YEDITEPE UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

SYNTHESIS OF A SERIES OF NEW SULFONAMIDE SUBSTITUTED ISOINDOLO[2,1-a]QUINAZOLINE-6,12-DIONE DERIVATIVES AND EVALUATION OF THEIR BIOLOGICAL ACTIVITY

MASTER of SCIENCES THESIS

GÜNEŞ KAVALA

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SUPERVISOR

Assoc. Prof. Dr. FİLİZ ESRA ÖNEN BAYRAM

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APPROVAL

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 01/03/2021 and numbered 2021/02-15.

Prof. Dr. Bayram YILMAZ

Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

Date

Name Surname

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BHT	Butyl hydroxytoluene
CPT	Camphotectin
DABCO	1,4-diazabicyclo[2.2.2]octane
DMSO	Dimethyl sulfoxide
DPPH	1,1-diphenyl-2-picrylhydrazyl
EtOH	Ethanol
FDA	Food and Drug Administration
IL	Ionic liquid
LC-MS	Liquid chromatography–mass spectrometry
MH	Molecular hybridization
Mw	Molecular weight
NMR	Nuclear magnetic resonance
ppm	Parts per million
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TNF	Tumor necrosis factor
TopI	Topoisomerase I
TsOH	p-Toluenesulfonic acid
UV	Ultraviolet

ABSTRACT

KAVALA, G. Synthesis of a series of new sulfonamide substituted isoindolo[2,1-a]quinazoline-6,12-dione derivatives and evaluation of their biological activity, Yeditepe University Institute of Health Science, Thesis on Pharmaceutical Chemistry Master of Science Degree Programme, Istanbul, 2021.

Cancer and inflammatory diseases constitute two major public health concerns. Although radiotherapy and surgery are considered for the treatment of cancer cases, chemotherapy remains the most effective therapeutic strategy. However, because of its side effects, there is still an urgent need for the generation of more selective novel synthetic or natural bioactive molecules. Inflammation is a biological response for tissue damage and activities pathways that are involved in tissue repair. Chronic inflammation results from simultaneous and prolonged tissue destruction and repair and is often related to an infection. In diseases caused by chronic inflammation, as TNF-α immunomodulator is overproduced, the therapeutic strategy consists of using molecules that regulate the TNF-α levels. Moreover, since in chronic inflammative tissues, cells are repeatedly exposed to high levels of reactive oxygen and nitrogen species, there are many studies that suggest that their DNA is consequently damaged which results in cancer development. In this research, sulfonamide substituted isoindolo[2,1-a]quinazoline derivatives were synthesized via molecular hybridization, to develop new structures that can potentially show both anti-inflammatory and anticancer activity. Eleven new sulfonamide substituted isoindoloquinazoline were thus obtained. Their structure was elucidated via spectral analyses and the compounds were evaluated for their biological activity.

Keywords: isoindoloquinazoline, sulfonamides, molecular hybridization, cancer, inflammation

ÖZET

KAVALA, G. Yeni bir dizi sülfonamit süstitüe izoindolo[2,1-a]kinazolin-6,12-dion bileşiğinin sentezi ve biyolojik aktivite değerlendirmesi. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü Farmasötik Kimya Yüksek Lisans Programı Tezi, İstanbul 2021.

Kanser ve enflamasyon insan sağlığını tehdit eden hastalıkların başında gelmektedir. Kanser tedavisinde radyoterapi ve cerrahi müdahele yöntemlerine başvurulsa da ilaç tedavisi (kemoterapi) hala en etkili çözüm olarak kabul edilmektedir. Klinikte kulllanılan kanser ilaçlarının öne çıkan sorunu ise istenmeyen yan etkilerdir ve bu nedenle, yan etkisi azaltılmış ve daha seçici yeni bitkisel ve/veya sentetik molküllerin bulunmasına ihtiyaç duyulmaktadır. Enflamasyon doku zedelenmesine karşı geliştirilen bir cevaptır ve doku onarımını sağlayan yolakları aktive eder. Enflamasyona bağlı kronik hastalıklarda TNF-α imünomodülatörü aşırı salgılandığından, tedavilerinde TNF-α düzeylerini düzenleyici moleküller kullanılmaktadır. Ayrıca kronik enflamatif dokularda hücreler sürekli olarak yüksek oranda reaktif oksijen ve azot türevlerine maruz kaldığından, bu hücrelerde DNA hasarının gerçekleştiği ve takibinde ise bu dokularda kanserin geliştiği düşünülmektedir. Bu çalışmada, tübilin polimeraz ve topoizomeraz I'i inhibe ederek antikanser etki gösterdiği düşünülen izoindolo[2,1-a]kinazolin yapısı ile hücre içi TNF-α düzeylerini düşürüp anti-enflamatuvar etki gösterdiği belirtilen sülfonamit yapısının moküler hibritleşme stratejisince birleştirilerek hem anti-kanser hem de anti-enflamatuvar etki gösteren bileşiklerin sentezlenmesi hedeflenmiştir. Bu kapsamda on bir yeni sülfonamit sübstitüe izoindolokinazolin bileşiği elde edilmiş, oluşturulan bileşiklerin yapıları spektral analiz yöntemleri ile aydınlatılmış ve bileşiklerin biyolojik aktiviteleri değerlendirilmiştir.

Anahtar kelimeler: izoindolokinazolin, sülfonamit, hibrit ilaç, kanser, enflamasyon

1. INTRODUCTION AND PURPOSE

Cancer plays a crucial role in public health concerns. Even if radiotherapy and surgery are also considered for the treatment of cancer, chemotherapy remains the most effective therapeutic strategy.

Cancer and inflammation are related to each other as it has been reported that chronic inflammation is a hallmark for the generation and development of cancer. Inflammation is the biological response of tissue damage and involves pathways for tissue repair. An important release of reactive oxygen and nitrogen species results from this process with the migration of leukocytes and phagocytic cells to the inflammatory tissue. Prolonged tissue destruction related to an infection can lead to chronic inflammation and overproduction of the TNF-α immunomodulator with repeated exposure to high reactive oxygen and nitrogen species levels lead to DNA damage and thus cancer development.

Free radicals are highly reactive molecules that bear at least one unpaired electron. They can be generated in cells either from metabolic transformations mainly taking place in mitochondria[1], microsomes[1] or macrophages[1], from exposure to toxic xenobiotics[2] or exposure to UV, X-ray or gamma rays.[3] The main free radicals present in cells are reactive oxygen or nitrogen species also known as ROS or RNS respectively. The ROS and RNS presence can be both beneficial and harmful according to the concentrations at which they can be found. Indeed, while at low levels they act as messengers in many cellular pathways[4] and can have a mitogenic activity[5], at high concentrations, they are reacting and damaging the structure of proteins, lipids and even the DNA molecule[4] .

ROS correspond to the superoxide radical ion O_2 or the hydroxyl radical OH. O_2 is considered as a primary radical as it is further converted either directly or *via* metal-catalyzed reactions into the hydroxyl radical. This radical is the toxic one, with a half-life of less than 1 ns, that damages the structure of biomolecules, especially the DNA structure [6] [7].

Concerning RNS, NO is the most abundant nitrogen radical in cells that is involved as an oxidative messenger in pathways that are controlling many physiological mechanisms involved in either the vascular system, host defense mechanism or neuronal signaling. In

high levels NO can be converted into the harmful peroxynitrite anion (ONOO), an oxidizing free radical that can cause DNA fragmentation or lipid oxidation [8].

Damages resulting from the presence and reactivity of ROS and RNS have been related to the development of diseases such as cancer, inflammatory diseases, neurodegenerative diseases[9] such as Alzheimer's disease or diabetes[5]. Preventing the formation of such radicals in high amounts thus constitute an important feature for inhibiting the tumor formation or progression processes [5]. Designing thus drug molecules that would also present antioxidant activity would thus be beneficial for the modulation of inflammation and slowing down cancer development.

Furthermore, it has been proven that inflammation can lead to neoplastic development by supplying bioactive molecules to the tumor microenvironment, as well as growth factors, proangiogenic factors, or factors that can assist invasion and/or metastasis.

Given the interrelationship of cancer and inflammation, we aimed in this study to develop new structures that can show both anti-inflammatory and anticancer activity. Indeed, combining pharmacophores with different bioactivities known as molecular hybridization (MH) in drug design allows the generation of hybrid compounds with improved properties.[10] (Figure 1.1) As some recent publications have shown that isoindolo[2,1-α]quinazoline derivatives can present some anticancer activity by inhibiting tubulin polymerase and topoisomerase I and sulfonamide derivatives are described for decreasing TNF-α levels, we chose to develop novel sulfonamide substituted isoindoloquinazoline derivatives for the development of potent anticancer and anti-inflammatory agents.

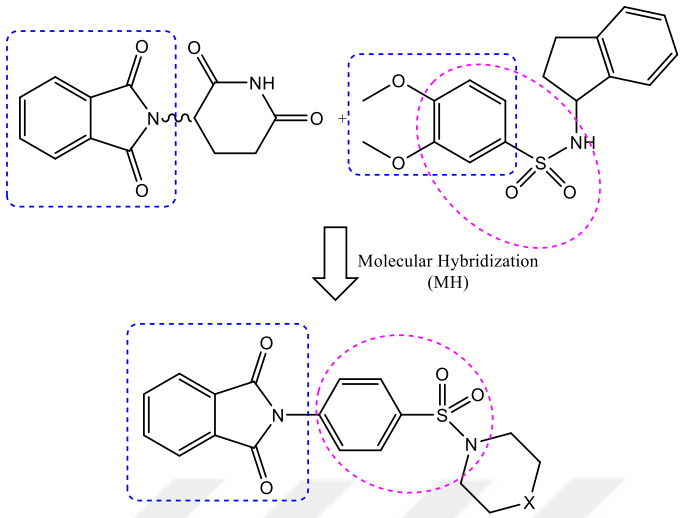


Figure1.1. Molecular Hybridization

2. GENERAL INFORMATION

2.1. Inflammation and Cancer

Inflammation is a defense mechanism of the body that is involved in wound healing but it also plays a crucial role in the development of some chronic diseases.[11] It is *via* this process that the body signals the immune system to heal and repair damaged tissue and defend itself against foreign invaders such as viruses and bacteria. There are two types of inflammation: acute and chronic. The comparison of both of the types is given in the following table 2.1.

Table 2.1. Types of inflammation

	Chronic	**Acute**
Caused by	Pathogens Some type of virus Foreign bodies	Harmful bacteria Tissue injury
Onset	Slow	Rapid
Duration	Months to years	A few days
Outcomes	Tissue death Thickening and scarring of tissue	Inflammation Abscess or becomes chronic

According to the work of Virchow in 1863 the functional relationship between inflammation and cancer was hypothesized and based on that the origin of cancer was at sites of chronic inflammation.[12] Recent works in the cancer research area have been shown

that TNF-α is one of the major mediators of inflammation. On the one hand, TNF-α induces other inflammatory mediators and proteases that organize an inflammatory response, on the other hand, it is produced by tumors and can take effect as an endogenous tumor promoter. The dysregulation of TNF-α can cause many diseases including cancers and a variety of inflammatory conditions like rheumatoid arthritis and inflammatory bowel disease called Crohn's disease.[13] According to this informations, anti-TNF agents targeting TNF-α have been developed to neutralize the deleterious effects of this inflammatory cytokine. In order to therapeutic strategies for TNF-α inhibition several drugs are used as immunosuppressants, such as cyclosporine A (1), dexamethasone (2), however, their effects are associated with remarkable toxicity. (Figure 2.1)

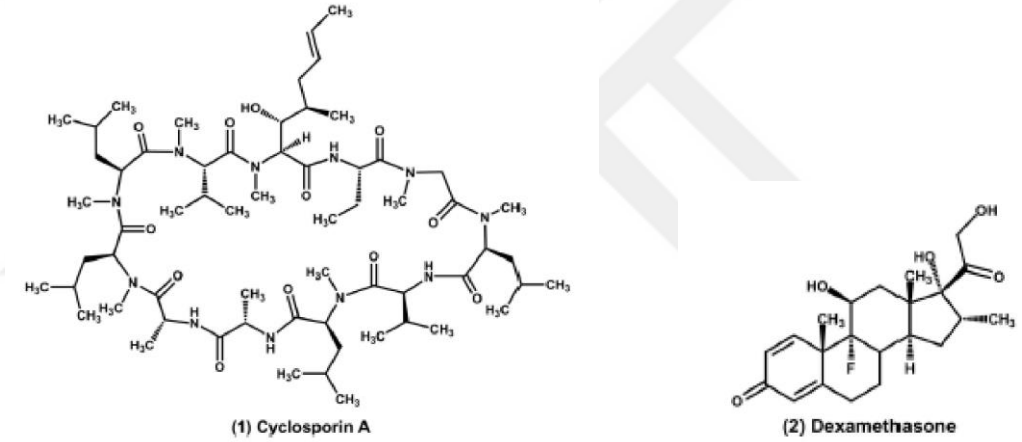


Figure 2.1. Molecular structure of Cyclosporin A and Dexamethasone

2.2. Isoindoloquinazoline Derivatives

2.2.1. Quinazoline derivatives as anticancer agents

There are several quinazoline derivatives for clinical use as anticancer drugs, which are approved by the Food and Drug Administration (FDA), such as gefitinib (Iressa®, 2003), erlotinib (Tarceva®, 2004), vandetanib (Caprelsa®, 2011), lapatinib (Tykreb®, 2012) and afatinib (Gilotrif®, 2013).[19] (Figure 2.2)

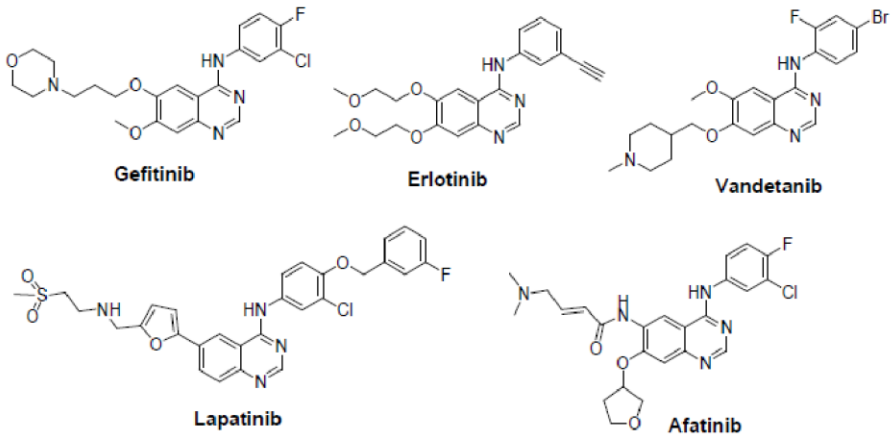


Figure 2.2. Quinazoline derivatives as anticancer drugs

Isoindoloquinazoline derivatives are an important class of quinazoline containing heterocyclic compounds. Heterocycles are a site of special scientific interest in organic chemistry, especially in the examination of bioactive scaffolds in the pharmaceutical industry. Nitrogen-containing heterocycles with integrated pharmacophoric units have an important place in medicinal chemistry. Between these heterocycles quinazoline-, quinazolinone-structures and their derivatives have been of great interest to biomaterial scientists for many years, because of their important and influential therapeutic and pharmacological activities such as antimicrobial[14], anticonvulsant[15], anticancer[16], antimalaria[17], antihypertensive[18], anti-inflammatory[19], anti-diabetic[20], antitumor[21], dihydrofolate reductase inhibition[22], anti-cholinesterase[23], kinase inhibitory[24], and cellular phosphorylation inhibition[25].

Quinazoline is also known as 1,3-diazanaphthalene and its 4-oxo derivative is named as quinazoline-4(3H)-one. The molecular structure of the compound can be seen in the following figure 2.3 below.

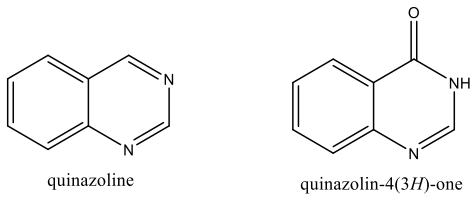


Figure 2.3. Molecular structure of quinazolines

Isoindole is benzo-fused pyrrole and an isomer of indole. The core structure of isoindole and the main framework of isoindoloquinazoline derivative can be seen in figure 2.4 below.

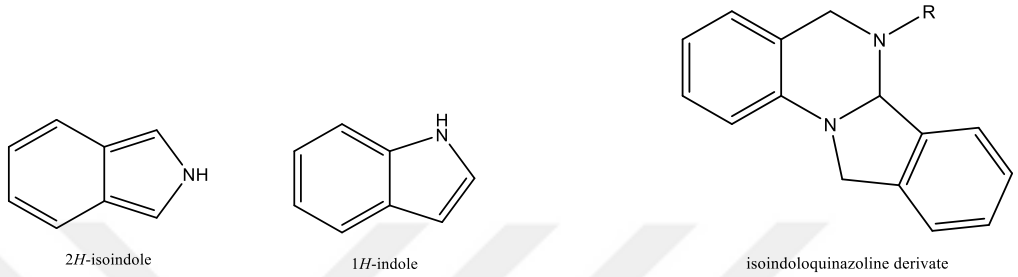


Figure 2.4. Structure of isoindoloquinazoline derivatives

Isoindoloquinazoline derivatives are frequently contained in natural products and synthetic compounds. They have important medicinal properties like:

- Potent inhibitors of TNF-α = anti-inflammatory effect
- Potent inhibitors cancer cell proliferation = anticancer effect
- Efficient stabilizers of organic materials against degradation caused by heat, light, and oxides[26]

Furthermore, isoindoles are the most common heterocyclic structures, which are encountered also in natural products and bioactive compounds, which show bioactivities against human ovarian cancer cell lines and play a role as EP4 receptor agonists in pain therapy. [27]

2.2.2. Quinazoline derivatives as topoisomerase I inhibitors

DNA topoisomerases are very important enzymes for regulating of the conformational changes in DNA topology and for reuniting of DNA strands during cellular processes like replication, transcription, and repair. They are able to catalyze the isomerization reactions between different three-dimensional DNA forms. Depending on their functionalities by the catalyzation reactions there are two major groups of DNA

topoisomerases like topoisomerase I (TopI) and topoisomerase II (TopII). TopI cleaves temporarily only one DNA strand whereas Top II temporarily double DNA strands.[28,29] The previous researches have shown that TopI enzyme inhibitors have a big role as a new group of anticancer agents in hematological and solid tumors.[30] The first clinically approved TopI inhibitor is camptothecin (CPT). Even though their promising activity in colon, lung, and ovarian cancers CPTs have shown high toxicity.[28] Therefore noncamptothecin TopI inhibitors were also synthesized such as indenoisoquinoline, indolocarbazoles, and phenanthridines. (Figure 2.5)

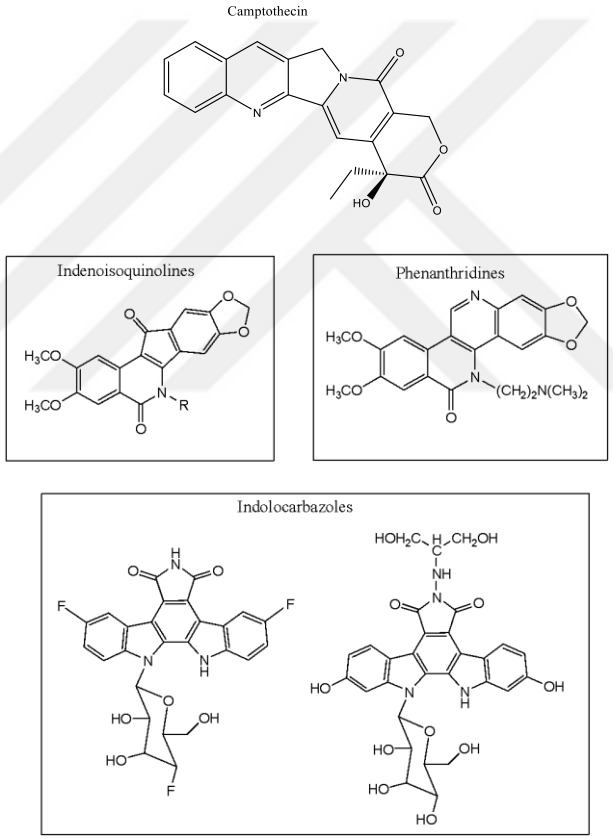


Figure 2.5. TopI inhibitors

2.2.3. Quinazoline derivatives as tubulin polymerization inhibitors

Tubulins, α- and β- heterodimers, are highly protected proteins in all eukaryotic cytoskeleton and through the polymerization of these heterodimers, microtubules are formed. Microtubules are involved in cell division, cell mobility, mitosis, cell shape, intracellular organelle transportation, and cell-cell interactions.[31] Tubulin-binding drugs like certain anticancer drugs, can bind to and block the function of tubulin, which can inhibit the propagation of cancer cells. The most examined binding sites are vinca- and colchicine-sites for tubulin polymerization inhibitors and the taxane-site for tubulin depolymerization inhibitors. (Figure 2.6)

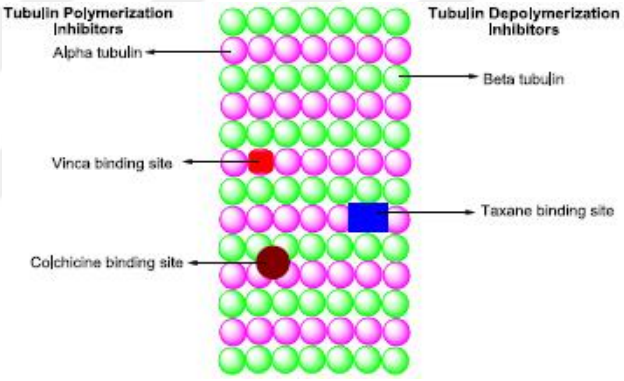


Figure 2.6.The binding sites of tubulin

According to recent researches, the most preferred binding site of quinazoline derivatives is the colchicine binding site. In this manner, they inhibit tubulin polymerization. Colchicine and its analogs with promising activity are shown in figure 2.7 below.[30]

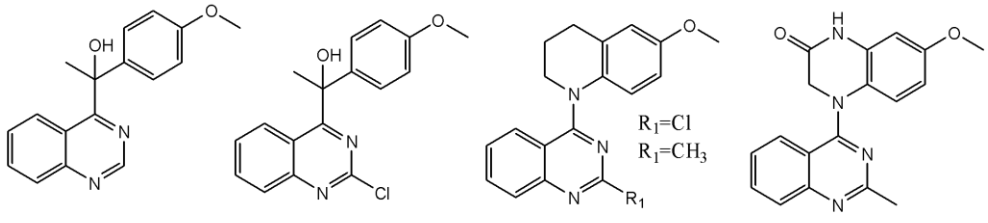


Figure 2.7. Colchicine and its analog

2.2.4. Quinazoline-Sulfonamide Hybrids

Sulfonamides are important pharmacophores with attractive pharmacological properties like antibacterial, diuretic, anti-viral, anti-inflammatory. There are more than 112 marketed drug molecules with sulfonamide functionality. According to the researches it was stated that sulfonamide makes hydrogen bonds in the binding site of TNF-α with polar residues (Figure 2.8) and in that way the TNF-α inhibition ability of compounds was increased.[32]

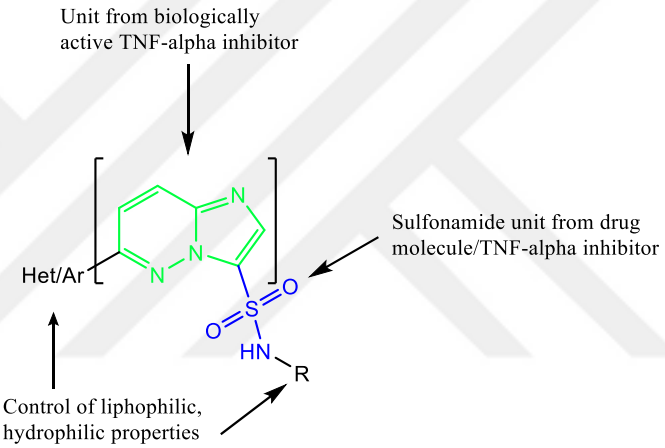
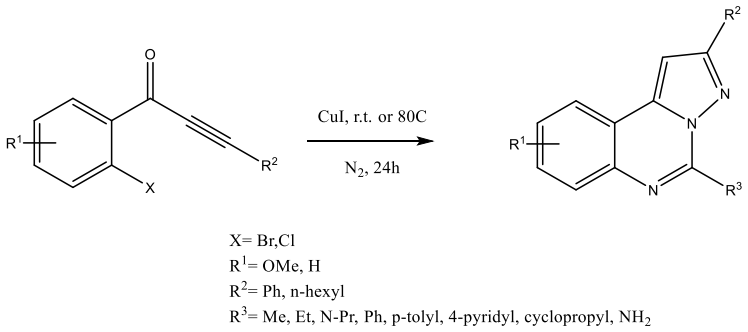


Figure 2.8. Binding sites of sulfonamides

2.2.5. Synthetic pathways for obtaining isoindoloquinazoline heterocycles

Within the past years, many synthetic methods have been developed for the generation of functionalized quinazoline and its derivatives. A various number of publications have shown the interesting properties of these compounds in the pharmaceutical industry. There is a high output of the presence of these compounds in marketed medicines as core structures.

In a report, Yang and co-workers[33] developed an efficient method for the synthesis of pyrazolo[1,5-c]quinazolines. This was a one-pot synthesis with substituted 1-(2-halophenyl)-3-alkylprop-2-yn-1-ones, hydrazine hydrochloride, and amide hydrochlorides, also with CuI catalyst in good yields. (Scheme 2.1) This pathway affords a new approach for N-fused heterocyclic compounds for medicinal chemistry.



