T.C.

YEDITEPE UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

SYNTHESIS AND BIOLOGIC ACTIVITY STUDIES OF SOME SUBSTITUED *N*-(1,3-DIOXOHEXAHYDRO-2*H*-ISOINDOL-2-YL) BENZENESULFONAMIDE DERIVATIVES

MASTER OF SCIENCE THESIS

BETÜL KAYA

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SUPERVISOR

PROF. DR. HÜLYA AKGÜN

STANBUL 2018

APPROVAL

Institute : Yeditepe University Institute of Health Sciences
Programme : Pharmaceutical Chemistry
Title of the Thesis : Synthesis and Biologic Activity Studies of Some Substitue *N*-(1,3-Dioxohexahydro-2*H*-isoindol-2-yl)benzenesulfonamide Derivatives

Owner of the Thesis : BETÜL KAYA
Examination Date : 14.03.2018
This study has been approved as a Master Thesis in regard to content and quality by the Jury.

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Prof. Dr. Bayram Yılmaz
Director of Institute of Health Sciences

v

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Kurum : Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü

Program : Farmasötik Kimya Programı

Tez Başlığı: Bazı Sübstitüe N-(1,3-Dioxohekzahidro-2H-isoindol-2-il)benzensülfonamit Türevlerinin Sentezi ve Biyolojik Aktivitelerinin İncelenmesi

Tez Sahibi : Betül Kaya

Sınav Tarihi : 14.03.2018

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Prof. Dr. Bayram Yılmaz

Sağlık Bilimleri Enstitüsü Müdürü

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 acknowledgement has been made in the text.

14.03.2018

Betül Kaya

I dedicate this thesis to my mother and my father.

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I would like to express my gratitude to Prof. Dr. Hülya Akgün for her guidance, supervision and understanding in every phase of the thesis. I faithfully appreciate her enthusiastic help and all she has taught me. In the past two years, the training that I have received from Prof. Dr. Hülya Akgün has helped me in developing into a junior researcher. I am also deeply grateful to members of pharmaceutical chemistry department; Prof. Dr. Mine Yarım Yüksel and Prof. Dr. Meriç Köksal Akkoç for their guidance and help.

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Finally, I specially thank my dear family for their love and encouragement.

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LIST OF ABBREVIATIONS

CTD	Cantharidin
NCTD	Norcantharidin
Hep3B	Human liver cancer cell line
HepG2	Human liver cancer cell line
HCT-8	Hematocrit (volume percentage of red blood in blood)
HCT-15	Human colon cancer cell line
WI-38	Human fetus lung cancer cell line
HeLa	Human cervical cancer cell line
HONE-1	Human epithelial tumor cell line
NUGC	Human gastric adenocarcinoma cell line
MDA-MB231	Human breast adenocarcinoma cell line
KG1a	Human leukemic cell line
AML	Human acute myeloid leukemia cancer cell line
HL-60	Human promyelocytic leukemia cell line
HT29	Human colon cancer cell line
SW480	Human colorectal cancer cell line
MCF-7	Human breast cancer cell line
SCM-1	Human gastric cancer cell line
SMMC-7721	Human hepatocellular carcinoma cell line
H22	Human hepatocellular carcinoma cell line
SC558	COX-2 inhibitor
COX-1	Cyclooxygenase enzyme inhibitor
COX-2	Cyclooxygenase enzyme inhibitor
SI	Selectivity index
SBO	Stilbenylbenzoxazole
SBT	Stilbenylbenzothiazole
AD	Alzheimer's disease
HBV	Hepatit B
HCV	Hepatit C
HPV	Human papiloma virus

HIV	Human immunodeficiency virus
59T	Human lung cancer cell line
H460	Human lung cancer cell line
A431	Human skin cancer cell line
DU145	Human prostate cancer cell line
SJ-G2	Human brain cancer cell line
BE2-C	Human neuroblastoma cancer cell line
SKHep1	Human hepatic adenocarcinoma cell line
CA46	Burkitt's lymphoma cell line (Bcell Type)
CML	Human chronic myelogenous cancer cell line
K562	Human erythroleukemia type cancer cell line
A2780	Human ovarian cancer cell line
ECV304	Human bladder cancer cell line
T47D	Human breast cancer cell line
PP1, PP2A	Protein phosphatase
PPP1-PPP6	Protein phosphatase
IC_{50}	Half maximal inhibitory concentration
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DNA	Deoxyribonucleic acid
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
MeOH	Methanol
IR	Infrared
NMR	Nuclear Magnetic Resonance
UV	Ultraviolet
MS	Mass spectrometry
s	Singlet
d	Doublet
t	Triplet
q	Quartet

m	Multiplet
ppm	Parts per million
R_f	Retention factor
ND:	Non detectable
ASA:	Acetylsalicylic acid
NO:	Nitric oxide

SUMMARY

Kaya, B. Studies on Novel Substitued *N*-(1,3-Dioxohexahydro-2*H*-isoindol-2-yl)benzenesulfonamide Derivatives and Biological Activities. Yeditepe University Institue of Health Science, Department of Pharmaceutical Chemistry, M.Sc. Thesis, İstanbul, 2018.

In the course of this study five out of ten novels substituted *N*-(1,3-dioxohexahydro-2*H*-isoindol-2-yl)benzenesulfonamide (compound **1-10**) were synthesized byusing two different methods. In the first method, the cis-1,2-cyclohexanecarboxylic anhydride and sulfa derivatives were conventionally heated in acetic acid for 4 hours In the second method, the *cis*-1,2-cyclohexanecarboxylic anhydride and sulfa derivatives were dissolved in DMF and radiated by microwave.

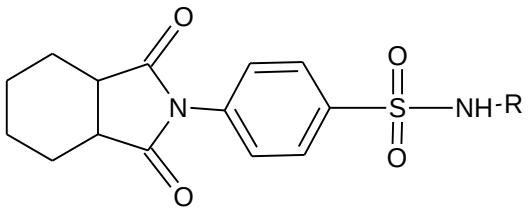
Structure elucidation of the synthesized compounds were confirmed by UV, IR ^{1}H-NMR,^{13}C-NMR with mass spectral methods.

Anticancer activities of the compounds were studied on human breast cancer (MCF7) cell lines by MTT assay. Anti-inflammatory activities of the compounds were examined by measuring nitrite concentrations by using a colorimetric method based on the Griess reaction on RAW 264.7 macrophage cells.

Synthesized compounds generally showed moderate or no cytotoxic activity against MCF7 cell line. Among them, 4-(1,3-dioxohexahydro-2*H*-isoindol-2-yl)-*N*-(1,3-thiazol-2-yl)benzenesulfonamide (compound **3**), 4-(1,3-dioxohexahydro-2*H*-isoindol-2-yl)-N-(4,5-dimethoxypyrimidin-2-yl)benzenesulfonamide (compound **7**), and 4-(1,3-dioxohexahydro-2*H*-isoindol-2-yl)-N-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide (compound **10**) presented activity against MCF7 cancer cell lines with IC_{50} values of 87.9 ± 2.34 μM, 71.5 ± 3.01 μM, and 89.3 ± 2.05 μM, respectively.

Proposed compounds were also analyzed for their anti-inflammatory activity on RAW 264.7 macrophage cells. Among them, 4-(1,3-dioxohexahydro-2H-isoindol-2-yl)-N-(6-mmethoxypyridazin-3-yl)benzenesulfonamide (compound **4**), 4-(1,3-dioxohexahydro-2H-isoindol-2-yl)-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide (compound **1**) and 4-(1,3-dioxohexahydro-2H-isoindol-2-yl)benzenesulfonamide (compound **9**) presented activity against RAW 264.7 macrophages with NO inhibition (% of control) values of 24.43 ± 3.16 μM, 9.73 ± 1.04 μM and 6.44 ± 2.48 μM,

respectively. No significant cytotoxic activities on RAW 264.7 macrophage cells were observed under all tested concentrations and IC_{50} values of the tested compounds were higher than 500 μM.



Compounds **1-10**

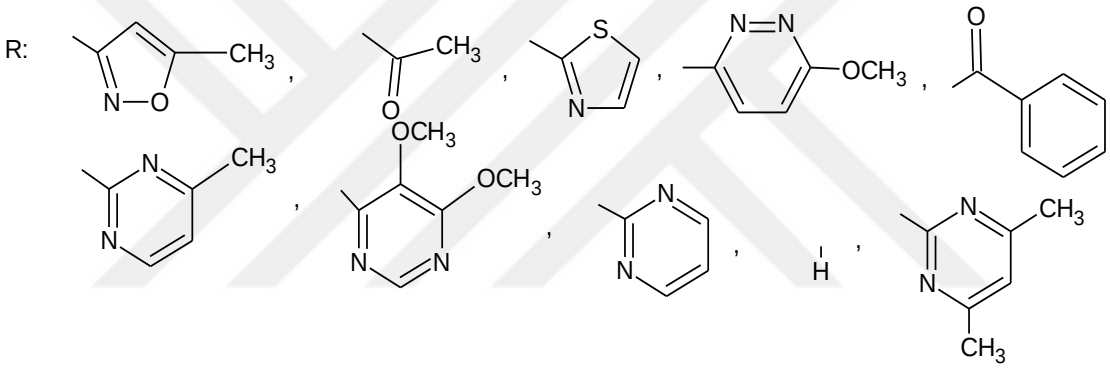


Figure 1: Structures of the synthesized compounds **(1-10)**

Keywords: Hexahydroisoindol, cyclohexylanhydride, sulfa drugs, anticancer, anti-inflammatory activity.

ÖZET

Kaya, B. Bazı Sübstitüe *N*-(1,3-Dioksohekzahidro-2*H*-isoindol-2-il)benzensülfonamit Türevlerinin Sentezi ve Biyolojik Çalışmaları. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Farmasötik Kimya Programı Yüksek Lisans Tezi, İstanbul, 2018.

Bu tez çalışmasında, beş bileşik orjinal olmak üzere on adet sübstitüe *N*-(1,3-Dioksohekzahidro-2*H*-isoindol-2-il)benzensülfonamit yapısında (bileşik **1**-**10)** bileşik iki farklı yöntem kullanılarak sentezlenmiştir. İlk yöntemde, *cis* -1,2- siklohekzan karboksilik anhidrit ve sülfa türevleri asetik asitli ortamda geri çeviren soğutucu altında 4 saat ısıtılarak bileşikler elde edilmiştir. İkinci yöntemde *cis*-1,2- sikolohekzan karboksilik anhidrit ve sülfa türevleri DMF içinde çözülerek mikro dalga kullanılarak sentezlenmişlerdir.

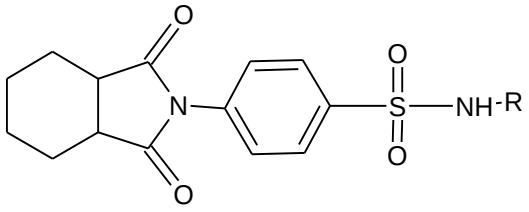
Elde edilen bileşiklerin yapıları UV, IR ^{1}H-NMR, ^{13}C-NMR ile kütle spektroskopisi kullanılarak doğrulanmıştır.

Bileşiklerin antikanser aktivite testleri MCF7 hücre hatlarında MTT testi ile çalışılmıştır. Bileşiklerin anti-inflamatuvar aktivite çalışmaları ise RAW 264.7 makrofaj hücreleri üzerinde Griess reaksiyonu uygulaması ile kolorimetrik yöntem uygulanarak nitrit konsantrasyonu ölçülerek saptanmıştır.

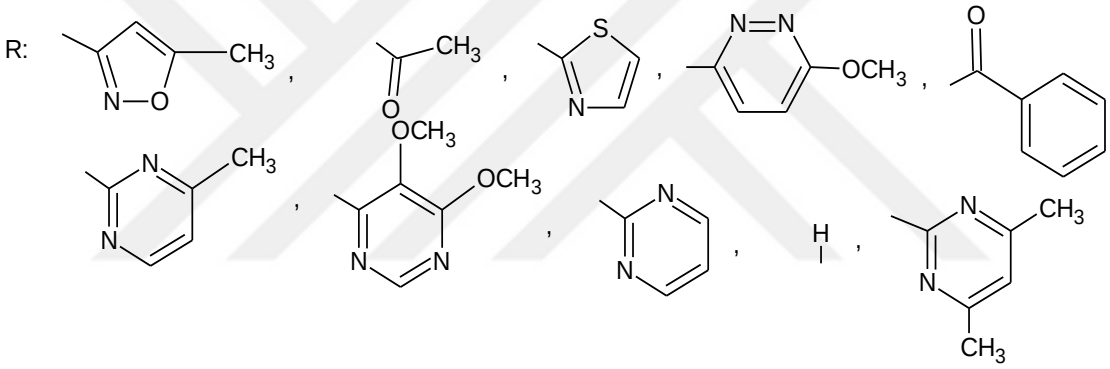
Sitotoksisite sonuçları incelendiğinde sentezlenen bileşiklerin MCF7 hücre hattında orta derecede aktivite gösterdikleri veya aktivite göstermedikleri belirlenmiştir. 4-(1,3-Dioksoohekzahidro-2*H*-isoindol-2-il)-N-(1,3-tiyazol-2-il)benzensülfonamit (bileşik 3,) 4-(1,3-dioksohekzahidro-2*H*-isoindol-2-il)–N-(4,5–dimetoksipirimidin-2-il)benzensülfonamit (bileşik 7), ve 4-(dioksoohekzahidro-2*H*-isoindol-2-il)-N-(4,6-dimetilpirimidin-2-il)benzensülfonamit (bileşik 10), sırasıyla 87.9 ± 2.34 μM, 71.5 ± 3.01 μM ve 89.3 ± 2.05 μM IC_{50} değerleri ile MCF7 kanser hücre hattına karşı aktivite gösteren bileşiklerdir.

Sentezlenen bileşiklerden 4-(1,3-dioksohekzahidro-2*H*-isoindol-2-il)-N-(6-metoksipiridazin-3-il) benzensülfonamit (bileşik 4), 4-(1,3-dioksohekzahidro-2*H*-isoindol-2-il)-*N*-(5-metil-1,2-okzazol-3-il)benzensülfonamit (bileşik l) ve 4-(1,3-dioksohekzahidro-2*H*-isoindol-2-il) benzensülfonamit (bileşik 9)'ın ölçülen NO

değerleri, sırasıyla 24.43 ± 3.16 μM, 9.73 ± 1.04 μM ve 6.44 ± 2.48 μM olmuştur. NO inhibisyonu değerleri RAW 264.7 makrofaj hücreleri (% kontrol) varlığında test edilmiştir. Diğer bileşiklerin test edilen konsantrasyonlarda IC_{50} değerleri 500 uM'den yüksek bulunmuştur.



Bileşikler **1-10**



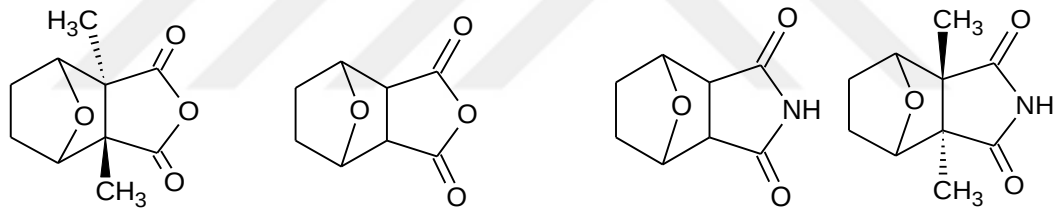
Figür 1: Sentezlenen bileşiklerin yapıları **(1-10)**

Anahtar kelimeler: Hekzahidroisoindol, sikloheksilanhidrit, sulfa ilaçlar, antikanser, antiinflamatuvar aktivite.

1. INTRODUCTION AND AIM

Cancer and inflammatory diseases are the most important health problems around the world today. The functional relationship between inflammation and cancer is not new. In 1863, Virchow hypothesized that the origin of cancer was at sites of chronic inflammation [1]. Today, the relationship between inflammation- immunity and cancer is more widely accepted. Several excellent reviews are found about acquired immune response to cancer which is related to the inflammatory response [2, 3].These evidences are forcing the scientists to design the new anti-inflammatory drugs may be useful for cancer treatment.

Several mechanisms of action were offered for clinically used anticancer drugs. One of them was the connection between protein phosphatase inhibition PP1 and PP2A and anticancer activity [4-8]. The oldest known compounds which inhibit protein phosphatase inhibition PP1 and PP2A are Cantharidin (CTD), Norcantharidin (NCTD), Cantharimide and Norcantharimide.



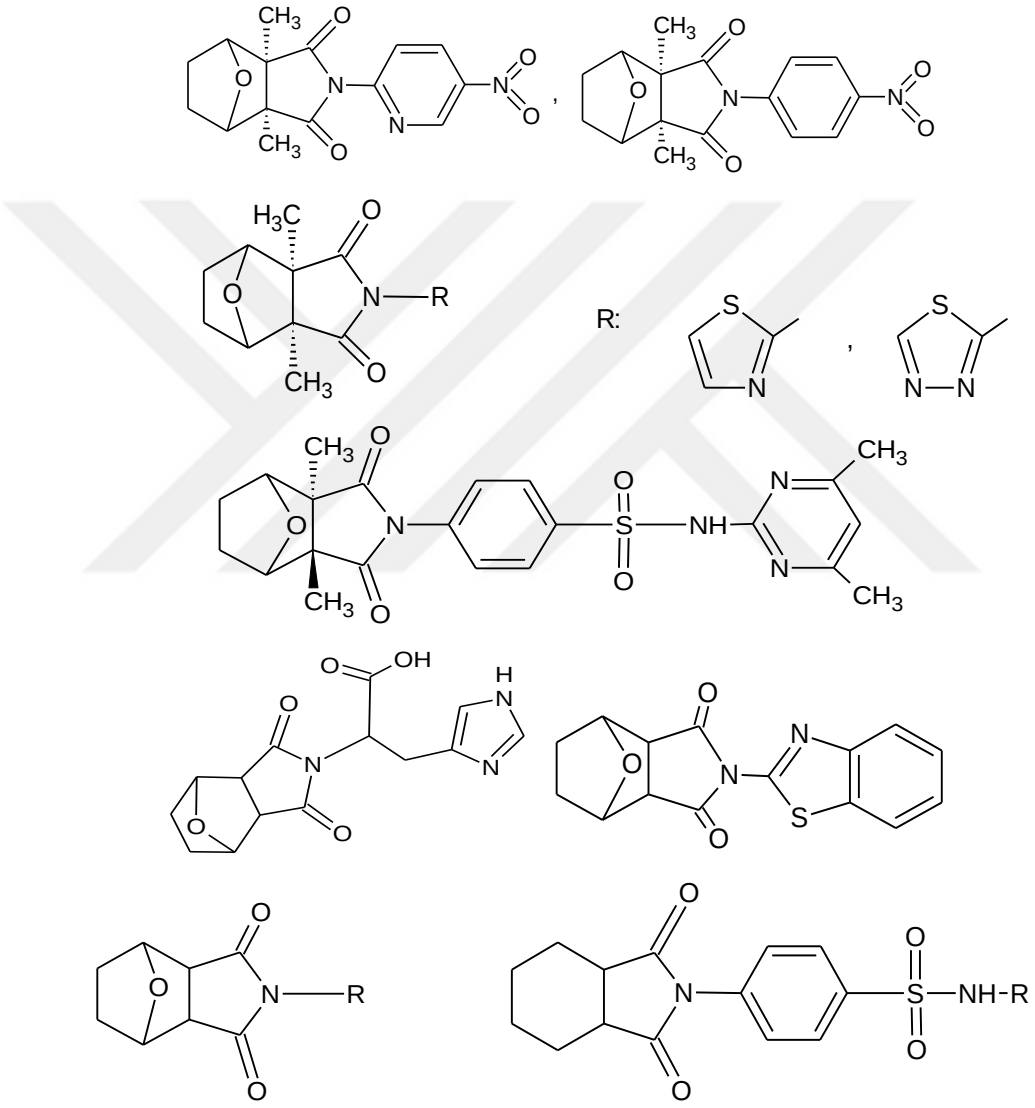
Cantharidin (CTD) Norcantharidin (NCTD) Cantharimide Norcantharimide

These compounds were isolated from Mylabris which is the dried body of the blister beetle. Mylabris has been used in Chinese medicine for thousands of years for the treatment of malignant tumors of breast, colorectal, hepatoma and abdominal cancer [9].

Scheme 1: Blister Beetle Types [10].

NCTD is demethylated form of CTD, appears to have less nephrotoxicity and liver toxicity, however the demethylation lowers its bioactivity [9].

Various CTD and NCTD analogues bearing several different substituent at *N*-position, have been synthetized and screened for their anticancer activity as seen below [5, 9-11].



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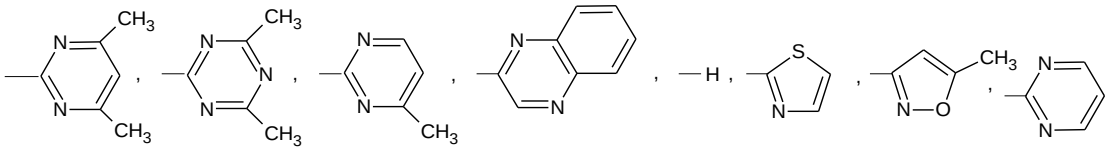
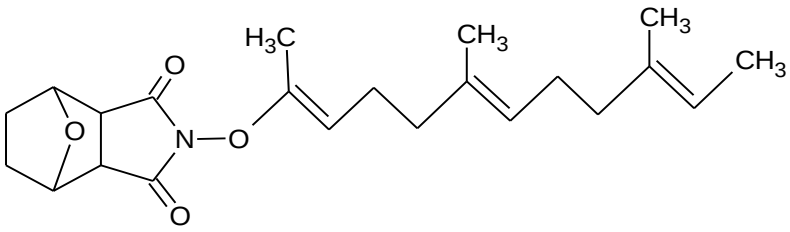
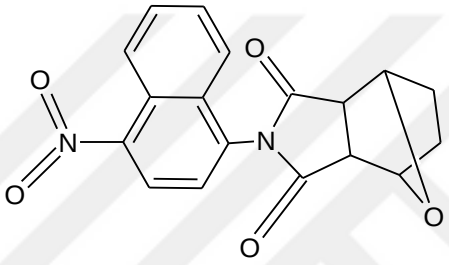
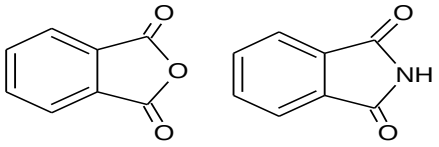


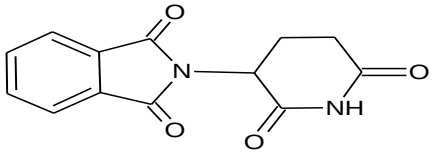




Phthalic anhydride and phthalimide are the aromatic derivatives of cyclohexanedicarboxylic anhydride/imide. They are good starting materials for many drug molecules. The most important derivative is thalidomide and has antitumor, anti-inflammatory, antimicrobial and immunomodulatory activities [12-15].

