Synthesis and transformation of functionalized kynurenic acid derivatives

Ph.D. Thesis

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“If you want your children to be intelligent, read them fairy tales. If you want them to be more intelligent, read them more fairy tales.”

Albert Einstein

PUBLICATIONS

Papers related to the thesis

1. Mándi, Y.; Endrész, V.; Mosolygó, T.; Burián, K.; Lantos, I.; Fülöp, F.; Szatmári, I.; **Lőrinczi, B.**; Balog, A.; Vécsei, L.
The opposite effects of kynurenic acid and different kynurenic acid analogues on tumor necrosis factor-α (TNF-α) production and tumor necrosis factor-stimulated gene-6 (TSG-6) expression in U-937 cells.
Front. Immunol. **2019**, *10*, 1406, DOI: 10.3389/fimmu.2019.01406 **IF.:5.085**

2. Fehér, E.; Szatmári, I.; Dudás, T.; Zalatnai, A.; Farkas, T.; **Lőrinczi, B.**; Fülöp, F.; Vécsei, L.; Toldi, J.
Structural evaluation and electrophysiological effects of some kynurenic acid analogs.
Molecules **2019,** *24*, 3502, DOI: 10.3390/molecules24193502 **IF.:3.267**

3. **Lőrinczi, B.**; Csámpai, A.; Fülöp, F.; Szatmári, I.
Synthesis of New C-3 Substituted Kynurenic Acid Derivatives.
Molecules **2020**, *25*, 937, DOI: 10.3390/molecules25040937 **IF.:3.267**

4. Molnár, K.; **Lőrinczi, B**.; Fazakas, C.; Szatmári, I.; Fülöp, F.; Kmetykó, N.; Berkecz, R.; Ilisz, I.; Krizbai, A. I.; Wilhelm, I.; Vécsei, L.
SZR-104, a novel kynurenic acid analogue with high permeability through the blood–brain barrier.
Pharmaceutics **2021**, *13*, 61, DOI: 10.3390/pharmaceutics13010061 **IF.:4.421**

5. **Lőrinczi, B**.; Csámpai, A.; Fülöp, F.; Szatmári, I.
Synthetic- and DFT modelling studies on regioselective modified Mannich reactions of hydroxy-KYNA derivatives.
RSC Adv. **2021,** *11*, 543. DOI: 10.1039/d0ra08325a **IF.:3.119**

6. Lo, Y-C.; Lin, C-L.; Fang, W-Y.; **Lőrinczi, B**.; Szatmári, I.; Chang, W-H.; Fülöp, F.; Wu, S-N.
Effective activation by kynurenic acid and its aminoalkylated derivatives on M-type K+ current.
Int. J. Mol. Sci. **2021**, *22*, 1300. DOI: 10.3390/ijms22031300 **IF.:4.556**

Conference lectures

7. **Bálint Lőrinczi**
   Kinurénsav-származékok továbbalakítása ortokinon-metid köztiterméken keresztül;
   XIII. Clauder Ottó memorial
   November 22. – 23. 2018. Budapest, Hungary

8. **Bálint Lőrinczi**, Ferenc Fülöp and István Szatmári
   A kinurénsav C-3 helyzetének szubsztitúciója módosított Mannich-reakció segítségével
   Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése
   June 3. – 5. 2019. Balatonszemes, Hungary

9. **Bálint Lőrinczi**, István Szatmári and Ferenc Fülöp
   Synthesis and transformation of kynurenic acid derivatives
   19th Tetrahedron Symposium
   June 26. – 29. 2018. Riva del Garda, Italy

10. **Bálint Lőrinczi**, Ferenc Fülöp and István Szatmári
    Synthesis of pyrroloquinolinone derivatives via ortho-quinonemethides
    26th Young Research Fellow Meeting
    February 20. – 22. 2019. Paris, France

11. **Bálint Lőrinczi**, Ferenc Fülöp and István Szatmári
    Transformation of substituted kynurenic acid derivatives in modified Mannich reaction
    25th International Conference on Chemistry
    October 24. – 26. 2019. Cluj-Napoca, Romania

Abbreviations

Tryptophan ----------TRP

Nicotinamide adenine dinucleotide ----------NAD

Modified Mannich reaction ----------*m*Mr

Diphenyl ether ----------Ph_2O

Dimethylacetylene dicarboxylate ----------DMAD

N,*N*-Dimethylformamide ----------DMF

1,2-Dichlorobenzene ----------DCB

p-Toluenesulfonic acid ----------*p*TsOH

Electron-donating groups----------EDG

Diethyl fumarate ----------DEF

Polyphosphoric acid----------PPA

Diethylacetylene dicarboxylate ----------DEAD

Tetraphenylporphin ----------tPorphin

Acetonitrile ----------MeCN

N,*N*′-Diisopropylcarbodiimide----------DIC

1-Hydroxybenzotriazole hydrate ----------HOBt

Blood-brain barrier ----------BBB

Tumor necrosis factor alpha ----------TNF-α

TNF-stimulated gene 6 protein ----------TSG-6

1. Introduction

KYNA (kynurenic acid) is an endogenous product of the tryptophan (TRP) metabolism, a pathway known to be responsible for the production of nicotinamide adenine dinucleotide (NAD) and NAD phosphate.[1,2] In this pathway, TRP is converted into various compounds, including L-kynurenine, which can be metabolized in two separate ways. One furnishes KYNA, whereas the other gives 3-hydroxykynurenine and quinolinic acid, the precursors of NAD.[3,4]

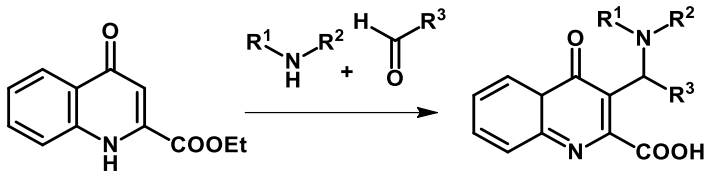
Among the important features of KYNA, one is that it is one of the few known endogenous excitatory amino acid receptor blockers with a broad spectrum of antagonistic properties in supraphysiological concentrations. One of its confirmed sites of action is the α-7-nicotinic acetylcholine receptor and, interestingly, the other, identified recently, is a higher-affinity positive modulatory binding site at the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor.[5]

Since KYNA is a neuroprotective agent able to prevent neuronal loss following excitotoxic, ischemia-induced, and infectious neuronal injuries,[6,7] there has recently been increasing interest in the synthesis and pharmacological studies of KYNA derivatives. The substitution of KYNA at positions 5–8 was achieved by starting from the corresponding aniline via the modified Conrad–Limpach method.[8-10] The hydroxy group at position 4 was transformed to ether[10–12] or amine functions,[13–15] while the carboxylic function at position 2 was mostly modified by synthesizing the corresponding esters[10–12] or amides.[16–21]

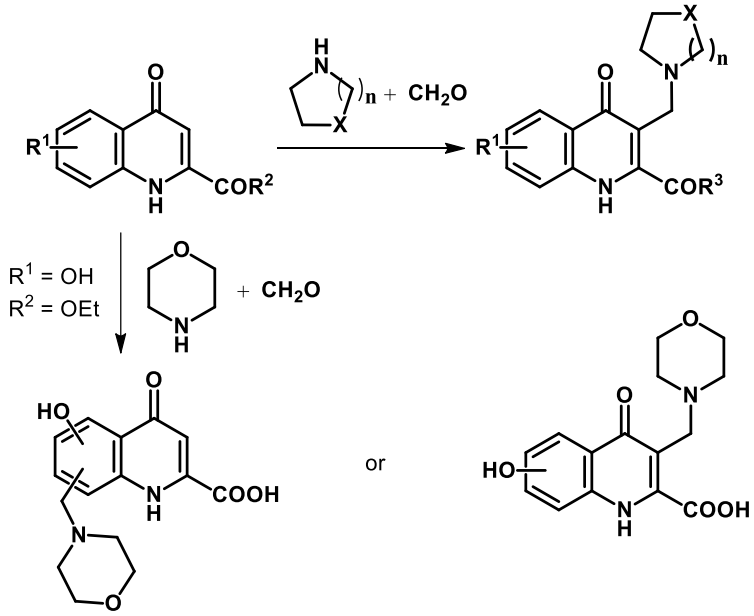
Formally, KYNA can be considered to be a nitrogen-containing 1-naphthol derivative. In our previous studies, 1-naphthol and its *N*-containing analogues were successfully applied in the modified Mannich reaction (*m*Mr)[22] leading to the corresponding aminonaphthols,[23] aminoquinolinols or aminoisoquinolinols.[24,25] A similar transformation starting from xanthurenic acid has been described by Schmitt et al.[26] They managed to perform regioselective aminoalkylation at position 3 on this substrate, by using benzyl protection of the C-8 hydroxyl group.

Based on the evaluations of previous KYNA amides, a tertiary nitrogen is needed for biological activity towards the central nervous system.[27–30] Derivatives bearing such functional groups can be synthesized by various methods, such as carboxyl amidation mentioned previously.[16–21] An alternative route could be the transformation of the 4-hydroxy group into an amino function and its subsequent alkylation with an appropriate nitrogen-containing haloalkylamine.[13]

My PhD work has been planned to accomplish two major goals. The first aim was to investigate the reactivity of kynurenic acid in a modified Mannich-type reaction. In the original Mannich reaction a C–H acid, formaldehyde and a secondary amine forms a so-called Mannich base in a relatively easy, one-pot reaction. Recently, one of its special variations, the *m*Mr has gained ground, in which the C–H acid is replaced by electron-rich aromatic compounds such as 1- and 2-naphthols as active hydrogen sources.[31] As KYNA can be considered to be a nitrogen-containing 1-naphthol derivative, its reaction in the *m*Mr also emerges as a straightforward version of functionalization. Using an array of amines and aldehydes, the reaction can yield the corresponding targeted aminoalkylated derivatives with the desired cationic center.



The second aim of my work was to investigate the scope and limitations of the *m*Mr on KYNA by reacting a representative amine and aldehyde with different functionalized or amide derivatives of KYNA. Reactions of a few selected amides and derivatives hydroxy-substituted at the B ring were studied further either by comparing different synthetic routes or through systematic investigations supported by DFT calculations.



2. Literature background

The modifications carried out on the 4-hydroxyquinoline-2-carboxylic acid skeleton can either be (i) additions of functional groups at different positions or (ii) modifications in the benzo-fused 4-oxo-1,4-dihydropyridine-2-carboxylic acid skeleton itself, meaning the exchange of carbons to heteroatoms or the change of B ring size. While aspect (i) was already reviewed in 2009,[21] the modifications so far done on the B ring have not yet been collected. The aim of this section is to summarize the syntheses and use of KYNA derivatives modified at the B ring that are either containing a heteroatom in the B ring and/or have a different ring size (Figure 1). In the future, these compounds may provide a basis for the synthesis of new KYNA derivatives and thus a variety of candidates for medicinal use.

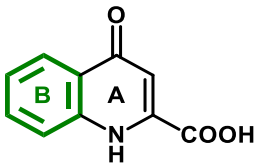


Figure 1.

The synthesis of modified quinoline structures has a wide variety of possible methods.[32] However, with the 2-carboxylic and 4-oxo functions taken into consideration, the possible routes are narrowed down to a small range of nucleophilic substitutions or additions followed by an intramolecular ring closure. Since the syntheses, from this aspect, differ only in a few cases, they have been categorized on the basis of the type and position of the heteroatom in the modified skeletons.

2.1. Nitrogen-containing ring systems

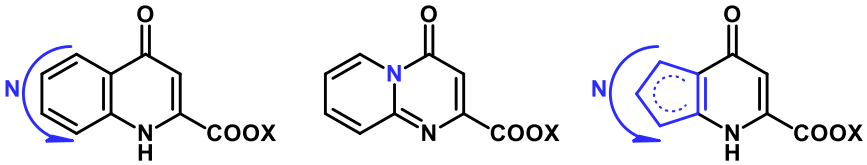
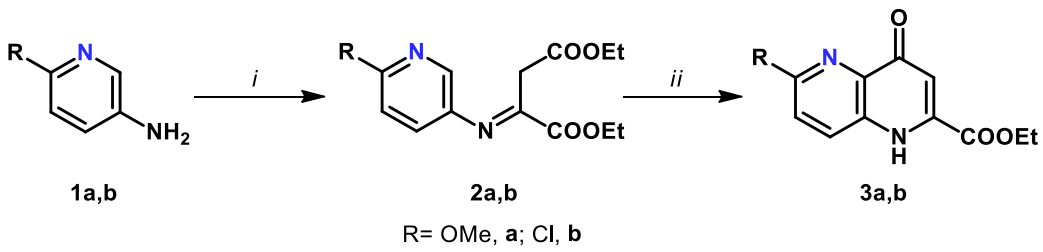


Figure 2.

2.1.1. Pyridine- and pyrimidine-fused ring systems

A possible route for the preparation of functionalized quinoline structures is the Conrad–Limpach synthesis. During the procedure described by Max Conrad and Leonhard Limpach, an aniline is reacted with β-keto esters to form an intermediate Schiff-base. In the second step, this intermediate undergoes a thermal intramolecular ring closure yielding the final quinolone derivative.[33–35] As the synthesis of the intermediate can be achieved with several methods, variations of the reaction using different aniline derivatives or electrophiles to synthesize the intermediate have been disclosed.

One of the first synthesis concerning the synthesis of KYNA derivatives bearing modified B rings has been published by Williamson *et al.* Presumably, since the described ethyl ethoxalylacetate would not yield the mentioned naphthyridine derivative, diethyl oxalacetate was used with aniline derivative **1a** to carry out the synthesis of **2a**. Then the product was stirred in boiling diphenyl ether (Ph_2O) to achieve ring closure yielding 1,5-naphthyridine derivative **3a** (Scheme 1, Table 1).[36]



Scheme 1. Synthesis of 1,5-naphthyridine derivatives **3a,b**

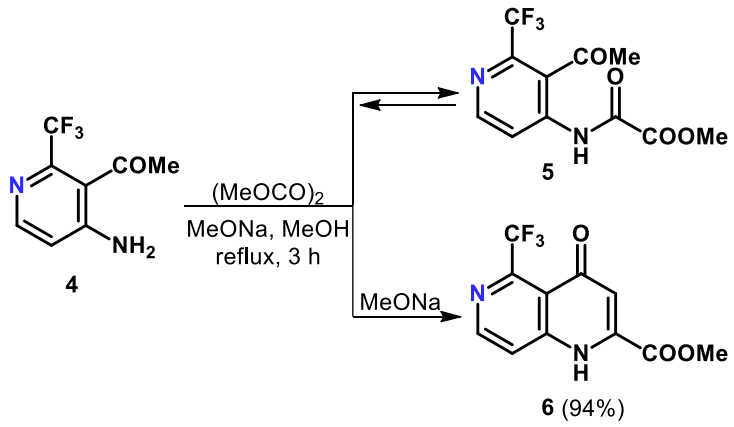
Table 1. Reaction conditions for the preparation of 1,5-naphthyridine derivatives

Reaction	Reagent	Solvent	Temperature (°C)	Reaction time	Yield (%)
i	diethyl oxalacetate	glacial acetic acid	40-50 °C; r.t.	4 h; over night	**2a** 43%[36]
		neat	90 °C	7 h	**2b** 21%[37]
	sodium (*Z*)-1,4-diethoxy-1,4-dioxobut-2-en-2-olate	glacial acetic acid	r.t.	2.5 days	**2b** 23%[38]
ii	-	Ph_2O	reflux	10+10 min	**3a** 84%[36]
		Downtherm A	210 °C	5 h	**3b** 34%[37]
		Ph_2O	reflux	15+20 min	**3b** 29%[38]

The same method was utilized 50 years later by Nakamoto *et al.*[37] and Feng *et al.*[38] in two distinct patents to synthesize the Schiff base intermediate of **3b** by either using diethyl oxalacetate

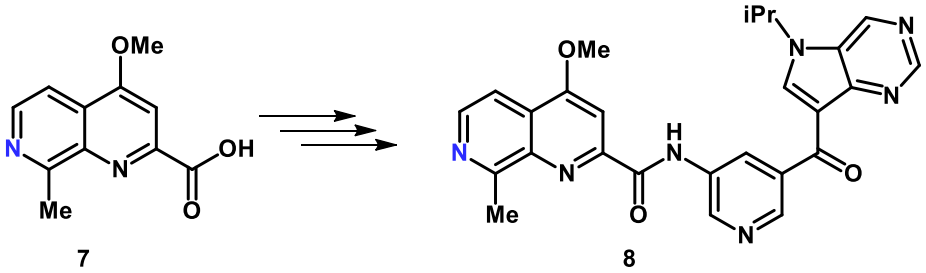
or its alkali salt, sodium (*Z*)-1,4-diethoxy-1,4-dioxobut-2-en-2-olate, respectively. The synthesized compounds were later transformed to potential antifungal[37] and antimalarial[49] or potential farnesoid X receptor modulators (Scheme 1 and Table 1).[38,40]

In the case of the synthesis of 1,6-naphthyridine derivatives, the method presented in the literature, involves a procedure different from the one described by Conrad and Limpach. Pyridine with an active α-methyl function (**4**), that can be regarded as a acetophenone derivative,[41] is reacted with dimethyl oxalate in the presence of excess MeONa needed to shift the reaction toward the ring closure (Scheme 2).[42]

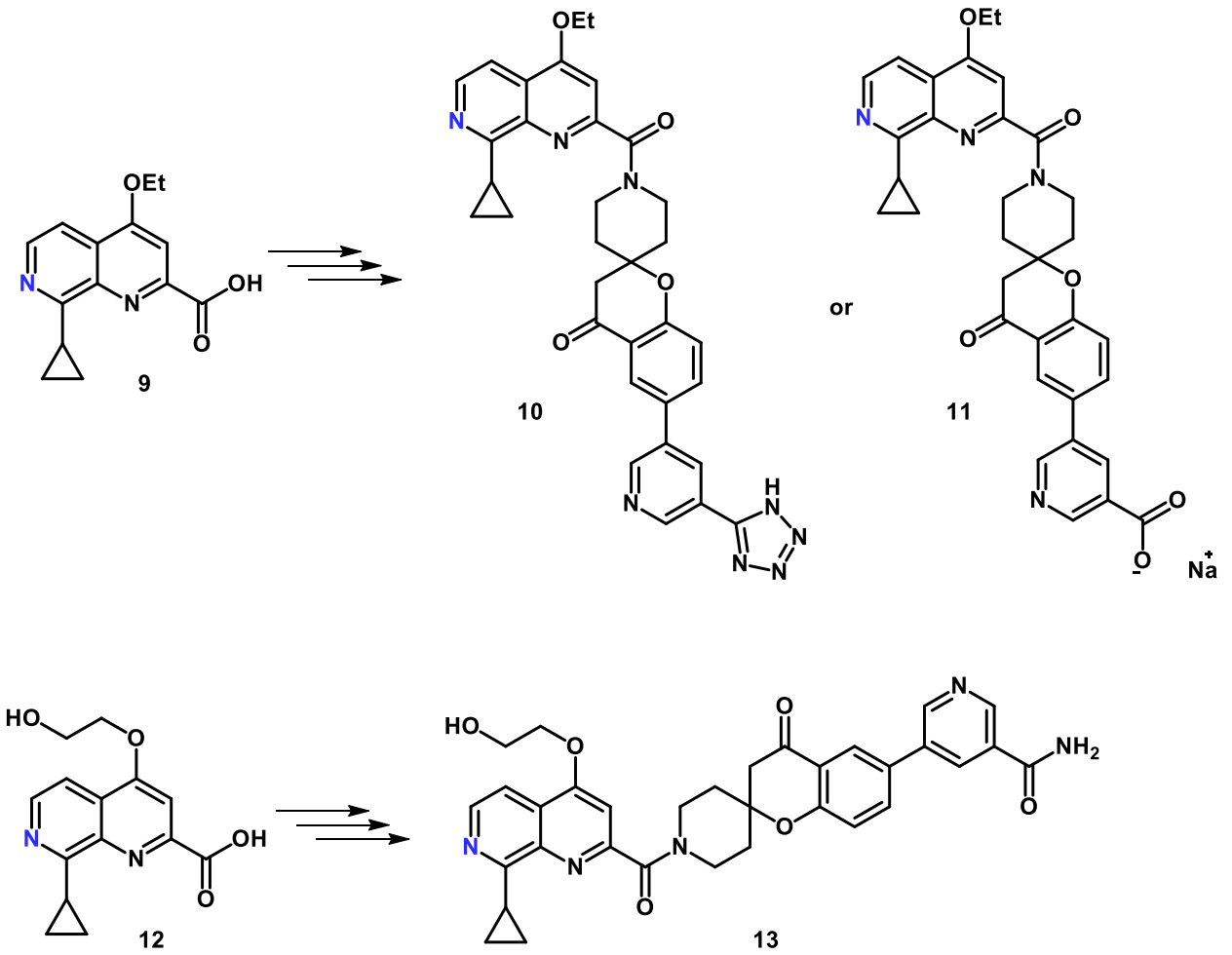


Scheme 2. Synthesis of naphthyridine derivative **6** via acetophenone analogue **4**

Regarding the synthesis of 1,7-naphthyridine derivatives, the literature is scarce: only two patents mention these compounds. In both cases, the derivatives were used to broaden the 4-hydroxyquinoline-2-carboxylic acid scaffold of the final bioactive compound with an additional heteroatom-containing skeleton (**8**, **10**, **11**, **13**, Scheme 3 and 4).[43,44] However, the patents neither described the synthesis of the naphthyridine skeleton nor cited an appropriate reference.

