TECHNISCHE UNIVERSITÄT WIEN
Vienna University of Technology

DIPLOMARBEIT

SYNTHESIS OF PYRAZOLOQUINOLINONES AND IMIDAZOQUINOLINES AS POTENTIAL $GABA_A$ RECEPTOR LIGANDS

ausgeführt zum Zwecke der Erlangung des akademischen Grades eines Diplomingenieurs der technischen Wissenschaften unter der Leitung von

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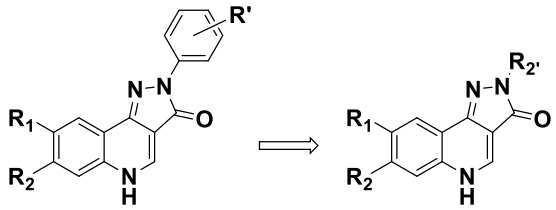
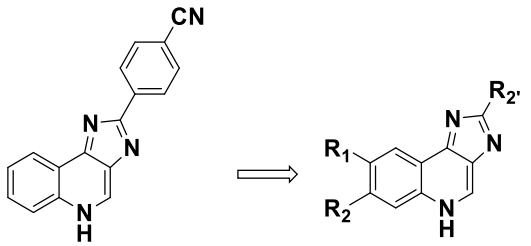
Abstract

$GABA_A$ receptors are the major inhibitory neurotransmitter receptors in the central nervous system (CNS). They are the target of many clinically important drugs, like benzodiazepines. Different binding sites have already been discovered on the receptor. Ligands bind specifically to the receptor on these binding sites, e.g. the endogenous ligand γ-aminobutyric acid (GABA) or above mentioned benzodiazepines (BZ). Recent studies have shown that pyrazolo[4,3-*c*]quinolinones bind to the BZ binding site, but elicit their activity from another interface.



To increase the selectivity of this substance class towards the novel binding site, structural modifications were carried out in a small library synthesis. In a first approach, the substituent ($R_{2'}$) on the pyrazolo system was replaced by smaller and more hydrophilic substituents. The role of the substituents (R_1 & R_2) on the quinoline system was also investigated.

Another promising compound, which also showed activity on the $GABA_A$ receptor was LAU 197, featuring an imidazo[4,5-*c*]quinoline scaffold.

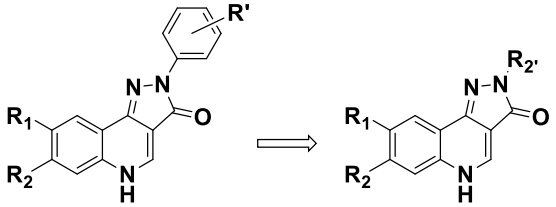


To expand the library of substances, the introduction of various substituents was planned. After finding a suitable synthetic route, which tolerates different substitution patterns, the influence on the biological activity of diverse substituents (R_1 & R_2) on the quinoline and as well on the imidazo system ($R_{2'}$) were of interest.

The synthesis of both pyrazolo[4,3-*c*]quinolinones and imidazo[4,5-*c*]quinolines was successful, yielding 14 different substances, which have been submitted to biological testing. Preliminary results of tested pyrazolo[4,3-*c*]quinolinones are already available and show biological activity on $GABA_A$ receptors.

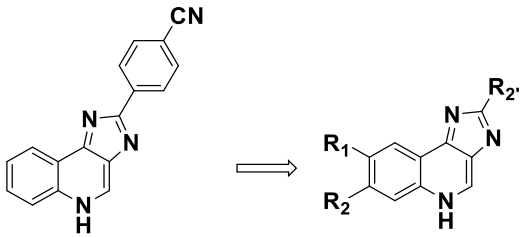
Kurzfassung

$GABA_A$ Rezeptoren sind die am häufigsten vorkommenden inhibitorischen Rezeptoren im zentralen Nervensystem (ZNS). Sie sind das Ziel von vielen klinisch wichtigen Medikamenten, z.B. Benzodiazepinen. Am Rezeptor wurden bereits verschiedene Bindungsstellen entdeckt. Es zeigt sich, dass Liganden an einer spezifischen Stelle an dem Rezeptor binden, z.B. der endogene Neurotransmitter γ-Aminobuttersäure (GABA) oder die oben erwähnten Benzodiazepine (BZ). Neueste Studien zeigen, dass Pyrazolo[4,3-*c*]chinolinone an der BZ Bindungsstelle binden, aber ihre Aktivität über eine andere Schnittstelle entfalten.



Um die Selektivität dieser Substanzklasse zu der kürzlich entdeckten Bindungstelle zu erhöhen, wurden strukturelle Modifikationen in einer kleinen Bibliothekssynthese ausgeführt. Zuerst wurde der Substituent ($R_{2'}$) am Pyrazol durch einen sterisch weniger anspruchsvollen und hydrophileren Substituenten ersetzt. Die Rolle der Substituenten (R_1 & R_2) am Chinolin wurde ebenfalls untersucht.

Eine weitere Verbindung, die ebenfalls Aktivität am $GABA_A$ Rezeptor zeigte, war LAU 197, ein Imidazo[4,5-*c*]chinolin.



LAU 197

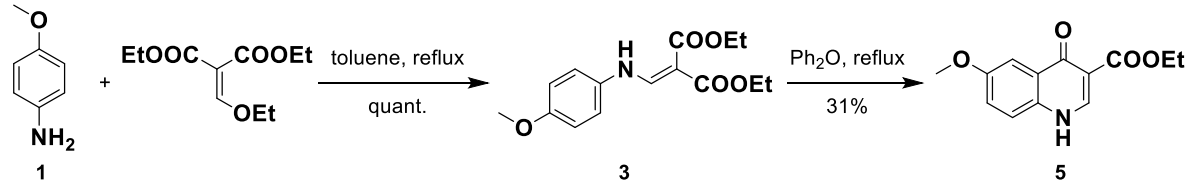
Um die Substanzbibliothek zu erweitern, war die Einführung von Substituenten geplant. Nachdem ein geeigneter Syntheseweg entwickelt wurde, der das Substitutionsmuster toleriert, wurde der Effekt von unterschiedlichen Substituenten auf die biologische Aktivität untersucht. Für diesen Zweck wurden verschiedene Substituenten sowohl am Chinolin (R_1 & R_2) als auch am Imidazol ($R_{2'}$) eingeführt.

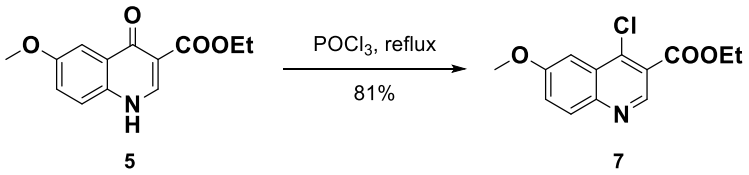
Die Synthese von beiden, Pyrazolo[4,3-*c*]chinolinonen und Imidazo[4,5-*c*]chinolinen wurde erfolgreich durchgeführt und es wurden 14 unterschiedliche Verbindungen erhalten. Diese werden bereits auf ihre biologische Aktivität getestet, welche im Fall der Pyrazolo[4,3-*c*]quinolinonen bereits gezeigt werden konnte.

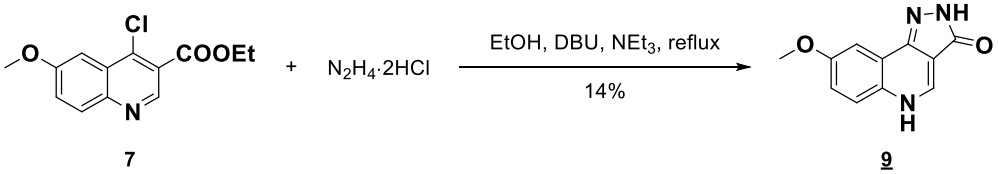
A Synthetic schemes

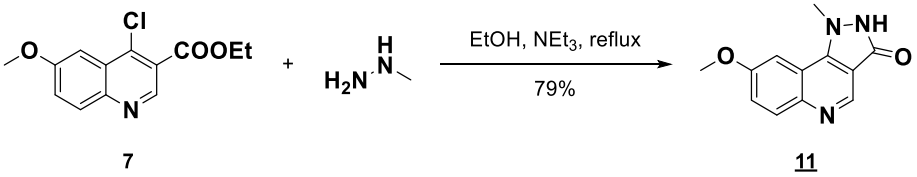
All compounds prepared or used as starting materials in this thesis are numbered in bold Arabic numerals. Compounds unknown to the literature are additionally underscored.

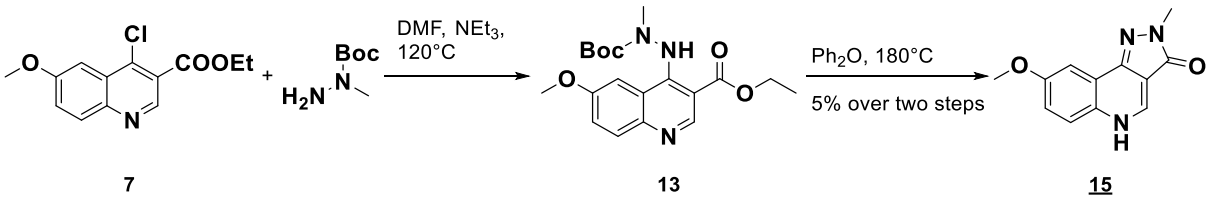
Synthesis of methoxy substituted pyrazolo[4,3-*c*]quinolinones

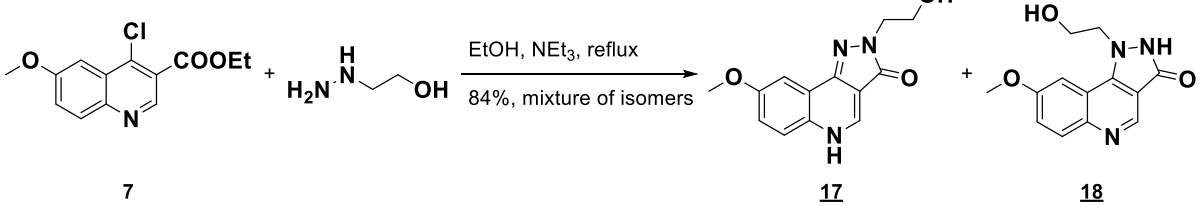




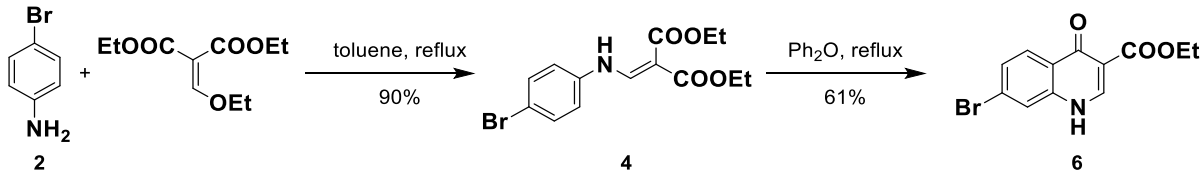








Synthesis of bromo substituted pyrazolo[4,3-*c*]quinolinones



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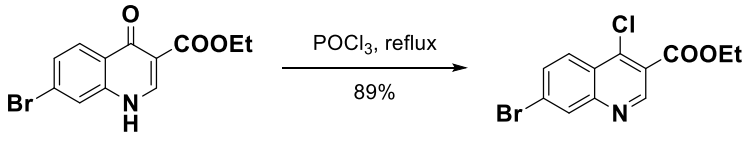

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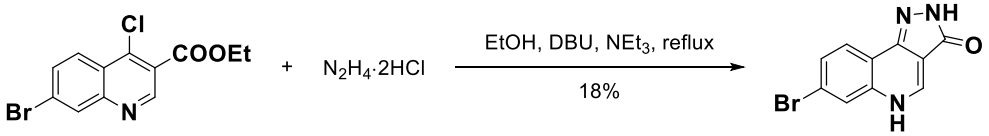

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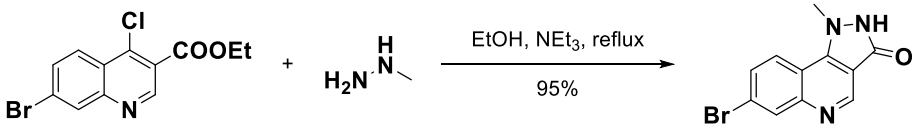

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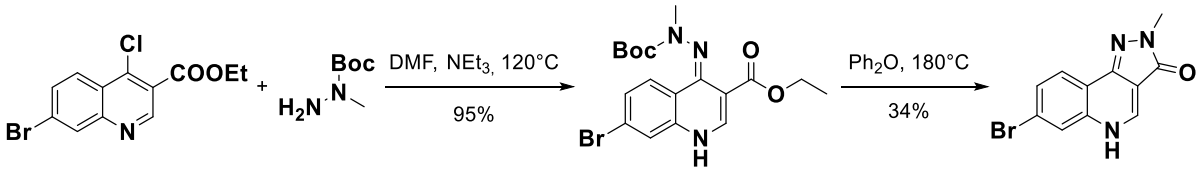

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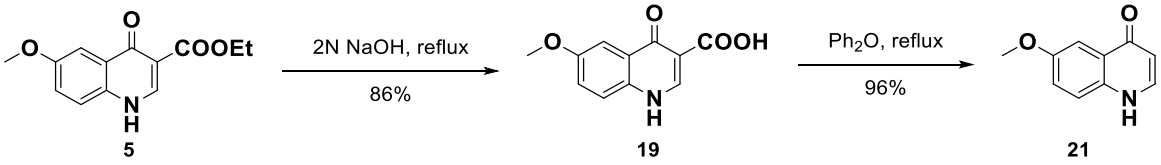
Synthesis of methoxy substituted imidazo[4,5-*c*]quinolines



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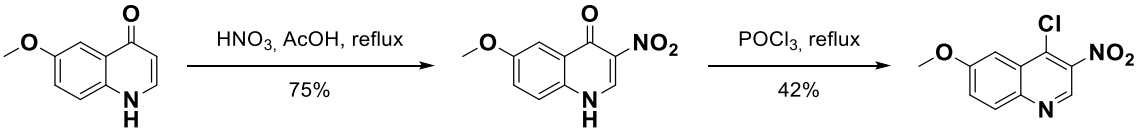

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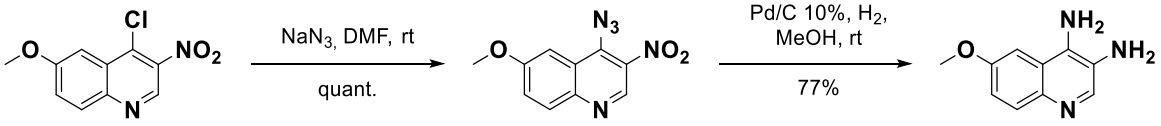

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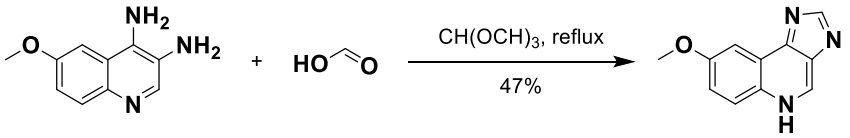

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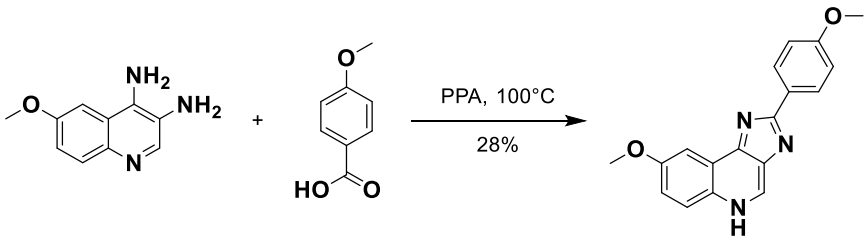

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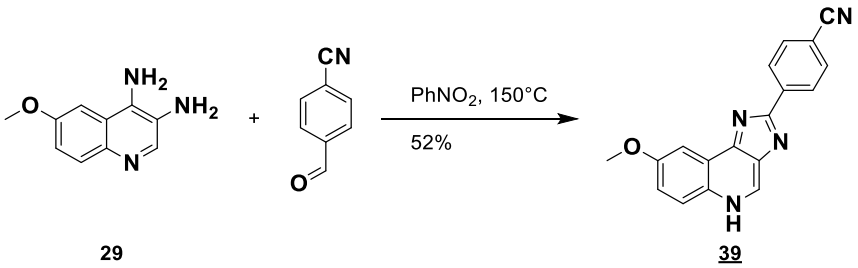

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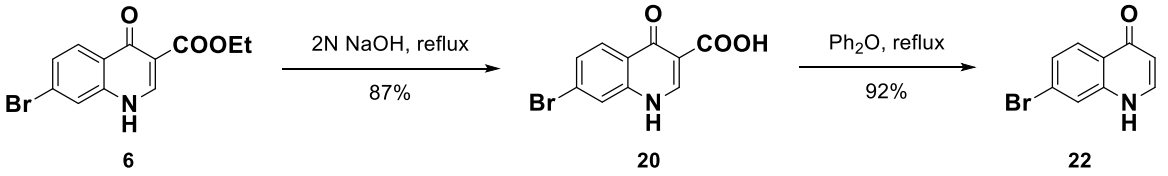
Synthesis of bromo substituted imidazo[4,5-*c*]quinolines



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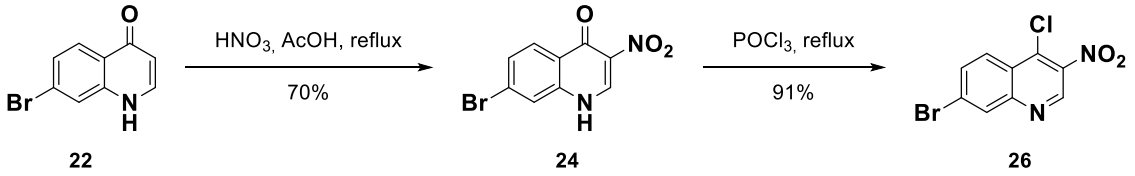

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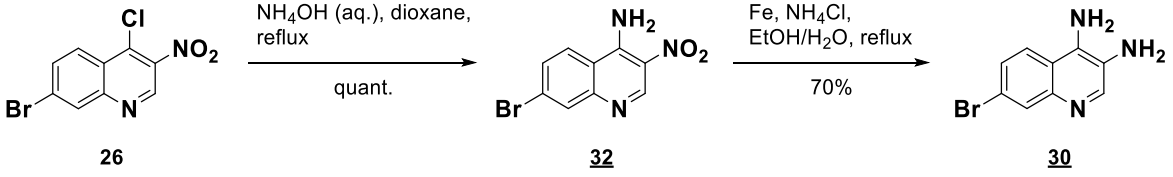

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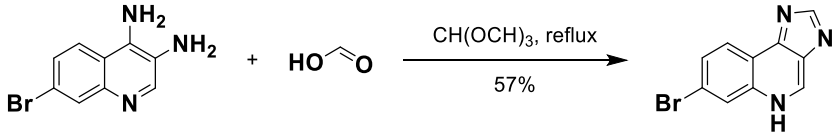

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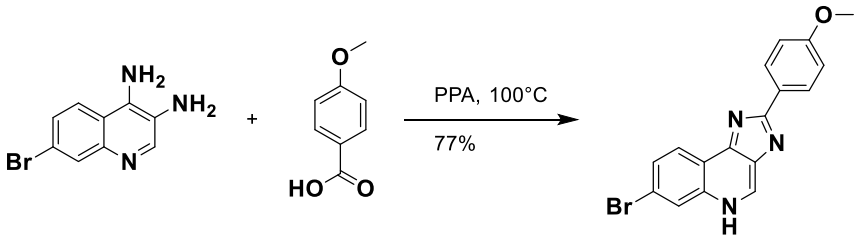

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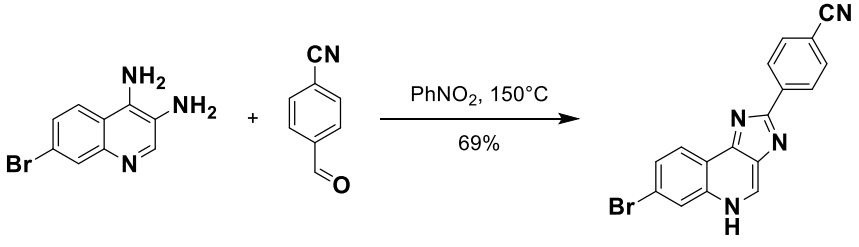

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B Introduction

B I Structure and function of the $GABA_A$ receptor

B I.1 Function

γ-Aminobutyric acid (GABA), the major inhibitory neurotransmitter in the mammal brain, binds specifically to the $GABA_A$ receptor, giving the receptor its name. The receptor is a transmembrane protein located in the nervous cells in the central nervous system (CNS) of mammals. It plays a major role in the signal transduction in the synapse. In a synapse, a signal is passed in an electrical or chemical way to another neuron. In the case of a chemical synapse, neurotransmitters are released in the presynaptic neuron and trigger an effect in the postsynaptic neuron by binding to a receptor[1]. In this way, a signal can be passed on or, as it is the case of GABAergic receptors, inhibited. The inhibitory effect is caused by conduction of chloride ions through the channel located in the center of the protein. This leads to hyperpolarization of the neuron, which then increases the threshold of the action potential (see Figure 1)[2].

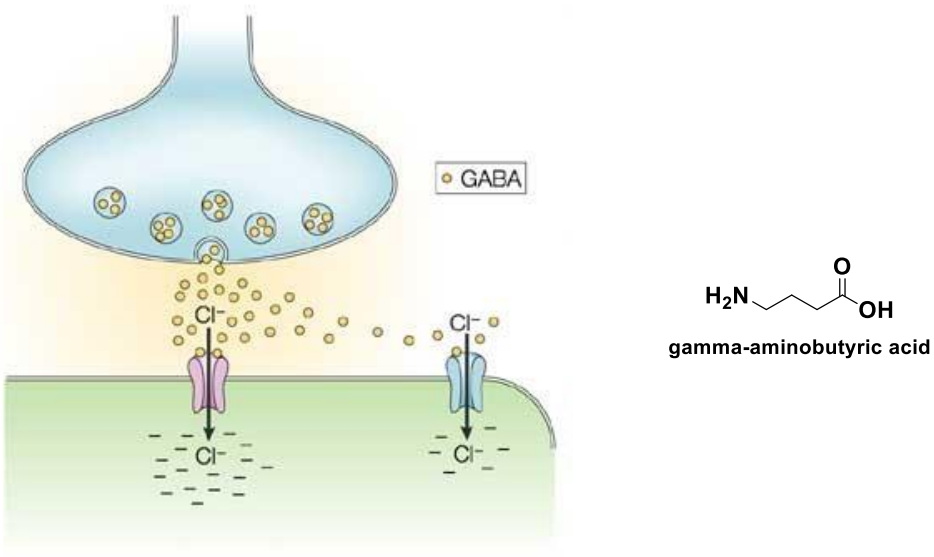


Figure 1 Synaptic cleft with $GABA_A$ receptors[3], structure of GABA

Such inhibitory effects have great medical relevance, since they trigger a sedative or anxiolytic behavior (among others). Drugs, such as benzodiazepines, propofol or muscimol (Figure 2), which target the $GABA_A$ receptor, can either compete with GABA by binding to the same position or they modulate allosterically the protein structure and consequently the biological function[4, 5].

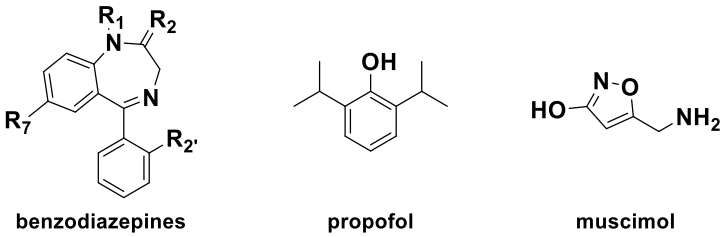


Figure 2 Strucutre of benzodiazepines, propofol and muscimol

B I.2 Structure

The structure of the $GABA_A$ receptor is pentameric, i.e. it consists of five subunits[6] (see Figure 3). Recently, the crystallization of a homopentamer was achieved, proving the structure[7]. As depicted, the subunits form a channel in the middle, where Cl^- can pass through the membrane. In the human brain the $GABA_A$ receptor usually consists of two α, two β and one γ subunit. The γ subunit can also be replaced by a δ, ε, θ or π one. Furthermore, there are more subtypes known of one subunit, e.g. six different subtypes of the α subunit[8]. That implies many different variations of the receptor composition, which all show different selectivity towards target molecules[9].

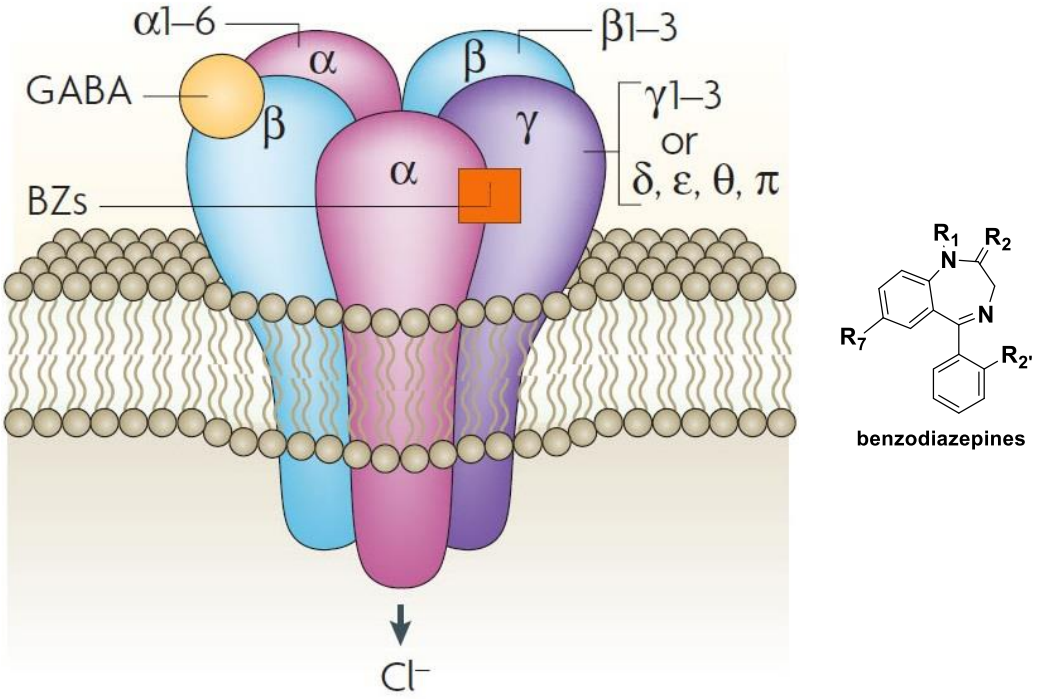


Figure 3 $GABA_A$ receptor structure and general structure of benzodiazepines[8]

The binding sites of GABA and clinically important benzodiazepines (BZ) is in the extracellular domain of the receptor[5]. The subunits of the $GABA_A$ receptor have a + (plus) and – (minus) site, as depicted in Figure 4[10].