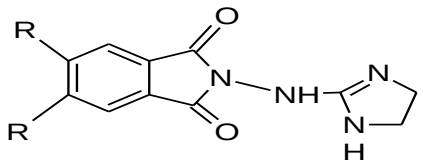


Phthalic anhydride Phthalamide

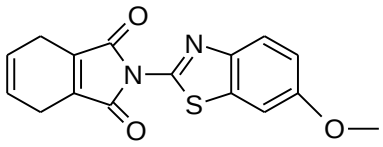


Thalidomide

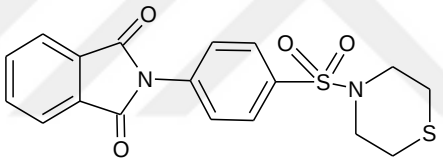
Recently, it was reported that *N*-substituted cyclic imide derivatives possess inflammatory activity on inhibition of tumor necrosis factor-α (TNF-α) [16,17].



R = -H and -OCH_3

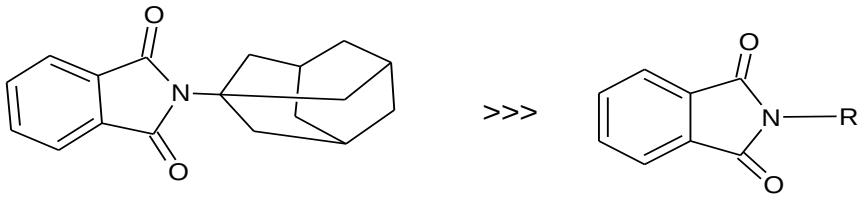


Compound LASSBio468 was found to have a sulfonyl-thiomorpholine moiety, and it showed potent inhibitory activity on LPS-induced neutrophile recruitment with ED_{50}=2.5 mg/kg, which was correlated with its inhibitory effect on TNF-α level [18].



LASSBio 468

N-alkylated phthalimide analogues bearing adamantyl and several R groups at *N*-position showed very potent bi-directional TNF-α production-regulating activity. Among these series 4-pentylphenyl, 1-adamantyl and 2,4- dimethylphenyl substituted compounds gave the best activity [19].

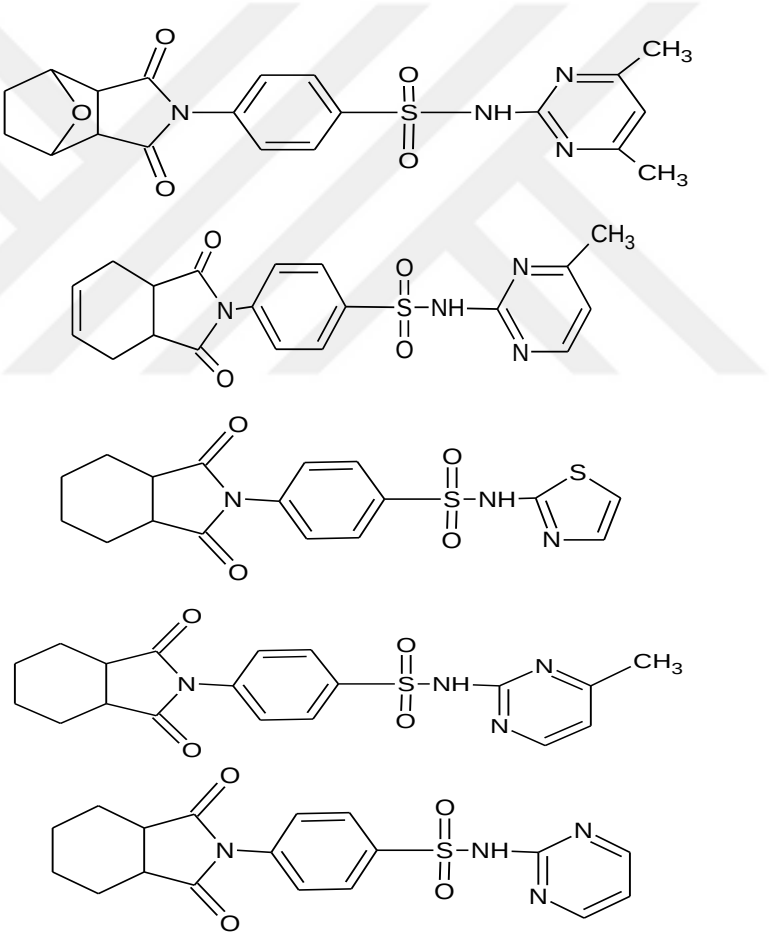


R = 3-penthyl > R = cyclohexyl > R = t-butyl > R = n-butyl

As it is seen in below examples, a series of norcantharimide and phthalimide analogs bearing a long alkyl chain, cyclic saturated rings, alkyl amines or aromatic rings at *N*-position were synthetized. These hydride structures have enhanced bioavailability and transportability through cell membrane when compared with the norcantharimide and phthalimide [20].

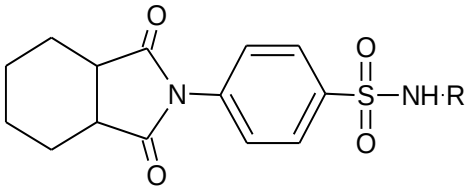
Sulphonamides are interesting aryl amines showing antibacterial, carbonic anhydrase inhibitor, hypoglycemic and antithyroid activities [21-26].

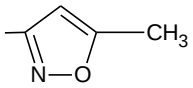
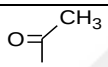
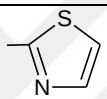
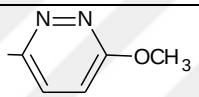
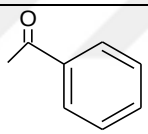
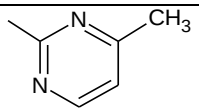
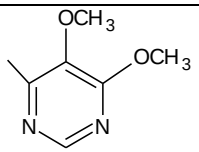
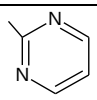
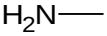
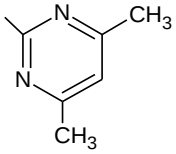
The analogues of norcantharimide and phthalimides with sulphonamides were also prepared and were found to promote antiinflammatory and anticancer activity [17, 27].



Under the light of these studies, we aimed to synthetize a series of *N*-(1,3-dioxohexahydro-2*H*-isoindol-2-yl)benzenesulfonamide derivatives which were expected to show anticancer and antiinflammatory activity.

Table 1. Formula and physicochemical properties of the synthesized compounds **1-10**

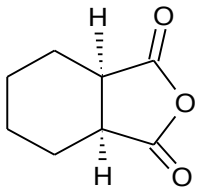


COMPOUND		% YIELD		M.P. (^{0}C)
No	**R**	**RFLX**	**MW**	
1		31	97	213
2		98	98	178
3		100	97	230
4		60	100	240
5		100	97	245
6		100	100	318
7		100	99	190
8		90	100	254
9		40	95	256
10		90	90	213

2. GENERAL DESCRIPTIONS

2.1. *Cis*-1,2-cyclohexanedicarboxylic Anhydride

Hexahydrophthalic acid (*cis*-hexahydro-2-benzofuran-1,3-dion) is the anhydride form of 1,2-cyclohexanedicarboxylic acid.

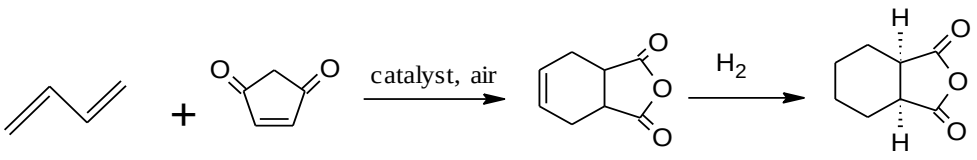


Cis-1,2 cyclohexanedicarboxylic anhydride

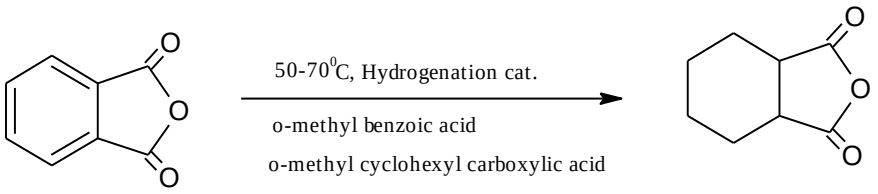
It is a white moisture sensitive solid crystalline compound. Melting point: 32-34 C, density: 1.23 and boiling point: 158°C. *Cis*-1,2-cyclohexanedicarboxylic anhydride is a useful reagent in variety of polymers and organic synthesis [28].

2.1.1. Synthesis of *cis*-1,2-Cyclohexanedicarboxylic Anhydride

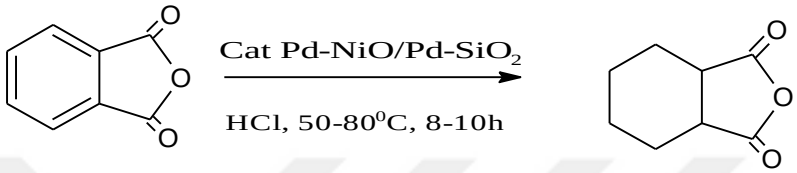
In the literature, several methods were used for the synthesis of *cis*-1,2-cyclohexanedicarboxylic anhydride. The oxidation of 1,4- butadiene and maleic acid anhydride with air, molybdenum-, bismuth-, or nickel-based catalyst gave *cis*-1,2,3,6-tetrahydrophthalic anhydride with high yield. Then, hydrogenation of the intermediate product to obtain *cis*- 1,2-cyclohexanedicarboxylic anhydride [29].



Catalytic hydrogenation of phthalic anhydride was carried out with catalyst o-methyl benzoic acid/ o-methylcyclohexanecarboxylic acid gave hexahydrophthalic anhydride [30].



Other alternative synthesis of *cis*-1,2-cyclohexanedicarboxylic anhydride was hydrogenation reaction of phthalic anhydride with some catalysts (Pd-NiO/Pd-SiO_2) [31].

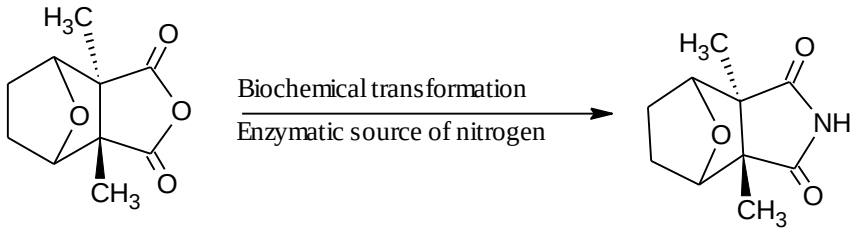


2.2. Cantharidin, Cantharimide and Similar Natural Compounds

2.2.1. Synthesis of Cantharidin and Cantharimide Derivatives

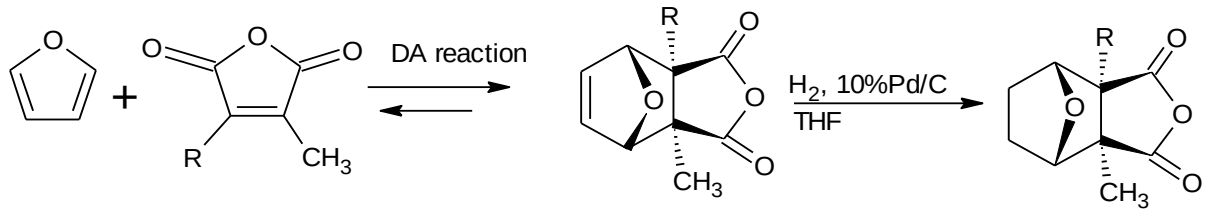
Cantharidin (CTD) (exo-2,3-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid anhydride) was first obtained from Mylabris. Mylabris is dried body of blister beetle and has been used in Chinese medicine due to its anticancer activity since 13th century. Although it is found to be toxic to the kidney and liver, CTD possess anticancer activity due to the the inhibition of serine/threonine protein phoshatase1 and 2 (PP1 and PP2) [32-38] CTD analogues compounds NCTD, cantharimide, and norcantharimide may show similar pharmacologic activity like CTD [5, 10, 39].

Cantharimide was synthesized through a biochemical transformation of CTD in which oxygen atom was replaced by nitrogen, by a natural source of nitrogen [10].

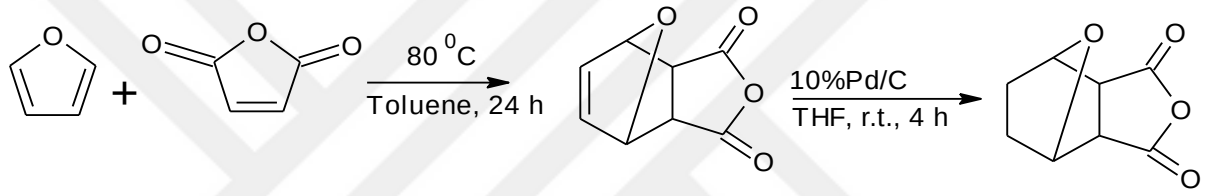


Cantharidin Cantharimide

NCTD and CTD were prepared as Diels-Alder cyclo addition adducts of furan derivatives and maleic anhydride and presented a well-defined exo-stereochemistry of oxygenated ring and in the 7-oxo-norborn-2-ene system by Galvis *et al.* in 2013 [10].

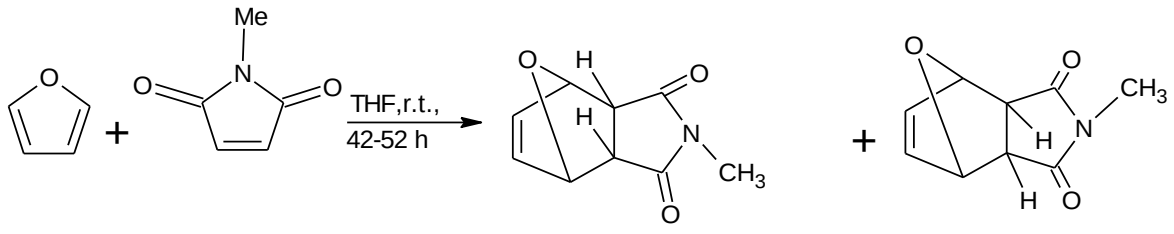


Furan R= CH_3, H R= CH_3, H R=CH_3, H



Furan Maleic anhydride 5,6-dehydronorcantharidin Norcantharidin

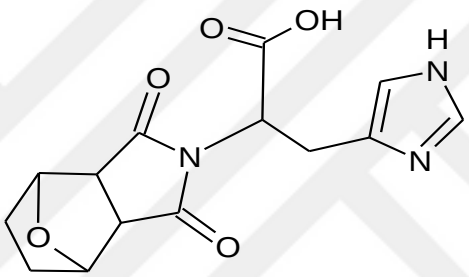
Diels-Alder cyclo addition adducts of furan and *N*-methylmaleimide anhydride was offered for the synthesis of *N*-methylnorcantharimide with the yield of 31-98%.



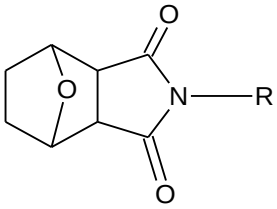
Furan *N*-methylmaleimide A B

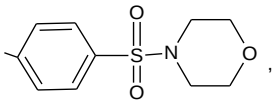
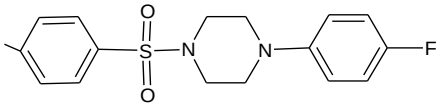
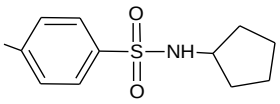
2.2.1.Cantharimide and Norcantharimide Derivatives Possessing Anticancer Activity

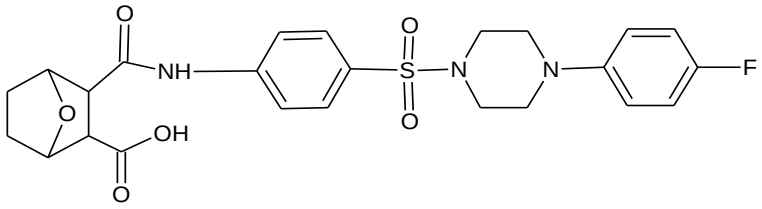
In recent years, there is an intense interest in the development of potent inhibitors of protein phosphatase PP1 and PP2A [4, 38]. The modified CTD analogues target for inhibition of PP1 and PP2A were reported by McCluskey in 2001 [4]. In this study CTD and NCTD and amino acids were reacted in basic media using toluene. These derivatives have been screened for phosphoprotein phosphatase inhibitory activity (PPP1-PPP6). Among them, cantharimide D- or L-histidine hybrides, *N*-histidin-7-oxa-bicyclo[2.2.1]heptanes-2,3-dicarboximide are more potent inhibitors of PP1 and PP2A (PP1 IC_{50}=3.22+/-0.7 μM; PP2A IC_{50}=0.81+/-0.1 μM and PP1 IC_{50}=2.82+/-0.6 μM; PP2A IC_{50}=1.35+/-0.3 μM), respectively.



Some of NCTD derivatives were synthesized as protein phospatase-1 inhibitors by the Zhao et. al. *N*-(4-morpholin-1-yl-sulfonylphenyl)-7-oxa-bicyclo[2.2.1]heptanes-2,3-dicarboximide, *N*-(4-(4-(4-fluorophenyl)piperazin-1-yl-sulfonyl)phenyl)-7-oxabicyclo [2.2.1]heptanes-2,3-dicarboximide and ring open analogue 3-((4-(4-(4-flurophenyl) piperazin-yl-sulfonyl)phenyl)carbamoyl)-7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid were obtained by reacting furane and maleic anhydride in toluene with higher than 60% yield [40].

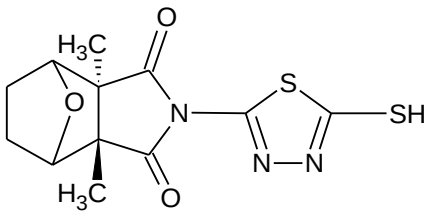


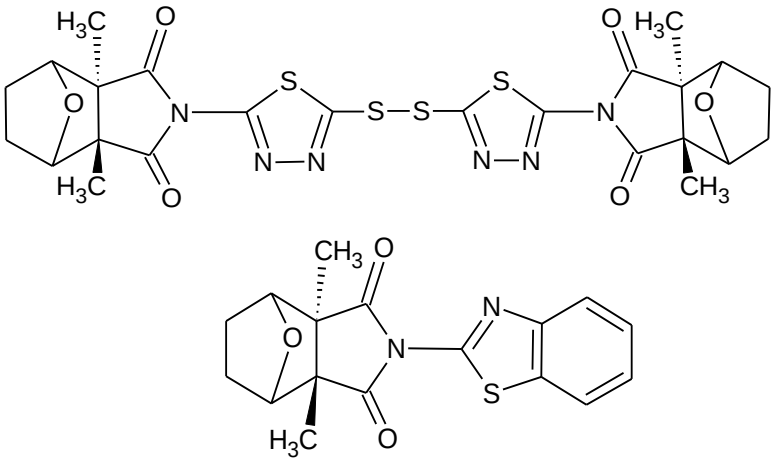
R:  ,  , 



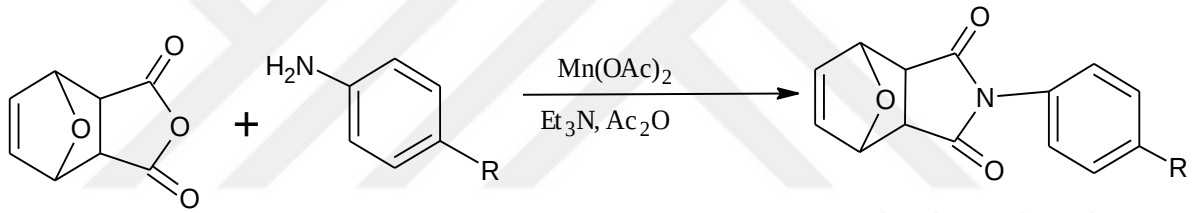
Especially 3-((4-(4-(4-flurophenyl)piperazin-1ylsulfonyl)phenyl)carbamoyl)-7-oxo-bicyclo[2.2.1]heptane-2-carboxylic acid exhibited potent cytotoxic effects on the tumor cell lines A-549, HepG2, HeLa, and HCT-8, whereas it was less toxic to WI-38 cells than its parent compound NCTD. This compound inhibited protein phosphatase-1 activity and microtubule formation in HeLa cells, and it also interacts with calf thymus DNA [41].

Hybrid structure of CTD with 1,3,4-thiadiazole- 2-thiol/ 2-aminobenzothiazole were prepared with high yield by using CTD and thiazole amines in toluene [33]. The corresponding compounds were tested on the Hep3B (hepatocellular carcinoma), MDA-MB231 (breast cancer), A549 (non-small cell lung carcinoma) and KG1a (acute myelogenous leukemia) (AML) cell lines by monitoring the intracellular adenosine triphosphate level [32]. Bis[*N*-(5-sulfonyl-1,3,4-thiadiazol-2-yl)cantharidin] showed more specific inhibitory and cytotoxic activity on both the Hep3B, HCC and the KG1a AML cell lines. *N*-(1,3-benzothiazol-2-yl)cantharidin was reported to possess anti-cancer activity on HCC, Hep3B and SK-Hep-1 cell lines.

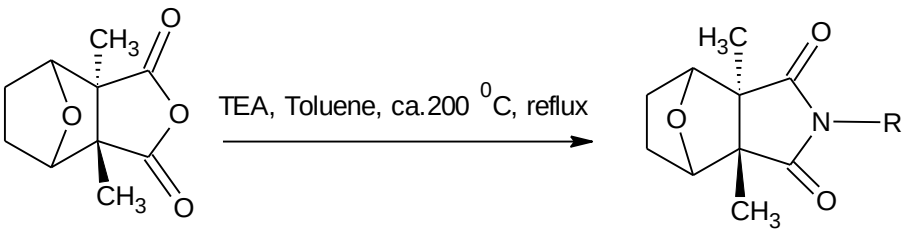




$Mn(OAc)_2$ catalyzed synthesis of fourteen novel *N*-phenyl-norcantharimides were also performed by reacting *exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride and anilines derivatives, with moderate -to-excellent yields [42].



Thiazol- and thiadiazol- containing cantharidinimides, caused cytotoxic effects on 59T, SCM-1, Hep3B, HONE-1 and NUGC human carcinoma cell lines [43].