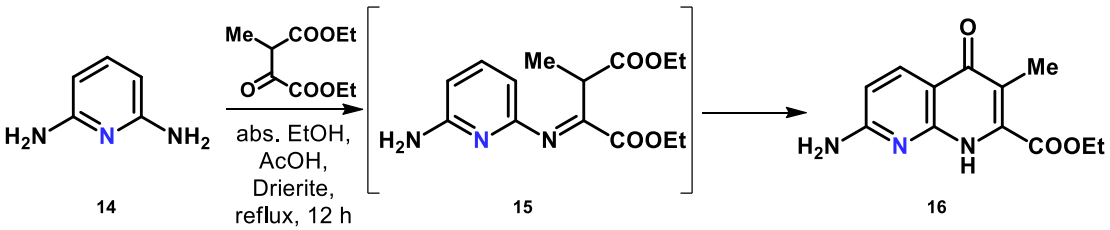


Scheme 3. 1,7-Naphthyridine derivative with possible kinase-inhibiting effect



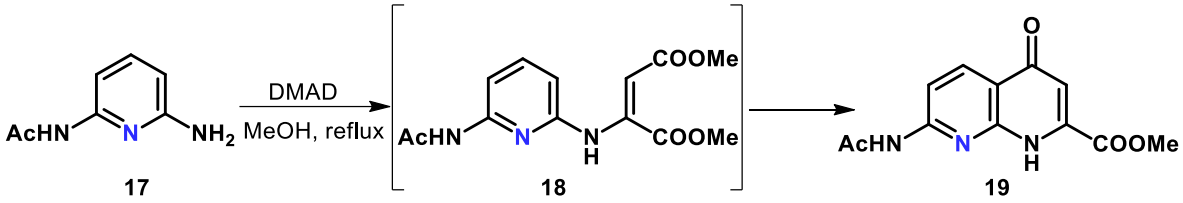
Scheme 4. 1,7-Naphthyridine derivatives (**10**, **11**, **13**) with possible acetyl coenzyme A inhibiting effect

The synthesis of 1,8-naphthyridine, containing a carboxylic function at position C-2 and a hydroxy function at position C-4, was first carried out by Weiss *et al.* The reaction is a perfect example of Conrad–Limpach procedure using a substituted β-keto ester. For the formation of the Schiff base, α-ethoxalylpropionate (a β-keto ester) was applied yielding **16**; however, its isolation in high purity was unsuccessful (Scheme 5).[45]



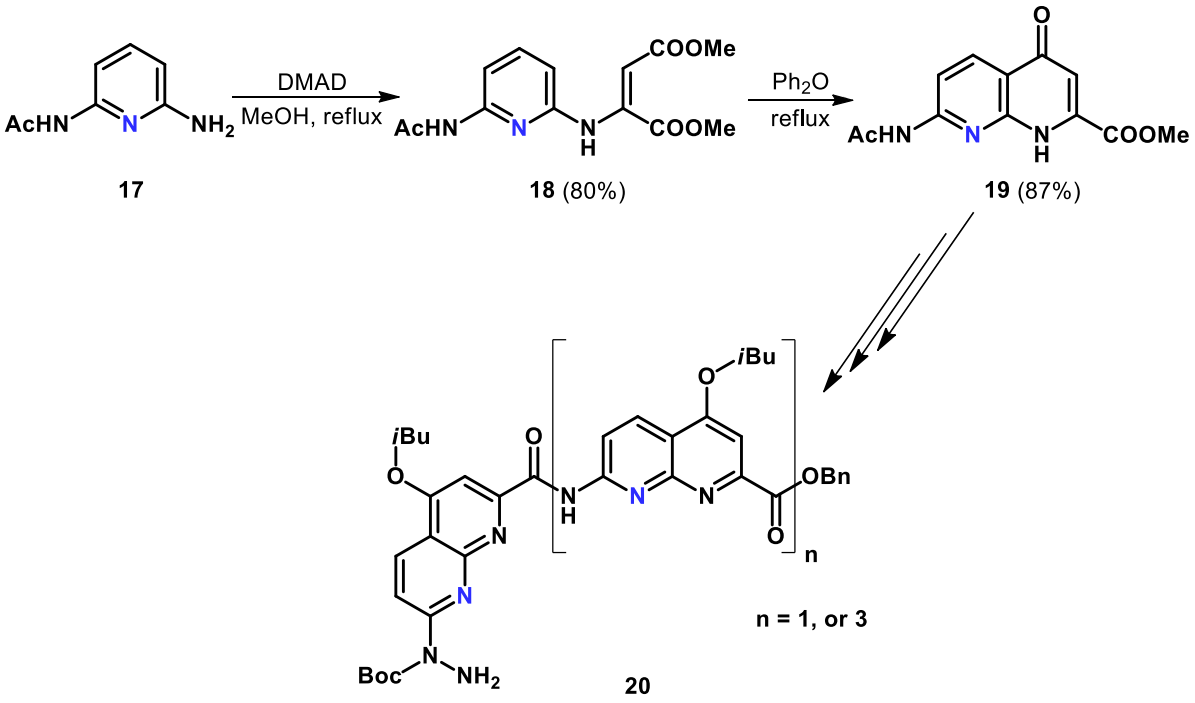
Scheme 5. Synthesis of C-3 methyl-substituted 1,8-naphthyridine derivative **16**

The modification used in the synthesis of many KYNA derivatives[46] was also applied for the synthesis of 1,8-naphthyridine derivatives. This method involves the use of acetylene derivatives for the synthesis of enamine intermediates through ~~a~~ Michael addition. Tonetti *et al.* utilized this method to synthesize **19** by applying dimethylacetylene dicarboxylate (DMAD) as an electrophilic reagent. However, the precise conditions of the synthesis are unclear, as the original paper could not be found and the reaction scheme was put forward based on the information gathered from Chemical Abstracts (Scheme 6).[47]



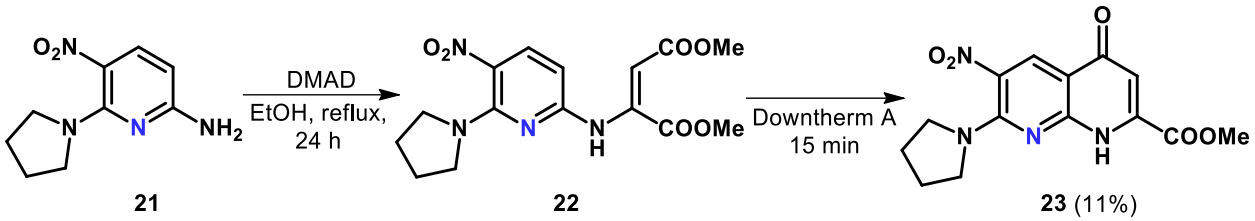
Scheme 6. Proposed synthesis of 1,8-naphthyridine derivative **19**

However, judging from the work of Huc *et al.*, it is safe to conclude that the reaction depicted in Scheme 7 is correct, since the research group managed to synthesize the same compound from the same starting materials (Scheme 7).[48] Derivative **19** was later used to create oligomers, that were investigated in the hope of finding foldamers with a chemical space as vast as their aliphatic counterparts (namely, α-peptides).[49–52]



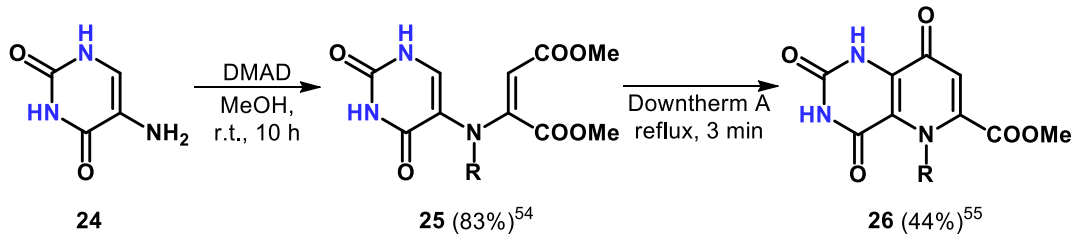
Scheme 7. Synthesis of 1,8-naphthyridine derivative **19** and its use as a foldamer building block

The same method was applied by Dohmori *et al.* during the synthesis of different potentially antimicrobial agents (against *Trichomonas vaginalis*). Pyrrolidine-substituted 1,8-naphthyridine **23** was synthesized using functionalized aminopyridine **21** and DMAD, albeit, a with a low yield of 11%. Nevertheless, it further supports the idea that these compounds can be synthesized with this modified Conrad–Limpach procedure (Scheme 8).[53]



Scheme 8. Synthesis of pyrrolidine substituted 1,8-naphthyridine **23**

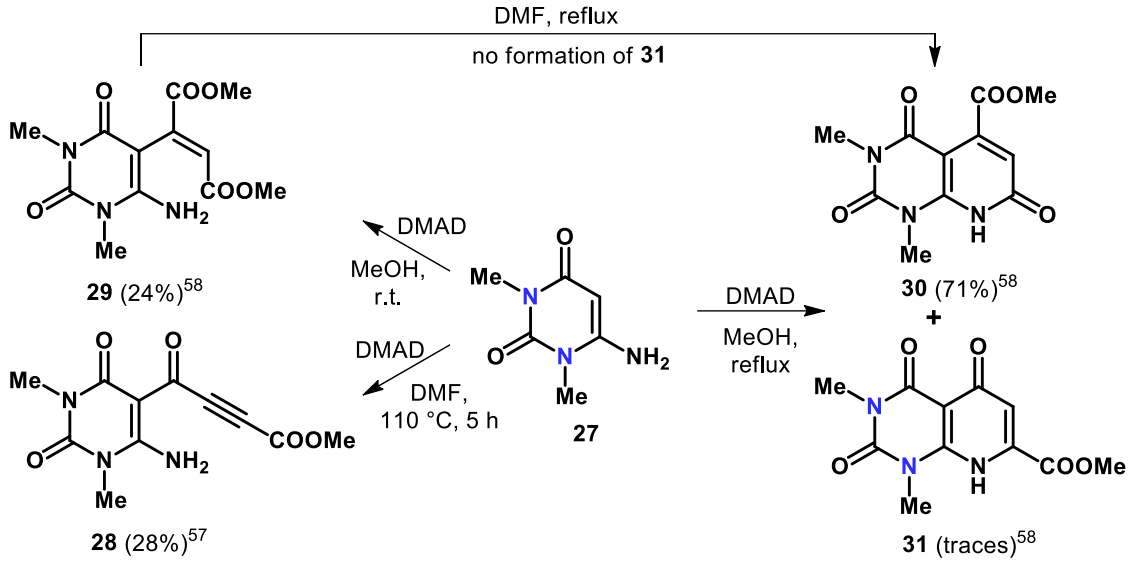
The use of DMAD to carry out the Michael addition and subsequently the synthesis of different pyrimidine-containing skeletons has also been investigated. The first method published describes the synthesis of pyrido[3,2-*d*]pyrimidine skeleton.[54] This method was later optimized by Rosowsky *et al.* by decreasing the reaction time of the intramolecular ring closure from 45 minutes to a reaction time as short as 3 minutes (Scheme 9).[55] The method was later cited by patents for the synthesis of compounds exhibiting PI3K inhibitory activities.[56]



Scheme 9. Synthesis of pyrido[3,2-*d*]pyrimidine derivative **26**

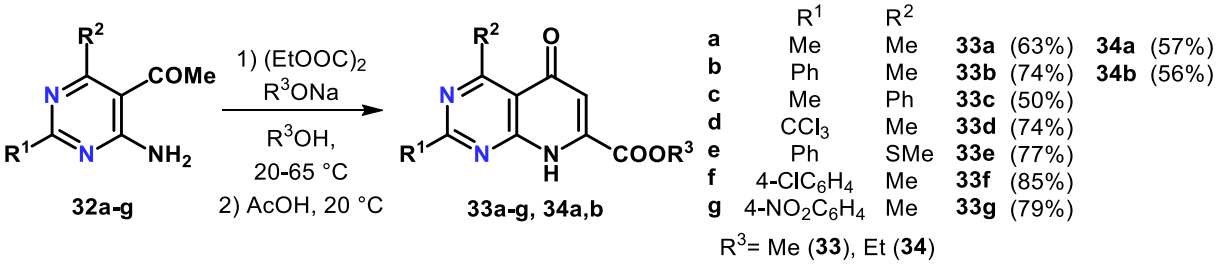
The synthesis of pyrido[2,3-*d*]pyrimidine skeleton has also been described; however, in the first publication it was only mentioned as a desired product. The research group tried to apply the same Michael addition with DMAD. However, the reaction yielded the C-5 carbonylated derivative of 6-aminouracil (**28**, Scheme 10).[57] Sakaguchi *et al.* tried to achieve the ring closure by changing *N*,*N*-dimethylformamide (DMF) to MeOH. Still, both at room temperature and under reflux conditions, the formation of the same maleate intermediate **29** was observed. Subsequently, it was transformed in either DMF or ~~in~~ MeOH under reflux to 2-oxo-4-carboxylic acid derivative **30**. However, in MeOH the formation of a minimal amount of the 4-oxo derivative **31** also took place. This observation, in combination with the fact that solvents used for other Conrad–Limpach

ring closure steps [Downtherm A, 1,2-dichlorobenzene (DCB)] were not investigated, indicates that the synthesis with a better yield may be possible (Scheme 10).[58]



Scheme 10. Possible methods for the synthesis of pyrido[2,3-*d*]pyrimidine derivative **31**

Dorokhov *et al.* used their method described for the synthesis of 1,6-naphthyridine **6**[42] to synthesize a wide variety of pyrimidine analogues (**33a,b**, **34a–g**) as well (Scheme 11).[59]



	R^1	R^2		
a	Me	Me	**33a** (63%)	**34a** (57%)
b	Ph	Me	**33b** (74%)	**34b** (56%)
c	Me	Ph	**33c** (50%)	
d	CCl_3	Me	**33d** (74%)	
e	Ph	SMe	**33e** (77%)	
f	4-ClC_6H_4	Me	**33f** (85%)	
g	4-$NO_2C_6H_4$	Me	**33g** (79%)	

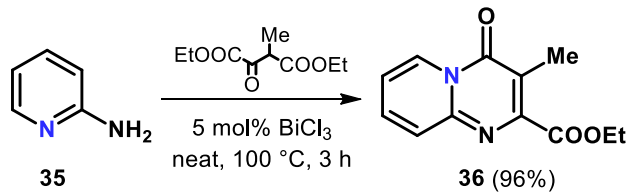
Scheme 11. Synthesis of pyrido[2,3-*d*]pyrimidine derivatives **33a–g**, **34a,b** *via* acetophenone derivatives **32a–g**

2.1.2. *N*-Bridgehead annulations

For the synthesis of 4*H*-pyrido[1,2-*a*]-pyrimidin-4-one skeleton, several methods have been described. Earlier reports include the reaction of 2-aminpyridine with different reagents under varied conditions ranging from reactions at 250 °C using Meldrum's acid[60] through reactions in AcOH (under reflux conditions)[61] or with the use of metal catalysts[62] to milder reactions in ethanol at reflux temperature.[63] However, the synthesis of quinoline compounds bearing both the same bridgehead nitrogen and the 4-oxo and 2-carboxylic functions required different conditions.

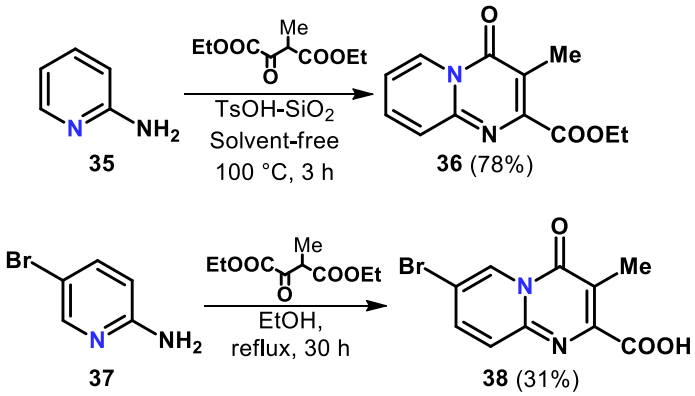
In this subsection, methods available for the synthesis of the specific skeleton until now are collected.

As a straightforward approach, the Conrad–Limpach procedure can also be applied to synthesize the required derivatives. Diethyl 2-methyl-3-oxosuccinate, as a functionalized β-keto ester was used by Jaenicke *et al.* along with bismuth trichloride as catalyst to synthesize KYNA analogue **36**, methyl-substituted at C-3 containing bridgehead nitrogen (Scheme 12). Regarding catalysts, the reaction showed high selectivity toward bismuth trichloride compared to other salts, such as zinc chloride or iridium chloride.[64]



Scheme 12. Synthesis of C-3 functionalized *N*-bridgehead derivative **36** using metal catalyst

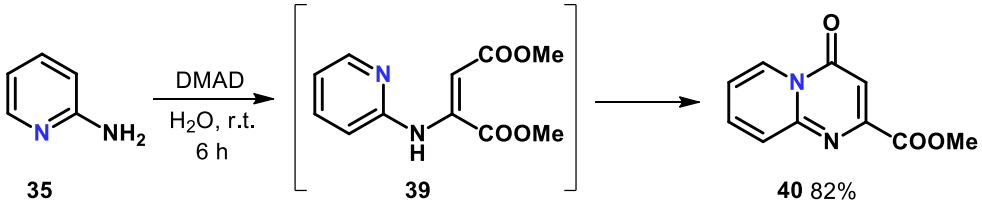
It has been shown in two articles that the synthesis can also be carried out without the use of a toxic metal catalyst. Starting from the same β-keto ester, the synthesis of both **36** and the 6-bromo-substituted derivative **38** could be achieved (Scheme 13).[65,66] Though the inaccurate description of the methods (not given w/w% and mol% of TsOH-SiO_2 catalyst) and the lower yields overshadow the results.



Scheme 13. Metal-free synthesis of compound **36** and its bromo-substituted derivative **38**

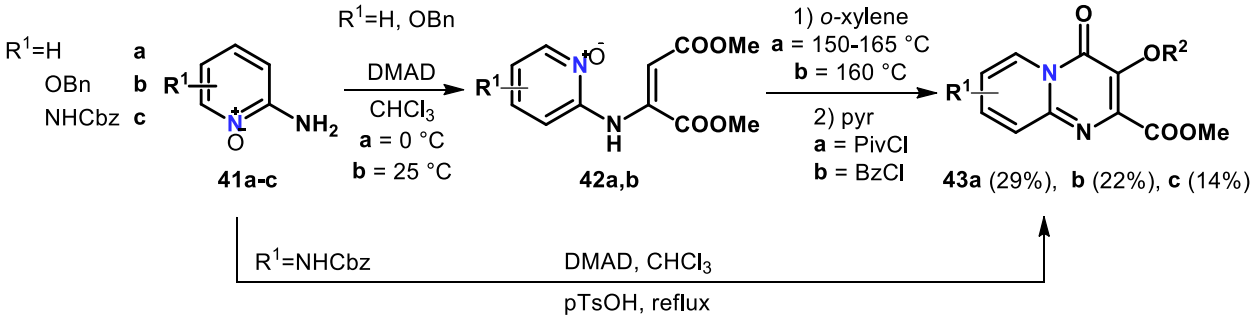
Similar to the synthesis of pyridine-fused derivatives, the β-keto ester electrophile can be replaced by other electron-deficient reagents, such as DMAD. One of the reactions described used the acetylene derivative in water. The reaction carried out at room temperature yielded the unsubstituted *N*-bridgehead derivative **40** in 6 hours (Scheme 14).[67] This procedure was later

applied in a patent to create derivatives inhibiting MeTTL3 activity, and in this way proliferative disorders, such as cancer, autoimmune, infectious or inflammatory diseases.[68]



Scheme 14. Synthesis of unsubstituted *N*-bridgehead derivative **40**

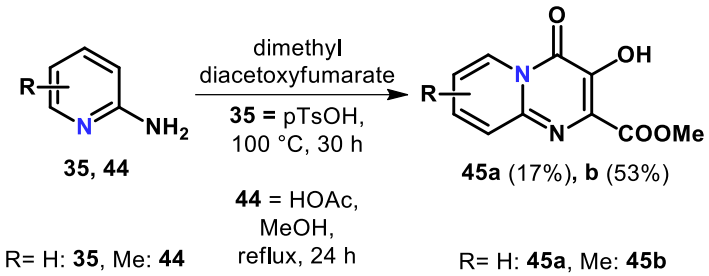
A similar procedure was used by Summa *et al.*[69,70] to synthesize 3-hydroxy-containing 9a*H*-pyrido[1,2-*a*]pyrimidine derivatives (Scheme 15). Based on the Conrad–Limpach procedures using DMAD as described in previous paragraphs, it is expected that the first step would involve the enamine formation of the acetylene and the amino group. However, based on their previous experiences with the synthesis of hydroxypyrimidinones, the formation of an *O*-adduct intermediate was expected. This could possibly be a 2-aminopyridine-*N*-oxide regarded as the tautomer of amidoxime. These derivatives are known to react with DMAD to form *O*-adducts (vinylhydroxylamines).[71,72] The subsequent rearrangement/cyclization of **42a,b** took place in *o*-xylene under reflux. However, the isolation of the product was not described, since it was further transformed without isolation into its pivalate derivative (**43a**) in order to facilitate its purification. The procedure was successfully broadened to other aminopyridine derivatives as well, with *N*-oxidation of the aminopyridines. It is interesting to mention that in the case of **43c**, the described final synthesis using *p*-toluenesulfonic acid (*p*TsOH) as catalyst, was performed in a single step, providing the lowest yield.



