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Synthetic- and DFT modelling studies on regioselective modified Mannich reactions of hydroxy-KYNA derivatives†

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The syntheses of hydroxy-substituted kynurenic acid (KYNA) derivatives have been achieved by an optimised Conrad–Limpach procedure. The derivatives were then reacted with morpholine and paraformaldehyde, as a representative amine and aldehyde, in a modified Mannich reaction. The newly introduced substituents altered the preferred reaction centre of the KYNA skeleton. A systematic investigation of substitutions was carried out, using different reaction conditions, resulting in mono- or disubstituted derivatives. Product selectivity and regioselectivity were rationalised by DFT calculations disclosing HOMO distribution and NBO charges on the potential nucleophilic centres in the anion of the appropriate KYNA ester assumed to be active components towards the iminium ion intermediate.

Introduction

Kynurenic acid (KYNA) or 4-hydroxyquinoline-2-carboxylic acid is an endogenous substance, produced during the metabolism of tryptophan (TRP), *via* a pathway that is also responsible for the production of nicotinamide adenine dinucleotide (NAD^+) and NAD phosphate ($NADP^+$).[1,2] This metabolism is involved in the generation of a variety of other compounds and also leads to the formation of L-kynurenine (L-KYN), which can be further metabolised in two separate ways. One of them constructs KYNA, while the other gives 3-hydroxykynurenine (3-OH-KYN) and quinolinic acid (QUIN), the latter being the precursor of NAD.[3,4]

KYNA is one of the few endogenous excitatory amino acid receptor blockers with a broad spectrum of antagonistic properties in supraphysiological concentrations. It is well established that KYNA has high affinity towards *N*-methyl-D-aspartate (NMDA) receptors. Moreover, it has recently been disclosed that KYNA shows an even higher affinity towards positive modulatory binding site at α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor.[5]

Recently there has been increasing interest in the synthesis and pharmacological studies of KYNA derivatives. This is due to its neuroprotective ability, as it can prevent neuronal loss following excitotoxic, ischemia-induced and infectious neuronal injuries.[6,7] Several papers have been published on different alterations of the KYNA skeleton. Substitutions at positions 5–8 were 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] while the carboxyl group at position 2 was mostly modified into esters[10–12] or amides.[14–16]

Based on the evaluations of previous KYNA amides, a tertiary nitrogen is needed for biological activity towards the central nervous system.[17–20] Derivatives bearing such functional groups can be synthesised by various methods, such as carboxyl amidation[14–16] or by the transformation of the 4-hydroxy group.[13] The substitution of KYNA in the modified Mannich reaction (mMr) also emerges as a straightforward version of functionalisation. In this one-pot reaction KYNA, used as a CH acid, can be reacted with an array of amines and aldehydes giving the corresponding targeted aminoalkylated derivatives with the desired cationic centre.[21]

Our present aim was to carry out a systematic series of investigations with respect to the aminoalkylation of 5-, 6-, 7- and 8-hydroxykynurenic acid ethyl ester derivatives. According to our hypothesis, the newly introduced hydroxy groups may override the effect of the pre-existing 4-hydroxy function, allowing the substitution to take place on ring B. The experimental results of the systematic investigation were planned to be rationalised by comparative DFT modelling studies.

Results and discussion

Synthesis of 5-, 6- and 7-hydroxykynurenic acid derivatives

In order to compare the results of the present study with those of previous mMr investigations, the esters of hydroxykynurenic

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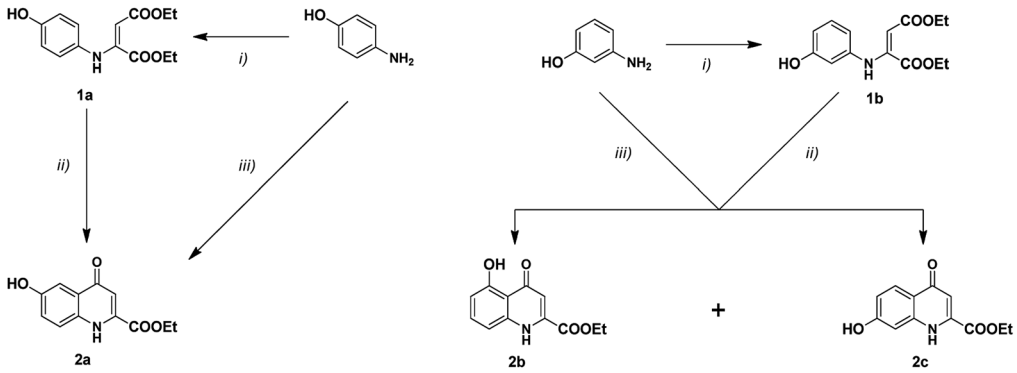


Scheme 1 Synthesis of hydroxykynurenic acid derivatives; (i) DEAD, EtOH, reflux; (ii) DCB, 180 °C; **2a** (55%), **2b** (16%), **2c** (39%); (iii) DEAD, *p*-TsOH, DCB, 190 °C, MW; **2a** (62%), **2b** (20%), **2c** (43%).

acids were selected as substrates for the reactions. Although the syntheses of the methyl esters of hydroxy derivatives are described in the literature,[22,23] the ethyl esters were chosen for several reasons. By carrying out their mMr, they provide a better basis for comparison with previous derivatives. Furthermore, using diethyl acetylenedicarboxylate (DEAD), faster formation of the enamine intermediate was observed. The desired derivatives (**2a–c**) were synthesised with the Conrad–Limpach method applying the optimisations refined in previous investigations (Scheme 1).[21]

As a last step, an additional column chromatographic purification of the esters was also required. The reason is that during the syntheses, 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.[24–26] However, in our case, probably due to the higher reactivity of hydroxyanilines, the syntheses were tilted toward the formation of the maleimides. After purification, the desired hydroxy esters were isolated in moderate yields. It is interesting to note that the synthesis starting from *m*-aminophenol allowed the isolation of the two possible regioisomers with a final ratio 1 : 2 (**2b** : **2c**).

Based on the work of Sutherland *et al.*,[27] *p*-toluenesulfonic acid (*p*-TsOH) as catalyst was investigated in a one-pot version of the Conrad–Limpach procedure applying microwave irradiation (Scheme 1). 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.

Table 1 Screening of solvents in the case of **2a**

Entry	Amine equiv.	Solvent	T (°C)	t	Conv.[a]	**3a** : **3b**[a]
1	1.0	EtOH	80	30′	10%	**3a**
2	1.0	EtOH	80	1 h	50%	**3a**
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 crude NMR spectra.

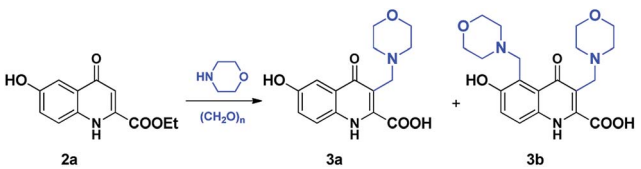
6-Hydroxykynurenic acid ethyl ester

Based on preceding works, 1,4-dioxane seemed to be the optimal media for the substitution reactions. However, after achieving only moderate conversion (Table 1, entry 4) during the mMr of **2a** (Scheme 2), the reaction was conducted in other solvents including acetonitrile (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 1).

We aimed to investigate the selectivity of the synthesis in ethanol, as this solvent enabled the formation of the most homogeneous reaction mixture presumably facilitating to achieve the highest yield. Although the higher temperature accelerated the reactions, conversions were maximised at around 85% (Table 2, entry 3 and 6). While higher temperature and longer reaction promoted the formation of **3b**, its share in the isolated products remained low this showing high selectivity towards C-3 substitution.