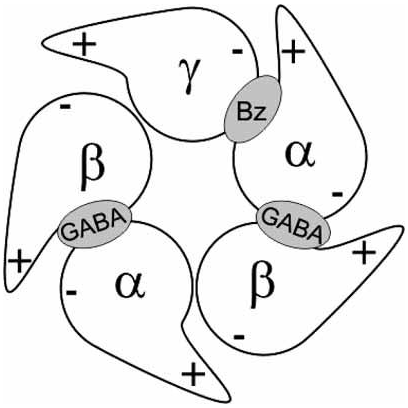


Figure 4 Schematic top view of an aβγ $GABA_A$ receptor[11]

The specific binding sites of GABA and benzodiazepines (BZs) are also shown in Figure 4. GABA binds at the α-β+ interface, whereas the BZ-binding site is located at the α+γ- site[12]. Benzodiazepines are an important class of drugs, which have been used in medicine since the 1960s[13]. BZs allosterically modulate the receptor, which leads to an enhancement of the Cl^- current, causing an anxiolytic, muscle relaxant or sedative effect. However, they show this behavior only after GABA has already bound to its binding site and has activated the receptor[14].

B I.3 Subunit isoforms

The large number of subunits, which have been identified so far, leads to a huge diversity of $GABA_A$ receptors in the mammal brain. There are eight different subunit classes, which further have multiple forms of subtypes: α(1-6), β(1-3), γ(1-3), δ, ε, ρ(1-3), θ and π[9]. In total, this adds up to nineteen heterogeneous subunits, which leads to a large set of possible combinations. The major isoform consists of two α, two β and one γ subunit. The two α subunits are usually of one kind, α1, but examples where one α1 subunit is replaced with α2/3/5 one are known[14]. Hence, the number of theoretically possible subunit compositions is around 150 000[15].

As mentioned above, the binding site of BZ is located at the α/γ interface. It has been shown that the subtype of the α subunit has to be α1/2/3/5 to show a high BZ sensitivity[9]. If α4/6 is present in the receptor, the corresponding protein pentamer will not bind traditional benzodiazepines, like quazepam or non-traditional BZ like zolpidem (see Figure 5). On the other hand, imidazobenzodiazepines like flumazenil (see Figure 5) show high affinity towards receptors containing α4 or α6 subunits. Moreover, to enable binding of BZ to the receptor, a γ subunit has to be present in it. Although the most common form of $GABA_A$ receptors contain at least a γ subunit, there are receptor compositions known, which do not contain any[15].

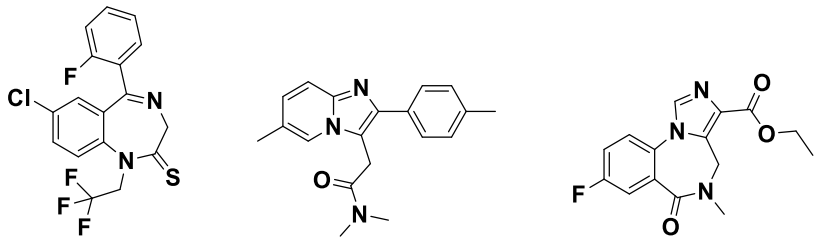


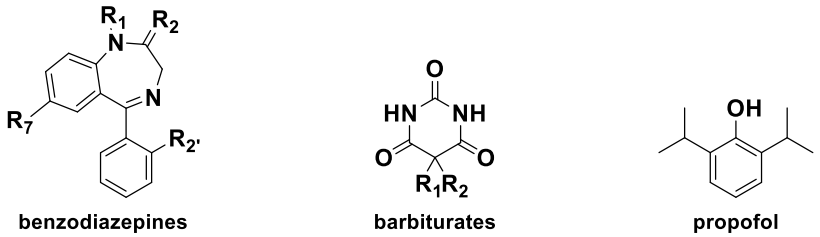
Figure 5 Traditional and non-traditional benzodiazepines quazepam, zolpidem and flumazenil

To conclude, the subunit composition is of great importance for the biological activity of agonists. The potency and efficacy of agonists depend highly on the presence or absence of certain subunits. It has been shown that there is a regional distribution of individual subunits in the mammal brain[15]. On one hand, the most abundant subunit, α1, can be found throughout the brain. On the other hand, subtypes, like the α2 subunit, are less

frequently found all over the brain, but show a higher concentration in the forebrain area. Also, the other subtypes α3/4/5/6 show a higher presence in certain particular areas of the brain. The same case applies to the other subunits β, γ, δ, ε, θ and π. Subunits of type ρ form homopentameres, which are called $GABA_C$ and are only found in the retina[16]. The distribution and localization of individual subunits can be an approach for novel agonists, which are sensitive to a certain subunit and therefore, only interact in a particular area in the brain leading to specific biological responses.

B I.4 Physiologically and clinically relevant compounds

Many drugs target the $GABA_A$ receptor, e.g. benzodiazepines, barbiturates or propofol (see Figure 6). To achieve an interaction with the receptor possible, GABA has to bind to its binding site first, activating the receptor. Afterwards, these compounds bind to a different site and allosterically modulate the receptor, which leads either to an increase in the Cl^- flux (positive allosteric modulation, PAM), to a decrease (negative allosteric modulation, NAM) or to no change at all (silent allosteric modulation, SAM). BZ, the most important class of drugs, are typically PAMs[17]. The triggered effects can be anxiolytic, anticonvulsant, hypnotic, muscle relaxant or sedative[18]. Besides these very useful effects, addictive behavior and withdrawal syndromes can also be observed as unwanted side effects[19].



benzodiazepines barbiturates propofol

Figure 6 Structure of BZs, barbiturates and propofol

B II Alpha/beta binding site

In addition to the binding sites of BZs at the α+γ- interface and GABA at α-β+, a novel binding site has recently been discovered at the α+β- interface[20]. The compound CGS 9895, a pyrazoloquinolinone, binds to the BZ-site, but acts only as a SAM, whereas on the α+β- interface, slightly positive allosteric modulation can be observed (see Figure 7)[21]. Hence, this novel binding site is referred to as CGS-site.

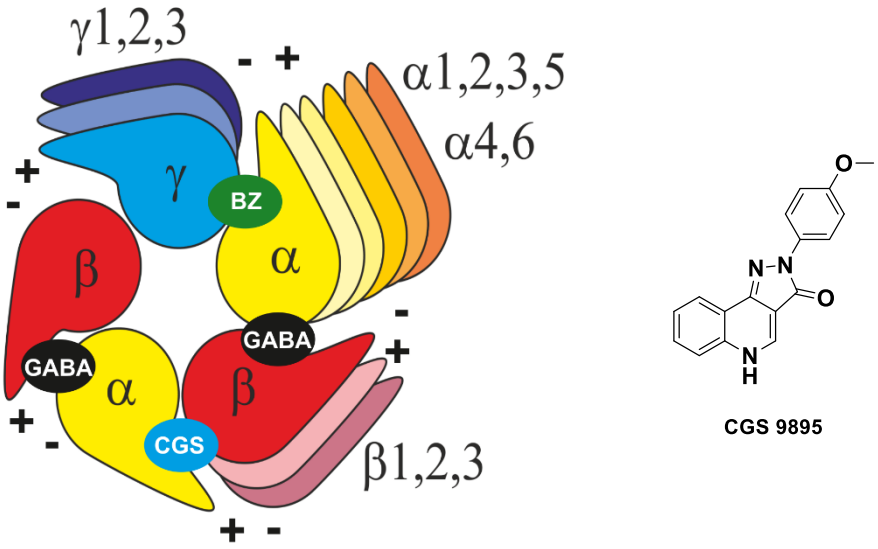


Figure 7 Top view onto the extracellular domain of $GABA_A$ receptor, structure of CGS9895[22]

Compounds that target the α+β- interface in a αβγ receptor should also interact with αβ, αβδ, αβε, αβπ and αβθ receptors, which are not targeted by BZs, because of the lacking γ subunit. Therefore, the range of possible actions should be broader[23]. Further studies by Varagic *et al.*[21] focused on the identification of novel allosteric modulators, which target the α+β- interface. The tested compounds were mostly pyrazoloquinolinones with varying substituents (see Figure 8).

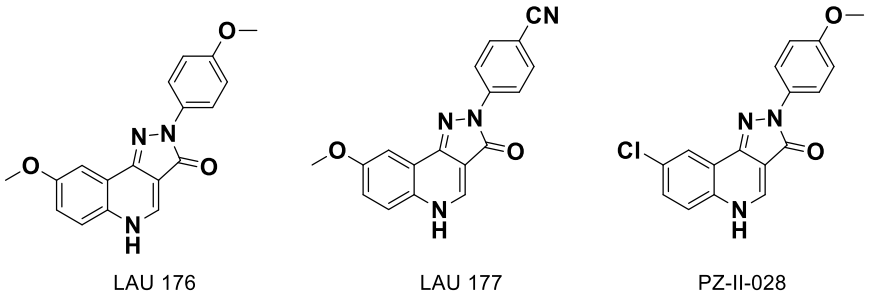


Figure 8 Tested compounds in studies of Varagic et al.[21]

In their studies, Varagic *et al.* could prove that many structural analogues of CGS9895 enhance the Cl^- flow by more than 1000% at a 10 µM concentration. Although these compounds also bind to the BZ-site, their activity mainly results from binding to the novel binding site at α+β-. This indicates that pyrazoloquinolinones can act as silent allosteric modulators at the BZ site. To increase the affinity towards the α+β- site, further structural modifications on the tested compounds were planned.

In a structure-activity approach, one of the tested 2-phenylpyrazoloquinolinones was analyzed in a docking study[24]. Recently, Miller and Aricescu were able to crystallize a β3 homopentamer[7]. Based on the obtained X-ray structure, a homology model was created and the pyrazoloquinolinone was placed in the proposed binding pose at both binding sites, α+γ- and α+β- (see Figure 9 and Figure 10).

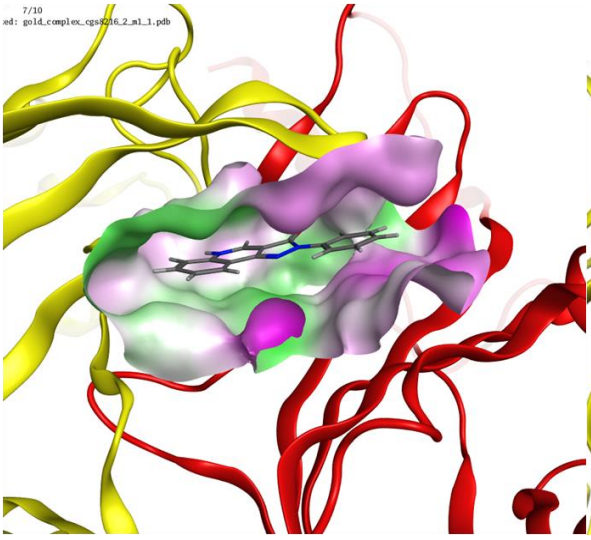


Figure 9 α+γ- binding site, with a 2-phenylpyrazolo[4,3-*c*]quinolinone in the binding pose

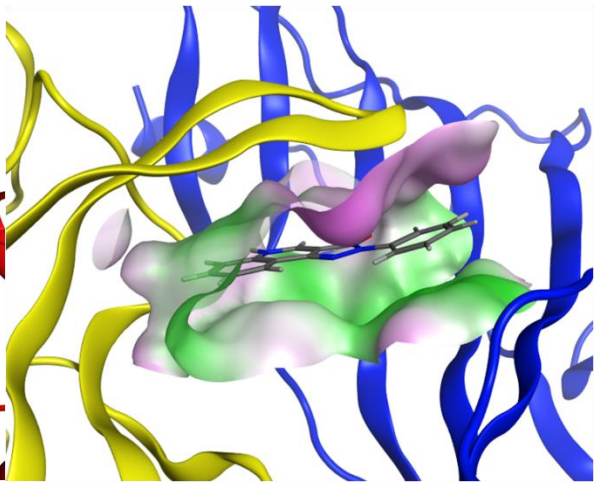
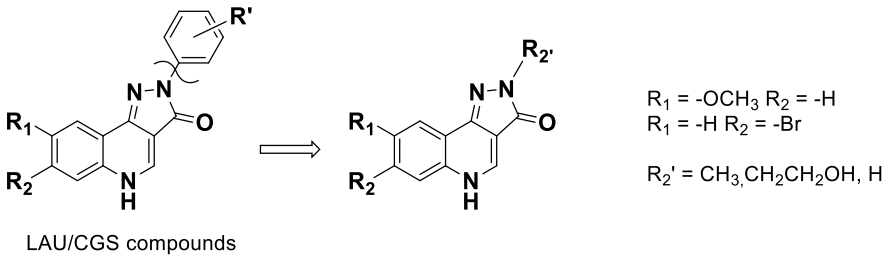


Figure 10 α+β- binding site, with a 2-phenylpyrazolo[4,3-*c*]quinolinone in the binding pose

In these figures the lipophilic (purple) and hydrophilic (green) areas of the binding site are illustrated. The tertiary structure of the α (yellow), γ (red) and β (blue) subunits are also shown. The difference between the two binding sites can be seen where a γ subunit is replaced with a β one, which leads to change from a lipophilic into a hydrophilic area. In this area, the phenyl ring of above mentioned LAU/CGS compounds is placed in the proposed binding pose. This might explain why the tested pyrazolo[4,3-*c*]quinolinones also exert activity on the α+γ-binding site, since they all have a phenyl substituent in position two. Replacing this substituent with something more hydrophilic might increase the selectivity towards the required α+β- binding site.

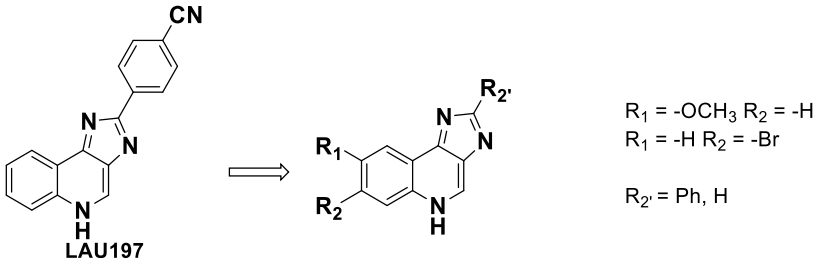
B III Objective

The novel binding site on the $GABA_A$ receptor at the α+β- interface would be an interesting target for new compounds. Already tested 2-phenylpyrazolo[4,3-*c*]quinolinones showed excellent activity on αβ-receptors (>1000% of GABA control current at 10 µM). Nevertheless, the tested compounds also showed affinity to the BZ-site. Results from the molecular docking studies suggest a replacement of the phenyl ring to an aliphatic or hydrophilic substituent due to the more hydrophilic areas from the β subunit in the binding pose, whereas the γ subunit features lipophilicity. A new series of pyrazoloquinolinones will feature polar and apolar aliphatic substituents as well as an unsubstituted one with the intention to provide further information if the phenyl substituent is necessary for the biological activity and if the selectivity towards the novel α+β- binding site can be increased (see Scheme 1).



Scheme 1 Proposed modifications on the pyrazolo[4,3-*c*]quinolinone

In an additional exploratory study, development of a small compound library based on the structure of already tested imidazo[4,5-*c*]quinoline LAU 197 was of interest. The different scaffold may display another subtype selectivity, which would be difficult to achieve by solely exchanging substituents. To compare the results of this novel compounds to already tested pyrazolo[4,3-*c*]quinolinones, similar substituents were chosen (see Scheme 2).

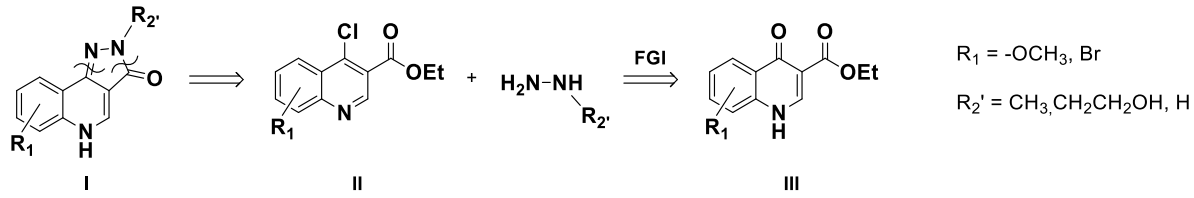


Scheme 2 Proposed expansion of the library of imidazo[4,5-*c*]quinoline

C Results and Discussion

C I Retrosynthetic analysis

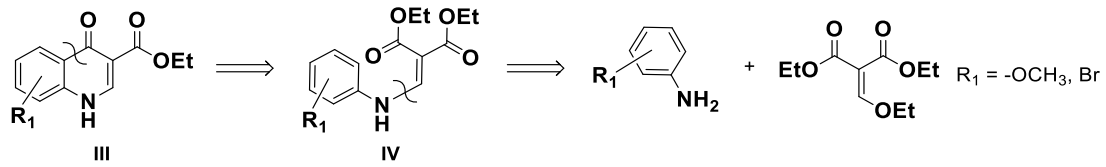
C I.1 Pyrazolo[4,3-*c*]quinolinones



Scheme 3 Retrosynthetic analysis of pyrazolo[4,3-*c*]quinolinones

The target structure **I** (

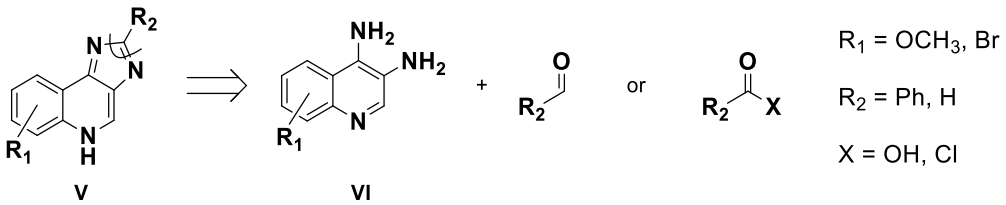
Scheme 3) is a pyrazolo[4,3-*c*]quinolinone. The two depicted retrosynthetic cuts divide the molecule into two parts, which will allow the introduction of different substituents on the pyrazolo system, upon choosing a properly substituted hydrazine. The two nucleophilic attacks of the hydrazine are facilitated by the introduction of good leaving groups, which leads to a chlorinated quinolinecarboxylate **II**. Functional group interconversion (FGI) of the chlorine group in structure **II** into a carbonyl group gives precursor structure **III** (Scheme 3). This route towards pyrazolo[4,3-*c*]quinolinones has already been reported in the literature and was therefore chosen for the synthesis[25].



Scheme 4 Retrosynthetic analysis of hydroxyquinolines

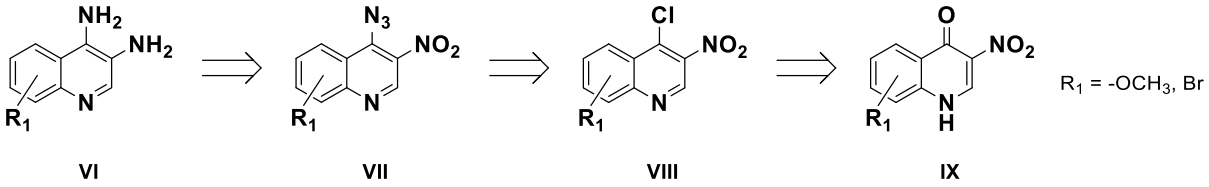
Such quinolonecarboxylates are accessible in two steps from commercially available anilines and ethoxymethylenemalonate (Scheme 4). This kind of reaction is called Gould-Jacobs reaction[26]. First, the nucleophilic attack of the nitrogen from the aniline gives the malonate **IV**. Then, a benzoannulation takes places, which requires a lot of energy, because of the intermediate loss of aromaticity.

C I.2 Imidazo[4,5-*c*]quinolines



Scheme 5 Retrosynthetic analysis of imidazo[4,5-*c*]quinolines

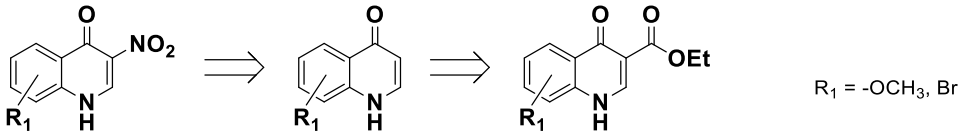
Structure **V**, an imidazo[4,5-*c*]quinolone, can be obtained in a cyclization step from a diaminoquinoline **VI** and the corresponding carboxylic acid (derivative) or aldehyde (Scheme 5)[27, 28]. Commercially available carbonyl compounds will allow the introduction of different substituents R_2.



VI **VII** **VIII** **IX**

Scheme 6 Retrosynthetic analysis of diaminoquinoline

Structure **VI**, a diaminoquinoline can be obtained by reduction of a nitro and an azide group. The azide can be introduced easily in two steps from nitroquinolone **IX** (Scheme 6)[29].

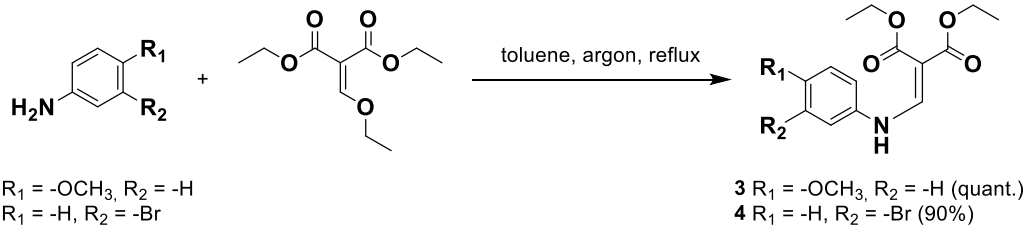


IX **X** **III**

Scheme 7 Retrosynthetic analysis of nitroquinolone IX

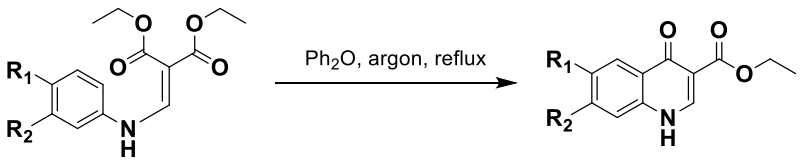
The nitroquinolone **IX** is accessible by nitration of quinolone **X**, which should selectively substitute in position 3, given the directing effect of the ketone[30]. The quinolone **X** will be obtained by decarboxylation of the carboxylate **III**, which convergently leads to the same intermediate already envisioned in the retrosynthetic analysis of pyrazolo[4,3-*c*]quinolinones (Scheme 7).

C II Synthesis of pyrazolo[4,3-*c*]quinolinones



Scheme 8 First step of the Gould-Jacobs reaction towards malonates 3 and 4

In the first synthetic step, commercially available anilines **1** and **2** were transformed in a Gould-Jacobs reaction into malonates **3** and **4** by adding ethoxymethylenemalonate and refluxing in toluene (Scheme 8)[31]. The obtained methoxy substituted crude product was of high purity and crystallized already upon removal of the solvent. The bromo substituted compound was recrystallized to obtain a pure product. In case of the anisidine a quantitative yield was obtained and 90% in case of the 3-bromoaniline.



3 R_1 = -OCH_3, R_2 = -H
4 R_1 = -H, R_2 = -Br

5 R_1 = -OCH_3, R_2 = -H (31%)
6 R_1 = -H, R_2 = -Br (61%)

Scheme 9 Second step of the Gould-Jacobs reaction towards quinolonecarboxylates

In the second step of the Gould-Jacobs reaction malonates **3** and **4** were submitted to ring closure at 250°C to form quinolones **5** and **6** (Scheme 9). The reaction was carried out under an argon atmosphere to avoid decomposition. The formed quinolone was insoluble in the reaction mixture, therefore it was easily collected by filtration. The low yield of the methoxy substituted derivative **5** (31%) was caused due to decomposition, which led to the generation of black tar and the formation of side products, from which only the decarboxylated quinolinone **21** could be identified. The bromo substituted quinolone **6** was formed in a better yield of 61%. The formation of any regioisomer (Figure 11) was not observed, which might be caused by steric repulsion of the bromine substituent.

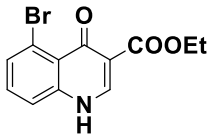
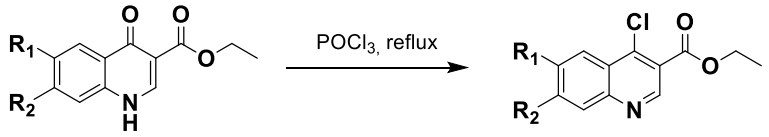


Figure 11 Possible regioisomer of 6



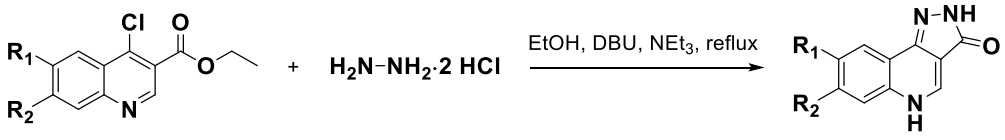
5 R_1 = -OCH_3, R_2 = -H
6 R_1 = -H, R_2 = -Br

7 R_1 = -OCH_3, R_2 = -H (81%)
8 R_1 = -H, R_2 = -Br (89%)

Scheme 10 Chlorination of quinolones

Chlorination of quinolones was carried out by adding an excess of $POCl_3$ to **5** or **6** (Scheme 10). The reagent converts the oxygen of the carbonyl group into a good leaving group, which favors the aromatic nucleophilic substitution[30]. Chlorinated quinolinecarboxylates **7** and **8** were synthesized in excellent yields of 81% and 89%, respectively.

With the chlorinated quinolines in hand, the synthesis of the first pyrazolo[4,3-*c*]quinolinones was attempted. For this purpose adequately substituted hydrazines were reacted with the quinoline precursor.

