Preparation and Study of Photoprotective and Antimicrobial Properties of Novel Materials Based on 1,2,3-Triazole

David Milićević, Ph.D.

Doctoral Thesis Summary

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Faculty of Technology

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Příprava a stadium fotoprotektivních a antimikrobiálních vlastností nových látek na bázi 1,2,3-triazolu

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ABSTRACT

A class of novel 1,2,3-triazole functionalised quinoline-2,4-diones was synthesized using multi-step reaction approach, starting with the synthesis of suitable organic azides that served as precursors for introduction of the first 1,2,3-triazole ring to the quinoline-2,4-dione framework.

Afterwards, desirable bis-triazole esters were obtained by acetylation of mono-triazole alcohols, subsequent introduction of propargyl group to the position N1 of quinolone heterocycle, and finally employment of copper(I)-catalysed »click« reaction using three different organic azides. Resulted bis- as well as mono-triazole acetates were then deprotected using acidic alcoholysis, while provided alcohols were further oxidized to suitable aldehydes and carboxylic acids. While crystallization of synthesized compounds was normally performed in case of mono-triazoles, it was seldom successful for bis-triazole species. Consequently, quinolones with two 1,2,3-triazole rings were purified mostly by silica-gel column chromatography. During the fulfilment of outline transformation scheme, various reaction conditions and synthetic routes were tested, monitored, and finally optimised. Apart from mainstream reaction pathway, some focus was also devoted to a few accompanying transformations that either highlighted interesting behaviour of the studied systems (quinoline-2,4-dione ring cleavage), or could be exploited as an alternative approach to anthranilic acid derivatives preparation.

Several synthesized materials were also evaluated for their potential ligand-to-metal coordination abilities, as well as antimicrobial activities against ten microbial strains, including bacteria, yeast and fungi. Additionally, their potential photoprotective characteristics were also briefly examined. Regrettably, no interesting physical properties or biological activities were detected for any of the tested compounds.

The vast majority of results obtained throughout my doctoral studies and presented in this dissertation, have already been published or will be published in scientific journals with the impact factors.

ABSTRAKT

V rámci disertační práce byla vícestupňovou syntézou, jež vycházela z vhodných organických azidů, připravena skupina nových chinolin-2,4-dionů substituovaných 1,2,3-triazolovými kruhy.

Bis-triazolové estery byly po acetylaci mono-triazolových alkoholů získány zavedením propargylové skupiny do polohy N1 chinolonového heterocyklu a následnou "click" reakcí s třemi různými organickými azidy za katalytického účinku měďných kationtů. Z výsledných bis-triazolových, stejně jako z mono-triazolových acetátů byly poté kyselou alkoholýzou odstraněny chránící skupiny a získané alkoholy byly dále oxidovány na příslušné aldehydy a karboxylové kyseliny. Zatímco krystalizace syntetizovaných mono-triazolových sloučenin byla prakticky vždy úspěšná, u bis-triazolových derivátů byla úspěšná pouze zřídka, a proto byly chinolony se dvěma 1,2,3-triazolovými kruhy purifikovány převážně chromatografií na sloupci silikagelu. V průběhu řešení podle naplánovaného schématu byly testovány, sledovány a nakonec optimalizovány reakční podmínky jednotlivých syntetických kroků. Kromě hlavních produktů reakcí byla věnována také pozornost několika vedlejším přeměnám výchozích látek, které buď zdůraznily zajímavé chování studovaných systémů (štěpení chinolin-2,4-dionového kruhu), nebo by mohly být využity jako alternativní přístup k přípravě derivátů kyseliny anthranilové.

U několika syntetizovaných derivátů byla sledována jejich potenciální schopnost koordinovat kovy, a také byly testovány jejich antimikrobiální účinky vůči deseti druhům mikroorganismů zahrnujících zástupce bakterií, kvasinek a hub. Navíc byly okrajově zkoumány jejich potenciální fotoprotektivní vlastnosti. Bohužel nebyly zjištěny žádné zajímavé fyzikální vlastnosti nebo biologické aktivity u žádné z testovaných sloučenin.

Naprostá většina výsledků získaných v rámci doktorského studia, které jsou prezentovány v této disertační práci, byla již publikována v impaktovaném vědeckém časopisu nebo je obsažena v rukopisu publikace zaslaném do redakce impaktovaného časopisu.

TABLE OF CONTENTS

ABBREVIATIONS

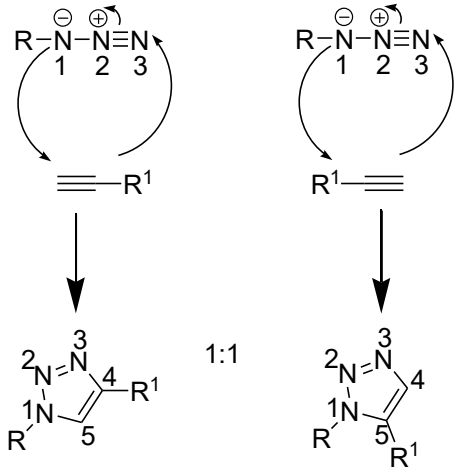
ACN – acetonitrile
Bn – benzyl
CCM – Czech collection of microorganisms
COSY – correlation spectroscopy
CuAAC – copper(I)-catalysed azide-alkyne cycloaddition
DCM – dichloromethane
DIPEA – *N*,*N*-diisopropylethylamine
DMF – dimethylformamide
DMSO – dimethyl sulfoxide
EA – elemental analysis
HMBC – heteronuclear multiple bond correlation
HOMO – highest occupied molecular orbital
HRMS – high resolution mass spectrometry
HSQC – heteronuclear single quantum correlation
IR – infrared (spectroscopy)
LUMO – lowest unoccupied molecular orbital
Me – methyl
NMR – nuclear magnetic resonance
OAc – acetoxy
PCC – pyridinium chlorochromate
Ph – phenyl
pKa – negative base-10 logarithm of the acid dissociation constant of a solution
ppm – parts per million
Py – pyridyl
rt – room temperature
Ru–Cym – (cymene)ruthenium dichloride dimer
SEM – scanning electron microscope
SN2 – bimolecular nucleophilic substitution
t-BuOH – *tert*-butyl alcohol
THF – tetrahydrofuran
TLC – thin-layer chromatography
TMS – trimethylsilane
TOF – time of flight
TSG – tryptone–soya peptone–glucose (broth)
UV-VIS – ultraviolet-visible
V/*V* – volume/volume percent

1. INTRODUCTION

1,2,3-Triazole species are five-membered heterocyclic compounds consisting of three consecutive linked nitrogen and two carbon atoms. They can be derived from three conceivable tautomeric basic compounds: 1*H*-1,2,3-triazole, 2*H*-1,2,3-triazole, and 4*H*-1,2,3-triazole. They possess strong dipole moment that polarizes the proton on carbon to such a rate that it can act as a hydrogen-bond donor.[1-3] However, because of their low expressed basic character, protonation of 1,2,3-triazoles at physiological pH is suppressed.[1,2] Furthermore, they are practically inert even at elevated temperatures and stable in oxidizing, reducing, as well as hydrolytic media.[2,4] Even though, 1,2,3-triazoles exhibit a large variety of desirable biological activities and physical properties, they do not occur in nature and thus can be obtained only synthetically.[5]

Despite the fact that the cyclisation reaction between the triple-bond compounds and azides was established more than 125 years ago,[6] the synthesis of 1,2,3-triazoles and their characterization had remained considerably unexplored for more than a century.[7] Relatively limited interest in the last-mentioned reaction was due to the fact that traditional Huisgen 1,3-dipolar cycloaddition not only requires severe reaction conditions but also results in hardly separable mixture of 1,4- and 1,5-disubstituted 1,2,3-triazole species.[4,7,8] The 1:1 blend of both compounds (Scheme 1) appears because of their comparable HOMO and LUMO energy levels, and therefore practically equal probability of 1,4- and 1,5-disubstituted adducts emergences.[9-11]

Scheme 1

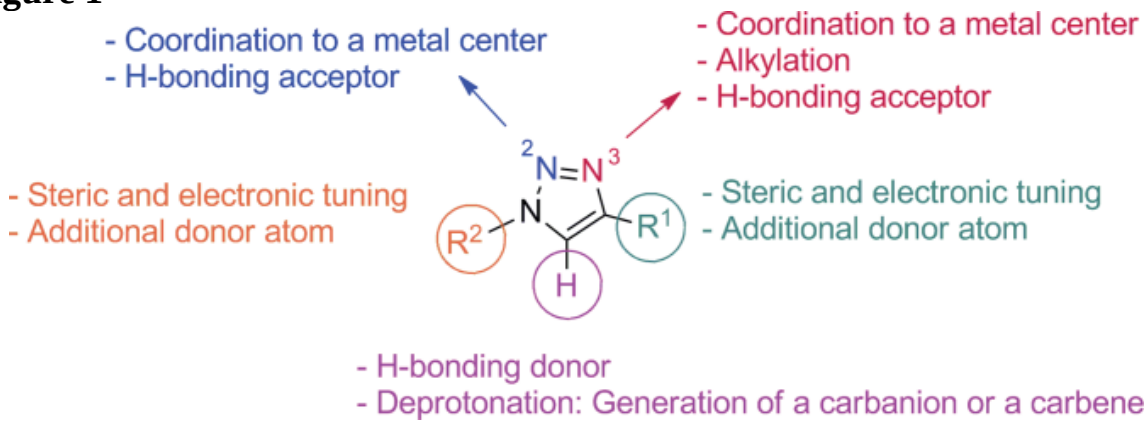


However, the situation dramatically changed in 2002, when Sharpless[8] and Meldal[12] independently discovered and published copper(I) catalysed alkyne-azide cycloaddition (CuAAC) as a 1,2,3-triazoles preparation approach. At the same time, Sharpless introduced and precisely defined the expression »click chemistry«[13] to designate a series of reactions that are simple to perform,

stereoselective, wide in scope, highly-yielding, generate solely inoffensive products (purification limited to crystallization or distillation) and use only readily accessible reagents.[13,14] Furthermore, transformations should proceed in environmentally friendly conditions and they need to be thermodynamically favourable.[10,14] The most common »click reaction« types are additions to carbon-carbon multiple bonds, carbonyl chemistry of the non-aldol type, nucleophilic substitution chemistry and cycloadditions of unsaturated hydrocarbons.[15]

Although, more than 15 years passed from the Sharpless and Meldal's discovery,[8,12] scientists all around the world still admire desirable characteristics of copper catalysed 1,3-dipolar cycloaddition – an effective synthetic pathway for 1,2,3-triazole species preparation. Sarkar et al. emphasized the rarity in chemistry that so much attention is devoted to one single reaction approach, as it is obvious in the case of last-mentioned transformation.[16] At the first sight, simple heterocyclic compound 1,2,3-triazole possesses many versatile properties (Figure 1) that could be effectively exploited in the fields of synthetic,[16-18] medicinal,[17] physical,[19] organometallic,[16] and coordination[16,20] chemistry, as well as in polymer[19] and life[21,22] science.

Figure 1[16]



1.1 Copper catalysed synthesis

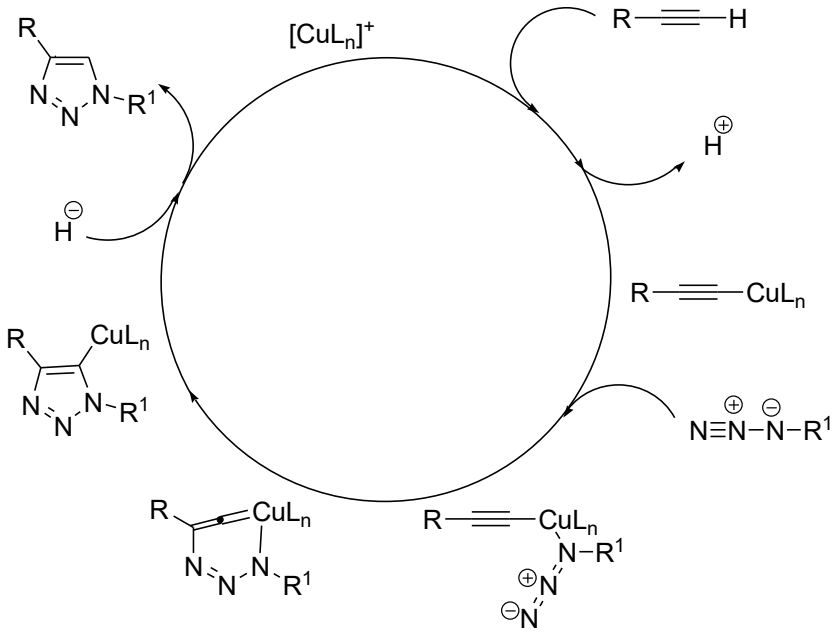
Copper catalysed 1,3-dipolar (Huisgen) cycloaddition is a greatly appreciated and well-investigated method for 1,4-disubstitued 1,2,3-triazoles preparation,[9,23] and therefore a representative example of a »click reaction«.[8,12,18]

Fully converted and highly pure products could be obtained in various solvents including *N*,*N*-dimethylformamide,[12,23] acetonitrile,[12] *N*-ethyldiisopropylamine,[12] dichloromethane,[12] toluene[12] tetrahydrofuran[12] and *tert*-butyl alcohol solution in water.[8,24] Even though, Cu(I) cations as a conversion driving force could be utilized directly from the copper monovalent sources such as copper(I) chloride, copper(I) iodide or copper(I) bromide dimethyl sulphide complex, it is more convenient to prepare them *in situ* by the reduction of Cu(II)

salts that are frequently more pure and less expensive than their univalent counterparts.[8,12] Some simple reducing agents including metallic copper,[25-27] glucose in the presence of Fehling's reagent[28], sodium ascorbate[29] or cuprous cyanide obtained by the NaCN reduction of copper(II) sulphate,[23] are regularly used. The acceleration rates of 10^5–10^8 in comparison to non-catalysed transformation, quantitative regioselectivity, high degree of oxygen and water tolerance, as well as smooth and simple realisation are just few of numerous advantages that make CuAAC reaction widely popular,[9,23] and consequently »the best click reaction to date«.[30] Furthermore, the stability of resultant 1,2,3-triazole species under many reaction conditions and their compatibility with a broad range of functional groups enable formation of triazole consisting peptides, oligosaccharides and other macromolecules.[10]

An acknowledged mechanism of copper catalysed reaction (Scheme 2) starts with the formation of a copper acetylide between Cu(I) and a terminal alkyne. As a result, pKa of alkyne is lowered enough to be able to get deprotonated in aqueous solution. This copper complex undergoes azido-compound attack that is followed by intramolecular cyclisation reaction and further formation of copper-including triazole. Finally, an adduct is protonated, resulting in formation of 1,4-disubstitued 1,2,3-triazole and regeneration of a catalyst.[10,23]

Scheme 2



1.2 Practical applications

Approximately half a century ago, a strong tendency to connection between the synthetic organic chemistry and biosciences, such as biology, medicine and biophysics appeared. As a result, many theoretical findings and facts got applicable values that resulted in sudden upswing of preliminary and practically oriented studies that have been continuously and exponentially growing until today.[31]

