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JULIUS-MAXIMILIAN-UNIVERSITÄT WÜRZBURG

FAKULTÄT FÜR CHEMIE UND PHARMAZIE

Institut für Pharmazie und Lebensmittelchemie

Design and synthesis of potential M_1 muscarinic acetylcholine receptor dualsteric modulator

DIPLOMA THESIS

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Hradec Králové 2015 **Petr Průša**

DECLARATION

I declare that this thesis is my original work. All used literature and sources are listed in the list of used literature at the end of the thesis and are properly cited. This work has not been used to gain equal or different degree.

Hradec Králové 2015

Signature:

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Abstrakt

Univerzita Karlova v Praze, Farmaceutická fakulta v Hradci Králové

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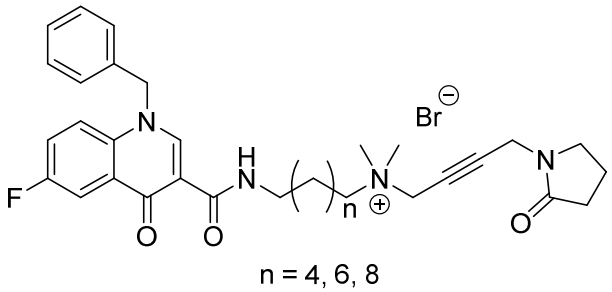
Název diplomové práce: Návrh a syntéza potenciálních dualsterických modulátorů M_1 muskarinového acetylcholinového receptoru

Dualsterické ligandy modulující aktivitu receptorů spojených s G-proteiny jsou zajímavé nejenom jako farmakologické nástroje pro objasnění aktivity receptorů, ale také jako koncept pro návrh nových léků. Již bylo dokázáno, že dualsterické sloučeniny složené z orthosterického ligandu, který může působit jako agonista nebo antagonista, a alosterické části, která slouží jako adresa určující cílový receptor pomocí vlastní selektivity, mají pozměněnou afinitu k receptoru. Navíc dualsterismus může sloužit ke vnesení selektivity k určitému podtypu daného receptoru.

S G-proteiny jsou spojeny muskarinové acetylcholinové receptory. Doposud bylo rozpoznáno pět podtypů těchto receptorů (značeny M_1 – M_5). Aktivace jednotlivých subtypů těchto receptorů má v organismu rozdílné projevy. M receptory jsou široce zastoupeny v celém organismu a jsou to důležité cíle některých léků. Bohužel, kvůli podobné struktuře vazebných míst těchto receptorů je obtížné dosáhnout funkční selektivity, což vede k různým vedlejším účinkům. Zatím se podařilo připravit pouze selektivní agonisty M_2 receptoru – iper-6-phtp a iper-6-naph.

Ve své diplomové práci jsem se zabýval přípravou dualsterických látek, které by byly selektivní k M_1 receptoru a na těchto receptorech působili jako agonisté. Jako vhodné látky pro takovouto strukturu byly zvoleny fluorovaný derivát benzyl chinolonové karboxylové kyseliny a derivát oxotremorinu spojené vhodným řetězcem (Obr. 1).



Obr. 1: Struktura potenciálních M_1 selektivních dualsterických modulátorů.

Předpokládá se, že selektivní agonisté M_1 receptoru by mohly mít využití v terapii schizofrenie a narušených kognitivních funkcí u Alzheimerovi choroby, kde by mohly působit zlepšení symptomů těchto onemocnění. Navíc jsou předpoklady, že tito agonisté mohou také pozitivně ovlivňovat patofyziologické procesy spojené s Alzheimerovou chorobou.

Abstract

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Title of Thesis: Design and synthesis of potential M_1 muscarinic acetylcholine receptor dualsteric modulator

Dualsteric ligands can modulate activity of receptors connected with G-proteins, also known as G-protein coupled receptor. These ligands are interesting for their use as pharmacological tools to clarify activity-induced conformational transitions. Moreover, dualsteric ligands can serve like a concept for design of new drugs. It was already prove that dualsteric compounds containing orthosteric ligand, which can act as agonist or antagonist, and allosteric part, which act as address determining target receptor via own selectivity, have affected affinity to receptor. Further, dualsteric binding can serve to introduce receptor subtype selectivity.

Muscarinic acetylcholine receptors are related to G-proteins. Until now five subtypes of these receptors (usually marked as M_1 – M_5) are known. Activation of single muscarinic acetylcholine receptors subtypes induce different replies. Muscarinic acetylcholine receptors are widely represent in entire organism and are important targets for some drugs. Unfortunately due to similar structure of binding sites it is hard to achieve functional selectivity, which causes different side effects. So far selective agonists of M_2 muscarinic acetylcholine receptor – iper-6-phtp and iper-6-naph - were prepared.

In my diploma thesis I dealt with the preparation of compounds selective to M_1 receptor and which affects this receptor as agonist. As suitable structures for this compound were chosen fluorinated derivate of benzyl quinolone carboxylic acid and derivate of oxotremorine linked with appropriate spacer (Fig. 1).

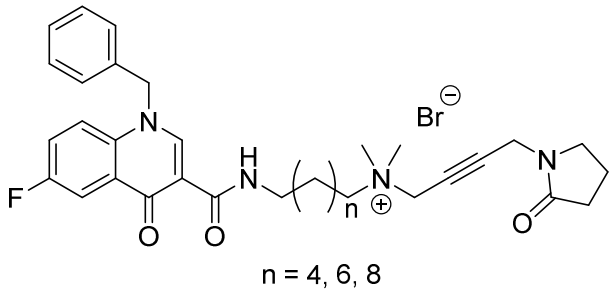


Fig. 1: Structure of potential M_1 selective dualsteric modulators.

There are assumptions that selective agonists of M_1 muscarinic acetylcholine receptor could be used in therapy of schizophrenia and impaired cognitive functions such as in Alzheimer's disease, where these compounds can improve symptoms of these diseases. Moreover there are assumptions that these agonists can positively affect pathophysiological processes related with Alzheimer's disease.

Content

1 Muscarinic acetylcholine receptors

Muscarinic acetylcholine receptors (mAChRs) are responsible for different physiological functions. There are 5 scientifically approved subtypes of mAChRs M_1 – M_5. These subtypes have same architecture in helical parts of the structure. Helical part of the receptor contains orthosteric acetylcholine binding site.[1] Because mAChRs modulate variety of physiological functions, they are important target for drugs. But due to 5 different subtypes of mAChRs, where each have different function and stimulation of each have different response in organism, it is highly important to have compounds with high selectivity toward certain subtypes of mAChRs. Experimental compounds with high selectivity to some subtype of mAChRs are already described, i.e. allosteric agonists and positive allosteric modulators for subtype M_1 and subtype M_4.[1]

1.1 Subtypes of muscarinic acetylcholine receptors

Muscarinic acetylcholine receptors are part of A group G protein-coupled receptors (GPCRs). Answer to the stimulation of certain subtype of mAChRs depends on G-protein connected to it. It can cause activation or inhibition effect. Receptors from this group has helical structure and seven transmembrane domains (7TMRs) (Fig. 2). This type of receptors are the most common superficial cell receptor in humans. 7TMRs are involved in modulation of variety of physiological processes. That made them interesting in the research of new drugs. Recently, there are drugs used to treatment diseases like chronical obstructive pulmonary disease, overactive bladder, bronchial asthma or glaucoma. According the literature almost one third of currently used medicaments have mechanism of action through this protein group.[1] In fact, 7TMRs serve as structures which translate extracellular impulses. Structural studies revealed that orthosteric binding site is located in third transmembrane domain and for binding is important negatively charged aspartate residue which interact with positively charged nitrogen of endogenous ligand.[2] This is done especially via GPCRs and β-arrestins.[1]

It was discovered that there are 5 specific subtypes of mAChRs named M_1 – M_5. But only M_1 – M_4 of them are approved pharmacologically. Genetic engineers and

molecular biologists revealed five different genes, which encode mAChRs and so there is assumption that fifth subtype also exist.[3]

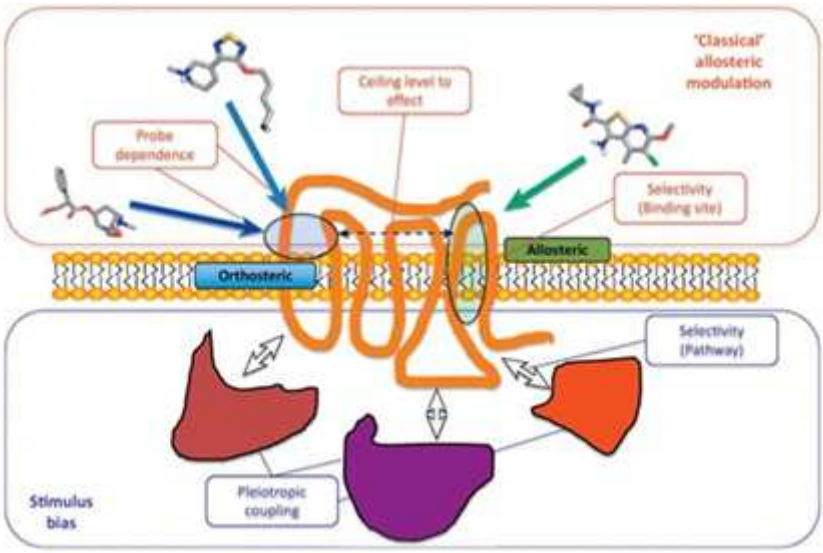
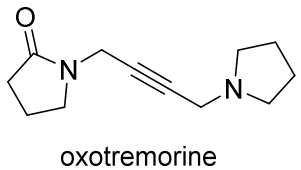


Fig. 2: Schematic structure of mAChRs.[4]

1.2 Muscarinic acetylcholine receptor M_1

Muscarinic acetylcholine receptor M_1 can be found primarily in central nervous system (CNS) especially in cortex, striatum and hippocampus, in peripheral neurons and parietal cells of stomach. M_1 receptor is also situated in other exocrine glands. Its interaction with ACh causes excitation – e.g. stimulation of CNS or increase secretion of HCl in stomach. Nonspecific agonist of M_1 receptors is oxotremorine (Fig. 3) and specific antagonist of peripheral M_1 receptor is pirenzepin.



oxotremorine

Fig. 3: Structure of oxotremorine.

This type of the mAChRs is coupled with G protein of class $G_{q/11}$ (such as M_3 muscarinic acetylcholine receptor) and stimulation of phospholipase C (PLC) releases intracellular messengers IP_3 and DAG. This leads to increase of intracellular

concentration of K^+ and depolarization of plasmatic membrane. This all evoke excitation – contraction of smooth muscles, increase secretion etc. (depends on certain mAChRs subtype). It is also often called neuronal muscarinic acetylcholine receptor. There are certain proves that deficiency of M_1 receptor and the lack of acetylcholine interaction can caused some types of dementia such as Alzheimer's disease. Hence, specific agonists of M_1 can be used in the treatment of these diseases.

1.3 Muscarinic acetylcholine receptor M_2

It is also called cardiac subtype of muscarinic receptors. This type of receptor is situated mostly in heart and neuronal tissues, where its interaction with ACh causes inhibition – e.g. vagus inhibition of heart rate, presynaptic inhibition in central and peripheral nervous system. M_2 muscarinic acetylcholine receptor is connected with $G_{i/0}$ (inhibition G-protein). Its stimulation leads to decrease activity of adenylyl cyclase. It activate K^+ receptor related canal which slow down the speed of depolarization.

1.4 Muscarinic acetylcholine receptor M_3

M_3 muscarinic acetylcholine receptors are situated in glands and smooth muscles and have excitation activity – e.g. increase secretion of salivary and bronchial glands and contraction of smooth muscles. But in smooth muscle of blood-vessels it mediates relaxation. Even if most of the blood-vessels don't have any cholinergic innervation, they respond on stimulation via acetylcholine and other parasympathomimetics through vasodilatation. Vasodilatation effect of ACh is indirect via presynaptic autoreceptors (heterotrophy inhibition, decrease releasing of noradrenaline) and also stimulation of endothelial cells and increase production and realising of NO (NO diffuse to blood-vessels smooth muscles and evoke their relaxation).

1.5 Muscarinic acetylcholine receptors M_4 and M_5

Function of muscarinic acetylcholine receptors M_4 and M_5 haven't been clarify until now. There are proves/assumes that receptor M_4 is mostly situated in glands and CNS and have certain similarities with muscarinic acetylcholine receptor M_2. Receptor M_5 is known even less. It still isn't know, where this subtype is located, only one what is known is that it has certain structural similarities with muscarinic acetylcholine receptor M_1.

2 Dualsteric targeting and functional selectivity

In last years were discovered ligands, which simultaneously bind into ACh binding site, which is orthosteric, and at the same time into allosteric binding site. This so called dualsteric ligands can be targeted to specific mAChRs subtype via its substructures. Dualsteric targeting can present new strategy in creating functional selectivity.

2.1 Models of dualsteric targeting

How it was said above, structural heterogeneity between allosteric domains can be used to create subtype mAChRs selectivity. We can distinguish three models of a dualsteric targeting.

2.1.1 Binary complex

Allosteric ligand binds to vacant receptor and modulates receptor activity through allosteric binding site. It can effect as allosteric agonist, neutral antagonist or inverse agonist itself.