Scheme 2.1. Cu-catalyzed synthesis

Pal and co-workers developed a new one-pot Cu-mediated synthetic method for the generation of fused N-heterocyclic ring, which is 5H-isoquinolino[2,3-a]quinazoline-5,12(6H)dione via coupling reaction between carboxylic acid chloride, amino carboxylate ester, and ethyl cyanoacetate in good yields. (Figure 2.9)

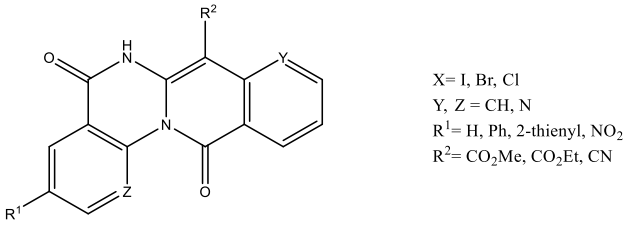
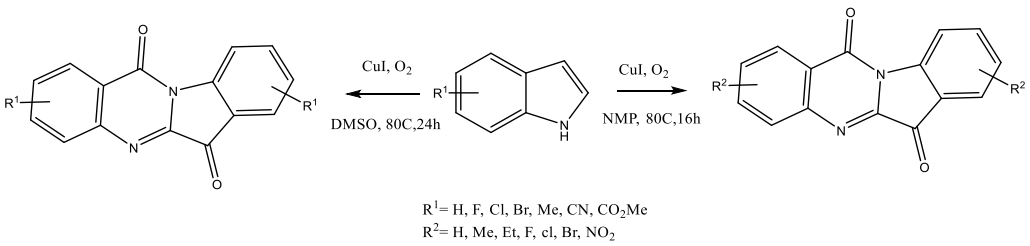


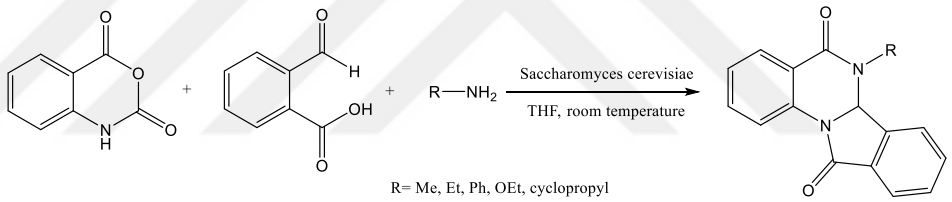
Figure 2.9. Isoindoloquinazoline derivative with different substituents

Wang et al.[34] reported an efficient and simple method for the synthesis of tryptanthrins started from indoles under mild reaction conditions with different functional groups. (Scheme 2.2) The basic pathway is based on aerobic oxidation in presence of a copper catalyst, intramolecularly nucleophilic addition, and finally oxidative aromatization.



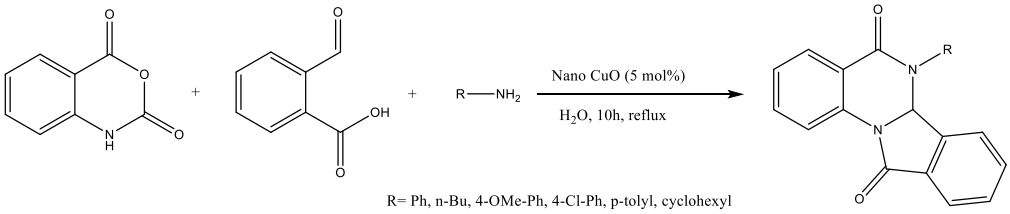
Scheme 2.2. Cu-catalyzed reaction pathways with different solvents

Avalani and co-workers[27] established a convenient and efficient simple one-pot synthesis to produce isoindolo[2,1-a]quinazolines using *Saccharomyces cerevisiae* also known as baker's yeast as biocatalyst at root temperature with ultrasonic irradiation. (Scheme 2.3)



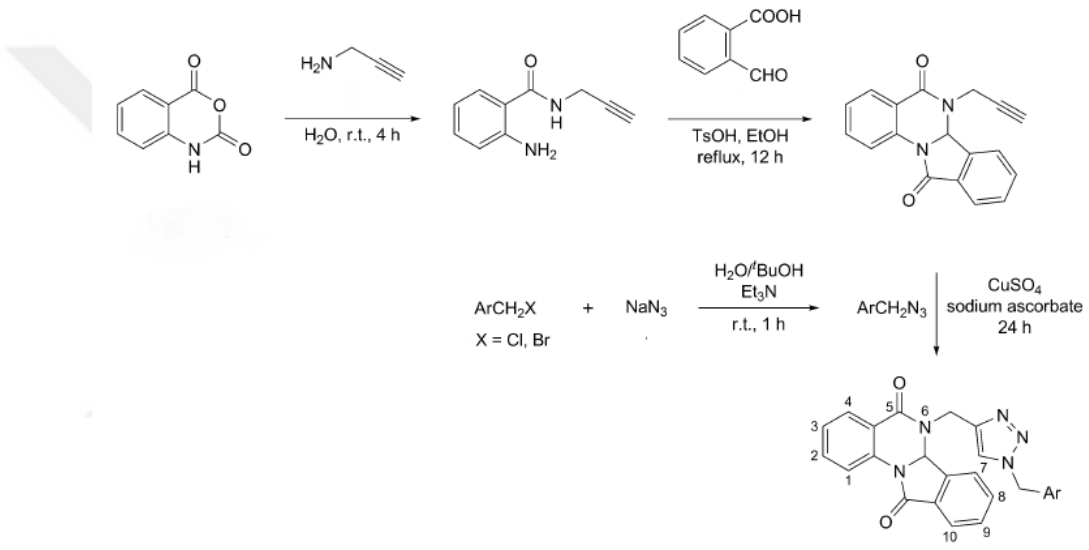
Scheme 2.3. Synthesis pathway with Saccharomyces cerevisiae

Moreover, Santra et al.[35] performed a nano CuO-catalyzed 'on-water' method as a one-pot synthesis for isoindolo[2,1-a]quinazolines by coupling of isatoic anhydride, 2-carboxybenzaldehyde, and various amines. (Scheme 2.4) In this case of the reaction, water is responsible for the acceleration of this transformation due to hydrogen bonds.



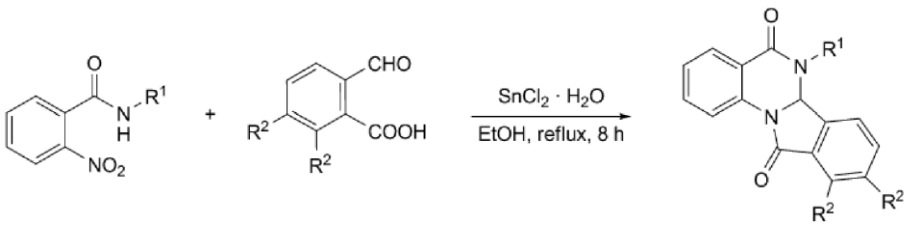
Scheme 2.4. CuO -catalyzed reaction 'on water'

Esmaeili-Marandi et al.[36] developed a straightforward approach for the generation of isoindolo[2,1-a]quinazoline coupled 1,2,3-triazoles, based on isatoic anhydride and 2-formylbenzoic acid as starting materials in presence of primary amines. The reaction was performed under various conditions to obtain the desired product, 6-(prop-2-yn-1-yl)-6,6a-dihydroisoindolo[2,1-a]quinazoline-5,11-dione. (Scheme 2.5)



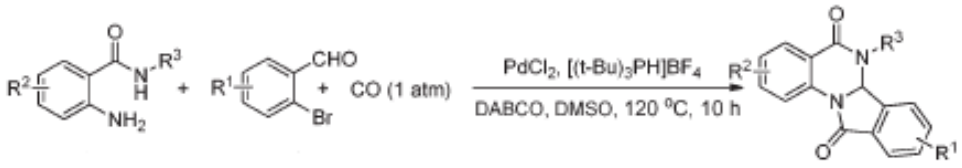
Scheme 2.5. Synthesis of 1,2,3-triazole derivatives

Another interesting method has been established by Mahdavi et al.[37] for the synthesis of the fused isoindoloquinazolinone derivatives because of their important biological properties. The main pathway for the generation of the desired compounds remains a reductive catalytical reaction with $SnCl_2$ H_2O, also 2-nitrobenzamide, and 2-formylbenzoic acid as starting materials. The reaction was performed under reflux conditions in EtOH as the solvent. (Scheme 2.6)



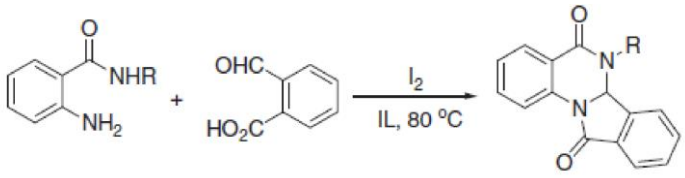
Scheme 2.6. Reductive catalyzed reaction with $SnCl_2$ H_2O

Guo and co-workers performed a series of isoindolo[2,1-a]quinazoline derivatives.[26] The reaction method is based on palladium-catalyzed carbonylative cyclization under an atmospheric pressure of carbon monoxide. 2-aminobenzamide and 2-bromobenzaldehyde were used as starting materials. (Scheme 2.7)



Scheme 2.7. Pd-catalyzed carbonylative cyclization reaction

As an easly and mild method for the generation of isoindolo[2,1-a]quinazoline derivatives, Lu and co-workers [38] developed a reaction between 2-aminobenzamides and 2-formylbenzoic acid using ionic liquids as 'green conditions'. The reaction was catalyzed by iodine. (Scheme 2.8)



Scheme 2.8. Iodine-catalyzed reaction in ionic liquid

Sulfonamide derivatives are playing a crucial role as an interesting target for the drug design of anti-inflammatory and anticancer drugs. Also, they constitute a remarkable important class of drugs with various types of pharmacological activities. Some

sulfonamide derivatives with anti-inflammatory, anticancer, and antiviral effects are shown in figure 2.10 below.

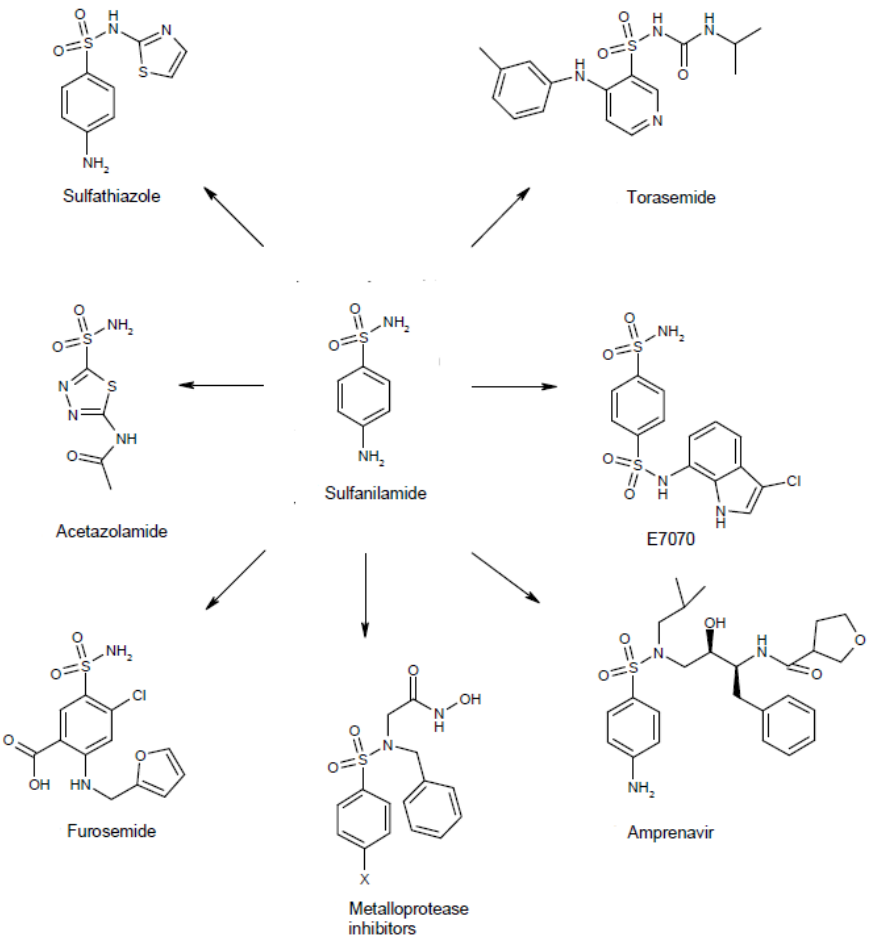


Figure 2.10. Sulfonamide derivatives with anti-inflammatory, anticancer, and antiviral effects

Thus in this project, we aimed to synthesize a series of sulfonamide substituted isoindoloquinazoline derivatives as novel structures demonstrating potentially dual bioactivity.

3. MATERIALS AND METHODS

3.1. Chemistry

3.1.1. Materials

For this work, isatoic anhydride, 2-formylbenzoic acid, 2,5-dichlorobenzenesulfonyl chloride, 2,4,5-trichlorobenzenesulfonyl chloride, 3,4-dichlorobenzenesulfonyl chloride, 2-(trifluoromethyl)benzenesulfonyl chloride, 3-(trifluoromethyl)benzenesulfonyl chloride, 2-fluorobenzenesulfonyl chloride, 4-fluorobenzenesulfonyl chloride, N-acetylbenzenesulfonyl chloride, 4-bromobenzenesulfonyl chloride, 2-thiophenebenzenesulfonyl chloride and 2,4-dichlorobenzenesulfonyl chloride were purchased from Sigma-Aldrich.

3.1.2. Methods of synthesis

3.1.2.1. General procedure A: Synthesis of compound 1

One equivalent of isatoic anhydride, one equivalent of formylbenzoic acid, and 2 equivalent of ethylenediamine were stirred in 30 ml of acetic acid, refluxed at 110°C for 2 hours, and stirred following 24 h at room temperature. After that cold water was poured into the reaction mixture and the obtained precipitation was filtered off. The mixture was extracted with diethyl ether and the aqueous layer was evaporated by rotary evaporator. The crude product was recrystallized from ethanol.

3.1.2.2. General procedure B: Synthesis of compounds 2-6

First of all, dichloromethane was distilled over P_2O_5. One equivalent of amine (compound 1) and 2 equivalents of triethylamine were mixed in 30 ml freshly distilled dichloromethane. The reaction temperature was decreased at 0°C and one equivalent of the corresponding sulfonyl chloride in absolute DMSO was added into the reaction mixture dropwise. The reaction mixture was stirred at room temperature under a nitrogen atmosphere. Depending on the sulfonyl chloride the reaction mixture was stirred 1h to 24h, which was controlled by TLC. After the completion of the reaction, cold distilled water was

poured into the mixture and both layers were extracted with water. The combined organic layers were washed with brine and dried over Na_2SO_4. Finally, the organic phase was evaporated and the crude product was purified by column chromatography. (Ethylacetate:Hexane= 70:30)

3.1.2.3. General procedure C: Synthesis of compounds 9-12

One equivalent of amine (compound **1**) and one equivalent of the corresponding sulfonyl chloride were refluxed in ethanol for 2h and finally stirred one week at room temperature. The reaction was controlled by TLC. After completion of the reaction, the solvent was evaporated. The crude product was dissolved in DMSO and the product was precipitated by water and isolated with filtration.

3.1.3. Analytical methods

3.1.3.1. Melting point determination

Melting points of the compounds were determined by an electrothermal melting point apparatus (Mettler Toledo FP62) in open capillary tubes.

3.1.3.2. Thin-layer chromatography

Plates: During this work, kieselgel 60 F_{254} (Merck) silica gel plates were used for thin-layer chromatography. Completion of the reactions was observed by the chromatographic monitoring of amines and sulfonamides.

Solvent systems: Ethyl acetate: n-hexane (70: 30) solvent system was used for chromatographic controls.

Elution conditions: The solvent system was poured into chambers and waited for saturation. Synthesized compounds and their starting materials were dissolved in appropriate solvents, applications were made on thin-layer chromatography (TLC) plates and dragged along at room temperature.

Identification of TLC spots: UV light (254 nm) was used for the detection of the spots

3.1.3.3. ^{1}H-NMR spectra

The NMR spectra were recorded with a Bruker AC 400 Hz spectrometer using tetramethylsilane as the internal reference, dimethylsulfoxide-d_6 (d_6-DMSO) as the solvent, and chemical shifts were reported in parts per million (ppm).

3.1.3.4. ^{13}C-NMR Spectra

^{13}C-NMR spectra of the compounds were recorded with a Bruker AC 400 Hz spectrometer.

3.1.3.5. Mass spectra

Mass spectra were recorded with a Waters Alliance HPLC ve ZQ mikromass (Waters Corporation, Milford, MA, USA). For the LC-MS analysis, electro spray method ESI (+) or (-) was used.

3.1.3.6. Elemental Analysis

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

3.2. Biological activity studies

3.2.1. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The assay was performed in 96-well plates. The DPPH solution was prepared (0.1 mM or 0.004 g / 0.1 L) and sample solutions were prepared by appropriate serial dilution within a range from 1000 µM to 6.75 µM. As a reference solution, butyl hydroxytoluene (BHT) was prepared and used at a concentration range varying between 2000 and 125 µM.

In each well, 250 µL of DPPH solution and 50 µL of sample or reference solutions were added and incubated in dark at RT for 50 minutes. Each sample was evaluated in triplicate.

Absorbance was measured at 517 nm and the radical scavenging ability of the molecules were calculated as follows;

DPPH radical - scavenging activity % = $[1-(A_{sample} - A_{blank}) / (A_{control}-A_{blank})] \times 100$

$A_{control}$ is the absorbance value of the BHT reference solutions and A_{sample} is the absorbance of the samples.

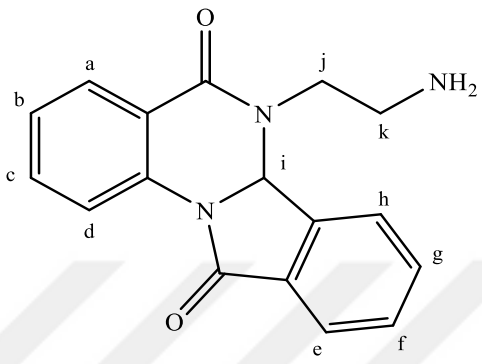
3.2.2. Cupper Reducing Activity (CUPRAC) Assay

The cupric ion reducing capacities of the compounds were determined on 96-well plates[39]. Briefly, 45 µl standard/sample and 50 µl distilled water were mixed. Subsequently, equal volumes of copper sulfate, neocuproine, and ammonium acetate were added. After 20 minutes of incubation period at 50°C, the absorbance was measured at 450 nm. Ascorbic acid was used as standard and the results were expressed as mg ascorbic acid equivalent per g compound.

4. RESULTS

4.1. Chemical Data

6-(2-aminoethyl)-6,6a-dihydroisoindolo[2,1-a]quinazoline-5,11-dione (1)



One equivalent (3.1 mmol, 0.5 g) of isatoic anhydride, one equivalent (3.1 mmol, 0.47 g) of formylbenzoic acid, and 2 equivalent (6.1 mmol, 0.4 ml) of ethylenediamine were stirred in 30 ml of acetic acid according to the general procedure A at 3.1.2.1.

Molecular weight [g/mol]: 293.33

Molecular formula: $C_{17}H_{15}N_3O_2$

Yield [%]: 73

Retention factor (R_f): 0.00 (Ethylacetate:n-Hexane=70:30)

Physical appearance: white powder

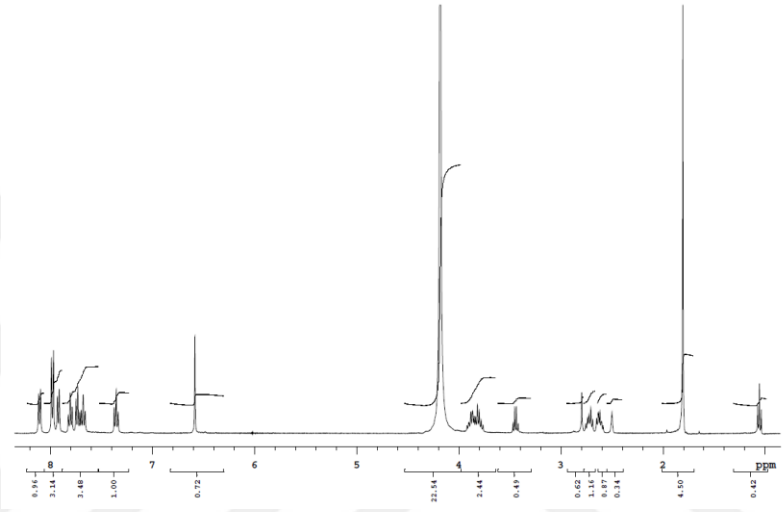
Melting point [°C]: 197.5

Solubility: DMSO

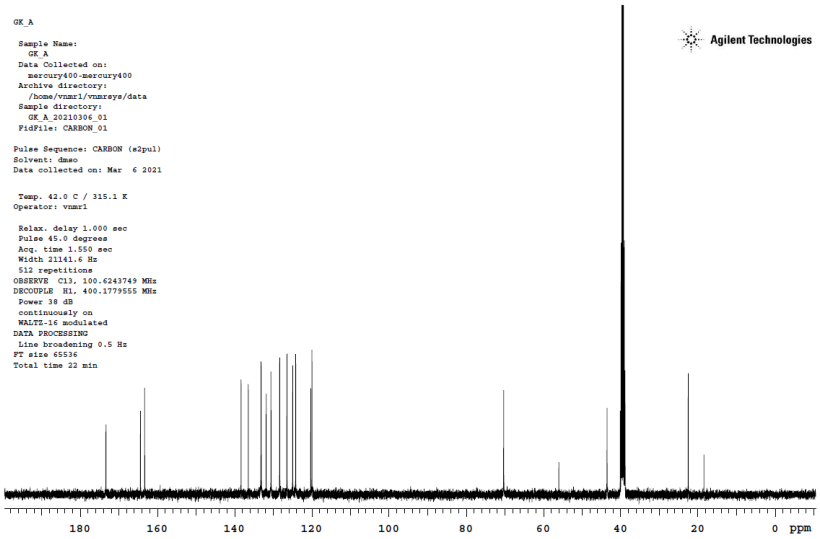
Elemental Analysis:

	%C	%H	%N	%S
Calculted	69.61	5.15	14.33	-
Found	56.53	6.357	10.96	-

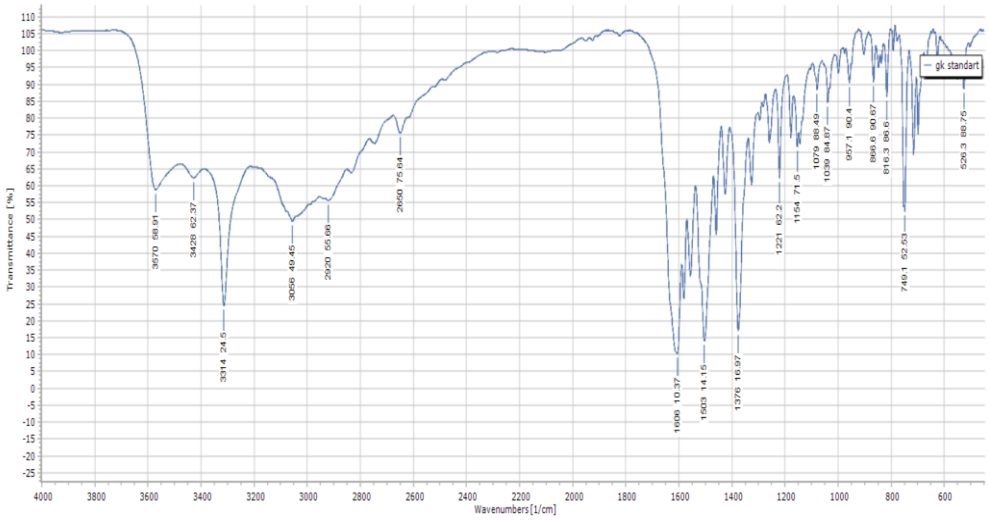
^{1}H NMR (400MHz, d_6-DMSO): 7.99-7.91 (m, 4H, H_{arom}); 7.82-7.66 (m, 5H, H_{arom}); 7.38-7.34 (t, 1H, H_b); 6.59 (s, 1H, H_i); 4.18-3.76 (m, 1H, H_k); 3.47-3.42 (q, 1H, H_k); 2.76-2.69 (m, 1H, H_j); 2.64-2.59 (m, 1H, H_j)



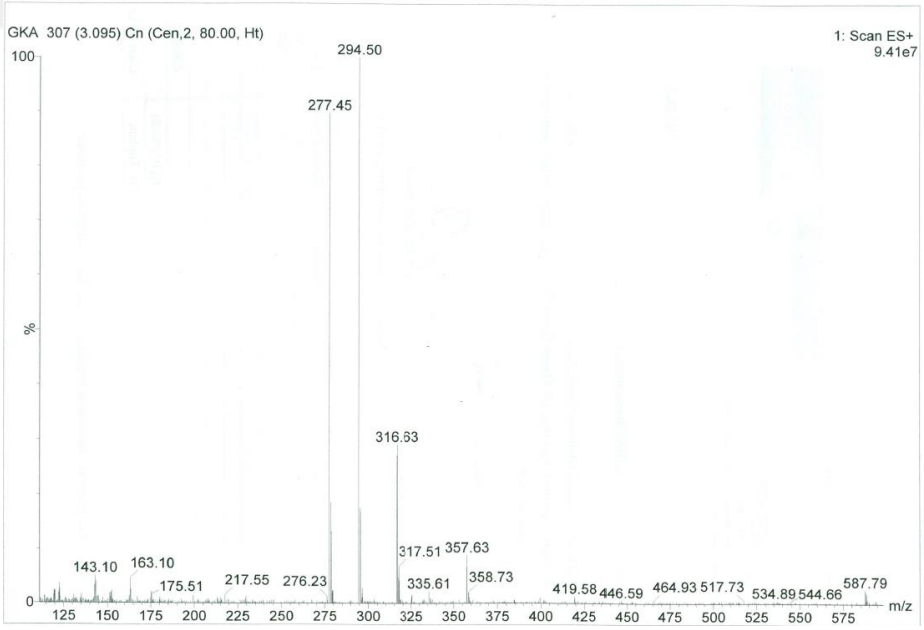
^{13} C NMR (400MHz, d_6-DMSO): 173.4; 164.4 (N-CO-O); 163.3 (C-CO-N); 138.4; 136.5; 133.2; 133.1; 131.9; 130.6; 128.4; 126.5; 124.9; 124.2; 120.3; 119.9; 70.3 (N-CH-C); 56.0; 43.5 (N-CH_2-CH_2); 22.5 (CH_2-CH_2-NH_2); 18.5



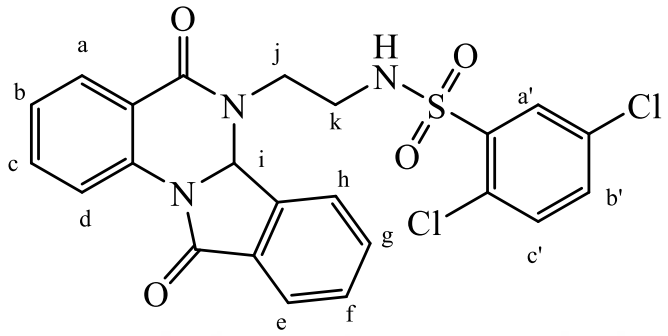
IR (ν_{max} [cm^{-1}]): 3570 and 3428 (-NH2-stretching); 3056 (-C=C-stretching); 2920 (aromatic -C-H-stretching); 1606 (aromatic -C=C stretching)



LC-MS [m/z]: [M+1]= 294.50



2,5-dichloro-N-[2-(5,11-dioxo-6a,11-dihydroisoindolo[2,1-a]quinazoline-6(5H)-yl)ethyl]benzenesulfonamide (2)



One equivalent (1.02 mmol, 0.30 g) of compound **1**, one equivalent (1.02 mmol, 0.25 g) of 2,5-dichlorobenzenesulfonyl chloride, and 2 equivalents (2.05 mmol, 0.21 g) of triethylamine were reacted in 30 ml freshly distilled dichloromethane according to the general procedure B at 3.1.2.2.

