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Synthesis and transformation of functionalized kynurenic acid derivatives

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A. INTRODUCTION AND AIMS

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. 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.

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.

Since KYNA is a neuroprotective agent able to prevent neuronal loss following excitotoxic, ischemia-induced, and infectious neuronal injuries, 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. The hydroxy group at position 4 was transformed to ether or amine functions, while the carboxylic function at position 2 was mostly modified by synthesizing the corresponding esters or amides.

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) leading to the corresponding aminonaphthols, aminoquinolinols or aminoisoquinolinols. A similar transformation starting from xanthurenic acid has been described by Schmitt et al. 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. Derivatives bearing such functional groups can be synthesized by various methods, such as carboxyl amidation

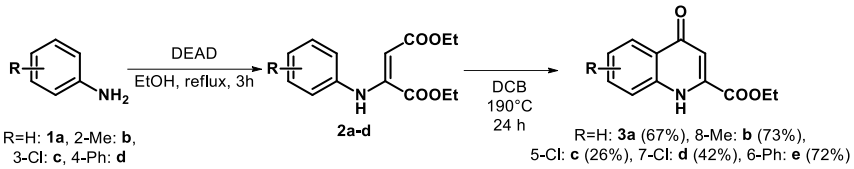
mentioned previously. An alternative route could be the transformation of the 4-hydroxy group into an amino function and its subsequent alkylation with an appropriate nitrogen-containing haloalkylamine.

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. 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.

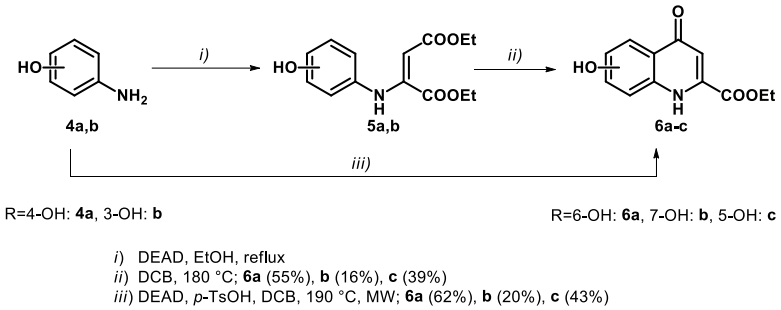
B. RESULTS AND DISCUSSION

1. The Conrad–Limpach procedure used in the synthesis of the ethyl ester of KYNA and its alkyl-, aryl-, and halogen-substituted derivatives (**3a-e**) 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 (Scheme 1).



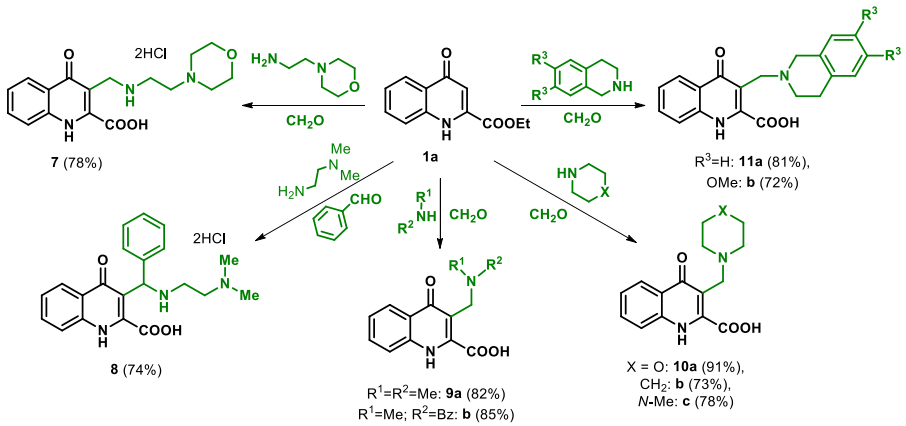
Scheme 1

For the synthesis of hydroxylated KYNA derivatives (**6a-c**), beyond extending the reaction and using the optimized Conrad–Limpach procedure, a microwave-assisted alternative procedure catalyzed by *p*-TsOH was also applied (Scheme 2).



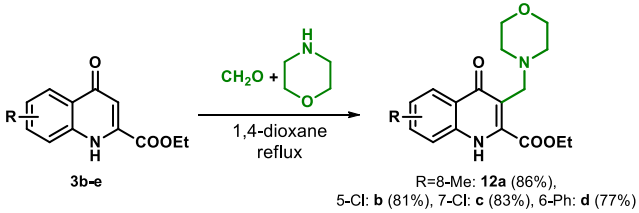
Scheme 2

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 (Scheme 3).



Scheme 3

This synthesis method was then extended by using the alkyl-, aryl-, and halogen-substituted KYNA derivatives (**3b-e**) in *m*Mr with morpholine and formaldehyde as representative amine and aldehyde, respectively (Scheme 4). The reactions resulted in the formation of C-3-substituted derivatives (**12a-d**). It was also concluded that the substituents at the B ring do not influence significantly the reactivity of KYNA ester precursors.



Scheme 4

3. The scope and limitations of *m*Mr were also studied starting from hydroxy-functionalized derivatives (**6a-c**, **13**). Through a systematic investigation of substitutions applying morpholine and paraformaldehyde as representative reagents, mono- and disubstituted derivatives (**14a-d**, **15a-d**) were synthesized (Table 1). 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.