2.1.2 Ternary complex

Allosteric ligand co-binds with ACh to receptor and modulate binding of ACh and/or affect the effect of binding ACh in positive, neutral or negative way.

2.1.3 Bitopic allosteric/orthosteric ligand binding to both binding sites

Dualsteric targeting is considered for special subtype of bitopic binding. Bitopic literally means “two places”. In fact for dualsteric targeting are important heterobivalent ligands – molecules containing two structural different pharmacophores. These structure then can undergo different types of bitopic binding (Fig. 4 and Fig. 5). Firstly, bitopic ligand can be connected by monovalent interaction with each of possible targets. Secondly, bitopic ligand can interfere with two neighboring target proteins like receptor – heterodimer. Compounds used to address to target multiple proteins are also called “designed multiple ligands”.[1] Thirdly and most importantly in context with my diploma thesis, bitopic ligand binds to allosteric and orthosteric binding site of one receptor at the same time: dualsteric binding.

Orthosteric part of dualsteric ligand can encode the message for activation of the receptor, and meanwhile allosteric part of the structure can serve as address for specific receptor subtype. In cases, where allosteric part of the structure is the carrier of any modulation activity, the situation will be more complex.[1]

Dualsteric targeting was firstly described in mAChRs, subtype M_2, which become archetypal for study allosteric/orthosteric interaction.[1]

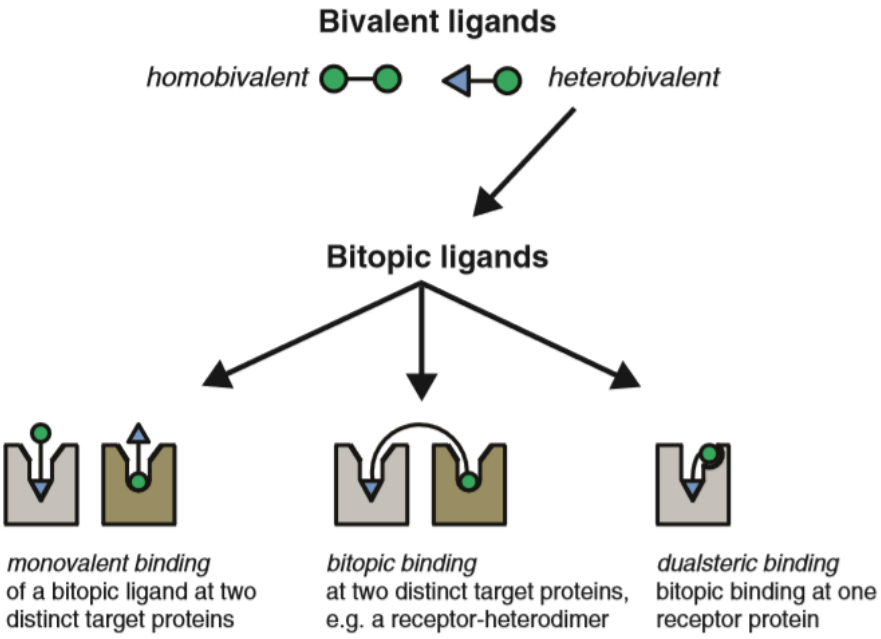


Fig. 4: Types of bitopic binding.[1]

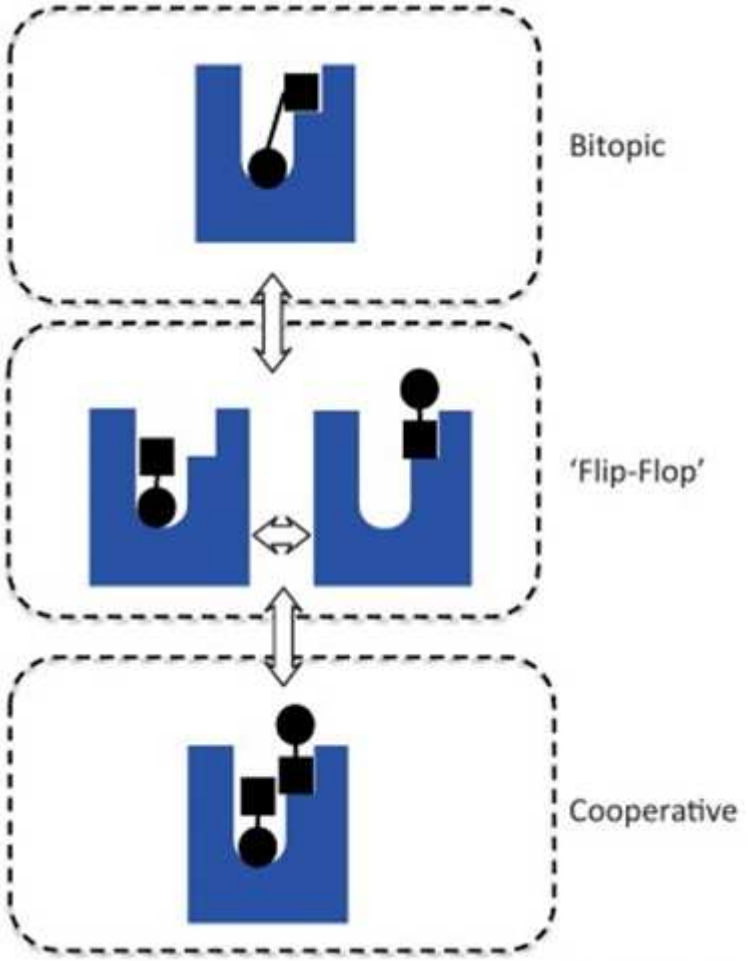


Fig. 5: Modes of dualsteric bindings. Potential modes of putative bitopic ligand interactions. A true bitopic mode involves the ligand making simultaneous interaction with orthosteric and an allosteric site. Alternatively, ligands may distribute between orthosteric or allosteric orientations via "flip-flop" mechanism, or concomitantly by binding cooperatively to each site on a single receptor.[4]

2.2 Oxotremorine M

Oxotremorine M is analog of orthosteric muscarinic agonist oxotremorine. Both are nonselective orthosteric muscarinic agonists (Fig. 6).

Fig. 6: Orthosteric muscarinic agonists.

2.3 Benzyl quinolone carboxylic acid

The exact chemical name for this structure is 1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (Fig. 7), also known as benzyl quinolone carboxylic acid (BQCA). BQCA is selective allosteric modulator of M_1 receptor and acts as partial agonist[2] with positive cooperativity with orthosteric agonists (including ACh) and is orally bioavailable.[5] Due to this properties BQCA was selected as address part of dualsteric targeting structure linked via spacer to nonselective agonist. The carboxylic group of BQCA was utilized to connect to spacer.

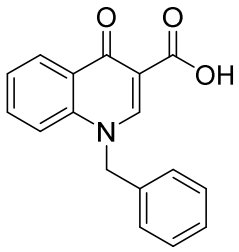


Fig. 7: Selective M_1 allosteric modulator.

2.4 Spacer

Among allosteric and orthosteric parts of structure, length of the spacer is equally important, because it can affect efficacy of dualsteric binding. In the previous studies

it was proved that prolongation from C4 to C6 length can have positive effect on dualsteric binding.[2]

3 Alzheimer's disease

How it was written above, specific M_1 receptor agonist could be used in the treatment of Alzheimer's disease.

3.1 Definition and epidemiology

Alzheimer's disease (AD) is neurodegenerative illness of the brain which causes incurable form of dementia. It is chronical progressive disease of the nervous system during which the destruction of neurons occurs. This process is accompanied with characteristic histopathological changes. Today AD is the most common cause of dementia. Its' prevalence is 1% of population and it is cause of 60% of all dementia. It belongs also in to the 5 most common worldwide reasons of the death.[6]

3.2 Risk factors

Without the rare cases of AD, which are caused by genetic mutations, the development of this disease is a result of multiple factors.[7]

3.2.1 Age

Age is the major risk factor for the development of AD. Most diagnosis of AD can be found in persons at age 65 or older. The incidence of AD rises exponentially with age. While the incidence of AD between 65 and 74 years is around 3%, between 75 and 84 years is already 19% and after 85 year it is even 47%.[8,9]

3.2.2 Family anamnesis

Persons, whose first-degree relative (brother or sister, parent) suffers from AD, more likely develop AD and even those who have more than one are at higher risk. But this can be caused by shared environmental and lifestyle factors. The second option to explain this connection is via the inherited apolipoprotein E-ε4 gene.[8]

3.2.2.1 Apolipoprotein E-ε4 gene

Apolipoprotein E (APOE) is the second most important risk factor. This gene is important for a protein that carries cholesterol in the bloodstream. There are three subtypes of the APOE gene which can be inherited from parents – ε2, ε3 and ε4. Mostly population dispose of the ε3 form of this gene. It is believed that this subtype

has no effect on the risk of the development of AD. Individuals with ε2 subtype display moderate decrease of the risk. Vice versa, disposing of the ε4 form induces an increase of the risk. And also it can cause the development of AD at a younger age. According to studies, between 40 and 80 percent of people suffering from AD dispose of at least one APOE-ε4 allele.[10] The risk of the development of AD is three times higher in heterozygotes disposing of this gene and even fifteen times higher in homozygotes individuals.[8]

3.2.3 Cardiovascular diseases

It is well known, that brain is one of the organs with the richest networks of blood vessels which brain needs to secure sufficient supply with the oxygen and necessary nutrients. From that we can suggest, that health of cardiovascular system is linked with the possibility of brain issues development which includes AD too. There are several risk factors which are common for both – cardiovascular diseases and AD. Among them belong smoking, midlife obesity, diabetes, midlife high cholesterol and also midlife hypertension. All these risk factors can negatively affect the supply of the brain. In connection with that, factors like physical activity and appropriately composed diet can act preventively against the developing of AD.[8]

3.2.4 Education

The education also belongs to risk factors. Several studies proved the relationship between risk of developing AD and achieved education. Result of these studies indicated that people with more years of formal education have smaller chance for the developing of AD than those with less years. Researchers attribute that the creation of "cognitive reserves" can help to compensate changes in the brain which can result in the symptoms of AD. The explanation for this considers that the process of the education increases quantity of the connections between neurons in the brain. Some other scientists believe that this is the result of socioeconomics factors which are highly connected with the level of the education.[8]

3.2.5 Other risk factors

To the risk factors of the development of AD belong traumatic brain injuries and psychological factors such as depression, anxiety disorders, psychological distress and sleep disorders.[8]

3.3 Etiopathogenesis

Still there isn't any clear evidence what cause AD. Only several things are known, which seems to be connected with suffering from AD.

3.3.1 Amyloid hypothesis

Full pathogenesis is unknown but protein amyloid β plays an important role. It shapes from amyloid precursor protein (APP). This protein belongs to transmembrane proteins which penetrates the neuron's membrane and is necessary for neuron growth, survival and post-injury repair.[11] It is usually degraded via enzyme α-secretase which cutting it on 40 fragments. But in some pathological occasions APP is degraded via β secretase or γ secretase. Formed fragments have different size than the original one and are insoluble. Result of this act is coagulation and polymerization into the β-amyloid. Thus formed β-amyloid is stored in the neuropil and forming Alzheimer's plaque (AP). Inside of these AP passes off the neurodegeneration during which the necrosis of the neurons occurs, creating the glial hem and forming the sterile inflammation.[12] Interference to these processes could be used in the therapy.

3.3.2 τ-protein hypothesis

Second important protein in the developing of AD is τ-protein. Normally τ-protein binds to the microtubules. For binding it is important that τ-protein is phosphorylated and the binding is making the site of the microtubules more stable. In pathological occasions amino acids are cleaved from peripheral locations of τ-protein and they are hyperphosphorylated. This change leads to the creation of neurofibrillary tangles which affects neuron's transport system. This leads to the apoptosis of the cells.[13]
Actually everybody develop some plaques and neurofibrillary tangles during ageing, but in brains of individuals suffering from AD much more of them could be find and

they are located in specific locations such as the temporal lobe. APOE is also stored in AP and neurofibrillary tangles.

3.3.3 Cholinergic hypothesis

Majority of the treatment of AD is based on this hypothesis. Cholinergic hypothesis presumes that AD is caused by decreased availability of the ACh which is caused by reduced activity of enzyme acetylcholinetransferase.[15] Treatment based on this hypothesis is only symptomatic and cures only symptoms resulting from the deficit of ACh in synapses (Fig. 8).

Next factors co-operating on the neural degeneration are reactive oxygen species which causes peroxidation of lipids in cell membranes. Further, there is the influence of the enormous release of excitatory amino acids. They binds in ionotropic receptors thereby increases the influx of calcium in to the neuron which started the signalling cascade leading to the apoptosis of the cells.[14]

All aforementioned factors cause neural degeneration and loss of the synapses. It affects both afferent and efferent routs of the hippocampus. There is also corrupted production of the ACh in presynaptic area of the neurons[14]. Further, in AD, there are deficits of serotonin, noradrenalin, GABA, glutamate, somatostatin, substance P and neuropeptide.