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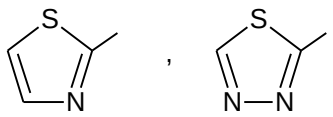
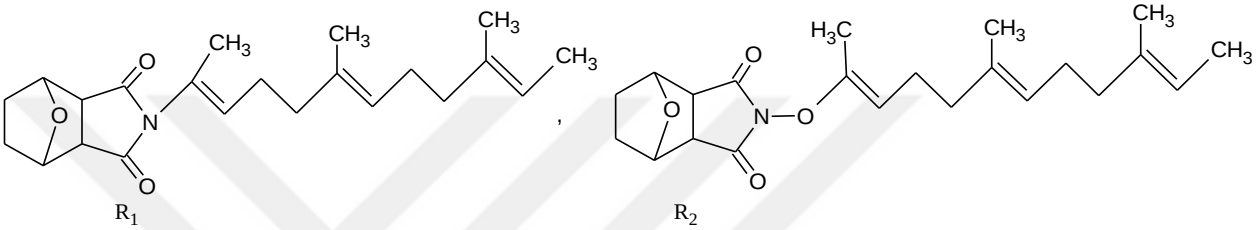
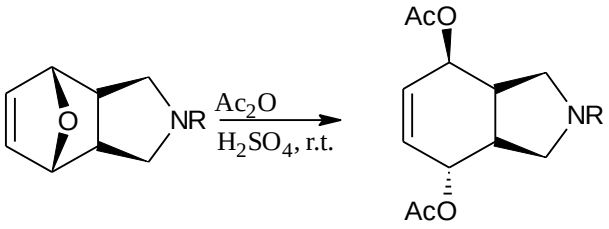


Jin-Yi Wu *et al.* prepared several NCTD analogues having long chain alkyls at *N*-position. Long alkyl chains at *N*-position may improve bioavailability and uptake through cell line. The *N*-farnesyloxy derivatives were prepared by the reaction of *N*-hydroxy cantharimide with alkylbromide in dry acetone and K_2CO_3. Among them, compounds *N*-farnesyloxy-7-oxabicyclo[2.2.1]heptanes-2,3-dicarboximide and *N*-farnesyl-7-oxabicyclo[2.2.1]heptanes-2,3-dicarboximide showed the highest cytotoxicity, anti-proliferative and apoptotic effect against human liver carcinoma HepG2 cell lines [9].

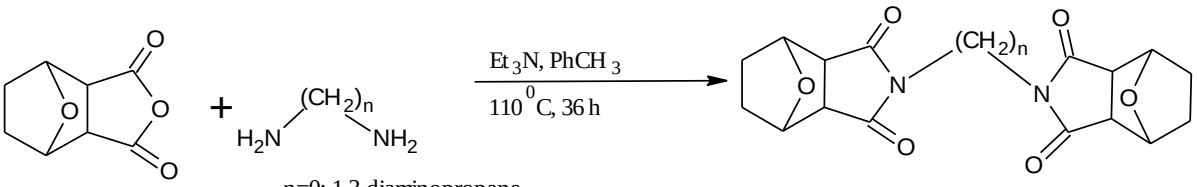


New isoindole analogues were synthesized whose the epoxide rings were opened with Ac_2O in the catalytic amount of H_2SO_4. *N*-2- acetoxyethyl and *N*-methyl derivatives displayed cytotoxicity against A549 and MCF-7 cell lines [44].

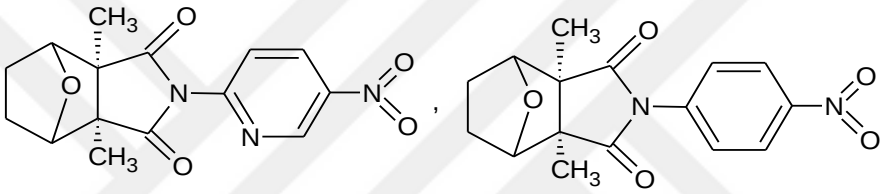


R = $(CH_2)_2OAc$ and CH_3

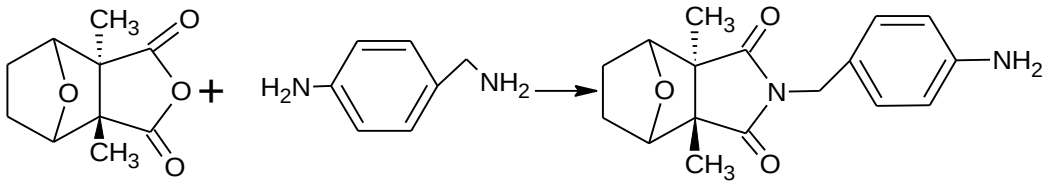
Hill *et al*. reported analogues bis-norcantharidimides by using NCTDs and 1,3-diaminopropan. This groups of compounds showed potent PP1 (IC_{50} = 9.0 ± 1.4 μM) and PP2A (IC_{50} = 3.0 ± 0.4 μM) inhibitor activity and induced growth inhibition (GI_{50} ~45 μM) across a range of human cancer cell lines including those of colorectal (HT29, SW480), breast (MCF-7), ovarian (A2780), lung (H460), skin (A431), prostate (DU145), neuroblastoma (BE2-C), and glioblastoma (SJ-G2) [45].



Lin *et al.* prepared new cantharidinimides using CTD and primary amines, aniline derivatives and aminopyridines in toluene with high yields. The potential cytotoxicity of prepared compounds were investigated against hepatocellular carcinoma cell (Hep G2) and human myeloid leukemia cell (HL-60) lines using MTT cell viability assays. The following compounds were the most active derivatives [41].

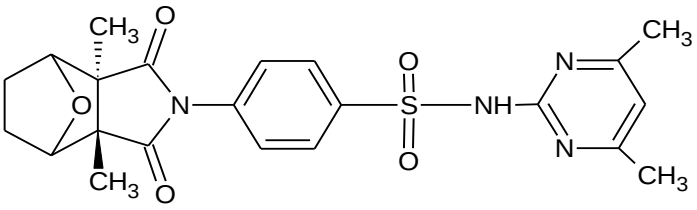


The *para*-aminobenzylic imides were synthesized by the reaction of CTD with aminopyridines, 4-aminomethylaniline, in triethylamine and toluene at 200^0C. Tseng et al. reported the compound, *para*-aminobenzylic norbonane-imide, had the most potent effect on inducible NOS among the tested compounds and showed %35 inhibition [43].



CTD *para*-aminobenzylamine *para*-aminobenzylic norbonane-imide

Tseng et al. also reported some cantharidimido-sulfo analogues. Among the Catharidinimido-sulfo analogues *N*- (Cantharidinimido)sulfamathazine was showed more potent activity than the parent compound CTD and other sulfonamide derivatives on HL-60 and Hep3B cell line [20, 44].



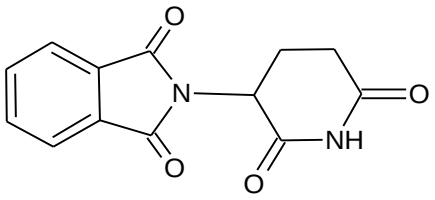
N-cantharidinimido-sulfamethazine

2.3. Phthalic anhydride, Phthalimide and Their Biological Properties

Phthalic anhydride is aromatic derivative of cyclohexanedicarboxylic anhydride. Phthalimide was first synthesized by Vogel in 1967 with phthalic anhydride and concentrated ammonia solution [45]. Since then many similar synthetic procedures were applied for phthalimide and derivatives.

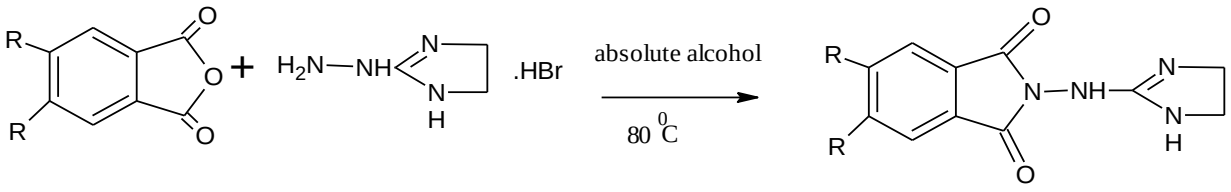
2.3.1. *N*-Substitued Phthalimide Derivatives and Their Biological Properties

One of the important phthalimide derivatives is thalidomide. After the dramatic disaster of thalidomide, they found that the compound has a selectivity of blocking tumor TNF-α production. Therefore thalidomide was suggested to use as antitumor, anti-inflammatory, antimicrobial and immunomodulatory [12-14, 46].



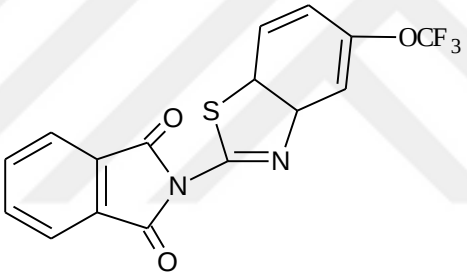
Thalidomide

Phthalimide derivatives were usually prepared by reaction of phthalic anhydride and aromatic amines. Phthalic anhydride and dimethoxyphthalic anhydride were reacted with some imidazole hydrazone derivatives in absolute alcohol to obtain following structure and screened for antineoplastic activity. These derivatives exhibited weak cytotoxic activity [17].

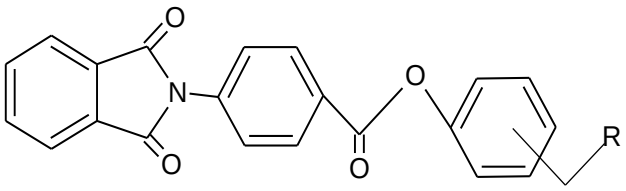


R= -OCH $_3$ and -H

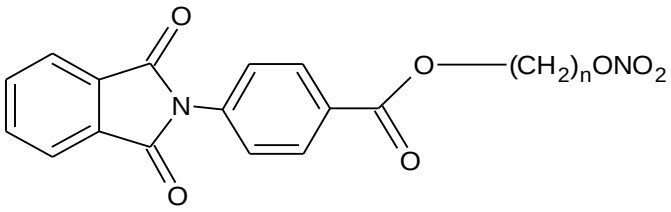
Stanton Hon Lung Kok *et al.* synthetized some phthalimide derivatives bearing benzothiazole ring system at *N*-position and screened *in vitro* cytotoxic potential on human cancer cell lines as hepatoma cell line SKHep1, the Burkitt's lymphoma cell line (B cell type) CA46 and K562 cell line (chronic myelogenous leukemia 'CML'). Among them, 2-amino-6-trifluoromethoxy-benzothiazole phthalimide showed the best anti-cancer activity [16].



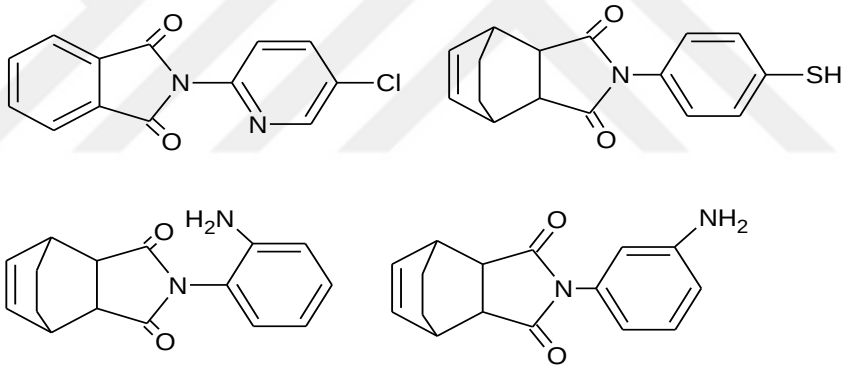
Several phthalimide derivatives were synthesized which include p- and m-nitrooxymethyl substituents on the aromatic ring with enhanced activity against ECV304 and HepG2 cells, on the other hand removal of phenyl ring created more potent compound against HepG2 cells [46].



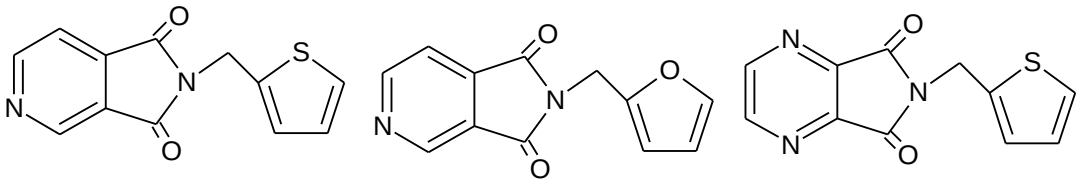
R = para and meta -CH_2ONO_2

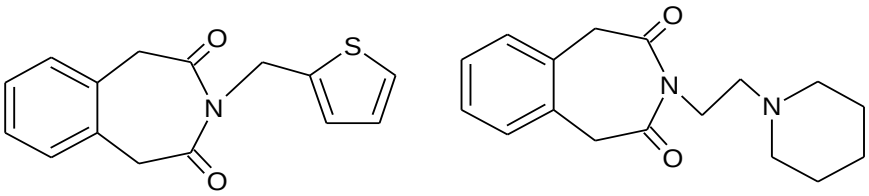


Yeh *et al.* prepared the new series phenylphthalimide analogues in TEA and toluene high pressure sealed tube. Among them, *N*-(5-chloro-2-pyridyl)phthalimide, *N*-(4-mercapto-phenyl)-4,7-ethano-3a*H*,7a*H*-cis-3a,4,7,7a-tetrahydro-isoindolin-1,3-dion, *N*-(2-amino-phenyl)-4,7-ethano-3a*H*,7a*H*-cis-3a,4,7,7a-tetrahydro-isoindolin-1,3-dion, *N*-(3-amino-phenyl)-4,7-ethano-3a*H*,7a*H*-cis-3a,4,7,7a-tetrahydro-isoindolin-1,3-dion were found to have antitumor activity in Hep2 and HL-60 cell lines [47].

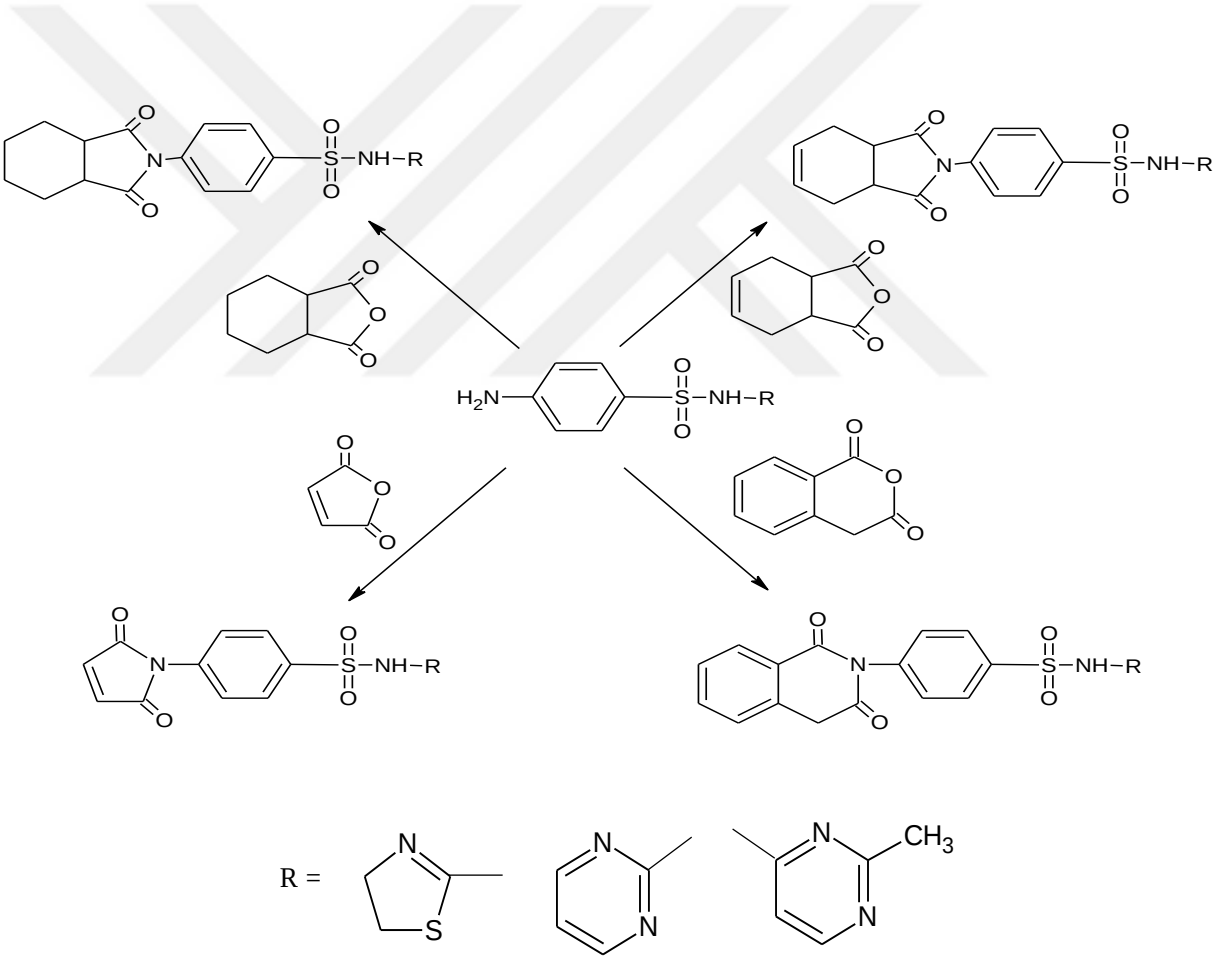


In 2009 Sondhi et al. synthesized some *N*-substituted bicycloimides by condensation of various diacids with different amines under microwave irradiation. The following all compounds showed activity against colon cancer [48].

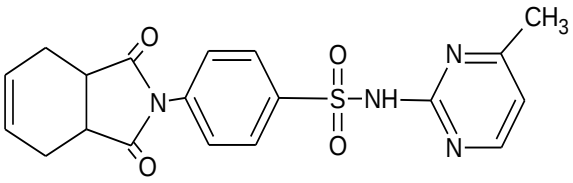




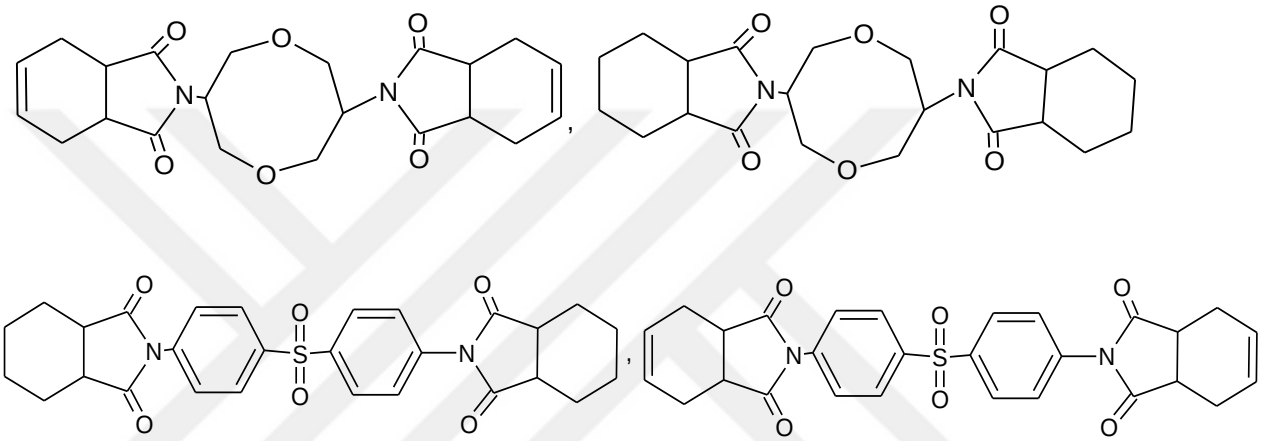
Kumar *et al.* prepared some cyclicimide hybride molecules using condensation of benzene sulfonamide with cis-1,2-cyclohexanecarboxylic anhydride, hexahydroisobenzofuran-1,3-dione, 3a,4,7,7a-tetrahydroisobenzofuran-1,3-dione, furan-2,5-dione, furan-2,5-dione, 1*H*-2-benzopyran-1,3(4*H*)-dione respectively. All these compounds showed moderate anticancer activities against breast (T47D), lung (NCI H-522), colon (HCT-522), ovary (PA-1) and liver (Hep G2) cell lines [49].



Among them 4-(1,3-dioxo-1,3,3a,4,7,7a–tetrahydro-2*H*-isoindol-2-yl)-*N*-(4-methylpyrimidin-2-yl)benzenesulfonamide exhibited better anticancer activity against liver cancer [49].

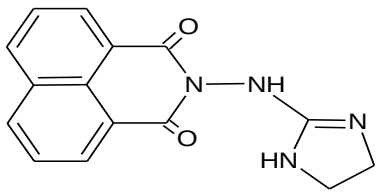


In 2017, bis-cyclic imide derivatives were prepared by two moles of corresponding anhydride and diamines under microwave irradiation condition. The below compounds were reported to be inhibitors against(breast T47D), (breast T47D, liver HepG2), (breast T47D, liver HepG2), (colon HCT-15) cell lines in good range [50].

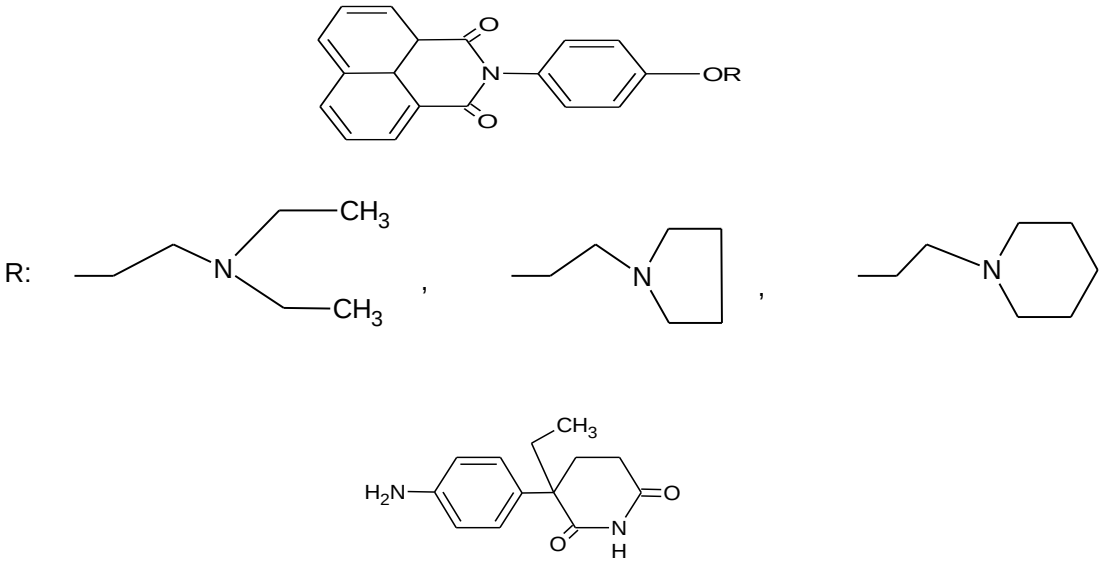


2.3.2. Naphthylimide Derivatives

Some naphthylimide derivatives were reported synthesized by the reaction of naphthalic anhydride, with 2-hydrazino-1-imidazoline hydrobromide, various para substituted aryl amines, aminoglutethimide and 2,4-dinitrophenyl hydrazine [17]. Among them, the following compound was found to be active on 3-cell lines as MCF-7, NCI-H460 and SF-268.

