectively. Each of these procedures requires, for oxidation with air, a specific catalyst with a vanadium pentoxide and titanium dioxide. These specific catalysts have a relatively brief life, and require continuous checks, changes in reaction conditions, and especially in reaction temperature, and cause the

formation of pollutants which can only be tolerated in extremely low percentages . It is also obtained by catalytic oxidation of o-xylene [20].



Scheme 2.2. Synthetic pathway of phthalic anhydride from o-xylene and naphthalene

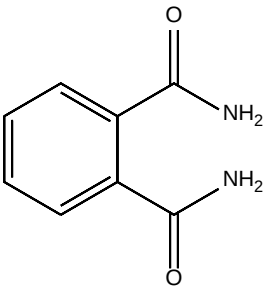
But the common way of the synthesis of phthalic anhydride was to prepare from phthalic acid by heating at high temperature such as 120 to 200 ºC [21].



Scheme 2.3. Synthetic pathway of phthalic anhydride from phthalic acid

2.2. Phthalamide

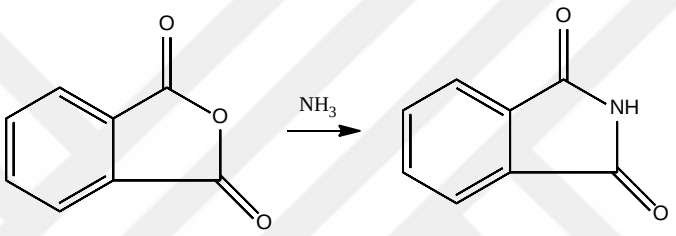
Phthalamide is a compound with the two amide groups and benzene ring. It is also known as phthalic acid diamide or 1,2-benzenedicarboxamide [19].



Phthalamide

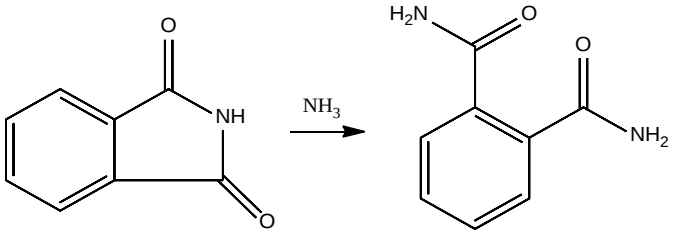
2.2.1. Synthesis of Phthalamide

Phthalimide was first synthesized by Vogel in 1967 with phthalic anhydride and concentrated ammonia solution with the yield of 75% [19].



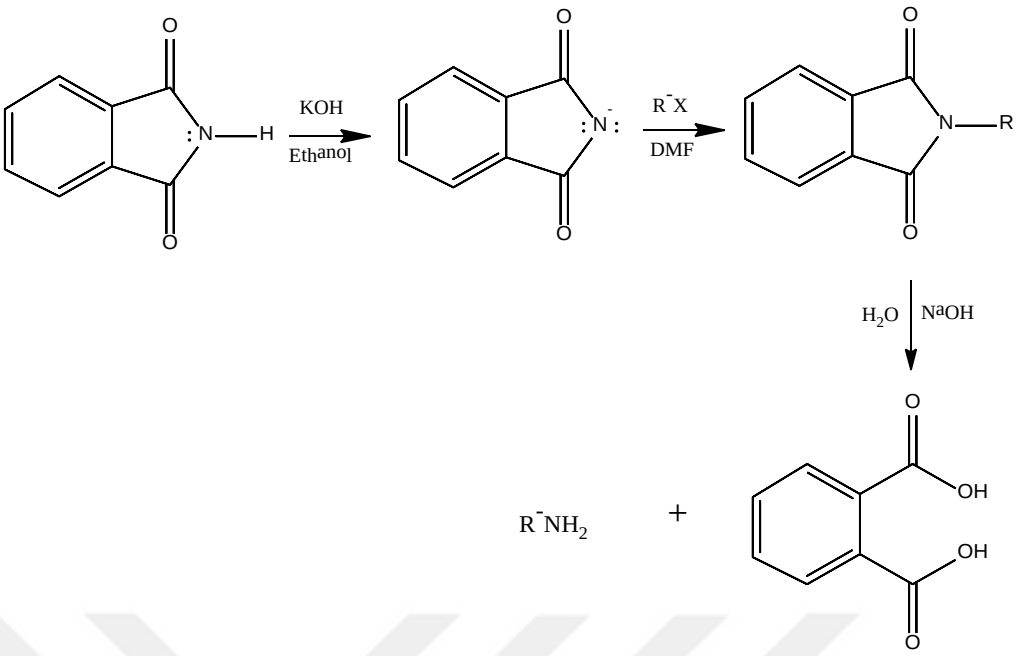
Scheme 2.4. Synthetic pathway of phthalimide

The mixture of phthalimide and concentrated ammonia solution in a beaker were stirred mechanically for 24 hours to yield phthalamide [19].



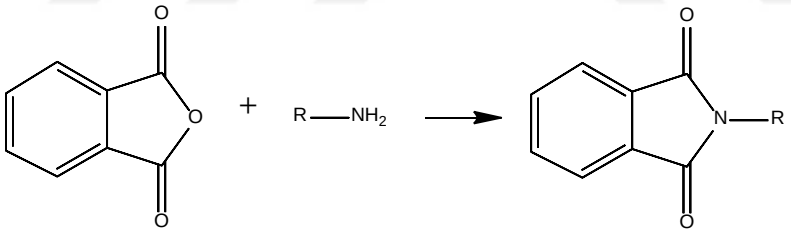
Scheme 2.5. Synthetic pathway of phthalamide

The Gabriel synthesis is used to obtain primary amine from phthalimide. The obtained product from this reaction is phthalic acid which is used for the preparation of phthalic anhydride [22].



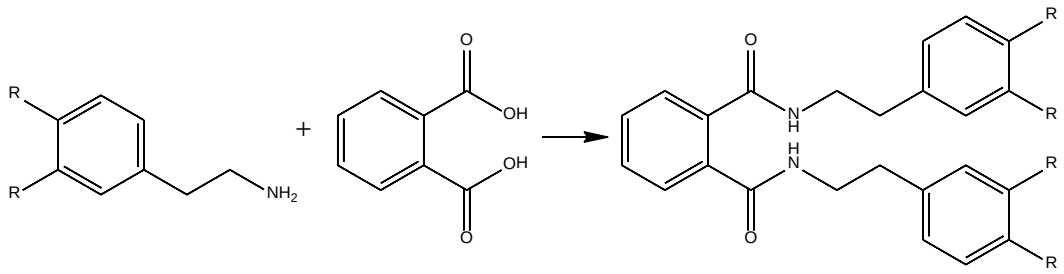
Scheme 2.6. The Gabriel synthesis

N-substituted phthalimide was synthesized with phthalic anhydride and substituted amine by using almost stoichiometric quantities of the reactants [23].



Scheme 2.7. Synthesis of N-alkylphthalimide

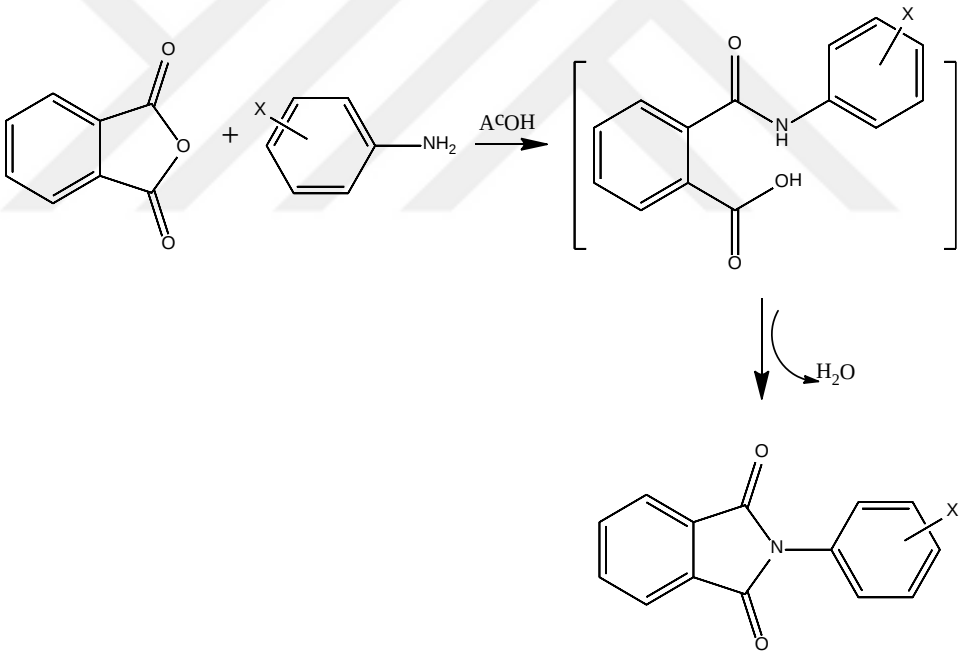
The overwhelming majority of phthalimides were prepared using the well-known Bischler–Napieralski reaction. It was studied with the reaction of homoveratrylamine with dicarboxylic acids in order to prepare phthalamide derivatives. Amides were obtained readily upon heating the salt to 175°C for 1–2 h [24].



Scheme 2.8. Synthesis of N-alkylphthalamide

Phthalimides were obtained by the reaction of phthalic anhydride and an appropriate amine following the procedure described earlier [25].

The ring opening took place by nucleophilic attack of the amine nitrogen atom on carbonyl carbon of phthalic anhydride with the formation of N-substituted phthalic acids as an intermediate which lose water under heating conditions to give products. The yields were between 69 and 99% [26].

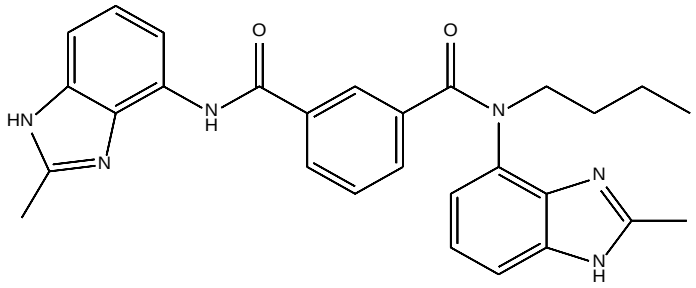


Scheme 2.9 Synthetic pathway of N-alkylphthalimide

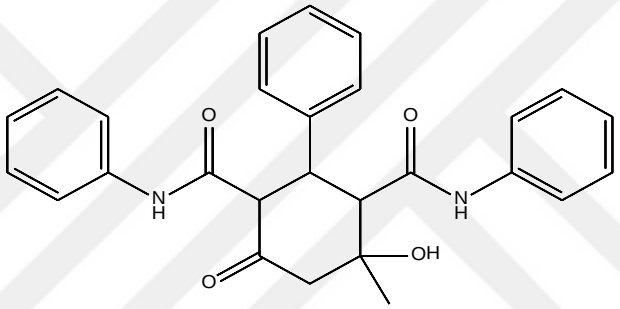
2.2.2. Biological Properties of Phthalamide Derivatives

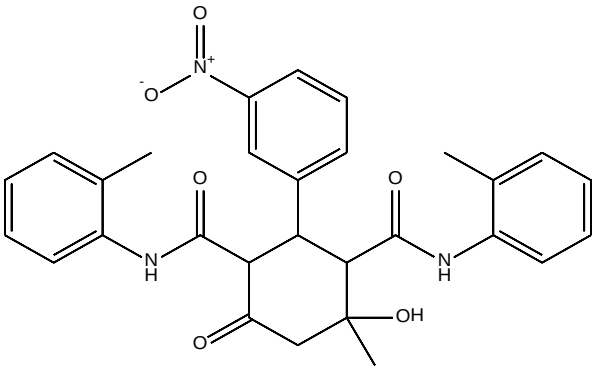
2.2.2.1. Antimicrobial Activity

Prasad *et al.* synthesized N^1-butyl-$N^1N,^3$-bis(2-methyl-1H-benzo[d]imidazol-4-yl)phthalamide with highly potent antibacterial activity with MIC of 6 and 4 µg/ml against *Staphylococcus aureus* and *Escherichia coli* [31].

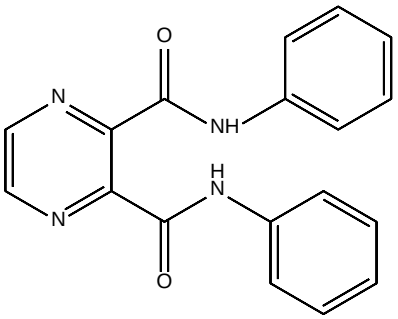


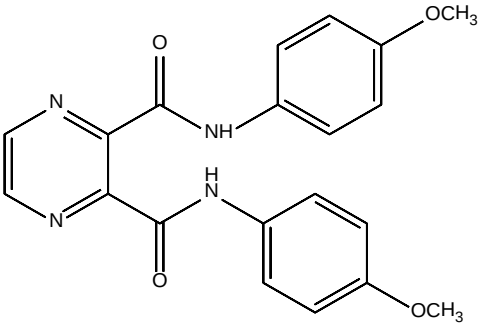
Gein *et al*. prepared 4-hydroxy-4-methyl-6-oxo-N^1,N^3-2-triphenylcyclohexane-1,3-dicarboxamide and 4-hydroxy-4-methyl-2-(3-nitrophenyl)-6-oxo-N^1,N^3-di-o-tolylcyclohexane-1,3-dicarboxamide with weak antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* with MIC of 1000 and 1000 µg/ml [27].



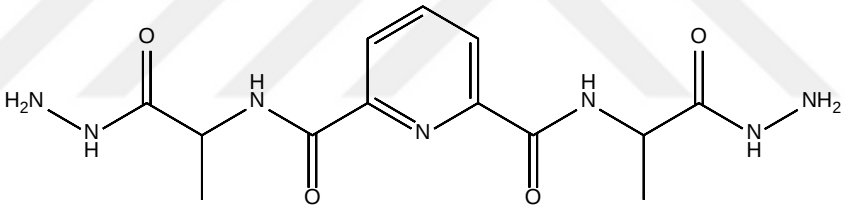


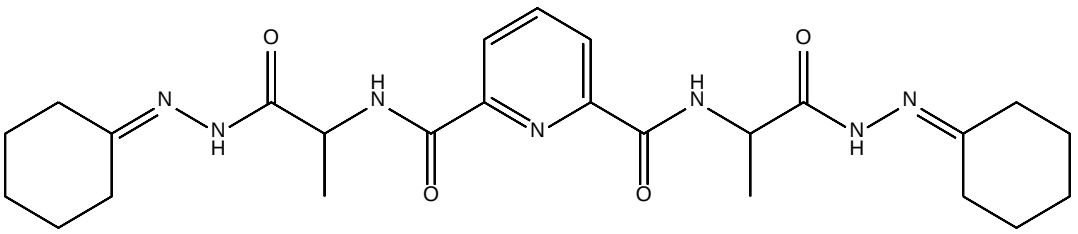
N^2,N^3-diphenylpyrazine-2,3-dicarboxamide and N^2,N^3-bis(4-methoxyphenyl)pyrazine-2,3-dicarboxamide were reported by Rao and found to show significant antimicrobial activities against *Bacillus subtilis* and *Escherichia coli* [28].



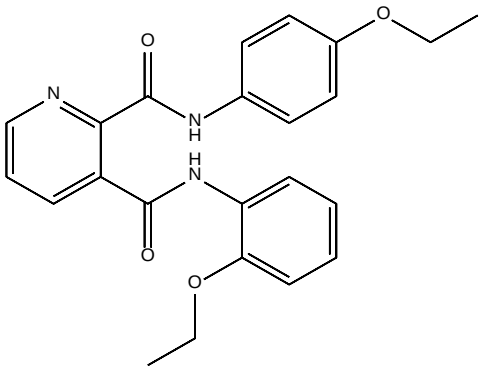


Salahi *et al*. synthesized N^2,N^6-bis(1-hydrazinyl-1-oxopropan-2-yl)pyridine-2,6-dicarboxamide and N^2,N^6-bis(1-(2-cyclohexylidenehydrazinyl)-1-oxopropan-2-yl)pyridine-2,6-dicarboxamide which were effective against gram-positive bacteria and gram-negative bacteria. The minimum inhibitory concentrations of these compounds were 14 and 23 µg/ml against *Bacillus subtilis,* 18 and 25 µg/ml against *Staphylococcus aureus* 19 and 20 µg/ml against *Escherichia coli* respectively. Ciprofloxacin was used as antibacterial reference drug [29].

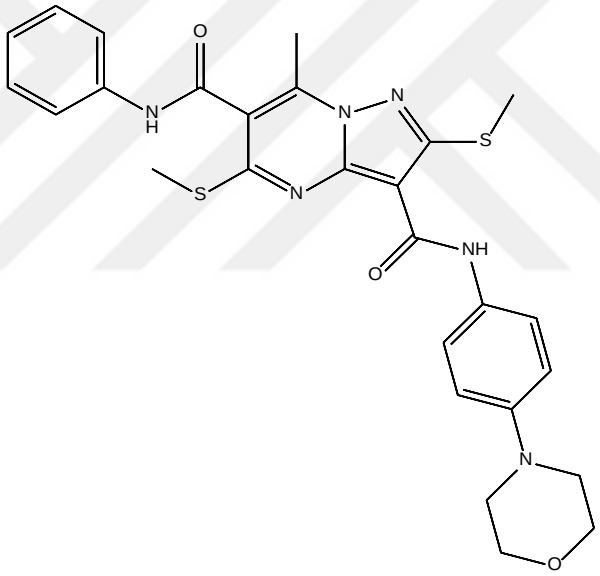




A series of novel N^3-(2-ethoxyphenyl)-N^2-(4-ethoxyphenyl)pyridine-2,3-dicarboxamide derivatives were synthesized by Ammar *et al*. and reported for their potent antibacterial activities against *Sarcina genus*, *Bacillus subtilis, Klebsiella pneumoniae* and *Salmonella typhi* at the minimum inhibitory concentrations of 7, 6 and 6 µg\ml. Erythromycin was used as antibacterial reference drug [30].



7-Methyl-2,5-bis(methylthio)-N^3-(4-morpholinophenyl)-N^6-(p-tolyl)pyrazolo[1,5-a]pyrimidine-3,6-dicarboxamide derivatives were described to have potent antibacterial activity against *Bacillus megaterium, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aerugino* with MIC of 500, 500, 250 and 250 µg\ml. Streptomycin and Ampicillin was used as antibacterial reference drug [32].

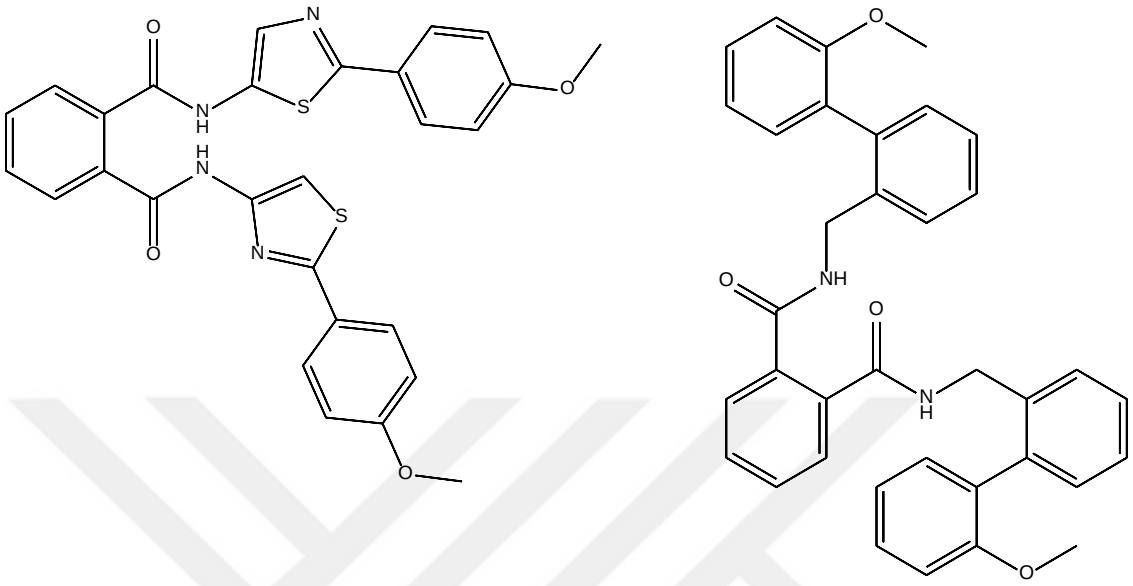


Other compounds reported in the literature which possessed anticancer and antiviral agents involved bioisoteric replacement of benzene ring of phthalamide structure.

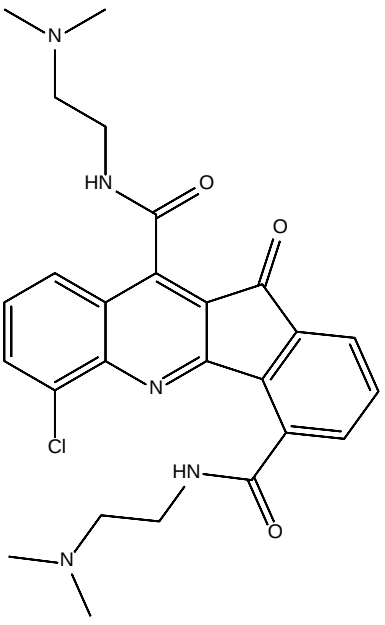
2.2.2.2. Anticancer Activity

N^1-[2-(4-methoxyphenyl)thiazol-4-yl]-*N^2*-[2-(4-methoxyphenyl)thiazol-5-yl] phthalamide and *N^1*,*N^2*-bis[(2'-methoxy-(1-1'-biphenyl)-2-yl)methyl]phthalamide were synthesized by Sankappa. *In vitro* anticancer activities of these phthalamide derivatives

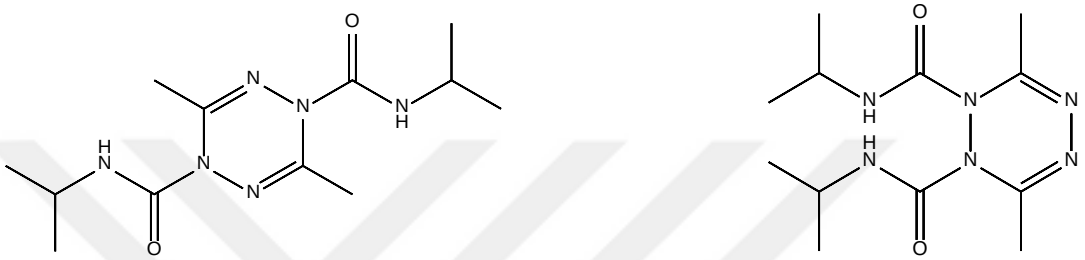
were described by MTT method on two cancer cell lines MCF7 and HeLa in the literature. The compounds showed moderate inhibition in human MCF7 and HeLa cell lines with the IC_{50} values of 81.46, 106.5, 1.41 and 4.37 µM, respectively [35].



N[1],*N*[2]-bis[2-(dimethylamino)ethyl]-6-chloro-11-oxo-11H-indeno[1,2-b]quinoline-4,10-dicarboxamide were prepared by Deady and showed cytotoxic activity on murine leukaemia (P388), lewis lung carcinoma (LLC), jurkat leukaemia (JL) cancer cell lines. In general, this compound presented higher cytotoxicity against cancer cell lines than reference drug Doxorubicin with the IC_{50} values of 27, 15 and 35µM [33].