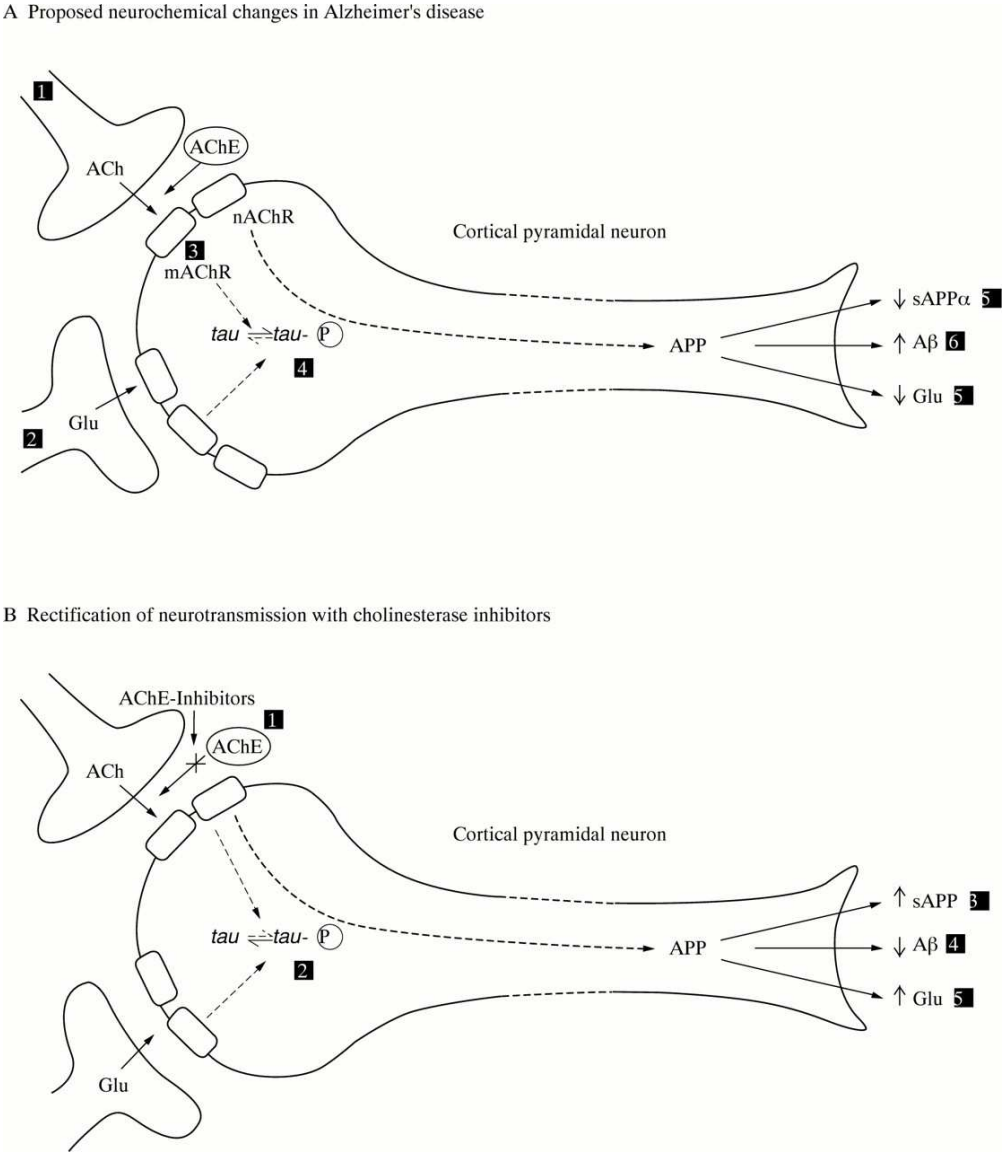


Fig. 8: Schematic diagram of a neuron representing (A) alterations in neurotransmission in Alzheimer's disease and (B) the hypothetical mode of action of AChE inhibitors. Key to figure (A): (1) reduced cortical cholinergic innervation; (2) reduced corticocortical glutamatergic neurotransmission due to neuron or synapse loss; (3) reduced coupling of muscarinic M1 receptors to second messenger system; (4) shift of tau to the hyperphosphoryalted state—precursor of neurofibrillary tangles; (5) reduced secretion of soluble APP; (6) increased production of β-amyloid protein; (7) decreased glutamate production. *It is hypothesised that these changes give rise to the clinical symptoms of Alzheimer's disease and contribute to the spread of pathology. Key to figure B: (1) AChE inhibitors reduce the breakdown of endogenously released ACh, resulting in greater activation of postsynaptic ACh receptors; hypothesised consequences: (2) reduced phosphorylation of tau; (3) secretion of sAPP returned towards normal; (4) reduced β-amyloid production; (5) glutamatergic neurotransmission returns towards normal, possibly due to activation of muscarinic and nicotinic receptors. ACh=acetylcholine; mAChR=ACh muscarinic receptor; APP=amyloid precursor protein; AChE=acetylcholinesterase; nAChR=ACh nicotinic receptor; Glu=glutamate.[15]

3.4 Histopathology

Mostly noticeable macroscopically change is atrophy of the brain tissue which leads to expend of the sulci and brain chambers. This is evident especially in the cortex.

There is visible deficit of the neurons and the occurrence of neurotic plaques and neurofibrillary tangles in the microscopy. Except these main characteristics of AD we can observe the granulovacuolary degeneration, neuropil threads and amyloid angiopathy. There are two types of the neurotic plaques. Both of them have core created β-amyloid, but so-called Diffuse plaques have unsharp limitation of the core and they are surrounded with dystrophic neurites. Vice versa Burnt-out type of the plaques has visible border, the core is terse and there are no dystrophic neurites.

Neurofibrillary tangles are situated intracellularly and are formed from hyperphosphorylated τ-protein connected with the microtubules.[14]

3.5 Forms of Alzheimer's disease

Development of AD is slow and inconspicuous. AD can be divided to one pre-stage and three main stages.

3.5.1 Pre-dementia

Certain neuropsychological tests can detect some symptoms of AD like cognitive difficulties up to eight years before establishment of clinical criteria of AD. Unfortunately the first symptoms can be counted to ageing or stress. This include the short term memory loss which is most striking symptom affecting daily living activities. The biggest problem for individuals suffering with pre-dementia is remembering recently learned facts and difficulty with process of acquiring of the new information.[16]

To the pre-dementia symptoms connected with developing of AD belong problems with executive functions, planning, flexibility, abstract thinking and semantic memory. And already in this stadium, we can observe apathy which is symptom which could be observe across whole stages of AD.[16] Of course that this all we can observe as a result of ageing, but the mainly different is in quantity. The difference between effects of ageing on memory and AD: forgetting things occasionally, misplacing items

sometimes, minor short-term memory loss, forgetting that memory lapses happened.[16] Previous mentioned is normal situation for ageing.

3.5.2 Early stage Alzheimer's disease

Main symptoms for early stage of AD are: absent-mindedness, forgetting appointments, slight changes seen by close loved ones, some confusion in situations outside the familiar.

Progressing degradation of learning and memory and development of all main symptoms leads to definitive diagnosis. But there is also small part of patients suffering from AD who has bigger difficulties with language, executive functions, perception or execution of movements and thus degradation of learning and memory is only minor problem for them. There is also visible difference in affecting different parts of the patient's memory. Short term memory is affected much more than episodic, semantic and implicit memory.

Difficulties with language take shape via decreased vocabulary and continuity of speaking. Patient is still able to communicate and express ideas and thoughts adequately. Also some movement coordination and planning difficulties (symptoms of apraxia) could be displayed but due to its dimness it usually stays unnoticed. With progress of AD decrease of independence appears and persons suffering from AD may need assistance or supervision with harder tasks and cognitively demanding activities.

3.5.3 Middle stage Alzheimer's disease

Main symptoms for middle stage AD are: deeper difficulty with remembering recently learned information, deepening confusion in many circumstances, speech impairment, repeatedly initiating the same conversation.

As progression continue patients become unable to perform common activities of daily living. Problems with speech escalate due to inability to remember vocabulary which leads to paraphrasing and incorrect word substitution. The ability of writing and reading also decreased and could be lost definitely. Movement coordination become worse too. It leads to heightened danger of falling. Furthermore, problems with recognising of close relatives occur and even long term memory is affected.

Changes in behaviour are common at this stage. Patients start to wander around places which they used to know, they become irritabile and labile which is accompanied with

unpredictable crying, aggression or even resistance to caregiving which is probably due to lose of insight into the disease process and limitations. As the means of defence, approximately 30% of the patients build themselves illusionary misidentifications and other delusional symptoms. In some cases urinary incontinence can develop.

3.5.4 Late stage Alzheimer's disease

Main symptoms for late stage of AD are: aggressivity or passivity, some loss of self-awareness, debilitating cognitive deficit, abusiveness, anxiousness or paranoia.
In final stage the patient totally lost independence and his survive fully depend upon caregivers. Problems with speech escalate in to almost or totally full loss of communication ability. Patients are able to communicate only via simple phrases or single words or at least they can return emotional signals. Patients become extremely apathy and exhausted, aggression ebb. They are not able to perform even the simplest and basic task needed to live independently. They are permanently bedridden and full dependent on care from others. AD isn't usually the reason of death. Patients usually die due to external reasons like infection of pressure ulcers or pneumonia.[17]

3.6 Diagnosis

Diagnosis of AD is based on fulfilling the criteria of dementia and simultaneously elimination of any other causes of dementia. This is so-called diagnosis per exclusionem. Diagnostic criteria for dementia are:

1. Defect of memory
2. At least one of next:
    a. Failure of abstract thinking
    b. Failure of opinion and planning
    c. Aphasia, apraxia, agnosia and/or failure in space orientation
    d. Change in personality
3. Important cognitive decrease which can interrupt normal daily activity
4. Decrease of abilities – attention, constructive abilities, problem solving, orientation and functional abilities.[18]

When anybody fulfil at least one point from aforementioned, it doesn't mean that the individual has to suffer from AD, but there is a risk of that. For the definitive diagnosis advanced medical imaging is needed. Computed tomography or magnetic resonance imaging is often used. Imaging of brain tissue proves cortex atrophy and eliminates other causes (e.g. tumors, vascular lesions etc.). Neuropsychological testing serves for indication of AD stage. This testing contains activities such as copying of drawings, remembering words, reading or solving of subtract serial numbers.

3.7 Therapy

Therapy of AD should be complex and contains pharmacotherapy and non-biological treatment.

3.7.1 Non-biological treatment

This type of treatment is an integral part in caregiving to patient due to his self-insufficient. Caregivers can be relatives or professionals who act according the medicians.[8]

3.7.2 Pharmacotherapy

Unfortunately there are no medicaments which can definitively cure AD or even halt the progression of AD. How it was proven in the studies, patients suffering from AD have decreased activity of the cholinergic neurons. For their proper function sufficient concentration of ACh is needed, which is degraded via enzyme acetylcholinesterase. To slow down the degradation of ACh, inhibitors of acetylcholinesterase (AChEI) are used.

3.7.2.1 Inhibitors of acetylcholinesterase

AChEI are sort of medicaments which don't bind to cellular receptors but inhibit activity of acetylcholinesterase. This enzyme is responsible for breaking down ACh. There are two groups of known AChEI which are used in the therapy of diseases – reversible and irreversible inhibitors.

3.7.2.1.1 Reversible inhibitors of acetylcholinesterase

Mechanism of action of these inhibitors is the competition for binding site of the AChE. Among reversible AChEI used in the treatment of AD belong rivastigmine, galantamine, donepezil and in Czech republic not used tacrine. They are used in therapy of early and middle stages of AD.

3.7.2.1.2 Irreversible inhibitors of acetylcholinesterase

The difference between reversible and irreversible AChEI is the phosphorylation of AChE which permanently deactivate the enzyme. None of irreversible AChEI is used in any treatment.

3.7.2.2 Antagonist of NMDA receptors

Noncompetitive NMDA receptor antagonist memantin is used in the therapy of AD. Its binding into the receptor causes disabling binding of glutamate which is one of the main neurological inhibitor. Memantin is used in the therapy of middle and late stages. It is used as monotherapy or could be used in combination with donepezil.

4 Aim of work

The aim of this work was to develop a new potent dualsteric modulator of M_1 receptors. Both parts of the designed structure – allosteric fluorinated derivate of BQCA and orthosteric derivative of oxotremorine-M – show required properties for creating dualsteric selective agonist of M_1 muscarinic acetylcholine receptor. Selective agonism is important for remove side effects of insufficiently selective agonists. It is important to say that the design structures probably could not cross the blood-brain barrier. Hence, we didn't intend to find new drug but more likely the proof-of-concept regarding bipharmacophoric partial M_1 agonists.

