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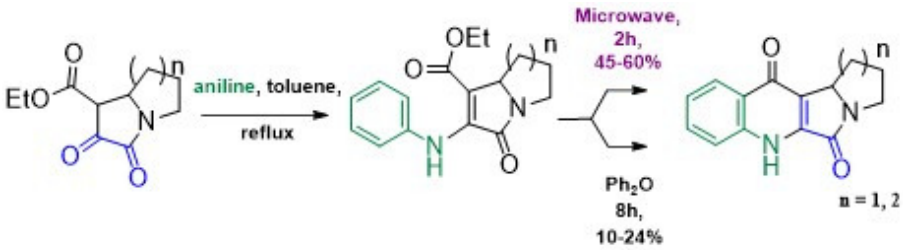

Total synthesis of tetracyclic kynurenic acid analogues isolated from chestnut honey

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ABSTRACT

A short and efficient synthesis of novel tetracyclic Kynurenic acid analogues, isolated from chestnut honey, is described. The crucial step of the strategy was a MW-assisted cyclization of enamines of ethyl dioxohexahydropyrrolizine and 2,3-dioxooctahydroindolizine carboxylates to obtain 2,3,6,11b-tetrahydro-1H-pyrrolizino[2,1-b]quinoline-5,11-dione and 5,8,9,10,11,11a-hexahydroindolizino[2,1-b]quinoline-6,12-dione, respectively. Because of its modular nature, the synthetic strategy can have value as a general method for the preparation of compounds containing these new heterocyclic scaffolds.

Keywords: Kynurenic acid analogues, chestnut honey, natural products, microwave assisted synthesis

Kynurenic acid (KYNA), an endogenous, non-selective antagonist of ionotropic glutamate receptors, is commonly considered as a final metabolite of tryptophan via the kynurenine pathway (KP).

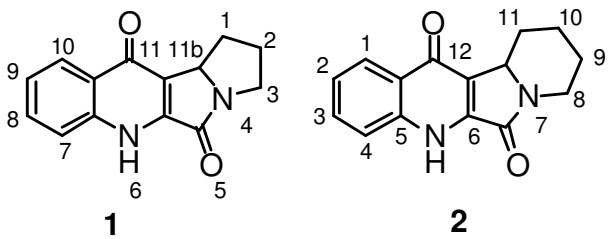
Recent studies provided evidence for an important role of kynurenine pathway metabolites in several neurological, psychiatric and neurodegenerative diseases, with particular emphasis on the putative neuroprotective activity exerted by KYNA.[1] The biological activity of KYNA is due to its

antagonizing capacity toward the glycine site of the NMDA (N-methyl-D-aspartate) receptors and/or the cholinergic α7 nicotine receptors. However, concomitant or alternative involvement of other pharmacological targets responsible for its electrophysiological and behavioral actions cannot be excluded.[2]

KYNA is present in several food products, with the highest known concentrations found in chestnut honey.[3] 6-hydroxy-KYNA and KYNA are also present in the plant kingdom with a taxa-specific distribution of the former.[4] The observation of significant levels of KYNA in different medicinal plants suggested that it may contribute to their therapeutic potential, especially in the digestive system.[5]

Interestingly, recent studies consistently demonstrated that chestnut honey also contains tetracyclic KYNA derivatives, with a tetrahydropyrrolizinoquinolinedione (compound **1**, figure 1) or a hexahydroindolizinoquinolinedione (compound **2**, figure 1) core.[6] These substances originate mainly from the nectar of male flowers of chestnut.[7] Their biosynthesis is also intriguing, as they probably derive from KYNA,[7] differently from the isomeric fungal metabolite tetrahydroquinolactacide,[8] whose biosynthesis most likely starts from anthranilic acid.[9]