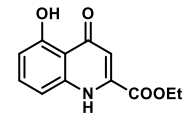
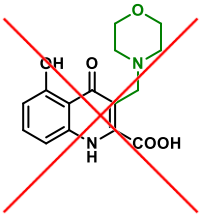
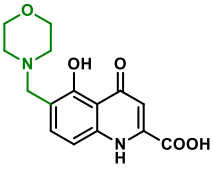
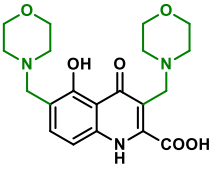
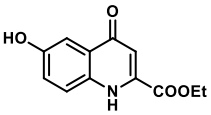
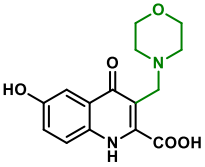
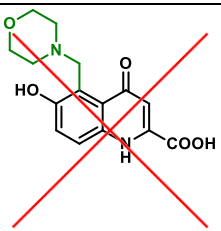
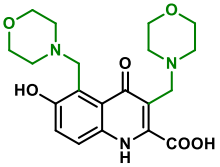
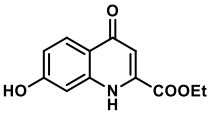
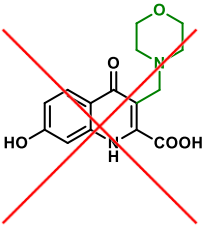
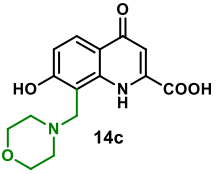
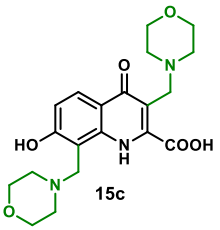
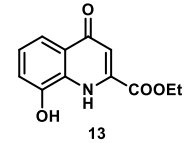
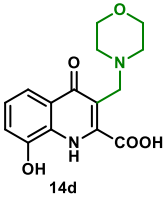
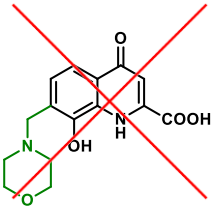
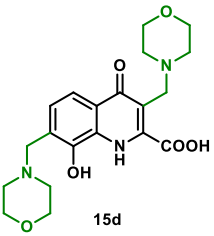
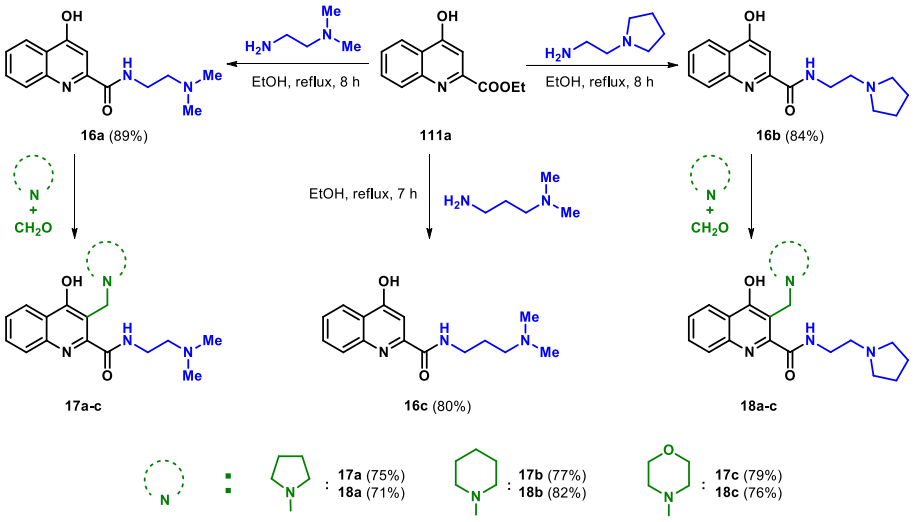
Ester derivative	C-3 substituted derivative	B-ring substituted derivative	Disubstituted derivative
 6a		 14a	 15a
 6b	 14b		 15b
 6c		 14c	 15c
 13	 14d		 15d

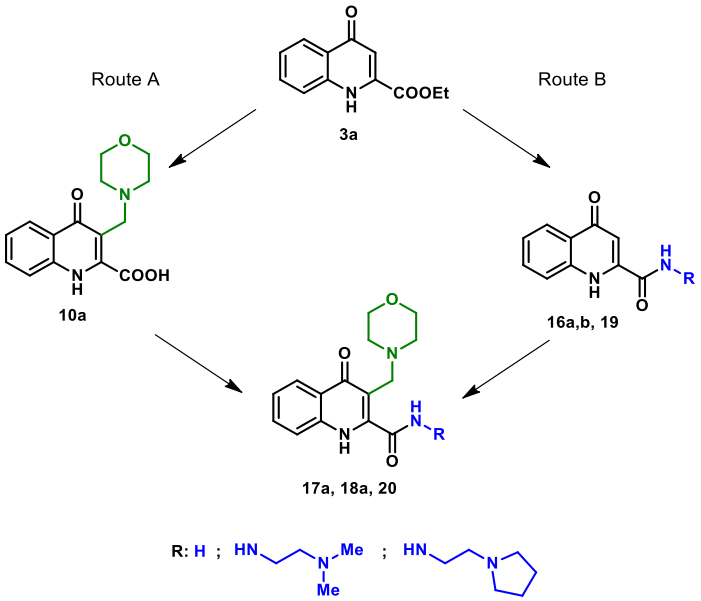
Table 1

4. Amines bearing tertiary nitrogen needed for biological activity towards the central nervous system were used to synthesize amide derivatives of KYNA (**16a-c**). These amides were then used to further extend C-3 aminoalkylations using cyclic and acyclic secondary amines and formaldehyde (**17a-c**, **18a-c**, Scheme 5). 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 showed the most promising properties for further investigations.



Scheme 5

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.



Scheme 6

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.

C. PUBLICATIONS

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**

D. CONFERENCE LECTURES

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

2. **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

3. **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

4. **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

5. **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