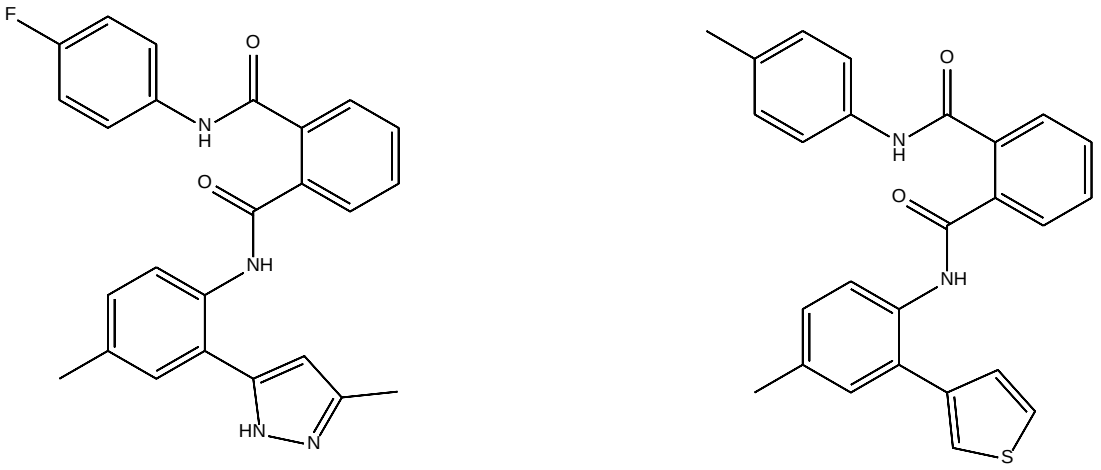


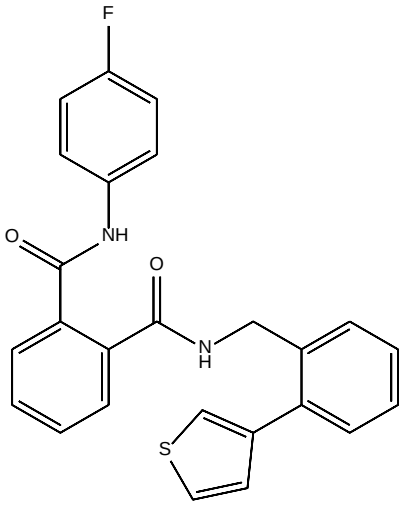
Rao *et al.* reported the potential cytotoxic effect of 3,6-dimethyl-1,2,4,5-tetrazine-1,4-dicarboxamide derivatives. Compounds were found to have high antitumor activities against MCF7, SGC-7901 (cellosaurus cancer cell line), HO-8910 (human ovarian carcinoma cell line) and A-549 (adenocarcinoma human alveolar basal epithelial cancer cell line). Variation in the functional group at carboxamide showed good antiproliferative activity versus above cell lines with the IC_{50} values of 0.45, 0.72, 0.90 and 0.57 µM, respectively [34].



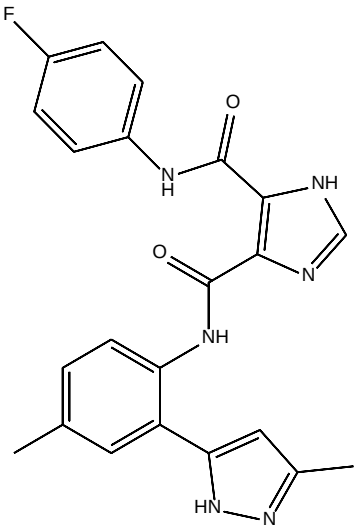
2.2.2.3. Antiviral Activity

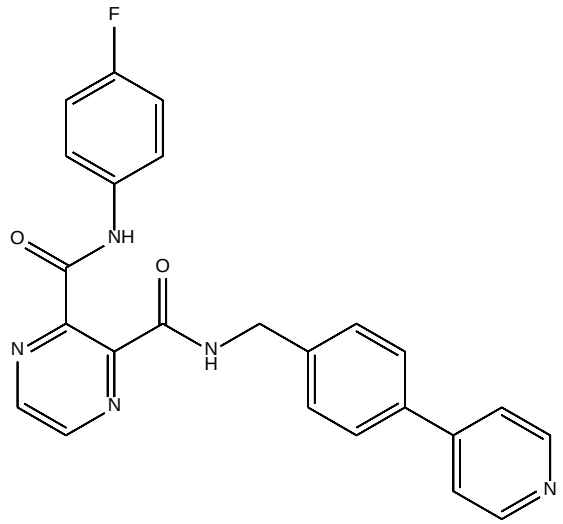
Zmurko *et al*. synthesized N^1-(4-fluorophenyl)-N^2-(4-methyl-2-(3-methyl-1H-pyrazol-5-yl)phenyl)phthalamide, N^1-(4-methyl-2-(thiophen-3-yl)phenyl)-N^2-(p-tolyl)phthalamide and N^1-(4-fluorophenyl)-N^2-(2-(thiophen-3-yl)benzyl)phthalamide which were evaluated in cell-based assays for antiviral activity against the dengue virus and yellow fever virus. Compounds exhibited anti-dengue virus activity with EC_{50} of 0.5, 0.7 and 3 µM, respectively. Compounds demonstrated strong inhibition of yellow fever virus growth with EC_{50} of 23.2, 1.9 and 1.4 µM, respectively [36].

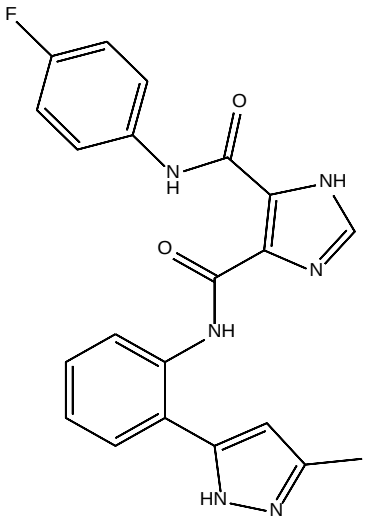




Saudi *et al.* reported the compounds N^5-(4-fluorophenyl)-N^4-(4-methyl-2-(3-methyl-1H-pyrazol-5-yl)phenyl)-1H-imidazole-4,5-dicarboxamide, N^2-(4-fluorophenyl)-N^3-(4-(pyridin-4-yl)benzyl)pyrazine-2,3-dicarboxamide and N^5-(4-fluorophenyl)-N^4-(2-(3-methyl-1H-pyrazol-5-yl)phenyl)-1H-imidazole-4,5-dicarboxamide which were evaluated in cell-based assays for antiviral activity against the dengue virus and yellow fever virus. Compounds exhibited anti-dengue virus activity with EC_{50} of 2.5, 0.94 and 6.03 µM, respectively. Compounds demonstrated strong inhibition of yellow fever virus growth with EC_{50} of 3.47, 7.49 and 1.85 µM, respectively [37].

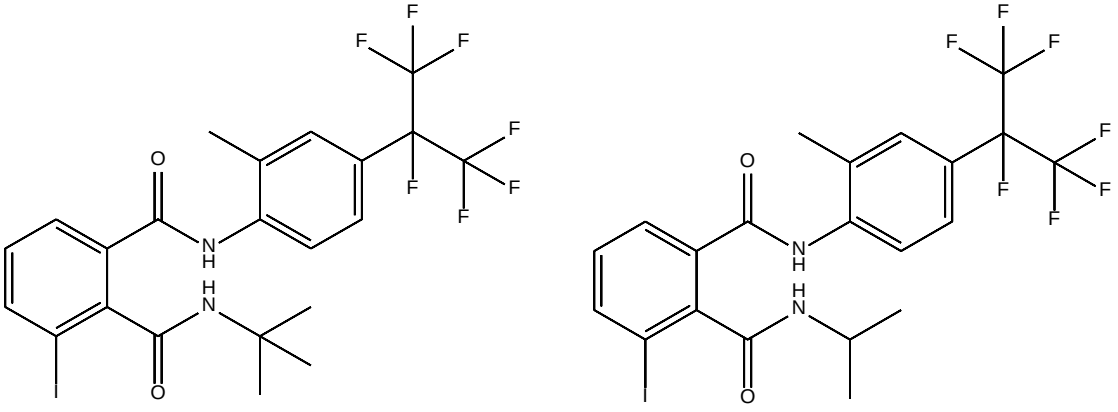




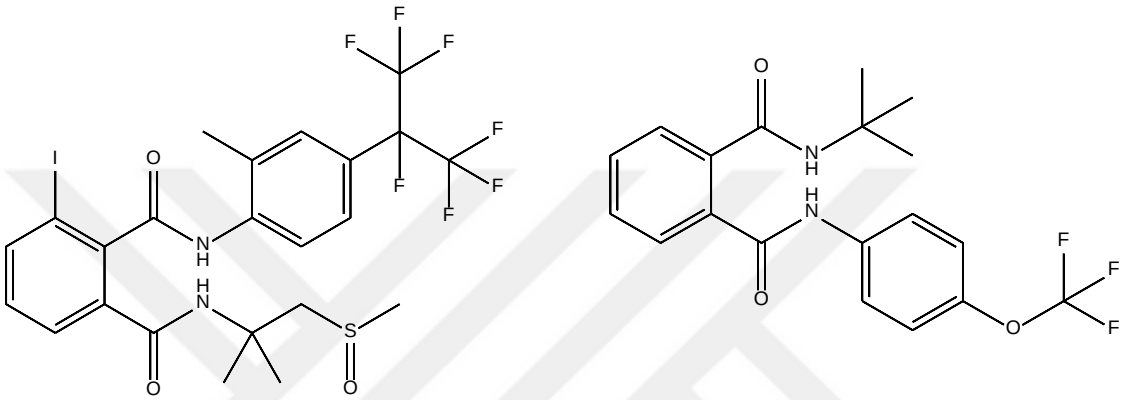


2.2.2.4. Insecticidal Activity

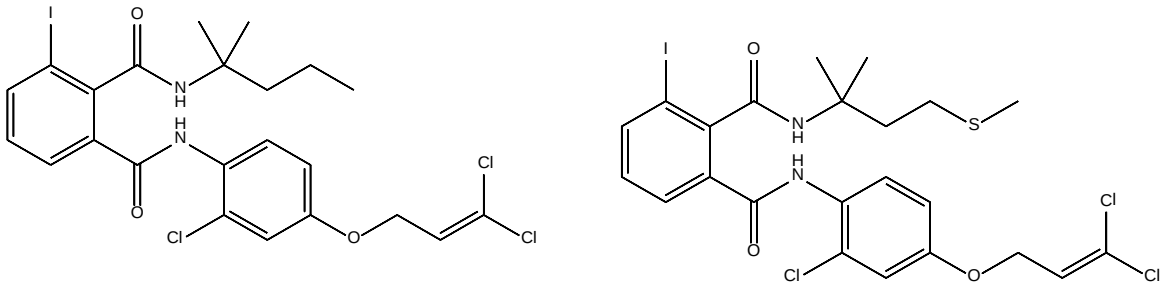
Tosnishi *et al*. prepared N^2-(*tert*-butyl)-3-iodo-N^1-(2-methyl-4-(perfluoropropan-2-yl)phenyl)phthalamide and 3-iodo-N^2-isopropyl-N^1-(2-methyl-4-(perfluoropropan-2-yl)phenyl)phthalamide. The compounds showed the insecticidal activity against *Spodoptera litura* and *Plutella xylostella* larvae. LC_{50} of compounds against *Spodoptera litura* were 0.3 and 0.3 mg/l which were near to Flubendiamide that was 0.03 mg/L. LC_{50} of compounds against *Plutella xylostella* were 0.1 and 0.3 mg/l that were near to Flubendiamide that was 0.01 mg/L [16].

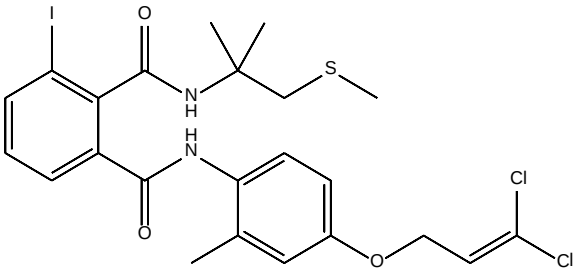


3-iodo-N^1-[(2-methyl-1-(methylsulfinyl)propan-2-yl)-N^2-(2-methyl-4-(perfluoro)propan-2-yl)phenyl]phthalamide and N^1-(tert-butyl)-N^2-(4-(trifluoromethoxy)phenyl]phthalamide were synthesized by Kintscher. The compounds showed the insecticidal activity against *Heliothis virescens* and *Drosophila* larvae. LC_{50} of compounds against *Heliothis virescens* were 30 µM which were similar to Flubendiamide that was 0.3 µM. LC_{50} of compounds against *Drosophila* were 30 µM that were similar to Flubendiamide that was 0.1 µM [11].

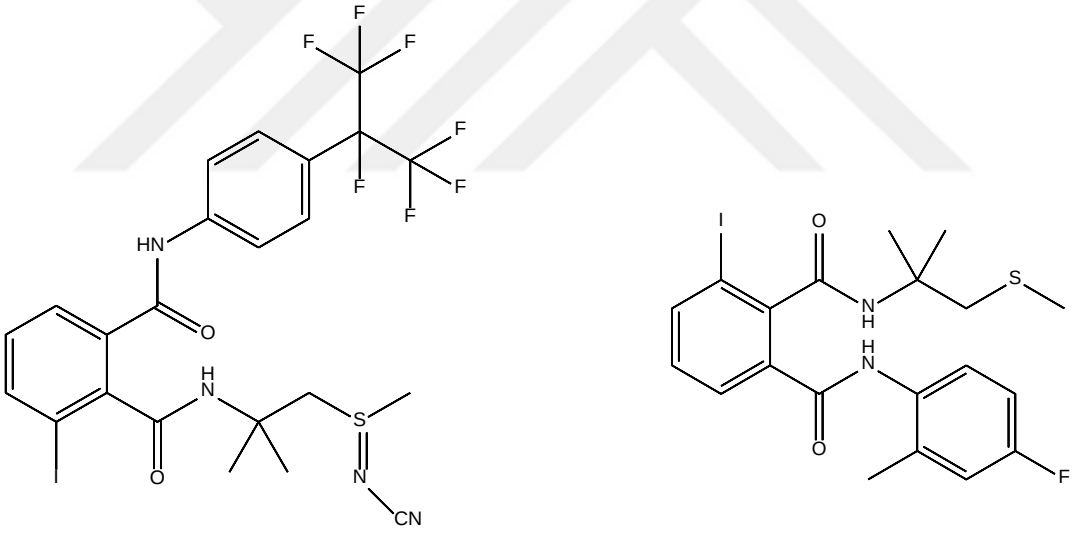


N^1-[2-chloro-4-((3,3-dichloroallyl)oxy)phenyl)-3-iodo-N^2-(2-methylpentan-2-yl]phthalamide, N^1-[2-chloro-4-((3,3-dichloroallyl)oxy)phenyl)-3-iodo-N^2-(2-methyl-4-(methylthio)butan-2-yl]phthalamide and N^1-[4-((3,3-dichloroallyl)oxy)-2-methylphenyl)-3-iodo-N^2-(2-methyl-1-(methylthio)propan-2-yl)]phthalamide were reported by Feng. LC_{50} of compounds against *Plutella xylostella* were 100, 90 and 90 µg/L that were similar to Flubendiamide that was 100 µg/L [38].





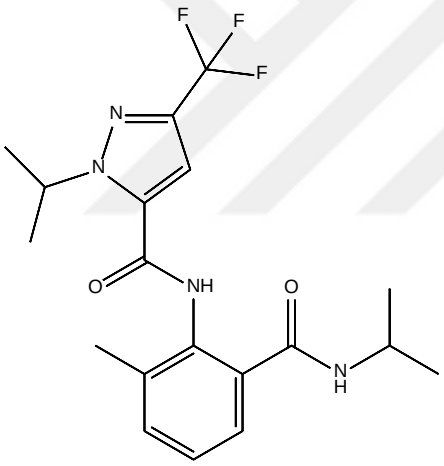
Youwei *et al*. synthesized N^1-[4-(perfluoropropan-2-yl)phenyl)-3-iodo-N^2-2-methyl-1-(((N-methylenecyanamidyl)-methylthio)propan-2-ylphthalamide and N^1-(4-fluoro-2-methylphenyl)-3-iodo-N^2-(2-methyl-1-(methylthio)propan-2-yl)phthalamide. The insecticidal activity of the compounds was tested against *Plutella xylostella* and *Mythimna separata*. LC_{50} of compounds against *Plutella xylostella* were 100 and 90 mg/L, (Flubendiamide was 100 mg/L). LC_{50} of compounds against oriental armyworm were 1.018 and 1.225 mg/L (Flubendiamide was 0.123 mg/L) [14].

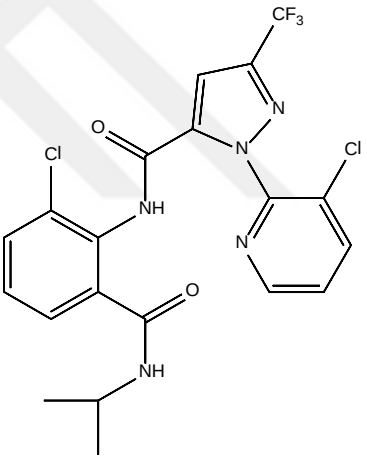


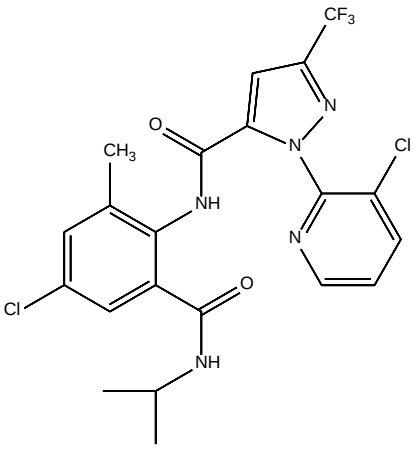
Youwei *et al*. was also applied the calcium-imaging technique to investigate the effects of compounds on the intracellular calcium ion concentration (Ca^{2+}), which indicated that they released stored calcium ions from endoplasmic reticulum, which denoted that some compounds are potential modulators of the insect ryanodine receptor (RyR). Compared with the control, the calcium concentrations in the neurons elevated by the compounds are well correlated with the insecticidal against oriental armyworms. The compounds were examined on central neurons of *Spodoptera exigua* larvae on the calcium homeostasis with calcium imaging technique after neuron loading with Fluo-3

AM according to previous method. Treated with 10 mg/L of the compounds and Flubendiamide to isolated *Spodoptera exigua* neurons caused an increase in the cytosolic calcium concentration. The peak of Ca^{2+} was increased to, 110.7 %, 113.1 %, 103.4 % and 120.3 % of the initial value after the neurons were treated with compounds and Flubendiamide for 30 s, respectively [14].

Lahm *et al*. studied the insecticidal activity of 1-isopropyl-N-(2-(isopropylcarbamoyl)-6-methylphenyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide and N-(4-chloro-2-(isopropylcarbamoyl)-6-methylphenyl)-1-(3-chloropyridin-2-yl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide. The compounds showed the insecticidal activity against *Plutella xylostella*, *Spodoptera frugiperda* and *Heliothis virescens*. LC_{50} of compounds against *Plutella xylostella* were 11.1, 0.1 and 0.01 ppm. LC_{50} of compounds against *Spodoptera frugiperda* were 22.8, 0.1 and 0.03 ppm. LC_{50} of compounds against *Heliothis virescens* were 36.4, 0.4 and 0.02 ppm, respectively [6].

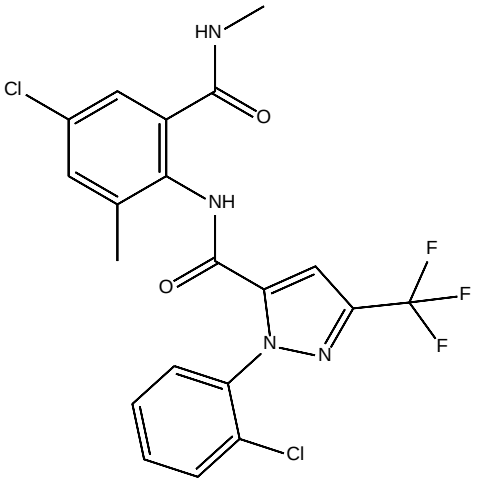


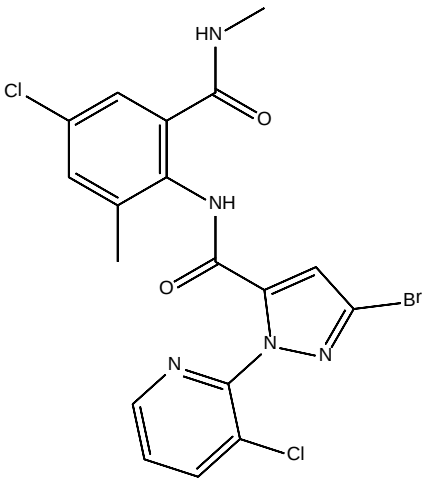


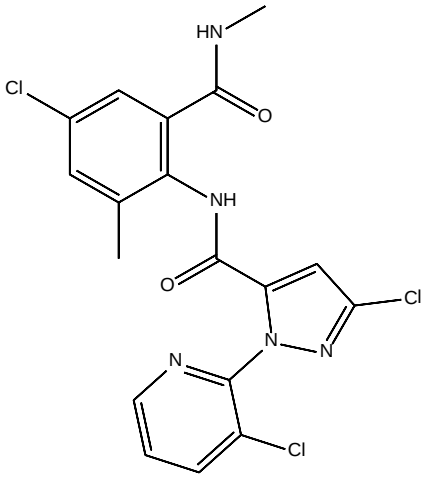


Furthermore, Lahm *et al*. was applied to exhibit the compounds action by release of intracellular Ca^{2+} stores mediated by the ryanodine receptor. A subset of compounds was tested in a calcium mobilization assay, using neurons from the American cockroach, Periplaneta Americana, to assess activity at the insect ryanodine receptor. This assay demonstrates the ability of anthranilic diamide to release internal calcium stores while failing to activate voltage-gated calcium channels. Furthermore, the calcium mobilization induced by anthranilic diamide was blocked following ryanodine treatment, consistent with action at the ryanodine receptor. Insecticidal activity was found to correlate with potency in mobilizing calcium. These studies have confirmed the mode of action for the compounds to be ryanodine activation and this chemistry has been found to exhibit selectivity for insect receptors [6].

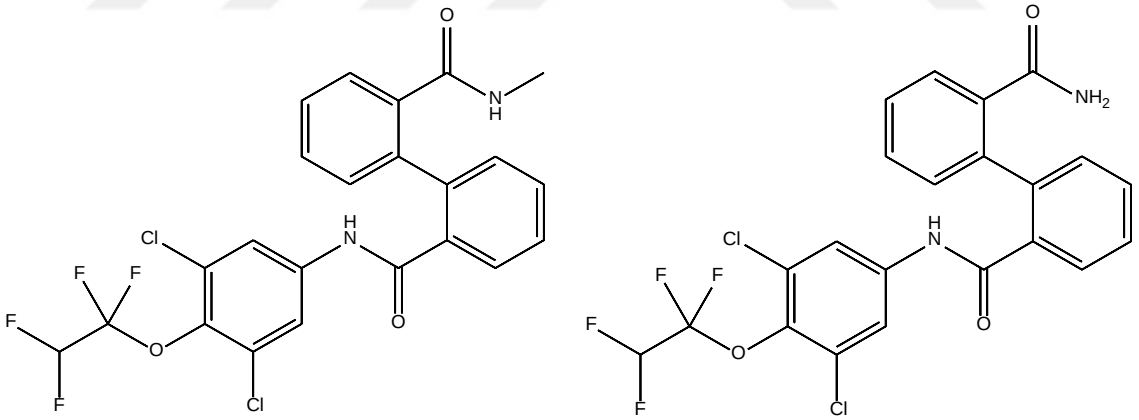
Lahm *et al*. also reported the insecticidal activity of N-(4-chloro-2-methyl-6-(methylcarbamoyl)phenyl)-1-(2-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide, 3-bromo-N-(4-chloro-2-methyl-6-(methylcarbamoyl)phenyl)-1-(3-chloropyridin-2-yl)-1H-pyrazole-5-carboxamide and 3-chloro-N-(4-chloro-2-methyl-6-(methylcarbamoyl)phenyl)-1-(3-chloropyridin-2-yl)-1H-pyrazole-5-carboxamide. The compounds showed the insecticidal activity against fall armyworm, diamondback moth, and tobacco budworm on a broad spectrum of Lepidoptera. LC_{50} of compounds against *Spodoptera frugiperda* were 0.02, 0.02 and 0.03 ppm. LC_{50} of compounds against *Plutella xylostella* were 0.01, 0.02 and 0.03 ppm. LC_{50} of compounds against *Heliothis virescens* were 0.05, 0.04 and 0.07 ppm, respectively [18].