1.2.1 Coordination properties and catalytic activities

Several journals reported that nitrogen based compounds, such as triethylamine,[32,33] proline,[32,34] diisopropylethylamine,[32,35] 2,6-lutidine[8,24,32] and many others not only stabilize copper(I) species but also quicken the catalytic process. Similarly, numerous 1,2,3-triazole species exhibit excellent coordination abilities towards various metal centres, including late transition elements,[36] and form different complexes of miscellaneous geometrical structures such as tetrahedral, square-planar and octahedral.[37-40] Taking into account the number of available coordination sites, monodentate,[36] bidentate,[41] tridentate,[42] as well as tetradentate[43] 1,2,3-triazole-based ligands have been developed. Those attached to the metal centre through the more basic N3 1,2,3-triazole nitrogen were labelled as »regular«,[44] while rarer N2-coordinated[41] were designated as »inverse«[44] ligands. In addition, high catalytic activities and great tuning capabilities of bidentate phosphor-containing 1,2,3-triazole-based P,N-chelators were also observed.[45]

1.2.2 Photophysical and electrochemical characteristics

Some 1,2,3-triazole ligands possess specific coordination abilities that could be further utilised for detection of particular cationic or/and anionic species, and consequently exploited in the field of optical and electrical applications.[46] For example, some iridium(III)-1,2,3-triazole complexes were found to exhibit strong phosphorescence, as well as spectral blue-shifting behaviour.[46] Similarly, fluorescent 1,2,3-triazole-based detector for Cu(II) and Hg(II) species was also developed.[47] In addition, recently described »click« reaction approach could be efficiently exploited for bivalent copper cations detection. As dispersed golden nanoparticles, modified with azide and terminal alkyne functional groups, aggregate in the presence of Cu^{2+} ions and sodium ascorbate, practically instant discolouration of pink solution occurs as a visible change.[48] Apart from cationic compounds, anions such as pyrophosphate ($HP_2O_7^{3-}$),[49] chloride[50] and bromide[50] could also be effectively detected by appropriate 1,2,3-triazole-based chemosensors. Finally, simultaneous perception of Zn^{2+} and $H_2PO_4^-$ species was reported as well.[51]

1.2.3 Polymerization and gelation

1,2,3-Triazole scaffolds could also serve as bridging blocks and connectors in the process of polymerization. Using terminal alkyne and azido groups containing monomeric units, 1,2,3-triazole based linear chains,[52] as well as cross-linked networks[53] might be prepared by step-growth polymerization. Additionally, copper(I) catalysed cross-linking concept could also be utilised for the synthesis of bifunctional dendrimers[54] and hydrogels[55] that were already recognised as highly promising materials due to their great usefulness in numerous applications, enviable biocompatibility and preferable water-swelling characteristics.[56]

1.2.4 Medical activities

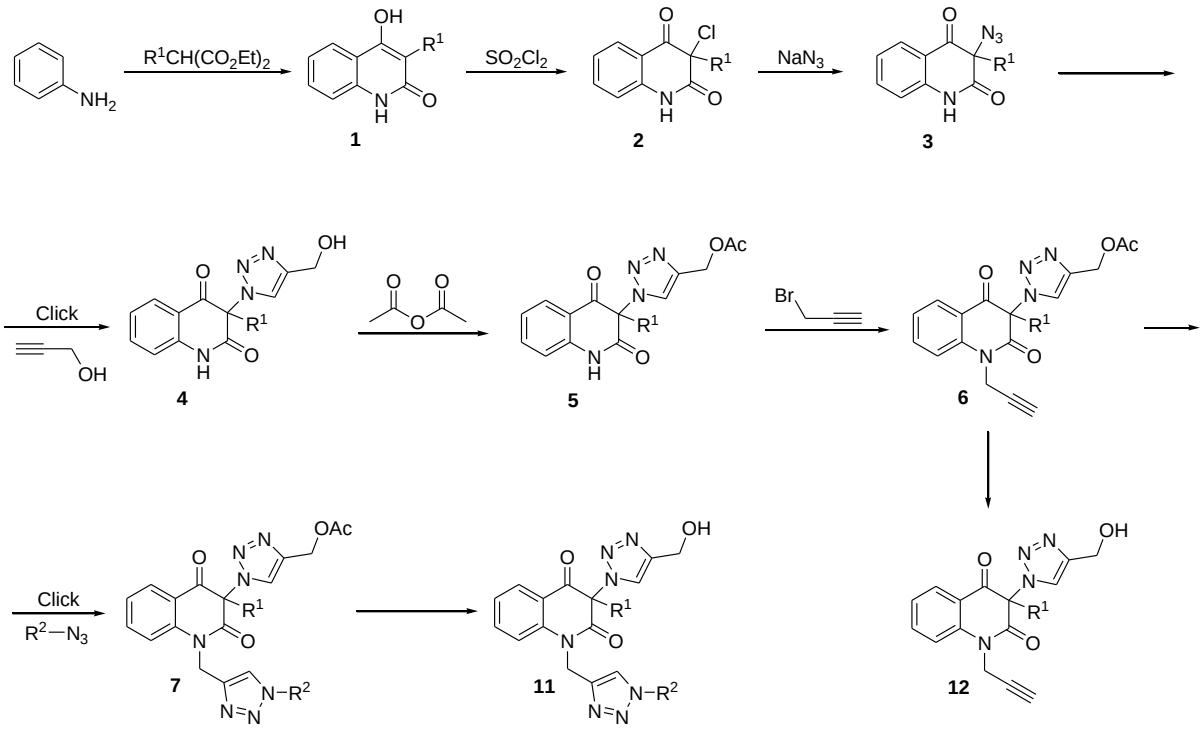
Apart from recently mentioned physical properties, 1,2,3-triazole bearing compounds also exhibit a large variety of medicinal activities. The primary clues of 1,2,3-triazole examination in medicinal purposes dated back in the first half of 20th century, when 1,2,3-triazolo[4,5-*d*] pyrimidine was mentioned as potential anticancer and anti-malignant agent. Because of its desirable and perspective biological characteristics, 1,2,3-triazole based heterocyclic chemistry was designated as »the corner stone« of the medicinal chemistry.[57] Until nowadays, numerous compounds with 1,2,3-triazole scaffolds have been synthesized, examined and found effective against bacterial and fungal strains including *Aspergillus fumigatus*,[58] *Bacillus megaterium*,[59] *Bacillus subtilis*,[60] *Candida albicans*,[61] *Candida mycoderma*,[62] *Chrysosporium keratinophilum*,[60] *Escherichia coli*,[61] *Klebseilla pneumonia*,[59] *Mycobacterium tuberculosis*,[63] *Penicillium marneffei*,[58] *Saccharomyces cerevisiae*,[59] *Salmonella typhosa*,[62] *Shigella dysenteriae*,[62] *Staphylococcus aureus*,[58] *Staphylococcus pyogenes*[58] and *Trichophyton mentagrophytes*.[58] In addition, antiviral,[64] anti-inflammatory,[65] anti-tubercular,[63,66-69] anticancer,[70,71] antitumor,[70,72] anti-oxidative,[73,74] as well as Anti-Alzheimer's disease[75] characteristics were also detected and successfully evaluated for 1,2,3-triazole materials. Finally, 1,2,3-triazole species exhibit high stability towards reductive, oxidative, acidic and also basic environments.[57] Because of their well-expressed dipole moment, they are able to take part in π-stacking and dipole-dipole interactions, and consequently facilitate their potential bindings to the target molecules.[57]

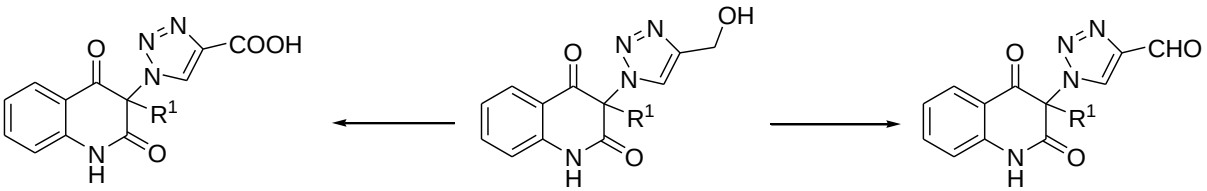
2. AIMS AND OBJECTIVES

As it is already summarized in the title, the primary consideration of my doctoral studies was preparation of various 1,2,3-triazole functionalised quinoline-2,4-dione derivatives, and according to possibilities, evaluation of their potential antimicrobial activities and photoprotective properties.

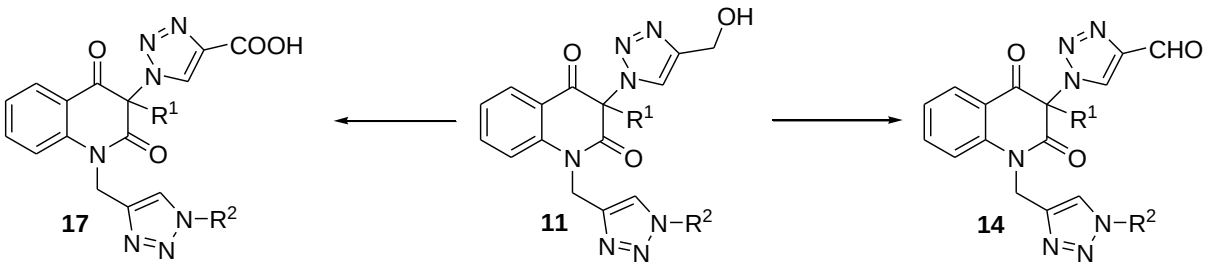
The fundamentals of dissertation topic date back in 2011, when premiere merging of 1,2,3-triazole scaffold with quinoline-2,4-dione framework was published.[76] The idea was to upgrade mono-triazole species to the appropriate bis-triazoles by the introduction of propargyl group to quinolone nitrogen, and subsequent formation of the second 1,2,3-triazole ring. As anticipated, three different organic azides were synthesized and further combined with mono-triazole propargyl derivatives, providing novel bis-triazole counterparts. According to plan, that way obtained collection of mono- and bis-triazole species was efficiently extended by preparation of different functional derivatives bearing hydroxymethyl, formyl or carboxylic group in the position four of the first 1,2,3-triazole scaffold. Establishment and optimisation of reaction conditions for alcohols deprotection and their subsequent oxidation to suitable aldehydes and carboxylic acids were also intended. The main synthetic route is presented in Scheme 3.

Scheme 3. *Synthesis of (1,2,3-triazole)-functionalised quinoline-2,4-diones.*

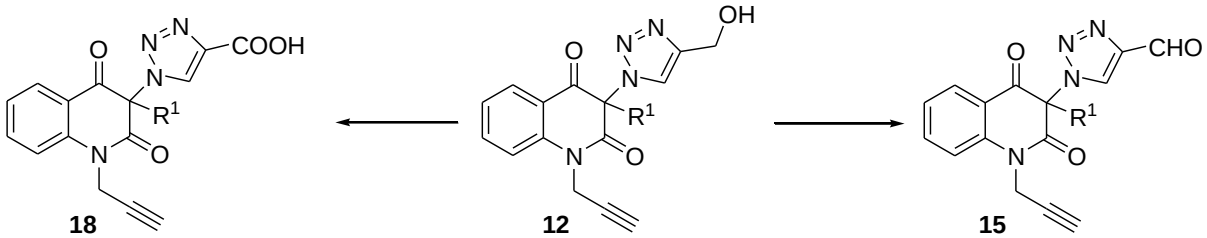




16 **4** **13**



17 **11** **14**



18 **12** **15**

R^1 = Me, Ph R^2 = Bn, Ph, 2-Py

3. RESULTS AND DISCUSSION

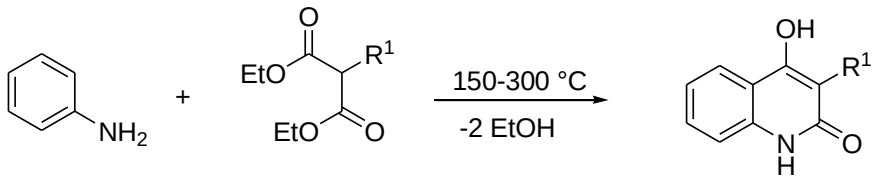
The synthesis of 1,2,3-triazole-functionalised quinolone derivatives was carried out in two parallels, performing each reaction step on both, 3-methyl and 3-phenyl quinoline-2,4-dione counterparts.[77] 3-Azidoquinoline-2,4-dione species were obtained throughout three-step,[77-79] slightly modified literature procedure, and were utilised as precursors for the following »click« reactions. The obtained yields of particular compounds are collected in tables or could be found in schemes. In addition, some accompanying experiments were carried out, to either highlight interesting properties of studied structures or to suggest and evaluate alternative synthetic ways for target compounds acquisition.

3.1 Synthesis of mono-triazole compounds

3.1.1 Precursors

The reaction sequence started with the known condensation reaction between aniline and diethyl methylmalonate or diethyl phenylmalonate, providing 4-hydroxy-3-methylquinolin-2(1*H*)-one[77] (**1a**) and 4-hydroxy-3-phenylquinolin-2(1*H*)-one[77] (**1b**), respectively. At the beginning, the reaction temperature was kept relatively low (up to 150 °C) to avoid distillation of malonate, especially in the case of diethyl methylmalonate (b.p. 200 °C / 760 mm Hg).[80] For this reason, appropriate ester was also added in 10% excess to the reaction mixture. When more than 50% of theoretical mass of ethanol was collected, and therefore amide bond between both reactants was formed, the temperature was gradually raised up to 300 °C. The yields of prepared compounds **1** are given in Scheme 4. Due to the fact that considerably lower yield was observed in the case of methyl derivative (**1a**), the transformation was also performed in the presence of nitrogen, however the obtained yield (53%) was not improved.

Scheme 4. *Preparation of compounds **1a** and **1b**.*

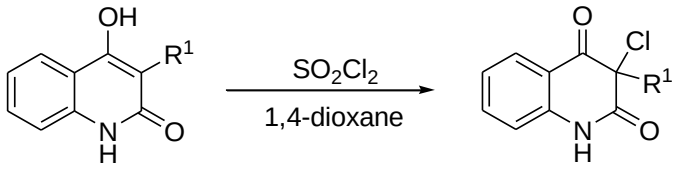


1a (R^1 = Me), 59%
1b (R^1 = Ph), 83%

Synthesized materials were then subjected to chlorination at slightly elevated temperature (40–50 °C), using sulfuryl chloride as a source of chlorine in 1,4-dioxane. During the addition of sulfuryl chloride to the reaction mixture,

white suspension of insoluble starting compound was gradually transformed into vivid yellow solution, indicating formation of 1,4-dioxane soluble chlorinated product. Desired compounds **2** were prepared in excellent yields during the time period of 30 minutes. (Scheme 5).