Molecular weight [g/mol]: 502.37

Molecular formula: $C_{23}H_{17}Cl_2N_3O_4S$

Yield [%]: 16

Retention factor (R_f): 0.73 (Ethylacetate:n-Hexane=70:30)

Physical appearance: white powder

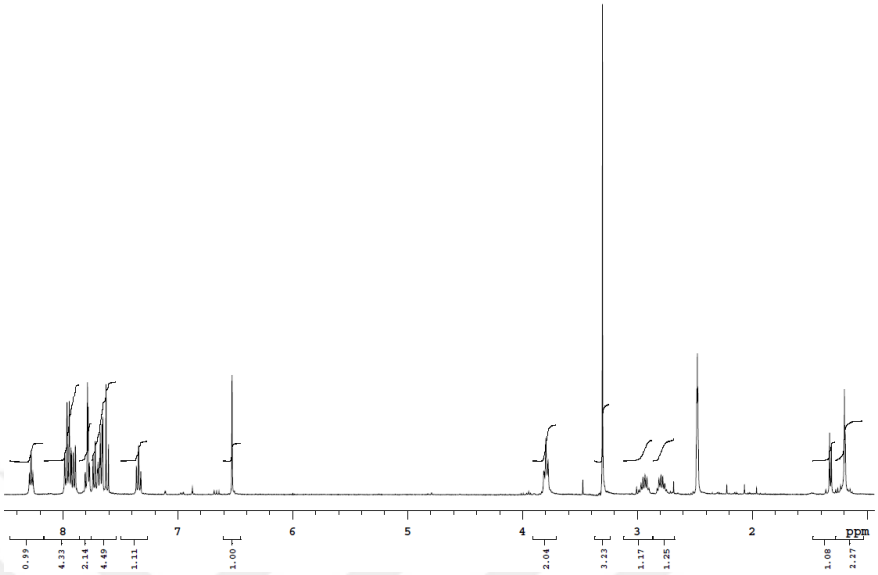
Melting point [°C]: 197.5

Solubility: DMSO

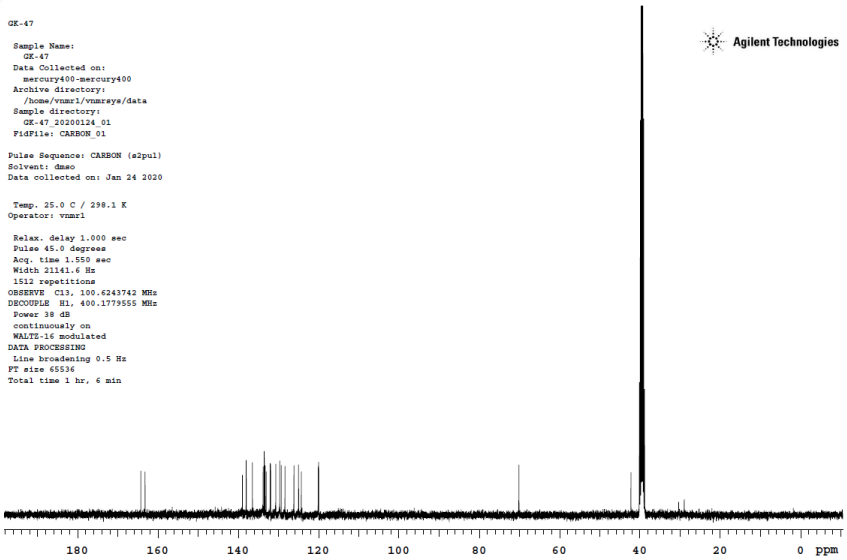
Elemental Analysis:

	%C	**%H**	**%N**	**%S**
Calculted	54.99	3.41	8.36	6.38
Found	55.02	3.72	8.03	6.58

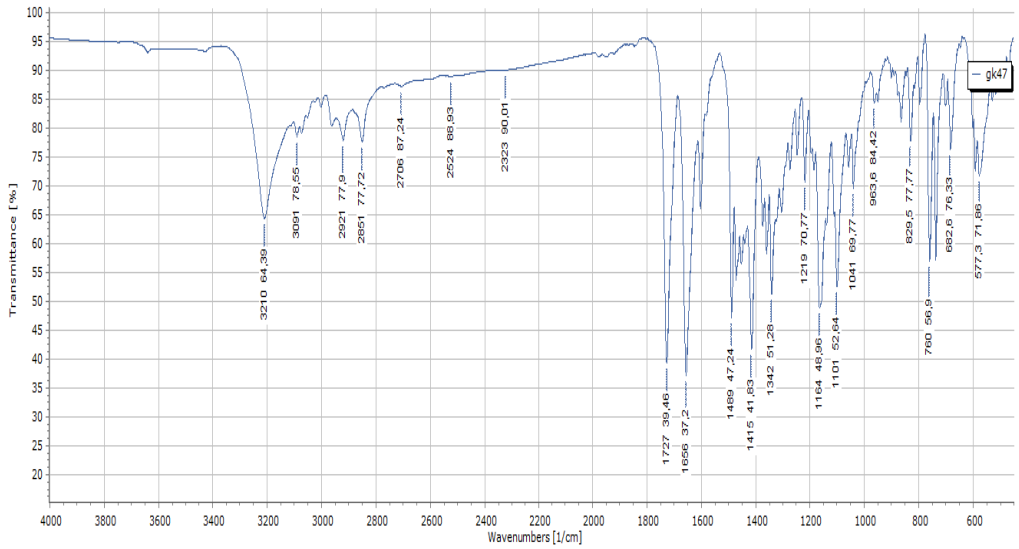
^{1}H NMR (400MHz, d_6-DMSO): 8.28 (t, 1H, *J=6.0Hz*, H_e.); 7.98-7.91 (m, 4H,H_{arom}); 7.81-7.60 (m, 6H, H_{arom} + $H_{a'}$ + $H_{b'}$); 7.34 (td, 1H, *J_1=7.68Hz, J_2=0.95 Hz*, H_b); 6.53 (s, 1H, H_i); 3.78 (t, 2H, *J=7.74 Hz*, H_k); 3.00-2.90 (m, 1H, H_j); 2.81-2.74 (m, 1H, H_j).



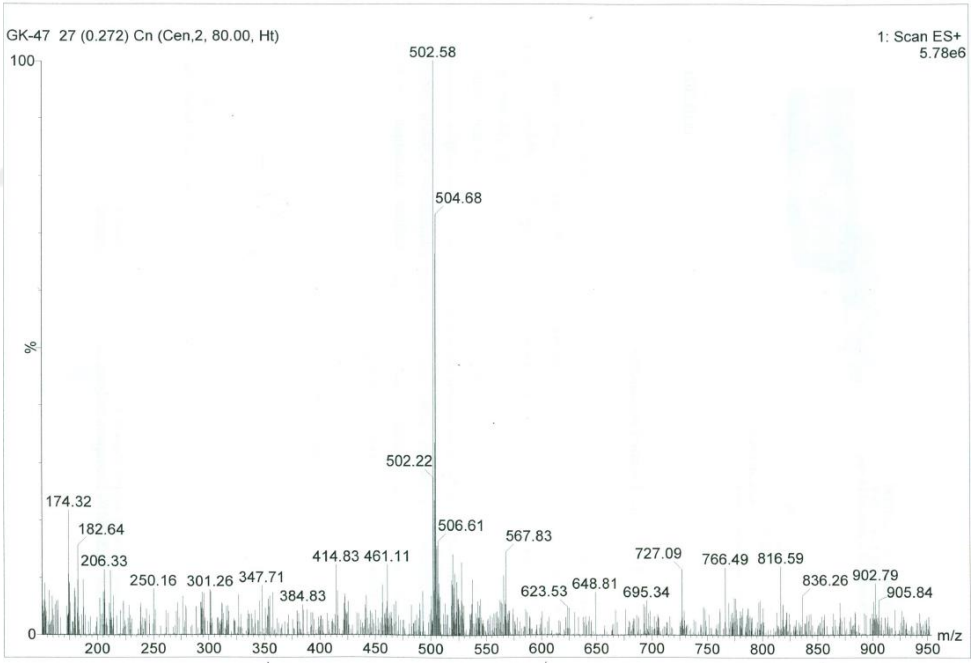
^{13} C NMR (400MHz, d_6-DMSO): 164.3 (N-CO-C); 163.2 (C-CO-N); 138.9; 138.0; 136.5; 133.8; 133.5; 133.3; 133.1; 131.9; 130.6; 129.7; 129.3; 128.3; 126.1; 125.0; 124.3; 120.0; 70.1 (N-CH-C); 42.2 (N-CH_2-CH_2); 30.3 (CH_2-CH_2-NH); 28.9



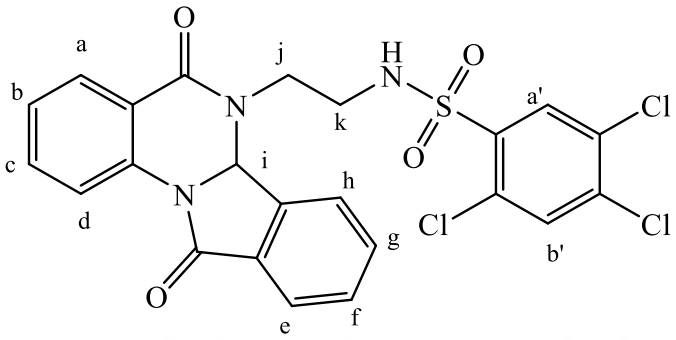
IR (ν_{max} [cm^{-1}]): 3210 (-N-H stretching); 3091 (-C=C stretching); 2921 (aromatic -C-H stretching); 1727 (-N-H bending); 1658 (C=O stretching); ~1600 (aromatic -C=C stretching); 1489 and 1415 (O=S=O stretching)



LC-MS [m/z]: [M]= 502.58



2,4,5-trichloro-N-[2-(5,11-dioxo-6a,11-dihydroisoindolo[2,1-a]quinazoline-6(5H)-yl)ethyl]benzenesulfonamide (3)



One equivalent (1.02 mmol, 0.30 g) of compound **1**, one equivalent (1.02 mmol, 0.25 g) of 2,4,5-trichlorobenzenesulfonyl chloride, and 2 equivalents (2.05 mmol, 0.21 g) of triethylamine were reacted in 30 ml freshly distilled dichloromethane according to the general procedure B at 3.1.2.2.

Molecular weight [g/mol]: 536.81

Molecular formula: $C_{23}H_{16}Cl_3N_3O_4S$

Yield [%]: 89

Retention factor (R_f): 0.73 (Ethylacetate:n-Hexane=70:30)

Physical appearance: white powder

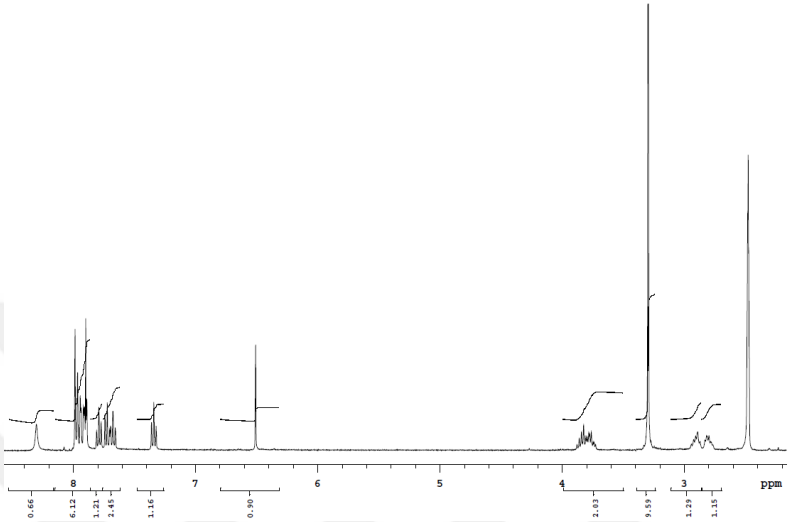
Melting point [°C]: 224.3

Solubility: DMSO

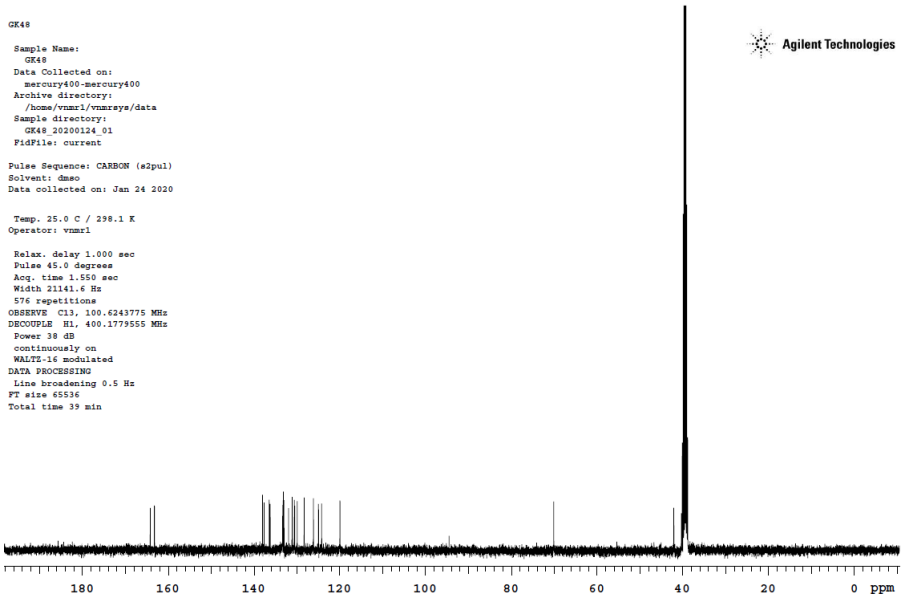
Elemental analysis:

	%C	%H	%N	%S
Calculted	51.46	3.00	7.83	5.97
Found	50.80	2.88	7.91	6.37

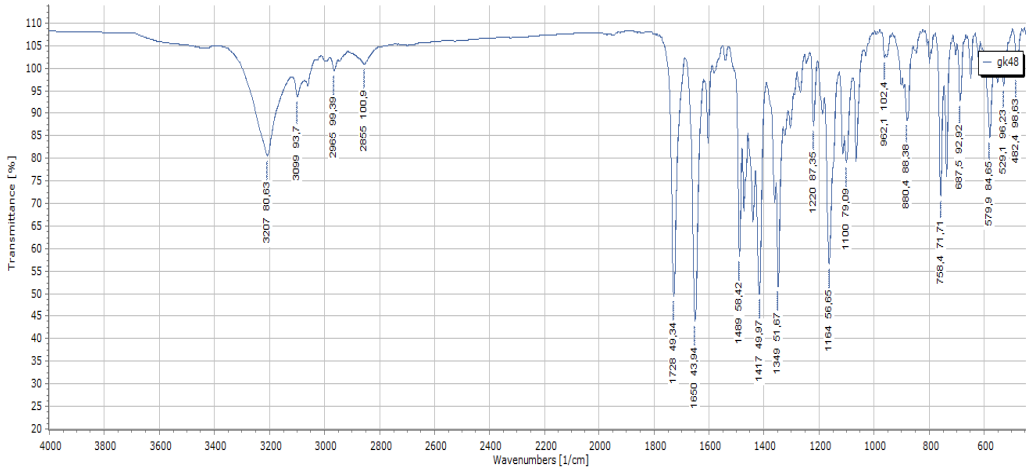
^{1}H NMR (400MHz, d_6-DMSO): 8.30 (s, 1H, $H_{e.}$); 7.98-7.89 (m, 6H, H_{arom} + H_e + $H_{a'}$); 7.80 (t, 1H, *J=7.46Hz*, $H_{arom.}$); 7.74-7.65 (m, 2H, H_{arom}); 7.34 (t, 1H, *J=7.61Hz*, H_b); 6.51 (s, 1H, H_i); 3.88-3.72 (m, 2H, H_k); 2.94-2.87 (m, 1H, H_j); 2.83-2.746(m, 1H, H_j).



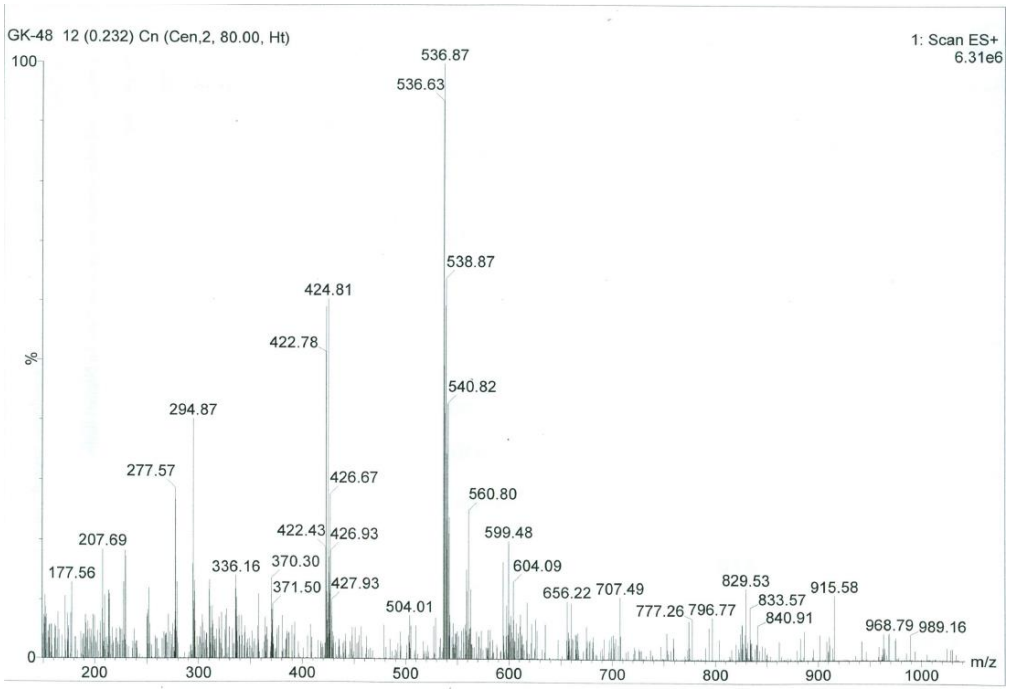
^{13} C NMR (400MHz, d_6-DMSO): 164.2 (N-CO-C); 163.2 (C-CO-N); 138.0; 137.6; 136.4; 136.2; 133.3; 133.2; 133.0; 131.9; 131.1; 130.6; 130.5; 129.9; 128.3; 126.1; 124.9; 124.2; 119.9; 70.1 (N-CH-C); 42.1 (N-CH_2-CH_2)



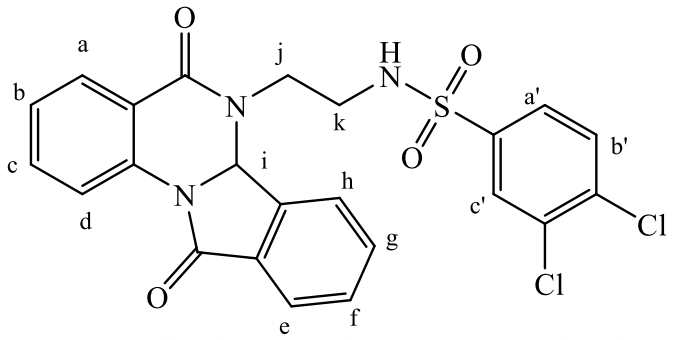
IR (ν_{max} [cm^{-1}]): 3207 (-N-H stretching); 3099 (-C=C stretching); 2965 (aromatic -C-H stretching); 1728 (-N-H bending); 1650 (C=O stretching); ~1600 (aromatic -C=C stretching); 1489 and 1417 (O=S=O stretching)



LC-MS [m/z]: [M]= 536.87



3,4-dichloro-N-[2-(5,11-dioxo-6a,11-dihydroisoindolo[2,1-a]quinazoline-6(5H)-yl)ethyl]benzenesulfonamide (4)



One equivalent (1.12 mmol, 0.33 g) of compound **1**, 1 equivalent (1.22 mmol, 0.28 g) of 3,4-dichlorobenzenesulfonyl chloride, and 2 equivalents (2.24 mmol, 0.23 g) of triethylamine were reacted in 30 ml freshly distilled dichloromethane according to the general procedure B at 3.1.2.2.

Molecular weight [g/mol]: 502.37

Molecular formula: $C_{23}H_{17}Cl_2N_3O_4S$

Yield [%]: 22

Retention factor (R_f): 0.73 (Ethylacetate:n-Hexane=70:30)

Physical appearance: white powder

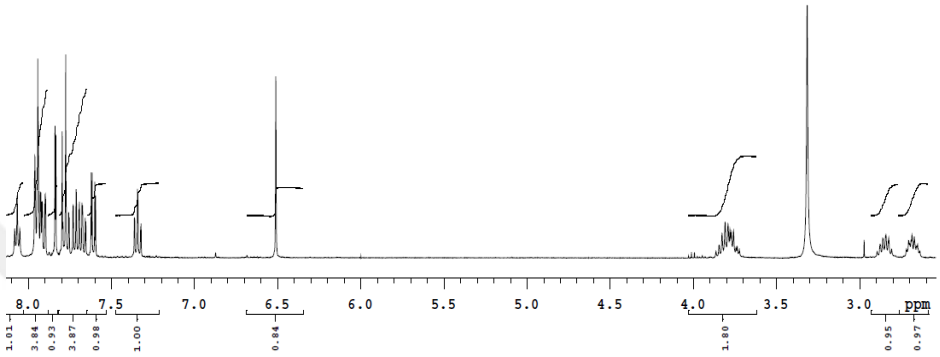
Melting point [°C]: 183.1

Solubility: DMSO

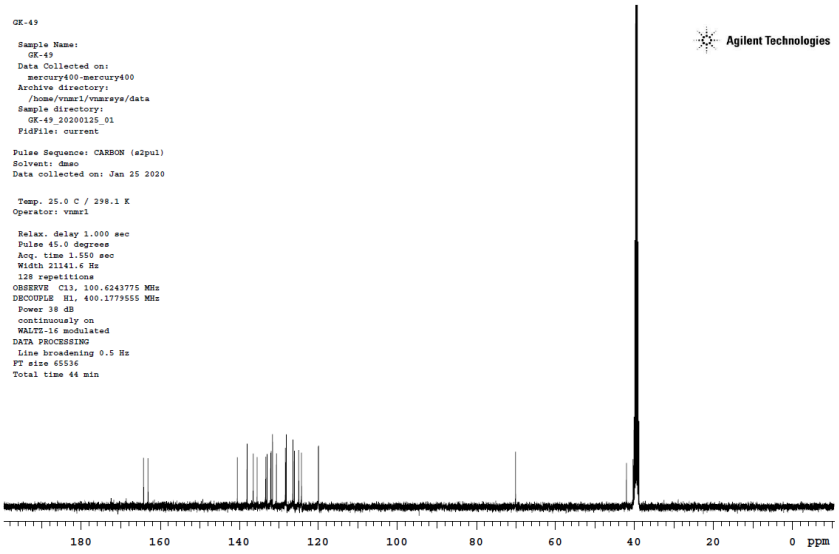
Elemental Analysis:

	%C	%H	%N	%S
Calculted	54.99	3.41	8.36	6.38
Found	55.04	3.80	8.48	6.52

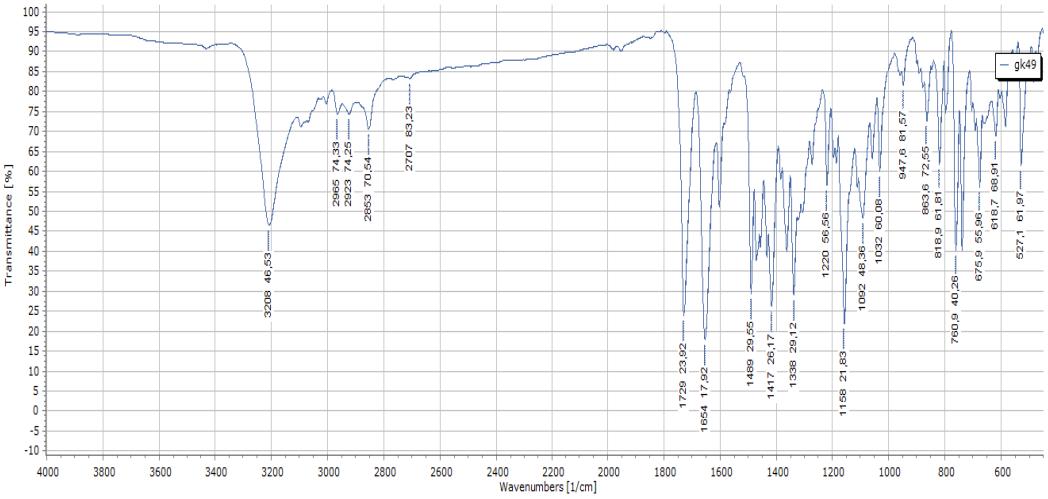
^{1}H NMR (400MHz, d_6-DMSO): 8.07 (t, 1H, *J=6.14Hz*, H_e); 7.96-7.89 (m, 4H, $H_{arom.}$); 7.83 (d, 1H, *J=2.11Hz*, $H_{a'}$); 7.79-7.65 (m, 4H, H_{arom}); 7.60 (dd, 1H, J_1*= 8.44Hz*, J_2*=2.14z*, $H_{b'}$); 7.34 (td, 1H, J_1*=7.68Hz*, J_2*=0.95 Hz*, H_b); 6.51 (s, 1H, H_i); 3.86-3.71 (m, 2H, H_k); 2.89-2.80 (m, 1H, H_j); 2.70-2.63 (m, 1H, H_j).



