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Selective heterofunctionalization of kynurenic acid derivatives†

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The latest findings in the literature show that kynurenic acid and its analogues are potent drug candidates against numerous neurological diseases. In this article, kynurenic acid derivatives were treated with NaOCl and NaOBr solutions and yielded the corresponding 3-halogeno compounds. The reaction is fast and conducted under mild conditions, and the yields are efficient just like the previous methods available for the same transformation. These newly synthesized halogeno compounds can serve as starting materials for the synthesis of 3-aminokynurenic acid analogues by treating the 3-bromokynurenic acid analogue with NaN_3. The solvent effect of this reaction was also examined. These reactions are suitable for the synthesis of 3-heterosubstituted kynurenic acid analogues.

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Introduction

Kynurenic acid (KYNA) is a biologically active metabolite of the essential amino acid tryptophan, of which the major part is metabolized through the kynurenine pathway. In this pathway, tryptophan first forms kynurenine and then kynurenic acid.[1–3] KYNA has various biological effects and acts as a ligand of numerous receptors, *i.e.* NMDA receptors, α7-nAch receptors, GPR35, AHR and more.[4–11] The kynurenic acid level or the dysregulation of the kynurenine pathway is associated with numerous diseases. Moreover, treatment with exogenous KYNA or its analogues has been found to be efficient in treating neurological and other diseases in animal models.[3,12–22] Seeing these promising results, kynurenic acid and its derivatives have big potential in drug discovery.

The limitation of exogenous kynurenic acid therapy is the poor water solubility and inadequate penetration through the blood–brain barrier (BBB) of the molecule. Previous studies have shown that both solubility and BBB penetration can be enhanced with the incorporation of a tertiary amine function-containing side chain to the molecule. This modification was implemented by the amidation of the carboxylic acid function with alkyl diamines.

Another possibility for altering the pharmaceutical properties of KYNA is the synthesis of substituted analogues *via* the Conrad–Limpach method. According to this method, an aromatic amine reacts with oxo-dicarboxylic acid or acetylene dicarboxylic acid derivatives, and kynurenic acid derivative is formed in a two-step reaction. First, an enamine is formed then an intramolecular cyclization yields the target molecule. Using this method various kynurenic acid analogues can be synthesized from substituted anilines resulting in C5–C8 substituted KYNA compounds.[23–26]

Another possibility to enhance the BBB penetration of KYNA analogues was found by the synthesis of 3-aminoalkyl derivatives *via* the modified-Mannich reaction using paraformaldehyde and secondary amines such as morpholine, piperidine or pyrrolidine. These two modifications can also be combined with each other resulting in aminoalkylated amide compounds.[2,27–29]

There are known ways to synthesize 3-chloro- and 3-bromokynurenic acids using *N*-chlorosuccinimide (NCS) or *N*-bromosuccinimide (NBS) as halogenating agents. Another way to get the same compounds is by treating the KYNA with inorganic reagents like SO_2Cl_2 or Br_2.[30–32] However, the work of Suzuki *et al.*[33] has shown that kynurenic acid can react with hypochlorous and hypobromous acid. Their study highlighted that due to inflammation in the human body, HOCl and HOBr form, which can further react with kynurenic acid yielding the 3-halogeno KYNA derivatives.

Our plan was to treat kynurenic acid derivatives on a 50–150 milligram scale with NaOCl and NaOBr solutions, expecting the 3-halogenated compounds, thus representing a biomimetic method to synthetize KYNA compounds. Our further plan was the substitution of the halogeno compounds with various nucleophiles (Fig. 1).

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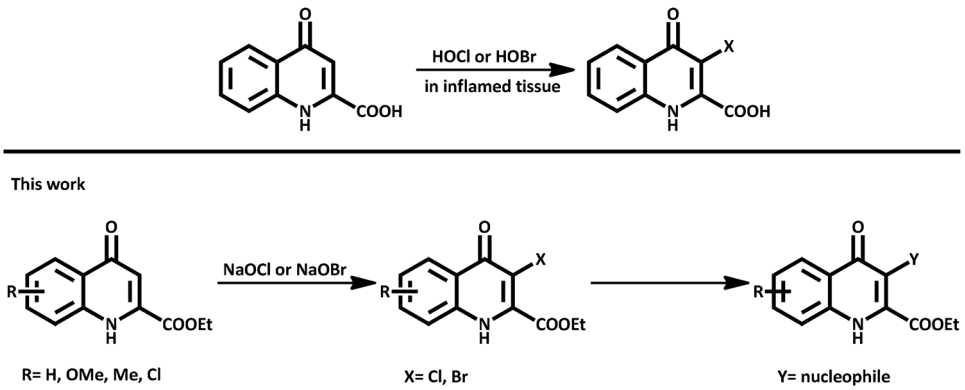


Fig. 1 *In vivo* and *in vitro* findings of Suzuki *et al.* and our current synthetic work.