Scheme 5. *Preparation of compounds **2a** and **2b**.*

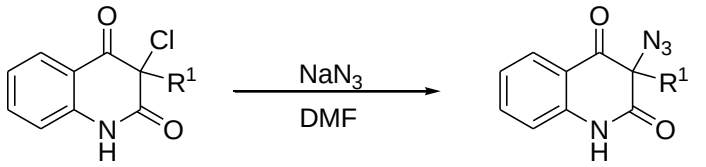


1a (R^1 = Me)
1b (R^1 = Ph)

2a (R^1 = Me), 91%
2b (R^1 = Ph), 93%

Organic azides are widely recognised as valuable partners to acetylenic compounds in 1,3-dipolar cycloaddition reactions. Although, they are assigned as hazardous and harmful species, they play an irreplaceable role in 1,2,3-triazole preparation process.[8] 3-Aziodquinoline-2,4-diones **3** were synthesized by nucleophilic displacement, where chlorine atom of a particular chloro-derivative was exchanged with the azido group. The transformation and isolation processes were carried out in darkness, as organic azides are quite unstable on light. The reactions were completed in approximately three hours, providing yields presented in Scheme 6.

Scheme 6. *Preparation of compounds **3a** and **3b**.*



2a (R^1 = Me)
2b (R^1 = Ph)

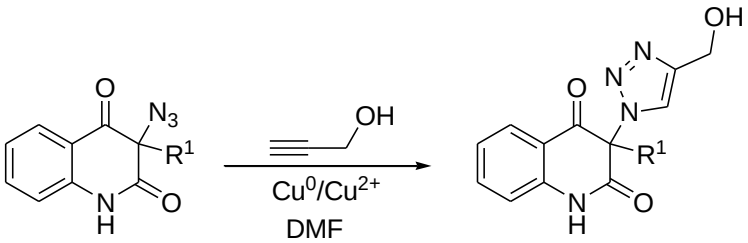
3a (R^1 = Me), 95%
3b (R^1 = Ph), 97%

3.1.2 First 1,2,3-triazole ring

Synthesized organic azides served as precursors in the reaction scheme, as they were further combined with propargyl alcohol to give suitable mono-triazoles. The transformations were carried out in the presence of metal copper and $CuSO_4 \cdot 5H_2O$ in DMF (Scheme 7) and were completed within 30 minutes. Although, relatively large quantities of copper species were added to the reaction mixtures, their residues were in most cases successfully eliminated using filtration through the silica-gel column, subsequent extraction with saturated aqueous solution of NH_4Cl, and finally crystallization from the suitable solvent.

In addition, DMF was efficiently removed by multiple dilutions with toluene and following co-distillations *in vacuo* at 50 °C. Yields of pure compounds **4** could be found in Scheme 7.

Scheme 7. *Preparation of compounds **4a** and **4b**.*

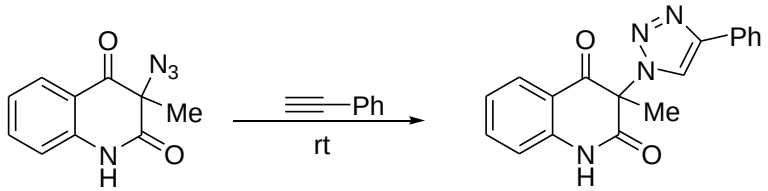


3a (R^1 = Me)
3b (R^1 = Ph)

4a (R^1 = Me), 99%
4b (R^1 = Ph), 98%

Even though, sodium ascorbate or L-ascorbic acid in the presence of Cu^{2+} ions in *t*-BuOH/H_2O 1:1 (*V*/*V*) solvent mixture is the most commonly utilised system for the purpose of 1,2,3-triazole preparation, Cu^0/Cu^{2+} catalytic pair in DMF was recognised as more convenient method for the synthesis of 1,2,3-triazole functionalised quinoline-2,4-diones. To confirm the hypothesis, 3-azido-3-methylquinoline-2,4(1*H*,3*H*)-dione was combined with phenyl acetylene in the presence of Cu^{2+} ions and L-ascorbic acid in CH_2Cl_2/H_2O 1:1 (*V*/*V*) solvent system. Although, the reaction mixture was stirred nearly 100 times longer (48 hours) than in case of Cu^0/Cu^{2+} (30 min), the obtained yield of the former (83%) was still obviously lower than in case of the latter (95%) (Table 1). Taking into account results from 2011,[76] where DMSO was also successfully used as a solvent, it was concluded that the main drawback of quinoline-2,4-dione based materials is their very low solubility in most organic solvent–water systems.

Table 1. *Preparation of compound **4c**.*



3a **4c**

Entry	Time (h)	Yield of **4c** (%)[a]
1	0.5	95[b]
2	48	83[c]

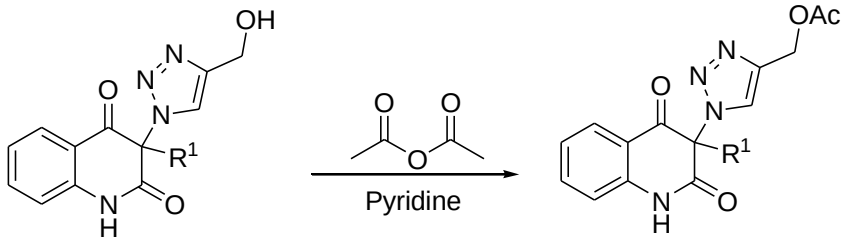
[a] Refers to percent yield of pure (by TLC and IR) isolated product. [b] Employing DMF / Cu^0 / $CuSO_4 \cdot 5H_2O$ conditions. [c] Employing CH_2Cl_2 / water / $CuSO_4 \cdot 5H_2O$ / L-ascorbic acid conditions.

3.2 Synthesis of bis-triazole compounds

3.2.1 Acetylation and propargylation

After mono-triazole alcohols were successfully synthesized, introduction of the second 1,2,3-triazole segment was taken into consideration. The idea was to introduce a propargyl group to the quinolone N1 nitrogen. Even though, *N*-alkylation of the lactam group takes place predominantly, *O*-alkylation has also been reported for similar systems.[81] To avoid formation of undesirable ethers, primary alcohol group was protected using acetic anhydride in pyridine and appropriate acetates were formed. Pyridine not only served as a solvent, but also acted as a base that neutralized the formed acidic by-product, namely acetic acid. After completion of reaction, the content of the flask was poured onto ice-cooled water to remove all inorganic impurities. In case of phenyl derivative, precipitated sand-like solid product was simply collected by filtration through the sintered glass filter, while methyl counterpart (in a contact with water) turned into a sticky material that was firstly exposed to the temperature of 4 °C to partially solidify and then filtered. The yields of pure products are given in Scheme 8.

Scheme 8. *Preparation of compounds **5a** and **5b**.*

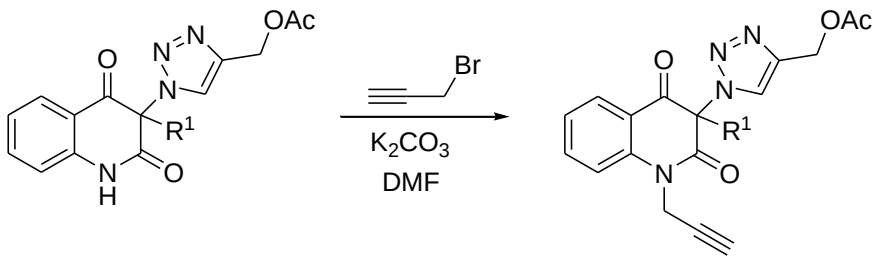


4a (R^1 = Me)
4b (R^1 = Ph)

5a (R^1 = Me), 84%
5b (R^1 = Ph), 85%

Obtained esters **5** were then exposed to nucleophilic displacement with propargyl bromide. Apart from the last-mentioned reactants and DMF as solvent, K_2CO_3 (3 equiv.) was also added to the reaction mixture. Using basic potassium carbonate, relatively acidic proton from the position one of quinoline-2,4-dione framework was extracted, and thus quinolone nitrogen became more nucleophilic. Afterwards, propargyl bromide (1.5 equiv.) was added and SN2 nucleophilic substitution took place, resulting in gradual disappearance of starting compound and arising of more non-polar propargyl derivative **6**. The reactions were completed within 45 minutes, providing moderate to very good yields (Scheme 9).

Scheme 9. *Preparation of compounds **6a** and **6b**.*

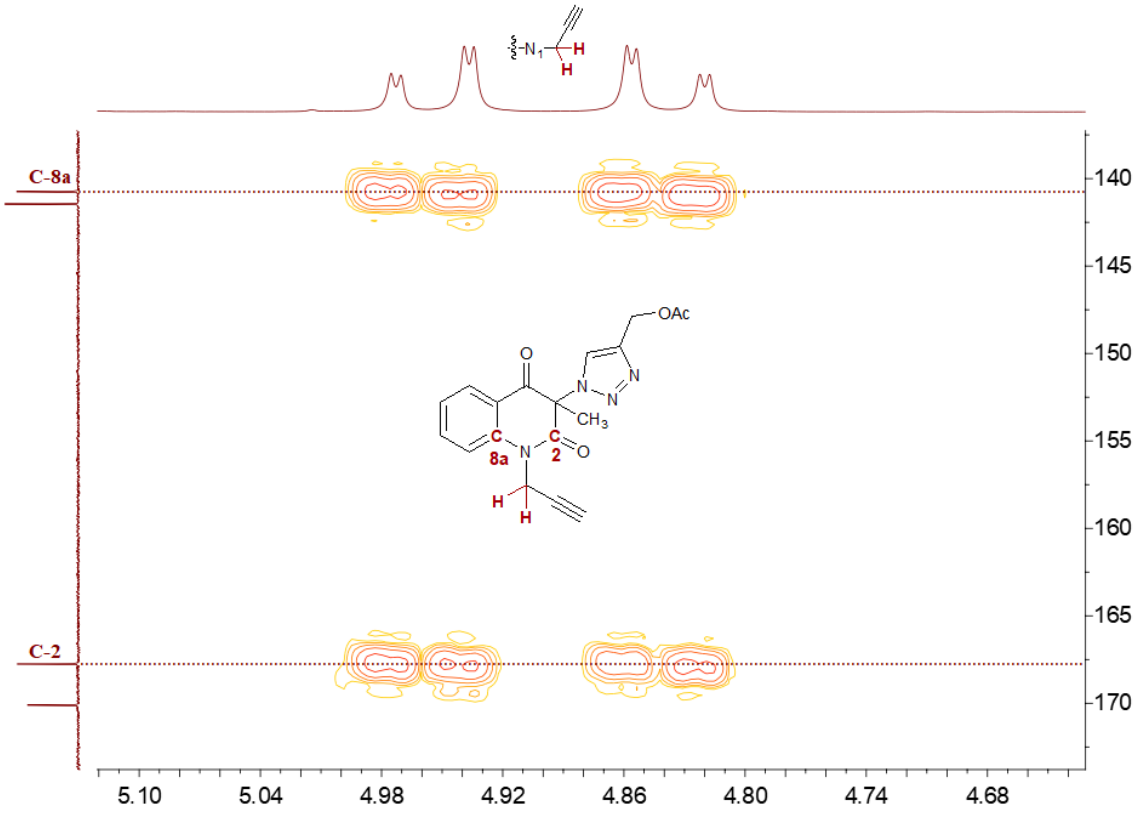


5a (R^1 = Me)
5b (R^1 = Ph)

6a (R^1 = Me), 81%
6b (R^1 = Ph), 63%

Expected products were further confirmed by 2D NMR, more precisely, $^{1}H–^{13}C$ *gs*-HMBC and $^{1}H–^{15}N$ *gs*-HMBC. As can be seen in Figure 2, two hydrogen atoms from N_1-CH_2 group strongly correlate with C-8a and C-2 carbons of quinolone scaffold, and therefore unambiguously determine the proposed structure of synthesized compounds **6**.

Figure 2. *$^{1}H–^{13}C$ gs-HMBC NMR spectrum of propargyl derivative.*

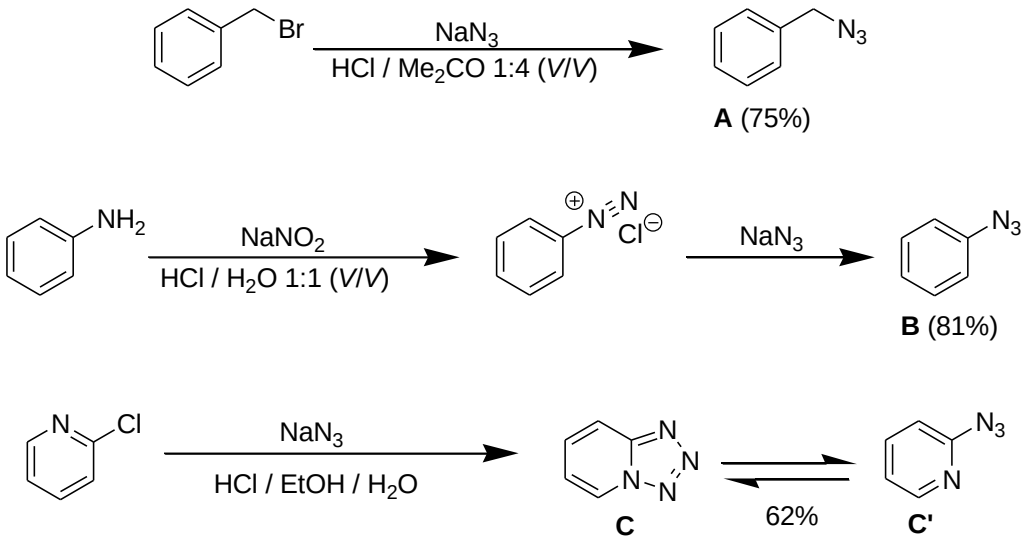


3.2.2 Organic azides

In the next step, each of both last-mentioned propargyl derivatives was combined with three different organic azides, namely benzyl azide **A**, phenyl azide **B** and tetrazolo(1,5-*a*)pyridine **C** to gain bis-triazole counterparts. All three

A, **B** and **C** azido compounds were synthesized from benzyl bromide, aniline and 2-chloropyridine, respectively. While for liquid azides **A** and **B** no additional purification was needed, solid tetrazolo(1,5-*a*)pyridine was chromatographed on silica-gel column using chloroform as mobile phase, and subsequently crystalized from acidified ethanol. Purity above 95% for all three azido compounds was established by gas chromatography (**A** and **B**) or ^{1}H NMR spectroscopy (**C**). Obtained yields are presented in Scheme 10.