To further investigate the reactivity of compound **2a**, 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 **3a** and **3b** (Table 2, entry 12 and 15) further suggesting that the substitution at C-5 is much less preferred relative to that taking place at C-3.

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 **3b**. This indicates a possible basic inhibition of the reaction preventing the formation of the reactive iminium ion. This view is in accord



Scheme 2 mMr of **2a** using morpholine and paraformaldehyde.

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Table 2 Screening of temperature and reagent equivalents in the case of 2a

Entry	Amine equiv.	Solvent	T (°C)	t	Conv.[a]	3a : 3b[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%	3a
14	3.0	EtOH	80	1 h	99%	10 : 1
15[c]	3.0	EtOH	80	2 h	99%	1 : 1

[a] Determined from crude NMR spectra. [b] Work-up performed to isolate **3a**. [c] Work-up performed to isolate **3b**.

Table 4 Screening of temperature and reagent equivalents in the case of 2b

Entry	Amine equiv.	Solvent	T (°C)	t	Conv.[a]	4a : 4b[a]
1	1.0	EtOH	100	30′	40%	**4a**
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%	**4a**
5[b]	1.0	EtOH	150	1 h	95%	**4a**
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 crude NMR spectra. [b] Work-up performed to isolate **4a**. [c] Work-up performed to isolate **4b**.

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 referring to the involvement of ionic species in the crucial regioselective coupling as discussed later (Table 3).

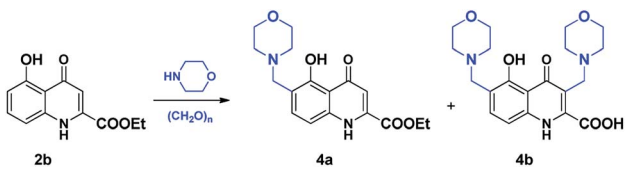
5-Hydroxykynurenic acid ethyl ester

On the basis of the results of the reactions performed with compound **2a**, the first mMr starting from **2b** was carried out in EtOH. As the reaction featured moderate conversion (Table 3, entry 3), the mMr was repeated using the other three solvents, showing lower conversion rates. Considering similarity in selectivity with **2a**, a detailed investigation with EtOH as protic polar solvent was performed. During the reactions, C-6 aminoalkylated derivative **4a** 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 **4a**, the hydrolysis of the ester function did not take place, while in the case of compound **4b** the free acid was isolated (Scheme 3).

Table 3 Screening for solvent in the case of 2b

Entry	Amine equiv.	Solvent	T (°C)	t	Conv.[a]	4a : 4b[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 crude NMR spectra.



Scheme 3 mMr of compound **2b** using morpholine and paraformaldehyde.

Table 5 Screening of temperature and reagent equivalents in the case of 2c

Entry	Amine equiv.	Solvent	T (°C)	t	Conv.[a]	5a : 5b[a]
1	1.0	EtOH	80	30′	80%	**5a**
2	1.0	EtOH	80	1 h	95%	**5a**
3[c]	1.0	EtOH	80	1.5 h	99%	**5a**
4	1.0	EtOH	100	30′	75%	**5a**
5	1.0	EtOH	100	1 h	80%	**5a**
6	1.0	EtOH	100	1.5 h	90%	**5a**
7	1.0	EtOH	150	30′	—[b]	—
10	1.5	EtOH	80	30′	85%	**5a**
11	1.5	EtOH	80	1 h	95%	**5a**
12	1.5	EtOH	80	1.5 h	99%	**5a**
13	2.0	EtOH	80	30′	85%	**5a**
14	2.0	EtOH	80	1 h	90%	**5a**
15	2.0	EtOH	80	1.5 h	99%	18 : 1
16[d]	2.0	EtOH	80	56 h	99%	3 : 10
17	3.0	EtOH	80	15′	99%	**5a**
18	3.0	EtOH	80	1 h	99%	**5a**
19	3.0	EtOH	80	1.5 h	99%	18 : 1

[a] Determined from crude NMR spectra. [b] Work-up performed to isolate **5a**. [c] Multicomponent reaction, conversion could not be determined. [d] Work-up performed to isolate **5b**.

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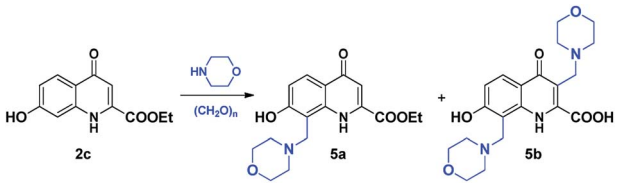


Scheme 4 mMr of compound **2c** using morpholine and paraformaldehyde.

Table 6 Screening for solvent in the case for **2c**

Entry	Amine equiv.	Solvent	T (°C)	t	Conv.[a]	**5a** : **5b**[a]
1	1.0	1,4-Dioxane	80	2 h	45%	1 : 5
2	1.0	Toulene	80	2 h	0%	—
3	1.0	MeCN	80	2 h	70%	2 : 1

[a] determined from crude NMR spectra.

The formation of **4a** can be promoted by using higher temperature and shorter reaction, as a prolonged reaction led to the appearance of **4b**, even using equivalent amounts of reagents. When using 3 equivalents of reagents, full conversion of **2b** was achieved after 30 minutes with a **4a** : **4b** distribution of 4 : 1 (Table 4, entry 13) that could be increased to 1 : 4 after 2 hours reaction time (Table 4, entry 15). Concluding the outcome of the reactions, **2b** features higher reactivity at position C-6 than position C-3. However, similar to **2a**, disubstitution of **2b** can also be promoted by using longer reactions regardless of the polarity of the solvent.

7-Hydroxykynurenic acid ethyl ester

The modified Mannich reactions of **2c** carried out in EtOH at 80 °C proved to be highly facile as indicated by the 80% conversion achieved even in 30 minutes (Table 5, entry 1). In this acidic phenol derivative, the reactivity of C-8 proved to be substantially higher than that of C-3 as indicated by the exclusive formation of **5a** observed in all experiments using one equivalent of morpholine. It is of note that the hydrolysis of the ester function in **5a** did not take place similar to that of **4a** (Scheme 4).

During reactions carried out using higher equivalents of reagents, the formation of **5b** 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 **5b** in sufficient amount (Table 5, entry 16).

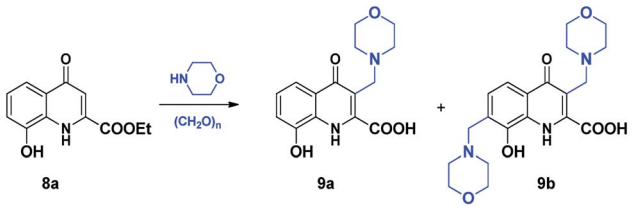
As the formation of **5b** was low even upon using higher temperature or increased amount of reagents, solvents tested previously were also investigated. In the case of 1,4-dioxane (as an aprotic solvent with moderate polarity), a selectivity towards the formation of disubstituted derivative **5b** was observed (Table 6, entry 1), while in MeCN both **5a** and **5b** were formed with selectivity slightly tilted towards **5a** (Table 6, entry 3).

8-Hydroxykynurenic acid ethyl ester

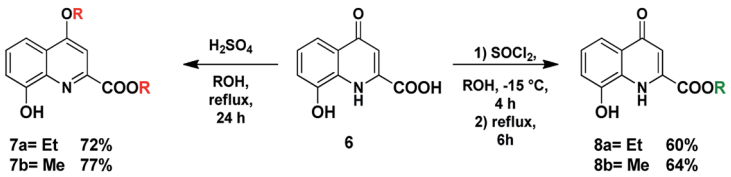
Compound **8** was synthesised by the esterification of 8-hydroxykynurenic acid (xanthurenic acid, **6**) as it was commercially available (Scheme 5). This step was needed to avoid the direct use of poorly soluble xanthurenic acid and to obtain information from the mMr comparable to that resulted from the experiments previously carried out with hydroxylated KYNA derivatives.

