University of Szeged

Doctoral School of Pharmaceutical Sciences

Educational Program:	Pharmaceutical Chemistry and Drug Research
Programme director:	Prof. Dr. István Szatmári
Institute:	Institute of Pharmaceutical Chemistry
Supervisor:	Prof. Dr. István Szatmári

dr. Péter Simon

Sustainable methods for the synthesis and functionalization of KYNA derivatives

Final examination committee:

Head:	Prof. Dr. Zsolt Szakonyi
Members:	Prof. Dr János. Wölfling
	Prof. Dr. Anikó Borbás

Reviewer committee:

Head:	Prof. Dr. György Dombi
Reviewers:	Dr. Balázs Bognár
	Dr. Iván Kanizsai
Members:	Dr. Anita Sztojkov-Ivanov (secretary)
	Dr. Tivadar Kiss

A. INTRODUCTION AND AIMS

Kynurenic acid (KYNA) is an endogenous quinoline derivative, biosynthesized from the essential amino acid L-triptophane via the kynurenine pathway. The compound is of great importance due to its neuroprotective properties as aphysiological levels are measured in several neurological disorders such as Parkinson and Huntington disease, epilepsy, and migraine. The total synthesis of kynurenic acid and its 5–8 substituted derivatives is conducted most commonly *via* non eco-friendly methods and types of the two-step Conrad–Limpach method. As the method consists of an enamine formation and subsequent cyclization steps, utilizing traditional solvents of high boiling pont such as diphenyl ether, halogenated arenes or catalysts such as polyphosphoric acid or *p*TsOH are crucial factors regarding the conduction of the syntheses. In most recent literature, optimalization has been conducted focusing on the heat source or additional work-up processes. Last milestones of research focused on the further modification of the KYNA skeleton in order to enhance its effectivity and penetrability through the blood–brain barrier. Transformation of its ester precursors towards various amides bearing tertiary nitrogen residues was a versatile means of structural fine tuning. Certain amides of kynurenic acid are reported to show inhibitory properties on NMDAR related excitotoxicity exerting protective effects in cerebral ischemia and transgenic mouse models of Alzheimers's disease and transgenic mouse models of Huntington disease. However, KYNA being an *aza*-analogue of 1-naphtol, its transformation *via* the Mannich reaction at the C–3 site or at *ortho*-position to its occurring hydroxysubstituents was possible and cleared the way towards its further modifications. Certain Mannich-bases of KYNA derivatives are reported to show significant inhibiton on field excitatory postsynaptic potentials in the CA1 hippocampal region of rats.

The first aim of my PhD work was the design of a sustainable method for the precursor synthesis of kynurenic acid. The Conrad–Limpach method – able to yield various KYNA analogs such as ethyl esters of the biologically and chemically representative methyl-, methoxy- and halogeno derivatives – was aimed to be transferred into green chemical processes utilizing microwave irradiation as heat source on grounds of former improvements in the field. A two-step, one batch method without purification of the

intermediates was our objective in order to reduce solvent consumption. The traditional reaction media were targeted to be exchanged to green solvents. Selection of green solvents is a multifactored task as ESH (Environment – Health – Safety) and LCA (Life Cycle Assessment) analysis is required. These classification methods take the environmental impact of the solvent synthesis, eradication, solvent usage in preparative work (*e.g.* boiling point, volatility) and effect on humans into consideration. As a wide-palette of eco-friendly solvents are available nowadays, gamma-valerolactone (GVL) and diethyl carbonate (DEC) seemed prosperous for the further optimization as these bear the optimal properties to make the solvent for the synthesis of kynurenic acid analogs. Scale-up of the optimized reactions was a further aim regarding the design of a green chemical process for the synthesis of KYNA analogs. Not only the synthetic method itself, but the analytical technique was also targeted to follow the concept of green chemistry.

The second group of aims were the broadening of the scope of the C–3 functionalization of the kynurenic acid *via* the modified Mannich-reaction. Amino acid fragments were chosen to be incorporated in the KYNA scaffold in order to yield zwitterionic Mannich-bases. However, on basis of preliminary research C–3 alkoxymethyl derivatives formed. Since there is a wide array of literature of alkoxyalkylation; the elaboration, optimization and overall investigation of the direct and *N*-nucleophile catalyzed alkoxymethylation of KYNA was targeted by utilizing various cyclic amines, corresponding acetates and amino acids. Broadening the scope of the aforementioned transformation towards different benzyl-ether type KYNA analogs was also plotted. As direct and *retro*-Mannich based alkoxyalkylations are an emerging field of organic syntheses, it was chosen to make the literature background of my thesis.

