SYNTHESIS OF 1,2,3-TRIAZOLE SUBSTITUTED AZACOUMARIN DERIVATIVES AS POTENTIAL ANTIPROLIFERATIVE COMPOUNDS

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İZMİR

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ABSTRACT

SYNTHESIS OF 1,2,3-TRIAZOLE SUBSTITUTED AZACOUMARIN DERIVATIVES AS POTENTIAL ANTIPROLIFERATIVE COMPOUNDS

Cancer is one of the deadly diseases that affects millions of people every year and causes death. Although studies toward its treatment are very promising, they are not sufficient. Therefore, the need for new and powerful molecules with less side effects is increasing day by day. 1-Azacoumarin derivatives are molecules whose potential biological activity has just begun to be understood, but not enough research has been done on them. 1,2,3-Triazole structures, on the other hand, are a very important family of molecules in some drugs, whose biological effects have been known for many years. It is known that they have important roles in cancer-preventing mechanisms in various types of cancer.

In this study, two different 1,2,3-triazole 1-azacoumarin derivatives were tried to be synthesized. For structure **149**, it was aimed to form yinon first and then formation of 1,2,3-triazole was studied but failed. Afterwards, the emphasis was placed on the production of 1-azacoumarin, and then click chemistry experiments were carried out in the presence of copper (I) catalyst. Finally, click chemistry studies were tested in the presence of a nickel catalyst for structure **150**. Triazole formation experiments were carried out by click chemistry in the presence of copper catalyst after 1-azacoumarin was obtained.

ÖZET

POTANSİYEL ANTİPROLİFERATİF ETKİYE SAHİP 1,2,3-TRİAZOL SÜBSTİTÜELİ AZOKUMARİN TÜREVLERİNİN SENTEZİ

Kanser her yıl milyonlarca insanı etkileyen ve ölümüne sebep olan başlıca hastalıklardan biridir. Tedavisine yönelik çalışmalar oldukça umut vaat edici olsa da yeterli düzeyde değildir. Bu bağlamda yeni daha az yan etkiye sahip ve güçlü moleküllere olan ihtiyaç her geçen gün artmaktadır. 1-Azakumarin türevlerinin biyolojik aktivite potansiyelleri yeni anlaşılmaya başlanmış, ancak üzerinde yeterince araştırma yapılmamış moleküllerdir. 1,2,3-Triazol yapıları ise uzun yıllardan beri çeşitli biyolojik etkileri bilinen, bazı ilaçların içerinde yer alan oldukça önemli bir molekül ailesidir. Çeşitli kanser türlerinde kanser yapıcı ve kanser engelleyici mekanizmalarda önemli görevleri olduğu bilinmektedir.

Bu çalışmada hedeflenen iki ayrı 1,2,3-triazol 1-azakumarin türevi sentezlenmeye çalışılmıştır. **149** Numaralı yapı için önce yinon oluşturup sonrasında 1,2,3-triazol oluşturmak hedeflenmiş ancak başarısız olunmuştur. Sonrasında 1-azakumarin elde edilmesine ağırlık verilmiş ve arkasından bakır (I) katalizörlüğünde klik kimyası denemeleri yapılmıştır. Son olarak ise klik kimyası çalışmaları nikel katalizörü varlığında denenmiştir. **150** Numaralı yapı için, 1-azakumarin elde edilmesinden sonra bakır katalizörü varlığında, klik kimyası ile triazol oluşturma denemeleri yapılmıştır.

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ABBREVATIONS

DNA	Deoxyribonucleic Acid
DMSO	Dimethyl Sulfoxide
DMF	Dimethylformamide
THF	Tetrahydrofuran
MW	Microwave
p-TsOH	p-Toluenesulfonic Acid
TsN_3	p-Toluene Sulfonyl Azide
CuAAC	Copper(I)-catalyzed Azide-Alkyne Cycloaddition
NiAAC	Nickel Catalyzed Azide-Alkyne Cycloaddition
RuAAC	Ruthenium Azide-Alkyne Cycloaddition
rt	Room Temperature
ATP	Adenosine Triphosphate
MOLT-4	Lymphoblastic Leukemia
MCF-7	Breast Cancer
HepG2	Hepatoma Carcinoma
CEM	T-lymphoblastic Carcinoma
HeLa	Cervical Carcinoma
A549	Lung Cancer
MIA-PaCa-2	Pancreatic Cancer
HT-29	Colon Cancer
DU-145	Prostate Cancer

s	Singlet
d	Doublet
t	Triplet
m	Multiplet
dd	Doublet of Doublet

CHAPTER 1

INTRODUCTION

Cancer is the general name of more than 100 different diseases caused by cells that grow and spread uncontrollably due to damage to cell DNA. Cancer, whose history dates to ancient times, is one of the most common diseases, along with cardiovascular diseases, with the advancement of medicine and longer life spans. Cancer is the leading cause of death in developed countries. It is also in second place in developing countries.[1] Radiation, some chemicals, living carcinogens (viruses, parasites, bacteria and fungi), alcohol and smoking, high body mass index, some hormones and heredity can be counted as the main causes of cancer.

Surgery, radiotherapy, chemotherapy, immunotherapy and stem cell treatments are used in cancer treatment. Small molecule drugs have been used as an adjunct to chemotherapy in targeted therapies. The general purpose may be to inhibit cancer formation mechanisms, prevent cancerous cells' growth, or kill cancer cells. However, because the disease is very systematic and challenging, there are cases where even these treatment methods and their combinations cannot be satisfactory to cure the disease. Therefore, finding new chemotherapic drugs or new cytotoxic molecules can prevent millions of deaths every year.

1,2,3-Triazoles

5-Membered heterocyclic azole structures can be broadly divided into three classes (Figure 1.1). In the nitrogen class, heterocyclic compounds with 3 nitrogen atoms called as triazoles. They have 2 tautomeric forms, 1,2,3-triazole and 1,2,4-triazole, depending on where the nitrogen is attached.[2] Triazole structures have been used for many years as pioneer structures in many fields.

Triazole nucleus is found in the structure of many biological active classes such as anticancer[3], antiviral[4], anti-inflammatory[5], antiepileptic[6], antidepressant[7], antidiabetic[8], antianxiety[9], antitubercular[10], antibacterial[11], antifungal[12], antioxidant[13]. Also, some triazole compounds are commercially available as medication (Figure-1.2).[14]

Until today, studies show that 1,2,4-triazole is an essential and more promising antiproliferative effect, but as the results of new studies increase, 1,2,3-triazole will also prove to be quite essential as 1,2,4-triazole.[15]

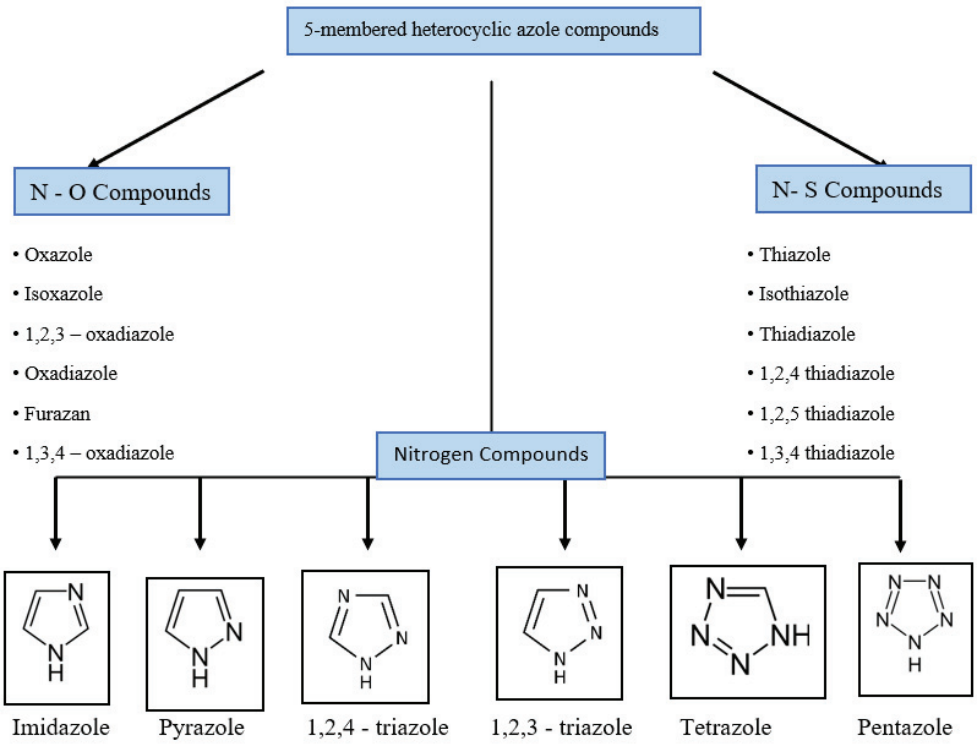


Figure 1.1. 5-membered heterocyclic azole compounds

The reason why triazole structures have such an effective drug potential, they can bind enzymes and receptors via weak interactions like hydrogen bonds, ion-dipole, hydrophobic effect, and Van der Waals forces.[16]

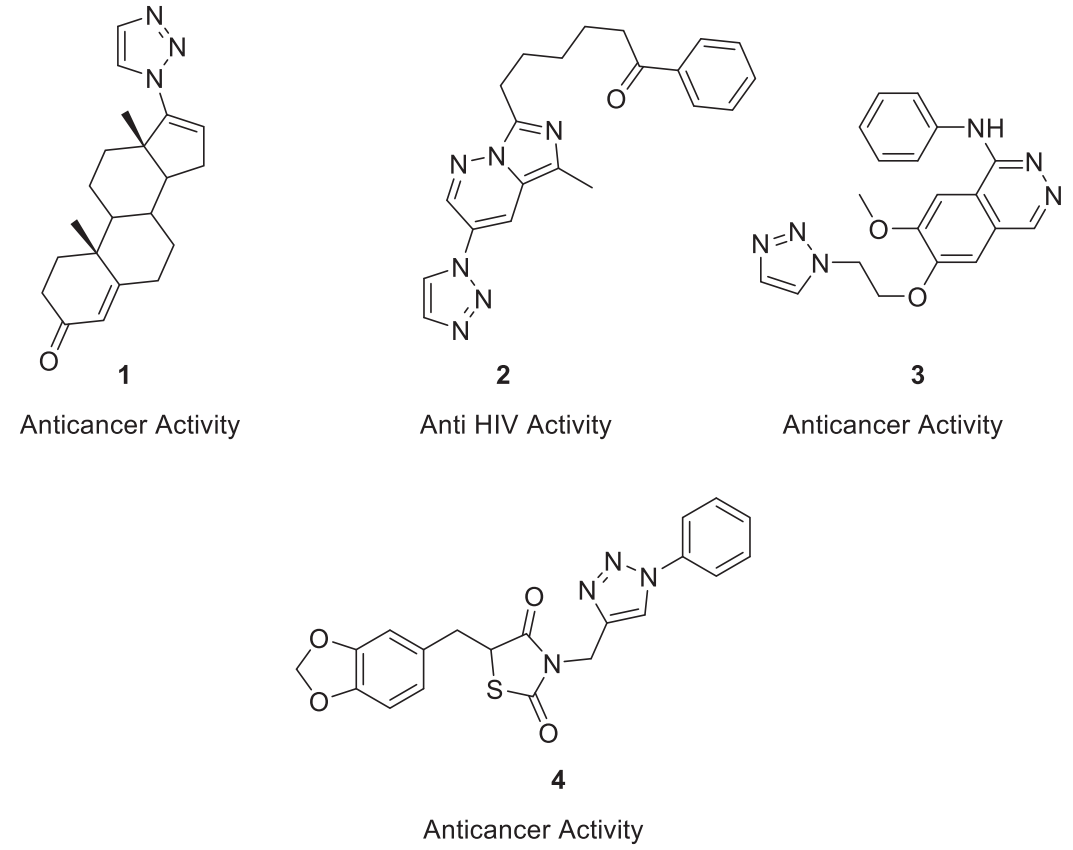


Figure 1.2. Examples of 1,2,3-triazole containing compounds as commercial drugs and bioactive molecules

As studies on triazole derivatives increase, the clinical and therapeutic properties will become more evident. Thus, there will be treatments with less toxic effects, reduced side effects, high bioavailability, and less resistance.[14]

1.1.1. Metal Free Synthesis Methods of 1,2,3-Triazole Derivatives

Studies on the synthesis of 1,2,3-triazole derivatives can be divided into metal-free and metal-catalyzed methods. Both classes contain essential and groundbreaking reactions. Paixao – Alves and coworkers synthesized selenium-containing 1,2,3-triazoles via β-enaminone azide cycloaddition. As a result of their optimization studies, it was observed that the reaction did not proceed without an amine catalyst, and it was

observed that the yield was low in the presence of proline, pyrrolidine, morpholine and triethylamine, and the best results were obtained in the presence of diethylamine. Their reaction works well β-keto acetates (**6**) containing alkyl, benzyl and propargyl groups. In addition, it was determined that the experiments made in the microwave shorten the time.[17] (Figure 1.3)

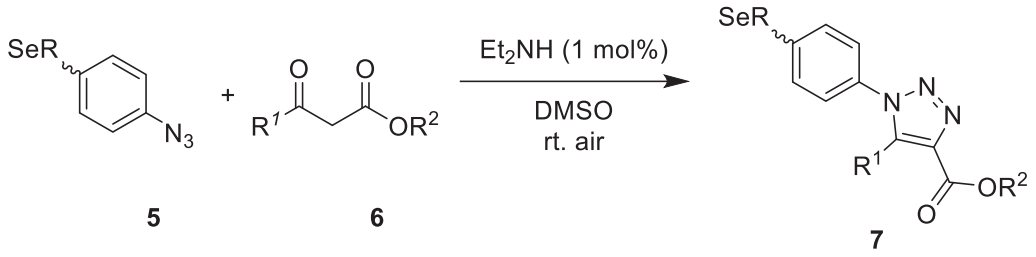


Figure 1.3. Cycloaddition of azido phenyl selenium compounds **5** with β-ketoesters **6**

Similarly, in another base catalyzed reaction used for Azide Zwitterion Cycloadditions of α,β–unsaturated esters with azides by Li and Wang. They used 1,8-diazabicyclo[5.4.0]undec-7-ene as Lewis base. In this type of reaction, the azide group is aryl, hetoaryl or alkyl linked while the ester side can be COR, CO_2R, CHO, CN, $CONR_2$ etc. Chloroform solvent showed higher yields than DMSO.[18] (Figure 1.4)

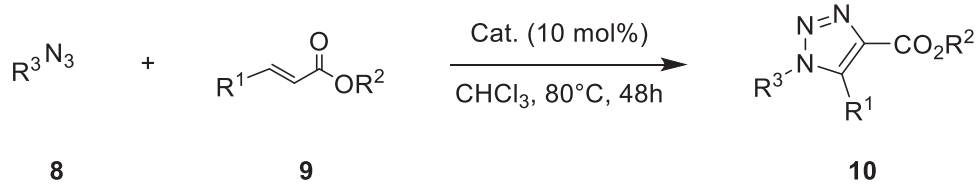
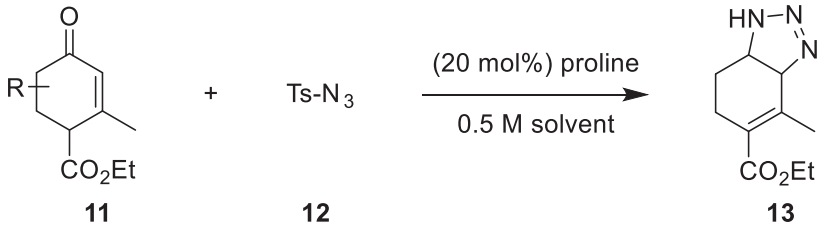


Figure 1.4. Cycloaddition of α,β–unsaturated esters with azides

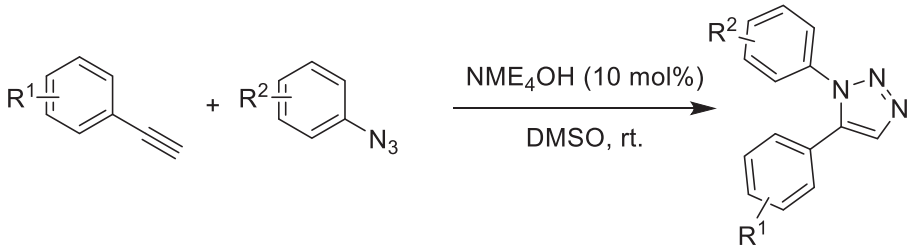
Ramachary and coworkers; synthesized NH-1,2,3-triazole by amino acid catalyzed cascade [3+2] cycloaddition from Hagemann's ester. They showed that amino acid-catalyzed cascade reactions depend on solvents. These reactions give good or moderately yields in aprotic polar solvents like; NMP, DMSO or DMF. When acetonitrile, THF or H_2O were used as solvent the yield of the products were little in quantity.[19] (Figure 1.5)



11 12 13

Figure 1.5. Amino acid catalyzed cascade [3+2] cycloaddition

1,5-Diarlysubstituted 1,2,3-triazoles are synthesized by Kwork and coworkers by base catalyzed reactions in DMSO. Since the alkene is highly acidic in DMSO, the reaction was performed successful at room temperature.[20] (Figure 1.6)



14 15 16

R^1 = Alkyl, OMe, F, Br, NO_2, CN
R^2 = Alkyl, OMe, Br, NO_2, CO_2R

Figure 1.6. Base catalyzed 1,5-diarlysubstituted 1,2,3-triazole derivatives

While previously working only with activated ketones, Belkheira and coworkers successfully synthesized 1,2,3-triazole structures from unactivated ketones. In the optimization studies, it has been observed that proline works appropriately. Higher yields were obtained with MW and sealed tube experiments.[21] (Figure 1.7)

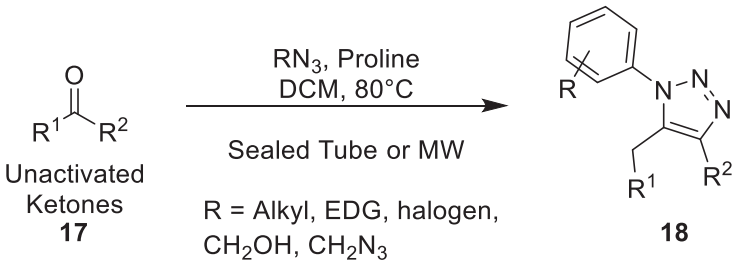


Figure 1.7. Amino acid catalyzed 1,2,3-triazoles from unactivated ketones

Paixao and coworkers have reported the synthesis of 1,4-substituted 1,2,3-triazole with the assistance of active alkylidenes. It was observed that the synthesis method they established as a one-pot strategy is very suitable for aldehydes, malononitrile, phenyl azide to produce 1,2,3-triazoles in high yields. They also used their strategy to synthesize optically active triazoles and obtained a high stereoselectivity.[22] (Figure 1.8)

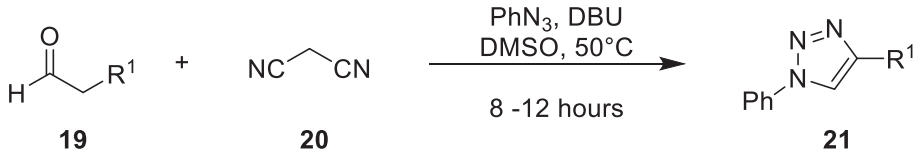


Figure 1.8. One pot strategy for the regioselective synthesis of 1,4-disubstituted 1,2,3-triazole derivatives

In another one-pot experiment by Lin, aryl azides (**23**) and β- or α-vinyl bromides (**22**) were used, and 1,5-disubstituted triazoles were synthesized under base-catalyzed. Because of the expensiveness and the difficulties of using the terminal alkynes, they were synthesized in the experimental environment and aimed to be used directly in cyclization. Their work reveals that the selection of the appropriate base and solvent is vital.[23] (Figure 1.9)

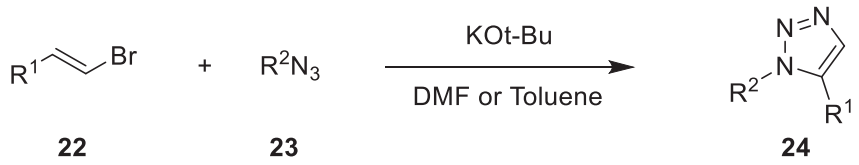


Figure 1.9. Synthesis of base mediated one pot 1,5-disubstituted 1,2,3-triazole derivatives

Guan and coworkers discovered that p-TsOH is a vital additive in 1,3-dipolar cycloaddition. They synthesized rapidly the N1-substituted 1,2,3-triazole in high yield, which is difficult to synthesize with Huisgen 1,3-dipolar cycloaddition and Cu-Ru catalyzed cycloaddition. Nitroolefins (**25**) with electron neutral or donating groups on

aryl rings (methyl, methoxyl, hydroxyl) give excellent yields. Nitroolefins with electron with-drawing groups on aryl rings (fluoro, chloro, bromo and nitro) give moderate yields but aliphatic nitroolefins inactive in this reaction.[24] (Figure 1.10)

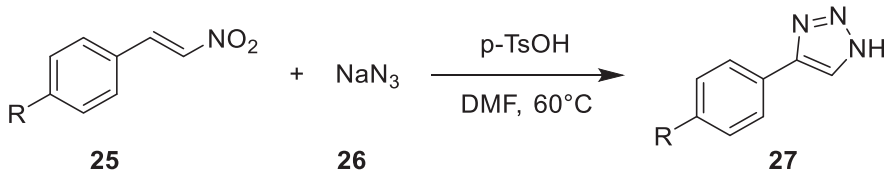


Figure 1.10. p-TsOH mediated 1,3-dipolar cycloaddition of nitroolefins (**25**) to azide ion

1,5-Disubstituted 1,2,3-triazoles were synthesized by multicomponent cascade reactions by Cui and coworkers. They assumed that with suitable bases, iminoenol could react with TsN_3 to give diazoimine intermediate, which could cyclize to triazole. Electron donating groups gave higher yields than electron drawing groups in this reaction.[25] (Figure 1.11)

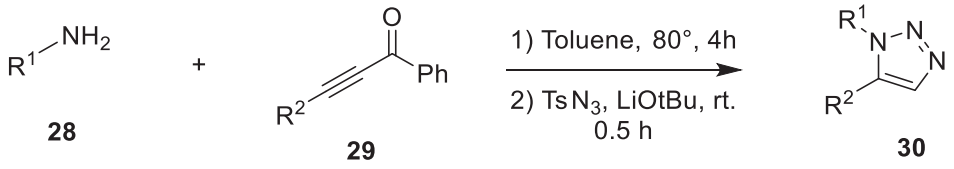


Figure 1.11. Synthesis of multicomponent cascade reaction for 1,5 disubstituted 1,2,3 triazole derivatives

1.1.2. Metal Catalyzed Synthesis Methods of 1,2,3-Triazoles

Huisgen 1,3-dipolar cycloaddition reactions of azides and alkynes require long reaction times and high temperatures. In addition, the probability of regioisomeric mixture formation of 1,4-disubstituted and 1,5-disubstituted triazoles is very high. For these reasons, there is a need for robust, reliable and selective new reactions.