The insecticidal activity of N^2-(3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl)-$N^{2'}$-methyl-(1,1'-biphenyl)-2,2'-dicarboxamide and N^2-(3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl)-(1,1'-biphenyl)-2,2'-dicarboxamide were found against *Mythimna sepatara* by Liu. LC_{50} of compounds were 100 and 75 mg/l which were similar to Flubendiamide that was 10 mg/l [9].



3. MATERIALS AND METHODS

3.1. Chemistry

3.1.1. Materials

All materials were commercially available and used without further purification. 3-nitrophthalic anhydride, 2-phenylethylamine, 2-chlorophenylethylamine, 3-chlorophenylethylamine, 4-chlorophenylethylamine, 2,4-dichlorophenylethylamine, 2-fluorophenylethylamine, 3-fluorophenylethylamine, 4-fluorophenylethylamine, 2-(4-methoxy)phenylethylamine, 2-(4-morpholino)phenylethylamine, 1-phenylethylamine were purchased from Sigma Aldrich Chemical Corporation.

3.1.2. Methods of Synthesis

3.1.2.1. General Procedure A: Synthesis of Phthalamide Derivatives

The compounds were prepared through condensation reaction between 1 mmol phthalic anhydride and 2 mmol phenylethylamine derivatives in 20 ml ethanol under reflux. After 6 hours, the reaction mixture wass evaporated under reduced pressure. The obtained crude products were crystallized in ethanol then filtered and dried in vacuum [39].

3.1.2.2. General Procedure B: Synthesis of Nitrophthalamide Derivatives

The compounds were prepared through condensation reaction between 2 mmol 3-nitrophthalic anhydride and 4 mmol phenylethylamine derivatives in 20 ml methanol under reflux. After 8 hours, the reaction mixture was evaporated under reduced pressure. The solid was dissolved in 10 mL $CHCl_3$, and washed with HCl solution (3%), NaOH solution (2%), and H_2O until neutral. The $CHCl_3$ was distilled off. The obtained products were filtered and dried in vacuum.

3.1.3. Analytical Methods

3.1.3.1. Melting Point Determination

Melting points of the compounds were determined by an electro thermal melting point apparatus (Mettler Toledo FP62) in open capillary tubes and are uncorrected.

3.1.3.2. Controls by Thin Layer Chromatography (TLC) Solvent Systems

Merck Kieselgel 60 F_{254} silica gel plaques were used for thin layer chromatography in this thesis.

Solvent systems were used for the chromatographic controls of the compounds are given below with their codes which given in the results part.

S_1: Ethyl acetate : Methanol (50: 50)

S_2: Benzene : Methanol (50: 50)

S_3: Chloroform : Methanol (60: 40)

S_4: Dichloromethane : Methanol (60: 40)

The saturation of the tanks with solvent vapors was achieved after 24 hours from first addition of the solvents. All compounds were dissolved in dimethylsulfoxide (DMSO) then applied on silica gel. After dragging the compounds for 10 cm at 20°C, R_f values were calculated. UV light at 254 nm was used for the detection of the spots.

3.1.3.3. Purification by Column Chromatography

System was used for the column chromatography of the compounds are given below which given in the results part.

Stationary phase: Silica gel - 60 mesh

Mobile phase: Ethyl acetate : Methanol (50:50)

Column was filled in accordance with wet method. Elution was controlled with TLC using silica gel plates and ethyl acetate: methanol (50:50).

3.1.3.4. Spectrometric Analysis

3.1.3.4.1. Infrared Spectroscopy

Infrared (IR) spectra with $10T/cm^2$ pressure applied potassium bromide pellets were recorded on a Perkin Elmer FT-IR 1720X spectrometer and the frequencies were expressed in cm^{-1}.

3.1.3.4.2. UV Spectroscopy

UV spectra were recorded at concentration of 2×10^{-5} M in methanol with quartz cell of path length 1 cm by UV-VIS Agilent 8453 spectrometer.

3.1.3.4.3. ^{1}H-NMR Spectroscopy

^{1}H-NMR spectra was obtained from 10% solution of the compounds in deutrated-dimethylsulphoxide (DMSO-d_6) Bruker AC 400 MHz spectrometer. All chemical shift values were given in parts per million (ppm) relative to a tetramethylsilane (TMS) reference.

3.1.3.4.4. ^{13}C-NMR Spectroscopy

^{13}C-NMR spectra were recorded with a Varian Mercury-400 FT-NMR spectrometer with dimethyl sulfoxide (DMSO-d_6) as solvent. All chemical shift values were given in parts per million (ppm) relative to a tetramethylsilane (TMS) reference.

3.1.3.4.5. Elemental Analysis

Elemental analysis was performed on LECO 932 CHNS (Leco-932, St. Joseph, MI, USA) instrument.

3.2. Biological Assays

3.2.1. Antimicrobial Activity Test Procedure

The minimum inhibitory concentrations (MICs) of compounds were determined by the conventional agar dilution method. The quinolone antibacterial agent Ofloxacin was used as reference drug. The test compounds (10.0 mg) were dissolved in DMSO (1 mL) and then diluted with distilled water (9 mL) for preparation of stock solution. Further progressive two-fold serial dilution with molten sterile Mueller–Hinton agar was performed to obtain the required concentrations of 100, 50, 25, 12.5, 6.25, 3.13, 1.56 μg/mL. The medium containing the test compounds was dispensed into a sterile Petri-dish and allowed to solidify. Petri-dishes were inoculated with 1.5×10^4 CFU and incubated at 37 °C for 18 h. The MIC was defined as the lowest concentration of the test compound, which resulted in no visible growth.

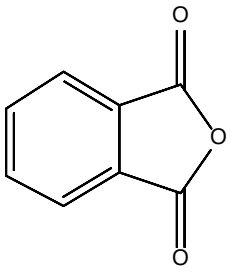
3.2.2. Anticancer Activity Test Procedure

Anticancer activity of compounds was tested against 2 different cancer cell lines. Liver (Hep3B) and breast (MCF7) cells (ATCC, USA) were maintained in DMEM (Gibco, England), supplemented with 10% FBS (Gibco, USA) and 1% streptomycin and penicillin (Gibco, USA) at 37°C in 5% CO_2.Cells were seeded on 48-well plates at the density of $2x10^4$ cell per mL in a 48 well-plate and incubated for 24 hours at 37°C in 5% CO_2. All compounds have been solubilized in dimethyl sulfoxide (DMSO) and kept protected from sunlight in room temperature. As reference compound, doxorubicin (DOX) was used. Cells were exposed to different concentrations of compounds and DOX and incubated for 72 hours in 5% CO_2 and at 37ºC. At the end of the incubation period, supernatants were discarded and MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) was applied as 0.5 µg/mL. After 2 hours, MTT was discarded from the plate wells and isopropanol was added to dissolve blue formazan. Absorbance of the MTT formazan was determined at 540 nm by a UV-spectrophotometric plate reader (Thermo Multiscan Spectrum, Finland). Viability was defined as the ratio (expressed as a percentage) of absorbance of the cells exposed to compounds to the cells treated with 0.5% DMSO (as control). All measurements were done in triplicates.

4. EXPERIMENTAL

4.1. Chemical Data

4.1.1. Synthesis of Phthalic Anhydride (CAS Registry Number: 85-44-9)



5 g (33 mmol) Phthalic Acid was heated in the beaker at approximately 180 ºC. When the temperature raises some of the phthalic anhydride was vaporized forming filamentous crystals around the wall of the beaker to yield phthalic anhydride. Phthalic anhydride was obtained with the yield of 65%. Phthalic anhydride is white powdered compound. Melting point of the compound is 131 ºC (Lit: 130.8 ºC).

IR (KBr, V_{max}, cm^{-1}): 3092 (C-H, Aromatic), 1900 and 1850 (C=O, Anhydride), 1258 and 1112 (C-O).

UV (MeOH, λ_{max}, nm): 287 (log ε: 6.4), 295 (log ε: 6.6).

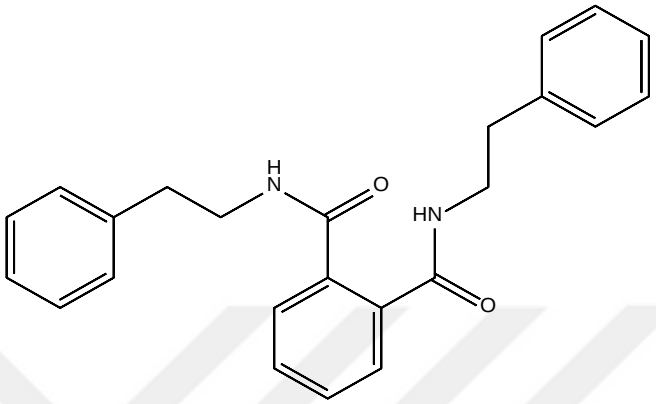
^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 7.92-8.04 (m, 4H, Aromatic **CH**).

Elemental analysis for $C_8H_4O_3$ (148.12 g/mol);

	%C	%H	%O
Calculated	64.87	2.72	32.41
Found	64.80	2.65	32.55

4.1.2. Synthesis of Phthalamide Derivatives

N^1,N^2-bis[(2-phenyl)ethyl]phthalamide (Compound 1) (CAS Registry Number: 38229-00-4)



0.150 g (1 mmol) Phthalic Anhydride and 0.182 g (2 mmol) [(2-phenyl)ethyl]amine were reacted as described in the general procedure A. The yield is 0.265 g (79%). The compound is a white crystal, soluble in cold methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.87 and 0.90 in the solvent systems S_1 and S_2, respectively. Melting point of the compound is 163.2 ºC.

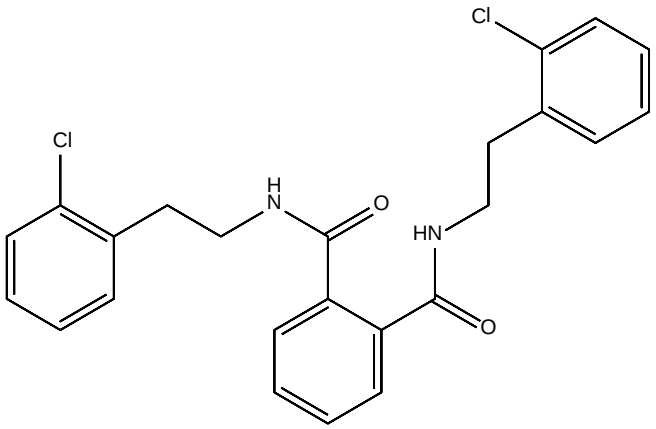
IR (KBr, V_{max}, cm^{-1}): 3256 (N-H), 3074 (C-H, Aromatic), 2919 (C-H, Aliphatic), 1595 and 1575 (C=O, Amide).

UV (MeOH, λ_{max}, nm): 204 (log ε: 6.90), 254 (log ε: 6.99).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.83 (t, 4H, CH_2-**CH_2**-Ar, *J*=6.8 Hz), 3.45 (q, 4H, NH-**CH_2**-CH_2, *J*=7.2 Hz), 7.20-7.50 (m, 14H, Aromatic **CH**), 8.35 (t, 2H, **NH**-C=O, *J=5.6* Hz).

^{13}C-NMR (DMSO, δ, ppm): 136.3 (C^1), 136.3 (C^2), 127.5 (C^3), 129.2 (C^4), 129.2 (C^5), 127.5 (C^6), 168.0 (C^7), 40.7 (C^8), 34.9 (C^9), 139.5 (C^{10}), 128.3 (C^{11}), 128.6 (C^{12}), 126.0 (C^{13}), 128.6 (C^{14}), 128.3 (C^{15}), 168.0 (C^{16}), 40.7 (C^{17}), 34.9 (C^{18}), 139.5 (C^{19}), 128.6 (C^{20}), 128.6 (C^{21}), 126.0 (C^{22}), 128.6 (C^{23}), 128.6 (C^{24}).

***N*[1],*N*[2]-bis[2-(2-chlorophenyl)ethyl]phthalamide (Compound 2)**



0.150 g (1 mmol) Phthalic Anhydride and 0.233 g (2 mmol) [2-(2-chlorophenyl)ethyl]amine were reacted as described in the general procedure A. The yield is 0.133 g (35%). The compound is a white powder, soluble in cold methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.88 and 0.91 in the solvent systems S_1 and S_2, respectively. Melting point of the compound is 198.2 ºC.

IR (KBr, V_{max}, cm^{-1}): 3236 (N-H), 3073 (C-H, Aromatic), 2930 (C-H, Aliphatic), 1591 and 1571 (C=O, Amide).

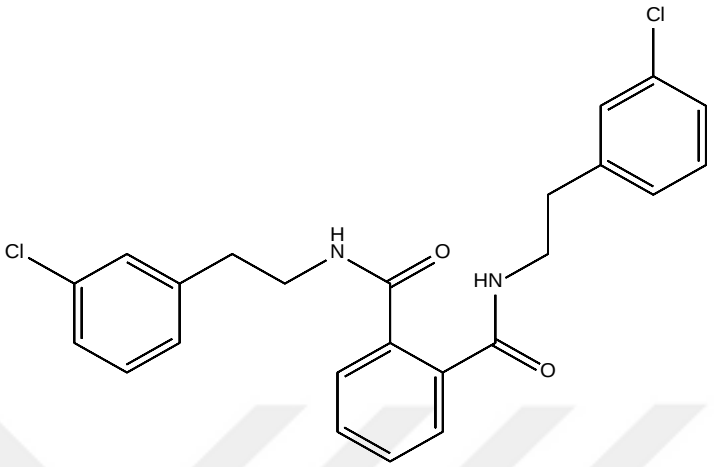
UV (MeOH, λ_{max}, nm): 203 (log ε: 7.17), 280 (log ε: 7.32).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.9 (t, 4H, CH_2-**CH_2**-Ar, *J*=7.2 Hz), 3.47 (q, 4H, NH-**CH_2**-CH_2, *J*=7.6 Hz), 7.15-7.5 (m, 12H, Aromatic **CH**), 8.4 (t, 2H, **NH**-C=O, *J*=5.6 Hz).

Elemental analysis for $C_{24}H_{22}Cl_2N_2O_2$ (441.35 g/mol);

	%C	%H	%N
Calculated	65.31	5.02	6.35
Found	65.12	5.08	6.32

***N*[1],*N*[2]-bis[2-(3-chlorophenyl)ethyl]phthalamide (Compound 3) (CAS Registry Number: 548439-88-9)**



0.150 g (1 mmol) Phthalic Anhydride and 0.233 g (2 mmol) [2-(3-chlorophenyl)ethyl]amine were reacted as described in the general procedure A. The yield is 0.284 g (73%). The compound is a white powder, soluble in cold methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.86 and 0.88 in the solvent systems S_1 and S_2, respectively. Melting point of the compound is 159.5 ºC.

IR (KBr, V_{max}, cm^{-1}): 3237 (N-H), 3073 (C-H, Aromatic), 2935 (C-H, Aliphatic), 1590 and 1551 (C=O, Amide).

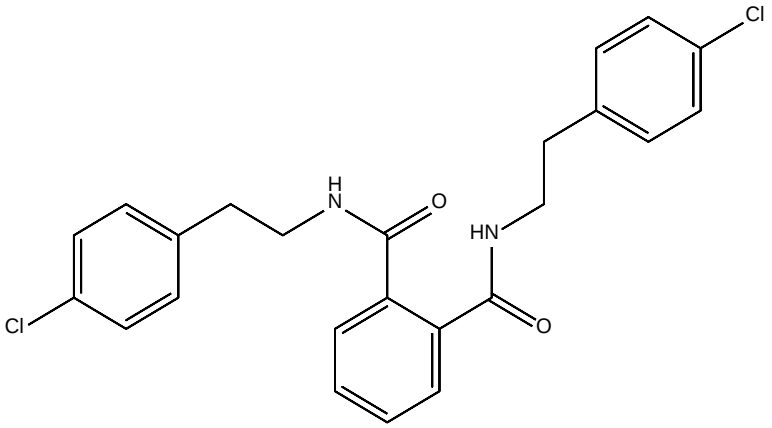
UV (MeOH, λ_{max}, nm): 203 (log ε: 7.17), 272 (log ε: 7.30).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.8 (t, 4H, CH_2-**CH_2**-Ar, *J*=7.8 Hz), 3.4 (q, 4H, NH-**CH_2**-CH_2, *J*=8.0 Hz), 7.2-7.48 (m, 12H, Aromatic **CH**), 8.32 (t, 2H, **NH**-C=O, *J*=4.0 Hz).

Elemental analysis for $C_{24}H_{22}Cl_2N_2O_2$ (441.35 g/mol);

	%C	%H	%N
Calculated	65.31	5.02	6.35
Found	65.11	4.89	6.54

N^1,N^2-bis[2-(4-chlorophenyl)ethyl]phthalamide (Compound 4)



0.150 g (1 mmol) Phthalic Anhydride and 0.233 g (2 mmol) [2-(4-chlorophenyl)ethyl]amine were reacted as described in the general procedure A. The yield is 0.182 g (47%). The compound is a white crystal, soluble in cold methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.79 and 0.76 in the solvent systems S_1 and S_2, respectively. Melting point of the compound is 143.1 ºC.

IR (KBr, V_{max}, cm^{-1}): 3237 (N-H), 3075 (C-H, Aromatic), 2975 (C-H, Aliphatic), 1590 and 1551 (C=O, Amide).

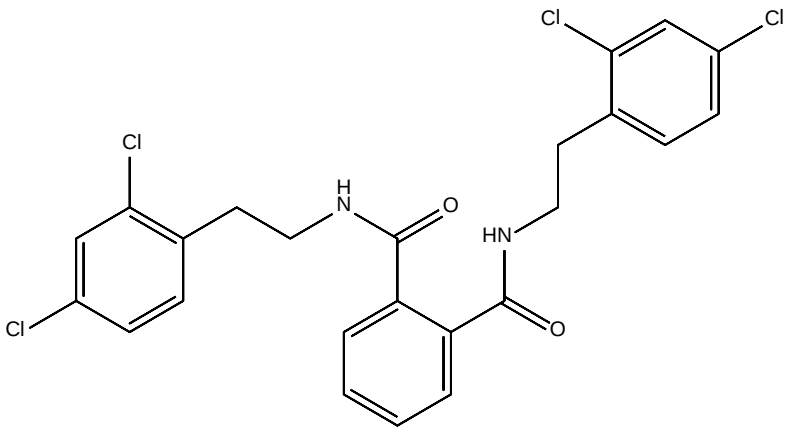
UV (MeOH, λ_{max}, nm): 204 (log ε: 7.18), 276 (log ε: 7.31).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.81 (t, 4H, CH_2-**CH_2**-Ar, *J*=7.8 Hz), 3.38 (q, 4H, NH-**CH_2**-CH_2, *J*=8.0 Hz), 7.2-7.48 (m, 12H, Aromatic **CH**), 8.35 (t, 2H, **NH**-C=O, *J*=5.6 Hz).

Elemental analysis for $C_{24}H_{22}Cl_2N_2O_2$ (441.35 g/mol);

	%C	%H	%N
Calculated	65.31	5.02	6.35
Found	65.87	5.42	6.11

***N*[1],*N*[2]-bis[2-(2,4-dichlorophenyl)ethyl]phthalamide (Compound 5)**



0.150 g (1 mmol) Phthalic Anhydride and 0.285 g (2 mmol) [2-(2,4-dichlorophenyl)ethyl]amine were reacted as described in the general procedure A. The yield is 0.308 g (70%). The compound is a white powder, soluble in cold methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.885 and 0.895 in the solvent systems S_1 and S_2, respectively. Melting point of the compound is 164.6 ºC.

IR (KBr, V_{max}, cm^{-1}): 3235 (N-H), 3081 (C-H, Aromatic), 2935 (C-H, Aliphatic), 1585 and 1551 (C=O, Amide).

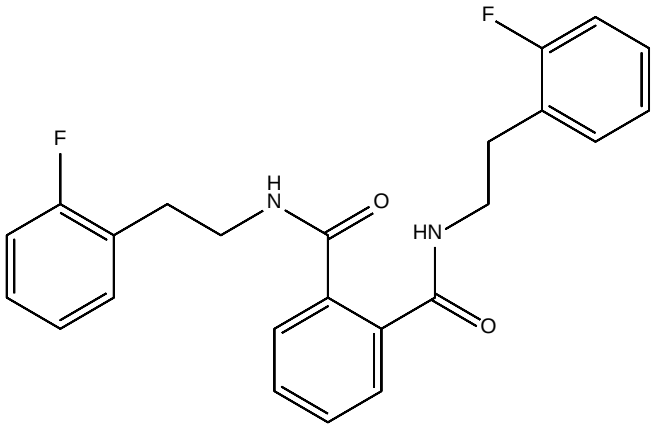
UV (MeOH, λ_{max}, nm): 203 (log ε: 7.24), 220 (log ε: 7.27).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.92 (t, 2H, CH_2-**CH_2**-Ar, *J*=4.0 Hz), 3.03 (t, 2H, CH_2-**CH_2**-Ar, *J*=8.0 Hz), 3.42 (q, 2H, NH-**CH_2**-CH_2, *J*= 8.0 Hz), 3.84 (q, 2H, NH-**CH_2**-CH_2, *J*=8.0 Hz), 7.2-7.84 (m, 10H, Aromatic **CH**), 8.2 (t, 2H, **NH**-C=O, *J*=5.6 Hz).

Elemental analysis for $C_{24}H_{20}Cl_4N_2O_2$ (441.35 g/mol);

	%C	%H	%N
Calculated	64.7	4.92	17.98
Found	65.54	4.69	17.33

***N*^{1},*N*^{2}-bis[2-(2-fluorophenyl)ethyl]phthalamide (Compound 6)**



0.150 g (1 mmol) Phthalic Anhydride and 0.208 g (2 mmol) [2-(2-fluorophenyl)ethyl]amine were reacted as described in the general procedure A. The yield is 0.268 g (74%). The compound is a white powder, soluble in cold methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.88 and 0.865 in the solvent systems S_1 and S_2, respectively. Melting point of the compound is 200.7 ºC.

IR (KBr, V_{max}, cm^{-1}): 3238 (N-H), 3079 (C-H, Aromatic), 2880 (C-H, Aliphatic), 1592 and 1556 (C=O, Amide).

