T.C.

YEDİTEPE UNIVERSITY

INSTITUTE OF HEALTH SCIENCE

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

SYNTHESIS AND BIOLOGICAL STUDIES OF SOME 3-SUBSTITUTED-2,4(1*H*,3*H*)-QUINAZOLINEDIONE DERIVATIVES

DOCTOR OF PHILOSOPHY THESIS

DEMET YILMAZ, B.Pharm

ADVISOR

Prof. Dr. HÜLYA AKGÜN

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Doktora öğrencisi Ecz. Demet Us YILMAZ'ın çalışması jürimiz tarafından Farmasötik Kimya Anabilim Dalı Doktora tezi olarak uygun görülmüştür.

İMZA

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ONAY

Yukarıdaki jüri kararı Enstitü Yönetim Kurulu'nun 23/05/2014 tarih ve ..10..2..... sayılı kararı ile onaylanmıştır.

Prof. Dr. Bayram YILMAZ
Müdür V.

ÖZET

Yılmaz, D. Bazı 3-Sübstitüe-2,4(1*H*,3*H*)-kinazolindion Türevlerinin Sentezi ve Biyolojik Çalışmaları. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Farmasötik Kimya Programı Doktora Tezi, İstanbul, 2014.

Bu tez çalışmasında, yirmi sekiz adet yeni 3-{2-[4-(sübstitüe)piperazin-1-il]-2-oksoetil}kinazolin-2,4(1*H*,3*H*)-dion türevi bileşiğin sentezi yapılarak biyolojik aktiviteleri incelenmiştir. Başlangıç maddesi olarak kullanılan 4,5-disübstitüe-2-aminobenzoik asit türevleri ile etil isosiyanatoasetat bazik ortamda reaksiyona sokularak 4,5-disübstitüe-2-[3-(2-etoksi-2-oksoetil)üreido]benzoik asit türevleri (bileşik **1**-**3**) elde edilmiştir. Bu bileşiklerin derişik hidroklorik asitle muamelesi sonucu halka kapama reaksiyonuna uğraması ile (6,7-disübstitüe-2,4-diokso-1,4-dihidrokinazolin-3(2*H*)-il)asetik asit türevleri (bileşik **4**-**6**) sentezlenmiştir. Bu bileşiklerin çeşitli piperazin türevleri ile amidasyon reaksiyonu sonucu 6,7-disübstitüe-3-{2-[4-sübstitüepiperazin-1-il]-2-oksoetil}kinazolin-2,4(1*H*,3*H*)-dion türevleri (bileşik **7**-**34**) elde edilmiştir.

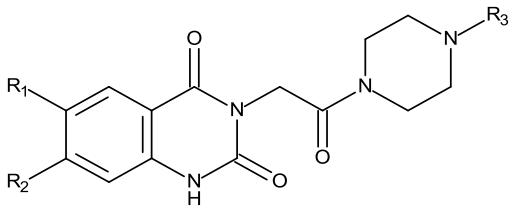
Elde edilen bütün bileşiklerin yapıları ^{1}H-NMR, UV ve IR spektral metodları ve elemental analiz yöntemi ile doğrulanmıştır. Bileşik **11**, **20** ve **32** için ayrıca ^{13}C-NMR ve kütle spektral teknikleri uygulanmıştır. Sentezlenen bileşiklerin antimikrobiyal ve antikanser aktivite çalışmaları ise agar disk diffüzyon ve Sulforodamin B testleri ile yapılmıştır.

Sentezlenen bileşiklerin antimikrobiyal aktiviteleri gram pozitif bakterilerden *Bacillus subtilis* ve *Staphylococcus aureus*'a karşı incelenmiştir. Bileşikler standard ampisilin ile karşılaştırıldığında genellikle orta veya düşük seviyede etki göstermişlerdir. 3-[2-(4-Siklohekzilpiperazin-1-il)-2-oksoetil]-6,7-dimetoksikinazolin-2,4(1*H*,3*H*)-dion *S. Aureus*'a karşı 14 mm ile en yüksek inhibisyon zon çapını vermiştir.

Sitotoksisite sonuçları incelendiğinde, sentezlenen bileşiklerin genellikle aktivite göstermedikleri veya orta seviyede aktivite gösterdikleri belirlenmiştir. Bileşiklerden 3-{2-[4-(4-klorobenzil)piperazin-1-il]-2-oksoetil}kinazolin-2,4(1*H*,3*H*)-dion'un (bileşik **7**) hepatoselüler karsinoma (HUH-7), meme kanseri (MCF-7) ve kolorektal karsinoma

(HCT-116) hücre hatlarına karşı iyi ve orta seviyede etkili olduğu belirlenmiştir (IC_{50} = 2.5, 6.8 ve 4.9 μM).

Tablo: Sentezlenen bileşiklerin genel formülleri.



Bileşik 7-34

Bileşik	R_1	R_2	R_3
7	-H	-H	4-klorobenzil
8	-H	-H	benzo[d][1,3]dioksol-5-il
9	-H	-H	2-furoil
10	-H	-H	siklohekzil
11	-H	-H	2-siyanofenil
12	-H	-H	difenilmetil
13	-H	-H	benzoil
14	-H	-H	piridin-4-il
15	-Cl	-H	4-klorobenzil
16	-Cl	-H	3-(triflorometil)fenil
17	-Cl	-H	benzo[d][1,3]dioksol-5-il

18	-Cl	-H	2-furoil
19	-Cl	-H	siklohekzil
20	-Cl	-H	2-siyanofenil
21	-Cl	-H	difenilmetil
22	-Cl	-H	4-metoksifenil
23	-Cl	-H	benzoil
24	-Cl	-H	piridin-4-il
25	$-OCH_3$	$-OCH_3$	4-klorobenzil
26	$-OCH_3$	$-OCH_3$	3-(triflorometil)fenil
27	-OCH3	$-OCH_3$	benzo[d][1,3]dioksol-5-il
28	$-OCH_3$	$-OCH_3$	2-furoil
29	$-OCH_3$	$-OCH_3$	siklohekzil
30	-OCH3	$-OCH_3$	2-siyanofenil
31	$-OCH_3$	$-OCH_3$	difenilmetil
32	$-OCH_3$	$-OCH_3$	4-metoksifenil
33	$-OCH_3$	$-OCH_3$	benzoil
34	$-OCH_3$	$-OCH_3$	piridin-4-il

Anahtar kelimeler: Kinazolin, kinazolin-2,4(1*H*,3*H*)-dion, antikanser, antimikrobiyal, sitotoksisite.

SUMMARY

Yılmaz, D. Synthesis and Biological Studies of Some 3-Substituted-2,4(1*H*,3*H*)-quinazolinedione Derivatives. Yeditepe University Institute of Health Science, PhD Thesis on Pharmaceutical Chemistry Programme, İstanbul, 2014.

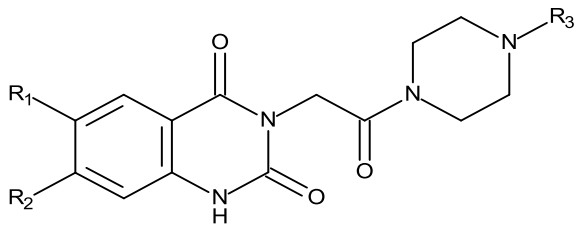
In this thesis, twenty eight novel derivatives of 6,7-disubstituted-3-{2-[4-(substituted)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1*H*,3*H*)-dione (compound **7-34**) have been synthesized and their antimicrobial and anticancer activities were evaluated. Synthesis of the target compounds were started by the reaction of 4,5-disubstituted-2-aminobenzoic acid derivatives with ethyl isocyanatoacetate in basic media to form 4,5-disubstituted-2-[3-(2-ethoxy-2-oxoethyl)ureido]benzoic acid derivatives (compound **1**-**3**). These products were submitted to the ring closure reaction by refluxing in concentrated hydrochloric acid to prepare 2-(6,7-disubstituted-2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)acetic acid derivatives (compound **4**-**6**). The target compounds, 6,7-disubstituted-3-{2-[4-substitutedpiperazin-1-yl]-2-oxoethyl} quinazoline-2,4(1*H*,3*H*)-dione derivatives (compound **7**-**34**) were obtained by the amidation reaction of (6,7-disubstituted-2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)acetic acid derivatives (compound **4**-**6**) with various 1-substitutedpiperazines in the presence of DCC coupling reagent.

Structure identifications of the compounds were done by ^{1}H-NMR, UV and IR spectral methods and elemental analysis. ^{13}C-NMR and mass spectral techniques were also applied for compounds **11**, **20** and **32**. Antimicrobial and anticancer activities of the compounds were examined by using disc diffusion and Sulforhodamine B test, respectively.

Synthesized compounds were screened against two gram positive bacteria strains, *Bacillus subtilis* and *Staphylococcus aureus*, by using agar-based disc diffusion method. The compounds generally showed mild to moderate activity compared with ampicillin as standard. Among them, 3-[2-(4-Cyclohexylpiperazin-1-yl)-2-oxoethyl]-6,7-dimethoxyquinazoline-2,4(1*H*,3*H*)-dione (compound **29**) exhibited the highest zone inhibition value of 14 mm against *S. Aureus*.

According to the cytotoxicity screening results, the compounds generally showed moderate or no cytotoxic activity. 3-{2-[4-(4-Chlorobenzyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1*H*,3*H*)-dione (compound **7**) presented the highest activity against hepatoma (HUH-7), breast cancer (MCF-7) and colorectal cancer cell line (HCT-116) with the IC_{50} values of 2.5, 6.8 and 4.9 μM, respectively.

Table: General formula of the synthesized compounds.



Compound 7-34

Compound	R_1	R_2	R_3
7	-H	-H	4-chlorobenzyl
8	-H	-H	benzo[d][1,3]dioxol-5-yl
9	-H	-H	2-furoyl
10	-H	-H	cyclohexyl
11	-H	-H	2-cyanophenyl
12	-H	-H	diphenylmethyl
13	-H	-H	benzoyl
14	-H	-H	pyridine-4-yl
15	-Cl	-H	4-chlorobenzyl

16	-Cl	-H	3-(trifluoromethyl)phenyl
17	-Cl	-H	benzo[d][1,3]dioxol-5-yl
18	-Cl	-H	2-furoyl
19	-Cl	-H	cyclohexyl
20	-Cl	-H	2-cyanophenyl
21	-Cl	-H	diphenylmethyl
22	-Cl	-H	4-methoxyphenyl
23	-Cl	-H	benzoyl
24	-Cl	-H	pyridine-4-yl
25	$-OCH_3$	$-OCH_3$	4-chlorobenzyl
26	$-OCH_3$	$-OCH_3$	3-(trifluoromethyl)phenyl
27	$-OCH_3$	$-OCH_3$	benzo[d][1,3]dioxol-5-yl
28	$-OCH_3$	$-OCH_3$	2-furoyl
29	$-OCH_3$	$-OCH_3$	cyclohexyl
30	$-OCH_3$	$--OCH_3$	2-cyanophenyl
31	$-OCH_3$	$-OCH_3$	diphenylmethyl
32	$-OCH_3$	$-OCH_3$	4-methoxyphenyl
33	$-OCH_3$	$-OCH_3$	benzoyl
34	$-OCH_3$	$-OCH_3$	pyridine-4-yl

Keywords: Quinazoline, quinazoline-2,4(1*H*,3*H*)-dione, anticancer, antimicrobial, cytotoxicity.

dedicated to my father, İsmail Us.

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I appreciate my family especially my brother Hakan for keeping me encouraged from the beginning of my PhD program. I also thank my mother for looking after my lovely son Yiğit Eray who is joy of my life. Lastly, I would like to thank my husband, Dr. Özgür Yılmaz, who gave me the strength with his great patience to complete this study. I hope we will keep on our talks about chemistry and life for many years.

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ABBREVIATIONS

5-HT_3	5-Hydroxytryptamine 3
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATP	Adenosine triphosphate
CaIPDE	Calcium-independent phosphodiesterase enzyme
cAMP	cyclic-Adenosine monophosphate
CDI	1,1'-Carbonyldiimidazole
CDK5	Cyclin-dependent kinase 5
CNS	Central nervous system
COX-2	Cyclooxygenase-2
cPLA2a	Cytosolic phospholipase A2
d_6	Deuterated
DBN	1,5-Diazabicyclo[4.3.0]non-5-ene
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N'*-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DCU	*N*,*N'*-Dicyclohexylurea
DIO	Diet induced obesity
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid

EGFR	Epidermal growth factor receptor
EI	Electron ionization
ESI	Electrospray ionization
FDA	Food and Drug Administration
FT	Farnesyl transferase
GI	Growth inhibition
Gly	Glycine
HCV	Hepatitis C virus
IC_{50}	Half maximal inhibitory concentration
IL-2	Interleukin-2
IMPDH	Inosine monophosphate dehydrogenase
IR	Infrared
KA	Kainate
LC	Liquid chromatography
m-CPMA	*m*-Chloroperbenzoic acid
MCH	Melanin concentrating hormone
MES	Maximal electroshock
MIC	Minimum inhibitory concentration
MMP	Matrix metalloproteinase
MS	Mass spectrometry
MTHP	*N*-methyltetrahydropyrimidine
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

NCI	National Cancer Institute
NFAT	Nuclear factor of activated T-cells
NMDA	*N*-methyl-D-aspartate
NMR	Nuclear magnetic resonance
PARP	Poly(ADP-ribose) polymerase
PDE	Phosphodiesterase
PDGFR	Platelet-derived growth factor receptor
PGGTase-1	Protein geranylgeranyltransferase-1
Ppm	Parts per million
PSA	Puromycin-sensitive aminopeptidase
PTZ	Pentylene tetrazole
PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
rDA	retro-Diels-Alder
R_f	Retention factor
RSV	Respiratory syncytial virus
STK	Serine-threonine kinase
t-BuOK	*tert*-Butoxide
TGF-β	Transforming growth factor beta
THF	Tetrahydrofurane
TK	Tyrosine kinase
TLC	Thin layer chromatography

TMS	Tetramethylsilane
TNF-α	Tumor necrosis factor
TSHR	Thyroid-stimulating hormone receptor
UHP	Urea-hydrogen peroxide adduct
UV	Ultraviolet
VEGFR-2	Vascular endothelial growth factor receptor-2

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1.INTRODUCTION AND AIM

In 1928, Alexander Fleming observed that colonies of the bacteria *Staphylococcus aureus* could be destroyed by a common mold fungus *Penicillium notatum*. Purification of the β-lactam antibiotic penicillin from this fungus species was achieved by Florey and Chain in 1940s. Florey and his team lead to decrease the number of deaths and amputations with using penicillin during World War II [1].

Sulfa drugs are the first class of synthetic antibiotics. Prontosil, discovered in 1935, was the first sulfa drug that was used in clinical practice. In 1943 Waksman isolated the first drug of the aminoglycosides, streptomycin, from soil bacteria. [2]. Quinolones, the second synthetic antibiotic family, were discovered in 1962 by empirical screening of the by-product of chloroquine synthesis. Oxazolidinones are the third class of synthetic antibiotic produced in 1979 that led to Food and Drug Administration (FDA) approval of linezolid in 1999 [3].

Nowadays, antibiotic resistance is a serious health problem. In other words, multidrug resistant infections are becoming increasingly common in public. Antibiotic resistance can result from modification of an antibacterial's target or it can be dependent on active efflux, drug impermeability, or enzymatic inactivation [4]. Traditional therapies are proving to be ineffective due to the repeated appearance of bacterial strains that demonstrate drug resistance. Thus, antimicrobial agents effective against drug-resistant pathogens are need to be developed urgently [5–7].

On the other hand, cancer is the second most common reason of death following cardiovascular diseases in many countries. Cancer is characterized by the loss of control of the growth, division and spread of a group of cells, leading to a primary tumor that invades and destroys adjacent tissues. Cancer cells are rapidly divided by synthesizing new deoxyribonucleic acid (DNA). There are mainly five group of cytotoxic drugs which interfere with DNA replication resulting with cell death as follows:

1) Antimetabolites: e.g., 5-fluorouracil, gemcitabine, methotrexate, raltitrexed.

2) Alkylating agents: e.g., chlorambucil, procarbazine, carboplatin, cisplatin.

3) Topoisomerase inhibitors: e.g., etoposide, doxorubicin and teniposide [8].

4) Anti-microtubule agents: Vinca Alkaloids and Taxanes [9].

5) Cytotoxic antibiotics: e.g., mitomycin, doxorubicin and daunorubicin [10].

These cytotoxic agents affect cell proliferation which leads to toxicity to healthy cells as well as acquired resistance in cancer cells. Recently, several molecular target-based compounds (such as; imatinib, gefitinib, bortezomib, erlotinib) have emerged with the aim of minimizing side effects of cytotoxic agents. These molecularly targeted agents are predesigned to inhibit or modify a molecular marker considered to be important in prognosis, growth or metastasis occurring only in cancer cells. Thus, they exhibit better toxicity profiles than cytotoxic drugs [11, 12].

Quinazoline ring resembles both the purine and the pteridine nucleus. Therefore, some quinazoline derivatives which inhibit the purinic or folate metabolic pathways were discovered. Quinazolines may also exhibit their anticancer activity by both tyrosine kinase (TK) and serine-threonine kinase (STK) inhibition, in besides of p53 modulation and thyroid-stimulating hormone receptor (TSHR) agonistic activity [13].

Since the discovery of gefitinib (**Figure 1.1.**), first marketed kinase inhibitor, the quinazoline derivatives particularly 4-anilinoquinazolines have attracted much attention for their anticancer properties. Some of the 4-anilinoquinazoline derivatives have been marketed for their anticancer properties (i.e., erlotinib, vantetanib, lapatinib, etc). These adenosine triphosphate (ATP)-mimic compounds show their TK enzyme inhibition activities by occupying the ATP-binding pocket with high affinity [8, 12, 13].

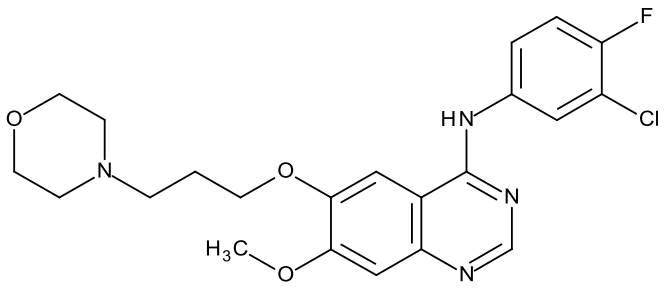
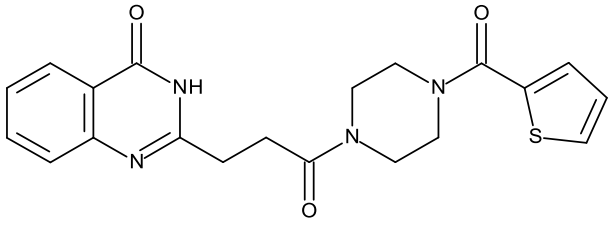
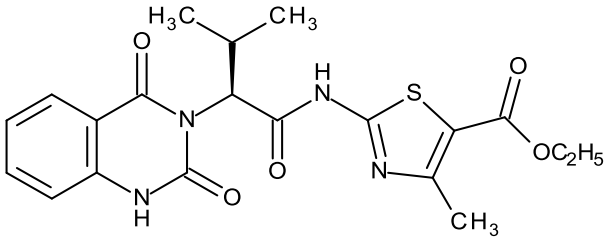


Figure 1.1. Molecular structure of gefitinib.

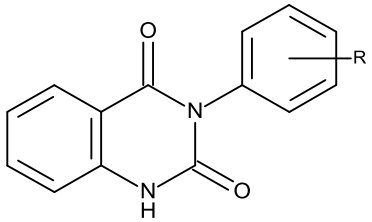
Some 4-oxoquinazoline and 2,4-dioxoquinazoline derivatives possessing anticancer activity were recently reported in the literature. Giannini *et al.* reported an piperazinylamide derivative of 3-(4-oxo-3,4-dihydro-quinazolin-2-yl)-propionic acid derivative showing inhibitory activity over the tumor volume of breast carcinoma [14].



Another study was done by Good *et al.* presenting anticancer activity of 3-substituted-2,4(1*H*,3*H*)-quinazolinedione derivative with IC_{50} value of $\leq$ 10 nM and GI_{50} value of $\leq$ 10 nM against human ovarian SKOV3 cancer cells [15].

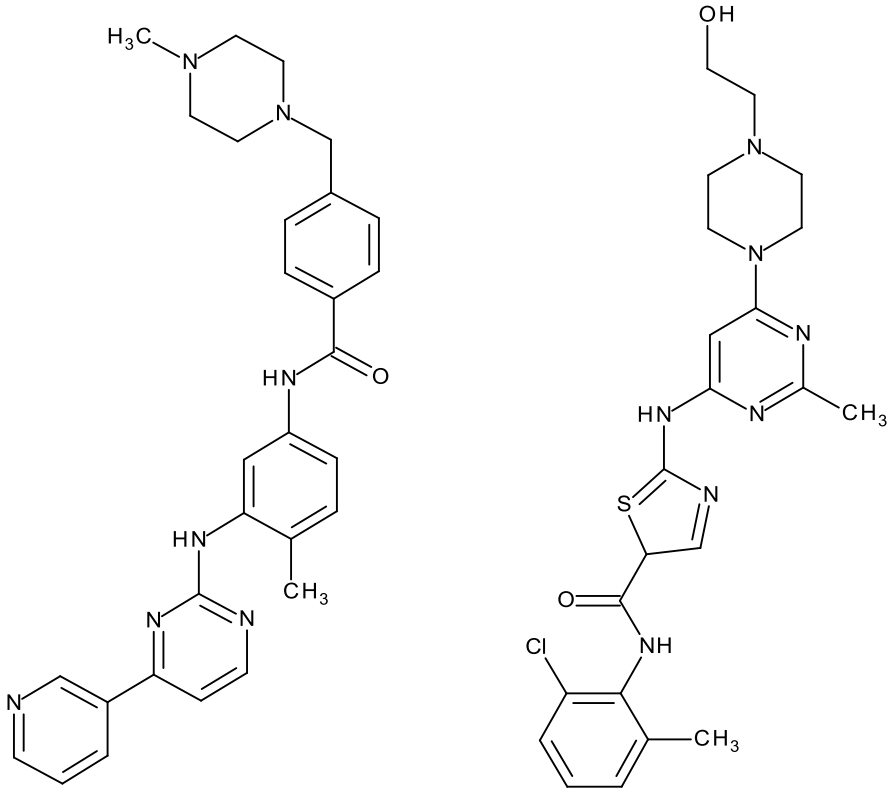


There is a study presenting 3-phenyl-2,4(1*H*,3*H*)-quinazolinediones had moderate to excellent antibacterial activity against *B. subtillis*, *S. Aureus*, *E. coli* and *P. aeruginosa* [16].



The increasing role of piperazine moiety in drug design and discovery was reviewed by Patel and Park recently [17]. Piperazine ring has attracted much attention because of being embedded in variety of biologically active molecules. Many piperazine containing compounds such as imatinib**,** dasatinib (anticancer), fluphenazine (antipsychotic), sildenafil (treatment of erectile dysfunction) and setirizine

(antihistaminic) were successfully marketed [18]. Imatinib (Gleevec®) and dasatinib (Sprycel®) (**Figure 1.2.**) are Bcr-Abl TK enzyme inhibitors used in the treatment of multiple type of cancers. The presence of piperazine ring provides enhanced physical properties such as lipophilicity and solubility. In the crystal structure of imatinib with the enzyme it was also shown that piperazine ring made a key connection with the backbone of the inactive form of the kinase enzyme [19]. Some studies bringing out the cytotoxicity of piperazine bearing compounds have been also reported recently [20–24].



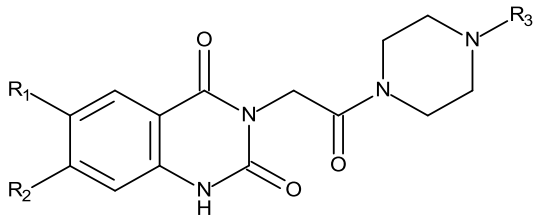
Imatinib Dasatinib

Figure 1.2. Structure of some piperazine bearing anticancer drugs.

In the light of these observations, novel hybrides of quinazoline-2,4(1*H*,3*H*)-dione and piperazine moieties which are linked through an amide group were designed with the aim of getting biologically active molecules. We synthesized twenty eight 3-{2-[4-(substituted)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1*H*,3*H*)-dione derivatives

(**Table 1.1.)** which of twenty six are novel and screened their antimicrobial and cytotoxic activities by using disc diffusion and Sulforhodamine B test, respectively.

Table 1.1. Formula of the synthesized target compounds **7-34.**



Compound 7-34

Compound	R_1	R_2	R_3
7	-H	-H	4-chlorobenzyl
8	-H	-H	benzo[d][1,3]dioxol-5-yl
9	-H	-H	2-furoyl
10	-H	-H	cyclohexyl
11	-H	-H	2-cyanophenyl
12	-H	-H	diphenylmethyl
13	-H	-H	benzoyl
14	-H	-H	pyridine-4-yl
15	-Cl	-H	4-chlorobenzyl
16	-Cl	-H	3-(trifluoromethyl)phenyl
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22	-Cl	-H	4-methoxyphenyl
23	-Cl	-H	benzoyl
24	-Cl	-H	pyridine-4-yl
25	$-OCH_3$	$-OCH_3$	4-chlorobenzyl
26	$-OCH_3$	$-OCH_3$	3-(trifluoromethyl)phenyl
27	$-OCH_3$	$-OCH_3$	benzo[d][1,3]dioxol-5-yl
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29	$-OCH_3$	$-OCH_3$	cyclohexyl
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31	$-OCH_3$	$-OCH_3$	diphenylmethyl
32	$-OCH_3$	$-OCH_3$	4-methoxyphenyl
33	$-OCH_3$	$-OCH_3$	benzoyl
34	$-OCH_3$	$-OCH_3$	pyridine-4-yl

2. GENERAL DESCRIPTIONS

2.1. Quinazolines

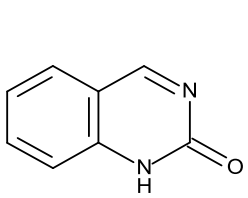
Quinazoline is a heterocyclic compound with the two fused aromatic rings benzene and pyrimidine. Although 2-cyano-4(3*H*)-quinazolinone was the first quinazolinone derivative to be synthesized by Griess in 1869, quinazoline ring was prepared by Gabriel in 1903 [25].

The name of quinazoline which is universally accepted for this compound was proposed by Weddige. The currently used numbering of quinazoline ring system was suggested by Paal and Bush. The other less frequently used names for this ring system are 5,6-benzopyrimidine and phenmiazine. Keto-quinazolines also called as quinazolinones, are the most significant derivatives of this structure. Depending upon the position of the oxo group, these compounds may be categorized into three types [25];

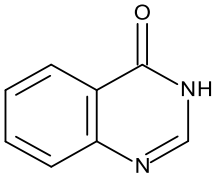
a) 2(1*H*)-Quinazolinones (1,2-dihydro-2-oxoquinazolines),

b) 4(3*H*)-Quinazolinones (3,4-dihydro-4-oxoquinazolines),

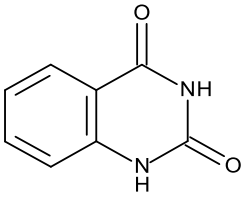
c) 2,4(1*H*,3*H*)-Quinazolinediones (1,2,3,4-tetrahydro-2,4-dioxoquinazoline or benzoylene urea).



2(1*H*)-Quinazolinone



4(3*H*)-Quinazolinone



2,4(1*H*,3*H*)-Quinazolinedione

These keto derivatives show lactam-lactim tautomerism. There are three possible tautomers for 2-oxo and 4-oxoquinazolines, and six possible tautomers for 2,4-dioxoquinazoline as shown in **Figure 2.1.**

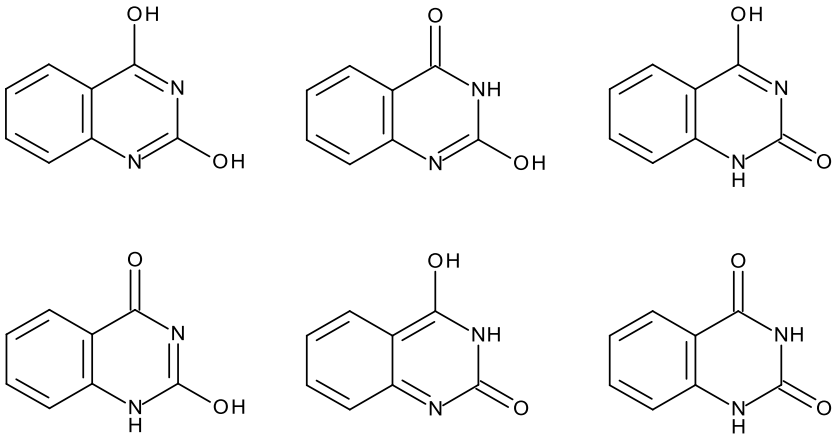
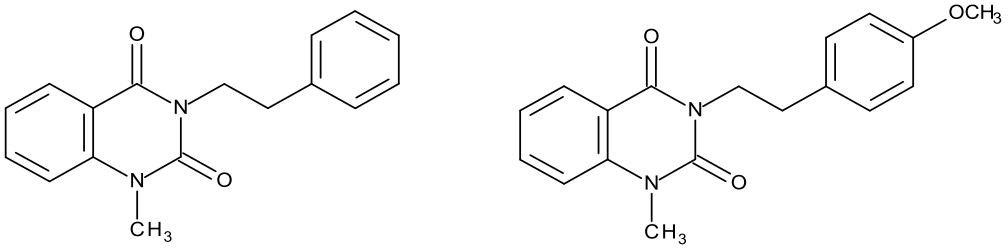


Figure 2.1. Six possible tautomers for 2,4-dioxoquinazoline.

The quinazoline skeleton exists in many alkaloids. Two 2,4(1*H*,3*H*)-quinazolinedione alkaloids which were assigned as 1-methyl-3-(2-phenylethyl)-quinazoline-2,4(1*H*,3*H*)-dione and 1-methyl-3-[2-(4-methoxyphenyl)ethyl]-quinazoline-2,4(1*H*,3*H*)-dione were isolated from seeds of *Zanthoxylum arborescens* [26].



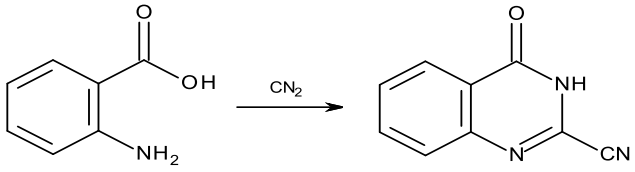
Quinazolines and quinazolinones are of considerable interest because of their various range of biological properties, for example, antimicrobial [27–40], anticancer [13, 41–64], anticonvulsant [65]–[70], anti-inflammatory [72–76], antihypertensive [77–81], antiobesity [81], antiplatelet [82], antipsychotic [83], aldose reductase enzyme inhibition [84], diuretic [85], antidiabetic [86], antioxidant [87] activities and antiproliferative activity against benign prostatic hyperplasia (BPH) [78–81].

2,4-Dioxoquinazolinediones also have various biological activities, such as, antimicrobial [16, 89–104], anticancer [15, 105–117], antihypertensive [8, 106–114], anticonvulsant [127–135], anti-inflammatory [136–139], 5-hydroxytryptamine 3 (5-

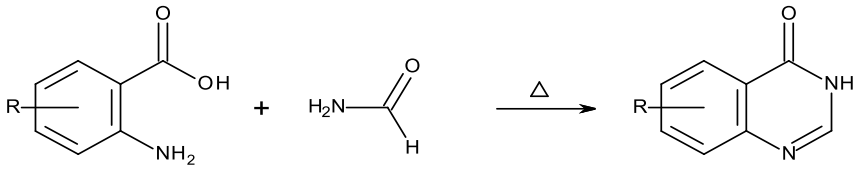
HT_3) receptor antagonist [139], phosphodiesterase (PDE) 4 inhibition [141, 142], PDE7 inhibition [142], calcium-independent phosphodiesterase enzyme inhibition (CaIPDE) [143], cyclin-dependent kinase 5 (CDK5) inhibition [144], Poly(ADP-ribose) polymerase (PARP) inhibition [146, 147], antipsychotic [147], 5-HT_{3A} receptor antagonist [148], glucokinase activation [149], antioxidant [150], antiplatelet [151] and nonsteroidal progesterone receptor antagonist activities [152], and they can be used for the prevention of graft rejection [153], treatment of systemic lupus erythematosus, psoriasis [139, 155] and diabetic neuropathy diseases [156, 157].

2.1.1. Synthesis of Quinazolines and 4(3*H*)-Quinazolineones

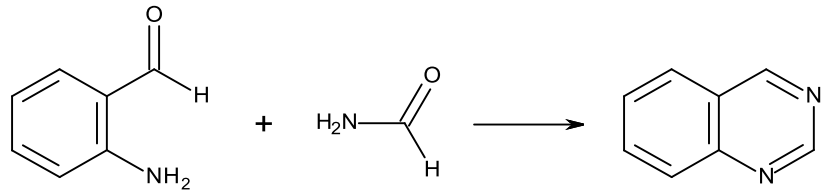
The first quinazoline derivative, 2-cyano-3,4-dihydroquinazoline-4-one, was synthesized with the reaction of cyanogen with anthranilic acid by Griess in 1869 [25].



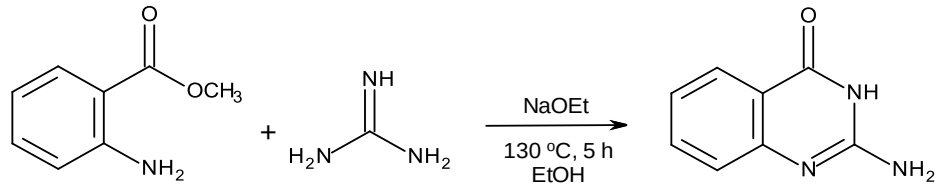
The synthetic method of the 4(3*H*)-quinazolinone ring was published by Stefan Niementowski in 1895 and named as “Niementowski quinazolinone synthesis” which includes the fusion of anthranilic acid or 2-aminobenzonitrile with formamide at hard conditions (i.e., 130-150 °C, 6 hours) [157]. Alexandre *et al.* reported a modern alternative to conventional procedure of Niementowski synthesis by using microwave irradiation under milder conditions (150 °C, 20 minutes) [158].



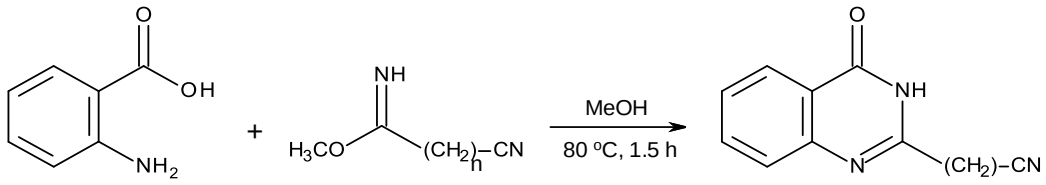
Gabriel devised a more detailed synthesis of quinazoline and its derivatives in 1903. 2-Aminobenzaldehyde was treated with formamide to form quinazoline ring [159].



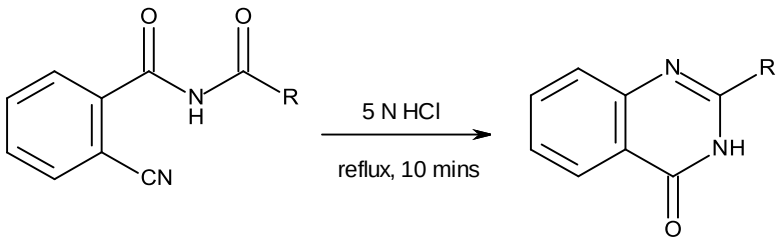
Hess treated methyl anthranilate with excess guanidine in the presence of sodium ethoxide in ethanol to yield 2-aminoquinazolin-4(3*H*)-one [160].



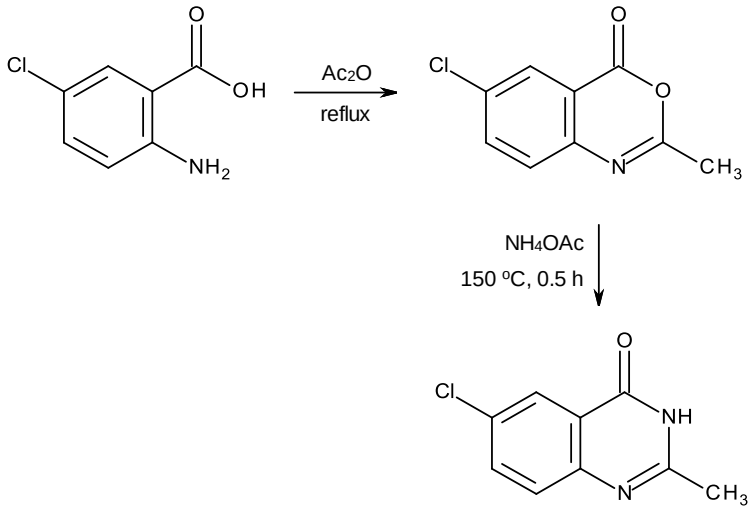
Hennequin *et al.* reported the synthesis of 2-substituted-quinazoline-4(3*H*)-one by the condensation of imidates and anthranilic acid in methanol at 80 °C [161].



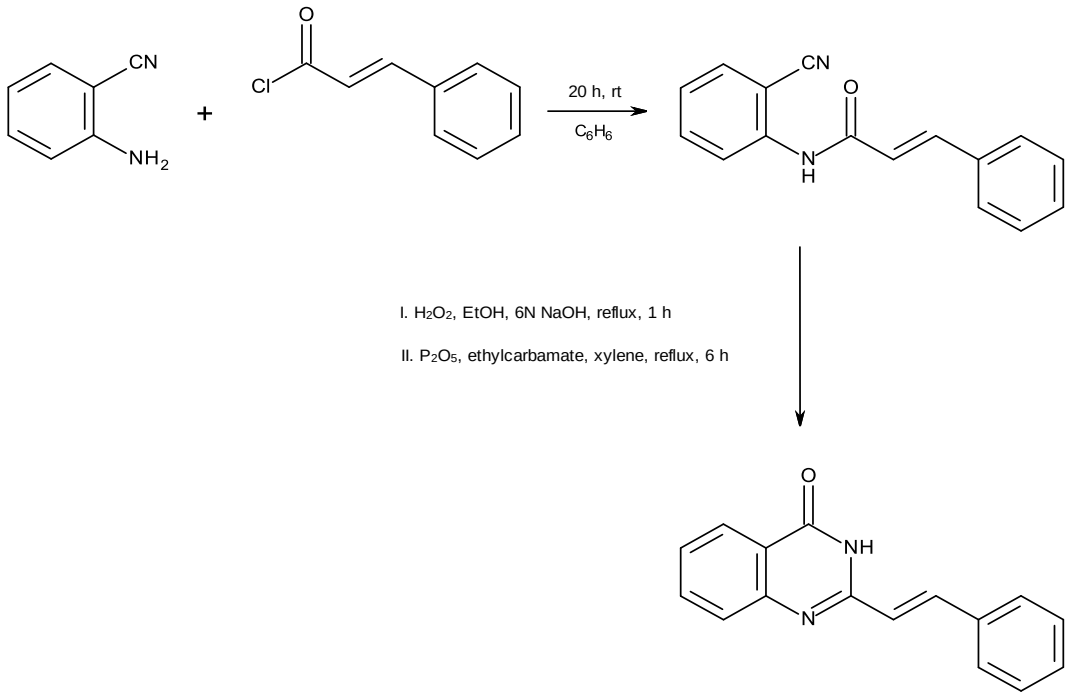
2-Substitutedquinazolin-4(3*H*)-one derivatives were synthesized under acidic conditions by using the starting material of imido containing benzonitrile [162].



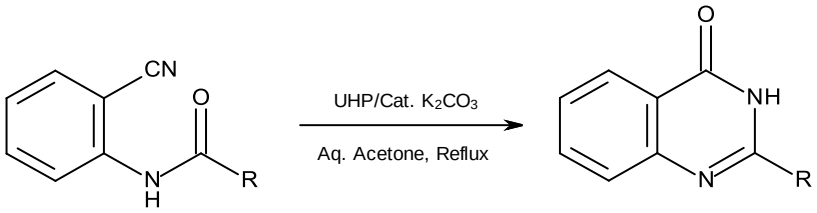
Jiang *et al.* treated 5-chloroanthranilic acid with acetic anhydride to afford the benzoxazinone. Then, benzoxazinone was reacted with ammonium acetate at an elevated temperature to yield 6-chloro-2-methylquinazolin-4(3*H*)-one [163].



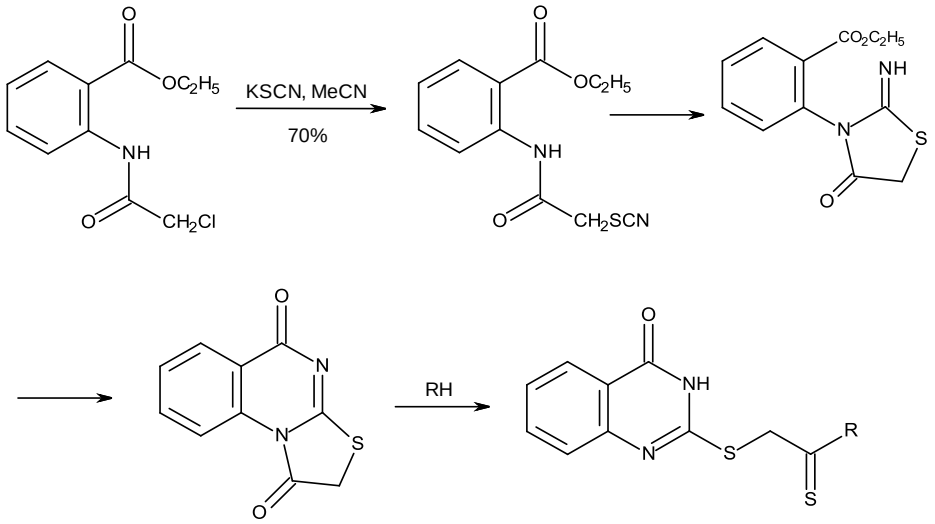
The most frequently used method includes amidation of 2-aminobenzonitrile with 3-phenylacryloyl chloride followed by oxidative ring closure under basic conditions. The cinnamide compound also refluxed with ethyl carbamate in the presence of P_2O_5 for 6 hours to give 2-styryl-4(3*H*)-quinazolinone [163, 164].



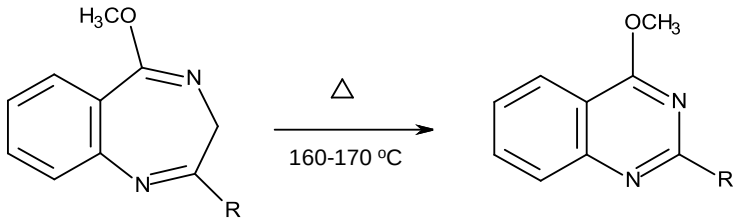
Bandgar and Bavetsias developed a method starting from 1-cyanobenzamide by using urea-hydrogen peroxide (UHP) and potassium carbonate to yield 2-substituted quinazolinones in high yields ranging from 86 to 98% [165, 166].



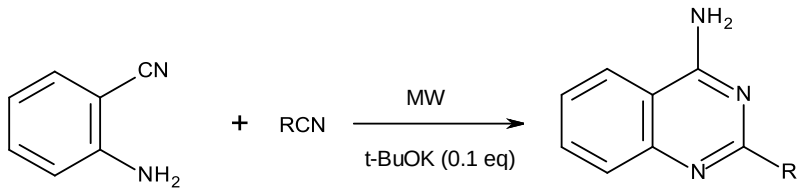
Gruner *et al.* obtained 2-alkylthioquinazolines via heating *N*-chloroacetyl-anthranilic acid ethyl ester with potassium thiocyanate in acetonitrile [166].



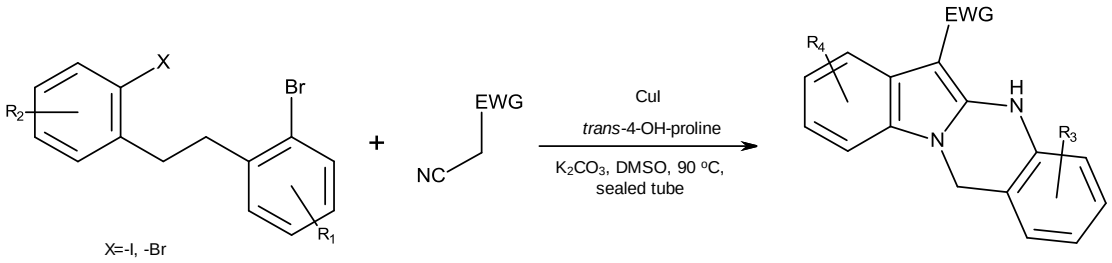
Kaname *et al.* presented a thermolysis reaction of 5-methoxy-(3*H*)-1,4-benzodiazepines in diphenyl ether at 160-170 °C to yield 4-methoxyquinazolines by a ring contraction mechanism [167].



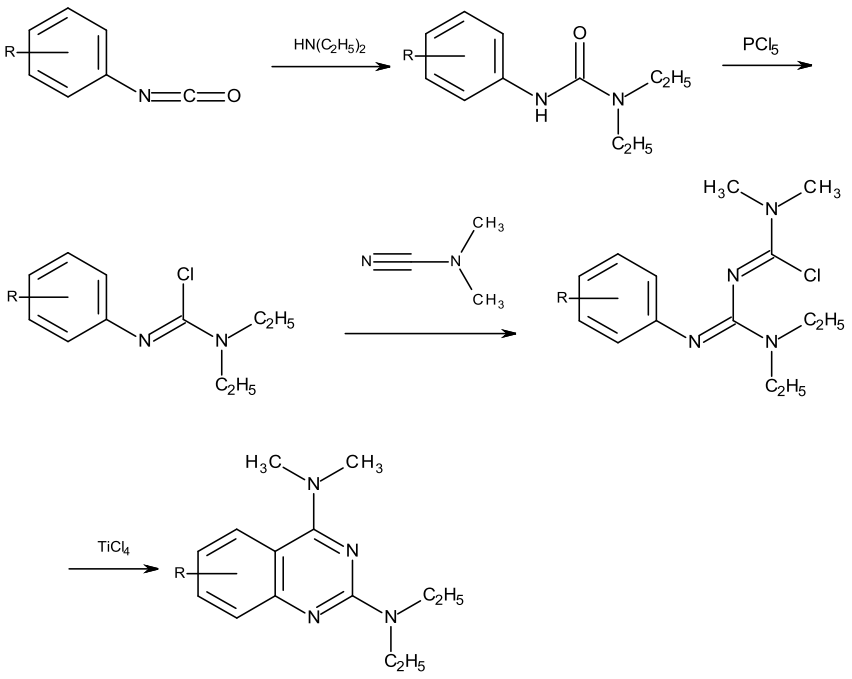
Mizuno *et al.* obtained 4-aminoquinazolines by the reaction of cyano compounds with 2-aminobenzonitrile in the presence of catalytic amount of potassium *tert*-butoxide under microwave irradiation for a few minutes [168].



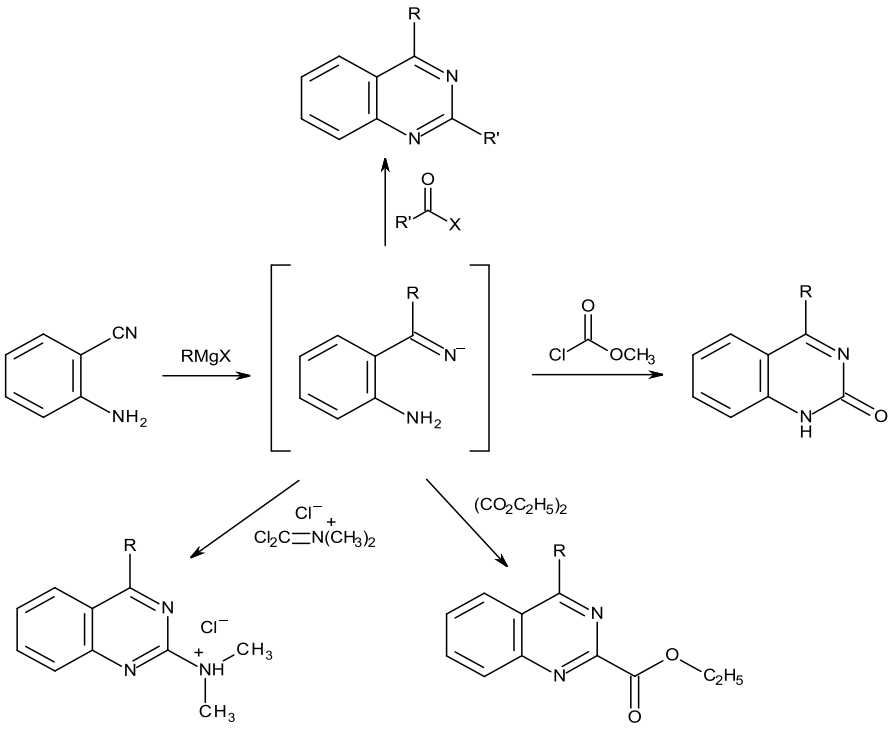
Classical copper-catalyzed Ullmann reaction was utilized for the convenient synthesis of quinazoline derivatives [170, 171]. One-pot synthesis of 5,12-dihydroindolo[2,1-*b*]quinazoline derivatives were synthesized by using copper-catalyzed Ullmann-type intermolecular C-C and intramolecular C-N coupling reaction as seen below [170].



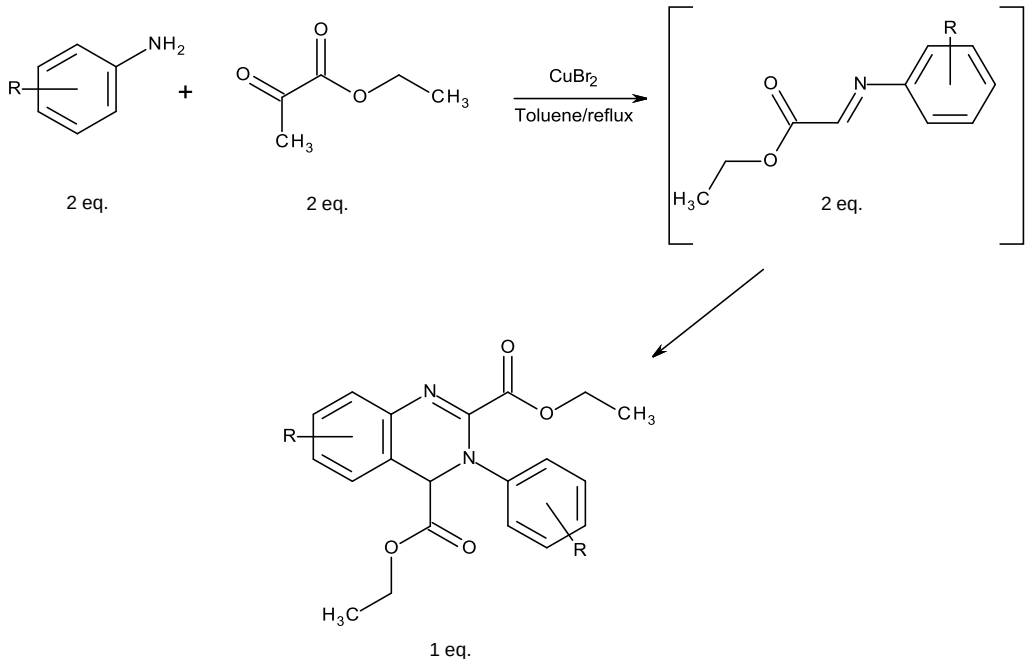
Zielinski *et al.* reported a method to enable the synthesis of 2,4-diaminoquinazolines by reacting chloroamidines with dialkylcyanamides. Phenyl isocyanate were reacted with *N*,*N*-diethylamine to afford urea compounds. Phosphorus pentachloride was utilized for the subsequent chlorination. The reaction of chloroamidine with *N*,*N*-dimethylcyanamide yielded an intermediate which gives 2-(*N*,*N*-diethylamino)-4-(*N*,*N*-dimethylamino)quinazoline with $TiCl_4$ [171].