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.[28] The use of EtOH as solvent led to the isolation of 4-ethoxy-substituted ethyl ester **7a**. Since methanol was used for the synthesis described in the aforecited literature, the reaction was repeated in this solvent. In a similar manner, under these conditions, methoxy-substituted methyl ester **7b** could be isolated. As suggested also by the literature, **7a** and **7b** should have been formed during the esterification by thionyl chloride in EtOH or MeOH.[29] In our hands, however employing these conditions both esters (**8a**,**b**) could be obtained in good yields, without being contaminated by the corresponding 4-alkoxyquinoline.

Compound **9a** has already been described in the literature, synthesised by employing benzyl protection of the 8-hydroxy function.[30] As there was no further information about the reactivity of the 8-hydroxy derivative, we planned to explore the



Scheme 6 Synthesis of aminoalkylated xanthurenic acid derivatives.



Scheme 5 Esterification of xanthurenic acid (**6**).

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Table 7 Screening of temperature and reagent equivalents in the case of **8a**

Entry	Amine equiv.	Solvent	T (°C)	t	Conv.[a]	**9a** : **9b**[a]
1	1.0	EtOH	80	1 h	—[b]	—[c]
2	1.0	EtOH	100	1 h	—[b]	—[c]
3	1.0	EtOH	150	1 h	—[b]	—[c]
4	1.5	EtOH	80	1 h	—[b]	**9b**[d]
5	2.0	EtOH	80	1 h	—[b]	**9b**[d]
6	3.0	EtOH	80	1 h	—[b]	**9b**[d]

[a] Determined from crude NMR spectra. [b] Conversion could not be determined. [c] No traces of **9a** or **9b** was detected. [d] Minimal amounts, could not be isolated.

effect of the unprotected function on the course of the reaction (Scheme 6).

Based on our experiences on the synthesis of the previously discussed hydroxy derivatives, aminoalkylations were first carried out in EtOH. All attempts at different temperatures yielded complex mixtures, but formation of **9a** could not be detected. The reactions, conducted in the presence of increased equivalents of reagents, also provided complex mixtures, only traces of **9b** could be detected in the crude product by ^{1}H-NMR (Table 7).

After these unsuccessful experiments carried out in EtOH, solvents tested previously were again investigated. In toluene and MeCN the sole formation of **9b** was observed, while in 1,4-dioxane the reaction yielded the C-3 substituted derivative (**9a**) as single product (Table 8).

DFT calculations

In order to rationalise the marked regioselectivity patterns observed in the modified Mannich reactions of the systematic selection of kynurenic acid esters, we undertook a series of comparative DFT calculations carried out with B3LYP functional[31–33] using 6–31 + G(d,p) basis set.[34] The computations were supported by IEFPCM solvent model[35] with dielectric constant $\varepsilon = 24.5$ to represent the polarity of ethanol employed as solvent in most experiments. However, the following characteristic features of the studied transformations seemed to be worth for primary consideration to create reliable models that will be analysed by theoretical methods: (i) no additional acidic component was added to the reaction mixtures; (ii) depending on the substitution pattern of the substrate, the Mannich-type coupling reactions might be accompanied by facile ester hydrolysis. Addressing the first point it can be assumed that the iminium ion and the amidate or phenolate anion derived from the condensation of morpholine, formaldehyde and the appropriate substrate of acidic character, are the actual coupling components involved in the Mannich reactions. It seems to be reasonable to assume that the formation of the major product can be interpreted by the coupling of iminium ion **10** to the most nucleophilic carbon centre of kynurenic anion derived from the deprotonation of the most acidic site of the neutral substrate. The possible sequences of the competitive Mannich condensations accompanied by the hydrolysis of the ester residue are exemplified by the conversions of 6-hydroxykynurenic acid ethyl ester **2a** (Fig. 1). Since **2a/I**, featuring the most pronounced nucleophilicity on C3, is more stable by 0.42 kcal mol^{-1} than **2a/II** with preferred nucleophilic site at position 5, the dominant coupling of iminium ion **10** must lead to intermediate **11** capable of undergoing either equilibrium isomerisation to quinolone ester **12** or transannular ethanol elimination to generate quinoidal ketene intermediate **14**. (The details of modelling studies disclosing relative energetics and local reactivity are discussed later.) Thus, carboxylic acid **3a**, identified as major product, can be formed by the addition of water to **14** or by the neighbouring amine-assisted ester hydrolysis proceeding *via* spirocyclic zwitterionic intermediate **13**. The regioselective iminium-mediated coupling of the alternative anion **2a/II** is supposed to construct ester **15**, which undergoes tautomerisation followed by transannular ethanol elimination (**15** ↔ **16** ↔ **17**). Subsequent hydration of the ketene intermediate might generate carboxylic acid **18** prone to undergo a further Mannich-type substitution affording minor product **3b**. On the other hand, this polyfunctionalised kynurenic acid can also be derived from a subsequent coupling of **3a**.

The mechanistic picture, accounting for the experimentally observed regioselectivity and ester hydrolysis, represented by the reaction pathways applied for the modified Mannich reactions of **2a**, can be extended to analogous multistep transformations of the 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 delocalisation and the local NBO charges[36] were also disclosed for the corresponding anion pairs type **2/I-2/II** as outlined on Fig. 2. Since the crucial coupling between iminium ion **10** 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 optimised 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 **4a** and **5a** do not allow the ester

Table 8 Screening for solvent in the case for **8a**

Entry	Amine equiv.	Solvent	T (°C)	t	Conv.[a]	**9a** : **9b**[a]
1	1.0	1,4-Dioxane	80	2 h	80%	**9a**
2	1.0	1,4-Dioxane	100	2 h	90%	**9a**
3[b]	1.0	1,4-Dioxane	150	2 h	99%	**9a**
4	2.0	1,4-Dioxane	150	2 h	99%	**9a**
5[c]	2.0	Toluene	80	1 h	99%	**9b**
6	2.0	MeCN	80	2 h	99%	**9b**

[a] Determined from crude NMR spectra. [b] Work-up performed to isolate **9a**. [c] Work-up performed to isolate **9b**.