Further modifications of KYNA derivatives towards indole based triarylmethanes was aimed. Based upon previous research two main routes incorporating *retro*-Mannich reactions were chosen as possible tools for the syntheses. Transformations of indole-based Mannich products using KYNA and also, on the contrary, the *retro*-Mannich reaction of Mannich bases of KYNA in the presence of indole was targeted. Optimization of the synthesis of KYNA-indole triarylmethanes and investigation of the scope and limitations of the reaction by utilizing an array of 5–8 substituted kynurenic acid analogs made a general objective of my PhD work.

B. RESULTS AND DISCUSSION

1. Synthesis of biologically prosperous kynurenic acid derivatives were investigated and optimized for one-batch, two-step microwave-assisted reactions. Utilizing both chemically and biologically representative non-, methyl-, methoxy- and chlorosubstituted aniline derivatives, in catalyst-free conditions, 2–3.5 hour timeframe syntheses of seven kynurenic acid derivatives were introduced. Crucial parameters including reaction time, reaction temperature, solubility, concentration and molar ratio of substrates along with overall reaction volume were determined, investigated and optimized (Figure 1).

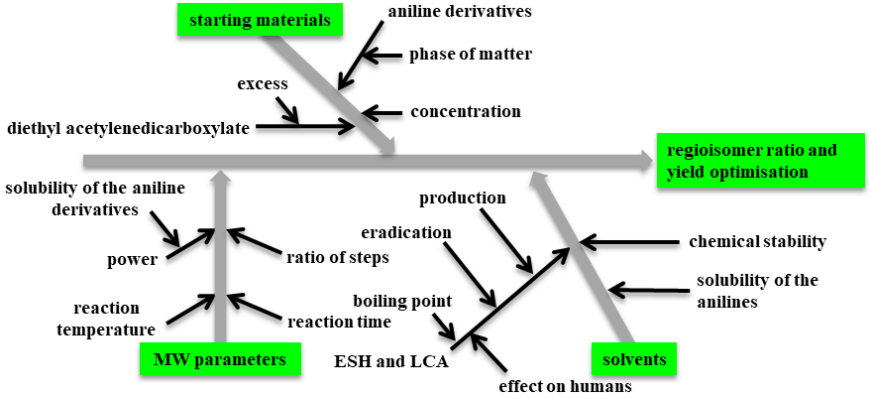
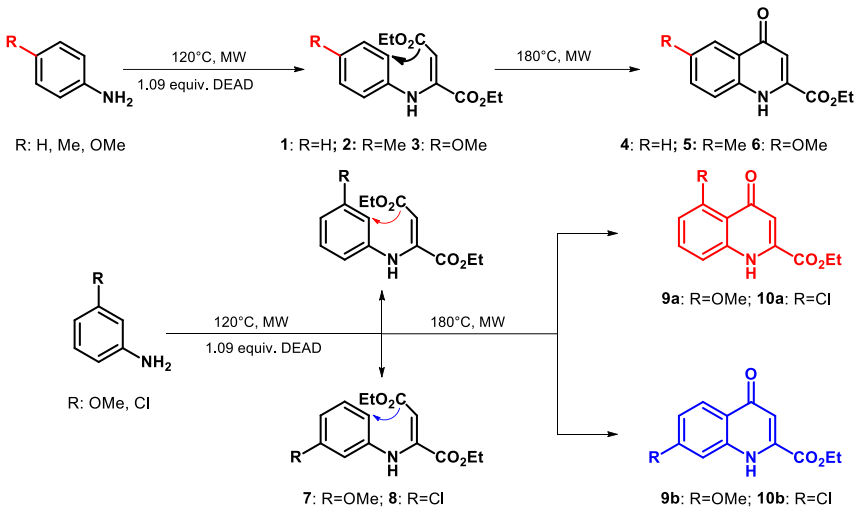


Figure 1. Factors taken into consideration while designing the MW-assisted reactions

Four green, tuneable solvent systems made of diethyl carbonate and gamma-valerolactone were tested as substitutes for 1,2-dichlorobenzene as reaction medium in each synthesis (Scheme 1). The performance of the green solvent systems was highlighted, because of their ability to be used in microwave-assisted reactions. Yields are not lower than those found in traditional solvents. Moreover, the potential of the tuneable solvent system to regulate regioisomeric ratios in CL synthesis was emphasised.