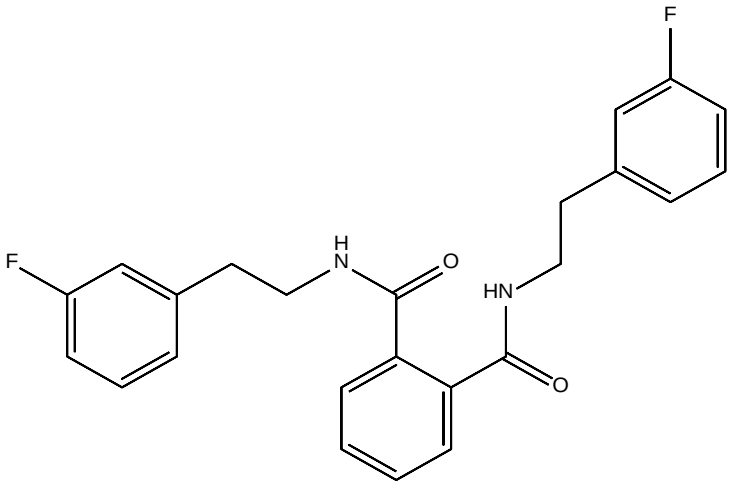
UV (MeOH, λ_{max}, nm): 204 (log ε: 7.14), 262 (log ε: 7.25).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.85 (t, 4H, CH_2-**CH_2**-Ar, *J*=7.2 Hz), 3.45 (q, 4H, NH-**CH_2**-CH_2, *J*=6.8 Hz), 7.15-7.5 (m, 12H, Aromatic **CH**), 8.4 (t, 2H, **NH**-C=O, *J*=6.0 Hz).

Elemental analysis for $C_{24}H_{22}F_2N_2O_2$ (408.44 g/mol);

	%C	%H	%N
Calculated	70.57	5.43	6.86
Found	70.52	5.42	6.85

N^1,N^2-bis[2-(3-fluorophenyl)ethyl]phthalamide (Compound 7)



0.150 g (1 mmol) Phthalic Anhydride and 0.208 g (2 mmol) [2-(3-fluorophenyl)ethyl]amine were reacted as described in the general procedure A. The yield is is 0.128 g (36%). The compound is a white crystal, soluble in cold methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.88 and 0.87 in the solvent systems S_1 and S_2, respectively. Melting point of the compound is 131 ºC.

IR (KBr, V_{max}, cm^{-1}): 3236 (N-H), 3080 (C-H, Aromatic), 2878 (C-H, Aliphatic), 1591 and 1554 (C=O, Amide).

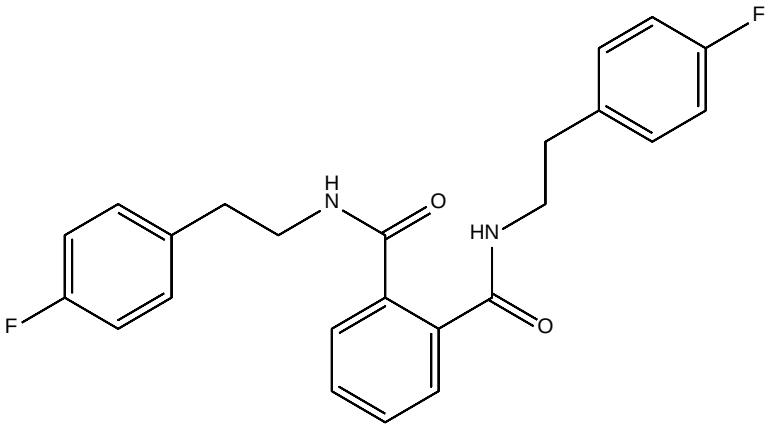
UV (MeOH, λ_{max}, nm): 204 (log ε: 7.14), 263 (log ε: 7.25).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.85 (t, 4H, CH_2-**CH_2**-Ar, *J*=7.2 Hz), 3.45 (q, 4H, NH-**CH_2**-CH_2, *J*=7.0 Hz), 7.1-7.5 (m, 12H, Aromatic **CH**), 8.4 (t, 2H, **NH**-C=O, *J*=6.0 Hz).

Elemental analysis for $C_{24}H_{22}F_2N_2O_2$ (408.44 g/mol);

	%C	%H	%N
Calculated	70.57	5.43	6.86
Found	70.46	5.31	6.73

***N*[1],*N*[2]-bis[2-(4-fluorophenyl)ethyl]phthalamide (Compound 8)**



0.150 g (1 mmol) Phthalic Anhydride and 0.208 g (2 mmol) [2-(4-fluorophenyl)ethyl]amine were reacted as described in the general procedure A. The yield is 0.262 g (72%). The compound is a white crystal, soluble in cold methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.875 and 0.89 in the solvent systems S_1 and S_2, respectively. Melting point of the compound is 123.4 ºC.

IR (KBr, V_{max}, cm^{-1}): 3259 (N-H), 3067 (C-H, Aromatic), 2877 (C-H, Aliphatic), 1538 and 1512 (C=O, Amide).

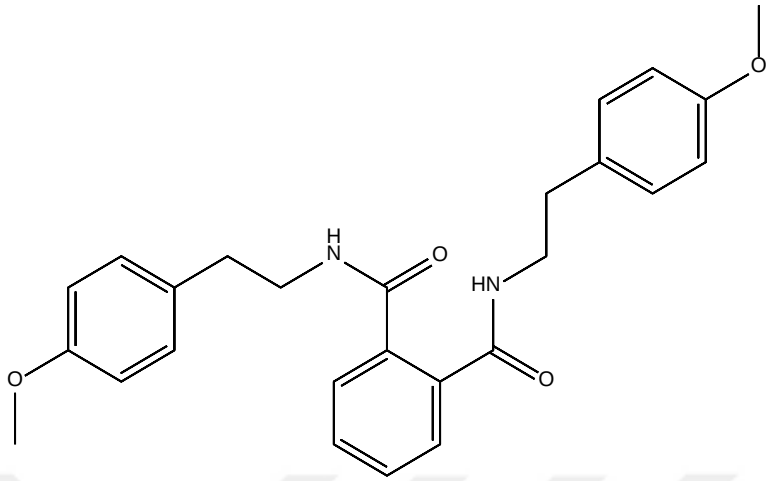
UV (MeOH, λ_{max}, nm): 204 (log ε: 7.14), 265 (log ε: 7.25).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.85 (t, 4H, CH_2-**CH_2**-Ar, *J*=6.8 Hz), 3.45 (q, 4H, NH-**CH_2**-CH_2, *J*=7.2 Hz), 7.25-7.6 (m, 12H, Aromatic **CH**), 8.03 (t, 2H, **NH**-C=O, *J*=6.0 Hz).

Elemental analysis for $C_{24}H_{22}F_2N_2O_2$ (408.44 g/mol);

	%C	%H	%N
Calculated	70.57	5.43	6.86
Found	69.79	5.83	6.83

***N*[1],*N*[2]-bis[2-(4-methoxyphenyl)ethyl]phthalamide (Compound 9) (CAS Registry Number: 547711-55-7)**



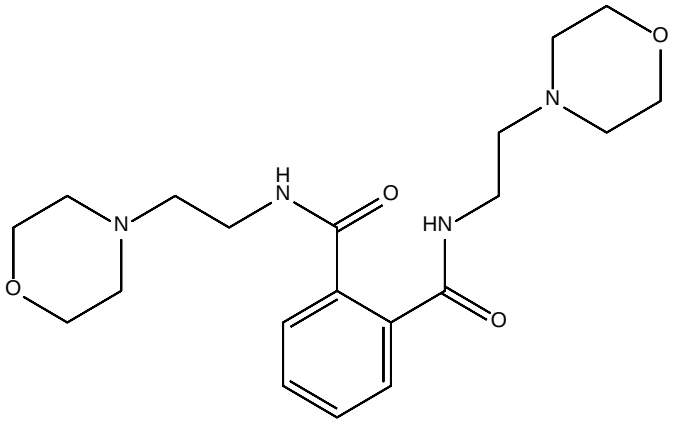
0.150 g (1 mmol) Phthalic Anhydride and 0.226 g (2 mmol) [2-(4-methoxyphenyl)ethyl]amine were reacted as described in the general procedure A. The yield is 0.207 g (55%). The compound is a white powder, soluble in cold methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.93 and 0.91 in the solvent systems S_1 and S_2, respectively. Melting point of the compound is 139.6 ºC.

IR (KBr, V_{max}, cm^{-1}): 3289 (N-H), 3060 (C-H, Aromatic), 2836 (C-H, Aliphatic), 1576 and 1544 (C=O, Amide).

UV (MeOH, λ_{max}, nm): 202 (log ε: 7.16), 225 (log ε: 7.21).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.75 (t, 4H, CH_2-**CH_2**-Ar, *J*=6.8 Hz), 3.4 (q, 4H, NH-**CH_2**-CH_2, *J*=7.2 Hz), 3.8 (s, 6H, O-**CH_3**), 7.2-7.5 (m, 12H, Aromatic **CH**), 8.35 (t, 2H, **NH**-C=O, *J*=5.2Hz).

***N*[1],*N*[2]-bis[2-(4-morpholino)ethyl]phthalamide (Compound 10) (CAS Registry Number: 570429-11-7)**



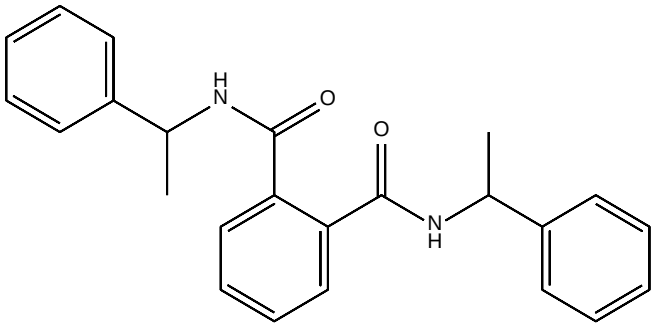
0.150 g (1 mmol) Phthalic Anhydride and 0.195 g (2 mmol) [2-(4-morpholino)ethyl]amine were reacted as described in the general procedure A. The product was purified by column chromatography. The yield is 0.072 g (21%). The compound is a white powder, soluble in cold methanol, ethanol, acetone, DMSO and DCM and it is insoluble in water. R_f values were calculated as 0.87 and 0.88 in the solvent systems S_1 and S_2, respectively. Melting point of the compound is 119.7 ºC.

IR (KBr, V_{max}, cm^{-1}): 3278 (N-H), 3054 (C-H, Aromatic), 2794 (C-H, Aliphatic), 1636 and 1543 (C=O, Amide).

UV (MeOH, λ_{max}, nm): 219 (log ε: 7.15), 240 (log ε: 7.19).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.3 (m, 8H, N-**CH_2**, *J*=4.4 Hz), 2.6 (t, 4H, CH_2-**CH_2**, *J*=6.8 Hz), 3.3 (q, 4H, NH-**CH_2**-CH_2, *J*=6.8 Hz), 3.7 (m, 8H, O-**CH_2**, *J*=6.4 Hz), 6.9-7.5 (m, 4H, Aromatic **CH**), 8.1 (t, 2H, **NH**-C=O, *J*=5.6 Hz).

N^1,N^2-bis[(1-phenyl)ethyl]phthalamide (Compound 11) (CAS Registry Number: 256417-20-6)



0.150 g (1 mmol) Phthalic Anhydride and 0.182 g (2 mmol) [(1-phenyl)ethyl]amine were reacted as described in the general procedure A. The yield is 0.180 g (48%). The compound is a transparent liquid, soluble in cold methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.92 and 0.89 in the solvent systems S_1 and S_2, respectively.

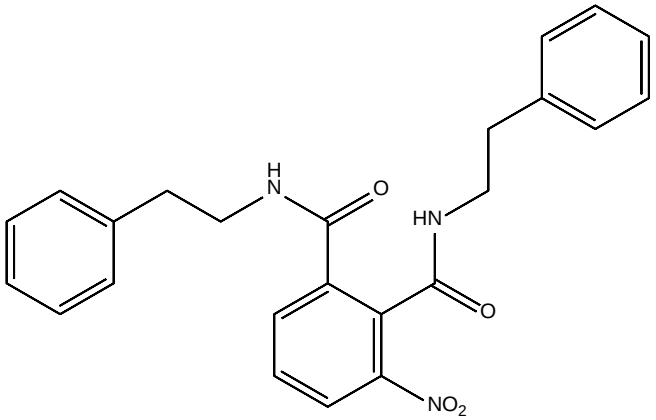
IR (KBr, V_{max}, cm^{-1}): 3256 (N-H), 3074 (C-H, Aromatic), 2919 (C-H, Aliphatic), 1595 and 1575 (C=O, Amide).

UV (MeOH, λ_{max}, nm): 204 (log ε: 6.89), 252 (log ε: 6.98).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 1.47 (d, 6H, CH-**CH_3**, *J*=4.0Hz), 4.95 (m, 2H, NH-**CH**, *J*=7.2 Hz), 7.26-7.41 (m, 14H, Aromatic **CH**), 8.07 (t, 2H, **NH**-C=O, *J*=8.0 Hz).

4.1.3. Synthesis of Nitrophthalamide Derivatives

3-nitro-N^1,N^2-bis[(2-phenyl)ethyl]phthalamide (Compound 12)



0.400 g (2 mmol) 3-Nitrophthalic Anhydride and 0.363 g (4 mmol) [(2-phenyl)ethyl]amine were reacted as described in the general procedure B. The yield is 0.1056 g (24%). The compound is a yellow powder, soluble in hot methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.91 and 0.95 in the solvent systems S_3 and S_4, respectively. Melting point of the compound is 177.2 ºC.

IR (KBr, V_{max}, cm^{-1}): 3236 (N-H), 3070 (C-H, Aromatic), 2930 (C-H, Aliphatic), 1579 and 1547 (C=O, Amide), 1571 and 1316 (NO_2).

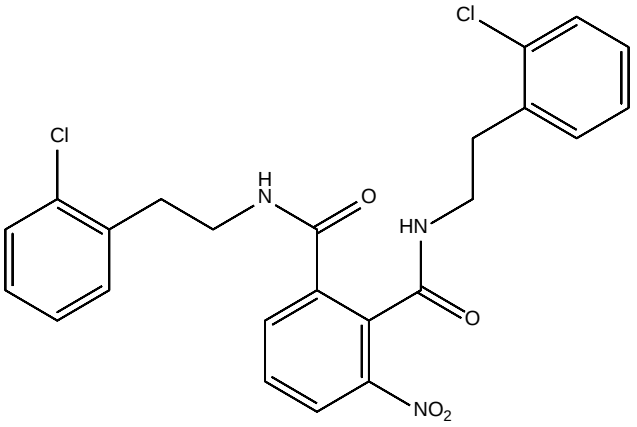
UV (MeOH, λ_{max}, nm): 207 (log ε: 7.16), 212 (log ε: 7.17).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.75 (t, 2H, CH_2-**CH_2**-Ar, *J*=7.6 Hz), 2.82 (t, 2H, CH_2-**CH_2**-Ar, *J*=8.0 Hz), 3.3 (q, 2H, NH-**CH_2**, *J*=6.8 Hz), 3.42 (q, 2H, NH-**CH_2**, *J*=6.8 Hz), 7.28-8.05 (m, 13H, Aromatic **CH**), 8.2 and 8.3 (t, 2H, **NH**-C=O, *J*=5.2 Hz and *J*=5.6 Hz).

Elemental analysis for $C_{24}H_{23}N_3O_4$ (417.46 g/mol);

	%C	%H	%N
Calculated	69.05	5.55	10.07
Found	69.00	5.54	10.06

3-nitro-N^1,N^2-bis[2-(2-chlorophenyl)ethyl]phthalamide (Compound 13)



0.400 g (2 mmol) 3-Nitrophthalic Anhydride and 0.466 g (4 mmol) [2-(2-chlorophenyl)ethyl]amine were reacted as described in the general procedure B. The yield is 0.3314 g (38%). The compound is a white crystal, soluble in hot methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.935 and 0.97 in the solvent systems S_3 and S_4, respectively. Melting point of the compound is 211.7 ºC.

IR (KBr, V_{max}, cm^{-1}): 3296 (N-H), 3013 (C-H, Aromatic), 2937 (C-H, Aliphatic), 1590 and 1564 (C=O, Amide), 1542 and 1330 (NO_2).

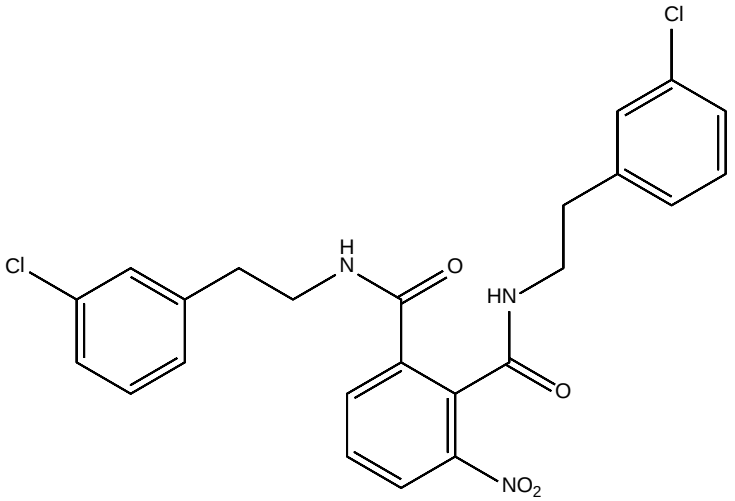
UV (MeOH, λ_{max}, nm): 202 (log ε: 7.21), 219 (log ε: 7.25).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.78 (t, 2H, CH_2-**CH_2**-Ar, *J*=7.2 Hz), 2.9 (t, 2H, CH_2-**CH_2**-Ar, *J*=7.2 Hz), 3.41 (q, 2H, NH-**CH_2**, *J*=6.0 Hz), 3.8 (q, 2H, NH-**CH_2**, *J*=6.8 Hz), 7.25-8.05 (m, 11H, Aromatic **CH**), 8.23 and 8.3 (t, 2H, **NH**-C=O, *J*=7.6 Hz and *J*=7.6 Hz).

Elemental analysis for $C_{24}H_{21}Cl_2N_3O_4$ (486.35 g/mol);

	%C	%H	%N
Calculated	59.27	4.35	8.64
Found	59.11	4.33	8.61

3-nitro-N^1,N^2-bis[2-(3-chlorophenyl)ethyl]phthalamide (Compound 14)



0.400 g (2 mmol) 3-Nitrophthalic Anhydride and 0.466 g (4 mmol) [2-(3-chlorophenyl)ethyl]amine were reacted as described in the general procedure B. The yield is 0.1996 g (23%). The compound is a white powder, soluble in hot methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.92 and 0.96 in the solvent systems S_3 and S_4, respectively. Melting point of the compound is 248.4 ºC.

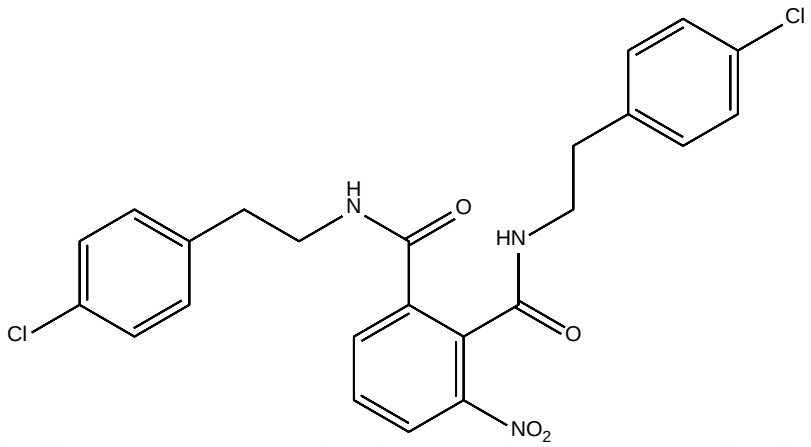
IR (KBr, V_{max}, cm^{-1}): 3329 (N-H), 3094 (C-H, Aromatic), 2963 (C-H, Aliphatic), 1586 and 1542 (C=O, Amide), 1540 and 1338 (NO_2).

UV (MeOH, λ_{max}, nm): 201 (log ε: 7.21), 212 (log ε: 7.23).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.72 (t, 2H, CH_2-**CH_2**-Ar, *J*=7.2 Hz), 2.83 (t, 2H, CH_2-**CH_2**-Ar, *J*=7.2 Hz), 3.45 (q, 2H, NH-**CH_2**, *J*=6.2 Hz), 3.65 (q, 2H, NH-**CH_2**, *J*=6.8 Hz), 7.2-8.05 (m, 11H, Aromatic **CH**), 8.45 and 8.6 (t, 2H, **NH**-C=O, *J*=7.6 Hz and *J*=7.2 Hz).

3-nitro-N^1,N^2-bis[2-(4-chlorophenyl)ethyl]phthalamide

(Compound 15)



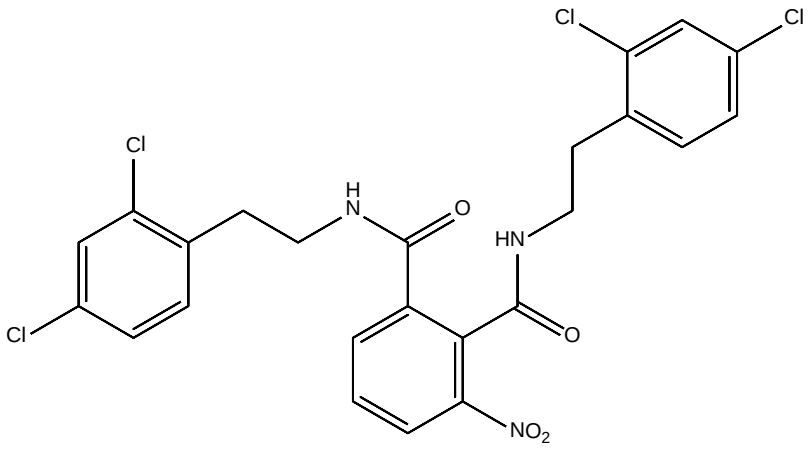
0.400 g (2 mmol) 3-Nitrophthalic Anhydride and 0.466 g (4 mmol) [2-(4-chlorophenyl)ethyl]amine were reacted as described in the general procedure B. The yield is 0.1976 g (22%). The compound is a light yellow powder, soluble in hot methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.94 and 0.97 in the solvent systems S_3 and S_4, respectively. Melting point of the compound is 208.3 ºC.

IR (KBr, V_{max}, cm^{-1}): 3292 (N-H), 3060 (C-H, Aromatic), 2960 (C-H, Aliphatic), 1570 and 1544 (C=O, Amide), 1548 and 1332 (NO_2).

UV (MeOH, λ_{max}, nm): 201 (log ε: 7.21), 220 (log ε: 7.25).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.7 (t, 2H, CH_2-**CH_2**-Ar, *J*=6.8 Hz), 2.85 (t, 2H, CH_2-**CH_2**-Ar, *J*=7.0 Hz), 3.48 (q, 2H, NH-**CH_2**, *J*=6.2 Hz), 3.6 (q, 2H, NH-**CH_2**, *J*=7.2 Hz), 7.2-7.7 (m, 11H, Aromatic **CH**), 8.4 and 8.7 (t, 2H, **NH**-C=O, *J*=7.6 Hz and *J*=7.2 Hz).

3-nitro-N^1,N^2-bis[2-(2,4-dichlorophenyl)ethyl]phthalamide (Compound 16)



0.400 g (2 mmol) 3-Nitrophthalic Anhydride and 0.570 g (4 mmol) [2-(2,4-dichlorophenyl)ethyl]amine were reacted as described in the general procedure B. The yield is 0.074 g (9%). The compound is a white powder, soluble in hot methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.95 and 0.98 in the solvent systems S_3 and S_4, respectively. Melting point of the compound is 213.8 ºC.

IR (KBr, V_{max}, cm^{-1}): 3234 (N-H), 3058 (C-H, Aromatic), 2937 (C-H, Aliphatic), 1590 and 1564 (C=O, Amide), 1549 and 1335 (NO_2).

