SYNTHESIS OF 1,5-DISUBSTITUTED 1,2,3-TRIAZOLE MODIFIED AZACOUMARINS

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ABSTRACT

SYNTHESIS OF 1,5-DISUBSTITUTED 1,2,3-TRIAZOLE MODIFIED AZACOUMARINS

Cancer is a deadly disease that threatens human health and all life, and it is still a serious problem despite all scientific studies for more than half a century. Pharmacophores are a part of the structure of a drug (or drug candidate) responsible from the biological activity. This thesis is related with the synthesis of novel compounds having two well-known pharmacophore structures, 1,2,3-triazole and 1-azacoumarin. Both structures can be found in the structure of many biologically active molecules. Triazole modified coumarin derivatives are scarce in the literature.

In this study, we aimed to improve the synthetic route toward the synthesis of 1-azacoumarin derivative modified by 1,2,3-triazole group at position 4-. Synthesis starts with the conversion of methyl 4-chloroanthranilate to the corresponding 4-OH azacoumarin. Then it is transferred into the 4-OTf group by simply addition of Tf group to OH under basic condition. After Sonogashira reaction and removal of TMS group 4-alkynyl-1-azacoumarin was produced. At this point, conversion of alkyne into 1,5-disubstituted 1,2,3-triazole was examined in the presence of Cp_2Ni-Xantphos and RuCl(COD)Cp* catalytic systems but all of trials were failed probably due to the presence of ester group close to the reaction site. In further studies, design of the molecule will be reperformed and ester group will be moved over phenyl rings in order to test its biological activity over cancer cell lines.

ÖZET

1,5-DİSÜBSTİTÜELİ 1,2,3-TRİAZOL TÜREVLENDİRİLMİŞ AZAKUMARİNLERİN SENTEZİ

Kanser, insan sağlığını ve tüm yaşamı tehdit eden ölümcül bir hastalıktır ve yarım asrı aşkın süredir yapılan tüm bilimsel çalışmalara rağmen hala ciddi bir sorundur. Farmakoforlar, biyolojik aktiviteden sorumlu bir ilacın (veya ilaç adayının) yapısının bir parçasıdır. Bu tez, iyi bilinen iki farmakofor yapısı olan 1,2,3-triazol ve 1-azacoumarini bünyesinde barındıran yeni molekülün sentezi ile ilgilidir. Her iki yapı da biyolojik olarak aktif birçok molekülün yapısında bulunabilir. Triazol modifiyeli kumarin türevleri literatürde azdır.

Bu çalışmada 4- pozisyonundaki 1,2,3-triazol grubu ile modifiye edilmiş 1-azakumarin türevinin sentezine yönelik sentez yolunu geliştirmeyi amaçladık. Sentez, metil 4-kloroantranilat'ın ilgili 4-OH azakumarine dönüştürülmesiyle başlar. Daha sonra bazik koşul altında OH grubuna kolayca Tf grubunun eklenmesiyle 4-OTf grubuna aktarılır. Sonogashira reaksiyonundan ve akabinde TMS grubunun ayrılmasından sonra 4-alkinil-1-azakumarin üretildi. Bu noktada, alkinin 1,5-disübstitüeli 1,2,3-triazole dönüşümü, Cp_2Ni-Xantphos ve RuCl(COD)Cp* katalitik sistemleri varlığında incelenmiştir, ancak muhtemelen reaksiyon bölgesine yakın ester grubundan dolayı tüm denemeler başarısız olmuştur. İleriki çalışmalarda molekülün tasarımı yeniden yapılacak ve ester grubu fenil halkaları üzerinden taşınarak biyolojik aktivitesi kanser hücre hatları üzerinde test edilecektir.

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LIST OF ABBREVIATIONS

A549	Lung Cancer Cell Line
Ar	Aryl
Bn	Benzyl
bs	Broad Singlet
d	Doublet
dd	Doublet of Doublets
DCE	1,2-Dichloroethane
DCM	Dichloromethane
DEM	Diethylmalonate
DMA	Dimethylacetamide
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid
DPEphos	Bis[(2-diphenylphosphino)phenyl]ether
Et_3N	Triethylamine
EtOH	Ethanol
Eq.	Equivalent
h	Hour
HepG-2	Hepatoma Cancer Cell Line
m	Multiplet
MCF-7	Breast Cancer Cell Line
MeOH	Methanol
MesCOOH	Mesitylene Carboxylic Acid
mg	Milligram
MGC-803	Gastric Cancer Cell Line
min	Minute
µL	Microliter
µg	Microgram
mL	Milliliter
MSAA	Methane Sulfonic Acid Anhydrous

NMR	Nuclear magnetic resonance
NiAAC	Nickel Catalyzed Azide-Alkyne Cycloaddition
PC-3 and DU-145	Prostate Cancer Cell Lines
PEG	Polyethyleneglycol
Ph	Phenyl
$PhN(Tf)_2$	N-Phenyl-bis(trifluoromethanesulfonimide)
ROS	Reactive Oxygen Species
RT	Room Temperature
RuAAC	Ruthenium Catalyzed Azide-Alkyne Cycloaddition
s	Singlet
TBAB	Tetrabutylammonium bromide
TBAF	Tetra-n-butylammonium fluoride
TBHP	Tert-butyl hydroperoxide
THF	Tetrahydrofuran
TMS	Trimethylsilane
U-937	Leukemia Cancer Cell Line

CHAPTER 1

INTRODUCTION

Uncontrolled cell division, which occurs as a result of mutations in DNA that play a role in the regulation of cell proliferation, creates cancer. Age, cigarette use, sun exposure, radiation exposure, chemicals and other substances, some viruses and bacteria, specific hormones, a family history of cancer, alcohol use, a poor diet, a lack of exercise, or being overweight are among the most prevalent risk factors for cancer.

Each year, there are more cancer cases and deaths worldwide. Despite enormous advancements in medical science and technology, there are few effective treatments for cancer. Some of the cancer treatment types are surgery, radiotherapy, chemotherapy, immunotherapy, extracellular vesicle (EV) therapy, and stem cell therapies. The aim of treatment is to either stop the growth of cancer cells or kill them. The current emphasis is on utilizing methods to improve drug delivery, strengthen cells' capacity to fight cancer, and get rid of tumor resistance and metastasis. Despite scientific advancements, there are still issues with successful cancer therapy, creating both hurdles and potential for further research. As a result, further research should be done, and new treatment strategies should be developed.[1]

1.1. 1,2,3-Triazoles and Their Structures

1,2,3-Triazole is an aromatic heterocycle with a five-membered ring structure containing two carbon atoms and three nitrogen atoms. Monocyclic 1,2,3-triazoles, benzotriazoles, and 1,2,3-triazolium salts are the three main subgroups of 1,2,3-triazoles (Figure 1.1). Depending on where the NH proton is located, the monocyclic 1,2,3-triazoles are further classified into three subclasses. While the *4H*-1,2,3-triazole is nonaromatic, the *1H*- and *2H*-1,2,3-triazoles are aromatic and are in equilibrium in both

the gas phase and solution. Two isomeric versions of the 1,2,3- triazolium salts are also available. A bicyclic nitrogen heterocycle called benzotriazole is created when the benzene ring and the 4,5-positions of *1H*-1,2,3-triazole are fused. There are two tautomeric forms of it: *1H* and *2H*.[2]

Also, because they readily establish hydrogen bonds and are resistant to metabolic degradation, 1,2,3-triazoles can improve solubility and facilitate binding to biomolecular targets. Therefore, 1,2,3-triazoles are important structures in drug discovery.[3]

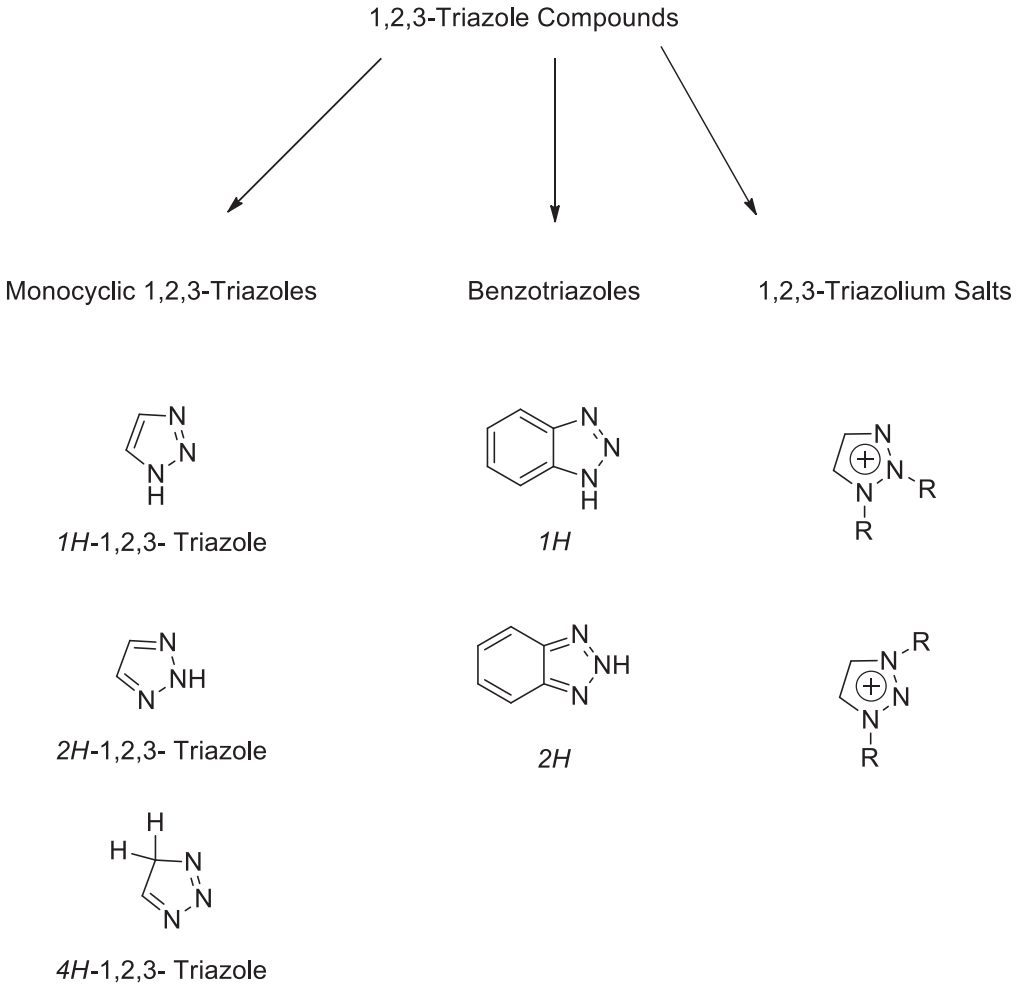


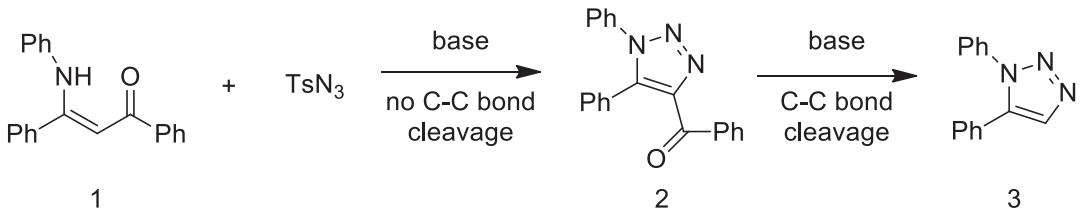
Figure 1.1. Classification of 1,2,3-triazole compounds

1.1.1. Preparation of 1,5-Disubstituted 1,2,3-Triazole Derivatives

In the literature, there are limited numbers of reactions for the preparation of 1,5-disubstituted 1,2,3-triazole derivatives. In this part of thesis some of these methods will be summarized.

1.1.1.1. Catalyst Free Synthesis Method

For the subsequent synthesis of 1,5-disubstituted 1,2,3-triazoles from 1,4,5-trisubstituted 1,2,3-triazoles via C-C bond cleavage, has been developed as an effective method. This methodology produced 1,2,3 triazoles with good substrate tolerance through Regitz diazo-transfer and C-C bond cleavage (Figure 1.2).[4]



Base: Cs_2CO_3, Yield (2): 65%, Yield (3): 19%
Base: DBU, Yield (2): 81%, Yield (3): trace
Base: KOt-Bu, Yield (2): 18%, Yield (3): 56%

Solvent: CH_3CN, Time: 30 min.

Figure 1.2. Base-promoted regiospecific synthesis of 1,4,5-trisubstituted 1,2,3-triazole and 1,5-disubstituted 1,2,3-triazole.

1.1.1.2. Catalyzed by $ZnEt_2$

The regioselective synthesis of 1,5-disubstituted 1,2,3-triazoles is also reported by using a gentle approach. At room temperature, the zinc-mediated reaction is effective on a variety of azido/alkynyl substrates. The alkyne (5) reacts with diethylzinc, and terminal H is replaced with an ethyl zinc group in *N*-methylimidazole. The reaction of this with azide (4) produces 1,5-disubstituted triazole (6) product (Figure 1.3).[5]

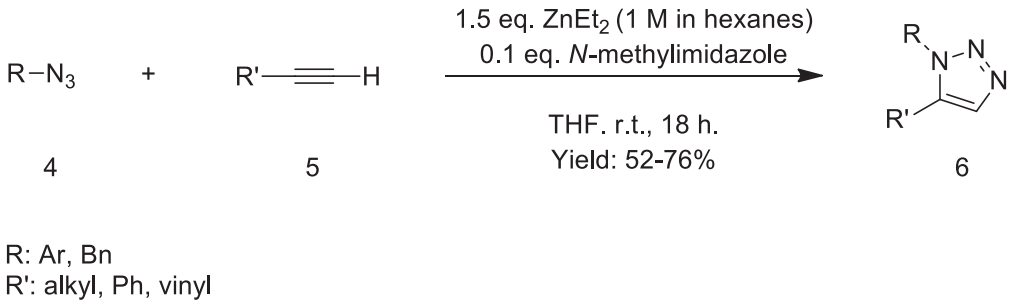


Figure 1.3. $ZnEt_2$ mediated azide-alkyne ligation to produce 1,5-disubstituted 1,2,3-triazoles.

1.1.1.3. Catalyzed by Cp_2Ni/Xantphos

Another methodology is reactions that are catalyzed by nickel complexes. Variety of reaction conditions on the model substrates 4-tert-butylbenzyl bromide (7) and phenylacetylene (8) were tried. Reaction conditions were applied with the combination of compound 7 (1.0 eq.), sodium azide (1.0 eq.), compound 8 (1.2 eq.) in the presence of nickelocene (Cp_2Ni) precatalyst (20 mol%), bidentate Xantphos ligand (20 mol%) and Cs_2CO_3 (1.0 eq.) at room temperature for 2 h in water. Azide was formed from benzyl bromide (7) in these conditions, but the cycloaddition was not observed. DMF (2 hours at room temperature), often used as a solvent for azide formation from bromide and shown to be efficient for the NiAAC, was evaluated. This solvent allowed the triazole formation with a good yield 71%, contrary to other solvents such as DCM, ethanol and THF under

the same reaction conditions. Similar reactions were performed in DMF solvent at higher temperatures. When the reaction was heated to 50 °C under microwave radiation for 1 hour, the yield was calculated as 73%, while when heated to higher temperatures such as 80 °C and 100 °C (again, for 1 hour), the yield decreased, possibly due to catalyst degradation. And finally, increasing the reaction time to 4 hours increased the yield, which was calculated as 86% and 89% at 50 °C (Figure 1.4).[6]

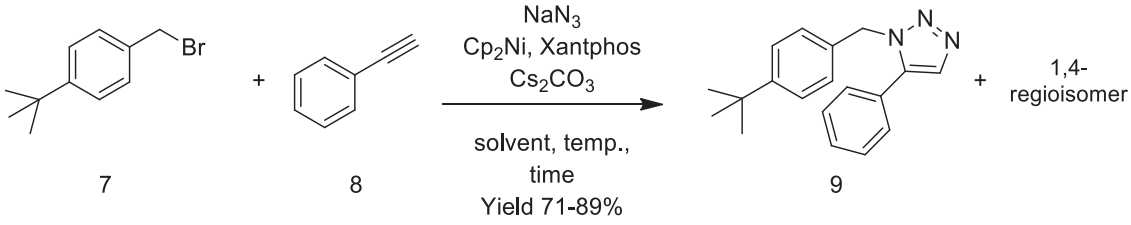


Figure 1.4. Formation of 1,5-disubstituted 1,2,3-triazoles by azides and NiAAC.