Scheme 1. Regiodivergent syntheses of KYNA analogs **4–6, 9,10** *via* the CL method

2. The benefits of TLC-densitometry (Figure 2), a rapid, eco-friendly, inexpensive analytical technique, were revealed in the case of reaction monitoring and conversion analysis compared to quantitative NMR.

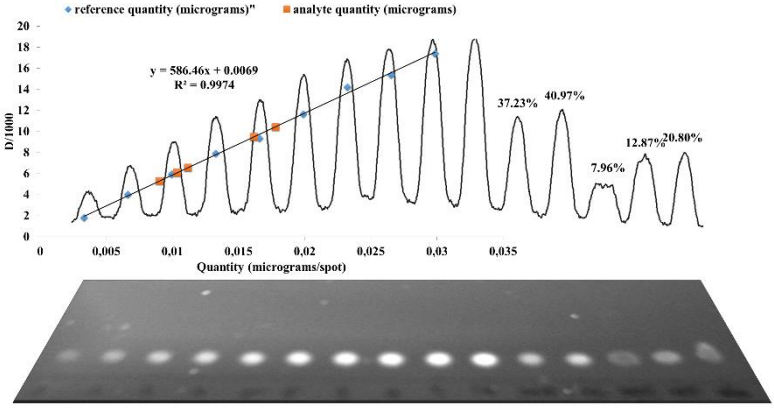
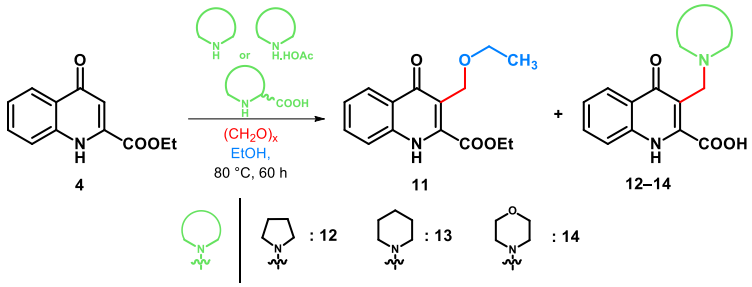


Figure 2. TLC densitometric analysis of compound **4**

Gram-scale scale-up was accomplished in the halogenated solvent DCB and, most importantly, in green substitutes with the solvent-performance similar to preliminary

experiments. These synthetic methods facilitated the CL synthesis of unsubstituted and several substituted kynurenic acid analogues.

3. Novel C–3 alkoxymethylated derivatives of kynurenic acid were synthesized with cyclic secondary alpha amino acid additives *via* the hypothesized *retro*-Mannich cleavage of the intermediates. The importance of a secondary acidic *N*-nucleophile was proven. Test reactions were conducted using common organic additives, including cyclic amines and their acetate salts, as well as using other acidic *N*-nucleophiles. Result showed the importance of the local acidic conditions, particularly when using amino acids. The importance of the corresponding heterocycle and its ability to coordinate the *O*-nucleophile were also highlighted (Scheme 2).



Scheme 2. C–3 functionalization of **4** with cyclic secondary amines, their acetates or corresponding amino acid derivatives

4. A mechanism of the alkoxyalkylation of kynurenic acid ethyl esters was proposed on basis of the literature of *retro*-Mannich C–*N* cleavage of Mannich bases moreover, via the conduction of analogous reactions, indirect proof was presented regarding the reaction mechanism. The optimal equivalence of the amino acid was found considering both economic and preparative reasons. The findings supported that the used amino acid (L-proline, D-pipecolic acid, (*S*)-3-morpholinecarboxylic acid) should be in the additive-range (*i.e.* 0.5 equiv.) which both elevates conversion and selectivity towards the desired products, also subdue side-reactions encumbering the work-up processes (Figure 3).

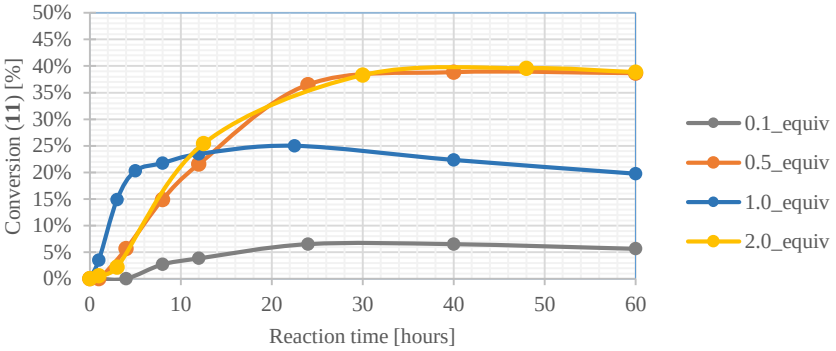
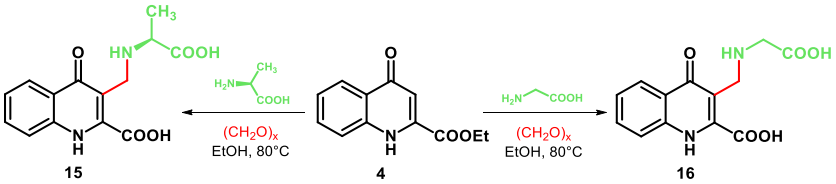