This work presents a new halogenation method to synthesize 3-halogenated KYNA derivatives, with inorganic reagents that are easy to handle during the reactions. Moreover, the reaction conditions are milder compared with previous works.[30–32]

Results and discussion

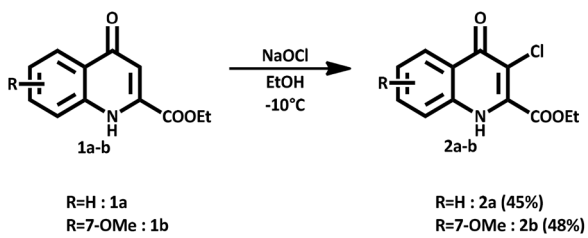
Synthesis of substituted KYNA derivatives

The starting materials used in this article (**1a–g**) were synthesized *via* the Conrad–Limpach method by treating the appropriate substituted anilines with diethyl acetylene dicarboxylic acid according to experimental methods already published in the literature.[34]

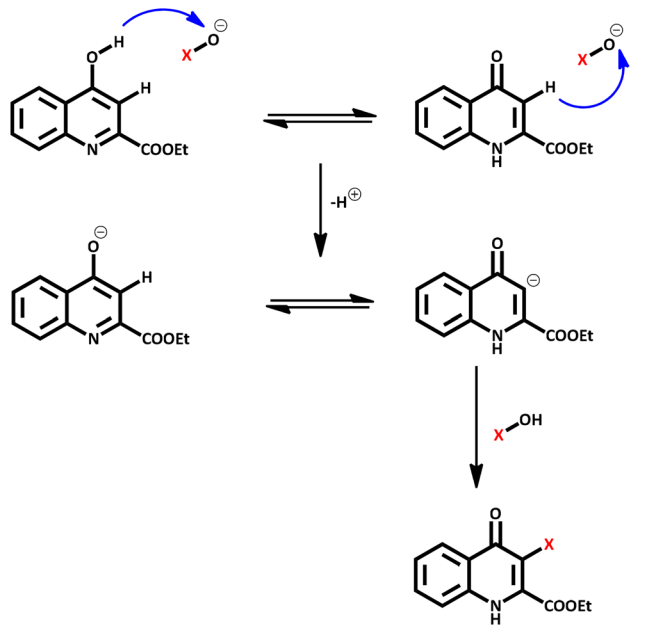
Chlorination of KYNA derivatives

First, kynurenic acid ethyl ester (**1a**) was treated with 150 g L^{-1} NaOCl aqueous solution in ethanol (EtOH) at room temperature, and the reaction mixture turned yellowish. The reaction was followed by thin layer chromatography (TLC), and the mixture was worked up when the starting compound was no longer detectable by TLC. The reaction mixture was diluted with methylene chloride and washed with distilled water twice. The organic layer was dried with sodium sulphate and evaporated *in vacuo*. Crystals were formed and were washed with diethyl ether and filtrated. The isolated product **2a** was the 3-chlorokynurenic acid ethyl ester with 25% yield. The next step was the fine-tuning of the reaction to achieve higher yields. The optimal temperature was found to be −10 °C, and the concentration of the NaOCl solution decreased to 50 g L^{-1} to achieve the highest yield. Despite the approximately 50% yield, there was no sign of any other product in the organic fraction after the extractions. It is very likely that the NaOCl would oxidize the molecules during the reaction,[33] making them so polar that they can be removed by washing with water. Later, 7-methoxy kynurenic acid ethyl ester (**1b**) was also treated with the reagent NaOCl to yield the expected compound **2b**. Although **1b** did not totally dissolve in EtOH, we insisted on using it as the solvent because of the ethyl ester function of the molecule and its miscibility with aqueous solutions (Scheme 1).

Our hypothesis regarding the mechanism of this halogenation is quite similar to the α-halogenation of ketones. In the case of KYNA, the hydrogen in position 3 is activated. We assume that the hydrogen in C3 or the phenolic hydrogen (depending on the tautomeric form) can be easily removed by the basic hypohalogenite ion. The formed anion also has a tautomeric equilibrium. Probably, the halogeno part of the hypohalogenite acts as an electrophilic agent and can attack this anion, forming the halogenated product (Scheme 2).

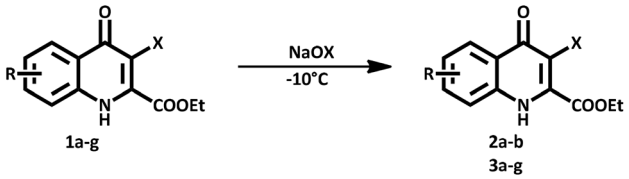


Scheme 1 Chlorination of kynurenic acid ethyl ester derivatives.