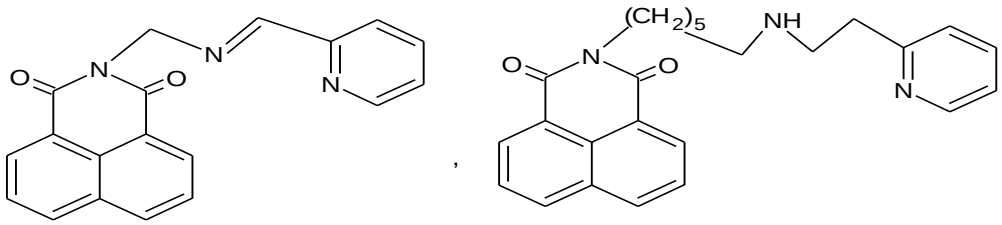


On the other hand, these compounds have exhibited weak inhibition of human placental aromatase activity when compared to aminoglutethimide [17].

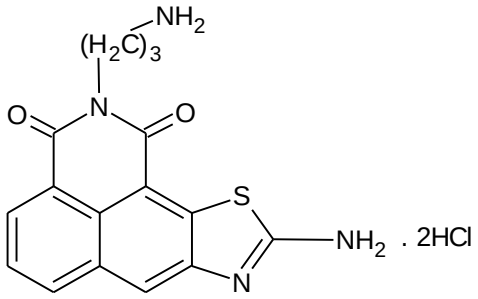


Aminoglutethimide

Naphthalimide polyamine conjugates were synthesized for screening *in vitro* antiproliferative activity against Jurkat, HeLa, MCF-7 and A549 cell lines in 2013. Among them conjugates 2-(1-{[1-pyridin-2-yl-meth-(E)-ylidene]-amino}-hexyl)-benzo[d,e]isoquinoline-1,3-dione and 2-{6-[(pyridin-2-yl-methyl)-amino]-hexyl}-benzo[d,e]isoquinoline-1,3-dione showed the highest antiproliferative activity with IC_{50} values of between 5-11 μmol/L on the cycle of Jurkat cells [51].

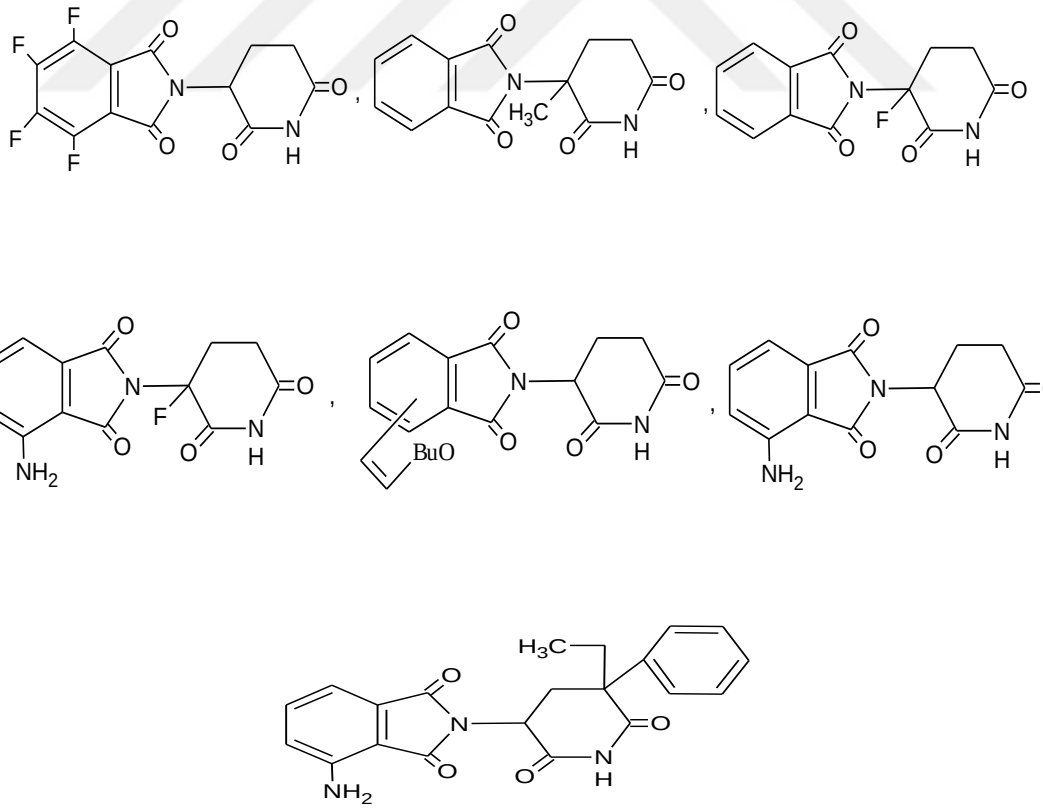


Several naphthalimide analogs were synthesized and evaluated for their *in vitro* their anti-hepatocellular carcinoma properties. Among them compounds 9-amino-2-(3-aminopropyl)-1H-benzo[de]thiazolo[4,5-h]isoquinoline-1,3(2H)-dione showed inhibition cell migration of SMMC-7721 and HepG2, partly inhibited primary H22 tumor growth and potently interrupted lung metastasis [52].

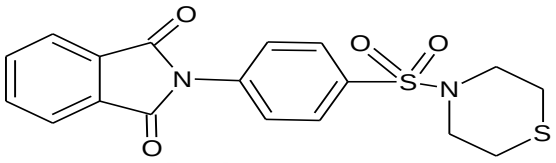


2.4. Anti-inflammatory Activity of Imide Derivatives

Cyclic imides, such as phthalimide and succinimide have structural properties that confer potential biological activity and pharmaceutical use. The several classes of cyclic imides have received perfect attention due to their anti-inflammatory, antitumor and antihyperlipidemic activities. Since thalidomide is a phthalimide derivative, has a selectivity of blocking TNF-α production as mentioned earlier, several thalidomide derivatives have been synthesized and screened for this activity. [16, 17, 53].

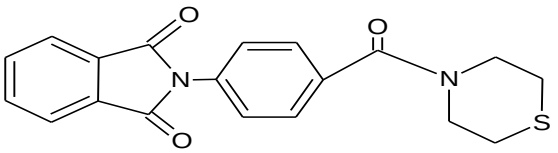


Several *N*-phenyl-phthalimide sulfonamides were synthesized for their anti-inflammatory activity. Compound LASSBio468 [4-(1,4-Thiazinan-4-ylsulfonyl)phenyl]-1,3-isoindoline-dione] having a sulfonyl-thiomorpholine moiety was found potent inhibitory activity on LPS-induced neutrophil recruitment with ED_{50}=2.5 mg/kg, which was correlated with its inhibitory effect on TNF-α level [27]. It was also found to inhibit the neutrophil infiltration induced by LPS with ED_{50} = 2.5 mg/kg [54].

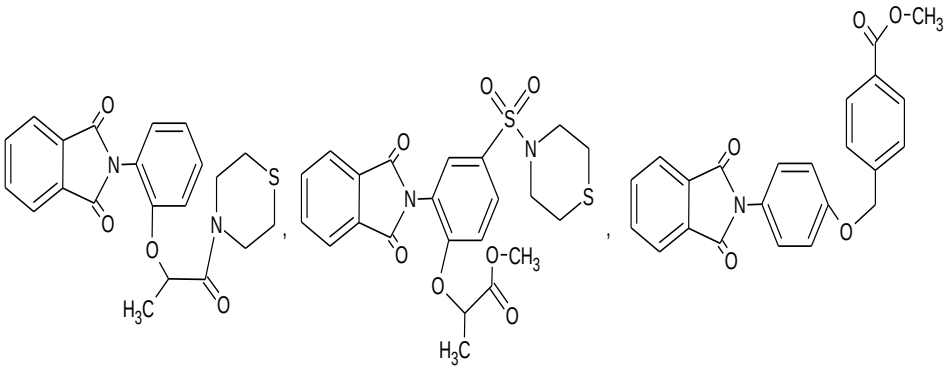


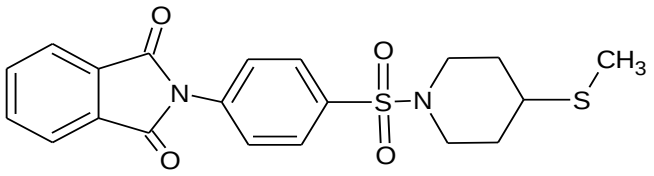
LASSBio 468

After the prototypes LASSBio 468, LASSBio 595, were designed as hybrid analogues of thalidomide and aryl-amide, presented anti-inflammatory properties acting on TNF-α production [54]. Anti-inflammatory activity of new *N*-phenyl-phthalimide sulfonamides and the isosters *N*-phenyl-phthalimide amides, designed as hybrids of thalidomide and aryl sulfonamide phosphodiesterase inhibitor [54].

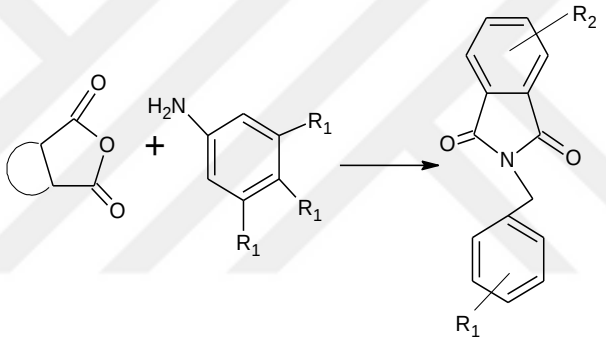


LASSBio-595



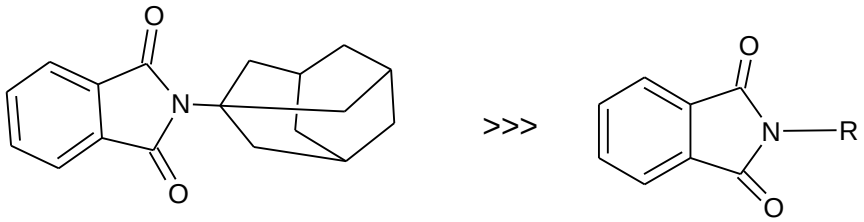


Abdel-Aziz et al. prepared a new group of imide derivatives to screen anti-inflammatory activity. The compounds 5-nitro-2-(3,4,5-trimethoxybenzyl)isoindoline-1,3-dione, 5-nitro-2-(4-methoxybenzyl)isoindoline-1,3-dione, 5,6-dichloro-2-(3,4,5-trimethoxybenzyl)isoindoline-1,3-dione, 5,6-dichloro-2-(4-methoxybenzyl)isoindoline-1,3-dione were proved to be potent COX-2 inhibitors with IC_{50} ranged from 0.1-1.0 μM. 5-nitro-2-(3,4,5-trimethoxybenzyl)isoindoline-1,3-dione is a highly potent (IC_{50} = 0,1 μM) and COX-2 inhibitor showed superior anti-inflammatory activity relative to diclofenac (ED_{50} = 114 mg/kg) [55].



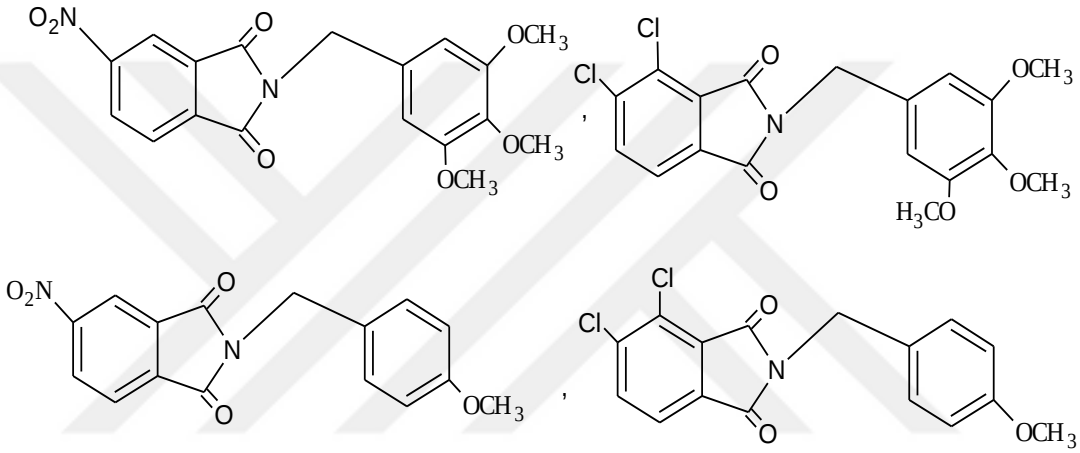
R_1 = MeO, Cl R $_2$ = NO_2, 5,6-dichloro

N-Alkylated phthalimide analogues revealed that phthalimides bearing a spherical alkyl group, such as an adamantyl and a carbonyl group, possessed very potent bi-directional TNF-α production-regulating activity. Among those series 4-pentylphenyl, 1-adamantyl and 2,4-dimethylphenyl substituted compounds were the most active compounds [19].

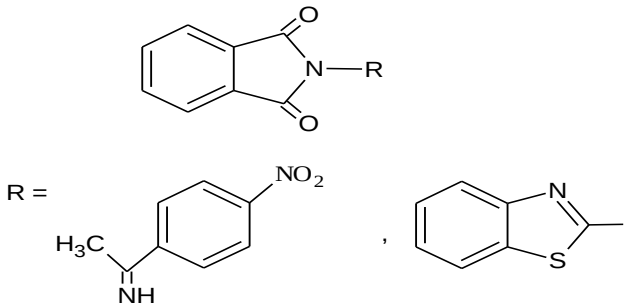


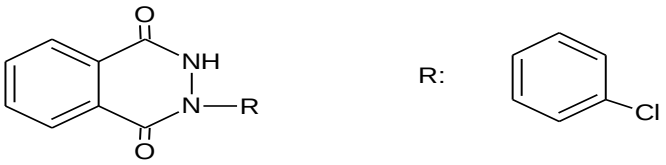
R = 3-penthyl > R = cyclohexyl > R = t-butyl > R = n-butyl

A group of cyclic imides were synthesized by reacting phthalic anhydride with several amines by Alanazi et al. and were screened for COX-1/COX-2 inhibition, analgesic and anti-inflammatory activities. The compounds exhibit optimal COX-2 inhibitory potencies (IC_{50} = 0.18, 0.24, 0.28 and 0.36 μM; respectively) and selectivity (SI) 363-668) comparable with celecoxib and better ED_{50} than diclofenac. Compound 5-nitro-2-(3,4,5-trimethoxybenzyl)isoindole-1,3-dione having NO_2 group on the phythalimide ring possessed highly potent in *in vitro* COX-1/COX-2 inhibition when search structure-activity studies [56].

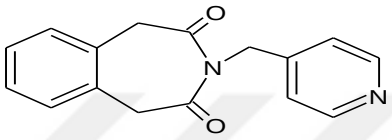


N-substituted (aryl, allyl and heteroaryl) phthalimides derivatives were prepared. Compounds 2-((4-nitrobenzylidene)amino)isoindoline-1,3-dione, 2-(benzo(d)tiazol-2-yl)isoindolin-1,3-dione and 2-(4-chlorophenyl)2,3-dihydrophthalazin-1,4-dione showed inhibition of TNF-α production. Among them, 2-(4-chlorophenyl)2,3-dihydrophthalazin-1,4-dione showed the highest *in vivo* anti-inflammatory activity [57].





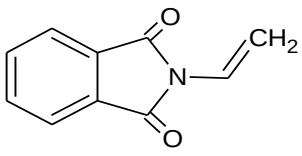
N-Substitued benzo-3-azepan-2,4-dione type cyclic imides were synthesized, by condensation of various diacids with different amines under microwave irradiation. The compound 3-(*N*-piperidin-4-yl)benzo-3-azepan-2,4- dione exhibited anti-inflammatory activity [49].



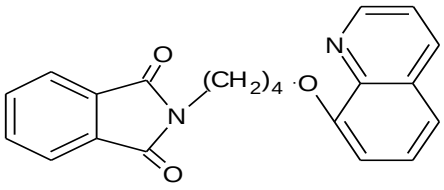
2.5. Other Activities

2.5.1.Antifungal Activity

A series of *N*-substituted phthalimides were designed by Pan et al. in 2016. 8-[4-(phthalimide-2-yl)butyloxyl]quinoline (IC_{50} = 10.85 μg/mL) and *N*-vinylphthalimide (IC_{50} = 7.92 μg/mL) were determined as the most promising candidates against *B. cinera and A.solani*. The structure-activity relationships have clarified that quinolyl, bromide alkyl, vinyl and benzyl substitutions were proper substituents [58].



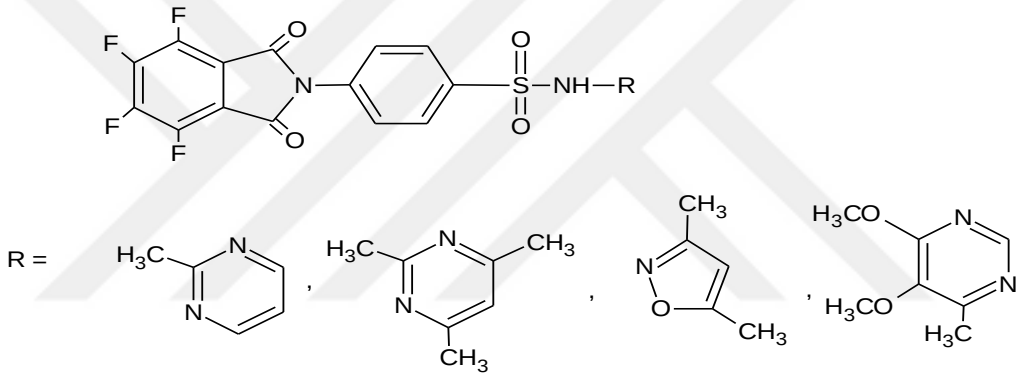
N-vinylphthalimide



8-[4-(phthalimide-2-yl)butyloxyl]quinoline

2.5.2.Antimycobacterial Activity

Akgün *et al.* synthesized phthalimide derivatives via condensation of phthalic and tetrafluorophthalic anhydride with selected sulfonamides with variable yields. 4-(4,5,6,7-tetrafluoro-1,3-dioxo-isoindolin-2-yl)-*N*-pyrimidin-2-yl-benzenesulfonamide, *N*-(4,6-dimethylpyrimidin-2-yl)-4-(4,5,6,7-tetrafluoro-1,3-dioxo-isoindolin-2-yl)benzenesulfonamide, *N*-(4-methylisoxazol-3-yl)-4-(4,5,6,7-tetrafluoro-1,3-dioxo-isoindolin-2-yl)benzenesulfonamide and *N*-(5,6-dimethoxypyrimidin-4-yl)-4-(4,5,6,7-tetrafluoro-1,3-dioxo-isoindolin-2-yl)benzenesulfonamide possessed good minimum inhibitory concentration (MIC) over *Mycobacterium* species compared to isoniazid (MIC<0.02 μg/mL) and pyrazinamide (MIC 50-100 μg/mL) [59].



3. MATERIALS AND METHODS

3.1. Chemistry

3.1.1. Materials

All materials were commercially available and used without further purification. Sulfacetamide and sulfamethoxazole were purchased from Fluka. Sulfathiazole and sulfamethazine were purchased from Alfa Aesar GmbH & Co. *Cis*-1,2-cyclohexane carboxylic anhydride, acetic acid, dimethylformamide, sulfadoxine, sulfamethoxypyridazine, sulfamerazine and sulfadiazine were purchased from Sigma-Aldrich. Sulfabenzamide was purchased from Acros Organics. Sulfanilamide was purchased from Merck.

3.1.2. Methods of Synthesis

3.1.2.1. General Procedure A: Conventional synthesis of Compounds 1-10

0,0013 mol (0.20g) of *cis* -1,2-cyclohexane carboxylic anhydride and 0,0013 mol of corresponding sulfa derivatives were stirred in 10 ml of acetic acid under reflux for four hours. After that 20 ml of distillated water was added to the solution at room temperature and filtered. The precipitate was crystallized from ethanol.

3.1.2.2. General Procedure B: Microwave-assisted synthesis of Compounds 1-10

0,0013 mol (0.20g) of c*is*- 1,2-cyclohexane carboxylic anhydride and 0.0013 mol of sulfa derivatives were stirred in 0,4 ml of dimethylformamide until dissolve at room temperature. Then the mixture was subjected to at a power of 200-250 Watt in a Microwave for 4-5 minutes at 90 ^{0}C. After, the mixture was cooled, 20 ml of distillated water was added on to mixture. The precipitates were filtered and crystallized from ethanol.

3.1.3. Analytical Methods

3.1.3.1. Melting Point Determination

Melting Points of the compounds were determined in Celcius (°C) by using a Mettler Toledo FP81HT MBC Cell.

Microwave-assisted synthesis

Microsynth Microwave Labstation was used for the synthesis of the compounds.

3.1.3.2. Controls by Thin Layer Chromatography Material:

Plates: TLC aluminum sheets 20×20 cm Silica gel 60 F254 (Merck).

Solvent systems: Two different solvent systems were prepared to be used in chromatographic controls of compounds.

S.1: Chloroform:Methanol (95:5)

S.2:Dichloromethane:Methanol (80:20)

Method:

Dragging conditions: Solvent systems were poured to chambers and waited for 24 hours for saturation.

Synthesized compounds and their starting materials dissolved in suitable solvents were applied to thin layer chromatography (TLC) plates and waited to drag 10 cm at room temperature. Retention factor (Rf) values of compounds were determined.

Stain determination: Stains of the synthesized compounds and their starting materials were determined by UV light (254/365 nm).

3.1.3.3. Spectrometric Analysis

3.1.3.3.3.1. 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.

3.1.3.3.2. Infrared Spectra

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}.

3.1.3.3.3. ^{1}H-NMR Spectra

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

3.1.3.3.4. ^{13}C-NMR Spectra

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

3.1.3.3.5. Mass Spectra

M+1 peaks were determined by Shimadzu LC/MS ITTOF system (Shimadzu, Tokyo, Japan).