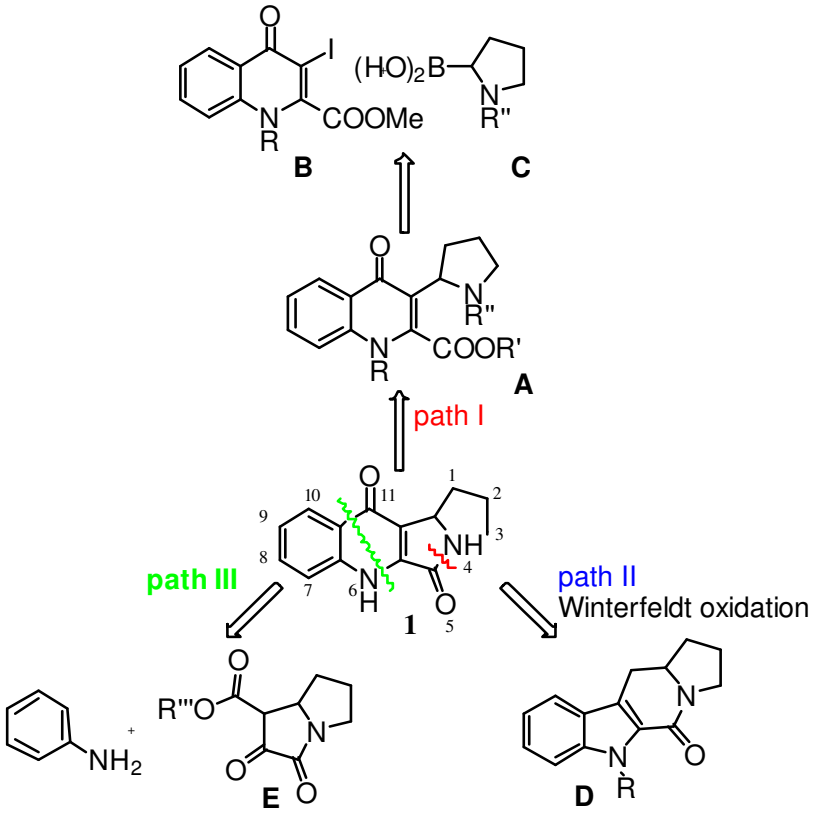
Currently, the biological function(s) and therapeutic potentiality of derivatives **1** and **2** are unknown. Hence, the aim of the present study was to set up a synthetic procedure for these new quinolinone alkaloids which may - in principle - have value in the preparation of the natural compounds themselves as well as in synthesizing analogues for structure-activity relationship studies.



1 **2**

Figure 1. Structures of compounds **1** and **2** isolated from chestnut honey

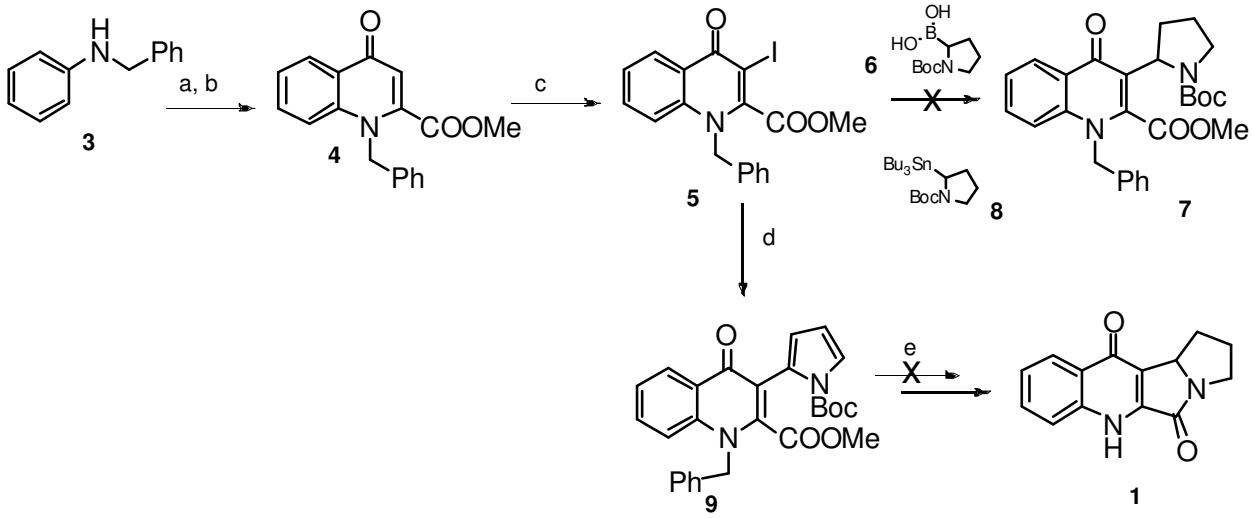
Initially, we focused on the synthesis of compound **1.** We envisioned that the most straightforward approach could be based on the construction of the tetracyclic system starting from kynurenic acid. Thus, disconnection at the amide bond level of the compound **1** gave as precursor the key intermediate **A**, which should be accessible by metal-catalyzed cross-coupling reaction between a functionalized kynurenic acid **B** and a boronic acid **C** (Scheme 1, path I)