Corona et al. proposed a new inexpensive and highly efficient synthesis method using copper nanoparticles. They used nanoparticles made of charcoal and Cu_2O to catalyze the reaction of various alkynes (**32**) with alkyl azides to produce corresponding 1,2,3-triazoles in high yields in the presence of triethyl amine. Also, they figured out that their catalyst is so robust, after filtration, it gave nearly similar yields. Considering the explosion risks of organic azides, they tried this reaction in one pot and again obtained high yields.[26] (Figure 1.12)

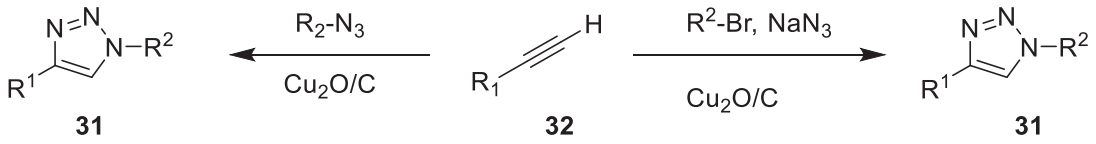


Figure 1.12. Click reactions catalyzed by Cu2O/C to produce1,4-disubstituted 1,2,3-triazole derivatives

Yu Ji and coworkers used Cu_2O as the catalyst to perform cycloaddition reactions of various terminal alkynes (**32**) with alkyl or arylazides (**23**) to produce 1,5-disubstituted 1,2,3-triazoles (**31**) in the presence of water. They found that water is quite essential. Even on a ppm scale, reaction works very well. To understand how water accelerates the reaction, they used D_2O. They observed that deuterium binds to the 5-position of the triazole. Thus, they concluded that water is both an activator for Cu_2O and participates in the reaction. [27] (Figure 1.13)

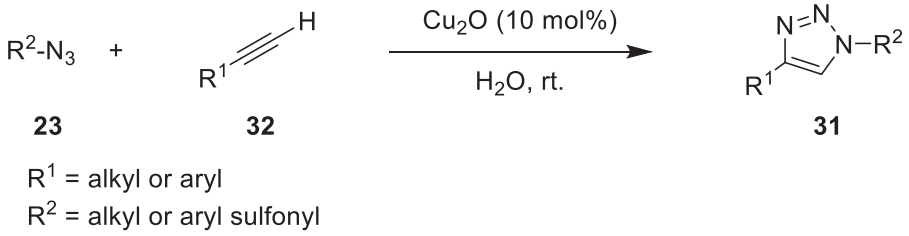


Figure 1.13. Click reactions catalyzed by Cu_2O to prepare 1,4-disubstituted 1,2,3-triazole derivatives

Fukazawa and coworkers used CuCl as a cupper catalyst for the cycloaddition of terminal benzyl or arylalkynes (**32a**) to benzyl or mesitylene azides (**23a**), however they observed low yields. In the experiment they carried with 2-ethynylpyridine under the same conditions, they observed 45% efficiency. They thought that the reason for this high yield might be the complex formation of azide, CuCl and 2-ethynylpyridine structures. To prove that they used a catalytic amount of 2-ethynylpyridine in each reaction, and the efficiency increased considerably. Thus, they proved that using 2-ethynylpyridine and CuCl is a catalyst in itself and can be applied to various functional groups and crowded structures. [28] (Figure 1.14)

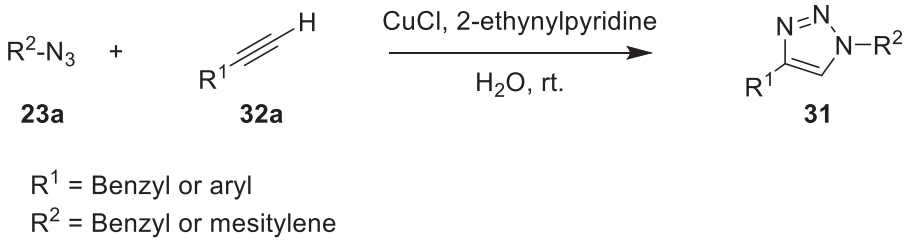


Figure 1.14. 2-ethynylpyridine promoted synthesis of 1,4-disubstituted 1,2,3-triazole derivatives

CuAAC reactions were mostly performed in water. Besides there are examples showing that these reactions can also be performed in DMF and other organic solvents. However, in mechanochemistry, without any solvent, click type reaction can also be achieved. Mack and coworkers reported that the first copper vial catalyzed CuAAC. In this method, the copper vessel was charged with alkyne, azide and copper milling ball. After 15 minutes to 20 hours of milling the components, desired products can be synthesized in high yields without any purification.[29] (Figure 1.15)

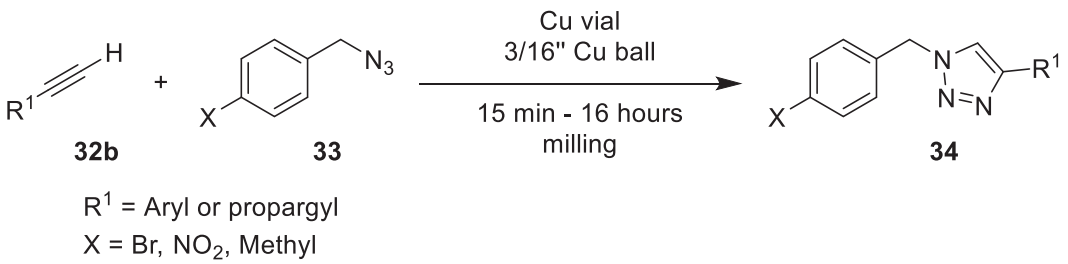


Figure 1.15. Green synthesis of 1,2,3-triazole derivatives by using copper reaction vial

Lee and coworkers used Cyclodextrins as a phase transfer catalyst for the first time in CuAAC reactions. Cyclodextrins are oligosaccharides of D-(+)-glucopyranosyl with a hydrophilic outer surface and a hydrophobic central cavity in their structure. They take the water-insoluble organic structures into the central cavity so enhancing the dissolution properties of poorly soluble organic materials. Water was used as the test solvent because it is ecological and non-toxic.[30] (Figure 1.16)

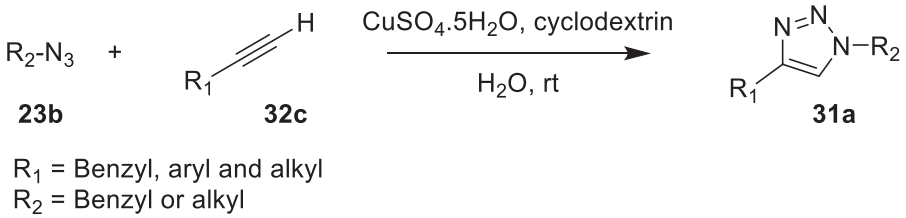


Figure 1.16. Cyclodextrins catalyzed synthesis of 1,4-disubstituted 1,2,3-triazole derivatives

No work has been reported to catalyze the catalytic cycle alone with silver(I). Vemula research group focused on the silver(I) complexes to produce 1,2,3-triazoles (**31a**). They synthesized the crystalline 1:1 complex of the diisopropylamide ligand with silver acetate. In the experiment with silver alone, yields of the reactions were around 10%. Excellent yields were obtained after combining Ag complex (10 mol%) with benzoic acid (10 mol %) and excess azide. [31] (Figure 1.17)

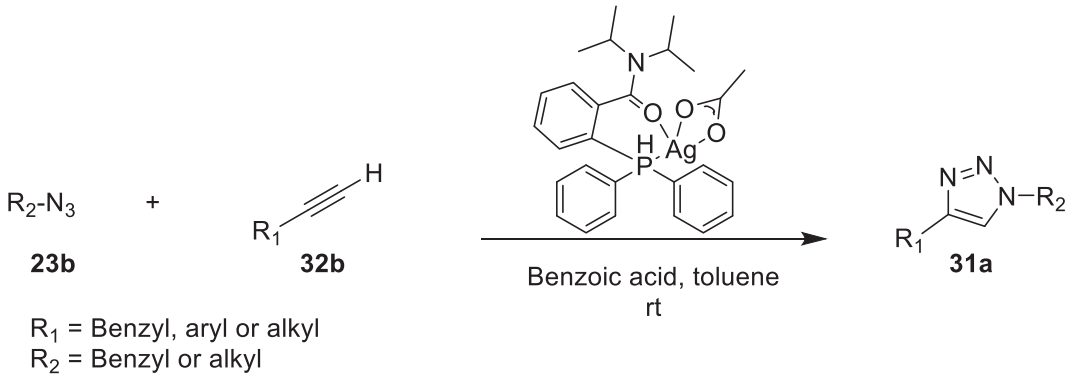
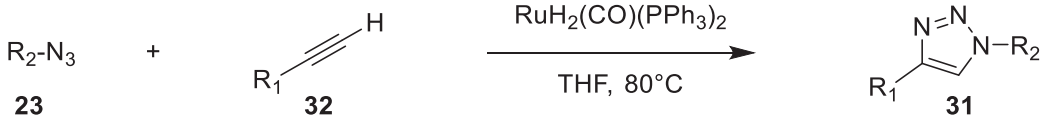


Figure 1.17. First silver catalyzed synthesis of 1,2,3-triazole derivatives

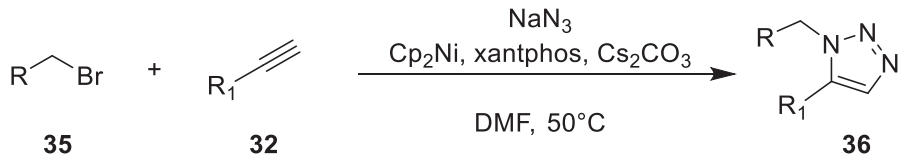
Previously $RuCl_2(PPh_3)_3$, $RuHCl(CO)(PPh_3)_3$, $Ru(OAc)_2(PPh_3)_3$ was reported as catalyzed of cycloaddition reactions of alkane and azides in low yields. With the catalytic amount of $RuH_2(CO)(PPh_3)_2$ in THF at 80°C, Jio and coworkers achieved the cycloaddition of azides - alkynes with high selectivity and excellent yields. They observed that the presence of an electron-withdrawing groups such as acetyl and ester in alkynes has a negative effect on the yield. [32] (Figure 1.18)



23 32 31

Figure 1.18. $RuH_2(CO)(PPh_3)_2$ catalyzed synthesis of 1,2,3-triazole derivatives

Bosch and coworkers achieved the synthesis of 1,5-disubstituted 1,2,3-triazoles by the NiAAC method. Their methodology has the advantage of in situ generations of azides from bromides available with a large diversity. Nickel catalyzed the N-promoted amines, hydroxyls and esters. Benzyl bromides having electron with drawing or electron donating substituents and aromatic or aliphatic alkynes can be used successfully in this method.[33] (Figure 1.19)



35 32 36

Figure 1.19. Nickel catalyzed synthesis of 1,5-disubstituted 1,2,3-triazole derivatives

Huang and coworkers investigated the inorganic nanocubes, octahedral and rhombic dodecahedrons for click chemistry. They observed the formation of the Au-acetylene intermediate, which is the most vital step for click chemistry. They found that RD32, the smallest among the nanoparticles, was the most catalytic active and stereoselective for 1,4-disubstituted 1,2,3-triazole derivatives. And other particles gave

a mixture of 1,4-disubstituted 1,2,3-triazole and 1,5-disubstituted 1,2,3-triazole. Rhombic dodecahedrons are so strong because they thought that the maximum number of unsaturated coordination bonds could be formed between the three surfaces to bind the phenylacetylene species.[34] (Figure 1.20)

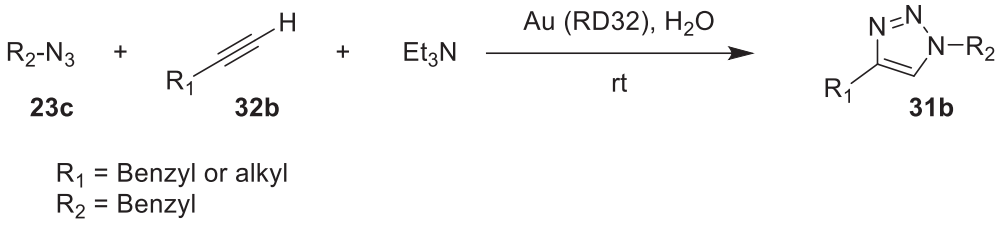


Figure 1.20. Synthesis of 1,4-disubstituted 1,2,3-triazoles with Au nanoparticles

For fully substituted 1,2,3-triazoles, previous methods based on the oxidative coupling, but 1,3-diynes and bistriazoles could be a problem as side products. Even if palladium containing methods were available for this synthesis, it was very costly. Xu and coworkers have achieved a one-pot synthesis of three-component click chemistry catalyzed by copper(I). They trapped the cuprate-triazole intermediate A. Then by binding electrophilic alkynyl on intermediate **A**, three substituted 1,2,3-triazole was synthesized successfully. In the studies carried out to prevent bromoalkyne and terminal alkyne from competing, it was seen that the most appropriate result was achieved by the use of DCE and lithium tert-butoxide.[35] (Figure 1.21)

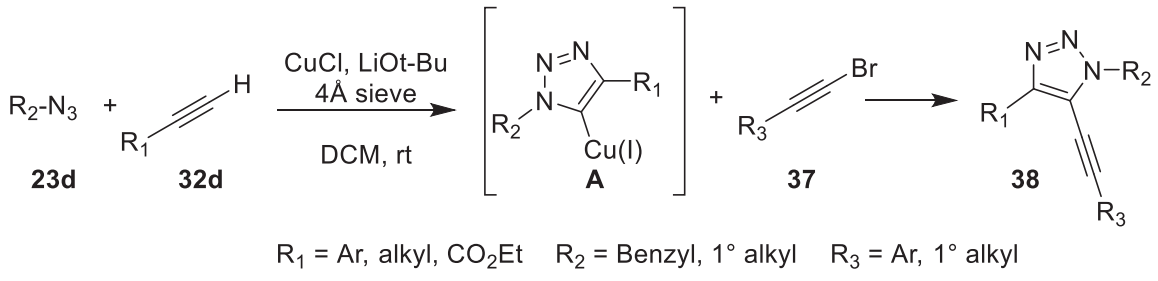


Figure 1.21. Copper(I) catalyzed one-pot synthesis of 5-alkynyl 1,2,3-triazole

After investigations of several ruthenium(II) complexes, CpRu's are showed excellent catalytic effect in click chemistry by Baren et al. The reason why Cp is so important is that it stabilizes higher formal oxidation states of the metal center. 1,5-Disubstituted triazole compounds are synthesized starting from terminal alkynes and azides by using $CpRuCl(PPh_3)_2$ as catalyst. When using CpRuCl(COD), it showed higher activity and was very suitable for room temperature experiments. Especially it useful for the reaction of internal alkynes to produce fully substituted triazoles. CpRu catalyzed reactions are selective to primary azides than secondary azides because of steric effects. [36] (Figure 1.22)

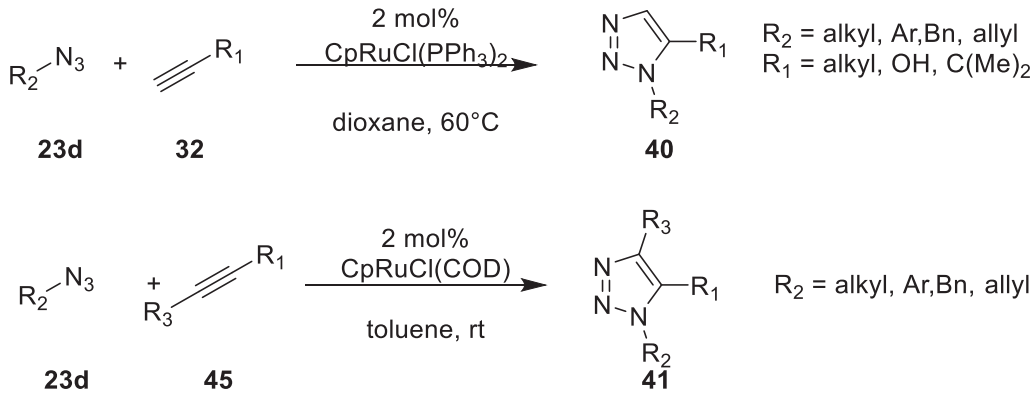


Figure 1.22. Ruthenium(II) catalyzed 1,5-disubstituted and fully substituted 1,2,3-triazole derivatives

Feng et al. synthesized fully substituted *N*2-aryl-1,2,3-triazole derivatives by Cu catalyzed regiospecific annulation of azirines and aryldiazonium salts. Electron-rich and electron-withdrawing aryl groups, heteroaryl groups on both 2 and 3 positions of azirine rings can be used. But monoalkyl or dialkyl substituted azirines are unsuitable for this methodology. [37] (Figure 1.23)

1.1.3. Anticancer Activities of 1,2,3-Triazole Derivatives

In the literature there are many examples of small molecules having 1,2,3-triazole moiety in their structures showing good anticancer activity by interacting with

diverse enzymes or receptors. They can induce cell cycle arrest or apoptosis in cancer cells. They can be used as a pharmacophore themselves or they can be used as just as linker between two pharmacophores.[38]

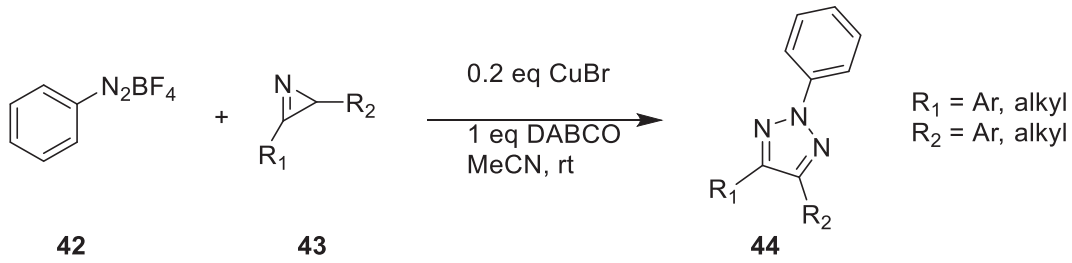


Figure 1.23. Cupper catalyzed synthesis of fully substituted N^2-aryl substituted 1,2,3-triazole derivatives

1.1.3.1. Amino Acid and 1,2,3-Triazole Substituted 3-Methoxy-4-phenoxybenzoic Acid Derivatives

3-Methoxy-4-phenoxybenzoic acid is an important scaffold and exist in the structure of many biologically active molecules. Substitution of this scaffolds with amino acids in carboxylic acid side and 1,2,3-triazole in the second benzene gave promising antiproliferative activity against MCF-7 and HepG2 cell lines. (Figure 1.24) Both of the compounds (46 and 47) have comparable IC_{50} values while compound 47 is a little bit more potent than the compound 46 in MCF-7 cells. Compound 47 is much more antiproliferative in HepG2 cell lines. [39]

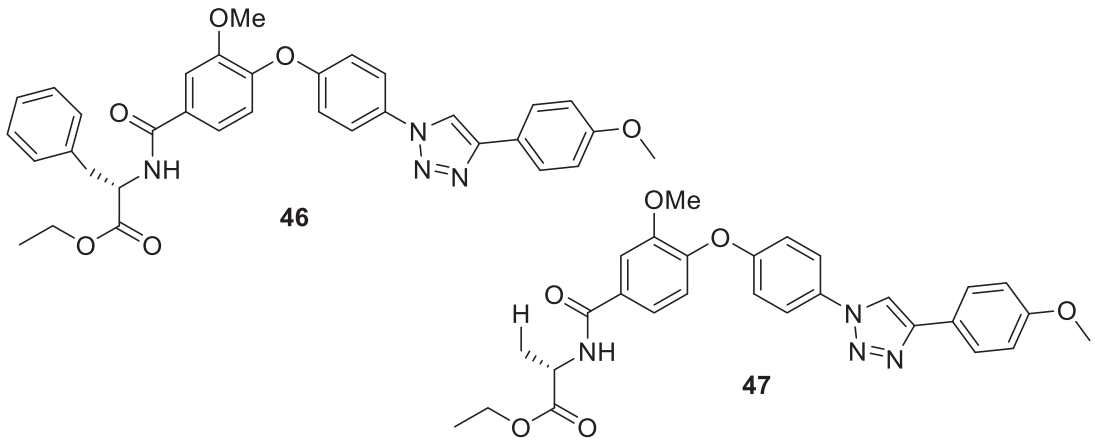


Figure 1.24. Amino acid and 1,2,3-triazole substituted 3-methoxy-4-phenoxybenzoic acid derivatives

1.1.3.2. Etodolac – 1,2,3-Triazole Hybrids

Kummari et al. identified promising drug candidates for lung cancer by preparing the hybrid structure of Etodolac and 1,2,3-triazole. Among the 12 molecules, five structures (>70% inhibitions are selected) were chosen for MTT assay in A547 cell lines. Compound 52 showed very good anticancer activity with IC_{50} value 3.29 μM. [40] (Figure 1.25)

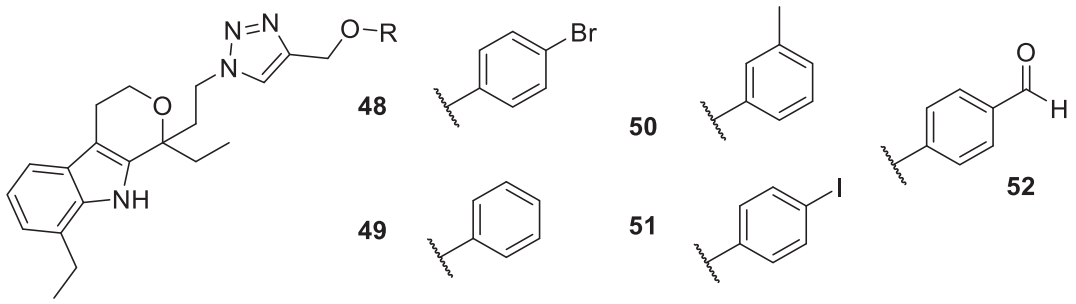


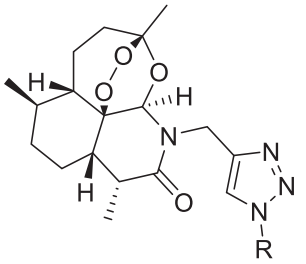
Figure 1.25. Etodolac-1,2,3-triazole conjugates