3.2. Biologic Activity Studies

3.2.1. Cytotoxicity Analysis of the Compounds

Cytotoxic activities of the synthesized compounds were investigated on breast (MCF-7) cancer cell lines by MTT assays in triplicate [61]. Serial dilutions from 100 µM to 2.5 µM were used, 5-fluorouracil (5-FU) was the reference compound for the cytotoxic effect.

Cell Culture

Human cancer cell lines were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin. Each cell line was maintained in an incubator at 37 °C supplied with 5% CO_2 and 95% air. All cell culture reagents were from Gibco in UK. Penicillin, streptomycin, MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazoliumbromide) [Roche, Cell Proliferation KIT I.], cell culture grade DMSO, 5- fluorouracil (5-FU) were from Roche in Germany.

3.2.2. Anticancer Activity Test Procedure:

Cancer cells were seeded into 96-well plates and allowed to adhere for 24 h before drugs were introduced. Following a 48-h incubation, drugs and media were removed, and each well was treated with 100 µL of 500 µg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in culture medium. Following a 4h incubation period to allow the metabolism of MTT by mitochondrial dehydrogenases of viable cells to form an insoluble formazan product, the plates were centrifuged at 450 x g for 10 min, and supernatants were removed and replaced with 100 µL DMSO. The plates were shaken to maximize solubility of the formazan crystals. The absorbance, as a measure of viable cell numbers, was read the following day at a wavelength of 550 nm. It was previously shown that viable cell numbers are correlated with the optical density as determined in the MTT assay. IC_{50} values were obtained by a linear regression analysis of the percent absorbance vs. the log of the drug concentration.

Statistics

Complete solubilisation of the purple formazan crystals were checked and absorbance of the samples were measured using a microplate (ELISA) reader between 550 and 600 nm. The data were considered statistically significant of the reference wavelength should be more than 650 nm according to MTT assay.

3.2.3. Anti-inflammatory Activity Test Procedure:

In vitro Anti-inflammatory Activity Assay

Cell culture

RAW 264.7 macrophages were kindly provided by Yeditepe University, Faculty of Engineering, Department of Genetics and Bioengineering (İstanbul, Turkey). The cells were cultured in DMEM (Gibco, UK), supplemented with 10% FBS (Gibco, USA) and 1% streptomycin and penicillin (Gibco, USA) at 37°C in 5% CO_2.

Cell cytotoxicity

RAW 264.7 cells at the density of 1 x 10^5 cells per well and incubated for 24 h. The cells were treated with compounds for 24 hours in the presence of LPS (1µg/ml) and the medium was removed and 100µl of 0.5 mg/ml MTT (AppliChem, Germany) was added and incubated for 2 h. The MTT solution was removed and 100 µL of isopropanol (Sigma-Aldrich, Germany) were added in each well and optical absorbance was measured at 570 nm.

Nitrite Assay

The nitrite inhibition activity of the tested compounds were evaluated by measuring nitrite concentrations by using a colorimetric method based on the Griess reaction. RAW 264.7 cells were seeded into a 48-well culture plate at the density of 1 x 10^5 cells per well and incubated for 24 h. The cells were then pretreated with compounds and the reference molecule, acetylsalicylic acid (500 µM). 2 hours later, cells were stimulated with LPS (1µg/ml) and 22 h later the nitrite concentration in the medium was measured by adding 50 µl Griess reagent [1% sulfanilamide (Sigma-Aldrich, USA) and 0.1% N-(1-naphthyl)ethylendiamine dihydrochloride (Sigma-Aldrich, USA) in 5 % phosphoric acid (Mettler, Switzerland)] to 50 µl of medium for 10 min. The absorbance was measured at 570 nm, using a microplate reader (Microplate photometer, Multiskan Ascent, Finland). A sodium nitrite (Fluka Chemika, Germany) standard curve was used to calculate the amount of nitrite in the test samples.

Statistics

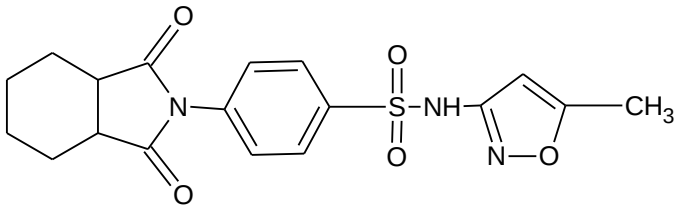
All results were expressed as the mean ± SD of experiments. Statistical significance was determined by one-way ANOVA followed by Turkey's test using a computerized statistical program. The data were considered statistically significant if $p<0.05$.

4. EXPERIMENTAL SECTION

4.1. Chemical Data

4-(1,3-dioxohexahydro-2*H*-isoindol-2-yl)-*N*-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide

(Compound 1)



0.0013 mol of *cis*-1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfamethoxazole (0.33 gr) and 10 ml of acetic acid were reacted as described in the general procedure A. The compound was crystallized from ethanol. The compound is soluble in acetone, hot ethanol, methanol and DMSO, it is insoluble in water. The yield is 31% and the form of compound is white crystals.

0,0013 mol of c*is*- 1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfamethoxazole (0.33 gr), in 0,4 ml of dimethylformamide Radiated as described in the general procedure B. The compound was crystallized from ethanol. The yield is 97% and the form of compound is white crystals. The compound has a melting point of 213 °C.

Rf values: 0.6 (S.1), 0.9(S.2).

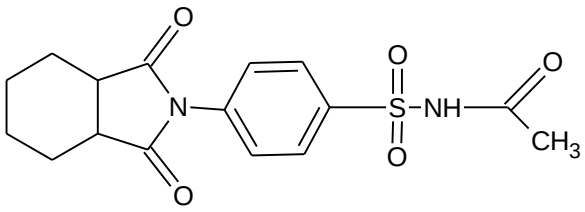
UV (MeOH, λ_{max}, nm): 240 (log ε : 6,08), 395 (log ε : 8,30).

FT-IR (KBr, ν_{max}, cm^{-1}): 3475 (N-H), 3075 (C-H, aromatic), 2934 (aliphatic C-H), 1702 (O=C-N-C=O), 1384 and 1170 (SO_2-NH).

^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 11.65 (s, 1H, SO_2-N**H**), 7.60-8.00 (m, 4H, Ar), 6.20 (s, 1H, C**H**, oxazol), 3.10 (m, 2H, C**H**) 2.30 (s, 3H, **CH_3**) 1.80 (m, 4H, C**H_2**) 1,33 (m, 4H, C**H_2**).

LC-MS (m/z): 390.43(M^+) ($C_{18}H_{19}N_3O_5S$), 254 $(C_{10}H_{12}N_3O_3S)^+$.

***N*-[4-(1,3-diioxohexahydro-2*H*-isoindol-2-yl)benzene-1-sulfonyl]acetamide (Compound 2)**



0.0013 mol of *cis*-1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfacetamide (0.28 gr), 10 mL of acetic acid were reacted as described in the general procedure A. The compound was crystallized from ethanol. The compound is soluble in acetone, hot ethanol, methanol and DMSO, it is insoluble in water. The yield is 98% and the form of compound is white crystals.

0.0013 mol of sulfacetamide (0.28 gr) and 0,0013 mol of c*is*- 1,2-cyclohexane carboxylic anhydride (0.20 gr) in 0,4 ml of dimethylformamide irradiated as described in the general procedure B. The compound was crystallized from ethanol. The yield is 98% and the form of compound is white crystals. The compound has a melting point of 178 °C.

Rf values: 0.22 (S.1), 0.08 (S.2).

UV (MeOH, λ_{max}, nm): 207 (log ε : 8,01), 239 (log ε : 8,08), 398(log ε : 8,30).

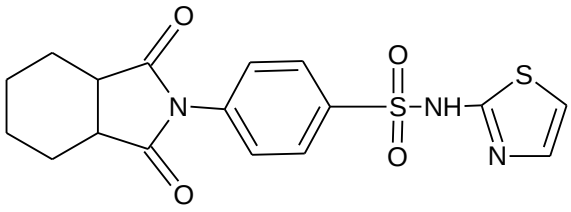
FT-IR (KBr, ν_{max}, cm^{-1}): 3273 (N-H), 3000 (C-H, aromatic), 2942 (C-H, aliphatic), 1703 (O=C-N-C=O), 1597 (HN-C=O), 1336 and 1167 (SO_2NH).

^{1}H-NMR (400 MHz) (DMSO-*d*6/TMS, δ, ppm): 12.76 (s, 1H, SO_2-N**H**), 7.40-8.00 (m, 4H, Ar), 3.05 (m, 2H, **CH**), 2.00 (s, 3H, -COC**H$_3$**), 1.80 (m, 4H, C**H$_2$**) , 1.40 (m, 4H, C**H$_2$**).

^{13}C-NMR (400 MHz) (DMSO-*d*6/TMS, δ, ppm): 178.13, 168.91, 138.51, 136.82, 128.30, 127.31, 39.42, 23.28, 23.23, 21.45.

LC-MS (m/z): 351.39 (M^+) ($C_{16}H_{18}N_2O_5S$).

4-(1,3-dioxohexahydro-2*H*-isoindol-2-yl)-*N*-(1,3-thiazol-2-yl)benzenesulfonamide (Compound 3) (CAS Registry Number: 1802658-09-8) [49]



0.0013 mol of *cis*-1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfathiazole (0.33 gr),10 ml of acetic acid were reacted as described in the general procedure A. The compound was crystallized from ethanol. The compound is soluble in acetone, hot ethanol, methanol and DMSO, it is insoluble in water. The yield is 100% and the form of compound is cream crystals.

0.0013 mol of c*is*- 1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfathiazole (0.33 gr) in 0,4 ml of dimethylformamide irradiated as described in the general procedure B. The compound was crystallized from ethanol. The yield is 98% and the form of compound is cream crystals. The compound has a melting point of 230 °C.

Rf values: 0.3 (S.1), 0.3 (S.2).

UV (MeOH, λ_{max}, nm): 284 (log ε : 8,15), 231 (log ε : 8,00).

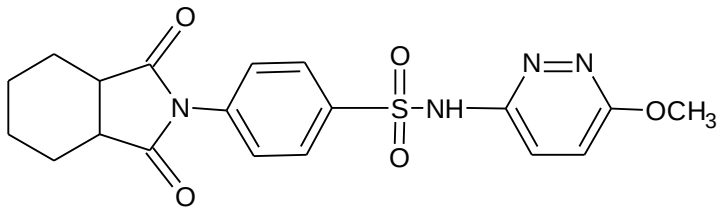
FT-IR (KBr, ν_{max}, cm^{-1}): 3321 (N-H), 3095-3142 (C-H, aromatic), 2856 (C-H, aliphatic), 1703 (O=C-N-C=O), 1313 and 1160 (SO_2NH).

^{1}H-NMR (400 MHz) (DMSO-*d*6/TMS, δ, ppm): 10.10 (s, 1H, SO_2-N**H),** 7.92-7.50 (m, 4H, Ar), 7.4 (s, 2H, 4, 5- thia.), 3.10 (m, 2H, C**H**), 1.80 (m, 4H, C$\mathbf{H_2}$), 1.40 (m, 4H, C$\mathbf{H_2}$).

^{13}C-NMR (400 MHz) (DMSO-d6/TMS, δ, ppm): [49].

LC-MS (m/z): 391.46 (M^+) (C_{17} H_{17} N_3 O_4 S_2), 256.32 $(C_9H_{10}N_3O_2S_2)^+$.

4-(1,3-dioxohexahydro-2*H*-isoindol-2-yl)-*N*-(6-methoxypyridazin-3-yl)benzenesulfonamide (Compound 4)



0.0013 mol of *cis*-1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfamethoxypyridazine (0.36 gr), 10 ml of acetic acid were reacted as described in the general procedure A. The compound was crystallized from ethanol. The compound is soluble in acetone, hot ethanol, methanol and DMSO, it is insoluble in water. The yield is 60% and the form of compound is white crystals.

0.0013 mol of c*is*- 1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfamethoxypyridazine (0.36 gr) in 0,4 ml of dimethylformamide irradiated as described in the general procedure B. The compound was crystallized from ethanol. The yield is 100% and the form of compound is white crystals. The compound has a melting point of 240 °C.

Rf values: 0.9 (S.1), 0.9(S.2).

UV (MeOH, λ_{max}, nm): 204 (log ε : 8,01), 222 (log ε : 8,05), 325 (log ε : 8,21).

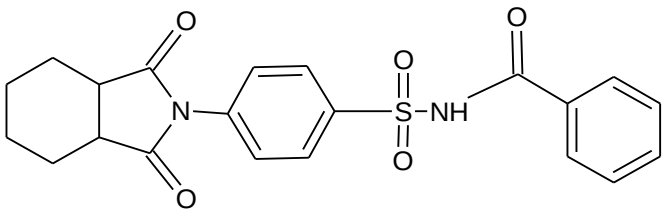
FT-IR (KBr, ν_{max}, cm^{-1}): 3466 (N-H), 3080 (C-H, aromatic), 2942 (C-H), 1702 (O=C-N-C=O), 1384 and 1168 (SO_2NH), 2855 (OCH_3).

^{1}H-NMR (400 MHz) (DMSO-*d*6/TMS, δ, ppm): 7.90 (m, 4H, Ar) 7.40 (m, 2H, Ar), 3.8 (s, 3H, -O-C**H$_3$**), 3.10 (m, 2H, C**H**), 1.77 (m, 4H, C**H$_2$**) 1.33 (m, 4H, C**H$_2$**).

^{13}C-NMR (400 MHz) (DMSO-d6/TMS, δ, ppm): 178.25, 135.31, 127.33, 126.72, 54.55, 39.50, 23.28, 21.43.

LC-MS (m/z): 417.45 (M^+) (C_{19} H_{20} N_4O_5S), 267.30 ($C_{11}H_{13}N_3O_3S$).

***N*-[4-(1,3-dioxohexahydro-2*H*-isoindol-2-yl)benzene-1-sulfonyl]benzamide (Compound 5)**



0.0013 mol of *cis*-1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfobenzamide (0.36 gr), 10 ml of acetic acid were reacted as described in the general procedure A. The compound was crystallized from ethanol. The compound is soluble in acetone, hot ethanol, methanol and DMSO, it is insoluble in water. The yield is 99 % and the form of compound is white crystals.

0,0013 mol of c*is*- 1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfobenzamide (0.36 gr) in 0,4 ml of dimethylformamide irradiated as described in the general procedure B. The compound was crystallized from ethanol. The compound was crystallized from ethanol. The yield is 97% and the form of compound is white crystals. The compound has a melting point of 245 °C.

Rf values: 0.18 (S.1), 0.8 (S.2).

UV (MeOH, λ_{max}, nm): 206 (log ε : 8,01), 238 (log ε : 8,07), 393 (log ε : 8,29).

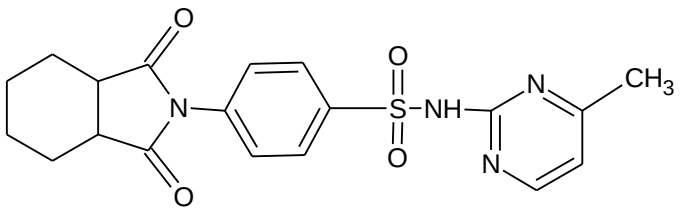
FT-IR (KBr, ν_{max}, cm^{-1}): 3296 (C-H, aromatic), 3218 (N-H), 3064 (C-H, aromatic), 2954 (aliphatic, C-H), 1781 (O=C-N-C=O), 1694 (C=O, amide), 1245 and 1166 (SO_2NH).

^{1}H-NMR (400 MHz) (DMSO-*d*6/TMS, δ, ppm): 12.60 (s, 1H, SO_2-N**H**), 8.10-7.40 (m, 9H, Ar), 3.10 (m, 2H, C**H**), 1.90 (m, 4H, C$\mathbf{H_2}$), 1.40 (m, 4H, C$\mathbf{H_2}$).

^{13}C-NMR (400 MHz) (DMSO-d6/TMS, δ, ppm): 178.12, 165.58, 138.62, 136.83, 133.36, 131.34, 128.61, 128.47, 128.45, 127.29, 39.50, 23.27, 21.44.

LC-MS (m/z): 413.45 (M^+) ($C_{21}H_{20}N_2O_5S$), 183.18 ($C_7H_5NO_3S$), 267.34 ($C_{13}H_{17}NO_3S$).

4-(1,3-dioxohexahydro-2*H*-isoindol-2-yl)-*N*-(4-methylpyrimidin-2-yl)benzenesulfonamide (Compound 6) (CAS Registry Number: 309267-54-7) [49]



0.0013 mol of *cis*-1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfomerazine (0.34 gr), 10 ml of acetic acid were reacted as described in the general procedure A. The compound was crystallized from ethanol. The compound is soluble in acetone, hot ethanol, methanol and DMSO, it is insoluble in water. The yield is 100% and the form of compound is cream crystals.

0.0013 mol of c*is*- 1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfomerazine (0.34 gr) in 0,4 ml of dimethylformamide irradiated as described in the general procedure B. The compound was crystallized from ethanol. The yield is 100% and the form of compound is cream crystals. The compound has a melting point of 318 °C.

Rf values: 0.52 (S.1), 0.6 (S.2).

UV (MeOH, λ_{max}, nm): 205 log ε : 8,01), 262 (log ε : 8,11), 246 (log ε : 8,09).

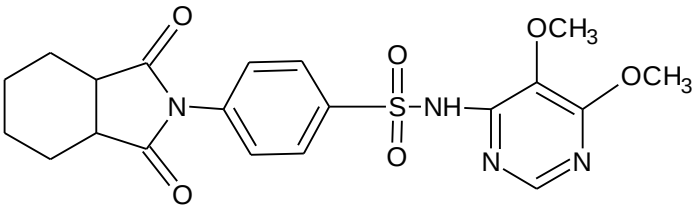
FT-IR (KBr, ν_{max}, cm^{-1}): 3300 (C-H, aromatic), 3220 (N-H), 3070 (C-H, aromatic), 2856 (C-H, aliphatic), 1781 (O=C-N-C=O), 1330 and 1250 (SO_2NH).

^{1}H-NMR (400 MHz) (DMSO-*d*6/TMS, δ, ppm): 11.90 (s, 1H, SO_2-N**H**), 8.20-6.90 (m, 6H, Ar), 3.10 (m, 2H, C**H**), 2.30 (s, 3H, C$\mathbf{H_3}$), 1.90 (m, 4H, C$\mathbf{H_2}$), 1.40 (m, 4H, C$\mathbf{H_2}$).

^{13}C-NMR (400 MHz) (DMSO-d6/TMS, δ, ppm): [49].

LC-MS (m/z): 401.45 (M^+) ($C_{19}H_{20}N_4O_4S$), 307 $(C_{14}H_{15}N_2O_4S)^-$, 265 $(C_{11}H_{13}N_4O_2S)^+$.

4-(1,3-dioxohexahydro-2*H*-isoindol-2-yl)-N-(5,6-dimethoxypyrimidin-4-yl)benzenesulfonamide (Compound 7)



0.0013 mol of *cis*-1,2-cyclohexane carboxylic anhydride (0.2gr) and 0.0013 mol of sulfadoxine (0.40 gr), 10 ml of acetic acid were reacted as described in the general procedure A. The compound was crystallized from ethanol. The compound is soluble in acetone, hot ethanol, methanol and DMSO, it is insoluble in water. The yield is 100 % and the form of compound is white crystals.

0.0013 mol of c*is*- 1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfadoxine (0.40 gr) in 0,4 ml of dimethylformamide irradiated as described in the general procedure B. The compound was crystallized from ethanol. The yield is 99% and the form of compound is white crystals. The compound has a melting point of 190 °C.

Rf values: 0.9 (S.1), 0.9 (S.2).

UV (MeOH, λ_{max}, nm): 240 (log ε : 8,08), 261 (log ε : 8,12).

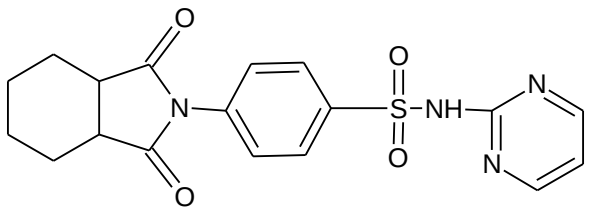
FT-IR (KBr, ν_{max}, cm^{-1}): 3260 (N-H), 3078 (C-H, aromatic), 2936 (C-H, aliphatic), 1783 (O=C-N-C=O), 1373 (OCH_3), 1305(OCH_3), 1346 and 1258 (SO_2NH).

^{1}H-NMR (400 MHz) (DMSO-*d*6/TMS, δ, ppm): 11.27 (s, 1H, SO_2-N**H**), 8.10 (m, 1H, Ar), 7.50 (m, 4H, Ar), 3.90 (s, 3H, -O-C**H$_3$**), 3.70 (s, 3H, -O-C**H$_3$**), 3.31 (m, 2H, C**H**), 1.80 (m, 4H, C**H$_2$**), 1.40 (s, 2H, C**H$_2$**).

^{13}C-NMR (400 MHz) (DMSO-d6/TMS, δ, ppm): 178.64, 162.18, 156.38, 151.98, 150.71, 149.34, 140.64, 136.59, 128.54, 127.97, 127.60, 125.76, 60.68, , 54.56, 39.55, 23.73, 21.90.

LC-MS (m/z): 447.48 (M^+) ($C_{20}H_{22}N_4O_6S$), 311.08 $(C_{12}H_{15}N_4O_4S)^+$.

4-(1,3-Dioxohexahydro-2*H*-2-yl)-*N*-(pyrimidin-2-yl)benzenesulfonamide (Compound 8) (CAS Registry Number: 431918-18-2) [49].



0.0013 mol of *cis*-1,2-cyclohexane carboxylic anhydride (0.2gr) and 0.0013 mol of sulfadiazine (0.33 gr), 10 ml of acetic acid were reacted as described in the general procedure A. The compound was crystallized from ethanol. The compound is soluble in acetone, hot ethanol, methanol and DMSO, it is insoluble in water. The yield is 90% and the form of compound is yellow crystals.

0.0013 mol of c*is*- 1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfadiazine (0.33 gr) in 0,4 ml of dimethylformamide irradiated as described in the general procedure B. The compound was crystallized from ethanol. The yield is 100% and the form of compound is yellow crystals. The compound has a melting point of 254 °C.

Rf values: 0 (S.1), 0.9 (S.2).

UV (MeOH, λ_{max}, nm): 203 (log ε : 8,01), 255 (log ε : 8,11).

FT-IR (KBr, ν_{max}, cm^{-1}): 3467 (N-H.), 3040 (C-H, aromatic), 2940 (C-H, aliphatic), 1783 (O=C-N-C=O), 1385 and 1261 (SO_2NH).