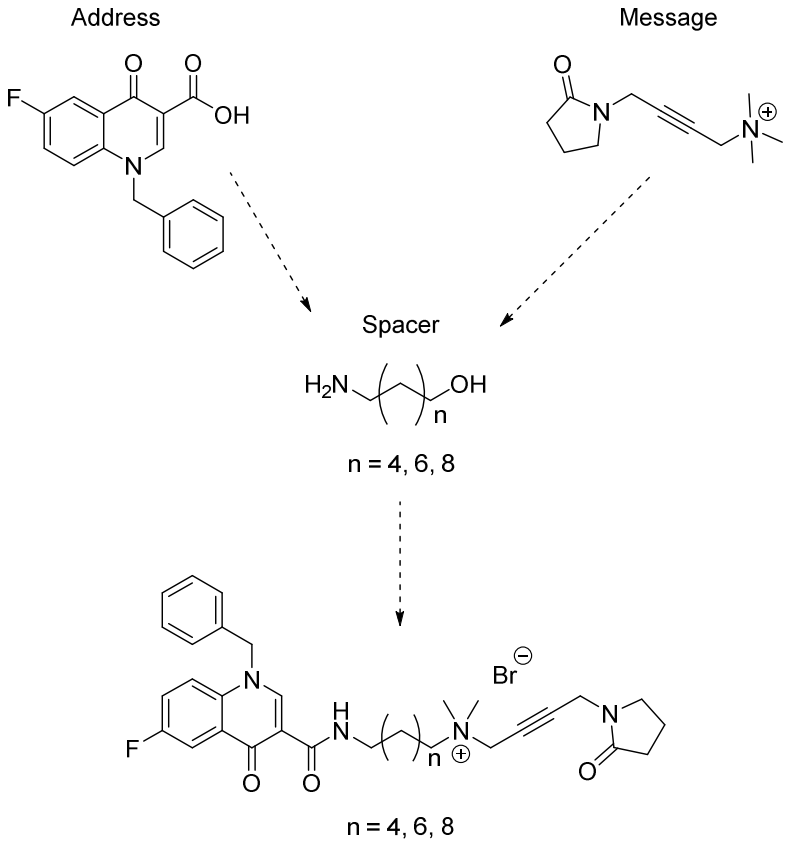
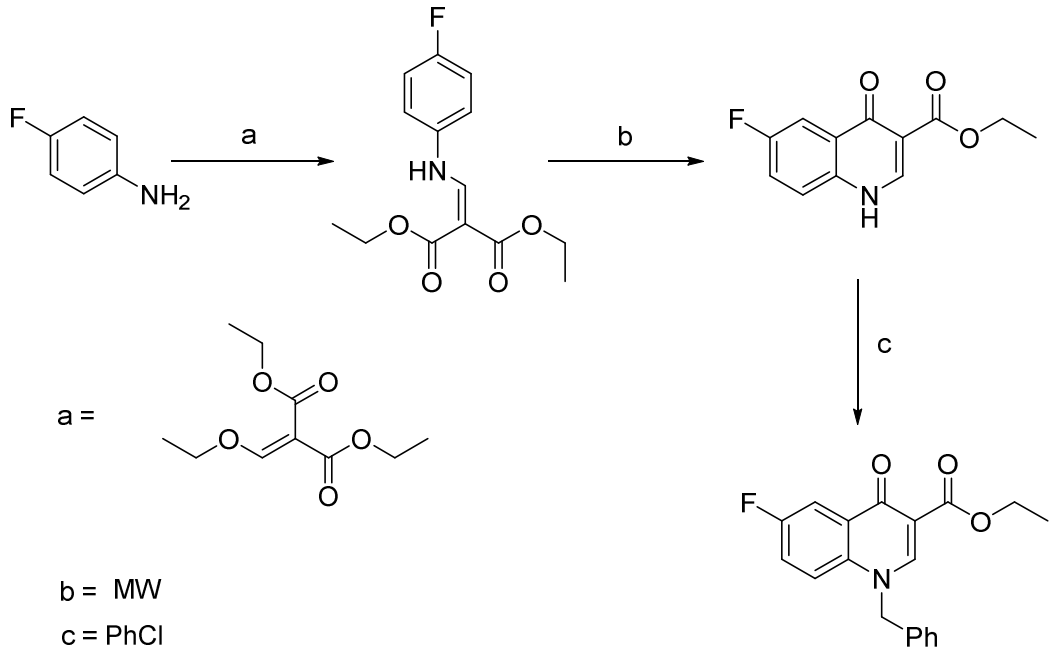


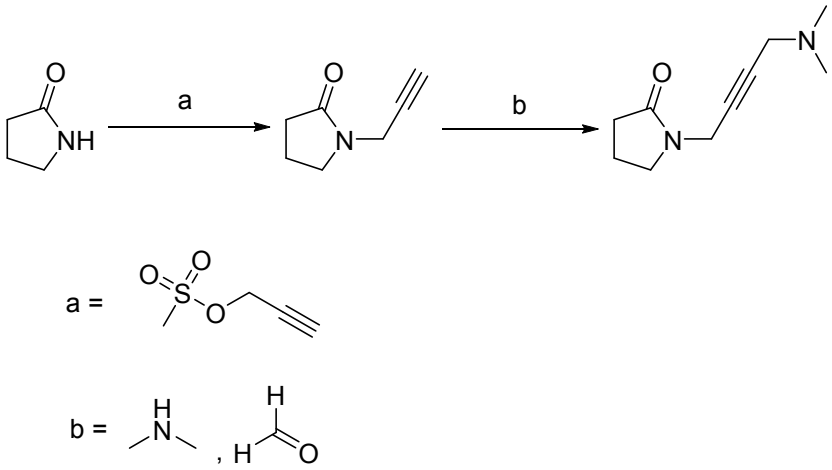
Fig. 9: Design of the target compounds.

5 Plan of synthesis

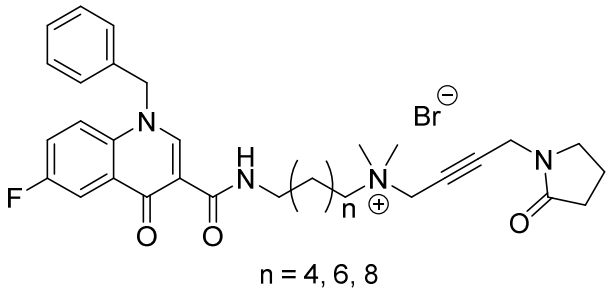
For the synthesis of BQCA fluorinated derivative Gould-Jacobs reaction was chosen, which is well known in research group of Prof. Holzgrabe. This reaction consist of two steps. The addition of benzyl to nitrogen in position 1 should provide the compound which is suitable for the connection to spacer (Scheme 1). For the preparation of oxotremorine-M analog literature approach was selected[19] (Scheme 2). Both products would be connected under microwave irradiation to give desired final compound Based on the previous studies C6, C8 and C10 lengths of the spacer were chosen (Scheme 3).



Scheme 1: Preparation of BQCA analog.



Scheme 2: Preparation of oxotremorine derivative.



Scheme 3: Structure of desired compounds.

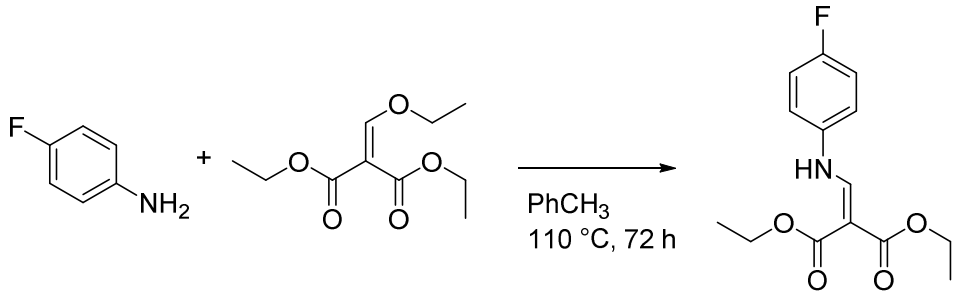
6 Experimental part

6.1 Synthesis of fluorinated derivative of BQCA

The reactions used to the preparation of fluorinated derivate of BCQA are described below.

6.1.1 Synthesis of diethyl 2-(((4-fluorophenyl)amino)methylene)malonate

Reaction



PP-1-1

Reactants

1. 4-fluoroaniline
    a. ME = 1.0; M_r = 111.12 g/mol, ρ = 1.173 g/ml
2. diethyl 2-(ethoxymethylene)malonate
    a. ME = 1.2; Mr = 216.23 g/mol, ρ = 1.08 g/ml
3. dry toluene
    a. solvent

Approach

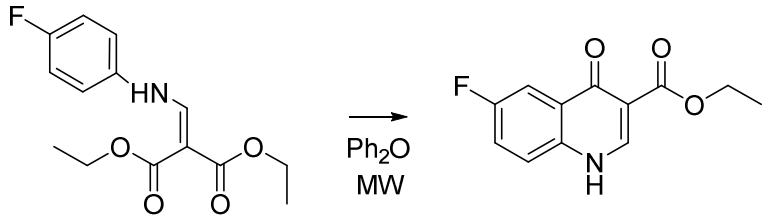
The reaction was done under the Argon atmosphere. 4-Fluoroaniline and diethyl 2-(ethoxymethylene)malonate were dissolved in dry toluene. The mixture was stirred and heated at 110 °C for 72 hours. Then the solvent was evaporated under reduced pressure to gain light yellow oil. To this oily residue was added pentane and the flask was stored in the fridge overnight. Formed crystals was filtered and washed with pentane to get white crystalline powder.

^{1}H NMR (400 MHz, DMSO) δ 10.69 (d, *J* = 13.6 Hz, 1H), 8.33 (d, *J* = 13.7 Hz, 1H), 7.43 (dd, *J* = 9.0, 4.6 Hz, 2H), 7.23 (t, *J* = 8.8 Hz, 2H), 4.21 (q, *J* = 7.1 Hz, 2H), 4.12 (q, *J* = 7.1 Hz, 2H), 1.30 – 1.19 (m, 6H).

Entry	**4-Fluoroaniline**	**Diethyl 2-(ethoxymethylene)malonate**	**Yield of PP-1-1**
1	9.47 ml; (0.10 mol)	24.25 ml; (0.12 mol)	82.8% (23.30g)
2	1.23 ml; (13.05 mmol)	3.15 ml; (15.58 mmol)	84.2% (3.09g)

6.1.2 Synthesis of ethyl 6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate

Reaction



PP-1-1 PP-1-2

Reactants

1. diethyl 2-(((4-fluorophenyl)amino)methylene)malonate (**PP-1-1)**
    a. ME = 1.0; M_r = 281.21 g/mol
2. diphenyl ether
    a. solvent

Approach

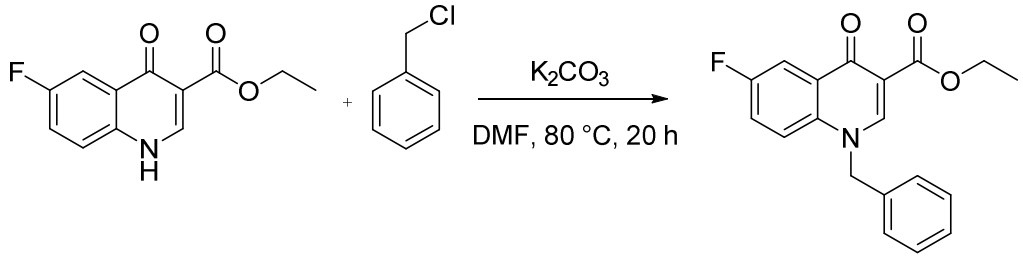
Diethyl 2-(((4-fluorophenyl)amino)methylene)malonate (PP-1-1) was inserted into the flask for microwave reaction. Then was added diphenyl ether and the mixture was stirred in MW for 1 hour at 210 °C. After that diethyl ether was added and the product started to precipitate from the solution. Formed solid was filtered and washed with diethyl ether to gain white crystalline powder.

Entry	PP-1-1	Yield of PP-1-2	Note
1	1.0 g; (3.55 mmol)	-	*
2	6.0 g; (21.33 mmol)	82.9% (4.16 g)	
3	3.09 g; (10.99 mmol)	77.3% (1.98 g)	

*The first attempt was not successful, because the reaction mixture did not reached required temperature. In next two attempts was added more passive heaters.

6.1.3 Synthesis of ethyl 1-benzyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate

Reaction



PP-1-2 PP-1-3

Reactants

1. ethyl 6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**PP-1-2**)
    a. ME = 1.0; M_r = 235.21 g/mol
2. benzyl chloride
    a. ME = 5.0; M_r = 126.58 g/mol, ρ = 1.10 g/ml
3. potassium carbonate
    a. ME = 2.5; M_r = 138.21 g/mol
4. dry dimethylformamide
    a. solvent

Approach

The reaction was done under the Argon. Ethyl 6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (PP-1-2) and K_2CO_3 was dissolved in dry DMF and was stirred for 20 minutes. Then benzyl chloride was added dropwise and the solution was stirred for 20 h at 80 °C. Then the mixture was filtered and stored in the fridge overnight. Formed crystals was filtered and recrystallize from EtOH. I gained white crystalline powder.

^{1}H NMR (400 MHz, DMSO) δ 8.94 (s, 1H), 7.90 (dd, *J* = 9.1, 3.1 Hz, 1H), 7.73 (dd, *J* = 9.3, 4.3 Hz, 1H), 7.63 – 7.57 (m, 1H), 7.41 – 7.34 (m, 2H), 7.33 – 7.27 (m, 1H), 7.27 – 7.21 (m, 2H), 5.71 (s, 2H), 4.26 (q, *J* = 7.1 Hz, 2H), 1.30 (t, *J* = 7.1 Hz, 3H).