1.1.3.3. Artemisin – 1,2,3-Triazole Hybrids

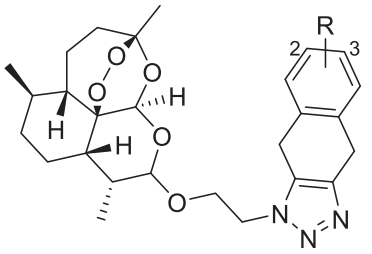
Artemisin is known for its antimalarial activity. Also new results show that it possess anticancer activities at low concentrations. Jana et al. synthesized different 1,4-disubstituted, 1,5-disubstituted and fused 1,2,3-triazole artemisin hybrids. The compounds were tested for anticancer activity against 2 cancer cell lines (CEM: T-lymphoblastic leukemia, HeLa: Cervical carcinoma) and 1 endothelial healthy cell line. According to dose dependent cell proliferation experiments compound 53d showed the highest activity. Also, it shows 30-fold more active in tumor versus healthy endothelial cells. It proves that compound 53d is tumor selective agent. [41] (Figure 1.26)

1.1.3.4. Chalcone – 1,2,3-Triazole Hybrids

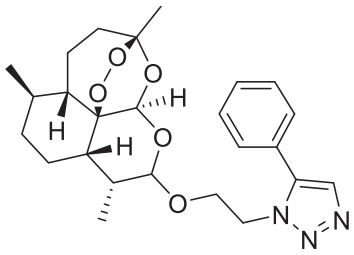
Chalcones (1,3-diaryl-2-propenones) are flavonoids which have 2 aromatic rings in its structure. Chalcone-triazole derivatives were prepared and evaluated for their anticancer activities against 4 cancer cell lines (MCF7, MIA-Pa-Ca-2, A549, HepG2) and 1 normal cell (fR2) by MTT assay (calorimetric cell proliferation assay). Among the 24 compounds tested, compound 60 was found to be the most effective one with a range of IC_{50} values between 4-11 μM in cancer cells and no observed toxicity in healthy cell line. Compound 60 mainly induces apoptosis by causing the loss of membrane potential in mitochondria. When mitochondria damaged it triggered apoptosis by activating procaspases. Also, studies showed that compound 60 causes G2/S phase cell cycle arrest.[42] (Figure 1.27)



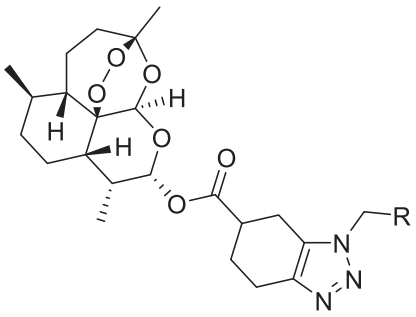
53a : R=H, **53b**: R=3.4.5-triOMe
53c : R=4-CN **53d**: R=3-i-Pr



54a : R=H, **54b**: R=2.3-diOMe
54c : R=2-OMe **54d**: R=2-i-NO_2

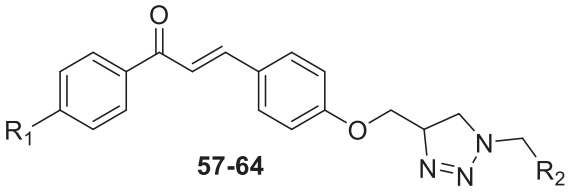


55a : R=H, **55b**: R=3.4-diBr
55c : R=3-OMe-4-OH



56a : R=4-MePh, **56c**: R=3.4.5-triOMePh
56b : R=benzo[d][1,3]dioxol-5-yl
56d: R=indole-3-methylene

Figure 1.26. Artemisin-1,2,3-triazole hybrids



57-64

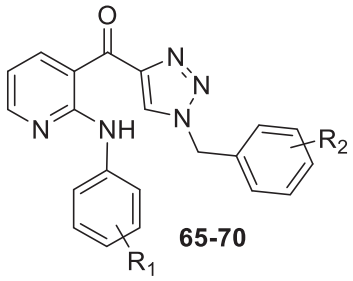
	R_1	R_2
57	Br	2-BrC_6H_4
58	Br	C_6H_5
59	NO_2	C_6H_5
60	OCH_3	C_6H_5
61	OCH_3	2-BrC_6H_4
62	OCH_3	3-BrC_6H_4
63	OCH_3	4-BrC_6H_4
64	OCH_3	2-FC_6H_4

Figure 1.27. Chalcone-1,2,3-triazole hybrids

1.1.3.5. Pyridine – 1,2,3-Triazole Hybrids

Kamal et al. synthesized dix different 2-anilinopyridyl triazole conjugates. (Figure 1.28) The compounds were evaluated for anticancer activities against 3 cancer cell line, HT-29 (colon cancer), DU-145 (prostate cancer), A549 (lung cancer) and one

normal human embryonic kidney (HEK-293) by MTT assay. Surprisingly all the compounds showed very good cytotoxic activity and very selective to the tumor cells. All of them showed activity at low micromolar concentrations. According to results, electron-withdrawing in R1 and 3-OPh group in R1 were optimal for better cytotoxic activity. Also flow cytometric analysis showed that compounds cause the cell cycle arrest at G1/M phase and triggered apoptosis [43]



65-70

	R_1	R_2
65	4-OMe	4-OMe
66	4-OMe	3.4-diOMe
67	4-OMe	3-OPh
68	4-F	3.4-diOMe
69	4-F	3.5-diOME
70	4-F	3-OPh

Figure 1.28. Pyridine-1,2,3-triazole hybrids

1.1.3.6. Quinone – 1,2,3-Triazole Hybrids

Naphthoquinone scaffolds are quite effective to produce ROS and oxidative stress that triggers apoptosis in cells. Seven new promising quinone – triazole structures were synthesized by Gholampour and coworkers. (Figure 1.29) The compounds were evaluated for their cytotoxic activity against MCF-7 (breast cancer), HT-29 (colon cancer) and MOLT-4 (lymphoblastic leukemia) cancer cell lines. Compound **77** especially showed the most potent cytotoxic activity against MCF-7 with IC_{50} value of 6.8 μM. It is also shown that compounds **71**, **77** and **78** arrested cell cycle at G0/G1 phase at 10-20 μM. [44]

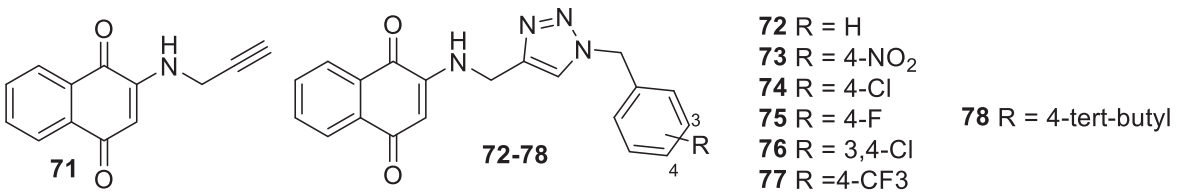


Figure 1.29. Quinone-1,2,3-triazole hybrids

1.1.3.7. Steroid – 1,2,3-Triazole Hybrids

Steroids are lipids which have four rings in carbon skeleton. There are hundreds of derivatives of steroids in animal, fungus and plants. The most important role of steroids in humans, be hormone. Betulin and betulinic acid is a triterpene and exhibit antiproliferative activity in cancer cells. Chrobak et al. synthesized a series of 3-phosphate-1,2,3-triazole derivatives of Betulin. (Figure 1.30) All of the compounds were examined against MV-4-11 (human leukemia). Compounds **79a** and **79e** were showed less activity than the others. Then just compounds **79b**, **79c** and **79d** were evaluated against A549 (lung cancer), DU-145 (prostate cancer), Hs 294T (melanoma) and MCF-10A (human mammary gland). Compound **79c** was the most potent agent with IC50 values 6.15 μM and 1.74 μM in DU-145 and A549 cell lines respectively. [45]

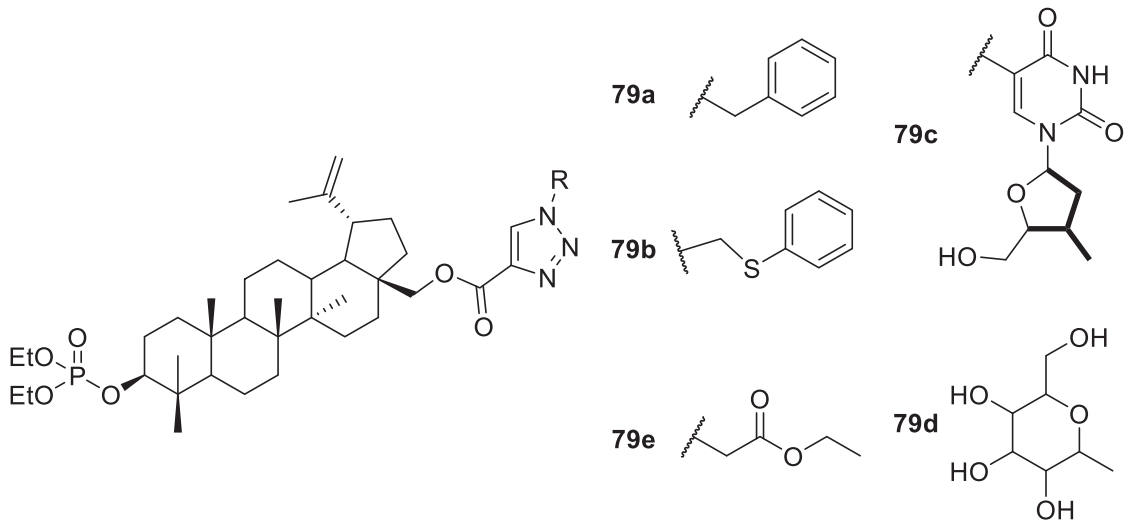
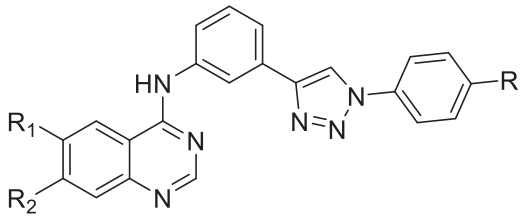


Figure 1.30. Steroid-1,2,3-triazole hybrids

1.1.3.8. Quinazoline – 1,2,3-Triazole Hybrids

Quinazoline is an aromatic group with a bicyclic structure of benzene and pyrimidine ring. Quinazoline derivatives are powerful inhibitors of EGFRs (Epidermal growth factor receptor) of tyrosine kinases. 15 Different 4-anilinoquizalione substituted triazole hybrids were synthesized by Le-Nhat Thuy. (Figure 1.31) Compounds were evaluated in vitro for anticancer activities by MTT assay in three different human cancer cell lines; KB (epidermoid carcinoma), HepG2 (hepatoma carcinoma) and SK-Lu1(non-small lung cancer). Among the tested candidates, compound **82a** shows promising anticancer effect with IC_{50} values 0.04 μM and 0.14 μM in KB and HepG2 cell lines respectively. Docking studies imply the binding of compounds **80d**, **82a**, **82b** and **83c** to the ATP binding site of EGFR.[46]



R= 2-NO_2 for 80a, 81a, 82a, 83a
R= 3-NO_2 for 80b, 81b, 82b, 83b
R= 4-NO_2 for 80c, 81c, 82c
R= 3-CN-4-CF_3 for 80d, 81d, 82d, 83c

80 R_1=R_2=H
81 R_1,R_2=-OCH_2O-
82 R_1,R_2=-$O(CH_2)_2O$-
83 R_1,R_2=-$O(CH_2)_3O$-

Figure 1.31. Quinazoline-1,2,3-triazole hybrids

1.2. 1-Azacoumarins

Coumarins are organic compounds which can be present in the structure of naturally occurring various herbals. The first coumarin was isolated from tanka beans in 1820. First synthetic approach dates to 1868 by William Henry Perkin. 1-Azacoumarins are structurally related to coumarins, but it has nitrogen in their structure instead of oxygen. (Figure 1.32)

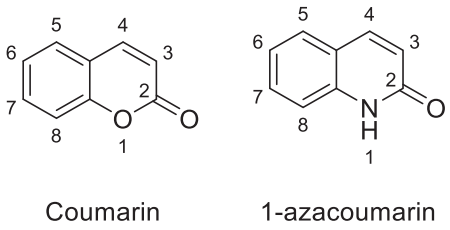


Figure 1.32. Coumarin and 1-azacoumarin

1.2.1. Synthesis of 1-Azacoumarin Derivatives

Wu et al. reported 1-azacoumarin synthesis starting from anilines and α,β-unsaturated esters via catalytic amount of $Pd(OAc_2)$. To maintain Pd(II)-Pd(0) catalytic cycle $Pd(OAc_2)$ used as catalyst and $Na_2S_2O_8$ used as oxidant. The best condition for C-H activation was using $TsOH{\cdot}H_2O$ in toluene. There wasn't product formation with electron withdrawing groups in anilines such as nitro, carboxyl or trifluoromethyl groups. (Figure 1.33)[47]

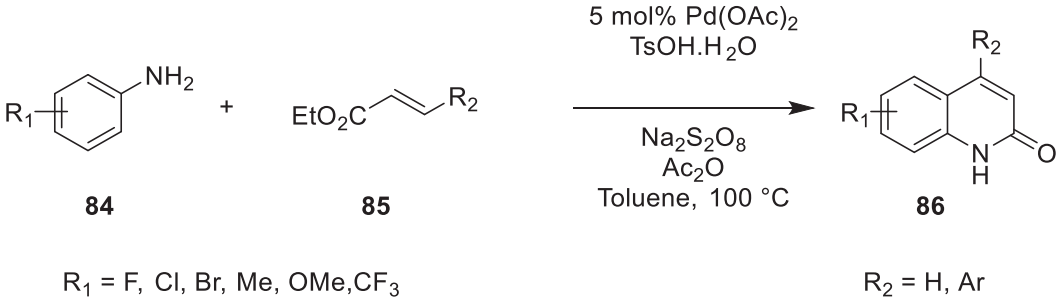


Figure 1.33. Pd catalyzed synthesis of 1-azacoumarin

Another Pd catalyzed reaction for synthesis of 1-azacoumarin were studied by Corpas et al. (Figure 1.34) Their strategy based on the cascade reaction between an internal alkyne and boronic acid substituted aniline. [48]

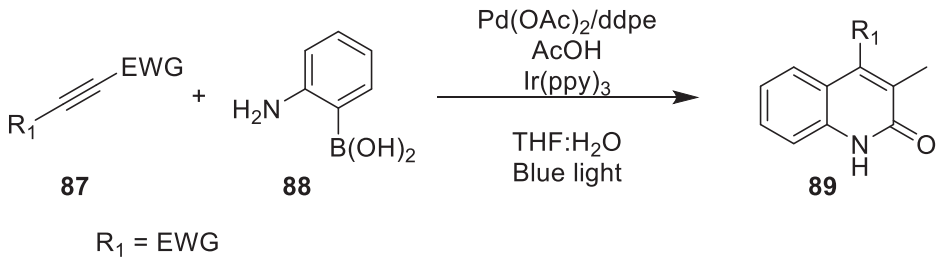


Figure 1.34. Synthesis of 1-azacoumarin starting from (2-aminophenyl)boronic acid

Heck type reactions generally needs hydrobromic acid and high temperatures (100-150 °C). In metal-catalyzed methods, the release of CO and transition metals are serious problem for the environment. Fan et al. proposed first metal free chemo selective intramolecular decarboxylative Heck type reaction at room temperature with oxamic acid. Oxamic acid was used as carbonyl source and hypervalent iodine (III) was used as promotor to lactonization of 2-vinylanilines. Reaction proceeds well with substituents that are weak electron donating groups. Strong electron donating groups can be problem to give oxidative side reactions. [49] (Figure 1.35)

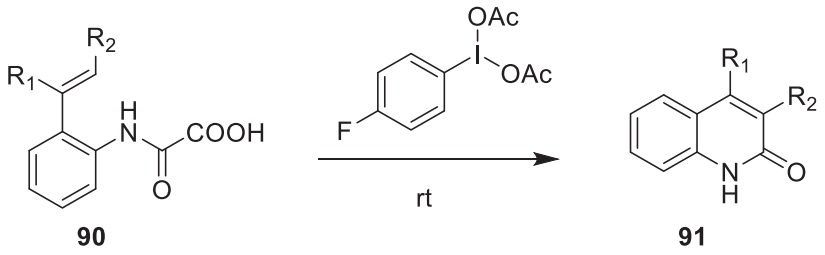


Figure 1.35. Synthesis of 1-azacoumarin by Heck reaction

Liu et al. proposed another iodine(III) mediated rearrangement reaction for synthesis of 1-azacoumarins. N-methyl-N-phenylcinnamamides used as substrate and PIFA used as promotor. This reaction provides both C-C bond formation and 1,2-arly shift. [50] (Figure 1.36)

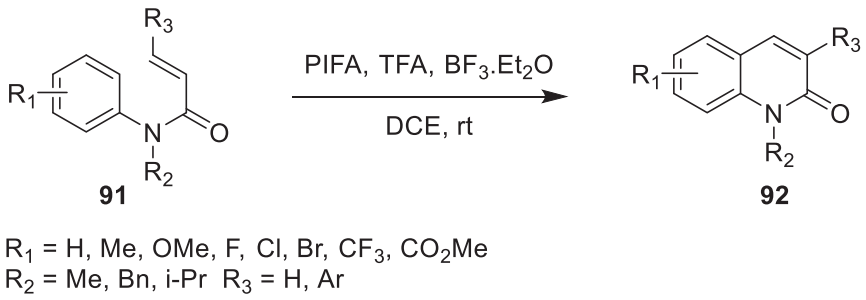


Figure 1.36. Synthesis of 1-azacoumarin from N-phenylcinnamamides

In general, 2-quinolinone are prepared by acid mediated cyclization of β-keto anilides and base mediated aldol condensation. Later Pd catalyzed reactions have also been produced. Manikandan et al. has produced Ru catalyzed cyclization of anilines with different propiolates. (Figure 1.37) [51]

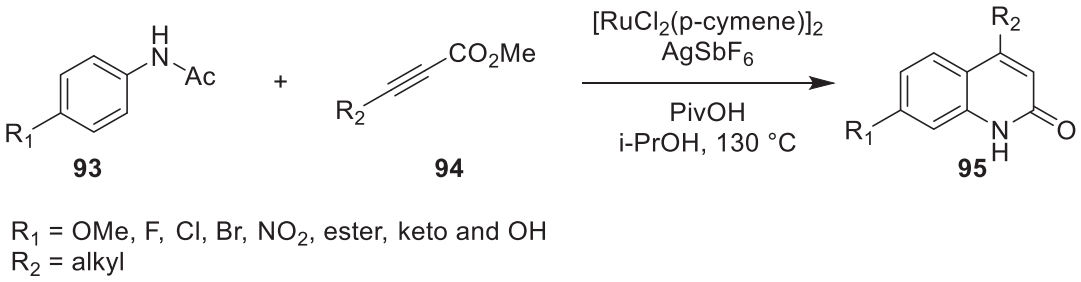
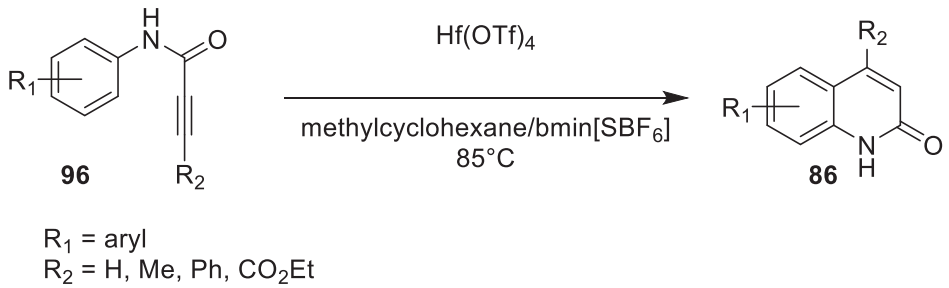


Figure 1.37. Ru catalyzed synthesis of 1-azacoumarin

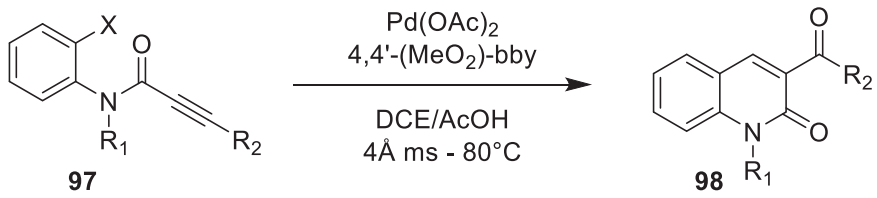
Sang et al. synthesized 1-azacoumarin derivatives with intramolecular Friedel Crafts alkenylation. Reaction catalyzed by $Hf(OTf)_4$ in methylcyclohexane and in the presence of bmim[SBF_6]. (Figure 1.38) [52]



R_1 = aryl
R_2 = H, Me, Ph, CO_2Et

Figure 1.38. Synthesis of 1-azacoumarin with intramolecular Friedel Crafts alkenylation