Scheme 15. Synthesis of C-3 aryl- and alkoxy-substituted derivatives **43a–c**

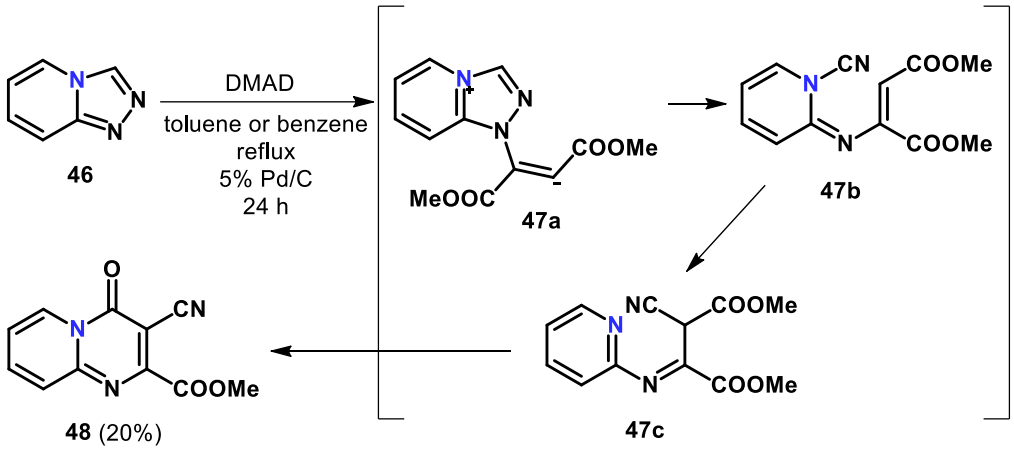
Furthermore, in addition to DMAD, the synthesis of the same C-3 hydroxy-functionalized compounds can be achieved through the use of a different electrophile, namely, dimethyl diacetoxyfumarate. Upon reacting with substituted 2-aminopyridines, it yields

2-hydroxy-pyrido[1,2-*a*]pyrimidin-4-on-2-carboxylic acid derivatives (**45a,b**, Scheme 16).[73] The method was later applied for the synthesis of different HIV-1 integrase inhibitors.[74–76]



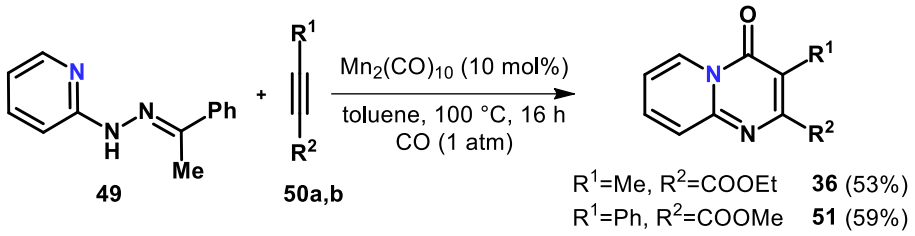
Scheme 16. Synthesis of C-3 hydroxy-substituted *N*-bridgehead derivatives **45a,b**

In continuing the discussion of reactions utilizing acetylenes to yield the desired pyrido[1,2-*a*]pyrimidin skeleton, DMAD can also be reacted with nucleophiles derived from unique compounds. An interesting alternative method was investigated by Rees *et al.* using 1,2,4-triazolo[4.3-*a*]pyridine as nucleophile (Scheme 17).[77] Among other reaction routes yielding different products, DMAD can react with the *N*-1 atom of the triazole ring, yielding intermediate **47a** delivering **48** through a ring-opening and subsequent intramolecular ring-closure.



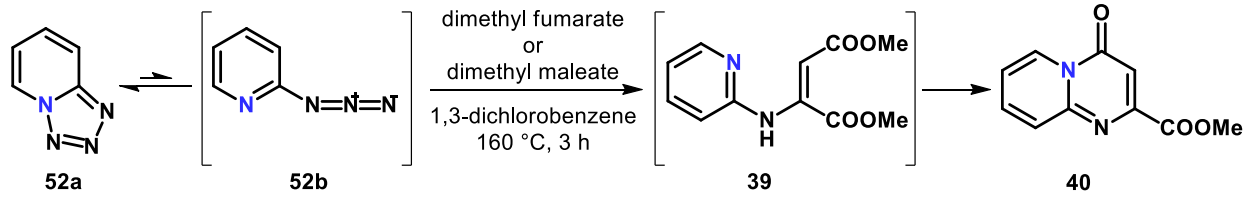
Scheme 17. Synthesis of *N*-bridgehead KYNA derivative **48** *via* ring-member extension

Even though previous methods described above give possibilities for the synthesis of C-3-substituted derivatives, further functionalization can be achieved through the method reported by Ackermann *et al.*[78] Starting from 2-pyridylhydrazone **49** and alkyne **50a,b**, manganese-catalyzed carbonylative annulations yielding compounds **36** and **51** were carried out (Scheme 18). The method was mainly used for the synthesis non-carboxylic derivatives; however, judging from the number of available acetylene derivatives, this reaction may provide a useful approach to synthesize new, C-3 functionalized *N*-bridgehead KYNA derivatives.



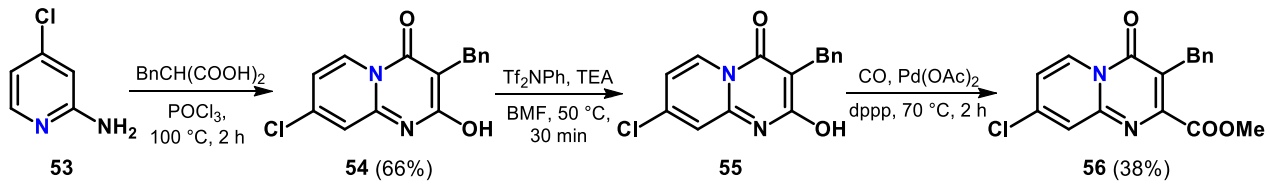
Scheme 18. Synthesis of derivatives **36** and **51** *via* a manganese-catalyzed reaction

One of the first publications regarding the synthesis of the pyrido[1,2-*a*]-pyrimidine skeleton was published in 1969 by Sturm *et al.*[79] An azide derivative, a special nucleophile, was used as starting compound upon reacting with either dimethyl fumarate (64% product yield) or dimethyl maleate (59% yield). The following steps were proposed for a possible mechanism. First, tautomer **52b** reacts with one of the electrophiles through a Diels–Alder type forming a 5-membered ring. In subsequent ring opening and elimination of N_2, enamine intermediate **39** is formed which, after a thermally driven ring closure, gives **40** as the final product (Scheme 19). The reaction between aminopyridine and acetylenedicarboxylic acid esters resulting in the same enamine (**39**) and giving the same product (**40**) after cyclization at 140 °C, was also mentioned. However, neither its synthesis was described nor a related literature reference was cited.



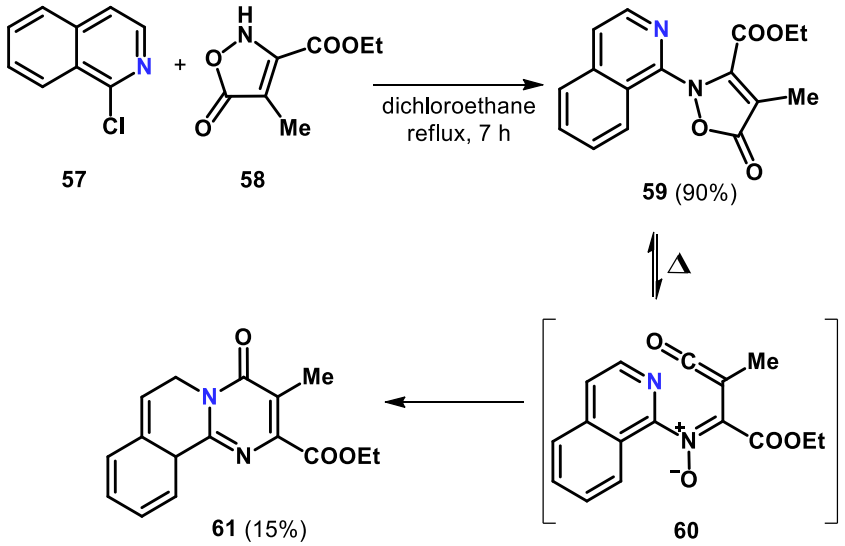
Scheme 19. Synthesis of **40** from an azide starting compound

The use of malonic acid derivatives was also applied to synthesize derivatives bearing C-3 substitutions. However, this method requires further modifications, since ring closure yields only 2-hydroxy derivative **54**, that needs to be oxidized (Scheme 20).[80] Even though the procedure itself is not as elegant as the previous ones, the use of functionalized anilines and malonic acids may yield a wide range of derivatives.



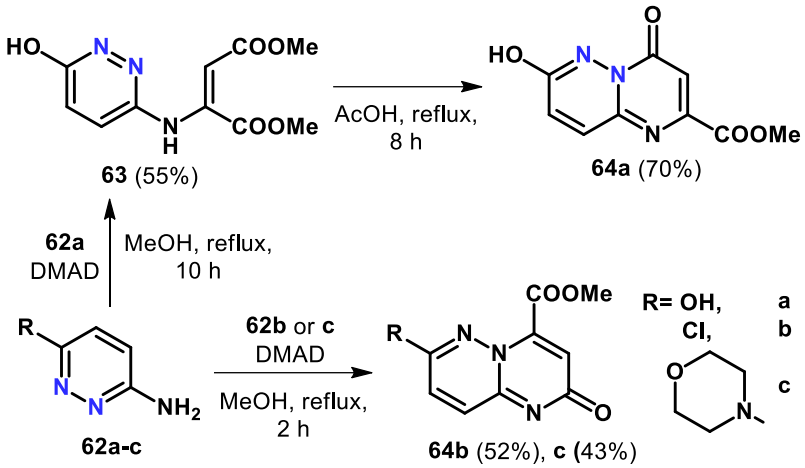
Scheme 20. Further method for the synthesis of C-3 substituted *N*-bridgehead derivatives

It is worth mentioning that the formation of the pyridine-fused pyrimidine skeleton has also been observed during the pyrolysis of an isoxazoline-substituted isoquinoline (**59**). The research group investigated the photolysis of different isoxazoline-substituted derivatives. When the photolysis of **59** was changed to pyrolysis in a scale-up study, the formation of **61** was observed (Scheme 21).[81]



Scheme 21. Pyrolytic decomposition of compound **59** yielding *N*-bridgehead derivative **61** of KYNA

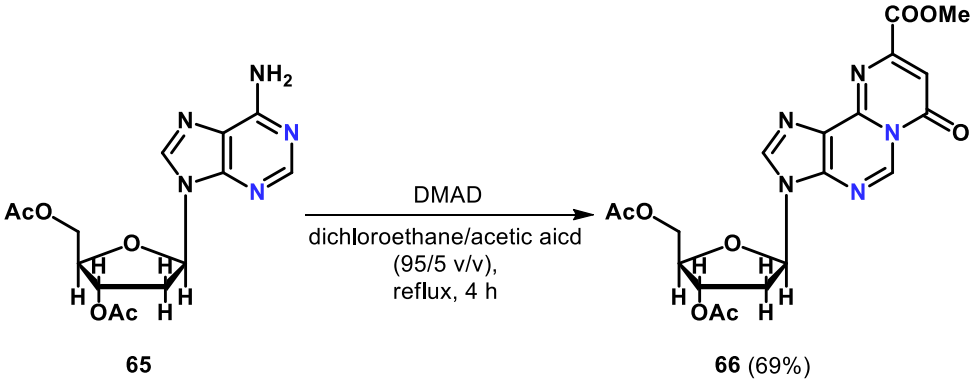
Six-membered heterocycles containing more heteroatoms have reduced reactivity toward electrophiles. As a consequence, during the synthesis of KYNA skeletons with pyridazine, pyrimidine or pyrazine moieties, aniline starting materials may require further modifications. One such modified method applied by Mátyus *et al.* uses DMAD and the accompanying aminopyridazine is substituted with electron-donating groups (EDG). However, beside electron donation, further factors may be present, as ring closure led to the formation of the desired pyrimido[1,2-*b*]pyridazin-2-one derivative only in the case of the hydroxy-substituted starting compound (**64a**). The morpholino group yielded 2-oxo-4-carboxylate analogues, similar to the chloro-substituted derivative (**64b,c**, Scheme 22).[82,83]



Scheme 22. Synthesis of pyrimido[1,2-*b*]pyridazine derivative (**64a**) of KYNA

The research group also noted this difference as an anomaly, based on a previous work.[84] These pieces of information and results with electron-density calculations support the conclusion that the favored reaction would be a nucleophilic addition on C-3 of DMAD.

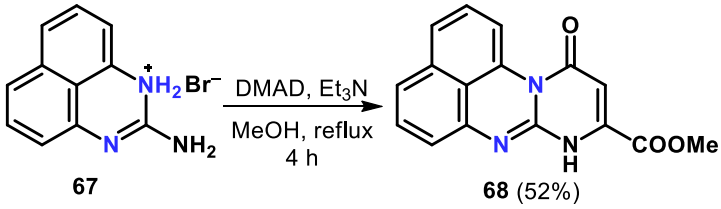
Koomen *et al.*[85] also used DMAD to synthesize adenosine derivatives bearing fluorescent attributes (Scheme 23). The starting 2'-deoxyadenosine compound **65** can be considered to be an imidazole-fused 6-aminopyrimidine. As previously mentioned, pyrimidine derivatives should be less reactive and thus unwilling to undergo the Michael addition; however, the imidazole ring may induce an excess of electron of the pyrimidine ring and promoting the reaction.



Scheme 23. Synthesis of adenosine derivative **66**

The work of Kang-Chien *et al.*[86] also seems to support the hypothesis that an electron-donating substituent favors the Michael addition. The synthesis of a special tetracyclic quinoline derivative [4(1*H*)-oxopyrimido[1,2-*a*]perimidine-2-carboxylate, **68**] was carried out starting form 2-aminoperimidine that can be considered ~~as~~ ~~a~~ to be 2-aminopyrimidine fused with naphthalene. Compared to the imidazole ring discussed previously, the naphthalene ring has a ~~of~~ more

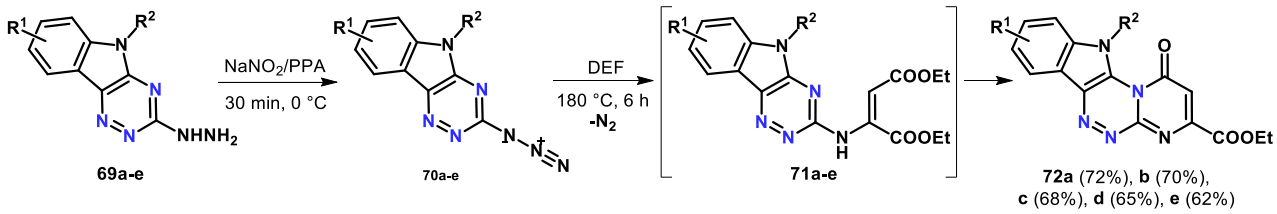
prominent electron-donating quality. Consequently, the reaction could be carried out in MeOH (Scheme 24).



67 **68** (52%)

Scheme 24. Synthesis of perimidine derivative **68**

The work of Jain *et al.* can be regarded as a further development of the study by Sturm *et al* described in Scheme 19 except that it was implemented in the synthesis of bridgehead derivatives.[87] Though via a different procedure, an azide intermediate was generated in both studies and then it was reacted further with a fumaric acid ester (in this case diethyl fumarate, DEF). Beside the hypothesized steps described by Sturm *et al.*, an alternative route have also been proposed. First, the decomposition of the azide function takes place and then a subsequent DEF attack provides an aziridine ring that opens up to give the enamine intermediates **71a–c** (Scheme 25).



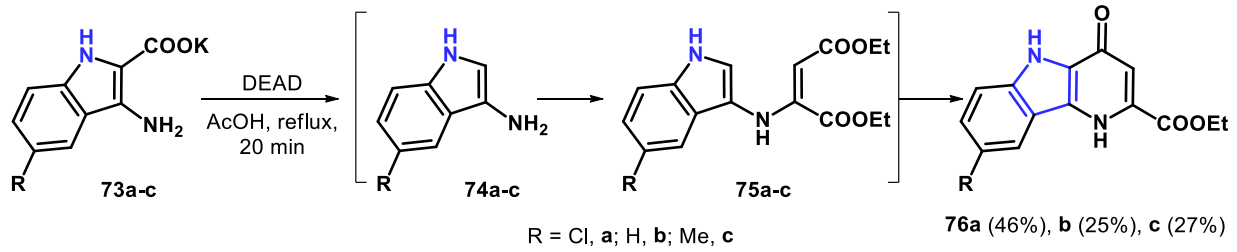
69a-e **70a-e** **71a-e** **72a** (72%), **b** (70%), **c** (68%), **d** (65%), **e** (62%)

R^1=R^2=H: **a**; R^1=7-F, R^2=H: **b**; R^1=8-F, R^2=H: **c**; R^1=H, R^2=Me: **d**; R^1=9-CF_3, R^2=H: **e**

Scheme 25. Synthesis of indole-fused *N*-bridgehead derivative **72a–e** from azide starting compounds

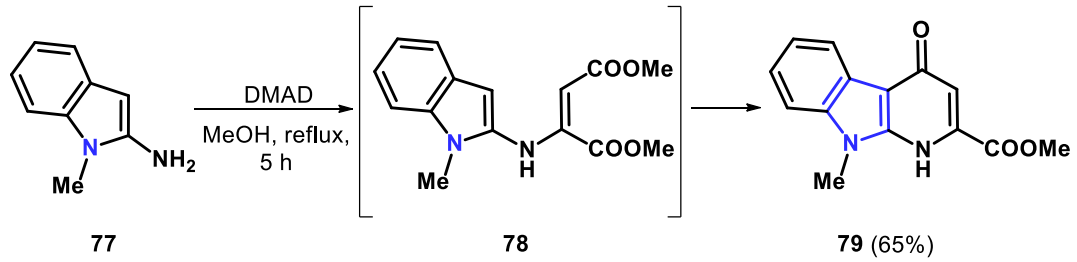
2.1.3. Five-membered B-ring derivatives

Since five-membered heterocyclic compounds have an excess of electrons, the modified Conrad–Limpach procedure applying DMAD or DEAD (diethyl acetylenedicarboxylate) for intermediate formation and its subsequent ring closure can be easily carried out. Both pyrrolo[2,3-*b*]pyridine and pyrrolo[3,2-*b*]pyridine ring systems have been synthesized in a similar way. Grinev *et al.* used the potassium salt of 3-aminoindole-2-carboxylic acid in glacial acetic acid yielding 3-aminoindole *in situ* that subsequently reacted with DEAD (Scheme 26).[88] The reaction yields δ-carbolines **76a–c** similar to the reactions described by Guyot *et al.* reporting the synthesis of α-carboline derivatives **79** (Scheme 27).[89]



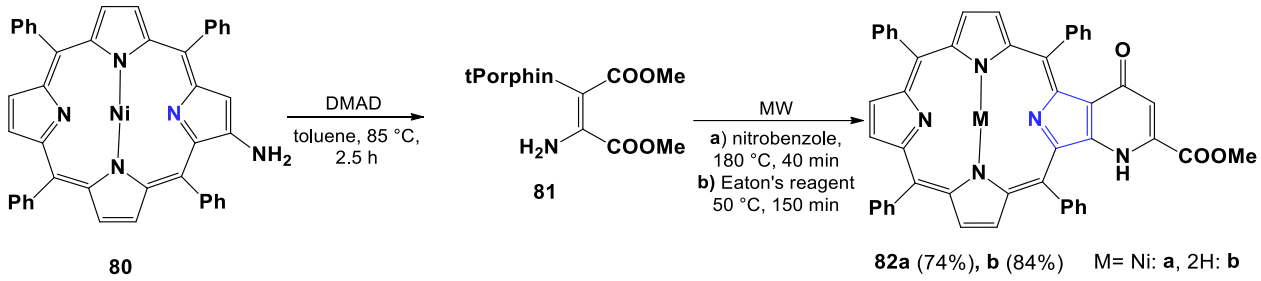
Scheme 26. Synthesis of δ-carboline KYNA derivatives **76a–c**

It is interesting to note that in both cases, probably as the consequence of the high nucleophility of indole, formation of side-products was also reported. In the synthesis of δ-carbolines, pentacyclic aromatic compounds appeared bearing an additional indole structure. In the case of α-carboline derivatives, in turn, Guyot *et al.* used *N*-methyl-2-aminoindole to presumably inhibit the formation of bridge-head nitrogen-containing derivatives. It was not directly specified by Guyot *et al.* whether it was for this reason. Nevertheless, in the following paragraphs, they described the synthesis of a derivatives, where the ring closure takes place at the unsubstituted N-1 atom.



Scheme 27. Synthesis of α-carboline KYNA derivative **79**

The same Conrad–Limpach procedure can also be used for the synthesis of the pyrrolo[3,4-*b*]pyridine skeleton. Silva *et al*. prepared a tetraphenylporphin (tPorphin) compound containing the pyridine-2-carboxylic acid moiety fused to one of the pyrrole rings of the porphin skeleton (**82a,b**, Scheme 28).[90]

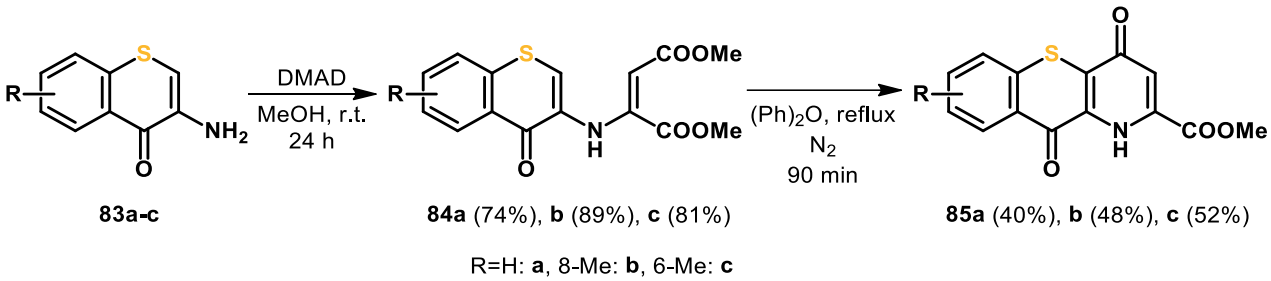


Scheme 28. Synthesis of tetraphenylporphin derivatives **82a,b**

2.2. Sulfur-containing ring systems

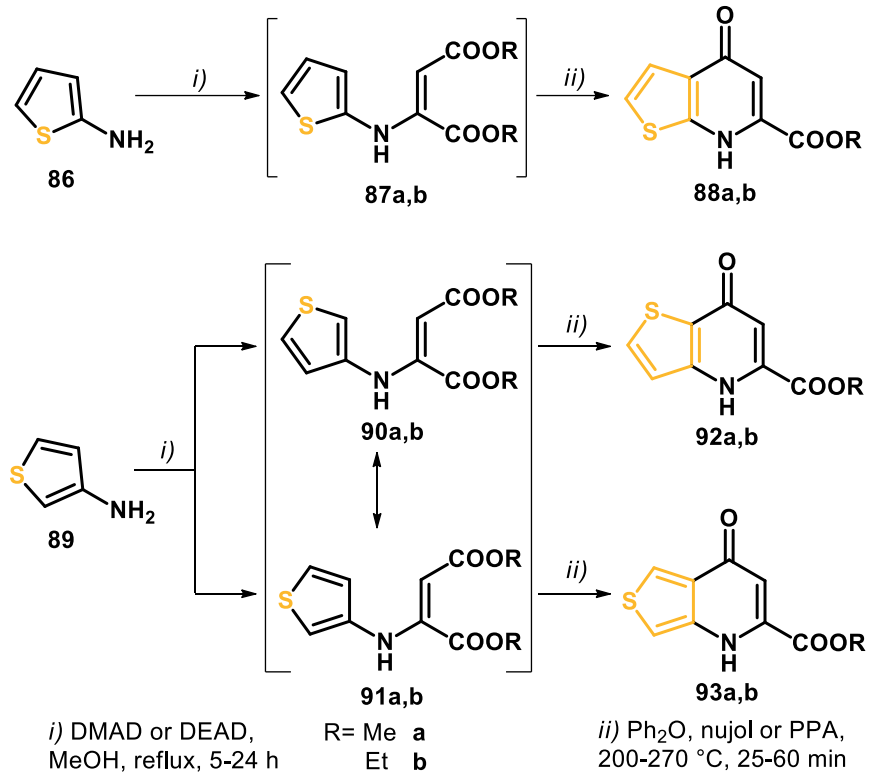
Figure 3.