Scheme 2 Proposed mechanism of halogenation.

Table 1 Halogenation of kynurenic acid analogues in EtOH and water : acetone 1 : 1



Starting compound	Product	R	X	Solvent	Yield	Solvent	Yield
1a	**2a**	H	Cl	EtOH	45%		
1b	**2b**	7-OMe			48%		
1a	**3a**	H	Br		78%	Water : Acetone 1 : 1	60%
1b	**3b**	7-OMe			67%	[a]38%	
1c	**3c**	7-Cl			60%		51%
1d	**3d**	7-Me			72%		65%
1e	**3e**	6-OMe			65%		51%
1f	**3f**	6-Cl			83%		70%
1g	**3g**	6-Me			72%		58%

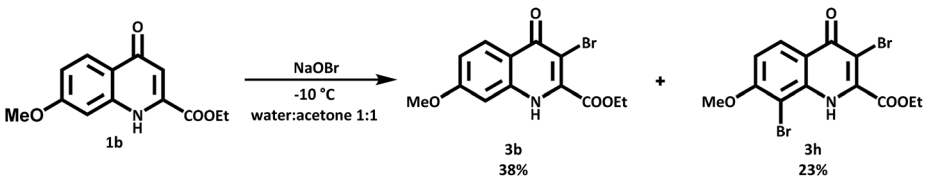
[a] Lower yield is experienced because a side product is formed in the reaction.

Bromination of KYNA derivatives

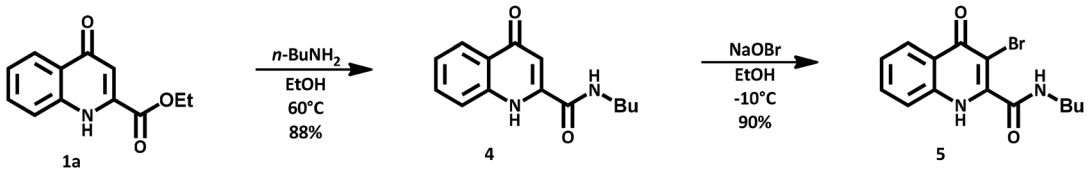
After the successful chlorination, the next step was to broaden the scope of halogenation with bromination. First, the NaOBr solution, a brominating reagent, was prepared by the dropwise addition of bromine to an aqueous NaOH solution at −10 °C, yielding a clear orange solution. NaOBr is prone to disproportion, so the reagent was stored below 0 °C and kept in an ice bath during the reaction setup. This solution was used as the brominating reagent of the further conducted reactions.

The bromination was performed following the optimized reaction parameters found for chlorination. Using the exact same parameters, a higher yield was observed in the case of the unsubstituted KYNA derivative (**3a**). This higher yield value can be explained by the lower oxidizing effect of bromine compared to chlorine. The reaction was repeated with multiple substituted kynurenic acid ethyl esters. Each time, the 3-brominated KYNA derivatives (**3b–g**) were isolated with good yields. According to these tests, the yields do not correlate with the quality of the substituents in ring B, nor their position (Table 1).

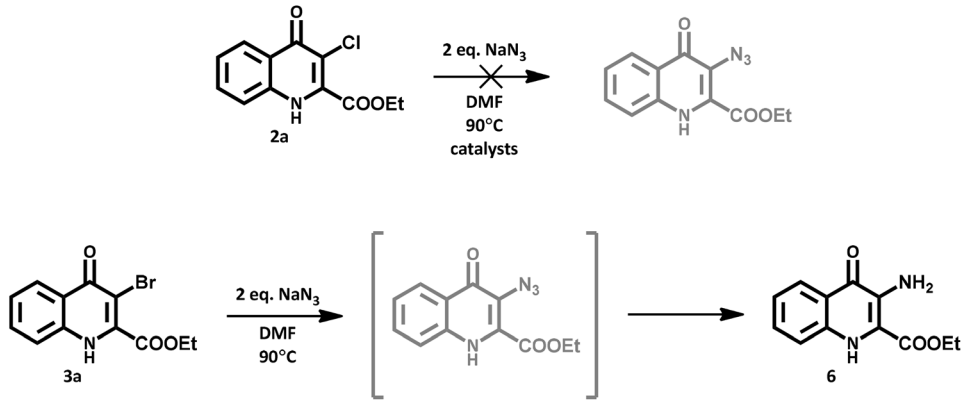
During the bromination of **1b**, the formation of a second product was detectable by TLC, but after the workup only **3b** was isolated. We found this interesting, so we decided to investigate what the side product was. The reaction was repeated, and before the workup, a sample was taken and examined with mass spectrometry (MS). According to the results of MS, the side product was a dibrominated KYNA ethyl ester derivative. To describe this side product, we changed the matrix of the reaction. The limitation of this change is the solubility of **1b** and the aqueous solution of the brominating reagent. We supposed that a polar mixture of solvents could possibly yield the side product in a higher ratio. Repeating the reaction using water : acetone 1 : 1 instead of EtOH was found to be sufficient and both products were synthesized. They were isolated by column chromatography. The exact structure of the side product (**3h**, Scheme 3) was determined with nuclear magnetic resonance (NMR) spectroscopy. The second bromine was found in the *ortho* position of the methoxy substituent in ring B. The position of the second bromine could be determined from the J-couplings (doublets), which proved that the two remaining aromatic hydrogens are vicinal to each other. Additionally, the COSY spectrum clearly showed cross peaks between the protons of ring B, which can only happen if the bromine is in position 8. The dibromination can be explained with the electronic effect of the methoxy group, and it activates ring B toward electrophilic aromatic substitution (S_EAR). To investigate the solvent effect, some other substituted KYNA derivatives were reacted using the water-acetone mixture, but no other cases gave side products, although all yields were lower than before (Table 1). An explanation of this particular dibromination can be found in the oxo form of the molecule. In this case, the second bromine is in the *ortho* position both to the methoxy and the NH in ring A. According to our hypothesis, the electron donating effect of