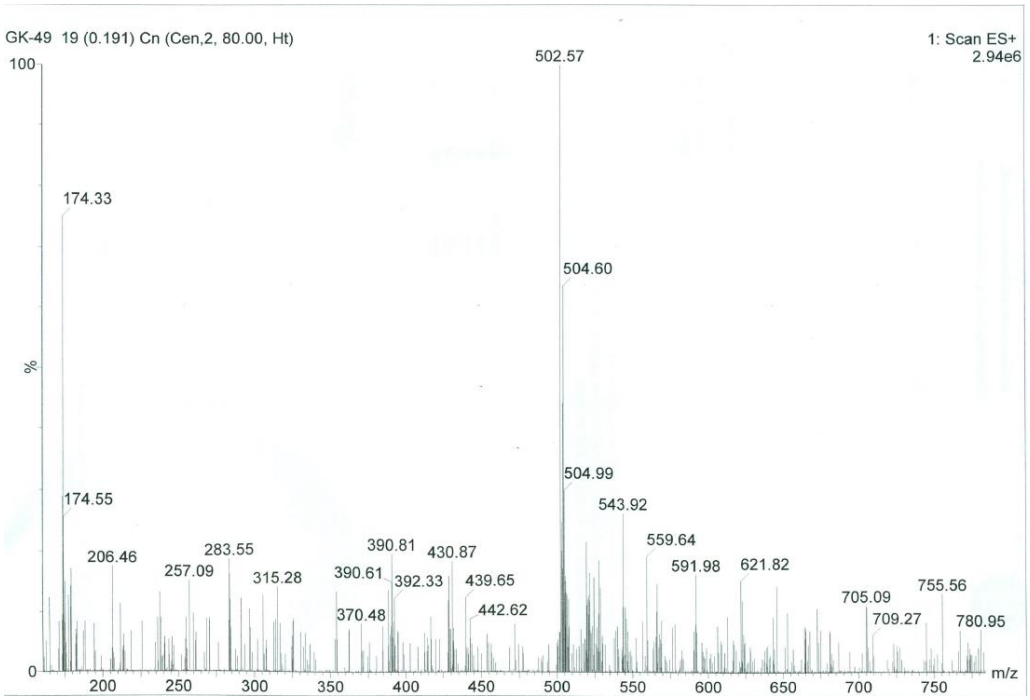
^{13}C NMR (400MHz; d_6-DMSO): 164.2 (N-CO-C); 163.1 (C-CO-N); 140.5; 138.0; 136.4; 135.5; 133.3; 132.9; 132.0; 131.9; 131.6; 130.6; 128.3; 128.1; 126.4; 126.1; 124.9; 124.2; 120.0; 119.9; 70.1 (N-CH-C); 42.0 (N-CH_2-CH_2)



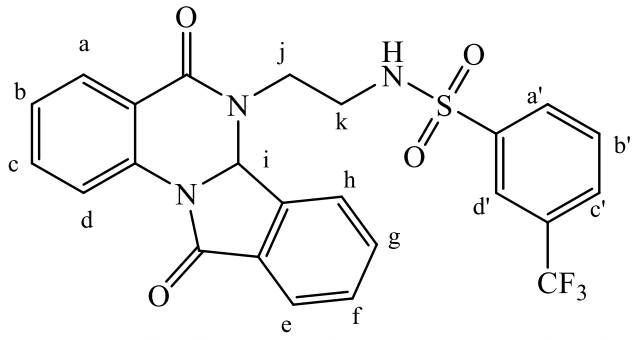
IR (ν_{max} [cm^{-1}]): 3208 (-N-H stretching); 3100 (-C=C stretching); 2965 (aromatic -C-H stretching); 1729 (-N-H bending); 1654 (C=O stretching); ~1600 (aromatic -C=C stretching); 1489 and 1417 (O=S=O stretching)



LC-MS [m/z]: [M]= 502.57



N-[2-(5,11-dioxo-6a,11-dihydroisoindolo[2,1-a]quinazoline-6(5H)-yl)ethyl)-3-(trifluoromethyl]benzenesulfonamide (5)



One equivalent (1.02 mmol, 0.30 g) of compound **1**, one equivalent (1.02 mmol, 0.25 g) of 3-(Trifluoromethyl)benzenesulfonyl chloride, and 1.2 equivalents (1.23 mmol, 0.13 g) of triethylamine were reacted in 30 ml freshly distilled dichloromethane according to the general procedure B at 3.1.2.2.

Molecular weight [g/mol]: 501.48

Molecular formula: $C_{24}H_{18}F_3N_3O_4S$

Yield [%]: 21

Retention factor (R_f): 0.67 (Ethylacetate:Hexane=70:30)

Physical appearance: white powder

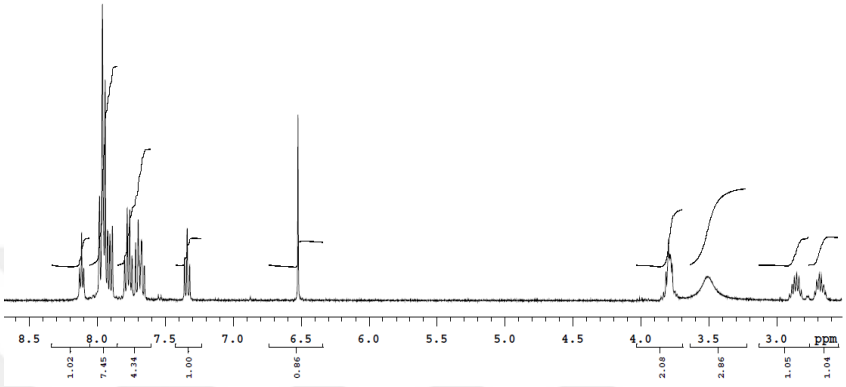
Melting point [°C]: 186.9

Solubility: DMSO

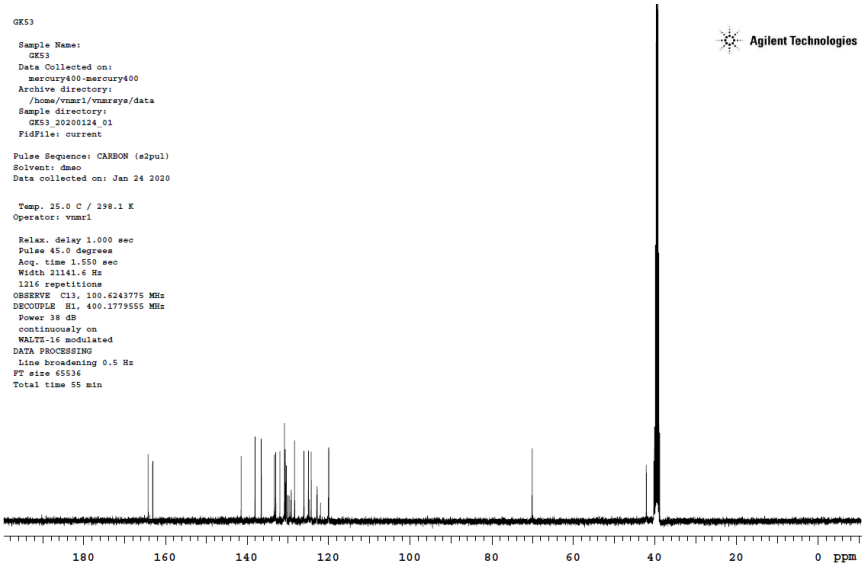
Elemental Analysis:

	%C	%H	%N	%S
Calculted	57.48	3.62	8.38	6.39
Found	56.57	3.71	8.31	6.47

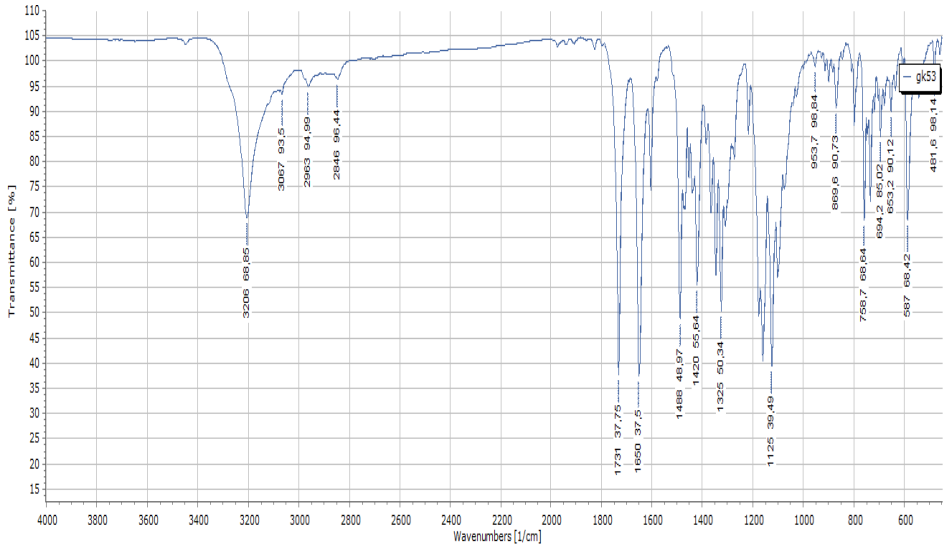
^{1}H NMR (400MHz, d_6-DMSO): 8.12 (t, 1H, *J=6.05Hz*, H_e); 7.98-7.88 (m, 7H, H_{arom} + $H_{a'}$); 7.79-7.65 (m, 4H, H_{arom}); 7.34 (td, 1H, J_1*=7.48Hz*, J_2*=0.76 Hz*, H_b); 6.52 (s, 1H, H_i); 3.81-3.77 (m, 2H, H_k); 2.90-2.82 (m, 1H, H_j); 2.72-2.64 (m, 1H, H_j).



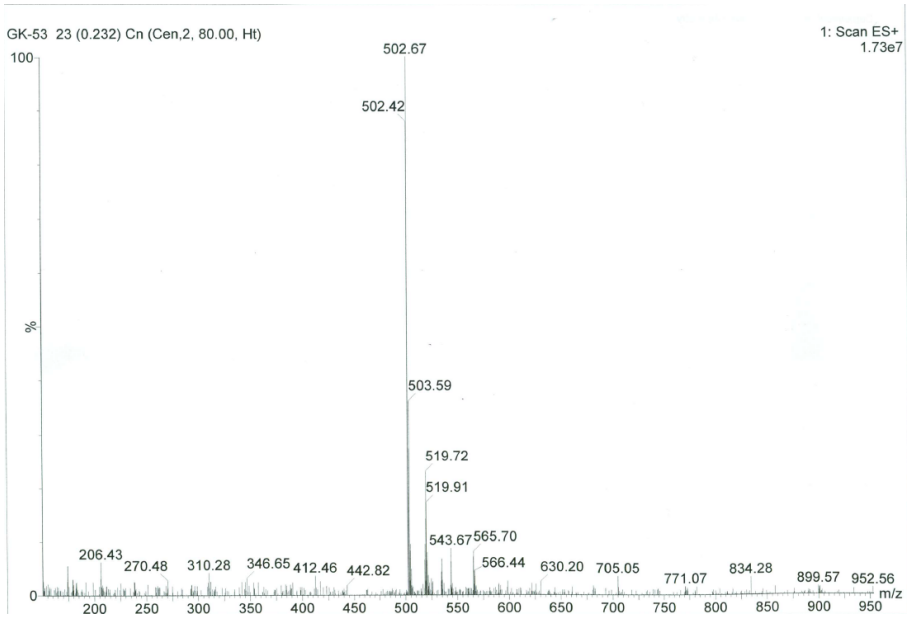
^{13}C NMR (400MHz, d_6-DMSO): 164.2 (N-CO-C); 163.1 (C-CO-N); 141.4; 138.0; 136.5; 133.3; 132.9; 131.9; 130.8; 139.6; 130.3; 129.9; 129.6; 129.2; 129.1; 128.3; 126.0; 124.9; 124.7; 124.3; 122.8; 122.4 (C-CF_3); 120.0; 119.9; 70.1 (N-CH-C); 42.1 (N-CH_2-CH_2)



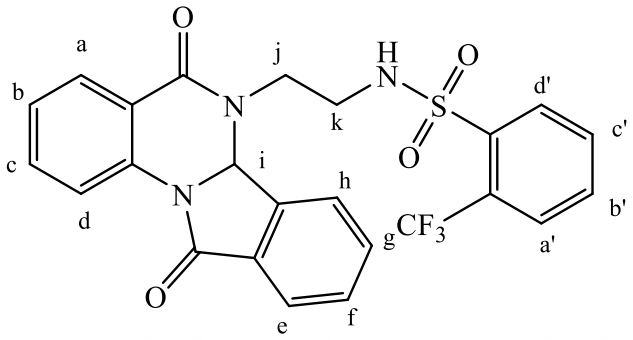
IR (ν_{max} [cm^{-1}]): 3206 (-N-H stretching); 3067 (-C=C stretching); 2963 (aromatic -C-H stretching); 1731 (-N-H bending); 1650 (C=O stretching); ~1600 (aromatic -C=C stretching); 1488 and 1420 (O=S=O stretching)



LC-MS [m/z]: [M+1]= 502.42



N-[2-(5,11-dioxo-6a,11-dihydroisoindolo[2,1-a]quinazoline-6(5H)-yl)ethyl)-2-(trifluoromethyl)]benzenesulfonamide (6)



One equivalent (1.02 mmol, 0.30 g) of compound **1**, one equivalent (1.02 mmol, 0.25 g) of 2-(Trifluoromethyl)benzenesulfonyl chloride, and 1.2 equivalents (1.23 mmol, 0.13 g) of triethylamine were reacted in 30 ml freshly distilled dichloromethane according to the general procedure B at 3.1.2.2.

Molecular weight [g/mol]: 501.48

Molecular formula: $C_{24}H_{18}F_3N_3O_4S$

Yield [%]: 68

Retention factor (R_f): 0.73 (Ethylacetate:Hexane=70:30)

Physical appearance: white powder

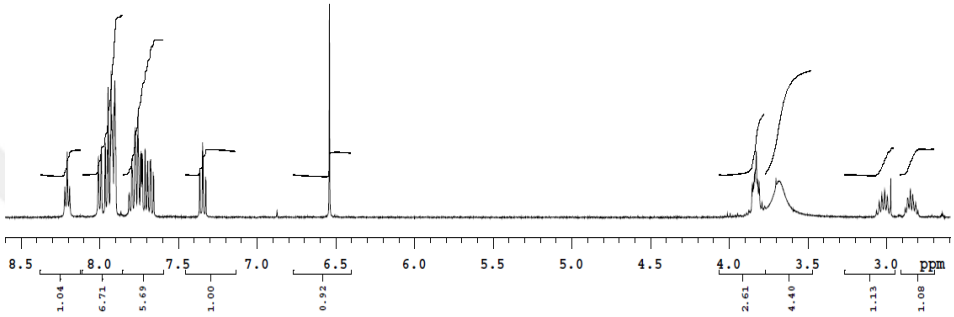
Melting point [°C]: 198.5

Solubility: DMSO

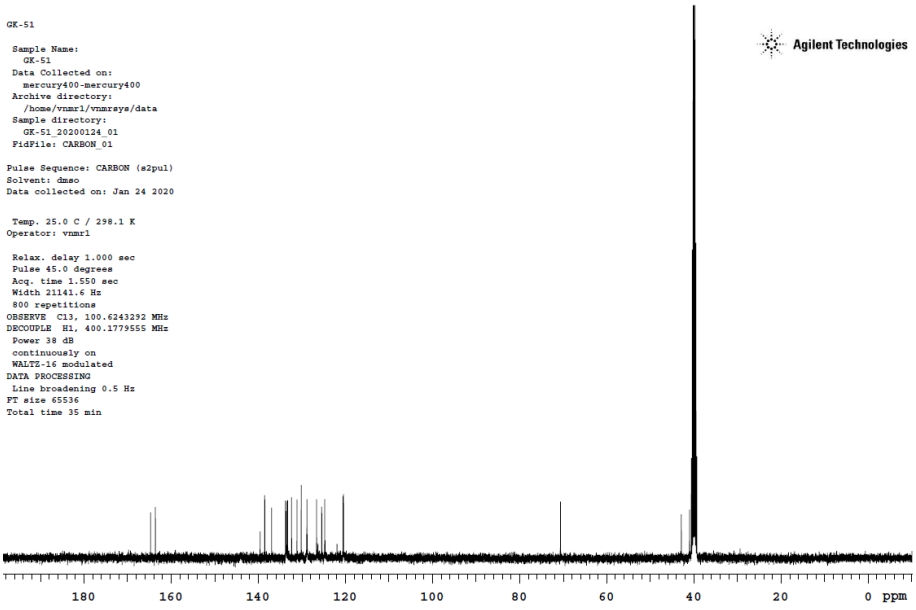
Elemental analysis:

	%C	**%H**	**%N**	**%S**
Calculted	57.48	3.62	8.38	6.39
Found	55.65	3.89	8.01	6.86

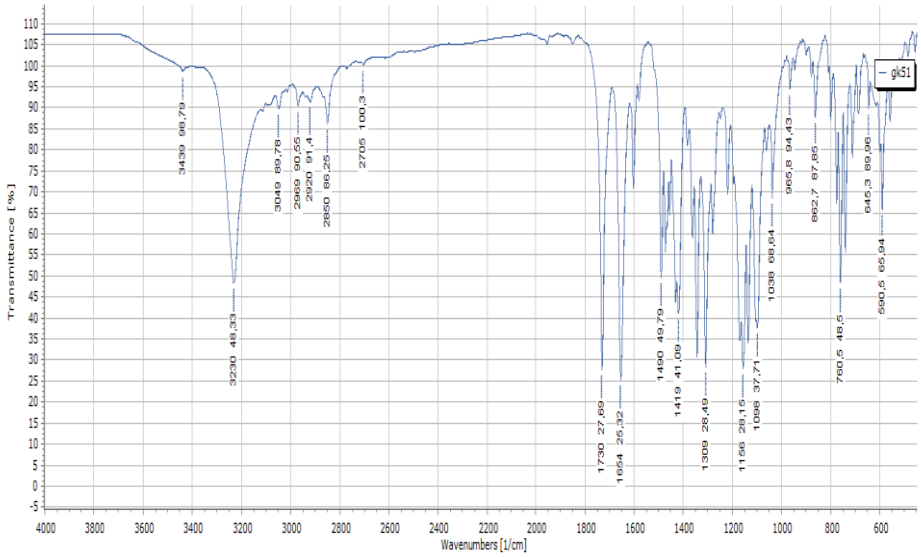
^{1}H NMR (400MHz, d_6-DMSO): 8.21 (t, 1H, *J=6.05Hz*, He.); 8.00-7.89 (m, 6H, H_{arom} + He + Ha'); 7.81-7.65 (m, 5H, H_{arom} + Hb'); 7.35 (td, 1H, J_1*=7.63Hz,* J_2*=1.11 Hz*, Hb); 6.54 (s, 1H, Hi); 3.85-3.81 (m, 2H, Hk); 3.06-2.97 (m, 1H, Hj); 2.88-2.79 (m, 1H, Hj).



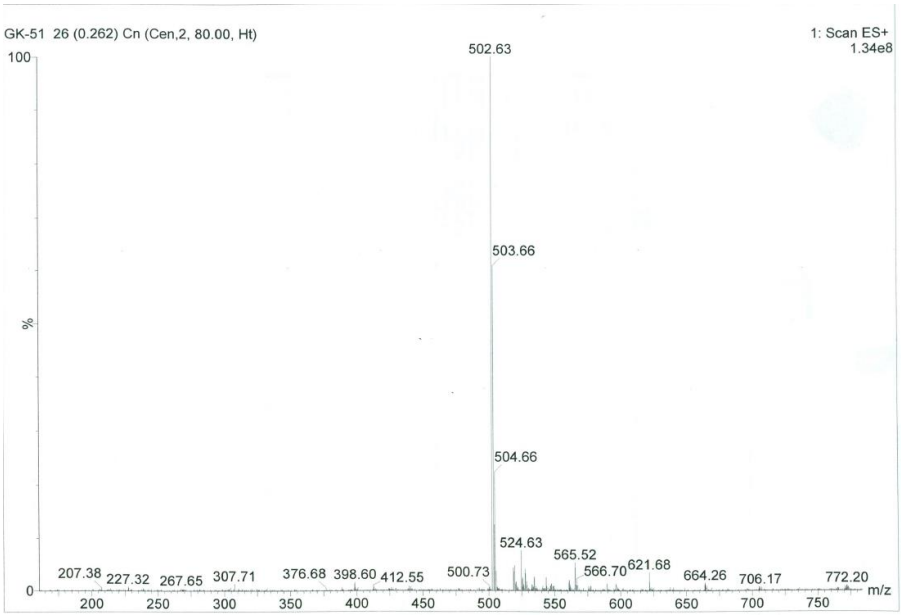
^{13}C NMR (400MHz, d_6-DMSO): 164.7 (N-CO-C); 163.6 (C-CO-N); 139.5; 138.5; 136.9; 133.8; 133.6; 133.5; 133.2; 132.3; 131.1; 130.1; 128.9; 128.8; 126.6; 125.4; 124.7; 120.5; 120.4; 70.6 (N-CH-C); 42.9 (N-CH_2-CH_2)



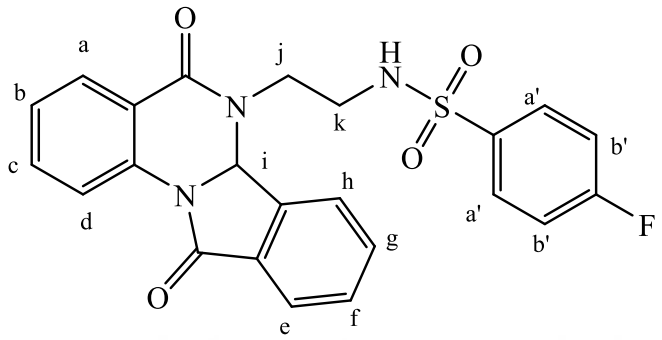
IR (ν_{max} [cm^{-1}]): 3230 (-N-H stretching); 3049 (-C=C stretching); 2969 (aromatic -C-H stretching); 1730 (-N-H bending); 1654 (C=O stretching); ~1600 (aromatic -C=C stretching); 1490 and 1419 (O=S=O stretching)



LC-MS [m/z]: [M+1]= 502.68



N-[2-(5,11-dioxo-6a,11-dihydroisoindolo[2,1-a]quinazolin-6(5H)-yl)ethyl]-4-fluorobenzenesulfonamide (7)



One equivalent (1.36 mmol, 0.40g) of compound **1**, one equivalent (1.36 mmol, 0.27 g) of 4-fluorobenzenesulfonyl chloride, and 6 equivalents (8.16mmol, 0.83 g) of triethylamine were reacted in 30 ml freshly distilled dichloromethane according to the general procedure B at 3.1.2.2.