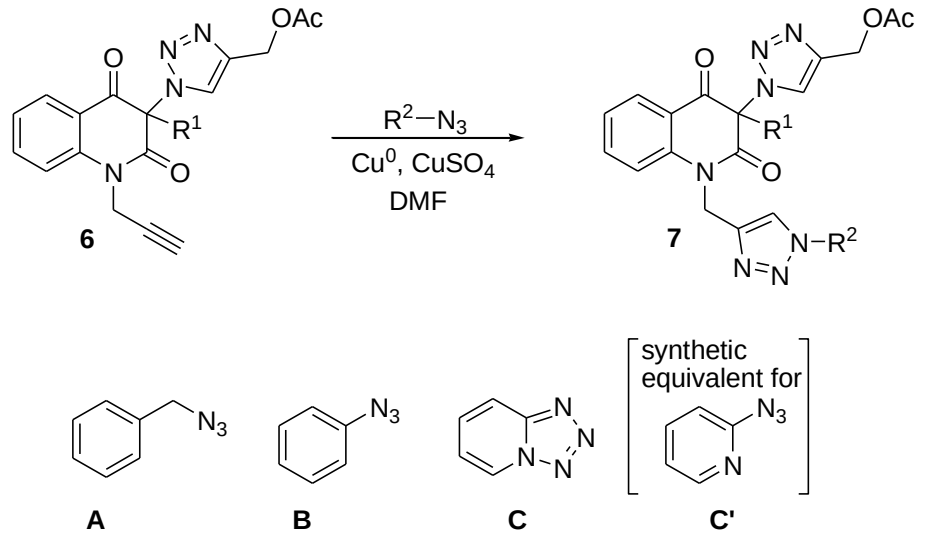
Scheme 10. *Preparation of compounds **A**, **B** and **C**.*



3.2.3 Second 1,2,3-triazole ring

Synthesis of bis-triazole species was carried out using very similar conditions than in case of the first »click«. Both, benzyl and phenyl azides (**A** and **B**) were combined with acetylenic compounds **6** at the laboratory temperature, however elevated temperature was utilized in case of the tetrazolo(1,5-*a*)pyridine **C**, which is a synthetic equivalent and an equilibrium form of 2-azidopyridine **C'**. While the former is »click-inactive« and predominant at laboratory temperature, the latter could be acquired at elevated temperature and readily used as an azide partner for preparation of 2-pyridyl substituted 1,2,3-triazoles. Consequently, transformations with tetrazolo(1,5-*a*)pyridine (**C**) were performed at 100 °C under inert atmosphere. Apart from traditional heating on oily bath, syntheses in microwave reactor were also carried out and yields of isolated compounds from both synthetic approaches were compared. While in the case of phenyl derivative, a bit higher yield was obtained from the reaction in microwave reactor, an opposite trend was observed for methyl counterpart, exhibiting slightly better performance when conventional heating was utilised. Nevertheless, all yields of isolated 2-pyridyl derivatives **7c** and **7f** were quite similar, and therefore comparable. (Table 2).

Table 2. *Preparation of compounds **7a**–**f**.*

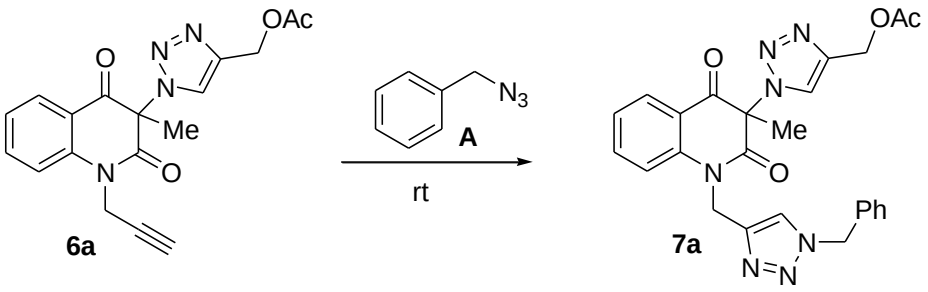


Entry	**6**	R^1	R^2	T (°C)	Time (h)	Yield of **7** (%)[a]
1	**a**	Me	Bn	23	0.5	96
2	**b**	Me	Ph	23	2	92
3	**c**	Me	2-Py	100	1	85
4	**c**	Me	2-Py	90	0.25	79[b]
5	**d**	Ph	Bn	23	2	97
6	**e**	Ph	Ph	23	0.5	93
7	**f**	Ph	2-Py	100	0.5	85
8	**f**	Ph	2-Py	90	0.25	92[b]

[a] Refers to percent yield of pure (by TLC and IR) isolated product. [b] Transformation was carried out in a microwave reactor T = 90 °C, t = 15 min, P = 150 W.

Apart from $Cu^0/CuSO_4 \cdot 5H_2O$ in *N*,*N*-dimethylformamide, some more standard solvent systems in the presence of sodium ascorbate or L-ascorbic acid, as well as $CuSO_4 \cdot 5H_2O$ were further investigated. In these experiments, compound **6a** and benzyl azide (**A**) were used as dienophile and diene, respectively. Three different solvent mixtures, namely *t*-BuOH/H_2O, *t*-BuOH/H_2O/CH_3CN and CH_2Cl_2/H_2O were studied. In comparison with Cu^0/Cu^{2+} in DMF, the reactions times were significantly longer, while the yields of isolated compounds were considerably lower for all of three organic solvent/water combinations. (Table 3). As the main reason, highly hydrophobic nature of both, reactants and products was established. The presence of water in the reaction mixture, especially in the case of *t*-BuOH/H_2O 1:1 (*V*/*V*), turned compounds into sticky resin-like materials that stuck to the magnetic stirring bar as well as flask walls, and therefore prevented reaction from proceeding to the completion.

Table 3. *Preparation of compound **7a**.*



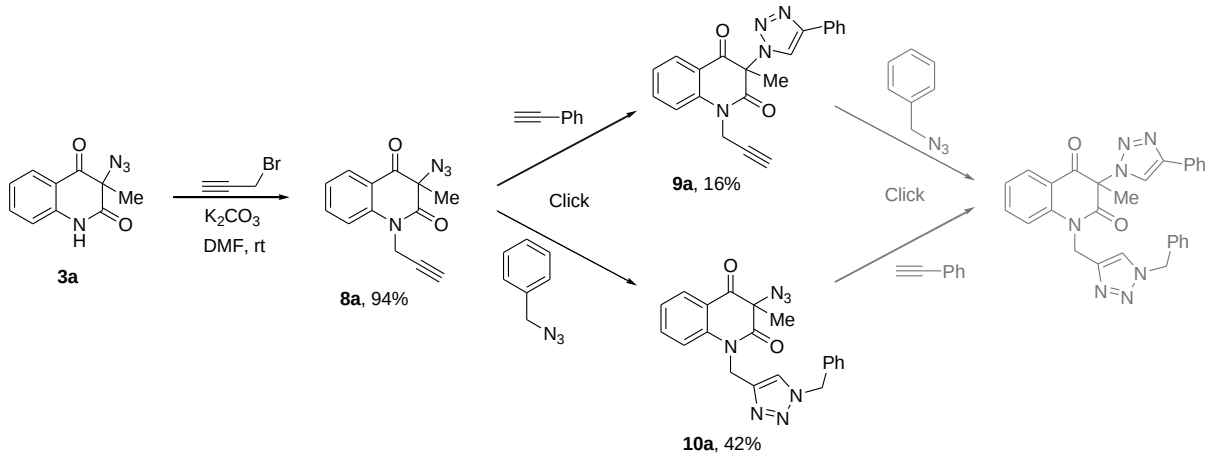
Entry	Time (h)	Yield of **7a** (%)[a]
1	0.5	96[b]
2	45	45[c]
3	48	81[d]
4	4	85[e]

[a] Refers to percent yield of pure (by TLC and IR) isolated product. [b] Employing DMF / Cu^0 / $CuSO_4 \cdot 5H_2O$ conditions. [c] Employing *t*-BuOH / water / $CuSO_4 \cdot 5H_2O$ / L-ascorbic acid conditions. [d] Employing *t*-BuOH / water / CH_3CN / $CuSO_4 \cdot 5H_2O$ / Na-ascorbate conditions. [e] Employing CH_2Cl_2 / water / $CuSO_4 \cdot 5H_2O$ / Na-ascorbate conditions.

3.2.4 Alternative reaction pathway

Apart from already presented »click-propargylation-click« synthetic approach, »propargylation-click-click« strategy was also briefly examined. As a result, 3-azido-3-methylquinoline-2,4(1*H*,3*H*)-dione (**3a**) was exposed to the *N*-alkylation reaction using propargyl bromide and K_2CO_3 in DMF, providing 3-azido-3-methyl-1-(prop-2-ynyl)quinoline-2,4(1*H*,3*H*)-dione (**8a**) as a desired product. The transformation proceeded smoothly, providing an excellent yield, however, manipulation of obtained product was not very convenient due to its tendency to be in an oily state. Expectedly, all attempts of its crystallization failed, and therefore silica-gel column chromatography was performed as a purification method, giving pure oily material. After several additions of diethyl ether and its subsequent evaporation using rotary evaporator, the compound was somehow transformed into sticky, kneadable, partially solid state. Obtained azide-propargyl bifunctional species (**8a**) was then combined with phenyl acetylene and benzyl azide (**A**) in two separate experiments, using $Cu^0/CuSO_4 \cdot 5H_2O$ catalytic system in DMF, providing 3-methyl-3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-1-(prop-2-ynyl)quinoline-2,4(1*H*,3*H*)-dione (**9a**) and 3-azido-1-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-3-methylquinoline-2,4(1*H*,3*H*)-dione (**10a**), respectively. The former was prepared in a poor reaction yield of only 16%, while the latter was obtained in 42% (Scheme 11).

Scheme 11. *Alternative »propargylation-click-click« reaction approach.*



Taking into account all last-mentioned facts, classical »click-propargylation-click« approach is definitely more reasonable and rational than its modification in »propargylation-click-click« form. While throughout the former, only one single product is expected in each transformation step, in case of the latter, competitive reaction of 3-azido-1-propargyl-quinolone derivative self-polymerization takes place. Moreover, light-sensitivity, toxicity and in some cases also explosiveness of organic azides are unwanted characteristics that should be definitely considered during the reaction scheme planning. Consequently, many »one-pot« 1,2,3-triazole synthetic approaches have already been developed and published throughout the last fifteen years.[82-85] Even though, in this study no »one-pot« reactions were performed and organic azides were prepared, isolated, purified, crystalized, characterized and even chromatographed, minimization of manipulation with the currently mentioned species seems to be prudent decision. As a result, more convenient »click-propargylation-click« strategy was not only found more practical and higher yielding, but also a wise choice when taking into account impacts on health and environment.

3.3 Synthesis of mono- and bis-triazole functional derivatives

3.3.1 Removal of acetyl protecting group

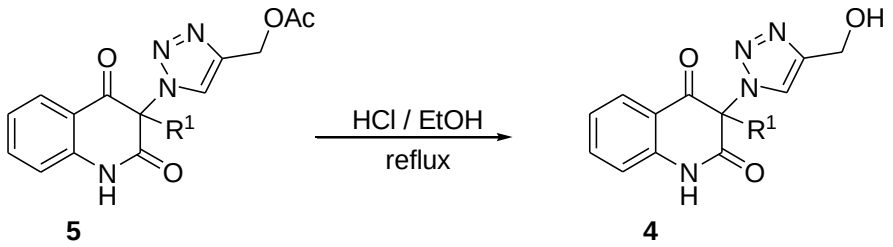
Due to already mentioned well-know and highly applicable physical properties and biological activities of 1,2,3-triazole bearing compounds, as well as quinolone based materials, maximization of number of mono- and bis-triazole functionalised quinoline-2,4-dione counterparts was carried out by the synthesis of different functional derivatives. Consequently, synthesized acetates **6** and **7** were firstly deacetylated to gain alcohols **12** and **11**, respectively. Before the transformations were performed on previously mentioned compounds **6** and **7**,

reaction conditions were studied and optimised on more accessible *N*-unsubstituted mono-triazole functionalised quinoline-2,4-diones (**5**).

Three different re-esterification approaches in basic, as well as acidic environments were tested. The reaction with catalytic quantities of sodium methoxide in dry methanol gave a mixture of products, as undesirable quinolone framework opening took place. Furthermore, cleavage of quinolone scaffold was also observed in case, when transformation was carried out in alkaline ethanol, using potassium hydroxide as a source of base. Finally, acidic alcoholysis resulted in formation of expected derivatives.

Additionally, optimisation of acidic deacetylation conditions was also briefly investigated and obtained results are presented in Table 4.

Table 4. *Re-esterification of compounds **5a** and **5b**.*



5 **4**

Entry	**5**	R^1	HCl/EtOH *V*/*V*(%)	Time (h)	Yield of **4** (%)[a]
1	**a**	Me	2.0	1.5	89
2	**a**	Me	1.0	3	92
3	**a**	Me	0.5	11	76
4	**b**	Ph	1.0	3.5	93

[a] Refers to percent yield of pure (by TLC and IR) isolated product.

The reaction was extremely slow when carried out at laboratory temperature, using 2% (*V*/*V*) solution of concentrated hydrochloride acid in ethanol as a solvent, however at elevated temperature, it proceeded smoothly in the time period of 90 minutes. Keeping the reflux temperature constant, the volume percent of HCl in ethanol was halved twice and reaction times, as well as yields were monitored. While in the case of 1% (*V*/*V*) HCl in ethanol, the transformation was finished in 3 hours, the time period of 11 hours was needed when only 0.5% (*V*/*V*) solution of hydrochloric acid in EtOH was utilised. Taking into account reaction yields, 1% (*V*/*V*) solution of hydrochloric acid in ethanol and reflux temperature were recognised optimal for the primary alcohols preparation.

Propargyl derivatives **6** and bis-triazoles **7** were then subjected to the optimised alcoholysis conditions, providing expected alcohols **12** and **11** within the time period of 4 hours (Table 5).

Table 5. *Preparation of compounds **11a–f**, **12a** and **12b**.*

Entry	Acetate	R^1	R^2	Time (h)	Alcohol	Yield (%)
1	**7a**	Me	Bn	3.5	**11a**	86[a]
2	**7b**	Me	Ph	3.5	**11b**	98[a]
3	**7c**	Me	2-Py	2.5	**11c**	80[a]
4	**7d**	Ph	Bn	4	**11d**	89[a]
5	**7e**	Ph	Ph	3	**11e**	97[a]
6	**7f**	Ph	2-Py	3	**11f**	87[a]
7	**6a**	Me	-	3	**12a**	83[b]
8	**6b**	Ph	-	3	**12b**	87[b]

[a] Refers to percent yield of pure (by TLC and IR) isolated product. [b] Refers to percent yield of crystallized product.

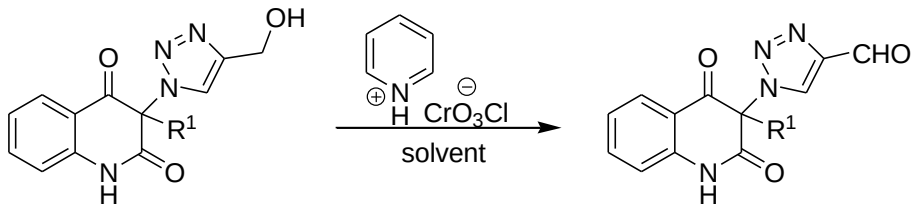
3.3.2 Oxidations

All three groups of alcohols **4**, **11** and **12** were subjected to oxidation reactions in the presence of chromium-based reagents to provide appropriate aldehydes and carboxylic acids. In addition, compounds **4** and **12**, as well as one representative of compounds **11** were further oxidized to corresponding aldehydes, using more benign MnO_2 as an oxidant.