In a similar approach, Cp_2Ni-Xantphos catalytic system was used to react benzyl azide (10) and phenyl acetylene (8) to produce corresponding 1,5- and 1,4-disubstituted 1,2,3-triazole mixture (Figure 1.5). The yield is highest at 94% at the conditions specified in Figure 1.5. In addition, the yield is 91% when the reaction time is 1.5 hours and 90% when DMF and DCM are used instead of toluene.[7]

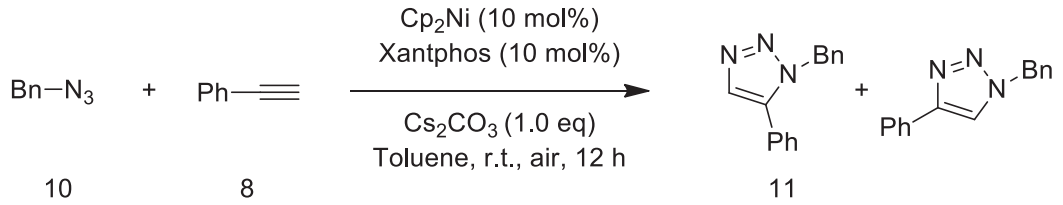


Figure 1.5. Synthesis of 1,4- and 1,5-disubstituted 1,2,3-triazole compounds via NiAAC in toluene at room temperature.

1.1.1.4. Catalyzed by Cp*RuCl(COD)

In the literature, one of the best well known reaction is the ruthenium-based catalyst to modulate the selectivity of the reactions between azide and alkyne functional groups. As shown in Figure 1.6 phenylacetylene (8) can be reacted with benzyl azide (10) in the presence of chloro(1,5-cyclooctadiene)(pentamethylcyclopentadiene)ruthenium and 1,5-disubstituted 1,2,3-triazole (11) can selectively be formed in good yields in DCE at 45 °C and in 30 minutes only.[8]

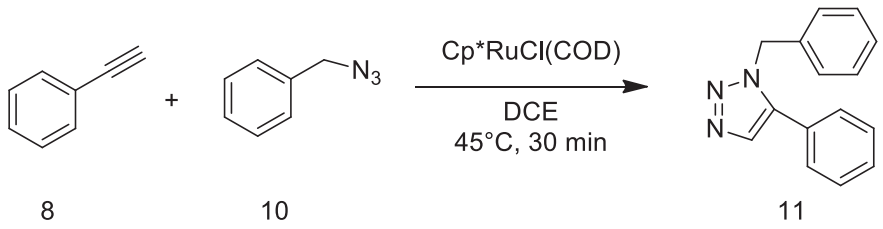


Figure 1.6. Preparation of 1-benzyl-5-phenyl-*1H*-1,2,3-triazole (11) via Cp*RuCl(COD) catalyzed benzyl azide (10) and phenylacetylene (8) cycloaddition reaction.

1.1.1.5. Catalyzed by Cp*RuCl(PPh$_3$)$_2$

Ortho-substituted 2-methoxyphenyl azide (12) reacted with four different alkynes (13) and contributed to the formation of 1,5-disubstituted 1,2,3-triazole (14) (Figure 1.7). When the R group was *i*-penthyl or cyclohexyl, it gave the desired 1-(2-methoxyphenyl)-5-alkylsubstituted-1,2,3-triazoles in 87% and 78% yields, respectively (over 12 hours). Next, compound 12 was reacted with the more steric groups such as 2-methoxyphenylacetylene (36 hours) and 1-naphthylacetylene (48 hours), and yields decreased to 58% and 7%, respectively, due to the steric nature.[9]

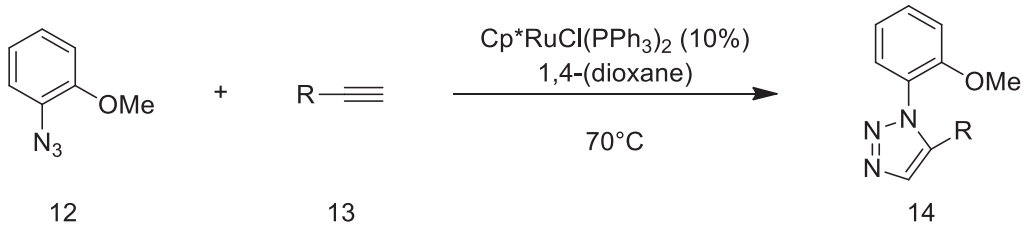


Figure 1.7. Synthesis of 1,5-disubstituted 1,2,3-triazole (14) with 2-methoxyphenyl azide (12) and alkyne derivatives (13).

An experimentally straightforward sequential one-pot RuAAC reaction is also described that produces 1,5-disubstituted *1H*-1,2,3-triazoles from an alkyl halide, sodium azide, and an alkyne (Figure 1.8). The organic azide is formed in-situ by treating the primary alkyl halide with sodium azide in DMA (95% conversion) under microwave heating. After additional microwave irradiation, the combination of [RuClCp*(PPh_3)$_2$] and the alkyne produced the required cycloaddition product. In the beginning, the RuAAC reaction between benzyl azide (10) and 3-ethynylpyridine (15) was investigated in various solvents while being heated in a microwave. Solvents such as dioxane, THF, and 2-MeTHF are ethers that can be used for completely conversion of alkyne to triazole (16).[10]

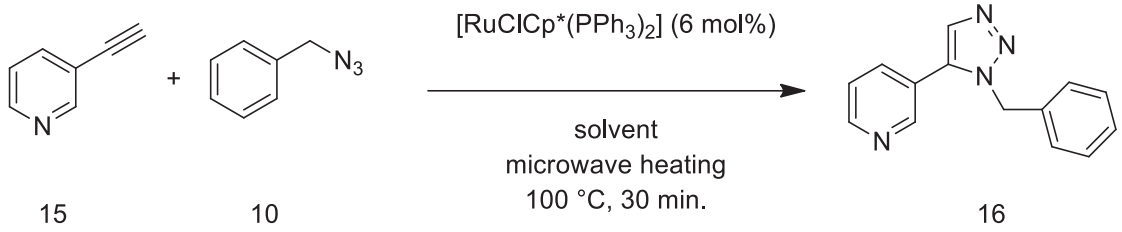


Figure 1.8. Sequential one-pot [RuClCp*(PPh_3)$_2$] catalyzed azide-alkyne cycloaddition from primary alkyl halides and sodium azide.

1.1.1.6. Catalyzed by $Pd(OAc)_2$

A highly efficient procedure is described for the arylation of N-aryl 1,2,3-triazole at the C-5 position using the direct $Pd(OAc)_2$ catalyzed arylation process. The reactions were carried out successfully in the presence of catalytic amounts of $Pd(OAc)_2$ and tris(o-tolyl)phosphine and produced the required product, C-5 substituted N-aryl 1,2,3-triazole, in 76% yield. (Figure 1.9)[11]

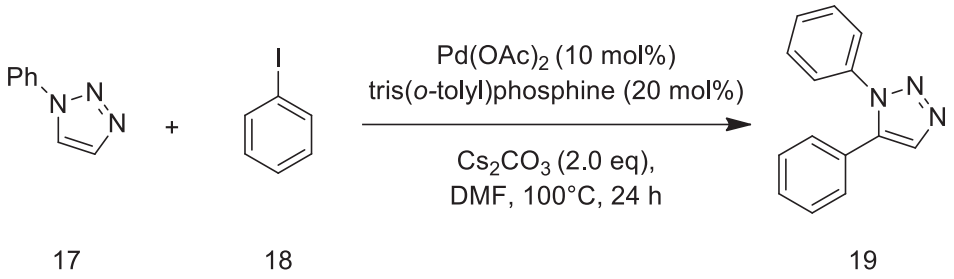


Figure 1.9. Synthesis of C-5 substituted 1,2,3-triazole by $Pd(OAc)_2$ catalysis.

1.1.2. Biological Activities of 1,2,3-Triazoles

There are many 1,2,3-triazole modified small molecules that possess biological activities like antibacterial, anti-allergic, analgesic, anti-HIV, anti-inflammatory, cytotoxic, anti-malarial, and anti-tuberculosis effects.[12] Similarly, benzotriazole ring systems are very well-known pharmacophore systems. There are many biologically active triazole-based compounds (20-27) in the literature for the treatment of numerous diseases, including anticancer, antifungal, and antibacterial ones. For the molecules shown in Figure 1.10, compound 20 has an IC_{50} value (5.2-7.8 μmol/L) and chlorohexidine (4.4-11.0 μmol/L) against two cancer cell lines.[13] Compound 21 has an IC_{50} value (1.2-1.5 nM) compared to doxorubicin (0.19-0.38 nM) in three cancer cell lines.[14] Compound 22 showed very strong activity against etoposide (0.3-1.2 μM) in five cancer cell lines with IC_{50} values in the range of 0.1-0.6 μM.[15] Compound 23 has MICs values of 1.6 μg/mL and 3.1 μg/mL against to *Candida albicans, C.a,* and *Candida parapsilosis, C.p,*

respectively. Two controls were used in this test, these are fluconazole and amphotericinB, with MIC values of 0.8 and 0.4 μg/mL, respectively both in *C.a.* and *C.p.*[16] Compound 24 was found to have in vitro activity comparable to fluconazole and clotrimazole against *Microsporum canis*, *Trichophyton mentagrophyte, Trichophyton rubrum, Epidermophyton floccosum,* and *Candida albicans.*[17] Molecules with antitumor activity were obtained by derivatizing the compound 25.[18] Compound 26 is used a lead molecule to prepare a list of derivatives having amino and nitro substituents to improve the antiproliferative properties.[19] The minimum inhibitory concentrations (MIC) of compound 27 was investigated for its antibacterial and antileishmanial properties. It was discovered to be effective against eleven bacterial species at various concentrations, according to the test results (MIC: 312-5000 μg/mL).[20]

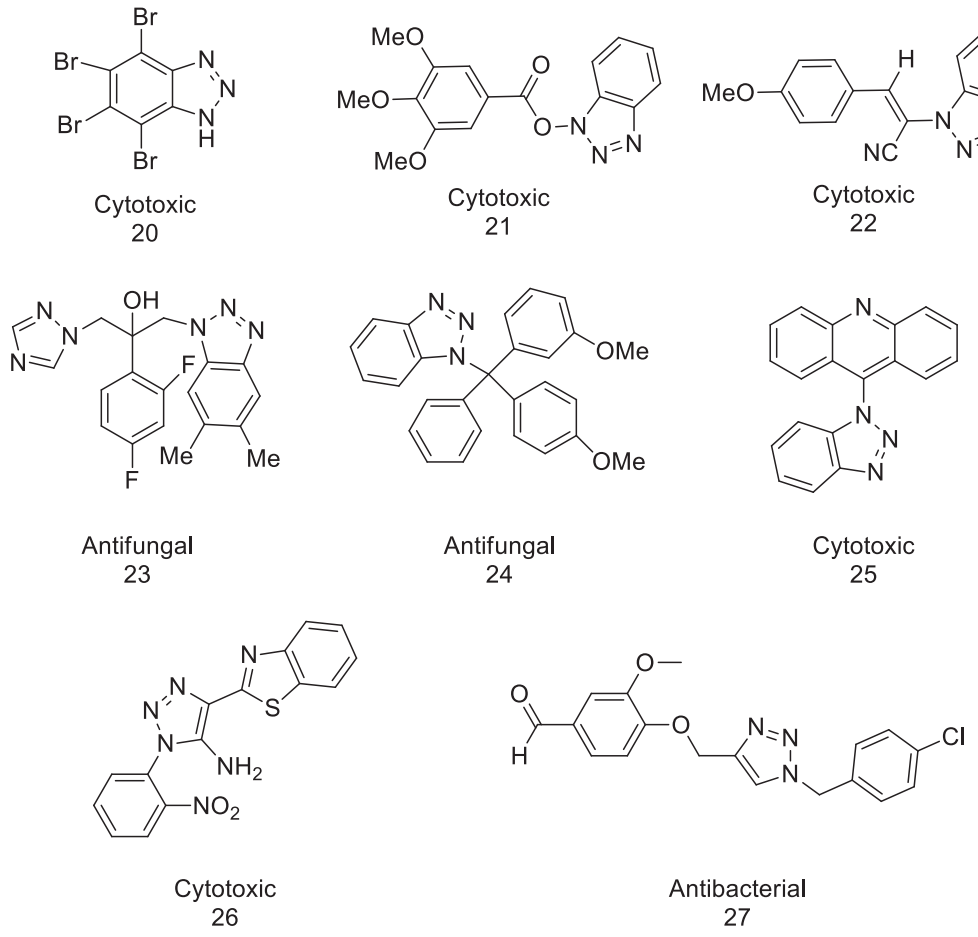
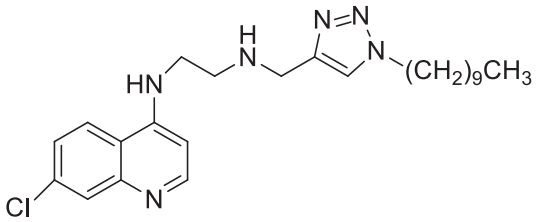


Figure 1.10. Biological activities of compounds 20-27.

Leishmania amazonensis (L. amazonensis) is a parasitic disease found in tropical regions responsible for leishmaniasis. Antileishmanial used or effective against leishmaniasis by destroying protozoa of the genus *Leishmania*. Although their antileishmanial activity is still underutilized, quinoline and 1,2,3-triazoles are well-known nitrogen-based heterocycles with a variety of pharmacological characteristics. In one of the studies, several compounds bearing 4-aminoquinoline and 1,2,3-triazole were synthesized, and biological studies utilizing *L. amazonensis* species were carried out. The results indicate that compound 28, shown in Figure 1.11 a 4-aminoquinoline/1,2,3-triazole bifunctionalized, demonstrated the best antileishmanial activity, with 50% inhibitory concentration (IC_{50}) values of ~ 1 μM against intramacrophage amastigotes of *L. amazonensis* and being 16-fold more active to parasites than to the host cell. Compound 28 did not promote the up-regulation of reactive oxygen and nitrogen species in *L. amazonensis*-infected macrophages. With IC_{50} values of 5.7 μM and 1.1 μM against promastigotes and amastigotes of *L. amazonensis*, respectively, compound 28 had quite effective antileishmanial activity, outperforming miltefosine has an IC_{50} values of 22.0 μM and 4.2 μM, respectively. According to data, compound 28 has a multi-targeted, selective antileishmanial activity that may be considered for future research on this field. The tested substance's cytotoxicity was assessed against murine peritoneal macrophages.[21]



28

Figure 1.11. Structure of potent antileishmanial drug candidate compound 28.

Along with lung cancer, breast cancer is the most common type of cancer in women. High levels of estrogen and progesterone hormones are among the potential risks

that increase the possibility for developing breast cancer. The most prevalent type of breast cancer in which the presence of estrogen plays a role in its development and spread is known as estrogen receptor-positive (ER+) breast cancer. Estrogen Receptor Alpha (Erα) regulates the growth of breast tissue and may play an important role in the development of breast cancer. Estrogen binds to the ERα and Erβ, receptors to act in other tissues. The primary ERα implicated in breast cancer has been identified as a key target for endocrine therapy in clinical settings. Several derivatives of metacetamol (Figure1.12) have been synthesized, and tested for possible cytotoxicity, antibacterial activity, and antioxidative activity. Twelve 1,2,3-triazole functionalized metacetamol derivatives (Figure 1.12) 30 a-l were tested. Among the tested compounds 30a, 30g, 30h, and 30i were found to be the most cytotoxic compounds with an IC_{50} values between 15.84-22.83 μg/mL. Besides compounds 30c, 30d, and 30e showed promising cytotoxic activity with IC_{50} values between 30.10-62.91 μg/mL range. The characteristics of 1,2,3-triazoles, which include medium dipole qualities, hydrogen bonding ability, rigidity, and stability, make them more than merely passive linkers.[22]

Compound 30i which contains 3,4-dichlorobenzyl, 30h which contains 2,4-dichlorobenzyl, 30g which contain 4-chlorobenzyl, and 30a which contain 1-benzyl groups on the triazole ring at *N*-1 position were most active and approximately half as active as the positive control drug tamoxifen. The triazole ring-containing compounds 30c with 3-fluorobenzyl groups, 30d with 4-fluorobenzyl groups, and 30e with 2-chlorobenzyl groups have good cytotoxic activity, whereas compounds 30b with 2-fluorobenzyl groups, 30f with 2-chlorobenzyl groups, and methylated compounds 30j, 30k, and 30l were the least active ones. The cytotoxic and antioxidant activity of compounds 30a, 30h, and 30i has proven to be the most promising ones. According to SAR analyses, mono-chloro substitution at p-position improves activity, while di-chloro substitution at positions 2-, 3-, and 4- decreases activity. While methyl substitution on benzyl groups at positions 2-, 3-, and 4- kills activity, fluoro substitution on benzyl groups at positions 2-, 3-, and 4- improves activity.