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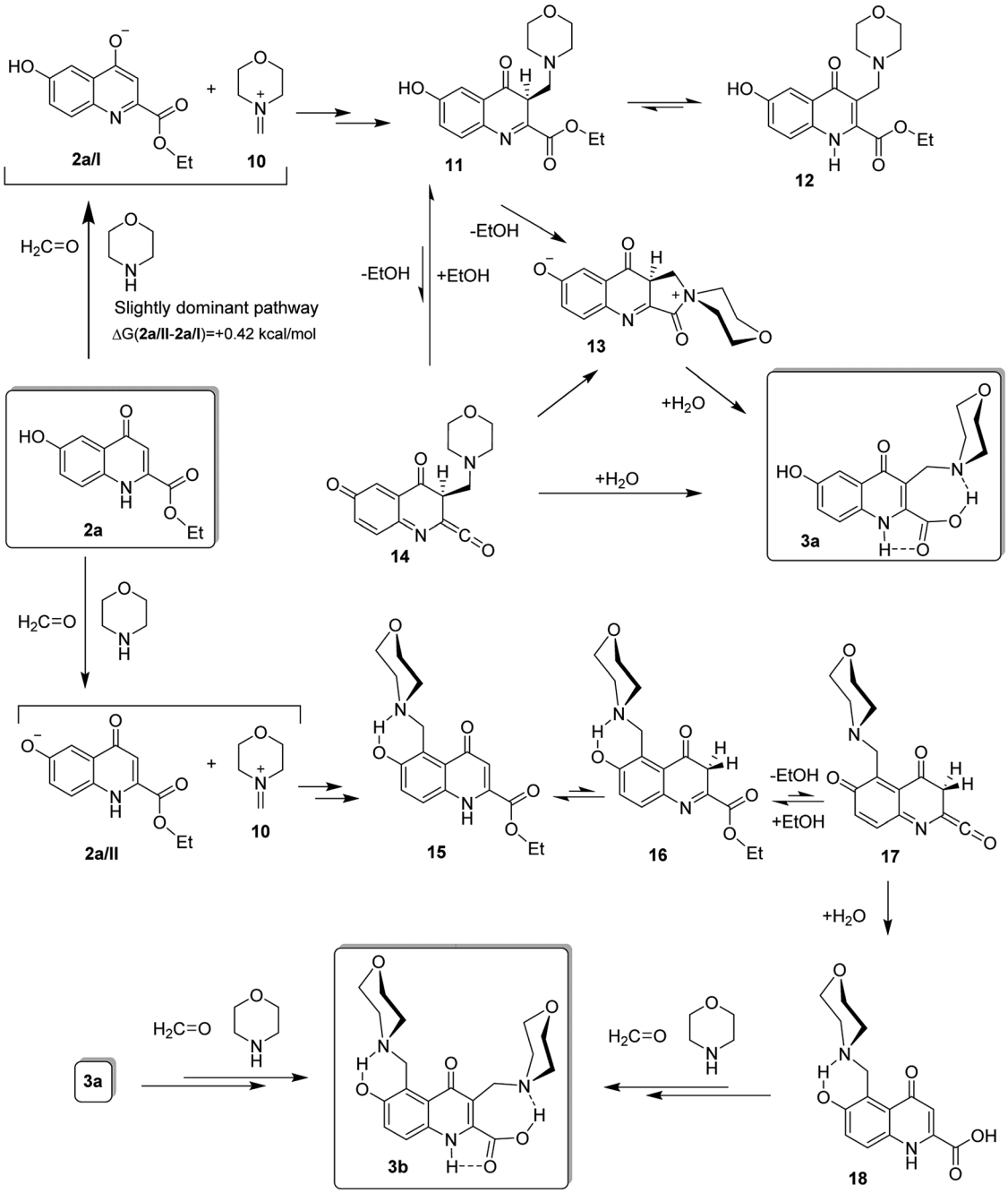

Fig. 1 The possible sequences of the competitive Mannich condensations.

hydrolysis taking place through the corresponding quinoidal ketene intermediates. However, regardless of the position of the OH group, the neighbouring group assistance from the 3-morpholinomethyl substituent obviously promotes this hydrolysis as discussed above.

Since it is reasonable to assume that besides the electronic properties of the active intermediates the overall tendency of isomers **2a–c** to undergo Mannich-type reactions must also be strongly influenced by the concentration of the actual coupling partners, the changes in the free energy accompanying the generation of the appropriate ion pairs (types **2/I-10** and **2/II-10**) were calculated by the DFT method used for the structural analysis of the anions (Fig. 2). In this regard, conversion **2c** → **5a** proceeding *via* **2c/II** seems to be partly facilitated by the enhanced OH-acidity of the kynurenic precursor as reflected from the ΔG value calculated for ion-pair generation **2c** + morpholine + CH_2O → **2c/II+10** + H_2O which is substantially smaller than those calculated for the other related processes studied in this work. It is of note that the relatively low HOMO energy level disclosed for anion **2c/II** might suggest its decreased tendency to participate in orbital controlled interactions however, the significant electron density in position 8

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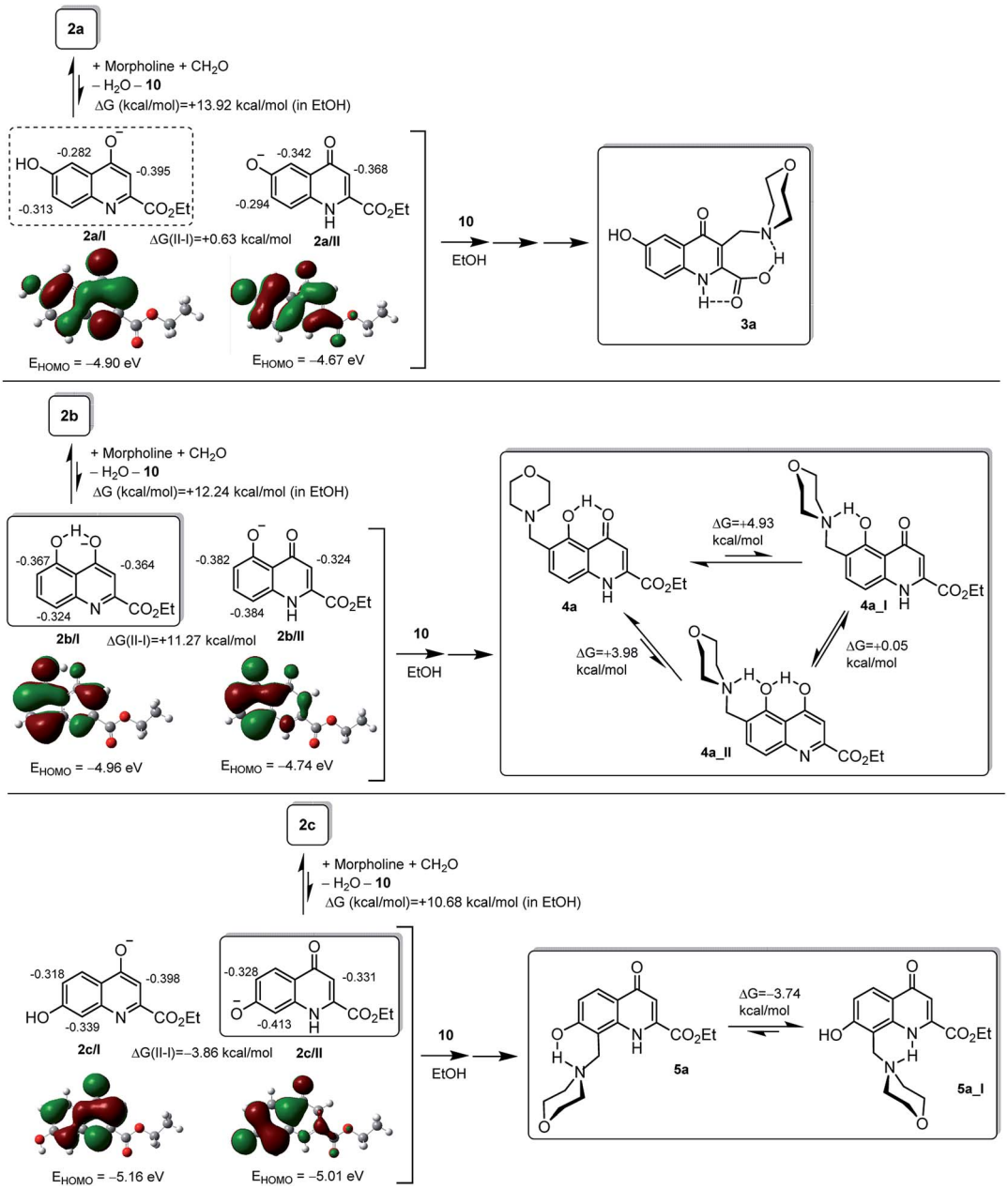

Fig. 2 Rationalisation of the feasibility and regioselectivity observed in the Mannich reactions of **2a–c** in terms of the relative thermodynamics, HOMO-energy, HOMO-delocalisation and the local NBO charges of the possible alternative anions generated by morpholine and formaldehyde along with the relative thermodynamics of the chelate-stabilized rotamer products.