Molecular weight [g/mol]: 451.47

Molecular formula: $C_{24}H_{18}F_3N_3O_4S$

Yield [%]: 29

Retention factor (R_f): 0.65 (Ethylacetate:Hexane=70:30)

Physical appearance: white powder

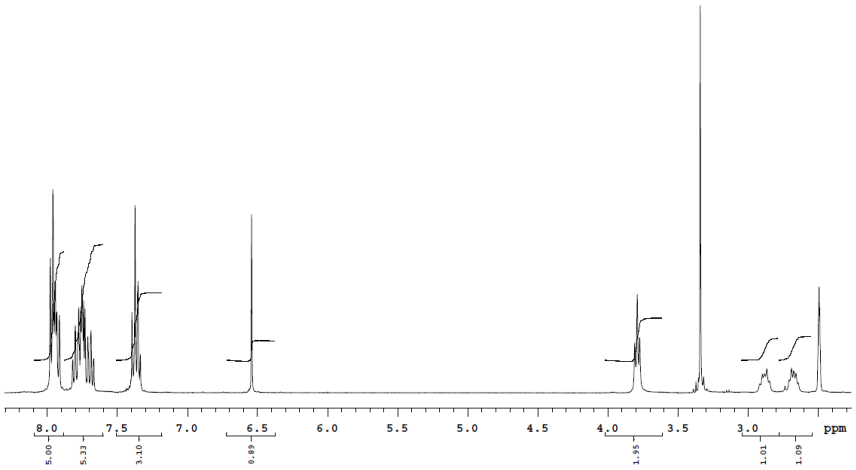
Melting point [°C]: 208.8

Solubility: DMSO

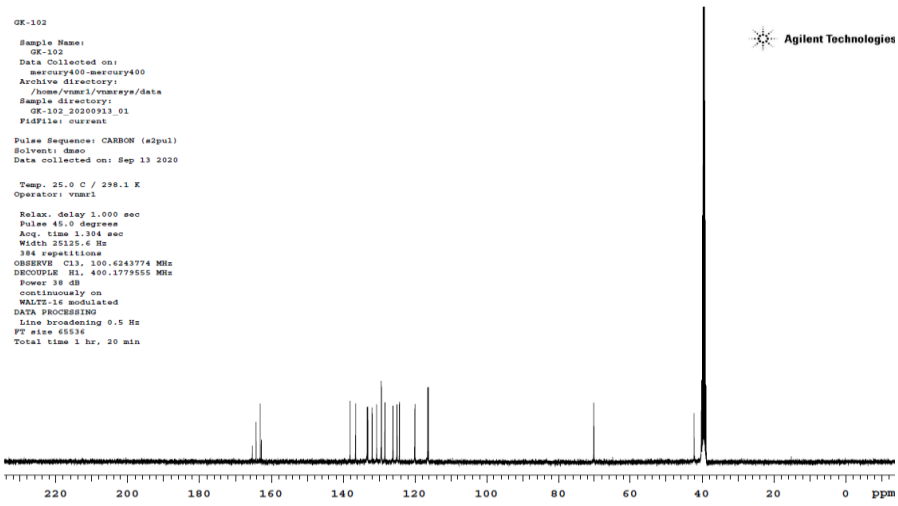
Elemental analysis:

	%C	%H	%N	%S
Calculted	61.19	4.02	9.31	7.10
Found	61.02	3.92	9.29	7.17

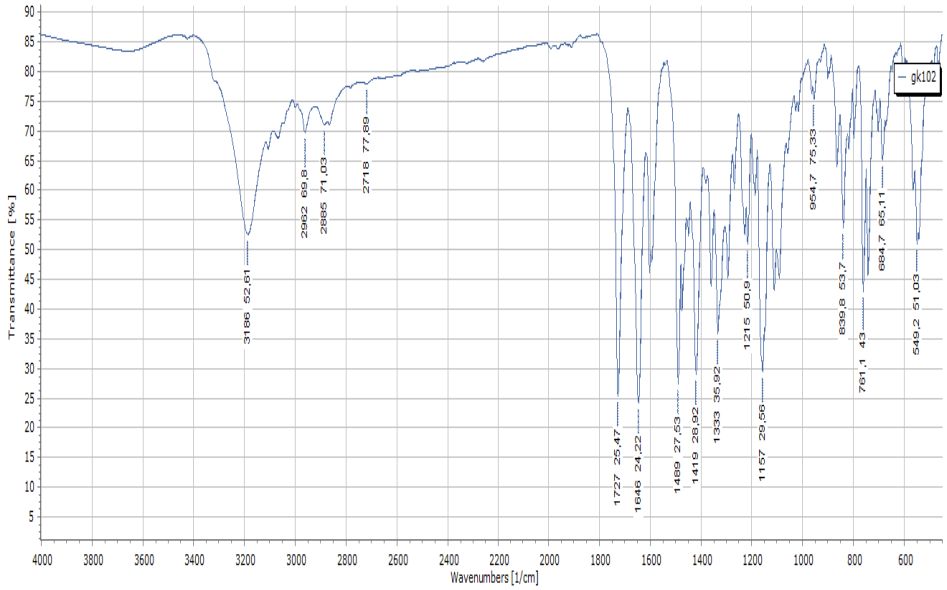
^{1}H NMR (400MHz, d_6-DMSO):7.98-7.91 (m, 6H, H_{arom} + H_e + $H_{a'}$); 7.82-7.66 (m, 4H, H_{arom}); 7.40-7.39 (m, 3H, H_b+$H_{b'}$); 6.54 (s, 1H, H_i); 3.79 (t, 2H, *J=7.27 Hz*, H_k); 2.92-2.83 (m, 1H, H_j); 2.71-2.65 (m, 1H, $H_{j'}$)



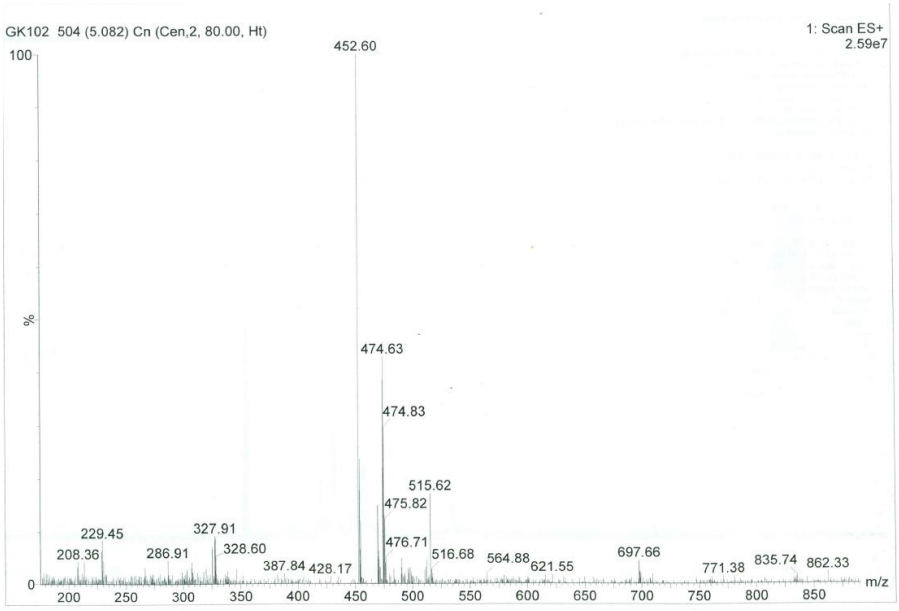
^{13}C NMR (400MHz, d_6-DMSO): 165.3 (C-F); 164.3 (N-CO-C); 163.1 (C-CO-N); 162.8; 138.1; 136.6; 136.5; 136.5; 133.3; 133.1; 131.9; 130.7; 129.4; 129.3; 128.4; 126.1; 124.9; 124.3; 120.1; 119.9; 116.5 (CH-C(F)); 116.2; 70.2 (N-CH-C); 42.2; 40.2; 40.1; 39.9; 39.7; 39.5; 39.3; 39.1; 38.9



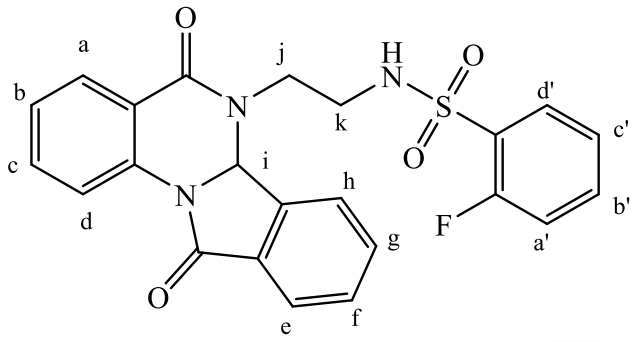
IR (ν_{max} [cm^{-1}]): 3186 (-N-H stretching); 2962 (aromatic -C-H stretching); 1727 (-N-H bending); 1646 (C=O stretching); ~1600 (aromatic -C=C stretching); 1489 and 1419 (O=S=O stretching)



LC-MS [m/z]: [M+1]= 451.47



N-[2-(5,11-dioxo-6a,11-dihydroisoindolo[2,1-*a*]quinazoline-6(5*H*)-yl)ethyl]-2-fluorobenzenesulfonamide (8)



One equivalent (1.70 mmol, 0.50 g) of compound **1**, one equivalent (1.70 mmol, 0.33 g) of 2-fluorobenzenesulfonyl chloride, and 1.2 equivalents (2.04 mmol, 0.21 g) of triethylamine were reacted in 30 ml freshly distilled dichloromethane according to the general procedure B at 3.1.2.2.

Molecular weight [g/mol]: 451.47

Molecular formula: $C_{23}H_{18}FN_3O_4S$

Yield [%]: 26

Retention factor (R_f): 0.85 (Ethylacetate:Hexane=70:30)

Physical appearance: white powder

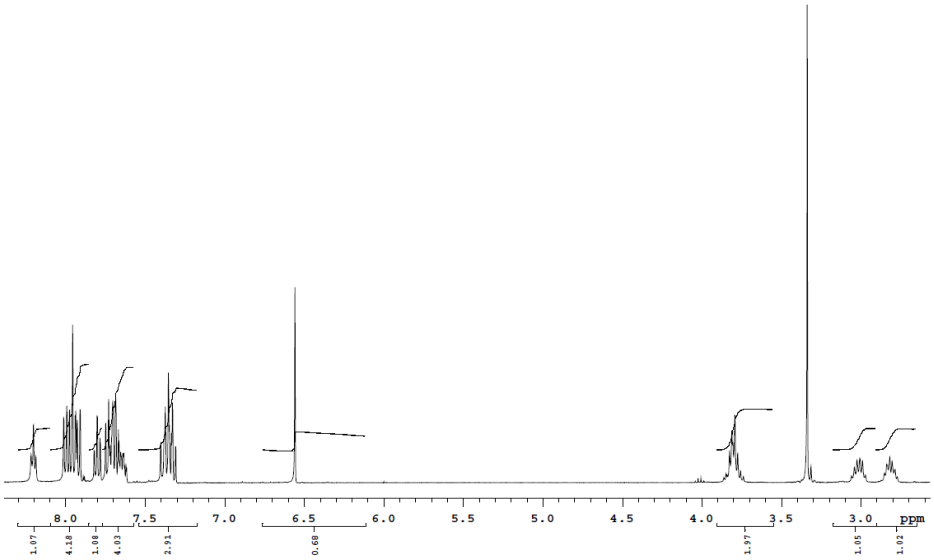
Melting point [°C]: 176.6

Solubility: DMSO

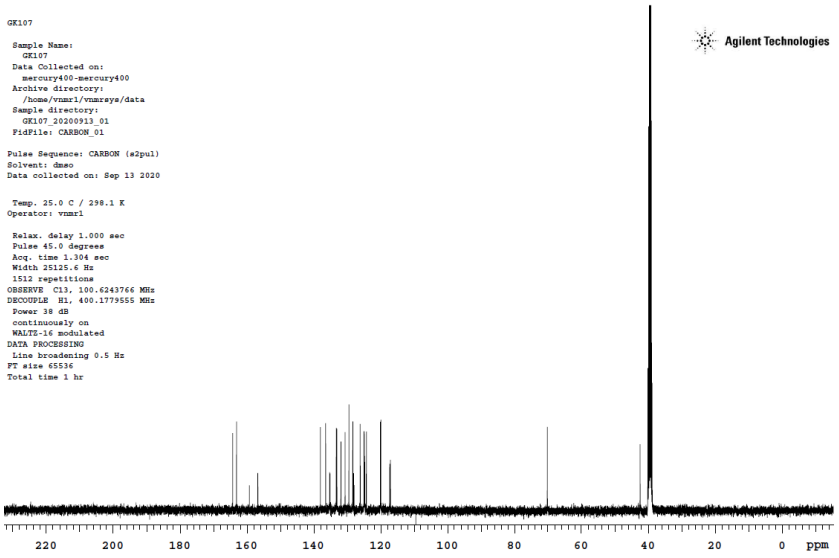
Elemental analysis:

	%C	**%H**	**%N**	**%S**
Calculted	61.19	4.02	9.31	7.10
Found	61.22	4.13	9.29	7.00

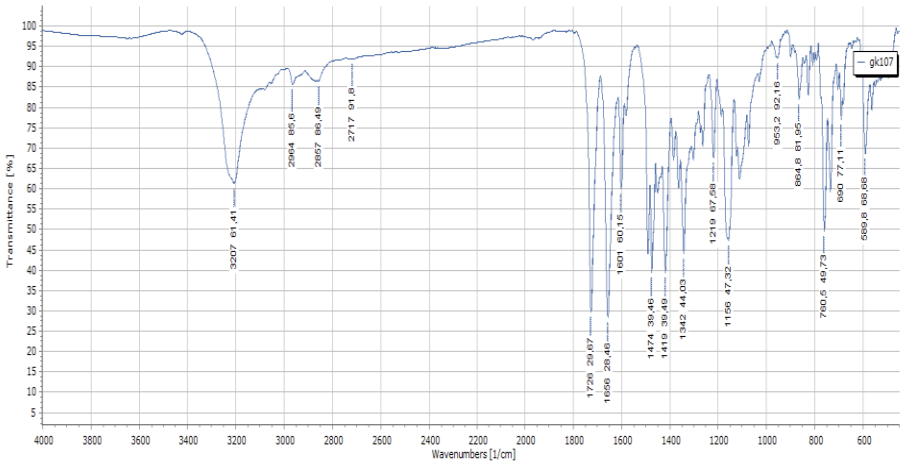
^{1}H NMR (400MHz, d_6-DMSO): 8.20 (t, 1H, *J=6.0Hz*, H_e); 8.10-7.90 (m, 4H, H_{arom}); 7.80 (ddd, 1H, J_1*=7.47Hz*, J_2*=6.30Hz*, J_3*=1.1Hz*, H_{arom}); 7.74-7.62 (m, 3H, H_{arom}); 6.56 (s, 1H, H_i); 3.86-3.74 (m, 2H, H_k); 3.07-2.97 (m, 1H, H_j); 2.85-2.77 (m, 1H, H_j).



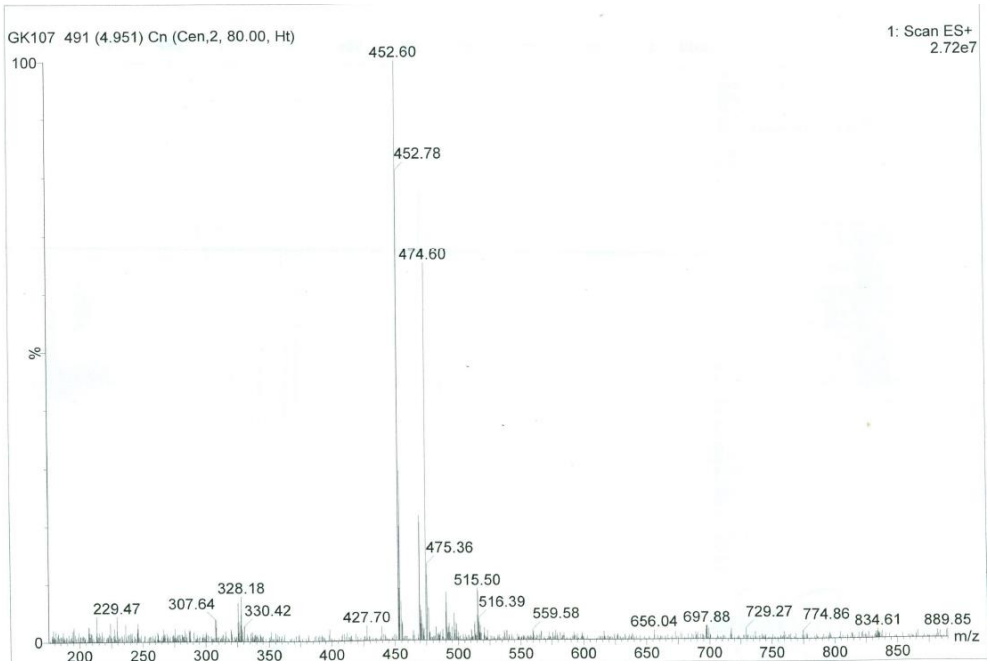
^{13}C NMR (400MHz, d_6-DMSO): 164.7 (N-CO-C); 163.6 (C-CO-N); 159.7 (C-F), 157. 2, 138.5,; 136.9, 135.7, 132.4, 131.1; 128.8, 128.6, 126.6, 125.4, 125.2, 124.5, 120.5; 120.4; 117.8 (CH-C(F)); 70.6 (N-CH-C); 42.9 (N-CH_2-CH_2); 40.7 (CH_2-CH_2-NH).



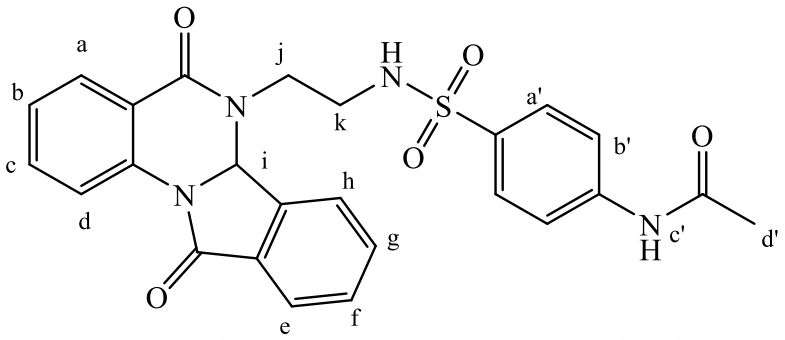
IR (ν_{max} [cm^{-1}]): 3207 (-N-H stretching); 2964 (aromatic -C-H stretching); 1726 (-N-H bending); 1658 (C=O stretching); 1601 (aromatic -C=C stretching); 1474 and 1419 (O=S=O stretching)



LC-MS [m/z]: [M+1]= 452.60



N-[4-(N-(2-(5,11-dioxo-6a,11-dihydroisoindolo[2,1-a]quinazoline-6(5H)-yl)ethyl)sulfamoyl)phenyl]acetamide (9)



2 equivalents (1.70 mmol, 0.50g) of compound **1** and one equivalent (0.85mmol, 0.20 g) of N-acetylbenzenesulfonyl chloride were reacted in 30 ml EtOH according to the general procedure C at 3.1.2.3.

Molecular weight [g/mol]: 490.53

Molecular formula: $C_{25}H_{22}N_4O_5S$

Yield [%]: 47

Retention factor (R_f): 0.80 (Ethylacetate:n-Hexane=70:30)

Physical appearance: white powder

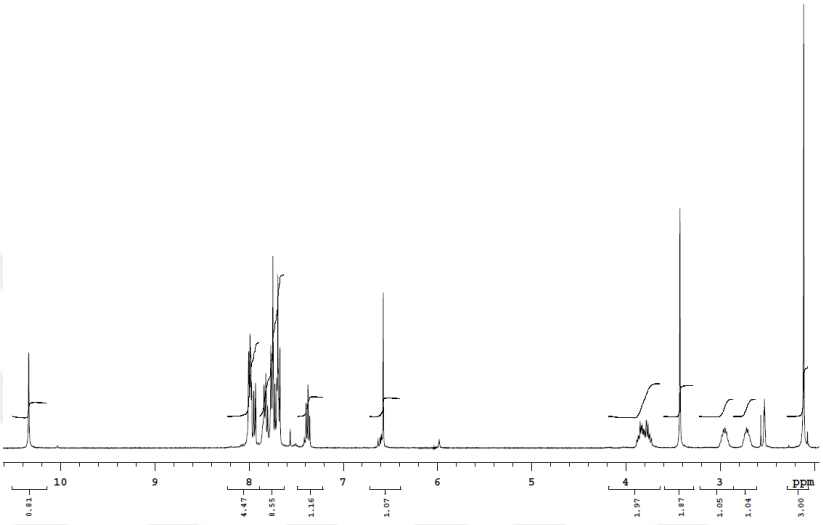
Melting point [°C]: 211.8

Solubility: DMSO

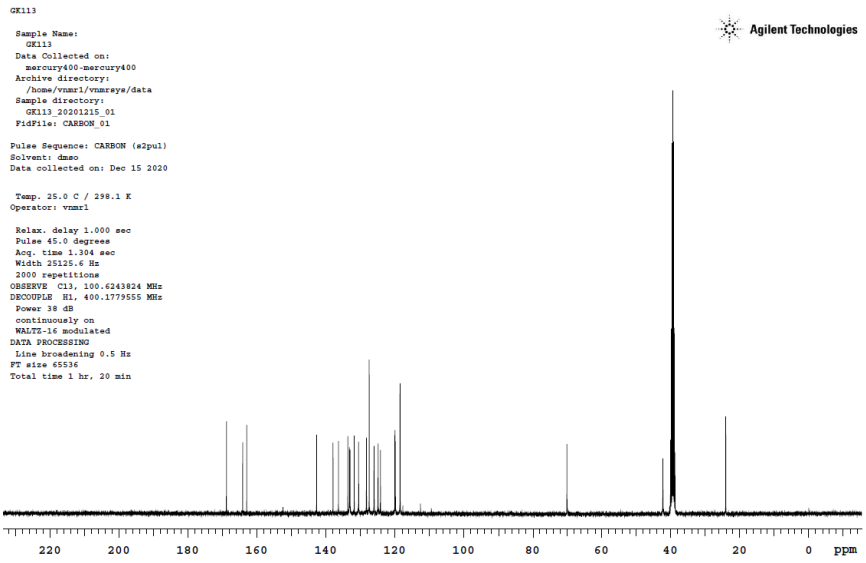
Elemental analysis:

	%C	**%H**	**%N**	**%S**
Calculted	61.19	4.02	9.31	7.10
Found	60.68	4.62	11.21	6.56

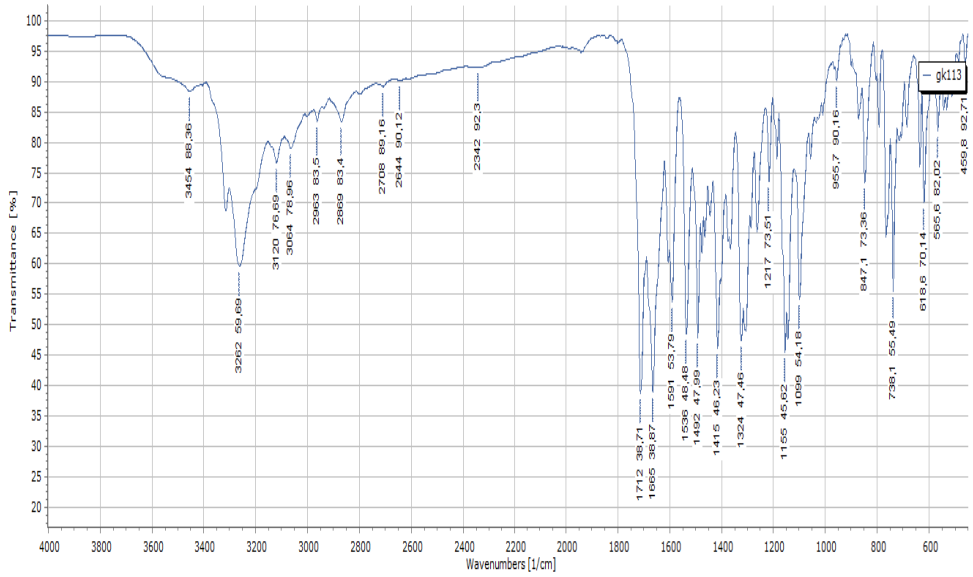
^{1}H NMR (400MHz, d_6-DMSO): 10.30 (s, 1H, $H_{c'}$); 7.97-7.88 (m, 4H, H_{arom}); 7.80-7.63 (m, 7H, H_{arom} +$H_{a'}$+$H_{b'}$); 7.34 (ddd, 1H, J_1=7.6Hz, J_2=6.78Hz, J_3=1.0Hz, H_b); 6.54 (s, 1H, H_i); 3.83-3.69 (m,2H, H_k); 2.94-2.83 (m, 1H, H_j); 2.71-2.63 (m, 1H, H_j); 2.07 (s, 3H, $H_{d'}$).



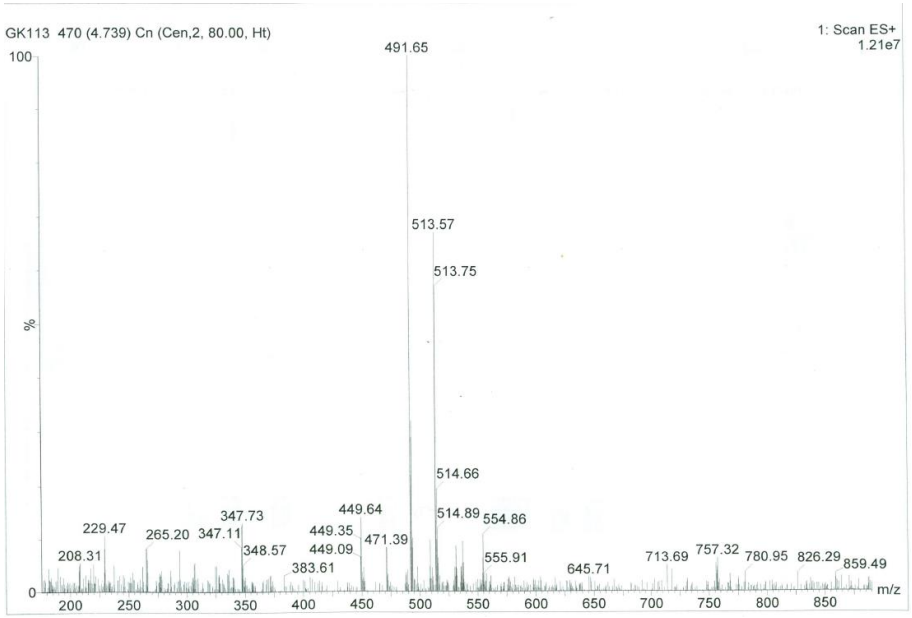
^{13} C NMR (400MHz, d_6-DMSO): 169.4 (N-<u>C</u>O-CH_3); 164.5 (N-<u>C</u>O-C); 163.6 (C-<u>C</u>O-N); 143.2; 138.5; 136.9; 134.2 (CH-<u>C</u>-NH); 132.4; 131.1; 128.8; 128.0 (SO_2-<u>C</u>-CH); 126.6; 125.4; 124.5; 120.5; 120.4; 119.0 (CH-<u>C</u>H-C); 70.6 (N-<u>C</u>H-C); 42.9 (N-<u>C</u>H_2-CH_2); 40.7 (CH_2-<u>C</u>H_2-NH); 24.6 (CO-<u>C</u>H_3).



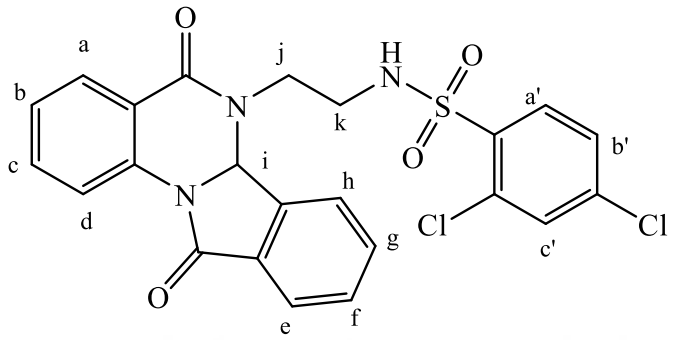
IR (ν_{max} [cm^{-1}]): 3262 (-N-H stretching); 3120 and 3064 (-C=C stretching); 2963 (aromatic -C-H stretching); 1712 (-N-H bending); 1665 (C=O stretching); 1591 (aromatic -C=C stretching); 1492 and 1415 (O=S=O stretching)



LC-MS [m/z]: [M+1]= 491.65



2,4-dichloro-N-[2-(5,11-dioxo-6a,11-dihydroisoindolo[2,1-a]quinazolin-6(5H)-yl)ethyl]benzenesulfonamide (10)



2 equivalents (1.70 mmol, 0.50 g) of compound **1** and one equivalent (0.85 mmol, 0.21 g) of 2,4-dichlorobenzenesulfonyl chloride were reacted in 30 ml EtOH according to the general procedure C at 3.1.2.3.