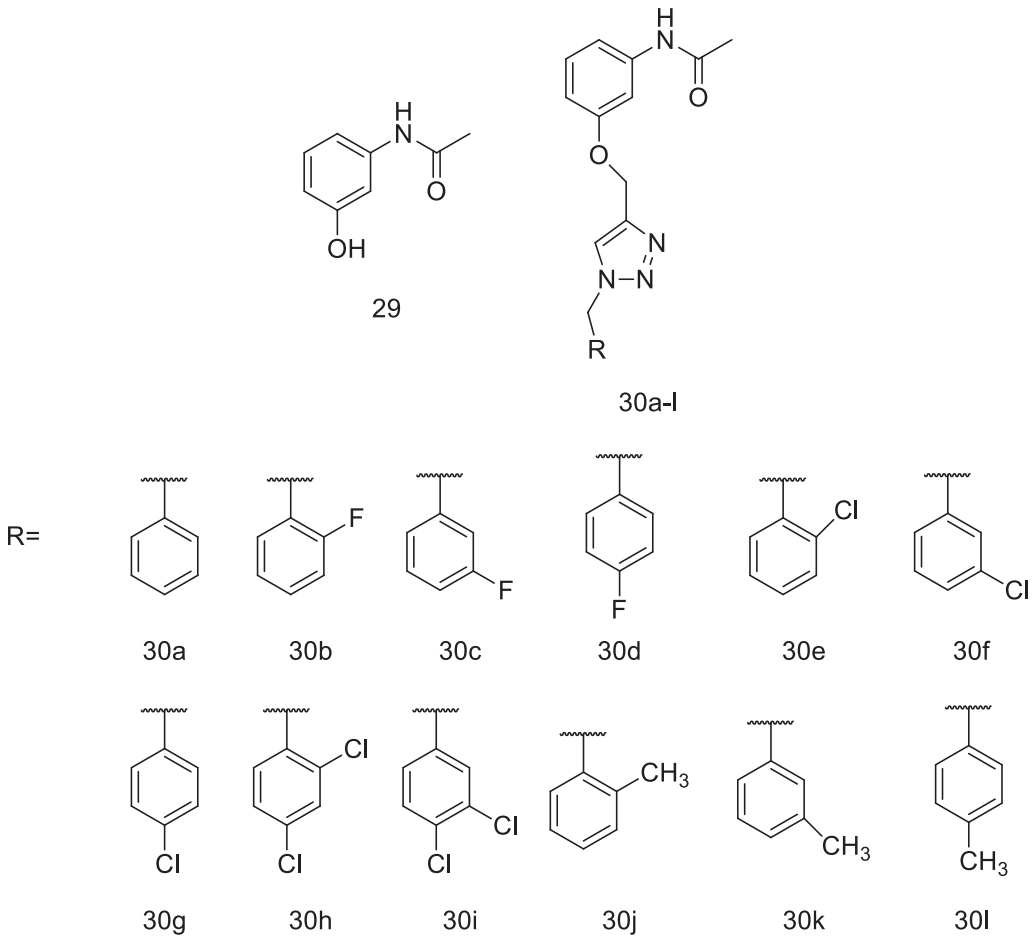


Figure 1.12. Structures of metacetamol (29), and its 1,2,3-triazole modified derivatives (30a-l).

In another study, Cao, and coworkers[23] prepared a list of 1,4-disubstituted 1,2,3-triazole modified indazole derivatives 31a-e binded through an amide linker as shown in Figure 1.13. Prostate cancer (PC) is the most prevalent kind of cancer in males among the several types of skin cancer. By using the MTT assay, the antiproliferative activity of compounds 31a-e, shown in Figure 1.14 was evaluated in four human cancer cells: MGC-803 (gastric cancer cell), HepG-2 (hepatoma cell), PC-3 (prostate cancer cell), and MCF-7 (breast cancer cell). In screening for antiproliferative activity, most compounds were observed to have specific cytotoxicity to PC-3 cancer cells, especially compound 31a with an IC_{50} value of 4.42 ± 0.06 μmol/L. It was discovered that the chemical 31a can arrest the cell cycle in the G2/M phase and reduce cell growth through a cell cycle arrest assay. Overall, analogue 31a can be seen as a possible lead molecule for further development of

a novel anti-prostate cancer medication. The 1,2,3-triazole N-heterocyclic structural unit is crucial for the synthesis and design of pharmaceuticals. The moiety of the 1,2,3-triazole is a valuable linking unit that can establish a hydrogen bond, which is advantageous for attaching to biomolecular targets and improving solubility. It is also stable for metabolic breakdown. Due to its low basicity, the 1,2,3-triazole ring is not protonated at physiological pH, unlike other azaheterocycles. Numerous substances containing the 1,2,3-triazole moiety exhibit a variety of biological activity, including those that are antibacterial, anti-HIV, anti-tuberculosis, and anti-tumor. As a result, 1,2,3-triazole can serve as a tiny drug molecule's pharmacophore. Compound 31a shown cytotoxic activity in four different cancer cell lines (PC-3, MCF-7, HepG-2, and MGC-7) by IC_{50} values in the range of 4.42-14.29 μM. In the same study they used 5-fluorourasil, 5-Fu, as positive control and it has IC_{50} values in the range of 8.41-15.14 μM for the same cancer cell lines.[23]

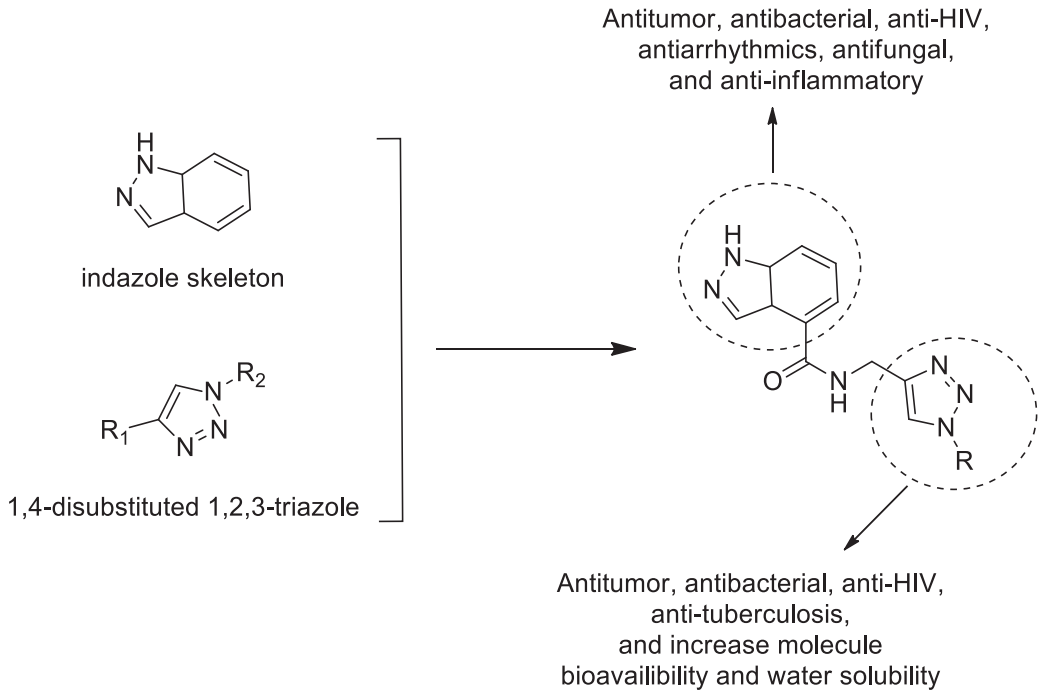
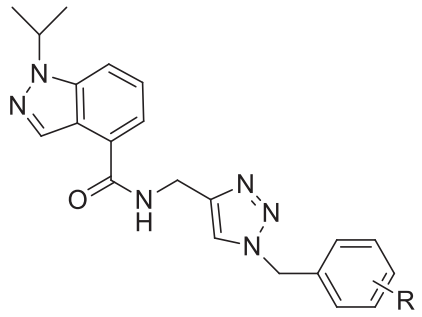


Figure 1.13. Design of derivatives containing 1,2,3-triazole modified indazole derivatives.



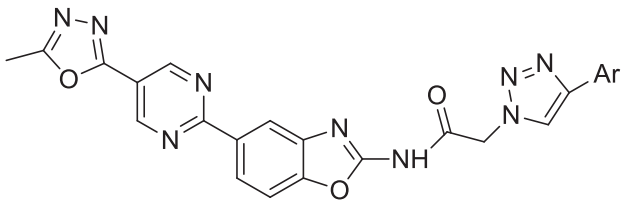
31a-31e

R=

31a: benzyl
31b: 4-trifluoromethylbenzyl
31c: 4-cyanobenzyl
31d: 4-methoxybenzyl
31e: 4-tert-butylbenzyl

Figure 1.14. Structures of compound 31a-e.

1,2,3-triazole linked pyrimidine-benzoxazole compounds (32a–j) were synthesized and designed by Sudhakar and coworkers (Figure 1.15). These compounds' anticancer activity was tested on four human cancer cell lines such as prostate cancer (PC3 and DU-145), lung cancer (A549), and breast cancer (MCF-7) by using the MTT method and compared with clinical drug etoposide. Among the synthesized compound derivatives, 32a, 32b, 32c, 32d, and 32j showed good activity, of which 32b had the most promising activity. Compounds (32a-32j) were tested in four cancer cell lines and etoposide was used as a positive control. Most of the investigated substances had significant activity on four cell lines, according to the data. Etoposide revealed IC_{50} values between 1.97-3.08 μM, whereas compounds 32a-d and 32j demonstrated cytotoxic activity with IC_{50} values ranging from 0.01 μM to 17.5 μM. Five compounds, 32a, 32b, 32c, 32d, and 32j showed more strong anti-cancer activity when compared to etoposide among the tested compounds. Compound 32b displayed the most cytotoxic activity in four cancer cell lines by IC_{50} values in the range of 0.01-0.07 μM. Compound 32a**,** showed also very potent cytotoxic activity, has 4-pyridyl moiety attached to triazole nucleus IC_{50} values in the range of 0.17-1.33. Compound 32c, which replaced the 2-pyrimidyl ring with a 3,4,5-trimethoxyphenyl ring, displayed slightly lower activity with the IC_{50} values in the range of 0.08-0.15 μM when compared to compound 32b. Therefore, compound 32b which has 2-pyrimidyl ring showed highest cytotoxic activity than with other compounds and etoposide.



32a-32-j

32a; Ar = pyridine-4-yl
32b; Ar = pyrimidine-2-yl
32c; Ar = 3,4,5-trimethoxyphenyl
32d; Ar = 3,5-dimethoxyphenyl
32e; Ar = 4-methoxyphenyl
32f; Ar = 4-chlorophenyl
32g; Ar = 4-bromophenyl
32h; Ar = 4-nitrophenyl
32i; Ar = 3,5-dinitrophenyl
32j; Ar = 3,5-dimethylphenyl

Figure 1.15. Structures of 1,2,3-triazole modified pyrimidine-benzoxazole compounds (32a-j).

Structural activity relationship of these derivatives indicates that presence of more than one methoxy substituted phenyl group enhances the cytotoxic activity. Also, the presence of two nitro group at 3,5-positions of the phenyl group yields the acceptable biological activities for these derivatives.[24]

1.2. 1-Azacoumarin and Coumarin Structures

Coumarins are organic substances that can be found in tonka beans and other naturally occurring herbal products. The basic structure of coumarin, benzopyrone, (Figure 1.16) has different reactive sites. Azacoumarins share structural similarities with coumarins, however they have a nitrogen atom in place of an oxygen atom. (Figure 1.16) Many coumarin derivatives are shown to be as anti-microbial, anti-cancer, and anti-HIV properties.[25] In the literature, it was shown that water soluble coumarin derivatives can be used to generate singlet oxygen specie and can be useful for photodynamic therapy.[26] Also, antioxidant properties of some of the coumarins against the ROS were also shown.[27] Recently, their antioxidant activities were thoroughly investigated in relation to potential methods of oxidative stress regulation.[28]

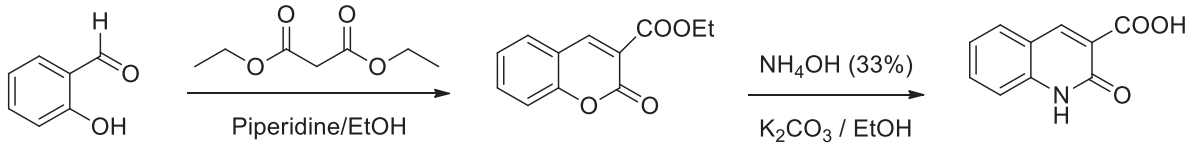
Coumarin 1-Azacoumarin

Figure 1.16. General structures of coumarin and 1-azacoumarin compounds.

There are many synthetic routes for the preparation of coumarins and 1-azacoumarin derivatives in the literature. In the following past several preparation methods will be summarized.

1.2.1. Preparation of 1-Azacoumarin Derivatives

Condensation of 2-hydroxy-benzaldehyde (33) and diethyl malonate in the presence of a base catalyst, ethyl coumarin-3-carboxylate (34) can be produced as an intermediate product. Compound 34 is dissolved in ethanol and treated with anhydrous potassium carbonate under reflux for 30 min. Finally, the ammonia solution is added the reaction medium and heated under pressure for 3 hours to produce the corresponding 1-azacoumarin (35) with 63% yield (Figure 1.17).[29]



33 34 35

Figure 1.17. Preparation of 1-azacoumarin-3-carboxylic acid starting from salicylic acid (33) and diethyl malonate.

Reyes-Batlle and coworkers reported that, aniline (36) can be converted into quinolin-2(1H)-one (38) structure (92% yield) by acylating it with *(2E)*-3-phenylacryloyl chloride, followed by $AlCl_3$ aided cyclization and dearylation sequence (Figure 1.18).[30]

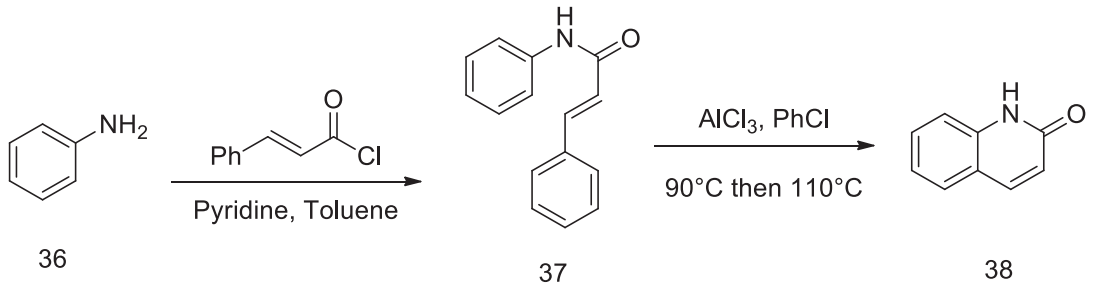


Figure 1.18. Synthesis of azacoumarin (38) starting from aniline (36).

In another alternative study, 4-methyl substituted-2-quinolinone (41) was produced in 86% isolated yield by cyclizing 3,4-dimethoxy acetanilide (39) with ethyl-2-butynoate (40) in the presence of [{$RuCl_2$(*p*-cymene)}$_2$], $AgSbF_6$, and pivalic acid in *i*-PrOH at 130 °C after 24 h. of stirring (Figure 1.19).[31]

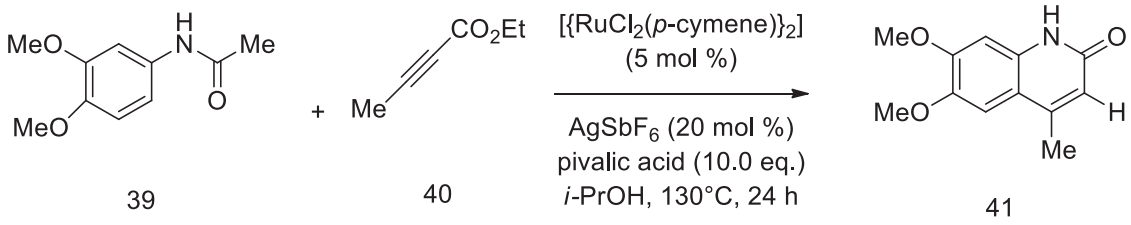


Figure 1.19. Cyclization of 3,4-dimethoxy acetanilide (39) with ethyl-2-butynoate (40).