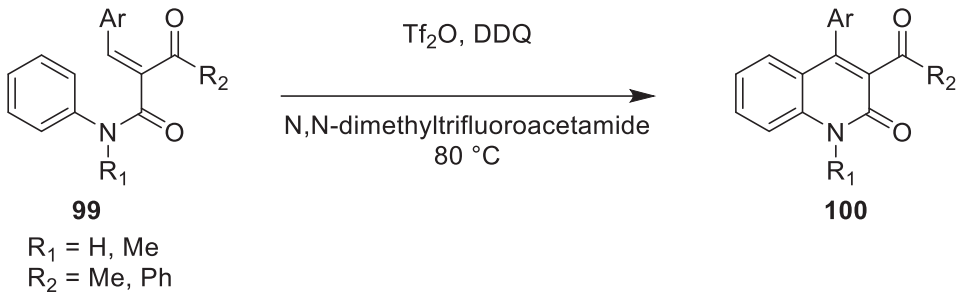
1-azacoumarin derivatives were prepared with transition metal catalyzed cascade reaction. $Pd(OAc_2)$ catalyzed cyclization were studied by Zhang et al. As a new strategy, reaction based on the oxypalladation of alkynes. Also, as an advantage Pd(II)/Pd(II) process are utilized instead of Pd(II)/Pd(0) or Pd(II)/Pd(IV) redox systems. It provides more efficient and economical way. (Figure 1.39) [53]



R_1 = H, Me, Bn R_2 = 1° alkyl, Ph X= CHO

Figure 1.39. Synthesis of 1-azacoumarin with palladium catalyst

Methodology for the synthesis of polysubstituted quinolines were developed by using triflic anhydride to perform intramolecular cyclization of N-aryl cinnamides. First attempt to synthesis of azacoumarin with Tf_2O was failed without additive of DDQ. DDQ is required to perform smoothly due to its oxidative properties. [54] (Figure 1.40)

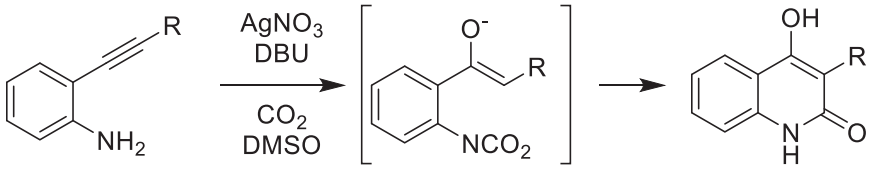


99 **100**

R_1 = H, Me
R_2 = Me, Ph

Figure 1.40. Triflic anhydride catalyzed synthesis of 1-azacoumarin

Silver catalyzed synthesis of 1-azacoumarin derivatives starting from alkynylaniline and carbon dioxide was reported in the literature. Amino and carbon dioxide form carbamite and then cyclization with Ag catalyst gave product. For primary aniline DABCO base is more suitable. CO_2 was used to promote the formation of C-C bond. Different metal salts were tried but just $AgNO_3$ was successful.[55] (Figure 1.41)



101 **102** **103**

R = Ar, Bu, H, COPh

Figure 1.41. Silver catalyzed synthesis of 1-azacoumarin

Metal free novel one pot regioselective reaction was proposed by Tangella et al for the synthesis of 1-azacoumarin derivatives. In this reaction both electron donating and electron with drawing substituted isatin can be used to react with aldehydes in the presence of tosylhydrazine. Also the absence of metal is an advantage in the environment. [56] (Figure 1.42)

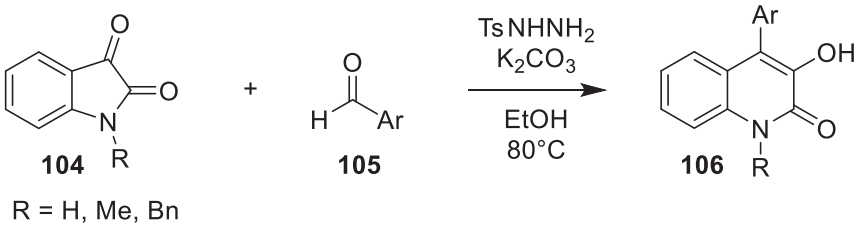


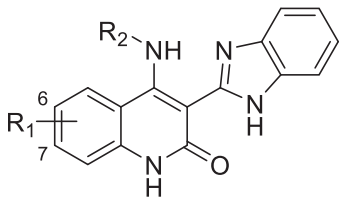
Figure 1.42. Metal free synthesis of 1-azacoumarin from isatin

1.2.2. Anticancer Activities of 1-Azacoumarins

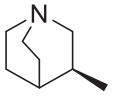
Azacoumarin derivatives are found to be very potent anti-inflammatory, anticancer and analgesic agents in previous studies. They can act as aromatase inhibitors, nuclear factor kappaB inhibitors and CHK-1 inhibitor. [57]

1.2.2.1. Benzimidazole Substituted 1-Azacoumarins

CHK-1 (Serine/threonine checkpoint kinase) is an enzyme to regulate the checkpoints of G1/S, intra-S and G2/M. To cure cancer CHK-1 inhibitors promise future. Ni et al. synthesized 10 different novel 4-(aminoalkylamino)-3-benzimidazole-azacoumarins. (Figure 1.43) The compounds were tested against MDA-MB-435 (Breast Cancer Cell Line). Compounds **109** and **114** were more potent than the remaining compounds with IC_{50} values 0.35 μM and 0.32 μM respectively. Combinational application of compound **114** with CPT (Camptothecin, topoisomerase I poison) accelerates the death of MDA-MB-435 cells.[58]



107-116

	R1	**R2**
107	H	

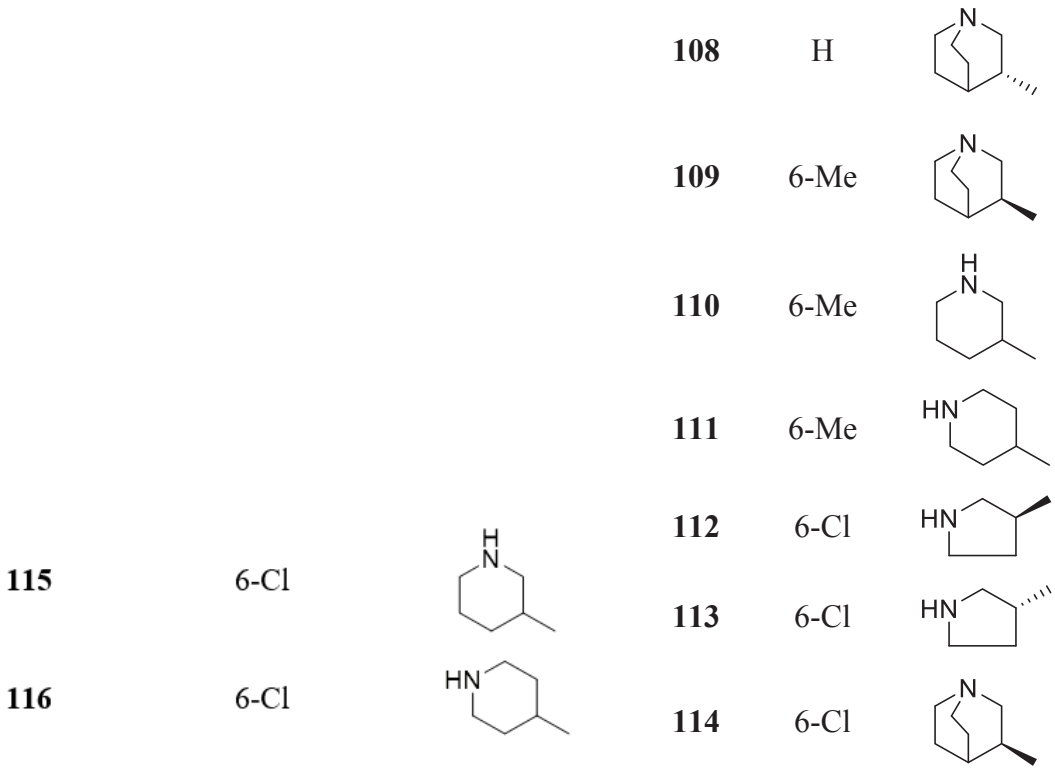
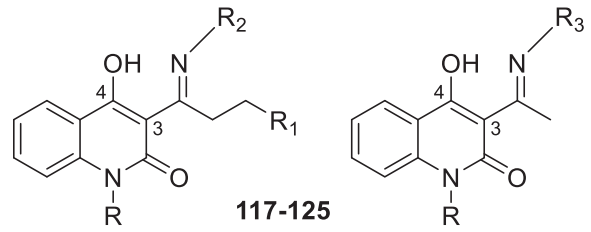


Figure 1.43. Benzimidazole substituted 1-azacoumarins

1.2.2.2. 4-Hydroxy-3-Substituted 1-Azacoumarins

Desai et al. synthesized series of 4-hydroxy-3-substituted 1-azacoumarins. These derivatives were tested in vitro against K562 (Human myelogenous leukemia) and Hep3b (human hepatocellular carcinoma) for anticancer activities by MTT assay. Compound **117** was most potent compound for K562 and **120** was most potent compound for Hep3b cell lines. [59] (Figure 1.44)



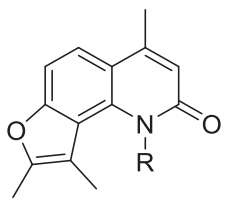
R **R1** **R2** **R3**

117	$-C_6H_5$	Morpholine	C_6H_5-NH-	-
118	$-C_6H_5$	N-Methyl piperazine	4-NO_2-C_6H_4-NH-	-
119	$-CH_3$	Morpholine	C_6H_5-NH-	-
120	$-C_6H_5$	-	-	C_6H_5-NH-
121	$-CH_3$	-	-	C_6H_5-NH-
122	$-C_6H_5$	-	-	4-Cl-C_6H_4-NH-
123	$-CH_3$	-	-	4-Cl-C_6H_4-NH-
124	$-C_6H_5$	-	-	4-Br-C_6H_4-NH-
125	$-CH_3$	-	-	4-Br-C_6H_4-NH-

Figure 1.44. 4-Hydroxy-3-substituted 1-azacoumarins

1.2.2.3. Furoquinoline Derivatives

Furoquinoline derivatives were synthesized by Liguan Xie et al. The compounds were evaluated against five different cancer cell lines: QGY (Human endocervical adenocarcinoma), K562 (Human myelogenous leukemia), HeLa (Cervical cancer), P388 (Mouse leukemia), A549 (Lung cancer). According to results of MTT assay, basic amino substituted furoquinolines are more potent against cancer cells. The most potent compound is **133** with IC50 values between 14 μM-21 μM in three cancer cell lines. Intercalators are the molecules inserted between the DNA bases. The resulting structures can be used as cancer drug. By DNA binding experiment, it has been proven that Furoquinoline derivatives intercalates between the DNA bases. [60] (Figure 1.45)



126-134

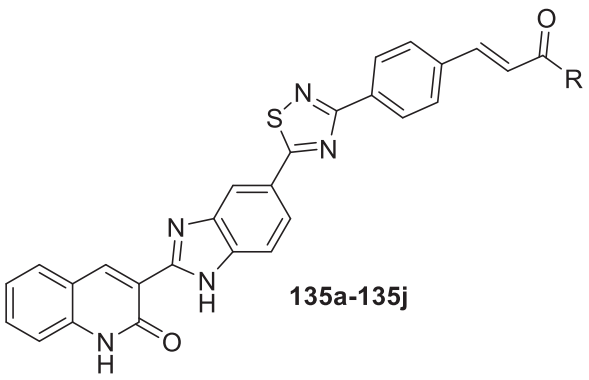
	R
126	H
127	C_2H_5
128	CH_2CN
129	$CH_2C_6H_5$
130	$CH_2CO_2C_2H_5$
131	C_2HNMe_2

132	$C_3H_6NMe_2$
133	$CH_2CONH(CH_2)_2NMe_2$
134	CH_2CO_2H

Figure 1.45. Furoquinoline derivatives

1.2.2.4. 1,2,4-Thiadiazol Substituted 1-Azacoumarin Derivatives

Novel chalcone incorporated 1,2,4 thiadiazol substituted azacoumarins were synthesized and tested in vitro against MCF-7 (breast cancer), A549 (lung cancer), Colo-205 (colon cancer) and A2780 (ovarian cancer) by Pragotti et al. The compounds showed excellent cytotoxic activity against cancer cells. Especially compound **135d** was the most potent compound and can be a good candidate for in vivo studies.[61] (Figure 1.46)

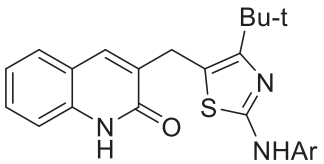


	R
135a	3,4,5-trimethoxyphenyl
135b	3,5-dimethoxyphenyl
135c	4-methoxyphenyl
135d	4-pyridyl
135e	2-thionyl
135f	4-nitrophenyl
135g	3,5-dinittrophenyl
135h	4-chlorophenyl
135i	4-bromophenyl
135j	4-(dimethylamino)phenyl

Figure 1.46. Chalcone incorporated 1.2.4-thiadiazol substituted 1-azacoumarins

1.2.2.5. Aryl Aminothiazole Substituted 1-Azacoumarin Derivatives

Microtubules are the components of the cytoskeletons. The main functions of microtubules are the formation of cell shape, cell signals and intracellular transport. Tubulin inhibitors play an important role in cancer treatment because of the dynamics of the microtubules is quite important in the division of cancer cells. As candidates of tubulin inhibitors, novel aryl aminothiazole substituted 1-azacoumarin derivatives were synthesized by Fang et al. The compounds were tested against HeLa (Cervical cancer), NCI-H460 (Lung cancer), T24 (Bladder cancer) and SKOV3 (Ovarian cancer) cancer cell lines. Compound **136f** showed excellent cytotoxic effect in the tumor cells with IC50 values in the range of 4.4 μM to 8.7 μM. With tubulin polymerization assay experiment, compound **136f** also inhibited the tubulin polymerization. Besides, compound **136f** induced apoptosis and cell cycle arrest in G2/M phase. (Figure 1.47) [62]



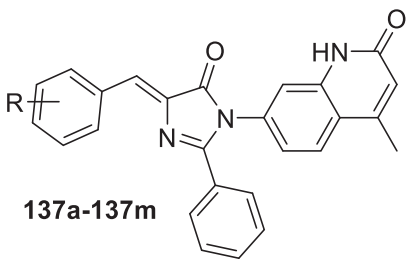
136a-136m

	Ar
136a	C_2H_5
136b	3-$CH_3C_6H_4$
136c	4-$CH_3C_6H_4$
136d	4-$CH_3OC_6H_4$
136e	2-FC_6H_4
136f	4-FC_6H_4
136g	2-ClC_6H_4
136h	3-ClC_6H_4
136j	4-ClC_6H_4
136k	2-BrC_6H_4
136l	4-BrC_6H_4
136m	3-$CF_3C_6H_4$

Figure 1.47. Aryl aminothiazole substituted 1-azacoumarins

1.2.2.6. Imidazol-5(4H)-one Substituted 1-Azacoumarin Derivatives

Nuclear Factor kappa B (NF-KB) is a protein and activates the transcription of DNA. Some studies show that NF-KB directly related with tumorigenesis. As NF-KB inhibitor novel imidazol-5(4H)-one substituted 1-azacoumarin derivatives were synthesized by Kumar et al. The compounds were evaluated in vitro for anticancer activities against BT-549 (Breast cancer) and HeLa (Cervical carcinoma) cell lines. Compounds **137c** and **137m** showed moderate cytotoxic effect to the BT-549 and Hela cells. Also docking studies imply that the compounds can bind to the active site of NF-KB. [63] (Figure 1.48)



	R
137a	H
137b	4-fluoro
137c	3-fluoro
137d	2-chloro
137e	4-chloro
137f	4-bromo
137g	2-bromo
137h	4-methoxy
137j	2-methoxy
137k	3,4-dimethoxy
137l	4-ethoxy
137m	4-phenoxy

Figure 1.48. Imidazol-5(4H)-one substituted 1-azacoumarin derivatives

1.3. 1,2,3-Triazole Substituted 1-Azacoumarins

In literature 1-azacoumarin substituted 1,2,3-triazoles synthesis based on the click chemistry. First road is copper catalyzed azide alkyne cycloaddition. This method well understood and robust for very broad range of functional groups. Second one is Ruthenium catalyzed azide alkyne cycloaddition. Although RuAAC is not robust as

CuAAC, it has very useful with internal alkynes to produce fully substituted triazole derivatives.

1.3.1. Cupper Catalyzed Synthesis of 1,2,3-Triazole Substituted 1-Azacoumarins

For synthesis of 1,2,3-triazole substituted 1-azacoumarin, a short route was developed by Majumdar. Firstly, NH_2 group over 1-azacoumarin derivative **138** was converted to diazonium salt and then treatment with azide afforded azide substituted 1-azacoumarin (**139**). Then 4-chlorobenzaldeyde (**140**) was converted to dibromo substituted styrene derivative (**141**) via a phosphorous ylide produced from CCl_4 and PPh_3. Elimination and metal-halogen exchange reaction of geminal dihalide substituted styrene gave a terminal alkyne (**142**). Copper (I) catalyzed click chemistry of azide **139** and **142** gave the target product.[64] (Figure 1. 49)

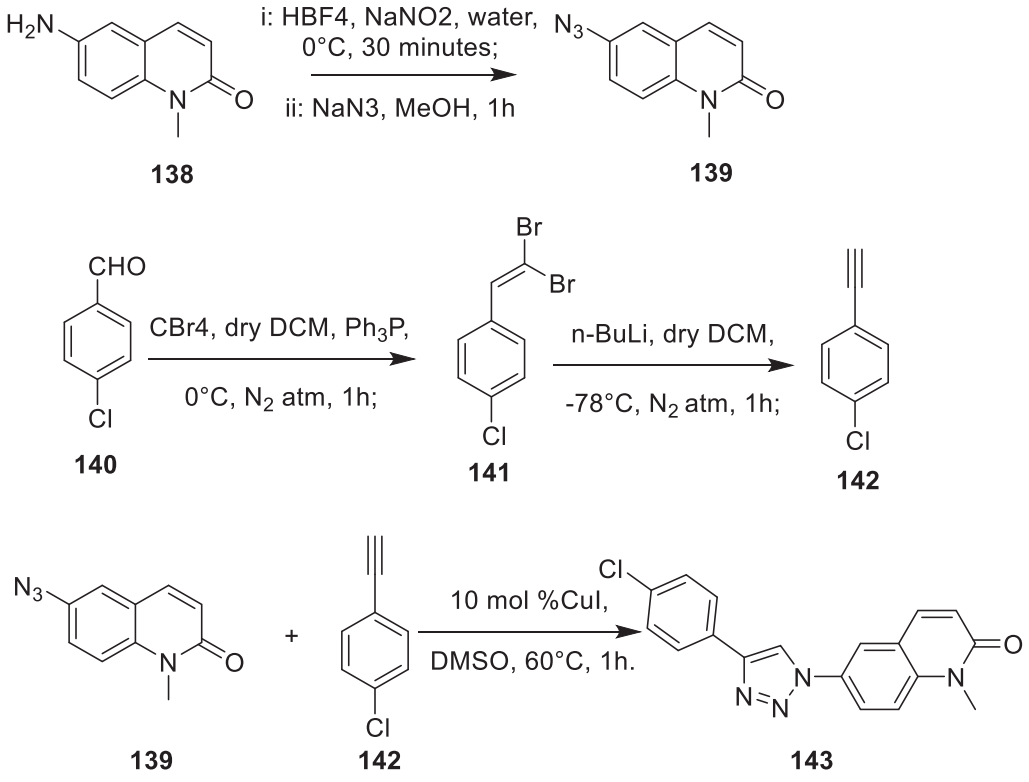


Figure 1.49. Cupper catalyzed synthesis of 1,2,3-triazole substituted 1-azacoumarin

1.3.2. Ruthenium Catalyzed Synthesis of 1,2,3-Triazole Substituted 1-Azacoumarin Derivatives

Ruthenium complex was used as catalyst by Oakdale et al. for the synthesis of 1,2,3-triazole substituted 1-azacoumarin. CpRuCl(cod) very useful catalyst in the reaction between nitrile oxides or azide and electron deficient chloro, bromo and iodo alkynes to cyclize triazoles.

Azide substituted azacoumarin (**145**) was synthesized starting from amine substituted azacoumarin (**144**) with transfer reagent. (Figure 1.50) Chlorinated acetylene (**147**) was synthesized with CCl_4 in the presence of TBAF. Finally, 1,2,3-triazole substituted 1-azacoumarin (**148**) was synthesized by click chemistry with ruthenium catalyzed.[65] (Figure 1.51)

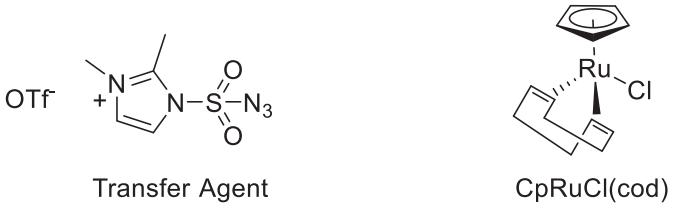


Figure 1.50. Transfer reagent and Ruthenium complex

1.3.3. Anticancer Activities of 1,2,3-Triazole Substituted 1-Azacoumarins

Up to date the anticancer activities of 1,2,3-triazole substituted 1-azacoumarins as well as its synthesis methods have not been studied or developed sufficiently. In general, biological activity studies have mainly focused on anti-microbial and anti-fungal properties of the hybrid structures.

Only anti-cancer study in the literature was tried by Audisio et al. Novel 1-azacoumarins with 1,2,3-triazole substituted at position 3 were synthesized. Their

antiproliferative activities were tested in MCF-7 cancer cell lines. However due to the solubility problems, there isn't any reported antiproliferative activity so far in the literature.[66]

1.4. Aim of the Study

In this study we aimed to synthesized novel 1,2,3-triazole substituted 1-azacoumarin derivatives. (Figure 1.52) Their anticancer studies against cancer cell lines will be investigated in the future.