($\rho_{NBO} = -0.413$) refers to an increased coulombic contribution in promoting the coupling with iminium ion **10**.

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 (**4a**/**4a_I**/**4a_II** and **5a**/**5a_I**: Fig. 2) that identified **4a** and **5a**, respectively, as the preferred isomers in ethanolic solution.

The sluggish reactivity of **8a** experienced in EtOH can be associated with the HOMO energy level of **8a**/**III** found to be the lowest one in the series of the modelled nucleophilic anions and with the electron density in position 3 ($\rho_{NBO} = -0.382$) decreased relative to that identified in position 8 for anion **2c**/**II**, the model with somewhat lowered HOMO energy level that presented an increased reactivity in EtOH (Fig. 3).

Finally, we assume that the spectacular solvent–dependence observed in the transformations of **8a** can be rationalized by the polarity-controlled feasibility of the ion pairs **8a**/**III-10** and **9a**/**III-10**, the active coupling components of the first and second Mannich-like reactions, respectively. Accordingly, by means of a series of further B3LYP/6-31+G(d,p) calculations supported by IEFPCM method with the dielectric constants of the solvents used in the experiments, we assessed the changes in free energy associated with the primary ion-pair generating equilibrium

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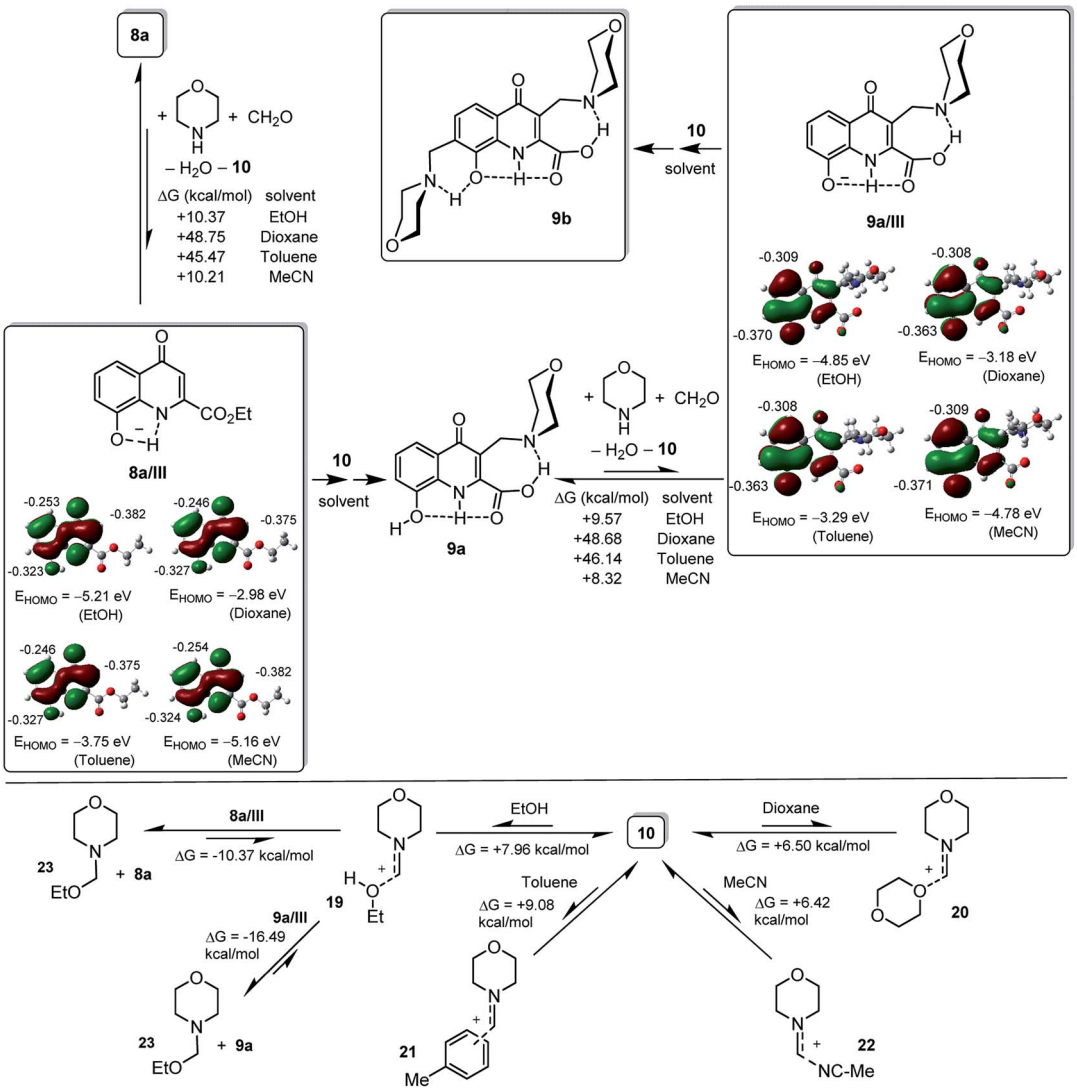

Fig. 3 Rationalisation of the marked solvent effect observed for the regioselective sequential Mannich reactions of **8a** in terms of: (i) the solvent-dependent changes in free energy accompanying the conversions leading active ion pairs **8a/III-10** and **9a/III-10**, respectively; (ii) HOMO-energy of the anions; (iii) interactions of the iminium ion **10** with the solvent molecules.

condensation steps that involve **8a** and **9a**, respectively (Fig. 3). In the course of the modelling studies both the geometry optimisation and the subsequent frequency calculation were performed using IEFPCM solvent model. In accord with the general expectations the results (Fig. 3) indicate that in the relatively polar ethanol and acetonitrile the formation of reactive ion pairs **8a/III-10** and **9a/III-10** is substantially more favourable 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 **9a** and with the ready formation of **9b** *via* **9a** 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 **10** with the solvent molecules into account (Fig. 3). Such interactions simply modelled by the involvement of one solvent molecule are presumably responsible for at least a partial deactivation of the electrophilic component, as reflected from the comparison of the ΔG values calculated for the formation of bimolecular complexes. Thus, among the solvents tested toluene seems to be the one with the most decreased capability to deactivate iminium ion **10** allowing a relatively facile Mannich-like couplings of the ion pairs. It is also noteworthy that in both anions the HOMO delocalisation 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. Highlighting the significance of HOMO level influencing anion reactivity and consequent chemoselectivity, the HOMO energy calculated in dioxane for **8a_III** is markedly higher

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compared to that disclosed for **9a_III** (−2.98 eV and −3.18 eV, respectively: Fig. 3). In accord with the outcome of the reaction conducted in dioxane selectively affording **9a**, this finding refers to a decreased nucleophilicity of **9a_III** relative to that of **8a_III**, while in toluene the reversed order of HOMO levels was disclosed for these anions (−3.75 eV and −3.29 eV for **8a_III** and **9a_III**, respectively) plausibly reasoning the facile formation of **9b** when the reaction was carried out in this solvent. In acetonitrile the relatively high concentration of ion pairs **8a/III-10** and **9a/III-10** might be responsible for the facilitation of the sequential coupling steps finally constructing **9b**, as the HOMO levels of anions calculated in this polar solvent are lower (−5.16 eV and −4.78 eV for **8a_III** and **9a_III**, respectively) referring to their decreased donor character. Finally, the highly decreased reactivity of the ion pairs in ethanol can also be attributed to the deactivation of anions **8a/III** and **9a/III** by hydrogen bonds with the solvent molecules and to the favoured formation of **23** (Fig. 3).