Another alternative preparation starting from aniline and α,β-unsaturated esters, $Pd(OAc)_2$ was used as catalyst and $Na_2S_2O_8$ was used as the oxidant for synthesis of azacoumarin. It is understood that the reaction is performed when Pd (II) is reduced to Pd(0). Although the reaction was carried out in many different solvents such as

chloroform, DCE, DMF, DMSO and DMA, toluene is found to be the most effective solvent (Figure 1.20).[32]

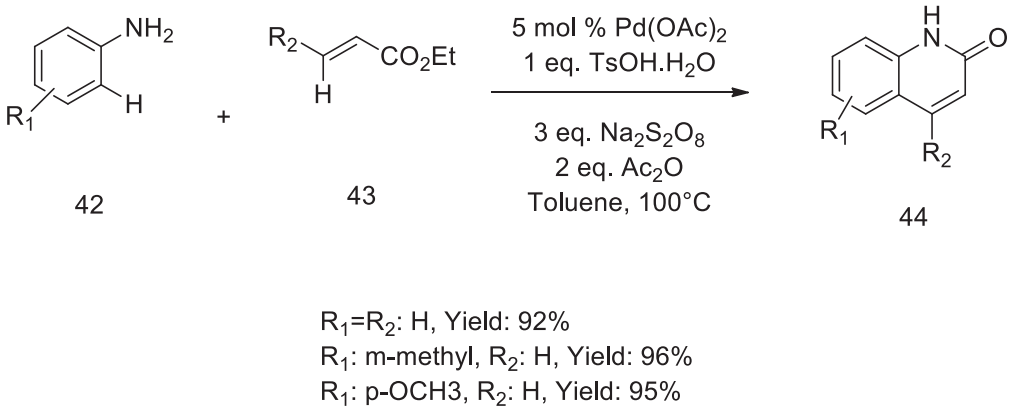


Figure 1.20. Synthesis of azacoumarin (44) starting from aniline via $Pd(OAc)_2$ catalyst.

Another interesting synthesis is the solvent-free condensation reaction. Starting with substituted anilines (45), Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione) was applied to produce malonic acid monoanilide (46) (Figure.1.21). Without using a solvent, the reactions were carried out at 60 °C for four hours. 4-hydroxy-2-quinolinones were produced in excellent yields under mild reaction conditions for the N-acylation and using an effective reagent (MSAA) for the intramolecular Friedel-Crafts cyclization steps. If the R group is 4-methoxy and reaction time for 3 h, the reaction had highest yield as 91%. On the other hand, if the R group is H, and 2-methyl (3 and 6 hours, respectively) reaction yield became 90%.[33]

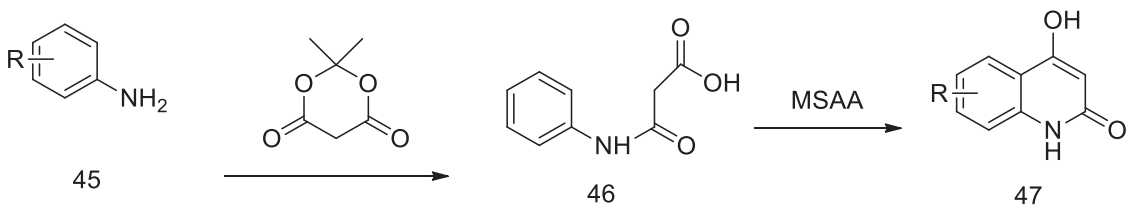


Figure 1.21. Synthesis of 4-hydroxy-1-azacoumarin via solvent-free intramolecular Friedel-Crafts cyclization starting from aniline.

According to another $Pd(OAc)_2$ catalyzed preparation of 1-azacoumarin, ortho-iodoacetanilide (48) was selected as the model iodoarene to undergo acylation with benzaldehyde (49) under $Pd(OAc)_2$ catalysis as the first step. At a high temperature of 120 °C, the cyclization reaction in the presence of NaH in DMF produced the 1-azacoumarin (51) with a 67% yield (Figure 1.22).[34]

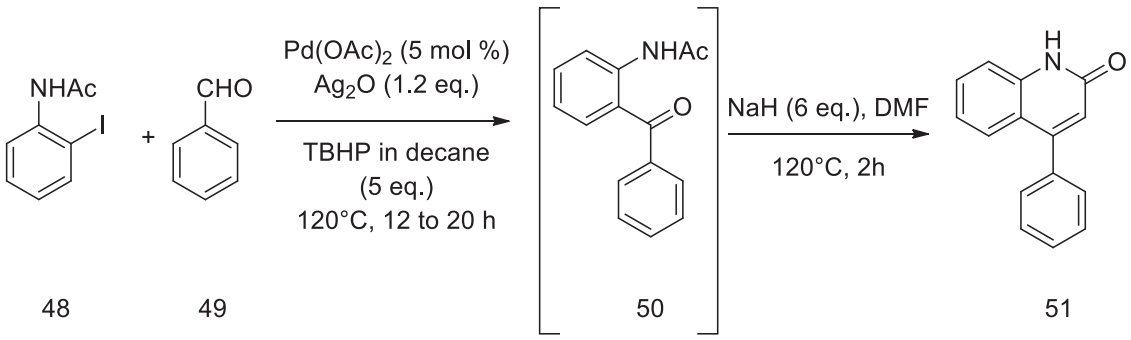


Figure 1.22. Synthesis of 1-azacoumarin derivative with $Pd(OAc)_2$ catalyzed direct acylation of iodo acetanilides/iodo-phenyl acetates.

Alternatively, preparation of 7-amino 4-methylquinolin-2(*1H*)-one (54) was performed with 65% yield by reacting *m*-phenylene diamine (52) and ethylacetoacetate (53) under refluxed at 150 °C for 18 h in an oil bath (Figure1.23).[35]

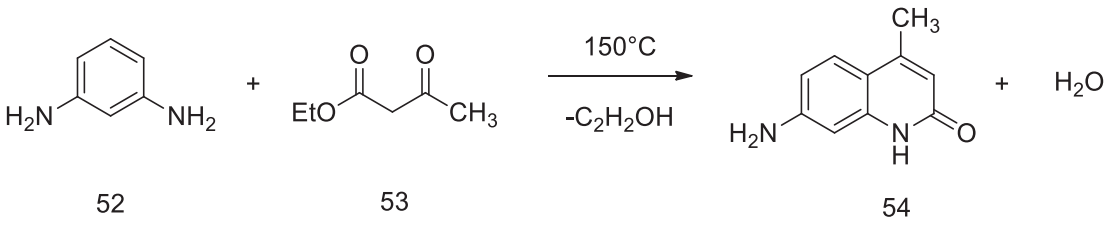


Figure 1.23. Preparation of 7-amino 4-methyl substituted 1-azacoumarin.

In the last example different o-alkynyl anilines (55) were treated with carbon dioxide and a base under mild reaction conditions in the presence of a catalytic quantity of silver salt to produce the appropriate 4-hydroxyquinolin-2(*1H*)-one derivatives. When DMSO was used as the solvent and the R group is Ph, the reaction gave the highest yield with 97% (Figure 1.24).[36]

Figure 1.24. Synthesis of azacoumarin derivative (56) via silver (I) nitrate catalyzed carbonylation reaction.

1.2.2. Preparation of Coumarin Derivatives

Low loadings of cheap $ZnBr_2$ efficiently catalyzed the preparation of coumarins from sulfonamide moiety of ynamides and salicylaldehyde in excellent yields. For strong regioselectivity, the sulfonamide moiety works well as a traceless directing group. The protocol allows for strong functional group tolerance, a wide range of substrates, and sulfonamide recovery and reuse (Figure 1.25).[37]

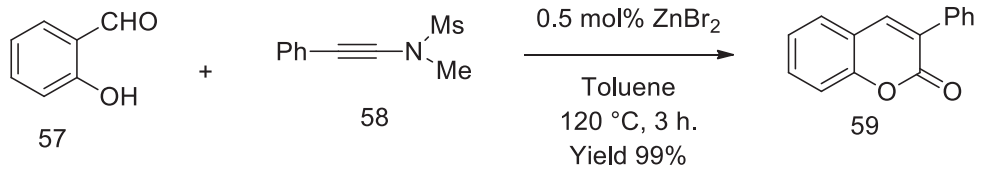
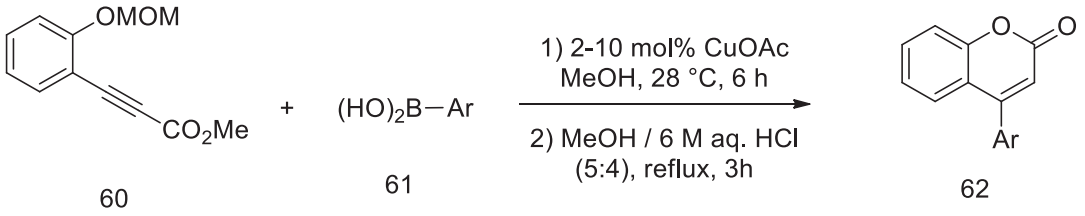


Figure 1.25. Synthesis of coumarins from sulfonamide moiety of ynamides and salicylaldehyde via Zn(II)-catalysis.

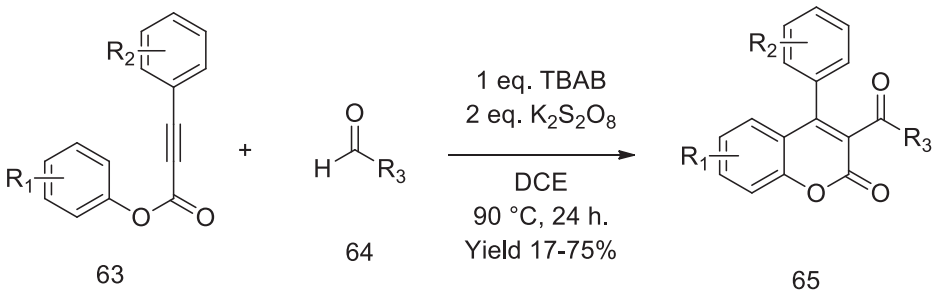
When different arylboronic acids were hydroarylated with arylpropionic acid methyl esters that had a MOM-protected hydroxy group at the ortho position at room temperature with a catalytic quantity of CuOAc, 4-arylcoumarins were produced after the acidic workup in high yields (Figure 1.26).[38]



Ar: C_6H_5, Yield: 89%
Ar: *m*-MeC_6H_4, Yield: 93%
Ar: *p*-MeC_6H_4, Yield: 90%
Ar: *o*-MeC_6H_4, Yield: 89%

Figure 1.26. Synthesis of 4-arylcoumarins via CuOAc catalyzed addition of arylboronic acids to arylpropionic acid esters.

The synthesis of 3-acyl-4-arylcoumarins (65) is possible by the effective tandem acylation/cyclization of alkynoates (63) with aldehydes (64) without the use of metal catalyst, which involves addition of an acyl radical to alkynes and functionalizing the C-H link to simultaneously produce two new C-C bonds (Figure 1.27).[39]



R_1 = H, Me, *t*Bu, OMe, OPh, F, Cl, Br, CF_3
R_2 = H, Me, OMe, $C(O)CH_3$
R_3 = aryl or alkyl

Figure 1.27. Preparation of 3-acyl-4-aryl-coumarins via metal-free reaction.

Under visible light irradiation, 3-arylpropiolates ester can be converted into 3-trifluoromethyl coumarins (68) with a high degree of efficiency by using CF_3SO_2Cl as the source of the trifluoromethyl radical. The process includes cascades of cyclization, dearomatization, ester migration, oxidation, and rearomatization (Figure 1.28).[40]

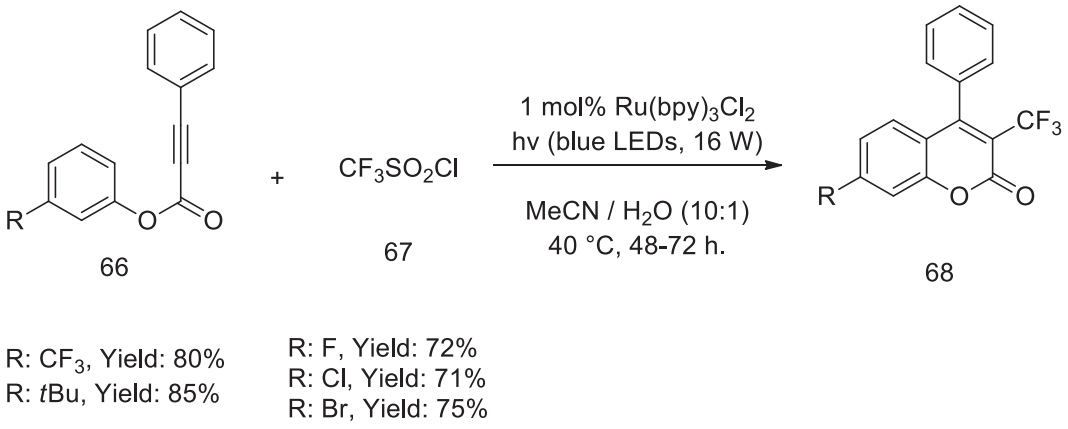
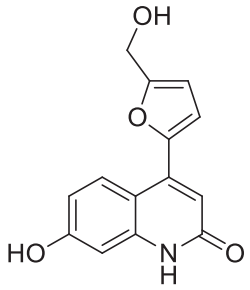


Figure 1.28. Synthesis of 3-trifluoromethyl coumarins under visible light irradiation.

1.2.3. Biological Activities of Coumarin and 1-Azacoumarin Derivatives

The family of naturally occurring lactones and lactams include coumarins, also known as benzopyran-2-ones, and their nitrogen counterparts, 1-azacoumarins. The biological activities of the plant extracts containing coumarin-related heterocycles, which were used as herbal treatments in the past, have recently been thoroughly investigated. A 6-functionalized 1-azacoumarin that is a component of quinoline alkaloids is currently undergoing human clinical trials as an orally effective anti-tumor medication due to its farnesyl protein-inhibiting activity in the nanomolar range. 1-Azacoumarins are known for their wide biological activity. Numerous synthetic coumarins have undergone extensive testing for anti-microbial, anti-HIV, anti-cancer, lipid-lowering, antioxidant, and anti-coagulation actions. These coumarins may have different pharmacophoric groups at the C-3, C-4, and C-7 locations.[41]

A 4-2'-furyl carbostyril compound 69 was isolated from the ethyl acetate extraction of the Chinese medicinal plant *Aquilegia ecalcarata*, and it was found to be cytotoxic to two human cancer cell lines with IC_{50} values in the 8.8–10.1 mM range (Figure 1.29).[42]

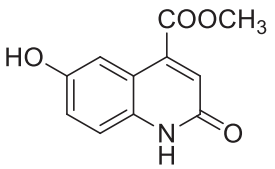


69

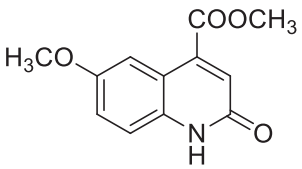
Figure 1.29. Structure of compound 69.

From the rice variety *Oryzae sativa*, two novel alkaloids, 70 and 71, were discovered that contain an ester group at C-4. Compound 70, which has a free phenolic OH group, was found to have a moderate amount of antioxidant activity (IC_{50} = 36.4 μg/mL) in a radical scavenging assay (Figure 1.30).[43]

Human leukemia cell line U-937 was exposed to the similar methoxy compound 71, which shown considerable anti-neoplastic action (IC_{50} = 118.1 μg/mL) (Figure 1.30).[44]



70

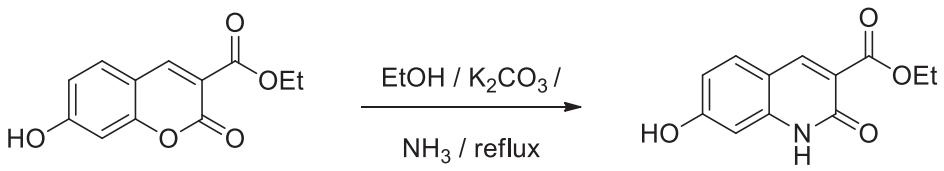


71

Figure 1.30. Structures of compounds 70 and 71.