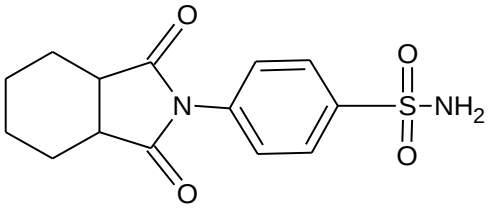
^{1}H-NMR (400 MHz) (DMSO-*d*6/TMS, δ, ppm): 11.90 (s, 1H, SO_2-N**H**), 8.10-7.50 (m, 6H, Ar), 3.10 (m, 2H, C**H**), 2.10 (s, H, C**H**), 1.90 (m, 4H, C$\mathbf{H_2}$), 1.40 (m, 4H, C$\mathbf{H_2}$).

^{13}C-NMR (400 MHz) (DMSO-d6/TMS, δ, ppm): [49].

LC-MS (m/z): 387.42 (M^+) ($C_{18}H_{18}N_4O_4S$).

4-(1,3-Dioxohexahydro-2*H*-isoindol-2-yl)benzenesulfonamide (Compound 9)

(CAS Registry Number: 301173-22-8)



0.0013 mol of *cis*-1,2-cyclohexane carboxylic anhydride (0.2gr) and 0.0013 mol of sulfanilamide (0.22 gr), 10 ml of acetic acid were reacted as described in the general procedure A. The compound was crystallized from ethanol. The compound is soluble in acetone, hot ethanol, methanol and DMSO, it is insoluble in water. The yield is 40% and the form of compound is white crystals.

0.0013 mol of c*is*- 1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfanilamide (0.22 gr) in 0,4 ml of dimethylformamide irradiated as described in the general procedure B. The compound was crystallized from ethanol. The yield is 95% and the form of compound is bright white crystals. The compound has a melting point of 256 °C.

Rf values: 0.44 (S.1), 0.44 (S.2).

UV (MeOH, λ_{max}, nm): 205 (log ε : 8,01), 234 (log ε : 7,77).

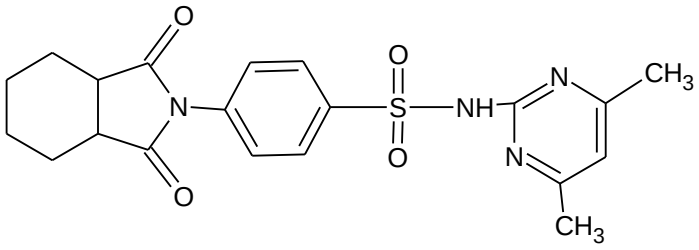
FT-IR (KBr, ν_{max}, cm^{-1}): 3258 (N-H), 3096 (C-H, aromatic), 2928 (C-H, aliphatic), 1783 (O=C-N-C=O), 1347 and 1167 (SO_2NH).

^{1}H-NMR (400 MHz) (DMSO-*d*6/TMS, δ, ppm): 7.90-7.50 (m, 4H, Ar), 3.50 (s, 2H, N**H$_2$**), 3.30 (m, 2H, C**H**), 1.90 (m, 4H, C**H$_2$**), 1.40 (m, 4H, C**H$_2$**).

^{13}C-NMR (400 MHz) (DMSO-d6/TMS, δ, ppm): 178.26, 143.52, 135.28, 127.40, 126.35, 39.50, 23.28, 21.40.

LC-MS (m/z): 309.35 (M^+) ($C_{14}H_{16}N_2O_4S$).

4-(1,3-Dioxohexahydro-2*H*-2-yl)-*N*-(4,6-dimethylpyrimidin-2-yl)benzene1sulfonamide (Compound 10) (CAS Registry Number: 850782-33-1)



0.0013 mol of *cis*-1,2-cyclohexane carboxylic anhydride (0.2gr) and 0.0013 mol of sulfamethazine (0.22 gr), 10 ml of acetic acid were reacted as described in the general procedure A. The compound was crystallized from ethanol. The compound is soluble in acetone, hot ethanol, methanol and DMSO, it is insoluble in water. The yield is 90% and the form of compound is gray crystals.

0.0013 mol of c*is*- 1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfamethazine (0.22 gr) in 0,4 ml of dimethylformamide irradiated as described in the general procedure B. The compound was crystallized from ethanol. The yield is 90% and the form of compound is gray crystals. The compound has a melting point of 213 °C.

Rf values: 0 (S.1), 0.9 (S.2).

UV (MeOH, λ_{max}, nm): 311 (log ε : 8,19).

FT-IR (KBr, ν_{max}, cm^{-1}): 3464 (N-H), 3056 (C-H, aromatic), 2941 (C-H, aliphatic), 1781 (O=C-N-C=O),1386 and 1163 (SO_2NH).

^{1}H-NMR (400 MHz) (DMSO-*d*6/TMS, δ, ppm): 12.0 (s, 1H, SO_2-N**H**), 7.70-8.20 (m, 4H, Ar), 6.80 (d, 1H, pyr.), 3.05 (m, 2H, C**H**), 2.20 (s, 6H, C$\mathbf{H_3}$), 1.90 (m, 4H, C$\mathbf{H_2}$), 1.60 (m, 4H, C$\mathbf{H_2}$).

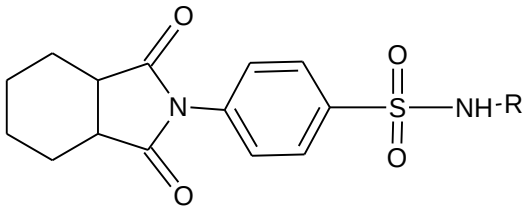
^{13}C-NMR (400 MHz) (DMSO-d6/TMS, δ, ppm): 178.22, 155.94, 135.65, 128.57, 126.66, 39.50, 23.27, 22.64, 21.43.

LC-MS (m/z): 415.49 (M^+) ($C_{18}H_{24}N_4O_4S$), 267.34 ($C_{12}H_{17}N_3O_2S$).

4.2. Biological Data

4.2.1. Anticancer Activity Data

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



Compounds **1-10**

Table 4.2. IC_{50} values of synthesized compounds 1-10 against human breast cancer cell line (MCF7) by MTT assay.

		Cancer Cell Lines (IC_{50}, µM)
Compound	**R**	**MCF7**
1	5-methyl-1,2-oxazol-3-yl	>100
2	acetyl	>100
3	1,3-thiazol-2-yl	87.9 ± 2.34
4	1,2-diazine-6-methoxy-3-yl	>100
5	phenylcarbonyl	>100
6	1,3-diazine-6-methyl-2-yl	>100
7	1,3-diazine-5,6-dimethoxy-4yl	71.5 ± 3.01
8	1,3-diazine-2-yl	>100
9	H	>100
10	1,3-diazine-4,6-dimethyl-2-yl	89.3 ± 2.05
	5-fluorouracil	3.51

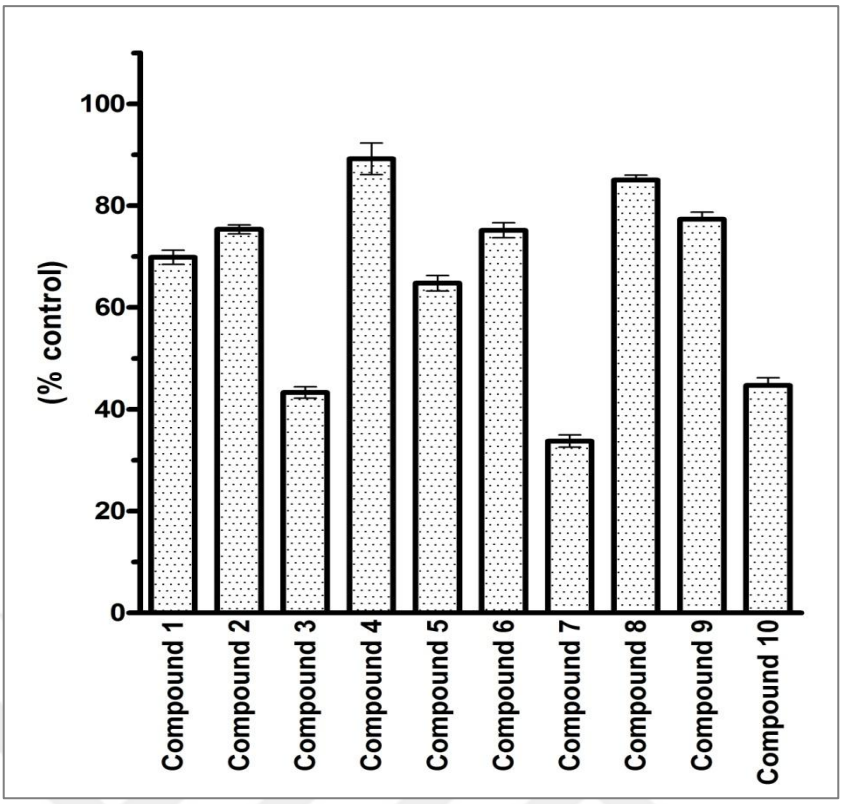


Figure 4.2.1. % viability of compounds applied to MCF 7 cell line

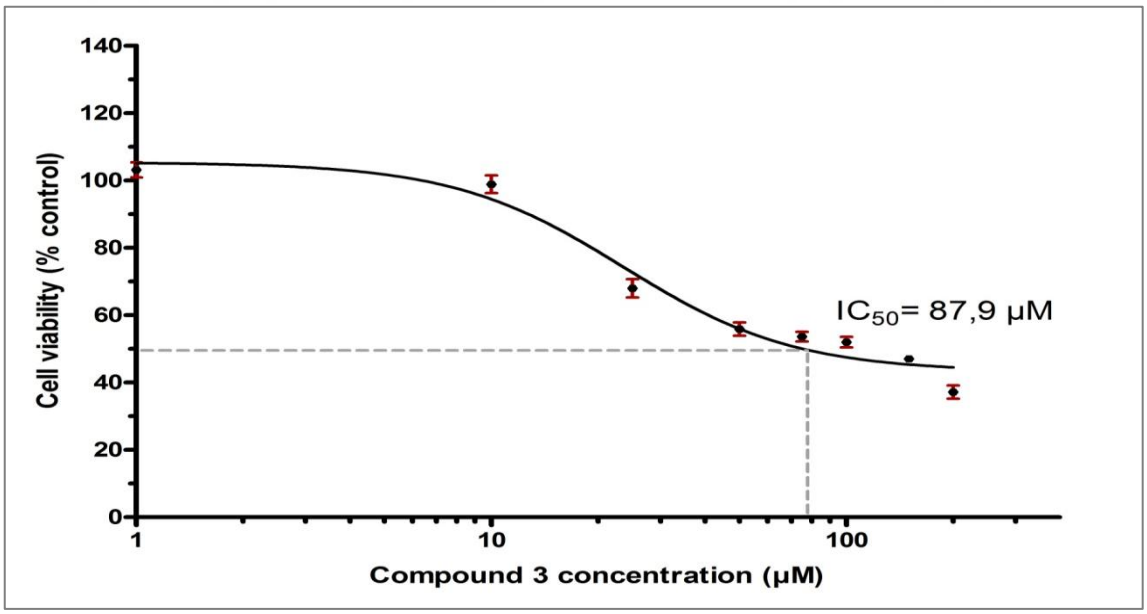


Figure 4.2.2. Sigmoidal graph of decreasing concentrations and IC50 value belong to Compound 3

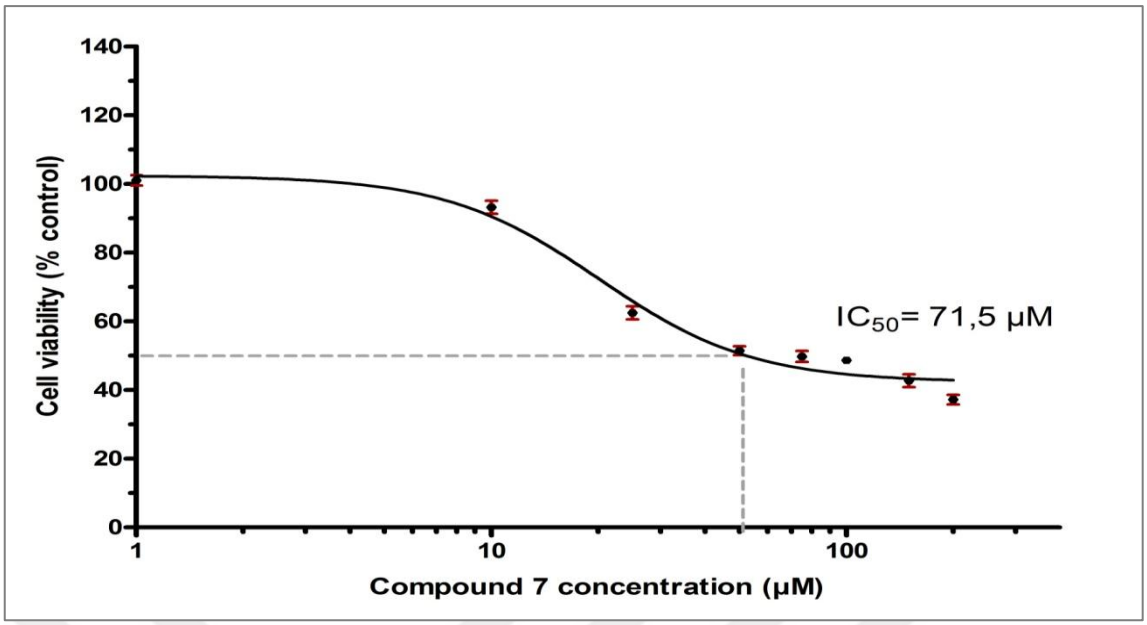


Figure 4.2.3. Sigmoidal graph of decreasing concentrations and IC50 value belong to Compound 7

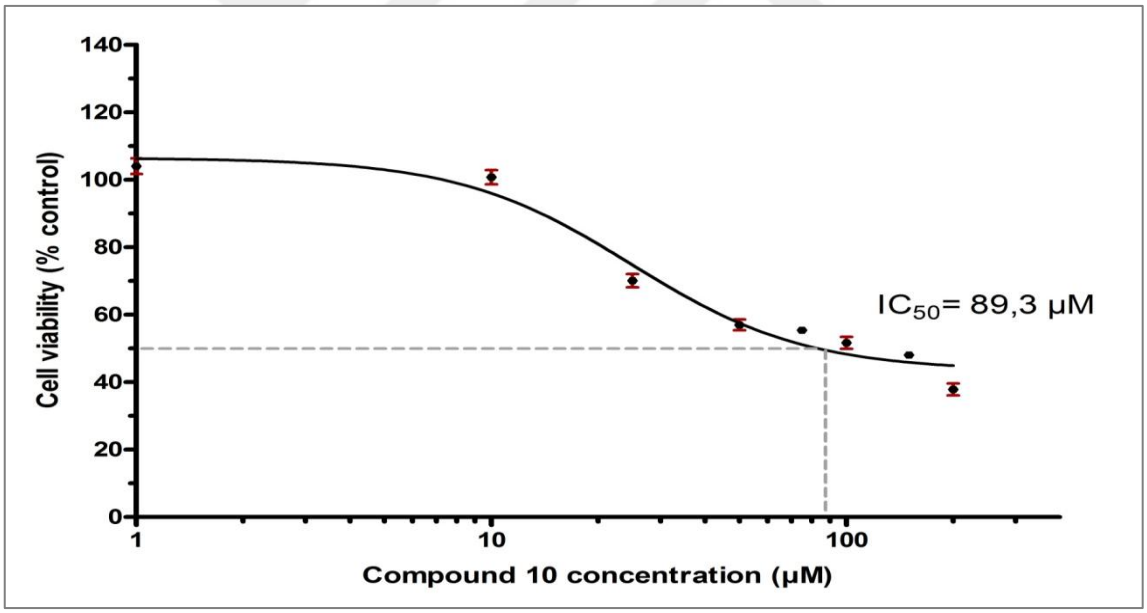
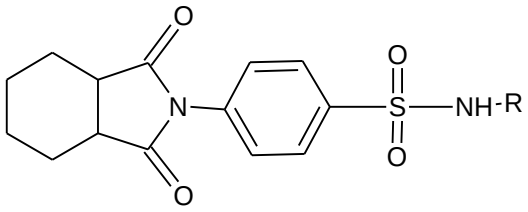


Figure 4.2.4. Sigmoidal graph of decreasing concentrations and IC50 value belong to Compound 10

4.2.2. Anti-inflammatory Activity Data

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



Compounds **1-10**

Table 4.3. Inhibitory effect of compounds **1-10** and reference molecule ASA on nitric oxide (NO) levels in LPS-stimulated macrophage cells.

			NO Inhibition (% of Control)
Compound	**R**	**Cell Viability**	**NO Inhibition**
1	5-methyl-1,2-oxazol-3-yl	90.63 ± 7.77	9.73 ± 1.04
2	acetyl	89.44 ± 6.18	ND
3	1,3-thiazol-2-yl	93.00 ± 4.38	ND
4	1,2-diazine-6-methoxy-3-yl	88.52 ± 4.15	24.43 ± 3.16
5	phenylcarbonyl	96.03 ± 4.07	ND
6	1,3-diazine-6-methyl-2-yl	87.30 ± 8.39	ND
7	1,3-diazine-5,6-dimethoxy-4yl	91.22 ± 3.00	ND
8	1,3-diazine-2-yl	93.10 ± 4.69	ND
9	H	87.82 ± 6.71	6.44 ± 2.48
10	1,3-diazine-4,6-dimethyl-2-yl	90.23 ± 6.31	ND
	ASA (500μM)	100.22 ± 10.07	40.89 ± 3.36

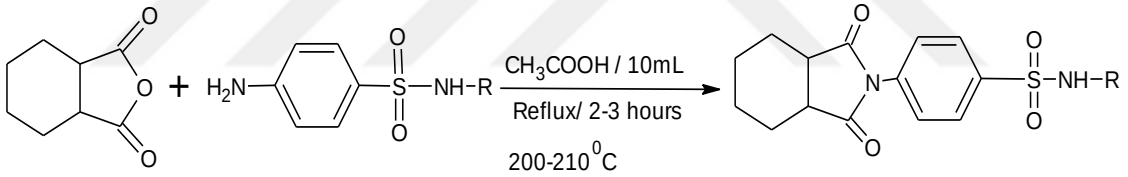
ASA: Acetylsalicylic acid, ND: Non detectable, *: $p<0.05$

5. DISCUSSION AND CONCLUSION

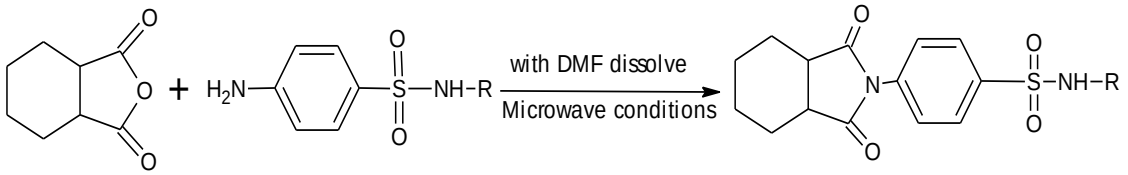
In this study, ten compounds having *N*-(1,3-dioxohexahydro-2*H*-isoindol-2-yl)benzenesulfonamide derivatives which seven of them novel were prepared and evaluated their anticancer and anti-inflammatory activities against MCF-7cell lines and their nitric oxide (NO) inhibitions anti-inflammatory respectively. UV, IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectra were used for structures elucidation.

Two synthetic procedure were applied to the target compounds which were synthesized in this study. In the first method, the compounds were prepared by the reaction of *cis*-1,2-cyclohexane carboxylic anhydride with corresponding sulfa derivatives in acetic acid under reflux for 2-3 hours. In the second method, the *cis*-1,2-cyclohexanecarboxylic anhydride and sulfa derivatives were dissolved in DMF and radiated by microwave by given conditions in table 5.1. The yield of the compounds were between 30% - 100% in the either methods.

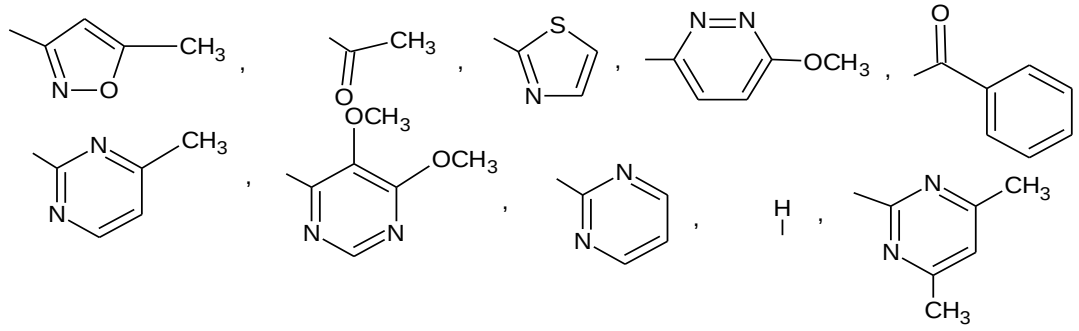
1. Method



2. Method



R:

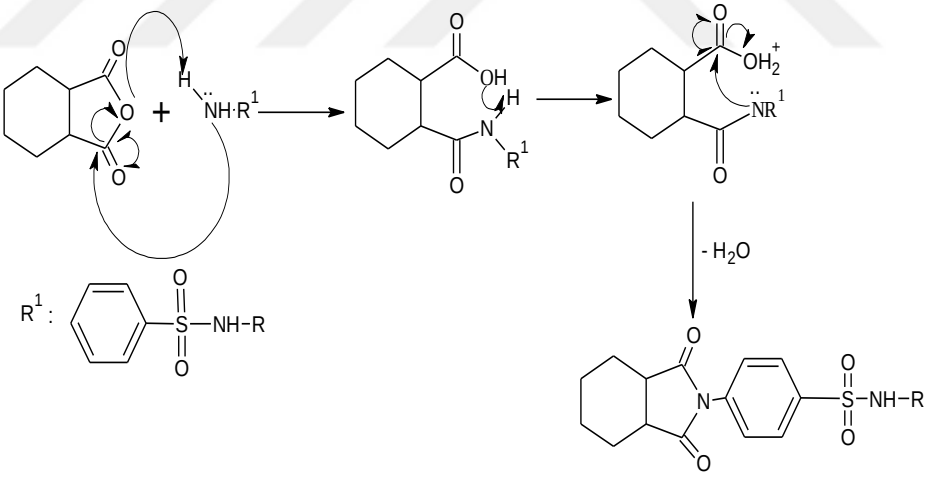


Scheme 5.1. General synthesis pathway of compounds.