Scheme 3 Treating **1b** with NaOBr in a water : acetone 1 : 1 mixture.



Scheme 4 Synthesis and bromination of kynurenic acid butyl amide.



Scheme 5 Reaction of **2a** and **3a** with NaN_3.

both functional groups is necessary for the dibromination. In the case of **3a**, ring B is not activated enough for a second bromination. At **3c** the chlorine is rather a deactivating functional group for S_EAR reactions, while the methyl group of **3d** is an activating substituent, but it is not powerful enough. When the methoxy group is in the C6 position, its activated *ortho* positions do not overlap with the activated position of the NH group.

In order to examine the effect of the ester group on the halogenation, an amide derivative was also synthesized by treating **1a** with *n*-butyl amine, and *via* direct amidation, **4** was formed. This **4** was treated with the brominating reagent using the same conditions as for previous halogenating reactions. The yield of this reaction was higher than in the case of ethyl ester (Scheme 4). This suggests that the ester functional group participates in side reactions. Most probably, it is hydrolyzed to carboxylic acid due to the basic environment, and according to the work of Suzuki *et al.*,[33] the molecule is oxidized by the hypohalogenites. As amides are less susceptible to hydrolysis than the esters, compound **5** was observed in higher yield.

Synthesis of 3-amino kynurenic acid derivatives

Furthermore, we planned to treat the 3-halogeno derivatives with *N*-nucleophiles in order to substitute the halogens. Our first aim was to synthesize 3-amino kynurenic acid derivatives. Using ammonia as a nucleophile typically resulted in carboxamide compounds; therefore, an azide should be synthetized first, and further reduced to an amine. Using this method, the ester function could remain intact. The chosen reagent and solvent were NaN_3 in *N*,*N*-dimethylformamide (DMF).

When **2a** was treated with NaN_3 in DMF, there was no reaction. However, the reaction of **3a** yielded a product that was isolated by column chromatography. Interestingly, this product was not the azido but the amino derivative (Scheme 5). This can be explained by an *in situ* reduction of the azide to amine. This reduction could have happened due to the reducing ability of DMF.[35] The originally planned azido compound could not be isolated at any point during the reaction.

Due to the complexity of the reaction mixture on TLC and investigating the effect of DMF, the reaction was also conducted using various solvents (Table 2). In the case of abs. EtOH or an EtOH-water mixture, no reaction was observed. In pure acetonitrile (MeCN), the reaction yielded compound **6**; however, the isolated yield was not found to be adequate meaning that the reduction is not strictly related to the DMF. Then, DMF/MeCN mixtures were tested with different ratios to enhance the yield. The highest yield was found using a MeCN : DMF 9 : 1 mixture.

Table 2 Solvent effect on the yield of 3-amino kynurenic acid ethyl ester (**6**)

Solvent	Yield (%)
DMF	35
EtOH	No reaction
50% EtOH + 50% H_2O	No reaction
MeCN	43
50% MeCN + 50% DMF	50
90% MeCN + 10% DMF	55

Conclusions

Novel and known C3 chlorinated and brominated kynurenic acid analogues were synthesized using sodium hypochlorite and hypobromite solutions as reagents. This novel rapid synthetic method requires mild conditions and facile workup by only an extraction and crystallization. Therefore, we think that this method is a great replacement of using other reagents (*e.g.* NCS, NBS, SO_2Cl_2), which are less eco-friendly. The reaction mechanism was proposed for the halogenations, which is analogous to the α-halogenations of ketones considering the active hydrogen in position 3 of KYNA.