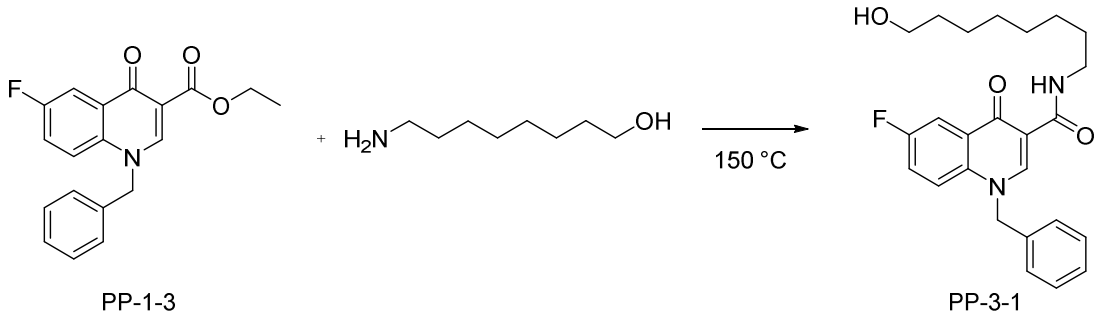
Entry	PP-1-2	Benzylchlorid	K_2CO_3	Yield of PP-1-3
1	6.0 g; (25.51 mmol)	16.14 g; (0.13 mol)	8.81 g; (63.74 mmol)	67.7% (5.62 g)

6.2 Connecting with spacer

In the next part of my diploma thesis connecting of fluorinated BQCA derivative with spacers with different length will be described. For the connection of spacer was utilized carboxylic functional group of BQCA. As starting material for creating spacers were chosen commercial available aminoalcohols with required length of carbon chain.

6.2.1 Synthesis of 1-benzyl-6-fluoro-*N*-(8-hydroxyoctyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide

Reaction



PP-1-3 PP-3-1

Reactants

1. ethyl 1-benzyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**PP-1-3**)
    a. ME = 1.0; M_r = 325.33 g/mol
2. 8-amino-1-octanol
    a. ME = 3.0; M_r = 145.25 g/mol

Approach

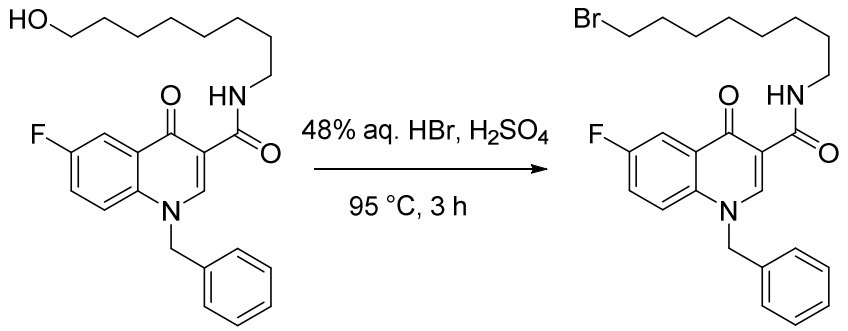
This reaction was performed without the solvent.Ethyl 1-benzyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (PP-1-3) and 8-amino-1-octanol was heated at 150 °C and stirred. The reaction was monitored via TLC every 30 minutes (eluent: dichlormethan/methanol = 20/1 $Rf_{PP\text{-}1\text{-}3}$ = 0.59; $Rf_{PP\text{-}3\text{-}1}$ = 0.49). After 90 minutes the starting material was not detected. . After cooling was the mixture dissolved in MeOH and stored in the freezer overnight. Formed crystals was filtered and dissolved in the EtOH and stored in the freezer again. This approach was selected to secure purity of product (PP-3-1). Formed crystals was filtered to gain white crystalline powder.

^{1}H NMR (400 MHz, DMSO) δ 9.88 (t, *J* = 5.7 Hz, 1H), 9.08 (s, 1H), 8.00 (dd, *J* = 9.0, 3.1 Hz, 1H), 7.85 (dd, *J* = 9.5, 4.3 Hz, 1H), 7.74 – 7.61 (m, 1H), 7.42 – 7.26 (m, 3H), 7.27 – 7.17 (m, 2H), 5.81 (s, 2H), 4.31 (t, *J* = 5.2 Hz, 1H), 3.44 – 3.33 (m, 4H, overlaped with H_2O), 1.60 – 1.50 (m, 2H), 1.45 – 1.24 (m, 10H).

Entry	**PP-1-3**	**8-Amino-1-octanol**	**Yield of PP-3-1**
1	0.21 g; (0.63 mmol)	0.27 g; (1.89 mmol)	41.1% (0.11 g)
2	0.43 g; (1.32 mmol)	0.57 g; (3.95 mmol)	44.3% (0.25 g)

6.2.2 Synthesis of 1-benzyl-*N*-(8-bromooctyl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxamide

Reaction



PP-3-1 PP-3-2

Reactants

1. 1-benzyl-6-fluoro-*N*-(8-hydroxyoctyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**PP-3-1**)
    a. ME = 1.0; M_r = 424.51 g/mol
2. 48% hydrobromic acid in H_2O
    a. ME = 20.0; M_r = 80.90 g/mol, ρ = 1.48 g/ml
3. concentrated sulphuric acid
    a. ME = 4.25; M_r = 98.08 g/mol, ρ = 1.83 g/ml

Approach

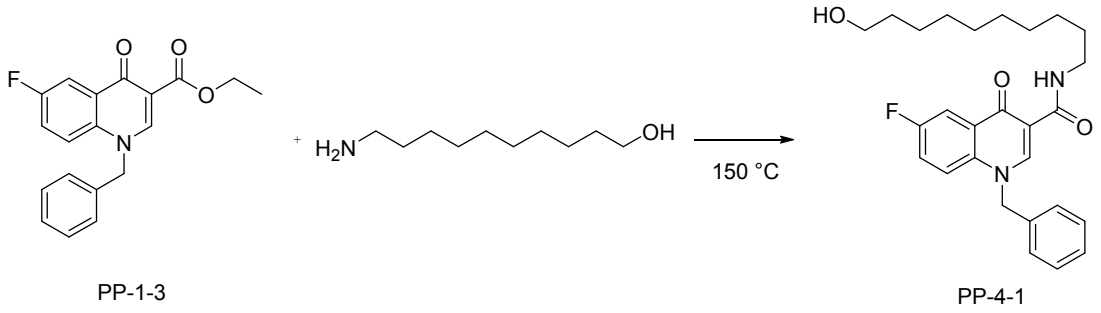
1-benzyl-6-fluoro-*N*-(8-hydroxyoctyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (PP-3-1) was placed into the flask, then was added 48% HBr in H_2O and concentrated H_2SO_4. The mixture was heated at 95 °C under stirring. After 3 hours any starting material was not detected. TLC control was performed (eluent: dichlormethan/methanol = 20/1 Rf_{PP-3-1} = 0.75; Rf_{PP-3-2} = 0.28). After that water was added and the extraction was made with chloroform. The organic phase was neutralized with K_2CO_3, dried with Na_2SO_4, filtered and evaporated to gain white crystalline powder.

^{1}H NMR (400 MHz, DMSO) δ 9.88 (t, *J* = 5.6 Hz, 1H), 9.08 (s, 1H), 8.00 (dd, *J* = 9.0, 3.1 Hz, 1H), 7.85 (dd, *J* = 9.4, 4.3 Hz, 1H), 7.73 – 7.61 (m, 1H), 7.40 – 7.25 (m, 3H), 7.25 – 7.18 (m, 2H), 5.81 (s, 2H), 3.52 (t, *J* = 6.7 Hz, 2H), 3.38 – 3.33 (m, 2H), 1.85 – 1.74 (m, 2H), 1.60 – 1.50 (m, 2H), 1.44 – 1.25 (m, 8H).

Entry	**PP-3-1**	**48%HBr in H_2O**	**conc. H_2SO_4**	**Yield of PP-3-2**
1	0.04 g; (0.09 mmol)	0.10 ml; (1.88 mmol)	0.02 ml; (0.38 mmol)	86.6 % (0.04 g)
2	0.23 g; (0.54 mmol)	0.59 ml; (10.75 mmol)	0.12 ml; (2.16 mmol)	87.1 % (0.23 g)

6.2.3 Synthesis of 1-benzyl-6-fluoro-N-(10-hydroxydecyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide

Reaction



PP-1-3 PP-4-1

Reactants

1. ethyl 1-benzyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**PP-1-3**)
    a. ME = 1.0; M_r = 325.33 g/mol
2. 10-amino-1-decanol
    a. ME = 3.0; M_r = 173.30 g/mol

Approach

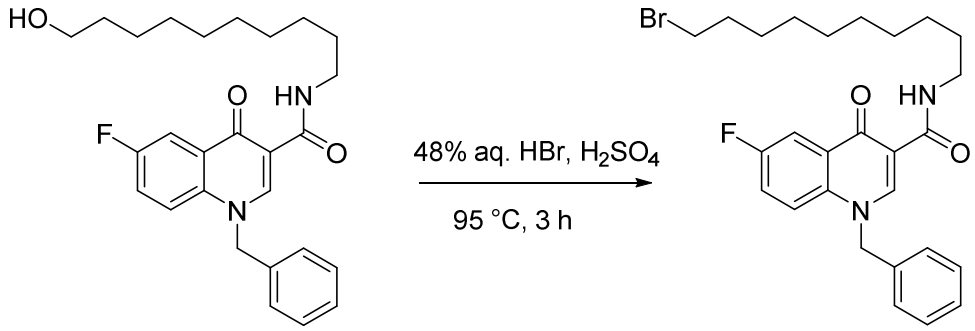
This reaction was performed without the solvent. Ethyl 1-benzyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (PP-1-3) and 10-amino-1-decanol was heated at 150 °C under stirring. The reaction was monitored via TLC every 30 minutes (eluent: dichlormethan/methanol = 20/1 $Rf_{PP\text{-}1\text{-}3}$ = 0.59; $Rf_{PP\text{-}4\text{-}1}$ = 0.38). After 90 minutes any starting material was not detected. After cooling the mixture was dissolved in MeOH and stored in the freezer overnight. Formed crystals was filtered and dissolved in the EtOH and stored in the freezer again. This approach was selected to secure purity of product (PP-4-1). Formed crystals was filtered to gain white crystalline powder.

^{1}H NMR (400 MHz, DMSO) δ 9.88 (t, *J* = 5.6 Hz, 1H), 9.08 (s, 1H), 8.00 (dd, *J* = 9.0, 3.1 Hz, 1H), 7.85 (dd, *J* = 9.4, 4.3 Hz, 1H), 7.72 – 7.63 (m, 1H), 7.42 – 7.27 (m, 3H), 7.26 – 7.19 (m, 2H), 5.81 (s, 2H), 4.30 (t, *J* = 5.1 Hz, 1H), 3.42 – 3.32 (m, 4H), 1.64 – 1.46 (m, 2H), 1.45 – 1.17 (m, 14H).

Entry	PP-1-3	10-Amino-1-decanol	Yield of PP-4-1
1	0.61 g; (1.87 mmol)	0.97 g; (5.61 mmol)	29.3% (0.23 g)

6.2.4 Synthesis of 1-benzyl-*N*-(10-bromodecyl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxamide

Reaction



Reactants

1. 1-benzyl-6-fluoro-*N*-(10-hydroxydecyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**PP-4-1**)
    a. ME = 1.0; M_r = 452.57 g/mol
2. 48% hydrobromic acid in H_2O
    a. ME = 20.0; M_r = 80.90 g/mol, ρ = 1.48 g/ml
3. concentrated sulphuric acid
    a. ME = 4.25; M_r = 98.08 g/mol, ρ = 1.83 g/ml

Approach

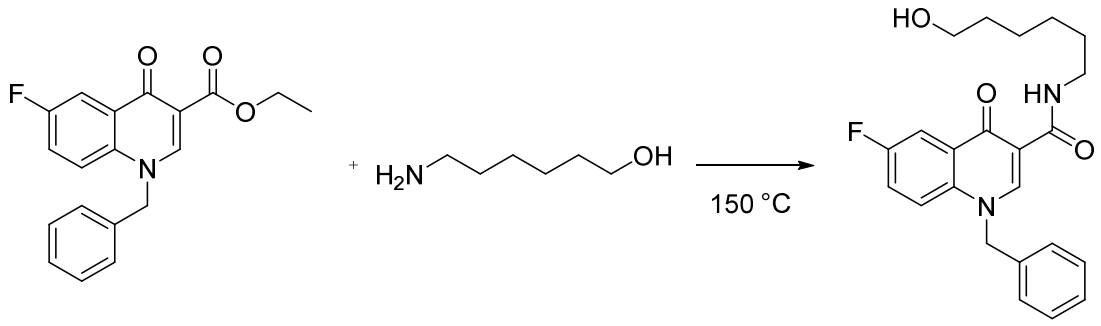
1-benzyl-6-fluoro-N-(10-hydroxydecyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (PP-4-1) was placed into the flask, then was added 48% HBr in H_2O and concentrated H_2SO_4. The mixture was heated at 95 °C under stirring. After 3 hours any starting material was not detected. TLC control was performed (eluent: dichlormethan/methanol = 20/1 $Rf_{PP\text{-}4\text{-}1}$ = 0.82; $Rf_{PP\text{-}4\text{-}2}$ = 0.33). After that water was added and the extraction was made with chloroform. The organic phase was neutralized with K_2CO_3, dried with Na_2SO_4, filtered and evaporated to gain white crystalline powder.