Scheme 1. Retrosynthetic analyses of compound **1**

The N-benzyl protected coupling partner **B** (compound **5**) was easily obtained by treatment of *N*-benzylaniline **3** with DMAD (dimethyl acetylenedicarboxylate) in methanol at reflux, followed by cyclization with Eaton's reagent[10] to give *N*-benzyl kynurenic acid methyl ester (**4**). Iodination[11] provided intermediate **5** in good yield.

The N-Boc protected partner **C** (compound **6**) was obtained from commercially available *N*-Boc pyrrole-2- boronic acid by hydrogenation with PtO_2 in ethyl acetate at room temperature (Scheme 2). Once prepared the two fragments, we had to couple them to obtain compound **7**.



Scheme 2. Synthesis of compound **9**. Reagents and conditions: a) DMAD, MeOH, reflux, 85%; b) $P_2O_5/MeSO_3H$, N_2, 50 °C, 82%; c) I_2, CAN, N_2, 50-80 °C, 98%; d) $Pd(PPh_3)_4$, $NaHCO_3$, DME, 2-pyrrol boronic acid, N_2, reflux, 64%; e) H_2 40 atm, Pd/C 10%, MeOH, rt.

Initial efforts to effect the carbon-carbon coupling reaction, carried out using $Pd(PPh_3)_4$ and aqueous Na_2CO_3 in toluene at 80 °C, were unsuccessful. Various attempts of coupling made with different bases (K_2CO_3, K_3PO_4, $CsCO_3$) different phosphine ligands (Pd(dppf), $Pd(dba)_2$) and a variety of solvents (dioxane, THF and DME) always gave complex reaction mixtures.

Stille cross coupling reaction was also unsuccessful. Stannane **8** was prepared from N-Boc pyrrolidine and n-Bu_3SnCl with *sec*BuLi in THF. Reaction of this latter with **5** in the presence of $Pd(PPh_3)_4$ in dioxane[12] did not provide the expected product (Scheme 2).

A possible explanation of these failures in coupling reactions is the poor reactivity of alkyl boronic acids. Aryl-alkyl Suzuki-Miyaura cross-couplings are often complicated by reduction and isomerization side reactions as a result of the presence of β-hydrogen atoms in the alkyl group.[13]

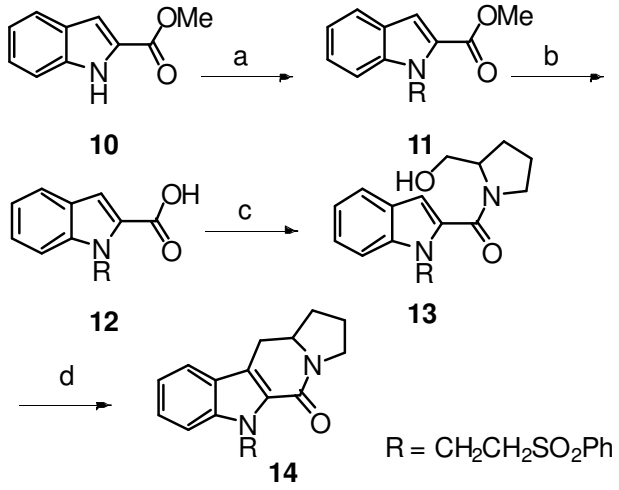
The use of *N*-Boc-2-pyrroleboronic acid instead of the corresponding alkyl boronic acid **6** easily afforded the coupling product **9**,[14] but the successive reduction of the ring together with the removal of the protecting benzyl group failed, even by hydrogenation at high pressure[15] (Scheme 2).