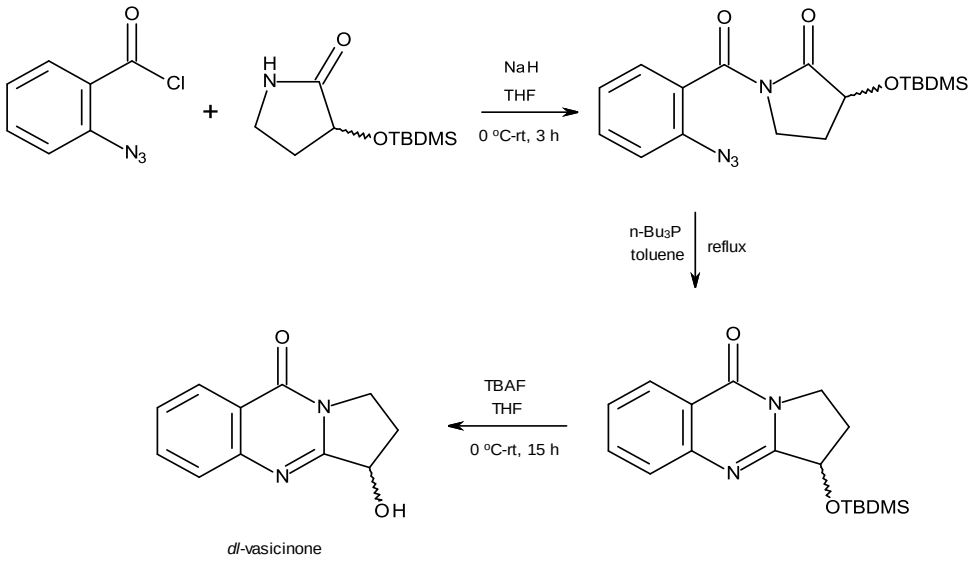
According to the Wiklund and Bergman *et al.*, reaction of 2-aminobenzonitrile with Grignard reagents resulted in an intermediate, that could form various quinazolines by reacting different reagents, as shown in below [172].



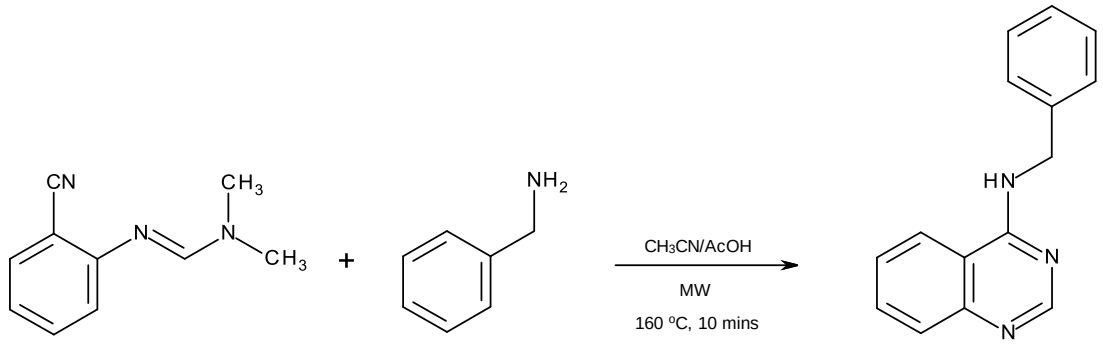
Aniline and ethyl glyoxalate were refluxed with catalyst $CuBr_2$ in toluene for 24 hours to give α-iminoester that forms quinazoline derivative in high yields [173].



The racemic vasicinone, an alkaloid having pyrrolo[2,1-b]-quinazolinone structure, was obtained from the reaction of γ-lactam derivative with 2-azidobenzoyl chloride [174].



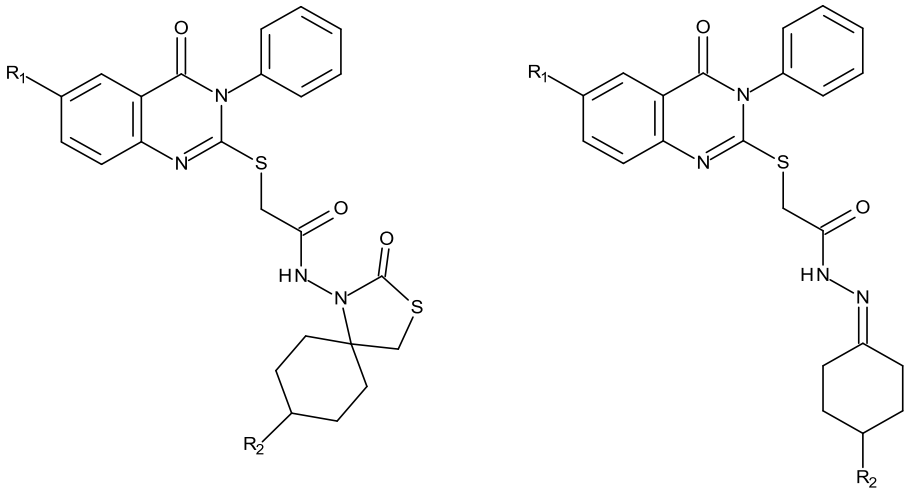
A simple and efficient synthesis of quinazolin-4-ylamine was reported by Yoon *et al.* [175]. The reaction of *N*,*N*-dimethylamidinobenzamide and benzylamine under microwave irradiation conditions gave the target compound in good yield (69-97%).



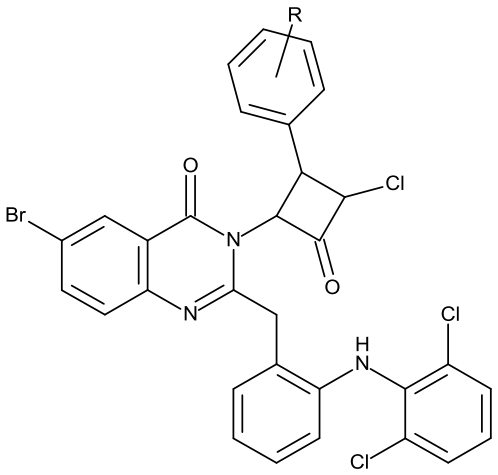
2.1.2. Biological Properties of Quinazoline Derivatives

2.1.2.1. Antimicrobial Activity

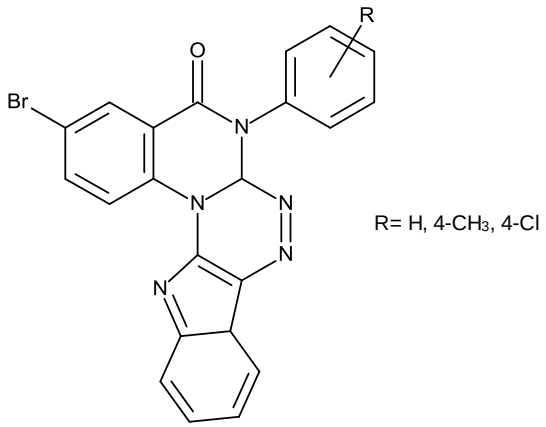
Karalı *et al.* reported quinazoline-4(3*H*)-one derivatives displaying antifungal activity against *Microsporum gypseum* (NCPF-580), *M. canis*, *Trichophyton mentagrophytes var. erinacei* (NCPF-375) and *T. rubrum* [27].



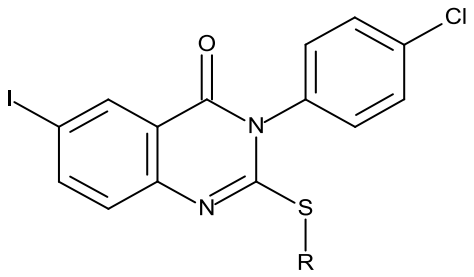
It was reported that some 2-azetidinone derivatives exhibit antibacterial activity against *S. aureus*, *B. subtilis* and *Escherichia coli* and antifungal activity against *Candida albicans* [28].



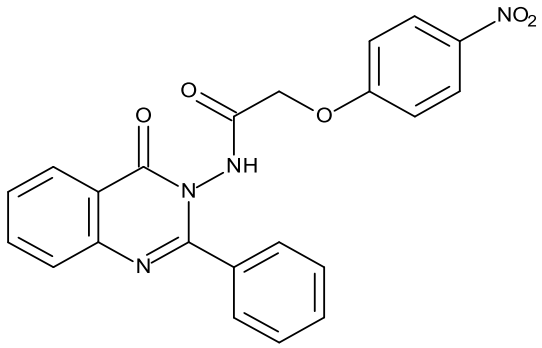
Rajput and Pandey *et al.* presented some indolo[2,3-*c*][1,2,4]-triazino[4,3-*a*]-quinazolin-8-one derivatives showing antifungal activity against *C. albicans*, *Aspergillus fumigatus*, *A. flavus* and *A. niger* [29, 30].



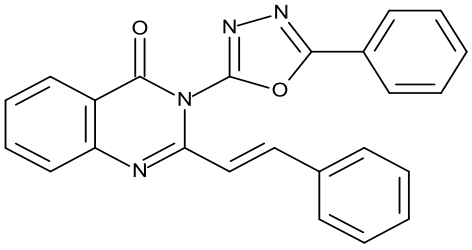
Some of the 2,3-disubstituted-6-iodo-4(3*H*)-quinazolinone derivatives exhibited remarkable antimicrobial activity [31].



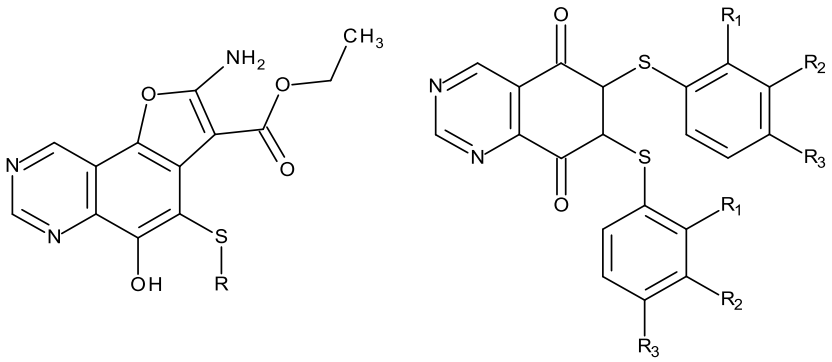
Kohli *et al.* reported a 4(3*H*)-quinazolinone derivative showing antibacterial activity against *S. aureus* and *E. coli* [32].



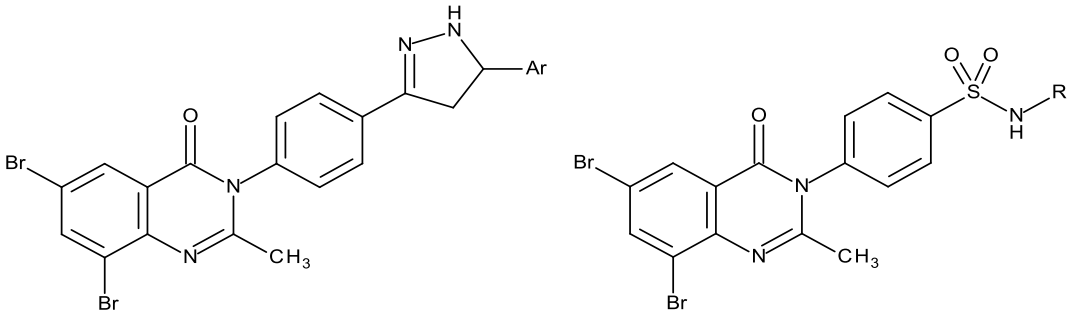
An unsubstituted styryl derivative showed significant activity against bacterial strains of *B. subtilis*, *S. aureus*, *E. coli* and *Proteus vulgaris* [33].



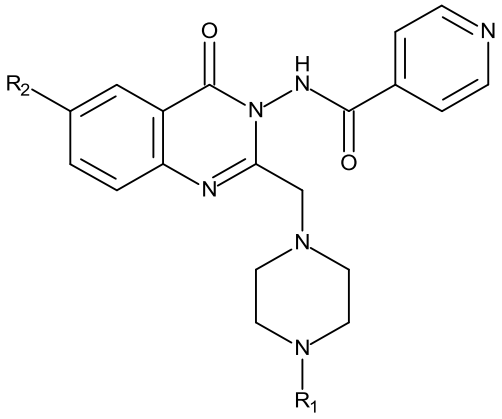
Ryu *et al.* reported some furo[2,3-*f*]quinazolin-5-ol and 6,7-bis(arylthio)-quinazolin-5,8-dione derivatives with good antifungal activity against *C. albicans*, *Aspergillus species* and *Cryptococcus neoformans* [34].



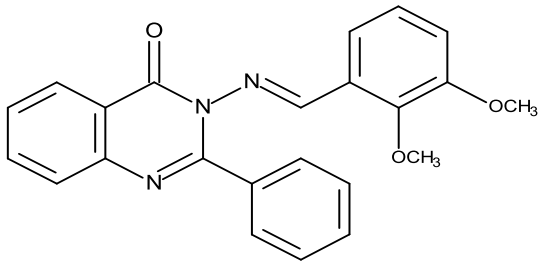
3-(4-Substitutedphenyl)-4(3*H*)-quinazolinone derivatives showed mild to good activities against bacterias; *S. Aureus*, *B. cereus*, *Serratia marcescens, Proteus mirabilis* and fungis; *Aspergillus chraceus Wihelm* and *Penicillium Chrysogenum Thom* in disc diffusion test [35].



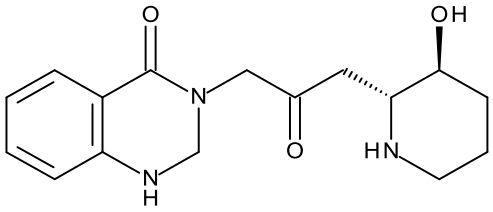
Piperazinylmethyl-4(3*H*)-quinazolinone derivatives were screened for their antibacterial and antifungal activity by using broth dilution method against bacteria strains; *E. coli*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *S. aureus*, *S. pyogenus*, *B. subtilis* and fungis; *C. albicans*, *A. niger*, *A. clavatus*. Compounds displayed comparable activity against all standard antibiotics such as gentamycin, ampicillin, chloramphenicol [36].



Krishnan *et al.* reported some hydrazone derivatives inhibiting viral replication of parainfluenza virus, reovirus-1, Sindbis virus, Coxsackie B4 virus, Punta Toro virus in cell cultures [37].



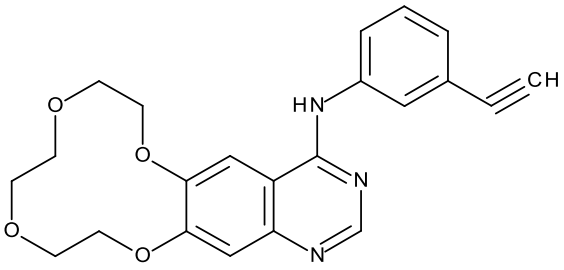
Febrifugine, 3-{3-[(2*S*,3*R*)-3-hydroxypiperidin-2-yl]-2-oxopropyl}quinazolin-4(*3H*)-one, is a quinazolinone alkaloid isolated from Chinese herb *Dichroa febrifuga* and garden plant *Hydrangea*, is marketed as an antimalarial agent [38].



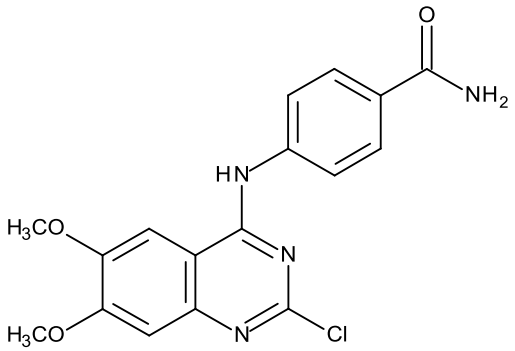
Febrifugine

2.1.2.2. Anticancer Activity

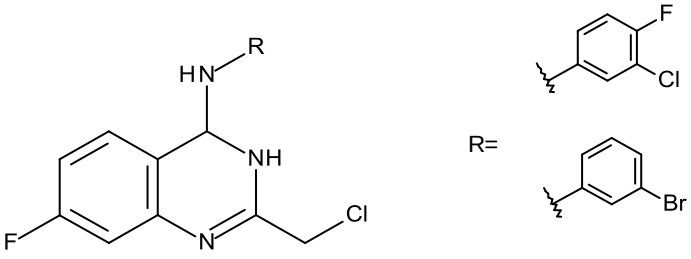
Hu *et al.* reported some epidermal growth factor receptor (EGFR) kinase inhibitors bearing crown ether on its structure with IC_{50} values of 2-150 nM. 2-Ethynlphenyl derivative also showed good pharmacokinetic properties [41].



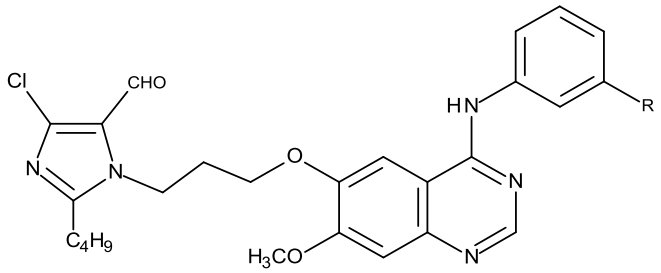
Significant EGFR and vascular endothelial growth factor receptor-2 (VEGFR-2) inhibitiory activities were obtained for the compound of 4-((2-chloro-6,7-dimethoxyquinazolin-4-yl)amino)benzamide hydrochloride with IC_{50} values of 0.90 and 1.17 μM, respectively [42].



Li *et al.* reported some 4-anilinoquinazoline derivatives exhibiting cytotoxicity with IC_{50} values of 3.2-20 μM in three different types of cancer cell lines including human hepatoma cell line (HepG2), breast cancer cell line (MDA-MB-468), and colorectal cancer cell line (HCT-116) [46].

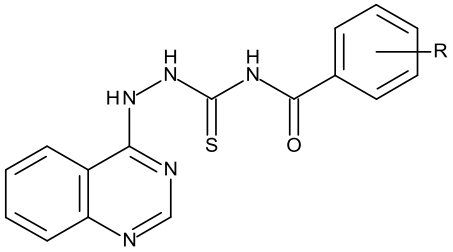


Chandregowda *et al.* synthesized novel 4-anilinoquinazolines and evaluated their cytotoxicity on EGFR over-expressing skin epidermoid carcinoma cell line (A431). 3-Trifluoromethylphenyl and 3-iodophenyl derivatives showed IC_{50} values of 3.5 and 3 mM, respectively [49].

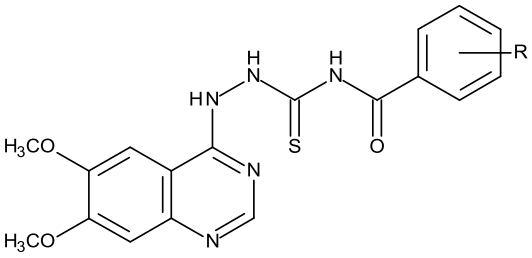


R=CF_3, I

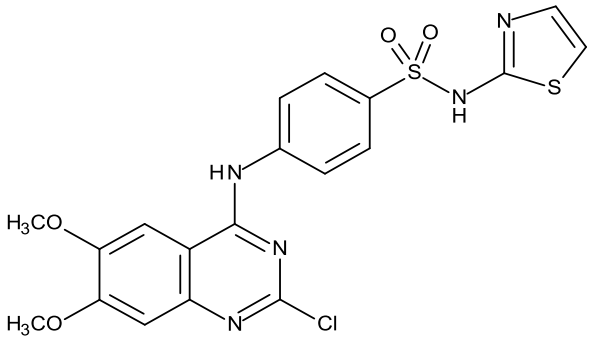
Some 4-substituted-thiosemicarbazidequinazoline derivatives showed better anticancer activity against five human cancer cell lines than standard drug 5-fluorouracil [50].



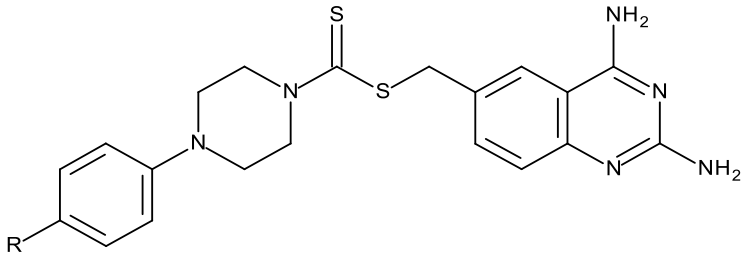
R= -H, 3-Cl, 4-Cl, 2-F



Abouzid *et al.* presented *N*-thiazol-2-ylsulphonamide derivative exhibiting antitumor activity on human breast carcinoma cell line (MCF-7) with IC_{50} value of 0.13 nmol [51].

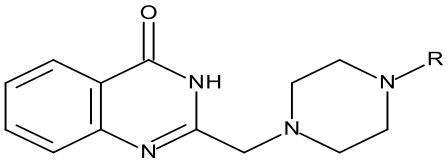


4-Methoxyphenyl, 4-fluorophenyl and 4-nitrophenylpiperazine-1-carbodithioate derivatives of 2,4-diaminoquinazoline showed high activity against five human cancer cell lines including lung (A549), breast (MCF-7), cervical (HeLa), colorectal cancer cell line (HT29 and HCT-116) with IC_{50} values in the range of 1.58-2.27, 1.84-3.27 and 1.47-4.68 μM, respectively [53].



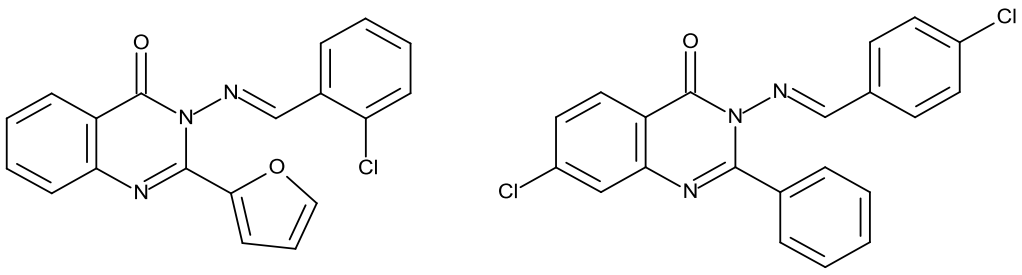
R= -OCH_3, -F, -NO_2

Manikanta *et al.* reported some 4-substitutedpiperazine derivatives exhibiting potent anticancer activity with MTT cytotoxicity assay [43].

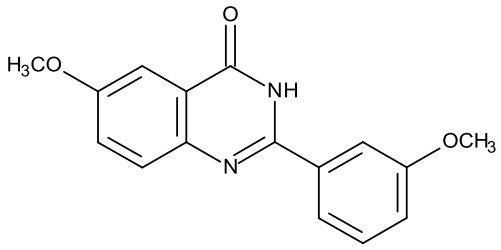


Some hydrazone derivatives of 4-quinazolinone were evaluated for their anticancer activities. 2-Furyl derivative was reported to have anticancer activity against ovarian (OVCAR-4) and non-small cell lung cancer cell line (NCI-H522) with GI_{50} value of 1.82 and 2.14 μM, respectively [44]. In another study, 2-phenyl derivative of 4-

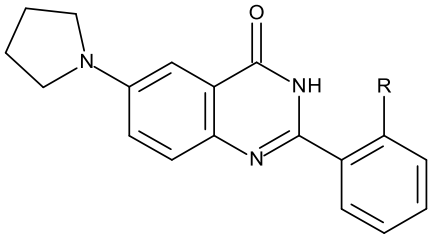
quinazolinone was presented cytotoxicity with IC_{50} value of 2.57 μM against central nervous system (CNS) cancer cell line (SNB-75) [48].



Xia *et al.* reported 2-phenyl derivative of 4-quinazolinone showing significant growth inhibition over human epidermoid carcinoma of the nasopharynx [45].

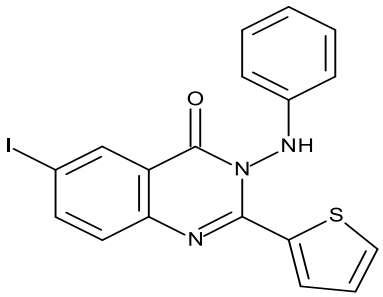
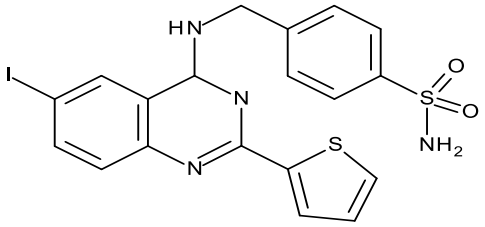


2-Phenyl-6-pyrrolidinyl-4(3*H*)-quinazolinone derivatives exhibited cytotoxicity with the IC_{50} values of 0.30-10.10 μM against human monocytic leukemia cells (U937), mouse monocytic leukemia cell line (WEHI-3), human hepatoma cell line (HepG2, Hep3B) and human lung carcinoma cell line (A549, CH27) [47].

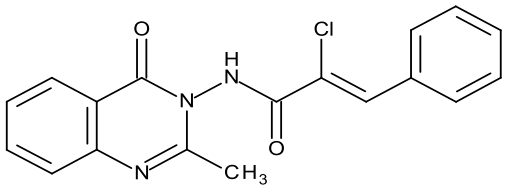
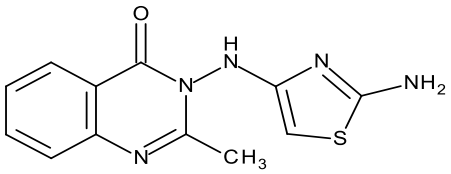


R= -F, -Br, -OH, $-OCF_3$, $-OC_2H_5$, -Cl, $-N(CH_3)_2$

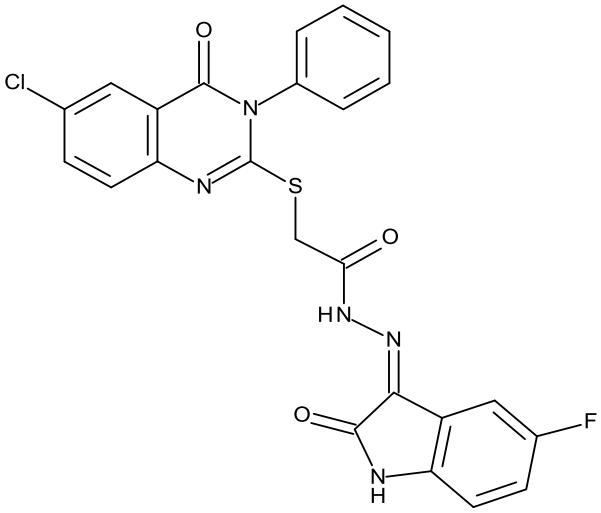
Al-Obaid *et al.* evaluated cytotoxic activities of 2-thienoquinazoline derivatives by using breast (MCF-7), lung (NCI-H460), and CNS (SF-268) cancer cell lines. Two derivatives that are given below showed high activity with the IC_{50} values of 10.3 and 16.9 μM, respectively [52].

Benzylidene derivative of 4(3*H*)-quinazolinone showed remarkable activity against cancer cell lines including breast (MCF-7), non-small cell lung (NCI-H460), and CNS (SF-268) cancer cell lines. 2-Aminothiazole derivative presented IC_{50} value of 0.01 nM against breast adenocarcinoma (MCF-7) [54].

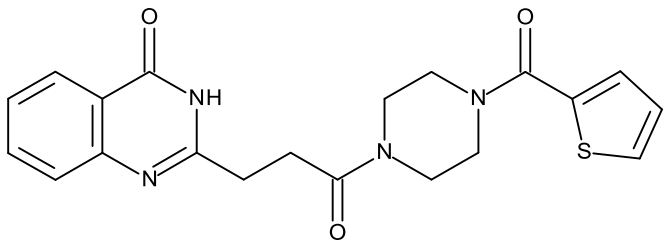



Gürsoy and Karalı presented 3-[[(6-chloro-3-phenyl-4(3*H*)-quinazolinone-2-yl)mercaptoacetyl]hydrazono]-5-fluoro-1*H*-2-indolinone derivative showing high cytotoxicity against renal cancer cell line (UO-31) (IC_{50}= 0.21 μM) [55].



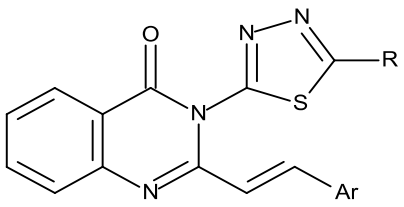
PARP-1 enzyme is extremely expressed in a variety of cancers, such as breast, hepatocellular carcinoma and non-small cell lung cancer. Some acylpiperazinylamides

of 3-(4-oxo-3,4-dihydro-quinazolin-2-yl)-propionic acid were presented as PARP-1 inhibitors *in vitro*; moreover thiophene derivative showed inhibitory activity over the tumor volume of breast carcinoma [14].

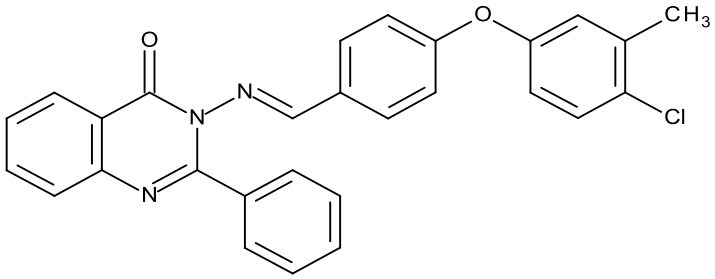


2.1.2.3. Anticonvulsant Activity

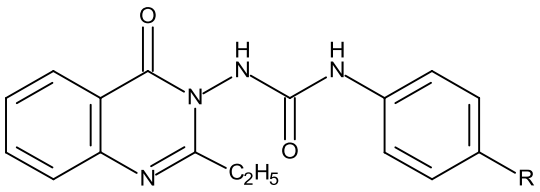
Jatav *et al.* reported some 1,3,4-thiadiazole derivatives of 4-quinazolinone with anticonvulsant, sedative-hypnotic and CNS depressant activities [65].



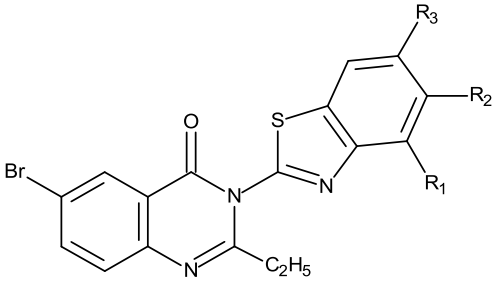
Kumar *et al.* reported phenyloxy derivative with high anticonvulsant activity in mice model [66].



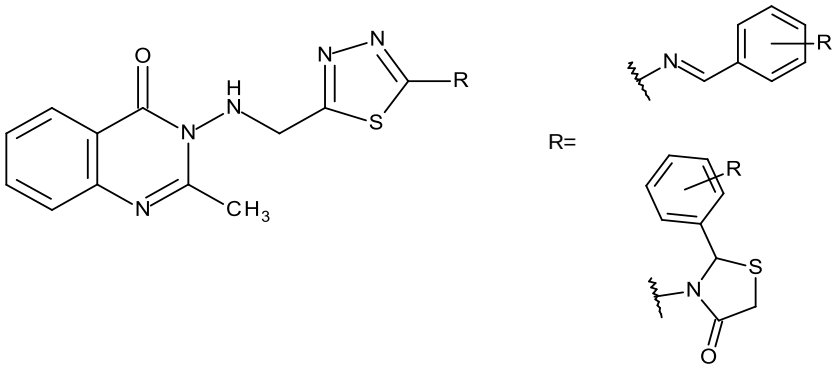
Some urea derivatives of 4-quinazolinone exhibited potent anticonvulsant activity in maximal electroshock (MES) induced seizures and subcutaneous pentylene tetrazole (PTZ) induced seizure models in mice [67].



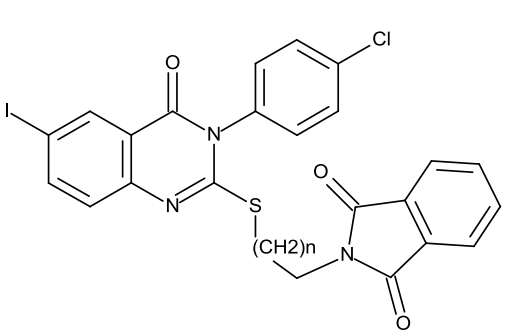
Ugale *et al.* presented benzo[*d*]thiazol derivative of 4-quinazolinone with high activity against tonic seizure induced by the MES [68].

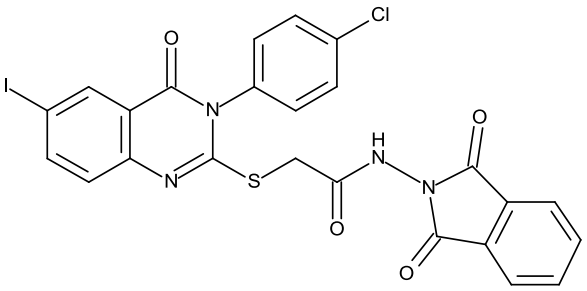


Some 1,3,4-thiadiazole derivatives of 4-quinazolinone were examined by using MES test and PTZ seizure test. Moderate to good anticonvulsant activities were obtained for thiazolidinone bearing derivatives [69].



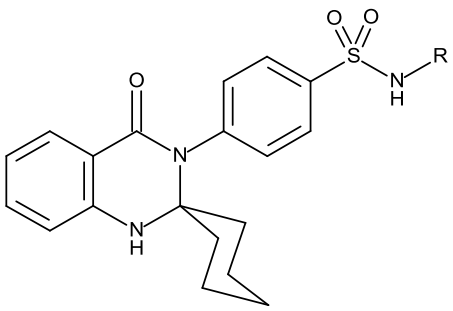
2-Mercapto-3-(4-chlorophenyl)-6-iodo-4(*3H*)-quinazolineone derivatives were examined for their anticonvulsant activity with PTZ seizure threshold test [70].





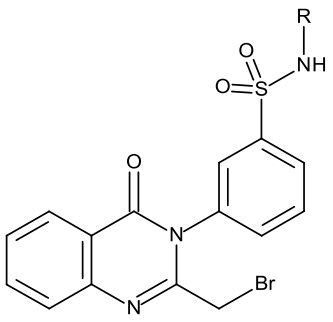
2.1.2.4. Anti-inflammatory Activity

Some spiro derivatives exhibited considerable anti-inflammatory and analgesic activity with gastrointestinal safety profile in experimental rats compared to indomethacin and tramadol [71].



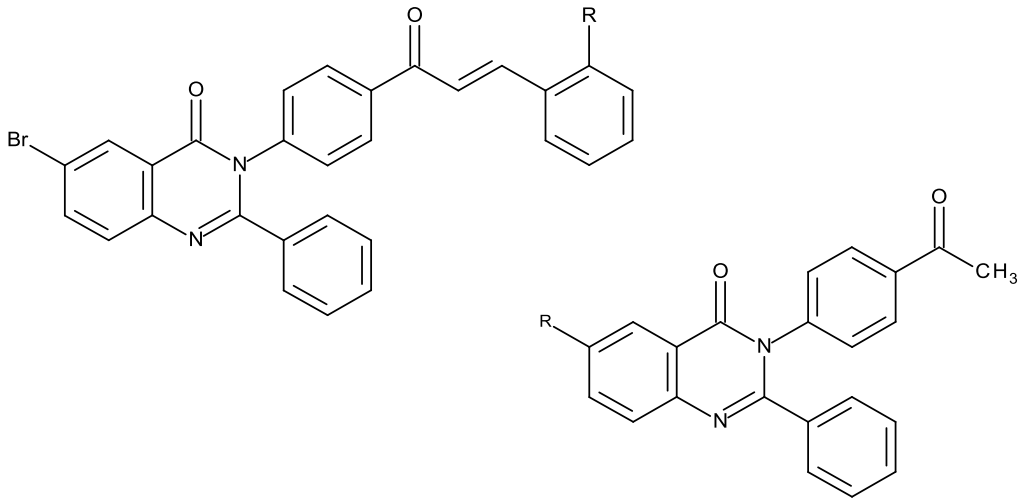
R= -H, -CH3

A series of sulphonamide derivatives showed potent anti-inflammatory and analgesic activity [72].



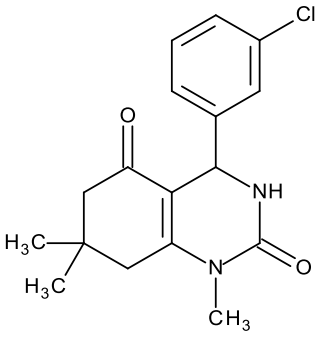
R= -H, -Br, -Cl

Mohamed *et al.* presented a series of 2-phenyl-4(3*H*)-quinazolinone derivatives with significant analgesic and anti-inflammatory activity in experimental rat models when compared to indomethacin [74, 75].

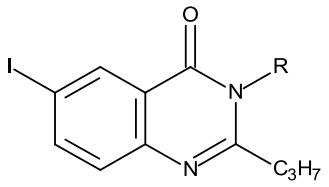


2.1.2.5. Miscellaneous

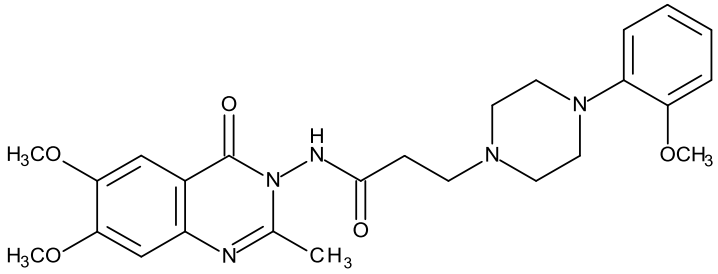
Yarım *et al.* synthesized some 4-aryl-7,7-dimethyl/1,7,7-trimethyl-1,2,3,4,5,6,7,8-octahydroquinazoline-2,5-dione and 4-aryl-7,7-dimethyl-5-oxo-1,2,3,4,5,6,7,8-octahydroquinazoline-2-thione derivatives. It was reported that 4-(3-chlorophenyl)-1,7,7-trimethyl-1,2,3,4,5,6,7,8-octahydroquinazoline-2,5-dione showed the calcium antagonist activity on isolated aortic strips of lamb as high as the reference drug nicardipine [176, 177].



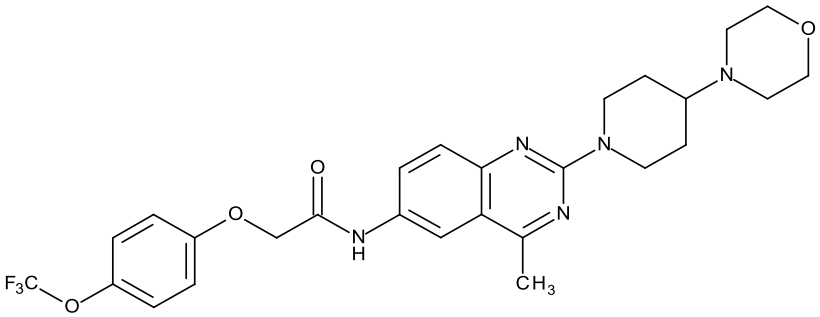
A series of 6-iodo-2-propyl-4(3*H*)-quinazolinone derivatives were reported to have antioxidant activity [87].



Abou-Seri *et al.* presented some 4(3*H*)-quinazolinone derivatives showing α_1-adrenoceptor antagonist activity with IC_{50} values of 0.2-0.4 mM less than prazosin (IC_{50}=0.487 mM). 2-Methoxyphenyl derivative exhibited the highest activity *in vitro* (IC_{50}=0.201 mM), also hypotensive activity *in vivo* (40.00 and 42.22% reduction of diastolic and systolic blood pressure) [76].

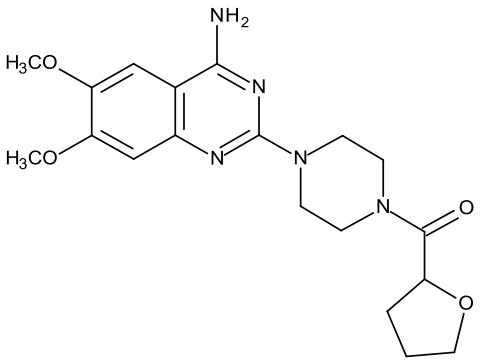


Sasmal *et al.* evaluated quinazoline derivatives in terms of melanin concentrating hormone (MCH) receptor antagonistic activity. 4-Morpholinyl derivative showed an obvious antiobesity effect in a diet induced obesity (DIO) mice model *in vivo* [81].

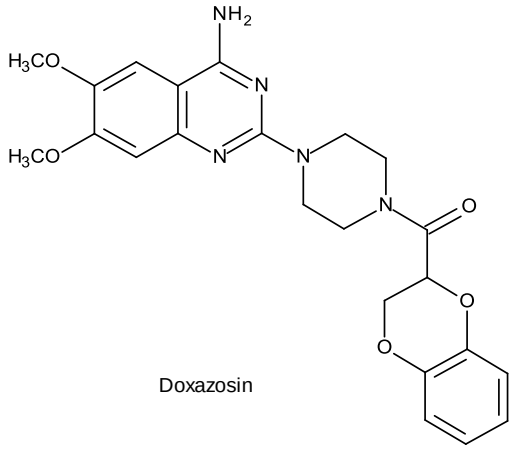


Tahmatzopoulos *et al.* reported that the doxazosin and terazosin (**Figure 2.2.**), quinazoline-based α_1-adrenoceptor antagonists, could generate apoptosis in benign and malignant prostate cells, also decrease tumor vascularity in prostate tumors and

suppress prostate tumorigenic growth *in vivo* through activation of transforming growth factor beta (TGF-β) signaling [56].



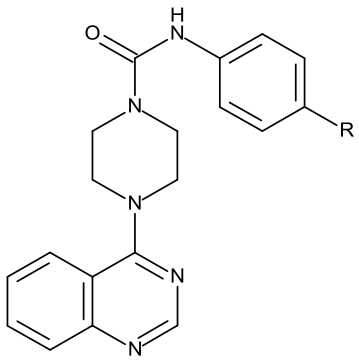
Terazosin



Doxazosin

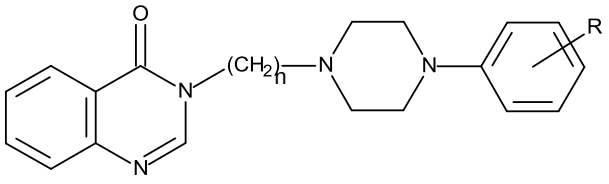
Figure 2.2. α_1-Adrenoceptor antagonists, terazosin and doxazosin.

Matsuno *et al.* screened 4-piperazinylquinazoline derivatives for platelet-derived growth factor receptor (PDGFR) phosphorylation inhibitory activity. 4-Chloro, 4-bromo and 4-isopropyloxy analogs were shown to have obvious inhibitory activity against neointima formation in the carotid artery of the balloon catheter deendothelialized vessel in the rats [82].



R= -Cl, -Br, isopropyloxy

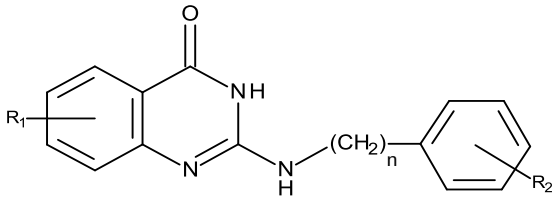
3-Arylpiperazine-4(3*H*)-quinazolinone derivatives were tested for dual 5-HT_{1A}/5-HT_{2A} receptor antagonist and psychotropic activity [83].



n=3-4

R= 2-OCH_3, 3-Cl,

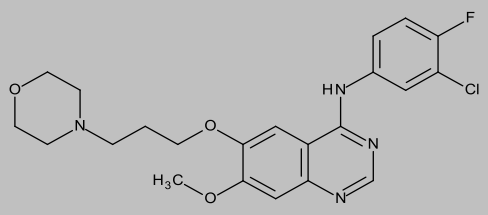
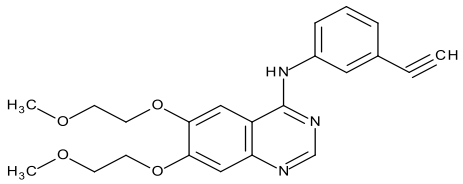
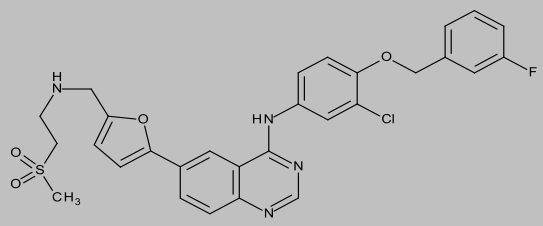
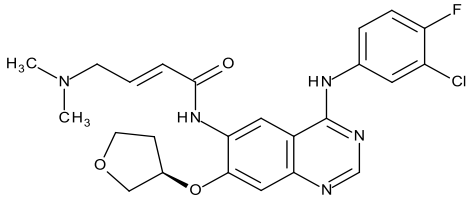
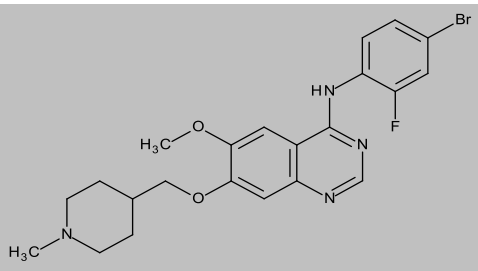
DeRuiter *et al.* reported a series of 2-(arylalkylamino)-4(3*H*)-quinazolinone derivatives showing moderate inhibition activity against aldose reductase enzyme with IC_{50} values in the range of 34-75 μM [84].

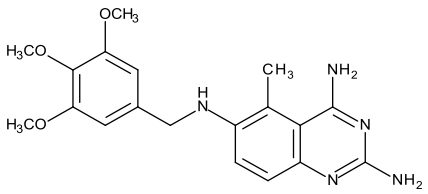
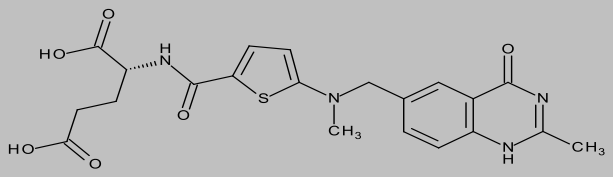
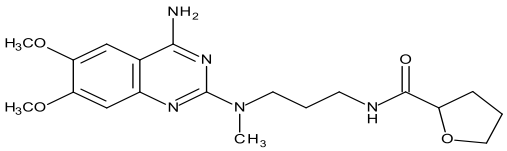
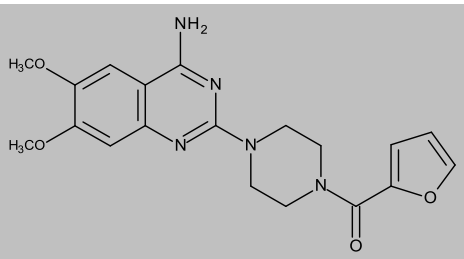
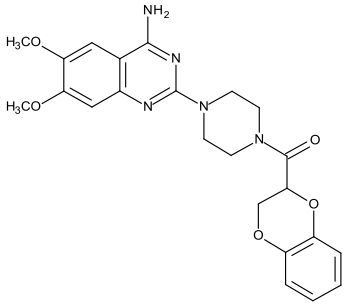


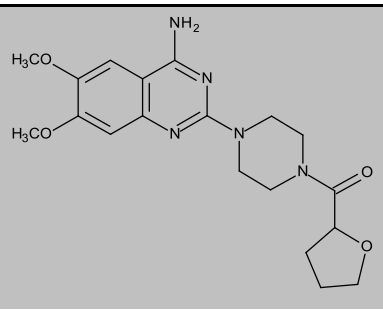
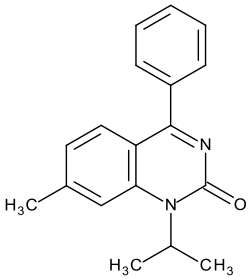
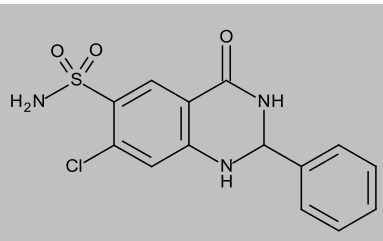
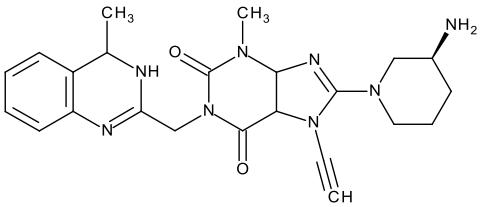
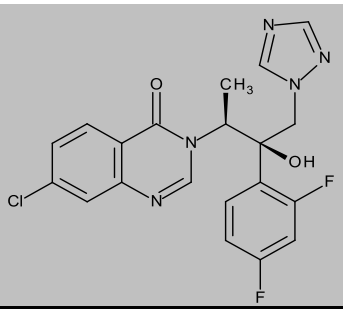
2.1.2.6. Quinazoline Marketed Drugs

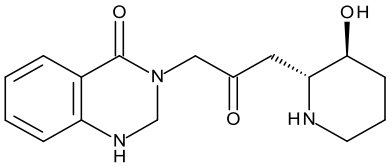
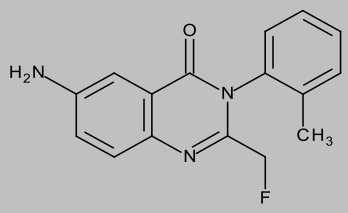
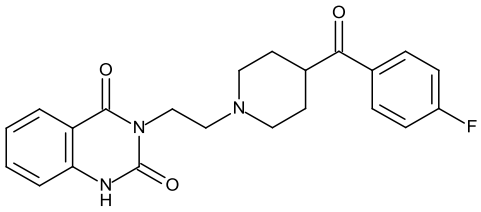
Some quinazoline-bearing compounds were marketed in different countries for their various usage as shown in **Table 2.1.**.

Table 2.1. Quinazoline-bearing marketed drugs.