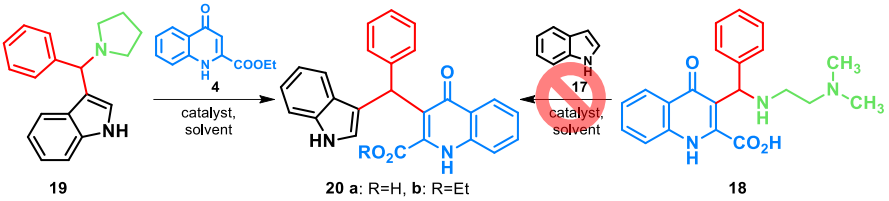
Figure 3. Conversion curves (**11**) of the C–3 ethoxymethylation of **4** using different equivalents of S3MCA

5. Two novel kynurenic acid derivatives, containing glycine or L-alanine fragments were synthesized, thus proving the scope and limitations of the aminoalkylation reaction of kynurenic acid ethyl ester using alpha amino acids (Scheme 3). Limitations were set of alkoxyalkylation reactions and two novel kynurenic acid derivatives were synthesized bearing ethoxymethyl or isopropoxymethyl moieties at the site C–3.



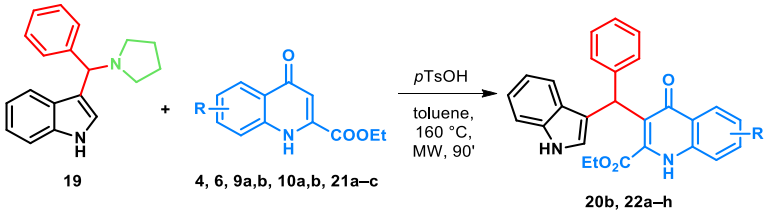
Scheme 3. C–3 functionalization of **4** with glycine and L-alanine

6. The synthesis of TRAM bioconjugates consisting of indole and the ethyl ester of kynurenic acid has been accomplished (Scheme 4). The reactions took place through the cleavage of the C–*N* bond of indole Mannich bases and subsequent C–C bond formation between the benzylidene intermediate and KYNA. On the basis of acid and base catalysis, two possible catalytic pathways are hypothesized, both promoting the elimination of the amine moiety. An alternative reaction route starting with the Mannich bases of KYNA and its structural analogue 1-naphthol was also investigated showing a prominent tilt toward the synthesis applying the Mannich base of indole involving the benzylidene intermediate.



Scheme 4. Synthesis of triarylmethanes of KYNA and indole fragments

7. To further investigate the scope and limitations of the reaction, KYNA derivatives bearing chemically and biologically prosperous chloro and methoxy groups were also reacted yielding a wide variety of new TRAM KYNA derivatives with possible bioactivities (Scheme 5).



R=H: **4**, **20b**; 5-Cl: **10a, 22a**; 6-Cl: **21a, 22b**; 7-Cl: **10b, 22c**; 8-Cl: **21b, 22d**;
5-OMe: **9a, 22e**; 6-OMe: **6, 22f**; 7-OMe: **9b, 22g**; 8-OMe: **21c, 22h**

Scheme 5. Broadening the scope of the synthesis of KYNA-indol based triarylmethane derivatives

C. PUBLICATIONS

Papers related to the thesis

I. **Simon, P.**; Lőrinczi, B.; Szatmári, I.
Alkoxyalkylation of Electron-Rich Aromatic Compounds
Int. J. Mol. Sci. **2024**, *25*, 6966.
https://doi.org/10.3390/ijms25136966 **IF.: 4.9**

II. **Simon, P.**; Lőrinczi, B.; Szatmári, I.
Novel Eco-friendly, One-Pot Method for the Synthesis of Kynurenic Acid Ethyl Esters
ACS Omega **2023**, *8*, 17966.
https://doi.org/10.1021/acsomega.3c01170 **IF.: 3.7**