3.3.3 Preparation of aldehydes 13a, 13b, 14a–f, 15a and 15b

A set of reactions (Table 6) was performed, searching for optimal transformation conditions for aldehydes preparation, using well-known pyridinium chlorochromate (PCC) as a reagent. Transformation parameters were again optimised, utilizing more available *N*-unsubstituted mono-triazole species. According to literature, great majority of primary alcohol oxidations with PCC were carried out in dichloromethane, obtaining even quantitative reaction yields in some cases.[86] Due to excellent solubility of phenyl derivative, last-mentioned solvent was assumed as a prime choice for the synthesis of compound **13b**. Conversely, low solubility of counterpart **4a** in dichloromethane was a reason to consider acetone as an alternative.

Table 6. *Preparation of compounds **13a** and **13b** using PCC.*



4 **13**

Entry	**4**	R^1	PCC (eqv.)	Time (h)	T (°C)	Solvent	Yield of **13** (%)[a]
1	**a**	Me	1.7	22	23	Me_2CO	36[b]
2	**a**	Me	1.2	22	23	CH_2Cl_2	31[b]
3	**a**	Me	1.2	MW[c]	40	CH_2Cl_2	16
4	**a**	Me	1.2	1.5	40	CH_2Cl_2	15
5	**a**	Me	1.2	5	56	Me_2CO	23[b]
6	**b**	Ph	1.7	1.5	23	CH_2Cl_2	35
7	**b**	Ph	1.7	22	23	Me_2CO	26[b]
8	**b**	Ph	1.7	0.5	40	CH_2Cl_2	34
9	**b**	Ph	2.0	1	23	CH_2Cl_2	31
10	**b**	Ph	1.5	4	23	CH_2Cl_2	41
11	**b**	Ph	1.2	1.5	40	CH_2Cl_2	44
12	**b**	Ph	1.2	MW[c]	40	CH_2Cl_2	42

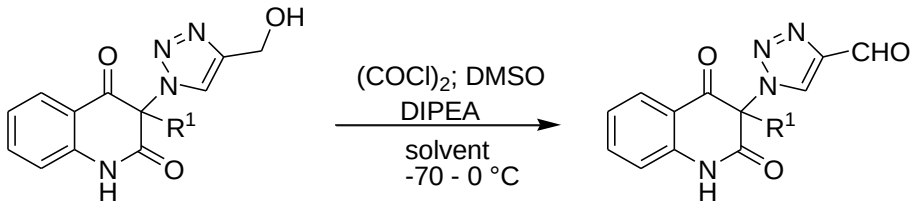
[a] Refers to percent yield of pure (by TLC and IR) isolated product. [b] Complete consumption of **4** was not reached. [c] Transformation in microwave reactor – T = 40 °C, t = 10 min, P = 150 W.

According to collected data, obtained yields for the phenyl analogue (**13b**) were significantly higher, than in the case of its methyl partner (**13a**). In addition, 1.2 equivalents of PCC in refluxing dichloromethane (40 °C) were found optimal reaction conditions for preparation of **13b**. On the other hand, quite opposite is true for the methyl derivative (**13a**). Despite the fact that transformations never proceeded to completion in acetone, slightly higher yields were observed when last-mentioned solvent was used. Similarly, better results were gained when reactions were carried out at lower – laboratory temperature. Apart from conventional heating on oil bath, microwave-induced syntheses of aldehydes **13a** and **13b** were also considered. By comparing both synthetic approaches, it turned out that no worth mentioning difference in reaction yields was observed for methyl (*Entries 3 and 4*), as well as phenyl (*Entries 11 and 12*) analogues.

Additionally, Swern oxidation using oxalyl chloride and *N*,*N*-diisopropylethylamine (DIPEA) in dimethyl sulfoxide (DMSO) was also briefly studied, however obtained results were unsatisfactory for both, phenyl and methyl counterparts (Table 7). While the former resulted in the yield of 33%, no pure product was isolated in case of the latter. The main drawback of this reaction

approach was the presence of hardly removable dimethyl sulfoxide that remained in products, despite the fact that they were intensively washed with ice-cold water. Apparently, utilization of relatively large quantities of water also caused significant loses of target compounds that were much more obvious in the case of methyl analogue. Finally, according to our previous observations, some 1,2,3-triazole bearing quinoline-2,4-dione species are not very stable in DMSO, and therefore usage of last-mentioned solvent could be troublesome also from that point of view.

Table 7. *Preparation of compounds **13a** and **13b** using Swern reaction approach.*



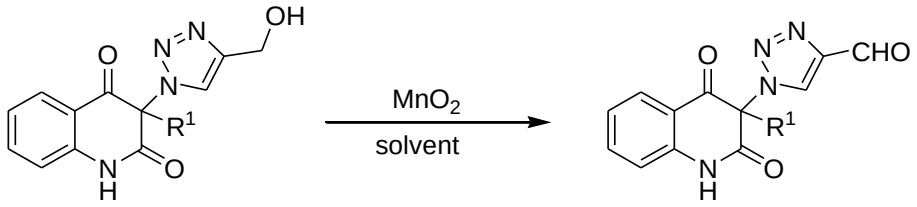
4 **13**

Entry	**4**	R^1	DMSO (eqv.)	Time (h)	Solvent	Yield of **13** (%)[a]
1	**a**	Me	2.6	3.5	Me_2CO	0
2	**b**	Ph	2.6	3.5	CH_2Cl_2	33

[a] Refers to percent yield of pure (by TLC and IR) isolated product.

As the third option, oxidation of primary alcohols **4** with MnO_2 was further examined. Taking into account reaction parameters such as transformation times and quantities of reagent (Table 8), acetone was recognized superior in comparison with dichloromethane, while obtained yields were practically the same in both cases (*Entries 1 and 2*).

Table 8. *Preparation of compounds **13a** and **13b** using MnO_2.*



4 **13**

Entry	**4**	R^1	MnO_2 (eqv.)	Time (h)	T (°C)	Solvent	Yield of **13** (%)[a]
1	**a**	Me	10	1.25	56	Me_2CO	60
2	**b**	Ph	10	1.5	56	Me_2CO	58
3	**b**	Ph	10+5[b]	3	40	CH_2Cl_2	62
4	**b**	Ph	10	96	23	CH_2Cl_2	44

[a] Refers to percent yield of pure (by TLC and IR) isolated product. [b] After 2 hours of stirring in the presence of 10 eqv. of MnO_2, another 5 eqv. of MnO_2 were added.

Optimised reaction conditions were then applied for preparation of aldehydes **14** and **15**. Oxidation of all compounds from the series **11** and **12** was performed by 1.2 equivalents of PCC in refluxing dichloromethane, while materials **14b**, **15a** and **15b** were also synthesized using MnO_2 in acetone. As solubility of *N*-substituted 3-methyl-quinoline-2,4-dione alcohols **11a–c** and **12a** in dichloromethane is significantly better than in the case of *N*-unsubstituted species **4a**, obtained yields for compounds **14a–c** and **15a** were considerably higher (up to 48%) than in the case of **13a**. Poor to moderate yields gained in reactions with PCC are most probably caused by formation of tar-like sediment in the reaction mixture. Even though, chromium sticky material was intensively triturated and washed during the isolation process, non-negligible part of product could still be trapped into the gluey residue, and consequently lost.

Considering toxicity of both utilised oxidants (PCC and MnO_2), as well as achieved yields, MnO_2 was recognised as more convenient reagent for 1,2,3-triazole bearing quinoline-2,4-dione aldehydes preparation. Obtained results are collected in Table 9.

Table 9. *Preparation of compounds **14a–f**, **15a** and **15b**.*

Entry	Alco.	R^1	R^2	Reag.	t (h)	T (°C)	Solv.	Aldeh.	Yield (%)
1	**11a**	Me	Bn	PCC	0.5	40	CH_2Cl_2	**14a**	41[a]
2	**11b**	Me	Ph	PCC	0.5	40	CH_2Cl_2	**14b**	40[a]
3	**11b**	Me	Ph	MnO_2	0.75	56	Me_2CO	**14b**	51[a]
4	**11c**	Me	2-Py	PCC	0.75	40	CH_2Cl_2	**14c**	48[a]
5	**11d**	Ph	Bn	PCC	0.5	40	CH_2Cl_2	**14d**	41[a]
6	**11e**	Ph	Ph	PCC	0.5	40	CH_2Cl_2	**14e**	45[a]
7	**11f**	Ph	2-Py	PCC	0.5	40	CH_2Cl_2	**14f**	41[a]
8	**12a**	Me	-	PCC	1	40	CH_2Cl_2	**15a**	41[b]
9	**12a**	Me	-	MnO_2	1.25	56	Me_2CO	**15a**	40[b]
10	**12b**	Ph	-	PCC	0.75	40	CH_2Cl_2	**15b**	38[b]
11	**12b**	Ph	-	MnO_2	2	56	Me_2CO	**15b**	38[b]

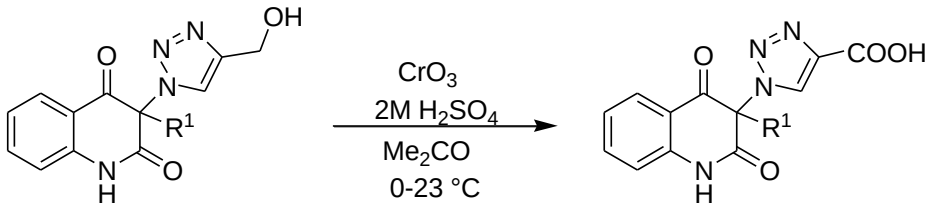
[a] Refers to percent yield of pure (by TLC and IR) isolated product. [b] Refers to percent yield of crystalized product.

3.3.4 Preparation of carboxylic acids 16a, 16b, 17a–f, 18a and 18b

Alcohols (**4**, **11** and **12**) were also transformed to suitable carboxylic acids, using CrO_3 in 2M H_2SO_4 and acetone as a solvent. Although, up to 9-fold molar excess,[87-89] of hexavalent chromium compound is usually added to the reaction mixture, utilization of 24 proportional parts of CrO_3 was found reasonable in our case (Table 10). When only 6 equivalents of CrO_3 were used, the transformation was significantly slower and achieved yield was drastically lower, as mixture of carboxylic acid and appropriate aldehyde was isolated after three hours of stirring

at laboratory temperature. Although, large quantities of potentially toxic chromium species were added to the reaction mixture, performed isolation process enabled efficient separation of desirable carboxylic acids from chromium residues, providing chromatographically pure products.

Table 10. *Preparation of compounds **16a** and **16b**.*



4 **16**

Entry	**4**	R^1	CrO_3 (eqv.)	H_2SO_4 (eqv.)	Time (h)	Yield of **16** (%)[a]
1	**a**	Me	24	48	2.75	33
2	**b**	Ph	6	12	3	40[b]
3	**b**	Ph	24	48	3.25	71

[a] Refers to percent yield of crystalized product. [b] Complete consumption of the intermediate (aldehyde) was not reached.

After the establishment of satisfactory reaction conditions, bis-triazoles **11** and *N*-propargyl substituted alcohols **12** were subjected to the oxidation process. Due to excellent solubility of all starting materials in acetone, the transformations proceeded smoothly providing only desirable products. In all cases, oxidations were completed within the time period of three hours, indicating no starting compounds, intermediates or any other impurities according to TLC (Table 11). Carboxylic acids were then isolated in two portions of equal quality, gaining the first crude product by precipitation from the ice-cold water and the second one by extraction from filtrate. It is also worth mentioning that synthesized species possess low solubility in chloroform, however presence of acetone in mostly aqueous filtrate enables facile transition of desirable compound residues into organic ($CHCl_3$) phase. Due to the fact that isolated bis-triazoles **17** were TLC and NMR pure, no silica-gel column purification was performed. While crystallization of mono-triazole species **18** was carried out with the ease, it was unsuccessful in the case of bis-triazole carboxylic acids (**17**).

Table 11. *Preparation of compounds **17a–f**, **18a** and **18b**.*

Entry	Alcohol	R^1	R^2	Time (h)	Carbox. acid	Yield (%)
1	**11a**	Me	Bn	3	**17a**	88[a]
2	**11b**	Me	Ph	2.5	**17b**	92[a]
3	**11c**	Me	2-Py	2.5	**17c**	84[a]
4	**11d**	Ph	Bn	2	**17d**	75[a]
5	**11e**	Ph	Ph	2.25	**17e**	77[a]
6	**11f**	Ph	2-Py	2.5	**17f**	69[a]
7	**12a**	Me	-	3	**18a**	55[b]
8	**12b**	Ph	-	3	**18b**	68[b]

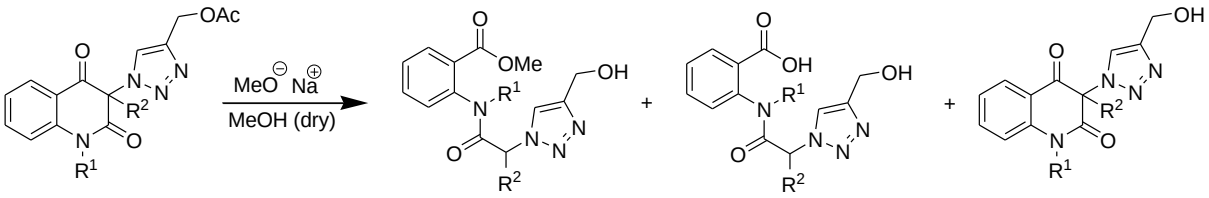
[a] Refers to percent yield of pure (by TLC and IR) isolated product. [b] Refers to percent yield of crystalized product.

3.3.5 Ring-opening studies

As already mentioned, deprotection of synthesized acetates in basic environments resulted in partial or complete quinoline-2,4-dione framework cleavage. Due to the potentially interesting synthetic route for 1,2,3-triazole bearing anthranilic acid derivatives preparation, the quinolone ring opening was also taken into consideration. The transformations were mostly carried out in dry methanol and in the presence of sodium methoxide, however potassium hydroxide in ethanol was also tested as reaction medium. The main difference between phenyl and methyl derivatives is in the nature of both substituents. While the former could be cleaved considerably faster and at laboratory temperature, heating on oil bath is mandatory for ring opening of the latter. The electron-withdrawing character of phenyl group pulls the electrons from C3 carbon of quinolone ring, making it electron-deficient, and therefore more exposed to the nucleophilic attack of methoxide or hydroxide anion. Conversely, electron-donating methyl group increases the electron density on C3 carbon, and consequently harsher reaction conditions are necessary for quinoline-2,4-dione cleavage.

Throughout the study of quinoline-2,4-dione ring opening process, three different acetates **5a**, **5b** and **6b** were deacetylated using sodium methoxide in dry methanol. The transformations were carried out at laboratory temperature (23 °C) or at the temperature of reflux (65 °C). Partial or complete ring-opening, as well as appearance of pure anthranilic acid derivative or mixture with its ester were detected. The reaction conditions are collected in Table 12.