^{1}H NMR (400 MHz, DMSO) δ 9.88 (t, *J* = 5.6 Hz, 1H), 9.08 (s, 1H), 8.00 (dd, *J* = 9.0, 3.1 Hz, 1H), 7.86 (dd, *J* = 9.4, 4.3 Hz, 1H), 7.71 – 7.63 (m, 1H), 7.42 – 7.27 (m, 3H), 7.25 – 7.18 (m, 2H), 5.81 (s, 2H), 3.47 – 3.33 (m, 4H), 1.51 – 1.39 (m, 2H), 1.42 – 1.14 (m, 14H).

Entry	**PP-4-1**	**48% HBr in H_2O**	**conc. H_2SO_4**	**Yield of PP-4-2**
1	0.19 g; (0.42 mmol)	0.46 ml; (8.48 mmol)	0.10 ml; (1.78 mmol)	81.8 % (0.18 g)

6.2.5 Synthesis of 1-benzyl-6-fluoro-*N*-(6-hydroxyhexyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide

Reaction



PP-1-3 PP-5-1

Reactants

1. ethyl 1-benzyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**PP-1-3**)
    a. ME = 1.0; M_r = 325.33 g/mol
2. 6-amino-1-hexanol
    a. ME = 3.0; M_r = 117.19 g/mol

Approach

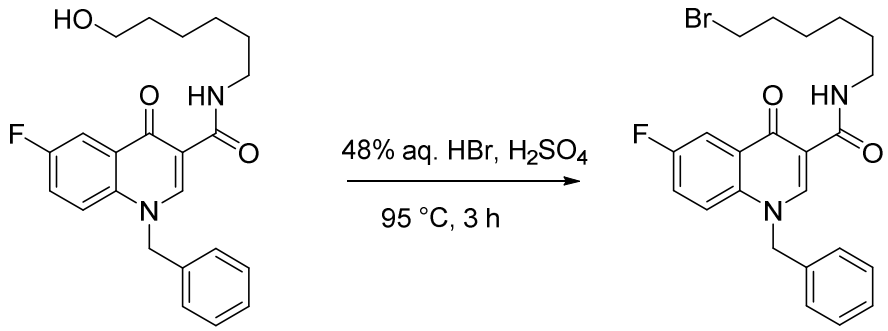
This reaction was performed without the solvent. Ethyl 1-benzyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (PP-1-3) and 6-amino-1-hexanol was heated at 150 °C under stirring. The reaction was monitored via TLC every 30 minutes (eluent: dichlormethan/methanol = 20/1 $Rf_{PP\text{-}1\text{-}3}$ = 0.59; $Rf_{PP\text{-}4\text{-}1}$ = 0.27). After 90 minutes was not any starting material detected. . After cooling the mixture was dissolved in MeOH and stored in the freezer overnight. Formed crystals was filtered and dissolved in the EtOH and stored in the freezer again. This approach was selected to secure purity of product (PP-5-1). Formed crystals was filtered to gain white crystalline powder.

^{1}H NMR (400 MHz, DMSO) δ 9.88 (t, *J* = 5.7 Hz, 1H), 9.09 (s, 1H), 8.00 (dd, *J* = 9.0, 3.1 Hz, 1H), 7.84 (dd, *J* = 9.4, 4.3 Hz, 1H), 7.71 – 7.63 (m, 1H), 7.41 – 7.24 (m, 3H), 7.24 (s, 2H), 5.81 (s, 2H), 4.34 (t, *J* = 5.1 Hz, 1H), 3.45 – 3.29 (m, 4H), 1.59 – 1.48 (m, 2H), 1.47 – 1.28 (m, 6H).

Entry	PP-1-3	6-Amino-1-hexanol	Yield of PP-5-1
1	0.84 g; (2.59 mmol)	0.91 g; (7.76 mmol)	29.3% (0.30 g)

6.2.6 Synthesis of 1-benzyl-*N*-(6-bromohexyl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxamide

Reaction



PP-5-1 PP-5-2

Reactants

1. 1-benzyl-6-fluoro-*N*-(6-hydroxyhexyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**PP-5-1**)
    a. ME = 1.0; M_r = 396.46 g/mol
2. 48% hydrobromic acid in H_2O
    a. ME = 20.0; M_r = 80.90 g/mol, ρ = 1.48 g/ml
3. concentrated sulphuric acid
    a. ME = 4.25; M_r = 98.08 g/mol, ρ = 1.83 g/ml

Approach

1-benzyl-6-fluoro-*N*-(6-hydroxyhexyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (PP-5-1) was placed into the flask, then was added 48% HBr in H_2O and concentrated H_2SO_4. The mixture was heated at 95 °C under stirring. After 3 hours was not any starting material detected. TLC control was performed (eluent: dichlormethan/methanol = 20/1 $Rf_{PP\text{-}5\text{-}1}$ = 0.81; $Rf_{PP\text{-}5\text{-}2}$ = 0.30). After that water was added and the extraction was made with chloroform. The organic phase was neutralized with K_2CO_3, dried with Na_2SO_4, filtered and evaporated to gain white crystalline powder.

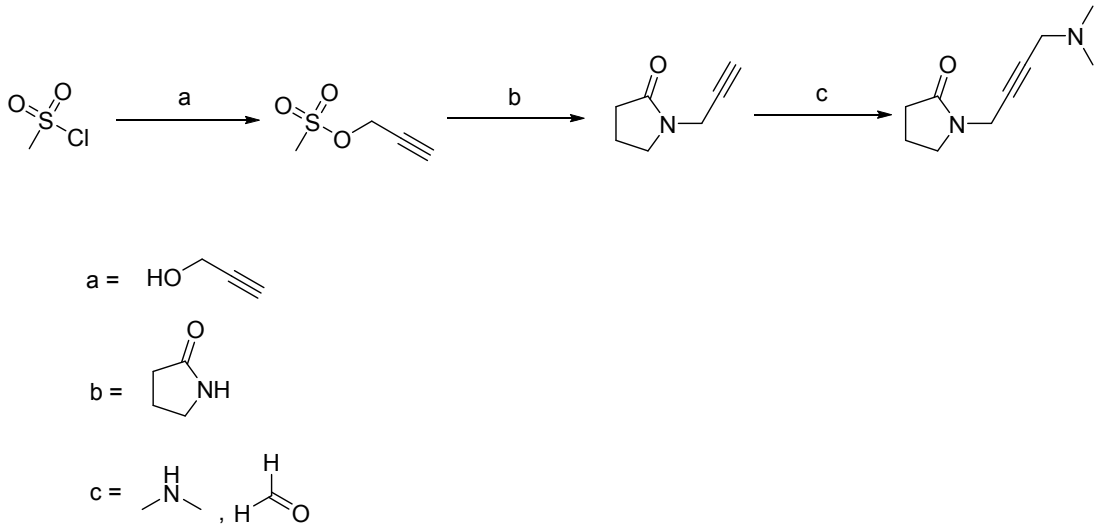
^{1}H NMR (400 MHz, DMSO) δ 9.88 (t, *J* = 5.7 Hz, 1H), 9.09 (s, 1H), 7.99 (dd, *J* = 9.1, 3.1 Hz, 1H), 7.85 (dd, *J* = 9.4, 4.3 Hz, 1H), 7.71 – 7.63 (m, 1H), 7.40 – 7.26 (m, 3H), 7.26 – 7.18 (m, 2H), 5.81 (s, 2H), 3.54 (t, *J* = 6.7 Hz, 2H), 3.39 – 3.30 (m, 2H), 1.89 – 1.75 (m, 2H), 1.61 – 1.51 (m, 2H), 1.49 – 1.31 (m, 4H).

Entry	**PP-5-1**	**48% HBr in H_2O**	**conc. H_2SO_4**	**Yield of PP-5-2**
1	0.24 g; (0.61 mmol)	0.66 ml; (12.11 mmol)	0.14 ml; (2.59 mmol)	79.6 % (0.22 g)

6.3 Synthesis of oxotremorine-M derivate

In the last part of the experimental part of my diploma thesis synthesis of oxotremorine-M derivative is described.

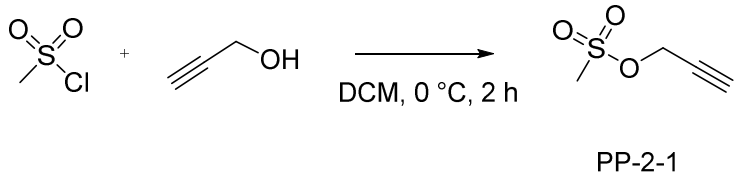
6.3.1 Approach 1 via Mannich reaction – Attempt 1



Scheme 4: General scheme of approach 1 via Manich reaction.

6.3.1.1 Synthesis of propargyl methanesulfonate

Reaction



PP-2-1

Reactants

1. methanesulfonyl chloride
    a. ME = 1.1; M_r = 114.56 g/mol, ρ = 1.48 g/ml
2. 2-propyn-1-ol
    a. ME = 1.0; M_r = 56.06 g/mol, ρ = 0.97 g/ml
3. triethylamine
    a. ME = 1.1; M_r = 101.19 g/mol, ρ = 0.73 g/ml
4. dry dichloromethane
    a. solvent

Approach

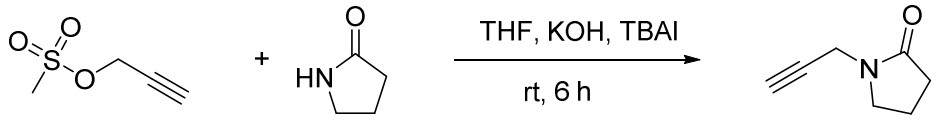
The reaction was performed under the Argon atmosphere. 2-Propyn-1-ol was inserted in to the flask together with dry DCM. The mixture was cooled to 0 °C. Then TEA was added and the mixture was cooled again to 0 °C. After that methansulfonyl chloride was added dropwise. Then the mixture was stirred at 0 °C for 2 hours. Formed mixture was extracted with water, organic phase was dried with sodium sulfate, filtered and evaporated to gain yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.79 (d, *J* = 2.5 Hz, 2H), 3.07 (s, 3H), 2.64 (t, *J* = 2.5 Hz, 1H).

Entry	**Methanesulfonyl chloride**	**2-Propyn-1-ol**	**TEA**	**Yield of PP-2-1**
1	3.38 ml; (44.0 mmol)	2.31 ml; (40.0 mmol)	6.10 ml; (44.0 mmol)	72 % (3.86 g)
2	20.44 ml; (0.26 mol)	13.87 ml; (0.24 mol)	36.59 ml; (0.26 mol)	79.5 % (25.59 g)

6.3.1.2 Synthesis of *N*-propargyl-2-pyrrolidinone

Reaction



PP-2-1 PP-2-2

Reactants

1. propargyl methanesulfonate (**PP-2-1**)
    a. ME = 1.0; M_r = 134.15 g/mol
2. 2-pyrrolidinone
    a. ME = 1.1; M_r = 85.11 g/mol, ρ = 1.11 g/ml
3. tetrabutylammonium iodide
    a. ME = 0.2; M_r = 369.38 g/mol
4. potassium hydroxide
    a. ME = 1.3; M_r = 56.11 g/mol
5. dry tetrahydrofuran
    a. solvent

Approach

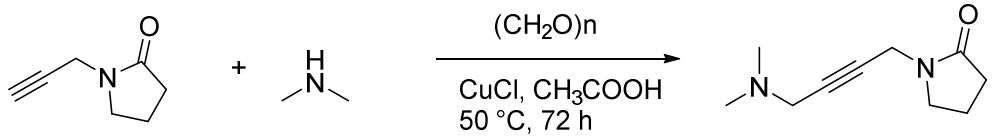
The reaction was prepared under Argon. KOH and TBAI was inserted in to the flask and then dry THF was added. The mixture was stirred for 30 minutes at room temperature. After that the mixture was cooled to 0 °C and 2-pyrrolidinone and propargyl methanesulfonate ester were added dropwise (PP-2-1). Then the mixture was stirred for 6 hours at room temperature. Formed mixture was filtered, washed with diethyl ether and evaporated to gain yellow oil. TLC control was performed and for the detection of spots was used Dragendorff reagent (eluent: EA/PE = 7/3 $Rf_{PP\text{-}2\text{-}2}$ = 0.57).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.04 (d, *J* = 2.5 Hz, 2H), 3.48 – 3.37 (m, 2H), 2.36 – 2.28 (m, 2H), 2.16 (t, *J* = 2.5 Hz, 1H), 2.05 – 1.94 (m, 2H).