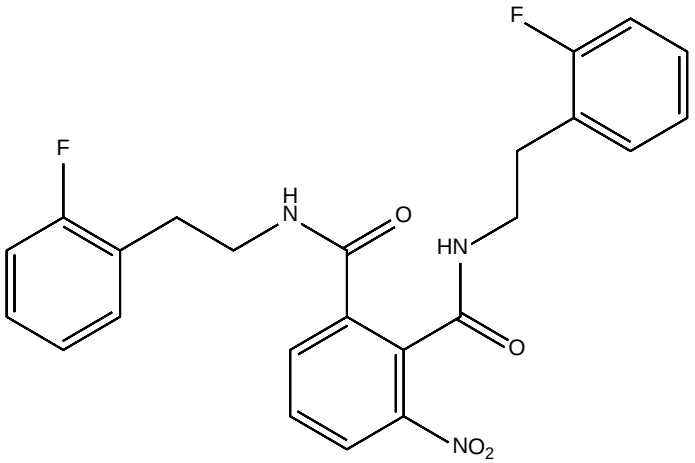
UV (MeOH, λ_{max}, nm): 202 (log ε: 7.27), 219 (log ε: 7.30).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.65 (t, 2H, CH_2-**CH_2**-Ar, *J*=6.8 Hz), 2.78 (t, 2H, CH_2-**CH_2**-Ar, *J*=7.2 Hz), 3.35 (q, 2H, NH-**CH_2**, *J*=6.0 Hz), 3.7 (q, 2H, NH-**CH_2**, *J*=7.2 Hz), 7.3-7.9 (m, 9H, Aromatic **CH**), 8.2 and 8.5 (t, 2H, **NH**-C=O, *J*=7.6 Hz and *J*=7.2 Hz).

Elemental analysis for $C_{24}H_{19}Cl_4N_3O_4$ (555.24 g/mol);

	%C	%H	%N
Calculated	51.92	3.45	7.57
Found	51.71	3.42	7.53

3-nitro-N^1,N^2-bis[2-(2-fluorophenyl)ethyl]phthalamide (Compound 17)



0.400 g (2 mmol) 3-Nitrophthalic Anhydride and 0.417 g (4 mmol) [2-(2-fluorophenyl)ethyl]amine were reacted as described in the general procedure B. The yield is 0.166 g (19%). The compound is a dark yellow powder, soluble in hot methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.95 and 0.98 in the solvent systems S_3 and S_4, respectively. Melting point of the compound is 143.2 ºC.

IR (KBr, V_{max}, cm^{-1}): 3267 (N-H), 3092 (C-H, Aromatic), 2937 (C-H, Aliphatic), 1573 and 1545 (C=O, Amide), 1525 and 1318 (NO_2).

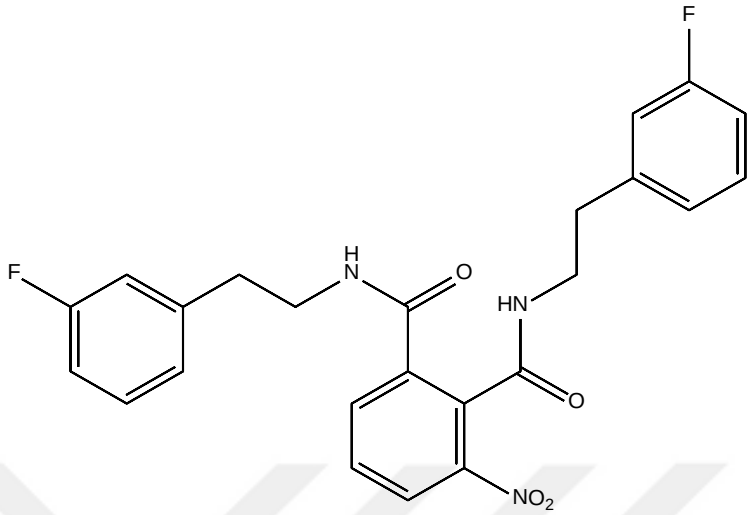
UV (MeOH, λ_{max}, nm): 202 (log ε: 7.18), 220 (log ε: 7.22).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.62 (t, 2H, CH_2-**CH_2**, *J*=7.0 Hz), 2.83 (t, 2H, CH_2-**CH_2**, *J*=7.2 Hz), 3.57 (q, 2H, NH-**CH_2**, *J*=6.8 Hz), 3.7 (q, 2H, NH-**CH_2**, *J*=7.2 Hz) 7.2-8.1 (m, 11H, Aromatic **CH**), 8.3 and 8.6 (t, 2H, **NH**-C=O, *J*=7.6 Hz and *J*=7.2 Hz).

Elemental analysis for $C_{24}H_{21}F_2N_3O_4$ (453.44 g/mol);

	%C	%H	%N
Calculated	63.53	4.67	9.27
Found	63.57	4.60	9.26

3-nitro-N^1,N^2-bis[2-(3-fluorophenyl)ethyl]phthalamide (Compound 18)



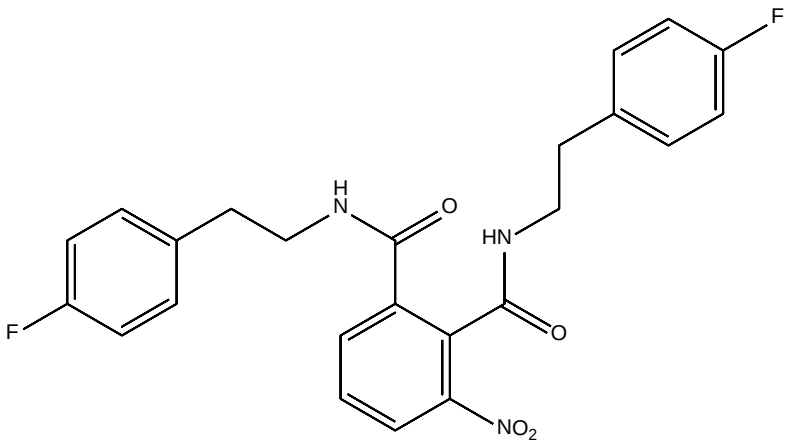
0.400 g (2 mmol) 3-Nitrophthalic Anhydride and 0.417 g (4 mmol) [2-(3-fluorophenyl)ethyl]amine were reacted as described in the general procedure B. The yield is 0.080 g (9%). The compound is a yellow powder, soluble in hot methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.95 and 0.98 in the solvent systems S_3 and S_4, respectively. Melting point of the compound is 139.8 ºC.

IR (KBr, V_{max}, cm^{-1}): 3279 (N-H), 3074 (C-H, Aromatic), 2924 (C-H, Aliphatic), 1586 and 1545 (C=O, Amide), 1522 and 1326 (NO_2).

UV (MeOH, λ_{max}, nm): 202 (log ε: 7.18), 229 (log ε: 7.24).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.76 (t, 2H, CH_2-**CH_2**, *J*=7.2 Hz), 2.85 (t, 2H, CH_2-**CH_2**, *J*=6.8 Hz), 3.43 (q, 2H, NH-**CH_2**, *J*=6.8 Hz), 3.79 (q, 2H, NH-**CH_2**, *J*=7.2 Hz), 7.25-8.05 (m, 11H, Aromatic **CH**), 8.15 and 8.32 (t, 2H, **NH**-C=O, *J*=7.6 Hz and *J*=7.2 Hz).

3-nitro-N^1,N^2-bis[2-(4-fluorophenyl)ethyl]phthalamide (Compound 19)



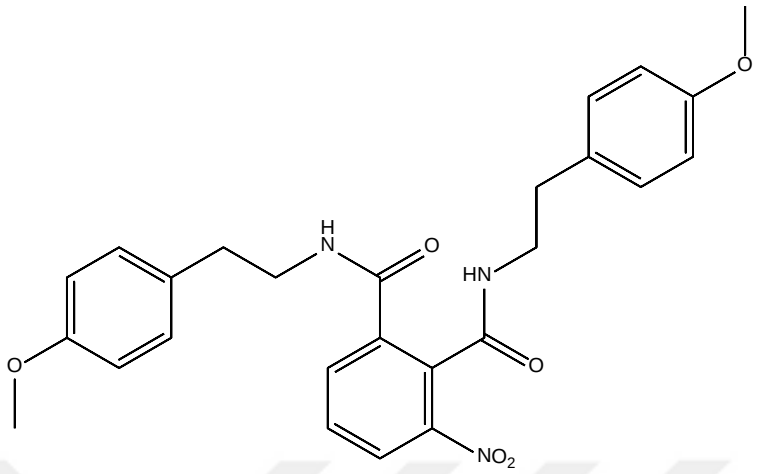
0.400 g (2 mmol) 3-Nitrophthalic Anhydride and 0.417 g (4 mmol) [2-(4-fluorophenyl)ethyl]amine were reacted as described in the general procedure B. The yield is 0.1404 g (16%). The compound is a light yellow powder, soluble in hot methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.94 and 0.98 in the solvent systems S_3 and S_4, respectively. Melting point of the compound is 147.6 ºC.

IR (KBr, V_{max}, cm^{-1}): 3288 (N-H), 3070 (C-H, Aromatic), 2935 (C-H, Aliphatic), 1579 and 1550 (C=O, Amide), 1519 and 1323 (NO_2).

UV (MeOH, λ_{max}, nm): 202 (log ε: 7.18), 221 (log ε: 7.22).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.64 (t, 2H, CH_2-**CH_2**, *J*=7.2 Hz), 2.82 (t, 2H, CH_2-**CH_2**, *J*=6.8 Hz), 3.47 (q, 2H, NH-**CH_2**, *J*=6.8 Hz), 3.72 (q, 2H, NH-**CH_2**, *J*=7.2 Hz), 7.2-8.1 (m, 11H, Aromatic **CH**), 8.29 and 8.5 (t, 2H, **NH**-C=O, *J*=7.6 Hz and *J*=7.2 Hz).

3-nitro-N^1,N^2-bis[2-(4-methoxyphenyl)ethyl]phthalamide (Compound 20)



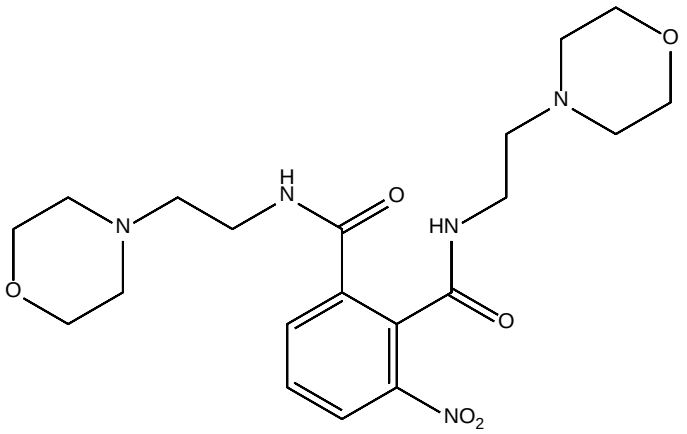
0.400 g (2 mmol) 3-Nitrophthalic Anhydride and 0.453 g (4 mmol) [2-(4-methoxyphenyl)ethyl]amine were reacted as described in the general procedure B. The yield is 0.099 g (10%). The compound is a white powder, soluble in hot methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.95 and 0.98 in the solvent systems S_3 and S_4, respectively. Melting point of the compound is 158.8 ºC.

IR (KBr, V_{max}, cm^{-1}): 3288 (N-H), 3070 (C-H, Aromatic), 2935 (C-H, Aliphatic), 1579 and 1550 (C=O, Amide), 1538 and 1349 (NO_2).

UV (MeOH, λ_{max}, nm): 201 (log ε: 7.18), 211 (log ε: 7.20).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.6 (t, 2H, CH_2-**CH_2**, *J*=6.8 Hz), 2.86 (t, 2H, CH_2-**CH_2**, *J*=6.8 Hz), 3.44 (q, 2H, NH-**CH_2**, *J*=7.2 Hz), 3.62 (q, 2H, NH-**CH_2**, *J*=7.2 Hz), 3.82 (s, 6H, O-**CH_3**, *J*=7.6 Hz), 7.2-8.1 (m, 11H, Aromatic **CH**), 8.2 and 8.7 (t, 2H, **NH**-C=O, *J*=8.0 Hz and *J*=7.2 Hz).

3-nitro-N^1,N^2-bis[2-(4-morpholino)ethyl]phthalamide (Compound 21)



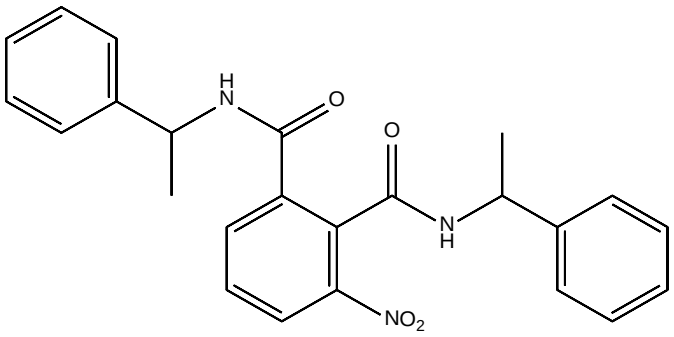
0.400 g (2 mmol) 3-Nitrophthalic Anhydride and 0.390 g (4 mmol) [2-(4-morpholino)ethyl]amine were reacted as described in the general procedure B. The yield is is 0.063 g (7%). The compound is a yellow liquid, soluble in hot methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.94 and 0.97 in the solvent systems S_3 and S_4, respectively.

IR (KBr, V_{max}, cm^{-1}): 3256 (N-H, s), 3050, (C-H, Aromatic), 2848 (C-H, Aliphatic), 1789 and 1708 (C=O, Amide), 1498 and 1315 (NO_2).

UV (MeOH, λ_{max}, nm): 203 (log ε: 7.17), 219 (log ε: 7.20).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 2.32 (m, 8H, N-**CH_2**, *J*=4.4 Hz), 2.61 (t, 2H, CH_2-**CH_2**, *J*=6.8 Hz), 2.78 (t, 2H, CH_2-**CH_2**, *J*=7.2 Hz), 3.49 (q, 2H, NH-**CH_2**, *J*=6.4 Hz), 3.60 (q, 2H, NH-**CH_2**, *J*=6.4 Hz), 3.65 (t, 8H, O-**CH_2**, *J*=6.8 Hz), 8.0-8.1 (m, 3H, Aromatic **CH**), 8.2 and 8.7 (t, 2H, **NH**-C=O, *J*=5.6 Hz and *J*=5.2 Hz).

3-nitro-N^1,N^2-bis[(1-phenyl)ethyl]phthalamide (Compound 22)



0.400 g (2 mmol) 3-Nitrophthalic Anhydride and 0.363 g (4 mmol) [(1-phenyl)ethyl]amine were reacted as described in the general procedure B. The yield is 0.121 g (14%). The compound is a light yellow powdered, soluble in hot methanol, ethanol, acetone, DMSO and DCM and insoluble in water. R_f values were calculated as 0.92 and 0.95 in the solvent systems S_3 and S_4, respectively. Melting point of the compound is 180.1 ºC.

IR (KBr, V_{max}, cm^{-1}): 3263 (N-H), 3081 (C-H, Aromatic), 2924 (C-H, Aliphatic), 1557 and 1533 (C=O, Amide), 1549 and 1350 (NO_2).

UV (MeOH, λ_{max}, nm): 202 (log ε: 7.15), 219 (log ε: 7.17).

^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 1.49 (d, 6H, CH-**CH_3**, *J*=4.0 Hz), 4.98 (m, 2H, NH-**CH**, *J*=7.2 Hz), 7.31-7.45 (m, 10H, Aromatic **CH**), 7.3-7.72 (m, 3H, Aromatic **CH**), 8.07 and 8.23 (t, 2H, **NH**-C=O, *J*=5.2 Hz and *J*=5.6 Hz).

4.2. Biological Data

4.2.1. Antimicrobial Activity Data

Synthesized compounds were screened against gram positive bacteria strain of *Staphylococcus aureus* and gram negative bacteria strains of *Escherichia coli* and *Pseudomonas aeruginosa* with using the conventional agar dilution method. Ofloxacin was used as reference. Activity results of the compounds are shown in Table 4.1.

Table 4.1. The minimum inhibitory concentrations (MIC, µg/ml) of synthesized compounds 1-22 against different bacteria

Compound	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
1	100	100	100
2	100	100	100
3	50	50	50
4	25	25	25
5	100	100	100
6	-	-	-
7	100	100	100
8	-	-	-
9	-	-	-
10	-	-	-
11	100	100	100
12	-	-	-
13	25	25	25
14	100	100	100

15	25	25	25
16	25	50	25
17	100	100	100
18	-	-	-
19	50	50	50
20	100	100	100
21	-	-	-
22	100	100	100
Ofloxacin	0.78	0.78	0.78

4.2.2. Anticancer Activity Data

Cytotoxic activity results of synthesized compounds are given at Table 4.2.

Table 4.2. IC_{50} values of synthesized compounds 1-22 against human breast cancer cell line (MCF7) and human liver cancer cell line (Hep3B) by MTT assay

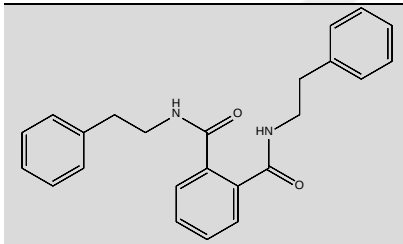
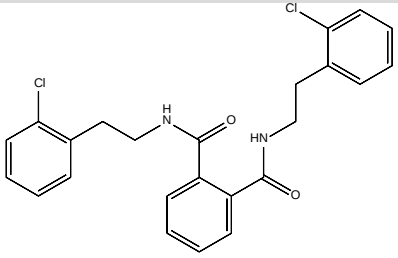
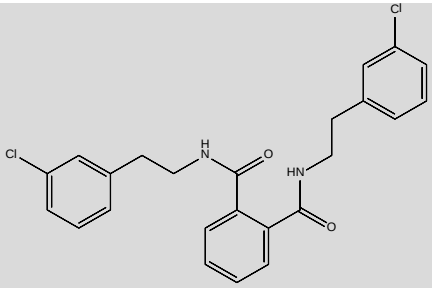
		Cancer Cell Lines (IC_{50}, µM)	
Compound	**R_3**	**MCF7**	**Hep3B**
1	phenyl	>100	>100
2	2-chlorophenyl	>100	>100
3	3-chlorophenyl	>100	>100
4	4-chlorophenyl	>100	>100
5	2,4-dichlorophenyl	87.6 ± 5.3	48.9 ± 5.4
6	2-fluorophenyl	>100	>100
7	3-fluorophenyl	>100	>100
8	4-fluorophenyl	>100	>100

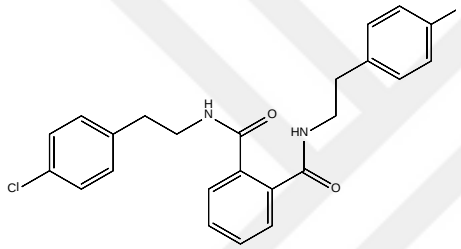
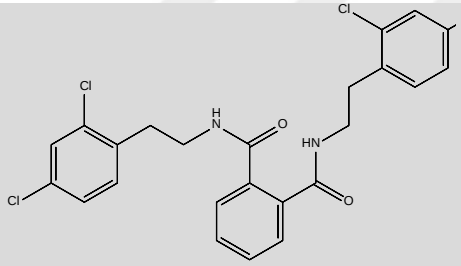
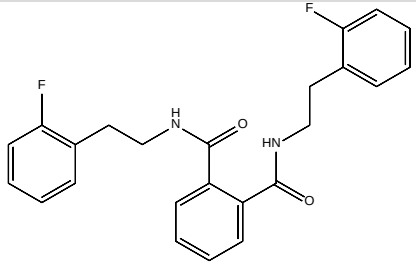
9	4-methoxyphenyl	>100	>100
10	morpholine	>100	>100
11	phenyl	>100	>100
12	phenyl	>100	>100
13	2-chlorophenyl	54.5 ± 7.1	46.2 ± 6.9
14	3-chlorophenyl	>100	>100
15	4-chlorophenyl	84.3 ± 2.2	>100
16	2,4-dichlorophenyl	46.0 ± 2.6	45.1 ± 2.9
17	2-fluorophenyl	50.5 ± 3.3	37.6 ± 4.7
18	3-fluorophenyl	92.6 ± 5.8	97.2 ± 0.3
19	4-fluorophenyl	>100	>100
20	4-methoxyphenyl	>100	>100
21	morpholine	98.2 ±2.5	>100
22	phenyl	84.6 ± 8.3	95.0 ± 4.1
Doxorubicin	-	0.035 ± 0.001	0.020 ± 0.001

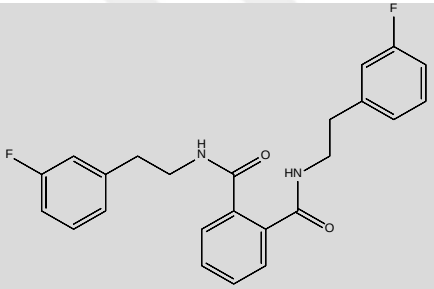
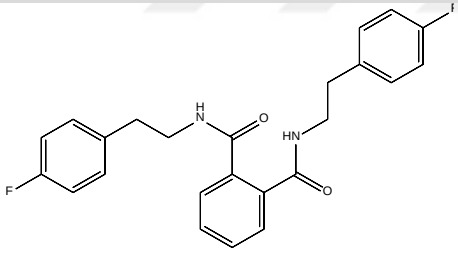
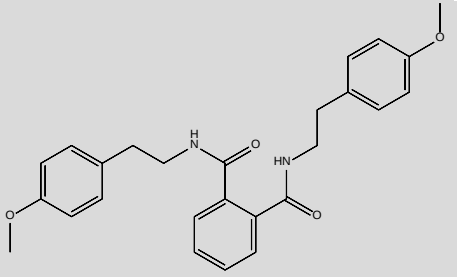
4.3. ADME Prediction Data

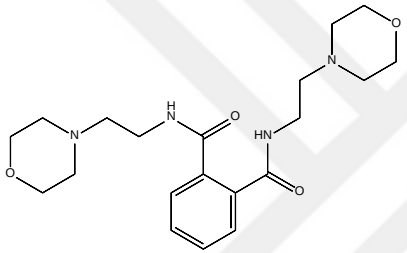
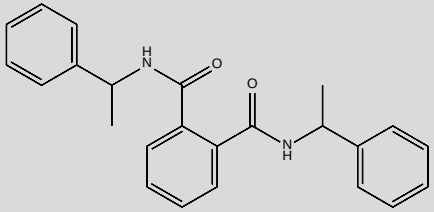
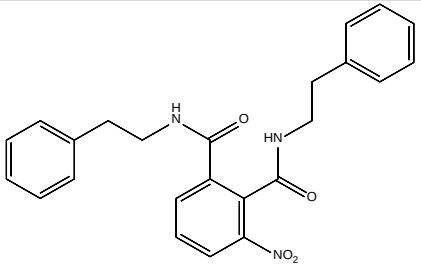
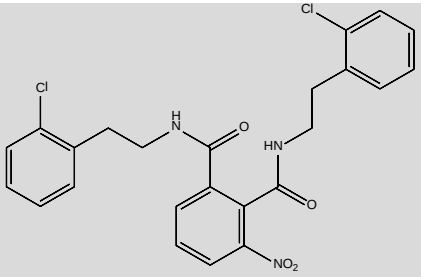
Prediction of drug-likeness, molecular and ADME properties of synthesized compounds were evaluated. In this thesis, physicochemical parameters of synthesized compounds were generated in order to evaluate their drug-likeness properties using QikProp module of Schrödinger mentioned in Table 4.3.