In one instance, a solvent change led to a 3,8-dibrominated side product, for which a possible explanation was proposed for the dibromination. These synthesized halogeno compounds could be precursors for other new kynurenic acid derivatives.

The 3-aminokynurenic acid analogue was synthesized in a one-pot reaction by treating the 3-bromokynurenic acid with NaN_3. The solvent effect was examined and the catalytic role of DMF in the yield was also clarified. We believe that the 3-amino derivative of the kynurenic acid ethyl ester could be a valuable synthon for the synthesis of pharmacologically upgraded KYNA derivatives, due to its hydrogen bond donor and acceptor properties and broad transformability.

Materials and methods

The ^{1}H and ^{13}C-NMR spectra were recorded in DMSO-d_6 and $CDCl_3$ solutions in 5 mm tubes at room temperature (*RT*), on a Bruker DRX-500 spectrometer (Bruker Biospin, Karlsruhe, Baden Württemberg, Germany) at 500 (^{1}H) and 125 (^{13}C) MHz, with the deuterium signal of the solvent as the lock and TMS as the internal standard (^{1}H, ^{13}C).

Melting points were determined using a Hinotek X-4 melting point apparatus. Merck Kieselgel 60F254 plates were used for TLC.

The HRMS flow injection analysis using positive heated electrospray ionisation mode (HESI) was performed with a Thermo Scientific Exploris™ 240 hybrid quadrupole-Orbitrap (Thermo Fisher Scientific, Waltham, MA, USA) mass spectrometer coupled to a Waters Acquity I-Class UPLC™ (Waters, Manchester, UK).

General method of C3 chlorination of KYNA analogues (2a–b)

0.45 mmol of kynurenic acid ethyl ester analogues were added to a round bottom flask and dispersed with 20 mL of abs. EtOH and cooled to −10 °C with ice and acetone. To this mixture, 50 g L^{-1} NaOCl aqueous solution was added dropwise. The reaction was monitored using TLC. Once the starting material was no longer observed, the mixture was immediately diluted with 5 mL of brine and 10 mL of distilled water and extracted with 30 mL of methylene chloride twice. The organic layer was separated, dried with Na_2SO_4 and evaporated *in vacuo*. Crystals were formed, which were washed with diethyl ether and filtered.

Ethyl 3-chloro-4-oxo-1,4-dihydroquinoline-2-carboxylate (2a)

Yield: 51 mg (45%),white solid, Mp.: 214–217 °C (Lit.:[30] 217–217,5 °C) ^{1}H-NMR δ_H (500.20 MHz, DMSO-d_6, 30 °C, Me_4Si) 1.35 (3H, t, $J = 7.1$ Hz), 4.42 (2H, q, $J = 7.1$ Hz), 7.36 (1H, t, $J = 7,4$ Hz), 7.76–7.71 (2H, m), 8.13 (1H, d, $J = 8.21$ Hz), ^{13}C-NMR δ_C (125.62 MHz, DMSO-d_6, 30 °C, Me_4Si) 14.4, 62.9, 112.3, 121.3, 124.3, 125.4, 125.5, 126.3, 132.1, 141.4, 163.3, 171.1. HRMS (HESI) calcd for $[M + H]^+ = 252.04220$ m/z, found: 252.04220 m/z ($\Delta mi = -0.91$ ppm).

Ethyl 3-chloro-7-methoxy-4-oxo-1,4-dihydroquinoline-2-carboxylate (2b)

Yield: 61 mg (48%), beige solid, Mp.: 192–195 °C ^{1}H-NMR δ_H (500.20 MHz, DMSO-d_6, 30 °C, Me_4Si) 1.32 (3H, t, $J = 7.0$ Hz), 3.81 (3H, s), 4,34 (2H, q, $J = 7.0$ Hz), 6.85 (1H, d, $J = 8.86$ Hz), 7.0 (1H, s), 7.98 (1H, d, $J = 8.98$ Hz) ^{13}C-NMR δ_C (125.62 MHz, DMSO-d_6, 30 °C, Me_4Si) 14.5, 55.7, 61.9, 103.9, 112.8, 113.4, 114.2, 121.3, 126.7, 146.3, 161.3, 165.2, 169.7. HRMS (HESI) calcd for $[M + H]^+ = 282.05276$ m/z, found: 282.05238 m/z ($\Delta mi = -1.4$ ppm).

Preparation of the brominating reagent

10 mL of distilled water was added to a round bottom flask, and 1.5 g of NaOH was dissolved in it at 0 °C. The solution was cooled to −10 °C using ice and acetone. To the solution, 1.0 mL of bromine was added dropwise under vigorous stirring, yielding a yellow liquid. The solution was diluted up to 30 mL with distilled water. The reagent was sealed and stored in a refrigerator between experiments. Before each experiment, the reagent was brought to room temperature and allowed to melt before use.