A series of coumarin and 1-aza coumarin-derived molecules were synthesized and investigated for biological activities, as shown in Figure 1.31. These molecules were

tested in the MCF-7 human breast cancer cell line. Ethyl-7-hydroxy coumarin-3-carboxylate and ammonium solution were used as the primary synthons in the development of the synthesis of ethyl 7-hydroxy-1-azacoumarin-3-carboxylate (73). Also reported herein is the use of a simple method for the preparation of 7-hydroxy-1-aza coumarins from 7-hydroxy coumarin derivative analogues.[45]



72 73

Figure 1.31. Synthesis of ethyl 7-hydroxy-1-azacoumarin-3-carboxylate.

N-Substituted-7-hydroxy-1-azacoumarin-3-carboxamide (74, 75 and 76) and N-phenyl substituted 2,7-diacetoxy-quinoline-3-carboxamides (77 and 78) were designed and synthesized (Figure 1.32). The *in vitro* antiproliferative activities of the synthesized compounds 74–78 against the MCF-7 cancer cell line was evaluated (Figure 1.32). Compound 76's cell cycle research revealed G2/M phase cell cycle arrest and Pre-G1 death. Compound 76 is the most cytotoxic compound (IC_{50} = 10.12 μM). Doxorubicin was used as a positive control in this study (IC_{50} = 3.17 μM). While compounds 74, 75 and 78 were also quite effective over MCF-7 cell line.

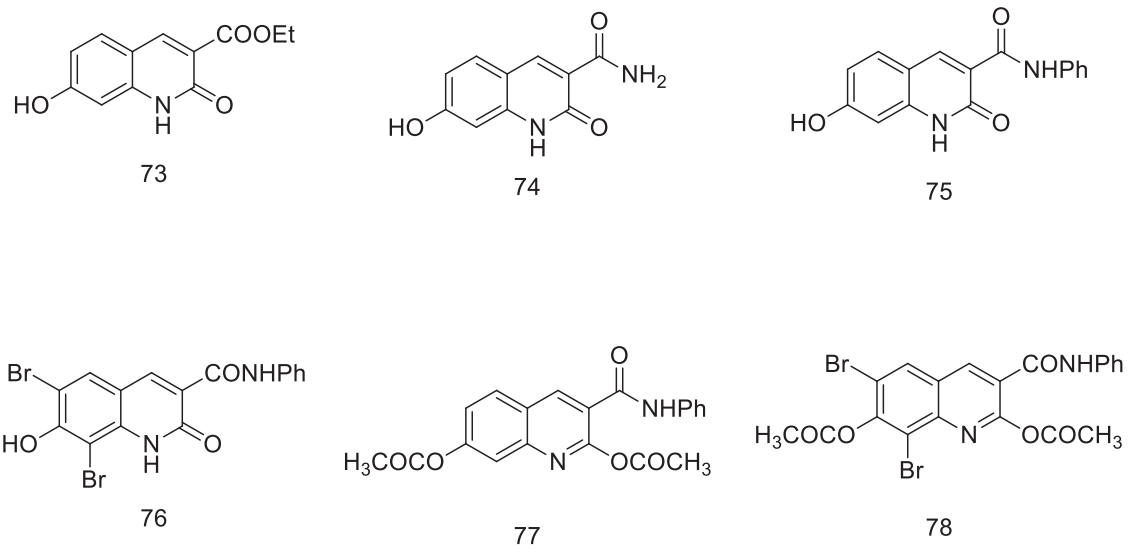


Figure 1.32. Structures of biologically active 1-azacoumarin 3-carboxamide derivatives.

According to another study, compounds bearing coumarin pharmacophore (Figure 1.33) were synthesized and their biological activities were examined against two human tumor cell lines, breast cancer MCF-7 and hepatocellular carcinoma HePG-2. Compound 79, 80, 81, 82, 83 and 84 showed strong activity against both MCF-7 and HepG-2 cell lines. 5-Fluorouracil was used as a positive control in this study (IC_{50} = 5.4-7.9 μg/mL). Compound 83 was shown best cytotoxic activity in two cancer cell lines by IC_{50} values in the range of 5.5-6.9 μg/mL. Especially, compounds 79, 82 and 83 were quite promising with IC_{50} values between 6.9-9.0 μg/mL in MCF-7 cell line and 5.5-12.5 μg/mL in HePG-2 cell lines.[46]

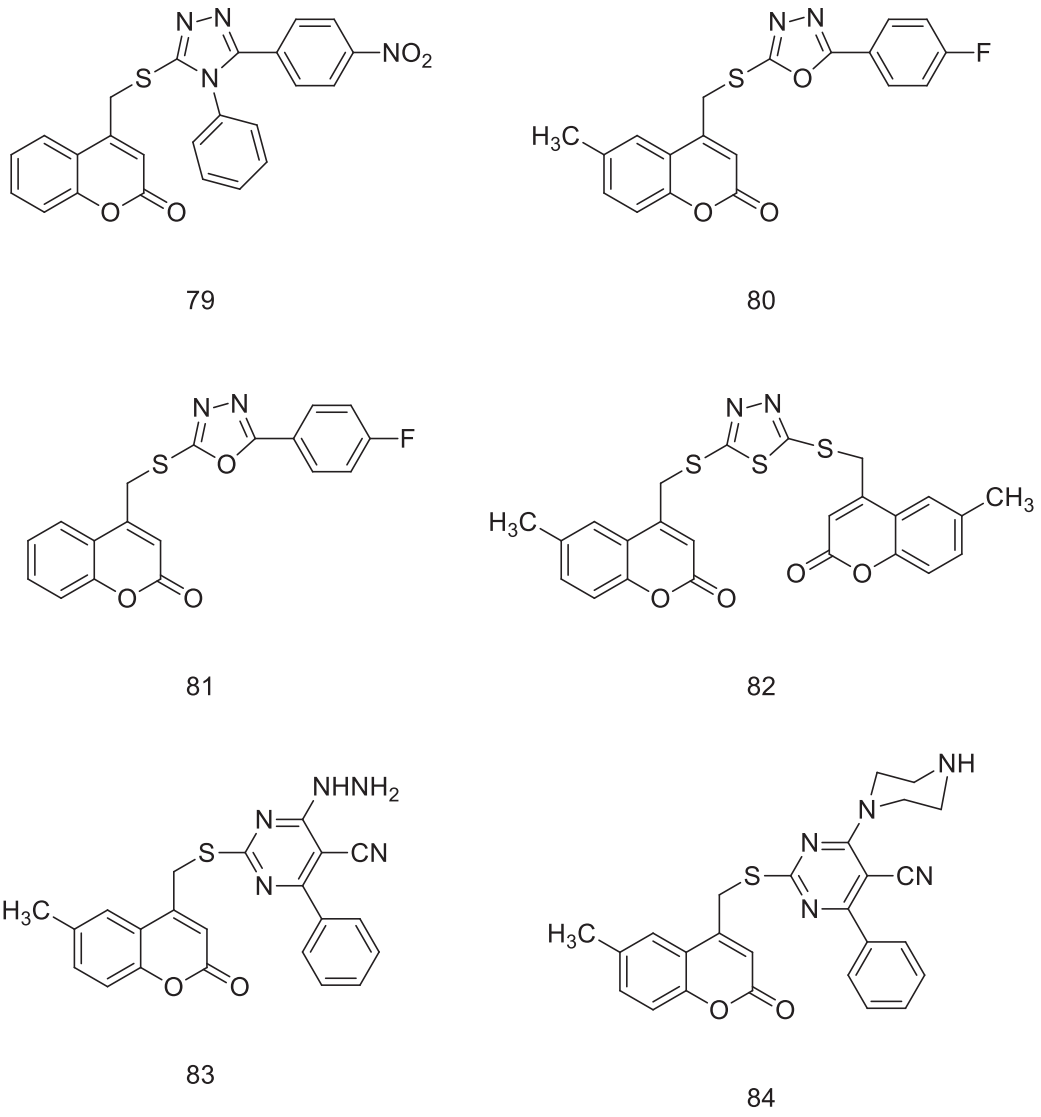


Figure 1.33. Structures of biologically active pyrimidine modified coumarin derivatives (79-84).

1.3. Triazole Substituted Coumarin and 1-Azacoumarin Derivatives

There is a limited number of studies for the preparation of 1,2,3-triazole substituted azacoumarin derivatives. In this part two examples were be given as summary.

1.3.1. Preparation of Triazole Substituted Coumarin and 1-Azacoumarin Derivatives

When Cu(I) salts were used, the azide alkyne cycloaddition—originally studied as a two component, 1,3-dipolar cycloaddition—was regioselectivity transformed into the production of 1,4-substituted triazoles under the so-called click chemistry conditions. First, azide will replace the position of Cl atom in the coumarin derivative (85) then it will be reacted with alkyne modified coumarin to form compound 87 in 65% yield (Figure 1.34).[47]

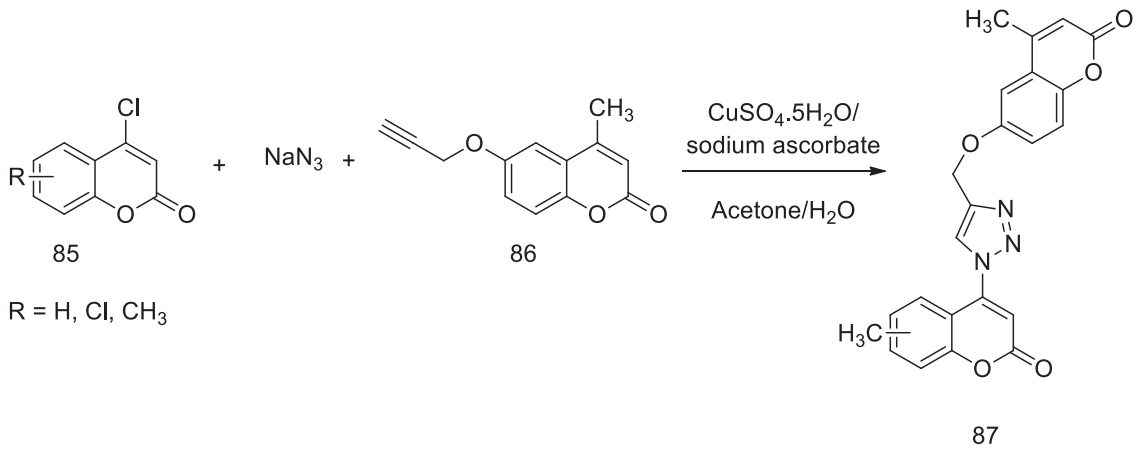
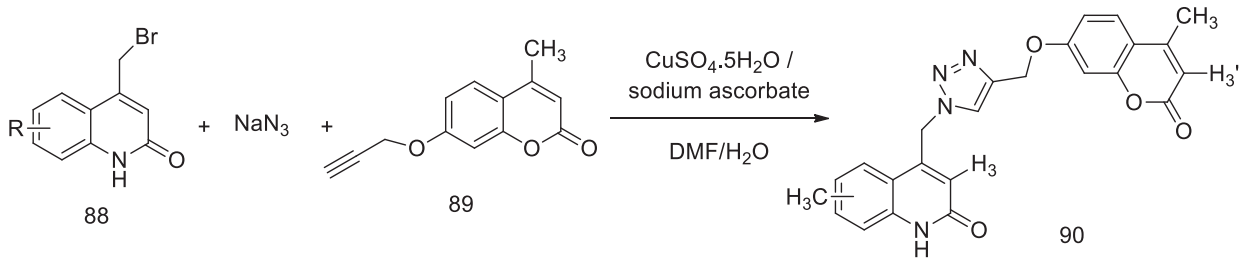


Figure 1.34. Synthesis of 1,4-disubstituted triazole substituted coumarin via click chemistry.

In another study, 4-bromomethyl substituted 1-azacoumarin (88) was reacted with alkyne modified coumarin (89) in the presence of Cu(I) ions to give compound 90 in 63% yield (Figure 1.35).



R = -H; 6-Cl; 7-Cl; 8-Me

Figure 1.35. Synthesis of 1,4-disubstituted triazole modified 1-azacoumarin via click chemistry.

1.3.2. Anticancer Activity of 1,2,3-Triazole Substituted 1-Aza-coumarin

The anticancer activities of 1,2,3-triazole substituted 1-azacoumarins are not yet available in the literature.

1.4. Aim of the Study

In this thesis, optimization of a synthetic method for the preparation of 1,5-disubstituted 1,2,3-triazole modified 1-azacoumarin derivative starting from anthranilic acid as shown in Figure 1.36 which can be used as precursor for the preparation of compound 92. The biological activity for this molecule targeted to be synthesized would be examined.

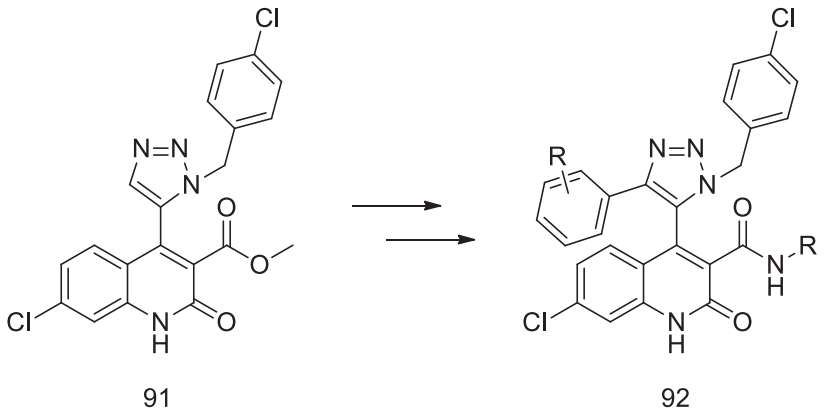


Figure 1.36. Novel 1,5-disubstituted 1,2,3-triazole modified 1-azacoumarin (91).

CHAPTER 2

RESULTS AND DISCUSSION

The target compound 91, Figure 1.36, was previously synthesized by Erdoğmuş M.[48] with a very low yield (4 mg) and the process was not reproducible for the last step of the synthesis shown in Figure 2.1. In this thesis we aimed to try to optimize the reaction condition to increase the yield this click chemistry reaction and then to transform compound 91 into amide derivatives at position 3- of coumarin.

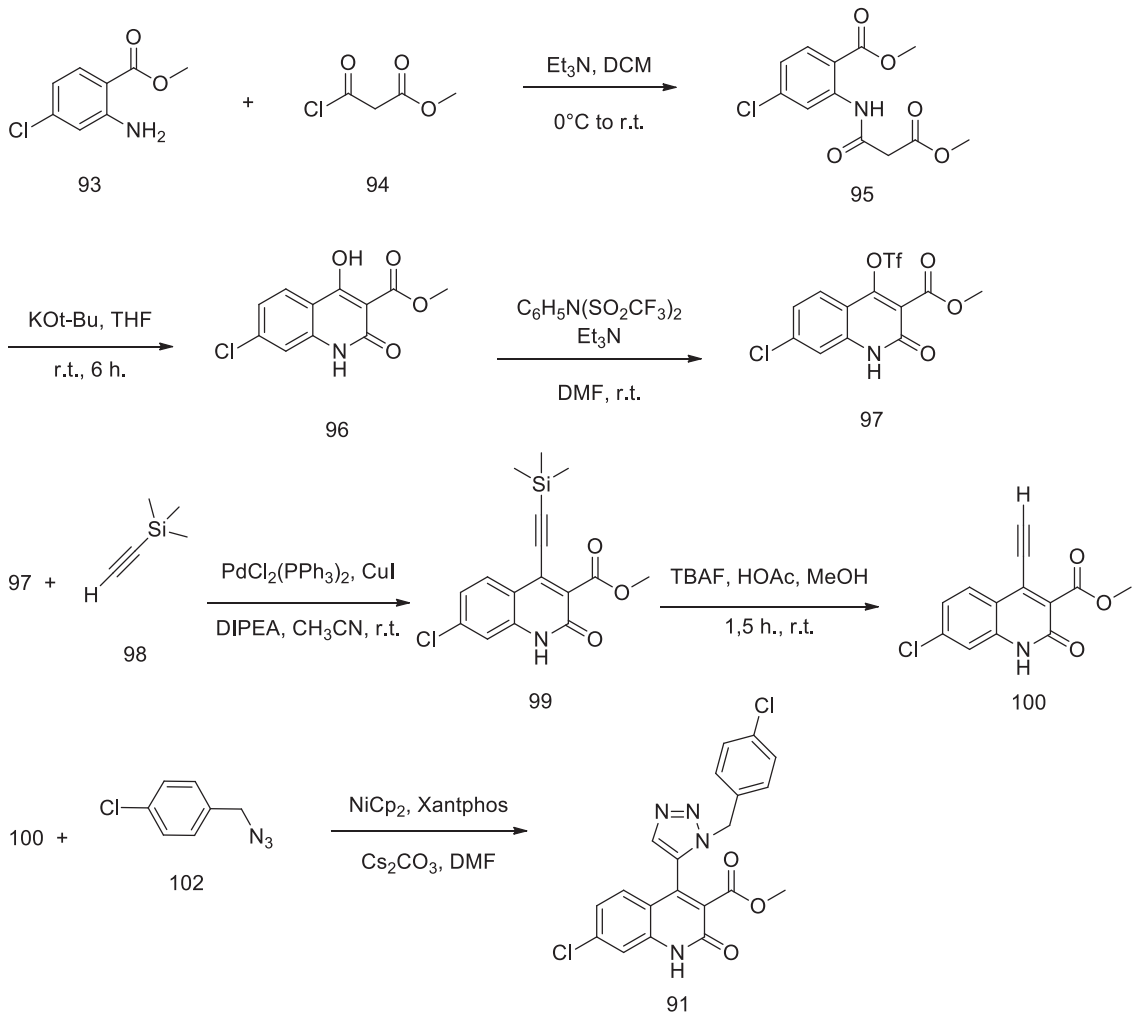
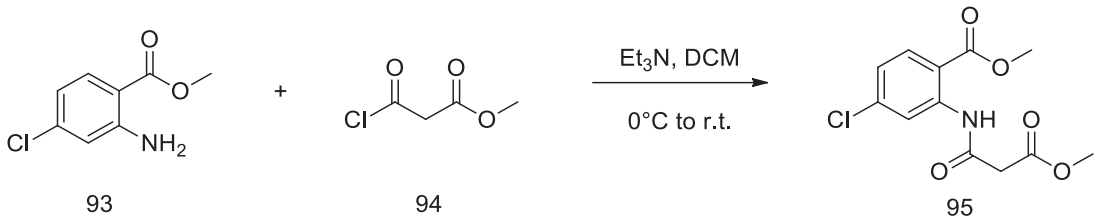


Figure 2.1. Synthetic route for the preparation of compound 91.