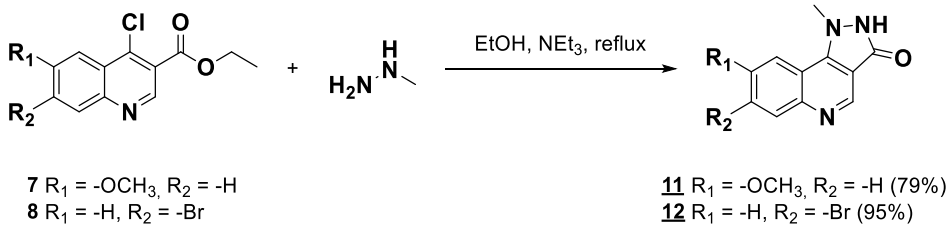


7 R_1 = -OCH_3, R_2 = -H
8 R_1 = -H, R_2 = -Br

9 R_1 = -OCH_3, R_2 = -H (14%)
10 R_1 = -H, R_2 = -Br (18%)

Scheme 11 Synthesis of pyrazolo[4,3-*c*]quinolinones

The first set of pyrazlolo[4,3-*c*]quinolones **9** and **10** were obtained by using hydrazine dihydrochloride (Scheme 11). Due to the low purity of the obtained crude product, which was obtained in a crude yield of approximately 40%, various purification steps were carried out, which led to a low isolated yield of 14% and 18%, respectively. The purification was further complicated by the low solubility of the compounds.

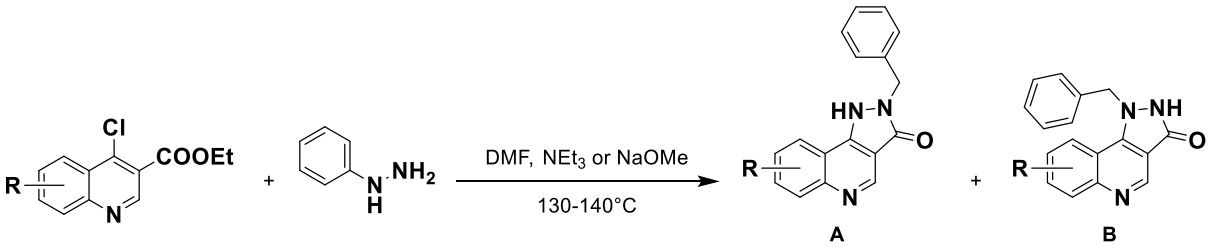


7 R_1 = -OCH_3, R_2 = -H
8 R_1 = -H, R_2 = -Br

11 R_1 = -OCH_3, R_2 = -H (79%)
12 R_1 = -H, R_2 = -Br (95%)

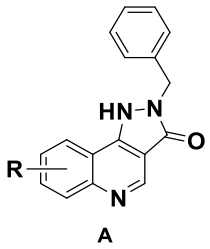
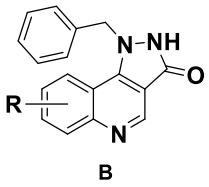
Scheme 12 Synthesis of 1-methylpyrazolo[4,3-*c*]quinolinones

The reaction with methylhydrazine did not form the desired pyrazolone with the substituent in position 2, as observed in the synthesis of LAU compounds, when using phenylhydrazines. The reaction led only to 1-methylpyrazolo[4,3-*c*]quinolinones in excellent yields of 79% and 95%, respectively (Scheme 12). The position of the methyl group was determined using 2D-NMR techniques, which will be discussed in detail further below. The reason for the different reactivity of the hydrazine can be explained by comparing the nucleophilicity of methylhydrazine to phenylhydrazine. When using methylhydrazine the secondary amine is a better nucleophile and attacks first, whereas in the case of phenylhydrazine the secondary amine is less nucleophilic, due to mesomeric effects, therefore favoring the attack of the primary amine first. This is even more evident, if we consider the difference of the pKs values of aniline (pK_s = 25) and methylamine (pK_s = 35)[32]. The aliphatic amine is a stronger base than the aromatic one and therefore a better nucleophile. It has been reported that variation of reaction conditions could shift the ratio between the isomers, but a complete inversion of the regioselectivity was not observed (see Scheme 13)[33]. Table 1 shows the effect of different bases on the ratio of formed regioisomers. The ratio varies when using different bases and is also dependent on the amount of added base. The formation of desired isomer **A** is favored when using NaOMe instead of NEt_3. Nevertheless, the formation of only isomer **A** was not observed. Therefore, a new approach to obtain the 2-methylated product was needed.

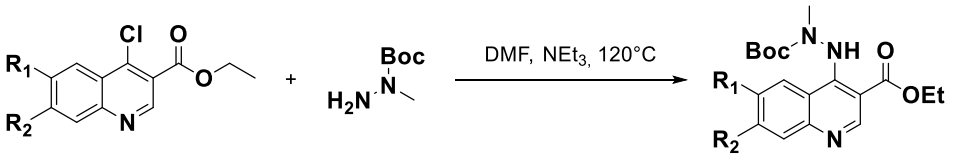


Scheme 13 Formation of regioisomers A and B

Table 1 Relative ratio of isomers A and B, determined from ^{1}H-NMR[33]

Substrate: R = 7-Br	Base, equivalents	 A	 B
	NaOMe, 2	**43**	**57**
	NaOMe, 1	**31**	**69**
	TEA, 2	**28**	**72**
	TEA, 1	**-**	**100**

To block the reactivity of the secondary amine, protection as *tert*-butylcarbamate was chosen. This should allow the primary nitrogen to react exclusively. After the cleavage of the *tert*-butyloxycarbonyl (Boc) group and subsequent ring formation, this synthetic route was expected to make 2-methlyated pyrazolo[4,3-*c*]quinolinones accessible.



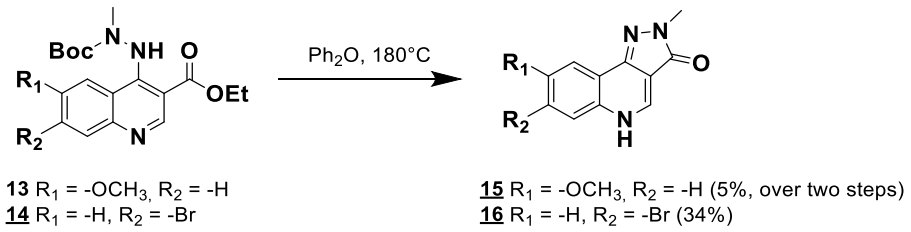
7 R_1 = -OCH_3, R_2 = -H
8 R_1 = -H, R_2 = -Br

13 R_1 = -OCH_3, R_2 = -H (-)
14 R_1 = -H, R_2 = -Br (95%)

Scheme 14 Introduction of boc-protected hydrazine

The aromatic nucleophilic substitution with Boc-protected methylhydrazine led to the formation of the quinolines **13** and **14** under slightly modified reaction conditions (Scheme 14). The derivative bearing the methoxy substituent was not isolated due to decomposition in the purification process, which might be caused due to partial loss of the protective group. Hence, it was used as a crude mixture in the next reaction step.

Deprotection of Boc groups is usually carried out by acidic cleavage (in the absence of water), using trifluoroacetic acid or etherial hydrochloric acid[34]. The work-up process usually includes neutralization of the reagent followed by extraction of the product. In the case of the desired products, 2-methylpyrazolo[4,3-*c*]quinolinone **15** and **16**, which show low solubility in organic solvents, the extraction did not lead to any product isolation. Moreover, the usage of acids leads to a protonation of the quinoline and even in basic conditions the product could not be isolated from the aqueous layer. Only the corresponding trifluoroacetate salts of **13** and **14** were isolated, which could not be converted further to the closed pyrazoloquinolinone. Therefore, another approach by thermal cleavage of the protection group was chosen[35].

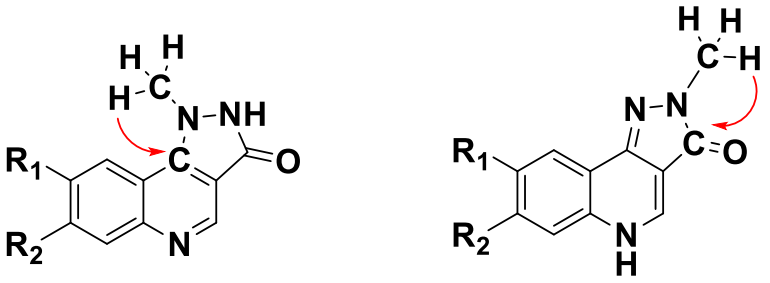


13 R_1 = -OCH_3, R_2 = -H
14 R_1 = -H, R_2 = -Br

15 R_1 = -OCH_3, R_2 = -H (5%, over two steps)
16 R_1 = -H, R_2 = -Br (34%)

Scheme 15 Synthesis of 2-methylpyrazolo[4,3-*c*]quinolinones

The deprotection of the boc group also led to the formation of the pyrazolo[4,3-*c*]quinolinone by spontaneous cyclization in the same reaction step. The yield of the methoxy substituted derivative was not determined, since the starting material was used as a crude, over two steps it can be estimated around 5%. The bromine derivative was formed in a better yield of 34% (Scheme 15). The poor overall yield might be explained due to decomposition of the hydrazine group, which is very likely to occur at such high temperatures[36]. Nevertheless, the required product was obtained and structural assignment was based on 2D-NMR in comparison with previously synthesized **11** and **12** (Figure 12). The heteronuclear multiple-bond correlation spectroscopy (HMBC) detects correlations between protons and carbons which are two to four bonds separated[37]; complementary to heteronuclear single-quantum correlation spectroscopy (HSQC), which detects correlation of nuclei, which are separated by only one bond. In the experiment where only methylhydrazine was used to form products **11** and **12** protons from the methyl group correlated to the carbon in position 9b and no correlation towards the carbonyl carbon was observed. On the other hand, products **15** and **16** showed no correlation between the protons from the methyl group and the carbon in position 9b, but a good correlation to the carbonyl carbon.

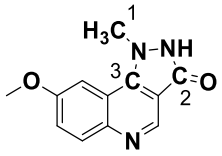


11 R_1 = -OCH_3, R_2 = -H
12 R_1 = -H, R_2 = -Br

15 R_1 = -OCH_3, R_2 = -H
16 R_1 = -H, R_2 = -Br

Figure 12 Observed ^3J coupling in HMBC 2D-NMR

Figure 13 shows a section from the HMBC-NMR of product **11**. The protons of methyl group 1 do not correlate with the carbonyl carbon 2, but with the carbon 3 in the quinoline.



11

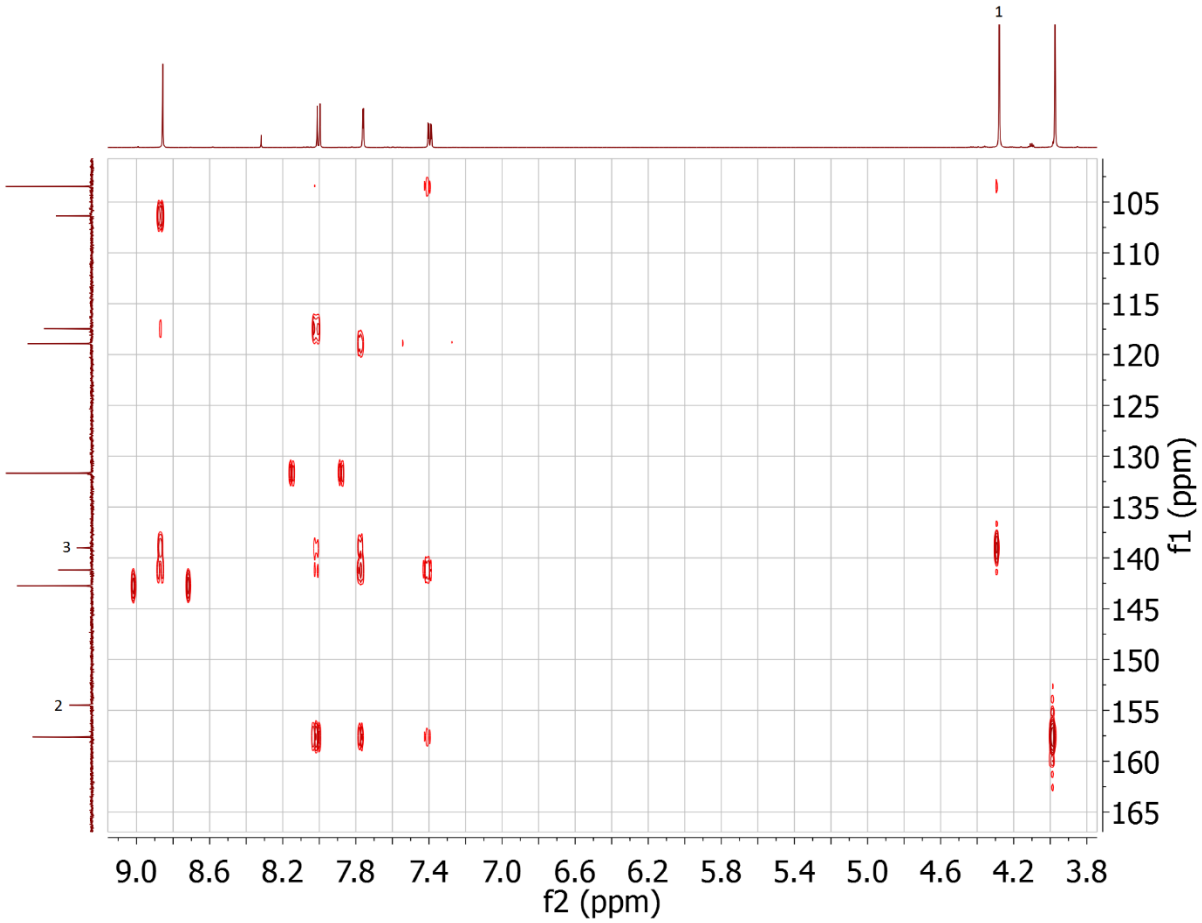
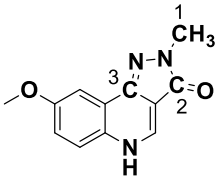


Figure 13 Section of HMBC-NMR of 8-methoxy-1-methyl-1,2-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one (11)

Figure 14 shows the same section of the HMBC-NMR of product **15**. Now, the methyl group 1 shows a good correlation with the carbonyl carbon 2 and no correlation with the carbon 3 in the quinoline.



15

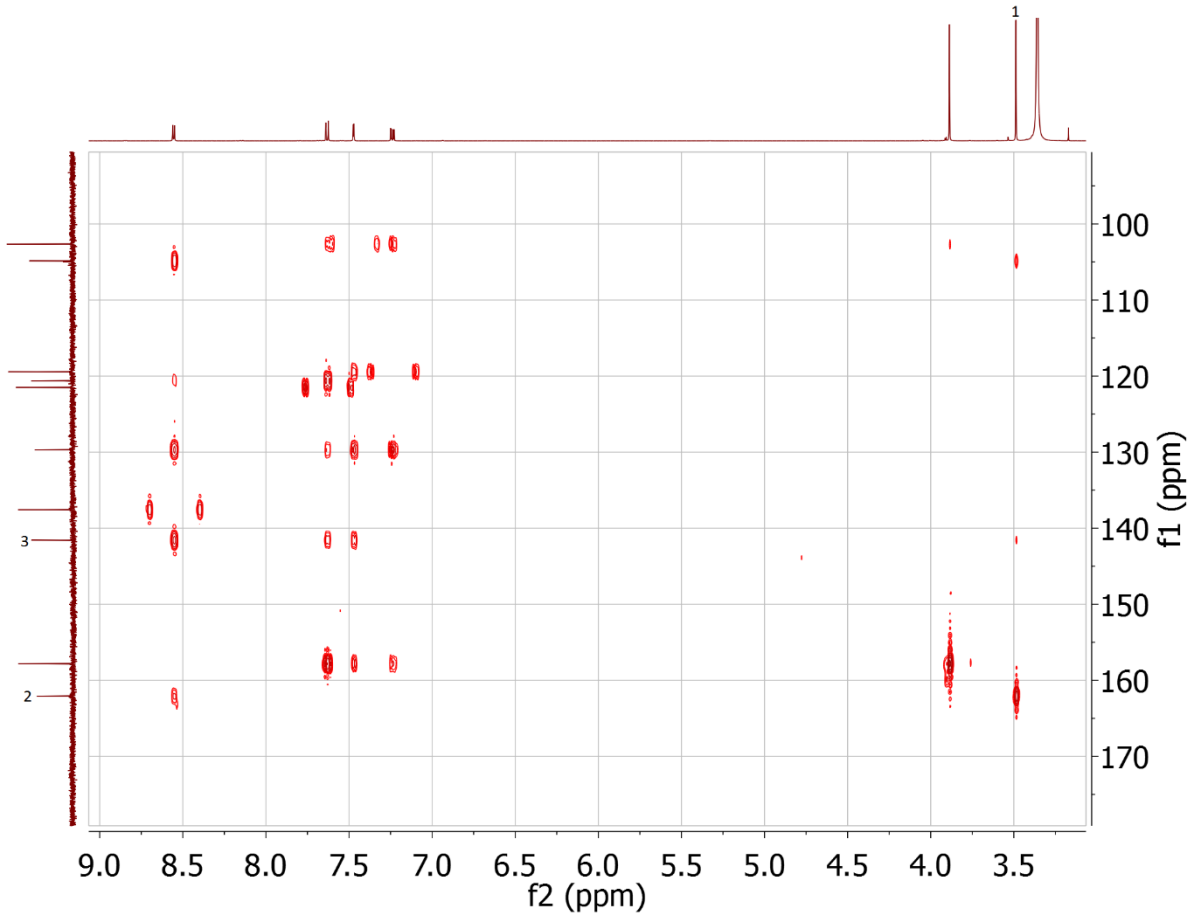
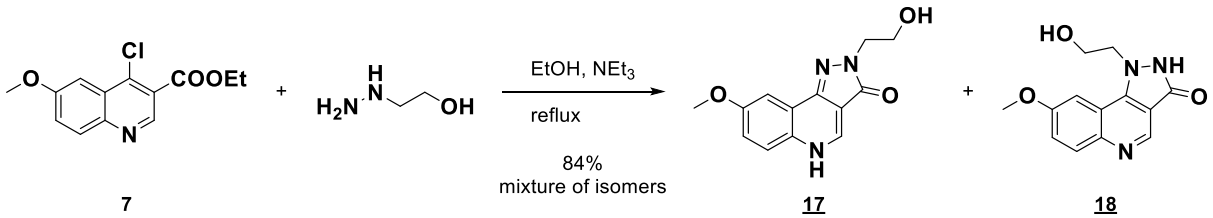


Figure 14 Section of HMBC NMR of 8-methoxy-2-methyl-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one (15)

As a last example, hydroxyethylhydrazine was used as a reaction partner to introduce a more polar, hydrophilic group onto the pyrazolo system.



7 **17** **18**

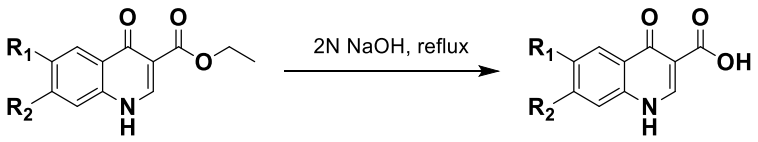
Scheme 16 Synthesis of (hydroxyethyl)-8-methoxypyrazolo[4,3-*c*]quinolinones

Only the methoxy substituted derivative was used in this reaction as quinoline reaction partner. In comparison to the experiment employing methylhydrazine, a mixture of both regioisomers in the ratio of **17**:**18**= 1:10 (based on ^{1}H-NMR) in a total yield of 84% was observed (Scheme 16). Using a Waters© preparative HPLC system made the difficult separation of both isomers possible and products **17** and **18** were isolated using a C18-column and

an eluent gradient of water and MeOH. Structural assignment of both isolated isomers **17** and **18** was achieved using the same 2D-NMR techniques as mentioned above.

C III Synthesis of imidazo[4,5-*c*]quinolines

The synthesis of the imidazo[4,5-*c*]quinolines was started from the same intermediates **5** and **6**, which were already used in the synthetic route towards pyrazolo[4,3-*c*]quinolinones.

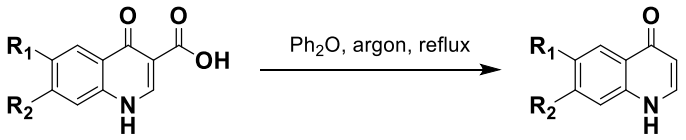


5 R_1 = -OCH_3, R_2 = -H
6 R_1 = -H, R_2 = -Br

19 R_1 = -OCH_3, R_2 = -H (86%)
20 R_1 = -H, R_2 = -Br (87%)

Scheme 17 Saponification of carboxylates 5 and 6

Quinolonecarboxylates **5** and **6** were saponified using 2N NaOH at reflux temperature to obtain the corresponding carboxylic acids **19** and **20** in excellent yields of 86% and 87%, respectively (Scheme 17).

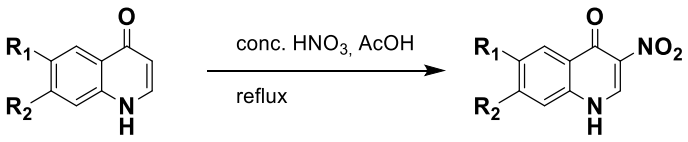


19 R_1 = -OCH_3, R_2 = -H
20 R_1 = -H, R_2 = -Br

21 R_1 = -OCH_3, R_2 = -H (96%)
22 R_1 = -H, R_2 = -Br (92%)

Scheme 18 Decarboxylation of carboxylic acids 19 and 20

The carboxyl acids were decarboxylated in refluxing diphenylether, according to a modified literature procedure[38]. Quinolones **21** and **22** were formed in excellent yields of 96% and 92%, respectively (Scheme 18). The reaction apparatus had to be carefully purged with argon, otherwise decomposition of the product at such high temperatures would be likely.

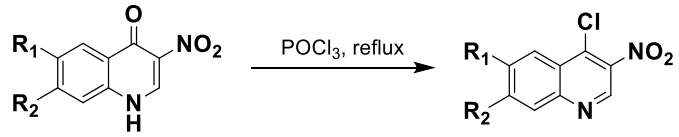


21 R_1 = -OCH_3, R_2 = -H
22 R_1 = -H, R_2 = -Br

23 R_1 = -OCH_3, R_2 = -H (75%)
24 R_1 = -H, R_2 = -Br (70%)

Scheme 19 Nitration of quinolones

Nitration of quinolones **21** and **22** was carried out according to a modified literature procedure[39], whereas acetic acid and concentrated nitric acid were used to form nitro quinolones **23** and **24** in good yields of 75% and 70%, respectively (Scheme 19). Only substitution in position three is observed, which is in agreement with expectations based on the directing effect of the carbonyl group in position four[30].

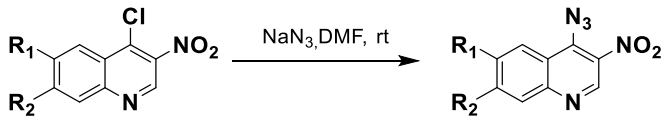


23 R_1 = -OCH_3, R_2 = -H
24 R_1 = -H, R_2 = -Br

25 R_1 = -OCH_3, R_2 = -H (42%)
26 R_1 = -H, R_2 = -Br (91%)

Scheme 20 Chlorination of nitro quinolones

Chlorination of quinolones was carried according to a modified literature procedure[40], using phosphorus oxychloride in neat conditions. The formation of chlorinated quinolines from quinolones in a nucleophilic substitution reaction is known to proceed very fast and selectively at the position of the carbonyl group[41]. The low yield of 42% of the methoxy derivative **25** is due to problems in the work-up process, whereas the analog bearing a bromine substituent (**26**) was formed in excellent yield of 91%.

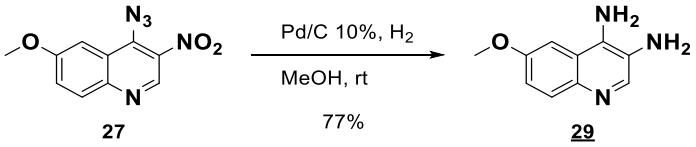


25 R_1 = -OCH_3, R_2 = -H
26 R_1 = -H, R_2 = -Br

27 R_1 = -OCH_3, R_2 = -H (quant.)
28 R_1 = -H, R_2 = -Br (quant.)

Scheme 21 Substitution of chloride to an azide

Formation of azides by substitution of the chloride in quinolines **25** and **26** was carried out according to a modified literature procedure[40] under mild conditions to obtain products **27** and **28** in quantitative yields (Scheme 21). The products did not display a high stability, especially when elevated temperatures were applied. This was already observed upon the removal of DMF from the reaction mixture. Therefore, compounds were used as quickly as possible for the next reaction step, the catalytic hydrogenation.

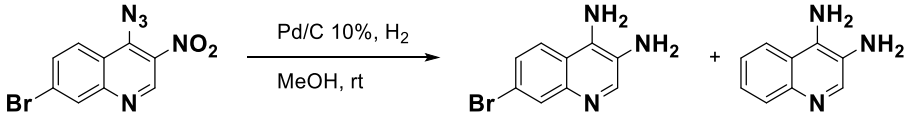


27 **29**

Scheme 22 Catalytic hydrogenation of 4-azido-6-methoxy-3-nitroquinoline

Diamine **29** was prepared according to a modified literature procedure[40]. Catalytic hydrogenation of methoxy derivative **27** was accomplished using palladium on charcoal (10%) and hydrogen at atmospheric pressure. The product, 6-methoxyquinolin-3,4-diamine **29**, was obtained in a good yield of 77%.

Upon reduction of the bromo derivative with the same protocol, formation of two products was observed.

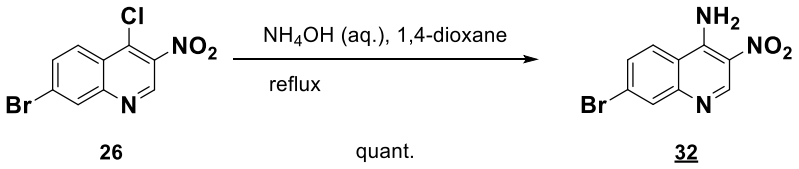


28 **30** **31**

Scheme 23 Catalytic hydrogenation of 4-azido-7-bromo-3-nitroquinoline

Hydrodehalogenation is known to occur using Pd/C and hydrogen[42]. Due to the difficult separation of both formed products **30** and **31**, another procedure to reduce the azido and nitro group to the diamine was required. Several methods are available for the reduction of aromatic nitro compounds, which are known to leave aromatic

halides untouched, e.g. by using iron in hydrochloric acid (a Béchamp reduction)[43] or in less acidic environment like acetic acid[44] or aqu. ammonium chloride[45]. Furthermore, the usage of stannous chloride is also reported to reduce a nitro group to an amine[46]. To sum up, for the reduction of the nitro group under mild conditions many literature protocols are available, whereas the reduction of an azide under the same conditions is not known. Therefore, it was necessary to introduce the amino group in position four directly in order to avoid the detour *via* an azide. It has been reported that a nucleophilic substitution using aqu. NH_4OH is possible, if the aromatic system is activated by electron withdrawing groups, which is the case in **26**[47].