General method A: C3 bromination of KYNA analogues in EtOH (3a–3g)

0.45 mmol of kynurenic acid ethyl ester analogues were added to a round bottom flask and dispersed with 20 mL of abs. EtOH and cooled to −10 °C with ice and acetone. To this mixture, brominating reagent solution was added dropwise. The reaction was monitored using TLC. Once the starting material was no longer observed, the mixture was immediately diluted with 5 mL of brine and 10 mL of distilled water and extracted with 30 mL of methylene chloride twice. The organic layer was separated, dried with Na_2SO_4 and evaporated *in vacuo*. Crystals were formed, which were washed with diethyl ether and filtered.

General method B: C3 bromination of KYNA analogues in water-acetone mixture (3a–h)

0.45 mmol of kynurenic acid ethyl ester analogues were added to a round bottom flask and dispersed with 20 mL of water and acetone 1 : 1 ratio mixture and cooled to −10 °C with ice and acetone. To this mixture, the brominating reagent solution was added dropwise. The reaction was monitored using TLC. Once the starting material was no longer observed, the mixture was immediately diluted with 5 mL of brine and 10 mL of distilled water and extracted with 30 mL of methylene chloride twice.

The organic layer was separated, dried with Na_2SO_4 and evaporated *in vacuo*. Crystals were formed, which were washed with diethyl ether and filtered.

Ethyl 3-bromo-4-oxo-1,4-dihydroquinoline-2-carboxylate (3a)

Yield: 104 mg (78%), white solid, Mp.: 251–253 °C (Lit.:[30]:250–251 °C) ^{1}H-NMR δ_H (500.20 MHz, DMSO-d_6, 30 °C, Me_4Si) 1.39 (3H, t, J = 7.0 Hz), 4.48 (2H, q, J = 7.0 Hz), 7.45 (1H, t, J = 7.61 Hz), 7.70 (1H, d, J = 8.57 Hz), 7.76 (1H, t, J = 7.5 Hz), 8.14 (1H, d, 8.4 Hz), 12.73 (1H, brs), ^{13}C-NMR δ_C (125.62 MHz, DMSO-d_6, 30 °C, Me_4Si) 14.3, 63.7, 103.3, 119.2, 119.3, 123.9, 125.3, 125.8, 133.4, 139.0, 141.3, 172.2. HRMS (HESI) calcd for $[M + H]^+$ = 295.99168 m/z, found: 295.99134 m/z (Δmi = −1.2 ppm).

Ethyl 3-bromo-7-methoxy-4-oxo-1,4-dihydroquinoline-2-carboxylate (3b)

Yield: 98 mg (67%), beige solid, Mp.: 206–210 °C. ^{1}H-NMR δ_H (500.20 MHz, DMSO-d_6, 30 °C, Me_4Si) 1.38 (3H, t, J = 7.1 Hz), 3.87 (3H, s), 4.46 (2H, q, J = 7.0 Hz), 7.04 (1H, d, J = 9.2 Hz), 7.08 (1H, s), 8.04 (1H, d, 9.2 Hz), 12.46 (1H, brs), ^{13}C-NMR δ_C (125.62 MHz, DMSO-d_6, 30 °C, Me_4Si) 14.3, 56.1, 63.6, 99.8, 103.8, 115.7, 118.3, 127.8, 140.6, 140.9, 162.3, 163.1, 171.6. HRMS (HESI) calcd for $[M + H]^+$ = 326.00225 m/z, found: 326.00185 m/z (Δmi = −1.2 ppm).

Ethyl 3-bromo-7-chloro-4-oxo-1,4-dihydroquinoline-2-carboxylate (3c)

Yield: 89 mg (60%), beige solid, Mp.: 249–251 °C (Lit.:[30] 244–245 °C) ^{1}H-NMR δ_H (500.20 MHz, DMSO-d_6, 30 °C, Me_4Si) 3.39 (3H, t, J = 7.3 Hz), 4.48 (2H, q, J = 7.3 Hz), 7.46 (1H, d, J = 8.6 Hz), 7.75 (1H, s), 8.13 (1H, d, J = 8.6 Hz), 12.76 (1H, brs), ^{13}C-NMR δ_C (125.62 MHz, DMSO-d_6, 30 °C, Me_4Si) 14.3, 63.8, 104.8, 118.6, 122.4, 125.8, 128.3, 137.9, 141.4, 162.1, 171.9. HRMS (HESI) calcd for $[M + H]^+$ = 329.95271 m/z, found: 329.95247 m/z (Δmi = −0.7 ppm).