Conclusions

Through an optimised Conrad–Limpach procedure, the syntheses of hydroxy-substituted kynurenic acid derivatives were achieved. In order to investigate the substitution affecting effect of the new hydroxy groups in modified Mannich reaction, a systematic investigation was carried out on the synthesised derivatives. Hydroxykynurenic acids were reacted with morpholine and paraformaldehyde – as representative amine and aldehyde – under different reaction conditions. These reactions resulted in varied substitution positions.

Employing 5-, 6- and 7-hydroxy derivatives as substrates, higher conversions and, consequently, higher yields could be achieved in EtOH. It is hypothesised that this protic, polar solvent can contribute to higher intermediate stability by solvating the iminium ion formed during the reaction. Higher temperatures allowed higher conversions, while giving appearance to disubstituted derivatives. Decreasing the solvent polarity resulted in increased amounts of disubstituted derivatives. This may be attributed to a decreased stability and enhanced reactivity of the iminium ion capable of aminoalkylating the less reactive positions as well. In the case of 8-hydroxykynurenic acid, comparative DFT calculations suggested that the overall low reactivity might be due to the low energy level of HOMO in the chelate-stabilised anion.

By means of DFT calculations it was also disclosed that the regioselectivity of the modified Mannich reactions of hydroxykynurenic acids can reasonably be rationalised on the basis of the relative acidity of the potential nucleophilic sites as well as the HOMO delocalisation and the local NBO charges in the resulting anions. The spectacular solvent-dependent reactivity of 8-hydroxykynureic acid ester was also rationalised by a series of comparative DFT calculations revealing the relative thermodynamics of a polarity-controlled formation of the ion pair involved in the crucial C_C coupling, the iminium-deactivation by the interaction with the solvent used and the polarity-dependent HOMO energy level of the anion component.

Experimental

General

The ^{1}H and ^{13}C-NMR spectra were recorded in DMSO d6, $CDCl_3$ and D_2O 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 internal standard (^{1}H, ^{13}C).

The HRMS flow injection analysis was performed with Thermo Scientific Q Exactive Plus hybrid quadrupole-Orbitrap (Thermo Fisher Scientific, Waltham, MA, USA) mass spectrometer coupled to a Waters Acquity I-Class UPLC™ (Waters, Manchester, UK).

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

All calculations were carried out by using the Gaussian 09 software (Gaussian Incorporation, Pittsburgh, U.S.) package.[37] The optimised structures are available from the authors.

General procedure for the synthesis of aminoalkylated 6-hydroxykynurenic acid derivatives (3a,b)

Ethyl 6-hydroxy-4-oxo-1,4-dihydroquinoline-2-carboxylate (**2a**, 117 mg, 0.5 mmol), morpholine (131 mg, 1.5 mmol) and paraformaldehyde (70 mg, 2.3 mmol) were placed in a pressure-resistant 10 mL vessel with EtOH (5 mL). The mixture was kept at 80 °C for the corresponding reaction time in a microwave reactor (300 W). Following the removal of the solvent, the residue was crystallised as detailed below.

6-Hydroxy-3-(morpholinomethyl)-4-oxo-1,4-dihydroquinoline-2-carboxylic acid (3a). Reaction time in microwave reactor: 30 min. Crystallisation from DCM (5 mL). Yield: 123 mg (81%); M.p. 220–225 °C. ^{1}H NMR (DMSO-d_6); 2.99–3.15 (2H, m); 3.16–3.27 (2H, m); 3.53–3.74 (2H, m); 3.81–4.04 (2H, m); 4.38 (2H, s); 7.17 (1H, d, J = Hz); 7.41 (1H, s); 7.87 (1H, d, J = Hz); 9.74 (1H, brs); 11.70 (1H, brs); 12.87 (1H, brs); ^{13}C NMR (DMSO-d6); 49.8; 50.8; 64.3; 106.4; 107.8; 121.6; 123.0; 126.0; 132.9; 134.4; 147.0; 154.7; 165.0; 176.8; HRMS calcd for [M + H$^+$] m/z = 305.1132, found m/z = 305.1138.

6-Hydroxy-3,5-bis(morpholinomethyl)-4-oxo-1,4-dihydroquinoline-2-carboxylic acid (3b). Reaction time in microwave reactor: 2 h. Crystallisation from DCM (5 mL) – removal of **3a**; evaporation of DCM from the mother liquor, crystallisation from EtOAc (5 mL). Yield: 59 mg (39%); M.p. >325 °C. ^{1}H NMR (DMSO-d_6); 2.50–2.58 (4H, m); 3.09–3.19 (4H, m); 3.59–3.66 (4H, m); 3.68–3.87 (4H, m); 4.34 (2H, s); 4.77 (2H, m); 7.10 (1H, d, J = 8.4 Hz); 7.85 (1H, d, J = 8.9 Hz); ^{13}C NMR (DMSO-d6); 49.9; 50.9; 52.9; 64.3; 66.4; 108.0; 120.8; 123.0; 123.8; 134.4; 145.9; 155.6; 164.8; 179.3; HRMS calcd for [M + H$^+$] m/z = 404.1816, found m/z = 404.1810.

Ethyl 5-hydroxy-3-(morpholinomethyl)-4-oxo-1,4-dihydroquinoline-2-carboxylate (4a)

Ethyl 5-hydroxy-4-oxo-1,4-dihydroquinoline-2-carboxylate (**2b**, 117 mg, 0.5 mmol), morpholine (48 mg, 0.5 mmol) and

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paraformaldehyde (45 mg, 1.5 mmol) were placed in a pressure-resistant vessel of 10 mL with 5 mL EtOH. The mixture was kept at 150 °C for 1 h in a microwave reactor. Following the removal of the solvent, the residue was crystallised from EtOAc (5 mL). Yield: 132 mg (80%); M.p. 160–164 °C. ^{1}H NMR (DMSO-d$_6$); 1.37 (3H, t, J = 7.4 Hz); 2.37–2.47 (4H, m); 3.48–3.61 (6H, m); 4.44 (2H, q, J = 7.1 Hz); 6.65 (1H, s); 7.35 (1H, d, J = 8.7 Hz); 7.62 (1H, d, J = 8.6 Hz); 12.46 (1H, brs); 14.45 (1H, brs); ^{13}C NMR (DMSO-d6); 14.3; 53.5; 54.9; 108.1; 108.9; 114.0; 136.6; 139.5; 161.9; 183.3; HRMS calcd for [M + H$^+$] m/z = 333.1445, found m/z = 333.1444.

5-Hydroxy-3,6-bis(morpholinomethyl)-4-oxo-1,4-dihydroquinoline-2-carboxylic acid (4b)

Ethyl 5-hydroxy-4-oxo-1,4-dihydroquinoline-2-carboxylate (**2b**, 117 mg, 0.5 mmol), morpholine (131 mg, 1.5 mmol) and paraformaldehyde (70 mg, 2.3 mmol) were placed in a pressure-resistant vessel of 10 mL with 5 mL EtOH. The mixture was kept at 80 °C for 2 h in a microwave reactor. Following the removal of the solvent, the residue was crystallised from EtOAc (5 mL) and was recrystallised from EtOAc (10 mL). Yield: 137 mg (68%); M.p. 218–220 °C. ^{1}H NMR (DMSO-d$_6$); 2.40 (4H, s); 3.18 (2H, m); 3.50 (2H, s); 3.56 (4H, m); 3.59–4.05 (4H, m); 4.37 (2H, s); 7.37 (1H, d, J = 8.7 Hz); 7.55 (1H, d, J = 8.5 Hz); 12.16 (1H, brs); 12.48 (1H, brs); 14.62 (1H, brs); ^{13}C NMR (DMSO-d6); 49.7; 50.1; 53.6; 55.1; 64.3; 65.8; 66.7; 107.9; 108.0; 108.1; 112.1; 122.5; 136.1; 143.8; 149.8; 163.9; 182.2; HRMS calcd for [M + H$^+$] m/z = 404.1816, found m/z = 404.1817.