Scheme 24 Amination of chlorinated quinoline

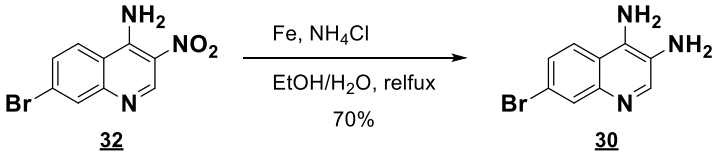
The title compound **32** was prepared according to a modified literature procedure in a quantitative yield using aqu. NH_4OH as a reagent (Scheme 24)[47].

Several attempts were now made to reduce the nitro group in **32** to obtain diamine **30**. Either $SnCl_2$ or Fe were used as reducing agent. The reduction was usually fast and could be carried out under mild conditions, nevertheless the work-up procedures described in the literature did not lead to isolation of the desired product in acceptable yields. The used methods are summed up in Table 2.

Table 2 Conditions and results of various reduction attempts

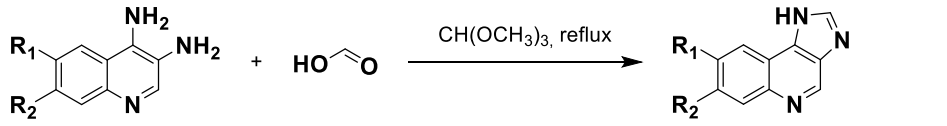
Cond.	Observed results
$SnCl_2$*2 H_2O, EtOH; work-up with activated charcoal	No product isolated
$SnCl_2$*2 H_2O, EtOH; work-up without activated charcoal	60% crude yield
Fe, HCl, EtOH; extractive work-up	59% crude yield
Fe, NH_4Cl (aqu.), EtOH, extractive work-up	28% crude yield
Fe, AcOH; extractive work-up	No product isolated
Fe, NH_4Cl, EtOH/H_2O; isolation of product *via* column chromatography[48]	70% yield

The problem was either due to using charcoal to remove any metals or salts thereof, which also adsorbed the very polar diamine **30**, or extraction of the reaction mixture was not feasible, due to the high polarity and low solubility of the desired product. A satisfying procedure was established by using iron and ammonium chloride and directly concentrating the reaction mixture on silica and isolating title compound **30** by flash chromatography. This led to the isolation of diamine **30** in a good yield of 70% (Scheme 25).



Scheme 25 Reduction of 7-bromo-3-nitroquinolin-4-amine

With the two diamines **29** and **30** in hand, the synthesis of imidazo[4,5-*c*]quinolines was targeted next. It is known that this reaction can be accomplished using carboxylic acids[29] or their derivatives[39] under acid catalysis as well as using aldehydes[28] with an oxidizing agent.



29 R_1 = -OCH_3, R_2 = -H
30 R_1 = -H, R_2 = -Br

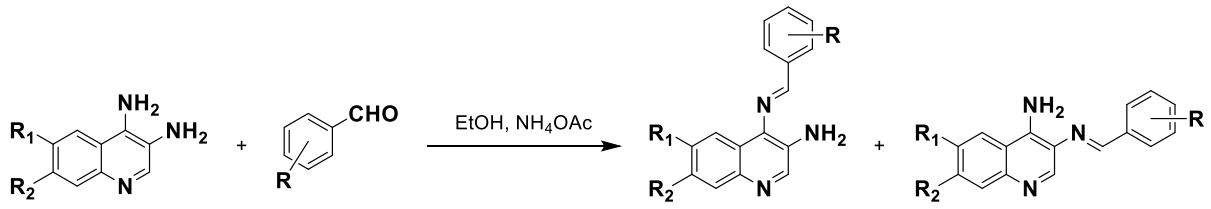
33 R_1 = -OCH_3, R_2 = -H (47%)
34 R_1 = -H, R_2 = -Br (57%)

Scheme 26 Synthesis of imidazo[4,5-*c*]quinolines

First, imidazo[4,5-*c*]quinolines **33** and **34**, which do not feature a substituent on the imidazo system, were prepared according to a modified literature protocol, with acceptable yields of 47% and 57%, respectively (Scheme 26)[49]. On one hand, these unsubstituted imidazo[4,5-*c*]quinolines will be used for biological testing, on the other hand, they represent a potential precursor for the modular introduction of various substituents *via* C-H activation[50].

Synthesis of imdazo[4,5-*c*]quinolines bearing a phenyl ring in position 2 was also carried out with the same protocol used above in the case of unsubstituted products employing carboxylic acid reagents, which did not lead to full consumption of starting material even after longer reaction time. Therefore, another procedure was needed.

In an alternative approach, the diamine was reacted with the corresponding benzaldehyde using ammonium acetate in EtOH, which are reported conditions for the synthesis of benzimidazoles[28]. Applying this procedure for the synthesis of the desired imidazo[4,5-*c*]quinolines led to full consumption of the starting material, but the reaction stopped at the stage of the open chain imine, in the form of two regioisomers (Scheme 27).



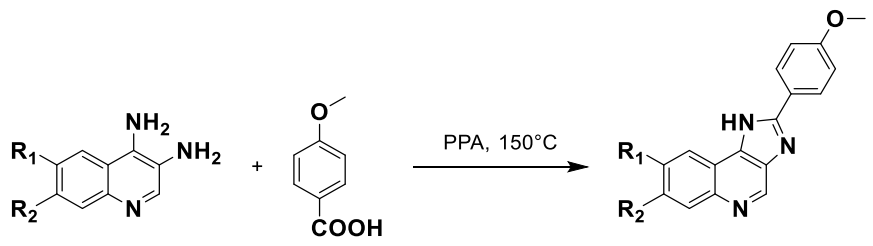
29 R_1 = -OCH_3, R_2 = -H
30 R_1 = -H, R_2 = -Br

R_1 = -OCH_3, R_2 = -H
R_1 = -H, R_2 = -Br
R = CN, OCH_3

Scheme 27 Formation of open chained imines

The ring closure was attempted using DMSO at an elevated temperature of 150°C and under acid catalysis with acetic acid at reflux, which did not lead to any conversion.

Therefore, polyphosphoric acid (PPA) was used with the corresponding carboxylic acids as reagents, which are well known conditions for the formation of imidazoles[49].

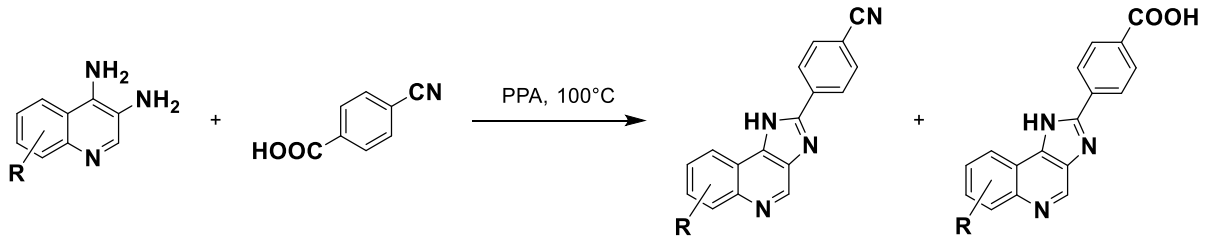


29 R_1 = -OCH_3, R_2 = -H
30 R_1 = -H, R_2 = -Br

35 R_1 = -OCH_3, R_2 = -H (24%)
36 R_1 = -H, R_2 = -Br (77%)

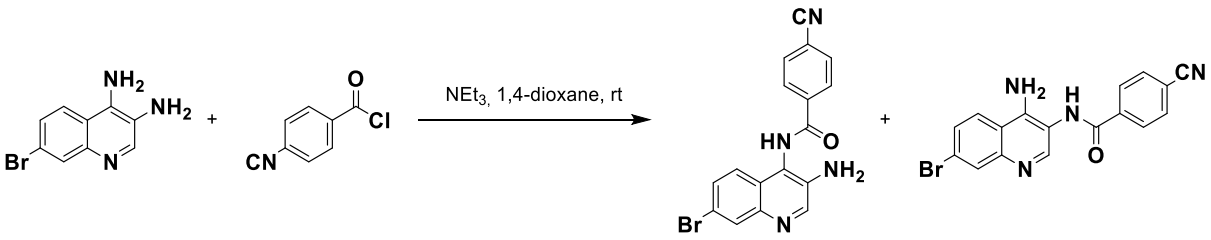
The imidazo[4,5-*c*]quinolines bearing a *p*-methoxy substituted phenylsubstituent at the imidazo system were synthesized using *p*-methoxybenzoic acid and polyphosphoric acid under neat conditions. The low isolated yield of 24% for methoxy derivative **35** was due to a difficult work-up procedure, attributed to the high viscosity of the used polyphosphoric acid. Nevertheless, enough material was isolated for biological testing. Bromo derivative **36** was isolated in a good yield of 77%.

As a last example, a nitrile group was planned in *para* position on the phenyl ring. As mentioned above, the usage of the corresponding aldehyde, 4-formylbenzonitrile, in EtOH with NH_4OAc did not lead to the formation of the desired product. Applying the protocol with PPA as reagent led to full consumption of the starting material, but also decreased the formation of wanted product (less than 10%), mainly because the cyano group could also react to form a side product, which was not isolated (see Scheme 28). Therefore, the procedure had to be adjusted again.



Scheme 28 Formation of two products when using 4-cyanobenzoic acid

In another attempt, more reactive 4-cyanobenzoyl chloride was used instead of 4-cyanobenzoic acid, which diminished the side reaction of the cyano group. But then again, the reaction stopped at the stage of the amide and did not undergo ring closure towards the wanted imidazole (Scheme 29).



30 **37** **38**

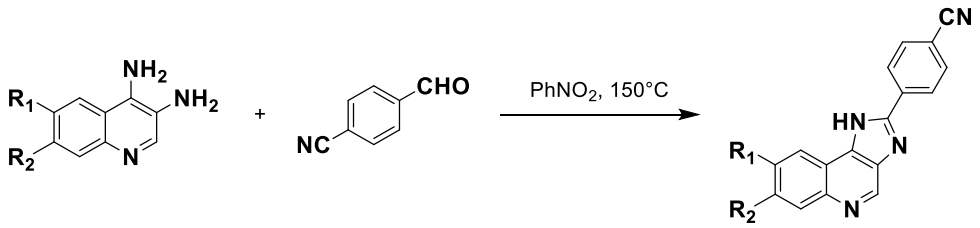
Scheme 29 Reaction with acid chloride led to the formation of two amides

Various attempts were made to complete the ring closure, using different conditions, which are summarized in Table 3.

Table 3 Attempts on ring closure of amides towards imidazo[4,5-*c*]quinolines

Conditions	Observed results
AcOH, reflux	No conversion
Polyphosphoric acid, 100°C	No conversion
$BF_3 \cdot OEt_2$, dioxane, reflux	No conversion

It has been reported that Brønsted or Lewis acids facilitate the formation of imidazole[51]. Nevertheless, in the present case no product formation was observed. The low reactivity of the formed amides **37** and **38**, in comparison to the imine, were expected to require very harsh reaction conditions to form the wanted imidazole. Therefore, this route was not further pursued and another procedure was used for the synthesis.



29 R_1 = -OCH_3, R_2 = -H
30 R_1 = -H, R_2 = -Br

35 R_1 = -OCH_3, R_2 = -H (52%)
36 R_1 = -H, R_2 = -Br (69%)

Scheme 30 Synthesis of imidazo[4,5-*c*]quinolines using $PhNO_2$

In the protocol using an aldehyde as reaction partner, molecular oxygen (from the air) is used as an oxidizing reagent. Apparently, this is not sufficient to oxidize the formed dihydroimidazoquinoline intermediate. Therefore, a stronger oxidizing agent should accomplish the reaction towards the desired imidazoquinolines. Nitrobenzene has already been used in such ring closure reactions[52]. At a temperature of 150°C, the synthesis of imidazo[4,5-*c*]quinolines **39** and **40** was successful. The desired products were isolated in good yields of 52% and 69%, respectively.

C IV Biological results

The previously synthesized compounds were now available to be tested on $GABA_A$ receptors. The tests were carried out in the laboratory of cooperation partner Prof. Margot Ernst at the Medical University of Vienna.

Two-electrode voltage clamp methodology was used for the assessment of currents of heterologously expressed $GABA_A$ receptor pentamers in *Xenopus laevis* oocytes. In this technique, oocytes are excised from adult female *Xenopus laevis* and injected with mRNA of the wanted $GABA_A$ receptor subunit combination. After the expression of the receptors, the oocytes are then investigated electrophysiologically by placing two microelectrodes into the oocyte and applying a current, which can range from 5 nA to 100µA. The current increases upon opening of the channel, since Cl^- can pass through the membrane. First, only GABA is added, which gives the base value in further experiments. Then, test compounds are co-applied in solution with GABA, which can now increase or decrease the current of Cl^- through the channel. If the compound increases the GABA control current, it is a positive allosteric modulator (PAM). If the GABA control current is decreased, the compound acts as a negative allosteric modulator (NAM) (see Figure 15)[53, 54].

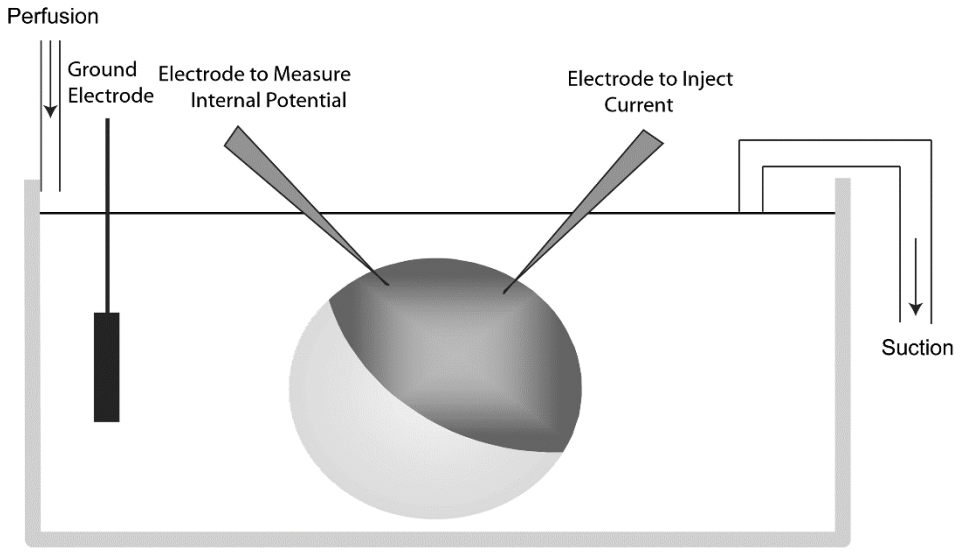


Figure 15 Diagram of the two-electrode voltage clamp[53]

All synthesized pyrazolo[4,3-*c*]quinolinones and imidazo[4,5-*c*]quinolines (Figure 16) were submitted to the cooperation partner and tested for their activity on the $GABA_A$ receptor.

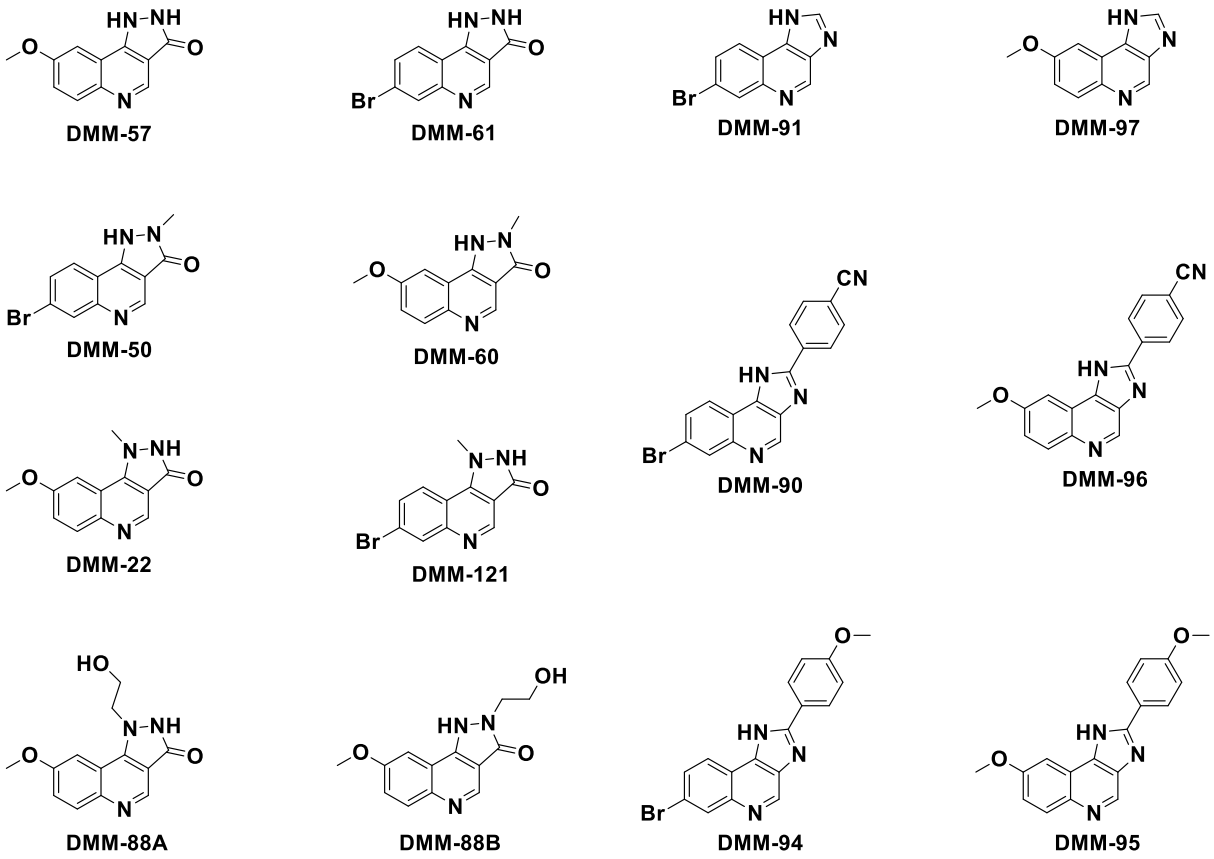
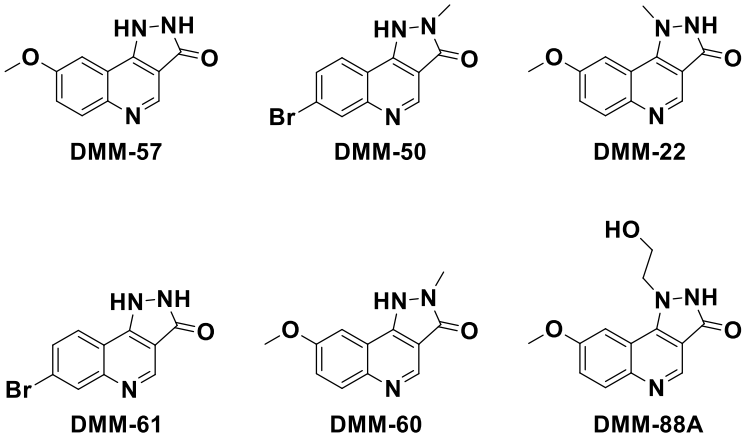


Figure 16 Compounds submitted for biological testing

At the point of compilation of this thesis, not all compounds have been fully tested, but first tests showed activity of the newly synthesized compounds at several $GABA_A$ receptors (see Figure 17).



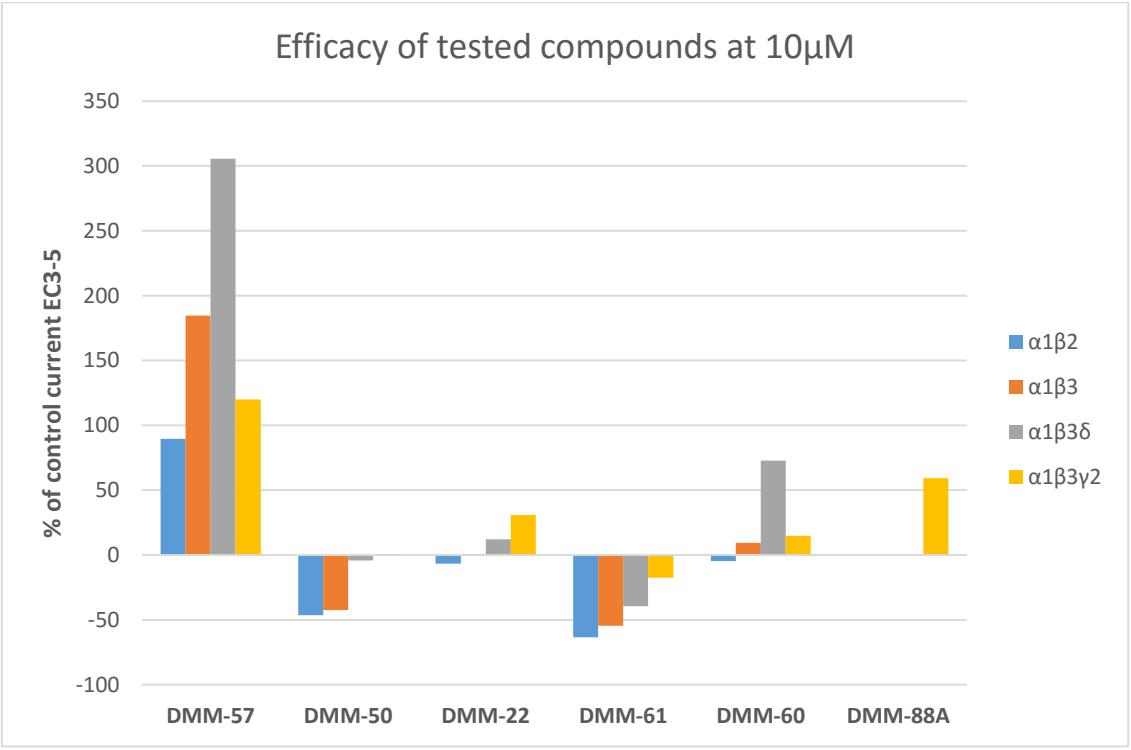


Figure 17 Efficacy of tested pyrazolo[4,3-*c*]quinolinones

It can be observed that all tested compounds show activity on at least one receptor. They exert positive as well as negative allosteric modulation. Compounds bearing a bromo substituent DMM-50 and DMM-61 show negative allosteric modulation, whereas methoxy substituted compounds DMM-57/22/60/88A show positive allosteric modulation. In comparison to the parent LAU compounds, which increased the control current by up to 3000%, the effect is significantly less. Nevertheless, in this preliminary tests it has been shown that pyrazolo[4,3-*c*]quinolinones still exert activity on the $GABA_A$ receptor, even if the phenyl ring in position 2 is missing. Moreover, a subunit selectivity can also be observed, e.g. DMM-57/60 show higher efficacy in α1β3δ receptors. Also, the NAM DMM-50/61 show a higher activity in $GABA_A$ receptors containing only α and β subunits. Nevertheless, more tests have to be carried out to determine if the compounds only bind to the newly discovered α+β- interface, or also bind to the BZ-binding site.

The rest of the submitted compounds (Figure 16) are still being tested on their biological activity.

D Conclusion and Perspective

The synthesis of 14 novel potential $GABA_A$ receptor modulators was successfully conducted, from which six already showed to possess biological activity on the investigated receptors. In the case of pyrazolo[4,3-*c*]quinolinones, the introduction of aliphatic substituents on the pyrazolo system was feasible. Preliminary tests also showed that biological activity was still in the range of +300% to -50%, in comparison to >1000% as in the case of 2-phenylpyrazolo[4,3-*c*]quinolinones (of GABA control current at 10µM). Further tests have to be carried out to obtain more information on the selectivity towards the α+β- binding site. If the tests show that the affinity towards the novel binding site is increased, the assumptions made in the homology model and docking studies may be considered as relevant and can be used to design novel compounds for the α+β- binding site.

A synthetic route for imidazo[4,5-*c*]quinolines with a versatile substitution pattern on the quinoline as well as on the imidazo system was established. The route was also adjusted to tolerate halides as substituents. Different protocols for the ring closure towards imidazo[4,5-*c*]quinolines using aldehydes or carboxylic acids as reagents were established. Further biological data, especially of imidazo[4,5-*c*]quinolines will demonstrate whether the introduction of new substituents may have the same changes in biological activity as seen in the pyrazolo[4,3-*c*]quinolinones.

E Experimental part

E I Materials and methods – chemical synthesis

Unless noted otherwise, all reagents were purchased from commercial suppliers and used without further purification. DCM, Et_2O, dioxane, MeOH, THF and toluene intended for water-free reactions were pre-distilled and then desiccated on Al_2O_3 columns (PURESOLV, Innovative Technology). Chromatography solvents were distilled prior to use. For all other solvents quality grade is given in the reaction procedures.

Column chromatography was performed on a Büchi Sepacore Flash System (2 x Büchi Pump Module C-605, Büchi Pump Manager C-615, Büchi UV Photometer C-635, Büchi Fraction Collector C-660) or standard manual glass columns using silica gel from Merck (40-63 µm) using LP/EtOAc or DCM/MeOH mixtures.

HPLC chromatography was carried out with an Autopurification system of Waters using an ACQUITY QDa MS-Detector in combination with a 2998 Photodiode Array Detector. Analytical separation was made using XSELECT CSH Fluoro-Phenyl 5 µm 4.6 x 150 mm and XSELECT CSH C18 5 µm 4.6 x 150 mm columns. Preparative separation was made using XSELECT CSH Prep Fluoro-Phenyl 5 µm 30 x 150 mm and XSELECT CSH Prep C18 5 µm OBD 30 x 150 mm columns. As solvents HPLC grade methanol and HPLC grade water were used containing 0.1 % formic acid.

Table E-1. Recipes for TLC staining solutions used in this thesis.

TLC staining solution 1 (general purpose)		**TLC staining solution 2 (general purpose)**		**TLC staining solution for acidic compounds**		**TLC staining solution for carbonyl compounds**	
6 g	$KMnO_4$	10 g	phosphomolybdic acid hydrate	40 mg	bromocresol green	0.8 g	2,4-dinitrophenylhydrazine
0.5 g	KOH	1 g	cerium ammonium nitrate	100 mL	dry EtOH	200 mL	2N HCl
40 g	K_2CO_3	20 g	H_2SO_4 conc.	0.1 M	NaOH until blue color appears	2 mL	EtOH
600 mL	deion. H_2O	300 mL	EtOH				

Desiccation of organic solvents after extraction in reaction workup was performed using anhydrous sodium sulfate and subsequent filtration.

Melting points were determined by a Leica Galen III Kofler and a Büchi Melting Point B-545.

NMR spectra were recorded from $CDCl_3$, d_6-DMSO or d_4-MeOH solutions on a Bruker AC 200 (200 MHz), Avance UltraShield 400 (400 MHz) or Avance III HD 600 (600 MHz) spectrometer and chemical shifts are reported in ppm using tetramethylsilane as an internal standard. Whenever possible calibration via residual solvent peaks was performed. Peak assignment is based on correlation experiments or software prediction.