Ethyl 3-bromo-7-methyl-4-oxo-1,4-dihydroquinoline-2-carboxylate (3d)

Yield: 100 mg (72%), beige solid, Mp.: 248–252 °C ^{1}H-NMR δ_H (500.20 MHz, DMSO-d_6, 30 °C, Me_4Si) 1.38 (3H, t, J = 7.1 Hz), 2.45 (3H, s), 4.46 (2H, q, J = 7.1 Hz), 7.27 (1H, d, J = 8.4 Hz), 7.45 (1H, s), 8.03 (1H, d, J = 8.4 Hz), 12.58 (1H, brs), ^{13}C-NMR δ_C (125.62 MHz, DMSO-d_6, 30 °C, Me_4Si) 14.3, 21.8, 63.6, 103.3, 118.4, 121.9, 125.8, 127.1, 139.2, 141.0, 143.8, 162.4, 171.9. HRMS (HESI) calcd for $[M + H]^+$ = 310.00733 m/z, found: 310.00703 m/z (Δmi = −1.0 ppm).

Ethyl 3-bromo-6-methoxy-4-oxo-1,4-dihydroquinoline-2-carboxylate (3e)

Yield: 95 mg (65%), beige solid, Mp.: 236–239 °C ^{1}H-NMR δ_H (500.20 MHz, DMSO-d_6, 30 °C, Me_4Si) 1.38 (3H, t, J = 7.1 Hz), 3.86 (3H, s), 4.46 (2H, q, J = 7.1 Hz), 7.41 (1H, d, J = 9.2 Hz), 7.51 (1H, s), 7.68 (1H, d, J = 9.2 Hz), 12.71 (1H, brs), ^{13}C-NMR δ_C (125.62 MHz, DMSO-d_6, 30 °C, Me_4Si) 14.3, 56.0, 63.6, 102.6, 104.7, 121.3, 124.1, 125.1, 133.6, 139.9, 157.1, 162.4, 171.5. HRMS (HESI) calcd for $[M + H]^+$ = 326.00225 m/z, found: 326.00217 m/z (Δmi = −0.2 ppm).

Ethyl 3-bromo-6-chloro-4-oxo-1,4-dihydroquinoline-2-carboxylate (3f)

Yield: 123 mg (83%), beige solid, Mp.: 258–260 °C ^{1}H-NMR δ_H (500.20 MHz, DMSO-d_6, 30 °C, Me_4Si) 1.38 (3H, t, J = 7.1 Hz), 4.47 (2H, q, J = 7.1 Hz), 7.75 (1H, d, J = 8.9 Hz), 7.80 (1H, d, J = 8.9 Hz), 8.07 (1H, s), 12.90 (1H, brs), ^{13}C-NMR δ_C (125.62 MHz, DMSO-d_6, 30 °C, Me_4Si) 14.3, 63.8, 102.5, 121.9, 124.6, 124.7, 129.8, 133.5, 138.3, 162.3, 171.4. HRMS (HESI) calcd for $[M + H]^+$ = 329.95271 m/z, found: 329.95251 m/z (Δmi = −0.6 ppm).

Ethyl 3-bromo-6-methyl-4-oxo-1,4-dihydroquinoline-2-carboxylate (3g)

Yield: 100 mg (72%), beige solid, Mp.: 219–219 °C ^{1}H-NMR δ_H (500.20 MHz, DMSO-d_6, 30 °C, Me_4Si) 1.38 (3H, t, J = 7.1 Hz), 2.43 (1H, s), 4.46 (2H, q, J = 7.1 Hz), 7.60 (2H, m), 7.93 (1H, s), 12.66 (1H, brs), ^{13}C-NMR δ_C (125.62 MHz, DMSO-d_6, 30 °C, Me_4Si) 14.3, 21.3, 63.6, 103.0, 119.3, 123.9, 124.9, 134.8, 134.9, 137.1, 140.8, 162.4, 171.2. HRMS (HESI) calcd for $[M + H]^+$ = 310.00733 m/z, found: 310.00710 m/z (Δmi = −0.7 ppm).

Ethyl 3,8-dibromo-7-methoxy-4-oxo-1,4-dihydroquinoline-2-carboxylate (3h)

Yield: 41 mg (23%), white solid, Mp.: 206–209 °C. Isolated by column chromatography, eluent: *n*-hexane : ethyl-acetate 1 : 1. ^{1}H-NMR δ_H (500.20 MHz, DMSO-d_6, 30 °C, Me_4Si) 1.36 (3H, *t*, J = 7.1 Hz), 4.02 (3H, s), 4.42 (2H, q, J = 7.6 Hz), 7.35 (1H, d, J = 8.9 Hz), 8.19 (1H, d, J = 8.9 Hz), 11.53 (1H, brs), ^{13}C-NMR δ_C (125.62 MHz, DMSO-d_6, 30 °C, Me_4Si). HRMS (HESI) calcd for $[M + H]^+$ = 403.91276 m/z, found: 403.91238 m/z (Δmi = −0.9 ppm).