Thus, an alternative route was pursued. We envisioned that the functional quinolone skeleton of compounds **1** and **2** might be assembled by the application of the Winterfeldt oxidation of indoles.[16] This reaction has provided an efficient method for indole-quinolone transformations in the total synthesis of the potent antitumor agent camptothecin [17] and of the antibiotics quinolactacine A[18] and B.[19]

As reported by Husson and coworkers,[20] pyrrolo[3,4-b]-quinoline-3,9-diones can be obtained by Winterfeldt oxidation (O_2, *t*-BuO^-K^+, DMF) of *N*(b) substituted tetrahydropyrido[3,4-b]indol-1-ones. Thus, we identified compound **D** as a new key intermediate (Scheme 1, path II).

As the removal of benzyl group had been troublesome (see scheme 2), we chose the (phenylsulfonyl)ethyl group for N-protection. Indeed, the group can be easily removed by treatment with strong bases such as *t*-BuOK,[21] which is also used to perform Winterfeldt oxidation.

Thus, commercially available indole-2-carboxylic acid methyl ester **10** was treated with 2-phenylsulfonylethyl chloride in presence of NaH and DMF to obtain compound **11**. Hydrolysis of the ester to the corresponding acid **12** was achieved under basic conditions using LiOH in THF/H_2O 1:1 (Scheme 3).



Scheme 3. Synthesis of compound **14**. Reagents and conditions: a) $ClCH_2CH_2SO_2Ph$, NaH, DMF, N_2, rt, 61%; b) $LiOH.H_2O$, THF/H_2O 1:1, 98%; c) EDCI (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride), HOBt (hydroxybenzotriazole), prolinol, THF, N_2, rt, 86%; d) TFA, 1,2-dichloromethane, 50 °C, N_2, 12%.

The condensation of **12** with prolinol gave compound **13**, which was cyclized by TFA in 1,2-dichloroethane[22] (Scheme 3) to obtain compound **14**. However, the very poor yield of this reaction prevented us to pursue this strategy.

In our last approach (Scheme 1, path III), retrosynthetic cleavage at the C4/C5a and NH/C2 bonds dissected the target molecule **1** into an aniline and the intermediate **E**.[23]

Ethyl-2-pyrrolidinylacetate **17** was prepared in two steps in 32% overall yield by radical alkylation of pyrrole with ethyl iodoacetate followed by reduction with rhodium on alumina in glacial acetic acid. Alternatively, the compound was obtained in three steps from commercially available 2-pyrrolidinone in an improved 53% yield.[24] The synthesis of 1-carbethoxy-2,3-dioxopyrrolizidine **19** was carried out using Adams' method.[25] Reaction with diethyl oxalate in presence of sodium ethoxide gave compound **19** in 80% yield. Condensation with aniline in toluene produced compound **21** in good yield. Intramolecular cyclization of the enamine performed under various conditions (P_2O_5/Me_3SO_3H, H_2SO_4 and PPA) did not allow us to obtain the desired compound, giving only decomposition products, most probably due to the instability of enamines in an acidic environment. When the reaction was performed thermally in a neutral medium (boiling diphenyl ether) the desired compound **1** was obtained in low yield (24%) after a long reaction time (Scheme 4). To overcome this limitation we shifted to microwave heating. After several attempts (solvent free[26] and in acetonitrile at various temperatures between 130 °C and 180 °C), we found that the desired compound could be obtained in a satisfactory 45% yield heating at 240 °C in DMF for 2h.

The compound was fully characterized by MS and NMR spectroscopy. Disappointingly, when we compared the ^{1}H NMR spectrum of compound **1** dissolved in $CDCl_3$ (20 mg/mL) with the spectrum of the natural compound reported in the literature in the same solvent,[6] we found some discrepancies. Specifically, the reported chemical shift of the aromatic proton on carbon 7 was 7.57 ppm, whereas in our compound the chemical shift of the same proton was 7.79 ppm. Analogously, the signal of the NH proton was 9.74 ppm in the literature paper and 11.15 ppm in our spectrum. In ^{13}C NMR spectrum

of the isolated compound the signals of C6a and C10a were overlapped at 139.8 ppm, whereas the same signals in our spectrum were at 141.1 and 140.2 ppm.