Generic name	Chemical structure	Usage
Gefitinib		Anticancer [57]
Erlotinib		Anticancer [58]
Lapatinib		Anticancer [59]
Afatinib		Anticancer [60]
Vandetanib		Anticancer [61]

Trimetrexate		Anticancer and antiparasitic against pneumocystis pneumonia [62, 63]
Raltitrexed		Anticancer [64]
Alfuzosin		Treatment of benign prostatic hyperplasia (BPH) [77]
Prazosin		Treatment of benign prostatic hyperplasia and hypertension [78]
Doxazosin		Treatment of benign prostatic hyperplasia and hypertension [80, 81]

Terazosin		Treatment of benign prostatic hyperplasia and hypertension [80, 177]
Proquazone		Non-steroidal anti-inflammatory drug [75]
Fenquizone		Diuretic [85]
Linagliptin		Antidiabetic [86]
Albaconazole		Antifungal [39]

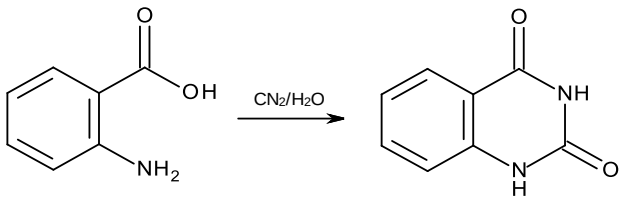
Febrifugine		Antimalarial [40]
Afloqualone		Sedative and muscle relaxant [179]
Ketanserin		Antihypertensive [180]

2.2. 2,4(1*H*,3*H*)-Quinazolinediones

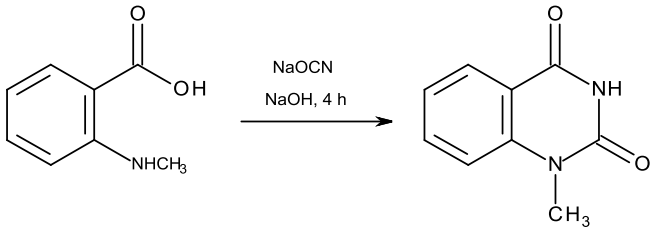
2.2.1. Synthesis of 2,4(1*H*,3*H*)-Quinazolinediones

2.2.1.1. From Anthranilic Acid Derivative and Cyanides/Isocyanates

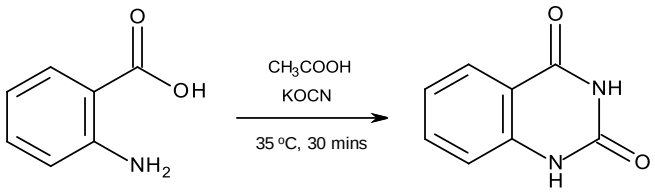
In 1872 Griess obtained 2,4(1*H*,3*H*)-quinazolinedione by fusing anthranilic acid with urea and also showed that it could be prepared directly from the reaction of anthranilic acid with cyanogen in water as shown below [181];



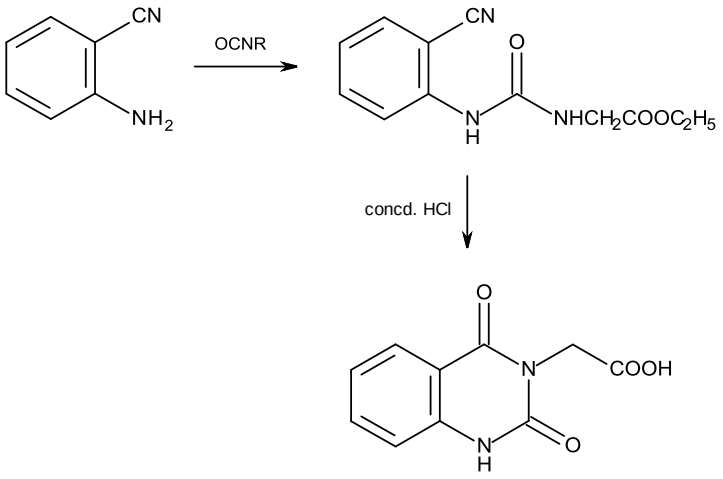
Glycosmicine, 1-methylquinazoline-2,4(1*H*,3*H*)-dione, is naturally occurring compound isolated from the *Gmelina arborea*. The glycosmicine was prepared by the reaction of *N*-methylanthranilic acid with sodium cyanate in basic media [128].



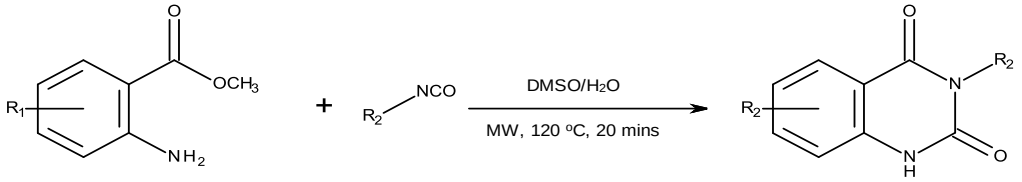
2,4(1*H*,3*H*)-Quinazolinedione was obtained in the presence of glacial acetic acid and potassium cyanate at 35 °C. The reaction was affected by manipulation of the pH of the reaction mixture [42, 157, 181, 182].



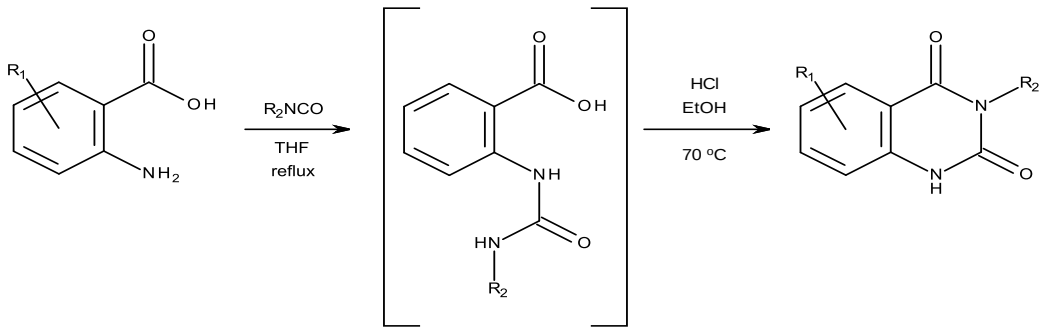
Papadopoulos reported 1,4-dihydro-2,4-dioxo-3(2*H*)-quinazoline acetic acid synthesis through the following steps. Anthranilonitrile reacted with ethyl isocyanatoacetate to afford 2-(3-ethoxycarbonylmethylureido)benzonitrile which was converted into 1,4-dihydro-2,4-dioxo-3-(2*H*)quinazoline acetic acid by heating with concentrated hydrochloric acid [184].



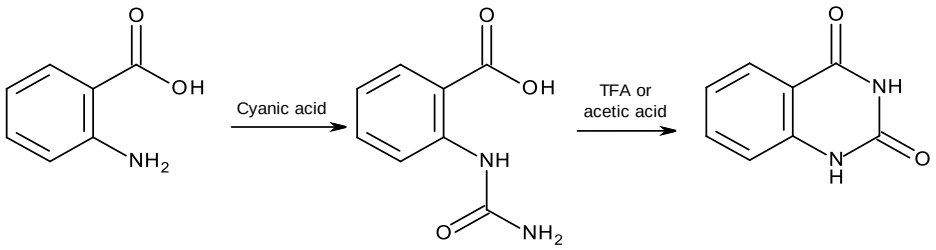
Li *et al.* obtained 2,4(1*H*,3*H*)-quinazolinediones by the reaction of substituted methyl anthranilate with various isocyanates under microwave irradiation for 20 minutes [185].



Koay *et al.* developed one-pot synthesis for the preparation of 3-substituted quinazolinediones by using anthranilic acids and isocyanates [186].

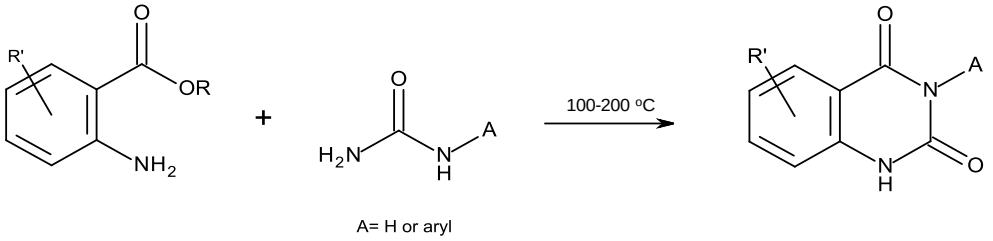


Michel *et al.* presented synthesis of 2,4(1*H*,3*H*)-quinazolinediones. Firstly, *o*-ureido benzoic acid was obtained by the reaction of anthranilic acid with cyanic acid. Cyclocondensation reaction of *o*-ureidobenzoic acid with trifluoroacetic acid or acetic acid yielded 2,4(1*H*,3*H*)-quinazolinediones [187].

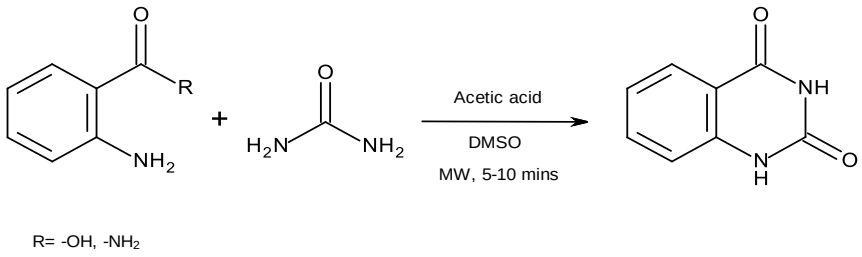


2.2.1.2. From Anthranilic Acid Derivatives and Urea

Quinazoline-2,4*(*1*H*,3*H)*-diones were prepared by fusing anthranilic acid or esters with urea at high temperatures [106, 186, 187].

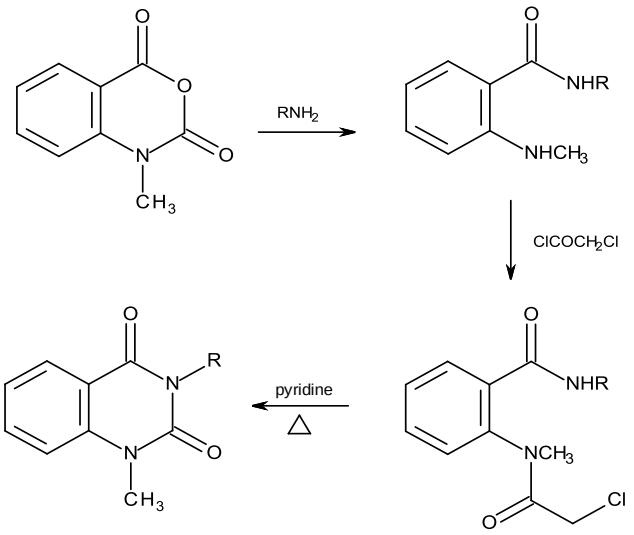


Acid-catalyzed coupling of antranilamide/anthranilic acid with urea under microwave irradiation gave the 2,4(1*H*,3*H*)-quinazolidinediones in high yields ranging from 66 to 88% [189].

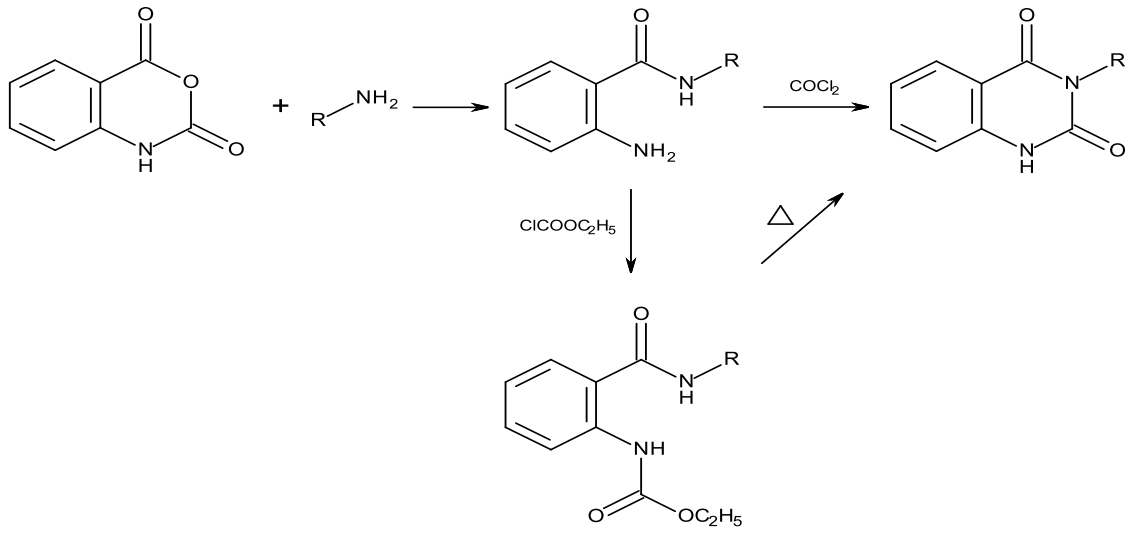


2.2.1.3. From Isatoic Anhydride

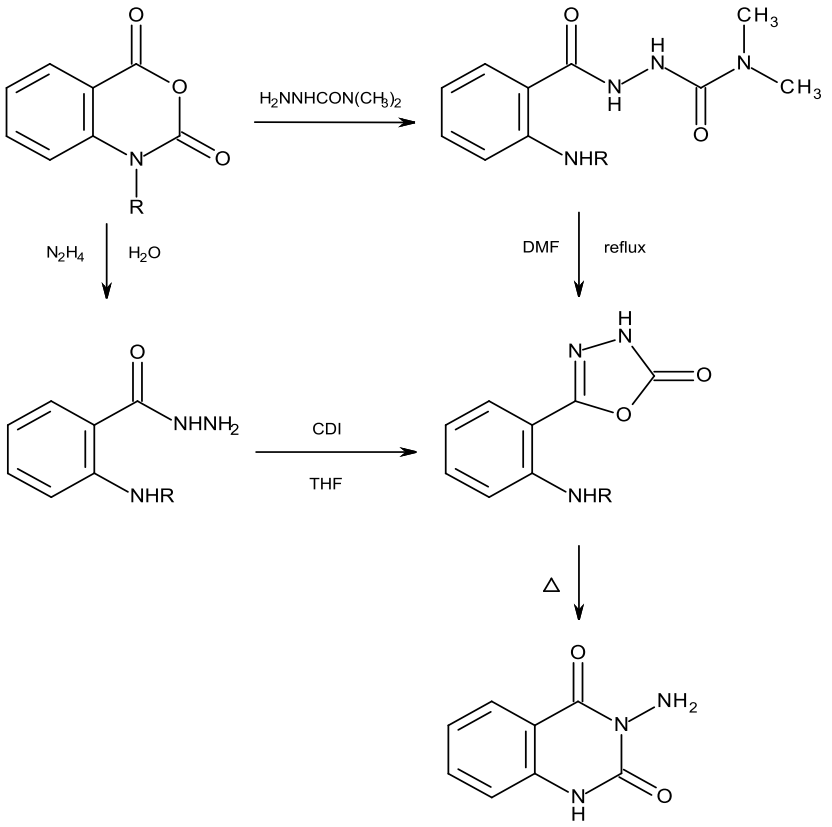
Lee reacted isatoic anhydride with amines to afford *N*-substituted antranilamides. Resulting compound was treated with α-chloroacetylchloride and subsequently refluxed in pyridine to yield 2,4(1*H*,3*H*)-quinazolinediones [190].



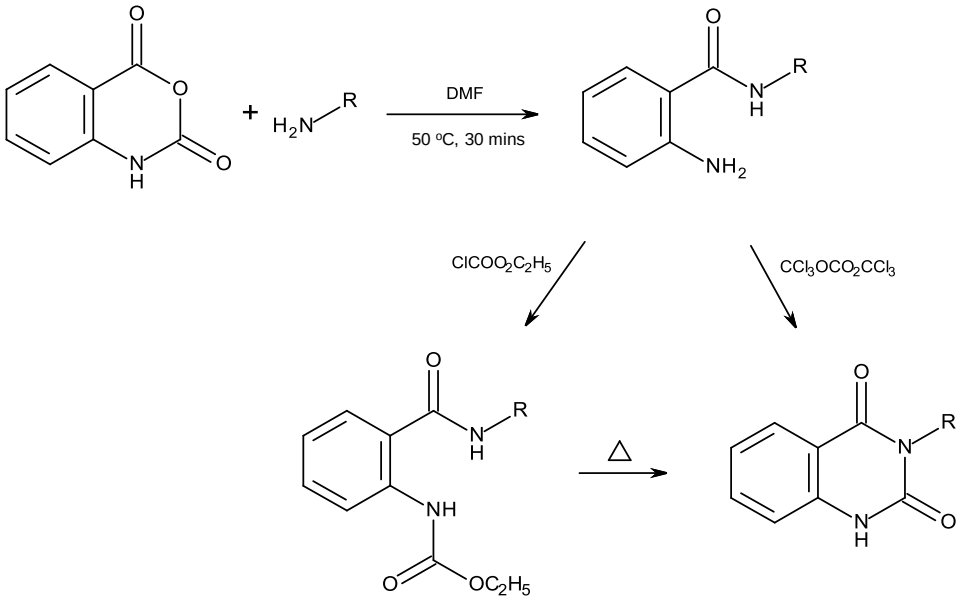
The synthesis of 3-alkyl-2,4(1*H*,3*H*)-quinazolinedione from the isatoic anhydride was patented as follows; isatoic anhydride was reacted with alkylamine to form *o*-aminobenzamide which was subsequently reacted with ethyl chloroformate or phosgene to afford 3-alkyl-2,4(1*H*,3*H*)-quinazolinediones [191, 192].



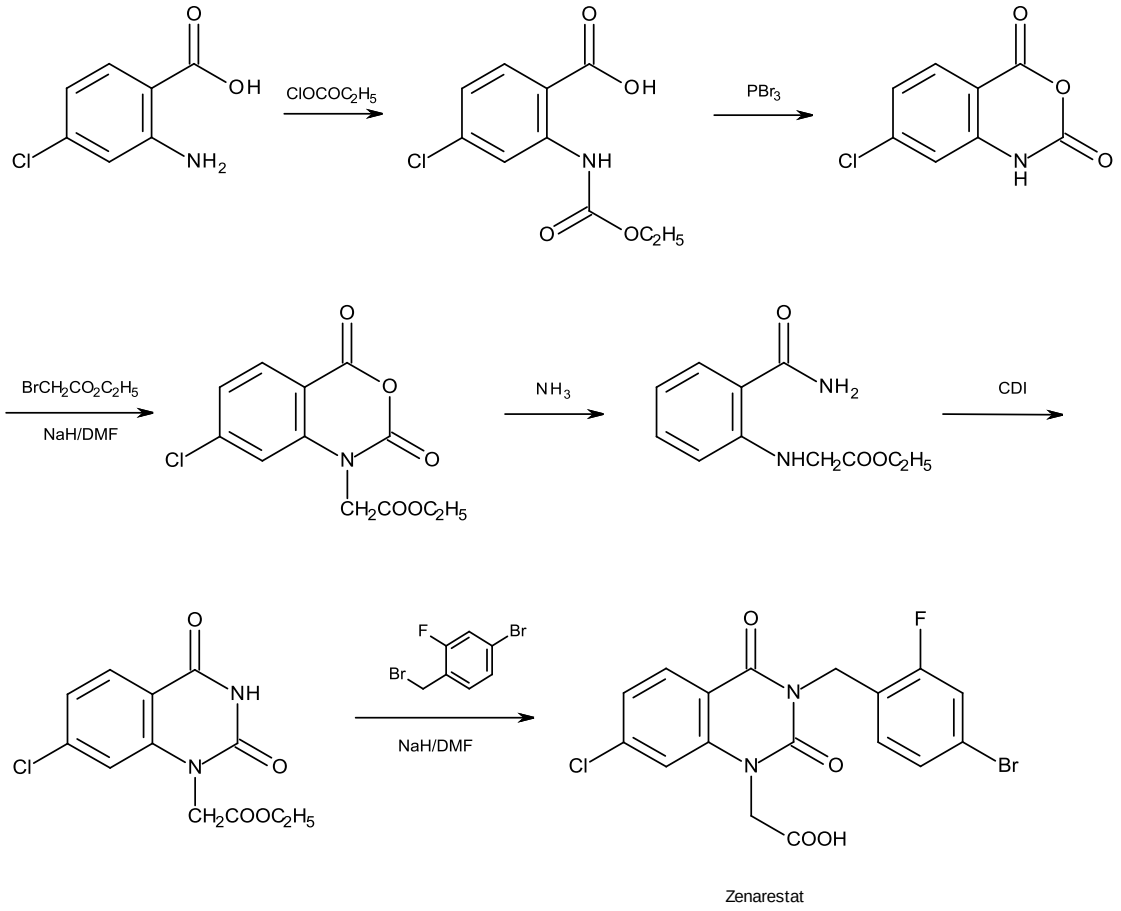
Rearrangement of 5-(2-aminophenyl)-1,3,4-oxadiazole-2(3*H*)-one to 3-amino-2,4(*1H,3H*)-quinazolinedione structure was subjected by Davidson. 5-(2-Aminophenyl)-1,3,4-oxadiazole-2(3*H*)-one was obtained from the cyclization of isatoic anhydride in either by refluxing 1-anthraniloyl-4,4-dimethylsemicarbazide or treating *o*-aminobenzhydrazide with 1,1'-carbonyldiimidazole (CDI) as shown below [193].



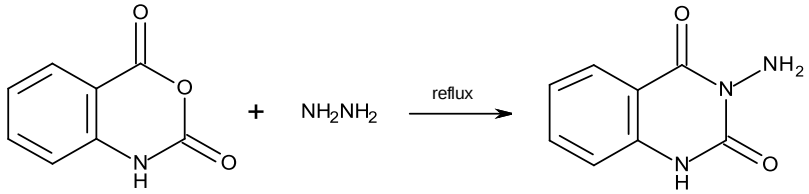
Cortez *et al.* and Lee *et al*. reported the similar synthesis of 2,4(1*H*,3*H*)-quinazolinedione by using isatoic anhydride, suitable amine and ethyl chloroformate to make the *o*-carboethoxybenzamide which is subsequently pyrolysed to give 2,4(1*H*,3*H*)-quinazolinedione [194, 195].



Synthesis of zenarestat (FK366) was reported in the literature. Subjecting anthranilic acid to ethyl chloroformate followed by the phosphorus tribromide afforded isatoic anhydride. 2,4(1*H*,3*H*)-Quinazolinedione was obtained through the ring closure of benzamide derivative with CDI. N^3-alkylation of the ring was carried out by using 2-fluoro-4-bromo-benzylbromide in the presence of sodium hydride which yielded zenarestat [156].

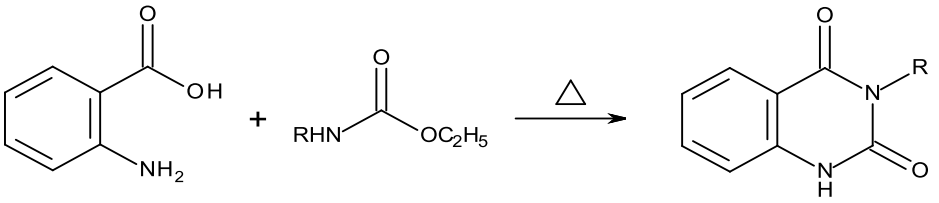


3-Amino-2,4*(*1*H*,3*H)*-quinazolinedione was synthesized by refluxing isatoic anhydride with hydrazine hydrate for 8 hours [150].

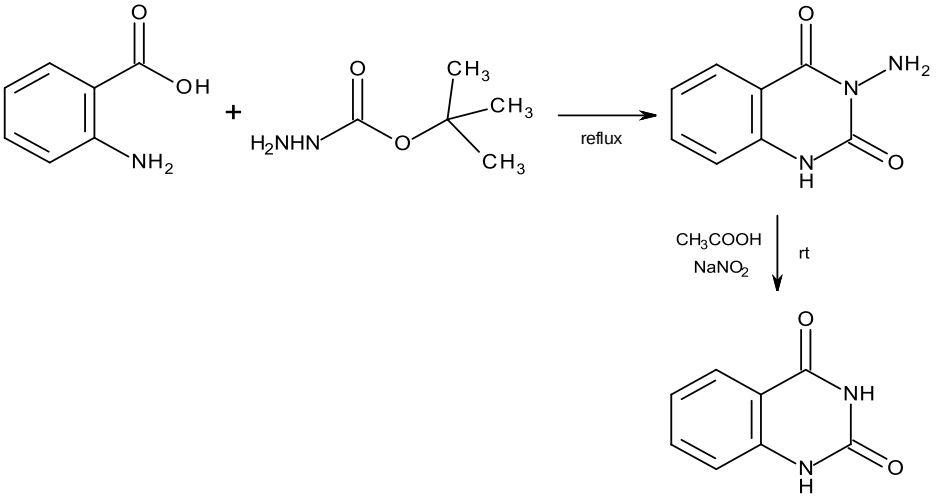


2.2.1.4. From Anthranilic Acid and Carbamate

Michman *et al.* synthesized 2,4*(*1*H*,3*H)*-quinazolinediones by fusing carbamates with anthranilic acid [196].

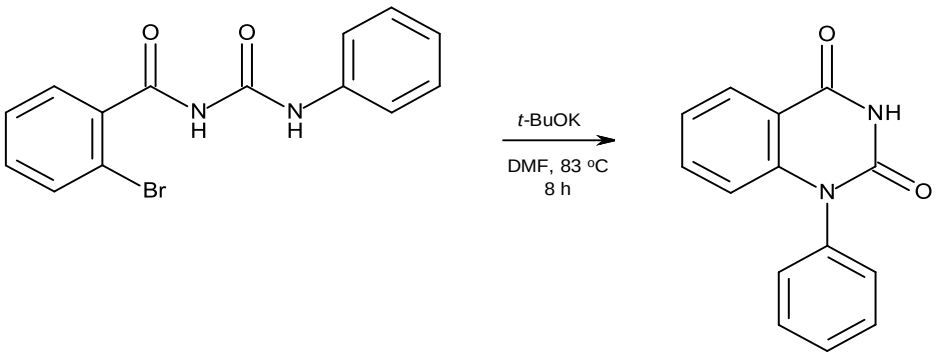


Lalezari and Golgolab prepared 3-amino-2,4*(*1*H*,3*H)*-quinazolinedione by refluxing antranilic acid with *tert*-butyl carbazate. Subsequent deamination of the product by treating acetic acid and sodium nitrite at room temperature yielded 2,4*(*1*H*,3*H)*-quinazolinedione [197].

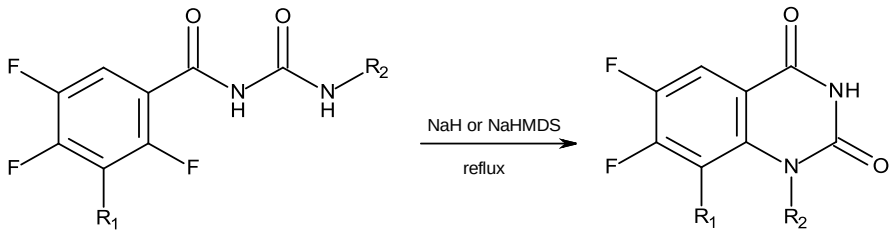


2.2.1.5. From *N*-Benzoylurea

When 1-(2-bromobenzoyl)-3-phenylurea was heated in the presence of potassium *tert*-butoxide (t-BuOK), 1-phenyl-2,4(1*H*,3*H*)-quinazolinedione was afforded in high yield [198].

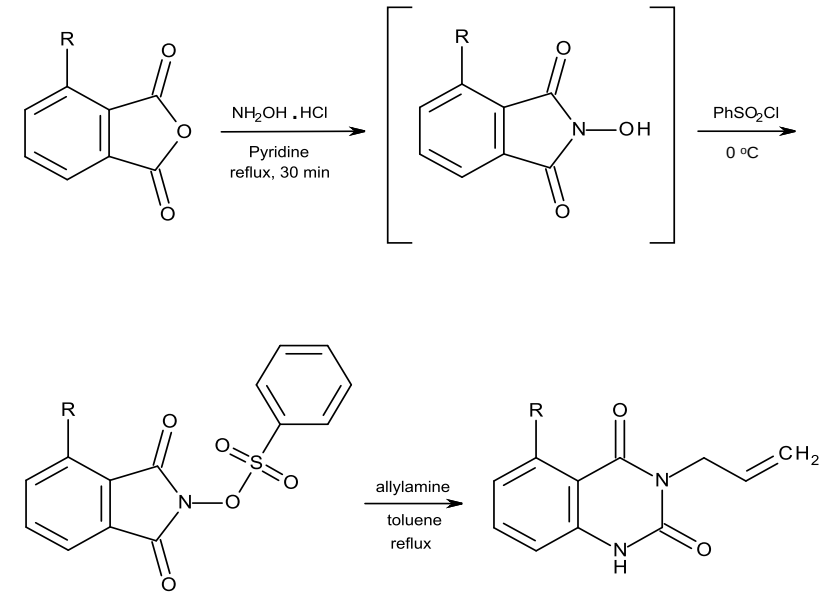


Tran *et al.* synthesized N^1-substituted 2,4(*1H,3H*)-quinazolinedione from the starting material of 1-aryl/alkyl-3-benzoylurea by using potassium bis(trimethylsilyl)amide (KHMDS), strong base, along with a catalytic amount of 18-crown-6 in tetrahydrofuran. It was stated that there were no significant differences between the ring-closure reaction of the alkyl and aromatic substituents; moreover both of them were formed in high yields (88% and 93%, respectively). Beylin *et al.* also utilized from strong bases (e.g., NaH, NaHMDS, KHMDS) to carry out the same cyclization in toluene/glyme mixture [199, 200].



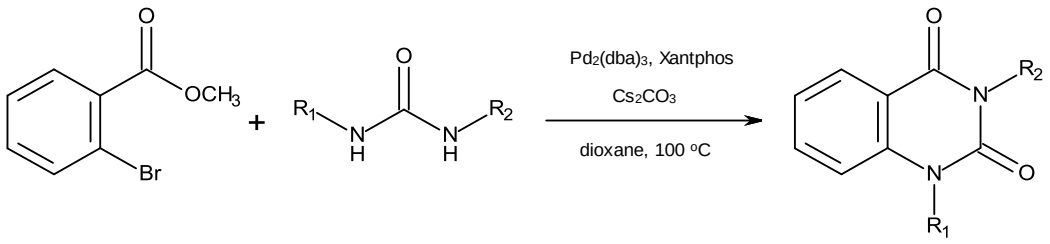
2.2.1.4. Miscelleneaous

Another way for the synthesis of 3-substituted-2,4(*1H,3H*)-quinazolinediones is to start from phthalimide. *N*-(phenylsulphonyloxy)phthalimide was obtained from the reaction of phthalic anhydride with hydroxylamine hydrochloride. After treatment of phthalimide with phenylsulphonylchloride resulted in *O*-sulphonyl derivative of phathalimide. Following rearrangement of this compound gave the target compound [201, 202].

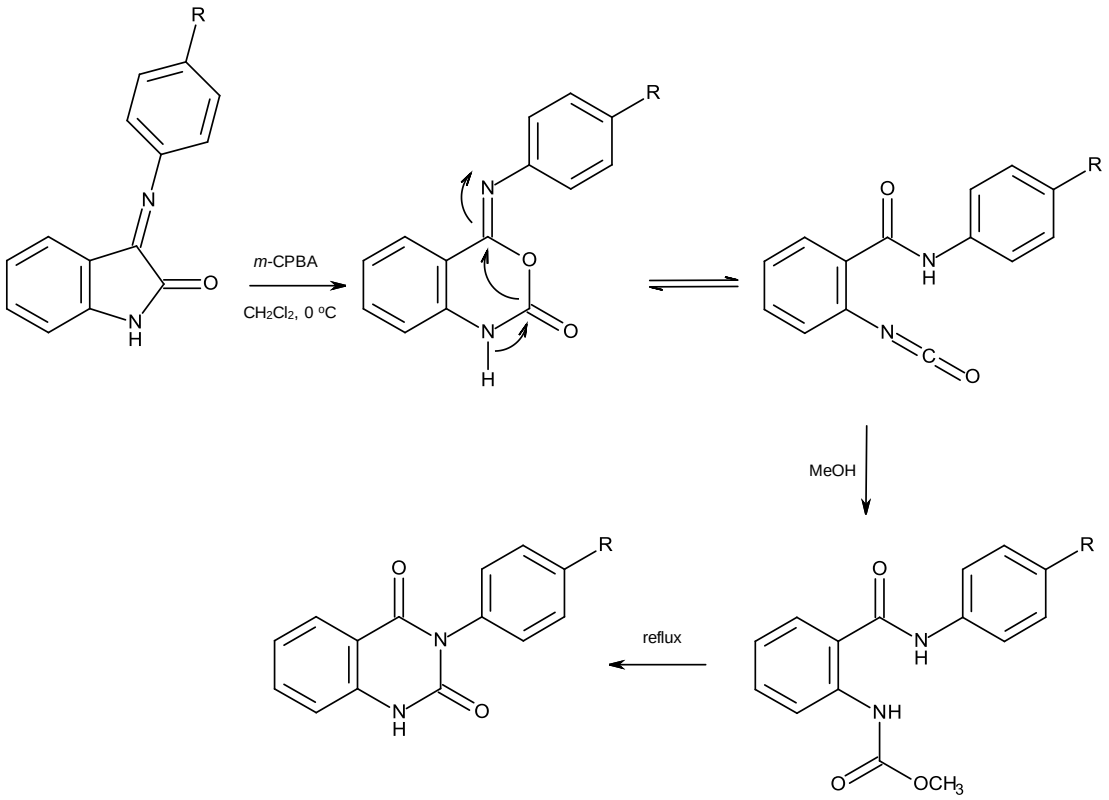


R= -NO_2, -H

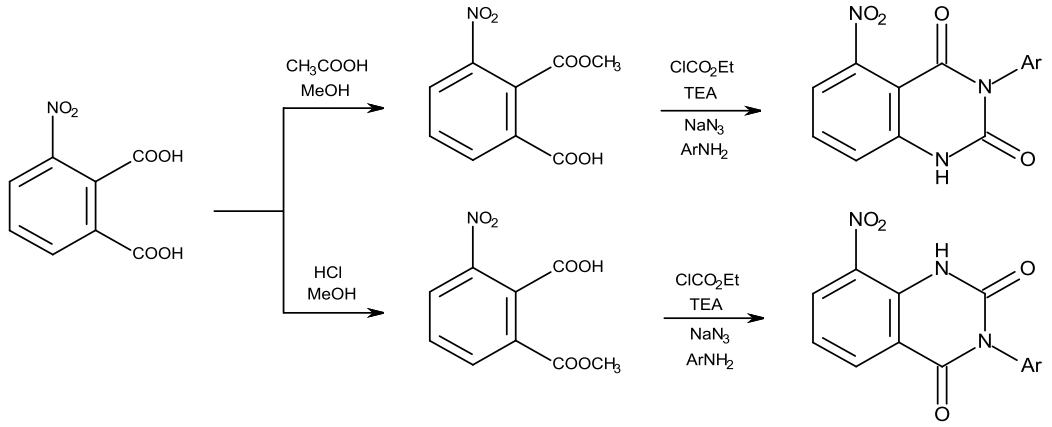
Willis *et al.* reported a new method to yield 2,4(1*H*,3*H*)-quinazolinedione ring system starting with methyl *o*-bromobenzoate and disubstituted urea in the presence of palladium catalyst [203].



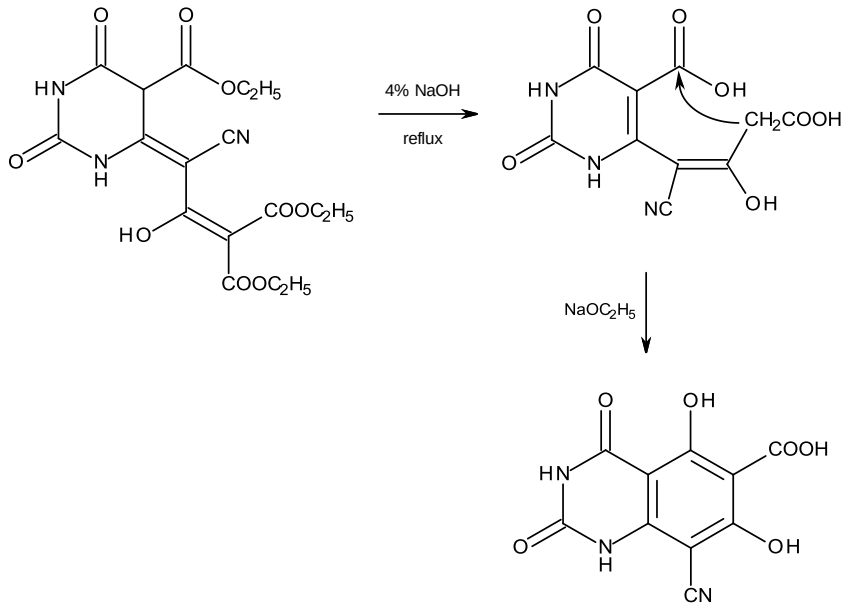
Azizian *et al.* reported Baeyer-Villiger oxidation of 3-arylimino-2-indolinones. The reaction of 3-arylimino-2-indolinones with *m*-chloroperbenzoic acid (*m*-CPMA) at 0 °C gave the corresponding benzoxazinone intermediate. This intermediate gave related carbamate derivatives, which could be altered into the 2,4(*1H,3H*)-quinazolinedione by refluxing in methanol [204].



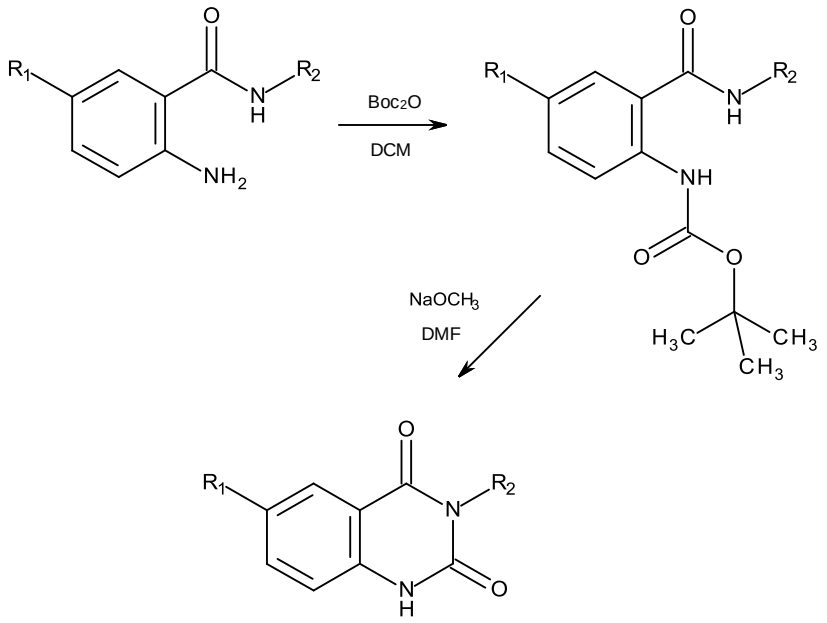
Aziane *et al.* converted 3-nitrophthalic acid to the two regioisomeric monoesters, which were subsequently transformed via Curtius rearrangement to the corresponding 5-nitro and 8-nitro-2,4(1*H*,3*H*)-quinazolinediones [205].



Quinazoline-2,4(1*H*,3*H*)-dione derivative bearing various substituents on aromatic ring were afforded by refluxing pyrimidine-2,4(1*H*,3*H*)-dione derivative in basic media followed by the treatment with sodium ethoxide [206].

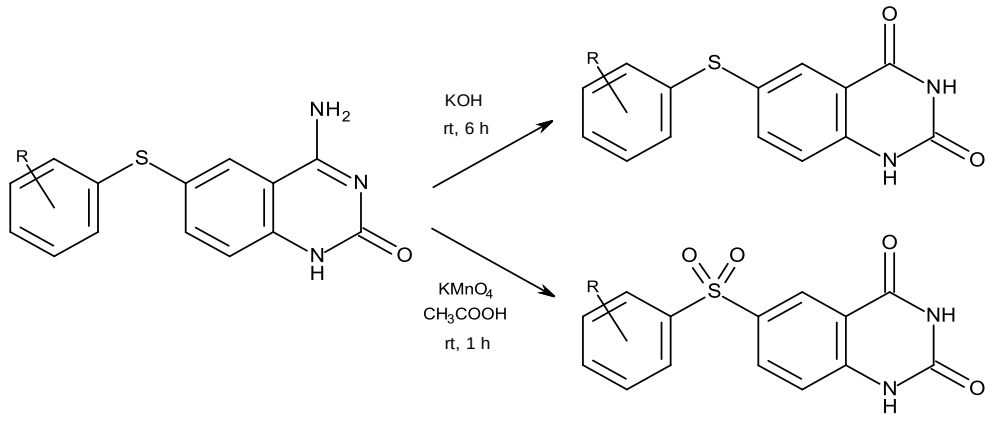


Li *et al.* developed a synthetic pathway to 2,4(1*H*,3*H*)-quinazolinedione derivatives from substituted anthranilamide via carbamate formation with di-*tert*-butyl dicarbonate (Boc anhydride) which then cyclized in the presence of sodium methoxide [207].

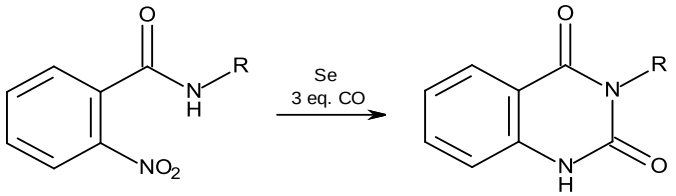


4-Amino-6-phenylthio-2(1*H*)-quinazolinones were hydrolyzed to 2,4(1*H*,3*H*)-quinazolinediones by refluxing the compounds in concentrated potassium hydroxide for

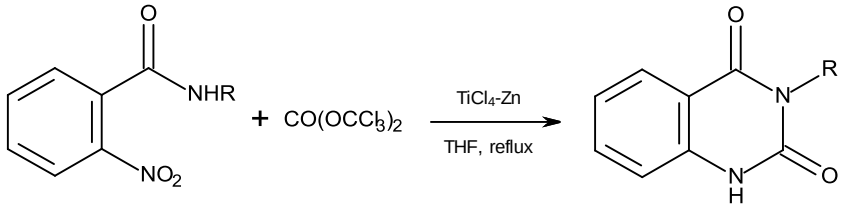
4 hours and oxidized to 6-sulphonylquinazoline-2,4(1*H*,3*H*)-diones using potassium permanganate in acetic acid [106].



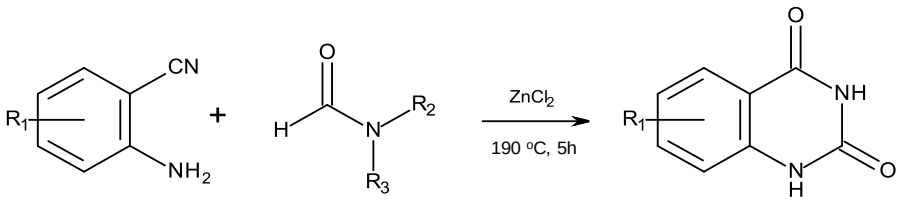
Palladium and selenium-catalyzed synthesis of quinazoline-2,4(1*H*,3*H*)-diones in the presence of carbonmonoxide were detailed [207, 208]. 2-Nitrobenzamides were subjected to the selenium-catalyzed carbonylation under relatively mild conditions to afford quinazoline-2,4(1*H*,3*H*)-diones [209].



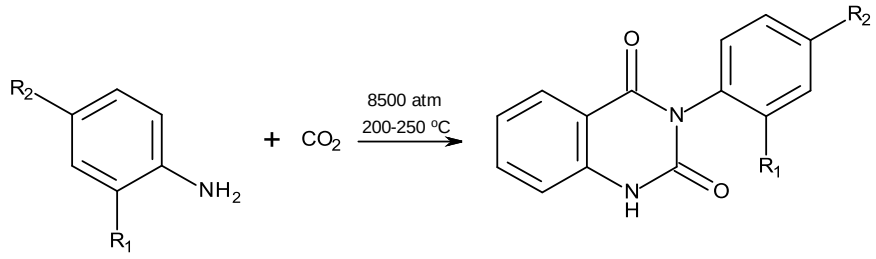
Shi *et al.* reported a method in which cyclization of 2-nitrobenzamide and triphosgene to quinazoline-2,4(1*H*,3*H*)-dione was promoted by $TiCl_4$/Zn system [210].



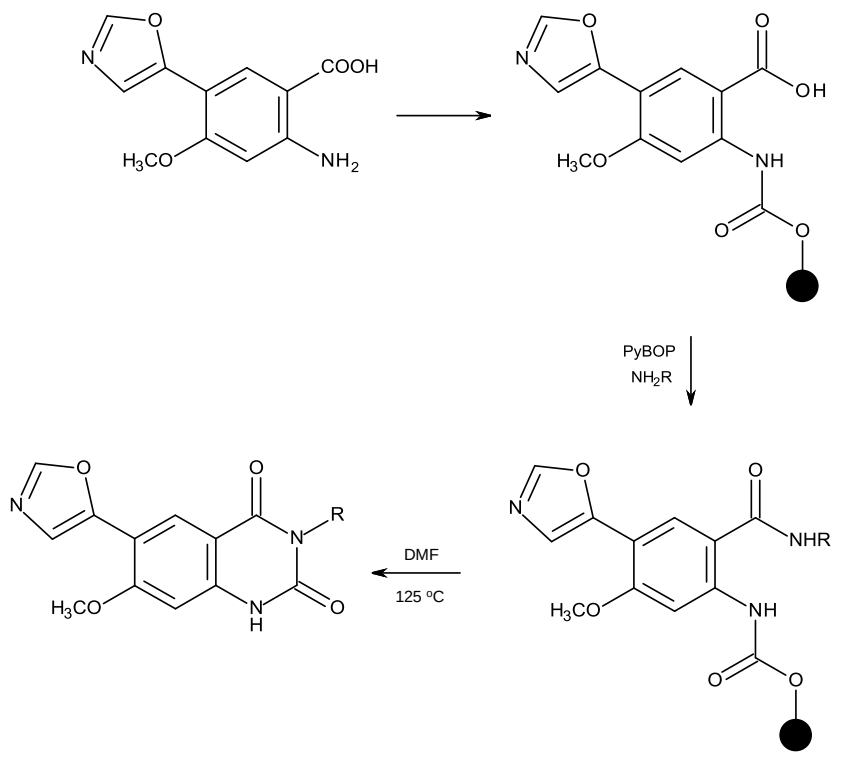
The condensation of *o*-aminobenzonitriles with $ZnCl_2$ in dimethylformamide (DMF) at 190-200 °C resulted in quinazoline-2,4(1*H*,3*H*)-diones [211].



Several methods have been reported for the synthesis of quinazoline-2,4-dione with the fixation of carbondioxide under milder conditions in the presence of catalytic bases; such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) [212], polyamidine and *N*-methyltetrahydropyrimidine (MTHP) [213], basic ionic liquid ([BMIM]OH) [213–215], MgO/ZrO_2 heterogeneous system [217], tetramethylguanidine (TMG), triethylamine [217, 218]. Cairns *et al.* also synthesized 3-aryl-2,4(1*H*,3*H*)-quinazolinediones by the reaction of carbon dioxide with primary aromatic amines at 8500 atm and 200-250 °C [220].



Several methods for the solid phase synthesis of quinazoline-2,4(1*H*,3*H*)-diones have been also developed [109, 220–222]. Buckley was utilised a solid phase synthesis in which anthranilic acid derivative was loaded onto Merrifield resin as the carbamate. Amidation was carried out with benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), then cyclisation and release of the final compound was afforded 2,4(1*H*,3*H*)-quinazolinediones at 125 °C without any purification [154].

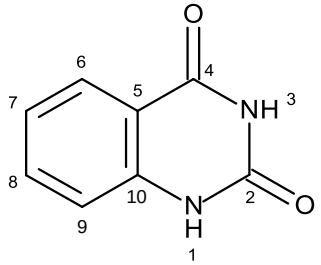


2.2.2. Physical Properties of 2,4(1*H*,3*H*)-Quinazolinediones

2,4(1*H*,3*H*)-Quinazolinedione which is light-yellow solid gives light blue fluorescence on thin layer chromatography (TLC) at 366 nm and yellow spots on spraying with anisaldehyde/sulfuric acid, which turned to blue-violet and then yellowish after heating [224]. The compound has a melting point of 354-356 °C (dec) [225].

2.2.3. Spectral Properties of 2,4(1*H*,3*H*)-Quinazolinediones

2.2.3.1. ^{1}H-NMR Spectrum of 2,4(1*H*,3*H*)-Quinazolinedione



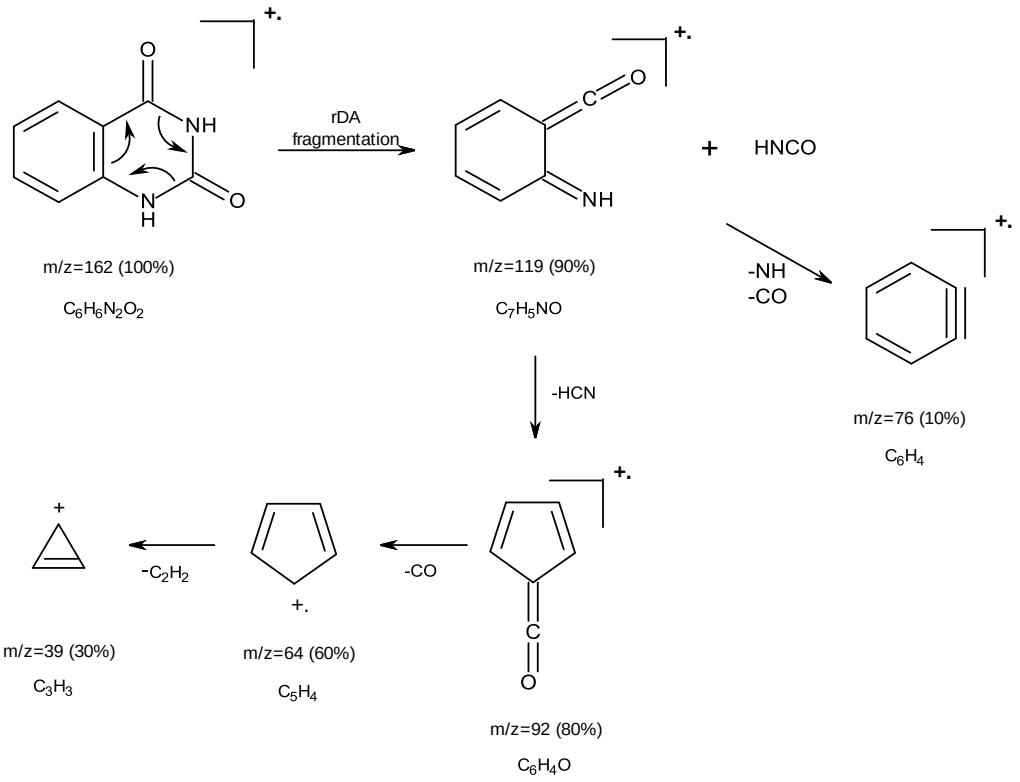
In ^{1}H-NMR spectrum of 2,4(1*H*,3*H*)-quinazolinedione, H^{7} and H^{9} protons give signal at 7.15 ppm as multiplet. H^{8} proton gives one signal at 7.62 ppm as doublet of triplet integrating one proton. Signal of H^{6} can be seen at 7.87 ppm as doublet of doublet. H^{1} and H^{3} give signal as broad singlet at 11.28 ppm and 11.40 ppm, respectively [201, 223–227].

2.2.3.2. ^{13}C-NMR Spectrum of 2,4(1*H*,3*H*)-Quinazolinedione

In ^{13}C-NMR spectrum of 2,4(1*H*,3*H*)-quinazolinedione, C=O carbons (C^{2} and C^{4}) of the ring can be generally observed as singlets at 151.1 and 162.5 ppm, respectively. Benzene carbons give signals at (C^{5}) 121.1, (C^{6}) 129.0, (C^{7}) 113.0, (C^{8}) 132.5, (C^{9}) 112.0 and (C^{10}) 140.5 ppm [223, 225–228].