3-Bromo-*N*-butyl-4-oxo-1,4-dihydroquinoline-2-carboxamide (5)

Yield: 130 mg (90%), beige solid, Mp.: 226–230 °C ^{1}H-NMR δ_H (500.20 MHz, DMSO-d_6, 30 °C, Me_4Si) 0.92 (3H, t, 7.3 Hz), 1.36–1.44 (2H, m), 1.51–1.57 (2H, m), 3.28 (2H, q, J = 6.5 Hz), 7.41 (1H, t, J = 7.6 Hz), 7.63 (1H, d, J = 8.3 Hz), 7.72 (1H, t, J = 7.6 Hz), 8.13 (1H, d, J = 8.1 Hz), 8.97 (1H, brs), 12.61 (1H, brs), ^{13}C-NMR δ_C (125.62 MHz, DMSO-d_6, 30 °C, Me_4Si) 14.1, 20.0, 31.1, 39.2, 102.0, 118.9, 123.8, 124.9, 125.7, 132.9, 139.1, 146.4, 162.1, 172.1. HRMS (HESI) calcd for $[M + H]^+$ = 323.03897 m/z, found: 323.03872 m/z (Δmi = −0.8 ppm).

N-butyl-4-oxo-1,4-dihydroquinoline-2-carboxamide (4)

1 mmol of kynurenic acid ethyl ester was added to a round bottom flask and dissolved in 15 mL of ethanol. 2 mL of butyl amine was added to the solution and refluxed for 8 h. The reaction mixture was concentrated *in vacuo* and with diethyl ether greenish crystals were formed. The crystals were filtrated and washed with diethyl ether.

Yield: 214 mg (88%), greenish solid, Mp.: 287–290 °C ^{1}H-NMR δ_H (500.20 MHz, DMSO-d_6, 30 °C, Me_4Si) 0.91 (3H, t, J = 7.5 Hz), 1.30–1.38 (2H, m), 1.51–1.57 (2H, m), 3.29–3.32 (under

water), 6.68 (1H, s), 7.33 (1H, t, $J = 7.0$ Hz), 7.66 (1H, t, $J = 7.3$ Hz), 7.93 (1H, d, $J = 8.2$ Hz), 8.05 (1H, d, 7.8 Hz), 8.97 (1H, brs), 11.77 (1H, brs), ^{13}C-NMR δ_C (125.62 MHz, DMSO-d_6, 30 °C, Me_4Si) 14.1, 20.0, 31.3, 40.2, 107.3, 119.9, 124.1, 125.3, 126.0, 132.7, 140.3, 142.2, 162.2, 178.1. HRMS (HESI) calcd for $[M + H]^+$ = 245.12845 *m*/*z*, found: 245.12805 *m*/*z* ($\Delta mi = -1.7$ ppm).

Ethyl 3-amino-4-oxo-1,4-dihydroquinoline-2-carboxylate (6)

0.35 mmol of **3a** was measured into a round bottom flask and dissolved in a mixture of 9 mL of acetonitrile and 1 mL of *N*,*N*-dimethylformamide. 2 equivalent NaN_3 was added to the solution and stirred at 90 °C for 12 hours. The reaction mixture was extracted with methylene chloride and distilled water. The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The compounds were separated by column chromatography (eluent: CH_2Cl_2 : MeCN 5 : 1), crystallized with *n*-hexane and filtered on a glass filter.

Yield: 44 mg (55%), Mp.: 170–173 °C (Lit.36: 174–175 °C) 1H-NMR δ_H (500.20 MHz, DMSO-d6, 30 °C, Me4Si) 1.40 (3H, *t*, $J = 7.1$ Hz), 4.46 (2H, q, $J = 7.1$ Hz), 6.03 (2H, brs), 7.14 (1H, *t*, $J = 7,4$ Hz), 7.53 (1H, *t*, $J = 7.4$ Hz), 7.83 (1H, d, $J = 8.6$ Hz), 8.05 (1H, d, 8.3 Hz), 11.04 (1H, brs), ^{13}C-NMR δ_C (125.62 MHz, DMSO-d_6, 30 °C, Me_4Si) 14.8, 62.1, 111.7, 119.3, 119.4, 121.6, 125.2, 131.8, 136.6, 138.2, 164.8, 171.4. HRMS (HESI) calcd for $[M + H]^+$ = 233.09207 *m*/*z*, found: 233.09190 *m*/*z* ($\Delta mi = -0.7$ ppm).

Data availability

We declare that the data supporting this article have been included as part of the ESI.†

Author contributions

Levente Törteli: writing – review & editing, writing – original draft, investigation. Péter Simon: writing – review & editing, writing – original draft, investigation. Róbert Berkecz: HRMS measurements. István Szatmári: writing – review & editing, conceptualization.

Conflicts of interest

There are no conflicts to declare.

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