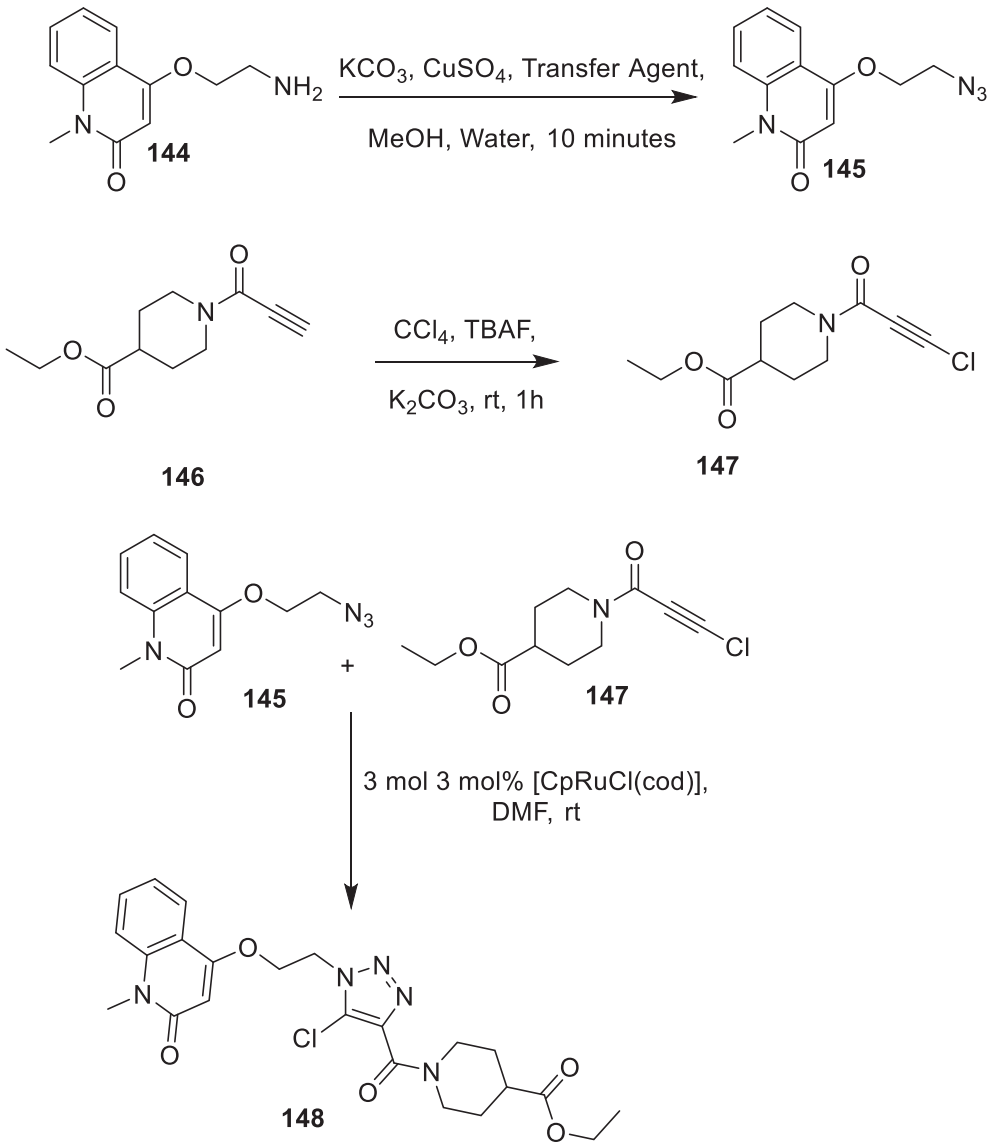


Figure 1.51. Ruthenium catalyzed synthesis of 1,2,3-triazole substituted 1-azacoumarin

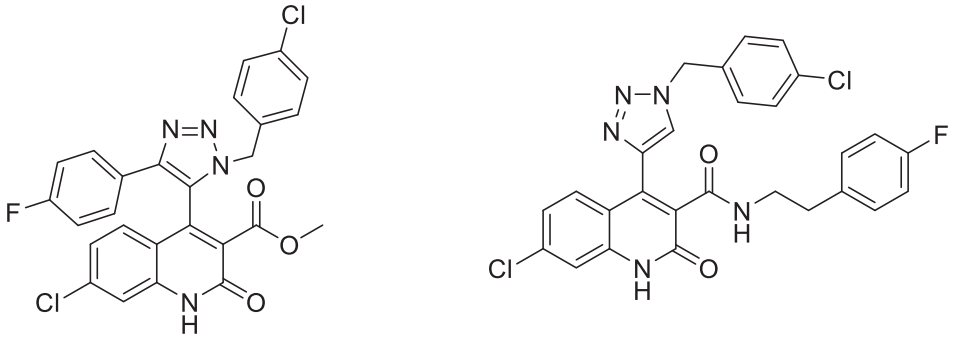


Figure 1.52. Novel 1,2,3-triazole substituted 1-azacoumarin derivatives

CHAPTER 2

RESULT AND DISCUSSION

2.1. First Attempt to Synthesize of compound 149 from Ynone Intermediate

In this part of the thesis early synthetic attempts toward the preparation of compound **149** was shown in Figure 2.1. Ynone **154** was the key intermediate for the preparation of two isomeric triazoles (**157** and **158**). All attempts for this synthetic route will be discussed at below.

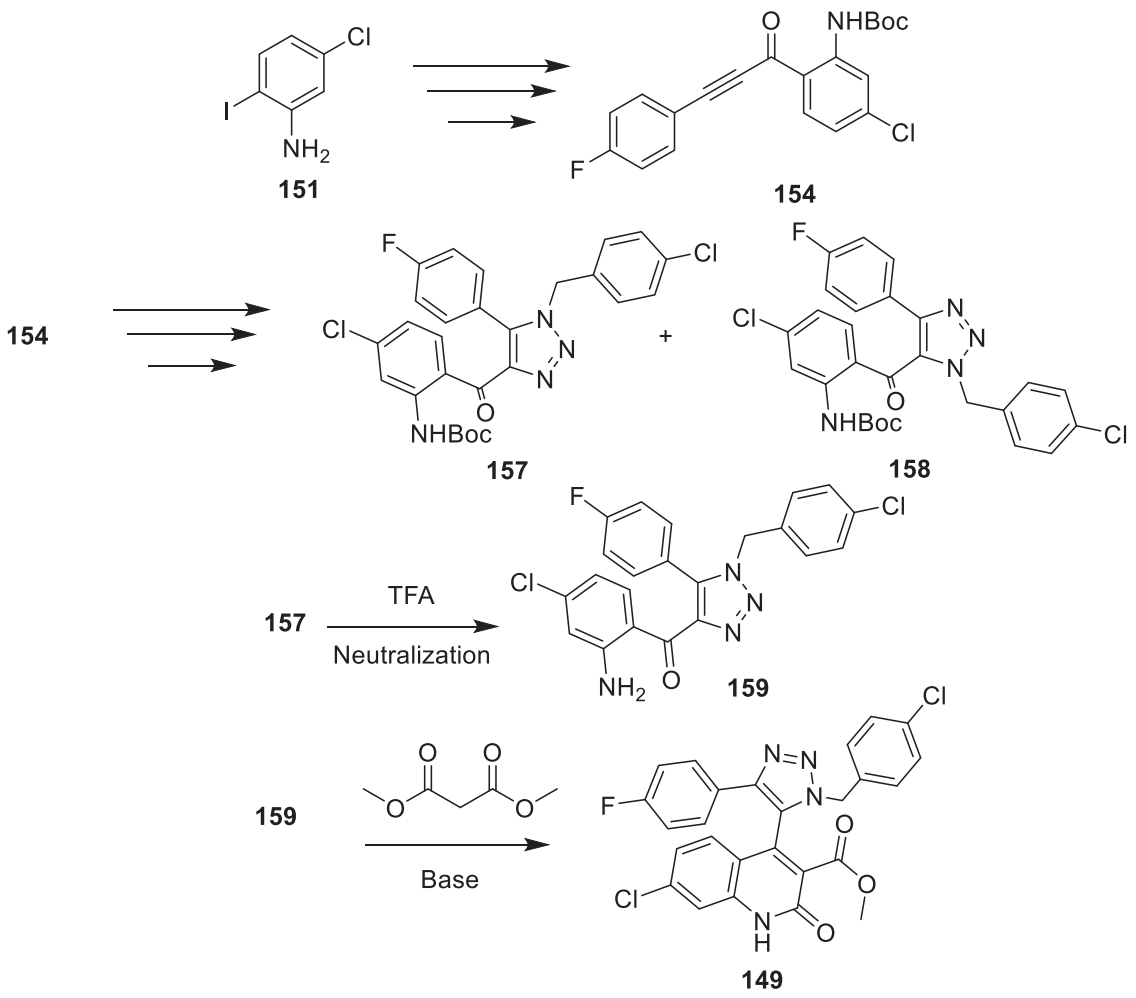


Figure 2.1. Proposed route for synthesis of compound **149**

2.1.1. Boc Protection Studies for 5-Chloro-2-Iodoaniline (151)

To obtain N-Boc protected aniline (**152**), compound **151** was reacted with 1 eq. Boc_2O in THF. However, formation of an inseparable mixture of mono and di-Boc aniline (**152** and **160**) was observed. The attempts to separate this mixture by column chromatography was unsuccessful. The same results were seen when MeOH and K_2CO_3 were used instead of THF. In this case, an attempt was made to convert di-Boc protected aniline to mono NH-Boc protection, which has examples in the literature. [67] N-Boc protected aniline was synthesized %25 yield. (Figure 2.2)

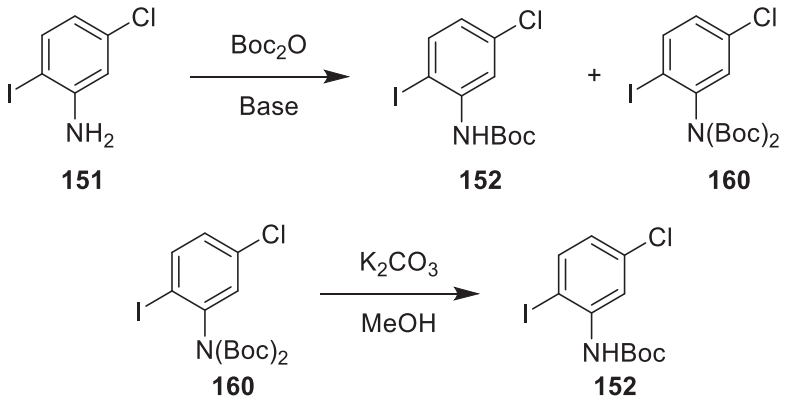
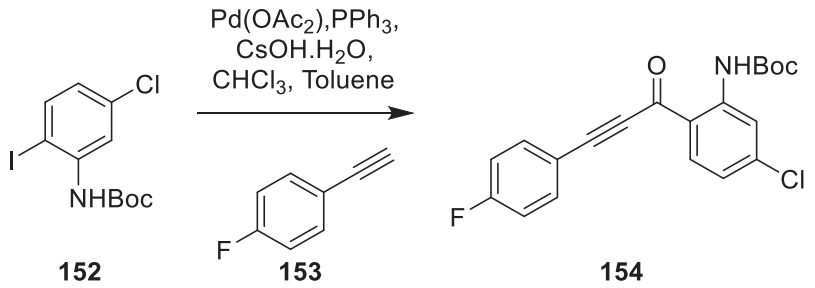


Figure 2.2. Synthesis of NH(Boc) protected aniline

2.1.2. Studies Toward to Synthesis of Ynone

In the next step, conversion of compound **152** to the ynone derivative (**154**) was tried by adding 1-ethynyl-4-fluorobenzene and chloroform in the presence of CsOH and $Pd(OAc)_2$ as reported in the literature.[68] However, this was not successful in repeated experiments (Table 2.1). At this stage, the same reaction was tried without protecting the aniline group and promising results were obtained. As a result of the optimization studies, ynone synthesis was carried out with yields between 25% and 46%. Interestingly, the reaction did not work at all in dry toluene, but when a small amount of water was added to the reaction, it was observed to have a positive effect on the reaction. (Table 2.2)

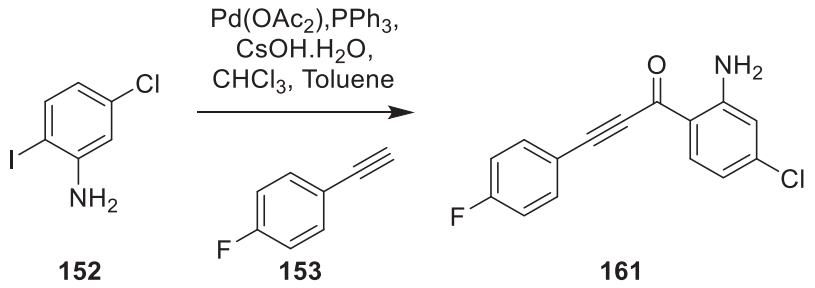
Table 2.1 Synthesis studies of ynone from Boc protected aniline



152 **153** **154**

Entry	$CHCl_3$ (eq)	Toluene (mL)	Conditions	Result
1	3	8	80 °C - reflux	No rxn
2	15	2.5	80 °C – sealed tube	No rxn

Table 2.2 Synthesis studies of ynone from aniline



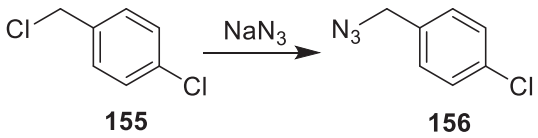
152 **153** **161**

Entry	Chloroform (eq)	Solvent (mL)	Conditions	Yield
1	3	THF – 5	70 °C – reflux – 8 hours	No rxn
2	4.5	Toluene – 5	80°C – sealed tube – 8 hours	Trace
3	15	Toluene – 5	80°C – sealed tube – 8 hours	25%
4	15	Toluene – 3	80°C – sealed tube – 8 hours	Trace
5	15	Toluene – 3	80°C – sealed tube – 8 hours – 75 µL water	44%
6	15	Toluene – 3	80 °C - sealed tube – 8 hours – 75 µL water	49%

2.1.3. 1-(Azidomethyl)-4-Chlorobenzene (159) from 4-Chlorobenzyl Chloride

At this step, 4-chlorobenzyl chloride was reacted with the azide ion in the presence of DMSO. In repeated experiments, it was observed that using DMF instead of DMSO increased efficiency.[69] However, due to the low boiling point of the 4-chlorobenzyl azide obtained, it should not be kept under reduced vacuum for a long time. Therefore, the yields given are not represent the actual yields. (Table 2.3)

Table 2.3 Studies towards to 1-(azidomethyl)-4-chlorobenzene (**156**)



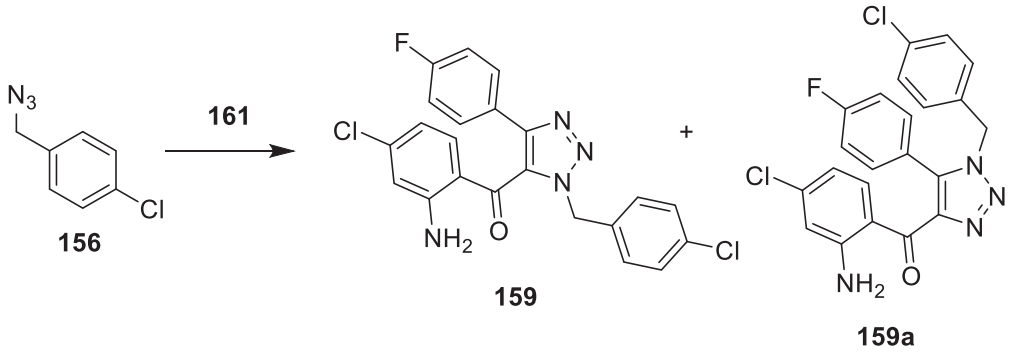
155 **156**

Entry	Solvent (mL)	Temperature (°C)	Time	Yield (%)
1	DMSO – 30	80	Overnight	87
2	DMF – 2.5	60	6 hours	69
3	DMF – 2.5	60	6 hours	81
4	DMF – 3	60	6 hours	87

2.1.4. Cupper Catalyzed Click Chemistry Reaction with Ynone Intermediate and 1-(Azidomethyl)-4-Chlorobenzene

CuAAC reaction was tried by CuI catalyzed to obtain 1,4,5-trisubstituted 1,2,3-triazole derivative from the ynone (**161**) and benzyl azide (**156**) intermediates. The first attempt was failed in the presence of 5 mol% CuI and DMSO was used as solvent. Then other attempts carried out in THF, and different temperatures were also failed. (Table 2.4)

Table 2.4 First attempt for click chemistry with ynone intermediate (**161**)



Entry	161 (eq)	156 (eq)	CuI (mol %)	Solvent (mL)	Conditions	Results
1	1	1.5	5	DMSO 2	Rt. Overnight	No rxn.
2	1	1.5	5	THF 3 H_2O 1.5	Rt. Overnight	No rxn.
3	1	1.5	6	THF 3 H_2O 1.5	80°C Overnight	No rxn.

Considering whether this procedure wasn't working with benzyl azide (**156**) and ynone intermediate (**161**), an alternative procedure for CuAAC reaction was tried but again the reaction was failed. (Table 2.5)

Table 2.5 Alternative approach for click chemistry with ynone intermediate (**161**)

Entry	161 (eq)	156 (eq)	CuI (eq)	Dipea (eq)	HOAc (eq)	Solvent (mL)	Results
1	1	1.05	0.02	0.04	0.04	DCM – 2	No rxn.
2	1	1.05	0.02	0.1	0.1	DCM – 2	No rxn.
3	1	1.05	0.02	0.1	0.1 eq.	$CHCl_3$ – 12	No rxn.

2.1.5. Attempts to Synthesize Amide Substituted Ynone Intermediate

At this stage, it is aimed to form amide by adding monomethyl malonate (**162**) to the amino group on the ynone intermediate (**161**). After converting the amide structure to 1-azacoumarin structure, it is aimed to synthesized 1,4,5-trisubstituted 1,2,3-triazole by click chemistry from the alkyne. However, attempts to form amides on ynone have been unsuccessful. (Figure 2.3)

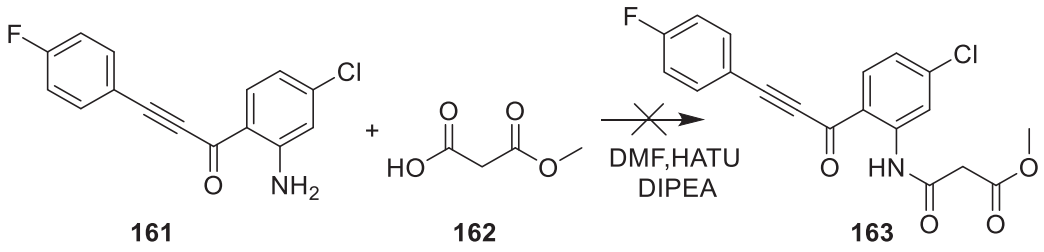


Figure 2.3. Amide substituted ynone intermediate

2.2. Studies Toward 1,4,5-Trisubstituted 1,2,3-Triazole (149) From 1-Azacoumarin

Since the desired 1,4,5-trisubstituted 1,2,3-triazole (**149**) could not be obtained from the trials with ynone intermediate so far, an alternative method has been examined as reported below. First, 1-azacoumarin was formed, and then possible coupling reactions with 1,2,3-triazoles were studied. (Figure 2.4)

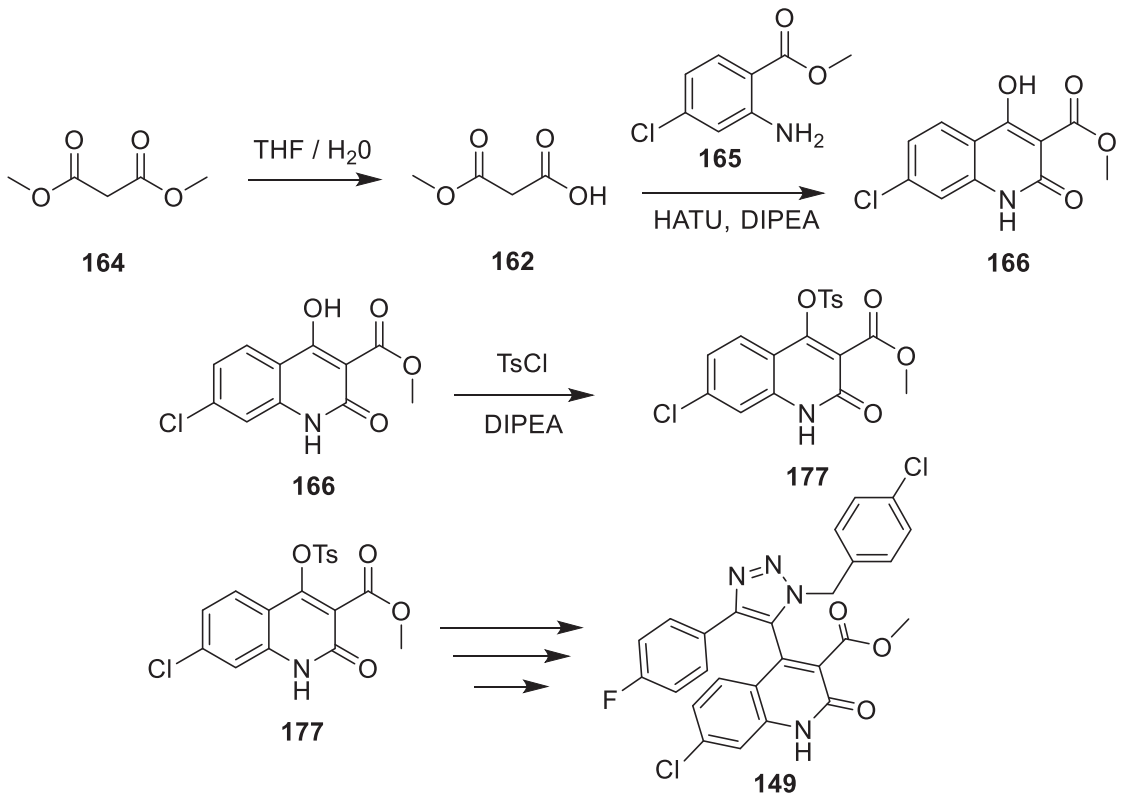


Figure 2.4. Compound **149** from OTs modified 1-azacoumarin

2.2.1. Attempt to Synthesis of 1-Azacoumarin (169) From Methyl-2-Amino-4-Chlorobenzoate (165) and Dimethyl Malonate (164)

A single step reaction was tried with Dean-Stark apparatus in methanol in the presence of NaH base for the synthesis of 1-azacoumarine (**166**), but it was not successful. (Figure 2.5)

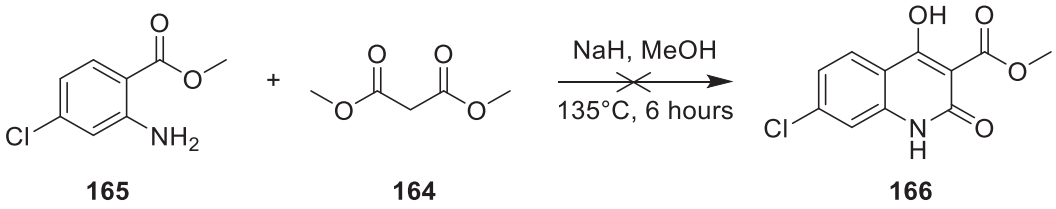
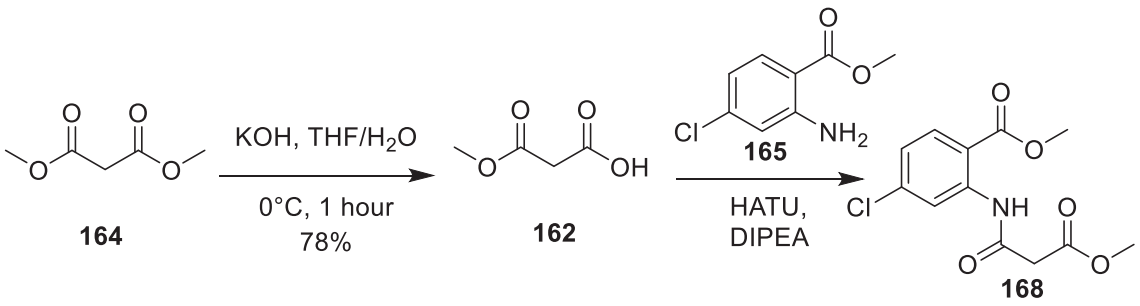


Figure 2.5. Single step attempt to synthesis of 1-azacoumarin (**166**)

2.2.2. Synthesis of 1-Azacoumarin (166) From Methyl-2-Amino-4-Chlorobenzoate (165) and Monomethyl Malonate (162)

As a precursor, to obtain 1-azacoumarin (**166**), monomethyl malonate (**162**) was obtained with hydrolysis of dimethyl malonate (**164**) with KOH in THF and water. Afterwards, it was converted to amide structure with methyl-2-amino-4-chlorobenzoate (**165**) by HATU. (Table 2.6)

Table 2.6 Synthesis of amide intermediate (**168**) by HATU



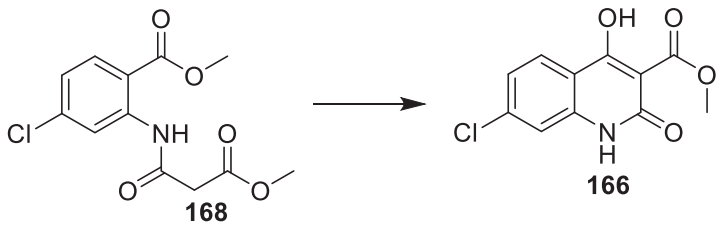
164 162 165 168

Entry	162 (eq)	165 (eq)	HATU (eq)	DIPEA (eq)	Solvent (mL)	Yield
1	1.2	1.2	1.2	2.4	DMF 8	No rxn.
2	1.1	1.1	1.1	2	DCM 3	%76

The amide intermediate (**168**) was converted to 1-azacomarin (**166**) under basic conditions. When the reaction is carried out in methanol, sodium hydride base works more effectively than potassium tertiary-butoxide base. On the other hand, in the reactions carried out in THF, potassium tertiary-butoxide gives the targeted product with very high yields. (Table 2.7)

The solubility of the 1-azacomarin (**166**) molecule obtained from the reaction is low. So, purification by column chromatography is quite problematic. Therefore, the crude product from the reaction is used without purification, and the product from the reactions carried out in THF appears to be relatively pure by TLC.