2.1. Addition of Methyl Malonyl Chloride to Methyl 2-Amino-4-Chlorobenzoate

In the first step of synthesis, methyl malonyl chloride (94) was dissolved in dry DCM and reated methyl-2-amino-4-chlorobenzoate (93), in dry DCM and triethylamine. A dry environment is very important for efficiency of the reaction because methyl malonyl chloride is very sensitive to moisture (Table 2.1). This process is very reproducible and yields of the reactions can be in the range of 49-88%. The high yields reported in the first reaction in Table 2.1 indicates that reaction mostly depends on the amount of moisture in the reaction solvent rather than the amount of methyl malonyl chloride or base. In Table 2.1, the average yield of first five trials is 68±17.5% at the same conditions.

Table 2.1. Addition of methyl malonyl chloride (94) to methyl 2-amino-4 chlorobenzoate (93)



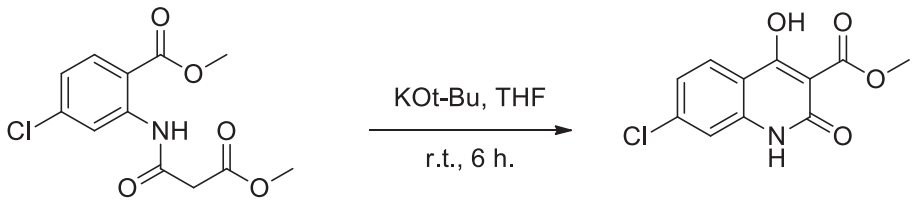
93 94 95

Entry	93 (mmol)	94 (mmol)	Et_3N (mmol)	DCM (mL)	Time (h)	Yield (%)
1	1.08	1.19	3.23	5	4	88
2	1.08	1.19	3.23	5	4	85
3	1.08	1.19	3.23	5	4	62
4	1.08	1.19	3.23	5	4	56
5	1.08	1.19	3.23	5	4	49
6	2.69	2.96	8.07	12.5	4	53
7	2.69	2.96	8.07	6	overnight	60
8	2.69	2.96	8.07	6	overnight	65
9	8.08	8.89	24.2	11	overnight	70
10	8.08	8.89	24.2	9	overnight	72

2.2. Preparation of 4-Hydroxy 1-Azacoumarin Derivative (96) from Malonylamide of Methylanthranilate (95)

In the second step, cyclization of malonylamide of methylanthranilate (95) into the corresponding 1-azacoumarin derivative (96) was studied. Reproducibility of this process was quite good and yields were in the range of 66-98% (Table 2.2). Because the solubility of the product was very low it could not be purified in column chromatography and used as crude product in the following step.

Table 2.2. Preparation of 4-hydroxy 1-azacoumarin derivative (96)



95 96

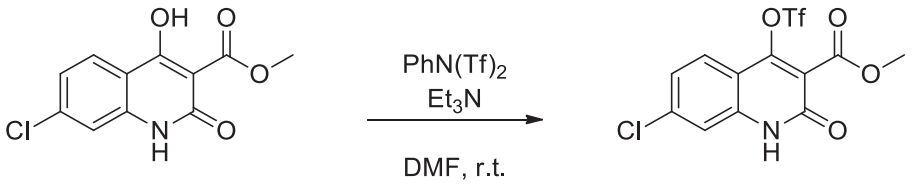
Entry	95 (mmol)	KOt-Bu (mmol)	THF (mL)	Yield* (%)
1	0.94	2.07	15	86
2	0.53	1.17	11	66
3	0.91	2.00	20	98
4	0.60	1.32	13	85
5	1.41	3.11	30	82
6	1.60	3.50	35	85
7	1.80	3.97	40	83
8	1.57	3.46	40	86
9	1.64	3.61	50	79
10	2.10	4.62	55	89

* Yields are calculated from crude product.

2.3. Preparation of 4-OTf 1-Azacoumarin Derivative (97) from 4-OH-1-Azacoumarin (96)

In this step of the synthesis 4-OH substituted 1-azacoumarin (96) is transformed into 4-OTf modified coumarin derivative by simply treatment with $PhN(Tf)_2$ under basic condition. Results of these transformations were summarized in Table 2.3. Previously the similar reaction was also performed by Erdoğmuş M.[48] for the preparation of 4-OTs, 4-ONs and 4-OTf 1-azacoumarin derivatives. It was observed that yields for the preparation of 4-OTs 1-azacoumarin was higher than the yields of 4-ONs 1-azacoumarin products, although 4-ONs derivatives were more effective in the next step. It was also seen that purification of these in column chromatography does not improving the yield in the next step. Lastly, the yields of the reactions were mostly depending on the time. Yields of the reactions were getting lower at longer reaction times. Similarly, Table 2.3 shows that, reaction can be carried out in better yields at shorter reaction times. It seems that the product is decomposing in the reaction mixture by time.

Table 2.3. Preparation of 4-OTf 1-azacoumarin derivative (97)



96 97

Entry	96 (mmol)	$PhN(Tf)_2$ (mmol)	Et_3N (mmol)	DMF (mL)	Time	97* (mmol)
1	0.60	0.73	1.80	5	4 h	0.24
2	0.80	0.96	2.40	6	4 h	0.25
3	0.51	0.61	1.53	4	4 h	0.23
4	0.80	0.96	2.40	6	5 h	0.23
5	0.60	0.73	1.80	3	1.5 h	0.25

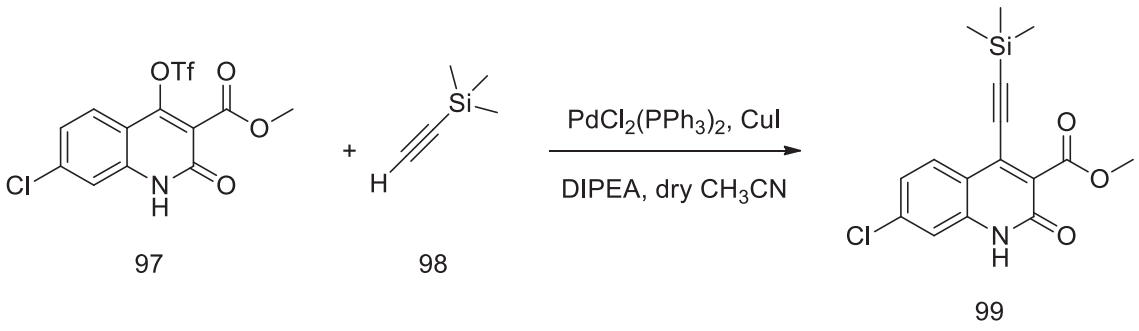
6	0.67	0.80	2.01	3	1.5 h	0.24
7	0.60	0.73	1.80	3	40 min	0.29
8	0.63	0.76	1.89	3	40 min	0.28
9	0.63	0.76	1.89	3	30 min	0.27
10	0.63	0.76	1.89	3	30 min	0.28

*Due to the stability issue, crude products were used immediately in the next step without further purification.

2.4. Preparation of Trimethylsilylacetylene Substituted 1-Azacoumarin Derivative (99) from Compound 97

Reproducibility of the next reaction, conversion of 4-OTf substituted 1-azacoumarin (97) to trimethylacetylene substituted 1-azacoumarin (99) was not so good. Under varying amount of catalyst and base treatment (reactions 1, 3, 4, 5, and 6 in Table 2.4) the yields of the reactions were moderate (50-75%) for two steps. On the other hand, the yields of the reactions 7-10 in Table 2.4 were quite low although there were high amounts of catalyst. We believe that reactions reproducibility is mostly depends on the amount of the moisture present in the solvent.

Table 2.4. Preparation of trimethylsilylacetylene substituted 1-azacoumarin



97 98 99

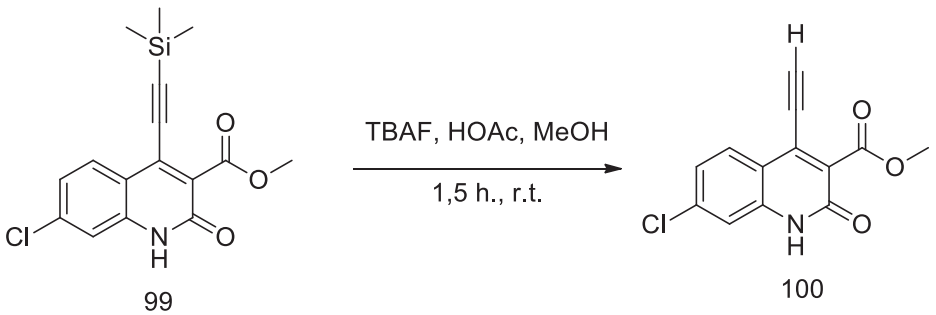
Entry	97 (eq.)	98 (eq.)	$PdCl_2(PPh_3)_2$ (eq.)	CuI (eq.)	DIPEA (eq.)	CH_3CN (mL)	Time (h)	Yield* (%)
1	1.0	3.2	0.3	0.6	8.0	8	3.5	51
2	1.0	1.5	0.2	0.4	4.0	8	5.5	-[a]
3	1.0	1.2	0.1	0.2	3.0	8	4	50
4	1.0	1.2	0.1	0.2	3.0	9	4	75
5	1.0	1.2	0.1	0.2	3.0	9	4	68
6	1.0	3.2	0.3	0.5	8.0	8	3.5	50
7	1.0	3.0	0.2	0.4	4.0	8	3.5	19
8	1.0	3.0	0.2	0.4	4.0	8	3.5	25
9	1.0	3.0	0.2	0.4	4.0	8	3.5	18
10	1.0	3.0	0.2	0.4	4.0	8	3.5	15

*Yields are given for two steps. a: no reaction.

2.5. Removal of Trimethylsilane to Prepare Acetylene Substituted 1-Azacoumarin Derivative (100)

In the next step, TMS group from the compound 99 was removed in the presence of TBAF and acetic acid in methanol. Results are summarized in Table 2.5 and this reaction is quite reproducible and works better in the presence of more acid in more diluted reaction mixtures.

Table 2.5. Preparation of acetylene substituted 1-azacoumarin (100)



Entry	99 (eq.)	TBAF (eq.)	MeOH (mL)	HOAc (mL)	Time (h)	Yield (%)
1	1.0	3.0	5	1	1	64
2	1.0	3.0	5	1	2.5	58
3	1.0	3.0	5	1	1	58
4	1.0	4.5	7.5	1.5	4	-[a]
5	1.0	3.0	10	2	1.5	73
6	1.0	3.0	10	2	1.5	81
7	1.0	3.0	10	2	1.5	83
8	1.0	3.0	5	1	1.5	75
9	1.0	3.0	10	2	1.5	70
10	1.0	3.0	5	1	1.5	56

a: no reaction.

2.6. Synthesis of 4-Chlorobenzyl Azide (102)

To use in click chemistry, 4-chlorobenzyl azide was prepared starting from 4-chlorobenzyl chloride (101) by simply after heating at 60 °C with sodium azide in dry DMF (Figure 2.2). This reaction was quite reproducible and yields were in the range of (73-94%).

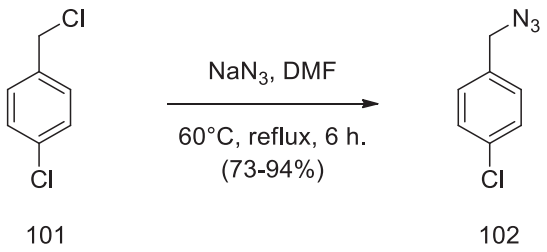


Figure 2.2. Preparation of 4-chlorobenzyl azide (102).

2.7. Attempts for the Preparation of 1,5-Disubstituted 1,2,3-Triazole Modified 1-Azacoumarin (91) via Cp_2Ni-Xantphos Catalyst

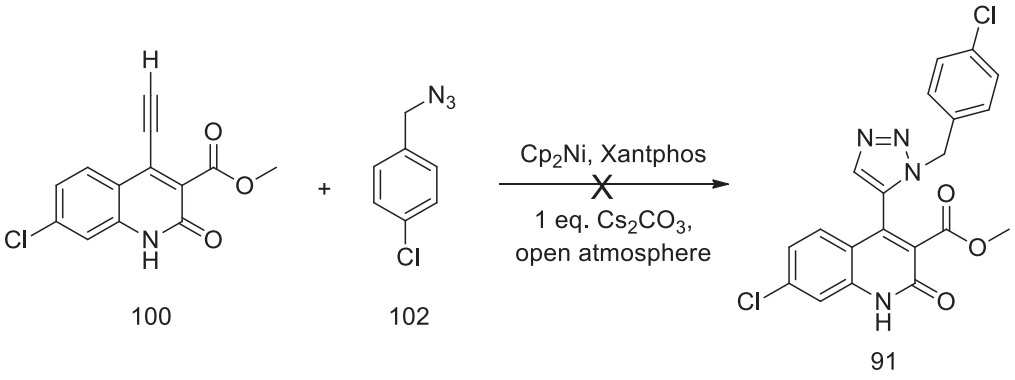
As it was mentioned above, click chemistry step of this synthesis was the most problematic and irreproducible part. The product of this reaction, compound 91, could be prepared only in 4 mg by Erdoğmuş M.[48] To increase the yield of this reaction, parameters of the reaction conditions such as different solvent systems, amount of catalyst or benzyl azide and temperature were studied. The results of these trials were summarized in Table 2.6.

In trial 1, DCM-MeOH mixture was used as solvent system to give a proton source in order to protonate position 4- of 1,2,3-triazole moiety but no product formation was observed. Because no positive effect of MeOH was seen in the reaction rest of the experiments were carried out either in DCM or DMF.

At low temperature experiments (Entries 2-6), no product formation was observed in both DCM and DMF solution. Also, increment in the amount of catalyst did not change the result of the reaction.

Because the solubility of the starting materials is better in DMF. Next reactions were carried out in DMF by increasing the heat up to 50 °C (Entries 8-10). High amount of catalyst loading (Entry 8) or longer reaction times (Entries 9-10) could not improve the click chemistry for this alkyne (100). It seems that either the presence of 1-azacoumarin or ester next to the reaction center may cause the failure of this reaction. Also, it may be the possible failure during the preparation of Cp_2Ni-Xantphos complex may be the lack of reaction.