HR-MS analysis was carried out from methanol solutions (concentration: 10 µM) by using an HTC PAL system autosampler (CTC Analytics AG, Zwingen, Switzerland), an Agilent 1100/1200 HPLC with binary pumps, degasser

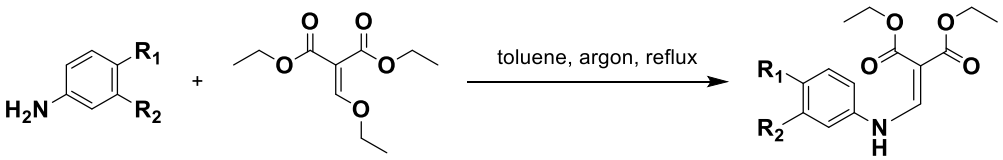
and column thermostat (Agilent Technologies, Waldbronn, Germany) and Agilent 6230 AJS ESI–TOF mass spectrometer (Agilent Technologies, Palo Alto, United States).

LogP values were calculated using ChemDraw® Professional 15.0.

E II General procedures

This section gives general descriptions of repeatedly used protocols in chemical synthesis experiments within this work.

E II.1 General procedure A for synthesis of malonates



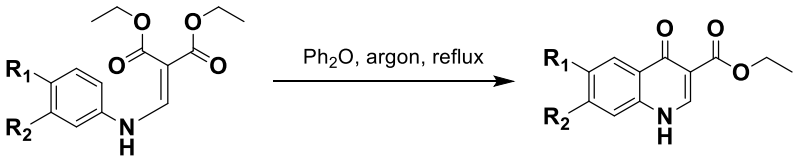
1 R_1 = -OCH_3, R_2 = -H
2 R_1 = -H, R_2 = -Br

3 R_1 = -OCH_3, R_2 = -H
4 R_1 = -H, R_2 = -Br

Malonates **3** and **4** were synthesized according to a modified literature procedure[55]

A three-necked flask equipped with a reflux condenser and a balloon was charged with the corresponding aniline **1** or **2** (1 equiv.) and diethyl (ethoxymethylene)malonate (1 equiv.). The reactants were dissolved in toluene (c ≈ 0.5 M) and the whole reaction apparatus was carefully purged with argon. Consecutively, it was heated to reflux for up to 24 h. The reaction mixture was cooled to room temperature after full consumption of starting material was observed by TLC. After evaporation of solvent the products (**3** or **4**) could be obtained in adequate purity. If the purity of the crude product was too poor, which did not lead to crystallization of the desired product, recrystallization with iso-octane was performed.

E II.2 General procedure B for ring closure towards quinolones



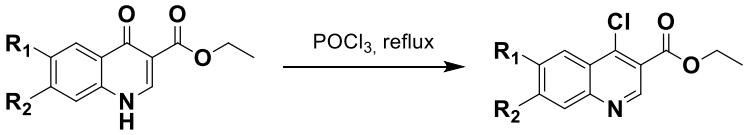
3 R_1 = -OCH_3, R_2 = -H
4 R_1 = -H, R_2 = -Br

5 R_1 = -OCH_3, R_2 = -H
6 R_1 = -H, R_2 = -Br

Quinolones **5** and **6** were prepared according to a modified literature procedure[56].

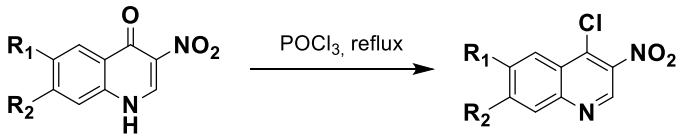
In a three-necked flask equipped with a reflux condenser and a balloon malonate **3** or **4** was dissolved in diphenylether (c ≈ 0.5M). The reaction apparatus was carefully purged with argon and heated to reflux, whereupon the desired product precipitated in the reaction solution. After full consumption of starting material was observed, which took up to 24h, the reaction mixture was cooled to room temperature and poured onto LP. The desired products (**5** or **6**) were obtained by filtration, washing with LP and drying *in vacuo*.

E II.3 General procedure C for chlorination of quinolones and nitroquinolones



5 R_1 = -OCH_3, R_2 = -H
6 R_1 = -H, R_2 = -Br

7 R_1 = -OCH_3, R_2 = -H
8 R_1 = -H, R_2 = -Br



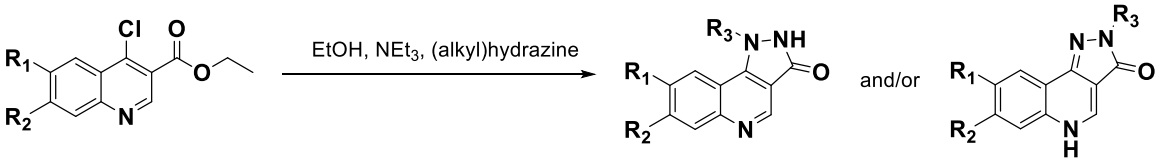
23 R_1 = -OCH_3, R_2 = -H
24 R_1 = -H, R_2 = -Br

25 R_1 = -OCH_3, R_2 = -H
26 R_1 = -H, R_2 = -Br

Chlorination of quinolones and nitroquinolones was carried out according to a modified literature protocol[40, 57].

A three-necked flask equipped with a reflux condenser was charged with quinolone **5, 6, 23** or **24** (1 equiv.). Phosphorus oxychloride (4 equiv.) was added and the reaction mixture was heated to reflux up to four hours until full consumption of starting material was observed *via* TLC. The reaction mixture was poured onto ice and satd. aqu. $NaHCO_3$ was added until a neutral pH was reached, whereupon precipitation occurred. The products (**7, 8, 25** or **26**) were extracted with EtOAc several times and the combined organic layers were dried over $MgSO_4$. After evaporation of the solvent *in vacuo*, the products were obtained.

E II.4 General procedure D for synthesis of pyrazolo[4,3-*c*]quinolin-3-ones



7 R_1 = -OCH_3, R_2 = -H
8 R_1 = -H, R_2 = -Br

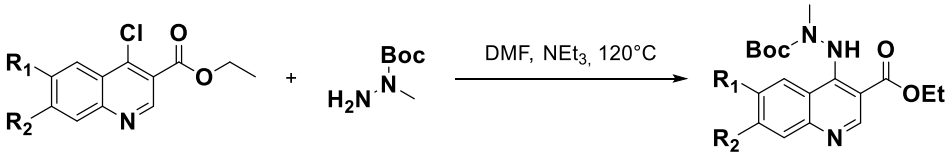
R_1 = -OCH_3, -H
R_2 = -Br, -H
R_3 = -H, CH_3, -CH_2CH_2OH

R_1 = -OCH_3, -H
R_2 = -Br, -H
R_3 = -H, -CH_2CH_2OH

Pyrazolo[4,3-*c*]quinolin-3-ones were prepared according to a modified literature procedure[58].

Activated quinoline **7** or **8** was dissolved in EtOH in a screw cap vial and after addition of NEt_3 and the corresponding hydrazine (in cases where the hydrazine was added as a hydrochloride, DBU was added to form the hydrazine *in situ*) the reaction mixture was heated to reflux, whereupon the product precipitated. The reaction was heated for up to 24 h, until full consumption of starting material was observed *via* TLC. The reaction mixture was poured into LP and the products were collected by filtration. After washing with EtOH and LP, the products were dried *in vacuo* and obtained in good purity.

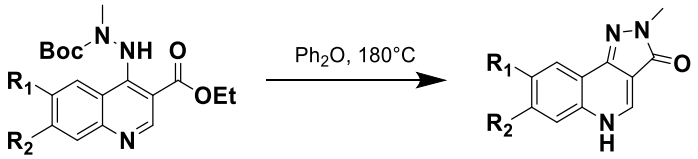
E II.5 General procedure E for synthesis of 2-methylated pyrazolo[4,3-*c*]quinolin-3-ones



7 R_1 = -OCH_3, R_2 = -H
8 R_1 = -H, R_2 = -Br

13 R_1 = -OCH_3, R_2 = -H (-)
<u>14</u> R_1 = -H, R_2 = -Br (95%)

Activated quinoline **7** or **8** (1 equiv.) was dissolved in DMF in a screw cap vial, 1-Boc-1-methylhydrazine (1.2 equiv.) and NEt_3 (1 equiv.) were added and the reaction mixture was heated to 120°C for up to 24 h, until full consumption of starting material was observed *via* TLC. The solvent and excess reactants were evaporated *in vacuo* and after absorbing the residue on silica, the products (**13** or **<u>14</u>**) were isolated by flash chromatography using a manual glass column with a gradient of 5-20% MeOH in CH_2Cl_2.

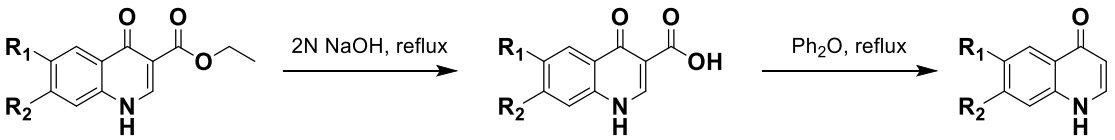


13 R_1 = -OCH_3, R_2 = -H
<u>14</u> R_1 = -H, R_2 = -Br

<u>15</u> R_1 = -OCH_3, R_2 = -H (-)
<u>16</u> R_1 = -H, R_2 = -Br (34%)

Protected quinoline **13** or **<u>14</u>** was dissolved in Ph_2O in a screw cap vial, flushed with argon and heated to reflux for 24 h, whereupon the product precipitated. The conversion of the reaction was monitored by TLC. After full consumption of starting material the reaction mixture was poured into LP and the products were collected by filtration and washing with large amounts of LP. After drying *in vacuo*, the products were usually obtained in high purity, otherwise recrystallization in MeOH was performed.

E II.6 General procedure F for decarboxylation of quinolonecarboxylates



5 R_1 = -OCH_3, R_2 = -H
6 R_1 = -H, R_2 = -Br

19 R_1 = -OCH_3, R_2 = -H (86%)
20 R_1 = -H, R_2 = -Br (87%)

21 R_1 = -OCH_3, R_2 = -H (96%)
22 R_1 = -H, R_2 = -Br (92%)

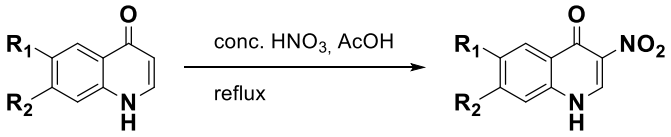
Quinolones **21** and **22** were prepared according to a modified literature procedure[38].

Quinolonecarboxylate **5** or **6** was placed in a round bottom flask, equipped with a reflux condenser and 2N NaOH (c(carboxylate) ≈ 0.05 M) was added. The reaction mixture was heated to reflux for up to four hours, until full consumption of starting material was observed by TLC. Then, the reaction mixture was neutralized with 2N HCl and precipitation occurred. The precipitate was collected by filtration, washed with water and dried *in vacuo*.

Decarboxylation was performed in Ph_2O (c ≈ 0.05 M). A three-neck flask was equipped with a reflux condenser and a balloon and charged with carboxylic acid **19** or **20**. Ph_2O was added and the whole reaction apparatus was purged carefully with argon. Then it was heated to reflux for up to four hours, until full consumption of starting

material was observed via TLC. The reaction mixture was cooled to room temperature and poured on LP, whereupon the desired products precipitated. Product **21** or **22** were collected by filtration, washed with large amounts of LP and dried *in vacuo*.

E II.7 General procedure G for nitration of quinolones



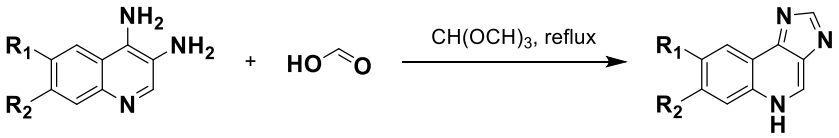
21 R_1 = -OCH_3, R_2 = -H
22 R_1 = -H, R_2 = -Br

23 R_1 = -OCH_3, R_2 = -H
24 R_1 = -H, R_2 = -Br

Nitration of quinolones was carried out according to a modified literature procedure[39].

Quinolone **21** or **22** (1 equiv.) was dissolved in AcOH (c ≈ 0.1 M) in a three-neck flask equipped with a reflux condenser and a dropping funnel. Concentrated HNO_3 (2.2 equiv.) was diluted with conc. AcOH (approx. 1:10) and added to the reaction dropwise. The reaction mixture was heated to reflux for up to one hour until full consumption of starting material was observed by TLC. The reaction mixture was cooled to rt and poured on EtOH, whereupon the product precipitated. Product **23** or **24** was filtered off and washed with small amounts of EtOH and water and dried.

E II.8 General procedure H for synthesis of imidazo[4,5-*c*]quinolines



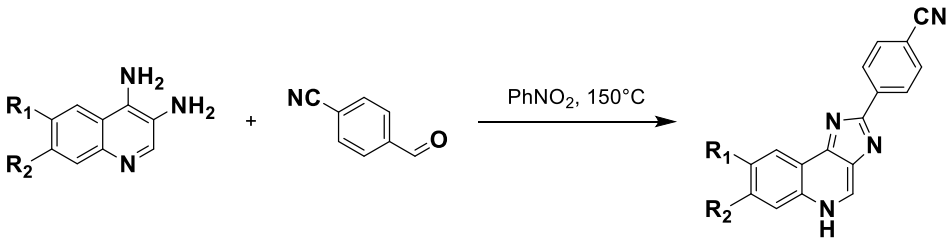
29 R_1 = -OCH_3, R_2 = -H
30 R_1 = -H, R_2 = -Br

33 R_1 = -OCH_3, R_2 = -H
34 R_1 = -H, R_2 = -Br

Imidazo[4,5-*c*]quinolines **33** and **34** were prepared according to a modified literature procedure[49].

Diamine **29** or **30** (1 equiv.) was dissolved in trimethylorthoformiate (c ≈ 0.05 M) in a screw-cap vial and heated until a clear solution was observed. Then, formic acid (1.3 equiv.) was added, whereupon precipitation occurred. The reaction mixture was refluxed until full consumption of starting material was observed by TLC, usually up to five hours. After cooling to rt, Et_2O:EtOH 4:1 was added and the precipitated product was collected by filtration. After washing with several portions of Et_2O, the product was dried *in vacuo*. Purification of products **33** and **34** was purified by column chromatography, using SiO_2 and a gradient of MeOH in CH_2Cl_2 from 5-20%.

E II.9 General procedure I for synthesis of 2-substituted imidazo[4,5-*c*]quinolines using benzaldehydes



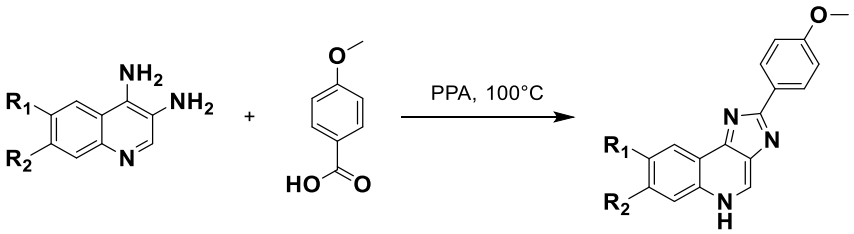
29 R_1 = -OCH_3, R_2 = -H
30 R_1 = -H, R_2 = -Br

39 R_1 = -OCH_3, R_2 = -H
40 R_1 = -H, R_2 = -Br

Imidazo[4,5-c]quinolines **39** and **40** were prepared according to a modified literature procedure[59].

A screw-cap vial was charged with diamine **29** or **30** (1 equiv.) and 4-formylbenzonitrile (1.05 equiv.). After addition of $PhNO_2$ (c ≈ 0.1 M), the reaction mixture was heated to 150°C for up to 24 h, until full consumption of starting material was observed by TLC. Then, the reaction mixture was concentrated on silica and product **39** or **40** was obtained by flash chromatography using SiO_2 and a gradient of MeOH in CH_2Cl_2 from 5-20%.

E II.10 General procedure J for synthesis of 2-substituted imidazo[4,5-*c*]quinolines using benzoic acids



29 R_1 = -OCH_3, R_2 = -H
30 R_1 = -H, R_2 = -Br

35 R_1 = -OCH_3, R_2 = -H
36 R_1 = -H, R_2 = -Br

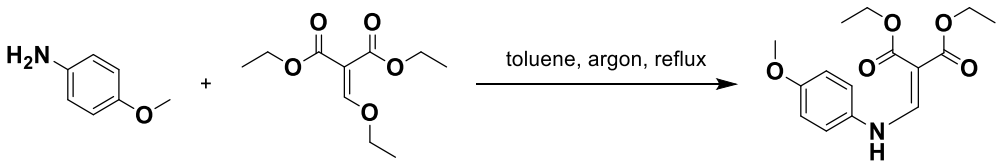
Imidazo[4,5-c]quinolines **35** and **36** were prepared according to a modified literature procedure[49].

A screw-cap vial was charged with diamine **29** or **30** (1 equiv.) and *p*-anisic acid (1.2 equiv.). Then, PPA (1.5 ml/mmol) was added and the reaction mixture was heated to 100°C for up to 24 h, until full consumption of starting material was observed by TLC. The reaction mixture was then poured on H_2O and adjusted to a pH of 9-10 with aqu. NH_4OH (25%), whereupon precipitation occurred. Product **35** or **36** was collected by filtration, washed with H_2O and dried.

E III Chemical synthesis

E III.1 Synthesis of quinolones

E III.1.1 Diethyl 2-(((4-methoxyphenyl)amino)methylene)malonate (3)



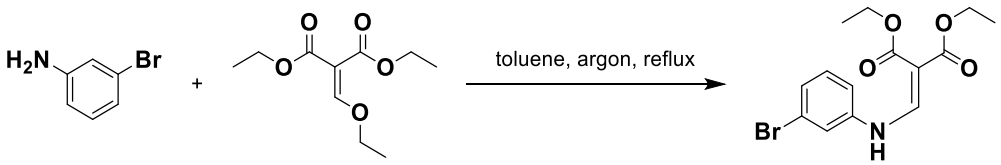
1
M = 123.16

3
M = 293.32

General procedure	A
Reaction scale	15 g (122 mmol) of **1**
Reaction time	8 h
Yield	35.7 g (quant.)
Appearance	yellow-brown solid
Purification	recrystallization with iso-octane
TLC	R_f (LP:EtOAc = 3:1) = 0.34
Sum formula, m.w.	$C_{15}H_{19}NO_5$, 293.32
M.p.	30 – 32°C (lit.[55]: 38 – 40°C)
^{1}H-NMR (400 MHz, $CDCl_3$)	δ = 1.31 (t, *J* = 7.1 Hz, 3H, CH_2-C<u>H</u>$_3$), 1.37 (t, *J* = 7.1 Hz, 3H, CH_2-C<u>H</u>$_3$), 3.79 (s, 3H, -O-C<u>H</u>$_3$), 4.23 (q, *J* = 7.1 Hz, 2H, -C<u>H</u>$_2$-CH_3), 4.29 (q, *J* = 7.1 Hz, 2H, -C<u>H</u>$_2$-CH_3), 6.85 – 6.94 (m, 2H, H3' & H5'), 7.02 – 7.11 (m, 2H, H2' & H6'), 8.43 (d, *J* = 13.8 Hz, 1H, -CH=), 10.98 (d, *J* = 13.8 Hz, 1H, -NH-) ppm.
^{13}C-NMR (101 MHz, $CDCl_3$)	δ = 14.3 (q, CH_2-<u>C</u>H$_3$), 14.4 (q, CH_2-<u>C</u>H$_3$), 55.6 (q, -O-<u>C</u>H$_3$), 60.0 (t, -<u>C</u>H$_2$-CH_3), 60.2 (t, -<u>C</u>H$_2$-CH_3), 92.5 (s, -C=), 115.0 (d, C3' & C5'), 118.8 (d, C2' & C6'), 132.8 (s, C1'), 152.6 (d, N-CH=), 157.2 (s, C4'), 165.8 (s, COOEt), 169.2 (s, COOEt) ppm.

Spectral data are in accordance with the literature[55].

E III.1.2 Diethyl 2-(((3-bromophenyl)amino)methylene)malonate (4)



2
M = 172.03

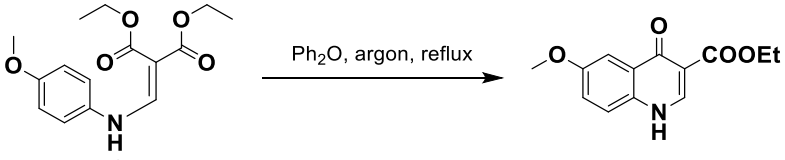
4
M = 342.19

General procedure	A
Reaction scale	15 g (87 mmol) of **2**
Reaction time	24 h
Yield	26 g (90%)
Appearance	yellow needles
TLC	R_f (LP:EtOAc = 3:1) = 0.74

Sum formula, m.w.	$C_{14}H_{16}BrNO_4$, 342.19
M.p.	70 – 71°C (lit.[60]: 70 – 71°C)
^{1}H-NMR (400 MHz, $CDCl_3$)	δ = 1.36 (q, *J* = 7.1 Hz, 6H, CH_2-CH_3), 4.29 (m, 4H, -CH_2-CH_3), 7.03-7.25 (m, 4H, Ar), 8.45 (d, *J* = 13.5 Hz, 1H, -CH=), 10.98 (d, *J* = 13.6 Hz, 1H, -NH-) ppm.
^{13}C-NMR (101 MHz, $CDCl_3$)	δ = 14.3 (q, CH_2-CH_3), 14.4 (q, CH_2-CH_3), 60.3 (t, CH_2-CH_3), 60.6 (t, CH_2-CH_3), 94.7 (s, -C=), 115.8 (d, C6'), 120.1 (d, C2'), 123.5 (s, C3'), 127.7 (d, C4'), 131.1 (d, C5'), 140.6 (s, C1'), 151.2 (d, N-CH=), 165.5 (s, COOEt), 168.9 (s, COOEt) ppm.

Spectral data are in accordance with the literature[60].

E III.1.3 Ethyl 6-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (5)



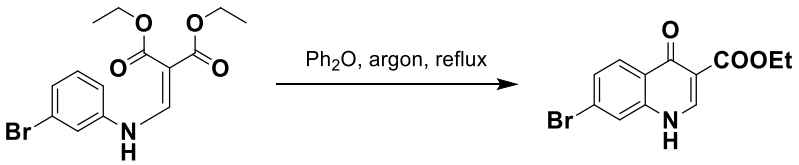
3
M = 293.32

5
M = 247.25

General procedure	B
Reaction scale	14.3 g (49 mmol) of **3**
Reaction time	24 h
Yield	3.79 g (31%)
Appearance	brown solid
Purification	washing with LP
TLC	R_f (CH_2Cl_2 + 2% MeOH) = 0.33
Sum formula, m.w.	$C_{13}H_{13}NO_4$, 247.26
M.p.	258 – 260°C (lit.[61]: 274 – 276°C)
^{1}H-NMR (200 MHz, DMSO-d_6)	δ = 1.28 (t, *J* = 7.1 Hz, 3H, CH_2-CH_3), 3.84 (s, 3H, O-CH_3), 4.21 (q, *J* = 7.1 Hz, 2H, CH_2-CH_3), 7.34 (dd, *J* = 8.9, 2.9 Hz, 1H, H7), 7.51 – 7.65 (m, 2H, H5 & H8), 8.49 (s, 1H, H2), 12.29 (bs, 1H, NH) ppm.
^{13}C NMR (50 MHz, DMSO-d_6)	δ = 14.3 (q, CH_2-CH_3), 55.4 (q, O-CH_3), 59.4 (t, CH_2-CH_3), 105.5 (d, C5) , 118.3 (s, C3), 120.5 (d, C7/8), 122.1 (d, C7/8), 128.4 (s, C4a), 133.3 (s, C8a), 143.6 (d, C2), 156.5 (s, C6), 172.8 (s, COOEt), 214.9 (s, C4) ppm.
^{13}C-NMR (151 MHz, DMSO-d_6) δ =	14.4 (q, CH_2-CH_3), 55.5 (q, O-CH_3), 59.5 (t, CH_2-CH_3), 105.5 (d, C5), 108.7 (s, C3), 120.6 (d, C7/8), 122.2 (d, C7/8), 128.5 (s, C4a), 133.4 (s, C8a), 143.7 (d, C2), 156.6 (s, C6), 165.0 (s, COOEt), 172.9 (s, C4) ppm.

Spectral data are in accordance with the literature[61].

E III.1.4 Ethyl 7-bromo-4-oxo-1,4-dihydroquinoline-3-carboxylate (6)



4
M = 342.19

6
M = 296.12

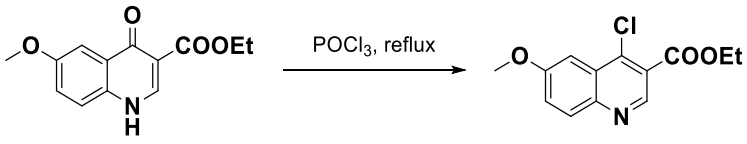
General procedure	B

Reaction scale	26.9 g (79 mmol) of **4**
Reaction time	24 h
Yield	14 g (61%)
Appearance	brown solid
Purification	washing with LP
TLC	R_f (EtOAc) = 0.39
Sum formula, m.w.	$C_{12}H_{10}BrNO_3$, 296.12
M.p.	296.5°C (decomp.) (lit.[62]: 334 – 335°C (decomp.))
^{1}H-NMR (400 MHz, DMSO-d_6)	δ = 1.24 - 1.31 (t, 3H, *J* = 6.9 Hz, CH_2-C<u>H</u>$_3$), 4.20 (q, 2H, *J* = 6.9 Hz, C<u>H</u>$_2$-CH_3), 7.56 (d, 1H, *J* = 8.2 Hz, H6), 7.82 (s, 1H, H8), 8.05 (d, 1H, *J* = 8.5 Hz, H5), 8.58 (s, 1H, CH, H2), 12.30 (s, 1H, NH) ppm.
^{13}C NMR (101 MHz, DMSO-d_6)	14.8 (q, CH_2-<u>C</u>H_3), 60.2 (t, <u>C</u>H_2-CH_3), 110.9 (s, C3), 121.5 (d, C8), 126.2 (s, C4a), 126.6 (s, C7), 128.2 (d, C6), 128.4 (d, C5), 140.4 (s, C8a), 145.9 (d, C2), 165.0 (s, COOEt), 173.3 (s, C4)

Spectral data are in accordance with the literature[62]

E III.2 Synthesis of pyrazolo[4,3-*c*]quinoline-3-ones

E III.2.1 Ethyl 4-chloro-6-methoxyquinoline-3-carboxylate (7)



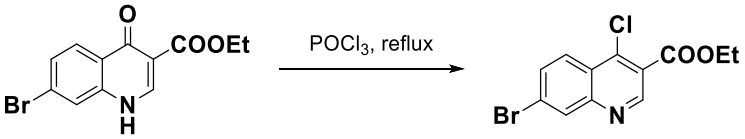
5
M = 247.25

7
M = 265.69

General procedure	C
Reaction scale	1.6 g (6.5 mmol) of **5**
Reaction time	4 h
Yield	1.4 g (81%)
Appearance	yellow-brown solid
Purification	product was obtained pure after work-up
TLC	R_f (LP:EtOAc = 4:1) = 0.29
Sum formula, m.w.	$C_{12}H_9BrClNO_2$, 265.69
M.p.	86 – 87°C (lit.[55]: 84 – 86 °C)
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 1.46 (t, *J* = 7.1 Hz, 3H, CH_2-C<u>H</u>$_3$), 3.99 (s, 3H, O-CH_3), 4.49 (q, *J* = 7.1 Hz, 2H, C<u>H</u>$_2$-CH_3), 7.47 (dd, *J* = 9.1, 2.8 Hz, 1H, H7), 7.59 (d, *J* = 2.8 Hz, 1H, H5), 8.02 (d, *J* = 9.2 Hz, 1H, H8), 9.04 (s, 1H, H2) ppm.
^{13}C NMR (50 MHz, $CDCl_3$)	δ = 14.2 (q, CH_2-<u>C</u>H_3), 55.8 (q, O-CH_3), 62.1 (t, <u>C</u>H_2-CH_3), 102.7 (d, C5), 123.3 (s, C3), 124.9 (d, C7), 127.5 (s, C4a), 131.0 (d, C8), 145.3 (s, C8a), 147.3 (d, C2), 159.4 (s, C6), 164.6 (s, C4), 175.5 (s, COOEt) ppm.