III. **Simon, P.**; Lőrinczi, B.; Szatmári, I.
C–3 alkoxymethylation of 4-oxo-1,4-dihydroquinoline 2-carboxylic acid esters *via* organic additives
Heliyon **2024**, *10*, e32188.
https://doi.org/10.1016/j.heliyon.2024.e32188 **IF.: 3.4**

IV. Lőrinczi, B.; **Simon, P.**; Szatmári, I.
Synthesis of Indole-Coupled KYNA Derivatives *via C-N* Bond Cleavage of Mannich Bases.
Int. J. Mol. Sci. **2022**, *23*, 7152.
https://doi.org/10.3390/ijms23137152 **IF.: 5.6**

Other publications

V. Gombár, G.; **Simon, P.**; Ungor, D.; Szatmári, I.; Csapó, E.
Histidinehydroxamic acid as new biomolecule to produce molecular-like fluorescent gold nanoclusters: Possible mechanisms for metal ion sensing
J. Mol. Liq. **2023**, *387*, 122597.
https://doi.org/10.1016/j.molliq.2023.122597 **IF.: 5.3**

VI. Ibos, K.E.; Bodnár, É.; Dinh, H.; Kis, M.; Márványkövi, F.; Kovács, Z.; Siska, A.; Földesi, I.; Galla, Z.; Monostori, P.; Szatmári, I.; **Simon, P.**; Sárközy, M.; Csabafi, K.
Chronic kidney disease may evoke anxiety by altering CRH expression in the amygdala and tryptophan metabolism in rats
Pflüg. Arch. **2024**, *476*, 179.
https://doi.org/10.1007/s00424-023-02884-y **IF.: 2.9**

D. CONFERENCE LECTURES

1. **Péter Simon**
   Szubsztituált kinurénsavszármazékok mikrohullám-asszisztált zöldkémiai előállítása
   A Szegedi Ifjú Szerves KémikusokTámogatásáért Alapítvány a SZAB Szerves és Gyógyszerkémiai Munkabizottsággal és az MKE Csongrád Megyei Csoportjával közösen rendezett 22. tudományos előadóülése
   May 18. 2022. Szeged, Hungary
2. **Péter Simon,** Bálint Lőrinczi, István Szatmári
   Új környezetbarát, one-batch eljárások kidolgozása kinurénsav-származékok szintézisére
   Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése
   May 23–25. 2022. Balatonszemes, Hungary
3. **Péter Simon,** Bálint Lőrinczi, István Szatmári
   A novel eco-friendly, one-pot method for the synthesis of kynurenic acid ethyl esters
   22nd Tetrahedron Symposium: Catalysis for a Sustainable World
   June 28.–July 1. 2022. Lisbon, Portugal
4. **Péter Simon**
   Kinurénsavszármazékok egylombikos, környezetbarát előállítása és továbbalakításai
   A Magyar Gyógyszerésztudományi Társaság Gyógyszerkutatási Szakosztálya „Fiatal Kutatók Fóruma”
   November 25. 2022. Budapest, Hungary
5. **Péter Simon,** Bálint Lőrinczi, István Szatmári
   Új, C-3 alkoximetil-kinurénsavszármazékok szintézise
   Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése
   May 31–June 2. 2023. Balatonszemes, Hungary
6. **Péter Simon,** Bálint Lőrinczi, István Szatmári
   Kinurénsavszármazékok organokatalitikus C–3 alkoximetilezési reakcióinak feltérképezése és kiterjesztése
   XXIX. Nemzetközi Vegyészkonferencia
   October 23–25. 2023. Targu Mures, Romania
7. **Péter Simon**
   Kinurénsavszármazékok zöldkémiai előállítása és orto-kinonmetid köztiterméken való funkcionalizálása organoadditívek alkalmazásával
   MTA Heterociklusos és Elemorganikus Kémiai Munkabizottságának Patonay Tamás-díj átadásával egybekötött nyílt ülése
   December 18. 2023. Budapest, Hungary
8. **Péter Simon,** Bálint Lőrinczi, István Szatmári
   Organoadditive facilitated C–3 alkoxymethylation of 4-oxo-1,4-dihydroquinoline 2-carboxylic acid esters
   16th Congress of the International Society for Tryptophan Research
   April 24–26. 2024. Jena, Germany
9. **Péter Simon,** Bálint Imre Modok, István Szatmári
   Synthesis and transformation of novel sulfur-containing kynurenic acid heteroanalogues
   24th Tetrahedron Symposium
   June 18–21. 2024. Montpellier, France