Molecular weight [g/mol]: 502.37

Molecular formula: $C_{23}H_{17}C_{l2}N_3O_4S$

Yield [%]: 44

Retention factor (R_f): 0.73 (Ethylacetate:n-Hexane=70:30)

Physical appearance: white powder

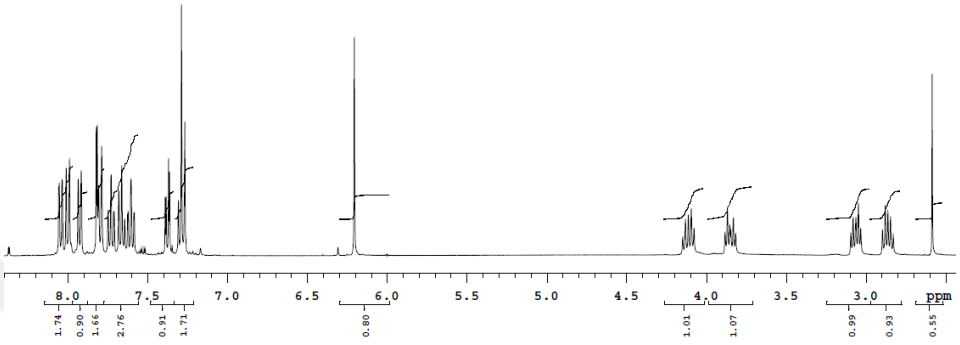
Melting point [°C]: 225.3

Solubility: DMSO

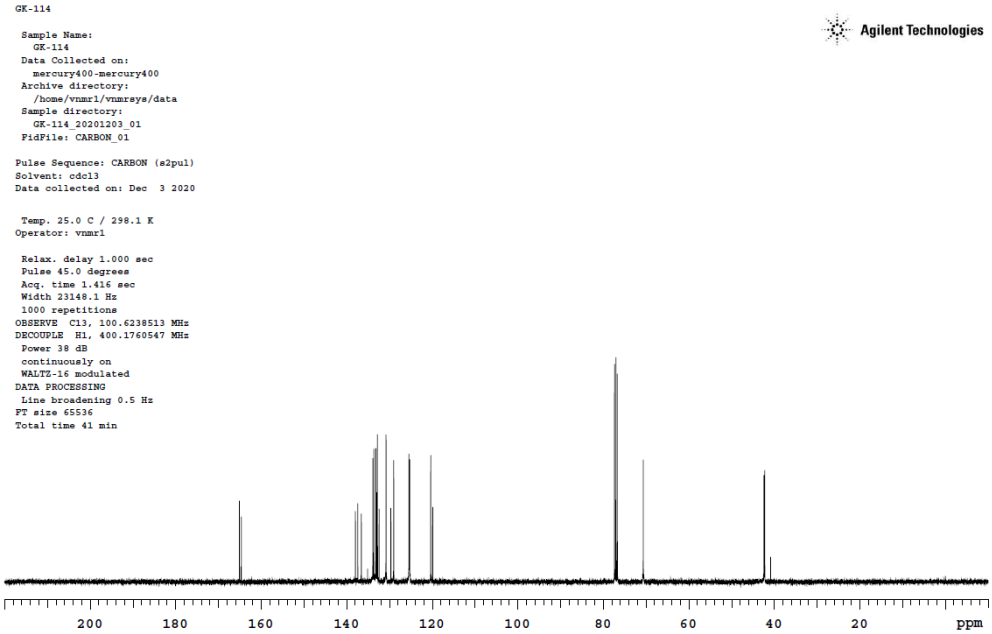
Elemental analysis:

	%C	%H	%N	%S
Calculted	46.63	4.59	7.09	5.41
Found	47.80	3.63	7.03	5.47

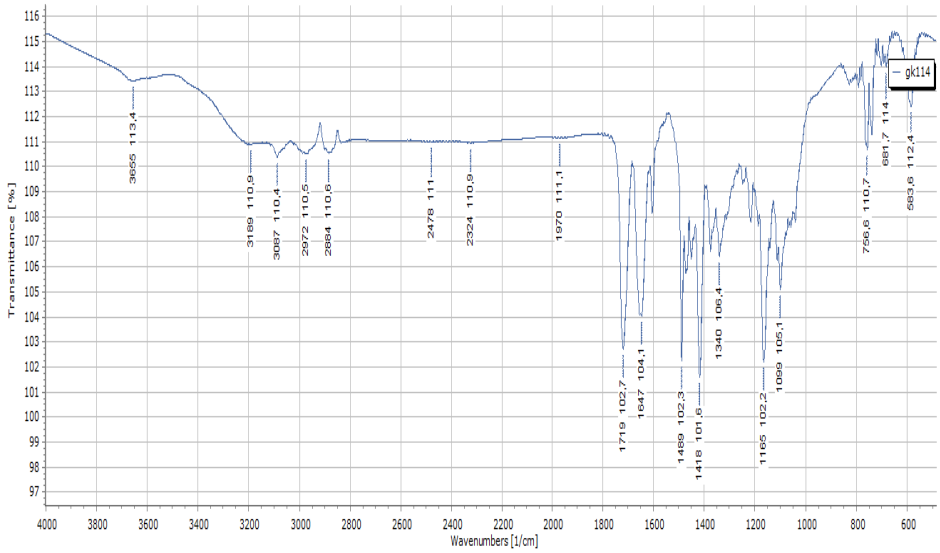
^{1}H NMR (400MHz, $CDCl_3$): 8.06-7.99 (m, 2H, H_{arom}); 7.93 (d, 1H, *J=7.4314 Hz*, H_{arom}); 7.87-7.58 (m, 5H, H_{arom}); 7.38 (dd, 1H, J_1*=8.53 Hz*, J_2*=2.5 Hz*, H_{arom} m); 7.31-7.27 (m, 2H, H_{arom}); 6.21 (s, 1H, H_i); 4.15-4.07 (m, 1H, H_k); 3.81-3.88 (m, 1H, H_k); 3.09-3.03 (m, 1H, H_j); 2.90-2.83 (m, 1H, H_j)



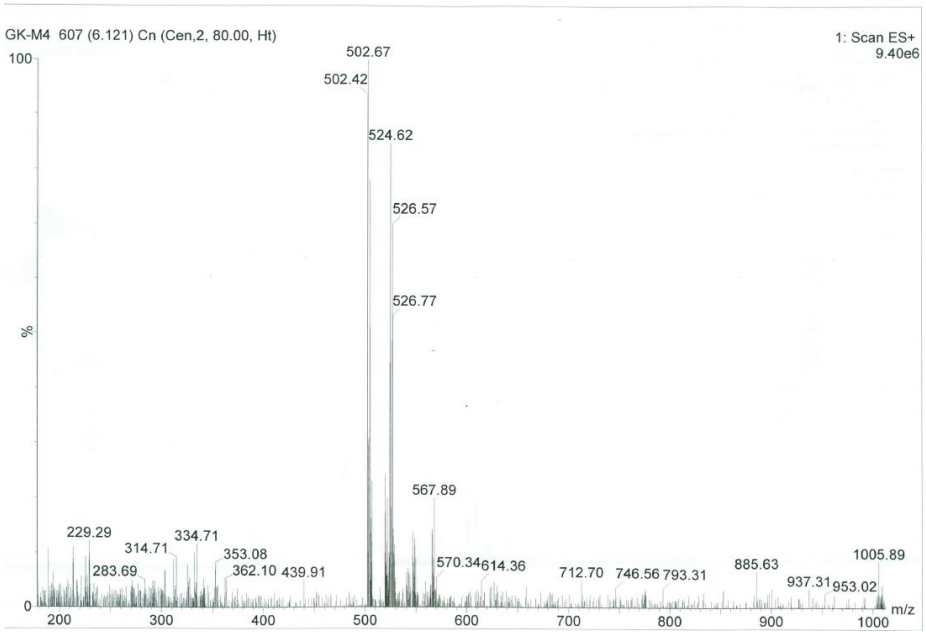
^{13}C NMR (400MHz, $CDCl_3$): 165.1 (N-CO-C); 164.7 (C-CO-N); 137.9; 137.4; 136.6; 133.9; 133.7; 133.2; 132.9; 132.9; 132.8; 132.4; 130.8; 130.8; 129.7; 129.0; 125.5; 125.3; 125.3; 120.4; 119.9; 77.4; 77.1; 76.8; 70.7 (N-CH-C); 42.4; 42.3; 40.9 (CH_2-CH_2-NH)



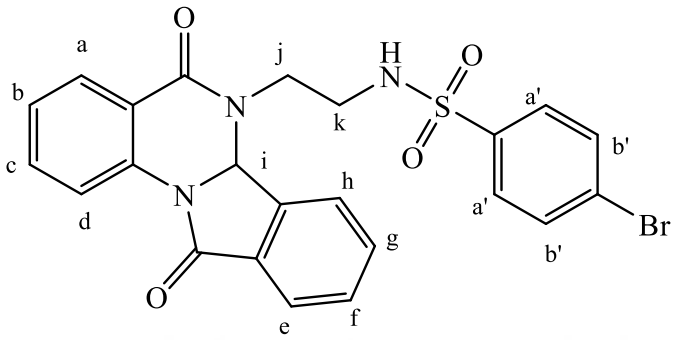
IR (ν_{max} [cm^{-1}]): 3189 (-N-H stretching); 3087 (-C=C stretching); 2972 (aromatic -C-H stretching); 1719 (-N-H bending); 1647 (C=O stretching); ~1600 (aromatic -C=C stretching); 1489 and 1418 (O=S=O stretching)



LC-MS [m/z]: [M]= 502.42



4-bromo-N-[2-(5,11-dioxo-5H,6H,6aH,11H-isoindolo[2,1-a]quinazolin-6-yl)ethyl]benzene-1-sulfonamide (11)



2 equivalents (1.70 mmol, 0.50 g) of compound **1** and one equivalent (0.85 mmol, 0.22 g) of 4-bromobenzenesulfonyl chloride were reacted in 30 ml EtOH according to the general procedure C at 3.1.2.3.

Molecular weight [g/mol]: 512.38

Molecular formula: $C_{23}H_{18}BrN_3O_4S$

Yield [%]: 23

Retention factor (R_f): 0.65 (Ethylacetate:n-Hexane=70:30)

Physical appearance: white powder

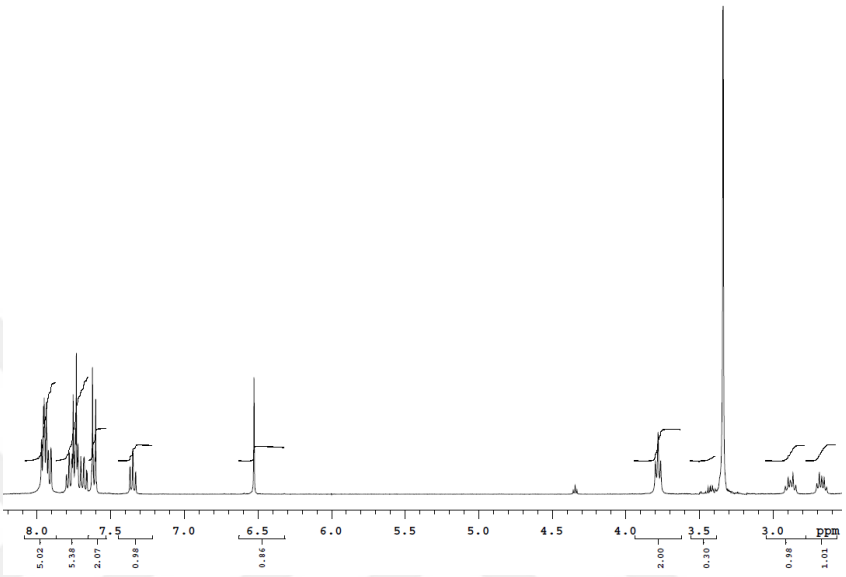
Melting point [°C]: 198.8

Solubility: DMSO

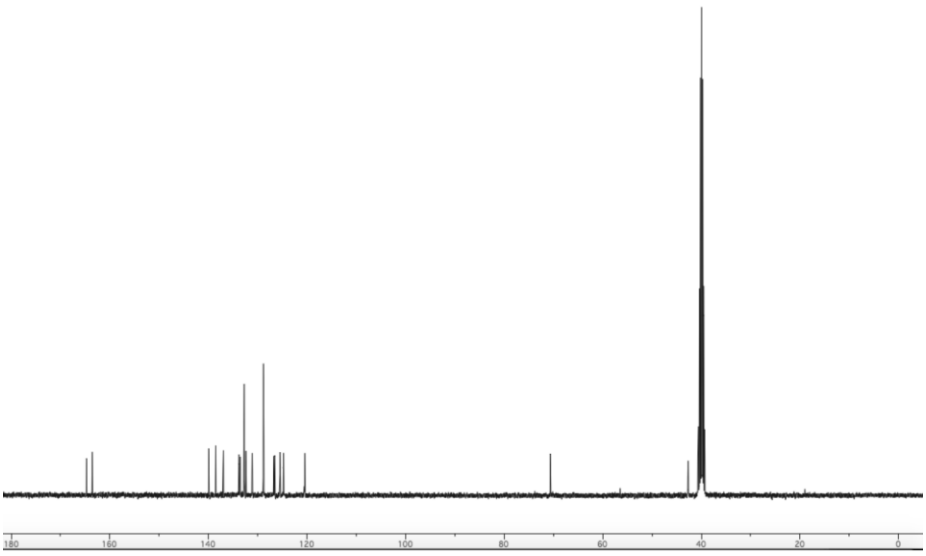
Elemental analysis:

	%C	**%H**	**%N**	**%S**
Calculted	53.92	3.54	8.20	6.26
Found	54.32	4.07	8.25	6.19

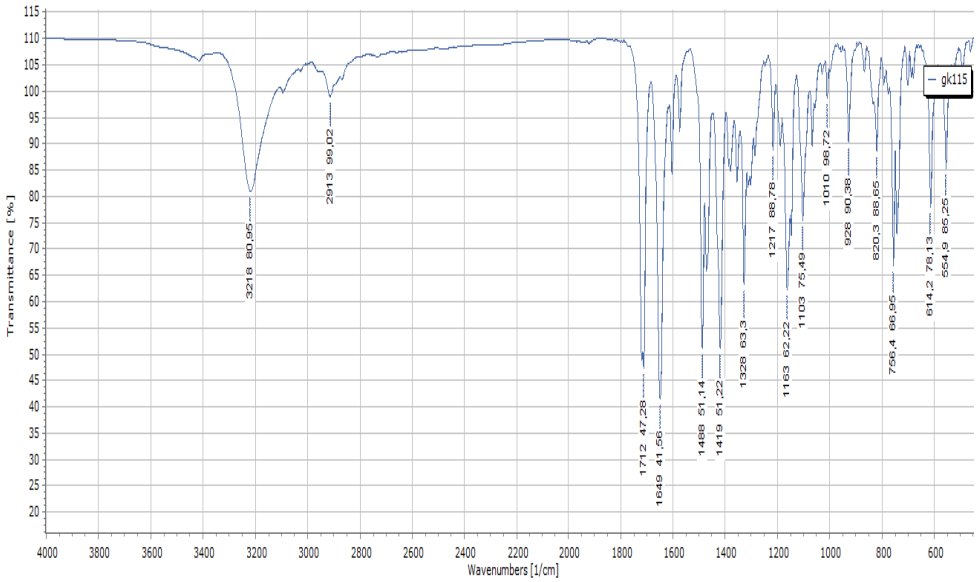
^{1}H NMR (400MHz, d_6-DMSO): 7.96-7.90 (m, 4H, H_{arom} + H_e); 7.81-7.66 (m, 5H, H_{arom} + $H_{a'}$); 7.61 (d, 2H, *J=8.6 Hz*, $H_{b'}$); 7.35 (ddd, 1H, H_b, *J_1=7.76 Hz, J_2=6.76 Hz, J_3= 1.0 Hz*); 6.53 (s, 1H, H_i); 3.79 (d, 2H, *J=7.12 Hz*, H_k); 2.89-284 (m, 1H, H_j); 2.70-2.69 (m, 1H, H_j)



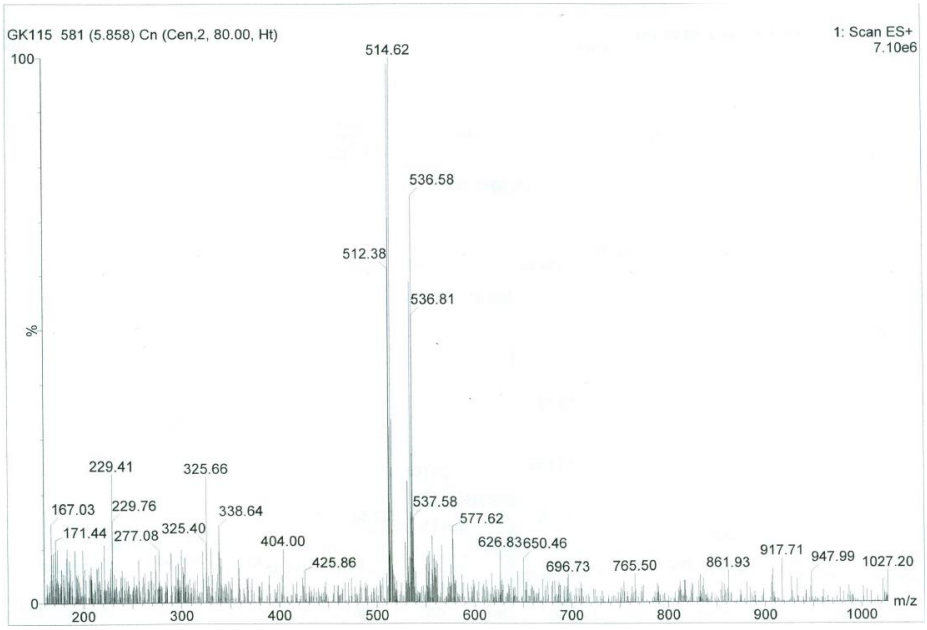
^{13}C NMR (400MHz, d_6-DMSO): 164.5 (N-CO-C); 163.6 (C-CO-N); 139.9; 138.5; 136.9; 132.7; 132.4; 131.1; 128.8; 126.6; 125.4; 124.5;120.5; 120.4; 70.6 (N-CH-C) ; 42.9 (N-CH_2-CH_2) ; 40.7 (CH_2-CH_2-NH).



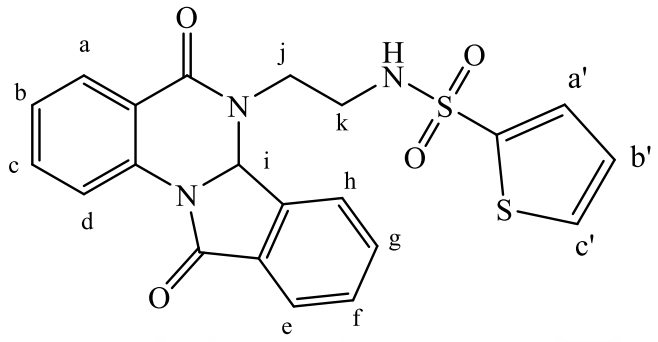
IR (ν_{max} [cm^{-1}]): 3218 (-N-H stretching); 2913 (aromatic -C-H stretching); 1712 (-N-H bending); 1649 (C=O stretching); ~1600 (aromatic -C=C stretching); 1488 and 1419 (O=S=O stretching)



LC-MS [m/z]: [M]= 512.38



N-[2-(5,11-dioxo-5H,6H,6aH,11H-isoindolo[2,1-a]quinazolin-6-yl)ethyl]thiophene-2-sulfonamide (12)



One equivalent (1.00 mmol, 0.26 g) of compound **1** and one equivalent (1.00 mmol, 0.18 g) of Thiophenebenzenesulfonyl chloride were reacted in 30 ml EtOH according to the general procedure C at 3.1.2.3.

Molecular weight [g/mol]: 439.50

Molecular formula: $C_{21}H_{17}N_3O_4S_2$

Yield [%]: 27

Retention factor (R_f): 0.77 (Ethylacetate:n-Hexane=70:30)

Physical appearance: white powder

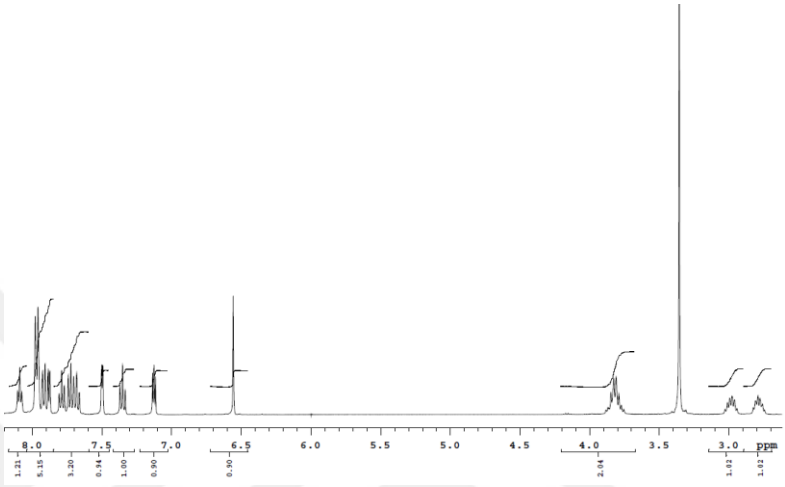
Melting point [°C]: 227.3

Solubility: DMSO

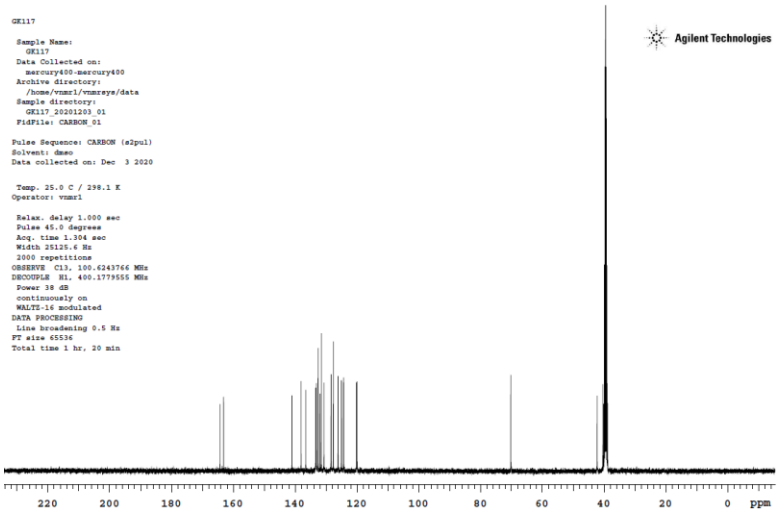
Elemental analysis:

	%C	**%H**	**%N**	**%S**
Calculted	57.39	3.90	9.56	14.59
Found	57.49	4.26	9.64	14.58

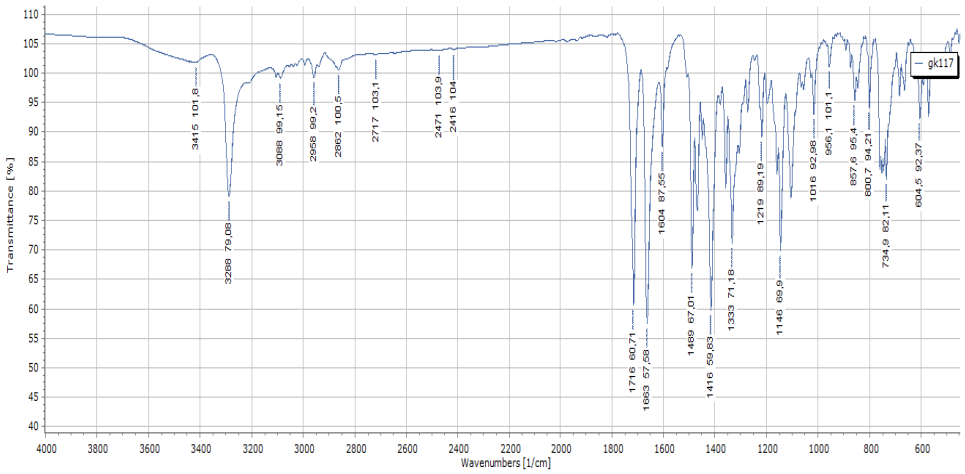
^{1}H NMR (400MHz, d_6-DMSO): 8.1 (t, 1H, *J=6.1 Hz*, H_e); 7.99-7.98 (m, 3H, H_{arom}); 7.88 (dd, 1H, J_1*= 4.9 Hz,* J_2*= 1.1 Hz*, $H_{c'}$); 7.80-7.66 (m, 2H, H_{arom}); 7.50 (dd, 1H, J_1*=3.6 Hz,* J_2*=1.1 Hz*, $H_{a'}$); 7.35 (t, 1H, *J=7.3 Hz*, $H_{b'}$); 7.12 (dd, 1H, J_1*=4.9 Hz*, J_2*=3.6 Hz*, H_b); 6.56 (s, 1H, H_i); 3.87-3.78 (m, 2H, H_k); 3.11-2.96 (m, 1H, H_j); 2.88- 2.75 (m, 1H, H_j)