Table 12. *Cleavage of quinoline-2,4-dione using sodium methoxide in methanol.*



5a (R^1 = H, R^2 = Me)
5b (R^1 = H, R^2 = Ph)
6b (R^1 = CH_2CCH, R^2 = Ph)

19a (R^1 = H, R^2 = Me)
19b (R^1 = H, R^2 = Ph)
19c (R^1 = CH_2CCH, R^2 = Ph)

20a (R^1 = H, R^2 = Me)
20b (R^1 = H, R^2 = Ph)
20c (R^1 = CH_2CCH, R^2 = Ph)

4a (R^1 = H, R^2 = Me)

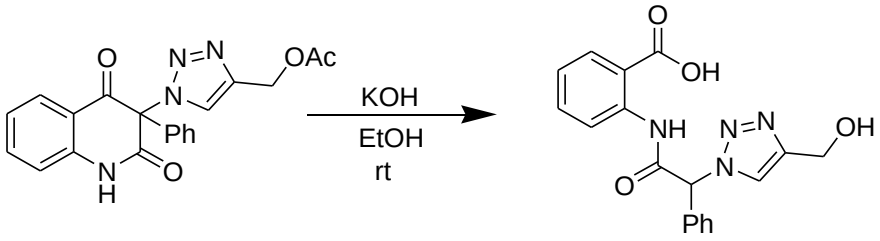
Entry	Acetate	R^1	R^2	Time (h)	Temp. (°C)	Yield (%)[a] **19 20 4**
1	**5b**	H	Ph	10	23	0 + 33 + 0
2	**5b**	H	Ph	0.5	65	26 + 28 + 0
3	**6b**	$CH_2C{\equiv}CH$	Ph	1.5	23	0 + 69 + 0
4	**5a**	H	Me	1	65	23 + <5[b] + 29

[a] Refers to percent yield of crystalized product. [b] No pure compound was isolated.

As can be seen from the collected data, solely open-ring species were isolated in the case of phenyl-bearing quinoline-2,4-dione esters. Conversely, closed form (**4a**) appeared predominantly throughout the methyl derivative deacetylation. During the isolation process, reaction mixtures were neutralised with 1.0 M HCl. Consequently, hydrolysis took place that turned appropriate methyl esters **19a**, **19b** and **19c** into carboxylic acids **20a**, **20b** and **20c**, respectively. According to data in Table 12, the acidic hydrolysis of open-ring phenyl derivatives **19b** and **19c** seems to be considerably faster than for methyl counterpart **19a**. While the major products of the former are carboxylic acids **20b** and **20c**, respectively, no pure derivative **20a** was isolated in case of the latter.

Furthermore, compound **5b** was also subjected to basis alcoholysis using potassium hydroxide in ethanol. Expectedly, complete ring-opening occurred and only one product appeared that was identified as a derivative of anthranilic acid **20b** (Scheme 12).

Scheme 12. *Cleavage of quinoline-2,4-dione using KOH in ethanol.*



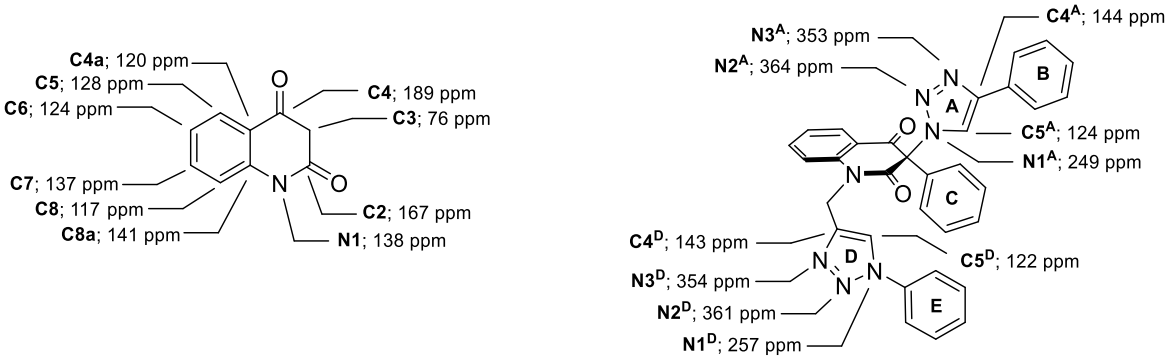
5b **20b** (75%)

Beside the preliminary results presented in this chapter, the research on synthesis of 1,2,3-triazole substituted anthranilic acid derivatives could still be significantly broadened by introduction of new substituents on the position 3 of quinoline-2,4-dione molecule, as well as utilization of different bases for intentional ring-opening. Nevertheless, a ratio between obtained esters and carboxylic acids could also be potentially regulated.

3.4 NMR chemical shifts and single-crystal structure

All prepared compounds were assigned by ^{1}H and ^{13}C, while most of them also by ^{15}N NMR spectroscopy. The corresponding resonances were characterized on the basis of gradient-selected 2D NMR experiments including $^{1}H–^{1}H$ gs-COSY, $^{1}H–^{13}C$ gs-HSQC, $^{1}H–^{13}C$ gs-HMBC and $^{1}H–^{15}N$ gs-HMBC. The ring and atom numbering system, as well as their chemical shift data are presented in Figure 3.

Figure 3. *Ring and atom numbering system, including chemical shift data.*



Selected ^{1}H, ^{13}C and ^{15}N NMR chemical shifts of synthesized compounds are presented in Tables 13–17.

Table 13. *Selected ^{1}H, ^{13}C and ^{15}N NMR chemical shifts in ppm for mono-triazoles **4**, **5**, **13** and **16**.*

	4a	**4b**	**5a**	**5b**	**13a**	**13b**	**16a**	**16b**
Quinolone								
N1	–	–	133.5	–	133.4	134.8	133.2	–
C2	168.7	166.8	168.6	166.8	168.3	166.6	168.5	166.8
C3	71.9	79.7	72.4	79.9	73.6	80.7	73.4	80.6
C4	190.8	189.0	190.7	188.8	190.3	188.4	190.5	188.6
C4a	117.5	119.2	117.5	119.3	117.6	119.5	117.7	119.6
C5	127.6	127.5	127.6	127.5	127.6	127.5	127.6	127.4
C6	123.3	123.4	123.3	123.4	123.5	123.5	123.4	123.4

	4a	4b	5a	5b	13a	13b	16a	16b
C7	137.1	136.9	137.1	136.9	137.2	136.8	137.2	136.7
C8	116.9	116.7	116.9	116.7	117.0	116.7	117.0	116.7
C8a	141.6	140.6	141.6	140.5	141.4	140.4	141.5	140.4
Ring A								
$N1^{A}$	–	–	248.7	–	252.0	249.8	250.2	–
$N2^{A}$	–	–	362.9	–	367.9	356.4	367.5	–
$N3^{A}$	–	–	354.1	–	358.6	351.6	357.1	–
$C4^{A}$	147.4	146.8	141.4	140.8	146.6	146.2	139.5	139.2
$C5^{A}$	123.7	124.8	125.8	127.0	129.6	130.7	130.4	131.1
$H5^{A}$	8.26	7.77	8.45	8.07	9.18	8.93	8.99	8.71

Table 14. *Selected ^{1}H, ^{13}C and ^{15}N NMR chemical shifts in ppm for bis-triazoles **7**.*

	7a	7b	7c	7d	7e	7f
Quinolone						
N1	138.7	138.7	135.3	140.4	140.4	138.9
C2	168.2	168.3	168.6	166.6	166.9	166.6
C3	71.6	71.5	73.3	79.6	79.6	79.7
C4	189.4	189.4	189.9	187.9	187.9	187.9
C4a	119.2	119.2	119.4	120.9	120.9	121.0
C5	129.3	129.4	127.9	129.0	129.1	129.1
C6	124.6	124.7	123.8	124.6	124.7	124.6
C7	137.8	137.8	137.0	137.2	137.4	137.2
C8	116.9	116.8	116.5	116.8	116.7	116.6
C8a	141.7	141.7	141.3	141.1	140.9	141.2
Ring A						
$N1^{A}$	248.4	248.8	247.6	249.8	249.9	249.7
$N2^{A}$	361.6	–	363.7	365.1	–	–
$N3^{A}$	355.2	355.5	353.4	356.9	357.2	357.1
$C4^{A}$	142.3	142.3	141.6	140.9	140.9	140.9
$C5^{A}$	124.2	124.1	126.1	126.4	126.4	126.4
$H5^{A}$	7.78	7.86	8.47	7.08	7.14	7.13
Ring D						
$N1^{D}$	250.4	256.3	260.0	250.4	256.3	261.2
$N2^{D}$	362.6	–	361.9	362.9	–	–
$N3^{D}$	350.0	351.9	356.5	350.5	352.9	355.8
$C4^{D}$	142.9	143.2	143.2	142.9	143.2	143.0
$C5^{D}$	123.5	121.7	120.6	123.5	121.8	121.0
$H5^{D}$	7.55	8.10	8.82	7.58	8.05	8.63

Table 15. *Selected ^{1}H, ^{13}C and ^{15}N NMR chemical shifts in ppm for bis-triazoles **11**.*

	11a	**11b**	**11c**	**11d**	**11e**	**11f**
Quinolone						
N1	138.6	138.5	137.7	140.4	139.5	–
C2	168.3	168.4	168.3	166.6	166.9	166.7
C3	71.7	71.6	72.0	79.6	79.6	79.7
C4	189.6	189.5	189.6	188.0	188.0	188.0
C4a	119.2	119.2	119.3	120.9	120.9	121.0
C5	129.0	129.4	129.3	129.0	129.1	129.0
C6	124.6	124.6	124.6	124.5	124.6	124.5
C7	137.7	137.8	137.6	137.2	137.3	137.1
C8	116.9	116.8	116.6	116.8	116.7	116.6
C8a	141.7	141.7	141.6	141.1	140.9	141.2
Ring A						
$N1^{A}$	247.1	247.4	246.7	248.9	249.0	–
$N2^{A}$	362.1	–	–	364.9	–	–
$N3^{A}$	350.4	350.8	350.3	352.8	353.0	–
$C4^{A}$	147.3	147.3	147.4	145.8	145.8	145.9
$C5^{A}$	122.1	122.1	122.3	124.5	124.6	124.5
$H5^{A}$	7.70	7.76	7.76	7.03	7.09	7.09
Ring D						
$N1^{D}$	250.4	256.2	259.9	250.6	256.2	–
$N2^{D}$	361.6	–	–	362.8	–	–
$N3^{D}$	349.3	351.6	355.0	350.0	352.6	–
$C4^{D}$	142.9	143.2	143.0	142.9	143.2	143.0
$C5^{D}$	123.5	121.8	120.9	123.6	121.8	121.0
$H5^{D}$	7.56	8.09	8.58	7.59	8.07	8.61

Table 16. *Selected ^{1}H, ^{13}C and ^{15}N NMR chemical shifts in ppm for bis-triazoles **14**.*

	14a	**14b**	**14c**	**14d**	**14e**	**14f**
Quinolone						
N1	138.4	138.5	137.8	140.0	139.3	139.2
C2	167.7	167.8	167.7	165.9	166.2	165.9
C3	72.6	72.5	72.9	80.1	80.1	80.2
C4	188.9	188.8	189.0	187.3	187.2	187.3
C4a	119.0	119.0	119.1	120.7	120.7	120.8
C5	129.3	129.4	129.4	129.0	129.3	129.1
C6	124.8	124.9	124.8	124.8	124.8	124.8
C7	138.0	138.1	137.9	137.5	137.6	137.4

	14a	14b	14c	14d	14e	14f
C8	117.0	117.0	116.8	117.0	116.8	116.7
C8a	141.5	141.5	141.6	141.0	140.9	141.1
Ring A						
$N1^A$	251.7	251.6	251.1	253.7	254.3	254.0
$N2^A$	361.8	362.2	361.8	–	363.4	363.1
$N3^A$	–	–	–	–	–	–
$C4^A$	147.0	147.0	147.1	145.8	145.8	145.8
$C5^A$	126.3	126.2	126.4	128.4	128.4	128.4
$H5^A$	8.30	8.36	8.35	7.58	7.64	7.63
Ring D						
$N1^D$	250.6	256.2	261.0	250.6	256.1	260.2
$N2^D$	362.6	–	–	362.5	–	–
$N3^D$	350.0	352.0	355.3	350.8	352.7	355.5
$C4^D$	142.7	143.0	142.8	142.7	143.0	142.8
$C5^D$	123.4	121.8	120.8	123.5	121.8	120.9
$H5^D$	7.53	8.05	8.59	7.58	8.06	8.63

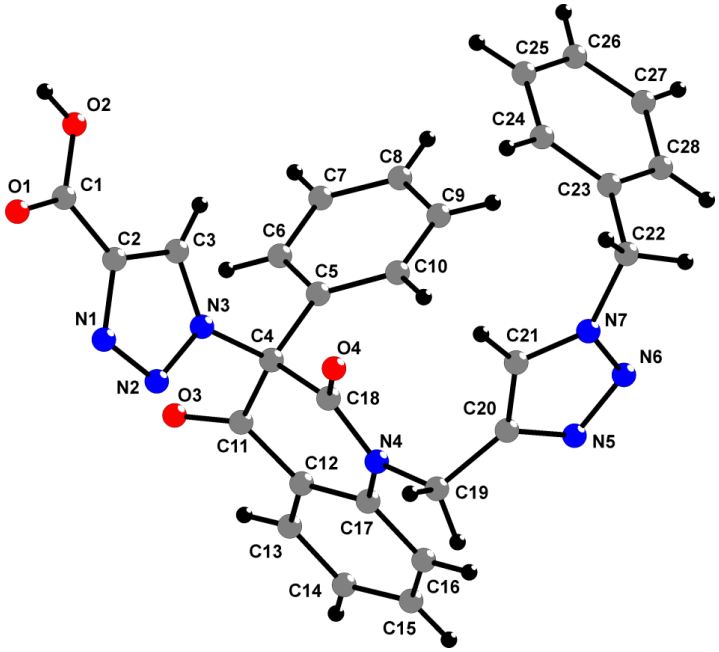
Table 17. *Selected ^1H, ^{13}C and ^{15}N NMR chemical shifts in ppm for bis-triazoles **17**.*

	17a	17b	17c	17d	17e	17f
Quinolone						
N1	136.8	135.9	–	–	139.5	137.8
C2	168.2	168.3	168.5	166.1	166.3	166.7
C3	74.0	74.1	74.2	80.5	80.7	80.8
C4	189.8	189.9	189.8	187.9	188.0	187.9
C4a	119.3	119.4	119.4	121.1	121.1	121.2
C5	128.2	128.0	128.0	127.7	127.8	127.9
C6	123.9	124.0	123.9	124.0	124.1	124.0
C7	137.1	137.2	137.1	136.6	136.8	136.7
C8	116.7	116.8	116.6	116.6	116.7	116.5
C8a	141.4	141.5	141.2	140.8	140.7	140.4
Ring A						
$N1^A$	249.3	249.4	249.2	249.8	249.8	249.7
$N2^A$	367.7	367.8	–	–	372.6	–
$N3^A$	357.4	357.0	–	356.4	357.5	356.8
$C4^A$	139.6	139.7	139.7	139.3	139.3	139.2
$C5^A$	130.6	130.6	130.6	131.2	131.3	131.3
$H5^A$	8.96	8.97	8.99	8.76	8.79	8.86
Ring D						
$N1^D$	251.2	255.7	260.3	250.9	255.6	260.4
$N2^D$	362.4	–	–	362.7	–	–

	17a	17b	17c	17d	17e	17f
$N3^D$	351.1	353.2	–	351.6	354.2	357.1
$C4^D$	142.3	143.3	143.2	141.9	142.9	143.0
$C5^D$	123.9	121.8	120.7	124.3	122.4	120.9
$H5^D$	8.16	8.74	8.83	8.24	8.81	8.83

From solution of **17d** in deuterated chloroform originally intended to NMR measurements, the crystal had grown. Using X-ray diffraction, the structure of solvate **12d**·2$CDCl_3$ was unambiguously determined (Figure 4).