Table 2.7 Synthesis toward of 1-azacoumarin (**166**)



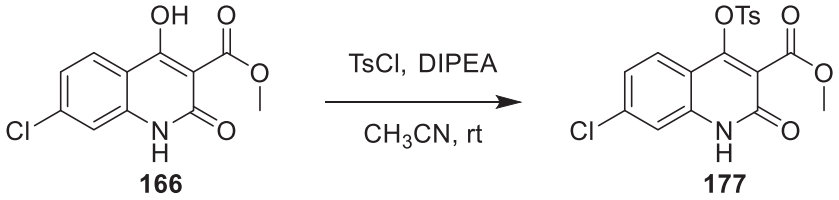
Entry	168 (eq)	Base (eq)	Solvent (mL)	Conditions	Yield* (%)
1	1 eq.	NaH – 10	MeOH 4	rt. 2 hours	84
2	1 eq.	KOt-Bu 2.2	MeOH 7	rt. 2 hours	26
3	1 eq.	KOt-Bu 2.2	THF 6	rt. 5 hours	98
4	1 eq.	KOt-Bu 2.2	THF 7	rt. 5 hours	90
5	1 eq.	KOt-Bu 2.2	THF 25	rt. 6 hours	73

* Yields are given without purification

2.2.3. Tosylation of 1-Azacoumarin

The OTs-azacoumarin (**167**) that needed in the next steps was synthesized repeatedly in moderate yields. Tosylation experiments were monitored by TLC control. It has been observed that the yield decreases in the experiments held for a long time (Table 2.8)

Table 2.8 Tosylation studies of 1-azacoumarin (166)



Entry	166 (eq)	TsCl (eq)	DIPEA (eq)	CH_3CN (mL)	Time (hours)	Yield (%)
1	1	1.1	3	12	3	43
2	1	1.1	3	11	4	36
3	1	1.1	3	14	2	59
4	1	1.1	3	10	2.5	68

2.2.4. 1,2,3-Triazole Addition to 167 in One-Pot Experiment

In the literature, addition of electrophilic species to a 1,2,3-triazole in the presence of LiO^tBu and CuI was reported.[70] The similar methodology was tried in order to prepare compound **149** as shown in Figure 2.13. In the presence of CuI, 1,2,3-triazole-5-Cu intermediate is formed. Then the reaction with the electrophiles in the environment is bound to the 5 position. However, all the performed studies were unsuccessful. (Figure 2.6)

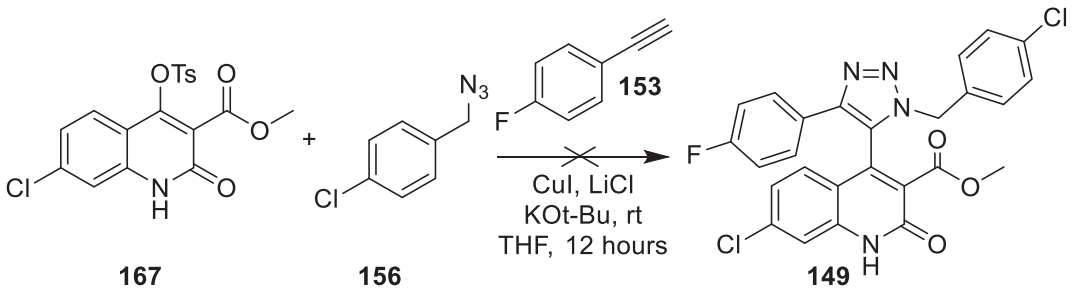
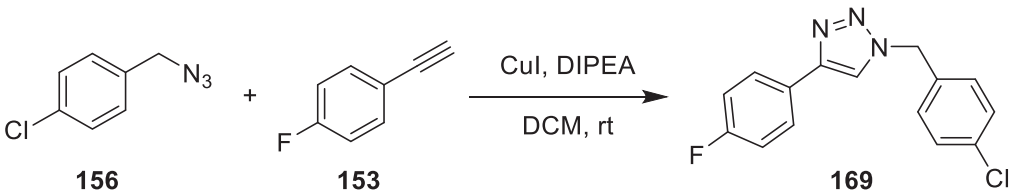


Figure 2.6. One pot experiment with **167**

2.2.5. Synthesis of 1,4-Disubstituted 1,2,3-Triazole

1,4-Disubstituted 1,2,3-triazole was synthesized from 1-ethynyl-4-fluorobenzene (**153**) and 1-(azidomethyl)-4-chlorobenzene (**156**) by CuAAC reaction. In the repeated experiment, the yield increased considerably when DIPEA and HOAc were doubled and time increased. (Table 2.9)

Table 2.9 Studies towards 1,4-disubstituted 1,2,3-triazole (**169**)



Entry	156 (eq)	153 (eq)	CuI (eq)	DIPEA (eq)	HOAc (eq)	DCM (mL)	Time	Yield (%)
1	1.05	1	0.02	0.04	0.04	2	3 hours	23
2	1.05	1	0.02	0.08	0.08	4.5	Overnight	80

2.2.6. Pd-Catalyzed OTs-Azacoumarin (167) Addition Experiment to the 4-Position of 1,4-Disubstituted 1,2,3-Triazole Structure (169)

After all attempts were failed, as an alternative 1,4-disubstituted 1,2,3-triazole (**169**) was prepared and then coupling with 4-OTs modified 1-azacoumarin (**167**) was tried in the presence of Pd catalysis. A similar approach was used in the coupling of benzothiazoles to ariyltriflates in the literature.[71] However, all attempts again failed as shown in Figure 2.7

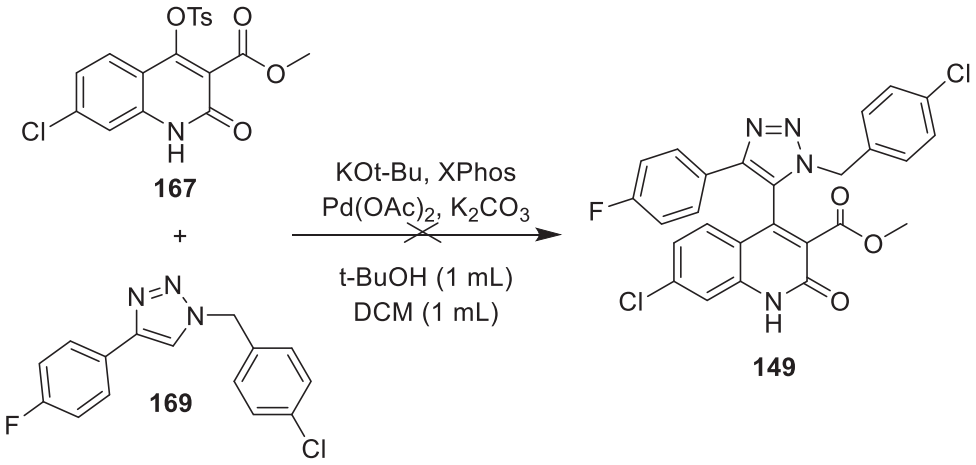


Figure 2.7. Pd catalyzed (**167**) addition to (**169**)

2.3. Studies Toward 1,4-Disubstituted 1,2,3-Triazole (150) From 1-Azacoumarin (163)

After all the efforts to bind OTs-azacoumarin (**164**) directly to 1,2,3-triazole or form 1,2,3-triazole were failed, the Sonogashira reaction of 4-OTs modulated 1-azacomarin derivative (**167**) with Trimethylsilylacetylene was studied as an alternative method. (Figure 2.8)

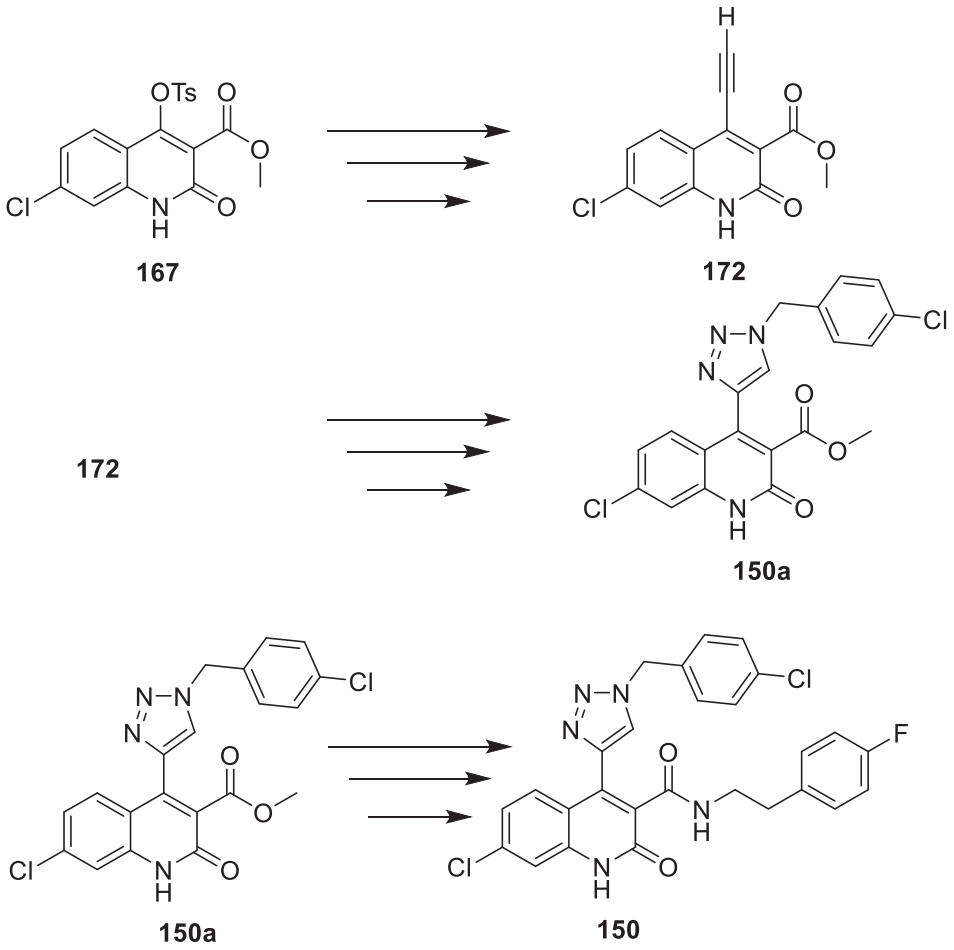


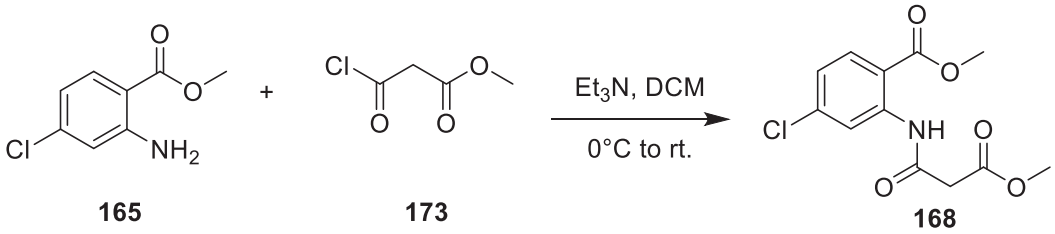
Figure 2.8. **150** from 1-azacoumarin (**166**)

2.3.1. New Method for Amid Intermediate (168)

An alternative to the amide formation experiment reaction, was considered because HATU was expensive. Methyl malonyl chloride (**173**) was added to methyl-2-

amino-4-chlorobenzoate (**165**) in dry dichloromethane in the presence of triethylamine and amide intermediate (**168**) was obtained repeatedly. If dry dichloromethane is not used in the reaction the reaction does not take place. Because it is very sensitive to even small amounts of moisture. (Table 2.10)

Table 2.10 Addition of methyl malonyl chloride to aniline (**165**)



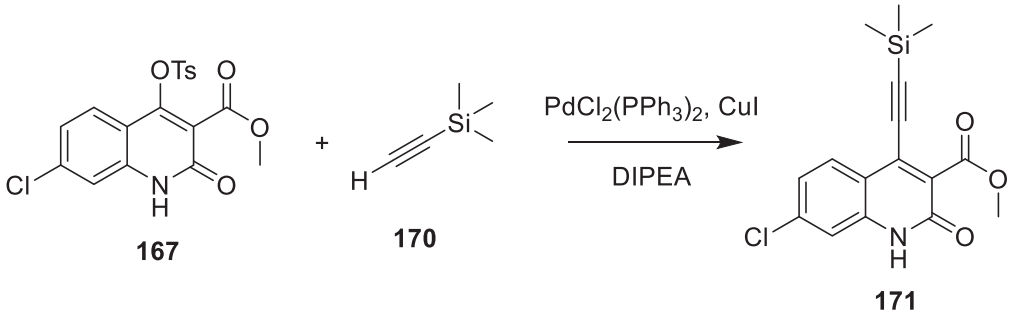
165 **173** **168**

Entry	165 (eq)	173 (eq)	Et_3N (eq)	DCM (mL)	Time (hours)	Yield
1	1	1.1	3	3.5	3	92%
2	1	2	3	8	4	94%
3	1	2	3	10	5.5	94%

2.3.2. Sonogashira Reactions with Trimethylsilylacetylene (170)

For trimethylsilylacetylene substituted 1-azacoumarin (**168**) the targeted coumarin derivative (**171**) was obtained with 30% yield in the reaction carried out at 60°C under the catalysis of $PdCl_2(PPh_3)_2$ and CuI. Due to the low yield in this reaction, it was tried to increase the yield by changing the reaction conditions. The biggest difficulty in this reaction is the low solubility of OTs-azacoumarin (**167**) in acetonitrile. However, solubility increases with temperature. And lower yields were obtained in the reactions carried out in acetonitrile at lower temperatures for 5 hours - 1 night. Although the starting material **167** is completely dissolved in THF, it has been observed that it turns into unwanted side products in a short time. Although starting material **167** is soluble in DMF, no reaction formation was observed during overnight in TLC control. (Table 2.11)

Table 2.11 Studies for trimethylsilylacetylene substituted 1-azacoumarin (**171**) from **167**



Entry	167 (eq)	170 (eq)	$PdCl_2(PPh_3)_2$ (eq)	CuI (eq)	DIPEA (eq)	Solvent (mL)	Conditions	Yield
1	1	1.2	0.05	0.05	1.25	CH_3CN 10	60 °C Overnight	30%
2	1	1.2	0.05	0.05	1.25	CH_3CN 8	45°C – 5 hours	6%
3	1	1.5	0.05	0.05	1.5	CH_3CN 6	40 °C Overnight	No rxn.
4	1	1.2	0.05	0.05	1.25	CH_3CN 5	rt. Overnight	14%
5	1	1.2	0.05	0.05	1.25	THF 5	rt. 2.5 hours	No rxn.
6	1	1.2	0.1	0.2	3	DMF 3.5	rt. Overnight	No rxn.

When the Sonogashira reaction with trimethylsilylacetylene (**170**) over OTs-azacoumarin (**167**) gave low yields of product, a stronger leaving group, ONs-azacoumarin (**174**) was synthesized in similar way (yield 14% - 20%). (Figure 2.9)

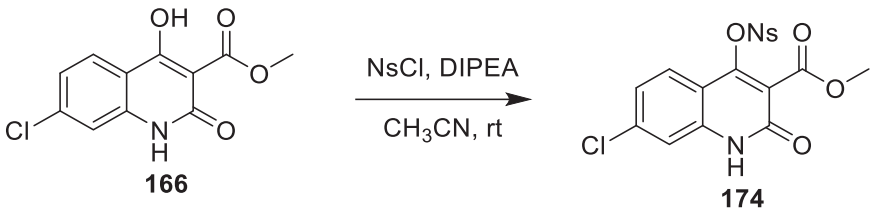


Figure 2.9. Obtaining of ONs-azacoumarin (**174**)

The desired product trimethylsilylacetylene substituted 1-azacoumarin (**171**) was obtained in 65% yield from Sonogashira reaction of ONs-azacoumarin (**174**). (Figure 2.10)

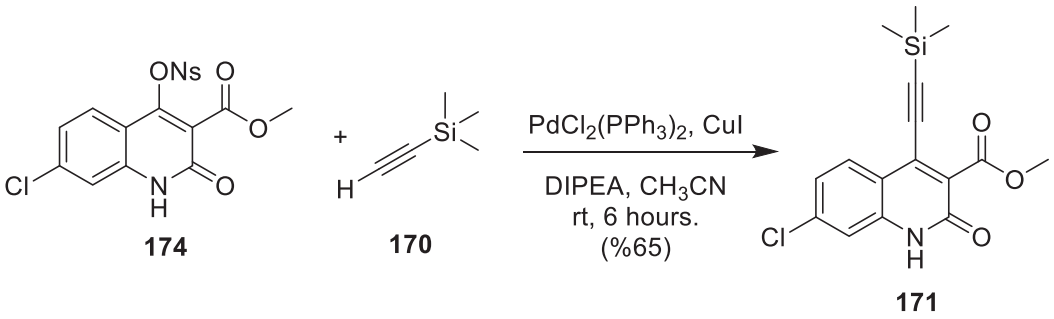


Figure 2.10. Sonogashira reaction of ONs-coumarin (**174**) with trimethylsilylacetylene (**170**)

2.3.3. Studies Towards Acetylene Substituted 1-Azacoumarin (172)

In the next step, it was tried to remove the Me_3Si group on the alkyne in trimethylsilylacetylene substituted 1-azacoumarin (**171**). Firstly, the reaction with K_2CO_3 in dichloromethane and methanol was tried, but only 15% yield was obtained. (Figure 2.11)

In the next methodology, the reaction with TBAF in dry THF was tried and the desired product was **(172**) obtained with a yield of 77%. (Table 2.12)

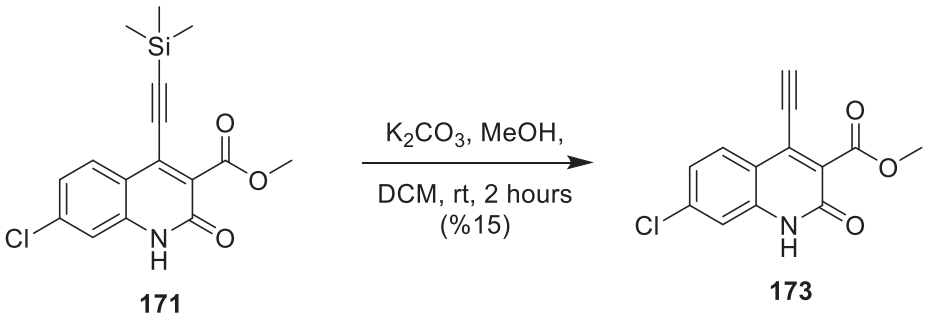
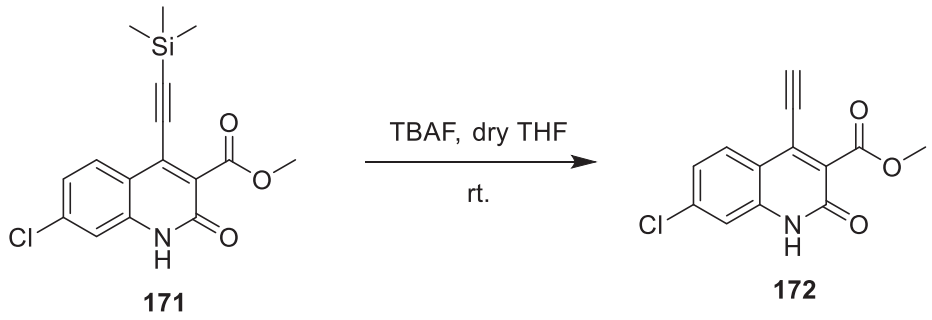