However, we noticed that increasing the dilution of the sample (5mg/mL), the above reported signals shifted upfield and at a concentration of 1mg/mL, the spectra of compound **1** completely matched those reported in the literature (Figure 2). Moreover, the signal of the NH proton, sharp at high concentration (20 mg/mL), became progressively broader during the dilution process, a behaviour indicative of the reduced ability of the NH proton to form hydrogen bonds.

This evidence suggested that compound **1** in concentrated $CDCl_3$ solution gives strong intermolecular hydrogen bond interactions, which progressively weaken with the dilution of the sample. The formation of hydrogen bonds was confirmed by the shift of NH and aromatic hydrogen signals in the NMR spectra of compound **1** (10 mg/mL) run at different temperatures (10 °C, 15 °C, 25 °C). (see SI, figures 1S and 2S).

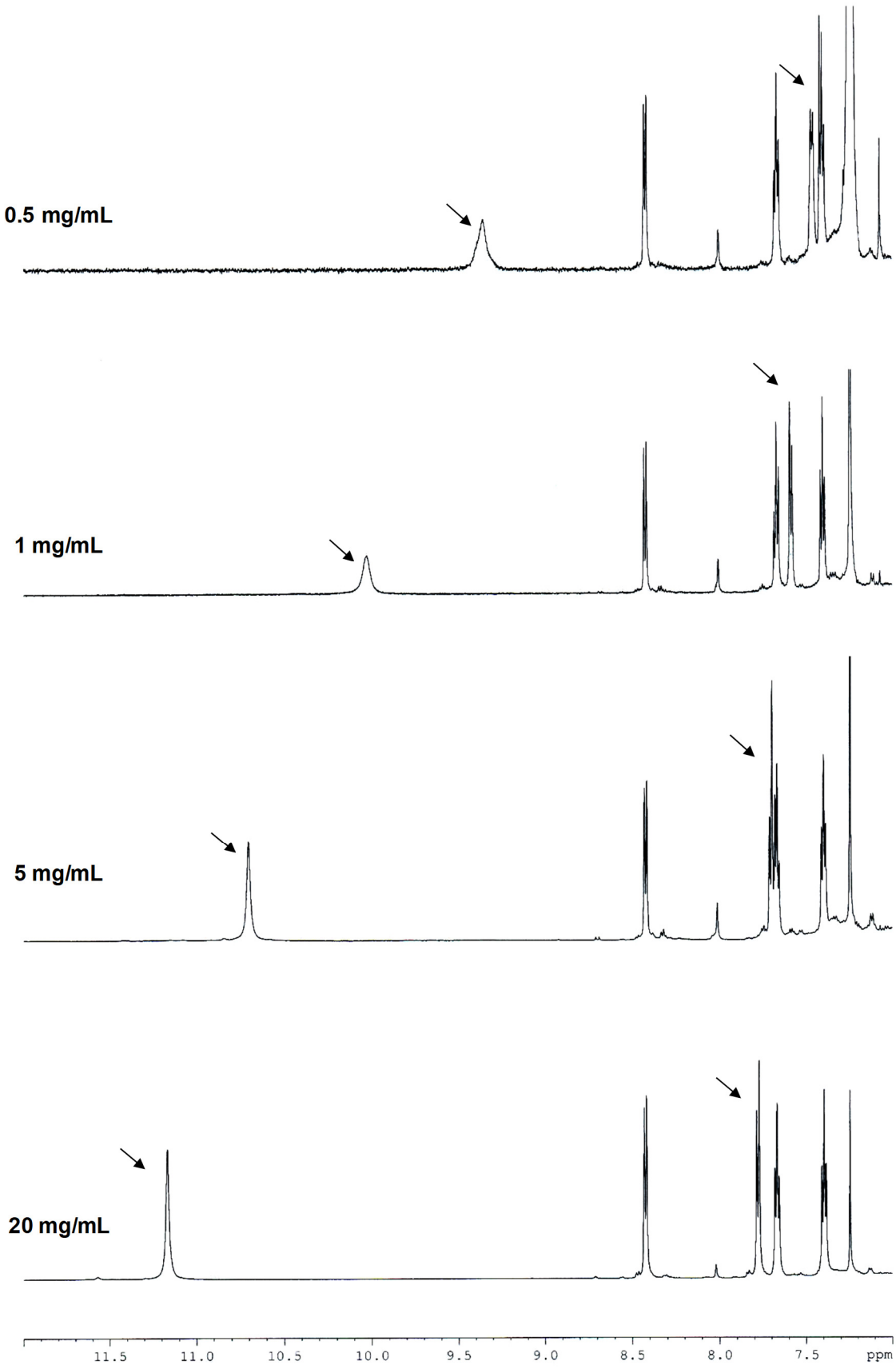
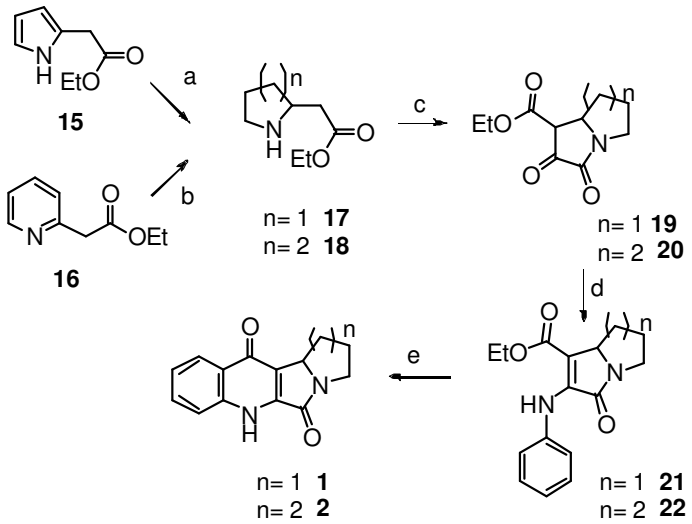