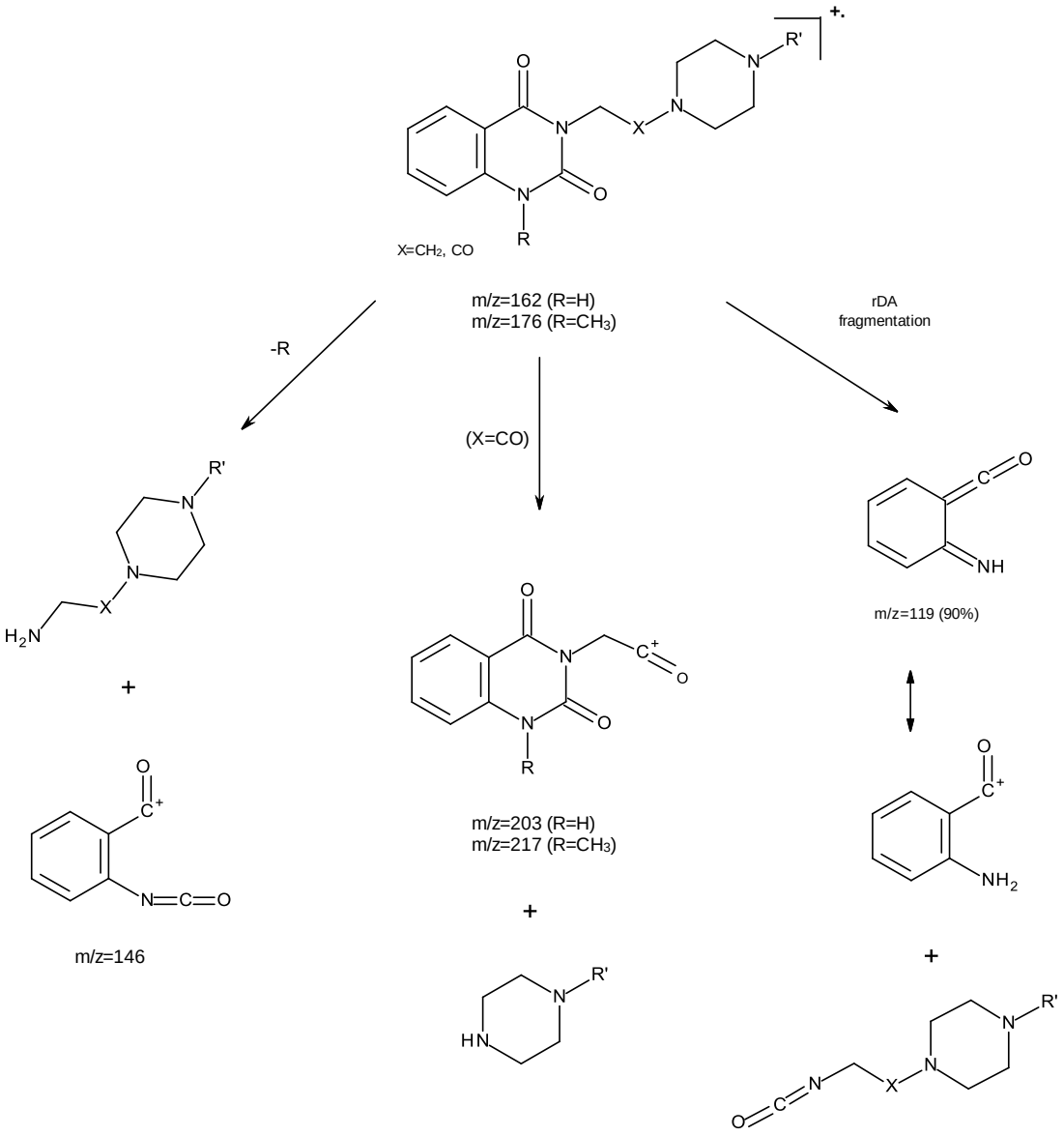
2.2.3.3. Mass Spectrum of 2,4(*1H,3H*)-Quinazolinedione

The electron ionization-mass (EI-MS) spectrum of 2,4(*1H,3H*)-quinazolinedione diplayed the molecular ion peak [M^{+}] at m/z = 162 (100%), which fragmented according to the retro-Diels-Alder (rDA) giving a fragment at m/z = 119 (90%) as shown in the **Scheme 2.1.** [224, 225, 229];



Scheme 2.1. Mass fragmentations of 2,4(1*H*,3*H*)-quinazolinedione.

Mass fragmentations of some 3-substituted-2,4(1*H*,3*H*)-quinazolinedione compounds were reported by Akgün and Hollstein [231]. Loss of piperazine ring fragment gave the acylium ion at 203 (m/z) for 3-(4-substitutedpiperazine)-2,4(1*H*,3*H*)-quinazolinedione and *N*-methylated acylium ion at 217 (m/z) for 1-methyl-3-(4-substitutedpiperazine)-2,4(1*H*,3*H*)-quinazolinedione. It was concluded that all 2,4(1*H*,3*H*)-quinazolinedione rings were break up in two ways; the rings converted into the 2-isocyanatobenzoylium ion (m/z=146), or fragmentation was occured by rDA cleavage into the 2-iminobenzoylium ion (m/z=119) as shown in **Scheme 2.2.**



Scheme 2.2. Mass fragmentations of 3-substituted-2,4(1*H*,3*H*)-quinazolinediones.

2.2.3.4. Infrared Spectrum of 2,4(1*H*,3*H*)-Quinazolinedione

In the infrared (IR) spectrum of the compound, absorption bands at 3128-3228 cm^{-1} are attributed to the N-H stretching of the secondary amine group. Streching bands of C=O at position 4 and C=O bonds at position 2 for 2,4(1*H*,3*H*)-quinazolinedione ring

are seen at 1703 and 1674 cm^{-1}. Streching band of C=C bond can be observed at 1610 cm^{-1} [201, 205, 225].

2.2.3.5. Ultraviolet Spectrum of 2,4(1*H*,3*H*)-Quinazolinedione

In the ultraviolet (UV) spectrum of 2,4(1*H*,3*H*)-quinazolinedione, measured in ethanol solution (5×10^{-5} M), there are mainly three absorption bands at 217 (log ε: 4,39), 242 (log ε: 3.76, as shoulder) and 311 nm (log ε: 3.37) which represent $\pi\rightarrow\pi^*$, $n\rightarrow\pi^*$ transitions of C=O and aromatic groups in 2,4(1*H*,3*H*)-quinazolinedione (**Figure 2.3.**) [232].

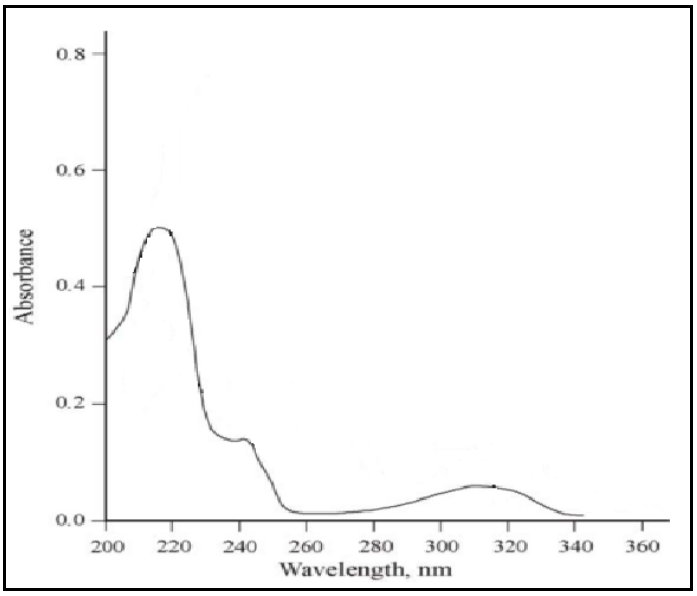


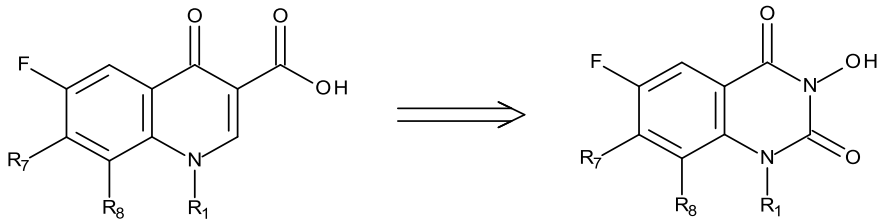
Figure 2.3. UV spectrum of 2,4(1*H*,3*H*)-quinazolinedione.

2.2.4. Biological Properties

2.2.4.1. Antimicrobial Activity

Quinolone-like agents (i.e.,quinazoline-2,4-diones) have attracted much attention for their activity against fluoroquinolone resistant bacterias [233]. Tran *et al.* presented

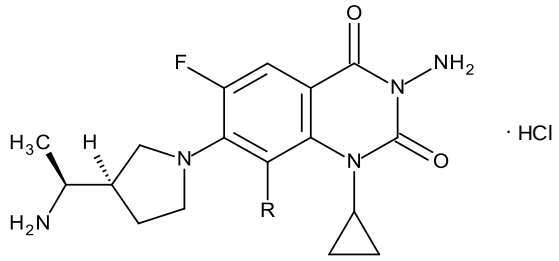
some 3-hydroxyquinazoline-2,4(1*H*,3*H*)-diones showing lower antibacterial activity than ciprofloxacin [88].



Fluoroquinolone

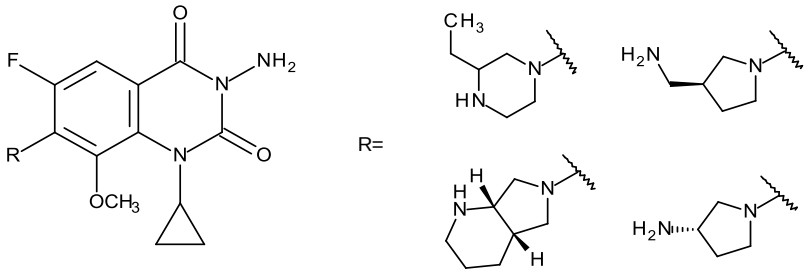
3-Hydroxyquinazoline-2,4(1*H*,3*H*)-diones

3-Aminoquinazoline-2,4(1*H*,3*H*)-diones inhibited *E. coli* DNA gyrase and exhibited significant activity against *S. pyogenes* and *E. faecalis* models *in vivo*, showing the potential of the 3-amino-2,4(1*H*,3*H*)-quinazolinediones as antibacterial agents [233, 234]. Antibacterial properties of the 2,4(1*H*,3*H*)-quinazolinediones versus *S. pneumoniae* were defined as similar or superior to those of fluoroquinolones with low minimum inhibitory concentration (MIC) values, low frequency of resistance development, and high *in vivo* potency [89].

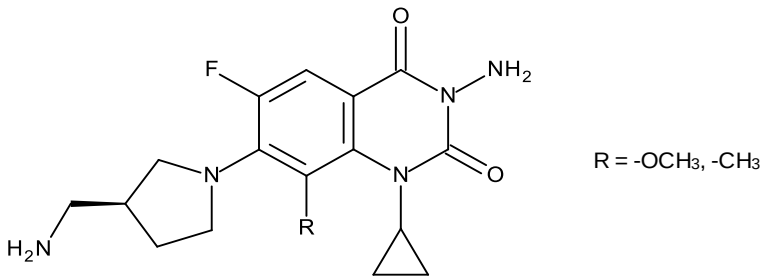


R= -OCH_3 , -CH_3

3-Aminomethylpyrrolidinyl derivatives were presented with low $MIC_{mutant}/MIC_{wild\ type}$ ratio against gyrA mutants. It was also reported that 3-amino-2,4(1*H*,3*H*)-quinazolinediones had high MIC values against *Mycobacterium smegmatis* compared to fluoroquinolones [91–93].

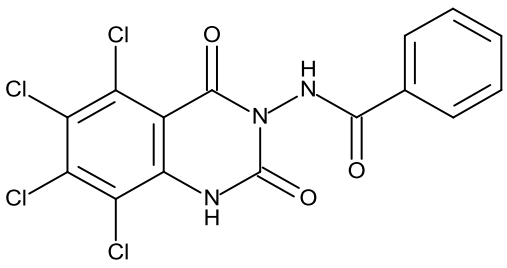


8-Methoxy and 8-methyl derivatives showed inhibition against DNA gyrase, topoisomerase IV enzymes and it was also proposed that quinazoline-2,4(1*H*,3*H*)-diones might act as dual-targeting agents against *S. aureus* [93].

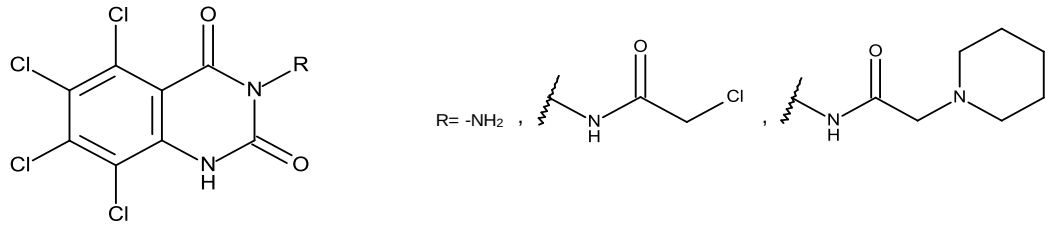


It is clarified that although quinazolinediones and fluoroquinolones display structural similarity, there are differences in target enzyme responses that have implications for the mechanism [94]. Dual-targeting activity of the quinazoline-2,4(1*H*,3*H*)-diones against DNA gyrase and topoisomerase IV is expected to slow the emergence of drug-resistant mutants [96–98].

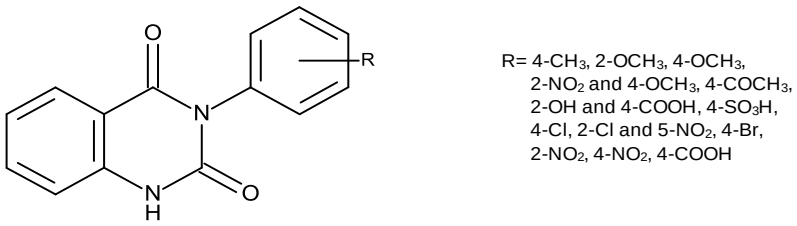
Tetrachloroquinazolin-2,4(1*H*,3*H*)-dione derivatives showed mild to moderate antibacterial activity against *Salmonella typhi*, *S. aureus*, *B. cereus* and *B.subtilis*. Benzohydrazide derivative exhibited the highest zone inhibition against *B.subtilis* [98].



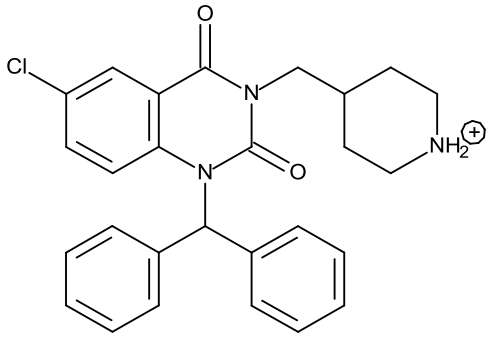
Hassan *et al.* presented a series of tetrachloroquinazolin-2,4(1*H*,3*H*)-dione derivatives showing mild to moderate antibacterial activity against *S. typhi*, *S. aureus*, and *B.cereus* with zone inhibition test [99].



3-Phenyl-2,4(1*H*,3*H*)-quinazolinedione derivatives exhibited moderate to excellent antibacterial activity against gram-positive organisms; *B. subtillis*, *S. aureus* and gram-negative organisms; *E. coli*, *P. aeruginosa* with zone inhibition test [16].

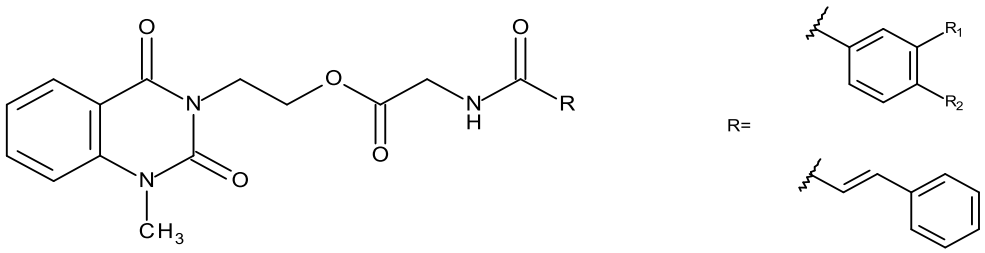


6-Chloro-1-biphenylmethyl derivative was reported to have the MIC value of 0.97 μM against *Trypanosoma brucei*, a causative parasite of Human African Trypanosomiasis (HAT) [100].

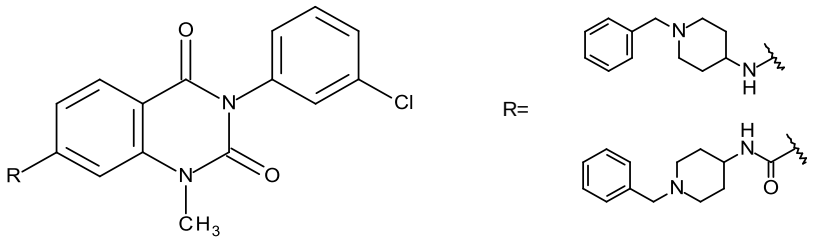


A series of 1-methyl-3-substituted-2,4(1*H*,3*H*)-quinazolinedione derivatives were evaluated for antimicrobial activities against *S. aureus*, *B. subtilis*, methicillin-resistant

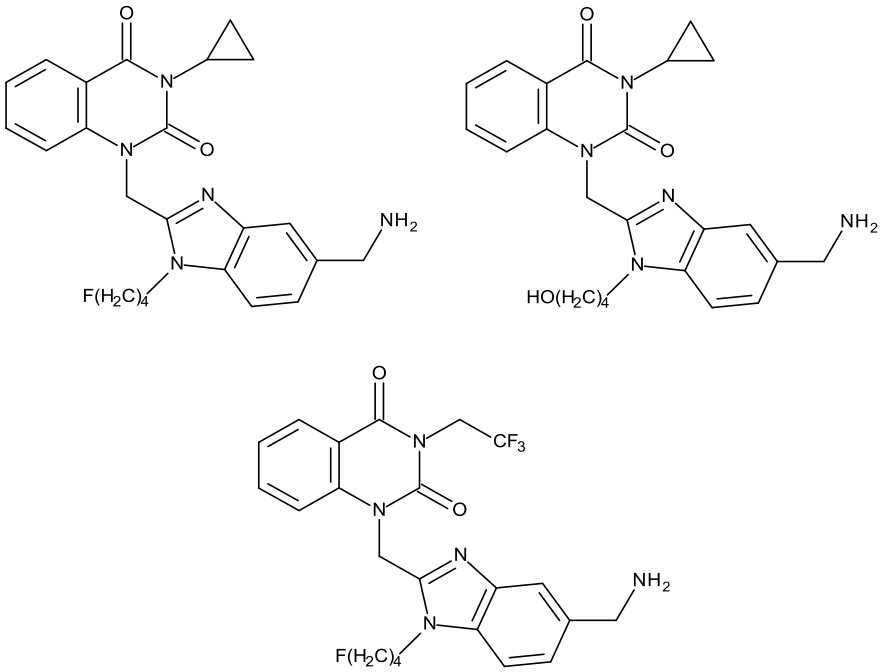
S. aureus (MRSA), *B. proteus*, *E. coli*, and *P. aeruginosa*; and antifungal activity against *Cryptococcus neoformans*, *Candida mycoderma*, *C. albicans*, *Saccharomyces cerevisiae*, and *Aspergillus flavus* by disc diffusion method. Especially, 4-fluorophenyl, 3-nitrophenyl and benzylidene derivatives exhibited good activity against MRSA with the MIC values of 4 μg/mL. When compared to fluconazole, almostly all compounds showed antifungal activity against *A. flavus* [101].



The NS5B polymerase of hepatitis C virus (HCV), playing a main role in virus replication, is a major target for the anti-HCV agents. 2,4(1*H*,3*H*)-Quinazolinediones with benzylpiperidinyl fragment exhibited inhibitory activity over NS5B polymerase enzyme in a nanomolar range [102].



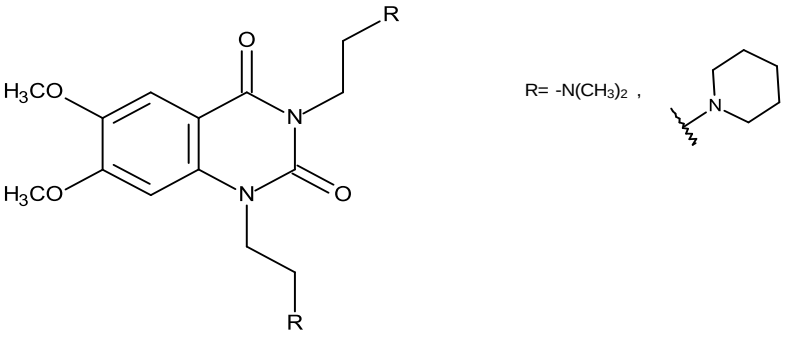
2,4(1*H*,3*H*)-Quinazolinedione derivatives were presented as a series of potent respiratory syncytial virus (RSV) fusion inhibitors. Three derivatives of them also demonstrated antiviral activity in a mouse model of RSV infection [103].



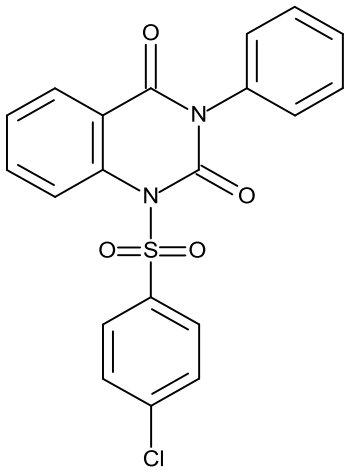
2.2.4.2. Anticancer Activity

2.2.4.2.1. Cytotoxic Activity

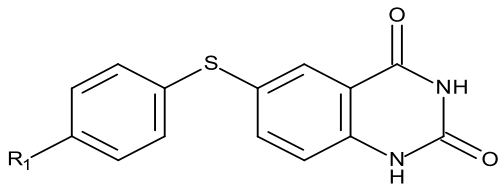
6,7-Dimethoxyquinazoline-2,4(1*H*,3*H*)-dione derivatives were tested for their cytotoxic properties in leukemia (K562) and cervix carcinoma (HeLa) cell lines. Compounds exhibited very limited anticancer activity with IC_{50} values of 100-400 μM [104].



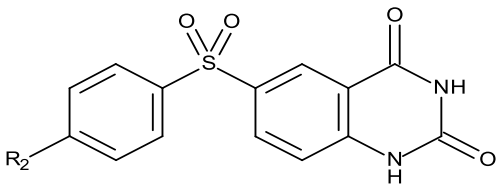
3-(4-Substituted)phenyl-1-(4-substituted)phenylsulfonylquinazoline-2,4(1*H*,3*H*)-diones were subjected to *in vitro* anticancer assay against 60 tumor cell lines taken from 9 different organs (lung, colon, breast, ovary, blood, kidney, skin, prostate and brain). 4-Chlorophenylsulphonyl derivative presented good inhibitory activity at the ovarian cancer cells and melanoma cells with the growth inhibition of 91.42% and 79.27%, respectively [105].



Some derivatives of 2,4(1*H*,3*H*)-quinazolinediones were evaluated *in vitro* for their cytotoxicity against the NCI-60 cancer cell lines. 4-Fluorophenylmercapto derivative showed growth inhibition (GI) value of 27% against non-small cell lung cancer cell line (NCI-H55). 4-Methylphenylsulphonyl derivative was the most active on the A549/ATCC cell line of the same panel (GI=19.3%). The CNS cancer cell line (SNB-75) exhibited moderate sensitivity against 4-chlorophenylmercapto derivative (GI=27.3%) [106].

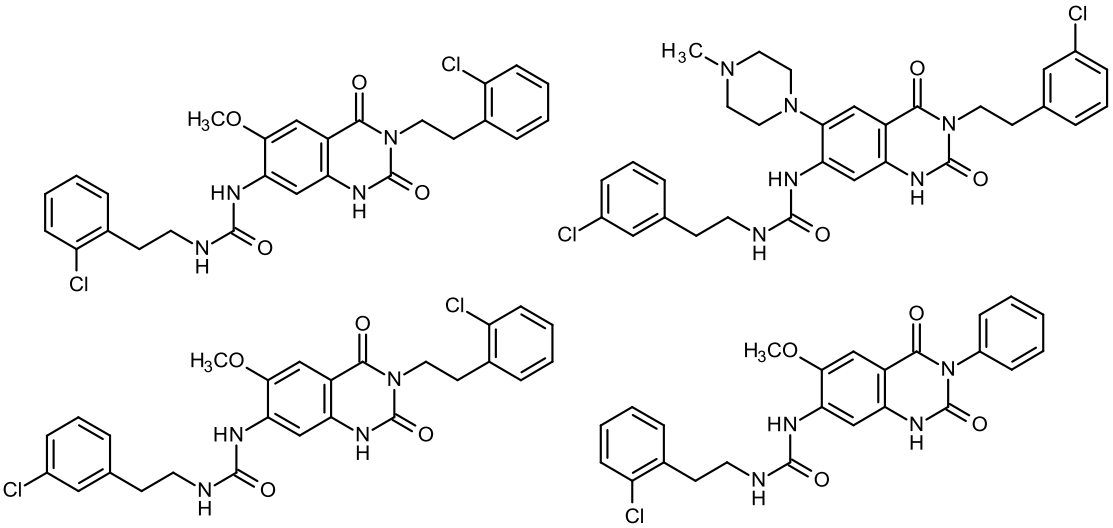


R_1= -H, -CH_3, -Cl, -F

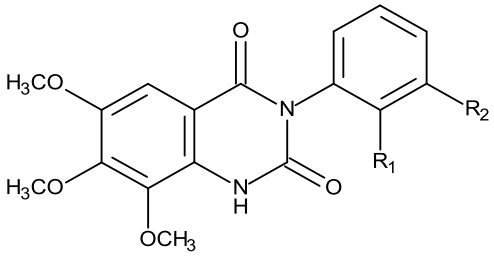


R_2=Cl, CH_3

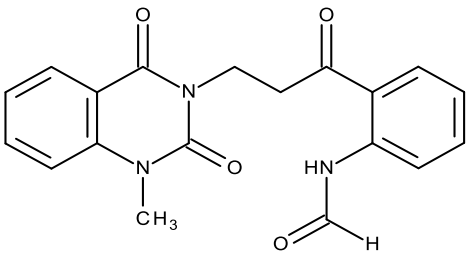
Some 2,4(1*H*,3*H*)-quinazolinedione derivatives were submitted to the NCI antitumor screening program. Four compounds significantly inhibited growth of 60 human tumor cells *in vitro* with IC_{50} values ranging from 0.4 to 0.8 μM [107].



Choo *et al.* synthesized a series of 3-aryl-2,4(1*H*,3*H*)-quinazolinediones with low cytotoxic activity on colon carcinoma in sulforhodamine B assay. IC_{50} values of 3-hydroxyphenyl and 2-hydroxyphenyl were 11.41 and 16.5 mg/mL, respectively whereas that of ellipticine was 0.5 mg/mL [108].

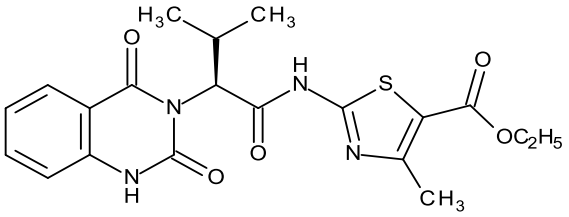


Wuchuyuamide IV, a quinazolinedione alkaloid, was isolated from the fruits of *Evodia officinalis*. The compound presented moderate cytotoxicity with IC_{50} values of 31.91 μM and 24.52 μM in Hela and HT1080 cell lines, respectively by using MTT assay [109].



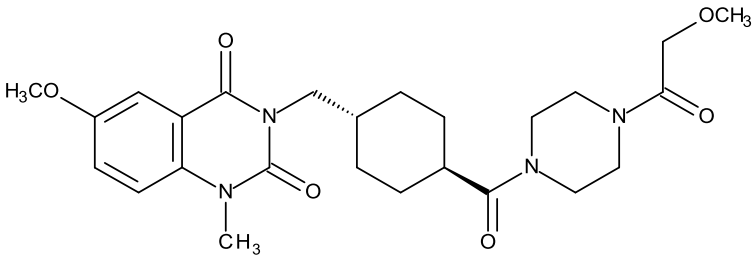
Wuchuyuamide Ⅳ

2,4(1*H*,3*H*)-Quinazolinedione derivative which was presented in a patent showed anticancer activity with IC_{50} values of ≤ 10 nM and GI_{50} values of ≤ 10 nM against human ovarian (SKOV3) cancer cell line [15].



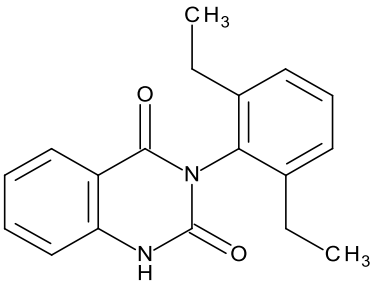
2.2.4.2.2. Targeted (Non-cytotoxic) Anticancer Activity

Up-regulation of the Wnt pathway exist in various types of cancer (e.g., lung, breast, pancreatic, gastric, colorectal, hepatocellular carcinoma, medulloblastoma, glioblastoma). Drug discovery research program was set up to identify new inhibitors of the β-catenin-dependent Wnt pathway for the treatment of glioblastoma multiforme. Primary hit series were discovered; followed by the optimization studies led to the identification of an advanced lead compound SEN461 [110].



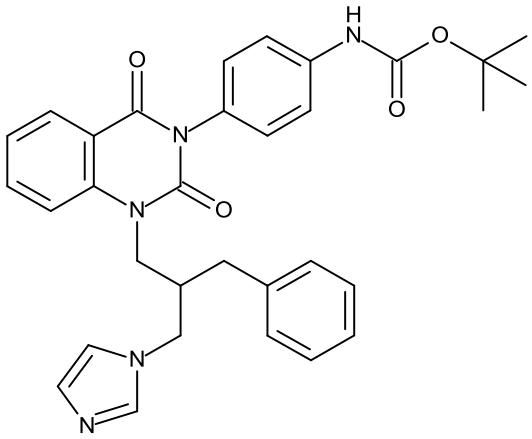
SEN461

It was declared that the 2,4(1*H*,3*H*)-quinazolinediones had inhibitory activities towards some amino peptidases, such as puromycin-sensitive aminopeptidase (PSA) and aminopeptidase N. These PSA inhibitors exhibited potent and dose-dependent cell invasion-inhibitory activity in a Matrigel assay using mouse melanoma cells, despite their low cell toxicity [111]. PAQ-22 exhibited potent and specific PSA inhibitory activity with an IC_{50} of 0.09 μg/mL [112].

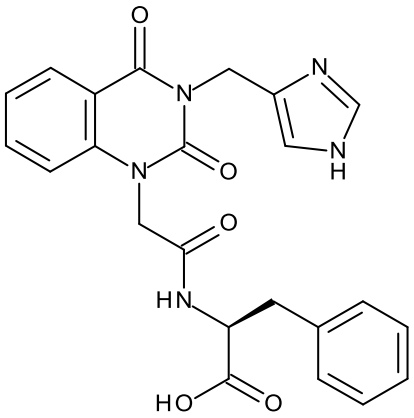


PAQ-22

It has been clarified that enzyme Farnesyl Transferase (FT) catalyses the phenylation of cysteine residues of several proteins associated with cancer progression. Thus, FT inhibitors were investigated for their activity of blocking tumour evolution [113]. The 2,4(1*H*,3*H*)-quinazolinedione derivative showed the highest FT inhibitory activity with IC_{50} value of 19 nM, as well as good *in vivo* activity when given orally to Swiss nude mice which were transplanted with oncogenic H-Ras-transfected cells [114].

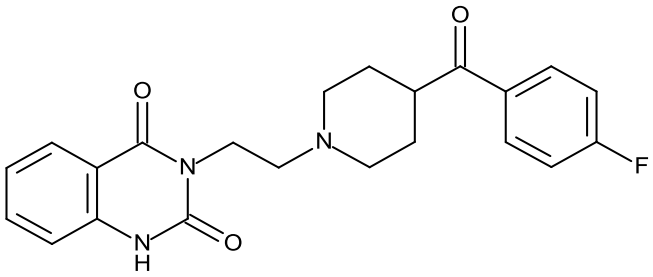


Potent and selective inhibitors of protein geranylgeranyltransferase-1 (PGGTase-1) enzyme are reported to be therapeutic agents against cancer [115]. 2,4(1*H*,3*H*)-Quinazolinedione derivative with L-phenylalanine exhibited the highest inhibition activity against PGGTase-I with an IC_{50} value of 170 nM [116].



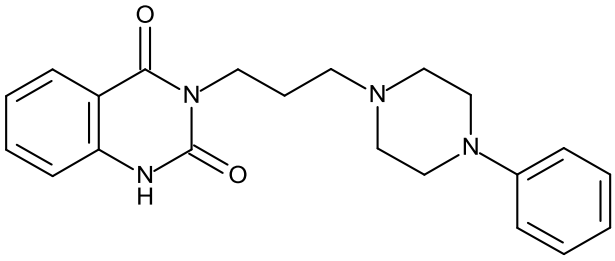
2.2.4.3. Antihypertensive Activity

Ketanserin (R41468), 3-[2-(4-(4-fluorobenzoyl)piperidin-1-yl)ethyl]-2,4(1*H*,3*H*)-quinazolinedione, was identified as a specific 5-HT_2 receptor antagonist [117]. *In vivo* studies showed that ketanserin decreases blood pressure following acute treatment [119, 120].



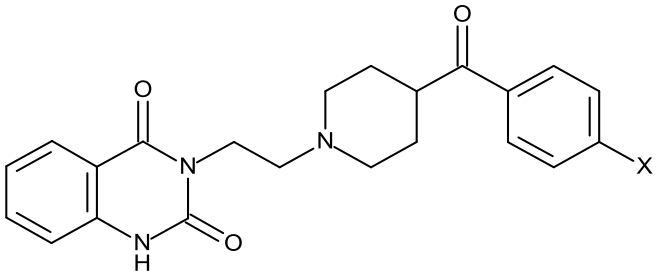
Ketanserin

Pelanserin (TR2515), 3-[3-(4-phenylpiperazin-1-yl)-propyl]-2,4(1*H*,3*H*)-quinazolinedione, is an antihypertensive agent showing activity comparable to ketanserin. The compound interacts with 5-HT_2 receptors which also makes pelanserin have the properties of an α-adrenergic antagonist [24, 121].



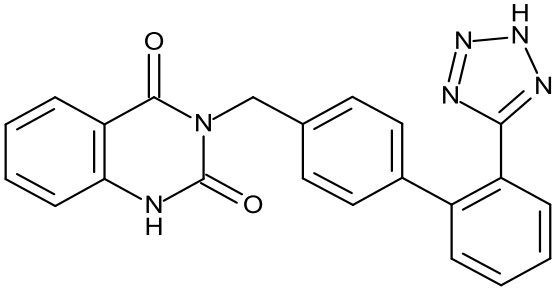
Pelanserin

A series of 3-substituted-2,4(1*H*,3*H*)-quinazolinediones displayed lower affinity at 5-HT_{1c} compared to ketanserin. Whereas benzoyl derivativatives exhibited high 5-HT_2 Ki values of 3.5-6.5 nM [121].

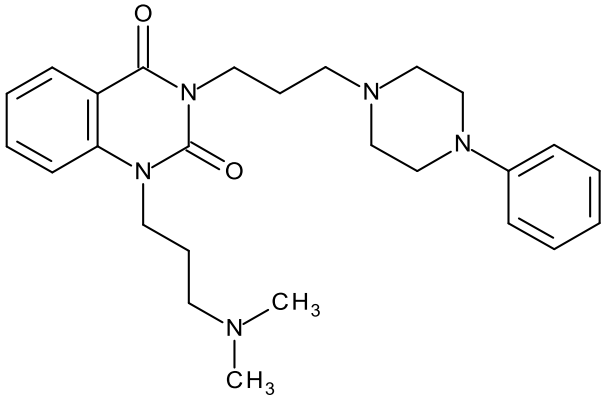


X=H, F

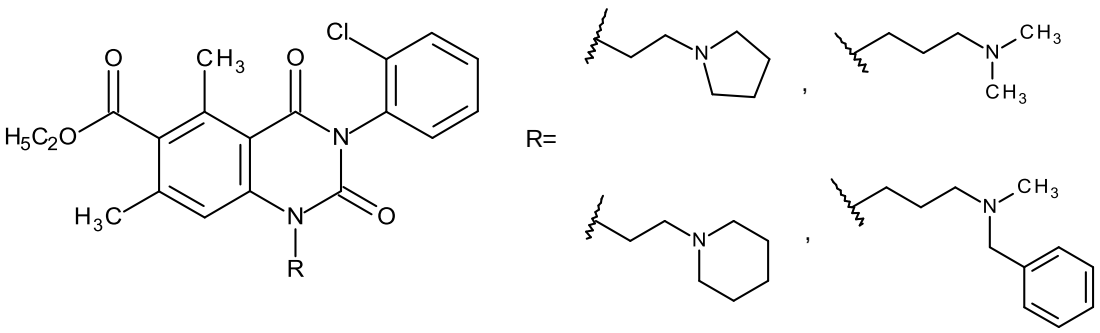
Tetrazole derivative, angiotensin II receptor antagonist, exhibited moderate competition when compared to losartan by using angiotensin II receptor binding assay [122].



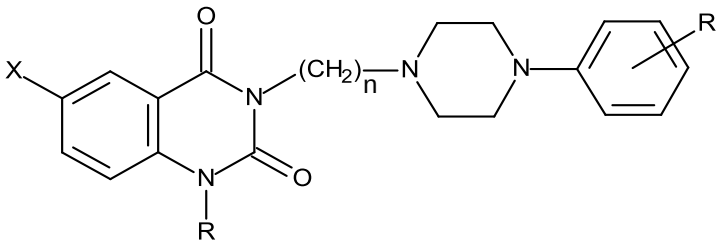
A series of 1,3-disubstituted-2,4(1*H*,3*H*)-quinazolinediones showed varying degrees of vasodilation and antihypertensive activity without significant blockade of α-adrenergic receptors. 1-[3-(*N*,*N*-dimethylamino)propyl]-3-[3-(4-phenyl-1-piperazinyl)propyl]-2,4(1*H*,3*H*)-quinazolinedione was more potent than papaverine in stimulating vasodilation of hypertensive rats upon oral administration [123].



Some 1-substituted-2,4(1*H*,3*H*)-quinazolinediones presented significant hypotensive activity [124].



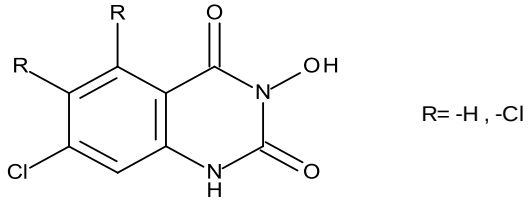
3-Phenylpiperazinylalkyl derivatives demonstrated varying degrees of sedative and hypotensive efficacy in experimental animal models (n=0-6) [125].



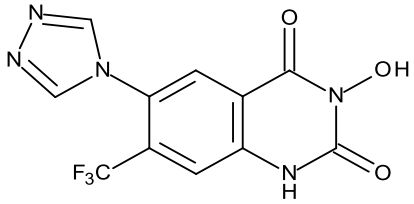
2.2.4.4. Anticonvulsant Activity

Some 3-hydroxy-quinazoline-2,4(1*H*,3*H*)-dione derivatives showed an affinity towards Gly/NMDA (Glycine/*N*-methyl-D-aspartate) receptor in low micromolar range

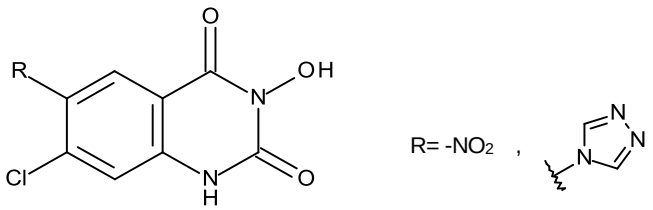
also good selectivity over both AMPA (α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainate (KA) receptors [132].



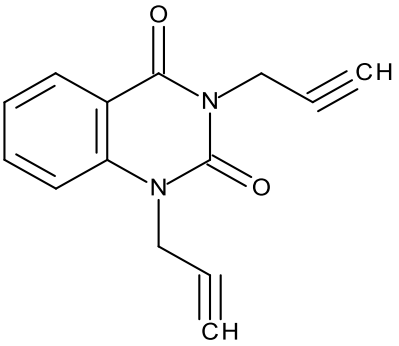
3-Hydroxy-6-substituted-7-trifluoromethyl-2,4(1*H*,3*H*)-quinazolinedione derivatives were evaluated for their Gly/NMDA, AMPA and KA receptor binding. 6-(1,2,4-Triazol-4-yl) derivative exhibited the highest affinity and selectivity for AMPA receptor *in vitro*; furthermore, it showed anticonvulsant activity against pentylenetetrazole (PTZ)-induced convulsions *in vivo* [126].



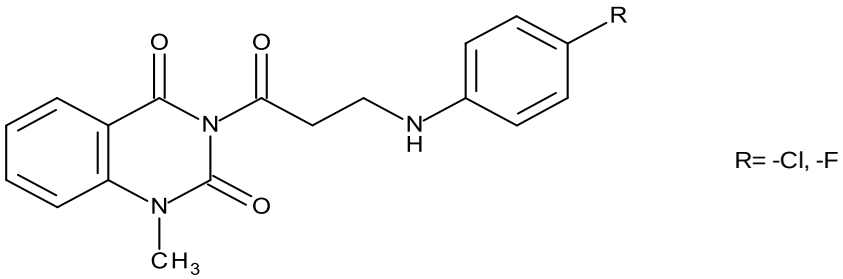
6-Nitro and 6-(1,2,4-triazole-4-yl) derivatives of 3-hydroxyquinazoline-2,4(1*H*,3*H*)-dione showed good potencies in the binding assay for the AMPA receptor, also anticonvulsant properties in mice when administered intra-peritoneal (i.p.) [131].



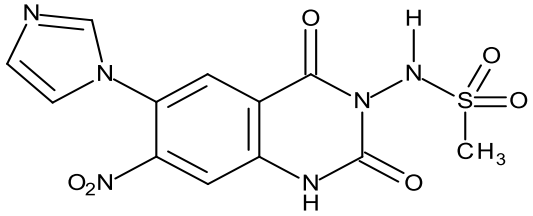
It was reported that, 1,3-bis-(prop-2-ynyl)-quinazoline-2,4(1*H*,3*H*)-dione was about ten-fold less active than carbamazepine or phenytoin but as active as mesuximide in MES test [127].



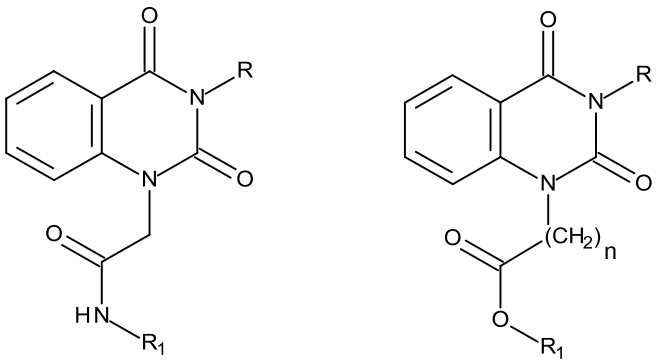
3-[*N*-(4-chlorophenyl)-β-alanyl]-1-methylquinazoline-2,4(1*H*,3*H*)-dione and 3-[*N*-(4-fluorophenyl)-β-alanyl]-1-methylquinazoline-2,4(1*H*,3*H*)-dione presented an optimal anticonvulsant efficacy with no neurological toxicity in MES test [128].



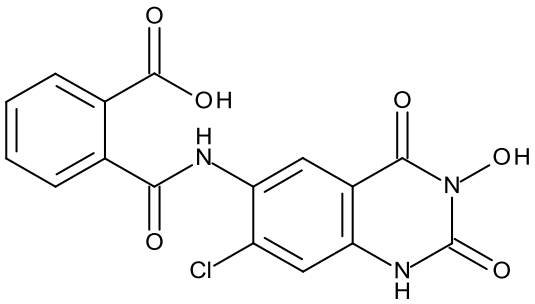
Quinazoline-2,4(1*H*,3*H*)-diones bearing a sulfonamide group were evaluated for their AMPA receptor antagonist activity. 6-(Imidazol-1-yl)-7-nitro derivative displayed activity with IC_{50} value of 82 nm for AMPA receptor, whereas the others displayed oral anticonvulsant activity against MES-induced seizures in mice [129].



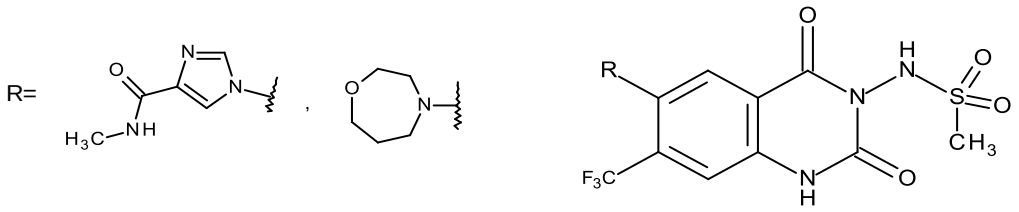
Some 1-substituted-2,4(1*H*,3*H*)-quinazolinediones were exhibited low anticonvulsant effect as compared to phenobarbitone [130].



6-(2-Carboxybenzoylamino)-3-hydroxy-1*H*-quinazolin-2,4-dione presented good affinity for high-affinity and low-affinity KA receptors with IC_{50} values of 0.62 μM and 1.6 μM, respectively [133].



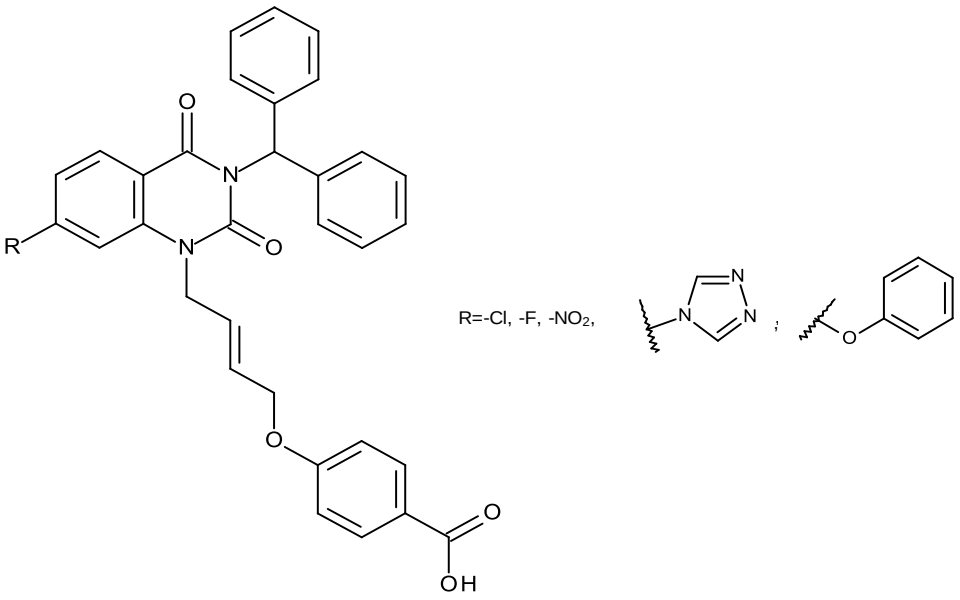
4-(*N*-methylamido)imidazol-1-yl derivative with good *in vitro* AMPA receptor antagonist activity (IC_{50}= 14 nM) but poor *in vivo* efficacy after oral dosage, was identified by screening of diverse 6-*N*-heteroaromatic fragments. After tuning the physicochemical properties, 6-homomorpholine derivative with good oral activity (ED_{50}= 5.5 mg/kg - in the mouse audiogenic seizure test) was achieved [134].



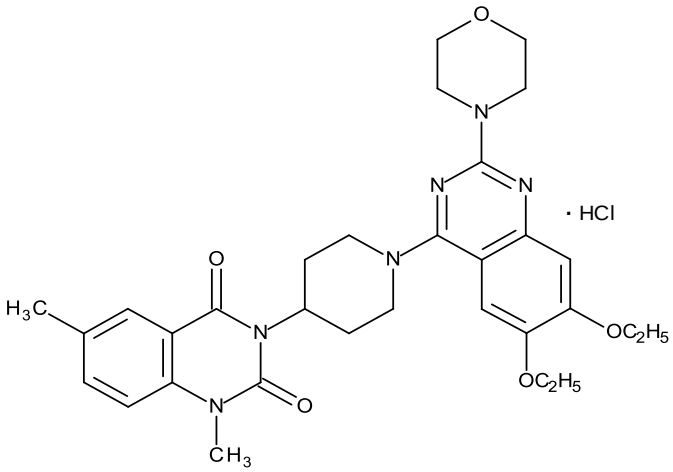
2.2.4.5. Anti-inflammatory Activities

The cytosolic phospholipase A2 (cPLA2a) inhibitors, blocking both prostaglandin and leukotriene production, are expected to be more effective than

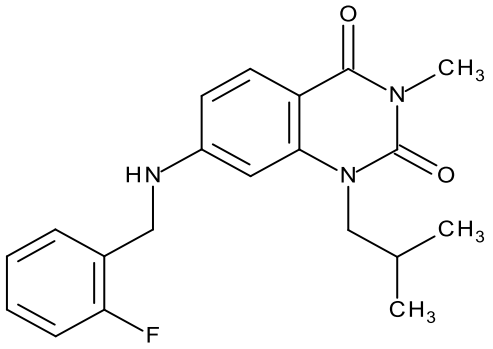
cyclooxygenase-2 (COX-2) inhibitors. Kirincich et al. reported 4-({(2*E*)-4-[3-(diphenylmethyl)-7-substituted-2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl]but-2-en-1-yl}oxy)benzoic acid showing inhibitory activity against cPLA2a in cell-based assay [135].



Adenosine uptake inhibitors show anti-inflammatory effects by means of inhibiting lipopolysaccharide-induced TNF-α (tumor necrosis factor) production and leukopenia [136]. 3-[1-(6,7-Diethoxy-2-morpholinoquinazolin-4-yl)piperidin-4-yl]-1,6-dimethyl-2,4(1*H*,3*H*)-quinazolinedione hydrochloride (KF24345), orally active adenosine uptake inhibitor, exhibited antipancreatitis effect on experimental acute pancreatitis induced by choline-deficient and ethionine-supplemented diet in mice [137].

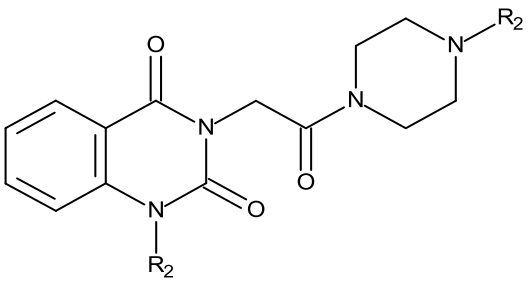


Michne *et al.* reported 7-[(2-fluorobenzyl)amino]-1-isobutyl-3-methylquinazoline-2,4(1*H*,3*H*)-dione as a potential immuno-suppressive and anti-inflammatory agent with the inhibition of nuclear factor of activated T-cells (NFAT) mediated ß-galactosidase with IC_{50} value of 1.32 μM [153].