Both six- and five-membered derivatives have been synthesized. Kitao *et al*. used a modified Conrad–Limpach procedure, utilizing DMAD to form enamine intermediates (**84a–c**). Ring formation was carried out in Ph_2O (Scheme 29).[91]



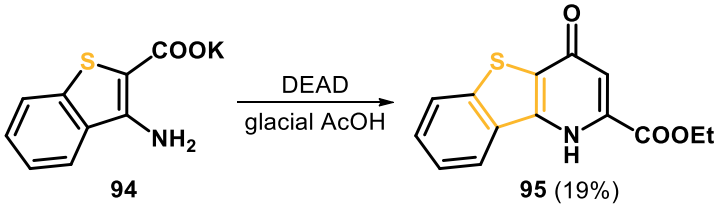
Scheme 29. Synthesis of 4*H*-thiopyrano[3,2-*b*]pyridine derivatives **85a–c**

Baron *et al*. fabricated a wide variety of excitatory amino acid antagonists with the six-membered B ring of KYNA changed to a five-membered sulfur-containing ring. The synthesis of these compounds is similar to that of the six-membered skeleton, utilizing DMAD or DEAD in the first step with the corresponding aminothiophenes (**86,89**). The subsequent intramolecular ring closure was carried out in Ph_2O, nujol or polyphosphoric acid (Scheme 30).[92] Unfortunately, no yields have been published for compounds **88a,b**, **92a,b**, **93a,b**.



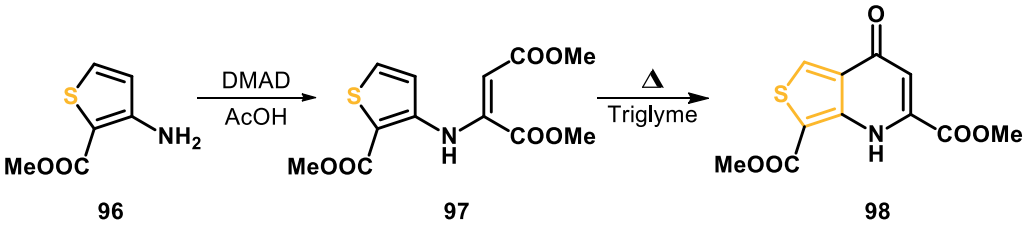
Scheme 30. Synthesis of thiophene derivatives **88a,b**, **92a,b** and **93a,b**

Though the method described in Scheme 30 has the most detailed description, it is not the only one. The synthesis of a thieno[3,2-*b*]pyridine system was already published earlier. Here, similar to the δ-carboline derivatives,[88] DEAD was used yielding the thieno analogue (**95**) of δ-carboline derivative **76b** (Scheme 31).[93]



Scheme 31. Synthesis of the thieno analogue of δ-carboline derivative **76b**

The thieno[3,4-*b*]pyridine skeleton was already mentioned earlier. However, in this work, the synthesis yield was not reported (Scheme 32).[94]



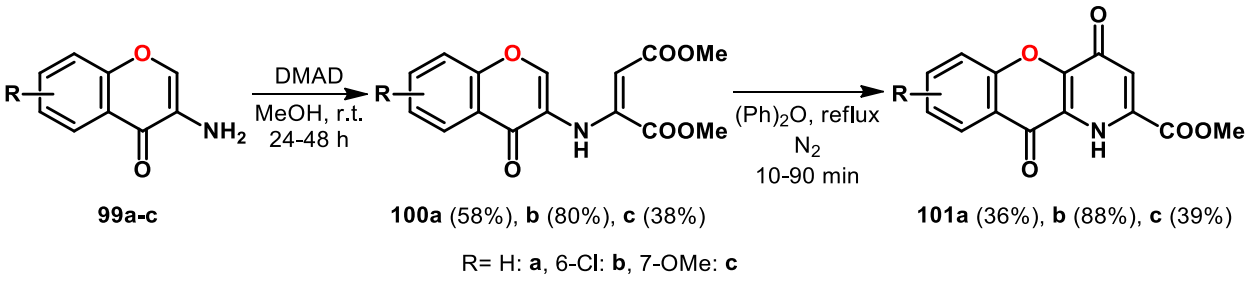
Scheme 32. Synthesis of thieno[3,4-*b*]pyridine derivative **98**

2.3. Oxygen-containing ring systems

Figure 4.

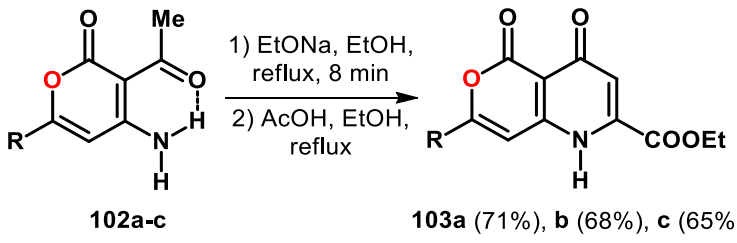
Regarding pyrano ring systems, it must be emphasized that in contrary to nitrogen-bearing systems, the aromaticity of the compounds is lost. Whereas the procedures described in the following paragraphs indicate that efficiency of the synthesis is not affected, the potential biological activity may vary substantially in comparison with KYNA.

Kitao *et al.* based their procedure for the thiopyrano derivatives[92] on the work of Strandtmann *et al.* who synthesized 1,5-dihydro-4,10-dioxobenzopyranopyridine-2-carboxylic acid derivatives **101a–c** (Scheme 33).[95]



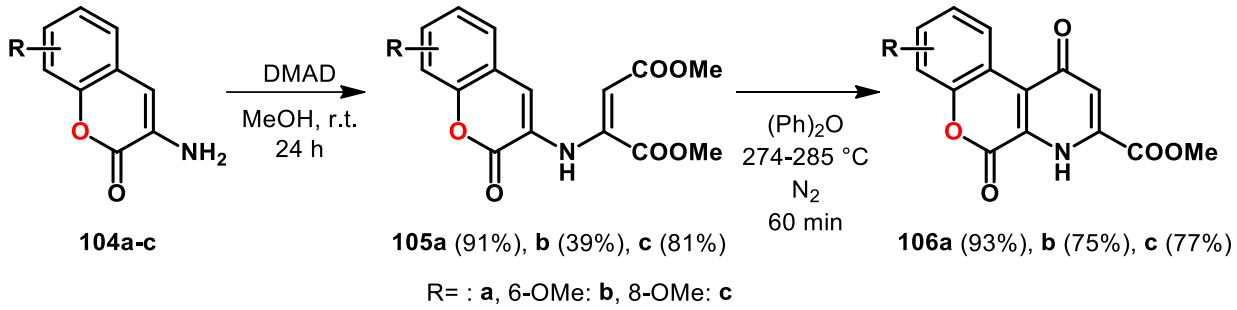
Scheme 33. Synthesis of benzopyranopyridine derivatives **101a–c**

Dorokhov *et al.*, beside synthesizing the pyridine- and pyrimidine-fused derivatives with the use of acetophenones,[41,42] also achieved the synthesis of pyranone derivatives. Pyranones **102a–c** bearing the crucial active α-methyl function, similar to the ones used for pyridine- and pyrimidine-fused derivatives synthesized previously, were reacted with diethyl oxalate in the presence of sodium ethoxide (Scheme 34).[96]



Scheme 34. Synthesis of pyrano[4,3-*b*]pyridine compounds **103a–c** *via* acetophenone derivatives

The method used by Strandtmann *et al*. in the synthesis of benzopyrano[2,3-*b*]pyridine derivatives mentioned above was extended by the research group to synthesize benzopyrano[3,4-*b*]pyridine derivatives (**106a–c**, Scheme 35).[95] The triazol derivatives of these compounds have been patented for their possible useful application in allergic manifestations such as bronchial asthma, hay fever, etc.[97]

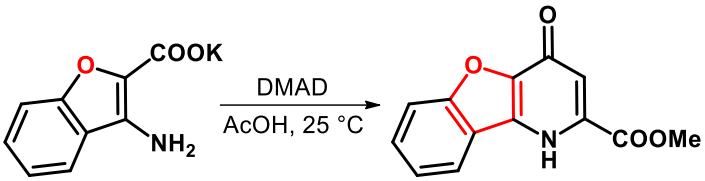


104a-c **105a** (91%), **b** (39%), **c** (81%) **106a** (93%), **b** (75%), **c** (77%)

R= : **a**, 6-OMe: **b**, 8-OMe: **c**

Scheme 35. Synthesis of pyrano[3,4-*b*]pyridine derivatives **106a–c**

Regarding the furan-fused 4-oxopyridine-2-carboxylic acid derivatives, the only synthesis mentioned describes a procedure similar to that applied in the synthesis of δ-carbolines reported earlier.[89] Using a potassium salt of 3-aminobenzofuran-2-carboxylic acid (**107**) and DMAD, compound **108**, the furan analogue of δ-carboline **76b** could be synthesized (Scheme 36).[98]



107 **108**

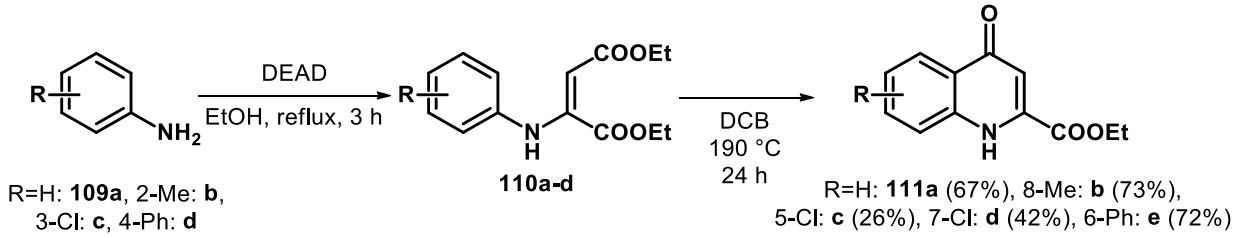
Scheme 36. Synthesis of **108** the furan analogue of δ-carboline **76b**

3. Results and discussions

3.1. Synthesis of substituted KYNA derivatives

3.1.1. Preparation of KYNA esters *via* the optimized Conrad–Limpach procedure

For the modified Mannich reactions to be carried out, KYNA and its derivatives were needed. In the case of KYNA, the synthesis of the required ethyl 4-hydroxyquinoline-2-carboxylate (**111a**) derivative has already been published.[99] In the described process, aniline was reacted with diethyl acetylenedicarboxylate forming the intermediate enamine. The second ring-closing reaction in Ph_2O at 250 °C led to the formation of the ethyl ester in an overall yield of 58%. In our case, the following optimizations were applied: (i) after the formation of enamine **110a**, column chromatography was used to purify the intermediate; (ii) Ph_2O was replaced by DCB that has lower boiling point (180 °C) allowing an easier work-up procedure. These modified conditions led to the formation of **111a** in an overall yield of 67% (Scheme 37). The same procedure was used for the synthesis of KYNA derivatives, alkyl-, aryl-, and halogen-substituted at the B ring (**111b–e**) as well. It is interesting to mention that in the case of starting material **109b**, two regioisomers formed with a ratio of 1:2 (**111c**:**111d**).

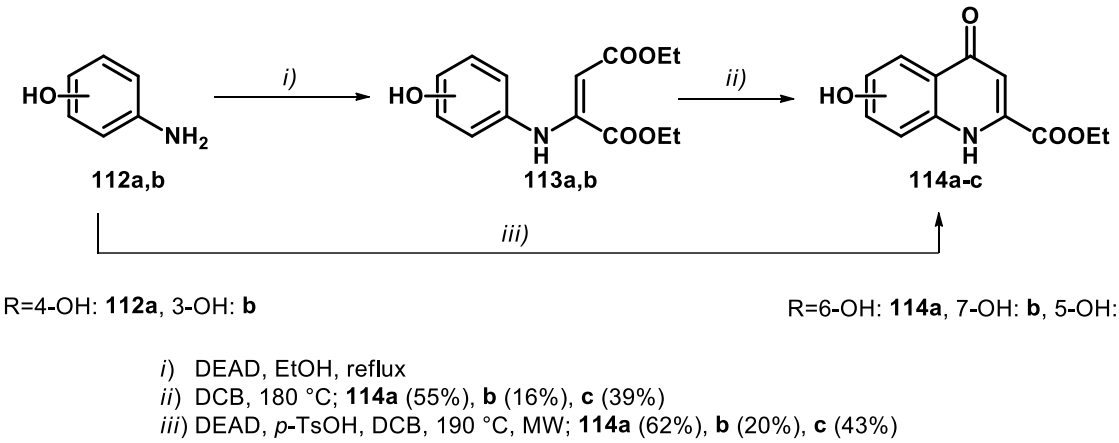


Scheme 37. Synthesis of KYNA ethyl ester.

3.1.2. Synthesis of hydroxy-functionalized KYNA derivatives

Although the synthesis of the methyl esters of hydroxy derivatives are described in the literature,[100,101] ethyl esters were chosen for several reasons. By carrying out their *m*Mr, they provided a better basis for comparison with previous derivatives. Furthermore, by using DEAD, faster formation of the enamine intermediate was observed. The desired

derivatives (**114a–c**) were synthesized with the Conrad–Limpach method applying the optimizations refined in previous investigations (Scheme 38).

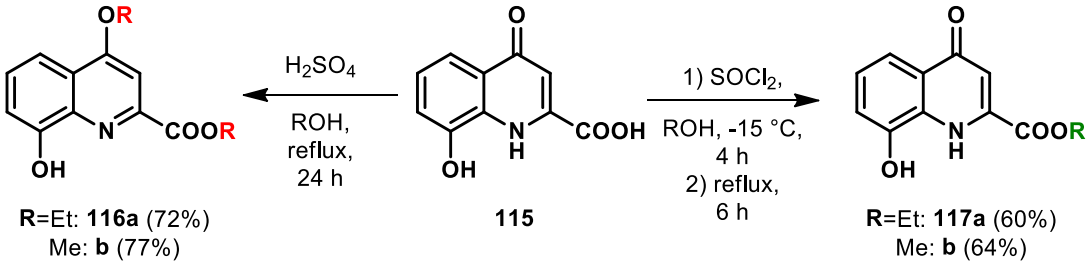


Scheme 38. Synthesis of hydroxykynurenic acid derivatives

As a last step, an additional column chromatographic purification of the esters was also required. The reason is that during the synthesis, even with the application of DEAD instead of the methyl derivative, maleimide side-products were formed in high yields. The literature describes the formation of these compounds as side-products during certain Conrad–Limpach reactions.[102–104] However, in our case, probably due to the higher reactivity of hydroxyanilines, the synthesis were shifted toward the formation of the maleimides. After purification, the desired hydroxy esters were isolated in moderate yields. Note that the synthesis starting from *m*-aminophenol allowed the isolation of the two possible regioisomers with a final ratio of 1:2 (**114b**:**114c**) similar to that of derivatives **111c**:**111d**.

Based on the work of Sutherland *et al.*,[105] *p*-TsOH as catalyst was investigated in a one-pot version of the Conrad–Limpach procedure applying microwave irradiation (Scheme 38). The synthesis provided the appropriate hydroxy derivatives in increased yields with diminished maleimide formation and the work-up could be carried out without time-consuming chromatography.

Compound **117a** was synthesized by the esterification of 8-hydroxykynurenic acid (xanthurenic acid, **115**) as it was commercially available (Scheme 39). This step was needed to avoid the direct use of poorly soluble xanthurenic acid and to obtain information for the *m*Mr comparable to that of **111a**–**e** and **114a**–**c**.



Scheme 39. Esterification of xanthurenic acid (**115**)

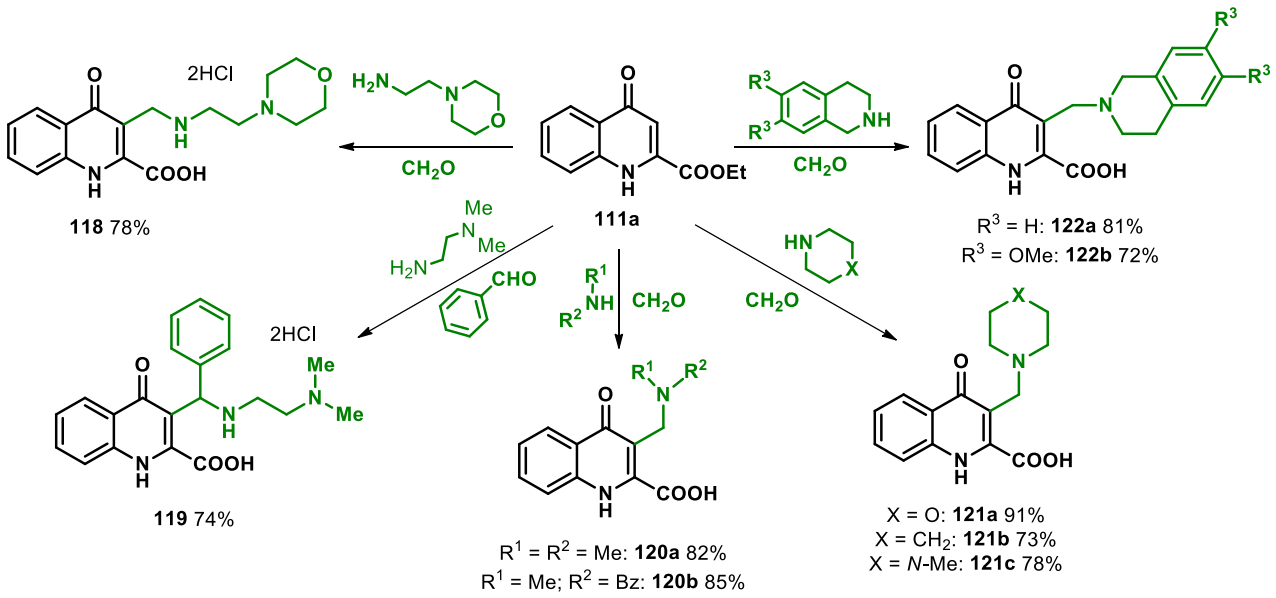
It is interesting to mention that the esterification was first carried out according to a literature method used for the synthesis of the methyl ester.[106] The use of EtOH as solvent led to the isolation of 4-ethoxy-substituted ethyl ester **116a**. Since methanol was used for the synthesis described in the literature cited above, the reaction was repeated in this solvent. In a similar manner, under these conditions, methoxy-substituted methyl ester **116b** could be isolated. As suggested also by the literature, **116a** and **116b** should have been formed during the esterification by thionyl chloride in EtOH or MeOH.[107] In our hands, however, employing these conditions, both esters (**117a**,**b**) could be obtained in good yields, without being contaminated by the corresponding 4-alkoxyquinoline.

3.2. Application of synthesized KYNA derivatives in *m*Mr

3.2.1. Transformations of unsubstituted KYNA ethyl ester

The ethyl ester of 4-oxo-1,4-dihydroquinoline-2-carboxylate (**111**) was first reacted with 2-morpholinoethylamine in the presence of aqueous formaldehyde (22% solution). The reaction was conducted in different solvents [acetonitrile (MeCN), DMF, EtOH, and 1,4-dioxane] at different temperatures (60 °C, 80 °C, and 110 °C). The optimized reaction conditions were found to be reflux temperature, using 1,4-dioxane as solvent, and 5 h reaction time. All reaction conditions led to the formation of **118** as the single product. It is interesting to note that the medium was basic enough to lead to the hydrolysis of the ester function (Scheme 40).

To explore the scope and limitation of the transformation, **111** was reacted with *N*,*N*-dimethylethane-1,2-diamine in the presence of benzaldehyde. The desired amino acid derivative **119** was isolated in a yield of 74%. Next, starting from dimethylamine or *N*-benzylmethylamine in the *m*Mr, the insertion of cationic centers in one-carbon distance at position 3 could be achieved.