Figure 2.11. Removal of TMS group with K_2CO_3 in dichloromethane and methanol

Table 2.12 Removal of TMS group with TBAF in dry THF



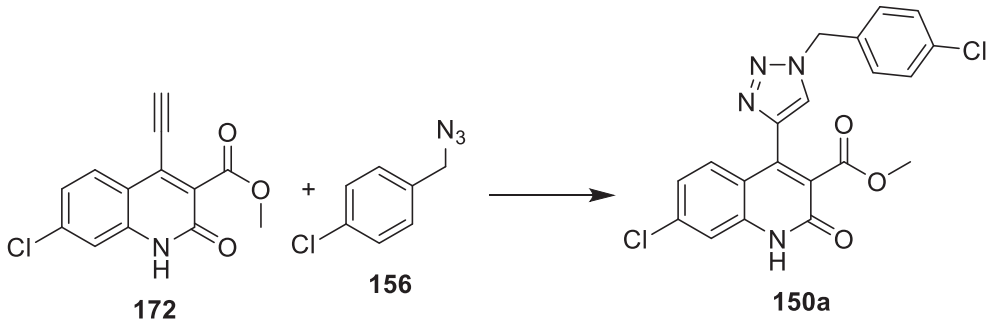
Entry	171 (eq)	TBAF (eq)	THF (mL)	Time	Yield (%)
1	1	2	2	45 minutes	77
2	1	1	2	1 hour	62
3	1	3	3	1 hour	80

2.3.4. Triazole Studies from the Acetylene Substituted 1-Azacoumarin (172) By Click Chemistry

After forming the compound of **172**, click chemistry was tried with Cu (I) catalyst. In the first experiments, it was thought that the efficiency of the experiments was low because it was studied on a small scale, but later it was understood that DCM

was not a suitable solvent. The yield increased dramatically when switching to EtOAc as the solvent. (Table 2.13)

Table 2.13 CuI catalyzed click chemistry for the synthesis of **150a**

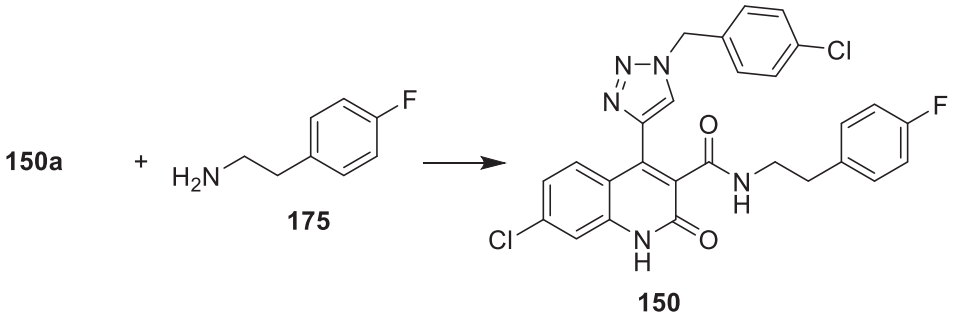


Entry	172 (eq)	156 (eq)	CuI (eq)	Dipea (eq)	HOAc (eq)	Solvent (mL)	Yield (%)
1	1	1	0.02	0.08	0.08	DCM 3	No rxn.
2	1	1.05	0.02	0.08	0.08	DCM 5	22
3	1	1.05	0.02	0.08	0.08	EtOAc 10	66
4	1	1.05	0.02	0.08	0.08	EtOAc 11	37
5	1	1.05	0.02	0.08	0.08	EtOAc 11	81

2.3.5. Studies Towards 150

After the triazole structure (**150a**) was formed, we aimed to form amide by heating 4-(fluorophenyl)ethylamine to the structure. When we could not see the product in the experiments carried out in reflux, it was thought that the amine evaporates without reacting because it is very volatile. Later the reaction was repeated in a closed reaction tube, but the results was same (Table 2.14)

Table 2.14 Studies towards the preparation of compound **150**

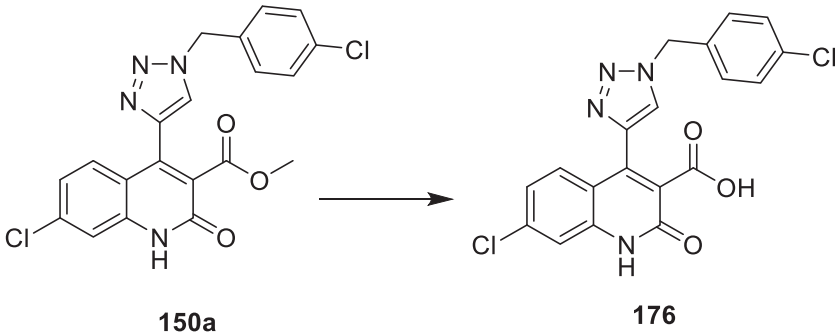


Entry	150a (eq)	175 (eq)	Solvent (mL)	Conditions	Result
1	1	1.2	MeOH 12	65 °C reflux	No rxn.
2	1	1.2	MeOH 3	65 °C reflux	No rxn.
3	1	4	DMF 2	75 °C reflux	No rxn.
4	1	4	DMF 2	65 °C closed	No rxn.

2.3.6. Hydrolysis of compound 150a

When amine could not be attached to the ester, it was aimed to convert it to a carboxylic acid first and then to form an amide by using a coupling agent like HATU. But all attempts failed. The reaction does not proceed, probably the H atom of methylene between triazole and 4-chlorophenyl group is quite acidic. (Table 2.15)

Table 2.15 Studies for hydrolysis of compound **150a**



Entry	150a (mg)	Base	Solvent	Condition	Result
1	30	1 mL of 2M NaOH	1 mL THF	rt	Side Products
2	12	0.16 mL of 1M LiOH	1 mL Ethanol 0.5 ml H_2O	rt	Trace
3	50	0.6 mL of 1M NaOH	2 mL Ethanol 0.5 ml H_2O 2 ml THF	rt	Trace

2.3.7. Alternative Synthetic Route for Compound 150

Since it was not possible to hydrolyze the ester in the presence of triazole (**176**), the order of the reactions were switched and click chemistry was tried after hydrolysis of the ester group. (Figure 2.12)

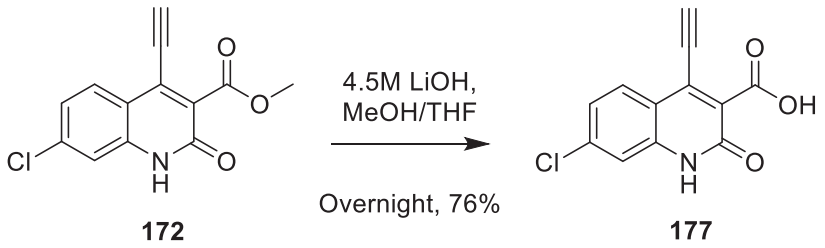
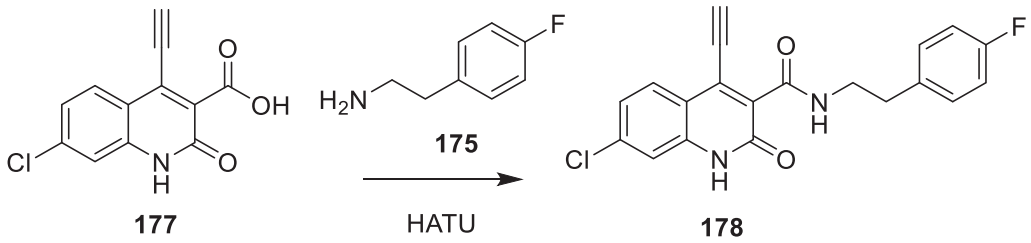


Figure 2.12. Hydrolysis of acetylene substituted 1-azacoumarin (**172**)

After succeeding in hydrolysis of acetylene substituted 1-azacoumarin (**172**), conversion of (**177**) to corresponding amide (**178**) was attempted in the presence of HATU. (Figure 2.13)

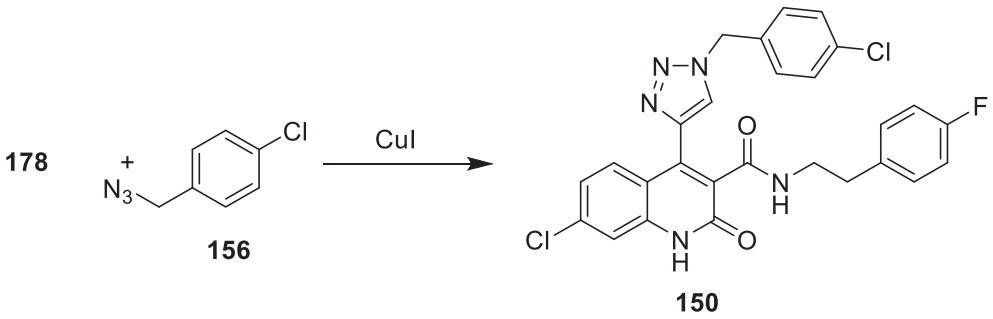


Reagents and Conditions: 1 eq of **177**, 4 eq of **175**, 2.2 eq of HATU, 4 eq of DIPEA in rt, overnight

Figure 2.13. Synthesis of 178 from Acetylene Substituted 1-Azacoumarin (**172**)

As a result of the experiment, 30 mg of crude product was collected by workup. The crude NMR shows the signals from the amide. However, due to the small amount of substance, the next step was tried without column purification. (Table 2.16)

Table 2.16 Final product (**150**) from **178**



Entry	178 (eq)	156 (eq)	CuI (eq)	Dipea (eq)	HOAc (eq)	Solvent	Conditions	Result
1	1	1.05	0.02	0.08	0.08	1 mL DMSO 2 mL EtOAc	rt – closed Overnight	No rxn.
2	1	2.1	0.02	0.08	0.08	1.5 mL DMSO	rt – closed Overnight	No rxn.
3	1	2.1	0.02	0.08	0.08	1 mL DMSO	rt – closed Overnight	No rxn.

2.4. Studies Towards 149 by Nickel Catalyzed Cycloaddition Reaction

As an alternative route to prepare compound **149** was shown in Figure 2.29. In this synthesis 4-acetylene substituted coumarin (**172**) will be reacted with benzylazide **156** to give 1,5-disubstituted triazole derivative (**179**) and then coupling with aryl iodide in the presence of Pd catalyst should give compound **149**.[33]

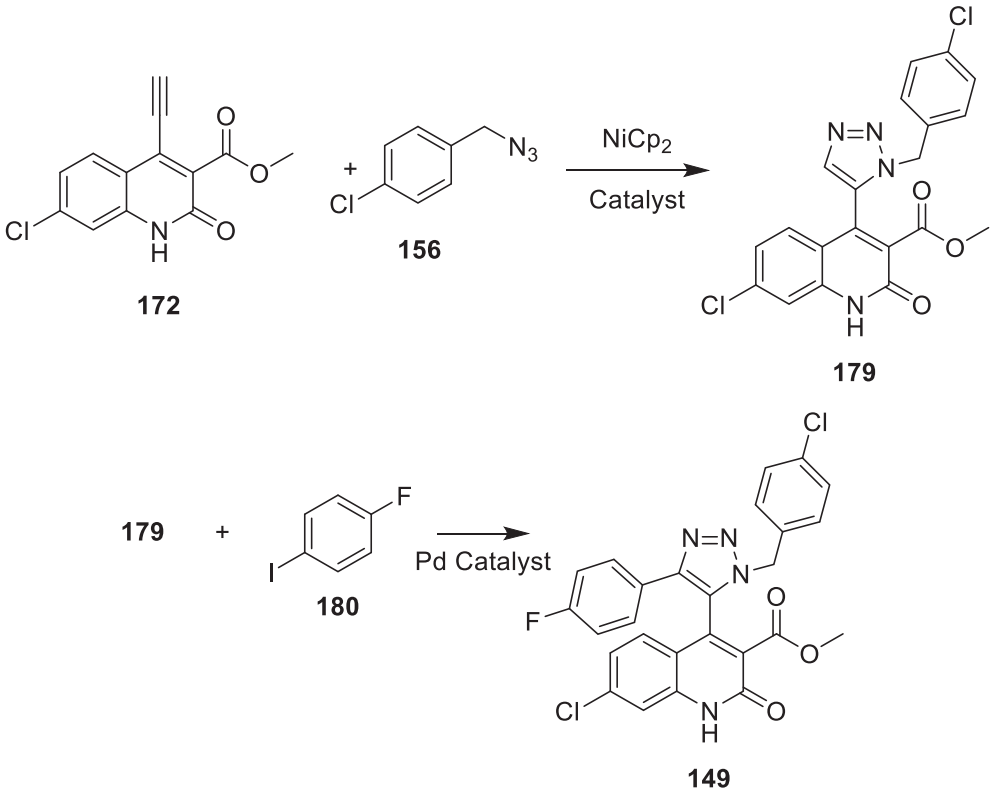


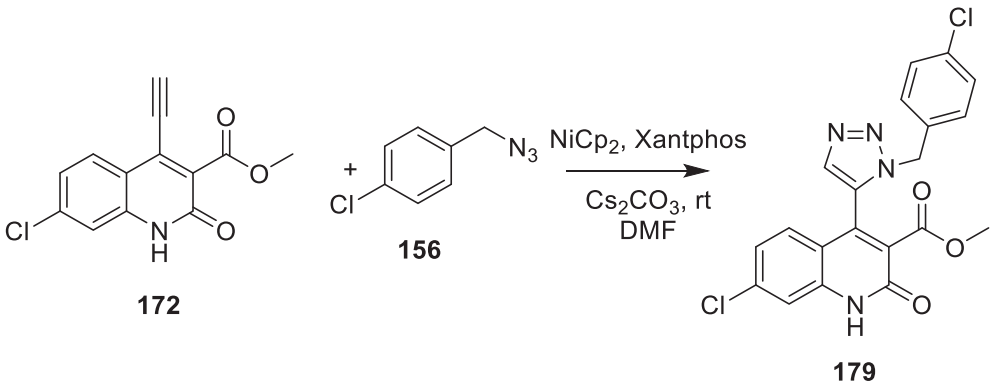
Figure 2.14. Studies Towards **149** by Nickel Catalyzed Triazole Formation

2.4.1. Nickel Catalyzed 1,2,3-Triazole Formation

During the early trials for the preparation of 1,5-disubstituted 1,2,3-triazole, sequential addition of niclocene and xantphos and then other reagents were performed in one product. (Table 2.17 Entry 1 and 2) In these trials no product formation was observed in TLC and crude H-NMR. Later, in the third trial niclocene and xantphos were mixed and stirred vigorously in DMF to produce dark green solution at 0 °C before addition to the mixture of other reagents. Reaction was carried out in open

atmosphere at room temperature and 4.5 mg (6%) of target compound **179** was purified successfully.

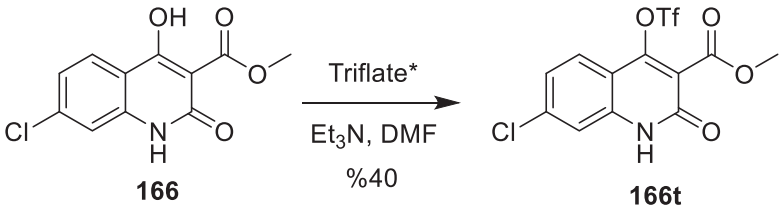
Table 2.17 Studies of Nickel catalyzed Cycloaddition



Entry	Niclocene (mg)	Xantphos (mg)	Cs_2CO_3 (mg)	172 (mg)	156 (µL)	Yield (%)
1	3	10	52	50	30	No rxn.
2	3	10	52	50	60	No rxn.
3	7	20	52	50	30	6

2.4.2. Triflate Modulated 1-Azacoumarin

Due to the need for a stronger leaving group for the Sonogashira reaction, the triflate modulated 1-azacoumarin structure was attempted to be synthesized as shown in Figure 2.15.



* Triflate : n-phenyl-bis(trifluoromethanesulfonimide)

Figure 2.15. Triflate modulated 1-azacoumarin

CHAPTER 3

EXPERIMENTAL

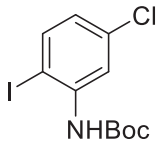
3.1. General Methods

The chemicals and solvents were commercial quality and used as purchased from Carlo Erba, Sigma-Aldrich and ABCR. Tetrahydrofuran, dichloromethane and acetonitrile were dried with 4Å molecular sieve. DMF were dried with the MBraun solvent purification system. Unless stated otherwise, all experiments were conducted under a nitrogen atmosphere. Reactions were monitored by thin layer chromatography by using Merck TLC plates (Silica gel 60 F254). Chromatographic purification processes were performed with silica gel of 70-230 mesh size. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian 400-MR (400 MHz) spectrometer. Chemical shifts were reported in δ (ppm). $CDCl_3$ peaks were used as reference for ^{1}H NMR (7.26 ppm) and 77.36 spectra for ^{13}C NMR. DMSO peaks were used as reference for ^{1}H NMR (2.5 ppm) and 39.5 spectra for ^{13}C NMR.

3.1.1. Tert-butyl (5-chloro-2-iodophenyl)carbamate (152)

In a two-necked reaction flask, 460 mg of 5-chloro-2-iodoaniline (1.82 mmol, 1 eq.) is dissolved in THF to a final concentration of 0.15 M and heated to 70 °C. 25 mg of DMAP (0.2 mmol, 0.1 eq.) and 915 mg of Boc_2O (4.19 mmol, 2.3 eq.) are added over it. Experiment is stirred at 70 °C under nitrogen atmosphere. After overnight stirring the reaction is quenched by addition of 50 mL of 0.05 M HCl. The crude product is extracted with ethyl acetate (3x30 mL) and then the combined organic phase was dried over anhydrous $MgSO_4$. Organic layer is filtered and concentrated under reduced pressure. The crude product is dissolved in 15 mL of MeOH and 0.15 M K_2CO_3 (0.76 mmol, 0.4 eq.) is added in it. The mixture is stirred at 70 °C under nitrogen atmosphere until all di-Boc product is consumed (Checking by TLC). After the completion of the reaction, same workup procedure is repeated one more time.

Purification by column chromatography (EtOAc/Hex; 1:14) resulted 158 mg of product desired as white solid with 25% yield.

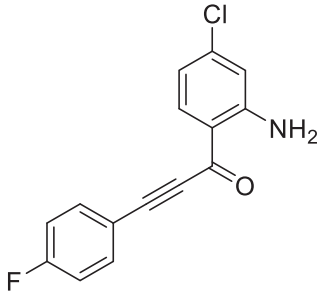


Rf: 0.83 (EtOAc-Hexane, 1:10)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.55 (d, *J* = 8.5 Hz, 1H), 6.81 (s, 1H), 6.70 (d, *J* = 8.5 Hz, 1H), 1.49 (s, 9H).

3.1.2. 1-(2-Amino-4-chlorophenyl)-3-(4-fluorophenyl)prop-2-yn-1-one (161)

In a heat gun dried and cooled, sealable reaction tube, 100 mg of 5-chloro-2-iodoaniline (0.394 mmol, 1 eq.) is dissolved in 5 mL of toluene. Then 57 mg of 1-ethynyl-4-fluorobenzene (0.474 mmol, 1.2 eq.), 591 mg of $CsOH.H_2O$ (3.94 mmol, 10 eq.), 2.2 mg of $Pd(OAc)_2$ (0.001 mmol, 2.5% eq.), 10.5 mg of PPh_3 (0.04 mmol, 10% eq.) and 470 μL of (15 eq.) chloroform are added, and the lid is closed. The mixture is stirred at 70 °C under nitrogen atmosphere then reaction mixture is filtered off over celite-silica and concentrated under reduced pressure. Purification by column chromatography (EtOAc/Hex; 1:12) resulted 26 mg of desired product as yellow oily with 25% yield.



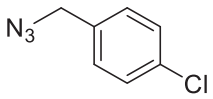
Rf: 0.19 (EtOAc-Hexane, 1:10)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 9.1 Hz, 1H), 7.65 (dd, *J* = 8.9, 5.3 Hz, 2H), 7.11 (t, *J* = 8.7 Hz, 2H), 6.67 (dd, *J* = 4.5, 2.6 Hz, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 178.60, 165.23, 162.71, 151.74, 141.73, 135.89, 135.25, 135.16, 117.47, 116.88, 116.42, 116.20, 116.15, 91.72, 86.79.

3.1.3. 1-(Azidomethyl)-4-chlorobenzene (156)

In a two-necked flask, 150 mg of sodium azide (2.31 mmol, 1.1 eq.) is dissolved in 3 mL of DMF. 336 mg of 4-chlorobenzylchloride (237 µL, 2.1 mmol, 1 eq.) is added on it. Experiment is stirred at 60 °C under nitrogen atmosphere. After 6 hours of stirring, reaction is quenched with 300 mL water. The crude product is extracted with ethyl acetate (3x50 mL) and then the combined organic phase is dried over anhydrous $MgSO_4$. The desired product is obtained without any purification in a yield of 69% as colorless oil.

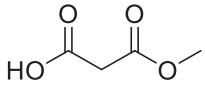


Rf: 0.65 (EtOAc-Hexane, 1:10)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, *J* = 8.5 Hz, 2H), 7.23 (d, *J* = 8.6 Hz, 2H), 4.29 (s, 2H).

3.1.4. 3-Methoxy-3-oxopropanoic acid (162)

In a two-neck flask, 160 mg of dimethyl malonate (139 µL, 1.2 mmol) is dissolved in 2 mL of THF and cooled to 0 °C by adding 20 mL of water. Then 8 mL of 0.25 M KOH is added dropwise and mixed for 1 hour at 0 °C. At the end of the period, 1M HCl solution is added to the mixture and acidified until pH = 2-3. The mixture is then saturated with NaCl and extracted with 80 mL of EtOAc (4x20 mL). The target product is obtained in a yield of 125 mg (87%) without further purification.



Rf: 0.13 (1% MeOH containing EtOAc-Hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.27 (s, 1H), 3.75 (s, 3H), 3.44 (s, 2H).