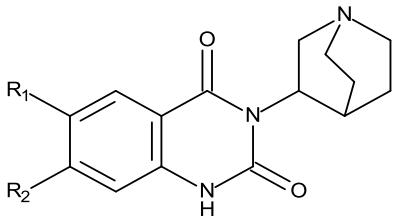


2.2.4.6. Miscellaneous

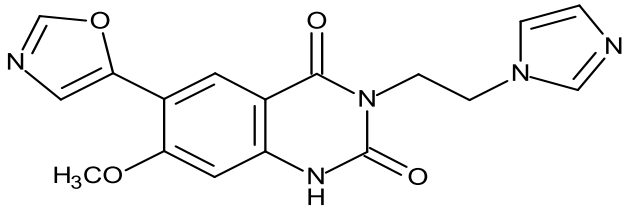
Akgün *et al.* reported a series of 3-substituted and 1-methyl-3-substituted 2,4(1*H*,3*H*)-quinazolinediones showing inhibitory action on contractile function of smooth muscles isolated from longitudinal muscles from the ginea pig ileum and tracheal muscles from the guinea pig [236].



Langloisl *et al.* reported 3-(1-azabicyclo[2.2.2]oct-3-yl)-6,7-disubstituted-quinazoline-2,4(1*H*,3*H*)-dione to have 5-HT_3 receptor antagonistic acitivity [139].



Inosine monophosphate dehydrogenase (IMPDH) enzyme directs the guanine nucleotide pool, which organizes proliferation and many other physiological routes. This feature of IMPDH enzyme makes it an important target for cancer and antiviral chemotherapy [237]. Buckley et al. presented 3-[2-(1*H*-imidazol-1-yl)ethyl]-7-methoxy-6-(1,3-oxazol-5-yl)quinoline-2,4(1*H*,3*H*)-dione as novel inhibitor of IMPDH enzyme with IC_{50} value of 86 nM for the treatment of diseases such as systemic lupus erythematosus and psoriasis [154].



The phosphodiesterase type 4 (PDE4) enzyme hydrolyzes cyclic adenosine monophosphate (cAMP) within both immune cells and cells in the central nervous system. Inhibitors of this enzyme type can be used for the treatment of respiratory diseases including asthma and chronic obstructive pulmonary diseases [238]. Generally, PDE4 enzyme inhibitors are grouped into three main classes: catechol ethers (e.g.,

rolipram), xanthines (e.g., denbufylline) and quinazolinediones (e.g., nitraquazone) (**Figure 2.4.**) [140].

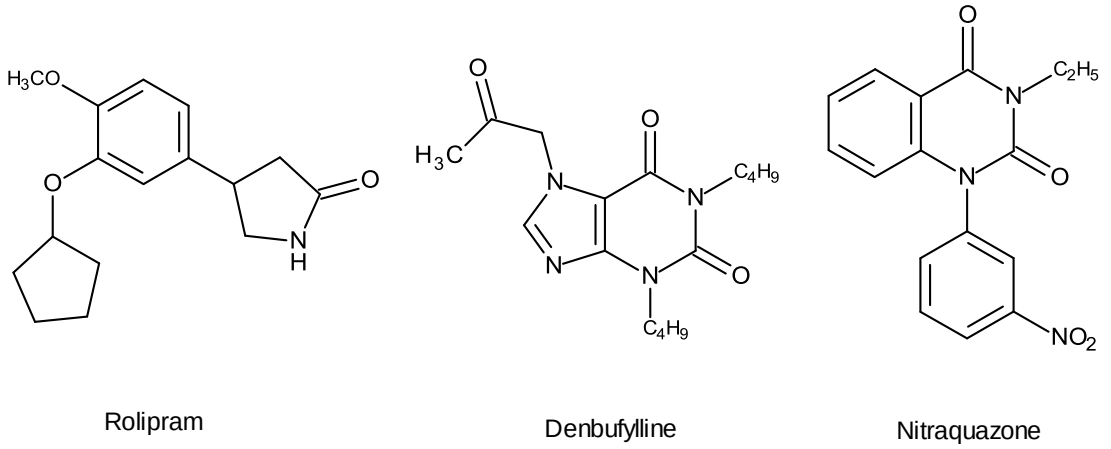
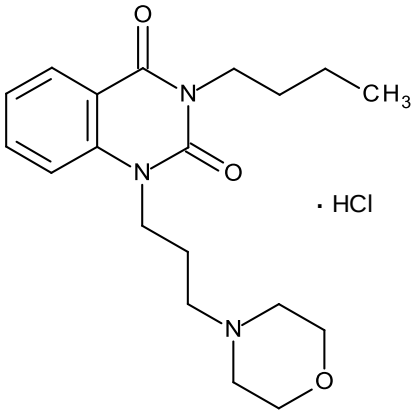
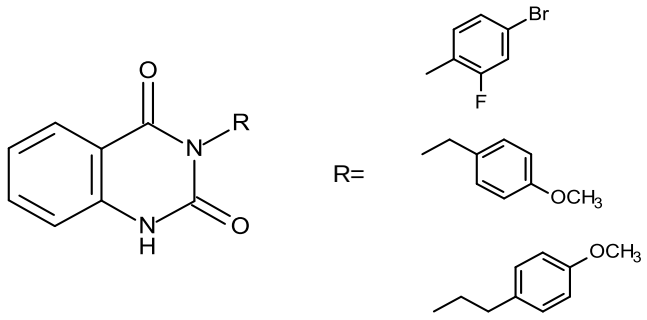


Figure 2.4. PDE4 enzyme inhibitors.

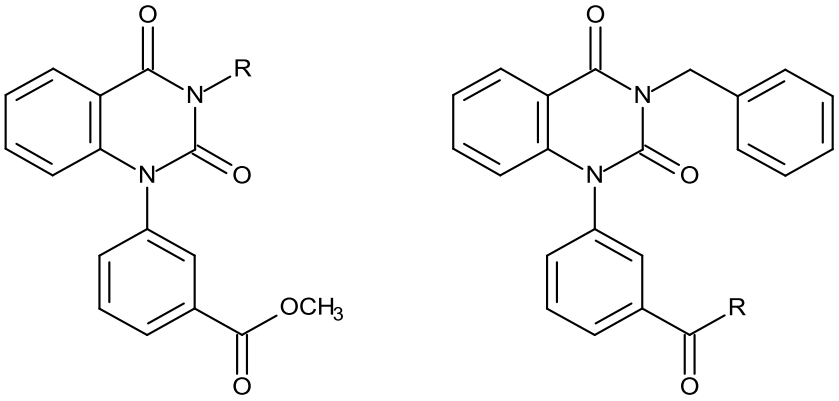
A series of 3-butylquinazolinedione derivatives were evaluated for their inhibitory activity of PDE4B enzyme. 3-butyl-1-(3-morpholin-4-ylpropyl)quinazoline-2,4(1*H*,3*H*)-dione hydrochloride was the most active compound showing inhibition of 100% better than rolipram [141].



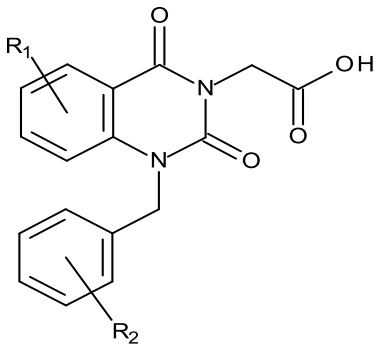
PDE7 enzyme inhibitors are potent anti-inflammatory and neuroprotective agents in primary cultures of neural cells. Redondo *et al.* presented a series of 2,4(1*H*,3*H*)-quinazolinedione derivatives with low IC_{50} values against PDE7 [142].

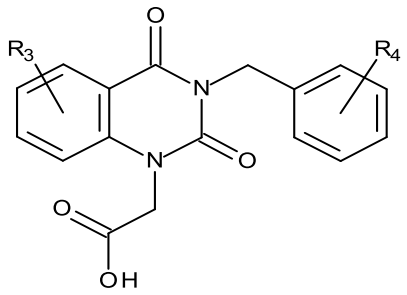


Inhibition of calcium-independent phosphodiesterase enzyme (CaIPDE) may be effective treatment for chronic diseases such as depression and inflammation. Some 1-(3-substitutedphenyl)-3-substitutedquinazoline-2,4(1*H*,3*H*)-dione showed inhibitory activity towards the CaIPDE with the IC_{50} values of less than 10 μM [143].

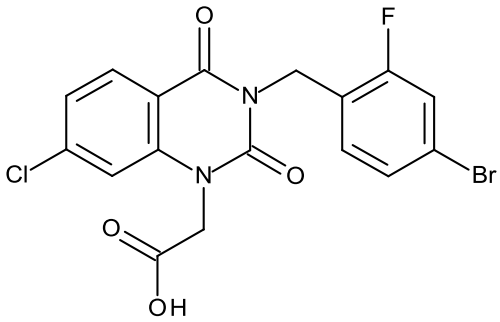


Aldose reductase is a key enzyme in pathogenesis of diabetic neuropathy. Inhibition of this enzyme prevents the progression of peripheral nerve's damage [239]. 2,4(1*H*,3*H*)-Quinazolinedione derivatives were tested for their inhibitory activity of bovine lens aldose reductase *in vitro* and their efficiency to reduce galactitol accumulation in the galactosemic rat model *in vivo*. Quinazolinedione acetic acid derivatives exhibited high *in vitro* activity (IC_{50}= 40 nM), also good oral potency [155].



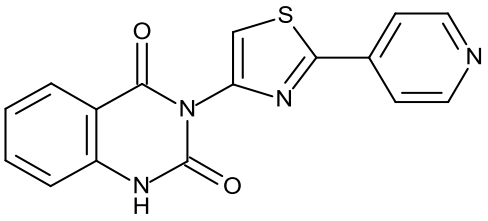


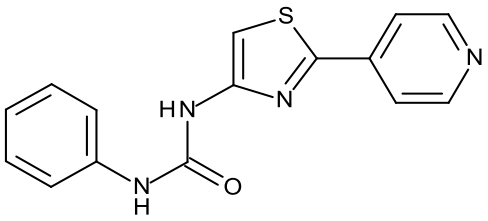
Zenarestat (FK366), 3-(4-bromo-2-fluorobenzyl)-7-chloro-3,4-dihydro-2,4-dioxo-1(2*H*)-quinazoline-1-acetic acid, is a potent aldose reductase inhibitor [156].



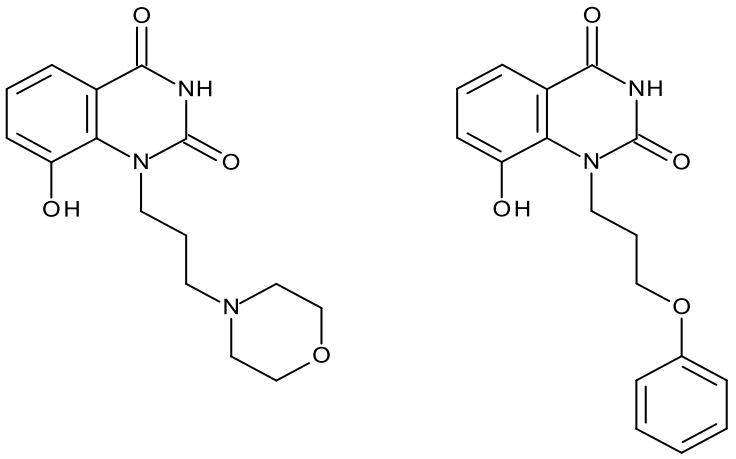
Zenarestat

2,4(1*H*,3*H*)-Quinazolinedione derivative showed an inhibition of cyclin-dependent kinase 5 (CDK5) with IC_{50} value of higher than 10.000 nM and was 3-fold less potent than its cognate acyclic urea compound [144].

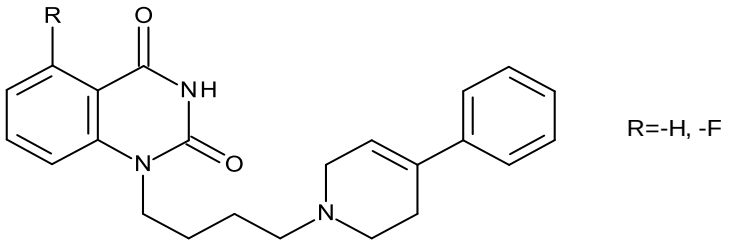




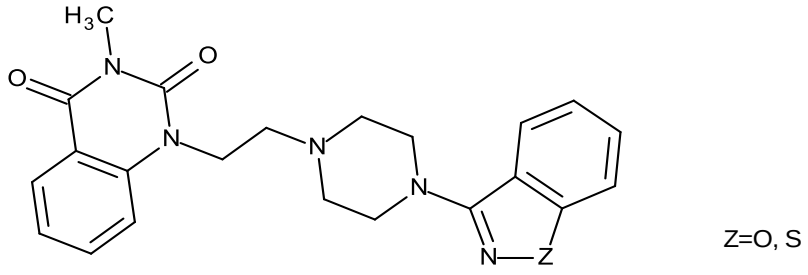
PARP is a key enzyme in many cellular processes, such as DNA replication, repair, cell proliferation and death, gene transcription, inflammation, and carcinogenesis [240]. Matsumoto *et al.* reported 3-morpholinopropyl and 3-phenoxypropylquinazoline-2,4(1*H*,3*H*)-dione compounds as good inhibitors of PARP enzyme with IC_{50} value of 85 nM and 46 nM, respectively [145].



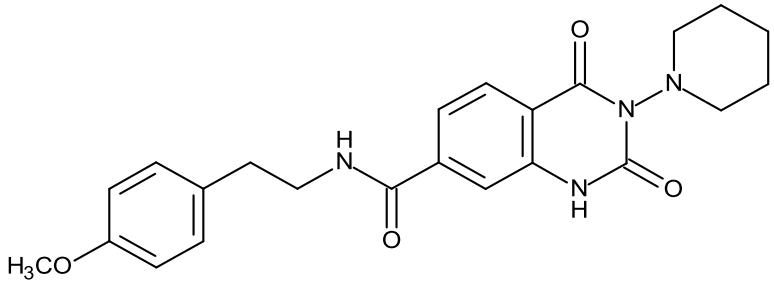
PARP-1 enzyme inhibitors were reported to be benefit neurodegenerative disorders such as cerebral ischemia or Parkinson disease. PARP-1 and PARP-2 selective inhibitors were examined with PARP enzyme assays using recombinant PARP-1 and PARP-2. Quinazolinedinone derivatives did not show any selectivity for PARP-1 over PARP-2 [146].



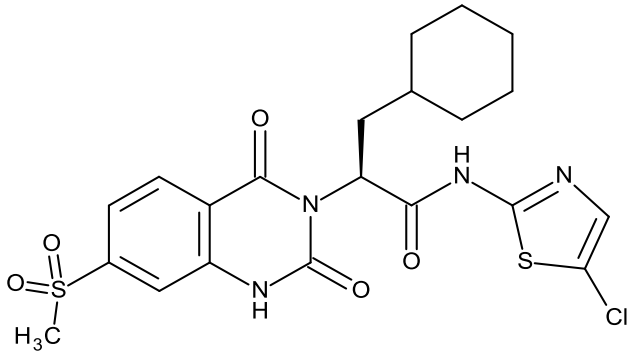
3-Benzisothiazolyl and 3-benzisoxazolylpiperazine derivatives demonstrated high affinity for the 5-HT_{2A} receptor combined with moderate to low 5-HT_{1A} and dopamine receptor D_2 affinities (5-HT_{2A}; $Ki_{isoxazol}$=11,99 nm, $Ki_{isothiazol}$=0,8 nm and $Ki_{risperidon}$=0,22 nm). Compounds were selected for further studies to be examined as potential atypical antipsychotics [147].



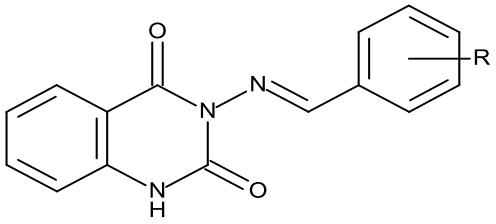
N-[2-(4-methoxyphenyl)ethyl]-2,4-dioxo-3-piperidin-1-yl-1,2,3,4-tetrahydroquinazoline-7-carboxamide showed high activity against 5-HT_{3A} receptor with the IC_{50} value of 0.8 μM and selectivity towards T-type calcium channel. 5-HT_{3A} receptor antagonists are used especially for the treatment of nausea and vomiting [148].



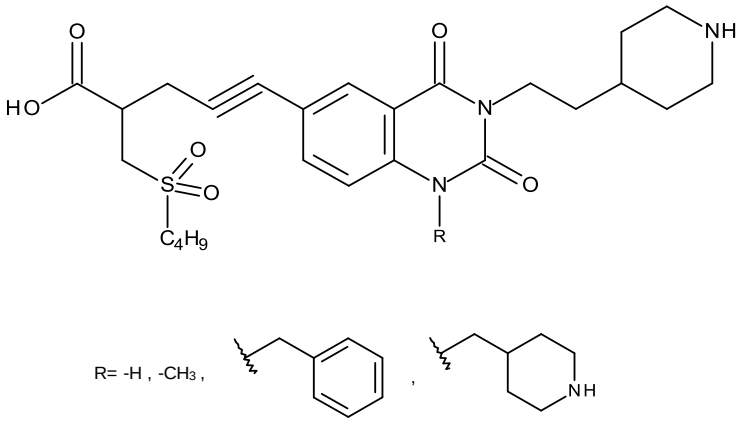
Molecules that activate glucokinase enzyme can be useful in the treatment of type-2 diabetes [241]. 5-Chlorothiazole derivative showed high activation potencies of glucokinase in enzyme and cell assays [149].



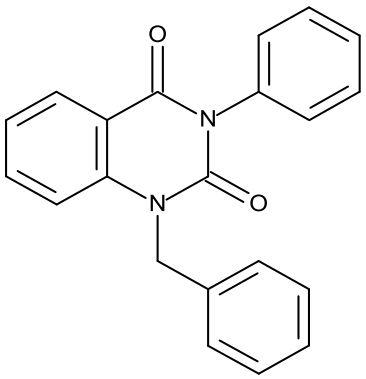
Hydrazone derivatives were exhibited moderate to good free radical scavenging activity [150].



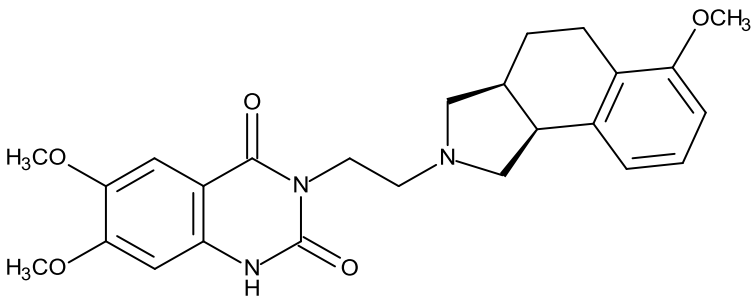
2-[(Butylsulfonyl)methyl]-6-[1-substituted-2,4-dioxo-3-(2-piperidin-4-ylethyl)-1,2,3,4-tetrahydroquinazolin-7-yl]hex-4-ynoic acid showed potent activity as platelet aggregation inhibitors [151].



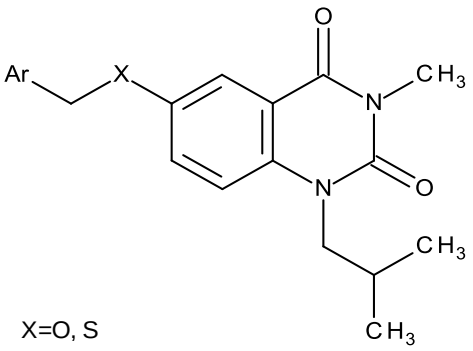
3-Phenylquinazoline-2,4(1*H*,3*H*)-dione derivatives were evaluated for their nonsteroidal progesterone receptor antagonistic activity. 1-Benzyl derivative exhibited moderate activity with IC_{50} values of 11-15 μM in alkaline phosphatase activity and reporter gene assays [152].



The alpha-1A adrenergic receptor (α_{1A} adrenoreceptor) is the most prevalent subtype of the human α_1 adrenoreceptor in the prostate. α_{1B} Adrenoreceptor also has important role in the regulation of blood pressure. With the aim of reducing side effects, finding high uroselective α_{1A}/α_{1B} agent was targeted. 6-Methoxyhexahydrobenz[*e*]isoindole derivative exhibited high selectivity on α_{1A} adrenoreceptor [242].



2,4(1*H*,3*H*)-Quinazolinedione derivatives which identified from random screening showed low micromolar (1.3-4.4 μM) potency in the nuclear factor of activated T cells-1-regulated β-galactosidase expression assay which shows interleukin-2 (IL-2) gene transcription activity. Potential agents for IL-2 gene transcription is therapeutically useful in cases of graft rejection and autoimmune diseases [153].



3. MATERIALS AND METHODS

3.1.Chemistry

3.1.1. Materials

Anthranilic acid (Riedel-de Haën), ethyl isocyanatoacetate (Sigma-Aldrich), 1-(4-chlorobenzyl)-piperazine (Sigma-Aldrich), 1-piperonylpiperazine (Alfa aesar), 1-(2-furoyl)piperazine (Sigma-Aldrich), 1-cyclohexylpiperazine (Fluka), 1-(2-cyanophenyl) piperazine (Sigma-Aldrich), 1-(diphenylmethyl)piperazine (Sigma-Aldrich), 1-benzoylpiperazine (Sigma-Aldrich), 1-(4-pyridyl)piperazine (Sigma-Aldrich), 2-amino-5-chlorobenzoic acid (Fluka), 1-(3-trifluoromethylphenyl)piperazine (Alfa aesar), 2-amino-4,5-dimethoxybenzoic acid (Sigma-Aldrich), 1-(4-methoxyphenyl)piperazine (Sigma-Aldrich) were purchased.

3.1.2. Methods of Synthesis

3.1.2.1. General Procedure A: Preparation of 2-(3-ethoxycarbonylmethylureido)-benzoic acid derivatives (Compound 1-3)

0.0265 mol of anthranilic acid or substituted anthranilic acid was dissolved in 35 ml of saturated potassium bicarbonate ($KHCO_3$) solution and stirred with 3.3 ml (0.0294 mol) of ethyl isocyanatoacetate for an hour at room temperature. The solution was acidified with concentrated hydrochloric acid and and obtained residue was filtered. The precipitate was crystallized from ethanol [236].

3.1.2.2. General Procedure B: Preparation of (substituted-2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)acetic acid derivatives (Compound 4-6)

0.0113 mol of compound **1-3** was refluxed in 30 ml of concentrated HCl for 2 hours. The mixture was cooled and diluted with water. The obtained precipitate was filtered and washed with cold water.

3.1.2.3. General Procedure C: Preparation of 3-{2-[4-(substituted)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1*H*,3*H*)-dione (Compound 7-34)

A mixture of 1 mmol compound **4-6** in 5 ml of dry dichloromethane (DCM) and 1 mmol of appropriate piperazine derivative was cooled in an ice bath. Then, a solution of 1.1 mmol of *N*,*N'*-dicyclohexylcarbodiimide (DCC) in 5 ml of dry dichloromethane was added to the mixture under nitrogen (N_2) atmosphere. Reaction mixture was stirred for 0.5 hour in an ice bath, followed by 10-16 hours at room temperature. Reaction solvent was evaporated to the dryness. Obtained residue was dissolved in hot acetonitrile then cooled in refrigerator to get the DCU (*N,N'*-dicyclohexylurea) precipitated. White crystalline DCU was removed by filtration. Liquid part was evaporated in vacuo and precipitate was crystallized from appropriate solvents.

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 FP62 capillary melting apparatus and are uncorrected.

3.1.3.2. Controls by Thin Layer Chromatography

Material:

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

Solvent systems: Three different solvent systems were prepared to use in chromatographic controls of compounds.

S.1: Ethyl acetate : n-hexane (4:1)

S.2: n-Hexane : ethanol (7:3)

S.3: Benzene : methanol (1:1)

Method:

Dragging conditions: Solvent systems were poured into chambers and waited for 24 hours to 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 Analyses

3.1.3.3.1. Infrared Spectra

Infrared Spectras were recorded on a Perkin-Elmer Spectrum One series FT-IR apparatus (Version 5.0.1), using potassium bromide pellets, the frequencies were expressed in cm^{-1}.

3.1.3.3.2. ^{1}H-NMR Spectra

The ^{1}H-NMR spectras were recorded with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA, USA), using tetramethylsilane (TMS) as the internal reference, with deuterated-dimethyl sulfoxide (DMSO-d_6) as solvent, the chemical shifts were reported in parts per million (ppm).

3.1.3.3.3. ^{13}C-NMR Spectra

^{13}C-NMR spectras were recorded with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA, USA), with dimethyl sulfoxide (DMSO) as solvent.

3.1.3.3.4. Elemental Analyse

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

3.1.3.3.5. UV Spectra

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

3.1.3.3.6. Mass Spectra

Liquid chromatography-mass spectrometry (LC-MS) spectras were recorded with a Waters 2695 Alliance Micromass ZQ instrument using electrospray ionization (ESI) technique.

3.2. Biological Assays

3.2.1. Antimicrobial Activity Test Procedure: General Disc Diffusion (Agar-Based) Method

In vitro antibacterial activities of the synthesized compounds against two gram positive bacteria *S. aureus* and *B. subtilis* were investigated by the agar-based disc diffusion method. Standard disc of ampicillin served as positive control and reference. Filter discs impregnated with 10 µl of dimethyl sulfoxide solvent were used as a negative control. The solutions of synthesized compounds (0.5 mg/ml) and ampicillin (0.01 mg/ml) were prepared in DMSO, sterilized by filtration using glass filter, and stored at 4 °C. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains and allowed to stay at 37 °C for 3 hours. Blank paper discs with a diameter of 8.0 mm were impregnated with 10 µl of the stock solutions and placed on agar. The zones of growth inhibition around the discs were measured after 18 to 24 hours of in incubation at 37 °C for bacteria. The sensitivities of the microorganism species to the compounds were determined by measuring the sizes of inhibitory zones on the agar surface around the discs [243].

3.2.2. Anticancer Activity Test Procedure: Sulforhodamine B Assay

Cells were plated in 96-well plates (1000-5000 cell/well in 200 µl) and grown for 24 hours at 37 °C before being treated with various concentrations of the tested compounds (from 2.5 to 40 µM). After 72 hours of incubation the medium was aspirated, washed once with PBS ($CaCl_2$-, $MgCl_2$- free) (Gibco, Invitrogen), and then

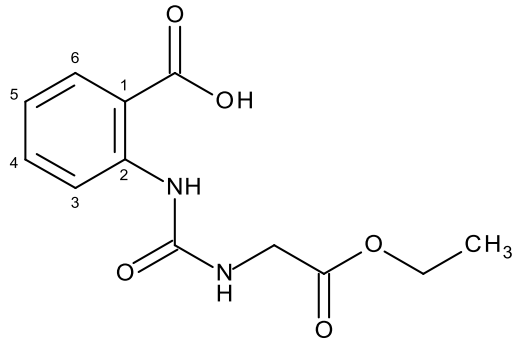
50 µl of a cold (4 °C) solution of 10% (v/v) trichloroacetic acid (Merck) was added. Microplates were left for 1 hour at 4°C. After aspiration of the solution, plates were washed five times with deionized water and left to dry. 50 µl of a 0.4% (w/v) of sulforhodamine B solution was removed and the plates were washed five times with 1% acetic acid before air-drying. Bound sulforhodamine B solubilize in a 200 µl 10 mM Tris-base solution and the plates were left on a plate shaker for 10 minutes. The absorbance was read in a 969-well plate reader at 515 nm.

4. EXPERIMENTAL SECTION

4.1. Chemical Data

2-[3-(2-Ethoxy-2-oxoethyl)ureido]benzoic acid (Compound 1)

(CAS Registry Number: 78754-95-7)



A mixture of 2-aminobenzoic acid (7.056 g, 0.026 mol) and ethyl isocyanatoacetate (3.3 ml, 0.0294 mol) were reacted as described in the general procedure A to yield 2.117 g (30%) of white needle crystals. The compound is soluble in cold acetone, DMSO, DCM and hot ethanol.

M.p.: 170 °C (171-172.5 °C [184]).

Rf values: 0.09 (S.1), 0.27 (S.2), 0.79 (S.3).

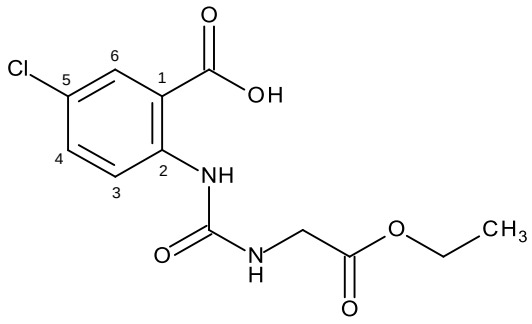
UV (MeOH, λ_{max}, nm): 224 (log ε : 4.48), 249 (log ε : 4.11), 312 (log ε : 3.62).

IR (KBr, ν_{max}, cm^{-1}): 3398 (N-H str.), 2993 (aliphatic C-H str.), 1725 (ester C=O str.), 1683 (carboxylic acid C=O str.), 1663 (amide I band, C=O str.), 1588 (amide II band, N-H bending), 1536 (C=C str.).

^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 1.17-1.19 (t, 3H, CH_3, J=6.8 Hz), 3.8 (dd, 2H, NH**C**$\mathbf{H_2}$), 4.1 (q, 2H, **C**$\mathbf{H_2}$$CH_3$), 6.95 (m, 1H, H^5), 7.45 (m, 1H, H^4), 7.85 (t, 1H, CO**N**$\mathbf{H}$$CH_2$), 7.9 (dd, 1H, H^3), 8.35 (d, 1H, H^6), 10.2 (s, 1H, C**N**$\mathbf{H}$CO), 13.2 (s, 1H, COOH).

5-Chloro-2-[3-(2-ethoxy-2-oxoethyl)ureido]benzoic acid (Compound 2)

(CAS Registry Number: 1308637-76-4)



A mixture of 2-amino-5-chlorobenzoic acid (4.547 g, 0.026 mol) and ethyl isocyanatoacetate (0.0294 mol, 3.3 ml) were reacted as described in the general procedure A to yield 2.950 g (37%) of light yellow needle crystals. The compound is soluble in cold acetone, DMSO, DCM and hot ethanol.

M.p.: 178.9 °C.

Rf values: 0.06 (S.1), 0.19 (S.2), 0.82 (S.3).

UV (MeOH, λ_{max}, nm): 219 (log ε : 4.42), 257 (log ε : 4.09), 344 (log ε : 3.52).

IR (KBr, ν_{max}, cm^{-1}): 3336 (N-H str.), 3041 (aromatic C-H str.), 2980, 2928 (aliphatic C-H str.), 1732 (ester C=O str.), 1690 (carboxylic acid C=O str.), 1650 (amide I band, C=O str.), 1582 (amide II band, N-H bending).

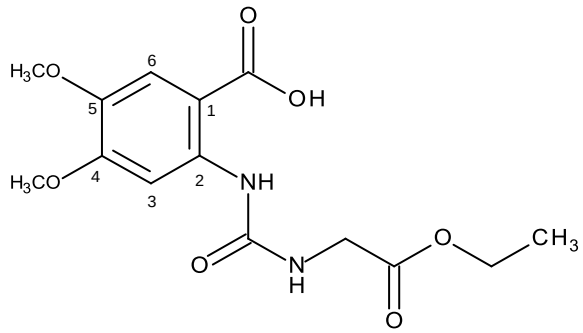
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 1.18 (t, 3H, CH_3, J=7.2 Hz), 3.81(d, 2H, NH**C**$\mathbf{H_2}$CO, J=6.0 Hz), 4.10 (q, 2H, **C**$\mathbf{H_2}$$CH_3$, J=7.2 Hz), 7.53 (q, 1H, H^4, J=8.8 Hz, J=2.8 Hz), 7.83 (d, 1H, H^6, J=2.4 Hz), 7.95 (s, 1H, CO**NH**CH_2), 8.37 (d, 1H, H^3, J=8.8 Hz), 10.16 (s, 1H, C**NH**CO), 13.60 (s, 1H, COOH).

Elemental analysis for $C_{12}H_{13}ClN_2O_5$ (300.6948 g/mol);

Calculated: 47.93 (C%), 4.36 (H%), 9.32 (N%).

Found: 47.53 (C%), 4.00 (H%), 9.39 (N%).

2-[3-(2-Ethoxy-2-oxoethyl)ureido]-4,5-dimethoxybenzoic acid (Compound 3)



A mixture of 2-amino-4,5-dimethoxybenzoic acid (5.226 g, 0.026 mol) and ethyl isocyanatoacetate (0.0294 mol, 3.3 ml) were reacted as described in the general procedure A to yield 4.688 g (54%) of light brown colored needle shaped crystals. The compound is soluble in cold acetone, DMSO, DCM and hot ethanol.

M.p.: 172.5 °C.

Rf values: 0.03 (S.1), 0.29 (S.2), 0.85 (S.3).

UV (MeOH, λ_{max}, nm): 219 (log ε : 4.41), 257 (log ε : 4.08), 344 (log ε : 3.52).

IR (KBr, ν_{max}, cm^{-1}): 3397 (N-H str.), 3020 (aromatic C-H str.), 2912 (aliphatic C-H str.), 1738 (ester C=O str.), 1684 (carboxylic acid C=O str.), 1615 (amide I band, C=O str.), 1539 (C=C str.).

^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 1.20 (t, 3H, $OCH_2C\mathbf{H_3}$, *J*=7.2 Hz), 3.73 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.82 (d, 2H, $NH\mathbf{CH_2}$, *J*=6.0 Hz), 4.11 (q, 2H, $OC\mathbf{H_2}CH_3$, *J*=7.2 Hz), 7.37 (s, 1H, H^6), 7.92 (s, 1H, $\mathbf{NH}CH_2$), 8.16 (s, 1H, H^3), 10.34 (s, 1H, C**NH**CO), 13.07 (s, 1H, COOH).

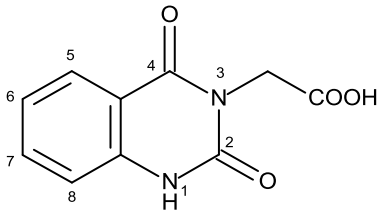
Elemental analysis for $C_{14}H_{18}N_2O_7.1/2H_2O$ (326.302 g/mol);

Calculated: 50.15 (C%), 5.71 (H%), 8.35 (N%).

Found: 49.73 (C%), 5.30 (H%), 8.39 (N%).

2-(2,4-Dioxo-1,2-dihydroquinazolin-3(4*H*)-yl)acetic acid (Compound 4)

(CAS Registry Number: 78754-94-6)



It was obtained by starting compound **1** (3 g, 0.011 mol) as described in the general procedure B to yield 1.742 g (73%) of white powdered compound. The compound is soluble in cold acetone, ethanol, DMSO and DCM.

M.p.: 295 °C (290-292 °C [184], 297-299 °C [244]).

Rf values: 0.01 (S.1), 0.07 (S.2), 0.50 (S.3).

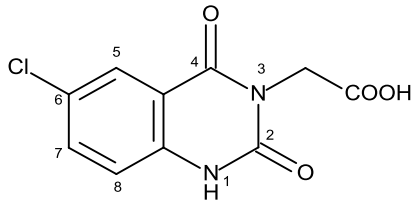
UV (MeOH, λ_{max}, nm): 220 (log ε : 4.43), 241 (log ε : 3.86), 311 (log ε : 3.43).

IR (KBr, ν_{max}, cm^{-1}): 3285 (hydrogen bonded N-H str.), 3010 (aromatic C-H str.), 2945 (aliphatic C-H str.), 1716 (carboxylic acid C=O str.), 1657, 1625 (amide I band, C=O str.), 1494 (C=C str.).

^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 4,56 (s, 2H, CH_2), 7.21-7.27 (m, 2H, H^6 and H^8), 7.87-7.73 (m, 1H, H^7), 7.95 (dd, 1H, H^5, *J*=8.0 Hz, *J*=1.2 Hz), 11.62 (s, 1H, NH).

2-(6-Chloro-2,4-dioxo-1,2-dihydroquinazolin-3(4*H*)-yl)acetic acid (Compound 5)

(CAS Registry Number: 81438-22-4)



Compound **2** (3.4 g, 0.011 mol) was reacted as described in the general procedure B to yield 1.027 g (36%) of light yellow needle shaped crytalline compound. The compound is soluble in cold acetone, DMSO, DCM and hot methanol, and ethanol.

M.p.: 300 °C (dec) (327-329 °C [245][244]).

Rf values: 0.01 (S.1), 0.02 (S.2), 0.55 (S.3).

UV (MeOH, λ_{max}, nm): 222 (log ε : 4.51), 245 (log ε : 3.95), 322 (log ε : 3.60).

IR (KBr, ν_{maks}, cm^{-1}): 3071 (aromatic C-H str.), 2930 (aliphatic C-H str.), 1716 (carboxylic acid C=O str.), 1656 (amide I band, C=O str.).

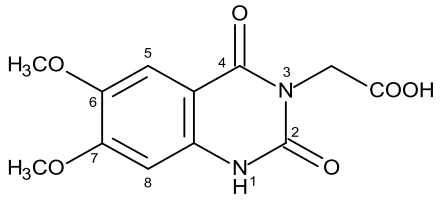
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 4.56 (s, 2H, CH_2), 7.25 (d, 1H, H^8, *J*=8.8 Hz), 7.77 (dd, 1H, H^7, *J*=8.8 Hz, *J*=2.8 Hz), 7.89 (d, 1H, H^5, *J*=2.8 Hz), 11.78 (s, 1H, NH), 13.09 (s, 1H, COOH).

Elemental analysis for $C_{10}H_7ClN_2O_4$ (254.6264 g/mol);

Calculated: 47.17 (C%), 2.77 (H%), 11.00 (N%).

Found: 47.13 (C%), 3.06 (H%), 10.98 (N%).

2-(6,7-Dimethoxy-2,4-dioxo-1,2-dihydroquinazolin-3(4*H*)-yl)acetic acid (Compound 6)



Compound **3** (3.69 g, 0.0113 mol) was reacted as described in the general procedure B to give 1.251 g yield (40 %) of light creamy colored powdered crystalline compound. The compound is soluble in cold acetone, ethanol, DMSO and DCM.

M.p.: 300 °C (dec).

Rf values: 0.01 (S.1), 0.08 (S.2), 0.66 (S.3).

UV (MeOH, λ_{max}, nm): 235 (log ε : 4.49), 259 (log ε : 3.86), 344 (log ε : 3.72).

IR (KBr) v_{maks} (cm^{-1}): 3017 (aromatic C-H str.), 2959 (aliphatic C-H str.), 1702 (carboxylic acid C=O str.), 1620 (amide I band, C=O str.), 1513 (C=C str.), 1468 (aliphatic C-H bending).

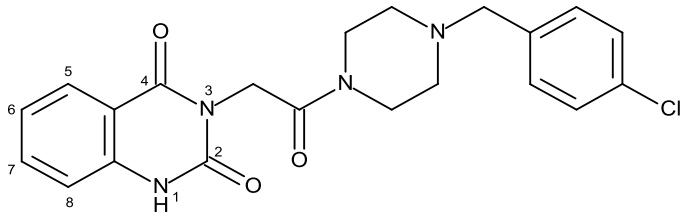
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.54 (s, 2H, CH_2), 6.71 (s, 1H, H^8), 7.29 (s, 1H, H^5), 11.38 (s, 1H, NH), 12.96 (s, 1H, COOH).

Elemental analysis for $C_{12}H_{12}N_2O_6$ (280.2336 g/mol);

Calculated: 51.43 (C%), 4.32 (H%), 10.00 (N%).

Found: 51.24 (C%), 4.03 (H%), 9.94 (N%).

3-{2-[4-(4-Chlorobenzyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1*H*,3*H*)-dione (Compound 7)



Compound **4** (0.220 g, 1 mmol) and 1-(4-chlorobenzyl)piperazine (0.211 g, 1 mmol) were reacted as described in the general procedure C and crystallized from methanol:ether mixture to yield 0.091 g (11%) of white powdered compound. The compound is soluble in cold acetone, ethanol, methanol, DMSO, DCM and unsoluble in water.

M.p.: 300 °C (dec).

Rf values: 0.01 (S.1), 0.02 (S.2), 0.55 (S.3).

UV (MeOH, λ_{max}, nm): 219 (log ε : 4.73), 242 (log ε : 4.40), 311 (log ε : 3.88).

IR (KBr, ν_{max}, cm^{-1}): 3276 (hydrogen bonded N-H str.), 3030 (aromatic C-H str.), 2928, 2850 (aliphatic C-H str.), 1729, 1639 (amide I band, C=O str.), 1491 (C=C str.).

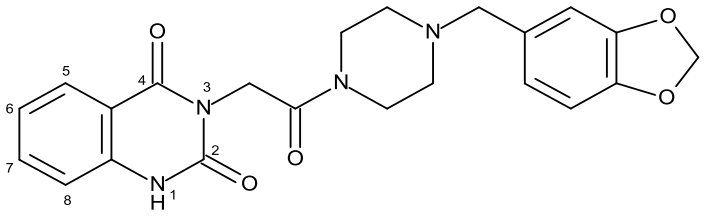
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.34 (t, 2H, CH_2 piperazine, *J*=4.4 Hz), 2.43 (t, 2H, CH_2 piperazine, *J*=4.4 Hz), 3.44 (t, 2H, CH_2 piperazine, *J*=4.4 Hz), 3.52 (s, 2H, CH_2 benzyl), 3.57 (t, 2H, CH_2 piperazine, *J*=4.4 Hz), 4.74 (s, 2H, CH_2CO), 7.20-7.24 (m, 2H, H^6 and H^8), 7.35-7.42 (dd, 4H, *J*=8.4 Hz, *J*=1.6 Hz, aromatic CH benzyl), 7.67-7.71 (m, 1H, H^7), 7.90-7.93 (m, 1H, H^5), 11.53 (bs, 1H, NH).

Elemental analysis for $C_{21}H_{21}ClN_4O_3$ (412.8693 g/mol);

Calculated: 61.09 (C%), 5.13 (H%),13.57 (N%).

Found: 60.98 (C%), 5.013 (H%),13.52 (N%).

3-{2-[4-(Benzo[d][1,3]dioxol-5-ylmethyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1*H*,3*H*)-dione (Compound 8) (CAS Registry Number: 879570-04-4)



Compound **4** (0.220 g, 1 mmol) and 1-piperonylpiperazine (0.220 g, 1 mmol) were reacted as described in the general procedure C and crystallized from ethanol to yield 0.028 g (7%) of white powdered compound. The compound is soluble in cold acetone, ethanol, methanol, DMSO, DCM and unsoluble in water.

M.p.: 218.2 °C.

Rf values: 0.01 (S.1), 0.75 (S.2), 0.98 (S.3).

UV (MeOH, λ_{max}, nm): 219 (log ε : 4.77), 241 (log ε : 4.06), 311 (log ε : 3.34).

IR (KBr, ν_{max}, cm^{-1}): 3292 (hydrogen bonded N-H str.), 3071, 3009 (aromatic C-H), 2928, 2850 (aliphatic C-H str.), 1731 and 1643 (amide I band, C=O str.), 1494 (C=C str.).

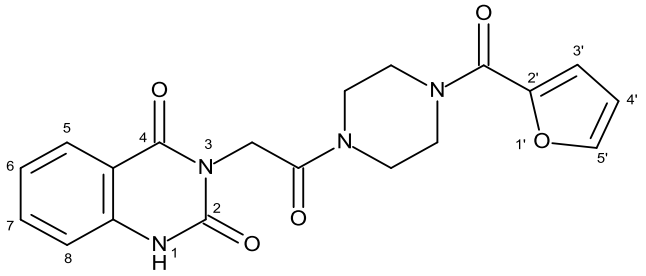
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.32 (t, 2H, CH_2 piperazine, J=4.8 Hz), 2.42 (t, 2H, CH_2 piperazine, J=4.8 Hz), 3.44 (m, 4H, CH_2 piperazine and OCH_2O), 3.56 (t, 2H, CH_2 piperazine, J=4.8 Hz), 4.73 (s, 2H, CH_2CO), 6.0 (s, 2H, N**CH_2**C_6H_5), 6.76-7.25 (m, 3H, CH benzodioxol), 7.20-7.25 (m, 2H, H^2 and H^4), 7.67-7.71 (m, 1H, H^3), 7.90-7.93 (m, 1H, H^1), 11.53 (bs, 1H, NH).

Elemental analysis for $C_{22}H_{22}N_4O_5$. 1/2 H_2O (422.434 g/mol);

Calculated: 61.24 (C%), 5.37 (H%),12.99 (N%).

Found: 61.13 (C%), 5.064 (H%),12.88 (N%).

3-{2-[4-(2-Furoyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1*H*,3*H*)-dione (Compound 9)



Compound **4** (0.220 g, 1 mmol) and 1-(2-furoyl)piperazine (0.180 g, 1 mmol) were reacted as described in the general procedure C and crystallized from ethanol:n-hexane mixture to yield 0.320 g (84%) of light yellow powdered compound. The compound is soluble in cold acetone, ethanol, methanol, DMSO, DCM and unsoluble in water.

M.p.: 228 °C.

Rf values: 0.06 (S.1), 0.32 (S.2), 0.96 (S.3).

UV (MeOH, λ_{max}, nm): 219 (log ε : 4.88), 243 (log ε : 4.48), 310 (log ε : 3.82).

IR (KBr, ν_{max}, cm^{-1}): 3327 (hydrogen bonded N-H str.), 3061 (aromatic C-H str.), 2928, 2851 (aliphatic C-H str.), 1718, 1655, 1625 (amide I band, C=O str.), 1571 (amide II band, N-H bending), 1492 (C=C str.), 1438 (aliphatic C-H bending), 1244 (C-O str.).

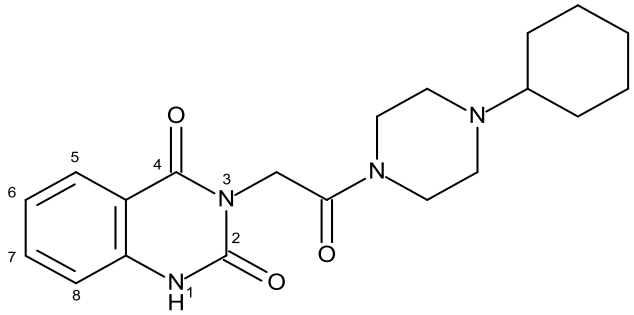
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.54 (t, 4H, CH_2 piperazine), 3.70 (t, 4H, CH_2 piperazine), 4.80 (s, 2H, CH_2CO), 6.65 (t, 1H, $H^{4'}$, J=1.6 Hz), 7.05-7.06 (m, 1H, $H^{3'}$), 7.21-7.25 (m, 2H, H^6 and H^8), 7.68-7.71 (m, 1H, H^7), 7.88-7.94 (m, 2H, H^5 and $H^{5'}$), 11.55 (bs, 1H, NH).

Elemental analysis for $C_{19}H_{18}N_4O_5$. 1/9 H_2O (382.3702 g/mol);

Calculated: 59.37 (C%), 4.78 (H%),14.58 (N%).

Found: 59.18 (C%), 4.20 (H%),14.35 (N%).

3-[2-(4-Cyclohexylpiperazin-1-yl)-2-oxoethyl]quinazoline-2,4(1*H*,3*H*)-dione (Compound 10) (CAS Registry Number: 1235121-27-3)



Compound **4** (0.220 g, 1 mmol) and 1-cyclohexylpiperazine (0.168 g, 1 mmol) were reacted as described in the general procedure C and crystallized from methanol:ether mixture to yield 0.023 g (6%) of white powdered compound. The compound is soluble in cold acetone, ethanol, methanol, DMSO, DCM and unsoluble in water.

M.p.: 260.1 °C.

Rf values: 0.01 (S.1), 0.41 (S.2), 0.84 (S.3).

UV (MeOH, λ_{max}, nm): 218 (log ε : 4.77), 242 (log ε : 4.02), 309 (log ε : 3.71).

IR (KBr) ν_{max}, cm^{-1}): 3327 (hydrogen bonded N-H str.), 3060 (aromatic C-H), 2928, 2852 (aliphatic C-H str.), 1719, 1657, 1623 (amide I band, C=O str.), 1493 (C=C str.).

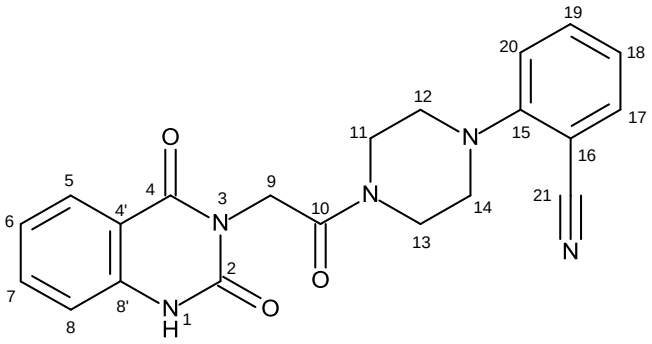
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 1.06-1.21 (m, 5H, CH_2 cyclohexyl), 1.54-1.72 (m, 5H, CH_2 cyclohexyl), 2.26-2.31 (m, 1H, CH cyclohexyl), 2.42 (t, 2H, CH_2 piperazine, *J*=4.8 Hz), 2.52 (t, 2H, CH_2 piperazine, *J*=4.8 Hz), 3.38 (t, 2H, CH_2 piperazine, *J*=4.8 Hz), 3.49 (t, 2H, CH_2 piperazine, *J*=4.8 Hz), 4.70 (s, 2H, CH_2CO), 7.17-7.22 (m, 2H, H^6 and H^8), 7.64-7.68 (m, 1H, H^7), 7.89 (dd, 1H, H^5, *J*=7.6 Hz, *J*=1.6 Hz), 11.49 (bs, 1H, NH).

Elemental analysis for $C_{20}H_{26}N_4O_3$ (370.4456 g/mol);

Calculated: 64.84 (C%), 7.07 (H%),15.12 (N%).

Found: 65.26 (C%), 7.28 (H%),15.35 (N%).

2-{4-[2-(2,4-Dioxo-1,2-dihydroquinazolin-3(4*H*)-yl)acetyl]piperazin-1-yl}benzonitrile (Compound 11)



Compound **4** (0.220 g, 1 mmol) and 1-(2-cyanophenyl)piperazine (0.187 g, 1 mmol) were reacted as described in the general procedure C and crystallized from methanol:ether mixture to yield 0.173 g (45%) of yellow powdered compound. The compound is soluble in cold acetone, ethanol, methanol, DMSO, DCM and unsoluble in water.

M.p.: 256.4 °C.

Rf values: 0.32 (S.1), 0.46 (S.2), 0.97 (S.3).

UV (MeOH, λ_{max}, nm): 219 (log ε : 4.83), 242 (log ε : 4.10), 312 (log ε : 3.72).

IR (KBr, ν_{maks}, cm^{-1}): 3273 (hydrogen bonded N-H str.), 3072, 3003 (aromatic C-H str.), 2962, 2872, 2824 (aliphatic C-H str.), 2214 (aromatic C≡N str.), 1727, 1670, 1640 (amide I band, C=O str.), 1489 (C=C str.), 1455 (aliphatic C-H bending).

^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.13 (t, 2H, CH_2 piperazine), 3.25 (t, 2H, CH_2 piperazine), 3.63 (t, 2H, CH_2 piperazine), 3.77 (t, 2H, CH_2 piperazine), 4.81 (s, 2H, CH_2CO), 7.13 (t, 1H, H^{18}, *J*=7.6 Hz), 7.19-7.23 (m, 3H, H^6, H^8 and H^{20}), 7.59-7.73 (m, 3H, H^7, H^{17} and H^{19}), 7.92 (dd, 1H, H^5, *J*=7.2 Hz, *J*=1.2 Hz), 11.47 (bs, 1H, NH).