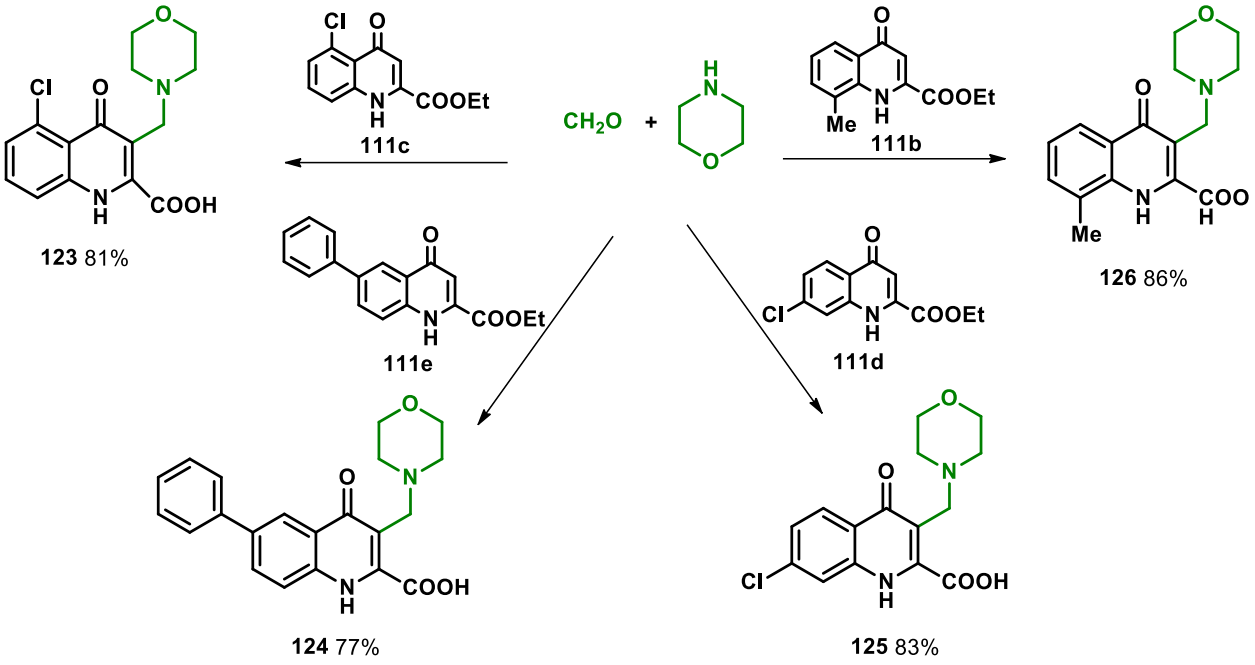


Scheme 40. Synthesis of kynurenic acid (KYNA) Mannich derivatives.

The extension possibility of the reaction was further tested by starting from cyclic secondary amines such as morpholine, piperidine, and *N*-methylpiperazine leading to **121a**, **121b**, and **121c**, respectively. As the last representative amines 1,2,3,4-tetrahydroisoquinoline and its dimethoxy analogue were chosen as aromatic fused cyclic secondary amines. In these cases, relatively long reactions (8 h and 10 h) led to the formation of **122a** and **122b**.

3.2.2. Substituted KYNA ethyl esters applied in *m*Mr

To test the effect of substituents at positions 5, 6, 7 or 8 on the *m*Mr, derivatives **111b–e** were reacted with morpholine, as a representative secondary cyclic amine, in the presence of formaldehyde. Reactions were carried out in 1,4-dioxane at reflux, yielding **123**–**126** (Scheme 41). Based on the yields, it can be concluded that aryl/alkyl substituents at position 6 or 8 and the halogen at position 5 or 7 have no significant influence on the aminoalkylation at position C-3.

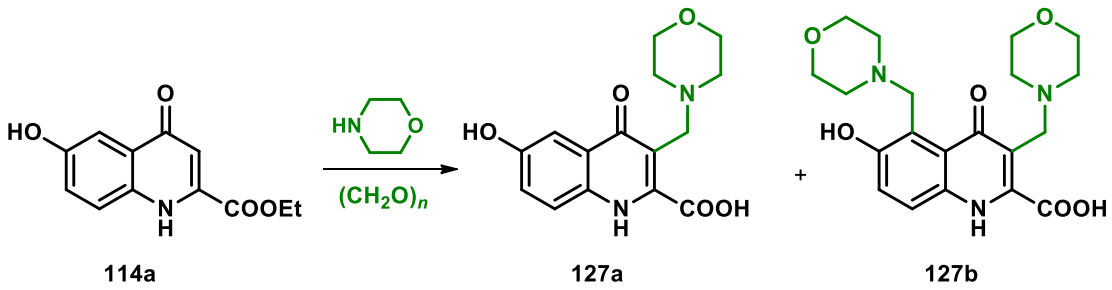


Scheme 41. Synthesis of 5-, 6-, 7-, and 8-substituted KYNA Mannich derivatives.

3.2.3. Diverse aminoalkylations of the hydroxylated KYNA derivatives

3.2.3.1. 6-Hydroxykynurenic acid ethyl ester

Based on preceding works, 1,4-dioxane seemed to be the optimal media for the substitution reactions of **114a**. However, after achieving only moderate conversion (Table 2, Entry 4) during the *m*Mr of **114a** (Scheme 42), the reaction was conducted in other solvents including MeCN as aprotic polar solvent, EtOH as protic polar solvent, and toluene as aprotic apolar solvent at 80 °C, using 1 equivalent of morpholine and 3 equivalents of paraformaldehyde (Table 2).



Scheme 42. *m*Mr of **114a** using morpholine and paraformaldehyde.

Table 2. Screening of solvents in the case of **114a**

Entry	Amine equiv.	Solvent	T (°C)	t	conv.[a]	127a:127b[a]
1	1.0	EtOH	80	30’	10%	**127a**
2	1.0	EtOH	80	1 h	50%	**127a**
3	1.0	EtOH	80	2 h	85%	4:1
4	1.0	1,4-dioxane	80	2 h	70%	1:1
5	1.0	toluene	80	2 h	~1%	–
6	1.0	MeCN	80	2 h	70%	1:2

[a]: determined from the crude NMR spectra

We aimed to investigate the selectivity of the synthesis in ethanol, since this solvent enabled the formation of the most homogeneous reaction mixture presumably facilitating the highest yield. Although the higher temperature accelerated the reactions, conversions were maximized at around 85% (Table 3, Entries 3 and 6). While higher temperature and longer reaction promoted the formation of **127b**, its share in the isolated products remained low this showing high selectivity towards C-3 substitution.

To further investigate the reactivity of compound **114a**, reactions using 2 and 3 equivalents of morpholine were carried out in EtOH. Faster reactions were observed in both cases, reaching a final ratio of 1:1 between **127a** and **127b** (Table 3, Entries 12 and 15) further suggesting that the substitution at C-5 is much less preferred relative to that taking place at C-3.

Table 3. Screening of temperature and reagent equivalents in the case of **114a**

Entry	Amine equiv.	Solvent	T (°C)	t	conv.[a]	127a:127b[a]
1	1.0	EtOH	100	30’	45%	4:1
2	1.0	EtOH	100	1 h	55%	4:1
3	1.0	EtOH	100	2 h	85%	7:2
4	1.0	EtOH	150	30’	60%	4:1
5	1.0	EtOH	150	1 h	65%	7:2
6	1.0	EtOH	150	2 h	85%	13:2
7	1.5	EtOH	80	30’	60%	4:1
8	1.5	EtOH	80	1 h	65%	3:1
9	1.5	EtOH	80	2 h	90%	2:1
10	2.0	EtOH	80	30’	60%	15:8
11	2.0	EtOH	80	1 h	70%	10:9
12	2.0	EtOH	80	2 h	90%	1:1
13[b]	3.0	EtOH	80	30’	99%	**128a**
14	3.0	EtOH	80	1 h	99%	10:1
15[c]	3.0	EtOH	80	2 h	99%	1:1

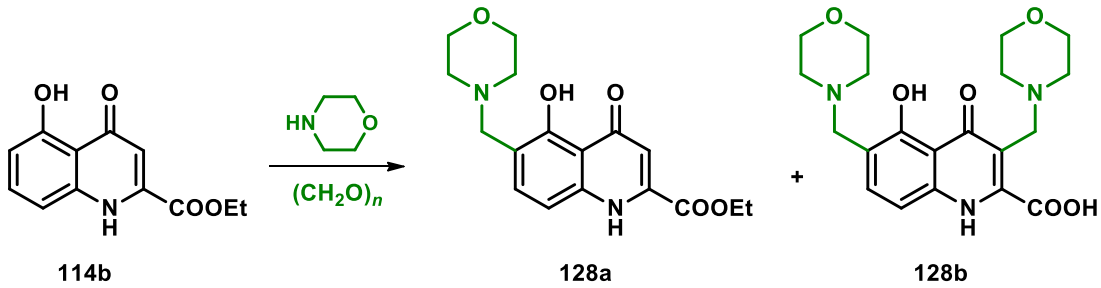
[a]: determined from the crude NMR spectra
[b]: work-up performed to isolate **127a**
[c]: work-up performed to isolate **127b**

It is worth mentioning that even though the reactions stopped after reaching a ratio of 1:1 after 2 hours, using increased amounts of amine halted the formation of **127b**. This indicates a possible basic inhibition of the reaction preventing the formation of the reactive iminium ion. This view is in accord with the mechanism proposed for the Mannich-type condensation studied in this contribution. On the basis of solvent screening, the selectivity towards the formation of the disubstituted derivative can be increased by using aprotic solvents. Comparing the effect of MeCN to that of toluene and 1,4-dioxane suggests that an increased polarity might also increase this selectivity indicating the involvement of ionic species in the crucial regioselective coupling as discussed below.

3.2.3.2. 5-Hydroxykynurenic acid ethyl ester

On the basis of the results of the reactions performed with compound **114a**, the first *m*Mr starting from **114b** was carried out in EtOH (Scheme 43). Since the reaction featured moderate conversion (Table 4, Entry 3), the *m*Mr was repeated using the other three solvents, showing lower conversion rates. Considering similarity in selectivity with **114a**, a detailed investigation with EtOH as protic polar solvent was performed. During the reactions, C-6 aminoalkylated derivative **128a** appeared to be the primary product with the

C-3, C-6 disubstituted derivative formed only upon using prolonged reactions. It is interesting to note that in the case of **128a**, the hydrolysis of the ester function did not take place, while in the case of compound **128b** the free acid was isolated.



Scheme 43. *m*Mr of compound **114b** using morpholine and paraformaldehyde

Table 4. Screening for solvent in the reaction of **114b**

Entry	Amine equiv.	Solvent	T (°C)	t	conv.[a]	128a:128b[a]
1	1.0	EtOH	80	30’	50%	3:1
2	1.0	EtOH	80	1 h	70%	2:1
3	1.0	EtOH	80	2 h	70%	2:1
4	1.0	1,4-dioxane	80	2 h	60%	1:1
5	1.0	toluene	80	2 h	55%	5:3
6	1.0	MeCN	80	2 h	65%	2:1

[a]: determined from the crude NMR spectra

Formation of **128a** can be promoted by using higher temperature and shorter reaction, as a prolonged reaction led to the appearance of **128b**, even using equivalent amount of reagents. With 3 equivalents of amine reagent, full conversion of **114b** was achieved after 30 minutes with a **128a**:**128b** distribution of 4:1 (Table 5, Entry 13), that could be increased to 1:4 in a 2-hour reaction (Table 5, Entry 15). Concluding the outcome of the reactions**, 114b** features higher reactivity at position C-6 than at position C-3. However, similar to **114a**, disubstitution of **114b** can also be promoted by using longer reactions regardless of the polarity of the solvent.

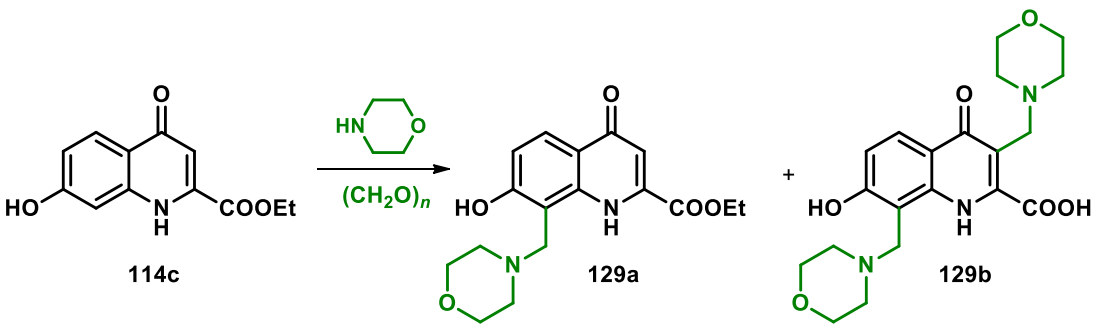
Table 5. Screening of temperature and reagent equivalents in the case of **114b**

Entry	Amine equiv.	Solvent	T (°C)	t	conv.[a]	128a:128b[a]
1	1.0	EtOH	100	30’	40%	**128a**
2	1.0	EtOH	100	1 h	70%	12:1
3	1.0	EtOH	100	2 h	70%	6:1
4	1.0	EtOH	150	30’	90%	**128a**
5[b]	1.0	EtOH	150	1 h	95%	**128a**
6	1.0	EtOH	150	2 h	99%	10:1
7	1.5	EtOH	80	30’	40%	10:3
8	1.5	EtOH	80	1 h	80%	7:1
9	1.5	EtOH	80	2 h	90%	2:1
10	2.0	EtOH	80	30’	90%	4:1
11	2.0	EtOH	80	1 h	95%	2:1
12	2.0	EtOH	80	2 h	99%	1:1
13	3.0	EtOH	80	30’	99%	4:1
14	3.0	EtOH	80	1 h	99%	1:1
15[c]	3.0	EtOH	80	2 h	99%	1:4

[a]: determined from the crude NMR spectra
[b]: work-up performed to isolate **128a**
[c]: work-up performed to isolate **128b**

3.2.3.3. 7-Hydroxykynurenic acid ethyl ester

The modified Mannich reactions of **114c** carried out in EtOH at 80 °C proved to be highly facile as indicated by the 80% conversion achieved even in 30 minutes (Scheme 44, Table 6, Entry 1). In this acidic phenol derivative, the reactivity of C-8 was found to be substantially higher than that of C-3 as indicated by the exclusive formation of **129a** observed in all experiments using one equivalent of morpholine. It is of note that the hydrolysis of the ester function in **129a** did not take place similar to that of **128a**.



Scheme 44. *m*Mr of compound **114c** using morpholine and paraformaldehyde

During reactions carried out employing higher equivalents of reagents, the formation of **129b** was also observed. However, the formation of this product was slow, becoming

detectable only after approximately 1.5–2 hours at 80 °C. As the formation of the products seemed to proceed under kinetic control, we attempted to perform a reaction using conventional heating for 56 hours that afforded **129b** in sufficient amount (Table 6, Entry 16).

As the formation of **129b** was low even upon using higher temperature or increased amount of reagents, solvents tested previously were also investigated. In the case of 1,4-dioxane (as an aprotic solvent with moderate polarity), a selectivity towards the formation of disubstituted derivative **129b** was observed (Table 7, Entry 1), while in MeCN both **129a** and **129b** were formed with selectivity slightly shifted towards **129a** (Table 7, Entry 3).

Table 6. Screening of temperature and reagent equivalents in the case of **114c**

Entry	Amine equiv.	Solvent	T (°C)	t	conv.[a]	129a:129b[a]
1	1.0	EtOH	80	30’	80%	**129a**
2	1.0	EtOH	80	1 h	95%	**129a**
3[b]	1.0	EtOH	80	1.5 h	99%	**129a**
4	1.0	EtOH	100	30’	75%	**129a**
5	1.0	EtOH	100	1 h	80%	**129a**
6	1.0	EtOH	100	1.5 h	90%	**129a**
7	1.0	EtOH	150	30’	–*	–
10	1.5	EtOH	80	30’	85%	**129a**
11	1.5	EtOH	80	1 h	95%	**129a**
12	1.5	EtOH	80	1.5 h	99%	**129a**
13	2.0	EtOH	80	30’	85%	**129a**
14	2.0	EtOH	80	1 h	90%	**129a**
15	2.0	EtOH	80	1.5 h	99%	18:1
16[c]	2.0	EtOH	80	56 h	99%	3:10
17	3.0	EtOH	80	15’	99%	**129a**
18	3.0	EtOH	80	1 h	99%	**129a**
19	3.0	EtOH	80	1.5 h	99%	18:1

[a]: determined from the crude NMR spectra
–*: multicomponent reaction, conversion could not be determined
[b]: work-up performed to isolate **129a**
[c]: work-up performed to isolate **129b**

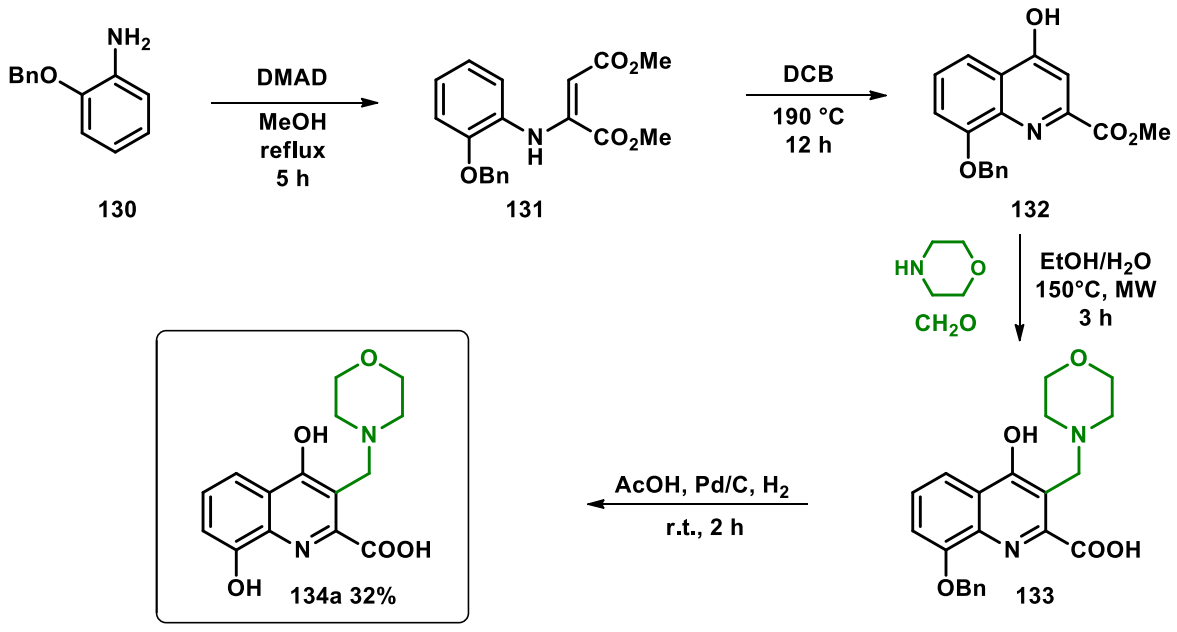
Table 7. Screening for solvent in the case for **114c**

Entry	Amine equiv.	Solvent	T (°C)	t	conv.[a]	129a:129b[a]
1	1.0	1,4-dioxane	80	2 h	45%	1:5
2	1.0	toluene	80	2 h	0%	–
3	1.0	MeCN	80	2 h	70%	2:1

[a]: determined from the crude NMR spectra

3.2.3.4. 8-Hydroxykynurenic acid ethyl ester

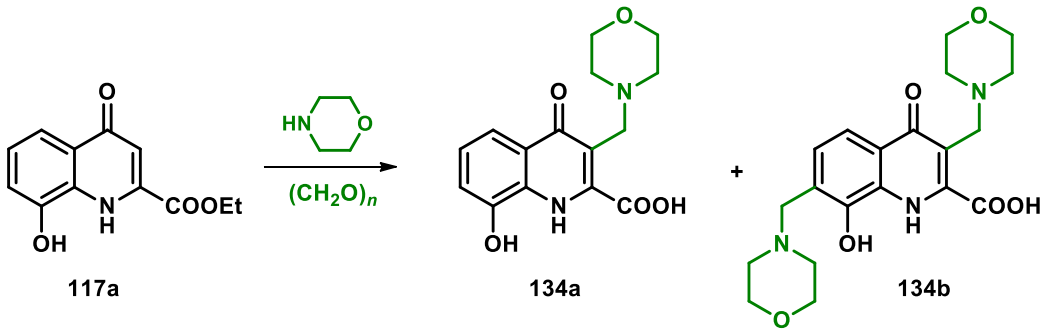
Compound **134a** has already been described in the literature, synthesized by employing benzyl protection of the 8-hydroxy function.[108] As a preliminary experiment, this reaction has also been carried out, implementing the following optimizations: (i) the enamine formation required longer reaction time; (ii) Ph_2O as solvent was changed to 1,2-dichlorobenzene for easier work-up; (iii) the aminoalkylation reaction required much higher reaction temperature; (iv) during the reduction step with platinum/carbon catalyst, if the reaction was kept under H_2 gas as long as described, a decrease in yield was observed. This might have been due to the adsorption of the product on the carbon surface that could be eluded with shorter reaction time and an immediate work-up (Scheme 45).



Scheme 45. Synthesis of morpholinomethylated xanthurenic acid **130a**

Without having any further information about the reactivity of the 8-hydroxy derivative, we decided to explore the effect of the unprotected function on the course of the reaction.

Based on our experiences on the synthesis of hydroxy derivatives discussed previously, aminoalkylations were first carried out in EtOH (Scheme 46). In all attempts at different temperatures complex mixtures were detected ~~but~~ without the formation of **134a**. Reactions, conducted in the presence of increased equivalents of reagents, also provided complex mixtures with only traces of **134b** detected in the crude product by ^{1}H-NMR (Table 8).