Figure 4. *Crystallographic view of molecule **17d**. $CDCl_3$ molecules are omitted.*



It turned out that compound **17d** crystalize in monoclinic $P2_1/n$ space group. Intermolecular hydrogen bonds of O–H---N type were found in the crystal structure of bis-triazole species **17d**. While O2 atom acts as hydrogen bond donor, N5 atom of symmetry related molecule was recognised as hydrogen bond acceptor. Consequently, two dimensional chain extension along *b*-axis was established (Figure 5). Selected bonds' lengths and angles are presented in Tables 18–20.

Figure 5. *Hydrogen bonding interactions resulting in polymeric chain.*

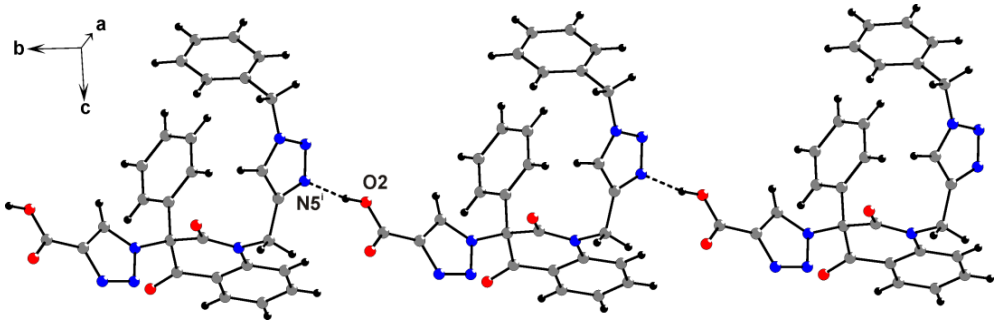


Table 18. *Crystal data and structure refinement details for compound **17d**.*

formula	$C_{30}H_{21}Cl_6D_2N_7O_4$
Fw (g mol–1)	760.29
crystal size (mm)	0.50×0.30×0.10
crystal color	colourless
crystal system	monoclinic
space group	P21/n
a (Å)	13.5462(6)
b (Å)	11.9884(9)
c (Å)	20.8335(10)
β (º)	92.823(4)
V (Å3)	3379.2(3)
Z	4
calcd density (g cm-3)	1.494
F(000)	1544
no. of collected reflns	29191
no. of independent reflns	7754
Rint	0.0563
no. of reflns observed	3853
no. parameters	438
R[I> 2σ (I)]a	0.0974
wR2(all data)b	0.3413
Goof , Sc	1.092

Table 19. *Selected bond lengths and angles for compound **17d**.*

Bond	Length (Å)	Bonds	Angle (°)
N1–N2	1.298(5)	N1–N2–N3	106.8(3)
N2–N3	1.359(5)	N5–N6–N7	106.8(4)
N5–N6	1.310(5)	N2–N3–C3	110.7(3)

Bond	Length (Å)	Bonds	Angle (°)
N6–N7	1.328(6)	N6–N7–C21	111.2(4)
N1–C2	1.353(5)	N1–C2–C3	108.3(4)
N3–C3	1.330(5)	N3–C3–C2	104.9(3)
N3–C4	1.456(5)	N4–C17–C12	119.9(4)
N4–C17	1.424(5)	N4–C18–C4	118.0(3)
N4–C18	1.358(5)	N4–C19–C20	112.1(3)
N4–C19	1.475(5)	N5–C20–C21	107.0(4)
N5–C20	1.348(6)	N7–C21–C20	105.4(4)
N7–C21	1.328(6)	C17–N4–C18	123.2(3)
N7–C22	1.481(6)	C19–N4–C17	121.9(4)

Table 20. *Hydrogen bonding geometry for compound **17d**.*

D–H···A	D–H (Å)	H···A (Å)	D···A (Å)	D–H···A (°)	Sym. code
O2–H2···N5	0.82	1.90	2.700(5)	166.7	x, y+1, z

3.5 Practical applications of synthesized compounds

3.5.1 Coordination abilities

The purpose of preparing bis-triazole species was also interrogation of their potential coordination abilities to the metal centre, and consequently possible usage in various practical applications. As a result, some synthesized materials with two 1,2,3-triazole rings on quinolone framework were subjected to NMR experiment, where particular derivatives were mixed with equimolar amounts of ruthenium species $[RuCl(\mu\text{-}Cl)(\eta^6\text{-}p\text{-cymene})]_2$ in deuterated chloroform. While in the case of bis-triazole compounds with oxygen-containing functional groups on the first 1,2,3-triazole ring no coordination was detected to the ruthenium-cymene system, weak metal-to-ligand interactions were observed for three compounds with the phenyl segment on the position four of the first 1,2,3-triazole scaffold. Due to overlap as well as lack of several indicative cross-peaks in the spectra, especially in $^{1}H–^{15}N$ gs-HMBC, an explicit structure determination of obtained [Ru–Cym]–bis-triazole complex through the 2D NMR techniques was unsuccessful. However, the analysis of the available NMR data (Figure 6) tentatively suggested the coordination of both 1,2,3-triazole rings to the Ru–Cym unit as presented in Scheme 13. Even though, three previously mentioned compounds that exhibited some mild affinities towards ruthenium

metal centre were synthesized in our laboratory, they were not prepared as a part of my dissertation, and therefore their assignment, preparation procedure, as well as detailed spectral data are not described in this work.

Scheme 13. *Proposed structure of [Ru–Cym]–(bis-triazole) complex.*

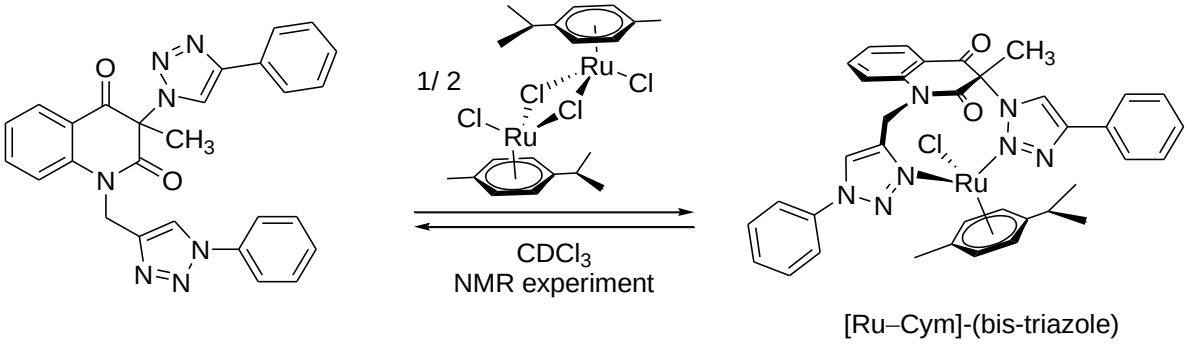
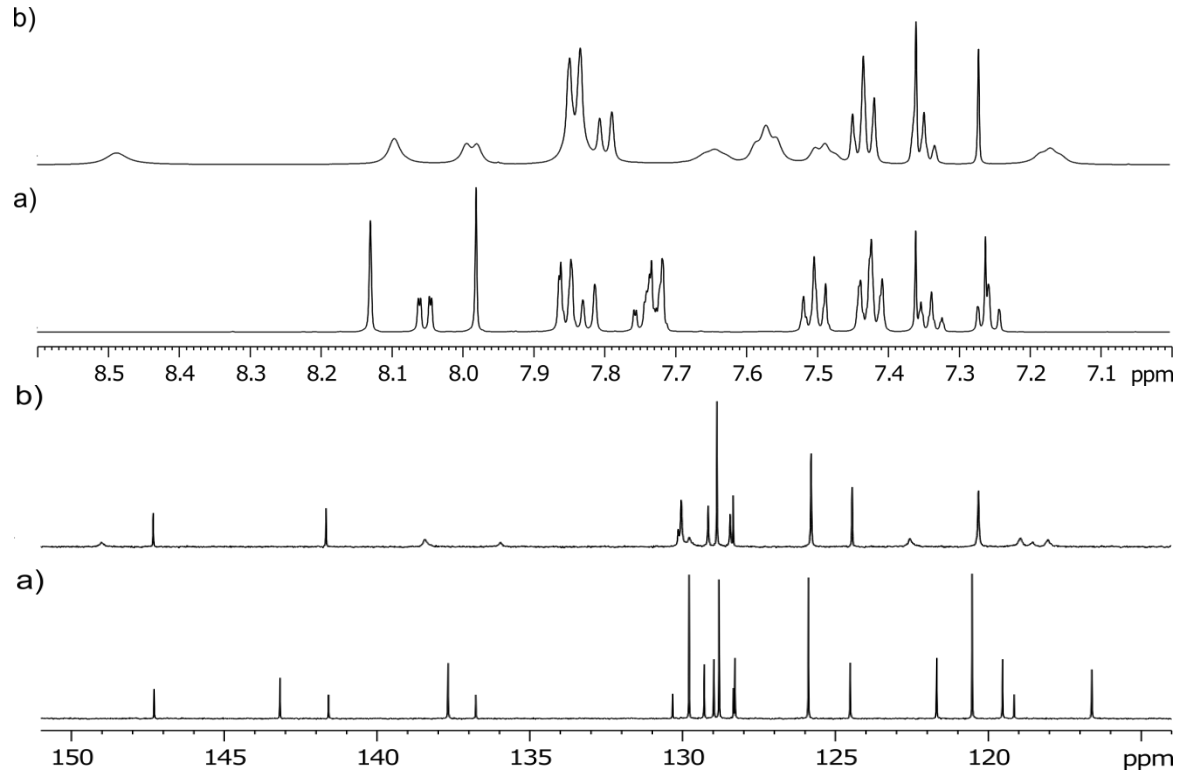


Figure 6. *Aromatic region of ^{1}H (above) and ^{13}C (bellow) NMR spectra of: a.) bis-triazole in $CDCl_3$, and b.) a mixture of bis-triazole (42 mM) and $[RuCl(\mu\text{-}Cl)(\eta^6\text{-}p\text{-}cymene)]_2$ (21 mM) in $CDCl_3$ immediately after dissolution.*



3.5.2 Evaluation of prepared compounds as potential photoprotective agents

Utilization of 1,2,3-triazole species as photoprotective and antiphotoaging agents has already been reported.[84] Even though, investigation of their photoprotective characteristics was mainly focused on human dermal fibroblast

cells protection, materials with similar properties might also be potentially used as additives to polymers for preservation purposes.

To evaluate possible photoprotective activities of synthesized compounds, their UV spectra were recorded. The measuring range was set to 200–400 nm. Four different solvents including ethanol, acetonitrile, chloroform and dimethyl sulfoxide were taken into consideration. In comparison with chloroform and dimethyl sulfoxide, the background of absorption spectra was considerably lower, when ethanol or acetonitrile were used. In contrast to ethanol, all studied compounds were sufficiently soluble in acetonitrile, making it suitable solvent for preparation of appropriate stock solutions.

In Table 21, UV spectral data of recorded species are collected. Relatively large deviations in attenuation coefficients for quite similar derivatives could be caused by various amounts of solvents presented in the samples. While negligible quantities of solvents are expected in crystalized compounds, the opposite could be true for amorphous, foamy solids. Nevertheless, multiple measurements of the particular specimens gave reproducible results.

Table 21. *Ultra-violet spectral data measured in acetonitrile.*

Compound	c [μM]	λ_{max} [nm]	A	10^{-3} ε [$M^{-1}cm^{-1}$]
4a	26.23	232	1.004	38.3
		346	0.074	2.8
4b	22.86	235	0.899	39.3
		348	0.066	2.9
5a	27.68	232	1.047	37.8
		347	0.081	2.9
5b	28.64	235	1.063	37.1
		348	0.078	2.7
6a	23.61	234	0,937	39.7
		343	0.072	3.1
6b	22.78	236	0.868	38.1
		343	0.062	2.7
7a	25.95	235	0.996	38.4
		343	0.080	3.1
7b	23.71	235	1.047	44.2
		342	0.071	3.0
7c	25.19	235	1.074	42.6
		271	0.312	12.4
		344	0.075	3.0
7d	24.84	237	0.882	35.5
		346	0.062	2.5
7e	23.92	237	0.992	41.5
		343	0.059	2.5

Compound	c [μM]	λ_{max} [nm]	A	10^{-3} ε [$M^{-1}cm^{-1}$]
7f	25.26	236	1.064	42.1
		271	0.231	9.1
		344	0.073	2.9
9a	22.62	203	0.764	33.8
		237	1.017	45.0
		344	0.069	3.1
10a	23.23	234	0.752	32.4
		343	0.065	2.8
11b	24.45	235	1.036	42.4
		342	0.069	2.8
11c	23.70	234	1.007	42.5
		271	0.294	12.4
		342	0.071	3.0
11d	25.28	237	0.851	33.7
		342	0.061	2.4
11e	22.09	237	0.811	36.7
		344	0.045	2.0
11f	25.14	237	1.018	40.5
		272	0.338	13.4
		343	0.062	2.5
12a	21.76	234	0,762	35.0
		341	0,059	2.7
12b	24.65	236	0.841	34.1
		343	0.060	2.4
13a	23.16	234	0.996	43.0
		342	0.064	2.8
13b	24.13	237	1.048	43.4
		346	0.062	2.6
14a	24.56	238	0.994	40.5
		346	0.068	2.8
14b	24.05	238	1.122	46.7
		347	0.065	2.7
14c	24.98	237	1.164	46.6
		271	0.311	12.5
		347	0.069	2.8
14d	24.75	239	0.983	39.7
		346	0.058	2.3
14e	25.82	239	1.170	45,3
		343	0.061	2,4
14f	26.59	239	1.196	45.0
		272	0.341	12.8
		344	0.059	2.2

Compound	c [μM]	λ_{max} [nm]	A	10^{-3} ε [$M^{-1}cm^{-1}$]
15a	26.40	236	1.226	46.4
		341	0.081	3.1
15b	27.59	238	1.260	45.7
		341	0.066	2.4
16a	26.27	232	1.073	40.8
		344	0.075	2.9
16b	25.95	235	1.037	40.0
		348	0.065	2.5
17a	25.31	235	0.927	36.6
		343	0.064	2.5
17b	27.69	235	1.325	47.9
		342	0.081	2.9
17c	24.53	234	1.016	41.4
		272	0.277	11.3
		342	0.065	2.7
17d	25.72	237	0.912	35.5
		344	0.060	2.3
17e	25.04	237	1.020	40.7
		345	0.052	2.1
17f	27.80	237	1.029	37.0
		272	0.326	11.7
		345	0.058	2.1
18a	27.44	234	1.112	40.5
		342	0.077	2.8
18b	26.56	236	1.068	40.2
		343	0.071	2.7

For the majority of tested species, two absorbance maximums and one inflection point were observed in UV spectra. In the case of bis-triazoles with 2-pyridyl segment in a molecule, the third absorbance maximum appeared in the place of the inflection point. Additional absorption of derivative **9a** most probably belongs to π-conjugated system of the phenyl ring attached to 1,2,3-triazole scaffold. For the compounds **4–18**, the most intense absorption peak (maximum at 230–240 nm) was quite narrow, covering the zone of approximately 25 nm in UV-C range. On the other hand, wide absorption band (maximum at 340–350 nm) was spread along roughly 80 nm of UV-B and UV-A regions, however its intensity was low. Finally, the eventual third absorption maximum in UV-C area, as well as absorptions of anthranilic acid derivatives **19** and **20** were found both, relatively narrow and weak. According to literature,[90] intensive and wide absorption bands in UV spectra, especially in UV-A (315–400 nm) and UV-B (280–315 nm) areas, are considered the key characteristic of efficient photoprotective agents. Consequently, measured mono- and bis-triazole

compounds could be designated as unpromising candidates for the prevention of photodamaging and photoaging processes on the surfaces of materials or tissues.