^{13}C-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 41.91 (C^{11}), 42.12 (C^{13}), 44.77 (C^9), 51.31 (C^{12}), 51.98 (C^{14}), 105.59 (C^{16}), 113.90 (C^{20}), 115.68 (C^8), 118.57 ($C^{4'}$),

119.88 (C^{18}), 122.95 (C^{21}), 123.11 (C^{6}), 127.85 (C^{5}), 134.67 (C^{17}), 134.84 (C^{19}), 135.67 (C^{7}), 139.88 ($C^{8'}$), 150.48 (C^{2}), 155.32 (C^{15}), 162.19 (C^{4}), 165.41 (C^{10}).

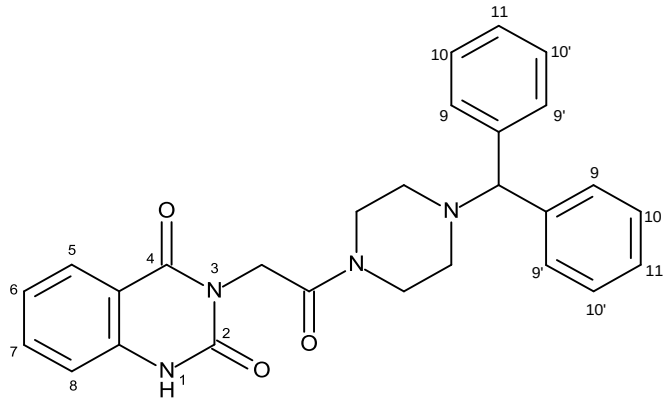
MS (ESI+, m/z): 390.2 ([M^{+}], base peak), 162.7 ($C_8H_6N_2O_2$), 229.2 ($C_{13}H_{15}N_3O$).

Elemental analysis for $C_{21}H_{19}N_5O_3$ (389.4075 g/mol);

Calculated: 64.77 (C%), 4.92 (H%), 17.98 (N%).

Found: 65.54 (C%), 4.70 (H%), 17.64 (N%).

3-{2-[4-(Diphenylmethyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1*H*,3*H*)-dione (Compound 12)



Compound **4** (0.220 g, 1 mmol) and 1-(diphenylmethyl)piperazine (0.252 g, 1 mmol) were reacted as described in the general procedure C and crystallized from acetone:ether mixture to yield 0.080 g (18%) of white powdered compound. The compound is soluble in cold ethanol, methanol, DMSO, DCM, hot acetone and unsoluble in water.

M.p.: 300 °C (dec).

Rf values: 0.58 (S.1), 0.74 (S.2), 0.98 (S.3).

UV (MeOH, λ_{max}, nm): 219 (log ε : 4.80), 242 (log ε : 4.04), 311 (log ε : 3.47).

IR (KBr, v_{max}, cm^{-1}): 3207 (hydrogen bonded N-H str.), 3061, 3025 (aromatic C-H str.), 2924, 2859, 2805 (aliphatic C-H str.), 1727, 1655 (amide I band, C=O str.), 1492 (C=C str.).

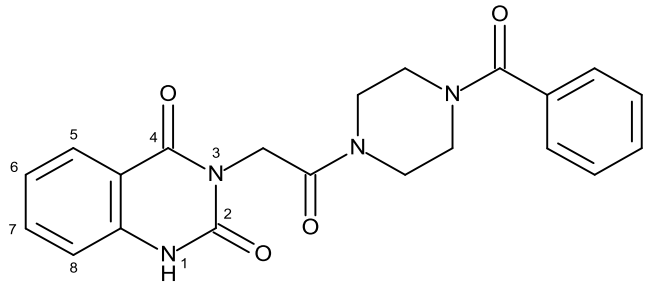
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.29 (t, 2H, CH_2 piperazine), 2.37 (t, 2H, CH_2 piperazine), 3.47 (t, 2H, CH_2 piperazine), 3.60 (t, 2H, CH_2 piperazine), 4.38 (s, 1H, CH), 4.71 (s, 2H, CH_2CO), 7.19-7.24 (m, 2H, H^6 and H^8), 7.30-7.34 (m, 6H, H^{10}, $H^{10'}$ and H^{11}), 7.46 (d, 4H, H^9 and $H^{9'}$, *J*=7.6 Hz), 7.66-7.70 (m, 1H, H^7), 7.91 (q, 1H, H^5, *J*=8.0 Hz, *J*=1.2 Hz), 11.51 (bs, 1H, NH).

Elemental analysis for $C_{27}H_{26}N_4O_3$. 1/3 H_2O (454.5205 g/mol);

Calculated: 70.42 (C%), 5.84 (H%), 12.17 (N%).

Found: 70.37 (C%), 5.81 (H%), 12.12 (N%).

3-[2-(4-Benzoylpiperazin-1-yl)-2-oxoethyl]quinazoline-2,4(1*H*,3*H*)-dione (Compound 13)



Compound **4** (0.220 g, 1 mmol) and 1-benzoylpiperazine (0.190 g, 1 mmol) were reacted as described in the general procedure C and crystallized from methanol:ether mixture to yield 0.110 g (28%) of white powdered compound. The compound is soluble in cold acetone, DMSO, DCM and hot methanol, ethanol and unsoluble in water.

M.p.: 257 °C.

Rf values: 0.07 (S.1), 0.39 (S.2), 0.95 (S.3).

UV (MeOH, λ_{max}, nm): 219 (log ε : 4.97), 240 (log ε : 4.16), 310 (log ε : 3.73).

IR (KBr, ν_{max}, cm^{-1}): 3274 (hydrogen bonded N-H str.), 3065, 3001 (aromatic C-H str.), 2959, 2911, 2860 (aliphatic C-H str.), 1726, 1676, 1617 (amide I band, C=O str.), 1492 (C=C str.).

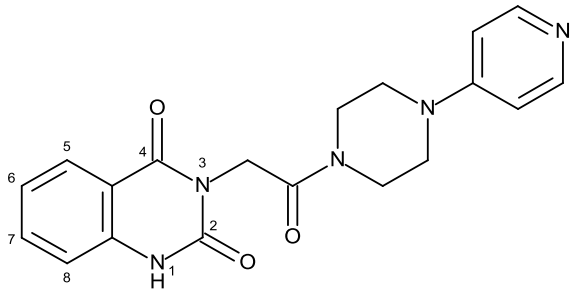
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS) δ ppm: 3.30-3.60 (m, 8H, CH_2 piperazine), 4.79 (s, 2H, CH_2CO), 7.20-7.25 (m, 2H, H^6 and H^8), 7.45-7.49 (m, 5H, CH benzoyl), 7.67-7.72 (m, 1H, H^7), 7.93 (dd, 1H, H^5, J=4 Hz, J=1.2 Hz), 11.55 (bs, 1H, NH).

Elemental analysis for $C_{21}H_{20}N_4O_4$ (392.4081 g/mol);

Calculated: 64.28 (C%), 5.14 (H%), 14.28 (N%).

Found: 64.12 (C%), 4.75 (H%), 14.08 (N%).

3-[2-Oxo-2-(4-pyridin-4-ylpiperazin-1-yl)ethyl]quinazoline-2,4(1*H*,3*H*)-dione (Compound 14)



Compound **4** (0.220 g, 1 mmol) and 1-(4-pyridyl)piperazine (0.163 g, 1 mmol) were reacted as described in the general procedure C and crystallized from methanol:ether mixture to yield 0.024 g (7%) of white powdered compound. The compound is soluble in cold acetone, DMSO, DCM and hot methanol, ethanol and unsoluble in water.

M.p.: 300 °C (dec).

Rf values: 0.01 (S.1), 0.01 (S.2), 0.25 (S.3).

UV (MeOH, λ_{max}, nm): 218 (log ε : 4.70), 242 (log ε : 4.19), 308 (log ε : 3.52).

IR (KBr, ν_{maks}, cm^{-1}): 3429 (free N-H str.), 3178 (hydrogen bonded N-H str.), 3089, 3003 (aromatic C-H str.), 2944 (aliphatic C-H str.), 1708, 1659 (amide I band, aliphatic C=O str.), 1514 (amide II band, N-H bending), 1514 (C=C str.).

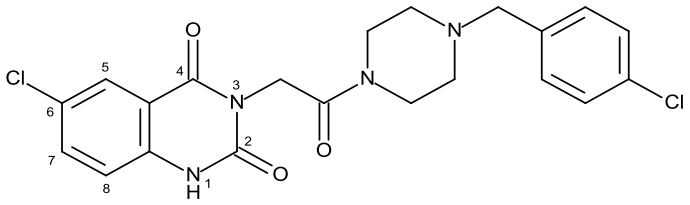
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.35 (t, 2H, CH_2 piperazine), 3.45 (t, 2H, CH_2 piperazine), 3.58 (t, 2H, CH_2 piperazine), 3.75 (t, 2H, CH_2 piperazine), 4.80 (s, 2H, CH_2CO), 6.85 (dd, 2H, pyridine CH, J=4.0 Hz, J=1.2 Hz), 7.23 (m, 2H, H^6 and H^8), 7.70 (m, 1H, H^7), 7.95 (dd, 1H, H^5), 8.20 (dd, 2H, pyridine CH), 11.55 (bs, 1H, NH).

Elemental analysis for $C_{19}H_{19}N_5O_3$. 2/3 H_2O (365.3861 g/mol);

Calculated: 60.47 (C%), 5.43 (H%), 18.56 (N%).

Found: 60.27 (C%), 5.49 (H%), 18.65 (N%).

3-{2-[4-(4-Chlorobenzyl)piperazin-1-yl]-2-oxoethyl}-6-chloro-quinazoline-2,4(1*H*,3*H*)-dione (Compound 15)



Compound **5** (0.255 g, 1 mmol) and 1-(4-chlorobenzyl)piperazine (0.211 g, 1 mmol) were reacted as described in the general procedure C and the crude product was crystallized from methanol:ether mixture to yield 0.039 g (9%) of white powdered compound. The compound is soluble in cold acetone, DMSO and hot methanol, ethanol and unsoluble in water.

M.p.: 300 °C (dec).

Rf values: 0.15 (S.1), 0.78 (S.2), 0.95 (S.3).

UV (MeOH, λ_{max}, nm): 223 (log ε : 4.56), 246 (log ε : 4.10), 323 (log ε : 3.57).

IR (KBr) v_{maks} (cm^{-1}): 3503 (free N-H str.), 3060 (aromatic C-H str.), 2916 (aliphatic C-H str.), 1726, 1662 (amide I band, C=O str.), 1457 (aliphatic C-H bending).

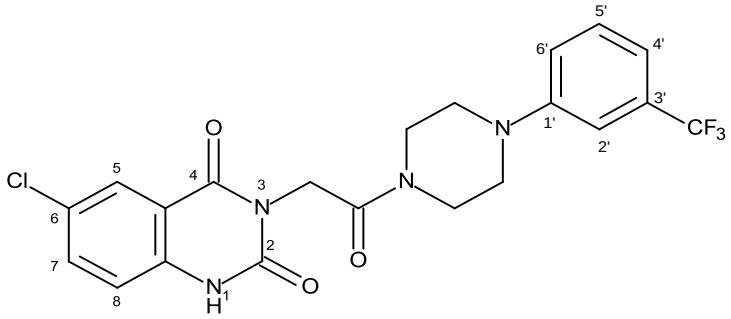
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.32 (t, 2H, CH_2 piperazine, *J*=4.4 Hz), 2.41 (t, 2H, CH_2 piperazine, *J*=4.4 Hz), 3.42 (t, 2H, CH_2 piperazine, *J*=4.8 Hz), 3.50 (s, 2H, CH_2 benzyl), 3.54 (t, 2H, CH_2 piperazine, *J*=4.4 Hz), 4.71 (s, 2H, CH_2CO), 7.22 (d, 1H, H^8, *J*=9.2 Hz), 7.33-7.40 (m, 4H, aromatic CH benzyl), 7.75 (dd, 1H, H^7, *J*=8.8 Hz, *J*=2.8 Hz), 7.84 (d, 1H, H^5, *J*=2.4 Hz), 11.67 (bs, 1H, NH).

Elemental analysis for $C_{21}H_{20}Cl_2N4O_3$. 1/2 H_2O (447.3141 g/mol);

Calculated: 55.27 (C%), 4.64 (H%), 12.28 (N%).

Found: 55.00 (C%), 4.55 (H%), 12.29 (N%).

6-Chloro-3-(2-oxo-2-{4-[3-(trifluoromethyl)phenyl]piperazin-1-yl}ethyl)quinazoline-2,4(1*H*,3*H*)-dione (Compound 16)



Compound **5** (0.255 g, 1 mmol) and 1-(3-trifluoromethylphenyl)piperazine (0.230 g, 1 mmol) were reacted as described in the general procedure C and the crude product was crystallized from methanol:ether mixture to yield 0.038 g (8%) of white powdered compound. The compound is soluble in cold acetone, DMSO and hot methanol, ethanol and unsoluble in water.

M.p.: 300 °C (dec).

Rf values: 0.61 (S.1), 0.94 (S.2), 0.98 (S.3).

UV (MeOH, λ_{max}, nm): 222 (log ε : 4.68), 253 (log ε : 4.33), 320 (log ε : 3.57).

IR (KBr, ν_{max}, cm^{-1}): 3067 (aromatic C-H str.), 2927 (aliphatic C-H str.), 1725, 1668 (amide I band, C=O str.), 1461 (aliphatic C-H bending), 1232 (C-N str.), 1118 (C-F str.), 1075 (C-Cl str.).

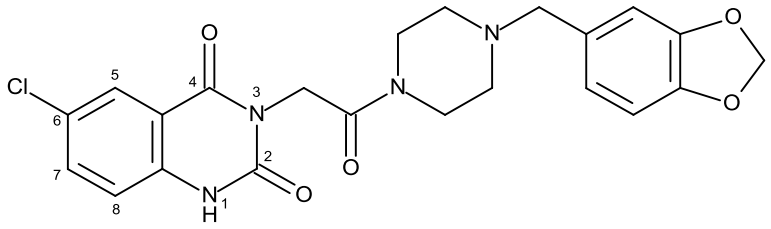
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.23 (t, 2H, CH_2 piperazine, *J*=4.8 Hz), 3.35 (t, 2H, CH_2 piperazine), 3.59 (t, 2H, CH_2 piperazine, *J*=4.8 Hz), 3.73 (t, 2H, CH_2 piperazine, *J*=4.8 Hz), 4.80 (s, 2H, CH_2CO), 7.10 (d, 1H, $H^{6'}$, *J*=7.2 Hz), 7.21-7.26 (m, 2H, $H^{5'}$ and $H^{2'}$), 7.23 (d, 1H, H^8, *J*=8.8 Hz), 7.44 (t, 1H, $H^{4'}$, *J*=8.0 Hz), 7.74 (dd, 1H, H^7, *J*=8.8 Hz, *J*=2.4 Hz), 7.85 (d, 1H, H^5, *J*=2.4 Hz), 11.70 (bs, 1H, NH).

Elemental analysis for $C_{21}H_{18}ClF_3N_4O_3$ (466.8407 g/mol);

Calculated: 54.03 (C%), 3.89 (H%), 12.00 (N%).

Found: 53.75 (C%), 3.77 (H%), 12.07 (N%).

3-{2-[4-(Benzo[d][1,3]dioxol-5-ylmethyl)piperazin-1-yl]-2-oxoethyl}-6-chloroquinazoline-2,4(1*H*,3*H*)-dione (Compound 17)



Compound **5** (0.255 g, 1 mmol) and 1-piperonylpiperazine (0.220 g, 1 mmol) were reacted as described in the general procedure C and crude product was washed with acetone and hot methanol to yield 0.022 g (5%) of white powdered compound. The compound is soluble in DMSO, and hot acetone and insoluble in cold acetone, ethanol, methanol and unsoluble in water.

M.p.: 300 °C (dec).

Rf values: 0.12 (S.1), 0.73 (S.2), 0.97 (S.3).

UV (MeOH, λ_{max}, nm): 222 (log ε : 4.75), 245 (log ε : 4.23), 322 (log ε : 3.72).

IR (KBr, ν_{max}, cm^{-1}): 3058 (aromatic C-H str.), 2915 (aliphatic C-H str.), 1719, 1659 (amide I band, C=O str.), 1491 (C=C str.).

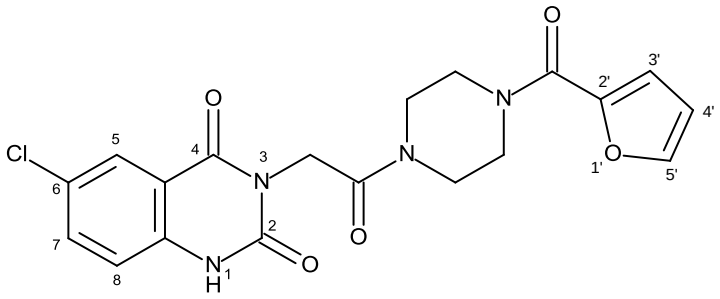
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.31 (t, 2H, CH_2 piperazine), 2.40 (t, 2H, CH_2 piperazine), 3.42 (s, 4H, CH_2 piperazine and OCH_2O), 3.53 (t, 2H, CH_2 piperazine), 4.71 (s, 2H, CH_2CO), 5.97 (s, 2H, N**CH$_2$**C_6H_5), 6.74-6.87 (m, 3H, CH benzodioxol), 7.22 (d, 1H, H^8, *J*=8.4 Hz), 7.72 (dd, 1H, H^7, *J*=8.8 Hz, *J*=2.4 Hz), 7.84 (d, 1H, H^5, *J*=2.0 Hz), 11.70 (bs, 1H, NH).

Elemental analysis for $C_{22}H_{21}ClN_4O_5$. 2/3 H_2O (456.8788 g/mol);

Calculated: 54.61 (C%), 5.00 (H%), 11.58 (N%).

Found: 54.46 (C%), 5.19 (H%), 11.25 (N%).

6-Chloro-3-{2-[4-(2-furoyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1*H*,3*H*)-dione (Compound 18)



Compound **5** (0.255 g, 1 mmol) and 1-(2-furoyl)piperazine (0.180 g, 1 mmol) were reacted as described in the general procedure C and crude product was washed with hot methanol to yield 0.089 g (21%) of white powdered compound. The compound is soluble in cold acetone, DMSO, hot acetone and unsoluble in hot methanol, DCM and water.

M.p.: 294.5 °C.

Rf values: 0.11 (S.1), 0.76 (S.2), 0.95 (S.3).

UV (MeOH, λ_{max}, nm): 223 (log ε : 4.81), 247 (4.39), 322 (log ε : 3.97).

IR (KBr) v_{maks} (cm^{-1}) : 3054 (aromatic C-H str.), 2931 (aliphatic C-H str.), 1718, 1655 (amide I band, C=O str.), 1486 (C=C str.).

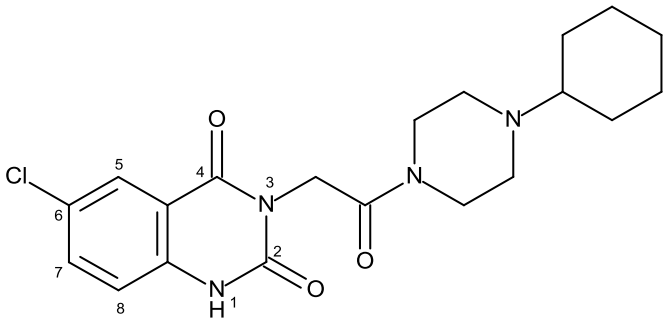
^{1}H-NMR (400 MHz) (DMSO-d_6 / TMS) δ ppm: 3.28 (t, 2H, CH_2 piperazine), 3.52 (t, 2H, CH_2 piperazine), 3.67-3.75 (m, 4H, CH_2 piperazine), 4.78 (s, 2H, CH_2CO), 6.64 (dd, 1H, $H^{4'}$, *J*=3.6 Hz, *J*=2.0 Hz), 7.04 (d, 1H, $H^{3'}$, *J*=2.8 Hz), 7.23 (d, 1H, H^8, *J*=8.4 Hz), 7.74 (dd, 1H, H^7, J_1=8.8 Hz, J_2=2.4 Hz), 7.85-7.86 (m, 2H, H^5 and $H^{5'}$), 11.75 (bs, 1H, NH).

Elemental analysis for $C_{19}H_{17}ClN_4O_5$ (416.8149 g/mol);

Calculated: 53.97 (C%), 4.21 (H%), 13.25 (N%).

Found: 54.18 (C%), 4.12 (H%), 13.35 (N%).

6-Chloro-3-[2-(4-cyclohexylpiperazin-1-yl)-2-oxoethyl]quinazoline-2,4(1*H*,3*H*)-dione (Compound 19)



Compound **5** (0.255 g, 1 mmol) and 1-cyclohexylpiperazine (0.168 g, 1 mmol) were reacted as described in the general procedure C and crude product was washed with hot methanol to yield 0.036 g (9%) of white powdered compound. The compound is soluble in cold acetone, ethanol, methanol, DMSO, DCM and unsoluble in water.

Rf values: 0.03 (S.1), 0.40 (S.2), 0.89 (S.3).

M.p.: 300 °C (dec).

UV (MeOH, λ_{max}, nm): 222 (log ε : 4.55), 245 (log ε : 3.92), 322 (log ε : 3.39).

IR (KBr, ν_{max}, cm^{-1}): 3068 (aromatic C-H str.), 2925 (aliphatic C-H str.), 1722, 1660 (amide I band, C=O str.).

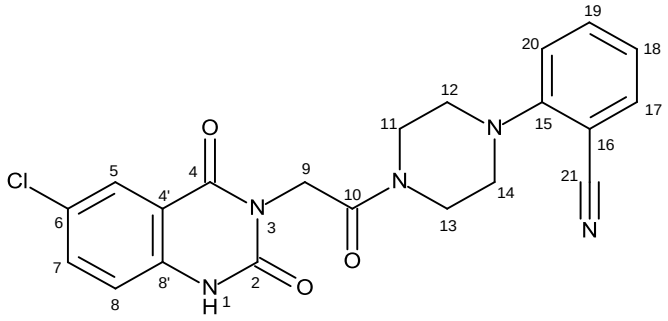
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 1.16-1.22 (m, 5H, cyclohexyl), 1.54-1.73 (m, 5H, cyclohexyl), 2.26 (1H, m, CH cyclohexyl), 2.43 (t, 2H, CH_2 piperazine, J=4.4 Hz), 2.53 (t, 2H, CH_2 piperazine, J=4.8 Hz), 3.39 (t, 2H, CH_2 piperazine, J=4.8 Hz), 3.50 (t, 2H, CH_2 piperazine, J=4.8 Hz), 4.71 (s, 2H, CH_2CO), 7.22 (d, 1H, H^8, J=8.8 Hz), 7.72 (dd, 1H, H^7, J=8.8 Hz, J=2.4 Hz), 7.83 (d, 1H, H^5, J=2.0 Hz), 11.68 (bs, 1H, NH).

Elemental analysis for $C_{20}H_{25}ClN_4O_3$ .1/3 H_2O (404.8904 g/mol);

Calculated: 58.46 (C%), 6.30 (H%), 13.64 (N%).

Found: 58.02 (C%), 6.00 (H%), 13.53 (N%).

2-{4-[2-(6-Chloro-2,4-dioxo-1,2-dihydroquinazolin-3(4*H*)-yl)acetyl]piperazin-1-yl}benzonitrile (Compound 20)



Compound **5** (0.255 g, 1 mmol) and 1-(2-cyanophenyl)piperazine (0.187 g, 1 mmol) were reacted as described in the general procedure C and crude product was washed with hot methanol to yield 172 mg (41%) of white powdered compound. The compound is soluble in cold acetone, ethanol, methanol, DMSO and DCM.

M.p.: 300 °C (dec).

Rf values: 0.51 (S.1), 0.91 (S.2), 0.96 (S.3).

UV (MeOH, λ_{max}, nm): 224 (log ε : 4.82), 247 (log ε : 4.28), 321 (log ε : 3.81).

IR (KBr, ν_{max}, cm^{-1}): 3439 (free N-H str.), 3188 (hydrogen bonded N-H str.), 3069 (aromatic C-H str.), 2961, 2928, 2860 (aliphatic C-H str.), 2224 (C≡N str.), 1714, 1663 (amide I band, C=O str.), 1489 (C=C str.).

^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.14 (t, 2H, CH_2 piperazine), 3.24 (t, 2H, CH_2 piperazine), 3.64 (t, 2H, CH_2 piperazine), 3.78 (t, 2H, CH_2 piperazine), 4.83 (s, 2H, CH_2CO), 7.15 (t, 1H, H^{18}, *J*=7.6 Hz), 7.21-7.26 (m, 2H H^8 and H^{20}), 7.62-7.66 (m, 1H, H^7), 7.74-7.78 (m, 2H, H^{17} and H^{19}), 7.87 (d, 1H, H^5, *J*=2.8 Hz), 11.72 (s, 1H, NH).

^{13}C-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 42.06 (C^{11}), 42.17 (C^{13}), 44.81 (C^9), 51.28 (C^{12}), 51.94 (C^{14}), 105.58 (C^{16}), 115.33 (C^{20}), 117.98 ($C^{4'}$), 119.88 (C^8), 118.54 (C^{18}), 122.92 (C^{21}), 126.74 (C^6), 127.12 (C^5), 134.66 (C^{17}), 134.80 (C^{19}), 135.58 (C^7), 138.76 ($C^{8'}$), 150.17 (C^2), 155.29 (C^{15}), 161.21 (C^4), 165.22 (C^{10}).

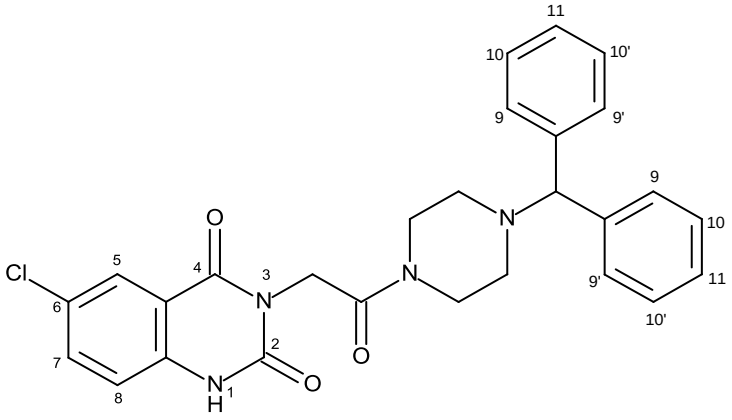
MS (ESI+, m/z): 426.2 ($[M+2]^+$) 424.1 ($[M^+]$, base peak).

Elemental analysis for $C_{21}H_{18}ClN_5O_3$. 1/5 H_2O (423.8522 g/mol);

Calculated: 59.01 (C%), 4.34 (H%), 16.38 (N%).

Found: 58.97 (C%), 4.26 (H%), 16.30 (N%).

6-Chloro-3-{2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1*H*,3*H*)-dione (Compound 21)



Compound **5** (0.255 g, 1 mmol) and 1-(diphenylmethyl)piperazine (0.252 g, 1 mmol) were reacted as described in the general procedure C and crude product was washed with hot methanol to yield 0.045 g (18%) of white powdered compound. The compound is soluble in cold DMSO and acetone and and unsoluble in cold methanol.

M.p.: 300 °C (dec).

Rf values: 0.76 (S.1), 0.95 (S.2), 0.96 (S.3).

UV (MeOH, λ_{max}, nm): 223 (log ε : 4.95), 245 (log ε : 4.15), 322 (log ε : 3.46).

IR (KBr, ν_{max}, cm^{-1}): 3327 (free N-H str.), 3187 (hydrogen bonded N-H str.), 3062 (aromatic C-H), 2928, 2851 (aliphatic C-H str.), 1727, 1655 (amide I band, C=O str.), 1491 (aromatic C=C str.).

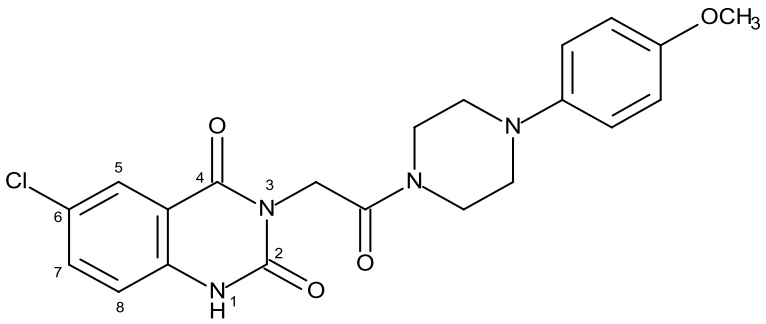
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.29 (t, 2H, CH_2 piperazine), 2.37 (t, 2H, CH_2 piperazine), 3.46 (t, 2H, CH_2 piperazine), 3.59 (t, 2H, CH_2 piperazine), 4.38 (s, 1H, CH), 4.71 (s, 2H, CH_2CO), 7.19-7.24 (m, 1H, H^8), 7.30-7.34 (m, 6H, H^{10}, $H^{10'}$ and H^{11}), 7.45-7.47 (m, 4H, H^9 and $H^{9'}$), 7.74 (dd, 1H, H^7, *J*=8.8 Hz, *J*=2.4 Hz), 7.86 (d, 1H, H^5, *J*=2.8 Hz), 11.69 (bs, 1H, NH).

Elemental analysis for $C_{27}H_{25}ClN_4O_3$. 2/3 H_2O (488.9653 g/mol);

Calculated: 64.73 (C%), 5.30 (H%), 11.18 (N%).

Found: 64.86 (C%), 5.29 (H%), 10.85 (N%).

6-Chloro-3-{2-[4-(4-methoxyphenyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1*H*,3*H*)-dione (Compound 22)



Compound **5** (0.255 g, 1 mmol) and 1-(4-methoxyphenyl)piperazine (0.192 g, 1 mmol) were reacted as described in the general procedure C and crude product was washed with acetone and hot methanol to yield 0.102 g (24%) of white powdered compound. The compound is soluble in cold DMSO and hot acetone and and unsoluble in cold methanol and acetone.

M.p.: 300 °C (dec).

Rf values: 0.42 (S.1), 0.88 (S.2), 0.95 (S.3).

UV (MeOH, λ_{max}, nm): 223 (log ε : 4.44), 244 (log ε : 4.07), 320 (log ε : 3.31).

IR (KBr, ν_{max}, cm^{-1}): 3067 (aromatic C-H str.), 2928 (aliphatic C-H str.), 1724, 1655 (amide I band, C=O str.), 1512 (C=C str.).

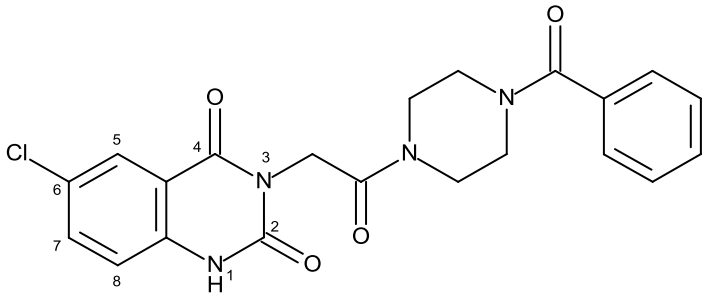
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.97 (t, 2H, CH_2 piperazine), 3.01 (t, 2H, CH_2 of piperazine), 3.57 (t, 2H, CH_2 piperazine), 3.68 (s, 3H, OCH_3), 3.70 (t, 2H, CH_2 piperazine), 4.78 (s, 2H, CH_2CO), 6.82-6.94 (m, 4H, methoxyphenyl CH), 7.23 (d, 1H, H^8, *J*=8.8 Hz), 7.73 (dd, 1H, H^7, *J*=8.8 Hz, *J*=2.4 Hz), 7.84 (d, 1H, H^5, *J*=2.8 Hz), 11.63 (bs, 1H, NH).

Elemental analysis for $C_{21}H_{21}ClN_4O_4$. 2/3 H_2O (428.8687g/mol);

Calculated: 57.21 (C%), 5.11 (H%), 12.71 (N%).

Found: 56.95 (C%), 4.84 (H%), 12.68 (N%).

3-[2-(4-Benzoylpiperazin-1-yl)-2-oxoethyl]-6-chloroquinazoline-2,4(1*H*,3*H*)-dione (Compound 23)



Compound **5** (0.255 g, 1 mmol) and 1-benzoylpiperazine (0.190 g, 1 mmol) were reacted as described in the general procedure C and crude product was washed with acetone and hot methanol to yield 0.035 g (8%) of white powdered compound. The compound is soluble in cold DMSO and hot acetone and and unsoluble in cold methanol and acetone.

M.p.: 300 °C (dec).

Rf values: 0.18 (S.1), 0.85 (S.2), 0.94 (S.3).

UV (MeOH, λ_{max}, nm): 223 (log ε : 4.59), 245 (log ε : 4.08), 323 (log ε : 3.38).

IR (KBr, ν_{max}, cm^{-1}): 3057 (aromatic C-H str.), 2930 (aliphatic C-H str.), 1715, 1660 (amide I band, C=O str.), 1618 (C=C str.).

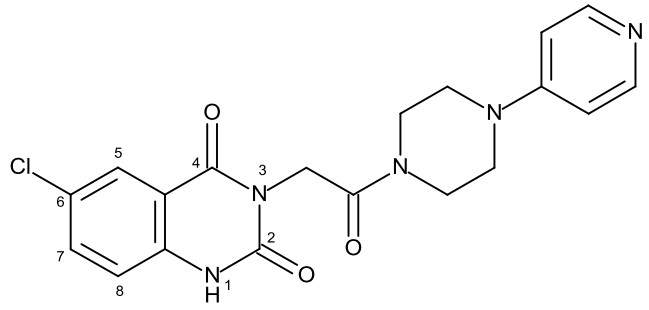
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.50 (t, 4H, CH_2 piperazine), 3.63 (t, 4H, CH_2 piperazine), 4.76 (s, 2H, CH_2CO), 7.23 (d, 1H, H^8, J=8.8 Hz), 7.42-7.46 (m, 5H, benzoyl CH), 7.73 (dd, 1H, H^7, J=8.8 Hz, J=2.4 Hz), 7.84 (d, 1H, H^5, J=2.4 Hz), 11.64 (bs, 1H, NH).

Elemental analysis for $C_{21}H_{19}ClN_4O_4$. 2/3 H_2O (426.8528 g/mol);

Calculated: 57.47 (C%), 4.67 (H%), 12.77 (N%).

Found: 57,05 (C%), 4,62 (H%), 12,69 (N%).

6-Chloro-3-[2-oxo-2-(4-pyridin-4-ylpiperazin-1-yl)ethyl]quinazoline-2,4(1*H*,3*H*)-dione (Compound 24)



Compound **5** (0.255 g, 1 mmol) and 1-(4-pyridyl)piperazine (0.163 g, 1 mmol) were reacted as described in the general procedure C and crude product was washed with hot acetonitrile and crystallized from methanol:ether mixture to yield 0.024 g (6%) of white powdered compound. The compound is soluble in cold DMSO and hot acetone and and unsoluble in cold methanol and acetone.

M.p.: 300 °C (dec).

Rf values: 0.01 (S.1), 0.01 (S.2), 0.29 (S.3).

UV (MeOH, λ_{max}, nm): 222 (log ε : 4.78), 255 (log ε : 4.38), 322 (log ε : 3.63).

IR (KBr, ν_{max}, cm^{-1}): 3068 (aromatic C-H str.), 2932 (aliphatic C-H str.), 1715, 1657 (amide I band, C=O str.), 1595 (C=C str.), 1456 (aliphatic C-H bending).

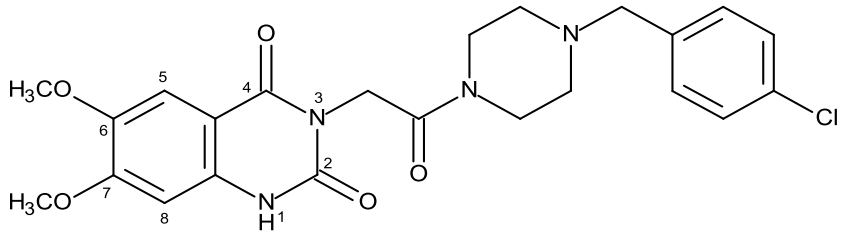
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.34 (t, 2H, CH_2 piperazine), 3.44 (t, 2H, CH_2 piperazine, *J*=4.8 Hz), 3.56 (t, 2H, CH_2 piperazine, *J*=4.8 Hz), 3.71 (t, 2H, CH_2 piperazine, *J*=4.8 Hz), 4.80 (s, 2H, CH_2CO), 6.83 (dd, 2H, CH pyridine, *J*=5.2 Hz, *J*=1.2 Hz), 7.23 (d, 1H, H^8, *J*=8.4 Hz), 7.75 (dd, 1H, H^7, *J*=8.8 Hz, *J*=2.4 Hz), 7.85 (d, 1H, H^5, *J*=2.8 Hz), 8.18 (d, 2H, CH pyridine, *J*=6.8 Hz), 11.70 (bs, 1H, NH).

Elemental analysis for $C_{19}H_{18}ClN_5O_3$ (399.8308 g/mol);

Calculated: 57.07 (C%), 4.54 (H%), 17.52 (N%).

Found: 57.49 (C%), 4.45 (H%), 17.13 (N%).

3-{2-[4-(4-Chlorobenzyl)piperazin-1-yl]-2-oxoethyl}-6,7-dimethoxyquinazoline-2,4(1*H*,3*H*)-dione (Compound 25)



Compound **6** (0.280 g, 1 mmol) and 1-(4-chlorobenzyl)piperazine (0.211 g, 1 mmol) were reacted as described in the general procedure C and the crude product was crystallized from methanol:ether mixture to yield 0.025 g (5%) of white powdered crystalline compound. The compound is soluble in cold acetone, DMSO, DCM and hot methanol, and ethanol.

M.p.: 300 °C (dec).

Rf values: 0.03 (S.1), 0.51 (S.2), 0.93 (S.3).

UV (MeOH, λ_{max}, nm): 235 (log ε : 4.42), 257 (log ε : 3.58), 320 (log ε : 3.61).

IR (KBr, v_{max}, cm^{-1}): 3380 (free N-H str.), 3096 (aromatic C-H str.), 2955 (aliphatic C-H str.), 1718, 1657 (amide I band, C=O str.), 1513 (C=C str.), 1460 (aliphatic C-H bending).

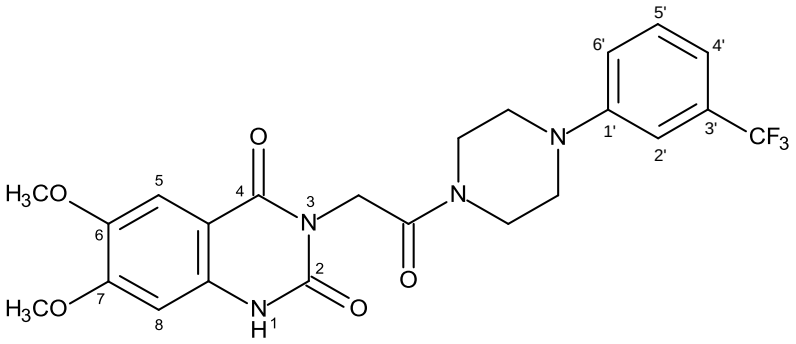
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.30 (t, 2H, CH_2 piperazine), 2.40 (t, 2H, CH_2 piperazine), 3.41 (t, 2H, CH_2 piperazine), 3.49 (s, 2H, CH_2 benzyl), 3.54 (t, 2H, CH_2 piperazine), 3.76 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.68 (s, 2H, CH_2CO), 6.68 (s, 1H, H^8), 7.24 (s, 1H, H^5), 7.35 (dd, 4H, *J*=17.6 Hz, *J*=8.8 Hz, aromatic CH benzyl), 11.27 (s, 1H, NH).

Elemental analysis for $C_{23}H_{25}ClN_4O_5$. 2/3 H_2O (472.9213 g/mol);

Calculated: 56.97 (C%), 5.47 (H%), 11.55 (N%).

Found: 56.88 (C%), 5.85 (H%), 11.38 (N%).

6,7-Dimethoxy-3-(2-oxo-2-{4-[3-(trifluoromethyl)phenyl]piperazin-1-yl}ethyl)quinazoline-2,4(1*H*,3*H*)-dione (Compound 26)



Compound **6** (0.280 g, 1 mmol) and 1-(3-trifluoromethylphenyl)piperazine (0,230 g, 1 mmol) were reacted as described in the general procedure C and the crude product was crystallized from methanol:ether mixture to yield 0.041 g (8%) of white powdered crystalline compound. The compound is soluble in cold acetone, DMSO, DCM and hot methanol, and ethanol.

M.p.: 228 °C.

Rf values: 0.14 (S.1), 0.80 (S.2), 0.94 (S.3).

UV (MeOH, λ_{max}, nm): 236 (log ε : 4.52), 256 (log ε : 4.21), 319 (log ε : 3.67).

IR (KBr, ν_{max}, cm^{-1}): 3437 (free N-H str.), 3079 (aromatic C-H str.), 2958 (aliphatic C-H str.), 1705, 1671, 1646 (amide I band, C=O str.), 1512 (C=C str.), 1440 (aliphatic C-H bending).

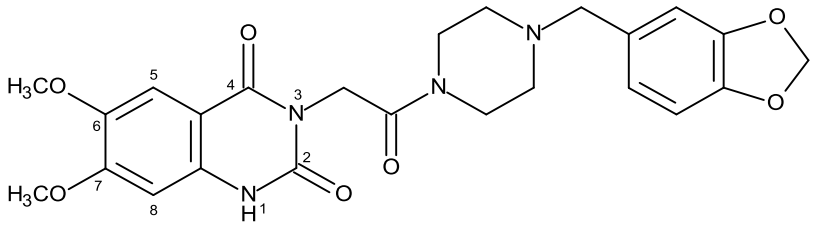
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.24 (t, 2H, CH_2 piperazine, J=4.8 Hz), 3.32 (t, 2H, CH_2 piperazine), 3.60 (t, 2H, CH_2 piperazine), 3.75 (t, 2H, CH_2 piperazine), 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.80 (s, 2H, CH_2CO), 6.71 (s, 1H, H^8), 7.12 (d, 1H, $H^{2'}$, J=8.0 Hz), 7.23 (s, 1H, H^5), 7.26-7.29 (m, 2H, $H^{4'}$ and $H^{6'}$), 7.46 (t, 1H, $H^{5'}$, J=8.0 Hz), 11.34 (s, 1H, NH).

Elemental analysis for $C_{23}H_{23}F_3N_4O_5$ (492.4479 g/mol);

Calculated: 56.10 (C%), 4.71 (H%), 11.38 (N%).

Found: 56.13 (C%), 4.80 (H%), 11.30 (N%).

3-{2-[4-(Benzo[d][1,3]dioxol-5-ylmethyl)piperazin-1-yl]-2-oxoethyl}-6,7-dimethoxy quinazoline-2,4(1*H*,3*H*)-dione (Compound 27)



Compound **6** (0.280 g, 1 mmol) and 1-piperonylpiperazine (0.220 g, 1 mmol) were reacted as described in the general procedure C and the crude product was crystallized from methanol:ether mixture to yield 0.064 g (15%) of white powdered crystalline compound. The compound is soluble in cold acetone, DMSO, DCM and hot methanol, and ethanol.

M.p.: 211.3 °C.

Rf values: 0.03 (S.1), 0.42 (S.2), 0.96 (S.3).

UV (MeOH, λ_{max}, nm): 236 (log ε : 4.61), 258 (log ε : 3.94), 321 (log ε : 3.69).

IR (KBr, ν_{max}, cm^{-1}): 3092 (aromatic C-H str.), 2946 (aliphatic C-H str.), 1709, 1662 (amide I band, aliphatic C=O str.), 1469 (C=C str.), 1440 (aliphatic C-H bending).

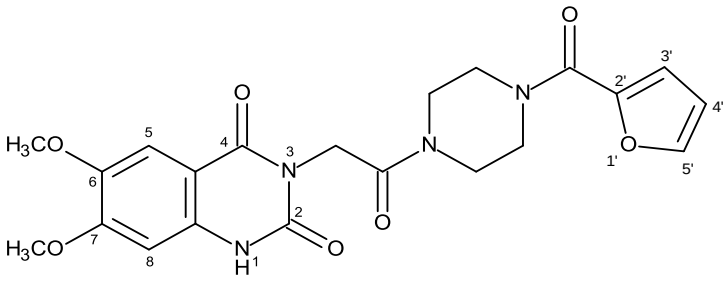
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.31 (t, 2H, CH_2 piperazine, J=4.8 Hz), 2.41 (t, 2H, CH_2 piperazine), 3.31 (t, 2H, CH_2 piperazine), 3.43 (s, 2H, OCH_2O), 3.55 (t, 2H, CH_2 piperazine), 3.79 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.70 (s, 2H, CH_2CO), 6.00 (s, 2H, N**CH_2**-benzene), 6.70 (s, 1H, H^8), 6.76-6.89 (m, 3H, CH benzodioxol), 7.27 (s, 1H, H^5), 11.30 (s, 1H, NH).

Elemental analysis for $C_{22}H_{22}N_4O_5$ (422.434 g/mol);

Calculated: 59.74 (C%), 5.43 (H%), 11.61 (N%).

Found: 59.87 (C%), 5.20 (H%), 11.59 (N%).

3-{2-[4-(2-Furoyl)piperazin-1-yl]-2-oxoethyl}-6,7-dimethoxyquinazoline-2,4(1*H*,3*H*)-dione (Compound 28)



Compound **6** (0.280 g, 1 mmol) and 1-(2-furoyl)piperazine (0.180 g, 1 mmol) were reacted as described in the general procedure C and the crude product was crystallized from ethanol to yield 0.079 g (18%) of white powdered crystalline compound. The compound is soluble in cold acetone, DMSO, DCM and hot methanol, and ethanol.

M.p.: 300 °C (dec).

Rf values: 0.01 (S.1), 0.39 (S.2), 0.93 (S.3).

UV (MeOH, λ_{max}, nm): 236 (log ε : 4.26), 258 (log ε : 3.82), 321 (log ε : 2.86).

IR (KBr, ν_{max}, cm^{-1}): 3437 (free N-H str.), 3017 (aromatic C-H str.), 2934 (aliphatic C-H str.), 1714, 1658, 1624 (amide I band, C=O str.), 1514 (C=C str.), 1438 (aliphatic C-H bending).

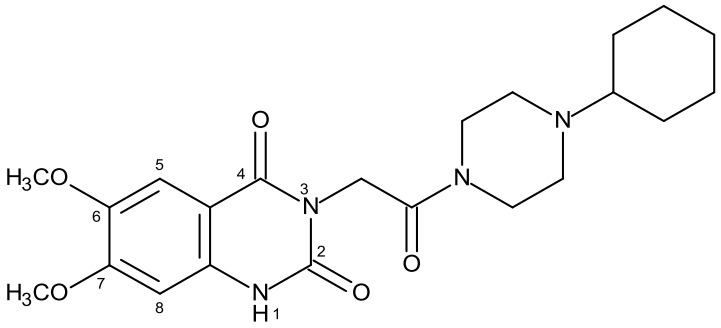
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS) δ ppm: 3.54 (t, 4H, CH_2 piperazine), 3.70 (t, 4H, CH_2 piperazine), 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.78 (s, 2H, CH_2CO), 6.65-6.66 (m, 1H, $H^{4'}$), 6.71 (s, 1H, H^8), 7.06 (d, 1H, $H^{3'}$, *J*=3.6 Hz), 7.28 (s, 1H, H^5), 7.88 (d, 1H, $H^{5'}$, *J*=0.8 Hz), 11.34 (s, 1H, NH).

Elemental analysis for $C_{21}H_{22}N_4O_7.3H_2O$ (442.4221 g/mol);

Calculated: 50.80 (C%), 5.68 (H%), 11.29 (N%).

Found: 51.08 (C%), 5.77 (H%), 11.34 (N%).

3-[2-(4-Cyclohexylpiperazin-1-yl)-2-oxoethyl]-6,7-dimethoxyquinazoline-2,4(1*H*,3*H*)-dione (Compound 29)



Compound **6** (0.280 g, 1 mmol) and 1-cyclohexylpiperazine (0.168 g, 1 mmol) were reacted as described in the general procedure C and the crude product was crystallized from methanol:ether mixture to yield 0.074 g (17%) of white powdered crystalline compound. The compound is soluble in cold acetone, DMSO, DCM and hot methanol, and ethanol.

M.p.: 239.7 °C.

Rf values: 0.01 (S.1), 0.20 (S.2), 0.83 (S.3).

UV (MeOH, λ_{max}, nm): 236 (log ε : 4.62), 259 (log ε : 3.92), 321 (log ε : 3.77).

IR (KBr, ν_{max}, cm^{-1}): 3446 (free N-H str.), 3084, 3008 (aromatic C-H str.), 2926, 2856 (aliphatic C-H str.), 1702, 1661 (amide I band, C=O str.), 1512 (C=C str.).

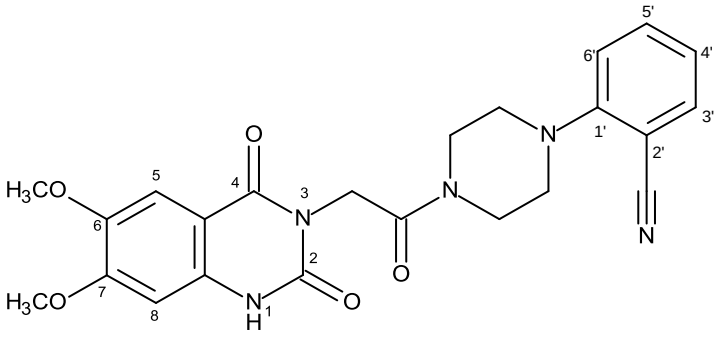
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 1.09-1.74 (m, 10H, CH_2 cyclohexyl), 2.30 (m, 1H, CH cyclohexyl), 2.45 (t, 2H, CH_2 piperazine, *J*=4.0 Hz), 2.54 (t, 2H, CH_2 piperazine), 3.41 (t, 2H, CH_2 piperazine *J*=3.6 Hz), 3.52 (t, 2H, CH_2 piperazine), 3.79 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.70 (s, 2H, CH_2CO), 6.70 (s, 1H, H^8), 7.26 (s, 1H, H^5), 11.30 (s, 1H, NH).

Elemental analysis for $C_{22}H_{30}N_4O_5$. 1/4 H_2O (430.4976 g/mol);

Calculated: 60.74 (C%), 7.07 (H%), 12.88 (N%).

Found: 60.57 (C%), 7.19 (H%), 12.79 (N%).

2-{4-[2-(6,7-Dimethoxy-2,4-dioxo-1,2-dihydroquinazolin-3(4*H*)-yl)acetyl]piperazin-1-yl}benzonitrile (Compound 30)



Compound **6** (0.280 g, 1 mmol) and 1-(2-cyanophenyl)piperazine (0.187 g, 1 mmol) were reacted as described in the general procedure C and the crude product was crystallized from methanol:ether mixture to yield 0.021 g (5%) of white powdered crystalline compound. The compound is soluble in cold acetone, DMSO, DCM and hot methanol, and ethanol.

M.p.: 300 °C (dec).

Rf values: 0.10 (S.1), 0.68 (S.2), 0.97 (S.3).

UV (MeOH, λ_{max}, nm): 227 (log ε : 4.20), 259 (log ε : 3.57), 320 (log ε : 3.23).

IR (KBr, ν_{max}, cm^{-1}): 3206 (N-H str.), 3094 (aromatic C-H str.), 2955 (aliphatic C-H str.), 2214 (C≡N str.), 1720, 1663 (amide I band, C=O str.), 1514 (C=C str.), 1440 (aliphatic C-H bending).