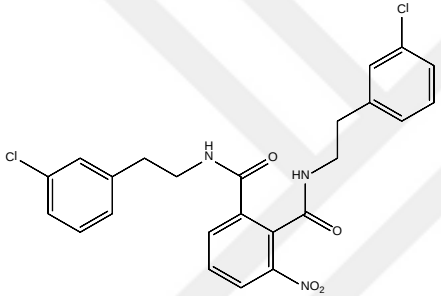
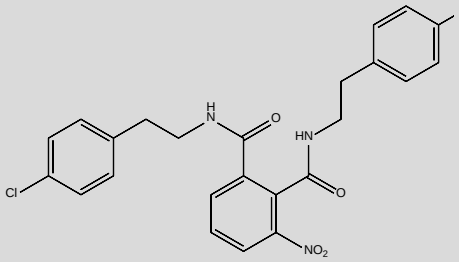
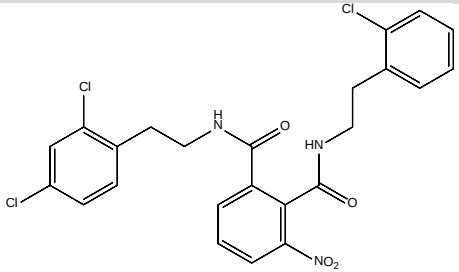
Table 4.3. Prediction of drug-likeness, molecular and ADME properties

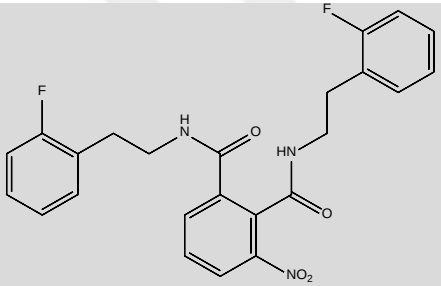
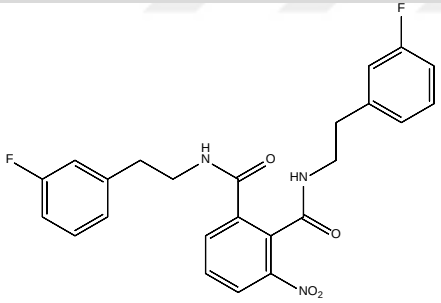
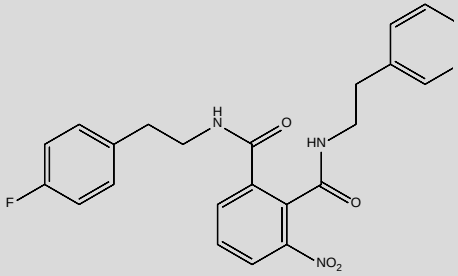
Structure	Compound	CNS	Qplog Po/w	QPlog HERG	QPP Caco	Qplog BB	QPP MDCK	QPlog Khsa	HOA	PHOA	ROF	ROT	J_M
	**1**	0	4.929	-6.936	2195	-0.61	1157.2	0.62	3	100	0	0	0.695
	**2**	0	5.696	-6.487	2532	-0.297	4639.0	0.809	1	100	1	1	0.091
	**3**	0	6.141	-7.196	2219	-0.31	7126.9	0.922	1	100	1	1	0.006

	**4**	0	6.075	-7.295	1884	-0.403	5973.7	0.91	1	100	1	1	0.004
	**5**	1	7.384	-7.455	3800	0.168	10000	1.227	1	100	2	1	0.0003
	**6**	0	5.304	-6.627	2188	-0.44	2705.2	0.705	3	100	1	1	0.127

	**7**	0	5.627	-7.13	2216	-0.41	3823.6	0.776	1	100	1	1	0.037
	**8**	0	5.496	-5.739	2405	-0.168	8516.5	0.703	3	100	1	1	0.064
	**9**	0	5.255	-6.655	3874	-0.484	2139.8	0.626	3	100	1	1	0.845

	**10**	1	0.713	-6.634	164.2	0.274	85.92	0.644	2	70.8	0	0	0.196
	**11**	0	5.019	-6.351	3828	-0.21	2111.3	0.699	3	100	1	0	1.002
	**12**	-2	4.781	-7.688	429.8	-1.592	198.62	0.704	1	100	0	1	0.003
	**13**	-2	5.156	-6.591	462.3	-1.17	705.40	0.762	1	91.87	1	1	0.003

	**14**	-2	5.512	-7.024	345.5	-1.30	954.76	0.867	1	91.69	1	1	0.001
	**15**	-2	5.763	-7.463	429.8	-1.30	1208.8	0.933	1	94.862	1	1	0.043
	**16**	-1	6.135	-6.418	462.4	-0.88	4292.8	0.987	1	84.647	2	1	0.044

	**17**	-2	4.861	-6.884	427.7	-1.33	428.90	0.688	1	100	0	1	0.003
	**18**	-1	4.787	-5.988	639.4	-0.96	972.39	0.603	3	100	0	0	0.018
	**19**	-2	5.201	-7.233	457.3	-1.31	694.46	0.763	1	92.05	1	1	0.001

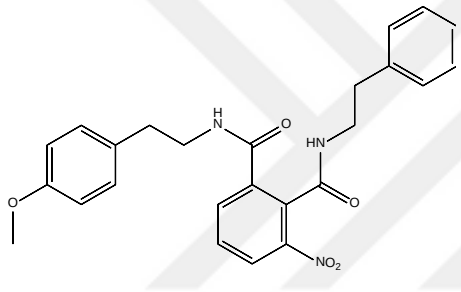
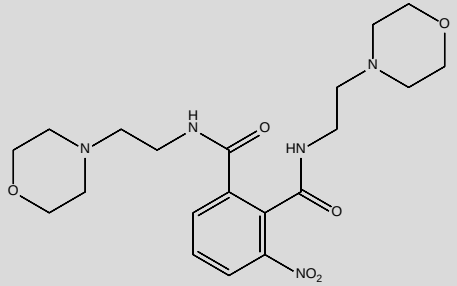
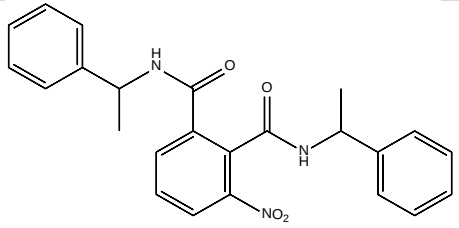
	**20**	-2	4.862	-7.307	434.4	-1.75	200.88	0.68	1	100	0	1	0.001
	**21**	1	0.109	-6.626	28.07	-0.62	12.734	-0.72	2	40.54	1	0	0.007
	**22**	-2	4.581	-6.775	706,3	-1.08	339.73	0.68	3	100	0	1	0.018

Table 4.4. ADME properties descriptor

Property or Descriptor	Description	Range or Recommended values
CNS	Predicted central nervous system activity on a –2 (inactive) to +2 (active) scale	–2 (inactive), +2 (active)
QPlogPo/w	Predicted octanol/water partition coefficient	–2.0 – 6.5
QPlogHERG	Predicted IC_{50} value for blockage of HERG K+ channels	below –5
QPPCaco	Predicted apparent Caco-2 cell permeability in nm/sec. Caco-2 cells are a model for the gut-blood barrier	<25 poor, >500 great
QPlogBB	Predicted brain/blood partition coefficient	–3.0 – 1.2
QPPMDCK	Predicted apparent MDCK cell permeability in nm/sec. MDCK cells are considered to be a good mimic for the bloodbrain barrier	<25 poor, >500 great
QPlogKhsa	Prediction of binding to human serum albumin	–1.5 – 1.5
Human Oral Absorption (HOA)	Predicted qualitative human oral absorption: 1, 2, or 3 for low, medium, or high	

Percent Human Oral Absorption (PHOA)	Predicted human oral absorption on 0 to 100% scale	>80% is high <25% is poor
Rule Of Five (ROF)	Number of violations of Lipinski's rule of five [3]. The rules are: mol_MW < 500, QPlogPo/w < 5, donorHB ≤ 5, accptHB ≤ 10	maximum is 4
Rule Of Three (ROT)	Number of violations of Jorgensen's rule of three. The three rules are: QPlogS > -5.7, QPPCaco > 22 nm/s, Primary Metabolites < 7	maximum is 3
Jm	Predicted maximum transdermal transport rate, *K*p×MW × S (μg cm–2 hr–1). *K*p and S are obtained from the aqueous solubility and skin permeability, QPlogKp and QPlogS.	

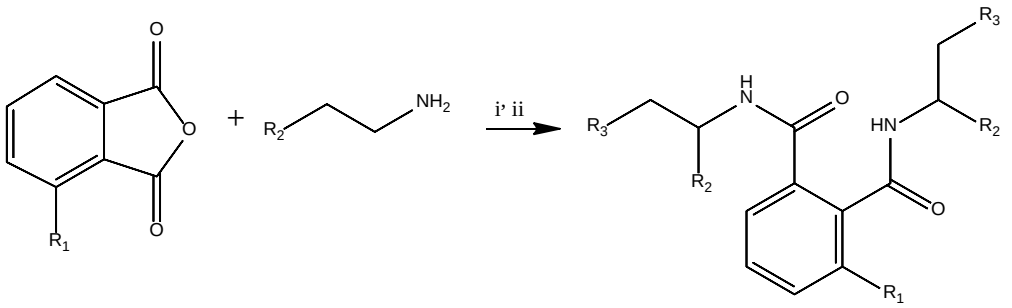
5. DISCUSSION AND CONCLUSION

In this thesis, eighteen novel derivatives of N^1,N^2-bis[(2-substitutedphenyl)ethyl]phthalamide and 3-nitro-N^1,N^2-bis[(2-substitutedphenyl) ethyl]phthalamide were synthesized and evaluated for their antimicrobial and anticancer activities. Structures of the compounds were clarified by UV, IR, ^{1}H-NMR, ^{13}C-NMR, elemental analysis and the prediction of drug-likeness, molecular and ADME properties.

The non-nitro carrying compounds (compounds 1-11) were synthesized by the reaction of phthalic anhydride with corresponding phenylethylamine derivatives. Reactions were carried out in ethanol by refluxing for 6 hours. Reaction solvent was removed by the rotary evaporator. Residues were dissolved in hot ethanol then cooled at the room temperature to get the precipitation. White crystalline products were removed by filtration. Compounds 1-11 were obtained in moderate yields (21-79%).

The nitro carrying compounds (compounds 12-22) were synthesized by the reaction of 3-nitrophthalic anhydride with corresponding phenylethylamine derivatives in methanol under reflux for 8 hours. Reaction solvent was removed by the rotary evaporator. The products were dissolved in $CHCl_3$ then organic layer extracted with (3%) HCl solution and (2%) NaOH solution and distilled water. Organic layer was dried on Sodium Sulphate anhydrous, evaporated to obtain the crystalline products. Compounds 12-22 were obtained in low yields (7-38%).

The target compounds mentioned in this thesis were prepared according to the synthetic pathway shown in Scheme 5.1.



i = Ethanol, 6h

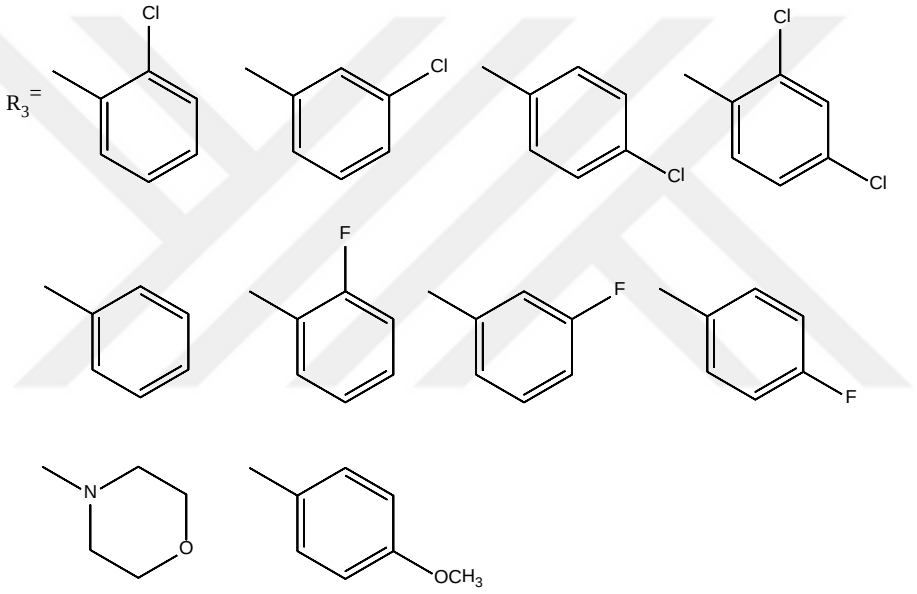
ii = Methanol, 8h

R_1 = H (compounds 1-11)

NO_2 (compounds 12-22)

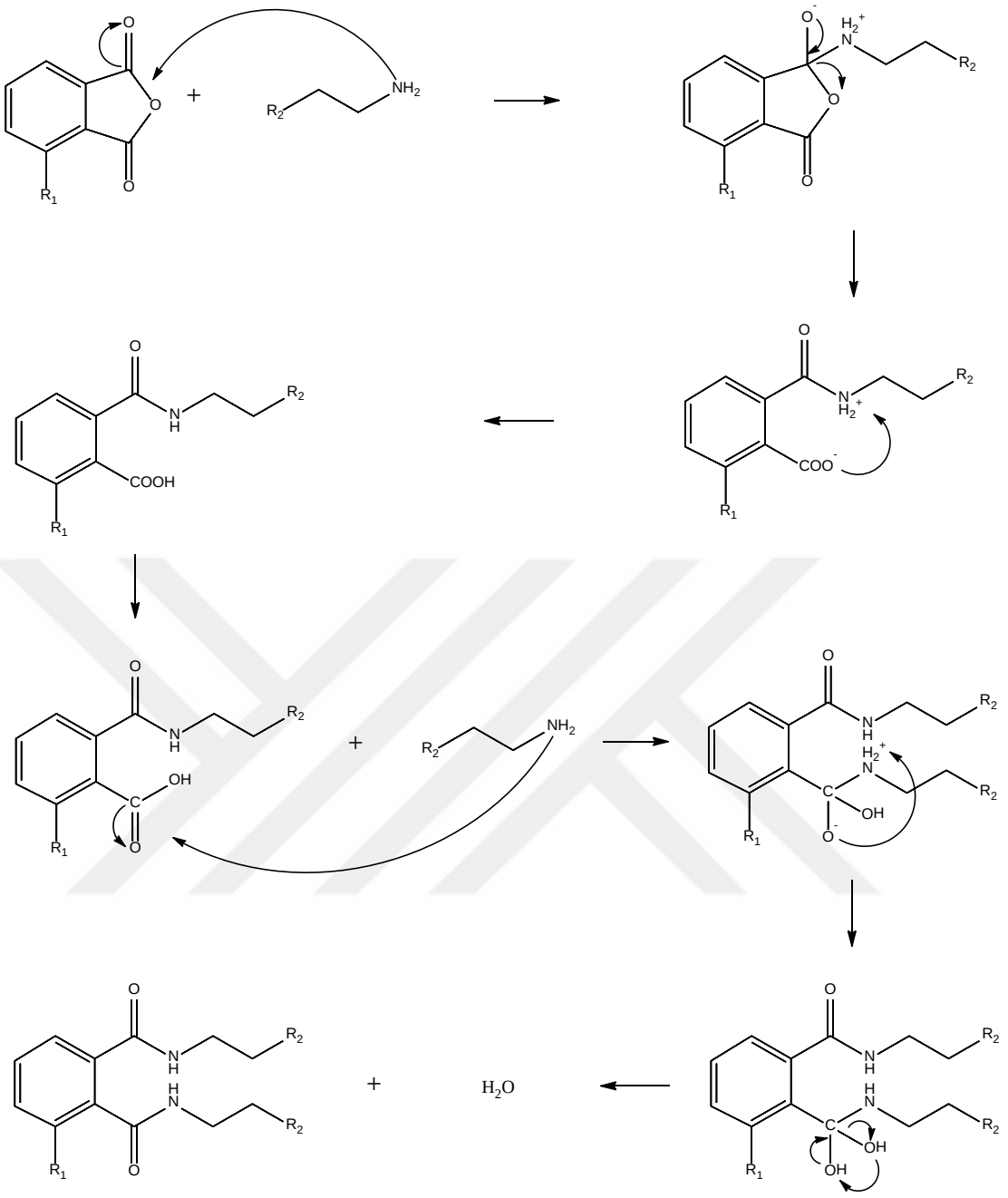
R_2 = H

Phenyl



Scheme 5.1. General synthetic pathway of the target compounds 1-11 and 12-22

The mechanism of phthalamide formation for this synthesis is depicted in Scheme 5.2.



Scheme 5.2. Reaction mechanism of phthalamide formation

Following the synthesis of the compounds, structure elucidation was carried out with spectral analysis. All spectral data are in accordance with the proposed structure. UV spectral data of synthesized compounds were examined in methanol. The compounds showed maximum absorbance at range of 201-220 nm which represent $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ transitions of phthalamides. NO_2 group did not affect the maximum absorbance bands scientifically. UV spectra of compounds 5 and 15 were given below.

The compound 5 gave mainly two absorption bands at 203 and 220 which represent π→π*, n→π* transitions of C=O and aromatic groups in N^1,N^2-bis[(2-substitutedphenyl)ethyl]phthalamide derivatives in Figure 5.1.

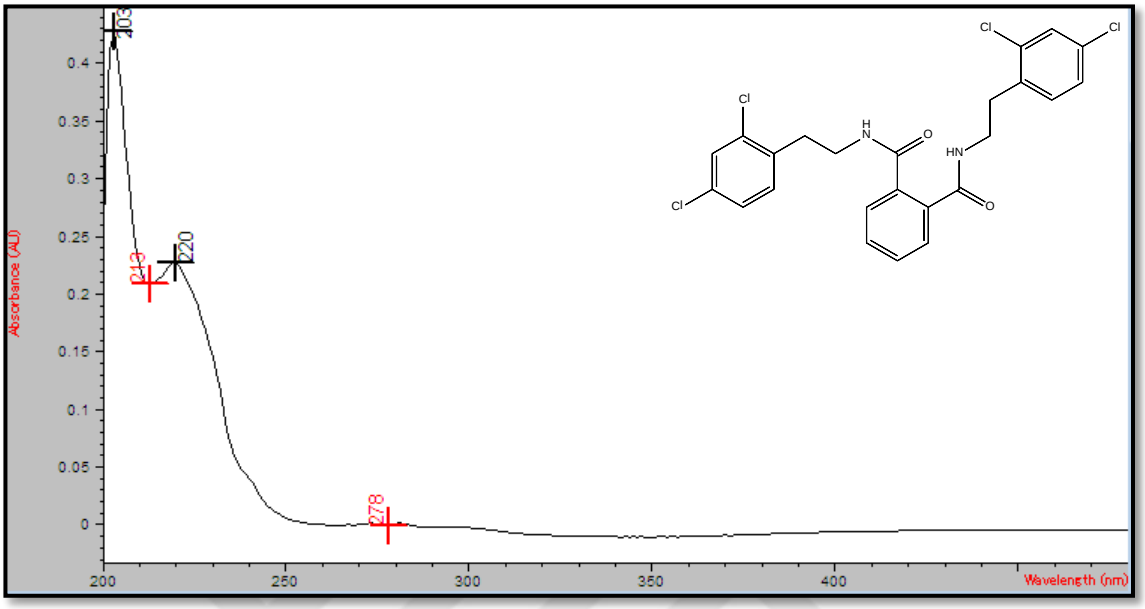


Figure 5.1. UV spectrum of the compound 5; (MeOH, $\lambda_{max,}$ nm); 203 (log ε : 7.24), 220 (log ε :7.27)

UV spectrum of the compound 15 gave mainly two absorption bands at 201 and 220 which represent π→π*, n→π* transitions of C=O and aromatic groups N^1,N^2-bis[(2-substitutedphenyl)ethyl]phthalamide derivatives in Figure 5.2.

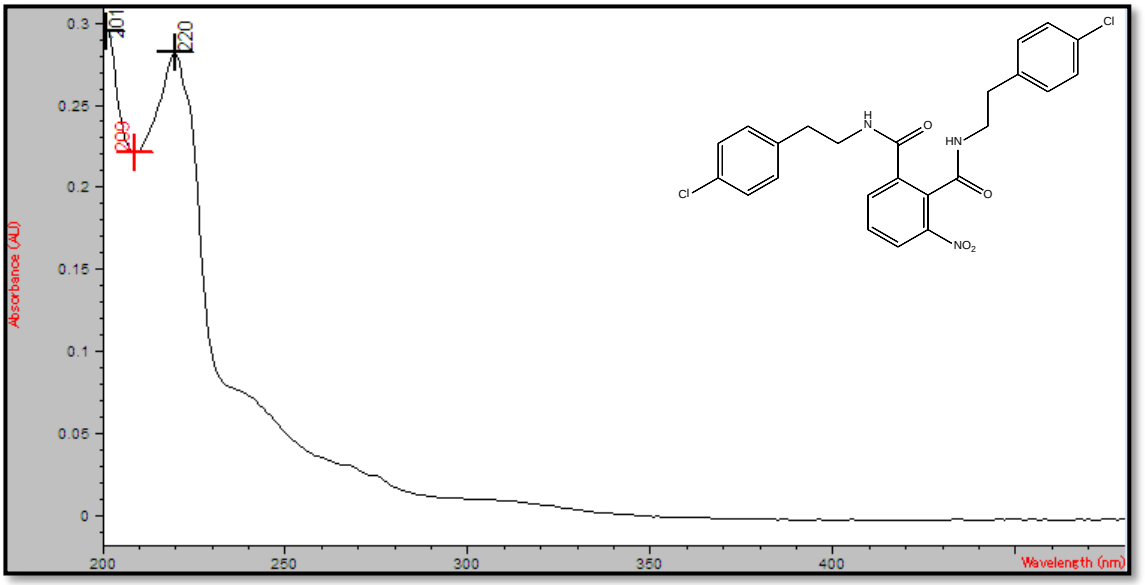


Figure 5.2. UV spectrum of the compound 15; (MeOH, $\lambda_{max,}$ nm); 201 (log ε : 7.21), 220 (log ε : 7.25)

FT-IR spectral data of synthesized compounds were taken by KBr tablets. Stretching bands of aromatic hydrogen gave peaks at over 3070 while C=O stretching bands 1590 and 1550 cm^{-1}. NO_2 stretching bands were shown at 1519 and 1315 cm^{-1}. FT-IR spectra of compounds 1 and 12 were given below.

The characteristic stretching bands of the compound 1 are observed as follows: 3256 cm^{-1} (N-H), 3074 cm^{-1} (C-H, Aromatic), 2919 cm^{-1} (C-H, Aliphatic), 1595 cm^{-1} and 1575 cm^{-1} (C=O, Amide) in Figure 5.3.