3.5.3 Antimicrobial activities

Several mono- and bis-triazole compounds were tested against Gram-positive bacteria (*Staphylococcus aureus* CCM 3953), Gram-negative bacteria (*Escherichia coli* CCM 3954 and *Pseudomonas aeruginosa* CCM 3955), yeast (*Candida albicans* CCM 8275) and fungi (*Trichoderma viride* CCM F-486 and *Aspergillus niger* CCM 8155). All microbes were obtained from Czech Collection of Microorganisms in Brno (CCM). While testing against bacteria and yeast took place on 96-well microtiter plates using Broth microdilution method, interrogation of antifungal characteristics of chosen materials was carried out in Petri plates utilizing Disc diffusion test.

a.) Broth dilution method

All tested compounds exhibited excellent solubility in DMSO, and thus were fully dissolved for the purpose of stock solutions (20 g/L, 10 g/L, 5 g/L and 1 g/L) preparation. However, after applying them into the particular wells with nutrient media, precipitation occurred in some cases. The actual concentrations in the wells were 500 mg/L, 250 mg/L, 125 mg/L and 25 mg/L. Due to drastically reduced solubility in mostly aqueous media (wells) in comparison with pure DMSO (stock solutions), the most intense precipitation was expectedly observed at the highest concentrations (500 mg/L). Consequently, non-inoculated microtiter plate was also prepared and incubated as a reference to determine either turbidity in a particular well was caused by the growth of microbes or precipitation of tested compound. Furthermore, it also served as a control that utilized accessories and nutrient media were indeed sterile before usage. In most cases, precipitation in non-inoculated microplate disappeared during 24-hour incubation at 37 °C. Based on the differences in absorbances between inoculated and non-inoculated microtiter plates, the growth of microbes was unambiguously confirmed in almost all cases. Only for one compound, the obtained results were not completely obvious. As a result, contents of the wells in question were transferred to the agar nutrient media in Petri plates. After 24 hours of incubation at 37 °C, the growth of bacteria was explicitly established also in this case.

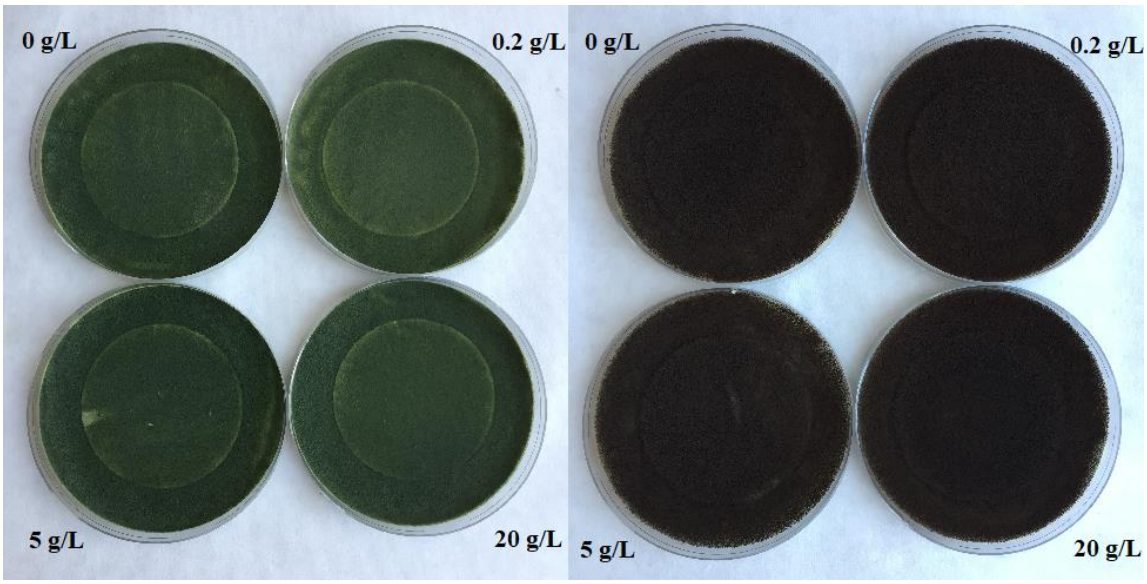
Even though, the minimum inhibitory concentration of all evaluated compounds is evidently higher than 500 mg/L, very slight suppression of microbes' growth was detected for the highest concentrations of three interrogated materials after 24 hours of incubation. While compounds **13a**, **13b** and **15a** (500 mg/L) exhibited weak repression against *Staphylococcus aureus*, similar could be said for compound **13b** (500 mg/L) against *Escherichia coli*. Although, lastly mentioned species slightly slow down the microbes' growth, they still do

not show any considerable antimicrobial activities, and thus could be designated as unpromising antimicrobial agents against all four tested strains.

b.) Disk diffusion test

A series of synthesized compounds was also tested against fungal strains *Trichoderma viride* and *Aspergillus niger*. Stock solutions of three different concentrations (20 g/L, 5 g/L and 0.2 g/L) in acetone were utilised. While majority of interrogated candidates were highly soluble in last-mentioned solvent, some of them showed poor solubility at the highest tested concentration (20 g/L). In cases, where suspension in acetone was obtained, it was firstly vigorously stirred to gain uniform distribution of present particles in liquid medium, and then immediately applied to the paper discs that served as carriers for tested materials. After 7 day of incubation, it was established that the surface of paper discs, as well as surrounding agar media were completely covered by the fungal species (Figure 7), and consequently all tested materials were characterised as totally inactive against both fungal strains.

Figure 7. *Growth of Trichoderma viride (left) and Aspergillus niger (right).*



c.) Testing against Mycobacterium strains

Ten acetates (**5a**, **5b**, **6a**, **6b**, **7a–f**), five alcohols (**4a**, **4b**, **11c**, **12a**, **12b**), ten aldehydes (**13a**, **13b**, **14a–f**, **15a**, **15b**), ten carboxylic acids (**16a**, **16b**, **17a–f**, **18a**, **18b**), as well as compounds **4c**, **8a**, **10a** and **20b** were also tested against four *Mycobacterium* strains including *Mycobacterium tuberculosis*, *Mycobacterium marinum*, *Mycobacterium kansasii* and *Mycobacterium smegmatis*. Similar to the previously described results, no worth mentioning activities against any of *Mycobacterium* species were detected for any of screened compounds.

4. CONCLUSIONS

Throughout my doctoral studies, a collection of quinoline-2,4(1*H*,3*H*)-dione species with one and two 1,2,3-triazole rings was prepared. The multi-step transformation scheme started with condensation reactions between aniline and suitably substituted diethyl malonates to give appropriate 4-hydroxyquinolin-2(1*H*)-ones **1** that were further transformed into 3-chloroquinoline-2,4(1*H*,3*H*)-diones **2**, and finally into 3-azidoquinoline-2,4(1*H*,3*H*)-diones **3**.

Using copper-catalysed 1,3-dipolar cycloaddition between azides **3** and propargyl alcohol, mono-triazole alcohols **4** were formed. Compounds **4** were further acetylated to provide corresponding esters **5**. By protecting primary alcohol group, potential *O*-alkylation during the propargylation reaction was effectively avoided, and therefore only *N*-alkylation of synthesized acetates **5** took place. As a result, *N*-propargyl derivatives **6** were obtained in very good yields.

In the next step, second 1,2,3-triazole ring was introduced to the quinolone segment, as materials **6** were further combined with three organic azides, namely benzyl azide **A**, phenyl azide **B** and tetrazolo[1,5-*a*]pyridine **C**, a synthetic equivalent for 2-azidopyridine **C'**. Consequently, six different bis-triazole acetates were synthesized, employing similar reaction conditions to those for the first »click«.

Apart from Cu^0/Cu^{2+} catalytic system in DMF, more commonly used bivalent copper in the presence of L-ascorbic acid or its sodium salt in various organic solvent(s)/water systems were also studied. Due to highly hydrophobic nature of synthesized compounds, the presence of water turned reactants and products into sticky, gummy materials that stuck on the flask's wall, as well as magnetic stirring bar and thus prevented reactions from going to completion. As a result, considerably lower yields and longer transformation times were established, when water was added to the reaction mixture. Consequently, Cu^0/Cu^{2+} catalytic pair in DMF was recognised superior for 1,2,3-triazole-bearing quinoline-2,4-dione species preparation.

Beside presented »click–propargylation–click« synthetic approach, its »propargylation–click–click« modification was also briefly evaluated. While the former provided only one expected product in each reaction step, 3-azido-1-propargyl bifunctional quinolone (**8a**) self-polymerization was detected during the latter. Consequently, observed reaction yields were significantly lower than in the case of »click–propargylation–click« reaction sequence.

Due to the fact that numerous materials with 1,2,3-triazole and/or quinolone building blocks exhibit large variety of desirable physical properties and biological activities, the number of synthesized compounds was maximized, using chemical functionality as an efficient tool for various derivatives preparation. Appropriate mono- and bis-triazole esters (**5**–**7**) were firstly deacetylated, and subsequently oxidized to suitable aldehydes and carboxylic acids. In all cases, reaction conditions were firstly optimised on more accessible *N*-unsubstituted

mono-triazole species, and then successfully utilised for bis-triazoles and propargyl derivatives preparation.

Three different deprotection approaches were evaluated for preparation of alcohols **4**, **11** and **12**. While basic reaction environments resulted in quinoline-2,4-dione ring-opening, desirable products were obtained by acidic alcoholysis of appropriate acetates. In addition, last-mentioned quinolinedione framework cleavage, using sodium methoxide in dry methanol, as well as potassium hydroxide in ethanol was also taken into consideration. While mixture of anthranilic acid derivatives commonly arose during the former, only one product was expectedly isolated throughout the latter.

Comparing PCC and MnO_2 as reagents for aldehydes **13**–**15** preparation, slightly higher reaction yields were gained, when manganese dioxide was used as an oxidant. In both cases, desirable aldehydes were prepared in only moderate yields. Relatively low transformation yields, especially in the case of pyridinium chlorochromate, were most probably caused by reagents' residues that apparently encaged formed products, preventing them from being transferred into organic solvent. In addition, Swern oxidation approach was found unsuitable for 1,2,3-triazole-bearing quinoline-2,4-dione aldehydes preparation.

Hexavalent chromium CrO_3 in sulphuric(VI) acid and acetone was proved to be an efficient reagent for the oxidation of primary alcohols **4**, **11** and **12** to carboxylic acids **16**–**18** that were obtained in very good to excellent yields. Even though, quite large quantities of toxic chromium-based oxidant were added to the reaction mixtures, pure carboxylic acids were provided, as reagent residuals were effectively removed during the isolation process.

Finally, several synthesized materials were interrogated for their coordination properties to ruthenium metal centre, as well as for antimicrobial activities against ten microbial strains including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Mycobacterium tuberculosis*, *Mycobacterium marinum*, *Mycobacterium kansasii*, *Mycobacterium smegmatis*, *Trichoderma viride* and *Aspergillus niger*. In addition, prepared species were also briefly screened for their potential photoprotective properties. Unfortunately, none of the tested compounds synthesized in the context of this dissertation exhibited any promising characteristics at all.

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1.) **Milićević, D**.; Kimmel, R.; Urankar, D.; Pevec, A.; Košmrlj, J.; Kafka, S. Preparation of Quinoline-2,4-dione Functionalized 1,2,3-Triazol-4-ylmethanols, 1,2,3-Triazole-4-carbaldehydes and 1,2,3-Triazole-4-carboxylic Acids.
*J. Heterocycl. Chem. (**Submitted Manuscript***)

2.) **Milićević, D.**; Kimmel, R.; Gazvoda, M.; Urankar, D.; Kafka, S.; Košmrlj, J. Synthesis of Bis(1,2,3-Triazole) Functionalized Quinoline-2,4-Diones.
Molecules **2018**, *23*(9), 2310.

3.) De Macedo, M. B.; Kimmel, R.; Urankar, D.; Gazvoda, M.; Peixoto, A.; Cools, F.; Torfs, E.; Verschaeve, L.; Lima, E. S.; Lyčka, A.; **Milićević, D.**; Klásek, A.; Cos, P.; Kafka, S.; Košmrlj, J.; Cappoen, D. Design, synthesis and antitubercular potency of 4-hydroxyquinolin-2(1*H*)-ones. *Eur. J. Med. Chem.* **2017**, *138*, 491–500.

CONFERENCE CONTRIBUTIONS

1.) **Milićević D.**, Kimmel R., Košmrlj J., Kafka S.: New Compounds with 1,2,3-Triazole Moiety. *70th Congress of the Czech and Slovak Chemical Societies,* Zlín, 9-12 September 2018. *Czech Chemical Society Symposium Series* **2018**, *16*, 342–343. ISSN: 2336-7202 *(Presentation)*

2.) **Milićević D.**, Kimmel R., Košmrlj J., Kafka S.: Synthesis and Analysis of 3-Azidoquinoline-2,4-diones. *46th EuroCongress on Drug Synthesis and Analysis*, Bratislava, 5-8 September 2017. Planková A., Ježko P., Maráková K. (editors): *Book of Abstracts*, p. 163. ISBN: 978-80-223-4388-6 *(Poster)*

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Preparation and Study of Photoprotective and Antimicrobial Properties of Novel Materials Based on 1,2,3-Triazole

Příprava a stadium fotoprotektivních a antimikrobiálních vlastností nových látek na bázi 1,2,3-triazolu

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