Spectral data are in accordance with the literature[55].

E III.2.2 Ethyl 7-bromo-4-chloroquinoline-3-carboxylate (8)

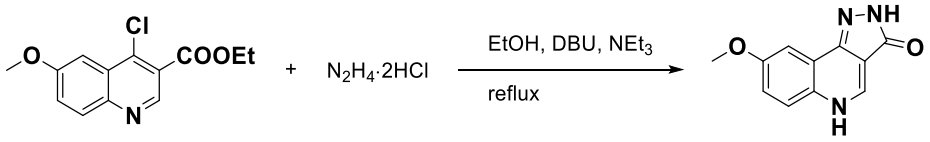


6
M = 296.12

8
M = 314.56

General procedure	C
Reaction scale	296 mg (1.0 mmol) of **6**
Reaction time	4 h
Yield	283 mg (89%)
Appearance	yellow solid
Purification	product was obtained pure after work-up
TLC	R_f (LP:EtOAc = 6:1) = 0.51
Sum formula, m.w.	$C_{12}H_9BrClNO_2$, 314.56
M.p.	91 – 92°C
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 1.46 (t, *J* = 7.2 Hz, 3H, CH_2-C<u>H</u>$_3$), 4.50 (q, *J* = 7.1 Hz, 2H, C<u>H</u>$_2$-CH_3), 7.78 (dd, *J* = 8.9, 2.0 Hz, 1H, H6), 8.19 – 8.38 (m, 2H, H5 & H8), 9.14 – 9.24 (m, 1H, H2) ppm.
^{13}C NMR (101 MHz, $CDCl_3$)	δ = 14.4 (q, CH_2-<u>C</u>H_3), 62.4 (t, <u>C</u>H_2-CH_3), 123.4 (s, C3), 125.2 (s, C4a), 126.9 (s, C7), 127.0 (d, C5), 132.1 (d, C6/C8), 132.3 (d, C6/C8), 143.8 (s, C8a), 150.1 (s, C4), 151.4 (d, C2), 164.3 (s, COOEt) ppm.
HR-ESI-MS	m/z 313.9568 $[M+H]^+$ (calc. 313.9585, diff. -12.86 ppm)

E III.2.3 8-Methoxy-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one (<u>9</u>)



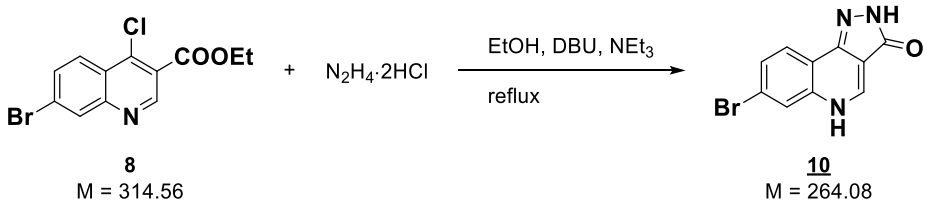
7
M = 265.69

<u>9</u>
M = 215.21

General procedure	D
Reaction scale	quinoline 200 mg (0.76 mmol, 1 equiv.) hydrazine dihydrochloride 95 mg (0.91 mmol, 1.2 equiv.) DBU 138 mg (0.91 mmol, 1.2 equiv.) NEt_3 76 mg (0.76 mmol, 1 equiv.) EtOH 8ml
Reaction time	4 h
Yield	22 mg (14%)
Appearance	yellow solid
Purification	washing with EtOH and LP
TLC	R_f (EtOAc + 30% MeOH) = 0.34
Sum formula, m.w.	$C_{11}H_9N_3O_2$, 215.21
M.p.	325°C (decomp.)
Log P	0.28

^{1}H-NMR (600 MHz, DMSO-d_6)	δ = 3.87 (s, 3H, O-CH_3), 7.21 (d, *J* = 11.6 Hz, 1H, H7), 7.46 (d, *J* = 2.9 Hz, 1H, H9), 7.59 (d, *J* = 9.0 Hz, 1H, H6), 8.45 (s, 1H, H4), 11.35 (s, 1H, NH), 12.43 (s, 1H, NH) ppm.
^{13}C NMR (151 MHz, DMSO-d_6)	δ = 55.6 (q, O-CH_3), 102.5 (d, C9), 104.7 (s, C3a), 118.6 (d, C7), 120.9 (d, C6), 121.0 (s, C9a), 129.3 (s, C5a), 137.2 (d, C4), 142.7 (s, C9b), 157.2 (s, C8), 164.5 (s, C3) ppm.
HRMS	m/z 216.0775 $[M+H]^+$ (calc. 216.0767, diff. -3.53 ppm)

E III.2.4 7-Bromo-2,5-dihydro-3H-pyrazolo[4,3-*c*]quinolin-3-one (10)

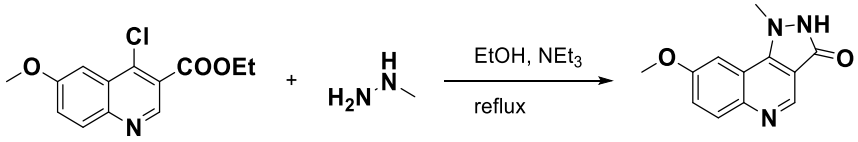


8
M = 314.56

10
M = 264.08

General procedure	D
Reaction scale	quinoline 200 mg (0.64 mmol, 1 equiv.) hydrazine dihydrochloride 80 mg (0.76 mmol, 1.2 equiv.) DBU 116 mg (0.76 mmol, 1.2 equiv.) NEt_3 64 mg (0.64 mmol, 1 equiv.) EtOH 8 ml
Reaction time	24 h
Yield	30 mg (18%)
Appearance	yellow solid
Purification	washing with EtOH and LP
TLC	R_f ($CHCl_3$ + 20% MeOH) = 0.42
Sum formula, m.w.	$C_{10}H_6BrN_3O$, 264.08
M.p.	409°C (decomp.)
Log P	1.24
^{1}H-NMR (600 MHz, DMSO-d_6)	δ = 7.62 (dd, *J* = 8.5, 1.9 Hz, 1H, H8), 7.81 (d, *J* = 1.9 Hz, 1H, H6), 7.98 (d, *J* = 8.5 Hz, 1H, H9), 8.55 (d, *J* = 6.2 Hz, 1H, H4), 11.45 (s, 1H, H2), 12.41 (d, *J* = 6.3 Hz, 1H, H5) ppm.
^{13}C NMR (151 MHz, DMSO-d_6)	δ = 106.4 (s, C3a), 118.7 (s, C9a), 121.4 (d, C9), 121.8 (s, C7), 123.7 (d, C6), 128.8 (d, C8), 136.8 (s, C5a/C9b), 138.8 (d, C5), 142.0 (s, C5a/C9b), 164.4 (s, C3) ppm
HRMS	m/z 263.9773 $[M+H]^+$ (calc. 263.9767, diff. -2.17 ppm)

E III.2.5 8-Methoxy-1-methyl-1,2-dihydro-3H-pyrazolo[4,3-*c*]quinolin-3-one (11)



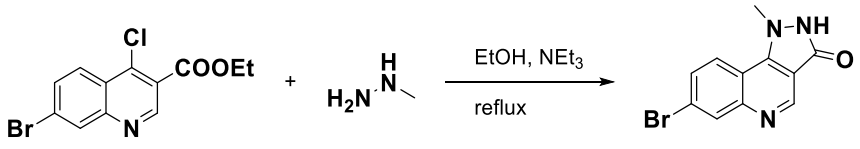
7
M = 265.69

11
M = 229.24

General procedure	D
Reaction scale	quinoline 27 mg (0.1 mmol, 1 equiv.)

	methylhydrazine 8 mg (0.17 mmol, 1.7 equiv.)
	NEt_3 10 mg (0.1 mmol, 1 equiv.)
	EtOH 4 ml
Reaction time	2 h
Yield	18 mg (79%)
Appearance	colorless solid
Purification	washing with EtOH and LP
TLC	R_f (CH_2Cl_2 + 20% MeOH) = 0.23
Sum formula, m.w.	$C_{12}H_{11}N_3O_2$, 229.24
M.p.	324°C (decomp.)
Log P	0.85
^{1}H-NMR (600 MHz, DMSO-d_6)	δ = 3.97 (s, 3H, O-CH_3), 4.28 (s, 3H, N-CH_3), 7.40 (dd, *J* = 9.1, 2.8 Hz, 1H, H7), 7.76 (d, *J* = 2.7 Hz, 1H, H9), 8.00 (d, *J* = 9.1 Hz, 1H, H6), 8.86 (s, 1H, H4), 11.14 (s, 1H, H2) ppm.
^{13}C-NMR (151 MHz, DMSO-d_6)	δ = 38.4 (q, N-CH_3), 55.6 (q, O-CH_3), 103.0 (d, C9), 105.9 (s, C3a), 117.0 (s, C9a), 118.5 (d, C7), 131.2 (d, C6), 138.6 (s, C9b), 140.7 (s, C5a), 142.3 (d, C4), 154.0 (s, C3), 157.2 (s, C8) ppm.
HRMS	m/z 230.0935 $[M+H]^+$ (calc. 230.0924, diff. -4.76 ppm)

E III.2.6 7-Bromo-1-methyl-1,2-dihydro-3H-pyrazolo[4,3-*c*]quinolin-3-one (12)

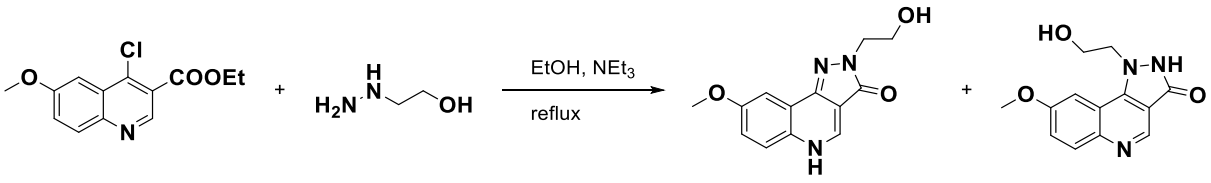


8
M = 314.56

12
M = 278.11

General procedure	D
Reaction scale	quinoline 50 mg (0.16 mmol, 1 equiv.)
	methylhydrazine 12 mg (0.27 mmol, 1.7 equiv.)
	NEt_3 16 mg (0.16 mmol, 1 equiv.)
	EtOH 8 ml
Reaction time	24 h
Yield	42 mg (95%)
Appearance	colorless solid
Purification	washing with EtOH and LP
TLC	R_f (CH_2Cl_2 + 20% MeOH) = 0.45
Sum formula, m.w.	$C_{11}H_8BrN_3O$, 278.11
M.p.	347°C (decomp.)
Log P	1.61
^{1}H-NMR (400 MHz, DMSO-d_6)	δ = 4.24 (s, 3H, -CH_3), 7.82 (dd, *J* = 8.8, 2.2 Hz, 1H, H8), 8.27 (d, *J* = 2.1 Hz, 1H, H6), 8.41 (d, *J* = 8.8 Hz, 1H, H9), 9.03 (s, 1H, H4), 11.35 (bs, 1H, NH) ppm.
^{13}C-NMR (101 MHz, DMSO-d_6)	δ = 38.8 (q, -CH_3), 106.2 (s, C3a), 115.2 (s, C9a), 121.4 (s, C7), 124.5 (d, C9), 129.0 (d, C8), 131.6 (d, C6), 138.3 (s, C5a/C9b), 146.1 (d, C4), 146.5 (s, C5a/C9b), 154.5 (s, C3) ppm.
HRMS	m/z 277.9939 (calc. 277.9923, diff. -5.73 ppm).

E III.2.7 1-(2-Hydroxyethyl)-8-methoxy-1,2-dihydro-3H-pyrazolo[4,3-*c*]quinolin-3-one (<u>17</u>) and 2-(2-hydroxyethyl)-8-methoxy-1,2-dihydro-3H-pyrazolo[4,3-*c*]quinolin-3-one (<u>18</u>)



7
M = 265.69

<u>17</u>
M = 259.27

<u>18</u>
M = 259.27

The isomers were formed in a ratio of **<u>17</u>**:**<u>18</u>**= 1:10, according to ^{1}H-NMR and were separated on a Waters preparative HPLC system.

General procedure	D
Reaction scale	quinoline 270 mg (1 mmol, 1 equiv.) hydroxyethylhydrazine 85 mg (1.12 mmol, 1.1 equiv.) NEt_3 205 mg (2.04 mmol, 2 equiv.) EtOH 20 ml
Reaction time	3 h
Yield	220 mg (84%), mixture of isomers 10:1

2-(2-Hydroxyethyl)-8-methoxy-1,2-dihydro-3H-pyrazolo[4,3-*c*]quinolin-3-one (<u>17</u>):

Appearance	yellow solid
TLC	R_f (CH_2Cl_2 + 20% MeOH) = 0.34
Sum formula, m.w.	$C_{13}H_{13}N_3O_3$, 259.27
M.p.	320°C (decomp.)
Log P	0
^{1}H-NMR (400 MHz, DMSO-d_6)	δ = 3.69 (t, *J* = 6.4 Hz, 2H, C<u>H</u>$_2$-OH), 3.88 (s, 3H, O-CH_3), 3.94 (t, *J* = 6.4 Hz, 2H, N-C<u>H</u>$_2$), 7.21 (dd, *J* = 9.0, 2.9 Hz, 1H, H7), 7.46 (d, *J* = 2.8 Hz, 1H, H9), 7.64 (d, *J* = 9.0 Hz, 1H, H6), 8.34 (s, 1H, H5), 8.52 (s, 1H, H4) ppm.
^{13}C-NMR (151 MHz, DMSO-d_6)	δ = 47.0 (t, N-CH_2), 55.6 (q, O-CH_3), 59.7 (t, CH_2-OH), 102.2 (d, C9), 104.5 (s, C3a), 118.8 (d, C7), 120.4 (s, C9a), 121.6 (d, C6), 130.0 (s, C5a), 137.7 (d, C4), 141.4 (s, C9b), 157.2 (s, C8), 161.8 (s, C3) ppm.
HRMS	m/z 260.1033 $[M+H]^+$ (calc. 260.1030, diff. -1.36 ppm)

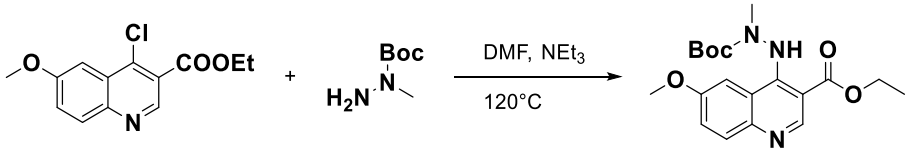
1-(2-Hydroxyethyl)-8-methoxy-1,2-dihydro-3H-pyrazolo[4,3-*c*]quinolin-3-one (<u>18</u>):

Appearance	yellow solid
TLC	R_f (CH_2Cl_2 + 20% MeOH) = 0.13
Sum formula, m.w.	$C_{13}H_{13}N_3O_3$, 259.27
M.p.	320°C (decomp.)
Log P	-0.78
^{1}H-NMR (600 MHz, DMSO-d_6)	δ = 3.88 (q, *J* = 4.8 Hz, 2H, C<u>H</u>$_2$-OH), 3.96 (s, 3H, O-CH_3), 4.65 (t, *J* = 5.8 Hz, 2H, N-CH_2), 5.08 (s, 1H, OH), 7.43 (dd, *J* = 9.1, 2.7 Hz, 1H, H7), 7.86 (d, *J* = 2.8 Hz, 1H, H9), 8.02 (d, *J* = 9.1 Hz, 1H, H6), 8.93 (s, 1H, H4), 11.39 (s, 1H, H2) ppm.

^{13}C-NMR (151 MHz, DMSO-d_6) δ = 54.1 (t, N-CH_2), 56.1 (q, O-CH_3), 60.6 (t, CH_2-OH), 104.2 (d, C9), 106.4 (s, C3a), 117.2 (s, C9a), 119.2 (d, C7), 130.8 (d, C6), 139.6 (s, C5a/C9b*), 140.1 (s, C5a/C9b*), 142.3 (d, C4), 155.5 (s, C3), 157.6 (s, C8) ppm.

HRMS m/z 260.1033 $[M+H]^+$ (calc. 260.1030, diff. -1.36 ppm)

E III.2.8 Ethyl-4-(2-(tert-butoxycarbonyl)-2-methylhydrazono)-6-methoxy-1,4-dihydroquinoline-3-carboxylate (13)



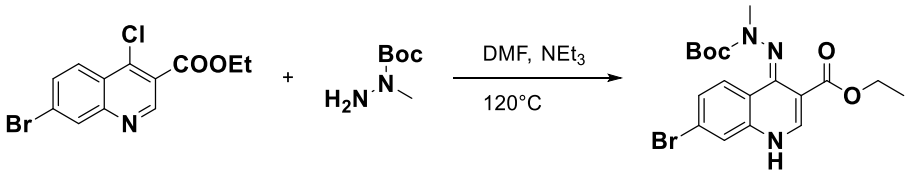
7
M = 265.69

13
M = 375.43

Compound **13** was prepared according to the general procedure. The work-up of the reaction led to decomposition of the product, therefore it was used as crude for the next reaction step.

General procedure	D
Reaction scale	quinoline 200 mg (0.75 mmol, 1 equiv.) 1-boc-1-methylhydrazine 132 mg (0.9 mmol, 1.2 equiv.) NEt_3 74 mg (0.75 mmol, 1 equiv.) DMF 6 ml
Reaction time	24 h
Yield	0.289 g (quantitative, crude)

E III.2.9 Ethyl-7-bromo-4-(2-(tert-butoxycarbonyl)-2-methylhydrazono)-1,4-dihydroquinoline-3-carboxylate (<u>14</u>)



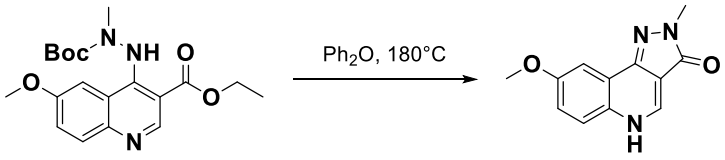
8
M = 314.56

<u>14</u>
M = 424.30

General procedure	D
Reaction scale	quinoline 460 mg (1.5 mmol, 1 equiv.) 1-boc-1-methylhydrazine 256 mg (1.75 mmol, 1.2 equiv.) NEt_3 147 mg (1.5 mmol, 1 equiv.) DMF 250 ml
Reaction time	24 h
Yield	585 mg (95%)
Appearance	yellow solid
Purification	column chromatography using DCM:MeOH as eluent
TLC	R_f (CH_2Cl_2 + 5% MeOH) = 0.52
Sum formula, m.w.	$C_{18}H_{22}BrN_3O_4$, 424.30
M.p.	81.8 – 81.9°C
^{1}H-NMR (400 MHz, $CDCl_3$)	δ = 1.43 (t, *J* = 7.1 Hz, 3H,), 3.33 (s, 3H), 4.42 (q, *J* = 7.1 Hz, 2H), 7.50 (dd, *J* = 9.3, 2.1 Hz, 1H), 8.14 – 8.22 (m, 2H), 9.17 (s, 1H), 10.78 (s, 1H) ppm.

^{13}C-NMR (101 MHz,$CDCl_3$)	δ = 14.4 (q, CH_2-$\underline{C}H_3$), 28.1 (q, C-($\underline{C}H_3)_3$), 38.3 (q, N-CH_3), 61.5 (t, $\underline{C}H_2$-CH_3), 82.3 (s, $\underline{C}$-$(CH_3)_3$), 103.1 (s, C3), 117.0 (s, C4a), 126.0 (d, C5), 126.2 (s, C7), 129.0 (s, C6), 132.5 (s, C8), 151.4 (s, C8a), 152.3 (d, C2), 155.2 (s, C4), 155.4 (s, Boc-C=O), 168.3 (s, C=O) ppm.
HRMS	424.0858 $[M+H]^+$ (calc. 424.0866, diff. 1.89 ppm)

E III.2.10 8-Methoxy-2-methyl-2,5-dihydro-3H-pyrazolo[4,3-*c*]quinolin-3-one (<u>15</u>)

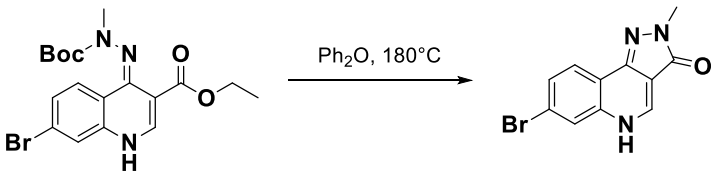


13
M = 375.43

<u>15</u>
M = 229.24

General procedure	E
Reaction scale	**13** used as a crude (m = 162 mg)
Reaction time	24 h
Yield	12 mg (5% over two steps)
Appearance	yellow solid
Purification	washing with EtOH and LP
TLC	R_f (CH_2Cl_2 + 20% MeOH) = 0.40
Sum formula, m.w.	$C_{12}H_{11}N_3O_2$, 229.24
M.p.	323°C (decomp.)
Log P	0.52
^{1}H-NMR (600 MHz, DMSO-d_6)	δ = 3.49 (s, 3H, N-CH_3), 3.89 (s, 3H, O-CH_3), 7.24 (dd, *J* = 9.0, 2.9 Hz, 1H, H7), 7.47 (d, *J* = 2.8 Hz, 1H, H9), 7.63 (d, *J* = 9.0 Hz, 1H, H6), 8.55 (d, *J* = 6.4 Hz, 1H, H4), 12.64 (d, *J* = 6.5 Hz, 1H, H5) ppm.
^{13}C-NMR (151 MHz, DMSO-d_6)	δ = 31.6 (q, N-CH_3), 55.6 (q, O-CH_3), 102.2 (d, C9), 104.4 (s, C3a), 119.0 (d, C7), 120.2 (s, C9a), 121.1 (d, C6), 129.3 (s, C5a), 137.1 (d, C4), 141.1 (s, C9b), 157.4 (s, C8), 161.6 (s, C3) ppm.
HRMS	m/z 230.0931 $[M+H]^+$ (calc. 230.0924, diff. -3.18 ppm)

E III.2.11 7-Bromo-2-methyl-2,5-dihydro-3H-pyrazolo[4,3-*c*]quinolin-3-one (<u>16</u>)



<u>14</u>
M = 424.30

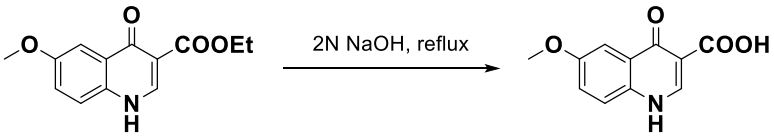
<u>16</u>
M = 278.11

General procedure	E
Reaction scale	80 mg (0.19 mmol) of **<u>14</u>** Ph_2O (8 ml)
Reaction time	24 h
Yield	18 mg (34%)
Appearance	yellow solid
Purification	washing with EtOH and LP

TLC	R_f (CH_2Cl_2 + 5% MeOH) = 0.42
Sum formula, m.w.	$C_{11}H_8BrN_3O$, 278.11
M.p.	323°C (decomp.)
Log P	1.47
^{1}H-NMR (600 MHz, DMSO-d_6)	δ = 3.46 (s, 3H, N-CH_3), 7.64 (dd, *J* = 8.5, 1.9 Hz, 1H, H8), 7.83 (d, *J* = 1.9 Hz, 1H, H6), 8.00 (d, *J* = 8.6 Hz, 1H, H9), 8.65 (s, 1H, H4), 12.60 (s, 1H, H5) ppm.
^{13}C-NMR (151 MHz, DMSO-d_6)	δ = 31.6 (q, N-CH_3), 106.0 (s, C3a), 118.0 (s, C9a), 121.5 (d, C9), 121.9 (s, C7), 123.7 (d, C6), 129.0 (d, C8), 136.1 (s, C5a/C9b), 138.9 (d, C4), 140.5 (s, C5a/C9b), 161.6 (s, C=O) ppm.
HRMS	m/z 277.9915 [M+H]+ (calc. 277.9923, diff. 3.13 ppm)

E III.3 Synthesis of imidazo[4,5-*c*]quinoline

E III.3.1 6-Methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (19)



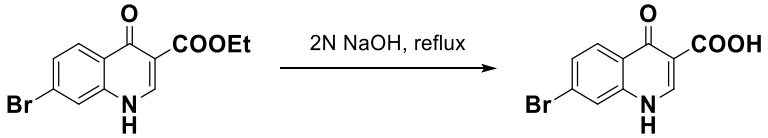
5
M = 247.25

19
M = 219.20

General procedure	F
Reaction scale	3.79g (15 mmol) of **5**
Reaction time	4 h
Yield	2.9 g (86%)
Appearance	colorless solid
Purification	washing with H_2O
TLC	R_f (CH_2Cl_2 + 20% MeOH + 1% AcOH) = 0.78
Sum formula, m.w.	$C_{11}H_9NO_4$, 219.20
M.p.	278 – 279°C (lit.[63]: 278 – 279°C (decomp.))
^{1}H-NMR (400 MHz, DMSO-d_6)	δ = 3.90 (s, 3H, O-CH_3), 7.52 (dd, *J* = 9.1, 2.9 Hz, 1H, H7), 7.63 (d, *J* = 2.9 Hz, 1H, H5), 7.78 (d, *J* = 9.1 Hz, 1H, H8), 8.81 (s, 1H, H2), 13.43 (s, 1H, NH), 15.54 (s, 1H, COOH) ppm.
^{13}C-NMR (101 MHz, DMSO-d_6)	δ = 55.7 (q, O-CH_3), 104.1 (d, C5), 106.9 (s, C3), 121.4 (d, C7), 124.4 (d, C8), 125.7 (s, C4a), 134.1 (s, C8a), 143.4 (d, C2), 157.4 (s, C6), 166.6 (s, COOH), 177.5 (s, C4) ppm.