0.5 mg/mL
1 mg/mL
5 mg/mL
20 mg/mL
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
ppm

Figure 2. Effect of the concentration of compound **1** on the chemical shift of aromatic and N-H protons (^{1}H NMR, 600 MHz, $CDCl_3$, expanded region 7-12 ppm)

The developed synthetic protocol was exploited for the synthesis of the six-membered ring compound **2**. Ethyl 2-piperidinylacetate **18** was prepared by hydrogenation of the commercially available 2-pyridylacetate **16** with PtO_2 in ethanol and 6M HCl. Reaction with diethyl oxalate gave the cyclized compound **20**, which was then converted into the corresponding enamine **22** in 54% yield. (Scheme 4). Microwave cyclization afforded compound **2**, whose spectroscopic properties matched with those of the natural compound isolated from chestnut honey.



Scheme 4. Synthesis of compounds **1** and **2.** Reagents and conditions: a) Rh/Al_2O_3 5%, CH_3COOH, H_2, 1 atm, 79% b) PtO_2, EtOH, HCl 6M, H_2 1 atm; 74% c) diethyl oxalate, EtONa, EtOH, reflux, N_2, 80% for **19** and 70% for **20**; d) aniline, toluene, reflux, 70% for **21** and 53% for **22**; e) DMF, 245 °C, MW, 45% for **1** and 60% for **2**.

To summarise, the first total synthesis of two novel tetracyclic KYNA derivatives isolated from chestnut honey was designed and carried out. The crucial step of our strategy was a MW-assisted cyclization of enamines of ethyl 2,3-dioxooctahydroindolizine and dioxohexahydropyrrolizine carboxylates. Further synthetic studies and evaluation of biological activity of the obtained tetracyclic compounds are in progress.

A. Supplementary data Supplementary data associated with this article, including experimental procedures and copies of NMR spectra of the new compounds, can be found, in the online version, at

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