Table 2.6. Cp_2Ni-Xantphos catalytic conditions for prepare 1,5-disubstituted 1,2,3-triazole modified 1-azacoumarin



Entry	100 (eq.)	102 (eq.)	Cp_2Ni (eq.)	Xantphos (eq.)	Solvent (mL)	Conditions
1	1.2	1.0	0.2	0.2	DCM:MeOH (6:1)	0°C to r.t. 6 h
2	1.2	1.0	0.2	0.2	DCM (2)	0°C to r.t. 2 h
3	1.2	1.0	0.2	0.2	DCM (9)	0°C to r.t. 4 h
4	1.0	1.0	0.34	0.34	DCM (2)	0°C to r.t. 5 h
5	1.0	1.0	0.34	0.34	DMF (2)	0°C to r.t. 6 h
6	1.0	2.09	0.34	0.34	DMF (2)	0°C to r.t. overnight
7	1.2	1.0	0.2	0.2	DMF (2)	50 °C 4 h
8	1.0	2.09	0.34	0.34	DMF (2)	50 °C 5 h
9	1.2	1.0	0.2	0.2	DMF (2)	50 °C overnight
10	1.2	1.0	0.2	0.2	DMF (2)	50 °C overnight

2.8. Preparation of 1-(4-Chlorobenzyl)-5-(4-fluorophenyl)-*1H*-1,2,3-Triazole (104)

To investigate these possible effects, a model reaction was performed as shown in Figure 2.3. Preparation of Cp_2Ni-Xantphos catalytic system was performed in a vessel under air and then used for click chemistry between 4-fluorophenyl acetylene and 4-chlorobenzyl azide. Even at first trials, 62% product formation was observed at room temperature. This result indicates that we successfully prepared the Cp_2Ni- Xantphos

catalyst in all of our previous experiments and problem mainly caused from either the presence of 1-azacoumarin or ester functional groups.

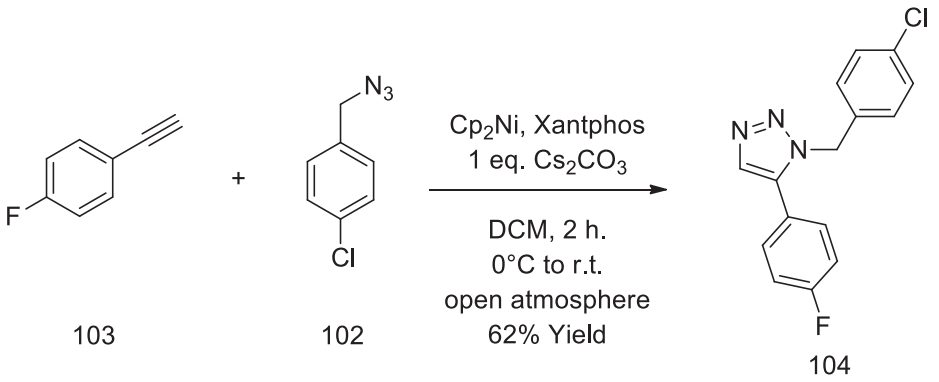
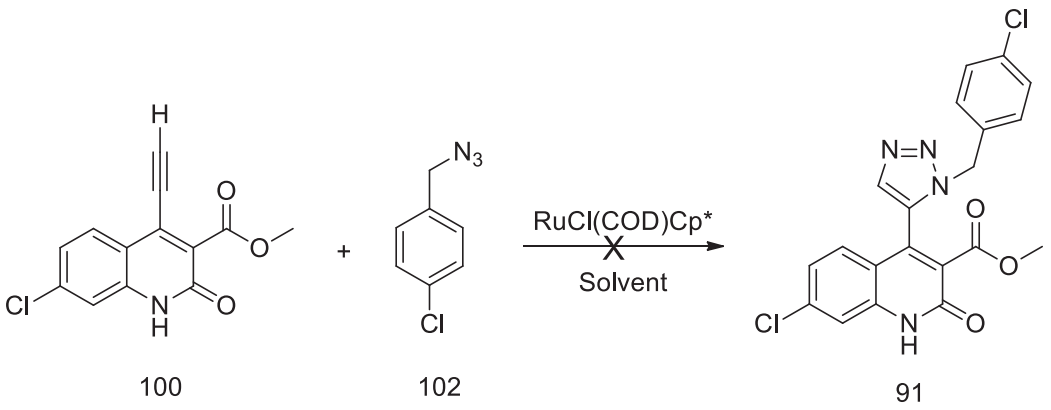


Figure 2.3. Preparation of 1-(4-chlorobenzyl)-5-(4-fluorophenyl)-*1H*-1,2,3-triazole (104) by Cp_2Ni-Xantphos catalytic system.

2.9. Attempts for the Preparation of 1,5-Disubstituted 1,2,3-Triazole Substituted 1-Azacoumarin via RuCl(COD)Cp* Catalyst

Cp_2Ni-Xantphos catalytic system works only in the presence of Cs_2CO_3 which may be also the cause of lack of reaction via the hydrolysis of ester group. Hence another well-known catalyst, RuCl(COD)Cp*, was used to eliminate the effect of possible hydrolysis of ester under basic conditions. As it can be seen from Table 2.7, all trials were inconclusive in THF and DMF solvents. It seems that the presence of an ester group next to the reaction centre may cause the lack of reaction for alkyne substituted 1-azacoumarin derivative (100).

Table 2.7. RuCl(COD)Cp* catalytic conditions to prepare 1,5-disubstituted 1,2,3-triazole modified 1-azacoumarin (91)



100 102 91

Entry	100 (eq.)	102 (eq.)	RuCl(COD)Cp* (eq.)	Solvent (mL)	Time
1	1.00	2.00	0.01	THF (3)	overnight
2	1.00	2.00	0.01	THF (3)	1 h
3	1.05	2.00	0.01	DMF (2)	2 h

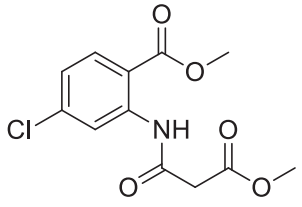
CHAPTER 3

EXPERIMENTAL

3.1. General Methods

All chemicals and solvents used were purchased from Carlo Erba, Sigma-Aldrich and ABCR and are commercial grade. By using a 4Å molecular sieve, tetrahydrofuran, dichloromethane, and acetonitrile were dried. Using the MBraun solvent purification method, DMF was dried. All experiments, unless otherwise noted, were carried out in a nitrogen atmosphere. Merck TLC plates were used to perform thin layer chromatography and monitor reactions (Silica gel 60 F254). Processes for chromatographic purification were carried out using silica gel with a mesh size range of 70–230. With the use of a Varian 400-MR (400 MHz) spectrometer, ^{1}H NMR spectra were acquired and chemical shifts were reported in δ (ppm). $CDCl_3$ peaks were used as reference for ^{1}H NMR (7.26 ppm), DMSO peaks were used as reference for ^{1}H NMR (2.5 ppm).

3.1.1. Methyl 4-Chloro-2-(3-methoxy-3-oxopropanamido)benzoate (95)



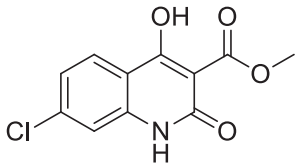
$C_{12}H_{12}ClNO_5$

The experiment is set up in a 25 mL double necked bottom reaction flask. Under nitrogen atmosphere, 200 mg (1.07 mmol, 1.0 eq.) of methyl-2-amino-4-chlorobenzene and 327 mg (450 µL, 3.23 mmol, 3.0 eq.) of triethylamine is dissolved in 3 mL dry DCM

at 0 °C. 162 mg (130 µL, 1.18 mmol, 1.1 eq.) methyl malonyl chloride is dissolved in 2 mL dry DCM and added drop by drop to the reaction flask, and the reaction was warmed up to room temperature. After stirring three hours at 0 °C, 30 mg (25 µL, 0.2 mmol, 0.2 eq.) of methyl malonyl chloride is added. The reaction mixture, which is stirred at room temperature for 4 hours, is quenched with 100 mL of 5% $NaHCO_3$ and extracted with 3x25 mL of DCM. The organic phase is washed once with 50 mL of concentrated NaCl solution, the organic phase is dried with $MgSO_4$ organic solvent is removed under reduced pressure. The crude product is purified by SiO_2 column chromatography with a 1:2 (EtOAc/Hex) solvent system. The pure product obtained in 270 mg (88 %) as a white solid. R_f = 0.40 (1:2, EtOAc:Hexane)

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.54 (s, 1H), 8.79 (d, *J* = 2.1 Hz, 1H), 7.97 (d, *J* = 8.6 Hz, 1H), 7.09 (dd, *J* = 8.6, and 2.1 Hz, 1H), 3.94 (s, 3H), 3.81 (s, 3H), 3.54 (s, 2H).

3.1.2. Methyl 7-Chloro-4-hydroxy-2-oxo-1,2-dihydroquinoline-3 carboxylate (96)



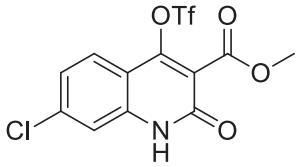
$C_{11}H_8ClNO_4$

Under nitrogen atmosphere, 225 mg (2.00 mmol, 2.2 eq.) of potassium tert-butoxide is added to one necked bottom flask which is containing 260 mg (0.91 mmol, 1.0 eq.) of compound 95. Then, 20 mL of dry THF is added to the reaction flask. The reaction flask is closed with septum. The experiment is stirred for 6 h at room temperature. After that, THF is removed from reaction flask under reduced atmosphere. To adjust the pH of reaction mixture to 2, 1 M HCl solution was added dropwise. Mixture is extracted

with 50 mL of EtOAc. After collecting the water phase, the organic solid phase remaining above is filtered through filter paper and dried for one day. The product was obtained in 227 mg (98% yield) as white solid. R_f = 0.23 (3% MeOH in chloroform)

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.26 (s, 1H), 11.63 (s, 1H), 7.91 (d, *J* = 8.6 Hz, 1H), 7.26 (d, *J* = 1.8 Hz, 1H), 7.23 (dd, *J* = 8.6 Hz and 2.0 Hz, 1H), 3.83 (s, 3H).

3.1.3. Methyl 7-Chloro-2-oxo-4-(((trifluoromethyl)sulfonyl)oxy)-1,2-dihydroquinoline-3-carboxylate (97)

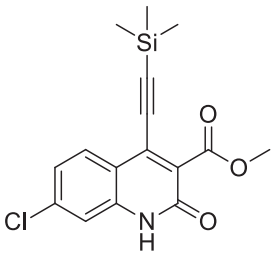


$C_{12}H_7ClF_3NO_6S$

The experiment is set up in a 25 mL one neck round reaction flask. 150 mg (0.60 mmol, 1.0 eq.) of 4-OH-coumarin (96) is added to a solution of 250 µL (1.80 mmol, 3.0 eq.) of triethylamine in 1 mL of dry DMF under nitrogen atmosphere. In another flask, 260 mg (0.73 mmol, 1.2 eq.) of *N*-phenyl-bis(trifluoromethanesulfonimide) is dissolved in 1 mL of dry DMF and transferred to the reaction flask. Then, 1 mL of dry DMF is added. The experiment, which is stirred for 30 minutes, is stopped with 300 mL of distilled water, and extracted with 3x100 mL of EtOAc. The organic phase is washed once with 100 mL of concentrated NaCl solution. The collected organic phase is dried with $MgSO_4$ and organic solvent is removed under reduced pressure. The crude product is purified by SiO_2 column chromatography with 1:1 EtOAc-Hexane solvent system. The pure yellow liquid product is obtained as 70 mg (37% yield). R_f = 0.67 (1:1, EtOAc:Hexane)

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.25 (s, 1H), 7.73 (d, *J* = 8.8 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.29 (d, *J* = 7.7 Hz, 1H), 3.97 (s, 3H).

3.1.4. Methyl 7-Chloro-2-oxo-4-((trimethylsilyl)ethynyl)-1,2-dihydroquinoline-3-carboxylate (99)

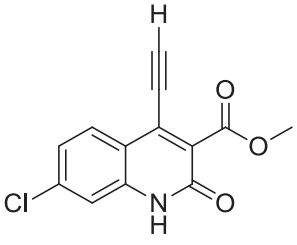


$C_{16}H_{16}ClNO_3Si$

The crude product of 4-triflatecoumarin (97) (0.24 mmol, 1 eq.) is dissolved in 9 mL of dry acetonitrile and degassing with nitrogen atmosphere. Then 17 mg of bis(triphenylphosphine)palladium (II) dichloride (0.024 mmol, 0.1 eq.), 10 mg of copper (I) iodide (0.048 mmol, 0.2 eq.), 30 mg of trimethylsilylacetylene (98) (40 µL, 0.30 mmol, 1.2). eq.) and 93 mg of DIPEA (125 µL, 0.72 mmol, 3.0 eq.) are added and closed with a septum. The reaction is stirred for 4 hours at room temperature and filtered off from a 1:1 silica gel-celite column. The crude product is purified by SiO_2 column chromatography with 1:2 EtOAc-Hexane solvent system. The pure dark brown liquid product is obtained as 60.2 mg (75% yield). R_f = 0.37 (1:2, EtOAc:Hexane)

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.76 (s, 1H), 7.88 (d, *J* = 8.7 Hz, 1H), 7.45 (d, *J* = 1.8 Hz, 1H), 7.24 (dd, *J* = 8.7 and 2.0 Hz, 1H), 4.00 (s, 3H), 0.31 (s, 9H).

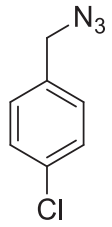
3.1.5. Methyl 7-Chloro-4-ethynyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (100)



$C_{13}H_8ClNO_3$

Under a nitrogen atmosphere, 78 mg (0.24 mmol, 1.0 eq.) of compound 99 is dissolved in 10 mL of methanol in a one-neck reaction flask followed by the addition of 2 mL of acetic acid. Then, 675 mg (710 µL, 0.71 mmol, 3 eq.) of TBAF is added and the reaction is closed with septum. The experiment, which is stirred at room temperature for 1.5 hours, is quenched with 50 mL of saturated $NaHCO_3$ and 50 mL of distilled water. Then reaction mixture is extracted with 3x30 mL of EtOAc. The collected organic phase is dried with $MgSO_4$ and organic solvent is removed under reduced pressure. The product, which is purified by 1:3 EtOAc-Hexane SiO_2 column chromatography and pure yellow liquid product is weighed as 51.5 mg (83% yield). R_f = 0.48 (1:2, EtOAc:Hexane)
^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 7.83 (d, *J* = 8.6 Hz, 1H), 7.25 - 7.24 (m, 1H), 7.13 (dd, *J* = 8.7 and 2.0 Hz, 1H), 3.91 (s, 1H), 3.86 (s, 3H).

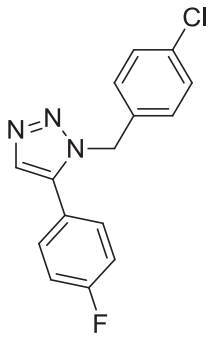
3.1.6. 1-(Azidomethyl)-4-chlorobenzene (102)



$C_7H_6ClN_3$

The experiment is set up on a double-necked reaction flask. To a solution of 673 mg (560 µL, 4.18 mmol, 1.0 eq.) of 4-chlorobenzyl chloride (101) in 2 mL of dry DMF 300 mg (4.61 mmol, 1.1 eq.) of sodium azide is added under reflux at 60 °C. The experiment, which is stirred for 6 hours, is quenched with 200 mL of distilled water, and extracted with 3x100 mL of EtOAc. The collected organic phase is dried with $MgSO_4$, and organic solvent is removed under reduced pressure. After the reaction is dried in the vacuum pump for 30 minutes, the oily liquid is weighed 653.8 mg (3.9 mmol) and the yield is calculated as 93%. R_f = 0,60 (1:10, EtOAc:Hexane)
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.33 (m, 2H), 7.26 – 7.22 (m, 2H), 4.30 (s, 2H).

3.1.7. 1-(4-Chlorobenzyl)-5-(4-fluorophenyl)-*1H*-1,2,3-triazole (104)



$C_{15}H_{11}ClFN_3$

In 4 mL vial, 13 mg of (0.07 mmol, 0.20 eq.) bis(cyclopentadienyl)nickel(II), 40 mg of (0.07 mmol, 0.20 eşd.) 4,5-bis(diphenylphosphino)-9,9-dimethyl xanthene, and 114 mg of (0,35 mmol, 1,00 eq.) Cs_2CO_3 are dissolved in 1 mL of DCM at 0 °C. In another 4 mL vial, 50 mg of (0.42 mmol, 1.20 eq.) compound 103 is dissolved 1 mL of DCM at 0 °C and transferred to first reaction vial. When the reaction mixture wormed up to the room temperature, 58 mg of (55 μL, 0.35 mmol, 1.00 eq.) compound 102 is added. The reaction is stirred for 2 hours in open atmosphere. The reaction mixture is filtered off from a 1:1 silica gel-celite column. The product, which is purified by 1:4 EtOAc-Hexane SiO_2 column chromatography, and pure transparent liquid product is weighed as 74.7 mg (62% yield). R_f = 0.50 (1:1, EtOAc:Hexane)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (bs, 1H), 7.25 – 7.14 (m, 4H), 7.12 – 7.06 (m, 2H), 6.96 (d, *J* = 8.4 Hz, 2H), 5.46 (s, 2H).