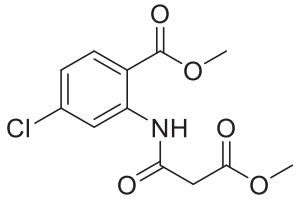
3.1.5. Methyl 4-chloro-2-(3-methoxy-3-oxopropanamido)benzoate (168)

In a two-neck heart-shaped reaction flask, 200 mg of methyl-2-amino-4-chlorobenzoate (1.08 mmol, 1 eq) is dissolved in 3 mL of dry DCM. 140 mg of

monomethyl malonate (1.2 mmol, 1.1 eq), 465 mg of HATU (1.2 mmol, 1.1 eq) and 280 mg of DIPEA (2.16 mmol, 2 eq) are added under nitrogen atmosphere and it is closed with septa. After stirring at room temperature for 20 hours, the solvent is evaporated under vacuum. Purification by column chromatography (EtOAc/Hex; 1:3) resulted desired 220 mg product as white solid with 71% yield.

Alternative Method of Methyl 4-Chloro-2-(3-methoxy-3-oxopropanamido)benzoate (168)

In a two-neck heart-type reaction flask, under a nitrogen atmosphere, dissolve 200 mg of methyl-2-amino-4-chlorobenzoate (1.08 mmol, 1 eq.) and 450 µL of triethylamine (3.23 mmol, 3 eq.) in 3 mL of dry DCM at 0°C. 162 mg of methylmalonate (1.18 mmol, 1.1 eq.) is dissolved in 2 mL of dry DCM and added to the reaction mixture. The reaction flask is closed with septa. After the mixture is stirred for 10 minutes, it is brought to room temperature and then the reaction is completed after three hours under TLC control, it is stopped by adding 100 mL 5% $NaHCO_3$. The resulting mixture is extracted with 3x25 mL of DCM. After the combined organic phase is washed with 100 mL of concentrated NaCl solution, the organic phase is dried with $MgSO_4$, and the solvent is removed under vacuum. Purification by column chromatography (EtOAc/Hex; 1:2) resulted 240 mg of desired product as white solid with 92% yield.

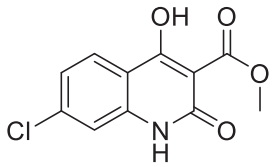


Rf: 0.33 (EtOAc-Hexane, 1:2)

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.52 (s, 1H), 8.78 (d, *J* = 2.1 Hz, 1H), 7.96 (d, *J* = 8.6 Hz, 1H), 7.08 (dd, *J* = 8.6, 2.1 Hz, 1H), 3.94 (s, 3H), 3.81 (s, 3H), 3.53 (s, 2H).

3.1.6. Methyl 7-chloro-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylate (166)

Under nitrogen atmosphere, 143 mg of potassium tert-butoxide (1.28 mmol, 2.2 eq) in a single neck reaction flask dissolved with 4 mL of dry THF. Then 165 mg of malonilamide (**171**) (0.58 mmol, 1 eq.) dissolved in 2 mL of THF is added. The reaction flask is closed with septa. After stirring for five hours, the experiment is stopped by adding HCl solution to adjust pH=2. Mixture was extracted with 50 mL of EtOAc. The solids formed in the organic phase is filtered through filter paper. Water phase is extracted 50 mL of EtOAc once more. The combined organic phases are washed with 100 ml of concentrated NaCl. The pure product, which has very low solubility in EtOAc and DCM on filter paper, is obtained as 145 mg (98% yield) white solid.



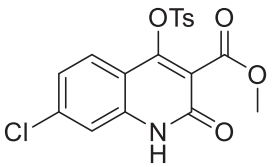
Rf: 0.28 (3% MeOH in chloroform)

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 13.24 (s, 1H), 11.58 (s, 1H), 7.90 (d, *J* = 8.6 Hz, 1H), 7.27-7.18 (m, 2H), 3.82 (s, 3H)

3.1.7. Methyl 2-Oxo-4-(tosyloxy)-1,2-dihydroquinoline-3-carboxylate (167)

200 mg of 1-azacoumarin (**166**) (0.79 mmol, 1 eq.) is dissolved in 18 mL of dry acetonitrile and then 166 mg of TsCl (0.87 mmol, 1.1 eq.) and 306 mg of DIPEA (2.37 mmol, 3 eq.) are added. It is shaken every 10 minutes until there is no insoluble substance present in a closed vessel under nitrogen gas. Reaction is quenched with 100 mL $NaHCO_3$, 1.5 hours later. Then mixture is extracted 3 times (40 mL) with EtOAc. Purification by column chromatography (EtOAc/Hex; 1:1) resulted 110 mg of desired product as white solid with 34% yield.

Rf: 0.40 (EtOAc-Hexane, 1:1)

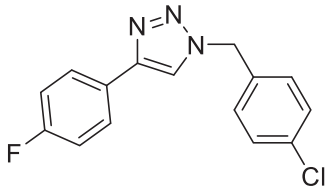


^{1}H NMR (400 MHz, $CDCl_3$) δ 12.45 (s, 1H), 7.87 (d, *J* = 8.3 Hz, 2H), 7.62 (d, *J* = 8.8 Hz, 1H), 7.44-7.37 (m, 3H), 7.15 (dd, *J* = 8.8, 1.9 Hz, 1H), 3.77 (s, 3H), 2.49 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 162.52, 165.23, 161.76, 153.86, 146.57, 139.56, 139.00, 132.35, 130.15, 128.53, 126.17, 124.25, 116.04, 114.34, 52.87, 21.81.

3.1.8. 1-(4-Chlorobenzyl)-4-(4-fluorophenyl)-1H-1,2,3-triazole (169)

In a two-necked flask, 237 mg of 1-ethinyl-4-fluorobenzene (1.97 mmol, 1 eq.) is dissolved with 10 mL of dry DCM and then 350 mg of 4-chlorobenzyl (2.07 mmol, 1.05 eq.) in 3 mL of dry DCM. Finally, 1 mL of the stock solution (300 µL DIPEA, 90 µL HOAc, 10 mL DCM) and 7.5 mg of CuI (0.04 mmol, 0.02 eq.) are added to the reaction mixture. After stirring the reaction mixture under nitrogen atmosphere for 16 hours at room temperature, 100 mL of distilled water is added to it and transferred to the separatory funnel. The mixture is extracted with 150 mL of DCM (3x50 mL). The combined organic phases are washed with 100 ml of concentrated NaCl solution, and the organic phase is dried with $MgSO_4$, and the solvent is removed under vacuum. The target product is obtained in a yield of 500 mg (88%) without further purification.



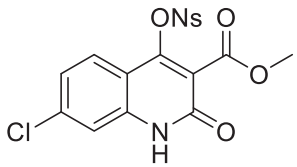
Rf: 0.40 (EtOAc-Hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (dd, *J* = 7.8, 5.4 Hz, 2H), 7.61 (s 1H), 7.35 (d, *J* = 7.6 Hz, 2H), 7.23 (d, *J* = 8.5 Hz, 2H), 7.08 (t, *J* = 8.2 Hz, 2H), 5.52 (s, 1H).

3.1.9. Methyl 7-chloro-4-(((4-nitrophenyl)sulfonyl)oxy)-2-oxo-1,2-dihydroquinoline-3 carboxylate (174)

Under nitrogen atmosphere, 210 mg of 4-hydroxycoumarin (0.83 mmol, 1 eq.) is dissolved in 10 mL of acetonitrile in a one-necked reaction flask. After 10 minutes, 202

mg of nosy chloride (0.91 mmol, 1.1 eq.) and 321 mg of DIPEA (1.71 mmol, 3 eq.) are added. Then the flask is closed with septa and stirred for three hours. The experiment is stopped by adding 100 mL of saturated $NaHCO_3$. The resulting mixture is extracted with 3x40 mL of ethyl acetate. The combined organic phase is washed with 100 mL of brine and dried with $MgSO_4$ and then concentrated. When the crude product is purified by SiO_2 column chromatography with a 1:1 EtOAc-Hexane solvent system, 50 mg of desired product is obtained in 14% yield.



Rf: 0.70 (EtOAc-Hexane, 1:1)

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 12.59 (s, 1H), 8.49 (dt, J = 9.5, 2.2 Hz, 2H), 8.27 (dt, J = 9.5, 2.3 Hz, 2H), 7.47 (dd, J = 8.8, 1.7 Hz, 1H), 7.37 (d, J = 1.9 Hz, 1H), 7.29 – 7.23 (m, 1H), 3.57 (s, J = 1.9 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d$_6$) δ 162.07, 158.79, 151.49, 151.28, 139.67, 139.37, 137.93, 130.08, 125.80, 125.30, 123.26, 119.46, 115.13, 112.88, 52.71.

3.1.10. Methyl 7-chloro-2-oxo-4-((trimethylsilyl)ethynyl)-1,2-dihydroquinoline-3-carboxylate (171)

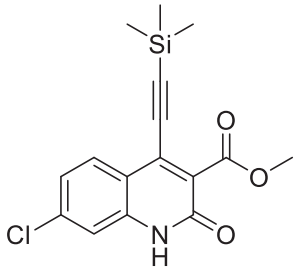
50 mg of 4-tosyl coumarin (0.123 mmol, 1 eq.) (**167**) in a single neck reaction flask under a nitrogen atmosphere dissolved in degassed acetonitrile. 5 mg of bis(triphenylphosphine)palladium chloride (0.006 mmol, 0.005 eq.), 2 mg of copper (I) iodide (0.006 mmol, 0.005 eq.) and 15 mg of trimethylsilylacetylene (0.148 mmol, 1.2 eq.) are added. Finally, 21 mg of DIPEA (0.154 mmol, 1.25 eq.) is added dropwise. The reaction flask is closed with septa. After mixing for one hour, it is observed that there is not enough conversion according to TLC. Due to the solubility problem, 4.5 mg of bis(triphenylphosphine)palladium chloride, 3 mg of copper (I) iodide and 60 μL of DIPEA are added together with 4 mL of dry acetonitrile to keep the catalyst concentrations constant. After mixing for four hours, it is heated to 60° and stirred under nitrogen gas overnight, since there is not enough change by TLC. The next day, 40 μL of trimethylsilylacetylene, 5 mg of bis(triphenylphosphine)palladium chloride

and 2.6 mg of copper(I) iodide was added. And the reaction mixture is mixed one more night. Then the reaction is quenched by adding water (100 mL) and extracted with 3x50 mL of EtOAc. The combined organic phase is washed with 100 mL of brine. The crude product is purified by SiO_2 column chromatography by using a 1:5 EtOAc-Hexane solvent system to obtain 13 mg (30% yield) of the desired product.

Alternative Method of Methyl 7-chloro-2-oxo-4-((trimethylsilyl)ethynyl)-1,2-dihydroquinoline-3-carboxylate (171)

7 mL of 50 mg of 4-nosylcoumarin (0.114 mmol, 1 eq.) (**174**) in a screw cap reaction tube under a nitrogen atmosphere dissolved in degassed acetonitrile. Then 4 mg of bis(triphenylphosphine)palladium chloride (0.006 mmol, 0.005 eq.), 1 mg of copper (I) iodide (0.006 mmol, 0.005 eq.) and 14 mg of trimethylsilylacetylene (20 µL, 0.138 mmol, 1.2 eq.), 19 mg of DIPEA (25 µL, 0.143 mmol, 1.25 eq.) are added and the lid is closed. After stirring at room temperature for 4 hours, 50 µL (0.345 mmol) of trimethylsilylacetylene is added to the reaction followed by TLC. After stirring overnight, the reaction is stopped by adding 10 mL diethyl ether. The final reaction mixture is filtered on a 1:1 silica gel-celite column. The crude product is purified by SiO_2 column chromatography with a 1:3 EtOAc-Hexane solvent system to obtain 25 mg (65% yield) of the desired product.

Rf: 0.50 (EtOAc-Hexane, 1:1)



^{1}H NMR (400 MHz, $CDCl_3$) δ 12.45 (s, 1H), 7.90 (d, J = 8.6 Hz, 1H), 7.41 (d, J = 1.7 Hz, 1H), 7.24 (dd, J = 8.6, 1.9 Hz, 1H), 4.00 (s, 3H), 0.30 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.26, 160.91, 138.83, 138.52, 132.18, 129.12, 128.64, 124.33, 117.18, 116.48, 112.16, 96.32, 53.01, -0.31.

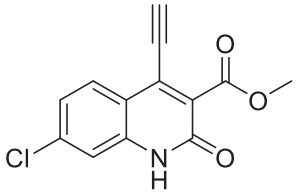
3.1.11. Methyl 7-chloro-4-ethynyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (172)

Under nitrogen atmosphere, in a single neck reaction flask, 13 mg compound of **171** (0.039 mmol, 1 eq.) is dissolved in 1.3 mL of methanol and 0.7 mL of DCM. In ice bath, 13.5 mg of potassium carbonate (0.0975 mmol, 2.5 eq.) was added then the flask is allowed warm to room temperature. After stirring for two hours at room temperature, the experiment is quenched by adding 5 mL of chloroform and filtered. After adding 100 mL of concentrated NaCl solution to the obtained solution, it is extracted with 3x50 mL of EtOAc. 1.5 mg (15% yield) of pure product is obtained without further purification.

Alternative Method of Methyl 7-chloro-4-ethynyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (172)

Under nitrogen atmosphere, 25 mg of compound **171** (0.075 mmol, 1 eq.) is dissolved in 8 mL of THF in a one-necked reaction flask then 1.5 mL acetic acid is added. Finally, 40 mg of TBAF (43 µL, 0.15 mmol, 2 eq.) is added then the flask is closed with septa. After stirring for 45 minutes at room temperature, the reaction is quenched by adding 100 mL of brine solution and extracted with 3x30 mL of ethyl acetate. 15 mg (77% yield) of pure product is obtained without further purification.

Rf: 0.51 (EtOAc-Hekzan, 1:1)

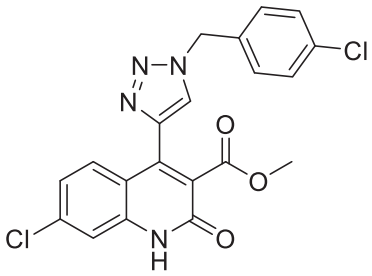


^{1}H NMR (400 MHz, $CDCl_3$) δ 12.14 (s, 1H), 7.91 (d, *J* = 8.7 Hz, 1H), 7.38 (d, *J* = 2.0 Hz, 1H), 7.22 (dd, *J* = 8.6, 2.0 Hz, 1H), 3.98 (s, 3H), 3.90 (s, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.24, 159.90, 138.84, 138.71, 131.22, 130.05, 128.60, 124.28, 117.10, 116.01, 92.34, 75.72, 53.15.

3.1.12. Methyl 7-chloro-4-(1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)-2-oxo-1,2 dihydroquinoline-3-carboxylate (150a)

Under nitrogen atmosphere, into a single neck reaction flask, 15 mg of 4-acetylenecoumarin (**172**) (0.057 mmol, 1 eq.), 13 mg of 4-chlorobenzylazide (0.059 mmol, 1.05 eq.), and 3 mg of copper (I) iodide (0.001 mmol, 0.02 eq.) are added. Then 1 mL of the stock solution obtained by dissolving 300 µL of DIPEA and 90 µL of HOAc in 10 mL of dichloromethane is added. The flask is closed with septa. After stirring under gas for 2.5 hours at room temperature, the reaction is quenched by adding 30 mL of water and extracted with 3x40 mL of DCM. The combined organic phase is washed with 100 mL of brine solution, then dried with $MgSO_4$ and concentrated. The crude product is purified by SiO_2 column chromatography with a 5:1 EtOAc-Hexane solvent system to obtain 6 mg (25% yield) of the desired product.



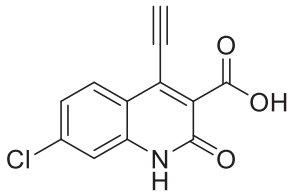
Rf: 0.57 (EtOAc-Hexane, 5:1)

^{1}H NMR (400 MHz, DMSO) δ 12.45 (s, 1H), 8.55 (s, 1H), 7.73 (d, *J* = 8.8 Hz, 1H), 7.49 (d, *J* = 8.5 Hz, 2H), 7.42 (d, *J* = 2.0 Hz, 1H), 7.38 (d, *J* = 8.5 Hz, 2H), 7.28 (dd, *J* = 8.8, 2.1 Hz, 1H), 5.75 (s, 2H), 3.56 (s, 3H).

^{13}C NMR (101 MHz, DMSO) δ 165.90, 158.78, 140.14, 139.99, 137.38, 136.69, 135.22, 133.43, 130.23, 129.55, 129.26, 127.31, 126.48, 123.20, 116.17, 115.45, 52.66, 52.62.

3.1.13. 7-Chloro-4-ethynyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (177)

In a single neck reaction flask, 18 mg of acetylene-substituted 1-azacoumarin (**172**) (0.068 mmol, 1eq.) is dissolved in 5 mL of THF. Then 1.5 mL of MeOH is added 282 mg of LiOH is dissolved in 1.5 mL of water and it is added to the reaction. The reaction, stirred at room temperature, is quenched the next day with 100 mL of water. 1 M HCl acid solution is added until pH = 2-3. The mixture is washed 3 times with 50 mL of EtOAc. 13 mg (76%) of the desired product is obtained without further purification.

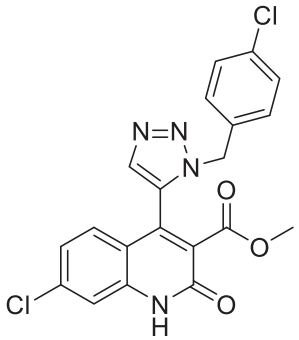


Rf: 0.1 (1% Acetic acid in EtOAc-Hexane, 1:1)

3.1.14. Methyl 7-Chloro-4-(1-(4-chlorobenzyl)-4-(4-fluorophenyl)-1H-1,2,3-triazol-5-yl)-2-oxo-1,2-dihydroquinoline-3-carboxylate (149)

In a beaker, 7 mg of Bis(cyclopentadienyl)nickel (0.032 mmol, 20 mol %) , 20 mg of 4,5bis(diphenylphosphino)-9,9 dimethylxanthene (0.032 mmol, 20 mol %)) and 2 mL DMF are added. It is stirred very quickly and strongly. When the characteristic green color is obtained it is added on the single-necked reaction flask which has 50 mg of compound **172** (0.19 mmol, 1.2 eq). Then 55 mg of Cs_2CO_3 (0.16 mmol, 1 eq) and 30 µL of 4-chlorobenzylazide (0.16 mmol, 1 eq) are added. After stirring in open atmosphere for two nights, the reaction is quenched by adding 200 mL of water and extracted with 3x50 mL of EtOAc. The combined organic phase is washed with 100 mL of brine solution, then dried with $MgSO_4$ and concentrated. The crude product is purified by SiO_2 column chromatography with a 1:1 EtOAc-Hexane solvent system to obtain 4.5 mg (4% yield) of the desired product.

Rf: 0.45 (EtOAc-Hexane 1:1)

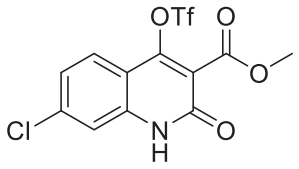


^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.70 (d, *J* = 8.9 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.29 (s, 1H), 7.22 (d, *J* = 8.3 Hz, 2H), 7.16 – 7.10 (m, 1H), 5.55 (s, 2H), 3.55 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.23, 160.43, 139.05, 138.45, 138.40, 135.40, 132.75, 129.70, 129.53, 129.48, 124.84, 124.31, 116.77, 116.13, 53.90, 53.59, 53.02, 29.85.

3.1.15. Methyl 7-Chloro-2-oxo-4-(((trifluoromethyl)sulfonyl)oxy)-1,2-dihydroquinoline-3-carboxylate (166t)

150 mg of OH-coumarin (0.6 mmol, 1 eq.) is added to a one-neck reaction flask and dissolved in 4 mL of dry DMF. 250 µL of triethylamine (1.8 mmol, 3 eq.) and 260 mg of n-phenyl-bis(trifluoromethanesulfonimide) (0.72 mmol, 1.2 eq.) are dissolved in 2 mL of dry DMF and added to the flask. The experiment which closed with septa, is quenched with 600 mL of water after stirring for four hours at room temperature. Reaction mixture is extracted 3 times (100 mL) with EtOAc. Then washed with 100 mL of concentrated NaCl solution. The crude product is purified by SiO_2 column chromatography with a 1:1 EtOAc-Hexane solvent system to obtain 90 mg (40% yield) of the desired product.



Rf: 0.55 (EtOAc-Hexane, 1:1)

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.65 (s, 1H), 7.76 (d, *J* = 8.8 Hz, 1H), 7.55 (d, *J* = 1.8 Hz, 1H), 7.35 (dd, *J* = 8.8, 1.9 Hz, 1H), 4.02 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 161.69, 161.18, 152.70, 140.79, 139.26, 125.27, 124.81, 123.22, 120.03, 118.38, 116.84, 116.75, 113.65, 112.64, 53.48.

CHAPTER 4

CONCLUSION

Cancer is a complex disease that needs highly advanced treatments, called the plague of our age so new molecules are needed for treatment. 1-Azacoumarin and 1,2,3-triazoles are two important skeletons that can be found in many valuable biological active compounds.

In this thesis 2 different novel 1,2,3-triazole substituted 1-azacoumarin derivatives were tried to be synthesized. Many different synthetic approach was tried during these synthetic pathways. One of the approach was producing diarlyl substituted ynone which can be transferred into a triazole substituted coumarine. In this trials transformation of ynone to triazole were failed. As an alternative acetylene substituted 1-azacoumarine was prepared and then click chemistry was used to prepare 1,2,3-triazole substituted 1-azcoumarine. Afterwards, conversion of ester at position 3- of 1-azacoumarin to amide by refluxing with 2-arylethylamine was failed.

As alternative conversion of ester to carboxylic acid was also failed in the presence of 1,2,3-triazole. Therefore, ester of substituted coumarine was hydrolyzed to carboxylic acid and then transformed into corresponding amide by adding 2-arylethylamine in the presence of HATU. Then it was tried to produce 1,2,3-triazole by click chemistry. Although ^{1}H NMR of the crude product indicate the formation of triazole structure could not be proven by ^{1}H NMR pure compound due to the presence of low amount of crude product.

For product **149**, formation of 1,5-disubstituted 1,2,3-triazole studies were performed under niclocene catalyst. Although this compound was isolated, we couldn't start to the aryl coupling reaction in the presence of low amount of 1,5-disubstituted 1,2,3-triazole substituted 1-azacoumarine derivative. Further studies are required to finish the synthesis.

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