Ethyl 7-hydroxy-8-(morpholinomethyl)-4-oxo-1,4-dihydroquinoline-2-carboxylate (5a)

Ethyl 7-hydroxy-4-oxo-1,4-dihydroquinoline-2-carboxylate (**2c**, 117 mg, 0.5 mmol), morpholine (48 mg, 0.5 mmol) and paraformaldehyde (45 mg 1.5 mmol) were placed in a pressure-resistant vessel of 10 mL with 5 mL EtOH. The mixture was kept at 80 °C for 1.5 h in a microwave reactor. Following the removal of the solvent, the residue was crystallised from EtOH (5 mL). Yield: 144 mg (87%); M.p. 228–231 °C. ^{1}H NMR (DMSO-d$_6$); 1.37 (3H, t, J = 7.2 Hz); 2.45–2.50 (4H, m); 3.65–3.75 (4H, m); 3.94 (2H, s); 4.45 (2H, q, J = 7.1 Hz); 6.55 (1H, s); 6.95 (1H, d, J = 8.5 Hz); 7.88 (1H, d, J = 8.8 Hz); 10.55 (1H, brs); 13.13 (1H, brs); ^{13}C NMR (DMSO-d6); 14.9; 53.6; 54.0; 63.5; 66.8; 107.8; 110.5; 115.1; 120.7; 126.2; 137.7; 142.4; 159.8; 163.3; 178.1; HRMS calcd for [M + H$^+$] m/z = 333.1445, found m/z = 333.1445.

7-Hydroxy-3,8-bis(morpholinomethyl)-4-oxo-1,4-dihydroquinoline-2-carboxylic acid (5b)

Ethyl 7-hydroxy-4-oxo-1,4-dihydroquinoline-2-carboxylate (**2c**, 233 mg, 1.0 mmol), morpholine (174 mg, 2.0 mmol) and paraformaldehyde (120 mg, 4.0 mmol) were placed in a 50 mL round-bottom flask with 20 mL EtOH. The mixture was let stir for 56 h at reflux temperature using conventional heating. Following the evaporation of the solvent, **5a** was removed by crystallizing the residue from EtOH (10 mL). The solvent from the mother liquor was evaporated and the residue was crystallised from EtOAc (10 mL) yielding **5b**. Yield: 242 mg (60%); M.p. 239–241 °C. ^{1}H NMR (DMSO-d$_6$); 2.40–2.48 (4H, m); 2.97–3.11 (2H, m); 3.15–3.25 (2H, m); 3.55–3.63 (2H, m); 3.71 (4H, t, J = 4.5 Hz); 3.87 (2H, s); 3.90–4.03 (2H, m); 4.39 (2H, s); 6.91 (1H, d, J = 8.8 Hz); 7.88 (1H, d, J = 8.8 Hz); 10.39 (1H, brs); 12.89 (1H, brs); 13.13 (1H, brs); ^{13}C NMR (DMSO-d6); 50.0; 50.3; 53.4; 53.4; 64.3; 66.1; 107.7; 108.0; 114.4; 118.4; 126.2; 140.6; 146.4; 159.2; 164.8; 177.3; HRMS calcd for [M + H$^+$] m/z = 404.1816, found m/z = 404.1812.

General procedure for the synthesis of methyl and ethyl esters of 4-alkoxy substituted xanthurenic acid derivatives (7a,b)

Xanthurenic acid (6, 1 g, 4.9 mmol) was put in a 100 mL round bottom flask with 40 mL MeOH or EtOH and 1 mL cm^3 H_2SO_4. The reaction mixture was treated at reflux temperature for 24 h. The H_2SO_4 was neutralised using $NaHCO_3$ (using litmus paper as indicator). After the evaporation of the solvent, the mixture was dissolved in H_2O (30 mL) and was extracted with DCM (3 × 25 mL). The collected organic phases were dried (Na_2SO_4), the solvent was evaporated and the residue was crystallised from n-hexane : EtOAc (1 : 9) mixture (15 mL).

Ethyl 4-ethoxy-8-hydroxyquinoline-2-carboxylate (7a). Solvent: EtOH. Yield: 685 mg (60%); M.p. 144–147 °C. ^{1}H NMR (DMSO-d$_6$); 1.11 (3H, t, J = 7.1 Hz); 1.38 (3H, t, J = 7.2 Hz); 3.75 (2H, q, J = 7.1 Hz); 4.43 (2H, q, J = 7.1 Hz); 6.81 (1H, s); 7.20 (1H, d, J = 7.7 Hz); 7.30 (1H, t, J = 8.0 Hz); 7.57 (1H, d, J = 8.3 Hz); ^{13}C NMR (DMSO-d6); 14.8; 63.7; 109.1; 114.9; 116.4; 126.2; 126.7; 131.8; 139.8; 140.7; 162.9; 175.5.

Methyl 8-hydroxy-4-methoxyquinoline-2-carboxylate (7b). Solvent: MeOH. Yield: 687 mg (64%); M.p. 275–277 °C. ^{1}H NMR (DMSO-d$_6$); 3.96 (3H, s); 4.11 (1H, s); 7.17 (1H, d, J = 7.7 Hz); 7.50 (1H, t, J = 8.2 Hz); 7.53 (1H, s); 7.59 (1H, d, J = 8.4 Hz); 9.73 (1H, brs); ^{13}C NMR (DMSO-d6); 53.1; 56.9; 101.0; 111.8; 113.3; 123.0; 129.2; 139.3; 147.5; 148.2; 154.5; 163.2.

8-Hydroxy-3-(morpholinomethyl)-4-oxo-1,4-dihydroquinoline-2-carboxylic acid (9a)

Ethyl 8-hydroxy-4-oxo-1,4-dihydroquinoline-2-carboxylate (**8a**, 117 mg, 0.5 mmol), morpholine (48 mg, 0.5 mmol) and paraformaldehyde (45 mg, 1.5 mmol) were placed in a pressure-resistant vessel of 10 mL with 5 mL 1,4-dioxane. The mixture was kept at 150 °C for 2 h in a microwave reactor. Following the removal of the solvent, the residue was crystallised from EtOAc (5 mL). Yield: 53 mg (35%); M.p. 290–295 °C (decomposition). ^{1}H NMR (DMSO-d$_6$); 3.03–3.23 (2H, m); 3.25–3.43 (2H, m); 3.54–3.74 (2H, m); 3.85–4.05 (2H, m); 4.51 (2H, s); 7.12 (1H, d, J = 7.5 Hz); 7.19 (1H, t, J = 8.0 Hz); 7.55 (1H, d, J = 8.2 Hz); 10.27 (1H, brs); 12.13 (1H, brs); ^{13}C NMR (DMSO-d6); 50.3; 50.6; 64.1; 108.9; 115.6; 115.7; 124.6; 125.5; 128.9; 145.0; 147.0; 163.9; 178.0; HRMS calcd for [M + H$^+$] m/z = 305.1132, found m/z = 305.1139.