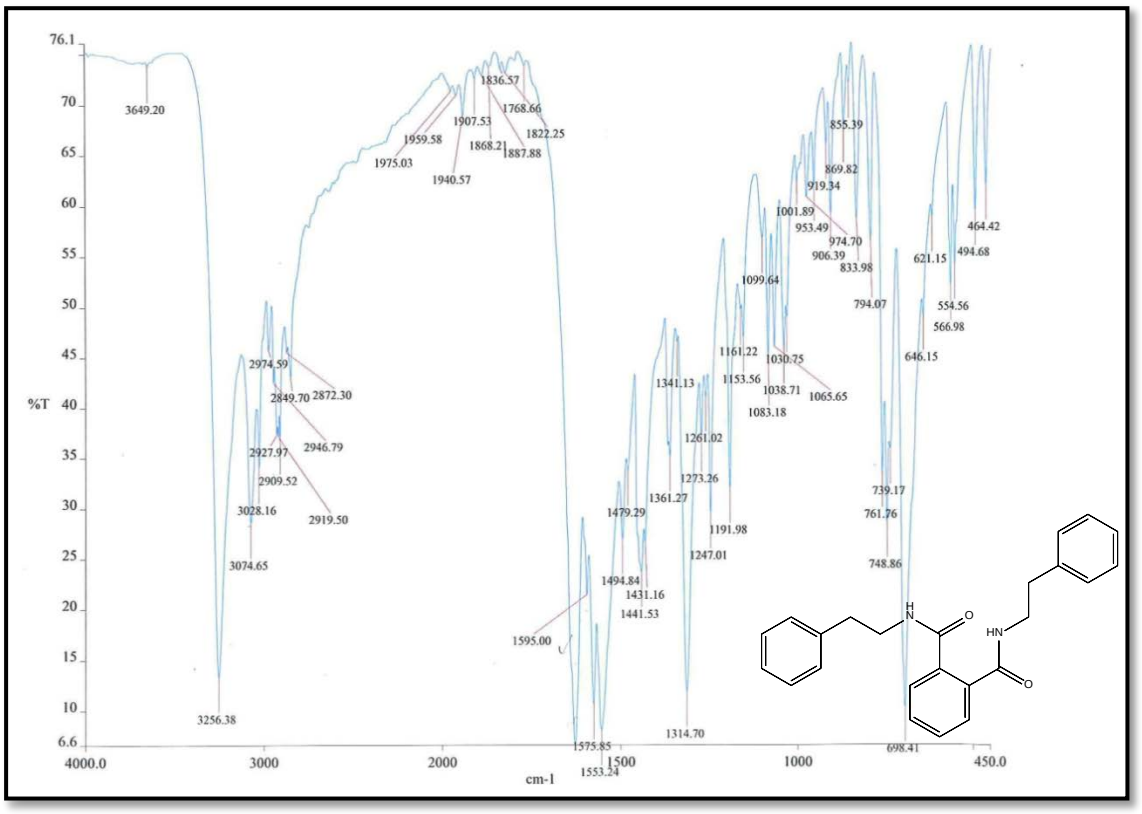


Figure 5.3. IR spectrum of the compound 1

The characteristic stretching bands of the compound 12 are illustrated in Figure 5.4. N-H stretching band is observed at 3236 cm^{-1}. Other stretching bands are observed at 3073 cm^{-1} (C-H, Aromatic), 2930 cm^{-1} (C-H, Aliphatic), 1579 cm^{-1} and 1547 cm^{-1} (C=O, Amide) and 1571 and 1316 (NO_2).

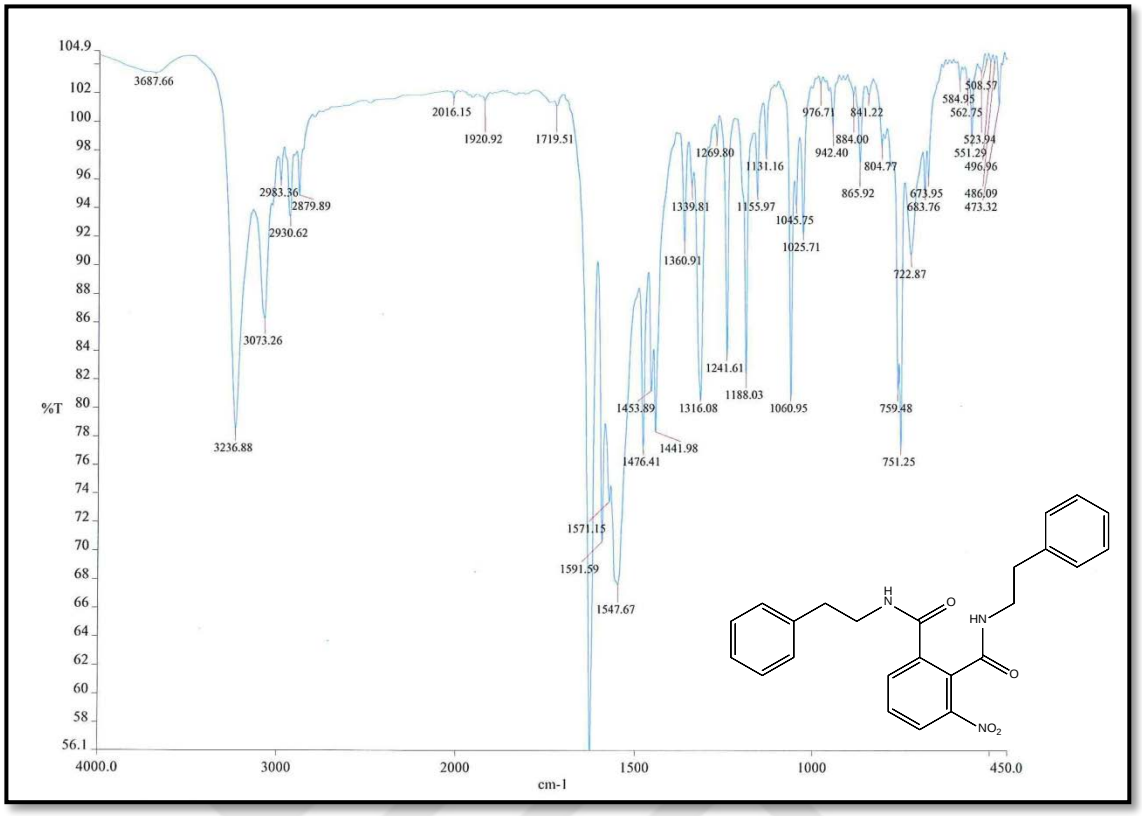


Figure 5.4. IR spectrum of the compound 12

^{1}H-NMR spectra of all compounds were taken using DMSO-*d*6 as solvent.

Because of the symmetry of the compounds 1-11, ^{1}H-NMR spectra of the synthesized compounds displayed as follows: Triplet at 2.75-2.9 ppm was related to CH_2-**CH_2**-Ar. The signal originated from CH_2 of NH-**CH_2** resonated at 3.40-3.47 ppm as quartet. The chemical shift range of aromatic **CH**'s of the phthalamide ring varied from 7.1 to 7.8 ppm as multiplet. **NH** of amide structure appeared overlap furthest downfield at 8.1-8.4 ppm as triplet. These signals were similar for all derivatives (compounds 1-11). ^{1}H-NMR spectra of compounds are presented here with compounds 1, 2, 6 and 9.

^{1}H-NMR spectra of compound 1 displayed a triplet due to CH_2-**CH_2**-Ar groups at 2.83 ppm. The signal originated from CH_2 of NH-**CH_2** resonated at 3.45 ppm as quartet. The chemical shift range of aromatic **CH**'s of the phthalamide ring varied from 7.2 to 7.5 ppm as multiplet. The signal originated from **NH** of *N*^{1},*N*^{2}-bis[(2-phenyl)ethyl]phthalamide appeared overlap furthest downfield at 8.35 ppm as triplet (Figure 5.5).

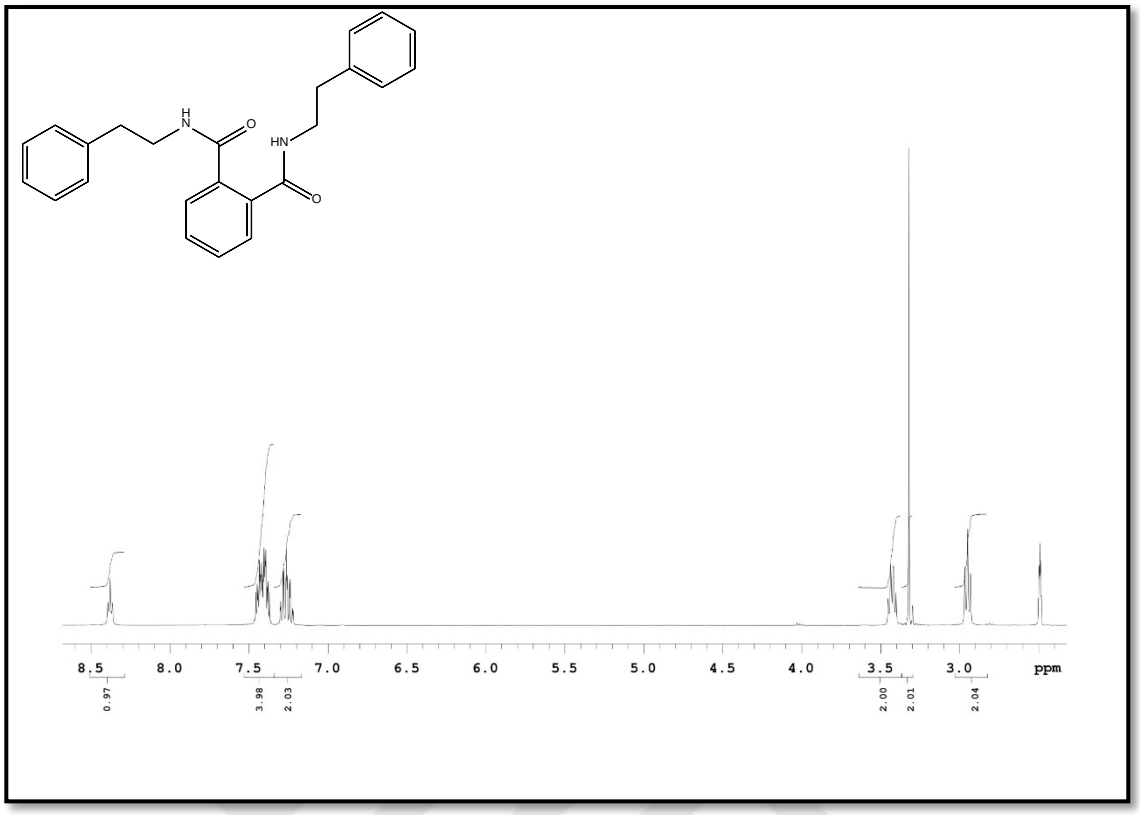


Figure 5.5. ^{1}H-NMR spectrum of the compound 1

^{1}H-NMR spectra of compound 2 displayed a triplet due to CH_2-**CH_2**-Ar groups at 2.9 ppm. The signal originated from CH_2 of NH-**CH_2** resonated at 3.47 ppm as quartet. The chemical shift range of aromatic **CH**'s of the phthalamide ring varied from 7.15 to 7.5 ppm as multiplet. The signal originated from **NH** of *N*^{1},*N*^{2}-bis[2-(2-chlorophenyl)ethyl]phthalamide appeared overlap furthest downfield at 8.4 ppm as triplet (Figure 5.6).

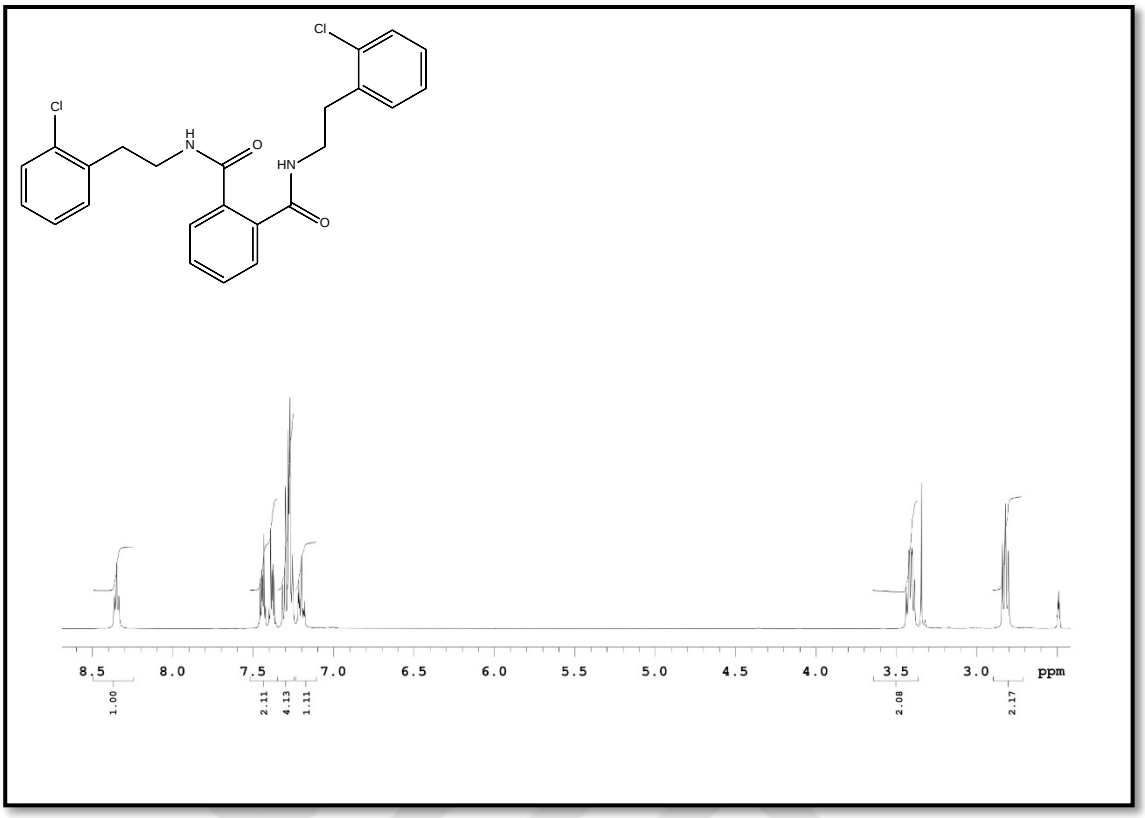


Figure 5.6. ^{1}H-NMR spectrum of the compound 2

^{1}H-NMR spectra of compound 6 displayed a triplet due to CH_2-**CH_2**-Ar groups at 2.85 ppm. The signal originated from CH_2 of NH-**CH_2** resonated at 3.45 ppm as quartet. The chemical shift range of aromatic **CH**'s of the phthalamide ring varied from 7.15 to 7.5 ppm as multiplet. The signal originated from **NH** of *N*^{1},*N*^{2}-bis[2-(2-fluorophenyl)ethyl]phthalamide appeared overlap furthest downfield at 8.4 ppm as triplet (Figure 5.7).

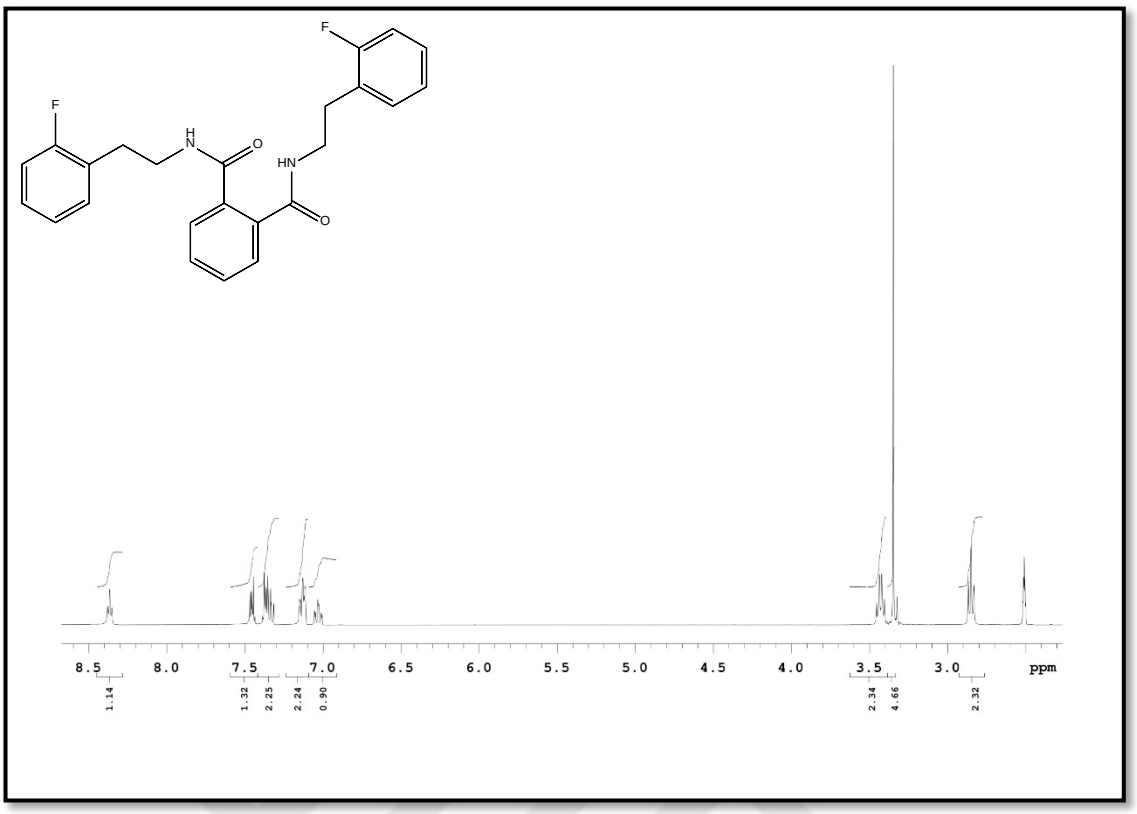


Figure 5.7. ^{1}H-NMR-Spectrum of compound 6

^{1}H-NMR spectra of compound 9 displayed a triplet due to CH_2-**CH_2**-Ar groups at 2.75 ppm. The signal originated from CH_2 of NH-**CH_2** resonated at 3.40 ppm as quartet. The chemical shift range of O-**CH_3** resonated at 3.80 ppm as singlet. The chemical shift range of aromatic **CH**'s of the phthalamide ring varied from 7.15 to 7.50 ppm as multiplet. The signal originated from **NH** of *N*^{1},*N*^{2}-bis[(2-(4-methoxyphenyl)ethyl]phthalamide appeared overlap furthest downfield at 8.35 ppm as triplet (Figure 5.8).

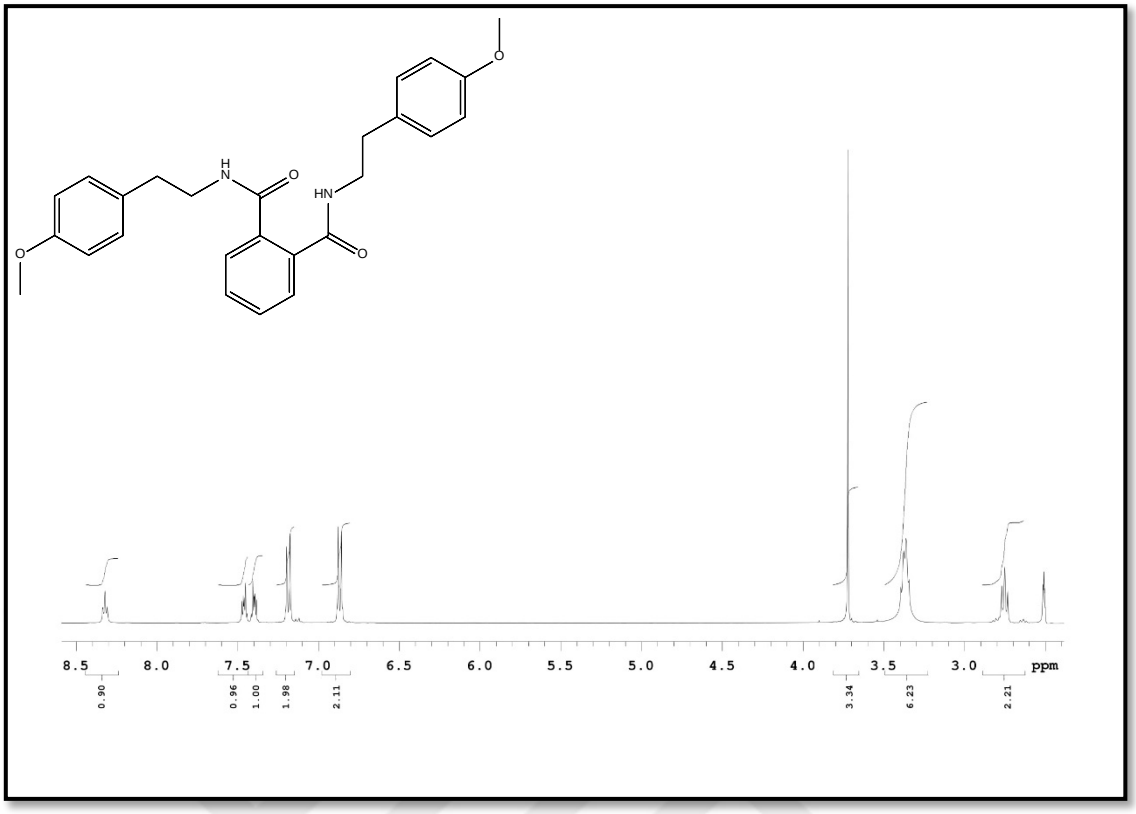


Figure 5.8. ^{1}H-NMR-Spectrum of compound 9

^{1}H-NMR spectra of the compounds which are having a NO_2 substituent on aromatic rings (compounds 12-22) displayed as follows: Triplet due to CH_2-**CH_2**-Ar groups at 2.6-2.9 ppm. The signal originated from CH_2 of NH-**CH_2** resonated at 3.30-3.80 ppm as quartet. The chemical shift range of aromatic **CH**'s of the phthalamide ring varied from 7.2 to 8.15 ppm as multiplet. Two **NH** of amide structures appeared furthest downfield at 8.2 and 8.4 ppm as triplet due to the NO_2. These signals were similar for all nitro derivatives.

^{1}H-NMR spectra of compound 12 gave peaks as follows, the peak at 2.75 and 2.82 ppm displayed as triplet due to CH_2-**CH_2**-Ar groups. The signal originated from CH_2 of NH-**CH_2** resonated at 3.30 and 3.42 ppm as quartet. The chemical shift range of aromatic **CH**'s of the phthalamide ring varied from 7.28 to 8.05 ppm as multiplet. The signal originated from two **NH** of 3-nitro-*N*^{1},*N*^{2}-bis[(2-phenyl)ethyl]phthalamide appeared furthest downfield at 8.2 and 8.3 ppm as triplet (Figure 5.9).

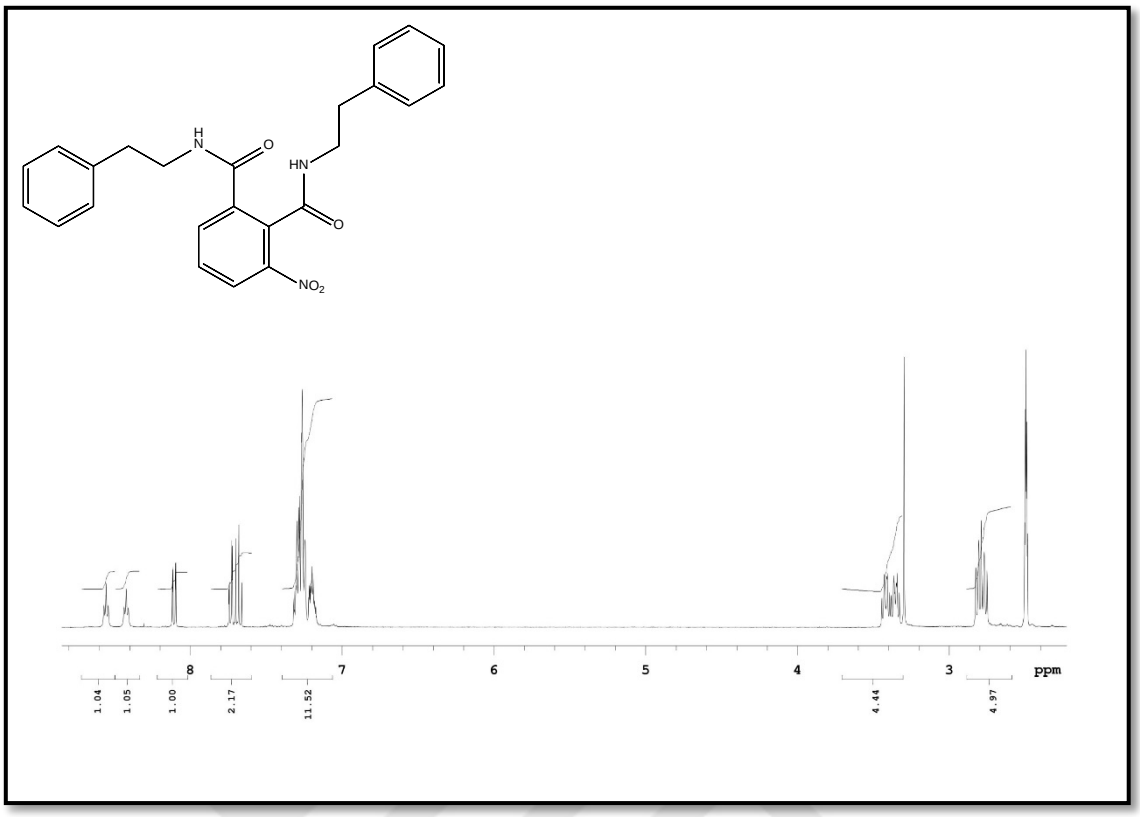


Figure 5.9. ^{1}H-NMR spectrum of the compound 12

^{1}H-NMR spectra of compound 13 displayed triplet due to CH_2-**CH_2** groups at 2.78 and 2.90 ppm. The signal originated from CH_2 of NH-**CH_2** resonated at 3.41 and 3.80 ppm as quartet. The chemical shift range of aromatic **CH**'s of the phthalamide ring varied from 7.25 to 8.05 ppm as multiplet. The signal originated from two **NH** of 3-nitro-N^1,N^2-bis[2-(2-chlorophenyl)ethyl]phthalamide appeared furthest downfield at 8.23 and 8.3 ppm as triplet (Figure 5.10).