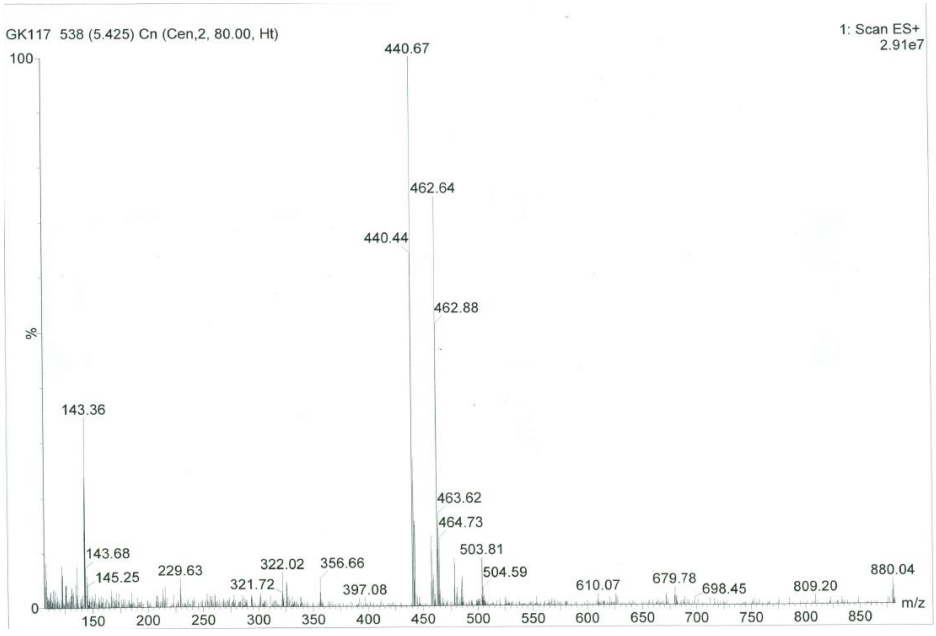
^{13}C NMR (400MHz, d_6-DMSO): 164.3 (N-CO-C); 163.2 (C-CO-N); 141.0; 138.1; 136.5; 133.3; 133.1; 132.5; 131.9; 131.5; 130.7; 128.4; 127.7; 126.1; 125.0; 124.3; 120.1; 119.9; 70.2 (N-CH-C); 42.3; 40.4; 40.1; 39.9; 39.7; 39.5; 39.3; 39.1; 38.9



IR (ν_{max} [cm^{-1}]): 3288 (-N-H stretching); 3088 (-C=C stretching); 2958 (aromatic -C-H stretching); 1716 (-N-H bending); 1663 (C=O stretching); 1604 (aromatic -C=C stretching); 1489 and 1416 (O=S=O stretching)



LC-MS [m/z]: [M+1]= 440.67



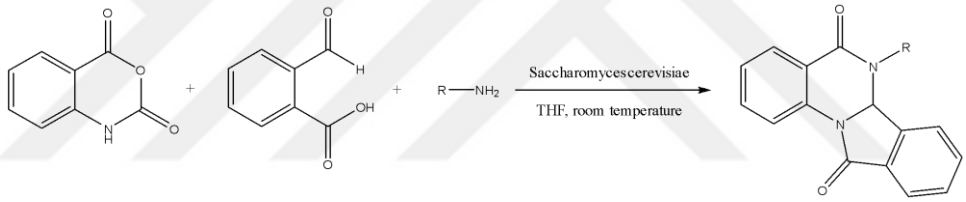
5. DISCUSSION AND CONCLUSION

5.1. Chemistry

5.1.1. One-pot synthesis using *Saccharomyces Cerevisiae* (baker's yeast)

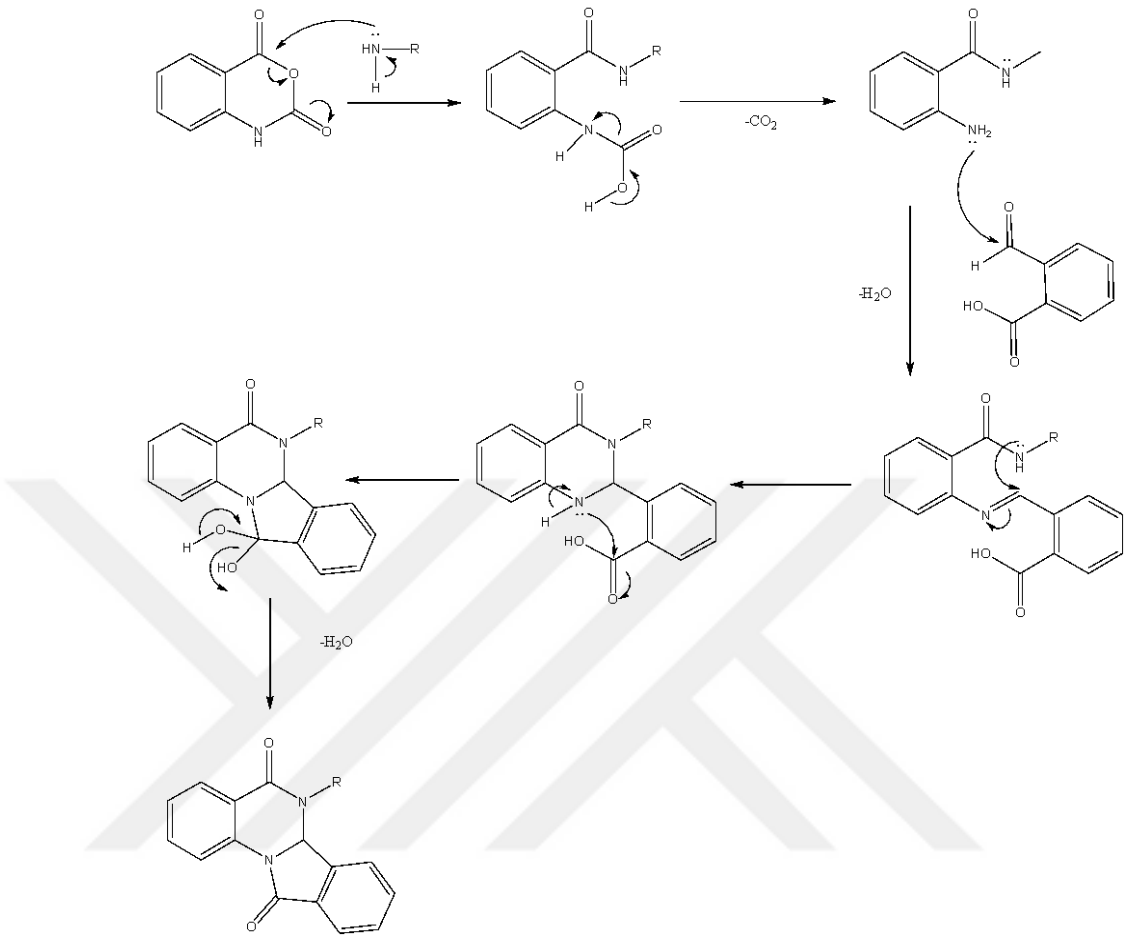
This study consisted of the synthesis of novel isoindolo[2,1-α]quinazoline derivatives combined with sulfonamide moiety and their analogs, in order to investigate their possible biological activity on human tissue.

The first attempt of the 'one-pot' synthesis to obtain 6,6a-dihydroisoindolo[2,1-a]quinazoline-5,11-dione derivatives were performed according to the procedure previously described by Avalani et al.[27] in the presence of *Saccharomyces cerevisiae* also known as Baker's yeast as the catalyst. For this reaction isatoic anhydride, formyl benzoic acid, and sulfonamide derivatives were used simultaneously as shown in figure 5.1.



Scheme 5.1. Reaction pathway with *Saccharomyces cerevisiae*

A possible reaction mechanism for the formation of isoindolo[2,1-a]quinazoline derivatives in these conditions has been proposed by Avalani et al.[27] and is shown in the figure below.



Scheme 5.2. Reaction mechanism for the forming of isoindolo[2,1-a]quinazoline derivatives

According to this mechanism, the reaction occurs by the attack of the amine derivative to the anhydride and followed by the attack of the aniline derivative to the formylbenzoic acid to obtain the corresponding imine compound. The ring-closure reaction takes place through the nucleophilic attack of the imine function to the carboxylic acid. Then a final dehydration leads to the four cyclic isoindoloquinazoline structure.

In our study, to begin with, sulfacetamide, sulfathiazole, and sulfabenzamide (Figure 5.1) were chosen for the generation of isoindoloquinazoline derivatives.

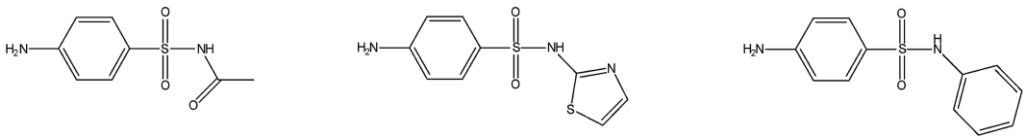


Figure 5.1. Structures of sulfacetamide, sulfathiazole, and sulfabenzamide

For this purpose, experiments were carried out at room temperature and with ultrasonic irradiation. As the reaction solvent, dry THF was used. Data obtained with LCMS and ^{1}H-NMR spectroscopy indicated that the target product was not observed, because the molecular peak in the mass spectrum was not observed and instead of the singlet proton peak of the ring closure, a triplet peak has been occurred.

As desired the synthesis of desired products was not achieved, alternative methods were developed to obtain the isoindoloquinazoline heterocycles.

5.1.2. Isoindoloquinaqzoline heterocycle synthesis with alternative methods

The several conditions given in the literature [27,35,40] were performed to obtain the four cyclic structure. Performed conditions are given in the following table (Table 5.1).

Table 5.1. The performed conditions for the synthesis

	1	**2**	**3**	**4**	**5**	**6**	**7**
Solvent	THF	THF	THF	THF	CH_3COOH	THF	H_2O
Reaction Time	2h	2-7d	2-7d	2-7d	2h	2-7d	10h
Conditions	Ultrasonic irritation	Room temperature	Room temperature	Room temperature	110°C	Reflux	Reflux
Catalyst	Saccharomyces cerevisiae	Saccharomyces cerevisiae	ZnO	CuO		ZnO	ZnO

The isoindolo[2,1-a]quinazoline derivatives were not synthesized under these conditions. Because of the water sensitivity of anhydrides, further reactions were performed under anhydrous conditions and nitrogen atmosphere. On the other hand, due to the limited solubility of the sulfonamides in THF, DMSO was tested as a solvent. All the procedures, which were carried out, are shown in the table below (Table 5.2).

Table 5.2. Several conditions to obtain the product

#	1	2	3	4	5	6	7
Solvent	Anhydrous THF	Anhydrous THF	Anhydrous THF	Anhydrous DMSO	Anhydrous DMSO	Anhydrous DMSO	Anhydrous DMSO/THF
Reaction Time	2h	24h	24h	24h	24h	24h	24h
Conditions	Ultrasonic irritation	Room temperature	Room temperature	Room temperature	Room temperature	Room temperature	Room temperature
Catalyst	Saccharomyces cerevisiae	Saccharomyces cerevisiae	ZnO	Saccharomyces cerevisiae	ZnO	CuO	CuO

The analysis with thin layer chromatography demonstrated a new spot on the chromatogram that was isolated for analysis. A ^{1}H-NMR spectrum and a LC-MS analysis were performed on the obtained product. Results are shown in the following figure 5.2.

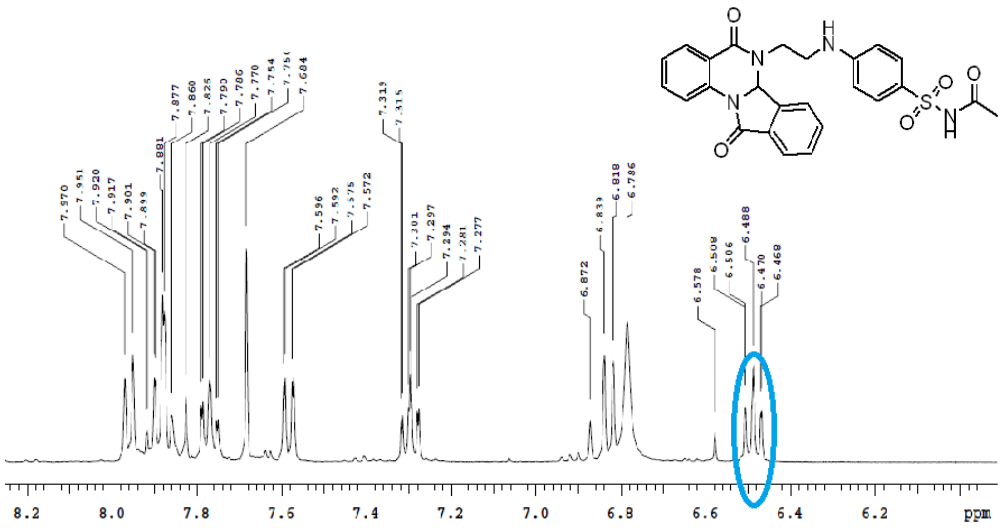


Figure 5.2. ^{1}H-NMR spectrum of reaction with sulfacetamide

According to data analysis, the corresponding proton peak at 6.5 ppm on the isoindoloquinazoline ring shows a triplet signal instead of a singlet as reported in the literature. In addition to that, by the reaction with sulfacetamide, the peaks of the acetyl group expected approximately at 3 ppm were not observed (Figure 5.3).

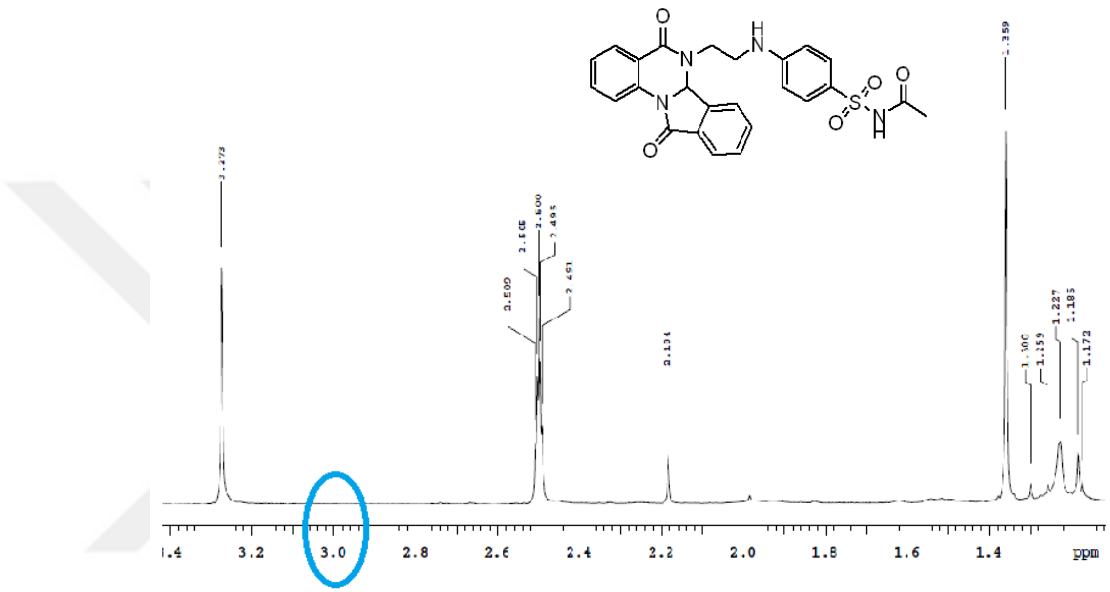


Figure 5.3.[1]H-NMR spectrum of the reaction with sulfacetamide

Another reaction with sulfadoxine was intended for the identification of the protons of the methoxy groups at 3-4 ppm by [1]H-NMR, however again a triplet signal at 6.5 ppm was observed and no signals for the methoxy groups have occurred (Figure 5.3). Moreover, in the LC-MS spectrum of this compound, there was no M^+ -peak as expected. (Figure 5.4)

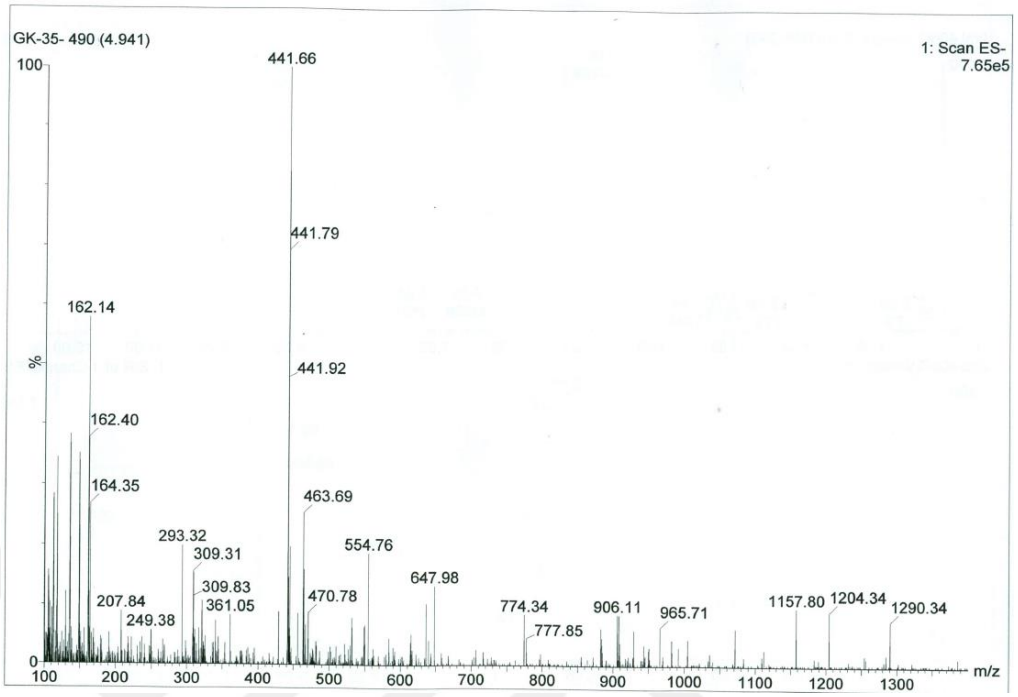


Figure 5.4. Mass spectrum of sulfadoxine derivatives

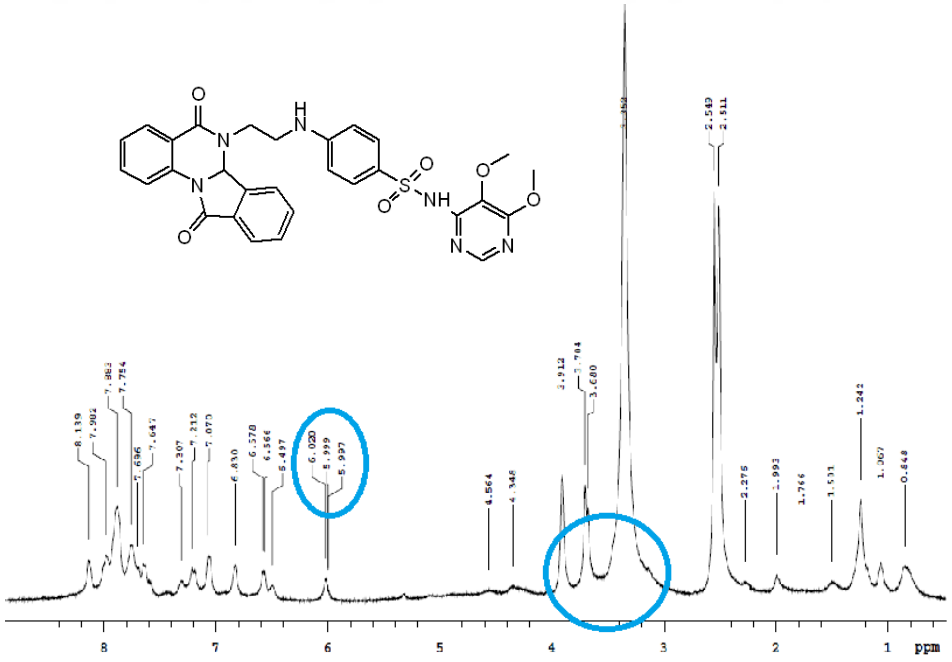


Figure 5.5. [1]H-NMR spectrum of the reaction with sulfadoxine

To determine the structure of compounds that were found to not correspond to the expected products, the reaction mechanism was analyzed. It seems that under these conditions sulfonamides were hydrolyzed to form sulfonic acid derivatives and the final dehydration step was not obtained to lead to the following structure (Figure 5.6).

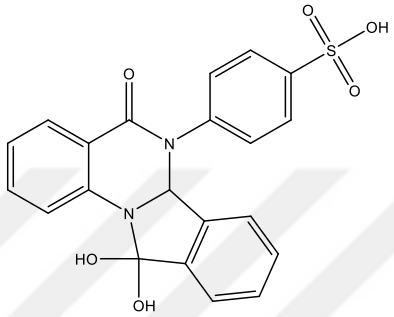
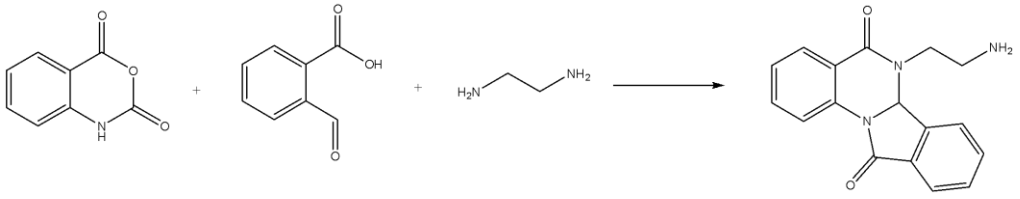


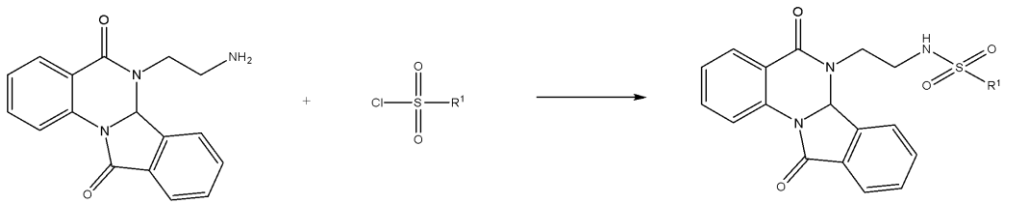
Figure 5.6. The obtained product at the first approach

All the conducted experiments have shown that these reaction conditions were not suitable for the synthesis of isoindoloquinazoline ring structures *via* aryl sulfonamide compounds. For this reason, another method was developed for the generation of sulfonamide substituted isoindoloquinazoline derivatives.

5.1.3. Isoindoloquinaqzoline heterocycle synthesis using an ethylene diamine spacer

As the synthesis of the isoindoloquinazoline cycle was not achieved using complex amines, we decided to first synthesize the heterocycle in the presence of a simple amine, the ethylenediamine molecule. In addition to the generation of the heterocycle, such a structure will allow the introduction of a spacer with a terminal amine function that will be later derivatized with a series of sulfonyl chlorides in order to give sulfonamide structures (Scheme 5.3).

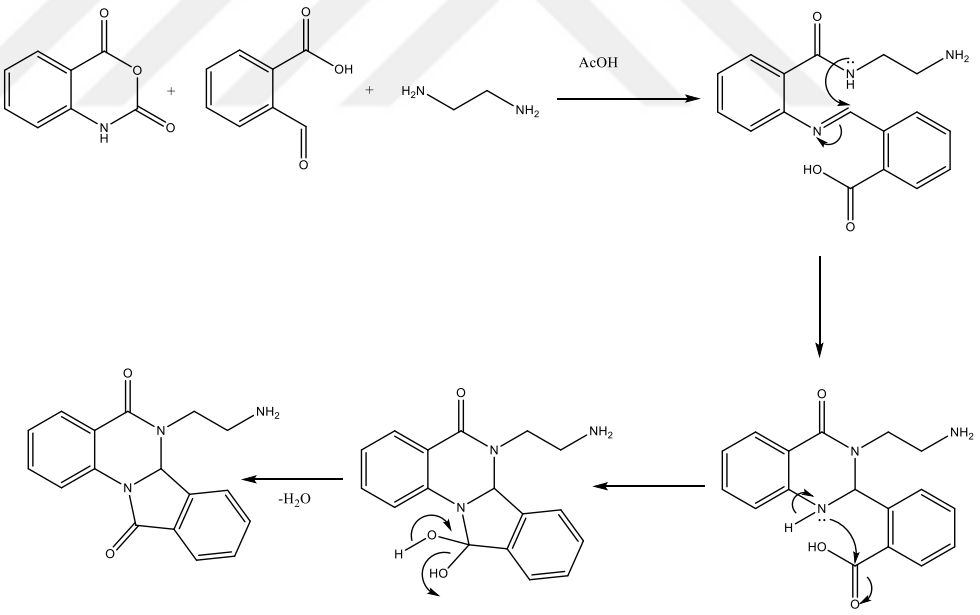




Scheme 5.3. The first and second reaction steps to obtain sulfonamide derivatives

5.1.4. Synthesis of the starting compound: 6-(2-aminoethyl)-6,6a-dihydroisoindolo[2,1-a]quinazoline-5,11-dione (1)

A plausible reaction mechanism for the formation of 6-(2-aminoethyl)-6,6a-dihydroisoindolo[2,1-a]quinazoline-5,11-diones is shown in the following scheme (Scheme 5.4)



Scheme 5.4. Reaction mechanism for the formation of 6-(2-aminoethyl)-6,6a-dihydroisoindolo[2,1-a]quinazoline-5,11-dione

The reaction was performed in different solvents. With freshly distilled dichloromethane, distilled water, or dry dimethylsulfoxide the product didn't occur. The

best results were obtained in acetic acid as solvent. This result can indicate the importance of the acidic reaction medium caused by acetic acid.

The product obtained with acetic acid as a solvent was analyzed by the ^{1}H-NMR and LC-MS. Data obtained showed a singlet peak at 6.59 ppm corresponding to the heterocyclic proton and also peaks at 4.18-3.76 ppm, 3.47-3.42 ppm, 2.76-2.69 ppm and 2.64-2.59 ppm for the ethylene spacer, indicating the ring closure reaction (Figure 5.7). In addition to the carbonyl and aromatic peaks on the ^{13}C-NMR, the peak at 70.3 ppm confirmed the presence of the ring system as well as the peaks of ethylene amine structure at 43.5 and 22.5 ppm. Furthermore, the LC-MS analysis indicates the M+1 -peak at the value of m/z=294.5 (Figure 5.9).