8-Hydroxy-3,7-bis(morpholinomethyl)-4-oxo-1,4-dihydroquinoline-2-carboxylic acid (9b)

Ethyl 8-hydroxy-4-oxo-1,4-dihydroquinoline-2-carboxylate (**8a**, 117 mg, 0.5 mmol), morpholine (87 mg, 1.0 mmol) and paraformaldehyde (60 mg, 2.0 mmol) were placed in a pressure-

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resistant vessel of 10 mL with 5 mL toluene. The mixture was kept at 80 °C for 1 h in a microwave reactor. Following the removal of the solvent, the residue was crystallised from toluene (5 mL). Yield: 168 mg (83%); M.p. 218–224 °C. ^{1}H NMR (DMSO-d_6); 2.53–2.58 (4H, m); 3.03–3.20 (2H, m); 3.20–3.37 (2H, m); 3.64–3.69 (4H, m); 3.65–3.75 (2H, m) 3.89 (2H, s); 3.91–4.01 (2H, m); 4.50 (2H, s); 7.10 (1H, d, $J = 8.5$ Hz); 7.52 (1H, d, $J = 8.5$ Hz); 10.25 (1H, brs); 12.08 (1H, brs); ^{13}C NMR (DMSO-d6); 24.0; 50.3; 50.5; 52.9; 60.0; 64.1; 66.4; 108.9; 115.3; 115.4; 124.4; 124.9; 145.0; 146.5; 159.6; 163.9; 177.9; HRMS calcd for $[M + H^+]$ $m/z = 404.1816$, found $m/z = 404.1817$.

Conflicts of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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Notes and references

1 É. Rózsa, H. Robotka, L. Vécsei and J. Toldi, *J. Neural Transm.*, 2008, **115**, 1087–1091.
2 T. W. Stone, *Expert Opin. Invest. Drugs*, 2001, **10**, 633–645.
3 H. Németh, J. Toldi and L. Vécsei, *Curr. Neurovasc. Res.*, 2005, **2**, 249–260.
4 H. Németh, J. Toldi and L. Vécsei, *J. Neural Transm., Suppl.*, 2006, **70**, 285–304.
5 K. Sas, H. Robotka, J. Toldi and L. Vécsei, *J. Neurol. Sci.*, 2007, **257**, 221–239.
6 G. Gigler, G. Szénási, A. Simó, G. Lévay, L. G. Hársing Jr, K. Sas, L. Vécsei and J. Toldi, *Eur. J. Pharmacol.*, 2007, **564**, 116–122.
7 E. Luchowska, P. Luchowski, A. Sarnowska, M. Wielosz, W. A. Turski and E. M. Urbańska, *Pol. J. Pharmacol.*, 2003, **55**, 443–447.
8 B. L. Harrison, B. M. Baron, D. M. Cousino and I. A. McDonald, *J. Med. Chem.*, 1990, **12**, 3130–3132.
9 D. Edmont, R. Rocher, C. Plisson and J. Chenault, *Bioorg. Med. Chem. Lett.*, 2000, **16**, 1831–1834.
10 F. P. Bonina, L. Arenare, R. Ippolito, G. Boatto, G. Battaglia, V. Bruno and P. de Caprariis, *Int. J. Pharm.*, 2000, **202**, 79–88.
11 S. Manfredini, B. Pavan, S. Vertuani, M. Scaglianti, D. Compagnone, C. Biondi, A. Scatturin, S. Tanganelli, L. Ferraro, P. Prasad and A. Dalpiaz, *J. Med. Chem.*, 2002, **45**, 559–562.
12 S. Manfredini, S. Vertuani, B. Pavan, F. Vitali, M. Scaglianti, F. Bortolotti, C. Biondi, A. Scatturin, P. Prasad and A. Dalpiaz, *Bioorg. Med. Chem.*, 2004, **12**, 5453–5463.
13 A. C. Nichols and K. L. Yielding, *Mol. Chem. Neuropathol.*, 1998, **35**, 1–12.
14 A. Brik, Y. C. Lin, J. Elder and C. H. Wong, *Chem. Biol.*, 2002, **9**, 891–896.
15 E. Knyihár-Csillik, A. Mihály, B. Krisztin-Péva, H. Robotka, I. Szatmári, F. Fülöp, J. Toldi, B. Csillik and L. Vécsei, *Neurosci. Res.*, 2008, **61**, 429–432.
16 F. Fülöp, I. Szatmári, E. Vámos, D. Zádori, J. Toldi and L. Vécsei, *Curr. Med. Chem.*, 2009, **16**, 4828–4842.
17 D. Zádori, G. Nyíri, A. Szőnyi, I. Szatmári, F. Fülöp, J. Toldi, T. F. Freund, L. Vécsei and P. Klivényi, *J. Neural Transm.*, 2011, **118**, 865–875.
18 D. Zádori, G. Veres, L. Szalárdy, P. Klivényi, J. Toldi and L. Vécsei, *J. Alzheimer's Dis.*, 2014, **42**, S177–S187.
19 R. Greco, C. Demartini, A. M. Zanaboni, E. Redavide, S. Pampalone, J. Toldi, F. Fülöp, F. Blandini, G. Nappi, G. Sandrini, L. Vécsei and C. Tassorelli, *Cephalalgia*, 2017, **37**, 1272–1284.
20 A. Fejes-Szabó, Z. Bohár, E. Vámos, G. Nagy-Grócz, L. Tar, G. Veres, D. Zádori, M. Szentirmai, J. Tajti, I. Szatmári, F. Fülöp, J. Toldi, A. Paardutz and L. Vecsei, *J. Neural. Transm.*, 2014, **121**, 725–738.
21 B. Lőrinczi, A. Csámpai, F. Fülöp and I. Szatmári, *Molecules*, 2020, **25**, 937.
22 M. C. Hall, G. H. Johnson and B. J. Wright, *J. Med. Chem.*, 1974, **17**, 685–690.
23 J. A. Bryant, B. O. Buckman, I. Islam, R. Mohan, M. M. Morrissey, G. P. Wei, X. Wei and S. Yuan, *US Pat.*, 16374202A, 2002.
24 N. D. Heindel, *J. Org. Chem.*, 1970, **35**, 3138–3140.
25 K. Kizaki, H. Imoto, T. Kato and K. Naka, *Tetrahedron*, 2015, **71**, 643–647.
26 M. Boominathan, V. Sathish, M. Nagaraj, N. Bhuvanesh, S. Muthusubramanian and S. Rajagopal, *RSC Adv.*, 2013, **3**, 22246–22252.
27 N. L. Sloan, S. K. Luthra, G. McRobbie, S. L. Pimlott and A. Sutherland, *RSC Adv.*, 2017, **7**, 54881–54891.
28 B. H. Aktas, M. Chorev, J. A. Halperin and G. Wagner, *US Pat.*, 2011042139W, 2011.
29 H. Kikuchi, T. Suzuki, M. Ogura, M. K. Homma, Y. Homma and Y. Oshima, *Bioorg. Med. Chem.*, 2015, **23**, 66–72.
30 M. Abarghaz, J.-J. Bourguignon, E. Klotz, J.-P. Macher, G. Ronsin, M. Schmitt and P. Wagner, *US Pat.*, 49940305A, 2005.
31 A. D. Becke, *J. Chem. Phys.*, 1998, **98**, 5648–5652.
32 C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B: Condens. Matter Mater. Phys.*, 1988, **37**, 785–789.
33 P. J. Stephens, F. J. Devlin, C. F. Chahalowsky and M. J. Frisch, *J. Phys. Chem.*, 1994, **98**, 11623–11627.
34 J. W. Hehre, L. Radom, P. V. R. Schleyer and J. A. Pople, *Ab initio molecular orbital theory*, Wiley, New York, NY, USA, 1986.

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35 J. Tomasi, B. Mennucci and É. Cancés, *J. Mol. Struct.*, 1999, **464**, 211–216.
36 F. Weinhold and C. R. Landis, *Valency and Bonding: A Natural Bond Orbital Donor-Acceptor Perspective*, Cambridge University Press, 2005.
37 *Gaussian 09, Revision A.02*, ed. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson and H. Nakatsuji, *et al.*, Gaussian, Inc., Wallingford, CT, USA, 2016.

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