Spectral data is in accordance with the literature[64].

E III.3.2 7-Bromo-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (20)



6
M = 296.12

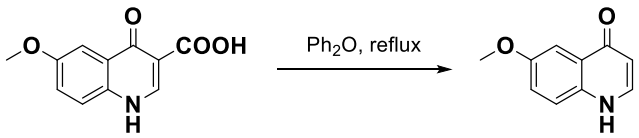
20
M = 268.07

General procedure	F
Reaction scale	8.0 g (27 mmol) of **6**

Reaction time	4 h
Yield	6.3 g (87%)
Appearance	colorless solid
Purification	washing with H_2O
TLC	R_f (CH_2Cl_2 + 20% MeOH + 1% AcOH) = 0.77
Sum formula, m.w.	$C_{10}H_6BrNO_3$, 268.07
M.p.	287.5 – 289.7 °C (lit.[65]: 296°C (decomp.))
^{1}H-NMR (400 MHz, DMSO-d_6)	δ = 7.73 (dd, *J* = 8.7, 1.9 Hz, 1H, H6), 8.00 (d, *J* = 1.8 Hz, 1H, H8), 8.18 (d, *J* = 8.7 Hz, 1H, H5), 8.93 (s, 1H, H2), 13.39 (s, 1H, NH), 15.05 (s, 1H, COOH) ppm.
^{13}C-NMR (101 MHz, DMSO-d_6)	δ = 108.1 (s, C3), 121.9 (d, C8), 123.4 (s, C7), 127.2 (d, C5), 127.4 (s, C4a), 129.2 (d, C6), 140.3 (s, C8a), 146.0 (d, C2), 166.0 (s, COOH), 178.0 (C4) ppm.

Spectral data is in accordance with the literature[66].

E III.3.3 6-Methoxyquinolin-4(1H)-one (21)



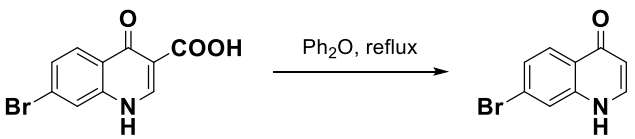
19
M = 219.20

21
M = 175.19

General procedure	F
Reaction scale	2.9 g (13 mmol) of **19**
Reaction time	4 h
Yield	2.23 g (96%)
Appearance	colorless solid
Purification	washing with LP
TLC	R_f (CH_2Cl_2 + 20% MeOH) = 0.48
Sum formula, m.w.	$C_{10}H_9NO_2$, 175.19
M.p.	250 – 251°C (lit.[67]: 251 – 252°C)
^{1}H-NMR (400 MHz, DMSO-d_6)	δ = 3.82 (s, 3H, O-CH_3), 6.00 (d, *J* = 7.3 Hz, 1H, H3), 7.28 (dd, *J* = 9.0, 3.0 Hz, 1H, H7), 7.46 – 7.54 (m, 2H, H5 & H8), 7.85 (d, *J* = 7.2 Hz, 1H, H2), 11.75 (s, 1H, NH) ppm.
^{13}C-NMR (101 MHz, DMSO-d_6)	δ = 55.3 (q, O-CH_3), 104.2 (d, C5), 107.5 (d, C3), 120.0 (d, C7), 122.1 (d, C8), 126.8 (s, C4a), 134.7 (s, C8a), 138.4 (d, C2), 155.4 (s, C6), 176.2 (s, C4) ppm.

Spectral data is in accordance with the literature[67].

E III.3.4 7-Bromoquinolin-4(1H)-one (22)



20
M = 268.07

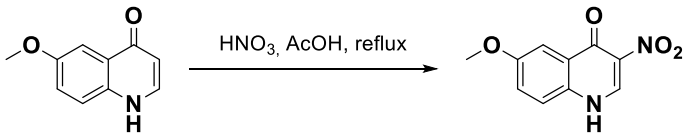
22
M = 224.06

General procedure	F
Reaction scale	10.4 g (39 mmol) of **20**
Reaction time	4 h

Yield	8 g (92%)
Appearance	colorless solid
Purification	washing with LP
TLC	R_f ($CHCl_3$ + 5% MeOH) = 0.17
Sum formula, m.w.	C_9H_6BrNO, 224.06
M.p.	345°C (decomp.) (lit.[60]: 289 – 291°C)
^{1}H-NMR (400 MHz, DMSO-d_6)	δ = 6.06 (d, *J* = 7.4 Hz, 1H, H3), 7.45 (dd, *J* = 8.6, 1.9 Hz, 1H, H6), 7.76 (d, *J* = 1.9 Hz, 1H, H8), 7.90 – 8.05 (m, 2H, H2 & H5), 11.85 (s, 1H, NH) ppm.
^{13}C-NMR (101 MHz, DMSO-d_6)	δ = 109.3 (d, C3), 120.5 (d, C8), 124.6 (s, C4a/C7), 125.0 (s, C4a/C7), 126.1 (d, C5/C6), 127.3 (d, C6/C5), 139.9 (d, C2), 141.0 (s, C8a), 176.3 (s, C4) ppm.

Spectral data is in accordance with the literature[66].

E III.3.5 6-Methoxy-3-nitroquinolin-4(1H)-one (23)



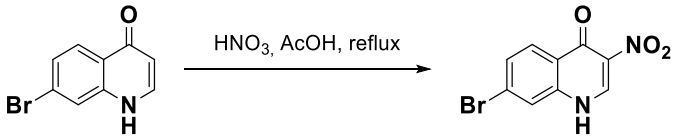
21
M = 175.19

23
M = 220.18

General procedure	G
Reaction scale	2.2 g (13 mmol) of **21**
Reaction time	1 h
Yield	2.1 g (75 %)
Appearance	yellow-brown solid
Purification	washing with EtOH
TLC	R_f (CH_2Cl_2 + 20% MeOH) = 0.22
Sum formula, m.w.	$C_{10}H_8N_2O_4$, 220.18
M.p.	320°C (decomp.) (lit.[68]: >325°C)
^{1}H-NMR (200 MHz, DMSO-d_6)	δ = 3.88 (s, 3H, O-CH_3), 7.42 (dd, *J* = 9.0, 3.0 Hz, 1H, H7), 7.59 – 7.75 (m, 2H, H5 & H8), 9.12 (s, 1H, H2), 12.99 (s, 1H, NH) ppm.
^{13}C-NMR (101 MHz, DMSO-d_6)	δ = 55.6 (q, O-CH_3), 106.0 (d, C5), 121.2 (d, C7/C8), 122.9 (d, C7/C8), 129.5 (s, C4a), 130.3 (s, C3), 132.6 (s, C8a), 140.9 (d, C2), 157.4 (s, C6), 167.0 (s, C4) ppm.

Spectral data is in accordance with the literature[69].

E III.3.6 7-Bromo-3-nitroquinolin-4(1H)-one (24)



22
M = 224.06

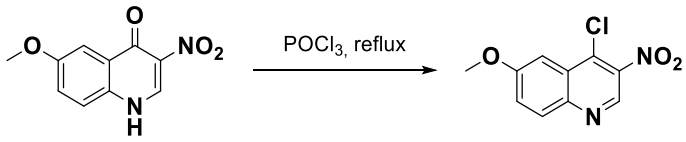
24
M = 269.05

General procedure	G
Reaction scale	5.0 g (22 mmol) of **22**
Reaction time	1 h
Yield	4.2 g (70%)

Appearance	yellow-brown solid
Purification	washing with EtOH
TLC	R_f (CH_2Cl_2 + 20% MeOH) = 0.14
Sum formula, m.w.	$C_9H_5BrN_2O_3$, 269.05
M.p.	384.3°C (decomp.)
^{1}H-NMR (200 MHz, DMSO-d_6)	δ = 7.68 (dd, *J* = 8.7, 1.9 Hz, 1H, H6), 7.91 (d, *J* = 1.8 Hz, 1H, H8), 8.16 (d, *J* = 8.7 Hz, 1H, H5), 9.24 (s, 1H, H2), 12.98 (s, 1H, NH) ppm.
^{13}C-NMR (101 MHz, DMSO-d_6)	δ = 121.8 (d, C8), 126.6 (s, C7), 127.1 (s, C4a), 128.2 (d, C5/C6), 128.8 (d, C5/C6), 131.4 (s, C3), 139.3 (s, C8a), 143.0 (d, C2), 167.2 (C4) ppm.

Spectral data is in accordance with the literature[70].

E III.3.7 4-Chloro-6-methoxy-3-nitroquinoline (25)



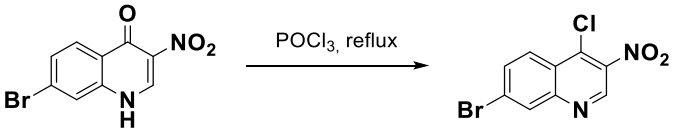
23
M = 220.18

25
M = 238.63

General procedure	H
Reaction scale	2.00 g (9.1 mmol) of **23**
Reaction time	30 min.
Yield	0.9 g (42%)
Appearance	yellow solid
Purification	was obtained pure after work-up
TLC	R_f (CH_2Cl_2 + 20% MeOH) = 0.74
Sum formula, m.w.	$C_{10}H_7ClN_2O_3$, 238.63
M.p.	113 – 114°C (lit.[69]: 281 – 283°C)
^{1}H-NMR (400 MHz, $CDCl_3$)	δ = 4.03 (s, 3H, O-CH_3), 7.52 – 7.61 (m, 2H, H5 & H8), 8.10 (dd, *J* = 8.7, 1.0 Hz, 1H, H7), 9.09 (s, 1H, H2) ppm.
^{13}C-NMR (151 MHz, $CDCl_3$)	δ = 56.1 (q, O-CH_3), 103.1 (d, C5), 126.3 (d, C7), 127.2 (s, C4a), 131.9 (d, C8), 134.3 (s, C3), 141.9 (d, C2), 145.4 (s, C8a), 160.5 (s, C6) ppm. Signal of C3 not detectable.

Spectral data is in accordance with the literature[69].

E III.3.8 7-Bromo-4-chloro-3-nitroquinoline (26)



24
M = 269.05

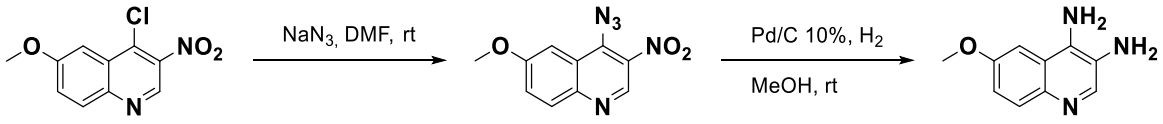
26
M = 287.50

General procedure	H
Reaction scale	700 mg (2.6 mmol) of **24**
Reaction time	4 h
Yield	680 mg (91%)
Appearance	yellow-brown solid
Purification	was obtained pure after work-up

TLC	R_f (CH_2Cl_2 + 5% MeOH) = 0.89
Sum formula, m.w.	$C_9H_4BrClN_2O_2$, 287.50
M.p.	150.9 – 151.1°C
^{1}H-NMR (400 MHz, $CDCl_3$)	δ = 7.90 (dd, *J* = 9.0, 1.9 Hz, 1H, H6), 8.30 (d, *J* = 9.0 Hz, 1H, H5), 8.41 (d, *J* = 1.9 Hz, 1H, H8), 9.26 (s, 1H, H2) ppm.
^{13}C-NMR (101 MHz, CDl_3)	δ = 124.54 (s, C7), 127.33 (d, C5), 128.41 (s, C4a), 132.75 (d, C6/C8), 133.47 (d, C6/C8), 136.95 (s, C4), 145.77 (d, C2), 149.72 (s, C8a) ppm. Signal of C3 not detectable.

Spectral data is in accordance with the literature[71].

E III.3.9 6-Methoxyquinoline-3,4-diamine (29)



25 M = 238.63 **27** M = 245.20 **29** M = 189.22

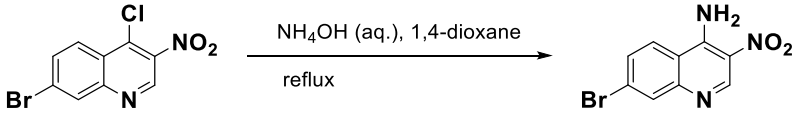
The title compound was prepared according to a modified literature procedure[40].

A round bottom flask was charged with 4-chloro-6-methoxy-3-nitroquinoline (0.8 g, 3.3 mmol, 1 equiv.) and dissolved in DMF (25 ml). NaN_3 (0.44 g, 6.6 mmol, 2 equiv.) was added and the reaction mixture was stirred 20 min. at rt. The solvent was removed in vacuo and the residue was dissolved in DCM. The organic layer was washed with water and brine several times and dried with $MgSO_4$. The solvent was removed in vacuo and the product was obtained in good purity and quantitative yield. Due to the instability of the azide group it was immediately used for the next reaction step.

4-Azido-6-methoxy-3-nitroquinoline (0.84 g, 3.4 mmol) was dissolved in MeOH and after addition of Pd/C (10% Pd, 0.084 g) the reaction was set under a hydrogen atmosphere (1 bar) and stirred at rt for 24h. The reaction mixture was filtered through a bed of Celite. The product was obtained after evaporation of the solvent.

Yield	0.5 g (77%)
Appearance	brown high viscous oil
Purification	column chromatography with DCM:MeOH as eluent
TLC	R_f (CH_2Cl_2 + 20% MeOH) = 0.1
Sum formula, m.w.	$C_{10}H_{11}N_3O$, 189.22
^{1}H-NMR (400 MHz, DMSO-d_6)	δ = 3.85 (s, 3H, O-CH_3), 4.69 (s, 2H, NH_2), 5.67 (s, 2H, NH_2), 6.95 (dd, *J* = 9.1, 2.7 Hz, 1H, H7), 7.30 (d, *J* = 2.8 Hz, 1H, H5), 7.54 (d, *J* = 9.1 Hz, 1H, H8), 8.06 (s, 1H, H2) ppm.
^{13}C-NMR (101 MHz, CD_3OD)	δ = 56.0 (q, O-CH_3), 100.1 (d, C5), 119.8 (d, C7), 120.7 (s, C4a), 125.4 (s, C3), 130.1 (d, C8), 137.9 (s, C4a), 140.3 (d, C2), 140.5 (s, C8a), 158.4 (s, C6) ppm.
HRMS	190.0982 $[M+H]^+$ (calc. 190.0975, diff. -3.7 ppm)

E III.3.10 7-Bromo-3-nitroquinolin-4-amine (32)



26
M = 287.50

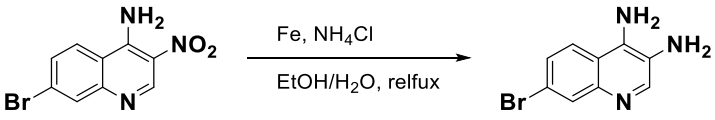
32
M = 268.07

The title compound was prepared according to a modified literature procedure[47].

7-Bromo-4-chloro-3-nitroquinolin (**26**) (0.679 g, 2.4 mmol) was dissolved in 1,4-dioxane (5 ml) and after addition of aqu. NH_4OH (25%, 5 ml) the reaction mixture was heated to reflux for 1h. Then, the reaction mixture was concentrated *in vacuo* and dissolved in EtOAc (100 ml) and washed with water and brine (100 ml each). The organic layer was dried over $MgSO_4$ and after evaporation of the solvent the product **32** was obtained.

Yield	0.64 g (quant.)
Appearance	yellow solid
Purification	was obtained pure after work-up
TLC	R_f ($CHCl_3$ + 5% MeOH) = 0.44
Sum formula, m.w.	$C_9H_6BrN_3O_2$ 268.07
M.p.	245 – 246°C
^{1}H-NMR (400 MHz, DMSO-d_6)	δ = 7.76 (dd, *J* = 8.9, 2.1 Hz, 1H, H6), 8.05 (d, *J* = 2.1 Hz, 1H, H8), 8.51 (d, *J* = 9.0 Hz, 1H, H5), 9.05 (s, 2H, NH_2), 9.15 (s, 1H, H2) ppm.
^{13}C-NMR (101 MHz, DMSO-d_6)	δ = 117.8 (s, C4a), 123.4 (s, C7), 126.4 (s, C3), 126.4 (d, C5), 129.2 (d, C6), 131.4 (d, C8), 148.2 (d, C2), 148.6 (s, C4/C8a), 149.1 (s, C4/8a) ppm.
HRMS	m/z 267.9724 $[M+H]^+$ (calc. 267.9716, diff.: -2.96 ppm)

E III.3.11 7-Bromoquinoline-3,4-diamine (30)



32
M = 268.07

30
M = 238.09

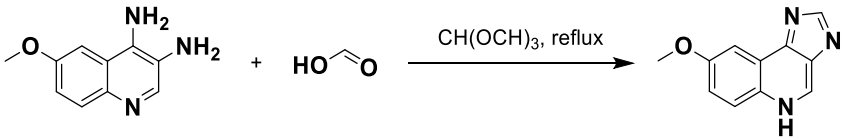
The title compound was prepared according to a modified literature protocol[48].

7-Bromo-3-nitro-quinolin-4-amine (**32**) (0.51 g, 1.9 mmol, 1 equiv.) was dissolved in EtOH:H_2O (4:1, 20 ml) and after addition of Fe (1.05 g, 19 mmol, 10 equiv.) and NH_4Cl (0.1 g, 1.9 mmol, 1 equiv.) heated to reflux for 4 h. The reaction mixture was filtered through a bed of Celite and adsorbed on silica. The product **30** was obtained by flash chromatography using 30 g of SiO_2 and a gradient of EtOAc:MeOH 10:1-4:1 (+3% NEt_3).

Yield	0.32 g (70%)
Appearance	yellow-brown solid
Purification	column chromatography with EtOAc:MeOH as eluent
TLC	R_f (EtOAc:MeOH = 10:1, +3% NEt_3) = 0.25
Sum formula, m.w.	$C_9H_8BrN_3$, 238.09
M.p.	126 – 128°C
^{1}H-NMR (400 MHz, DMSO-d_6)	δ = 4.84 (bs, 2H, NH_2), 6.03 (s, 3H, NH_2), 7.39 (dd, *J* = 9.0, 2.1 Hz, 1H, H6), 7.81 (d, *J* = 2.1 Hz, 1H, H8), 7.97 (d, *J* = 9.0 Hz, 1H, H5), 8.19 (s, 1H, H2) ppm.

^{13}C-NMR (101 MHz, DMSO-d_6)	δ = 117.2 (s, C4a/C7), 117.4 (s, C4a/C7)), 123.4 (d, C5), 125.8 (s, C3), 126.2 (d, C6), 130.2 (d, C8), 134.3 (s, C4), 141.6 (d, C2), 143.4 (s, C8a) ppm.
HRMS	m/z 237.9983 $[M+H]^+$ (calc. 237.9974, diff.: -3,71 ppm)

E III.3.12 8-Methoxy-5H-imidazo[4,5-*c*]quinoline (33)

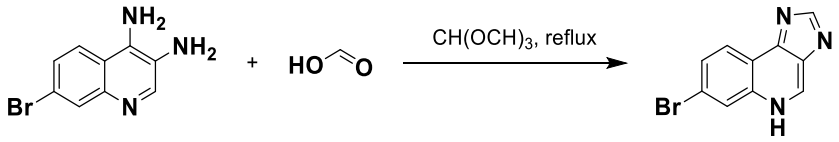


29
M = 189.22

33
M = 199.21

General procedure	I
Reaction scale	100 mg (0.53 mmol) of **29**
Reaction time	5 h
Yield	50 mg (47%)
Appearance	red solid
Purification	column chromatography with DCM:MeOH as eluent
TLC	R_f (CH_2Cl_2 + 20% MeOH) = 0.52
Sum formula, m.w.	$C_{11}H_9N_3O$, 199.21
M.p.	256 – 258°C
Log P	1.36
^{1}H-NMR (400 MHz, Methanol-d_4)	δ = 3.99 (s, 3H, O-CH_3), 7.32 (dd, *J* = 9.2, 2.8 Hz, 1H, H7), 7.74 (s, 1H, H9), 8.00 (d, *J* = 9.2 Hz, 1H, H6), 8.41 (s, 1H, H2), 8.97 (s, 1H, H4) ppm.
^{13}C-NMR (101 MHz, Methanol-d_4)	δ = 56.17 (q, O-CH_3), 101.35 (d, C9), 120.65 (d, C7), 131.06 (d, C6), 140.16 (s, C8a), 143.62 (d, C2), 160.03 (s, C8) ppm. The signals of C3a, C4, C9a and C9b were not detectable[1].
HRMS	m/z 200.0826 $[M+H]^+$ (calc. 200.0818, diff.: -3.97 ppm)

E III.3.13 7-Bromo-5H-imidazo[4,5-*c*]quinoline (34)



30
M = 238.09

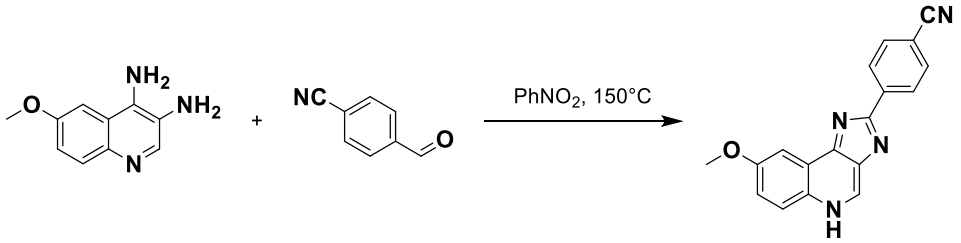
34
M = 248.08

General procedure	I
Reaction scale	20 mg (0.08 mmol) of **30**
Reaction time	5 h
Yield	14 mg (57%)
Appearance	colorless solid
Purification	column chromatography with DCM:MeOH as eluent
TLC	R_f (CH_2Cl_2 + 20% MeOH) = 0.59
Sum formula, m.w.	$C_{10}H_6BrN_3$, 248.08
M.p.	312 – 313°C

[1] Various ^{13}C-NMR experiments were carried out, using different relaxation times (T1). Nevertheless, the mentioned signals were not detectable.