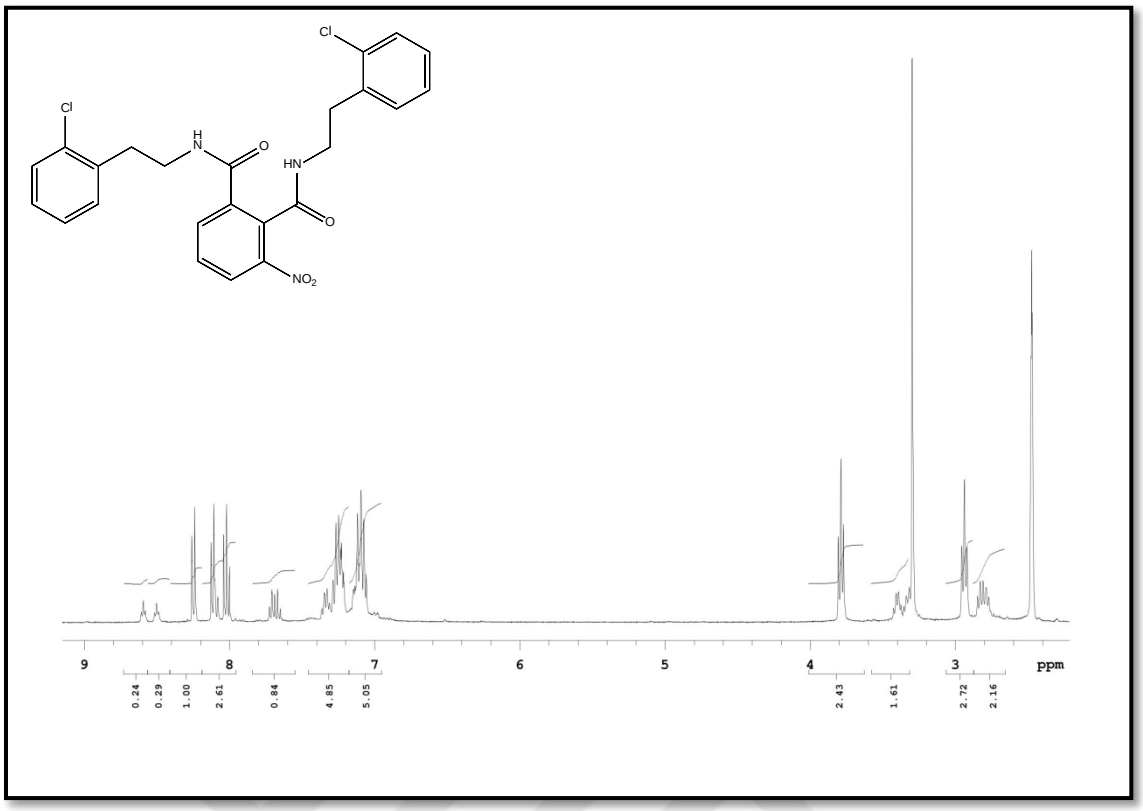


Figure 5.10. ^{1}H-NMR spectrum of the compound 13

^{1}H-NMR spectra of compound 18 displayed triplet due to CH_2-**CH_2** groups at 2.76 and 2.85 ppm. The signal originated from CH_2 of NH-**CH_2** resonated at 3.43 and 3.79 ppm as quartet. The chemical shift range of aromatic **CH**'s of the phthalamide ring varied from 7.25 to 8.05 ppm as multiplet. The signal originated from two **NH** of 3-nitro-*N*^{1},*N*^{2}-bis[2-(3-fluorophenyl)ethyl]phthalamide appeared furthest downfield at 8.15 and 8.32 ppm as triplet (Figure 5.11).

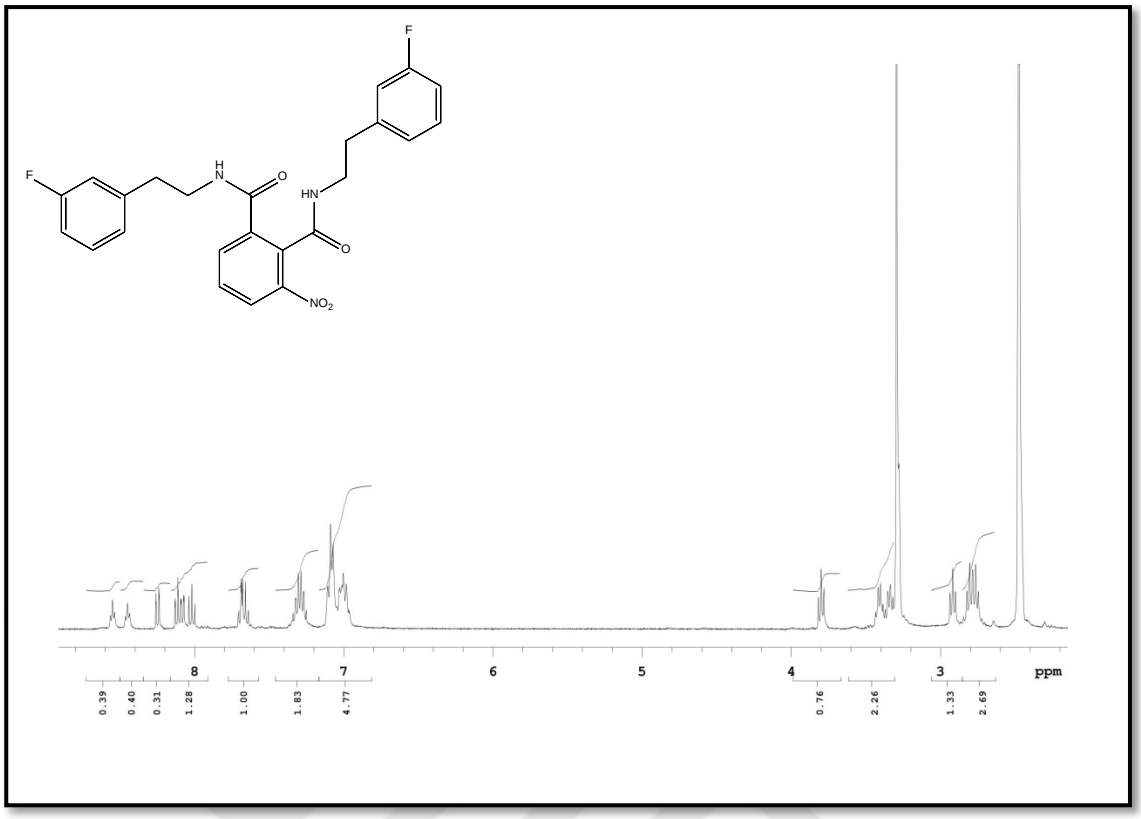


Figure 5.11. ^{1}H-NMR spectrum of the Compound 18

Compounds 12-22 gave different ^{1}H-NMR spectral pattern when compare with compounds 1-11. Since NO_2 group on the aromatic ring change the symmetry of the molecules, all overlapping peaks have seen separately.

^{13}C-NMR spectrum of the compound 1 was also taken by using DMSO-*d*6 as solvent by using TMS standard and spectrums were recorded in ppm.

^{13}C-NMR spectrum of the compound 1 gave peaks at 136.3 ppm (C^{1}) and 136.3 ppm (C^{2}) which belongs to the phthalamide ring carbons. The aromatic carbons of the phthalamide ring gave resonance at 127.5 (C^{3}), 129.2 (C^{4}), 129.2 (C^{5}) and 127.5 (C^{6}) ppm. Amide carbons C^{7} and C^{16} gave resonance at 168.0 ppm while aliphatic carbons at 40.7 (C^{8}), 34.9 (C^{9}), 40.7 (C^{17}) and 34.9 (C^{18}) ppm. The aromatic carbons of the benzene ring gave resonance at 139.5 (C^{10}), 128.3 (C^{11}), 128.6 (C^{12}), 126.0 (C^{13}), 128.6 (C^{14}), 128.3 (C^{15}), 139.5 (C^{19}), 128.6 (C^{20}), 128.6 (C^{21}), 126.0 (C^{22}), 128.6 (C^{23}) and 128.6 (C^{24}) which belongs to the benzene ring carbons (Figure 5.12).

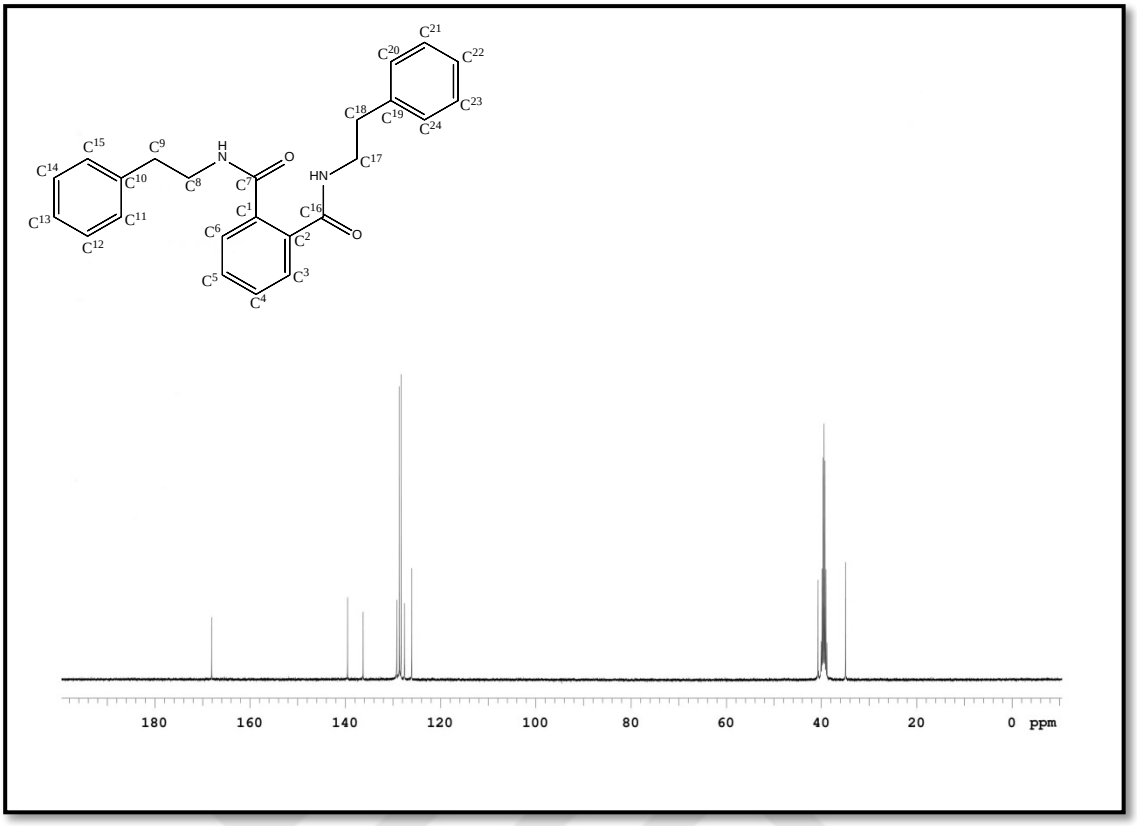


Figure 5.12. ^{13}C-NMR spectrum of the compound 1

All spectral data were in accordance with assumed structures.

In this thesis, synthesized compounds were evaluated for the antimicrobial and anticancer activities. The results are shown in Table 4.1 and 4.2.

Synthesized compounds were screened against gram positive bacteria strain *Staphylococcus aureus* and gram negative bacteria strains *Escherichia coli* and *Pseudomonas aeruginosa* with using the conventional agar dilution method. It was observed that compounds generally showed moderate activity compared with Ofloxacin. The compounds *N*^{1},*N*^{2}-bis[2-(3-chlorophenyl)ethyl]phthalamide (compound 3) and *N*^{1},*N*^{2}-bis[2-(4-chlorophenyl)ethyl]phthalamide (compound 4) exhibited the highest inhibition against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. MIC of compounds 3 and 4 are the 50 and 25 µg\ml, respectively. Compound 4 has the highest activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* with inhibition of 25 µg\ml. Compounds 1, 2 and 7 show low activities with inhibition of 100 µg\ml.

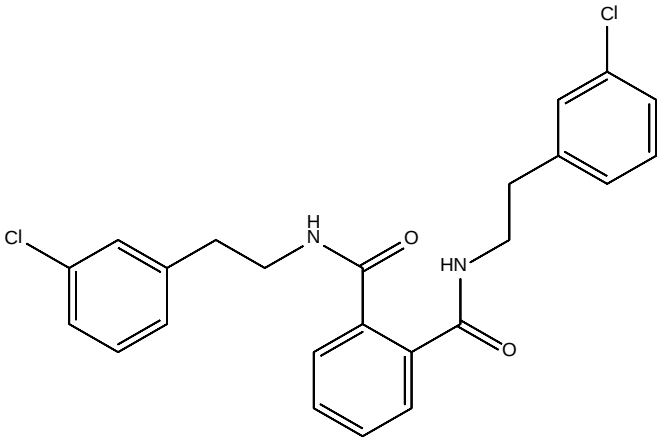


Figure 5.13. Structure of Compound 3

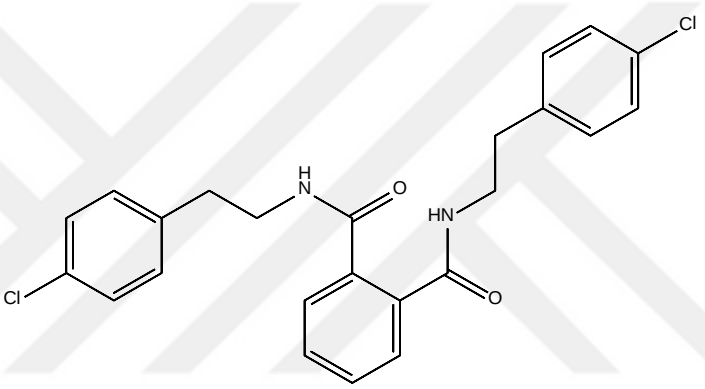


Figure 5.14. Structure of Compound 4

Nitro substituted derivatives (compounds 12-22) were also observed that compounds generally showed moderate activity compared with Ofloxacin. 3-nitro-N^1,N^2-bis[2-(2-chlorophenyl)ethyl]phthalamide (compound 13), 3-nitro-N^1,N^2-bis[2-(4-chlorophenyl)ethyl]phthalamide (compound 15), 3-nitro-N^1,N^2-bis[2-(2,4-dichlorophenyl)ethyl]phthalamide (compound 16) and 3-nitro-N^1,N^2-bis[2-(4-fluorophenyl)ethyl]phthalamide (compound 19) exhibited the highest inhibition value against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. Compounds 13, 15 and 16 were the most active compounds with the MIC of 25 µg\ml. Compound 19 also show moderate activity with the inhibition of 50 µg\ml.

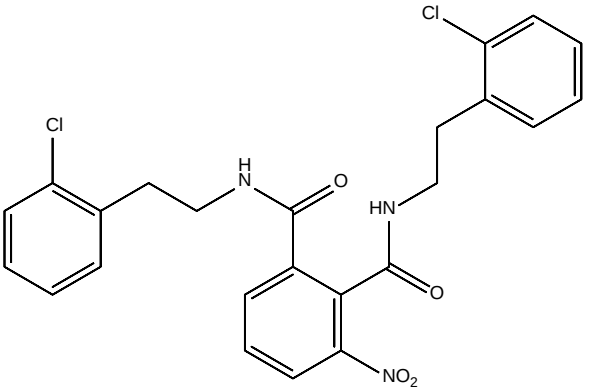


Figure 5.15. Structure of Compound 13

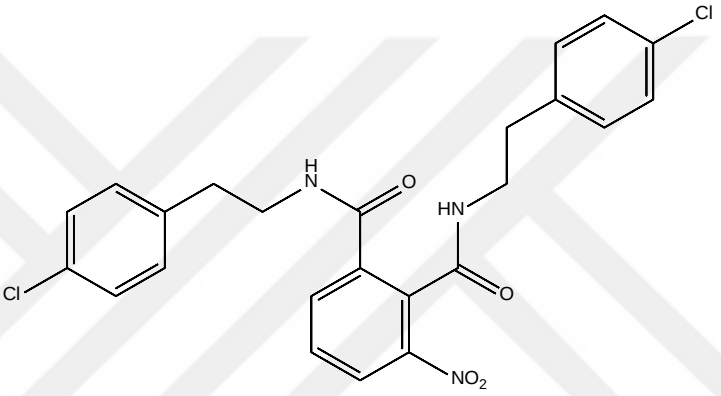


Figure 5.16. Structure of Compound 15

According to these results; it seemed that halogens (2-chloro and 4-chloro) carrying compounds generally showed better antimicrobial activity than the other compounds and 3-nitro moiety increased the antimicrobial activity of phthalamide derivatives.

All of synthesized compounds have been evaluated for the *in vitro* cytotoxic activity against MCF7 and Hep3B cancer cell lines by MTT assay. The results are given in Table 4.2. All of the compounds showed less activity than Doxorubicin against MCF7 and Hep3B.

The most active compound on MCF7 cell line is 3-nitro-N^1,N^2-bis[2-(2,4-dichlorophenyl)ethyl]phthalamide (compound 16) with the IC_{50} value of 46.0 µM. Other compounds with IC_{50} values lower than 100 µM are 3-nitro-N^1,N^2-bis[(2-

chlorophenyl)ethyl]phthalamide derivatives (compounds 13) and 3-nitro-N^1,N^2-bis[(2-fluorophenyl)ethyl]phthalamide (compound 17).

On liver (Hep3B) cancer cell line, the most active compound is 3-nitro-N^1,N^2-bis[2-(2-fluorophenyl)ethyl]phthalamide (compound 17) with the IC_{50} value of 37.6 µM. Other compounds with IC_{50} values lower than 50 µM are N^1,N^2-bis[(2-(2,4-dichlorophenyl)ethyl]phthalamide (compound 5) 3-nitro-N^1,N^2-bis[(2-(2,4-dichlorophenyl)ethyl]phthalamide (compound 16) and 3-nitro-N^1,N^2-bis[(2-chlorophenyl)ethyl]phthalamide (compound 13).

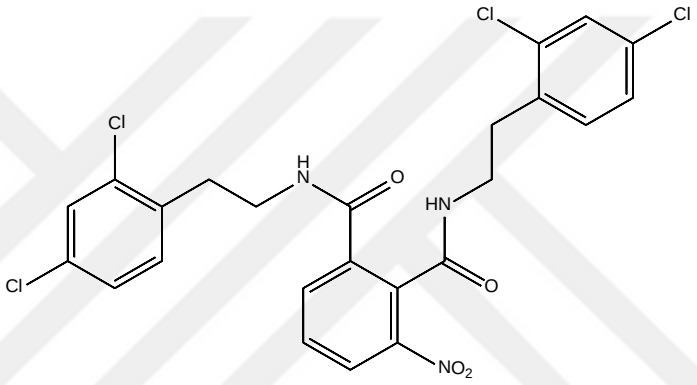


Figure 5.17. Structure of Compound 16

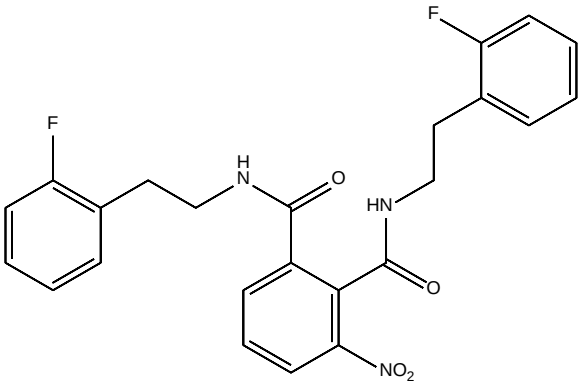


Figure 5.18. Structure of Compound 17

Additionally, prediction of drug-likeness, molecular and ADME properties of synthesized compounds were evaluated. ADME (Absorption, Distribution, Metabolism

and Excretion) parameters play a crucial role in the discovery and development of novel drugs. Currently available drugs in the market possess a balance of desirable ADME properties and actual potency. Lipinski's rule of five helps to predict the presence of drug-like physicochemical properties in a given compound, these properties affect as drug's pharmacokinetics in the human body. Drug candidates that comply with the Lipinski's rule of five have less failure rate during the clinical trial. Physicochemical parameters of synthesized compounds were generated in order to evaluate their drug likeness properties using QikProp module of Schrödinger mentioned in Table 4.3. General range of physicochemical parameters for drug-likeness varies as: CNS (-2 to 2), QPlogPo/w (−2 to 6.5), QPlogHERG (below to −5), QPPCaco (<25, >500), QPlog BB (−3.0 to 1.2), QPPMDCK (<25 poor, >500 great), QPlogKhsa (-1.5 to 1.5), HOA (1, 2 or 3) PHOA (≥80 % is high and ≤25 % is poor), ROF (max. 4), ROT (max. 3) [40]. Results obtained from this study showed that the parameters of all synthesized derivatives were within the acceptable range of drug-likeness.

In conclusion, seven original N^1,N^2-bis[(2-substitutedphenyl)ethyl]phthalamide derivatives and eleven original 3-nitro-N^1,N^2-bis[(2-substitutedphenyl)ethyl]phthalamide derivatives have been synthesized and tested for the antimicrobial and anticancer activities on cancer cell lines of breast (MCF7) and liver (Hep3B) under *in vitro* conditions. In order to obtain a rational structure activity relationship, compound set should be enlarged as a future plan. In addition, the cytotoxicity mechanism will be enlightened for the active compounds.

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7. CURRICULUM VITAE

Kişisel Bilgiler

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Lisans	Kimya Mühendisliği	Ege Üniversitesi	2010
Lise	Fen	Marmaris Sabancı Anadolu Lisesi	2005

Yabancı Diller

Bildiği Yabancı Diller	**Yabancı Dil Sınav Notu**
İngilizce	71

İş Deneyimi

Görev	**Kurum**	**Yıl**
Kalite Güvence	Mefar	2012
Kalite Kontrol	Ali Raif İlaç Sanayi	2010
Üretim	Ali Raif İlaç Sanayi	2010
Kalite Kontrol	Pulcra	2011

Bilgisayar Bilgisi

Program	**Kullanma Derecesi**
Microsoft Word	Çok iyi
Microsoft Excel	Çok iyi
Microsoft Powerpoint	Çok iyi
ChemDraw	Çok iyi