Entry	PP-2-1	2-Pyrrolidinone	TBAI	KOH	Yield of PP-2-2
1	4.43 g; (33.0 mmol)	2.76 ml; (36.0 mmol)	2.21 g; (6.0 mmol)	2.37 g; (0.04 mol)	4.3 % (0.17 g)
2	24.78 g; (0.18 mol)	15.33 ml; (0.20 mol)	13.29 g; (0.04 mol)	13.13 g; (0.23 mol)	10.3 % (2.28 g)

6.3.1.3 Synthesis of 1-(4-dimethylamino-but-2-yn-1-yl)-pyrrolidin-2-one

Reaction



PP-2-2 PP-2-3

Reactants

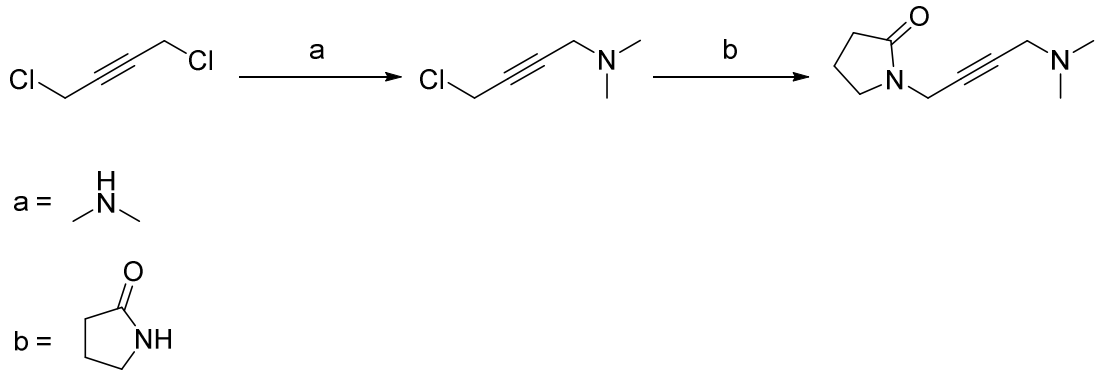
1. *N*-propargyl-2-pyrrolidinone (**PP-2-2**)
    a. ME = 1.0; M_r = 123.16 g/mol
2. dimethylamine
    a. ME = 1.2; M_r = 45.09 g/mol, ρ = 0.68 g/ml
3. paraformaldehyde
    a. ME = 1.25; M_r = 30.03 g/mol
4. acetic acid
    a. ME = 1.1; M_r = 60.05 g/mol, ρ = 1.05 g/ml
5. copper(I) chloride
    a. catalyst
6. dry dioxane
    a. solvent

Approach

The reaction was made under the Argon atmosphere. AcOH and CuCl were added to the suspension of dry dioxan and paraformaldehyd. After that *N*-propargyl-2-pyrrolidinone (PP-2-2) was inserted and dimethylamin was added dropwise. The mixture was stirred at 50 °C for 72 hours. Then solvent was evaporated, the residue dissolved in water, which was acidified with HCl and extracted to diethyl ether. In the next step water phase was neutralized with K_2CO_3 and extracted with DCM. The organic phase was dried with Na_2SO_4 and evaporated. The desired product wasn't detected.

Entry	PP-2-2	Dimethylamine	Paraformaldehyde	AcOH	Yield of PP-2-3
1	0.15g; (1.20 mmol)	0.10 ml; (1.44 mmol)	0.05g; (1.5 mmol)	74 µl; (1.33 mmol)	-

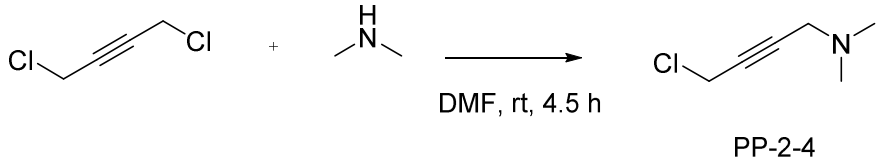
6.3.2 Approach 2



Scheme 5: General scheme of approach 2.

6.3.2.1 Synthesis of 4-chloro-*N*,*N*-dimethylbut-2-yn-1-amine

Reaction



Reactants

1. 1,4-dichloro-2-butyne
    a. ME = 1.11; M_r = 122.98 g/mol, ρ = 1.26 g/ml
2. dimethylamine
    a. ME = 1.2; M_r = 45.09 g/mol, ρ = 0.68 g/ml
3. dry dimethylformamide
    a. solvent

Approach

The reaction was made under the Argon atmosphere. 1,4-Dichloro-2-butyne was dissolved in dry DMF and at room temperature dimethylamin was added dropwise. The mixture was stirred at room temperature for 4.5 hours. After that the solvent was evaporated, the residue dissolved in water, which was acidified with HCl and extracted to diethyl ether. Then the water phase was neutralize with K_2CO_3 and extracted with DCM. The organic phase was dried with Na_2SO_4 and evaporated. The product wasn't detected.

Entry	1,4-Dichloro-2-butyne	Dimethylamine	Yield of PP-2-4	Note
1	3.22 g; 0.026 mol)	1.56 ml; (0.023 mol)	-	*
2	3.22 g; (0.026 mol)	1.56 ml; (0.023 mol)	-	**

*According NMR spectra we suggested that dimmer linked across nitrogen from dimethylamin was performed.

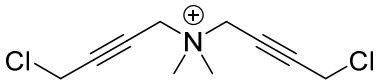


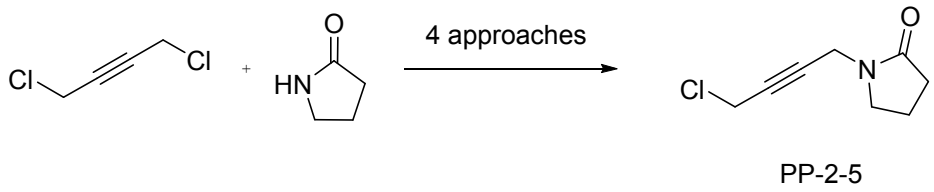
Fig. 9: Structure of isolated dimmer.

** Reaction time was decreased to 3 hours.

6.3.2.2 Synthesis of 1-(4-chlorobut-2-yn-1-yl)pyrrolidin-2-one

Once we discovered that in previous two reactions the dimmer was formed, we decided to firstly connect alkynyl chain to 2-pyrrolidinon and reaction with dimethylamin performed after that, what we assumed should solve the problem with dimmer.

Reaction



PP-2-5

Reactants A

1. 1,4-dichloro-2-butyne
    a. ME = 1.0; M_r = 122.98 g/mol, ρ = 1.26 g/ml
2. 2-pyrrolidinone
    a. ME = 1.0; M_r = 85.11 g/mol, ρ = 1.11 g/ml
3. potassium carbonate
    a. ME = 2.0; M_r = 138.21 g/mol
4. dimethylformamide
    a. solvent

Conditions – temperature: 100 °C; reaction time: 5 hours

Reactants B

1. 1,4-dichloro-2-butyne
    a. ME = 1.0; M_r = 122.98 g/mol, ρ = 1.26 g/ml
2. 2-pyrrolidinone
    a. ME = 1.0; M_r = 85.11 g/mol, ρ = 1.11 g/ml
3. natrium hydride
    a. ME = 1.0; M_r = 24.0 g/mol
4. tetrahydrofuran
    a. solvent

Conditions – temperature: reflux; reaction time: 5 hours

Reactants C

1. 1,4-dichloro-2-butyne
    a. ME = 1.0; M_r = 122.98 g/mol, ρ = 1.26 g/ml
2. 2-pyrrolidinone
    a. ME = 1.0; M_r = 85.11 g/mol, ρ = 1.11 g/ml
3. potassium carbonate
    a. ME = 2.0; M_r = 138.21 g/mol
4. acetonitrile
    a. solvent

Conditions – temperature: reflux; reaction time: 24 hours

Reactants D

1. 1,4-dichloro-2-butyne
    a. ME = 1.0; M_r = 122.98 g/mol, ρ = 1.26 g/ml
2. 2-pyrrolidinone
    a. ME = 1.0; M_r = 85.11 g/mol, ρ = 1.11 g/ml
3. triethylamin
    a. ME = 2.0; M_r = 101.19 g/mol
4. patassium iodide
    a. ME = 1.0; M_r = 166.0 g/mol
5. acetonitrile
    a. solvent

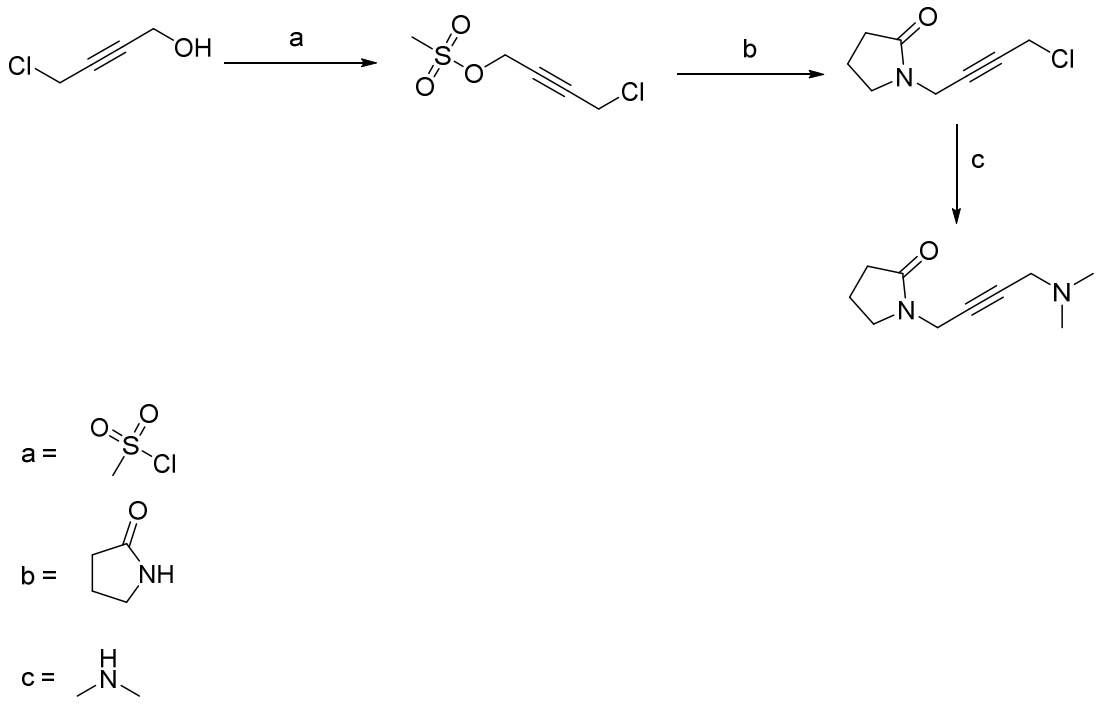
Conditions – temperature: reflux; reaction time: 24 hours

Approach

At all circumstances 2-pyrrolidinon was inserted into the flask with solvent and appropriate base. Formed mixture was always stirred at least for 1 hour (except attempt A, where it was only 20 minutes) and after that time 1,4-dichloro-2-butyne was added dropwise. Then the reactions were performed at described conditions. After cooling was the mixture extracted with DCM and water. The organic phase was dried with Na_2SO_4, filtered and the solvents were evaporated.

Attempt	1,4-Dichloro-2-butyne	2-Pyrrolidinone	base	KI	Yield of PP-2-5
A	0.24 ml; (2.44 mmol)	0.14 ml; (2.44 mmol)	0.67 g; (4.88 mmol)	-	-
B	0.24 ml; (2.44 mmol)	0.14 ml; (2.44 mmol)	0.06 g; (2.44 mmol)	-	-
C	0.24 ml; (2.44 mmol)	0.14 ml; (2.44 mmol)	0.67 g; (4.88 mmol)	-	-
D	0.24 ml; (2.44 mmol)	0.14 ml; (2.44 mmol)	0.49 g; (4.88 mmol)	0.41 g; (2.44 mmol)	-

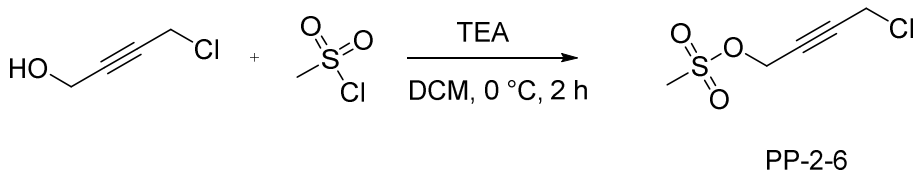
6.3.3 Approach 3



Scheme 6: General scheme of the approach 3.

6.3.3.1 Synthesis of 4-chlorobut-2-yn-1-yl methanesulfonate

Reaction



Reactants

1. 4-chloro-2-butyn-1-ol
    a. ME = 1.0; M_r = 104.53 g/mol, ρ = 1.21 g/ml
2. methanesulfonyl chloride
    a. ME = 1.1; M_r = 114.56 g/mol, ρ = 1.48 g/ml
3. triethylamine
    a. ME = 1.1; M_r = 101.19 g/mol, ρ = 0.73 g/ml
4. dry dichloromethane
    a. solvent

Approach

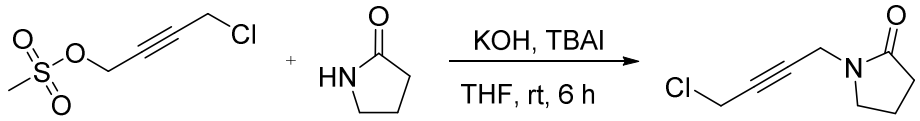
The reaction was made under the Argon. 4-Chloro-2-butyn-1-ol and dry DCM were inserted in to the flask and cooled to 0 °C. Then TEA was added and the mixture was again cooled to 0 °C and after this methanesulfonyl chloride was added dropwise. The mixture was stirred at 0 °C for 2 hours. Then the mixture was extracted with water, organic phase was dried with Na_2SO_4, filtered and evaporated to gain white oil.