Log P	2.31
^{1}H-NMR (600 MHz, DMSO-d_6)	mixture of isomers, δ = 7.85 (d, *J* = 8.3 Hz, 1H,), 8.32 (d, *J* = 2.0 Hz, 1H, H6), 8.35 (d, *J* = 8.7 Hz, 1H,), 8.55 (s, 1H,H2), 9.25 (s, 1H, H4), 13.40, 13.89 (s, 1H, H1 or H5) ppm.
^{13}C-NMR (151 MHz, DMSO-d_6)	mixture of isomers, δ = 119.8 (s, C9a), 123.6 (d, C2), 126.9 (d, C9), 128.2 (d), 129.4 (d, C8), 131.4 (d, C6), 142.8 (s, C3a/9b), 143.9 (s, C3a/9b), 144.2 (s, C5a) ppm.
HRMS	m/z 247.9819 $[M+H]^+$ (calc. 247.9818, diff.: -0.59 ppm)

E III.3.14 4-(8-Methoxy-5H-imidazo[4,5-*c*]quinolin-2-yl)benzonitrile (39)

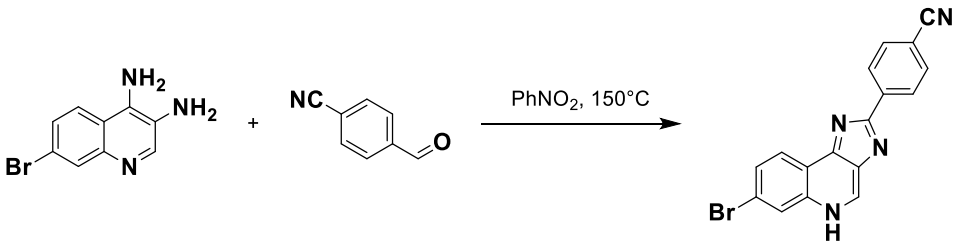


29
M = 189.22

39
M = 300.32

General procedure	J
Reaction scale	45 mg (0.24 mmol) of **29**
Reaction time	24 h
Yield	37 mg (52%)
Appearance	red solid
Purification	column chromatography with DCM:MeOH as eluent
TLC	R_f (CH_2Cl_2 + 20% MeOH) = 0.76
Sum formula, m.w.	$C_{18}H_{12}N_4O$, 300.32
M.p.	143 – 145°C
Log P	3.96
^{1}H-NMR (600 MHz, DMSO-d_6)	δ = 3.98 (s, 3H, O-CH_3), 7.32 (dd, *J* = 9.1, 2.9 Hz, 1H, H7'), 7.84 (d, *J* = 2.8 Hz, 1H, H9'), 8.02 (d, *J* = 9.1 Hz, 1H, H6'), 8.08 (d, *J* = 8.5 Hz, 2H, H2 & H6), 8.41 (d, *J* = 8.2 Hz, 2H, H4 & H5), 9.09 (s, 1H, H4') 13.93 (s, 1H, H5') ppm.
^{13}C-NMR (151 MHz, DMSO-d_6)	mixture of regioisomers δ = 55.60 (q, O-CH_3), 100.80 (d, C9'), 112.19 (s, C1), 118.59 (s, CN), 118.82 (d, C7'), 123.75 (d, C6'), 127.21 (d, C3 & C5), 129.28 (s, C4), 131.13 (d, C4'), 133.11 (d, C2 & C6), 133.89 (s, C3a'), 139.20 (s, C4a'), 149.80 (s, C9b'), 157.60 (s, C8') ppm.
HRMS	m/z 301.1088 $[M+H]^+$ (calc. 301.1084, diff.: -1.32 ppm)

E III.3.15 4-(7-Bromo-5H-imidazo[4,5-*c*]quinolin-2-yl)benzonitrile (40)

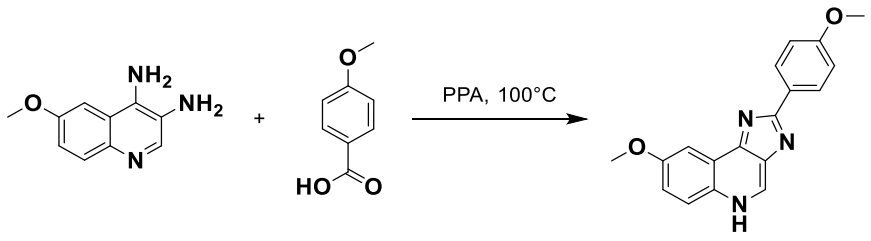


30
M = 238.09

40
M = 349.19

General procedure	J
Reaction scale	50 mg of **30**
Reaction time	4 h
Yield	52 mg (69 %)
Appearance	yellow solid
Purification	column chromatography with DCM:MeOH as eluent
TLC	R_f (CH_2Cl_2 + 5% MeOH) = 0.39
Sum formula, m.w.	$C_{17}H_9BrN_4$, 349.19
M.p.	350°C (decomp.)
Log P	4.91
^{1}H-NMR (400 MHz, DMSO-d_6)	δ = 7.82 (dd, *J* = 8.7, 2.0 Hz, 1H, H8'), 8.03 (d, *J* = 8.2 Hz, 2H, H3 & H5), 8.26 (d, *J* = 1.9 Hz, 1H, H6'), 8.36 (d, *J* = 8.5 Hz, 3H, H2, H6 & H9'), 9.22 (s, 1H, H4') ppm.
^{13}C-NMR (101 MHz, DMSO-d_6)	δ = 112.7 (s, C1), 118.5 (s, CN), 119.0 (s, C7'), 120.5 (s, C9a'), 124.3 (d, C4'), 127.7 (d C3 & C5), 129.9 (d, C9'), 131.9 (d, C2 & C6), 133.5 (d, C8'), 134.3 (s, C4), 139.2 (s, C3a'), 143.8 (s, C5a'), 144.8 (s, C2'), 151.3 (s, C9b') ppm.
HRMS	m/z 349.0092 $[M+H]^+$ (calc. 349.0083, diff.: -2.53 ppm)

E III.3.16 8-Methoxy-2-(4-methoxyphenyl)-5H-imidazo[4,5-*c*]quinoline (35)



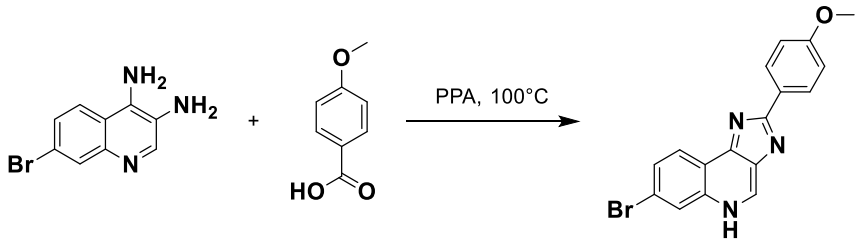
29
M = 189.22

35
M = 305.34

General procedure	K
Reaction scale	54 mg (0.29 mmol) of **29**
Reaction time	7 h
Yield	24 mg (28%)
Appearance	red oil
Purification	column chromatography with DCM:MeOH as eluent
TLC	R_f (CH_2Cl_2 + 20% MeOH) = 0.72
Sum formula, m.w.	$C_{18}H_{15}N_3O_2$, 305.34
M.p.	143 – 145°C

Log P	3.8
^{1}H-NMR (600 MHz, DMSO-d_6)	δ = 3.86 (s, 3H, O-CH_3'), 3.98 (s, 3H, O-CH_3), 7.17 (d, *J* = 8.8 Hz, 2H, H3' & H5'), 7.29 (dd, *J* = 9.1, 2.9 Hz, 1H, H7), 7.82 – 7.87 (m, 1H, H9), 7.99 (d, *J* = 9.1 Hz, 1H, H), 8.21 (d, *J* = 8.3 Hz, 2H, H2' & H6'), 9.02 (s, 1H, H4), 13.55 (s, 1H, H5) ppm.
^{13}C-NMR (151 MHz, DMSO-d_6)	δ = 55.43 (q, O-CH_3'), 55.55 (q, O-CH_3), 100.66 (d, C9), 114.54 (d, C3' & C5'), 118.42 (d, C6/C7), 122.21 (s, C9a), 128.30 (d, C2' & C6'), 131.14 (d, C4), 134.66 (s, C1'), 137.7 (s, C5a), 139.18 (s, C3a), 141.39 (s, C2), 151.61 (s, C9b), 157.34 (s, C8), 160.91 (s, C4') ppm.
HRMS	m/z 306.1231 $[M+H]^+$ (calc. 306.1237, diff.: 1.82 ppm)

E III.3.17 7-bromo-2-(4-methoxyphenyl)-5H-imidazo[4,5-*c*]quinoline (36)



30
M = 238.09

36
M = 354.21

General procedure	K
Reaction scale	50 mg (0.29 mmol) of **30**
Reaction time	5 h
Yield	57 mg (77%)
Appearance	brown solid
Purification	column chromatography with DCM:MeOH as eluent
TLC	R_f (CH_2Cl_2 + MeOH) = 0.76
Sum formula, m.w.	$C_{17}H_{12}BrN_3O$, 354.21
M.p.	108 – 110°C
Log P	4.75
^{1}H-NMR (400 MHz, DMSO-d_6)	mixture of isomers, δ = 3.85 (s, 3H, O-CH_3'), 7.15 (d, *J* = 8.8 Hz, 2H, H3' & H5'), 7.80 (m, 1H, H7), 8.19 (m, 2H, H2' & H6'), 8.27 (d, *J* = 2.0 Hz, 1H, H5), 8.40 (d, *J* = 8.8 Hz, 1H, H8), 9.15 or 9.21 (s, 1H, H4), 13.62 or 13.75 (s, 1H, H1/2 or H5) ppm.
^{13}C-NMR (101 MHz, DMSO-d_6)	mixture of isomers, δ = 55.39 (q, O-CH_3'), 113.72 (s, C3' & C5'), 114.51 (d, C6), 119.61 (s, C7), 121.86 (s, C9a), 123.72 (d, C4), 128.31 (d, C2' & C6'), 129.08 (d, C9), 131.44 (d, C8), 137.81, 138.33 (s, C1'), 144.18 (s, C3a), 145.08 (s, C5a), 151.97 (s, C2), 153.06 (s, C9b), 161.02 (s, C4') ppm.
HRMS	m/z 354.0237 $[M+H]^+$ (calc. 354.0236, diff.: 0.01 ppm)

F Appendix

F I Curriculum vitae

Personal Information

Date of birth	25. November 1989
Place of birth	Oberpullendorf
Adress	Kolonitzgasse 10/11, 1030 Wien, Austria
Mobile	+436645403703
E-Mail	markus.draskovits@gmx.net

Work Experience

May - June 2015, September 2015 - January 2016	Tutor in laboratory courses for undergraduates; TU Wien
July 2010, Augst 2011	Sailing teacher, Segelschule Kempf; Breitenbrunn
October 2008 - July 2009	Community Service, Red Cross; Mattersburg
September 2008	Internship, Agrana; Pischelsdorf
August 2007	Internship, Austrian Airlines; London
July 2006	Internship, SOT Süd-Ost Treuhand; Eisenstadt

Education

January - June 2014	Exchange semester at Universitat Polytècnica de València as an Erasmus student
from October 2013	Master's Program in Technical Chemistry with focus on synthetic organic chemistry at the TU Wien, Austria
October 2009 – October 2013	Bachelor's Program in Technical Chemistry at the TU Wien, Austria
	Title of Bachelor Thesis: Optimization of a synthetic method and synthesis of asymmetric derivatives of magnolol, potential anti-inflammatory agents and $GABA_A$ modulators. supervisor: Prof. Mihovilovic
September 2000 - June 2008	Gymnasium Kurzwiese Eisenstadt, Austria

Skills

Languages

German as mother tongue

Good level of spoken and written English and Spanish

Able to translate Latin

Computing

Good skills in MS Office

Interests

sailing, squash, tennis and soccer

travelling, visiting other countries

F II List of abbreviations

2D-NMR	two-dimensional nuclear magnetic resonance spectroscopy
AcOH	acetic acid
aqu.	aqueous
$BF_3{\cdot}OEt_2$	boron trifluoride diethyl etherate
Boc	tert-butyloxycarbonyl
bs	broad singlet (NMR)
d	doublet (NMR)
DBU	1,8-diazabicycloundec-7-ene
DCM	dichloromethane
dd	doublet of doublets (NMR)
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
ESI-TOF	electrospray ionization time-of-flight
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
equiv.	equivalent
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectroscopy
J	coupling constant (NMR)
LP	light petroleum (boiling point approx. 40 – 60 °C)
m	multiplet (NMR)
µA	microampere
MeOH	methanol
m.w.	molecular weight
nA	nanoampere
NMR	nuclear magnetic resonance
NEt_3	triethylamine
NH_4OAc	ammonium acetate
Pd/C	palladium on charcoal
$PhNO_2$	nitrobenzene
Ph_2O	diphenylether
PPA	polyphosphoric acid
ppm	parts per million (NMR)
R_f	retention factor (TLC)
rt	room temperature
s	singlet (NMR)
satd.	saturated
t	triplet (NMR)
TFA	trifluoroaetic acid
TLC	thin layer chromatography
q	quartet (NMR)

F III Reference

1. Sherwood, L., *Human Physiology: From Cells to Systems*. Cengage Learning2012.
2. Siegel, G. J.; Agranoff, B. W., *Basic Neurochemistry: Molecular, Cellular, and Medical Aspects*. Lippincott Williams & Wilkins1999.
3. Rudolph, U.; Antkowiak, B., Molecular and neuronal substrates for general anaesthetics. *Nat Rev Neurosci* **2004,** *5*, 709-720.
4. Bente Frolund, B. E., Uffe Kristiansen, Tommy Liljefors and Povl Krogsgaard-Larsen, GABA-A Receptor Ligands and their Therapeutic Potentials. *Current Topics in Medicinal Chemistry* **2002,** *2*, 817-832.
5. Wang, Q.; Han, Y.; Xue, H., Ligands of the GABAA Receptor Benzodiazepine Binding Site. *CNS Drug Reviews* **1999,** *5*, 125-144.
6. Sigel, E.; Steinmann, M. E., Structure, Function, and Modulation of GABA(A) Receptors. *The Journal of Biological Chemistry* **2012,** *287*, 40224-40231.
7. Miller, P. S.; Aricescu, A. R., Crystal structure of a human GABA(A) receptor. *Nature* **2014,** *512*, 270-275.
8. Jacob, T. C.; Moss, S. J.; Jurd, R., GABAA receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat Rev Neurosci* **2008,** *9*, 331-343.
9. Olsen, R. W.; Sieghart, W., International Union of Pharmacology. LXX. Subtypes of γ-Aminobutyric Acid(A) Receptors: Classification on the Basis of Subunit Composition, Pharmacology, and Function. Update. *Pharmacological reviews* **2008,** *60*, 243-260.
10. Smith, G. B.; Olsen, R. W., Functional domains of GABAA receptors. *Trends in Pharmacological Sciences* **1995,** *16*, 162-168.
11. Clayton, T.; Chen, J. L.; Ernst, M.; Richter, L.; Cromer, B. A.; Morton, C. J.; Ng, H.; Kaczorowski, C. C.; Helmstetter, F. J.; Furtmuller, R.; Ecker, G.; Parker, M. W.; Sieghart, W.; Cook, J. M., An Updated Unified Pharmacophore Model of the Benzodiazepine Binding Site on γ-Aminobutyric Acida Receptors: Correlation with Comparative Models. *Current Medicinal Chemistry* **2007,** *14*, 2755-2775.
12. Sieghart, W., Chapter Three - Allosteric Modulation of GABAA Receptors via Multiple Drug-Binding Sites. In *Advances in Pharmacology*, Uwe, R., Ed. Academic Press2015; Vol. Volume 72, pp 53-96.
13. Jackson, W. C., A Historical Dictionary of Psychiatry. *Primary Care Companion to The Journal of Clinical Psychiatry* **2006,** *8*, 313-313.
14. D'Hulst, C.; Atack, J. R.; Kooy, R. F., The complexity of the GABAA receptor shapes unique pharmacological profiles. *Drug Discovery Today* **2009,** *14*, 866-875.
15. Sieghart, W.; Sperk, G., Subunit composition, distribution and function of GABA-A receptor subtypes. *Current topics in medicinal chemistry* **2002,** *2*, 795-816.
16. Bormann, J., The 'ABC' of GABA receptors. *Trends in Pharmacological Sciences* **2000,** *21*, 16-19.
17. Nogrady, T.; Weaver, D. F., *Medicinal Chemistry: A Molecular and Biochemical Approach*. Oxford University Press, USA2005.
18. Sieghart, W., Structure and pharmacology of gamma-aminobutyric acidA receptor subtypes. *Pharmacological Reviews* **1995,** *47*, 181-234.
19. Lieberman, J. A.; Tasman, A., *Handbook of Psychiatric Drugs*. Wiley2006.
20. Ramerstorfer, J.; Furtmüller, R.; Sarto-Jackson, I.; Varagic, Z.; Sieghart, W.; Ernst, M., The GABA(A) Receptor α+β− Interface: A Novel Target for Subtype Selective Drugs. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **2011,** *31*, 870-877.
21. Varagic, Z.; Wimmer, L.; Schnürch, M.; Mihovilovic, M. D.; Huang, S.; Rallapalli, S.; Cook, J. M.; Mirheydari, P.; Ecker, G. F.; Sieghart, W.; Ernst, M., Identification of novel positive allosteric modulators and null modulators at the GABAA receptor α+β− interface. *British Journal of Pharmacology* **2013,** *169*, 371-383.
22. Simeone, X.; Draskovits, M.; Siebert, D.; Schnürch, M.; Mihovilovic, M. D.; Willeit, M.; Ernst, M., Novel GABAA Receptor Ligands Poster session presented at: 5th Neuroscience Vienna Network Meeting; 26.5.2015; University of Vienna
23. Sieghart, W.; Ramerstorfer, J.; Sarto-Jackson, I.; Varagic, Z.; Ernst, M., A novel GABA(A) receptor pharmacology: drugs interacting with the α(+)β(−) interface. *British Journal of Pharmacology* **2012,** *166*, 476-485.
24. Siebert, D., Dissertation, unpublished data, TU Wien, 2016
25. Yokoyama, N.; Ritter, B.; Neubert, A. D., 2-Arylpyrazolo[4,3-c]quinolin-3-ones: a novel agonist, a partial agonist and an antagonist of benzodiazepines. *Journal of Medicinal Chemistry* **1982,** *25*, 337-339.
26. Gould, R. G.; Jacobs, W. A., The Synthesis of Certain Substituted Quinolines and 5,6-Benzoquinolines. *Journal of the American Chemical Society* **1939,** *61*, 2890-2895.
27. Hanan, E. J.; Chan, B. K.; Estrada, A. A.; Shore, D. G.; Lyssikatos, J. P., Mild and General One-Pot Reduction and Cyclization of Aromatic and Heteroaromatic 2-Nitroamines to Bicyclic 2H-Imidazoles. *Synlett* **2010,** *2010*, 2759-2764.
28. Sharghi, H.; Asemani, O.; Khalifeh, R., New One-Pot Procedure for the Synthesis of 2-Substituted Benzimidazoles. *Synthetic Communications* **2008,** *38*, 1128-1136.
29. Göblyös, A.; Gao, Z.-G.; Brussee, J.; Connestari, R.; Santiago, S. N.; Ye, K.; Ijzerman, A. P.; Jacobson, K. A., Structure−Activity Relationships of New 1H-Imidazo[4,5-c]quinolin-4-amine Derivatives as Allosteric Enhancers of the A3 Adenosine Receptor. *Journal of Medicinal Chemistry* **2006,** *49*, 3354-3361.
30. Clayden, J.; Greeves, N.; Warren, S., *Organic Chemistry*. OUP Oxford2012.
31. Li, J. J., *Name Reactions: A Collection of Detailed Mechanisms and Synthetic Applications*. Springer2007.
32. Schwetlick, K., *Organikum*. Wiley2004.
33. López Rivilli, M. J.; Moyano, E. L.; Yranzo, G. I., An alternative approach toward 2-aryl-2H-pyrazolo[4,3-c]-quinolin-3-ones by a multistep synthesis. *Tetrahedron Letters* **2010,** *51*, 478-481.
34. Wuts, P. G. M., *Greene's Protective Groups in Organic Synthesis*. 5 ed.; Wiley2014.
35. Wasserman, H. H.; Berger, G. D.; Cho, K. R., Transamidation reactions using β-lactams. The synthesis of homaline. *Tetrahedron Letters* **1982,** *23*, 465-468.
36. Lucien, H. W., Thermal Decomposition of Hydrazine. *Journal of Chemical & Engineering Data* **1961,** *6*, 584-586.
37. Keeler, J., *Understanding NMR Spectroscopy*. Wiley2011.
38. Yang, D.; Arifhodzic, L.; Ganellin, C. R.; Jenkinson, D. H., Further studies on bis-charged tetraazacyclophanes as potent inhibitors of small conductance Ca2+-activated K+ channels. *European Journal of Medicinal Chemistry* **2013,** *63*, 907-923.
39. Gerster, J. F.; Lindstrom, K. J.; Miller, R. L.; Tomai, M. A.; Birmachu, W.; Bomersine, S. N.; Gibson, S. J.; Imbertson, L. M.; Jacobson, J. R.; Knafla, R. T.; Maye, P. V.; Nikolaides, N.; Oneyemi, F. Y.; Parkhurst, G. J.; Pecore, S. E.; Reiter, M. J.; Scribner, L. S.; Testerman, T. L.; Thompson, N. J.; Wagner, T. L.; Weeks, C. E.; Andre, J.-D.; Lagain, D.; Bastard, Y.; Lupu, M., Synthesis and Structure−Activity-Relationships of 1H-Imidazo[4,5-c]quinolines That Induce Interferon Production. *Journal of Medicinal Chemistry* **2005,** *48*, 3481-3491.

40. Shukla, N. M.; Kimbrell, M. R.; Malladi, S. S.; David, S. A., Regioisomerism-dependent TLR7 agonism and antagonism in an imidazoquinoline. *Bioorganic & Medicinal Chemistry Letters* **2009,** *19*, 2211-2214.
41. Fiala, W.; Stadlbauer, W., Nucleophilic Chlorination of 3-Formyl-4-hydroxy-quinolin-2(1H)-ones. *Journal für Praktische Chemie/Chemiker-Zeitung* **1993,** *335*, 128-134.
42. Cellier, P. P.; Spindler, J.-F.; Taillefer, M.; Cristau, H.-J., Pd/C-catalyzed room-temperature hydrodehalogenation of aryl halides with hydrazine hydrochloride. *Tetrahedron Letters* **2003,** *44*, 7191-7195.
43. Wang, Z., Béchamp Reduction. In *Comprehensive Organic Name Reactions and Reagents*, John Wiley & Sons, Inc.2010.
44. Frank, R.; Christoph, T.; Lesch, B.; Lee, J., Substituted bicyclic aromatic carboxamide and urea derivatives as vanilloid receptor ligands. Google Patents2013.
45. Shiro, T.; Takahashi, H.; Kakiguchi, K.; Inoue, Y.; Masuda, K.; Nagata, H.; Tobe, M., Synthesis and SAR study of imidazoquinolines as a novel structural class of microsomal prostaglandin E2 synthase-1 inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2012,** *22*, 285-288.
46. Gamble, A. B.; Garner, J.; Gordon, C. P.; O'Conner, S. M. J.; Keller, P. A., Aryl Nitro Reduction with Iron Powder or Stannous Chloride under Ultrasonic Irradiation. *Synthetic Communications* **2007,** *37*, 2777-2786.
47. LIOUX, T.; Drocourt, D.; Vernejoul, F.; Tiraby, G.; Perouzel, E., Conjugated TLR7 and/or TLR8 and TLR2 polycationic agonists. Google Patents2014.
48. Seixas, J. D.; Luengo-Arratta, S. A.; Diaz, R.; Saldivia, M.; Rojas-Barros, D. I.; Manzano, P.; Gonzalez, S.; Berlanga, M.; Smith, T. K.; Navarro, M.; Pollastri, M. P., Establishment of a Structure–Activity Relationship of 1H-Imidazo[4,5-c]quinoline-Based Kinase Inhibitor NVP-BEZ235 as a Lead for African Sleeping Sickness. *Journal of Medicinal Chemistry* **2014,** *57*, 4834-4848.
49. GÖBLYÖS, A.; Brussee, J.; Ijzerman, A. P.; Gao, Z. G.; Jacobson, K., A3 adenosine receptor allosteric modulators. Google Patents2013.
50. David Siebert: Dissertation, unpublished data; TU Wien
51. Charton, J.; Girault-Mizzi, S.; Debreu-Fontaine, M.-A.; Foufelle, F.; Hainault, I.; Bizot-Espiard, J.-G.; Caignard, D.-H.; Sergheraert, C., Synthesis and biological evaluation of benzimidazole derivatives as potent AMP-activated protein kinase activators. *Bioorganic & Medicinal Chemistry* **2006,** *14*, 4490-4518.
52. Yadagiri, B.; Lown, J. W., Convenient Routes to Substituted Benzimidazoles and Imidazolo[4,5-b]pyridines Using Nitrobenzene as Oxidant. *Synthetic Communications* **1990,** *20*, 955-963.
53. Goldin, A. L., Expression of Ion Channels in Xenopus Oocytes. In *Expression and Analysis of Recombinant Ion Channels*, Wiley-VCH Verlag GmbH & Co. KGaA2006; pp 1-25.
54. Rudolph, U.; Knoflach, F., Beyond classical benzodiazepines: novel therapeutic potential of GABAA receptor subtypes. *Nat Rev Drug Discov* **2011,** *10*, 685-697.
55. Kermack, W. O.; Storey, N. E., 115. Attempts to find new antimalarials. Part XXIX. The synthesis of various derivatives of 2 : 3-benz-[gamma]-carboline. *Journal of the Chemical Society (Resumed)* **1950**, 607-612.
56. Stern, E.; Muccioli, G. G.; Millet, R.; Goossens, J.-F.; Farce, A.; Chavatte, P.; Poupaert, J. H.; Lambert, D. M.; Depreux, P.; Hénichart, J.-P., Novel 4-Oxo-1,4-dihydroquinoline-3-carboxamide Derivatives as New CB2 Cannabinoid Receptors Agonists: Synthesis, Pharmacological Properties and Molecular Modeling. *Journal of Medicinal Chemistry* **2006,** *49*, 70-79.
57. Kaslow, C. E.; Clark, W. R., QUINOLINEMETHANOLS. *The Journal of Organic Chemistry* **1953,** *18*, 55-58.
58. Ghotekar, B.; Ghagare, M.; Toche, R.; Jachak, M., Synthesis of new quinoline fused heterocycles such as benzo[h]-1,6-naphthyridines and pyrazolo[4,3-c]quinolines. *Monatsh Chem* **2010,** *141*, 169-175.
59. Singh, M. P.; Joseph, T.; Kumar, S.; Bathini, Y.; Lown, J. W., Synthesis and sequence-specific DNA binding of a topoisomerase inhibitory analog of Hoechst 33258 designed for altered base and sequence recognition. *Chemical Research in Toxicology* **1992,** *5*, 597-607.
60. Conroy, A. E.; Mosher, H. S.; Whitmore, F. C., Heterocyclic Basic Compounds. XII. 7-Bromo-and 7-Iodo-quinolines. *Journal of the American Chemical Society* **1949,** *71*, 3236-3237.
61. Medapi, B.; Suryadevara, P.; Renuka, J.; Sridevi, J. P.; Yogeeswari, P.; Sriram, D., 4-Aminoquinoline derivatives as novel Mycobacterium tuberculosis GyrB inhibitors: Structural optimization, synthesis and biological evaluation. *European Journal of Medicinal Chemistry* **2015,** *103*, 1-16.
62. Chen, Y.-L.; Zacharias, J.; Vince, R.; Geraghty, R. J.; Wang, Z., C-6 aryl substituted 4-quinolone-3-carboxylic acids as inhibitors of hepatitis C virus. *Bioorganic & Medicinal Chemistry* **2012,** *20*, 4790-4800.
63. Schofield, K.; Simpson, J. C. E., 226. The preparation of quinoline derivatives from aromatic amines and ethyl ethoxymethylenemalonate. *Journal of the Chemical Society (Resumed)* **1946**, 1033-1035.
64. Zhu, J.; He, L.; Ma, L.; Wei, Z.; He, J.; Yang, Z.; Pu, Y.; Cao, D.; Wu, Y.; Xiang, M.; Peng, A.; Wei, Y.; Chen, L., Synthesis and biological evaluation of 4-oxoquinoline-3-carboxamides derivatives as potent anti-fibrosis agents. *Bioorganic & Medicinal Chemistry Letters* **2014,** *24*, 5666-5670.
65. Büyükbingöl, E.; Daş, N.; Klopman, G., Synthesis of 1,4-Dihydro-4-oxo-quinoline-3-carboxylic Acid Derivatives as Inhibitors of Rat Lens Aldose Reductase. *Archiv der Pharmazie* **1994,** *327*, 129-131.
66. Devine, W.; Woodring, J. L.; Swaminathan, U.; Amata, E.; Patel, G.; Erath, J.; Roncal, N. E.; Lee, P. J.; Leed, S. E.; Rodriguez, A.; Mensa-Wilmot, K.; Sciotti, R. J.; Pollastri, M. P., Protozoan Parasite Growth Inhibitors Discovered by Cross-Screening Yield Potent Scaffolds for Lead Discovery. *Journal of Medicinal Chemistry* **2015,** *58*, 5522-5537.
67. Hirano, J.; Hamase, K.; Zaitsu, K., Evaluation of a simple and novel fluorescent anion sensor, 4-quinolone, and modification of the emission color by substitutions based on molecular orbital calculations. *Tetrahedron* **2006,** *62*, 10065-10071.
68. Bachman, G. B.; Welton, D. E.; Jenkins, G. L.; Christian, J. E., Quinoline Derivatives from 3-Nitro-4-hydroxyquinoline1. *Journal of the American Chemical Society* **1947,** *69*, 365-371.
69. Capraro, H. G.; Furet, P.; Garcia-Echeverria, C.; Stauffer, F., 1h-imidazo[4,5-c]quinoline derivatives in the treatment of protein kinase dependent diseases. Google Patents2005.
70. Heppner, P. D.; Kshirsagar, T. A.; Lundquist, G. D.; Merrill, B. A.; Prince, R. B., Aryl and arylalkylenyl substituted thiazoloquinolines and thiazolonaphthyridines. Google Patents2007.
71. Amos, D. T.; Dellaria, J. F.; Heppner, P. D.; Kshirsagar, T. A.; Lundquist, G. D.; Zimmermann, B. M., Oxime substituted imidazo ring compounds. Google Patents2005.