Scheme 46. Synthesis of aminoalkylated xanthurenic acid derivatives

Table 8. Screening of temperature and reagent equivalents in the case of **117a**

Entry	Amine equiv.	Solvent	T (°C)	t	conv.[a]	134a:134b[a]
1	1.0	EtOH	80	1 h	–*	–[b]
2	1.0	EtOH	100	1 h	–*	–[b]
3	1.0	EtOH	150	1 h	–*	–[b]
4	1.5	EtOH	80	1 h	–*	**134b**[c]
5	2.0	EtOH	80	1 h	–*	**134b**[c]
6	3.0	EtOH	80	1 h	–*	**134b**[c]

[a]: determined from the crude NMR spectra
–*: conversion could not be determined
[b]: no traces of **134a** or **134b** was detected
[c]: minimal amounts, could not be isolated

After these unsuccessful experiments carried out in EtOH, solvents tested previously were investigated as well. In toluene and MeCN the sole formation of **134b** was observed, while in 1,4-dioxane the reaction yielded the C-3 substituted derivative (**134a**) as the single product (Table 9).

Table 9. Screening for solvent in the case for **117a**

Entry	Amine equiv.	Solvent	T (°C)	t	conv.[a]	134a:134b[a]
1	1.0	1,4-dioxane	80	2 h	80%	**134a**
2	1.0	1,4-dioxane	100	2 h	90%	**134a**
3[b]	1.0	1,4-dioxane	150	2 h	99%	**134a**
4	2.0	1,4-dioxane	150	2 h	99%	**134a**
5[c]	2.0	toluene	80	1 h	99%	**134b**
6	2.0	MeCN	80	2 h	99%	**134b**

[a]: determined from the crude NMR spectra
[b]: work-up performed to isolate **134a**
[c]: work-up performed to isolate **134b**

3.2.3.5. DFT calculations on the reactivity of HO-KYNA derivatives

In order to rationalize the marked regioselectivity patterns observed in the modified Mannich reactions of a systematic selection of kynurenic acid esters, we undertook a series of comparative DFT calculations carried out with B3LYP functional[109–111] using 6–31 +G(d,p) basis set.[112] Computations were supported by the IEFPCM solvent model[113] with dielectric constant ε=24.5 to represent the polarity of ethanol employed as solvent in most experiments. The possible sequences of the competitive Mannich condensations accompanied by the hydrolysis of the ester residue are exemplified by the transformations of 6-hydroxykynurenic acid ethyl ester **114a** (Figure 5).

The mechanistic picture, accounting for the experimentally observed regioselectivity and ester hydrolysis, represented by the reaction pathways applied for the modified Mannich reactions of **114a**, can be extended to analogous multistep transformations of other kynurenic esters investigated in this work. However, searching for a reliable interpretation of the characteristic dependence of the regioselectivity on the substitution pattern, besides the relative thermodynamic stability, the HOMO delocalization and the local NBO charges[114] were also disclosed for the corresponding anion pairs type **114/I-114/II** as outlined on Figure 5. Since the crucial coupling between iminium ion **135** and the appropriate anion presumably takes places under simultaneous controls of orbital overlap and the electrostatic interaction between the ionic coupling partners, it can be established that – in excellent correlation with the structures of the major products of primary Mannich-type coupling – the most nucleophilic regions in the optimized structures of the more stable anions (framed structures) can be considered as reliably identified reactive sites on the basis of NBO charge and HOMO distribution, if they are taken together into account. It must also be noted here that the positions of the OH group on the

fused benzene ring in **128a** and **129a** do not allow the ester hydrolysis taking place through the corresponding quinoidal ketene intermediates. However, regardless of the position of the OH group, the neighboring group assistance from the 3-morpholinomethyl substituent obviously promotes this hydrolysis.

Comparative DFT calculations were also performed for two sets of alternative chelate-stabilized rotamers of Mannich products with the morpholinomethyl group attached on the fused benzene ring (**128a**/**128a_I**/**128a_II** and **129a**/**129a_I**: Figure 6) that identified **128a** and **129a**, respectively, as the preferred isomers in ethanolic solution.

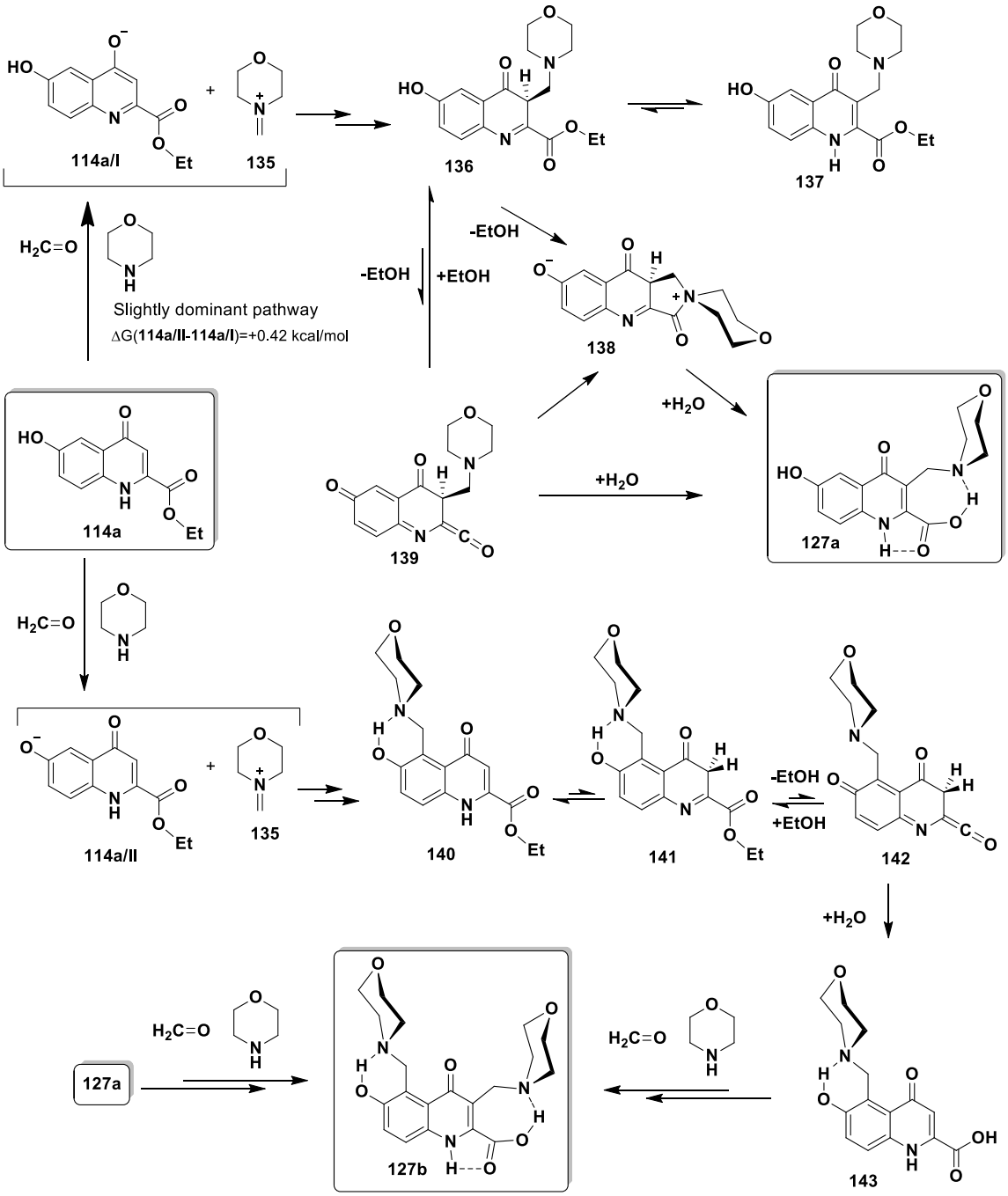


Figure 5. The possible sequences of the competitive Mannich condensations.

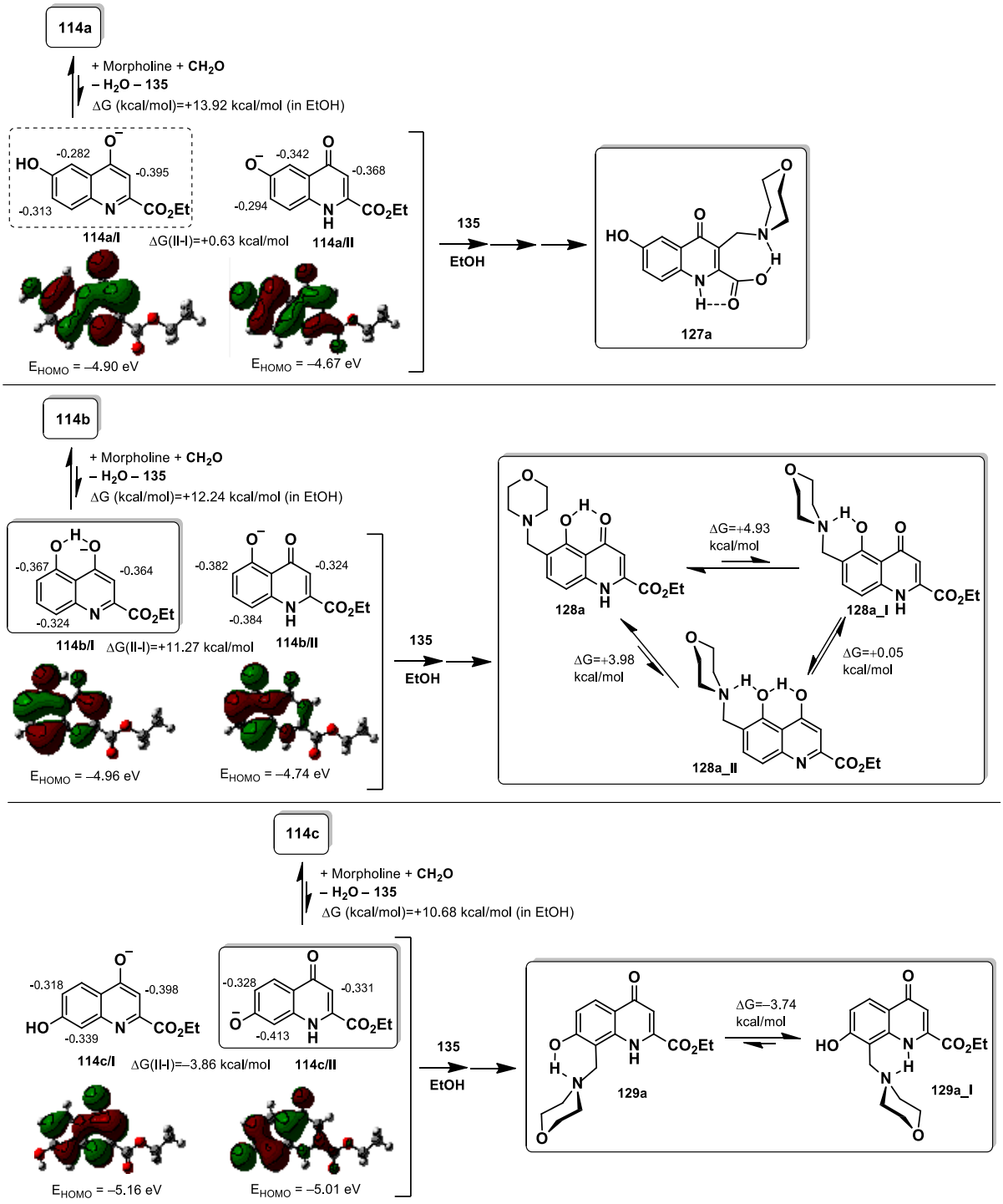


Figure 6. Rationalization of the feasibility and regioselectivity observed in the Mannich reactions of **114a**–**c** in terms of the relative thermodynamics, HOMO energy, HOMO delocalization, and the local NBO charges of the possible alternative anions generated by morpholine and formaldehyde along with the relative thermodynamics of the chelate-stabilized rotamer products.

The sluggish reactivity of **117a** experienced in EtOH can be associated with the HOMO energy level of **117a/III** found to be the lowest one in the series of the modelled nucleophilic anions and with the electron density in position 3 (ρ_{NBO}= –0.382) decreased relative to that identified in position 8 for anion **114c/II**, the model with somewhat lowered HOMO energy level that presented an increased reactivity in EtOH (Figure 7).

Finally, we assume that the spectacular solvent dependence observed in the transformations of **117a** can be rationalized by the polarity-controlled feasibility of the ion pairs **117a/III-135** and **134a/III-135**, the active coupling components of the first and second Mannich-like reactions, respectively. Accordingly, the changes in free energy associated with the primary ion pair generating equilibrium condensation steps that involve **117a** and **134a**, were assessed (Figure 7). In accord with the general expectations, the results (Figure 7) indicate that in the relatively polar ethanol and acetonitrile the formation of reactive ion pairs **117a/III-135** and **134a/III-135** is substantially more favorable than in the much less polar dioxane and toluene. Although this view is in accord with the failure of the second coupling step of the sequential Mannich reactions, attempted in dioxane affording **134a** and with the ready formation of **134b** *via* **134a** in acetonitrile, the ΔG values calculated for the ion-pair-forming condensations taking place in ethanol and toluene apparently contradict to the experimental results referring to a definite inhibitory effect of the more polar solvent and to the facilitating effect of the less polar one. This apparent contradiction can partly be resolved by taking donor–acceptor interactions of iminium cation **135** with the solvent molecules into account (Figure 7). Thus, among the solvents tested, toluene seems to be the one with the most decreased capability to deactivate iminium ion **135** allowing a relatively facile Mannich-like couplings of the ion pairs. It is also noteworthy that in both anions the HOMO delocalization and the local NBO charges are practically invariant to the polarity of the environment, while the HOMO energy level is substantially higher in the less polar solvents than in the more polar ones suggesting that these species, present even in low concentration in dioxane and toluene, display significant nucleophilicity enhanced relative to that predictable in ethanol and acetonitrile. These conclusions highlight the significance of HOMO level influencing anion reactivity and consequent chemoselectivity.(Figure 7)

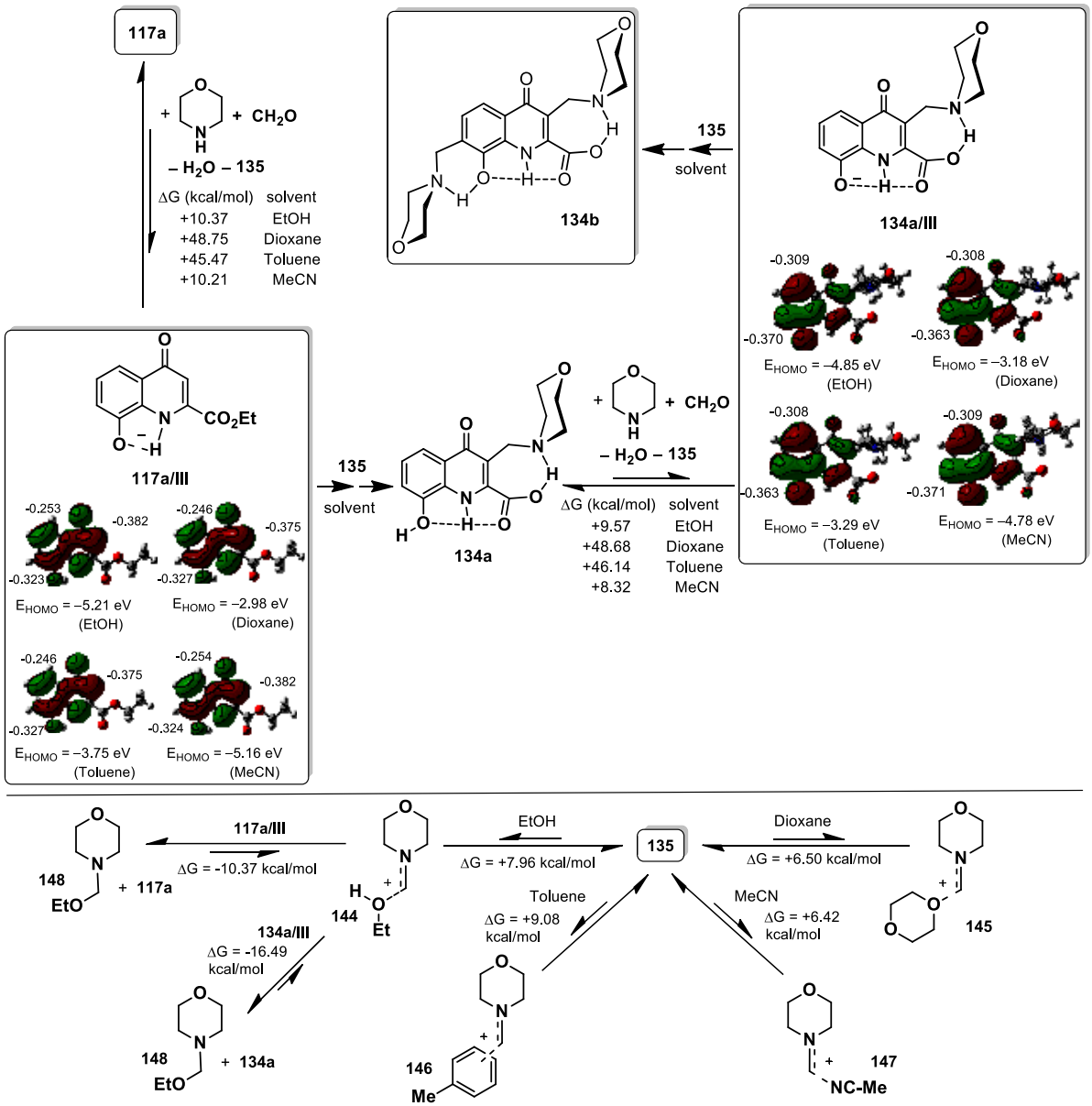


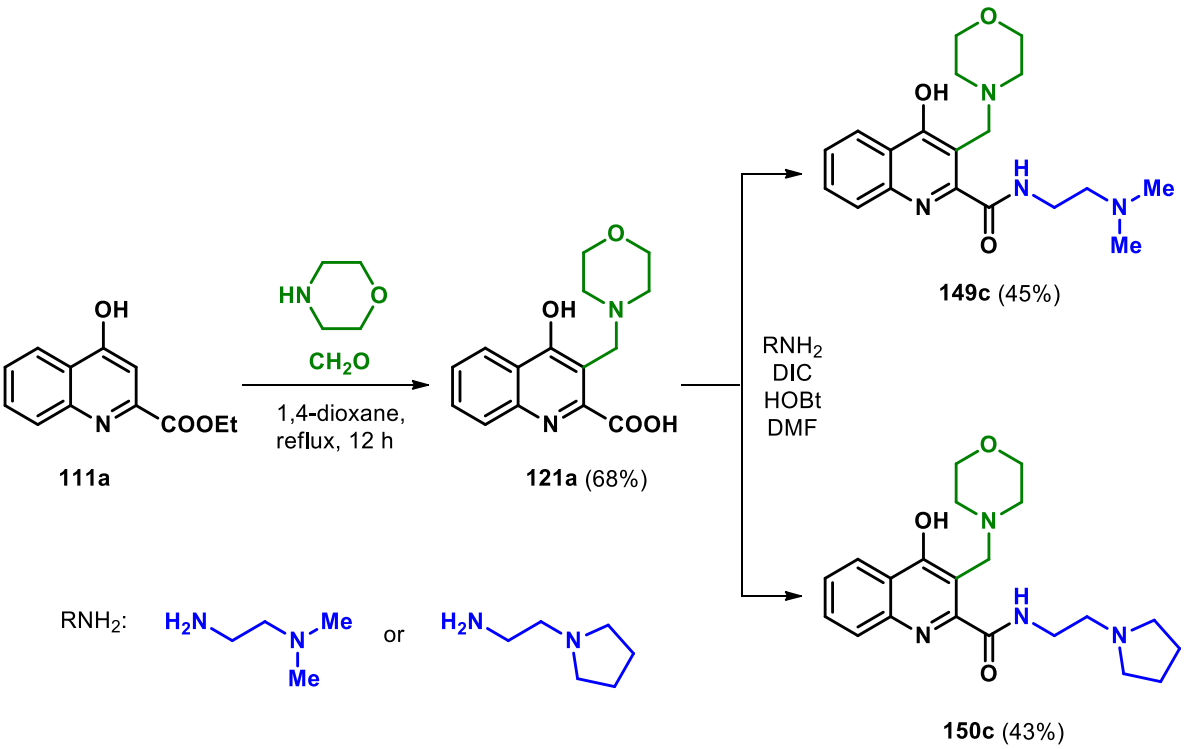
Figure 7. Rationalization of the marked solvent effect observed for the regioselective sequential Mannich reactions of **134a** in terms of: (i) the solvent-dependent changes in free energy accompanying the conversions leading active ion pairs **117a/III-135** and **134a/III-135**, respectively; (ii) HOMO energy of the anions; (iii) interactions of ~~the~~ iminium ion **135** with the solvent molecules.

3.2.4. Extending the *m*Mr to amide derivatives

It has been suspected that multiple cationic centers together will contribute to the biological effect with a greater magnitude. Thus, the synthesis of **149c** and **150c** containing two such functional groups were planned. As amidation and the *m*Mr could be carried out in exchanged

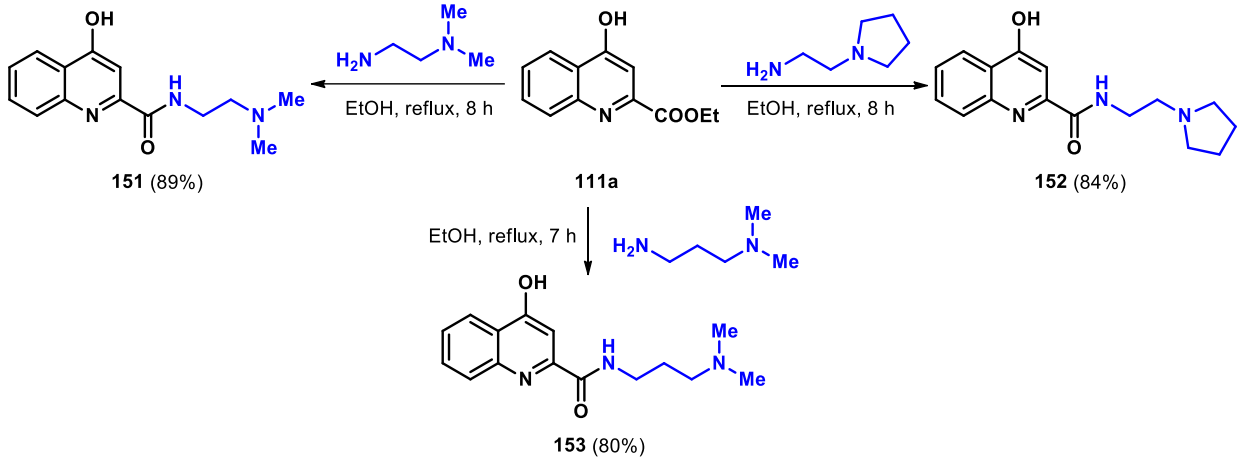
orders, the synthesis can be achieved by applying two different synthetic pathways: aminoalkylation followed by amidation (route A) and a reverse reaction sequence (route B).