Table 5. Microwave condition

Number	t(minute)	E (watt)	T1 (^{0}C)	T2 (^{0}C)	P (Bar)
1	5	250	0	90	0
2	4	200	0	90	0

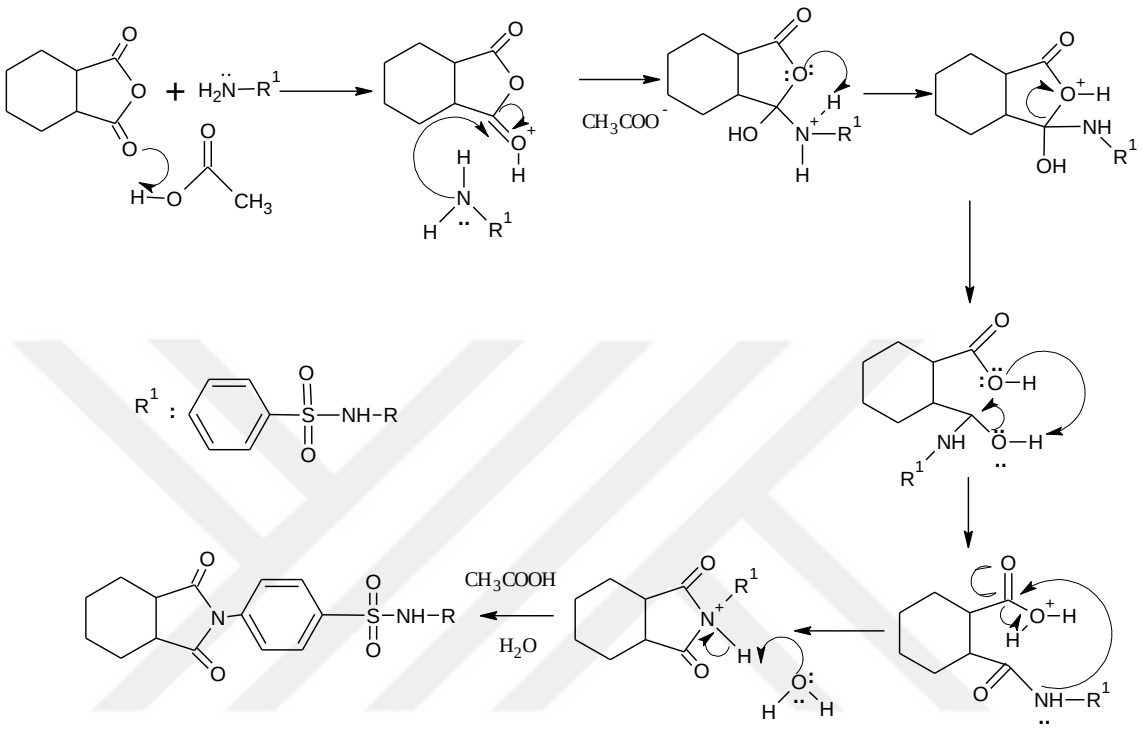
The below scheme shows the reaction mechanism of *N*-(1,3-dioxohexahydro-2*H*-isoindol-2-yl)benzenesulfonamide derivatives. The compounds were obtained by nucleophilic substitution of corresponding sulfonamides to *cis*-1,2-cyclohexanecarboxylic anhydride. Reaction started via attack of lone-pairs of amino group of sulfonamide structure which acts as a nucleophile, to one of the carbonyl carbon (electrophilic portion) of *cis*-1,2-cyclohexanecarboxylic anhydride by microwave. As a result ring opening occured. After hydroxonium elimination from the intermediate molecule, follows ring closing yielded the target compounds.



Scheme 5.2. Reaction mechanism of *N*-(1,3-Dioxohexahydro-2*H*-isoindol-2-yl)benzenesulfonamide formation by microwave.

Reaction started via attack of one of the carbonyl oxygen (electrophilic portion) of *cis*-1,2-cyclohexanecarboxylic anhydride to hydrogen atom of acetic acide structure which acts as a weak acid by under reflux. It goes on via attack of lone-pairs of amino group of sulfonamide structure which acts as a nucleophile, to one of the carbonyl carbon (electrophyllic portion) of *cis*-1,2-cyclohexanecarboxylic anhydride. As a result

ring opening occured. After hydroxonium elimination from the intermediate molecule, follows ring closing yielded the target compounds.



Scheme 5.2.1. Reaction mechanism of *N*-(1,3-Dioxohexahydro-2*H*-isoindol-2-yl)benzenesulfonamide formation by under reflux.

In the literature, *N*-([1,3-dioxoindolin-2H-2-yl)phenyl]sulfonyl and *N*-([1,3-dioxohexahydroindolin-2-yl)phenyl]sulfonyl derivatives were prepared by using phthalic anhydrate or hexahydrodicarboxylic anhydride and sulfa drugs in toluene under reflux. The reaction yields were moderate [49]. In 2015 compounds 3, 6 and 8 were synthetized by the microwave irradiation without solvent at a power of 850 Watt for 2 minutes at 150 ^{0}C . The yields of compounds were 80-90% [49].

In this study, dimethylformamide used as solvent for the synthesis of compounds 3, 6 and 8 while microwave irradiation was subjected at a power of 250 watt, for 4- 5 minutes at 90 ^{0}C. The yields of target compounds were also very high (Table 1.).

Structure elucidation of the synthesized compounds was carried out with UV, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra. All spectral data were in relevance with the predicted structure.

UV spectral data of synthesized compounds were examined in methanol. The compounds showed maximum absorbance at range of 395-398 nm which represent n→π* and π→π*, transitions of dioxohexahydoisoindole and benzene sulfonamide structures.

In UV spectrum of the compound 5 gave mainly two absorption bands at 206 (log ε : 8.01), and 238 (log ε : 8.07) which represent π→π* and n→π* transitions of C=O, aromatic groups and cyclohexyl moiety of the compound(Figure 5.1.).

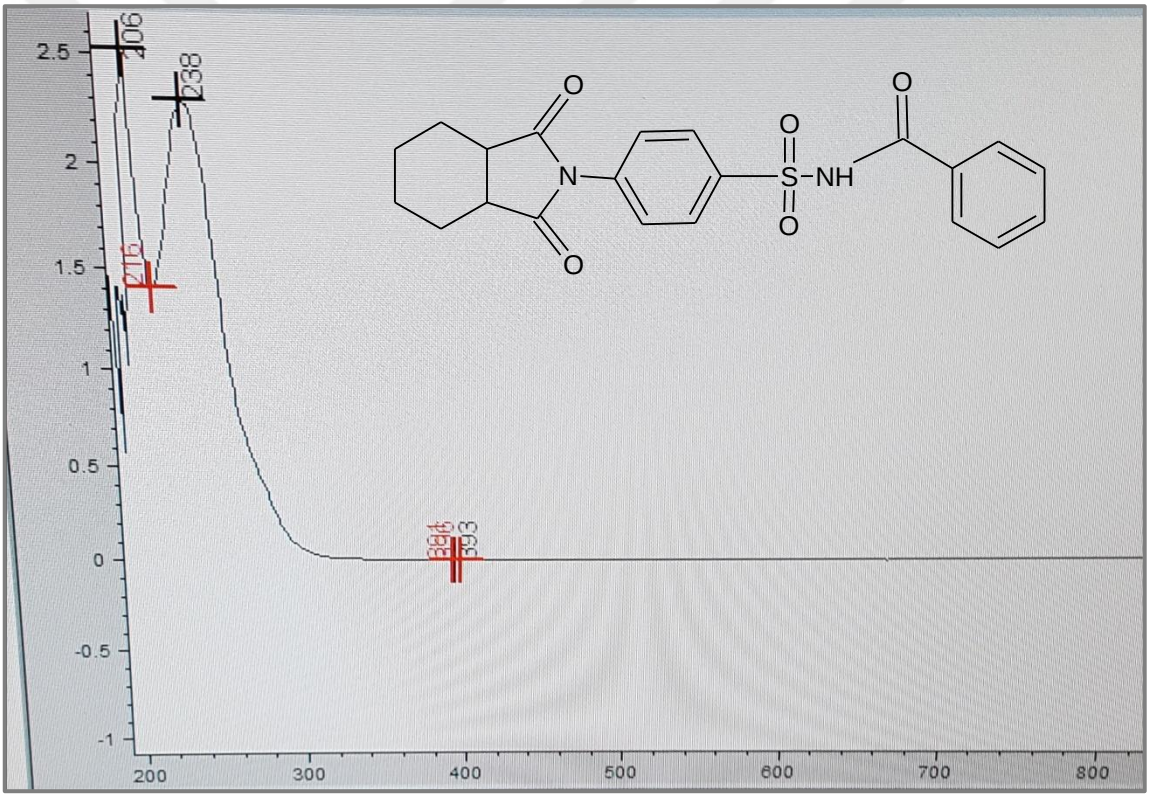


Figure 5.1. UV spectrum of the compound 5; (MeOH, $\lambda_{max,}$ nm); 206 (log ε : 8.01), 238 (log ε : 8.07),

In UV spectrum of the compound 9 gave mainly two absorption bands at at 205 (log ε : 8.01), 234 (log ε : 7.77) which represent π→π* and n→π* transitions of C=O, aromatic groups and aliphatic structures in *N*-(1,3-Dioxohexahydro-2*H*-isoindol-2-yl)benzenesulfonamide derivatives (Figure 5.2.)

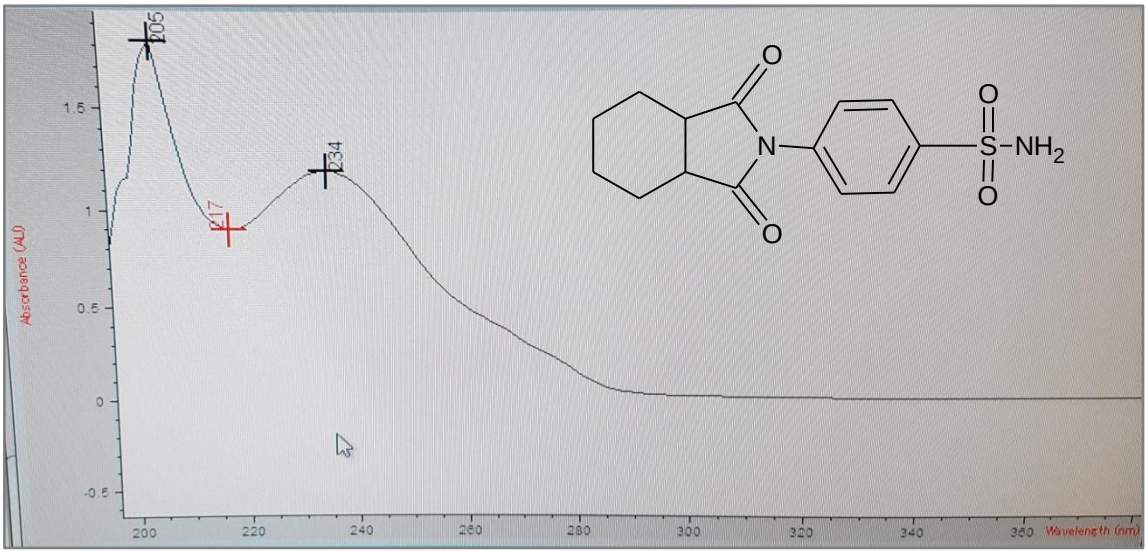


Figure 5.2. UV spectrum of the compound 9; (MeOH, $\lambda_{max,}$ nm); 205 (log ε : 8.01), 234 (log ε : 7.77).

FT-IR spectral data of synthesized compounds were taken by KBr tablets. In general, stretching bands of sulfonamide N-H and aromatic C-H were observed 3400 and 3000 cm^{-1} respectively.

FT-IR spectra of compound 1 gave mainly sulfonamide N-H and aromatic C-H stretching bands at 3475 cm^{-1} and 3075 cm^{-1}respectively. Other stretching bonds were observed as following: 2934 cm^{-1} (aliphatic; C-H), 1702 cm^{-1} (O=C-N-C=O), 1384 cm^{-1} and 1170 cm^{-1} (SO_2) (Figure 5.3.).

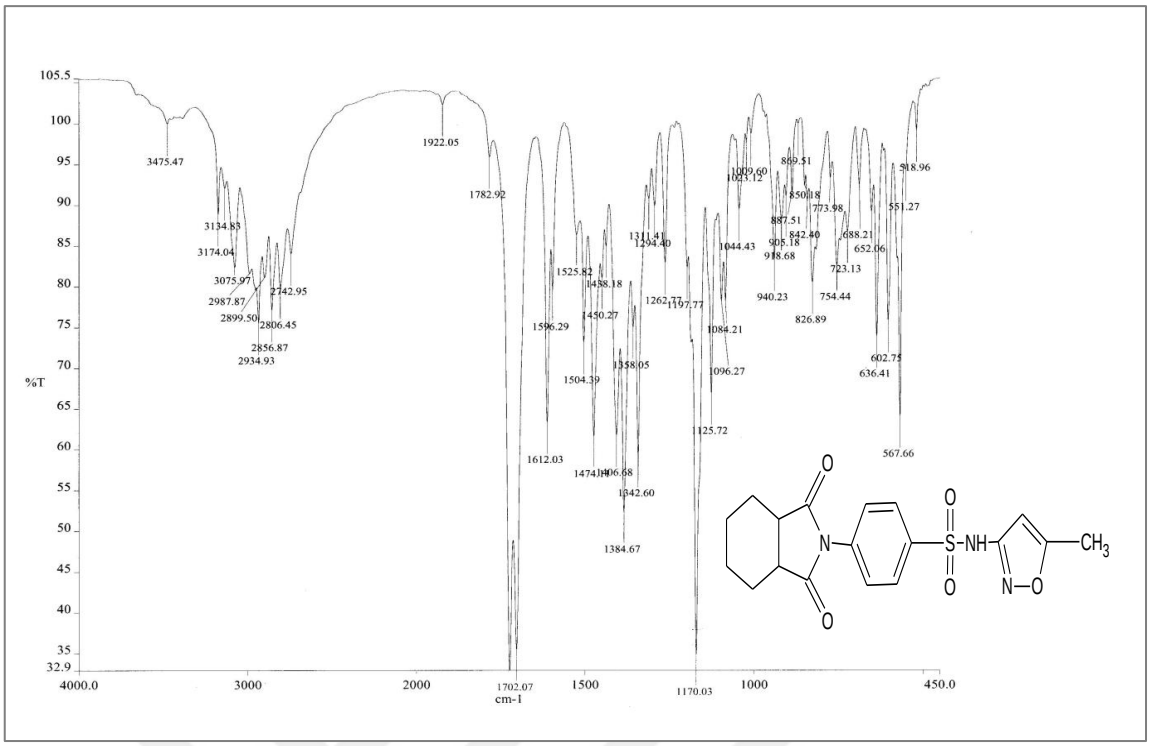


Figure 5.3. IR spectrum of the compound 1.

Stretching bands of sulfonamide N-H 3273 $cm^{-1}$and aromatic C-H stretching bands of compound 2 showed up at 3103 and 3077 cm^{-1}. Other stretching bonds were observed as following: 2942 cm^{-1} (aliphatic; C-H), 1703 cm^{-1} (O=C-N-C=O), 1336 cm^{-1} and 1167 cm^{-1} (SO_2).

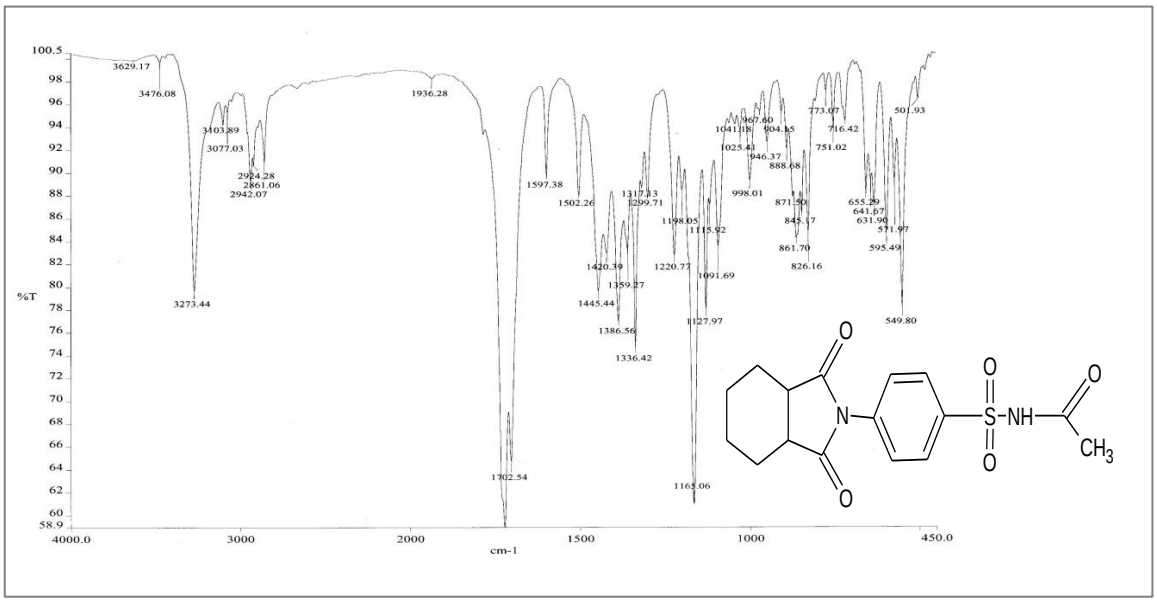


Figure 5.4. IR spectrum of the compound 2.

^{1}H-NMR spectra of all compounds were taken DMSO-d_6 using tetramethylsilane is an internal standard. SO_2-NH of amide structure appeared furthest downfield at 12.76 - 10.10 ppm as singlet. Para positions at 8.20-7.40 ppm was related to CH-Aromatics. The signal originated from CH of cyclohexane at 3.10- 3.05-ppm as multiplet. Multiplet at 1.90-1.77-ppm and at 1.40-1.33 ppm were related to respectively C**H$_2$**-CH_2 and CH_2-C**H$_2$** of cyclohexane ring. ^{1}H-NMR spectra of compounds are presented here with compounds 2 and 10.

^{1}H-NMR spectra of compound 2 displayed peaks belong to SO_2-**NH** of amide structure appeared at 12.10 ppm as singlet. The chemical shift range of aromatic **CH$_2$**'s of the benzenesulfonamide ring varied from 7.40 to 8.00 ppm. The signal originated from CH of cyclohexane part of isoindole at 3.10 ppm as multiplet. Singlet at 2.00 ppm was related to COC**H$_3$**. Multiplet at 1.90-1.80-ppm and at 1.4-1.3 ppm were related to CH_2-C**H$_2$** and C**H$_2$**-CH_2- on cyclohexane ring respectively (Figure 5.5.).

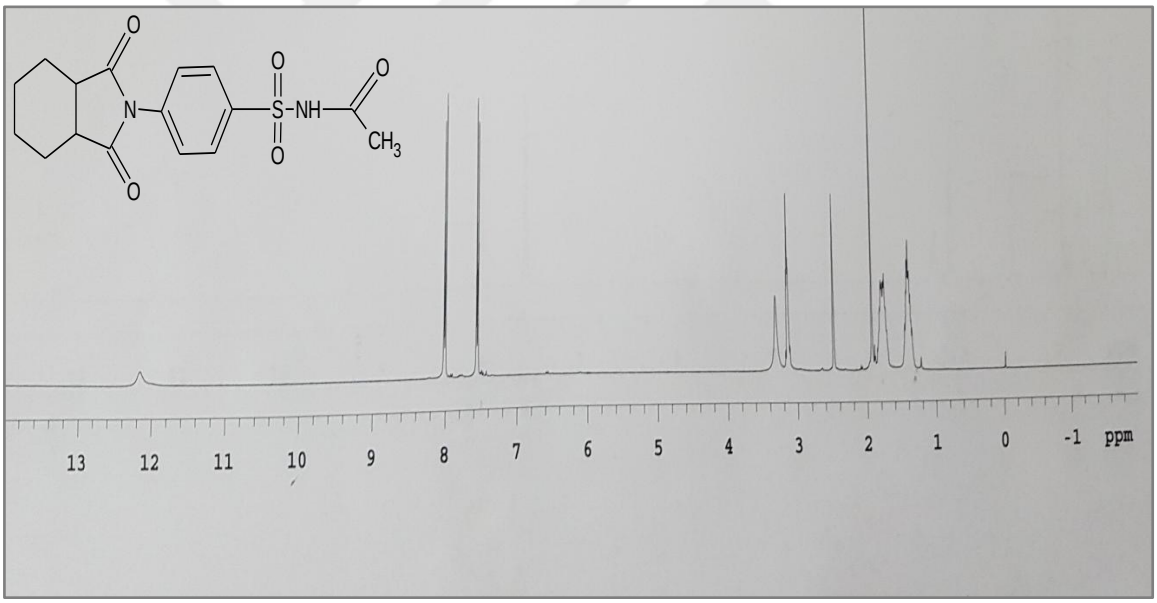


Figure 5.5. ^{1}H-NMR spectrum of the compound 2.

^{1}H-NMR spectra of compound 10 displayed peaks belong to SO_2-**NH** of amide structure appeared at 12.00 ppm as singlet. The chemical shift range of aromatic **CH$_2$**'s of the benzenesulfonamide ring varied from 7.70 to 8.20 ppm as multiplet. Doublet at 6.80 ppm was related to pyrimidine. The signal originated from C**H** of cyclohexane part of isoindole at 3.05 ppm as multiplet. Singlet at 2.20 ppm was related to -C**H$_3$** on pyrimidine ring. Multiplet at 1.90-1.60-ppm were related to CH_2-C**H$_2$** and C**H$_2$**-CH_2- on cyclohexane ring respectively (Figure 5.6.).

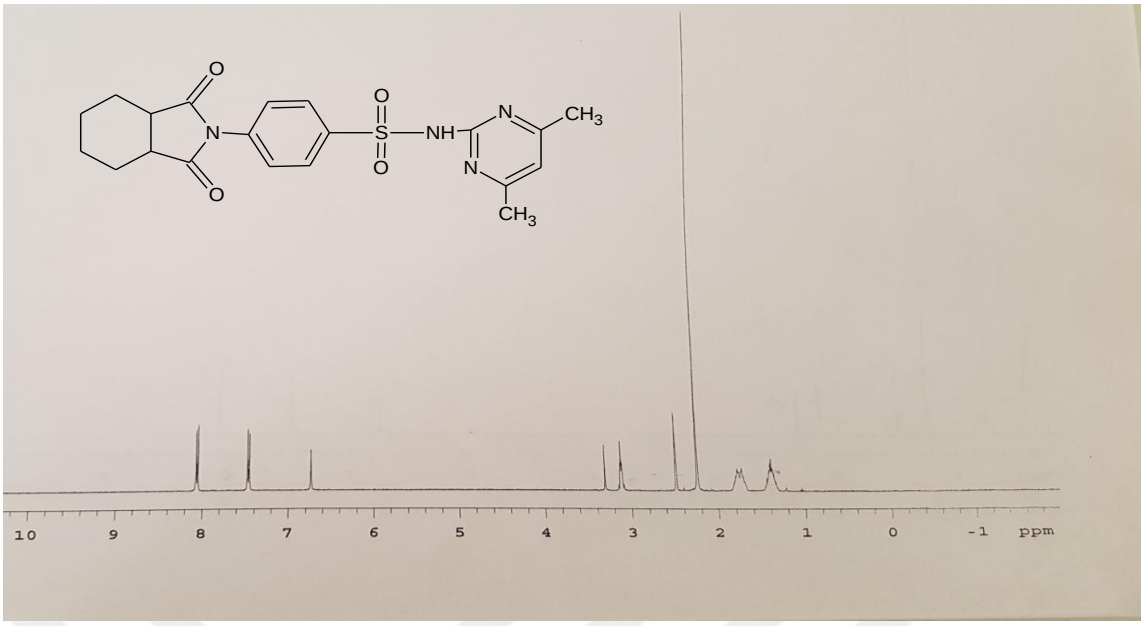


Figure 5.6. ^{1}H-NMR spectrum of the compound 10.