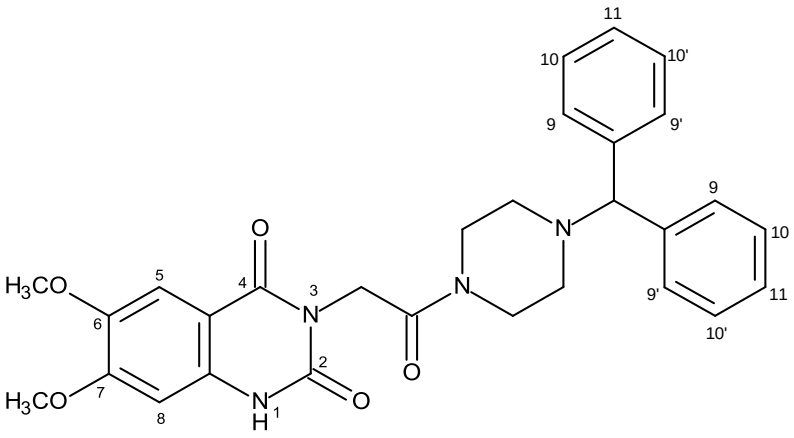
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ ppm): 3.13 (t, 2H, CH_2 piperazine), 3.24 (t, 2H, CH_2 piperazine), 3.64 (t, 2H, CH_2 piperazine), 3.78 (t, 2H, CH_2 piperazine), 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.80 (s, 2H, CH_2CO), 6.72 (s, 1H, H^8), 7.15 (t, 1H, $H^{4'}$, *J*=7.6 Hz), 7.22 (d, 1H, $H^{6'}$, *J*=8.0 Hz), 7.28 (s, 1H, H^5), 7.62-7.66 (m, 1H, $H^{5'}$), 7.76 (dd, 1H, $H^{3'}$, *J*=8.0 Hz, *J*=1.6 Hz), 11.33 (s, 1H, NH).

Elemental analysis for $C_{23}H_{23}N_5O_5$. H_2O (449.4594 g/mol);

Calculated: 59.09 (C%), 5.39 (H%), 14.98 (N%).

Found: 58.85 (C%), 5.24 (H%), 14.84 (N%).

3-{2-[4-(Diphenylmethyl)piperazin-1-yl]-2-oxoethyl}-6,7-dimethoxyquinazoline-2,4(1*H*,3*H*)-dione (Compound 31)



Compound **6** (0.280 g, 1 mmol) and 1-(diphenylmethyl)piperazine (0.252 g, 1 mmol) were reacted as described in the general procedure C and the crude product was crystallized from methanol:ether mixture to yield 0.021 g (4%) of white powdered crystalline compound. The compound is soluble in cold acetone, DMSO, DCM and hot methanol, and ethanol.

M.p.: 262.4°C.

Rf values: 0.19 (S.1), 0.87 (S.2), 0.98 (S.3).

UV (MeOH, λ_{max}, nm): 232 (log ε : 4.57), 259 (log ε : 3.77), 321 (log ε : 3.59).

IR (KBr, ν_{max}, cm^{-1}): 3057 (aromatic C-H str.), 2961, 2889 (aliphatic C-H str.), 1709, 1666 (amide I band, C=O str.), 1578 (amide II band, N-H bending), 1511 (C=C str.), 1435 (aliphatic C-H bending).

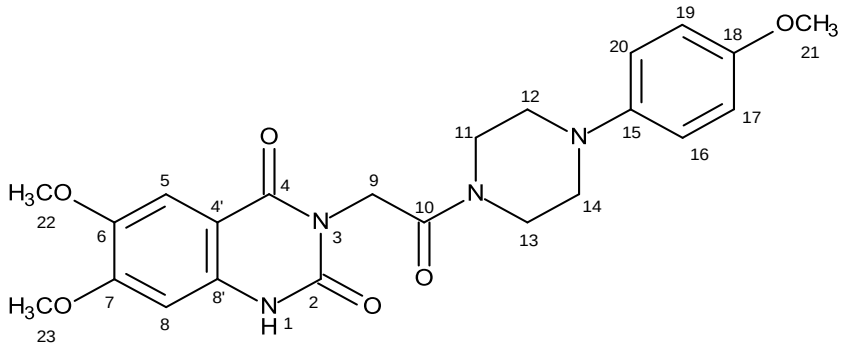
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.29 (t, 2H, CH_2 piperazine), 2.36 (t, 2H, CH_2 piperazine), 3.46 (t, 2H, CH_2 piperazine), 3.60 (t, 2H, CH_2 piperazine), 3.79 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.38 (s, 1H, CH diphenyl), 4.68 (s, 2H, CH_2CO), 6.70 (s, 1H, H^8), 7.20 (t, 2H, H^{11}, *J*=7.2 Hz), 7.26 (s, 1H, H^5), 7.32 (t, 4H, H^{10} and $H^{10'}$, *J*=7.6 Hz), 7.46 (d, 4H, H^9 and $H^{9'}$, *J*=7.2 Hz), 11.29 (s, 1H, NH).

Elemental analysis for $C_{29}H_{30}N_4O_5$ (514.5725 g/mol);

Calculated: 67.69 (C%), 5.88 (H%), 10.89 (N%).

Found: 67.33 (C%), 6.01 (H%), 11.01 (N%).

6,7-Dimethoxy-3-{2-[4-(4-methoxyphenyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1*H*,3*H*)-dione (Compound 32)



Compound **6** (0.280 g, 1 mmol) and 1-(4-methoxyphenyl)piperazine (0.192 g, 1 mmol) were reacted as described in the general procedure C and the crude product was washed with hot acetonitrile and methanol to yield 0.120 g (26%) of white powdered crystalline compound. The compound is soluble in cold acetone, DMSO, DCM and hot methanol, and ethanol.

M.p.: 283.5 °C.

Rf values: 0.06 (S.1), 0.67 (S.2), 0.97 (S.3).

UV (MeOH, λ_{max}, nm): 236 (log ε : 4.64), 258 (log ε : 3.94), 319 (log ε : 3.72).

IR (KBr, ν_{max}, cm^{-1}): 3441 (free N-H str.), 3194 (hydrogen bonded N-H str.), 3065, 3013 (aromatic C-H str.), 2948, 2886, 2830 (aliphatic C-H str.), 1723, 1659 (amide I band, C=O str.), 1513 (C=C str.), 1471 (aliphatic C-H bending).

^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.99 (t, 2H, CH_2 piperazine, J=3.6 Hz), 3.09 (t, 2H, CH_2 piperazine, J=3.6 Hz), 3.59 (t, 2H, CH_2 piperazine, J=3.6 Hz), 3.70 (s, 3H, OCH_3 methoxyphenyl), 3.73 (t, 2H, CH_2 piperazine, J=3.6 Hz), 3.80 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.79 (s, 2H, CH_2CO), 6.72 (s, 1H, H^8), 6.91 (dd, 4H, CH phenyl), 7.28 (s, 1H, H^5), 11.33 (s, 1H, NH).

^{13}C-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 41.72 (C^{11}), 42.05 (C^{13}), 44.62 (C^9), 50.27 (C^{12}), 50.71 (C^{14}), 55.62 (C^{21}), 56.19 (C^{22}), 56.31 (C^{23}), 97.99 (C^5), 105.98 ($C^{4'}$), 107.92 (C^8), 114.75 (C^{17},C^{19}), 118.58 (C^{16},C^{20}), 135.63 ($C^{8'}$), 145.58 (C^6), 145.68 (C^{15}), 150.58 (C^2), 153.85 (C^{18}), 155.56 (C^7), 161.69 (C^4), 165.34 (C^{10}).

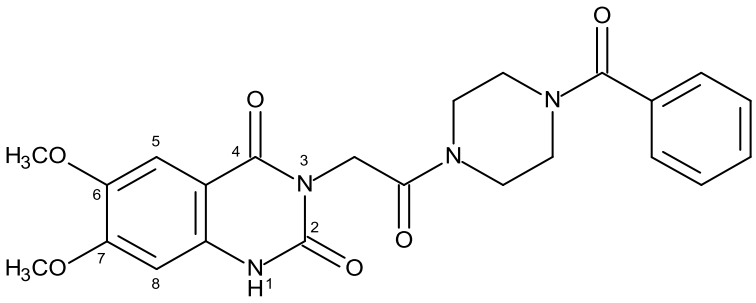
MS (ESI+, m/z): 455.2 ([M^+], base peak), 207.2 ($C_{10}H_9NO_4$), 249.2 ($C_{13}H_{19}N_3O_2$), 262.9 ($C_{12}H_{11}N_2O_5$).

Elemental analysis for $C_{23}H_{26}N_4O_6$. 1/4 H_2O (454.4759 g/mol);

Calculated: 60.19 (C%), 5.82 (H%), 12.21 (N%).

Found: 60.17 (C%), 5.84 (H%), 12.20 (N%).

3-[2-(4-Benzoylpiperazin-1-yl)-2-oxoethyl]-6,7-dimethoxyquinazoline-2,4(1*H*,3*H*)-dione (Compound 33)



Compound **6** (0.280 g, 1 mmol) and 1-benzoylpiperazine (0.190 g, 1 mmol) were reacted as described in the general procedure C and the crude product was crystallized from methanol:ether mixture to yield 0.032 g (7%) of white powdered crystalline compound. The compound is soluble in cold acetone, DMSO, DCM and hot methanol, and ethanol.

M.p.: 168.5 °C.

Rf values: 0.03 (S.1), 0.21 (S.2), 0.97 (S.3).

UV (MeOH, λ_{max}, nm): 236 (log ε : 4.60), 257 (log ε : 3.93), 321 (log ε : 3.68).

IR (KBr, ν_{max}, cm^{-1}): 3443 (free N-H str.), 3010 (aromatic C-H), 2958 (aliphatic C-H str.), 1712, 1660, 1623 (amide I band, C=O str.), 1513 (C=C str.), 1461 (aliphatic C-H bending).

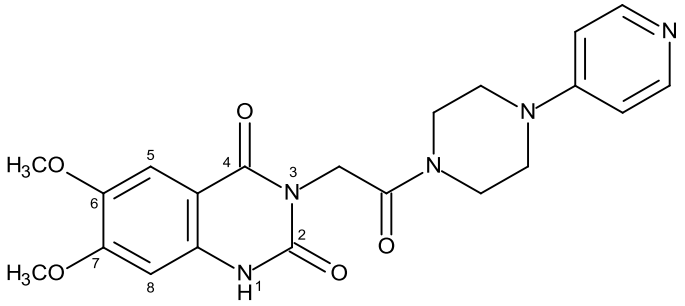
^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.51 (t, 4H, CH_2 piperazine), 3.66 (t, 4H, CH_2 piperazine), 3.79 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.76 (s, 2H, CH_2CO), 6.71 (s, 1H, H^8), 7.27 (s, 1H, H^5), 7.45-7.49 (m, 5H, CH benzoyl), 11.32 (s, 1H, NH).

Elemental analysis for $C_{23}H_{24}N_4O_6$. 3 H_2O (452.46 g/mol);

Calculated: 54.54 (C%), 5.97 (H%), 11.06 (N%).

Found: 54.68 (C%), 5.99 (H%), 11.15 (N%).

6,7-Dimethoxy-3-[2-oxo-2-(4-pyridin-4-ylpiperazin-1-yl)ethyl]quinazoline-2,4(1*H*,3*H*)-dione (Compound 34)



Compound **6** (0.280 g, 1 mmol) and 1-(4-pyridyl)piperazine (0.163 g, 1 mmol) were reacted as described in the general procedure C and the crude product was crystallized from methanol:ether mixture to yield 0.032 g (8%) of white powdered crystalline compound. The compound is soluble in colCNd acetone, DMSO, DCM and hot methanol, and ethanol.

M.p.: 300 °C (dec).

Rf values: 0.01 (S.1), 0.49 (S.2), 0.93 (S.3).

UV (MeOH, λ_{max}, nm): 236 (log ε : 4.44), 260 (log ε : 4.20), 321 (log ε : 4.00).

IR (KBr, ν_{max}, cm^{-1}): 3068 (aromatic C-H str.), 2932 (aliphatic C-H str.), 1715, 1657 (amide I band, C=O str.), 1510 (C=C str.), 1456 (aliphatic C-H bending).

^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.33 (t, 2H, CH_2 piperazine), 3.43 (t, 2H, CH_2 piperazine, *J*=4.4 Hz), 3.54 (t, 2H, CH_2 piperazine, *J*=4.8 Hz), 3.70 (t, 2H, CH_2 piperazine, *J*=4.8 Hz), 3.77 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.76 (s, 2H, CH_2CO), 6.68 (s, 1H, H^8), 6.82 (dd, 2H, CH pyridine, *J*=5.2 Hz, *J*=1.6 Hz), 7.25 (s, 1H, H^5), 8.16 (dd, 2H, CH pyridine, *J*=5.2 Hz, *J*=1.6 Hz), 11.30 (s, 1H, NH).

Elemental analysis for $C_{21}H_{23}N_5O_5$. 2 H_2O (425.438 g/mol);

Calculated: 54.66 (C%), 5.90 (H%), 15.18 (N%).

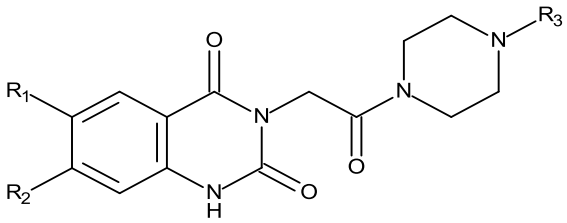
Found: 54.72 (C%), 5.79 (H%), 15.16 (N%).

4.2. Biological Data

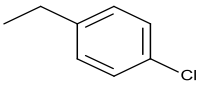
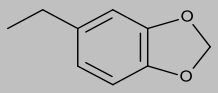
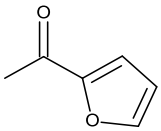
4.2.1. Antimicrobial Activity Data

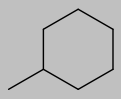
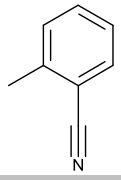
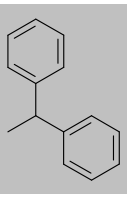
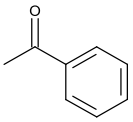
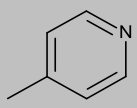
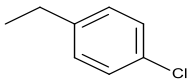
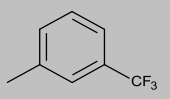
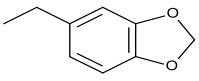
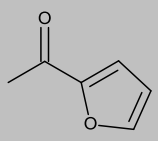
Antimicrobial activity results of synthesized compounds (**7-34**) are given at **Table 4.1.**

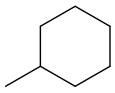
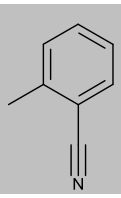
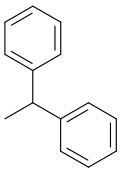
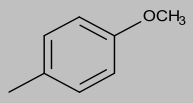
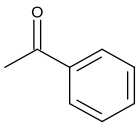
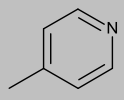
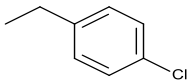
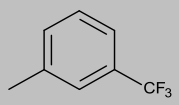
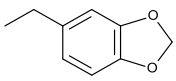
Table 4.1. Inhibitory zone of synthesized compounds against *B. subtilis* and *S. aureus* bacterias using disc diffusion method.

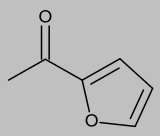
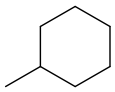
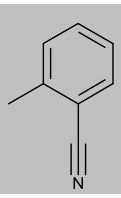
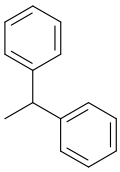
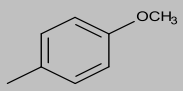
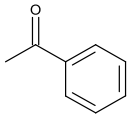
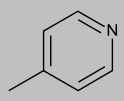


Compound 7-34

				Zone of inhibition (mm)	
Compound	**R_1**	**R_2**	**R_3**	***B. subtilis***	***S. aureus***
7	-H	-H		7	8
8	-H	-H		7	7
9	-H	-H		6	7

10	-H	-H		5	8
11	-H	-H		5	7
12	-H	-H		5	7
13	-H	-H		7	7
14	-H	-H		6	8
15	-Cl	-H		8	8
16	-Cl	-H		6	6
17	-Cl	-H		6	-
18	-Cl	-H		8	-

19	-Cl	-H		10	8
20	-Cl	-H		5	5
21	-Cl	-H		7	7
22	-Cl	-H		10	6
23	-Cl	-H		10	10
24	-Cl	-H		10	10
25	$-OCH_3$	$-OCH_3$		6	8
26	$-OCH_3$	$-OCH_3$		5	5
27	$-OCH_3$	$-OCH_3$		6	6

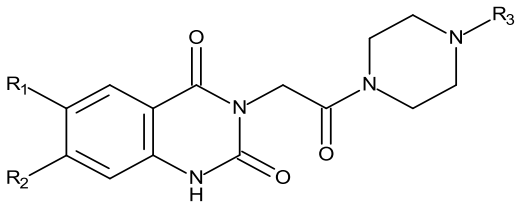
28	-OCH_3	-OCH_3		5	5
29	-OCH_3	-OCH_3		-	14
30	-OCH_3	-OCH_3		-	-
31	-OCH_3	-OCH_3		8	8
32	-OCH_3	-OCH_3		5	5
33	-OCH_3	-OCH_3		12	10
34	-OCH_3	-OCH_3		8	8
	Ampicillin			12	11

(Concentration was 0.5 mg/ml for the synthesized compounds and 0.01 mg/ml for ampicillin.)

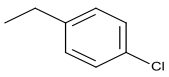
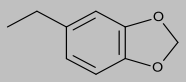
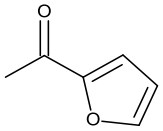
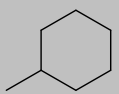
4.2.2. Cytotoxicity Data

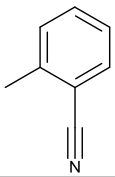
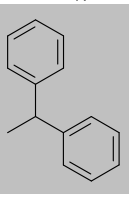
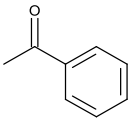
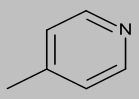
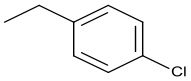
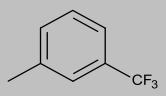
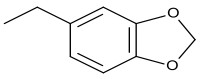
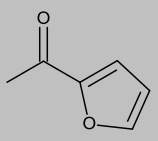
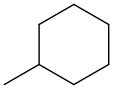
Cytotoxicity results of synthesized compounds (**7-34**) were given at **Table 4.2.**

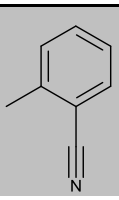
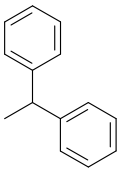
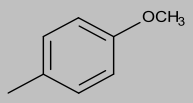
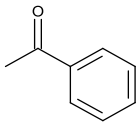
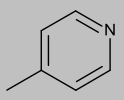
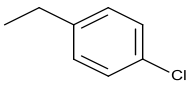
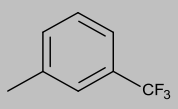
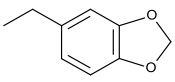
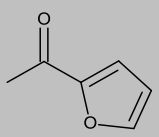
Table 4.2. IC_{50} values for tested compounds **7-34** against hepatoma cell line (HUH-7), breast cancer cell line (MCF-7) and colorectal cancer cell line (HCT-116) using Sulforhodamine B assay.

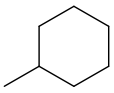
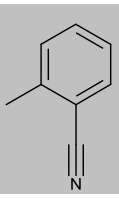
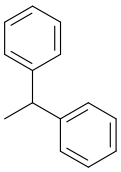
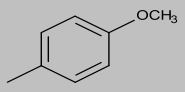
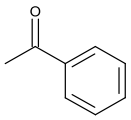
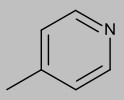


Compound 7-34

				IC_{50} Values (μM)		
Compound	**R_1**	**R_2**	**R_3**	**HUH-7**	**MCF-7**	**HCT-116**
7	-H	-H		2.5	6.8	4.9
8	-H	-H		11.5	12.2	35.3
9	-H	-H		-	-	-
10	-H	-H		-	-	-

11	-H	-H		-	-	-
12	-H	-H		-	15.2	-
13	H	H		-	-	-
14	-H	-H		-	-	-
15	-Cl	-H		7.0	13.1	9.4
16	-Cl	-H		12.8	18.6	-
17	-Cl	-H		-	-	-
18	-Cl	-H		-	-	-
19	-Cl	-H		-	-	-

20	-Cl	-H		-	-	-
21	-Cl	-H		9.2	13.0	9.0
22	-Cl	-H		-	-	-
23	-Cl	-H		-	-	-
24	-Cl	-H		-	-	-
25	$-OCH_3$	$-OCH_3$		-	-	16.9
26	$-OCH_3$	$-OCH_3$		-	-	6.0
27	$-OCH_3$	$-OCH_3$		-	-	-
28	$-OCH_3$	$-OCH_3$		-	-	-

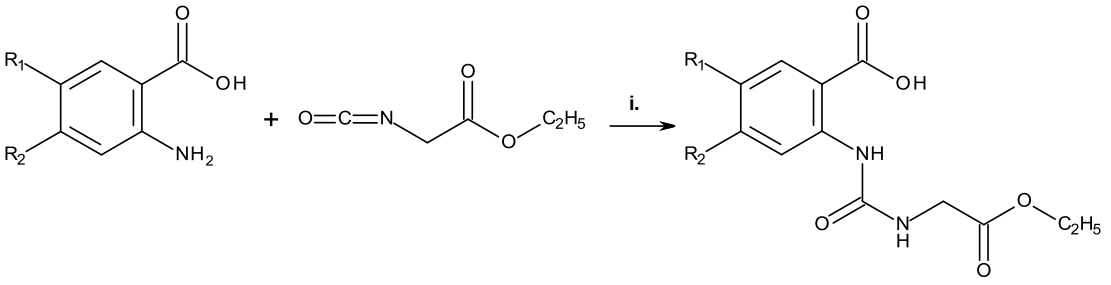
29	$-OCH_3$	$-OCH_3$		-	-	11.4
30	$-OCH_3$	$-OCH_3$		-	-	19.0
31	$-OCH_3$	$-OCH_3$		-	-	9.9
32	$-OCH_3$	$-OCH_3$		-	-	-
33	$-OCH_3$	$-OCH_3$		-	-	-
34	$-OCH_3$	$-OCH_3$		-	-	-
	Camptothecin*			0.00131	2.4×10^{-0}	$9,2\times10^{-07}$

(*=Camptothecin was positive control, -: no inhibition)

5. DISCUSSION AND CONCLUSION

In this study, twenty eight 3-substituted-2,4(1*H*,3*H*)-quinazolinedione derivatives which of twenty six are novel were synthesized and evaluated for their antimicrobial and cytotoxic activity. Structures of the compounds were clarified by UV, IR, ^{1}H-NMR, ^{13}C-NMR, mass spectral techniques and elemental analysis.

The target compounds presented in this study were prepared according to the synthetic pathways shown in **Scheme 5.1.**



i. saturated $KHCO_3$ solution, rt, 2h. **ii.** concd. HCl, reflux, 2h.

iii. 1-substitutedpiperazine, DCC, DCM, 0°C (0.5h) - rt (12h)

Compound 1, 4 : R_1, R_2 = H

Compound 2, 5 : R_1=Cl, R_2 = H

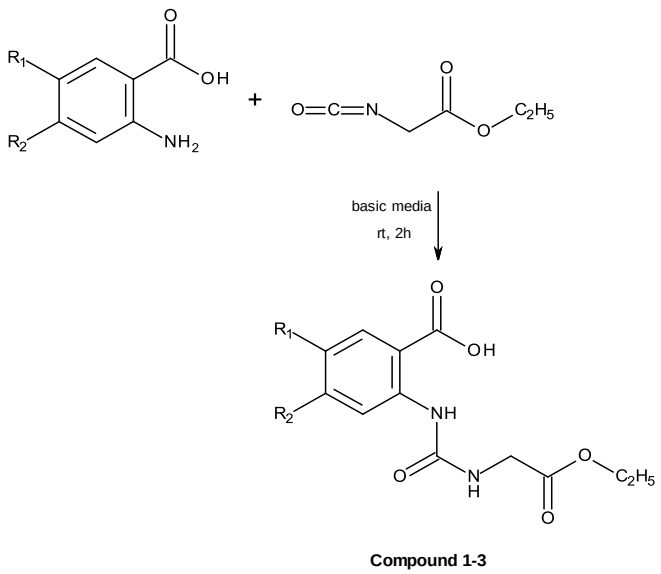
Compound 3, 6 : R_1, R_2 = OCH_3

Compound 7-34 : See **Table 1.1.**

Scheme 5.1. General synthetic pathway of the target compound **7-34.**

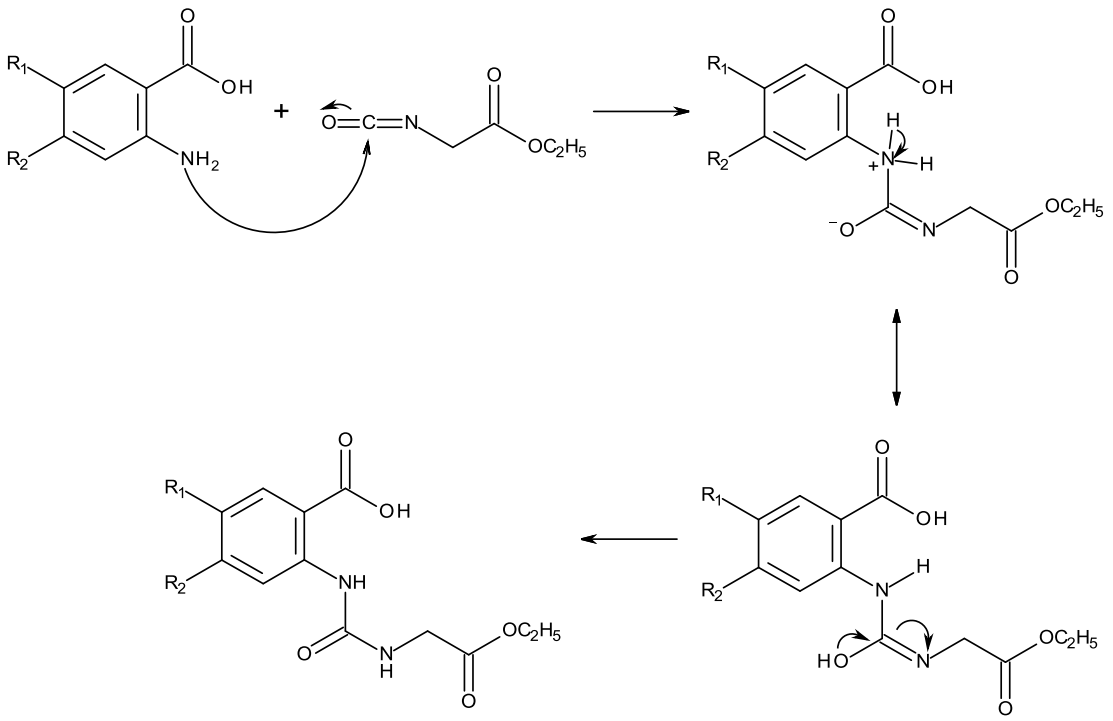
2-Aminobenzoic acid derivatives were treated with ethyl isocyanatoacetate in basic media to obtain subsequent product (compound **1**-**3**). Then these compounds were submitted to the ring closure reaction to form (6,7-disubstituted-2,4-dioxo-1,2-dihydroquinazolin-3(4*H*)-yl)acetic acid derivatives (compounds **4**-**6**) by refluxing in concentrated hydrochloric acid. The final compounds, 3-{2-[4-substitutedpiperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1*H*,3*H*)-dione derivatives (compound **7**-**34**) were prepared by the amidation reaction of (2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)acetic acid derivatives with various 1-substitutedpiperazines.

Synthesis of 2,4(1*H*,3*H*)-quinazolinedione ring derivatives started with the nucleophilic addition reaction of the 2-aminobenzoic acid derivatives to the ethyl isocyanatoacetate leading to the formation of corresponding 2-(3-ethoxycarbonylmethylureido)benzoic acid derivatives. Reactions were carried out by stirring the reactants in saturated potassium bicarbonate solution at room temperature to give moderate yields of urea derivatives (30-54%, compound **1**-**3**) (**Scheme 5.2.**). Spectral data and melting point of the compound **1** were in accordance with the values of published before [184].



Scheme 5.2. Synthetic pathway of the compound **1**-**3.**

Mechanism for this reaction can be proposed as nucleophilic addition which is shown in **Scheme 5.3.**

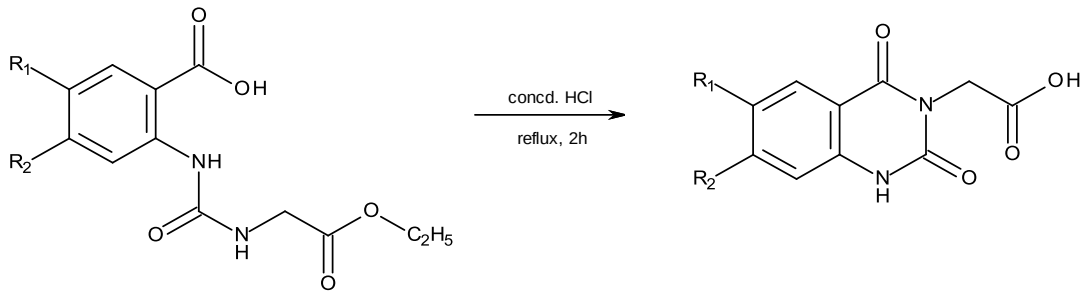


Scheme 5.3. Reaction mechanism of nucleophilic addition.

Isocyanate O=C=N double bonds have large differences in electronegativity charging the carbon atom partially positive. In the nucleophilic addition reaction, active nitrogen atom in nucleophilic amine attacks the electrophilic isocyanate carbon atom that leads to the formation of a new C-N bond. Reaction is completed by the transfer of active hydrogen to the isocyanate nitrogen atom.

Synthesis of 2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)acetic acid derivatives (compound **4-6**) were carried out by refluxing the 2-(3-ethoxycarbonylmethylureido) benzoic acid derivatives (compound **1-3**) in concentrated hydrochloric acid for 2 hours to give the products in moderate to good yields of 36-73% (**Scheme 5.4.**). The compounds were easily seperated from the reaction media without using any purification techniques. Ring closure reaction was carried out by heating compound **1-3**

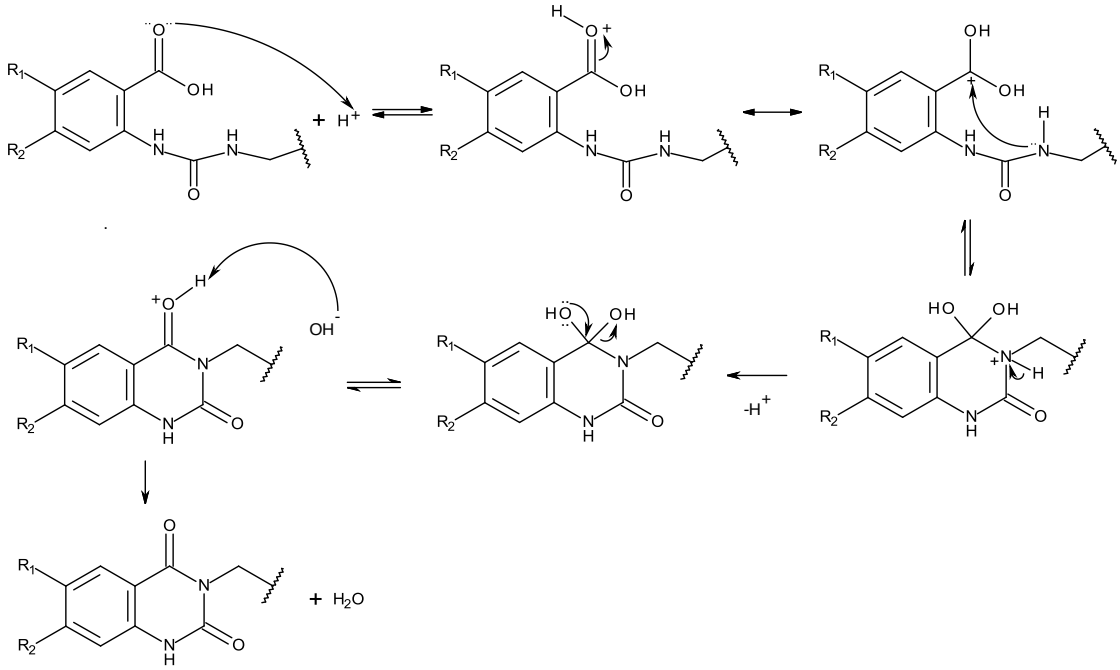
in acidic media, as well as the ester functional group were hydrolysed to carboxylic acid to yield compound **4-6**.



Compound 1-3 **Compound 4-6**

Scheme 5.4. Synthetic pathway of the compound **4-6.**

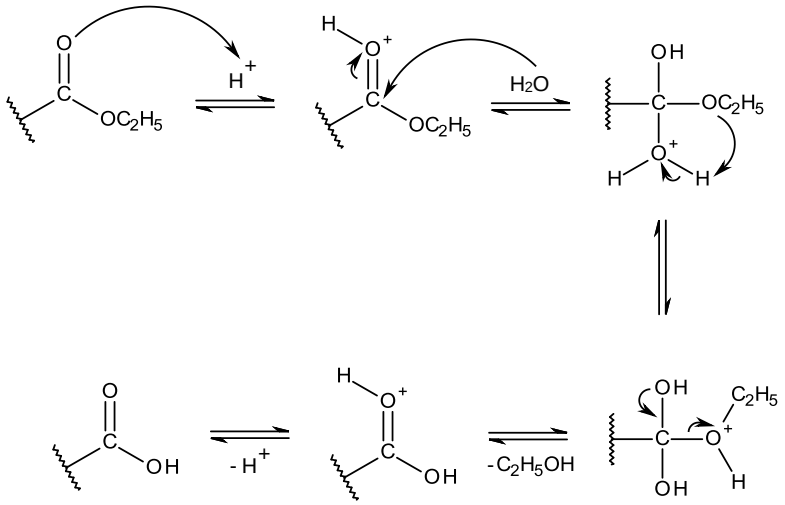
Mechanism for this reaction may be proposed as nucleophilic substitution which is depicted in **Scheme 5.5.**



Scheme 5.5. Reaction mechanism of nucleophilic substitution.

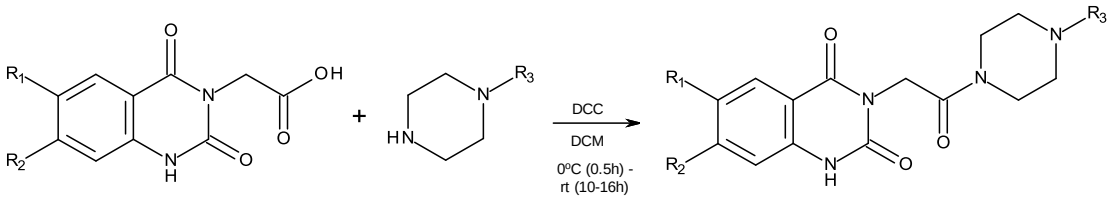
Nucleophilic substitution reaction starts with the attack of urea nitrogen atom to the carbon atom of carboxylic acid, subsequent leaving of the water molecule leads to the formation of 2,4(1*H*,3*H*)-quinazolinedione ring.

The mechanism of acid catalyzed hydrolysis of ester is depicted in **Scheme 5.6.**



Scheme 5.6. Reaction mechanism of acid catalyzed hydrolysis.

Final compounds (compound **7**-**34**) were prepared by the amidation reaction of 2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)acetic acid derivatives (compound **4**-**6**) with various 1-substitutedpiperazines. Reactions were carried out by stirring the reactants with the coupling reagent DCC in nitrogen atmosphere at cold (0-5 °C) to room temperature for 10-16 hours. Reaction solvent was evaporated to dryness. The residue was dissolved in hot acetonitrile then cooled in refrigerator to get the DCU precipitated. White crystalline DCU was removed by filtration. The liquid part was evaporated and crystallized from appropriate solvents. Separating DCU from the product was a time consuming process. Compound **7**-**34** were obtained in varied yields (5-84%) (**Scheme 5.7.**).

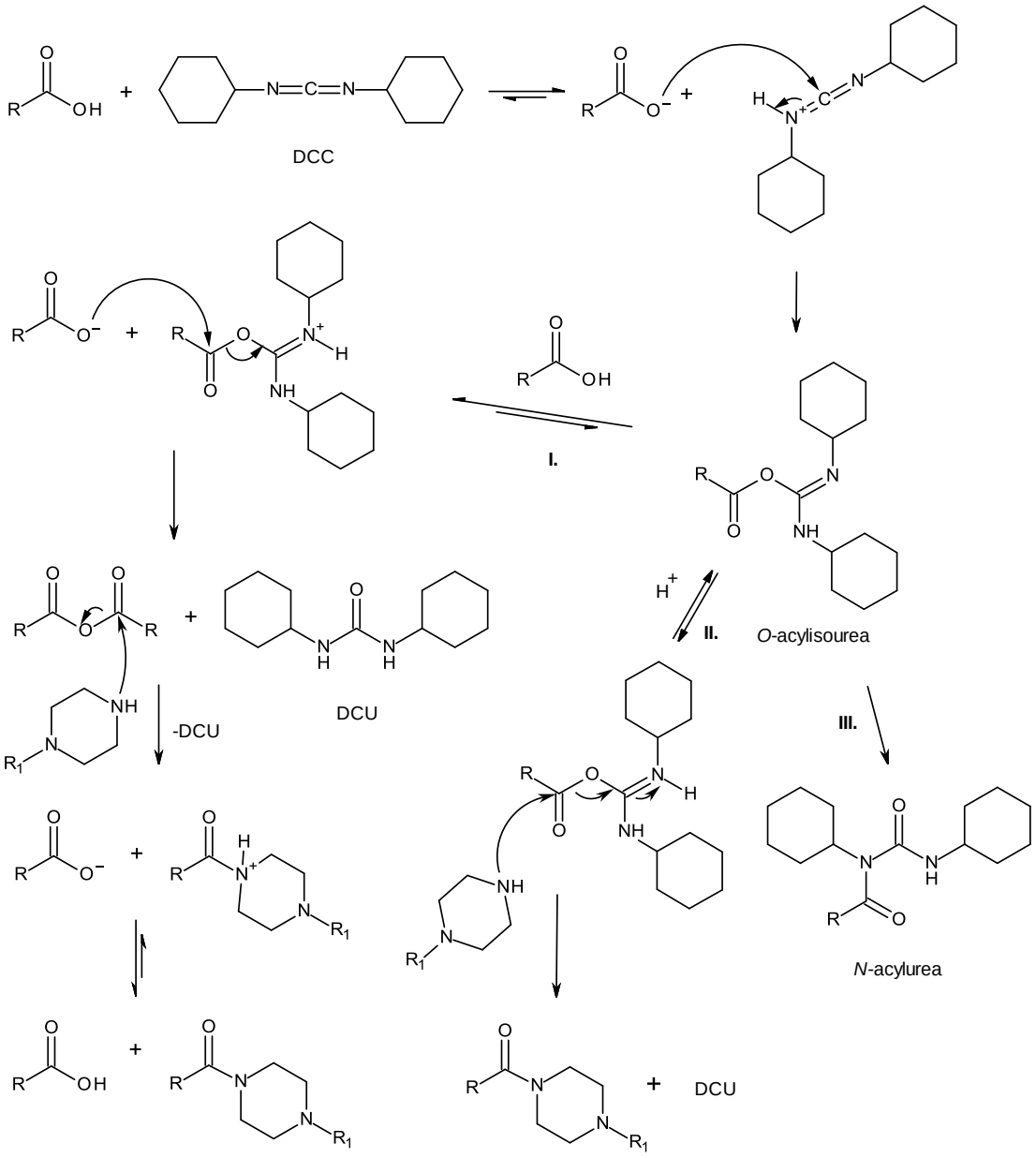


Compound 4-6

Compound 7-34

Scheme 5.7. Synthetic pathway of the compound **7-34.**

The mechanism of DCC-mediated amide formation for this reaction is depicted in **Scheme 5.8.**



Scheme 5.8. Reaction mechanism of DCC-mediated amide formation.

Amide bond formation with using different coupling reagents were well reviewed by Montalbetti [246]. The cheap and soluble carbodiimide coupling reagent DCC was used for the activation of carboxylic acid moiety. The diimide moiety of DCC which contains an electron deficient central carbon atom is attacked by the carboxylate anion generated *in situ*. The highly reactive *O*-acylisourea is subsequently formed

which could go on with different pathways to obtain amide product, DCU and *N*-acylurea as explained below;

I. If excess amount of carboxylic acid is found in the reaction media, reaction yields DCU by-product with symetric anhydride which can couple with the amine to form the amide product.

II. Reaction of *O*-acylisourea with the amine give the amide product and DCU by-product.

III. Acyl transfer from oxygene to nitrogen give side product *N*-acylurea.

These reaction pathways may be the explanation of the moderate yields that we obtained during the synthesis.

5.1. Spectral Datas

After the synthesis of the compounds, structure elucidation was carried out by ^{1}H-NMR, ^{13}C-NMR and mass spectral methods. UV and IR spectral datas also supported informations about chromophore and functional groups.

5.1.1. UV Spectrum

UV spectrums of the synthesized compounds are consistent with those of similar compounds stated in the literature [180, 231].

UV spectrum of the compound **2** gave mainly three absorption bands at 219 (strong), 257 and 344 nm (weak) which represent $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ transitions of C=O and aromatic groups in 2-[3-(2-ethoxy-2-oxoethyl)ureido]benzoic acid derivatives (**Figure 5.1.**).

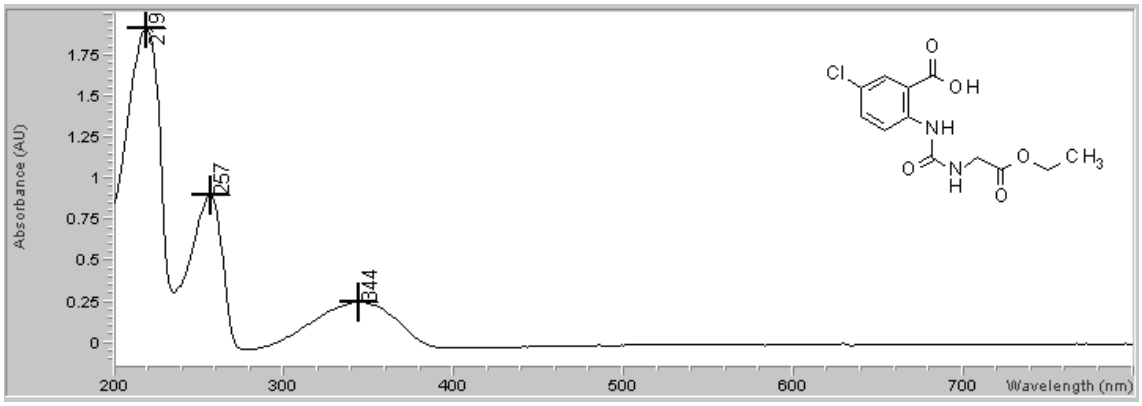


Figure 5.1. UV spectrum of the compound **2**.

UV spectrum of the compound **5** shows three absorption bands at 222 (strong), 245 (shoulder) and 322 nm (weak) which represent π→π*, n→π* transitions of C=O and aromatic groups in (2,4-dioxo-1,2-dihydroquinazolin-3(4*H*)-yl)acetic acid derivatives (**Figure 5.2.**).

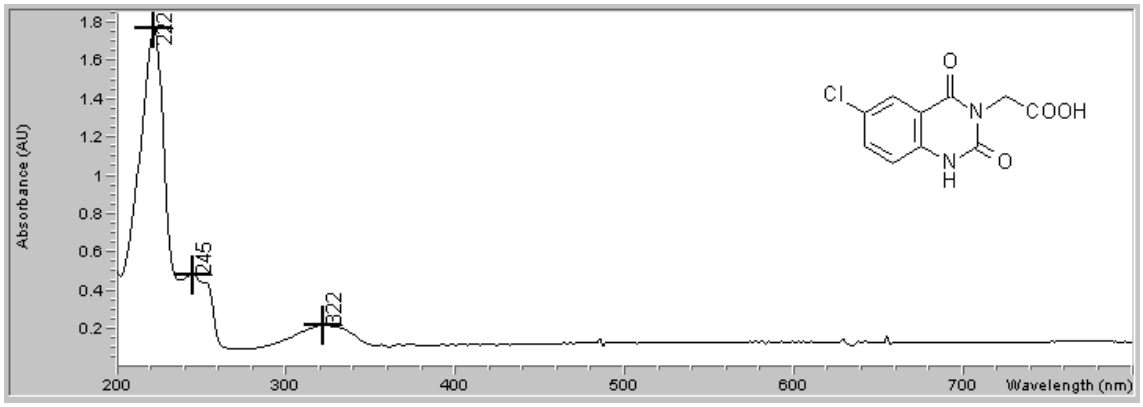


Figure 5.2. UV spectrum of the compound **5**.

UV spectrum of the compound **19** presents absorption bands, same with the compound **5**, at 222, 245 and 322 nm which represent π→π*, n→π* transitions of C=O and aromatic groups in 3-substitutedquinazoline-2,4(1*H*,3*H*)-dione derivatives (**Figure 5.3.**).

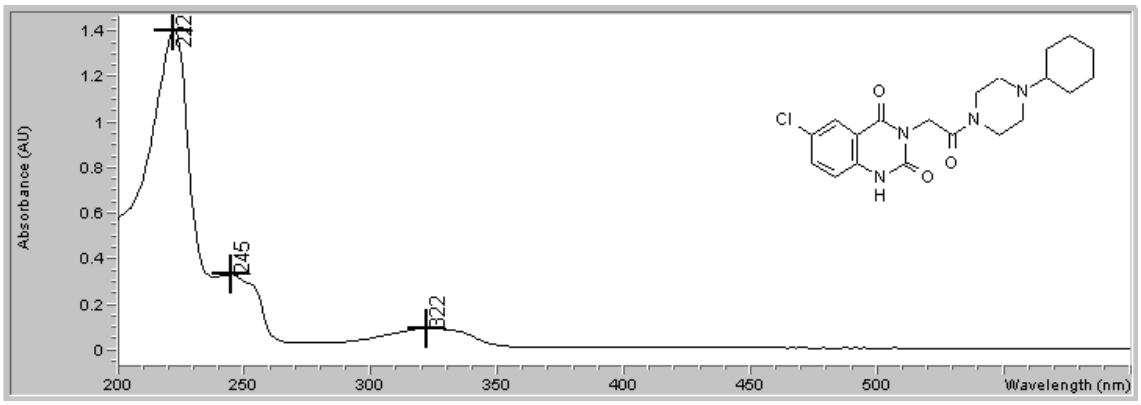


Figure 5.3. UV spectrum of the compound **19**.

Four absorption bands are seen in UV spectrum of the compound **29** (**Figure 5.4.**) at 208, 236, 259, and 321 nm which represent π→π*, n→π* transitions of C=O and aromatic groups in 3-[2-(4-substitutedpiperazin-1-yl)-2-oxoethyl]-6,7-dimethoxyquinazoline-2,4(1*H*,3*H*)-dione derivatives. All 3,4-dimethoxyquinazoline-2,4-dione derivatives, except compound **30**, showed four absorption bands ranging between 201-212, 232-236, 256-260 and 319-321 nm. It is assumed that addition of electron donating methoxy groups to the aromatic ring caused greater bathochromic shift of the bands compared to chloro and non-substituted derivatives [247].

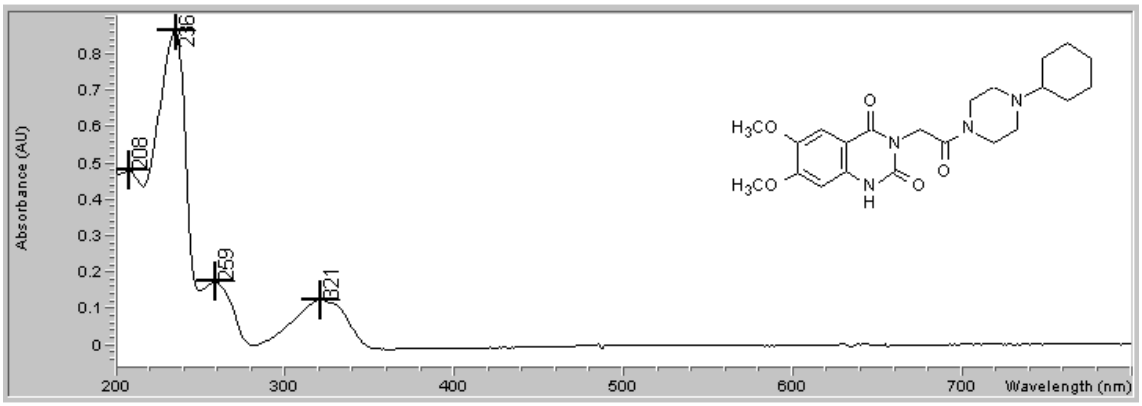


Figure 5.4. UV spectrum of the compound **29**.

5.1.2. IR Spectrum

In IR spectrum of compound **1**, **2** and **3**, C=O streching bands of the ester group were seen at 1725-1738 cm^{-1} (**Figure 5.5.**). Disappearence of these bands for compound **4**, **5** and **6** confirmed the hydrolysis of ester to carboxylic acid. The C=O stretching of aryl carboxylic acids for compound **1**, **2** and **3** appeared as an intense band at 1683-1690 cm^{-1}. Shift of these bands to higher frequencies, 1702-1716 cm^{-1}, for the compound **4**, **5** and **6** which contain aliphatic acid is an evidence for the ring closure reaction of aryl acid to aliphatic acid. Strong C=O stretching bands of compound **4**, **5** and **6** were replaced with the amide I band (C=O streching) of target compound (**7**-**34**) after the amidation reaction (**Figure 5.6.** and **Figure 5.7.**).