^{1}H NMR (400 MHz, DMSO) δ 5.05 (t, *J* = 2.0 Hz, 2H), 4.57 (s, 2H), 3.26 (s, 3H).

Entry	**4-Chloro-2-butyn-1-ol**	**Methanesulfonyl chloride**	**TEA**	**Yield of PP-2-6**
1	0.25 ml; (2.87 mmol)	0.24 ml; (3.16 mmol)	0.44 ml; (3.16 mmol)	87.2% (0.46 g)

6.3.3.2 Synthesis of 1-(4-chlorobut-2-yn-1-yl)pyrrolidin-2-one

Reaction



PP-2-6 PP-2-7

Reactants

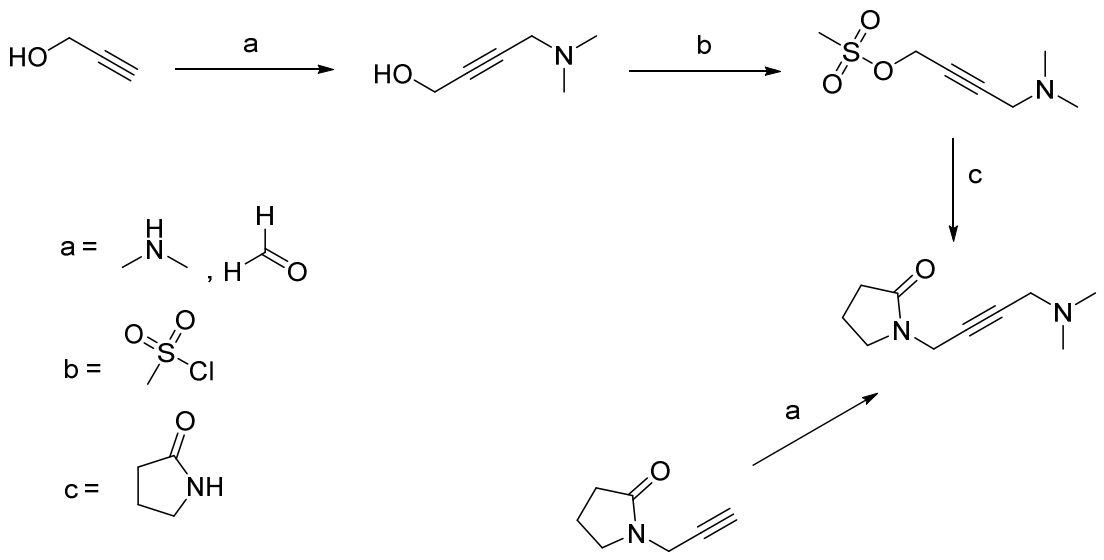
1. 4-chlorobut-2-yn-1-yl methanesulfonate (**PP-2-6**)
    a. ME = 1.0; M_r = 182.62 g/mol
2. 2-pyrrolidinone
    a. ME = 1.0; M_r = 85.11 g/mol, ρ = 1.11 g/ml
3. tetrabutylammonium iodide
    a. ME = 0.2; M_r = 369.38 g/mol
4. potassium hydroxide
    a. ME = 1.3; M_r = 56.11 g/mol
5. dry tetrahydrofuran
    a. solvent

Approach

The reaction was made under the Argon atmosphere. KOH and TBAI were inserted in to the flask, then dry THF was added and the mixture was stirred for 30 minutes at room temperature. After that the mixture was cooled to 0 °C and 2-pyrrolidinone and 4-chlorobut-2-yn-1-yl methanesulfonate were added dropwise (PP-2-5). The mixture was stirred for 6 hours at room temperature. Reaction didn't work so more base was added (3 fold amount) and the mixture was stirred for next 20 hours. The mixture was filtered and washed with diethyl ether. Product wasn't detected.

Entry	**PP-2-6**	**2-Pyrrolidinone**	**TBAI**	**KOH**	**Yield of PP-2-7**
1	0.39 g; (2.15 mmol)	0.16 ml; (2.15 mmol)	0.16 g; (0.43 mmol)	0.16 g; (2.80 mmol)	-

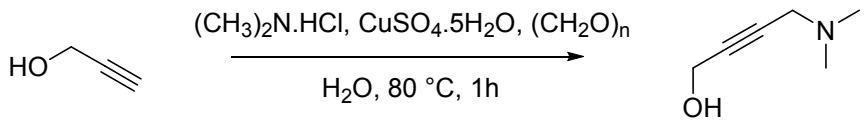
6.3.4 Approach 4 via Mannich reaction – Attempt 2



Scheme 7: General scheme of the approach 4 via Mannich reaction.

6.3.4.1 Synthesis of 4-(dimethylamino)-2-butyn-1-ol

Reaction



PP-2-8

Reactants

1. 2-propyn-1-ol
    a. ME = 1.0; M_r = 56.06 g/mol, ρ = 0.97 g/ml
2. paraformaldehyde
    a. ME = 1.7; M_r = 30.03 g/mol
3. dimethylamine hydrochloride
    a. ME = 1.2; M_r = 81.54 g
4. copper(II) sulfate
    a. catalyst
5. water
    a. solvent

Approach

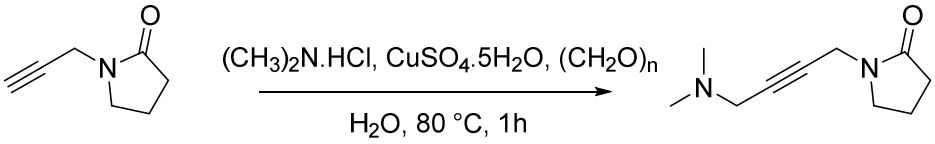
Dimethylamine hydrochloride was dissolved in water and NaOH was added to reach pH = 9. Then paraformaldehyd, 2-propyn-1-ol and $CuSO_4$ were added. After that NaOH was added to the mixture to reach pH = 8. The mixture was stirred at 80 °C for 1 hour. Then 25% aqueous ammonia solution was added and the reaction mixture was continuously extracted to diethyl ether for 2 days. Organic phase was dried with Na_2SO_4, filtered and evaporate to get yellow-brown oil.

^{1}H NMR (400 MHz, DMSO) δ 5.11 (t, *J* = 5.9 Hz, 1H), 4.09 (dt, *J* = 5.9, 2.0 Hz, 2H), 3.21 (t, *J* = 2.0 Hz, 2H), 2.17 (s, 6H).

Entry	**2-Propyn-1-ol**	**Paraformaldehyde**	**Dimethylamine hydrochloride**	**Yield of PP-2-8**
1	5.27 ml; (0.09 mol)	4.50 g; (0.15 mol)	8.72 g; (0.11 mol)	31.9 % (3.22 g)

6.3.4.2 Synthesis of oxotremorine-M derivative

Reaction



PP-2-2 PP-2-9

Reactants

1. *N*-propargyl-2-pyrrolidinone (**PP-2-2**)
    a. ME = 1.0; M_r = 123.16 g/mol
2. dimethylamine hydrochloride
    a. ME = 1.2; M_r = 45.09 g/mol, ρ = 0.68 g/ml
3. paraformaldehyde
    a. ME = 1.7; M_r = 30.03 g/mol
4. copper(II) sulfate
    a. catalyst
5. water
    a. solvent

Approach

Dimethylamine hydrochloride was dissolved in water and NaOH was added to reach pH = 9. Then paraformaldehyd, PP-2-2 and $CuSO_4$ were added. After that more NaOH was added to the mixture to reach pH = 8. The mixture was stirred at 80 °C for 1 hour. Then 25% aqueous ammonia solution was added and the reaction mixture was continuously extracted to diethyl ether for 2 days. Organic phase was dried with Na_2SO_4, filtered and evaporate. The reaction did not work, only starting material (PP-2-2) was obtained.

Entry	**PP-2-2**	**Paraformaldehyde**	**Dimethylamine hydrochloride**	**Yield of PP-2-9**
1	2.36 g; (0.02 mol)	1.02 g; (0.03 mol)	1.94 g; (0.02 mol)	-

7 Discussion and conclusion

At Julius-Maximilians-universität Würzburg I acquired knowledge about quinolones chemistry, which I used in the synthesis of fluorinated derivatives of BQCA. These derivatives were prepared via Gould-Jacobs reaction. In the first attempt of the second step of this reaction I had problems with precipitation of the final product. Formed crystals were very tiny and also the amount was to low. While searching for the reason why the reaction did not work it was discovered that even if the power of microwave irradiation was set up properly, the temperature of the reaction was low. In the next approach more passive heaters were used and immediately after cooling the mixture started to precipitate. The measurement of the NMR spectra of this product (PP-1-2) was impossible due to its insolubility. After one hour of reflux with methanol the crystalline powder dissolved, but it was more likely due to hydrolysis of ester bond then dissolving of PP-1-2.

In the reactions, where the spacers were connected to fluorinated derivatives of BCQA via utilizing carboxylic function groups (PP-3-1, PP-4-1 and PP-5-1), it was really important to secure accurate temperature. If the temperature was lower than how it was given, the compounds did not melt down. And if the temperature overstep given temperature aminoalcohols started to evaporate and condensate in the condenser. To secure properly cleanliness of the products, dual recrystallization was performed. Firstly with methanol and secondly with ethanol. In the next step – substitution of hydroxy functional group with bromine (PP-3-2, PP-4-2 and PP-5-2) – increased caution was needed due to quantity of the bromine vapours.

Further I got familiar with the system and mechanism of Mannich reaction, which I also performed. In the first attempt, 1-(4-dimethylamino-but-2-yn-1-yl)-pyrrolidin-2-one (PP-2-3) was not isolated and due to previous experiences of my supervisor in Würzburg with Mannich reactions, we decided to try another approach. The decision to choose another approach was also made on the assumption, that even if the next attempt of Mannich reaction would be successful, there is the risk that the yield will not be sufficient. Therefore we decided to circumvent the Mannich reaction and prepare 1-(4-dimethylamino-but-2-yn-1-yl)-pyrrolidin-2-one (PP-2-3) in way how it

was described in chapters 6.3.2 and 6.3.3. Unfortunately also in this approach we were not successful. In the synthesis of 4-chloro-*N*,*N*-dimethylbut-2-yn-1-amine we observed the formation of the dimmer (Fig. 9). We tried to decrease reaction time but it did not help to gain desired structure. Maybe increase of molar amount of dimethylamine would help. In the preparation of 1-(4-chlorobut-2-yn-1-yl)pyrrolidin-2-one we were not able to attach the propylene chain to 2-pyrrolidin-2-one. Despite we tried several bases it seems that they were not strong enough to deprotonate hydrogen from nitrogen of 2-pyrrolidin-2-one. And even if we prepared 4-chlorobut-2-yn-1-yl methanesulfonate (PP-2-6) and tried to attach it to 2-pyrrolidin-2-one via same way like in synthesis PP-2-2, it did not result in the desired structure. In my last attempt to prepare derivative of oxotremorine-M, preparation via Mannich reaction was chosen, but with modified reaction conditions. Firstly 4-(dimethylamino)-2-butyn-1-ol (PP-2-8) was prepared. This reaction was successful and we wanted to continue in the same way how *N*-propargyl-2-pyrrolidinone (PP-2-2) was prepared. Unfortunately, due to lack of time, we decided to do Mannich reaction using these conditions with *N*-propagryl-2-pyrrolidinone (PP-2-2). Unfortunately, preparation of 1-(4-dimethylamino-but-2-yn-1-yl)-pyrrolidin-2-one in that way was unsuccessful.

In summary, I prepared three different derivatives of fluorinated BCQA linked with spacers with variety length. Derivative of oxotremorine-M was prepared according to available literature. Unfortunately, reproduction of the reaction protocol was unsuccessful. Three more approaches were chosen to gain the desired compound, but none of them led to positive result. Regrettably, I was not able to prepare desired structures. In the next attempts the preparation 1-(4-dimethylamino-but-2-yn-1-yl)-pyrrolidin-2-one in the way as shown in Scheme 7 should be tried or the proper conditions for Mannich reaction with structures like *N*-propagryl-2-pyrrolidinone (PP-2-2) should be found.

8 List of abbreviations

7TMRs	seven transmembrane domains
ACh	acetylcholine
AChEI	inhibitors of acetylcholinesterase
AcOH	acetic acid
AD	Alzheimer's disease
APOE	Apolipoprotein E
AP	Alzheimer's plaque
APP	amyloid precursor protein
BCQA	benzyl quinolone carboxylic acid
CNS	central nervous system
DAG	1,2-diacylglycerol
DCM	dichlormethan
DMF	*N*,*N*-dimethylformamide
EA	ethyl acetate
GPCRs	G protein-coupled receptors
IP3	inositol 1,4,5-triphosphate
mAChRs	muscarinic acetylcholine receptors
PE	petrolether
PLC	phospholipase C
TBAI	tetrabutylamonium iodide
TEA	triethylamine
THF	tetrahydrofuran

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