^{13}C-NMR spectrums of the compounds 5 and 9 were 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 5 gave peaks at 178.12 ppm indicated amide carbons C^{1} and C^{2} . The aromatic carbons of the sulfonamide and pyrimidine ring gave resonance at 165.58 (C^{15}), 138.62 (C^{12}), 136.83 (C^{9}), 133.36 (C^{16}), 131.34 ($C^{11, 13}$), 128.61 ($C^{10, 14}$), 128.47 ($C^{17, 21}$), 128.45 ($C^{18, 20}$), 127.29 (C^{19}) ppm while 39.50 ($C^{4, 7}$) , 23.27 ($C^{3, 8}$), 21.44 ($C^{5, 6}$) (Figure 5.7.).

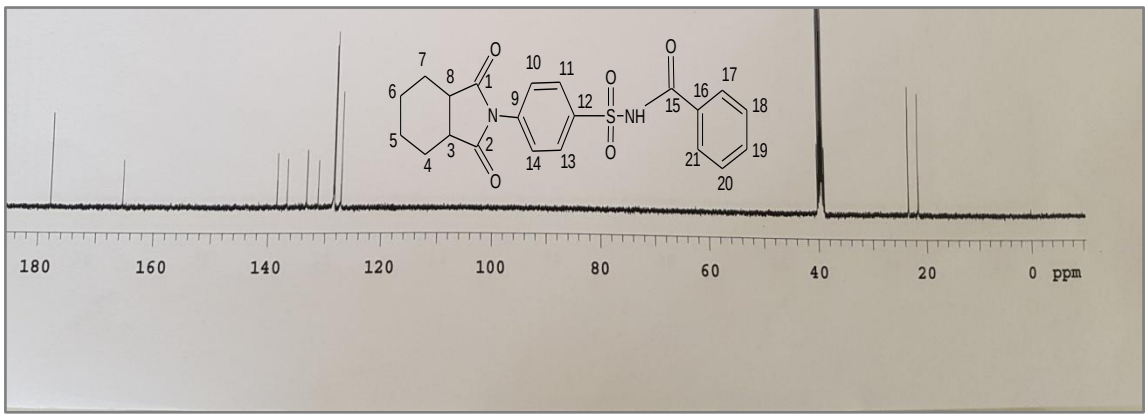


Figure 5.7. ^{13}C-NMR spectrum of compound 5.

^{13}C-NMR spectrum of the compound 9 gave peaks at 178.6 ppm indicated amide carbons C^{1} and C^{2} . The aromatic carbons of the sulphonamide and pyrimidine ring gave resonance at 143.52 (C^{12}), 135.28 (C^{9}), 127.40 ($C^{11, 13}$), 126.35 ($C^{10, 14}$) ppm while 39.50 ($C^{4, 7}$) , 23.28 ($C^{3, 8}$), 21.40 ($C^{5,6}$).

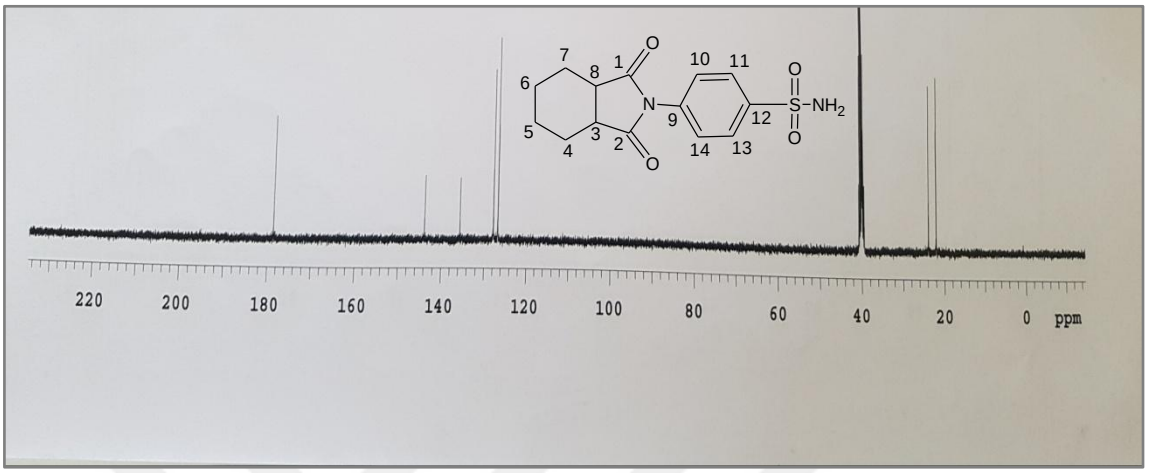
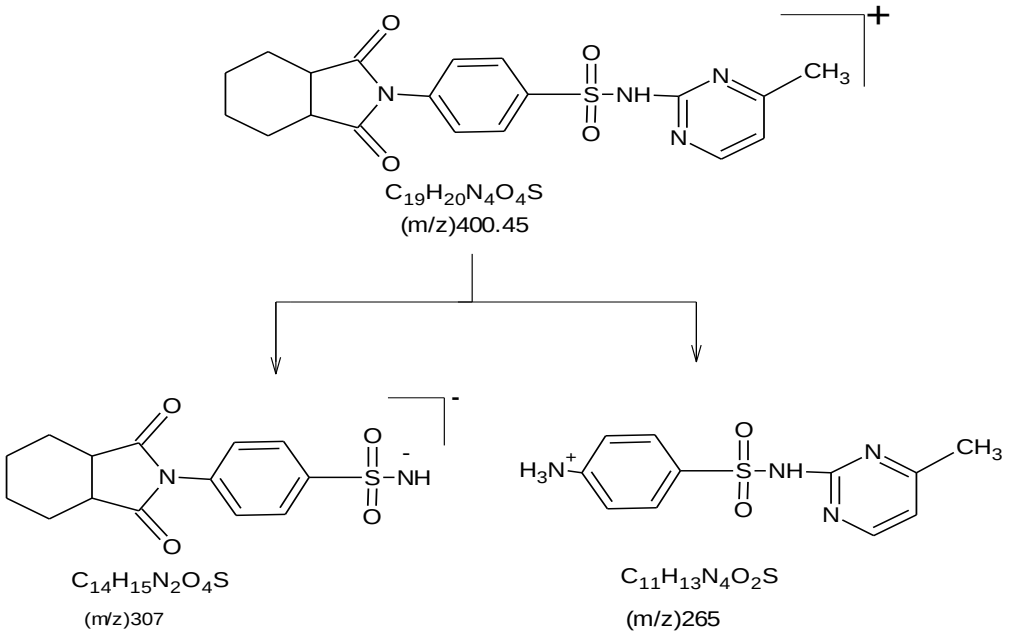


Figure 5.8. ^{13}C-NMR spectrum of compound 9.

Mass spectra of *N*-(1,3-Dioxohexahydro-2*H*-isoindol-2-yl)benzenesulfonamide derivatives are illustrated with compound **6.** Molecular ion peak [M^{+}] observed as base peak at 401.45 (m/z), verified the molecular mass (400.45 g/mol) of the compound. Fragmentation products give peaks at 265 (m/z) and 307 (m/z). The fragmentation pattern is seen bellow Scheme.5.9.



Scheme.5.3. Mass fragmentation pattern of compound 6.

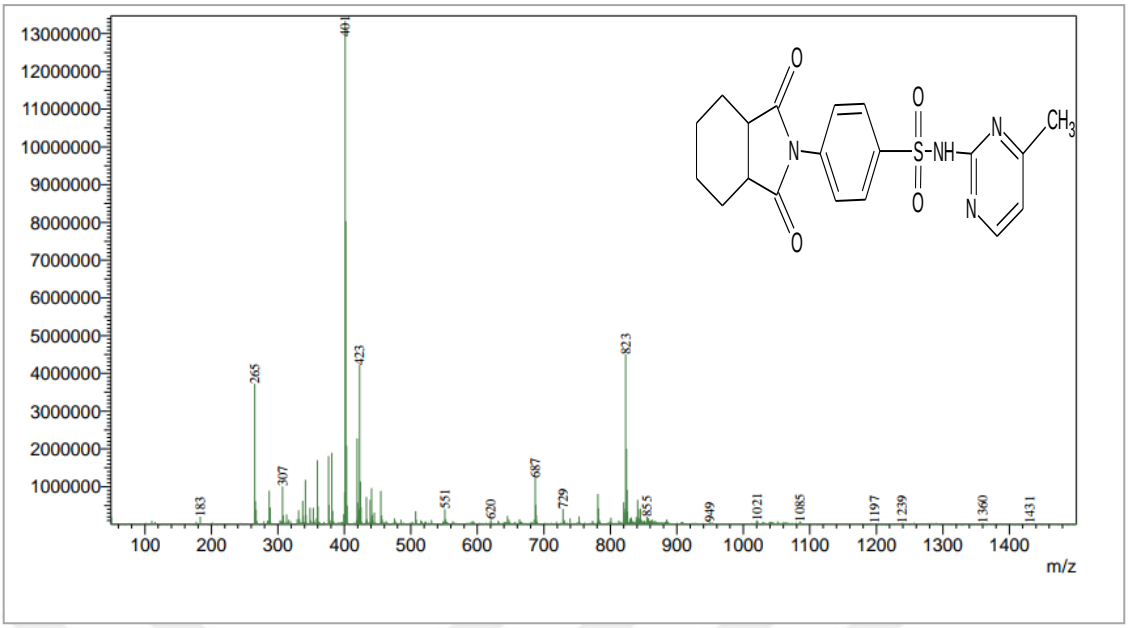
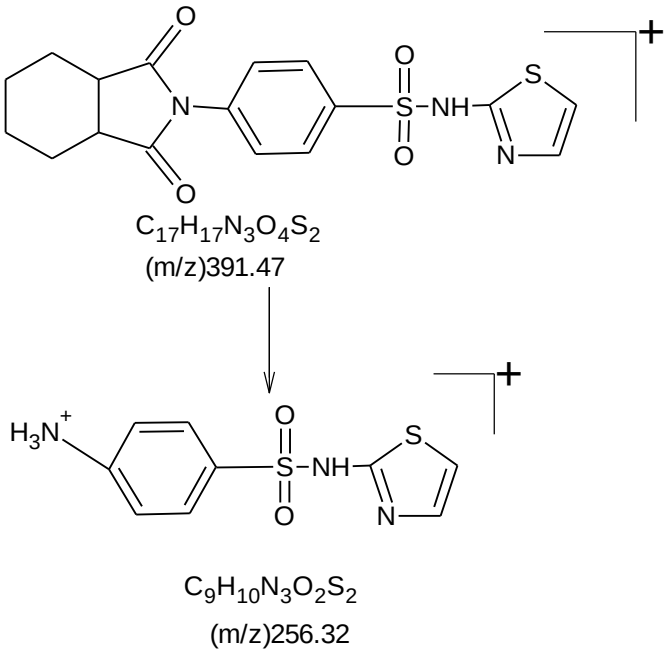


Figure 5.9. Fragmentation pattern of the compound 6.

Mass spectra of *N*-(1,3-Dioxohexahydro-2*H*-isoindol-2-yl)benzenesulfonamide derivatives are illustrated with compound **3.** Molecular ion peak [M^+] observed as base peak at 392.47 (m/z), verified the molecular mass (391.47 g/mol) of the compound. Fragmentation products give peaks at 256 (m/z). The fragmentation pattern is seen bellow Scheme.5.10.



Scheme.5.4. Mass fragmentation pattern of compound 3.

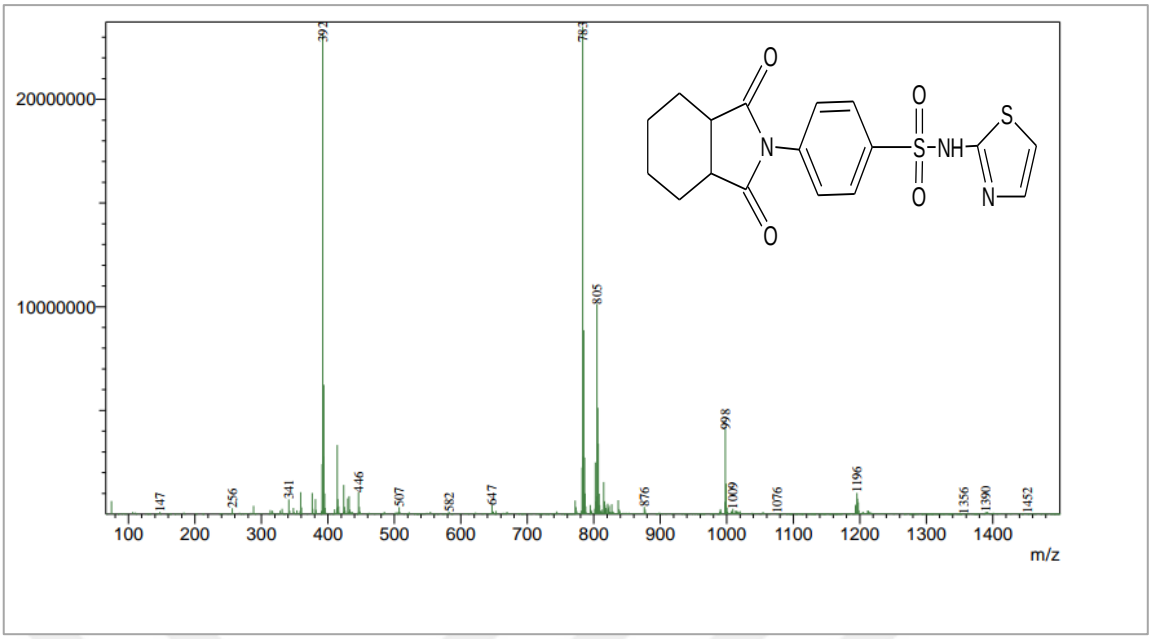
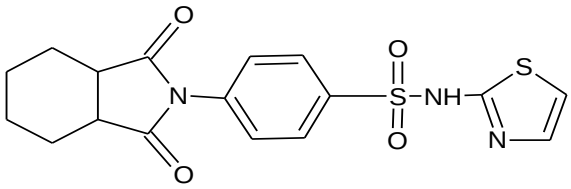


Figure 5.10. Fragmentation pattern of the compound 3.

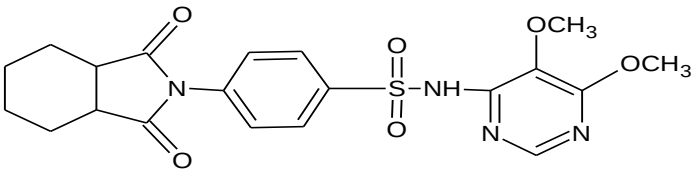
Anticancer activities of the compounds were studied on human breast line (MCF7) cell lines by MTT assay. Anti-inflammatory activities of the compounds were examined by measuring nitrite concentrations by using a colorimetric method based on the Griess reaction on RAW 264.7 macrophage cells.

In the previous literatures, compounds 3, 6 and 8 were screened for *in vitro* anticancer activity against five human cancer cell lines T47D, NCI H-522, HCT-15, PA-1, Hep G2. Among them Compound 3 were exhibited anticancer activity against NCI H-522 and Hep G2 with IC_{50} values 30 µM and 36 µM, respectively [49].The compounds 6 and 8 were not active.

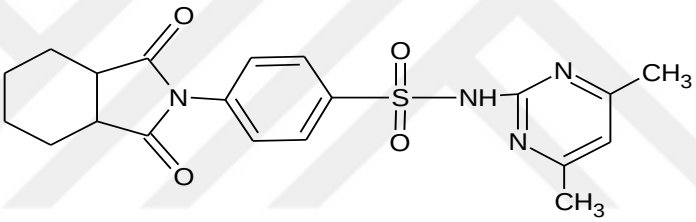
All our compounds 1-10 were screened for *in vitro* anticancer activity against human breast cancer cell line MCF7 by MTT test. Compounds 3,7 and 10 possessed anticancer activity with IC_{50} values 87.9 ± 2.34, 71.5 ± 3.01 and 89.3 ± 2.05 µM, respectively.



Compound 3

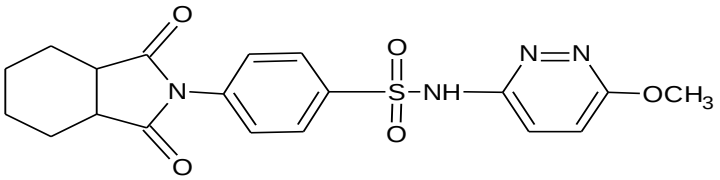


Compound 7

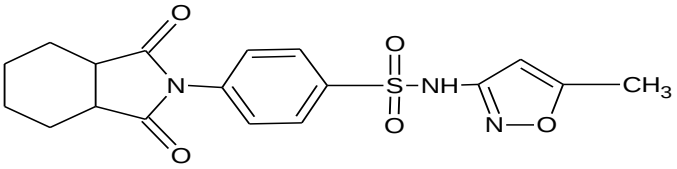


Compound 10

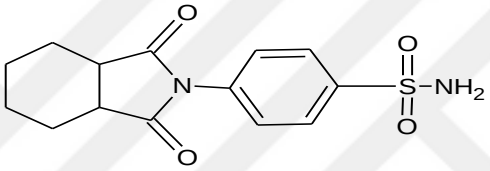
There were no research related to the anti-inflammatory activity of *N*-(1,3-dioxohexahydro-2*H*-isoindol-2-yl)benzenesulfonamide derivatives. In this study, compounds were tested for their inhibitory activities against LPS-induced nitrite production in RAW 264.7 cells, which are summarized in Table.1. Among the tested compounds, 1, 4 and 9 showed nitrite production inhibitory activity while compound 4 exhibited the highest anti-inflammatory activity by suppressing the NO production. Compounds were also analyzed for their cytotoxicity against RAW 264.7 macrophages by using the MTT assay. No significant cytotoxic activities were observed under all tested concentrations. IC_{50} values of the tested compounds were higher than 500 μM. Compounds 4,1 and 9 possessed anti-inflammatory activity with NO inhibition (% of control) values 24,43 ± 3,16, 9,73 ± 1,04 and 6,44 ± 2,48 μM, respectively.



Compound 4



Compound 1



Compound 9

In summary, the compounds have been synthesized by using conventional reflux and microwave irradiation technique and screened for anticancer and anti-inflammatory activity on cell lines of breast (MCF-7) and RAW 264.7 macrophage cells respectively. Compared to synthesis methods, microwave irradiation results high yields, less solvent consuming and short reaction time [Table 1.].

Compounds 4 and 7 which carries the methoxy group on their structures show more inhibition ability on cancer cells and RAW 264.7 macrophage cells than other compounds. 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

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LICENCE	B Class
	2014 November -2015 May Nebahat Cinarli Explosion Protection
EXPERIENCES	Document Processing Specialist Traniee
	Occupational Health and Safety Specialist
	2013 April- 2014 April Helen Yapi END. LTD.ŞTI. Project/Sales/Product/ Purshasing Manager (Tubitak)
	2010 December – 2011 August Soap Company Research and Development Project Consultant About Cleaning and Cosmetic Products
	Purshasing and Planning Manager / Research
	2009 April- 2010 August Gokay Paint Factory Quality Management System Internal Audit Manager Research and Development Manager
	2008 - 2013 (Weekend Parttime) Reks Agency Saleswoman and Introducer Worker

2008 November Maraton Classroom- Chemistry Teacher
2005 August- September Fako Actavis Medicine Factory
Laboratory Trainee
2004 August- September Fako Actavis Medicine Factory
Production Trainee

SEMINARS ATTENDED

C of Level Occupational Health and Safety Specialist 18 August, 2013

TS ISO/EN 17025-2005 Laboratory Accreditation of Laboratory Quality Management System Education and Internal Programme Audit Certification Organized by Association for Chemists
10-11 of December, 2011

Patent Education, 2016

IVEK 3rd International Convention of Pharmaceuticals and Pharmacies, 2017

TS EN ISO 17025 Method Validation and Testing and Analiysis with Statistical Calculations in Excel Educations Certification Programme Organized by Association for Chemists
17/24/31 of December, 2011

BRC Global Standard for Food Safety/Issue- 6 8 of October, 2012

TS ISO 22000:2005 Food Safety Management Systems 6 of October, 2012
TS ISO 22000:2005 Food Safety Management Systems Internal Auditor
7 of October, 2012

GC-MS AND ICP-MS Systems Seminars by Bruker 18 of October, 2012

TS EN ISO 17025 Chemical Analyses of Uncertainty Education Certification Programme Organized by Association for Chemists
7 of January, 2012

Chemistry of Career 2011- 5/7 of December, 2011

TURCHEM 2010 AND 2012 FUAR, FOOD FUAR 2012, PAINT FUAR 2012

HPLC,GC,UV etc. Education by Abem Kimya

III.Laboratory Quality Conference by The EUropen Union
13 OF December,2011

IV.Laboratory Quality Conference by The Europen Union
19 OF September,2012

Good Manufacturing Practices Certification of
Trakya Universty and Association for Chemists on 23-24 OF April, 2009 (GMP)

TS 18001:2005 Occupational Health Safety Assessment System
Certification Programme Organized by Chemistry Department of
Trakya University and Association for Chemists on 20-21 OF December, 2008
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TSE EN ISO 9001:2008 Quality Management System Certification
Programme Organized by Association for Chemists
on 12-13 December of, 2009

TSE EN ISO 9001:2008 Quality Management System Internal Audit
Certification Programme Organized by Berrin Yildiz on March of, 2010

Enocta Microsoft Office 2007 (March, 2009)

Upper Intermediate Level English Course, British English, 2011

Beginner Level French Course, French Culture, 2004

SEMINARS GIVEN

Management of Project TÜBİTAK about Fire and Sound Insulation Construction.

COMPUTER PROGRAMS

Microsoft Office 2010/ Navisson Programe/ SAP

TECHNICAL

Microwave, Melting Point, HPLC, GC, Atomic Absorption,
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