During route A, for the aminoalkylation of **111a**, morpholine as a representative secondary cyclic amine with formaldehyde was used to form the iminium salt that would attack the C-3 position on the KYNA skeleton. Afterward, the amidations of **121a** were achieved in dimethylformamide through the activation of the free carboxylic group with *N*,*N*′-diisopropylcarbodiimide (DIC). During the reactions, 1-hydroxybenzotriazole hydrate (HOBt) was also used to generate an active ester intermediate and further improve the yield (Scheme 47).



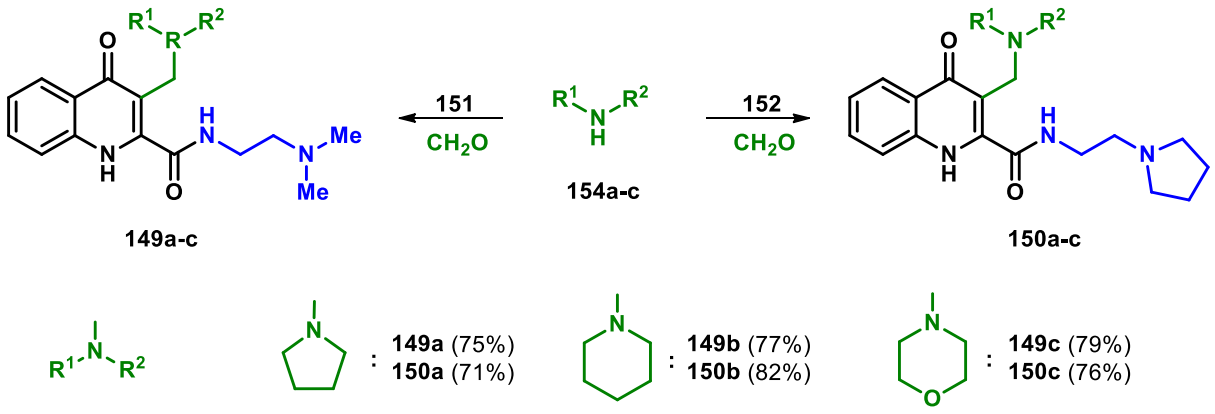
Scheme 47. Synthesis of aminoalkylated KYNA amide derivatives.

For route B, the starting amides **151** and **152** were synthesized by direct amidation of the ethyl ester of KYNA (**111a**) with *N*,*N*-dimethylethane-1,2-diamine (to achieve **151**) or with 2-(pyrrolidin-1-yl)ethanamine (to achieve **152**, Scheme 48). Furthermore, to suffice a wider evaluation of structure-effect connections, the synthesis of compound **153** was also carried out in a similar fashion using *N*,*N*-dimethylpropane-1,3-diamine.



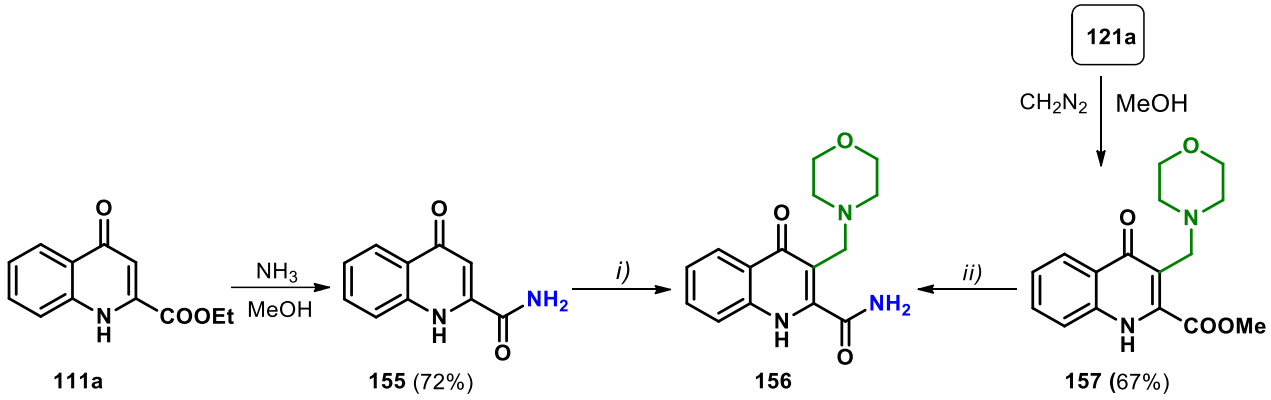
Scheme 48. Synthesis of KYNA amide derivatives.

During the *m*Mr, beside morpholine, the use of pyrrolidine and piperidine as secondary amines provided derivatives that could later be investigated *in vitro* for a deeper understanding of structure–activity relationships. Compounds **151** and **152** were aminoalkylated with **154a**–**c** in the presence of formaldehyde, resulting in aminoalkylated KYNA derivatives **149a**–**c** and **150a**–**c**, respectively (Scheme 49).



Scheme 49. Synthesis of C-3 aminoalkylated KYNA amides.

For further investigation, the synthesis of **156**, with an alternative amidation *via* the synthesis of ester **157** from **121a** and its further direct amidation, was carried out (Scheme 50).



Scheme 50. Synthetic pathways to obtain aminoalkylated KYNA amide **156**. *i)* morpholine, CH_2O, 1,4-dioxane, reflux (87%); *ii)* NH_3/MeOH, r.t. (88%).

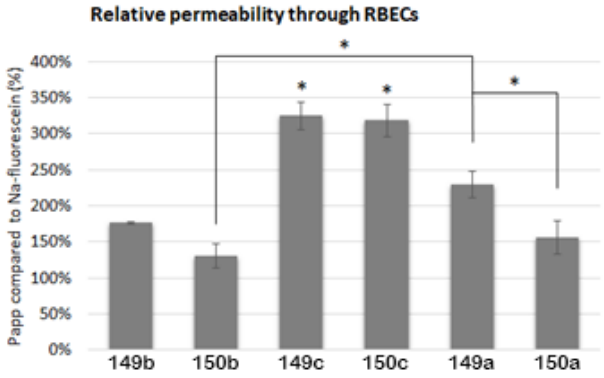
A comparison of the overall yields obtained for **156** and those found for **149c** and **150c** by using the two approaches shows that amidation followed by aminoalkylation (route B) resulted in the formation of the desired compounds in higher yield.

3.3. Biological evaluations on synthesized amides and aminoalkylated derivatives

The biological evaluations described in this section were carried out by our co-operating and co-author partners. The acquired results show the importance of the synthetic transformations carried out and were deemed worthy of mentioning in a separate paragraph.

As KYNA has poor central nervous system penetration, novel strategies are needed to take advantage of its important neuroprotective effects. Among several possible approaches[115] the synthetic method, *i.e.*, chemical modification of KYNA to obtain compounds with similar biological effects, but significantly improved ability to cross the cerebral endothelial cells forming the blood–brain barrier, has been carried-out in cooperation with Vécsei *et al.* During an *in vitro* model system mimicking the *in vivo* anatomical structure of the blood–brain barrier (BBB) that is suitable for drug testing,[116,117] the permeability of selected, newly synthesized derivatives (**149a–c**, **150a–c**) have been assessed beside sodium fluorescein as a control compound.

A



B

60 min

	Papp%	stdev
149b	177%	2%
150b	131%	17%
149c	325%	20%
150c	319%	22%
149a	230%	18%
150a	156%	23%

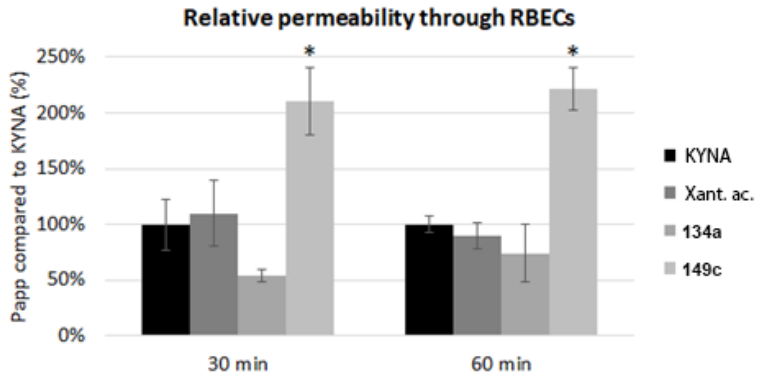
Figure 9. Penetration of KYNA analogues through the BBB. (A) Permeability of KYNA derivates after 60 min compared to sodium fluorescein. N = 2, average ± SD (ANOVA and Bonferroni's post hoc test). * $p < 0.05$ (**149c** and **150c**: significant difference compared to all other groups; significant difference between **149a** and **150b** or **150a**). (B) Permeability coefficients of KYNA analogues after 60 min compared to sodium fluorescein.

All of the above-mentioned analogues crossed the BBB more efficiently than sodium fluorescein. Furthermore, the permeability of **149c** and **150c** was significantly higher than the permeability of other analogues (Figure 9).

Next **149c**, a promising biologically active compound, was further investigated. Its permeability was compared with that of KYNA, xanthurenic acid, and **134a** its analogue, which is under patent protection.[118] **149c** had a significantly higher permeability through the *in vitro* BBB model than KYNA, xanthurenic acid or **134a** both at 30 min and 60 min time points. Differences among permeability of KYNA, xanthurenic acid, and **134a** were not statistically significant (Figure 10).

In line with this comparison, we hypothesize that aminoalkylation at C-3 facilitates BBB penetration with the morpholinomethyl functional group showing the best results. These results are in line with in vivo data showing that peripherally administered **149c** can reach sufficient concentration in the brain, since it is able to inhibit epileptiform activity.[119]

A



B

30 min

	Papp %	stdev
KYNA	100%	23%
Xant. ac.	110%	30%
134a	54%	6%
149c	210%	31%

60 min

	Papp %	stdev
KYNA	100%	8%
Xant. ac.	89%	12%
134a	74%	26%
149c	222%	19%

Figure 10. Penetration of **149c** through the BBB. (A) Permeability of **149c** after 30 and 60 min compared to KYNA, xanthurenic acid, and its analogue, **134a**. N = 3, average ± SD. * $p < 0.01$ (**149c**: significant difference compared to all other groups, in both time-points). (B) Permeability coefficients of **149c**, xanthurenic acid and **134a** after 30 and 60 min compared to KYNA.

Besides investigating their BBB penetration, several compounds have also been used in different biological studies. Compounds **151** and **149c** have played a major role in our

investigations aiming toward the study of their electrophysiological effects or their effects on tumor necrosis factor alpha (TNF-α) production.

In the case of compounds **149c**, **150c**, **152**, and **153** compared to KYNA, **151** showed similar effect on a model system of orthodromic stimulation of the Schaffer collateral/commissural pathway. The effect of **153** was fundamentally different. Its administration in higher concentration resulted in the facilitation of the amplitudes, while in lower concentration, its effect was slightly inhibitory. Aminoalkylation also resulted in different electrophysiological characteristics. In the case of **149c**, only a slight increase could be observed in higher concentration and a weak inhibition in low concentration, none of which was significant. In the case of compound **150c**, the results were not consistent, but in high concentration most of the results showed serious decrease in the amplitudes while in other cases the inhibition was smaller. In low concentration there was hardly any effect (Figure 11).

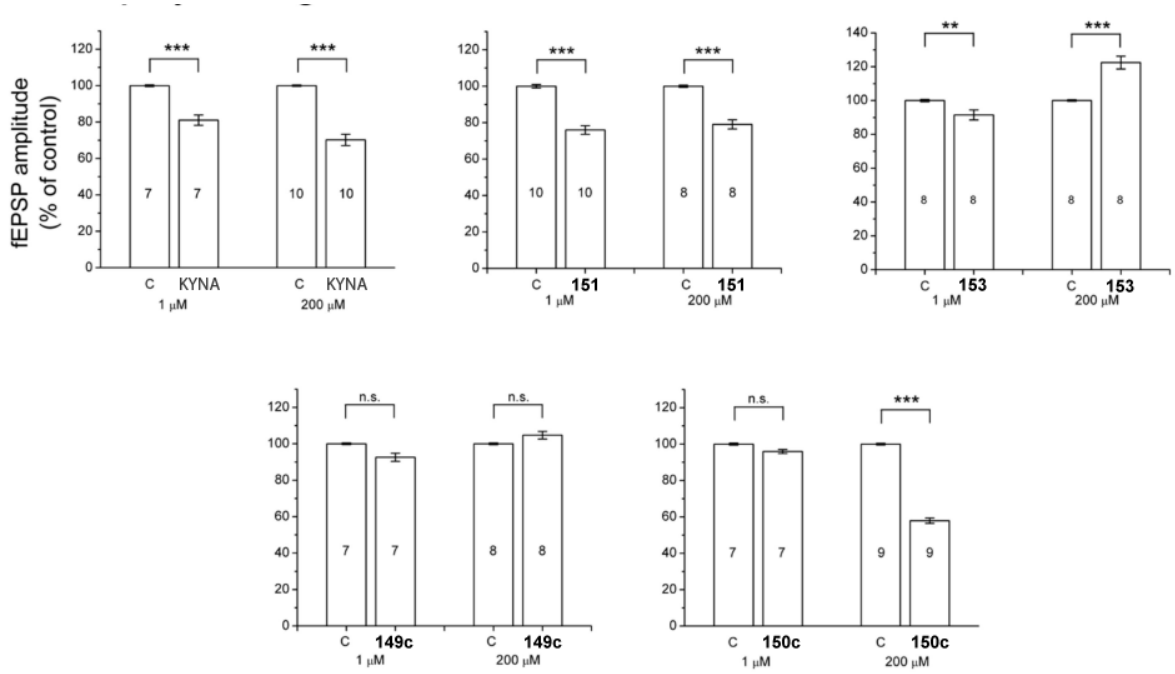


Figure 11. Effects of KYNA and its derivatives (**138**, **140**, **136c**, **137c**), respectively on the field excitatory postsynaptic potentials (fEPSPs) recorded in CA1 region of hippocampus.

In another study, the anti-inflammatory effect of KYNA and derivatives **149c**, **150c** and **151**–**153** was investigated. First the TNF-α production of U-937 cells stimulated with heat-inactivated *Staphylococcus aureus* was assessed showing inhibition in all cases (Figure 12). The effect of the analogues was also compared in equimolar concentration on the TNF-α production, when the inducer was *Chlamydia pneumoniae*. In these experiments, KYNA, **151**, and **152** increased TNF-α production by U-937 monocytic cells, while compounds **149c**, **150c**, and **153**

decreased it (Figure 13). The different behavior in the case of infection with *C. pneumonia* might be due to structural differences. Namely, the morpholinomethyl function at C-3 resulting in subgroups of (i) compounds with one cationic center (**151–153**) and (ii) compounds with two cationic centers (**149c**, **150c**) correlating with the difference in effect.

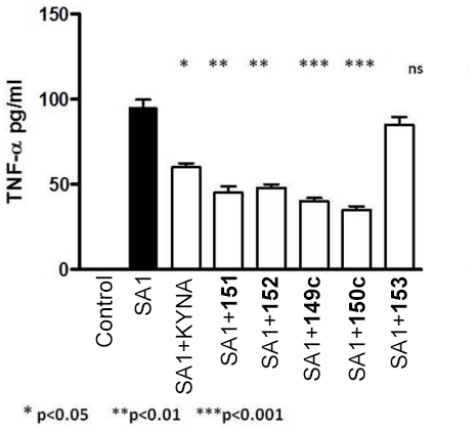


Figure 12. TNF-α levels attenuated by KYNA and KYNA analogues in SA1 stimulated U-937 cells.

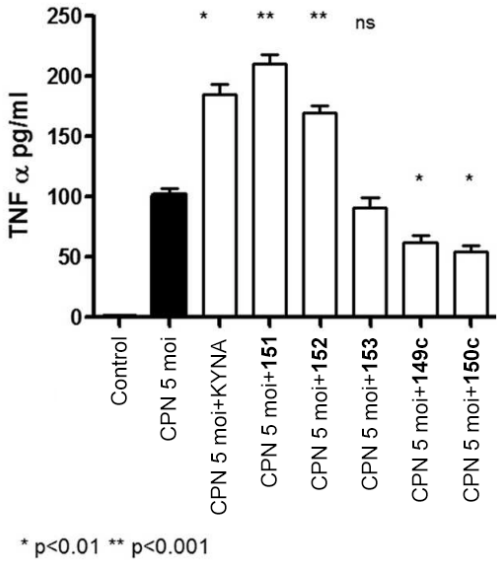


Figure 13. Effect of KYNA and KYNA analogues on TNF-α production in U-937 human monocytic cells stimulated by *Chlamydia pneumoniae.*

Finally, it has also been investigated by patch-clamp technique, how KYNA and derivatives **121a** and **149c** have any adjustments on different ionic currents in pituitary GH3 cells and hippocampal mHippoE-14 neurons. KYNA and **149c** increased the amplitude of M-type K+ current [IK(M)] and concomitantly enhanced the activation time course of the current. In cell-attached current recordings, addition of KYNA raised the open probability of M-type K+ channels, along with increased mean open time of the channel. In hippocampal mHippoE-14 neurons, the addition of KYNA also increased the IK(M) amplitude effectively. Taken together, the actions observed would be one of the noticeable mechanisms through which they modulate functional activities of excitable cells occurring *in vivo*.

Taking in consideration that, as mentioned above, compound **149c** achieved the highest permeability on a BBB model, it is reasonable to propose that these derivatives through their stimulation of IK(M) could be beneficial for the treatment of different psychiatric or neurological disorders.[120–123] This, however, requires further investigations.

TSG-6 expression following activation with bacterial components could participate in the suppression of inflammatory cytokines, such as TNF-α. As KYNA and, in particularly, KYNA analogues are able to enhance this effect, their further investigation in this direction may also yield interesting results.

4. Summary

1. The Conrad–Limpach procedure used in the synthesis of the ethyl ester of KYNA and its alkyl-, aryl-, and halogen-substituted derivatives has been optimized using two steps: (i) column-chromatographic purification of the intermediate enamines and (ii) using 1,2-dichlorobenzene for the ring-closure reaction resulting in an easier work-up. For the synthesis of hydroxylated KYNA derivatives, beyond extending the reaction and using the optimized Conrad–Limpach procedure, a microwave-assisted alternative procedure catalyzed by *p*-TsOH was also applied.

2. Based on the structural similarities between 1-naphthol and kynurenic acid, the reactivity of KYNA was investigated in a modified Mannich-type reaction. Aminoalkylations at the C-3 position have been achieved applying benzaldehyde and formaldehyde with different primary, secondary, cyclic and acyclic amines. This synthesis method was then extended by using the alkyl-, aryl-, and halogen-substituted KYNA derivatives in *m*Mr with morpholine and formaldehyde as representative amine and aldehyde, respectively. The reactions resulted in the formation of C-3-substituted derivatives. It was also concluded that the substituents at the B ring do not influence significantly the reactivity of KYNA ester precursors.

3. The scope and limitations of *m*Mr were also studied starting from hydroxy-functionalized derivatives. Through a systematic investigation of substitutions applying morpholine and paraformaldehyde as representative reagents, mono- and disubstituted derivatives were synthesized. Product selectivity and regioselectivity were rationalized by DFT calculations disclosing HOMO distribution and NBO charges on the potential nucleophilic centers in the anion of the appropriate KYNA ester assumed to be active components towards the iminium ion intermediate.

4. Amines bearing tertiary nitrogen needed for biological activity towards the central nervous system were used to synthesize amide derivatives of KYNA. These amides were then used to further extend C-3 aminoalkylations using cyclic and acyclic secondary amines and formaldehyde. The synthesized amides and aminoalkylated derivatives have been investigated in studies concerning their blood-brain-barrier penetration, their electrophysiological effects on different hippocampal cultures, and their effect on TNF-α production in the case of *S. aureus* and *C. pneumonia* induced U-937 monocytic cells. During these studies, the

morpholine-methylated *N*-2-(dimethyamino)ethylamide analogue **149c** showed the most promising properties for further investigations.

5. The two different synthetic pathways leading to aminoalkylated amides, namely aminoalkylation followed by amidation (route A) and a reverse reaction sequence (route B), have been investigated. A comparison of the overall yields to obtain three representative aminoalkylated amide derivatives showed that amidation followed by aminoalkylation (route B) resulted in the formation of the desired compounds in higher yields.