CHAPTER 4

CONCLUSION

In this thesis optimization of a synthetic route for the preparation of a 1,2,3-triazole modified 1-azacoumarin structure (91) was aimed in order to test its biological activities in cancer cells and then further derivatization to 1,4,5-trisubstituted triazole derivatives. In this synthetic route methyl 4-chloroanthranilate can be used as a starting material and target compound 91 can be prepared in six steps.

In the first step, methylmalonylchloride addition to aniline NH_2 group, and then the cyclization reaction to produce 4-OH 1-azacoumarin derivative was successfully achieved in moderate yield. After conversion of 4-OH group to a good leaving group by addition of triflate, Sonogashira reaction and then removal of the TMS group gave the 4-ethynyl substituted 1-azacoumarin derivative in moderate yields. All of these steps are quite reproducible. In the last step of the synthesis, click chemistry of 4-ethynyl 1-azacoumarin with 4-chlorobenzyl azide was carried out in the presence of either Cp_2Ni-Xantphos or RuCl(COD)Cp* catalytic systems. Both catalytic systems were failed in order to produce the final product 91. A model reaction between 4-fluorophenylacetylene and 4-chlorobenzyl azide produces the corresponding 1,5-disubstituted 1,2,3-triazole structure. This implies that the presence of an ester group close to the reaction center may be the reason for the lack of reaction for 4-ethynyl 1-azacoumarin. Hence, ester group should be eliminated before the click chemistry in order to synthesize triazole modified coumarin derivatives.

REFERENCES

(1) Najafi, M.; Majidpoor, J.; Toolee, H.; Mortezaee, K. The Current Knowledge Concerning Solid Cancer and Therapy. *J Biochem Mol Toxicol* **2021**, *35* (11), e22900.

(2) Ji Ram, V.; Sethi, A.; Nath, M.; Pratap, R. Chapter 5 - Five-Membered Heterocycles. In *The Chemistry of Heterocycles*; Ji Ram, V., Sethi, A., Nath, M., Pratap, R., Eds.; Elsevier, 2019; pp 149–478.

(3) Ali, A. A. 1,2,3-Triazoles: Synthesis and Biological Application. In *Azoles*; Kuznetsov, A., Ed.; IntechOpen: Rijeka, 2020.

(4) Zhang, X.; Cui, X.; Wang, W.; Zeng, T.; Wang, Y.; Tan, Y.; Liu, D.; Wang, X.; Li, Y. Base-Promoted Regiospecific Synthesis of Fully Substituted 1,2,3-Triazoles and 1,5-Disubstituted 1,2,3-Triazoles. *Asian J Org Chem* **2020**, *9* (12), 2176–2183.

(5) Smith, C. D.; Greaney, M. F. Zinc Mediated Azide–Alkyne Ligation to 1,5- and 1,4,5-Substituted 1,2,3-Triazoles. *Org Lett* **2013**, *15* (18), 4826–4829.

(6) Camberlein, V.; Kraupner, N.; Bou Karroum, N.; Lipka, E.; Deprez-Poulain, R.; Deprez, B.; Bosc, D. Multi-Component Reaction for the Preparation of 1,5-Disubstituted 1,2,3-Triazoles by in-Situ Generation of Azides and Nickel-Catalyzed Azide-Alkyne Cycloaddition. *Tetrahedron Lett* **2021**, *73*, 153131.

(7) Kim, W. G.; Kang, M. E.; Lee, J. bin; Jeon, M. H.; Lee, S.; Lee, J.; Choi, B.; Cal, P. M. S. D.; Kang, S.; Kee, J.-M.; Bernardes, G. J. L.; Rohde, J.-U.; Choe, W.; Hong, S. Y. Nickel-Catalyzed Azide–Alkyne Cycloaddition To Access 1,5-Disubstituted 1,2,3-Triazoles in Air and Water. *J Am Chem Soc* **2017**, *139* (35), 12121–12124.

(8) ’Oakdale, J. S. ’; ’Fokin, V. V. ’; ’Umezaki, S.; ’Fukuyama, T. Preparation of 1,5-Disubstituted 1,2,3-Triazoles via Ruthenium-Catalyzed Azide Alkyne Cycloaddition. *Organic Synth.* **2013**, *90*, 96–104.

(9) Mahadari, M. K.; Tague, A. J.; Keller, P. A.; Pyne, S. G. Synthesis of Sterically Congested 1,5-Disubstituted-1,2,3-Triazoles Using Chloromagnesium Acetylides and Hindered 1-Naphthyl Azides. *Tetrahedron* **2021**, *81*, 131916.

(10) Johansson, J. R.; Lincoln, P.; Nordén, B.; Kann, N. Sequential One-Pot Ruthenium-Catalyzed Azide−Alkyne Cycloaddition from Primary Alkyl Halides and Sodium Azide. *J Org Chem* **2011**, *76* (7), 2355–2359.

(11) Yamajala, K. D. B.; Patil, M.; Banerjee, S. Pd-Catalyzed Regioselective Arylation on the C-5 Position of N-Aryl 1,2,3-Triazoles. *J Org Chem* **2015**, *80* (6), 3003–3011.

(12) ’Celik, F.; ’Unver, Y.; ’Barut, B.; ’Ozel, A.; ’Sancak, K. Synthesis, Characterization and Biological Activities of New Symmetric Bis-1,2,3-Triazoles with Click Chemistry. *Med Chem (Los Angeles)* **2018**, *14* (3), 230–241.

(13) Kopańska (née Zastąpiło), K.; Najda, A.; Żebrowska, J.; Chomicz, L.; Piekarczyk, J.; Myjak, P.; Bretner, M. Synthesis and Activity of 1H-Benzimidazole and 1H-Benzotriazole Derivatives as Inhibitors of Acanthamoeba Castellanii. *Bioorg Med Chem* **2004**, *12* (10), 2617–2624.

(14) Fu, J.; Yang, Y.; Zhang, X.-W.; Mao, W.-J.; Zhang, Z.-M.; Zhu, H.-L. Discovery of 1H-Benzo[d][1,2,3]Triazol-1-Yl 3,4,5-Trimethoxybenzoate as a Potential Antiproliferative Agent by Inhibiting Histone Deacetylase. *Bioorg Med Chem* **2010**, *18* (24), 8457–8462.

(15) Carta, A.; Sanna, P.; Palomba, M.; Vargiu, L.; la Colla, M.; Loddo, R. Synthesis and Antiproliferative Activity of 3-Aryl-2-(1H-Benzotriazol-1-Yl)Acrylonitriles. Part III. *Eur J Med Chem* **2002**, *37*, 891–900.

(16) Patel, P. D.; Patel, M. R.; Kocsis, B.; Kocsis, E.; Graham, S. M.; Warren, A. R.; Nicholson, S. M.; Billack, B.; Fronczek, F. R.; Talele, T. T. Design, Synthesis and Determination of Antifungal Activity of 5(6)-Substituted Benzotriazoles. *Eur J Med Chem* **2010**, *45* (6), 2214–2222.

(17) Rezaei, Z.; Khabnadideh, S.; Pakshir, K.; Hossaini, Z.; Amiri, F.; Assadpour, E. Design, Synthesis, and Antifungal Activity of Triazole and Benzotriazole Derivatives. *Eur J Med Chem* **2009**, *44* (7), 3064–3067.

(18) Heald, R. A.; Modi, C.; Cookson, J. C.; Hutchinson, I.; Laughton, C. A.; Gowan, S. M.; Kelland, L. R.; Stevens, M. F. G. Antitumor Polycyclic Acridines. 8. Synthesis and Telomerase-Inhibitory Activity of Methylated Pentacyclic Acridinium Salts. *J Med Chem* **2002**, *45* (3), 590–597.

(19) Pokhodylo, N. T.; Obushak, M. D. 4-(Benzo[d]Thiazol-2-Yl)-1-(2-Nitrophenyl)-1H-1,2,3-Triazol-5-Amine. *Molbank* **2022**, *2022* (3).

(20) Çelik, F.; Ustabaş, R.; Süleymanoğlu, N.; Direkel, Ş.; Güler, H. İ.; Ünver, Y. 1,2,3-Triazole Derivative: Synthesis, Characterization, DFT, Molecular Docking Study and Antibacterial-Antileishmanial Activities. *Journal of the Indian Chemical Society* **2021**, *98* (8), 100105.

(21) Glanzmann, N.; Antinarelli, L. M. R.; da Costa Nunes, I. K.; Pereira, H. M. G.; Coelho, E. A. F.; Coimbra, E. S.; da Silva, A. D. Synthesis and Biological Activity of Novel 4-Aminoquinoline/1,2,3-Triazole Hybrids against Leishmania Amazonensis. *Biomedicine & Pharmacotherapy* **2021**, *141*, 111857.

(22) Joolakanti, H. B.; Kamepalli, R.; Miryala, J.; Battu, S. Synthesis, Docking, and Biological Activities of Novel Metacetamol Embedded [1,2,3]-Triazole Derivatives. *J Mol Struct* **2021**, *1242*, 130786.

(23) Cao, Y.; Yang, Y.; Ampomah-Wireko, M.; Obaid Arhema Frejat, F.; Zhai, H.; Zhang, S.; Wang, H.; Yang, P.; Yuan, Q.; Wu, G.; Wu, C. Novel Indazole Skeleton

Derivatives Containing 1,2,3-Triazole as Potential Anti-Prostate Cancer Drugs. *Bioorg Med Chem Lett* **2022**, *64*, 128654.

(24) Sudhakar, D. G. S.; Rao, A. S.; Reddy, Ch. V. R.; Somaiah, N. Design and Synthesis of 1,2,3-Triazole Incorporated Pyrimidine-Benzoxazole Derivatives as Anticancer Agents. *Chemical Data Collections* **2022**, *41*, 100906.

(25) Patil, S.; Jalde, S.; Patil, G. Medicinal Significance of Novel Coumarins: A Review. *Int J Curr Pharm Res* **2021**, *13*, 1–5.

(26) Çamur, M.; Durmuş, M.; Bulut, M. Highly Singlet Oxygen Generative Water-Soluble Coumarin Substituted Zinc(II) Phthalocyanine Photosensitizers for Photodynamic Therapy. *Polyhedron* **2012**, *41* (1), 92–103.

(27) Sersen, F.; Lácová, M. Antioxidant Activity of Some Coumarins. *Acta Fac Pharm Univ Comen* **2015**, *62*, 41–45.

(28) Traykova, M.; Kostova, I. Coumarin Derivatives and Oxidative Stress. *International Journal of Pharmacology* **2005**, *1*.

(29) Gaber, A.; Alsanie, W. F.; Alhomrani, M.; Alamri, A. S.; El-Deen, I. M.; Refat, M. S. Synthesis and Characterization of Some New Coumarin Derivatives as Probable Breast Anticancer MCF-7 Drugs. *Crystals (Basel)* **2021**, *11* (5).

(30) Reyes-Batlle, M.; Freijo, M. B.; López-Arencibia, A.; Lorenzo-Morales, J.; McNaughton-Smith, G.; Piñero, J. E.; Abad-Grillo, T. Identification of N-Acyl Quinolin-2(1H)-Ones as New Selective Agents against Clinical Isolates of Acanthamoeba Keratitis. *Bioorg Chem* **2020**, *99*, 103791.

(31) Manikandan, R.; Jeganmohan, M. Ruthenium-Catalyzed Cyclization of Anilides with Substituted Propiolates or Acrylates: An Efficient Route to 2-Quinolinones. *Org Lett* **2014**, *16* (13), 3568–3571.

(32) Wu, J.; Xiang, S.; Zeng, J.; Leow, M.; Liu, X.-W. Practical Route to 2-Quinolinones via a Pd-Catalyzed C–H Bond Activation/C–C Bond Formation/Cyclization Cascade Reaction. *Org Lett* **2015**, *17* (2), 222–225.

(33) Atalay, S. S.; Assad, M. Y.; Amagata, T.; Wu, W. Mild, Efficient, and Solvent-Free Synthesis of 4-Hydroxy-2-Quinolinones. *Tetrahedron Lett* **2020**, *61* (16), 151778.

(34) Basuli, S.; Satyanarayana, G. Palladium Catalyzed Direct Acylation of Iodo-Acetanilides/Iodo-Phenyl Acetates: Domino One-Pot Synthesis of 2-Quinolinones. *European J Org Chem* **2018**, *2018* (8), 957–970.

(35) Jayashree, B. S.; Thomas, S.; Nayak, Y. Design and Synthesis of 2-Quinolones as Antioxidants and Antimicrobials: A Rational Approach. *Medicinal Chemistry Research* **2010**, *19* (2), 193–209.

(36) Ishida, T.; Kikuchi, S.; Yamada, T. Efficient Preparation of 4-Hydroxyquinolin-2(1H)-One Derivatives with Silver-Catalyzed Carbon Dioxide Incorporation and Intramolecular Rearrangement. *Org Lett* **2013**, *15* (14), 3710–3713.

(37) Yoo, H. J.; Youn, S. W. Zn(II)-Catalyzed One-Pot Synthesis of Coumarins from Ynamides and Salicylaldehydes. *Org Lett* **2019**, *21* (9), 3422–3426.

(38) Yamamoto, Y.; Kirai, N. Synthesis of 4-Arylcoumarins via Cu-Catalyzed Hydroarylation with Arylboronic Acids. *Org Lett* **2008**, *10* (24), 5513–5516.

(39) Mi, X.; Wang, C.; Huang, M.; Wu, Y.; Wu, Y. Preparation of 3-Acyl-4-Arylcoumarins via Metal-Free Tandem Oxidative Acylation/Cyclization between Alkynoates with Aldehydes. *J Org Chem* **2015**, *80* (1), 148–155.

(40) Chen, L.; Wu, L.; Duan, W.; Wang, T.; Li, L.; Zhang, K.; Zhu, J.; Peng, Z.; Xiong, F. Photoredox-Catalyzed Cascade Radical Cyclization of Ester Arylpropiolates with CF3SO2Cl To Construct 3-Trifluoromethyl Coumarin Derivatives. *J Org Chem* **2018**, *83* (15), 8607–8614.

(41) Kulkarni, M. v.; Kulkarni, G. M.; Lin, C.-H.; Sun, C.-M. Recent Advances in Coumarins and 1-Azacoumarins as Versatile Biodynamic Agents. *Curr Med Chem* **2006**, *13* (23), 2795–2818.

(42) Lin, J.; Dong, H.-F.; Oppenheim, J.; Howard, O. Effects of Astragali Radix on the Growth of Different Cancer Cell Lines. *World J Gastroenterol* **2003**, *9* (4), 670–673.

(43) Kim, E. S.; Hyun, J.-W.; Shin, J.-C.; Chung, H.-S. New Alkaloids from Oryza Sativa Cv. Heugjinjubyeo Bran. *Bull Korean Chem Soc* **2009**, *30*, 739–741.

(44) Chung, H.-S. A Quinolone Alkaloid, from the Aleurone Layer of Oryza Sativa Cv. Mihyangbyo, Inhibits Growth of Cultured Human Leukemia Cell. *Prev Nutr Food Sci* **2002**, *7* (2), 119–122.

(45) Bakare, S. B. Anti-Tumor Agents: Design, Synthesis, and Biological Study of -Substituted-7-Hydroxy-1-Azacoumarin-3-Carboxamide Derivatives as Potent Cytotoxic Agents. *Polish Journal of Chemical Technology* **2021**, *23* (1), 53–59.

(46) Morsy, S. A.; Farahat, A. A.; Nasr, M. N. A.; Tantawy, A. S. Synthesis, Molecular Modeling and Anticancer Activity of New Coumarin Containing Compounds. *Saudi Pharmaceutical Journal* **2017**, *25* (6), 873–883.

(47) Revankar, H. M.; Kulkarni, M. v. Cu (I) Catalyzed One Pot SN-Click Reactions of Halogenated Coumarins and 1-Aza-Coumarins. *J Heterocycl Chem* **2018**, *55* (2), 537–544.

(48) Erdoğmuş, M. Synthesis of 1,2,3-Triazole Substituted Azacoumarin Derivatives as Potential Antiproliferative Compounds/ by Mustafa Erdoğmuş; Thesis Advisor Ali Çağır. January 1, 2021.