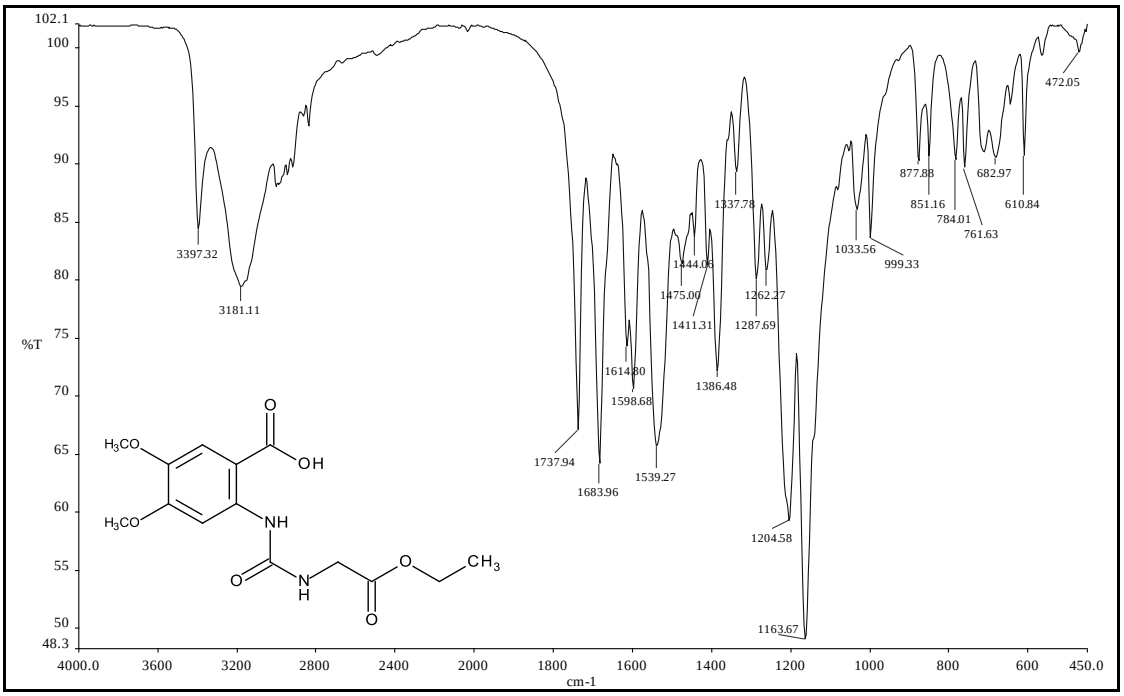


Figure 5.5. IR spectrum of the compound **3.**

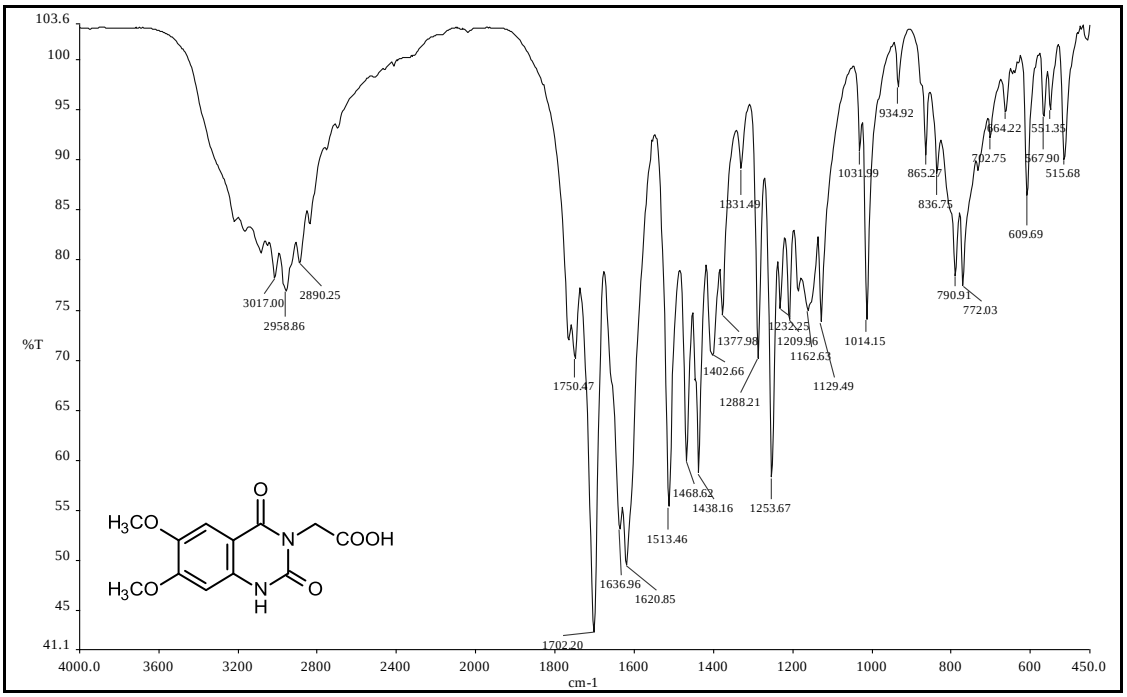


Figure 5.6. IR spectrum of the compound **6.**

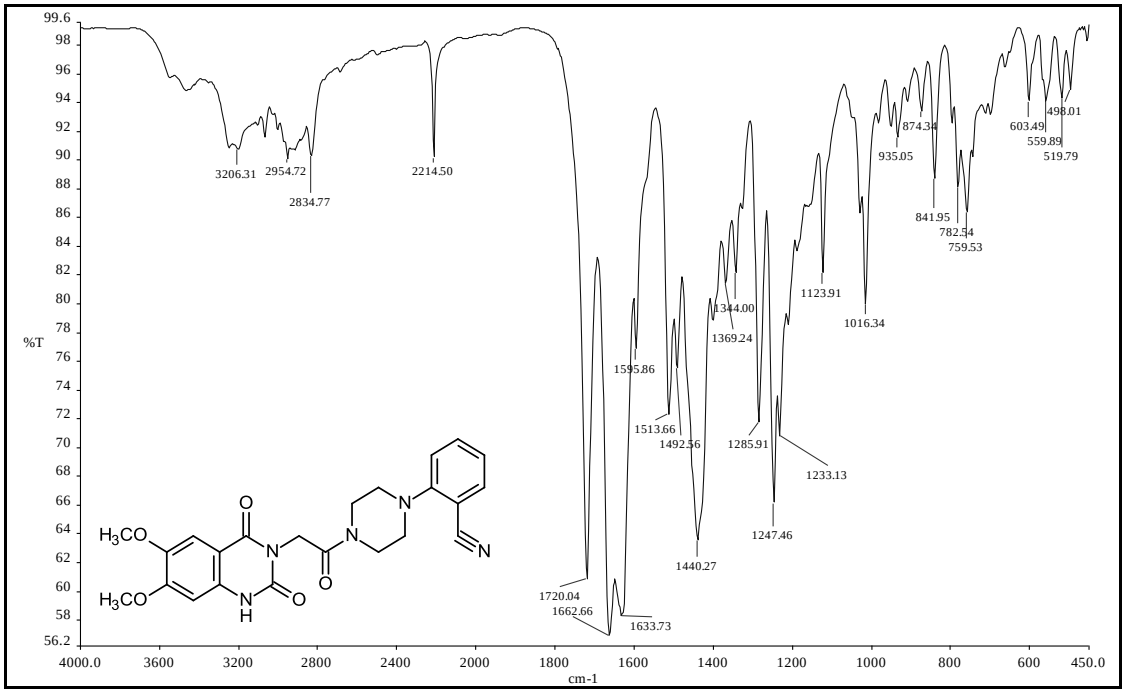


Figure 5.7. IR spectrum of the compound **30.**

C≡N streching bands of the compound **11**, **20** and **30** which contains 2-cyanophenyl structure were clearly seen at 2214-2224 cm^{-1}. Some of the other streching bands for the compound **30** are 3206 cm^{-1} (N-H), 3094 cm^{-1} (aromatic C-H), 2955 cm^{-1} (aliphatic C-H), 1720, 1663 and 1634 cm^{-1} (amide I band, C=O), 1514 cm^{-1} (C=C) (**Figure 5.7.**).

Strong amide I bands (C=O streching) of tertiary amide and cyclic ureide were generally detected as three medium to strong bands. Amide I bands of compound **21** were seen at 1727, 1655 and 1627 cm^{-1} as strong bands. Some of the other streching bands for compound **21** are 3327 cm^{-1} (free N-H), 3187 cm^{-1} (hydrogen bonded N-H), 3062 cm^{-1} (aromatic C-H), 2928, 2851 cm^{-1} (aliphatic C-H), 1491 cm^{-1} (aromatic C=C) (**Figure 5.8.**).

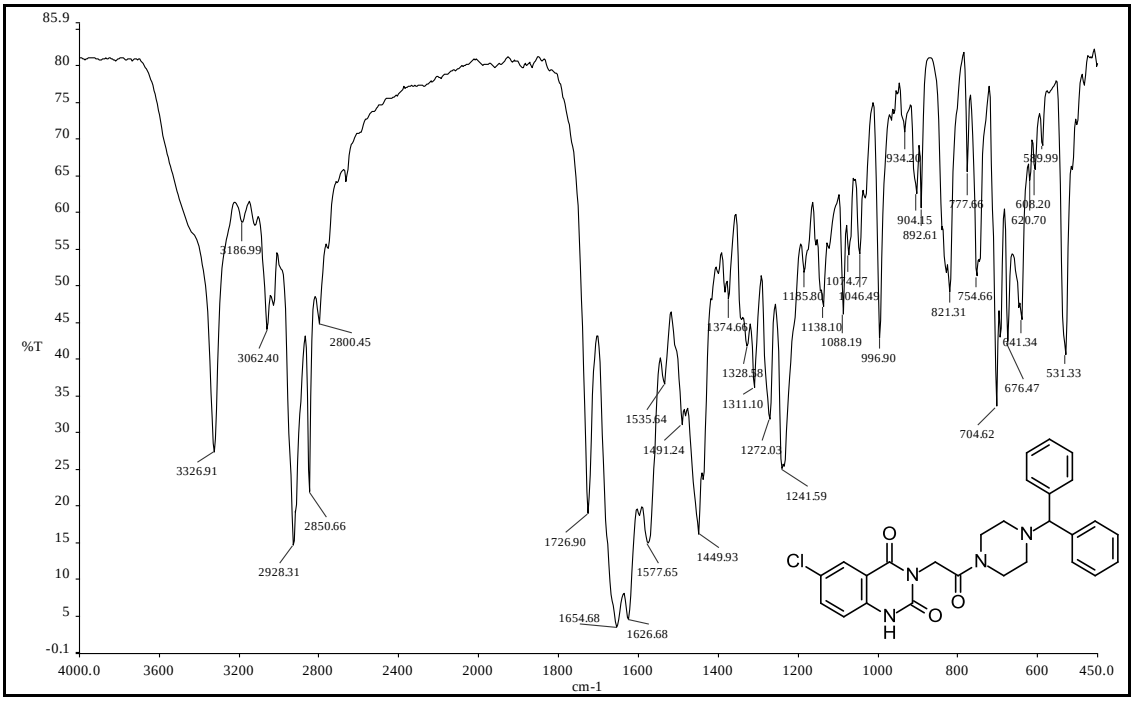


Figure 5.8. IR spectrum of the compound **21.**

5.1.3. Mass Spectrum

Mass spectrometry with electrospray ionization technique (ESI-MS) were performed for the compound **11**, **20** and **32**. ESI-MS is a soft ionization technique causing very little fragmentation of the analyte. Therefore, very little structural

information can be gained from the spectrums. On the other hand, molecular ion peak which carries information about the mass of the analyte is prominent in ESI-MS spectrums [248].

When mass spectrum of the compound **11** was examined (**Figure 5.9.**), molecular ion peak [M^+], observed as base peak at 390.2 (m/z), verified the molecular mass (389.4075 g/mol) of the compound. Peak at 162.7 (m/z) is referred to fragmentation pattern of $C_8H_6N_2O_2$ (162.1455) and peak at 229.2 (m/z) is referred to fragmentation pattern of $C_{13}H_{15}N_3O$ (229.2778) as shown in **Scheme 5.9.**.

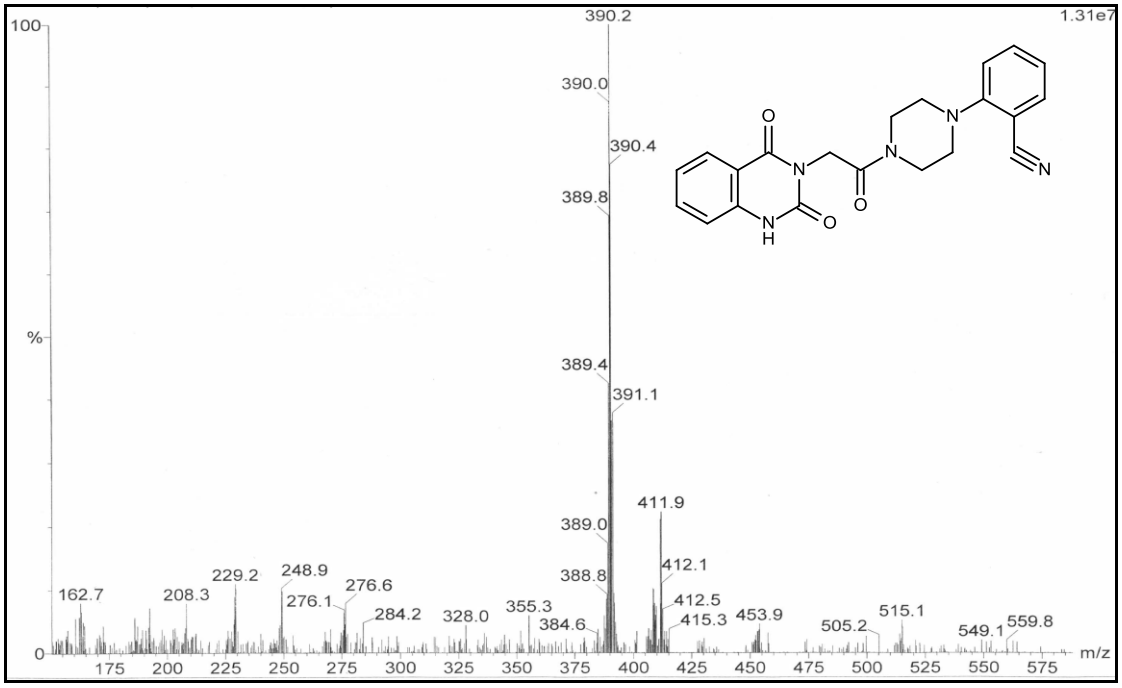
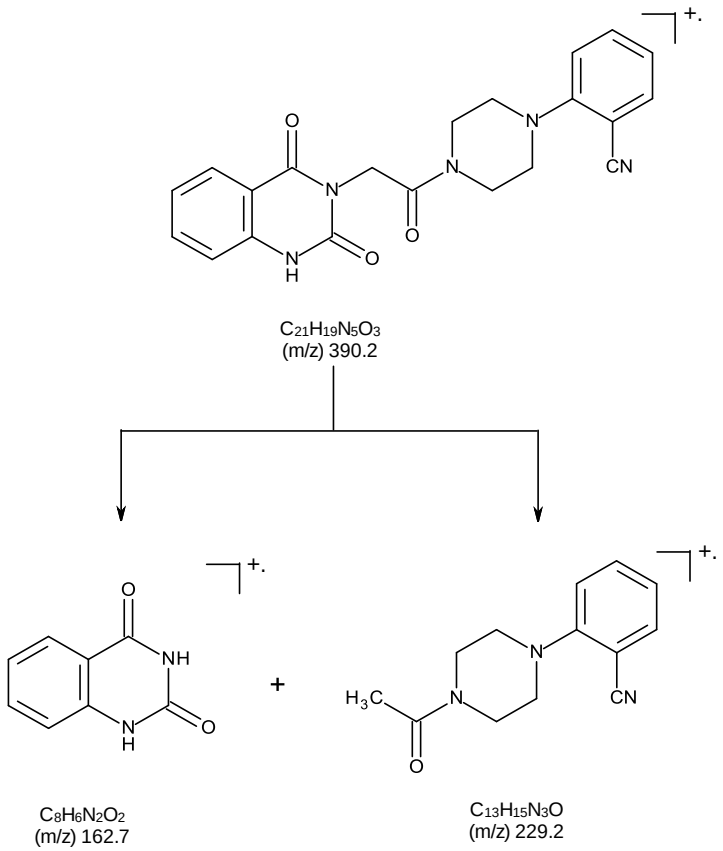


Figure 5.9. Mass spectrum of the compound **11.**



Scheme 5.9. Fragmentation pattern of the compound **11.**

In mass spectrum of compound **20** (**Figure 5.10.**), molecular ion peak [M^+] which was observed as a base peak at 424.1 (m/z) verified the molecular mass (423.8522 g/mol) of the compound. M+2 peak (m/z=426.2) which was about 1/3 intensity of molecular ion peak arised from the presence of chlorine atom in the compound (**Scheme 5.10.)**.

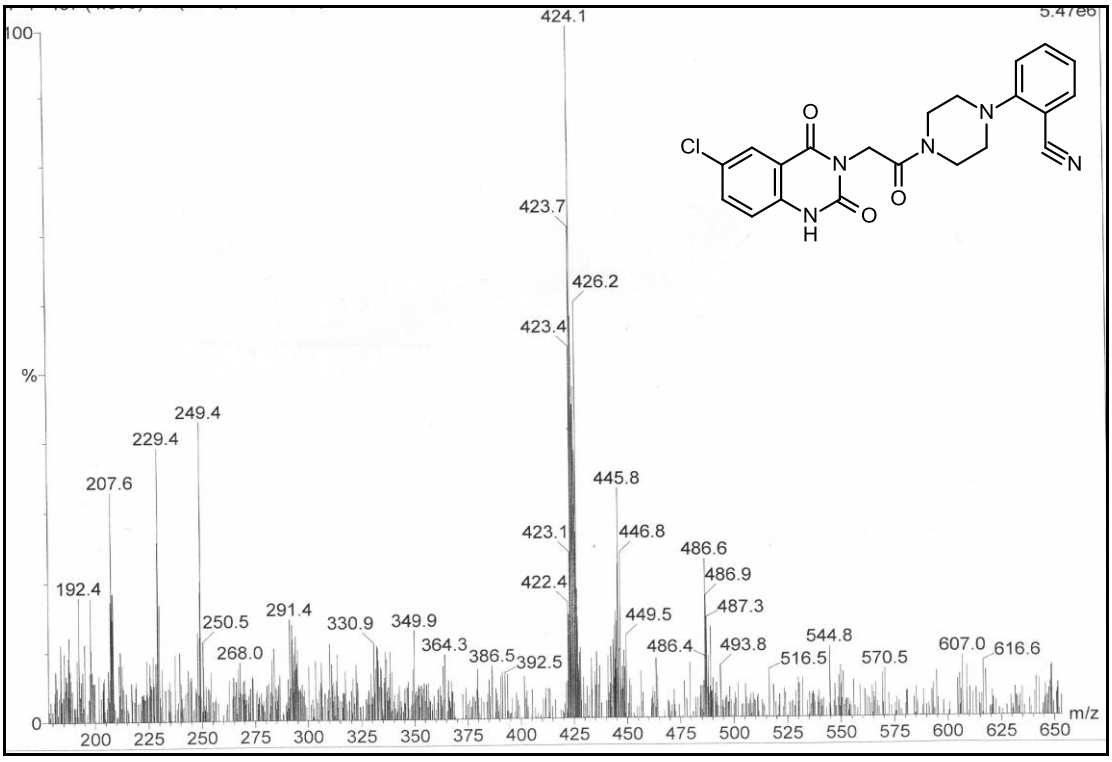
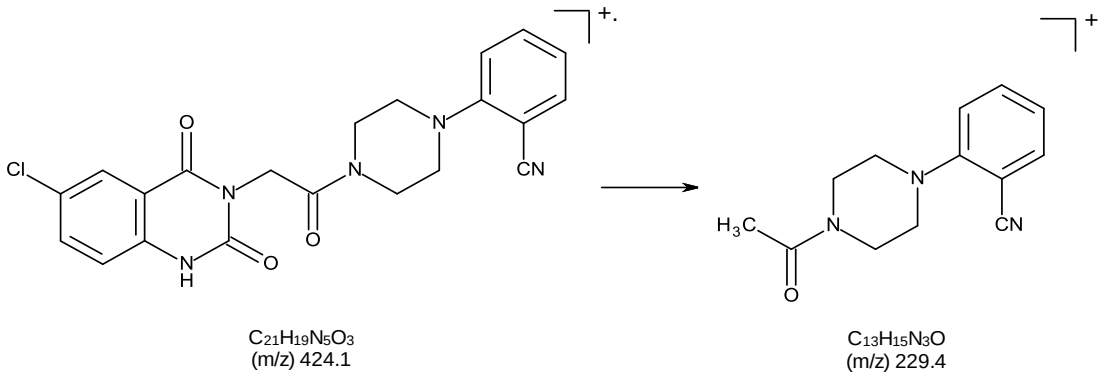


Figure 5.10. Mass spectrum of the compound **20.**



Scheme 5.10. Fragmentation pattern of the compound **20.**

Another example of the mass spectrum is given for the compound **32** (**Figure 5.11.**). The molecular ion peak [M^+] was observed as a base peak at 455.2 (m/z). The peaks at 207.2 (m/z), 249.2 (m/z) and 262.9 (m/z) correspond the fragments of

$C_{10}H_9NO_4$, $C_{13}H_{19}N_3O_2$ and $C_{12}H_{11}N_2O_5$, respectively (**Scheme 5.11.**). This fragmentation pattern is consistent with the fragmentations stated in the literature [231].

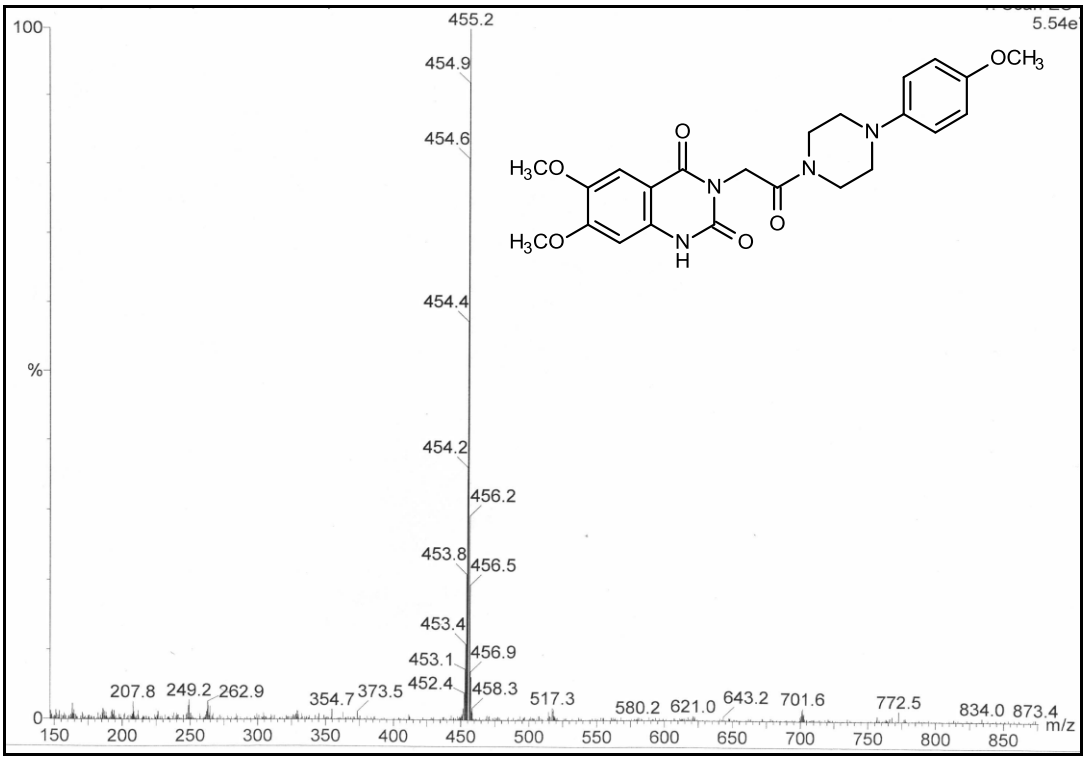
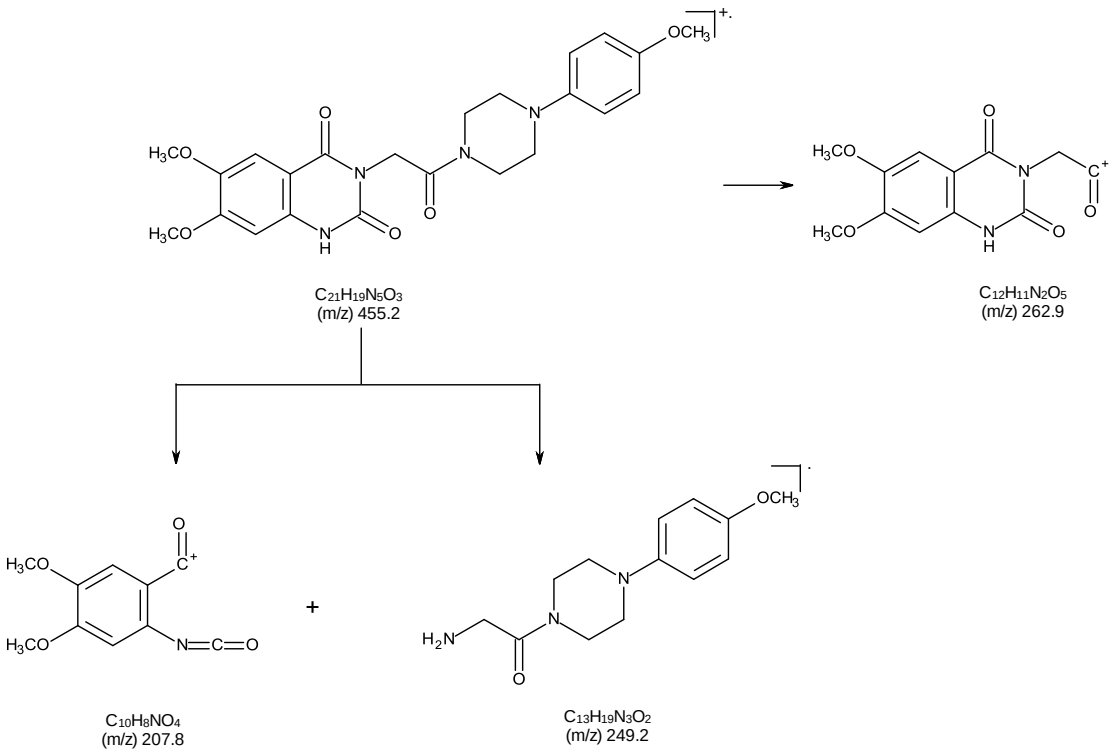


Figure 5.11. Mass spectrum of the compound **32.**



Scheme 5.11. Fragmentation pattern of the compound **32.**

5.1.4. ^{13}C-NMR Spectrum

^{13}C-NMR spectrum of the compound **11**, **20** and **32** were realized using DMSO-d_6 as solvent by using TMS standard.

The peaks for quinazoline-2,4(1*H*,3*H*)-dione ring carbons were consistent with the values stated in the literatures [223, 225–228]. Signals at 150.17-150.58 ppm and 161.21-162.19 ppm were assigned to C^2 and C^4 atom of C=O for quinazolinedione ring. Benzene carbons of quinazolinedione ring were observed as six signals between at 115.68-139.88 ppm, 117.98-138.76 ppm and 97.99-155.56 ppm for non-substituted, chloro-substituted and dimethoxy-substituted derivatives, respectively.

Signals which are shifted furthest downfield at 165.22-165.41 ppm are assigned to C^{10} atom of C=O, attached to piperazine, and were consistent with the values of similar structures stated in the literatures [248, 249].

The peaks for piperazine carbon atoms were ranging from 41.72 to 51.98 ppm. Signals of C^{12} and C^{14} atoms shifted downfield compared to C^{11} and C^{13} atoms due to the proximity with the phenyl ring [249–254]. Signals at 44.62-44.81 ppm are assigned to CH_2 of NCH_2CO linkage (C^9) which are shifted downfield because of the C=O and N- moieties.

^{13}C-NMR spectrum of the compound **11** gave peaks at 150.48 (C^2), 162.19 (C^4), and 165.41 (C^{10}) which belongs to the C=O carbons. The aromatic carbons ($C^{4'-8'}$ and C^{15-20}) gave resonance in the range of 105.59-155.32 ppm. Cyano carbon (C^{21}) gave resonance at 122.95 ppm, while aliphatic carbons at 44.77 (C^9), 41.91 (C^{11}), 42.12 (C^{13}), 51.31 (C^{12}) and 51.98 (C^{14}) ppm as seen in **Figure 5.12.**

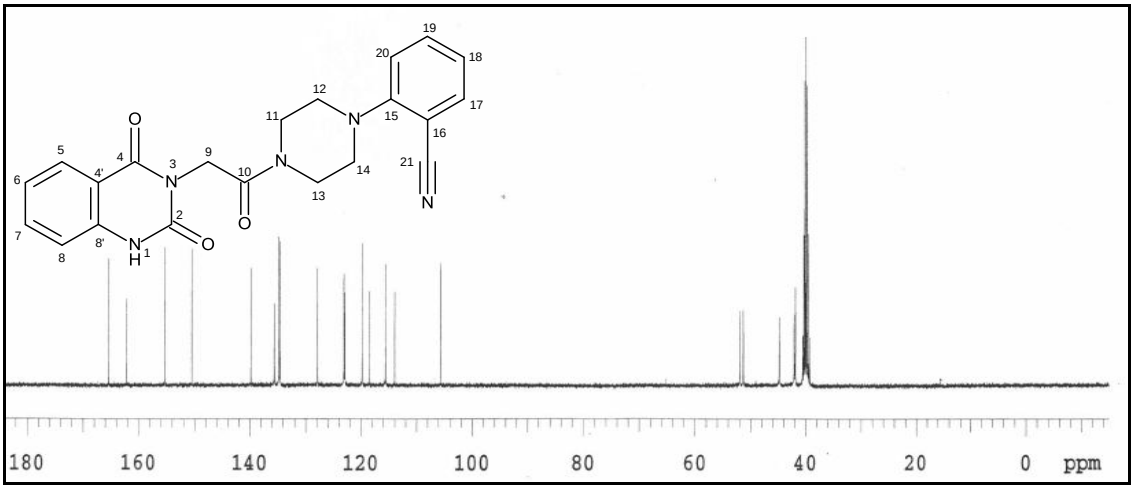


Figure 5.12. ^{13}C-NMR spectrum of the compound **11.**

Compound **20**, the chloro derivative of the compound **11**, gave the similar ^{13}C spectrum as seen in **Figure 5.13.**. Benzene carbons (C^{15-20}) gave peaks in the range of 105.58-155.29 ppm. C^{15} atom attached to the piperazine was the most deshielded atom for the phenyl ring and observed at 155.29 ppm. Cyano carbon (C^{21}) gave resonance at 122.92 ppm.

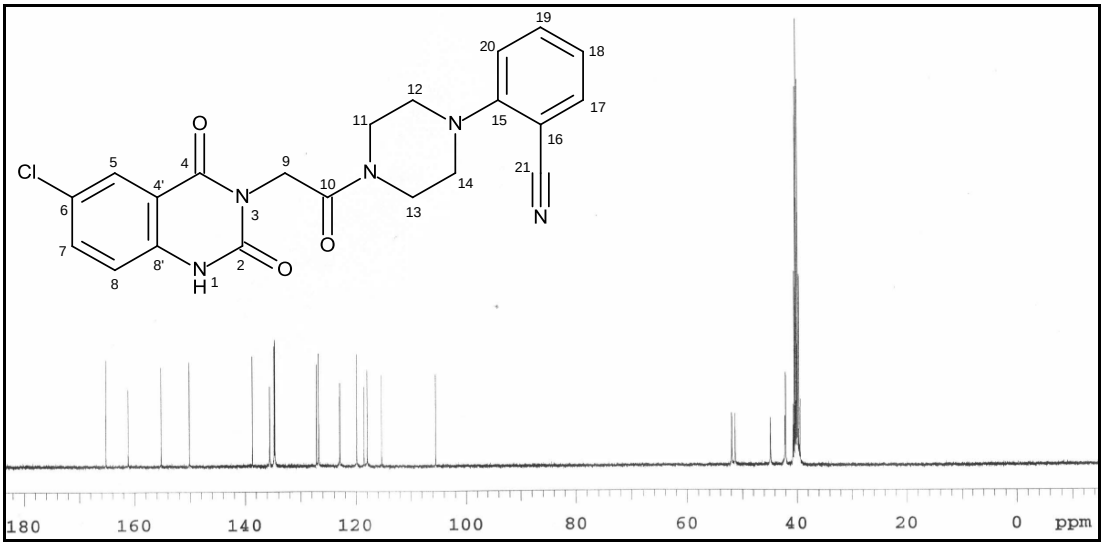


Figure 5.13. ^{13}C-NMR spectrum of compound **20.**

The ^{13}C-NMR spectrum of the compound **32** gave the peaks at 165.34 (C^{10}) 161.69 (C^{4}), 150.58 (C^{2}) ppm belong to the C=O carbons. Aromatic carbons $C^{15\text{-}20}$ and $C^{4'\text{-}8'}$ atoms were seen between at 114.75-153.85 ppm. Aliphatic carbons C^{21}, C^{9} and piperazine carbons were observed 55.62 (C^{21}), 44.62 (C^{9}), 41.72 (C^{11}), 42.05 (C^{13}), 50.27 (C^{12}), 50.71 (C^{14}), 56.19 (C^{22}), and 56.31 (C^{23}) ppm (**Figure 5.14.**).

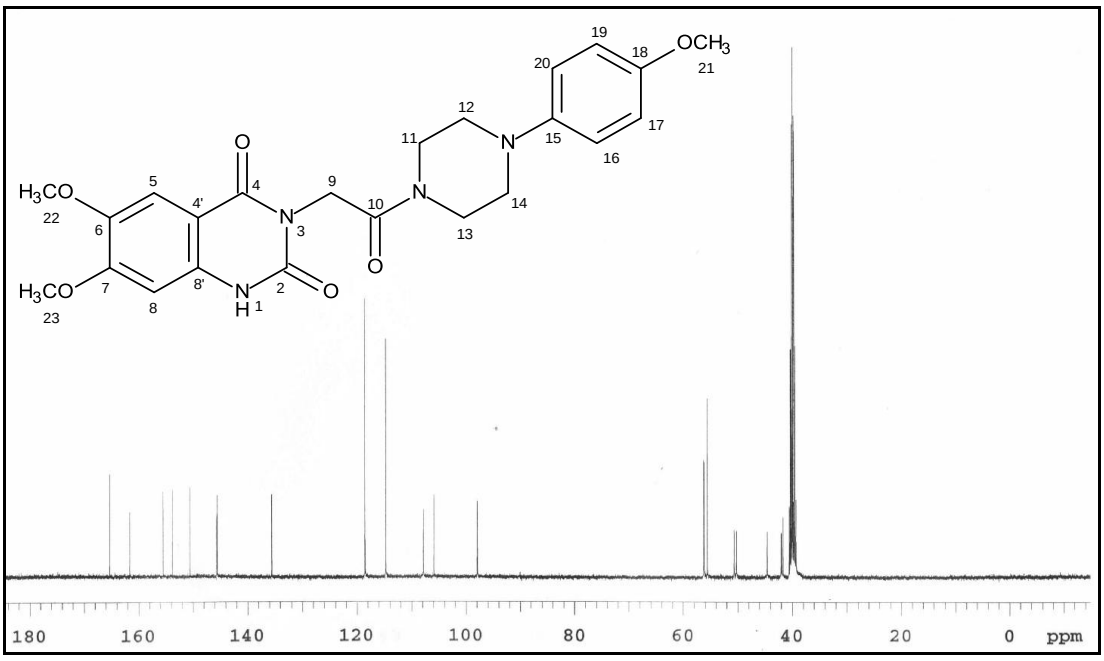


Figure 5.14. ^{13}C-NMR spectrum of the compound **32.**

5.1.5. ^{1}H-NMR Spectrum

^{1}H-NMR spectrum of all compounds were taken by using DMSO-d_6 as solvent by using TMS standard.

^{1}H-NMR spectrum of compound **7** displayed four triplet due to CH_2 groups of piperazine at 2.34-3.57 ppm. The signal originated from CH_2 protons of NCH_2CO resonated at 4.74 ppm as singlet. The chemical shift range of CH benzene of the quinazolinedione ring varied from 7.20 to 7.93 ppm. H^6 and H^8 protons of the benzene were observed as multiplets at 7.20-7.24 ppm. H^7 proton gave one signal at 7.67-7.71 as multiplet integrating one proton. H^5 proton was deshielded due to the proximity of C=O group and observed at 7.90-7.93 ppm as multiplet. The signal originated from NH of quinazoline-2,4(1*H*,3*H*)-dione appeared furthest downfield at 11.53 ppm as singlet (**Figure 5.15.**). These signals were similar for all analogs (compound **7**-**14**).

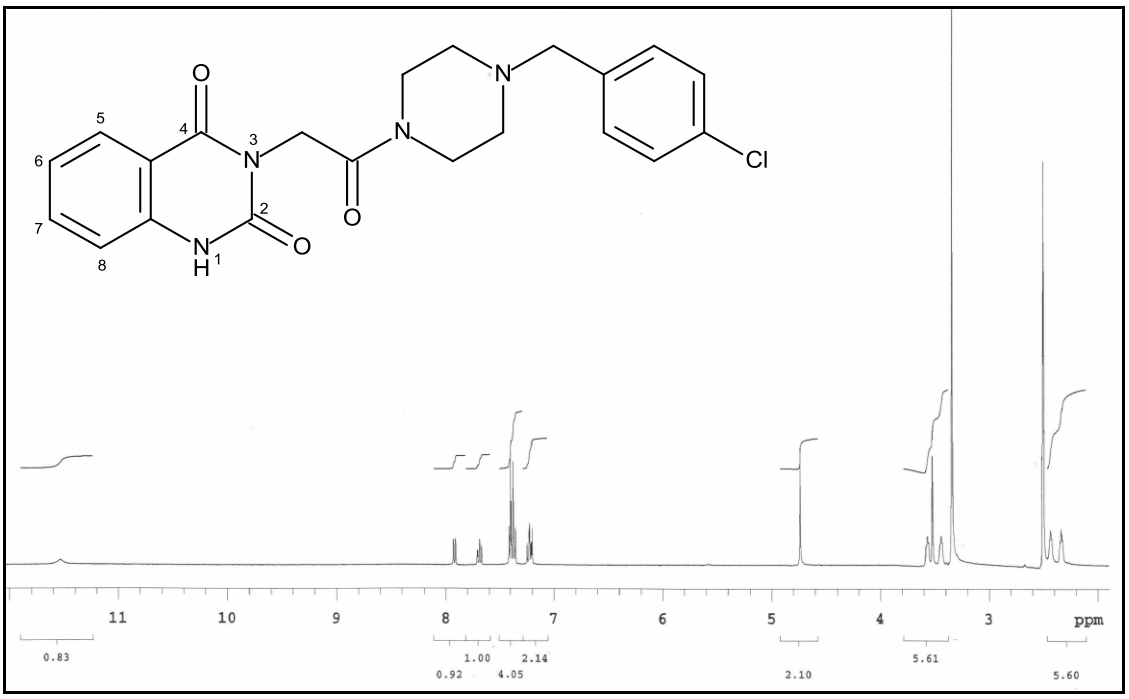


Figure 5.15. ^{1}H-NMR spectrum of the compound **7.**

^{1}H-NMR spectrum of the compound **21** displayed four triplet due to CH_2 groups of piperazine at 2.29-3.59 ppm. The signal originated from CH_2 of NCH_2CO resonated at 4.71 ppm as singlet. The chemical shift range of CH benzene of the quinazolinedione

ring varied from 7.19 to 7.86 ppm. H^8 proton of the benzene gave signal at 7.19-7.24 ppm as multiplet. H^7 proton was observed at 7.73-7.76 as doublet of doublet. H^5 proton was deshielded due to the proximity of C=O group and observed at 7.85-7.86 ppm as doublet. The signal originated from NH of quinazoline-2,4-dione appeared furthest downfield at 11.69 ppm as singlet (**Figure 5.16.)**. These signals were similar for all analogs (Compound **15-24**).

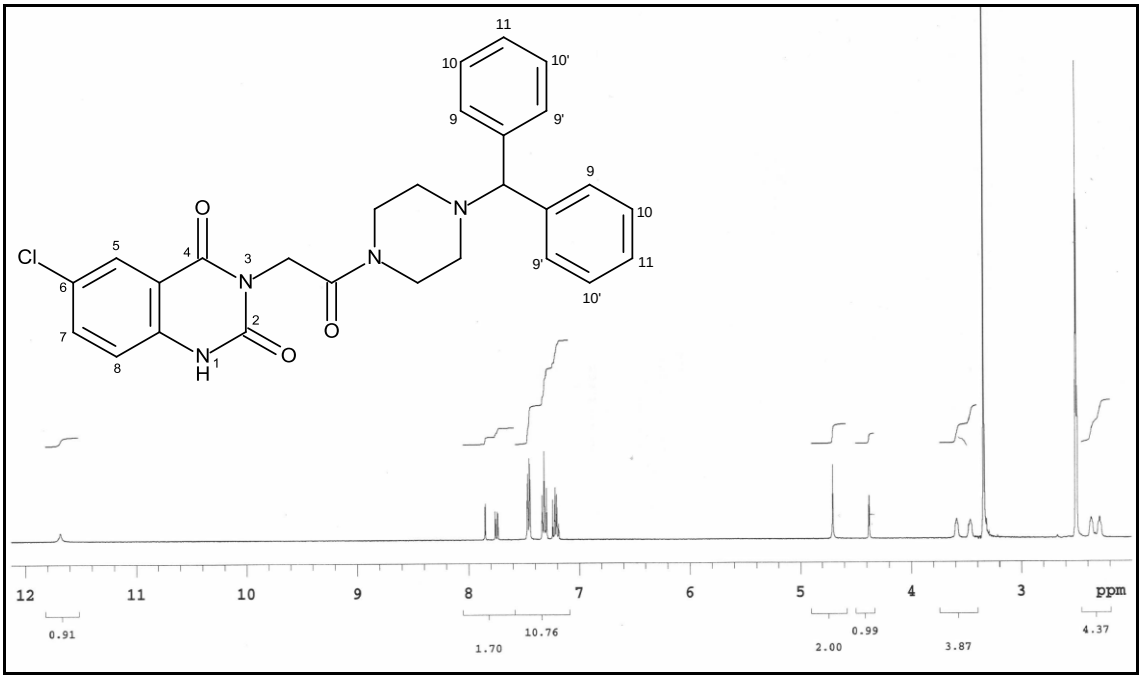


Figure 5.16. ^{1}H-NMR spectrum of the compound **21.**

^{1}H-NMR spectrum of compound **29** displayed four triplet due to CH_2 groups of piperazine at 2.45-3.41 ppm. Two methoxy group protons were observed as singlets at 3.79 and 3.85 ppm. The signal originated from CH_2 of NCH_2CO resonated at 4.70 ppm as singlet. The chemical shift range of CH benzene of the quinazolinedione ring varied from 6.70 to 7.26 ppm. H^8 proton was observed at 6.70 as singlet. H^5 proton was deshielded due to the proximity of C=O group and observed at 7.26 ppm as singlet. The signal originated from NH of quinazoline-2,4-dione appeared furthest downfield at 11.30 ppm as singlet (**Figure 5.17.)**. These signals were similar for all analogs (compound **25-34**).

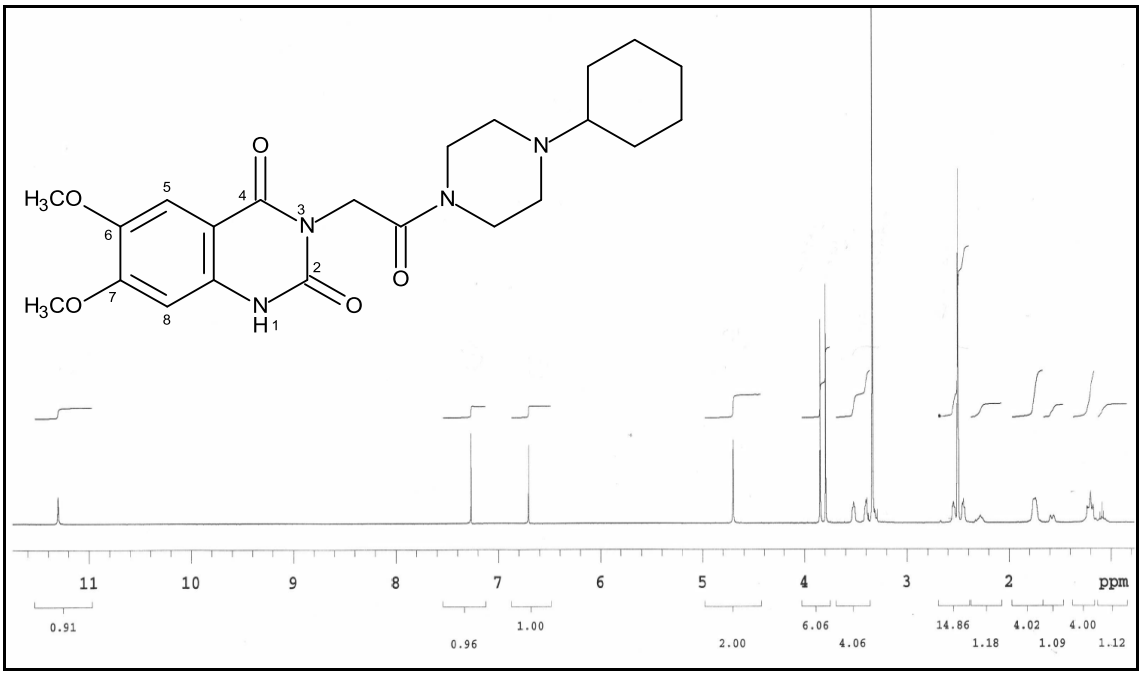
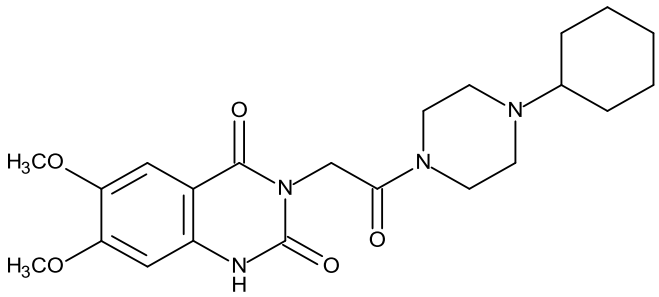


Figure 5.17. ^{1}H-NMR spectrum of the compound **29.**

5.1.5. Biological Activities

All synthesized compounds were evaluated for their antimicrobial and cytotoxic activity. The results are shown in **Table 4.1.** and **Table 4.2.**

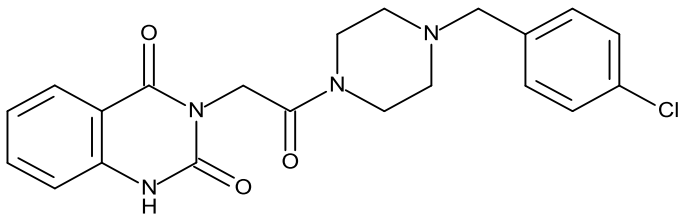
Synthesized compounds were screened against two gram positive bacteria strains, *Bacillus subtilis* and *Staphylococcus aureus*, with using agar-based disc diffusion method. When zone of inhibitions were examined, it was observed that compounds generally showed mild to moderate activity compared with ampicillin standard. 3-[2-(4-Cyclohexylpiperazin-1-yl)-2-oxoethyl]-6,7-dimethoxyquinazoline-2,4(1*H*,3*H*)-dione (compound **29**, **Figure 5.18.**) exhibited the highest zone of inhibition value against *S. Aureus.* Inhibition zone of compound **29** is 14 mm whereas that of ampicillin is 11 mm. Compound **33** has the highest activity against *B. Subtilis* with the inhibition zone of 12 mm. Compounds **19**, **22-24** also show moderate activity with the inhibition zone of 10 mm (**Table 4.1.**).



Zone of inhibition = 14 mm (*S. Aureus)*

Figure 5.18. Molecular structure of compound **29.**

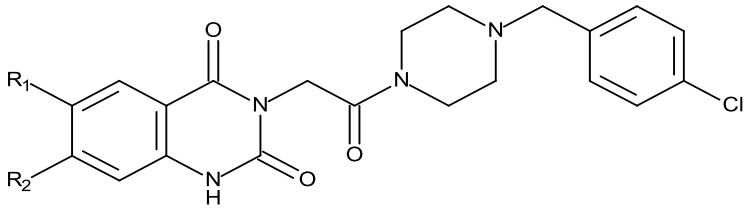
All synthesized compounds were also examined for their cytotoxic activity against hepatoma (HUH-7), breast cancer (MCF-7) and colorectal cancer cell line (HCT-116) with using Sulforhodamine B assay. According to the cytotoxicity data, all compounds generally exhibited moderate or no cytotoxic activity (**Table 4.2.**). 3-{2-[4-(4-Chlorobenzyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1*H*,3*H*)-dione (compound **7**, **Figure 5.19.**) presented the highest activity against HUH-7, MCF-7 and HCT-116 with the IC_{50} values of 2.5, 6.8 and 4.9 μM, respectively. 6-Chloro derivative (compound **15**) of this compound exhibited lower activity against these three cell lines than compound **7** with IC_{50} values of 7.0 μM, 13.1 μM and 9.4 μM, whereas 6,7-dimethoxy derivative (compound **25**) showed moderate cytotoxicity against HCT116 with IC_{50} value of 16.9 μM and no cytotoxicity against HUH-7 and MCF-7 cell lines **(Table 5.1.)**.



IC_{50} = 2.5 μM (HUH-7)

Figure 5.19. Molecular structure of compound **7**.

Table 5.1. IC_{50} values (μM) of compound **7**, **15** and **25**.



Compound	R_1	R_2	HUH-7	MCF-7	HCT-116
7	-H	-H	2.5	6.8	4.9
15	-Cl	-H	7.0	13.1	9.4
25	$-OCH_3$	$-OCH_3$	-	-	16.9

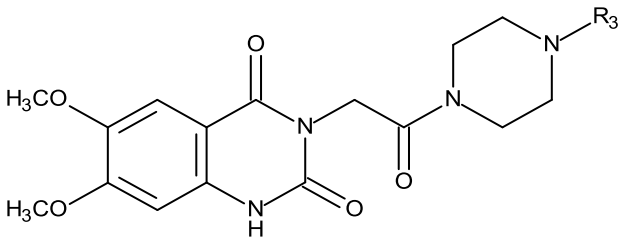
Compound **8** presented cytotoxicity over HUH-7, MCF-7 and HCT-116 cell lines with IC_{50} values of 11.5, 12.2 and 35.3 μM, respectively. But 6-chloro (compound **17**) and 6,7-dimethoxy derivative (compound **27**) of this compound did not show any activity against these three cell lines.

Compound **16** bearing 6-chloro atom on the 2,4-quinazolinedione ring exhibited moderate activity against HUH-7 and MCF-7 cell lines with IC_{50} values of 12.8 and 18.6 μM, whereas no activity against these cell lines was observed for the 6,7-dimethoxy derivative (compound **26**). Compound **26** only presented cytotoxic activity against HCT-116 with IC_{50} value of 6.0 μM.

Diphenylmethyl derivative of 6-chloro-2,4-quinazolinedione (compound **21**) showed cytotoxicity against HUH-7, MCF-7 and HCT-116 cell lines with IC_{50} values of 9.2 μM, 13 μM and 9 μM, respectively. Its non-substituted derivative (compound **12**) only exhibited cytotoxicity over MCF-7 cell line with IC_{50} value of 15.2 μM, whereas 6,7-dimethoxy derivative (compound **31**) only showed cytotoxicity over HCT-116 cell line with IC_{50} value of 9.9 μM.

Only the compounds **25**, **26** and **29**-**31** presented IC_{50} values ranging from 6 to19 μM against HCT-116 cell line. But generally, 6,7-dimethoxy derivatives (compound **25**-**34**) did not show any cytotoxic activity over HUH-7, MCF-7 cell lines (**Table 5.2.**).

Table 5.2. IC_{50} values (μM) of compound **25, 26** and **29**-**31.**



Compound	R_3	HUH-7	MCF-7	HCT-116
25	4-chlorobenzyl	-	-	16.9
26	3-(trifluoromethyl)phenyl	-	-	6.0
29	cyclohexyl	-	-	11.4
30	2-cyanophenyl	-	-	19.0
31	diphenylmethyl	-	-	9.9

In conclusion, we have prepared some 3-substituted-2,4(1*H*,3*H*)-quinazolinedione derivatives and evaluated their antimicrobial and cytotoxic activities under *in vitro* conditions. From the preliminary results of this study we could conclude that synthesized compounds did not show significant antimicrobial activity except compound **29**. The zone of inhibition value of compound **29** was 14 mm against *Staphylococcus aureus*, whereas that of ampicillin standart was 11 mm.

Cytotoxic activity of the compounds were also screened against hepatoma (HUH-7), breast cancer (MCF-7) and colorectal cancer cell lines (HCT-116). Compound **7** with 4-chlorobenzyl substituent on it is the most potent compound of this series against these three cell lines with IC_{50} values of below 7 μM.

In general, most of the synthesized compounds did not show significant cytotoxicity except compound 7, which emphasize the requirement of quinazoline ring modification. Our further studies may include derivatization of the compound **7** to obtain more potent compounds.

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