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References

1. Rózsa, E.; Robotka, H.; Vécsei, L.; Toldi, J. *J. Neural Transm.* **2008**, *115*, 1087–1091.
2. Stone, T.W. *Expert Opin. Inv. Drug.* **2001**, *10*, 633–645.
3. Németh, H.; Toldi, J.; Vécsei, L. *Curr. Neurovasc. Res.* **2005**, *2*, 249–260.
4. Németh, H.; Toldi, J.; Vécsei, L. *J. Neural Transm. Supp.* **2006**, *70*, 285–304.
5. Sas, K.; Robotka, H.; Toldi, J.; Vécsei, L. *J. Neurol. Sci.* **2007**, *257*, 221–239.
6. Gigler, G.; Szénási, G.; Simó, A.; Lévay, G.; Hársing, L.G., Jr.; Sas, K.; Vécsei, L.; Toldi, J. *Eur. J. Pharmacol.* **2007**, *564*, 116–122.
7. Luchowska, E.; Luchowski, P.; Sarnowska, A.; Wielosz, M.; Turski, W.A.; Urbańska, E.M. *Pol. J. Pharmacol.* **2003**, *55*, 443–447.
8. Harrison, B.L.; Baron, B.M.; Cousino, D.M.; McDonald, I.A. *J. Med. Chem.* **1990**, *12*, 3130–3132.
9. Edmont, D.; Rocher, R.; Plisson, C.; Chenault, J. *Bioorg. Med. Chem. Lett.* **2000**, *16*, 1831–1834.
10. Bonina, F.P.; Arenare, L.; Ippolito, R.; Boatto, G.; Battaglia, G.; Bruno, V.; de, C.P *Int. J. Pharm.* **2000**, *202*, 79–88.
11. Manfredini, S.; Pavan, B.; Vertuani, S.; Scaglianti, M.; Compagnone, D.; Biondi, C.; Scatturin, A.; Tanganelli, S.; Ferraro, L.; Prasad, P.; et al *J. Med. Chem.* **2002**, *45*, 559–562.
12. Manfredini, S.; Vertuani, S.; Pavan, B.; Vitali, F.; Scaglianti, M.; Bortolotti, F.; Biondi, C.; Scatturin, A.; Prasad, P.; Dalpiaz, A. *Bioorgan. Med. Chem.* **2004**, *12*, 5453–5463.
13. Yielding, K. L.; Nichols, A. C. *Mol. Chem. Neuropathol.* **1993**, *19*, 269–282.
14. Stone, T.W. *Eur. J. Med. Chem.* **2000**, *35*, 179–186.
15. Nichols, A.C.; Yielding, K.L. *Mol. Chem. Neuropathol.* **1998**, *35*, 1–12.
16. Füvesi, J.; Somlai, C.; Németh, H.; Varga, H.; Kis, Z.; Farkas, T.; Károly, N.; Dobszay, M.; Penke, Z.; Penke, B.; et al. *Pharmacol. Biochem. Behav.* **2004**, *77*, 95–102.
17. Zhang, L.; Sun, F.; Li, Y.; Sun, X.; Liu, X.; Huang, Y.; Zhang, L.H.; Ye, X.S.; Xiao, J. *ChemMedChem.* **2007**, *2*, 1594–1597.
18. Brik, A.; Lin, Y.C.; Elder, J.; Wong, C.H. *Chem. Biol.* **2002**, *9*, 891–896.
19. Tossi, A.; Benedetti, F.; Norbedo, S.; Skrbec, D.; Berti, F.; Romeo, D. *Bioorg. Med. Chem.* **2003**, *11*, 4719–4727.
20. Knyihár-Csillik, E.; Mihály, A.; Krisztin-Péva, B.; Robotka, H.; Szatmári, I.; Fülöp, F.; Toldi, J.; Csillik, B.; Vécsei, L. *Neurosci. Res.* **2008**, *61*, 429–432.
21. Fülöp, F.; Szatmári, I.; Vámos, E.; Zádori, D.; Toldi, J.; Vécsei, L. *Curr. Med. Chem.* **2009**, *16*, 4828–4842.
22. Szatmári, I.; Fülöp, F. *Tetrahedron* **2013**, *69*, 1255–1278.
23. Szatmári, I.; Fülöp, F. *Synthesis* **2009**, 775–778.
24. Szatmári, I.; Fülöp, F. *Tetrahedron Lett.* **2011**, *52*, 4440–4442.
25. Sas, J.; Szatmári, I.; Fülöp, F. *Curr. Org. Chem.* **2016**, *20*, 2038–2054.
26. Schmitt, M.; Klotz, E.; Macher, J.-P.; Bourguignon, J-J. *Patent application FR*2002002594, July **2007**.
27. Zádori, D.; Nyíri, G.; Szőnyi, A.; Szatmári, I.; Fülöp, F.; Toldi, J.; Freund, T. F.; Vécsei L.; Klivényi, P. *J. Neural Transm.*, **2011**, *118*, 865–875.
28. Zádori, D.; Veres, G.; Szalárdy, L.; Klivényi, P.; Toldi J.; Vécsei, L.; *J. Alzheimer's Dis.*, **2014**, *42*, S177–S187.
29. Greco, R.; Demartini, C.; Zanaboni, A. M.; Redavide, E.; Pampalone, S.; Toldi, J.; Fülöp, F.; Blandini, F.; Nappi, G.; Sandrini, G.; Vécsei L.; Tassorelli, C. *Cephalalgia*, **2017**, *37*, 1272–1284.
30. Fejes-Szabó, A.; Bohár, Z.; Vámos, E.; Nagy-Grócz, G.; Tar, L.; Veres, G.; Zádori, D.; Szentirmai, M.; Tajti, J.; Szatmári, I.; Fülöp, F.; Toldi, J.; Paardutz A.; Vecsei, L. *J. Neural. Transm.*, **2014**, *121*, 725–738.
31. Szatmári, I.; Fülöp, F. *Curr. Org. Synth.* **2004,** *1*, 155–165.
32. Sako, M. in *Category 2, Hetarenes and Related Ring Systems* eds. Yamamoto, Y.; Shinkai, I. *Thieme Verlagsgruppe*, Stuttgart, New York, Delhi, Rio; **2004**, *Product Class 19: Pyridopyrimidines*, 1155–1267.

33. Conrad, M.; Limpach, L. *Eur. J. Org. Chem.* **1887**, *20*, 944–948.
34. Conrad, M.; Limpach, L. *Eur. J. Org. Chem.* **1887**, *20*, 948–959.
35. Hauser, C. R.; Reynolds, G. A. *J. Am. Chem. Soc.* **1948**, *70*, 2402–2404.
36. Goldberg, A. A.; Theobald, R. S.; Williamson, W. *J. Chem. Soc.* **1954**, 2357–2361.
37. Abe, S.; Haneda, T.; Hata, K.; Inoue, S.; Matsukura, M.; Nakamoto, K.; Tanaka, K.; Tsukada, I.; Ueda, N.; Watanabe, N. *Patent Application* WO2005033079A1·14. April **2005**.
38. Broekema, M.; Carpenter, J.; Feng, J.; Liu, C.; Wang, W.; Wang, Y. *Patent Application* WO2019089670A1 09. May **2019**.
39. Abe, S.; Haneda, T.; Inoue, S.; Matsukura, M.; Nakamoto, K.; Sagane, K.; Tanaka, K.; Tsukada, I.; Ueda, N. *Patent Application* WO2006016548A1 16. February **2006**.
40. Feng, J.; Huang, Y.; Liu, C. *Patent Application* WO2019089665A1 09. May **2019**.
41. Vasil'ev, L. S.; Surzhikov, F. E.; Azarevich, O. G.; Bogdanov, V. S.; Dorokhov, V. A. *Russ. Chem. Bull.* **1996**, *45*, 2574–2577.
42. Vasil'ev, L. S.; Surzhikov, F. E.; Baranin, S. V.; Dorokhov, V. A. *Russ Chem Bull* **2013**, *62*, 1255–1261.
43. Andrews, M. D.; Bagal, S. K.; Gibson K. R.; Omoto K.; Ryckmans T.; Skerratt S. E.; Stupple P. A. *Patent Application* WO2012137089A1·11. October **2012**.
44. Andrews, M. D.; Bagal, S. K.; Gibson K. R.; Omoto K.; Ryckmans T.; Skerratt S. E.; Stupple P. A. *Patent Application* US8846698B2·30. September **2014**.
45. Hauser, C. R.; Weiss, M. J. *J. Org. Chem.* **1949**, *14*, 453–459.
46. Zhou, Y.; Li, W.; Liu, Y.; Zeng, L.; Su, W.; Zhou, M. *Dalton Trans.* **2012**, *31*, 9373–9381.
47. Carboni, S.; Da Settimo, A.; Bertini, D.; Ferrarini, P. L.; Livi, O.; Mori, C.; Tonetti, I. *Gazz. Chim. Ital.* **1972**, *102*, 253–264.
48. Ferrand, Y.; Kendhale, A. M.; Garric, J.; Kauffmann, B.; Huc, I. *Angew. Chem. Int. Ed.* **2010**, *49*, 1778–1781.
49. Mateus, P.; Chandramouli, N.; Mackereth, C. D.; Kauffmann, B.; Ferrand, Y.; Huc, I. *Angew. Chem. Int. Ed.* **2020**, *59*, 5797–5805.
50. Gole, B.; Kauffmann, B.; Maurizot, V.; Huc, I.; Ferrand, Y. *Angew. Chem. Int. Ed.* **2019**, *58*, 8063–8067.
51. Wang, X.; Gan, Q.; Wicher, B.; Ferrand, Y.; Huc, I. *Angew. Chem. Int. Ed.* **2019**, *131*, 4249–4253.
52. Saha, S.; Kauffmann, B.; Ferrand, Y.; Huc, I. *Angew. Chem. Int. Ed.* **2018**, *130*, 13730–13734.
53. Suzuki, N.; Kato, M.; Dohmori, R. *Yakugaku Zasshi.* **1979**, *99* (2), 155–164.
54. Srinivasan, A.; Broom, A. D. *J. Org. Chem.* **1979**, *44*, 435–440.
55. Rosowsky, A.; Forsch, R. A.; Queener, S. F. *J. Med. Chem.* **1995**, *38*, 2614–2620.
56. Desforges, G.; Gaillard, P.; Montagne, C.; Pomel, V.; Quattropani, A. *Patent Application* WO2010037765A2 08. April **2010**.
57. Shim, J. L.; Niess, R.; Broom, A. D. *J. Org. Chem.* **1972**, *37*, 578–581.
58. Ogura, H.; Sakaguchi, M. *Chem. Lett.* **1972**, *1*, 657–658.
59. Komkov, A. V.; Dorokhov, V. A. *Russ Chem Bull* **2002**, *51*, 1875–1878.
60. Cassis, R.; Tapia, R.; Valderrama, A. *Synth. Commun.* **1985**, *15*, 125–133.
61. Jadhav, S. B.; Fatema, S.; Patil, R. B.; Sangshetti, J. N.; Farooqui, M. *J. Heterocycl. Chem.* **2017**, *54*, 3299–3313.
62. Yang, Y.; Shu, W.-M.; Yu, S.-B.; Ni, F.; Gao, M.; Wu, A.-X. *Chem. Commun.* **2013**, *49*, 1729–1731.
63. Bonacorso, H. G.; Righi, F. J.; Rodrigues, I. R.; Cechinel, C. A.; Costa, M. B.; Wastowski, A. D.; Martins, M. A. P.; Zanatta, N. *J. Heterocycl. Chem.* **2006**, *43*, 229.
64. Roslan, I. I.; Lim, Q.-X.; Han, A.; Chuah, G.-K.; Jaenicke, S. *Eur. J. Org. Chem.* **2015**, 2351–2355.
65. Mathavan, S.; Durai Raj, A. K.; Yamajala R. B. R. D. *ChemistrySelect* **2019**, *4*, 10737–10741.

66. Yu, T.; Li, N.; Wu, C.; Guan, A.; Li, Y.; Peng, Z.; He, M.; Li, J.; Gong, Z.; Huang, L.; Gao, B.; Hao, D.; Sun, J.; Pan, Y.; Shen, L.; Chan, C.; Lu, X.; Yuan, H.; Li, Y.; Li, J.; Chen, S. *ACS Med. Chem. Lett.* **2018**, *9*, 256–261.

67. Yan, H.; Ma, Y.; Sun, Y.; Ma, C.; Wang, Y.; Ren, X.; Huang, G. *Tetrahedron* **2014**, *70*, 2761–2765.

68. Blackaby, W.; Brookfield, F.; Bubert, C.; Hardick, D.; Ridgill, M.; Shepherd, J.; Thomas, E. *Patent Application* WO2020201773A1 08. October·**2020.**

69. Kinzel, O. D.; Ball, R. G.; Donghi M.; Maguire C. K.; Muraglia, E.; Pesci, S.; Rowley, M.; Summa, V. *Tetrahedron* **2008**, *49*, 6556–6558.

70. Donghi, M..; Kinzel, O. D.; Summa, V. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1930–1934.

71. (a) Muraglia, E.; Kinzel, O.; Gardelli, C.; Crescenzi, B.; Donghi, M.; Ferrara, M.; Nizi, E.; Orvieto, F.; Pescatore, G.; Laufer, R.; Gonzalez-Paz, O.; Di Marco, A.; Fiore, F.; Monteagudo, E.; Fonsi, M.; Felock, P. J.; Rowley, M.; Summa, V. *J. Med. Chem.* **2008**, *514*, 861–874; (b) Kinzel, O. D.; Monteagudo, E.; Muraglia, E.; Orvieto, F.; Pescatore, G.; Rico Ferreira, M.; Rowley, M.; Summa, V. *Tetrahedron Lett*. **2007**, *48*, 6552–6555; (c) Crescenzi, B.; Kinzel, O.; Muraglia, E.; Orvieto, F.; Pescatore, G.; Rowley, M.; Summa, V. *Patent Application* WO2004058757A1·15. July **2004.**

72. (a) Koch, U.; Attenni, B.; Malancona, S.; Colarusso, S.; Conte, I.; Di Filippo, M.; Harper, S.; Pacini, B.; Giomini, C.; Thomas, S.; Incitti, I.; Tomei, L.; De Francesco, R.; Altamura, S.; Matassa, V. G.; Narjes, F. *J. Med. Chem.* **2006**, *49*, 1693–1707; (b) Zhong, Y.-L.; Zhou, H.; Gauthier, D. R., Jr.; Askin, D. *Tetrahedron Lett.* **2006**, *47*, 1315–1317.

73. Tisler, M.; Zupet, R. *Org. Prep. Proced. Int.* **1990,** *22*, 532–534.

74. Jones, E. D.; Vandegraaff, N.; Le, G.; Choi, N.; Issa, W.; Macfarlane, K.; Thienthong, N.; Winfield, L. J.; Coates, J. A. V.; Lu, L.; Li, X.; Feng, X.; Yu, C.; Rhodes, D. I.; Deadman, J. J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5913–5917.

75. Deadman, J. J.; Jones, E. D.; Le, G. T.; Rhodes, D. I.; Thienthong, N.; Vandegraff, N.; Winfield, L. J. *Patent Application* WO2010000032A1·07. January **2010**.

76. Le, G.; Vandegraff, N.; Rhodes, D. I.; Jones, E. D.; Coates, J. A. V.; Lu, L.; Li, X.; Yu, C.; Feng, X.; Deadman, J. J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5013–5018.

77. Heath, D. G.; Rees, C. W. *J. Chem. Soc. Chem. Comm.* **1982**, 21, 1280–1282.

78. Liang, Y.-F.; Steinbock, R.; Münch, A.; Stalke, D.; Ackermann, L. *Angew. Chem. Int. Ed.* **2018**, *57*, 5384–5388.

79. Huisgen, R.; Frauenberg, K.; Sturm, H. J. *Tetrahedron Lett.* **1969**, *30*, 2589–2594.

80. Bergnes, G.; Chabala, J. C.; Dhanak, D.; Knight, S. D.; Mcdonald, A.; Morgans, D. J., JR.; Zhou, H.-J. *Patent Application* WO2004064741A2 05. August·**2004.**

81. Ang, K. H.; Cox, M.; Lawrance, W. D.; Prager, R.; Smith, J. A.; Staker, W. *Aust. J. Chem.* **2004**, *57*, 101–105.

82. Mátyus, P.; Ősapay, K.; Gabányi, Z.; Kasztreiner, E.; Sohár, P. *Kem. Kozl.* **1986**, *66*, 203–212.

83. Mátyus, O.; Szilágyi, G.; Kasztreiner, E.; Rabloczky, G. *J. Heterocyclic Chem.* **1988**, *25*, 1535–1542.

84. Gerorge, V. M., Khetan, K. S., Gupta, K. R. in *Advances in Heterocyclic Chemistry*. eds. Keritzky, R. A., Boulton, J. A., *Academic Press*, New York-San Francisco-London, **1976**. *Vol. 19.* 280.

85. Boer, R. F.; Petra, D. G. I.; Wanner, M. J.; Boesaart, A.; Koomen, G. J. *Nucleos. Nucleot.* **1995**, *14*, 349–352

86. Liu, K.-C.; Huang, H.-S. *Arch. Pharm.* **1988**, *321*, 771–772.

87. Bhagat, S.; Jain, M.; Pandey, M. K.; Saxena, P. A.; Jain, S. C. *Heteroatom Chem.* **2006**, *17*, 272–276.

88. Boyarintseva, O. N.; Kurilo, G. N.; Anisimova, O. S.; Grinev, A. N. *Khim. Geterotsikl.* **1977**, *1*, 82–84

89. Meyer, M.; Guyot, M. *Tetrahedron Lett.* **1996**, *37*, 4931–4932

90. Silva, A. M. G.; Castro, B.; Rangel, M.; Silva, A. M. S.; Brandão, P.; Felix, V.; Cavaleiro, J. A. S. *Synlett* **2009**, *6*, 1009–1013.

91. Nakazumi, H.; Endo, T.; Nakaue, T.; Kitao, T. *J. Heterocyclic Chem.* **1985**, *22*, 89–92.

92. Baron, B. M.; Harrison, B. L. *Patent Application* US5026700A·25. June **1991**.

93. Goerlitzer, K.; Kramer, C. *Pharmazie* **2000**, *9*, 645–650.

94. Barker, J. M.; Huddleston, P. R.; Jones, A. W.; Edwards, M. *J. Chem. Res. S.* **1980**, *1*, 4–5.

95. Connor, D. T.; Young, P. A.; Strandtmann, M. *J. Heterocyclic Chem.* **1981**, *18*, 697–702.

96. Dorokhov, V. A.; Vasilyev, A. V.; Baranin, S. V. *Russ. Chem. Bull.* **2002**, *51*, 503–505.

97. Connor, D. T. *Patent Application* US4198511A·15. April **1980**.

98. Goerlitzer, K.; Kramer, C. *Pharmazie* **2000**, *8*, 587–594.

99. Zhou, Y.; Li,W.; Liu, Y.; Zeng, L.; Su,W.; Zhou, M. *Dalton Trans*. **2012**, *41*, 9373–9381.

100. Hall, M. C.; Johnson; G. H.; Wright, B. J. *J. Med. Chem.* **1974**, *17*, 685–690.

101. Bryant, J. A.; Buckman, B. O.; Islam, I.; Mohan, R.; Morrissey, M. M.; Wei, G. P.; Wei, X.; Yuan, S. *Patent Application* US2003060474A1·27. March **2003**.

102. Heindel, N. D. *J. Org. Chem*., **1970**, *35*, 3138–3140.

103. Kizaki, K.; Imoto, H.; Kato T.; Naka, K. *Tetrahedron***, 2015**, *71*, 643–647.

104. Boominathan, M.; Sathish, V.; Nagaraj, M.; Bhuvanesh, N.; Muthusubramanian, S.; Rajagopal, S. *RSC Adv.*, **2013**, *3*, 22246–22252.

105. Sloan, N. L.; Luthra, S. K.; McRobbie, G.; Pimlott, S. L.; Sutherland, A. *RSC Adv*. **2017**, *7*, 54881–54891.

106. Aktas, B. H.; Chorev, M.; Halperin, J. A.; Wagner, G. *Patent Application* WO2012006068A2·12. January 2012.

107. Kikuchi, H.; Suzuki, H.; Ogura, M.; Homma, M. K.; Homma, Y.; Oshima, Y. *Bioorg. Med. Chem.* **2015**, *23*, 66–72.

108. Abarghaz, M.; Bourguignon, J.-J.; Klotz, E.; Macher, J.-P.; Ronsin, G.; Schmitt, M.; Wagner, P. *Patent Application* US49940305A·01. March **2005**.

109. A. D. Becke, *J. Chem. Phys*., **1998**, *98*, 5648–5652.

110. C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B: Condens. Matter Mater. Phys.*, **1988**, *37*, 785–789.

111. Stephens, P. J.; Devlin, F. J.; Chahalowsky, C. F.; Frisch, M. J. *J. Phys. Chem.*, **1994**, *98*, 11623–11627.

112. Hehre, J. W.; Radom, L.; Schleyer, P. V. R.; Pople, J. A. in *Ab initio molecular orbital theory*, *Wiley*, New York, NY, USA, **1986**.

113. Tomasi, J.; Mennucci, B.; Cancés, É. *J. Mol. Struct*., **1999**, *464*, 211–216.

114. Weinhold, F.; Landis, C. R. in *Valency and Bonding: A Natural Bond Orbital Donor-Acceptor Perspective*, *Cambridge University Press*, Cambridge, **2005.**

115. Krizbai, I. A.; Nyul-Tóth, A.; Bauer, H. C.; Farkas, A. E.; Traweger, A.; Hasko, J.; Bauer, H.; Wilhelm I. *Curr. Pharm. Des.*, **2016**, *22*, 5442–5462.

116. Wilhelm, I.; Fazakas, C.; Krizbai, I. A. *Acta Neurobiol. Exp.*, **2011**, *71*, 113–128.

117. Wilhelm, I.; Krizbai, I. A. *Mol. Pharm.*, **2014**, *11*, 1949–1963.

118. Schmitt, M. K. E.; Macher, J.-P.; Bourguignon, J.-J. *Patent Application* WO2003010146A1, 6 February **2003**.

119. Demeter, I.; Nagy, K.; Gellért, L.; Vécsei, L.; Fülöp, F.; Toldi, J. *J. Neural Transm.* **2012**, *119*, 151–154.

120. Erhardt, S.; Olsson, S. K.; Engberg, G. *CNS Drugs*, **2009**, *23*, 91–101.

121. Erhardt, S.; Schwieler, L.; Nilsson, L.; Linderholm, K.; Engberg, G. *Physiol. Behav.* **2007**, *92*, 203–209.

122. Potter, M.C.; Elmer, G.I.; Bergeron, R.; Albuquerque, E.X.; Guidetti, P.; Wu, H.Q.; Schwarcz, R. *Neuropsychopharmacology*, **2010**, *35*, 1734–1742.

123. Vécsei, L.; Horváth, Z.; Tuka, B. Ideggyógyászati Szemle, **2014**, 67, 107–112.

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