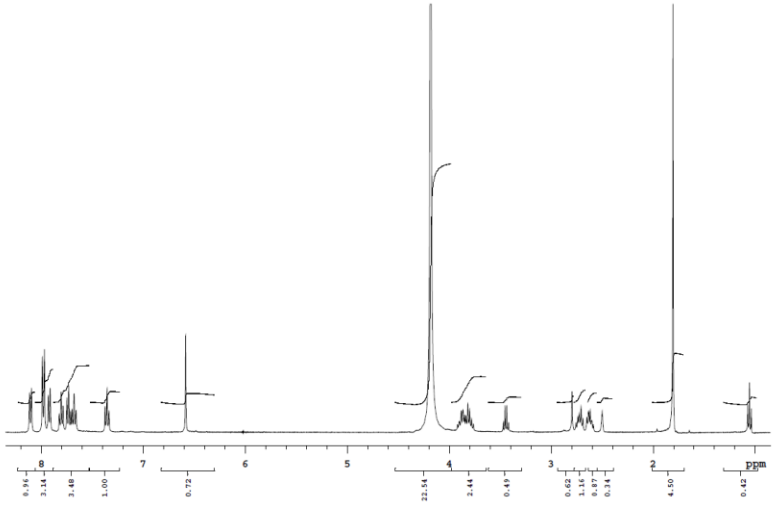


Figure 5.7. The characteristic ^{1}H-NMR spectrum of compound **1**.

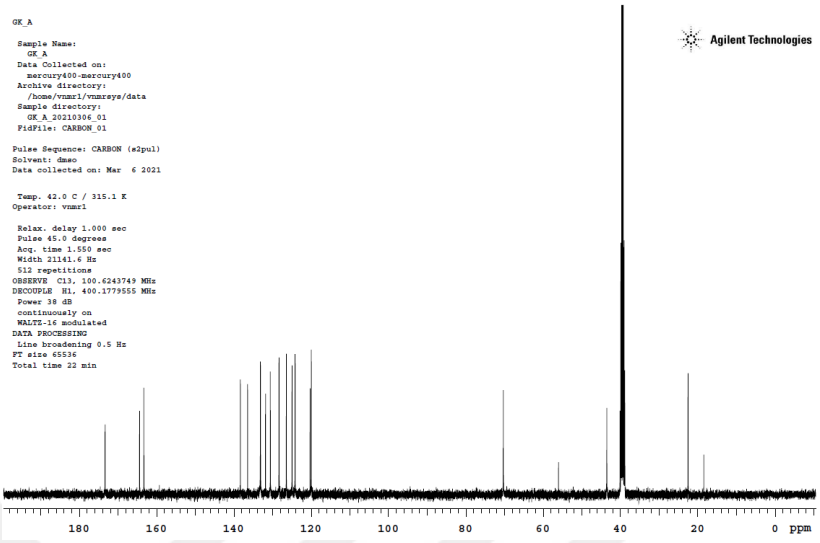


Figure 5.8. The characteristic ^{13}C-NMR spectrum of compound **1**.

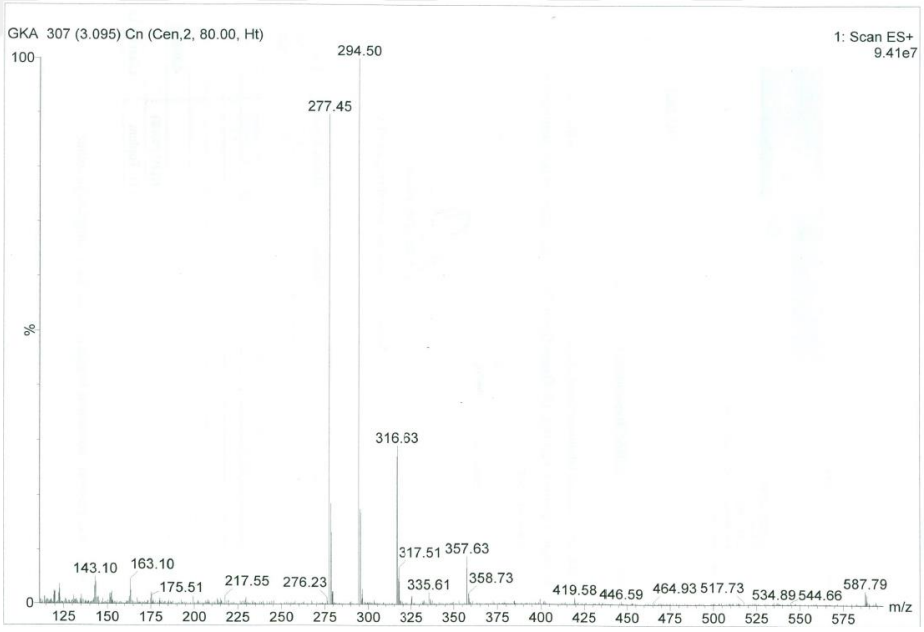
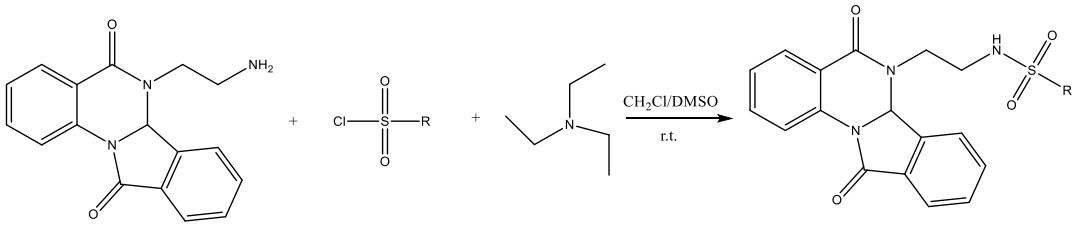


Figure 5.9. The mass-spectrum of compound **1**

5.1.5. Synthesis of sulfonamide derivatives

In order to get isoindolo[2,1-a]quinazoline-sulfonoamide derivatives a benzenesulfonyl chloride compound was introduced to the primary amine side of the starting molecule. The primary amine first reacts with benzenesulfonyl chloride and following that an addition-elimination reaction occurs on the highly electrophilic sulfonyl chloride derivative. For that purpose, the starting amine compound, triethylamine, and a benzenesulfonyl chloride compound were reacted under a nitrogen atmosphere in a solvent system like anhydrous CH_2Cl_2/anhydrous DMSO (10:1). Finally, the obtained products were purified and analyzed by ^{1}H-NMR and LC-MS. The singlet proton peak at 6.53 ppm, the aromatic proton peak in the aromatic region, and also the proton peaks of sulfonyl chloride have shown that the ring structure of the starting compound was preserved and the expected sulfonyl amide functionality was added, which is verified also with LC-MS spectrum with a corresponding M^+-peak. In that way, the compounds **2-6** were synthesized (Scheme 5.5).



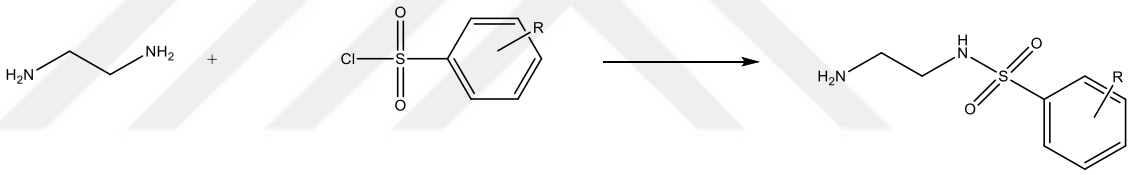
Scheme 5.5. Synthesis pathway of the compounds **2-6**

Analysis of the synthesis pathways of sulfonamide substituted isoindoloquinazoline derivatives, which were tried to be synthesized and obtained with ethylenediamine, showed that complex mixtures were formed with some sulfonyl chloride compounds, and causes low yields.

Table 5.3. The yields of compounds **2-6**

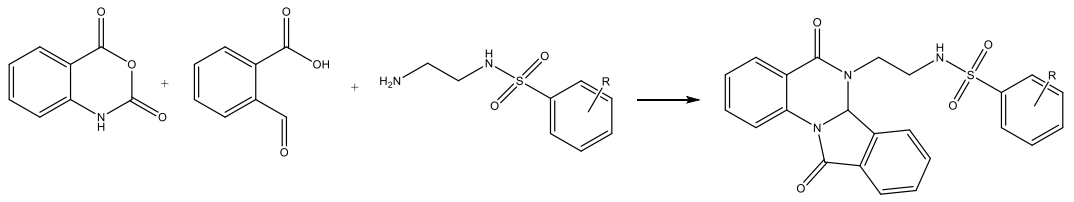
	Sulfonyl chloride	Yield [%]
2	2,5-Dichlorobenzenesulfonyl chloride	16
3	2,4,5-Trichlorobenzenesulfonyl chloride	89
4	3,4-Dichlorobenzenesulfonyl chloride	22
5	3-(Trifluoromethyl)benzenesulfonyl chloride	21
6	2-(Trifluoromethyl)benzenesulfonyl chloride	68

Therefore, a new synthetic method was used to obtain higher yields. Through this method, ethylenediamine was reacted with sulfonyl chloride to form sulfonamide derivative, which will react afterward with isatoic anhydride and formylbenzoic acid to form the desired products (Scheme 5.6).



Scheme 5.6. The new synthesis method with ethylenediamine and sulfonyl chloride

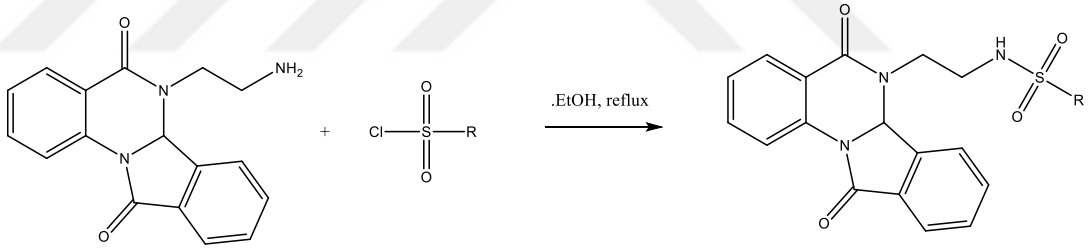
According to this method, 4-iodo-, 4-bromo-, 2-(trifluoromethyl)benzenesufonyl chloride and N-acetylbenzenesulfonyl chloride compounds were reacted with ethylenediamine in the basic medium in anhydrous dichloromethane. (Scheme 5.7) The resulting amine compounds were used for the second step, but target products were not obtained.



Scheme 5.7. The second step with isatoic anhydride and formylbenzoic acid

Thus, another synthetic method was developed to synthesize the compounds with satisfactory yields. Hereby, ethanol was used as solvent and the reaction was refluxed for one night and further stirred for an additional week before being directly evaporated. (Scheme 5.8)

The pure products were obtained by crystallization in H_2O. This strategy allowed the successful synthesis of compounds **7-12**. (Table 5.4).



Scheme 5.8. The synthetic method for the compounds **7-12**

Table 5.4. The yields of compounds **7-12**

	Sulfonyl chloride	**Yield [%]**
7	4-Fluorobenzenesulfonyl chloride	29
8	2-Fluorobenzenesulfonyl chloride	26
9	N-Acetylbenzenesulfonyl chloride	47
10	2,5-Dichlorobenzenesulfonyl chloride	44
11	4-Bromobenzenesulfonyl chloride	23
12	Thiophene sulfonyl chloride	27

For the exact interpretation of the spectral data of these compounds, the obtained spectra were compared with each other and with the spectrum of starting amine. The analysis showed that the compounds in the table above were pure and the detailed analysis is given below for compound **9** as an instance. The protons and carbons of the isoindoloquinazoline ring are named as a,b,c,… and those of the compound with sulfonamide functionalities are named as a', b', c', … (Figure 5.10)

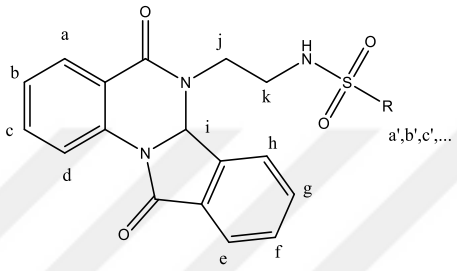


Figure 5.10. Isoindoloquinazoline derivatives **2-6**

In the ^{1}H-NMR spectra for all the compounds the H_i peak occurs at 6.53 ppm and is a singlet, furthermore, the proton peaks of ethylene compounds that are not equivalent are observed as multiplets at around 3.75 ppm for H_k and 3.0 ppm for H_j. Also, in addition to the peaks of eight aromatic protons of the isoindoloquinazoline ring, the aromatic protons of the sulfonamide rings have occurred in the same area. Concerning to compound **9**, the aromatic protons expected as doublets because of the p-disubstitution are observed in the aromatic area overlaying the other aromatic signals with a $J_{coupling}$ value of 8.85 Hz. Besides, the signal of the methyl group (H_d) of the compound **9** is at 2.07 ppm as a singlet with 3H.

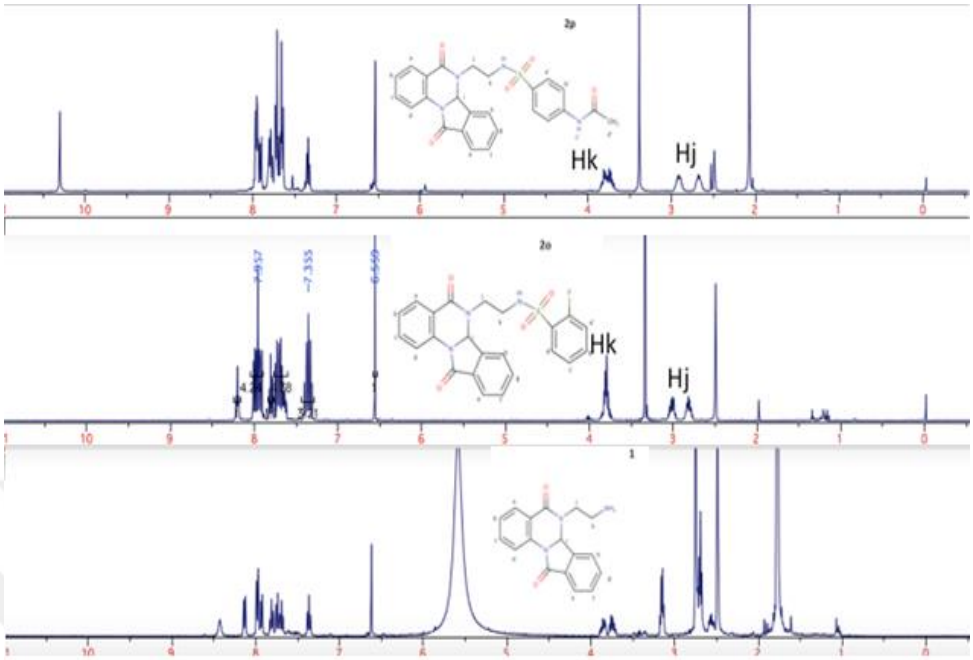


Figure 5.11. The comparative analysis of ^{1}H-NMR spectra of the compounds **8** and **9** with compound **1**

Concerning the ^{13}C-NMR spectrum, the signal of the aliphatic carbon at isoindoloquinazoline ring occurs about 70.6 ppm and those from ethylene compounds are at 42.9 ppm and 40.7 ppm. Furthermore, at 164.5 and 163.6 ppm the signals of the two carbonyl functionalities can be seen. Additionally, the aromatic carbons at the isoindoloquinazoline ring are obtained at 138.5, 136.9, 132.4, 131.1, 128.8, 126.6, 125.4, 124.5, 120.5, and 120.4 ppm. (Figure 5.13)

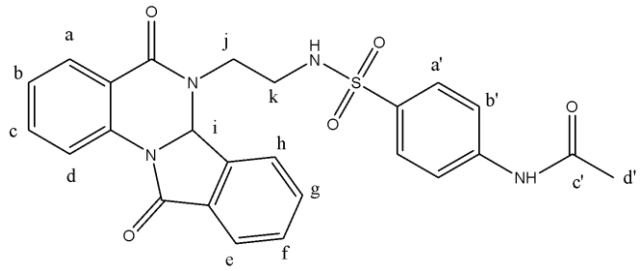


Figure 5.12. The molecular structure of the compound **9**

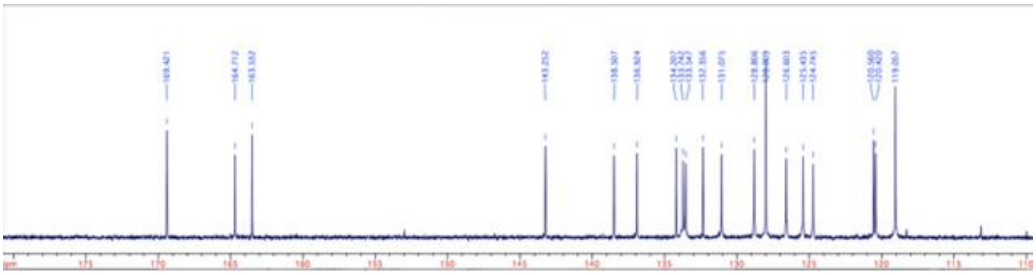
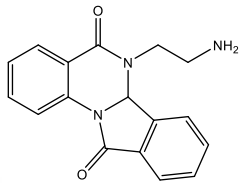
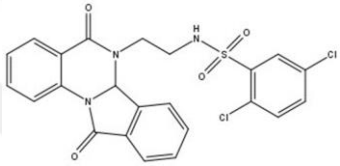
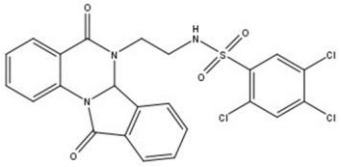
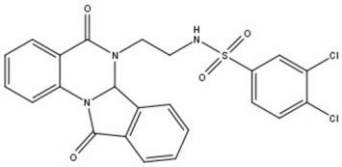
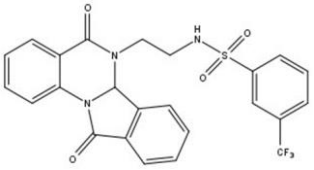
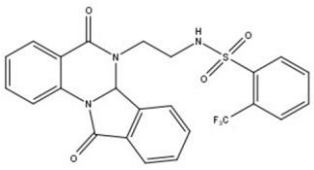


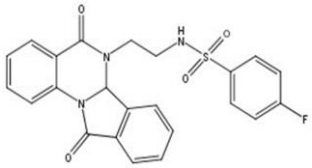
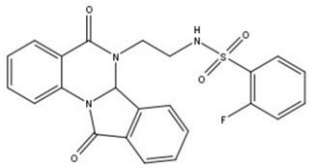
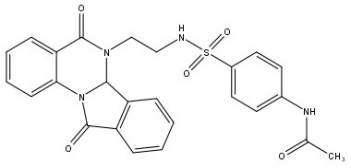
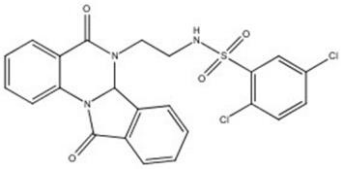
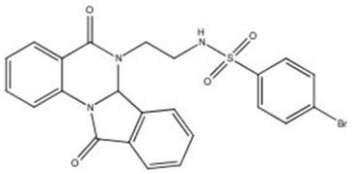
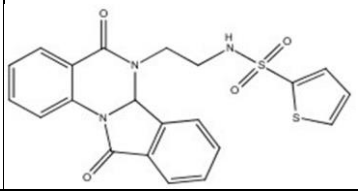
Figure 5.13. The ^{13}C-NMR spectrum of the compound **9**

Also, for the compound **9**, an extra signal appears at 169.4 ppm for the carbon of the carbonyl group of the acetamide group and the sp2 carbon peak next to the nitrogen is at 134.2 ppm. The a' carbons are obtained at 128 ppm, whereas the b' carbons are at 119 ppm. In addition to that, the carbon of the methyl group at acetyl functionality has occurred at 24.6 ppm.

To sum up, 11 novel isoindoloquinazoline molecules were successfully synthesized (Table 5.5) to be evaluated for their biological activity.

Table 5.5. Isoindoloquinazoline derivatives

#	Compound
1	
2	
3	
4	
5	
6	

#	Compound
7	
8	
9	
10	
11	
12	

5.2. Biological evaluation

In order to evaluate the biological activity of the synthesized isoindoloquinazoline derivatives their ability to scavenge free radicals was evaluated. Indeed, as the interrelationship between cancer and inflammation was mainly attributed to the long-term impact of the accumulation of reactive oxygen species, we chose to analyze the radical scavenging property of our molecules using the 2,2-diphenyl-1-picrylhydrazyl DPPH radical scavenging assay and also the cupric reducing antioxidant capacity (CUPRAC).

5.2.1. The DPPH assay

The DPPH assay consists of the determination of the capacity of a compound to reduce the stable free DPPH radical into its corresponding hydrazine, a reaction that mimics the capacity of a molecule to scavenge reactive oxygen species that are generated during an inflammative response in a tissue. The radical scavenging activity (RSA) of a molecule can be monitored by absorbance as the free DPPH absorbs at 517 nm whereas the reduced hydrazine does not give any signal at this wavelength. Thus, detecting a decrease in the absorbance of a solution where in addition to the free DPPH radical the isoindoloquinazoline molecule will be added will point out the ROS scavenging property of the evaluated heterocycle.

Because of the poor water solubility of the molecules, the assay was performed in DMSO. Butylated hydroxytoluene (BHT) was used as the reference molecule to determine the RSA of the synthesized isoindoloquinazolines (**2-12**). (Table 5.6) Compounds were evaluated at a concentration of 100 µM except for compound **3** that was tested at 50 µM because of its poor solubility even in DMSO

Table 5.6. DDPH Radical Scavenging

Compound	Substituent	DPPH RSA%
2	2,6-dichlorophenyl	29.40 ± 1.10
3	2,5,6-trichlorophenyl	29.09 ± 0.66
4	3,4-dichlorophenyl	28.47 ± 1.10
5	3-trifluoromethylphenyl	31.34 ± 0.11
6	2-trifluoromethylphenyl	35.14 ± 0.11
7	4-fluorophenyl	7.21 ± 0.51
8	2-fluorophenyl	4.51 ± 0.18
9	4-acetamidophenyl	1.63 ± 1.14
10	3,6-dichlorophenyl	14.20 ± 1.04
11	4-bromophenyl	36.23 ± 1.11
12	2-thiophenyl	37.37 ± 1.58

*Compound 3 was tested at 50 µM due to low solubility in DMSO while the others were tested at 100 µM. RSA: radical scavenging activity. BHT (0.015 mg-1 mg/mL) was used as reference for DPPH RSA.

Results indicate poor RSA for the isoindoloquinazoline structures as the RSA did not exceed 37% for these structures. The best activities were obtained for compounds **11** and **12** with RSA of 36.2 and 37.4% respectively, these compounds presenting as substituents a 4-bromophenyl for compound **11** and a 2-thiophenyl moiety for compound **12**. RSA values obtained for the other evaluated structures are not that different from the one obtained for these two compounds except for compounds **7**, **8,** and **9** that were found to be very poor radical scavengers with an RSA value of 7.2, 4.5, and 1.6% respectively. Interestingly two of these structures were found to present a fluor atom on their aromatic ring while the least reactive molecule was the one with the bulkiest substituent (4-acetamido).

This DPPH assay demonstrated thus that the 11 synthesized sulfonamide substituted isoindoloquinazoline derivatives do not to exhibit enough RSA.

5.2.2. The CUPRAC assay

As we were not able to detect a conclusive RSA with the DPPH assay, we also wanted to evaluate our compounds using a different assay, the CUPRAC assay. This method relies on the detection of the Cu(I)-neocuproine complex obtained from the reduction of the Cu(II)-neocuproine complex in the presence of a reducing agent, the eventual radical scavenger.

The compounds were tested in a range of concentrations (6.25-5 mg/mL) and results were given as ascorbic acid equivalent (AAE) (Table 5.7).

Best reducing activities were obtained for compounds **11** and **12**, a result consistent with the one found with the DPPH assay. Interestingly compounds **7**, **8,** and **9** were found to be similarly active with the other derivatives with this assay indicating that reducing capacity can this time be attributed to the isoindoloquinazoline heterocycle rather than the sulfonamide part of the molecules. Substitution of the 4th position of the phenyl moiety with a bulky group such as a bromine atom or an acetamido group seem also to enhance this activity.

Table 5.7. Cupper Reducing Activity (CUPRAC) Assay

Compound	Substituent	mg AAE/g compound
2	2,6-dichlorophenyl	34.52 ± 0.38
3	2,5,6-trichlorophenyl	37.90 ± 0.40
4	3,4-dichlorophenyl	37.62 ± 0.40
5	3-trifluoromethylphenyl	39.09 ± 0.40
6	2-trifluoromethylphenyl	50.62 ± 0.41
7	4-fluorophenyl	39.09 ± 0.35
8	2-fluorophenyl	36.42 ± 0.48
9	4-acetamidophenyl	50.32 ± 0.36
10	3,6-dichlorophenyl	29.79 ± 0.39
11	4-bromophenyl	77.06 ± 0.45
12	2-thiophenyl	76.63 ± 0.45

*Compounds were tested between 6.25-5 mg/mL. Ascorbic acid (6.25-200 µg/mL) was used as a reference for the CUPRAC assay.

These evaluations both indicate that the eleven synthesized isoindoloquinazoline compounds present modest RSA and that any anti-inflammatory or anticancer activity that may be later revealed should only be very poorly related to the scavenging of reactive oxygen species.

In this study, eleven novel sulfonamide substituted isoindoloquinazoline derivatives were synthesized with the moderate to good yields. As cancer development was related to inflammative sites where an accumulation of reactive oxygen species occurs, the ability of these species to scavenge radicals was evaluated with the DPPH and CUPRAC assays. Results obtained from these two assays were consistent and indicated the best activity with compounds **11** and **12** corresponding to structures presenting a bulky substitution on the 4th

position of their phenyl ring (a bromine atom and an acetamido group respectively). These structures may also be further analyzed for their anti-inflammatory and anticancer activity via other procedures such as a TNF-α inhibition assay or evaluation of cytotoxicity on cancer cell lines.

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